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Description

[0001] The present invention relates to a method, a kit and a device for diagnosing breast cancer. The method comprises determining panels of methylation and miRNA markers.

BACKGROUND

[0002] Cancer is one of the most important medical and health problems in the world. As the leading cause of death worldwide, there were 12.4 million new cancer cases and 7.6 million cancer related deaths in 2008. It has been predicted that the deaths from cancer worldwide is continuously rising and 12 million deaths would be caused by cancer in the year of 2030. Breast cancer is the most common cancer among women. About one out of nine women will develop breast cancer during her life (Feuer, E.J., et al., The lifetime risk of developing breast cancer. J Natl Cancer Inst 85, 892-897 (1993)). Worldwide approximately 1.3 million women develop breast cancer each year. Mortality rates have continued to decrease over the years due to all the efforts and advances made in early diagnosis and treatment (Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61:69-90). Nevertheless, thousands of women die from this disease each year. In US women the overall five-year survival is 98% when diagnosed at an early stage as opposed to 23% when the disease has already spread to distant organs. Thus, early breast cancer detection belongs to one of the major challenges in the struggle against this disease. Mammographic screening is currently applied as the diagnostic standard. However, it has limitations due to its use of ionizing radiation and a false positive rate of 8-10%, also depending on the age of the individuals to be screened (Taplin S, Abraham L, Barlow WE, Fenton JJ, Berns EA, Carney PA, Cutter GR, Sickles EA, Carl D, Elmore JG. Mammography facility characteristics associated with interpretive accuracy of screening mammography. J Natl Cancer Inst 2008; 100: 876-87).

[0003] Most of the breast cancers occur sporadic, whereas familial breast cancer accounts for about 10% of all breast cancer cases (Fackenthal, J.D. & Olopade, O.I. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. Nat Rev Cancer 7, 937-948 (2007)). Mutations in the main breast cancer related genes, BRCA1 and BRCA2 account for 25% and other intermediate- and low-penetrance genes for about 5% of all familial cases (Yang, R. & Burwinkel, B. (eds.). Familial risk in breast cancer, 251-256 (Springer, 2010)). Recent genome-wide association studies (GW AS) and single candidate gene approaches have been quite successful in detecting genetic low-risk variants for breast cancer (Thomas, G., et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1 p 11.2 and 14q24.1 (RAD51 L1). Nat Genet 41, 579-584 (2009); Cox, A., et al. A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet 39, 352-358 (2007); Stacey, S.N., et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 40, 703-706 (2008); Ahmed, S., et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. Nat Genet 41,585-590 (2009); Easton, D.F., et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447, 1087-1093 (2007); Milne, R.L., et al. Risk of estrogen receptor-positive and -negative breast cancer and single-nucleotide polymorphism 2q35-rs13387042. JNatl Cancer Inst 101, 1012-1018 (2009); Frank, B., et al. Association of a common AKAP9 variant with breast cancer risk: a collaborative analysis. J Natl Cancer Inst 100,437-442 (2008)). However, a large number of breast cancer risk factors remain to be explored.

[0004] Compared to BC, ovarian cancer (OvCa) is comparable rare in occurrence, but is the leading cause of death from gynecologic cancers because of its high malignancy. In 2008, 225,000 women were diagnosed with ovarian cancer worldwide, and 140,000 of these women died from the disease. Typically, women with the OvCa present with few early symptoms, and thus nearly three-quarters of ovarian cancer cases present at an advanced stage, with the disease spread well beyond the ovaries. Pancreatic cancer (PaCa) is the most aggressive of all epithelial malignancies. With 279,000 new diagnoses of PaCa worldwide, the 5-year overall survival rate of PaCa patients is less than 5%. Although recent genome-wide association studies (GWAS) have successfully detected several genetic variants associated with the risk of BC, OvCa and PaCa, no valuable marker for the early detection of BC has been identified.

[0005] Metastatic breast cancer (MBC) is a major health issue, worldwide. Current treatment strategies target primarily palliative care with very few cases being cured. An alternate approach of tackling MBC is development of screening methods and applying biomarkers to identify high risk groups and therapy response. This could facilitate decision making for clinicians and help them adopt the appropriate treatment regime for the patients.

[0006] Circulating tumor cells (CTC) have been proposed as an FDA approved independent prognostic marker for metastasis, specifically for progression-free survival and overall survival. A cardinal cut off of greater than 5 CTCs per 7.5ml of blood has been defined as CTC positive (Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, et al; Circulating tumor cells, disease progression, and survival in metastatic breast cancer; N Engl J Med. 2004 Aug 19;351(8):781-91). However, it is important to note that a significant fraction of patients with overt distant metastases are negative for CTCs. This could be partly contributed to the phenomenon of epithelial-mesenchymal transition in CTCs, in which case they can be missed by enumeration techniques that exploit the expression of epithelial markers such as EpCAM or cytokeratin-8, -18 and -19.

[0007] Beside CTCs, also protein based circulating tumor markers like carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) are widely used as prognostic markers, as well as in monitoring breast cancer treatment success and follow-up (Uehara M, Kinoshita T, Hojo T, Akashi-Tanaka S, Iwamoto E, Fukutomi T. Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer. Int J Clin Oncol 2008;13:447-51; Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC, Jr. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007; 25:5287-312) However, the sensitivity of these markers is low. Therefore, new sensitive and specific as well as minimally invasive markers are needed.

[0008] Epigenetic changes are defined as changes in gene expression that are not due to any alterations in the genomic DNA sequence. Aberrant epigenetic signatures have been considered as a hallmark of human cancer (Esteller, M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 8, 286-298 (2007).). One of the most important epigenetic signatures, DNA methylation, has critical roles in the control of gene activities and in the architecture of the nucleus of the cell Weber, M., et al. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 37, 853-862 (2005)). Furthermore, unlike genetic markers or variants, DNA methylation is principally reversible. Therefore, the methylation profile of specific genes are considered as therapeutic targets (Mack, G.S. Epigenetic cancer therapy makes headway. J Natl Cancer Inst 98, 1443-1444 (2006)). Meanwhile, due to the variable character, DNA methylation may serve as a link between environmental factors and the genome. DNA methylation modulated by environmental factors or aging may alter the expression of critical genes of cells and consequently induce malignant transformation of cells or even a cancer (Widschwendter, M., et al. Epigenotyping in peripheral blood cell DNA and breast cancer risk: a proof of principle study. P LoS One 3, e2656 (2008)).

[0009] As an early event in the development of cancer, changes of DNA methylation are particularly promising as markers for the early detection of cancer. Recent studies have shown that methylation analysis of blood cell DNA can serve as a reliable and robust marker. Intensive studies have disclosed altered DNA methylation signatures in cancer on the somatic level, whereas only a few studies with candidate-gene-approach have analysed methylation signatures in peripheral blood DNA in cancer.

[0010] Previous studies have explored hypermethylation in the promoter regions of tumor suppressor genes and hypomethylation in the promoter regions of oncogenes in breast cancer compared to their normal adjacent tissues (Ito, Y., et al. Somatically acquired hypomethylation of IGF2 in breast and colorectal cancer. Hum Mol Genet 17, 2633-2643 (2008); Potapova, A., Hoffman, A.M., Godwin, A.K., Al-Saleem, T. & Cairns, P. Promoter hypermethylation of the PALB2 susceptibility gene in inherited and sporadic breast and ovarian cancer. Cancer Res 68, 998-1002 (2008); Radpour, R., et al. Methylation profiles of 22 candidate genes in breast cancer using high-throughput MALDI-TOF mass array. Oncogene 28, 2969-2978 (2009); Widschwendter, M. & Jones, P.A. DNA methylation and breast carcinogenesis. Oncogene 21, 5462-5482 (2002)). Very few studies have focused on the methylation signatures in the peripheral blood DNA and-breast cancer risk. In these studies, only specific genes, like BRCA1 (Iwamoto, T., Yamamoto, N., Taguchi, T., Tamaki, Y. & Noguchi, S. BRCA1 promoter methylation in peripheral blood cells is associated with increased risk of breast cancer with BRCA1 promoter methylation. Breast Cancer Res Treat 129, 69-77 (2011)), ATM (Flanagan, J.M., et al. Gene-body hypermethylation of ATM in peripheral blood DNA of bilateral breast cancer patients. Hum Mol Genet 18, 1332-1342 (2009)), and genes in specific pathways (Widschwendter et al. (2008), loc. cit.) have been investigated. There is thus a need in the art for the identification of further epigenetic markers of breast cancer and other cancers, preferably allowing the identification of afflicted subjects by obtaining a sample by a means of low invasiveness, e.g. by taking a blood sample.

[0011] MiRNAs are small, non-coding RNAs (~18-25 nucleotides in length) that regulate gene expression on a post-transcriptional level by degrading mRNA molecules or blocking their translation (Bartel DP.: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97). Hence, they play an essential role in the regulation of a large number of biological processes, including cancer (Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002; 99:15524-9). Under the standard nomenclature system, names are assigned to experimentally confirmed miRNAs. The prefix "mir" is followed by a dash and a number. The uncapitalized "mir-" refers to the pre-miRNA, while a capitalized "miR-" refers to the mature form. MiRNAs with nearly identical sequences bar one or two nucleotides are annotated with an additional lower case letter. Species of origin is designated with a three-letter prefix, e.g. hsa for Homo sapiens (human). Two mature miRNAs originating from opposite arms of the same pre-miRNA are denoted with a -3p or -5p suffix.

[0012] Circulating miRNAs are defined as miRNAs present in the cell-free component of body fluids like plasma, serum, and the like. Lawrie et al. (Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008; 141:672-5) were among the first to demonstrate the presence of miRNAs in bodily fluids. Since then, circulating miRNAs have been reported as aberrantly expressed in blood plasma or serum in different types of cancer, e.g. prostate, colorectal or esophageal carcinoma (Brase JC,

Johannes M, Schlomm T, Falth M, Haese A, Steuber T, Beissbarth T, Kuner R, Sultmann H. Circulating miRNAs are correlated with tumor progression in prostate cancer. Int J Cancer 2011;128:608-16.; Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel markers for early detection of colorectal cancer. Int J Cancer 2010;127:118-26.; Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Chen L, Zhang Y, Li Y, et al. Expression profile of microRNAs in serum: a fingerprint for esophageal squamous cell carcinoma. Clin Chem 2010; 56:1871-9.). Their most important advantages include the possibility to be measured repeatedly in a minimally invasive manner as well as their remarkable stability in plasma/serum, where they circulate mostly outside of exosomes and are stable due to their binding to Argonaute proteins (Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513-8; Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223-33; Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 2011; 108:5003-8).

[0013] WO 2013/190091 A1 identifies miR-801, miR-148b, miR-376c, miR-376a, miR-652, miR-409 and miR-127 as breast cancer markers. In the disclosed method of diagnosing and prognosticating breast cancer, the amount of the individual markers is measured. Measuring the entire group of microRNA markers in combination with the methylation status of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4 is not disclosed, nor is any synergistic effect of measuring the miRNAs in combination with at least one of the methylation markers, which can be measured in healthy subjects and in BC patients.

[0014] There is thus an urgent need in the art for improved methods for the diagnosis and prognosis of breast cancer, in particular primarybreast cancer, and metastasizing breast cancer. These methods would preferably be also used in preventive screening of apparently healthy subjects, a low grade of invasiveness would be preferred.

SUMMARY OF THE INVENTION

[0015] In a first aspect, the present invention relates to a method of diagnosing breast cancer (BC) in a subject, comprising (a) determining the methylation status of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, and (b) determining the amount of the miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and miR-148b in a subject, wherein the methylation status of the at least one methylation marker and the presence of the miRNAs is indicative of the risk of said subject to suffer from BC.

[0016] In a second aspect, the present invention relates to the use of a kit for diagnosing BC, comprising

a) one or more means of detecting the methylation status of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and DYRK4, and
b) means of detecting the amount of miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 and miR-148b.

[0017] In a third aspect, the present invention relates to a device for identifying BC, comprising: (a) an analyzing unit comprising (i) a detection agent for determining the methylation status of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and DYRK4, and (ii) a detection agent for determining the presence of: miR-652, miR-801, miR-376c, miR-376a, miR-376a, miR-127, miR-409 and miR-148b in a sample of a subject; and (b) an evaluation unit comprising a data processor having tangibly embedded an algorithm for carrying out a comparison of the amount determined by the analyzing unit with a reference and which is capable of generating an output file containing a diagnosis established based on the said comparison.

LIST OF FIGURES

[0018]

Fig. 1: Sample Description of blood-based biomarker panel for the early detection of breast cancer
Fig. 2: Methylation differences of eight genes in three validation rounds
Fig. 3: The discriminatory power of DNA methylation marker sets to distinguish BC cases from healthy controls in samples of other centres
Fig. 4: The discriminatory power of DNA methylation marker sets and miRNA marker sets to distinguish BC cases from healthy controls in samples from our group
Fig. 5: The methylation level of the eight genes in sporadic BC patients with different clinical characteristics (cases

from the second validation round)

Fig. 6: The methylation level of the eight genes in sporadic BC patients with different clinical characteristics (cases from our group)

Fig. 7: Sample Description of blood-based biomarker panel for the early detection of pancreatic cancer

Fig. 8: Methylation differences in genes comparing PaCa cases and controls

Fig. 9: Methylation differences in genes comparing PaCa cases and controls stratified by gender

Fig. 10: The discriminatory power of the methylation in genes to distinguish PaCa cases from healthy controls

Fig. 11: The methylation of genes in PaCa patients with different clinical characteristics

Fig. 12: Sample Description of blood-based biomarker panel for the early detection of ovarian cancer

Fig. 13: Methylation differences in genes comparing OvCa cases and controls

Fig. 14: The discriminatory power of the methylation in genes to distinguish OvCa cases from healthy controls

Fig. 15: The determination of breast cancer related CpG island shore in HYAL2

Fig. 16: The inverse correlation between the methylation and expression of S100P, SLC22A18 and DYRK4 in leucocytes

Fig. 17: The methylation levels of HYAL2 CpG sites by Illumina 450K

Fig. 18: The methylation levels of S100P CpG sites by Illumina 450K

Fig. 19: The methylation levels of SLC22A18 CpG sites by Illumina 450K

Fig. 20: The methylation levels of DYRK4 CpG sites by Illumina 450K

Fig. 21: The methylation levels of FUT7 CpG sites by Illumina 450K

Fig. 22: The methylation levels of RAPSN CpG sites by Illumina 450K

Fig. 23: The methylation levels of RPTOR CpG sites by Illumina 450K

Fig. 24: The methylation levels of MGRN1 CpG sites by Illumina 450K

Fig. 25: The inverse correlation between the methylation and expression of HYAL2 in leucocytes, (a) The box plots show the methylation levels of cg27091787 and adjacent CpG sites in the HYAL2-A amplicon in leucocytes from 36 sporadic BC cases and 40 healthy controls. The box plot of cg27091787 is framed in box for emphasis. (b) The box plot shows the expression level of HYAL2 in leucocytes from sporadic BC cases and healthy controls. The presented *p*-values were calculated by Mann-Whitney U test. The circles indicate outliers. (c) The inverse correlation between the methylation level of cg27091787 and HYAL2 expression in leucocytes.

Fig. 26: The methylation levels of four CpG sites in HYAL2-A amplicon in sorted leucocytes fractions. The methylation levels were measured in triplicates in the samples (DNA from whole blood and from sorted leucocytes fractions) from seven sporadic BC cases and 14 healthy controls. The methylation difference between cases and controls was calculated by t-test. The methylation levels of cg27091787 are presented by box and whisker plot. The circle indicates an outlier.

LIST OF SEQUENCES

[0019]

SEQ ID NO: 1	hsa-miR-652-3p (MIMAT0003322): aauggcgccacuaggguugug
SEQ ID NO: 2	hsa-miR-652-5p (MIMAT0022709): caacccuaggagagggugccauuca
SEQ ID NO: 3	miR-801 located on chromosome 1: 28847698 - 28847793: gauugcucugcgugcggaaucgac
SEQ ID NO: 4	hsa-miR-376c-3p (MIMAT0000720): aacauagaggaaauuccacgu
SEQ ID NO: 5	hsa-miR-376c-5p (MIMAT0022861): gguggauauuccuucuauguu
SEQ ID NO: 6	hsa-miR-376a-3p (MIMAT0000729): aucauagaggaaaauccacgu
SEQ ID NO: 7	hsa-miR-376a-5p (MIMAT0003386): guagauucuccuucuaugagua
SEQ ID NO: 8	hsa-miR-127-3p (MIMAT0000446): ucggauccgucugagcuuggcu
SEQ ID NO: 9	hsa-miR-127-5p (MIMAT0004604): cugaagcucagagggcucugau
SEQ ID NO: 10	hsa-miR-409-3p (MIMAT0001639): gaauguugcucggugaaccccu
SEQ ID NO: 11	hsa-miR-409-5p (MIMAT0001638): agguuacccgagcaacuuugcau
SEQ ID NO: 12	hsa-miR-148b-3p (MIMAT0000759): ucagugcaucacagaacuuugu
SEQ ID NO: 13	hsa-miR-148b-5p (MIMAT0004699): aaguucuguuauacacucaggc
SEQ ID NO: 14	HYAL2 (NM_003773.4)
SEQ ID NO: 15	HYAL2 (NM_033158.4)
SEQ ID NO: 16	HYAL2 (NP_003764.3)
SEQ ID NO: 17	HYAL2 (NP_149348.2)
SEQ ID NO: 18	MGRN1 (NM_001142289.2)

(continued)

SEQ ID NO: 19	MGRN1 (NM_001142290.2)
SEQ ID NO: 20	MGRN1 (NM_001142291.2)
SEQ ID NO: 21	MGRN1 (NM_015246.3)
SEQ ID NO: 22	MGRN1 (NP_001135761.2)
SEQ ID NO: 23	MGRN1 (NP_001135762.1)
SEQ ID NO: 24	MGRN1 (NP_001135763.2)
SEQ ID NO: 25	MGRN1 (NP_056061.1)
SEQ ID NO: 26	RPTOR (NM_001163034.1)
SEQ ID NO: 27	RPTOR (NM_020761.2)
SEQ ID NO: 28	RPTOR (NP_001156506.1)
SEQ ID NO: 29	RPTOR (NP_065812.1)
SEQ ID NO: 30	SLC22A18 (NM_002555.5)
SEQ ID NO: 31	SLC22A18 (NM_183233.2)
SEQ ID NO: 32	SLC22A18 (NP_002546.3)
SEQ ID NO: 33	SLC22A18 (NP_899056.2)
SEQ ID NO: 34	FUT7 (NM_004479.3)
SEQ ID NO: 35	FUT7 (NP_004470.1)
SEQ ID NO: 36	RAPSN (NM_005055.4)
SEQ ID NO: 37	RAPSN (NM_032645.4)
SEQ ID NO: 38	RAPSN (NP_005046.2)
SEQ ID NO: 39	RAPSN (NP_116034.2)
SEQ ID NO: 40	S100P (NM_005980.2)
SEQ ID NO: 41	S100P (NP_005971.1)
SEQ ID NO: 42	DYRK4 (NM_001282285.1)
SEQ ID NO: 43	DYRK4 (NM_001282286.1)
SEQ ID NO: 44	DYRK4 (NM_003845.2)
SEQ ID NO: 45	DYRK4 (NP_001269214.1)
SEQ ID NO: 46	DYRK4 (NP_001269215.1)
SEQ ID NO: 47	DYRK4 (NP_003836.1)
SEQ ID NO: 33	sense sequence of HYAL2 primer
SEQ ID NO: 34	antisense sequence HYAL2 primer
SEQ ID NO: 33	sense sequence of HYAL2-is-310 primer
SEQ ID NO: 34	antisense sequence HYAL2-is-310 primer
SEQ ID NO: 33	sense sequence of HYAL2-is-325 primer
SEQ ID NO: 34	antisense sequence HYAL2-is-325 primer
SEQ ID NO: 35	sense sequence MGRN1 primer
SEQ ID NO: 36	antisense sequence MGRN1 primer
SEQ ID NO: 37	sense sequence RPTOR primer
SEQ ID NO: 38	antisense sequence RPTOR primer
SEQ ID NO: 39	sense sequence of SLC22A18 primer
SEQ ID NO: 40	antisense sequence SLC22A18 primer
SEQ ID NO: 41	sense sequence FUT7 primer
SEQ ID NO: 42	antisense sequence FUT7 primer
SEQ ID NO: 43	sense sequence RAPSN primer
SEQ ID NO: 44	antisense sequence RAPSN primer
SEQ ID NO: 45	sense sequence S100P primer
SEQ ID NO: 46	antisense sequence S100P primer
SEQ ID NO: 47	sense sequence DYRK4 primer
SEQ ID NO: 48	antisense sequence DYRK4 primer

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0020] Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

[0021] Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. In the event of a conflict between the definitions or teachings of such references and definitions or teachings recited in the present specification, the text of the present specification takes precedence.

[0022] In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

[0023] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0024] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents, unless the content clearly dictates otherwise.

[0025] The term "about" when used in connection with a numerical value is meant to encompass numerical values within a range having a lower limit that is 5% smaller than the indicated numerical value and having an upper limit that is 5% larger than the indicated numerical value.

[0026] "Nucleic acid molecules" are understood as a polymeric or oligomeric macromolecule made from nucleotide monomers. Nucleotide monomers are composed of a nucleobase, a five-carbon sugar (such as but not limited to ribose or 2'-deoxyribose), and one to three phosphate groups. Typically, a polynucleotide is formed through phosphodiester bonds between the individual nucleotide monomers. In the context of the present invention referred to nucleic acid molecules include but are not limited to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and mixtures thereof such as e.g. RNA-DNA hybrids. The terms "polynucleotide", "nucleic acid" and "nucleic acid molecule" are used interchangeably herein. The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584). Aptamers are nucleic acids which bind with high affinity to a polypeptide, here mir146-a. Aptamers can be isolated by selection methods such as SELEmir146-a (see e.g. Jayasena (1999) Clin. Chem., 45, 1628-50; Klug and Famulok (1994) M. Mol. Biol. Rep., 20, 97-107; US 5,582,981) from a large pool of different single-stranded RNA molecules. Aptamers can also be synthesized and selected in their mirror-image form, for example as the L-ribonucleotide (Nolte et al. (1996) Nat. Biotechnol., 14, 1116-9; Klussmann et al. (1996) Nat. Biotechnol., 14, 1112-5). Forms which have been isolated in this way enjoy the advantage that they are not degraded by naturally occurring ribonucleases and, therefore, possess greater stability. Nucleic acids may be degraded by endonucleases or exonucleases, in particular by DNases and RNases which can be found in the cell. It is, therefore, advantageous to modify the nucleic acids in order to stabilize them against degradation, thereby ensuring that a high concentration of the nucleic acid is maintained in the cell over a long period of time (Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO95/11910; WO98/37240; WO97/29116). Typically, such a stabilization can be obtained by introducing one or more internucleotide phosphorus groups or by introducing one or more non-phosphorus internucleotides. Suitable modified internucleotides are compiled in Uhlmann and Peyman (1990), supra (see also Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO95/11910; WO98/37240; WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid which can be employed in one of the uses according to the invention contain, for example, methyl phosphonate, phosphorothioate, phosphoramidate, phosphorodithioate and/or phosphate esters, whereas non-phosphorus internucleotide analogues contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also the intention that this modification should improve the durability of a pharmaceutical composition which can be employed in one of the uses according to the invention. Nucleic acids may be selected from the group consisting of, a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a glycol nucleic acid (GNA), a threose nucleic acid (TNA), a microRNA (miRNA), and a small interfering RNA (siRNA), a polynucleotide probe, a primer(s) (e.g. a primer pair), in particular a primer(s) for polymerase chain reaction (PCR), reverse transcription (RT) reaction, or DNA sequencing.

[0027] In the context of the different aspects of present invention, the term nucleic acid comprises genomic DNA, cDNA, recombinant DNA, cRNA, mRNA, microRNA (miRNA) and small interfering RNA (siRNA). A nucleic acid may consist of an entire gene, or a portion thereof. The nucleic acid can also be an artificial nucleic acid. Artificial nucleic acids include polyamide or peptide nucleic acid (PNA), morpholino and locked nucleic acid (LNA), as well as glycol nucleic acid (GNA) and threose nucleic acid (TNA). Each of these is distinguished from naturally-occurring DNA or RNA by changes to the backbone of the molecule as well known to the person skilled in the art.

[0028] As used herein, the term "microRNA" and variations such as "miRNA" and "miR" is understood by the skilled artisan and relates to a short ribonucleic acid (RNA) molecule found in eukaryotic cells and in body fluids of metazoan organisms. MiRNA include human miRNAs, mature single stranded miRNAs, precursor miRNAs (pre-miR), and variants thereof, which may be naturally occurring. In some instances, the term "miRNA" also includes primary miRNA transcripts (pri-miRNAs) and duplex miRNAs. Unless otherwise noted, when used herein, the name of a specific miRNA refers to the mature miRNA. MiRNA-precursor may consists of 25 to several thousand nucleotides, typically 40 to 130, 50 to 120, or 60 to 110 nucleotides. Typically, a mature miRNA consists of 5 to 100 nucleotides, often 10 to 50, 12 to 40, or 18 to 26 nucleotides. The term miRNA also includes the "guide" strand which eventually enters the RNA-induced silencing complex (RISC) as well as to the "passenger" strand complementary thereto.

[0029] The sequence of several miRNAs is known in the art and readily assessable to the skilled person via well-known sequence databases, such as e.g. miRBase (http://www.mirbase.org/), (Griffiths-Jones S., NAR 2004 32(Database Issue):D109-D111; Kozomara A, Griffiths-Jones S., NAR 2011 39(Database Issue):D152-D157). It is understood that below indicated database accession numbers of the individual miRNAs are those of miRNAs of human origin. However these database entries also provide the database accession numbers of the respective miRNA of different origin, such as e.g. mirNAs of any mammal, reptile, or bird origin, such as e.g. those selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, and gorillas miRNA. It is also understood that the reference to a specific miRNA by its number (e.g. miR-652) equally refers to the -3p and -5p sequence (miR-652-3p and miR-652-5p).

[0030] The sequence of miR-652 is deposited at miRBase ID MI0003667 which comprises hsa-miR-652-3p (MIMAT0003322) and hsa-miR-652-5p (MIMAT0022709), which corresponds to SEQ ID NO: 1 and 2, respectively, of the present invention.

[0031] The sequence of miR-801 was deposited at miRBase ID MI0005202: 5'-GAUUGCUCUGCGUGCGGAAUC-GAC-3', however, it is now considered as a fragment of U11 spliceosomal RNA and was thus remove from miRBase. The pre-miRNA-801 is located at chr1: 28847698 - 28847793. Its sequence corresponds to SEQ ID NO: 3 of the present invention.

[0032] miR-376c, also referred to as miR-368, is deposited at miRBase ID MI0000776, which comprises miR-376c-3p (MIMAT0000720) and hsa-miR-376c-5p (MIMAT0022861), which corresponds to SEQ ID NO: 4 and 5, respectively, of the present invention.

[0033] The sequence of miR-376a is deposited at miRBase ID MI0000784, which comprises hsa-miR-376a-3p (MIMAT0000729) and hsa-miR-376a-5p (MIMAT0003386), which corresponds to SEQ ID NO: 6 and 7, respectively, of the present invention.

[0034] The sequence of miR-127 is deposited at miRBase ID MI0000472, which comprises hsa-miR-127-3p (MIMAT0000446) and hsa-miR-127-5p (MIMAT0004604), which corresponds to SEQ ID NO: 8 and 9, respectively, of the present invention.

[0035] The sequence of miR-409 is deposited at miRBase ID MI0001735, which comprises hsa-miR-409-3p (MIMAT0001639) and hsa-miR-409-5p (MIMAT0001638), which corresponds to SEQ ID NO: 10 and 11, respectively, of the present invention.

[0036] The sequence of miR-148b is deposited at miRBase ID MI0000811, which comprises hsa-miR-148b-3p (MIMAT0000759) and hsa-miR-148b-5p (MIMAT0004699), which corresponds to SEQ ID NO: 12 and 13, respectively, of the present invention.

[0037] The term "combination of miRNAs" relates to combinations of the miRNAs of the present invention. The amount of a miRNA can be determined in a sample of a subject by techniques well known in the art. Depending on the nature of the sample, the amount may be determined by PCR based techniques for quantifying the amount of a polynucleotide or by other methods like mass spectrometry or (next generation) sequencing or one of the methods described in the examples (Cissell KA, Deo SK. Trends in microRNA detection. Anal Bioanal Chem. 2009;394(4):1109-1116 or de Planell-Saguer M, Rodicio MC. Analytical aspects of microRNA in diagnostics: a review. Anal Chim Acta 2011 Aug 12;699(2):134-52). The term "determining the amounts of at least the miRNAs of a combination of miRNAs", as used herein, preferably relates to determining the amount of each of the miRNAs of the combination separately in order to be able to compare the amount of each miRNA of the combination to a reference specific for said miRNA.

[0038] The term "probe" as used herein refers to a single-strand oligonucleotide which is typically used for the detection of target RNA and/or DNA sequences that is complementary to the sequence of the probe. A probe hybridizes to single-

stranded nucleic acid (DNA or RNA) whose nucleotide sequence allows for nucleotide pairing due to complementarity between the probe and the target sequence. The length of a probe depends on the intended use as well as the required specificity of the probe. Typically, a probe is 20-500 (i.e. 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500) nucleotides long, preferably 20-100 nucleotides, more preferably 20-50. For detection of microRNA probes are between 12 and 30 nucleotides. Probes are used in various experimental set ups such as but not limited to Southern and Northern Blots, for real-time PCR and In Situ Hybridization (ISH) as well as for microarray experiments. A probe may be unlabeled, directly labelled, or indirectly labelled, such as with biotin to which a streptavidin complex may later bind. Said label may be a molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, suitable labels include 32P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and other entities which are or can be made detectable. A label may be incorporated into nucleic acids at any position, e.g. at the 3' end, at the 5' end or internally. The term "probe" also encompasses nucleic acids differing in the composition of their backbone such as but not limited to peptide nucleic acids (PNAs), locked nucleic acids (LNAs), glycol nucleic acids (GNAs) and threose nucleic acids (TNAs).

[0039] The term "primer" as used herein refers to a single-strand oligonucleotide which typically serves as a starting point for DNA-replicating enzymes. A primer binds to or hybridises with a DNA template and typically comprises a sequence being complementary to the DNA sequence to which it is supposed to bind. A primer may also comprise additional sequences e.g. sequences serving as nuclease cleavage sites (e.g. Bam HI, Hind III, etc.). The length of a primer is chosen depending on the intended use. For instance, primers used for the amplification of DNA in Polymerase-Chain Reactions (PCR) typically have a length of at least 10 nucleotides, preferably between 10 to 50 (i.e. 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50) nucleotides, more preferably between 15 and 30 nucleotides. Shorter primers of at least 5 nucleotides are used for sequencing of DNA templates. Also encompassed in the term "primer" are "degenerate primers" which are a mixture of similar, but not identical primers. A primer may be tagged or labelled with a marker molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means.

[0040] The term "expression level" refers to the amount of gene product present in the body or a sample at a certain point of time. The expression level can e.g. be measured/quantified/detected by means of the protein or mRNA expressed from the gene. The expression level can for example be quantified by normalizing the amount of gene product of interest present in a sample with the total amount of gene product of the same category (total protein or mRNA) in the same sample or a reference sample (e.g. a sample taken at the same time from the same individual or a part of identical size (weight, volume) of the same sample) or by identifying the amount of gene product of interest per defined sample size (weight, volume, etc.). The expression level can be measured or detected by means of any method as known in the art, e.g. methods for the direct detection and quantification of the gene product of interest (such as mass spectrometry) or methods for the indirect detection and measurement of the gene product of interest that usually work via binding of the gene product of interest with one or more different molecules or detection means (e.g. primer(s), probes, antibodies, protein scaffolds) specific for the gene product of interest. The determination of the level of gene copies comprising also the determination of the absence or presence of one or more fragments (e.g. via nucleic acid probes or primers, e.g. quantitative PCR, Multiplex ligation-dependent probe amplification (MLPA) PCR) is also within the knowledge of the skilled artisan.

[0041] The terms "protein" and "polypeptide" are used interchangeably herein and refer to any peptide-linked chain of amino acids, regardless of length or post-translational modification. Proteins usable in the present invention (including protein derivatives, protein variants, protein fragments, protein segments, protein epitops and protein domains) can be further modified by chemical modification. This means such a chemically modified polypeptide comprises other chemical groups than the 20 naturally occurring amino acids. Examples of such other chemical groups include without limitation glycosylated amino acids and phosphorylated amino acids. Chemical modifications of a polypeptide may provide advantageous properties as compared to the parent polypeptide, e.g. one or more of enhanced stability, increased biological half-life, or increased water solubility. Chemical modifications applicable to the variants usable in the present invention include without limitation: PEGylation, glycosylation of non-glycosylated parent polypeptides, or the modification of the glycosylation pattern present in the parent polypeptide.

[0042] In the context of the different aspects of present invention, the term "peptide" refers to a short polymer of amino acids linked by peptide bonds. It has the same chemical (peptide) bonds as proteins, but is commonly shorter in length. The shortest peptide is a dipeptide, consisting of two amino acids joined by a single peptide bond. There can also be a tripeptide, tetrapeptide, pentapeptide, etc. Preferably, the peptide has a length of up to 8, 10, 12, 15, 18 or 20 amino acids. A peptide has an amino end and a carboxyl end, unless it is a cyclic peptide.

[0043] In the context of the different aspects of present invention, the term "polypeptide" refers to a single linear chain of amino acids bonded together by peptide bonds and preferably comprises at least about 21 amino acids. A polypeptide can be one chain of a protein that is composed of more than one chain or it can be the protein itself if the protein is composed of one chain.

[0044] In the context of the different aspects of present invention, the term "protein" refers to a molecule comprising one or more polypeptides that resume a secondary and tertiary structure and additionally refers to a protein that is made up of several polypeptides, i.e. several subunits, forming quaternary structures. The protein has sometimes non-peptide groups attached, which can be called prosthetic groups or cofactors. The primary structure of a protein or polypeptide is the sequence of amino acids in the polypeptide chain. The secondary structure in a protein is the general three-dimensional form of local segments of the protein. It does not, however, describe specific atomic positions in three-dimensional space, which are considered to be tertiary structure. In proteins, the secondary structure is defined by patterns of hydrogen bonds between backbone amide and carboxyl groups. The tertiary structure of a protein is the three-dimensional structure of the protein determined by the atomic coordinates. The quaternary structure is the arrangement of multiple folded or coiled protein or polypeptide molecules molecules in a multi-subunit complex. The terms "amino acid chain" and "polypeptide chain" are used synonymously in the context of present invention. The term "post-translational" used herein refers to events that occur after the translation of a nucleotide triplet into an amino acid and the formation of a peptide bond to the proceeding amino acid in the sequence. Such post-translational events may occur after the entire polypeptide was formed or already during the translation process on those parts of the polypeptide that have already been translated. Post-translational events typically alter or modify the chemical or structural properties of the resultant polypeptide. Examples of post-translational events include but are not limited to events such as glycosylation or phosphorylation of amino acids, or cleavage of the peptide chain, e.g. by an endopeptidase. The term "co-translational" used herein refers to events that occur during the translation process of a nucleotide triplet into an amino acid chain. Those events typically alter or modify the chemical or structural properties of the resultant amino acid chain. Examples of co-translational events include but are not limited to events that may stop the translation process entirely or interrupted the peptide bond formation resulting in two discreet translation products.

[0045] The term "segment" refers to any part of a macromolecule (e.g. a polypeptide, protein or polyprotein) into which this macromolecule can be divided. A macromolecule may consist of one or more segments. Such segmentation may exist due to functional (e.g. having immunoreactive features or membrane attachment functions) or structural (e.g. nucleotide or amino acid sequence, or secondary or tertiary structure) properties of the macromolecule and/or the individual segment. In the context of the present invention it is preferred that the term "segment" refers to a part of a protein or polyprotein. It is particularly preferred that such segment folds and/or functions independently of the rest of the protein or polyprotein.

[0046] An "epitope", also known as antigenic determinant, is the segment of a macromolecule that is recognized by the immune system, specifically by antibodies, B cells, or T cells. Such epitope is that part or segment of a macromolecule capable of binding to an antibody or antigen-binding fragment thereof. In this context, the term "binding" preferably relates to a specific binding. In the context of the present invention it is preferred that the term "epitope" refers to the segment of protein or polyprotein that is recognized by the immune system. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0047] As used herein, the term "domain" refers to the segment of a protein or polyprotein sequence or structure (or corresponding nucleotide sequence) that can evolve, function, and/or exist independently of the rest of the protein chain. Typically, a protein consists of one or several domains with each of them being three-dimensional structure that are stable and folded independently of the rest of the protein chain. Such domain typically forms an independent functional unit within the protein (e.g. transmembrane-domains, immunoglobulin-like domains, or DNA-binding domains).

[0048] The amino acid sequence of several peptides and proteins, as well as the nucleotide sequences encoding the respective peptides and proteins are well known in the art and readily assessable to the skilled person via well-known sequence databases, such as e.g. Genbank (http://www.ncbi.nlm.nih.gov/genbank/). It is understood that below indicated database accession numbers of the individual sequence are those of human origin. However these database entries also provide the database accession numbers of the respective nucleotide sequences of different origin, such as e.g. amino acid or nucleotides sequences of any mammal, reptile, or bird origin, such as e.g. those selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, and gorillas nucleotide amino acid or nucleotides sequences.

HYAL2:

Genbank Acc No: NM_003773.4 (GI:289802998) for transcript variant 1, which corresponds to SEQ ID NO:14 of the present invention

Genbank Acc No: NM_033158.4 (GI:289802999) for transcript variant 2, which corresponds to SEQ ID NO:15 of the present application;

Genbank Acc No: NP_003764.3 (GI:15022801), for the HYAL2 polypeptide encoded by transcript variant 1,

which corresponds to SEQ ID NO:16 of the present invention, and
Genbank Acc No: NP_149348.2 (GI:34304377), for the HYAL2 polypeptide encoded by transcript variant 2, which corresponds to SEQ ID NO:17 of the present invention

MGRN1:

Genbank Acc No: NM_001142289.2 for the transcript variant 2, which corresponds to SEQ ID NO:18 of the present invention, and
Genbank Acc No: NM_001142290.2 for the transcript variant 3, which corresponds to SEQ ID NO:19 of the present invention, and
Genbank Acc No: NM_001142291.2 for the transcript variant 4, which corresponds to SEQ ID NO:20 of the present invention, and
Genbank Acc No: NM_015246.3 for the transcript variant 1, which corresponds to SEQ ID NO:21 of the present invention, and
Genbank Acc No: NP_001135761.2 for the MGRN1 polypetide encoded by the transcript variant 2, which corresponds to SEQ ID NO:22 of the present invention;
Genbank Acc No: NP_001135762.1 for the MGRN1 polypetide encoded by the transcript variant 3, which corresponds to SEQ ID NO:23 of the present invention;
Genbank Acc No: NP_001135763.2 for the MGRN1 polypetide encoded by the transcript variant 4, which corresponds to SEQ ID NO:24 of the present invention;
Genbank Acc No: NP_056061.1 for the MGRN1 polypetide encoded by the transcript variant 1, which corresponds to SEQ ID NO:25 of the present invention;

RPTOR

Genbank Acc No: NM_001163034.1 for the transcript variant 2, which corresponds to SEQ ID NO:26 of the present invention
Genbank Acc No: NM_020761.2 for the transcript variant 1, which corresponds to SEQ ID NO:27 of the present invention
Genbank Acc No: NP 001156506.1 for the RPTOR polypetide encoded by the transcript variant 2, which corresponds to SEQ ID NO:28 of the present invention;
Genbank Acc No: NP_065812.1 for the RPTOR polypetide encoded by the transcript variant 1, which corresponds to SEQ ID NO:29 of the present invention;

SLC22A18

Genbank Acc No: NM_002555.5 for the transcript variant 1, which corresponds to SEQ ID NO:30 of the present invention
Genbank Acc No: NM_183233.2 for the transcript variant 2, which corresponds to SEQ ID NO:31 of the present invention
Genbank Acc No: NP_002546.3 for the SLC22A18 polypetide encoded by the transcript variant 1, which corresponds to SEQ ID NO:32 of the present invention;
Genbank Acc No: NP_899056.2 for the SLC22A18 polypetide encoded by the transcript variant 2, which corresponds to SEQ ID NO:33 of the present invention;

FUT7

Genbank Acc No: NM_004479.3 for the transcript, which corresponds to SEQ ID NO:34 of the present invention
Genbank Acc No: NP_004470.1 for the FUT7 polypetide encoded by the transcript, which corresponds to SEQ ID NO:35 of the present invention;

RAPSN

Genbank Acc No: NM_005055.4 for the transcript variant 1, which corresponds to SEQ ID NO:36 of the present invention
Genbank Acc No: NM_032645.4 for the transcript variant 2, which corresponds to SEQ ID NO:37 of the present invention
Genbank Acc No: NP_005046.2 for the RAPSN polypetide encoded by the transcript variant 1, which corre-

sponds to SEQ ID NO:38 of the present invention;
Genbank Acc No: NP_116034.2 for the RAPSN polypetide encoded by the transcript variant 1, which corresponds to SEQ ID NO:39 of the present invention;

S100P

Genbank Acc No: NM_005980.2 for the transcript, which corresponds to SEQ ID NO:40 of the present invention
Genbank Acc No: NP_005971.1 for the S100P polypetide encoded by the transcript, which corresponds to SEQ ID NO:41 of the present invention;

DYRK4

Genbank Acc No: NM_001282285.1 for the transcript variant 2, which corresponds to SEQ ID NO:42 of the present invention
Genbank Acc No: NM_001282286.1 for the transcript variant 3, which corresponds to SEQ ID NO:43 of the present invention
Genbank Acc No: NM_003845.2 for the transcript variant 1, which corresponds to SEQ ID NO:44 of the present invention
Genbank Acc No: NP_001269214.1 for the DYRK4 polypetide encoded by the transcript variant 2, which corresponds to SEQ ID NO:45 of the present invention;
Genbank Acc No: NP_001269215.1 for the DYRK4 polypetide encoded by the transcript variant 3, which corresponds to SEQ ID NO:46 of the present invention;
Genbank Acc No: NP_003836.1 for the DYRK4 polypetide encoded by the transcript variant 1, which corresponds to SEQ ID NO:47 of the present invention;

[0049] As used herein, the term "variant" is to be understood as a polynucleotide or protein which differs in comparison to the polynucleotide or protein from which it is derived by one or more changes in its length or sequence. The polypeptide or polynucleotide from which a protein or nucleic acid variant is derived is also known as the parent polypeptide or polynucleotide. The term "variant" comprises "fragments" or "derivatives" of the parent molecule. Typically, "fragments" are smaller in length or size than the parent molecule, whilst "derivatives" exhibit one or more differences in their sequence in comparison to the parent molecule. Also encompassed modified molecules such as but not limited to post-translationally modified proteins (e.g. glycosylated, biotinylated, phosphorylated, ubiquitinated, palmitoylated, or proteolytically cleaved proteins) and modified nucleic acids such as methylated DNA. Also mixtures of different molecules such as but not limited to RNA-DNA hybrids, are encompassed by the term "variant". Typically, a variant is constructed artificially, preferably by gene-technological means whilst the parent polypeptide or polynucleotide is a wild-type protein or polynucleotide. However, also naturally occurring variants are to be understood to be encompassed by the term "variant" as used herein. Further, the variants usable in the present invention may also be derived from homologs, orthologs, or paralogs of the parent molecule or from artificially constructed variant, provided that the variant exhibits at least one biological activity of the parent molecule, i.e. is functionally active.

[0050] A variant usable in the present invention exhibits a total number of up to 200 (up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200) changes in the amino acid or nucleotide sequence (i.e. exchanges, insertions, deletions, 5'-, 3'-, N-terminal, and/or C-terminal truncations). Amino acid exchanges may be conservative and/or non-conservative. A variant usable in the present invention differs from the protein or polynucleotide from which it is derived by up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid or nucleic acid exchanges. Alternatively or additionally, a "variant" as used herein, can be characterized by a certain degree of sequence identity to the parent polypeptide or parent polynucleotide from which it is derived. More precisely, a protein variant in the context of the present invention exhibits at least 80% sequence identity to its parent polypeptide. A polynucleotide variant in the context of the present invention exhibits at least 80% sequence identity to its parent polynucleotide. Preferably, the sequence identity of protein variants is over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids. Preferably, the sequence identity of polynucleotide variants is over a continuous stretch of 60, 90, 120, 135, 150, 180, 210, 240, 270, 300 or more nucleotides.

[0051] The term "at least 80% sequence identity" is used throughout the specification with regard to polypeptide and polynucleotide sequence comparisons. This expression preferably refers to a sequence identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide. Preferably, the polypeptide in question and the reference polypeptide exhibit the indicated sequence identity over a continuous stretch

of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids or over the entire length of the reference polypeptide. Preferably, the polynucleotide in question and the reference polynucleotide exhibit the indicated sequence identity over a continuous stretch of 60, 90, 120, 135, 150, 180, 210, 240, 270, 300 or more nucleotides or over the entire length of the reference polypeptide.

[0052] The terms "deletion variant" and "fragment" are used interchangeably herein. A fragment may be naturally occurring (e.g. splice variants) or it may be constructed artificially, preferably by gene-technological means. Preferably, a fragment (or deletion variant) has a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids or nucleic acids as compared to the parent polypeptide. In case where two sequences are compared and the reference sequence is not specified in comparison to which the sequence identity percentage is to be calculated, the sequence identity is to be calculated with reference to the longer of the two sequences to be compared, if not specifically indicated otherwise. If the reference sequence is indicated, the sequence identity is determined on the basis of the full length of the reference sequence indicated by SEQ ID, if not specifically indicated otherwise.

[0053] The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877), with hmmalign (HMMER package, http://hmmer.wustl.edu/) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673-80) available e.g. on http://www.ebi.ac.uk/Tools/clustalw/ or on http://www.ebi.ac.uk/Tools/clustalw2/index.html or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on http://www.ebi.ac.uk/Tools/clustalw/ or http://www.ebi.ac.uk/Tools/clustalw2/index.html. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12, to obtain homologous polynucleotide sequences.

[0054] "Hybridization" can also be used as a measure of sequence identity or homology between two nucleic acid sequences. A nucleic acid sequence encoding F, N, or M2-1, or a portion of any of these can be used as a hybridization probe according to standard hybridization techniques. The hybridization of an F, N, or M2-1 probe to DNA or RNA from a test source is an indication of the presence of the F DNA or RNA, N DNA or RNA, or M2-1 DNA or RNA, respectively, in the test source. Hybridization conditions are known to those skilled in the art and can be found, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y., 6.3.1-6.3.6, 1991. "Moderate hybridization conditions" are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1X SSC, 0.1% SDS at 50°C. "Highly stringent conditions" are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1 % SDS at 65°C.

[0055] Semi-conservative and especially conservative amino acid substitutions, wherein an amino acid is substituted with a chemically related amino acid are preferred. Typical substitutions are among the aliphatic amino acids, among the amino acids having aliphatic hydroxyl side chain, among the amino acids having acidic residues, among the amide derivatives, among the amino acids with basic residues, or the amino acids having aromatic residues. Typical semi-conservative and conservative substitutions are:

Amino acid	Conservative substitution	Semi-conservative substitution
A	G; S; T	N; V; C
C	A; V; L	M; I; F; G
D	E; N; Q	A; S; T; K; R; H
E	D; Q; N	A; S; T; K; R; H
F	W; Y; L; M; H	I; V; A
G	A	S; N; T; D; E; N; Q
H	Y; F; K; R	L; M; A
I	V; L; M; A	F; Y; W; G
K	R; H	D; E; N; Q; S; T; A
L	M; I; V; A	F; Y; W; H; C
M	L; I; V; A	F; Y; W; C;
N	Q	D; E; S; T; A; G; K; R
P	V; I	L; A; M; W; Y; S; T; C; F
Q	N	D; E; A; S; T; L; M; K; R
R	K; H	N; Q; S; T; D; E; A

(continued)

Amino acid	Conservative substitution	Semi-conservative substitution
S	A; T; G; N	D; E; R; K
T	A; S; G; N; V	D; E; R; K; I
V	A; L; I	M; T; C; N
W	F; Y; H	L; M; I; V; C
Y	F; W; H	L; M; I; V; C

[0056] Changing from A, F, H, I, L, M, P, V, W or Y to C is semi-conservative if the new cysteine remains as a free thiol. Furthermore, the skilled person will appreciate that glycines at sterically demanding positions should not be substituted and that P should not be introduced into parts of the protein which have an alpha-helical or a beta-sheet structure.

[0057] The term "tissue" as used herein, refers to an ensemble of cells of the same origin which fulfil a specific function concertedly. Examples of a tissue include but are not limited to connective tissue, muscle tissue, nervous tissue, and epithelial tissue. Multiple tissues together form an "organ" to carry out a specific function. Examples of an organ include but are not limited to glands, muscle, blood, brain, heart, liver, kidney, stomach, skeleton, joint, and skin.

[0058] The term "disease" and "disorder" are used interchangeably herein, referring to an abnormal condition, especially an abnormal medical condition such as an illness or injury, wherein a tissue, an organ or an individual is not able to efficiently fulfil its function anymore. Typically, but not necessarily, a disease is associated with specific symptoms or signs indicating the presence of such disease. The presence of such symptoms or signs may thus, be indicative for a tissue, an organ or an individual suffering from a disease. An alteration of these symptoms or signs may be indicative for the progression of such a disease. A progression of a disease is typically characterised by an increase or decrease of such symptoms or signs which may indicate a "worsening" or "bettering" of the disease. The "worsening" of a disease is characterised by a decreasing ability of a tissue, organ or organism to fulfil its function efficiently, whereas the "bettering" of a disease is typically characterised by an increase in the ability of a tissue, an organ or an individual to fulfil its function efficiently. A tissue, an organ or an individual being at "risk of developing" a disease is in a healthy state but shows potential of a disease emerging. Typically, the risk of developing a disease is associated with early or weak signs or symptoms of such disease. In such case, the onset of the disease may still be prevented by treatment. Examples of a disease include but are not limited to traumatic diseases, inflammatory diseases, infectious diseases, cutaneous conditions, endocrine diseases, intestinal diseases, neurological disorders, joint diseases, genetic disorders, autoimmune diseases, and various types of cancer.

[0059] "Cancer" refers to a proliferative disorder involving abnormal cell growth which may invade or spread to other tissues or organs of a subject. Cancers are classified by the type of cell that the tumor cells resemble and is therefore presumed to be the origin of the tumor. These types include but are not limited to carcinoma (cancers derived from epithelial cells) sarcoma (cancers arising from connective tissue such as e.g. bone, cartilage, fat, nerve), lymphoma and leukemia (cancer arising from hematopoietic cells that leave the marrow and tend to mature in the lymph nodes and blood), germ cell tumor (cancers derived from pluripotent cells), and blastoma (cancers derived from immature "precursor" cells or embryonic tissue). In particular, cancer includes but is not limited to acute lymphoblastic leukemia (ALL), acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytoma, childhood cerebellar or cerebral cancer, basal-cell carcinoma, bile duct cancer, extrahepatic, bladder cancer, bone tumor, osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain cancer, brain tumor (cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma), breast cancer, bronchial adenomas/carcinoids, Burkitt's lymphoma, carcinoid tumor, central nervous system lymphoma, cerebellar astrocytoma, Cervical cancer, Chronic bronchitis, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma in the Ewing family of tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer (intraocular melanoma, retinoblastoma), gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor (extracranial, extragonadal, or ovarian), gestational trophoblastic tumor, glioma of the brain stem, gastric carcinoid, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell carcinoma (endocrine pancreas), Kaposi sarcoma, kidney cancer (renal cell cancer), Laryngeal cancer, leukaemia (acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myelogenous), lip and oral cavity cancer, liposarcoma, liver cancer, lung cancer (non-small cell, small cell), lymphomas (AIDS-related, Burkitt, cutaneous T-Cell, Hodgkin, primary central nervous system), macroglobulinemia (Waldenström), male breast cancer, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanoma, Merkel cell cancer, Mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neo-

plasia syndrome, multiple myeloma/plasma cell neoplasm, Mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, chronic, myeloid leukemia, myeloma, myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, oligodendroglioma, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary adenoma, plasma cell neoplasia/Multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis and ureter, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma (Ewing family of tumors, Kaposi, soft tissue, uterine), Sezary syndrome, skin cancer (carcinoma, melanoma, non-melanoma, Merkel cell), small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, supratentorial primitive neuroectodermal tumor, testicular cancer, throat cancer, thymoma, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor, urethral cancer, uterine cancer, (endometrial, sarcoma), vaginal cancer, visual pathway and hypothalamic glioma, vulvar cancer, Wilms tumor (kidney cancer),

[0060] As used herein, the term "breast tumor" relates to an abnormal hyperproliferation of breast tissue cells in a subject, which may be a benign (non-cancerous) tumor or a malign (cancerous) tumor. Benign breast tumors, preferably, include fibroadenomas, granular cell tumors, intraductal papillomas, and phyllodes tumors. A malign tumor, is a breast cancer (BC) as specified herein above.

[0061] As used herein, the term "metastatic breast cancer" (MBC) relates to a breast cancer wherein cancer cells grow as a metastasis at least one secondary site, i.e. a non-adjacent organ or part of the body of a subject.

[0062] As used herein, the term "ovary tumor" relates to an abnormal hyperproliferation of ovary tissue cells in a subject, which may be a benign (non-cancerous) tumor or a malign (cancerous) tumor. A malign tumor is an ovary cancer (OvaCa) as specified herein above.

[0063] As used herein, the term "pancreatic tumor" relates to an abnormal hyperproliferation of ovary tissue cells in a subject, which may be a benign (non-cancerous) tumor or a malign (cancerous) tumor. A malign tumor is a pancreatic cancer (PaCa) as specified herein above.

[0064] The term "circulating tumor cell" or "CTC" is understood by the skilled artisan and relates to a tumor cell detached from the primary or metastatic tumor and circulating in the bloodstream. It is to be understood that the number of CTC is a prognostic marker for disease and therapy outcome in breast cancer, e.g. for overall survival. The term "CTC status" relates to the presence or absence of more than a reference amount of CTC in a sample. Preferably, the reference amount of CTC is 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, or 7.5 CTC / 7.5 ml blood, 5 CTC / 7.5 ml blood being more preferred. In subjects where a blood sample comprises more than said reference amount of CTC, the CTC status is unfavorable, indicating a low probability of successful treatment and a low progression-free and overall survival probability. Conversely, in subjects where a blood sample comprises less than said reference amount of CTC, the CTC status is favorable, indicating a high probability of successful treatment and a high progression-free and overall survival probability. Advantageously, it has been found in the present invention that the amounts of the miRNAs used for determining the CTC status of a subject as defined herein below are indicative of the CTC status of a subject. Thus, determining the CTC status in a subject as used herein relates to determining the amount or amounts of said miRNA or miRNAs and thus obtaining an indication of the subject's CTC status. Preferably, the status can be diagnosed to be "favorable" or "unfavorable"

[0065] "Symptoms" of a disease are implication of the disease noticeable by the tissue, organ or organism having such disease and include but are not limited to pain, weakness, tenderness, strain, stiffness, and spasm of the tissue, an organ or an individual. "Signs" or "signals" of a disease include but are not limited to the change or alteration such as the presence, absence, increase or elevation, decrease or decline, of specific indicators such as biomarkers or molecular markers, or the development, presence, or worsening of symptoms.

[0066] The term "indicator" and "marker" are used interchangeably herein, and refer to a sign or signal for a condition or is used to monitor a condition. Such a "condition" refers to the biological status of a cell, tissue or organ or to the health and/or disease status of an individual. An indicator may be the presence or absence of a molecule, including but not limited to peptide, protein, and nucleic acid, or may be a change in the expression level or pattern of such molecule in a cell, or tissue, organ or individual. An indicator may be a sign for the onset, development or presence of a disease in an individual or for the further progression of such disease. An indicator may also be a sign for the risk of developing a disease in an individual.

[0067] As used herein, the term "gene product" relates to a, preferably macromolecular, physical entity, the presence of which in a cell depends on the expression of said gene in said cell. The mechanisms of gene expression are well-known to the one skilled in the art to include the basic mechanisms of transcription, i.e. formation of RNA corresponding to the said gene or parts thereof, and translation, i.e. production of polypeptide molecules having an amino acid sequence encoded by said RNA according to the genetic code; it is well-known to the one skilled in the art that other cellular

processes may be involved in gene expression as well, e.g. RNA processing, RNA editing, proteolytic processing, protein editing, and the like. The term gene product thus includes RNA, preferably mRNA, as well as polypeptides expressed from said gene. It is clear from the above that the term gene product also includes fragments of said RNA(s), preferably with a length of at least ten, at least twelve, at least 20, at least 50, or at least 100 nucleotides, and fragments (peptides) from said polypeptides, preferably with a length of at least eight, at least ten, at least twelve, at least 15, at least 20 amino acids.

[0068] "Determining" the amount of a gene product relates to measuring the amount of said gene product, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly. Preferably, measuring is performed on a processed sample, said processing comprising extraction of polynucleotides or polypeptides from the sample. It is, however, also envisaged by the present invention that the gene product is determined in situ, e.g. by immunehistochemistry (IHC)

[0069] The amount of the polynucleotides of the present invention can be determined with several methods well-known in the art. Quantification preferably is absolute, i.e. relating to a specific number of polynucleotides or, more preferably, relative, i.e. measured in arbitrary normalized units. Preferably, a normalization is carried out by calculating the ratio of a number of specific polynucleotides and total number of polynucleotides or a reference amplification product. Methods allowing for absolute or relative quantification are well known in the art. E.g., quantitative PCR methods are methods for relative quantification; if a calibration curve is incorporated in such an assay, the relative quantification can be used to obtain an absolute quantification. Other methods known are, e.g. nucleic acid sequence-based amplification (NASBA) or the Branched DNA Signal Amplification Assay method in combination with dot blot or luminex detection of amplified polynucleotides. Preferably, the polynucleotide amounts are normalized polynucleotide amounts, i.e. the polynucleotide amounts obtained are set into relation to at least one reference amplification product, thereby, preferably, setting the polynucleotide amounts into relation to the number of cells in the sample and/or the efficiency of polynucleotide amplification. Thus, preferably, the reference amplification product is a product obtained from a polynucleotide known to have a constant abundancy in each cell, i.e. a polynucleotide comprised in most, preferably all, cells of a sample in approximately the same amount. More preferably, the reference amplification product is amplified from a chromosomal or mitochondrial gene or from the mRNA of a housekeeping gene. The amout of polynucleotides could be determined by Shotgun sequencing, Bridge PCR, Sanger sequencing, pyrosequencing, next-generation sequecing, Single-molecule real-time sequencing, Ion Torrent sequencing, Sequencing by synthesis, Sequencing by ligation, Massively parallel signature sequencing, Polony sequencing, DNA nanoball sequencing, Heliscope single molecule sequencing, Single molecule real time (SMRT) sequencing, Nanopore DNA sequencing, Tunnelling currents DNA sequencing, Sequencing by hybridization, Sequencing with mass spectrometry, Microfluidic Sanger sequencing, Transmission electron microscopy DNA sequencing, RNA polymerase sequencing, In vitro virus high-throughput sequencing, Chromatin Isolation by RNA Purification (ChIRP-Seq), Global Run-on Sequencing (GRO-Seq), Ribosome Profiling Sequencing (Ribo-Seq)/ARTseq, RNA Immunoprecipitation Sequencing (RIP-Seq), High-Throughput Sequencing of CLIP cDNA library (HITS-CLIP), Crosslinking and Immunoprecipitation Sequencing, Photoactivatable Ribonucleoside - Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP), Individual Nucleotide Resolution CLIP (iCLIP), Native Elongating Transcript Sequencing (NET-Seq), Targeted Purification of Polysomal mRNA (TRAP-Seq), Crosslinking, Ligation, and Sequencing of Hybrids (CLASH-Seq), Parallel Analysis of RNA Ends Sequencing (PARE-Seq), Genome-Wide Mapping of Uncapped Transcripts (GMUCT), Transcript Isoform Sequencing (TIF-Seq), Paired-End Analysis of TSSs (PEAT), Selective 2' -Hydroxyl Acylation Analyzed by Primer Extension Sequencing (SHAPE-Seq), Parallel Analysis of RNA Structure (PARS-Seq), Fragmentation Sequencing (FRAG-Seq), CXXC Affinity Purification Sequencing (CAP-Seq), Alkaline Phosphatase Calf Intestine-Tobacco Acid Pyrophosphatase Sequencing (CIP-TAP), Inosine Chemical Erasing Sequencing (ICE), m6A-Specific Methylated RNA Immunoprecipitation Sequencing (MeRIP-Seq), Digital RNA Sequencing, Whole-Transcript Amplification for Single Cells (Quartz-Seq), Designed Primer - Based RNA Sequencing (DP-Seq), Switch Mechanism at the 5' End of RNA Templates (Smart-Seq), Switch Mechanism at the 5' End of RNA Templates Version 2 (Smart-Seq2), Unique Molecular Identifiers (UMI), Cell Expression by Linear Amplification Sequencing (CEL-Seq), Single-Cell Tagged Reverse Transcription Sequencing (STRT-Seq), Single-Molecule Molecular Inversion Probes (smMIP), Multiple Displacement Amplification (MDA), Multiple Annealing and Looping - Based Amplification Cycles (MALBAC), Oligonucleotide-Selective Sequencing (OS-Seq), Duplex Sequencing (Duplex-Seq), Bisulfite Sequencing (BS-Seq), Post-Bisulfite Adapter Tagging (PBAT), Tagmentation-Based Whole Genome Bisulfite Sequencing (T-WGBS), Oxidative Bisulfite Sequencing (oxBS-Seq), Tet-Assisted Bisulfite Sequencing (TAB-Seq), Methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq), Methylation-Capture (MethylCap) Sequencing, Methyl-Binding-Domain - Capture (MBDCap) Sequencing, Reduced-Representation Bisulfite Sequencing (RRBS-Seq), DNase 1 Hypersensitive Sites Sequencing (DNase-Seq), MNase-Assisted Isolation of Nucleosomes Sequencing (MAINE-Seq), Chromatin Immunoprecipitation Sequencing (ChIP-Seq), Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE-Seq), Assay for Transposase-Accessible Chromatin Sequencing (ATAC-Seq), Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET), Chromatin Conformation Capture (Hi-C/3C-Seq), Circular Chromatin Conformation Capture (4-C or 4C-Seq), Chromatin Conformation Capture Carbon Copy (5-C), Retrotransposon Capture Sequencing (RC-Seq),

Transposon Sequencing (Tn-Seq) or Insertion Sequencing (INSeq), Translocation-Capture Sequencing (TC-Seq), fluorescence based methods (such as: mircoarray, real-time PCR), mass-based methods (mass spectrometry), restriction enzyme based methods, antibody-immunoprecipitation based methods, and digital PCR.

[0070] The amount of peptides or polypeptides of the present invention can be determined in various ways. Direct measuring relates to measuring the amount of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide present in the sample. Such a signal - sometimes referred to as intensity signal -may be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of the peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

[0071] Determining the amount of a peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Said means comprise immunoassay and / or immunohistochemistry devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass-spectrometers, NMR-analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays, Cobalt Binding Assays, and latex agglutination assays.

[0072] Determining the amount of a peptide or polypeptide comprises the step of measuring a specific intensity signal obtainable from the peptide or polypeptide in the sample. As described above, such a signal may be the signal intensity observed at an m/z variable specific for the peptide or polypeptide observed in mass spectra or a NMR spectrum specific for the peptide or polypeptide.

[0073] Determining the amount of a peptide or polypeptide may, preferably, comprise the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. The term "antibody" as used herein refers to secreted immunoglobulins which lack the transmembrane region and can thus, be released into the bloodstream and body cavities. Antibodies are typically made of four polypeptide chains comprising two identical heavy chains and identical two light chains which are connected via disulfide bonds and resemble a "Y"-shaped macro-molecule. Papain digestion of antibodies produces two identical antigen binding fragments, called "Fab fragments" (also referred to as "Fab portion" or "Fab region") each with a single antigen binding site, and a residual "Fc fragment" (also referred to as "Fc portion" or "Fc region") whose name reflects its ability to crystallize readily. The crystal structure of the human IgG Fc region has been determined (Deisenhofer (1981) Biochemistry 20:2361-2370). In IgG, IgA and IgD isotypes, the Fc region is composed of two identical protein fragments, derived from the CH2 and CH3 domains of the antibody's two heavy chains; in IgM and IgE isotypes, the Fc regions contain three heavy chain constant domains (CH2 - 4) in each polypeptide chain. In addition, smaller immunoglobulin molecules exist naturally or have been constructed artificially. The term "Fab' fragment" refers to a Fab fragment additionally comprising the hinge region of an Ig molecule whilst "F(a')2 fragments" are understood to comprise two Fab fragments being either chemically linked or connected via a disulfide bond. Whilst "single domain antibodies (sdAb)" (Desmyter et al. (1996) Nat. Structure Biol. 3:803-811) and "Nanobodies" only comprise a single VH domain, "single chain Fv (scFv)" fragments comprise the heavy chain variable domain joined via a short linker peptide to the light chain variable domain (Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85, 5879-5883). Divalent single-chain variable fragments (di-scFvs) can be engineered by linking two scFvs (scFvA-scFvB). This can be done by producing a single peptide chain with two VH and two VL regions, yielding "tandem scFvs" (VHA-VLA-VHB-VLB). Another possibility is the creation of scFvs with linkers that are too short for the two variable regions to fold together, forcing scFvs to dimerize. Usually linkers with a length of 5 residues are used to generate these dimers. This type is known as "diabodies". Still shorter linkers (one or two amino acids) between a VH and VL domain lead to the formation of monospecific trimers, so-called "triabodies" or "tribodies". Bispecific diabodies are formed by expressing to chains with the arrangement VHA-VLB and VHB-VLA or VLA-VHB and VLB-VHA, respectively. Single-chain diabodies (scDb) comprise a VHA-VLB and a VHB-VLA fragment which are linked by a linker peptide (P) of 12-20 amino acids, preferably 14 amino acids, (VHA-

VLB-P-VHB-VLA). "Bi-specific T-cell engagers (BiTEs)" are fusion proteins consisting of two scFvs of different antibodies wherein one of the scFvs binds to T cells via the CD3 receptor, and the other to a tumor cell via a tumor specific molecule (Kufer et al. (2004) Trends Biotechnol. 22:238-244). Dual affinity retargeting molecules ("DART" molecules) are diabodies additionally stabilized through a C-terminal disulfide bridge. The present invention also includes single chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody.

[0074] The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Nonspecific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

[0075] First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance. Second, if the ligand also serves as a substrate of an enzymatic activity of the peptide or polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/peptide or polypeptide" complex or the ligand which was bound by the peptide or polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for a detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured. Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labelling may be done by direct or indirect methods. Direct labelling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labelling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal intensity. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemo luminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include 35S, 1251, 32P, 33P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme- linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests, like e.g. reverse phase protein arrays or antibody arrays. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can

be used alone or in combination with labelling or other detection methods as described above.

[0076] The amount of a peptide or polypeptide may also be determined as follows: (a) contacting a solid support comprising a ligand for the peptide or polypeptide as specified above with a sample comprising the peptide or polypeptide and (b) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

[0077] As used herein, the term "CpG site" relates to a dinucleotide sequence 5'-CG-3' comprised in a polynucleotide, preferably comprised in DNA, more preferably comprised in genomic DNA of a subject. The CpG sites to be analyzed according to the present invention are the CpG sites located in the intron, exon or promoter region of a gene of interest. In case the CpG sites are located in the promoter region, said region is preferably 3000 nucleotides, 2500 nucleotides, 2100 nucleotides, or 1750 nucleotides upstream of the translation start site of the respective gene of interest. More preferably, the CpG sites to be analyzed according to the present invention are the CpG sites located in the region 1750-3000 nucleotides, 2100-3000 nucleotides, or 2500-3000 nucleotides upstream of the translation start site of the gene of interest gene.

[0078] Thus, analysis of a CpG site corresponding to a CpG site of the present invention is also encompassed by the present invention. The skilled person knows how to determine the CpG sites in a sample corresponding to the CpG sites detailed herein above, e.g. by determining the translation start site of the gene of interest and / or by aligning said sequence from a sample to the sequence of the gene of interest. Further, it is also envisaged by the present invention that the methylation status of other CpG sites is determined in addition to determining the methylation status of a CpG site of the present invention.

[0079] The term "determining the methylation status" relates to determining if a methyl group is present at the 5 position of the pyrimidine ring of a cytosine in a polynucleotide. Preferably, the cytosine residue is followed in 3' direction by a guanosine residue, the two residues forming a CpG site. The presence of said methyl group can be determined by various methods well known to the skilled person, including, e.g., methylation-specific PCR (MSP), whole genome bisulfite sequencing or other sequencing based methods (Bisulfite Sequencing (BS-Seq), Post-Bisulfite Adapter Tagging (PBAT), Tagmentation-Based Whole Genome Bisulfite Sequencing (T-WGBS), Oxidative Bisulfite Sequencing (oxBS-Seq), Tet-Assisted Bisulfite Sequencing (TAB-Seq), Methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq), Methylation-Capture (MethylCap) Sequencing, Methyl-Binding-Domain - Capture (MBDCap) Sequencing, Reduced-Representation Bisulfite Sequencing (RRBS-Seq)), real-time PCR based methods of bisulfite treated DNA, e.g. Methylight, restriction with a methylation-sensitive restriction enzyme, e.g. in the HpaII tiny fragment enrichment by ligation-mediated PCR (HELP)-Assay, pyrosequencing of bisulfite treated DNA, or the like AIMS, amplification of inter-methylated sites; BC-seq, bisulphite conversion followed by capture and sequencing; BiMP, bisulphite methylation profiling; BS, bisulphite sequencing; BSPP, bisulphite padlock probes; CHARM, comprehensive high-throughput arrays for relative methylation; COBRA, combined bisulphite restriction analysis; DMH, differential methylation hybridization; HELP, HpaII tiny fragment enrichment by ligation-mediated PCR; MCA, methylated CpG island amplification; MCAM, MCA with microarray hybridization; MeDIP, mDIP and mCIP, methylated DNA immunoprecipitation; MIRA, methylated CpG island recovery assay; MMASS, microarray-based methylation assessment of single samples; MS-AP-PCR, methylation-sensitive arbitrarily primed PCR; MSCC, methylation-sensitive cut counting; MSP, methylation-specific PCR; MS-SNuPE, methylation-sensitive single nucleotide primer extension; NGS, next-generation sequencing; RLGS, restriction landmark genome scanning; RRBS, reduced representation bisulphite sequencing; -seq, followed by sequencing; WGSBS, whole-genome shotgun bisulphite sequencing. (Manel Esteller, Cancer epigenomics: DNA methylomes and histone-modification maps, Nature, 2007, 8:286-298; Peter W. Laird, Principles and challenges of genome-wide DNA methylation analysis. Nature Review Genetics, 2010, 11: 191-203). Preferably, the methylation status is determined by the methods described in the examples herein below, e.g. the sequencing-based Infinium 27K methylation assay or the mass spectrometry based method of MALDI-TOF mass spectrometry. As such, the methylation status of a specific cytosine residue in a specific polynucleotide molecule can only be "unmethylated" (meaning 0% methylation) or "methylated" (meaning 100% methylation). In the case of a CpG site in a double-stranded DNA molecule, which comprises two cytosine residues, the

methylation status can be "unmethylated" (meaning 0% methylation, i.e. none of the two cytosine residues methylated), "hemimethylated" (meaning 50% methylation, i.e. one of the two cystosine residues methylated), or "methylated" or "fully methylated" (meaning 100% methylation, i.e. both cytosine residues methylated) It is, however, understood by the person skilled in the art that if polynucleotides from a multitude of cells are obtained and the methylation status of a specific cytosine residue within said multitude of polynucleotides is determined, an average methylation status is determined, which can e.g, preferably, be expressed as a percentage (% methylation), and which can assume any value between 0% and 100%. It is also understood by the skilled person, that the methylation status can be expressed as a percentage in case the average methylation of different cell populations is determined. E.g. the blood cells according to the present invention are a mixture of variant cell types. It is possible that certain cell types have high methylation levels whereas other cell types have lower methylation levels, and finally reach an average methylation of e.g. 50 %.

[0080] As used herein, the term "detection agent" relates to an agent specifically interacting with, and thus recognizing, the expression level of a gene of interest, the methylation status of a gene of interest, or the presence or amount of a miRNA of the present invention. Preferably, said detection agent is a protein, polypeptide, peptide, polynucleotide or an oligonucleotide. Preferably, the detection agent is labeled in a way allowing detection of said detection agent by appropriate measures. Labeling can be done by various techniques well known in the art and depending of the label to be used. Preferred labels to be used are fluorescent labels comprising, inter alia, fluorochromes such as fluorescein, rhodamin, or Texas Red. However, the label may also be an enzyme or an antibody. It is envisaged that an enzyme to be used as a label will generate a detectable signal by reacting with a substrate. Suitable enzymes, substrates and techniques are well known in the art. An detection agent to be used as label may specifically recognize a target molecule which can be detected directly (e.g., a target molecule which is itself fluorescent) or indirectly (e.g., a target molecule which generates a detectable signal, such as an enzyme). The labeled detection agents of the sample will be contacted to the sample to allow specific interaction. Washing may be required to remove non-specifically bound detection agent which otherwise would yield false values. After this interaction step is complete, a researcher will place the detection device into a reader device or scanner. A device for detecting fluorescent labels, preferably, consists of some lasers, preferably a special microscope, and a camera. The fluorescent labels will be excited by the laser, and the microscope and camera work together to create a digital image of the sample. These data may be then stored in a computer, and a special program will be used, e.g., to subtract out background data. The resulting data are, preferably, normalized, and may be converted into a numeric and common unit format. The data will be analyzed to compare samples to references and to identify significant changes.

[0081] "Comparing" as used herein encompasses comparing the presence, absence or amount of an indicator referred to herein which is comprised by the sample to be analyzed with the presence, absence or amount of said indicator in a suitable reference sample. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount of the indicator as referred to herein is compared to an absolute reference amount of said indicator; a concentration of the indicator is compared to a reference concentration of said indicator; an intensity signal obtained from the indicator as referred to herein in a sample is compared to the same type of intensity signal of said indicator in a reference sample. The comparison referred to may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison by means of an expert system. Accordingly, the result of the identification referred to herein may be automatically provided in a suitable output format.

[0082] The term "sample" or "sample of interest" are used interchangeably herein, referring to a part or piece of a tissue, organ or individual, typically being smaller than such tissue, organ or individual, intended to represent the whole of the tissue, organ or individual. Upon analysis, a sample provides information about the tissue status or the health or diseased status of an organ or individual. Examples of samples include but are not limited to fluid samples such as blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate, or tissue samples such as e.g. tissue extracts obtained from tumour tissue or tissue adjacent to a tumour. Further examples of samples are cell cultures or tissue cultures such as but not limited to cultures of various cancer cells.

[0083] Samples can be obtained by well known techniques and include, preferably, scrapes, swabs or biopsies from the digestive tract, liver, pancreas, anal canal, the oral cavity, the upper aerodigestive tract and the epidermis. Such samples can be obtained by use of brushes, (cotton) swabs, spatula, rinse/wash fluids, punch biopsy devices, puncture of cavities with needles or surgical instrumentation. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy or other surgical procedures. More preferably, samples are samples of body fluids, e.g., preferably, blood, plasma, serum, urine, saliva, lacrimal fluid, and fluids obtainable from the breast glands, e.g. milk. Most preferably, the sample of a body fluid comprises cells of the subject. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as filtration, centrifugation or cell sorting. Preferably, samples are obtained from those body fluids described herein below. More preferably, cells are isolated from said body fluids as described herein below.

[0084] Analysis of a sample may be accomplished on a visual or chemical basis. Visual analysis includes but is not limited to microscopic imaging or radiographic scanning of a tissue, organ or individual allowing for morphological evaluation of a sample. Chemical analysis includes but is not limited to the detection of the presence or absence of specific indicators or alterations in their amount or level.

[0085] The term "reference sample" as used herein, refers to a sample which is analysed in a substantially identical manner as the sample of interest and whose information is compared to that of the sample of interest. A reference sample thereby provides a standard allowing for the evaluation of the information obtained from the sample of interest. A reference sample may be derived from a healthy or normal tissue, organ or individual, thereby providing a standard of a healthy status of a tissue, organ or individual. Differences between the status of the normal reference sample and the status of the sample of interest may be indicative of the risk of disease development or the presence or further progression of such disease or disorder. A reference sample may be derived from an abnormal or diseased tissue, organ or individual thereby providing a standard of a diseased status of a tissue, organ or individual. Differences between the status of the abnormal reference sample and the status of the sample of interest may be indicative of a lowered risk of disease development or the absence or bettering of such disease or disorder. A reference sample may also be derived from the same tissue, organ, or individual as the sample of interest but has been taken at an earlier time point. Differences between the status of the earlier taken reference sample and the status of the sample of interest may be indicative of the progression of the disease, i.e. a bettering or worsening of the disease over time. A reference sample was taken at an earlier or later time point in case a period of time has lapsed between taking of the reference sample and taking of the sample of interest. Such period of time may represent years (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 years), months (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months), weeks (e.g. 1, 2, 3, 4, 5, 6, 7, 8 weeks), days (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 days), hours (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours), minutes (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 minutes), or seconds (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 seconds).

[0086] A reference sample may be "treated differently" or "exposed differently" than a sample of interest in case both samples are treated in a substantially identical way except from a single factor. Such single factors include but are not limited to the time of exposure, the concentration of exposure, or the temperature of exposure to a certain substance. Accordingly, a sample of interest may be exposed to a different dosage of a certain substance than the reference sample or may be exposed for a different time interval than the reference sample or may be exposed at a different temperature than the reference sample. Different dosages to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold increased or decreased dosage of the dosage the reference sample is exposed to. Different exposure times to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold longer or shorter time period than the exposure of the reference. Different temperatures of exposure to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold increased or decreased temperature than the exposure of the reference. In a nonlimiting example a sample of interest may be exposed to a 10-fold increased concentration of a substance than the reference sample. The analysis of both samples is then conducted in a substantially identical manner allowing determining the effects, i.e. a beneficial or an adverse effect, of the increased concentration of such substance on the sample of interest. The skilled person will appreciate that this example applies mutatis mutandis to different ranges of concentrations, different exposure times, and/or different temperatures at exposure.

[0087] The terms "lowered" or "decreased" level of an indicator refer to the level of such indicator in the sample being reduced in comparison to the reference or reference sample. The terms "elevated" or "increased" level of an indicator refer to the level of such indicator in the sample being higher in comparison to the reference or reference sample.

[0088] Reference amounts can, in principle, be calculated for a group or cohort of subjects as specified herein based on the average or median values for a given miRNA by applying standard methods of statistics. In particular, accuracy of a test such as a method aiming to diagnose an event, or not, is best described by its receiver-operating characteristics (ROC) (see especially Zweig 1993, Clin. Chem. 39:561-577). The ROC graph is a plot of all of the sensitivity versus specificity pairs resulting from continuously varying the decision threshold over the entire range of data observed. The clinical performance of a diagnostic method depends on its accuracy, i.e. its ability to correctly allocate subjects to a certain prognosis or diagnosis. The ROC plot indicates the overlap between the two distributions by plotting the sensitivity versus 1-specificity for the complete range of thresholds suitable for making a distinction. On the y-axis is sensitivity, or the true-positive fraction, which is defined as the ratio of number of true-positive test results to the sum of number of true-positive and number of false-negative test results. This has also been referred to as positivity in the presence of a disease or condition. It is calculated solely from the affected subgroup. On the x-axis is the false-positive fraction, or 1-specificity, which is defined as the ratio of number of false-positive results to the sum of number of true-negative and number of false-positive results. It is an index of specificity and is calculated entirely from the unaffected subgroup. Because the true- and false-positive fractions are calculated entirely separately, by using the test results from two different subgroups, the ROC plot is independent of the prevalence of the event in the cohort. Each point on the ROC plot

represents a sensitivity/-specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions of results) has an ROC plot that passes through the upper left corner, where the true-positive fraction is 1.0, or 100% (perfect sensitivity), and the false-positive fraction is 0 (perfect specificity). The theoretical plot for a test with no discrimination (identical distributions of results for the two groups) is a 45° diagonal line from the lower left corner to the upper right corner. Most plots fall in between these two extremes. If the ROC plot falls completely below the 45° diagonal, this is easily remedied by reversing the criterion for "positivity" from "greater than" to "less than" or vice versa. Qualitatively, the closer the plot is to the upper left corner, the higher the overall accuracy of the test. Dependent on a desired confidence interval, a threshold can be derived from the ROC curve allowing for the diagnosis or prediction for a given event with a proper balance of sensitivity and specificity, respectively. Accordingly, the reference to be used for the methods of the present invention can be generated, preferably, by establishing a ROC for said cohort as described above and deriving a threshold amount there from. Dependent on a desired sensitivity and specificity for a diagnostic method, the ROC plot allows deriving suitable thresholds. Preferably, the reference amounts lie within the range of values that represent a sensitivity of at least 75% and a specificity of at least 45%, or a sensitivity of at least 80% and a specificity of at least 40%, or a sensitivity of at least 85% and a specificity of at least 33%, or a sensitivity of at least 90% and a specificity of at least 25%.

[0089] Preferably, the reference amount as used herein is derived from samples of subjects obtained before treatment, but for which it is known if their donors were being afflicted with BC or MBC or not. This reference amount level may be a discrete figure or may be a range of figures. Evidently, the reference level or amount may vary between individual species of miRNA. The measuring system therefore, preferably, is calibrated with a sample or with a series of samples comprising known amounts of each specific miRNA. It is understood by the skilled person that in such case the amount of miRNA can preferably be expressed as arbitrary units (AU). Thus, preferably, the amounts of miRNA are determined by comparing the signal obtained from the sample to signals comprised in a calibration curve. The reference amount applicable for an individual subject may vary depending on various physiological parameters such as age or subpopulation. Thus, a suitable reference amount may be determined by the methods of the present invention from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample. Moreover, a threshold amount can be preferably used as a reference amount. A reference amount may, preferably, be derived from a sample of a subject or group of subjects being afflicted with BC or MBC which is/are known to be afflicted with BC or MBC. A reference amount may, preferably, also be derived from a sample of a subject or group of subjects known to be not afflicted with BC or MBC. It is to be understood that the aforementioned amounts may vary due to statistics and errors of measurement. A deviation, i.e. a decrease or an increase of the miRNA amounts referred to herein is, preferably, a statistically significant deviation, i.e. a statistically significant decrease or a statistically significant increase.

[0090] As used herein, "treat", "treating" or "treatment" of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in an individual that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in individuals that were previously symptomatic for the disorder(s).

[0091] As used herein, "prevent", "preventing", "prevention", or "prophylaxis" of a disease or disorder means preventing that such disease or disorder occurs in patient.

[0092] As used herein, the term "therapy" refers to all measures applied to a subject to ameliorate the diseases or disorders referred to herein or the symptoms accompanied therewith to a significant extent. Said therapy as used herein also includes measures leading to an entire restoration of the health with respect to the diseases or disorders referred to herein. It is to be understood that therapy as used in accordance with the present invention may not be effective in all subjects to be treated. However, the term shall require that a statistically significant portion of subjects being afflicted with a disease or disorder referred to herein can be successfully treated. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools discussed herein above.

[0093] The term "breast cancer therapy", as used herein, relates to applying to a subject afflicted with breast cancer, including metastasizing breast cancer, measures to remove cancer cells from the subject, to inhibit growth of cancer cells, to kill cancer cells, or to cause the body of a patient to inhibit the growth of or to kill cancer cells. Preferably, breast cancer therapy is chemotherapy, anti-hormone therapy, targeted therapy, immunotherapy, or any combination thereof. It is, however, also envisaged that the cancer therapy is radiation therapy or surgery, alone or combination with other therapy regimens. It is understood by the skilled person that the selection of the breast cancer therapy depends on several factors, like age of the subject, tumor staging, and receptor status of tumor cells. It is, however, also understood by the person skilled in the art, that the selection of the breast cancer therapy can be assisted by the methods of the present invention: if, e.g. BC is diagnosed by the method for diagnosing BC, but no MBC is diagnosed by the method for diagnosing MBC, surgical removal of tumor may be sufficient. If, e.g. BC is diagnosed by the method for diagnosing BC and MBC is diagnosed by the method for diagnosing MBC, therapy measures in addition to surgery, e.g. chemotherapy and / or targeted therapy, may be appropriate. Likewise, if, e.g. BC is diagnosed by the method for diagnosing BC, and

an unfavorable CTC status is determined by the method for determining the CTC status, e.g. a further addition of immunotherapy to the therapy regimen may be required.

[0094] As used herein, the term "chemotherapy" relates to treatment of a subject with an antineoplastic drug. Preferably, chemotherapy is a treatment including alkylating agents (e.g. cyclophosphamide), platinum (e.g. carboplatin), anthracyclines (e.g. doxorubicin, epirubicin, idarubicin, or daunorubicin) and topoisomerase II inhibitors (e.g. etoposide, irinotecan, topotecan, camptothecin, or VP16), anaplastic lymphoma kinase (ALK)-inhibitors (e.g. Crizotinib or AP26130), aurora kinase inhibitors (e.g. N-[4-[4-(4-Methylpiperazin-1-yl)-6-[(5-methyl-1H-pyrazol-3-yl)amino]pyrimidin-2-yl]sulfanylphenyl]cyclopropanecarboxamide (VX-680)), antiangiogenic agents (e.g. Bevacizumab), or Iodine 131-1-(3-iodobenzyl)guanidine (therapeutic metaiodobenzylguanidine), histone deacetylase (HDAC) inhibitors, alone or any suitable combination thereof. It is to be understood that chemotherapy, preferably, relates to a complete cycle of treatment, i.e. a series of several (e.g. four, six, or eight) doses of antineoplastic drug or drugs applied to a subject separated by several days or weeks without such application.

[0095] The term "anti-hormone therapy" relates to breast cancer therapy by blocking hormone receptors, e.g. estrogen receptor or progesterone receptor, expressed on tumor cells, or by blocking the biosynthesis of estrogen. Blocking of hormone receptors can preferably be achieved by administering compounds, e.g. tamoxifen, binding specifically and thereby blocking the activity of said hormone receptors. Blocking of estrogen biosynthesis is preferably achieved by administration of aromatase inhibitors like, e.g. anastrozole or letrozole. It is known to the skilled artisan that anti-hormone therapy is only advisable in cases where tumor cells are expressing hormone receptors.

[0096] The term "targeted therapy", as used herein, relates to application to a patient a chemical substance known to block growth of cancer cells by interfering with specific molecules known to be necessary for tumorigenesis or cancer or cancer cell growth. Examples known to the skilled artisan are small molecules like, e.g. PARP-inhibitors (e.g. Iniparib), or monoclonal antibodies like, e.g., Trastuzumab.

[0097] The term "immunotherapy" as used herein relates to the treatment of cancer by modulation of the immune response of a subject. Said modulation may be inducing, enhancing, or suppressing said immune response. The term "cell based immunotherapy" relates to a breast cancer therapy comprising application of immune cells, e.g. T-cells, preferably tumor-specific NK cells, to a subject.

[0098] The terms "radiation therapy" or "radiotherapy" is known to the skilled artisan. The term relates to the use of ionizing radiation to treat or control cancer. The skilled person also knows the term "surgery", relating to operative measures for treating breast cancer, e.g. excision of tumor tissue.

[0099] As used herein, the term "therapy monitoring" relates to obtaining an indication on the effect of a treatment against cancer on the cancer status of a subject afflicted with said cancer. Preferably, therapy monitoring comprises application of a method of the present invention on two samples from the same subject, wherein a first sample is obtained at a time point before the second sample. Preferably, the time point of obtaining the first sample is separated from the time point of obtaining the second sample by about one week, about two weeks, about three weeks, about for weeks, about five weeks, about, six weeks, about seven weeks, about two months, about three months, about five months, about six month, or more than about six months. It is, however, also envisaged by the present invention that the method of therapy monitoring is used for long-term monitoring of subjects, e.g. monitoring the time of relapse-free survival or the like. In such case, the time point of obtaining the first sample is separated from the time point of obtaining the second sample, preferably, by at least six months, at least one year, at least two years, at least three years, at least four years, at least five years, or at least six years. It is known to the person skilled in the art that the first sample is preferably obtained before cancer therapy is started, while the second sample is preferably obtained after therapy is started. It is, however, also envisaged by the present invention that both samples are obtained after therapy is started. The skilled artisan also understands that more than two successive samples may be obtained according to the method for therapy monitoring of the present invention and that in such case the sample obtained at the first point in time may be used as the first sample relative to the second sample as well as for a third sample. Mutatis mutandis, the sample obtained at the second point in time may nonetheless be used as a first sample relative to a third sample, and the like.

[0100] The term "treatment success", as used herein, preferably relates to an amelioration of the diseases or disorders referred to herein or the symptoms accompanied therewith to a significant extent. More preferably, the term relates to a complete cure of said subject, i.e. to the prevention of progression and/or relapse of metastasizing breast cancer for at least five years. Accordingly, "determining treatment success" relates to assessing the probability according to which a subject was successfully treated. Preferably, the term relates to predicting progression free survival and/or overall survival of the subject, more preferably for a specific period of time. The term "predicting progression free survival" relates to determining the probability of a subject surviving without relapse and/or progression of metastatic breast cancer for a specific period of time. Accordingly, the term "predicting overall survival" relates to determining the probability according to which a subject will survive for a specific period of time. Preferably, said period of time is at least 12 months, more preferably at least 24 months.

[0101] The terms "pharmaceutical", "medicament" and "drug" are used interchangeably herein referring to a substance and/or a combination of substances being used for the identification, prevention or treatment of a tissue status or disease.

[0102] The term "kit" as used herein refers to a collection of the aforementioned components, preferably, provided separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention. Examples for such the components of the kit as well as methods for their use have been given in this specification. The kit, preferably, contains the aforementioned components in a ready-to-use formulation. Preferably, the kit may additionally comprise instructions, e.g., a user's manual for adjusting the components, e.g. concentrations of the detection agents, and for interpreting the results of any determination(s) with respect to the diagnoses provided by the methods of the present invention. Particularly, such manual may include information for allocating the amounts of the determined a gene product to the kind of diagnosis. Details are to be found elsewhere in this specification. Additionally, such user's manual may provide instructions about correctly using the components of the kit for determining the amount(s) of the respective biomarker. A user's manual may be provided in paper or electronic form, e.g., stored on CD or CD ROM. The present invention also relates to the use of said kit in any of the methods according to the present invention.

[0103] The term "device" as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to allow the diagnosis. Preferred means for determining the methylation status or the amount of gene product and means for carrying out the comparison are disclosed above in connection with the methods of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the methylation status or the amount of a gene product are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to establish a diagnosis. Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for determining the methylation status or the amount of a gene product in a sample and an evaluation unit for processing the resulting data for the diagnosis. Preferred means for detection are disclosed in connection with embodiments relating to the methods of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further inventive skills. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of parametric diagnostic raw data, preferably, as absolute or relative amounts. It is to be understood that these data will need interpretation by the clinician. However, also envisaged are expert system devices wherein the output comprises processed diagnostic raw data the interpretation of which does not require a specialized clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the polypeptides, Plasmon surface resonance devices, NMR spectrometers, mass- spectrometers etc.) or evaluation units/devices referred to above in accordance with the methods of the invention.

Embodiments

[0104] In a first aspect the present invention relates to a method of diagnosing breast cancer (BC) in a subject, comprising

a) determining the methylation status of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and DYRK4, and
b) determining the amount of the miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 and miR-148b, in a subject.

[0105] The term miR-652 may refer to the sequence of the -3p or -5p strand (in particular miR-652-3p), the term miR-801 refers to the sequence of the -3p or -5p strand, the term miR-376c may refer to the sequence of the -3p or -5p strand (in particular miR-376c-3p), the term miR-376a may refer to the sequence of the -3p or -5p strand (in particular miR-376a-3p), the term miR-127 may refer to the sequence of the -3p or -5p strand (in particular miR-127-3p), the term miR-409 may refer to the sequence of the -3p or -5p strand (in particular miR-409-3p), and the term miR-148b may refer to the sequence of the -3p or -5p strand (in particular miR-148-3p).

[0106] An alteration in the methylation status and/or expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and/or DYRK4, may indicate a change in tissue status or disease such as the worsening or bettering of a tissue status or disease, in particular cancer. In particular, a decreased methylation status ofHYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of a worsening of a tissue status or disease. An increased methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of a bettering of a tissue status or disease. An alteration in the methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may also be indicative of the risk of developing

an altered tissue status or a disease, in particular cancer. More specifically, a decreased methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of the risk of developing a degenerative tissue status or disease, in particular cancer. An altered methylation status ofHYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, in particular decreased methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S 100P, and/or DYRK4, may also be indicative of an individual suffering from an altered tissue status or a disease, in particular cancer. Furthermore, an altered methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, e.g. an elevated or lowered level of methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may indicate the progression or a stage of a tissue status or a disease, in particular cancer, in a subject. In particular a decreased methylation status of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of a worsening of a tissue status or disease, in particular cancer.

[0107] In particular, an increased expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of a worsening of a tissue status or disease. A decreased expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of a bettering of a tissue status or disease. An alteration in the expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may also be indicative of the risk of developing an altered tissue status or a disease, in particular cancer. More specifically, an increased expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of the risk of developing a degenerative tissue status or disease, in particular cancer. An altered expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, in particular increased expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may also be indicative of an individual suffering from an altered tissue status or a disease, in particular cancer. Furthermore, an altered expression level of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, e.g. an elevated or lowered expression level ofHYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may indicate the progression or a stage of a tissue status or a disease, in particular cancer, in a subject. In particular, an increased expression level ofHYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4, may be indicative of a worsening of a tissue status or disease, in particular cancer.

[0108] An alteration in the miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b level may indicate a change in tissue status or disease such as the worsening or bettering of a tissue status or disease, in particular cancer.

[0109] In particular, an elevated level of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b may be indicative of a worsening of a tissue status or disease. A lowered level of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b may be indicative of a bettering of a tissue status or disease. An alteration in the miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b level may also be indicative of the risk of developing an altered tissue status or a disease, in particular cancer. More specifically an elevated level of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b may be indicative of the risk of developing a degenerative tissue status or disease, in particular cancer. An altered miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b level, in particular an elevated miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b level, may also be indicative of an individual suffering from an altered tissue status or a disease, in particular cancer. Furthermore, an altered miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b level, e.g. an elevated or lowered level of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b, may indicate the progression or a stage of a tissue status or a disease, in a subject. In particular an elevated miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and/or miR-148b level may be indicative of a worsening of a tissue status or disease, in particular cancer.

[0110] The methylation status and/or expression level of the at least one methylation marker and the presence, in particular the amount, of at least one miRNA is indicative of the prognosis and/or diagnosis of said subject. The prognosis and/or diagnosis of cancer includes

i. the risk of developing cancer,
ii. the presence of cancer, and/or
iii. the progression, in particular the worsening or bettering, of cancer.

[0111] The methylation status of at least 2, 3, 4, 5, 6, 7 or 8 different methylation markers can be determined. In particular, all 7 miRNA marker are determined. In case all seven miRNA marker are determined, this combination is reffered to as miR-7, i.e. miR-7 encompasses all seven miRNA marker miR-652, miR-801, miR-376c, miR-376a, miR-127p, miR-409, and miR-148b.

[0112] It is to be understood that various specific combination of methylation marker and miRNAs may be used for prognosing and/or diagnosing cancer.

[0113] In particular embodiments, the methylation status of the methylation markers RPTOR, MGRN1 and RAPSN,

is determined, and the presence, in particular the amount, of the miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127-3p, miR-409-3p and miR-148b is determined. Optionally, the methylation status and/or expression level of HYAL2 is also determined.

[0114] In particular embodiments, the methylation status of the methylation markerw DYRK4, S100P, FUT7 and SLC22A18 is determined, and the presence, in particular the amount, of the miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127-3p, miR-409-3p and miR-148b is determined. Optionally, the methylation status of HYAL2 is also determined.

[0115] In further embodiments, the methylation status of the methylation markers MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and DYRK4 is determined, and the presence of the miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127-3p, miR-409-3p and miR-148b is determined. Optionally, the methylation status of HYAL2 is also determined.

[0116] The determination of the methylation status may comprise determining methylation of at least one CpG site within the HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and/or DYRK4 gene. In particular, the methylation status of the promoter, intron and/or exon region of said genes is determined.

[0117] In particular, the HYAL2 gene is the human HYAL2 gene located on human chromosome 3 (Genbank Acc No: NC_000003.11 GI: 224589815). In particular, the methylation status of at least one of the CpG sites located between position 50334760 and position 50335700 on human chromosome 3 is determined. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position 50335694 (cg27091787), 50335584 (HYAL_CpG_1), 50335646 (HYAL_CpG_2), or 50335671 (HYAL_CpG_3), 50335166 (HYAL-is-310 CpG_1), 50335180 (HYAL-is-310 CpG_2), 50335192 (HYAL-is-310 CpG_3), 50335195 (HYAL-is-310 CpG_4), 50335227 (HYAL-is-310 CpG_5), 50335233 (HYAL-is-310 CpG_6), 50335300 (HYAL-is-310 CpG_7), 50335315 (HYAL-is-310 CpG_8), 50335375 (HYAL-is-310 CpG_9), 50335392 (HYAL-is-310 CpG_10), 50335401 (HYAL-is-310 CpG_11), 50334744 (HYAL2-is-325_CpG_1), 50334761 (HYAL2-is-325_CpG_2), 50334804 (HYAL2-is-325_CpG_3), 50334844 (HYAL2-is-325_CpG_4), 50334853 (HYAL2-is-325_CpG_5), 50334862 (HYAL2-is-325_CpG_6), 50334880 (HYAL2-is-325_CpG_7), 50334906 (HYAL2-is-325_CpG_8), 50334913 (HYAL2-is-325_CpG_9), 50334917 (HYAL2-is-325_CpG_10), 0334928 (HYAL2-is-325_CpG_11), 50334944 (HYAL2-is-325_CpG_12), 50334956 (HYAL2-is-325_CpG_13), 50334980 (HYAL2-is-325_CpG_14), 50334982 (HYAL2-is-325_CpG_15), 50335010 (HYAL2-is-325_CpG_16) 50335014 (HYAL2-is-325_CpG_17), 50331237 (cg08776109) and 50330420 (cg06721473) is determined.

[0118] Most specifically, at least one CpG site is selected from the list consisting of cg27091787 at position 50335694, HYAL_CpG_1 at position 50335584, HYAL_CpG_2 at position 50335646, and HYAL_CpG_3 at position 50335671. In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the HYAL2 promoter region comprised in the sample to be analyzed. E.g the HYAL2 gene is located on Chromosome 3: positions 50,355,221-50,360,337 in build37/hgl9, but on Chromosome 3: positions 50,330,244-50,335,146 in build36/hg18.

[0119] In particular, the MGRN1 gene is the human MGRN1 gene located at human chromosome 16 (Genbank Acc No: NC_000016.10, range: 4624824-4690974, Reference GRCh38 Primary Assembly; Genbank Acc No: NC_018927.2, range: 4674882-4741756, alternate assembly CHM1_1.1; Genbank Acc No: AC_000148.1, range: 4641815-4707494, alternate assembly HuRef). In particular, the methylation status of at least one of the CpG sites located between position 4654000 and position 4681000 on human chromosome 16 is determined. In particular, the CpG site(s) is/are located in one or more of the following regions of chromosome 16: 4670069-4670542, 4654000-4655000, 4669000-4674000, and 4678000-4681000. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position: 4670487 (MGRN1_CpG_1), 4670481 (MGRN1_CpG_2), 4670466 (MGRN1_CpG_3), 4670459 (MGRN1_CpG_4), 4670442 (MGRN1_CpG_5), 4670440 (MGRN1_CpG_6), 4670435 (MGRN1_CpG_7), 4670433 (MGRN1_CpG_8), 4670422 (MGRN1_CpG_9), 4670414 (MGRN1_CpG_10), 4670411 (MGRN1_CpG_11), 4670402 (MGRN1_CpG_12), 4670393 (MGRN1_CpG_13), 4670357 (MGRN1_CpG_14), 4670352 (MGRN1_CpG_15), 4670343 (MGRN1_CpG_16), 4670341 (MGRN1_CpG_17), 4670336 (MGRN1_CpG_18), 4670313 (MGRN1_CpG_19), 4670310 (MGRN1_CpG_20), 4670301 (MGRN1_CpG_21), 4670292 (MGRN1_CpG_22), 4670287 (MGRN1_CpG 23), 4670281 (MGRN1_CpG_24), 4670276 (MGRN1_CpG_25), 4670264 (MGRN1_CpG_26), 4670234 (MGRN1_CpG_27), 4670211 (MGRN1_CpG_28), 4670180 (MGRN1_CpG 29), 4670174 (MGRN1_CpG_30), 4670157 (MGRN1_CpG_31), 4670137 (MGRN1_CpG_32), 4670123 (MGRN1_CpG_33), 4670117 (MGRN1_CpG_34). In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the MGRN1 promoter region comprised in the sample to be analyzed.

[0120] In particular, the RPTOR gene is the human RPTOR gene located at human chromosome 17 (Genbank Acc No: NC_000017.11, range: 80544825-80966373, GRCh38 Primary Assembly; Genbank Acc No: NG_013034.1, range: 5001-426549, RefSeqGene; Genbank Acc No: NC_018928.2, range: 78604958-79026514, Alternate assembly CHM1_1.1; Genbank Acc No: NG_013034.1; Genbank Acc No: AC_000149.1,range: 73954508-74378467, alternate assembly HuRef). In particular, the methylation status of at least one of the CpG sites located between position 76.297.000 and position 76.416.000 on human chromosome 17 is determined. In particular, the CpG site(s) is/are located in one or more of the following regions of chromosome 17: 76.369.937-76.370.536. 76.297.000-76.310.000, 76.333.000-76.341.000, 76.360.000-76.380.000, and 76.411.000-76.416.000. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position: 76370001 (RPTOR_CpG_1), 76370037 (RPTOR_CpG_2), 76370073 (RPTOR_CpG_3), 76370092 (RPTOR_CpG_4), 76370172 (RPTOR_CpG_5), 76370199 (RPTOR_CpG_6), 76370220 (RPTOR_CpG_7), 76370253 (RPTOR_CpG_8), In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the RPTOR promoter region comprised in the sample to be analyzed.

[0121] In particular, the SLC22A18 gene is the human SLC22A18 gene located at human chromosome 11 (Genbank Acc No: NC_000011.10, range: 2899721-2925246, Reference GRCh38 primary assembly; Genbank Acc No: NG_011512.1, range: 5001-30526, RefSeqGene; Genbank Acc No: NT_187585.1, range: 131932-157362, Reference GRCh38 ALT_REF_LOCI_1; Genbank Acc No: AC_000143.1, range: 2709509-2734907, alternate assembly HuRef; Genbank Acc No: NC_018922.2, range:2919878-2945340, alternate assembly CHM1_1.1). In particular, the methylation status of at least one of the CpG sites located between position 2876000 and position 2883000 on human chromosome 11 is determined. In particular the CpG sites are located at 2.877.113-2.877.442. More specifically, chr11: 2.876.000 - chr11: 2.883.000, a 7000 bp cancer-associated, in particular BC, OvaCa, and/or PaCA-associated, differential methylation region covering, the promoter region, a CpG island and part of the gene body region of SLC22A18 (transcript variants). More specifically, in particular referring to build 36.1/hg 18 of the human genome, the methylation status of at least one of the CpG sites located at position: 2877395 (SLC22A18_CpG_1), 2877375 (SLC22A18_CpG_2), 2877365 (SLC22A18_CpG_3), 2877341 (SLC22A18_CpG_4), 2877323 (SLC22A18_CpG_5), 2877311 (SLC22A18_CpG_6), 2877193 (SLC22A18_CpG_7), 2877140 (SLC22A18_CpG_8). In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the SLC22A18 promoter region comprised in the sample to be analyzed.

[0122] In particular, the FUT7 gene is the human FUT7 gene located at human chromosome 9 (Genbank Acc No: NC_000009.12, range: 137030174-137032840, Reference GRCh38 primary assembly; Genbank Acc No: NG_007527.1, range: 5001-7667, RefSeqGene; Genbank Acc No: AC_000141.1, range: 109383478-109386144, Alternate assembly HuRef; Genbank Acc No: NC_018920.2, range: 140073389-140076055, Alternate assembly CHM1_1.1). In particular, the methylation status of at least one of the CpG sites located between position 139046000 and position 139048000 on human chromosome 9 is determined. More specifically, a 2000 bp BC, OvaCa, and/or PaCA-associated differential methylation region located at the promoter region of FUT7. In particular the CpG sites are located at 139.047.218-139.047.610, 139.046.000-139.048.000, and 139.045.065-139.045.817. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position: 139047253 (FUT_CpG_1), 139047314 (FUT_CpG_2), 139047346 (FUT_CpG_3), 139047427 (FUT_CpG_4), 139047445 (FUT_CpG_5), 139047467 (FUT_CpG_6), 139047483 (FUT_CpG_7), 139047566 (FUT_CpG_8). In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the FUT7 promoter region comprised in the sample to be analyzed.

[0123] In particular, the RAPSN gene is the human RAPSN gene located at human chromosome 11 (Genbank Acc No: NC_000011.10, range: 47437757-.47449178, Reference GRCh38 primary assembly; Genbank Acc No: NG_008312.1, range: 5001-16423, RefSeqGene; Genbank Acc No: NC_018922.2, range: 47458570-47469991, alternate assembly CHM1_1.1; Genbank Acc No: AC_000143.1, range: 47159075-47170494, alternate assembly HuRef). In particular, the methylation status of at least one of the CpG sites located between position 47427500 and position 47428500 on human chromosome 11 is determined. Preferably the CpG sites are located at 47427500 -47428300. More specificly, a 1000 bp cancer-associated, preferably BC, OvaCa, and/or PaCA-associated, differential methylation region located at the promoter region of RAPSN. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position: 47427787 (RAPSN_CpG_1), 47427825 (RAPSN_CpG_2), 47427883 (RAPSN_CpG_3), 47427915 (RAPSN_CpG_4), 47427930 (RAPSN_CpG_5), 47427976

(RAPSN_CpG_6), 47428029 (RAPSN_CpG_7), 47428110 (RAPSN_CpG_8). In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the RAPSN promoter region comprised in the sample to be analyzed.

[0124] In particular, the S100P gene is the human S100P gene located at human chromosome 4 (Genbank Acc No: NC_000004.12, range: 6693839-6697170, Reference GRCh38 primary assembly; Genbank Acc No: AC_000136.1, range: 6627254-6630595, alternate assembly HuRef; Genbank Acc No: NC_018915.2, range: 6693944-6697285, alternate assembly CHM1_1.1). In particular, the methylation status of at least one of the CpG sites located between position 6746000 and position 6747000 on human chromosome 4 is determined. More specifically, a 1000 bp cancer-associated (preferably BC, OvaCa, and/or PaCA-associated) differential methylation region located from the promoter region till the first exon of S100P. In particular the CpG sites are located at 6.746.537-6.746.823. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position: 6746565 (S100P_CpG_1), 6746599 (S100P_CpG_2), 6746609 (S100P_CpG_3), 6746616 (S100P_CpG_4), 6746623 (S100P_CpG_5), 6746634 (S100P_CpG_6), 6746710 (S100P_CpG_7), 6746728 (S100P_CpG_8), 6746753 (S100P_CpG_9), 6746779 (S100P_CpG_10), 6746788 (S100P_CpG_11), 6746791 (S100P_CpG_12). In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the S100P promoter region comprised in the sample to be analyzed.

[0125] In particular, the DYRK4 gene is the human DYRK4 gene located at human chromosome 12 (Genbank Acc No: NC_000012.12, range: 4590072-4613888, Reference GRCh38 primary assembly; Genbank Acc No: AC_000144.1, range: 4555932-4579747, Alternate assembly HuRef; Genbank Acc No: NC_018923.2, range: 4698860-4722666, alternate assembly CHM1_1.1). In particular, the methylation status of at least one of the CpG sites located between position 4569000 and position 4571000 on human chromosome 12 is determined. More specifically, a 2000 bp cancer-associated, preferably BC, OvaCa, and/or PaCA associated, differential methylation region located at the promoter region of DYRK4. In particular the CpG sites are located at 4569448 -4569945. More specifically, in particular referring to build 36.1/hg18 of the human genome, the methylation status of at least one of the CpG sites located at position: 4569879 (DYRK4_CpG_1), 4569809 (DYRK4_CpG_2), 4569707 (DYRK4_CpG_3), 4569493 (DYRK4_CpG_4), In particular, the methylation status of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or at least fifteen CpG sites of the present invention is determined. It is understood by the skilled person that the exact numbering of said CpG sites may depend on the specific genomic sequence and on the specific sequence of the DYRK4 promoter region comprised in the sample to be analyzed.

[0126] The method of prognosing and/or diagnosing cancer may further comprise the step of comparing the methylation status of the at least one methylation marker and the presence, in particular the amount, of the miRNA markers in said subject, to the methylation status of the at least one methylation marker and the presence, in particular the amount, of the miRNA markers in one or more reference(s). In particular, the reference is a threshold value, a reference value or a reference sample.

[0127] In cases, wherein the reference is a threshold value, a methylation status of the at least on methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, which is below a threshold value is indicative of a subject being afflicted with cancer, an increased risk of developing cancer, or a worsening of the disease; whereas a methylation status which is equal to or above the threshold value is indicative of a subject not afflicted with cancer, of a decreased risk of developing cancer, or of a bettering of the disease. It is to be understood that the aforementioned level may vary due to statistics and errors of measurement.

[0128] In cases, wherein the reference is a threshold value, an expression level of the at least on methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, which is equal to or above the threshold value is indicative of a subject being afflicted with cancer, an increased risk of developing cancer, or a worsening of the disease; whereas an expression level which is below the threshold value is indicative of a subject not being afflicted with cancer, of a decreased risk of developing cancer, or of a bettering of the disease. It is to be understood that the aforementioned level may vary due to statistics and errors of measurement.

[0129] In cases, wherein the reference is a threshold value, an amount of the miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 and miR-148b, which is equal to or above the threshold value is indicative of a subject being afflicted with cancer, an increased risk of developing cancer, or a worsening of the disease; whereas an amount which is below the threshold value is indicative of a subject not being afflicted with cancer, of a decreased risk of developing cancer, or of a bettering of the disease. It is to be understood that the aforementioned amounts may vary due to statistics and errors of measurement.

[0130] The threshold value for a subject being afflicted with cancer, an increased risk of developing cancer, or a

worsening of the disease for HYAL2 can be a methylation status of less than 90% of the controls and an expression level of more than 1.2 folds higher than the controls. The threshold value for MGRN1 can be a methylation status of less than 90% of the controls. The threshold value for RPTOR can be a methylation status of less than 95% of the controls. The threshold value for SLC22A18 can be a methylation status of less than 95% of the controls and an expression level of more than 1.1 folds higher than the controls. The threshold value for FUT7 can be a methylation status of less than 92% of the controls. The threshold value for RAPSN can be a methylation status of less than 98% of the controls. The threshold value for S100P can be a methylation status of less than 90% of the controls and an expression level of more than 2 folds higher than the controls. The threshold value for DYRK4 can be a methylation status of less than 85% of the controls.

[0131] The threshold level for miR-652 can be an amount of at least 0.5Ct value less than the controls (or more than 1.4 folds higher than the controls). The threshold level for miR-801 can be an amount of at least 0.6Ct value less than the controls (or more than 1.5 folds higher than the controls). The threshold level for miR-376c can be an amount of at least 0.5Ct value less than the controls (or more than 1.4 folds higher than the controls). The threshold level for miR-376a can be an amount of at least 0.6Ct value less than the controls (or more than 1.5 folds higher than the controls). The threshold level for miR-127 can be an amount of at least 0.5Ct value less than the controls (or more than 1.4 folds higher than the controls). The threshold level for miR-409 can be an amount of at least 0.4Ct value less than the controls (or more than 1.3 folds higher than the controls). The threshold level for miR-148b can be an amount of at least 0.3Ct value less than the controls (or more than 1.2 folds higher than the controls).

[0132] In cases, wherein the reference is a reference value, said reference value can be a representative value of the absence of cancer, of the presence of cancer, or of an increased or decreased risk of developing cancer.

[0133] The reference sample can be selected from the group consisting of a reference sample derived from a healthy individual, a reference sample derived from a diseased individual, a reference sample derived from the same individual as the sample of interest taken at an earlier or later time point, and a reference sample representative for a healthy individual or representative for the presence or absence of cancer or representative for an increased or decreased risk of developing cancer.

[0134] In cases, wherein the reference is a healthy subject or an subject with a decreased risk of developing cancer or a methylation status of a methylation marker or an amount of miRNA representative of the absence of cancer, a decreased methylation level and/or an increased expression of the at least one methylation marker and the presence or an increased amount of the at least one miRNA marker compared to the reference indicates

i. the risk of developing cancer, in particular BC, OvaCa, and/or PaCA,
ii. the presence of cancer, in particular BC, OvaCa, and/or PaCA, and/or
iii. the progression of cancer, in particular BC, OvaCa, and/or PaCA in the subject.

[0135] In cases, wherein the reference is a diseased subject or a subject with an increased risk of developing cancer or a methylation status of a methylation marker or an amount of miRNA representative of the presence of cancer, a similar methylation status or expression level of the at least one methylation marker and a similar amount of the at least one miRNA marker indicates

i. the risk of developing cancer, in particular BC, OvaCa, and/or PaCA,
ii. the presence of cancer, in particular BC, OvaCa, and/or PaCA, and/or
iii. the progression of cancer, in particular BC, OvaCa, and/or PaCA in the subject.

[0136] In cases, wherein the reference sample is derived from the same subject as the sample of interest and was taken at an earlier time point,

(i) a decreased methylation and/or an increased expression of the at least one methylation marker and the presence or an increased amount of the at least one miRNA marker compared to the reference indicates

i. the risk of developing cancer, in particular BC, OvaCa, and/or PaCA,
ii. the presence of cancer, in particular BC, OvaCa, and/or PaCA, and/or
iii. the progression of cancer, in particular BC, OvaCa, and/or PaCA,

(ii) an increased methylation and/or lower expression of the at least one methylation marker and the absence or a decreased amount of the at least one miRNA marker compared to the reference indicates

i. a decreased risk to develop cancer, in particular BC, OvaCa, and/or PaCA,
ii. the absence of cancer, in particular BC, OvaCa, and/or PaCA, and/or

iii. a declined progression of cancer, in particular BC, OvaCa, and/or PaCA,

and/or

(iii) a similar level of methylation and/or expression of the at least one methylation marker and a similar amount of the at least one miRNA marker compared to the reference indicates

i. a similar risk to develop cancer, in particular BC, OvaCa, and/or PaCA,
ii. a stagnation in the progression of cancer, in particular BC, OvaCa, and/or PaCA,
and/or
iii. a persistence of cancer, in particular BC, OvaCa, and/or PaCA,

in the subject.

[0137] According to the present invention, the amount of miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 and miR-148b is determined. Preferably, an amount of at least 0.5Ct value less than the controls (or more than 1.4 folds higher than the controls) miR-652 is indicative of cancer, an amount of at least 0.6Ct value less than the controls (or more than 1.5 folds higher than the controls) miR-801 is indicative of cancer, an amount of at least 0.5Ct value less than the controls (or more than 1.4 folds higher than the controls) miR-376c is indicative of cancer, an amount of at least 0.6Ct value less than the controls (or more than 1.5 folds higher than the controls) miR-376a is indicative of cancer, an amount of at least 0.5Ct value less than the controls (or more than 1.4 folds higher than the controls) miR-127 is indicative of cancer, an amount of at least 0.4Ct value less than the controls (or more than 1.3 folds higher than the controls) miR-409 is indicative of cancer, an amount of at least 0.3Ct value less than the controls (or more than 1.2 folds higher than the controls) miR-148b is indicative of cancer.

[0138] The sample of interest and/or the reference sample can be a body fluid sample or a tissue sample. The body fluid sample can be selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. Preferably, the body fluid is blood.

[0139] The tissue sample can be a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. The sample of interest and/or the reference sample can be a medium obtained from said cell cultures or tissue cultures.

[0140] The subject can be a mammal, reptile, or bird. In particular, the subject can be selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0141] Also disclosed is a method for determining the dosage of a pharmaceutical for the alteration of cancer or the prevention of cancer or the treatment of cancer in a subject, said method however not part of the invention, comprising the steps of

(a) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in a sample of a subject, and
(b) determining the dosage of a pharmaceutical depending on the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest.

[0142] In particular, the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, is determined in a reference for comparison with the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest.

[0143] In particular, the dosage of a pharmaceutical is determined depending on the comparison of the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker a in the sample of interest and the reference or reference sample.

[0144] In particular, the sample of interest and/or the reference sample is a body fluid samples or a tissue samples. In particular embodiments, the body fluid sample is selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. In particular, the body fluid is blood.

[0145] The tissue sample is preferably a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. The sample of interest and/or the reference sample can be a medium obtained from

said cell cultures or tissue cultures.

[0146] In particular, the subject can be a mammal, reptile, or bird. Preferably, the subject is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0147] Also disclosed is a method for adapting the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer, said method however not part of the invention comprising the steps of

(a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample,
(b) determining the methylation status and/or expression level of the at least one methylation marker and the amount of the at least one miRNA marker in one or more references or reference samples,
(c) examining the tested sample as to whether the methylation status and/or expression level of the at least one methylation marker and the amount of the at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, and
(d) adapting the dosage of a pharmaceutical depending on whether the methylation status and/or expression level of the at least one methylation marker and the amount of the at least one miRNA marker in the sample of interest is different from the level in the one or more references or reference samples.

[0148] In particular, the dosage of a pharmaceutical is increased if

a) the methylation status of the at least one methylation marker is decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.
b) the methylation status of the at least one methylation marker is equal to or decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.
c) the methylation status of the at least one methylation marker is equal to decreased in comparison to a reference sample obtained from said subject at an earlier time point.
d) the expression level of the at least one methylation marker is increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.
e) the expression level of the at least one methylation marker is equal to or increased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.
f) the expression level of the at least one methylation marker is equal to increased in comparison to a reference sample obtained from said subject at an earlier time point.
g) the amount of the at least one miRNA is increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.
h) the amount of the at least one miRNA marker is equal to or increased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.
i) the amount of the at least one miRNA marker is equal to or increased in comparison to a reference sample obtained from said subject at an earlier time point.

[0149] In particular, the dosage of a pharmaceutical is decreased if

a) the methylation status of the at least one methylation marker is equal to or increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.
b) the methylation status of the at least one methylation marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.
c) the methylation status of the at least one methylation marker is equal to decreased in comparison to a reference sample obtained from said subject at an earlier time point.

d) the expression level of the at least one methylation marker is equal to or decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

e) the expression level of the at least one methylation marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

f) the expression level of the at least one methylation marker is decreased in comparison to a reference sample obtained from said subject at an earlier time point.

g) the amount of the at least one miRNA is equal to or decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

h) the amount of the at least one miRNA marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

i) the amount of the at least one miRNA marker is decreased in comparison to a reference sample obtained from said subject at an earlier time point.

[0150] In particular, the sample of interest and/or the reference sample can be a body fluid sample or a tissue sample. The body fluid sample can be selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. In particular, the body fluid sample can be a blood sample.

[0151] The tissue sample can be a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. The sample of interest and/or the reference sample can be a medium obtained from said cell cultures or tissue cultures.

[0152] In particular, the subject is a mammal, reptile, or bird. In particular, the subject is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0153] Also disclosed is a method of determining the beneficial and/or adverse effects of a substance on cancer or the development of cancer, said method however not part of the invention, comprising the steps of

(a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S 100P, and DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample,

(b) determining the methylation status and/or expression level of the at least one methylation marker and the amount of the at least one miRNA marker in one or more references or reference samples, and

(c) examining the sample of interest as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples,

wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples.

[0154] The sample of interest can be exposed differently to said substance with regard to time and/or concentration. Thus, the sample of interest may be exposed to said substance for a longer or shorter time interval, and/or at a higher or lower concentration of said substance.

[0155] In cases, wherein the sample of interest is exposed to a higher concentration and/or for a longer time interval, an adverse effect of a substance is determined if

a) the methylation status of the at least one methylation marker is decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

b) the methylation status of the at least one methylation marker is equal to or decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

c) the methylation status of the at least one methylation marker is equal to decreased in comparison to a reference sample obtained from said subject at an earlier time point.

d) the expression level of the at least one methylation marker is increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

e) the expression level of the at least one methylation marker is equal to or increased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

f) the expression level of the at least one methylation marker is equal to increased in comparison to a reference sample obtained from said subject at an earlier time point.

g) the amount of the at least one miRNA is increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

h) the amount of the at least one miRNA marker is equal to or increased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

i) the amount of the at least one miRNA marker is equal to or increased in comparison to a reference sample obtained from said subject at an earlier time point.

[0156] In cases, wherein the sample of interest is exposed to a higher concentration and/or for a longer time interval, a beneficial effect of a substance is determined if

a) the methylation status of the at least one methylation marker is equal to or increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

b) the methylation status of the at least one methylation marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

c) the methylation status of the at least one methylation marker is equal to decreased in comparison to a reference sample obtained from said subject at an earlier time point.

d) the expression level of the at least one methylation marker is equal to or decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

e) the expression level of the at least one methylation marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

f) the expression level of the at least one methylation marker is decreased in comparison to a reference sample obtained from said subject at an earlier time point.

g) the amount of the at least one miRNA is equal to or decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

h) the amount of the at least one miRNA marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

i) the amount of the at least one miRNA marker is decreased in comparison to a reference sample obtained from said subject at an earlier time point.

[0157] In cases, wherein the sample of interest is exposed to a lower concentration and/or for a shorter time interval, no effect or an adverse effect of a substance is determined if

a) the methylation status of the at least one methylation marker is decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

b) the methylation status of the at least one methylation marker is equal to or decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

c) the methylation status of the at least one methylation marker is equal to decreased in comparison to a reference sample obtained from said subject at an earlier time point.

d) the expression level of the at least one methylation marker is increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject

or representative of the absence of the disease.

e) the expression level of the at least one methylation marker is equal to or increased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

f) the expression level of the at least one methylation marker is equal to increased in comparison to a reference sample obtained from said subject at an earlier time point.

g) the amount of the at least one miRNA is increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

h) the amount of the at least one miRNA marker is equal to or increased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

i) the amount of the at least one miRNA marker is equal to or increased in comparison to a reference sample obtained from said subject at an earlier time point.

[0158] In cases, wherein the sample of interest is exposed to a lower concentration and/or for a shorter time interval, a beneficial effect of a substance is determined if

a) the methylation status of the at least one methylation marker is equal to or increased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

b) the methylation status of the at least one methylation marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

c) the methylation status of the at least one methylation marker is equal to decreased in comparison to a reference sample obtained from said subject at an earlier time point.

d) the expression level of the at least one methylation marker is equal to or decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

e) the expression level of the at least one methylation marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

f) the expression level of the at least one methylation marker is decreased in comparison to a reference sample obtained from said subject at an earlier time point.

g) the amount of the at least one miRNA is equal to or decreased in comparison to a reference indicative of the absence of the disease or the decreased risk of developing a disease, or reference sample of a healthy subject or representative of the absence of the disease.

h) the amount of the at least one miRNA marker is decreased in comparison to a reference indicative of the presence of the disease or the increased risk of developing a disease, or reference sample of a diseased subject or representative of the presence of the disease.

i) the amount of the at least one miRNA marker is decreased in comparison to a reference sample obtained from said subject at an earlier time point.

[0159] In particular, the sample of interest and/or the reference sample is a body fluid samples or a tissue samples. The body fluid sample can be selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. The body fluid sample can be a blood sample.

[0160] The tissue sample can be a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. The sample of interest and/or the reference sample can be a medium obtained from said cell cultures or tissue cultures.

[0161] In particular, the subject is a mammal, reptile, or bird. In particular, the subject is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0162] Also disclosed but nor forming part of the present invention is a method for identifying a patient as a responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4,

as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken from the subject subsequently to the first sample, wherein an increased methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and the absence or decreased amount of the at least one miRNA marker indicates a response to the treatment.

[0163] The sample of interest and/or the reference sample may be a body fluid sample or a tissue sample. In particular, the body fluid sample can be selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. Preferably, the body fluid sample is a blood sample.

[0164] The tissue sample can be a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. The sample of interest and/or the reference sample can be a medium obtained from said cell cultures or tissue cultures.

[0165] The subject can be a mammal, reptile, or bird. In particular, the subject is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0166] Also disclosed but not forming part of the present invention is a method for identifying a patient as a non-responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and the presence or increased amount of the at least one miRNA marker indicates a lack of response to the treatment.

[0167] The sample of interest and/or the reference sample can be a body fluid sample or a tissue sample. In particular, the body fluid sample can be selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. The tissue sample can be a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. The sample of interest and/or the reference sample can be a medium obtained from said cell cultures or tissue cultures.

[0168] The subject can be a mammal, reptile, or bird. In particular, the subject can be selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0169] Also disclosed but not forming part of the present invention is a method for treating cancer, comprising the steps:

(i) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample of a subject;
(ii) starting treatment of said subject with a first treatment regimen comprising one or more anti-cancer agents or therapies,
(iii) determining the methylation status of the at least one methylation marker and/or the expression level of the at least one methylation marker, and the amount of the at least one miRNA in one or more subsequently taken second samples of said subject;
(iv) optionally repeating steps (ii) and (iii) one or more times;
(v) continuing treating the subject with the first treatment regimen if there is a substantial increase of the methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and a decreased amount or absence of the at least one miRNA marker, or
(vi) amending the treatment or terminating treating the subject with the first treatment regimen and treating the subject instead with a second treatment regimen comprising one or more anti-cancer agents or therapies not comprised in the first treatment regimen if there is a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and an increased amount or presence of the at least one miRNA marker.

[0170] The sample of interest and/or the reference sample can be a body fluid sample or a tissue sample. In particular, the body fluid sample can be selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva,

lymphatic fluid, lacrimal fluid, and fluid obtainable from the glands such as e.g. breast or prostate. In particular, the body fluid sample is a blood sample.

[0171] The tissue sample can be a tissue extract obtained from tumour tissue or tissue adjacent to a tumour. The sample of interest and/or the reference sample can be a cell culture or tissue culture such as but not limited to cultures of various cancer cells. IThe sample of interest and/or the reference sample can be a medium obtained from said cell cultures or tissue cultures.

[0172] The subject can be a mammal, reptile, or bird. In particular, the subject can be selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas, and human being. Human beings are particularly preferred.

[0173] The treatment regime can be selected from the list consisting of chemotherapy, anti-hormone therapy, immunotherapy, and radiation therapy.

[0174] Also disclosed are means for prognosing and/or diagnosing

i. the risk of developing cancer, in particular BC, OvaCa, and/or PaCA,
ii. the presence of cancer, in particular BC, OvaCa, and/or PaCA, and/or
iii. the progression of cancer, in particular BC, OvaCa, and/or PaCA, comprising

c) one or more means of detecting the methylation status and/or expression level of at least one methylation marker, and
d) one or more means of detecting the amount of at least one miRNA marker.

[0175] Said means may detect the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above.

[0176] The one or more means for detecting the methylation status of at least one methylation marker may comprise at least one methylation-specific polynucleotide. In particular, the methylation-specific polynucleotide can be a methylation-specific primer and/or a methylation-specific probe.

[0177] The one or more means for detecting the expression level of at least one methylation marker may comprise a binding moiety. Said binding moiety is in particular a polynucleotide, peptide, protein, or aptamer. The binding moiety may be selected from the group consisting of monoclonal antibodies, polyclonal antibodies, Fab fragments, Fc fragments, Fab' fragments, F(ab')2 fragments, single domain antibodies (sdAb), nanobodies, single chain Fv (scFv), divalent single-chain variable fragments (di-scFvs), tandem scFvs, diabodies, triabodies, bispecific diabodies, single-chain diabodies (scDb), bispecific T-cell engagers (BiTEs), and DART" molecules.

[0178] The binding moiety may bind to a part of the gene product of the methylation marker. Accordingly, in cases wherein the binding moiety is a polynucleotide, said polynucleotide binds to the mRNA transcribed from the gene of the respective methylation marker, i.e. the gene of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, or DYRK4.

[0179] In cases, wherein the binding moiety is a peptide, protein or aptamer, said peptide, protein, or aptamer, binds to a part, in particular an epitope, of the protein translated from the gene of the respective methylation marker, i.e. the gene of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, or DYRK4.

[0180] Said means may detect at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above.

[0181] The one or more means for detecting the amount of at least one miRNA marker may comprise at least one miRNA specific polynucleotide.

[0182] In particular, said at least one miRNA specific polynucleotide may have a sequence according to SEQ ID NO: 1-13

[0183] The said means may be for use in the method of specified in detail above. In particular, said means are for use in a method selected from the group consisting of:

(i) a method of prognosing and/or diagnosing cancer, in particular BC, OvaCa, and/or PaCA, in a subject, comprising (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, and (b) determining the presence, in particular the amount, of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, in a subject, wherein the methylation status and/or expression level of at least one methylation marker and the presence of at least one miRNA is indicative of the prognosis and/or diagnosis of said subject,
(ii) a method for determining the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer in a subject, comprising the steps of (a) determining the methylation status and/or expression level of at

least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample of a subject, and optionally determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in a reference for comparison with the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest, optionally depending on the comparison of the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker a in the sample of interest and the reference or reference sample,

(iii) a method for adapting the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample, (b) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in one or more references or reference samples, (c) examining the tested sample as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest is different from the level in the one or more references or reference samples,

(iv) a method of determining the beneficial and/or adverse effects of a substance on cancer or the development of cancer, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample of interest,(b) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in one or more references or reference samples, and (c) examining the sample of interest as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples,

(v) a method for identifying a patient as a responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein an increased methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and the absence or decreased amount of the at least one miRNA marker indicates a response to the treatment,

(vi) a method for identifying a patient as a non-responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and the presence or increased amount of the at least one miRNA marker indicates a lack of response to the treatment, and

(vii) a method for treating cancer, comprising the steps: (i) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample of a subject; (ii) starting treatment of said patient with a first treatment regimen comprising one or more anti-cancer agents or therapies, (iii) determining the methylation status of at least one methylation marker and/or the expression level of at least one methylation marker, and the amount of at least one miRNA in one or more subsequently taken further samples of said subject; (iv) optionally repeating steps (ii) and

(iii) one or more times; (v) continuing treating the patient with the first treatment regimen if there is a substantial increase of the methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and a decreased amount or absence of the at least one miRNA marker, or (vi) amending the treatment or terminating treating the patient with the first treatment regimen and treating the patient instead with a second treatment regimen comprising one or more anti-cancer agents or therapies not comprised in the first treatment regimen if there is a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and an increased amount or presence of the at least one miRNA marker.

[0184] In a fourth aspect, the present invention relates to the use of a kit for diagnosing BC, the kit comprising one or more means for detecting the methylation status of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and DYRK4, as specified in detail above, and means for detecting the amount of miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 and miR-148b, as specified in detail above.

[0185] The kit may further comprise

(a) a container, and/or
(b) a data carrier, wherein the data carrier comprises information such as

(i) instructions concerning methods for identifying the risk for developing and/or identifying the presence and/or monitoring progression of cancer
(ii) instructions for use of the means for detecting the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker, in particular in a sample, more specifically in a sample from an individual and/or of the kit,
(iii) quality information such as information about the lot/batch number of the means for detecting the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker and/or of the kit, the manufacturing or assembly site or the expiry or sell-by date, information concerning the correct storage or handling of the kit,
(iv) information concerning the composition of the buffer(s), diluent(s), reagent(s) for detecting the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker and/or of the means for detecting the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker,
(v) information concerning the interpretation of information obtained when performing the above-mentioned methods identifying and/or monitoring progression of cancer,
(vi) a warning concerning possible misinterpretations or wrong results when applying unsuitable methods and/or unsuitable means, and/or
(vii) a warning concerning possible misinterpretations or wrong results when using unsuitable reagent(s) and/or buffer(s).

[0186] The kit disclosed herein can be used in the method specified in detail above. In particular, the kit can be for use in a method selected from the group consisting of:

(i) a method of prognosing and/or diagnosing cancer, in particular BC, OvaCa, and/or PaCA, in a subject, comprising (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S 100P, DYRK4, and (b) determining the presence, in particular the amount, of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, in a subject, wherein the methylation status and/or expression level of at least one methylation marker and the presence of at least one miRNA is indicative of the prognosis and/or diagnosis of said subject,
(ii) a method for determining the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer in a subject, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample of a subject, and optionally determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in a reference for comparison with the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the methylation

status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest, optionally depending on the comparison of the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker a in the sample of interest and the reference or reference sample,

(iii) a method for adapting the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample, (b) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in one or more references or reference samples, (c) examining the tested sample as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest is different from the level in the one or more references or reference samples,

(iv) a method of determining the beneficial and/or adverse effects of a substance on cancer or the development of cancer, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample of interest,(b) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in one or more references or reference samples, and (c) examining the sample of interest as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples,

(v) a method for identifying a patient as a responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein an increased methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and the absence or decreased amount of the at least one miRNA marker indicates a response to the treatment,

(vi) a method for identifying a patient as a non-responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and the presence or increased amount of the at least one miRNA marker indicates a lack of response to the treatment, and

(vii) a method for treating cancer, comprising the steps: (i) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample of a subject; (ii) starting treatment of said patient with a first treatment regimen comprising one or more anti-cancer agents or therapies, (iii) determining the methylation status of at least one methylation marker and/or the expression level of at least one methylation marker, and the amount of at least one miRNA in one or more subsequently taken further samples of said subject; (iv) optionally repeating steps (ii) and (iii) one or more times; (v) continuing treating the patient with the first treatment regimen if there is a substantial increase of the methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and a decreased amount or absence of the at least one miRNA marker, or (vi) amending the treatment or terminating treating the patient with the first treatment regimen and treating the patient instead with a second treatment regimen comprising one or more anti-cancer agents or therapies not comprised in the first treatment regimen if there is a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and an increased amount or presence of the

at least one miRNA marker.

[0187] Also disclosed are means for detecting the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the presence, in particular the amount, of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, or the kit comprising said means, as specified in detail above, for prognosing and/or diagnosing

i. the risk of developing cancer, in particular BC, OvaCa, and/or PaCA,
ii. the presence of cancer, in particular BC, OvaCa, and/or PaCA, and/or
iii. the progression of cancer, in particular BC, OvaCa, and/or PaCA.

[0188] Said means and/or said kit can be used in one of the methods specified in detail above. In particular, they can be used in a method selected from the group consisting of:

(i) a method of prognosing and/or diagnosing cancer, preferably BC, OvaCa, and/or PaCA, in a subject, comprising (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, and (b) determining the presence, in particular the amount, of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, in a subject, wherein the methylation status and/or expression level of at least one methylation marker and the presence of at least one miRNA is indicative of the prognosis and/or diagnosis of said subject,
(ii) a method for determining the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer in a subject, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample of a subject, and optionally determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in a reference for comparison with the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest, optionally depending on the comparison of the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker a in the sample of interest and the reference or reference sample,
(iii) a method for adapting the dosage of a pharmaceutical for the alteration of cancer or the prevention or treatment of cancer, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample, (b) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in one or more references or reference samples, (c) examining the tested sample as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in the sample of interest is different from the level in the one or more references or reference samples,
(iv) a method of determining the beneficial and/or adverse effects of a substance on cancer or the development of cancer, comprising the steps of (a) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a sample of interest,(b) determining the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker in one or more references or reference samples, and (c) examining the sample of interest as to whether the methylation status and/or expression level of at least one methylation marker and the amount of at least one miRNA marker present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples.

(v) a method for identifying a patient as a responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein an increased methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and the absence or decreased amount of the at least one miRNA marker indicates a response to the treatment,

(vi) a method for identifying a patient as a non-responder to a cancer treatment, comprising determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample and in one or more further samples taken subsequently to the first sample, wherein a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and the presence or increased amount of the at least one miRNA marker indicates a lack of response to the treatment, and

(vii) a method for treating cancer, comprising the steps: (i) determining the methylation status and/or expression level of at least one methylation marker selected from the group consisting of HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, as specified in detail above, and the amount of at least one miRNA marker selected from the group consisting of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, miR-148b, as specified in detail above, in a first sample of a subject; (ii) starting treatment of said patient with a first treatment regimen comprising one or more anti-cancer agents or therapies, (iii) determining the methylation status of at least one methylation marker and/or the expression level of at least one methylation marker, and the amount of at least one miRNA in one or more subsequently taken further samples of said subject; (iv) optionally repeating steps (ii) and (iii) one or more times; (v) continuing treating the patient with the first treatment regimen if there is a substantial increase of the methylation status of the at least one methylation marker and/or a lower expression level of the at least one methylation marker, and a decreased amount or absence of the at least one miRNA marker, or (vi) amending the treatment or terminating treating the patient with the first treatment regimen and treating the patient instead with a second treatment regimen comprising one or more anti-cancer agents or therapies not comprised in the first treatment regimen if there is a decreased methylation status of the at least one methylation marker and/or an increased expression level of the at least one methylation marker, and an increased amount or presence of the at least one miRNA marker.

[0189] In an fifth aspect, the present invention relates to the device for identifying BC, comprising:

(a) an analyzing unit comprising

(i) one or more means for detecting the methylation status of at least one methylation marker selected from the group consisting of HY AL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P and DYRK4, and
(ii) means for detecting the amount of miRNA markers miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 and miR-148b in a sample of a subject; and

(b) an evaluation unit comprising a data processor having tangibly embedded an algorithm for carrying out a comparison of the amount determined by the analyzing unit with a reference and which is capable of generating an output file containing a diagnosis established based on the said comparison.

[0190] Particular devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of parametric diagnostic raw data, in particular, as absolute or relative amounts. It is to be understood that these data will need interpretation by the clinician. However, also envisaged are expert system devices wherein the output comprises processed diagnostic raw data the interpretation of which does not require a specialized clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the miRNAs of the present invention, Plasmon surface resonance devices, NMR spectrometers, mass-spectrometers etc.) or evaluation units/devices.

[0191] Aspects and embodiments of the present invention are also detailed in the accompanying claims.

[0192] The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

EXAMPLES

Study population

[0193] The present study was approved by the Ethics Committee of the University of Heidelberg (Germany). All the cancer patients and healthy controls were Caucasian. All the recruited cases and controls gave written informed consent for the study. Genomic DNA was isolated from peripheral whole blood using DNA isolation kits from Qiagen. The leucocytes were immediately frozen in liquid nitrogen after isolation and stored at -80°C until use. DNA and RNA were isolated from leucocytes using AllPrep DNA/RNA/Protein Mini Kit from Qiagen. Detailed information for the samples was shown in Table 1. Please see the clinical data of the sporadic BC patients in Table 5 and Table 6.

BC cases and matched controls

[0194] Peripheral blood samples from 270 BRCA1/2 mutation-negative index familial BC patients (first validation round) were collected by the centers of the German Consortium for Hereditary Breast and Ovarian Cancer in Heidelberg and Cologne. All the familial BC cases were recruited according to the criteria of family history. Peripheral blood samples from 350 sporadic BC patients (189 in the second validation round and 161 in the third validation round) were collected at the time point of first BC diagnosis before any BC treatment and surgery at the University Hospital of Heidelberg. The clinical characteristics of sporadic BC patients were defined according to the American Joint Committee on Cancer (AJCC) cancer staging manual. Peripheral blood samples from 459 healthy female controls (251 in the first validation round and 189 in the second validation round) were collected from blood donors by the German Red Cross Blood Service of Baden-Württemberg-Hessen. Peripheral blood samples from 151 healthy female controls (third validation round) were collected at the University Hospital of Heidelberg. All the cases and controls in the third validation round were processed with the same manner in parallel. Leucocytes were isolated from peripheral blood using red blood cell lysis buffer within four hours after blood collection at the University Hospital of Heidelberg. All the leucocytes from cases and controls were processed in parallel.

PaCa cases and matched controls

[0195] Peripheral blood samples from 147 sporadic PaCa patients (80 male cases and 67 female cases) were collected from multiple centers in Germany. The PaCa cases were specially selected with higher percentage of early stage cases. Peripheral blood samples from 191 healthy controls (115 male cases and 76 female cases) were collected from blood donors by the German Red Cross Blood Service of Baden - Württemberg-Hessen.

OvCa cases and matched controls

[0196] Peripheral blood samples from 84 sporadic OvCa patients were collected at the University Hospital of Heidelberg. The OvCa cases were specially selected with higher percentage of early stage cases. Peripheral blood samples from 148 healthy controls were collected at the University Hospital of Heidelberg.

Example 1: Analysis of Methylation Marker

Infinium 27k Methylation Assay and Infinium 450k Methylation Assay

[0197] In the discovery round, 500 ng genomic DNA from each sample was treated by EZ-96 DNA Methylation Kit (Zymo Research) for bisulfite conversion and subjected to genome-wide methylation screening by Human Methylation27 BeadChip (Illumina) and Infinium HumanMethylation450 BeadChip Kit (Illumina) according to the manufacturer recommendations (Steemers FJ, Chang W, Lee G, Barker DL, Shen R, Gunderson KL. Whole-genome genotyping with the single-base extension assay. Nat Methods 2006;3:31-3. Bork S, Pfister S, Witt H, et al. DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. Aging Cell 2009;9:54-63). All samples passed the quality control according to manufacturer instructions.

Methylation Analysis Via Maldi-TOF Mass Spectrometry

[0198] MALDI-TOF mass spectrometry (Sequenom) described by Breitling et al. (Breitling LP, Yang R, Korn B, Burwinkel B, Brenner H. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. Am J Hum Genet 2011;88:450-7.) was used in various verification rounds. DNA was bisulfite converted by EZ-96 DNA Methylation Gold Kit (Zymo Research) and amplified by bisulfite-specific primers (Fig. 1). The PCR products were treated according

to the standard protocol of Sequenom EpiTyper Assay and dispensed to a 384 SpectroCHIP by a Nanodispenser. The chips were read by a Sequenom Mass Spectrometer system. Data were collected by SpectroACQUIRE v3.3.1.3 software and visualized with MassArray EpiTyper v1.0 software. 5% samples were randomly chosen for the duplication analysis.

Quantitative Real-time PCR for RNA Expression

[0199] 100 ng of total RNA from each sample was transcribed to cDNA by TaqMan® Reverse Transcription Reagents (Applied Biosystems). Quantitative real-time PCR was performed using a LightCycler480 (Roche) in combination with TaqMan gene expression assays (Applied Biosystems) for HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, and DYRK4 gene and housekeeping gene HPRT1 as endogenous control. Crossing point values were calculated using the second-derivative maximum method by the LightCycler 480 basic software (Roche). Relative expression of genes for each sample was calculated according to the ΔΔCt method by normalization to HPRT1. All the cases and controls were processed in parallel.

Bisulfite-specific primers for different amplicons

Amplicons	Primers	Sequences
S100P	sense	aggaagagagGGAAGGTGGGTTTGAATTTAGTATT
	antisense	cagtaatacgactcactatagggagaaggctCTATCCCTCTTACCTCTAAACCCCT
SLC22A18	sense	aggaagagagTAAGTGGAATTTTGGTATTTTTGGA
	antisense	cagtaatacgactcactatagggagaaggctCACTCCAAACCTAAACTCACCTCTA
DYRK4	sense	aggaagagagGGTTTTTTTAAAATTGGTTTTGGAT
	antisense	cagtaatacgactcactatagggagaaggctAAACCCCATTTTTATTCCCATAAT
FUT7	sense	aggaagagagGAAGAGGAAGGGATTTAGTTTGAAG
	antisense	cagtaatacgactcactatagggagaaggctACAAACCTTAACCTCCCAAAATACT
RPTOR	sense	aggaagagagGTGGGGTTTTTGTAGTAGTTGAGA
	antisense	cagtaatacgactcactatagggagaaggctTAATAACCCAAAACCAAACCCTAAC
MGRN1	sense	aggaagagagTTTTGGGGTATAAGGGAAGTTTAAG
	antisense	cagtaatacgactcactatagggagaaggctCCTAACCAACAAAAAACCTAAAAAA
RAPSN	sense	aggaagagagGATTTTTAGTTGGTGAGAGGTTTGA
	antisense	cagtaatacgactcactatagggagaaggctAAAACCACTAAATTACCCAACCAAA
HYAL2	sense	aggaagagagTTTTAAATTTAGTAGGGTGTGAGAGGA
	antisense	cagtaatacgactcactatagggagaaggctCTCATCCATATTATAAAAAACCCCC
HYAL2-310	sense	aggaagagagTTTTTTTGGGGTGAGTTTTTTTAGT
	antisense	cagtaatacgactcactatagggagaaggctCACCTAATCCTAAACCCATAACCTT
HYAL2-325	sense	aggaagagagTTGTTTAGTTTTTGAGGTTTTTTGG
	antisense	cagtaatacgactcactatagggagaaggctATTACACTCCCTCCCTCTCCTAAC

Statistical Analysis

[0200] The Illumina 27K Array data were processed by the Illumina BeadStudio software with default settings. Probes with detection P-value > 0.01 were removed and samples were quantile-normalized. Association of probes with case/control status was assessed by beta-regression models with a logistic link and associated Wald tests using the R package betareg v2.2-3 30. Likelihood ratio tests were used to compare the case/control model with the nested model for chip differences in order to identify possible false hits due to confounding by chip effects. Multiple testing adjustments were done with the Benjamini-Hochberg method controlling the false discovery rate at the level of 0.05. All analysis was performed with the statistical software R v2.11.1.

All the statistical analyses of the gene expression data were conducted by SPSS Statistics 17.0 software. The correlations were assessed by Spearman's rank correlation coefficients. Logistic regression models and non-parametric tests were used for comparisons between two and multiple groups. The results of logistic regression were adjusted for possible confounding effects of age and different measurement batches by including additional co-variables in the logistic regression models. Receiver operating characteristic (ROC) curve analysis was performed to assess the discriminatory

power of methylation levels.

Example 2: Analysis of miRNA Marker

Blood processing and miRNA isolation from plasma

[0201] EDTA blood samples were collected from cases and control individuals and processed for plasma within 2 hours of collection. To avoid contamination with epithelial cells from the initial skin puncture the first blood tube collected during phlebotomy was not processed for plasma. Blood was centrifuged at 1300g for 20 minutes at 10°C. The supernatant (plasma) was transferred into microcentrifuge tubes followed by a second high-speed centrifugation step at 15500 g for 10 minutes at 10°C to remove cell debris and fragments. The plasma was aliquoted into cryo vials, snap-frozen in liquid nitrogen and stored at -80°C until use. Total RNA (including miRNAs) was extracted from 400 μL of plasma. Denaturation and phase separation were conducted using TRIzol LS (Invitrogen, Germany) according to manufacturer's protocol, with a minor modification: 10 fmol of a C. elegans miR-39/miR-238 mixture was spiked-in. The aqueous phase was transferred into another tube, 1.5 volumes of absolute ethanol were added and the mixture was applied to miRNeasy Mini kit columns (Qiagen, Germany). After washing miRNAs were eluted in 30 μL of RNase-free water.

Validation of selected marker candidates

[0202] Reverse transcription (RT) reactions were performed using TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Germany) and miRNA-specific RT primers for miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and miR-148b (Applied Biosystems, Germany). Singleplex (primary breast cancer) or multiplex (metastatic breast cancer) reactions were carried out in a volume of 7.5 μl or 15 μl, respectively. Each reaction comprised 1x RT buffer, 1 mM dNTPs, 0.3x miRNA-specific RT primers, 0.25 U RNase inhibitor, 3.3U Multiscribe Reverse Transcriptase and a fixed volume of miRNA template (2 or 1 μl, respectively). For benign and malignant breast cancer tissue samples the reactions were carried out in 15 μl and comprised the following: 1 x RT buffer, 1mM dNTPs, 0.6x miRNA-specific and RNU6B RT primers, 0.25U RNase inhibitor, 3.3U Multiscribe Reverse Transcriptase and 5 ng RNA. Blinding of samples and a randomized, simultaneous investigation of cases and controls on reaction plates was intended to minimize bias and batch effects during validation. RT was carried out in a G-STORM GS2 PCR cycler (Alphametrix, Germany) under the following conditions: 16°C for 30 min, 42°C for 30 min and 85°C for 5 min, followed by a hold at 4°C.
TaqMan real-time PCR reactions were performed in triplicates in scaled-down reactions comprising 2.5 μL TaqMan 2x Universal PCR Master Mix with No AmpErase UNG (Applied Biosystems, Germany), 0.25 μL 20x miRNA-specific primer/probe mix (Applied Biosystems, Germany) and 2.25 μL of the reverse transcription product (diluted 1:4). Real-time PCR was carried out in a LightCycler 480 thermocycler (Roche, Germany) under the following conditions: 95°C for 10 min, then 50 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s, followed by a hold at 4°C.
[0203] Raw data from validation studies in blood plasma was normalized to spiked-in cel-miR-39 as described in Kroh et al. (Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods 2010;50:298-301). Raw Ct values from breast tissue samples were normalized to RNU6B as described in User Bulletin #2: ABI PRISM 7700 Sequence Detection System (Applied Biosystems).

Comparison of cancer cases with controls

[0204] To evaluate the breast cancer and prostate cancer detection potential, receiver operating characteristic (ROC) curves were constructed and the areas under the curves (AUC) calculated. Based on ROC curves with 95% confidence intervals, lowest specificities at pre-defined sensitivities (75% to 90%) were computed for miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and miR-148b. Based on ROC curves, lowest specificities at pre-defined sensitivities (75% to 90%) were computed for the most informative and least redundant model of miRNAs as the lower bounds of the 95% confidence intervals (Tom Fawcett (2006) "An introduction to ROC analysis". Pattern Recognition Letters 27, 861-874. DOI: 10.1016/j.patrec.2005.10.010; using R package pROC v1.3.2).

Diagnostic potential of miR-652. miR-801, miR-376c, miR-376a, miR-127, miR-409, and miR-148b in plasma

[0205] ROC curve analysis was performed to evaluate the diagnostic potential of miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and miR-148b for breast cancer and prostate cancer detection in blood plasma. The discriminatory power between tumor and control samples is depicted by the areas under the curves (AUC).
By investigating different combinations of miR-148b, miR-376c, miR-409-3p and miR-801 we found that a combined ROC curve with all seven miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409, and miR-148b gave the most

informative and least redundant miRNA panel with an AUC of 0.89.

Example 3: Combination of Methylation and miRNA Marker

Sample perparation

[0206] Peripheral blood samples from 161 sporadic BC patients (the third validation round) were collected at the time point of first BC diagnosis before any BC treatment and surgery at the University Hospital of Heidelberg. The clinical characteristics of sporadic BC patients were defined according to the American Joint Committee on Cancer (AJCC) cancer staging manual. Peripheral blood samples from 151 healthy female controls (third validation round) were collected at the University Hospital of Heidelberg. All the cases and controls in the third validation round were processed with the same manner in parallel. The DNA from the whole blood and the miRNA from plasma were extracted from each sample.

[0207] Determination of DNA methylation level and miRNA level. The DNA methylation levels were determined by MALDI-TOF mass spectrometry (Sequenom) as described in Example 1. The miRNA levels from plasam were determined by real-time PCR as described in Exapmle 2.

Statistical Analysis

[0208] All the statistical analyses of the gene expression data were conducted by SPSS Statistics 17.0 software. Results of the marker set (combination of DNA methylation and miRNA markers) were generated with the use of a logistic-regression algorithm. Logistic regression models was used to and non-parametric tests were used for comparisons between two and multiple groups. The results of logistic regression were adjusted for possible confounding effects of age and different measurement batches by including additional co-variables in the logistic regression models. Receiver operating characteristic (ROC) curve analysis was performed to assess the discriminatory power of methylation levels.

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<400> 16

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Gly Arg Pro Phe Val Val Ala Trp Asp Val Pro Thr Gln Asp Cys Gly
35 40 45

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Pro Arg Leu Lys Val Pro Leu Asp Leu Asn Ala Phe Asp Val Gln Ala
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Ser Pro Asn Glu Gly Phe Val Asn Gln Asn Ile Thr Ile Phe Tyr Arg
65                  70                  75                  80

Asp Arg Leu Gly Leu Tyr Pro Arg Phe Asp Ser Ala Gly Arg Ser Val
                85                  90                  95

His Gly Gly Val Pro Gln Asn Val Ser Leu Trp Ala His Arg Lys Met
            100                 105                 110

Leu Gln Lys Arg Val Glu His Tyr Ile Arg Thr Gln Glu Ser Ala Gly
        115                 120                 125

Leu Ala Val Ile Asp Trp Glu Asp Trp Arg Pro Val Trp Val Arg Asn
    130                 135                 140

Trp Gln Asp Lys Asp Val Tyr Arg Arg Leu Ser Arg Gln Leu Val Ala
145                 150                 155                 160

Ser Arg His Pro Asp Trp Pro Pro Asp Arg Ile Val Lys Gln Ala Gln
                165                 170                 175

Tyr Glu Phe Glu Phe Ala Ala Gln Gln Phe Met Leu Glu Thr Leu Arg
            180                 185                 190

Tyr Val Lys Ala Val Arg Pro Arg His Leu Trp Gly Phe Tyr Leu Phe
        195                 200                 205

Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Glu Ser Tyr Thr
    210                 215                 220

Gly Arg Cys Pro Asp Val Glu Val Ala Arg Asn Asp Gln Leu Ala Trp
225                 230                 235                 240

Leu Trp Ala Glu Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asp Glu
                245                 250                 255

Thr Leu Ala Ser Ser Arg His Gly Arg Asn Phe Val Ser Phe Arg Val
            260                 265                 270

Gln Glu Ala Leu Arg Val Ala Arg Thr His His Ala Asn His Ala Leu
        275                 280                 285

Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Ser Arg Arg Leu Thr Gly
    290                 295                 300
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Leu Ser Glu Met Asp Leu Ile Ser Thr Ile Gly Glu Ser Ala Ala Leu
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Gly Ala Ala Gly Val Ile Leu Trp Gly Asp Ala Gly Tyr Thr Thr Ser
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Thr Glu Thr Cys Gln Tyr Leu Lys Asp Tyr Leu Thr Arg Leu Leu Val
            340                 345                 350

Pro Tyr Val Val Asn Val Ser Trp Ala Thr Gln Tyr Cys Ser Arg Ala
        355                 360                 365

Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asn Pro Ser Ala Ser
    370                 375                 380

Thr Phe Leu His Leu Ser Thr Asn Ser Phe Arg Leu Val Pro Gly His
385                 390                 395                 400

Ala Pro Gly Glu Pro Gln Leu Arg Pro Val Gly Glu Leu Ser Trp Ala
                405                 410                 415

Asp Ile Asp His Leu Gln Thr His Phe Arg Cys Gln Cys Tyr Leu Gly
            420                 425                 430

Trp Ser Gly Glu Gln Cys Gln Trp Asp His Arg Gln Ala Ala Gly Gly
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<212> PRT
<213> Homo sapiens

<400> 17

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Gly Arg Pro Phe Val Val Ala Trp Asp Val Pro Thr Gln Asp Cys Gly
        35                  40                  45
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Pro Arg Leu Lys Val Pro Leu Asp Leu Asn Ala Phe Asp Val Gln Ala
    50                  55                  60

Ser Pro Asn Glu Gly Phe Val Asn Gln Asn Ile Thr Ile Phe Tyr Arg
65                  70                  75                  80

Asp Arg Leu Gly Leu Tyr Pro Arg Phe Asp Ser Ala Gly Arg Ser Val
                85                  90                  95

His Gly Gly Val Pro Gln Asn Val Ser Leu Trp Ala His Arg Lys Met
            100                 105                 110

Leu Gln Lys Arg Val Glu His Tyr Ile Arg Thr Gln Glu Ser Ala Gly
        115                 120                 125

Leu Ala Val Ile Asp Trp Glu Asp Trp Arg Pro Val Trp Val Arg Asn
    130                 135                 140

Trp Gln Asp Lys Asp Val Tyr Arg Arg Leu Ser Arg Gln Leu Val Ala
145                 150                 155                 160

Ser Arg His Pro Asp Trp Pro Pro Asp Arg Ile Val Lys Gln Ala Gln
                165                 170                 175

Tyr Glu Phe Glu Phe Ala Ala Gln Gln Phe Met Leu Glu Thr Leu Arg
            180                 185                 190

Tyr Val Lys Ala Val Arg Pro Arg His Leu Trp Gly Phe Tyr Leu Phe
        195                 200                 205

Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Glu Ser Tyr Thr
    210                 215                 220

Gly Arg Cys Pro Asp Val Glu Val Ala Arg Asn Asp Gln Leu Ala Trp
225                 230                 235                 240

Leu Trp Ala Glu Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asp Glu
                245                 250                 255

Thr Leu Ala Ser Ser Arg His Gly Arg Asn Phe Val Ser Phe Arg Val
            260                 265                 270

Gln Glu Ala Leu Arg Val Ala Arg Thr His His Ala Asn His Ala Leu
        275                 280                 285

Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Ser Arg Arg Leu Thr Gly
    290                 295                 300
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Leu Ser Glu Met Asp Leu Ile Ser Thr Ile Gly Glu Ser Ala Ala Leu
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Gly Ala Ala Gly Val Ile Leu Trp Gly Asp Ala Gly Tyr Thr Thr Ser
                325                 330                 335

Thr Glu Thr Cys Gln Tyr Leu Lys Asp Tyr Leu Thr Arg Leu Leu Val
            340                 345                 350

Pro Tyr Val Val Asn Val Ser Trp Ala Thr Gln Tyr Cys Ser Arg Ala
        355                 360                 365

Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asn Pro Ser Ala Ser
    370                 375                 380

Thr Phe Leu His Leu Ser Thr Asn Ser Phe Arg Leu Val Pro Gly His
385                 390                 395                 400

Ala Pro Gly Glu Pro Gln Leu Arg Pro Val Gly Glu Leu Ser Trp Ala
                405                 410                 415

Asp Ile Asp His Leu Gln Thr His Phe Arg Cys Gln Cys Tyr Leu Gly
            420                 425                 430

Trp Ser Gly Glu Gln Cys Gln Trp Asp His Arg Gln Ala Ala Gly Gly
        435                 440                 445

Ala Ser Glu Ala Trp Ala Gly Ser His Leu Thr Ser Leu Leu Ala Leu
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Ala Ala Leu Ala Phe Thr Trp Thr Leu
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            260                 265                 270

Asp Asn Ser Asn Glu Cys Val Val Cys Leu Ser Asp Leu Arg Asp Thr
        275                 280                 285

Leu Ile Leu Pro Cys Arg His Leu Cys Leu Cys Thr Ser Cys Ala Asp
    290                 295                 300

Thr Leu Arg Tyr Gln Ala Asn Asn Cys Pro Ile Cys Arg Leu Pro Phe
305                 310                 315                 320

Arg Ala Leu Leu Gln Ile Arg Ala Val Arg Lys Lys Pro Gly Ala Leu
                325                 330                 335

Ser Pro Val Ser Phe Ser Pro Val Leu Ala Gln Ser Leu Glu His Asp
            340                 345                 350

Glu His Ser Asn Ser Asp Ser Val Pro Pro Gly Tyr Glu Pro Ile Ser
        355                 360                 365

Leu Leu Glu Ala Leu Asn Gly Leu Arg Ala Val Ser Pro Ala Ile Pro
    370                 375                 380

Ser Ala Pro Leu Tyr Glu Glu Ile Thr Tyr Ser Gly Ile Ser Asp Gly
385                 390                 395                 400

Leu Ser Gln Ala Ser Cys Pro Leu Ala Ala Ile Asp His Ile Leu Asp
                405                 410                 415

Ser Ser Arg Gln Lys Gly Arg Pro Gln Ser Lys Ala Pro Asp Ser Thr
            420                 425                 430

Leu Arg Ser Pro Ser Ser Pro Ile His Glu Glu Asp Glu Glu Lys Leu
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                435                 440                 445

Ser Glu Asp Val Asp Ala Pro Pro Pro Leu Gly Gly Ala Glu Leu Ala
    450                 455                 460

Leu Arg Glu Ser Ser Ser Pro Glu Ser Phe Ile Thr Glu Glu Val Asp
465                 470                 475                 480

Glu Ser Ser Ser Pro Gln Gln Gly Thr Arg Ala Ala Ser Ile Glu Asn
                485                 490                 495

Val Leu Gln Asp Ser Ser Pro Glu His Cys Gly Arg Gly Pro Pro Ala
            500                 505                 510

Asp Ile Tyr Leu Pro Gly Arg Pro Thr Ser Met Glu Thr Ala His Gly
        515                 520                 525

Leu Ala Thr Thr Ser Pro Thr Trp Pro Pro Leu Gly Gly Pro Ser Pro
    530                 535                 540

Asp Pro Ser Ala Ala Glu Leu Thr Pro Leu
545                 550
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<210> 23
<211> 552
<212> PRT
<213> Homo sapiens

<400> 23

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1               5                  10                  15

Ile Gln Ala Asn Ser Ala Tyr Arg Tyr Pro Pro Lys Ser Gly Asn Tyr
            20                  25                  30

Phe Ala Ser His Phe Phe Met Gly Gly Glu Lys Phe Asp Thr Pro His
        35                  40                  45

Pro Glu Gly Tyr Leu Phe Gly Glu Asn Met Asp Leu Asn Phe Leu Gly
    50                  55                  60

Ser Arg Pro Val Gln Phe Pro Tyr Val Thr Pro Ala Pro His Glu Pro
65                  70                  75                  80

Val Lys Thr Leu Arg Ser Leu Val Asn Ile Arg Lys Asp Ser Leu Arg
                85                  90                  95

Leu Val Arg Tyr Lys Asp Asp Ala Asp Ser Pro Thr Glu Asp Gly Asp
```

```
                100                 105                 110

Lys Pro Arg Val Leu Tyr Ser Leu Glu Phe Thr Phe Asp Ala Asp Ala
        115                 120                 125

Arg Val Ala Ile Thr Ile Tyr Cys Gln Ala Ser Glu Glu Phe Leu Asn
    130                 135                 140

Gly Arg Ala Val Tyr Ser Pro Lys Ser Pro Ser Leu Gln Ser Glu Thr
145                 150                 155                 160

Val His Tyr Lys Arg Gly Val Ser Gln Gln Phe Ser Leu Pro Ser Phe
                165                 170                 175

Lys Ile Asp Phe Ser Glu Trp Lys Asp Asp Glu Leu Asn Phe Asp Leu
            180                 185                 190

Asp Arg Gly Val Phe Pro Val Val Ile Gln Ala Val Val Asp Glu Gly
        195                 200                 205

Asp Val Val Glu Val Thr Gly His Ala His Val Leu Leu Ala Ala Phe
    210                 215                 220

Glu Lys His Met Asp Gly Ser Phe Ser Val Lys Pro Leu Lys Gln Lys
225                 230                 235                 240

Gln Ile Val Asp Arg Val Ser Tyr Leu Leu Gln Glu Ile Tyr Gly Ile
                245                 250                 255

Glu Asn Lys Asn Asn Gln Glu Thr Lys Pro Ser Asp Asp Glu Asn Ser
            260                 265                 270

Asp Asn Ser Asn Glu Cys Val Val Cys Leu Ser Asp Leu Arg Asp Thr
        275                 280                 285

Leu Ile Leu Pro Cys Arg His Leu Cys Leu Cys Thr Ser Cys Ala Asp
    290                 295                 300

Thr Leu Arg Tyr Gln Ala Asn Asn Cys Pro Ile Cys Arg Leu Pro Phe
305                 310                 315                 320

Arg Ala Leu Leu Gln Ile Arg Ala Val Arg Lys Lys Pro Gly Ala Leu
                325                 330                 335

Ser Pro Val Ser Phe Ser Pro Val Leu Ala Gln Ser Leu Glu His Asp
            340                 345                 350
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```
Glu His Ser Cys Pro Phe Lys Lys Ser Lys Pro His Pro Ala Ser Leu
        355                 360                 365

Ala Ser Lys Lys Pro Lys Arg Glu Thr Asn Ser Asp Ser Val Pro Pro
    370                 375                 380

Gly Tyr Glu Pro Ile Ser Leu Leu Glu Ala Leu Asn Gly Leu Arg Ala
385                 390                 395                 400

Val Ser Pro Ala Ile Pro Ser Ala Pro Leu Tyr Glu Glu Ile Thr Tyr
                405                 410                 415

Ser Gly Ile Ser Asp Gly Leu Ser Gln Ala Ser Cys Pro Leu Ala Ala
            420                 425                 430

Ile Asp His Ile Leu Asp Ser Ser Arg Gln Lys Gly Arg Pro Gln Ser
        435                 440                 445

Lys Ala Pro Asp Ser Thr Leu Arg Ser Pro Ser Ser Pro Ile His Glu
    450                 455                 460

Glu Asp Glu Glu Lys Leu Ser Glu Asp Val Asp Ala Pro Pro Pro Leu
465                 470                 475                 480

Gly Gly Ala Glu Leu Ala Leu Arg Glu Ser Ser Ser Pro Glu Ser Phe
                485                 490                 495

Ile Thr Glu Glu Val Asp Glu Ser Ser Ser Pro Gln Gln Gly Thr Arg
            500                 505                 510

Ala Ala Ser Ile Glu Asn Val Leu Gln Asp Ser Ser Pro Glu His Cys
        515                 520                 525

Gly Arg Gly Pro Pro Ala Asp Ile Tyr Leu Pro Ala Leu Gly Pro Asp
    530                 535                 540

Ser Cys Ser Val Gly Ile Asp Glu
545                 550
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<210> 24
<211> 530
<212> PRT
<213> Homo sapiens

<400> 24

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Met Gly Ser Ile Leu Ser Arg Arg Ile Ala Gly Val Glu Asp Ile Asp
1               5                   10                  15
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Ile Gln Ala Asn Ser Ala Tyr Arg Tyr Pro Pro Lys Ser Gly Asn Tyr
            20                  25                  30

Phe Ala Ser His Phe Phe Met Gly Gly Glu Lys Phe Asp Thr Pro His
        35                  40                  45

Pro Glu Gly Tyr Leu Phe Gly Glu Asn Met Asp Leu Asn Phe Leu Gly
    50                  55                  60

Ser Arg Pro Val Gln Phe Pro Tyr Val Thr Pro Ala Pro His Glu Pro
65                  70                  75                  80

Val Lys Thr Leu Arg Ser Leu Val Asn Ile Arg Lys Asp Ser Leu Arg
                85                  90                  95

Leu Val Arg Tyr Lys Asp Asp Ala Asp Ser Pro Thr Glu Asp Gly Asp
            100                 105                 110

Lys Pro Arg Val Leu Tyr Ser Leu Glu Phe Thr Phe Asp Ala Asp Ala
        115                 120                 125

Arg Val Ala Ile Thr Ile Tyr Cys Gln Ala Ser Glu Glu Phe Leu Asn
    130                 135                 140

Gly Arg Ala Val Tyr Ser Pro Lys Ser Pro Ser Leu Gln Ser Glu Thr
145                 150                 155                 160

Val His Tyr Lys Arg Gly Val Ser Gln Gln Phe Ser Leu Pro Ser Phe
                165                 170                 175

Lys Ile Asp Phe Ser Glu Trp Lys Asp Asp Glu Leu Asn Phe Asp Leu
            180                 185                 190

Asp Arg Gly Val Phe Pro Val Val Ile Gln Ala Val Val Asp Glu Gly
        195                 200                 205

Asp Val Val Glu Val Thr Gly His Ala His Val Leu Leu Ala Ala Phe
    210                 215                 220

Glu Lys His Met Asp Gly Ser Phe Ser Val Lys Pro Leu Lys Gln Lys
225                 230                 235                 240

Gln Ile Val Asp Arg Val Ser Tyr Leu Leu Gln Glu Ile Tyr Gly Ile
                245                 250                 255

Glu Asn Lys Asn Asn Gln Glu Thr Lys Pro Ser Asp Asp Glu Asn Ser
            260                 265                 270
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Asp Asn Ser Asn Glu Cys Val Val Cys Leu Ser Asp Leu Arg Asp Thr
        275                 280                 285

Leu Ile Leu Pro Cys Arg His Leu Cys Leu Cys Thr Ser Cys Ala Asp
    290                 295                 300

Thr Leu Arg Tyr Gln Ala Asn Asn Cys Pro Ile Cys Arg Leu Pro Phe
305                 310                 315                 320

Arg Ala Leu Leu Gln Ile Arg Ala Val Arg Lys Lys Pro Gly Ala Leu
                325                 330                 335

Ser Pro Val Ser Phe Ser Pro Val Leu Ala Gln Ser Leu Glu His Asp
            340                 345                 350

Glu His Ser Asn Ser Asp Ser Val Pro Pro Gly Tyr Glu Pro Ile Ser
        355                 360                 365

Leu Leu Glu Ala Leu Asn Gly Leu Arg Ala Val Ser Pro Ala Ile Pro
    370                 375                 380

Ser Ala Pro Leu Tyr Glu Glu Ile Thr Tyr Ser Gly Ile Ser Asp Gly
385                 390                 395                 400

Leu Ser Gln Ala Ser Cys Pro Leu Ala Ala Ile Asp His Ile Leu Asp
                405                 410                 415

Ser Ser Arg Gln Lys Gly Arg Pro Gln Ser Lys Ala Pro Asp Ser Thr
            420                 425                 430

Leu Arg Ser Pro Ser Ser Pro Ile His Glu Glu Asp Glu Glu Lys Leu
        435                 440                 445

Ser Glu Asp Val Asp Ala Pro Pro Pro Leu Gly Gly Ala Glu Leu Ala
    450                 455                 460

Leu Arg Glu Ser Ser Ser Pro Glu Ser Phe Ile Thr Glu Glu Val Asp
465                 470                 475                 480

Glu Ser Ser Ser Pro Gln Gln Gly Thr Arg Ala Ala Ser Ile Glu Asn
                485                 490                 495

Val Leu Gln Asp Ser Ser Pro Glu His Cys Gly Arg Gly Pro Pro Ala
            500                 505                 510

Asp Ile Tyr Leu Pro Ala Leu Gly Pro Asp Ser Cys Ser Val Gly Ile
        515                 520                 525
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Asp Glu
530

<210> 25
<211> 576
<212> PRT
<213> Homo sapiens

<400> 25

Met Gly Ser Ile Leu Ser Arg Arg Ile Ala Gly Val Glu Asp Ile Asp
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Ile Gln Ala Asn Ser Ala Tyr Arg Tyr Pro Pro Lys Ser Gly Asn Tyr
20 25 30

Phe Ala Ser His Phe Phe Met Gly Gly Glu Lys Phe Asp Thr Pro His
35 40 45

Pro Glu Gly Tyr Leu Phe Gly Glu Asn Met Asp Leu Asn Phe Leu Gly
50 55 60

Ser Arg Pro Val Gln Phe Pro Tyr Val Thr Pro Ala Pro His Glu Pro
65 70 75 80

Val Lys Thr Leu Arg Ser Leu Val Asn Ile Arg Lys Asp Ser Leu Arg
85 90 95

Leu Val Arg Tyr Lys Asp Asp Ala Asp Ser Pro Thr Glu Asp Gly Asp
100 105 110

Lys Pro Arg Val Leu Tyr Ser Leu Glu Phe Thr Phe Asp Ala Asp Ala
115 120 125

Arg Val Ala Ile Thr Ile Tyr Cys Gln Ala Ser Glu Glu Phe Leu Asn
130 135 140

Gly Arg Ala Val Tyr Ser Pro Lys Ser Pro Ser Leu Gln Ser Glu Thr
145 150 155 160

Val His Tyr Lys Arg Gly Val Ser Gln Gln Phe Ser Leu Pro Ser Phe
165 170 175

Lys Ile Asp Phe Ser Glu Trp Lys Asp Asp Glu Leu Asn Phe Asp Leu
180 185 190

Asp Arg Gly Val Phe Pro Val Val Ile Gln Ala Val Val Asp Glu Gly
195 200 205

```
Asp Val Val Glu Val Thr Gly His Ala His Val Leu Leu Ala Ala Phe
    210                 215                 220

Glu Lys His Met Asp Gly Ser Phe Ser Val Lys Pro Leu Lys Gln Lys
225                 230                 235                 240

Gln Ile Val Asp Arg Val Ser Tyr Leu Leu Gln Glu Ile Tyr Gly Ile
                245                 250                 255

Glu Asn Lys Asn Asn Gln Glu Thr Lys Pro Ser Asp Asp Glu Asn Ser
            260                 265                 270

Asp Asn Ser Asn Glu Cys Val Val Cys Leu Ser Asp Leu Arg Asp Thr
        275                 280                 285

Leu Ile Leu Pro Cys Arg His Leu Cys Leu Cys Thr Ser Cys Ala Asp
    290                 295                 300

Thr Leu Arg Tyr Gln Ala Asn Asn Cys Pro Ile Cys Arg Leu Pro Phe
305                 310                 315                 320

Arg Ala Leu Leu Gln Ile Arg Ala Val Arg Lys Lys Pro Gly Ala Leu
                325                 330                 335

Ser Pro Val Ser Phe Ser Pro Val Leu Ala Gln Ser Leu Glu His Asp
            340                 345                 350

Glu His Ser Cys Pro Phe Lys Lys Ser Lys Pro His Pro Ala Ser Leu
        355                 360                 365

Ala Ser Lys Lys Pro Lys Arg Glu Thr Asn Ser Asp Ser Val Pro Pro
    370                 375                 380

Gly Tyr Glu Pro Ile Ser Leu Leu Glu Ala Leu Asn Gly Leu Arg Ala
385                 390                 395                 400

Val Ser Pro Ala Ile Pro Ser Ala Pro Leu Tyr Glu Glu Ile Thr Tyr
                405                 410                 415

Ser Gly Ile Ser Asp Gly Leu Ser Gln Ala Ser Cys Pro Leu Ala Ala
            420                 425                 430

Ile Asp His Ile Leu Asp Ser Ser Arg Gln Lys Gly Arg Pro Gln Ser
        435                 440                 445

Lys Ala Pro Asp Ser Thr Leu Arg Ser Pro Ser Ser Pro Ile His Glu
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     450                 455                 460

Glu Asp Glu Glu Lys Leu Ser Glu Asp Val Asp Ala Pro Pro Pro Leu
465                 470                 475                 480

Gly Gly Ala Glu Leu Ala Leu Arg Glu Ser Ser Ser Pro Glu Ser Phe
                485                 490                 495

Ile Thr Glu Glu Val Asp Glu Ser Ser Ser Pro Gln Gln Gly Thr Arg
            500                 505                 510

Ala Ala Ser Ile Glu Asn Val Leu Gln Asp Ser Ser Pro Glu His Cys
        515                 520                 525

Gly Arg Gly Pro Pro Ala Asp Ile Tyr Leu Pro Gly Arg Pro Thr Ser
    530                 535                 540

Met Glu Thr Ala His Gly Leu Ala Thr Thr Ser Pro Thr Trp Pro Pro
545                 550                 555                 560

Leu Gly Gly Pro Ser Pro Asp Pro Ser Ala Ala Glu Leu Thr Pro Leu
                565                 570                 575
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<210> 26
<211> 6382
<212> DNA
<213> Homo sapiens

<400> 26

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<211> 6856
<212> DNA
<213> Homo sapiens

<400> 27

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<400> 28

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Asp Glu Ala Asp Leu Thr Asp Trp Asn Leu Pro Leu Ala Phe Met Lys
20 25 30

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Lys Arg His Cys Glu Lys Ile Glu Gly Ser Lys Ser Leu Ala Gln Ser
        35                  40                  45

Trp Arg Met Lys Asp Arg Met Lys Thr Val Ser Val Ala Leu Val Leu
    50                  55                  60

Cys Leu Asn Val Gly Val Asp Pro Pro Asp Val Val Lys Thr Thr Pro
65                  70                  75                  80

Cys Ala Arg Leu Glu Cys Trp Ile Asp Pro Leu Ser Met Gly Pro Gln
                85                  90                  95

Lys Ala Leu Glu Thr Ile Gly Ala Asn Leu Gln Lys Gln Tyr Glu Asn
            100                 105                 110

Trp Gln Pro Arg Ala Arg Tyr Lys Gln Ser Leu Asp Pro Thr Val Asp
        115                 120                 125

Glu Val Lys Lys Leu Cys Thr Ser Leu Arg Arg Asn Ala Lys Glu Glu
    130                 135                 140

Arg Val Leu Phe His Tyr Asn Gly His Gly Val Pro Arg Pro Thr Val
145                 150                 155                 160

Asn Gly Glu Val Trp Val Phe Asn Lys Asn Tyr Thr Gln Tyr Ile Pro
                165                 170                 175

Leu Ser Ile Tyr Asp Leu Gln Thr Trp Met Gly Ser Pro Ser Ile Phe
            180                 185                 190

Val Tyr Asp Cys Ser Asn Ala Gly Leu Ile Val Lys Ser Phe Lys Gln
        195                 200                 205

Phe Ala Leu Gln Arg Glu Gln Glu Leu Glu Val Ala Ala Ile Asn Pro
    210                 215                 220

Asn His Pro Leu Ala Gln Met Pro Leu Pro Pro Ser Met Lys Asn Cys
225                 230                 235                 240

Ile Gln Leu Ala Ala Cys Glu Ala Thr Glu Leu Leu Pro Met Ile Pro
                245                 250                 255

Asp Leu Pro Ala Asp Leu Phe Thr Ser Cys Leu Thr Thr Pro Ile Lys
            260                 265                 270

Ile Ala Leu Arg Trp Phe Cys Met Gln Lys Cys Val Ser Leu Val Pro
        275                 280                 285
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```
Gly Val Thr Leu Asp Leu Ile Glu Lys Ile Pro Gly Arg Leu Asn Asp
    290                 295                 300

Arg Arg Thr Pro Leu Gly Glu Leu Asn Trp Ile Phe Thr Ala Ile Thr
305                 310                 315                 320

Asp Thr Ile Ala Trp Asn Val Leu Pro Arg Asp Leu Phe Gln Lys Leu
                325                 330                 335

Phe Arg Gln Asp Leu Leu Val Ala Ser Leu Phe Arg Asn Phe Leu Leu
            340                 345                 350

Ala Glu Arg Ile Met Arg Ser Tyr Asn Cys Thr Pro Val Ser Ser Pro
        355                 360                 365

Arg Leu Pro Pro Thr Tyr Met His Ala Met Trp Gln Ala Trp Asp Leu
    370                 375                 380

Ala Val Asp Ile Cys Leu Ser Gln Leu Pro Thr Ile Ile Glu Glu Gly
385                 390                 395                 400

Thr Ala Phe Arg His Ser Pro Phe Phe Ala Glu Gln Leu Thr Ala Phe
                405                 410                 415

Gln Val Trp Leu Thr Met Gly Val Glu Asn Arg Asn Pro Pro Glu Gln
            420                 425                 430

Leu Pro Ile Val Leu Gln Val Leu Leu Ser Gln Val His Arg Leu Arg
        435                 440                 445

Ala Leu Asp Leu Leu Gly Arg Phe Leu Asp Leu Gly Pro Trp Ala Val
    450                 455                 460

Ser Leu Ala Leu Ser Val Gly Ile Phe Pro Tyr Val Leu Lys Leu Leu
465                 470                 475                 480

Gln Ser Ser Ala Arg Glu Leu Arg Pro Leu Leu Val Phe Ile Trp Ala
                485                 490                 495

Lys Ile Leu Ala Val Asp Ser Glu Leu Val Val Ala Leu Ser His Leu
            500                 505                 510

Val Val Gln Tyr Glu Ser Asn Phe Cys Thr Val Ala Leu Gln Phe Ile
        515                 520                 525

Glu Glu Glu Lys Asn Tyr Ala Leu Pro Ser Pro Ala Thr Thr Glu Gly
    530                 535                 540
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```
Gly Ser Leu Thr Pro Val Arg Asp Ser Pro Cys Thr Pro Arg Leu Arg
545                 550                 555                 560

Ser Val Ser Ser Tyr Gly Asn Ile Arg Ala Val Ala Thr Ala Arg Ser
                565                 570                 575

Leu Asn Lys Ser Leu Gln Asn Leu Ser Leu Thr Glu Glu Ser Gly Gly
            580                 585                 590

Ala Val Ala Phe Ser Pro Gly Asn Leu Ser Thr Ser Ser Ser Ala Ser
        595                 600                 605

Ser Thr Leu Gly Ser Pro Glu Asn Glu Glu His Ile Leu Ser Phe Glu
    610                 615                 620

Thr Ile Asp Lys Met Arg Arg Ala Ser Ser Tyr Ser Ser Leu Asn Ser
625                 630                 635                 640

Leu Ile Gly Val Ser Phe Asn Ser Val Tyr Thr Gln Ile Trp Arg Val
                645                 650                 655

Leu Leu His Leu Ala Ala Asp Pro Tyr Pro Glu Val Ser Asp Val Ala
            660                 665                 670

Met Lys Val Leu Asn Ser Ile Ala Tyr Lys Ala Thr Val Asn Ala Arg
        675                 680                 685

Pro Gln Arg Val Leu Asp Thr Ser Ser Leu Thr Gln Ser Ala Pro Ala
    690                 695                 700

Ser Pro Thr Asn Lys Gly Val His Ile His Gln Ala Gly Gly Ser Pro
705                 710                 715                 720

Pro Ala Ser Ser Thr Ser Ser Ser Ser Leu Thr Asn Asp Val Ala Lys
                725                 730                 735

Gln Pro Val Ser Arg Asp Leu Pro Ser Gly Arg Pro Gly Thr Thr Gly
            740                 745                 750

Pro Ala Gly Ala Gln Tyr Thr Pro His Ser His Gln Phe Pro Arg Thr
        755                 760                 765

Arg Lys Met Phe Asp Lys Gly Pro Glu Gln Thr Ala Asp Asp Ala Asp
    770                 775                 780

Asp Ala Ala Gly His Lys Ser Phe Ile Ser Ala Thr Val Gln Thr Gly
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785 790 795 800

Phe Cys Asp Trp Ser Ala Arg Tyr Phe Ala Gln Pro Val Met Lys Ile
805 810 815

Pro Glu Glu His Asp Leu Glu Ser Gln Ile Arg Lys Glu Arg Glu Trp
820 825 830

Arg Phe Leu Arg Asn Ser Arg Val Arg Arg Gln Ala Gln Gln Val Ile
835 840 845

Gln Lys Gly Ile Thr Arg Leu Asp Asp Gln Ile Phe Leu Asn Arg Asn
850 855 860

Pro Gly Val Pro Ser Val Val Lys Phe His Pro Phe Thr Pro Cys Ile
865 870 875 880

Ala Val Ala Asp Lys Asp Ser Ile Cys Phe Trp Asp Trp Glu Lys Gly
885 890 895

Glu Lys Leu Asp Tyr Phe His Asn Gly Asn Pro Arg Tyr Thr Arg Val
900 905 910

Thr Ala Met Glu Tyr Leu Asn Gly Gln Asp Cys Ser Leu Leu Leu Thr
915 920 925

Ala Thr Asp Asp Gly Ala Ile Arg Val Trp Lys Asn Phe Ala Asp Leu
930 935 940

Glu Lys Asn Pro Glu Met Val Thr Ala Trp Gln Gly Leu Ser Asp Met
945 950 955 960

Leu Pro Thr Thr Arg Gly Ala Gly Met Val Val Asp Trp Glu Gln Glu
965 970 975

Thr Gly Leu Leu Met Ser Ser Gly Asp Val Arg Ile Val Arg Ile Trp
980 985 990

Asp Thr Asp Arg Glu Met Lys Val Gln Asp Ile Pro Thr Gly Ala Asp
995 1000 1005

Ser Cys Val Thr Ser Leu Ser Cys Asp Ser His Arg Ser Leu Ile
1010 1015 1020

Val Ala Gly Leu Gly Asp Gly Ser Ile Arg Val Tyr Asp Arg Arg
1025 1030 1035

```
Met Ala  Leu Ser Glu Cys Arg  Val Met Thr Tyr Arg  Glu His Thr
    1040                1045                1050

Ala Trp  Val Val Lys Ala Ser  Leu Gln Lys Arg Pro  Asp Gly His
    1055                1060                1065

Ile Val  Ser Val Ser Val Asn  Gly Asp Val Arg Ile  Phe Asp Pro
    1070                1075                1080

Arg Met  Pro Glu Ser Val Asn  Val Leu Gln Ile Val  Lys Gly Leu
    1085                1090                1095

Thr Ala  Leu Asp Ile His Pro  Gln Ala Asp Leu Ile  Ala Cys Gly
    1100                1105                1110

Ser Val  Asn Gln Phe Thr Ala  Ile Tyr Asn Ser Ser  Gly Glu Leu
    1115                1120                1125

Ile Asn  Asn Ile Lys Tyr Tyr  Asp Gly Phe Met Gly  Gln Arg Val
    1130                1135                1140

Gly Ala  Ile Ser Cys Leu Ala  Phe His Pro His Trp  Pro His Leu
    1145                1150                1155

Ala Val  Gly Ser Asn Asp Tyr  Tyr Ile Ser Val Tyr  Ser Val Glu
    1160                1165                1170

Lys Arg  Val Arg
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Met Glu Ser Glu Met Leu Gln Ser Pro Leu Leu Gly Leu Gly Glu Glu
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Asp Glu Ala Asp Leu Thr Asp Trp Asn Leu Pro Leu Ala Phe Met Lys
            20                  25                  30

Lys Arg His Cys Glu Lys Ile Glu Gly Ser Lys Ser Leu Ala Gln Ser
        35                  40                  45

Trp Arg Met Lys Asp Arg Met Lys Thr Val Ser Val Ala Leu Val Leu
    50                  55                  60
```

```
Cys Leu Asn Val Gly Val Asp Pro Pro Asp Val Val Lys Thr Thr Pro
65                  70                  75                  80

Cys Ala Arg Leu Glu Cys Trp Ile Asp Pro Leu Ser Met Gly Pro Gln
                85                  90                  95

Lys Ala Leu Glu Thr Ile Gly Ala Asn Leu Gln Lys Gln Tyr Glu Asn
            100                 105                 110

Trp Gln Pro Arg Ala Arg Tyr Lys Gln Ser Leu Asp Pro Thr Val Asp
        115                 120                 125

Glu Val Lys Lys Leu Cys Thr Ser Leu Arg Arg Asn Ala Lys Glu Glu
    130                 135                 140

Arg Val Leu Phe His Tyr Asn Gly His Gly Val Pro Arg Pro Thr Val
145                 150                 155                 160

Asn Gly Glu Val Trp Val Phe Asn Lys Asn Tyr Thr Gln Tyr Ile Pro
                165                 170                 175

Leu Ser Ile Tyr Asp Leu Gln Thr Trp Met Gly Ser Pro Ser Ile Phe
            180                 185                 190

Val Tyr Asp Cys Ser Asn Ala Gly Leu Ile Val Lys Ser Phe Lys Gln
        195                 200                 205

Phe Ala Leu Gln Arg Glu Gln Glu Leu Glu Val Ala Ala Ile Asn Pro
    210                 215                 220

Asn His Pro Leu Ala Gln Met Pro Leu Pro Pro Ser Met Lys Asn Cys
225                 230                 235                 240

Ile Gln Leu Ala Ala Cys Glu Ala Thr Glu Leu Leu Pro Met Ile Pro
                245                 250                 255

Asp Leu Pro Ala Asp Leu Phe Thr Ser Cys Leu Thr Thr Pro Ile Lys
            260                 265                 270

Ile Ala Leu Arg Trp Phe Cys Met Gln Lys Cys Val Ser Leu Val Pro
        275                 280                 285

Gly Val Thr Leu Asp Leu Ile Glu Lys Ile Pro Gly Arg Leu Asn Asp
    290                 295                 300

Arg Arg Thr Pro Leu Gly Glu Leu Asn Trp Ile Phe Thr Ala Ile Thr
305                 310                 315                 320
```

```
Asp Thr Ile Ala Trp Asn Val Leu Pro Arg Asp Leu Phe Gln Lys Leu
                325                 330                 335

Phe Arg Gln Asp Leu Leu Val Ala Ser Leu Phe Arg Asn Phe Leu Leu
            340                 345                 350

Ala Glu Arg Ile Met Arg Ser Tyr Asn Cys Thr Pro Val Ser Ser Pro
        355                 360                 365

Arg Leu Pro Pro Thr Tyr Met His Ala Met Trp Gln Ala Trp Asp Leu
    370                 375                 380

Ala Val Asp Ile Cys Leu Ser Gln Leu Pro Thr Ile Ile Glu Glu Gly
385                 390                 395                 400

Thr Ala Phe Arg His Ser Pro Phe Phe Ala Glu Gln Leu Thr Ala Phe
                405                 410                 415

Gln Val Trp Leu Thr Met Gly Val Glu Asn Arg Asn Pro Pro Glu Gln
            420                 425                 430

Leu Pro Ile Val Leu Gln Val Leu Leu Ser Gln Val His Arg Leu Arg
        435                 440                 445

Ala Leu Asp Leu Leu Gly Arg Phe Leu Asp Leu Gly Pro Trp Ala Val
    450                 455                 460

Ser Leu Ala Leu Ser Val Gly Ile Phe Pro Tyr Val Leu Lys Leu Leu
465                 470                 475                 480

Gln Ser Ser Ala Arg Glu Leu Arg Pro Leu Leu Val Phe Ile Trp Ala
                485                 490                 495

Lys Ile Leu Ala Val Asp Ser Ser Cys Gln Ala Asp Leu Val Lys Asp
            500                 505                 510

Asn Gly His Lys Tyr Phe Leu Ser Val Leu Ala Asp Pro Tyr Met Pro
        515                 520                 525

Ala Glu His Arg Thr Met Thr Ala Phe Ile Leu Ala Val Ile Val Asn
    530                 535                 540

Ser Tyr His Thr Gly Gln Glu Ala Cys Leu Gln Gly Asn Leu Ile Ala
545                 550                 555                 560

Ile Cys Leu Glu Gln Leu Asn Asp Pro His Pro Leu Leu Arg Gln Trp
                565                 570                 575
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```
Val Ala Ile Cys Leu Gly Arg Ile Trp Gln Asn Phe Asp Ser Ala Arg
            580                 585                 590

Trp Cys Gly Val Arg Asp Ser Ala His Glu Lys Leu Tyr Ser Leu Leu
        595                 600                 605

Ser Asp Pro Ile Pro Glu Val Arg Cys Ala Ala Val Phe Ala Leu Gly
    610                 615                 620

Thr Phe Val Gly Asn Ser Ala Glu Arg Thr Asp His Ser Thr Thr Ile
625                 630                 635                 640

Asp His Asn Val Ala Met Met Leu Ala Gln Leu Val Ser Asp Gly Ser
                645                 650                 655

Pro Met Val Arg Lys Glu Leu Val Val Ala Leu Ser His Leu Val Val
            660                 665                 670

Gln Tyr Glu Ser Asn Phe Cys Thr Val Ala Leu Gln Phe Ile Glu Glu
        675                 680                 685

Glu Lys Asn Tyr Ala Leu Pro Ser Pro Ala Thr Thr Glu Gly Gly Ser
    690                 695                 700

Leu Thr Pro Val Arg Asp Ser Pro Cys Thr Pro Arg Leu Arg Ser Val
705                 710                 715                 720

Ser Ser Tyr Gly Asn Ile Arg Ala Val Ala Thr Ala Arg Ser Leu Asn
                725                 730                 735

Lys Ser Leu Gln Asn Leu Ser Leu Thr Glu Glu Ser Gly Gly Ala Val
            740                 745                 750

Ala Phe Ser Pro Gly Asn Leu Ser Thr Ser Ser Ser Ala Ser Ser Thr
        755                 760                 765

Leu Gly Ser Pro Glu Asn Glu Glu His Ile Leu Ser Phe Glu Thr Ile
    770                 775                 780

Asp Lys Met Arg Arg Ala Ser Ser Tyr Ser Ser Leu Asn Ser Leu Ile
785                 790                 795                 800

Gly Val Ser Phe Asn Ser Val Tyr Thr Gln Ile Trp Arg Val Leu Leu
                805                 810                 815

His Leu Ala Ala Asp Pro Tyr Pro Glu Val Ser Asp Val Ala Met Lys
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                820                 825                 830

Val Leu Asn Ser Ile Ala Tyr Lys Ala Thr Val Asn Ala Arg Pro Gln
        835                 840                 845

Arg Val Leu Asp Thr Ser Ser Leu Thr Gln Ser Ala Pro Ala Ser Pro
    850                 855                 860

Thr Asn Lys Gly Val His Ile His Gln Ala Gly Gly Ser Pro Pro Ala
865                 870                 875                 880

Ser Ser Thr Ser Ser Ser Ser Leu Thr Asn Asp Val Ala Lys Gln Pro
                885                 890                 895

Val Ser Arg Asp Leu Pro Ser Gly Arg Pro Gly Thr Thr Gly Pro Ala
            900                 905                 910

Gly Ala Gln Tyr Thr Pro His Ser His Gln Phe Pro Arg Thr Arg Lys
        915                 920                 925

Met Phe Asp Lys Gly Pro Glu Gln Thr Ala Asp Asp Ala Asp Asp Ala
    930                 935                 940

Ala Gly His Lys Ser Phe Ile Ser Ala Thr Val Gln Thr Gly Phe Cys
945                 950                 955                 960

Asp Trp Ser Ala Arg Tyr Phe Ala Gln Pro Val Met Lys Ile Pro Glu
                965                 970                 975

Glu His Asp Leu Glu Ser Gln Ile Arg Lys Glu Arg Glu Trp Arg Phe
            980                 985                 990

Leu Arg Asn Ser Arg Val Arg Arg  Gln Ala Gln Gln Val  Ile Gln Lys
        995                 1000                1005

Gly Ile  Thr Arg Leu Asp Asp  Gln Ile Phe Leu Asn  Arg Asn Pro
    1010                1015                1020

Gly Val  Pro Ser Val Val Lys  Phe His Pro Phe Thr  Pro Cys Ile
    1025                1030                1035

Ala Val  Ala Asp Lys Asp Ser  Ile Cys Phe Trp Asp  Trp Glu Lys
    1040                1045                1050

Gly Glu  Lys Leu Asp Tyr Phe  His Asn Gly Asn Pro  Arg Tyr Thr
    1055                1060                1065
```

```
Arg Val  Thr Ala Met Glu Tyr  Leu Asn Gly Gln Asp  Cys Ser Leu
    1070                1075                1080

Leu Leu  Thr Ala Thr Asp Asp  Gly Ala Ile Arg Val  Trp Lys Asn
    1085                1090                1095

Phe Ala  Asp Leu Glu Lys Asn  Pro Glu Met Val Thr  Ala Trp Gln
    1100                1105                1110

Gly Leu  Ser Asp Met Leu Pro  Thr Thr Arg Gly Ala  Gly Met Val
    1115                1120                1125

Val Asp  Trp Glu Gln Glu Thr  Gly Leu Leu Met Ser  Ser Gly Asp
    1130                1135                1140

Val Arg  Ile Val Arg Ile Trp  Asp Thr Asp Arg Glu  Met Lys Val
    1145                1150                1155

Gln Asp  Ile Pro Thr Gly Ala  Asp Ser Cys Val Thr  Ser Leu Ser
    1160                1165                1170

Cys Asp  Ser His Arg Ser Leu  Ile Val Ala Gly Leu  Gly Asp Gly
    1175                1180                1185

Ser Ile  Arg Val Tyr Asp Arg  Arg Met Ala Leu Ser  Glu Cys Arg
    1190                1195                1200

Val Met  Thr Tyr Arg Glu His  Thr Ala Trp Val Val  Lys Ala Ser
    1205                1210                1215

Leu Gln  Lys Arg Pro Asp Gly  His Ile Val Ser Val  Ser Val Asn
    1220                1225                1230

Gly Asp  Val Arg Ile Phe Asp  Pro Arg Met Pro Glu  Ser Val Asn
    1235                1240                1245

Val Leu  Gln Ile Val Lys Gly  Leu Thr Ala Leu Asp  Ile His Pro
    1250                1255                1260

Gln Ala  Asp Leu Ile Ala Cys  Gly Ser Val Asn Gln  Phe Thr Ala
    1265                1270                1275

Ile Tyr  Asn Ser Ser Gly Glu  Leu Ile Asn Asn Ile  Lys Tyr Tyr
    1280                1285                1290

Asp Gly  Phe Met Gly Gln Arg  Val Gly Ala Ile Ser  Cys Leu Ala
    1295                1300                1305
```

```
Phe His  Pro His Trp Pro His  Leu Ala Val Gly Ser  Asn Asp Tyr
    1310                1315                1320

Tyr Ile  Ser Val Tyr Ser Val  Glu Lys Arg Val Arg
    1325                1330                1335
```

<210> 30
<211> 1555
<212> DNA
<213> Homo sapiens

<400> 30

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cagcacccct gcccggatca actggacttt tgccccctgc tccgccagcc tcctgcttgg 180

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accagggccg gtcccccggc aggatgagcg ctctaggccg gtcctcggtc atcttgctta 300

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cctccagccc ggccctgccc ggggtctacc tgctcttcgc ctcgcgcctg cccggagcgc 600

tcatgcacac gctgccagcc gcccagatgg tcatcacgga cctgtcggca cccgaggagc 660

ggcccgcggc cctgggccgg ctgggcctct gcttcggcgt cggagtcatc ctcggctccc 720

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gacacagact ggcaataaac tcctactaaa tccctccgaa aaaaaaaaaa aaaaa 1555

<210> 31
<211> 1563
<212> DNA
<213> Homo sapiens

<400> 31

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cgctcatgca cacgctgcca gccgcccaga tggtcatcac ggacctgtcg gcacccgagg 660

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aaa 1563

<210> 32
<211> 424
<212> PRT
<213> Homo sapiens

<400> 32

```
Met Gln Gly Ala Arg Ala Pro Arg Asp Gln Gly Arg Ser Pro Gly Arg
1               5                  10                  15

Met Ser Ala Leu Gly Arg Ser Ser Val Ile Leu Leu Thr Tyr Val Leu
            20                  25                  30

Ala Ala Thr Glu Leu Thr Cys Leu Phe Met Gln Phe Ser Ile Val Pro
        35                  40                  45

Tyr Leu Ser Arg Lys Leu Gly Leu Asp Ser Ile Ala Phe Gly Tyr Leu
    50                  55                  60

Gln Thr Thr Phe Gly Val Leu Gln Leu Leu Gly Gly Pro Val Phe Gly
65                  70                  75                  80

Arg Phe Ala Asp Gln Arg Gly Ala Arg Ala Ala Leu Thr Leu Ser Phe
                85                  90                  95

Leu Ala Ala Leu Ala Leu Tyr Leu Leu Leu Ala Ala Ala Ser Ser Pro
            100                 105                 110

Ala Leu Pro Gly Val Tyr Leu Leu Phe Ala Ser Arg Leu Pro Gly Ala
        115                 120                 125

Leu Met His Thr Leu Pro Ala Ala Gln Met Val Ile Thr Asp Leu Ser
    130                 135                 140

Ala Pro Glu Glu Arg Pro Ala Ala Leu Gly Arg Leu Gly Leu Cys Phe
145                 150                 155                 160

Gly Val Gly Val Ile Leu Gly Ser Leu Leu Gly Gly Thr Leu Val Ser
                165                 170                 175

Ala Tyr Gly Ile Gln Cys Pro Ala Ile Leu Ala Ala Leu Ala Thr Leu
            180                 185                 190

Leu Gly Ala Val Leu Ser Phe Thr Cys Ile Pro Ala Ser Thr Lys Gly
        195                 200                 205

Ala Lys Thr Asp Ala Gln Ala Pro Leu Pro Gly Gly Pro Arg Ala Ser
```

```
                210                 215                 220

Val Phe Asp Leu Lys Ala Ile Ala Ser Leu Leu Arg Leu Pro Asp Val
225                 230                 235                 240

Pro Arg Ile Phe Leu Val Lys Val Ala Ser Asn Cys Pro Thr Gly Leu
                245                 250                 255

Phe Met Val Met Phe Ser Ile Ile Ser Met Asp Phe Phe Gln Leu Glu
            260                 265                 270

Ala Ala Gln Ala Gly Tyr Leu Met Ser Phe Phe Gly Leu Leu Gln Met
        275                 280                 285

Val Thr Gln Gly Leu Val Ile Gly Gln Leu Ser Ser His Phe Ser Glu
    290                 295                 300

Glu Val Leu Leu Arg Ala Ser Val Leu Val Phe Ile Val Val Gly Leu
305                 310                 315                 320

Ala Met Ala Trp Met Ser Ser Val Phe His Phe Cys Leu Leu Val Pro
                325                 330                 335

Gly Leu Val Phe Ser Leu Cys Thr Leu Asn Val Val Thr Asp Ser Met
            340                 345                 350

Leu Ile Lys Ala Val Ser Thr Ser Asp Thr Gly Thr Met Leu Gly Leu
        355                 360                 365

Cys Ala Ser Val Gln Pro Leu Leu Arg Thr Leu Gly Pro Thr Val Gly
    370                 375                 380

Gly Leu Leu Tyr Arg Ser Phe Gly Val Pro Val Phe Gly His Val Gln
385                 390                 395                 400

Val Ala Ile Asn Thr Leu Val Leu Leu Val Leu Trp Arg Lys Pro Met
                405                 410                 415

Pro Gln Arg Lys Asp Lys Val Arg
            420
```

<210> 33
<211> 1335
<212> PRT
<213> Homo sapiens

<400> 33

Met Glu Ser Glu Met Leu Gln Ser Pro Leu Leu Gly Leu Gly Glu Glu

```
1               5                   10                  15

Asp Glu Ala Asp Leu Thr Asp Trp Asn Leu Pro Leu Ala Phe Met Lys
            20                  25                  30

Lys Arg His Cys Glu Lys Ile Glu Gly Ser Lys Ser Leu Ala Gln Ser
        35                  40                  45

Trp Arg Met Lys Asp Arg Met Lys Thr Val Ser Val Ala Leu Val Leu
    50                  55                  60

Cys Leu Asn Val Gly Val Asp Pro Pro Asp Val Val Lys Thr Thr Pro
65                  70                  75                  80

Cys Ala Arg Leu Glu Cys Trp Ile Asp Pro Leu Ser Met Gly Pro Gln
                85                  90                  95

Lys Ala Leu Glu Thr Ile Gly Ala Asn Leu Gln Lys Gln Tyr Glu Asn
            100                 105                 110

Trp Gln Pro Arg Ala Arg Tyr Lys Gln Ser Leu Asp Pro Thr Val Asp
        115                 120                 125

Glu Val Lys Lys Leu Cys Thr Ser Leu Arg Arg Asn Ala Lys Glu Glu
    130                 135                 140

Arg Val Leu Phe His Tyr Asn Gly His Gly Val Pro Arg Pro Thr Val
145                 150                 155                 160

Asn Gly Glu Val Trp Val Phe Asn Lys Asn Tyr Thr Gln Tyr Ile Pro
                165                 170                 175

Leu Ser Ile Tyr Asp Leu Gln Thr Trp Met Gly Ser Pro Ser Ile Phe
            180                 185                 190

Val Tyr Asp Cys Ser Asn Ala Gly Leu Ile Val Lys Ser Phe Lys Gln
        195                 200                 205

Phe Ala Leu Gln Arg Glu Gln Glu Leu Glu Val Ala Ala Ile Asn Pro
    210                 215                 220

Asn His Pro Leu Ala Gln Met Pro Leu Pro Pro Ser Met Lys Asn Cys
225                 230                 235                 240

Ile Gln Leu Ala Ala Cys Glu Ala Thr Glu Leu Leu Pro Met Ile Pro
                245                 250                 255
```

```
Asp Leu Pro Ala Asp Leu Phe Thr Ser Cys Leu Thr Thr Pro Ile Lys
            260                 265                 270

Ile Ala Leu Arg Trp Phe Cys Met Gln Lys Cys Val Ser Leu Val Pro
        275                 280                 285

Gly Val Thr Leu Asp Leu Ile Glu Lys Ile Pro Gly Arg Leu Asn Asp
    290                 295                 300

Arg Arg Thr Pro Leu Gly Glu Leu Asn Trp Ile Phe Thr Ala Ile Thr
305                 310                 315                 320

Asp Thr Ile Ala Trp Asn Val Leu Pro Arg Asp Leu Phe Gln Lys Leu
                325                 330                 335

Phe Arg Gln Asp Leu Leu Val Ala Ser Leu Phe Arg Asn Phe Leu Leu
            340                 345                 350

Ala Glu Arg Ile Met Arg Ser Tyr Asn Cys Thr Pro Val Ser Ser Pro
        355                 360                 365

Arg Leu Pro Pro Thr Tyr Met His Ala Met Trp Gln Ala Trp Asp Leu
    370                 375                 380

Ala Val Asp Ile Cys Leu Ser Gln Leu Pro Thr Ile Ile Glu Glu Gly
385                 390                 395                 400

Thr Ala Phe Arg His Ser Pro Phe Phe Ala Glu Gln Leu Thr Ala Phe
                405                 410                 415

Gln Val Trp Leu Thr Met Gly Val Glu Asn Arg Asn Pro Pro Glu Gln
            420                 425                 430

Leu Pro Ile Val Leu Gln Val Leu Leu Ser Gln Val His Arg Leu Arg
        435                 440                 445

Ala Leu Asp Leu Leu Gly Arg Phe Leu Asp Leu Gly Pro Trp Ala Val
    450                 455                 460

Ser Leu Ala Leu Ser Val Gly Ile Phe Pro Tyr Val Leu Lys Leu Leu
465                 470                 475                 480

Gln Ser Ser Ala Arg Glu Leu Arg Pro Leu Leu Val Phe Ile Trp Ala
                485                 490                 495

Lys Ile Leu Ala Val Asp Ser Ser Cys Gln Ala Asp Leu Val Lys Asp
            500                 505                 510
```

```
Asn Gly His Lys Tyr Phe Leu Ser Val Leu Ala Asp Pro Tyr Met Pro
        515                 520                 525

Ala Glu His Arg Thr Met Thr Ala Phe Ile Leu Ala Val Ile Val Asn
    530                 535                 540

Ser Tyr His Thr Gly Gln Glu Ala Cys Leu Gln Gly Asn Leu Ile Ala
545                 550                 555                 560

Ile Cys Leu Glu Gln Leu Asn Asp Pro His Pro Leu Leu Arg Gln Trp
                565                 570                 575

Val Ala Ile Cys Leu Gly Arg Ile Trp Gln Asn Phe Asp Ser Ala Arg
            580                 585                 590

Trp Cys Gly Val Arg Asp Ser Ala His Glu Lys Leu Tyr Ser Leu Leu
        595                 600                 605

Ser Asp Pro Ile Pro Glu Val Arg Cys Ala Ala Val Phe Ala Leu Gly
    610                 615                 620

Thr Phe Val Gly Asn Ser Ala Glu Arg Thr Asp His Ser Thr Thr Ile
625                 630                 635                 640

Asp His Asn Val Ala Met Met Leu Ala Gln Leu Val Ser Asp Gly Ser
                645                 650                 655

Pro Met Val Arg Lys Glu Leu Val Val Ala Leu Ser His Leu Val Val
            660                 665                 670

Gln Tyr Glu Ser Asn Phe Cys Thr Val Ala Leu Gln Phe Ile Glu Glu
        675                 680                 685

Glu Lys Asn Tyr Ala Leu Pro Ser Pro Ala Thr Thr Glu Gly Gly Ser
    690                 695                 700

Leu Thr Pro Val Arg Asp Ser Pro Cys Thr Pro Arg Leu Arg Ser Val
705                 710                 715                 720

Ser Ser Tyr Gly Asn Ile Arg Ala Val Ala Thr Ala Arg Ser Leu Asn
                725                 730                 735

Lys Ser Leu Gln Asn Leu Ser Leu Thr Glu Glu Ser Gly Gly Ala Val
            740                 745                 750

Ala Phe Ser Pro Gly Asn Leu Ser Thr Ser Ser Ser Ala Ser Ser Thr
        755                 760                 765
```

```
Leu Gly Ser Pro Glu Asn Glu Glu His Ile Leu Ser Phe Glu Thr Ile
    770                 775                 780

Asp Lys Met Arg Arg Ala Ser Ser Tyr Ser Ser Leu Asn Ser Leu Ile
785                 790                 795                 800

Gly Val Ser Phe Asn Ser Val Tyr Thr Gln Ile Trp Arg Val Leu Leu
                805                 810                 815

His Leu Ala Ala Asp Pro Tyr Pro Glu Val Ser Asp Val Ala Met Lys
            820                 825                 830

Val Leu Asn Ser Ile Ala Tyr Lys Ala Thr Val Asn Ala Arg Pro Gln
        835                 840                 845

Arg Val Leu Asp Thr Ser Ser Leu Thr Gln Ser Ala Pro Ala Ser Pro
    850                 855                 860

Thr Asn Lys Gly Val His Ile His Gln Ala Gly Gly Ser Pro Pro Ala
865                 870                 875                 880

Ser Ser Thr Ser Ser Ser Ser Leu Thr Asn Asp Val Ala Lys Gln Pro
                885                 890                 895

Val Ser Arg Asp Leu Pro Ser Gly Arg Pro Gly Thr Thr Gly Pro Ala
            900                 905                 910

Gly Ala Gln Tyr Thr Pro His Ser His Gln Phe Pro Arg Thr Arg Lys
        915                 920                 925

Met Phe Asp Lys Gly Pro Glu Gln Thr Ala Asp Asp Ala Asp Asp Ala
    930                 935                 940

Ala Gly His Lys Ser Phe Ile Ser Ala Thr Val Gln Thr Gly Phe Cys
945                 950                 955                 960

Asp Trp Ser Ala Arg Tyr Phe Ala Gln Pro Val Met Lys Ile Pro Glu
                965                 970                 975

Glu His Asp Leu Glu Ser Gln Ile Arg Lys Glu Arg Glu Trp Arg Phe
            980                 985                 990

Leu Arg Asn Ser Arg Val Arg Arg  Gln Ala Gln Gln Val  Ile Gln Lys
        995                 1000                1005

Gly Ile  Thr Arg Leu Asp Asp  Gln Ile Phe Leu Asn  Arg Asn Pro
```

1010 1015 1020

Gly Val Pro Ser Val Val Lys Phe His Pro Phe Thr Pro Cys Ile
1025 1030 1035

Ala Val Ala Asp Lys Asp Ser Ile Cys Phe Trp Asp Trp Glu Lys
1040 1045 1050

Gly Glu Lys Leu Asp Tyr Phe His Asn Gly Asn Pro Arg Tyr Thr
1055 1060 1065

Arg Val Thr Ala Met Glu Tyr Leu Asn Gly Gln Asp Cys Ser Leu
1070 1075 1080

Leu Leu Thr Ala Thr Asp Asp Gly Ala Ile Arg Val Trp Lys Asn
1085 1090 1095

Phe Ala Asp Leu Glu Lys Asn Pro Glu Met Val Thr Ala Trp Gln
1100 1105 1110

Gly Leu Ser Asp Met Leu Pro Thr Thr Arg Gly Ala Gly Met Val
1115 1120 1125

Val Asp Trp Glu Gln Glu Thr Gly Leu Leu Met Ser Ser Gly Asp
1130 1135 1140

Val Arg Ile Val Arg Ile Trp Asp Thr Asp Arg Glu Met Lys Val
1145 1150 1155

Gln Asp Ile Pro Thr Gly Ala Asp Ser Cys Val Thr Ser Leu Ser
1160 1165 1170

Cys Asp Ser His Arg Ser Leu Ile Val Ala Gly Leu Gly Asp Gly
1175 1180 1185

Ser Ile Arg Val Tyr Asp Arg Arg Met Ala Leu Ser Glu Cys Arg
1190 1195 1200

Val Met Thr Tyr Arg Glu His Thr Ala Trp Val Val Lys Ala Ser
1205 1210 1215

Leu Gln Lys Arg Pro Asp Gly His Ile Val Ser Val Ser Val Asn
1220 1225 1230

Gly Asp Val Arg Ile Phe Asp Pro Arg Met Pro Glu Ser Val Asn
1235 1240 1245

```
Val Leu  Gln Ile Val Lys Gly  Leu Thr Ala Leu Asp  Ile His Pro
    1250                1255                1260

Gln Ala  Asp Leu Ile Ala Cys  Gly Ser Val Asn Gln  Phe Thr Ala
    1265                1270                1275

Ile Tyr  Asn Ser Ser Gly Glu  Leu Ile Asn Asn Ile  Lys Tyr Tyr
    1280                1285                1290

Asp Gly  Phe Met Gly Gln Arg  Val Gly Ala Ile Ser  Cys Leu Ala
    1295                1300                1305

Phe His  Pro His Trp Pro His  Leu Ala Val Gly Ser  Asn Asp Tyr
    1310                1315                1320

Tyr Ile  Ser Val Tyr Ser Val  Glu Lys Arg Val Arg
    1325                1330                1335
```

<210> 34
<211> 2428
<212> DNA
<213> Homo sapiens

<400> 34

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acatctgagc agtgaaaaaa aaaaaaaa 2428

<210> 35
<211> 342
<212> PRT
<213> Homo sapiens

<400> 35

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Met Asn Asn Ala Gly His Gly Pro Thr Arg Arg Leu Arg Gly Leu Gly
1               5                  10                  15

Val Leu Ala Gly Val Ala Leu Leu Ala Ala Leu Trp Leu Leu Trp Leu
            20                  25                  30
```

```
Leu Gly Ser Ala Pro Arg Gly Thr Pro Ala Pro Gln Pro Thr Ile Thr
        35                  40                  45

Ile Leu Val Trp His Trp Pro Phe Thr Asp Gln Pro Pro Glu Leu Pro
    50                  55                  60

Ser Asp Thr Cys Thr Arg Tyr Gly Ile Ala Arg Cys His Leu Ser Ala
65                  70                  75                  80

Asn Arg Ser Leu Leu Ala Ser Ala Asp Ala Val Val Phe His His Arg
                85                  90                  95

Glu Leu Gln Thr Arg Arg Ser His Leu Pro Leu Ala Gln Arg Pro Arg
            100                 105                 110

Gly Gln Pro Trp Val Trp Ala Ser Met Glu Ser Pro Ser His Thr His
        115                 120                 125

Gly Leu Ser His Leu Arg Gly Ile Phe Asn Trp Val Leu Ser Tyr Arg
    130                 135                 140

Arg Asp Ser Asp Ile Phe Val Pro Tyr Gly Arg Leu Glu Pro His Trp
145                 150                 155                 160

Gly Pro Ser Pro Pro Leu Pro Ala Lys Ser Arg Val Ala Ala Trp Val
                165                 170                 175

Val Ser Asn Phe Gln Glu Arg Gln Leu Arg Ala Arg Leu Tyr Arg Gln
            180                 185                 190

Leu Ala Pro His Leu Arg Val Asp Val Phe Gly Arg Ala Asn Gly Arg
        195                 200                 205

Pro Leu Cys Ala Ser Cys Leu Val Pro Thr Val Ala Gln Tyr Arg Phe
    210                 215                 220

Tyr Leu Ser Phe Glu Asn Ser Gln His Arg Asp Tyr Ile Thr Glu Lys
225                 230                 235                 240

Phe Trp Arg Asn Ala Leu Val Ala Gly Thr Val Pro Val Val Leu Gly
                245                 250                 255

Pro Pro Arg Ala Thr Tyr Glu Ala Phe Val Pro Ala Asp Ala Phe Val
            260                 265                 270

His Val Asp Asp Phe Gly Ser Ala Arg Glu Leu Ala Ala Phe Leu Thr
```

275 280 285

Gly Met Asn Glu Ser Arg Tyr Gln Arg Phe Phe Ala Trp Arg Asp Arg
290 295 300

Leu Arg Val Arg Leu Phe Thr Asp Trp Arg Glu Arg Phe Cys Ala Ile
305 310 315 320

Cys Asp Arg Tyr Pro His Leu Pro Arg Ser Gln Val Tyr Glu Asp Leu
325 330 335

Glu Gly Trp Phe Gln Ala
340

<210> 36
<211> 1671
<212> DNA
<213> Homo sapiens

<400> 36

cccaactggc agcgacagct gcagacgggc tgaaccagct ttgttcccag ggtggcgcct 60

gctctccatc caggccccat tccggctccc acccgacgct gcttttgttc ccacgtttcg 120

ggggggcagct ggcactgtga ttcctgcccc atgagtgcct agaggcacgg agccaccagg 180

gatcacccca cgtgggacac agggcttggg gaggatgggg caggaccaga ccaagcagca 240

gatcgagaag gggctccagc tgtaccagtc caaccagaca gagaaggcat tgcaggtgtg 300

gacaaaggtg ctggagaaga gctcggacct catggggcgc ttccgcgtgc tgggctgcct 360

ggtcacagcc cactcggaga tgggccgcta caaggagatg ctgaagttcg ctgtggtcca 420

gatcgacacg gcccgggagc tggaggatgc cgacttcctc ctggagagct acctgaacct 480

ggcacgcagc aacgagaagc tgtgcgagtt tcacaagacc atctcctact gcaagacctg 540

ccttgggctg cctggtacca gggcaggtgc ccagctcgga ggccaggtca gcctgagcat 600

gggcaatgcc ttcctgggcc tcagcgtctt ccagaaggcc ctggagagct tcgagaaggc 660

cctgcgctat gcccacaaca atgatgacgc catgctcgag tgccgcgtgt gctgcagcct 720

gggcagcttc tatgcccagg tcaaggacta cgagaaagcc ctgttcttcc cctgcaaggc 780

ggcagagctt gtcaacaact atggcaaagg ctggagcctg aagtaccggg ccatgagcca 840

gtaccacatg gccgtggcct atcgcctgct gggccgcctg ggcagtgcca tggagtgttg 900

tgaggagtct atgaagatcg cgctgcagca cggggaccgg ccactgcagg cgctctgcct 960

gctctgcttc gctgacatcc accggagccg tggggacctg gagacagcct tccccaggta 1020

cgactccgcc atgagcatca tgaccgagat cggaaaccgc ctggggcagg tgcaggcgct 1080

gctgggtgtg gccaagtgct gggtggccag gaaggcgctg gacaaggctc tggatgccat 1140

cgagagagcc caggatctgg ccgaggaggt ggggaacaag ctgagccagc tcaagctgca 1200

ctgtctgagc gagagcattt accgcagcaa agggctgcag cgggaactgc gggcgcacgt 1260

tgtgaggttc cacgagtgcg tggaggagac ggagctctac tgcggcctgt gcggcgagtc 1320

cataggcgag aagaacagcc ggctgcaggc cctaccttgc tcccacatct tccacctcag 1380

gtgcctgcag aacaacggga cccggagctg tcccaactgc cgccgctcat ccatgaagcc 1440

tggctttgta tgactcctgg cagcaggcgt gggcttcctc ctcgccactc ctgctctttc 1500

tccactgcac gccagaggcc catttactcc tggggcagct gccaggtcgt cctcaccata 1560

gccaaggcct tggggcctgc ccagggctgc tcccctgggc ccagctcccc tccctgcctc 1620

tttgtacttt gctctttata gaaaaataaa ctgtttgtac ctggtcccag g 1671

<210> 37
<211> 1494
<212> DNA
<213> Homo sapiens

<400> 37

cccaactggc agcgacagct gcagacgggc tgaaccagct ttgttcccag ggtggcgcct 60

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gggggcagct ggcactgtga ttcctgcccc atgagtgcct agaggcacgg agccaccagg 180

gatcacccca cgtgggacac agggcttggg gaggatgggg caggaccaga ccaagcagca 240

gatcgagaag gggctccagc tgtaccagtc caaccagaca gagaaggcat tgcaggtgtg 300

gacaaaggtg ctggagaaga gctcggacct catggggcgc ttccgcgtgc tgggctgcct 360

ggtcacagcc cactcggaga tgggccgcta caaggagatg ctgaagttcg ctgtggtcca 420

gatcgacacg gcccgggagc tggaggatgc cgacttcctc ctggagagct acctgaacct 480

ggcacgcagc aacgagaagc tgtgcgagtt tcacaagacc atctcctact gcaagacctg 540

ccttgggctg cctggtacca gggcaggtgc ccagctcgga ggccaggtca gcctgagcat 600

gggcaatgcc ttcctgggcc tcagcgtctt ccagaaggcc ctggagagct tcgagaaggc 660

cctgcgctat gcccacaaca atgatgacgc catgctcgag tgccgcgtgt gctgcagcct 720

gggcagcttc tatgcccagg tcaaggacta cgagaaagcc ctgttcttcc cctgcaaggc 780

ggcagagctt gtcaacaact atggcaaagg ctggagcctg aagtaccggg ccatgagcca 840

gtaccacatg gccgtggcct atcgcctgct gggccgcctg ggcagtgcca tggagtgttg 900

tgaggagtct atgaagatcg cgctgcagca cggggaccgg ccactgcagg cgctctgcct 960

gctctgcttc gctgacatcc accggagccg tggggacctg gagctgagcc agctcaagct 1020

gcactgtctg agcgagagca tttaccgcag caaagggctg cagcgggaac tgcgggcgca 1080

cgttgtgagg ttccacgagt gcgtggagga gacggagctc tactgcggcc tgtgcggcga 1140

gtccataggc gagaagaaca gccggctgca ggccctacct tgctcccaca tcttccacct 1200

caggtgcctg cagaacaacg ggacccggag ctgtcccaac tgccgccgct catccatgaa 1260

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atagccaagg ccttggggcc tgcccagggc tgctcccctg ggcccagctc ccctccctgc 1440

ctctttgtac tttgctcttt atagaaaaat aaactgtttg tacctggtcc cagg 1494

<210> 38
<211> 412
<212> PRT
<213> Homo sapiens

<400> 38

```
Met Gly Gln Asp Gln Thr Lys Gln Gln Ile Glu Lys Gly Leu Gln Leu
1               5                  10                  15

Tyr Gln Ser Asn Gln Thr Glu Lys Ala Leu Gln Val Trp Thr Lys Val
            20                  25                  30

Leu Glu Lys Ser Ser Asp Leu Met Gly Arg Phe Arg Val Leu Gly Cys
        35                  40                  45

Leu Val Thr Ala His Ser Glu Met Gly Arg Tyr Lys Glu Met Leu Lys
    50                  55                  60

Phe Ala Val Val Gln Ile Asp Thr Ala Arg Glu Leu Glu Asp Ala Asp
65                  70                  75                  80

Phe Leu Leu Glu Ser Tyr Leu Asn Leu Ala Arg Ser Asn Glu Lys Leu
                85                  90                  95

Cys Glu Phe His Lys Thr Ile Ser Tyr Cys Lys Thr Cys Leu Gly Leu
            100                 105                 110

Pro Gly Thr Arg Ala Gly Ala Gln Leu Gly Gly Gln Val Ser Leu Ser
        115                 120                 125

Met Gly Asn Ala Phe Leu Gly Leu Ser Val Phe Gln Lys Ala Leu Glu
    130                 135                 140

Ser Phe Glu Lys Ala Leu Arg Tyr Ala His Asn Asn Asp Asp Ala Met
145                 150                 155                 160

Leu Glu Cys Arg Val Cys Cys Ser Leu Gly Ser Phe Tyr Ala Gln Val
                165                 170                 175
```

```
Lys Asp Tyr Glu Lys Ala Leu Phe Phe Pro Cys Lys Ala Ala Glu Leu
            180                 185                 190

Val Asn Asn Tyr Gly Lys Gly Trp Ser Leu Lys Tyr Arg Ala Met Ser
        195                 200                 205

Gln Tyr His Met Ala Val Ala Tyr Arg Leu Leu Gly Arg Leu Gly Ser
    210                 215                 220

Ala Met Glu Cys Cys Glu Glu Ser Met Lys Ile Ala Leu Gln His Gly
225                 230                 235                 240

Asp Arg Pro Leu Gln Ala Leu Cys Leu Leu Cys Phe Ala Asp Ile His
                245                 250                 255

Arg Ser Arg Gly Asp Leu Glu Thr Ala Phe Pro Arg Tyr Asp Ser Ala
            260                 265                 270

Met Ser Ile Met Thr Glu Ile Gly Asn Arg Leu Gly Gln Val Gln Ala
        275                 280                 285

Leu Leu Gly Val Ala Lys Cys Trp Val Ala Arg Lys Ala Leu Asp Lys
    290                 295                 300

Ala Leu Asp Ala Ile Glu Arg Ala Gln Asp Leu Ala Glu Glu Val Gly
305                 310                 315                 320

Asn Lys Leu Ser Gln Leu Lys Leu His Cys Leu Ser Glu Ser Ile Tyr
                325                 330                 335

Arg Ser Lys Gly Leu Gln Arg Glu Leu Arg Ala His Val Val Arg Phe
            340                 345                 350

His Glu Cys Val Glu Glu Thr Glu Leu Tyr Cys Gly Leu Cys Gly Glu
        355                 360                 365

Ser Ile Gly Glu Lys Asn Ser Arg Leu Gln Ala Leu Pro Cys Ser His
    370                 375                 380

Ile Phe His Leu Arg Cys Leu Gln Asn Asn Gly Thr Arg Ser Cys Pro
385                 390                 395                 400

Asn Cys Arg Arg Ser Ser Met Lys Pro Gly Phe Val
                405                 410
```

<210> 39
<211> 353

<212> PRT
<213> Homo sapiens

<400> 39

```
Met Gly Gln Asp Gln Thr Lys Gln Gln Ile Glu Lys Gly Leu Gln Leu
1               5                  10                  15

Tyr Gln Ser Asn Gln Thr Glu Lys Ala Leu Gln Val Trp Thr Lys Val
            20                  25                  30

Leu Glu Lys Ser Ser Asp Leu Met Gly Arg Phe Arg Val Leu Gly Cys
        35                  40                  45

Leu Val Thr Ala His Ser Glu Met Gly Arg Tyr Lys Glu Met Leu Lys
    50                  55                  60

Phe Ala Val Val Gln Ile Asp Thr Ala Arg Glu Leu Glu Asp Ala Asp
65                  70                  75                  80

Phe Leu Leu Glu Ser Tyr Leu Asn Leu Ala Arg Ser Asn Glu Lys Leu
                85                  90                  95

Cys Glu Phe His Lys Thr Ile Ser Tyr Cys Lys Thr Cys Leu Gly Leu
            100                 105                 110

Pro Gly Thr Arg Ala Gly Ala Gln Leu Gly Gly Gln Val Ser Leu Ser
        115                 120                 125

Met Gly Asn Ala Phe Leu Gly Leu Ser Val Phe Gln Lys Ala Leu Glu
    130                 135                 140

Ser Phe Glu Lys Ala Leu Arg Tyr Ala His Asn Asn Asp Asp Ala Met
145                 150                 155                 160

Leu Glu Cys Arg Val Cys Cys Ser Leu Gly Ser Phe Tyr Ala Gln Val
                165                 170                 175

Lys Asp Tyr Glu Lys Ala Leu Phe Phe Pro Cys Lys Ala Ala Glu Leu
            180                 185                 190

Val Asn Asn Tyr Gly Lys Gly Trp Ser Leu Lys Tyr Arg Ala Met Ser
        195                 200                 205

Gln Tyr His Met Ala Val Ala Tyr Arg Leu Leu Gly Arg Leu Gly Ser
    210                 215                 220

Ala Met Glu Cys Cys Glu Glu Ser Met Lys Ile Ala Leu Gln His Gly
225                 230                 235                 240
```

```
Asp Arg Pro Leu Gln Ala Leu Cys Leu Leu Cys Phe Ala Asp Ile His
                245                 250                 255

Arg Ser Arg Gly Asp Leu Glu Leu Ser Gln Leu Lys Leu His Cys Leu
            260                 265                 270

Ser Glu Ser Ile Tyr Arg Ser Lys Gly Leu Gln Arg Glu Leu Arg Ala
        275                 280                 285

His Val Val Arg Phe His Glu Cys Val Glu Glu Thr Glu Leu Tyr Cys
    290                 295                 300

Gly Leu Cys Gly Glu Ser Ile Gly Glu Lys Asn Ser Arg Leu Gln Ala
305                 310                 315                 320

Leu Pro Cys Ser His Ile Phe His Leu Arg Cys Leu Gln Asn Asn Gly
                325                 330                 335

Thr Arg Ser Cys Pro Asn Cys Arg Arg Ser Ser Met Lys Pro Gly Phe
            340                 345                 350

Val
```

<210> 40
<211> 510
<212> DNA
<213> Homo sapiens

<400> 40

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tgaggctgcc ttataaagca ccaagaggct gccagtggga cattttctcg gccctgccag       60

cccccaggag gaaggtgggt ctgaatctag caccatgacg gaactagaga cagccatggg      120

catgatcata gacgtctttt cccgatattc gggcagcgag ggcagcacgc agaccctgac      180

caaggggggag ctcaaggtgc tgatggagaa ggagctacca ggcttcctgc agagtggaaa      240

agacaaggat gccgtggata aattgctcaa ggacctggac gccaatggag atgcccaggt      300

ggacttcagt gagttcatcg tgttcgtggc tgcaatcacg tctgcctgtc acaagtactt      360

tgagaaggca ggactcaaat gatgccctgg agatgtcaca gattcctggc agagccatgg      420

tcccaggctt cccaaaagtg tttgttggca attattcccc taggctgagc ctgctcatgt      480

acctctgatt aataaatgct tatgaaatga                                       510
```

<210> 41
<211> 95
<212> PRT
<213> Homo sapiens

<400> 41

```
Met Thr Glu Leu Glu Thr Ala Met Gly Met Ile Ile Asp Val Phe Ser
1               5                  10                  15

Arg Tyr Ser Gly Ser Glu Gly Ser Thr Gln Thr Leu Thr Lys Gly Glu
            20                  25                  30

Leu Lys Val Leu Met Glu Lys Glu Leu Pro Gly Phe Leu Gln Ser Gly
        35                  40                  45

Lys Asp Lys Asp Ala Val Asp Lys Leu Leu Lys Asp Leu Asp Ala Asn
    50                  55                  60

Gly Asp Ala Gln Val Asp Phe Ser Glu Phe Ile Val Phe Val Ala Ala
65                  70                  75                  80

Ile Thr Ser Ala Cys His Lys Tyr Phe Glu Lys Ala Gly Leu Lys
                85                  90                  95
```

<210> 42
<211> 968
<212> DNA
<213> Homo sapiens

<400> 42

tggagggtaa gaagtttgtc ccacgaggtt tctcttactt tgcctccgca gtatacacgt 60

acatccaaag ccggttctac cgatccccag aagtgatcct gggccacccc tacgacgtgg 120

ccattgacat gtggagcctg ggctgcatca cggcggagtt gtacacgggc tacccctgt 180

tccccgggga gaatgaggtg gagcagctgg cctgcatcat ggaggtgctg ggtctgccgc 240

cagccggctt cattcagaca gcctccagga gacagacatt ctttgattcc aaaggttttc 300

ctaaaaatat aaccaacaac agggggaaaa aaagataccc agattccaag gacctcacga 360

tggtgctgaa aacctatgac accagcttcc tggactttct cagaaggtgt ttggtatggg 420

aaccttctct tcgcatgacc ccggaccagg ccctcaagca tgcttggatt catcagtctc 480

ggaacctcaa gccacagccc aggccccaga ccctgaggaa atccaattcc tttttccct 540

ctgagacaag gaaggacaag gttcaaggct gtcatcactc gagcagaaaa gcagatgaga 600

tcaccaaaga gactacagag aaaacaaaag atagccccac gaagcatgtt cagcattcag 660

gtgatcagca ggactgtctc cagcacggag ctgacactgt tcagctgcct caactggtag 720

acgctcccaa gaagtcagag gcagctgtcg gggcggaggt gtccatgacc tccccaggac 780

agagcaaaaa cttctccctc aagaacacaa acgttttacc ccctattgta tgacctttgc 840

tgagggtatg tcctgctcct ttccaccagt gatttgtatt aagacagcac ttatattgta 900

caatacttca gactgttttt tttaaataca taaaacttta tgttaaaaaa ctctattaac 960

atggccaa 968

<210> 43
<211> 965
<212> DNA
<213> Homo sapiens

<400> 43

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acatccaaag ccggttctac cgatccccag aagtgatcct gggccacccc tacgacgtgg 120

ccattgacat gtggagcctg ggctgcatca cggcggagtt gtacacgggc tacccctgt 180

tccccgggga gaatgaggtg gagcagctgg cctgcatcat ggaggtgctg ggtctgccgc 240

cagccggctt cattcagaca gcctccagga gacagacatt ctttgattcc aaaggttttc 300

ctaaaaatat aaccaacaac agggggaaaa aaagataccc agattccaag gacctcacga 360

tggtgctgaa aacctatgac accagcttcc tggactttct cagaaggtgt ttggtatggg 420

aaccttctct tcgcatgacc ccggaccagg ccctcaagca tgcttggatt catcagtctc 480

ggaacctcaa gccacagccc aggccccaga ccctgaggaa atccaattcc tttttccccct 540

ctgagacaag gaaggacaag gttcaaggct gtcatcactc gagcagaaaa gatgagatca 600

ccaaagagac tacagagaaa acaaaagata gccccacgaa gcatgttcag cattcaggtg 660

atcagcagga ctgtctccag cacggagctg acactgttca gctgcctcaa ctggtagacg 720

ctcccaagaa gtcagaggca gctgtcgggg cggaggtgtc catgacctcc ccaggacaga 780

gcaaaaactt ctccctcaag aacacaaacg ttttaccccc tattgtatga cctttgctga 840

gggtatgtcc tgctcctttc caccagtgat ttgtattaag acagcactta tattgtacaa 900

tacttcagac tgtttttttt aaatacataa aactttatgt taaaaaactc tattaacatg 960

gccaa 965

<210> 44
<211> 1864
<212> DNA
<213> Homo sapiens

<400> 44

agagtcagtg tgagctgttg aaagcctgca gctaaacacc agtgttactt cactcccctt 60

tgtggacacc aaggggaaga agaatacggt aagcttccca cacattagca agaaagtcct 120

gctgaagtca tccctgctgt atcaggagaa tcaagctcac aatcagatgc cggcctcaga 180

gctcaaggct tcagaaatac ctttccaccc tagcattaaa acccaggatc ccaaggcaga 240

ggagaagtca ccaaagaagc aaaaggtgac tctgacagcg gcagaggccc taaagctttt 300

taagaaccag ctgtctccat atgaacaaag tgaaatcctg ggctacgcgg agctgtggtt 360

cctgggtctt gaagccaaga agctcgacac ggctcctgag aaatttagca agacgagttt 420

tgatgatgag catggcttct atctgaaggt cctgcatgat cacattgcct accgctatga 480

agttctggag acaatcggga aggggtcctt tggacaggtg gccaagtgct tggatcacaa 540

aaacaatgag ctggtggccc tgaaaatcat caggaacaag aagaggtttc accagcaggc 600

cctgatggag ctgaagatcc tggaagctct cagaaagaag gacaaagaca acacctacaa 660

tgtggtgcat atgaaggact ttttctactt tcgcaatcac ttctgcatca cctttgagct 720

cctgggaatc aacttgtatg agttgatgaa gaataacaac tttcaaggct tcagtctgtc 780

catagttcgg cgcttcactc tctctgtttt gaagtgcttg cagatgcttt cggtagagaa 840

aatcattcac tgtgatctca agcccgaaaa tatagtgcta taccaaaagg gccaagcctc 900

tgttaaagtc attgactttg gatcaagctg ttatgaacac cagaaagtat acacgtacat 960

ccaaagccgg ttctaccgat ccccagaagt gatcctgggc caccctacg acgtggccat 1020

tgacatgtgg agcctgggct gcatcacggc ggagttgtac acgggctacc ccctgttccc 1080

cggggagaat gaggtggagc agctggcctg catcatggag gtgctgggtc tgccgccagc 1140

cggcttcatt cagacagcct ccaggagaca gacattcttt gattccaaag gttttcctaa 1200

aaatataacc aacaacaggg ggaaaaaaag atacccagat tccaaggacc tcacgatggt 1260

gctgaaaacc tatgacacca gcttcctgga ctttctcaga aggtgtttgg tatgggaacc 1320

ttctcttcgc atgaccccgg accaggccct caagcatgct tggattcatc agtctcggaa 1380

cctcaagcca cagcccaggc cccagaccct gaggaaatcc aattcctttt tcccctctga 1440

gacaaggaag gacaaggttc aaggctgtca tcactcgagc agaaaagcag atgagatcac 1500

caaagagact acagagaaaa caaaagatag ccccacgaag catgttcagc attcaggtga 1560

tcagcaggac tgtctccagc acggagctga cactgttcag ctgcctcaac tggtagacgc 1620

tcccaagaag tcagaggcag ctgtcggggc ggaggtgtcc atgacctccc caggacagag 1680

caaaaacttc tccctcaaga acacaaacgt tttacccccc attgtatgac ctttgctgag 1740

ggtatgtcct gctcctttcc accagtgatt tgtattaaga cagcacttat attgtacaat 1800

acttcagact gtttttttta aatacataaa actttatgtt aaaaaactct attaacatgg 1860

ccaa 1864

<210> 45
<211> 234
<212> PRT
<213> Homo sapiens

<400> 45

Met Trp Ser Leu Gly Cys Ile Thr Ala Glu Leu Tyr Thr Gly Tyr Pro
1 5 10 15

```
Leu Phe Pro Gly Glu Asn Glu Val Glu Gln Leu Ala Cys Ile Met Glu
            20                  25                  30

Val Leu Gly Leu Pro Pro Ala Gly Phe Ile Gln Thr Ala Ser Arg Arg
        35                  40                  45

Gln Thr Phe Phe Asp Ser Lys Gly Phe Pro Lys Asn Ile Thr Asn Asn
    50                  55                  60

Arg Gly Lys Lys Arg Tyr Pro Asp Ser Lys Asp Leu Thr Met Val Leu
65                  70                  75                  80

Lys Thr Tyr Asp Thr Ser Phe Leu Asp Phe Leu Arg Arg Cys Leu Val
                85                  90                  95

Trp Glu Pro Ser Leu Arg Met Thr Pro Asp Gln Ala Leu Lys His Ala
            100                 105                 110

Trp Ile His Gln Ser Arg Asn Leu Lys Pro Gln Pro Arg Pro Gln Thr
        115                 120                 125

Leu Arg Lys Ser Asn Ser Phe Phe Pro Ser Glu Thr Arg Lys Asp Lys
    130                 135                 140

Val Gln Gly Cys His His Ser Ser Arg Lys Ala Asp Glu Ile Thr Lys
145                 150                 155                 160

Glu Thr Thr Glu Lys Thr Lys Asp Ser Pro Thr Lys His Val Gln His
                165                 170                 175

Ser Gly Asp Gln Gln Asp Cys Leu Gln His Gly Ala Asp Thr Val Gln
            180                 185                 190

Leu Pro Gln Leu Val Asp Ala Pro Lys Lys Ser Glu Ala Ala Val Gly
        195                 200                 205

Ala Glu Val Ser Met Thr Ser Pro Gly Gln Ser Lys Asn Phe Ser Leu
    210                 215                 220

Lys Asn Thr Asn Val Leu Pro Pro Ile Val
225                 230
```

<210> 46
<211> 233
<212> PRT
<213> Homo sapiens

<400> 46

```
Met Trp Ser Leu Gly Cys Ile Thr Ala Glu Leu Tyr Thr Gly Tyr Pro
1               5                  10                  15

Leu Phe Pro Gly Glu Asn Glu Val Glu Gln Leu Ala Cys Ile Met Glu
            20                  25                  30

Val Leu Gly Leu Pro Pro Ala Gly Phe Ile Gln Thr Ala Ser Arg Arg
        35                  40                  45

Gln Thr Phe Phe Asp Ser Lys Gly Phe Pro Lys Asn Ile Thr Asn Asn
    50                  55                  60

Arg Gly Lys Lys Arg Tyr Pro Asp Ser Lys Asp Leu Thr Met Val Leu
65                  70                  75                  80

Lys Thr Tyr Asp Thr Ser Phe Leu Asp Phe Leu Arg Arg Cys Leu Val
                85                  90                  95

Trp Glu Pro Ser Leu Arg Met Thr Pro Asp Gln Ala Leu Lys His Ala
            100                 105                 110

Trp Ile His Gln Ser Arg Asn Leu Lys Pro Gln Pro Arg Pro Gln Thr
        115                 120                 125

Leu Arg Lys Ser Asn Ser Phe Phe Pro Ser Glu Thr Arg Lys Asp Lys
    130                 135                 140

Val Gln Gly Cys His His Ser Ser Arg Lys Asp Glu Ile Thr Lys Glu
145                 150                 155                 160

Thr Thr Glu Lys Thr Lys Asp Ser Pro Thr Lys His Val Gln His Ser
                165                 170                 175

Gly Asp Gln Gln Asp Cys Leu Gln His Gly Ala Asp Thr Val Gln Leu
            180                 185                 190

Pro Gln Leu Val Asp Ala Pro Lys Lys Ser Glu Ala Ala Val Gly Ala
        195                 200                 205

Glu Val Ser Met Thr Ser Pro Gly Gln Ser Lys Asn Phe Ser Leu Lys
    210                 215                 220

Asn Thr Asn Val Leu Pro Pro Ile Val
225                 230
```

<210> 47
<211> 520

<212> PRT
<213> Homo sapiens

<400> 47

```
Met Pro Ala Ser Glu Leu Lys Ala Ser Glu Ile Pro Phe His Pro Ser
1               5                   10                  15

Ile Lys Thr Gln Asp Pro Lys Ala Glu Glu Lys Ser Pro Lys Lys Gln
            20                  25                  30

Lys Val Thr Leu Thr Ala Ala Glu Ala Leu Lys Leu Phe Lys Asn Gln
        35                  40                  45

Leu Ser Pro Tyr Glu Gln Ser Glu Ile Leu Gly Tyr Ala Glu Leu Trp
    50                  55                  60

Phe Leu Gly Leu Glu Ala Lys Lys Leu Asp Thr Ala Pro Glu Lys Phe
65                  70                  75                  80

Ser Lys Thr Ser Phe Asp Asp Glu His Gly Phe Tyr Leu Lys Val Leu
                85                  90                  95

His Asp His Ile Ala Tyr Arg Tyr Glu Val Leu Glu Thr Ile Gly Lys
            100                 105                 110

Gly Ser Phe Gly Gln Val Ala Lys Cys Leu Asp His Lys Asn Asn Glu
        115                 120                 125

Leu Val Ala Leu Lys Ile Ile Arg Asn Lys Lys Arg Phe His Gln Gln
    130                 135                 140

Ala Leu Met Glu Leu Lys Ile Leu Glu Ala Leu Arg Lys Lys Asp Lys
145                 150                 155                 160

Asp Asn Thr Tyr Asn Val Val His Met Lys Asp Phe Phe Tyr Phe Arg
                165                 170                 175

Asn His Phe Cys Ile Thr Phe Glu Leu Leu Gly Ile Asn Leu Tyr Glu
            180                 185                 190

Leu Met Lys Asn Asn Asn Phe Gln Gly Phe Ser Leu Ser Ile Val Arg
        195                 200                 205

Arg Phe Thr Leu Ser Val Leu Lys Cys Leu Gln Met Leu Ser Val Glu
    210                 215                 220

Lys Ile Ile His Cys Asp Leu Lys Pro Glu Asn Ile Val Leu Tyr Gln
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Lys Gly Gln Ala Ser Val Lys Val Ile Asp Phe Gly Ser Ser Cys Tyr
245 250 255

Glu His Gln Lys Val Tyr Thr Tyr Ile Gln Ser Arg Phe Tyr Arg Ser
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Pro Glu Val Ile Leu Gly His Pro Tyr Asp Val Ala Ile Asp Met Trp
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Ser Leu Gly Cys Ile Thr Ala Glu Leu Tyr Thr Gly Tyr Pro Leu Phe
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Lys Lys Arg Tyr Pro Asp Ser Lys Asp Leu Thr Met Val Leu Lys Thr
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Tyr Asp Thr Ser Phe Leu Asp Phe Leu Arg Arg Cys Leu Val Trp Glu
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Pro Ser Leu Arg Met Thr Pro Asp Gln Ala Leu Lys His Ala Trp Ile
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His Gln Ser Arg Asn Leu Lys Pro Gln Pro Arg Pro Gln Thr Leu Arg
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420 425 430

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435 440 445

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Asp Gln Gln Asp Cys Leu Gln His Gly Ala Asp Thr Val Gln Leu Pro
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Gln Leu Val Asp Ala Pro Lys Lys Ser Glu Ala Ala Val Gly Ala Glu
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Patentkrav

1. Fremgangsmåde til diagnosticering af brystcancer (BC) hos en person, hvilken fremgangsmåde omfatter

a) bestemmelse af methyleringsstatus for mindst én methyleringsmarkør valgt fra gruppen bestående af HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P og DYRK4, og

b) bestemmelse af mængden af miRNA-markørerne miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 og miR-148b hos en person,

hvor forringet methyleringsstatus for den mindst ene methyleringsmarkør og tilstedeværelse af miRNA'erne indikerer personens risiko for at lide af BC.

2. Fremgangsmåde ifølge krav 1, hvor

a) methyleringsstatus for methyleringsmarkøren RPTOR, MGRN1 og RAPSN, og eventuelt HYAL2 bestemmes, og

b) tilstedeværelse af miRNA-markørerne miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 og miR-148b bestemmes.

3. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 2, hvor

a) methyleringsstatus for methyleringsmarkøren DYRK4, S100P, FUT7 og SLC22A18, og eventuelt HYAL2 bestemmes, og

b) tilstedeværelse af miRNA-markørerne miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 og miR-148b bestemmes.

4. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 2, hvor

a) methyleringsstatus for methyleringsmarkøren MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P, DYRK4, og eventuelt HYAL2, bestemmes, og

b) tilstedeværelse af miRNA-markørerne miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 og miR-148b bestemmes.

5. Anvendelse af et kit til diagnosticering af BC

omfattende

a) ét eller flere midler til påvisning af methyleringsstatus for mindst én methyleringsmarkør valgt fra gruppen bestående af HYAL2, MGRN1, RPTOR, SLC22A18,

FUT7, RAPSN, S100P og DYRK4, og

b) midler til påvisning af mængden af miRNA-markører miR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 og miR-148b.

6. Anordning til identificering af BC, der omfatter:

(a) en analyseenhed, der omfatter

(i) et påvisningsmiddel til bestemmelse af methyleringsstatus for mindst én methyleringsmarkør valgt fra gruppen bestående af HYAL2, MGRN1, RPTOR, SLC22A18, FUT7, RAPSN, S100P og DYRK4, og

(ii) et påvisningsmiddel til bestemmelse af tilstedeværelsen af miRNA'erne iR-652, miR-801, miR-376c, miR-376a, miR-127, miR-409 og miR-148b i en prøve fra en person; og

(b) en evalueringsenhed, der omfatter en dataprocessor med en materielt indlejret algoritme til at foretage en sammenligning af mængden bestemt af analyseenheden med en reference, og som er i stand til at generere en uddatafil, der indeholder en diagnose udfærdiget på baggrund af sammenligningen.

Fig. 1

Blood-based biomarker panel for the early detection of breast cancer

Table 1. Sample description

Rounds		First validation round		Second validation round		Third validation round	
Sample types		Peripheral blood DNA		Peripheral blood DNA		Peripheral blood DNA	
Assays		MassARRAY		MassARRAY		MassARRAY	
Groups		Controls	FBC cases	Controls	SBC cases	Controls	SBC cases
Sample resourses		Blood donor from Mannheim	Breast cancer centers in Heidelberg and Cologne	Blood donor from Mannheim	University hospital of Heidelberg	University hospital of Heidelberg	University hospital of Heidelberg
Target N		251	270	189	189	151	161
Median of age		43.0	43.0	64.0	60.0	45.0	46.0
Rang of age		30-67	24-78	31-69	32-87	21-77	27-61
Assayed N (call rate %)	HYAL2	251 (100.0%)	270 (100.0%)	189 (100.0%)	188 (99.5%)	151 (100.0%)	161 (100.0%)
	DYRK4	247 (98.4%)	268 (99.3%)	189 (100.0%)	189 (100.0%)	150 (99.3%)	158 (98.1%)
	S100P	251 (100.0%)	270 (100.0%)	189 (100.0%)	189 (100.0%)	151 (100.0%)	161 (100.0%)
	FUT7	251 (100.0%)	270 (100.0%)	189 (100.0%)	189 (100.0%)	151 (100.0%)	161 (100.0%)
	SLC22A18	251 (100.0%)	270 (100.0%)	189 (100.0%)	189 (100.0%)	151 (100.0%)	161 (100.0%)
	RPTOR	251 (100.0%)	270 (100.0%)	189 (100.0%)	189 (100.0%)	151 (100%)	161 (100.0%)
	MGRN1	251 (100.0%)	270 (100.0%)	189 (100.0%)	189 (100.0%)	150 (99.3%)	158 (98.1%)
	RAPSN	251 (100.0%)	270 (100.0%)	189 (100.0%)	189 (100.0%)	150 (99.3%)	161 (100.0%)
	miR-127-3p	–	–	–	–	148 (98.0%)	153 (95.0%)
	miR-148b	–	–	–	–	148 (98.0%)	153 (95.0%)
	miR-376a	–	–	–	–	148 (98.0%)	153 (95.0%)
	miR-376c	–	–	–	–	148 (98.0%)	153 (95.0%)
	miR-409-3p	–	–	–	–	148 (98.0%)	153 (95.0%)
	miR-652	–	–	–	–	148 (98.0%)	153 (95.0%)
	miR-801	–	–	–	–	148 (98.0%)	153 (95.0%)

Fig. 2

Table 2. Methylation difference of eight genes in the three validation rounds

CpG sites	First validation round				Second validation round			
	Controls median (IQR)	FBC cases median (IQR)	OR per 10% methylation	*p*-value *	Controls median (IQR)	Sporadic BC cases median (IQR)	OR per 10% methylation	*p*-value *
HYAL2_CpG_1	0.38 (0.33-0.45)	0.30 (0.26-0.37)	1.92 (1.57-2.33)	8.92E-11	0.39 (0.32-0.48)	0.3 (0.26-0.37)	1.96 (1.56-2.45)	4.70E-09
HYAL2_CpG_2	0.25 (0.20-0.30)	0.18 (0.14-0.23)	3.10 (2.32-4.15)	2.88E-14	0.23 (0.18-0.29)	0.17 (0.13-0.2)	5.31 (3.49-8.06)	5.27E-15
HYAL2_CpG_3	0.41 (0.36-0.47)	0.32 (0.28-0.38)	4.24 (3.14-5.75)	8.22E-21	0.41 (0.36-0.47)	0.32 (0.28-0.36)	5.44 (3.69-8.03)	1.51E-17
HYAL2_CpG_4	0.65 (0.59-0.72)	0.53 (0.48-0.60)	4.53 (3.41-6.03)	2.78E-25	0.64 (0.58-0.70)	0.5 (0.46-0.54)	8.14 (5.37-12.33)	4.79E-23
DYRK4_CpG_1	0.38 (0.19-0.55)	0.25 (0.15-0.38)	1.25 (1.15-1.35)	1.20E-07	0.34 (0.23-0.46)	0.26 (0.19-0.33)	1.49 (1.28-1.73)	3.82E-07
DYRK4_CpG_3	0.29 (0.16-0.42)	0.20 (0.12-0.30)	1.25 (1.14-1.39)	7.33E-06	0.27 (0.18-0.37)	0.22 (0.17-0.27)	1.53 (1.27-1.85)	8.23E-06
S100P_CpG_2.3	0.68 (0.64-0.72)	0.63 (0.59-0.68)	3.07 (2.31-4.09)	1.63E-14	0.68 (0.64-0.72)	0.65 (0.61-0.69)	2.28 (1.59-3.27)	7.32E-06
S100P_CpG_4	0.89 (0.64-0.95)	0.67 (0.58-0.91)	1.32 (1.19-1.47)	1.81E-07	0.86 (0.61-0.94)	0.83 (0.60-0.93)	1.08 (0.96-1.21)	0.227
S100P_CpG_7	0.58 (0.47-0.72)	0.41 (0.31-0.54)	1.61 (1.43-1.82)	1.53E-14	0.49 (0.40-0.67)	0.43 (0.32-0.61)	1.34 (1.16-1.56)	7.93E-05
S100P_CpG_8	0.52 (0.45-0.59)	0.43 (0.37-0.51)	2.36 (1.93-2.90)	1.10E-16	0.48 (0.42-0.58)	0.45 (0.38-0.51)	1.71 (1.37-2.15)	2.77E-06
S100P_CpG_9	0.58 (0.52-0.64)	0.5 (0.45-0.56)	2.72 (2.14-3.45)	1.97E-16	0.56 (0.51-0.62)	0.52 (0.46-0.58)	2.07 (1.58-2.72)	1.45E-07
SLC22A18_CpG_3	0.21 (0.16-0.26)	0.18 (0.14-0.21)	2.70 (1.98-3.67)	2.67E-10	0.19 (0.15-0.24)	0.16 (0.13-0.18)	3.78 (2.46-5.81)	1.20E-09
SLC22A18_CpG_4	0.26 (0.21-0.35)	0.21 (0.17-0.26)	1.94 (1.57-2.40)	8.99E-10	0.26 (0.21-0.32)	0.19 (0.16-0.24)	2.96 (2.15-4.06)	1.94E-11
SLC22A18_CpG_6	0.29 (0.23-0.36)	0.25 (0.21-0.29)	2.02 (1.61-2.54)	1.79E-09	0.27 (0.22-0.32)	0.21 (0.18-0.25)	3.35 (2.34-4.80)	4.80E-11
SLC22A18_CpG_8	0.65 (0.59-0.70)	0.60 (0.55-0.65)	2.22 (1.73-2.85)	4.99E-10	0.62 (0.57-0.67)	0.58 (0.52-0.61)	2.61 (1.90-3.60)	3.44E-09

Fig. 2 (continued)

Table 2. Methylation difference of eight genes in the three validation rounds (continued)

CpG sites	First validation round				Second validation round			
	Controls median (IQR)	FBC cases median (IQR)	OR per 10% methylation	*p*-value *	Controls median (IQR)	Sporadic BC cases median (IQR)	OR per 10% methylation	*p*-value *
FUT7_CpG_1	0.43 (0.35-0.49)	0.31 (0.23-0.39)	2.23 (1.87-2.67)	9.32E-19	0.40 (0.30-0.51)	0.31 (0.26-0.38)	1.84 (1.52-2.23)	5.20E-10
FUT7_CpG_2	0.26 (0.21-0.32)	0.20 (0.15-0.25)	3.14 (2.36-4.17)	4.08E-15	0.21 (0.14-0.28)	0.16 (0.13-0.20)	2.04 (1.56-2.67)	2.26E-07
FUT7_CpG_3	0.18 (0.14-0.23)	0.13 (0.09-0.18)	3.18 (2.37-4.28)	2.14E-14	0.16 (0.10-0.27)	0.13 (0.09-0.17)	1.73 (1.36-2.21)	9.46E-06
FUT7_CpG_4	0.23 (0.19-0.30)	0.19 (0.13-0.26)	1.63 (1.34-1.99)	1.46E-06	0.24 (0.18-0.31)	0.21 (0.16-0.25)	1.55 (1.23-1.95)	1.75E-04
cg02679745	0.31 (0.25-0.38)	0.21 (0.15-0.26)	3.28 (2.59-4.16)	1.15E-22	0.28 (0.20-0.39)	0.21 (0.16-0.27)	1.98 (1.60-2.45)	4.02E-10
FUT7_CpG_7	0.13 (0.09-0.18)	0.08 (0.05-0.12)	3.87 (2.78-5.39)	1.35E-15	0.12 (0.06-0.17)	0.09 (0.06-0.13)	1.46 (1.13-1.88)	0.004
FUT7_CpG_8	0.40 (0.34-0.48)	0.31 (0.25-0.39)	1.89 (1.60-2.23)	3.73E-14	0.38 (0.30-0.49)	0.31 (0.24-0.38)	1.49 (1.27-1.75)	6.88E-07
RPTOR_CpG_1	0.11 (0.08-0.18)	0.10 (0.06-0.15)	1.50 (1.12-2.01)	0.007	0.05 (0.00-0.17)	0.05 (0.00-0.13)	1.17 (1.02-1.35)	0.028
RPTOR_CpG_2	0.34 (0.26-0.43)	0.29 (0.20-0.36)	1.67 (1.36-2.05)	1.32E-06	0.26 (0.11-0.45)	0.20 (0.11-0.32)	1.17 (1.05-1.30)	0.004
RPTOR_CpG_3	0.71 (0.62-0.80)	0.64 (0.55-0.72)	1.59 (1.31-1.92)	1.97E-06	0.70 (0.50-0.94)	0.68 (0.53-0.80)	1.03 (0.95-1.12)	0.511
RPTOR_CpG_4	0.97 (0.91-1.00)	0.91 (0.83-0.99)	2.11 (1.57-2.85)	7.74E-07	0.85 (0.65-1.00)	0.80 (0.68-0.97)	1.02 (0.92-1.13)	0.670
RPTOR_CpG_5	0.83 (0.75-0.90)	0.80 (0.73-0.85)	1.55 (1.21-2.00)	6.09E-04	0.85 (0.70-0.97)	0.80 (0.68-0.90)	1.06 (0.95-1.17)	0.288
RPTOR_CpG_8	0.78 (0.70-0.84)	0.72 (0.67-0.77)	2.00 (1.52-2.63)	8.73E-07	0.80 (0.62-0.96)	0.72 (0.59-0.84)	1.08 (0.98-1.19)	0.116
RAPSN_CpG_1	0.95 (0.92-0.96)	0.94 (0.92-0.96)	1.26 (0.81-1.95)	0.302	0.97 (0.94-0.99)	0.96 (0.92-0.98)	1.12 (0.91-1.36)	0.285
RAPSN_CpG_2	0.67 (0.58-0.78)	0.67 (0.61-0.74)	1.06 (0.90-1.25)	0.480	0.73 (0.39-0.90)	0.67 (0.46-0.87)	1.00 (0.93-1.07)	0.988
RAPSN_CpG_4	0.50 (0.39-0.63)	0.41 (0.34-0.52)	1.48 (1.26-1.73)	1.11E-06	0.48 (0.08-0.82)	0.36 (0.12-0.59)	1.09 (1.02-1.16)	0.013
RAPSN_CpG_5	0.82 (0.71-0.88)	0.79 (0.72-0.85)	1.03 (0.85-1.25)	0.757	0.91 (0.64-0.99)	0.80 (0.59-0.94)	1.07 (0.99-1.16)	0.077
RAPSN_CpG_6	0.67 (0.57-0.78)	0.58 (0.48-0.68)	1.42 (1.21-1.67)	1.68E-05	0.64 (0.19-0.95)	0.57 (0.32-0.79)	1.01 (0.95-1.08)	0.699
RAPSN_CpG_7	0.74 (0.66-0.83)	0.72 (0.63-0.79)	1.20 (1.00-1.43)	0.047	0.95 (0.63-1.00)	0.79 (0.55-0.96)	1.06 (0.99-1.14)	0.123
RAPSN_CpG_8	0.96 (0.93-0.97)	0.95 (0.92-0.96)	1.32 (0.79-2.20)	0.293	0.97 (0.97-0.98)	0.97 (0.96-0.98)	0.95 (0.79-1.13)	0.542

Fig. 2 (continued)

Table 2. Methylation difference of eight genes in the three validation rounds (continued)

CpG sites	First validation round				Second validation round			
	Controls median (IQR)	FBC cases median (IQR)	OR per 10% methylation	*p*-value *	Controls median (IQR)	Sporadic BC cases median (IQR)	OR per 10% methylation	*p*-value *
MGRN1_CpG_1	0.35 (0.23-0.52)	0.23 (0.10-0.35)	1.38 (1.22-1.56)	3.98E-07	0.33 (0.02-0.63)	0.09 (0.00-0.30)	1.25 (1.15-1.36)	1.24E-07
MGRN1_CpG_2	0.65 (0.59-0.73)	0.60 (0.56-0.66)	1.49 (1.19-1.87)	5.72E-04	0.58 (0.42-0.76)	0.47 (0.38-0.59)	1.23 (1.11-1.36)	1.10E-04
MGRN1_CpG_3	0.52 (0.32-0.67)	0.34 (0.22-0.49)	1.33 (1.19-1.49)	5.08E-07	0.48 (0.07-0.83)	0.19 (0.04-0.50)	1.17 (1.10-1.25)	1.78E-06
MGRN1_CpG_4	0.44 (0.31-0.63)	0.32 (0.22-0.44)	1.33 (1.19-1.50)	1.93E-06	0.45 (0.05-0.74)	0.18 (0.04-0.42)	1.22 (1.14-1.32)	9.33E-08
MGRN1_CpG_5.6.7.8	0.46 (0.33-0.56)	0.33 (0.24-0.44)	1.52 (1.30-1.77)	9.82E-08	0.46 (0.10-0.62)	0.23 (0.09-0.41)	1.25 (1.15-1.37)	5.38E-07
MGRN1_CpG_11	0.35 (0.22-0.50)	0.22 (0.10-0.35)	1.37 (1.21-1.56)	1.29E-06	0.31 (0.01-0.63)	0.11 (0.00-0.34)	1.21 (1.12-1.31)	2.22E-06
MGRN1_CpG_12	0.50 (0.35-0.66)	0.38 (0.27-0.50)	1.36 (1.21-1.54)	7.75E-07	0.51 (0.09-0.83)	0.23 (0.07-0.47)	1.19 (1.11-1.27)	6.44E-07
MGRN1_CpG_13	0.65 (0.59-0.73)	0.60 (0.56-0.66)	1.49 (1.19-1.87)	5.72E-04	0.58 (0.42-0.76)	0.47 (0.38-0.59)	1.23 (1.11-1.36)	1.10E-04
MGRN1_CpG_14	0.40 (0.33-0.53)	0.34 (0.24-0.41)	1.52 (1.28-1.79)	1.08E-06	0.41 (0.02-0.67)	0.25 (0.00-0.42)	1.21 (1.12-1.31)	1.53E-06
MGRN1_CpG_15	0.50 (0.34-0.66)	0.37 (0.26-0.46)	1.44 (1.26-1.64)	5.25E-08	0.53 (0.12-0.84)	0.24 (0.05-0.51)	1.20 (1.12-1.29)	8.96E-08
MGRN1_CpG_16.17.18	0.48 (0.35-0.62)	0.35 (0.27-0.46)	1.46 (1.27-1.68)	2.04E-07	0.49 (0.10-0.76)	0.23 (0.09-0.45)	1.22 (1.13-1.31)	2.78E-07
MGRN1_CpG_19.20	0.53 (0.40-0.67)	0.41 (0.33-0.53)	1.46 (1.26-1.69)	3.77E-07	0.52 (0.20-0.79)	0.33 (0.18-0.53)	1.20 (1.11-1.30)	5.55E-06
MGRN1_CpG_21	0.40 (0.33-0.53)	0.34 (0.24-0.41)	1.52 (1.28-1.79)	1.08E-06	0.41 (0.02-0.67)	0.25 (0.00-0.42)	1.21 (1.12-1.31)	1.53E-06
MGRN1_CpG_22.23	0.49 (0.37-0.59)	0.37 (0.29-0.47)	1.52 (1.30-1.77)	1.05E-07	0.46 (0.14-0.71)	0.26 (0.09-0.45)	1.23 (1.14-1.34)	7.12E-07
MGRN1_CpG_26	0.48 (0.36-0.61)	0.38 (0.29-0.48)	1.35 (1.17-1.55)	2.45E-05	0.44 (0.07-0.74)	0.26 (0.11-0.49)	1.16 (1.08-1.25)	7.97E-05
MGRN1_CpG_27	0.51 (0.35-0.65)	0.38 (0.28-0.50)	1.37 (1.20-1.55)	1.94E-06	0.47 (0.04-0.79)	0.19 (0.01-0.49)	1.16 (1.08-1.23)	1.09E-05
MGRN1_CpG_28	0.45 (0.35-0.56)	0.36 (0.27-0.45)	1.41 (1.22-1.63)	3.65E-06	0.44 (0.06-0.74)	0.21 (0.03-0.44)	1.20 (1.12-1.29)	1.25E-06
MGRN1_CpG_29	0.56 (0.43-0.69)	0.46 (0.36-0.57)	1.36 (1.19-1.56)	1.03E-05	0.52 (0.13-0.84)	0.33 (0.15-0.57)	1.14 (1.06-1.22)	1.68E-04
MGRN1_CpG_31	0.48 (0.35-0.61)	0.40 (0.29-0.50)	1.33 (1.15-1.53)	9.66E-05	0.43 (0.09-0.76)	0.26 (0.10-0.48)	1.16 (1.08-1.25)	4.75E-05
MGRN1_CpG_32	0.39 (0.28-0.51)	0.30 (0.22-0.39)	1.32 (1.15-1.52)	6.18E-05	0.38 (0.07-0.70)	0.17 (0.06-0.38)	1.20 (1.12-1.30)	2.04E-06
MGRN1_CpG_34	0.48 (0.41-0.60)	0.43 (0.34-0.53)	1.24 (1.07-1.44)	0.003	0.51 (0.10-0.76)	0.34 (0.10-0.56)	1.11 (1.04-1.19)	0.003

Fig. 2 (continued)

Table 2. Methylation difference of eight genes in the three validation rounds (continued)

CpG sites	Third validation round			
	Controls median (IQR)	Sporadic BC cases median (IQR)	OR per 10% methylation	*p*-value *
HYAL2_CpG_1	0.27 (0.24-0.31)	0.25 (0.21-0.28)	3.01 (1.88-4.85)	5.13E-06
HYAL2_CpG_2	0.19 (0.17-0.23)	0.16 (0.13-0.20)	3.04 (1.89-4.88)	4.48E-06
HYAL2_CpG_3	0.32 (0.28-0.36)	0.28 (0.24-0.32)	3.50 (2.24-5.48)	4.13E-08
HYAL2_CpG_4	0.52 (0.48-0.55)	0.47 (0.42-0.51)	3.28 (2.18-4.94)	1.19E-08
DYRK4_CpG_1	0.32 (0.21-0.40)	0.22 (0.14-0.33)	1.20 (1.06-1.36)	0.003
DYRK4_CpG_3	0.25 (0.17-0.33)	0.19 (0.13-0.28)	1.26 (1.06-1.51)	0.010
S100P_CpG_2.3	0.71 (0.66-0.73)	0.67 (0.62-0.72)	2.86 (1.79-4.55)	9.93E-06
S100P_CpG_4	0.91 (0.63-0.96)	0.89 (0.62-0.96)	1.05 (0.92-1.20)	0.464
S100P_CpG_7	0.54 (0.44-0.61)	0.47 (0.42-0.55)	1.40 (1.16-1.69)	5.60E-04
S100P_CpG_8	0.54 (0.46-0.60)	0.47 (0.39-0.56)	2.00 (1.52-2.64)	9.85E-07
S100P_CpG_9	0.58 (0.54-0.63)	0.52 (0.46-0.59)	2.33 (1.69-3.22)	2.61E-07
SLC22A18_CpG_3	0.18 (0.15-0.22)	0.14 (0.11-0.17)	4.25 (2.56-7.04)	1.95E-08
SLC22A18_CpG_4	0.22 (0.18-0.28)	0.17 (0.13-0.23)	2.60 (1.82-3.70)	1.16E-07
SLC22A18_CpG_6	0.26 (0.21-0.30)	0.20 (0.16-0.25)	2.43 (1.70-3.48)	1.20E-06
SLC22A18_CpG_8	0.64 (0.59-0.70)	0.61 (0.53-0.67)	1.64 (1.23-2.20)	8.40E-04
FUT7_CpG_1	0.38 (0.30-0.46)	0.35 (0.25-0.42)	1.38 (1.13-1.69)	0.002
FUT7_CpG_2	0.23 (0.17-0.30)	0.21 (0.13-0.27)	1.35 (1.04-1.76)	0.025
FUT7_CpG_3	0.16 (0.11-0.21)	0.14 (0.09-0.21)	1.20 (0.95-1.53)	0.132
FUT7_CpG_4	0.22 (0.16-0.29)	0.20(0.15-0.26)	1.28 (1.01-1.62)	0.037
cg02679745	0.26 (0.20-0.33)	0.23 (0.16-0.32)	1.36 (1.08-1.71)	0.009
FUT7_CpG_7	0.12 (0.08-0.18)	0.09 (0.05-0.14)	1.61 (1.20-2.16)	0.001
FUT7_CpG_8	0.37 (0.27-0.45)	0.31 (0.23-0.40)	1.34 (1.11-1.61)	0.002
RPTOR_CpG_1	0.09 (0.07-0.15)	0.07 (0.03-0.10)	3.42 (2.16-5.43)	1.63E-07
RPTOR_CpG_2	0.27 (0.20-0.35)	0.20 (0.15-0.28)	2.66 (1.96-3.61)	2.90E-10
RPTOR_CpG_3	0.66 (0.59-0.73)	0.60 (0.55-0.68)	1.66 (1.30-2.13)	5.11E-05
RPTOR_CpG_4	0.84 (0.72-0.97)	0.77 (0.68-0.93)	1.86 (1.41-2.45)	1.22E-05
RPTOR_CpG_5	0.78 (0.74-0.84)	0.74 (0.69-0.82)	1.87 (1.41-2.49)	1.74E-05
RPTOR_CpG_8	0.72 (0.66-0.76)	0.68 (0.62-0.73)	2.06 (1.49-2.85)	1.30E-05

Fig. 2 (continued)

Table 2. Methylation difference of eight genes in the three validation rounds (continued)

CpG sites	Third validation round			
	Controls median (IQR)	Sporadic BC cases median (IQR)	OR per 10% methylation	*p*-value *
RAPSN_CpG_1	0.96 (0.93-0.98)	0.95 (0.93-0.98)	1.16 (0.71-1.92)	0.551
RAPSN_CpG_2	0.66 (0.59-0.72)	0.62 (0.52-0.70)	1.44 (1.18-1.76)	4.15E-04
RAPSN_CpG_4	0.48 (0.37-0.54)	0.39 (0.32-0.47)	1.49 (1.23-1.80)	5.61E-05
RAPSN_CpG_5	0.79 (0.73-0.84)	0.74 (0.66-0.82)	1.57 (1.25-1.99)	1.34E-04
RAPSN_CpG_6	0.60 (0.51-0.67)	0.53 (0.44-0.61)	1.47 (1.22-1.77)	4.42E-05
RAPSN_CpG_7	0.75 (0.68-0.80)	0.72 (0.65-0.80)	1.19 (0.95-1.48)	0.128
RAPSN_CpG_8	0.95 (0.93-0.97)	0.96 (0.93-0.97)	0.95 (0.53-1.71)	0.871
MGRN1_CpG_1	0.23 (0.13-0.34)	0.14 (0.07-0.28)	1.31 (1.11-1.55)	0.002
MGRN1_CpG_2	0.64 (0.59-0.69)	0.61 (0.56-0.66)	1.46 (1.10-1.94)	0.008
MGRN1_CpG_3	0.40 (0.30-0.50)	0.30 (0.20-0.38)	1.63 (1.35-1.95)	1.85E-07
MGRN1_CpG_4	0.33 (0.25-0.44)	0.26 (0.19-0.35)	1.44 (1.20-1.72)	6.89E-05
MGRN1_CpG_5.6.7.8	0.35 (0.27-0.44)	0.28 (0.21-0.36)	1.74 (1.39-2.18)	1.65E-06
MGRN1_CpG_11	0.17 (0.11-0.31)	0.14 (0.07-0.21)	1.39 (1.15-1.68)	6.48E-04
MGRN1_CpG_12	0.38 (0.295-0.5)	0.32 (0.22-0.39)	1.56 (1.30-1.89)	2.83E-06
MGRN1_CpG_13	0.64 (0.59-0.69)	0.61 (0.56-0.66)	1.29 (1.00-1.67)	0.053
MGRN1_CpG_14	0.34 (0.28-0.42)	0.28 (0.19-0.34)	1.83 (1.45-2.31)	4.59E-07
MGRN1_CpG_15	0.41 (0.33-0.49)	0.32 (0.24-0.42)	1.62 (1.33-1.98)	1.33E-06
MGRN1_CpG_16.17.18	0.37 (0.31-0.47)	0.31 (0.25-0.38)	1.61 (1.29-2.01)	2.11E-05
MGRN1_CpG_19.20	0.44 (0.36-0.54)	0.36 (0.26-0.44)	1.74 (1.41-2.16)	2.56E-07
MGRN1_CpG_21	0.34 (0.28-0.42)	0.28 (0.19-0.34)	1.83 (1.45-2.31)	4.59E-07
MGRN1_CpG_22.23	0.39 (0.31-0.46)	0.33 (0.25-0.39)	1.72 (1.37-2.17)	2.76E-06
MGRN1_CpG_26	0.40 (0.32-0.48)	0.31 (0.25-0.39)	1.77 (1.43-2.20)	2.31E-07
MGRN1_CpG_27	0.40 (0.30-0.49)	0.30 (0.20-0.40)	1.70 (1.40-2.06)	7.99E-08
MGRN1_CpG_28	0.37 (0.29-0.46)	0.31 (0.22-0.38)	1.58 (1.28-1.95)	2.11E-05
MGRN1_CpG_29	0.47 (0.40-0.56)	0.38 (0.32-0.47)	1.83 (1.47-2.29)	7.69E-08
MGRN1_CpG_31	0.39 (0.33-0.48)	0.34 (0.27-0.42)	1.69 (1.35-2.10)	2.83E-06
MGRN1_CpG_32	0.34 (0.26-0.42)	0.27 (0.17-0.36)	1.44 (1.20-1.73)	1.18E-04
MGRN1_CpG_34	0.43 (0.35-0.52)	0.36 (0.29-0.44)	1.50 (1.25-1.81)	1.98E-05

Fig. 3

Table 3. The discriminatory power of DNA methylation marker sets to distinguish BC cases from healthy controls in samples from other centers (first and second validation rounds)

Marker sets	First validation round		Second validation round			
	All cases vs. All controls		All cases vs. All controls		Stage 0&I vs. All controls	
	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)
HYAL2	267 vs. 250	0.85 (0.82-0.89)	188 vs. 189	0.88 (0.85-0.92)	101 vs. 189	0.88 (0.84-0.92)
S100P, SLC22A18, DYRK4, FUT7	265 vs. 246	0.86 (0.83-0.89)	189 vs. 189	0.80 (0.75-0.84)	101 vs. 189	0.78 (0.73-0.83)
MGRN1, RPTOR, RAPSN	267 vs. 250	0.80 (0.76-0.84)	189 vs. 189	0.76 (0.71-0.81)	101 vs. 189	0.74 (0.69-0.80)
HYAL2, S100P, SLC22A18, DYRK4, FUT7	265 vs. 245	0.90 (0.88-0.93)	188 vs. 189	0.90 (0.87-0.93)	101 vs. 189	0.90 (0.87-0.94)
HYAL2, MGRN1, RPTOR, RAPSN	267 vs. 249	0.90 (0.87-0.92)	188 vs. 189	0.91 (0.88-0.94)	101 vs. 189	0.91 (0.87-0.94)
S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN	265 vs. 245	0.91 (0.88-0.93)	189 vs. 189	0.86 (0.83-0.90)	101 vs. 189	0.84 (0.80-0.89)
HYAL2, S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN	265 vs. 244	0.94 (0.92-0.96)	188 vs. 189	0.93 (0.91-0.96)	101 vs. 189	0.93 (0.90-0.96)

Fig. 3 (continued)

Table 3. The discriminatory power of DNA methylation marker sets to distinguish BC cases from healthy controls in samples from other centers (first and second validation rounds)(continued)

Marker sets	Samples from other centers (1st+2nd validation rounds)					
	All cases vs. All control		Age < 50		Age >=50	
	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)
HYAL2	455 vs. 439	0.86 (0.84-0.88)	251 vs. 226	0.86 (0.83-0.90)	204 vs. 213	0.86 (0.83-0.90)
S100P, SLC22A18, DYRK4, FUT7	454 vs. 435	0.81 (0.78-0.84)	251 vs. 223	0.88 (0.85-0.91)	203 vs. 212	0.77 (0.73-0.82)
MGRN1, RPTOR, RAPSN	456 vs. 439	0.74 (0.71-0.78)	252 vs. 226	0.81 (0.78-0.85)	204 vs. 213	0.74 (0.69-0.79)
HYAL2, S100P, SLC22A18, DYRK4, FUT7	453 vs. 434	0.89 (0.87-0.91)	250 vs. 222	0.92 (0.90-0.95)	203 vs. 212	0.89 (0.85-0.92)
HYAL2, MGRN1, RPTOR, RAPSN	455 vs. 438	0.88 (0.86-0.90)	251 vs. 225	0.90 (0.87-0.93)	204 vs. 213	0.89 (0.86-0.93)
S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN	454 vs. 434	0.85 (0.82-0.87)	251 vs. 222	0.92 (0.90-0.95)	203 vs. 212	0.84 (0.80-0.87)
HYAL2, S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN	453 vs. 433	0.91 (0.89-0.93)	250 vs. 221	0.95 (0.93-0.97)	203 vs. 212	0.92 (0.89-0.94)

Fig. 4

Table 4. The discriminatory power of DNA methylation marker sets and miRNA marker sets to distinguish BC cases from healthy controls in samples from the third validation round

Marker sets	All cases vs. All control		Stage 0&I vs. All controls		Age < 50		Age >=50	
	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)	N (case vs. control)	AUC (95% CI)
HYAL2	161 vs. 149	0.72 (0.67-0.78)	57 vs. 149	0.79 (0.72-0.85)	116 vs. 98	0.85 (0.80-0.90)	45 vs. 51	0.73 (0.63-0.83)
S100P, SLC22A18, DYRK4, FUT7	158 vs. 148	0.76 (0.71-0.82)	55 vs. 148	0.84 (0.77-0.90)	113 vs. 98	0.88 (0.84-0.93)	45 vs. 50	0.85 (0.76-0.93)
MGRN1, RPTOR, RAPSN	158 vs. 147	0.82 (0.77-0.87)	56 vs. 147	0.89 (0.84-0.94)	113 vs. 96	0.93 (0.89-0.96)	45 vs. 51	0.89 (0.82-0.95)
HYAL2, S100P, SLC22A18, DYRK4, FUT7	158 vs. 148	0.77 (0.72-0.83)	55 vs. 148	0.86 (0.80-0.92)	113 vs. 98	0.90 (0.86-0.94)	45 vs. 50	0.87 (0.80-0.95)
HYAL2, MGRN1, RPTOR, RAPSN	158 vs. 147	0.83 (0.78-0.88)	56 vs. 147	0.90 (0.85-0.94)	113 vs. 96	0.94 (0.91-0.97)	45 vs. 51	0.90 (0.84-0.96)
S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN	155 vs. 146	0.85 (0.81-0.90)	54 vs. 146	0.94 (0.90-0.97)	110 vs. 96	0.96 (0.94-0.99)	45 vs. 50	1.00 (1.00-1.00)
HYAL2, S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN	155 vs. 146	0.86 (0.82-0.90)	54 vs. 146	0.94 (0.91-0.97)	110 vs. 96	0.98 (0.96-1.00)	45 vs. 50	1.00 (1.00-1.00)
7miRNA	153 vs. 148	0.82 (0.77-0.86)	53 vs. 148	0.80 (0.73-0.87)	109 vs. 98	0.90 (0.85-0.94)	44 vs. 50	0.84 (0.76-0.92)
HYAL2, 7miRNA	153 vs. 148	0.86 (0.81-0.90)	53 vs. 148	0.86 (0.81-0.92)	109 vs. 98	0.93 (0.90-0.96)	44 vs. 50	0.86 (0.78-0.93)
S100P, SLC22A18, DYRK4, FUT7, 7miRNA	150 vs. 147	0.89 (0.86-0.93)	51 vs. 147	0.91 (0.86-0.96)	106 vs. 98	0.96 (0.94-0.98)	44 vs. 49	0.93 (0.87-0.98)
MGRN1, RPTOR, RAPSN, 7miRNA	149 vs. 144	0.91 (0.88-0.94)	52 vs. 144	0.95 (0.92-0.98)	106 vs. 96	0.97 (0.95-0.99)	44 vs. 50	0.97 (0.94-1.00)
HYAL2, S100P, SLC22A18, DYRK4, FUT7, 7miRNA	150 vs. 147	0.90 (0.86-0.93)	51 vs. 147	0.92 (0.88-0.97)	106 vs. 98	0.97 (0.94-0.99)	44 vs. 49	0.94 (0.89-0.98)
HYAL2, MGRN1, RPTOR, RAPSN, 7miRNA	149 vs. 144	0.91 (0.88-0.95)	52 vs. 144	0.96 (0.93-0.99)	106 vs. 96	0.98 (0.96-0.99)	44 vs. 50	1.00 (1.00-1.00)
S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR, RAPSN, 7miRNA	146 vs. 143	0.94 (0.91-0.97)	50 vs. 143	1.00 (1.00-1.00)	103 vs. 96	1.00 (1.00-1.00)	44 vs. 49	1.00 (1.00-1.00)
HYAL2, S100P, SLC22A18, DYRK4, FUT7, MGRN1, RPTOR,RAPSN, 7miRNA	146 vs. 143	0.94 (0.92-0.97)	50 vs. 143	1.00 (1.00-1.00)	103 vs. 96	1.00 (1.00-1.00)	44 vs. 49	1.00 (1.00-1.00)

Fig. 5

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round)

Clinical characteristics (N)	Group (N)	Median of age	Median of methylation levels					
			HYAL2_ CpG_1	HYAL2_ CpG_2	HYAL2_ CpG_3	HYAL2_ CpG_4	DYRK4_ CpG_1	DYRK4_ CpG_3
Tumour stage (188)	Stage 0&I (101)	59.23	0.30	0.17	0.33	0.50	0.25	0.23
	Stage II (61)	57.80	0.31	0.17	0.32	0.50	0.27	0.21
	Stage III (26)	68.11	0.29	0.13	0.28	0.51	0.26	0.21
	p-value (Kruskal Wallis Test)	0.166	0.937	0.008	0.001	0.846	0.726	0.407
Tumour size (188)	Tis&T1 (119)	59.23	0.30	0.17	0.33	0.50	0.26	0.22
	T2 (57)	60.33	0.32	0.16	0.32	0.50	0.26	0.21
	T3 and T4 (12)	71.04	0.26	0.12	0.27	0.52	0.24	0.22
	p-value (Kruskal Wallis Test)	0.191	0.637	0.041	0.001	0.983	0.514	0.681
Lymph node (LN) involvement (185)	no involved LN (132)	60.00	0.30	0.17	0.33	0.50	0.26	0.22
	1-3 involved LN (30)	55.59	0.30	0.18	0.32	0.52	0.28	0.20
	> 3 involved LN (23)	67.92	0.29	0.12	0.27	0.51	0.27	0.21
	p-value (Kruskal Wallis Test)	0.265	0.875	0.003	0.004	0.661	0.946	0.139
Grading (187)	Grade 1 (35)	58.78	0.30	0.15	0.32	0.51	0.24	0.24
	Grade 2 (114)	61.50	0.29	0.17	0.33	0.50	0.26	0.21
	Grade 3 (38)	60.42	0.32	0.16	0.32	0.50	0.26	0.21
	p-value (Kruskal Wallis Test)	0.731	0.262	0.592	0.970	0.949	0.617	0.777
ER status (185)	ER negative (23)	57.72	0.34	0.16	0.32	0.50	0.26	0.22
	ER positive (162)	60.67	0.29	0.17	0.32	0.50	0.26	0.22
	p-value (Mann-Whitney U)	0.150	0.017	0.371	0.862	0.489	0.303	0.397
PR status (186)	PR negative (38)	60.97	0.33	0.16	0.32	0.51	0.25	0.21
	PR positive (148)	59.24	0.29	0.17	0.32	0.50	0.26	0.22
	p-value (Mann-Whitney U)	0.797	0.014	0.142	0.495	0.124	0.973	0.626
Her2 status (185)	Her2 negative (166)	60.71	0.30	0.17	0.33	0.51	0.26	0.22
	Her2 positive (19)	51.52	0.30	0.17	0.31	0.49	0.27	0.21
	p-value (Mann-Whitney U)	0.357	0.855	0.386	0.225	0.451	0.504	0.724
Three receptor status (185)	Tripple negative (15)	59.67	0.40	0.14	0.32	0.51	0.26	0.22
	Others (170)	59.88	0.30	0.17	0.32	0.50	0.26	0.22
	p-value (Mann-Whitney U)	0.310	0.062	0.072	0.683	0.281	0.358	0.264
Menopause status (180)	premenopause (56)	47.19	0.30	0.17	0.33	0.50	0.27	0.22
	postmenopause (124)	67.64	0.31	0.17	0.32	0.51	0.26	0.22
	p-value (Mann-Whitney U)	—	0.678	0.627	0.558	0.157	0.756	0.294
BC family history (186)	with BC family history (30)	58.96	0.29	0.17	0.32	0.51	0.29	0.26
	without BC family history (156)	60.45	0.30	0.17	0.33	0.51	0.26	0.21
	p-value (Mann-Whitney U)	0.296	0.533	0.243	0.211	0.476	0.202	0.055

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels				
		S100P_CpG_2.3	S100P_CpG_4	S100P_CpG_7	S100P_CpG_8	S100P_CpG_9
Tumour stage (188)	Stage 0&I (101)	0.65	0.83	0.40	0.44	0.52
	Stage II (61)	0.64	0.83	0.45	0.45	0.52
	Stage III (26)	0.66	0.90	0.47	0.47	0.53
	p-value (Kruskal Wallis Test)	0.427	0.746	0.290	0.843	0.679
Tumour size (188)	Tis&T1 (119)	0.65	0.84	0.43	0.44	0.52
	T2 (57)	0.64	0.69	0.42	0.45	0.51
	T3 and T4 (12)	0.67	0.92	0.52	0.48	0.56
	p-value (Kruskal Wallis Test)	0.223	0.298	0.306	0.570	0.230
Lymph node (LN) involvement (185)	no involved LN (132)	0.65	0.82	0.41	0.44	0.52
	1-3 involved LN (30)	0.66	0.85	0.48	0.46	0.52
	> 3 involved LN (23)	0.66	0.91	0.47	0.47	0.53
	p-value (Kruskal Wallis Test)	0.572	0.549	0.205	0.355	0.429
Grading (187)	Grade 1 (35)	0.65	0.65	0.45	0.42	0.52
	Grade 2 (114)	0.65	0.84	0.41	0.44	0.52
	Grade 3 (38)	0.66	0.86	0.51	0.47	0.53
	p-value (Kruskal Wallis Test)	0.757	0.490	0.290	0.394	0.500
ER status (185)	ER negative (23)	0.64	0.89	0.40	0.45	0.52
	ER positive (162)	0.65	0.83	0.43	0.45	0.52
	p-value (Mann-Whitney U)	0.376	0.195	0.988	0.606	0.725
PR status (186)	PR negative (38)	0.65	0.86	0.42	0.45	0.52
	PR positive (148)	0.65	0.83	0.44	0.45	0.52
	p-value (Mann-Whitney U)	0.232	0.619	0.984	0.474	0.878
Her2 status (185)	Her2 negative (166)	0.66	0.85	0.43	0.45	0.52
	Her2 positive (19)	0.61	0.69	0.40	0.42	0.49
	p-value (Mann-Whitney U)	0.030	0.056	0.679	0.139	0.122
Three receptor status (185)	Tripple negative (15)	0.66	0.91	0.48	0.46	0.52
	Others (170)	0.65	0.83	0.43	0.44	0.52
	p-value (Mann-Whitney U)	0.801	0.172	0.697	0.749	0.774
Menopause status (180)	premenopause (56)	0.63	0.84	0.38	0.42	0.49
	postmenopause (124)	0.66	0.83	0.45	0.46	0.53
	p-value (Mann-Whitney U)	0.004	0.790	0.032	0.012	0.004
BC family history (186)	with BC family history (30)	0.64	0.65	0.42	0.42	0.52
	without BC family history (156)	0.66	0.84	0.44	0.45	0.52
	p-value (Mann-Whitney U)	0.407	0.198	0.932	0.586	0.405

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		FUT7_ CpG_2	FUT7_ CpG_3	FUT7_ CpG_4	FUT7_ CpG_6	FUT7_ CpG_7	FUT7_ CpG_8
Tumour stage (188)	Stage 0&I (101)	0.17	0.13	0.22	0.22	0.09	0.34
	Stage II (61)	0.17	0.14	0.21	0.21	0.10	0.31
	Stage III (26)	0.13	0.11	0.17	0.17	0.08	0.30
	p-value (Kruskal Wallis Test)	0.033	0.135	0.169	0.003	0.226	0.790
Tumour size (188)	Tis&T1 (119)	0.17	0.13	0.21	0.21	0.09	0.34
	T2 (57)	0.16	0.14	0.22	0.21	0.09	0.30
	T3 and T4 (12)	0.12	0.11	0.16	0.14	0.07	0.31
	p-value (Kruskal Wallis Test)	0.220	0.720	0.448	0.010	0.180	0.296
Lymph node (LN) involvement (185)	no involved LN (132)	0.17	0.13	0.22	0.21	0.09	0.31
	1-3 involved LN (30)	0.17	0.15	0.22	0.24	0.11	0.33
	> 3 involved LN (23)	0.13	0.11	0.16	0.17	0.08	0.32
	p-value (Kruskal Wallis Test)	0.044	0.207	0.080	0.017	0.194	0.957
Grading (187)	Grade 1 (35)	0.18	0.13	0.22	0.24	0.11	0.35
	Grade 2 (114)	0.15	0.13	0.21	0.20	0.09	0.30
	Grade 3 (38)	0.18	0.14	0.20	0.22	0.10	0.33
	p-value (Kruskal Wallis Test)	0.019	0.628	0.446	0.110	0.673	0.234
ER status (185)	ER negative (23)	0.16	0.13	0.19	0.20	0.08	0.29
	ER positive (162)	0.17	0.13	0.21	0.21	0.09	0.33
	p-value (Mann-Whitney U)	0.653	0.691	0.378	0.478	0.107	0.607
PR status (186)	PR negative (38)	0.17	0.14	0.22	0.22	0.09	0.32
	PR positive (148)	0.17	0.13	0.21	0.21	0.09	0.31
	p-value (Mann-Whitney U)	0.441	0.553	0.679	0.479	0.761	0.292
Her2 status (185)	Her2 negative (166)	0.17	0.13	0.21	0.21	0.09	0.32
	Her2 positive (19)	0.15	0.13	0.19	0.22	0.08	0.31
	p-value (Mann-Whitney U)	0.429	0.756	0.628	0.991	0.464	0.924
Three receptor status (185)	Tripple negative (15)	0.17	0.11	0.19	0.20	0.08	0.29
	Others (170)	0.17	0.13	0.21	0.21	0.09	0.33
	p-value (Mann-Whitney U)	0.397	0.254	0.327	0.371	0.036	0.323
Menopause status (180)	premenopause (56)	0.16	0.13	0.20	0.20	0.08	0.27
	postmenopause (124)	0.17	0.14	0.22	0.21	0.09	0.34
	p-value (Mann-Whitney U)	0.490	0.321	0.498	0.549	0.225	0.021
BC family history (186)	with BC family history (30)	0.15	0.13	0.23	0.21	0.08	0.29
	without BC family history (156)	0.17	0.13	0.21	0.21	0.10	0.32
	p-value (Mann-Whitney U)	0.628	0.662	0.817	0.884	0.064	0.347

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels			
		SLC22A18_ CpG_3	SLC22A18_ CpG_4	SLC22A18_ CpG_6	SLC22A18_ CpG_8
Tumour stage (188)	Stage 0&I (101)	0.16	0.21	0.22	0.58
	Stage II (61)	0.16	0.19	0.22	0.57
	Stage III (26)	0.13	0.17	0.20	0.54
	p-value (Kruskal Wallis Test)	0.005	0.010	0.048	0.031
Tumour size (188)	Tis&T1 (119)	0.16	0.21	0.22	0.58
	T2 (57)	0.16	0.19	0.21	0.59
	T3 and T4 (12)	0.14	0.17	0.20	0.53
	p-value (Kruskal Wallis Test)	0.111	0.022	0.270	0.078
Lymph node (LN) involvement (185)	no involved LN (132)	0.16	0.20	0.22	0.58
	1-3 involved LN (30)	0.16	0.22	0.22	0.59
	> 3 involved LN (23)	0.13	0.17	0.18	0.53
	p-value (Kruskal Wallis Test)	0.005	0.048	0.048	0.038
Grading (187)	Grade 1 (35)	0.16	0.19	0.21	0.60
	Grade 2 (114)	0.16	0.19	0.21	0.57
	Grade 3 (38)	0.16	0.22	0.22	0.58
	p-value (Kruskal Wallis Test)	0.292	0.152	0.533	0.158
ER status (185)	ER negative (23)	0.16	0.21	0.22	0.57
	ER positive (162)	0.16	0.19	0.21	0.58
	p-value (Mann-Whitney U)	0.647	0.558	0.764	0.877
PR status (186)	PR negative (38)	0.15	0.21	0.22	0.57
	PR positive (148)	0.16	0.19	0.21	0.58
	p-value (Mann-Whitney U)	0.787	0.761	0.996	0.837
Her2 status (185)	Her2 negative (166)	0.16	0.20	0.22	0.58
	Her2 positive (19)	0.15	0.19	0.19	0.57
	p-value (Mann-Whitney U)	0.606	0.700	0.188	0.809
Three receptor status (185)	Tripple negative (15)	0.16	0.21	0.22	0.56
	Others (170)	0.16	0.19	0.21	0.58
	p-value (Mann-Whitney U)	0.737	0.581	0.493	0.724
Menopause status (180)	premenopause (56)	0.15	0.18	0.21	0.59
	postmenopause (124)	0.16	0.21	0.22	0.57
	p-value (Mann-Whitney U)	0.408	0.325	0.236	0.327
BC family history (186)	with BC family history (30)	0.16	0.21	0.22	0.58
	without BC family history (156)	0.16	0.19	0.22	0.58
	p-value (Mann-Whitney U)	0.403	0.391	0.748	0.342

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		RPTOR_ CpG_1	RPTOR_ CpG_2	RPTOR_ CpG_3	RPTOR_ CpG_4	RPTOR_ CpG_5	RPTOR_ CpG_8
Tumour stage (188)	Stage 0&I (101)	0.07	0.22	0.70	0.80	0.82	0.74
	Stage II (61)	0.02	0.18	0.67	0.75	0.75	0.72
	Stage III (26)	0.02	0.17	0.61	0.92	0.80	0.69
	p-value (Kruskal Wallis Test)	0.026	0.173	0.197	0.109	0.138	0.260
Tumour size (188)	Tis&T1 (119)	0.06	0.22	0.72	0.80	0.83	0.74
	T2 (57)	0.02	0.19	0.62	0.76	0.74	0.69
	T3 and T4 (12)	0.03	0.17	0.62	0.92	0.77	0.69
	p-value (Kruskal Wallis Test)	0.095	0.341	0.036	0.171	0.028	0.406
Lymph node (LN) involvement (185)	no involved LN (132)	0.05	0.20	0.67	0.78	0.80	0.72
	1-3 involved LN (30)	0.04	0.20	0.75	0.83	0.82	0.76
	> 3 involved LN (23)	0.02	0.15	0.49	0.89	0.79	0.67
	p-value (Kruskal Wallis Test)	0.702	0.377	0.028	0.208	0.934	0.050
Grading (187)	Grade 1 (35)	0.08	0.18	0.66	0.78	0.82	0.70
	Grade 2 (114)	0.04	0.20	0.68	0.78	0.81	0.72
	Grade 3 (38)	0.05	0.21	0.66	0.93	0.79	0.69
	p-value (Kruskal Wallis Test)	0.231	0.798	0.666	0.052	0.812	0.919
ER status (185)	ER negative (23)	0.07	0.28	0.69	0.85	0.79	0.68
	ER positive (162)	0.04	0.19	0.68	0.79	0.80	0.72
	p-value (Mann-Whitney U)	0.272	0.057	0.705	0.158	0.868	0.557
PR status (186)	PR negative (38)	0.08	0.25	0.65	0.81	0.81	0.68
	PR positive (148)	0.04	0.20	0.68	0.79	0.79	0.73
	p-value (Mann-Whitney U)	0.195	0.471	0.623	0.334	0.241	0.511
Her2 status (185)	Her2 negative (166)	0.04	0.20	0.69	0.80	0.80	0.72
	Her2 positive (19)	0.08	0.22	0.63	0.84	0.79	0.68
	p-value (Mann-Whitney U)	0.294	0.305	0.632	0.633	0.885	0.561
Three receptor status (185)	Tripple negative (15)	0.07	0.28	0.69	0.85	0.75	0.67
	Others (170)	0.04	0.20	0.67	0.79	0.80	0.72
	p-value (Mann-Whitney U)	0.511	0.125	0.948	0.503	0.786	0.401
Menopause status (180)	premenopause (56)	0.05	0.19	0.69	0.80	0.74	0.70
	postmenopause (124)	0.04	0.22	0.67	0.80	0.83	0.72
	p-value (Mann-Whitney U)	0.896	0.243	0.697	0.555	0.006	0.651
BC family history (186)	with BC family history (30)	0.05	0.20	0.73	0.85	0.77	0.73
	without BC family history (156)	0.05	0.20	0.66	0.79	0.81	0.71
	p-value (Mann-Whitney U)	0.373	0.442	0.113	0.166	0.224	0.973

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		MGRN1_CpG_1	MGRN1_CpG_2	MGRN1_CpG_4	MGRN1_CpG_5.6.7.8	MGRN1_CpG_12	MGRN1_CpG_13
Tumour stage (188)	Stage 0&I (101)	0.12	0.50	0.22	0.23	0.26	0.50
	Stage II (61)	0.09	0.48	0.14	0.19	0.24	0.48
	Stage III (26)	0.12	0.57	0.27	0.33	0.29	0.57
	p-value (Kruskal Wallis Test)	0.553	0.307	0.208	0.126	0.777	0.307
Tumour size (188)	Tis&T1 (119)	0.12	0.50	0.22	0.24	0.27	0.50
	T2 (57)	0.07	0.50	0.14	0.23	0.23	0.50
	T3 and T4 (12)	0.17	0.54	0.37	0.39	0.39	0.54
	p-value (Kruskal Wallis Test)	0.234	0.960	0.331	0.546	0.786	0.960
Lymph node (LN) involvement (185)	no involved LN (132)	0.12	0.50	0.19	0.22	0.24	0.50
	1-3 involved LN (30)	0.06	0.46	0.19	0.25	0.33	0.46
	> 3 involved LN (23)	0.21	0.57	0.28	0.33	0.31	0.57
	p-value (Kruskal Wallis Test)	0.411	0.086	0.291	0.147	0.636	0.086
Grading (187)	Grade 1 (35)	0.11	0.47	0.18	0.23	0.24	0.47
	Grade 2 (114)	0.12	0.50	0.21	0.23	0.27	0.50
	Grade 3 (38)	0.12	0.52	0.27	0.29	0.34	0.52
	p-value (Kruskal Wallis Test)	0.826	0.609	0.587	0.500	0.877	0.609
ER status (185)	ER negative (23)	0.14	0.48	0.28	0.32	0.33	0.48
	ER positive (162)	0.11	0.50	0.20	0.23	0.26	0.50
	p-value (Mann-Whitney U)	0.568	0.909	0.335	0.730	0.688	0.909
PR status (186)	PR negative (38)	0.14	0.50	0.27	0.31	0.34	0.50
	PR positive (148)	0.11	0.50	0.20	0.23	0.24	0.50
	p-value (Mann-Whitney U)	0.207	0.307	0.081	0.193	0.249	0.307
Her2 status (185)	Her2 negative (166)	0.11	0.50	0.21	0.23	0.25	0.50
	Her2 positive (19)	0.12	0.52	0.22	0.26	0.27	0.52
	p-value (Mann-Whitney U)	0.620	0.496	0.991	0.729	0.998	0.496
Three receptor status (185)	Tripple negative (15)	0.23	0.50	0.28	0.32	0.33	0.50
	Others (170)	0.11	0.50	0.20	0.23	0.26	0.50
	p-value (Mann-Whitney U)	0.263	0.784	0.194	0.629	0.534	0.784
Menopause status (180)	premenopause (56)	0.08	0.49	0.17	0.20	0.21	0.49
	postmenopause (124)	0.12	0.50	0.22	0.25	0.29	0.50
	p-value (Mann-Whitney U)	0.252	0.348	0.384	0.431	0.207	0.348
BC family history (186)	with BC family history (30)	0.13	0.50	0.23	0.22	0.24	0.50
	without BC family history (15(	0.11	0.50	0.20	0.25	0.27	0.50
	p-value (Mann-Whitney U)	0.415	0.818	0.535	0.818	0.427	0.818

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		MGRN1_CpG_15	MGRN1_CpG_16.17.18	MGRN1_CpG_19.20	MGRN1_CpG_22.23	MGRN1_CpG_26	MGRN1_CpG_27
Tumour stage (188)	Stage 0&I (101)	0.26	0.28	0.35	0.27	0.30	0.28
	Stage II (61)	0.30	0.24	0.35	0.22	0.28	0.20
	Stage III (26)	0.42	0.32	0.41	0.30	0.39	0.36
	p-value (Kruskal Wallis Test)	0.296	0.355	0.594	0.386	0.356	0.368
Tumour size (188)	Tis&T1 (119)	0.27	0.29	0.35	0.27	0.30	0.28
	T2 (57)	0.30	0.25	0.33	0.22	0.22	0.20
	T3 and T4 (12)	0.39	0.38	0.46	0.33	0.46	0.45
	p-value (Kruskal Wallis Test)	0.962	0.811	0.655	0.597	0.117	0.717
Lymph node (LN) involvement (185)	no involved LN (132)	0.27	0.25	0.35	0.26	0.28	0.25
	1-3 involved LN (30)	0.29	0.26	0.36	0.29	0.32	0.32
	> 3 involved LN (23)	0.45	0.33	0.41	0.30	0.40	0.38
	p-value (Kruskal Wallis Test)	0.099	0.185	0.627	0.588	0.354	0.298
Grading (187)	Grade 1 (35)	0.28	0.28	0.35	0.24	0.27	0.21
	Grade 2 (114)	0.29	0.26	0.36	0.28	0.29	0.25
	Grade 3 (38)	0.34	0.30	0.36	0.25	0.33	0.34
	p-value (Kruskal Wallis Test)	0.590	0.386	0.805	0.999	0.890	0.381
ER status (185)	ER negative (23)	0.30	0.33	0.37	0.31	0.33	0.35
	ER positive (162)	0.29	0.26	0.35	0.26	0.28	0.26
	p-value (Mann-Whitney U)	0.680	0.545	0.855	0.745	0.907	0.867
PR status (186)	PR negative (38)	0.35	0.34	0.38	0.32	0.34	0.29
	PR positive (148)	0.29	0.26	0.34	0.26	0.28	0.22
	p-value (Mann-Whitney U)	0.166	0.232	0.345	0.183	0.292	0.387
Her2 status (185)	Her2 negative (166)	0.30	0.28	0.35	0.27	0.28	0.25
	Her2 positive (19)	0.26	0.26	0.36	0.28	0.30	0.29
	p-value (Mann-Whitney U)	0.464	0.844	0.977	0.386	0.755	0.922
Three receptor status (185)	Tripple negative (15)	0.31	0.34	0.41	0.38	0.34	0.35
	Others (170)	0.29	0.26	0.35	0.26	0.28	0.26
	p-value (Mann-Whitney U)	0.882	0.365	0.563	0.096	0.744	0.768
Menopause status (180)	premenopause (56)	0.18	0.19	0.29	0.23	0.22	0.17
	postmenopause (124)	0.33	0.29	0.38	0.29	0.32	0.29
	p-value (Mann-Whitney U)	0.023	0.082	0.187	0.251	0.090	0.141
BC family history (186)	with BC family history (30)	0.28	0.23	0.36	0.27	0.26	0.23
	without BC family history (156	0.30	0.29	0.35	0.27	0.31	0.28
	p-value (Mann-Whitney U)	0.663	0.764	0.913	0.939	0.425	0.390

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round)

Clinical characteristics (N)	Group (N)	Median of methylation levels				
		MGRN1_ CpG_28	MGRN1_ CpG_29	MGRN1_ CpG_31	MGRN1_ CpG_32	MGRN1_ CpG_34
Tumour stage (188)	Stage 0&I (101)	0.24	0.34	0.30	0.20	0.38
	Stage II (61)	0.25	0.35	0.25	0.12	0.33
	Stage III (26)	0.37	0.45	0.36	0.37	0.43
	p-value (Kruskal Wallis Test)	0.379	0.372	0.565	0.049	0.677
Tumour size (188)	Tis&T1 (119)	0.24	0.36	0.30	0.20	0.36
	T2 (57)	0.25	0.37	0.30	0.14	0.35
	T3 and T4 (12)	0.42	0.49	0.44	0.36	0.44
	p-value (Kruskal Wallis Test)	0.363	0.580	0.608	0.576	0.701
Lymph node (LN) involvement (185)	no involved LN (132)	0.23	0.35	0.30	0.18	0.36
	1-3 involved LN (30)	0.28	0.37	0.27	0.21	0.27
	> 3 involved LN (23)	0.38	0.45	0.37	0.38	0.44
	p-value (Kruskal Wallis Test)	0.235	0.199	0.495	0.017	0.504
Grading (187)	Grade 1 (35)	0.23	0.37	0.29	0.20	0.33
	Grade 2 (114)	0.25	0.35	0.31	0.18	0.35
	Grade 3 (38)	0.38	0.43	0.34	0.28	0.43
	p-value (Kruskal Wallis Test)	0.204	0.363	0.370	0.209	0.814
ER status (185)	ER negative (23)	0.38	0.42	0.36	0.27	0.42
	ER positive (162)	0.25	0.37	0.30	0.19	0.36
	p-value (Mann-Whitney U)	0.388	0.454	0.668	0.511	0.985
PR status (186)	PR negative (38)	0.32	0.43	0.34	0.28	0.43
	PR positive (148)	0.25	0.37	0.30	0.18	0.35
	p-value (Mann-Whitney U)	0.247	0.200	0.315	0.106	0.452
Her2 status (185)	Her2 negative (166)	0.25	0.37	0.30	0.19	0.36
	Her2 positive (19)	0.26	0.39	0.33	0.27	0.35
	p-value (Mann-Whitney U)	0.987	0.989	0.858	0.858	0.449
Three receptor status (185)	Tripple negative (15)	0.38	0.43	0.40	0.30	0.44
	Others (170)	0.25	0.37	0.30	0.19	0.36
	p-value (Mann-Whitney U)	0.271	0.295	0.306	0.365	0.682
Menopause status (180)	premenopause (56)	0.18	0.27	0.25	0.16	0.32
	postmenopause (124)	0.28	0.40	0.32	0.20	0.40
	p-value (Mann-Whitney U)	0.283	0.054	0.138	0.169	0.314
BC family history (186)	with BC family history (30)	0.20	0.33	0.28	0.18	0.43
	without BC family history (15(	0.28	0.39	0.32	0.21	0.36
	p-value (Mann-Whitney U)	0.222	0.414	0.522	0.212	0.353

Fig. 5 (continued)

Table 5. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from second validation round) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels						
		RAPSN_ CpG_1	RAPSN_ CpG_2	RAPSN_ CpG_4	RAPSN_ CpG_5	RAPSN_ CpG_6	RAPSN_ CpG_7	RAPSN_ CpG_8
Tumour stage (188)	Stage 0&I (101)	0.96	0.67	0.39	0.84	0.58	0.79	0.97
	Stage II (61)	0.95	0.71	0.36	0.78	0.64	0.79	0.98
	Stage III (26)	0.96	0.62	0.26	0.74	0.45	0.76	0.97
	p-value (Kruskal Wallis Test)	0.600	0.882	0.413	0.198	0.339	0.770	0.032
Tumour size (188)	Tis&T1 (119)	0.96	0.66	0.39	0.84	0.58	0.80	0.97
	T2 (57)	0.96	0.67	0.36	0.79	0.54	0.78	0.97
	T3 and T4 (12)	0.94	0.78	0.26	0.72	0.49	0.73	0.97
	p-value (Kruskal Wallis Test)	0.145	0.616	0.711	0.195	0.845	0.940	0.260
Lymph node (LN) involvement (185)	no involved LN (132)	0.96	0.69	0.39	0.84	0.60	0.79	0.97
	1-3 involved LN (30)	0.97	0.67	0.34	0.71	0.56	0.78	0.97
	> 3 involved LN (23)	0.95	0.58	0.26	0.73	0.50	0.76	0.97
	p-value (Kruskal Wallis Test)	0.992	0.628	0.544	0.212	0.771	0.961	0.727
Grading (187)	Grade 1 (35)	0.96	0.67	0.39	0.84	0.62	0.88	0.97
	Grade 2 (114)	0.96	0.74	0.36	0.80	0.57	0.77	0.97
	Grade 3 (38)	0.96	0.59	0.36	0.81	0.49	0.78	0.97
	p-value (Kruskal Wallis Test)	0.904	0.160	0.970	0.678	0.625	0.652	0.330
ER status (185)	ER negative (23)	0.95	0.63	0.36	0.82	0.50	0.78	0.97
	ER positive (162)	0.96	0.68	0.36	0.80	0.58	0.79	0.97
	p-value (Mann-Whitney U)	0.366	0.739	0.793	0.700	0.482	0.686	0.673
PR status (186)	PR negative (38)	0.94	0.60	0.36	0.85	0.60	0.80	0.97
	PR positive (148)	0.96	0.70	0.36	0.80	0.57	0.79	0.97
	p-value (Mann-Whitney U)	0.018	0.197	0.603	0.345	0.777	0.638	0.600
Her2 status (185)	Her2 negative (166)	0.96	0.67	0.36	0.80	0.57	0.79	0.97
	Her2 positive (19)	0.94	0.75	0.44	0.80	0.53	0.70	0.97
	p-value (Mann-Whitney U)	0.078	0.443	0.222	0.736	0.957	0.116	0.097
Three receptor status (185)	Tripple negative (15)	0.95	0.59	0.35	0.82	0.50	0.76	0.97
	Others (170)	0.96	0.68	0.36	0.80	0.58	0.79	0.97
	p-value (Mann-Whitney U)	0.599	0.323	0.362	0.726	0.213	0.882	0.755
Menopause status (180)	premenopause (56)	0.96	0.68	0.39	0.83	0.60	0.79	0.97
	postmenopause (124)	0.96	0.67	0.36	0.80	0.52	0.79	0.97
	p-value (Mann-Whitney U)	0.775	0.629	0.843	0.789	0.572	0.832	0.260
BC family history (186)	with BC family history (30)	0.96	0.71	0.38	0.76	0.62	0.72	0.97
	without BC family history (15(	0.96	0.67	0.36	0.82	0.57	0.81	0.97
	p-value (Mann-Whitney U)	0.809	0.721	0.657	0.372	0.749	0.305	0.586

Fig. 6

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study)

Clinical characteristics (N)	Group (N)	Median of age	Median of methylation levels					
			HYAL2_ CpG_1	HYAL2_ CpG_2	HYAL2_ CpG_3	HYAL2_ CpG_4	DYRK4_ CpG_1	DYRK4_ CpG_3
Tumour stage (143)	Stage 0&I (57)	46.22	0.24	0.16	0.27	0.45	0.20	0.18
	Stage II (68)	46.57	0.26	0.16	0.30	0.48	0.22	0.18
	Stage III&IV (18)	47.54	0.25	0.19	0.32	0.49	0.23	0.21
	p-value (Kruskal Wallis Test)	0.271	0.026	0.141	0.062	0.365	0.647	0.582
Tumour size (147)	Tis&T1 (74)	46.31	0.25	0.16	0.28	0.46	0.20	0.18
	T2 (60)	47.12	0.25	0.17	0.30	0.48	0.24	0.19
	T3 and T4 (13)	44.67	0.24	0.16	0.30	0.48	0.22	0.20
	p-value (Kruskal Wallis Test)	0.446	0.416	0.728	0.312	0.543	0.424	0.888
Lymph node (LN) involvement (139)	no involved LN (96)	46.54	0.24	0.16	0.27	0.45	0.22	0.18
	1-3 involved LN (37)	45.98	0.25	0.17	0.30	0.49	0.22	0.18
	> 3 involved LN (6)	52.38	0.25	0.19	0.31	0.47	0.34	0.31
	p-value (Kruskal Wallis Test)	0.171	0.180	0.717	0.158	0.364	0.492	0.127
Grading (187)	Grade 1 (20)	46.96	0.24	0.16	0.27	0.48	0.20	0.18
	Grade 2 (94)	46.87	0.25	0.17	0.30	0.48	0.23	0.19
	Grade 3 (33)	45.27	0.24	0.16	0.30	0.45	0.25	0.19
	p-value (Kruskal Wallis Test)	0.061	0.801	0.807	0.390	0.195	0.622	0.915
ER status (147)	ER negative (23)	46.19	0.23	0.16	0.27	0.44	0.19	0.19
	ER positive (125)	46.71	0.25	0.16	0.29	0.48	0.24	0.18
	p-value (Mann-Whitney U)	0.184	0.377	0.540	0.220	0.042	0.656	0.815
PR status (148)	PR negative (32)	45.32	0.23	0.15	0.28	0.43	0.23	0.20
	PR positive (116)	46.77	0.25	0.17	0.29	0.48	0.22	0.18
	p-value (Mann-Whitney U)	0.036	0.178	0.276	0.183	0.003	0.580	0.867
Her2 status (148)	Her2 negative (114)	46.64	0.25	0.16	0.28	0.46	0.22	0.19
	Her2 positive (34)	46.25	0.24	0.17	0.30	0.48	0.24	0.18
	p-value (Mann-Whitney U)	0.626	0.805	0.689	0.310	0.586	0.675	0.731
Three receptor status (148)	Tripple negative (17)	44.76	0.25	0.16	0.25	0.41	0.17	0.18
	Others (131)	46.72	0.25	0.16	0.29	0.48	0.23	0.19
	p-value (Mann-Whitney U)	0.052	0.486	0.332	0.082	0.010	0.308	0.658
Menopause status (148)	premenopause (105)	45.47	0.24	0.16	0.28	0.46	0.22	0.18
	postmenopause (43)	55.93	0.26	0.19	0.30	0.49	0.23	0.22
	p-value (Mann-Whitney U)	—	0.043	0.004	0.093	0.012	0.274	0.145

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels				
		S100P_CpG_2.3	S100P_CpG_4	S100P_CpG_7	S100P_CpG_8	S100P_CpG_9
Tumour stage (143)	Stage 0&I (57)	0.65	0.89	0.45	0.44	0.51
	Stage II (68)	0.69	0.90	0.48	0.50	0.54
	Stage III&IV (18)	0.67	0.88	0.52	0.47	0.57
	p-value (Kruskal Wallis Test)	0.145	0.910	0.049	0.179	0.129
Tumour size (147)	Tis&T1 (74)	0.66	0.90	0.45	0.46	0.51
	T2 (60)	0.67	0.87	0.48	0.46	0.53
	T3 and T4 (13)	0.68	0.92	0.52	0.50	0.56
	p-value (Kruskal Wallis Test)	0.702	0.460	0.078	0.785	0.597
Lymph node (LN) involvement (139)	no involved LN (96)	0.67	0.89	0.46	0.45	0.51
	1-3 involved LN (37)	0.67	0.90	0.50	0.47	0.54
	> 3 involved LN (6)	0.66	0.87	0.49	0.47	0.54
	p-value (Kruskal Wallis Test)	0.896	0.953	0.683	0.937	0.794
Grading (187)	Grade 1 (20)	0.63	0.91	0.45	0.42	0.49
	Grade 2 (94)	0.68	0.88	0.48	0.48	0.54
	Grade 3 (33)	0.66	0.91	0.49	0.46	0.51
	p-value (Kruskal Wallis Test)	0.234	0.807	0.676	0.368	0.294
ER status (147)	ER negative (23)	0.65	0.94	0.45	0.43	0.49
	ER positive (125)	0.67	0.89	0.48	0.47	0.52
	p-value (Mann-Whitney U)	0.268	0.270	0.361	0.129	0.070
PR status (148)	PR negative (32)	0.66	0.92	0.47	0.44	0.51
	PR positive (116)	0.67	0.89	0.48	0.47	0.52
	p-value (Mann-Whitney U)	0.707	0.177	0.993	0.525	0.296
Her2 status (148)	Her2 negative (114)	0.66	0.89	0.47	0.45	0.51
	Her2 positive (34)	0.69	0.90	0.50	0.51	0.55
	p-value (Mann-Whitney U)	0.169	0.534	0.549	0.118	0.188
Three receptor status (148)	Tripple negative (17)	0.65	0.91	0.46	0.43	0.49
	Others (131)	0.67	0.89	0.48	0.47	0.52
	p-value (Mann-Whitney U)	0.584	0.671	0.854	0.257	0.187
Menopause status (148)	premenopause (105)	0.67	0.91	0.46	0.46	0.51
	postmenopause (43)	0.66	0.85	0.50	0.46	0.54
	p-value (Mann-Whitney U)	0.666	0.189	0.037	0.980	0.597

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		FUT7_CpG_2	FUT7_CpG_3	FUT7_CpG_4	FUT7_CpG_6	FUT7_CpG_7	FUT7_CpG_8
Tumour stage (143)	Stage 0&I (57)	0.19	0.15	0.20	0.24	0.09	0.30
	Stage II (68)	0.21	0.14	0.19	0.22	0.10	0.35
	Stage III&IV (18)	0.22	0.13	0.22	0.23	0.10	0.25
	p-value (Kruskal Wallis Test)	0.895	0.805	0.346	0.956	0.815	0.420
Tumour size (147)	Tis&T1 (74)	0.21	0.15	0.19	0.24	0.09	0.30
	T2 (60)	0.20	0.13	0.20	0.22	0.10	0.32
	T3 and T4 (13)	0.22	0.17	0.22	0.20	0.11	0.31
	p-value (Kruskal Wallis Test)	0.917	0.427	0.340	0.975	0.616	0.695
Lymph node (LN) involvement (139)	no involved LN (96)	0.22	0.14	0.20	0.24	0.09	0.31
	1-3 involved LN (37)	0.20	0.14	0.19	0.22	0.09	0.34
	> 3 involved LN (6)	0.28	0.22	0.31	0.28	0.16	0.27
	p-value (Kruskal Wallis Test)	0.173	0.076	0.239	0.325	0.107	0.976
Grading (187)	Grade 1 (20)	0.16	0.13	0.18	0.20	0.06	0.26
	Grade 2 (94)	0.22	0.14	0.21	0.25	0.10	0.32
	Grade 3 (33)	0.18	0.14	0.18	0.20	0.10	0.31
	p-value (Kruskal Wallis Test)	0.150	0.574	0.327	0.338	0.243	0.098
ER status (147)	ER negative (23)	0.20	0.12	0.17	0.19	0.10	0.32
	ER positive (125)	0.21	0.14	0.20	0.24	0.09	0.31
	p-value (Mann-Whitney U)	0.793	0.562	0.208	0.204	0.793	0.564
PR status (148)	PR negative (32)	0.23	0.16	0.19	0.23	0.11	0.33
	PR positive (116)	0.21	0.14	0.20	0.23	0.09	0.30
	p-value (Mann-Whitney U)	0.519	0.348	0.744	0.578	0.260	0.285
Her2 status (148)	Her2 negative (114)	0.19	0.13	0.20	0.22	0.09	0.30
	Her2 positive (34)	0.26	0.18	0.19	0.27	0.12	0.36
	p-value (Mann-Whitney U)	0.012	0.019	0.495	0.028	0.157	0.172
Three receptor status (148)	Tripple negative (17)	0.18	0.11	0.17	0.17	0.09	0.26
	Others (131)	0.21	0.14	0.20	0.24	0.09	0.31
	p-value (Mann-Whitney U)	0.327	0.206	0.077	0.042	0.800	0.205
Menopause status (148)	premenopause (105)	0.21	0.14	0.20	0.23	0.09	0.31
	postmenopause (43)	0.17	0.14	0.19	0.22	0.11	0.31
	p-value (Mann-Whitney U)	0.167	0.666	0.719	0.944	0.319	0.751

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels			
		SLC22A18_CpG_3	SLC22A18_CpG_4	SLC22A18_CpG_6	SLC22A18_CpG_8
Tumour stage (143)	Stage 0&I (57)	0.13	0.15	0.19	0.61
	Stage II (68)	0.15	0.18	0.22	0.63
	Stage III&IV (18)	0.15	0.20	0.21	0.56
	p-value (Kruskal Wallis Test)	0.183	0.212	0.293	0.284
Tumour size (147)	Tis&T1 (74)	0.14	0.16	0.20	0.61
	T2 (60)	0.15	0.18	0.22	0.63
	T3 and T4 (13)	0.14	0.16	0.18	0.56
	p-value (Kruskal Wallis Test)	0.276	0.479	0.138	0.308
Lymph node (LN) involvement (139)	no involved LN (96)	0.14	0.16	0.20	0.61
	1-3 involved LN (37)	0.14	0.20	0.21	0.63
	> 3 involved LN (6)	0.16	0.20	0.21	0.54
	p-value (Kruskal Wallis Test)	0.491	0.116	0.550	0.115
Grading (187)	Grade 1 (20)	0.14	0.15	0.19	0.66
	Grade 2 (94)	0.14	0.18	0.21	0.61
	Grade 3 (33)	0.14	0.17	0.21	0.61
	p-value (Kruskal Wallis Test)	0.904	0.683	0.848	0.199
ER status (147)	ER negative (23)	0.14	0.16	0.20	0.60
	ER positive (125)	0.14	0.17	0.21	0.61
	p-value (Mann-Whitney U)	0.202	0.240	0.408	0.153
PR status (148)	PR negative (32)	0.14	0.17	0.20	0.60
	PR positive (116)	0.14	0.17	0.21	0.61
	p-value (Mann-Whitney U)	0.562	0.503	0.439	0.250
Her2 status (148)	Her2 negative (114)	0.14	0.17	0.21	0.61
	Her2 positive (34)	0.15	0.20	0.23	0.62
	p-value (Mann-Whitney U)	0.907	0.521	0.430	0.830
Three receptor status (148)	Tripple negative (17)	0.12	0.16	0.19	0.60
	Others (131)	0.14	0.17	0.21	0.61
	p-value (Mann-Whitney U)	0.274	0.391	0.421	0.218
Menopause status (148)	premenopause (105)	0.14	0.16	0.19	0.62
	postmenopause (43)	0.17	0.22	0.23	0.59
	p-value (Mann-Whitney U)	0.002	0.001	0.003	0.112

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		RPTOR_ CpG_1	RPTOR_ CpG_2	RPTOR_ CpG_3	RPTOR_ CpG_4	RPTOR_ CpG_5	RPTOR_ CpG_8
Tumour stage (143)	Stage 0&I (57)	0.05	0.18	0.59	0.77	0.73	0.67
	Stage II (68)	0.07	0.19	0.62	0.81	0.72	0.67
	Stage III&IV (18)	0.08	0.24	0.63	0.79	0.77	0.72
	p-value (Kruskal Wallis Test)	0.328	0.152	0.191	0.847	0.527	0.514
Tumour size (147)	Tis&T1 (74)	0.05	0.18	0.59	0.77	0.72	0.67
	T2 (60)	0.08	0.21	0.62	0.77	0.73	0.69
	T3 and T4 (13)	0.09	0.29	0.66	0.83	0.78	0.67
	p-value (Kruskal Wallis Test)	0.165	0.197	0.130	0.748	0.614	0.232
Lymph node (LN) involvement (139)	no involved LN (96)	0.07	0.20	0.60	0.77	0.74	0.68
	1-3 involved LN (37)	0.06	0.19	0.59	0.73	0.70	0.63
	> 3 involved LN (6)	0.06	0.23	0.62	0.76	0.80	0.73
	p-value (Kruskal Wallis Test)	0.955	0.177	0.374	0.487	0.080	0.008
Grading (187)	Grade 1 (20)	0.07	0.18	0.60	0.78	0.73	0.68
	Grade 2 (94)	0.07	0.20	0.60	0.77	0.74	0.68
	Grade 3 (33)	0.06	0.19	0.62	0.79	0.71	0.68
	p-value (Kruskal Wallis Test)	0.669	0.426	0.456	0.868	0.719	0.987
ER status (147)	ER negative (23)	0.04	0.19	0.59	0.75	0.71	0.69
	ER positive (125)	0.07	0.20	0.60	0.77	0.74	0.67
	p-value (Mann-Whitney U)	0.139	0.449	0.628	0.694	0.525	0.757
PR status (148)	PR negative (32)	0.06	0.19	0.61	0.79	0.72	0.69
	PR positive (116)	0.07	0.20	0.60	0.77	0.73	0.67
	p-value (Mann-Whitney U)	0.267	0.755	0.492	0.812	0.812	0.416
Her2 status (148)	Her2 negative (114)	0.07	0.19	0.60	0.75	0.72	0.68
	Her2 positive (34)	0.09	0.23	0.64	0.86	0.78	0.68
	p-value (Mann-Whitney U)	0.463	0.065	0.090	0.002	0.036	0.862
Three receptor status (148)	Tripple negative (17)	0.04	0.17	0.59	0.75	0.69	0.69
	Others (131)	0.07	0.20	0.60	0.77	0.74	0.68
	p-value (Mann-Whitney U)	0.102	0.178	0.496	0.564	0.068	0.817
Menopause status (148)	premenopause (105)	0.07	0.19	0.59	0.79	0.72	0.67
	postmenopause (43)	0.06	0.20	0.62	0.75	0.77	0.68
	p-value (Mann-Whitney U)	0.687	0.463	0.202	0.090	0.099	0.078

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		MGRN1_ CpG_1	MGRN1_ CpG_2	MGRN1_ CpG_4	MGRN1_CpG _5.6.7.8	MGRN1_ CpG_12	MGRN1_ CpG_13
Tumour stage (143)	Stage 0&I (57)	0.12	0.60	0.23	0.23	0.26	0.60
	Stage II (68)	0.14	0.62	0.27	0.29	0.35	0.62
	Stage III&IV (18)	0.21	0.63	0.31	0.34	0.37	0.63
	p-value (Kruskal Wallis Test)	0.136	0.057	0.002	0.002	0.001	0.050
Tumour size (147)	Tis&T1 (74)	0.14	0.59	0.25	0.26	0.30	0.59
	T2 (60)	0.12	0.61	0.26	0.28	0.32	0.61
	T3 and T4 (13)	0.29	0.69	0.38	0.36	0.46	0.69
	p-value (Kruskal Wallis Test)	0.005	0.003	0.002	0.004	0.001	0.003
Lymph node (LN) involvement (139)	no involved LN (96)	0.13	0.61	0.25	0.27	0.30	0.61
	1-3 involved LN (37)	0.14	0.61	0.29	0.29	0.34	0.61
	> 3 involved LN (6)	0.13	0.60	0.28	0.33	0.36	0.60
	p-value (Kruskal Wallis Test)	0.949	0.622	0.171	0.336	0.235	0.710
Grading (187)	Grade 1 (20)	0.10	0.61	0.25	0.24	0.28	0.61
	Grade 2 (94)	0.14	0.61	0.26	0.29	0.33	0.61
	Grade 3 (33)	0.12	0.62	0.26	0.27	0.31	0.62
	p-value (Kruskal Wallis Test)	0.296	0.815	0.209	0.255	0.350	0.907
ER status (147)	ER negative (23)	0.12	0.62	0.26	0.27	0.30	0.62
	ER positive (125)	0.14	0.61	0.25	0.28	0.33	0.61
	p-value (Mann-Whitney U)	0.187	0.739	0.470	0.331	0.577	0.782
PR status (148)	PR negative (32)	0.12	0.62	0.25	0.28	0.31	0.63
	PR positive (116)	0.14	0.61	0.26	0.28	0.33	0.61
	p-value (Mann-Whitney U)	0.318	0.815	0.365	0.381	0.666	0.789
Her2 status (148)	Her2 negative (114)	0.14	0.61	0.26	0.28	0.33	0.61
	Her2 positive (34)	0.14	0.61	0.25	0.29	0.32	0.61
	p-value (Mann-Whitney U)	0.306	0.950	0.535	0.612	0.909	0.669
Three receptor status (148)	Tripple negative (17)	0.13	0.62	0.27	0.28	0.34	0.62
	Others (131)	0.14	0.61	0.25	0.28	0.33	0.61
	p-value (Mann-Whitney U)	0.449	0.958	0.851	0.595	0.971	0.885
Menopause status (148)	premenopause (105)	0.14	0.61	0.25	0.27	0.32	0.61
	postmenopause (43)	0.15	0.62	0.27	0.29	0.33	0.64
	p-value (Mann-Whitney U)	0.541	0.259	0.190	0.324	0.373	0.127

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels					
		MGRN1_CpG_15	MGRN1_CpG_16.17.18	MGRN1_CpG_19.20	MGRN1_CpG_22.23	MGRN1_CpG_26	MGRN1_CpG_27
Tumour stage (143)	Stage 0&I (57)	0.29	0.29	0.32	0.29	0.28	0.26
	Stage II (68)	0.34	0.31	0.37	0.34	0.32	0.32
	Stage III&IV (18)	0.39	0.36	0.42	0.39	0.36	0.38
	p-value (Kruskal Wallis Test)	0.003	0.001	0.002	0.001	0.007	0.003
Tumour size (147)	Tis&T1 (74)	0.31	0.29	0.34	0.31	0.31	0.29
	T2 (60)	0.33	0.31	0.36	0.33	0.31	0.29
	T3 and T4 (13)	0.43	0.41	0.47	0.44	0.40	0.43
	p-value (Kruskal Wallis Test)	0.002	0.000	0.003	0.000	0.005	0.001
Lymph node (LN) involvement (139)	no involved LN (96)	0.31	0.30	0.35	0.31	0.31	0.28
	1-3 involved LN (37)	0.34	0.33	0.38	0.34	0.33	0.31
	> 3 involved LN (6)	0.38	0.36	0.38	0.34	0.33	0.33
	p-value (Kruskal Wallis Test)	0.070	0.179	0.191	0.276	0.494	0.258
Grading (187)	Grade 1 (20)	0.31	0.29	0.33	0.31	0.31	0.25
	Grade 2 (94)	0.34	0.32	0.37	0.34	0.31	0.30
	Grade 3 (33)	0.32	0.31	0.36	0.32	0.33	0.31
	p-value (Kruskal Wallis Test)	0.581	0.364	0.329	0.422	0.781	0.661
ER status (147)	ER negative (23)	0.32	0.31	0.36	0.32	0.31	0.31
	ER positive (125)	0.33	0.31	0.36	0.33	0.32	0.30
	p-value (Mann-Whitney U)	0.689	0.727	0.245	0.530	0.288	0.991
PR status (148)	PR negative (32)	0.33	0.31	0.36	0.33	0.31	0.32
	PR positive (116)	0.32	0.31	0.36	0.33	0.32	0.30
	p-value (Mann-Whitney U)	0.795	0.708	0.200	0.600	0.246	0.960
Her2 status (148)	Her2 negative (114)	0.33	0.31	0.36	0.33	0.31	0.29
	Her2 positive (34)	0.31	0.31	0.36	0.32	0.31	0.32
	p-value (Mann-Whitney U)	0.929	0.686	0.896	0.957	0.892	0.425
Three receptor status (148)	Tripple negative (17)	0.33	0.31	0.37	0.32	0.31	0.34
	Others (131)	0.32	0.31	0.36	0.33	0.32	0.30
	p-value (Mann-Whitney U)	0.810	0.801	0.576	0.763	0.381	0.949
Menopause status (148)	premenopause (105)	0.32	0.30	0.36	0.32	0.31	0.30
	postmenopause (43)	0.34	0.33	0.36	0.33	0.33	0.32
	p-value (Mann-Whitney U)	0.563	0.202	0.423	0.483	0.279	0.206

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels				
		MGRN1_CpG_28	MGRN1_CpG_29	MGRN1_CpG_31	MGRN1_CpG_32	MGRN1_CpG_34
Tumour stage (143)	Stage 0&I (57)	0.26	0.35	0.29	0.24	0.33
	Stage II (68)	0.32	0.40	0.36	0.28	0.39
	Stage III&IV (18)	0.33	0.46	0.40	0.34	0.41
	p-value (Kruskal Wallis Test)	0.006	0.001	0.008	0.007	0.005
Tumour size (147)	Tis&T1 (74)	0.28	0.37	0.32	0.26	0.35
	T2 (60)	0.31	0.39	0.31	0.27	0.38
	T3 and T4 (13)	0.41	0.49	0.45	0.40	0.42
	p-value (Kruskal Wallis Test)	0.007	0.001	0.004	0.004	0.123
Lymph node (LN) involvement (139)	no involved LN (96)	0.28	0.37	0.30	0.27	0.35
	1-3 involved LN (37)	0.31	0.40	0.37	0.27	0.39
	> 3 involved LN (6)	0.33	0.41	0.40	0.31	0.37
	p-value (Kruskal Wallis Test)	0.466	0.374	0.097	0.489	0.122
Grading (187)	Grade 1 (20)	0.26	0.35	0.29	0.24	0.34
	Grade 2 (94)	0.31	0.39	0.35	0.27	0.37
	Grade 3 (33)	0.29	0.38	0.29	0.28	0.35
	p-value (Kruskal Wallis Test)	0.254	0.319	0.468	0.702	0.422
ER status (147)	ER negative (23)	0.28	0.39	0.33	0.27	0.35
	ER positive (125)	0.31	0.38	0.34	0.27	0.37
	p-value (Mann-Whitney U)	0.537	0.835	0.933	0.976	0.254
PR status (148)	PR negative (32)	0.29	0.39	0.36	0.27	0.35
	PR positive (116)	0.30	0.38	0.33	0.27	0.37
	p-value (Mann-Whitney U)	0.675	0.656	0.788	0.797	0.240
Her2 status (148)	Her2 negative (114)	0.30	0.38	0.33	0.27	0.37
	Her2 positive (34)	0.30	0.38	0.35	0.26	0.37
	p-value (Mann-Whitney U)	0.619	0.674	0.678	0.910	0.700
Three receptor status (148)	Tripple negative (17)	0.31	0.40	0.33	0.28	0.36
	Others (131)	0.30	0.38	0.34	0.27	0.37
	p-value (Mann-Whitney U)	0.825	0.990	0.858	0.884	0.777
Menopause status (148)	premenopause (105)	0.29	0.38	0.33	0.26	0.36
	postmenopause (43)	0.31	0.38	0.36	0.29	0.39
	p-value (Mann-Whitney U)	0.502	0.594	0.652	0.131	0.172

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels						
		RAPSN _CpG_1	RAPSN _CpG_2	RAPSN _CpG_4	RAPSN _CpG_5	RAPSN _CpG_6	RAPSN _CpG_7	RAPSN _CpG_8
Tumour stage (143)	Stage 0&I (57)	0.95	0.61	0.38	0.73	0.49	0.73	0.95
	Stage II (68)	0.94	0.63	0.42	0.77	0.56	0.70	0.96
	Stage III&IV (18)	0.95	0.63	0.41	0.75	0.56	0.74	0.96
	p-value (Kruskal Wallis Test)	0.646	0.757	0.370	0.037	0.007	0.441	0.688
Tumour size (147)	Tis&T1 (74)	0.94	0.61	0.38	0.73	0.51	0.73	0.96
	T2 (60)	0.95	0.62	0.41	0.78	0.54	0.70	0.96
	T3 and T4 (13)	0.97	0.64	0.53	0.71	0.65	0.73	0.96
	p-value (Kruskal Wallis Test)	0.330	0.976	0.044	0.089	0.081	0.413	0.669
Lymph node (LN) involvement (139)	no involved LN (96)	0.95	0.62	0.40	0.74	0.51	0.73	0.96
	1-3 involved LN (37)	0.94	0.62	0.39	0.76	0.55	0.69	0.96
	> 3 involved LN (6)	0.97	0.59	0.41	0.75	0.59	0.77	0.98
	p-value (Kruskal Wallis Test)	0.501	0.729	0.880	0.619	0.206	0.051	0.493
Grading (187)	Grade 1 (20)	0.97	0.61	0.41	0.73	0.52	0.75	0.95
	Grade 2 (94)	0.94	0.62	0.41	0.75	0.53	0.71	0.96
	Grade 3 (33)	0.94	0.64	0.37	0.75	0.53	0.70	0.97
	p-value (Kruskal Wallis Test)	0.264	0.767	0.453	0.827	0.796	0.585	0.082
ER status (147)	ER negative (23)	0.94	0.64	0.35	0.73	0.47	0.71	0.96
	ER positive (125)	0.95	0.62	0.41	0.75	0.53	0.72	0.96
	p-value (Mann-Whitney U)	0.794	0.727	0.043	0.520	0.090	0.711	0.917
PR status (148)	PR negative (32)	0.93	0.66	0.38	0.74	0.49	0.73	0.96
	PR positive (116)	0.95	0.62	0.41	0.75	0.53	0.71	0.96
	p-value (Mann-Whitney U)	0.137	0.773	0.217	0.818	0.389	0.510	0.891
Her2 status (148)	Her2 negative (114)	0.95	0.62	0.39	0.75	0.53	0.72	0.96
	Her2 positive (34)	0.93	0.64	0.42	0.75	0.53	0.72	0.95
	p-value (Mann-Whitney U)	0.001	0.608	0.553	0.544	0.583	0.798	0.374
Three receptor status (148)	Tripple negative (17)	0.94	0.65	0.35	0.75	0.47	0.72	0.96
	Others (131)	0.95	0.62	0.41	0.75	0.53	0.71	0.96
	p-value (Mann-Whitney U)	0.990	0.888	0.041	0.734	0.214	0.520	0.281
Menopause status (148)	premenopause (105)	0.94	0.62	0.38	0.75	0.53	0.71	0.96
	postmenopause (43)	0.95	0.62	0.43	0.75	0.54	0.74	0.96
	p-value (Mann-Whitney U)	0.540	0.788	0.016	0.891	0.418	0.234	0.633

Fig. 6 (continued)

Table 6. The methylation leveles of the eight genes in sporadic BC patients with different clinical characteristics (cases from the third validation study) (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels						
		miR1273p	miR148b	miR376a	miR376c	miR4093p	miR652	miR801
Tumour stage (143)	Stage 0&I (57)	32.97	31.28	35.51	33.17	32.54	29.69	30.33
	Stage II (68)	32.86	31.11	35.28	33.11	32.67	29.48	30.40
	Stage III&IV (18)	32.92	31.23	35.07	33.17	32.74	29.71	30.17
	p-value (Kruskal Wallis Test)	0.996	0.571	0.578	0.853	0.736	0.268	0.611
Tumour size (147)	Tis&T1 (74)	32.88	31.26	35.34	33.07	32.57	29.66	30.32
	T2 (60)	32.93	31.15	35.19	33.13	32.76	29.55	30.43
	T3 and T4 (13)	32.99	31.23	35.34	33.50	32.74	29.64	30.18
	p-value (Kruskal Wallis Test)	0.821	0.907	0.604	0.569	0.578	0.503	0.720
Lymph node (LN) involvement (139)	no involved LN (96)	32.74	31.28	35.35	33.06	32.62	29.64	30.26
	1-3 involved LN (37)	33.13	31.16	35.34	33.24	32.86	29.56	30.46
	> 3 involved LN (6)	32.37	31.05	34.67	32.71	32.31	29.09	30.36
	p-value (Kruskal Wallis Test)	0.570	0.827	0.308	0.510	0.791	0.557	0.730
Grading (187)	Grade 1 (20)	32.84	31.43	35.54	33.37	32.30	29.62	30.75
	Grade 2 (94)	33.03	31.22	35.32	33.17	32.72	29.64	30.40
	Grade 3 (33)	32.56	31.10	35.08	32.78	32.35	29.51	30.14
	p-value (Kruskal Wallis Test)	0.766	0.402	0.988	0.883	0.443	0.607	0.191
ER status (147)	ER negative (23)	32.65	31.36	35.07	32.98	32.33	29.80	30.25
	ER positive (125)	32.98	31.22	35.35	33.18	32.71	29.59	30.39
	p-value (Mann-Whitney U)	0.657	0.973	0.316	0.706	0.325	0.897	0.460
PR status (148)	PR negative (32)	32.74	31.36	35.08	33.18	32.41	29.83	30.25
	PR positive (116)	32.97	31.22	35.34	33.17	32.69	29.57	30.39
	p-value (Mann-Whitney U)	0.955	0.732	0.536	0.990	0.788	0.496	0.313
Her2 status (148)	Her2 negative (114)	32.96	31.23	35.32	33.19	32.70	29.60	30.29
	Her2 positive (34)	32.82	31.24	35.34	33.07	32.62	29.64	30.58
	p-value (Mann-Whitney U)	0.528	0.816	0.698	0.693	0.889	0.643	0.276
Three receptor status (148)	Tripple negative (17)	32.65	31.22	34.86	33.03	31.89	29.88	30.25
	Others (131)	32.97	31.23	35.34	33.18	32.71	29.59	30.39
	p-value (Mann-Whitney U)	0.831	0.814	0.334	0.814	0.292	0.736	0.409
Menopause status (148)	premenopause (105)	32.81	31.32	35.32	33.19	32.56	29.72	30.45
	postmenopause (43)	32.99	31.10	35.36	33.16	33.03	29.52	30.14
	p-value (Mann-Whitney U)	0.776	0.063	0.792	0.711	0.205	0.331	0.024

Fig. 7

Table 7. Sample Description of blood-based biomarker panel for the early detection of pancreatic cancer

Cohort	Sample types	Assays	Groups	Target N	Mean of age (range)	Median of age	Assayed N (call rate %)				
							HYAL2	S100P	SLC22A18	DYRK4	FUT7
PaCa case and controls	Peripheral blood DNA	MassARRAY	All controls	191	58.7 (21-68)	61	191 (100%)	191 (100%)	190 (99.5%)	191 (100%)	191 (100%)
			All PaCa cases	147	62.9 (19-86)	64	147 (100%)	147 (100%)	147 (100%)	147 (100%)	147 (100%)
			Male controls	115	59.0 (27-67)	58	115 (100%)	115 (100%)	115 (100%)	115 (100%)	115 (100%)
			Male PaCa case	80	62.9 (39-86)	63.5	80 (100%)	80 (100%)	80 (100%)	80 (100%)	80 (100%)
			Femal controls	76	60.0 (21-68)	63	76 (100%)	76 (100%)	75 (98.7%)	76 (100%)	76 (100%)
			Femal PaCa cas	67	62.0 (19-79)	66	67 (100%)	67 (100%)	67 (100%)	67 (100%)	67 (100%)

Fig.8

Table 8. Methylation differences in genes comparing PaCa cases and controls

CpG sites	Differences in methylation levels			
	Controls median (IQR)	PaCa cases median (IQR)	OR (95 % CI) * per -10% methylation	*p*-value *
HYAL2_CpG_1	0.35 (0.31-0.40)	0.25 (0.19-0.32)	2.09 (1.61-2.71)	2.68E-08
HYAL2_CpG_2	0.27 (0.23-0.31)	0.14 (0.09-0.18)	7.06 (4.58-10.88)	8.01E-19
HYAL2_CpG_3	0.43 (0.38-0.46)	0.29 (0.23-0.34)	7.94 (5.04-12.50)	3.94E-19
HYAL2_CpG_4	0.61 (0.56-0.66)	0.48 (0.43-0.55)	4.24 (3.01-5.98)	1.37E-16
S100P_CpG_2&3	0.73 (0.69-0.77)	0.59 (0.54-0.63)	14.07 (8.04-24.61)	1.97E-20
S100P_CpG_4	0.74 (0.65-0.90)	0.62 (0.55-0.82)	1.55 (1.33-1.82)	4.90E-08
S100P_CpG_7	0.64 (0.54-0.73)	0.31 (0.21-0.42)	4.03 (3.01-5.39)	6.51E-21
S100P_CpG_8	0.54 (0.47-0.61)	0.33 (0.25-0.40)	3.85 (2.87-5.16)	2.45E-19
S100P_CpG_9	0.62 (0.56-0.68)	0.43 (0.36-0.50)	5.02 (3.55-7.09)	6.61E-20
SLC22A18_CpG_1	0.34 (0.26-0.39)	0.17 (0.13-0.23)	5.28 (3.60-7.57)	1.17E-19
SLC22A18_CpG_3	0.23 (0.17-0.27)	0.12 (0.09-0.17)	8.81 (5.48-14.18)	3.03E-19
SLC22A18_CpG_4	0.30 (0.23-0.38)	0.13 (0.08-0.20)	4.23 (3.07-5.83)	1.03E-18
SLC22A18_CpG_6	0.30 (0.25-0.36)	0.17 (0.11-0.23)	5.14 (3.59-7.35)	3.98E-19
SLC22A18_CpG_8	0.68 (0.62-0.74)	0.57 (0.49-0.65)	2.13 (1.70-2.68)	7.17E-11
DYRK4_CpG_1	0.59 (0.37-0.75)	0.18 (0.02-0.49)	1.46 (1.33-1.62)	3.85E-14
DYRK4_CpG_3	0.32 (0.25-0.35)	0.32 (0.14-0.35)	1.20 (1.02-1.41)	0.032
FUT7_CpG_1	0.49 (0.36-0.61)	0.21 (0.15-0.36)	1.99 (1.70-2.33)	1.63E-17
FUT7_CpG_2	0.42 (0.37-0.47)	0.27 (0.22-0.33)	6.95 (4.55-10.61)	2.77E-19
FUT7_CpG_3	0.21 (0.11-0.31)	0.06 (0.03-0.16)	2.77 (2.10-3.66)	6.21E-13
FUT7_CpG_4	0.40 (0.33-0.66)	0.26 (0.21-0.35)	2.00 (1.66-2.41)	3.13E-13
FUT7_CpG_6	0.31 (0.22-0.39)	0.14 (0.08-0.22)	2.36 (1.88-2.98)	3.12E-13
FUT7_CpG_7	0.15 (0.09-0.22)	0.05 (0.01-0.10)	3.65 (2.54-5.25)	2.28E-12
FUT7_CpG_8	0.30 (0.20-0.39)	0.15 (0.10-0.25)	2.05 (1.65-2.53)	4.25E-11
All CpG panel				2.57E-26

* logistic regression, adjusted for age and different batches for the measurements

Fig. 8 (continued)

Table 8. Methylation differences in genes comparing PaCa cases and controls (continued)

CpG sites	Early stage (Stage0&1&2) vs All controls			
	Controls median (IQR)	PaCa early cases median (IQR)	OR (95 % CI) * per -10% methylation	*p* -value *
HYAL2_CpG_1	0.35 (0.31-0.40)	0.27 (0.20-0.33)	2.04 (1.46-2.84)	2.93E-05
HYAL2_CpG_2	0.27 (0.23-0.31)	0.14 (0.08-0.18)	6.29 (3.88-10.18)	7.83E-14
HYAL2_CpG_3	0.43 (0.38-0.46)	0.28 (0.23-0.34)	7.37 (4.41-12.29)	2.01E-14
HYAL2_CpG_4	0.61 (0.56-0.66)	0.47 (0.42-0.54)	5.54 (3.54-8.70)	8.71E-14
S100P_CpG_2&3	0.73 (0.69-0.77)	0.59 (0.54-0.63)	15.48 (7.84-30.55)	2.86E-15
S100P_CpG_4	0.74 (0.65-0.90)	0.63 (0.56-0.81)	1.58 (1.29-1.94)	1.12E-05
S100P_CpG_7	0.64 (0.54-0.73)	0.31 (0.21-0.47)	3.77 (2.72-5.23)	1.50E-15
S100P_CpG_8	0.54 (0.47-0.61)	0.34 (0.26-0.40)	4.00 (2.80-5.71)	2.26E-14
S100P_CpG_9	0.62 (0.56-0.68)	0.43 (0.36-0.52)	5.17 (3.43-7.80)	4.15E-15
SLC22A18_CpG_1	0.34 (0.26-0.39)	0.18 (0.14-0.23)	5.48 (3.50-8.56)	8.53E-14
SLC22A18_CpG_3	0.23 (0.17-0.27)	0.11 (0.08-0.15)	13.25 (6.89-25.48)	9.63E-15
SLC22A18_CpG_4	0.30 (0.23-0.38)	0.10 (0.07-0.18)	5.49 (3.56-8.49)	1.64E-14
SLC22A18_CpG_6	0.30 (0.25-0.36)	0.14 (0.11-0.22)	5.47 (3.54-8.47)	2.28E-14
SLC22A18_CpG_8	0.68 (0.62-0.74)	0.57 (0.48-0.65)	2.35 (1.76-3.14)	7.50E-09
DYRK4_CpG_1	0.59 (0.37-0.75)	0.17 (0.02-0.49)	1.50 (1.33-1.70)	2.06E-10
DYRK4_CpG_3	0.32 (0.25-0.35)	0.32 (0.12-0.35)	1.32 (1.05-1.65)	0.019
FUT7_CpG_1	0.49 (0.36-0.61)	0.21 (0.12-0.31)	1.94 (1.61-2.34)	5.49E-12
FUT7_CpG_2	0.42 (0.37-0.47)	0.27 (0.22-0.35)	6.23 (3.87-10.04)	5.65E-14
FUT7_CpG_3	0.21 (0.11-0.31)	0.08 (0.04-0.18)	2.31 (1.69-3.16)	1.29E-07
FUT7_CpG_4	0.40 (0.33-0.66)	0.27 (0.21-0.36)	2.01 (1.59-2.55)	5.86E-09
FUT7_CpG_6	0.31 (0.22-0.39)	0.14 (0.09-0.26)	2.10 (1.61-2.75)	4.41E-08
FUT7_CpG_7	0.15 (0.09-0.22)	0.05 (0.01-0.09)	3.25 (2.14-4.95)	3.68E-08
FUT7_CpG_8	0.30 (0.20-0.39)	0.15 (0.10-0.26)	1.92 (1.50-2.46)	2.74E-07
All CpG panel				1.66E-18

* logistic regression, adjusted for age and different batches for the measurements

Fig. 9

Table 9. Methylation differences in genes comparing PaCa cases and controls stratified by gender

CpG sites	Male samples			
	male controls median (IQR)	male PaCa cases median (IQR)	OR (95 % CI) * per -10% methylation	*p* -value *
HYAL2_CpG_1	0.36 (0.31-0.41)	0.25 (0.20-0.32)	2.43 (1.68-3.53)	2.60E-06
HYAL2_CpG_2	0.28 (0.23-0.31)	0.14 (0.10-0.18)	20.70 (8.69-49.27)	7.56E-12
HYAL2_CpG_3	0.43 (0.39-0.47)	0.28 (0.23-0.34)	9.60 (5.00-18.43)	1.10E-11
HYAL2_CpG_4	0.63 (0.56-0.68)	0.47 (0.42-0.54)	4.95 (3.07-7.98)	5.35E-11
S100P_CpG_2&3	0.74 (0.69-0.77)	0.59 (0.54-0.63)	14.72 (6.98-31.04)	1.59E-12
S100P_CpG_4	0.78 (0.67-0.91)	0.63 (0.56-0.83)	1.63 (1.32-2.02)	6.10E-06
S100P_CpG_7	0.65 (0.59-0.76)	0.34 (0.21-0.43)	5.25 (3.24-8.48)	1.39E-11
S100P_CpG_8	0.56 (0.49-0.63)	0.35 (0.25-0.42)	4.27 (2.81-6.47)	8.94E-12
S100P_CpG_9	0.64 (0.58-0.68)	0.44 (0.36-0.51)	6.56 (3.85-11.19)	4.75E-12
SLC22A18_CpG_1	0.35 (0.27-0.40)	0.17 (0.12-0.25)	4.39 (2.86-6.75)	1.46E-11
SLC22A18_CpG_3	0.23 (0.17-0.27)	0.12 (0.08-0.17)	6.08 (3.52-10.52)	9.95E-11
SLC22A18_CpG_4	0.30 (0.23-0.39)	0.13 (0.09-0.22)	4.56 (2.94-7.08)	1.17E-11
SLC22A18_CpG_6	0.30 (0.25-0.37)	0.17 (0.13-0.26)	4.11 (2.66-6.34)	1.68E-10
SLC22A18_CpG_8	0.68 (0.63-0.76)	0.61 (0.52-0.67)	1.87 (1.41-2.47)	1.21E-05
DYRK4_CpG_1	0.62 (0.39-0.75)	0.24 (0.03-0.56)	1.38 (1.22-1.57)	2.77E-07
DYRK4_CpG_3	0.32 (0.26-0.35)	0.31 (0.09-0.35)	1.21 (0.99-1.49)	0.068
FUT7_CpG_1	0.52 (0.40-0.64)	0.21 (0.15-0.31)	2.14 (1.72-2.67)	8.28E-12
FUT7_CpG_2	0.44 (0.39-0.49)	0.27 (0.22-0.33)	6.44 (3.73-11.12)	2.26E-11
FUT7_CpG_3	0.22 (0.12-0.33)	0.07 (0.04-0.13)	3.15 (2.12-4.70)	1.58E-08
FUT7_CpG_4	0.42 (0.32-0.69)	0.26 (0.21-0.36)	2.12 (1.64-2.74)	1.08E-08
FUT7_CpG_6	0.32 (0.25-0.39)	0.14 (0.08-0.22)	2.27 (1.70-3.04)	3.40E-08
FUT7_CpG_7	0.17 (0.09-0.22)	0.05 (0.01-0.10)	3.32 (2.11-5.20)	1.91E-07
FUT7_CpG_8	0.33 (0.21-0.41)	0.15 (0.09-0.23)	2.21 (1.66-2.93)	5.82E-08
All CpG panel				3.28E-14

* logistic regression, adjusted for age and different batches for the measurements

Fig. 9 (continued)

Table 9. Methylation differences in genes comparing PaCa cases and controls stratified by gender (continued)

CpG sites	Female samples			
	female controls median (IQR)	female PaCa cases median	OR (95 % CI) * per -10% methylation	*p* -value *
HYAL2_CpG_1	0.35 (0.30-0.38)	0.25 (0.19-0.33)	1.76 (1.22-2.54)	0.002
HYAL2_CpG_2	0.25 (0.21-0.30)	0.14 (0.08-0.18)	3.44 (2.09-5.66)	1.00E-06
HYAL2_CpG_3	0.41 (0.37-0.44)	0.30 (0.23-0.34)	6.88 (3.52-13.46)	1.73E-08
HYAL2_CpG_4	0.60 (0.55-0.64)	0.48 (0.43-0.55)	3.41 (2.09-5.58)	1.02E-06
S100P_CpG_2&3	0.72 (0.68-0.75)	0.60 (0.53-0.63)	13.61 (5.72-32.39)	3.55E-09
S100P_CpG_4	0.69 (0.63-0.88)	0.61 (0.55-0.80)	1.45 (1.14-1.84)	0.003
S100P_CpG_7	0.60 (0.51-0.68)	0.29 (0.20-0.37)	3.23 (2.24-4.67)	4.19E-10
S100P_CpG_8	0.51 (0.46-0.58)	0.31 (0.25-0.39)	3.39 (2.23-5.15)	1.14E-08
S100P_CpG_9	0.59 (0.54-0.66)	0.43 (0.35-0.49)	3.84 (2.43-6.06)	8.97E-09
SLC22A18_CpG_1	0.32 (0.24-0.37)	0.17 (0.14-0.21)	8.59 (4.28-17.23)	1.44E-09
SLC22A18_CpG_3	0.22 (0.17-0.26)	0.11 (0.08-0.15)	19.70 (7.63-50.83)	7.22E-10
SLC22A18_CpG_4	0.29 (0.22-0.37)	0.13 (0.06-0.19)	4.24 (2.58-6.98)	1.25E-08
SLC22A18_CpG_6	0.28 (0.25-0.34)	0.14 (0.11-0.21)	8.75 (4.40-17.38)	6.06E-10
SLC22A18_CpG_8	0.65 (0.61-0.71)	0.55 (0.47-0.63)	2.76 (1.83-4.16)	1.32E-06
DYRK4_CpG_1	0.56 (0.35-0.73)	0.16 (0.02-0.37)	1.62 (1.36-1.92)	3.33E-08
DYRK4_CpG_3	0.32 (0.24-0.35)	0.32 (0.23-0.35)	1.20 (0.90-1.59)	0.215
FUT7_CpG_1	0.47 (0.30-0.54)	0.19 (0.09-0.36)	1.82 (1.44-2.30)	5.16E-07
FUT7_CpG_2	0.40 (0.35-0.45)	0.26 (0.22-0.33)	7.99 (4.03-15.86)	2.81E-09
FUT7_CpG_3	0.17 (0.09-0.25)	0.06 (0.03-0.17)	2.37 (1.58-3.53)	2.61E-05
FUT7_CpG_4	0.40 (0.34-0.60)	0.26 (0.19-0.35)	1.89 (1.44-2.48)	4.88E-06
FUT7_CpG_6	0.26 (0.18-0.39)	0.13 (0.08-0.20)	2.50 (1.71-3.68)	2.79E-06
FUT7_CpG_7	0.13 (0.09-0.22)	0.05 (0.02-0.09)	4.17 (2.27-7.68)	4.28E-06
FUT7_CpG_8	0.27 (0.18-0.36)	0.15 (0.10-0.26)	1.86 (1.34-2.57)	1.75E-04
All CpG panel				7.24E-12

* logistic regression, adjusted for age and different batches for the measurements

Fig. 10

Table 10. The discriminatory power of the methylation in genes to distinguish PaCa cases from healthy controls

CpG sites	Area under curve (AUC), 95% CI			
	all PaCa cases vs. all controls	stage 0&I&II PaCa cases vs. all controls	Male, PaCa cases vs. controls	Female, PaCa cases vs. controls
HYAL2_CpG_1	0.77 (0.72-0.83)	0.76 (0.68-0.83)	0.79 (0.72-0.86)	0.75 (0.66-0.83)
HYAL2_CpG_2	0.90 (0.86-0.94)	0.90 (0.85-0.95)	0.94 (0.90-0.97)	0.86 (0.79-0.92)
HYAL2_CpG_3	0.90 (0.86-0.94)	0.90 (0.85-0.94)	0.92 (0.88-0.96)	0.87 (0.81-0.94)
HYAL2_CpG_4	0.86 (0.82-0.90)	0.87 (0.82-0.93)	0.89 (0.83-0.94)	0.83 (0.75-0.90)
S100P_CpG_2&3	0.93 (0.89-0.96)	0.92 (0.87-0.96)	0.93 (0.89-0.97)	0.92 (0.87-0.97)
S100P_CpG_4	0.74 (0.68-0.79)	0.75 (0.67-0.82)	0.75 (0.68-0.83)	0.71 (0.63-0.80)
S100P_CpG_7	0.94 (0.91-0.96)	0.92 (0.88-0.96)	0.96 (0.93-0.98)	0.92 (0.87-0.97)
S100P_CpG_8	0.90 (0.86-0.94)	0.88 (0.83-0.94)	0.91 (0.86-0.96)	0.89 (0.83-0.96)
S100P_CpG_9	0.90 (0.86-0.94)	0.89 (0.84-0.94)	0.93 (0.88-0.97)	0.87 (0.80-0.93)
SLC22A18_CpG_1	0.88 (0.84-0.91)	0.87 (0.82-0.92)	0.87 (0.81-0.92)	0.90 (0.85-0.95)
SLC22A18_CpG_3	0.87 (0.84-0.91)	0.89 (0.84-0.93)	0.85 (0.80-0.91)	0.91 (0.86-0.96)
SLC22A18_CpG_4	0.88 (0.84-0.92)	0.89 (0.84-0.93)	0.88 (0.83-0.93)	0.90 (0.85-0.96)
SLC22A18_CpG_6	0.86 (0.82-0.90)	0.85 (0.80-0.90)	0.85 (0.79-0.90)	0.89 (0.84-0.95)
SLC22A18_CpG_8	0.77 (0.72-0.82)	0.77 (0.71-0.84)	0.76 (0.69-0.83)	0.80 (0.73-0.88)
DYRK4_CpG_1	0.79 (0.74-0.84)	0.79 (0.72-0.85)	0.78 (0.71-0.85)	0.82 (0.75-0.89)
DYRK4_CpG_3	0.67 (0.60-0.73)	0.67 (0.59-0.75)	0.69 (0.61-0.77)	0.63 (0.53-0.72)
FUT7_CpG_1	0.85 (0.81-0.89)	0.84 (0.78-0.90)	0.88 (0.83-0.93)	0.80 (0.73-0.88)
FUT7_CpG_2	0.90 (0.87-0.94)	0.88 (0.83-0.93)	0.90 (0.86-0.95)	0.90 (0.85-0.96)
FUT7_CpG_3	0.81 (0.77-0.86)	0.77 (0.71-0.84)	0.84 (0.79-0.90)	0.77 (0.69-0.85)
FUT7_CpG_4	0.83 (0.78-0.87)	0.82 (0.76-0.87)	0.85 (0.79-0.90)	0.81 (0.73-0.88)
FUT7_CpG_6	0.82 (0.77-0.86)	0.78 (0.72-0.85)	0.82 (0.76-0.88)	0.80 (0.72-0.87)
FUT7_CpG_7	0.83 (0.78-0.87)	0.80 (0.74-0.87)	0.83 (0.77-0.89)	0.81 (0.74-0.88)
FUT7_CpG_8	0.78 (0.74-0.83)	0.77 (0.71-0.83)	0.81 (0.75-0.87)	0.76 (0.68-0.83)
All CpG panel	0.98 (0.96-0.99)	0.98 (0.96-0.99)	0.99 (0.97-1.00)	0.98 (0.96-1.00)

Fig. 11

Table 11. The methylation of genes in PaCa patients with different clinical characteristics

Clinical characteristics (N)	Group (N)	Median of age	Median of methylation levels										
			HYAL2_ CpG_1	HYAL2_ CpG_2	HYAL2_ CpG_3	HYAL2_ CpG_4	DYRK4_ CpG_1	DYRK4_ CpG_3	S100P_C pG_2&3	S100P_ CpG_4	S100P_ CpG_7	S100P_ CpG_8	S100P_ CpG_9
Tumour stage (108)	Stage 0&I&2 (79)	63.56	0.27	0.14	0.28	0.47	0.17	0.32	0.59	0.63	0.31	0.34	0.43
	Stage III&IV (29)	67.55	0.25	0.14	0.30	0.48	0.18	0.29	0.59	0.61	0.34	0.35	0.45
	p-value*	0.294	0.635	0.477	0.519	0.703	0.903	0.873	0.771	0.558	0.718	0.972	0.642
Tumour size (98)	< T3 (10)	63.67	0.27	0.14	0.31	0.49	0.25	0.30	0.61	0.77	0.32	0.37	0.44
	T3 & T4 (88)	64.15	0.25	0.13	0.28	0.47	0.16	0.32	0.59	0.61	0.31	0.34	0.44
	p-value*	0.699	0.729	0.647	0.304	0.327	0.507	0.298	0.259	0.043	0.860	0.967	0.842
Lymph node (LN)	N0 (28)	63.67	0.27	0.15	0.29	0.47	0.22	0.32	0.61	0.61	0.35	0.37	0.44
	N1 (71)	63.92	0.24	0.13	0.28	0.47	0.16	0.33	0.58	0.63	0.30	0.31	0.42
	p-value*	0.907	0.189	0.834	0.292	0.602	0.750	0.362	0.423	0.386	0.950	0.771	0.809
metastasis status	M0 (91)	63.56	0.26	0.14	0.28	0.47	0.17	0.32	0.59	0.62	0.31	0.34	0.43
	M1 (20)	67.84	0.25	0.15	0.31	0.49	0.29	0.31	0.60	0.62	0.34	0.35	0.46
	p-value*	0.199	0.687	0.659	0.478	0.343	0.562	0.875	0.942	0.800	0.602	0.839	0.461
Grading (83)	Grade 1&2 (53)	64.20	0.26	0.14	0.28	0.45	0.16	0.32	0.57	0.63	0.29	0.31	0.44
	Grade 3 (30)	63.41	0.21	0.14	0.29	0.49	0.17	0.33	0.59	0.61	0.33	0.35	0.42
	p-value*	0.798	0.013	0.939	0.367	0.292	0.943	0.562	0.189	0.791	0.290	0.448	0.974
Gender (147) cases	Male (80)	63.50	0.25	0.14	0.28	0.47	0.24	0.31	0.59	0.63	0.34	0.35	0.44
	Female (67)	66.33	0.25	0.14	0.30	0.48	0.16	0.32	0.60	0.61	0.29	0.31	0.43
	p-value*	0.605	0.853	0.933	0.784	0.301	0.134	0.239	0.907	0.491	0.234	0.425	0.713

* The *p*-values are calculated by Mann-Whitney Test

Table 11. The methylation of genes in PaCa patients with different clinical characteristics (continued)

Clinical characteristics (N)	Group (N)	Median of methylation levels											
		SLC22A18 _CpG_1	SLC22A18 _CpG_3	SLC22A18 _CpG_4	SLC22A18 _CpG_6	SLC22A18 _CpG_8	FUT7_ CpG_1	FUT7_ CpG_2	FUT7_ CpG_3	FUT7_ CpG_4	FUT7_ CpG_6	FUT7_ CpG_7	FUT7_ CpG_8
Tumour stage (108)	Stage 0&I&2 (79)	0.18	0.12	0.13	0.16	0.57	0.22	0.27	0.08	0.27	0.14	0.05	0.15
	Stage III&IV (29)	0.19	0.12	0.15	0.17	0.59	0.17	0.24	0.05	0.25	0.11	0.03	0.13
	p-value*	0.830	0.555	0.910	0.586	0.182	0.272	0.419	0.047	0.952	0.114	0.340	0.298
Tumour size (98)	< T3 (10)	0.17	0.10	0.12	0.14	0.54	0.19	0.25	0.06	0.24	0.09	0.03	0.11
	T3 & T4 (88)	0.19	0.12	0.15	0.17	0.60	0.21	0.27	0.07	0.26	0.15	0.04	0.15
	p-value*	0.468	0.264	0.288	0.092	0.068	0.253	0.191	0.601	0.235	0.008	0.336	0.130
Lymph node (LN)	N0 (28)	0.17	0.12	0.14	0.17	0.61	0.17	0.22	0.08	0.27	0.12	0.04	0.13
	N1 (71)	0.19	0.12	0.14	0.17	0.58	0.23	0.27	0.07	0.25	0.14	0.04	0.17
	p-value*	0.789	0.235	0.793	0.879	0.954	0.039	0.106	0.837	0.227	0.189	0.634	0.098
metastasis status	M0 (91)	0.18	0.12	0.14	0.17	0.59	0.21	0.27	0.07	0.26	0.14	0.04	0.15
	M1 (20)	0.19	0.12	0.14	0.17	0.58	0.21	0.27	0.06	0.24	0.12	0.05	0.14
	p-value*	0.712	0.794	0.443	0.933	0.651	0.771	0.519	0.236	0.822	0.933	0.717	0.985
Grading (83)	Grade 1&2 (53)	0.17	0.11	0.14	0.14	0.57	0.18	0.25	0.06	0.25	0.13	0.03	0.14
	Grade 3 (30)	0.19	0.13	0.19	0.20	0.63	0.22	0.28	0.07	0.25	0.16	0.07	0.17
	p-value*	0.120	0.065	0.060	0.103	0.288	0.090	0.123	0.180	0.795	0.072	0.068	0.144
Gender (147) cases	Male (80)	0.17	0.13	0.13	0.17	0.61	0.21	0.27	0.07	0.26	0.14	0.05	0.15
	Female (67)	0.17	0.11	0.13	0.14	0.55	0.24	0.26	0.06	0.26	0.13	0.05	0.15
	p-value*	0.502	0.057	0.639	0.117	0.003	0.784	0.648	0.700	0.791	0.745	0.953	0.994

* The *p*-values are calculated by Mann-Whitney Test

Fig. 12

Table 12. Sample Description of blood-based biomarker panel for the early detection of ovarian cancer

Sample types	Assays	Sample resourses	Groups	Target N	mean of age (range)	median of age	Assayed N (call rate %)			
							HYAL2	*S100P*	*SLC22A18*	*FUT7*
DNA from blood pellet	MassARRAY	University hospital of Heidelberg	controls	148	37.9 (21-63)	38	147 (99.3%)	147 (99.3%)	147 (99.3%)	144 (97.3%)
		University hospital of Heidelberg	OvCa cases	84	61.8 (37-80)	61.5	84 (100.0%)	78 (92.9%)	84 (100.0%)	82 (97.6%)

Fig. 13

Table 13. Methylation differences in genes comparing OvCa cases and controls

CpG sites	All samples		
	controls median (IQR)	OvCa cases median (IQR)	*p*-value *
HYAL2_CpG_1	0.33 (0.29-0.37)	0.25 (0.20-0.32)	1.57E-08
HYAL2_CpG_2	0.21 (0.18-0.24)	0.15 (0.11-0.20)	1.57E-09
HYAL2_CpG_3	0.37 (0.32-0.40)	0.32 (0.25-0.36)	4.08E-07
HYAL2_CpG_4	0.53 (0.50-0.58)	0.47 (0.41-0.54)	2.51E-07
S100P_CpG_2&3	0.66 (0.63-0.69)	0.63 (0.58-0.67)	0.008
S100P_CpG_4	0.65 (0.60-0.86)	0.63 (0.57-0.86)	0.351
S100P_CpG_7	0.44 (0.38-0.51)	0.33 (0.23-0.41)	3.53E-09
S100P_CpG_8	0.43 (0.40-0.49)	0.39 (0.31-0.46)	3.92E-04
S100P_CpG_9	0.49 (0.44-0.52)	0.43 (0.37-0.48)	3.32E-06
SLC22A18_CpG_3	0.19 (0.16-0.23)	0.13 (0.10-0.18)	4.50E-11
SLC22A18_CpG_4	0.25 (0.22-0.30)	0.16 (0.12-0.25)	2.74E-10
SLC22A18_CpG_6	0.24 (0.22-0.28)	0.16 (0.12-0.24)	9.47E-12
SLC22A18_CpG_8	0.66 (0.62-0.70)	0.57 (0.48-0.62)	3.43E-12
FUT7_CpG_1	0.41 (0.34-0.46)	0.28 (0.20-0.39)	3.13E-10
FUT7_CpG_2	0.21 (0.17-0.25)	0.14 (0.11-0.19)	8.80E-09
FUT7_CpG_3	0.16 (0.11-0.20)	0.10 (0.05-0.16)	8.31E-08
FUT7_CpG_4	0.20 (0.16-0.23)	0.16 (0.11-0.22)	0.001
FUT7_CpG_6	0.28 (0.21-0.34)	0.14 (0.11-0.24)	2.79E-10
FUT7_CpG_7	0.14 (0.10-0.17)	0.07 (0.04-0.12)	2.02E-09
FUT7_CpG_8	0.38 (0.31-0.42)	0.24 (0.15-0.35)	2.57E-10
All CpG panel			**7.42E-16**

* logistic regression, adjusted for different batches for the measurements

Fig. 14

Table 14. The discriminatory power of the methylation in genes to distinguish OvCa cases from healthy controls

CpG sites	Area under curve (AUC), 95% CI
	All samples
HYAL2_CpG_1	0.77 (0.70-0.84)
HYAL2_CpG_2	0.77 (0.70-0.83)
HYAL2_CpG_3	0.72 (0.65-0.79)
HYAL2_CpG_4	0.70 (0.63-0.78)
S100P_CpG_2&3	0.62 (0.54-0.71)
S100P_CpG_4	0.56 (0.48-0.64)
S100P_CpG_7	0.77 (0.70-0.84)
S100P_CpG_8	0.66 (0.58-0.75)
S100P_CpG_9	0.70 (0.62-0.77)
SLC22A18_CpG_3	0.79 (0.72-0.85)
SLC22A18_CpG_4	0.77 (0.70-0.84)
SLC22A18_CpG_6	0.79 (0.72-0.86)
SLC22A18_CpG_8	0.82 (0.76-0.88)
FUT7_CpG_1	0.76 (0.69-0.83)
FUT7_CpG_2	0.76 (0.69-0.82)
FUT7_CpG_3	0.73 (0.66-0.80)
FUT7_CpG_4	0.64 (0.56-0.72)
FUT7_CpG_6	0.78 (0.71-0.85)
FUT7_CpG_7	0.77 (0.70-0.84)
FUT7_CpG_8	0.77 (0.70-0.84)
All CpG panel	0.91 (0.87-0.95)

Fig. 15

Table 15. The determination of breast cancer related CpG island shore in HYAL2

CpG sites	Differences in methylation levels				Correlations to HYAL2_CpG_4	
	Controls median (IQR)	Familial BC cases median (IQR)	OR (95 % CI) * per 10% methylation	*p*-value*	Spearman rho	*p*-value
HYAL2-C_CpG_1	0.66 (0.60-0.71)	0.63 (0.56-0.67)	1.66 (1.15-2.41)	0.007	0.393	< 0.0001
HYAL2-C_CpG_2	0.52 (0.47-0.59)	0.53 (0.46-0.61)	1.07 (0.81-1.43)	0.623	0.248	0.001
HYAL2-C_CpG_3	0.63 (0.58-0.66)	0.59 (0.56-0.64)	1.39 (0.93-2.08)	0.113	0.357	< 0.0001
HYAL2-C_CpG_4	0.63 (0.58-0.66)	0.59 (0.56-0.64)	1.39 (0.93-2.08)	0.113	0.357	< 0.0001
HYAL2-C_CpG_5	0.73 (0.70-0.76)	0.72 (0.69-0.76)	1.19 (0.71-2.00)	0.505	0.253	0.0005
HYAL2-C_CpG_6	0.63 (0.58-0.66)	0.59 (0.56-0.64)	1.39 (0.93-2.08)	0.113	0.357	< 0.0001
HYAL2-C_CpG_7	0.40 (0.34-0.45)	0.39 (0.35-0.44)	1.26 (0.82-1.95)	0.291	0.287	< 0.0001
HYAL2-C_CpG_8	0.82 (0.76-0.88)	0.79 (0.71-0.84)	1.63 (1.15-2.31)	0.007	0.410	< 0.0001
HYAL2-C_CpG_9.10	0.70 (0.51-0.77)	0.71 (0.57-0.76)	0.94 (0.79-1.12)	0.484	0.198	0.007
HYAL2-C_CpG_11	0.74 (0.70-0.77)	0.70 (0.66-0.75)	1.88 (1.16-3.03)	0.010	0.463	< 0.0001
HYAL2-C_CpG_12.13	0.90 (0.88-0.92)	0.89 (0.86-0.91)	1.21 (0.87-1.69)	0.258	0.270	0.0002
HYAL2-C_CpG_14.15	0.83 (0.78-0.86)	0.80 (0.75-0.84)	1.41 (0.93-2.12)	0.103	0.381	< 0.0001
HYAL2-C_CpG_16.17	0.75 (0.72-0.78)	0.73 (0.70-0.75)	2.24 (1.21-4.16)	0.011	0.348	< 0.0001
HYAL2-B_CpG_1	0.66 (0.62-0.69)	0.61 (0.59-0.65)	1.77 (1.11-2.84)	0.017	0.534	< 0.0001
HYAL2-B_CpG_2	0.56 (0.49-0.62)	0.49 (0.43-0.54)	2.16 (1.51-3.09)	< 0.0001	0.621	< 0.0001
HYAL2-B_CpG_3.4	0.63 (0.61-0.66)	0.59 (0.56-0.63)	3.50 (1.87-6.56)	< 0.0001	0.566	< 0.0001
HYAL2-B_CpG_5.6	0.77 (0.74-0.83)	0.71 (0.66-0.77)	3.52 (2.17-5.71)	< 0.0001	0.649	< 0.0001
HYAL2-B_CpG_7	0.57 (0.50-0.62)	0.50 (0.42-0.57)	2.08 (1.47-2.95)	< 0.0001	0.569	< 0.0001
HYAL2-B_CpG_8	0.66 (0.62-0.69)	0.61 (0.59-0.65)	1.77 (1.11-2.84)	0.017	0.534	< 0.0001
HYAL2-B_CpG_9	0.36 (0.31-0.41)	0.30 (0.26-0.37)	1.88 (1.28-2.76)	0.001	0.482	< 0.0001
HYAL2-B_CpG_10	0.63 (0.59-0.69)	0.58 (0.52-0.65)	1.94 (1.35-2.78)	0.0004	0.634	< 0.0001
HYAL2-B_CpG_11	0.53 (0.48-0.59)	0.47 (0.41-0.52)	3.05 (1.94-4.78)	< 0.0001	0.602	< 0.0001
HYAL2_CpG_1	0.36 (0.31-0.43)	0.31 (0.26-0.39)	1.41 (1.08-1.86)	0.013	0.584	< 0.0001
HYAL2_CpG_2	0.24 (0.19-0.28)	0.16 (0.13-0.21)	4.40 (2.56-7.57)	< 0.0001	0.691	< 0.0001
HYAL2_CpG_3	0.41 (0.36-0.46)	0.32 (0.28-0.38)	4.14 (2.51-6.85)	< 0.0001	0.774	< 0.0001
HYAL2_CpG_4	0.65 (0.59-0.69)	0.50 (0.47-0.57)	8.13 (4.53-14.59)	< 0.0001	1.000	—

* logistic regression, adjusted for age and different batches for the measurements

Fig 16.

Table 16. The inverse correlation between the methylation and expression of S100P, SLC22A18 and DYRK4 in leucocytes

CpG sites	Difference in methylation or expression levels			Correlations to expression	
	Controls	Sporadic BC	*p*-value *	Spearman rho	*p*-value
S100P_CpG_2&3	0.63 (0.58-0.65)	0.59 (0.56-0.62)	0.006	-0.551	1.12E-06
S100P_CpG_4	0.60 (0.55-0.87)	0.61 (0.53-0.83)	0.445	-0.147	0.226
S100P_CpG_7	0.36 (0.30-0.40)	0.29 (0.24-0.33)	0.001	-0.501	8.55E-06
S100P_CpG_8	0.44 (0.38-0.48)	0.35 (0.32-0.39)	7.76E-06	-0.653	9.27E-10
S100P_CpG_9	0.49 (0.44-0.54)	0.45 (0.40-0.47)	0.001	-0.555	5.07E-07
Relative expression of S100P	1.31 (0.72-2.07)	3.47 (1.23-20.77)	0.001	1.000	—
SLC22A18_CpG_3	0.19 (0.15-0.23)	0.16 (0.14-0.19)	0.006	-0.507	5.53E-06
SLC22A18_CpG_4	0.26 (0.20-0.31)	0.21 (0.17-0.24)	0.006	-0.536	1.21E-06
SLC22A18_CpG_6	0.25 (0.20-0.28)	0.19 (0.17-0.23)	0.001	-0.565	2.29E-07
SLC22A18_CpG_8	0.64 (0.60-0.70)	0.59 (0.53-0.63)	0.001	-0.525	2.15E-06
Relative expression of SLC22A18	0.69 (0.55-0.87)	0.76 (0.54-1.23)	0.311	1.000	—
DYRK4_CpG_1	0.33 (0.25-0.41)	0.26 (0.20-0.33)	0.018	-0.074	0.540
DYRK4_CpG_3	0.29 (0.22-0.34)	0.24 (0.18-0.28)	0.023	-0.075	0.536
Relative expression of DYRK4	0.36 (0.31-0.41)	0.37 (0.29-0.46)	0.919	1.000	—

* Mann-Whitney U test

Fig.17

Table 17. The methylation levels of HYAL2 CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg06721473	0.0008	-0.020	36	3	50330420	3'UTR;3'UTR	NA
cg08776109	0.0006	-0.022	36	3	50331237	Body;Body	NA
cg00575896	0.1133	-0.007	36	3	50332175	Body;Body	TRUE
cg25630588	0.9482	-0.001	36	3	50332326	Body;Body	TRUE
cg09412061	0.9466	-0.002	36	3	50332470	Body;Body	NA
cg26794477	0.8988	-0.001	36	3	50332596	Body;Body	NA
cg00467652	0.3265	-0.004	36	3	50332648	Body;Body	NA
cg05164052	0.2727	0.002	36	3	50333326	5'UTR;5'UTR	NA
cg16563178	0.4019	0.005	36	3	50333447	5'UTR;5'UTR	NA
cg10109442	0.9007	0.000	36	3	50333891	5'UTR;1stExon;5'UTR	NA
cg04884420	0.0674	0.003	36	3	50333976	TSS200;5'UTR	NA
cg22280173	0.3091	-0.001	36	3	50333985	TSS200;5'UTR	NA
cg08173110	0.5201	0.001	36	3	50334009	TSS200;5'UTR	NA
cg23515942	0.7374	0.001	36	3	50334023	TSS200;5'UTR	NA
cg06211164	0.7758	0.000	36	3	50334069	TSS200;5'UTR	NA
cg13580654	0.6497	0.001	36	3	50334101	TSS200;5'UTR	NA
cg07271561	0.7449	-0.003	36	3	50334367	5'UTR;TSS1500	NA
cg12976582	0.0253	-0.015	36	3	50334587	5'UTR;TSS1500	NA
cg12150256	0.4194	-0.004	36	3	50334853	5'UTR;TSS1500	NA
cg13341668	0.0938	-0.008	36	3	50334913	5'UTR;TSS1500	NA
cg05118960	0.1069	-0.005	36	3	50334982	5'UTR;TSS1500	NA
cg03721058	0.0046	-0.010	36	3	50335045	5'UTR;TSS1500	NA
cg00840516	0.0074	-0.014	36	3	50335101	5'UTR;1stExon;TSS1500	NA
cg03051392	0.0005	-0.017	36	3	50335180	5'UTR;1stExon;TSS1500	NA
cg26460678	0.0029	-0.020	36	3	50335671	TSS1500	NA
cg27091787	0.0044	-0.028	36	3	50335694	TSS1500	NA
cg24335984	0.0507	-0.007	36	3	50336558	TSS1500	NA

Fig. 18

Table 18. The methylation levels of S100P CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg02883621	0.6312	-0.003	36	4	6745824	TSS1500	NA
cg14323984	0.7019	-0.002	36	4	6746104	TSS1500	NA
cg27027375	0.0233	-0.017	36	4	6746220	TSS1500	NA
cg14900031	0.0010	-0.021	36	4	6746278	TSS200	NA
cg14140379	0.0011	-0.029	36	4	6746281	TSS200	NA
cg25083732	0.0534	-0.027	36	4	6746365	TSS200	NA
cg07210669	0.0117	-0.028	36	4	6746376	TSS200	NA
cg26233331	0.0002	-0.040	36	4	6746515	1stExon;5'UTR	NA
cg22266967	0.0014	-0.033	36	4	6746599	1stExon	NA
cg02104700	0.3792	-0.003	36	4	6749069	Body	NA

Fig. 19

Table 19. The methylation levels of SLC22A18 CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg23335134	0.8641	0.001	36	11	2866266	Body	NA
cg26874323	0.6378	0.001	36	11	2866285	Body	NA
cg08222610	0.0833	-0.001	36	11	2866291	Body	NA
cg12240761	0.6550	0.002	36	11	2869910	Body	TRUE
cg14449910	0.1298	-0.008	36	11	2876265	Body;TSS1500	TRUE
cg26665035	0.0426	-0.023	36	11	2876339	Body;TSS1500	TRUE
cg22040301	0.2417	-0.012	36	11	2876374	Body;TSS1500	TRUE
cg05457684	0.0562	-0.017	36	11	2876384	Body;TSS1500	TRUE
cg18419977	0.8110	0.000	36	11	2876628	Body;TSS1500	TRUE
cg24033661	0.4585	0.000	36	11	2876631	Body;TSS1500	TRUE
cg13485320	0.6519	0.003	36	11	2876704	Body;TSS1500	TRUE
cg21853021	0.9589	0.002	36	11	2876722	Body;TSS1500	TRUE
cg18458509	0.0170	-0.019	36	11	2876765	Body;TSS1500	TRUE
cg23190089	0.0047	-0.019	36	11	2876785	Body;TSS1500	TRUE
cg16587707	0.0004	-0.022	36	11	2876841	Body;TSS1500	TRUE
cg02462487	0.0404	-0.015	36	11	2876926	Body;TSS1500	TRUE
cg16129800	0.0008	-0.023	36	11	2876990	Body;TSS1500	TRUE
cg21599100	0.0018	-0.022	36	11	2877013	Body;TSS1500	TRUE
cg05752118	0.0310	-0.016	36	11	2877140	Body;TSS1500	TRUE
cg11785933	0.0023	-0.041	36	11	2877193	Body;TSS1500	NA
cg25427871	0.0137	-0.032	36	11	2877311	Body;TSS1500	NA
cg22315192	0.0044	-0.016	36	11	2877341	TSS200;Body	NA
cg21019522	0.0042	-0.018	36	11	2877365	TSS200;Body	NA
cg16346422	0.0001	-0.042	36	11	2877395	TSS200;Body	NA
cg16873863	0.1148	-0.009	36	11	2877752	5'UTR;5'UTR	NA
cg22680591	0.5778	-0.002	36	11	2878627	5'UTR;5'UTR;TSS1500	NA
cg15904130	0.1653	0.003	36	11	2878639	5'UTR;5'UTR;TSS1500	NA
cg25073813	0.0276	-0.008	36	11	2878644	5'UTR;5'UTR;TSS1500	NA
cg24205453	0.4111	-0.001	36	11	2878910	5'UTR;5'UTR;TSS1500	NA
cg18471235	0.0055	0.011	36	11	2878971	5'UTR;5'UTR;TSS1500	NA
cg15739881	0.0966	-0.008	36	11	2879040	5'UTR;5'UTR;TSS1500	NA
cg12563184	0.2446	-0.004	36	11	2879067	5'UTR;5'UTR;TSS1500	NA
cg04665867	0.3414	-0.003	36	11	2879093	5'UTR;5'UTR;TSS1500	NA
cg24041239	0.1391	-0.006	36	11	2879211	5'UTR;5'UTR;TSS1500	NA
cg09198782	0.3067	-0.004	36	11	2879338	5'UTR;5'UTR;TSS1500	NA
cg07291601	0.4683	-0.002	36	11	2879383	5'UTR;5'UTR;TSS1500	NA
cg25548316	0.7994	0.001	36	11	2879388	5'UTR;5'UTR;TSS1500	NA

Fig. 19 (continued)

Table 19. The methylation levels of SLC22A18 CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg10943932	0.5082	0.000	36	11	2879409	5'UTR;5'UTR;TSS1500	NA
cg02081198	0.6399	0.000	36	11	2879421	5'UTR;5'UTR;TSS1500	NA
cg05385260	0.0511	-0.005	36	11	2879428	5'UTR;5'UTR;TSS1500	NA
cg16035277	0.4744	0.005	36	11	2879840	5'UTR;5'UTR;TSS1500	NA
cg09781437	0.0128	0.002	36	11	2879986	5'UTR;5'UTR;TSS200	NA
cg02200456	0.4292	-0.001	36	11	2880012	5'UTR;5'UTR;TSS200	NA
cg13671930	0.6034	0.003	36	11	2880014	5'UTR;5'UTR;TSS200	NA
cg22132309	0.7142	0.001	36	11	2880066	5'UTR;5'UTR;TSS200	NA
cg16184736	0.2003	-0.001	36	11	2880074	5'UTR;5'UTR;TSS200	NA
cg03829241	0.4543	0.001	36	11	2880080	5'UTR;5'UTR;TSS200	NA
cg17992161	0.0850	0.006	36	11	2880101	5'UTR;1stExon;5'UTR;5'UTR	NA
cg12733707	0.0846	0.005	36	11	2880123	5'UTR;1stExon;5'UTR;5'UTR	NA
cg24139421	0.9971	0.001	36	11	2880154	5'UTR;1stExon;5'UTR;5'UTR	NA
cg06211616	0.8977	0.002	36	11	2880235	5'UTR;5'UTR;5'UTR	NA
cg24528523	0.7157	0.000	36	11	2880384	5'UTR;5'UTR;5'UTR	NA
cg12911952	0.0388	-0.007	36	11	2881099	5'UTR;5'UTR;5'UTR	NA
cg02719634	0.0932	-0.019	36	11	2881475	1stExon;Body;5'UTR;Body	TRUE
cg15729154	0.0364	-0.018	36	11	2881602	1stExon;Body;5'UTR;Body	TRUE
cg07161669	0.2605	0.000	36	11	2881763	Body;Body;TSS200	TRUE
cg24724917	0.4820	-0.002	36	11	2882015	Body;Body;TSS1500	TRUE
cg06495763	0.8721	-0.001	36	11	2882046	Body;Body;TSS1500	TRUE
cg06048910	0.2015	-0.001	36	11	2882049	Body;Body;TSS1500	TRUE
cg08472797	0.2778	-0.003	36	11	2882146	Body;Body;TSS1500	TRUE
cg14101500	0.4686	0.000	36	11	2882170	Body;Body;TSS1500	TRUE
cg22833478	0.5602	-0.001	36	11	2882199	Body;Body;TSS1500	TRUE
cg26137286	0.0522	-0.006	36	11	2882210	Body;Body;TSS1500	TRUE
cg02390725	0.0040	-0.010	36	11	2882344	Body;Body;TSS1500	TRUE
cg20716202	0.0779	-0.005	36	11	2882423	Body;Body;TSS1500	TRUE
cg08999895	0.2548	-0.005	36	11	2882445	Body;Body;TSS1500	TRUE
cg16530128	0.5740	-0.002	36	11	2882527	Body;Body;TSS1500	NA
cg08827700	0.0045	-0.008	36	11	2882545	Body;Body;TSS1500	NA
cg22272492	0.0009	-0.010	36	11	2882572	Body;Body;TSS1500	NA
cg21991825	0.6773	0.002	36	11	2882719	Body;Body;TSS1500	NA
cg23912877	0.6175	0.000	36	11	2882913	Body;Body;TSS1500	NA
cg06981073	0.5191	-0.001	36	11	2882920	Body;Body;TSS1500	NA
cg06669405	0.0170	-0.010	36	11	2882937	Body;Body;TSS1500	NA

Fig. 19 continued

Table 19. The methylation levels of SLC22A18 CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg09731124	0.4994	-0.004	36	11	2882998	Body;Body;TSS1500	NA
cg02025860	0.4916	-0.002	36	11	2883177	Body;Body;TSS1500	NA
cg14168614	0.6637	-0.005	36	11	2883823	Body;Body	NA
cg02660089	0.9102	0.003	36	11	2886017	Body;Body	NA
cg22858288	0.4478	0.002	36	11	2886898	Body;Body	NA
cg04726200	0.6860	-0.007	36	11	2887061	Body;Body	NA
cg19497444	0.7053	-0.009	36	11	2887370	Body;Body	NA
cg03336167	0.9821	-0.005	36	11	2887571	Body;Body	NA
cg23698969	0.3834	-0.003	36	11	2887741	Body;Body	NA
cg24409566	0.6945	-0.002	36	11	2890543	Body;Body	TRUE
cg05351334	0.6395	-0.003	36	11	2896968	Body;Body	NA
cg14275836	0.7743	-0.002	36	11	2897889	Body;Body	NA
cg19240938	0.2782	0.002	36	11	2898612	Body;Body	NA
cg18655584	0.9972	-0.002	36	11	2898669	Body;Body	NA
cg12510502	0.5312	-0.002	36	11	2898752	Body;Body	NA
cg13328151	0.2459	0.004	36	11	2899615	Body;Body	NA
cg03010425	0.9510	0.002	36	11	2901347	Body;Body	NA
cg26595893	0.8941	0.002	36	11	2903050	3'UTR;3'UTR	NA

Fig. 20

Table 20. The methylation levels of DYRK4 CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg24707294	0.8090	-0.003	36	12	4568380	TSS1500	NA
cg08977032	0.5921	-0.001	36	12	4568578	TSS1500	NA
cg06270401	0.0000	-0.047	36	12	4569346	TSS200	NA
cg09581911	0.8105	-0.014	36	12	4569493	TSS200	NA
cg09418321	0.0011	-0.031	36	12	4569879	5'UTR	NA
cg01218945	0.4078	0.001	36	12	4584370	Body	NA
cg24337818	0.0539	-0.002	36	12	4584440	Body	NA
cg00532413	0.2381	0.000	36	12	4584588	Body	NA

Fig. 21

Table 21. The methylation levels of FUT7 CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg02971262	0.0031	-0.011	36	9	139045216	5'UTR;Body	NA
cg14205519	0.5871	0.002	36	9	139045571	5'UTR;Body	NA
cg03630596	0.6632	-0.003	36	9	139045677	5'UTR;Body	NA
cg13757845	0.0082	-0.023	36	9	139046561	5'UTR;1stExon;5'UTR	NA
cg09305224	0.0001	-0.030	36	9	139047066	5'UTR;1stExon;5'UTR	NA
cg02679745	0.0000	-0.036	36	9	139047467	Body;TSS1500	NA

Fig. 22

Table 22. The methylation levels of RAPSN CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg26738160	0.6801	0.001	36	11	47416086	3'UTR;3'UTR	NA
cg24812582	0.3992	0.002	36	11	47419939	Body;Body	NA
cg17614165	0.8996	-0.001	36	11	47425239	Body;Body	NA
cg15270729	0.1291	0.006	36	11	47425474	Body;Body	NA
cg09163021	0.7317	-0.005	36	11	47427344	TSS200;TSS200	NA
cg07407499	0.0904	-0.008	36	11	47427367	TSS200;TSS200	NA
cg14407987	0.9251	-0.002	36	11	47427369	TSS200;TSS200	NA
cg26454662	0.7520	-0.006	36	11	47427379	TSS200;TSS200	NA
cg03400491	0.2005	-0.005	36	11	47427463	TSS200;TSS200	NA
cg19771781	0.9445	-0.001	36	11	47427476	TSS200;TSS200	NA
cg13047308	0.0021	-0.033	36	11	47427915	TSS1500;TSS1500	NA
cg27466532	0.0000	-0.045	36	11	47427976	TSS1500;TSS1500	NA
cg02321133	0.9396	-0.006	36	11	47428365	TSS1500;TSS1500	NA

Fig. 23

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg03140026	0.1675	0.002	36	17	76131746	TSS1500;TSS1500	NA
cg14406501	0.1416	-0.015	36	17	76132775	TSS1500;TSS1500	NA
cg02352203	0.2860	0.001	36	17	76133032	TSS200;TSS200	NA
cg13515774	0.0979	0.000	36	17	76133067	TSS200;TSS200	NA
cg15600835	0.0368	-0.001	36	17	76133151	TSS200;TSS200	NA
cg00815931	0.1649	-0.002	36	17	76133168	TSS200;TSS200	NA
cg03172060	0.0707	-0.007	36	17	76133208	TSS200;TSS200	NA
cg12045294	0.1752	0.002	36	17	76133211	TSS200;TSS200	NA
cg20758492	0.4765	0.000	36	17	76134834	Body;Body	NA
cg02082642	0.8956	-0.002	36	17	76137204	Body;Body	NA
cg22652378	0.0006	-0.032	36	17	76148437	Body;Body	TRUE
cg25514328	0.5802	0.004	36	17	76163919	Body;Body	NA
cg11329058	0.0369	0.012	36	17	76163953	Body;Body	NA
cg18576374	0.5514	-0.001	36	17	76163966	Body;Body	NA
cg06799305	0.3461	0.000	36	17	76165965	Body;Body	TRUE
cg08129331	0.0079	-0.022	36	17	76175073	Body;Body	NA
cg01561259	0.8979	-0.003	36	17	76175376	Body;Body	NA
cg09929238	0.0443	-0.024	36	17	76175511	Body;Body	NA
cg27210166	0.9688	0.001	36	17	76189287	Body;Body	NA
cg12088417	0.6881	0.012	36	17	76189311	Body;Body	NA
cg10162696	0.9502	0.002	36	17	76191173	Body;Body	NA
cg09133154	0.6328	-0.001	36	17	76191267	Body;Body	NA
cg03520496	0.2563	0.007	36	17	76191285	Body;Body	NA
cg08732594	0.0311	-0.003	36	17	76196984	Body;Body	NA
cg04687939	0.2909	-0.003	36	17	76197144	Body;Body	NA
cg22280406	0.8201	0.000	36	17	76197242	Body;Body	NA
cg16027727	0.7303	-0.006	36	17	76200923	Body;Body	NA
cg04951638	0.7431	0.000	36	17	76208376	Body;Body	TRUE
cg00143364	0.2604	-0.006	36	17	76219939	Body;Body	TRUE
cg20462129	0.1050	-0.002	36	17	76221690	Body;Body	TRUE
cg02462904	0.2293	-0.005	36	17	76221850	Body;Body	NA
cg21925688	0.3817	0.001	36	17	76221963	Body;Body	NA
cg03637703	0.1905	0.001	36	17	76232333	Body;Body	NA
cg27551440	0.1464	-0.001	36	17	76232340	Body;Body	NA
cg27313007	0.0351	-0.007	36	17	76232345	Body;Body	NA
cg08811817	0.0927	0.009	36	17	76237232	Body;Body	TRUE
cg18758433	0.0001	0.046	36	17	76238196	Body;Body	TRUE
cg26170499	0.4804	0.000	36	17	76249958	Body;Body	TRUE

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg17442961	0.0068	-0.005	36	17	76250023	Body;Body	TRUE
cg00460639	0.3183	-0.005	36	17	76250229	Body;Body	TRUE
cg01432609	0.0032	-0.026	36	17	76253149	Body;Body	TRUE
cg13303377	0.5077	-0.003	36	17	76254840	Body;Body	NA
cg17944774	0.7632	-0.002	36	17	76258841	Body;Body	NA
cg17051395	0.1054	0.002	36	17	76258887	Body;Body	NA
cg27454679	0.8016	-0.003	36	17	76259128	Body;Body	NA
cg04681879	0.9956	-0.002	36	17	76259241	Body;Body	NA
cg17434577	0.7843	-0.004	36	17	76259361	Body;Body	NA
cg09592546	0.0053	0.044	36	17	76267497	Body;Body	TRUE
cg22882460	0.4555	0.001	36	17	76269243	Body;Body	NA
cg13311292	0.2817	-0.004	36	17	76269258	Body;Body	NA
cg06443231	0.6011	-0.002	36	17	76269860	Body;Body	NA
cg22838354	0.7433	-0.002	36	17	76269879	Body;Body	NA
cg09596252	0.2242	-0.003	36	17	76270088	Body;Body	NA
cg11303920	0.5721	0.001	36	17	76276168	Body;Body	NA
cg23238734	0.3909	-0.003	36	17	76276202	Body;Body	NA
cg17956530	0.7764	-0.002	36	17	76282294	Body;Body	NA
cg01500570	0.0109	-0.003	36	17	76282362	Body;Body	NA
cg13102028	0.2568	0.002	36	17	76282603	Body;Body	NA
cg04136113	0.9874	0.000	36	17	76282617	Body;Body	NA
cg09141931	0.9642	0.000	36	17	76282804	Body;Body	TRUE
cg06673969	0.4171	-0.009	36	17	76283457	Body;Body	TRUE
cg15116918	0.1751	0.002	36	17	76284147	Body;Body	TRUE
cg20562478	0.6685	-0.003	36	17	76284834	Body;Body	TRUE
cg20937981	0.2068	0.004	36	17	76294225	Body;Body	TRUE
cg18780100	0.0267	-0.027	36	17	76297380	Body;Body	NA
cg01498832	0.0002	-0.042	36	17	76297529	Body;Body	NA
cg07786220	0.0000	-0.047	36	17	76297677	Body;Body	NA
cg02240665	0.5351	-0.001	36	17	76297867	Body;Body	NA
cg25985643	0.1559	-0.008	36	17	76298052	Body;Body	TRUE
cg27511181	0.0108	-0.037	36	17	76299819	Body;Body	TRUE
cg11790527	0.0069	-0.022	36	17	76300748	Body;Body	TRUE
cg26733897	0.0009	0.018	36	17	76309187	Body;Body	NA
cg16918327	0.0511	0.013	36	17	76309246	Body;Body	NA
cg25673241	0.0453	0.014	36	17	76309451	Body;Body	NA
cg24667756	0.1747	0.004	36	17	76313417	Body;Body	TRUE
cg18965980	0.5135	-0.004	36	17	76313643	Body;Body	TRUE

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg25288455	0.2248	0.009	36	17	76315501	Body;Body	NA
cg06975080	0.0168	0.012	36	17	76315523	Body;Body	NA
cg10790704	0.2922	0.000	36	17	76315679	Body;Body	NA
cg18406924	0.0116	0.018	36	17	76315852	Body;Body	NA
cg19857461	0.3144	0.002	36	17	76317643	Body;Body	NA
cg18026826	0.2440	0.001	36	17	76318962	Body;Body	NA
cg26723185	0.1380	0.001	36	17	76319016	Body;Body	NA
cg03513049	0.5379	0.003	36	17	76319516	Body;Body	TRUE
cg06872548	0.1960	-0.010	36	17	76331578	Body;Body	TRUE
cg11757444	0.0126	-0.074	36	17	76333768	Body;Body	NA
cg10281768	0.0040	0.010	36	17	76334821	Body;Body	NA
cg05337636	0.2022	0.002	36	17	76335132	Body;Body	NA
cg17060157	0.0009	-0.023	36	17	76338722	Body;Body	NA
cg00701918	0.3726	-0.004	36	17	76339590	Body;Body	NA
cg24394819	0.6679	-0.001	36	17	76339644	Body;Body	NA
cg00549398	0.9789	-0.002	36	17	76339796	Body;Body	TRUE
cg16841014	0.0766	-0.019	36	17	76339970	Body;Body	TRUE
cg21143224	0.0344	-0.026	36	17	76340065	Body;Body	TRUE
cg18951390	0.7521	0.000	36	17	76349863	Body;Body	TRUE
cg11499091	0.4517	0.006	36	17	76349897	Body;Body	TRUE
cg09516200	0.5475	-0.007	36	17	76349919	Body;Body	TRUE
cg06412669	0.5255	-0.006	36	17	76350145	Body;Body	TRUE
cg16565901	0.0031	-0.022	36	17	76350191	Body;Body	NA
cg20797905	0.8335	0.009	36	17	76361822	Body;Body	TRUE
cg02675920	0.6275	-0.006	36	17	76362529	Body;Body	NA
cg11222173	0.0048	-0.029	36	17	76362614	Body;Body	NA
cg11153071	0.0000	-0.048	36	17	76362672	Body;Body	NA
cg15096353	0.9324	0.000	36	17	76362886	Body;Body	NA
cg00523683	0.8102	0.016	36	17	76363066	Body;Body	NA
cg02185248	0.1758	-0.014	36	17	76363089	Body;Body	NA
cg14343513	0.0000	-0.046	36	17	76367868	Body;Body	NA
cg05098037	0.0000	-0.027	36	17	76367922	Body;Body	NA
cg22386583	0.0001	-0.043	36	17	76368351	Body;Body	TRUE
cg12654199	0.0000	-0.047	36	17	76368421	Body;Body	TRUE
cg15230985	0.0024	-0.014	36	17	76368482	Body;Body	TRUE
cg05651511	0.0000	-0.052	36	17	76368685	Body;Body	TRUE
cg04662369	0.0004	-0.059	36	17	76368913	Body;Body	TRUE
cg14780427	0.0171	-0.031	36	17	76368967	Body;Body	TRUE

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg06153925	0.0000	-0.061	36	17	76369974	Body;Body	NA
cg08454507	0.0000	-0.039	36	17	76370001	Body;Body	NA
cg06418238	0.0000	-0.075	36	17	76370037	Body;Body	NA
cg22878693	0.0079	-0.021	36	17	76370199	Body;Body	NA
cg18469159	0.1941	-0.003	36	17	76370436	Body;Body	NA
cg16115689	0.0015	0.021	36	17	76378851	Body;Body	TRUE
cg12592365	0.0061	0.012	36	17	76380543	Body;Body	TRUE
cg15547672	0.7579	-0.002	36	17	76383535	Body;Body	TRUE
cg04566233	0.5072	-0.005	36	17	76385838	Body;Body	TRUE
cg10693767	0.4266	0.001	36	17	76387719	Body;Body	NA
cg23463786	0.2379	0.001	36	17	76387740	Body;Body	NA
cg04803424	0.2824	-0.003	36	17	76387797	Body;Body	NA
cg10585621	0.7002	-0.002	36	17	76388048	Body;Body	NA
cg15946337	0.6325	-0.003	36	17	76388093	Body;Body	NA
cg15815120	0.4312	0.001	36	17	76388256	Body;Body	NA
cg01476242	0.2066	-0.002	36	17	76388625	Body;Body	NA
cg17888563	0.2956	0.000	36	17	76388653	Body;Body	NA
cg18605975	0.6097	-0.002	36	17	76389318	Body;Body	NA
cg06675781	0.4149	0.001	36	17	76390230	Body;Body	NA
cg05548508	0.3504	0.001	36	17	76390392	Body;Body	NA
cg13098428	0.1352	-0.053	36	17	76390409	Body;Body	NA
cg27025953	0.6318	-0.004	36	17	76391155	Body;Body	NA
cg17703078	0.8596	0.000	36	17	76391181	Body;Body	NA
cg14596352	0.5598	-0.002	36	17	76391776	Body;Body	NA
cg00463485	0.1882	-0.006	36	17	76392152	Body;Body	NA
cg16896879	0.8665	0.000	36	17	76392689	Body;Body	NA
cg06343673	0.5419	0.002	36	17	76392827	Body;Body	NA
cg03533386	0.8783	0.000	36	17	76393148	Body;Body	TRUE
cg01516792	0.0487	-0.012	36	17	76393735	Body;Body	TRUE
cg04162316	0.0005	0.018	36	17	76400950	Body;Body	NA
cg12028455	0.4921	-0.003	36	17	76403673	Body;Body	NA
cg23542426	0.2420	-0.002	36	17	76404215	Body;Body	NA
cg16980736	0.5232	0.001	36	17	76404301	Body;Body	NA
cg27460531	0.0717	-0.010	36	17	76406318	Body;Body	TRUE
cg16015295	0.0110	-0.021	36	17	76408074	Body;Body	TRUE
cg13526488	0.0051	-0.025	36	17	76408402	Body;Body	TRUE
cg15616522	0.6877	-0.004	36	17	76408843	Body;Body	TRUE
cg12785535	0.6830	0.000	36	17	76409987	Body;Body	NA

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg18430553	0.9679	0.001	36	17	76410136	Body;Body	NA
cg04295549	0.9501	0.000	36	17	76410264	Body;Body	NA
cg25705936	0.6954	-0.001	36	17	76410459	Body;Body	NA
cg06444734	0.3604	-0.004	36	17	76410674	Body;Body	NA
cg17602102	0.5131	0.000	36	17	76410754	Body;Body	NA
cg02910299	0.0594	-0.002	36	17	76410761	Body;Body	NA
cg14073057	0.1024	0.001	36	17	76410958	Body;Body	NA
cg03794617	0.0045	-0.025	36	17	76411594	Body;Body	NA
cg08329754	0.0008	-0.021	36	17	76411610	Body;Body	NA
cg09001356	0.0240	-0.023	36	17	76411656	Body;Body	NA
cg26419477	0.6306	-0.001	36	17	76413754	Body;Body	NA
cg23245933	0.0615	-0.007	36	17	76414205	Body;Body	NA
cg23261154	0.5801	0.000	36	17	76414316	Body;Body	NA
cg09175325	0.0019	-0.014	36	17	76414794	Body;Body	TRUE
cg14955617	0.5025	0.001	36	17	76414859	Body;Body	TRUE
cg21550504	0.6946	-0.003	36	17	76414916	Body;Body	TRUE
cg16636468	0.6225	0.000	36	17	76415169	Body;Body	TRUE
cg25337513	0.8629	0.000	36	17	76415226	Body;Body	TRUE
cg07126783	0.0130	-0.019	36	17	76415362	Body;Body	TRUE
cg16638092	0.0070	-0.021	36	17	76415369	Body;Body	TRUE
cg08939850	0.0289	-0.024	36	17	76415401	Body;Body	TRUE
cg23715732	0.9932	-0.004	36	17	76416446	Body;Body	NA
cg05113898	0.7910	-0.001	36	17	76416564	Body;Body	NA
cg08219486	0.2564	0.001	36	17	76416699	Body;Body	NA
cg26633077	0.9590	0.000	36	17	76417720	Body;Body	NA
cg22984380	0.1903	-0.010	36	17	76417744	Body;Body	NA
cg24155025	0.1009	0.000	36	17	76417774	Body;Body	NA
cg26332535	0.6365	0.006	36	17	76417779	Body;Body	NA
cg06919800	0.2502	-0.003	36	17	76417915	Body;Body	NA
cg18607849	0.2512	-0.001	36	17	76418030	Body;Body	NA
cg23210522	0.2427	0.001	36	17	76418069	Body;Body	NA
cg19287064	0.3106	0.002	36	17	76418916	Body;Body	NA
cg21876181	0.7520	-0.001	36	17	76419031	Body;Body	NA
cg15826479	0.0414	0.014	36	17	76421088	Body;Body	TRUE
cg02386420	0.3306	0.000	36	17	76421465	Body;Body	TRUE
cg18516619	0.5753	0.003	36	17	76421486	Body;Body	TRUE
cg11637695	0.6786	-0.002	36	17	76422655	Body;Body	NA
cg22644320	0.6042	-0.001	36	17	76422782	Body;Body	NA

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg07530194	0.8677	-0.003	36	17	76423045	Body;Body	NA
cg19122260	0.5822	0.000	36	17	76423165	Body;Body	NA
cg20502501	0.5128	-0.007	36	17	76423998	Body;Body	NA
cg23248537	0.2625	-0.004	36	17	76424293	Body;Body	NA
cg03443590	0.4310	-0.002	36	17	76424447	Body;Body	NA
cg01464730	0.8055	-0.001	36	17	76424468	Body;Body	NA
cg12100537	0.4647	-0.002	36	17	76426131	Body;Body	NA
cg27101023	0.9609	0.006	36	17	76426276	Body;Body	NA
cg07475546	0.9147	-0.002	36	17	76426423	Body;Body	NA
cg21005054	0.7539	-0.001	36	17	76426710	Body;Body	NA
cg11824764	0.3056	0.000	36	17	76427082	Body;Body	NA
cg26263310	0.4535	-0.004	36	17	76428708	Body;Body	NA
cg18173185	0.1652	-0.003	36	17	76429257	Body;Body	NA
cg12434898	0.0064	-0.004	36	17	76432237	Body;Body	NA
cg25899969	0.5357	-0.002	36	17	76432533	Body;Body	NA
cg01911440	0.0600	0.001	36	17	76432640	Body;Body	NA
cg18648066	0.7551	-0.001	36	17	76433036	Body;Body	NA
cg02284802	0.1548	-0.006	36	17	76433173	Body;Body	NA
cg20500836	0.2073	0.002	36	17	76433240	Body;Body	NA
cg21507958	0.9498	0.000	36	17	76433466	Body;Body	NA
cg18091083	0.0381	0.022	36	17	76433687	Body;Body	NA
cg22888023	0.8116	-0.003	36	17	76434599	Body;Body	NA
cg23019125	0.5344	0.002	36	17	76434986	Body;Body	NA
cg16826504	0.3110	-0.003	36	17	76435176	Body;Body	NA
cg06756931	0.7339	-0.001	36	17	76435488	Body;Body	NA
cg14202916	0.5049	0.001	36	17	76435519	Body;Body	NA
cg01767927	0.7192	-0.003	36	17	76435619	Body;Body	NA
cg26360197	0.0010	0.030	36	17	76436199	Body;Body	NA
cg16541275	0.0432	0.017	36	17	76436349	Body;Body	NA
cg24844295	0.7784	0.001	36	17	76436523	Body;Body	NA
cg01518942	0.2359	-0.003	36	17	76437001	Body;Body	NA
cg12284870	0.2283	-0.005	36	17	76437026	Body;Body	NA
cg17481637	0.9598	0.001	36	17	76437125	Body;Body	NA
cg13945540	0.4211	0.001	36	17	76437320	Body;Body	NA
cg04102793	0.9524	-0.002	36	17	76437518	Body;Body	NA
cg04515258	0.8550	-0.001	36	17	76440007	Body;Body	NA
cg05815404	0.4279	0.000	36	17	76440096	Body;Body	NA
cg14271651	0.0295	-0.009	36	17	76440964	Body;Body	TRUE

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg14647957	0.3152	-0.004	36	17	76442838	Body;Body	NA
cg25024459	0.7461	-0.003	36	17	76444221	Body;Body	NA
cg15097361	0.0070	-0.017	36	17	76444296	Body;Body	NA
cg15228441	0.9427	-0.002	36	17	76444525	Body;Body	TRUE
cg04470054	0.1395	-0.003	36	17	76445067	Body;Body	TRUE
cg25512107	0.0106	0.002	36	17	76445762	Body;Body	NA
cg17323298	0.7979	-0.001	36	17	76445825	Body;Body	NA
cg22809418	0.3980	0.000	36	17	76445905	Body;Body	NA
cg08905415	0.7213	-0.003	36	17	76446113	Body;Body	NA
cg04494230	0.6880	0.000	36	17	76446239	Body;Body	NA
cg00516616	0.8101	-0.004	36	17	76446458	Body;Body	NA
cg24832218	0.7079	-0.003	36	17	76447543	Body;Body	TRUE
cg04191427	0.7525	-0.005	36	17	76447854	Body;Body	NA
cg05707492	0.3740	0.000	36	17	76448079	Body;Body	TRUE
cg05249744	0.0233	-0.027	36	17	76449305	Body;Body	NA
cg23625086	0.0284	0.002	36	17	76450934	Body;Body	NA
cg15476425	0.2539	0.001	36	17	76450998	Body;Body	NA
cg22161269	0.9634	-0.002	36	17	76451108	Body;Body	NA
cg01886663	0.1208	-0.014	36	17	76460774	Body;Body	TRUE
cg02878831	0.2833	-0.010	36	17	76462347	Body;Body	NA
cg27129144	0.3659	-0.002	36	17	76463128	Body;Body	NA
cg27394817	0.6181	-0.001	36	17	76463274	Body;Body	NA
cg07434008	0.5516	0.000	36	17	76463361	Body;Body	NA
cg06617879	0.0058	0.005	36	17	76464026	Body;Body	NA
cg13979266	0.3848	-0.007	36	17	76464322	Body;Body	NA
cg15358690	0.9109	0.000	36	17	76464505	Body;Body	NA
cg16116279	0.1482	-0.004	36	17	76464511	Body;Body	NA
cg16721879	0.3823	-0.002	36	17	76464566	Body;Body	NA
cg03119454	0.7343	0.000	36	17	76465075	Body;Body	NA
cg16886414	0.1244	0.012	36	17	76465744	Body;Body	NA
cg08314949	0.1652	0.018	36	17	76465808	Body;Body	NA
cg12078154	0.3725	0.016	36	17	76465857	Body;Body	NA
cg02251850	0.0008	0.035	36	17	76466098	Body;Body	NA
cg22091236	0.0033	-0.028	36	17	76468561	Body;Body	TRUE
cg27457201	0.0153	-0.020	36	17	76468827	Body;Body	NA
cg09977718	0.0499	-0.016	36	17	76468868	Body;Body	NA
cg22255288	0.0954	0.003	36	17	76469013	Body;Body	NA
cg09964933	0.6074	0.000	36	17	76470841	Body;Body	NA

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg21343406	0.3031	-0.002	36	17	76470926	Body;Body	NA
cg09318637	0.8519	-0.001	36	17	76471009	Body;Body	NA
cg08588357	0.0125	0.003	36	17	76471270	Body;Body	NA
cg17872658	0.0631	0.006	36	17	76471456	Body;Body	NA
cg05887890	0.2151	-0.005	36	17	76471606	Body;Body	NA
cg17585356	0.7787	-0.002	36	17	76471716	Body;Body	NA
cg04166962	0.5127	-0.002	36	17	76471833	Body;Body	NA
cg19185574	0.8236	-0.002	36	17	76472226	Body;Body	NA
cg23630758	0.2275	-0.002	36	17	76472307	Body;Body	NA
cg13831388	0.4582	-0.002	36	17	76472454	Body;Body	NA
cg18224819	0.8864	0.002	36	17	76472571	Body;Body	NA
cg02933375	0.8242	-0.001	36	17	76473423	Body;Body	NA
cg08150315	0.5730	0.001	36	17	76473562	Body;Body	NA
cg11623293	0.2144	-0.004	36	17	76473655	Body;Body	NA
cg24181389	0.9718	-0.002	36	17	76474340	Body;Body	NA
cg02243479	0.6669	0.000	36	17	76474554	Body;Body	NA
cg16660971	0.8045	-0.003	36	17	76474624	Body;Body	NA
cg13549638	0.0781	-0.014	36	17	76474671	Body;Body	NA
cg26954228	0.5258	-0.002	36	17	76475078	Body;Body	NA
cg26714263	0.3528	-0.002	36	17	76475106	Body;Body	NA
cg06154633	0.7570	0.000	36	17	76475200	Body;Body	NA
cg18562896	0.3348	-0.002	36	17	76477462	Body;Body	NA
cg24963810	0.7351	0.000	36	17	76477488	Body;Body	NA
cg16018154	0.3427	-0.009	36	17	76478165	Body;Body	NA
cg09891288	0.9173	0.006	36	17	76478269	Body;Body	NA
cg25902229	0.7244	0.009	36	17	76478683	Body;Body	NA
cg05774614	0.0449	-0.019	36	17	76479134	Body;Body	NA
cg10035831	0.2491	0.010	36	17	76479682	Body;Body	NA
cg22636722	0.4223	0.008	36	17	76479858	Body;Body	TRUE
cg00704970	0.9120	0.003	36	17	76479963	Body;Body	TRUE
cg03502601	0.3925	0.014	36	17	76479968	Body;Body	TRUE
cg09803959	0.0945	-0.013	36	17	76480109	Body;Body	TRUE
cg24207068	0.7115	-0.004	36	17	76480257	Body;Body	TRUE
cg04658243	0.1329	0.011	36	17	76480350	Body;Body	TRUE
cg11476241	0.8042	-0.003	36	17	76480830	Body;Body	TRUE
cg24327522	0.5242	-0.004	36	17	76481174	Body;Body	NA
cg10490202	0.9245	-0.001	36	17	76482226	Body;Body	NA
cg17628491	0.1611	-0.072	36	17	76482417	Body;Body	NA

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg21289763	0.6645	-0.004	36	17	76483430	Body;Body	NA
cg17180011	0.9825	0.000	36	17	76483873	Body;Body	NA
cg15022015	0.3763	0.001	36	17	76484122	Body;Body	NA
cg05064567	0.2731	-0.006	36	17	76484733	Body;Body	NA
cg04919811	0.7690	0.000	36	17	76484897	Body;Body	NA
cg16578291	0.0447	0.003	36	17	76485004	Body;Body	NA
cg19292222	0.7272	-0.006	36	17	76486006	Body;Body	NA
cg23938645	0.5029	-0.001	36	17	76487158	Body;Body	NA
cg06053702	0.0003	-0.010	36	17	76487304	Body;Body	NA
cg10538214	0.7647	-0.003	36	17	76487470	Body;Body	NA
cg16780847	0.3163	-0.004	36	17	76488294	Body;Body	NA
cg10880603	0.7098	-0.002	36	17	76488349	Body;Body	NA
cg11274148	0.2864	-0.001	36	17	76491251	Body;Body	NA
cg01412400	0.5867	0.001	36	17	76491321	Body;Body	NA
cg12131324	0.2116	-0.005	36	17	76491389	Body;Body	NA
cg19494960	0.0803	-0.002	36	17	76491556	Body;Body	NA
cg00474943	0.7289	-0.001	36	17	76491671	Body;Body	NA
cg24683534	0.4077	-0.005	36	17	76491800	Body;Body	NA
cg04163696	0.8307	0.000	36	17	76492566	Body;Body	NA
cg16895810	0.0604	-0.003	36	17	76492646	Body;Body	NA
cg09173565	0.3509	0.000	36	17	76492873	Body;Body	NA
cg22673070	0.9268	-0.004	36	17	76493125	Body;Body	NA
cg16218910	0.6600	-0.002	36	17	76493174	Body;Body	NA
cg19296258	0.8778	-0.001	36	17	76493394	Body;Body	NA
cg05580441	0.3368	0.000	36	17	76493439	Body;Body	NA
cg08992574	0.3860	-0.002	36	17	76494240	Body;Body	NA
cg02257048	0.7990	0.000	36	17	76494626	Body;Body	NA
cg07870603	0.8592	0.001	36	17	76494739	Body;Body	NA
cg01000996	0.9733	-0.002	36	17	76494821	Body;Body	NA
cg00554570	0.9380	-0.001	36	17	76494823	Body;Body	NA
cg06908052	0.9305	-0.001	36	17	76494991	Body;Body	NA
cg10462529	0.4569	-0.001	36	17	76495343	Body;Body	NA
cg06485000	0.1252	-0.005	36	17	76496316	Body;Body	NA
cg10508138	0.5197	-0.003	36	17	76496608	Body;Body	NA
cg02671711	0.7700	0.000	36	17	76496795	Body;Body	NA
cg19984991	0.2472	0.002	36	17	76497089	Body;Body	NA
cg00248805	0.1761	0.002	36	17	76497144	Body;Body	NA
cg19707379	0.9561	0.000	36	17	76497288	Body;Body	NA

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg14003223	0.6147	0.000	36	17	76497315	Body;Body	NA
cg14289594	0.3493	-0.003	36	17	76497349	Body;Body	NA
cg16892887	0.8649	0.003	36	17	76506807	Body;Body	NA
cg04789650	0.6675	-0.003	36	17	76506890	Body;Body	NA
cg21000762	0.7766	-0.001	36	17	76507445	Body;Body	NA
cg14059665	0.9123	-0.002	36	17	76507480	Body;Body	NA
cg15331383	0.2774	-0.004	36	17	76507958	Body;Body	NA
cg01525498	0.5356	-0.003	36	17	76508024	Body;Body	NA
cg07078467	0.5535	0.002	36	17	76508207	Body;Body	NA
cg15694704	0.2755	-0.009	36	17	76508959	Body;Body	NA
cg06096901	0.9803	0.000	36	17	76508988	Body;Body	NA
cg21818807	0.4883	0.000	36	17	76509072	Body;Body	NA
cg09790523	0.4747	-0.003	36	17	76509659	Body;Body	NA
cg09794615	0.1099	0.010	36	17	76509801	Body;Body	NA
cg05395366	0.2359	0.005	36	17	76509868	Body;Body	NA
cg17052885	0.0947	-0.005	36	17	76510607	Body;Body	NA
cg21734751	0.4560	0.000	36	17	76511032	Body;Body	NA
cg12044293	0.4862	-0.001	36	17	76511264	Body;Body	NA
cg17144164	0.0500	-0.002	36	17	76511295	Body;Body	NA
cg24744721	0.5349	-0.001	36	17	76511564	Body;Body	NA
cg06358794	0.6246	0.001	36	17	76511697	Body;Body	NA
cg26290973	0.3413	0.001	36	17	76511732	Body;Body	NA
cg02346006	0.3890	-0.001	36	17	76512072	Body;Body	NA
cg21219851	0.7685	-0.001	36	17	76512784	Body;Body	NA
cg07450393	0.8688	0.000	36	17	76512857	Body;Body	NA
cg16732367	0.2475	0.001	36	17	76512964	Body;Body	NA
cg09139509	0.5410	0.001	36	17	76513611	Body;Body	NA
cg06091647	0.0260	0.005	36	17	76513987	Body;Body	NA
cg05814100	0.9118	-0.003	36	17	76514392	Body;Body	NA
cg25739309	0.9893	-0.007	36	17	76515047	Body;Body	TRUE
cg02638755	0.3795	-0.006	36	17	76516964	Body;Body	NA
cg11188237	0.5847	-0.001	36	17	76516991	Body;Body	NA
cg18802706	0.3898	-0.001	36	17	76517029	Body;Body	NA
cg18612040	0.0580	-0.005	36	17	76519080	Body;Body	TRUE
cg07584637	0.1946	-0.005	36	17	76526172	Body;Body	NA
cg19394169	0.2908	-0.005	36	17	76526269	Body;Body	NA
cg03641032	0.6363	-0.002	36	17	76526363	Body;Body	NA
cg11949518	0.3498	0.048	36	17	76527360	Body;Body	NA

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg24315876	0.3700	0.009	36	17	76527706	Body;Body	NA
cg02580745	0.1085	0.002	36	17	76528696	Body;Body	NA
cg02266055	0.7714	-0.001	36	17	76528924	Body;Body	NA
cg00648660	0.7319	-0.001	36	17	76528951	Body;Body	NA
cg06469955	0.3871	-0.002	36	17	76529741	Body;Body	NA
cg13643509	0.4705	0.001	36	17	76530250	Body;Body	TRUE
cg19060120	0.1430	-0.007	36	17	76530473	Body;Body	TRUE
cg23736297	0.5482	-0.004	36	17	76530476	Body;Body	TRUE
cg13005428	0.0522	-0.036	36	17	76530973	Body;Body	TRUE
cg16438182	0.5382	0.000	36	17	76531162	Body;Body	TRUE
cg02033669	0.6037	0.001	36	17	76531614	Body;Body	NA
cg21238376	0.2941	0.003	36	17	76534371	Body;Body	NA
cg08999272	0.7934	-0.002	36	17	76536073	Body;Body	NA
cg11782601	0.8057	-0.002	36	17	76536493	Body;Body	NA
cg26729320	0.7299	-0.002	36	17	76536639	Body;Body	NA
cg14600877	0.6821	-0.002	36	17	76536815	Body;Body	NA
cg13597013	0.8713	-0.001	36	17	76537122	Body;Body	NA
cg13762486	0.2658	-0.007	36	17	76537745	Body;Body	NA
cg06420480	0.9135	-0.001	36	17	76537839	Body;Body	NA
cg19443023	0.6000	0.000	36	17	76537939	Body;Body	NA
cg07081946	0.8550	-0.001	36	17	76538447	Body;Body	NA
cg26469982	0.7033	-0.003	36	17	76538527	Body;Body	NA
cg02254800	0.1718	-0.004	36	17	76538681	Body;Body	NA
cg24343322	0.9926	-0.002	36	17	76539767	Body;Body	NA
cg25057221	0.2770	0.002	36	17	76539827	Body;Body	NA
cg18425700	0.3988	-0.004	36	17	76539873	Body;Body	NA
cg03052541	0.7777	-0.001	36	17	76540138	Body;Body	NA
cg18815595	0.7748	-0.001	36	17	76540402	Body;Body	NA
cg17779026	0.8527	-0.001	36	17	76540405	Body;Body	NA
cg21831512	0.1568	-0.009	36	17	76540686	Body;Body	NA
cg26932839	0.7133	-0.001	36	17	76540725	Body;Body	NA
cg24180621	0.0940	-0.003	36	17	76540780	Body;Body	NA
cg17831694	0.3741	-0.003	36	17	76544495	Body;Body	NA
cg11762703	0.0912	0.011	36	17	76544533	Body;Body	NA
cg02403929	0.3404	0.001	36	17	76544901	Body;Body	NA
cg22486214	0.4842	-0.001	36	17	76545061	Body;Body	NA
cg02047211	0.9318	-0.002	36	17	76547292	Body;Body	NA
cg02864619	0.8175	0.000	36	17	76548556	Body;Body	TRUE

Fig. 23 (continued)

Table 23. The methylation levels of RPTOR CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg17906851	0.2149	-0.036	36	17	76549665	Body;Body	NA
cg21879029	0.7815	0.000	36	17	76549770	Body;Body	NA
cg09491897	0.2438	-0.001	36	17	76549864	Body;Body	NA
cg07964113	0.1756	0.001	36	17	76549884	Body;Body	NA
cg09361653	0.9428	0.000	36	17	76550887	Body;Body	NA
cg17408291	0.7710	-0.001	36	17	76550967	Body;Body	NA
cg04275040	0.2387	0.000	36	17	76551223	Body;Body	NA
cg03800447	0.4430	0.000	36	17	76551344	Body;Body	NA
cg08804421	0.4328	0.003	36	17	76551392	Body;Body	NA
cg26886231	0.4056	0.000	36	17	76551484	Body;Body	NA
cg13136721	0.5328	0.003	36	17	76551778	Body;Body	NA
cg10278297	0.3103	-0.003	36	17	76552433	Body;Body	NA
cg10752731	0.3004	0.000	36	17	76552729	3'UTR;3'UTR	NA
cg03890538	0.5621	-0.003	36	17	76552939	3'UTR;3'UTR	NA
cg18732855	0.0473	0.001	36	17	76553701	3'UTR;3'UTR	NA
cg03389944	0.4000	-0.002	36	17	76554181	3'UTR;3'UTR	NA
cg15432510	0.2292	-0.002	36	17	76554265	3'UTR;3'UTR	NA
cg09439604	0.9534	-0.002	36	17	76554624	3'UTR;3'UTR	NA
cg15406978	0.4216	0.002	36	17	76554688	3'UTR;3'UTR	NA
cg25290617	0.0035	-0.012	36	17	76554705	3'UTR;3'UTR	NA
cg23051282	0.0132	-0.002	36	17	76554756	3'UTR;3'UTR	NA

Fig. 24

Table 24. The methylation levels of MGRN1 CpG sites by Illumina 450K

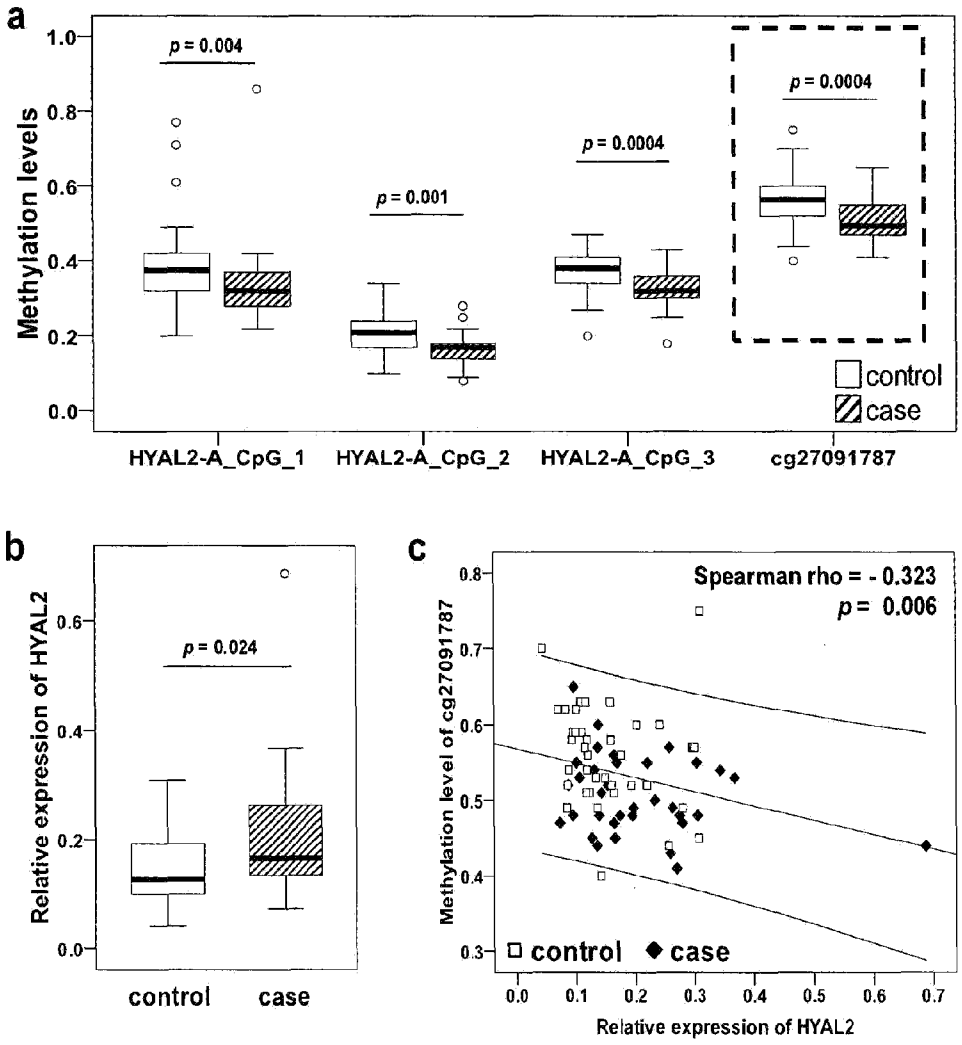
CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg03819286	0.0003	-0.024	36	16	4613975	N_Shore	NA
cg01678580	0.0839	-0.007	36	16	4614019	N_Shore	NA
cg08058836	0.9419	0.001	36	16	4614353	N_Shore	NA
cg00369126	0.7741	0.001	36	16	4614643	N_Shore	NA
cg00203035	0.9047	0.000	36	16	4614660	N_Shore	NA
cg02283436	0.3901	0.003	36	16	4614678	N_Shore	NA
cg04367464	0.9647	0.001	36	16	4614717	Island	NA
cg01482556	0.5192	0.001	36	16	4614719	Island	NA
cg00588858	0.2908	-0.001	36	16	4615007	Island	NA
cg10442572	0.4991	0.001	36	16	4615385	Island	NA
cg16118148	0.7137	0.001	36	16	4615388	Island	NA
cg08142943	0.8882	0.001	36	16	4615652	S_Shore	NA
cg08147187	0.9335	-0.001	36	16	4619397	S_Shelf	NA
cg00693240	0.6552	0.000	36	16	4624508		NA
cg02404489	0.9706	0.000	36	16	4624597		NA
cg08524372	0.8553	-0.002	36	16	4624732		NA
cg05459609	0.0097	-0.004	36	16	4629951		TRUE
cg07741192	0.8363	0.000	36	16	4630014		TRUE
cg02968175	0.4863	-0.001	36	16	4630021		TRUE
cg03336832	0.9156	-0.002	36	16	4636109	N_Shelf	NA
cg03427191	0.0004	0.024	36	16	4637113	N_Shore	NA
cg23233631	0.1891	-0.004	36	16	4638221	Island	NA
cg09440989	0.0021	-0.007	36	16	4639882	S_Shore	NA
cg04071866	0.0023	-0.020	36	16	4640738	N_Shore	NA
cg00639215	0.1100	0.001	36	16	4642770	Island	NA
cg05782454	0.2161	-0.005	36	16	4642835	Island	NA
cg26700932	0.2518	-0.004	36	16	4643004	S_Shore	NA
cg05287064	0.4451	-0.003	36	16	4645946	S_Shelf	NA
cg02647929	0.0001	-0.029	36	16	4654081		NA
cg06323332	0.0000	-0.032	36	16	4654230		NA
cg27193519	0.0001	-0.042	36	16	4654444		NA
cg01922891	0.0000	-0.031	36	16	4654648		NA
cg04962621	0.0001	-0.034	36	16	4654734		NA
cg01156249	0.0244	-0.018	36	16	4654795		NA
cg07635227	0.0124	-0.014	36	16	4654816		NA
cg09250423	0.2256	0.002	36	16	4657753		NA
cg03420907	0.4692	0.001	36	16	4657773		NA
cg08760128	0.3017	-0.001	36	16	4663508		NA

Fig. 24 (continued)

Table 24. The methylation levels of MGRN1 CpG sites by Illumina 450K (continued)

CpG ID	pvalWald_Group	meanDiff	BUILD	CHR	MAPINFO	LOCATION	ENHANCER
cg27004760	0.7278	-0.002	36	16	4663623		NA
cg03693714	0.1679	-0.004	36	16	4663817		NA
cg05383524	0.7179	-0.001	36	16	4666971	N_Shelf	NA
cg04087057	0.7062	-0.003	36	16	4668188	N_Shelf	NA
cg27436118	0.0002	-0.031	36	16	4669906	N_Shore	NA
cg08782022	0.0048	-0.048	36	16	4670137	N_Shore	TRUE
cg01662869	0.0000	-0.045	36	16	4670411	Island	TRUE
cg00736299	0.0000	-0.043	36	16	4670466	Island	TRUE
cg02074956	0.0000	-0.032	36	16	4670658	N_Shore	TRUE
cg10505257	0.0003	-0.030	36	16	4671640	Island	NA
cg07812289	0.6799	0.000	36	16	4671719	Island	NA
cg05901634	0.3634	0.003	36	16	4671822	Island	NA
cg00504410	0.0025	-0.014	36	16	4672263	Island	NA
cg03963853	0.1509	-0.002	36	16	4672370	Island	NA
cg07248377	0.0109	-0.015	36	16	4672407	Island	NA
cg10908196	0.0021	-0.018	36	16	4672912	Island	NA
cg01861603	0.8633	-0.002	36	16	4672974	Island	NA
cg16520815	0.0002	-0.030	36	16	4673182	Island	NA
cg09306188	0.0000	-0.057	36	16	4673254	S_Shore	NA
cg04208175	0.8742	-0.002	36	16	4674630	S_Shore	NA
cg04083430	0.0087	-0.035	36	16	4676064	S_Shelf	NA
cg16778018	0.0922	-0.006	36	16	4676226	S_Shelf	NA
cg26627888	0.1955	0.002	36	16	4676420	S_Shelf	NA
cg16576106	0.8006	0.000	36	16	4676779	S_Shelf	NA
cg02072002	0.8181	0.000	36	16	4676796	S_Shelf	NA
cg16420089	0.6085	-0.002	36	16	4676870	S_Shelf	NA
cg02352612	0.1408	-0.005	36	16	4677994		NA
cg00033551	0.0179	-0.012	36	16	4678569		NA
cg03157150	0.8802	-0.002	36	16	4678681		NA
cg09005651	0.0003	-0.013	36	16	4680695	N_Shelf	NA
cg10090769	0.0093	-0.013	36	16	4680792	N_Shelf	NA

Fig. 25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control
case
HYAL2-A_CpG_1
HYAL2-A_CpG_2
HYAL2-A_CpG_3
cg27091787
b
Relative expression of HYAL2
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0.4
0.2
0.0
p = 0.024
control
case
c
Methylation level of cg27091787
0.8
0.7
0.6
0.5
0.4
0.3
Spearman rho = - 0.323
p = 0.006
control
case
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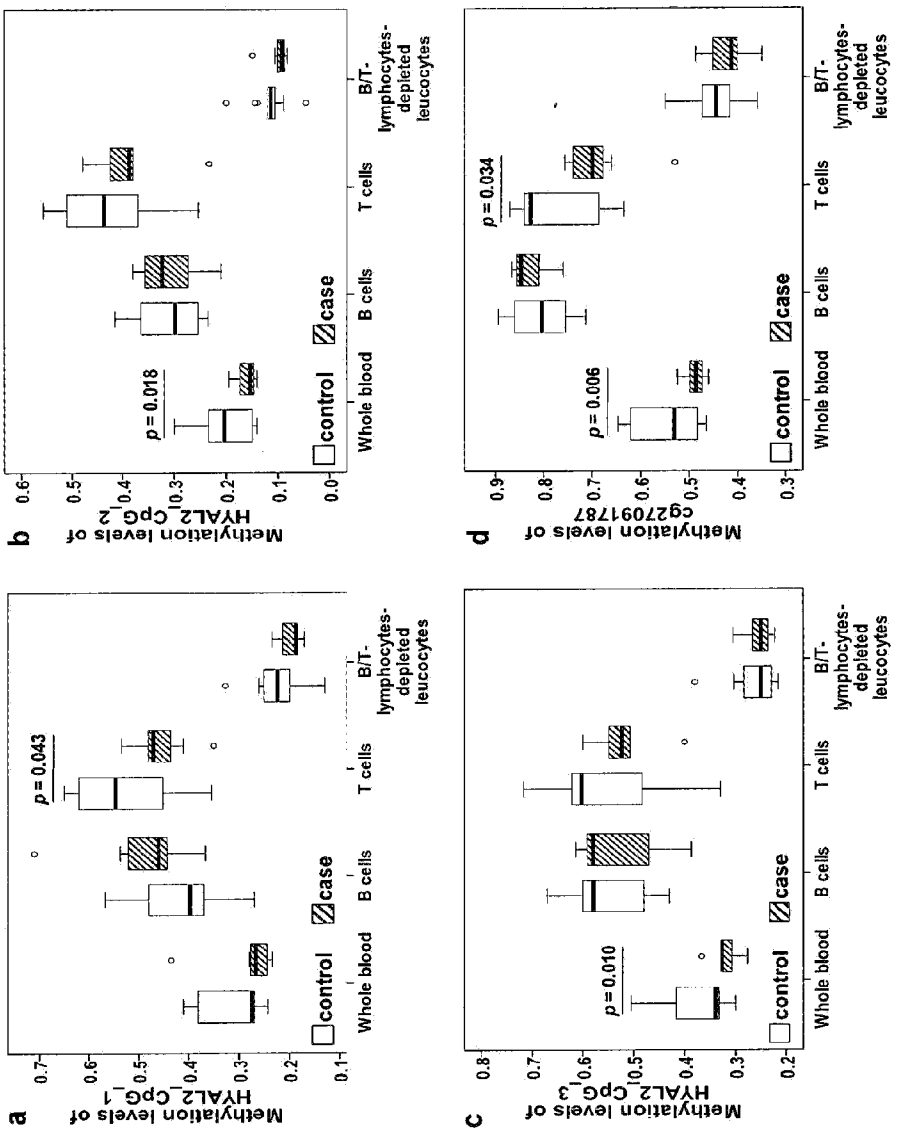
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Relative expression of HYAL2

Fig. 26


a
Methylation levels of HYAL2_CpG_1
p = 0.043
b
Methylation levels of HYAL2_CpG_2
p = 0.018
c
Methylation levels of HYAL2_CpG_3
p = 0.010
d
Methylation levels of cg27091787
p = 0.034
control
case
Whole blood
B cells
T cells
B/T-lymphocytes-depleted leucocytes