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(54) Title: METHODS FOR IDENTIFICATION OF TUMOR PHENOTYPE AND TREATMENT

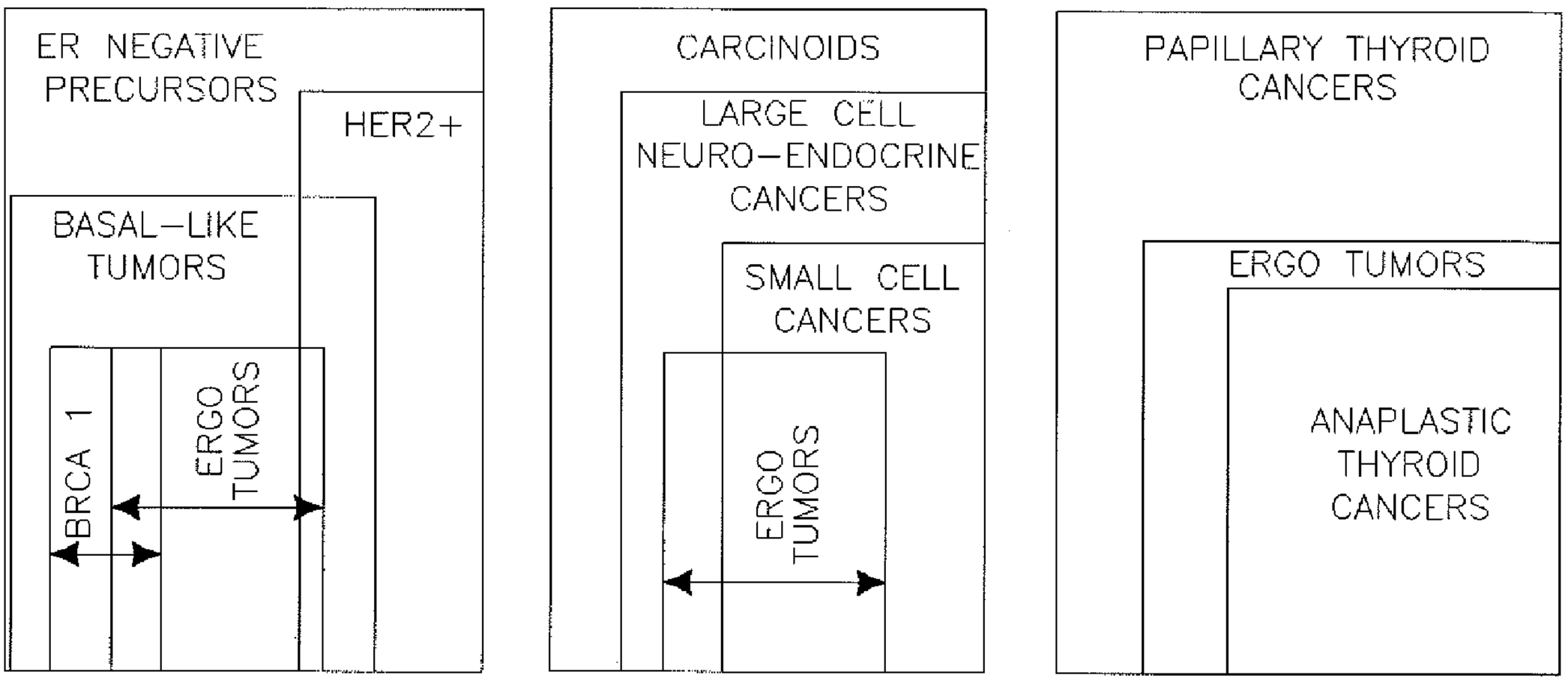

FIG.7

(57) Abrégé/Abstract:
The disclosure relates to methods for identifying a tumor as an E2F-responsive gene over-expressing (ERGO) tumor, methods of determining the likelihood that an ERGO tumor patient will survive to a future date, methods of treating an ERGO tumor in a patient, and methods of selecting patients diagnosed as ERGO tumor prostate cancer patients for aggressive clinical treatment. The methods of the disclosure are applicable to ERGO tumors present in different human organs and tissues such as breast, lung, thyroid, ovary, and prostate.

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(54) Title: METHODS FOR IDENTIFICATION OF TUMOR PHENOTYPE AND TREATMENT

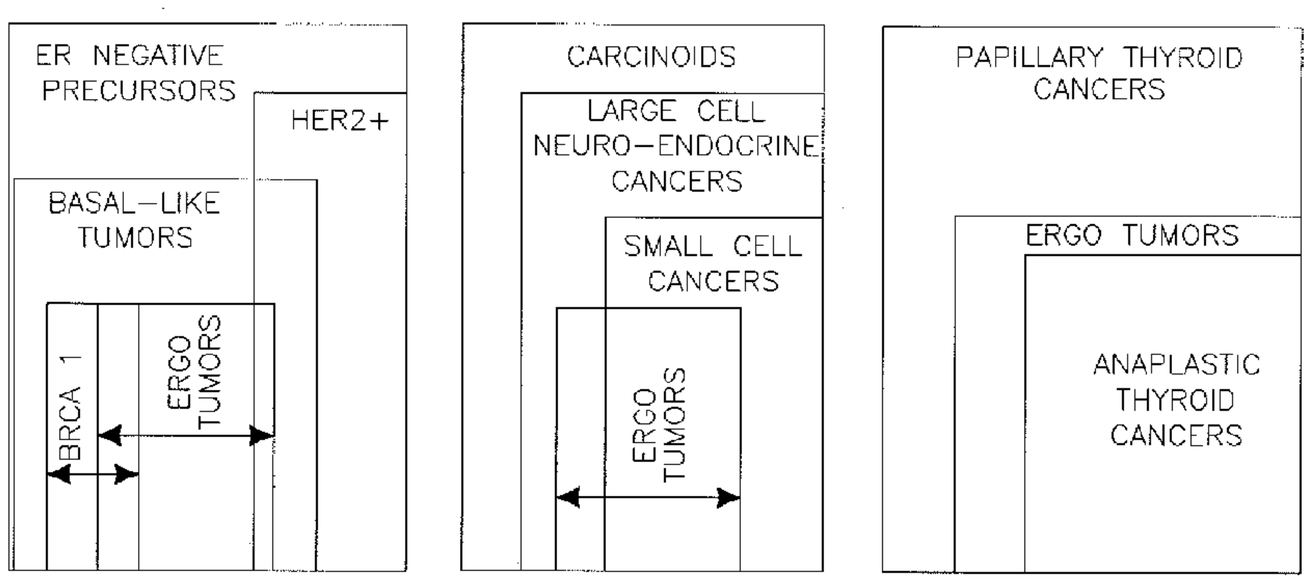

FIG.7

(57) Abstract: The disclosure relates to methods for identifying a tumor as an E2F-responsive gene over-expressing (ERGO) tumor, methods of determining the likelihood that an ERGO tumor patient will survive to a future date, methods of treating an ERGO tumor in a patient, and methods of selecting patients diagnosed as ERGO tumor prostate cancer patients for aggressive clinical treatment. The methods of the disclosure are applicable to ERGO tumors present in different human organs and tissues such as breast, lung, thyroid, ovary, and prostate.

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METHODS FOR IDENTIFICATION OF TUMOR PHENOTYPE AND TREATMENT

Related Application

This patent application claims the benefit of US Provisional Application No. 61/199,295 filed 14 November 2008.

Field of the Disclosure

The disclosure relates to methods for identifying a tumor as an E2F-responsive gene over-expressing (ERGO) tumor, methods of determining the likelihood that an ERGO tumor patient will survive to a future date, methods of treating an ERGO tumor in a patient, and methods of selecting patients diagnosed as ERGO tumor prostate cancer patients for aggressive clinical treatment. The methods of the disclosure are applicable to ERGO tumors present in different human organs and tissues such as breast, lung, thyroid, ovary, and prostate.

Background of the Disclosure

A major challenge in cancer treatment is to target specific therapies to distinct tumor types in order to maximize efficacy and minimize toxicity. Meeting this challenge requires that physicians and others involved in the treatment of cancer patients be able to identify distinct tumor types, determine the likelihood of patient survival given the patient's distinct tumor type, and be able to select appropriate treatments.

In the oncology field it is currently standard practice to identify tumor types using the microscopic histopathologic appearance of fixed and stained tumor samples, and to utilize the tumor-node-metastasis (TNM) system to determine the clinical extent of tumor spread. The TNM system uses the size of the tumor, the presence or absence of tumors in regional lymph nodes, and the presence or absence of distant metastases to assign a stage to the tumor. The tumor type and the stage assigned to a tumor are used as a basis for the selection of appropriate therapy and for prognostic purposes. However, this approach has serious limitations. This is because tumors with similar histopathologic appearance can exhibit significant variability in terms of clinical course and response to therapy. For example, some tumors spread early to distant sites and are rapidly progressive while others are not, and some tumors respond readily to hormonal therapy or chemotherapy while others are resistant.

Basal-like breast cancer tumors are one example of a tumor type that grows rapidly and which typically are not treated effectively with conventional adjuvant therapies such as

hormonal therapy or chemotherapy. Clinically, basal-like breast cancers behave aggressively, and while they may respond to chemotherapy initially, these responses are generally of brief duration, and survival times for most patients with these tumors are relatively short. Histologically, most basal-like breast cancers are poorly differentiated ductal carcinomas. Breast cancer tumors are normally classified as basal-like in the clinical setting based on whether the tumors have a so-called "triple-negative" phenotype characterized by a lack of over-expression of human epidermal growth factor receptor 2 (HER2), the estrogen receptor (ER), and the progesterone receptor (PR) and if the tumor over-expresses basal-like cytokeratins or other basal-like markers. Unfortunately, therapeutic options for patients with basal-like breast cancers are limited, because patients with such tumors are not candidates for hormonal therapy or targeted therapy against HER2 and there currently is no clear understanding of the underlying pathobiology of basal-like breast cancers. Importantly, basal-like tumors and distinct subsets of such tumors may also arise in other tissues and organs such as lung, thyroid, ovarian, and prostate tissues, but this has been poorly studied.

Furthermore, many tumors have aberrations in the expression or biological activity of proteins encoded by tumor suppressor genes. Aberrations in the biological activity of proteins encoded by tumor suppressor genes due to mutations in these genes or decreased gene transcription at the level of mRNA or protein expression can lead to unrestrained cell division. The Rb protein is an example of a protein encoded by a tumor suppressor gene. Normally, the Rb tumor suppressor protein binds to the E2F transcription factor and regulates E2F mediated gene transcription to negatively modulate and control cell proliferation. Importantly, the role of Rb and E2F in basal-like tumors in breast tissues and other tissues such as lung, thyroid, ovarian, and prostate tissues is also poorly understood.

Thus, there is a need for methods to identify tumors that over-express E2F responsive genes, methods for determining the likelihood that a patient diagnosed with a particular distinct tumor type will survive to a future date, methods for identifying and treating distinct tumor types, and methods for identifying target proteins expressed by a distinct tumor type in different tissues such as breast, lung, thyroid, ovarian prostate and other tissues.

Summary of the Disclosure

One aspect of the disclosure is a method of identifying a tumor as an ERGO tumor comprising the steps of providing a tumor sample; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of

the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed; whereby the tumor is identified as an ERGO tumor if at least 21 of these gene transcripts are over-expressed.

Another aspect of the disclosure is a method of determining the odds that an individual ERGO tumor patient will survive to a future date comprising the steps of providing a tumor sample from an individual patient; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor and the individual patient is diagnosed as an ERGO tumor patient if at least 21 of these gene transcripts are over-expressed; plotting the fraction of surviving patients in a population of patients diagnosed as ERGO tumor patients as a function of the time since diagnosis of the ERGO tumor to generate a survival plot; and selecting a future date after the individual patient is diagnosed as an ERGO tumor patient and determining the fraction of surviving patients in the population from the survival plot; whereby the fraction of surviving patients on the survival plot at the future date predicts the odds that an individual tumor patient will survive to the future date.

Another aspect of the disclosure is a method of treating an ERGO tumor in a patient comprising the steps of providing a tumor sample from a patient; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88,

91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor if at least 21 of these gene transcripts are over-expressed; selecting a drug capable of killing or inhibiting division of an ERGO tumor cell expressing at least one protein encoded by at least one gene transcript selected from the group consisting of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; and administering a pharmaceutically acceptable amount of the drug to the patient; whereby the ERGO tumor in the patient is treated.

Another aspect of the disclosure is a method of identifying an individual tumor in a population of tumors as an ERGO tumor comprising the steps of providing a population of tumor samples; providing a reference; measuring gene transcript levels in the tumor samples to produce a transcript value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 10-340; comparing the transcript value to the reference value for each tumor to identify the gene transcripts over-expressed by each tumor; ranking the tumors in the population with a rank ordering algorithm to order the tumors according to the number of the gene transcripts over-expressed by each tumor; and removing individual tumors from the population that over-express the smallest number of each gene transcript per cell and that have the lowest levels of over-expression of each gene transcript per cell until at least 20% of the individual tumors remaining in the population over-express at least 20% of the gene transcripts; whereby an individual tumor remaining in the population of tumors is identified as an ERGO tumor.

Another aspect of the disclosure is a method of identifying an individual tumor in a population of tumors as an ERGO tumor comprising the steps of providing a population of tumor samples; providing a reference; measuring gene transcript levels in the tumor samples to produce a transcript value for each of the following gene transcripts having the nucleic acid

sequence shown in SEQ ID NO:s 10-340; comparing the transcript value to the reference value for each tumor to identify the gene transcripts over-expressed by each tumor; and applying a principle component analysis algorithm in which the analyzed gene set is restricted to each gene transcript having the nucleic acid sequence shown in SEQ ID NO:s 10-340 to identify a tumor cluster over-expressing these E2F-responsive genes; whereby an individual tumor in the population of tumors in the cluster is identified as an ERGO tumor.

Another aspect of the disclosure is a method of selecting treatment for a prostate cancer patient comprising the steps of providing a tumor sample from a prostate cancer patient; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor and the prostate cancer patient is diagnosed as an ERGO tumor prostate cancer patient if at least 21 of these gene transcripts are over-expressed; and choosing at least one treatment selected from the group consisting of removal of at least one tumor and adjuvant therapy, if the patient is diagnosed as an ERGO tumor prostate cancer patient; whereby a treatment is selected for the prostate cancer patient.

Brief Description of the Figures

Fig. 1 shows microarray data of a selected gene expression profile for the ERGO, non-ERGO HER2-over-expressing, ER/PR over-expressing non-ERGO, and triple-non-positive non-ERGO tumor subsets identified in the Van't Veer human breast cancer microarray set (s1).

Fig. 2A shows a tumor cluster consisting of 38 samples identified by PCA of all tumors in the Van't Veer human breast cancer microarray set (s1).

Fig. 2B shows non-ERGO tumors clustered separately from the ERGO tumors identified by refined PCA analysis of basal-like tumors identified in the Van't Veer human breast cancer microarray set (s1).

Fig. 2C shows the transitional basal-like HER2 over-expressing tumors identified by refined PCA of all tumors in the purged Dai human breast cancer microarray set (s2).

Fig. 2D shows clusters comprising 30 ERGO and 36 non-ERGO tumor subsets identified by refined PCA of basal-like tumors in the purged Dai human breast cancer microarray set (s2).

Fig. 2E shows a cluster of tumors that contained carcinoids, large cell neuroendocrine tumors, and small cell lung cancers identified by PCA of all tumors in the Jones human lung cancer microarray set.

Fig. 2F shows carcinoids, large cell neuroendocrine tumors, and small cell lung cancer clusters identified by refined PCA of the neuroendocrine tumors in the Jones human lung cancer microarray set.

Fig. 2G shows anaplastic thyroid cancer clusters identified by PCA of all tumors in the Salvatore human thyroid cancer microarray set.

Fig. 3A shows a continuous strand of genes strung along the axis of the first principal component, rather than discrete gene clusters, identified by refined PCA gene clustering with the basal-like tumors from the Van't Veer microarray set as input.

Fig. 3B is a graph comparing the genes identified by refined PCA clustering of the basal-like tumors from the Van't Veer human breast cancer microarray set in Fig. 3A with the most frequently over-expressed E2F-responsive genes in the ERGO tumors identified by the weighted rank ordering of all tumors from the Van't Veer microarray set.

Fig. 3C shows a comparison of the frequency of over-expressed E2F-responsive genes per basal-like tumor sample identified by PCA gene clustering in Fig. 3A with the most frequently over-expressed E2F-responsive genes in ERGO tumors.

Fig. 4 shows microarray data for HER2 positive tumors identified by PCA of the purged Dai human breast cancer microarray set (s2) and per ERGO tumor sample identified by refined PCA of the basal-like tumors in this microarray set.

Fig. 5 shows a schematic diagram of genes that are targets of the dRM/dREAM/LINC complex and the downstream consequences of aberrations of the components of this complex (shown at far left).

Fig. 6 shows the number of over-expressed FOXM1-associated DNA replication genes and mitotic genes per ERGO tumor as a function of the relative level of FOXM1 over-expression per ERGO tumor.

Fig. 7 is a schematic diagram of the phylogenetic origin of ERGO tumors at different organ sites. The schematic is based on results obtained with the weighted rank ordering methods and PCA based methods described in the Examples below.

Fig. 8 shows the selected gene expression profile for the ERGO, non-ERGO HER2-over-expressing, ER/PR over-expressing non-ERGO, and triple-non-positive non-ERGO tumor subsets of Fig. 1 in greater detail.

Fig. 9 shows the clinical survival data for PCA basal-like breast cancers and non-basal-like tumors identified by PCA analysis. "Survival" on the Y-axis indicates the fraction of all patients diagnosed with PCA basal-like breast cancers that survive at a given time.

Fig. 10 shows the most frequently over-expressed E2F-responsive genes in ERGO tumors identified by weighted rank ordering of tumors in the Van't Veer set (s1) and purged Dai microarray set (s2).

Fig. 11 shows microarray data for HER2 positive basal-like tumors identified by PCA of the purged Dai human breast cancer microarray set (s2).

Fig. 12 shows a comparison of the most highly over-expressed E2F-responsive genes in ERGO tumors identified by PCA of the Van't Veer human breast cancer microarray set (s1) and ERGO tumors identified by refined PCA of the purged Dai human breast cancer microarray set (s2).

Fig. 13 shows a comparison of the most highly over-expressed E2F-responsive genes of ERGO tumors identified by refined PCA in the Van't Veer human breast cancer microarray set (s1), and ERGO tumors identified by refined PCA in the purged Dai human breast cancer microarray set (s2) with small cell lung cancer tumors identified by PCA in the Jones human lung cancer microarray set.

Fig. 14 shows microarray data for ERGO tumors identified by refined PCA, as well as large cell neuroendocrine tumors and carcinoid tumors identified by PCA of the Jones human lung cancer microarray set.

Fig. 15 shows a comparison of the most highly over-expressed E2F-responsive genes of ERGO tumors identified by refined PCA in the Van't Veer human breast cancer microarray set (s1) and ERGO tumors identified by refined PCA in the purged Dai human breast cancer microarray set (s2) with small cell lung cancer tumors identified by PCA in the Salvatore human thyroid cancer microarray set.

Fig. 16 shows microarray data for ERGO tumors identified by refined PCA, as well as large cell papillary cancer tumors identified by PCA of the Salvatore human thyroid cancer microarray set.

Fig. 17 shows the over-expressed genes in ERGO tumors identified by refined PCA analysis in the Van't Veer human breast cancer microarray set (s1), the purged Dai human breast cancer microarray set (s2), the Jones human lung cancer microarray set, and the Salvatore human thyroid cancer microarray set. Genes are further identified as belonging to a group comprising two prominent subclasses of E2F-responsive genes, a group that affects the G1/S cell cycle phase transition, and group that affects the G2M portion of the cell cycle.

Fig. 18 shows microarray data from basal-like and non-basal like tumors identified by PCA for the top 25 most highly over-expressed published basal-like marker genes and the top 50 most highly over-expressed basal-like marker genes.

Fig. 19 shows the clinical survival data for ERGO tumors, non-ERGO basal-like tumors, and HER2 over-expressing tumors (Panel A) subsets as well as the non-ERGO "triple non-positive" tumors subsets identified by PCA analysis. "Survival" on the Y-axis indicates the fraction of all patients diagnosed with PCA basal-like breast cancers that survive at a given time.

Fig. 20 shows microarray data from prostate cancer patients and the identification of ERGO genes and ERGO tumors in cancers from such patients.

Fig. 21 shows details of the positive feedback loops and regulatory aspects of the cascade of changes associated with dysregulation of the members of the Syn/MULVB/DRM/DREAM/LINC multiprotein repressor/activator complex.

Fig. 22 shows a tumor cluster identified by PCA of primary prostate cancer tumor without metastases in the Chandran microarray set human prostate cancer microarray set.

Fig. 23 shows microarray data from normal fallopian tube epithelium and high grade serous fallopian tube and ovarian carcinomas in both patients carrying BRCA1 gene mutations and patients that do not carry such mutations and the identification of ERGO genes and ERGO tumors in cancers from such patients as indicated.

Fig. 24 shows microarray data from the Van't Veer microarray set, showing the non-E2F responsive genes that are statistically significantly differentially under-expressed in basal-like breast cancers relative to non-basal-like breast cancers.

Fig. 25 shows microarray data from the Van't Veer microarray set, showing the non-E2F responsive genes that are statistically significantly differentially under-expressed in basal-like breast cancers relative to non-basal-like breast cancers.

Fig. 26 shows microarray data from bladder cancer patients and the identification of ERGO genes and ERGO tumors in cancers from such patients.

Fig. 27 shows microarray data from hepatoma patients and the identification of ERGO genes and ERGO tumors in such patients.

Detailed Description of the Disclosure

The term "agonist" as used herein means a molecule that partially or completely activates, by any mechanism, a biological activity or effect of another molecule or complex of molecules. As used herein, an "agonist" is a molecule that is capable of, directly or indirectly, substantially activating, stimulating, or increasing the biological activity or effects of another molecule or complex of molecules. Such agonists may be, for example, small organic molecules, peptides, antibodies, antibody fragments, or polynucleotides. Increasing the expression of a molecule or complex of molecules can be used to agonize the biological activities or effects of the molecule or complex of molecules. For example, an over-expressed molecule or a complex of molecules can be considered to be an "agonist."

The term "antagonist" as used herein means a molecule that partially or completely inhibits, by any mechanism, a biological activity or effect of another molecule or complex of molecules. As used herein, an "antagonist" is a molecule that is capable of, directly or indirectly, substantially counteracting, reducing or inhibiting the biological activity or effects of another molecule or complex of molecules. Such antagonists may be, for example, small organic molecules, peptides, antibodies, antibody fragments, or polynucleotides.

The term "aurora kinase" as used herein means a peptide chain which is at least 90% identical to the mature form of the *Homo sapiens* Aurora A kinase amino acid sequence shown in SEQ ID NO: 1 (described by Accession Number NP_003591), the mature form of the *Homo sapiens* Aurora B kinase amino acid sequence shown in SEQ ID NO: 2 (described by Accession Number NP_004208); or the *Homo sapiens* Aurora C kinase amino acid sequence shown in SEQ ID NO: 3 (described by Accession Number NP_001015878) as determined using the default settings of the CLUSTALW algorithm.

As used herein the term "date" as used herein means a specified time. For example, a date may be a specific time or day in the future.

The term "drug" as used herein means a substance which produces physiological effects in an organism, or effects on cells, that result in the cure, mitigation, treatment, or prevention of a pathological condition.

The term "E2F" as used herein means a peptide chain which is at least 90% identical to the mature form of the *Homo sapiens* E2F-1 amino acid sequence shown in SEQ ID NO: 6 (described by Accession Number NP_005216), the mature form of the *Homo sapiens* E2F-2 amino acid sequence shown in SEQ ID NO: 7 (described by Accession Number NP_004082); or the *Homo sapiens* E2F-3a amino acid sequence shown in SEQ ID NO: 8 (described by Accession Number NP_005215) as determined using the default settings of the CLUSTALW algorithm.

The term "FOXM1" as used herein means a peptide chain which is at least 90% identical to the mature form of the *Homo sapiens* FOXM1 amino acid sequence shown in SEQ ID NO: 5 (described by Accession Number NP_068772) as determined using the default settings of the CLUSTALW algorithm.

The term "indicator of gene transcript levels" as used herein means a measurable endpoint that correlates to the level of a gene transcript. Such indicators may be nucleic acids corresponding to mRNA gene transcripts (*e.g.* cDNAs) or peptide chains, such as proteins and other polypeptides, that correlate to a gene transcript from which they are translated. Indicators that correlate to a given gene transcript may also comprise other nucleic acids such as splice variants, nucleic acids encoding different peptide chain isoforms, or other nucleic acid sequence variants capable of hybridizing to any portion of a gene transcript including both non-coding sequences such as 5' and 3' untranslated regions or coding sequences.

The term "LINC protein complex" as used herein means a complex of proteins comprising a peptide chain which is at least 90% identical to the mature form of the *Homo sapiens* LIN-9 amino acid sequence shown in SEQ ID NO: 9 (described by Accession Number NP_775106) as determined using the default settings of the CLUSTALW algorithm and which has the biological activity of repressing gene transcription. The LINC multiprotein complex is also known to contain human LIN-37, RbAP48, which is a human homolog of LIN-53, B-MYB, and DKFZp686L1814, a human homolog of *C. elegans* LIN-54, and LIN-52. Tesmin is another human homolog of LIN-54 and Rb, p107, p130, and E2F isoforms also associate with the core complex in different phases of the cell cycle. *See Schmit et al.*, 6 Cell Cycle 1903 (2007).

The term "normalizing" as used herein means means placing a measured value in a first data set, and a measured value in a second data set, on a common scale to facilitate the comparison of the measured values in the first data set and the measured values in the second data set. Typically, normalization of measured values in different data sets (*e.g.* tumor sample data set and reference data) is performed by identifying a parameter common to both the first data set and second data sets, measuring the value of common parameter in both the first data set and second data set, dividing measured values in the first data set by the value of the common parameter in the first data set and dividing measured values in the second data set by the value of the common parameter in the second data set to place the data in the first data set and second data set on a common scale so they can be easily compared. For example, in the methods of the disclosure an indicator value can be measured for a first gene transcript (*e.g.* SEQ ID NO: 12) in a tumor sample, an indicator value can be measured for the first gene transcript (*e.g.* SEQ ID NO: 12) in a reference such as a normal tissue sample, an indicator value can be measured for a second gene transcript (*e.g.* a housekeeping gene such as SEQ ID NO: 162) in the tumor sample to produce a housekeeping value, an indicator value can be measured for the second gene transcript (*e.g.* a housekeeping gene such as SEQ ID NO: 162) in the normal tissue sample to produce a housekeeping value, the indicator value of the first gene transcript in the tumor sample can be divided by the housekeeping value for the tumor sample, the indicator value of the first gene transcript in the normal tissue sample can be divided by the housekeeping value for the normal tissue sample and the resulting normalized values, which are now on a common scale, can be compared. Importantly, as those of ordinary skill in the art will readily recognize a variety of approaches may be taken to normalize data such as, for example, normalizing to an aggregate of common parameter values (*e.g.* for multiple housekeeping genes). Normalization may also be performed across an entire data set (*e.g.* all indicator values for every gene transcript on an array) or for only a portion a data set (*e.g.* an individual indicator value for a single gene transcript on an array).

The term "nucleic acid" as used herein means a molecule comprising at least two nucleic acid residues linked to form a chain. Such nucleic acid residues may be those found in DNA or RNA. Small nucleic acids of less than 50 residues may be referred to as "oligonucleotides."

The term "over-expressed" as used herein means that a measured indicator of gene transcript levels is greater than a reference value. Over-expression occurs when an indicator

value is, for example, at least 1.5 times greater than a reference value or at least 1.8 times greater than a reference value.

The term "peptide chain" as used herein means a molecule comprising at least two amino acid residues linked by a peptide bond to form a chain. Large peptide chains of more than 50 amino acids may be referred to as "polypeptides" or "proteins." Small peptide chains of less than 50 amino acids may be referred to as "peptides."

The term "principal component analysis algorithm" as used herein mean an algorithm that performs a mathematical procedure that transforms a number of potentially correlated variables into a smaller number of uncorrelated variables called principal components. Principle component analysis (PCA) is mathematically defined as an orthogonal linear transformation that transforms data to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate which is called the first principal component, the second greatest variance on the second coordinate, and so on. PCA is theoretically the optimum transform for a given data in least square terms. Depending on the field of application, PCA is also named the discrete Karhunen-Loève transform (KLT), the Hotelling transform or proper orthogonal decomposition (POD). PCA and methods steps utilizing can be performed on a computer.

The term "rank ordering algorithm" as used herein mean an algorithm that performs a mathematical procedure that orders data based on use selected data. Data can be rank ordered in any coordinate system such as a graph or table using user defined criteria. In one application of such an algorithm varying degrees of stringency and criteria may be used such as the following:

a) Each tumor in a tumor subset to be ordered must over-express at least X percent of 325 E2F responsive genes having the nucleic acid sequence shown in SEQ ID NO:s 10-334; and

b) Each of the 325 E2F responsive genes having the nucleic acid sequence shown in SEQ ID NO:s 10-334 in the tumor subset must be over-expressed in at least X percent of the tumors in the tumor subset;

where X might be 14%, 17%, 20%, 25%, or 33% and the value of X reflects increasing degrees of stringency. Rank ordering algorithms and method steps using rank orderin algorithms can be performed on a computer.

The term "reference" as used herein means a standard of comparison. A reference may be normal cells or tissues. Alternatively, a reference may be the average of the signal intensity

values from all sample-probed gene spots on a microarray set. A reference may also be a signal corresponding to one or more gene transcripts, such as a housekeeping genes like GAPDH or others, which are assumed not to vary significantly in living cells.

The term "survival plot" as used herein means a plot in a coordinate system such as a graph or table in which an indicator of the number of surviving patients in a population is described as a function of the time after the patients in the population were diagnosed as having a particular condition.

The term "survivin" as used herein means a peptide chain which is at least 90% identical to the mature form of the *Homo sapiens* survivin amino acid sequence shown in SEQ ID NO: 4 (described by Accession Number NP_001012270) as determined using the default settings of the CLUSTALW algorithm.

The term "therapeutically effective amount" as used herein means those doses of a drug that, in a given individual patient, produce a response that results in the killing of an ERGO tumor cell or that inhibits the division of an ERGO tumor cell. Therapeutically effective amounts, or doses, appropriate for an individual patient can be readily determined using routine clinical techniques well known by those of skill in the art (*e.g.* dose response plots).

The term "tissue" as used herein means an aggregate of cells that form a structure in an organ or other part of an animal.

The term "tumor sample" as used herein means a portion of a tumor from a patient. Tumor samples can comprise individual cells isolated from a tumor, cell lines isolated from a tumor, or larger portions of a tumor comprising multiple different cells in the tumor. A tumor sample can also comprise a portion of molecules from a tumor such as a collection of peptide chain molecules expressed by a tumor or a collection of nucleic acids such as reverse transcribed mRNAs (*i.e.* cDNAs) from a tumor.

One aspect of the disclosure is a method of identifying a tumor as an ERGO tumor comprising the steps of providing a tumor sample; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value;

and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed; whereby the tumor is identified as an ERGO tumor if at least 21 of these gene transcripts are over-expressed.

Importantly, the methods of the disclosure and the steps of the disclosed methods can be performed on a specifically programmed computer including, for example, a personal computer or programmable logic controller. In particular, the comparison steps of the methods or the application of PCA or rank ordering algorithms can be performed using such a computer. The methods of the disclosure and steps of the disclosed methods may also be tied to a nucleic acid array analyzer, capable of detecting signals correlated to the amount of a particular probe nucleic acid on an nucleic acid array which has hybridized to a nucleic acid present in a sample such as a tumor sample. The GeneChip® Array Station manufactured by Affymetrix, Inc. (Santa Clara, CA) is one example of such a nucleic acid array analyzer. Typically, such nucleic acid array analyzers comprise a nucleic acid array, a fluid handler, an oven or other means such as a heat block for maintaining a specific temperature (*e.g.* a specific hybridization temperature), a hybridization signal detection means (*e.g.* photomultiplier, scintillation counter, phosphor imager *etc.*), and a data collection means such as a computer or other means for collecting data (*e.g.* conventional photographic film or digital photographic film). Optionally, such nucleic acid array analyzers can comprise one or more automated nucleic acid array autoloaders for providing nucleic acid arrays to be analyzed. The output representing the physical transformation associated with a nucleic acid hybridization or other result produced by the methods of the disclosure can also be displayed on an output display such as a video monitor or printer. The methods of the disclosure and the steps of these methods can also produce a physical transformation because nucleic acid hybridization can be a result of performing the steps of the methods. Last, any combination of the above machines and apparatuses or physical transformations may be used in performing the methods of the disclosure or a method step of the disclosure.

In the methods of the disclosure a tumor is identified as an ERGO tumor if at least 21 of gene transcripts of SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; are expressed. Alternatively, a tumor is may be as an ERGO tumor if from 20% to 100% of these gene

transcripts are overexpressed. Importantly, the recitation of this range is intended to support the recitation of any value in the range (*e.g.* 20%, 100% or all values between 20% and 100%).

In a method of the disclosure it is preferred that cDNA microarrays be used for the measurement of indicators of gene transcript levels and reference values. Importantly, gene transcript levels, as measured in microarray experiments, are generally regarded by those of ordinary skill in the art as a proxy for gene (protein) expression levels. Consequently, the measurement of gene transcripts is referred to in the art and in some instances herein as the measurement of gene expression, over-expression, or under-expression depending on the type of analysis being performed.

DNA microarrays consist of multiple of different DNAs, such as oligonucleotide DNA nucleic acid chains or cDNAs, spotted onto known locations on a solid support, such as a glass microscope slide. The cDNAs are typically obtained by PCR amplification of plasmid library inserts using primers complementary to the vector backbone portion of the plasmid or to the gene itself for genes where sequence is known. PCR products suitable for production of microarrays are typically between 0.5 and 2.5 kb in length. Full length cDNAs, expressed sequence tags (ESTs), or randomly chosen cDNAs from any library of interest can be chosen. ESTs are partially sequenced cDNAs as described. In general, the cDNAs are of sufficient length to hybridize to cDNAs obtained from mRNAs representing splice variants or nucleic acids encoding different peptide chain isoforms derived from a single gene or the polymorphs of a single gene under the hybridization conditions of a microarray experiment. The DNAs immobilized on an array may also be nucleic acid chains comprising less than 100 nucleic acid residues (*e.g.* oligonucleotides). Nucleic acids immobilized on an array may also comprise alternative backbone chemistries such as phosphothiorate bond based chemistries, and alternative nucleoside residues such as modified residues capable of pairing with multiple nucleoside base residues. Those skilled in the art will recognize a variety of chemical modifications that can be made to the basic nucleic acid structure to create non-naturally occurring molecules with improved properties which are still capable of hybridizing to other nucleic acid molecules. In general, such modifications are those that improve the stability of such molecules, the hybridization properties of such molecules, or the ability of such molecules to couple to a substrate such as an immobilized substrate.

In a typical microarray experiment, a microarray is hybridized with differentially labeled RNA or DNA populations derived from two different samples. Most commonly RNA is isolated from cells or tissues of interest and is reverse transcribed to yield cDNA. Such RNA

may be either total RNA or polyadenylated RNA. Labeling is usually performed during reverse transcription by incorporating a labeled nucleotide in the reaction mixture, but RNAs can also be labeled. Although various different labels can be used, most commonly the nucleotide is conjugated with fluorophores such as the fluorescent dyes Cy3 or Cy5 (*e.g.*, Cy5-dUTP and Cy3-dUTP). cDNA or RNA derived from one sample representing, for example, a particular cell type, tissue type or growth condition is labeled with one fluorophore while cDNA or RNA derived from a second sample representing, for example, a different cell type, tissue type, or growth condition is labeled with the second fluorophore. Similar amounts of labeled material from the two samples are hybridized to the microarray. In the case of a microarray experiment in which the samples are labeled with Cy5 which fluoresces red light and Cy3 which fluoresces green light, the primary data obtained by scanning the microarray using a detector capable of quantitatively detecting fluorescence intensity are ratios of red/green fluorescence intensity. These ratios represent the relative concentrations of labeled cDNA or RNA molecules that hybridized to the DNA on the microarray and thus reflect the relative expression levels of the mRNA corresponding to each cDNA or gene represented on the microarray.

Alternatively, different labeled probe samples can be hybridized to an array at different times by hybridizing a first sample, collecting fluorescence intensity data for the first sample, stripping the array, collecting background fluorescence intensity data, hybridizing the second sample, and then collecting fluorescence intensity data. This type of approach can be used to probe an array with a tumor sample and then a reference such as a sample from normal tissue to measure an indicator of gene transcript levels in a tumor sample or to measure a reference to produce a reference value. Those of ordinary skill in the art will recognize other strategies for measuring an indicator of gene transcript levels in a tumor sample and measuring a reference to produce a reference value. Additionally, those of ordinary skill in the art will recognize hybridization based analyses can be performed in other non-array based formats, and that when array based formats are used the array need not be on the micro-scale and can instead be provided in other formats.

Hybridizations to arrays can be performed under a variety of stringent conditions by varying temperature or salt, detergent, and crowding agent concentrations. Additionally, microarray hybridization reagents are commercially available such as, for example, the Agilent Gene Expression Hybridization Kit (part number 5188-5242; Agilent Technologies Inc. Santa Clara, CA) or Agilent Oligo aCGH Hybridization Kit (part number 5188-5220; Agilent Technologies Inc. Santa Clara, CA). The term "stringent hybridization conditions" as used

herein means those conditions which promote the hybridization of a given nucleic acid sequence to its sequence complement, without that sequence hybridizing significantly with sequences having a lesser degree of complementarity (*e.g.* having one or more mismatches or less than 85% or less than 90% sequence identity as measured by CLUSTALW alignment using the default settings of the CLUSTALW algorithm). More generally, "stringent hybridization conditions" means conditions which allow hybridization of a given sequence with its intended targets, without significant hybridization of the sequence with other, nucleic acid sequences having different nucleic acid sequences that may be present.

Tumor samples for use in the methods of the disclosure can be prepared by collecting a fresh portion of the tumor by core needle biopsy or surgery. Tumor samples to be used for microarray or other nucleic acid hybridization based analyses can then be placed in cold RNALATER™ (Applied Biosystems/Ambion Inc., Austin, TX) on ice for 30 minutes followed by freezing at -80°C until RNA extraction. RNA can be extracted from tumor samples using the RNEASY™ system (Qiagen Inc., Valencia, CA) and contaminating DNAs can be removed with TURBO™ DNAse (Applied Biosystems/Ambion Inc., Austin, TX). Each tumor sample RNA preparation can then be assayed for purity by PCR/reverse transcription-PCR differential amplification analysis to confirm satisfactory removal of contaminating DNAs from the preparation. Each tumor sample RNA preparation can also be assessed for RNA quality using capillary electrophoresis RNA 6000 Ladder LABCHIP™ kits and an Agilent 2100 Bioanalyzer system (Agilent Technologies Inc. Santa Clara, CA) to produce electropherograms for assessment of RNA quality. RNA quality can be evaluated by examining electropherograms to confirm the 18 S and 28 S rRNA subunit peaks are present and in the appropriate 1:2 ratio. Assessment of 18 S and 28 S rRNA by this and other techniques is accepted in the art as an indicator of the relative intactness or quality of other isolated RNAs in a sample such as mRNAs and as an indicator of RNAse degradation of such isolated RNAs.

The Agilent 2100 Bioanalyzer system can also assess RNA quality using software that produces a RNA integrity number (RIN) for estimating the integrity of total RNA samples. This software automatically assigns an integrity number to RNA sample. Such that sample integrity is no longer determined by the ratio of the ribosomal bands, but instead by the entire electrophoretic trace in an electropherogram prepared using the RNA sample. This includes the presence or absence of degradation products. RIN based analyses facilitates interpretation of an electropherogram comparison of samples and helps ensure the reproducibility of experiments or

analysis. Importantly, the assigned RIN is independent of sample concentration, instrument and analyst.

Only tumor sample RNA preparations satisfying the differential amplification assay and electropherogram assay or other RNA integrity analyses are used to generate probes for the microarray analysis or other hybridization based analyses. Tumor sample RNA preparations can be labeled for use as probes as described above, or by using a commercially available labeling kits according to the manufacturer's instructions. The Invitrogen SUPERSCRIPT™ Indirect cDNA Labeling System (Invitrogen Inc., Carlsbad, CA) is one example of such a commercially available kit. Those of ordinary skill in the art will readily recognize others. Alternatively, RNA preparations can be converted to DNA preparations by reverse transcription and RNAse treatment, labeled and used as probes as discussed above in more detail.

In the methods of the disclosure indicators of gene transcript levels and reference values can be determined using techniques such as multi-dimensional gel electrophoresis, chromatography based techniques for measuring expressed peptide chains encoded by a gene, and other techniques such as fluorescence activated cell sorting or protein array analyses. In chromatography based techniques the presence of a given protein in a sample can be determined by techniques such as mass spectroscopy or antibody based detection techniques (e.g. ELISA), which are well known in the art.

A protein microarray, sometimes referred to as a protein binding microarray, comprises a substrate such as glass on which different molecules of protein have been affixed at specific locations in an ordered manner to form an array. The most common protein microarray is the antibody microarray, where antibodies specific to different individual expressed peptide chains are spotted onto the substrate and are used as capture molecules to detect the level of the different individual expressed peptide chains (*e.g.*, specific proteins) cell lysate solutions. This is typically done by blocking the array to minimize non-specific signals, incubating a lysate sample with the array, washing the array, blocking again, and probing the array. Additional wash and blocking steps may be necessary depending on the strategy selected for probing the array and the method used to detect peptide chains bound to the probed array. Expressed peptide chains binding to antibody arrays may be detected directly or via a secondary antibodies in a sandwich type immuno-assay (*e.g.*, ELISA). Expressed peptide chains present in a lysate can be directly labeled with an appropriate chromophore or fluorophore using well known techniques. Where pairs of antibodies specific to the same expressed peptide chain are available, sandwich immunoassays provide high specificity and sensitivity. Additionally, label-

free methods for detecting expressed peptide chains binding to an antibody array are available and include mass spectrometry, surface plasmon resonance and atomic force microscopy. Multichannel fluorescence activated cell sorting can also be used in conjunction with a selected panel of peptide chain specific antibodies to identify cells expressing a given set of peptide chains. Those of ordinary skill in the art will recognize other techniques, as well as variations on the techniques described above, for measuring an indicator of gene transcript levels and reference values which are suitable for use in the methods of the invention.

In one embodiment of the methods of the disclosure, the tumor sample is from lung tissue.

In another embodiment of the methods of the disclosure, the tumor sample is from thyroid tissue.

In another embodiment of the methods of the disclosure, the tumor sample is from ovarian tissue.

In another embodiment of the methods of the disclosure, the tumor sample is from prostate tissue.

In another embodiment of the methods of the disclosure, the indicator value is at least 1.8 times greater than the reference value for each of the gene transcripts.

Another aspect of the disclosure is a method of determining the odds that an individual ERGO tumor patient will survive to a future date comprising the steps of providing a tumor sample from an individual patient; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor and the individual patient is diagnosed as an ERGO tumor patient if at least 21 of these gene transcripts are over-expressed; plotting the fraction of surviving patients in a population of patients diagnosed as ERGO tumor patients as a function of the time since diagnosis of the ERGO tumor to generate a survival plot; and selecting a future date after the individual patient is diagnosed as an ERGO tumor patient

and determining the fraction of surviving patients in the population from the survival plot; whereby the fraction of surviving patients on the survival plot at the future date predicts the odds that an individual tumor patient will survive to the future date.

In one embodiment of the methods of the disclosure the tumor sample is from lung tissue and the population of patients is diagnosed as ERGO lung tumor patients.

In another embodiment of the methods of the disclosure the tumor sample is from thyroid tissue and the population of patients is diagnosed as ERGO thyroid tumor patients.

In another embodiment of the methods of the disclosure the tumor sample is from ovarian tissue and the population of patients is diagnosed as ERGO ovarian tumor patients.

In another embodiment of the methods of the disclosure, the tumor sample is from prostate tissue and the population of patients is diagnosed as ERGO prostate tumor patients.

Another aspect of the disclosure is a method of treating an ERGO tumor in a patient comprising the steps of providing a tumor sample from a patient; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor if at least 21 of these gene transcripts are over-expressed; selecting a drug capable of killing or inhibiting division of an ERGO tumor cell expressing at least one protein encoded by at least one gene transcript selected from the group consisting of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; and administering a pharmaceutically acceptable amount of the drug to the patient; whereby the ERGO tumor in the patient is treated.

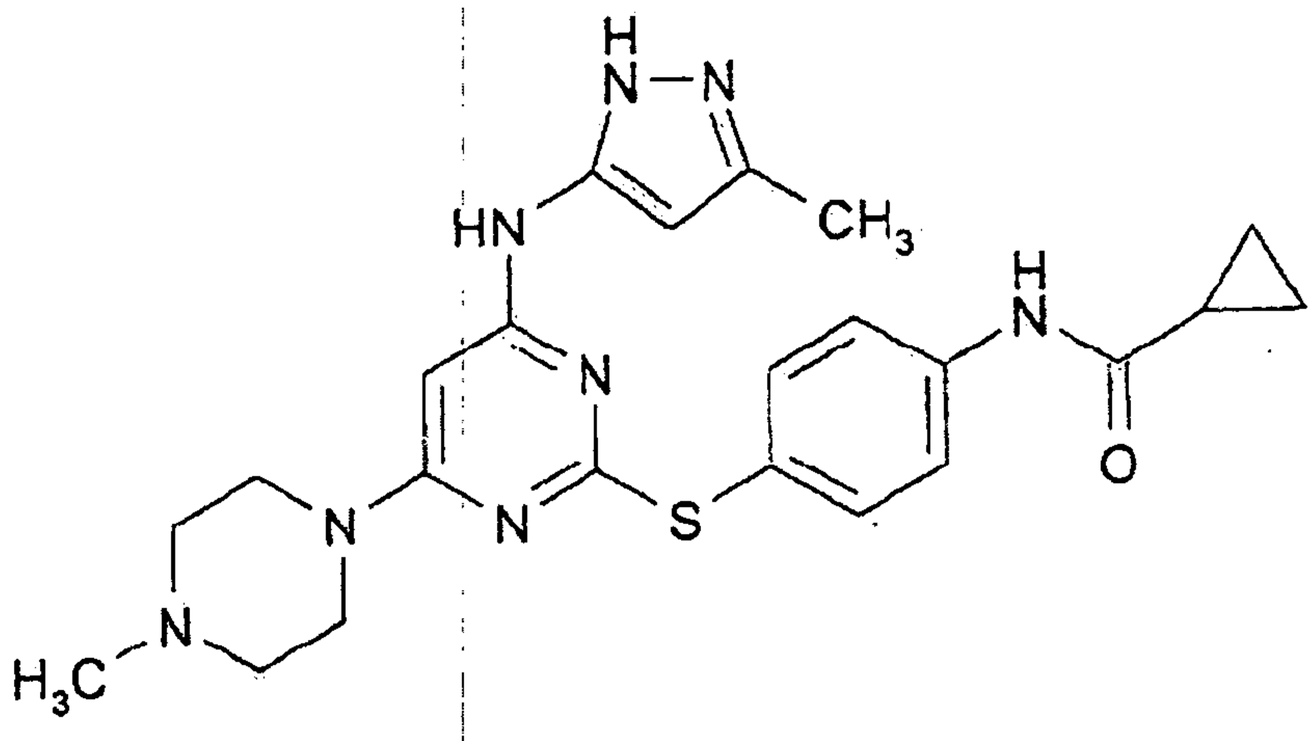
Drugs useful in the methods of the disclosure may be, for example, small organic molecules, peptides, antibodies, antibody fragments or polynucleotides. Such drugs may kill ERGO tumors or inhibit the division of an ERGO tumor cell. Division of an ERGO tumor cell is inhibited when the division of an ERGO tumor cell treated with a drug is decreased relative to an ERGO tumor cell which has not been treated with the compound.

In the methods of the disclosure, drugs may be administered by a variety of routes including, for example, orally, intravenously, intramuscularly, intra-arterially, subcutaneously, intraventricularly, transdermally, rectally, intravaginally, intraperitoneally, topically, or bucally, as a spray, aerosol, power, liquid or ointment. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the compound (*e.g.* its stability in the environment of the gastrointestinal tract), the condition of the patient (*e.g.* whether the patient is able to tolerate oral administration) *etc.* At present, the intravenous route is most commonly used to deliver therapeutic antibodies and nucleic acids. However, the disclosure encompasses the delivery of the pharmaceutical composition by any appropriate route. Those of ordinary skill in the art will recognize additional routes and techniques for the administration of drugs to a patient.

In the methods of the disclosure, drugs can be provided in a pharmaceutical composition. Such pharmaceutical compositions may comprise a number of different dosage forms such as solids or liquids or combinations of these. Such drugs can also be provided in pharmaceutically effective amounts sufficient to kill ERGO tumors or inhibit the division of an ERGO tumor cell. Pharmaceutically effective amounts of drugs can be readily identified using dose-response curves and other techniques well known the art.

In one embodiment of the methods of the disclosure the drug is an aurora kinase antagonist. A number of aurora kinase inhibitors have been in clinical phase I trial and results were reported in abstract form in 2008 for the SNS-314 aurora kinase inhibitor compound, Abstract #1462; the aurora kinase inhibitor compounds ZM 447439 (American Society of Clinical Oncology 2008 Meeting Abstract #2203), PF-03814735 (American Society of Clinical Oncology 2008 Meeting Abstract # 2517), PHA-739358 (American Society of Clinical Oncology 2008 Meeting Abstract # 3507), AS703569 (American Society of Clinical Oncology 2008 Meeting Abstract #14130), and MLN8054 (American Society of Clinical Oncology 2008 Meeting Abstract #3577). These drugs produced disease stabilization in some 20-40% of patients with tolerable toxicities and are suitable for use in the methods of the disclosure.

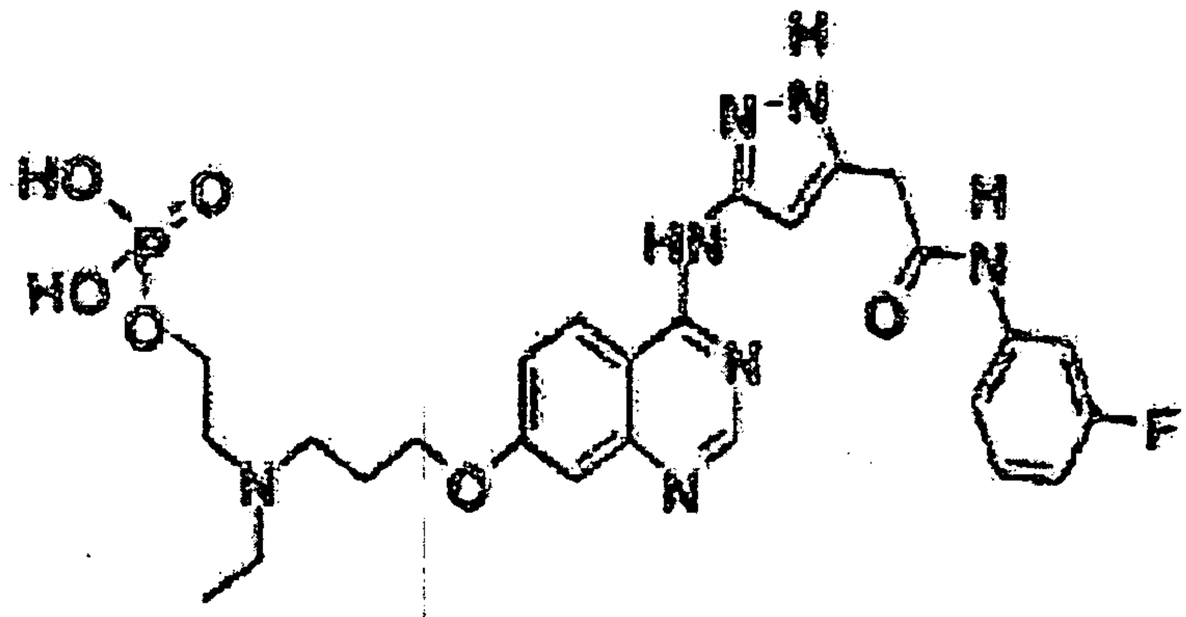
In another embodiment of the methods of the disclosure the aurora kinase antagonist is at least one molecule selected from the group consisting of VX-680, MLN8237, MLN8054, AZD1152, hesperadin, and ZM-447439. VX-680 has the structure shown below and includes the pharmaceutically acceptable derivatives of this compound.



VX-680

VX-680 is an aurora kinase inhibitor manufactured by Merck and Company Inc. (Whitehouse Station, NJ) which has been shown to block cell proliferation, disrupt bipolar spindle formation, and lead to accumulation of cells with 4N or greater numbers of chromosomal DNA copies and eventual cell death (reviewed in Agnese, V et al., Annals of Oncology 18: vi47–vi52, 2007). *In vivo*, VX-680 blocks cell-cycle progression and induces apoptosis in a wide range of human tumor types (Pan et al, Oral Oncol. 2008 Jul;44(7):639-45). VX-680 causes substantial inhibition of tumor growth in xenograft models, leading to regression of leukemia, colon and pancreatic tumors at well-tolerated doses (Harrington E, et al., Nat. Med. 2004 Mar;10(3):262-7). The results of a phase I clinical trial with VX-680 were reported at the ASCO 2008 Annual Meeting, in abstract #3009. In the trial three of 16 patients achieved stable disease at dose levels that were well below those which cause dose-limiting toxicity.

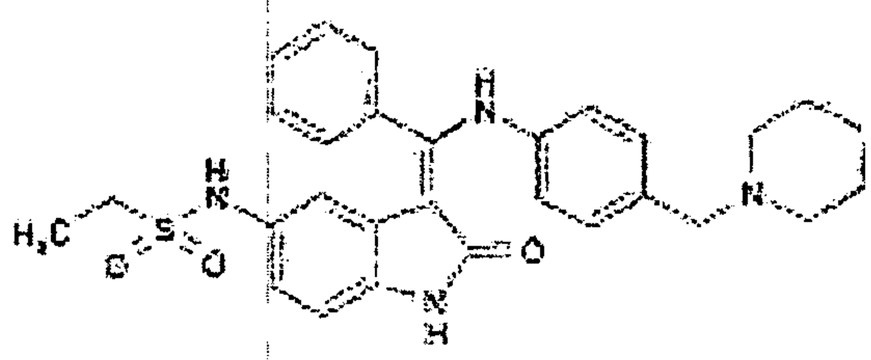
The compound AZD-1152 has the structure shown below and includes the pharmaceutically acceptable derivatives of this compound.



AZD1152

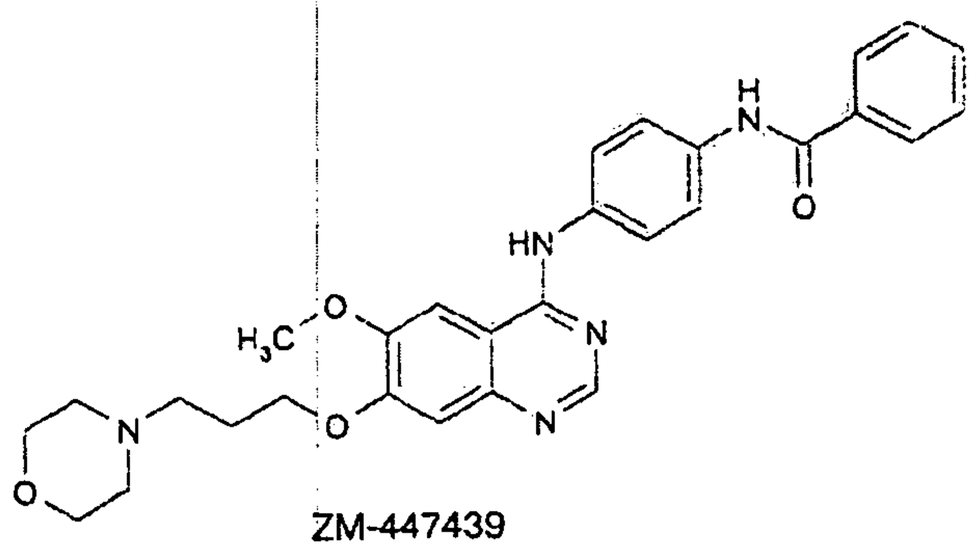
AD1152 is an aurora kinase inhibitor manufactured by AstraZeneca PLC (London, GB) that has been in phase I clinical trials. In the trial 5 of 12 patients had significant disease stabilization, with neutropenia as the only significant toxicity (Abstract #3008, American Society of Clinical Oncology (ASCO) 2008 Annual meeting).

The compound hesperadin has the structure shown below and includes pharmaceutically acceptable derivatives of this compound.



Hesperadin

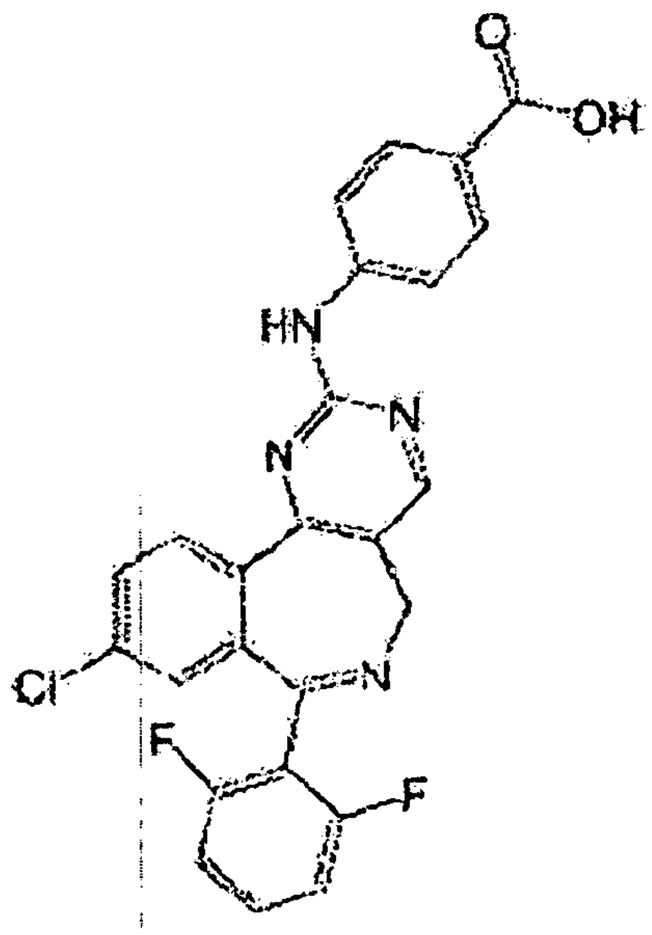
The compound ZM-447439 has the structure shown below and includes pharmaceutically acceptable derivatives of this compound.



ZM-447439

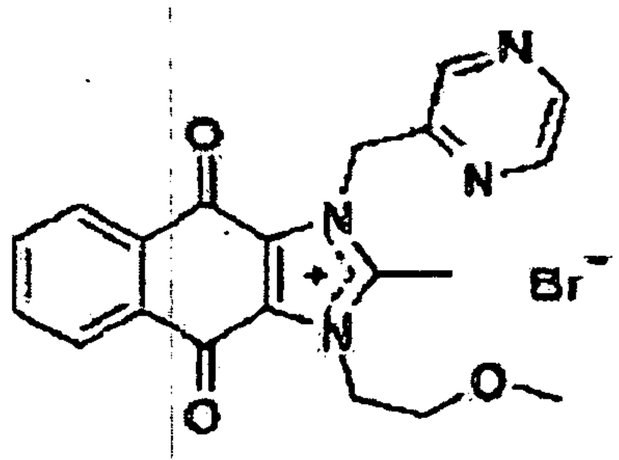
MLN8237 is an aurora A kinase inhibitor manufactured by Millenium Pharmaceuticals, Inc. (Cambridge, MA). The results of *in vivo* studies with MLN8237 were reported at the American Association for Cancer Research (AACR) 2008 Annual Meeting, in abstract #3237. The treatment of cultured human tumor cells with MLN8237 resulted in mitotic spindle abnormalities, mitotic accumulation, inhibition of cell proliferation and apoptosis. MLN8237 is orally bioavailable and has a favorable pharmacokinetic profile. A single oral administration of MLN8237 to nude mice bearing subcutaneous human tumor xenografts resulted in a time dependent accumulation of mitotic cells, consistent with the pharmacological effect being mediated through aurora A inhibition. Repeated oral administration of MLN8237 at well tolerated doses to nude mice bearing subcutaneous human tumor xenografts resulted in dramatic tumor growth inhibition in all tumor models evaluated. In these models MLN8237 induced mitotic accumulation and apoptosis. MLN8237 did not appreciably inhibit aurora B at efficacious concentrations, as indicated by measuring phosphorylated histone H3 Ser10 staining. MLN8237 is currently in Phase I clinical trials in patients with advanced malignancies.

MLN8054 is a selective small-molecule Aurora A kinase inhibitor that has entered Phase I clinical trials for advanced solid tumors. MLN8054 inhibits recombinant Aurora A kinase activity *in vitro* and is selective for Aurora A over the family member Aurora B in cultured cells. MLN8054 treatment results in G_2/M accumulation and spindle defects and inhibits proliferation in multiple cultured human tumor cells lines. Growth of human tumor xenografts in nude mice was dramatically inhibited after oral administration of MLN8054 at well tolerated doses. Moreover, the tumor growth inhibition was sustained after discontinuing MLN8054 treatment. In human tumor xenografts, MLN8054 induced mitotic accumulation and apoptosis, phenotypes consistent with inhibition of Aurora A. MLN8054 is a selective inhibitor of Aurora A kinase that robustly inhibits growth of human tumor xenografts and can be used in the treatment of cancers. The compound MLN8054 has the structure shown below and includes pharmaceutically acceptable derivatives of this compound. *See Manfredz et al.*, 104 Proc. Nat. Acad. *See* USA 4106 (2007).



In another embodiment of the methods of the disclosure the drug is a survivin antagonist.

In another embodiment of the methods of the disclosure the survivin antagonist is YM155. The compound YM155 has the structure shown below and includes pharmaceutically acceptable derivatives of this compound. YM155 is targeted therapy against survivin an E2F-responsive genes that is over-expressed in ERGO tumors. YM155 has also been in phase I clinical trials, and has shown clinical activity in solid tumors (American Society of Oncologist 2008 Meeting Abstracts # 3536, 5135, and 8538) with tolerable toxicity.



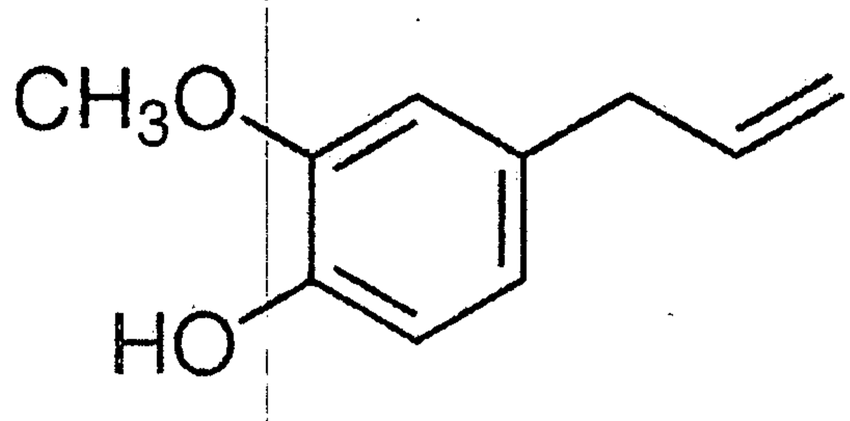
In another embodiment of the methods of the disclosure the drug is a FOXM1 antagonist. FOXM1 itself is a transcription factor that controls at least a dozen other E2F-responsive genes that are over-expressed ERGO tumors, including Aurora B kinase, survivin, and PLK1. A small peptide named $(D\text{-}Arg)_9$-p19ARF 26-44 has been developed that blocks

FOXM1, and is active both *in vitro* and *in vivo*, producing growth inhibition, reduction in levels of survivin, aurora B kinase, and PLK1 as well as inducing apoptosis (Gusarova, et al., J Clin Invest, 117: 99-111, 2007). Other small molecule inhibitors of FOXM1 have been found to be active *in vitro* and to down-regulate downstream targets of FOXM1 (American Association of Cancer Researchers 2008 Meeting Abstract #2663). These small molecule FOXM1 inhibitors can inducing apoptosis in breast cancer cell lines including the MDA-MB-231 basal-like breast cancer derived cell line (American Association of Cancer Researchers 2008 Meeting Abstract # 3280).

In another embodiment of the methods of the disclosure the FOXM1 antagonist is the $(D\text{-}Arg)_9$-p19ARF 26-44 peptide. The compound $(D\text{-}Arg)_9$-p19ARF 26-44 has the structure of rrrrrrrrrKFVRSRRPRTAS-CALAFVN containing nine D-Arg residues at the amino terminus and pharmaceutically acceptable derivatives of this compound.

In another embodiment of the methods of the disclosure the drug is an E2F antagonist.

In another embodiment of the methods of the disclosure the E2F antagonist is eugenol. Eugenol has the structure below and includes pharmaceutically acceptable derivatives of this compound.



Eugenol is the active agent in clove oil and has been reported to target E2F1 and kill melanoma cells *in vitro* (Ghosh, R., et al., J Biol Chem. 2005 Feb 18;280(7):5812-9).

In another embodiment of the methods of the disclosure, the drug is a LINC protein complex agonist. Such an agonist can be over-expressed LINC protein complexes.

Another aspect of the disclosure is a method of identifying an individual tumor in a population of tumors as an ERGO tumor comprising the steps of providing a population of tumor samples; providing a reference; measuring gene transcript levels in the tumor samples to produce a transcript value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 10-340; comparing the transcript value to the reference value for each tumor to identify the gene transcripts over-expressed by each tumor; ranking the tumors in the population with a rank ordering algorithm to order the tumors according to the number of the gene transcripts over-expressed by each tumor; and removing individual tumors

from the population that over-express the smallest number of each gene transcript per cell and that have the lowest levels of over-expression of each gene transcript per cell until at least 20% of the individual tumors remaining in the population over-express at least 20% of the gene transcripts; whereby an individual tumor remaining in the population of tumors is identified as an ERGO tumor.

Another aspect of the disclosure is a method of identifying an individual tumor in a population of tumors as an ERGO tumor comprising the steps of providing a population of tumor samples; providing a reference; measuring gene transcript levels in the tumor samples to produce a transcript value for each of the following gene transcripts having the nucleic acid sequence shown in SEQ ID NO:s 10-340; comparing the transcript value to the reference value for each tumor to identify the gene transcripts over-expressed by each tumor; and applying a principle component analysis algorithm in which the analyzed gene set is restricted to each gene transcript having the nucleic acid sequence shown in SEQ ID NO:s 10-340 to identify a tumor cluster over-expressing these E2F-responsive genes; whereby an individual tumor in the population of tumors in the cluster is identified as an ERGO tumor.

Another aspect of the disclosure is a method of selecting treatment for a prostate cancer patient comprising the steps of providing a tumor sample from a prostate cancer patient; providing a reference; measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; measuring the reference to produce a reference value; and comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor and the prostate cancer patient is diagnosed as an ERGO tumor prostate cancer patient if at least 21 of these gene transcripts are over-expressed; and choosing at least one treatment selected from the group consisting of removal of at least one tumor and adjuvant therapy, if the patient is diagnosed as an ERGO tumor prostate cancer patient; whereby a treatment is selected for the prostate cancer patient.

An important clinical problem for patients diagnosed with prostate cancer is that only about 15% of all such patients will ever die of their disease, but it is difficult to know whether

an individual patient is in this unfortunate population of 15% of all patients. Furthermore, many prostate tumors, including those in older patients, grow slowly and without metastasis. Consequently, patients diagnosed with prostate cancer and physicians have to select either of two approaches after a prostate cancer diagnosis. The first approach is known as the "watchful waiting" approach in which tumor growth and related indicators are monitored over time. In this approach no treatment is necessary until a change in disease severity or symptoms occurs. The second approach is to undergo a primary treatment such as conventional and other surgeries, cryosurgery, or radiation therapy to remove or kill the prostate cancer tumor and any metastasis. Additionally, as part of this approach adjuvant therapies such as chemotherapy or hormonal therapy may be necessary to help ensure effective treatment to prevent or postpone the prostate cancer from coming back after the primary treatment. Unfortunately, a number of side effects are potentially associated with the primary treatment and the adjuvant therapies of this second approach including impotence, urinary incontinence, nausea, fatigue, diarrhea, and infertility. Consequently, there is a need for methods of selecting treatment for patients diagnosed with prostate cancer to help insure a given treatment approach is appropriate for an individual patient and the patient's type of prostate tumor.

Importantly, the Applicant has discovered that about 20% of primary prostate cancers from individual patients are ERGO tumors irrespective of the Gleason score conventionally used to functionally classify prostate cancers assigned to these cancers. Furthermore, greater than 80% of all metastases associated with prostate cancer tumors are ERGO tumors. Consequently, the identification of prostate cancer ERGO tumors in patients diagnosed with prostate cancer provides a means for identifying those prostate cancer tumors that are most likely to metastasize or grow profusely and to select appropriate patient treatment.

In one aspect of the method of the disclosure the removal of at least one tumor is performed using at least one therapy selected from the group consisting of using focused energy, cryoablation, and radiation therapy. Examples of focused energy include light such as high intensity laser beams of appropriate wavelengths, and other forms of electromagnetic radiation such as microwaves, as well as X-rays or other types of ionizing radiation. In the methods of the disclosure, tumors are considered to be removed by these or other techniques such as cryoablation (freezing) and radiation therapy if the tumor or cells forming some portion of the tumor are killed, burned, or otherwise destroyed (*e.g.* by electromagnetic radiation).

In another aspect of the method of the disclosure the adjuvant therapy is at least one therapy selected from the group consisting of chemotherapy, hormone therapy, and

immunotherapy. Chemotherapy treatment with drugs such as small molecules that kill cancer cells. Examples of drugs useful in chemo-therapy include, for example, anthracyclines such as doxorubicin, epirubicin and liposomal doxorubicin; taxanes such as docetaxel, paclitaxel, and protein-bound paclitaxel; cyclophosphamide; capecitabine; 5-fluorouracil (5 FU); vinorelbine; emcitabine; mitoxantrone; estramustine; etoposide (VP-16); vinblastine; and carboplatin. These drugs, and others disclosed herein, may be used in the manufacture of medicaments for the treatment of ERGO tumors identified according to the disclosed methods. Hormone therapy is also called androgen deprivation therapy (ADT) or androgen suppression therapy. The goal of hormone therapy in prostate cancer patients is to reduce levels of the male hormones, called androgens, in the body. The main androgens are testosterone and dihydrotestosterone (DHT). Androgens, produced mainly in the testicles, stimulate prostate cancer cells to grow. Lowering androgen levels often makes prostate cancers shrink or grow more slowly. Drugs useful in such hormone therapy include, for example, luteinizing hormone-releasing hormone (LHRH) analogs including leuprolide, goserelin, and triptorelin; luteinizing hormone-releasing hormone (LHRH) antagonists such as abarelix; anti-androgens such as flutamide, bicalutamide, nilutamide; and ketoconazole. Hormone therapy may also require orchiectomy (surgical castration) to control androgen production. Immunotherapy treatments include treatments that stimulate a patient's own immune system to kill prostate tumors cells. The sipuleucel-T (PROVENGE™) treatment system (Dendreon Corp., Seattle, WA) is one example of an immunotherapy. In this system immune system white blood cells are removed from a prostate cancer patient, antigen presenting cells (APCs) are then isolated from these white blood cells by apheresis, and the patient's isolated APCs are co-cultured with a recombinant fusion protein antigen comprising prostatic acid phosphatase (PAP). The activated, antigen-loaded APCs at this point are designated as "sipuleucel–T" and injected into the prostate cancer patient. Once administered to the patient these APCs present the recombinant PAP antigen to T-cells and help stimulate the patient's own immune system to produce a killer T-cell response against his prostate cancer tumor cells. Those of ordinary skill in the art will recognize other chemotherapies, hormone therapies, and immunotherapies.

In another embodiment the methods of the disclosure further comprise measuring an indicator of gene transcript levels in at least one selected from the group consisting of the tumor sample and the reference to produce a housekeeping value for at least one gene transcript selected from the group consisting of the nucleic acid sequences shown in SEQ ID NO:s 162, 341-365 and 366; and normalizing at least one selected from the group consisting of the

indicator value and the reference value to the housekeeping value. This embodiment is particularly useful in relation to methods for the identification of ERGO tumors in prostate tissue and methods for the selection of treatment for a prostate cancer patient.

Another embodiment of the disclosure is the use of an aurora kinase antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of VX-680 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of MLN8237 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of MLN8054 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of AZD1152 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of hesperadin in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of ZM-447439 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of a survivin antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of YM155 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of a FOXM1 antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of the $(D\text{-}Arg)_9$-p19ARF 26-44 peptide in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of an E2F antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of eugenol in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is the use of a LINC protein complex agonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the methods of the disclosure.

Another embodiment of the disclosure is an apparatus comprising a specifically programmed computer in communication with a nucleic acid array analyzer and an output display, wherein the specifically programmed computer is adapted to compare indicator values to reference values and to determine which gene transcripts are over-expressed in a tumor sample; a nucleic acid array comprising at least 21 of the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340 adapted for hybridization to nucleic acids in a tumor sample; a memory containing an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340 produced by hybridization of the nucleic acid array to the nucleic acids in the tumor sample, and containing reference values for the gene transcripts; and an output display which shows the tumor sample is an ERGO tumor when the specifically programmed computer determines at least 21 of the gene transcripts are over-expressed in a tumor sample.

In another embodiment of the methods of the disclosure, the tumor sample is from liver tissue.

In another embodiment of the methods of the disclosure, the tumor sample is from bladder tissue.

In another embodiment of the methods of the disclosure, the tumor sample is from liver tissue and the population of patients is diagnosed as ERGO hepatoma tumor patients.

In another embodiment of the methods of the disclosure, the tumor sample is from bladder tissue and the population of patients is diagnosed as ERGO bladder tumor patients.

In another embodiment of the methods of the disclosure, the ERGO tumor is from a tissue selected from the group consisting of lung tissue, thyroid tissue, ovarian tissue, prostate tissue, liver tissue and bladder tissue.

In another embodiment of the methods of the disclosure, the tumor sample is from a tissue selected from the group consisting of lung tissue, thyroid tissue, ovarian tissue, prostate tissue, liver tissue and bladder tissue.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this disclosure are also included within the definition of the disclosure provided herein. Accordingly, the following examples are intended to illustrate but not limit the present disclosure.

Examples

Example 1

Identification of Human Breast Cancer E2F Responsive Gene Over-Expressing (ERGO) Tumors

Breast Cancer Microarray Sets

A gene expression microarray set from 117 different patient breast cancer samples described by Van't Veer *et al.* was analyzed. This microarray set was selected for analysis because it included 18 tumors obtained from patients with known BRCA1 mutations. The breast cancer microarray set described by Dai *et al.* was used to confirm the analytical results obtained using the Van't Veer microarray set. The gene expression microarray sets of Van't Veer and Dai both contained 57 samples that were identical and were excluded from the analyses of the Dai microarray set (also referred to herein as the "purged Dai microarray set") in the present study.

Weighted Rank Ordering Methods and Criteria for Identification of ERGO Tumors

First, a reference signal intensity value was obtained by averaging the signal intensity values from all sample probed gene spots on the Van't Veer microarray set to be analyzed. The reference signal intensity value was obtained by averaging the signal intensity values from all sample probed gene spots on the Van't Veer microarray set to be analyzed.

Then "over-expressed," "under-expressed," and non-over-expressed genes were identified. Gene "over-expression" was determined to occur when the signal intensity corresponding to a given gene transcript in the microarray set was 1.8 fold greater than the reference signal intensity value discussed above. Gene "under-expression" was determined to occur when the signal intensity corresponding to a given gene transcript in the microarray was 1.8 fold less than a reference signal intensity value. Gene "non-over-expression" was determined to occur when a gene was neither "over-expressed" nor "under-expressed."

Next, weighted rank ordering methods were performed using EXCEL™ software (Microsoft Corp., Redmond, WA) to rank the over-expressed genes by their frequency of expression among the tumors. In these rank ordering analyses the most highly over-expressed genes were placed closest to the origin and the contribution of each tumor to the ranking was weighted by its proximity to the origin of the tumor axis.

These weighted rank ordering methods were then used to rank the tumors by the number of the 325 specific E2F-responsive genes, shown in Table 1 (shown below), over-expressed per tumor ("1" or other value indicates gene functions).

Table 1

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
BCL2L11*	BCL2L11							
FOXO1A*	FOXO1A							
CCNE2*	CCNE2		1					
CDKN2C*	CDKN2C		1		1			
CCNE1*	CCNE1		1					
MYC*	MYC							
FGFR3*	FGFR3							1
MAP3K5*	MAP3K5						1	
BMP2*	BMP2	1						2
MYB*	MYB	1						
LHX2**	LHX2							
GCH1*	GCH1							1

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
MAPK9*	MAPK9							1
MAPK8*	MAPK8							1
MAPK3*	MAPK3				1			1
MAPK1*	MAPK1				1		1	1
MAPK4*	MAPK4							1
MAP2K1*	MAP2K1							1
MAP2K2*	MAP2K2							1
FOXO3A*	FOXO3A	1			1		1	
GADD45B*	GADD45B						1	
MCL1*	MCL1						1	
BCL2**	BCL2						1	
CCND3*	CCND3				1			
CHES1**	CHES1	1			1	1		
MKI67**	MKI67							1
CDKN1C**	CDKN1C				1			
KIFC1**	KNSL2							
PRG4**	PRG4							1
PMS2**	PMS2							
PLK2**	SNK					1		
HRK*	HRK						1	
CASP8**	CASP8							
TYMS*	TYMS			1	1			
TK1*	TK1			1				
DUT**	DUT			1				
RRM1*	RRM1			1				
RRM2*	RRM2			1				
CDK2*	CDK2			1		1		
MCM3*	MCM3			1				
MCM7*	MCM7	1		1				
PCNA*	PCNA			1	1			
RFC3*	RFC3			1				
PRIM1**	PRIM1			1				
TOP2A*	TOP2A			1	1	1		
LIG1**	LIG1			1	1			
FEN1**	FEN1			1				
RAD51**	RAD51				1			
CDC20**	CDC20					1		
CDC2*	CDC2					1	1	1	
CCNA2*	CCNA2	1				1		
CCNB1*	CCNB1					1		
CCNB2**	CCNB2					1		
SMC2**	SMC2L1					1		
STMN1*	STMN1					1		1

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
NDC80**	HEC					1		
BUB1**	BUB1					1		
KPNA2*	KPNA2							
HMGB2**	HMG2	1	1	1				
EZH2*	EZH2	1						
AURKB*	STK12					1		
PTTG1**	PTTG1	1		1		1		
SLBP*	SLBP		1					
RB1*	RB1	1			1	1		
ANXA8*	ANXA8							1
DCK**	DCK							1
CDC25A*	CDC25A				1	1		
EPS8**	EPS8							1
FST*	FST	1						3
TMPO*	TMPO	1						
RAD51AP1**	PIR51			1				
ASF1B**	FLJ10604							3
CDCA4*	FLJ20764							
RFC4**	RFC4		1	1				
BLM**	BLM		1	1				
VRK1**	VRK1							1
BARD1**	BARD1		1	1				
BTG3**	BTG3							1
CHAF1A**	CHAF1A	1	1	1				3
NPAT*	NPAT							3
HUNK*	HUNK							1
DEK**	DEK	1						1
EED**	EED							3
MCM4**	MCM4	1	1					
MELK**	KIAA0175							1
TCF19**	TCF19	1						
FANCL**	FLJ10335			1				
PBX3**	PBX3	1						1
EGR1*	EGR1	1						
CDCA7L**	DKFZp762L0311	1						
SKP2*	SKP2							1
CTGF*	CTGF							2
CITED2**	CITED2							
SERPINE1*	SERPINE1							2
CCND1*	CCND1				1			
UHRF1*	ICBP90			1				
MCM5*	MCM5	1	1					
HMGB3**	HMG4	1						

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
MCM6*	MCM6	1	1					
CDC45L**	CDC45L		1		1			
CDC6*	CDC6		1		1	1		
ORC6L*	ORC6L		1			1		
CKS2**	CKS2					1		
GMNN*	GEM		1		1			
PRIM2**	PRIM2A		1					
CENPK**	FKSG14					1		
NUP155**	NUP155							1
FIGNL1**	FIGNL1							1
MAD2L1*	MAD2L1				1	1		
CCNF**	CCNF					1		
DNMT1*	DNMT1	1						
RPA1**	RPA1		1	1				
PRC1**	PRC1					1		
RBL1**	RBL1	1						
BRCA1*	BRCA1	1	1	1	1			
H2AFZ**	H2AFZ							3
DTYMK**	DTYMK							1
PLK1**	PLK					1		
POLA2*	POLA2		1					
PBK**	SPK					1		
CASP7*	CASP7						1	
MCM2*	MCM2	1	1					
RPA3**	RPA3		1	1				
GJA7**	GJA7							1
USP1**	USP1			1				
DNA2L**	DNA2L		1					
CITED1**	CITED1	1						
NASP**	NASP		1					1
RFC5**	RFC5		1	1				
SMARCA5**	SMARCA5	1						1
SHCBP1**	FLJ22009							1
SSX2IP**	KIAA0923							
MFAP1**	MFAP1							1
ROD1**	ROD1							1
BMPR1A*	BMPR1A							2
E2F3**	E2F3	1						
UNG*	UNG			1				
ENO3**	ENO3							1
MSH2**	MSH2			1			1	
PLK4**	STK18							1
ACTA2**	ACTA2							1

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
TIMELESS**	TIMELESS	1						
BOK*	BOK						1	
KBTBD10**	SARCOSIN							1
BUB1B**	BUB1B				1	1		
NUP107**	NUP107							1
KIF2C**	KNSL6					1		
LMNB1*	LMNB1							1
RPA2**	RPA2		1					
CHEK2**	CDS1							1
COL11A1**	COL11A1							2
TGFB3**	TGFB3							2
CALR**	CALR	1					1	1
TTK*	TTK				1	1		
E2F2*	E2F2	1						
CKS1B**	CKS1							1
RFC2**	RFC2		1					
UMPS**	UMPS							1
DBF4*	ASK		1					
CHEK1**	CHEK1			1	1	1		
BUB3**	BUB3					1		
CENPE**	CENPE					1		
CSTF1**	CSTF1							1
RAD54L*	RAD54L			1				
POLD1**	POLD1			1				
MLH1**	MLH1			1				
CENPA**	CENPA					1		
SMC4**	SMC4L1					1		
HMGB1**	HMG1	1		1			1	
HIST1H3D**	H3FB							4
H2AFX**	H2AFX			1				1
CBX5**	CBX5							3
HIST1H2AC**	H2AFL							4
KIF22**	KNSL4					1		
NEK2*	NEK2					1		
KIF4A**	KIF4A					1		
HMMR**	HMMR							1
MTHFD1**	MTHFD1							1
GINS1*	KIAA0186		1					
SFPQ**	SFPQ	1		1				
HSP90B1*	TRA1	1		1				
MAP3K7**	MAP3K7							2
PLSCR1**	PLSCR1							1
ANLN**	ANLN					1		

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
SFRS2**	SFRS2	1						
ID3**	ID3	1						
TEAD4*	TEAD4	1						
SRPR**	SRPR							1
UBE2T**	HSPC150							1
INCENP**	INCENP					1		
CDC25B**	CDC25B					1		
AURKA*	STK15					1		
DHFR*	DHFR							1
CDKN3**	CDKN3		1					
CDC7**	CDC7L1		1					
RACGAP1**	ID-GAP					1		
CSRP2**	CSRP2							1
MAF**	MAF	1						
CBX3**	CBX3	1						3
CHAF1B*	CHAF1B	1	1		1			3
ADAMTS1**	ADAMTS1							1
TCOF1**	TCOF1							1
LSM5**	LSM5							1
HNRPC**	HNRPC							1
APAF1*	APAF1						1	
ASH2L*	ASH2L	1						
BCL3*	BCL3	1						1
CASP3*	CASP3						1	
CAV1*	CAV1							1
CD58*	CD58							1
DMRT1*	DMRT1	1						
DYRK1A*	DYRK1A							1
LIMA1*	EPLIN							1
FGFR2*	FGFR2							1
HEY1*	HEY1	1						
INHBA*	INHBA	1					1	2
TBC1D2B*	KIAA1055							1
OSMR*	OSMR							1
FURIN*	PACE							1
PRKAR2B*	PRKAR2B							1
PTPNS1*	PTPNS1							1
RANBP9*	RANBP9							1
SOX9*	SOX9							1
SPHK1*	SPHK1							1
TACC1*	TACC1							1
TGFA*	TGFA							1
TGFB2*	TGFB2							2

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
YY1*	YY1	1						
SNAPC1**	SNAPC1	1						
CCNG2**	CCNG2				1	1		
HOXA7**	HOXA7	1						
HOXA9**	HOXA9	1						1
PITX1**	PITX1	1						1
SMARCA2**	SMARCA2	1						
BACH1**	BACH1	1						
CBFB**	CBFB	1						
BIRC5*	BIRC5				1	1	1	
CDC25C**	CDC25C					1		
ORC3L**	ORC3L		1					
TOPBP1**	TOPBP1			1				
MRE11A**	MRE11A			1		1		
ATM*	ATM			1	1	1		
XRCC4**	XRCC4			1				
RECQL**	RECQL			1				
CENPF**	CENPF					1		
CENPH**	PMF1					1		
NOLC1**	NOLC1					1		
MPHOSPH1**	MPHOSPH1					1		
BMI1*	BMI1							3
HIST1H2AE**	H2AFA							3
HIST1H2AD**	H2AFG							4
HIST1H2BN**	H2BFD							4
HIST1H2BE**	H2BFH							4
HIST1H2BO**	H2BFN							4
HIST1H2BJ**	H2BFR							4
HIST1H3B**	H3FL							4
HIST2H4**	H4F2							4
HIST1H2BF**	H2BFG							4
HIST1H3E**	H3FD							4
KIF20A**	RAB6KIFL					1		3
FGFR1OP**	FOP							1
DHPS**	DHPS							1
PEX19**	PXF							1
PPM1D*	PPM1D					1		
E2F1*	E2F1	1					1	
PARP1**	ADPRT	1						
ORC2L**	ORC2L	1	1					
VEGFA**	VEGF						1	1
APOE**	APOE							3
PRPS1**	PRPS1							1

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
TGFB1I1*	TGFB1I1	1						
CXCR7*	RDC1							1
PDGFRA**	PDGFRA							1
SLPI*	SLPI							1
FAS**	TNFRSF6						1	
CPT1A*	CPT1A							1
PDCD4*	PDCD4	1					1	
TANK**	TANK							1
APBB2*	APBB2	1					1	1
GADD45A**	GADD45A			1			1	1
ACOX1**	ACOX1							1
CSDA*	CSDA	1				1		
UBE2C**	UBCH10					1		
TPX2**	C20orf1					1		
NUSAP1**	BM037					1		
SULT2A1*	SULT2A1							1
INMT*	INMT							1
ARHGAP4*	ARHGAP4							1
RAD52*	RAD52			1				
TNFSF9*	TNFSF9						1	
BAD*	BAD						1	
BAK1*	BAK1						1	
BID*	BID						1	
CFLAR*	CFLAR						1	
MAP3K14*	MAP3K14							1
PAWR*	PAWR	1					1	
FGF2•	FGF2						1	1
MMP16*	MMP16							1
TP53BP2*	TP53BP2						1	1
VEGFB*	VEGFB							1
IFNA2*	IFNA2						1	
SRGAP2*	KIAA0456							1
DIP*	KIAA0767						1	
SERPINF2*	SERPINF2							1
CCNU*	UNG2							1
TP73*	TP73	1		1	1		1	
POLE2*	POLE2		1					
RAD51C**	RAD51C			1				
PMS2L1**	PMS2L1			1				
DDB2*	DDB2			1				
NFKB2**	NFKB2	1						1
KIF11**	KNSL1					1		
FOXM1*	FOXM1	1						

gene-name	microaray name	trans-crip-tion, regu-lation	DNA repli-cation	DNA repair	check-point	mi-tosis	apop-tosis	other
PLAU*	PLAU							2
BBC3*	BBC3						1	
PMAIP1*	PMAIP1						1	
GAB2*	GAB2						1	1
SIVA1*	SIVA						1	
PPP1R13B*	PPP1R13B						1	
AXIN2*	AXIN2							1
DIABLO*	SMAC						1	
AR*	AR	1						1
NRP1*	NRP1							1
ECT2*	ECT2							1
ISYNA1*	ISYNA1							1

Tumors with the highest number of over-expressed genes per tumor were placed closest to the origin. The contribution of each gene to the ranking was positively weighted by its proximity to the origin of the gene axis.

Individual tumors with the lowest number of over-expressed genes per tumor, and individual genes with the lowest over-expression frequency among the tumors were then iteratively stripped out of the dataset, with recalculation of rankings, until predetermined stopping conditions were met. The stopping conditions were that at least 20% of the tumors remaining in the dataset over-express at least 20% of the remaining E2F-responsive genes. Tumors that remained in the data set after satisfaction of the stopping conditions were identified as ERGO tumors.

Identification of the ERGO Tumor Subset of Human Breast Cancers by Weighted Rank Ordering

The Van't Veer breast cancer microarray set was weighted rank ordered by the number of over-expressed E2F-responsive genes per tumor and by the number of tumors over-expressing E2F-responsive genes using the stopping criteria described above. These analyses resulted in identification of a group containing only 31 of the 117 tumors in the set analyzed and the identification of a set of 74 different E2F-responsive genes that met the stopping condition criteria that at least 20% of the tumors remaining in the set over-express at least 20% of the remaining E2F-responsive genes. These 31 tumors were identified as ERGO tumors because they remained in the data set after the criteria of the stopping conditions described above were satisfied.

The set of 74 different E2F-responsive genes identified are listed in Table 2 shown below.

Table 2

gene/protein name	P value, ERGO vs.non-ERGO	number of over-expressing tumors among total of 31 tumors
STK12	5.00E-16	23
KNSL6	5.00E-16	21
CENPA	5.00E-16	19
RAD54L	3.25E-12	18
FOXM1	3.25E-12	17
RAB6KIFL	0.00E+00	19
ANLN	5.21E-13	19
CDC20	0.00E+00	21
BTG3	1.10E-08	17
BUB1	1.11E-16	16
TYMS	3.25E-12	19
CCNB2	0.00E+00	16
STMN1	1.06E-13	17
C20orf1	0.00E+00	16
CDC2	3.25E-12	16
TTK	1.41E-12	16
BLM	3.31E-12	14
HRK	1.49E-06	15
PLSCR1	5.89E-07	15
EZH2	1.68E-12	16
HEC	3.26E-12	12
BIRC5	2.98E-13	13
CCNA2	3.04E-09	15
CDC25A	1.11E-16	14
MCM5	4.69E-11	15
MCM7	3.04E-11	15
PTTG1	2.10E-13	14
DEK	5.94E-06	15
KNSL2	1.22E-15	13
CDC45L	0.00E+00	15
UBCH10	1.44E-15	14
KIAA0175	3.68E-12	12
TEAD4	2.83E-07	13
CCNE1	2.59E-07	12
PRC1	3.25E-12	12
CHAF1B	2.03E-06	12
TK1	6.93E-08	12
SMC4L1	3.15E-08	13

gene/protein name	P value, ERGO vs.non-ERGO	number of over-expressing tumors among total of 31 tumors
RFC4	8.28E-11	10
MCM6	1.32E-11	12
CSRP2	3.07E-04	14
TGFA	2.10E-05	14
ORC6L	3.27E-12	11
PRIM2A	1.30E-09	10
KNSL1	6.25E-09	10
USP1	3.14E-06	13
MCM2	7.86E-09	11
MAD2L1	3.69E-12	14
TP53BP2	3.84E-07	9
CKS1	1.44E-09	9
NUP155	4.73E-09	8
NEK2	4.03E-13	10
STK15	2.34E-11	9
NASP	2.83E-08	10
DNA2L	2.22E-08	10
LMNB1	4.43E-09	13
NOLC1	1.03E-05	8
CKS2	3.29E-08	8
CSDA	3.35E-05	8
SOX9	6.86E-04	11
MYC	3.29E-05	9
KPNA2	4.49E-11	8
CCNE2	5.50E-08	11
FLJ22009	2.14E-09	8
KIF4A	8.99E-12	7
CCNB1	6.79E-12	9
RRM2	2.33E-11	8
CDC7L1	4.32E-08	6
CDC25B	2.86E-08	10
HMG4	6.95E-10	8
MAP3K14	8.30E-03	8
HMMR	5.27E-07	7
VEGF	3.75E-05	8
ANXA8	3.51E-05	9

This group of 74 different E2F-responsive genes was enriched for genes that are involved in the G_1/ S cell cycle transition stage and DNA replication (*e.g.* TYMS, TTK, ORC6L, MCM2, CCNE2MCM5, MCM6, MCM7, RFC4) and that are involved in the G_2/M cell cycle transition and mitosis (*e.g.* AURKB, KNSL6, CENPA, KIF20A, BUB1, CCNB2, STMN1, TPX2,

BIRC5, KNSL2). Differences between the frequencies of gene over-expression in ERGO and non-ERGO tumors were highly statistically significant for all genes in the ERGO subset and the P-values ranged from $1x10^{-5}$ to $< 1x10^{-16}$. However, these statistical comparisons are purely confirmatory in nature since the ERGO tumor subset was established by rank ordering.

In order to compare the ERGO tumors identified with other non-ERGO breast cancer tumor subsets, the non-ERGO tumors were subdivided into:

a) non-ERGO tumors over-expressing the HER2 receptor;

b) non-ERGO tumors over-expressing the estrogen receptor (ER) and/or the progesterone receptor (PR), and

c) non-ERGO tumors that do not over-express HER2, ER and/or PR.

Importantly, this last set of non-ERGO tumors which did not over-express HER2, ER and/or PR represents a residual "triple non-positive" tumor subset of non-HER2 over-expressing, non-ER over-expressing and/or non-PR over-expressing tumors. This tumor subset was designated the non-ERGO "triple non-positive" tumor subset. A selected gene expression profile for each of these subsets is shown in Fig. 1 and with more detail at Fig. 8.

The ERGO tumor subset is truly "triple-negative" or "triple non-positive," in that essentially all tumors in this set under-express the HER2 receptor, estrogen receptor (ER) and progesterone receptor (PR). This is significant because tumors over-expressing the HER2 receptor can be treated with trastuzumab, while tumors over-expressing the ER or PR receptors can be targeted with hormone therapy based treatment regimens using drugs that prevent estrogen and progesterone from stimulating these receptors. In contrast, "triple non-positive" tumors are unlikely to be responsive to treatments targeting over-expressed HER2, ER, or PR receptors.

Among the 31 ERGO tumors identified, 25 out of 31 (81%) of these tumors over-expressed cyclin E1, cyclin E2, and/or p16. However, over-expression of cyclin E1, cyclin E2, and/or p16 was observed in just 6 out of 46 (13%) of the non-ERGO "triple non-positive" tumors identified, 1 out of 26 (4%) of non-ERGO ER/PR over-expressing tumors, and 2 out of 14 (14%) of the non-ERGO HER2 over-expressing tumors. Importantly, this elevated expression of the cyclin E1, cyclin E2, and/or p16 genes in the ERGO tumor group indicates that E2F expression and E2F mediated regulation of the expression of these proteins is impaired in ERGO tumors, but not in most other tumors.

This is confirmed by the observation that among the 31 ERGO tumors identified 14 out of 31 (45%) of these tumors over-expressed E2F1, E2F2, and/or E2F3. In contrast, over-

expression of E2F1, E2F2, and/or E2F3 was observed in just 7 out 46 (15%) non-ERGO "triple non-positive" tumors identified, 8 out of 26 (31%) of the ER/PR over-expressing non-ERGO tumors identified (ER is known to induce E2F), and in none of the 14 HER2 over-expressing non-ERGO tumors identified. These findings are consistent with the conclusion that aberrant expression (*e.g.* under-expression) of E2Fs (such as E2F1) is occurring in the ERGO breast cancer tumors identified.

Additionally, 16 out of the 31 (52%) ERGO tumors identified over-expressed at least two basal-like tumor cytokeratin markers selected from the group consisting of the cytokeratin 5, cytokeratin 6A, cytokeratin 6B, cytokeratin 14, and cytokeratin 17 basal-like tumor markers. In contrast, over-expression of at least two of these basal-like tumor cytokeratin markers was observed in just 5 out of 46 (11%) non-ERGO "triple non-positive" tumors identified, 2 out of 26 (8%) of the ER/PR over-expressing non-ERGO tumors identified, and in none of the HER2 over-expressing non-ERGO tumors identified.

Importantly, over-expression of these cytokeratin markers is an important criterion for the identification of basal-like tumors. Consequently, the observation here that over-expression of at least two basal like cytokeratins occurred in both the ERGO tumors identified and non-ERGO "triple non-positive" tumor subset identified indicates that the basal-like tumors may represent a genus of tumors with a phenotype that includes both the "triple non-positive" ERGO tumor subset identified and the non-ERGO "triple non-positive" tumor subset identified.

The Van't Veer microarray set analyzed also included tumor samples from 18 patients with known hereditary BRCA1 mutations. Such BRCA1 mutations are believed to pre-dispose patients carrying such mutant genes to cancer. This is because the BRCA1 protein product is involved in DNA damage repair, ubiquitination, transcriptional regulation and other cellular functions. The BRCA1 protein and expression of the gene encoding BRCA1 is important because BRCA1 helps maintain genomic integrity by promoting high fidelity DNA repair when genomic DNA mutations occur. Mutations in the BRCA1 gene or defects in the expression of the gene encoding BRCA1 are believed to result in the accumulation of genomic DNA mutations that can lead to uncontrolled cell division and cancer.

Importantly, 11 of the 18 tumors (61%) from patients with BRCA1 mutations were ERGO tumors, and 8 of these 11 tumors (73%) over-expressed at least two basal-like tumor cytokeratin markers selected from the group consisting of cytokeratin 5, cytokeratin 6A, cytokeratin 6B, cytokeratin 14, and cytokeratin 17 basal-like tumor markers. The 7 other tumors (out of 18) from patients with BRCA1 mutations were non-ERGO "triple non-positive"

tumors, and only 2 of these 7 tumors (29%) over-expressed at least two of the basal-like tumor cytokeratin markers. These findings further confirm that the basal-like tumor genus includes both the ERGO tumor subset and the non-ERGO "triple non-positive" tumor subset. Additionally, these data also indicate that over-expression of basal-like cytokeratins alone is inadequate as a marker for identifying all members of the basal-like tumor genus. This is significant, because it indicates that the ability to identify ERGO tumors by using the gene expression profile of a tumor to determine if it is an ERGO tumor will permit the identification of appropriate tumor treatment regimens and survival predictions for an individual patient.

Last, ID4 which is a basal-like tumor marker was over-expressed in 10 out of 16 of the ERGO tumors (63%) identified, and 4 out of 4 of the non-ERGO "triple non-positive" tumors which over-expressed at least two of the basal-like tumor cytokeratins markers described above. However, ID4 was also over-expressed in 2 ERGO tumors and 3 non-ERGO BRCA1 tumors that did not over-express at least two basal-like cytokeratins. CDH3 which is another basal-like tumor marker was over-expressed in 14 out of 16 (88%) of the ERGO tumors over-expressing at least two basal-like cytokeratins, and in 3 out of 4 (75%) of the non-ERGO "triple non-positive" tumors identified that over-expressed at least two basal like cytokeratins. However, CDH3 was also over-expressed in 4 ERGO tumors and 4 non-ERGO tumors, including 3 non-ERGO tumors from patients with BRCA1 mutations that did not over-express at least two basal-like cytokeratins. Furthermore, ID4 and CDH3 over-expression was also observed in 3 non-ERGO tumors over-expressing the HER2 receptor. Together, these observations indicated that the basal-like tumor genus is not limited solely to ERGO tumors and non-ERGO "triple non-positive" tumors.

Principal component analysis and the basal-like subset.

Principal component analysis (PCA) was then performed on the Van't Veer microarray set using TM4 software version 4.1.01 (www.TM4.org; Dana-Farber Cancer Institute, Boston, MA, USA). This alternative methodology was used to confirm and validate the analytical results obtained with the weighted rank ordering method. PCA was performed using the default settings of the TM4 software unless otherwise indicated.

Principal component analysis of the Van't Veer microarray set revealed a tumor cluster consisting of 38 tumors (Fig. 2A) which all under-expressed the ER based on the criteria described above for "under-expression." 35 of these 38 tumors (92%) were "triple negative. Twenty (20) of these 38 tumors (53%) over-expressed at least two of the basal-like cytokeratins described above. Moreover, only 2 of the 79 tumors (3%) not included in this cluster over-

expressed at least two of these basal-like cytokeratins. Importantly, 91% of all tumors that over-expressed at least two basal-like cytokeratins were found in this cluster of 38 tumors.

Thirty-three (33) of the 38 tumors (87%) in the cluster over-expressed the basal-tumor markers ID4 and/or CDH3, but only 8 of the 79 (10%) tumors that were not in the cluster over-expressed these basal-tumor markers. Among 25 published markers for basal-like tumors (see Table 3 below) that are over-expressed in basal-like tumors, all members of the PCA cluster over-expressed at least 2 of these markers, and 35 out of 38 of the tumors in this cluster over-expressed at least 3 such markers (92%). Thus all members of this tumor cluster identified by PCA can be identified as basal-like tumors based on the over-expression of these markers.

Table 3

gene name	type	ref
CDH3	dx/prog	Ames,2005; Matos, 2005
KRT17	dx/prog	van de Rijn,2002
CRYAB	dx/prog	Moyano, 2006,2008
SNL/fascin	dx	Rodríguez-Pinilla, 2006
ID4	dx	turner, 2007
KRT5	dx/prog	van de Rijn,2002
VIM	dx/prog	Rodríguez-Pinilla, 2007
ACTG2	dx	livasy, 2006
CCNE1	dx/prog	Foulkes, 2004
EZH2	dx/prog	Collett,2006
LAMB3	dx	Rodríguez-Pinilla 2007
KRT6B	dx/prog	van de Rijn,2002
KRT14	dx/prog	Fulford, 2007
MSN	dx/prog	Charafe-Jauffret 2007
ANXA8	dx/prog	Stein, 2005
CCNE2	dx/prog	Foulkes, 2004
CD44	dx	Charafe-Jauffret, 2006
ITGB4	dx/prog	Lu, 2008
S100A9	dx/prog	Goncalves, 2008
MCAM/cd146	dx/prog	Garcia, 2007
KIT	dx	Nielsen, 2004
KRT6A	dx/prog	van de Rijn,2002
P63	dx	Matos, 2005
EGFR	dx	Nielsen, 2004
TGFBR2	prog	uck, 2004

This basal-like tumor cluster identified by PCA contained 27 of the 31 (87%) ERGO tumors identified by the rank ordering method and also included all 11 ERGO tumors from patients with BRCA1 mutations. Of the four ERGO tumors that were excluded from the basal-

like tumor cluster identified by PCA, three were borderline by the ranking method, and would have not been identified as ERGO tumors if the stopping criteria for the ranking method been more stringent (*e.g.*, requiring that ERGO tumors over-express at least 25% of over-expressed E2F-responsive genes instead of 20%). The basal-like tumor cluster identified by PCA also contained 6 out of 7 of the non-ERGO tumors identified from patients with BRCA1 mutations. Together, these results identify ERGO tumors as members of the basal-like breast cancer tumor subset identified by PCA, and confirms that both the ERGO tumors and non-ERGO tumors from patients with BRCA1 mutations identified by the rank ordering method are members of the basal-like tumor subset identified by the PCA method.

Refined Principal Component Analysis and the Identification of the ERGO Tumor Subset within the Basal-Like Tumor Subset Identified by PCA.

The basal-like tumor cluster identified by PCA as described above was further analyzed. This was done by using further refined PCA in which the analyzed gene set was restricted to the E2F-responsive genes listed in Table 1 shown above. In these analyses only those tumors in the basal-like tumor cluster identified by PCA as described above were used as input data. The result of this refined PCA analysis was that the non-ERGO tumors clustered separately from the ERGO tumors (Fig. 2B), although 2 of 27 ERGO tumors identified by the ranking method did not separate cleanly from the non-ERGO tumors.

This refined PCA gene clustering analysis revealed a continuous strand of genes strung along the axis of the first principal component, rather than discrete gene clusters (Fig. 3A). The group of genes shown in black in the upper left in Fig. 3A are the E2F-responsive genes over-expressed most frequently among the basal-like tumor cluster identified by PCA as described above. A list of the most frequently over-expressed genes in the basal-like tumors identified by PCA was then compared to a list of most frequently over-expressed E2F-responsive genes in the ERGO tumors identified by the weighted rank ordering method. As shown in Fig. 3B these two lists are essentially identical for genes that are over-expressed in greater than 35% of the basal-like tumors identified by PCA or the ERGO tumors identified by the weighted rank-ordering method. Very few of the genes that were over-expressed in less than 25% of the basal-like tumors identified by PCA were also over-expressed by the ERGO tumors identified by the rank ordering method. Importantly, it was the ERGO tumors identified by the refined PCA analysis of the basal-like tumor cluster identified by PCA that over-expressed the highest number of E2F-responsive genes per individual tumor (Fig. 3C).

Patterns of Over-Expression and Under-expression of Non-E2F-Responsive Genes in the Basal-Like Tumors Identified by PCA and the ERGO Tumors Identified by Refined PCA.

Over 850 non-E2F-responsive genes were over-expressed in at least 20% of PCA basal-like tumors identified by PCA, such that the differences in frequency of over-expression between the basal-like tumors identified by PCA and the non-basal-like tumors identified by PCA was statistically significant at the P <0.05 level. See Fig. 25 and Table 4 below.

Table 4

PCA Basal-Like Cancers

gene name	mean(1) > mean(2)
KIAA0514	0
BCL11A	1.11E-16
LDHB	1.11E-16
TONDU	2.22E-16
SH2D2A	2.22E-16
NCK1	5.55E-16
CLCN4	6.66E-16
C2orf2	6.66E-16
FOXC1	1.22E-15
CDH3	1.44E-15
MSN	1.67E-15
LAMP3	2.78E-15
PDI2	2.89E-15
IMPA2	3.55E-15
PFKP	6.99E-15
LOC51323	7.22E-15
DKFZP564M2423	8.10E-15
CTSL2	1.30E-14
ODC1	2.28E-14
NSEP1	4.60E-14
LOC56963	5.01E-14
TNFAIP3	6.56E-14
CEBPG	7.44E-14
UGT8	2.06E-13
RBMS1	2.24E-13
ST5	2.75E-13
H1F1	4.00E-13
BA395L14.2	4.70E-13
KIAA1209	5.83E-13
ASNS	7.26E-13
FLJ10540	9.04E-13
DKFZP586C1619	9.71E-13
SCYD1	1.09E-12

gene name	mean(1) > mean(2)
FLJ23399	1.12E-12
FLJ11413	1.13E-12
FLJ22833	1.18E-12
DKFZp762A227	1.21E-12
SH3BP1	1.50E-12
KIAA0042	1.71E-12
AF070552	2.66E-12
KCNK5	3.25E-12
LMO4	3.25E-12
KIAA1350	3.25E-12
SIAT8A	3.25E-12
PSA	3.25E-12
CTPS	3.25E-12
DSC2	3.25E-12
KIAA1140	3.25E-12
GABRP	3.25E-12
PHGDH	3.25E-12
DKFZp762E1312	3.25E-12
PIM1	3.25E-12
SIL	3.25E-12
TM4SF1	3.25E-12
ATDC	3.26E-12
DKFZp564A026	3.27E-12
NFIL3	3.27E-12
PROML1	3.29E-12
AF052117	3.29E-12
PRO1659	3.29E-12
DIAPH3	3.30E-12
SCYA18	3.32E-12
ICB-1	3.33E-12
IL6	3.34E-12
GBP1	3.35E-12
SERPINB5	3.44E-12
KCNN4	3.49E-12
MID1	3.49E-12
KIP2	3.54E-12
MYBL2	3.55E-12
FLJ20005	3.63E-12
KIAA0074	3.64E-12
HMGIY	3.68E-12
NDRG1	3.75E-12
KIAA1553	3.79E-12
EXO1	4.20E-12
SNL	4.23E-12
HSU54999	4.36E-12

gene name	mean(1) > mean(2)
GZMB	4.53E-12
PRO2249	4.65E-12
MCCC1	5.09E-12
KIAA1285	5.21E-12
SMOC1	5.43E-12
KIAA0481	5.45E-12
TMEFF1	5.46E-12
NSAP1	5.53E-12
VIM	5.58E-12
FLJ20354	6.05E-12
RUNX3	6.14E-12
CHIC2	6.45E-12
FLJ13154	6.84E-12
FLJ10468	7.28E-12
UQCRH	7.88E-12
PRKCN	8.01E-12
ME1	8.11E-12
MARCO	8.25E-12
PRKX	8.73E-12
FLJ20186	8.79E-12
FZD9	9.40E-12
GMPS	9.44E-12
DKFZP762N2316	9.90E-12
KIAA0159	1.07E-11
LOC51700	1.07E-11
NMB	1.13E-11
LOC64148	1.42E-11
HSPC159	1.42E-11
GAPDS	1.43E-11
TRIM2	1.49E-11
LAD1	1.54E-11
ARL7	1.55E-11
HSRNASEB	1.55E-11
NMT2	1.60E-11
AL110202	1.61E-11
FLJ10156	1.66E-11
KRT16	1.73E-11
SOAT1	1.75E-11
SCYA13	1.80E-11
KIAA0746	1.85E-11
FLJ21079	2.01E-11
ITM2C	2.51E-11
DKFZP434G032	2.82E-11
DKFZP586G1517	2.82E-11
FLJ12649	3.04E-11

gene name	mean(1) > mean(2)
MRAS	3.17E-11
NFE2L3	3.79E-11
FLJ20425	4.24E-11
AF131753	4.39E-11
PTX3	4.77E-11
KIAA1448	4.81E-11
HSU93243	5.16E-11
VRK2	5.23E-11
DUSP9	5.30E-11
FLJ23293	5.74E-11
LRP8	6.19E-11
TFDP1	6.74E-11
SCHIP1	6.88E-11
FLJ10549	7.01E-11
EGFL6	7.63E-11
PCDH8	7.84E-11
GAPD	7.92E-11
FLJ23414	8.75E-11
KIAA1069	8.88E-11
CHI3L1	8.96E-11
SCYA2	9.14E-11
MGC4090	9.16E-11
MPB1	9.21E-11
CSPG6	9.66E-11
NOGOR	1.04E-10
FLJ11252	1.08E-10
KIAA0077	1.10E-10
TTYH1	1.18E-10
DUSP2	1.20E-10
HHGP	1.26E-10
SRPK1	1.30E-10
CA9	1.41E-10
FLJ11029	1.42E-10
ZNF267	1.45E-10
KIAA0095	1.48E-10
LPIN1	1.58E-10
PGM1	1.62E-10
KIAA1357	1.65E-10
KLK6	1.72E-10
CHST2	1.75E-10
IFRD1	1.75E-10
CIN85	1.93E-10
AL050151	1.97E-10
JRKL	2.09E-10
ADM	2.10E-10

gene name	mean(1) > mean(2)
RIPK2	2.12E-10
DJ742C19.2	2.47E-10
KIAA0008	2.60E-10
ENO1	2.68E-10
LOC56938	2.72E-10
AMD1	2.87E-10
SNN	3.00E-10
UMPK	3.11E-10
PLD1	3.19E-10
FABP7	3.21E-10
POLR2F	3.49E-10
SOX11	3.57E-10
AK001295	4.06E-10
SOX10	4.17E-10
FLJ22341	4.24E-10
SOD2	4.40E-10
PLCG2	4.61E-10
FLJ10549	4.68E-10
MMP12	4.70E-10
CALB2	4.77E-10
DAPK1	4.80E-10
EBI2	5.53E-10
RAB6B	5.84E-10
MIA	5.92E-10
PTTG2	6.06E-10
MYO10	6.25E-10
LIPG	6.43E-10
S100A6	6.56E-10
ADAMTS7	6.64E-10
MMP7	7.37E-10
GDF5	7.90E-10
RARRES1	8.20E-10
ILF2	8.56E-10
EIF2C2	8.85E-10
S100A10	8.88E-10
FLJ10829	1.02E-09
KLF5	1.05E-09
PLA2G4A	1.08E-09
LOC55971	1.09E-09
TCN2	1.12E-09
BENE	1.19E-09
KIAA1424	1.23E-09
HOMER-3	1.44E-09
ADORA2B	1.59E-09
FABP5	1.69E-09

gene name	mean(1) > mean(2)
CLSP	2.00E-09
LCK	2.20E-09
ADFP	2.21E-09
STRIN	2.32E-09
INDO	2.33E-09
CDK2AP1	2.51E-09
ASS	2.51E-09
SBBI26	2.52E-09
LOC51765	2.60E-09
FLJ10901	2.60E-09
BCL2A1	2.83E-09
GSTP1	2.94E-09
BPI	3.21E-09
CYP39A1	3.41E-09
D123	3.46E-09
ETV6	3.51E-09
DKFZP564D0462	3.67E-09
SLC2A1	3.72E-09
HK3	3.81E-09
D21S2056E	4.02E-09
LOC51053	4.07E-09
PRO2013	4.08E-09
DC11	4.22E-09
KRT17	4.34E-09
EDN1	5.04E-09
UCHL3	5.29E-09
KIAA1089	5.35E-09
DSG3	5.40E-09
RNASEH1	5.44E-09
CP	5.55E-09
RELB	5.66E-09
MALT1	5.90E-09
ETS1	5.93E-09
KIAA0275	6.02E-09
DYSF	6.07E-09
PRG1	6.08E-09
KIAA1035	6.11E-09
AMY2B	6.11E-09
LBP-9	6.44E-09
LCP1	6.53E-09
PKP1	6.67E-09
TGM1	6.80E-09
RAP2A	7.18E-09
ZIC1	7.30E-09
RASAL1	7.50E-09

gene name	mean(1) > mean(2)
PDXK	7.51E-09
FLJ21324	7.80E-09
TIA1	7.84E-09
PIST	8.06E-09
ABP/ZF	8.18E-09
P5	8.47E-09
DEFB1	8.66E-09
DSCR1	8.90E-09
AMY1A	9.14E-09
LILRB2	9.91E-09
ICAM1	9.93E-09
HRB	1.00E-08
CXADR	1.01E-08
MT1L	1.02E-08
BCL11B	1.04E-08
FLJ10517	1.04E-08
BM039	1.06E-08
NMI	1.08E-08
FAT	1.13E-08
NUMBL	1.17E-08
LOC55862	1.18E-08
SLC6A14	1.20E-08
CCR1	1.23E-08
GPM6B	1.25E-08
E48	1.25E-08
IGHG3	1.31E-08
MGC3180	1.36E-08
C1GALT1	1.43E-08
DKFZP586F2423	1.44E-08
ART3	1.47E-08
B4-2	1.49E-08
DKFZP586E1621	1.51E-08
HDGF	1.52E-08
SLC2A5	1.53E-08
MMP1	1.56E-08
KPNA2	1.57E-08
COL9A3	1.62E-08
TRIP13	1.71E-08
GGH	1.72E-08
FLJ10706	1.79E-08
TEL2	1.87E-08
PLA2G7	1.98E-08
AKAP2	1.98E-08
MAPRE2	1.99E-08
TAP1	2.02E-08

gene name	mean(1) > mean(2)
KRT5	2.12E-08
ARP3BETA	2.32E-08
KRT6B	2.35E-08
DSCR2	2.55E-08
AK000660	2.70E-08
CSTB	2.71E-08
FLJ23518	2.82E-08
GNB4	2.86E-08
KIAA0197	2.95E-08
IL15RA	2.95E-08
DMD	2.98E-08
CXCR4	3.13E-08
DONSON	3.51E-08
S100A1	3.54E-08
SLC16A1	3.78E-08
WARS	3.87E-08
LTB	3.89E-08
TPI1	3.92E-08
EPHA2	3.93E-08
DD96	4.11E-08
KIAA0173	4.11E-08
UBD	4.13E-08
EBBP	4.26E-08
SCYA5	4.30E-08
CRYAB	4.35E-08
SEMA4D	4.49E-08
BRUNOL4	4.53E-08
ALY	4.62E-08
DDXL	4.69E-08
STAC	4.89E-08
LILRB3	4.93E-08
AMY2A	4.96E-08
AK001380	5.07E-08
CYBB	5.23E-08
FXYD5	5.33E-08
GRO1	5.34E-08
FLJ10407	5.49E-08
CCNC	5.56E-08
FLJ10709	5.94E-08
PHRET1	6.01E-08
PDL2	6.10E-08
ARHGEF4	6.17E-08
KIAA0353	6.24E-08
DHCR7	6.38E-08
AL137342	6.47E-08

gene name	mean(1) > mean(2)
PTGS2	6.59E-08
BIN1	6.62E-08
SCYB11	6.86E-08
KIAA0552	6.92E-08
CD163	6.97E-08
GPRC5B	7.04E-08
FLJ20287	7.09E-08
FBL	7.11E-08
AD024	7.13E-08
PTPN14	7.16E-08
SFRP1	7.25E-08
FOXD1	7.28E-08
PSMB9	7.54E-08
FLJ21069	7.75E-08
UBA2	7.76E-08
ANKRD3	7.77E-08
DSC3	7.95E-08
AK001942	8.06E-08
FLJ10262	8.10E-08
KIAA1609	8.21E-08
MX2	8.47E-08
FBXO5	9.27E-08
DKFZP564J0863	9.33E-08
DKFZp434F2322	9.33E-08
TEGT	9.60E-08
FIGN	1.04E-07
RDX	1.05E-07
FLJ10359	1.05E-07
SGK	1.06E-07
PPP1CB	1.07E-07
PRAME	1.13E-07
CKS1	1.13E-07
TMSNB	1.13E-07
KIAA0062	1.17E-07
BCE-1	1.19E-07
CKAP2	1.27E-07
KLK7	1.30E-07
ATF4	1.35E-07
SCYB10	1.38E-07
EPHB1	1.39E-07
AK001630	1.45E-07
KNSL5	1.46E-07
DAPP1	1.53E-07
PDE4B	1.55E-07
TC21	1.56E-07

gene name	mean(1) > mean(2)
SPIB	1.63E-07
PFDN2	1.69E-07
GATA6	1.77E-07
CD3Z	1.82E-07
ST14	1.86E-07
KIAA0179	1.94E-07
KIAA1392	1.96E-07
PIR	2.06E-07
KRTHB1	2.18E-07
MPZL1	2.19E-07
KIAA0668	2.20E-07
TRAF1	2.41E-07
LYZ	2.56E-07
APS	2.60E-07
TRB@	2.64E-07
TCF20	2.66E-07
AK000208	2.72E-07
NF2	2.74E-07
NK4	2.93E-07
TUBB4	2.95E-07
DNMT2	3.21E-07
PCOLCE2	3.35E-07
C6orf34	3.54E-07
CRABP1	3.66E-07
OTRPC4	3.76E-07
CD83	3.84E-07
FLJ10316	3.91E-07
SOD3	4.05E-07
FLJ12505	4.17E-07
CCR7	4.26E-07
HLA-F	4.38E-07
ribosomal protein L39	4.39E-07
PTPLA	4.45E-07
EPHB3	4.53E-07
IL1B	4.70E-07
FUT4	4.83E-07
PTPRCAP	5.06E-07
KIAA0007	5.07E-07
TNF	5.16E-07
MTAP44	5.20E-07
AK000954	5.28E-07
NOL1	5.32E-07
TCF7	5.42E-07
CDH19	5.77E-07
CDKN2A	5.97E-07

gene name	mean(1) > mean(2)
LOC51316	6.00E-07
AK001394	6.11E-07
CTSS	6.24E-07
FLJ20371	6.44E-07
ADORA2BP	6.60E-07
H1F5	6.88E-07
TNFRSF1B	6.92E-07
NDUFA9	7.03E-07
TNFRSF11A	7.31E-07
AIM2	7.41E-07
NMU	7.42E-07
SFN	7.43E-07
UGP2	7.44E-07
DKFZP761H171	7.86E-07
MTR1	8.03E-07
WDR5	8.09E-07
G2	8.14E-07
DKFZP564L0862	8.46E-07
TRIP7	8.48E-07
P450RAI-2	8.50E-07
PRO2000	8.56E-07
YES1	8.62E-07
KLK5	8.66E-07
KIAA0449	8.81E-07
KIAA0680	8.94E-07
LOC51203	9.62E-07
MRPL37	9.98E-07
CAPN6	1.01E-06
PGAR	1.03E-06
HRASLS	1.04E-06
DXS9928E	1.05E-06
FLJ10697	1.06E-06
FLJ20330	1.06E-06
TNFSF13B	1.08E-06
LBP-32	1.08E-06
SACS	1.10E-06
CEBPB	1.13E-06
KIAA0870	1.17E-06
KIAA0237	1.24E-06
SCYA4	1.39E-06
FLJ10895	1.41E-06
CD3G	1.47E-06
CORO1A	1.48E-06
CD72	1.49E-06
FZD7	1.52E-06

gene name	mean(1) > mean(2)
FLJ10665	1.62E-06
RPC32	1.66E-06
CD3D	1.66E-06
TERA	1.66E-06
AK000770	1.67E-06
LY64	1.72E-06
FOXG1B	1.74E-06
TBPL1	1.77E-06
SIRP-b2	1.80E-06
RGS2	1.92E-06
POMC	1.95E-06
MIG	1.96E-06
ROR1	1.99E-06
SCRG1	1.99E-06
MYCL1	2.04E-06
KIAA0020	2.04E-06
LOC51668	2.06E-06
FLJ20485	2.11E-06
PCDHB9	2.14E-06
LOC51630	2.15E-06
MRPL15	2.16E-06
ZNF313	2.18E-06
IL8	2.21E-06
NFIB	2.22E-06
FLJ20435	2.25E-06
CYBA	2.27E-06
SCYA7	2.28E-06
FLJ20105	2.28E-06
POU2AF1	2.33E-06
KRT15	2.43E-06
SSR1	2.53E-06
FLNA	2.55E-06
HLA-B	2.74E-06
IL12RB2	2.78E-06
FLJ10470	2.80E-06
CPA4	2.90E-06
NCF2	2.94E-06
IL10RA	3.00E-06
CHI3L2	3.03E-06
KRT7	3.04E-06
FLJ11296	3.07E-06
PSMB2	3.10E-06
KIAA1214	3.13E-06
NDRG2	3.13E-06
DDX21	3.29E-06

gene name	mean(1) > mean(2)
KIAA1678	3.54E-06
FADS2	3.57E-06
LRP6	3.75E-06
CNGA1	3.84E-06
KRT14	3.84E-06
RNASE1	3.86E-06
S100A2	3.93E-06
FLJ20500	3.95E-06
IRAK1	4.39E-06
CD2	4.55E-06
AK000106	4.69E-06
Immunoglobulin	4.78E-06
FLJ20116	5.16E-06
SOX8	5.17E-06
IGKV3D-15	5.18E-06
ID4	5.26E-06
FLJ20038	5.28E-06
CD19	5.32E-06
NPR3	5.42E-06
KIAA0637	5.61E-06
GPR37	6.23E-06
LCP2	6.39E-06
AF103458	6.41E-06
FOLR1	6.55E-06
BIRC3	6.56E-06
GNA15	6.60E-06
GPR9	6.61E-06
DKK1	6.73E-06
PCANAP8	6.79E-06
PTGFR	7.36E-06
AK000776	7.40E-06
ISG20	7.73E-06
FLJ10292	7.84E-06
KLK8	7.96E-06
PRRG1	8.07E-06
TNFAIP2	8.38E-06
CHORDC1	8.59E-06
VCAM1	9.46E-06
CD5	9.48E-06
DKFZp434B1222	9.86E-06
ZWINT	1.01E-05
DKFZP586N2124	1.03E-05
IL2RB	1.07E-05
GNG4	1.14E-05
AL080059	1.19E-05

gene name	mean(1) > mean(2)
P2RX5	1.22E-05
ZNFN1A1	1.24E-05
SLC15A1	1.29E-05
KLK10	1.30E-05
AF063725	1.33E-05
AL133101	1.36E-05
PIM2	1.36E-05
IGHM	1.41E-05
CYP27A1	1.47E-05
ALG6	1.54E-05
M12.219	1.59E-05
FLJ10637	1.62E-05
AGM1	1.62E-05
AARS	1.67E-05
SELL	1.78E-05
ECGF1	1.90E-05
MSLN	1.99E-05
EPS15R	2.06E-05
TYMSTR	2.14E-05
MT1G	2.15E-05
AK000933	2.16E-05
IFRG28	2.23E-05
MFGE8	2.26E-05
CLDN1	2.30E-05
CD53	2.38E-05
HLA-E	2.38E-05
TSPAN-2	2.39E-05
W61000_RC	2.61E-05
PLS3	2.96E-05
NET1	3.04E-05
SAR1	3.10E-05
TNFSF6	3.10E-05
U96394	3.34E-05
DSP	3.48E-05
GCNT1	3.53E-05
MS4A4A	3.54E-05
FLJ20360	3.60E-05
PCTK3	3.90E-05
RAB27A	3.91E-05
FLJ21129	4.00E-05
IGLL1	4.01E-05
PTPRK	4.11E-05
FLJ22408	4.18E-05
IMP-2	4.19E-05
HEM1	4.24E-05

gene name	mean(1) > mean(2)
CD69	4.26E-05
SPS	4.48E-05
PLEK	4.48E-05
AL353948	4.52E-05
MAL	4.72E-05
variable region	4.83E-05
AF097495	5.06E-05
OS4	5.40E-05
KIAA0036	5.70E-05
PTPRC	5.86E-05
HUMMHCW1A	5.92E-05
FLJ20260	5.93E-05
ANXA3	6.00E-05
AL137346	6.02E-05
CD24	6.68E-05
SECTM1	7.57E-05
FLJ22028	7.65E-05
AF058075	8.00E-05
GPI	8.14E-05
FMO2	8.95E-05
AK002088	8.95E-05
PLCB4	9.26E-05
TCF8	9.29E-05
RASGRP2	9.29E-05
S100A9	9.32E-05
RPP40	9.49E-05
BM-005	9.62E-05
SELE	0.00010074
AL137736	0.00010102
GALNT3	0.00010121
AL133611	0.00010218
TNFRSF12	0.00010285
WSX-1	0.00010743
ARHE	0.00010941
ITGB4	0.00011071
LAMB3	0.00011149
X79782	0.00011171
SCYA19	0.00011704
SLC5A6	0.00011832
PAPSS1	0.00012229
CLC	0.00012443
CHRM3	0.00012635
EPHB2	0.00012862
AIM1	0.00013146
KIAA0667	0.00013373

gene name	mean(1) > mean(2)
HN1	0.00013498
GS3686	0.00013717
MACROH2A2	0.00013758
LCN2	0.00014652
FLJ10110	0.00015325
PLCB3NP	0.00015669
PTGDS	0.0001567
MS4A2	0.00015756
FLJ22477	0.00015882
HLA-DRB6	0.00016152
FLJ13204	0.00017364
FLJ10408	0.00018014
AKR1B1	0.00018344
CSR1	0.00019574
KIAA0786	0.00020194
cig5	0.00020328
GYPC	0.00020494
MX1	0.0002066
PPARGC1	0.00020788
TU3A	0.00020804
ALDH1A3	0.00021256
KIAA0167	0.0002174
CSPG4	0.00021782
MYBL1	0.00022132
ETV5	0.00022218
NFIX	0.00022432
MGC3040	0.00023986
FLJ21212	0.00024208
DKFZP564D206	0.00025636
KIAA1229	0.00025668
LOC57823	0.00025934
STAF50	0.00026248
USP18	0.00026452
AF035318	0.00026674
RIG-I	0.00026756
APEG1	0.00027352
LOC51678	0.00027438
KIAA0965	0.00027974
ACTG2	0.00029244
EIF4EBP1	0.00029248
DSG2	0.0002974
GW112	0.00030546
RGN	0.00031526
CASP1	0.00031578
MCAM	0.00031884

gene name	mean(1) > mean(2)
SIX3	0.00032956
LY6E	0.0003374
TGM5	0.00033884
FLJ14054	0.00034114
H1F3	0.00034446
DNCI1	0.00034532
KIAA1566	0.00036884
IFI30	0.00038314
RNASEHI	0.00039798
ZRF1	0.00040436
LOC51056	0.00041266
ITGA9	0.00043048
KIAA0172	0.0004343
AF131837	0.00044782
ITGA6	0.00044988
FLJ11106	0.0004756
ARGBP2	0.0004899
CD48	0.00050636
SAA1	0.0005154
OPRK1	0.0005226
GP1BA	0.0005325
CD36L1	0.00053766
KIAA1453	0.00056168
CBS	0.00056362
KIAA0403	0.0005869
FLJ12691	0.00059502
VLDLR	0.00059822
KIAA0554	0.00061354
TIMP2	0.0006222
AL049266	0.00062746
CYP1B1	0.00063132
OAS2	0.0006379
BRDG1	0.00067598
PFN2	0.00068956
AL137332	0.00077032
EVI2B	0.00078094
FLJ10970	0.00081688
TCF3	0.00082408
MT1E	0.00086288
GPR64	0.0009027
RGS10	0.00091806
STAT4	0.00094354
KIAA0092	0.00097058
PDK3	0.00097262
HMGCS1	0.0010899

gene name	mean(1) > mean(2)
FLJ20048	0.00117324
KDELR3	0.0012341
HNF3A	0.00124078
BIN2	0.00128052
MDFI	0.0012817
PTB	0.00135722
PODXL	0.00141214
AK001536	0.00142296
FLJ20401	0.00146878
HSU15552	0.00147042
LILRB4	0.00147626
AL110125	0.00148956
HSPC156	0.00150478
ANXA1	0.00160068
TRD@	0.00185688
FGL2	0.00186104
TNFRSF10B	0.00187774
IGL@	0.00197044
KLRB1	0.0020324
CEP4	0.0023878
CD38	0.002417
SLC22A3	0.0024792
CRHSP-24	0.0024844
KIAA0820	0.00252
KIAA0027	0.00253
PLXNA2	0.0025982
ZNF215	0.0026394
WIF-1	0.0028488
AF1Q	0.0029846
SLN	0.0030636
EDN2	0.0030818
CRLF1	0.0031166
AF055007	0.003125
CYR61	0.0032168
FLJ23384	0.0032198
PIK3C2A	0.0034572
PYGB	0.0037392
ZNF179	0.0038464
AKR1C1	0.0039554
ITGB8	0.004776
ISG15	0.004782
PFC	0.004949
DKFZp761P1010	0.0051658
SERPINB2	0.0054208
SLC16A8	0.005542

gene name	mean(1) > mean(2)
FLJ20510	0.0055696
PAX6	0.0058166
LOX	0.0059874
HPRP3P	0.0060524
FLJ21841	0.0070238
TRAG3	0.007268
KIT	0.0073464
PTPRR	0.0076296
PSIP1	0.007736
BITE	0.0080652
SPRY2	0.0085948
KIAA0469	0.0090724
LOC56932	0.0091562
VNN1	0.0092706
DPYSL2	0.0094624
AF103591	0.0095962
DKFZp434P086	0.009694
AKR1C2	0.0097626
KIAA1118	0.009955
SCYA21	0.0100306
AK001932	0.0101928
DKFZP434I216	0.0106252
MAGEA1	0.010632
A2M	0.0115294
IDH2	0.0126046
FLJ22215	0.0129192
ORM1	0.0131094
FLJ22002	0.0133204
FLJ20733	0.0139614
FLJ10811	0.0144508
IDI1	0.0146528
NR4A1	0.0150532
LOC51237	0.0157056
IRF4	0.0159874
LIF	0.0162782
MAPK8IP3	0.0165006
HML2	0.0165248
TNRC3	0.0184704
NDST3	0.021434
TCL1A	0.021436
PNUTL1	0.026414
EMP1	0.027848
RASGRP1	0.028092
FLJ10143	0.028706
AL137343	0.030014

gene name	mean(1) > mean(2)
KIAA1473	0.030696
HSPB2	0.03185
MMP3	0.032616
SHC3	0.03665
STX4A	0.038144
ELF5	0.040156
DFFB	0.042634
HLA-G	0.04326
AK000125	0.043288
PP13	0.044702
FLJ10781	0.04918
PDE9A	0.049704

For the top 100 over-expressed genes, P values ranged from $1x10^{-11}$ to $<1x10^{-16}$ (See first 100 genes listed in Table 4). The basal-like tumors identified by PCA also under-expressed over 1000 non-E2F-responsive genes in at least 20% of PCA basal-like tumor, such that the differences in frequency of under-expression between the basal-like tumors identified by PCA and the non-basal-like tumors identified by PCA was statistically significant at the $P < 0.05$ level. See Fig. 24. This further establishes that the basal-like tumors identified by PCA are members of a separate and distinct subset of human breast cancers. Additionally, these patterns of gene over-expression and under-expression were shared by both the ERGO tumors identified by refined PCA and non-ERGO tumors identified by refined PCA. Altogether, the analyses here indicate that ERGO tumors developed in, and evolved from, basal-like tumor precursors.

Reproducibility of Findings Across Microarray Sets.

The Dai microarray set was purged of patient tumors that overlapped with those included the Van't Veer microarray set (*i.e.* indentical tumor samples from identical patients), and was used to evaluate the reproducibility of the analytical results in the Van't Veer microarray set. The Figures and Tables herein may refer to the Van't Veer microarray set as "s1" and the purged Dai microarray set as "s2." Application of the weighted rank ordering method identified 33 tumors out of 236 total tumors (14%) in the purged Dai microarray set that over-expressed at least 20% of the top over-expressed E2F-responsive genes, and 82 E2F-responsive genes that were over-expressed in at least 20% of the ERGO tumors identified by the weighted rank ordering method in the purged Dai microarray set. Importantly, the lower 26% frequency of ERGO tumors identified in the purged Dai microarray set relative to the Van't Veer microarray set may be more representative of the overall frequency of ERGO

tumors in the general population, since the purged Dai microarray set was not enriched with tumors from patients having known BRCA1 mutations.

As described above, 74 E2F-responsive genes were over-expressed in the ERGO tumors identified by the weighted rank ordering method using the Van't Veer microarray set. Importantly, 70 of these same E2F-responsive genes were also over-expressed in the ERGO tumors identified in the purged Dai microarray set by the weighted rank ordering method. Sixty-one (61) of these 70 genes (87%) were over-expressed in the ERGO tumors identified by the weighted rank ordering method in both the purged Dai and Van't Veer microarray sets. The frequencies of over-expression of these individual E2F-responsive genes in the ERGO tumors identified by weighted rank ordering methods and the level of statistical significance were comparable in the purged Dai microarray set (s2) and the Van't Veer microarray set (s1) and were preserved in the most highly over-expressed genes (Fig. 10).

PCA identified a cluster of 66 tumors in the purged Dai microarray set (s2) that exhibited the basal-like phenotype (Fig. 2C). These 66 tumors were then further analyzed. This was done by using refined PCA in which the analyzed gene set was restricted to the E2F-responsive genes listed in Table 1 shown above. In these analyses only those tumors in the basal-like tumor cluster identified by PCA were used as input data. Two clusters comprising 30 ERGO and 36 non-ERGO tumor subsets were identified by refined PCA analysis of these 66 tumors (Fig. 2D). In the purged Dai microarray set, HER2 positive tumors were among the basal-like tumors identified by refined PCA which clearly exhibited a basal-like phenotype (Fig. 4). These data can be viewed in greater detail in Supplementary Fig. 4. There was also a group of HER2 over-expressing basal-like tumors identified by refined PCA in the purged Dai set that exhibited a "transitional" phenotype intermediate to that of the basal-like tumors and that that of non-basal-like HER2 over-expressing tumors identified by PCA. In Fig. 2C the transitional HER2-over-expressing basal-like tumors identified are marked with crosses and are found within the basal-like cluster. The presence of HER2 over-expressing basal-like tumors and the transitional subgroup were not apparent from the analyses of the Van't Veer microarray set (s1).

In general, the gene expression profiles of ERGO tumors and the other breast cancer subtypes identified and analyzed in the purged Dai microarray set (s2) was quite similar to those observed after analysis of the Van't Veer microarray set (s1) (*see* Fig. 1 and Tables 6, 7 and 8). In both datasets, the ERGO tumors identified were almost entirely "triple-non-positive" for HER2, ER, and PR over-expression, and virtually all of the basal-like tumors identified

ınder-expressed ER and PR. The under-expression of Rb, the over-expression of cyclin E1 and :2, the over-expression of $p16^{Ink4a}$, the over-expression of E2F1, and the under-expression of :yclin D1 were prominent in ERGO tumors. However, this was less pronounced in non-ERGO)asal-like tumors, and rare in the other tumor subtypes. Together, these observations further ndicated that aberrations in E2F1 mediated control of transcription and the cell cycle is a 'eature of ERGO tumors. Last, the over-expression of multiple basal cytokeratins and of other)ublished basal-like tumor markers was restricted largely to ERGO tumors and non-HER2-)ver-expressing, non-ERGO basal-like tumors. Over-expression of the MYC proto-oncogene was more readily apparent as an ERGO tumor feature by inspection of the results from analysis)f the purged Dai microarray set than by inspection of the results from the analysis of the Van't Veer microarray set.

One hundred and one (101) E2F-responsive genes were over-expressed in at least 25%)f the ERGO tumors identified by refined PCA analysis of the purged Dai microarray set. In :ontrast, 86 E2F-responsive genes were over-expressed in at least 25% of the ERGO tumors dentified by refined PCA analysis of the Van't Veer microarray set (Fig. 12). The 101 E2F-'esponsive genes identified by refined PCA analysis of the purged Dai microarray set and the 36 E2F-responsive genes identified by refined PCA analysis of the purged Van't Veer nicroarray set were then compared. This comparison showed that 74 of the 86 genes (86%))ver-expressed in ERGO tumors identified by refined PCA in the purged Dai microarray set were also over-expressed in the ERGO tumors identified by refined PCA in the Van't Veer nicroarray set. Importantly, the relative frequencies of over-expression of individual genes among tumor were generally preserved in the two datasets especially among the most frequently over-expressed genes. In both the purged Dai microarray set and the Van't Veer nicroarray set the group of multiple E2F-responsive genes that were most frequently over-:xpressed in the ERGO tumor identified in these data sets by refined PCA were over-expressed)nly sporadically in PCA non-ERGO basal-like tumors identified in these microarray sets by refined PCA (Fig. 4).

In the Van't Veer microarray set both ERGO tumors identified by refined PCA and non-ERGO basal-like tumors identified by refined PCA differentially over-expressed and under-expressed hundreds of non-E2F-responsive genes. The top 50 most frequently over-expressed, and most frequently under-expressed, of these genes each are shown in Fig. 4. Surprisingly, these genes were reproducibly observed across both the purged Dai microarray set and the Van't Veer microarray sets. This observation was particularly apparent among the most

frequently over-expressed genes. For example, among the top 200 genes most frequently over-expressed genes in the basal-like tumor subset identified by PCA of the Van't Veer microarray set some 70% of these genes were also over-expressed in the purged Dai microarray set.

Patients with basal-like tumors as identified by PCA have an adverse clinical prognosis.

Detailed clinical survival data are available for 91 patients who provided tumor samples included in the Van't Veer microarray set. Clinical survival data are also available for the patients who provided tumor samples included in the Dai microarray set. The survival of patients with PCA basal-like breast cancer tumors, as identified by PCA, was shorter than for breast cancer patients identified by PCA as having other types of tumors ($P < 0.002$). Seventy five percent of patients with non-basal-like tumors as identified by PCA survived five years, whereas only fifty percent of patients with basal-like tumors as identified by PCA survived five years (Fig. 9). Patient survival data were plotted by the Kaplan-Meyer method and survival curves were compared using the Haentzel-Mantel statistic.

Detailed clinical survival data are also available for the 311 patients who provided tumor samples included in the Dai microarray set. Fig. 19 shows the clinical survival data for ERGO tumors, non-ERGO basal-like tumors, and the HER2 over-expressing tumors (Fig. 19A) subsets as well as the non-ERGO "triple non-positive" and all the remaining tumor subsets (Fig. 19B) identified by PCA analysis of the Dai microarray set. Patient survival data were plotted by the Kaplan-Meyer method and survival curves were compared using the Haentzel-Mantel statistic. Comparision of Figs 19A and 19B shows that patients with ERGO tumors, non-ERGO basal-like tumors, and the HER2 over-expressing tumors had a statistically significantly worse prognosis and odds of survival than all the remaining tumor subsets identified in the Dai microarray set by PCA analysis. These findings illustrate the point that different patient subsets with the same prognosis must be distinguished phenotypically for therapeutic purposes, even though the odds of survival are similar. This is because only HER2 over-expressing tumors can be treated with anti-HER2 therapeutics (*e.g.* trastuzumab) and only ERGO tumors can be considered for targeted therapy against ERGO tumor-associated genes. Stated differently, this data shows that individual HER2 over-expressing tumors and ERGO tumors must be classified and assigned to their appropriate treatment class followed by selection of an appropriate therapy for each individual patient having tumors in these clusters despite the similar survival prognosis for patients with tumors of either distinct type.

Example 2

Identification of E2F Responsive Gene Over-Expressing (ERGO) Tumors in Lung and Thyroid Organ Sites

Lung Cancer Microarray Set.

The lung cancer microarray set published by Jones *et al.* was used to investigate E2F-responsive gene expression in human lung cancers and human lung cancer derived cell lines. Gene expression data from tumor sample probed gene spots this microarray set was normalized to data from normal human lung tissue sample probed gene spots that were included in the microarray set. Preliminary analysis by rank ordering methods confirmed that results obtained from microarray samples identified as human small cell lung cancer clinical samples and from samples identified as human derived small cell lung cancer cell lines were sufficiently similar to justify including data from both sample types in the analyses here.

Thyroid cancer Microarray Set.

The gene expression microarray set published by Salvatore *et al.* was used to study E2F-responsive gene expression in human thyroid cancer. Microarray data from human thyroid cancer tumors were normalized to normal human thyroid tissue samples that were included in the microarray set. Gene expression data from tumor sample probed gene spots this microarray set was normalized to data from normal human thyroid tissue sample probed gene spots that were included in the microarray set.

Weighted Rank Ordering Methods and Criteria for Identification of ERGO Tumors in Lung and Thyroid Organ Sites.

First, a reference signal intensity value was obtained by determining the signal intensity values for the gene transcripts from normal lung or thyroid tissues samples as appropriate for a given array set to be analyzed. Then "over-expressed," "under-expressed," and non-over-expressed genes were identified. Gene "over-expression" was determined to occur when the signal intensity corresponding to a given gene transcript in the microarray set was 1.8 fold greater than the reference signal intensity value for the gene in normal lung or thyroid tissue as appropriate. Gene "under-expression" was determined to occur when the signal intensity corresponding to a given gene transcript in the microarray was 1.8 fold less than the reference signal intensity value for the gene in normal lung or thyroid tissue as appropriate. Gene "non-over-expression" was determined to occur when a gene was neither "over-expressed" nor "under-expressed."

Weighted rank ordering methods were performed using EXCEL™ software (Microsoft Corp., Redmond, WA) to rank the over-expressed genes by their frequency of expression

among tumors. In these rank ordering analyses the most highly over-expressed genes were placed closest to the origin and the contribution of each tumor to the ranking was weighted by its proximity to the origin of the tumor axis.

These weighted rank ordering methods were then used to rank the tumors by the number of the 325 specific E2F-responsive genes, shown above in Table 1, over-expressed per tumor. Tumors with the highest number of over-expressed genes per tumor were placed closest to the origin. The contribution of each gene to the ranking was positively weighted by its proximity to the origin of the gene axis.

Individual tumors with the lowest number of over-expressed genes per tumor, and individual genes with the lowest over-expression frequency among the tumors were then iteratively stripped out of the dataset, with recalculation of rankings, until predetermined stopping conditions were met. The stopping conditions were that at least 20% of the tumors remaining in the dataset over-express at least 20% of the remaining E2F-responsive genes. Tumors that remained in the data set after satisfaction of the stopping conditions were identified as ERGO tumors.

Identification of ERGO Tumors in Lung.

Microarray based studies of human lung cancer have been able to resolve adenocarcinomas, carcinoids, large cell cancers, and normal lung tissue, but have been unable to distinguish large cell neuroendocrine tumors from small cell lung cancers. The analyses here were undertaken because many small cell lung cancers are known to have Rb gene abnormalities. This is significant because normally the Rb tumor suppressor protein binds to E2F and regulates E2F mediated gene transcription. Thus, the analyses here sought to determine if some of these small cell lung cancers could be ERGO tumors and whether these ERGO tumors could be distinguished from non-ERGO neuroendocrine precursors by PCA based methods.

PCA analysis of the Jones lung cancer microarray set identified a cluster of tumors that contained carcinoids, large cell neuroendocrine tumors, and small cell lung cancers (Fig. 2E).

This lung cancer tumor cluster identified by PCA was then further analyzed. This was done by using further refined PCA in which the analyzed gene set was restricted to the E2F-responsive genes listed in Table 1 shown above. In these refined PCA analyses only those tumors in the lung cancer tumor cluster initially identified by PCA were used as input data.

The result of this refined PCA analysis was that three clusters corresponding to carcinoids, large cell neuroendocrine tumors, and small cell lung cancers were identified (Fig.

2F). The cluster consisting largely of small cell lung cancers over-expressed a number of E2F-responsive genes identical to those found in ERGO breast cancers such that over 50% of the E2F-responsive genes expressed in the ERGO breast cancer tumors identified by refined PCA and the E2F-responsive genes expressed in the small cell lung cancer cluster identified by refined PCA were identical (Fig. 13). This indicates that this sub-group of small cell lung cancers are ERGO tumors. The E2F-responsive genes that are over-expressed in ERGO lung cancers are over-expressed infrequently in most large cell neuroendocrine tumors and carcinoid tumors.

Small cell lung cancer ERGO tumors appear to have E2F regulatory defects (Fig. 14 and Table 8). Twenty-nine percent of small cell lung cancer ERGO tumors under-express Rb, and 36% over-express cyclin E1 or cyclin E2. This observation and the under-expression of cyclin D1 in 57% of small cell lung cancer ERGO tumors further indicates E2F dysregulation in ERGO tumors. There is no $p16^{ink4a}$ over-expression in small cell lung cancer ERGO tumors. However, p18 and p19, which like $p16^{ink4a}$ are cdk4 and cdk6 inhibitors that block phosphorylation of Rb by cyclin D1, are over-expressed in abundance and over-expression of p18 is found in 86% of the small cell lung cancer ERGO tumors 86% while over-expression of p19 is found in 71% of these small cell lung cancer ERGO tumors. p18 and p19 are believed to function analogously to $p16^{ink4a}$ and therefore their over-expression can be a marker for E2F dysregulation.

Additionally, the over-expression patterns of non-E2F–responsive genes in these small cell lung cancer ERGO tumors are shared with large cell neuroendocrine tumors, but not carcinoid tumors, indicating that these small cell lung cancers are derived from large-cell neuroendocrine tumors. In addition, there are non-E2F-responsive genes that are over-expressed in small cell tumors and large cell neuroendocrine tumors that are also over-expressed in carinoid tumors, indicating that both small cell tumors and large cell neuroendocrine tumors share a common heritage with carcinoids (Fig. 14).

Importantly, the E2F-responsive genes that are over-expressed in small cell lung cancer ERGO tumors identified by refined PCA and in breast cancer ERGO tumors identified by refined PCA strongly overlap, but the non-E2F responsive genes that are over-expressed in their respective precursor cells are mostly different, with several notable exceptions. Like breast cancer ERGO tumors, large cell neuroendocrine tumors over-express basal cytokeratins and other basal-like markers.

Microarray based studies have shown the phenotype of anaplastic thyroid cancers differs from that of papillary thyroid cancers and normal thyroid tissue with regard to the over-expression of genes involved in cell cycle control and chromosome segregation. The analyses here were undertaken because the transcriptional promoters of many of these cell cycle control and chromosome segregation genes are known to contain E2F binding sites.

Identification of ERGO Tumors in Thyroid.

The human thyroid cancer microarray set of Salvatore was also analyzed by PCA. Anaplastic thyroid cancers clustered separately by PCA (Fig. 2G), but this anaplastic thyroid cancer cluster also included two papillary thyroid cancers with transitional phenotypic patterns. There were 73 E2F-responsive genes that were over-expressed in at least 25% of the thyroid cancer samples in this anaplastic thyroid cancer cluster. Importantly, these 73 E2F-responsive genes were not over-expressed in those thyroid cancer samples that were not part of the anaplastic thyroid cancer cluster identified by PCA. Furthermore, fifty-one percent (51%) of these 73 E2F-responsive genes were identical to the over-expressed E2F-responsive genes that are characteristic of the ERGO breast cancer tumors identified by refined PCA (Fig. 15). A profile of selected over-expressed genes in thyroid cancer is provided in Fig. 16. There is also a group of E2F-responsive genes that was over-expressed in both anaplastic and papillary thyroid cancers, although the over-expression occurred at higher levels in the anaplastic thyroid cancer tumors which clearly identifies the papillary thyroid cancers as precursors of the ERGO anaplastic thyroid cancers. This conclusion is further supported by the observation that large numbers of both E2F-responsive and non-E2F-responsive genes were over-expressed in both tumor types, but without clear differences in levels of gene over-expression between the ERGO anaplastic thyroid cancers and papillary thyroid cancers (Fig. 16).

Importantly, the analyses here of thyroid and lung tumors show the frequency of over-expression of E2F-responsive genes that affect the G1/S cell cycle phase transition, and those that affect G2M were preserved in all ERGO tumors, regardless of the site of origin (*e.g.* breast, lung, or thyroid tissue) (Fig. 17). For example, in ERGO breast cancer tumors and ERGO thyroid cancer tumors, G2M-associated E2F-responsive genes were over-expressed more frequently than E2F-responsive genes associated with the G1/S phases. However, in ERGO lung cancer tumors identified by refined PCA the over-expression of G2M-associated E2F-responsive genes was less common and over-expression of E2F-responsive genes associated with DNA repair, was more common.

Example 3

Analysis of the Role of LINC Multiprotein Gene Transcription Repression Complex Function and FOXM1 Function in ERGO Tumors

The analyses above revealed that specific E2F-responsive genes were highly over-expressed in the ERGO tumors identified and that these specific E2F-responsive genes were over-expressed with high frequency among the different ERGO tumors identified. It was observed that many of these E2F responsive genes are targets of a multiprotein gene transcription repressing complex comprising both the Rb and E2F proteins that has been evolutionary conserved in *Caenorhabditis elegans* as the dRM protein complex, *Drosophila melanogaster* as the dREAM protein complex, and in mammals as the LINC protein complex. In *C. elegans* the dRM multiprotein complex represses transcription of genes that are required for DNA synthesis and mitosis, and produces controlled chromosomal aneuploidy to stop cell division and produce aneuploid differentiated cells as part of normal tissue differentiation of the hindgut in this organism.

Aberrations in at least four protein components of the dRM/dREAM/LINC multiprotein gene transcription repressing complex account for the majority of the abnormally over-expressed E2F-responsive genes in the ERGO tumors identified, and the over-expressed genes associated with DNA replication and mitosis. The downstream consequences of aberrations of the four components of this complex are shown in Fig. 5 and would produce a gene expression profile consistent with that observed in the ERGO tumors identified.

When the various components of these multiprotein repressor complexes, such as lin54, are lost these complexes fail to form correctly and no longer repress G2M-associated genes. Importantly, loss of TESMIN--the human homolog of the *C. Elegans* lin54 protein--results in the up-regulation of a number of known G2M-associated genes (Kittler 2007). Analysis of the complete Dai human breast cancer microarray set (s3) which consists of the entire group of 311 patients in the breast cancer microarray dataset revealed that TESMIN expression is deficient and apparently lost. This loss of TESMIN expression was observed in 25 out of 37 (68%) of ERGO tumors identified by refined PCA in the complete Dai human breast cancer microarray set and 25 out of 41 (61%) non-ERGO basal-like tumors identified. However, this loss of TESMIN expression occurred infrequently in the non-basal-like tumors and only 28 of 231 (12%) of the non-basal-like tumors identified by PCA of this microarray set had an apparent loss of TESMIN expression. Importantly, TESMIN-controlled genes account for 21 of the 107 E2F-responsive genes that are over-expressed in the ERGO tumors identified by refined PCA in

the complete Dai human breast cancer microarray set, and almost all of these 21 E2F-responsive genes are associated with mitosis.

The Rb protein is a component of the dRM/dREAM/LINC multiprotein gene transcription repressing complex. Loss of Rb expression in *Mus musculus* results in the over-expression of genes that are responsible for DNA replication and mitotic transit (Black and Nevins). Importantly, the analyses here found that RB1 is under-expressed in 19 of 37 (51%) ERGO tumors identified by refined PCA of the complete Dai human breast cancer microarray set, in 4 of 41 (10%) non-ERGO basal-like tumors identified, and in 23 of 231 (10%) of non-basal-like tumors identified in this microarray set. Twenty-four (24) of the genes that are over-expressed as a result of loss of Rb expression were also among the most highly over-expressed E2F-responsive genes in the ERGO tumors identified. These genes included mitosis-associated genes, and DNA replication-associated genes.

Some components of the dRM/dREAM/LINC multiprotein gene transcription repressing complex are associated with the complex only in quiescent cells. Examples of such proteins are E2F4 and p130 which is a "pocket" protein related to Rb. In proliferating cells the E2F4 and p130 components are shuttled out of the dRM/dREAM/LINC multiprotein gene transcription repressing complex and replaced by p107 which is another Rb-related protein, and B-Myb. When this occurs dRM/dREAM/LINC multiprotein complex becomes a gene transcription activating complex, and induces the transcription of genes that are associated predominantly with the G2M portion of the cell cycle. These induced genes minimally overlap with those induced by Rb loss.

B-myb induces transcription of cyclins A2, B1, and B2 which are also regulated by Rb. The analyses here found that B-myb is constitutively over-expressed in 10 of 37 (27%) ERGO tumors identified by refined PCA of the complete Dia human breast cancer microarray set, 4 of 41 (10%) non-ERGO basal-like tumors identified, and 8 of 231 (3%) of non-basal-like tumors identified. The analyses here also found that A-myb, another closely related member of the vertebrate myb gene family, is over-expressed in 12 of 37 (32%) ERGO tumors identified by refined PCA of the complete Dia human breast cancer microarray set, 2 of 41 (5%) of non-ERGO basal-like tumors identified, and 13 of 231 (6%) of non-basal-like tumors identified. Seven (7) genes that are regulated by B-myb were also over-expressed in the ERGO tumors identified and included cyclins A2, B1 and B2.

Over-expression of E2F1 and E2F2 by transfection of mouse embryo fibroblasts resulted in the identification of specific genes that are directly induced by E2F over-expression

(unlike a host of other E2F-responsive genes that might be indirectly induced as a result of downstream E2F-related effects on other genes) (Ishida *et al.*). The specific genes which are directly induced by E2F over-expression are predominantly genes associated with DNA replication and mitosis. The analyses here found that 15 of 37 (41%) ERGO tumors identified by refined PCA of the complete Dai human breast cancer microarray set over-expressed E2F1 and/or E2F2 (41%), while only 7 of 41 (17%) of non-ERGO basal-like tumors identified, and only 9 of 231 (4%) of non-basal-like tumors identified over-expressed E2F1 and/or E2F2. Twenty-one (21) genes that are governed by E2F are over-expressed in ERGO tumors and included cyclins A2, B1 and B2. This set of 21 genes had substantial overlap with those genes over-expressed as a result of an apparent loss of Rb expression.

Cyclins A2, B1, and B2 are over-expressed in response to derangement of multiple components of the human LINC multiprotein gene transcription repressing complex. These cyclins phosphorylate and activate the transcription factor FOXM1 (Major ML). FOXM1 is an E2F-responsive gene that is highly over-expressed in ERGO tumors, but is not a component of the LINC multiprotein repressor complex. Importantly, FOXM1 induces several DNA replication-associated and mitotic genes that do not overlap with those induced by derangements in components of the LINC multiprotein complex (Fig. 5). Activated FOXM1 does induce expression of cyclin A2, B1 and B2 which establishes a positive feedback loop between the cyclins and FOXM1. The analyses here found that FOXM1 is over-expressed in 23 of 37 (62%) ERGO tumors identified by refined PCA of the complete Dai human breast cancer microarray set, in 6 of 41 (15%) non-ERGO basal-like tumors identified, and in 2 of 231 (1%) non-basal-like tumors identified in this microarray set. Importantly, there is a direct relationship between the average level of FOXM1 gene expression per tumor and the number of over-expressed FOXM1-associated DNA replication genes and mitotic genes in that tumor (Fig. 6). Furthermore the highest average levels of FOXM1 and the greatest numbers of over-expressed FOXM1-associated genes per tumor are observed in ERGO tumors (Fig. 6).

Several additional over-expressed mitotic and DNA replication-associated genes that are over-expressed by the ERGO tumors identified have also been linked to the derangement of grouped components of LINC multiprotein repressor complex (Georlette). These four genes are identified in Fig. 5 by four short horizontal arrows.

The foregoing clearly indicates that derangements in four of the components of the LINC multiprotein gene transcription repressing complex and/or FOXM1 account for 55 out of 107 (51%) of the over-expressed E2F-responsive genes associated with ERGO tumors

identified in the complete Dai human breast cancer microarray set, and 50 of 67 (75%) of the mitotic and DNA replication-associated E2F-responsive genes that are over-expressed in these ERGO tumors. Most of the ERGO tumor-associated E2F-responsive genes that are aberrantly over-expressed in breast cancers, lung cancers, and thyroid cancers are DNA replication and mitosis associated genes that are linked to the Rb/E2F-containing multiprotein repressive complex. Together, these observations indicate that derangements of the human LINC multiprotein gene transcription repressing complex play a central role in the development of aggressive malignancies at multiple organ sites such as human breast, lung, and thyroid.

Example 4

Identification of E2F Responsive Gene Over-Expressing (ERGO) Tumors in a Prostate Cancer Microarray Set.

Prostate cancer is common among men over 60 years in age, but only about 15% of patients with invasive prostate cancer ultimately die of their disease. "Watchful waiting" is one option for management of prostate cancer, and could be more safely elected if robust markers for distinguishing aggressive prostate cancers from non-aggressive prostate cancers were available for patients with low to intermediate grade tumors (*e.g.* Gleason score of 4-7). Published studies also indicate that E2F3 over-expression may play some role in the development of prostate and bladder cancers. Here, prostate cancers were analyzed to determine if at least some prostate cancer tumors are ERGO tumors.

The prostate cancer microarray set published by Chandran *et al.* was used to investigate E2F-responsive gene expression in human prostate cancers and metastatic human prostate cancer samples. Gene expression data from tumor sample probed gene spots this microarray set was normalized to data from normal human prostate tissue sample probed gene spots that were included in the microarray set. The GSE6919 microarray set available from the NCBI GEO site was analyzed.

The Chandran microarray set includes multiple samples from metastatic prostate cancer tumors and androgen ablation-resistant prostate cancer tumors. Metastatic cancer samples in the same patient are known to be heterogeneous in their overall gene expression patterns, and this was confirmed in the Chandran microarray set. However, all metastatic cancer tumor samples in the Chandran microarry set showed fully developed ERGO tumor-associated gene expression patterns, and the homogeneity of ERGO gene expression patterns among different samples in each patient was striking. *See* Fig. 20. There is substantial overlap between the top

100 over-expressed E2F-responsive genes in metastatic prostate cancer, and the E2F-responsive genes over-expressed in ERGO tumors of the breast, lung, thyroid, and ovary.

In particular, E2F3 was over-expressed in most metastatic prostate cancer samples, as were cyclin A2, and cyclin E2. Cyclin B2 was was also prominently over-expressed. Many prostate cancer tumor samples also over-expressed EZH2 and FOXM1, as well as MYBL2 (B-MYB). It is noteworthy, that the cyclins FOXM1 and EZH2 are over-expressed, because these genes are members of potential positive feedback loops in the cascade of changes associated with dysregulation of the members of the Syn/MULVB/DRM/DREAM/LINC multiprotein repressor/activator complex. These potential positive feedback loops are shown in a larger context in Fig. 5, and are shown separately with more detail in Fig. 21.

This metastatic prostate cancer derived data indicates that these positive feedback loops are active in dangerous, advanced metastatic cancer, but not in the large subset of patients with non-metastatic primary tumors. Additionally, although the total number of cases included in the Chandran microarray set is relatively small, the fact that every one of these cases of metastatic prostate cancer exhibits over-expression of large numbers of over-expressed ERGO tumor-associated genes shows that E2F3-driven ERGO tumor development is a feature of most dangerous, advanced prostate cancers.

Identification of ERGO Tumors in Prostate by PCA.

The human prostate cancer microarray set of Chandran was analyzed by PCA. When PCA is applied to the primary prostate cancers without metastases identified in the Chandran microarray set using the limited subset of 325 E2F-responsive genes, a distinctive tumor cluster is apparent in which very few ERGO tumor-associated genes are over-expressed, but in which basal cytokeratin marker over-expression is relatively common. *See* Fig. 22 (spheres with crosses).

Among the remaining primary prostate cancer without metastases, there is a subset of primary prostate cancer tumors that exhibits many of the same features present in metastatic tumor samples. *See* Fig. 22 (clear white spheres). The primary prostate cancer tumors in this subset over-express large numbers of ERGO tumor-associated E2F responsive genes and frequently over-express E2F2 and E2F3, cyclin E2, cyclin A2, cyclin B2, EZH2, FOXM1 and MYBL2, suggesting that activation of the same positive feedback loops present in metastatic tumors. *See* Fig. 20 (tumors listed under "Advanced ERGO PCA Pattern"). Additionally, like the metastatic prostate cancer tumors identified, these tumors express basal cytokeratins only rarely. This subset of primary prostate cancer tumors also exhibits a wide range of Gleason

scores, ranging from 4 to 9. Consequently, patients with primary prostate cancer tumors in this subset should not be offered the "watchful waiting" option to manage their prostate cancer, and should instead be treated intensively at the time of diagnosis because the analyses here indicate these subsets of tumors have such extensive similarity with the dangerous metastatic prostate cancer tumors and are also ERGO tumors.

Example 5

Identification of E2F Responsive Gene Over-Expressing (ERGO) Tumors in a Ovarian Cancer Microarray Set.

Ovarian cancer, like breast cancer, arises in large proportions of patients with hereditary BRCA1 mutation (~ 50% of cases by age 70). Serous carcinomas is the predominant histological type of ovarian cancer occuring in BRCA1 patients. Patients carrying BRCA1 mutations that undergo prophylactic surgery are also frequently found to have occult serous cancers of the fallopian tube, suggesting that this is a preferred site of tumor formation in patients carrying BRCA1 mutations. Studies of normal fallopian tube epithelium and high grade serous fallopian tube and ovarian carcinomas in both patients carrying BRCA1 gene mutations and patients that do not carry such mutations were performed by microarray, and found that the gene expression profiles of the normal fallopian tube tissue in BRCA1 carriers clustered with the those of serous carcinomas not with normal fallopian tube epithelium from patients who do not carry BRCA1 mutations.

We have analyzed the normal fallopian tube epithelium, high grade serous fallopian tube and ovarian carcinoma microarray sets from these studies. These microarray sets are available as the GSE10971 series in the NCBI GEO data repository.

Theses analyses showed that high grade serous carcinomas from fallopian tube and ovary of both BRCA1 and non-BRCA1 mutation carriers were all advanced ERGO tumors. Results are shown in Fig. 23. The top 50 ERGO tumor-associated E2F-responsive genes were over-expressed in essentially every tumor. Every tumor over-expressed both E2F1 and E2F3, and also over-expressed CDKN2A and indicated a functional Rb defect. Essentially every tumor over-expressed at least one basal cytokeratin, and all but one expressed at least 2 basal cytokeratins, indicating that these cancers started out as basal like tumors, and that they retained their basal-like status in advanced malignancy--unlike the prostate cancers. All tumors over-expressed both EZH2 and FOXM1 plus at least two cyclins, indicating the activation of positive

feedback loops involving SYNMULVB/DRM/DREAM/LINC derangements of these components of the multiprotein repressor/activator complex. See Fig. 21.

In contrast, 10 of the 12 fallopian tube samples from the patients carrying BRCA1 mutations showed none of these patterns of gene over-expression. In the remaining two fallopian tube samples, approximately half of the top 50 ERGO tumor associated genes were over-expressed, and one over-expressed EZH2 and two cyclins. The findings in these two samples are interpreted as transitional evolutionary pattern phenotypes indicative of cells on the way toward developing into cells having an aggressive cancer phenotype in BRCA1 carriers.

The results here lead to the conclusion that all high grade serous ovarian and fallopian tube cancers, whether from patients carrying BRCA1 carriers or patients who do not carry such a mutation, are advanced ERGO tumors having patterns of gene over-expression consistent with the activation of positive feedback loops involving over-expression of EZH2, FOXM1, MYB2L (B-MYB), and mitotic cyclins. A minority of fallopian tube samples (<20%) from patients carrying BRCA1 mutation exhibit gene expression patterns consistent with early ERGO tumor-associated changes.

Example 6

Identification of ERGO Tumor Associated Over-Expressed Genes

A scoring system was developed to compile definitive master lists of ERGO tumor-associated over-expressed genes which took into account both the frequency of over-expression of a particular gene at any given organ site and the number of organ sites at which a particular gene is over-expressed. In this scoring system the "individual gene score" was determined using the following formula:

"Individual Gene Score" = fraction of ERGO breast cancers over-expressing the gene
+ fraction of ERGO lung cancers over-expressing the gene
+ fraction of ERGO thyroid cancers over-expressing the gene
+ fraction of ERGO ovarian cancers over-expressing the gene
+ fraction of metastatic ERGO prostate cancers over-expressing the gene.

A cutoff "individual gene score" of >1.2 was used to assign over-expressed E2F-responsive genes to the ERGO-tumor associated list. Genes with an "individual gene score" of greater than 1.2 were included in the list, and genes with an "individual gene score" of less than 1.2

were not included in the list. This insured that the gene was over-expressed in at least two organ sites, and that a frequency of over-expression of at least 0.6 was present at at least one organ site. Details concerning the frequencies of gene over-expression at each site and the individual gene scores for genes over-expressed in at least 20 percent of tumors at each site is shown in Table 5 below.

Table 5
ERGO tumor associated genes based on score cutoff > 1.2

	breast	lung	thyroid	ovary	prostate met	sum
BUB1	0.81081081	0.5	1	0.84615385	0.68	3.83696466
TPX2/C20ORF1	0.48648649	0.55555556	0.71428571	0.92307692	1	3.67940468
AURKA/STK15	0.45945946	0.5	0.71428571	1	1	3.67374517
CENPA	0.72972973	0.44444444	0.71428571	1	0.76	3.64845989
CKS2	0.32432432	0.33333333	1	1	0.92	3.57765766
AURKB/STK12	0.78378378	0.5	0.85714286	0.84615385	0.52	3.5070804[illegible]
UBE2C/UBCH10	0.62162162	0.38888889	0.28571429	0.92307692	0.96	3.17930172
KIF2C/KNSL6	0.75675676	0.38888889	0.71428571	0.76923077	0.48	3.10916213
STMN1	0.51351351	0.83333333	0.71428571	0.69230769	0.28	3.03344025
CDC45L	0.56756757	0.27777778	0.85714286	0.92307692	0.4	3.02556513
LMNB1	0.40540541		0.85714286	1	0.76	3.02254826
MAD2L1	0.48648649	0.66666667	0.71428571	0.53846154	0.56	2.96590041
KIFC1/KNSL2	0.81081081		0.57142857	0.92307692	0.56	2.86531631
HMMR	0.21621622	0.44444444	0.71428571	0.92307692	0.56	2.8580233
PTTG1	0.37837838	0.77777778		0.92307692	0.72	2.79923308
KIF11/KNSL1	0.32432432	0.5	0.42857143	0.92307692	0.6	2.77597268
CCNA2	0.62162162		0.71428571	0.76923077	0.64	2.74513811
UNG	0.24324324	0.72222222	0.57142857	0.61538462	0.52	2.67227865
CENPF	0.56756757	0.83333333	0.85714286	0.38461538		2.64265914
TTK	0.56756757	0.5	0.71428571	0.84615385		2.62800713
CCNB2	0.62162162	0.5	0.85714286		0.64	2.61876448
CDC2	0.37837838	0.61111111		0.92307692	0.68	2.59256641
EZH2	0.7027027			1	0.84	2.5427027
CDKN3		0.61111111	0.42857143	0.76923077	0.64	2.44891331

	breast	lung	thyroid	ovary	prostate met	sum
BUB1B			0.71428571	0.92307692	0.76	2.39736264
ASF1B/FLJ10604		0.44444444	1	0.92307692		2.36752137
MKI67			0.71428571	0.84615385	0.8	2.36043956
NDC80/HEC	0.59459459		0.42857143	0.92307692	0.4	2.34624295
FOXM1	0.62162162			1	0.72	2.34162162
BIRC5	0.54054054	0.38888889		0.92307692	0.48	2.33250635
CDC20/p55CDC	0.7027027			0.92307692	0.68	2.30577963
CDC25B	0.45945946	0.44444444	0.42857143	0.92307692		2.25555226
BLM	0.54054054		0.28571429	0.84615385	0.56	2.2324086
TIMELESS		0.66666667	0.28571429	1	0.24	2.19238095
MCM2	0.51351351		0.71428571	0.92307692		2.15087615
CENPE	0.21621622		0.71428571	0.92307692	0.28	2.13357885
KPNA2	0.43243243	0.33333333		1	0.36	2.12576577
TMPO		0.83333333		0.76923077	0.52	2.1225641
MCM7	0.43243243	0.5		0.84615385	0.32	2.09858628
RAD54L	0.64864865	0.55555556		0.69230769	0.2	2.0965119
CDC6			1	0.69230769	0.4	2.09230769
CHEK1	0.45945946		0.28571429	0.76923077	0.56	2.07440451
ANLN	0.43243243		0.85714286	0.76923077		2.05880606
CCNB1	0.40540541			0.92307692	0.72	2.04848233
TK1	0.40540541		1		0.64	2.04540541
TOP2A		0.94444444		0.23076923	0.84	2.01521368
NUSAP1/BM037	0.2972973		0.71428571	1		2.01158301
RFC4	0.43243243	0.72222222		0.84615385		2.0008085
DEK	0.43243243	0.72222222		0.84615385		2.0008085
SMC2/SMC2L1	0.21621622	0.66666667		0.61538462	0.48	1.9782675
TYMS	0.2972973	0.55555556	0.42857143	0.23076923	0.44	1.95219351

	breast	lung	thyroid	ovary	prostate met	sum
PBK			1	0.92307692		1.92307692
CCNE2	0.27027027			0.76923077	0.88	1.91950104
H2AFX	0.32432432		0.28571429	0.53846154	0.76	1.90850015
MCM6	0.43243243	0.27777778	0.42857143	0.76923077		1.90801241
ECT2	0.35135135	0.55555556		1		1.90690691
MELK/KIAA0175	0.62162162			0.92307692	0.36	1.90469854
CDC25C	0.24324324		0.57142857	0.46153846	0.6	1.87621028
NEK2	0.2972973	0.66666667		0.61538462	0.28	1.85934858
TOPBP1	0.21621622	0.66666667		0.61538462	0.36	1.8582675
CHAF1A	0.32432432	0.55555556		0.61538462	0.36	1.8552645
E2F1	0.35135135	0.57142857		0.92307692		1.84585685
PCNA	0.27027027	0.72222222		0.84615385		1.83864634
PRC1	0.40540541	0.5		0.92307692		1.82848233
HMGB3/HMG4	0.35135135	0.33333333		0.61538462	0.52	1.8200693
MSH2	0.27027027	0.38888889		0.69230769	0.44	1.79146685
E2F3				1	0.76	1.76
SRGAP2/KIAA0456	0.5		0.28571429		0.96	1.74571429
NUP155	0.21621622	0.27777778		0.76923077	0.48	1.74322476
RRM2			0.28571429	1	0.44	1.72571429
CHAF1B	0.48648649	0.61111111		0.61538462		1.71298221
UBE2T/HSPC150	0.21621622	0.77777778		0.92307692		1.7008547
RFC2		0.44444444		0.76923077	0.48	1.69367521
CDKN2C	0.21621622	0.72222222		0.30769231	0.4	1.64613075
SMC4/SMC4L1	0.37837838	0.33333333	0.28571429	0.30769231	0.30769231	1.61281061
UHRF1/ICBP90			0.57142857	1		1.57142857
PLAU			0.85714286	0.69230769		1.54945055
KIF20A/RAB6KIFL	0.62162162			0.92307692		1.54469854

	breast	lung	thyroid	ovary	prostate met	sum
GCH1		0.38888889	0.42857143	0.46153846	0.24	1.51899878
PARP1/ADPRT		0.72222222		0.76923077		1.49145299
TCF19		0.55555556		0.92307692		1.47863248
DNMT1	0.24324324	0.44444444		0.76923077		1.45691846
RACGAP1/ID-GAP	0.43243243			1		1.43243243
CCNF		0.55555556	0.85714286			1.41269841
DCK		0.38888889		0.61538462	0.4	1.4042735
TEAD4	0.45945946			0.92307692		1.38253638
MCM3		0.44444444		0.92307692		1.36752137
FEN1	0.40540541			0.92307692		1.32848233
GINS1/KIAA0186				0.92307692	0.4	1.32307692
RRM1			0.28571429	0.30769231	0.72	1.31340659
POLE2		0.38888889		0.92307692		1.31196581
BARD1	0.21621622	0.5		0.38461538	0.2	1.3008316
RAD51AP1/PIR51	0.2972973			1		1.2972973
PLK4/STK18	0.24324324	0.61111111			0.44	1.29435435
SERPINE1			0.85714286	0.23076923	0.2	1.28791209
POLA2	0.27027027			0.69230769	0.32	1.28257796
CDCA4/FLJ20764			0.42857143	0.84615385		1.27472527
CCND1			0.71428571		0.56	1.27428571
DTYMK				0.46153846	0.8	1.26153846
CCNE1	0.24324324			1		1.24324324
MCM4	0.27027027			0.76923077	0.2	1.23950104
H2AFZ		0.5		0.53846154	0.2	1.23846154
HIST1H2BF/H2BFG			0.57142857	0.46153846	0.2	1.23296703
LHX2	0.24324324	0.66666667			0.32	1.22990991
CKS1	0.37837838			0.84615385		1.22453222

Rejected based on score cutoff of 1.2 or less

	breast	lung	tyroid	ovary	Prostate met	sum
PMAIP1		0.38888889			0.8	1.18888889
MRE11A				0.69230769	0.48	1.17230769
USP1	0.35135135	0.27777778		0.53846154		1.16759067
SFPQ		0.44444444			0.72	1.16444444
ORC6L	0.37837838			0.76923077		1.14760915
MYC	0.56756757		0.57142857			1.13899614
CDC7L1	0.24324324			0.84615385		1.08939709
UMPS				0.76923077	0.32	1.08923077
PRIM2A	0.45945946			0.61538462		1.07484407
VRK1		0.33333333		0.53846154	0.2	1.07179487
DNA2L	0.37837838			0.69230769		1.07068607
VEGF	0.40540541			0.61538462		1.02079002
SLBP				0.61538462	0.4	1.01538462
MCL1				0.76923077	0.24	1.00923077
CDCA7L/DKFZp762L0311	0.2972973			0.69230769		0.98960499
DUT		0.5			0.48	0.98
AR					0.96	0.96
HMGB2/HMG2	0.21621622				0.72	0.93621622
PRIM1				0.69230769	0.24	0.93230769
RAD51				0.69230769	0.24	0.93230769
FANCL/FLJ10335	0.24324324				0.68	0.92324324
BAK1				0.92307692		0.92307692
KIF4A	0.21621622			0.69230769		0.90852391

	breast	lung	tyroid	ovary	Prostate met	sum
CDK2		0.44444444		0.46153846		0.90598291
RAD51C		0.66666667		0.23076923		0.8974359
MPHOSPH1				0.69230769	0.2	0.89230769
FGFR1OP					0.88	0.88
PRKAR2B				0.38461538	0.48	0.86461538
APOE				0.46153846	0.4	0.86153846
PBX3		0.5			0.36	0.86
DMRT1	0.24324324			0.61538462		0.85862786
HRK	0.56756757		0.28571429			0.85328185
FIGNL1				0.84615385		0.84615385
SHCBP1/FLJ22009				0.84615385		0.84615385
ORC3L	0.21621622	0.38888889		0.23076923		0.83587434
TP53BP2	0.48648649			0.30769231		0.79417879
ARHGAP4			0.28571429	0.30769231	0.2	0.79340659
MTHFD1	0.21621622		0.57142857			0.78764479
MAPK1				0.76923077		0.76923077
DHFR				0.76923077		0.76923077
CITED2					0.76	0.76
PRG4				0.46153846	0.28	0.74153846
RPA3				0.46153846	0.28	0.74153846
CSTF1				0.53846154	0.2	0.73846154
HOXA7		0.5		0.23076923		0.73076923
BTG3	0.48648649				0.24	0.72648649
MMP16			0.28571429		0.44	0.72571429
PLK1			0.71428571			0.71428571
INHBA			0.71428571			0.71428571
EED	0.24324324			0.46153846		0.7047817
SSX2IP/KIAA0923		0.38888889		0.30769231		0.6965812

	breast	lung	tyroid	ovary	Prostate met	sum
RFC3				0.69230769		0.69230769
FKSG14				0.69230769		0.69230769
NUP107				0.69230769		0.69230769
CASP8		0.44444444			0.24	0.68444444
ORC2L				0.46153846	0.2	0.66153846
FST			0.42857143	0.23076923		0.65934066
RPA1			0.42857143	0.23076923		0.65934066
COL11A1					0.64	0.64
DBF4/ASK				0.30769231	0.32	0.62769231
RDC1				0.61538462		0.61538462
BID	0.21621622			0.38461538		0.6008316
HIST1H2AE					0.6	0.6
PLK2/SNK			0.28571429	0.30769231		0.59340659
SPHK1			0.57142857			0.57142857
MCM5	0.56756757					0.56756757
EGR1			0.28571429		0.28	0.56571429
NPAT		0.55555556				0.55555556
PLSCR1	0.54054054					0.54054054
CSRP2	0.54054054					0.54054054
H2AFL				0.53846154		0.53846154
LSM5				0.53846154		0.53846154
H2AFA				0.53846154		0.53846154
RBL1					0.52	0.52
CBX5				0.23076923	0.28	0.51076923
		0.5				0.5
LIG1					0.48	0.48
PDGFRA					0.48	0.48
H3FB				0.46153846		0.46153846

	breast	lung	tyroid	ovary	Prostate met	sum
H2BFH				0.46153846		0.46153846
APBB2				0.46153846		0.46153846
SMARCA5		0.44444444				0.44444444
HIST1H3D					0.44	0.44
SOX9	0.43243243					0.43243243
NASP	0.43243243					0.43243243
HIST1H2BN/H2BFD			0.42857143			0.42857143
BACH1			0.42857143			0.42857143
TGFA	0.40540541					0.40540541
ROD1					0.4	0.4
KIF22					0.4	0.4
PTPNS1	0.37837838					0.37837838
CSDA	0.37837838					0.37837838
BMP2					0.36	0.36
SNAPC1					0.36	0.36
RAD52					0.36	0.36
PAWR					0.36	0.36
ANXA8	0.35135135					0.35135135
PRPS1		0.33333333				0.33333333
BUB3		0.33333333				0.33333333
HSP90B1					0.32	0.32
RECQL5					0.32	0.32
CFLAR					0.32	0.32
FGFR3				0.30769231		0.30769231
RFC5				0.30769231		0.30769231
BMPR1A				0.30769231		0.30769231
HNRPC				0.30769231		0.30769231
CD58				0.30769231		0.30769231

	breast	lung	tyroid	ovary	Prostate met	sum
PEX19/PXF				0.30769231		0.30769231
GAB2				0.30769231		0.30769231
NRP1				0.30769231		0.30769231
GMNN/GEM			0.28571429			0.28571429
RECQL			0.28571429			0.28571429
ADAMTS1			0.28571429			0.28571429
TGFB3			0.28571429			0.28571429
SERPINF2			0.28571429			0.28571429
HMGB1					0.28	0.28
HIST1H2AC					0.28	0.28
CASP3					0.28	0.28
ACOX1					0.28	0.28
MAP3K14	0.27027027					0.27027027
BCL2L11					0.24	0.24
SKP2					0.24	0.24
E2F2					0.24	0.24
SLPI					0.24	0.24
BBC3					0.24	0.24
PPP1R13B					0.24	0.24
MAP2K1				0.23076923		0.23076923
CASP7				0.23076923		0.23076923
ENO3				0.23076923		0.23076923
CALR				0.23076923		0.23076923
INCENP				0.23076923		0.23076923
YY1				0.23076923		0.23076923
NOLC1				0.23076923		0.23076923
H2BFN				0.23076923		0.23076923
H2BFR				0.23076923		0.23076923

	breast	lung	tyroid	ovary	Prostate met	sum
PMS2L1				0.23076923		0.23076923
POLD1					0.2	0.2
CDC7					0.2	0.2
ATM					0.2	0.2
SULT2A1					0.2	0.2

The working premise underlying the scoring system is that derangements of one or more of the components of the SYNMULVB/DRM/DREAM/LINC repressor/activator complex produces a cascade of gene over-expression that can occur at many different organ sites and produce ERGO tumors.

The final list of ERGO tumor associated over-expressed genes identified and their scores are summarized in Table 6 below.

Table 6

ERGO Gene List

gene/protein name	score
BUB1	3.83696466
TPX2/C20ORF1	3.67940468
AURKA/STK15	3.67374517
CENPA	3.64845989
CKS2	3.57765766
AURKB/STK12	3.50708049
UBE2C/UBCH10	3.17930172
KIF2C/KNSL6	3.10916213
STMN1	3.03344025
CDC45L	3.02556513
LMNB1	3.02254826
MAD2L1	2.96590041
KIFC1/KNSL2	2.86531631
HMMR	2.8580233
PTTG1	2.79923308
KIF11/KNSL1	2.77597268
CCNA2	2.74513811
UNG	2.67227865
CENPF	2.64265914
TTK	2.62800713
CCNB2	2.61876448
CDC2	2.59256641
EZH2	2.5427027
CDKN3	2.44891331
BUB1B	2.39736264
ASF1B/FLJ10604	2.36752137
MKI67	2.36043956
NDC80/HEC	2.34624295
FOXM1	2.34162162
BIRC5	2.33250635
CDC20/p55CDC	2.30577963
CDC25B	2.25555226

gene/protein name	score
BLM	2.23240867
TIMELESS	2.19238095
MCM2	2.15087615
CENPE	2.13357885
KPNA2	2.12576577
TMPO	2.1225641
MCM7	2.09858628
RAD54L	2.0965119
CDC6	2.09230769
CHEK1	2.07440451
ANLN	2.05880606
CCNB1	2.04848233
TK1	2.04540541
TOP2A	2.01521368
NUSAP1/BM037	2.01158301
RFC4	2.0008085
DEK	2.0008085
SMC2/SMC2L1	1.9782675
TYMS	1.95219351
PBK	1.92307692
CCNE2	1.91950104
H2AFX	1.90850015
MCM6	1.90801241
ECT2	1.90690691
MELK/KIAA0175	1.90469854
CDC25C	1.87621028
NEK2	1.85934858
TOPBP1	1.8582675
CHAF1A	1.8552645
E2F1	1.84585685
PCNA	1.83864634
PRC1	1.82848233
HMGB3/HMG4	1.8200693
MSH2	1.79146685
E2F3	1.76
SRGAP2/KIAA0456	1.74571429
NUP155	1.74322476
RRM2	1.72571429
CHAF1B	1.71298221
UBE2T/HSPC150	1.7008547
RFC2	1.69367521
CDKN2C	1.64613075
SMC4/SMC4L1	1.61281061
UHRF1/ICBP90	1.57142857
PLAU	1.54945055
KIF20A/RAB6KIFL	1.54469854

gene/protein name	score
GCH1	1.51899878
PARP1/ADPRT	1.49145299
TCF19	1.47863248
DNMT1	1.45691846
RACGAP1/ID-GAP	1.43243243
CCNF	1.41269841
DCK	1.4042735
TEAD4	1.38253638
MCM3	1.36752137
FEN1	1.32848233
GINS1/KIAA0186	1.32307692
RRM1	1.31340659
POLE2	1.31196581
BARD1	1.3008316
RAD51AP1/PIR51	1.2972973
PLK4/STK18	1.29435435
SERPINE1	1.28791209
POLA2	1.28257796
CDCA4/FLJ20764	1.27472527
CCND1	1.27428571
DTYMK	1.26153846
CCNE1	1.24324324
MCM4	1.23950104
H2AFZ	1.23846154
HIST1H2BF/H2BFG	1.23296703
LHX2	1.22990991
CKS1	1.22453222

This list of 105 ERGO tumor associated genes includes the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 43-44, 46-47, 49-51, 54, 56, 58-67, 69-71, 75, 79-84, 86, 88, 91, 93-95, 103-105, 107-110, 112, 116, 118-120, 122, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 163, 165-166, 171, 173, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 246-247, 249, 254, 267, 269, 274-275, 292-294, 311, 316, 231-323, and 333; and splice variants or nucleic acids encoding different peptide chain isoforms derived from these nucleic acids that are capable of hybridizing to the nucleic acid sequences shown in these SEQ ID NO:s, or the complement thereof, under the hybridization conditions of a microarray experiment. This list accounts for a common list, or basic "mosaic" of over-expressed genes that are observed in ERGO tumors including ERGO tumors occurring at different organ sites. Table 7 below provides a key and identifies the gene or protein name as appropriate, the accession number of each amino acid sequence or nucleic acid sequence as

appropriate, the SEQ ID NO:s for the 325 E2F response genes used in the analyses here and the SEQ ID NO:s for the final list of 105 ERGO tumor associated over-expressed genes identified. Astericks ("*") are used in Table 7 to identify the final list of 105 ERGO tumor associated over-expressed genes identified. Daggers ("†") are used in Table 7 to identify a refined list of 125 ERGO tumor associated over-expressed genes that were also identified. Double daggers ("‡") are used in Table 7 to identify housekeeping genes with transcript levels that do not appreciably vary in either normal or malignant tissue, such as normal or cancerous prostate tissue, and are useful for normalization of indicator values and references values. Genes having the sequences shown in SEQ ID NO:s 68 and 335-336 are E2F responsive genes with high sequence identity, or sequence similarity, to other ERGO genes identified. Genes having the sequences shown in SEQ ID NO:s 39, 72, 102, 123, 160, 164, 174, 217, 259, 295, 298, 307, 331 and 337-340 are E2F responsive genes that were identified by analyses of microarray data from prostate tissue samples and have been found to be useful in discriminating aggressive prostate cancers from non-aggressive prostate cancers.

Table 7

gene/protein name	accession number	SEQ ID NO:
BCL2L11	NM_006538	10
FOXO1//FOXO1A	NM_002015	11
CCNE2*,†	NM_057749	12
CDKN2C*,†	NM_001262	13
CCNE1*,†	NM_001238	14
MYC	NM_002467	15
FGFR3	NM_000142	16
MAP3K5	NM_005923	17
BMP2	NM_001200	18
MYB	NM_005375	19
LHX2*,†	NM_004789	20
GCH1*,†	NM_000161	21
MAPK9	NM_002752	22
MAPK8	NM_002750	23
MAPK3	X60188	24
MAPK1	NM_002745	25
MAPK4	NM_002747	26
MAP2K1	NM_002755	27
MAP2K2	NM_030662	28
FOXO3//FOXO3A	NM_001455	29

gene/protein name	accession number	SEQ ID NO:
GADD45B	NM_015675	30
MCL1	L08246	31
BCL2	NM_000633	32
CCND3	NM_00176	33
FOXN3//CHES1	NM_005197	34
MKI67*,†	NM_002417	35
CDKN1C	NM_000076	36
KIFC1*,†	D14678	37
PRG4	NM_005807	38
PMS2†	NM_000535	39
PLK2//SNK	NM_006622	40
HRK	NM_003806	41
CASP8	NM_001228	42
TYMS*,†	NM_001071	43
TK1*,†	NM_003258	44
DUT	NM_001948	45
RRM1*,†	NM_001033	46
RRM2*,†	NM_001034	47
CDK2	NM_001798	48
MCM3*,†	NM_002388	49
MCM7*,†	D55716	50
PCNA*,†	NM_002592	51
RFC3	NM_002915	52
PRIM1	NM_000946	53
TOP2A*,†	NM_001067	54
LIG1	NM_000234	55
FEN1*,†	NM_004111	56
RAD51	NM_002875	57
CDC20*,†	NM_001255	58
CDC2*,†	NM_001786	59
CCNA2*,†	NM_001237	60
CCNB1*,†	NM_031966	61
CCNB2*,†	NM_004701	62
SMC2//SMC2L1*,†	NM_006444	63
STMN1*,†	NM_005563	64
NDC80//HEC*,†	NM_006101	65
BUB1*,†	NM_004336	66
KPNA2*,†	NM_002266	67
HMGB2†	NM_001130688	68
EZH2*,†	NM_004456	69
AURKB//STK12*,†	NM_004217	70
PTTG1*,†	NM_004219	71
SLBP†	NM_006527	72
RB1	NM_000321	73
ANXA8	NM_001040084	74

gene/protein name	accession number	SEQ ID NO:
DCK*,†	NM_000788	75
CDC25A	NM_001789	76
EPS8	NM_004447	77
FST	NM_013409	78
TMPO*,†	NM_003276	79
RAD51AP1//PIR51*,†	NM_006479	80
ASF1B*,†	NM_018154	81
CDCA4*,†	NM_017955	82
RFC4*,†	NM_002916	83
BLM*,†	NM_000057	84
VRK1	NM_003384	85
BARD1*,†	NM_000465	86
BTG3	NM_006806	87
CHAF1A*,†	NM_005483	88
NPAT	NM_002519	89
HUNK	NM_014586	90
DEK*,†	NM_003472	91
EED	AF099032	92
MCM4*,†	X74794	93
MELK*,†	NM_014791	94
TCF19*,†	NM_007109	95
FANCL	NM_018062	96
PBX3	NM_006195	97
EGR1	NM_001964	98
CDCA7L	NM_001127370	99
SKP2	NM_005983	100
CTGF	NM_001901	101
CITED2†	NM_006079	102
SERPINE1*,†	M16006	103
CCND1*,†	NM_053056	104
UHRF1//ICBP90*,†	NM_013282	105
MCM5	NM_006739	106
HMGB3*,†	NM_005342	107
MCM6*,†	NM_005915	108
CDC45L*,†	NM_003504	109
CDC6*,†	NM_001254	110
ORC6L	NM_014321	111
CKS2*,†	NM_001827	112
GMNN	NM_015895	113
PRIM2	NM_000947	114
CENPK//FKSG14	NM_022145	115
NUP155*,†	NM_004298	116
FIGNL1	NM_001042762	117
MAD2L1*,†	NM_002358	118
CCNF*,†	NM_001761	119

gene/protein name	accession number	SEQ ID NO:
DNMT1*,†	NM_001379	120
RPA1	NM_002945	121
PRC1*,†	NM_003981	122
RBL1†	NM_002895	123
BRCA1	NM_007294	124
H2AFZ*,†	NM_002106	125
DTYMK*,†	NM_012145	126
PLK1	NM_005030	127
POLA2*,†	NM_002689	128
PBK*,†	NM_018492	129
CASP7	NM_001227	130
MCM2*,†	NM_004526	131
RPA3	NM_002947	132
GJC1//GJA7	NM_005497	133
USP1	NM_003368	134
DNA2L	D42046	135
CITED1	NM_004143	136
NASP	NM_002482	137
RFC5†	NM_007370, NM_001130112	138, 340
SMARCA5	NM_003601	139
SHCBP1//FLJ22009	NM_024745	140
SSX2IP	NM_014021	141
MFAP1	NM_005926	142
ROD1	NM_005156	143
BMPR1A	NM_004329	144
E2F3*,†	NM_001949	145
UNG*,†	NM_003362	146
ENO3	NM_001976	147
MSH2*,†	NM_000251	148
PLK4*,†	NM_014264	149
ACTA2	NM_001613	150
TIMELESS*,†	NM_003920	151
BOK	NM_032515	152
KBTBD10//SARCOSIN	NM_006063	153
BUB1B*,†	NM_001211	154
NUP107	NM_020401	155
KIF2C//KNSL6*,†	NM_006845	156
LMNB1*,†	NM_005573	157
RPA2	NM_002946	158
CHEK2//CDS1	NM_007194	159
COL11A1†	NM_001854	160
TGFB3	NM_003239	161
CALR‡	NM_004343	162
TTK*,†	NM_003318	163

gene/protein name	accession number	SEQ ID NO:
E2F2†	NM_004091	164
CKS1B//CKS1*†	NM_001826	165
RFC2*†	NM_002914	166
UMPS	NM_000373	167
DBF4//ASK	NM_006716	168
CHEK1*†	NM_001274	169
BUB3	NM_004725	170
CENPE*†	NM_001813	171
CSTF1	NM_001324	172
RAD54L*†	NM_003579	173
POLD1†	NM_002691	174
MLH1	NM_000249	175
CENPA*†	NM_001809	176
SMC4//SMC4L1*†	NM_005496	177
HMGB1//HMG1†	AL110194, NM_002128	178, 336
HIST1H3D//H3FB	NM_003530	179
H2AFX*†	NM_002105	180
CBX5	NM_012117	181
HIST1H2AC//H2AFL	NM_003512	182
KIF22//KNSL4	NM_007317	183
NEK2*†	NM_002497	184
KIF4A	NM_012310	185
HMMR*†	NM_012484	186
MTHFD1	NM_005956	187
GINS1*†	D80008	188
SFPQ	NM_005066	189
HSP90B1//TRA1	NM_003299	190
MAP3K7	NM_003188	191
PLSCR1	AB006746	192
ANLN*†	NM_018685	193
SFRS2	NM_003016	194
ID3	X69111	195
TEAD4*†	NM_003213	196
SRPR	NM_003139	197
UBE2T//HSPC150*†	NM_014176	198
INCENP	NM_020238	199
CDC25B*†	NM_004358	200
AURKA//STK15*†	NM_003600	201
DHFR	NM_000791	202
CDKN3*†	NM_005192	203
CDC7//CDC7L1	AF015592	204
RACGAP1//ID-GAP*†	NM_013277	205
CSRP2	NM_001321	206
MAF	NM_005360	207

gene/protein name	accession number	SEQ ID NO:
CBX3	NM_007276	208
CHAF1B*,†	NM_005441	209
ADAMTS1	NM_006988	210
TCOF1	NM_000356	211
LSM5	NM_012322	212
HNRNPC//HNRPC	NM_001077442	213
APAF1	NM_013229	214
ASH2L	NM_004674	215
BCL3	NM_005178	216
CASP3†	NM_004346	217
CAV1	AF070648	218
CD58	NM_001779	219
DMRT1	AF130728	220
DYRK1A	NM_001396	221
LIMA1//EPLIN	NM_016357	222
FGFR2	NM_000141	223
HEY1	NM_012258	224
INHBA	NM_002192	225
TBC1D2B//KIAA1055	AK000173	226
OSMR	NM_003999	227
FURIN//PACE	NM_002569	228
PRKAR2B	NM_002736	229
SIRPA//PTPNS1	NM_001040022	230
RANBP9	NM_005493	231
SOX9	NM_000346	232
SPHK1	AF238083	233
TACC1	NM_006283	234
TGFA	NM_003236	235
TGFB2	NM_003238	236
YY1	NM_003403	237
SNAPC1	NM_003082	238
CCNG2	NM_004354	239
HOXA7	NM_006896	240
HOXA9	NM_152739	241
PITX1	NM_002653	242
SMARCA2	NM_003070	243
BACH1	NM_001186	244
CBFB	NM_001755	245
BIRC5*,†	NM_001168	246
CDC25C*,†	NM_001790	247
ORC3L	NM_012381	248
TOPBP1*,†	NM_007027	249
MRE11A	NM_005590	250
ATM	NM_000051	251
XRCC4	NM_003401	252

gene/protein name	accession number	SEQ ID NO:
RECQL	NM_002907	253
CENPF*,†	NM_016343	254
CENPH//PMF1	NM_022909	255
NOLC1	NM_004741	256
KIF20B//MPHOSPH1	NM_016195	257
BMI1	NM_005180	258
HIST1H2AE//H2AFA†	NM_021052	259
HIST1H2AD//H2AFG	NM_021065	260
HIST1H2BN//H2BFD	NM_003520	261
HIST1H2BE//H2BFH	NM_003523	262
HIST1H2BO//H2BFN	NM_003527	263
HIST1H2BJ//H2BFR	NM_021058	264
HIST1H3B//H3FL	NM_003537	265
HIST2H4A//H4F2	NM_003548	266
HIST1H2BF//H2BFG*,†	NM_003522	267
HIST1H3E//H3FD	NM_003532	268
KIF20A//RAB6KIFL*,†	NM_005733	269
FGFR1OP//FOP†	AL117608, NM_007045	270, 337
DHPS	NM_013406	271
PEX19//PXF	NM_002857	272
PPM1D	NM_003620	273
E2F1*,†	M96577	274
PARP1//ADPRT*,†	NM_001618	275
ORC2L	NM_006190	276
VEGFA//VEGF	NM_003376	277
APOE	NM_000041	278
PRPS1	NM_002764	279
TGFB1I1	NM_015927	280
CXCR7//RDC1	U67784	281
PDGFRA	X76079	282
SLPI	NM_003064	283
FAS//TNFRSF6	NM_000043	284
CPT1A	NM_001876	285
PDCD4	NM_014456	286
TANK	NM_004180	287
APBB2	NM_173075	288
GADD45A	NM_001924	289
ACOX1	NM_007292	290
CSDA	NM_003651	291
UBE2C//UBCH10*,†	NM_007019	292
TPX2//C20orf1*,†	AB024704	293
NUSAP1//BM037*,†	NM_016359	294
SULT2A1†	NM_003167	295
INMT	NM_006774	296

gene/protein name	accession number	SEQ ID NO:
ARHGAP4	X78817	297
RAD52†	NM_134424	298
TNFSF9	NM_003811	299
BAD	U66879	300
BAK1	NM_001188	301
BID	NM_001196	302
CFLAR	NM_003879	303
MAP3K14	NM_003954	304
PAWR	NM_002583	305
FGF2	NM_002006	306
MMP16†	NM_005941	307
TP53BP2	NM_005426	308
VEGFB	NM_003377	309
IFNA2	M54886	310
SRGAP2//KIAA0456*†	AK000885	311
DIP//KIAA0767	AB018310	312
SERPINF2	D00174	313
CCNO//UNG2	NM_021147	314
TP73	NM_005427	315
POLE2*†	NM_002692	316
RAD51C	NM_002876	317
PMS2L1	D38435	318
DDB2	NM_000107	319
NFKB2	NM_002502	320
KIF11//KNSL1*†	NM_004523	321
FOXM1*†	U74612	322
PLAU*†	NM_002658	323
BBC3	U82987	324
PMAIP1	D90070	325
GAB2	NM_012296	326
SIVA1//SIVA	NM_006427	327
PPP1R13B	AB018314	328
AXIN2	NM_004655	329
DIABLO//SMAC	NM_019887	330
AR†	NM_000044	331
NRP1	NM_003873	332
ECT2*†	NM_018098	333
ISYNA1	NM_016368	334
MYBL1†	NM_001080416	335
MYBL2†	NM_002466	338
HIST1H2BK†	NM_080593	339
CUL4B‡	NM_001079872	341
OAZ2‡	NM_002537	342
TBCC‡	NM_003192	343
NUP214‡	NM_005085	344

gene/protein name	accession number	SEQ ID NO:
FBXW11‡	NM_012300	345
POP4‡	NM_006627	346
LPCAT3‡	NM_005768	347
ARSE‡	NM_000047	348
ARC‡	NM_015193	349
POLR3D‡	NM_001722	350
DLGAP4‡	NM_001042486	351
SLC22A5‡	NM_003060	352
BRMS1‡	NM_001024957	353
ZFAND5‡	NM_001102420	354
THOP1‡	NM_003249	355
DNPEP‡	NM_012100	356
USP20‡	NM_001008563	357
HMGN2‡	NM_005517	358
ARF3‡	NM_001659	359
PSMB1‡	NM_002793	360
CFL1‡	NM_005507	361
RPS25‡	NM_001028	362
MANF‡	NM_006010	363
HADHB‡	NM_000183	364
RPL22‡	NM_000983	365

Example 7

Bladder Cancer is an ERGO Tumor

Studies by Olsson *et al.* (2007) have indicated that E2F3 overexpression may play a role in the development of bladder cancers. We examined dataset GSE3167 (Dyrskjøt *et al.*, (2004)), available from the NCBI GEO site, which contains gene expression microarray data from patient samples of normal bladder tissues which were used for data normalization, patient samples of localized bladder cancers, and patient samples of muscle-invasive bladder cancers. Among 33 tumor samples that were histopathologically grade 3 or 4, 32 of these 33 samples simultaneously overexpressed at least 20 of a list of 93 E2F-responsive genes included in these analyses. *See* Fig. 26. All of the genes in the list were overexpressed in a least 20 percent of the 33 tumors. Fifty-five of these 93 E2F-responsive genes, or 59% of the 93 E2F-responsive genes, were amoung the 125 genes identified in the generic ERGO gene list shown in Table 8. In contrast, only 2 of 8 grade 2 bladder cancer samples, and none of the 7 normal bladder samples, overexpressed at least 20 of these 93 E2F responsive genes. *See* Fig. 26. These results demonstrate that bladder cancers are an ERGO tumor and that the overexpression of ERGO tumor genes is correlated to increasing bladder cancer grade.

Example 8

Hepatoma is an ERGO Tumor

Heptomas have been reported by Kalinichenko *et al.*, (2004) to overexpress FOXM1 which we know to be an ERGO tumor-associated gene. We examined dataset GSE1898, available from the NCBI GEO site, which contained gene expression microarray data from 182 patient samples of hepatoma. Sixty (60) of these samples simultaneously overexpressed at least 20% of the genes in a list of 107 E2F-responsive genes included in these analyses. *See* Fig. 27. Moreover, each one of the individual E2F-responsive genes in this list of 107 E2F-responsive genes was also overexpressed in at least 20% of the tumors. In fact, 58% of these genes were included in the list of 125 genes identified in the generic ERGO gene list shown in Table 8. These results demonstrate that approximately one third of hepatomas, or 60 out of 182 patient samples of hepatoma, are ERGO tumors.

All references (*e.g.* journal articles, patent documents, and accession numbers) cited herein are incorporated by reference in their entirety.

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Claims

What is claimed is:

1. A method of identifying a tumor as an ERGO tumor comprising the steps of:

a) providing a tumor sample;

b) providing a reference;

c) measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340;

d) measuring the reference to produce a reference value; and

e) comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed;

whereby the tumor is identified as an ERGO tumor if at least 21 of these gene transcripts are over-expressed.

2. The method of claim 1 wherein the tumor sample is from lung tissue.

3. The method of claim 1 wherein the tumor sample is from thyroid tissue.

4. The method of claim 1 wherein the tumor sample is from ovarian tissue.

5. The method of claim 1 wherein the tumor sample is from prostate tissue.

6. The method of claim 1 wherein the indicator value is at least 1.8 times greater than the reference value for each of the gene transcripts.

7. A method of determining the odds that an individual ERGO tumor patient will survive to a future date comprising the steps of:

a) providing a tumor sample from an individual patient;

b) providing a reference;

c) measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340;

d) measuring the reference to produce a reference value; and

e) comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor and the individual patient is diagnosed as an ERGO tumor patient if at least 21 of these gene transcripts are over-expressed;

f) plotting the fraction of surviving patients in a population of patients diagnosed as ERGO tumor patients as a function of the time since diagnosis of the ERGO tumor to generate a survival plot; and

g) selecting a future date after the individual patient is diagnosed as an ERGO tumor patient and determining the fraction of surviving patients in the population from the survival plot;

whereby the fraction of surviving patients on the survival plot at the future date predicts the odds that an individual tumor patient will survive to the future date.

8. The method of claim 7 wherein the tumor sample is from lung tissue and the population of patients is diagnosed as ERGO lung tumor patients.

9. The method of claim 7 wherein the tumor sample is from thyroid tissue and the population of patients is diagnosed as ERGO thyroid tumor patients.

10. The method of claim 7 wherein the tumor sample is from ovarian tissue and the population of patients is diagnosed as ERGO ovarian tumor patients.

11. The method of claim 7 wherein the tumor sample is from prostate tissue and the population of patients is diagnosed as ERGO prostate tumor patients.

12. The method of claim 7 wherein the indicator value is at least 1.8 times greater than the reference value for each of the gene transcripts.

13. A method of treating an ERGO tumor in a patient comprising the steps of:

a) providing a tumor sample from a patient;

b) providing a reference;

c) measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340;

d) measuring the reference to produce a reference value; and

e) comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor if at least 21 of these gene transcripts are over-expressed;

f) selecting a drug capable of killing or inhibiting division of an ERGO tumor cell expressing at least one protein encoded by at least one gene transcript selected from the group consisting of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188,

193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340; and

g) administering a pharmaceutically acceptable amount of the drug to the patient;

whereby the ERGO tumor in the patient is treated.

14. The method of claim 13 wherein the drug is an aurora kinase antagonist.

15. The method of claim 14 wherein the aurora kinase antagonist is at least one molecule selected from the group consisting of VX-680, MLN8237, MLN8054, AZD1152, hesperadin, and ZM-447439.

16. The method of claim 13 wherein the drug is a survivin antagonist.

17. The method of claim 16 wherein the survivin antagonist is YM155.

18. The method of claim 13 wherein the drug is a FOXM1 antagonist.

19. The method of claim 18 wherein the FOXM1 antagonist is the $(D\text{-}Arg)_9$-p19ARF 26-44 peptide.

20. The method of claim 13 wherein the drug is an E2F antagonist.

21. The method of claim 20 wherein the E2F antagonist is eugenol.

22. The method of claim 13 wherein the drug is a LINC protein complex agonist.

23. The method of claim 13 wherein the indicator value is at least 1.8 times greater than the reference value for each of the gene transcripts.

24. A method of identifying an individual tumor in a population of tumors as an ERGO tumor comprising the steps of:

a) providing a population of tumor samples;

b) providing a reference;

c) measuring gene transcript levels in the tumor samples to produce a transcript value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 10-340;

d) comparing the transcript value to the reference value for each tumor to identify the gene transcripts over-expressed by each tumor;

e) ranking the tumors in the population with a rank ordering algorithm to order the tumors according to the number of the gene transcripts over-expressed by each tumor; and

f) removing individual tumors from the population that over-express the smallest number of each gene transcript per cell and that have the lowest levels of over-expression of each gene transcript per cell until at least 20% of the individual tumors remaining in the population over-express at least 20% of the gene transcripts;

whereby an individual tumor remaining in the population of tumors is identified as an ERGO tumor.

25. The method of claim 24 wherein the indicator value is at least 1.8 times greater than the reference value for each of the gene transcripts.

26. A method of identifying an individual tumor in a population of tumors as an ERGO tumor comprising the steps of:

a) providing a population of tumor samples;

b) providing a reference;

c) measuring gene transcript levels in the tumor samples to produce a transcript value for each of the following gene transcripts having the nucleic acid sequence shown in SEQ ID NO:s 10-340;

d) comparing the transcript value to the reference value for each tumor to identify the gene transcripts over-expressed by each tumor; and

e) applying a principle component analysis algorithm in which the analyzed gene set is restricted to each gene transcript having the nucleic acid sequence shown in SEQ ID NO:s 10-340 to identify a tumor cluster over-expressing these E2F-responsive genes;

whereby an individual tumor in the population of tumors in the cluster is identified as an ERGO tumor.

27. The method of claim 26 wherein the indicator value is at least 1.8 times greater than the reference value for each of the gene transcripts.

28. A method of selecting treatment for a prostate cancer patient comprising the steps of:

a) providing a tumor sample from a prostate cancer patient;

b) providing a reference;

c) measuring an indicator of gene transcript levels in the tumor sample to produce an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340;

d) measuring the reference to produce a reference value; and

e) comparing the indicator value to the reference value to determine which of the gene transcripts is over-expressed, whereby the tumor is identified as an ERGO tumor and the prostate cancer patient is diagnosed as an ERGO tumor prostate cancer patient if at least 21 of these gene transcripts are over-expressed; and

f) choosing at least one treatment selected from the group consisting of removal of at least one tumor and adjuvant therapy, if the patient is diagnosed as an ERGO tumor prostate cancer patient;

whereby a treatment is selected for the prostate cancer patient.

29. The method of claim 28, wherein the removal of at least one tumor is performed using at least one therapy selected from the group consisting of using focused energy, cryoablation, and radiation therapy.

30. The method of claim 29, wherein the adjuvant therapy is at least one therapy selected from the group consisting of chemotherapy, hormone therapy, and immunotherapy.

31. A method as in claims 5, 11, 28, 29 or 30 further comprising measuring an indicator of gene transcript levels in at least one selected from the group consisting of the tumor sample and the reference to produce a housekeeping value for at least one gene transcript selected from the group consisting of the nucleic acid sequences shown in SEQ ID NO:s 162, 341-364 and 365; and normalizing at least one selected from the group consisting of the indicator value and the reference value to the housekeeping value.

32. The use of an aurora kinase antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

33. The use of VX-680 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

34. The use of MLN8237 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

35. The use of MLN8054 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

36. The use of AZD1152 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

37. The use of hesperadin in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

38. The use of ZM-447439 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

39. The use of a survivin antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

40. The use of YM155 in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

41. The use of a FOXM1 antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

42. The use of the $(D\text{-}Arg)_9$-p19ARF 26-44 peptide in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

43. The use of an E2F antagonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

44. The use of eugenol in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

45. The use of a LINC protein complex agonist in the manufacture of a medicament for the treatment of an ERGO tumor identified according to the method of claim 1.

46. An apparatus comprising:

a) a specifically programmed computer in communication with a nucleic acid array analyzer and an output display,

wherein the specifically programmed computer is adapted to compare indicator values to reference values and to determine which gene transcripts are over-expressed in a tumor sample;

b) a nucleic acid array comprising at least 21 of the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56,

58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340 adapted for hybridization to nucleic acids in a tumor sample;

c) a memory containing an indicator value for each of the following gene transcripts having the nucleic acid sequences shown in SEQ ID NO:s 12-14, 20-21, 35, 37, 39, 43-44, 46-47, 49-51, 54, 56, 58-72, 75, 79-84, 86, 88, 91, 93-95, 102-105, 107-110, 112, 116, 118-120, 122-123, 125-126, 128-129, 131, 145-146, 148-149, 151, 154, 156-157, 160, 163-166, 169, 171, 173-174, 176-177, 180, 184, 186, 188, 193, 196, 198, 200-201, 203, 205, 209, 217, 246-247, 249, 254, 259, 267, 269, 274-275, 292-295, 298, 307, 311, 316, 321-323, 331, 333, and 335-340 produced by hybridization of the nucleic acid array to the nucleic acids in the tumor sample, and containing reference values for the gene transcripts; and

d) an output display which shows the tumor sample is an ERGO tumor when the specifically programmed computer determines at least 21 of the gene transcripts are over-expressed in a tumor sample.

47. The method of claim 1 wherein the tumor sample is from liver tissue.

48. The method of claim 1 wherein the tumor sample is from bladder tissue.

49. The method of claim 7 wherein the tumor sample is from liver tissue and the population of patients is diagnosed as ERGO hepatoma tumor patients.

50. The method of claim 7 wherein the tumor sample is from bladder tissue and the population of patients is diagnosed as ERGO bladder tumor patients.

51. The method of claim 13 wherein the ERGO tumor is from a tissue selected from the group consisting of lung tissue, thyroid tissue, ovarian tissue, prostate tissue, liver tissue and bladder tissue.

52. The method of claim 24 wherein the tumor sample is from a tissue selected from the group consisting of lung tissue, thyroid tissue, ovarian tissue, prostate tissue, liver tissue and bladder tissue.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 4
CONTENANT LES PAGES 1 À 163

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

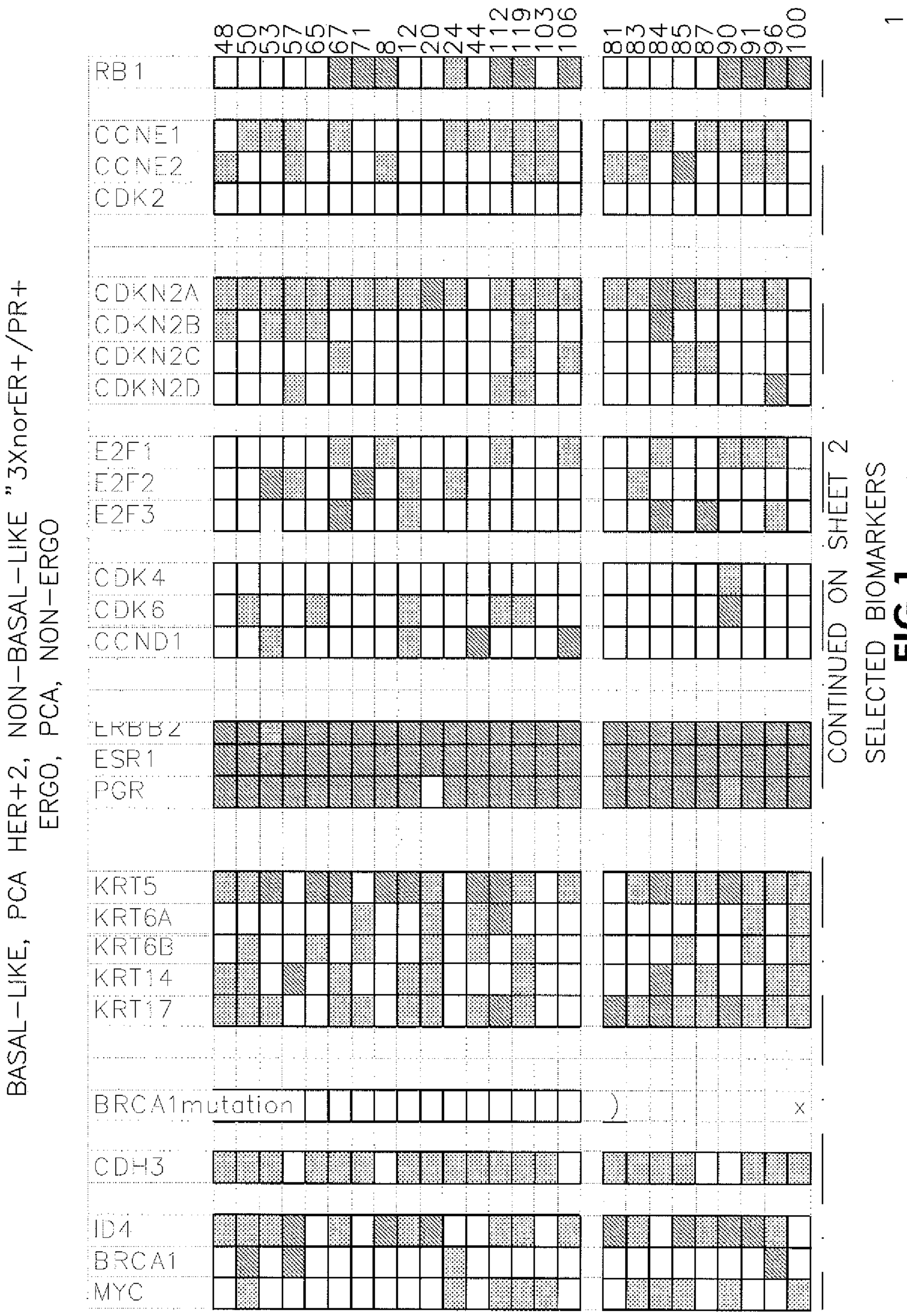
THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 4
CONTAINING PAGES 1 TO 163

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE: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RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
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CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mutation
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER+2, NON-ERGO, PCA, NON-ERGO "3XnorER+/PR+ -BASAL-LIKE
CONTINUED ON SHEET 2
SELECTED BIOMARKERS

FIG.1

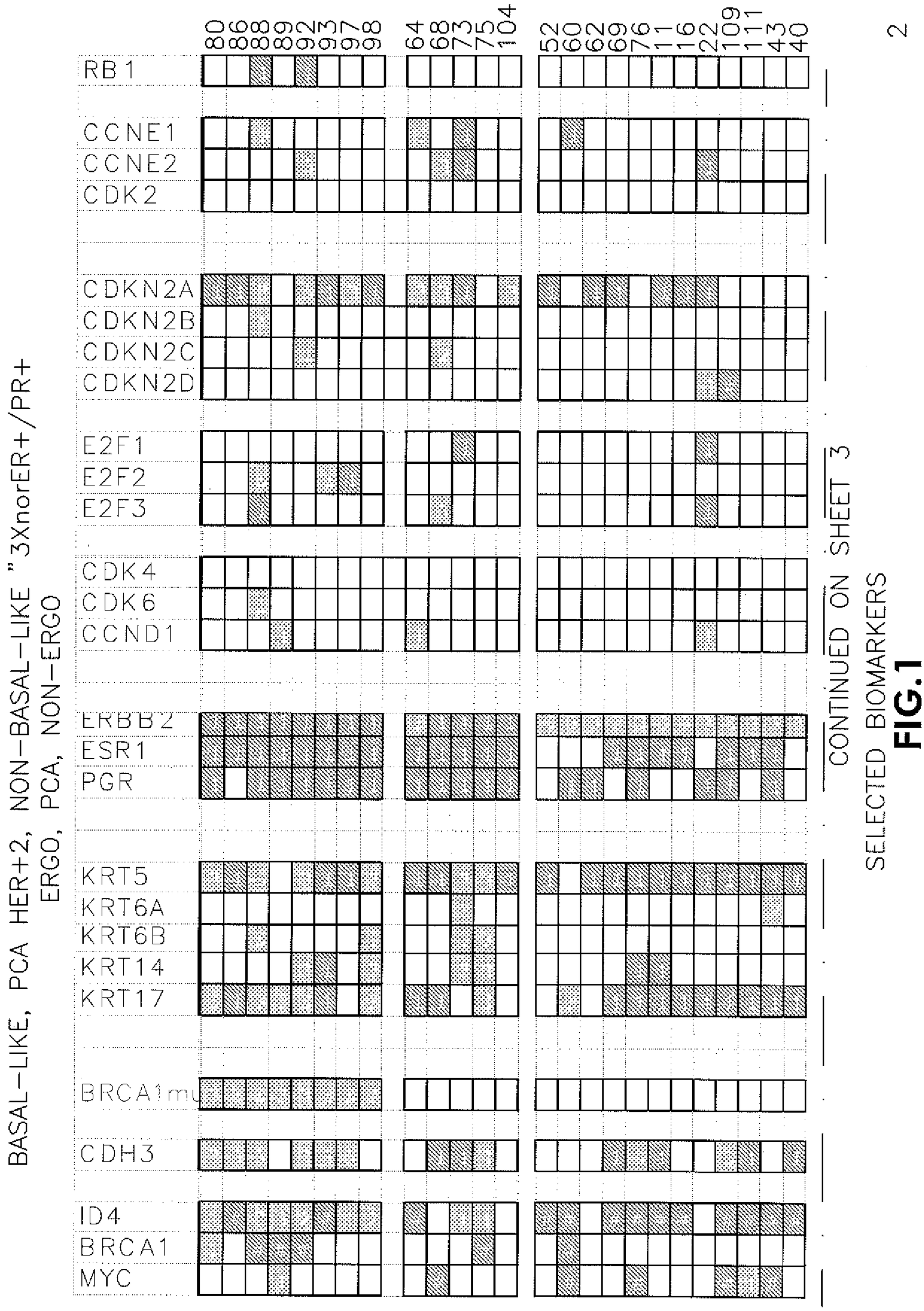
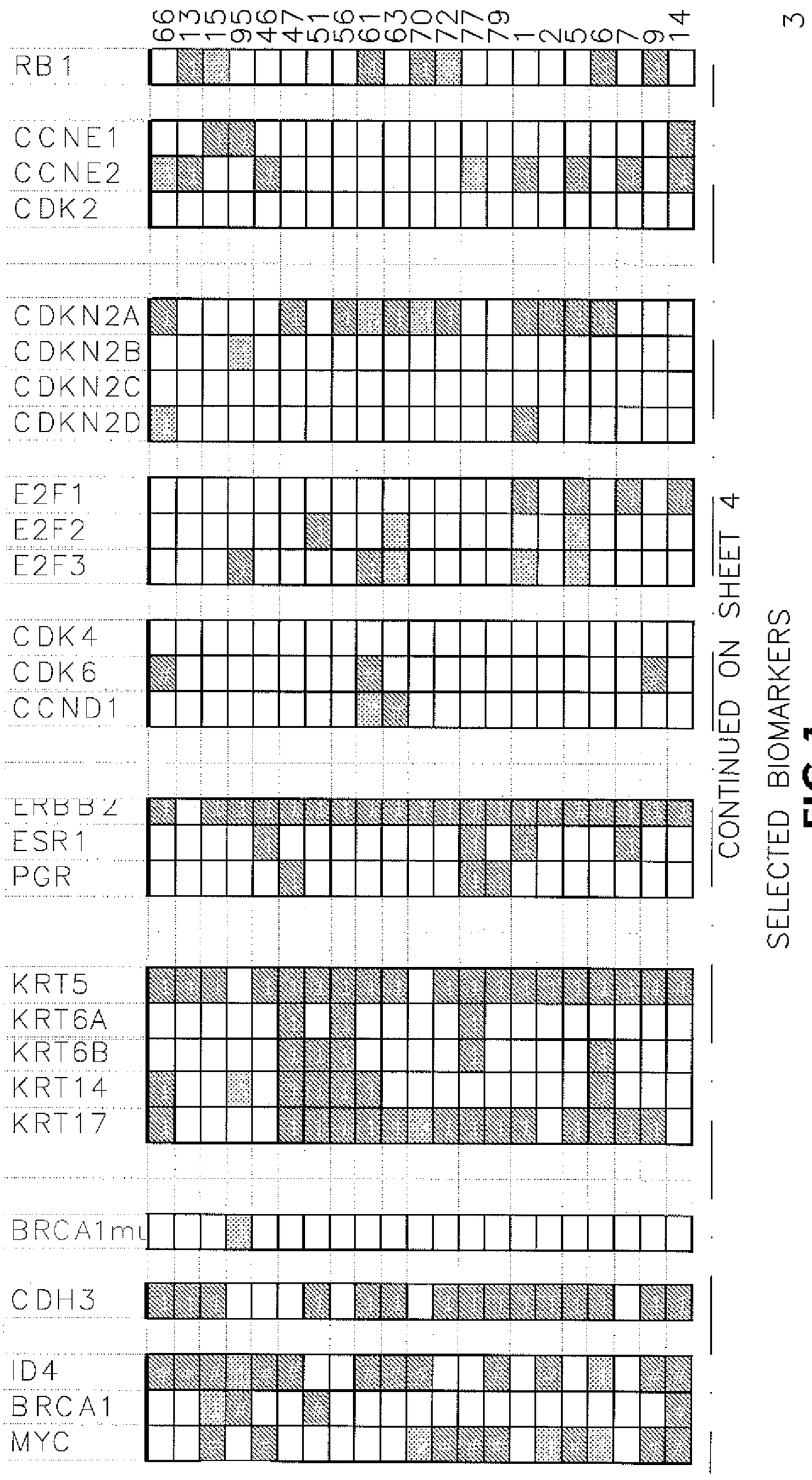

80 86 88 89 92 93 97 98 64 68 73 75 104 52 60 62 69 76 111 116 122 109 111 143 140
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
CONTINUED ON SHEET 3
SELECTED BIOMARKERS
2

FIG.1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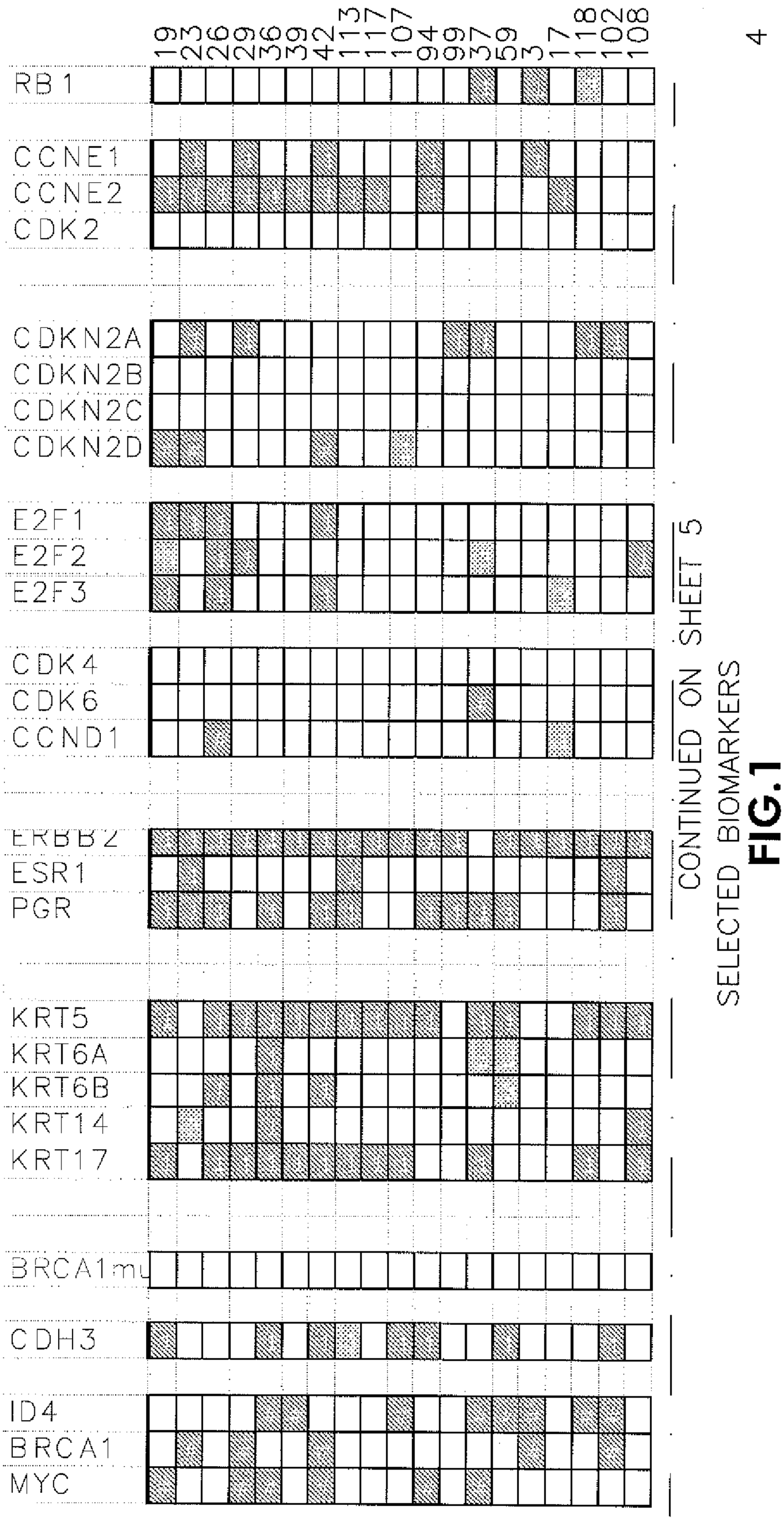
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
CONTINUED ON SHEET 4
SELECTED BIOMARKERS

FIG. 1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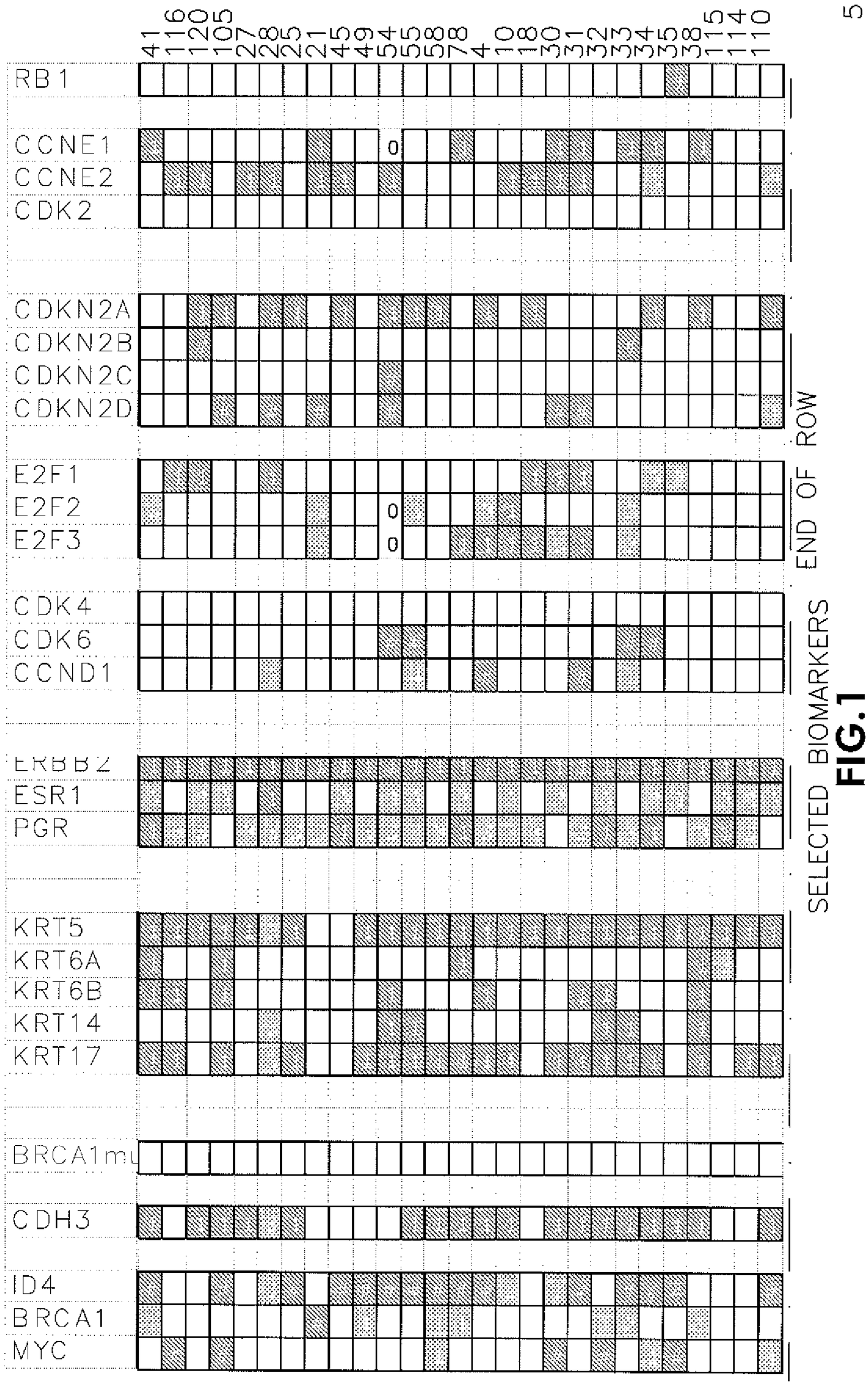
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
4
CONTINUED ON SHEET 5
SELECTED BIOMARKERS
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO

FIG.1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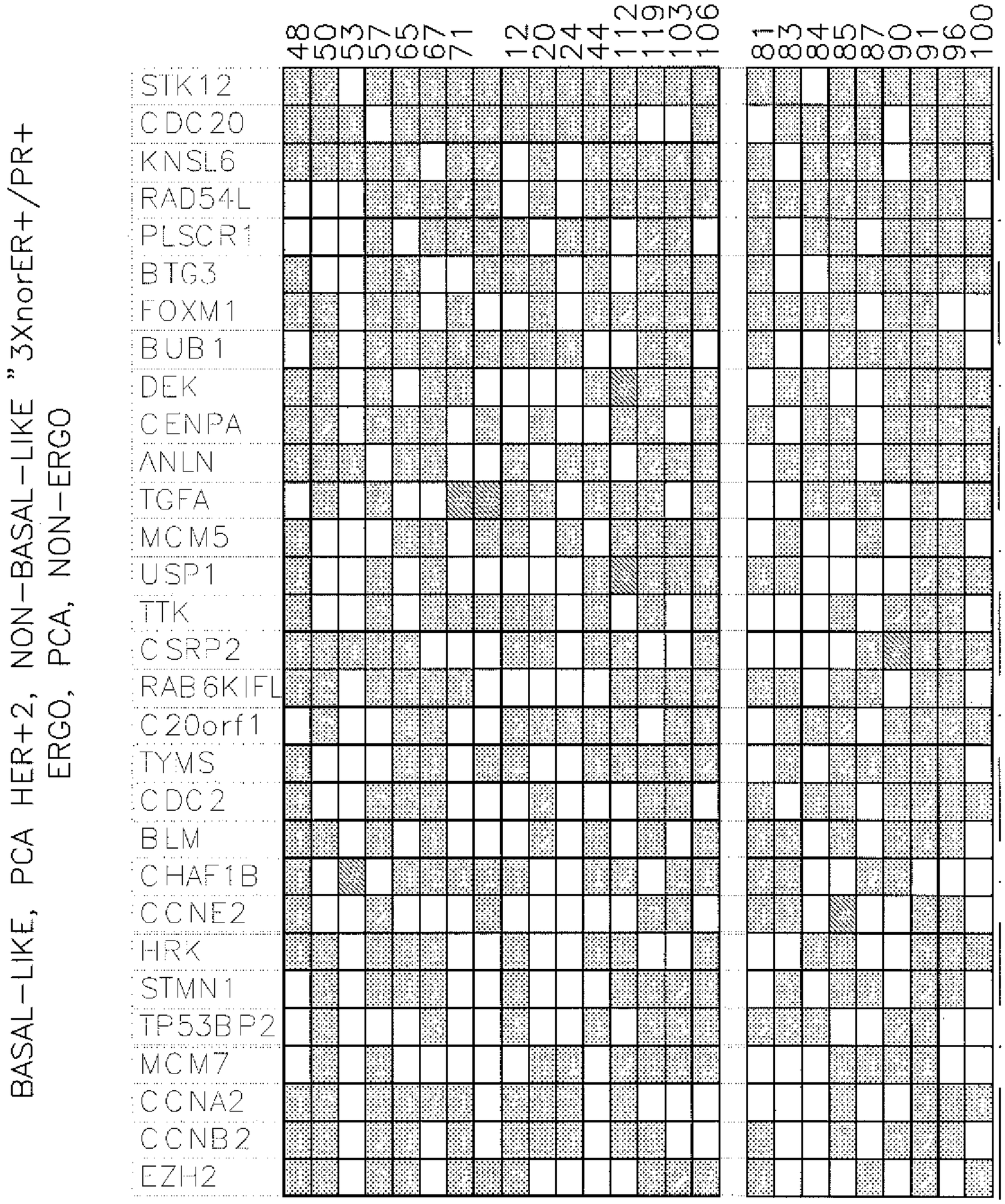
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
SELECTED BIOMARKERS
END OF ROW

FIG.1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STK12
CDC20
KNSL6
RAD54L
PLSCR1
BTG3
FOXM1
BUB1
DEK
CENPA
ANLN
TGFA
MCM5
USP1
TTK
CSRP2
RAB6KIFL
C20orf1
TYMS
CDC2
BLM
CHAF1B
CCNE2
HRK
STMN1
TP53BP2
MCM7
CCNA2
CCNB2
EZH2
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+ ERGO, PCA, NON-ERGO
CONTINUED ON SHEET 7
TOP 30 OVEREXPRESSED ERGO GENES

FIG.1

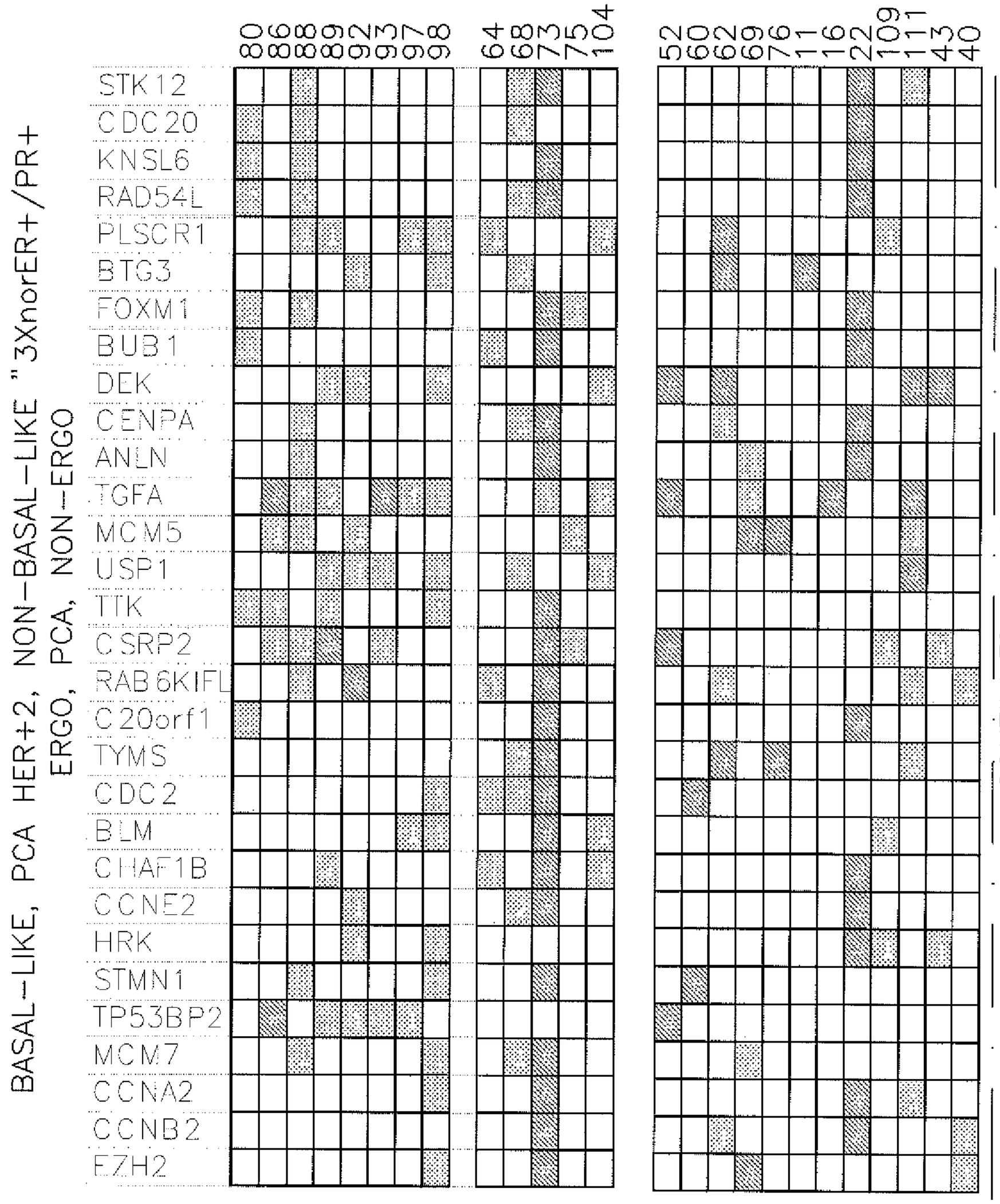

BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
80 86 88 89 92 93 97 98 64 68 73 75 104 52 60 62 69 76 11 16 22 109 111 43 40
STK12
CDC20
KNSL6
RAD54L
PLSCR1
BTG3
FOXM1
BUB1
DEK
CENPA
ANLN
TGFA
MCM5
USP1
TTK
CSRP2
RAB6KIFL
C20orf1
TYMS
CDC2
BLM
CHAF1B
CCNE2
HRK
STMN1
TP53BP2
MCM7
CCNA2
CCNB2
EZH2
CONTINUED ON SHEET 8
TOP 30 OVEREXPRESSED ERGO GENES

FIG.1

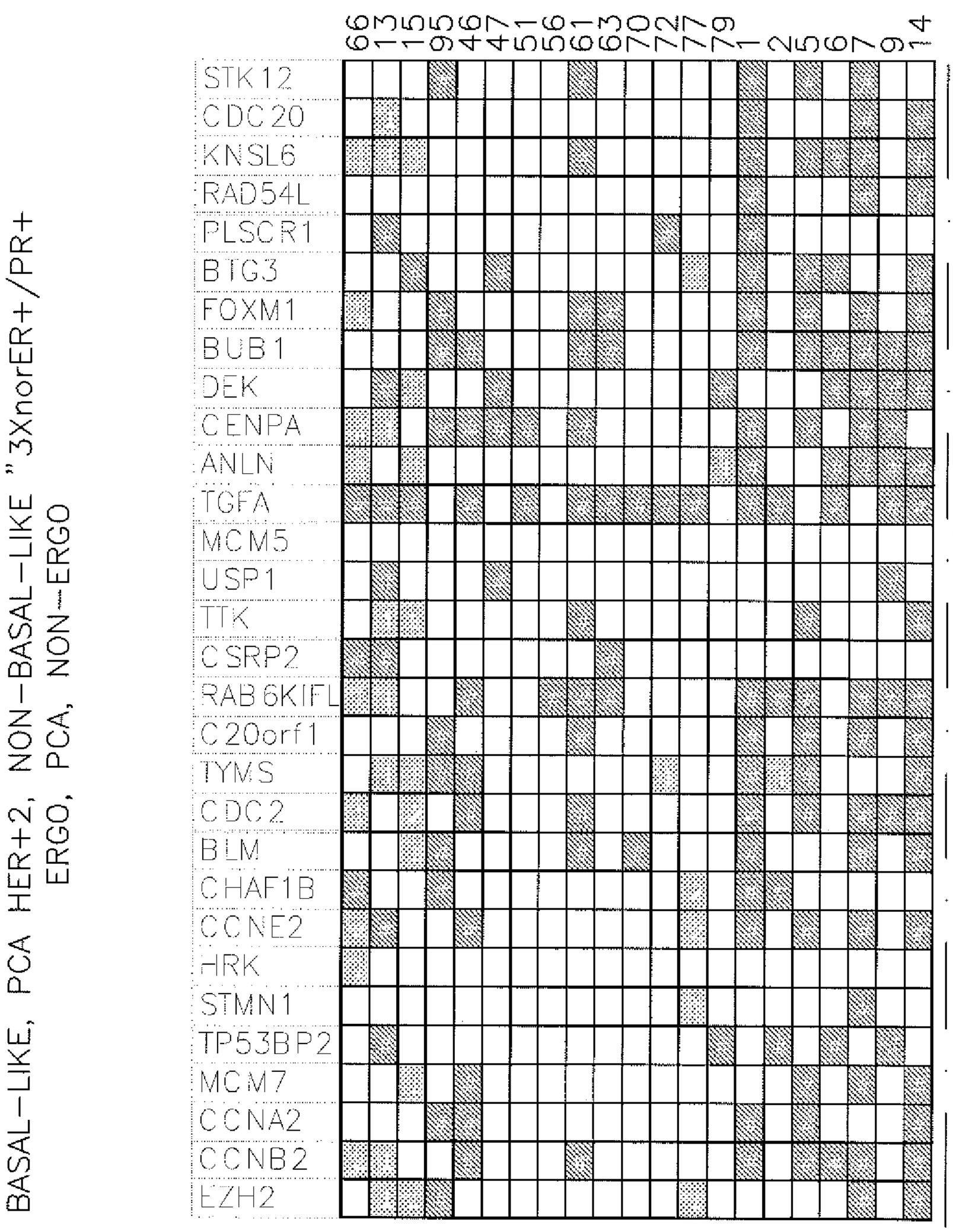

6 1 1 9 4 4 5 5 6 6 7 7 7 7 1 2 5 6 7 9 1
STK12
CDC20
KNSL6
RAD54L
PLSCR1
BTG3
FOXM1
BUB1
DEK
CENPA
ANLN
TGFA
MCM5
USP1
TTK
CSRP2
RAB6KIFL
C20orf1
TYMS
CDC2
BLM
CHAF1B
CCNE2
HRK
STMN1
TP53BP2
MCM7
CCNA2
CCNB2
EZH2
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
CONTINUED ON SHEET 9
TOP 30 OVEREXPRESSED ERGO GENES

FIG.1

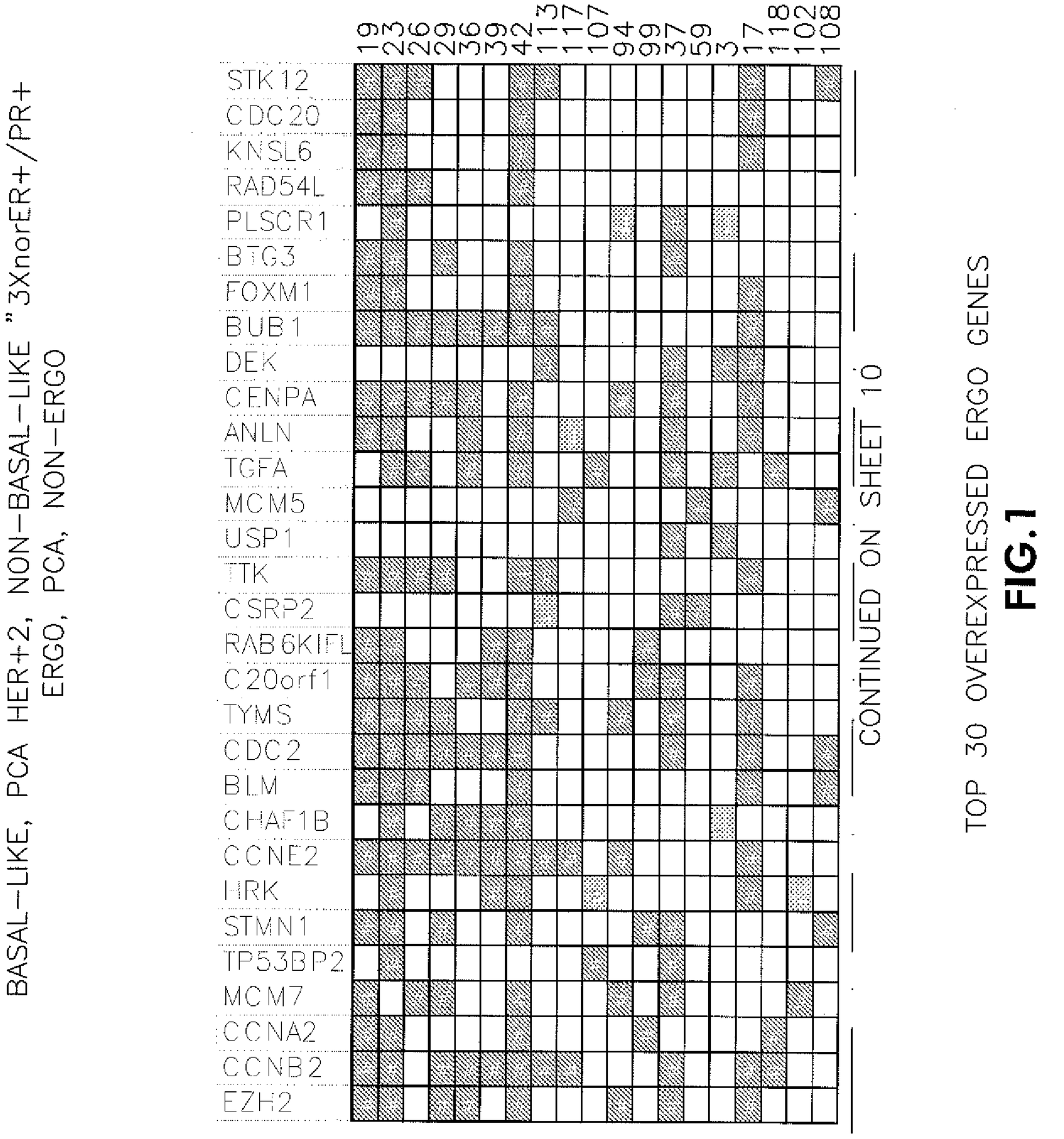

TOP 30 OVEREXPRESSED ERGO GENES
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
19 23 26 29 36 39 42 113 117 107 94 99 37 59 3 17 118 102 108
STK12
CDC20
KNSL6
RAD54L
PLSCR1
BTG3
FOXM1
BUB1
DEK
CENPA
ANLN
TGFA
MCM5
USP1
TTK
CSRP2
RAB6KIFL
C20orf1
TYMS
CDC2
BLM
CHAF1B
CCNE2
HRK
STMN1
TP53BP2
MCM7
CCNA2
CCNB2
EZH2
CONTINUED ON SHEET 10

FIG.1

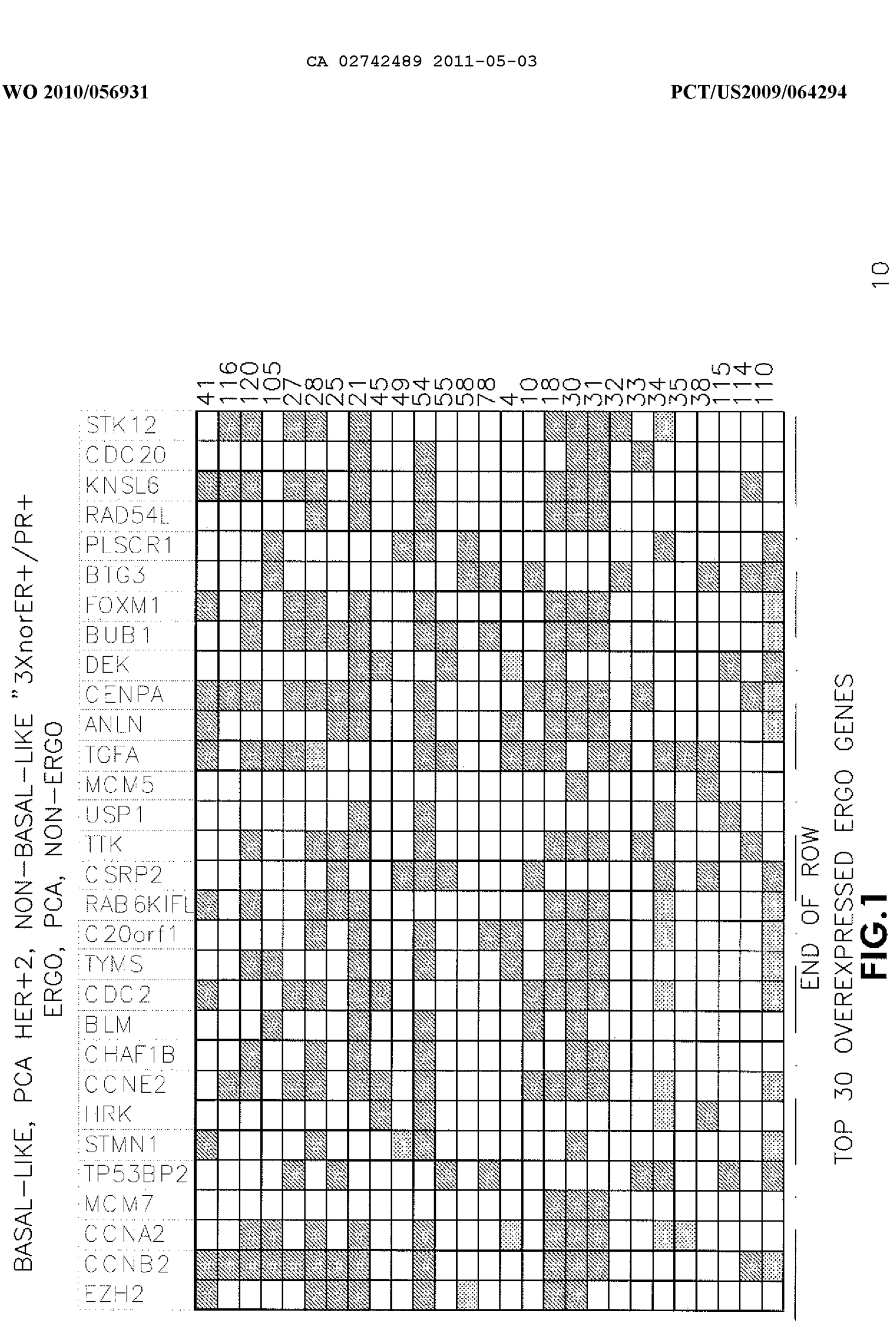

10
41
116
120
105
127
128
125
121
124
129
145
145
155
158
157
41
108
130
131
132
133
134
135
138
115
114
110
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+ ERGO, PCA, NON-ERGO
STK12
CDC20
KNSL6
RAD54L
PLSCR1
BTG3
FOXM1
BUB1
DEK
CENPA
ANLN
TGFA
MCM5
USP1
TTK
CSRP2
RAB6KIFL
C20orf1
TYMS
CDC2
BLM
CHAF1B
CCNE2
IIRK
STMN1
TP53BP2
MCM7
CCNA2
CCNB2
EZH2
END OF ROW
TOP 30 OVEREXPRESSED ERGO GENES

FIG.1

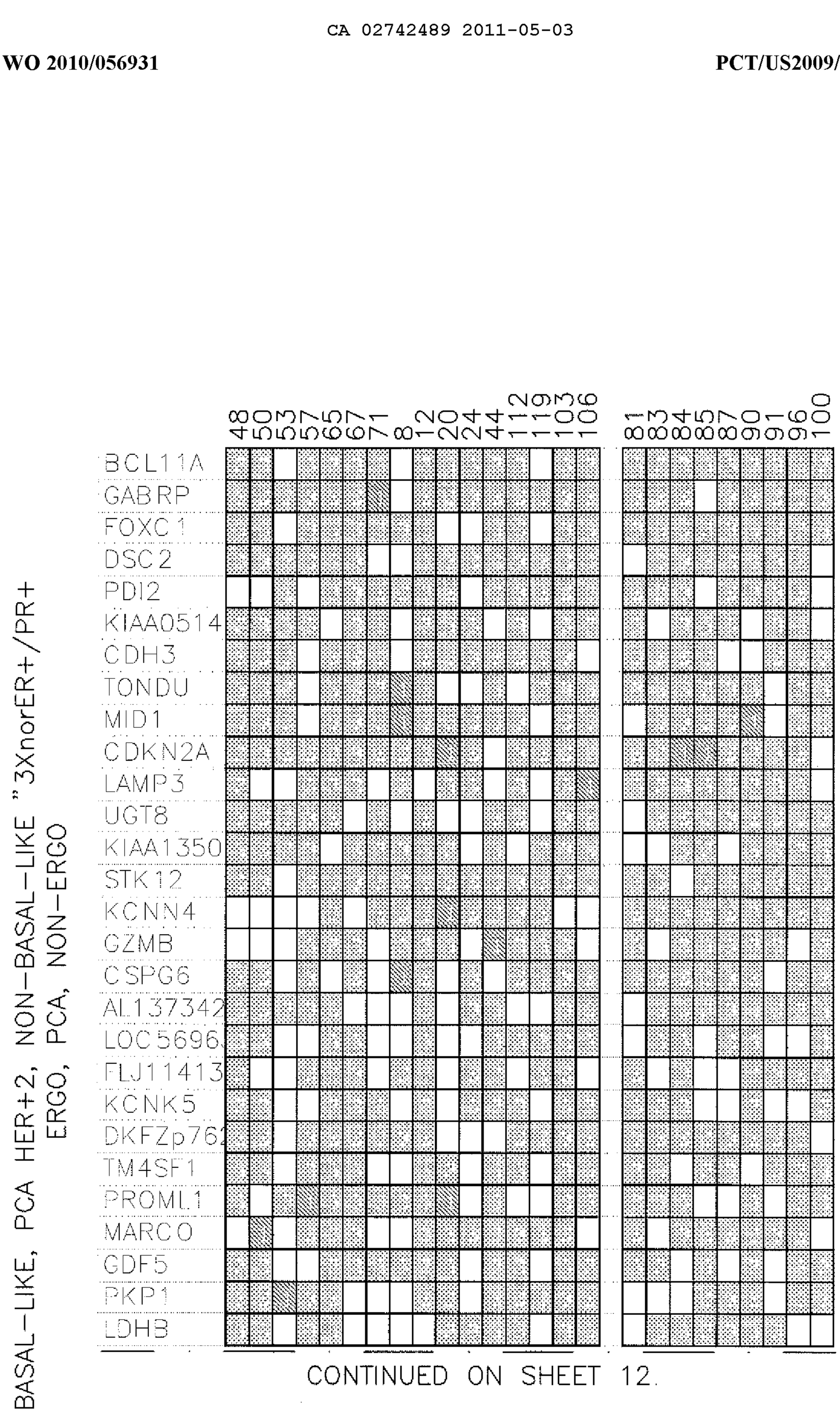
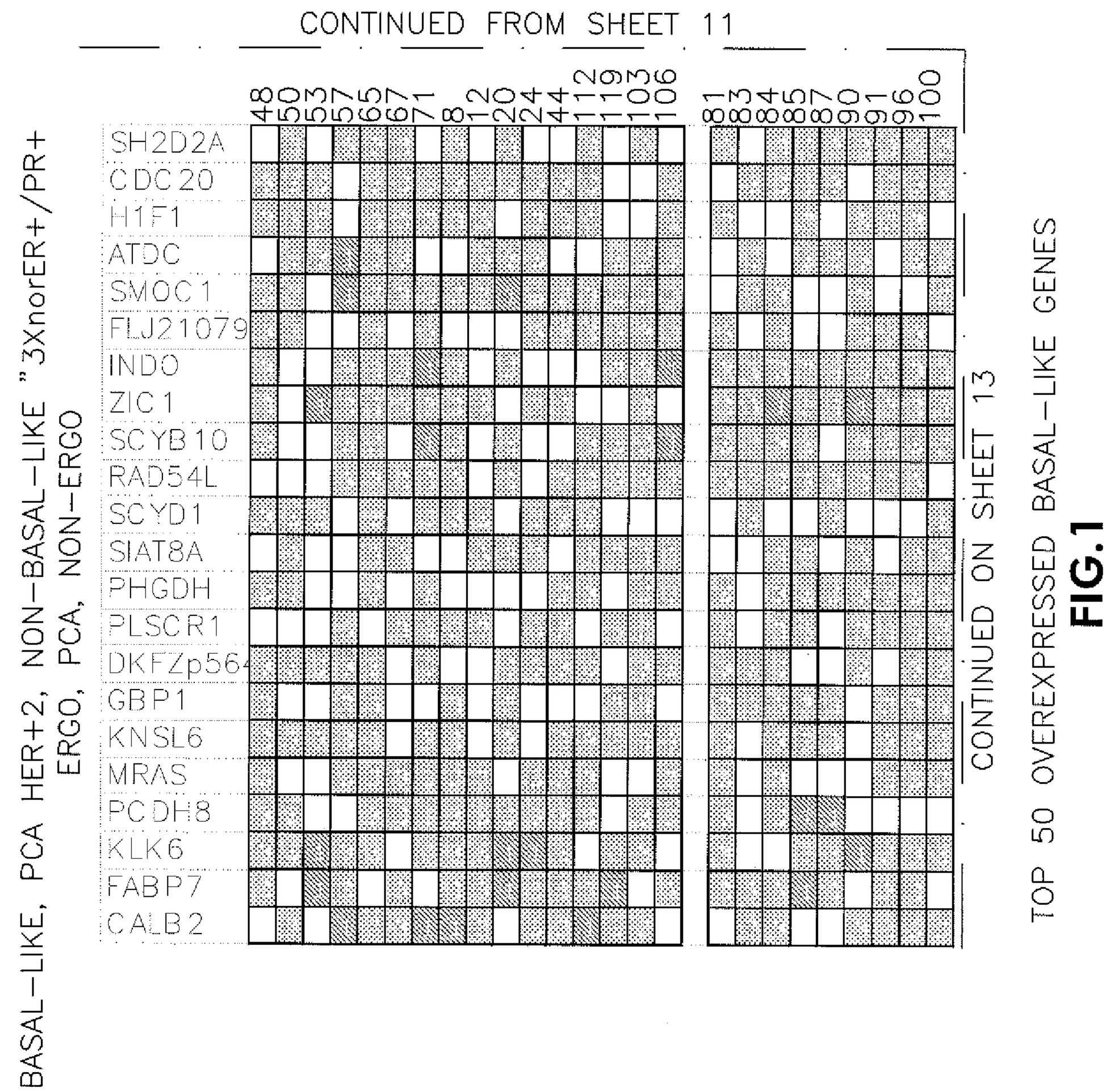


FIG.1

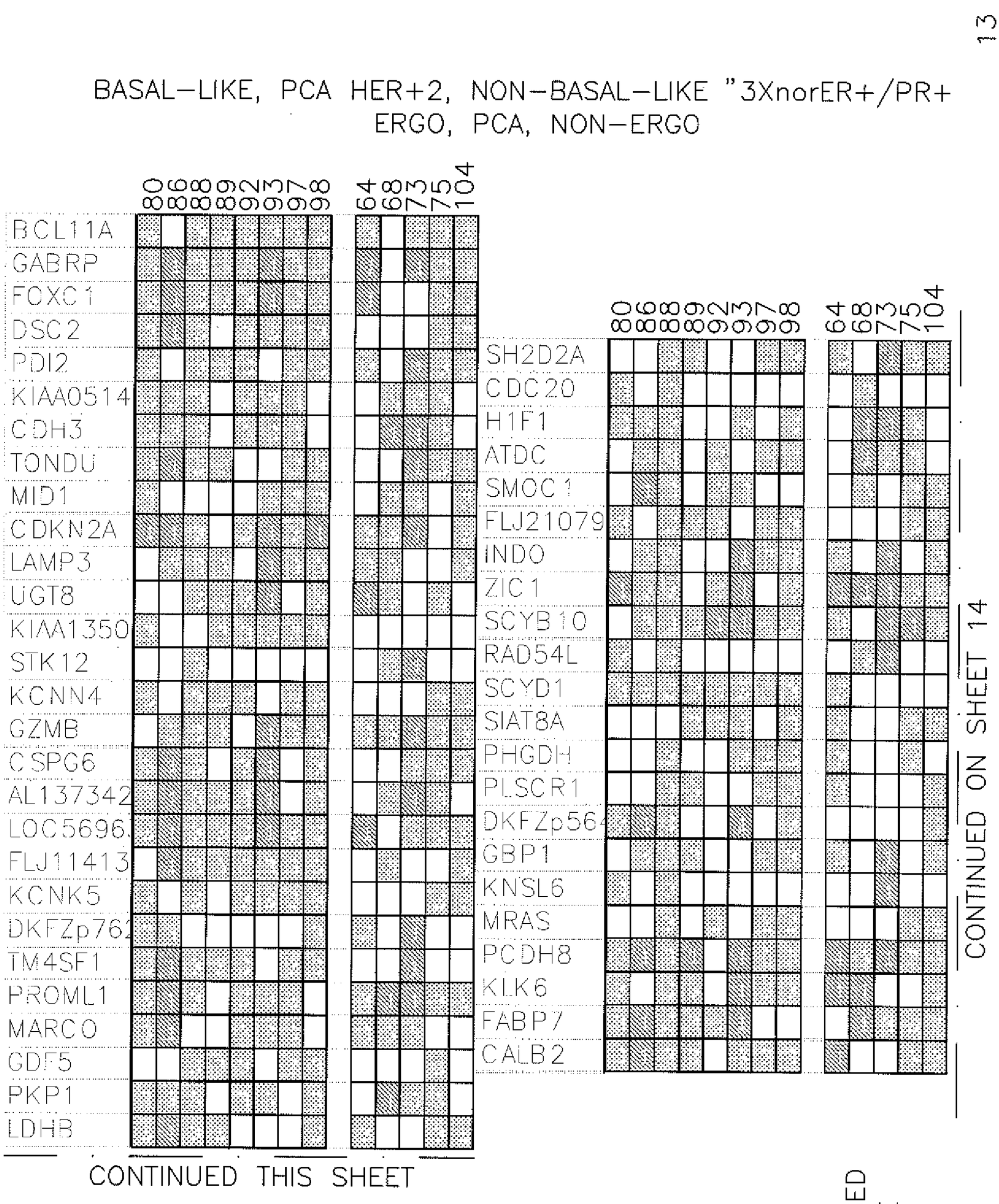
CONTINUED ON SHEET 12.



TOP 50 OVEREXPRESSED B

FIG.1

13



TOP 50 OVEREXPRESSED BASAL–LIKE GENES

FIG.1

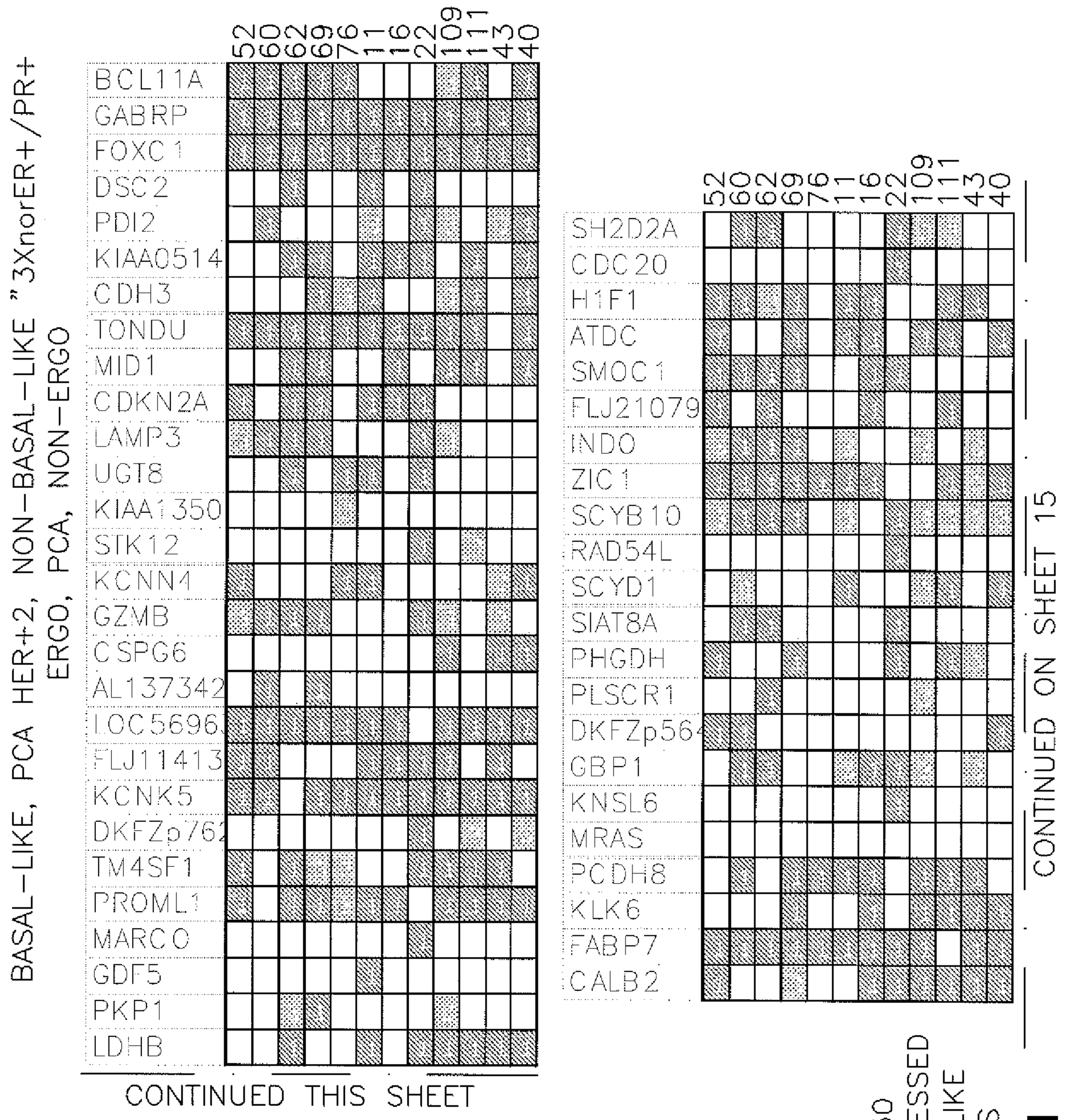

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+ ERGO, PCA, NON-ERGO
52 60 62 69 76 11 16 22 109 111 43 40
BCL11A
GABRP
FOXC1
DSC2
PDI2
KIAA0514
CDH3
TONDU
MID1
CDKN2A
LAMP3
UGT8
KIAA1350
STK12
KCNN4
GZMB
CSPG6
AL137342
IOC5696
FLJ11413
KCNK5
DKFZp762
TM4SF1
PROML1
MARCO
GDF5
PKP1
LDHB
CONTINUED THIS SHEET
52 60 62 69 76 11 16 22 109 111 43 40
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2
CONTINUED ON SHEET 15

FIG.1

15

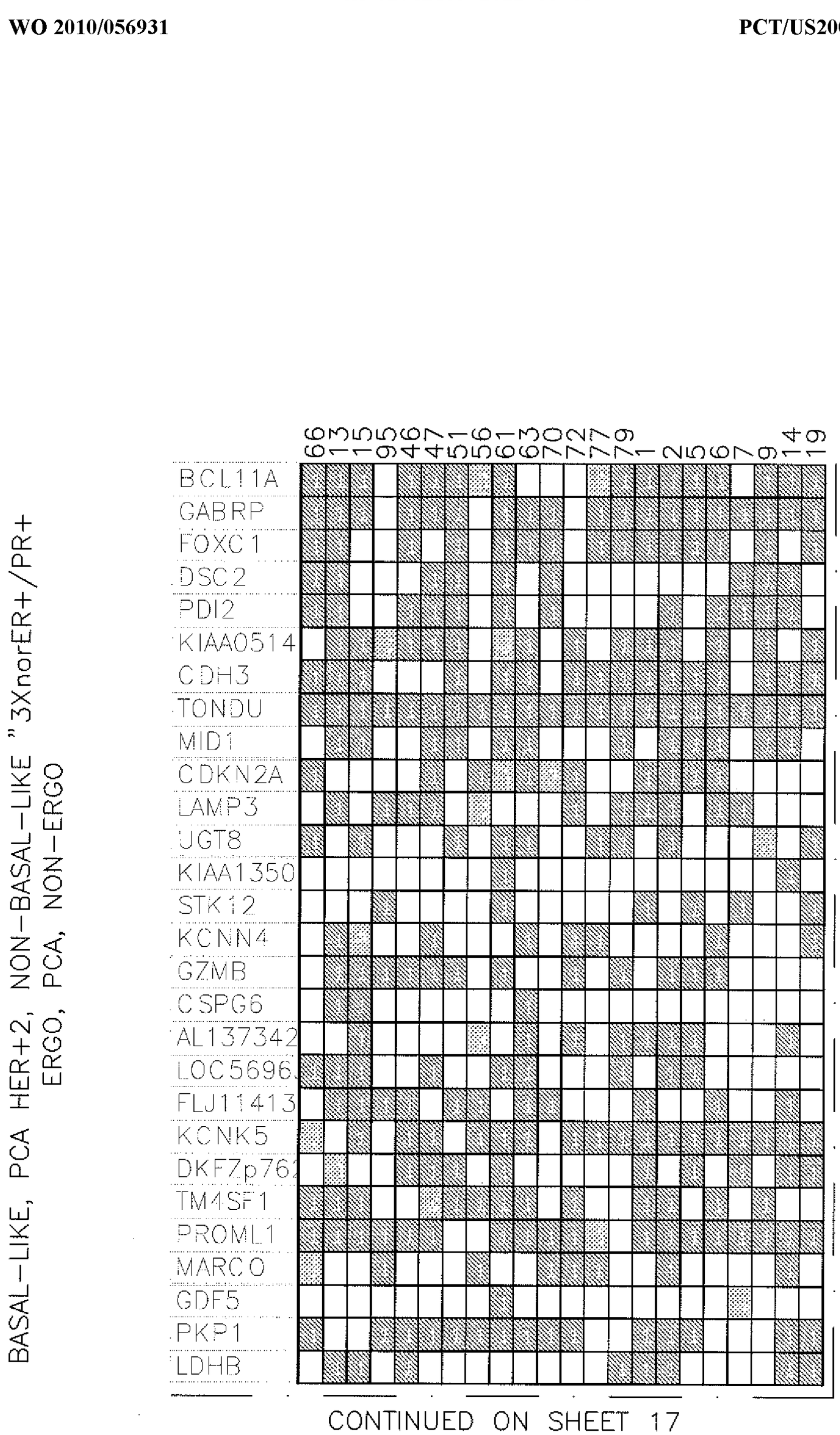

BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
66 13 15 95 46 47 51 56 61 63 70 72 77 79 1 2 55 67 9 14 19
BCL11A
GABRP
FOXC1
DSC2
PDI2
KIAA0514
CDH3
TONDU
MID1
CDKN2A
LAMP3
UGT8
KIAA1350
STK12
KCNN4
GZMB
CSPG6
AL137342
LOC5696
FLJ11413
KCNK5
DKFZp762
TM4SF1
PROML1
MARCO
GDF5
PKP1
LDHB
CONTINUED ON SHEET 16
TOP 50 OVEREXPRESSED BASAL-LIKE GENES
CONTINUED ON SHEET 17

FIG.1

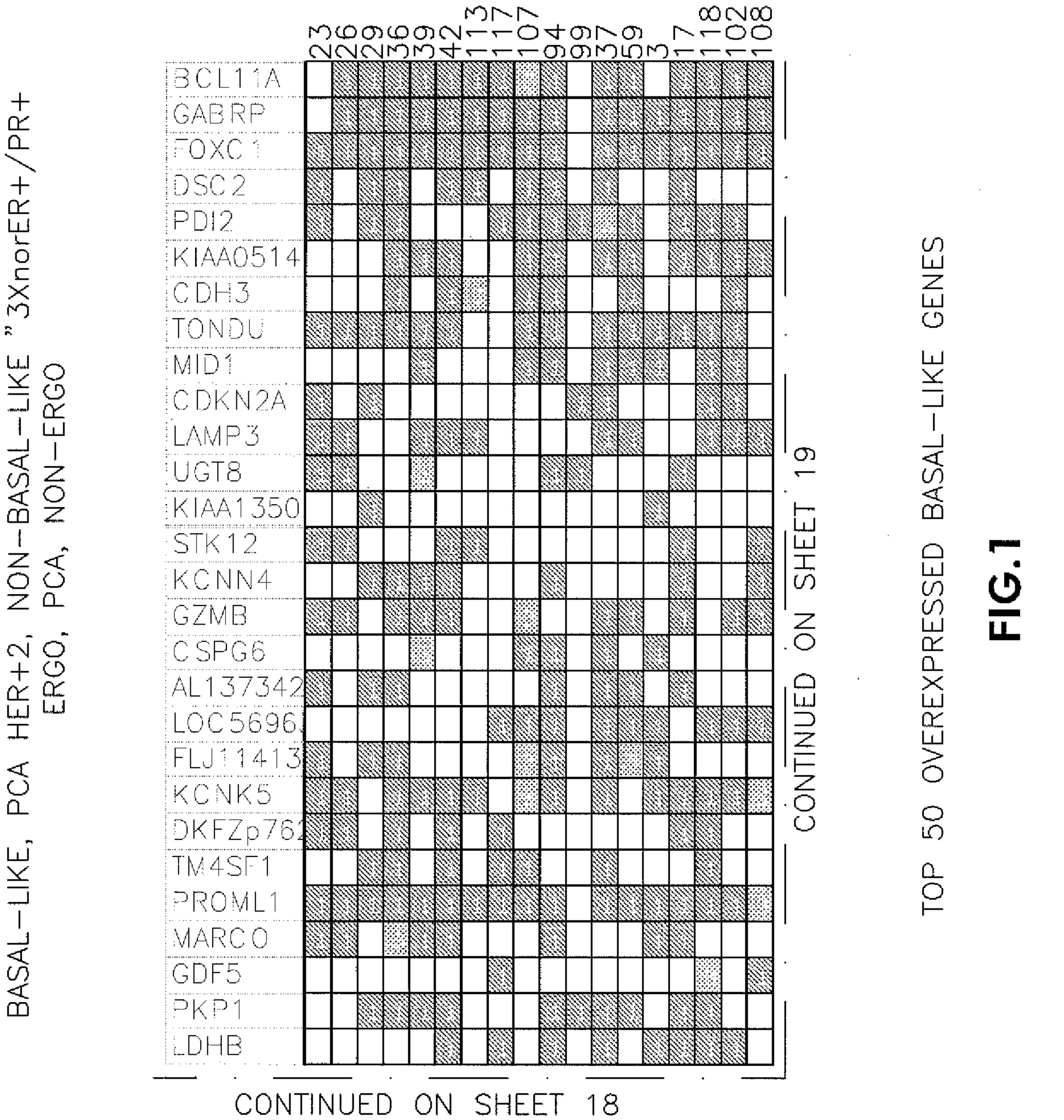

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
23 26 29 36 39 42 113 117 107 94 99 37 59 3 17 118 102 108
BCL11A
GABRP
FOXC1
DSC2
PDI2
KIAA0514
CDH3
TONDU
MID1
CDKN2A
LAMP3
UGT8
KIAA1350
STK12
KCNN4
GZMB
CSPG6
AL137342
LOC5696
FLJ11413
KCNK5
DKFZp762
TM4SF1
PROML1
MARCO
GDF5
PKP1
LDHB
CONTINUED ON SHEET 19
CONTINUED ON SHEET 18

FIG.1

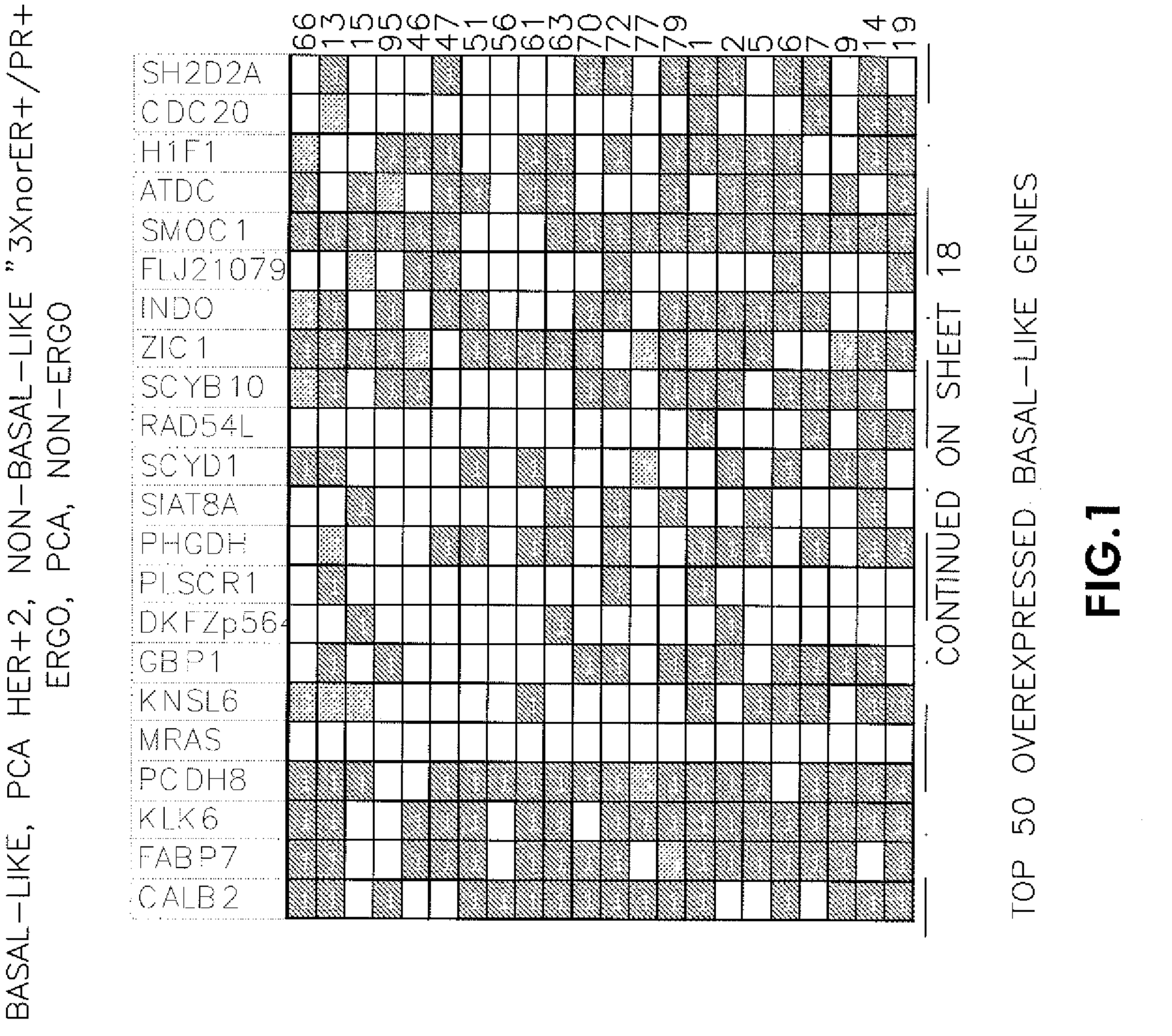

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
CONTINUED ON SHEET 18
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
66 13 15 95 46 47 51 56 61 63 70 72 77 79 1 2 5 6 7 9 14 19
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2

FIG.1

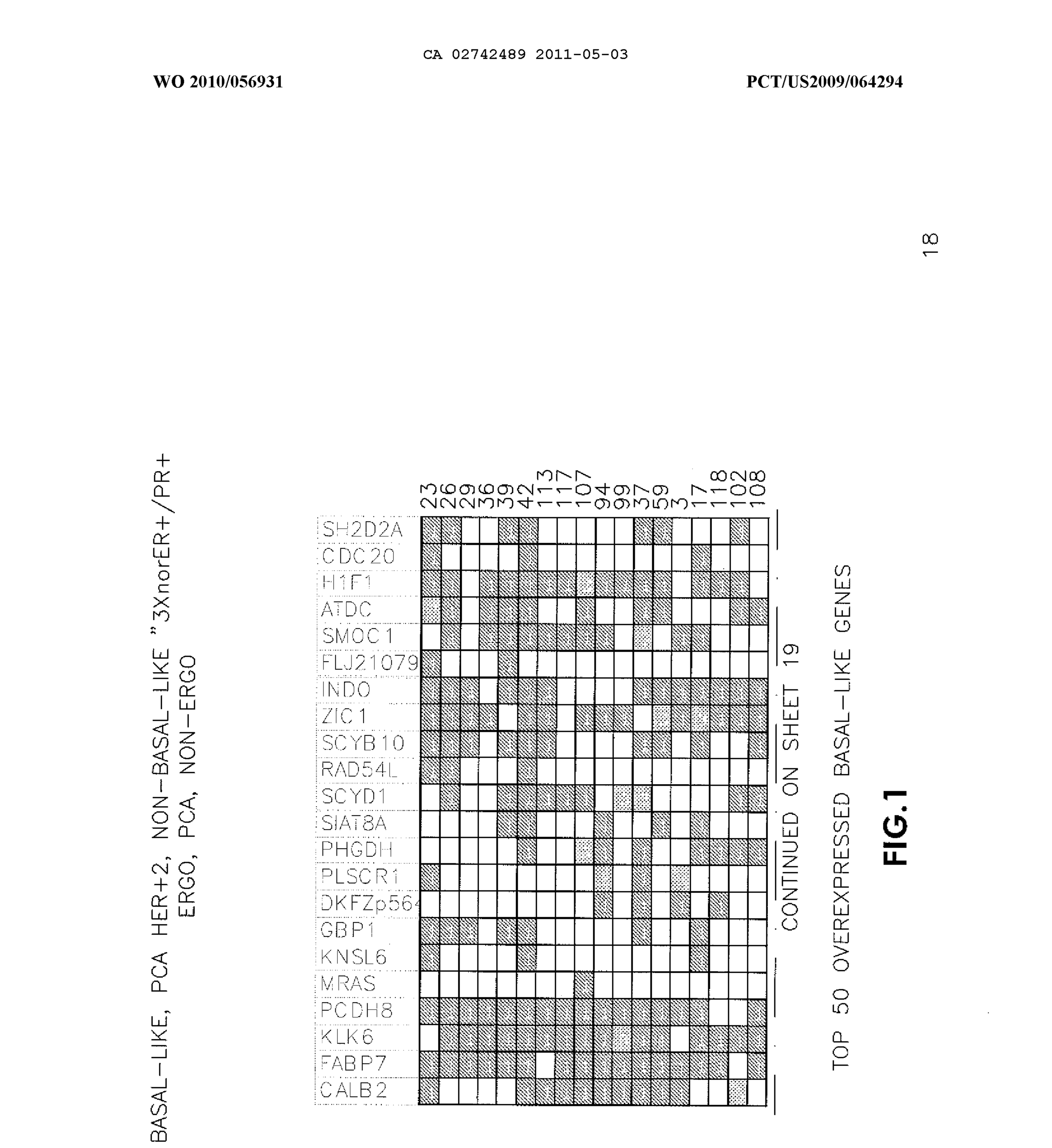

BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
23 26 29 36 39 42 113 117 107 94 99 37 59 3 17 118 102 108
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2
CONTINUED ON SHEET 19
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG.1

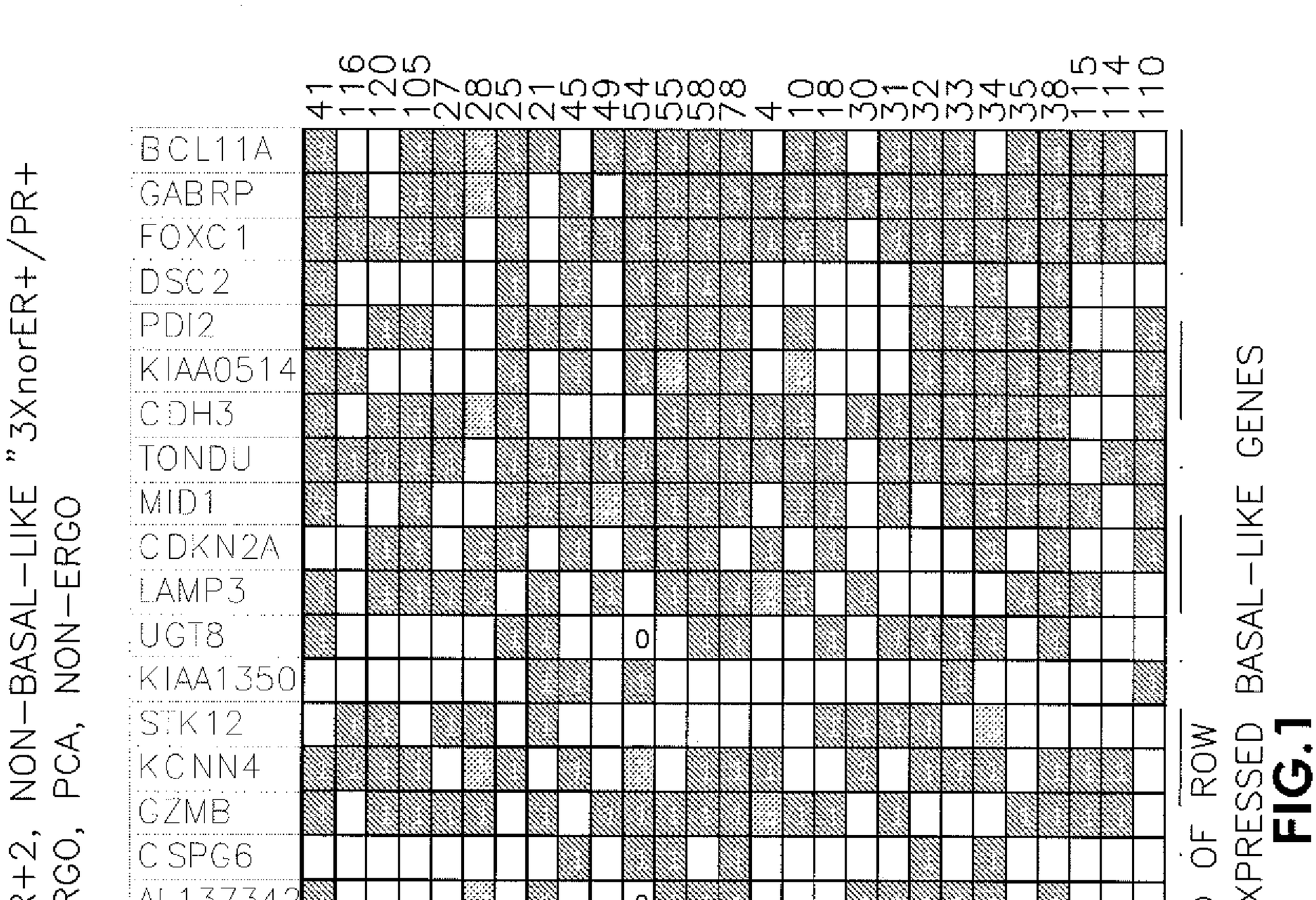


FIG.1

CONTINUED ON SHEET 20

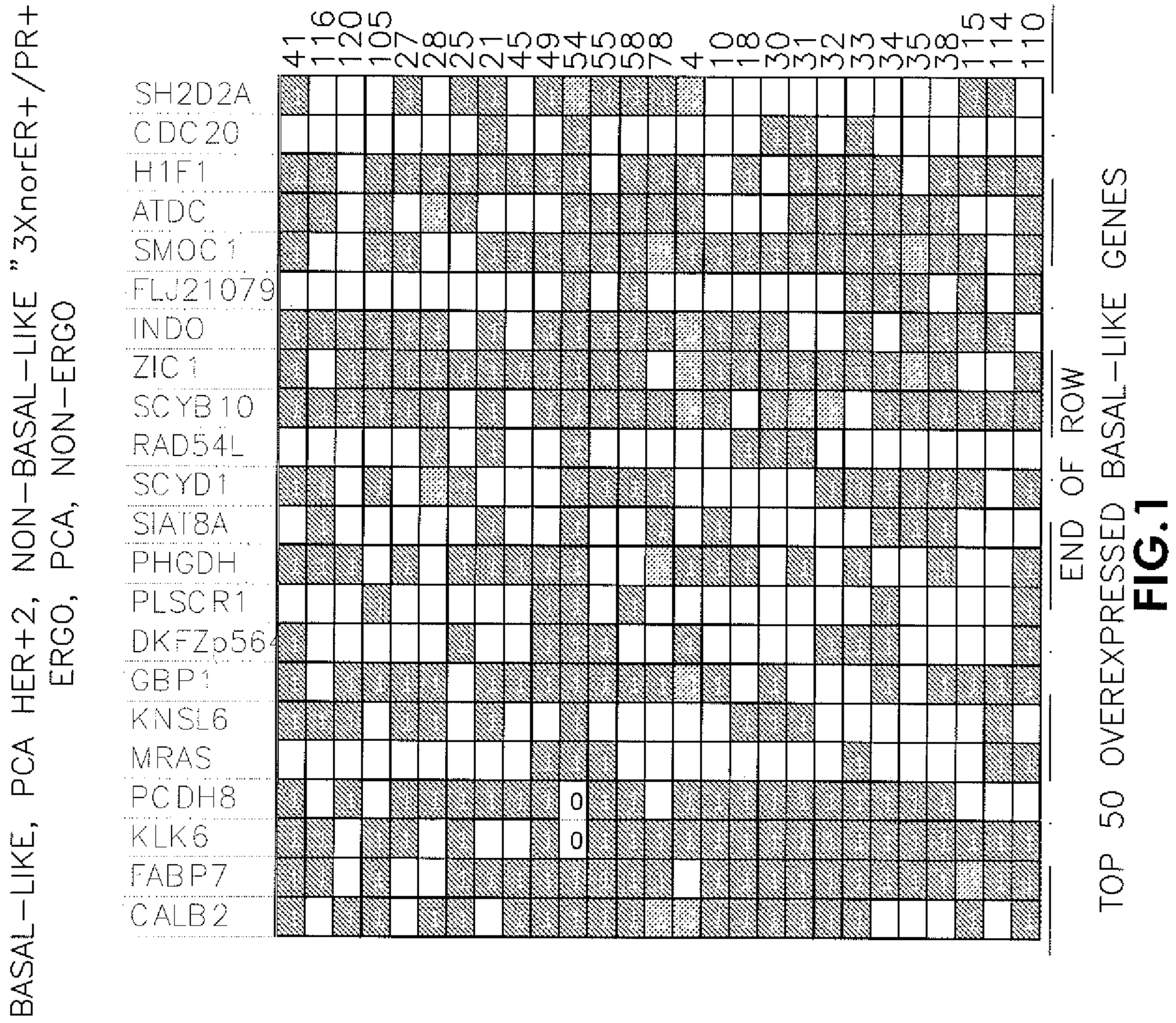

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
END OF ROW
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
41 116 120 105 27 28 25 21 45 49 54 55 58 78 4 10 18 30 31 32 33 34 35 38 115 114 110
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2

FIG.1

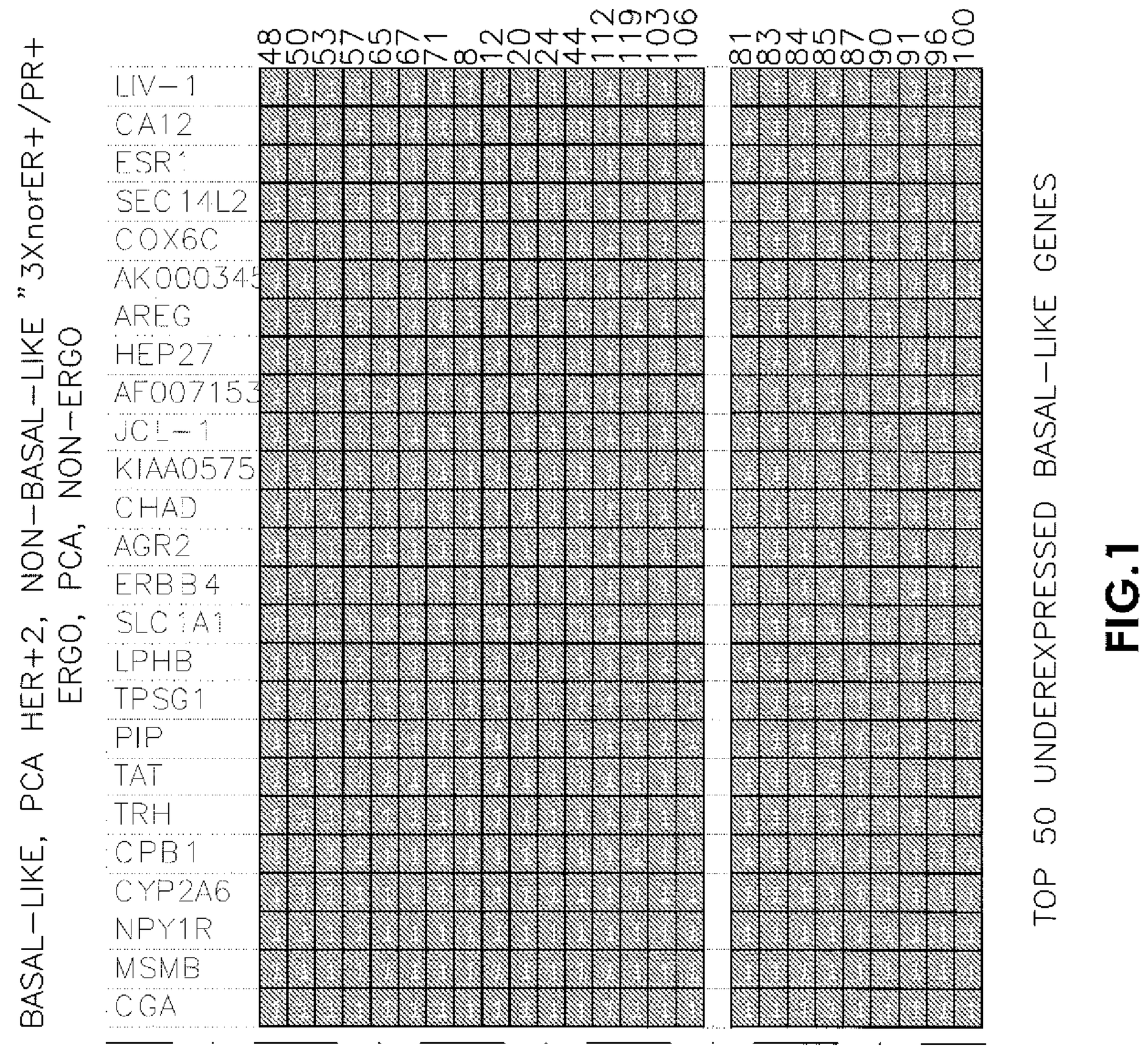


FIG.1

CONTINUED ON SHEET 22

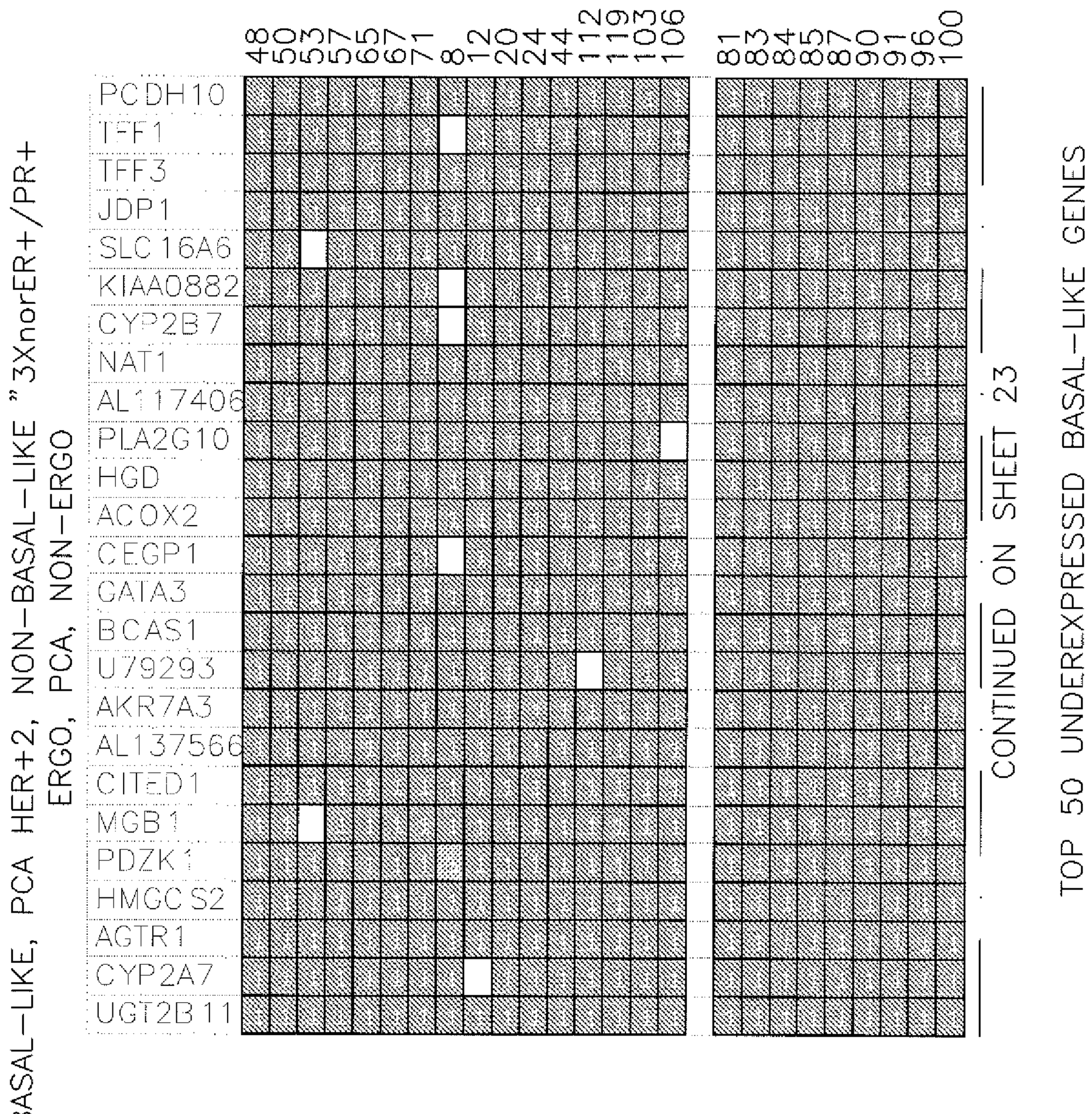

48 50 53 57 65 67 71 8 12 20 24 44 112 119 103 106
81 83 84 85 87 90 91 96 100
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AL137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UGT2B11
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+ ERGO, PCA, NON-ERGO
CONTINUED ON SHEET 23
TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG.1

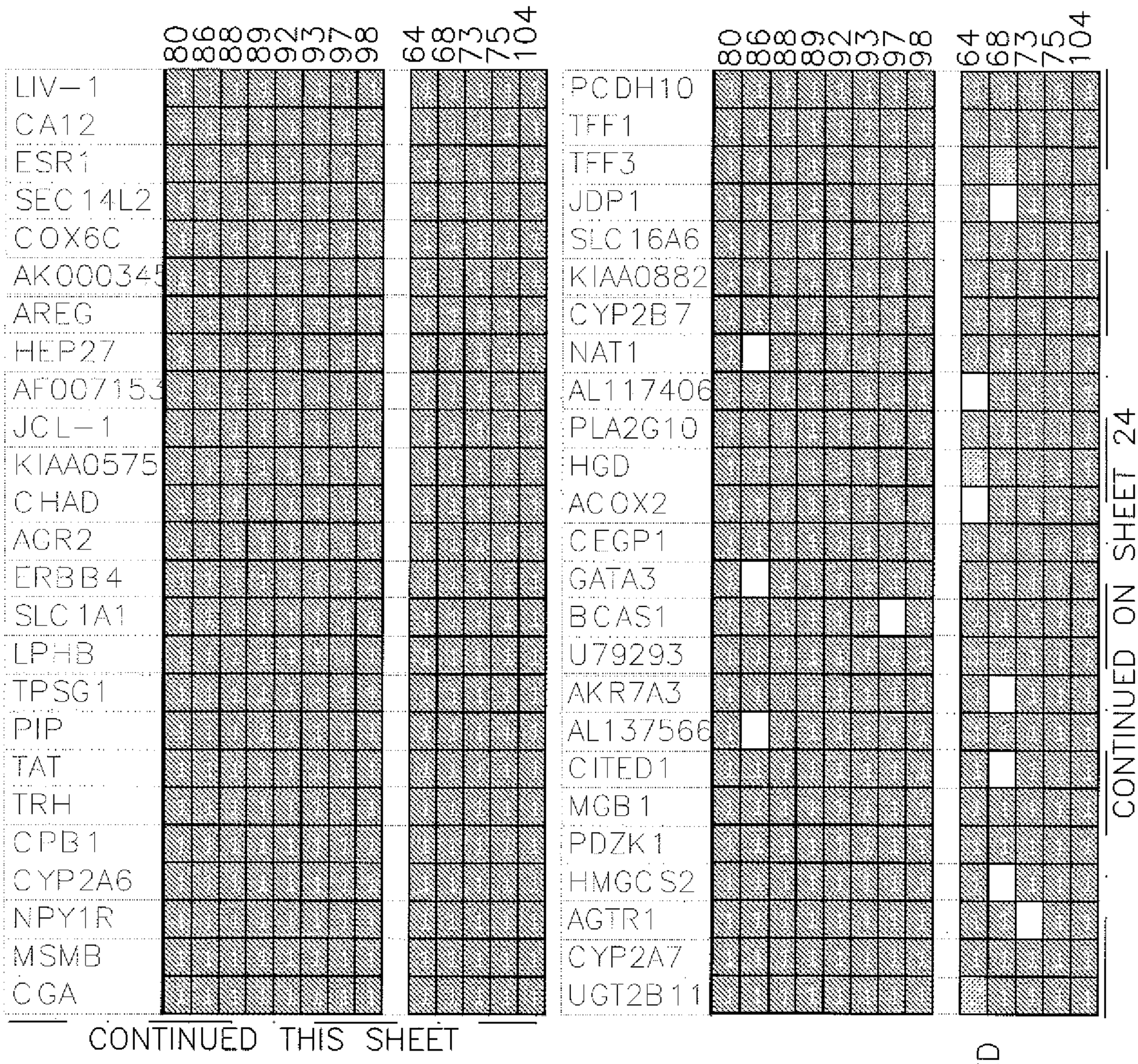

BASAL–LIKE, PCA HER+2, NON–BASAL–LIKE "3XnorER+/PR+
ERGO, PCA, NON–ERGO
80 86 88 89 92 93 97 98
64 68 73 75 104
LIV–1
CA12
ESR1
SEC14L2
COX6C
AK000345
AREG
HEP27
AF007153
JCL–1
KIAA0575
CHAD
AGR2
ERBB4
SLC1A1
LPHB
TPSG1
PIP
TAT
TRH
CPB1
CYP2A6
NPY1R
MSMB
CGA
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AL137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UGT2B11
CONTINUED THIS SHEET
CONTINUED ON SHEET 24
TOP 50
UNDEREXPRESSED
BASAL–LIKE
GENES

FIG. 1

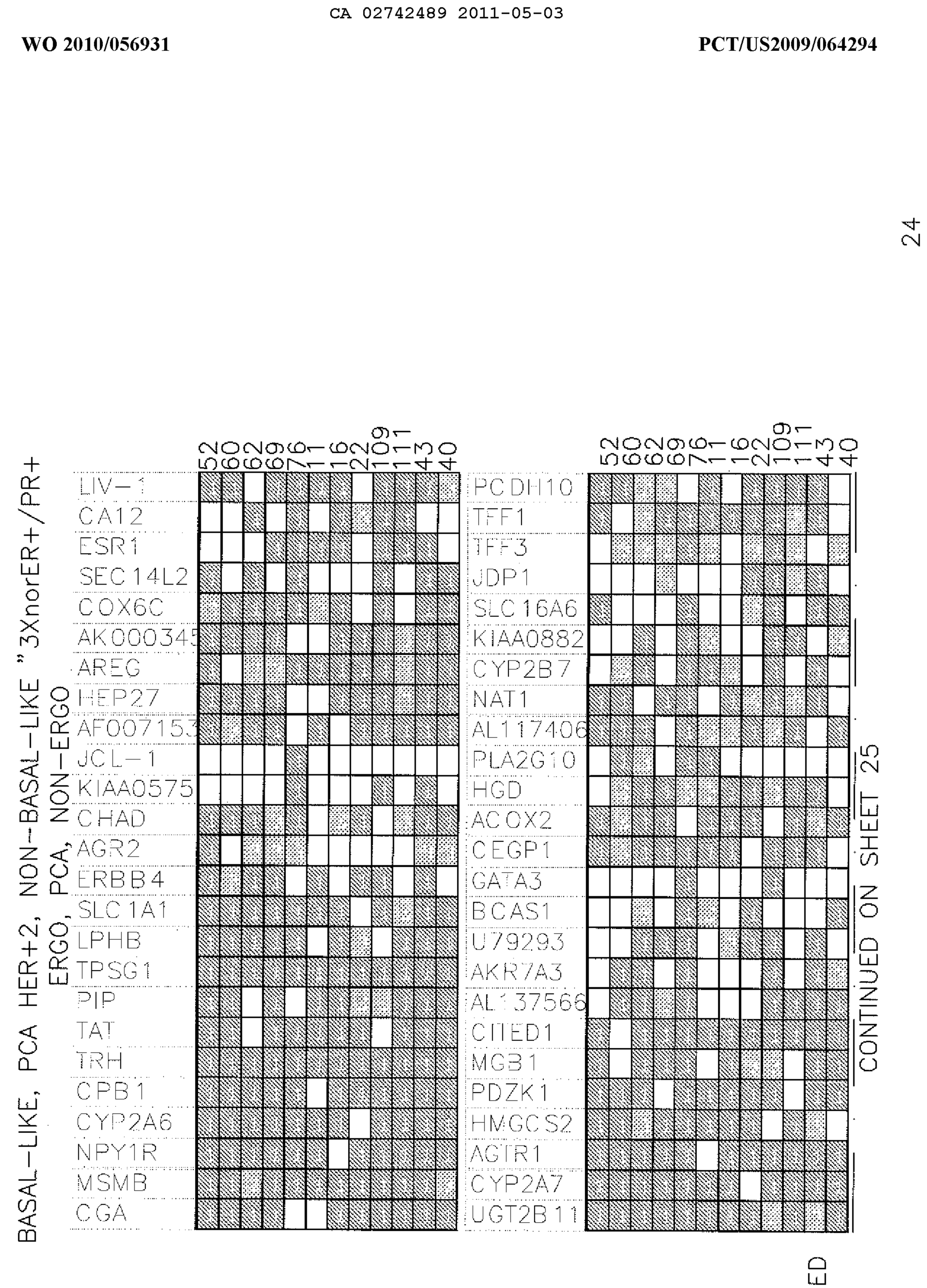

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+ ERGO, PCA, NON-ERGO
52 60 62 69 76 1 16 22 109 111 43 40
LIV-1
CA12
ESR1
SEC14L2
COX6C
AK00034
AREG
HEP27
AF00715
JCL-1
KIAA0575
CHAD
AGR2
ERBB4
SLC1A1
LPHB
TPSG1
PIP
TAT
TRH
CPB1
CYP2A6
NPY1R
MSMB
CGA
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AL137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UGT2B11
CONTINUED ON SHEET 25

FIG.1

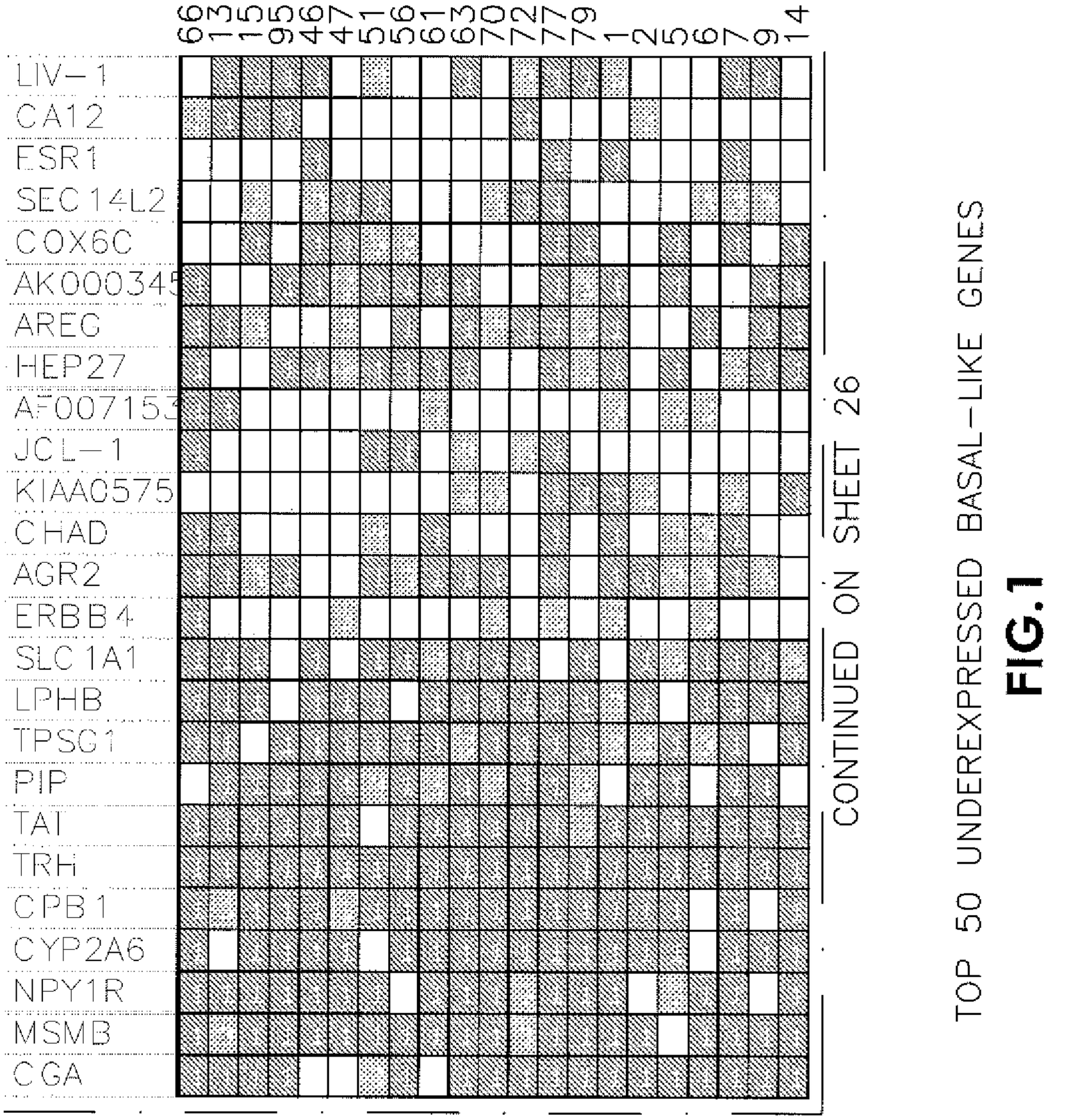


FIG. 1

CONTINUED ON SHEET 27

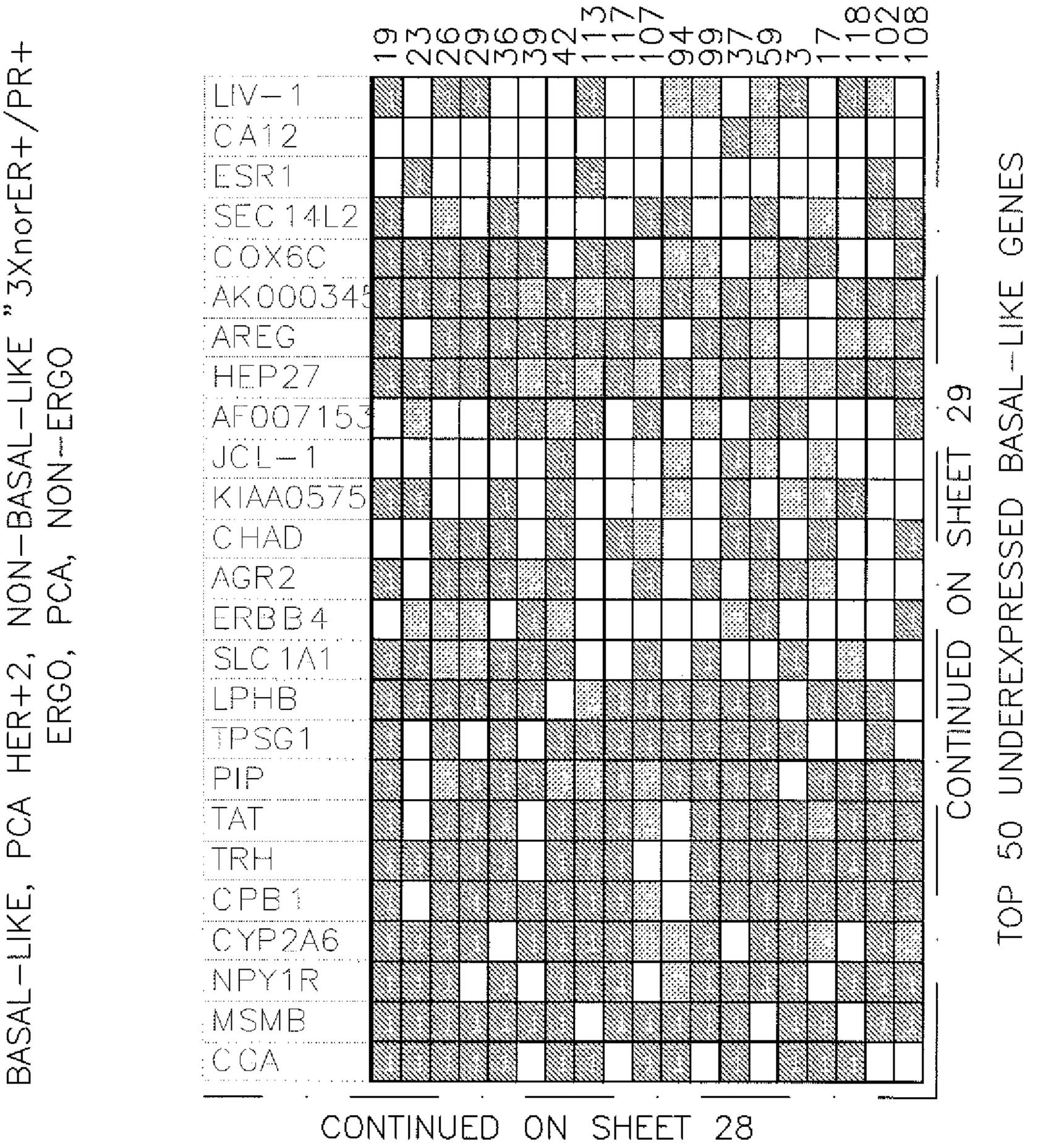

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
CONTINUED ON SHEET 29
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO
19 23 26 29 36 39 42 113 117 107 94 99 37 59 3 17 118 102 108
LIV-1
CA12
ESR1
SEC14L2
COX6C
AK000345
AREG
HEP27
AF007153
JCL-1
KIAA0575
CHAD
AGR2
ERBB4
SLC1A1
LPHB
TPSG1
PIP
TAT
TRH
CPB1
CYP2A6
NPY1R
MSMB
CCA
CONTINUED ON SHEET 28

FIG.1

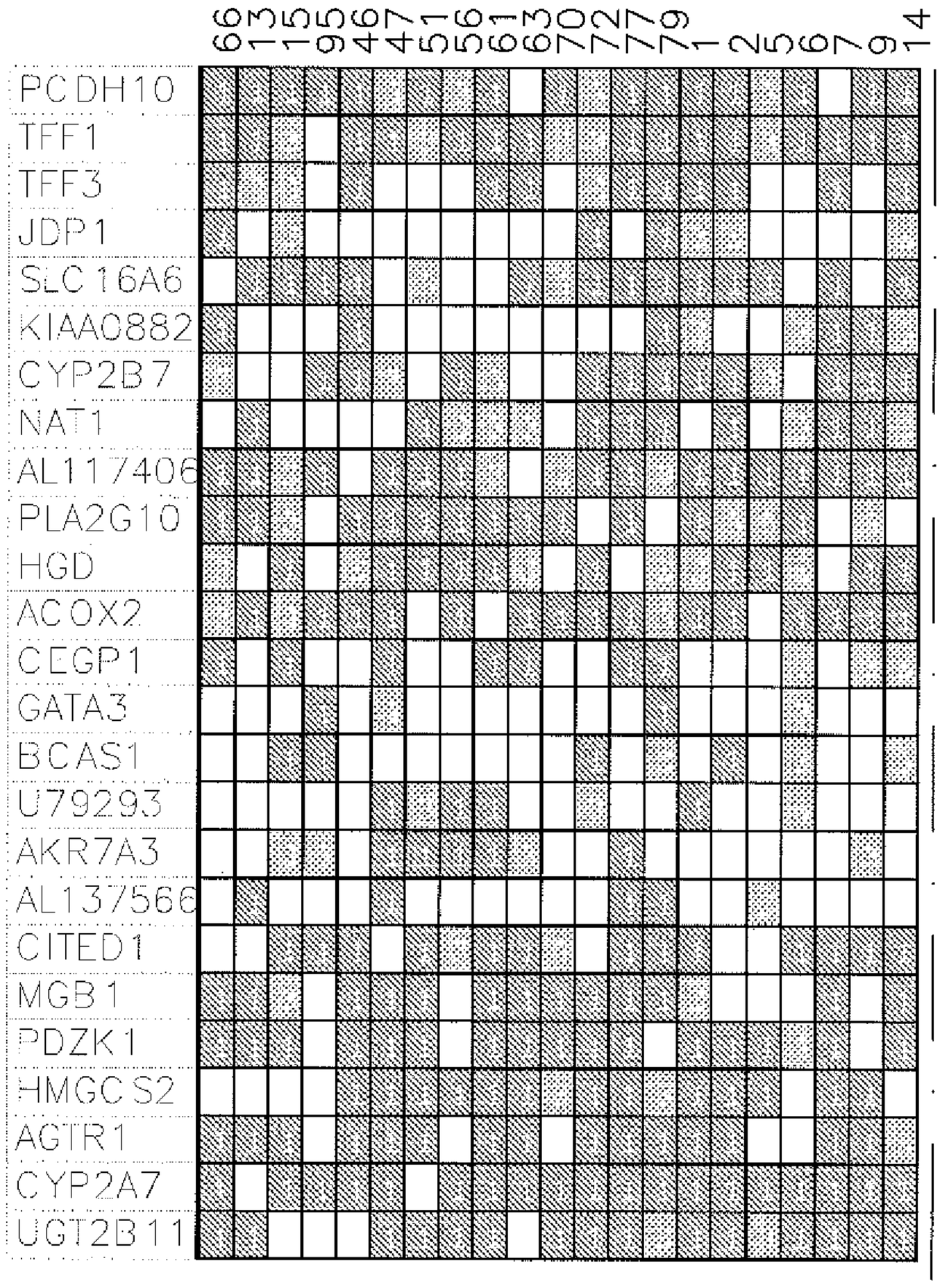

66
13
15
95
46
47
51
56
61
63
70
72
77
79
1
25
56
7
9
14
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AL137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UGT2B11
CONTINUED ON SHEET 28
TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO

FIG.1

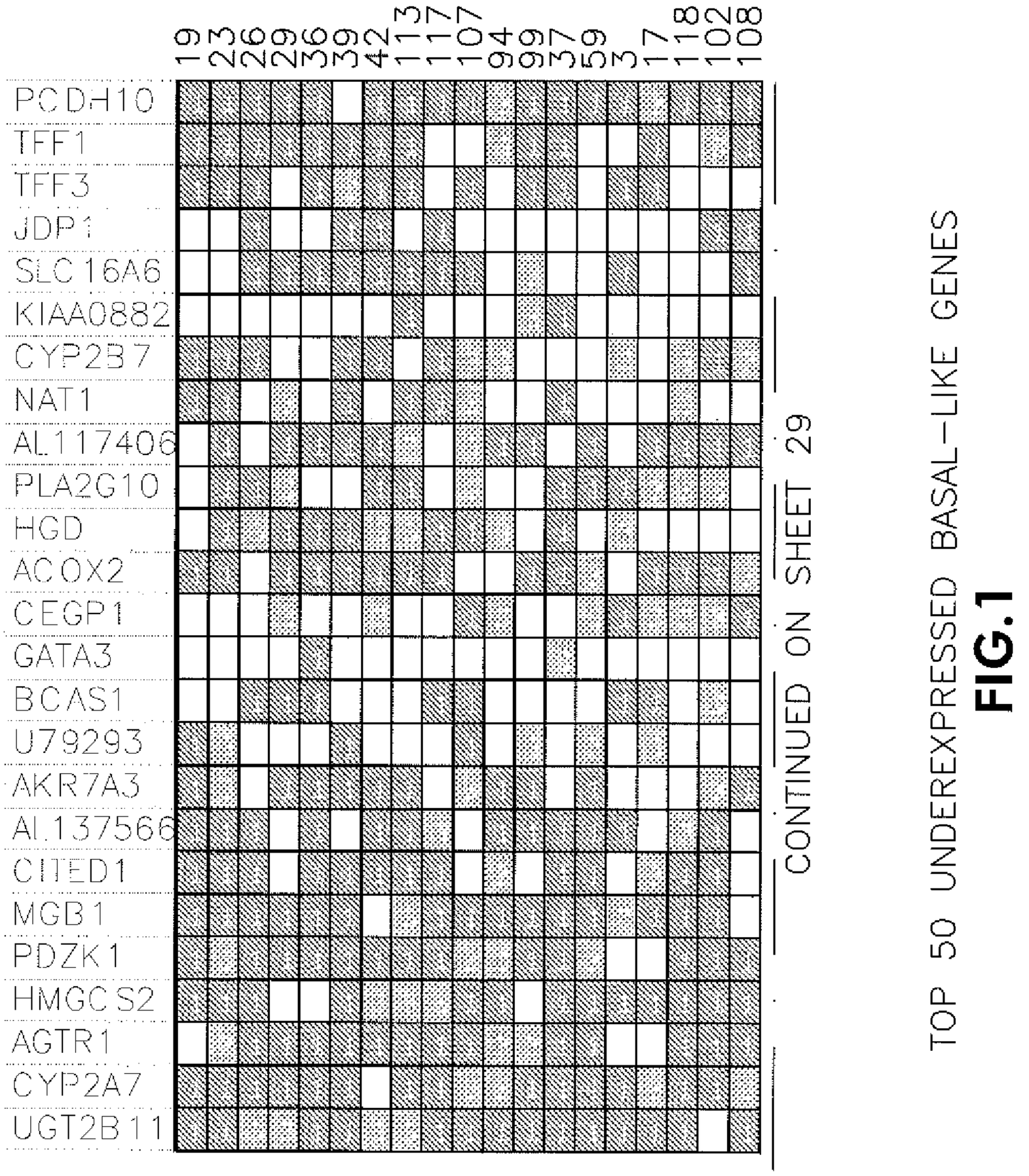

19 23 26 29 36 39 42 113 117 107 94 99 37 59 3 17 118 102 108
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AI.137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UGT2B11
CONTINUED ON SHEET 29
TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER+2, NON-BASAL-LIKE "3XnorER+/PR+
ERGO, PCA, NON-ERGO

FIG.1

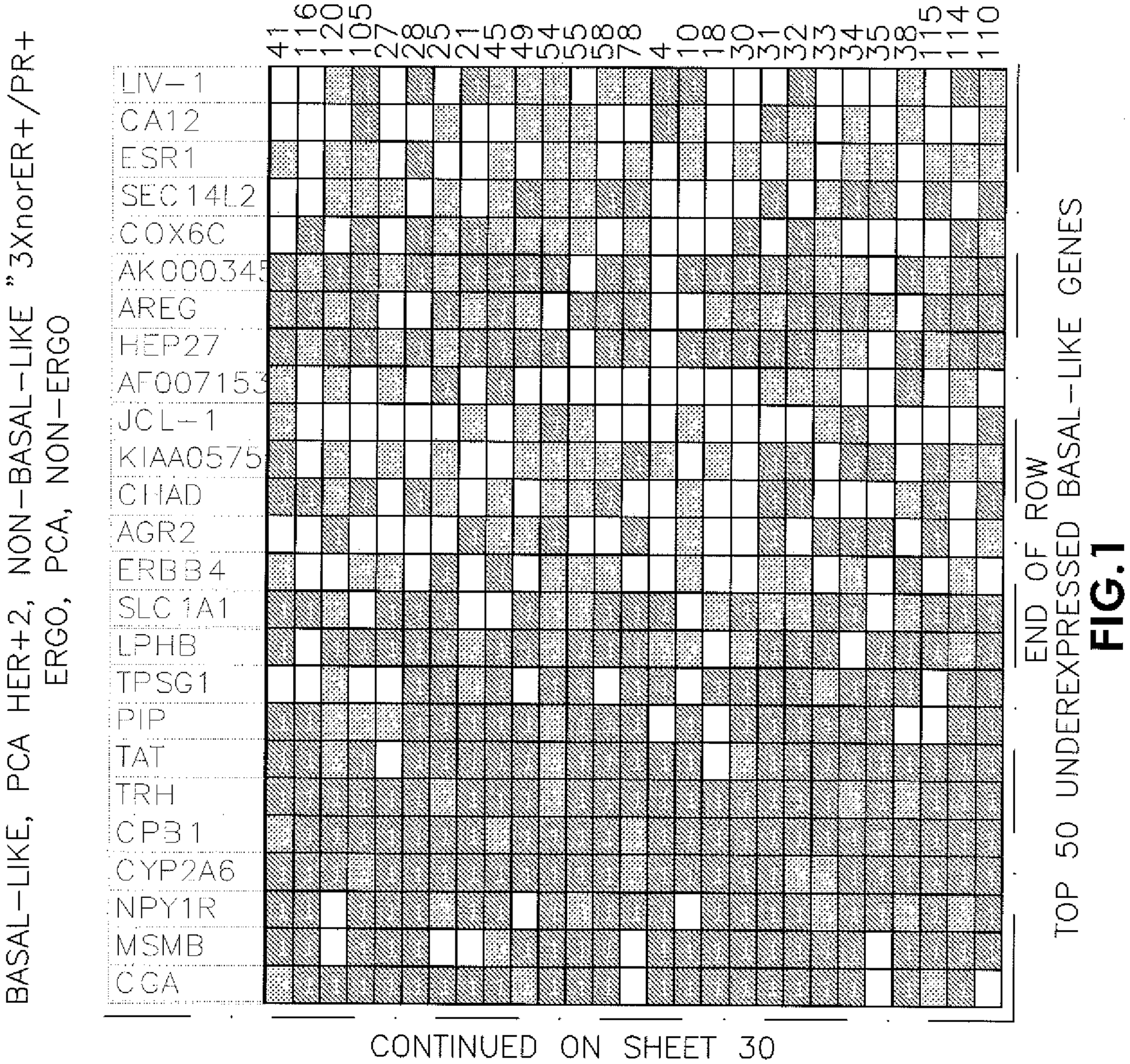


FIG.1

CONTINUED ON SHEET 30

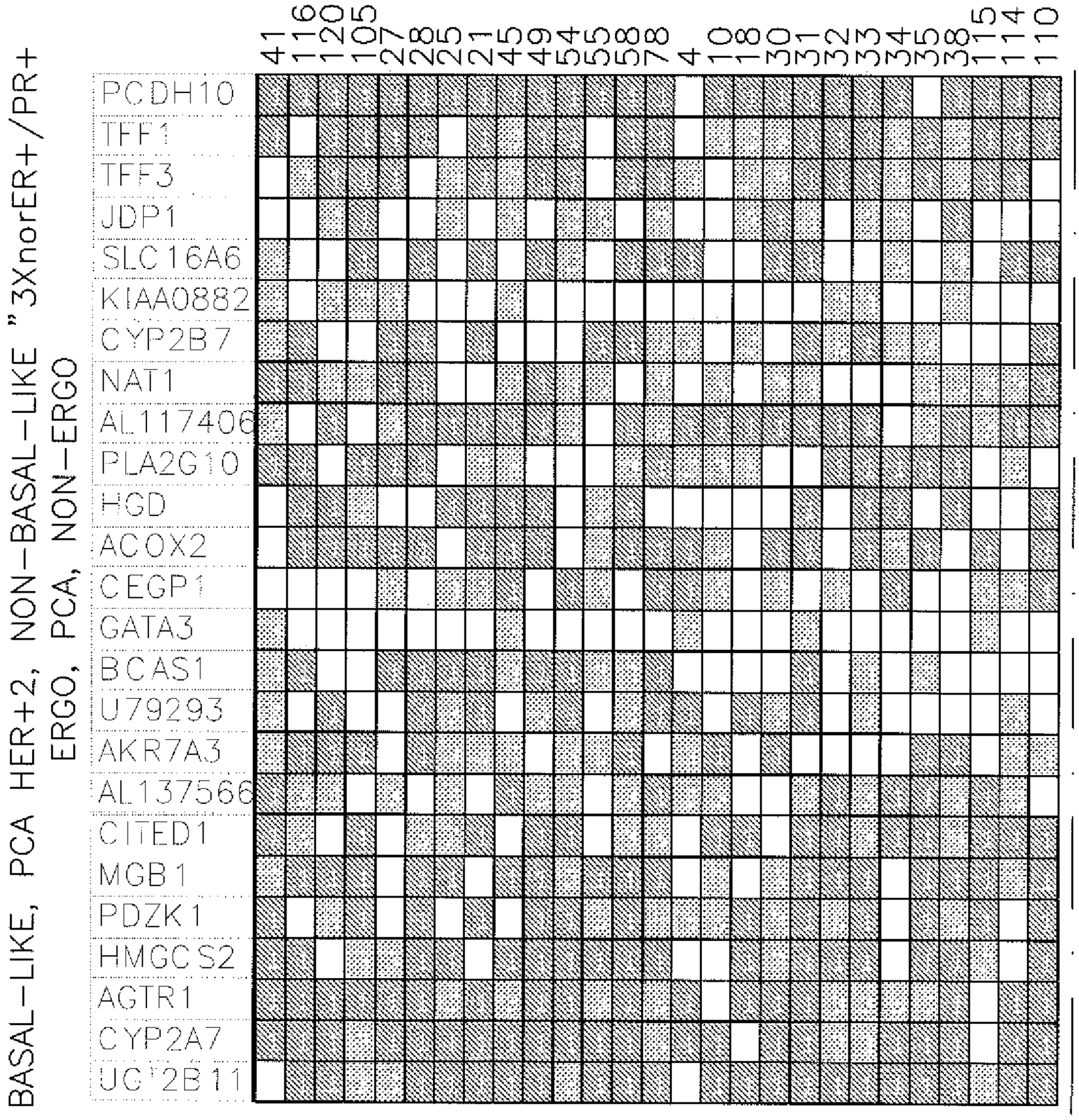

41 116 120 105 27 28 25 21 45 49 54 55 58 78 4 10 18 30 31 32 33 34 35 38 115 114 110
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AL137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UC 2B11
BASAL-LIKE, PCA HER+2, NON-ERGO, PCA, NON-ERGO
BASAL-LIKE "3XnorER+/PR+
END OF ROW
TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG.1

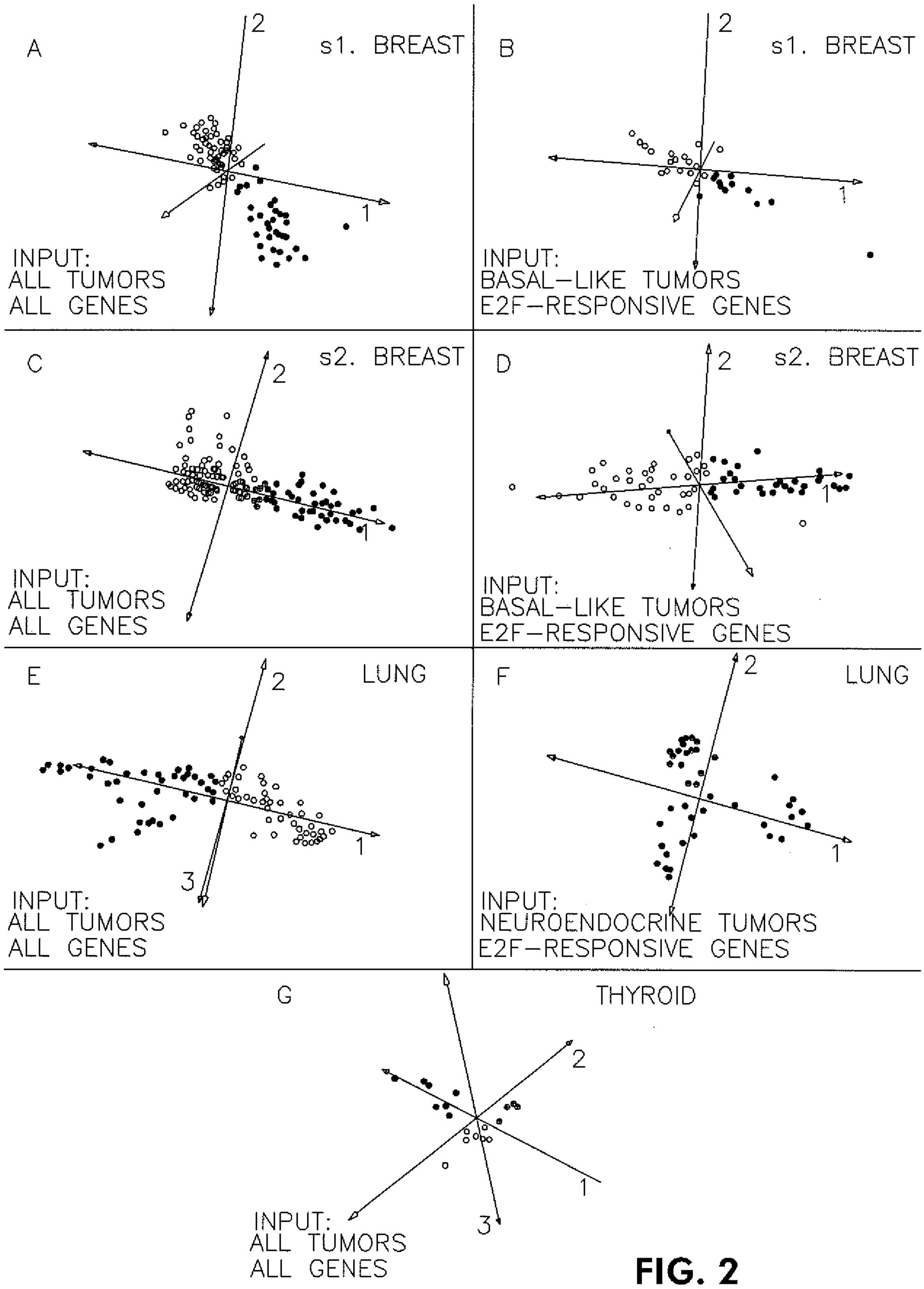

A
s1. BREAST
2
1
INPUT:
ALL TUMORS
ALL GENES
B
s1. BREAST
2
1
INPUT:
BASAL-LIKE TUMORS
E2F-RESPONSIVE GENES
C
s2. BREAST
2
1
INPUT:
ALL TUMORS
ALL GENES
D
s2. BREAST
2
1
INPUT:
BASAL-LIKE TUMORS
E2F-RESPONSIVE GENES
E
LUNG
2
1
3
INPUT:
ALL TUMORS
ALL GENES
F
LUNG
2
1
INPUT:
NEUROENDOCRINE TUMORS
E2F-RESPONSIVE GENES
G
THYROID
2
1
3
INPUT:
ALL TUMORS
ALL GENES

FIG. 2

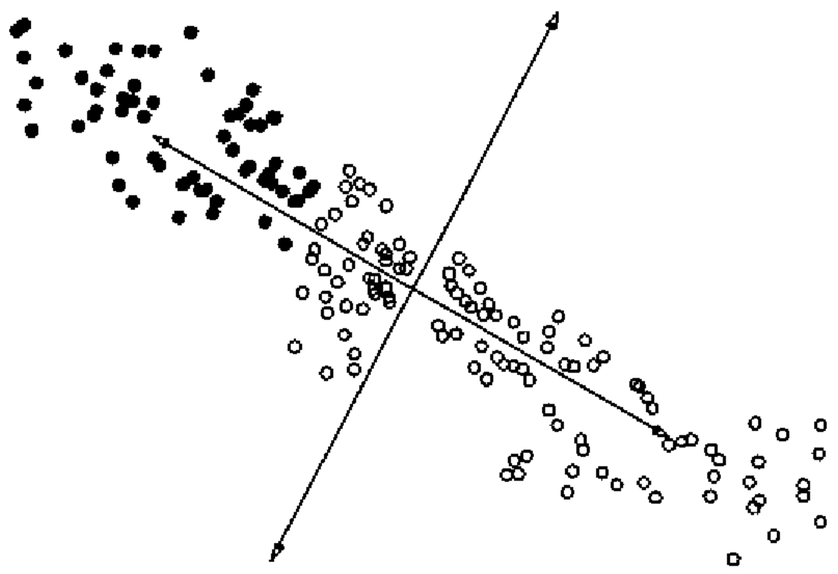


FIG. 3A

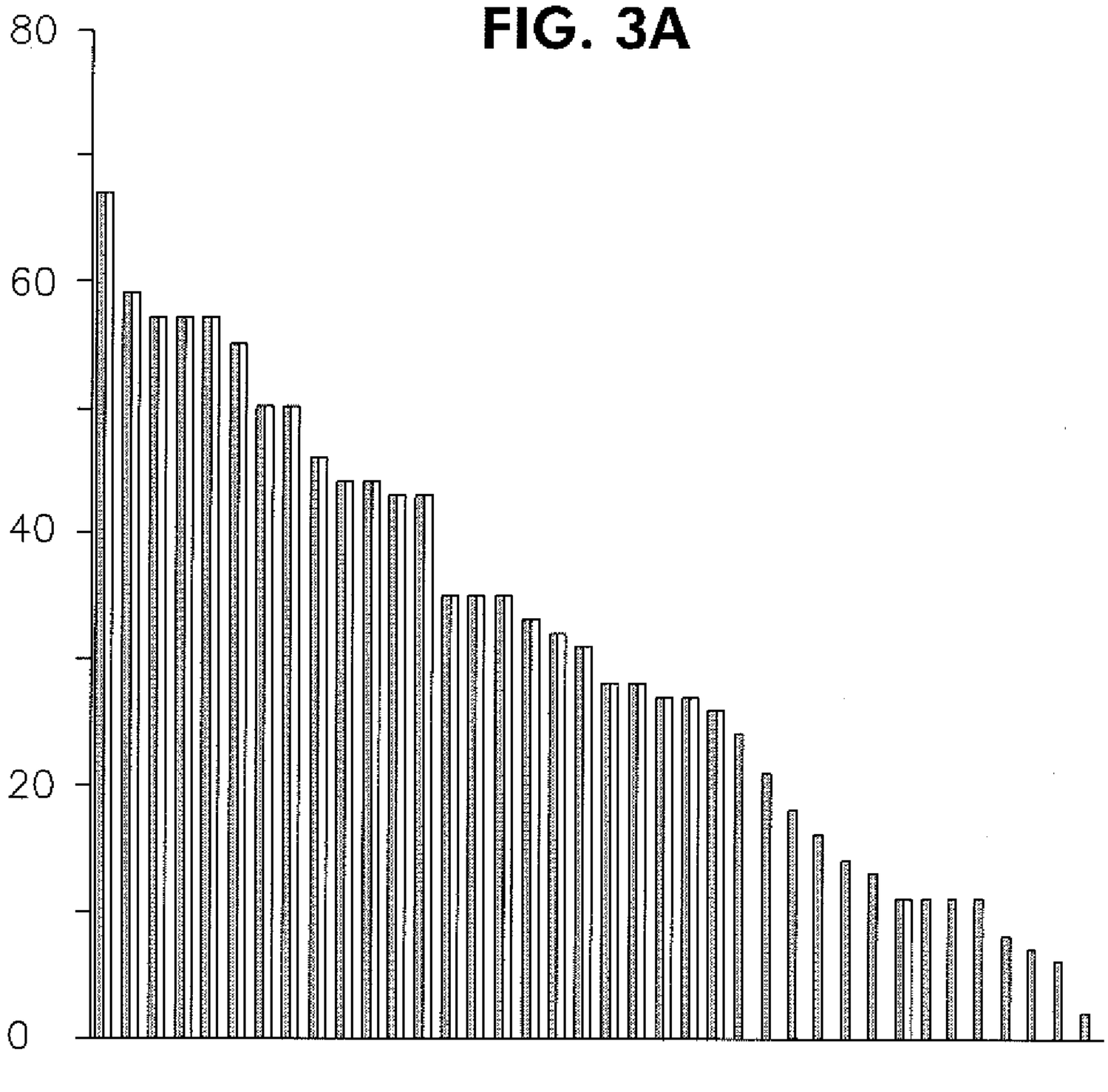

80
60
40
20
0
SAMPLES
ALL PCA BASAL-LIKE TUMORS
PCA ERGO TUMORS ONLY

FIG. 3C

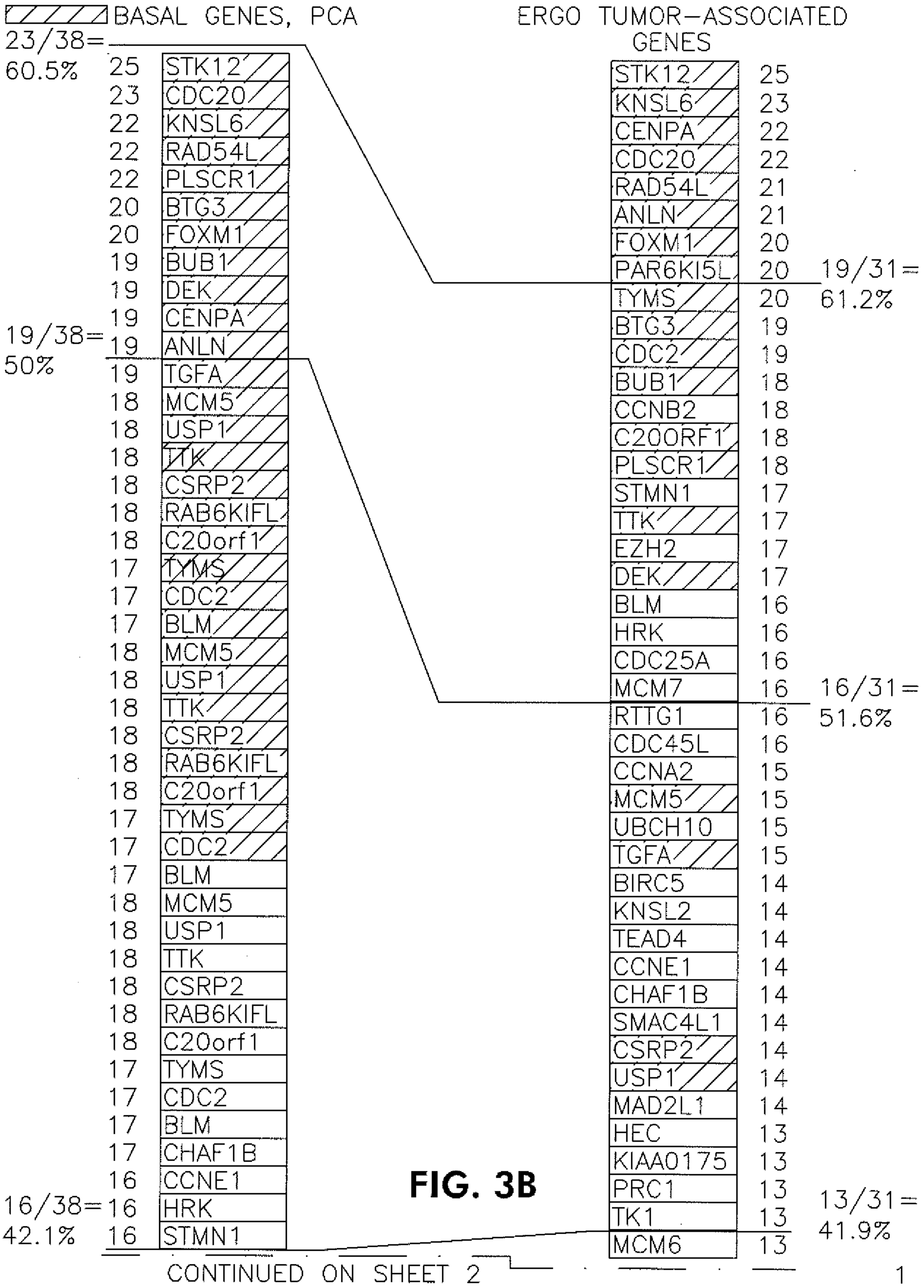

BASAL GENES, PCA
23/38=
60.5%
25 STK12
23 CDC20
22 KNSL6
22 RAD54L
22 PLSCR1
20 BTG3
20 FOXM1
19 BUB1
19 DEK
19 CENPA
19/38=
19 ANLN
50%
19 TGFA
18 MCM5
18 USP1
18 TTK
18 CSRP2
18 RAB6KIFL
18 C20orf1
17 TYMS
17 CDC2
17 BLM
18 MCM5
18 USP1
18 TTK
18 CSRP2
18 RAB6KIFL
18 C20orf1
17 TYMS
17 CDC2
17 BLM
17 CHAF1B
16 CCNE1
16/38= 16 HRK
42.1% 16 STMN1
ERGO TUMOR-ASSOCIATED GENES
STK12 25
KNSL6 23
CENPA 22
CDC20 22
RAD54L 21
ANLN 21
FOXM1 20
PAR6KI5L 20
19/31=
TYMS 20
61.2%
BTG3 19
CDC2 19
BUB1 18
CCNB2 18
C20ORF1 18
PLSCR1 18
STMN1 17
TTK 17
EZH2 17
DEK 17
BLM 16
HRK 16
CDC25A 16
MCM7 16
16/31=
RTTG1 16
51.6%
CDC45L 16
CCNA2 15
MCM5 15
UBCH10 15
TGFA 15
BIRC5 14
KNSL2 14
TEAD4 14
CCNE1 14
CHAF1B 14
SMAC4L1 14
CSRP2 14
USP1 14
MAD2L1 14
HEC 13
KIAA0175 13
PRC1 13
13/31=
TK1 13
41.9%
MCM6 13
CONTINUED ON SHEET 2

FIG. 3B

16	TP533BP2
15	MCM7
15	CCNA2
15	CCNB2
15	EZH2
15	TEAD4
14	KNSL2
14	PTTG1
14	CDC25A
14	FDJ22009
14	SMC4L1
14	MAP3K14
13	TK1
13	KIAA0175
13	CDC45L
13	MCM2
13	SOX9
13	VEGF
13	UBCH10
13	KNSL1
12	HEC
12	RFC4
12	RFC4
12	MCM6
12	ORC6L
12	PRIM2A
12	BIRC5
12	CSDA
11	MYC
11	KPNA2
11	MAD2L1
11	DNA2L
11	CKS1
11	CDC7L1
11	RTRSN1
11	ECT2
10	NUP155
10	PRC1
10	NASP
10	NEK2
9	PIR51

13/38= 34%

11/38= 28.9%

KNSL1	13
LMNB1	13
ORC6L	12
TP53BP2	12
NEK2	12
CCNE2	12
RFC4	11
MCM2	11
STK15	11
DNA2L	11
SOX9	11
MYC	11
PRIM2A	10
CKS1	10
NUP155	10
NASP	10
CSDA	10
KPNA2	10
CDC25B	10
MAP3K14	10
NOLC1	9
CKS2	9
FLJ22009	9
CCNB1	9
RRM2	9
ANXA8	9
KIF4A	8

11/31= 35.4%

9/31= 29%

CONTINUED ON SHEET 3

FIG. 3B

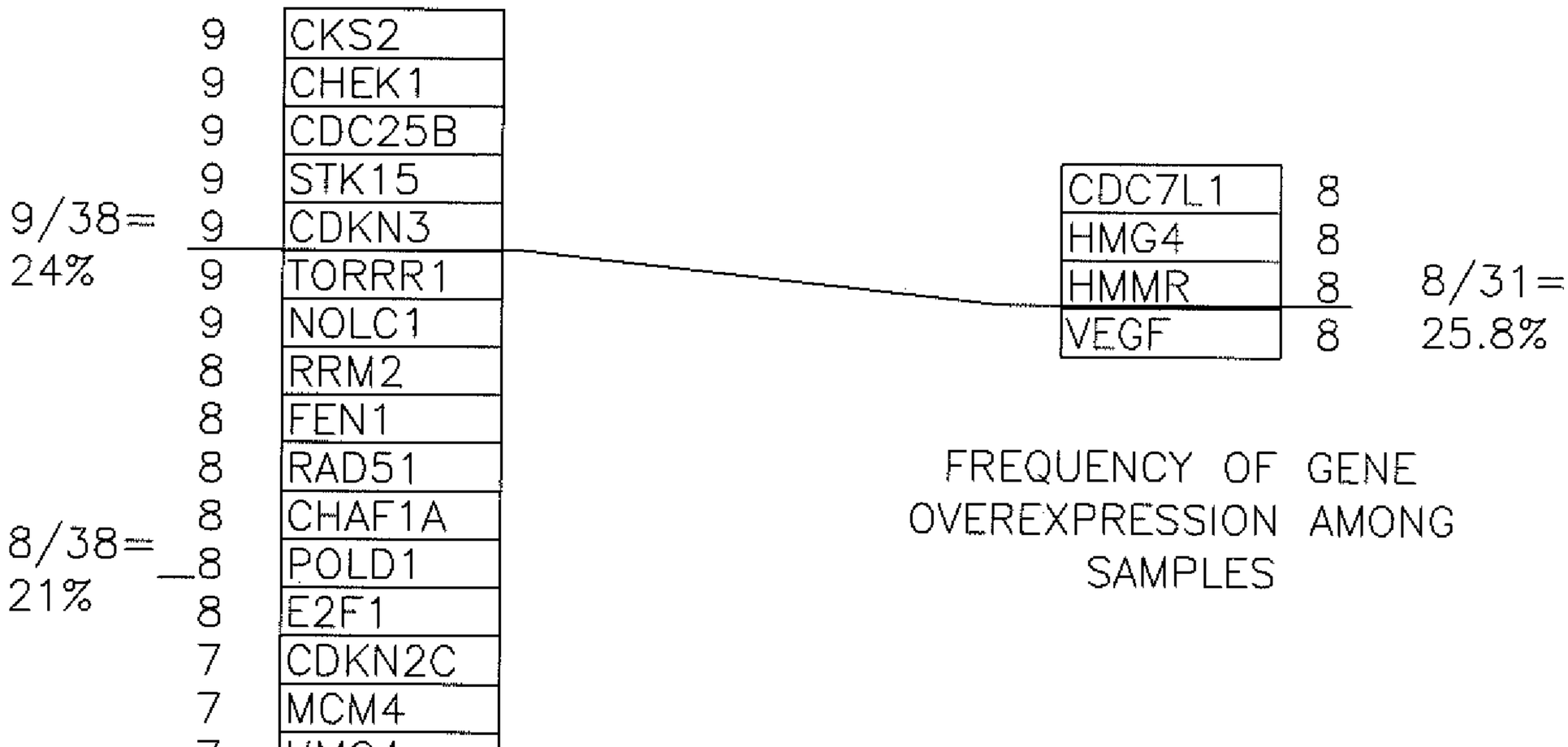
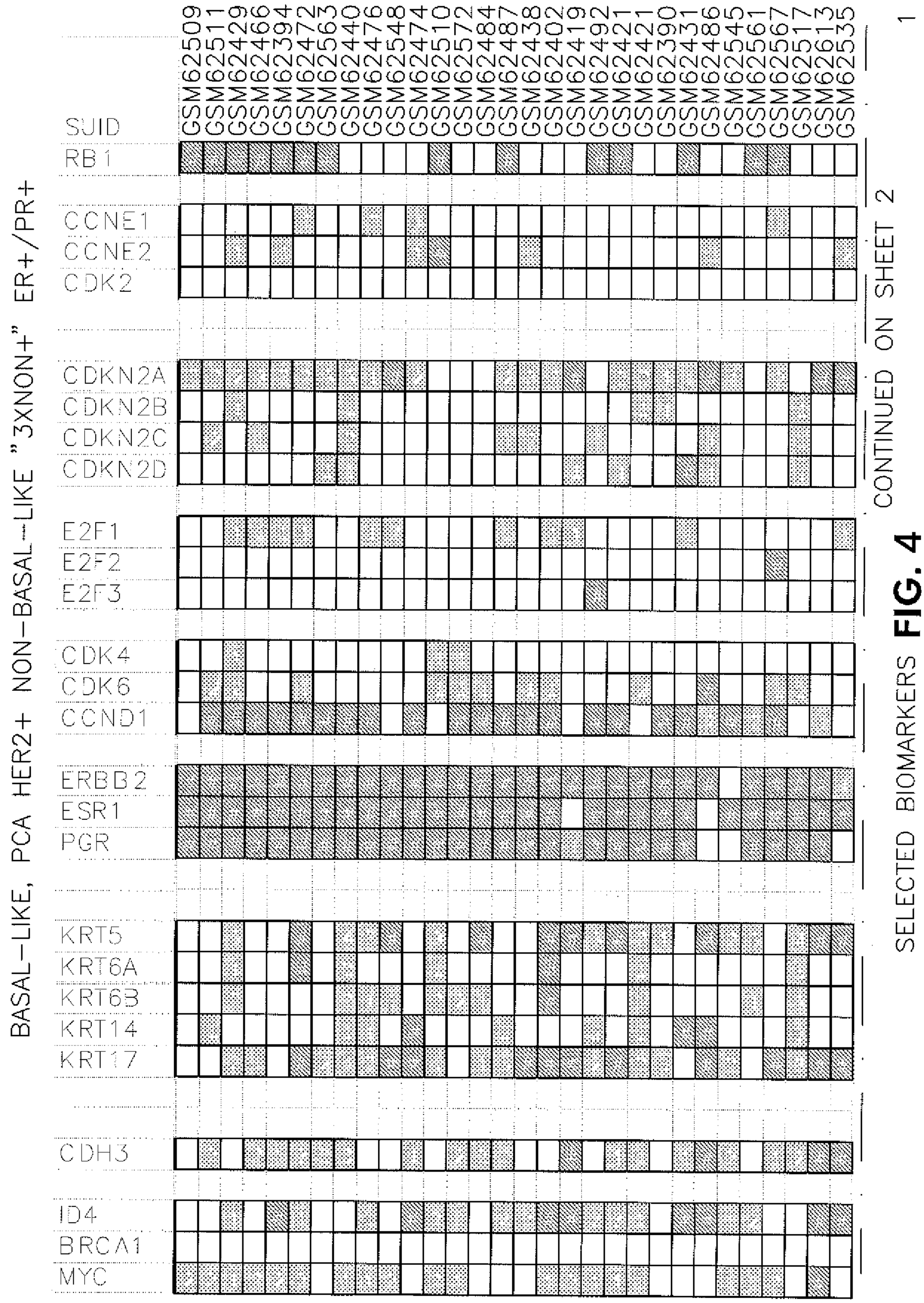

9/38= 24%
8/38= 21%
7/38= 18%
5/38= 13%
9 CKS2
9 CHEK1
9 CDC25B
9 STK15
9 CDKN3
9 TORRR1
9 NOLC1
8 RRM2
8 FEN1
8 RAD51
8 CHAF1A
8 POLD1
8 E2F1
7 CDKN2C
7 MCM4
7 HMG4
7 FIGNL1
7 MSH2
7 BUB1B
7 KIF4A
7 BM037
6 MCM3
6 TANK
5 FLJ10335
5 DNMT1
5 STK18
5 ID-GAP
5 CDC25C
5 BID
FREQUENCY OF GENE OVEREXPRESSION AMONG SAMPLES
CDC7L1 8
HMG4 8
HMMR 8
VEGF 8
8/31= 25.8%
FREQUENCY OF GENE OVEREXPRESSION AMONG SAMPLES

FIG. 3B



SELECTED BIOMARKERS **FIG. 4**

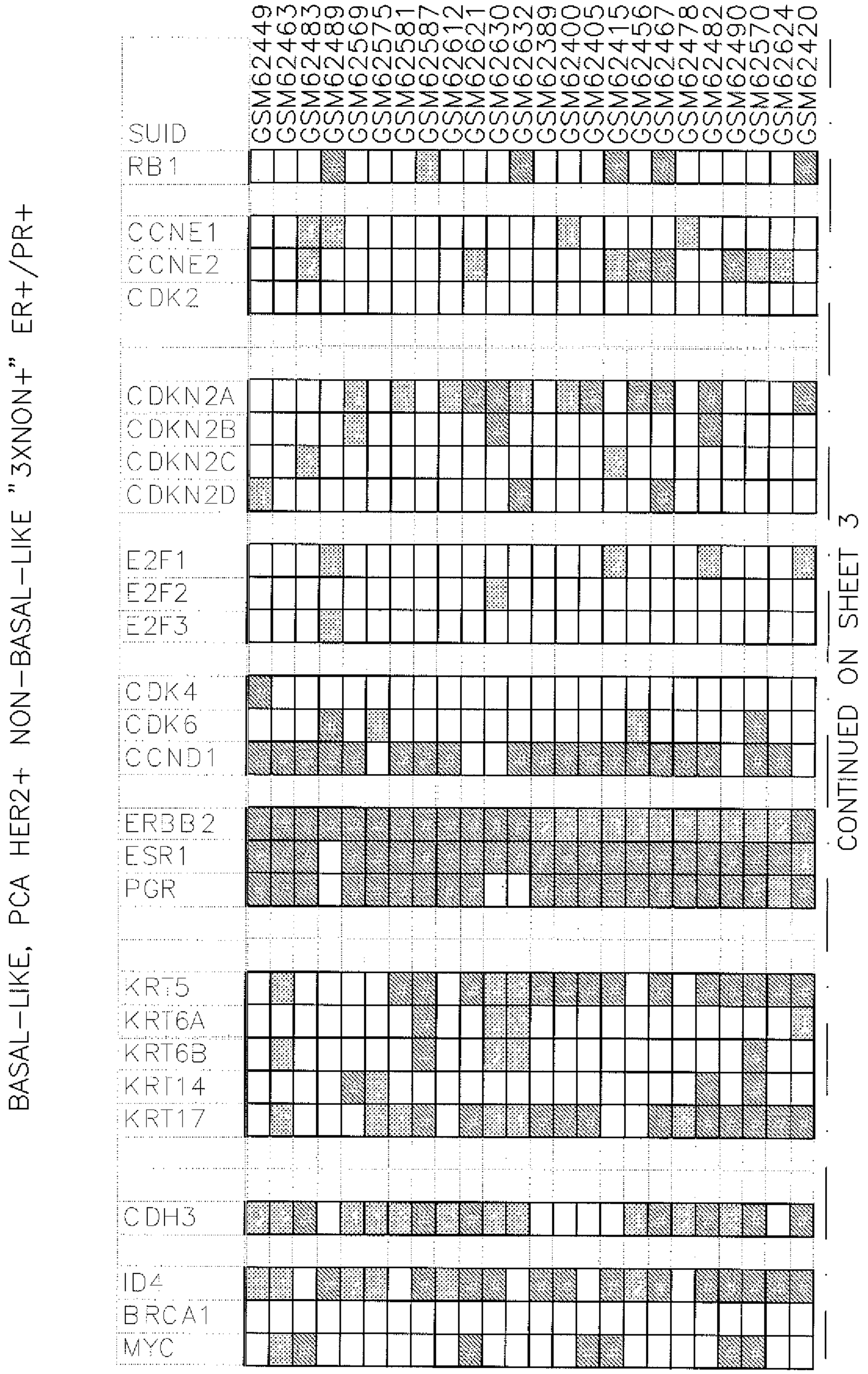
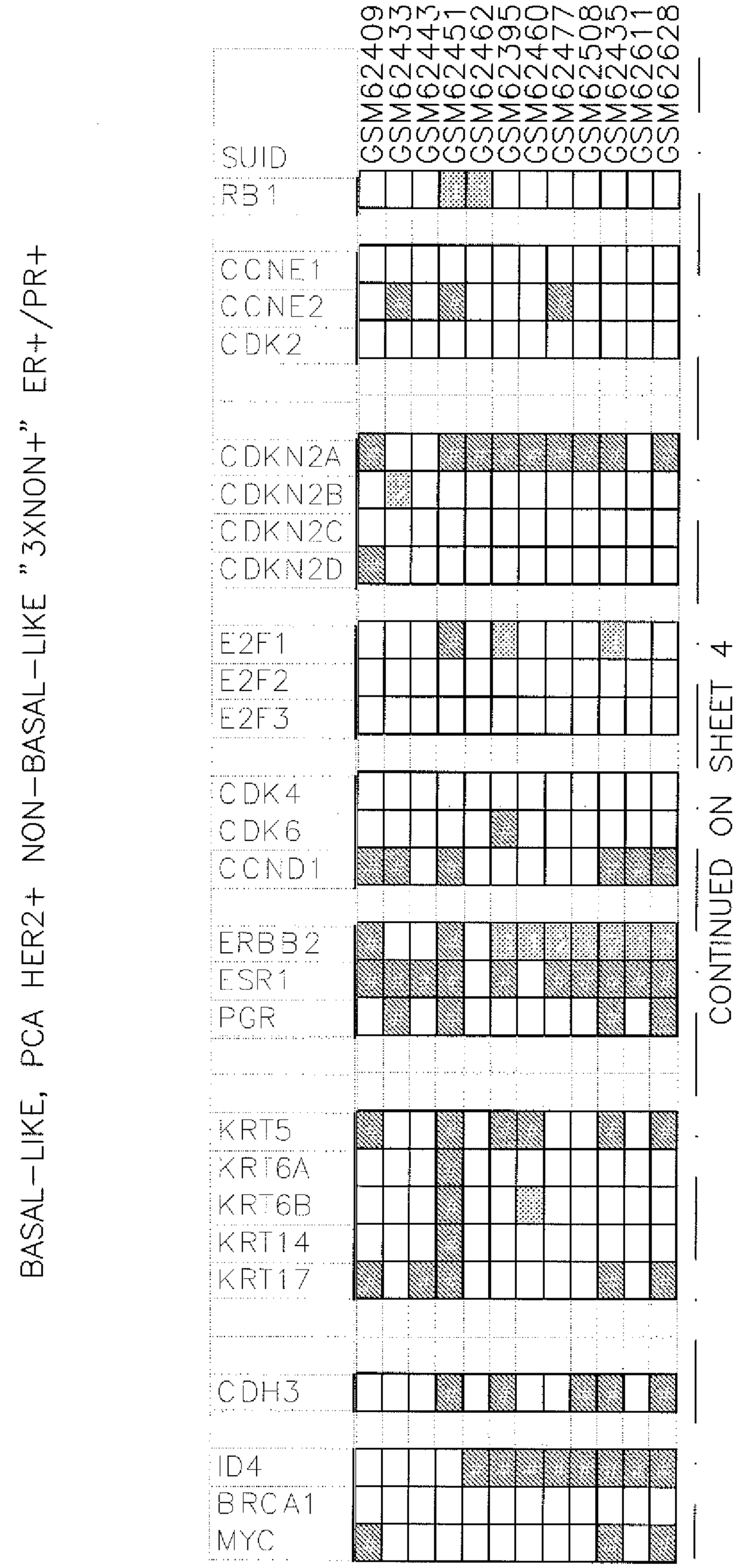


FIG. 4

SELECTED BIOMARKERS



SELECTED BIOMARKERS **FIG. 4**

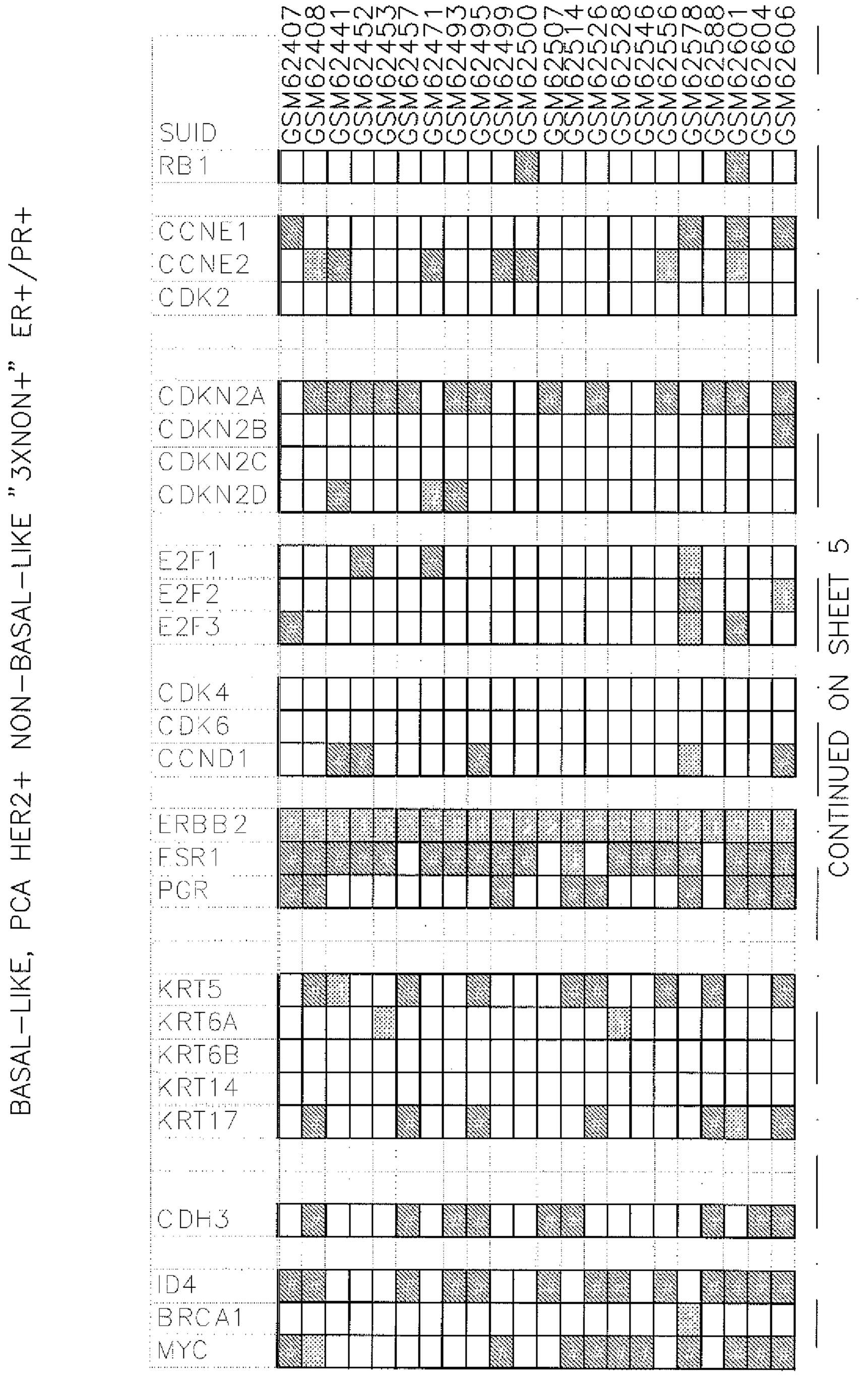


FIG. 4

SELECTED BIOMARKERS

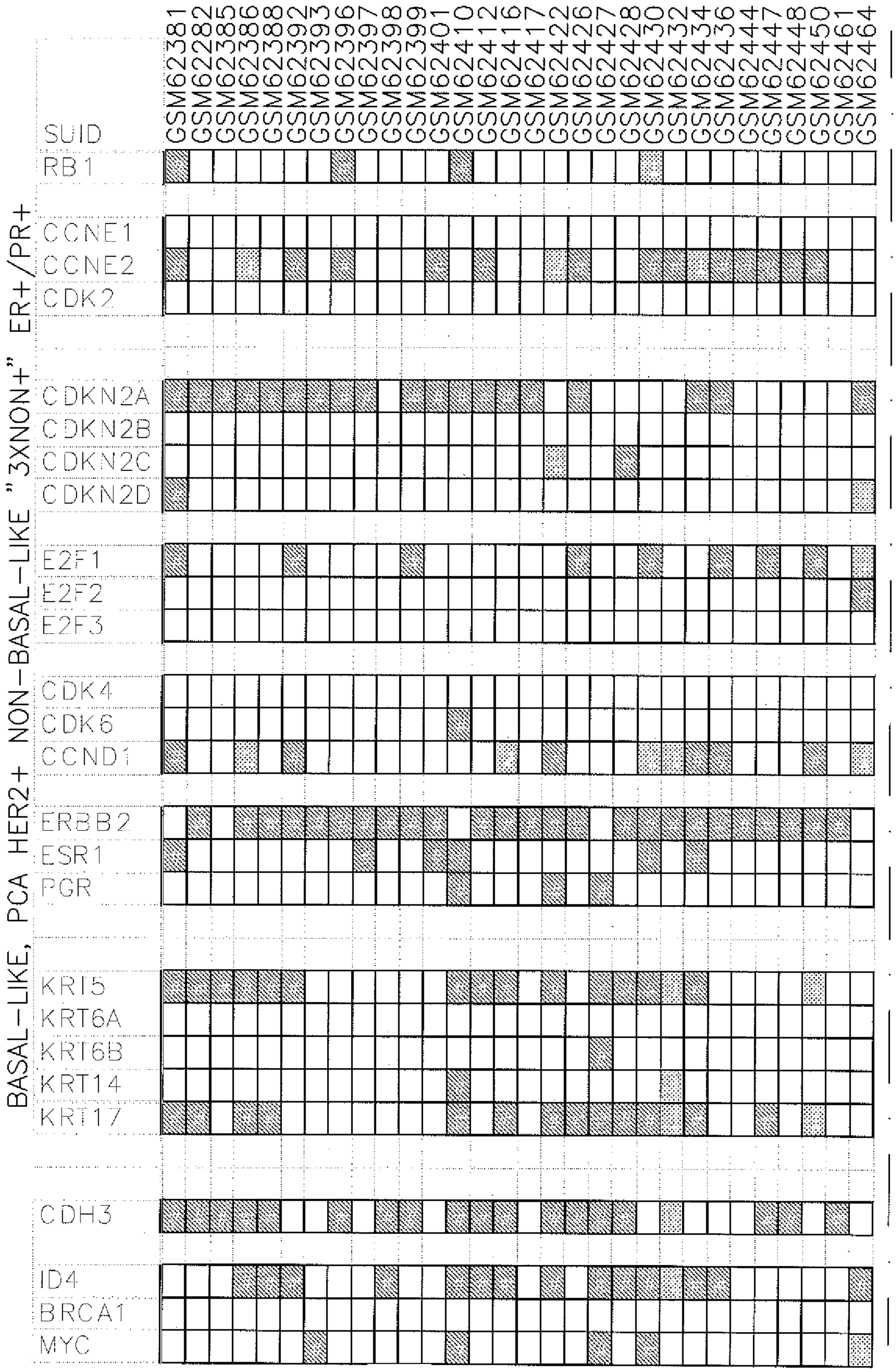
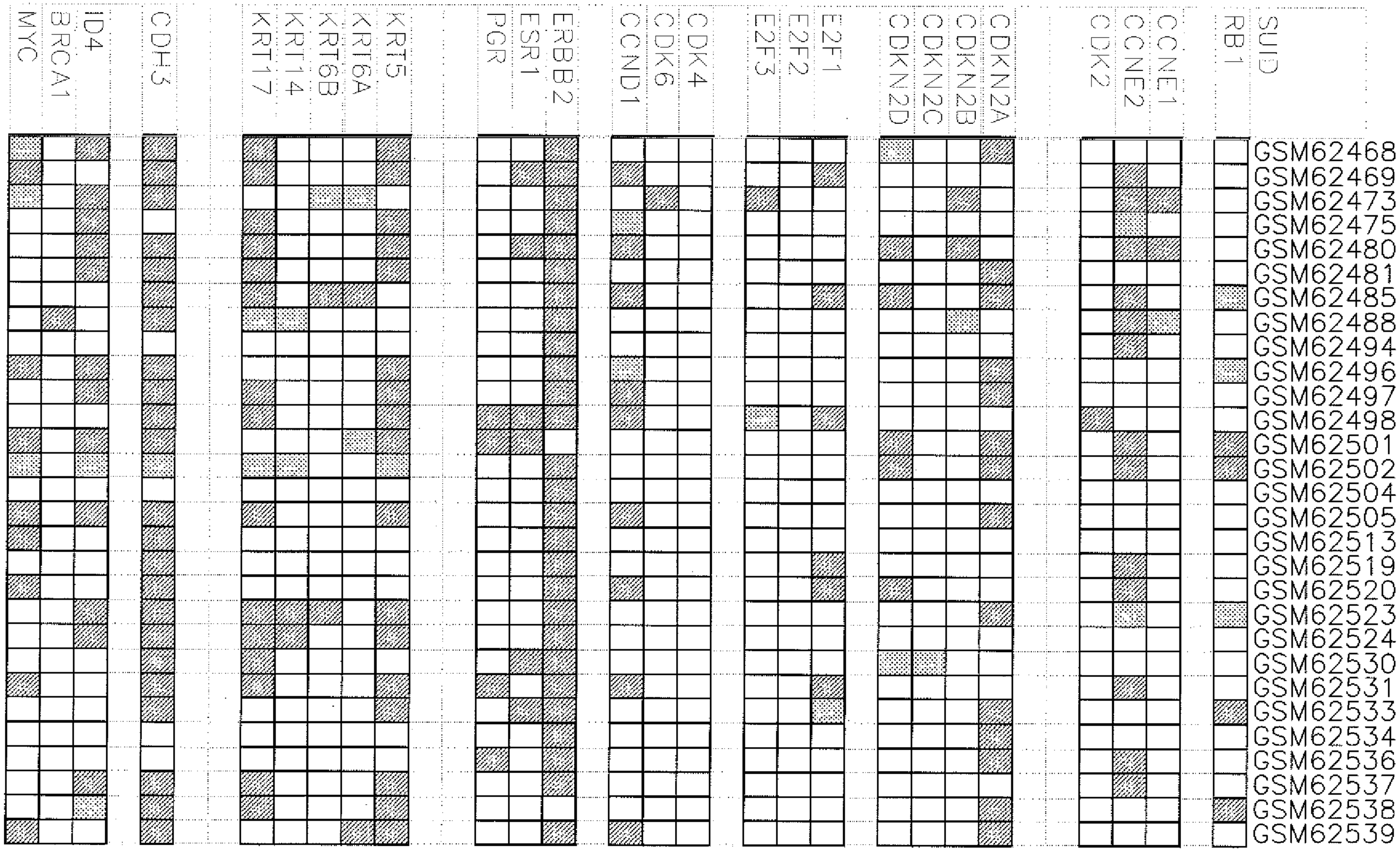


FIG. 4

SELECTED BIOMARKERS

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+



CONTINUED ON SHEET 7

SELECTED BIOMARKERS **FIG. 4**

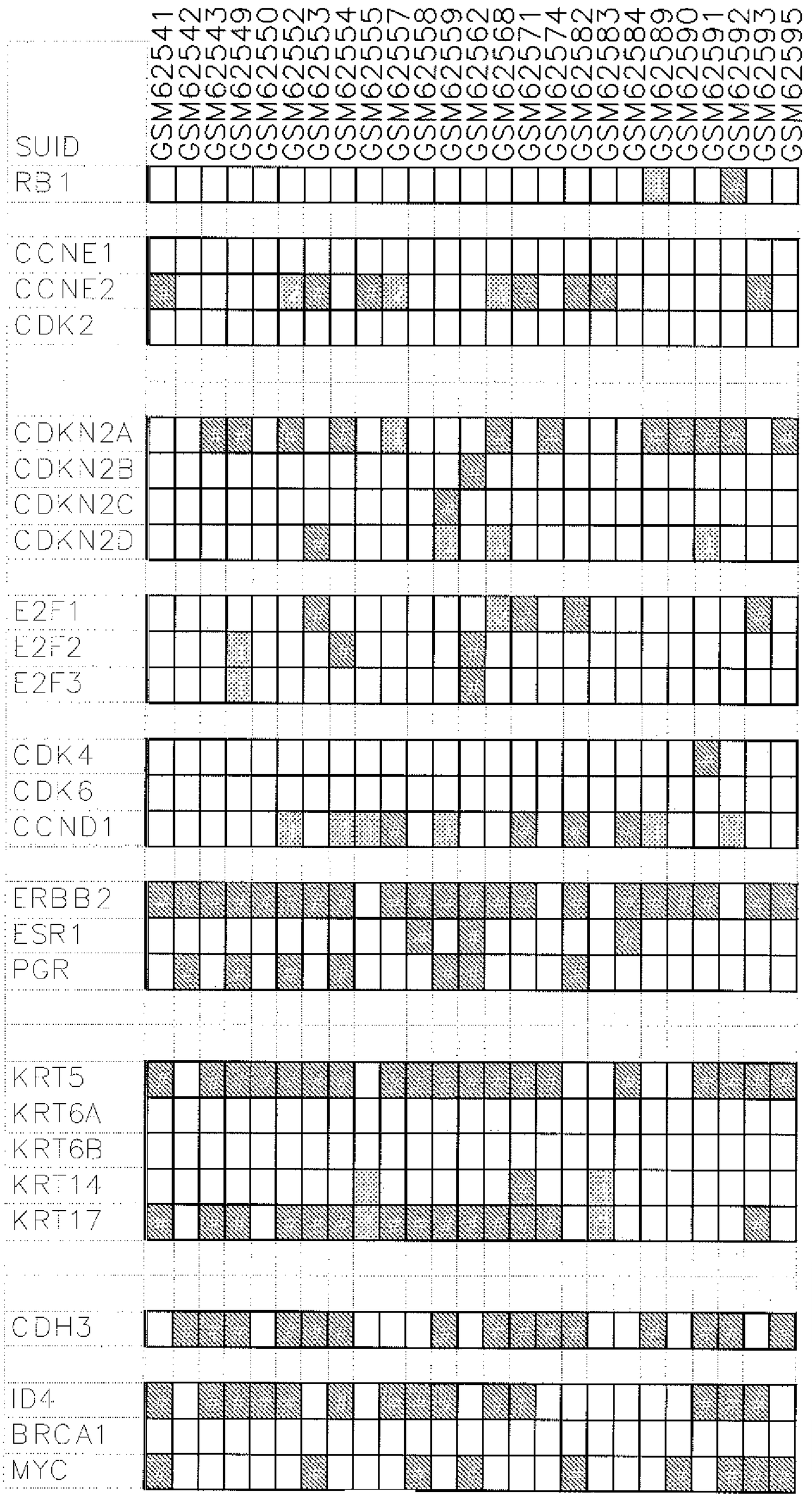


FIG. 4

SELECTED BIOMARKERS

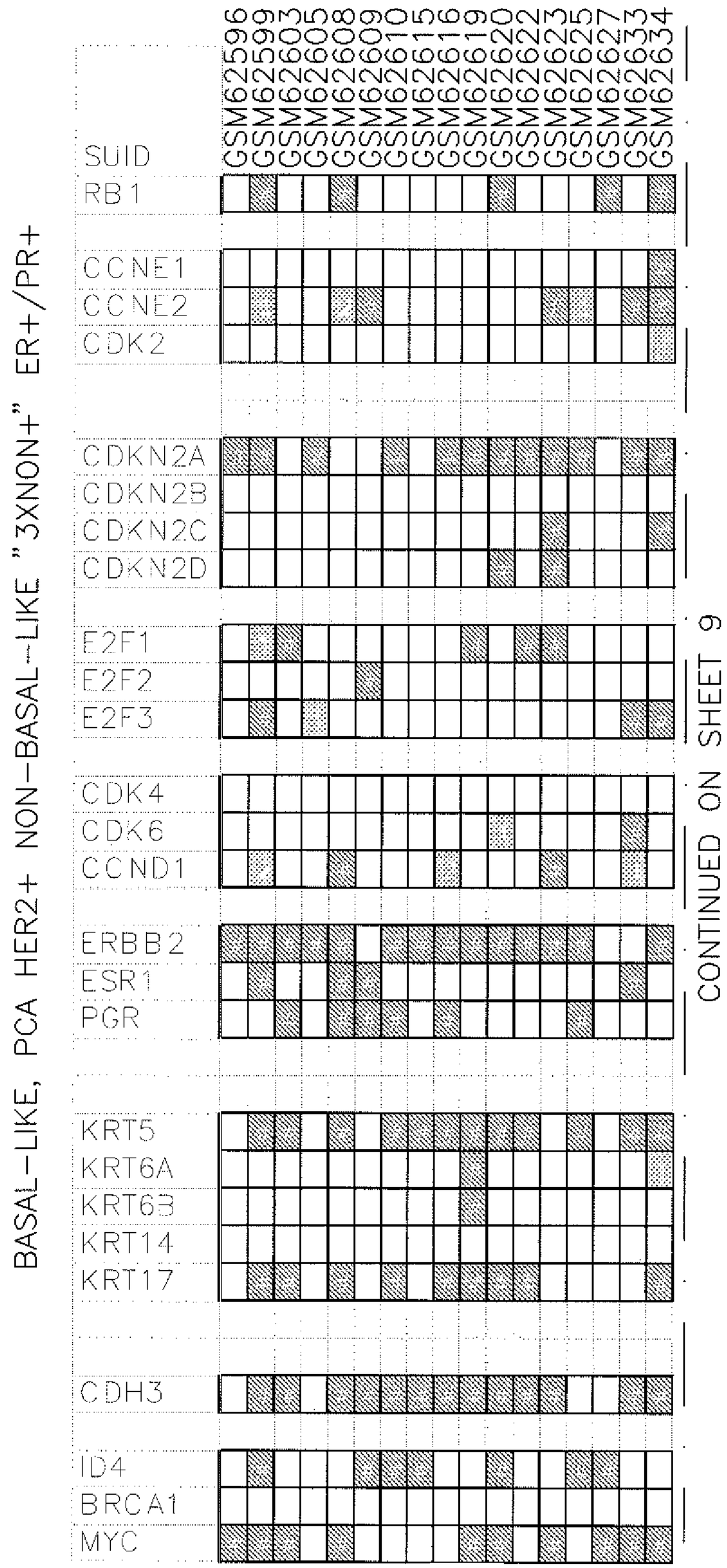


FIG. 4

SELECTED BIOMARKERS

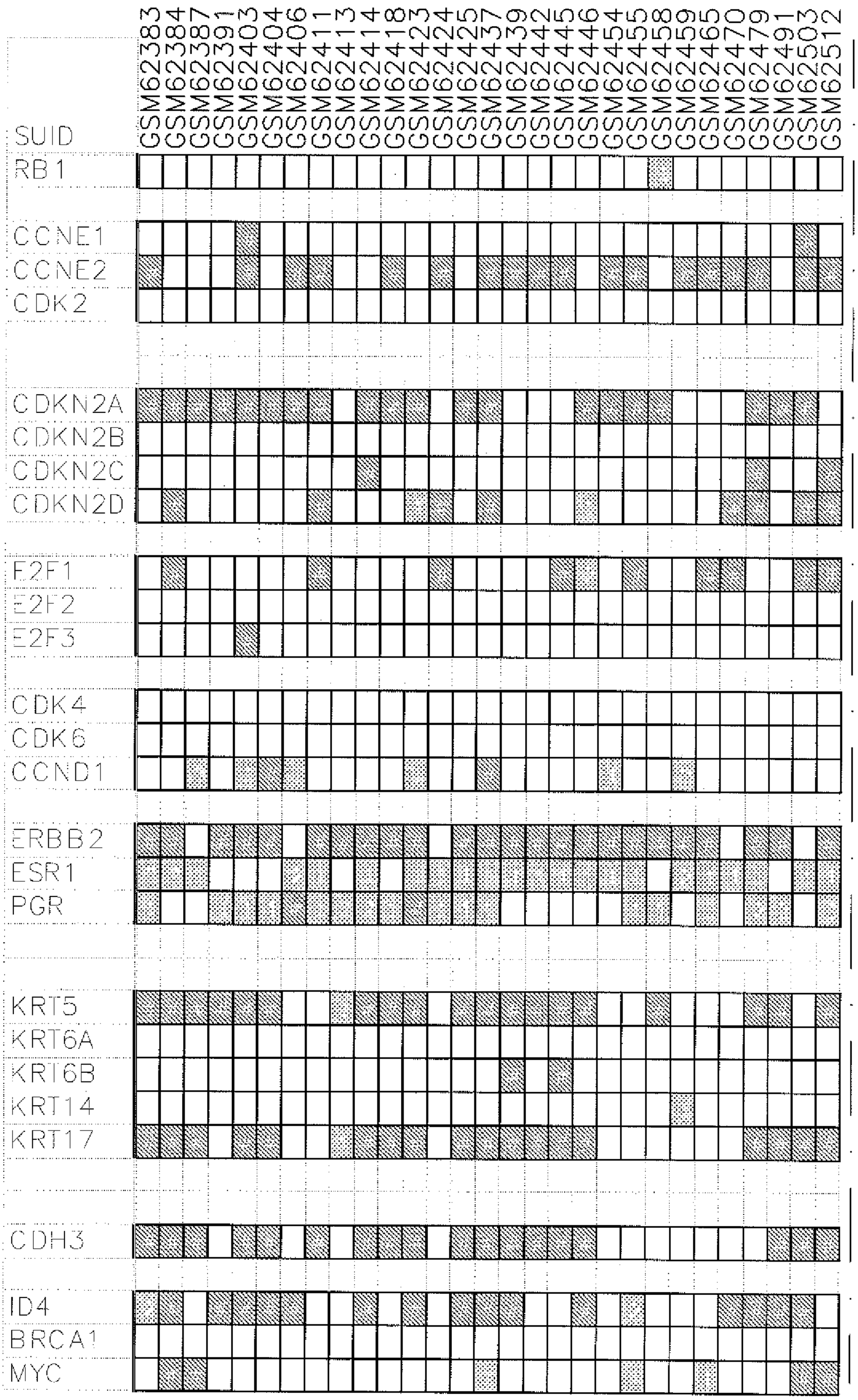

SUID
GSM623833
GSM623834
GSM623837
GSM623891
GSM624003
GSM624004
GSM624006
GSM624111
GSM624113
GSM624148
GSM624234
GSM624257
GSM624337
GSM624339
GSM624425
GSM624445
GSM624446
GSM624454
GSM624455
GSM624458
GSM624459
GSM624465
GSM624470
GSM624479
GSM624491
GSM624503
GSM625512
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
SELECTED BIOMARKERS
CONTINUED ON SHEET 10

FIG. 4

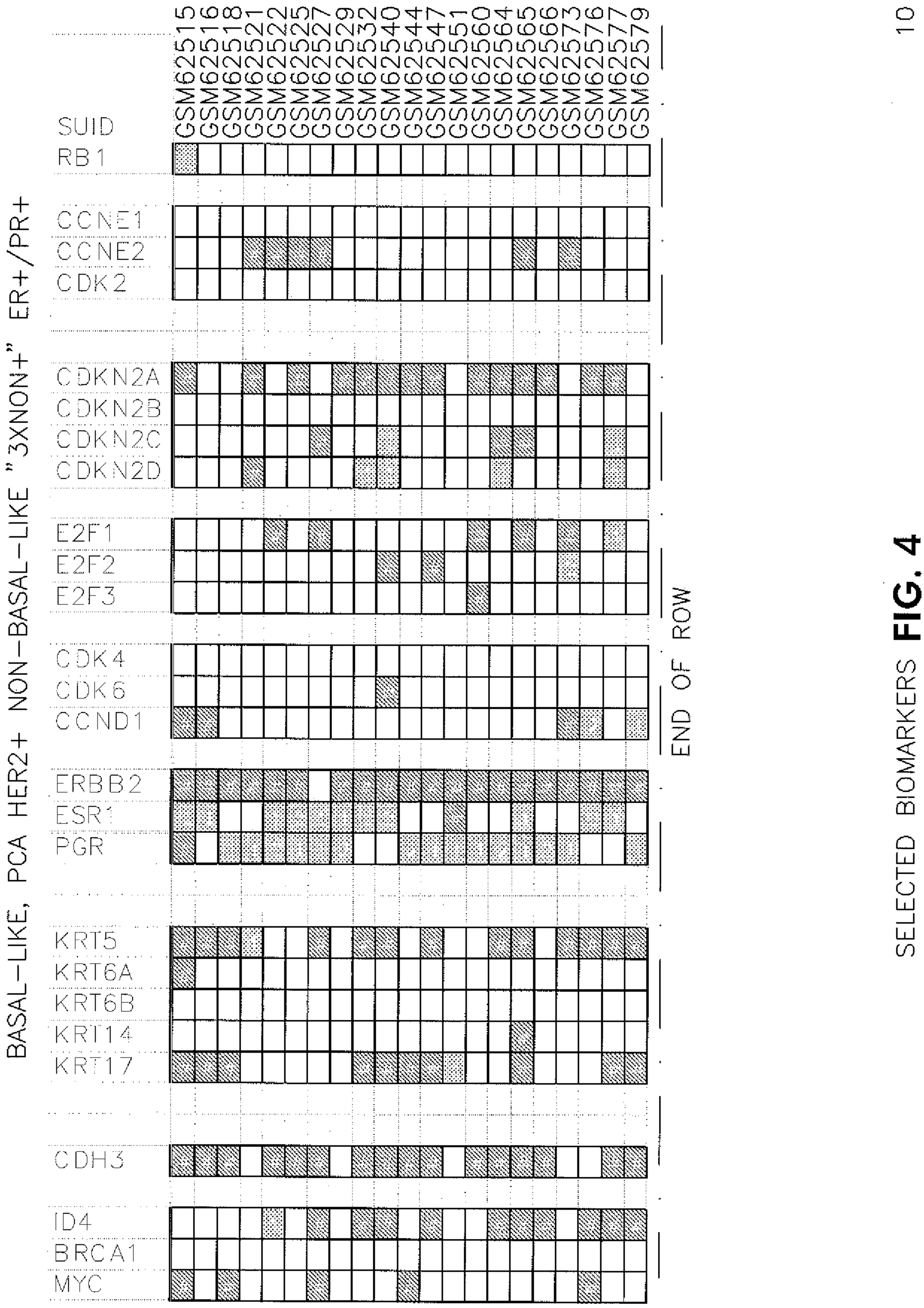
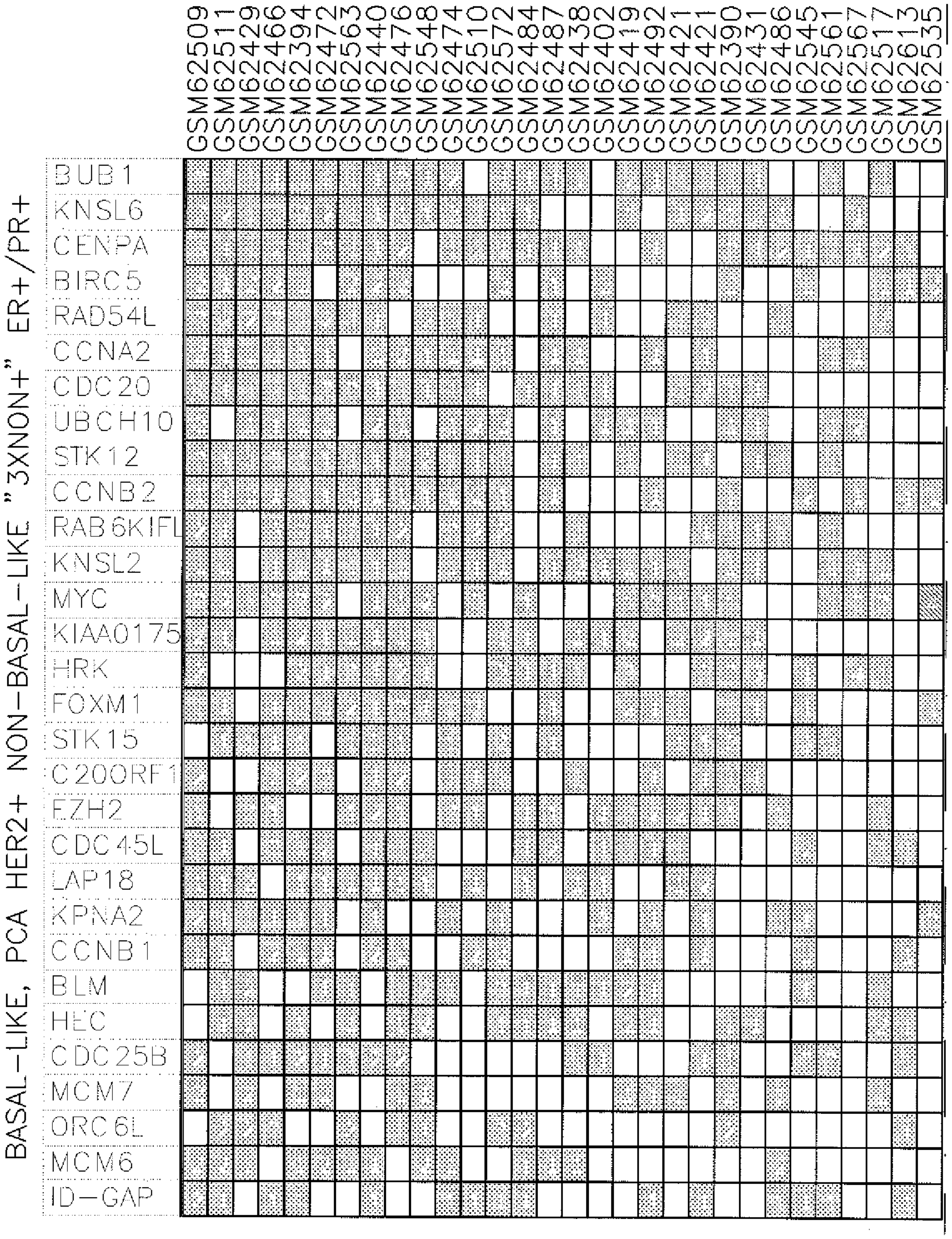


FIG. 4

SELECTED BIOMARKERS



CONTINUED ON SHEET 12

FIG. 4

TOP 30 OVEREXPRESSED ERGO GENES

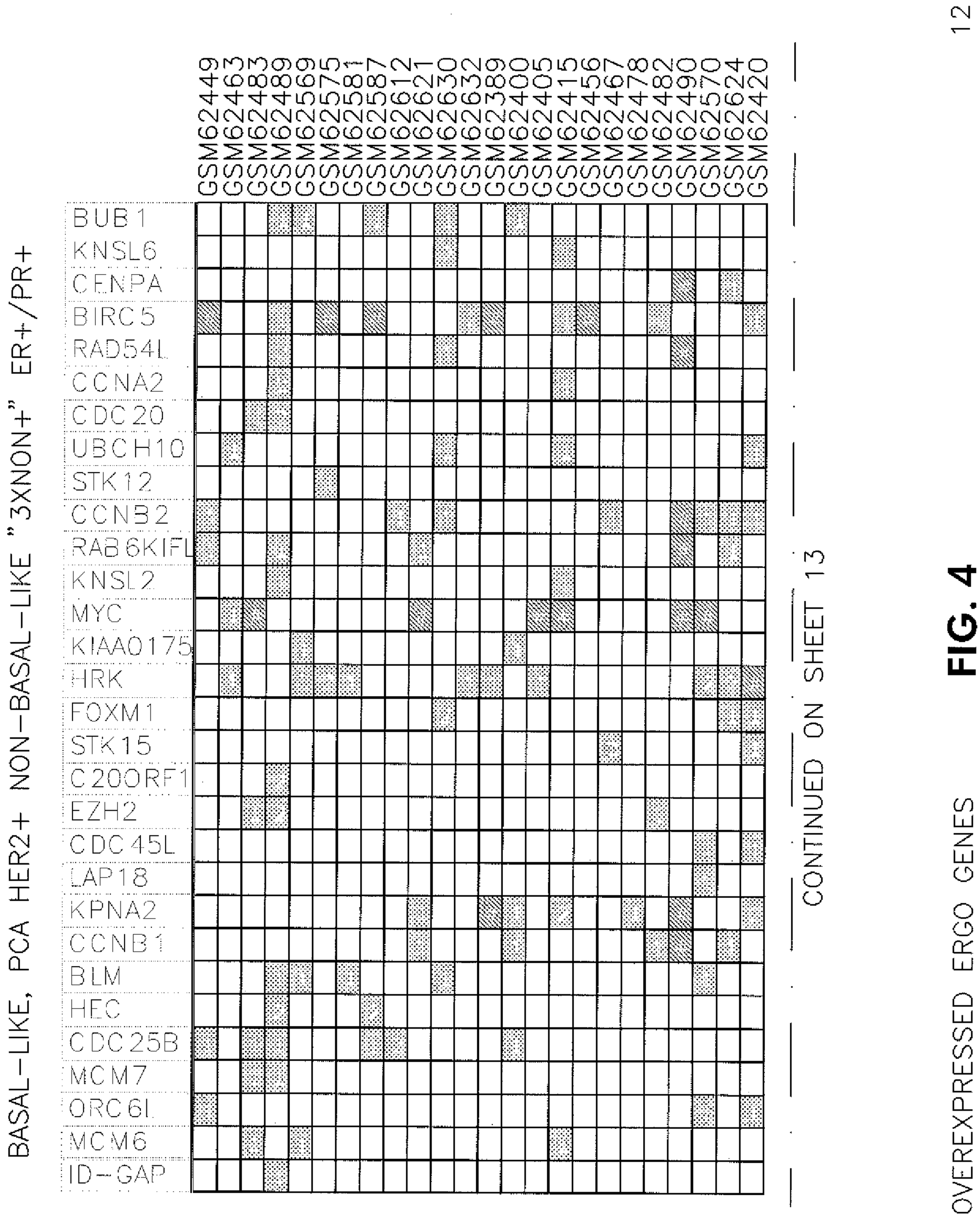

TOP 30 OVEREXPRESSED ERGO GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
CONTINUED ON SHEET 13
12

FIG. 4

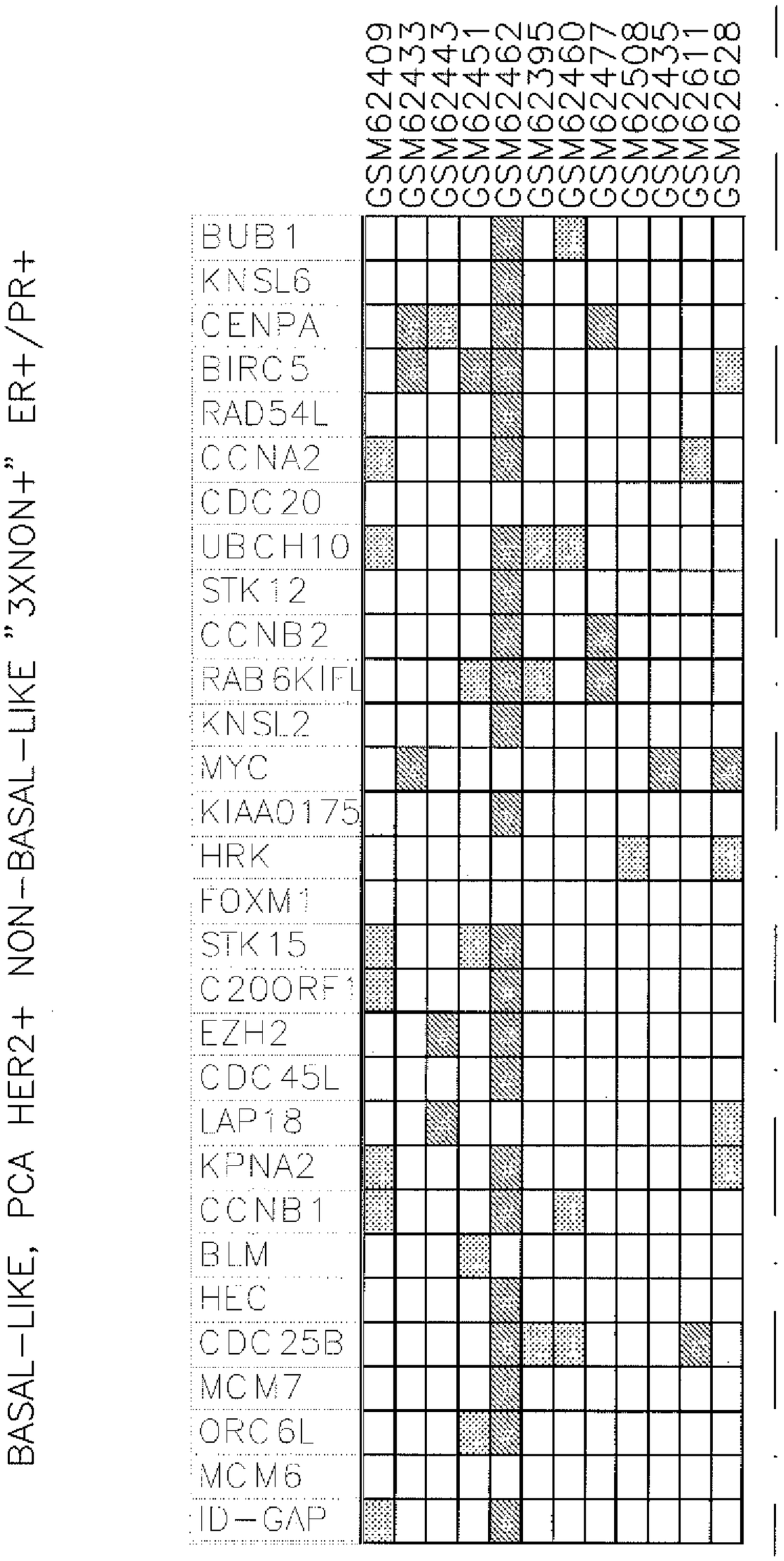


FIG. 4

TOP 30 OVEREXPRESSED ERGO GENES

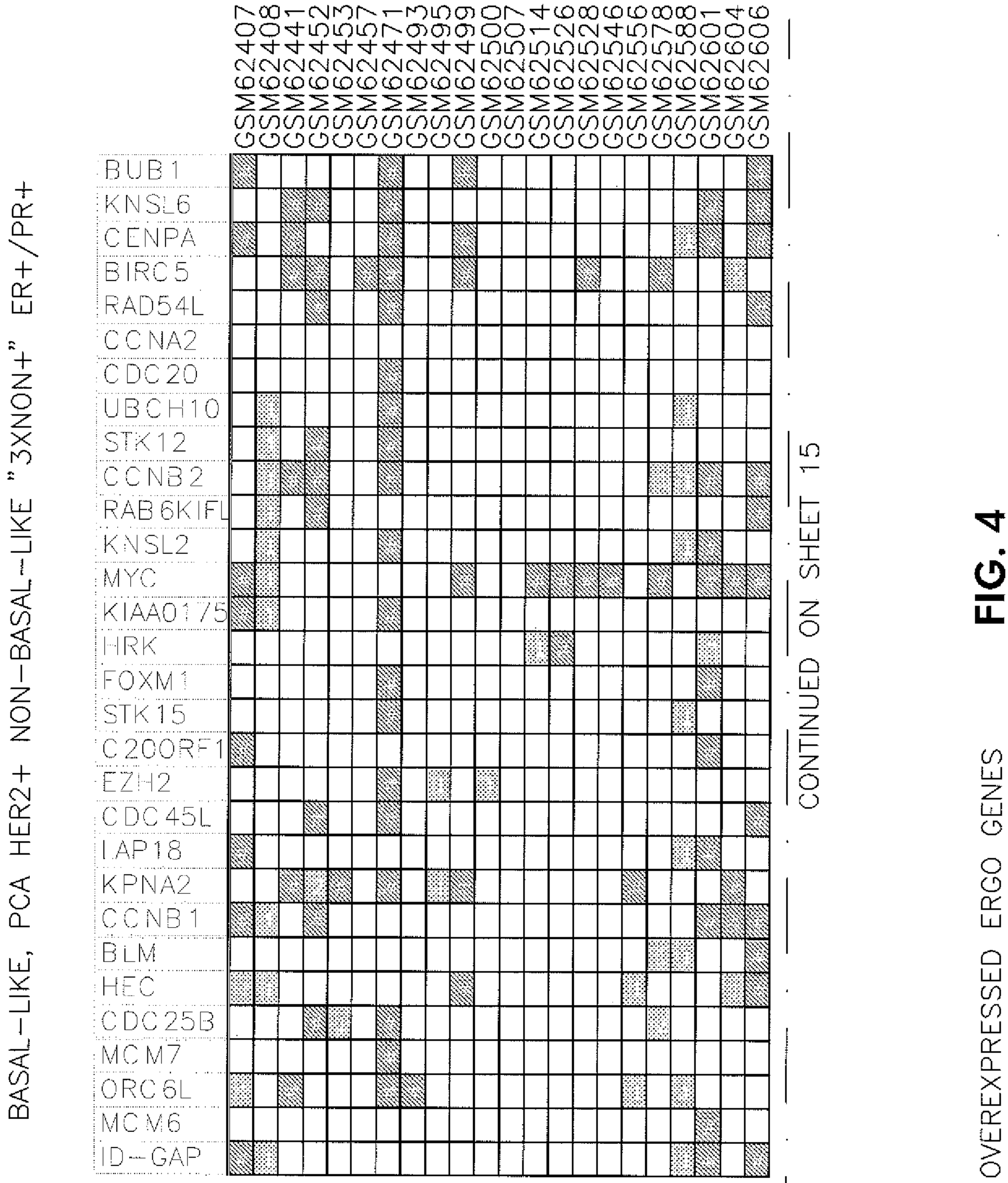

TOP 30 OVEREXPRESSED ERGO GENES
CONTINUED ON SHEET 15
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
M624407
M624408
M624441
M624452
M624453
M624457
M624471
M624493
M624499
M625007
M625014
M625026
M625028
M625046
M625556
M625578
M625588
M626601
M626604
M626606
GSS
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
IAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP

FIG. 4

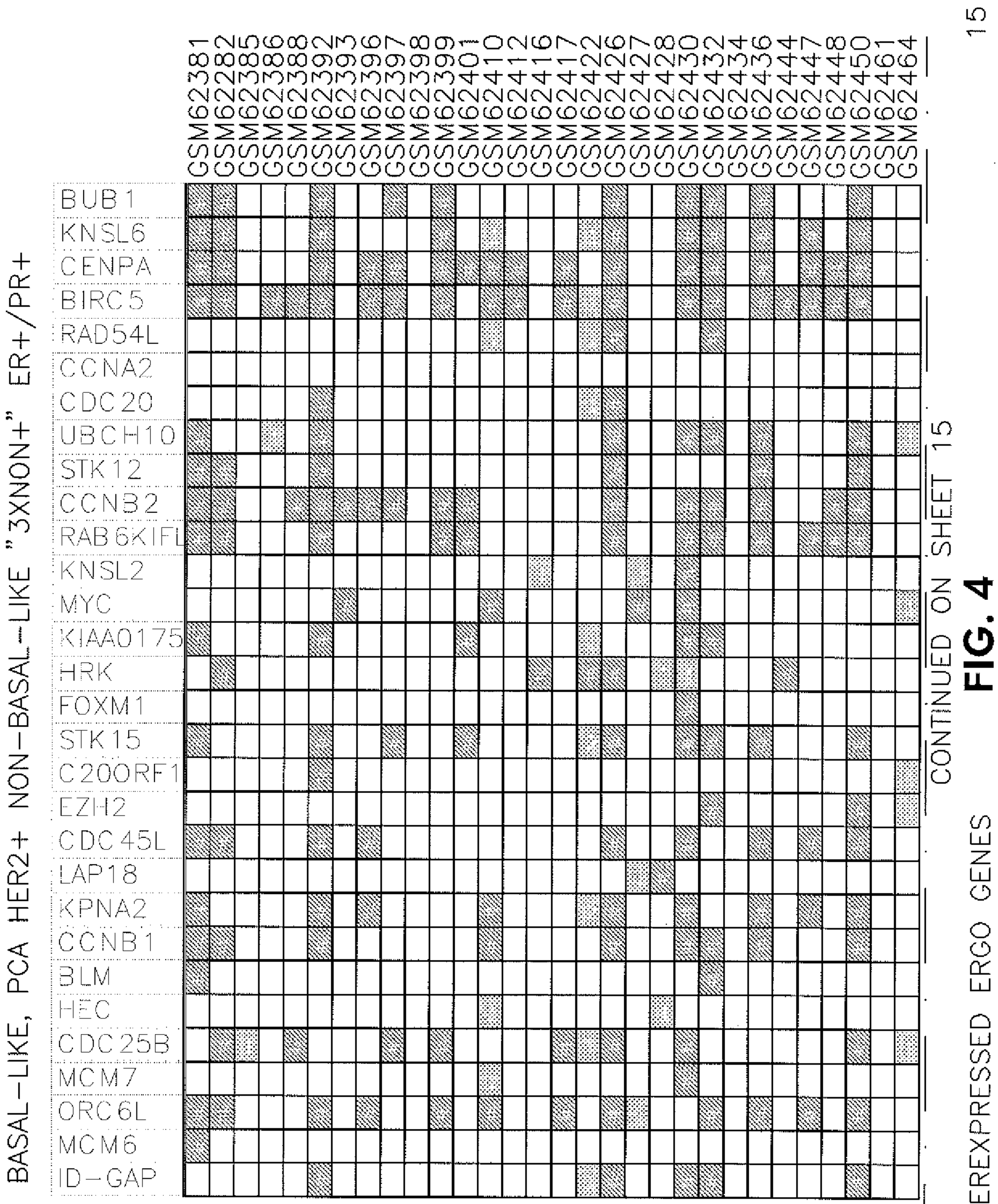
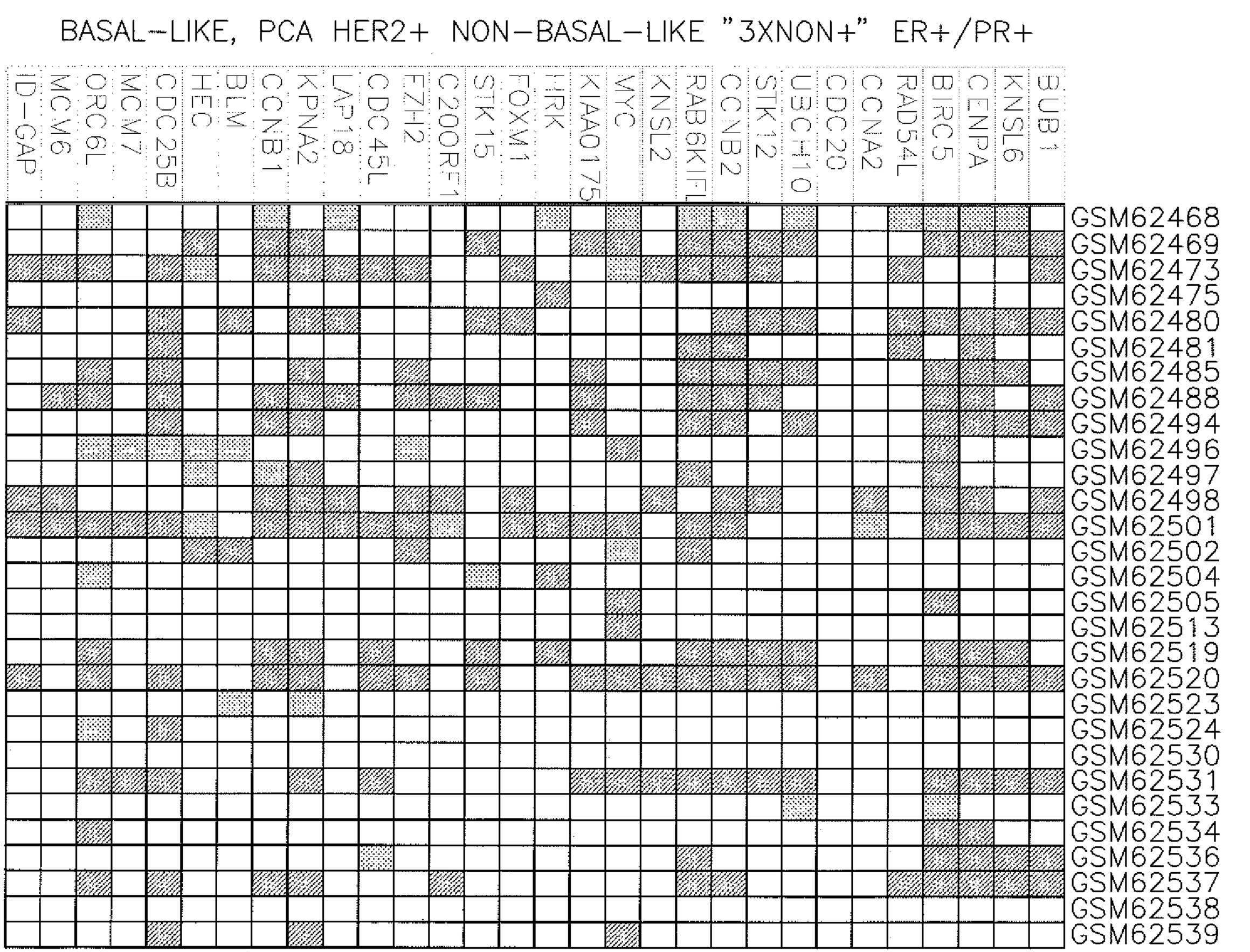

TOP 30 OVEREXPRESSED ERGO GENES
CONTINUED ON SHEET 15
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
M623811
M622882
M623885
M623886
M623888
M623892
M623967
M623997
M623998
M623999
M624010
M624126
M624167
M624226
M624267
M624278
M624304
M624332
M624344
M624436
M624447
M624448
M624450
M624461
M624641
15

FIG. 4



CONTINUED ON SHEET 17

TOP 30 OVEREXPRESSED ERGO GENES

FIG. 4

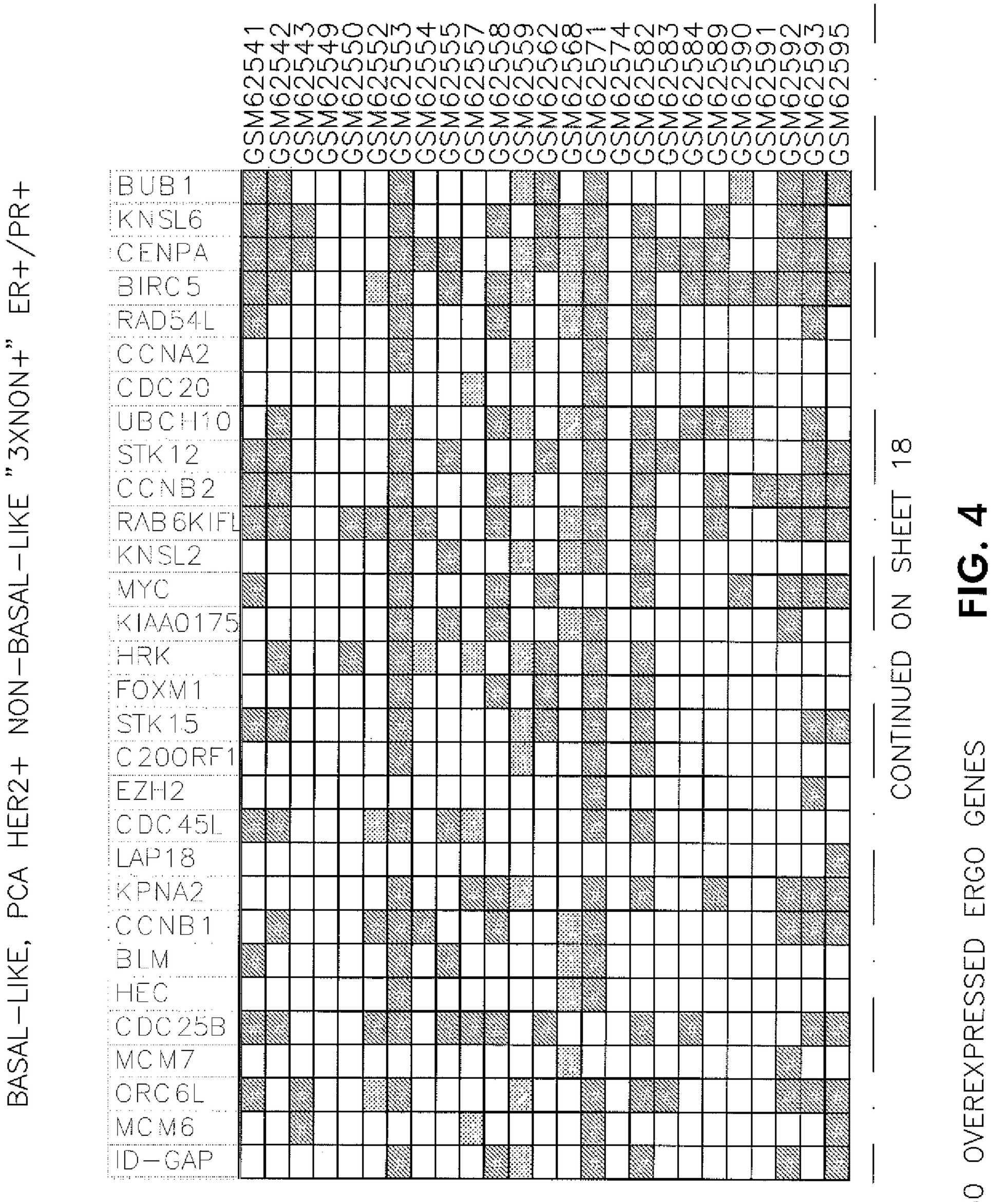

BASAL-LIKE "3XNON+" ER+/PR+
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE
TOP 30 OVEREXPRESSED ERGO GENES
CONTINUED ON SHEET 18
GSM625412
GSM625439
GSM625450
GSM625523
GSM625545
GSM625578
GSM625592
GSM625681
GSM625742
GSM625788
GSM625834
GSM625890
GSM625912
GSM625935
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP

FIG. 4

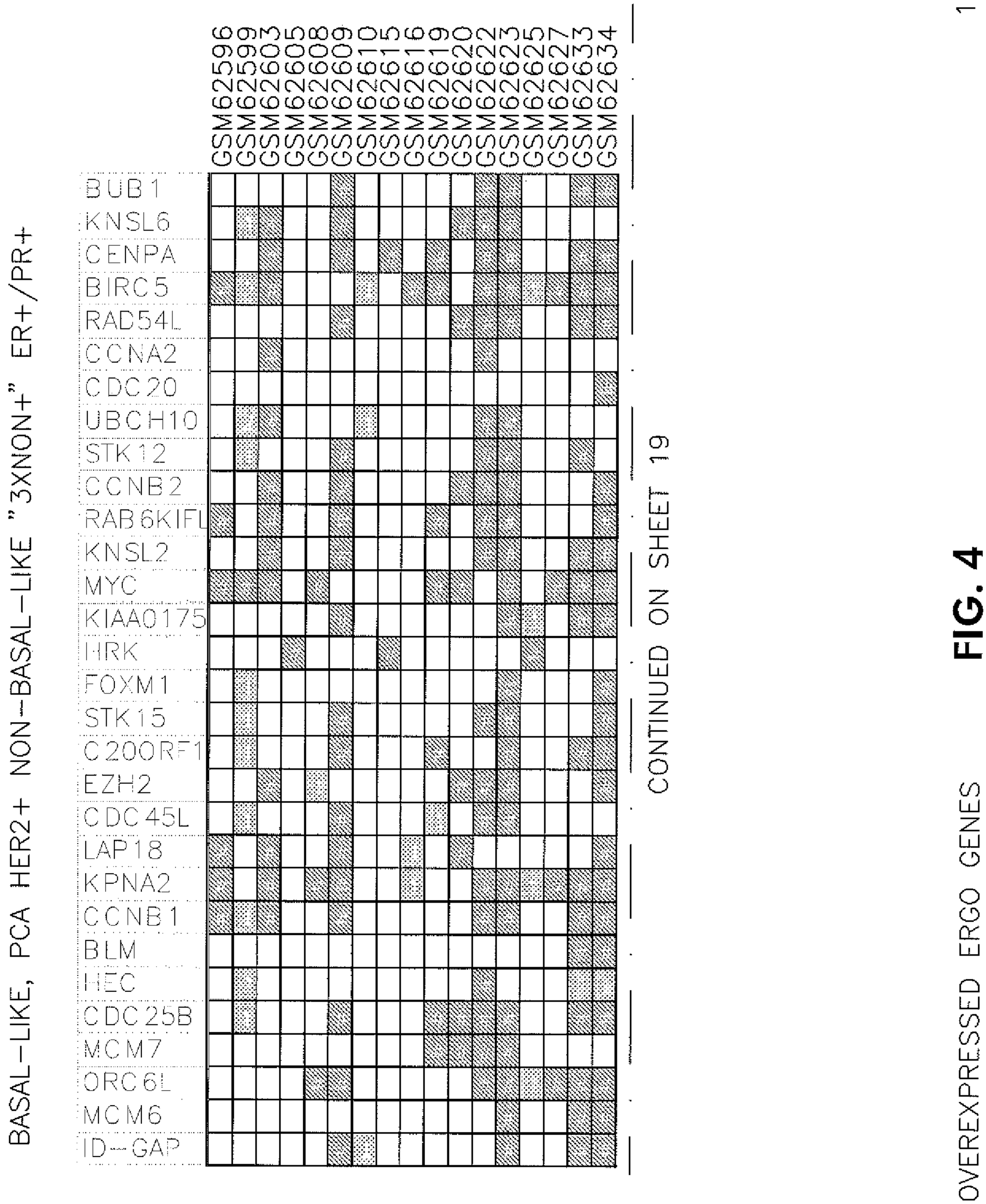
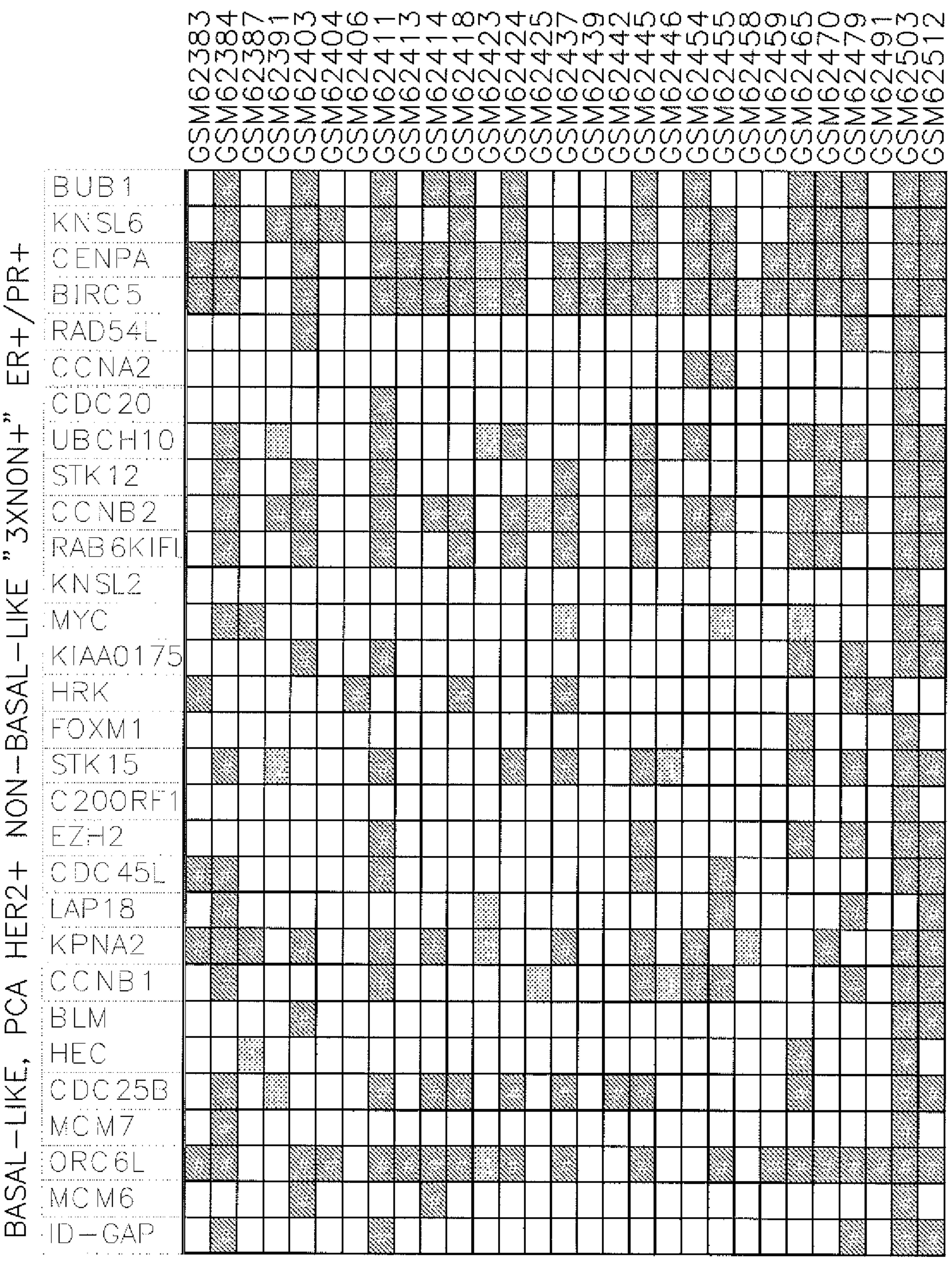


FIG. 4

TOP 30 OVEREXPRESSED ERGO GENES



CONTINUED ON SHEET 20

TOP 30 OVEREXPRESSED ERGO GENES

FIG. 4

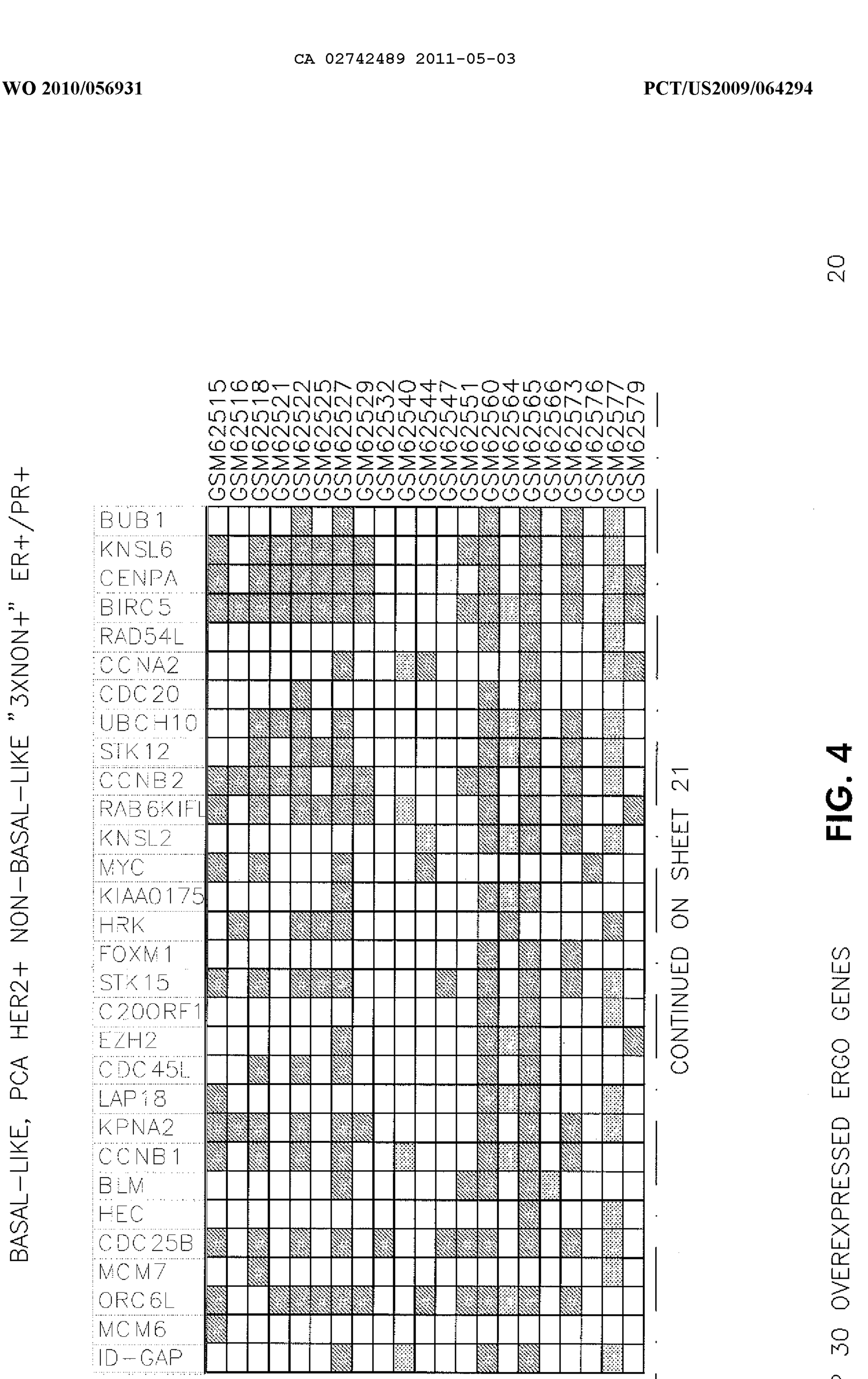
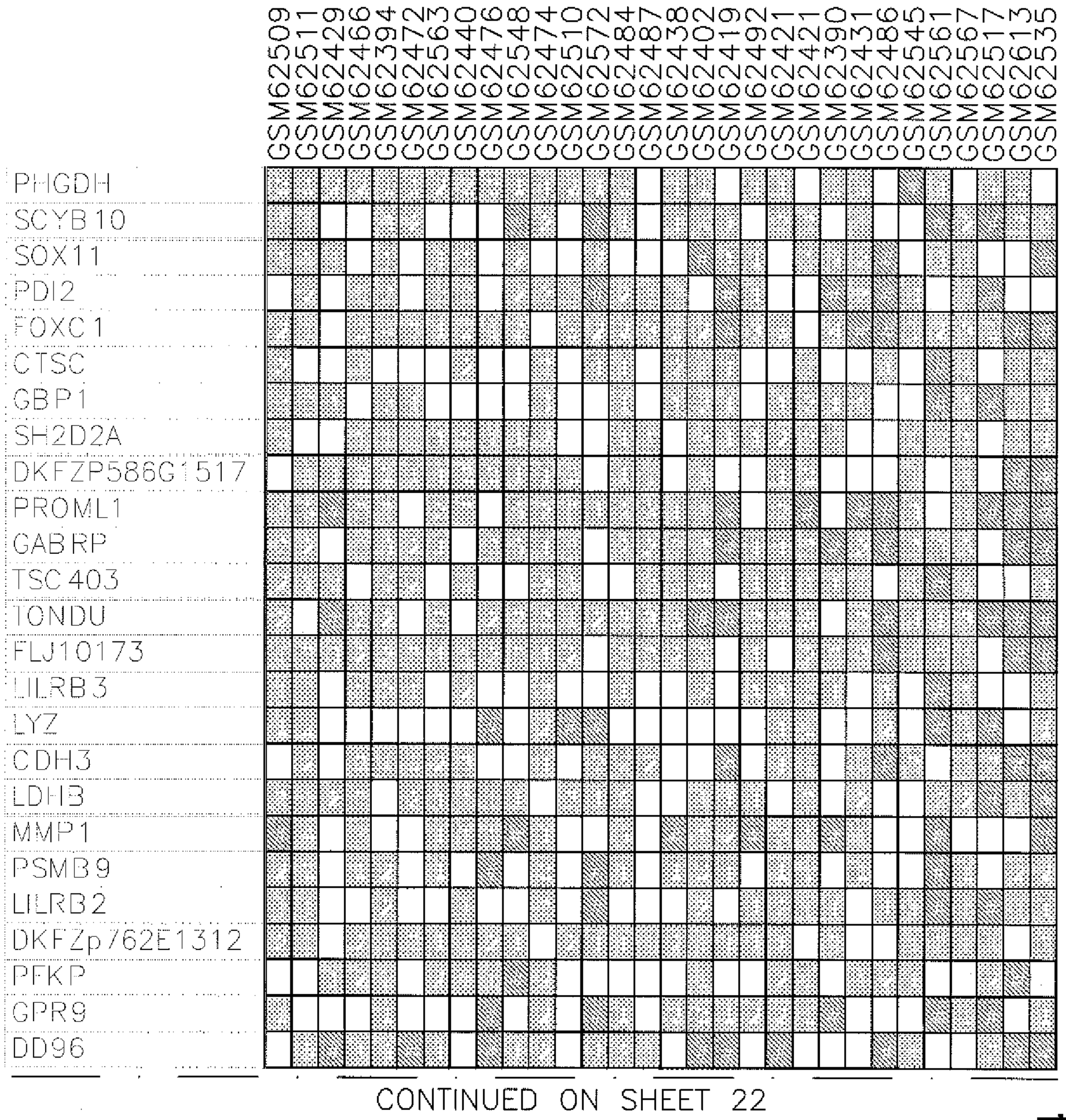

TOP 30 OVEREXPRESSED ERGO GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
CONTINUED ON SHEET 21
GSM625515
GSM625516
GSM625518
GSM625521
GSM625522
GSM625525
GSM625527
GSM625529
GSM625532
GSM625540
GSM625544
GSM625547
GSM625551
GSM625560
GSM625564
GSM625565
GSM625566
GSM625573
GSM625576
GSM625577
GSM625579
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP

FIG. 4

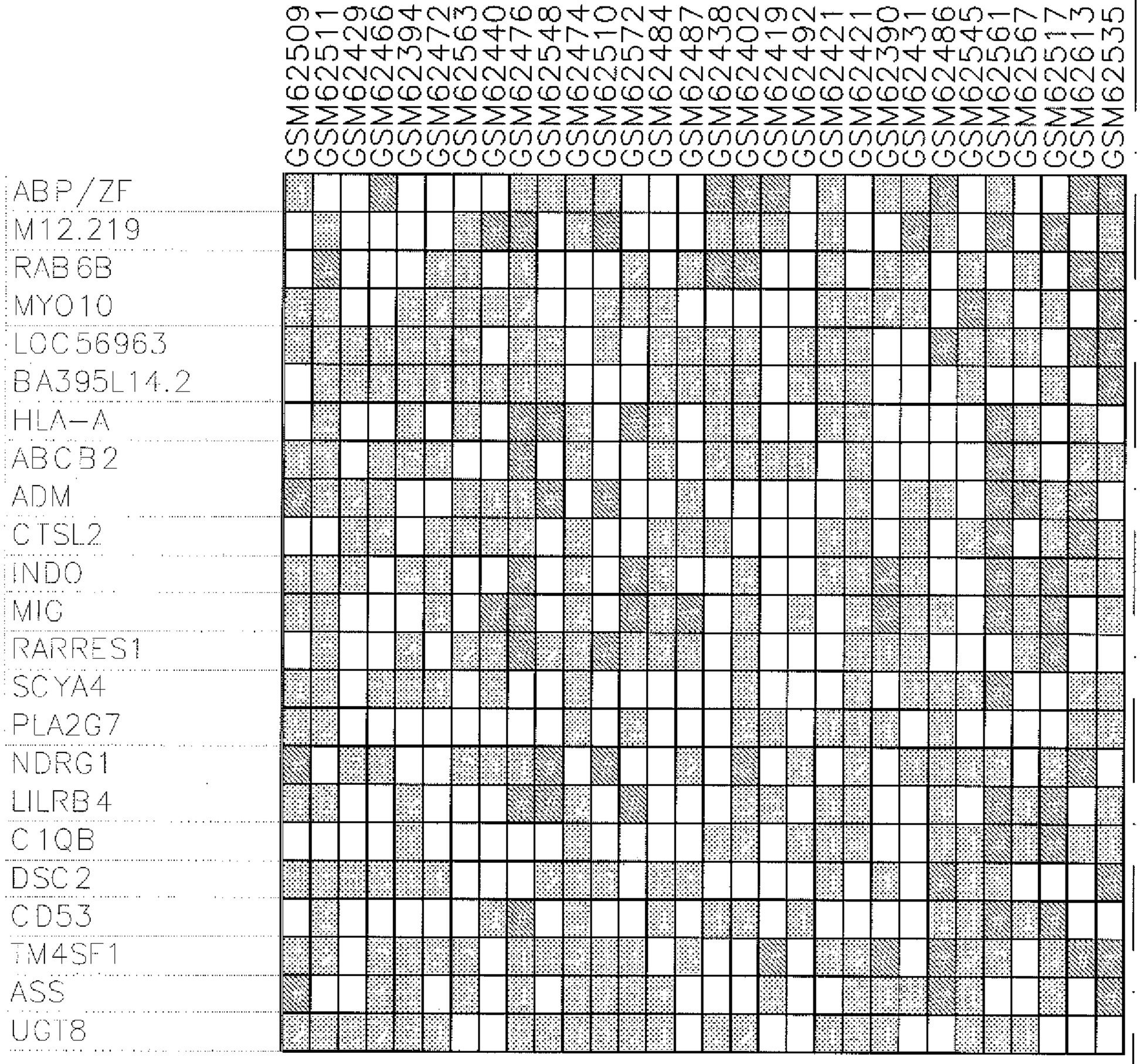


CONTINUED ON SHEET 22

FIG. 4

BASAL–LIKE, PCA HER2+ NON–BASAL–LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL–LIKE GENES

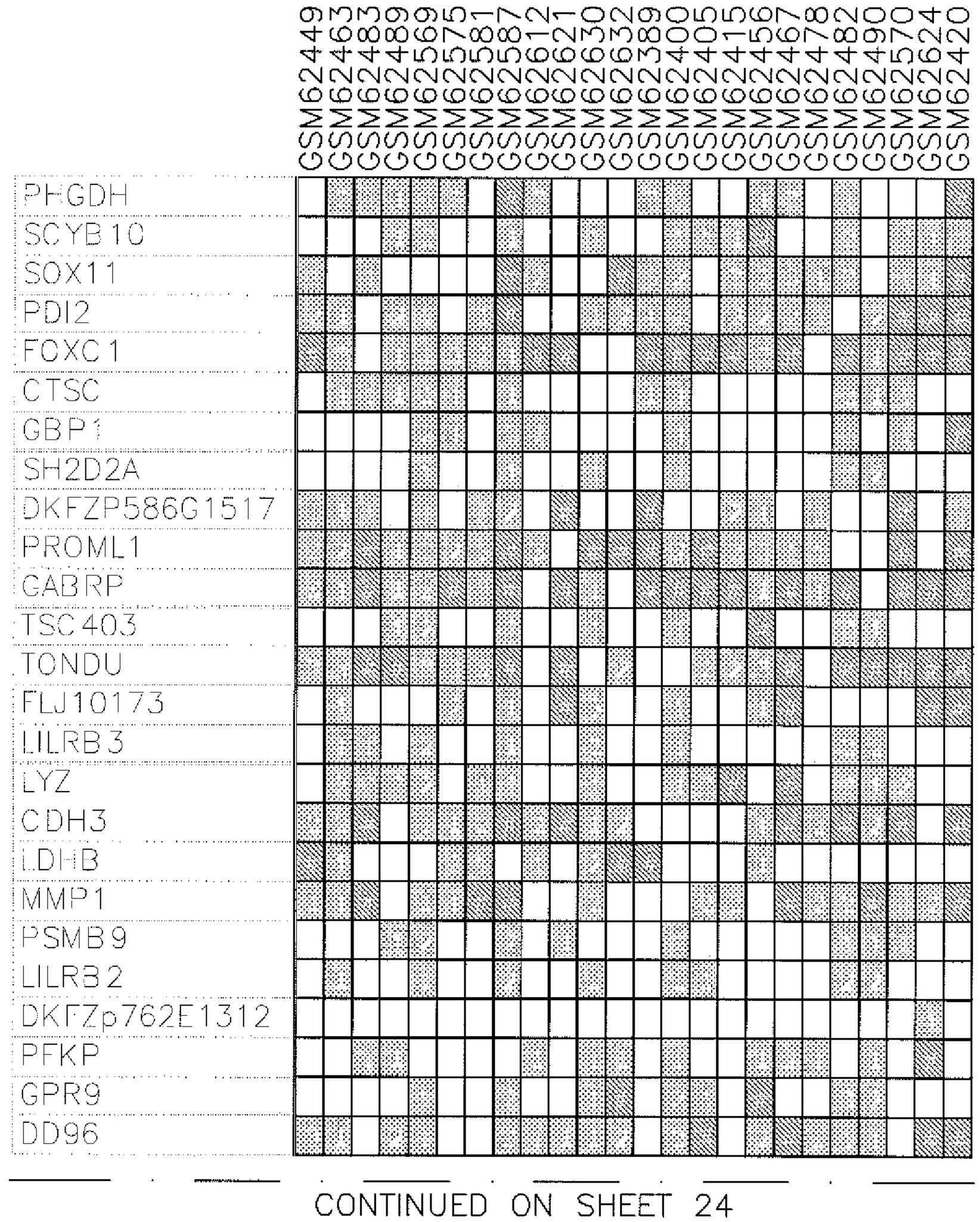
CONTINUED FROM SHEET 21



CONTINUED ON SHEET 3

FIG. 4

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

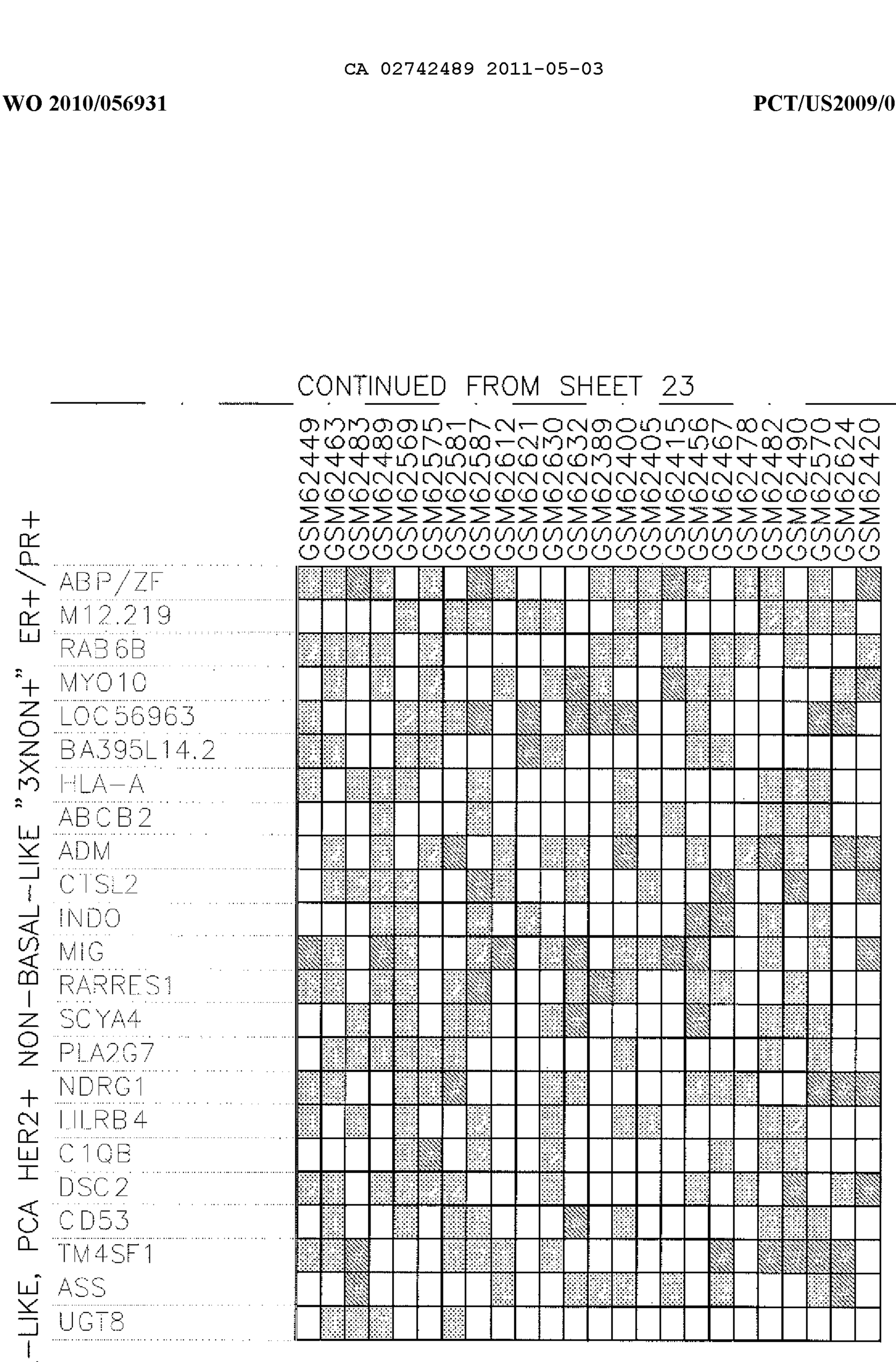


TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 4

CONTINUED ON SHEET 24

CONTINUED FROM SHEET 23



CONTINUED ON SHEET 25

TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 4

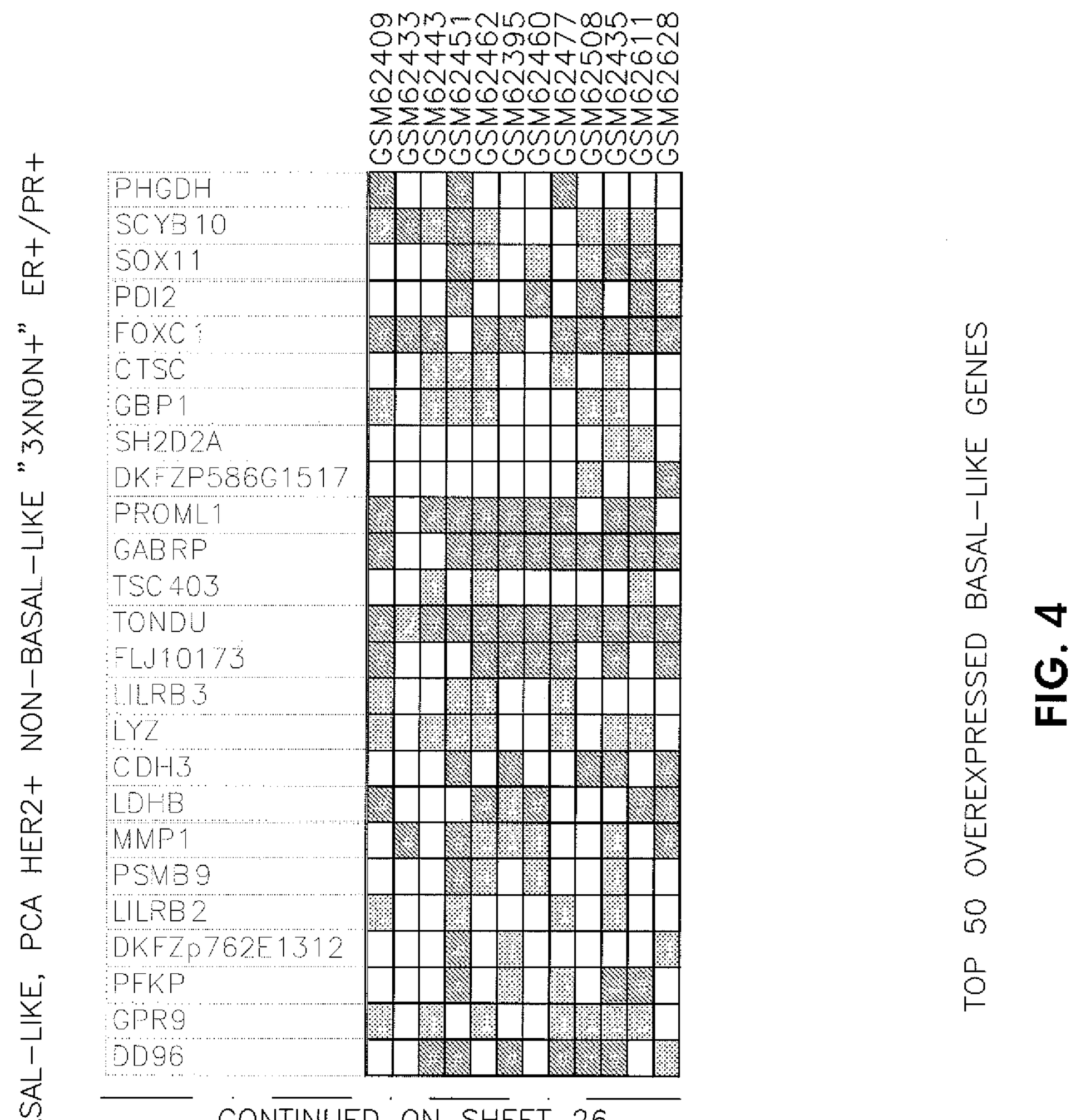
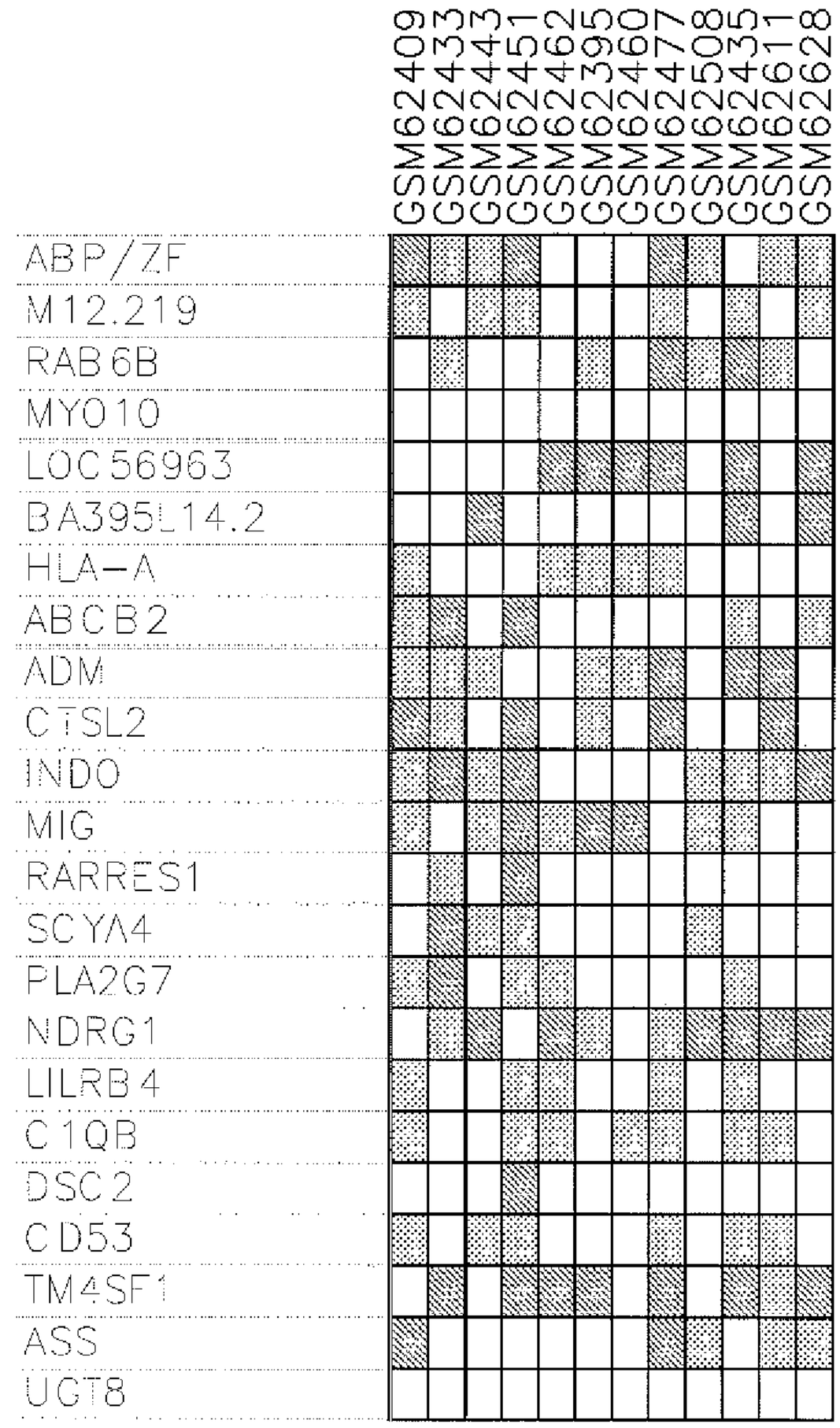

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
GSM624093
GSM624331
GSM624345
GSM624625
GSM624639
GSM624647
GSM624650
GSM624635
GSM624611
GSM624628
PHGDH
SCYB10
SOX11
PDI2
FOXC1
CTSC
GBP1
SH2D2A
DKFZP586G1517
PROML1
GABRP
TSC403
TONDU
FLJ10173
LILRB3
LYZ
CDH3
LDHB
MMP1
PSMB9
LILRB2
DKFZp762E1312
PFKP
GPR9
DD96
CONTINUED ON SHEET 26

FIG. 4

CONTINUED FROM SHEET 25



CONTINUED ON SHEET 27

TOP 50 OVEREXPRESSED BASAL-LIKE GENES

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

FIG. 4

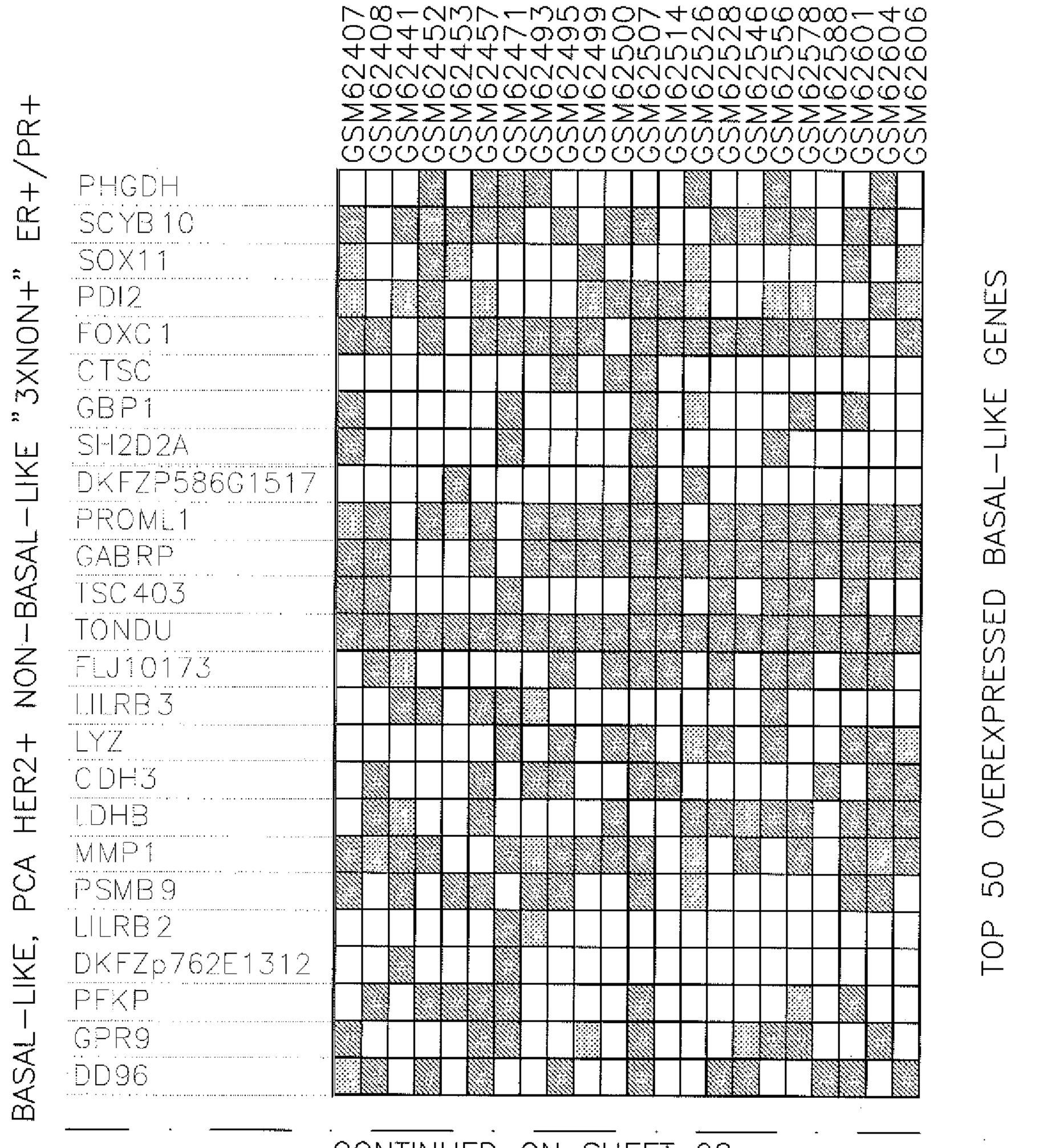
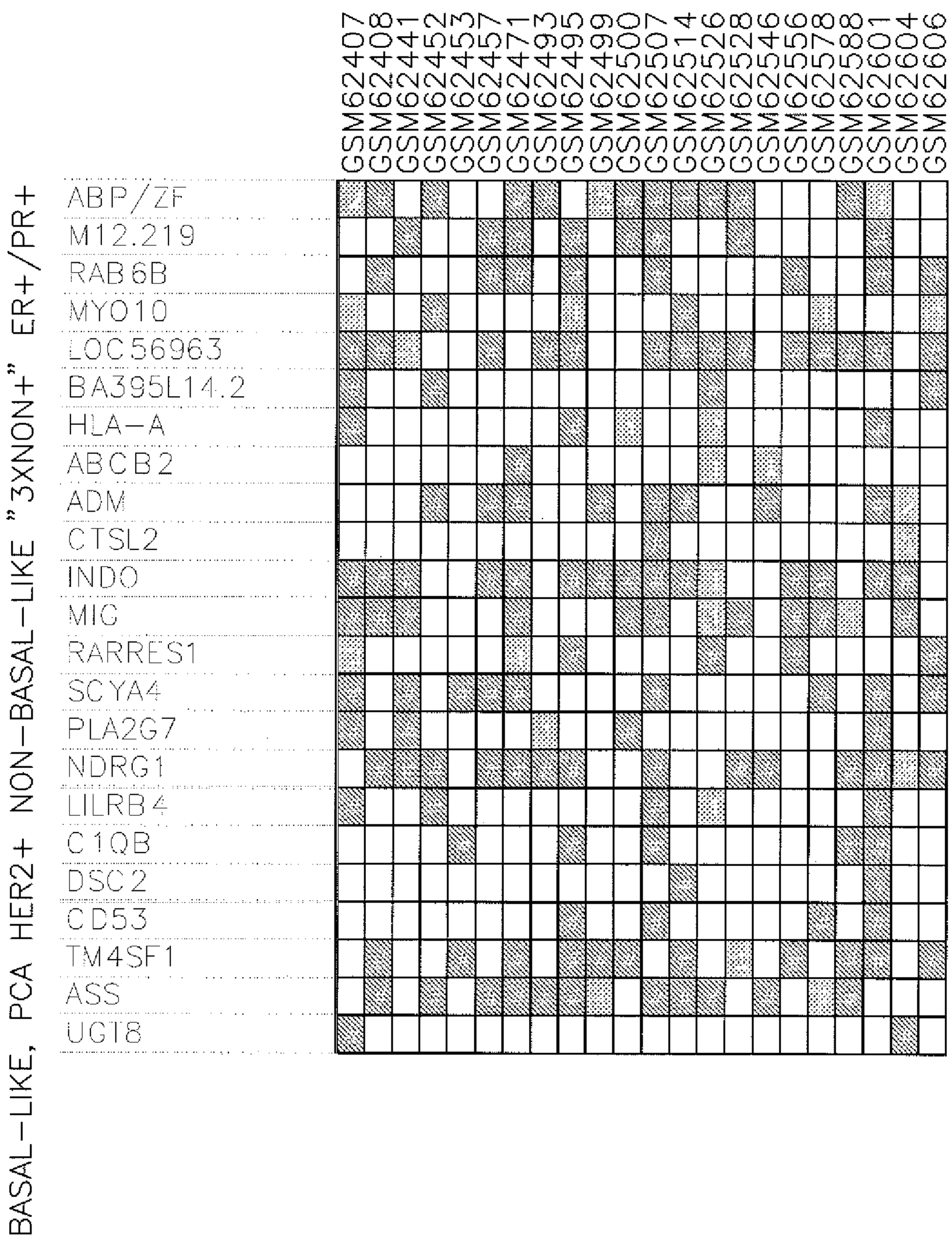


FIG. 4

CONTINUED ON SHEET 28

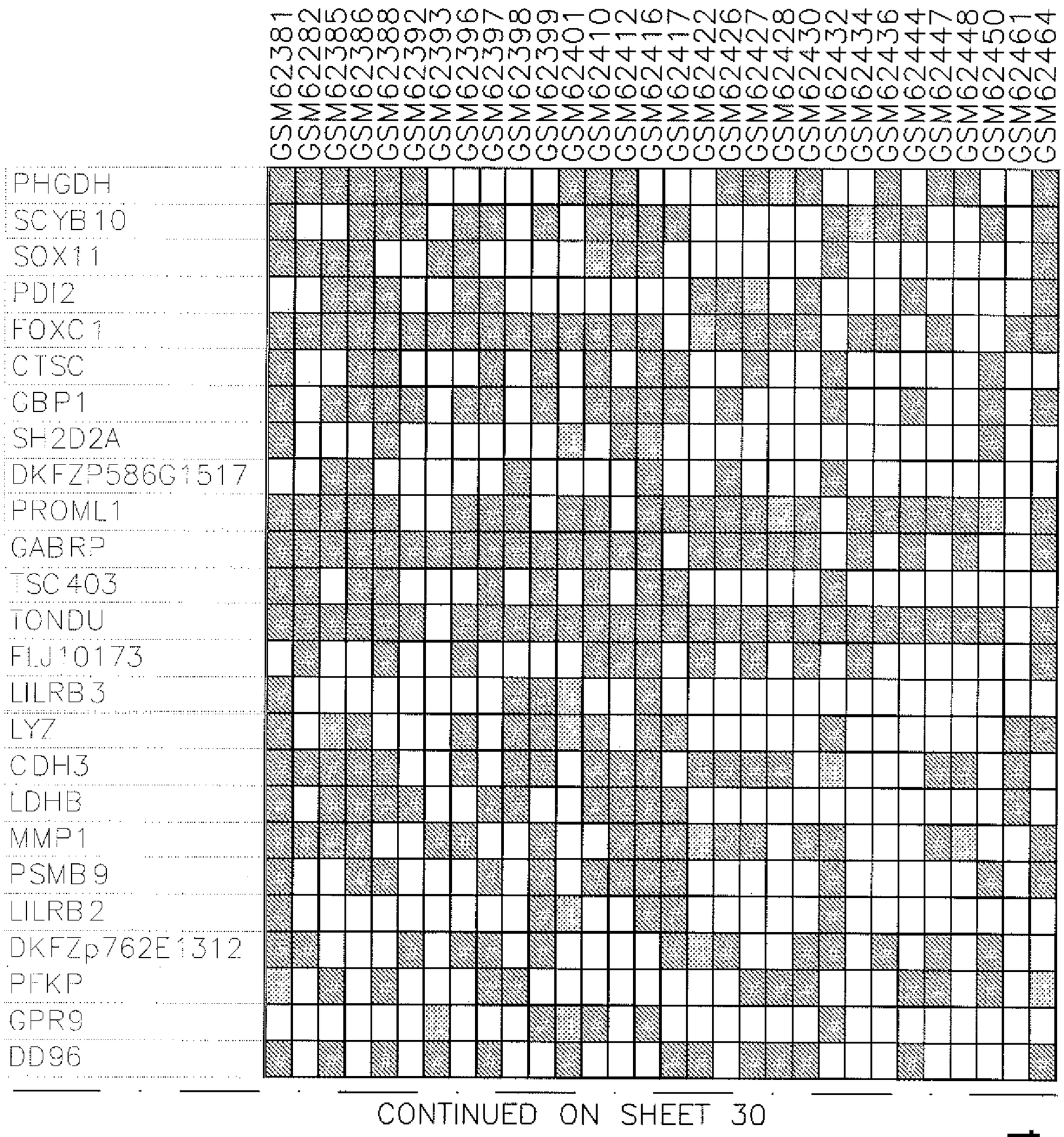
CONTINUED FROM SHEET 27



CONTINUED ON SHEET 29

TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 4

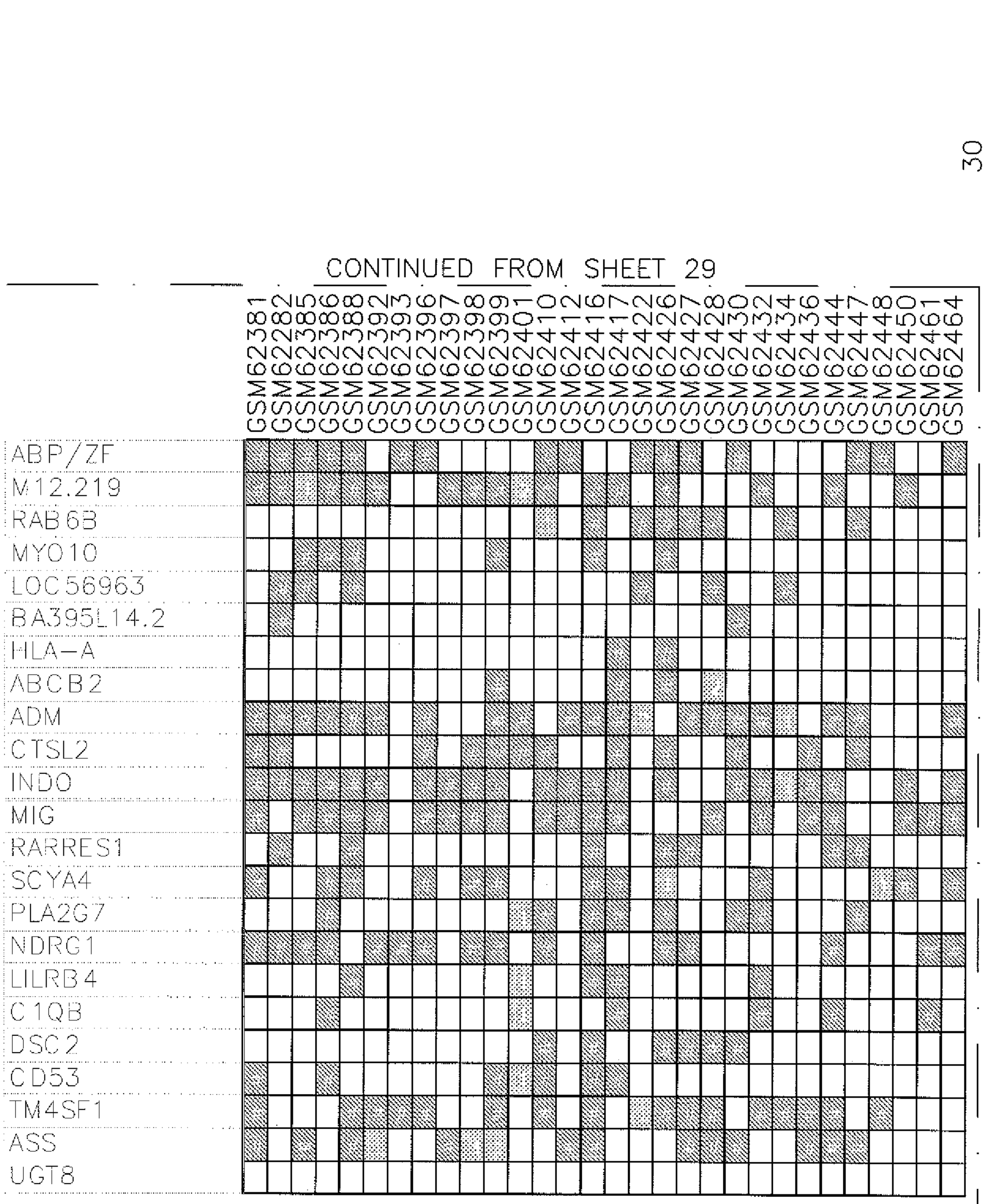


CONTINUED ON SHEET 30

FIG. 4

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

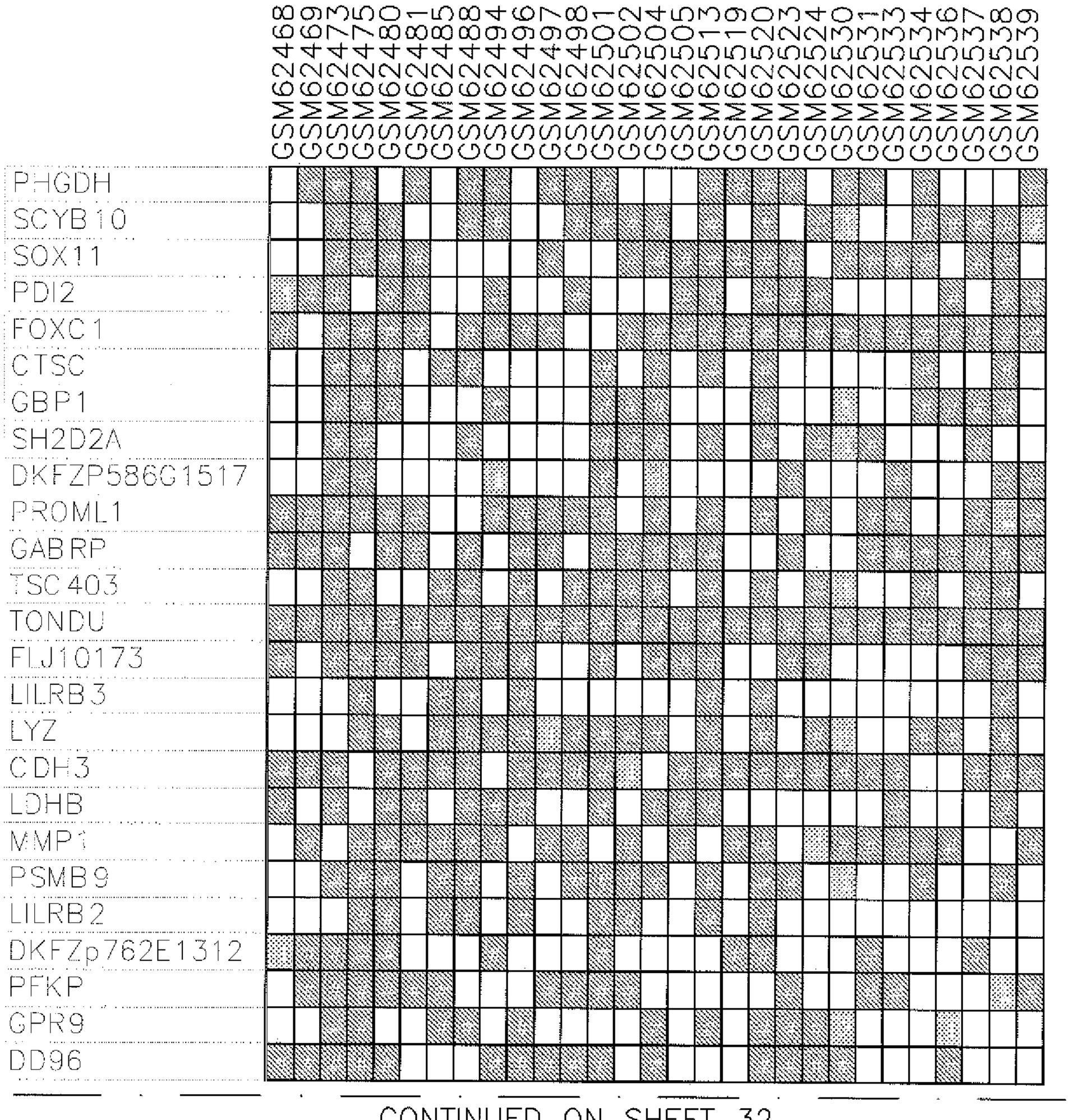
CONTINUED FROM SHEET 29



CONTINUE ON SHEET 31

FIG. 4

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

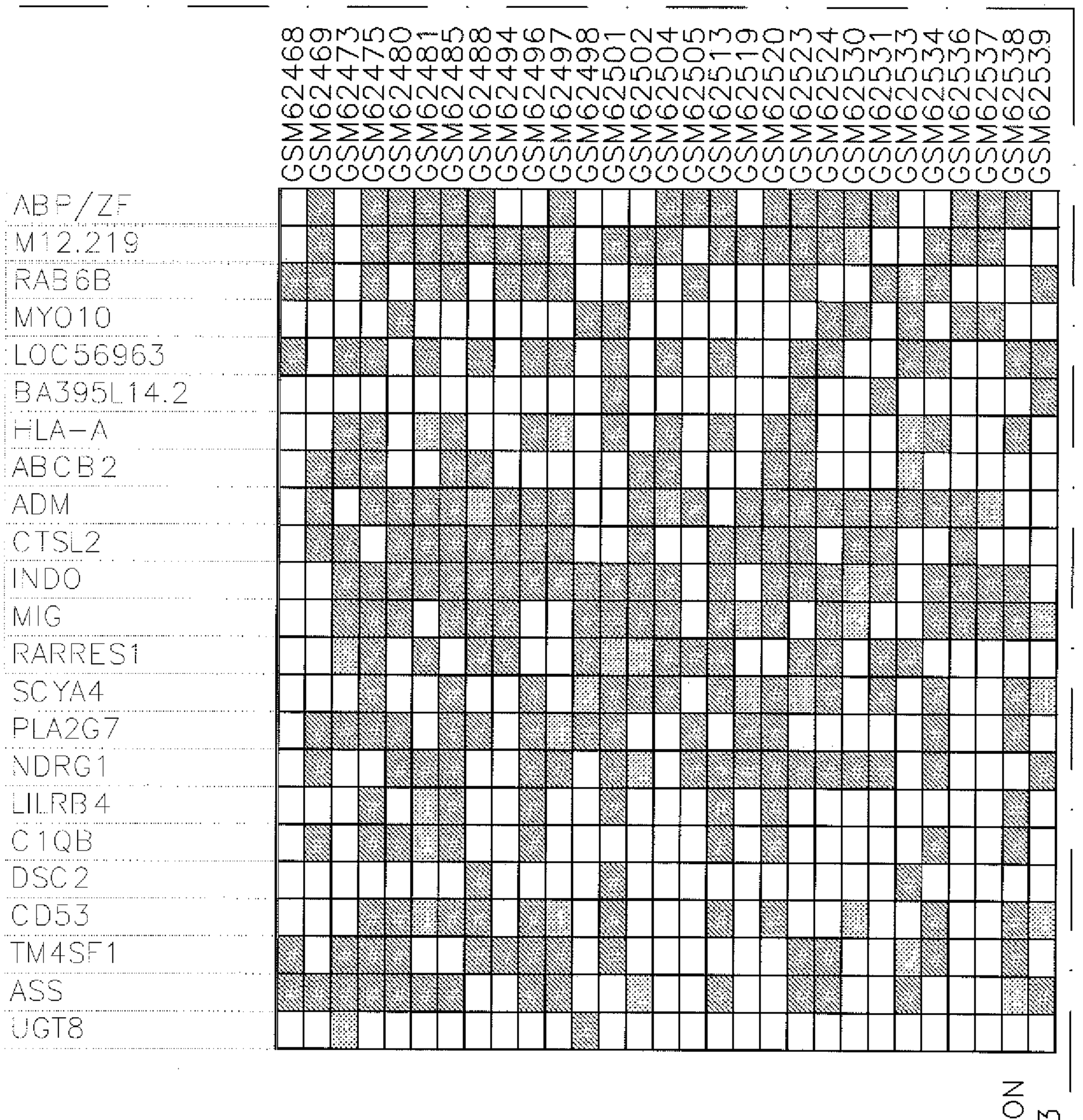


CONTINUED ON SHEET 32

FIG. 4

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

CONTINUED FROM SHEET 31



CONTINUE ON SHEET 33

FIG. 4

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

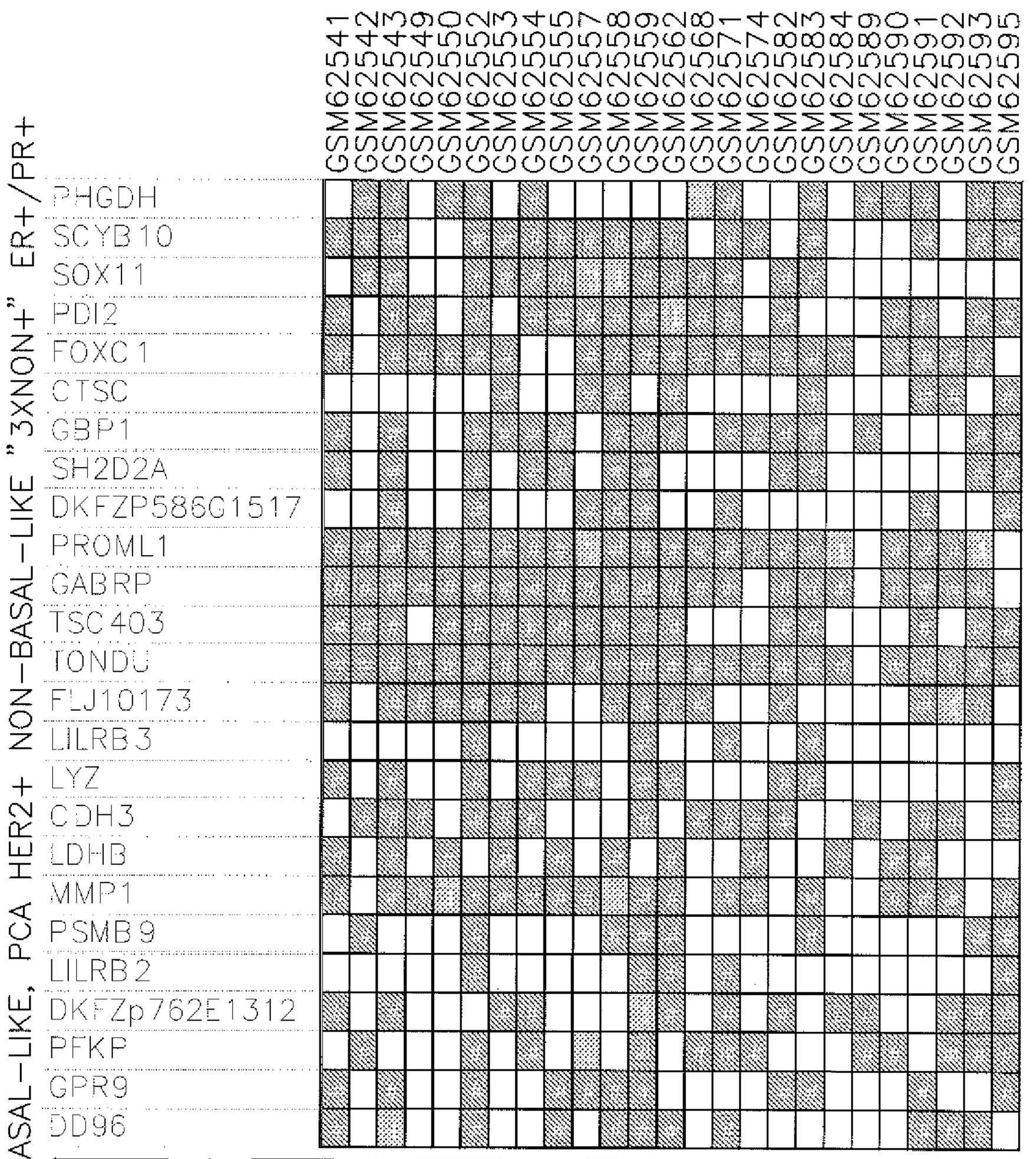


FIG. 4

CONTINUED ON SHEET 34

CONTINUED FROM SHEET 33

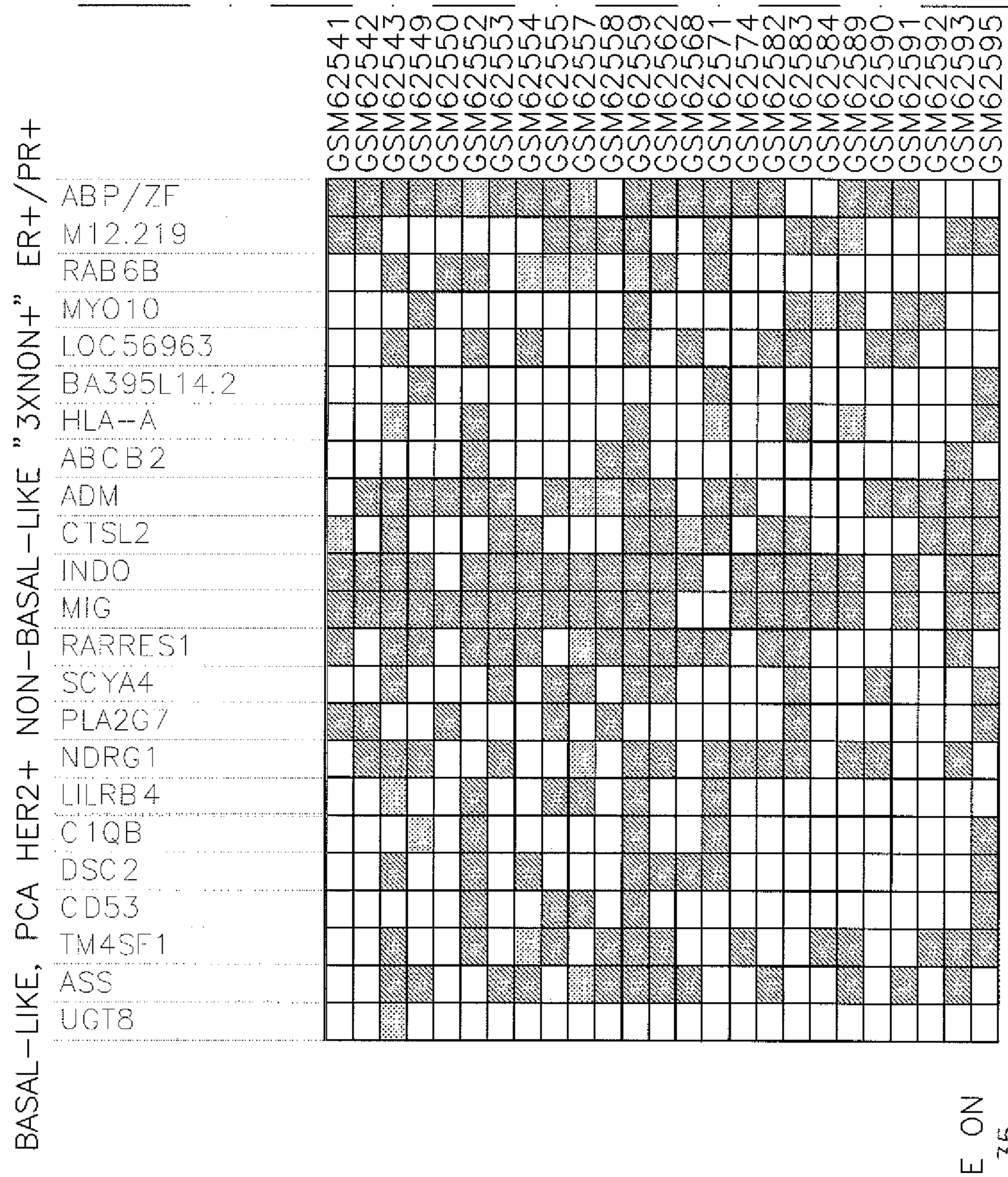
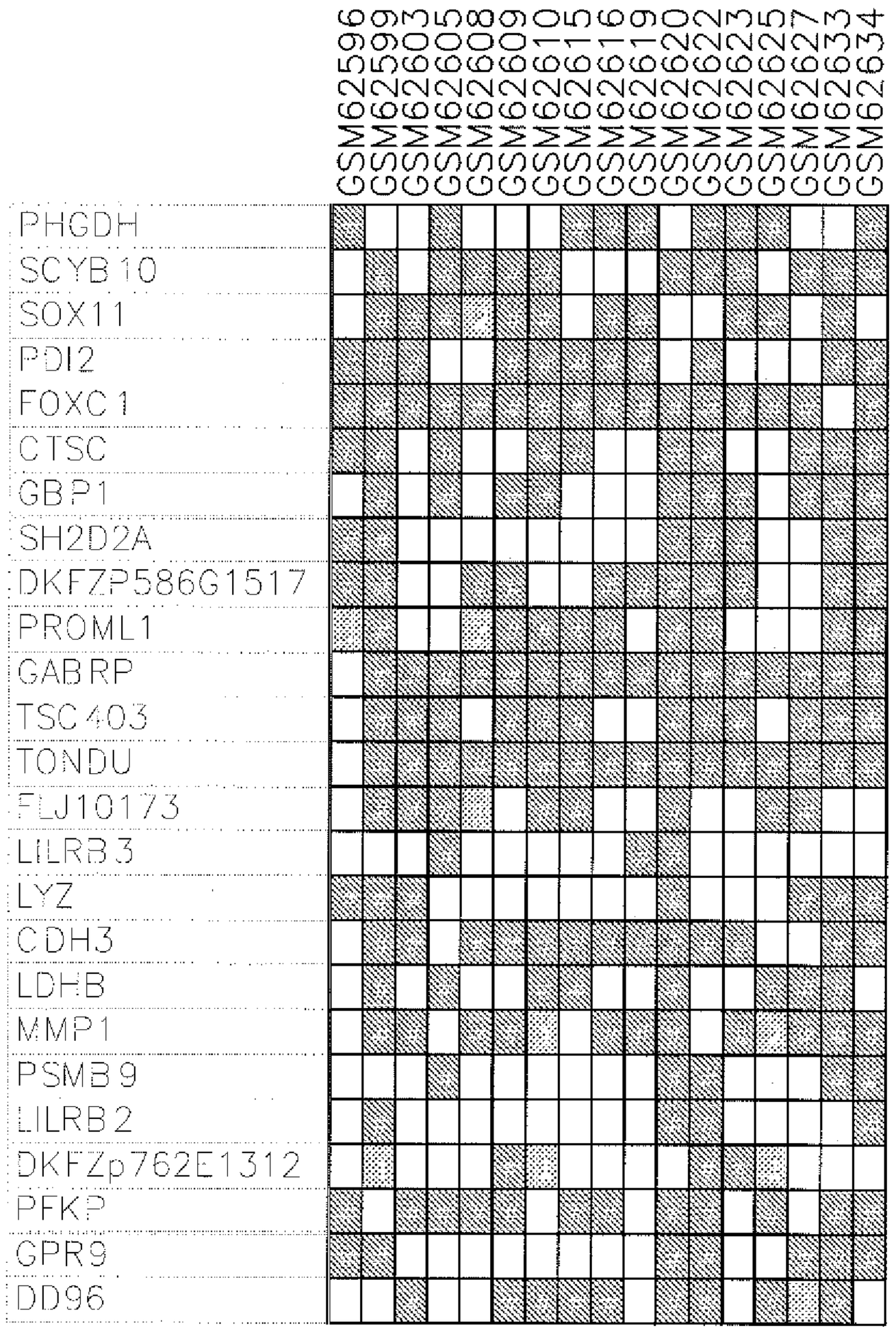


FIG. 4

CONTINUE ON SHEET 35



CONTINUED ON SHEET 36

FIG. 4

CONTINUED FROM SHEET 35

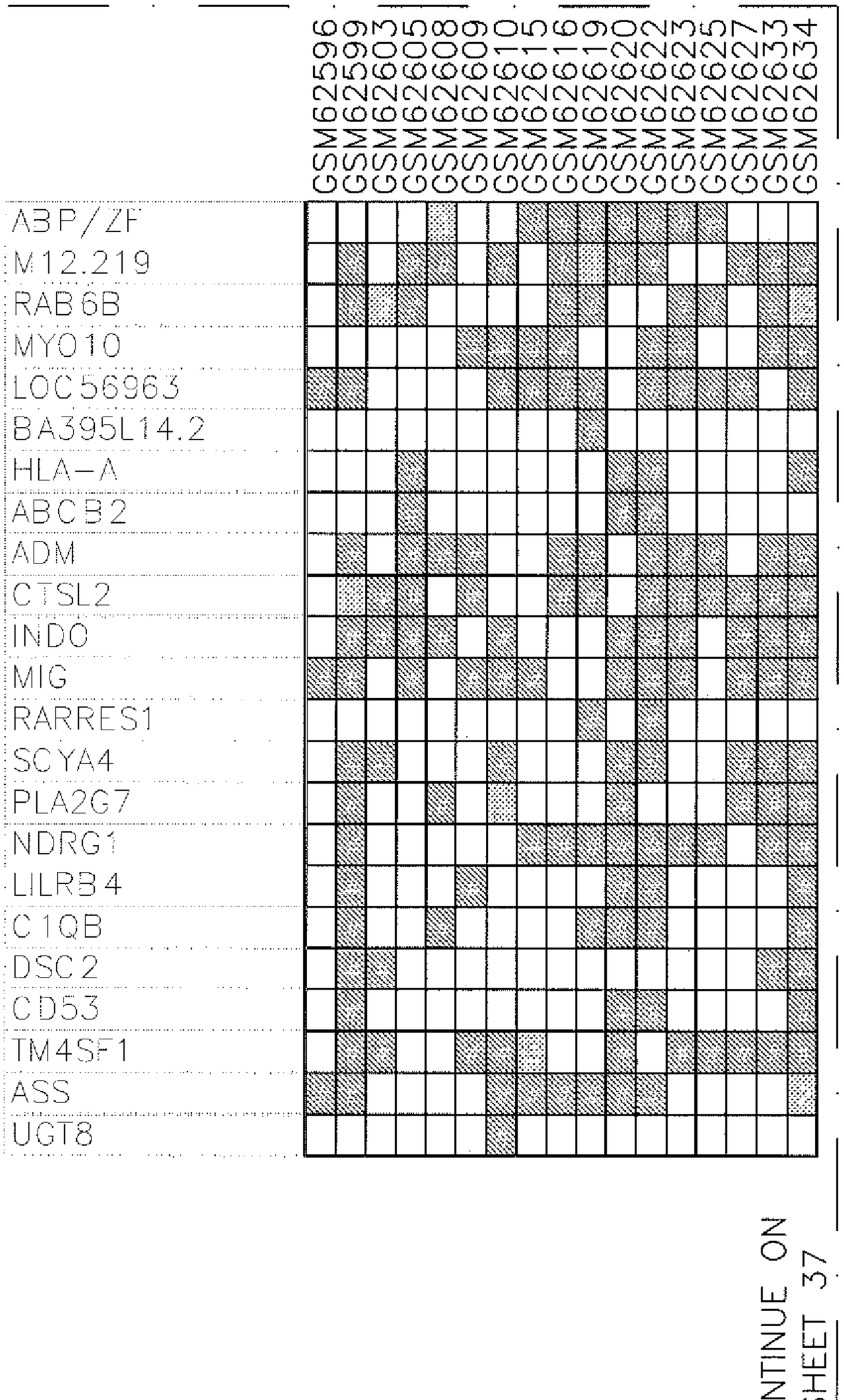


FIG. 4

CONTINUE ON SHEET 37

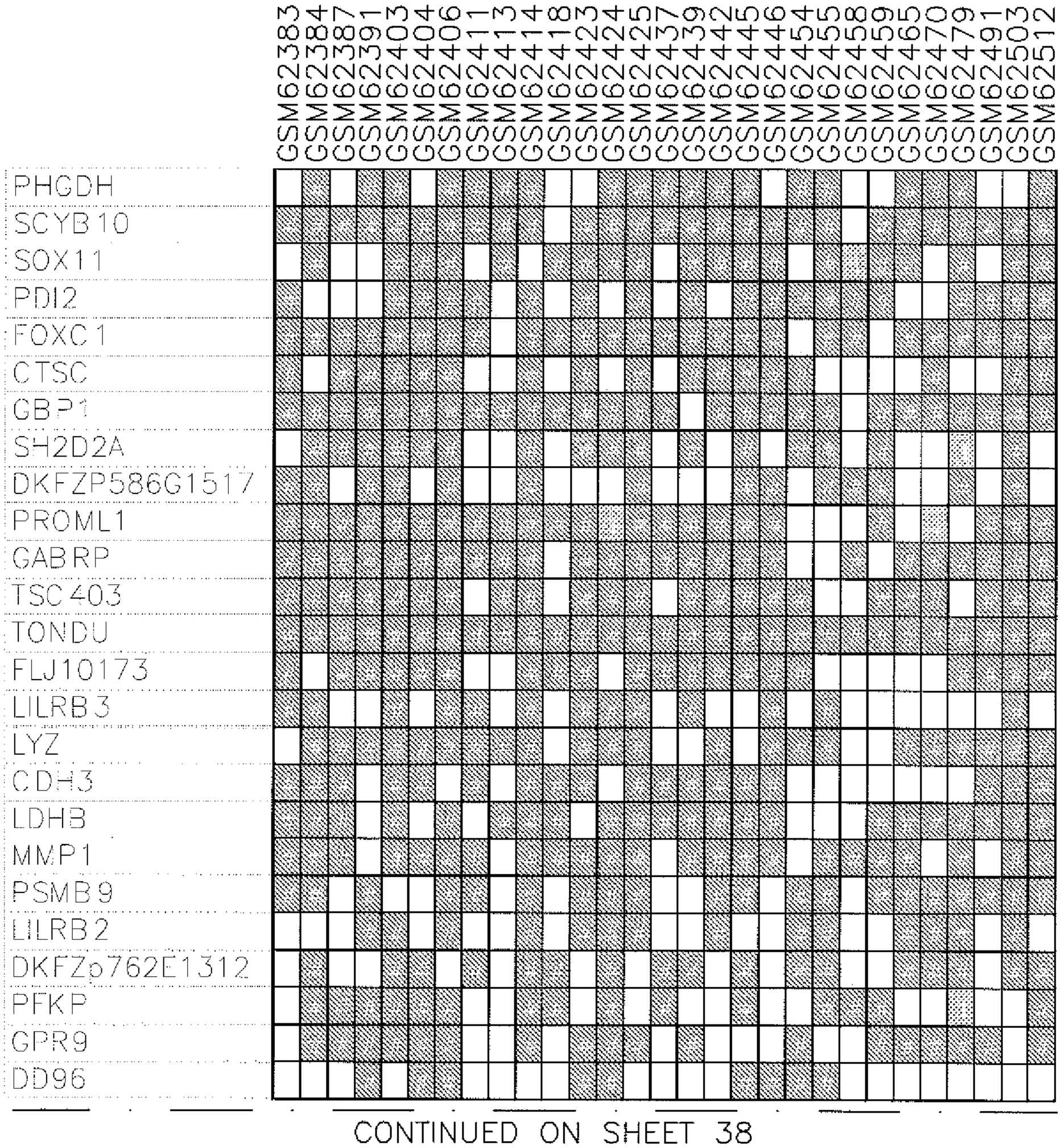


FIG. 4

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

CONTINUED FROM SHEET 37

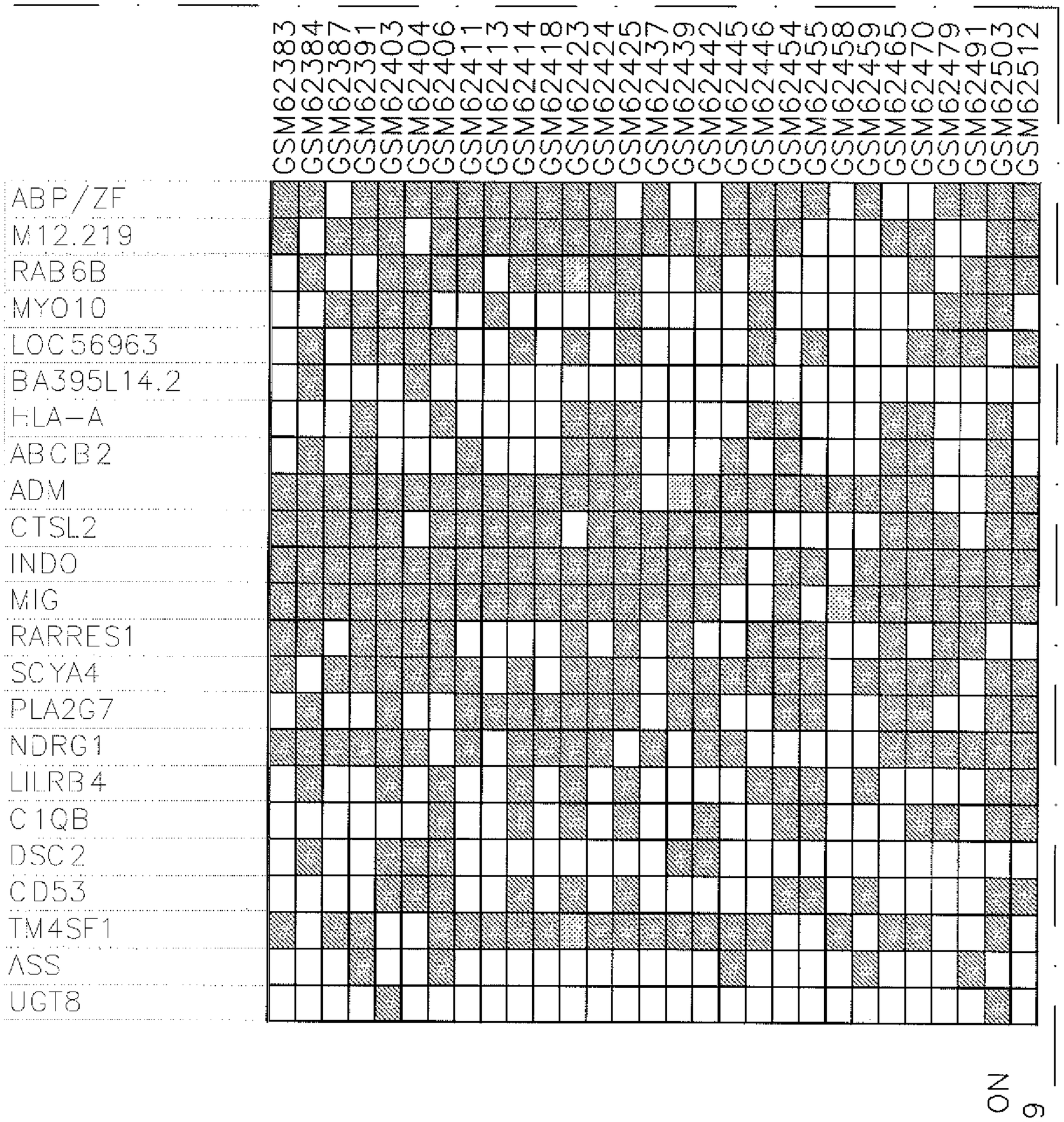
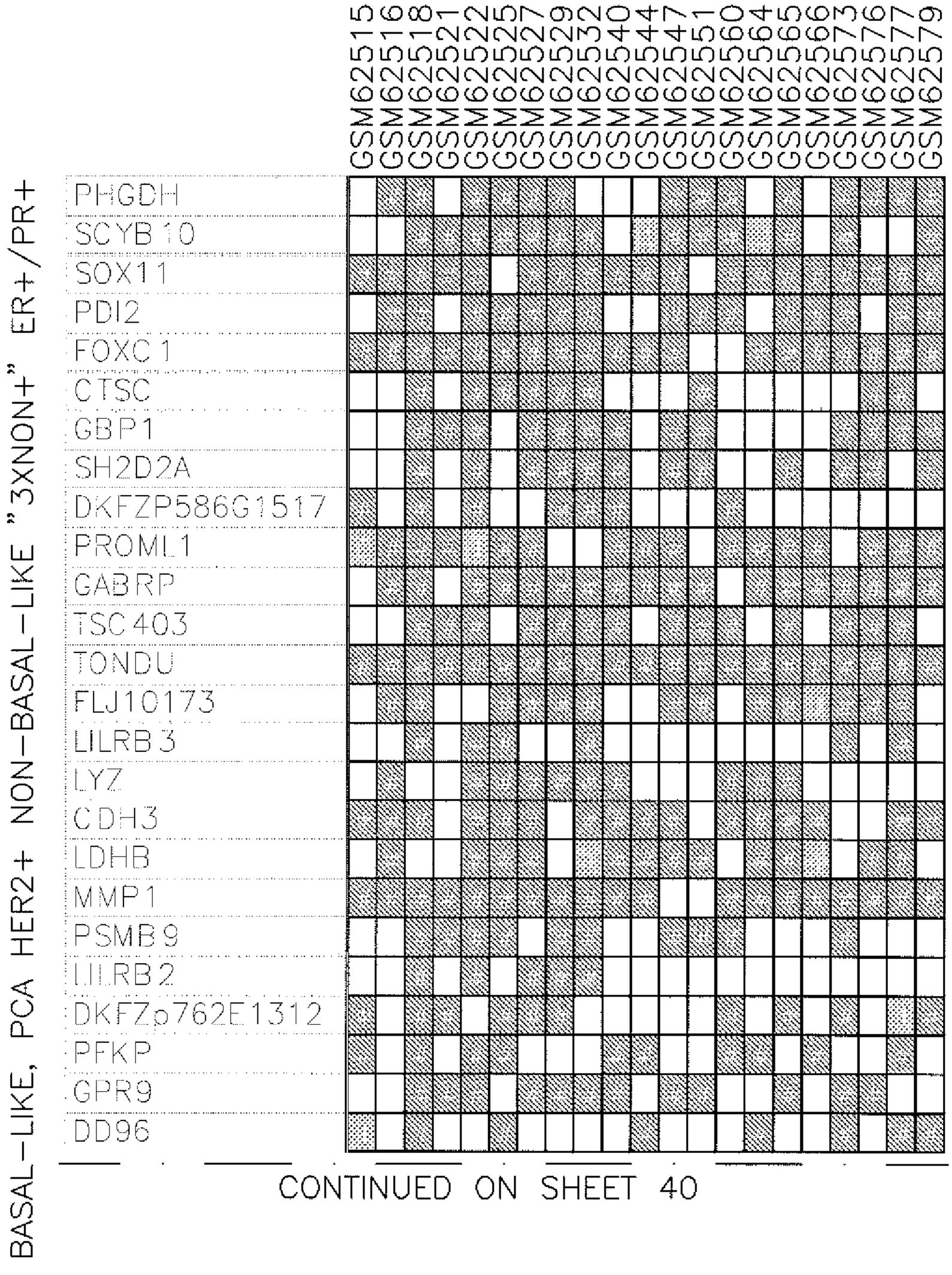


FIG. 4

CONTINUE ON SHEET 39

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

TOP 50 OVEREXPRESSED BASAL-LIKE GENES



TOP 50 OVEREXPRESSED BASAL-LIKE GENES.

FIG. 4

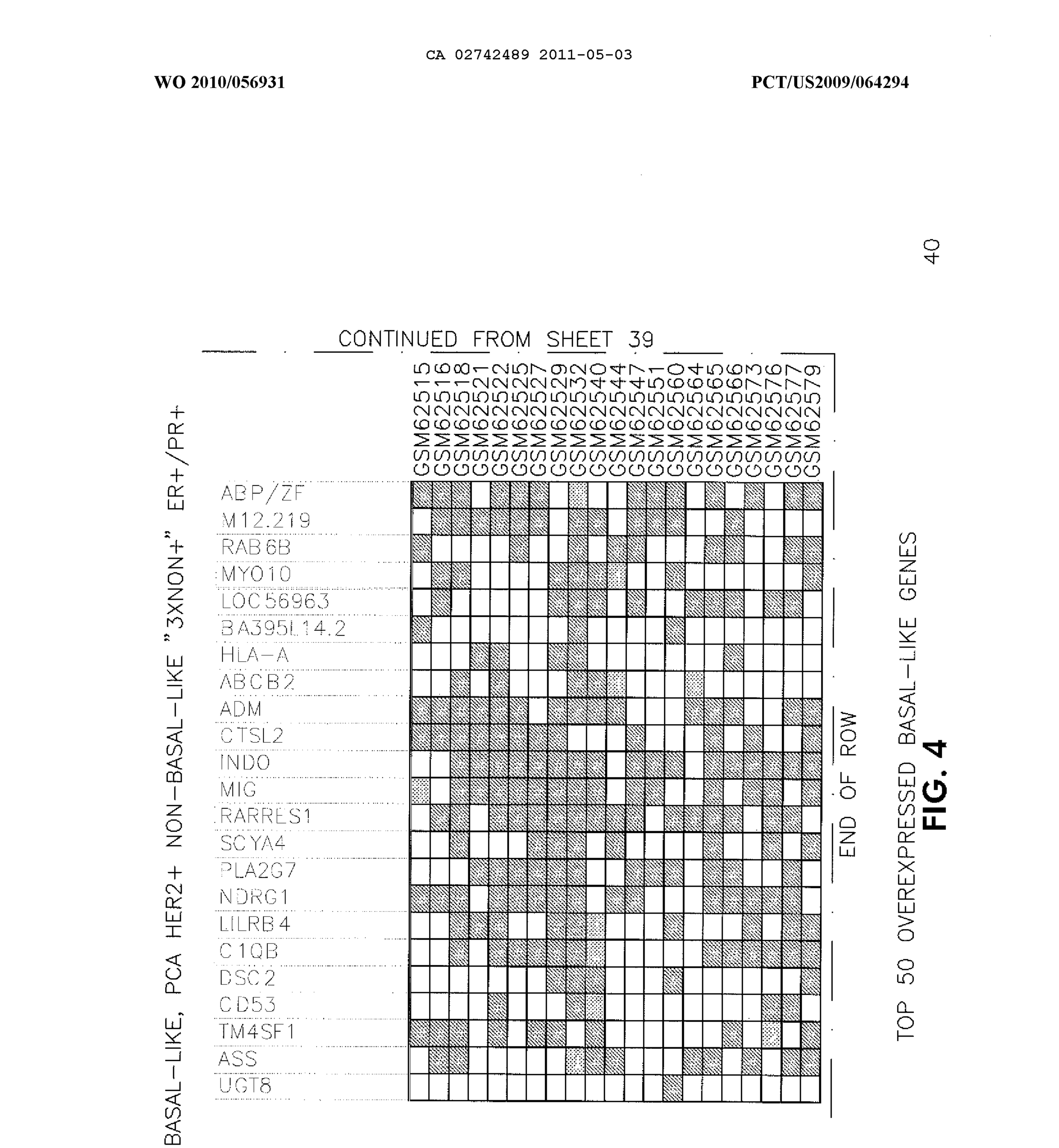
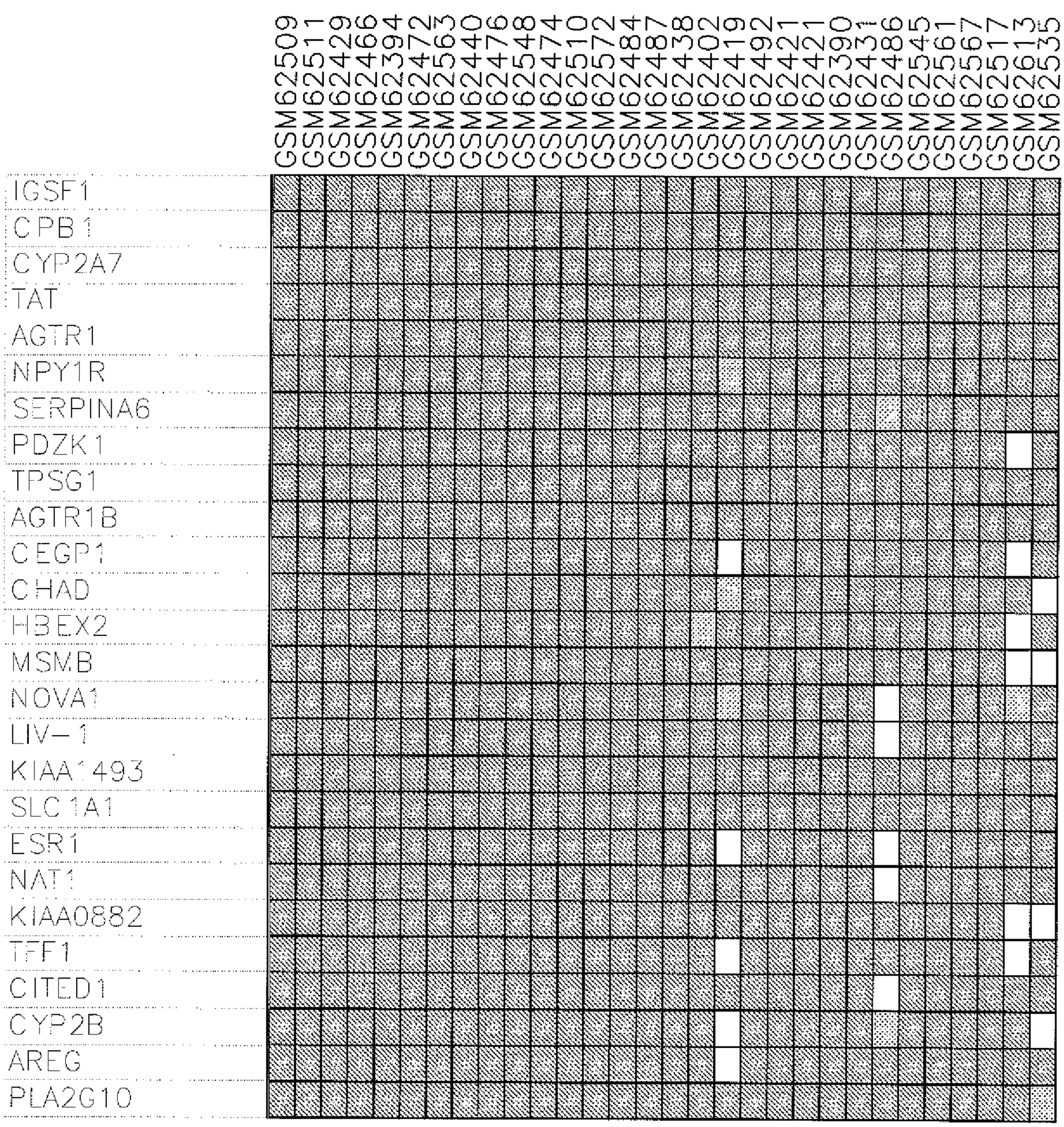

CONTINUED FROM SHEET 39
GSM625515
GSM625516
GSM625518
GSM625521
GSM625522
GSM625525
GSM625527
GSM625529
GSM625532
GSM625540
GSM625544
GSM625547
GSM625551
GSM625560
GSM625564
GSM625565
GSM625566
GSM625573
GSM625576
GSM625577
GSM625579
ABP/ZF
M12.219
RAB6B
MY010
LOC56963
BA395L14.2
HLA-A
ABCB2
ADM
CTSL2
INDO
MIG
RARRES1
SCYA4
PLA2G7
NDRG1
LILRB4
C1QB
DSC2
CD53
TM4SF1
ASS
UGT8
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+
END OF ROW
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 4

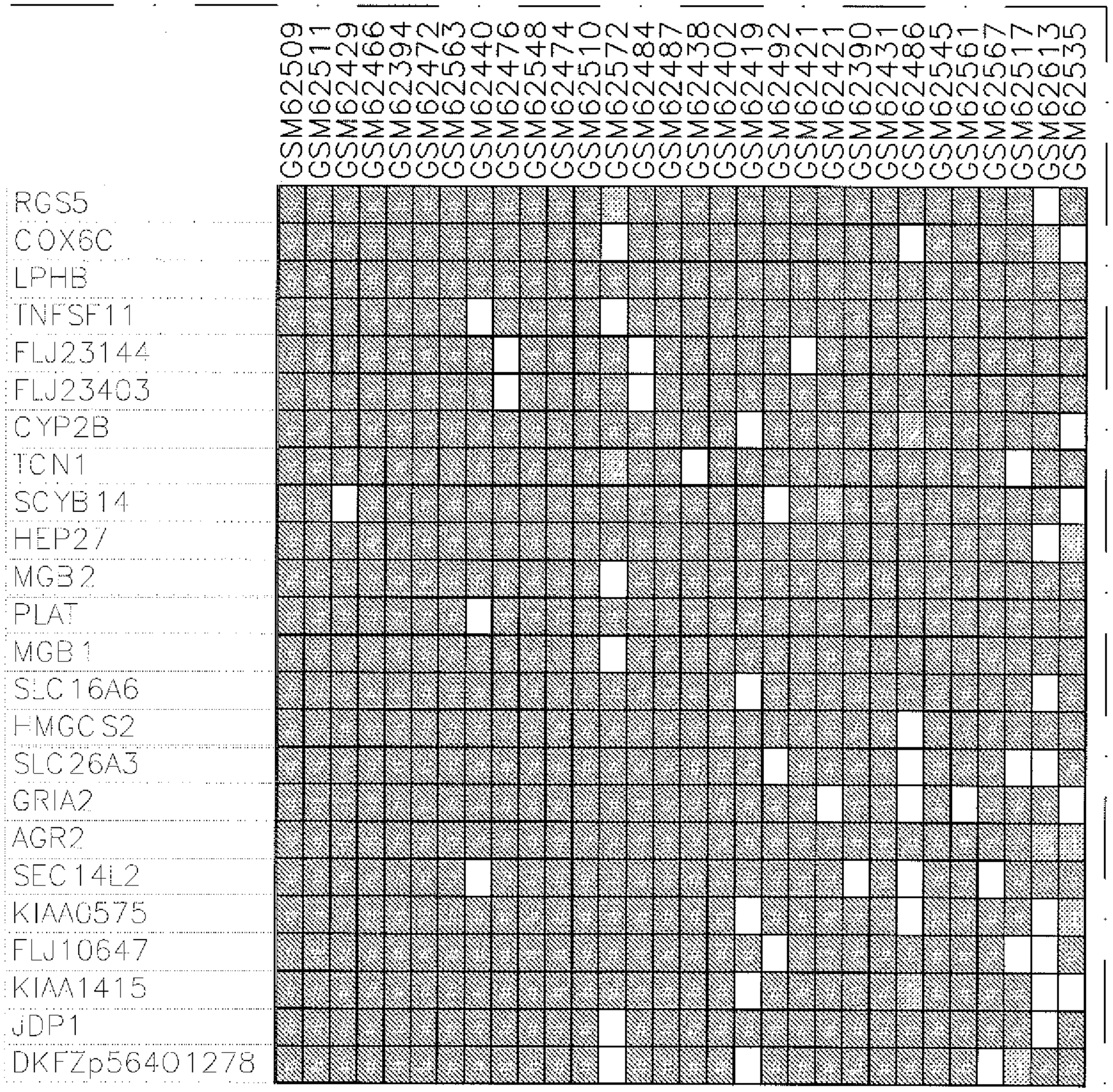


CONTINUED ON SHEET 42

FIG. 4

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

CONTINUED FROM SHEET 41



TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

FIG. 4

CONTINUE ON SHEET 43

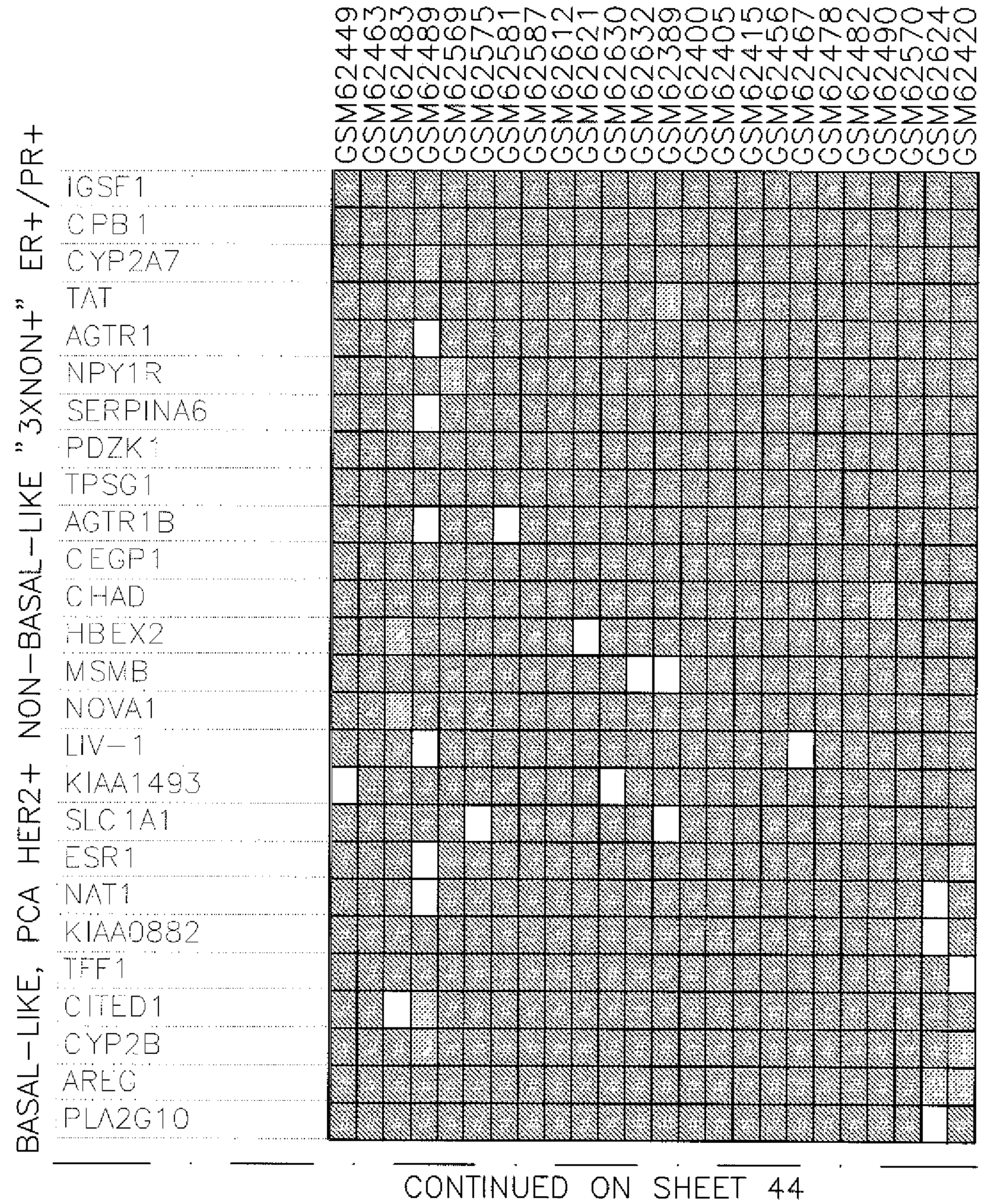
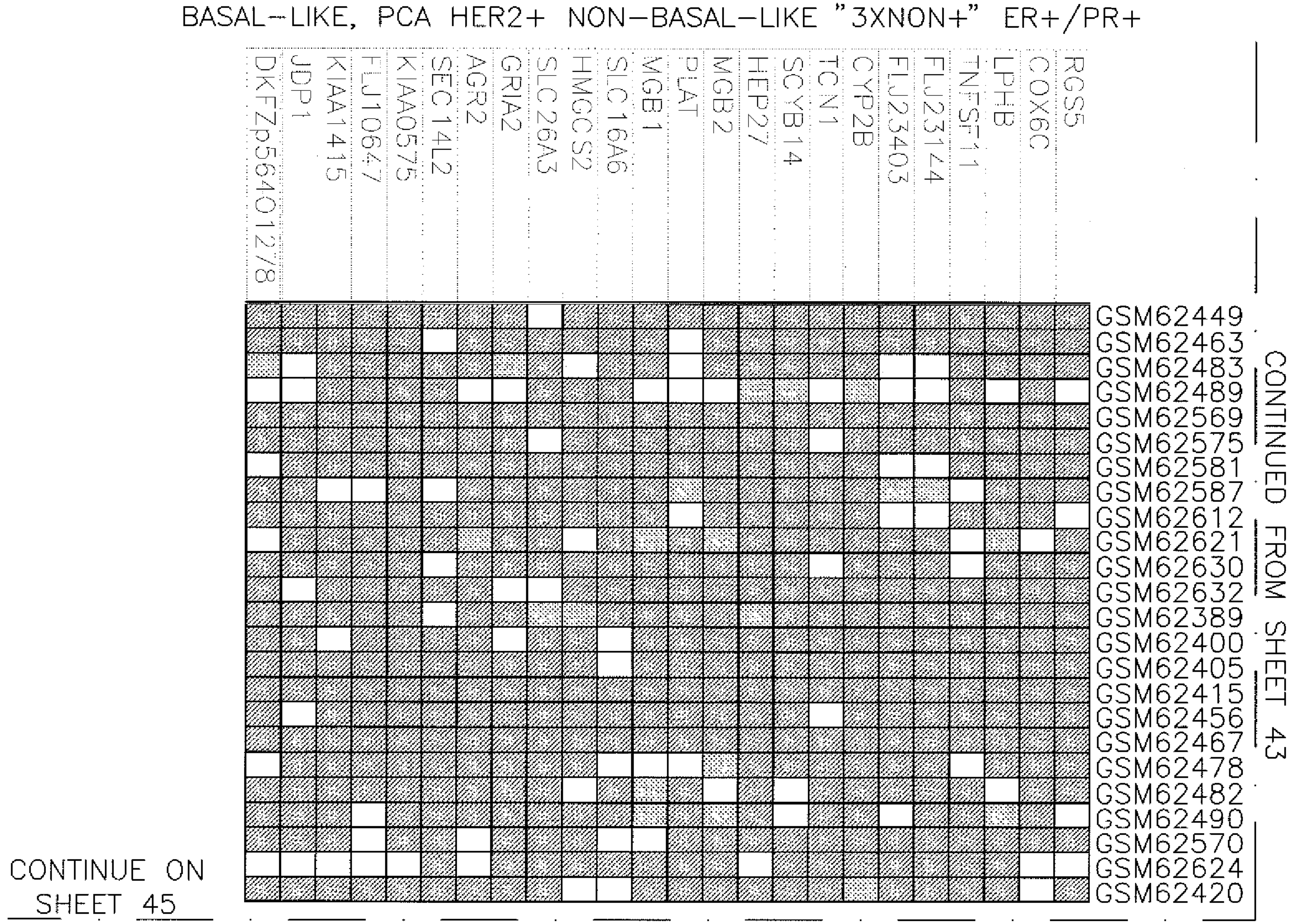


FIG. 4

CONTINUED ON SHEET 44



TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG. 4

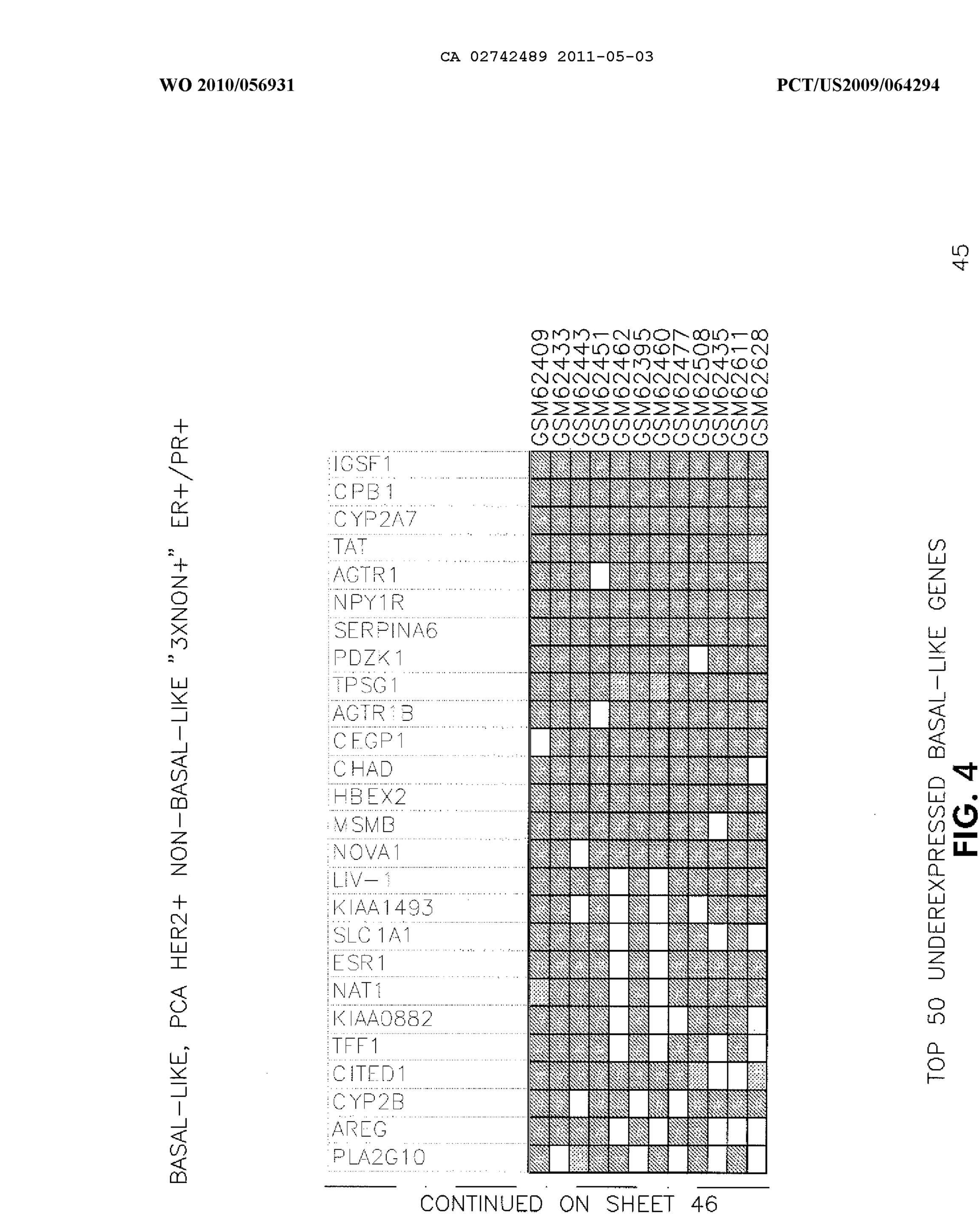


FIG. 4

CONTINUED ON SHEET 46

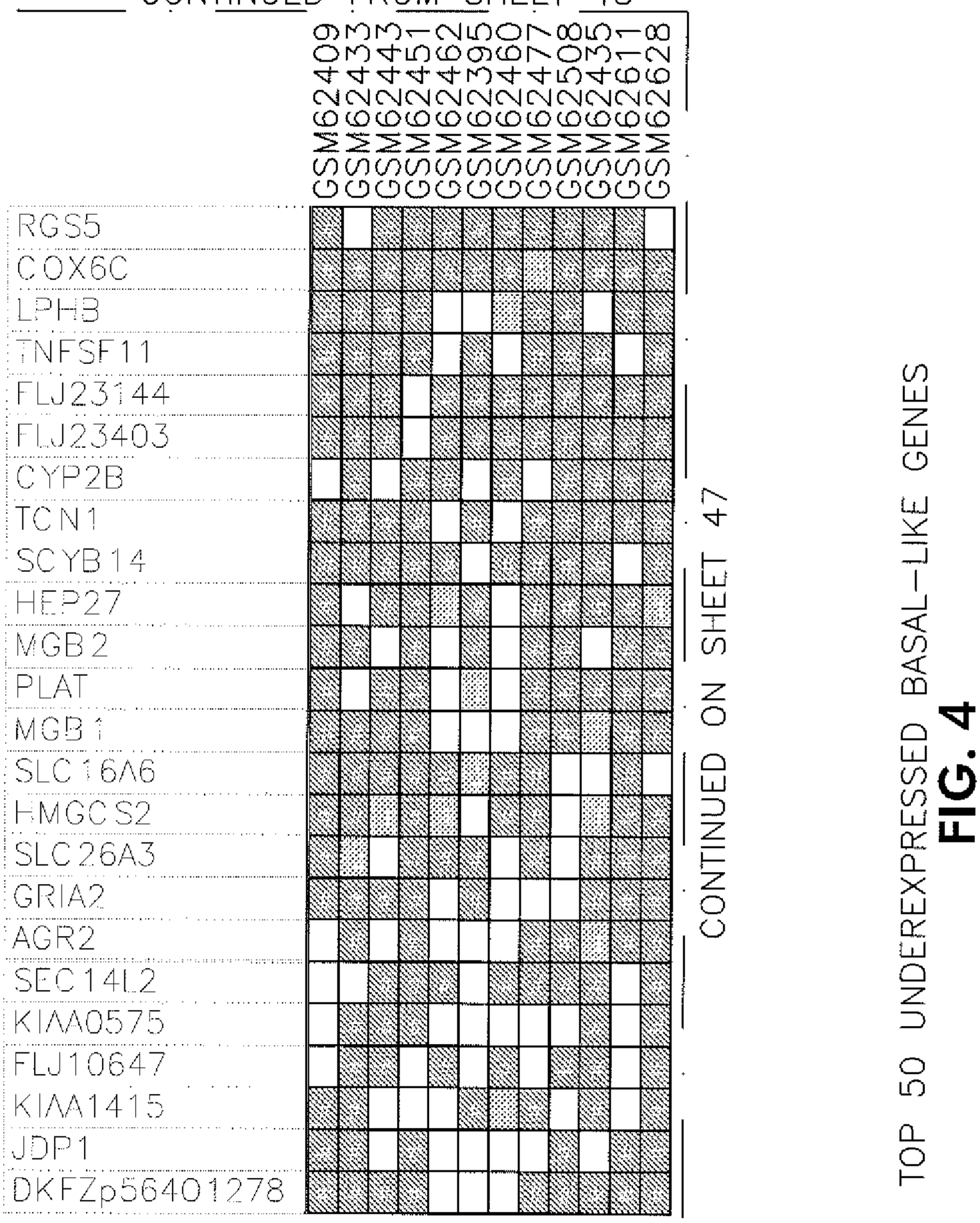

CONTINUED FROM SHEET 45
6240933-1
6243451-2
6246239
6246075
6250843
6243561
6261628
GSM
RGS5
COX6C
LPHB
TNFSF11
FLJ23144
FLJ23403
CYP2B
TCN1
SCYB14
HEP27
MGB2
PLAT
MGB1
SLC16A6
HMGCS2
SLC26A3
GRIA2
AGR2
SEC14L2
KIAA0575
FLJ10647
KIAA1415
JDP1
DKFZp56401278
CONTINUED ON SHEET 47
TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE "3XNON+" ER+/PR+ NON-BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE, PCA HER2+

FIG. 4

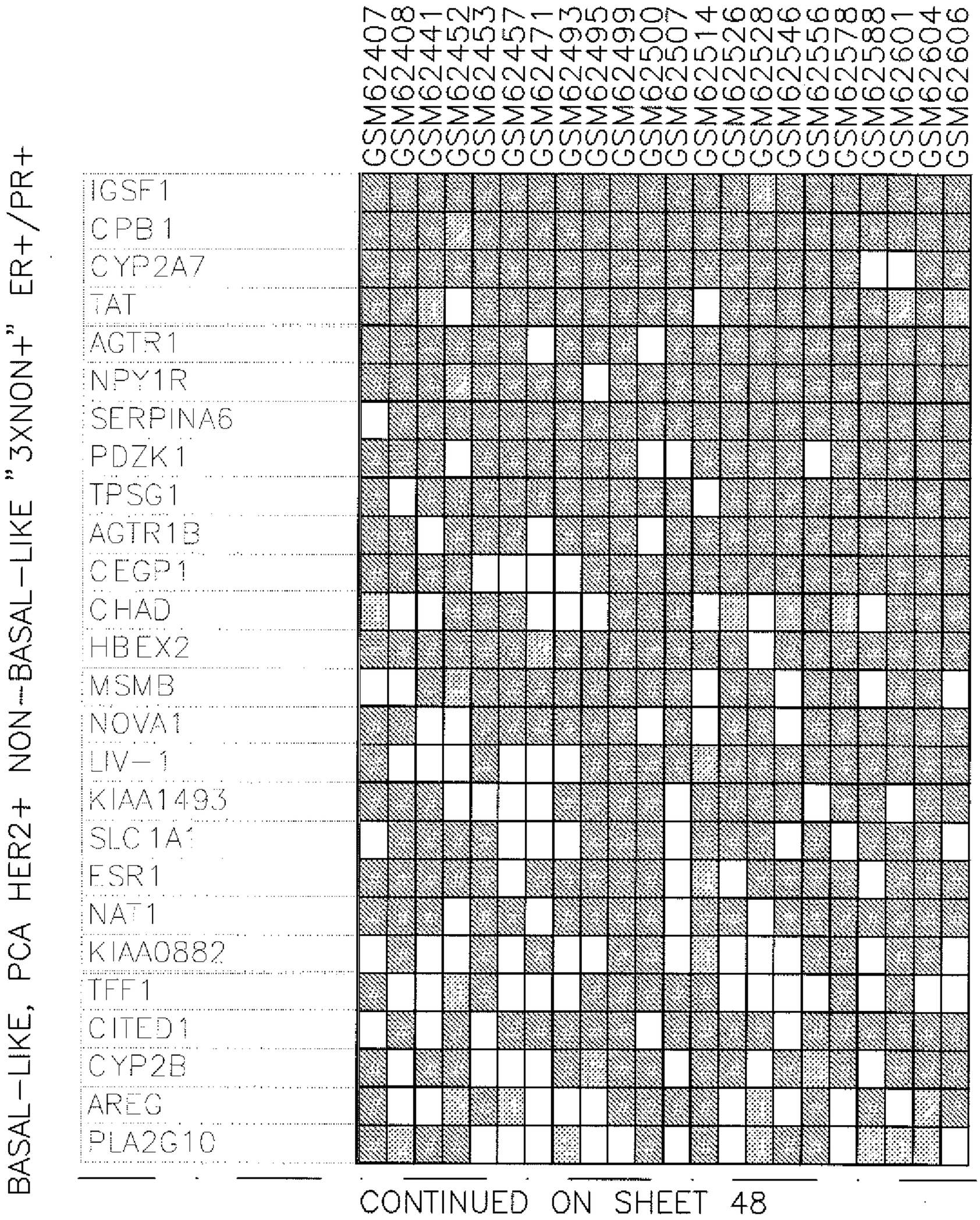
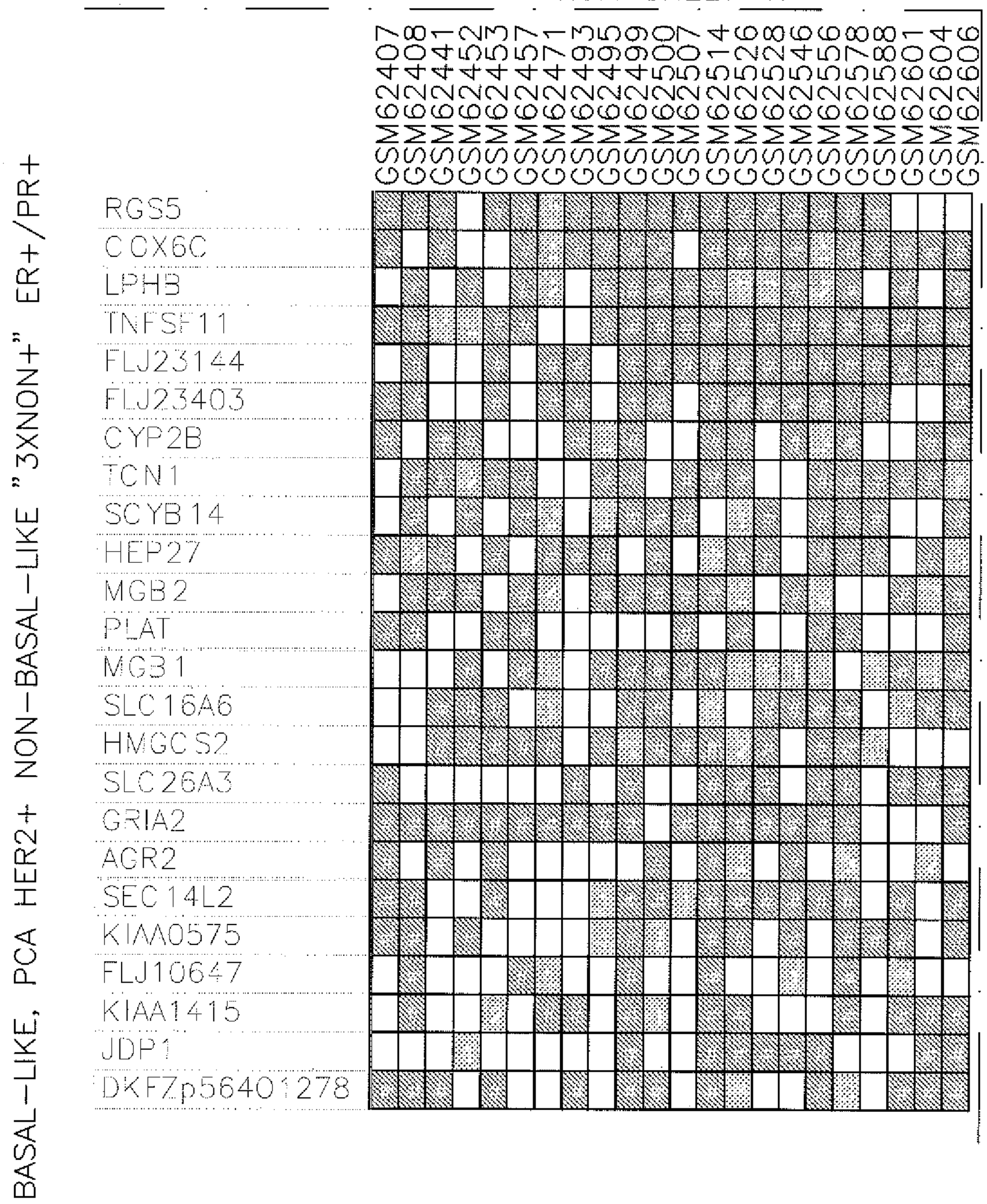


FIG. 4

CONTINUED ON SHEET 48

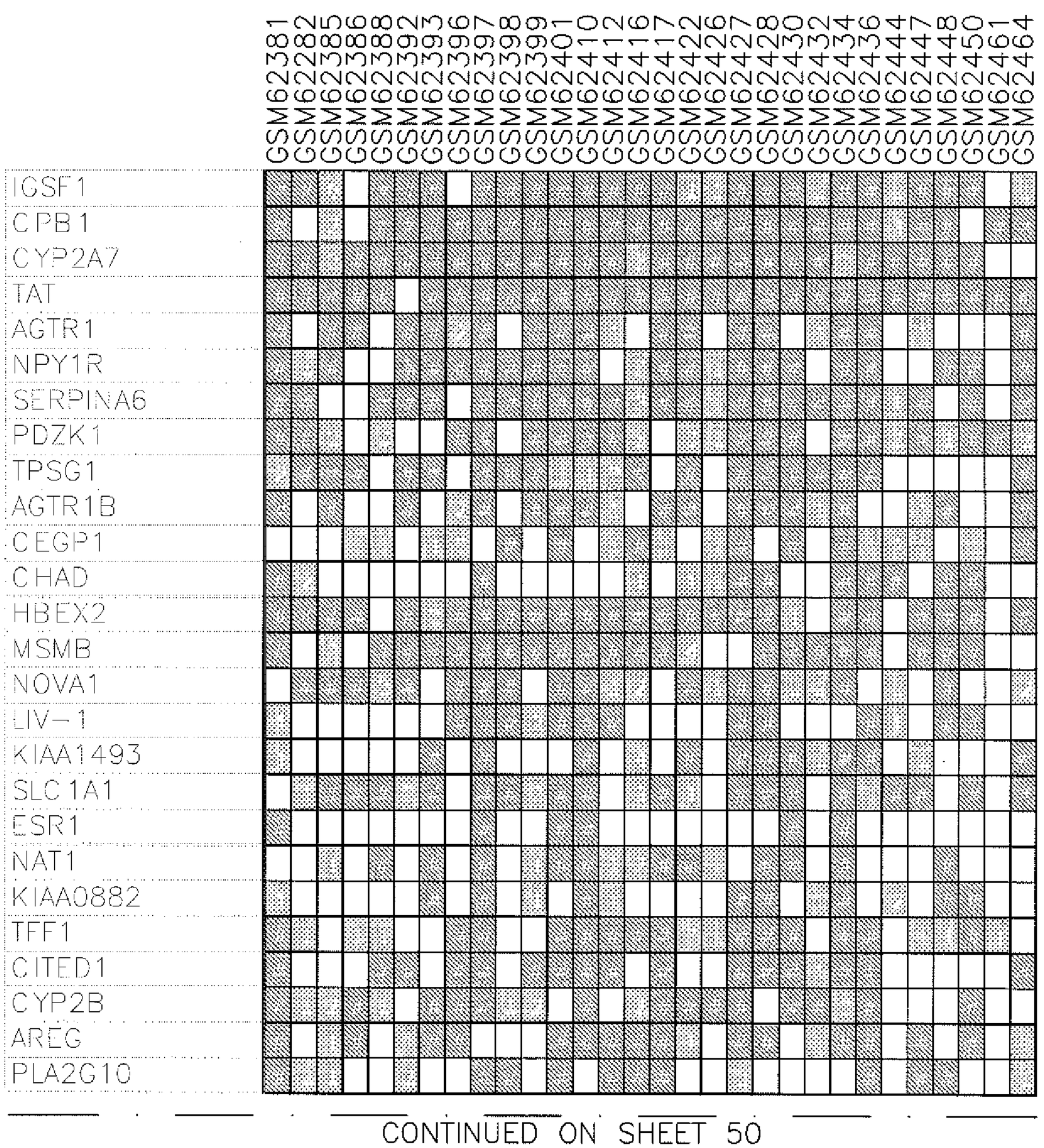
CONTINUED FROM SHEET 47



CONTINUED ON SHEET 49

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG. 4



CONTINUED ON SHEET 50

FIG. 4

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

CONTINUED FROM SHEET 49

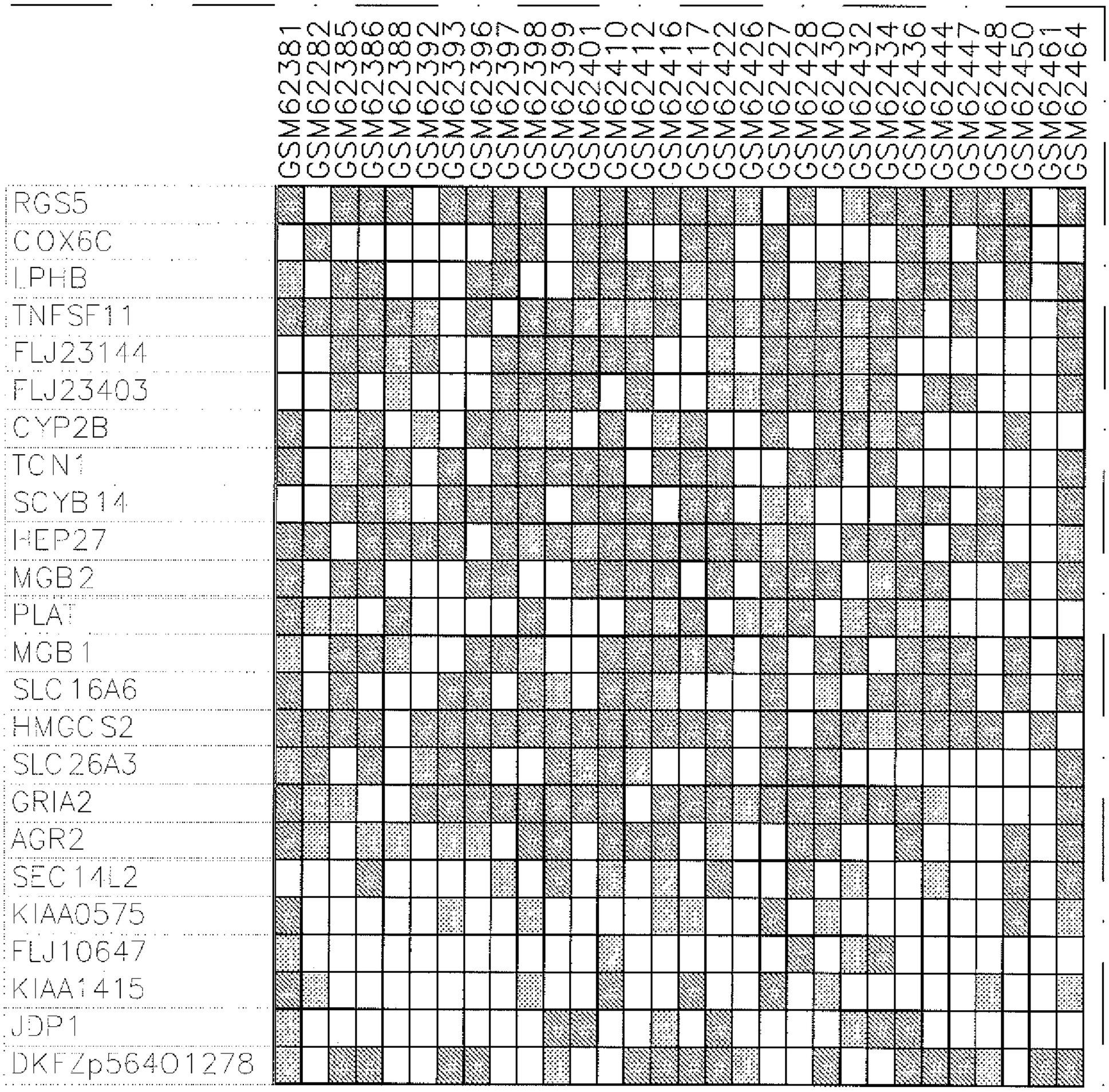
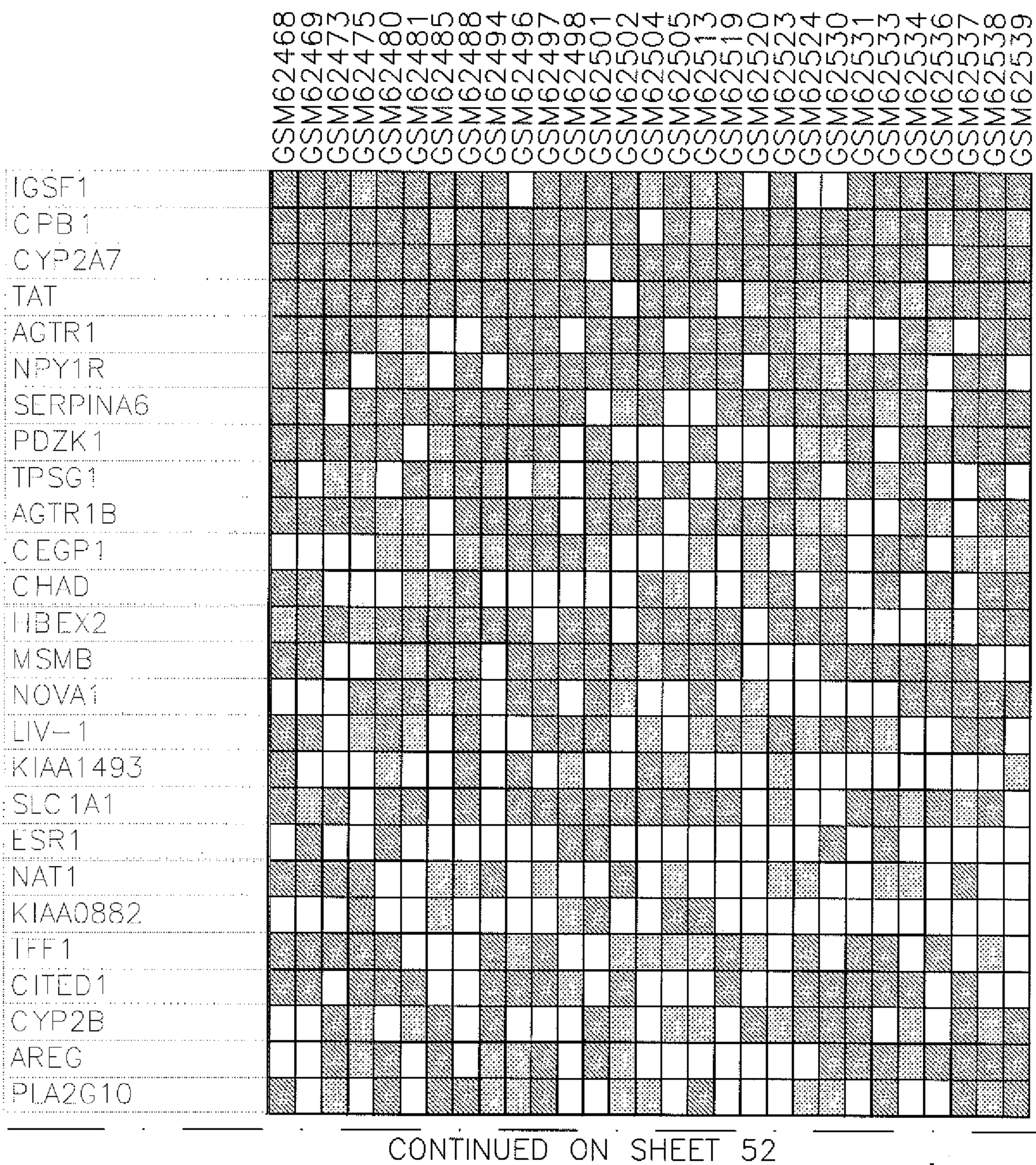


FIG. 4

CONTINUED ON SHEET 51

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+



CONTINUED ON SHEET 52

FIG. 4

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

CONTINUED FROM SHEET 51

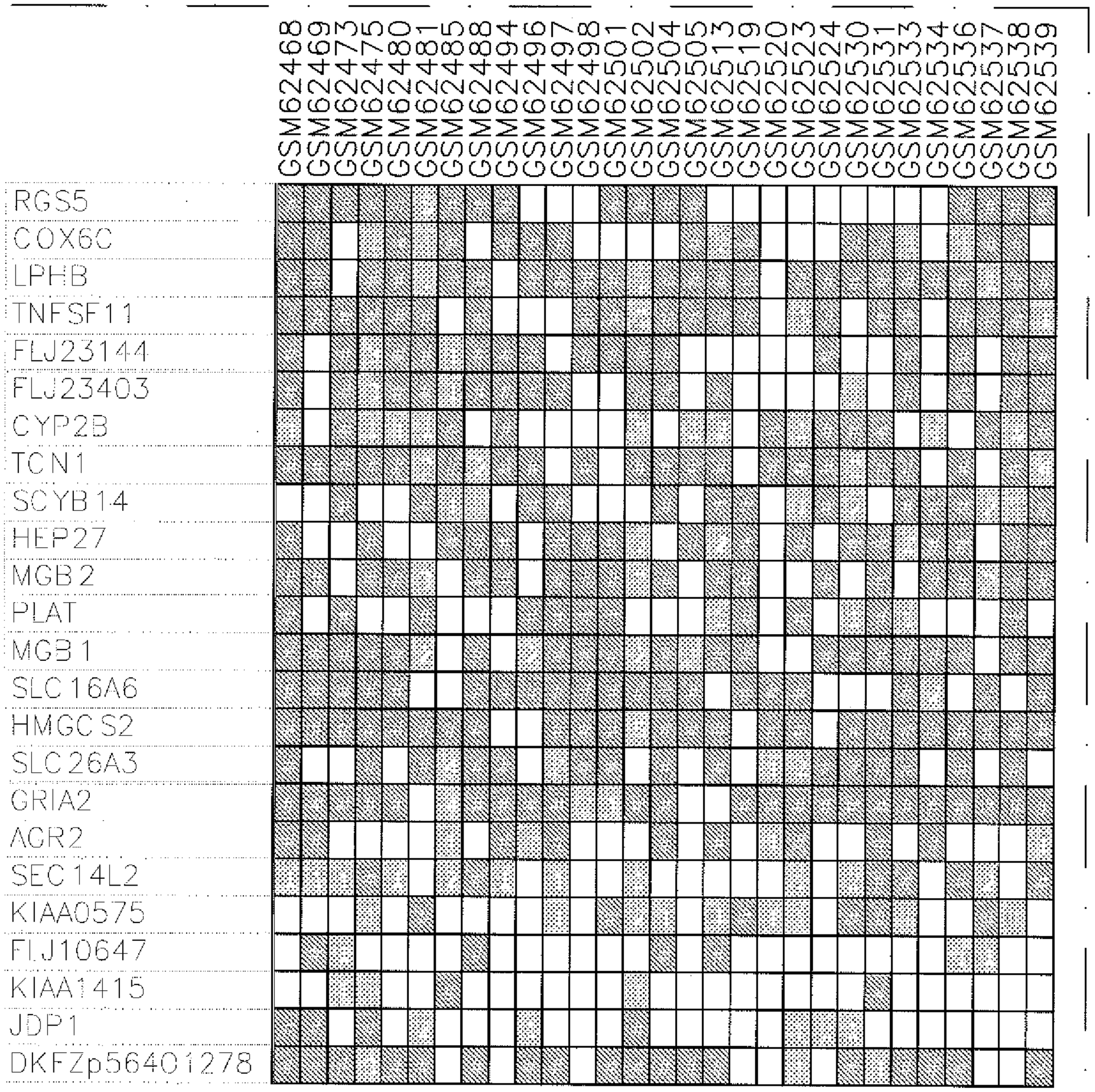


FIG. 4

CONTINUED ON SHEET 53

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

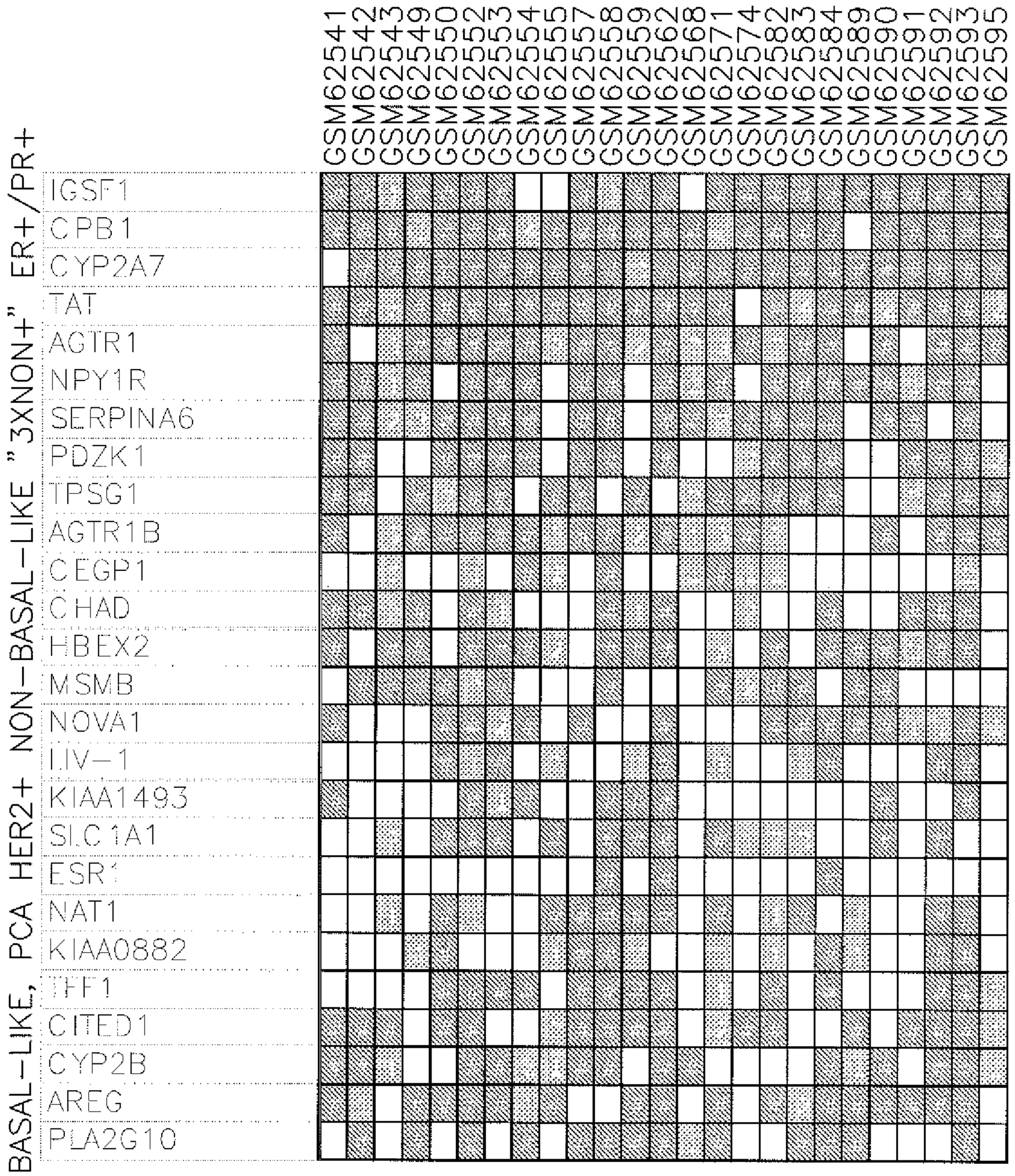


FIG. 4

CONTINUED ON SHEET 54

CONTINUED FROM SHEET 53

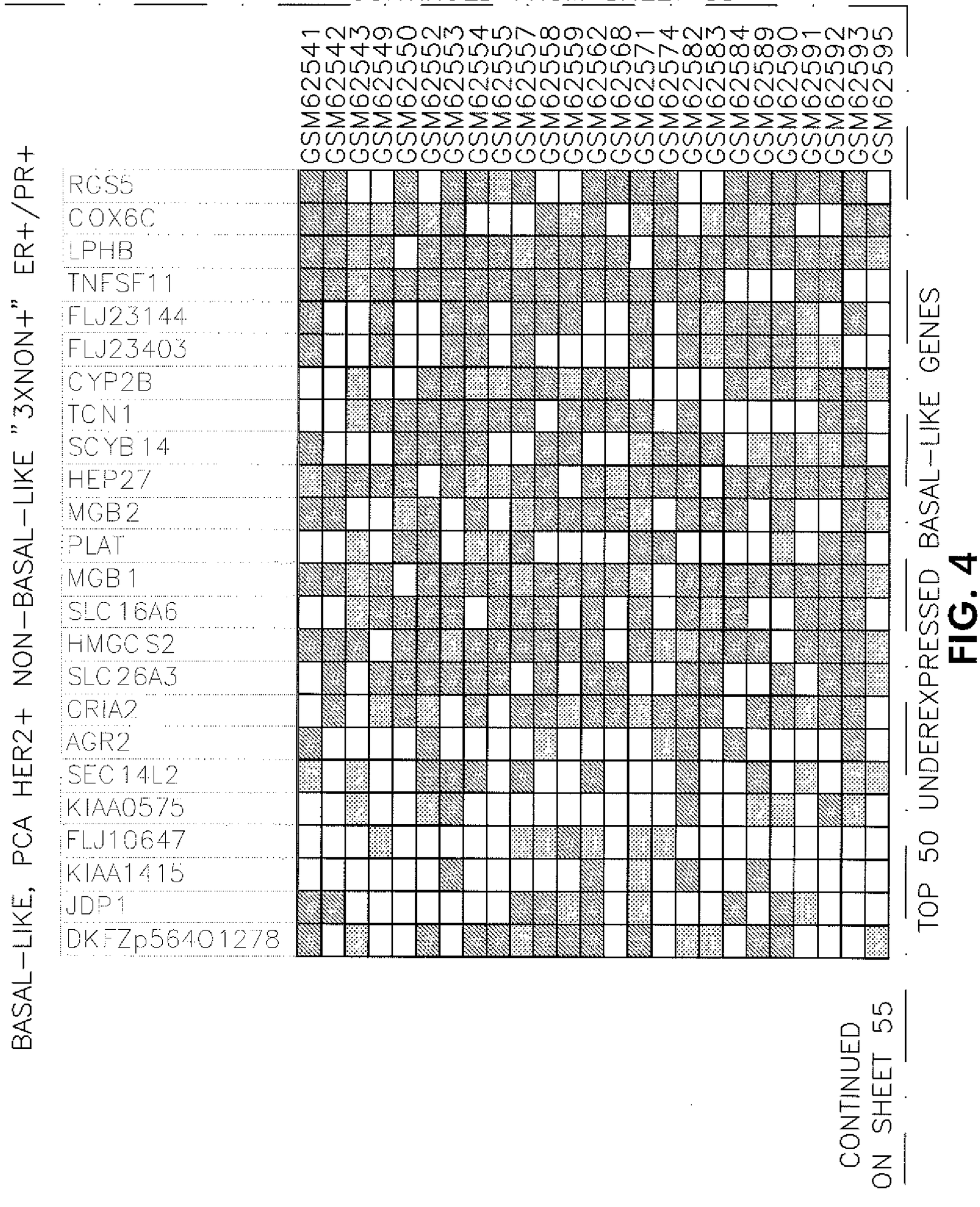


FIG. 4

CONTINUED ON SHEET 55

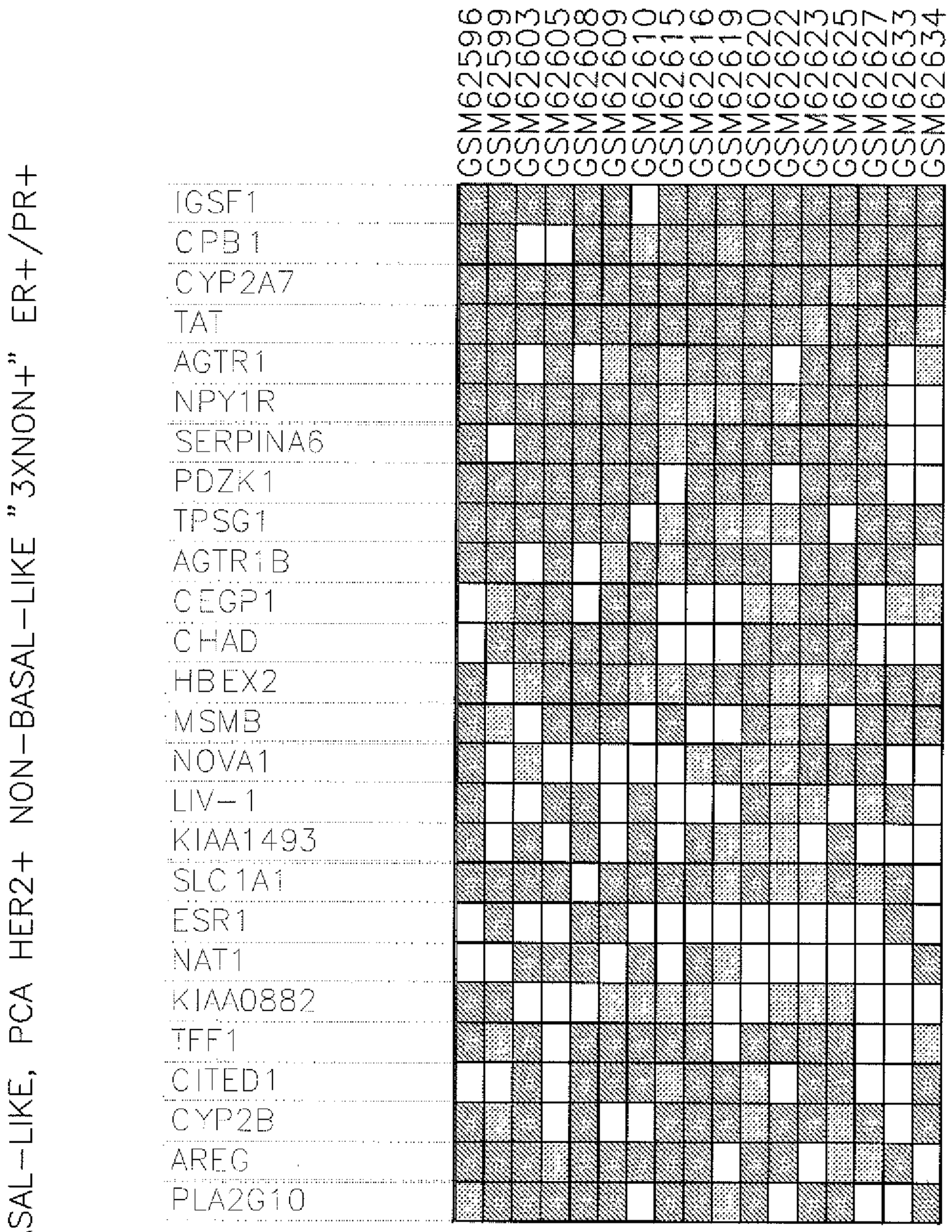
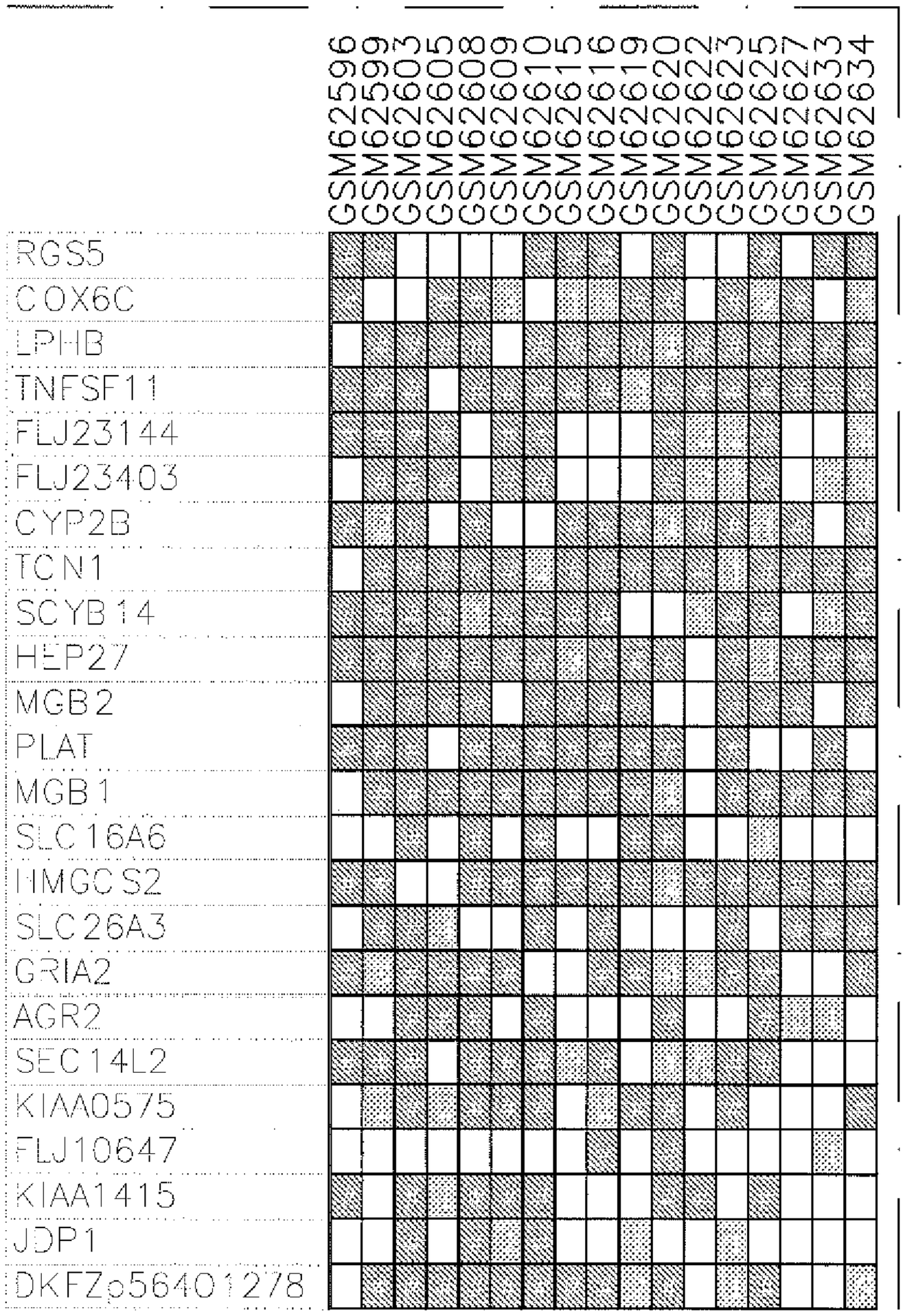


FIG. 4

CONTINUED ON SHEET 56

CONTINUED FROM SHEET 55

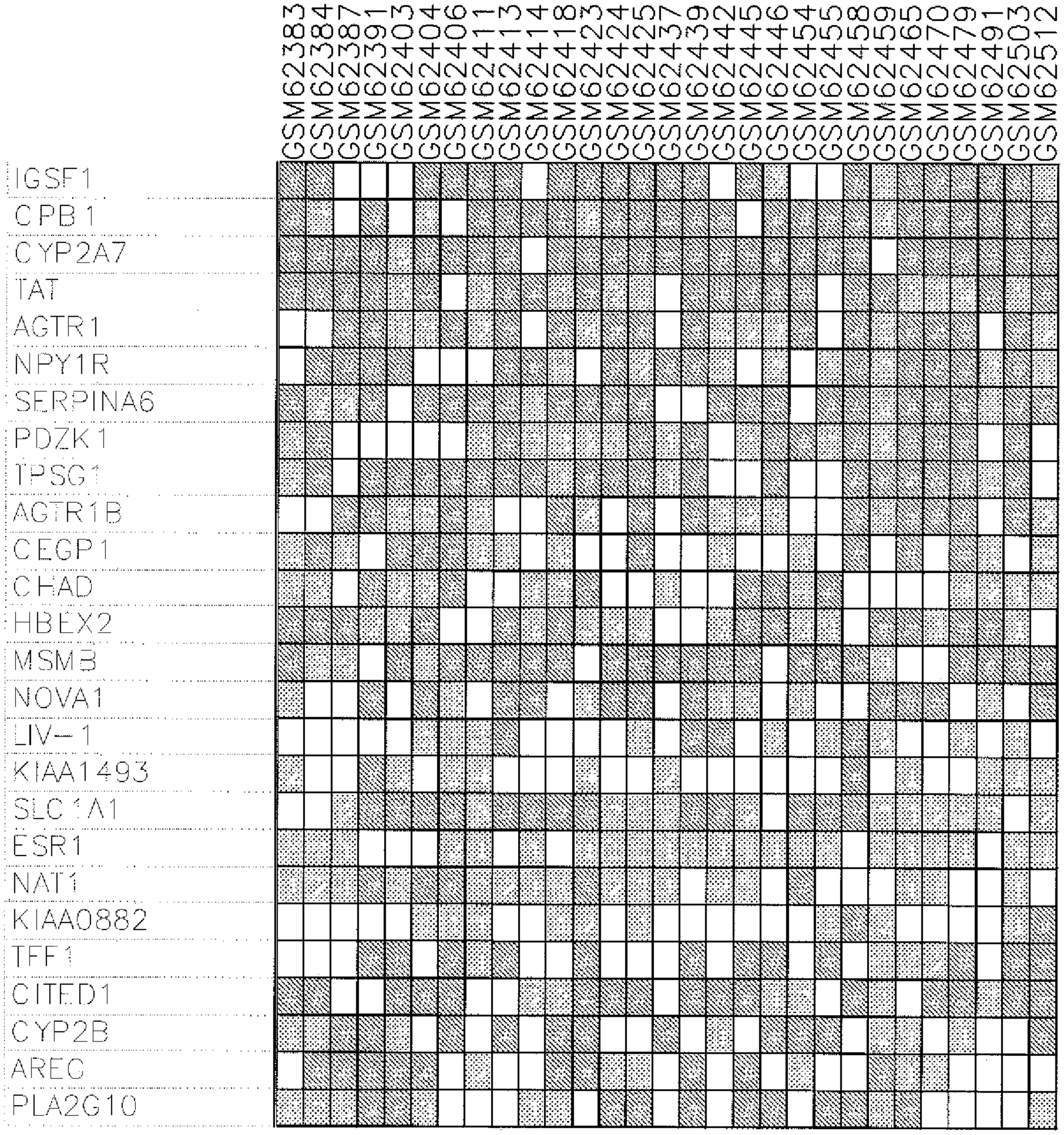


CONTINUED ON SHEET 57

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

FIG. 4



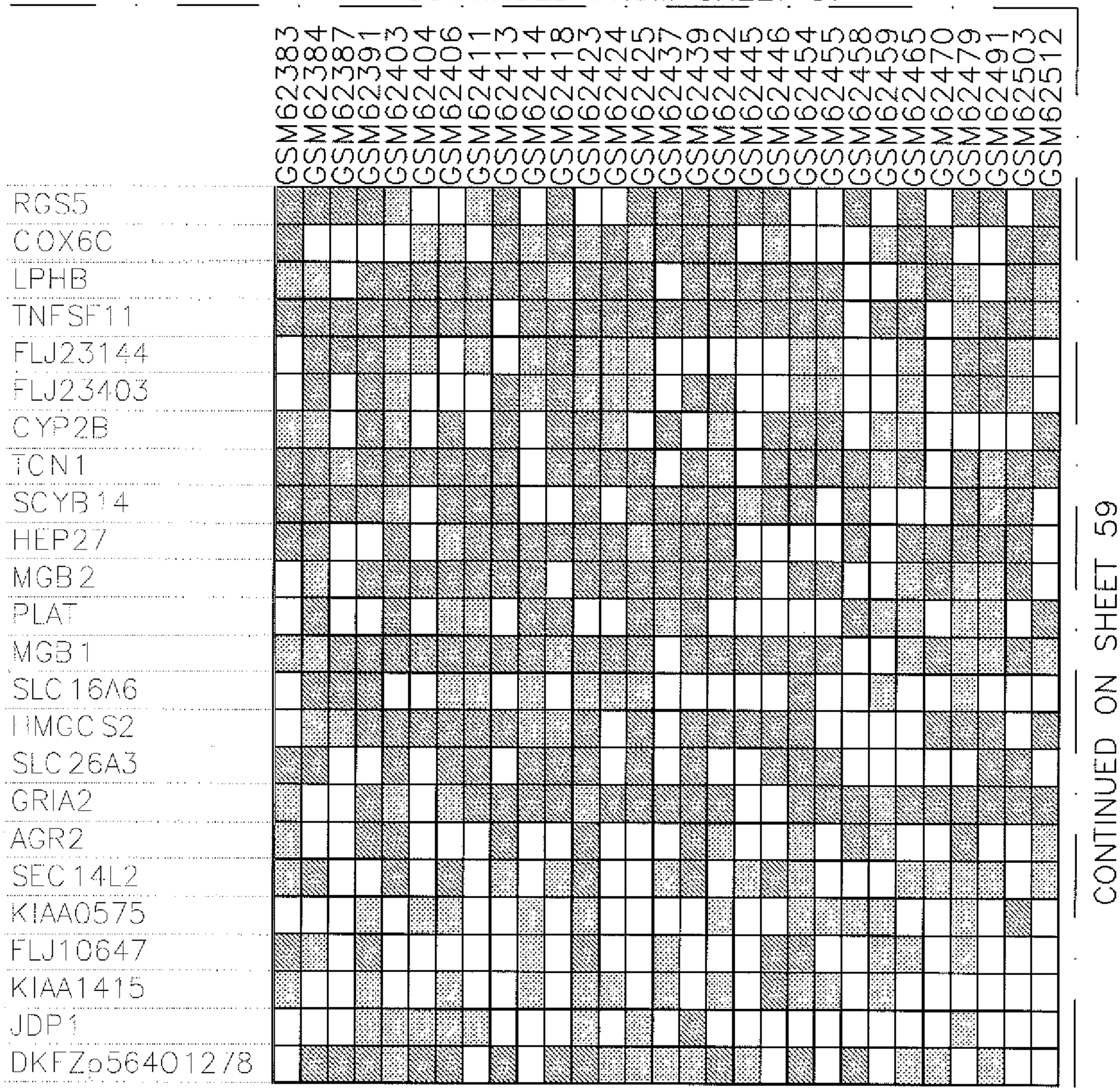
CONTINUED ON SHEET 58

FIG. 4

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

58

CONTINUED FROM SHEET 57



CONTINUED ON SHEET 59

FIG. 4

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2+ NON-BASAL-LIKE "3XNON+" ER+/PR+

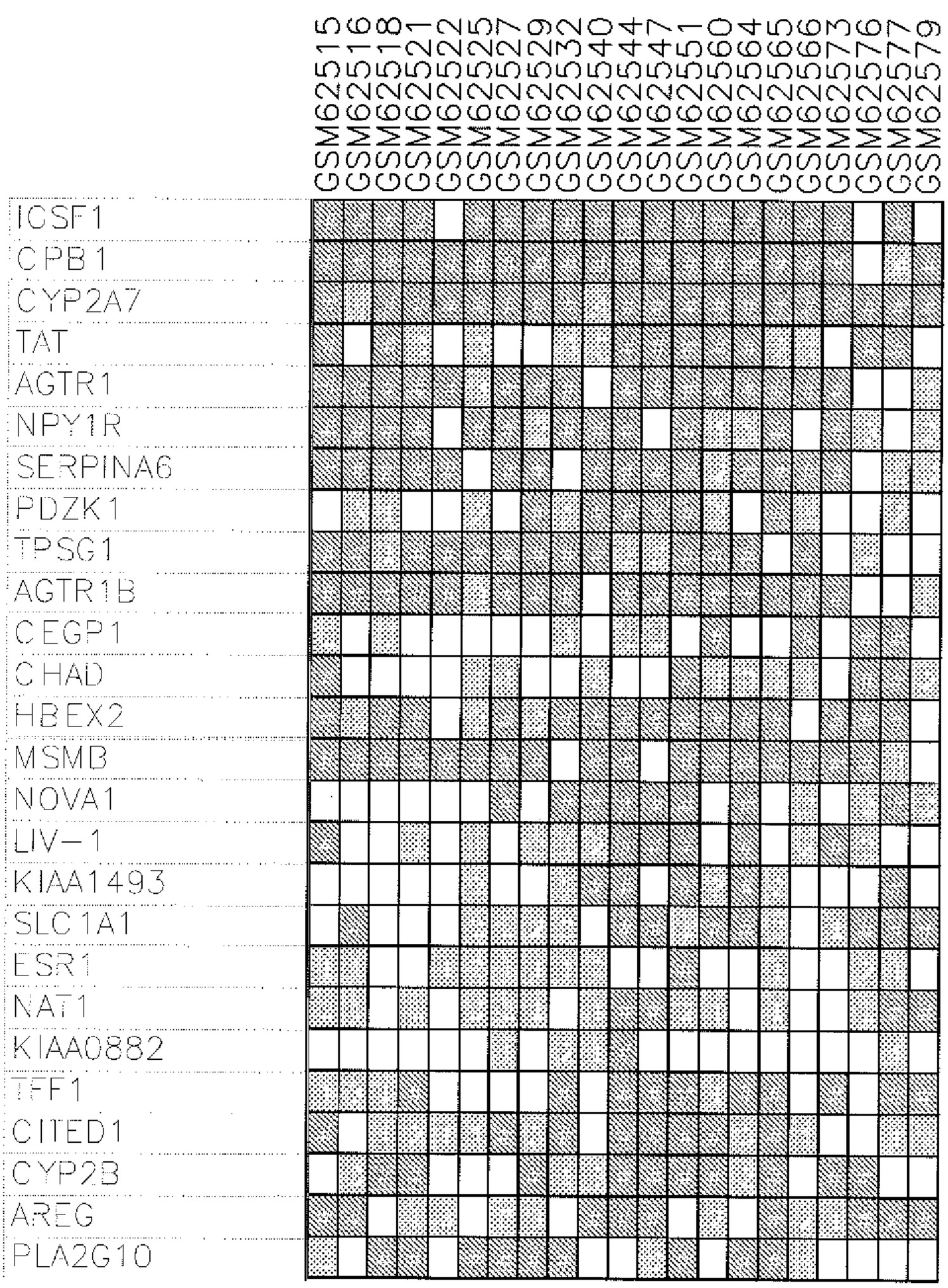


FIG. 4

CONTINUED ON SHEET 60

CONTINUED FROM SHEET 59

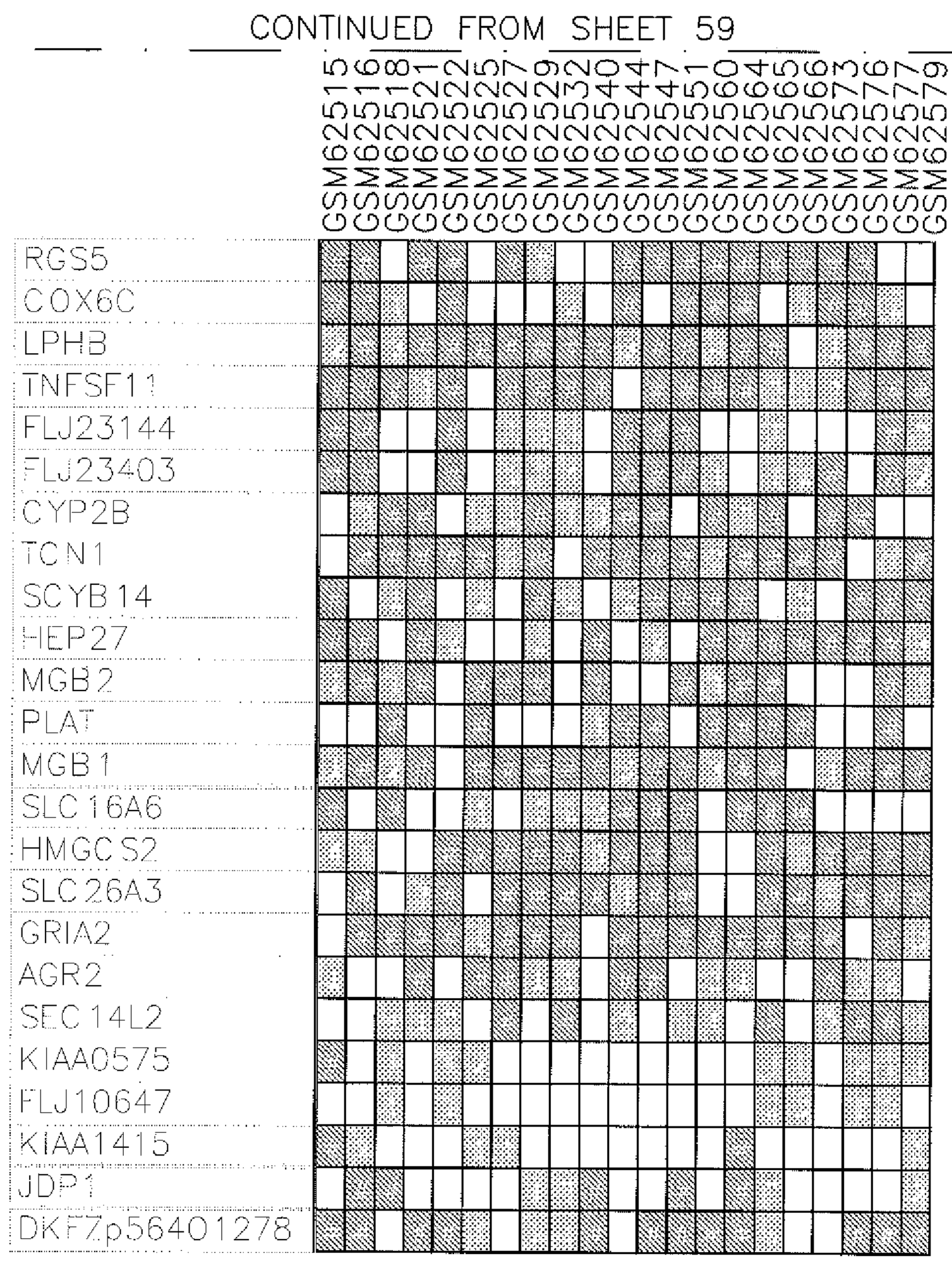


FIG. 4

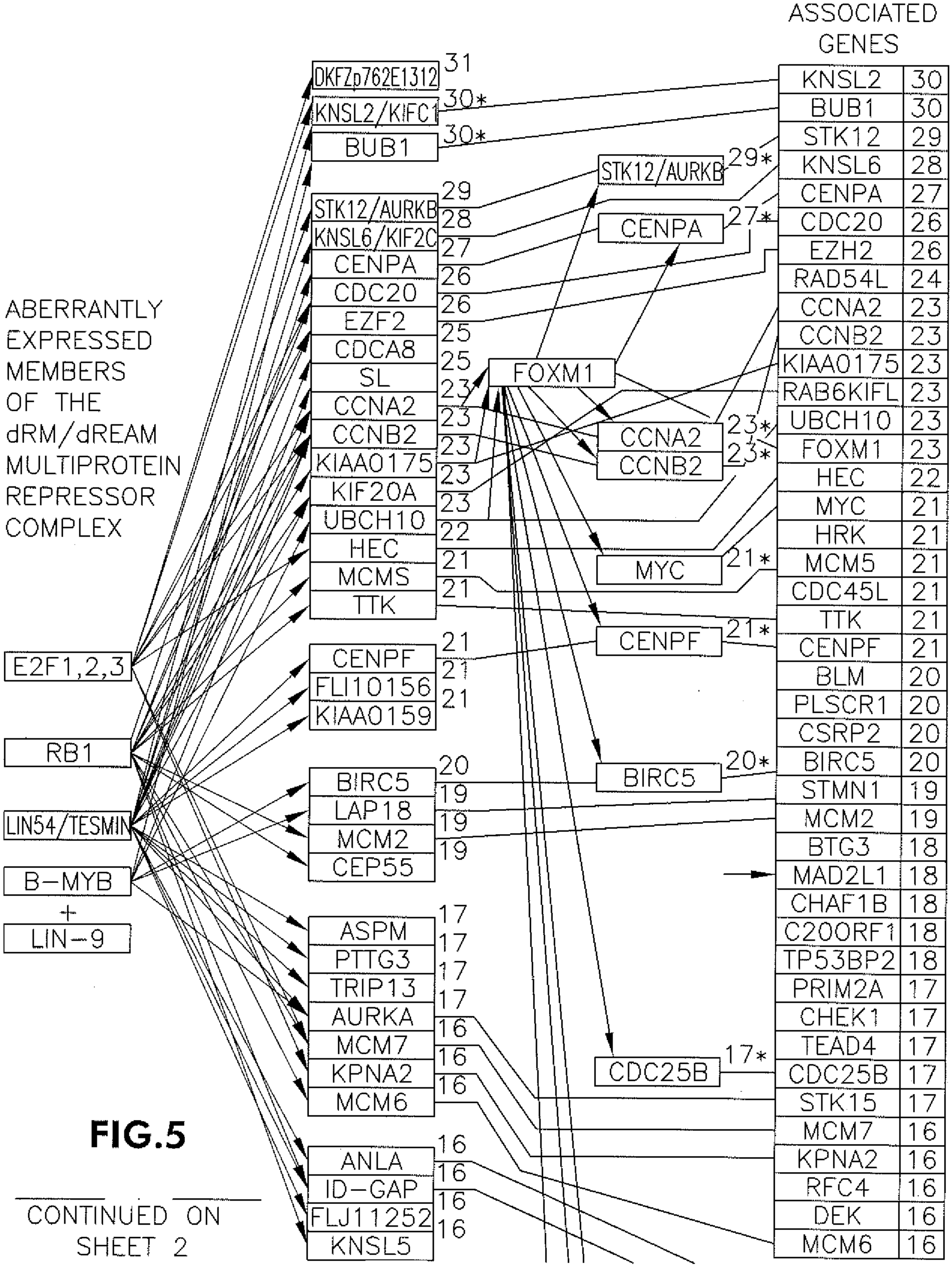


FIG.5

CONTINUED ON SHEET 2

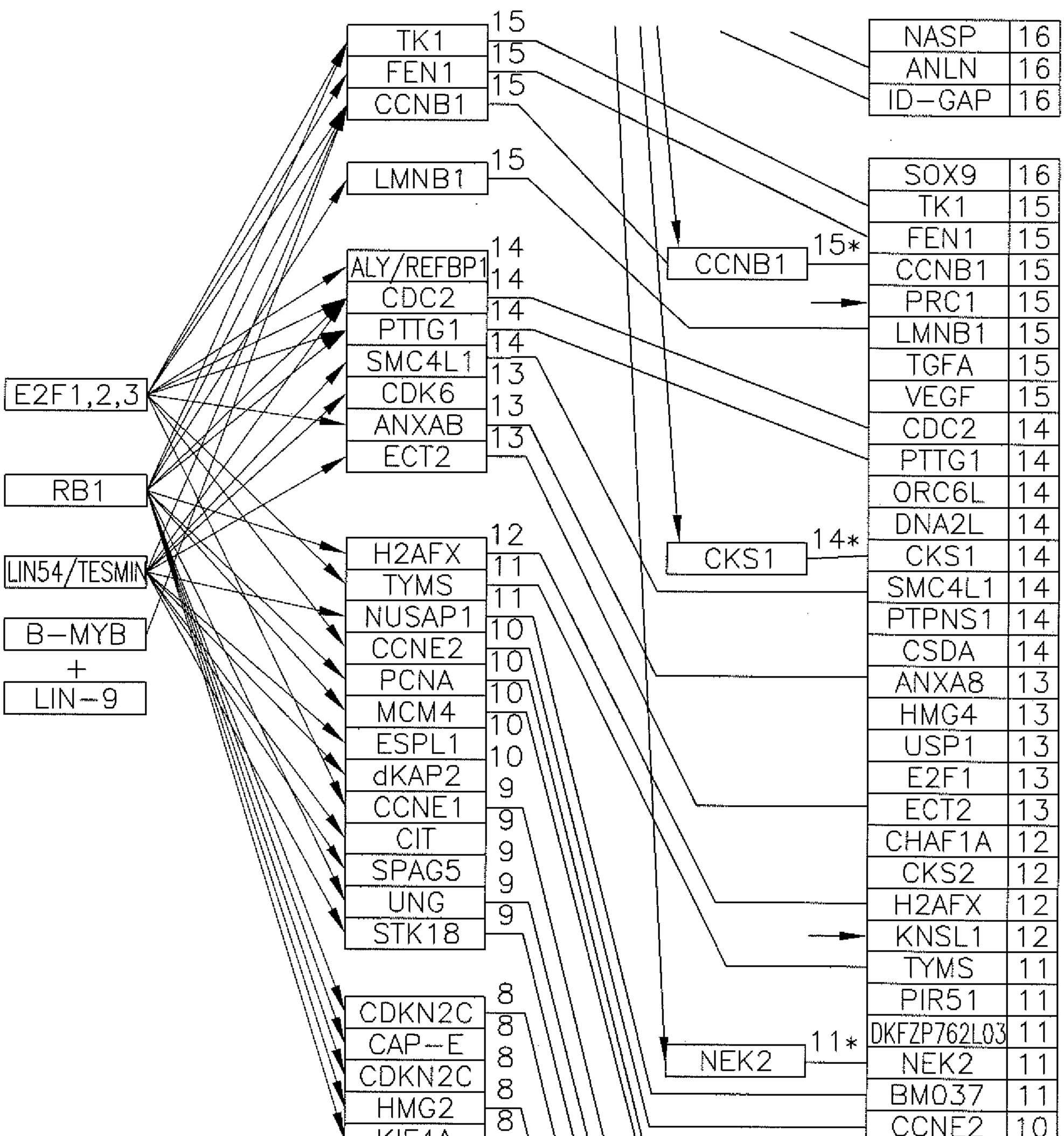
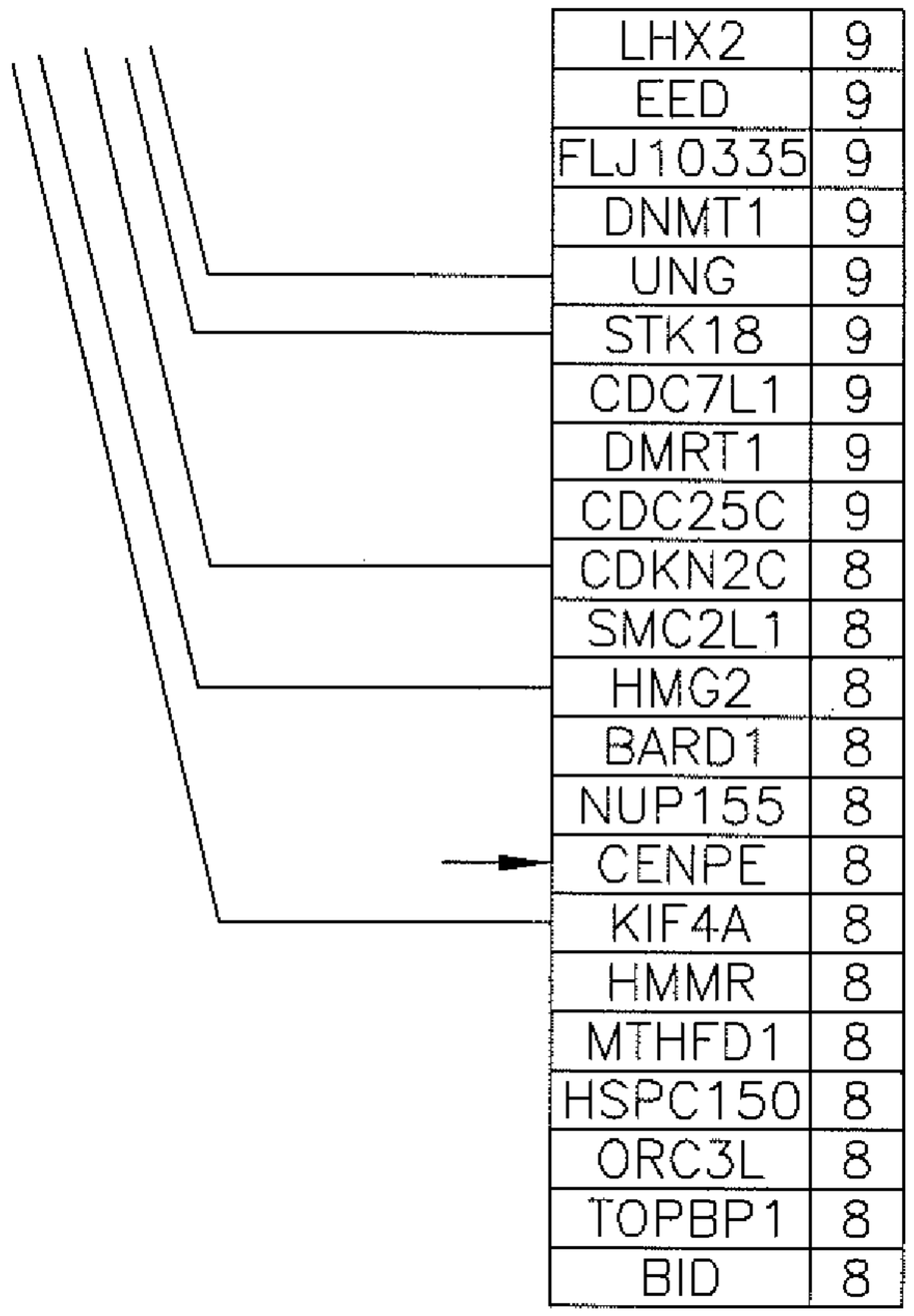


FIG.5

CONTINUED ON SHEET 3



LHX2	9
EED	9
FLJ10335	9
DNMT1	9
UNG	9
STK18	9
CDC7L1	9
DMRT1	9
CDC25C	9
CDKN2C	8
SMC2L1	8
HMG2	8
BARD1	8
NUP155	8
CENPE	8
KIF4A	8
HMMR	8
MTHFD1	8
HSPC150	8
ORC3L	8
TOPBP1	8
BID	8

FIG.5

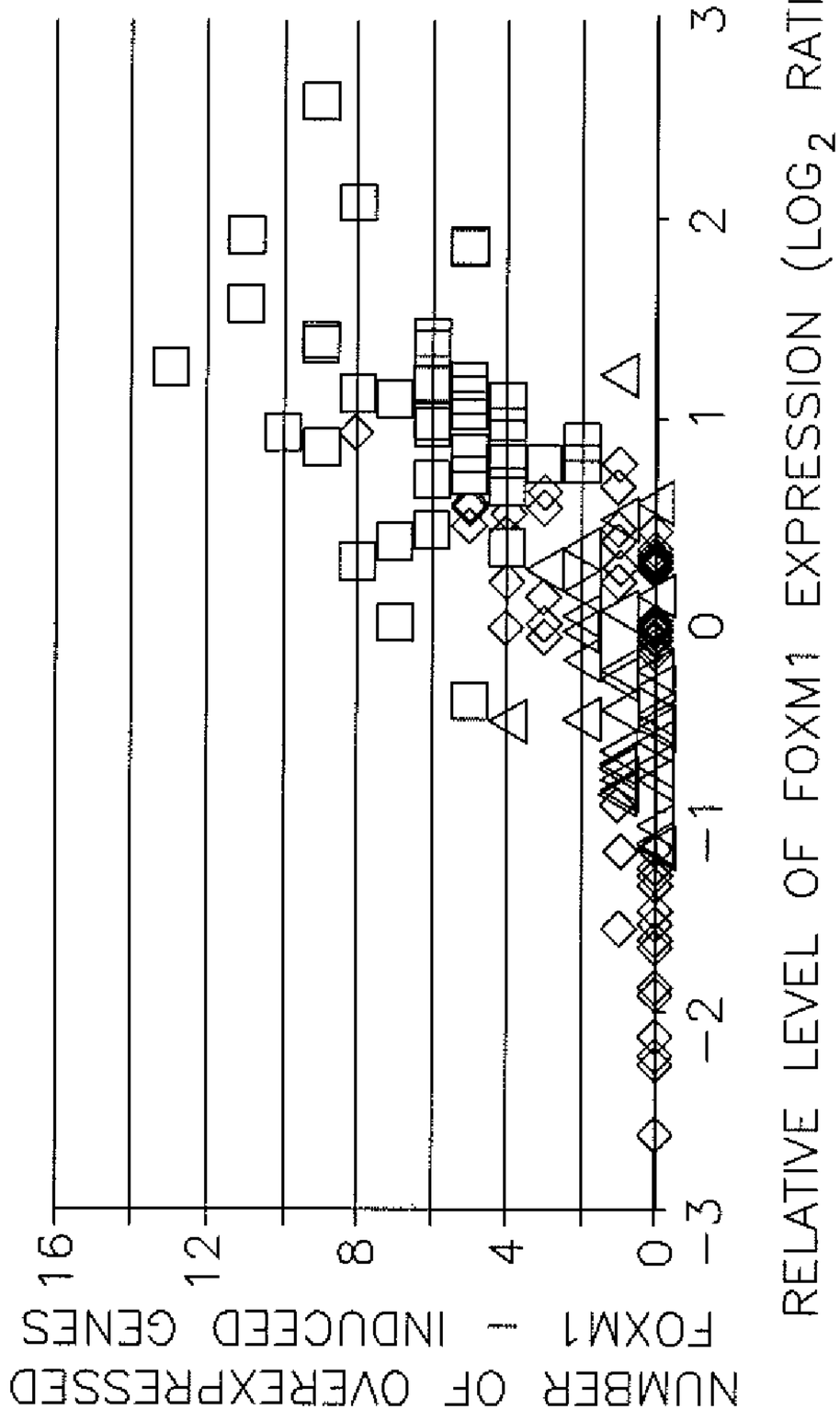

NUMBER OF OVEREXPRESSED FOXM1 - INDUCED GENES
16
12
8
4
0
−3
−2
−1
0
1
2
3
RELATIVE LEVEL OF FOXM1 EXPRESSION (LOG2 RATIO)
◇ NON−BASAL−LIKE TUMORS
△ NON−ERGO BASAL−LIKE TUMORS
□ ERGO TUMORS

FIG.6

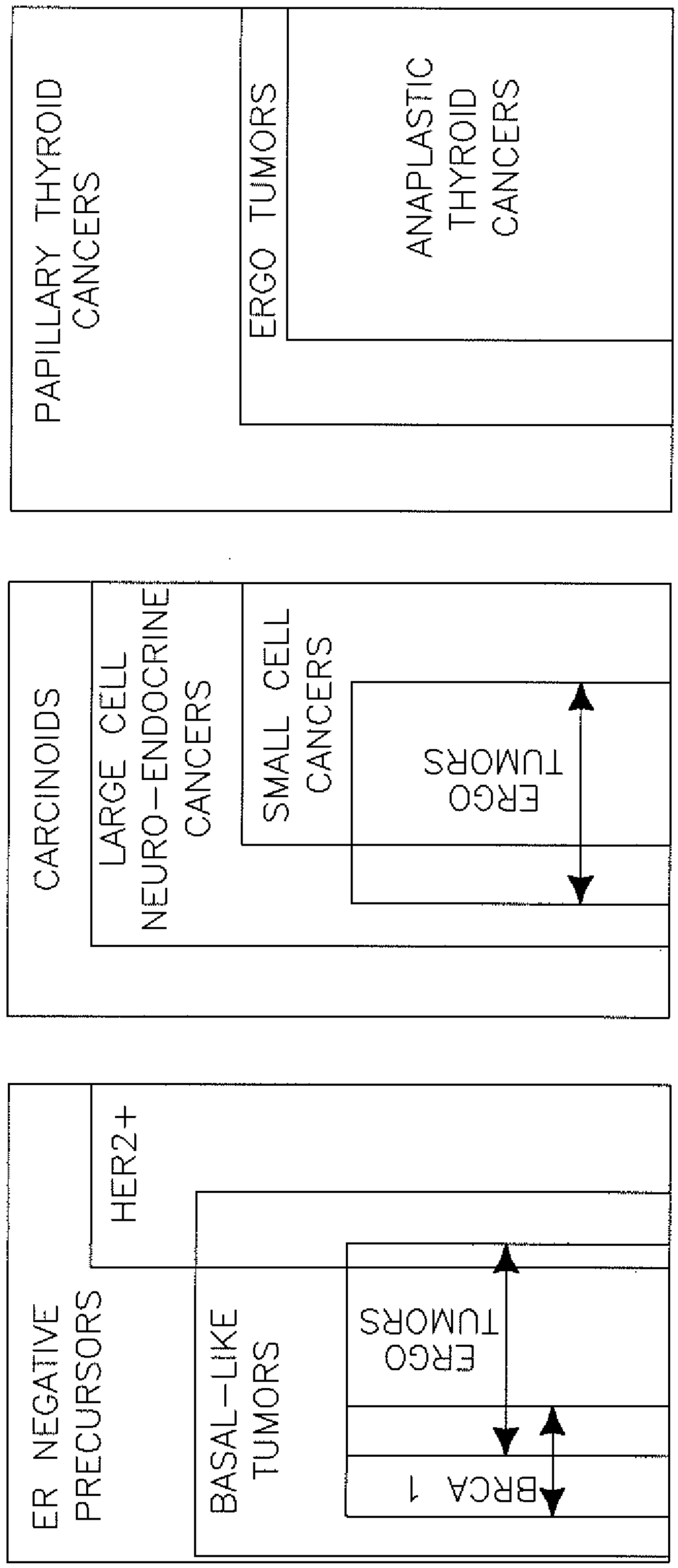

PAPILLARY THYROID CANCERS
ERGO TUMORS
ANAPLASTIC THYROID CANCERS
CARCINOIDS
LARGE CELL NEURO-ENDOCRINE CANCERS
SMALL CELL CANCERS
ERGO TUMORS
ER NEGATIVE PRECURSORS
HER2+
BASAL-LIKE TUMORS
ERGO TUMORS
BRCA 1

FIG.7

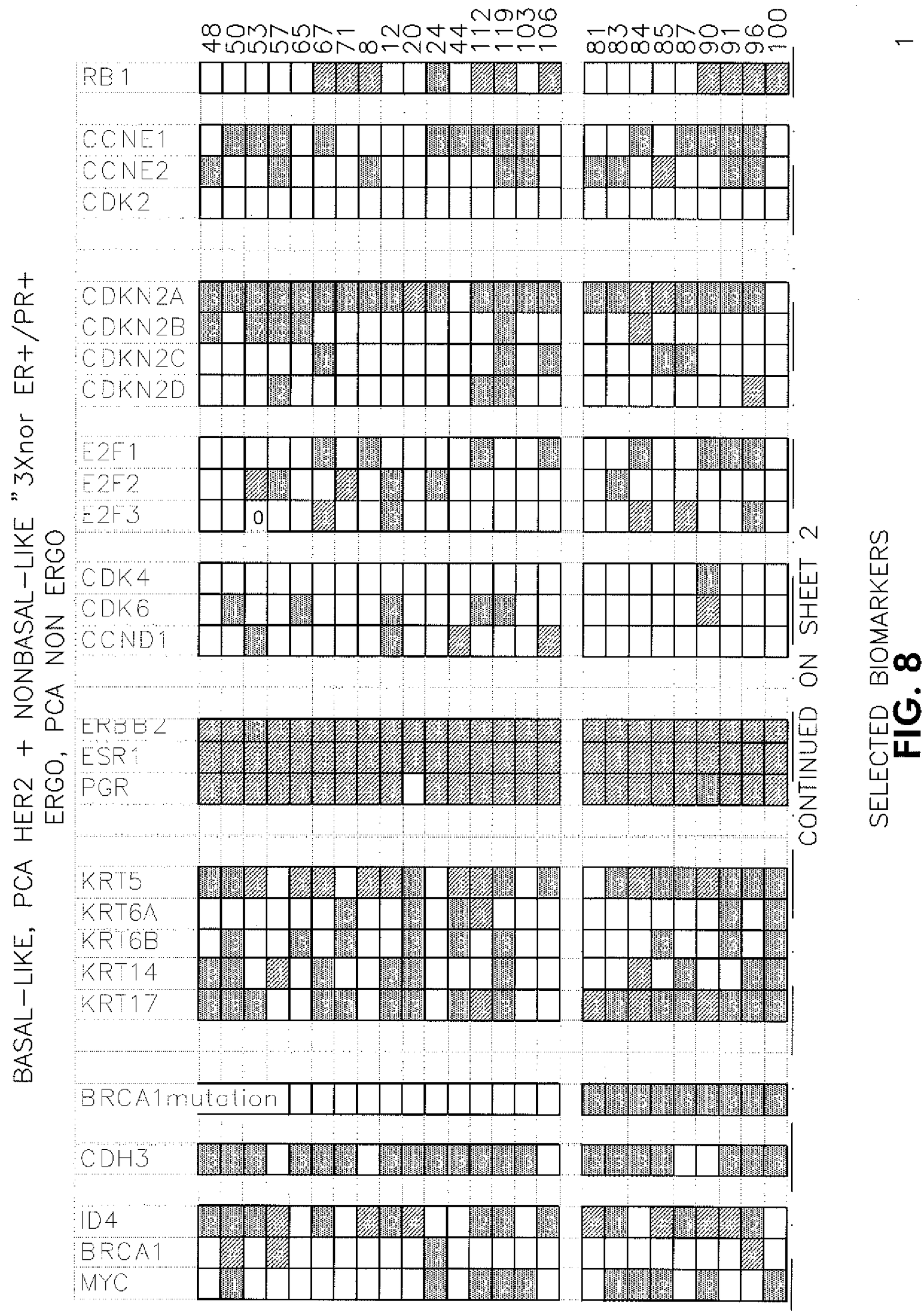

48 50 53 57 65 67 71 8 12 20 24 44 112 119 103 106 81 83 84 85 87 90 91 96 100
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mutation
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
CONTINUED ON SHEET 2
SELECTED BIOMARKERS

FIG. 8

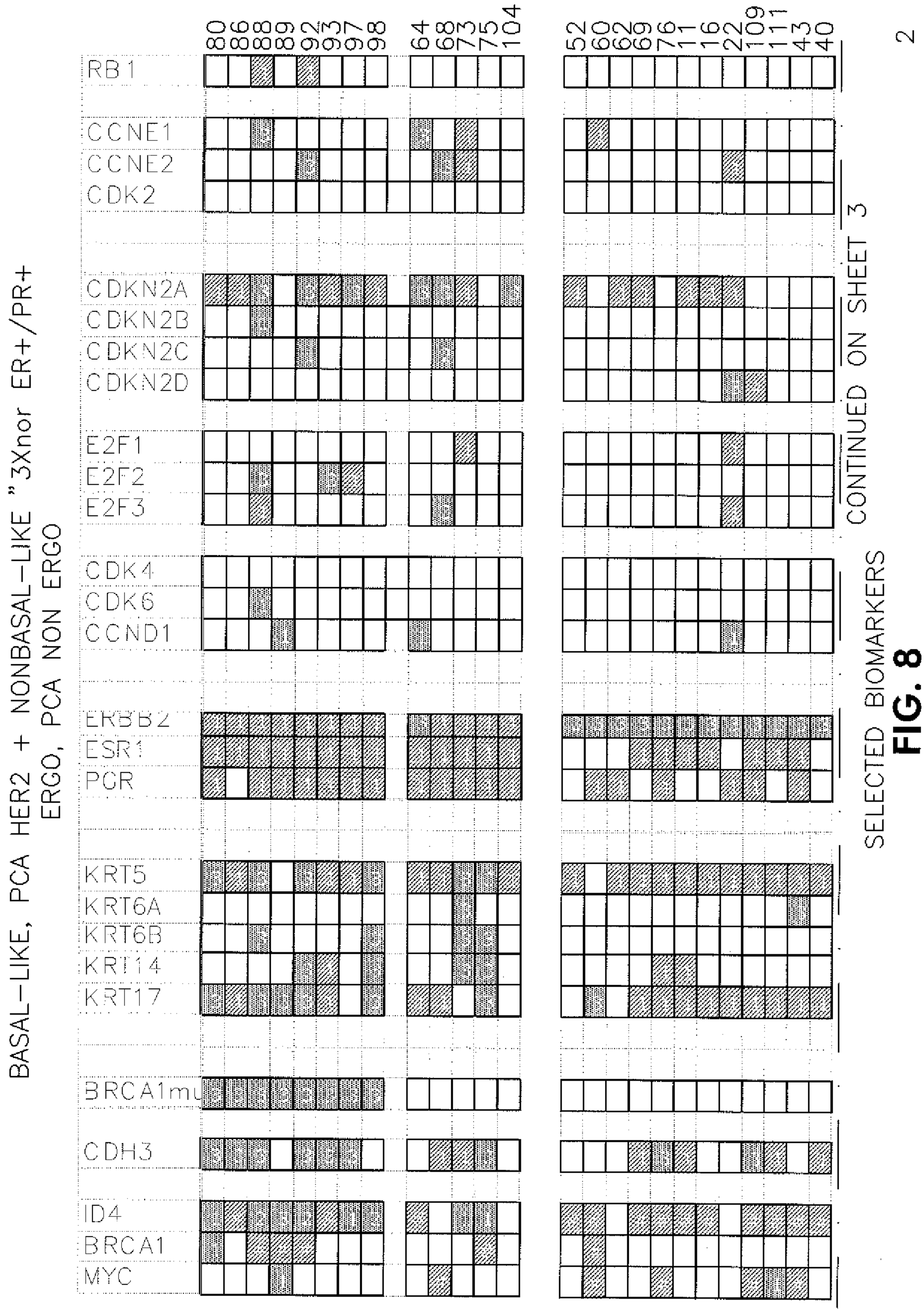

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
CONTINUED ON SHEET 3
SELECTED BIOMARKERS

FIG. 8

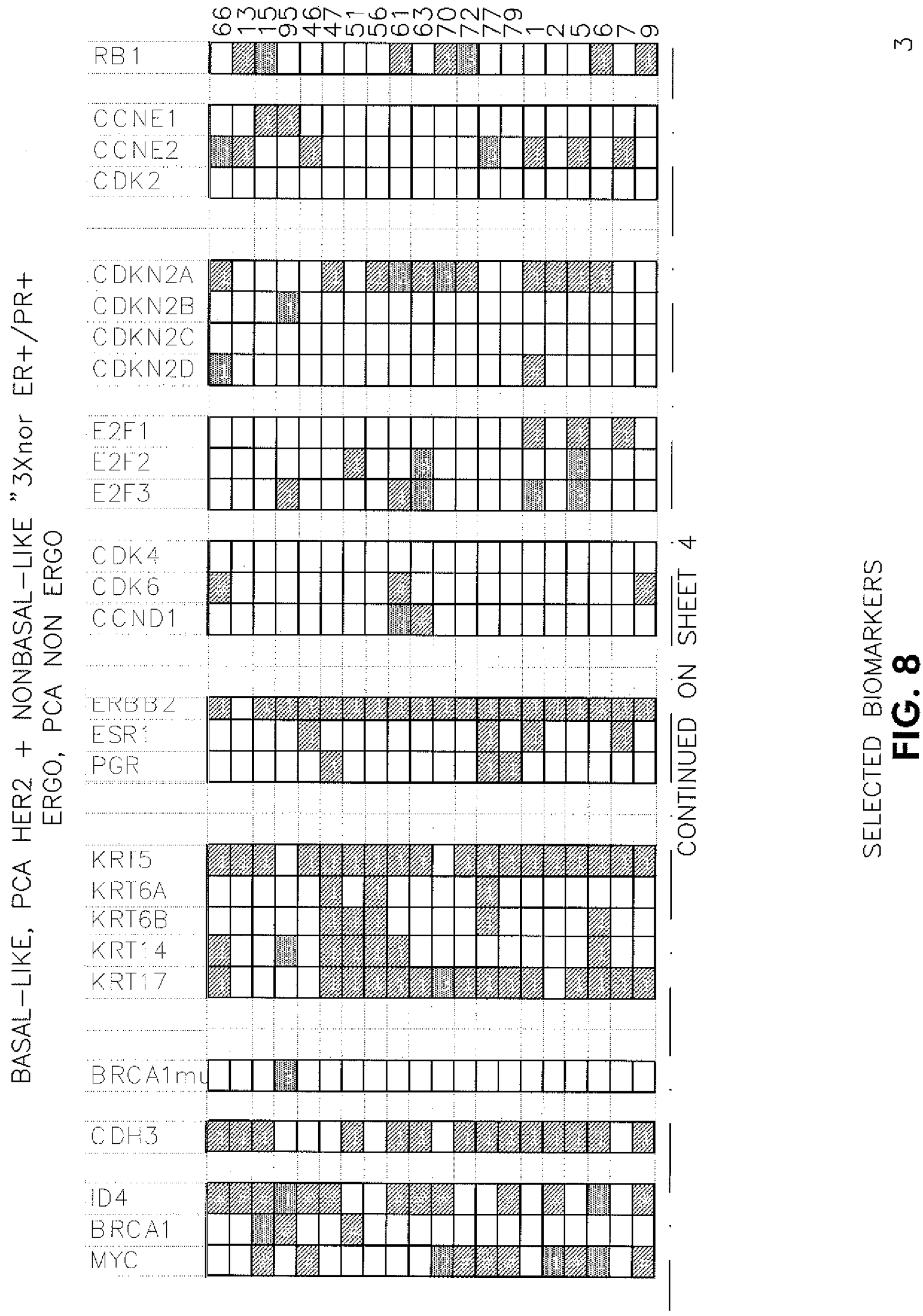

66 13 15 95 46 47 51 56 61 63 70 72 77 79 12 55 67 79
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
CONTINUED ON SHEET 4
SELECTED BIOMARKERS

FIG. 8

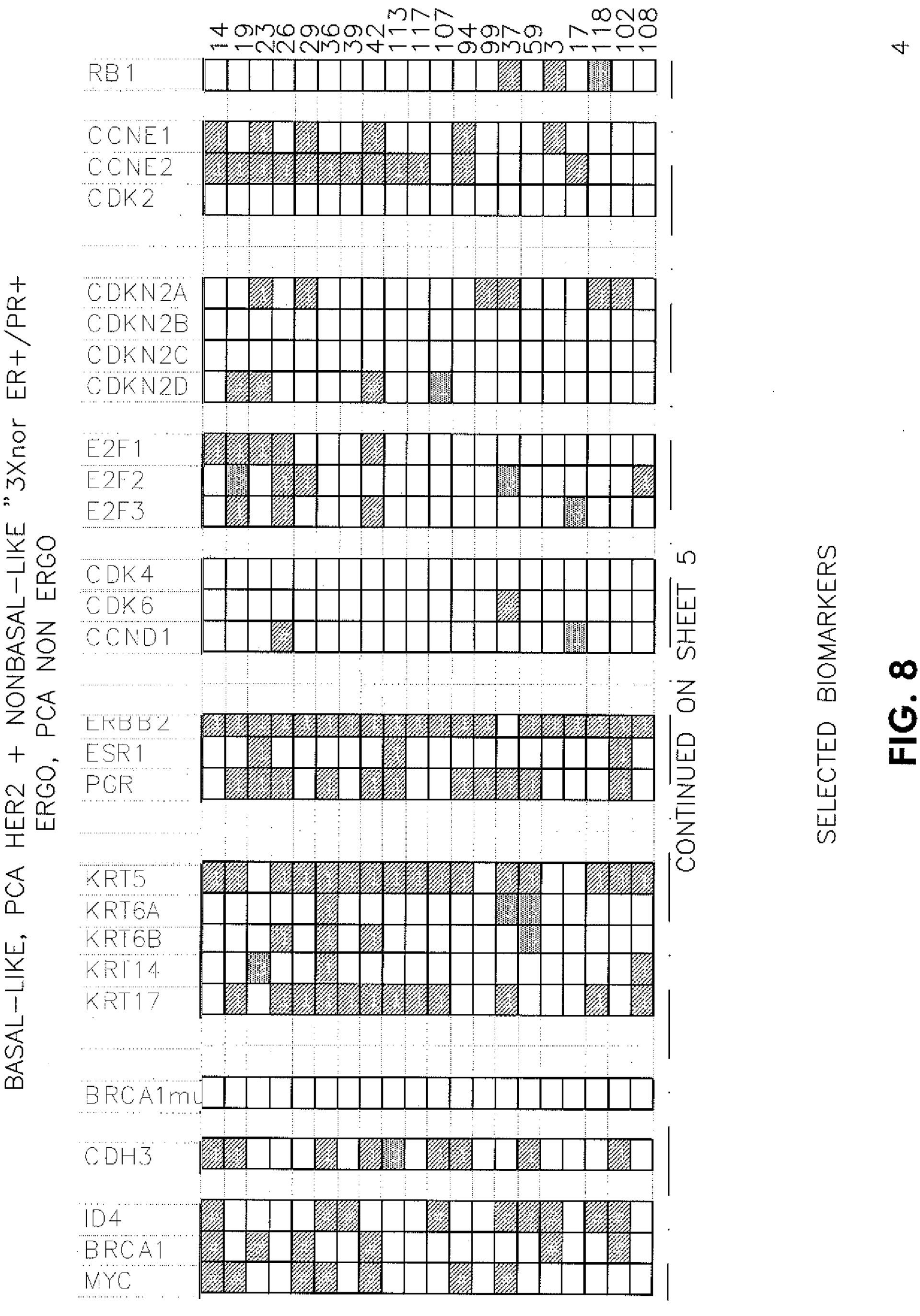

14 19 36 26 29 36 39 42 113 117 107 94 97 37 9 53 17 118 102 108
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
CONTINUED ON SHEET 5
SELECTED BIOMARKERS

FIG. 8

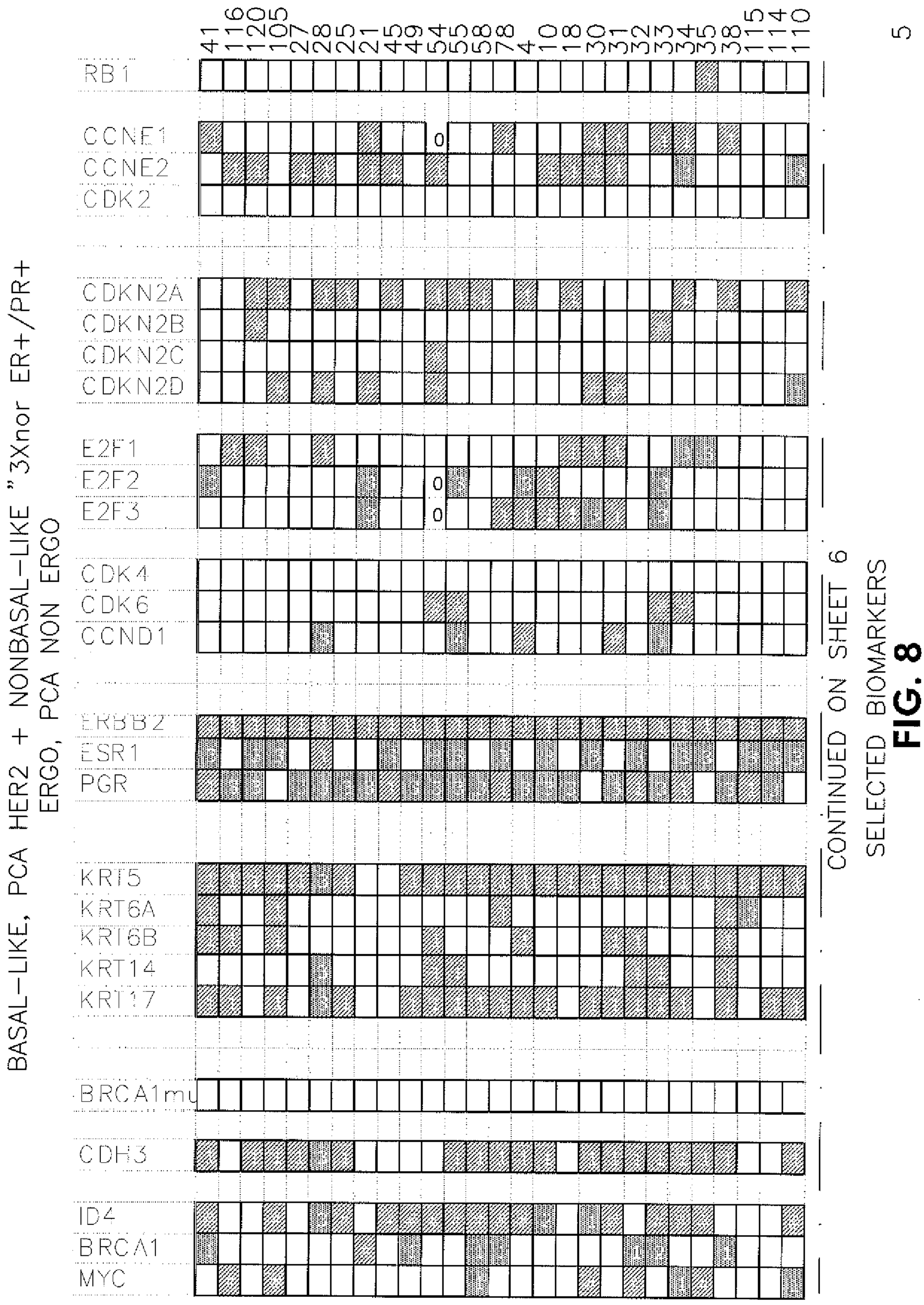

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
41 116 120 105 27 28 25 21 45 49 54 55 58 78 4 10 18 30 31 32 33 34 35 38 115 114 110
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
BRCA1mu
CDH3
ID4
BRCA1
MYC
CONTINUED ON SHEET 6
SELECTED BIOMARKERS

FIG. 8

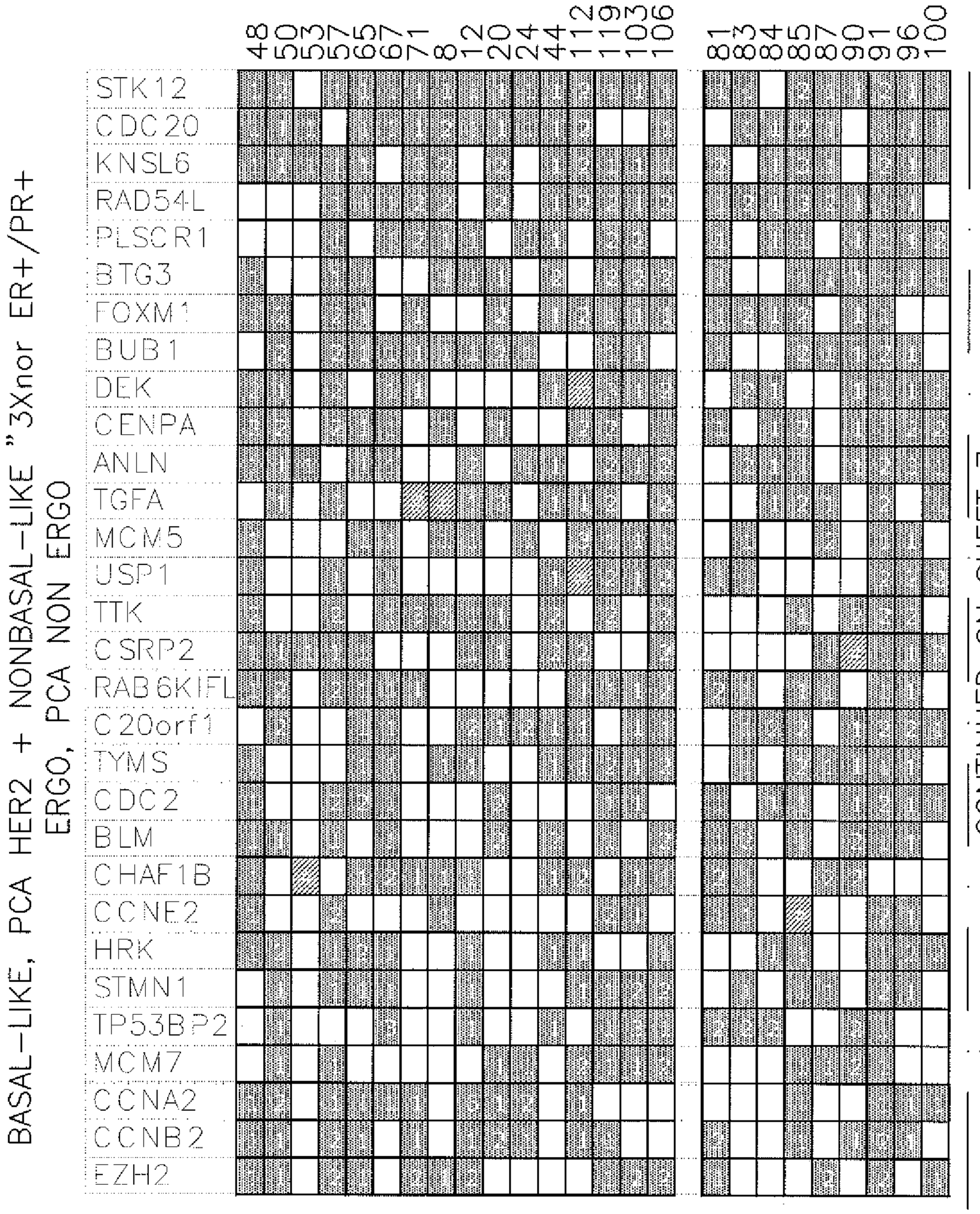
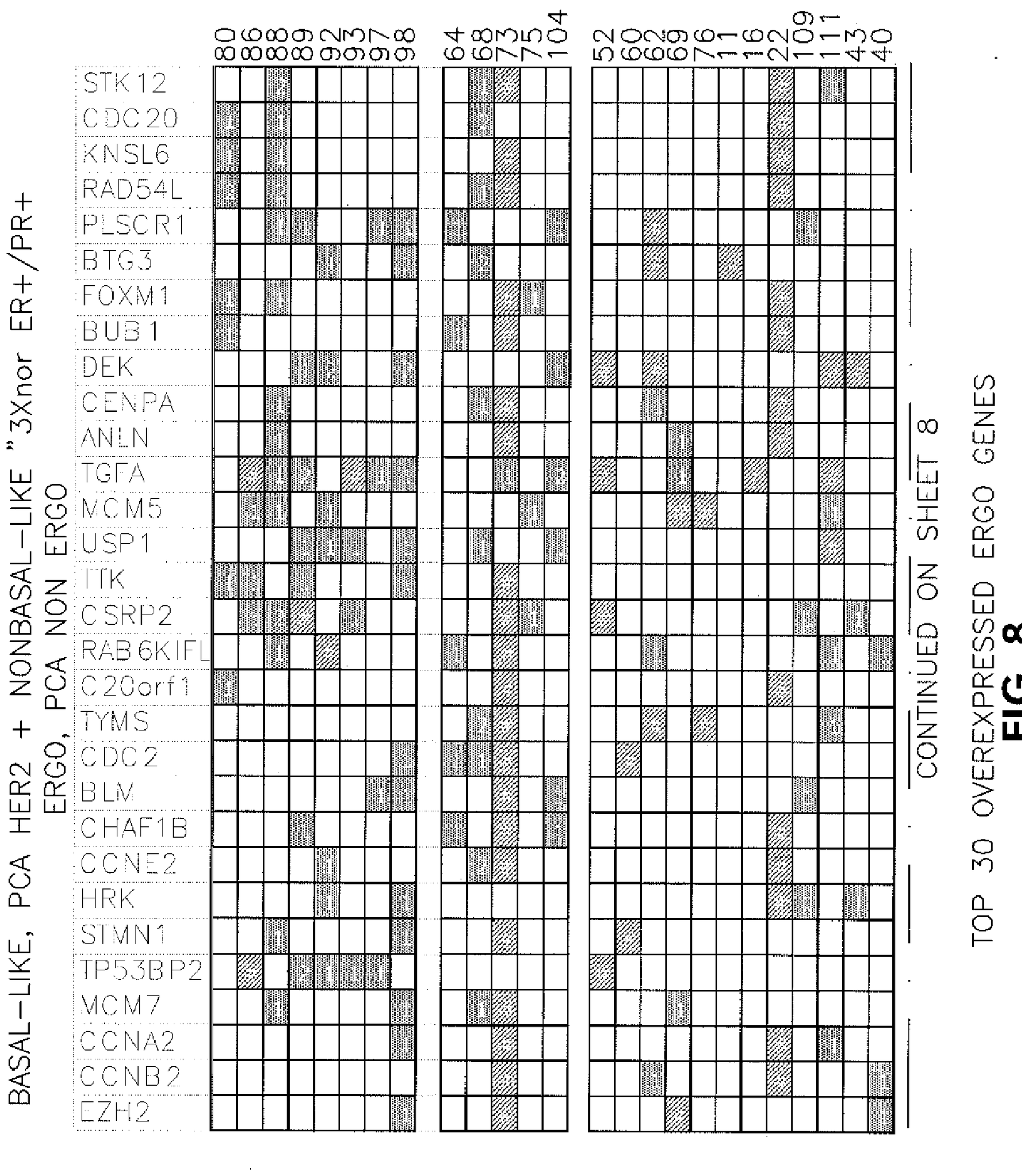

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
STK12
CDC20
KNSL6
RAD54L
PLSCR1
BTG3
FOXM1
BUB1
DEK
CENPA
ANLN
TGFA
MCM5
USP1
TTK
CSRP2
RAB6KIFL
C20orf1
TYMS
CDC2
BLM
CHAF1B
CCNE2
HRK
STMN1
TP53BP2
MCM7
CCNA2
CCNB2
EZH2
48 50 53 56 57 67 71 78 81 120 204 244 112 119 103 106 81 83 84 85 87 90 91 96 100
CONTINUED ON SHEET 7
TOP 30 OVEREXPRESSED ERGO GENES

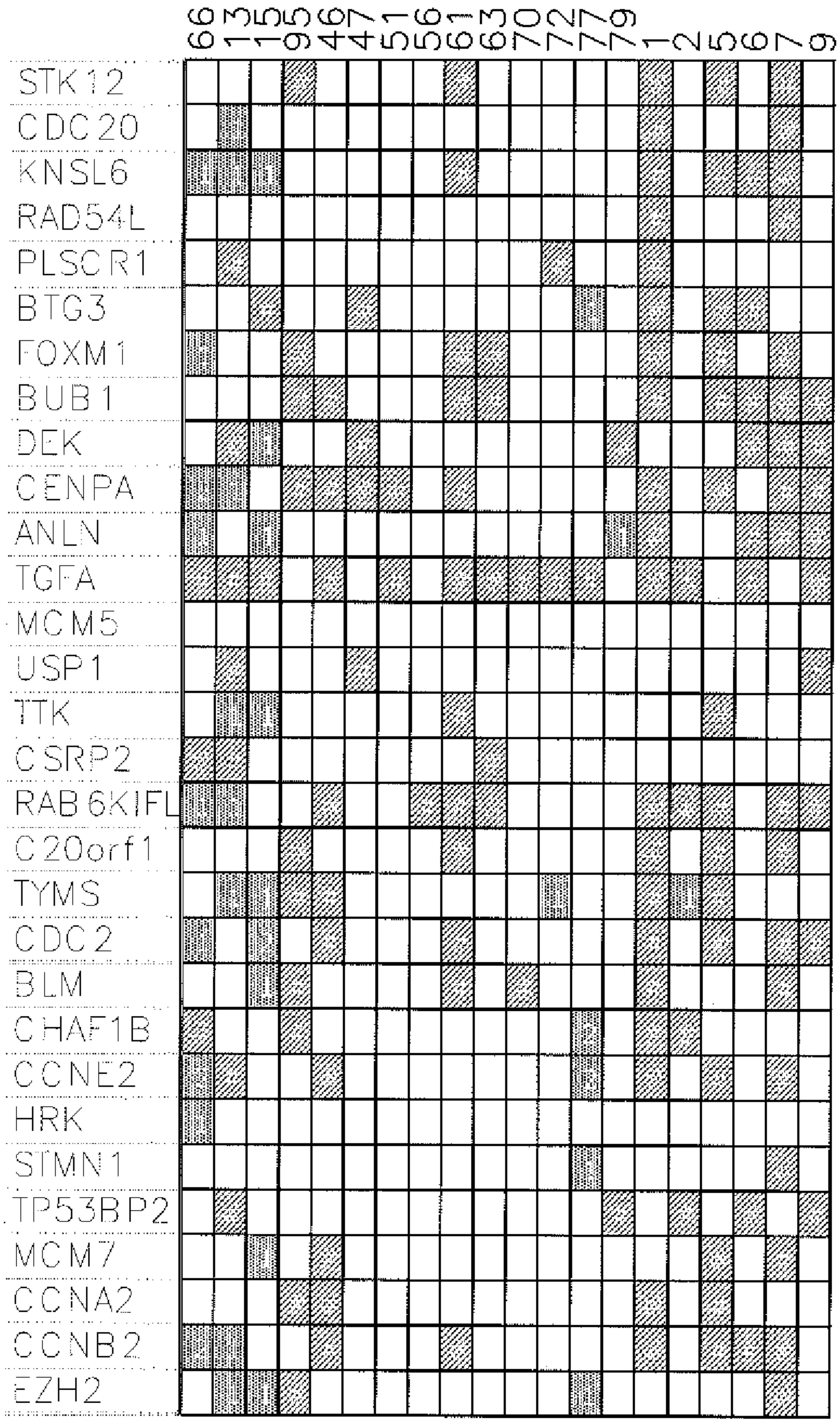
FIG. 8



TOP 30 OVEREXPRESSED ERGO GENES

FIG. 8

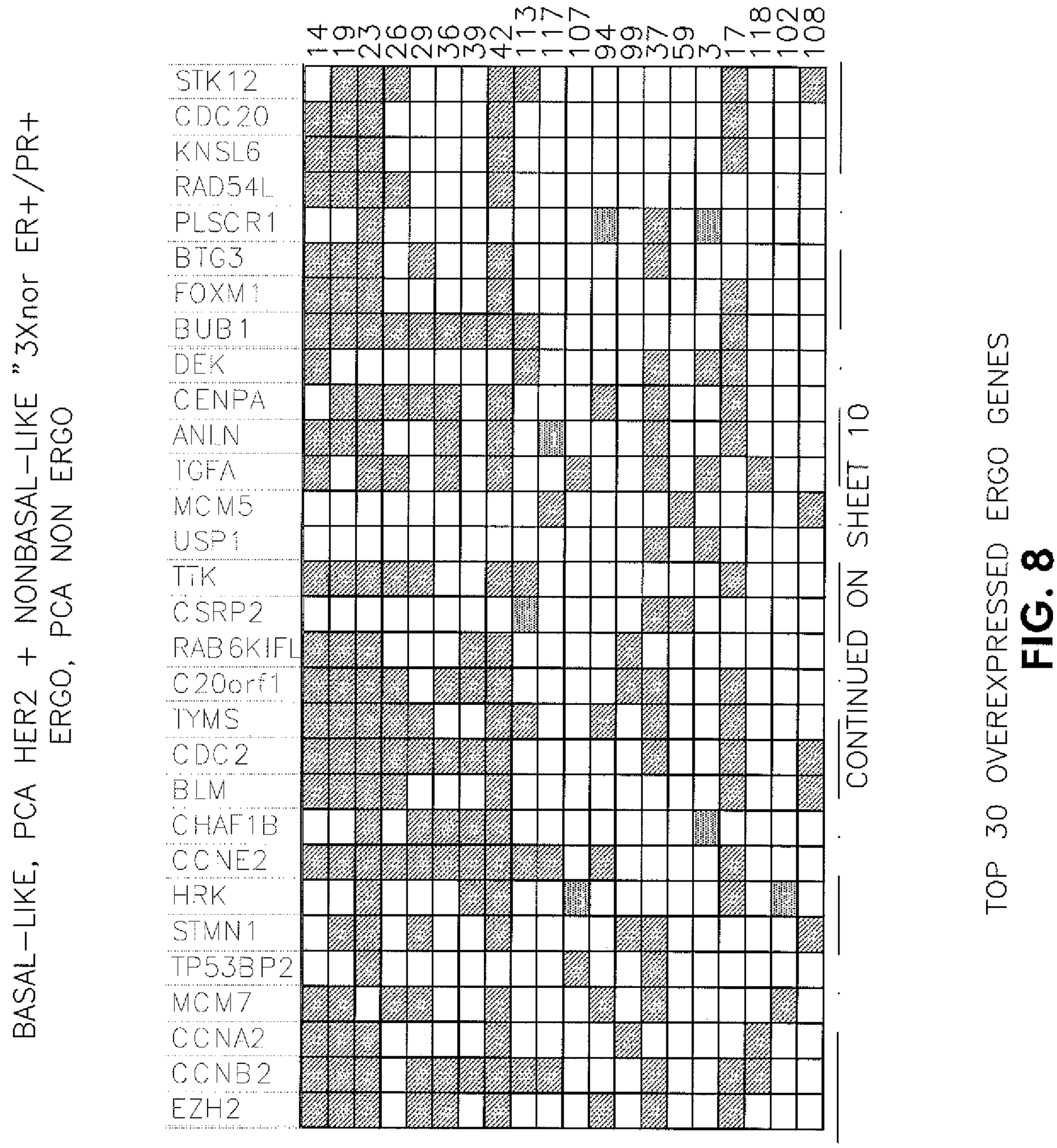
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO



CONTINUED ON SHEET 9

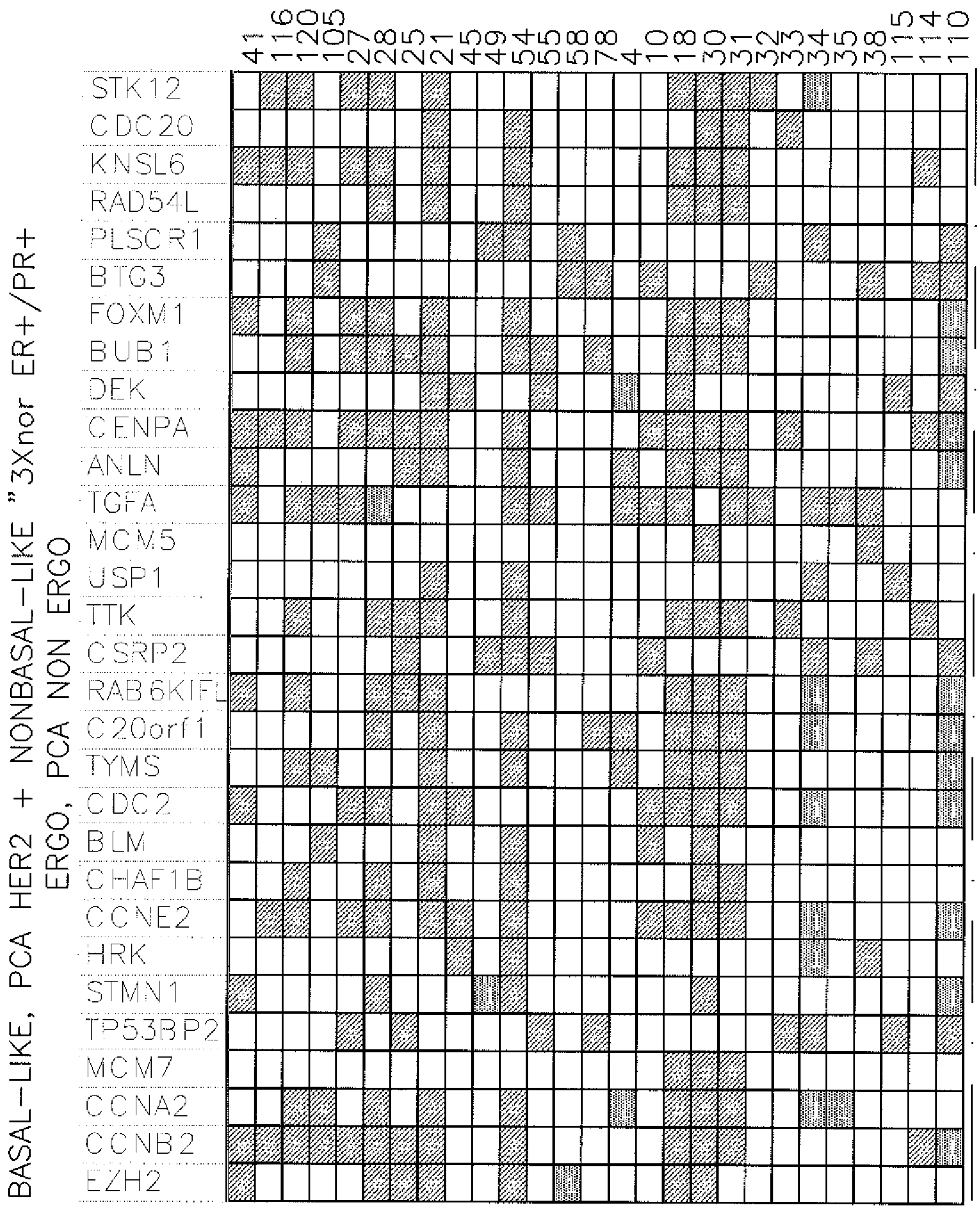
TOP 30 OVEREXPRESSED ERGO GENES

FIG. 8



TOP 30 OVEREXPRESSED ERGO GENES

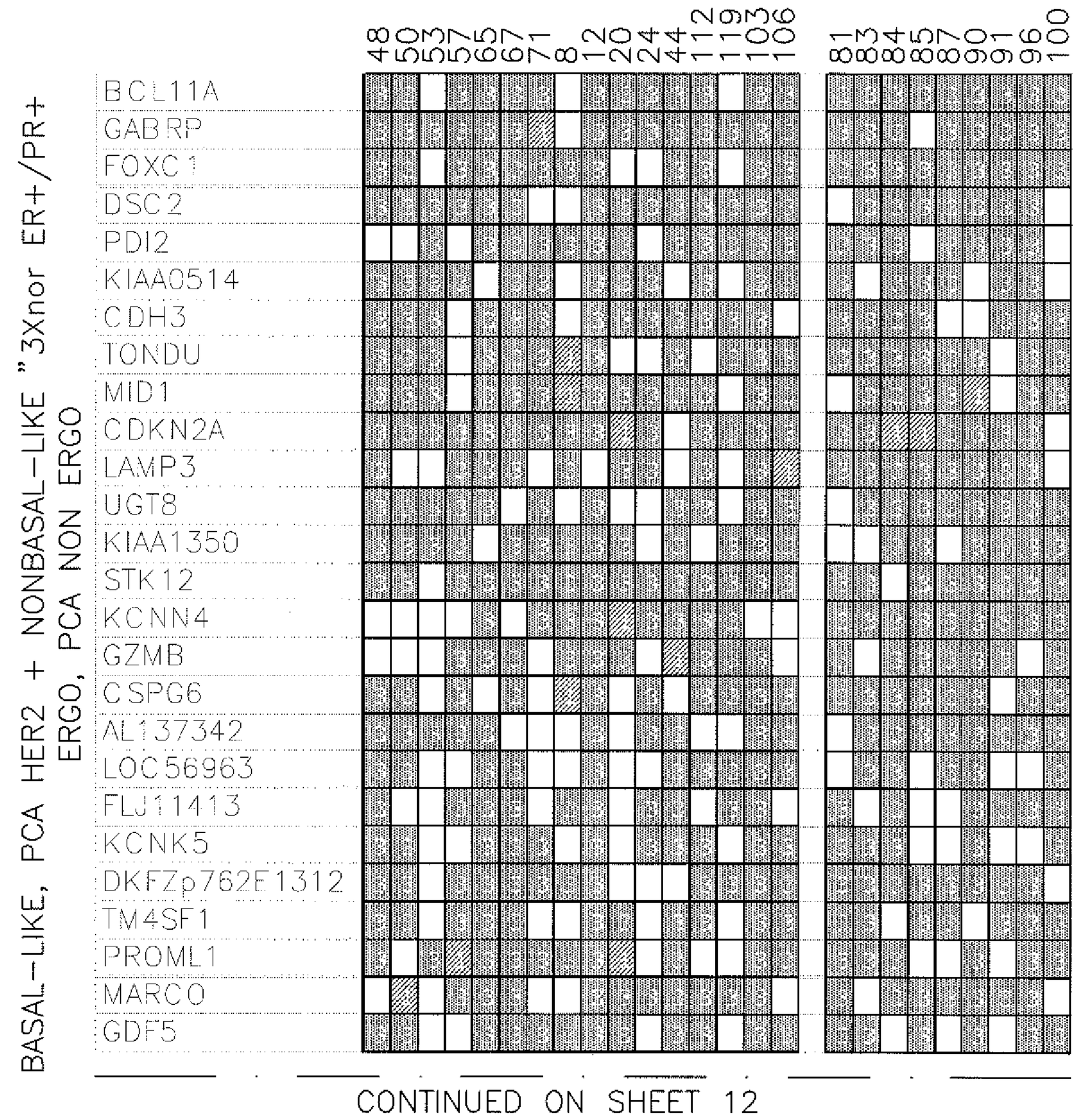
FIG. 8



CONTINUED ON SHEET 11

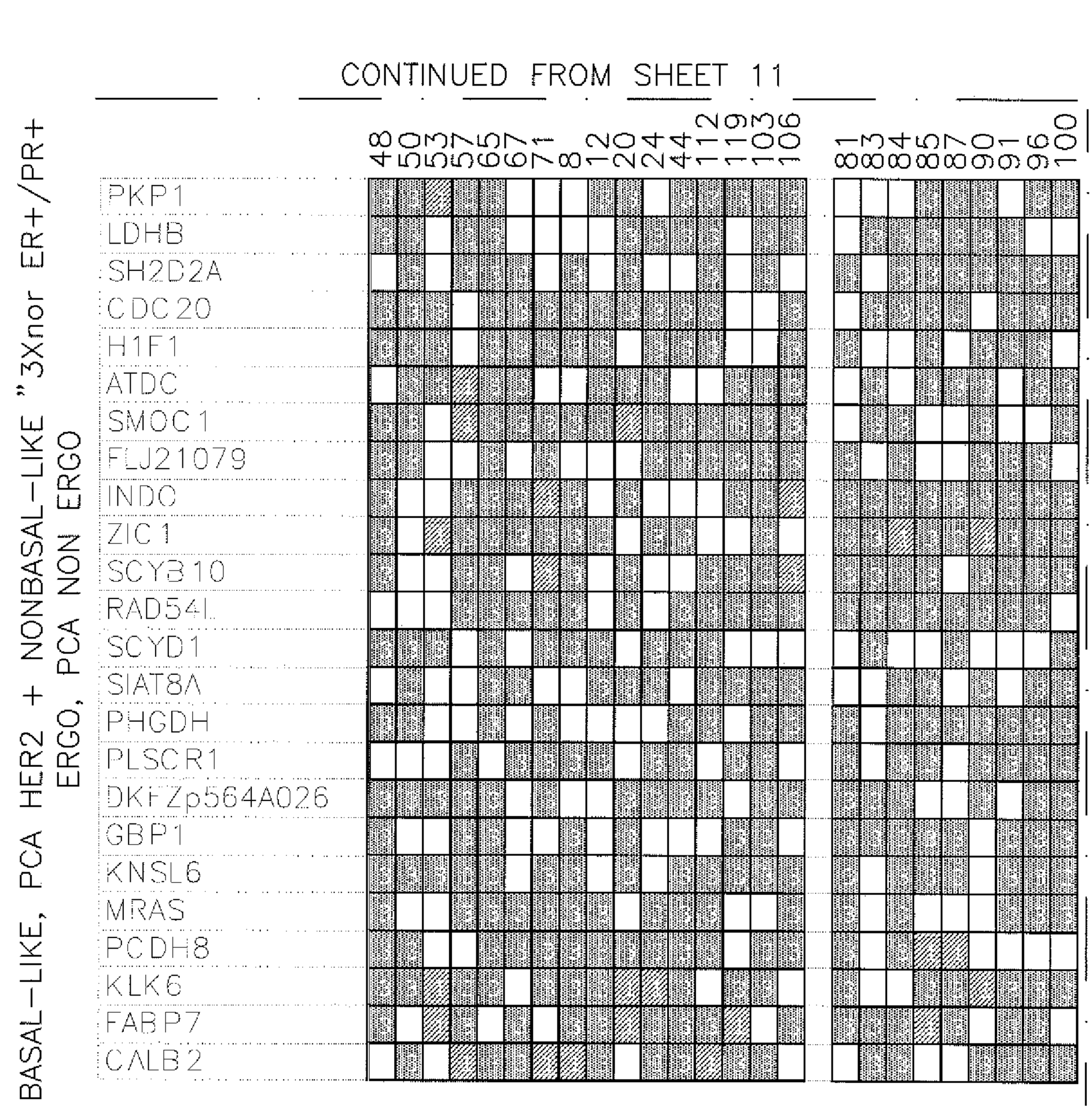
TOP 30 OVEREXPRESSED ERGO GENES

FIG. 8



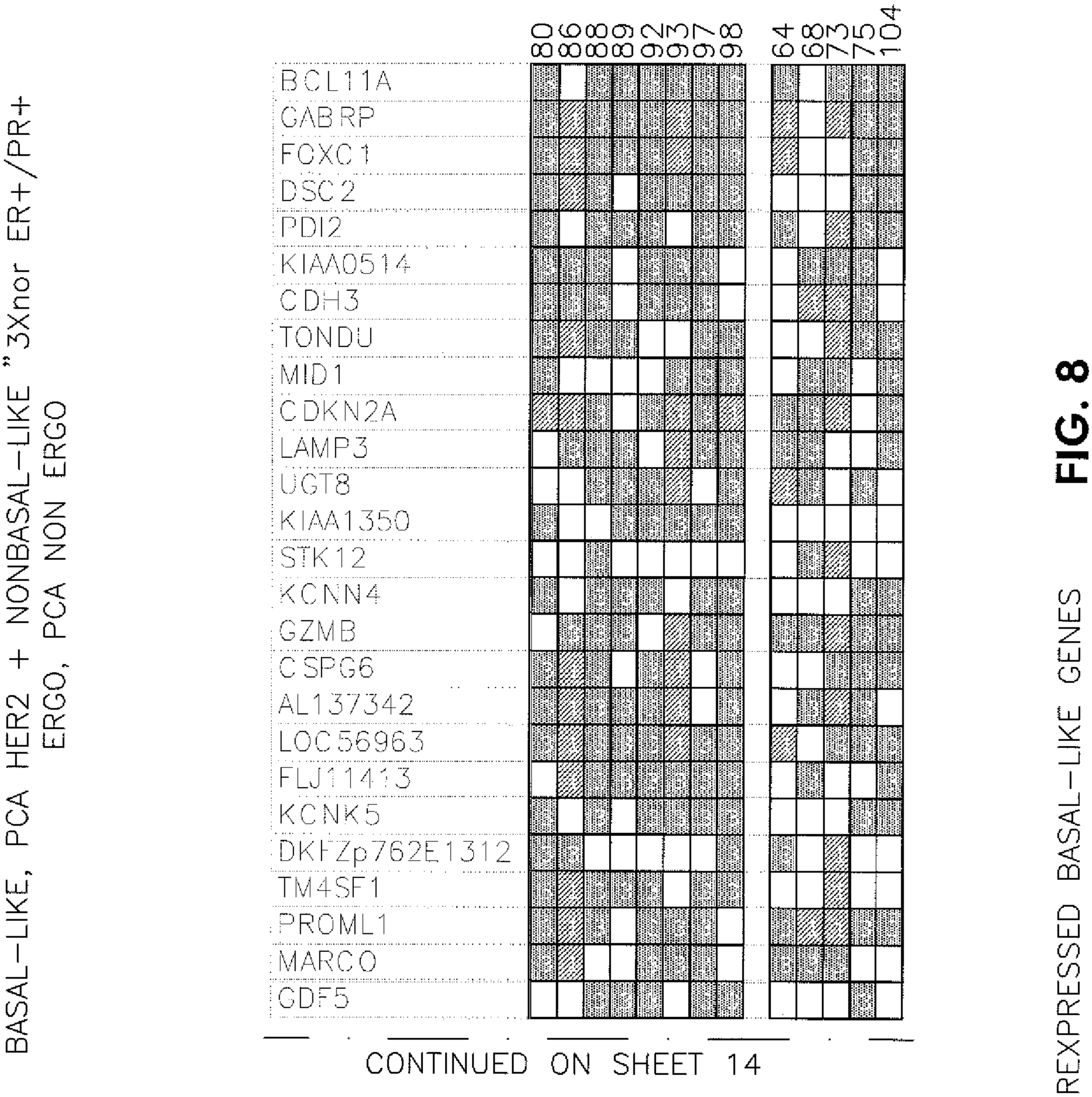
TOP 50 OVEREXPRESSED
BASAL-LIKE GENES

FIG. 8



TOP 50 OVEREXPRESSED
BASAL-LIKE GENES

FIG. 8

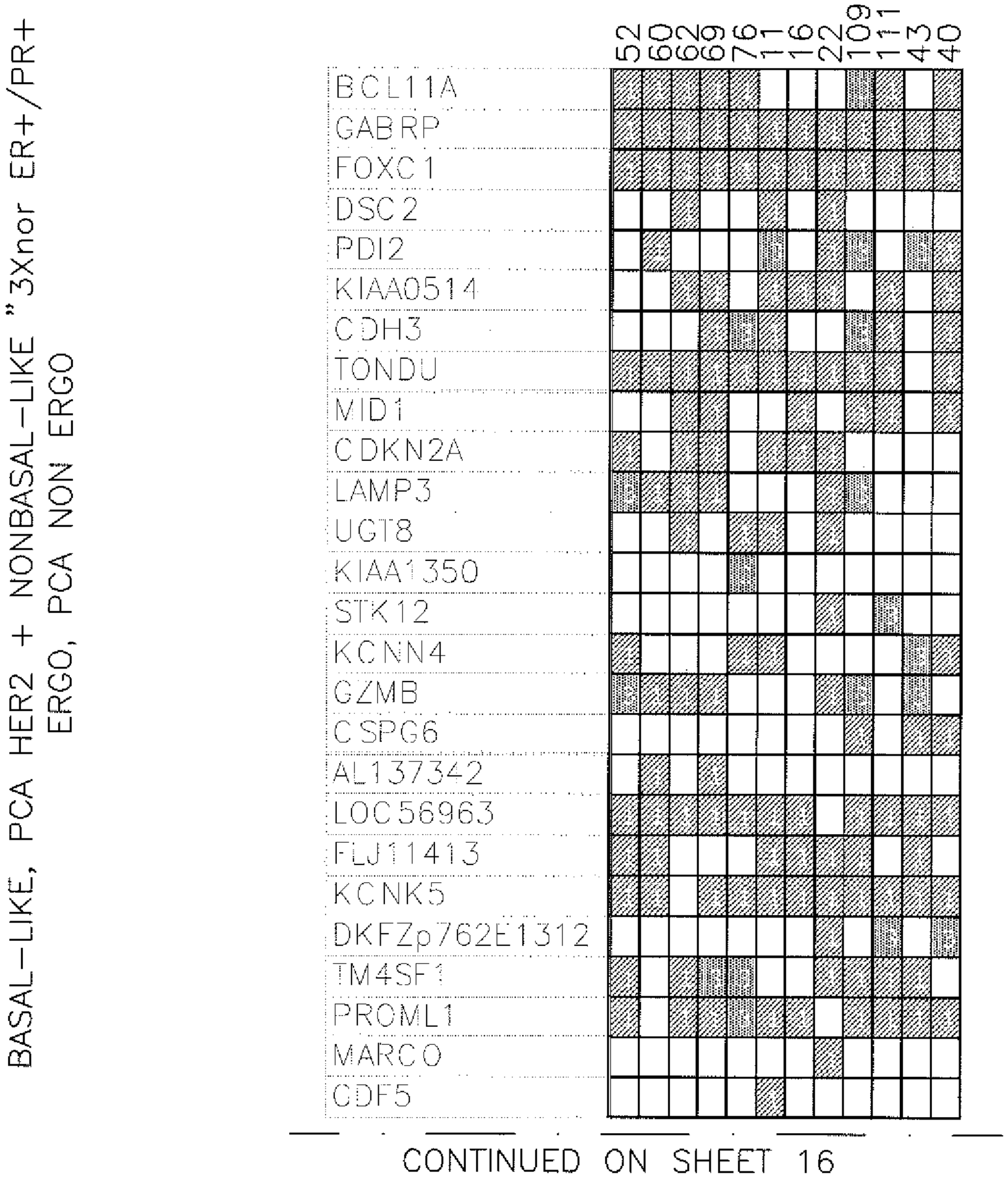


CONTINUED ON SHEET 14

FIG. 8

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NONERGO
CONTINUED FROM SHEET 13
CONTINUED ON SHEET 15
80 86 88 89 92 93 97 98 64 68 73 75 104
PKP1
LDHB
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564A026
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2

FIG. 8



TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 8

CONTINUED ON SHEET 16

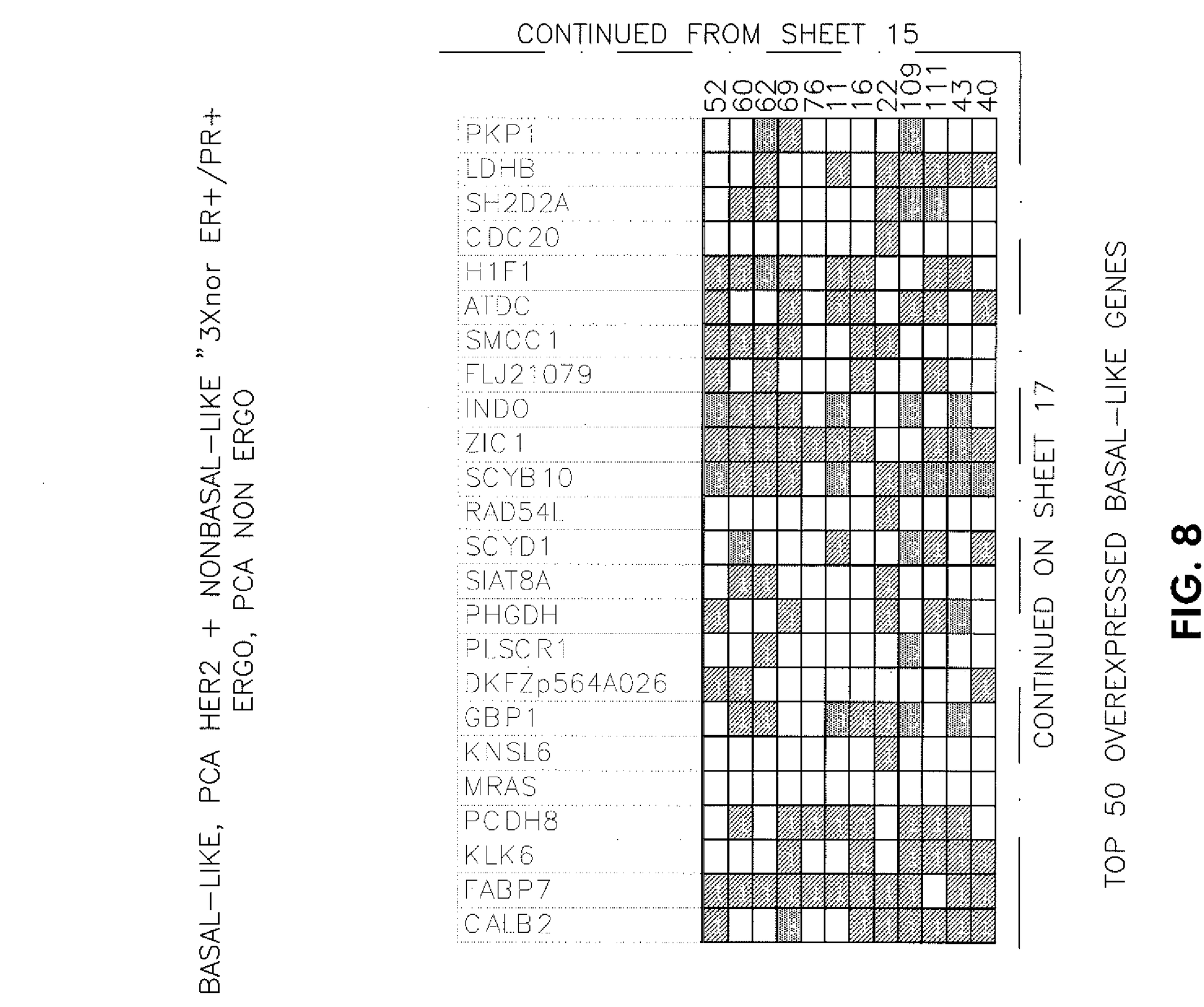

CONTINUED FROM SHEET 15
52 60 62 69 76 11 16 22 109 111 43 40
PKP1
LDHB
SH2D2A
CDC20
H1F1
ATDC
SMCC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564A026
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2
CONTINUED ON SHEET 17
TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO

FIG. 8

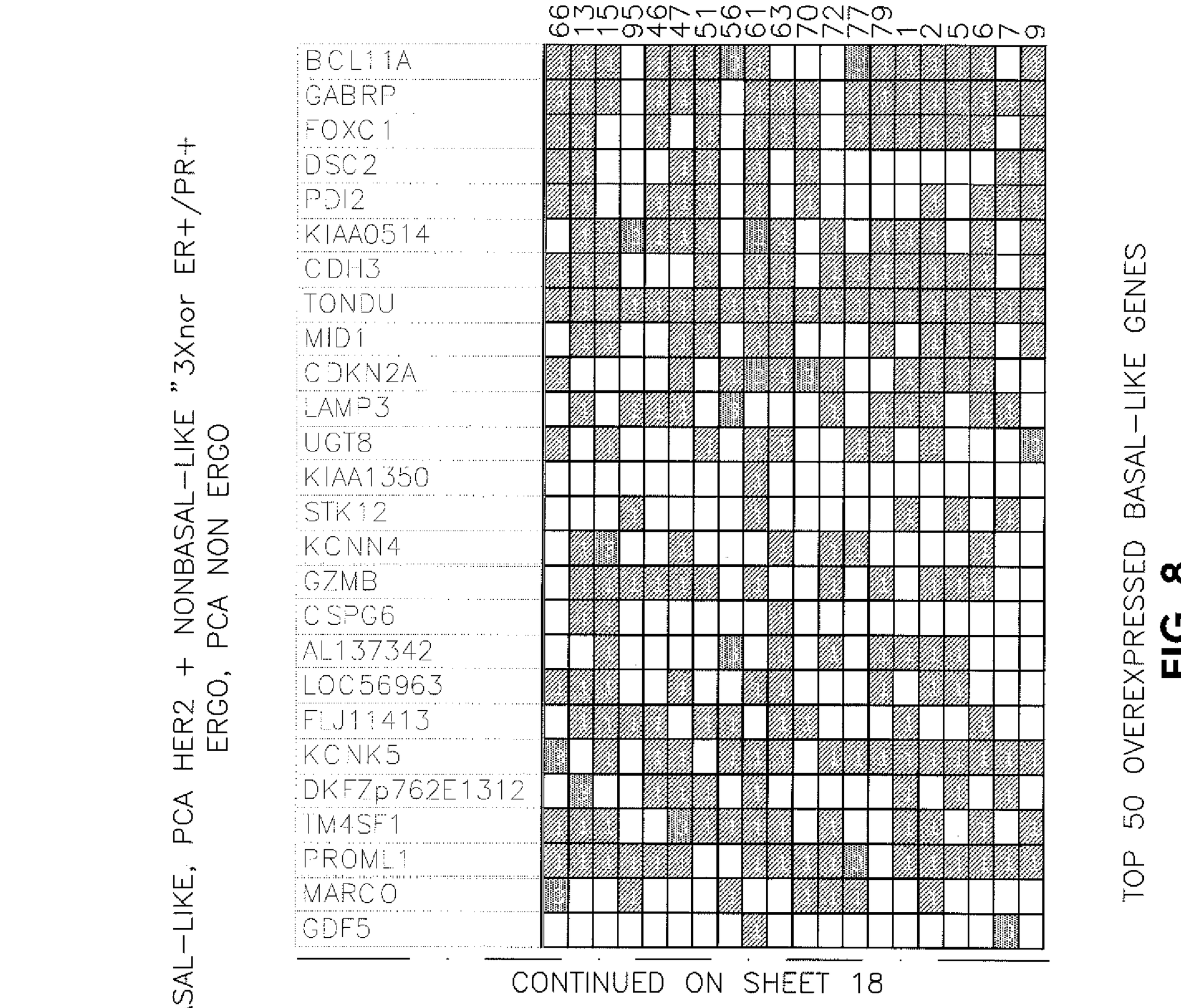

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BCL11A
GABRP
FOXC1
DSC2
PDI2
KIAA0514
CDH3
TONDU
MID1
CDKN2A
LAMP3
UGT8
KIAA1350
STK12
KCNN4
GZMB
CSPG6
AL137342
LOC56963
FLJ11413
KCNK5
DKFZp762E1312
TM4SF1
PROML1
MARCO
GDF5
CONTINUED ON SHEET 18

FIG. 8

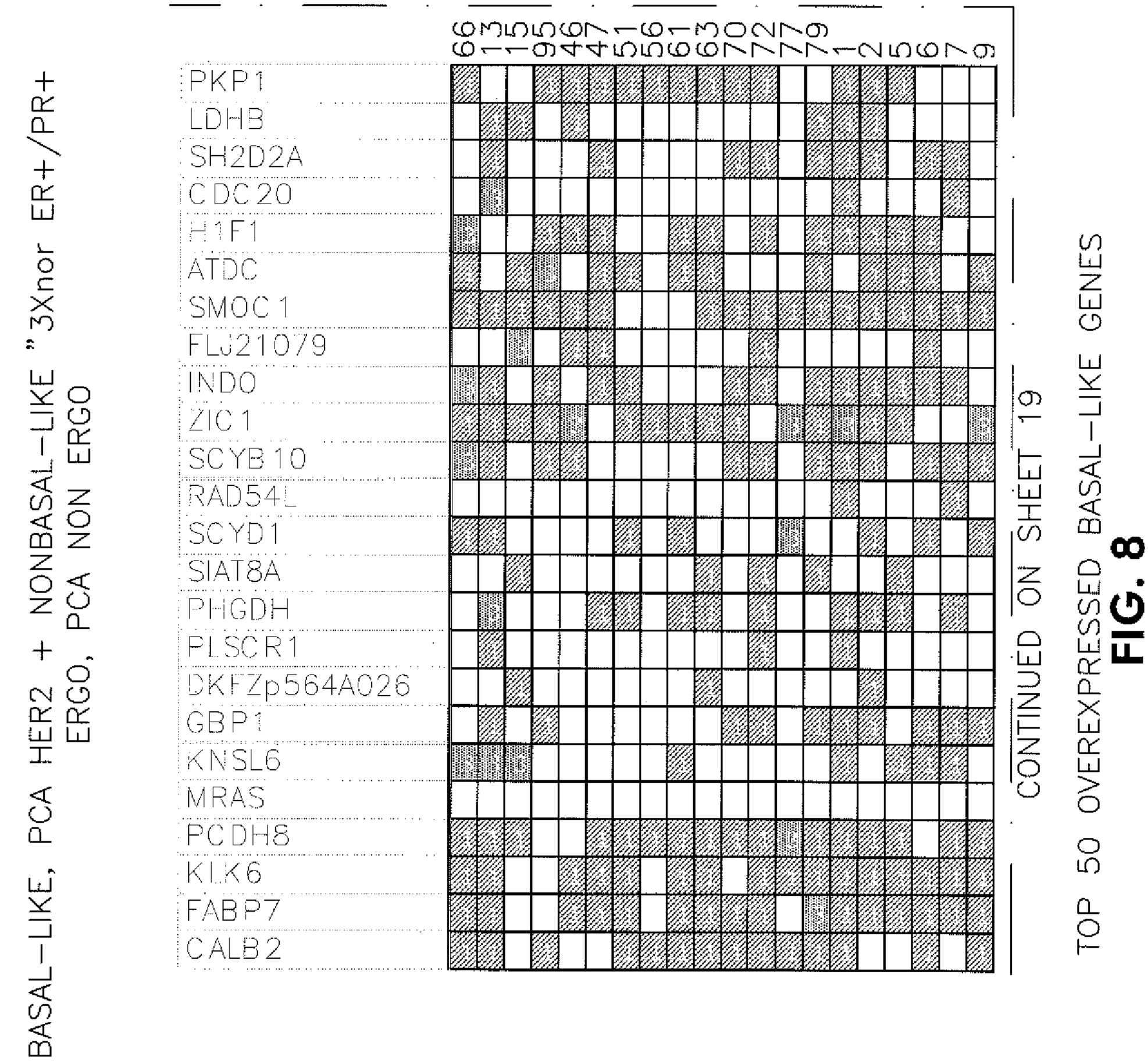

CONTINUED FROM SHEET 17
66 13 15 5 6 9 4 6 7 4 5 1 5 6 6 1 6 3 7 0 7 2 7 7 7 9 1 2 5 6 7 9
PKP1
LDHB
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564A026
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2
CONTINUED ON SHEET 19
TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERG0, PCA NONBASAL-LIKE ERG0

FIG. 8

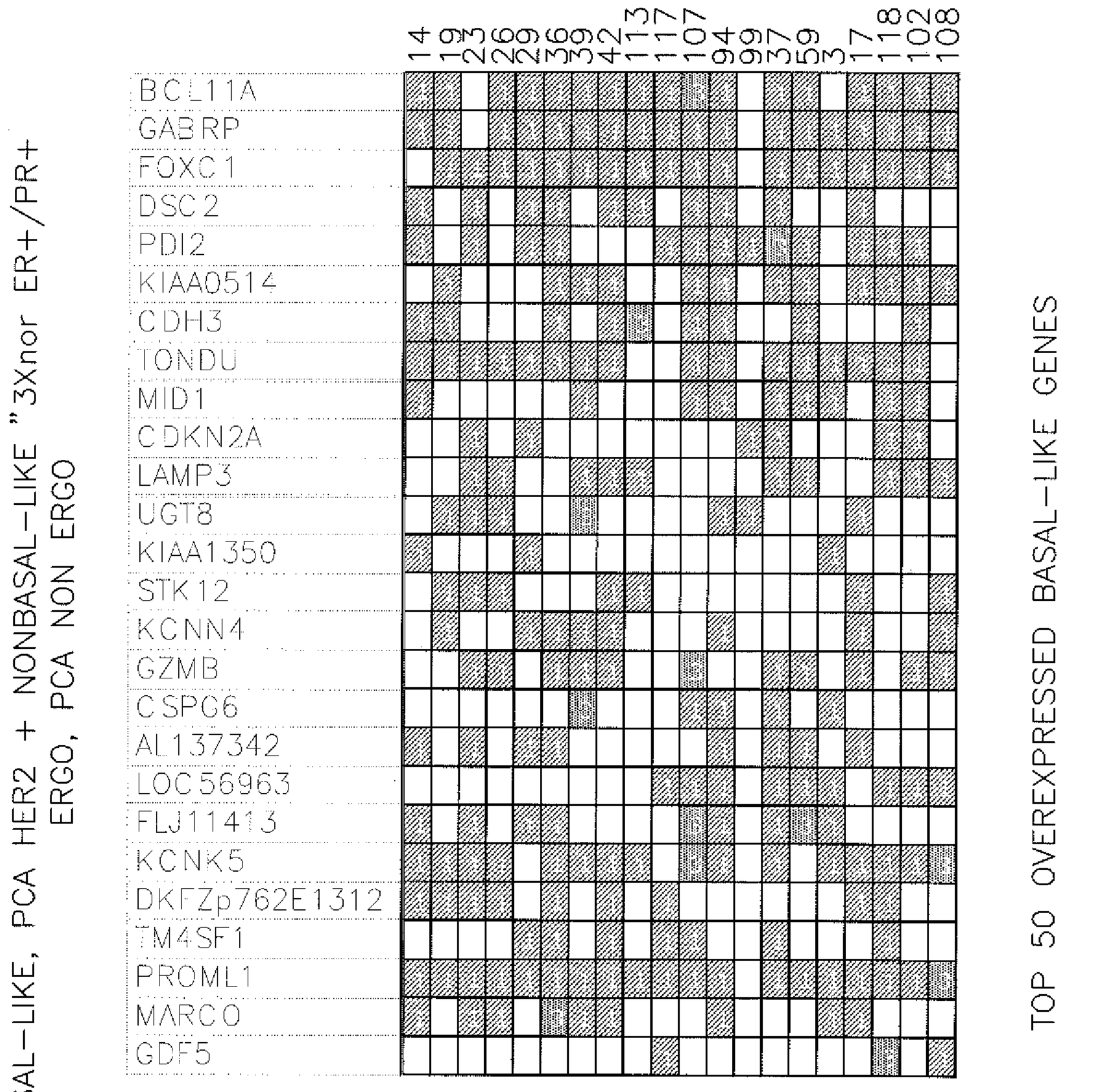


FIG. 8

CONTINUED ON SHEET 20

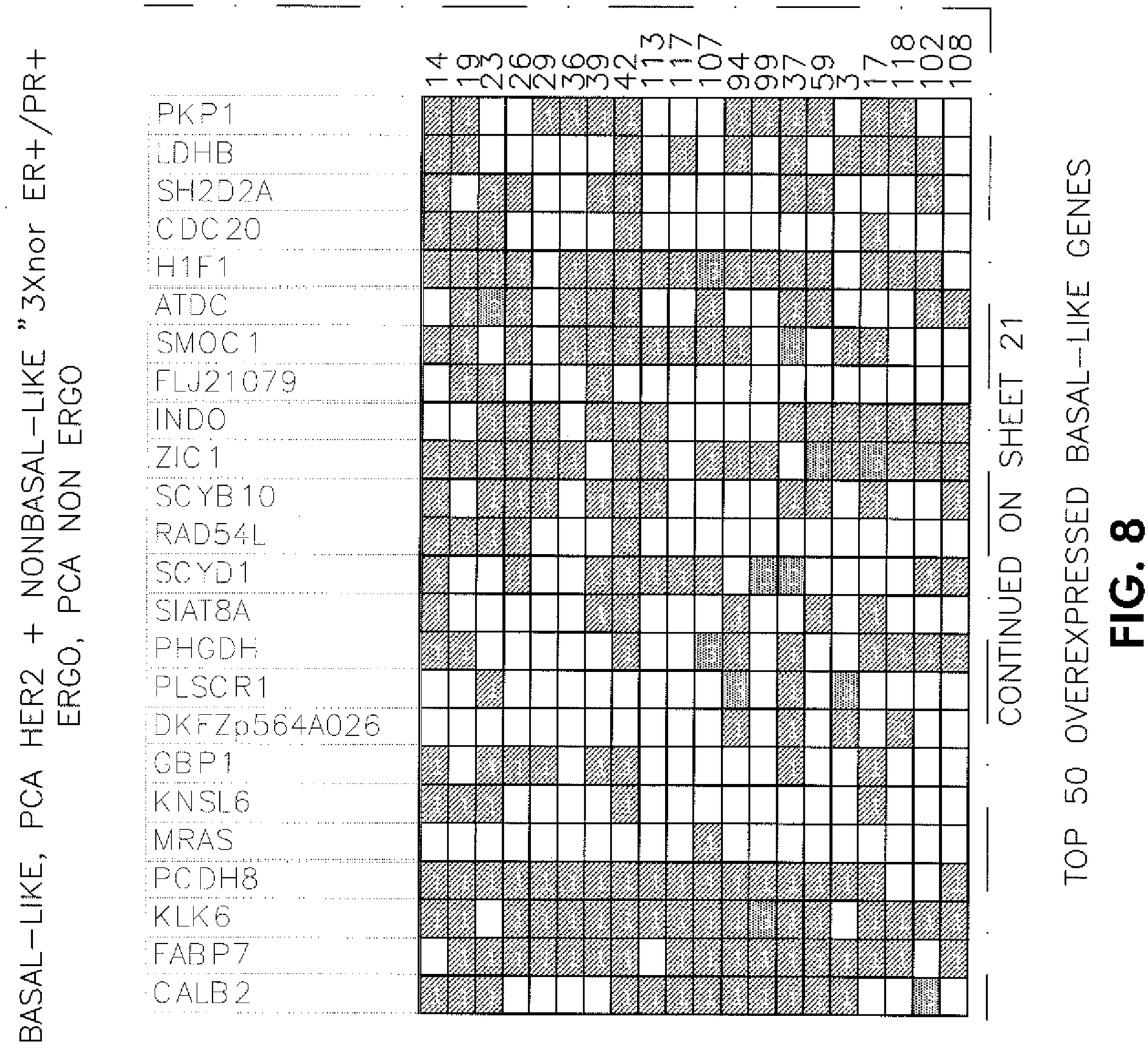

CONTINUED FROM SHEET 19
14 19 23 26 29 29 36 39 42 113 117 107 94 99 37 59 3 17 118 102 108
PKP1
LDHB
SH2D2A
CDC20
H1F1
ATDC
SMOC1
FLJ21079
INDO
ZIC1
SCYB10
RAD54L
SCYD1
SIAT8A
PHGDH
PLSCR1
DKFZp564A026
GBP1
KNSL6
MRAS
PCDH8
KLK6
FABP7
CALB2
CONTINUED ON SHEET 21
TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO

FIG. 8

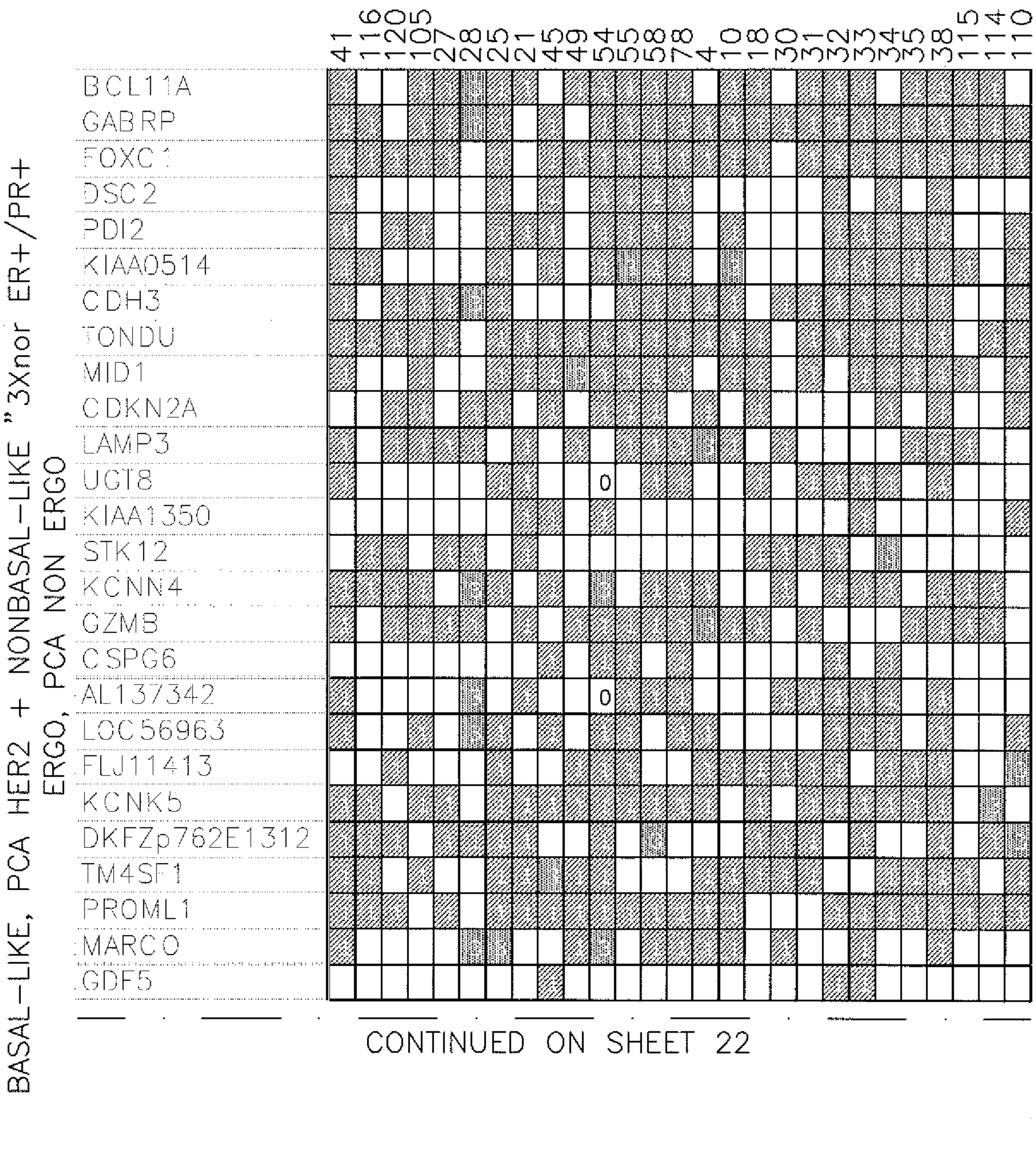

TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
BCL11A
GABRP
FOXC1
DSC2
PDI2
KIAA0514
CDH3
TONDU
MID1
CDKN2A
LAMP3
UGT8
KIAA1350
STK12
KCNN4
GZMB
CSPG6
AL137342
LOC56963
FLJ11413
KCNK5
DKFZp762E1312
TM4SF1
PROML1
MARCO
GDF5
CONTINUED ON SHEET 22

FIG. 8

CONTINUED FROM SHEET 21

FIG. 8

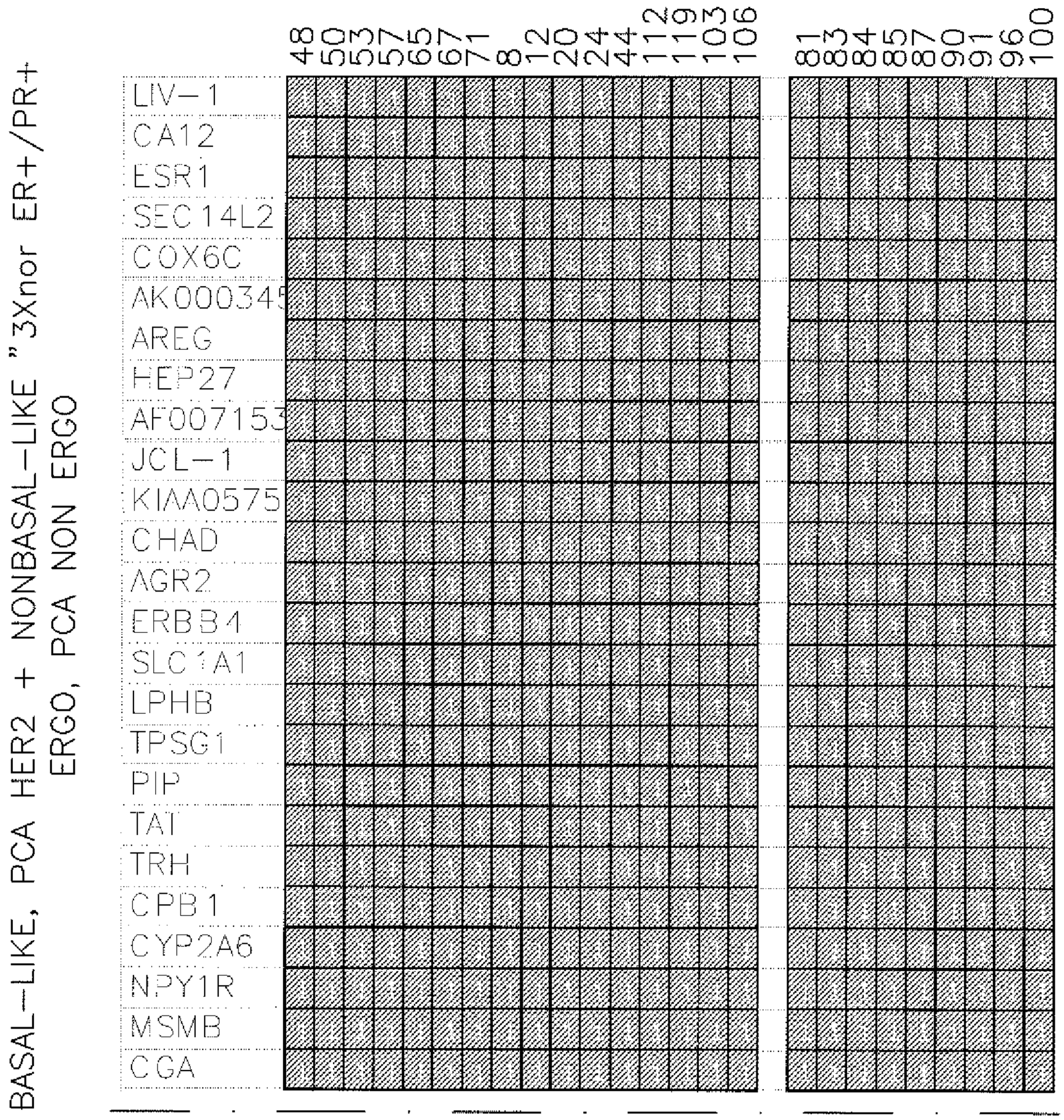
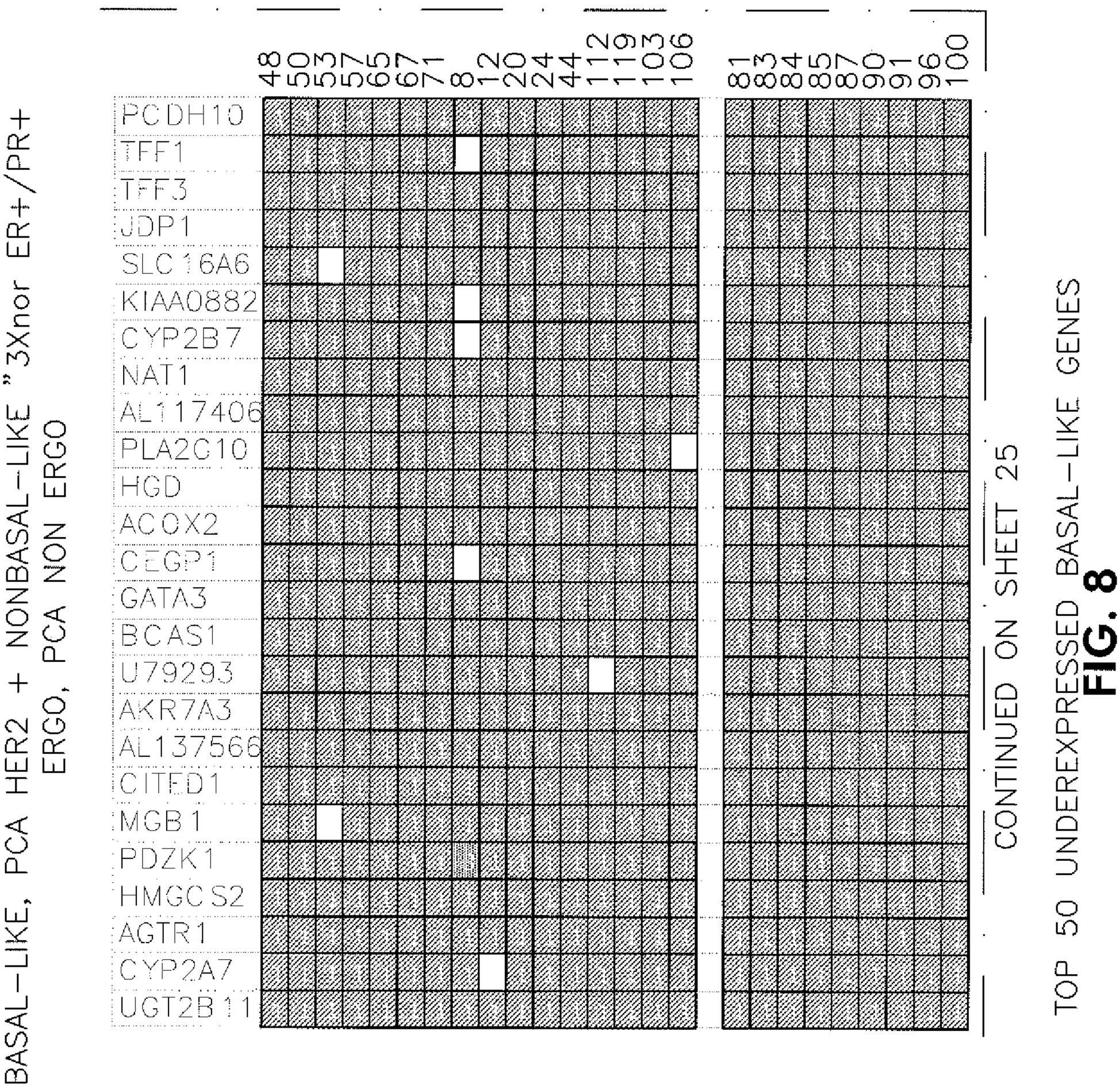


FIG. 8

CONTINUED ON SHEET 24

CONTINUED FROM SHEET 23



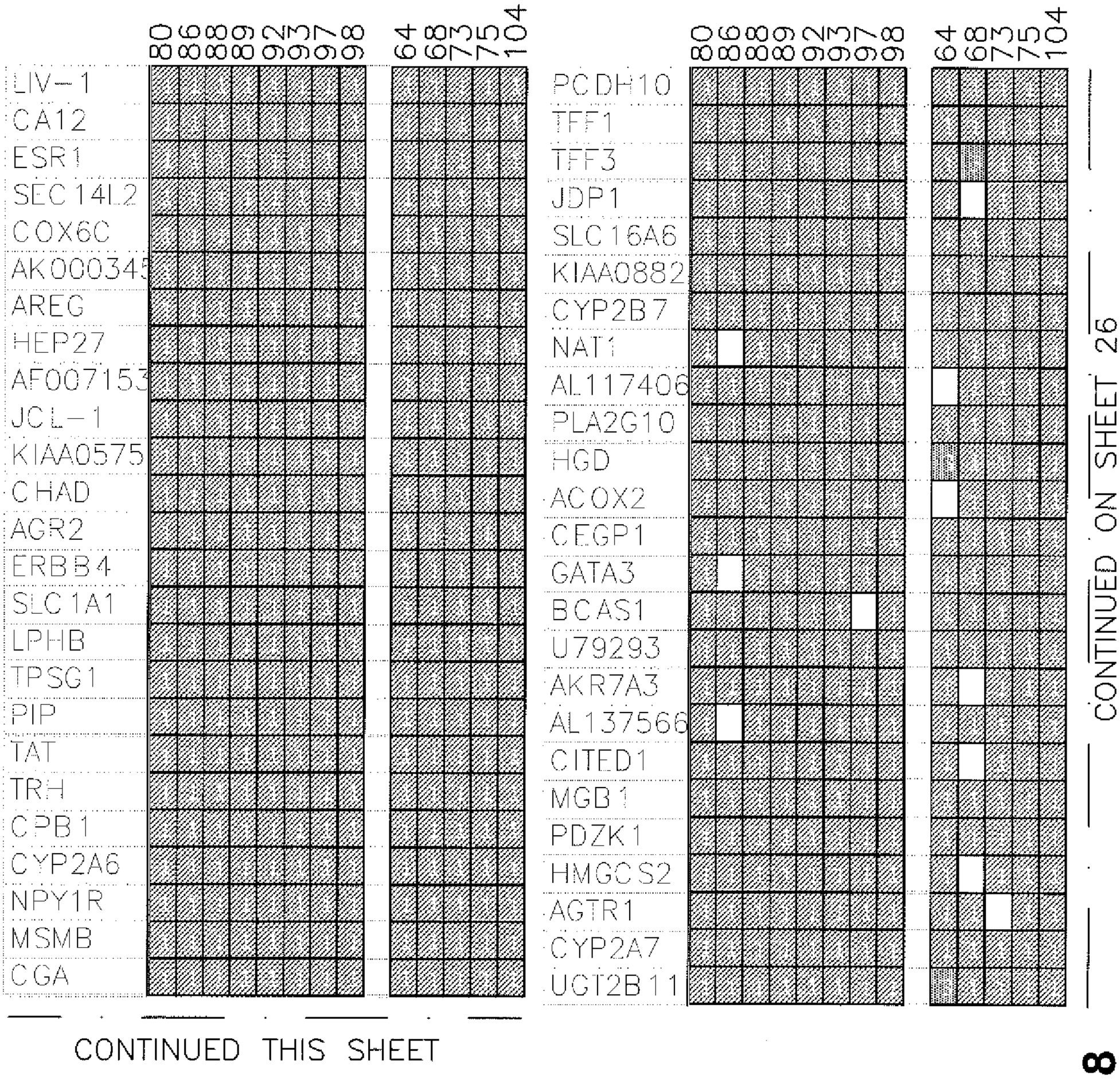
CONTINUED ON SHEET 25

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG. 8

25

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO



CONTINUED THIS SHEET

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG. 8

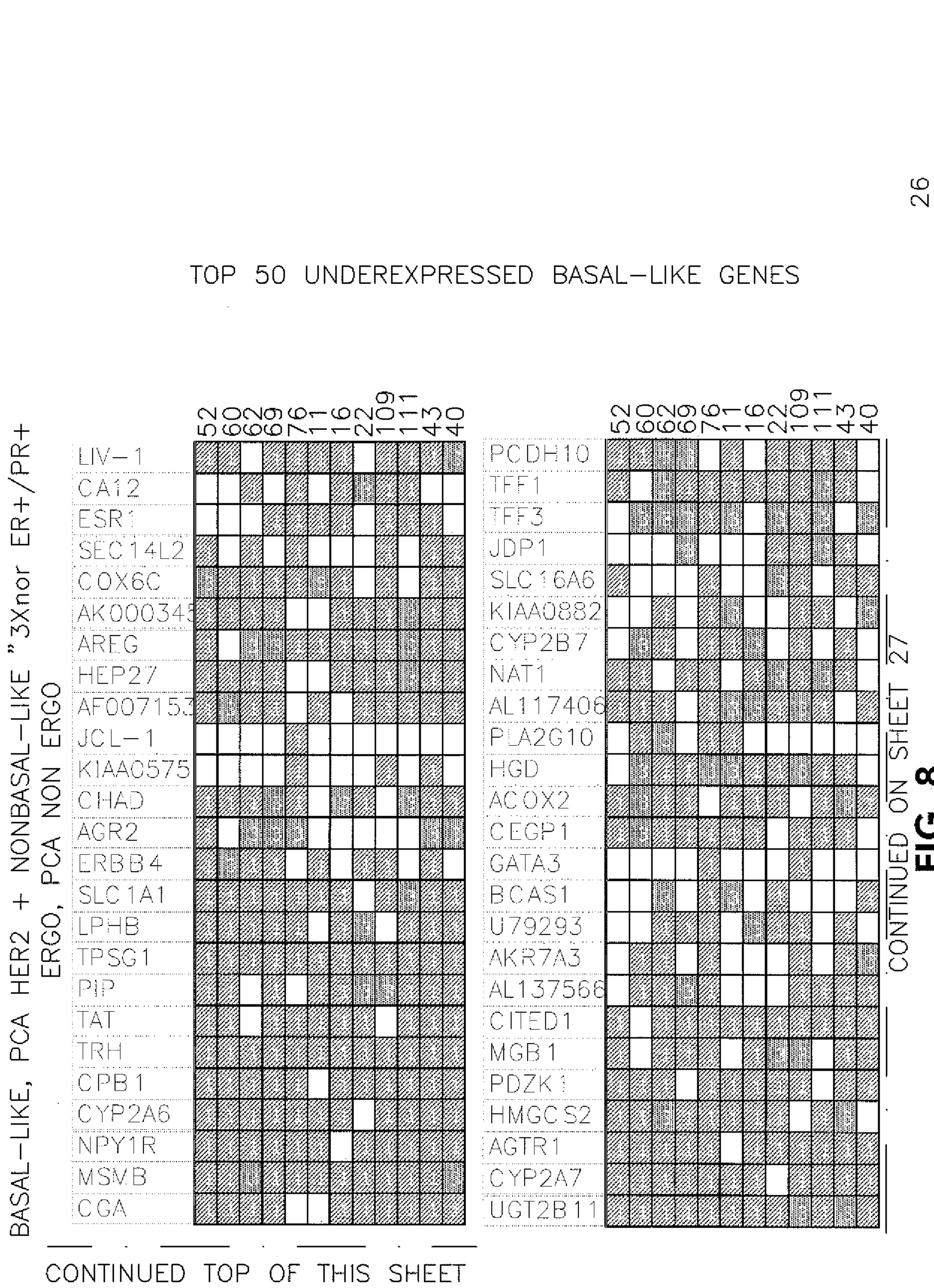
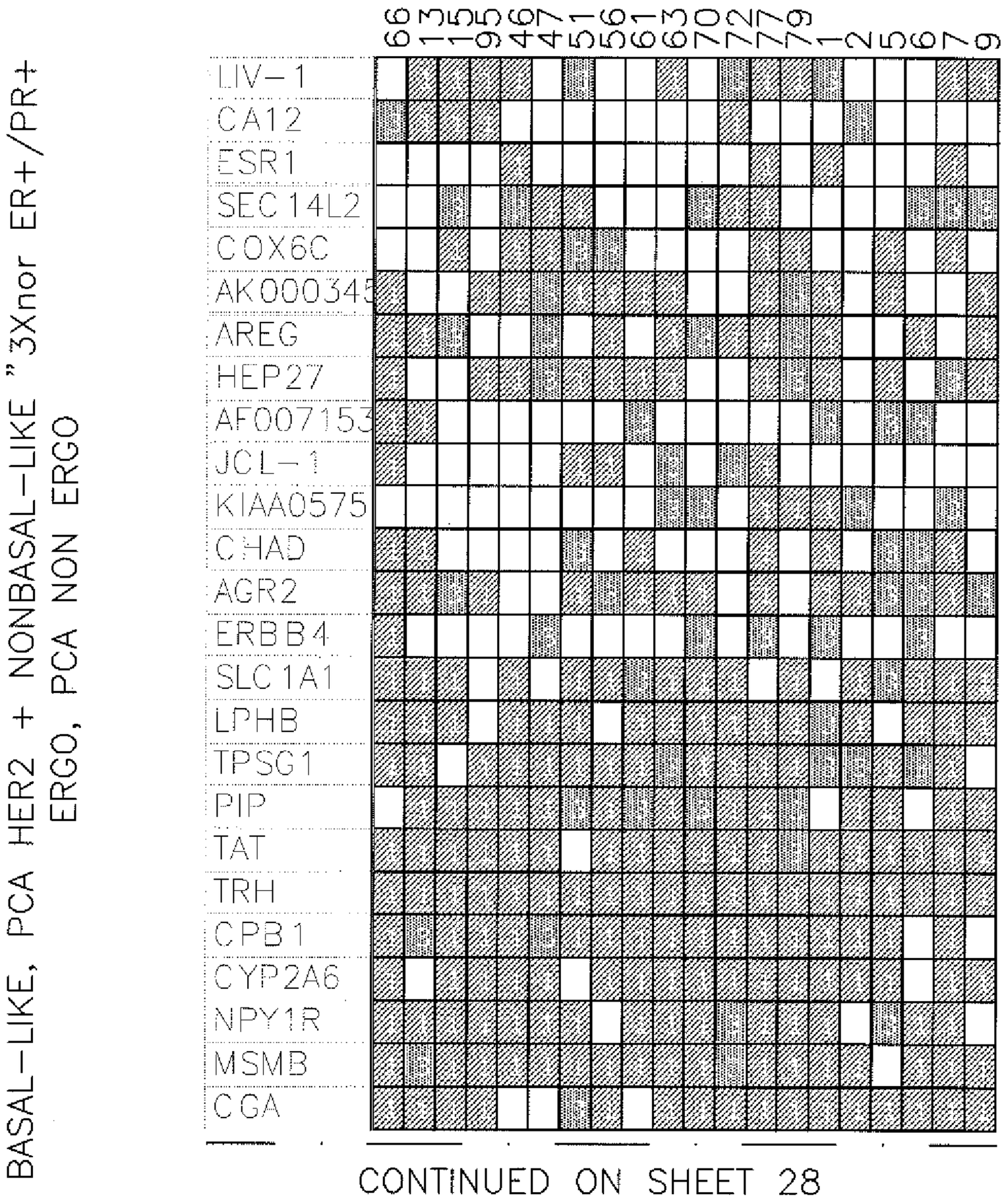

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
52 60 62 69 76 11 16 22 109 111 43 40
LIV-1
CA12
ESR1
SEC14L2
COX6C
AK000345
AREG
HEP27
AF007153
JCL-1
KIAA0575
CHAD
AGR2
ERBB4
SLC1A1
LPHB
TPSG1
PIP
TAT
TRH
CPB1
CYP2A6
NPY1R
MSMB
CGA
PCDH10
TFF1
TFF3
JDP1
SLC16A6
KIAA0882
CYP2B7
NAT1
AL117406
PLA2G10
HGD
ACOX2
CEGP1
GATA3
BCAS1
U79293
AKR7A3
AL137566
CITED1
MGB1
PDZK1
HMGCS2
AGTR1
CYP2A7
UGT2B11
CONTINUED ON SHEET 27
CONTINUED TOP OF THIS SHEET

FIG. 8

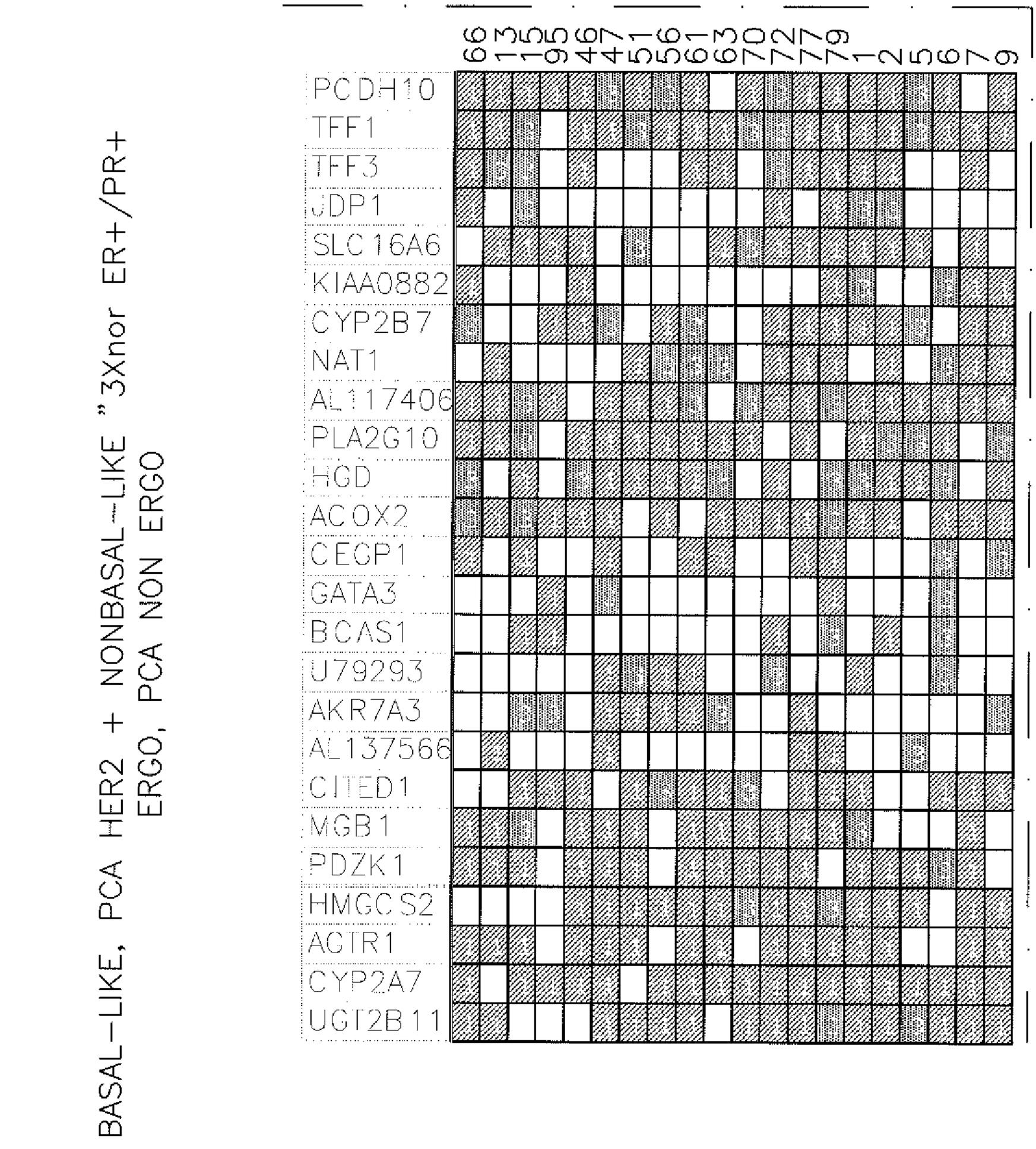


TOP 50 UNDEREXPRESSED E

FIG. 8

CONTINUED ON SHEET 28

CONTINUED FROM SHEET 27

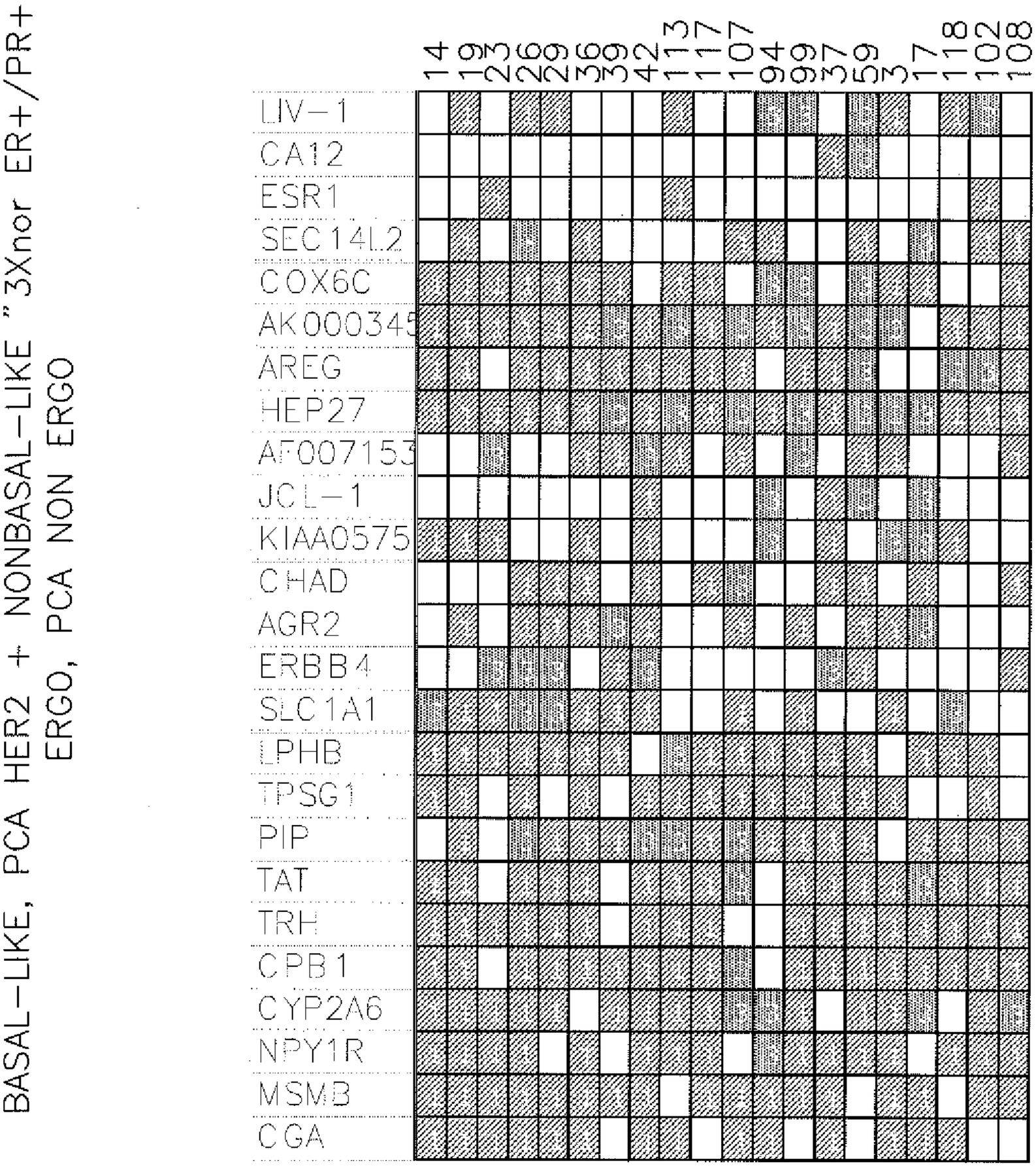


CONTINUED ON SHEET 29

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG. 8

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO

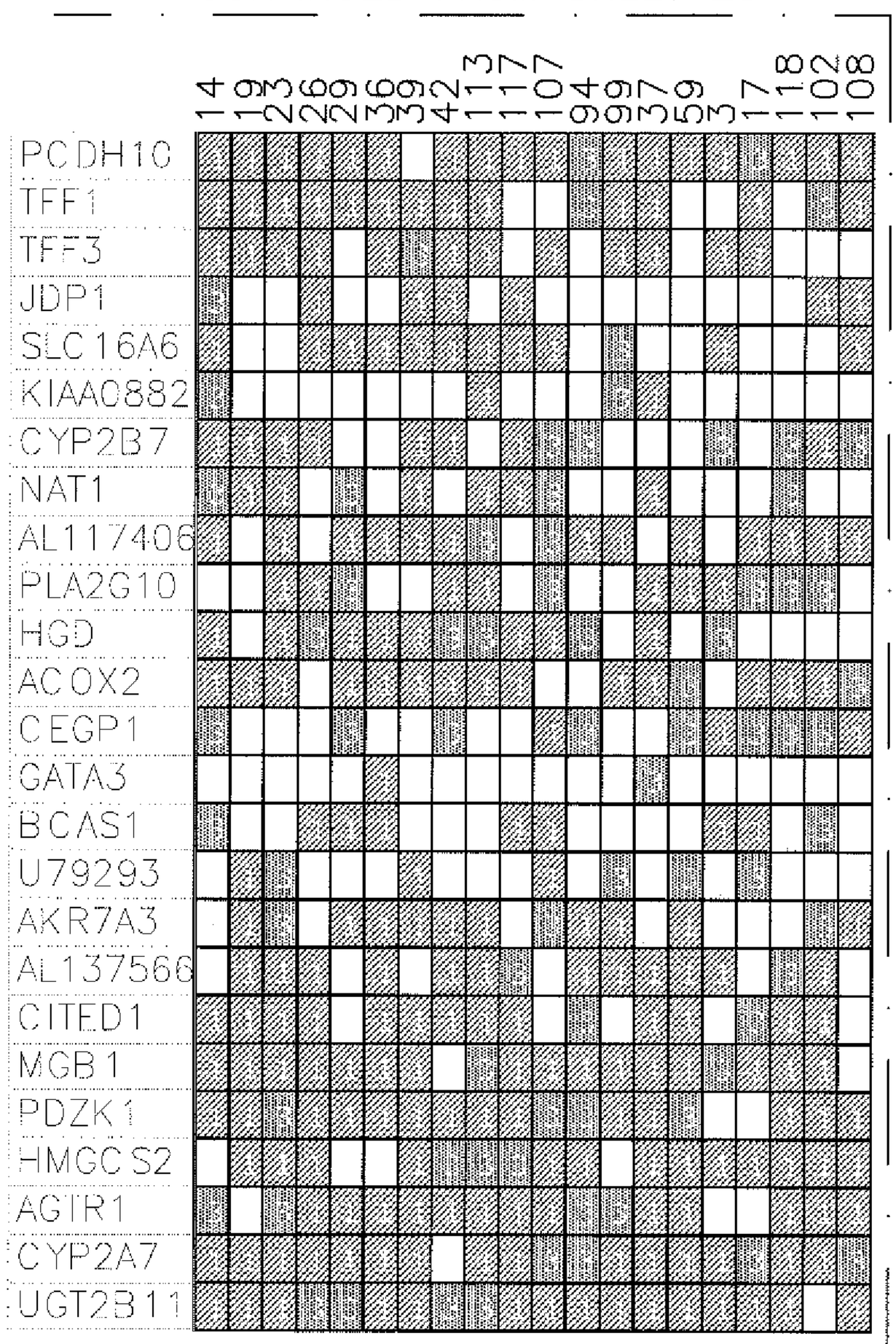


TOP 50 UNDEREXPRESSED BASAL-LIKE GENES

FIG. 8

CONTINUED ON SHEET 30

CONTINUED FROM SHEET 29



CONTINUED ON SHEET 31

TOP 50 UNDEREXPRESSED BASAL-LIKE GENES
ERGO, PCA NONBASAL-LIKE "3Xnor ER+/PR+
BASAL-LIKE, PCA HER2 + NONE

FIG. 8

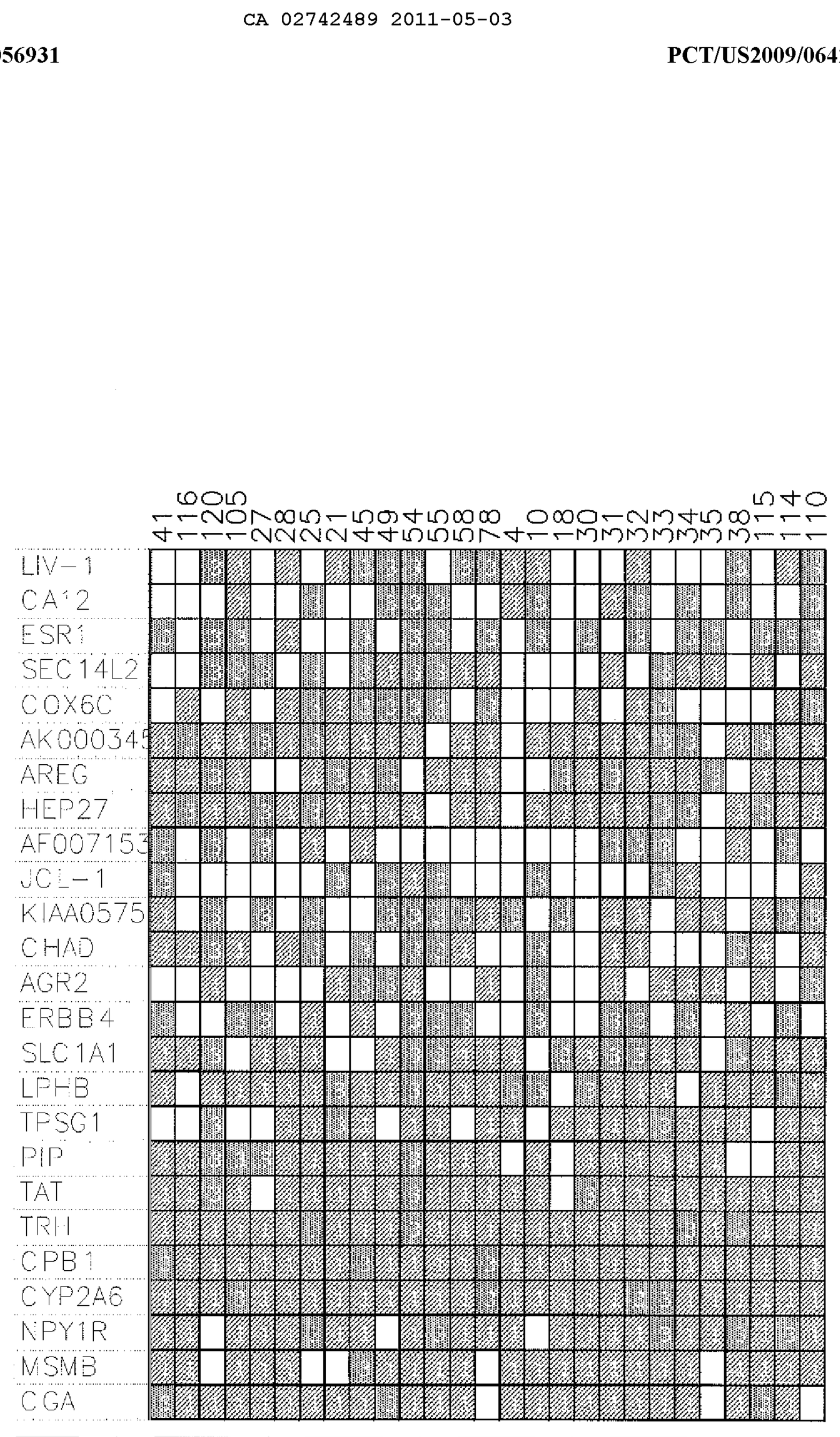


FIG. 8

CONTINUED ON SHEET 32

CONTINUED FROM SHEET 31

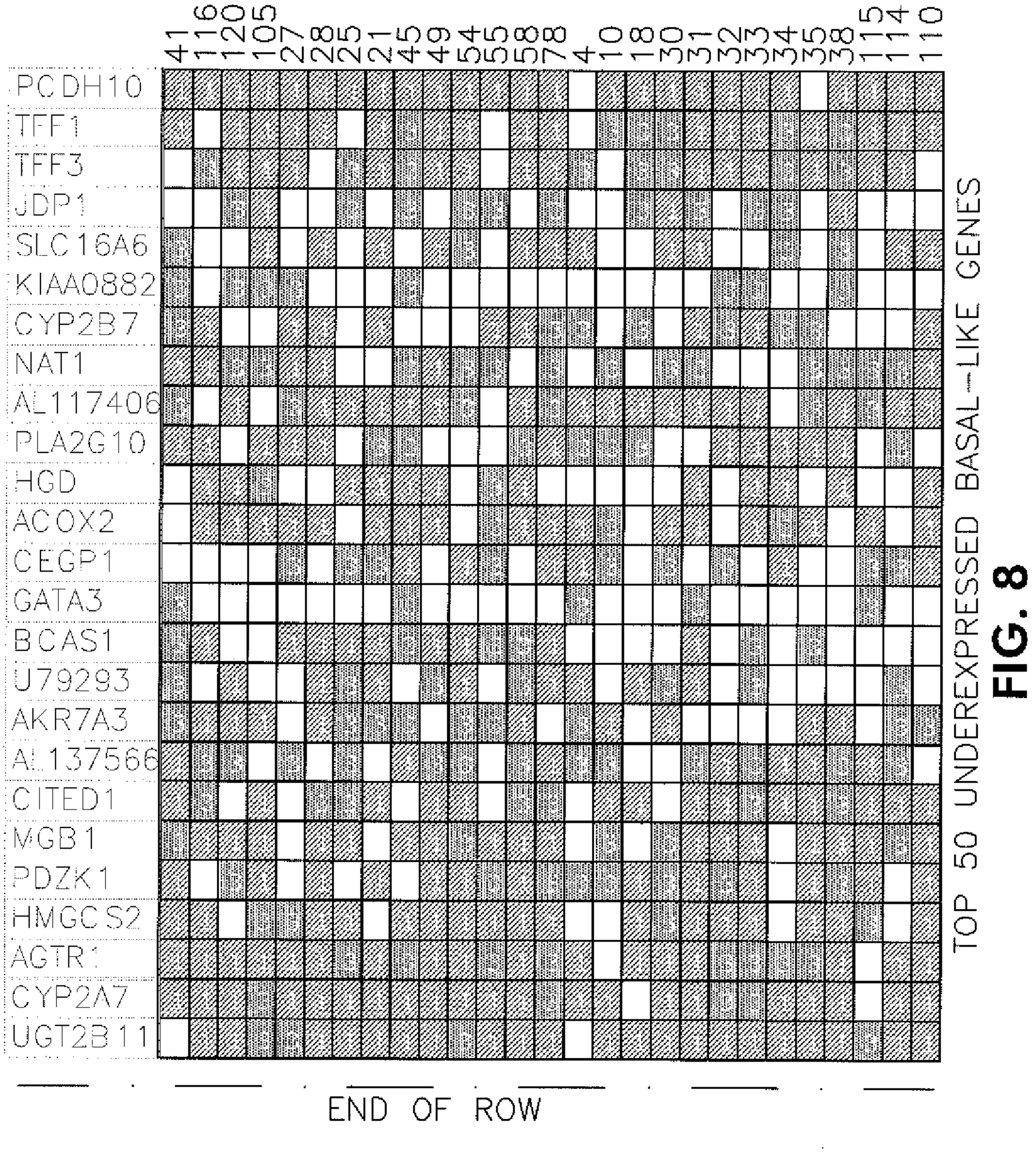


FIG. 8

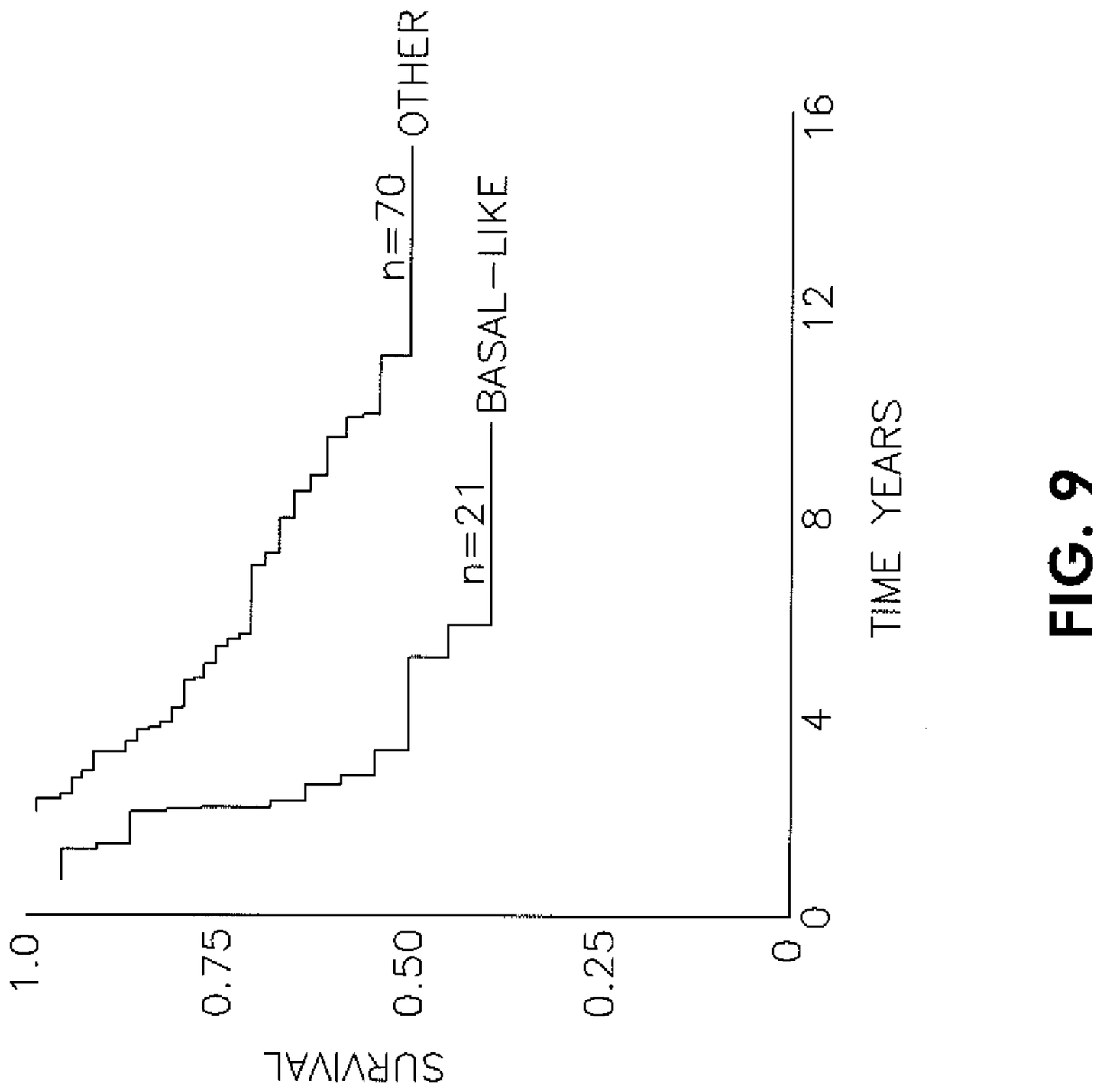

n=70
OTHER
n=21
BASAL-LIKE
SURVIVAL
1.0
0.75
0.50
0.25
0
0
4
8
12
16
TIME YEARS

FIG. 9

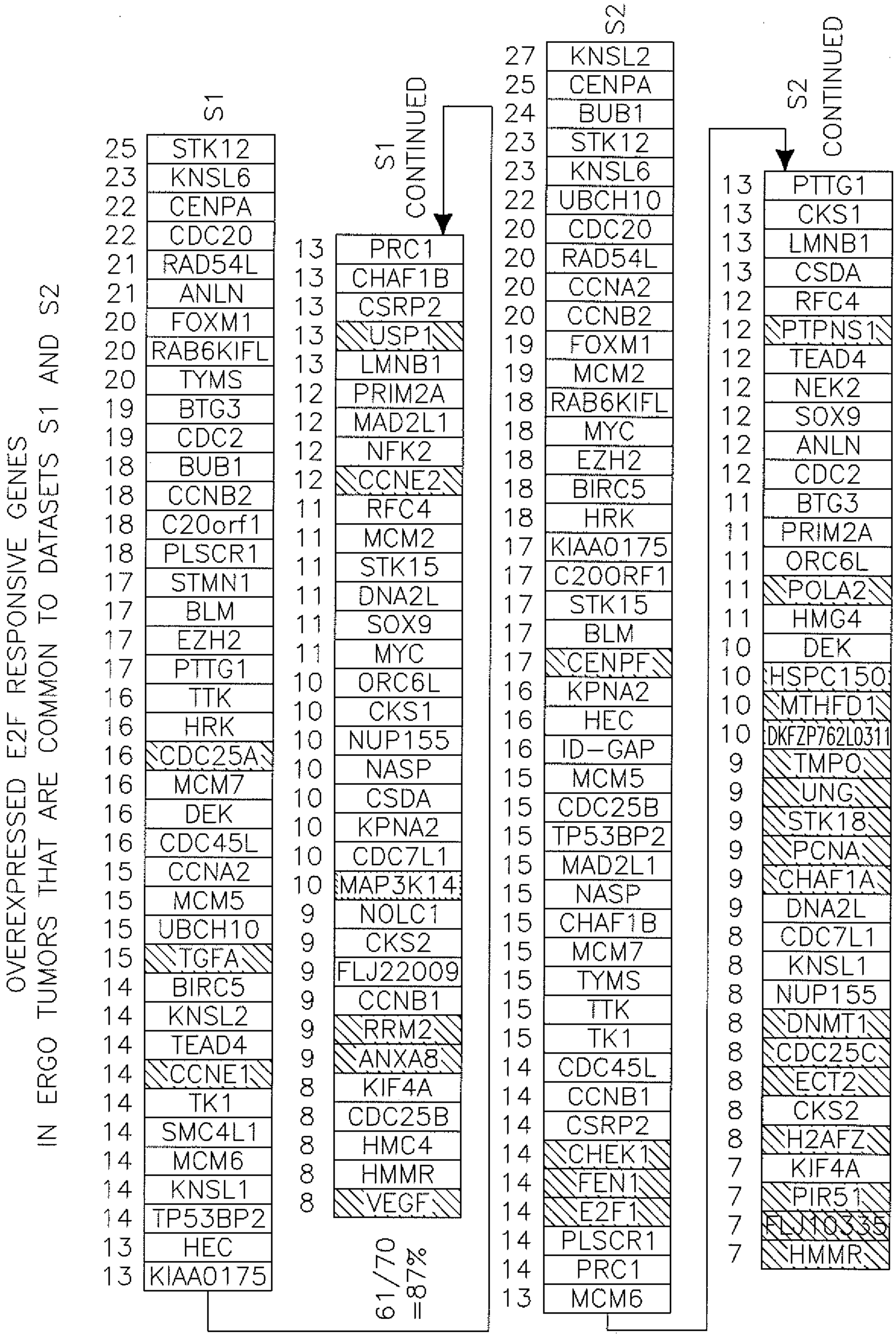

OVEREXPRESSED E2F RESPONSIVE GENES
IN ERGO TUMORS THAT ARE COMMON TO DATASETS S1 AND S2
S1
25 STK12
23 KNSL6
22 CENPA
22 CDC20
21 RAD54L
21 ANLN
20 FOXM1
20 RAB6KIFL
20 TYMS
19 BTG3
19 CDC2
18 BUB1
18 CCNB2
18 C20orf1
18 PLSCR1
17 STMN1
17 BLM
17 EZH2
17 PTTG1
16 TTK
16 HRK
16 CDC25A
16 MCM7
16 DEK
16 CDC45L
15 CCNA2
15 MCM5
15 UBCH10
15 TGFA
14 BIRC5
14 KNSL2
14 TEAD4
14 CCNE1
14 TK1
14 SMC4L1
14 MCM6
14 KNSL1
14 TP53BP2
13 HEC
13 KIAA0175
S1 CONTINUED
13 PRC1
13 CHAF1B
13 CSRP2
13 USP1
13 LMNB1
12 PRIM2A
12 MAD2L1
12 NFK2
12 CCNE2
11 RFC4
11 MCM2
11 STK15
11 DNA2L
11 SOX9
11 MYC
10 ORC6L
10 CKS1
10 NUP155
10 NASP
10 CSDA
10 KPNA2
10 CDC7L1
10 MAP3K14
9 NOLC1
9 CKS2
9 FLJ22009
9 CCNB1
9 RRM2
9 ANXA8
8 KIF4A
8 CDC25B
8 HMC4
8 HMMR
8 VEGF
61/70 = 87%
S2
27 KNSL2
25 CENPA
24 BUB1
23 STK12
23 KNSL6
22 UBCH10
20 CDC20
20 RAD54L
20 CCNA2
20 CCNB2
19 FOXM1
19 MCM2
18 RAB6KIFL
18 MYC
18 EZH2
18 BIRC5
18 HRK
17 KIAA0175
17 C20ORF1
17 STK15
17 BLM
17 CENPF
16 KPNA2
16 HEC
16 ID-GAP
15 MCM5
15 CDC25B
15 TP53BP2
15 MAD2L1
15 NASP
15 CHAF1B
15 MCM7
15 TYMS
15 TTK
15 TK1
14 CDC45L
14 CCNB1
14 CSRP2
14 CHEK1
14 FEN1
14 E2F1
14 PLSCR1
14 PRC1
13 MCM6
S2 CONTINUED
13 PTTG1
13 CKS1
13 LMNB1
13 CSDA
12 RFC4
12 PTPNS1
12 TEAD4
12 NEK2
12 SOX9
12 ANLN
12 CDC2
11 BTG3
11 PRIM2A
11 ORC6L
11 POLA2
11 HMG4
10 DEK
10 HSPC150
10 MTHFD1
10 DKFZP762L0311
9 TMPO
9 UNG
9 STK18
9 PCNA
9 CHAF1A
9 DNA2L
8 CDC7L1
8 KNSL1
8 NUP155
8 DNMT1
8 CDC25C
8 ECT2
8 CKS2
8 H2AFZ
7 KIF4A
7 PIR51
7 FLJ10335
7 HMMR
NON-SHARED GENES

FIG. 10

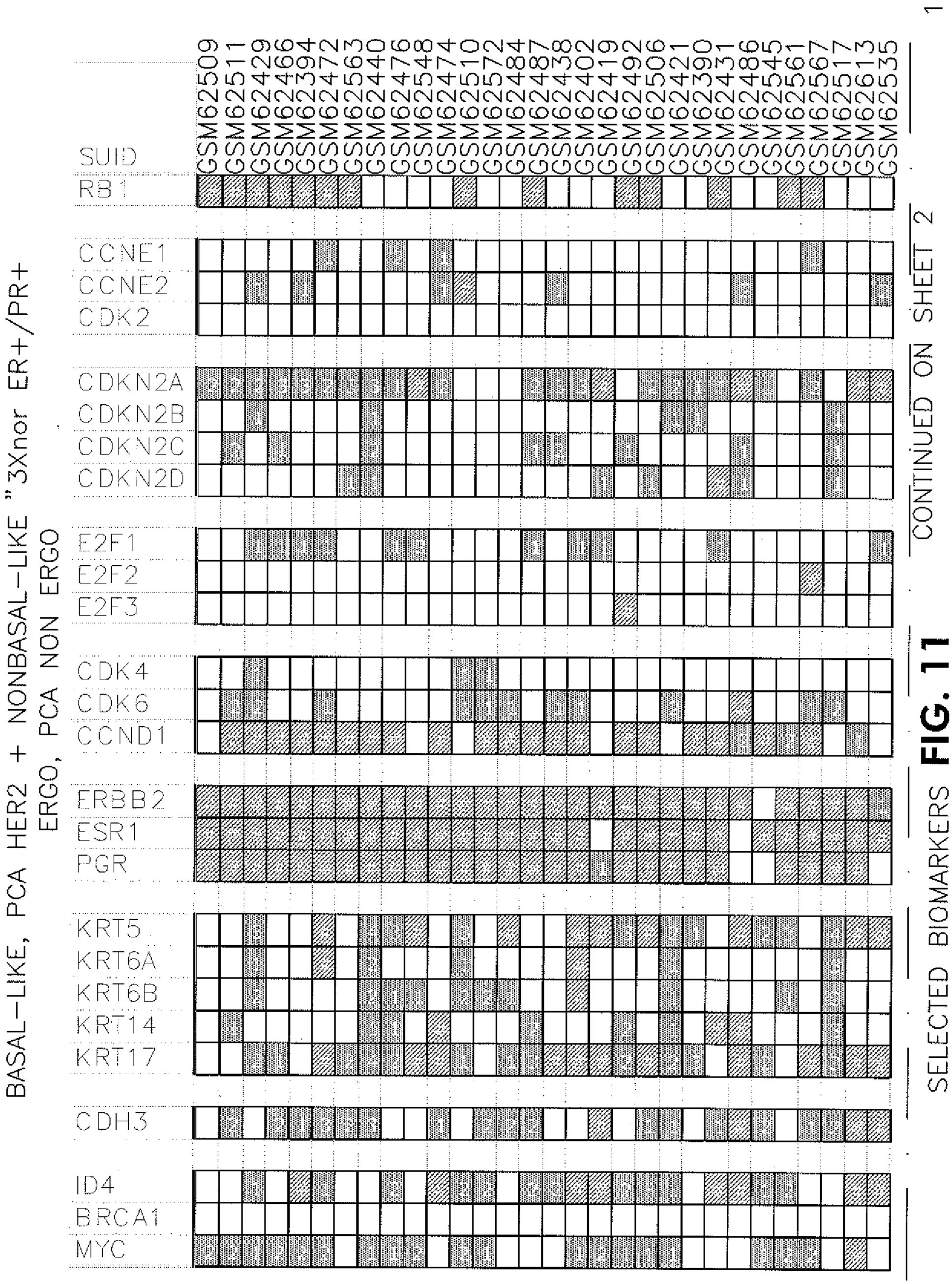


FIG. 11

SELECTED BIOMARKERS

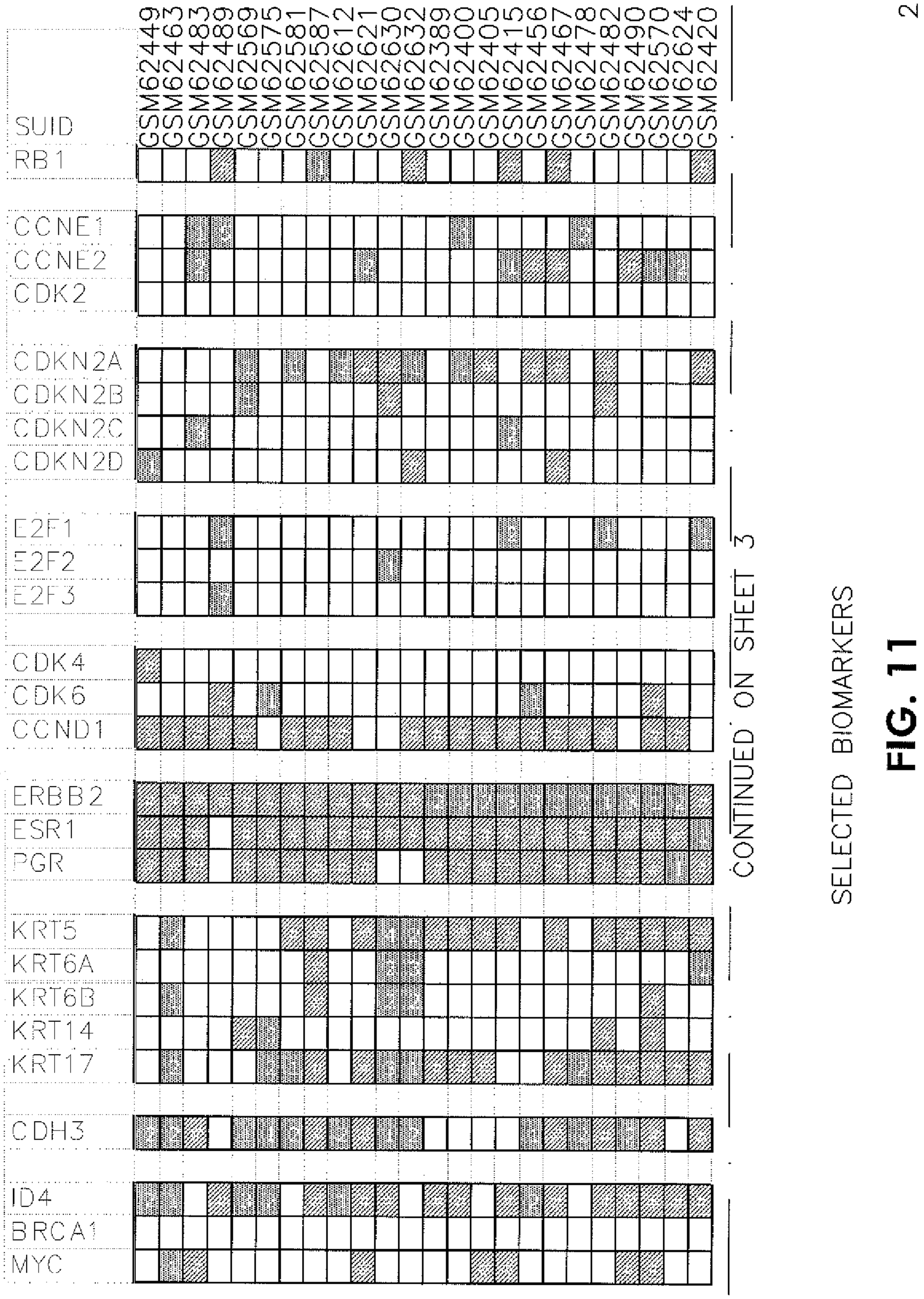

M62449
M62463
M62483
M62489
M62569
M62575
M62581
M62587
M62612
M62621
M62630
M62632
M62638
M62640
M62641
M62645
M62646
M62647
M62648
M62649
M62570
M62624
M62420
SUID
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
CONTINUED ON SHEET 3
SELECTED BIOMARKERS

FIG. 11

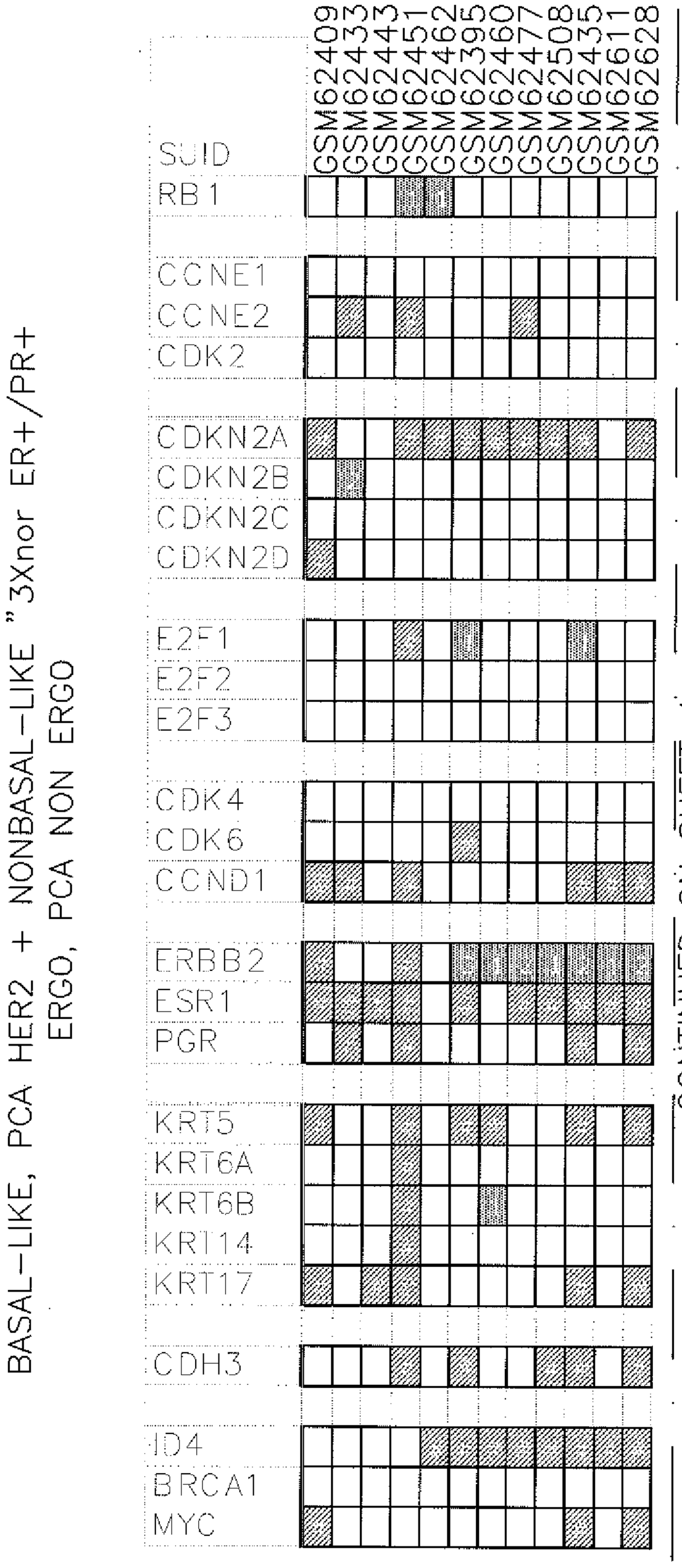

SUID
GSM624409
GSM624433
GSM624443
GSM624451
GSM624462
GSM623950
GSM624607
GSM624508
GSM624635
GSM626111
GSM626128
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO
CONTINUED ON SHEET 4
SELECTED BIOMARKERS

FIG. 11

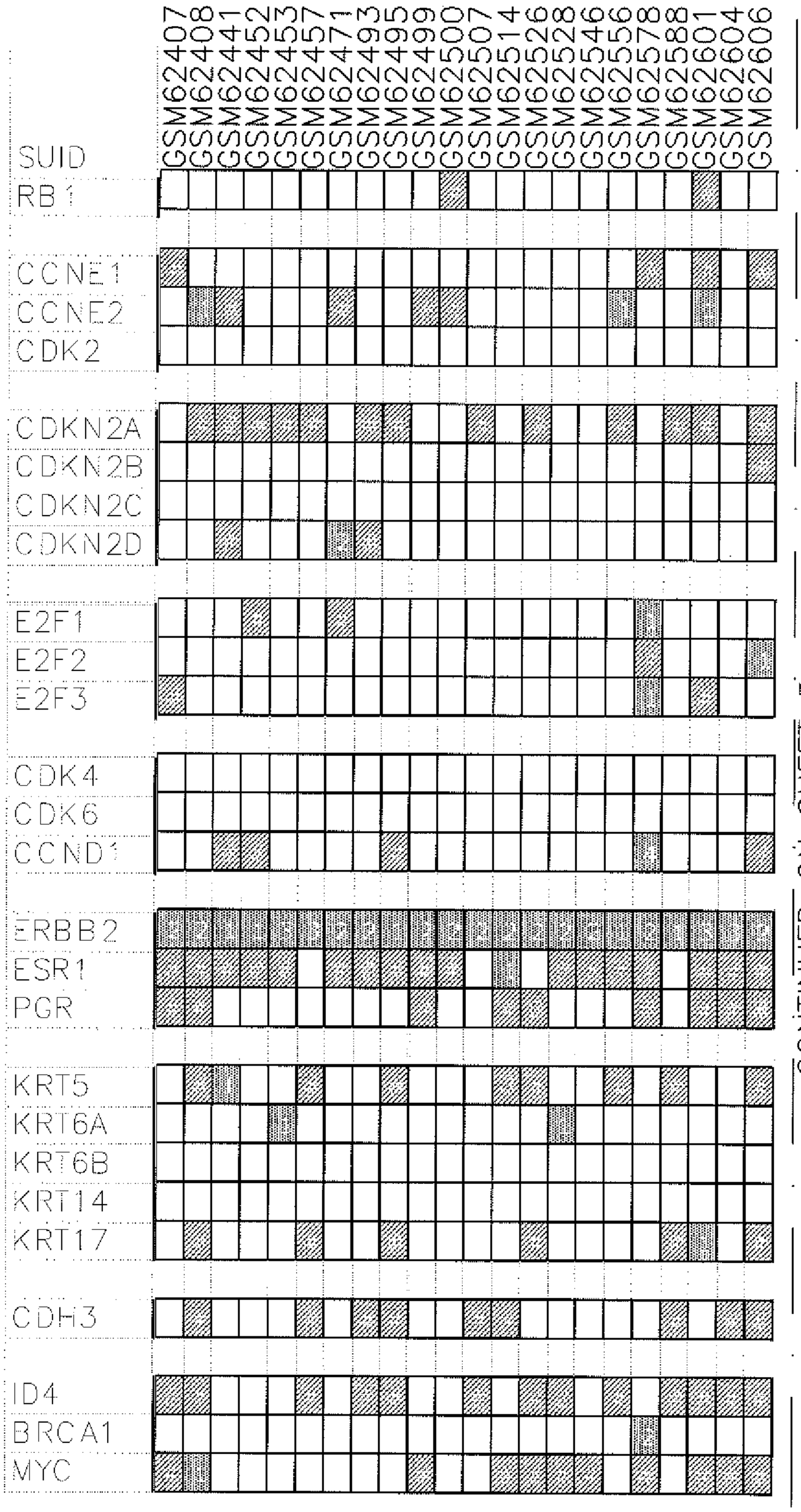

SUID
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO
CONTINUED ON SHEET 5
SELECTED BIOMARKERS

FIG. 11

5

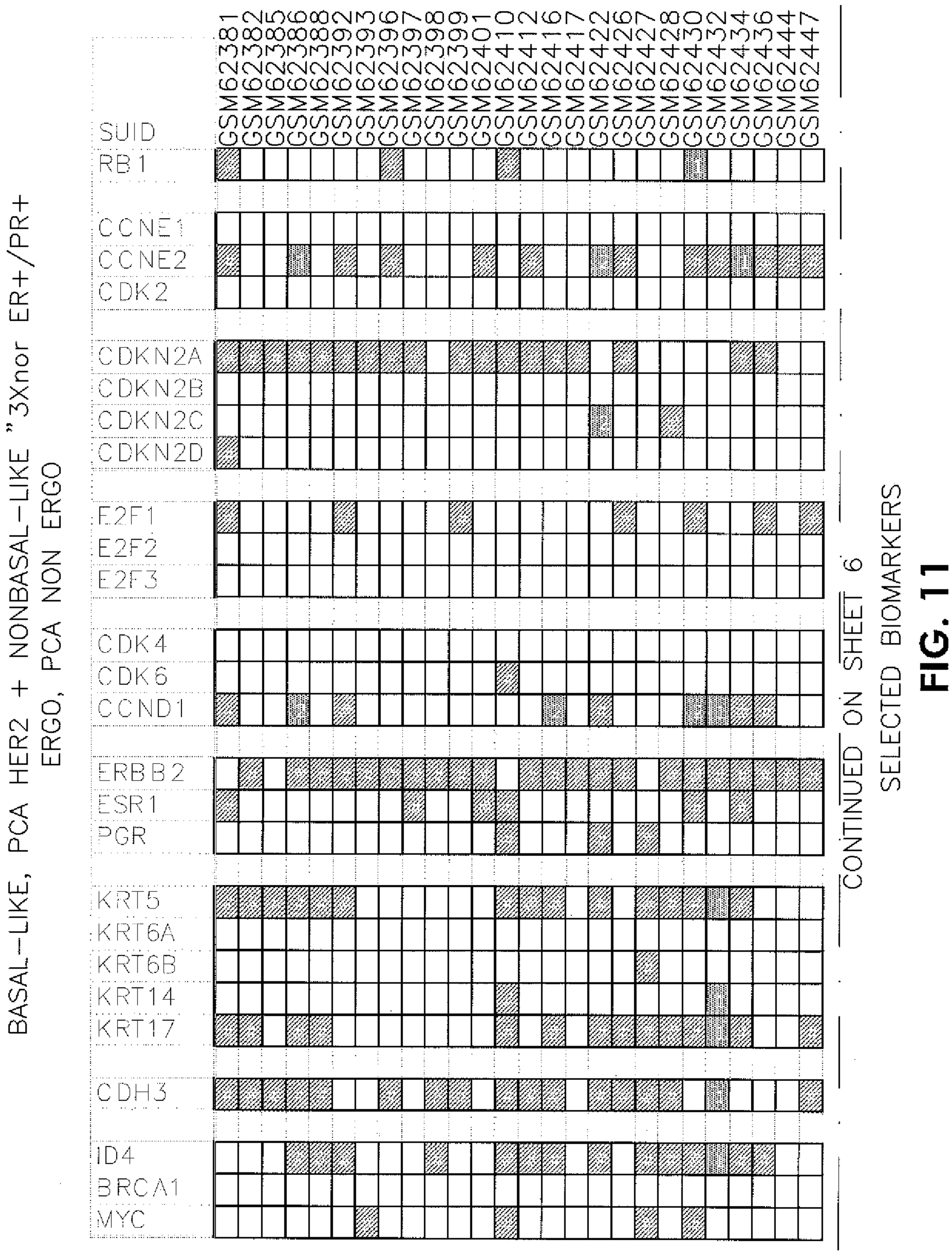

M623811
M623822
M623855
M623866
M623888
M623922
M623933
M623966
M623977
M623988
M623999
M624010
M624121
M624167
M624172
M624266
M624277
M624288
M624302
M624323
M624344
M624366
M624447
SUID
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
1D4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO
CONTINUED ON SHEET 6
SELECTED BIOMARKERS

FIG. 11

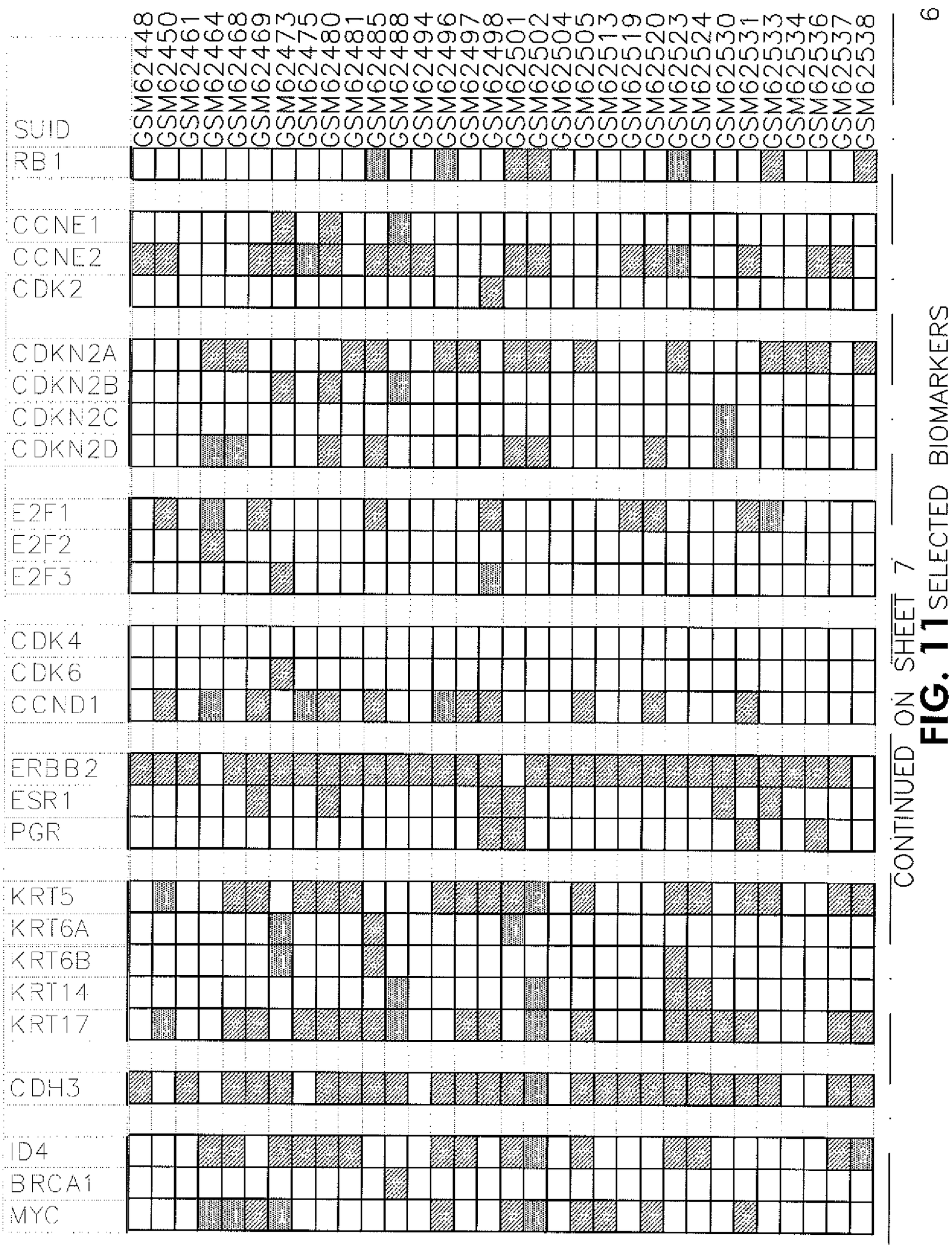
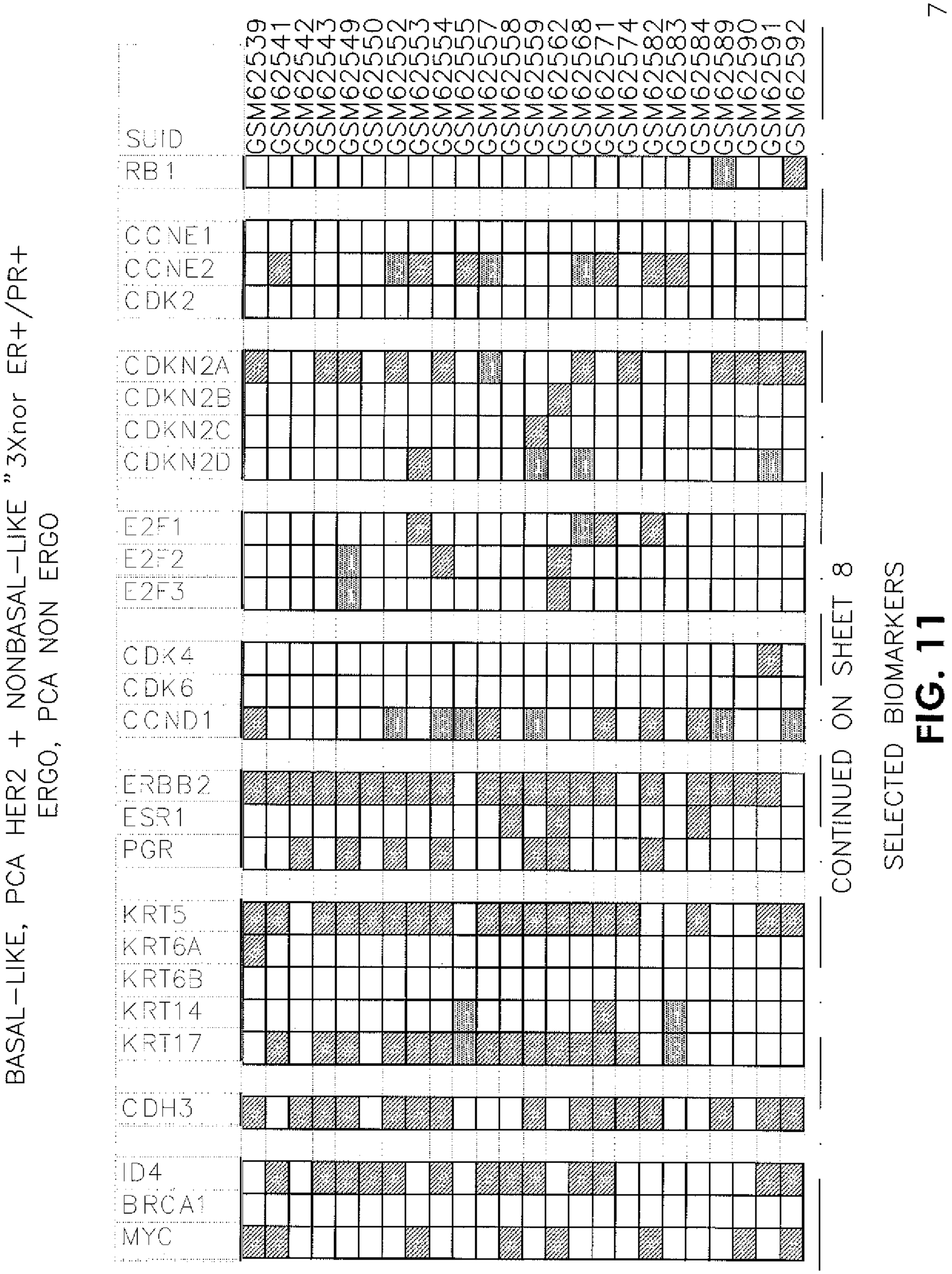
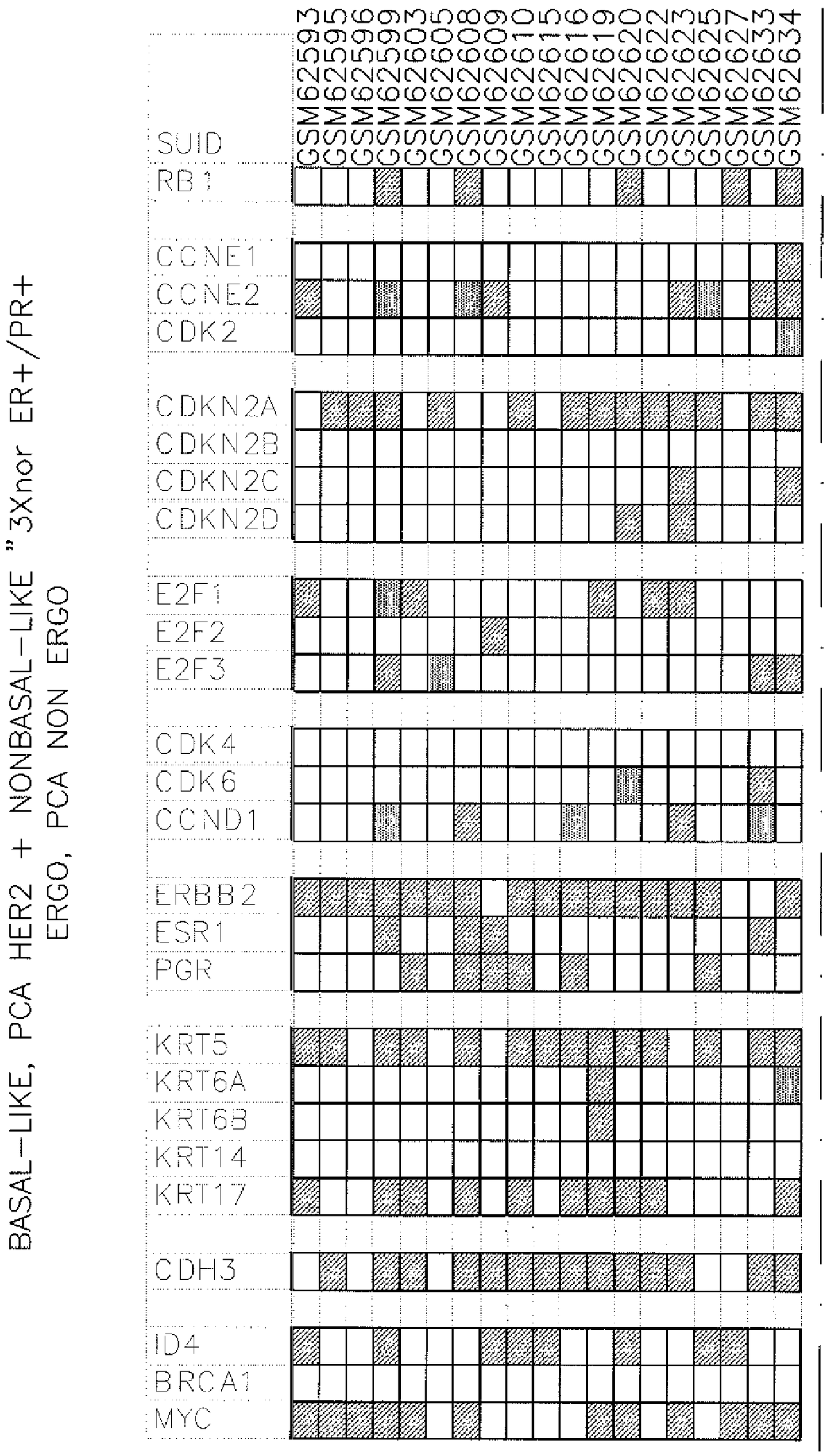

M62448
M62450
M62461
M62464
M62468
M62469
M62473
M62475
M62480
M62481
M62485
M62488
M62494
M62496
M62497
M62498
M62501
M62502
M62504
M62505
M62513
M62519
M62520
M62523
M62524
M62530
M62531
M62533
M62534
M62536
M62537
M62538
SUID
RB1
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC
CONTINUED ON SHEET 7
SELECTED BIOMARKERS
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO

FIG. 11 6



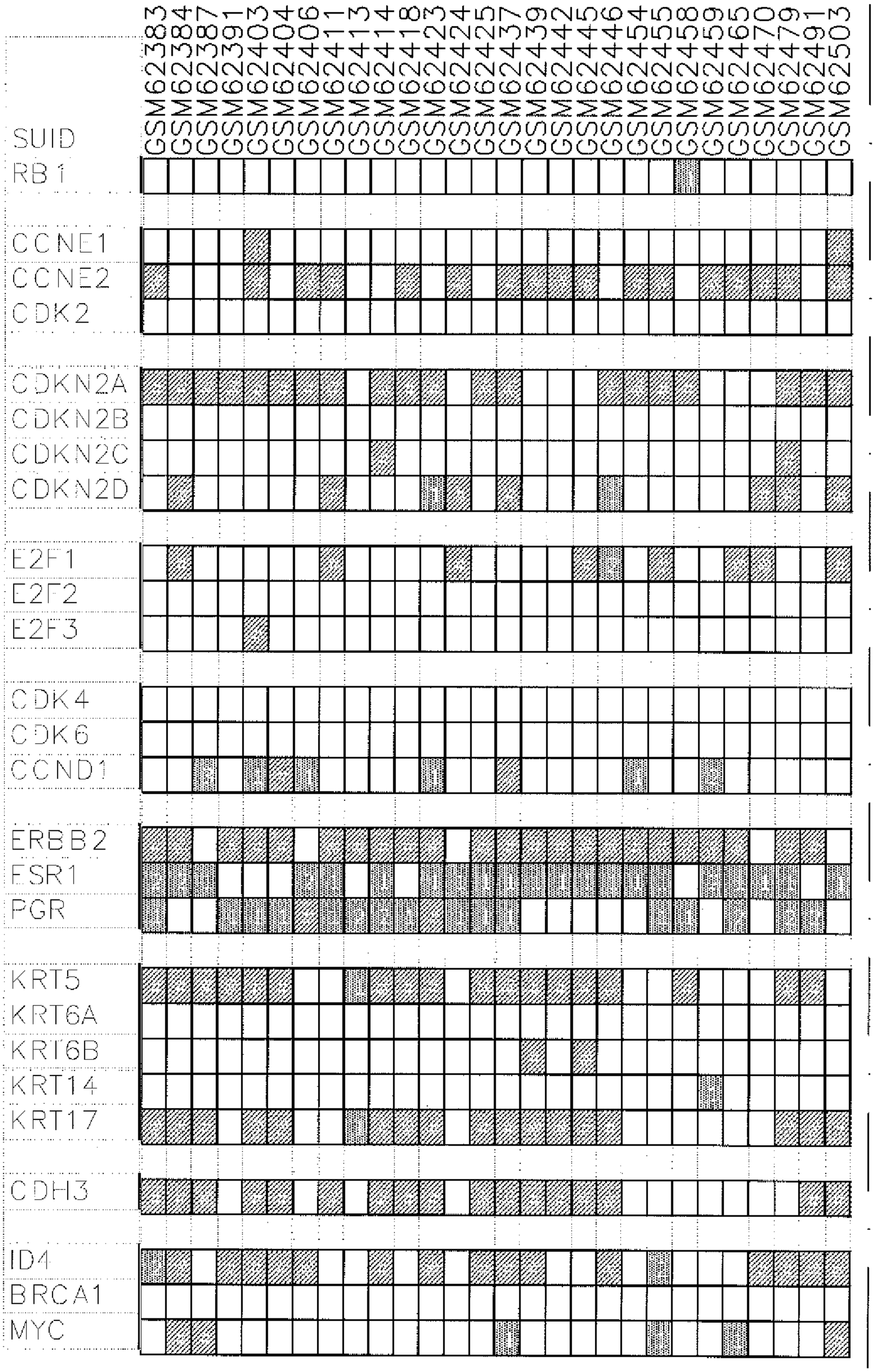
SELECTED BIOMARKERS

FIG. 11



SELECTED BIOMARKERS

FIG. 11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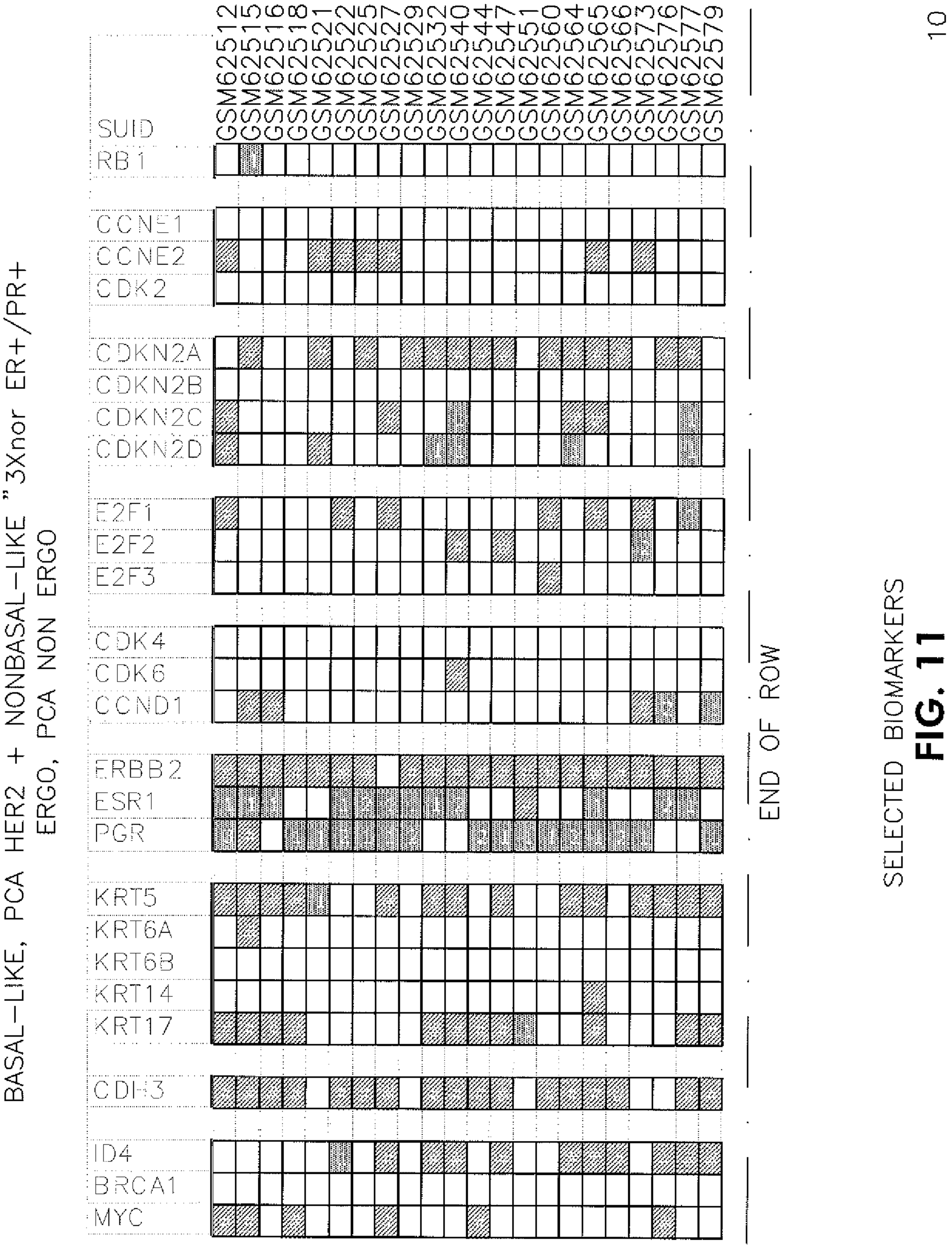
CCNE1
CCNE2
CDK2
CDKN2A
CDKN2B
CDKN2C
CDKN2D
E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
KRT5
KRT6A
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
CONTINUED ON SHEET 10
SELECTED BIOMARKERS

FIG. 11


BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
SELECTED BIOMARKERS
END OF ROW
10
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M625292
M625320
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M625510
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M625795
SUID
RB1
CCNE1
CCNE2
CDK2
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CDKN2B
CDKN2C
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E2F1
E2F2
E2F3
CDK4
CDK6
CCND1
ERBB2
ESR1
PGR
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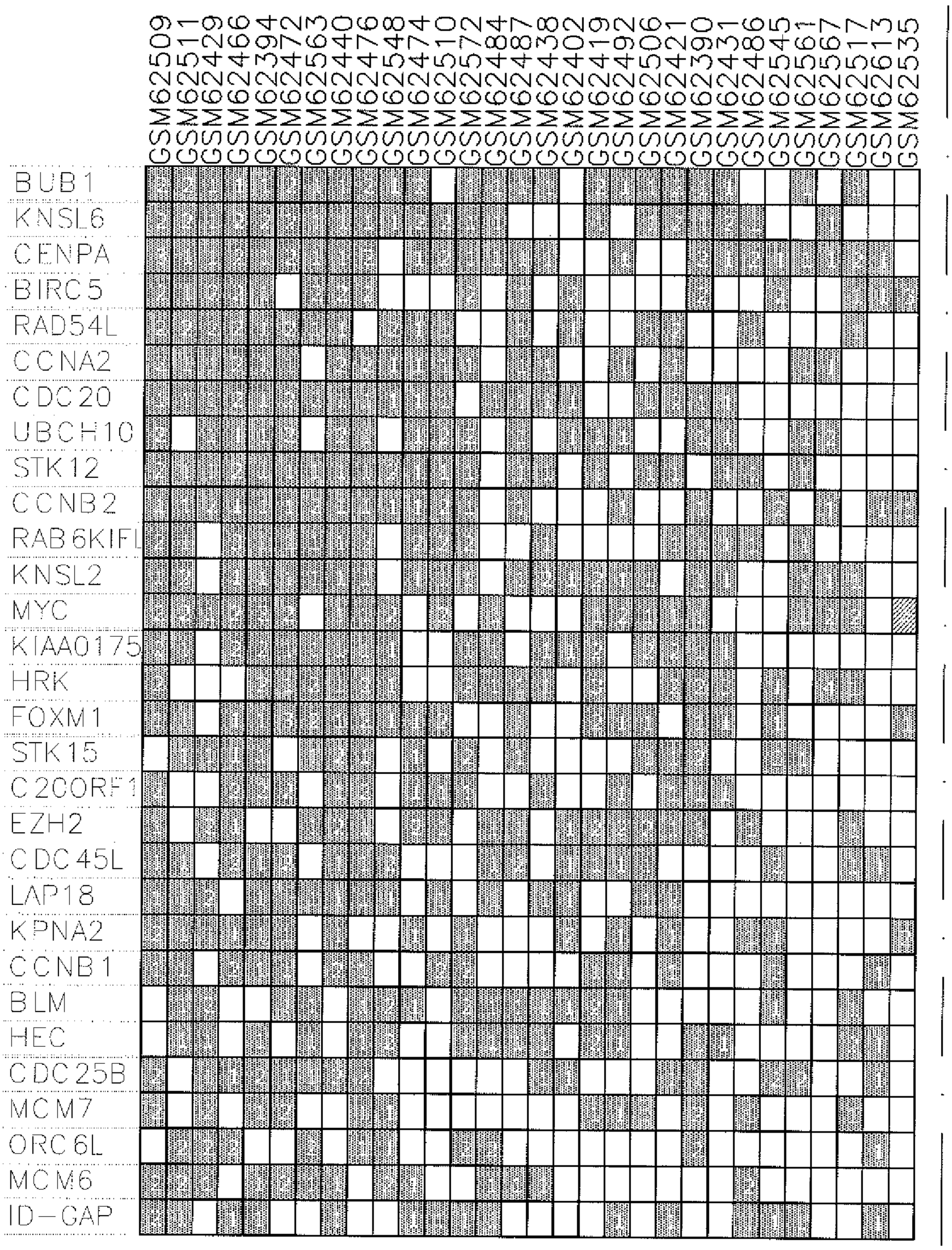
KRT6B
KRT14
KRT17
CDH3
ID4
BRCA1
MYC

FIG. 11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GSM624838
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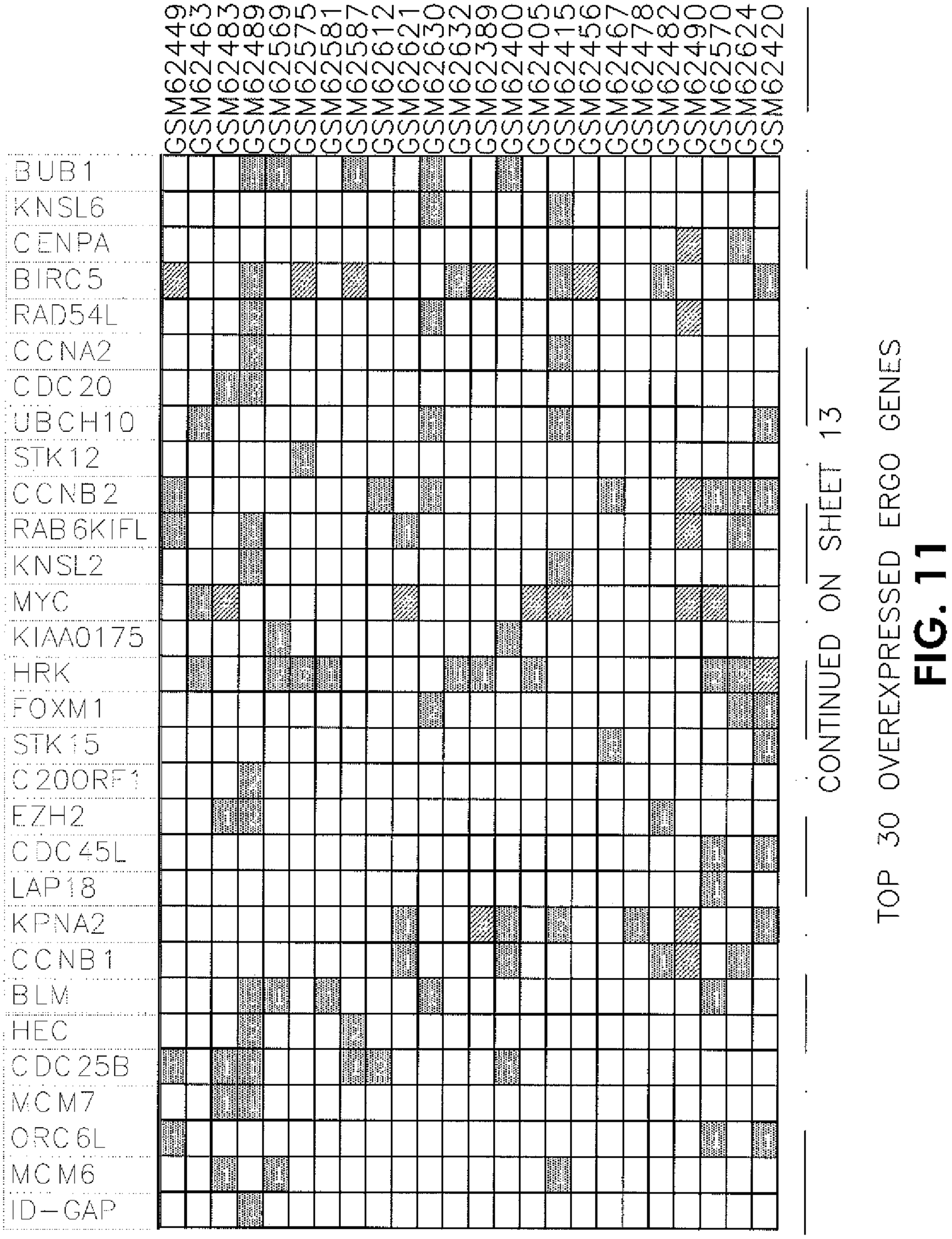
GSM625492
GSM624196
GSM625506
GSM624210
GSM623901
GSM624316
GSM624866
GSM625451
GSM625611
GSM625677
GSM625175
GSM626133
GSM625355
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO
CONTINUED ON SHEET 12
TOP 30 OVEREXPRESSED ERGO GENES

FIG. 11

12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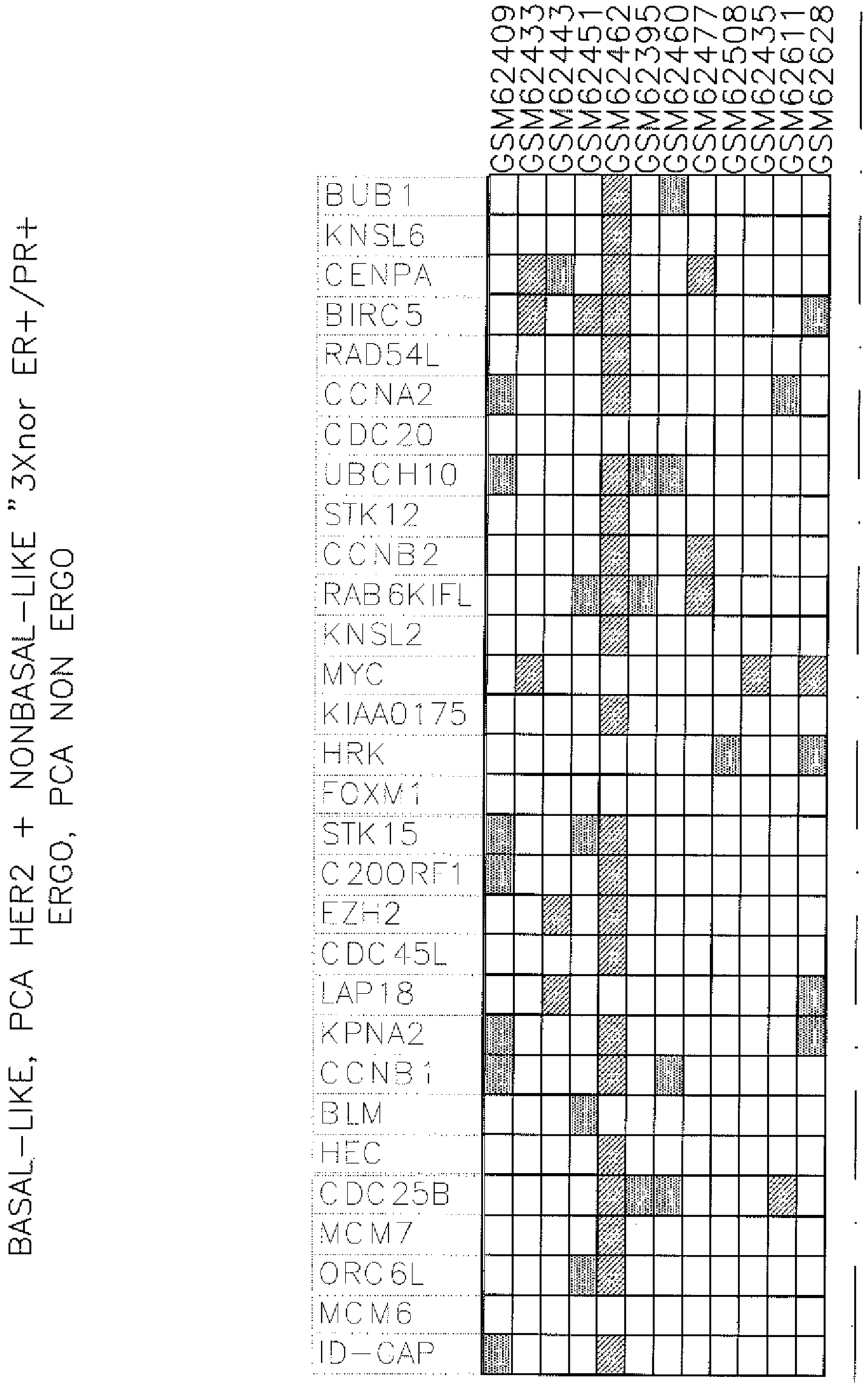
M62647
M62648
M62649
M62657
M62662
M62624
M62642
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NONERGO
CONTINUED ON SHEET 13
TOP 30 OVEREXPRESSED ERGO GENES

FIG. 11



TOP 30 OVEREXPRESSED ERGO GENES

FIG. 11

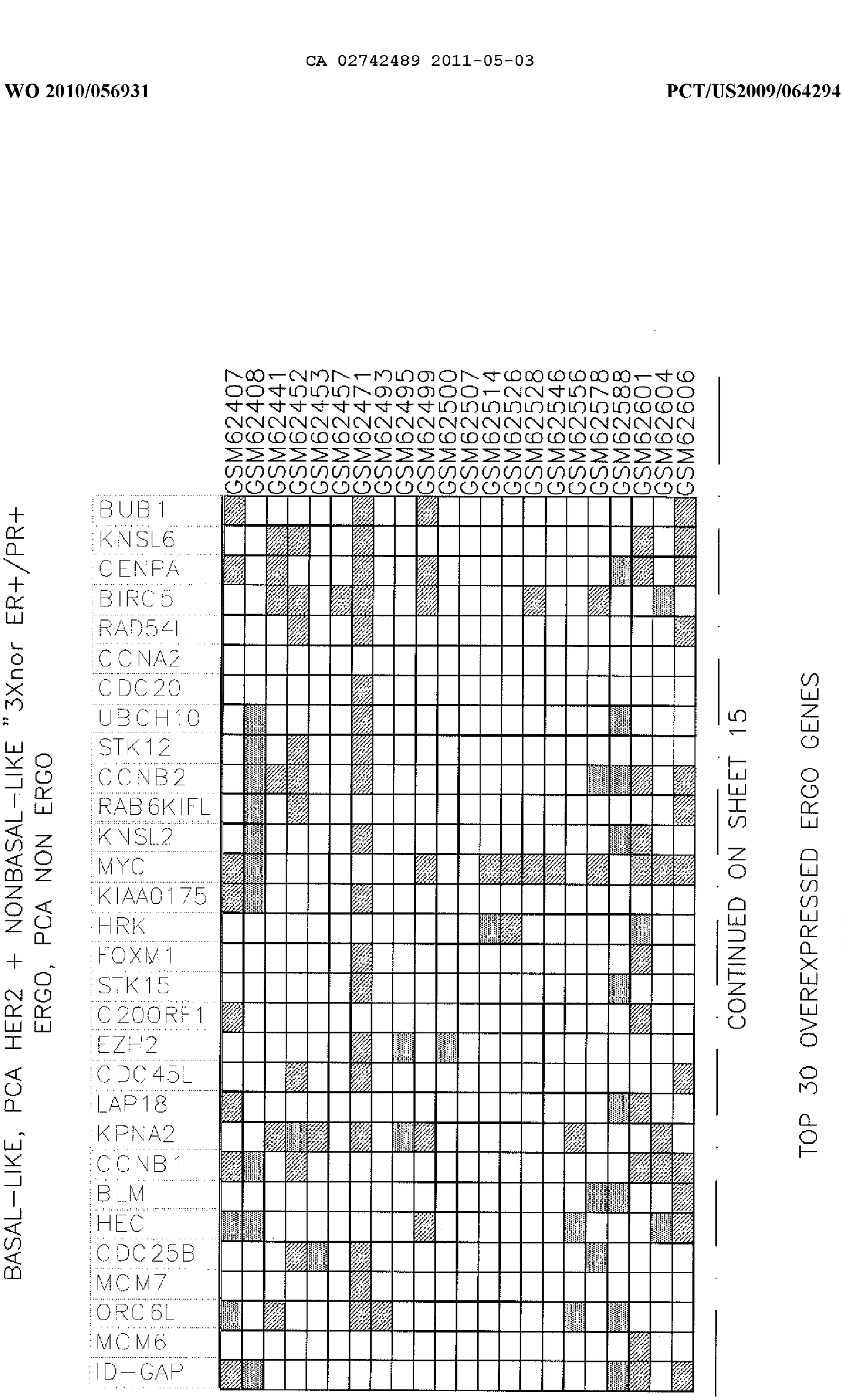
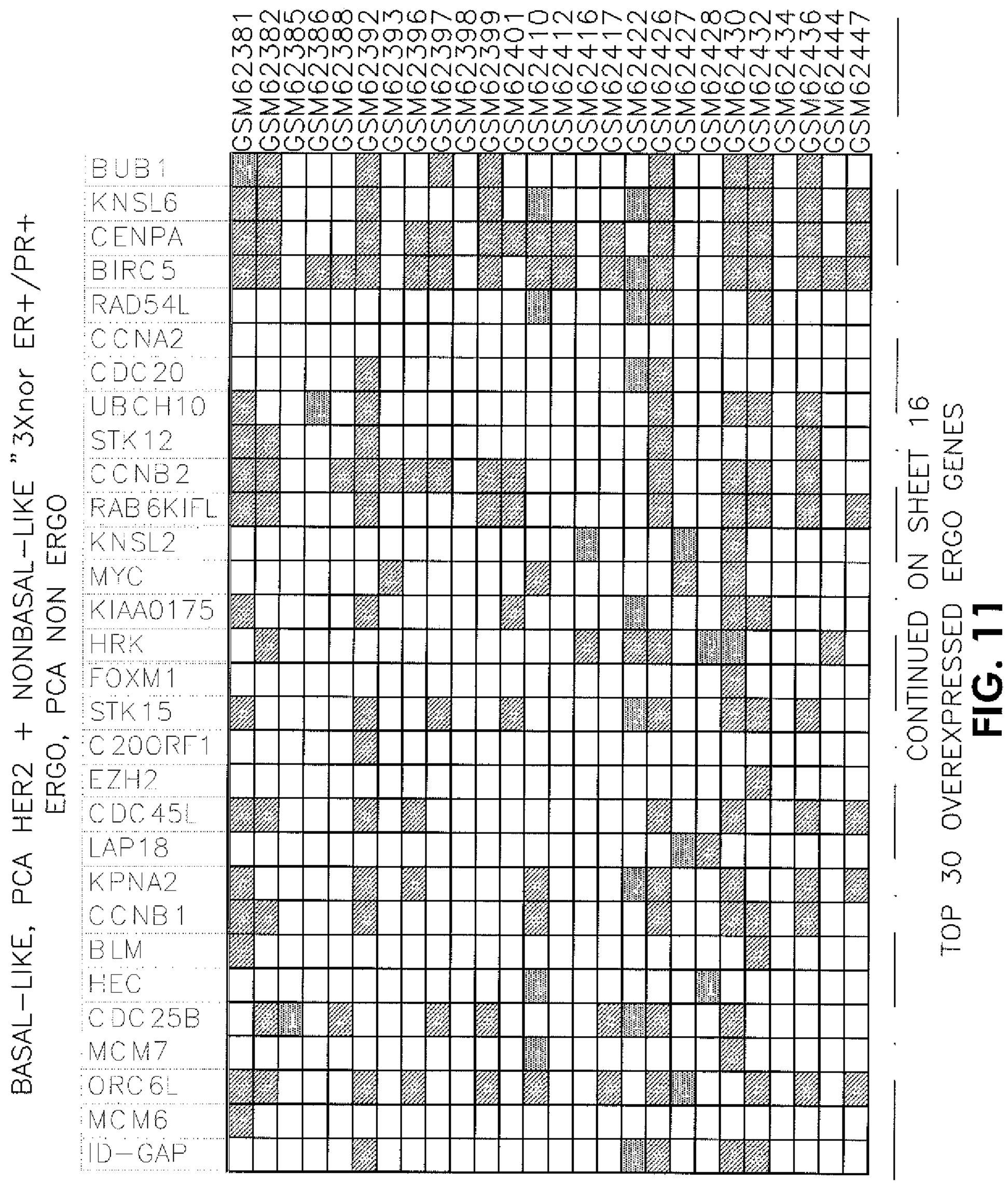

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
GSM624071
GSM624081
GSM624412
GSM624523
GSM624534
GSM624575
GSM624717
GSM624939
GSM624955
GSM624991
GSM625007
GSM625145
GSM625267
GSM625285
GSM625466
GSM625566
GSM625788
GSM626016
GSM626046
GSM626066
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
CONTINUED ON SHEET 15
TOP 30 OVEREXPRESSED ERGO GENES

FIG. 11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M62388
M62392
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M62397
M62398
M62399
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M62412
M62416
M62417
M62422
M62426
M62427
M62428
M62430
M62432
M62434
M62436
M62444
M62447
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
CONTINUED ON SHEET 16
TOP 30 OVEREXPRESSED ERGO GENES

FIG. 11

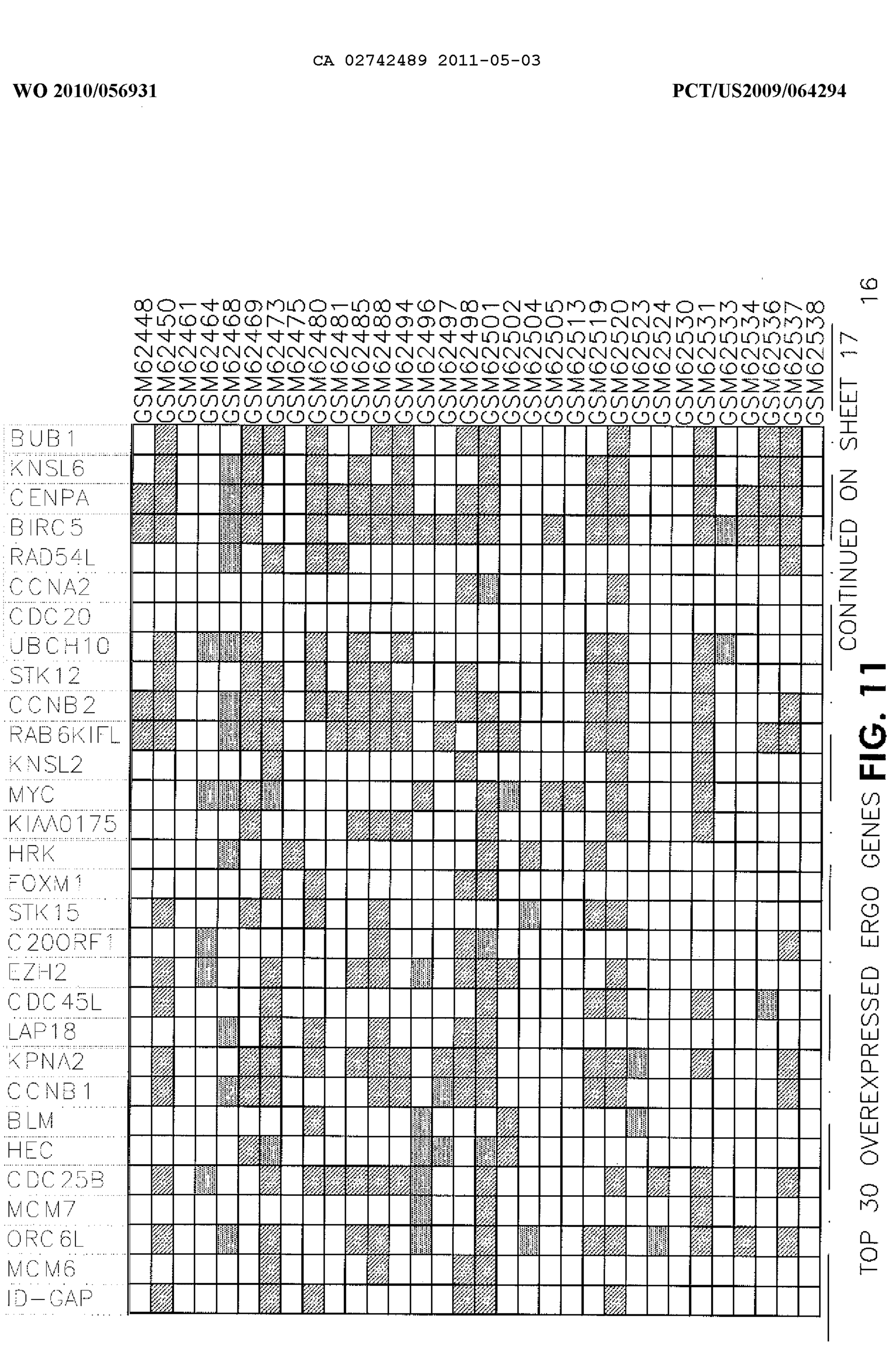
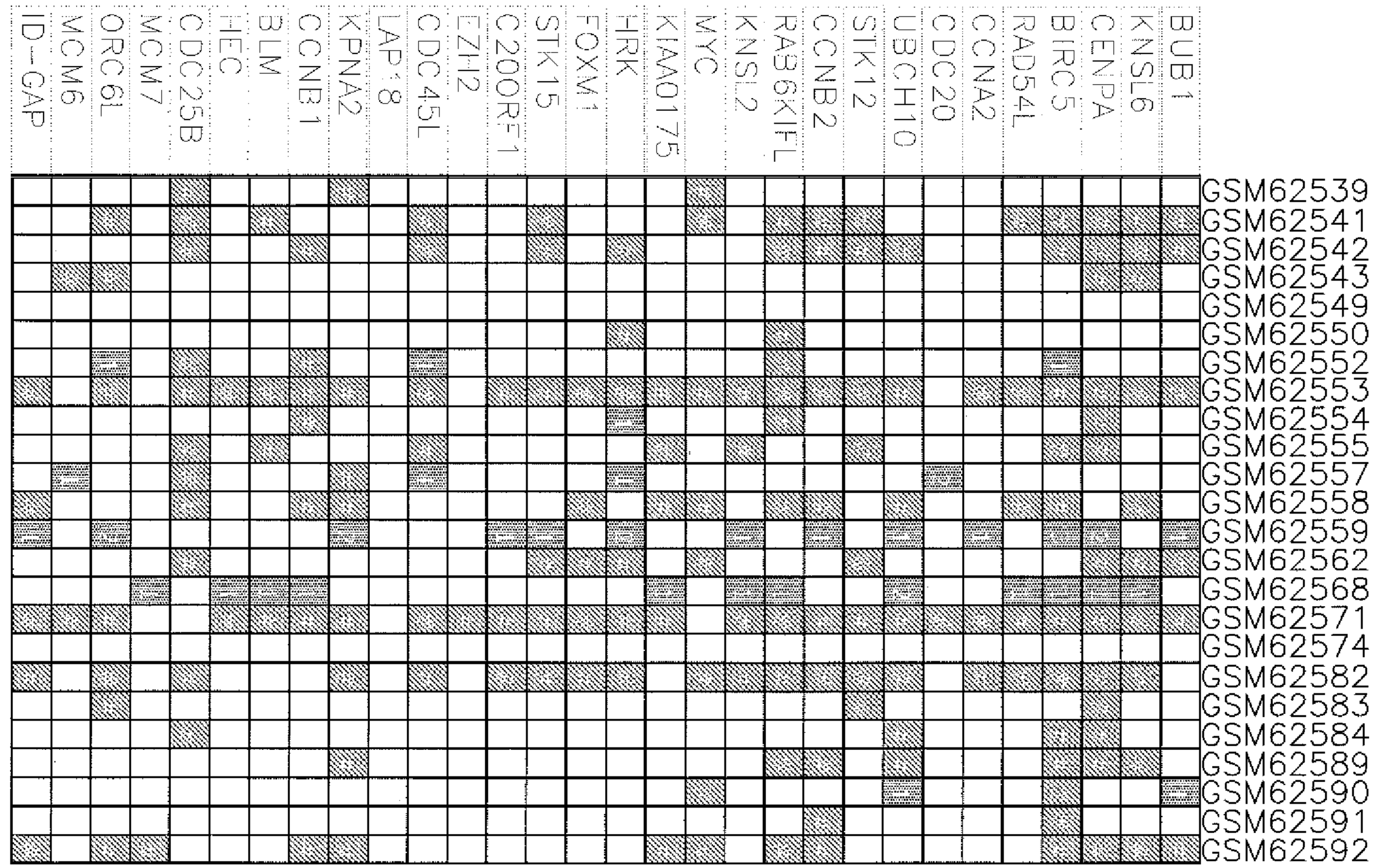


FIG. 11

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO



CONTINUED ON SHEET 18

TOP 30 OVEREXPRESSED ERGO GENES

FIG. 11

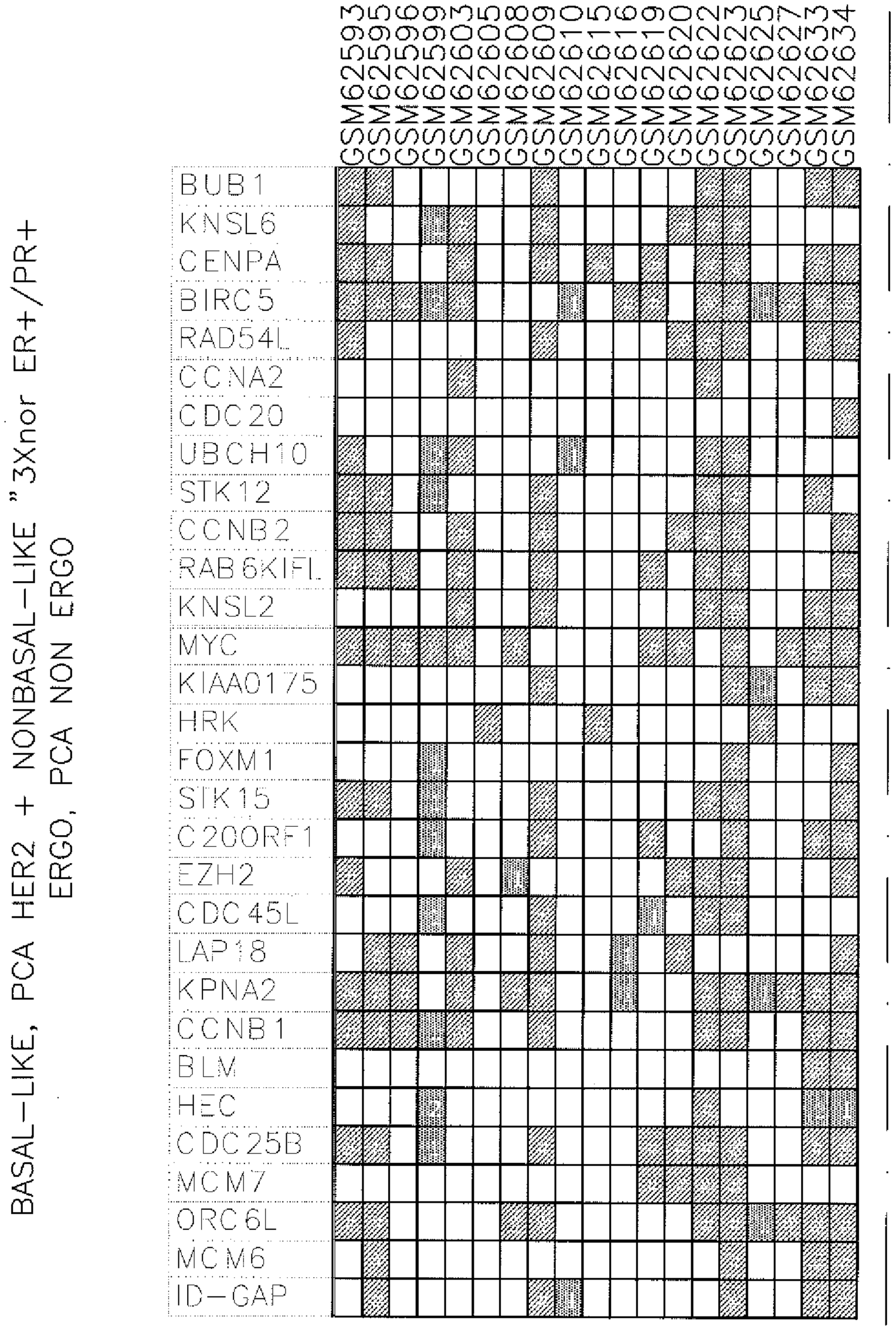
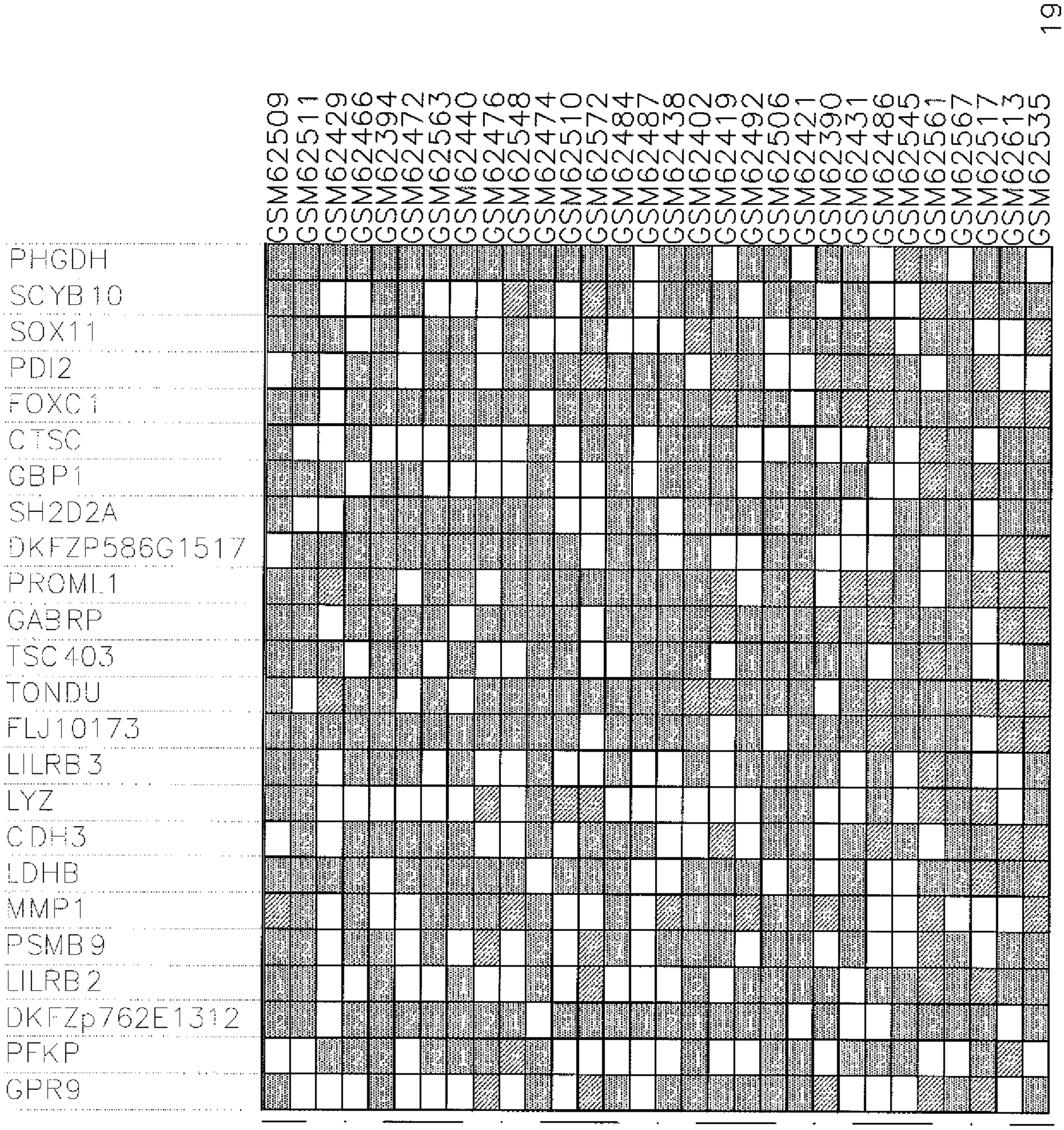

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
TOP 30 OVEREXPRESSED ERGO GENES
BUB1
KNSL6
CENPA
BIRC5
RAD54L
CCNA2
CDC20
UBCH10
STK12
CCNB2
RAB6KIFL
KNSL2
MYC
KIAA0175
HRK
FOXM1
STK15
C20ORF1
EZH2
CDC45L
LAP18
KPNA2
CCNB1
BLM
HEC
CDC25B
MCM7
ORC6L
MCM6
ID-GAP
END OF ROW

FIG. 11



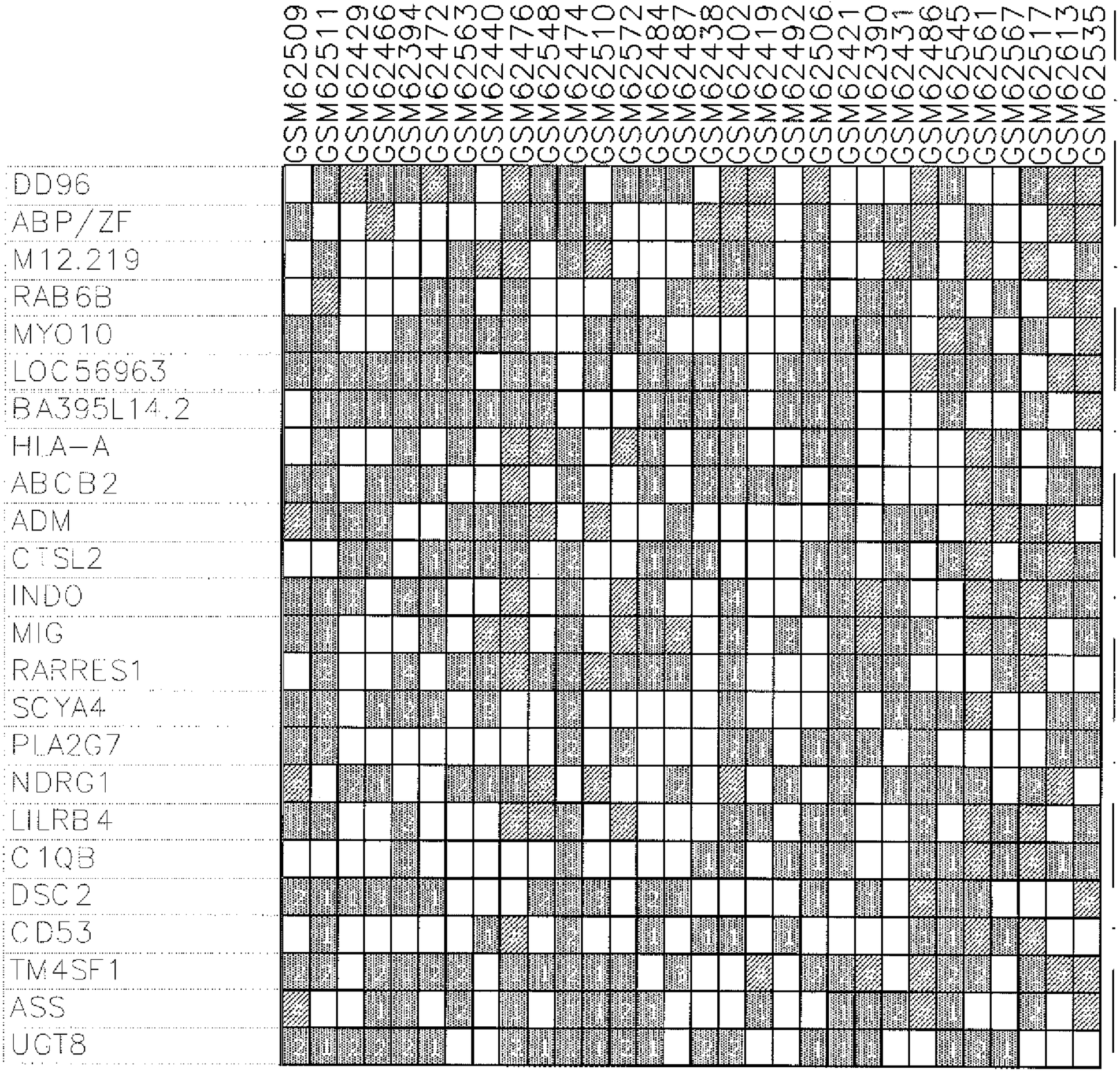
CONTINUED ON SHEET 20

BASAL–LIKE, PCA HER2 + NONBASAL–LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO

TOP 50
OVEREXPRESSED
BASAL–LIKE GENES

FIG. 11

CONTINUED FROM SHEET 19



BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO

TOP 50
OVEREXPRESSED
BASAL-LIKE GENES

FIG. 11

CONTINUED ON SHEET 21

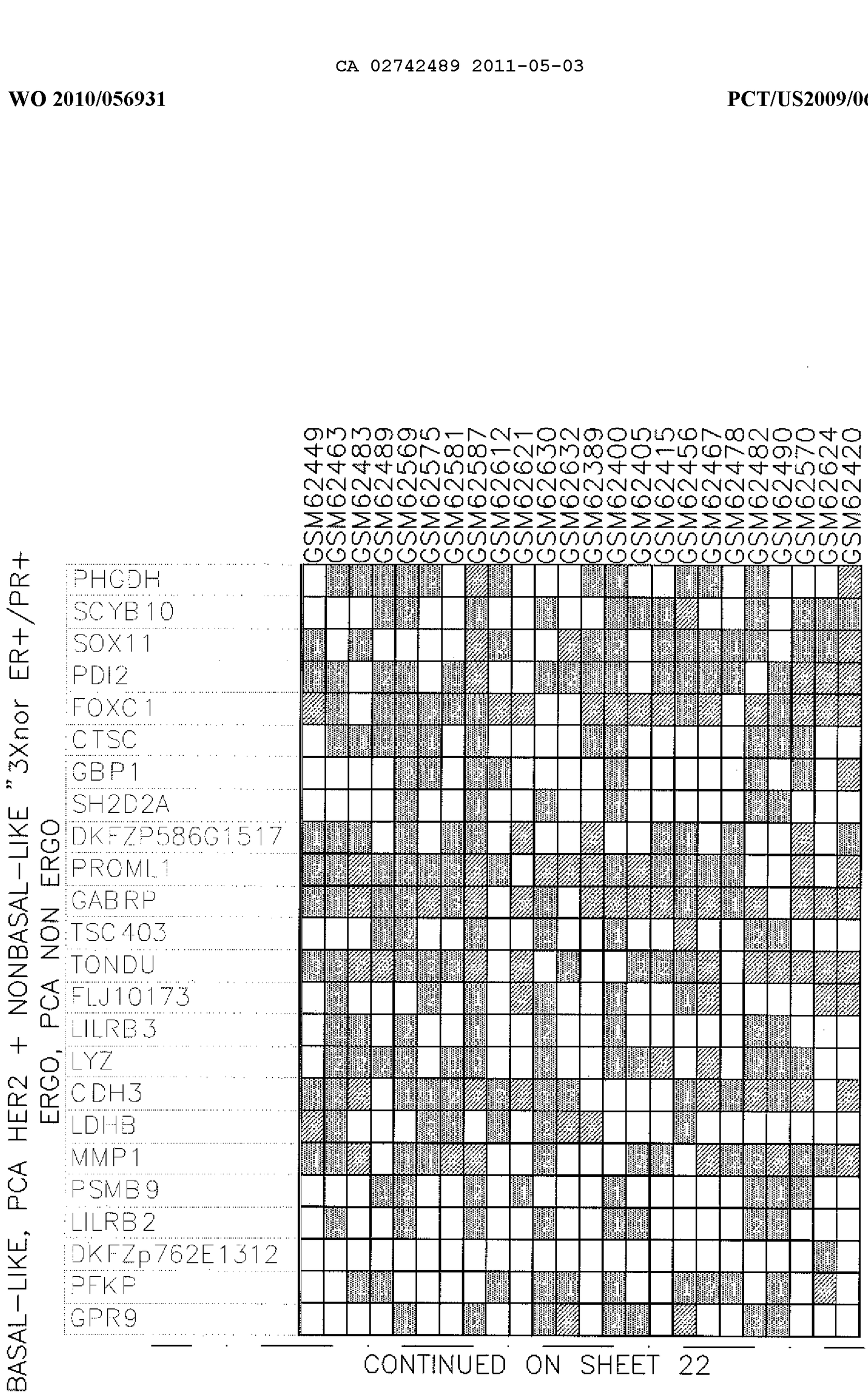


FIG. 11

CONTINUED ON SHEET 22

22

CONTINUED FROM SHEET 21

TOP 50 OVEREXPRESSED BASAL-LIKE GENES

CONTINUE ON SHEET 23

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NONERGO

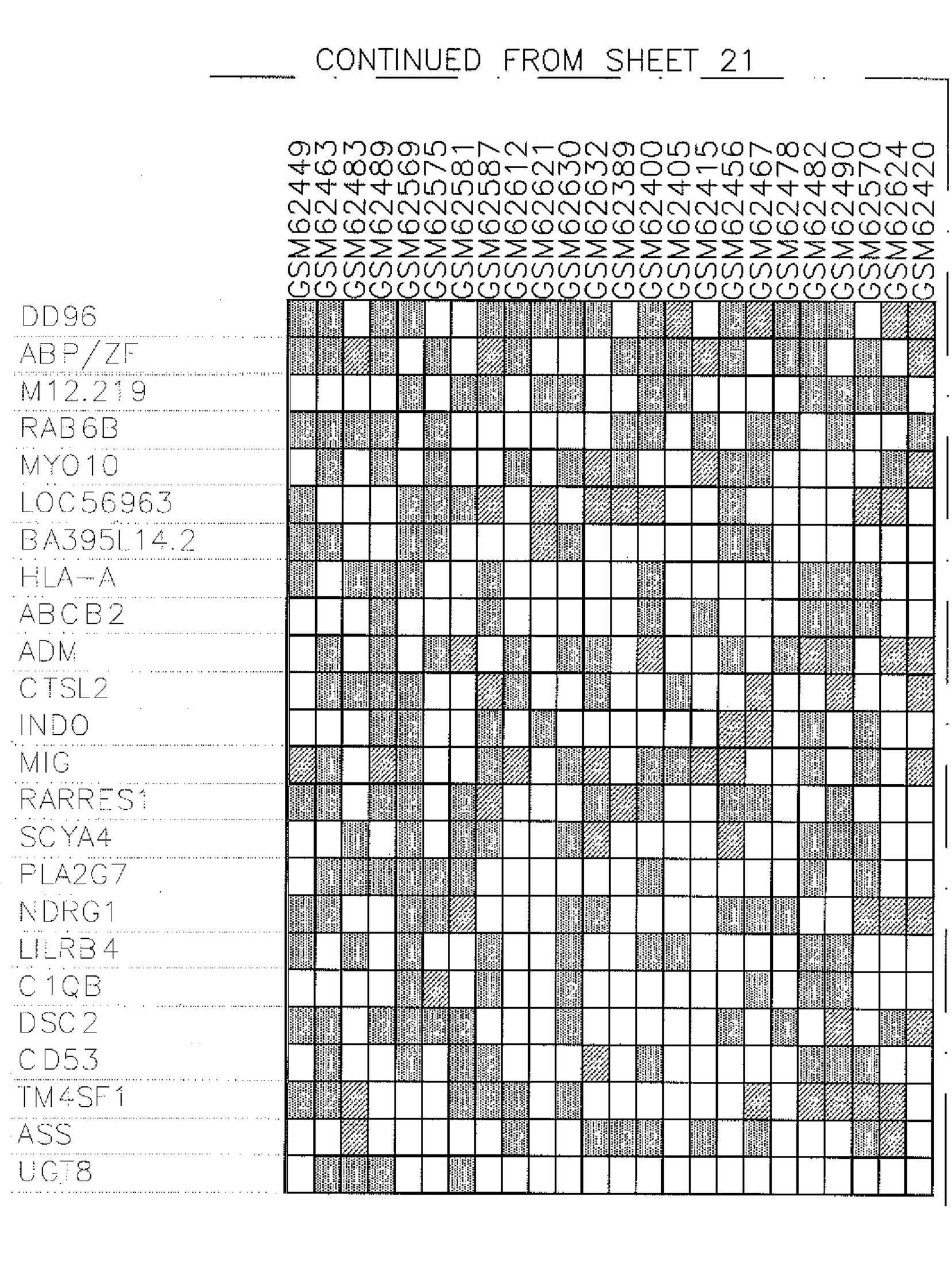
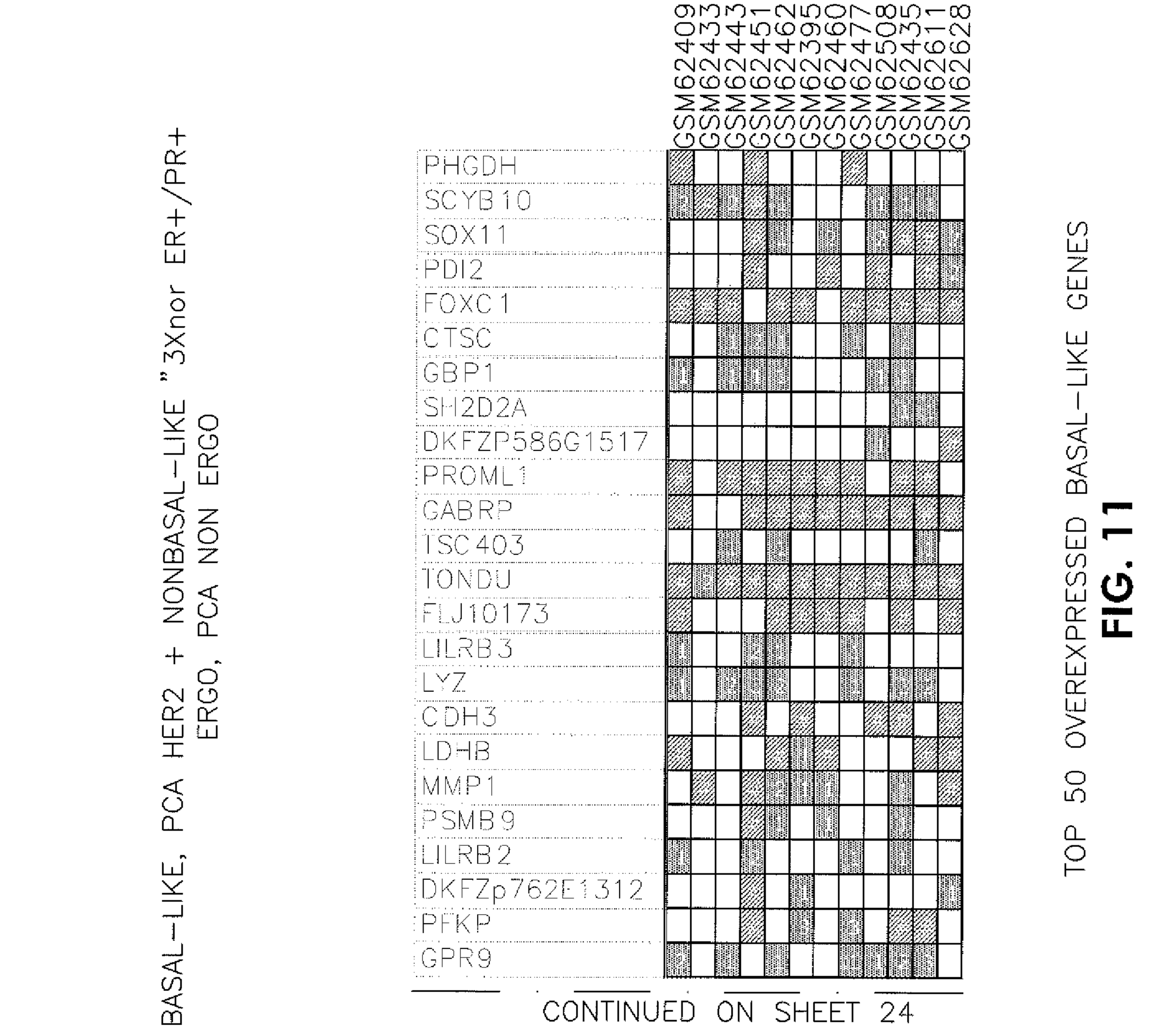


FIG. 11


TOP 50 OVEREXPRESSED BASAL-LIKE GENES
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
GSM624031-250708051-8
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GSM624044
GSM624045
GSM624046
GSM624039
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GSM624047
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GSM624043
GSM624061
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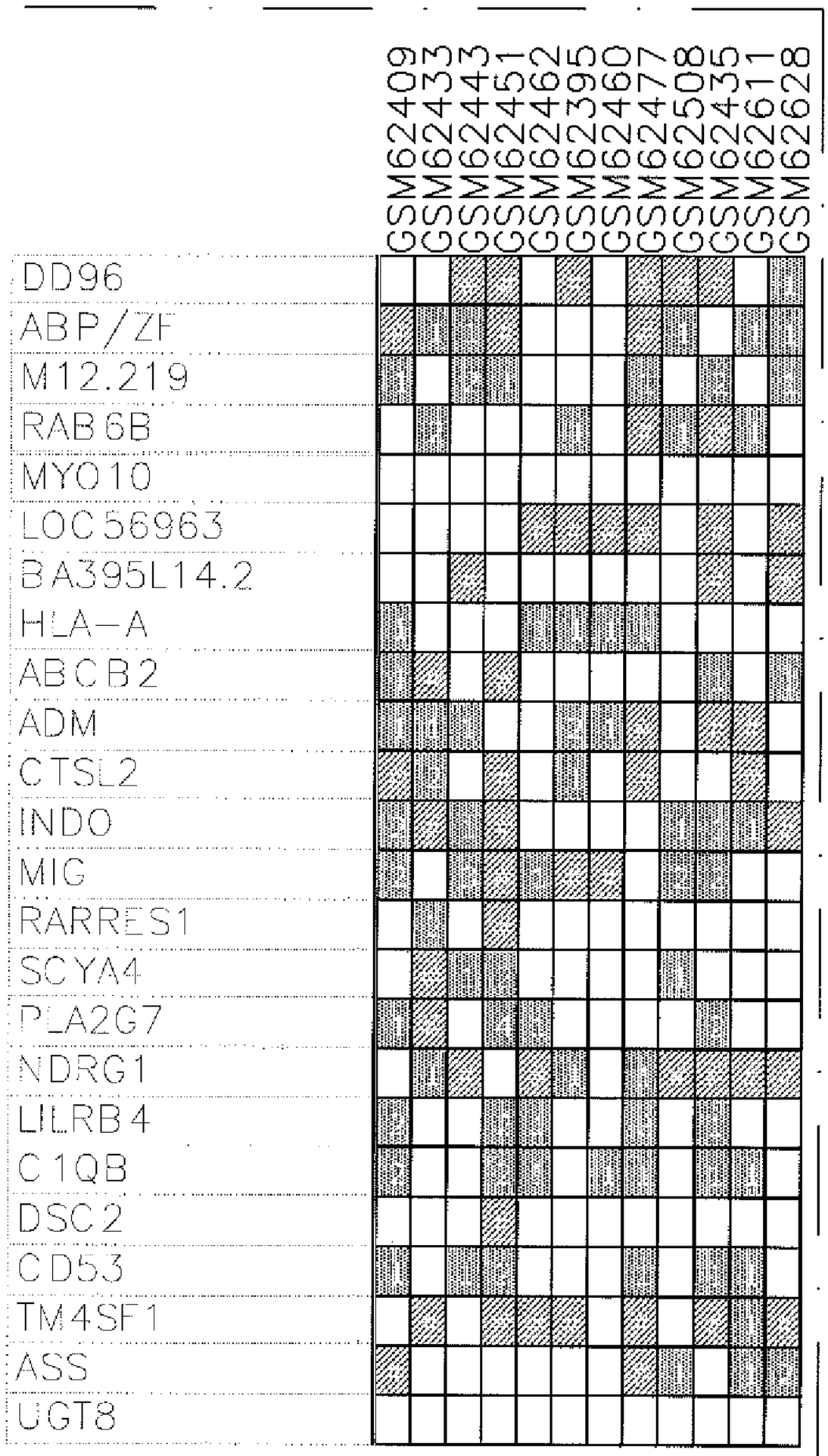
PHGDH
SCYB10
SOX11
PDI2
FOXC1
CTSC
GBP1
SH2D2A
DKFZP586G1517
PROML1
GABRP
TSC403
TONDU
FLJ10173
LILRB3
LYZ
CDH3
LDHB
MMP1
PSMB9
LILRB2
DKFZp762E1312
PFKP
GPR9
CONTINUED ON SHEET 24

FIG. 11

CONTINUED FROM SHEET 23



CONTINUE ON SHEET 25

TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 11

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO

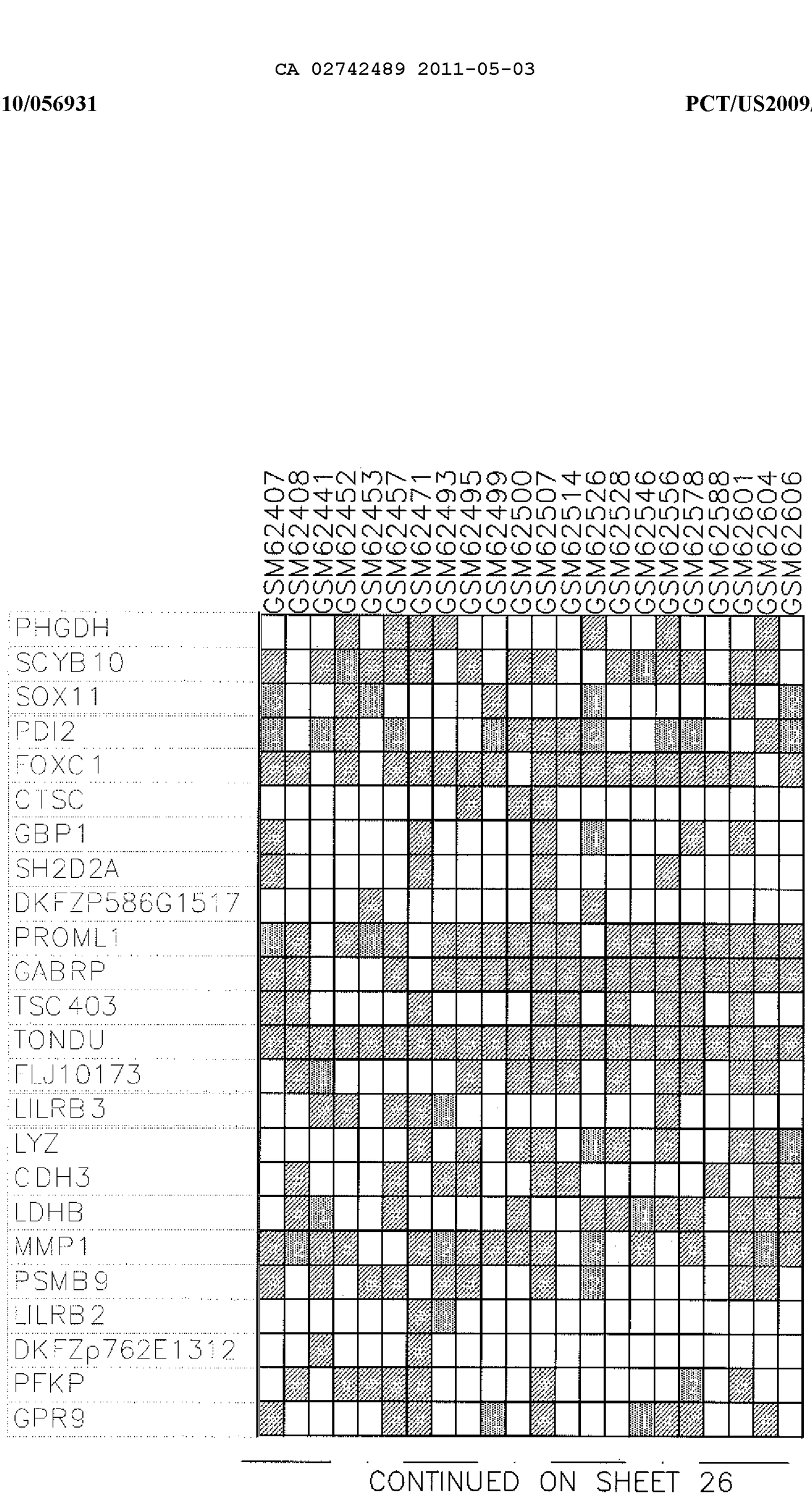
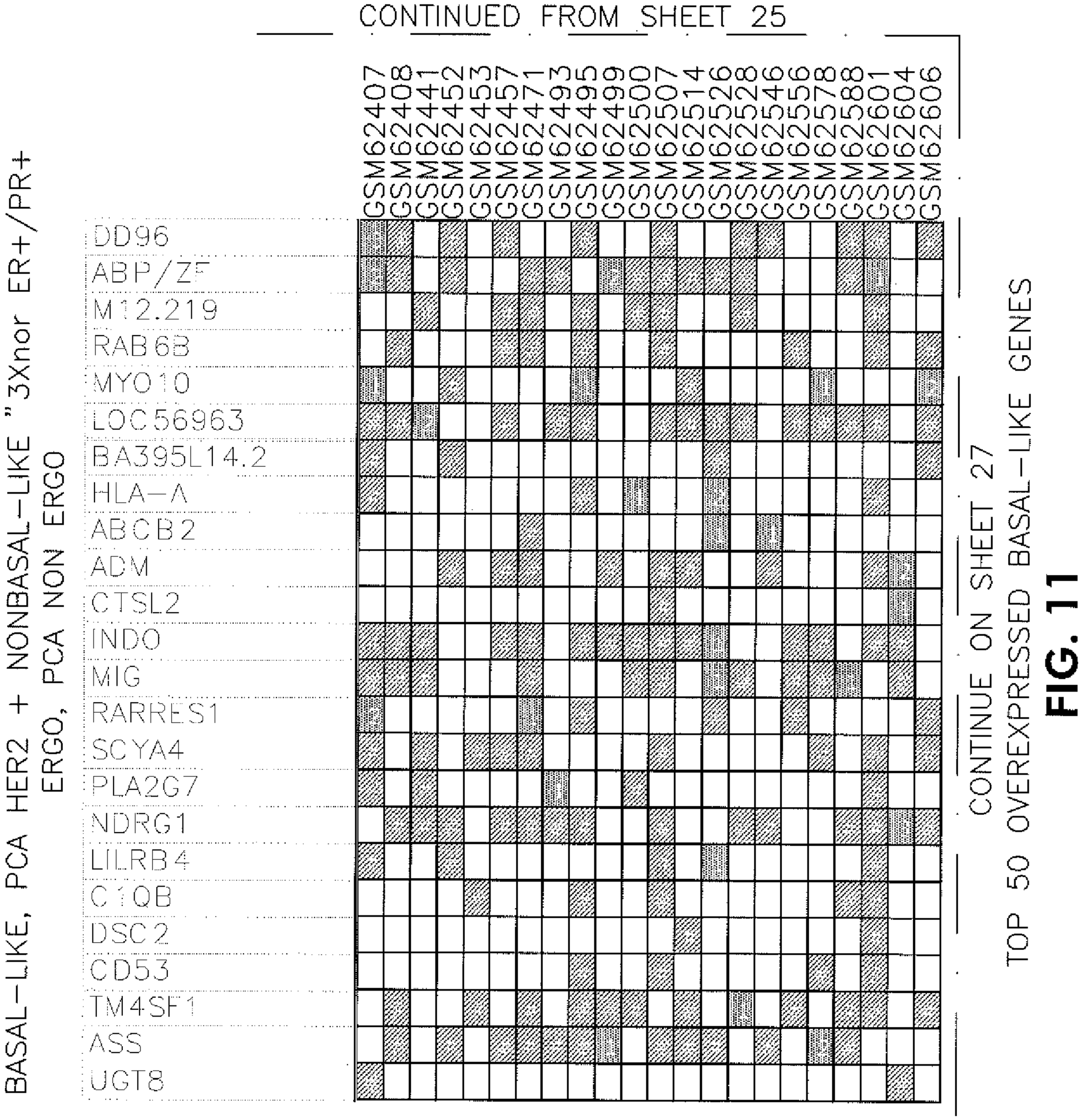


FIG. 11

CONTINUED ON SHEET 26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GSM624452
GSM624453
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GSM624471
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GSM625588
GSM626601
GSM626604
GSM626606
DD96
ABP/ZF
M12.219
RAB6B
MYO10
LOC56963
BA395L14.2
HLA-A
ABCB2
ADM
CTSL2
INDO
MIG
RARRES1
SCYA4
PLA2G7
NDRG1
LILRB4
C1QB
DSC2
CD53
TM4SF1
ASS
UGT8
BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+ ERGO, PCA NON ERGO
CONTINUE ON SHEET 27
TOP 50 OVEREXPRESSED BASAL-LIKE GENES

FIG. 11

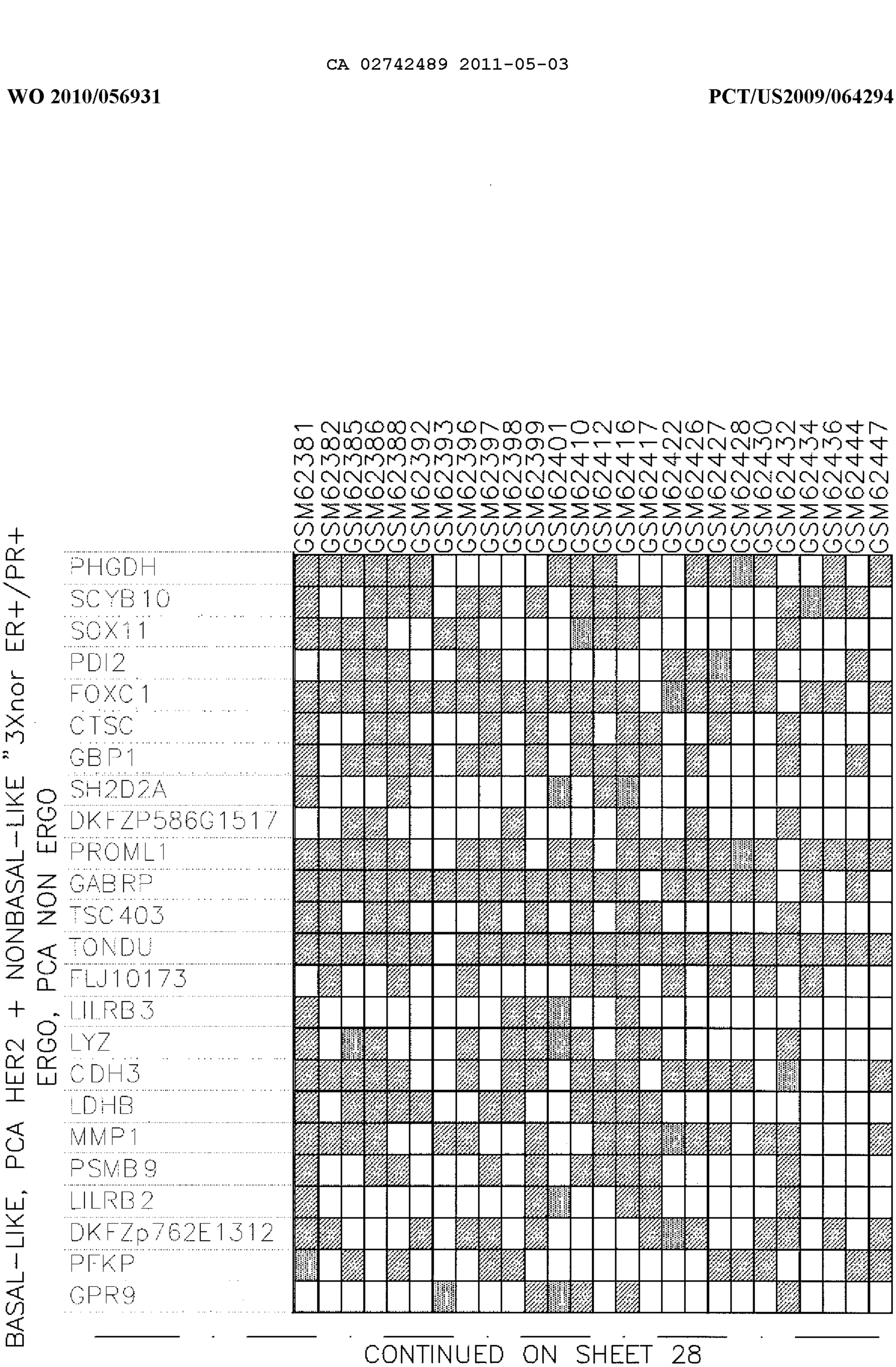

FIG. 11

CONTINUED ON SHEET 28

CONTINUED FROM SHEET 27

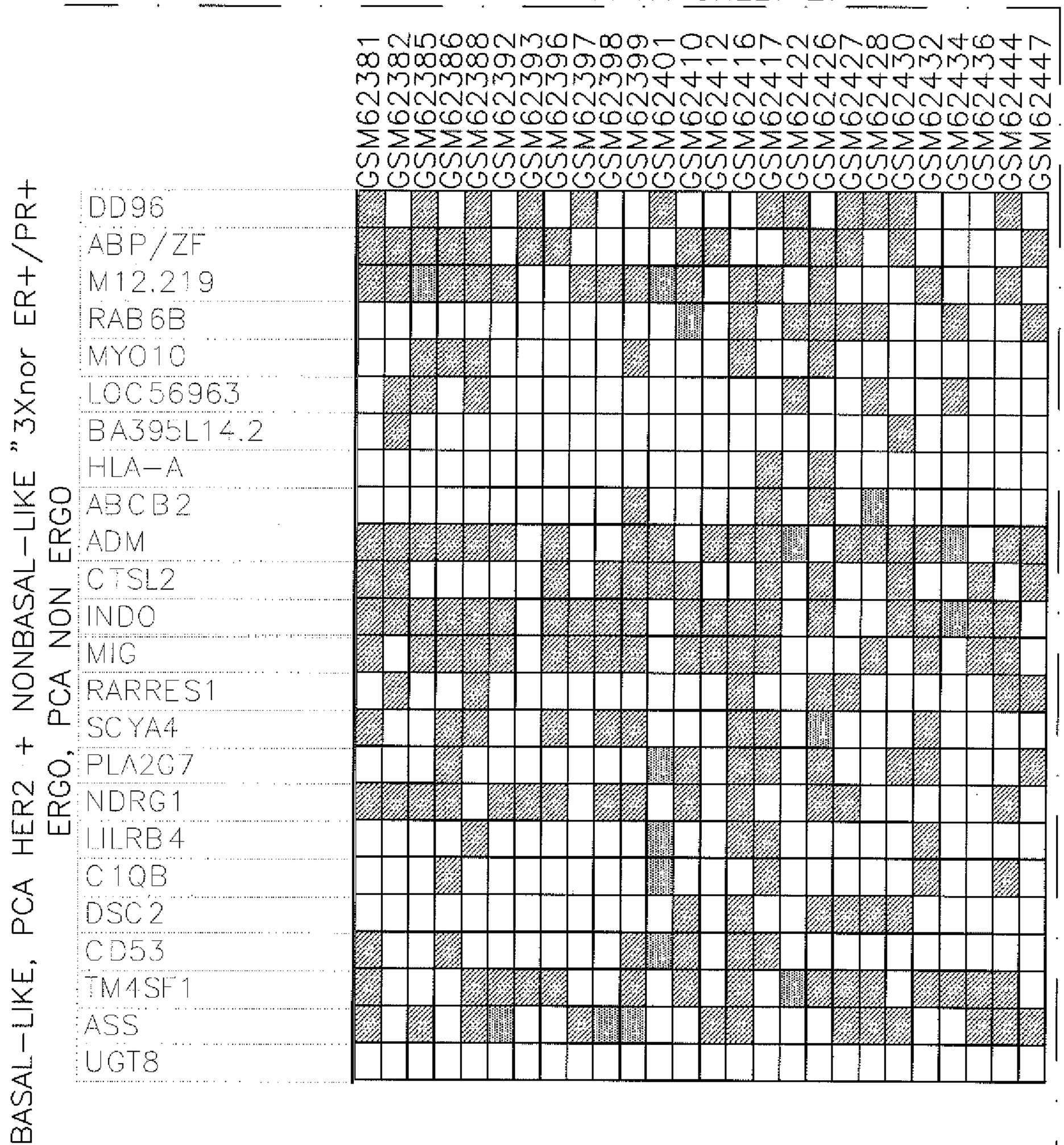

FIG. 11

TOP 50 OVEREXPRESSED BASAL-LIKE GENES

CONTINUE ON SHEET 29

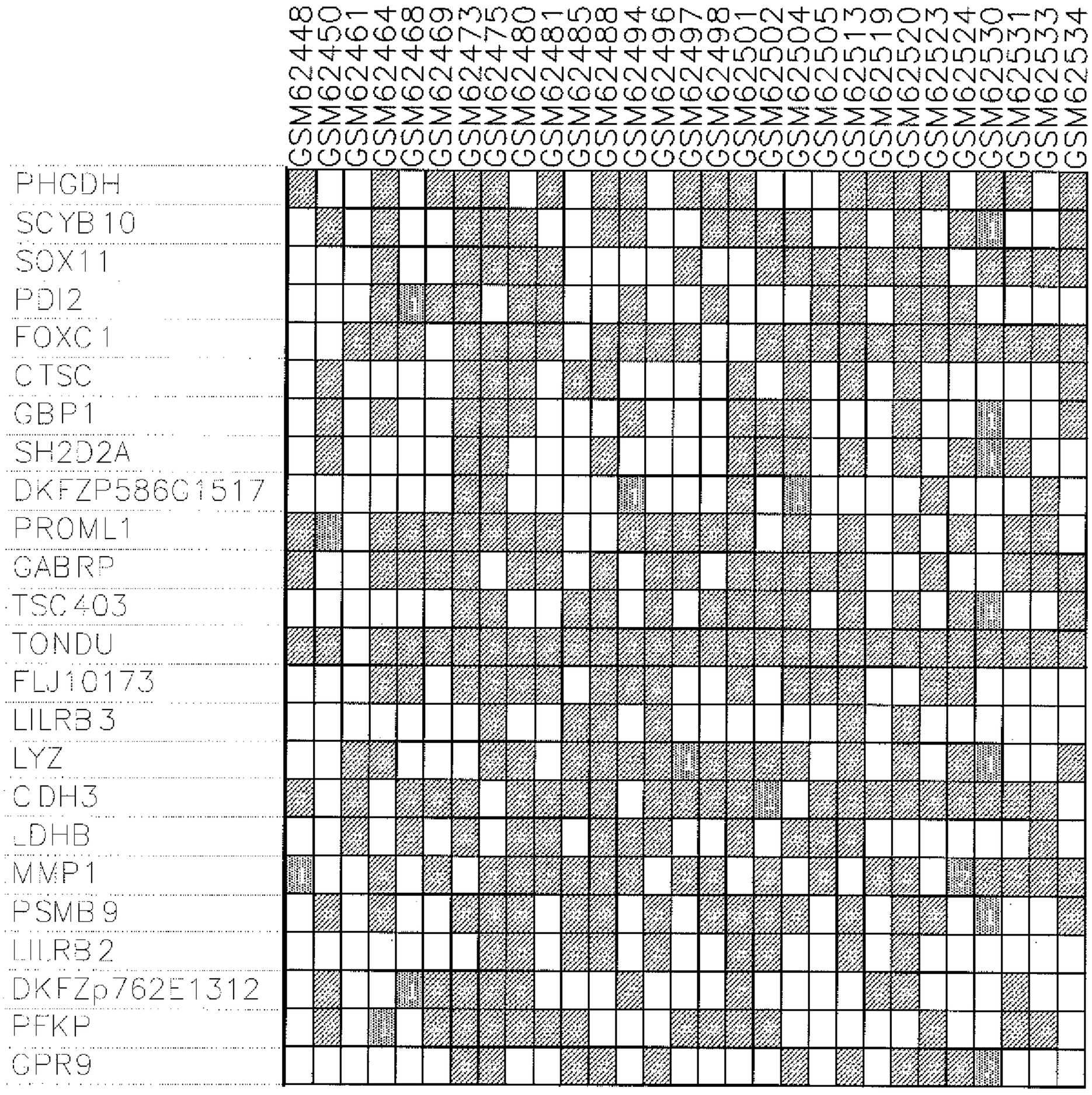

FIG. 11

CONTINUED ON SHEET 30

BASAL-LIKE, PCA HER2 + NONBASAL-LIKE "3Xnor ER+/PR+
ERGO, PCA NON ERGO

TOP 50
OVEREXPRESSED
BASAL-LIKE GENES

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 4
CONTENANT LES PAGES 1 À 163

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 4
CONTAINING PAGES 1 TO 163

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

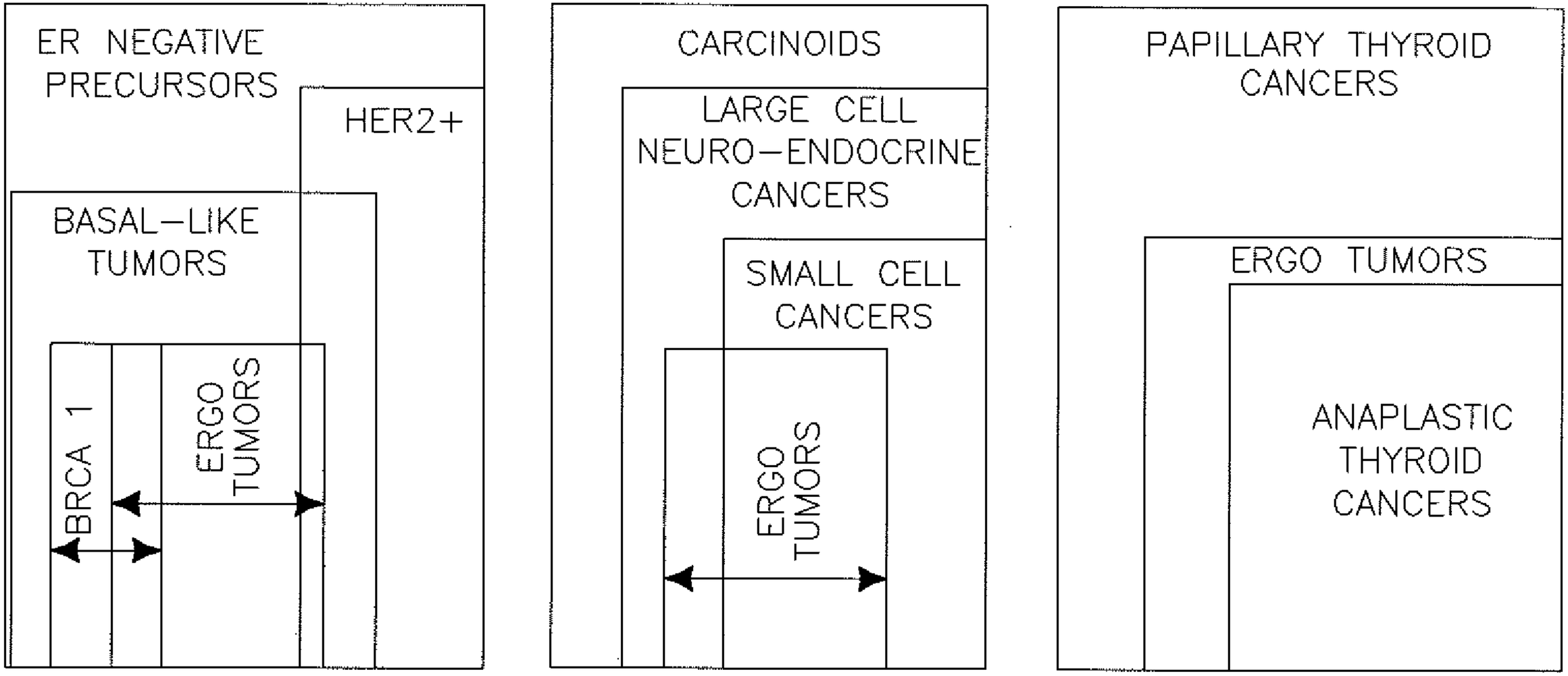
ER NEGATIVE PRECURSORS
HER2+
BASAL-LIKE TUMORS
BRCA 1
ERGO TUMORS
CARCINOIDS
LARGE CELL NEURO-ENDOCRINE CANCERS
SMALL CELL CANCERS
ERGO TUMORS
PAPILLARY THYROID CANCERS
ERGO TUMORS
ANAPLASTIC THYROID CANCERS

FIG.7