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(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US).

(72) Inventors: **SALLAM, Tamer**; 10889 Wilshire Blvd, Suite 920, Los Angeles, CA 90095-7191 (US). **ZHANG, Zhengyi**; 10889 Wilshire Blvd., Suite 920, Los Angeles, CA 90095-7191 (US).

(74) Agent: **HALSTEAD, David, P.** et al.; Foley Hoag LLP, 155 Seaport Boulevard, Boston, MA 02210-2600 (US).

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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING CARDIOMETABOLIC CONDITIONS

(57) **Abstract:** Disclosed herein are methods of treating a cardiometabolic condition, of lowering a cholesterol level, of lowering expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and of lowering expression of a gene coding for sterol regulatory element-binding protein 2. For use in such methods, also disclosed are mRNA-silencing nucleic acid molecules, which can act as down-regulators of RNA-binding protein Raly.

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COMPOSITIONS AND METHODS FOR TREATING CARDIOMETABOLIC CONDITIONS

RELATED APPLICATION

This application claims a right of priority from and the benefit of an earlier filing date of U.S. Provisional Application No. 62/954,993, filed on December 30, 2019, which is hereby incorporated by reference in its entirety.

STATEMENT OF RIGHTS

This invention was made with government support under Grant Number HL139549, awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a group of functionally versatile proteins that play critical roles in the biogenesis, cellular localization, and transport of RNA. Although several lines of evidence link hnRNP abnormalities to neurodegenerative diseases and cancer, their contributions to other conditions remain relatively unexplored.

Many cardiometabolic conditions relate to the accumulation of fat. For example, non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the United States. In NAFLD, excess fat accumulates in the liver due to reasons other than heavy alcohol use. Two forms of NAFLD are non-alcoholic fatty liver (NAFL), which is the less severe form, and non-alcoholic steatohepatitis (NASH), which is accompanied by liver inflammation. As another example, in dyslipidemia the level of cholesterol, triglycerides, or both is increased in the plasma, which can contribute to atherosclerosis. In atherosclerosis, fats such as cholesterol build inside of arteries and restrict blood flow.

Treatment of cardiometabolic conditions often relies on the use of drugs, such as statins for atherosclerosis, and on various surgical procedures. For NAFLD, there are still no drugs approved by the Food and Drug Administration. Because cardiometabolic conditions affect a large group of people and in many cases are still not effectively treatable, there is a need for additional treatment methods.

SUMMARY OF THE INVENTION

In some aspects, mRNA-silencing nucleic acid molecules include an antisense strand that has a nucleobase sequence at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead. As an example, SEQ ID NO: 1 provides this sequence: TTTGACACGC As an example, SEQ ID NO: 1 provides this sequence: TTTGACACGCCGGACCAAAG. When considering whether a test sequence is 90% identical with this reference sequence in which none, CGGACCAAAG. When considering whether a test sequence is 90% identical with this reference sequence in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead, one would compare the test sequence with the reference sequence that starts with "TTT" as well as with the alternative reference sequences that start with "UUU," "TUU," "UUT," "UTU," "UTT," "TUT," and "TTU."

In some embodiments of these aspects, the mRNA-silencing nucleic acid molecule is a single-stranded oligonucleotide in which the antisense strand includes the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69. The term "antisense strand" is used herein as a shared term among mRNA-silencing nucleic acid molecules; for an mRNA-silencing nucleic acid molecule that is a single-stranded oligonucleotide, the antisense strand is the same single-stranded oligonucleotide. In certain such embodiments, the antisense strand includes, linking each pair of neighboring sugars, a monothiophosphate group in which the sulfur atom is at a non-bridging position. An example of such single-stranded oligonucleotides are antisense oligonucleotides (e.g., with a phosphorothioate (PS) backbone). In some embodiments, the antisense strand includes 2'-O-(2-methoxyethyl)ribose groups as the sugars for residues 1–5 and 16–20, and 2'-deoxyribose groups as the sugars for residues 6–15 with respect to any one of SEQ ID NOs: 1–3 or 11–69. In certain embodiments, the nucleic acid molecule is a gapmer antisense oligonucleotide having the sequence of any one of SEQ ID NOs: 7–9.

In certain embodiments, the mRNA-silencing nucleic acid molecule includes a small interfering RNA (siRNA); a short hairpin RNA (shRNA) that is a precursor to said siRNA; or a double-stranded DNA that is a precursor to said shRNA or to said siRNA. The siRNA in these embodiments has a guide strand that includes the antisense strand, which has a nucleobase sequence at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil

nucleobases instead; and a passenger strand that is complementary to the guide strand with respect to at least 10 and at most 30 residues. In some embodiments, the guide strand includes the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead, and in which both the guide strand and the passenger strand have a 2-nucleotide 3'-overhang. In certain embodiments, at least one nucleobase, sugar, phosphate group, or a combination thereof is a non-canonical group instead.

The mRNA-silencing nucleic acid molecules of any of the aspects and embodiments described above can be used in any of the methods that are further described herein.

In some aspects, methods of treating a cardiometabolic condition of a subject include administering to the subject an effective amount of a down-regulator of a heterogeneous nuclear ribonucleoprotein. The heterogeneous nuclear ribonucleoprotein, in some embodiments, includes RNA-binding protein Raly. The cardiometabolic condition, in various embodiments, includes non-alcoholic fatty liver, non-alcoholic steatohepatitis, dyslipidemia, obesity, inflammation, or atherosclerosis. The down-regulator, in this as well as in any of the other methods disclosed herein, can include one or more of the disclosed mRNA-silencing nucleic acid molecules. The down-regulator, in this as well as in any of the other methods disclosed herein, can include an antibody, an antigen-biding fragment thereof, or a small molecule. In certain embodiments, the subject is a human. In some embodiments, the down-regulator is administered to the subject using a mode of administration that delivers the down-regulator to one or more hepatocytes of the subject.

In some embodiments, methods of lowering a cholesterol level in a subject include administering to the subject an effective amount of a down-regulator of RNA-binding protein Raly. When the down-regulator is one of the disclosed mRNA-silencing nucleic acid molecules, it can attenuate or prevent translation of messenger RNA of RNA-binding protein Raly. In certain embodiments, the cholesterol level includes the level of cholesterol in serum LDL. In some embodiments, the cholesterol level includes the level of cholesterol in hepatocytes of the subject. The lowering of the cholesterol level, in various embodiments, is accompanied by a lowering of an inflammation level in cells of the subject.

In some aspects, methods of lowering expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase include administering to the subject an effective amount of a down-regulator of RNA-binding protein Raly.

In certain aspects, methods of lowering expression of a gene coding for sterol regulatory element-binding protein 2 include administering to the subject an effective amount of a down-regulator of RNA-binding protein Raly. In some embodiments, the down-regulator lowers expression of a gene coding for sterol regulatory element-binding protein 2 by reducing an amount of nuclear transcription factor Y bound to RNA-binding protein Raly.

Additional aspects include an mRNA-silencing nucleic acid molecule for use in the treatment of a cardiometabolic condition of a subject; in the lowering of a cholesterol level in a subject; in the lowering of expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase; or in the lowering of expression of a gene coding for sterol regulatory element-binding protein 2.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A through 1H: Loss of RALY reduces Srebp2 and specifically enriches for metabolic pathways. 1A. Schematic of Raly conditional knockout strategy. 1B. Gene expression of RALY in liver from chow-fed mice *(n* = 8 per group). 1C. Protein level of RALY in liver from chow-fed mice *(n* = 8 per group). 1D. Volcano plot of RNA-seq results from liver with and without RALY *(n* = 4 per group). 1E. Enriched functional terms from RNA-seq of differentially regulated genes from mouse liver. Yellow bars (which are used for, from left to right, bars 1-9, 12, 15, 17, 20-23, 25, 27, 30, 35, 38, 43, 47, 49, and 53) indicate functional keyword is also enriched in liver SREBP2 ChIP. 1F. Gene expression in mice liver on chow diet *(n* = 8 per group). 1G. Total serum cholesterol levels isolated from chow-fed mice (n = 8 per group). 1H. Cholesterol levels in pooled fractionated serum from mice in 1G. All data are mean+/- SD. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ using two-tailed Student's t-test.

Fig. 2: *Raly*$^{flox/flox}$ genotyping strategy. Gel image of PCR genotyping showing WT and *Raly*$^{flox/flox}$.

Figs. 3A through 3H: Liver specific deletion of RALY alters hepatic lipid content. 3A-3B. Cholesterol level (3A) and Triglycerides level (3B) from primary hepatocytes relative to baseline levels (normalized to cell number). 3C-3D. Lipidomics investigation of cholesterol ester (CE) species from liver from L-Raly knockout mice and controls (n= 4 per group). 3E-3F. Lipidomics investigation of Triglycerides species from liver from L-Raly knockout mice and controls (n= 4 per group). Each line on heat map represents an individual triglyceride species of 411 different triglycerides detected (4 mice

per group). 3G. Oil Red O (ORO) staining of liver from NASH diet feed mice (scale bar 20 um). 3H. Quantification of positive ORO staining area from done with automated image j detection. Values are shown as means ± SD. * $P < 0.05$; ** $P < 0.01$; using two-tailed Student's t-test.

Figs. 4A through 4E: Loss of Raly reduces SREBPs and target genes in primary hepatocytes. 4A. Real-time PCR treated with GFP or Cre adenovirus. 4B. Western blot analysis of RALY in mouse primary hepatocytes treated with GFP or Cre adenovirus. 4C. Gene expression in mouse primary hepatocytes GFP or Cre adenovirus (n=3 per group). 4D. Western blot analysis of nuclear SREBP2 in mouse primary hepatocytes treated with GFP or Cre adenovirus. 4E. Western blot analysis of HMGCS and FDPS in mouse primary hepatocytes treated with GFP or Cre adenovirus. Values are shown as means ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and **** $P < 0.0001$.

Fig. 5: Lipidomics analysis from mouse livers comparing *L-RalyKO* and controls. Species abbreviations are SM = Sphingomyelins, CER = Ceramides, LPE = Lysoglycerophosphoethanolamines, LPC = Lysoglycerophosphocholines, PE = Glycerophosphoethanolamines, and PC = Glycerophosphocholines.

Fig. 6: Loss of RALY does not influence lipolysis. Gene expression in liver from chow-fed mice *(n* = 8 per group) (for each pair of bars, L-Cre- is on the left, and L-Cre+ is on the right).

Figs. 7A through 7K: Interrogation of the RALY *cistrome* and chromatin dynamics enriches for promoter binding and metabolic coregulators. 7A. Heat map of reproducible counts centered around a gene transcription start site for RALY ChIP-seq samples in hepa1-6 cells (n=4 per group). 7B. ChIP fragment depth enrichment for RALY across samples. 7C. Pie chart showing the binding pattern of RALY according to the location of a given peak. 7D. Representative RALY ChIP-seq profiles at *Srebfl* and *Klf6* loci. 7E. Motif analysis surrounding called peaks from RALY ChIP-seq in hepa1-6 cells. 7F. Schematic of ATAC-seq workflow. 7G. Metagene representation of the mean ATAC-seq signal. ATAC-seq was performed from mice liver with Raly f/f L-Cre+ or Cre- (n=4 per group). 7H-7I. Heat map shows the differential enrichment of ATAC peaks of Cre- or Cre+ livers and Gene ontology analysis. 7J. Representative ATAC-seq heat map in liver with Raly f/f L-Cre+ or Cre- at the *Srebf2* locus. 7K. Motif analysis showing top enriched factors at differentially regulated sites from Raly f/f L-Cre+ or L-Cre- liver.

Fig. 8: RALY does not bind Srebfl promoter. RALY ChIP-seq profiles at *Srebfl.*

Fig. 9: ATAC-seq profiles at cholesterogenic genes as a consequence of loss of RALY. ATAC-seq profiles at *cholesterol biosynthetic genes.*

Figs. 10A through 10I: The coactivator NFY is required for the transcriptional effects of RALY on cholesterogenesis. 10A-10B. Western blot from Co-IP studies performed in hepa1-6 cells. 10C. NFY ChIP-qPCR of the positive control NFY gene done from Raly f/f L-Cre+ or L-Cre- livers (n= 5 per group). 10D. ChIP-qPCR on Srebf2 promoter using various primers around NFY sites from ChIP in 10C. Data was normalized to negative control region. (n= 5 per group). Data are mean+/-SEM. 10E. Gene expression results from hepa1-6 cells treated with adenovirus GFP or RALY (n=4 per group) . 10F. Serum cholesterol levels from chow-fed mice treated with adenovirus GFP or RALY harvested after 6 days of injection. Data mean+/- SEM. (n=8 per group). 10G. Luciferase promoter assays of Srebp2 promoter region performed in hepa1-6 cells and with GFP or RALY overexpression. 10H. Srebp2 gene expression after NFY knockdown in control or RALY deficient primary hepatocytes (n=4 per group). values are Mean +/-SD. 10I. Gene expression results from METISM study showing correlation of metabolic traits with RALY. Values are inverse normal transformed. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

Figs. 11A and 11B: Validation of RALY overexpression. 11A. Western blot from Hepal-6 cells for RALY and V5 at increasing RALY-V5 expression. 11B. Quantitative PCR of RALY for experiments performed in 4B.

Fig. 12: Validation of NFY knockdown. Western blot of NFY from primary hepatocytes used in Fig 10H.

Figs. 13A and 13B: Correlation of hnRNPs with cholesterol level. Expression results from METISM study showing correlation of metabolic traits with hnRNPs. Values are inverse-normal transformed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, at least in part, on the discovery of a role for a heterogenous ribonucleoprotein (a type of RNA binding protein) known as RNA-binding protein Raly ("RALY") in cardiometabolic disease. Loss of RALY in mouse liver resulted in potent cholesterol lowering effects in the liver as well in serum. Loss of RALY also reduced key genes and factors involved in fatty liver pathogenesis and NASH. The lipid content in liver is key driver for fatty liver disease, NAFLD, and NASH. The serum cholesterol, particularly LDL cholesterol, is a direct regulator of heart disease. Thus, the

consequences of RALY inhibition would be highly beneficial for these diseases. In addition, RALY has been shown to be a GWAS hit for serum cholesterol in human studies, so this pathway should be important in humans as well. In fact, the experimental results provided herein show that RALY correlates with a number of cardiometabolic traits from human studies.

As one application of these discoveries, unique anti-sense oligonucleotides (ASOs) that target human RALY were designed and tested in a human cellular model, and showed that they reduce cholesterol. Further details of some of the experimental studies are in Examples 1 through 3.

Accordingly, the present invention provides methods of treating a cardiometabolic condition, of lowering a cholesterol level, of lowering expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and of lowering expression of a gene coding for sterol regulatory element-binding protein 2. For use in such methods, the present invention also provides mRNA-silencing nucleic acid molecules, which can act as down-regulators of RALY.

Definitions

As used in the description, the words “a” and “an” can mean one or more than one. As used in the claims in conjunction with the word “comprising,” the words “a” and “an” can mean one or more than one. As used in the description, “another” can mean at least a second or more.

The term “treating” includes curing, relieving, or ameliorating to any extent a symptom of an illness or medical condition, or preventing further worsening of such a symptom. For example, treating atherosclerosis includes making the atherosclerosis less severe.

A “biomarker” can be anything that can be used as an indicator of a particular physiological state of an organism. For example, a biomarker can be a level of a metabolite, by-product, mRNA, enzyme, peptide, polypeptide, or protein associated with a particular physiological state.

“Cholesterol” is an example of a biomarker. Cholesterol (e.g., in the form of *nat*-cholesterol, in the form of a cholesteryl ester, as incorporated into a lipoprotein) is a sterol that serves a variety of functions in animals. Cholesterol levels play a role in the homeostatic mechanisms that regulate biosynthesis of cholesterol. For example, intracellular cholesterol can be sensed by sterol regulatory element-binding protein 2

(SREBP2), which is another example of a biomarker. SREBP2 can act as a transcription factor (e.g., after its cleavage when cholesterol level is low) to stimulate expression of some genes, such as HMG-CoA reductase (HMGCR), which is yet another example of a biomarker.

The term "level," for example when forming a compound noun with a preceding word such as test or control, can denote a measurable value such as an amount, concentration, activity, maximum rate, Michaelis constant, half-maximal effective concentration, or half-maximal inhibitory concentration (e.g., of a biomarker or another tissue ingredient that is related to a biomarker). The term "level" also includes values such as presence or absence, which can be discrete when measured individually or can attain a more continuous character when measured collectively. The term "cholesterol level" can include, for any aspect or embodiment disclosed herein, a level of any one or more of the following types: plasma HDL, plasma LDL, plasma VLDL, the sum HDL + LDL + VLDL in plasma, intracellular (e.g., in hepatocytes).

The term "passes a control threshold" means that the measured value, such as a test level, differs by more than a certain amount (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%) from another value, such as a control threshold. In some embodiments, this difference is 20% (e.g., when the test value is P, the control threshold is Q, and the passing requires the test value to be higher than the control threshold, then (P-Q)/Q being higher than 20% would pass the control threshold).

The term "lowering," for example when referring to lowering a cholesterol level or lowering expression of a gene, means that the level (e.g., of cholesterol, of gene expression) becomes less as compared to a control. For example, an agent "lowers" the cholesterol level measured in the form of plasma LDL when the plasma LDL level in a test subject, to whom the agent is administered, has a lower plasma LDL level after the administration as compared to the plasma LDL level in a control subject after administration of a placebo to the control subject. Therefore, an agent may lower a level even if it actually causes an increase of the level in the subject as long as that increase is less than it would have been in the absence of the agent. Lowering expression of a gene includes lowering the level of mRNA transcribed from that gene, the level of polypeptide translated from the mRNA of the gene, as well as any other changes that would cause a lowering of the effects of the expression of that gene.

A "down-regulator" includes agents that lower a level, such as the level of an active protein (e.g., through lowering the expression of a gene that codes for the protein, through inhibiting the protein directly, through preventing the activation of the protein). As an example, a down-regulator of RALY can be an antibody, an antigen-biding fragment thereof, or a small molecule that inhibits the functioning of RALY. In some embodiments, the down-regulator is an antibody, a portion of an antibody, a mimetic of an antibody, or variants/combinations thereof (e.g., mAb, $F(ab')_2$, Fab, scFv, tandem di-scFv, tandem tri-scFv, diabody, tribody, sdAb (e.g.,V_{HH}, V_{NAR}), affilin, affimer, affitin, alphabody, anticalin, avimer, DARPin, monobody, nanoCLAMP). In some embodiments, the down-regulator includes an mRNA-silencing nucleic acid molecule (e.g., antisense oligonucleotide, small interfering RNA).

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, or encapsulating material. Each carrier must typically be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "subject" refers to a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Heterogeneous Nuclear Ribonucleoproteins and mRNA-silencing Nucleic Acid Molecules

In some aspects, disclosed are methods that include administering a down-regulator of a heterogeneous nuclear ribonucleoprotein (hnRNP). hnRNPs are proteins that are

involved in various aspects of nucleic acid metabolism, including regulation of gene expression. An exemplary hnRNP, down-regulators of which are used in the disclosed methods, is RNA-binding protein Raly (RALY). RALY is typically localized in the nucleus, and in humans, can exist as multiple isoforms (e.g., those produced via alternative splicing). One reference identifier for RALY, from the UniProtKB database, is Q9UKM9. Each of the isoforms of RALY is included among the embodiments of each of the aspects disclosed herein.

The down-regulator of RALY (or other hnRNPs), in some embodiments, is an mRNA-silencing nucleic acid molecule. Any nucleic acid molecule or a composition thereof can be used to down-regulate RALY. Two particular forms of mRNA-silencing nucleic acid molecules are antisense oliognucleotides (ASOs) and small interfering RNAs (siRNAs). Various features of these mRNA-silencing nucleic acid molecules have already been described in the literature (e.g., Mansoor and Melendez (2008) *Gene Regul. Syst. Bio.* 2: 275–295; Bilanges and Stokoe (2005) *Biochem. J.* 388: 573–583; Vickers *et al.* (2003) *J. Biol. Chem.* 278: 7108–7118; Watts and Corey (2012) *J. Pathol.* 226: 365–379).

ASOs are single-stranded oligonucleotides that are usually 18–21 nucleotides in length. They typically are synthetically prepared, and typically include deoxynucleotide sequences. ASOs can hybridize with their target mRNA sequences, for example through Watson-Crick base pairing, and thus inhibit gene expression. To improve ASO's stability and efficacy, researchers often use chemically modified versions of ASOs, which can be grouped into generations one through three: (1) those in which the phosphate backbone is altered (e.g., phosphorothioates, methylphosphonates, phosphoramidates); (2) those in which the sugars are modified (e.g., 2′-O-methyl, 2′-O-methoxyethyl; and (3) those in which further modifications can be made (e.g., locked nucleic acids, peptide nucleic acids, morpholino phosphoramidates). ASOs can affect variety of mechanisms, such as 5′-capping, polyadenylation, splicing, protein binding, and translation. In addition, ASOs can promote degradation by RNAse H (e.g., for gapmers with a phosphorothioate backbone and terminal stretches of 2′-O-methoxyethyl groups, RNAse H can act at the central portion of the mRNA).

In some embodiments, the ASOs have the sequences of any one of SEQ ID NOs: 1–3 and 11–69. In some embodiments, the ASOs have a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with of any one of SEQ ID NOs: 1–3 and 11–69. In some

embodiments, the ASOs have a sequence that has at least 13, 14, 15, 16, 17, 18, or 19 (or all) residues of the sequences of any one of SEQ ID NOs: 1–3 and 11–69. In some embodiments, the ASOs have a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with a sequence that has at least 13, 14, 15, 16, 17, 18, or 19 (or all) residues of any one of SEQ ID NOs: 1–3 and 11–69.

In some embodiments, the ASOs of any of the embodiments described in the paragraph above are modified, for example by having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all of their phosphate groups being replaced by phosphorothioate groups. In some embodiments, the ASOs of any of the embodiments described in the paragraph above are modified, for example by being constructed as gapmers with 2'-O-methoxyethyl groups on the sugars for some residues (e.g., 1 and 20, 1–2 and 19–20, 1–3 and 18–20, 1–4 and 17–20, 1–5 and 16–20, 1–6 and 15–20, 1–7 and 14–20, 1–8 and 13–20). The gapmers may also have phosphorothioate groups instead of some (e.g., all) of the phosphate groups. Additional embodiments include those in which the ASOs are modified in other suitable ways, for example according to the features described and referenced in this section for the three generations of ASOs.

siRNAs act through RNA interference (RNAi), during which a double-stranded RNA (typically 19–22 base pairs, which might have 3′-overhangs) associates with the RNA-induced silencing complex (which includes Argonaute), loses its passenger strand, binds with the remaining guide strand to RNA, and thus down-regulates gene expression (e.g., by causing degradation of the RNA). siRNAs can be introduced as precursors, for example as longer RNA duplexes (such as shRNA, which can be cleaved by Dicer to generate siRNA), or as DNA vectors (which can encode an shRNA). Since siRNAs include a duplex part, they can be stable as is, but they can also be chemically modified to improve their properties. Numerous such modifications exist; some have been compiled in a dedicated database that is available in World Wide Web at crdd.osdd.net/servers/sirnamod/.

Cardiometabolic Conditions and Methods of Treating a Cardiometabolic Condition

Nonalcoholic fatty liver disease (NAFLD) is a condition not caused by alcohol use, which is characterized by storage of too much fat in the liver. NAFLD can be classified as simple fatty liver (also known as non-alcoholic fatty liver (NAFL)) or as nonalcoholic steatohepatitis (NASH). NASH differs from NAFL mainly by the presence of inflammation, and potentially cell damage, in the liver.

Dyslipidemia is characterized by abnormal blood lipid levels. For example, the levels of triglycerides and cholesterol (e.g., as incorporated into LDL) may be too high in dyslipidemia. In some cases, dyslipidemia can lead to atherosclerosis. Atherosclerosis is characterized by narrowing and hardening of the arteries, which adversely affect the flow of blood.

Each of the cardiometabolic conditions described above as well as others and their symptoms (e.g., inflammation) can be treated with the methods described herein.

In some aspects, the disclosure relates to methods of treating a cardiometabolic condition (e.g., non-alcoholic fatty liver, non-alcoholic steatohepatitis, dyslipidemia, obesity, inflammation, atherosclerosis) of a subject by administering to the subject a down-regulator of RALY. In some embodimetns, the down-regulator includes an mRNA-silencing nucleic acid molecule, which can be an ASO or an siRNA.

In some aspects, the invention relates to a composition or a formulation including a down-regulator of RALY, which can be used in the methods described herein. The composition may include a pharmaceutically acceptable carrier. The pharmaceutical compositions disclosed herein may be delivered by any suitable route of administration (e.g., oral, intravenous), and can be supplied in various forms (e.g., powders, ointments, drops, liquids, gels, tablets, capsules, pills, or creams).

The selected dosage level, as can be determined by a medical practitioner, will depend upon a variety of factors including the activity of the particular agent employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

ASOs and siRNAs can be identified and designed via known algorithmic as well as experimental approaches. They can be introduced into cells or subjects by using suitable methods (e.g., directly in saline solution, by using cationic lipids). The amounts of ASOs and siRNAs can be varied. For example, the dose of each can be, in units of mg per meter-squared (body surface area), 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 950, 1000, or any of the values in between. Each dose can be delivered through various methods (e.g., as an intravenous injection, as an aerosol for inhalation).

Methods of Lowering a Cholesterol Level

In some aspects, the disclosure relates to methods of lowering a cholesterol level in a subject by administering to the subject a down-regulator of RALY. The down-regulator, in some embodiments, includes an mRNA-silencing nucleic acid molecule as described herein.

The cholesterol level can be any of the following: level of cholesterol in the form of LDL in the blood or plasma, level of cholesterol in the form of HDL + LDL + VLDL in the blood or plasma, level of cholesteryl esters in the form of LDL in the blood or plasma, level of cholesteryl esters in the form of HDL + LDL + VLDL in the blood or plasma, the level of cholesterol in hepatocytes.

Methods of Lowering Expression of a Gene Coding for 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase, or of a Gene Coding for Sterol Regulatory Element-Binding Protein 2

In some aspects, the disclosure relates to a method of lowering the expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). HMGCR is a transmembrane glycoprotein that is involved in cholesterol biosynthesis. While statins are known to inhibit this enzyme, the mRNA-silencing nucleic acid molecules disclosed herein provide a new approach of lowering the activity of this protein—by lowering the expression of the gene that codes for it. The methods include administering to a subject a down-regulator of RALY such as an mRNA-silencing nucleic acid molecule, which results in lowering of the expression of the *HMGCR* gene.

In some aspects, the disclosure relates to a method of lowering the expression of a gene coding for sterol regulatory element-binding protein 2 (SREBP2). SREBP2 is a transcriptional activator that is involved in cholesterol biosynthesis. The mRNA-silencing nucleic acid molecules disclosed herein provide a new approach of lowering the activity of this protein—by lowering the expression of the gene that codes for it. The methods include administering to a subject a down-regulator of RALY such as an mRNA-silencing nucleic acid molecule, which results in lowering of the expression of the *SREBF2* gene.

EXAMPLES

Example 1: Collaborative interactions of heterogenous ribonucleoproteins contribute to transcriptional regulation of sterol metabolism

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a group of functionally versatile proteins that play critical roles in the biogenesis, cellular localization and transport of RNA (Geuens *et al.* (2016) *Hum Genet* 135: 851-867). Although several lines of evidence link hnRNP abnormalities to neurodegenerative diseases and cancer, their contribution to metabolic control remains unexplored (Purice and Taylor (2018) *Front Neurosci* 12: 326; Cooper *et al.* (2009) *Cell 136:* 777-793). Herein is outlined a role for hnRNPs in gene regulatory circuits controlling sterol homoeostasis. Specifically, the findings disclosed here indicate that tissue-selective loss of the conserved hnRNP RALY enriches for metabolic pathways, arguing that hnRNPs can discriminately influence regulated gene expression programs. Liver-specific deletion of RALY alters hepatic lipid content and serum cholesterol level. *In vivo* interrogation of chromatin architecture and genome-wide RALY binding pattern reveal insights into its cooperative interactions and mode of action in regulating cholesterogenesis. Interestingly, the findings disclosed herein indicate that RALY binds the promoter region of the master metabolic regulator *Srebp2* and show that it directly interacts with coactivator NFY to influence cholesterogenic gene expression. The work disclosed here offers new insights into mechanisms orchestrating selective promoter activation in metabolic control and a model by which hnRNPs can impact health and disease states.

A number of Genome Wide Association Studies (GWAS) have linked variants at the hnRNP *RALY* (also known as Heterogeneous Nuclear Ribonucleoprotein C-Like 2) with cardiometabolic traits including total cholesterol and coronary artery disease, yet little is known about the function and mechanisms of actions of RALY (Howson *et al.* (2017) *Nat Genet* 49: 1113-1119; Nelson *et al.* (2017) *Nat Genet* 49: 1385-1391; Strachan *et al.* (2007) *Int J Epidemiol* 36: 522-531). Previous studies have shown that RALY interacts with *LeXis,* a noncoding RNA mediating crosstalk between the cholesterol biosynthesis and efflux pathways (Sallam *et al.* (2016) *Nature* 534: 124-128). *LeXis* is a direct transcriptional target of LXR, a sterol sensing nuclear receptor that triggers an "emergency response" to a lipid overload state. Activation of LXR induces the expression of genes involved in cholesterol efflux *(ABCA1* and *ABCG1),* limiting lipid uptake *(IDOL),* and promoting triglyceride-rich lipoprotein formation *(SREBP1 C* and *SCD1)* (Calkin and Tontonoz (2012) *Nat Rev Mol*

Cell Biol 13: 213-224). On the other hand, the SREBPs are master regulators of sterol metabolism, directly activating the expression of genes involved in cholesterol and fatty acid biosynthesis (Horton *et al.* (2002) *J Clin Invest* 109: 1125-1131). Although all SREBP isoforms can influence a large repertoire of genes at extreme perturbations, it is well established that SREBP1c preferentially activates genes involved in fatty acid biosynthesis whereas SREBP2 influences cholesterol biosynthetic machinery (Jeon and Osborne (2012) *Trends Endocrinol Metab* 23: 65-72). Consistent with unique epistatic relationship between various SREBPs, loss of SREBP2 in mouse liver reduces SREBP1c and triglyceride levels in addition to impacting cholesterol stores (Rong *et al.* (2017) *Elife* 6: 25015). Despite their unique activation signature both isoforms appear to bind similar DNA response elements and to partner with common transcriptional coactivators including NFYand SP1 (Ishimoto *et al.* (2010) *Biochem J*429: 347-357; Ericsson *et al.* (1996) *J Biol Chem* 271: 24359-24364).

To gain insights into the contribution of hnRNPs in metabolic disease, mice with LoxP sites flanking exons 3 and 4 of *Raly* were generated (Figs. 1A and 2). Administration of a Cre or control adenovirus to *Raly*$^{flox/flox}$ primary murine hepatocytes resulted in ablation of RALY transcript and protein levels (Figs. 4A and 4B). Since RALY is one of the few hnRNPs linked to human lipid traits, and since previous studies have shown that disruption of the *LeXis-RALY* axis perturbs cholesterogenic gene expression, the studies disclosed here sought to determine the effect of genetic deletion of *Raly* on *Srebp2* (official gene symbol Srebf2) gene expression. Deletion of *Raly* from primary murine hepatocytes led to a significant reduction of Srebp2 and its target genes involved in cholesterol biosynthesis including Hmgcr (Fig. 4C). Surprisingly, the deletion of *Raly* from mouse hepatocytes also led to a significant reduction in Srebp1c, the isoform responsible for triglyceride biosynthesis (Fig. 4C). The results confirmed reduced protein levels of a nuclear SREBP2 and a number of targets including HMGCS and FDPS (Figs. 4D and 4E). To explore the contributions of *Raly* in liver metabolism, liver specific *Raly* knockout mice were generated (referred to as *L-RalyKO* while *Raly*$^{flox/flox}$ Cre negative littermates are controls). Quantitative PCR analysis and western blotting confirmed significant and robust decrease in RALY in liver following Cre recombination (Figs. 1B and 1C). Previous studies have shown that at least a subset of hnRNP complexes influence gene expression in a non-discriminate fashion (Chaudhury *et al.* (2010) *RNA* 16: 1449-1462). To better define the range of RALY activities in liver, unbiased transcriptional profiling was performed of livers

from *L-RalyKO* and control mice on chow diet (Fig. 1D). Analysis of differentially regulated genes showed a significant and strong enrichment of lipid metabolic and related pathways (Fig. 1E) as well as substantial overlap with pathways known to be modulated by SREBP2 (Fig. lE) (Seo *et al.* (2011) *Cell Metab* 13: 367-375). Quantitative PCR showed a significant reduction in expression of Srebp2 and its target genes in L-RalyKO mice (Fig. IF). There was also a trend to reduced Srebp1c expression although did not reach significance (fasting mice). Consistent with gene expressing results loss of RALY was associated with a reduction in serum cholesterol level (Fig. 1G). Lipid fractionation analysis revealed a reduction in LDL and HDL fractions the predominant circulating pool in chow-few mice (Fig. 1H). Taken together, these results suggest that hnRNPs can regulate the activity of specific gene expression programs and that the effects of RALY on cholesterogenesis are non-redundant.

It was noted that loss of *Raly* from hepatocytes was associated with a decrease in cellular cholesterol and triglyceride content (Figs. 3A and 3B). To better define the contributions of RALY on hepatic lipid composition, unbiased shotgun lipidomics were performed on mouse liver comparing *L-RalyKO* and controls. It was observed that vast majority of lipid species were unchanged with the exception of hepatic cholesterol and triglyceride content (Figs. 3 and 5). Examination of cholesterol esters showed a reduction in most species in *L-RalyKO* although only a subset reached statistical significance (Figs. 3C and 3D). Similarly triglyceride content was significantly reduced in *L-RalyKO* livers (Figs. 3E and 3F). The results showed no changes in the expression of genes involved in lipolysis (Fig. 6). Intriguingly, these results partially phenocopy the chow-fed *Srebp2* liver-specific knockout mice, that exhibit mildly reduced liver cholesterol content as well altered SREBP1c and triglycerides levels (Rong *et al.* (2017) *Elife* 6: 25015). These results are also consistent with unique epistatic relationship between various SREBPs. Taken together, the lipidomics findings reinforce the gene expression results and support the notion that RALY may be affecting sterol metabolism through interaction with the *Srebp2* pathway. To more thoroughly investigate the contributions of RALY in chronic lipid abundance states, *L-RalyKO* mice and controls were fed a diet known to induce sterol accumulation and NASH. After 9 weeks of feeding, *L-RalyKO* showed significant reduction in Oil-Red-O staining (Figs. 3G and 3H). These results demonstrate that in a preclinical disease model the hepatic loss of RALY partially protects against lipid overload.

Previous studies have shown that *LeXis* and RALY are almost exclusively present in the cell nucleus in association with chromatin and that *LeXis* may be impacting cholesterogenic gene expression through transcriptional mechanisms (Sallam *et al.* (2016) *Nature* 534: 124-128). Thus, it was hypothesized that RALY may also be impacting *Srebp2* levels through nascent transcript production. To gain better insight as to how RALY may regulate gene expression, genome-wide RALY binding sites were mapped. ChIP-seq was performed to assess RALY DNA binding in murine hepatocytes and identified a total of 2950 RALY peaks that were independent identified by the same peak calling algorithm in at least two independent samples (Fig. 7A). Global analysis of fragment distribution around the peak summit showed overall agreement between samples and a broad peak contour profile spanning approximately 500 bps, a feature often associated with coregulators (Fig. 7B). Interestingly, RALY showed strong enrichment for promoter binding but was also in enriched in other parts of the genome including intronic and intergenic regions (Figs. 7C and 7D). Furthermore, unbiased peaking calling showed that RALY bound the *Srebp2* promoter region but not the *Srebp1* promoter (Figs. 7D and 8). These results reinforce the notion that RALY primarily affects Srebp2 and that perturbations in hepatic triglycerides content are likely a downstream consequence of reduced SREBP2 activity. In addition, motif discovery analysis of RALY bound peaks identified the transcription factor NFY as a highly enriched motif (Fig. 7E). NFY is a promoter-binding transcription factor (formed with trimeric complex of NFYA, NFYB, and NFYC with all subunits required for proper function) with an established role in mediating SREBP responses (Reed *et al.* (2008) *PLoS Genet* 4, e1000133). Taken together, these results hint that RALY may be influencing cholesterogenesis by modulating collaborative interactions with transcriptional coactivators at the *Srebp2* promoter.

A number of studies have shown that noncoding RNA-protein interactions can regulate gene activities by modulating a number of histone modifications at target genes (Davidovich and Cech (2015) *RNA* 21: 2007-2022; Wang and Chang (2011) *Molecular cell* 43: 904-914). To explore the possibility that RALY may influence gene expression by altering epigenetic states, Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) was performed (Buenrostro *et al.* (2015) *Curr Protoc Mol Biol* 109: 21 29 21-29), a method that allows unbiased interrogation of chromatin architecture, on livers from *L-RalyKO* and controls (Fig. 7F). Loss of RALY in mouse liver did not globally alter chromatin accessibility (Fig. 7G) arguing against the idea that RALY may be impacting

gene expression by impacting histone modifiers such as polycomb repressive complex (PRC) proteins or histone deacetylase (HDAC) (Margueron and Reinberg (2011) *Nature* 469: 343-349). In addition to directly interrogating changes in nucleosome rearrangements, ATAC-seq allowed us to infer transcriptional activity by examining changes in enhancer landscapes with targeted perturbations. Although globally most ATAC peaks did not change between controls and *L-RalyKO* samples, a number of peaks were differentially regulated (Fig. 7H). Intriguingly, many of these peaks clustered near genes involved in lipid metabolism (Figs. 7I, 7J, and 9). Furthermore, motif analysis of these differentially altered peaks showed specific enrichment for number of transcription factors known to impact metabolic regulation including NFY (Fig. 7K). Taken together, the above results suggest that RALY may be regulating cholesterol metabolism by impacting the coactivator NFY at the *Srebp2* gene.

To clarify whether RALY directly interacts with transcriptional machinery at SREBP2, Co-IP studies were performed in murine hepatocytes. Pulldown of RALY enriches for NFY and vice versa confirming a robust interaction between the two factors (Figs. 10A and 10B). To clarify whether RALY is required for NFY binding at promoter regions, ChIP was performed of NFY in *L-RalyKO* or control livers. NFY enrichment was confirmed at its known target gene Rnf5 (Fig. 10C) as well as the SREBP2 promoter region (Fig. 10D). The results suggest that NFY binding is minimally rearranged by loss of RALY consistent with the idea that RALY does not act as a guide to facilitate NFY complex DNA binding, rather it works cooperatively with NFY at select sites to influence its transcriptional activity. To determine if RALY was sufficient to induce the expression of cholesterogenic genes, RALY was overexpressed using an adenoviral vector in a murine hepatocyte cell line (Fig. 11). It was found that cholesterogenic gene expression was increased in response to RALY expression (Fig. 10E). In addition, hepatic RALY overexpression increased serum cholesterol in chow-fed mice (Fig. 10F). To more thoroughly investigate the cooperative relationship between RALY and cis/trans promoter factors, luciferase reporter assays were performed with an *Srebp2* promoter construct (Fig. 10G). Adenoviral expression of RALY in hepatocytes enhanced wild-type *Srebp2* promoter-driven luciferase activity, but failed to increase luciferase activity when either NFY or SRE sites were mutated (Fig. 10D). These results reinforce the idea that RALY is a coactivator that requires the binding of canonical transcriptional factors at the *Srebp2* promoter including SREBP itself. To better clarify the epistatic relationship between NFY

and RALY, a knockdown of NFY in RALY deficient cells was performed. The results show that reduction of NFY on a *Raly* deficient background no longer alters Srebp2 expression (Figs. 10H and 12). These results suggest that RALY is required for NFY dependent transcription of SREBP2 and is consistent with notion and RALY and NFY work cooperatively to influence gene expression.

To better explore the relationship between hnRNPs and human lipid traits, expression data from the *METISM* cohort was analyzed (Orozco *et al.* (2018) *Hum Mol Genet* 27: 1830-1846). Interestingly, expression of RALY was positively associated with a number of metabolic traits including total and LDL serum cholesterol levels though the strength of association was modest (Fig. 10I). Importantly, other hnRNPs, including ones that contribute to transcriptional control mechanisms (hnRNP K), were not significantly associated with total cholesterol or LDL cholesterol in this cohort (Figs. 13A and 13B). Although hnRNPs are known to form complexes with one another, these observations are consistent with the idea that individual hnRNPs may play functional roles favoring specific pathways

Previous work has identified important roles for hnRNPs in neurodegenerative disease, most prominently ALS and FTLD (Geuens *et al.* (2016) *Hum Genet* 135: 851-867; Purice and Taylor (2018) *Front Neurosci* 12: 326). The work disclosed herein expands the contributions of hnRNPs in health and disease by showing that a conserved hnRNP can help direct fundamental metabolic regulatory circuits. In addition, the work disclosed herein offers insights into the precise molecular mechanisms that link hnRNP abnormalities with pathologic states. The functional versatility of hnRNPs is thought to stem from their ability to "dance with different partners" to impact diverse biologic process such as RNA splicing, polyadenylation, export, and translation. Thus, there are potentially multiple ways by which hnRNPs abnormalities can lead to disease states. Defects in hnRNPs leading to stress granule changes and the accumulation of pathological inclusions are thought to be important in neurodegenerative states (Boeynaems *et al.* (2017) *Mol Cell* 65: 1044-1055; Alami *et al.* (2014) *Neuron* 81: 536-543). The work disclosed herein shows that hnRNPs proactively participate in transcriptional control mechanisms regulating cholesterol homoeostasis and that loss of a single hnRNP (RALY) influences hepatic lipid stores.

It is well-established that the predominantly cholesterogenic SREBP-2 and lipogenic SREBP1 transcription factors may be differentially processed depending on environment cues. However, both isoforms are capable of binding diverse SREs and

collaborate with common coactivators despite possessing distinct transcriptional effects once in the nucleus. In addition, it is unclear how generic collaborative partners such as NFY and SP1 are capable of turning on some but not all their target genes in response to specific environmental cues. The characterization of RALY offers some important clues into the selective promoter activities of different SREBPs. The fact that RALY binds the promoter of one isoform *(Srebp2)* and not the other hints that spatial collaborative interactions may favor a specific gene activation signature. The finding that RALY interacts with the SREBP coactivator NFY also raises a number of intriguing questions.

Example 2: Materials and Methods for Example 1

Study Approval

All experiments were approved by the UCLA Institutional Animal Care and Research Advisory Committee and performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Animals

All animals used in the study were in C57BL/6 background. The study disclosed herein used male mice unless otherwise specified. Mice were fed chow diet (Research Diet) and housed temperature-controlled room under a 12-h light/12-h dark cycle and pathogen-free conditions. *Raly*$^{flox/flox}$ mice were generated by Cyagen using the strategy outlined in Fig. 1A. To generated RALY liver specific knockout mice and littennate controls, *Raly*$^{flox/flox}$ were treated with adeno-associated virus (AAV) with TBG promoter (AAV8.TBG.Cre) or (AAV8.TBG.GFP) purchased from Penn Vector Core. AAV administered intraperitoneal injection at dose of 5x 10^{11} GC per mice. Mice were euthanized 4 weeks after AAV injection. Liver tissues were frozen in liquid nitrogen and stored at -80 °C or fixed in 10% formalin. Blood was collected by retro-orbital bleeding, and the plasma was separated by centrifugation. Plasma lipids were measured with the Wako L-Type TG M kit, the Wako Cholesterol E kit. All animal experiments were approved by the UCLA Institutional Animal Care and Research Advisory Committee.

Statistical analysis

A non-paired student t-test was used to determine statistical significance, defined at P-value < 0.05. For multiple group experiments ANOVA was used followed by multiple group analysis. Unless otherwise noted, error bars represent standard deviations.

Experiments were independently performed at least twice. Sample size is based on statistical analysis of variance and prior experience with similar *in vivo* studies.

Cells culture

Mouse primary hepatocytes were isolated as previously described and cultured in William's E medium with 5% BSA (Rong *et al.* (2013) *Cell Metab* 18: 685-697). Hepa1-6 cells were originally obtained from ATCC and cultured in DMEM medium with 10% FBS. Adenovirus studies were performed as previously described (Sallam *et al.* (2016) *Nature* 534: 124-128). RALY was cloned from mouse cDNA using a gateway cloning system and into the pAd/CMVN5-DEST Gateway vector by LR recombination according to the manufacturer's guidelines. NFY knockdown was done in primary hepatocytes using siRNA against nfya (Dharmacon^{TM} catalog number LQ-065522-00-0005). Transfection was proceeded using DharmaFECTTM 4 transfection reagent (Dharmacon^{TM}) according to the manufacturers recommendation. Cells were collected for RNA isolation or protein extraction 48 hours after transfection.

Gene expression analysis and immunoblot analysis

Total RNA was isolated using TRIzol reagent (Invitrogen) and reverse transcribed using a home made RT as was previously described (Sallam *et al.* (2018) *Nat Med* 24: 304-312). cDNA was quantified by real-time PCR using SYBR Green Master Mix (Diagenode) on BioRad Real-time PCR instrument. Gene expression levels were determined by using a standard curve. Each gene was normalized to the housekeeping gene 36B4. For immunoblot analysis, whole cell lysate or tissue lysate was extracted using RIPA lysis buffer (Boston Bioproducts) supplemented with complete protease inhibitor cocktail (Roche). For SREBP2 Proteins immunoblot analysis, nuclei from primary hepatocytes were prepared using Subcellular Protein Fractionation Kit for Cultured Cells (78840, Thermo Scientific). Proteins were diluted in Nupage loading dye (Invitrogen), heated at 95 C for 5 min, and run on 4-12% NuPAGE Bis-Tris Gel (Invitrogen). Proteins were transferred to hybond ECL membrane (GE Healthcare) blocked with 5% milk (or 5% BSA for anti-SREBP2) to quench nonspecific protein binding and blotted with the indicated primary antibody.

Dual luciferase assay

DNA transfection of Hepa1-6 cells was performed with LipofectamineTM 3000 (Invitrogen) according to user's manuscript on 24-well plates with a cell density of $1.5x10^5$ cells/well. The cells were transfected with 200 ng of the *Srebp2* promoter firefly reporter plasmid, 50 ng of *Renilla* reporter plasmid (Promega) and 200 ng of nSREBP2 expression

vector. At 12 h after transfection, the cells were cultured in DMEM supplemented with 1 % (v/v) FBS and adenovirus was administered. After 24 h, assays for both luciferase and Renilla activities were performed. The reporter activities were expressed as the relative firefly luciferase activity/Renilla luciferase activity.

RNA-seq

Libraries for RNA-Seq were prepared with KAPA Stranded RNA-Seq Kit on RNA isolated from livers of chow diet feeding mice with AAV-GFP or AAV-Cre transduction. The data was sequenced on Illumina HiSeq 3000 for a pair-end 150bp read run. Data quality check was done on Illumina SAV. Demultiplexing was performed with Illumina Bc12fastq2 v 2.17 program. RNA-seq reads were aligned with TopHatv2.0.2 to the mouse genome, version mm9 25. Transcripts were assessed and quantities were determined by Cufflinks v2.0.2, using a GTF file based on Ensembl mouse NCBI37. Comparison expression levels were made using FPKM values using Cuffdiff from the Cufflinks package 26.

NASH Diet feeding and Oil-Red-O staining

12-week -old male mice were fed with diet composition of 60 kcal% Fat with 0.1% Methionine and No Added Choline (A06071302, Research Diets) for 9 weeks. Oil Red O histochemistry staining were performed using frozen sections from mouse liver. Briefly, frozen sections were fixed in 10% neutral formalin for 10 minutes, followed by treatment in 60% isopropanol for 5 second and then staining in Oil Red O working solution (O-0625, Sigma Aldrich) for 15 minutes. This procedure was followed by washing with 60% isopropanol for 5 second and then water 1 minute. Finally, sections were contained with Mayer's Hematoxylin for 3 minutes.

Lipidomics

50-100 mg of frozen liver are homogenized in the Omni Bead Ruptor Elite with 2mL homogenizer tube system (Omni, 19-628D). Samples are homogenized in cold PBS for 3 cycles of 10 sec each at 5 m/s with a 10 second dwell time between cycles. 3-6 mg of homogenized material are applied to a modified Bligh and Dyer extraction (Bligh and Dyer (1959) *Can J Biochem Physiol* 37: 911-917). Prior to biphasic extraction, a 13-lipid class Lipidyzer Internal Standard Mix is added to each sample (AB Sciex, 5040156). Following two successive extractions, pooled organic layers are dried down in a Genevac EZ-2 Elite. Lipid samples are resuspended in 1:1 methanol/dichloromethane with 10mM Ammonium Acetate and transferred to robovials (Thermo 10800107) for analysis. Samples are analyzed

on the Sciex Lipidyzer Platform for targeted quantitative measurement of 1100 lipid species across 13 lipid sub-classes. Differential Mobility Device on Lipidyzer is tuned with SelexION tuning kit (Sciex 5040141). Data analysis performed on Lipidyzer software. Quantitative values are normalized to milligrams of material used.

Chromatin immunoprecipitation (ChIP)

ChIP experiments were performed as was previously described with exception of a few changes (Sallam *et al.* (2018) *Nat Med* 24: 304-312). For RALY ChIP, 20 million cells were used for each sample and four replicates were performed for each group. Sonication was performed using a M220 Focused-ultrasonicator (Covaris) according to the manufacturer's protocol (10 minutes for cells), and chromatin was immunoprecipitated with 4 μg antibodies against RALY (Ab170105, Abcam) overnight at 4 °C. ChIP-Seq libraries were prepared using the Kapa LTP Library Preparation Kit (Kapa Biosystems). ChIP-Seq was performed as described (Tong *et al.* (2016) *Cell* 165: 165-179). Bowtie2 was used for alignment, and Raly enriched peaks were identified using by Homer findPeaks (Heinz *et al.* (2010) *Mol Cell* 38: 576589) with FDR < 0.01. The called peaks were subsequently used for identifying the motifs enrichment by PscanChlP (Zambelli *et al.* (2013) *Nucleic Acids Res* 41: W535-543). For NFY ChIP, Chromatin samples from mice livers were prepared using truChlP Chromatin Shearing Tissue Kit (Covaris) according to manufacturer's recommendation. Sonication was performed using a M220 Focused-ultrasonicator (Covaris) according to the manufacturer's protocol for 12 minutes, and chromatin was immunoprecipitated with 6 μg antibodies against NFY-A subunit (sc-17753X, Santa Cruz Biotechnology) overnight at 4 °C. The ChIP samples were analyzed by real-time PCR using primers listed in table 1. All values obtained were normalized to the primers of negative control region.

ATAC-seq

ATAC-seq was optimized in liver after several modifications from original Buenstero protocol (Buenrostro *et al.* (2015) *Curr Protoc Mol Biol* 109: 2129). 100 mg of frozen liver were grinded to fine powder using cellcrusher and 1 mL of ice cold nuclei isolation buffer was added (20mM Tris-HC1, 50mM EDTA, 5mM Spermidine, 0.15mM Spermine, 0.1% mercaptoethanol, 40% Glycerol, pH 7.5, mM EGTA and 60mM KCl). After 5 min of cooling on ice, cell suspension was filtered through Miracloth (Calbiochem) followed by centrifugation at 1,100 x g for 10 min at 4°C. Pellet was resuspended with 50 u1 RSB buffer (10mM Tris-HC1, 10mM NaCl, 3mM MgCl2, pH 7.4) followed by

centrifugation at 500x g for 5 min at 4°C and resuspension in PBS. 75,000 nuclei pellet were used for transposase reaction. The rest of the protocol followed that described by Buenstero. ATAC-Seq libraries were prepared using the Nextera Tn5 Transposase and DNA library preparation kit (Illumina) as described (Buenrostro *et al.* (2015) *Curr Protoc Mol Biol* 109: 2129). Libraries were single-end sequenced (50bp) on an Illumina HiSeq 2000. Reads were mapped to the mouse genome (NCBI37/mm9) using Bowtie2 and were removed from the subsequent analysis if they were duplicated, mapped to mitochondrial genome, or aligned to unmapped contiguous sequences. Peak calling was performed using MACS2. The reads were converted to reads per thousand base pairs peak per million mapped reads (RPKM) by dividing by the total number of reads per sample. The average RPKM from four replicates was used to quantify the accessibility across all called peaks.

Antibodies

Antibody	**Company**	**Cat#**	**Utilization**	**Dilution**
anti-RALY	Abcam	ab170105	Western blot, ChIP	1:1000
Anti-NFYA	Santa Cruz Biotechnology	sc-17753X	Western blot, ChIP	1:2000
Anti-NFYB	Abcam	ab111577	Western blot	1:1000
Anti-SREBP2	Millipore	MABS1988	Western blot	1:1000
Anti-PD1	cell signaling	3501S	Western blot	1:1000
Anti-LaminA/C	Santa Cruz Biotechnology	sc-376248	Western blot	1:800
Anti-tubulin	Abcam	ab185224	Western blot	1:1000
Anti-HMGCS	Abcam	ab137043	Western blot	1:1000
Anti-FDPS	Abcam	ab15385	Western blot	1:1000

Primers

	Primer ID* Sequence: Forward (5'-3')	**Sequence: Reverse (5'-3')**
ChIP NC	TCCAGAAGAGGGCGTCAGAT (SEQ ID NO: 70)	GGCTCAGTGGGTAAGAGCAC (SEQ ID NO: 71)
ChIP Srebp2 Sitel	GCTTCTTTTCCTGCTCAGGCT (SEQ ID NO: 72)	GCTGTTGGGAAGCAGGTTA (SEQ ID NO: 73)
ChIP Srebp2 Site2	TGTTCTGGGAACTCCACCTTT (SEQ ID NO: 74)	TAGACCAGGCCAGCTTCAAAC (SEQ ID NO: 75)
ChIP Srebp2 Site3	TGAAAGATGGTGGAGGACGC (SEQ ID NO: 76)	AGAGACTGTGCTAAGTGACTGT (SEQ ID NO: 77)
ChIP Srebp2 Site4	AAACAGTTGCTTAGCCAGCC (SEQ ID NO: 78)	GGCAGTTGTTCTTAGGGGGT (SEQ ID NO: 79)
ChIP Srebp2 Site5	CCCGCTATGCAAATCTGAGC (SEQ ID NO: 80)	TTGTCAATGGGACCAGGCTT (SEQ ID NO: 81)
ChIP Srebp2	AGTCTTAGCCTGACCTGTCCT (SEQ ID NO: 82)	CTTGTCAGAGGATTCAGACACCA (SEQ ID NO: 83)

Site6 (not enriched)		
ChIP Srebp2 Site7 (not enriched)	AAAGGACGCAGTCGCATGT (SEQ ID NO: 84)	ATGGAGAACCCAAGAAGGG (SEQ ID NO: 85)
ChIP Srebp2 Site8 (not enriched)	TCCTTGGAACGTGGGAGTGGC (SEQ ID NO: 86)	TGGCCAGTCTGACATCCAT (SEQ ID NO: 87)

*For NFY positive control primers please see Yamanaka, T. et al. Nat. Commun. 5:3354 doi: 10.1038/ncomms4354 (2014).

Example 3: Antisense Oligonucleotides

Some exemplary sequences are provided below. SEQ ID NOs: 1-3 represent three different antisense oligonucleotides (named ASO-770, ASO-3943, ASO-5025, respectively), and show their nucleobase sequences, which in some embodiments can be modified. SEQ ID NOs: 4-6 have the same nucleobase sequences as SEQ ID NOs: 1-3, but they specify that the oligonucleotides have phosphorothioate groups (denoted by "*" in the sequence listing below) instead of some of the phosphate groups as shown. SEQ ID NOs: 7-9 also have the same nucleobase sequences as SEQ ID NOs: 1-3, but they instead are gapmers with 2'-O-Methoxyethyl (denoted by "MOE" in the sequence listing below) groups on the sugars for residues 1-5 and 16-20, and with phosphorothioate groups instead of internal phosphate groups throughout.

Additional exemplary sequences are represented by SEQ ID NOs: 11–69. As with SEQ ID NOs: 1-3, SEQ ID NOs: 11–69 can be modified to arrive at additional embodiments (e.g., by replacing phosphate groups with phosphorothioate groups, by converting them to gapmers with 2'-O-methoxyethyl groups on some of the sugars).

An exemplary sequence for RALY mRNA is also provided in the form of SEQ ID NO: 10, in which the "T" residues stand for the "U" residues that are normally part of the mRNA.

SEQ ID NO: 1
TTTGACACGCCGGACCAAAG

SEQ ID NO: 2
TGTCCAGCATATGACCCGGT

SEQ ID NO: 3
AATACCTGACGCTCACGTGC

SEQ ID NO: 4
T*T*T*G*A*C*A*C*G*C*C*G*G*A*C*C*A*A*A*G

SEQ ID NO: 5

T*G*T*C*C*A*G*C*A*T*A*T*G*A*C*C*C*G*G*T

SEQ ID NO: 6
A*A*T*A*C*C*T*G*A*C*G*C*T*C*A*C*G*T*G*C

SEQ ID NO: 7
/52MOErT/*/i2MOErT/*/i2MOErT/*/i2MOErG/*/i2MOErA/*
C*A*C*G*C*C*G*G*A*C*
/i2MOErC/*/i2MOErA/*/i2MOErA/*/i2MOErA/*/32MOErG/

SEQ ID NO: 8
/52MOErT/*/i2MOErG/*/i2MOErT/*/i2MOErC/*/i2MOErC/*
A*G*C*A*T*A*T*G*A*C*
/i2MOErC/*/i2MOErC/*/i2MOErG/*/i2MOErG/*/32MOErT/

SEQ ID NO: 9
/52MOErA/*/i2MOErA/*/i2MOErT/*/i2MOErA/*/i2MOErC/*
C*T*G*A*C*G*C*T*C*A*
/i2MOErC/*/i2MOErG/*/i2MOErT/*/i2MOErG/*/32MOErC/

SEQ ID NO: 10 = human RALY mRNA sequence from NM_016732.3
GTCAGTGCGGCGGGGCGCGCGAGCGGCGCCAGCTCGGGGCAGCGGAACCCAGAGAAGCTGAGGGGGCGGT
AGCGGCGGCGACGGCGACGACGACGACTCCCGCGCGTGTGCCCAGCCTCTTCCCGCCGCAGCCGCCCTTT
TCCTCCCTCCCTTACGTCCCCGAGTGCGGCAGTACCGCCTCCTTCCCAGCCGCGCGGCTTCCTCCAGACC
TCTCGGCGCGGGTGAGCCCTATTCCCAGAGGCAGGTGGTGCTGACCCTGTAACCCAAAGGAGGAAACAGC
TGGCTAAGCTCATCATTGTTACTGGTGGGCACCATGTCCTTGAAGCTTCAGGCAAGCAATGTAACCAACA
AGAATGACCCCAAGTCCATCAACTCTCGAGTCTTCATTGGAAACCTCAACACAGCTCTGGTGAAGAAATC
AGATGTGGAGACCATCTTCTCTAAGTATGGCCGTGTGGCCGGCTGTTCTGTGCACAAGGGCTATGCCTTT
GTTCAGTACTCCAATGAGCGCCATGCCCGGGCAGCTGTGCTGGGAGAGAATGGGCGGGTGCTGGCCGGGC
AGACCCTGGACATCAACATGGCTGGAGAGCCTAAGCCTGACAGACCCAAGGGGCTAAAGAGAGCAGCATC
TGCCATATACAGTGGCTACATCTTTGACTATGATTACTACCGGGACGACTTCTACGACAGGCTCTTCGAC
TACCGGGGCCGTCTGTCGCCCGTGCCAGTGCCCAGGGCGGTCCCTGTGAAGCGACCCCGGGTCACAGTCC
CTTTGGTCCGGCGTGTCAAAACTAACGTACCTGTCAAGCTCTTTGCCCGCTCCACAGCTGTCACCACCAG
CTCAGCCAAGATCAAGTTAAAGAGCAGTGAGCTGCAGGCCATCAAGACGGAGCTGACACAGATCAAGTCC
AATATCGATGCCCTGCTGAGCCGCTTGGAGCAGATCGCTGCGGAGCAAAAGGCCAATCCAGATGGCAAGA
AGAAGGGTGATGGAGGTGGCGCCGGCGGCGGCGGCGGTGGTGGTGGCAGCGGTGGCGGTGGCAGTGGTGG
TGGCGGTGGCGGTGGCAGCAGCCGGCCACCAGCCCCCCAAGAGAACACAACTTCTGAGGCAGGCCTGCCC
CAGGGGGAAGCACGGACCCGAGACGACGGCGATGAGGAAGGGCTCCTGACACACAGCGAGGAAGAGCTGG
AACACAGCCAGGACACAGACGCGGATGATGGGGCCTTGCAGTAAGCAGCCTGACAGGAGCAATGGCCACC
AGCAGGTGAAGGGCATCGCTGCCCCAGGCCTCAAGCCGGGCACCCAACCCTGGATGCCACCCCCCAGCGG
GTACCAGAGGAAAGCTGGCAGCAGGCGCCTCCTCCCCCAACGCATCCCAGCCAGTGCCATGTCCTCTGCA
GGTGGAGTTACTGGCCTACTCCTTCCCCATGAGCCCTCCCTGTCTGCACTGCCCAGGCCAGAGGGTAGAG
CACAGGGGTTTCCCCATACTACCTCCCCTCCCCAGGACACTCCCAGGCTTGGGTTTTTTCTATAGGTTTG
GCGGGGGGCCACAGGGAGGGGACCCTGACAATAAAGAGATTGGATCCCAACCTGTTCTGAGATGGGATGG
TTTGTGTTTTCTCATGAAGATATCCCGGCCCCTCTGCCAACCAAAAAGCTGGTCTAGGGTGCCTAATACT
GATCCATCTGGACCTTAGTGTCCTCAGTGGTGATTAAAATGGCCAGTGGGGCCACTGGGGAGGGTTGGAT
ATGCTGGCCCATGAGCATCTTGCTGGCTGAAGTGTCAAGCAGTTGTGACCCACTTGGTTTACCCCATAGT
AGGTCAAGACCTTATCTCTTTCCCCAGCTTCTAAGTCTGGTCTTTCCCAGCTCTTAAAAGGATTCTAGAG
TCTGCCAGTCTCTACCTTCTCTCTTCTGGCTTAGGACACTATAATTTTTTCATTTGGACGTTGTCCTCCC
ACCAGCCTCCCAGTCTTCTGCCTCTGCCCTGTACCATCTCCCAGCAGCCACGCAGTCTTTCTGAAACGTA
TCCCTTCCCTGCCTAAAATCCCTTTACTGACTCTTCATCATCAGGACAAAACCCTACCTCCTGAATGTAG
GGTGTAAGATCCTGCTTACTCCAGCCTCTCCTGTTTCTTGTCACCACCCCTTTCCTGCCCCTGACACAAA
CCCTACATCTCAGTCTCACAAAACACACAAGTTCTTCACACTCCGGGCAGGTTTGGTAGCAAGGAATAGA
GCGTACTCCAGTGACAGCAATCAGTAGGAAGTTTGCATCCGGAGACACGCAGCCTGTCTGCCCCTGTCTT
GGGCCACCCGTCTAGACATTTGTTCTCTAACAATCAGCCGGGTTCCTTCAAACGTGTGCTGCTTATACAT
GCTCATTGTGTACTGCTAATGTCACCTACTTCCCCCTTCATTCCCCACAATGAATGGATCCATTTTCCCC
ATGTTCAAGTTCCCAAGAGAGAATCGACTGCCACTGTAATTTTTTTTCCTAATCACTCATAGGTTACAAG
TACCTACCCTGGTCCAGTGAACTGCGGGATTCATATGTGGCCACTTAGGCTGGTCTCGTTGGTATGGAGC
TGTGGGAGGGCAGCACCCAGAAATAGGCTTTGTTCCAGGCCTGGCACGTACTCCTCCTGCTCCTACTTTC
TCTACCTGGGAAATAGCCGCTCAGGACGAAGCTGCTGCTGTGGTGTCGCCACCTCTGAGAAGTCTCCCAG
ACCCTCCGACTGCTCTTTAATTGCCTGTTCTCTCCTCTGGAAGCCTTCCCCCAACACAGAAAAATTCAAT
CCCATCATCAAAAAGGGCTTTAGGTTTTCCCTCCATCTTTACGTGTTGATCAAAGTTTGCAGATCTGCTG
GAATCCTTATTGAAAACTCCCCATTTCAAACCAACCCGGTCTACAGCCTCCAGGGAAGCTTCCTCCTGGG
CTTTGGCTCCATGTTTTGGTAGAGGGTGGAGGCTTTGGGCAGTGCTCAAACTCCACCGGGAAGTCTCTCT
CACTGAGCAGCCCTCCTGCCTGTCATCCTGGGCAGGCGAGCACTCTGAGGCCCCAGTGTAGCTCTGTGCT
TTGATATTCCCAAGCTCTGCTGGGGCCTGACTAGGCCAGCCCCAAGGTGGCCAGAGTTCTGGCTTCATAC
CTGAGCCAAAAGCCCCAATCCATGCTTGGCCATTGCCTGAGTATTAGCTGCCCCAGGGGGATCACGGTCC
CCATATATTTGCTTGCCATGGACCCTGGGCAGCAGGGAGAGAGTAGAGATTTGTCAAGAGCCCATGGTGG

AGGCTGAGGCCCTGAGGCCATGAGATGCAGGCATGGGGTGAGAAACAGGCCCCTTGGAATTGGGCTGGGC
CTTGGCCCAGCTTAGTCAAATCAAAAGGCTTCTATTTGGAGAGCTGAAGAGGGTGTACAGAGGAAGGGGC
TAGGTCTGCAAGGAGTGCCTCATCTCCCTGAAGAGCTCTCAGTGGAACATACTTCACCCATCCATGTACC
CACATCTTTCCTTGCCCAGAAGGCGAGAGCCAGCTATAACAGACCCATTTCAATACCCTGGCAAGTCATT
ACTGCCCTTAGCTCTTGGTGTCCCCATCTGTGAAACATGGGGGACAGCTGCTAGCCTGGATTGGAACTTG
GGCAAAGTCCAAAGAATGGGATTTAGAGCTGAATGAACCTCACACTGAGGGCACAATAGCACTAGGCACT
GCCCCCAGAGCCTAGTGCTATATGCCTGCTGCAGGTCCTACCCAAGCATGCTTTACTGAGGAACTCAACG
TTTCAGAGCTTGAGGGTCAGGTTGATCATGGGCTTGTGACCATAGGCTTCCTTAGTATGCCAGGCTTGGA
GAACCGTGAGAAAAGCAGGGAAGATACCTTCAAGGGTAGCAGGCATCAGCTCTACTCACCTTGGCCCATA
AGGCTTTGCCTGGTCATGCTGGCACCGGGTCATATGCTGGACAGGGAGAACGAGAGTCCCATCCTGGAAC
TCCAGAAAAGCCCCTGGATGCTCCAGCCCCTGGGAAAGCACACAGCCAGGCCCTTGGGTGGGAGGTTGGC
TTCTAACAGTGCATACACATGCCCTTCCTCTGAGTCGGGGCAGCAAAAACATCCATTCCGCTGCGCAACA
GTTGTCATTTTTCTAACATCTGAAAACTCCAGAAGGAGATGGTGATAAATGTGGTACCGGATTCTGCCTA
AAGGATCAGTCTTTAGATGTTTTCAGATTGAAAGCCTCATTTGTGATCCTCACAGCCATCTTGAAAGAAT
AGAGCAGCCAGTGGGTATACTGGATTGTGAGCTAAGAGGCCTGGGACTTTCCCCCTGTTGCTGCCAGCCA
GGTTGATGACCCTGGGCAAGTCTTTTTCCTTACCAGGTCTCAGTTTCCTCAGCTGTAAAATGAGAGGTTG
ATCTGGATCAGGGATAGTAAATGGGCCTTTGTTCAGTTACTGACTGTTGTATAACAAACCACCCCCAAAT
TTAGTAGCCTTAATAAACATTTATTAGCTCCTGAGTGAGTGGCTTGGCAGTTTGGGCTGTGTTCAGTGTG
GTGGTTCTTATCTCATCTGGGCCCTCGTGTGGCTGAAGTGAGCTGGCAGATCAGCTGGGGACTGGCTGGT
CTCAGATGGCCTCGCCCGCCTGTCAGGTGGTTGGCTGGGCAACTGGGCCACACGTTCCAGCATCTAGCAG
GCTGACCCAGGATTCTAGACATCATGGCTGAGTTCCAAAAACCGCAAGAGAAGACAAGCCCAGTATGCGA
GTGCTCTTGAGGATATTGCTATGACGTATCTTTTGGCCAAAGTAAGTCACATGGCTAAGCCCAAATTCTT
CTTAACAGGAGGAGCTTCAAAATATGGCCATATTTAAAAAATCTACCTCAGGACTCATTTCACTTGCCAA
CTCAGAACTTTTAGTAGCAGCTGCCTGGAGTGCCTTTTGAGGATCTGCAGCAATTTTCAGACTGTTTGGA
AATACTGACATGATAGTTTGGCAGTTTCTCTTATGAGCATGGAATTCAAGTGTGGGCACGTGAGCGTCAG
GTATTTGCCATCCCTGGCCAGGTTGACCCGTAAGGTCATAACCTGCCCCATAGGATGTTATATGCAGTAG
GCCTGTCTGACAAGACCACAGAAATAGAGAAGGCAAAGCAGAGCAAAAGATTCTGAGCCAGGCATCCTTG
GTTTTCATCCCAACCAGATCATTGATTGATTGAGGTTTTAGGCAAATCAAAATCCCTCCACCAGTAGCCA
GATTCTTCATAATAAACTTCCTCCATAACAGTAAACCATTTCCTGAACACCTGCTGTTAAAAAGCACATT
TTACATTTACTTAATCTCCACAACCACCCTCAGTAGGTGTAGTAAGGACTTCTGGTTACAAGTTACAGAA
ACTGAATTCAGTCCTGCTTAGTGGGAGGAAGTTTATTGGCTCACAAATTGTAAAGTCCAGGGATCTTCAG
ACATGGGGACTTGGGTTCAAAAGCTGCCATCGAGAATCCGGTTAGCCAGGCATGGTGACACGTGCCTGTA
GTCCCCGCTACTTGGGGGACTACAGTGGGAGATCATTTGCACCTAGGAGGTTAATGCTGCGGTGAGCCAT
GATCCTGCCGCTGCACTCCAGCCTGGGTGACAAAGCGAGACCCCAACTCAAGGGGAAAAAATCTGGTTTT
CGGGCAGGTTCCAAGAAGTGGTAGAGCATGGCCATAGGGCCTGTGAGAATAGAGGGACAGAGGGGAGAAT
CCCCAAAAGGAGAGCAAGGCTTGGCTAACAAAAACAGGCATCCACTGTGTGGTTATGTCTATTCTATAGA
TTTTGTAGATGAGGTTTAAAAGCATGGCCCCAAGTAAAGGTGGCATCTCTATCCTATCCTCGCCTTTATC
TAAACTCTGTGACCGATCAATAGCATATGATGGTGGTGGTGCTCTGCCAGTCTCCCAGTCCCAGACTGAA
GCACTAGCAGCTTCCACTTATTATCTCTTGGGACTCTAGCTCTTGGGACCCAACCACCATGCTGTGAAGC
AGCCCAGGCCACACAGAGAGGCCACGTGTACATGTTCAGCTGACAGCAGTATCAAATGTCAGACATGAGA
GAGGAAGGCTTCAAGATGACTCCAGCCCAGCCACCGTCTGAGAACAAAGTAAGAACTGCCTCACTGATGC
CATTCAAAGCCCAGCACCATGAGAGATAATAAAACGTTGTTTGAAGATGCTAA

SEQ ID NO: 11
TTCGTCCTGAGCGGCTATTT

SEQ ID NO: 12
TCGTCCTGAGCGGCTATTTC

SEQ ID NO: 13
GTAGTCGAAGAGCCTGTCGT

SEQ ID NO: 14
TATGACCTTACGGGTCAACC

SEQ ID NO: 15
ATGACCTTACGGGTCAACCT

SEQ ID NO: 16
TGTTATAGCTGGCTCTCGCC

SEQ ID NO: 17
TTTTGACACGCCGGACCAAA

SEQ ID NO: 18
TTTTGCTCCGCAGCGATCTG

SEQ ID NO: 19

AGACTTCCCGGTGGAGTTTG

SEQ ID NO: 20
ATCATCCGCGTCTGTGTCCT

SEQ ID NO: 21
GTTTTTGCTGCCCCGACTCA

SEQ ID NO: 22
CTGTCACTGGAGTACGCTCT

SEQ ID NO: 23
AGGGGCCGGGATATCTTCAT

SEQ ID NO: 24
TGGATTGGCCTTTTGCTCCG

SEQ ID NO: 25
AGTATACCCACTGGCTGCTC

SEQ ID NO: 26
GATCAACCTGACCCTCAAGC

SEQ ID NO: 27
AGAATCCGGTACCACATTTA

SEQ ID NO: 28
TATCCTCAAGAGCACTCGCA

SEQ ID NO: 29
TAGGCACCCTAGACCAGCTT

SEQ ID NO: 30
CATCCTATGGGGCAGGTTAT

SEQ ID NO: 31
GATGCTCATGGGCCAGCATA

SEQ ID NO: 32
TTTTTGCTGCCCCGACTCAG

SEQ ID NO: 33
ATTGCTGTCACTGGAGTACG

SEQ ID NO: 34
AATCCAGTATACCCACTGGC

SEQ ID NO: 35
GGACCTGCAGCAGGCATATA

SEQ ID NO: 36
TATGGTCACAAGCCCATGAT

SEQ ID NO: 37
GTGCTATTGTGCCCTCAGTG

SEQ ID NO: 38
TTTAGCCCCTTGGGTCTGTC

SEQ ID NO: 39
TAGTGCTATTGTGCCCTCAG

SEQ ID NO: 40
TTGTCAGACAGGCCTACTGC

SEQ ID NO: 41
GGCACCCTAGACCAGCTTTT

SEQ ID NO: 42
ATGCTCATGGGCCAGCATAT

SEQ ID NO: 43
TTTTGCTGCCCCGACTCAGA

SEQ ID NO: 44
TAGAGCTGATGCCTGCTACC

SEQ ID NO: 45
AGTTCACTGGACCAGGGTAG

SEQ ID NO: 46
AGGCACTCCTTGCAGACCTA

SEQ ID NO: 47
TCCTGAGCGGCTATTTCCCA

SEQ ID NO: 48
TATAGCTGGCTCTCGCCTTC

SEQ ID NO: 49
TAGGACCTGCAGCAGGCATA

SEQ ID NO: 50
AGTGCTATTGTGCCCTCAGT

SEQ ID NO: 51
GTGGCAGTCGATTCTCTCTT

SEQ ID NO: 52
TATACCCACTGGCTGCTCTA

SEQ ID NO: 53
CCTATGGCCATGCTCTACCA

SEQ ID NO: 54
ATTCTCACAGGCCCTATGGC

SEQ ID NO: 55
ACATGTACACGTGGCCTCTC

SEQ ID NO: 56
TTACTGCAAGGCCCCATCAT

SEQ ID NO: 57
TATTCTCACAGGCCCTATGG

SEQ ID NO: 58
CTTACTGCAAGGCCCCATCA

SEQ ID NO: 59
ATGTACACGTGGCCTCTCTG

SEQ ID NO: 60
TTGTTAGCCAAGCCTTGCTC

SEQ ID NO: 61
TAGCAGCTGTCCCCCATGTT

SEQ ID NO: 62
GTTCTCCAAGCCTGGCATAC

SEQ ID NO: 63
AAAGCCTTATGGGCCAAGGT

SEQ ID NO: 64
GTAGGCCAGTAACTCCACCT

SEQ ID NO: 65
ATGGGACTCTCGTTCTCCCT

SEQ ID NO: 66
CATGGTGCCCACCAGTAACA

SEQ ID NO: 67
CAGGCCTCTTAGCTCACAAT

SEQ ID NO: 68
CTGTGTCAGCTCCGTCTTGA

SEQ ID NO: 69
AAGCTGCTAGTGCTTCAGTC

INCORPORATION BY REFERENCE

Each publication and patent mentioned herein is hereby incorporated by reference in its entirety. In case of conflict, the present specification, including any definitions herein, will control.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of the preceding description and the following claims. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and by reference to the rest of the specification, along with such variations.

CLAIMS

What is claimed is:

1. An mRNA-silencing nucleic acid molecule comprising an antisense strand that comprises a nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead.

2. The mRNA-silencing nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a single-stranded oligonucleotide, and wherein said antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69.

3. The mRNA-silencing nucleic acid molecule of claim 1 or 2, wherein said antisense strand comprises, linking each pair of neighboring sugars, a monothiophosphate group in which the sulfur atom is at a non-bridging position.

4. The mRNA-silencing nucleic acid molecule of any one of claims 1 to 3, wherein said antisense strand comprises 2'-O-(2-methoxyethyl)ribose groups as the sugars for residues 1–5 and 16–20, and 2'-deoxyribose groups as the sugars for residues 6–15 with respect to any one of SEQ ID NOs: 1–3 or 11–69.

5. The mRNA-silencing nucleic acid molecule of any one of claims 1 to 4 for use in the treatment of a cardiometabolic condition of a subject, wherein the mRNA-silencing nucleic acid molecule acts as a down-regulator of a heterogeneous nuclear ribonucleoprotein.

6. The use according to claim 5, wherein the heterogeneous nuclear ribonucleoprotein comprises RNA-binding protein Raly.

7. The use according to claim 5 or 6, wherein the cardiometabolic condition comprises non-alcoholic fatty liver, non-alcoholic steatohepatitis, dyslipidemia, obesity, inflammation, or atherosclerosis.

8. The mRNA-silencing nucleic acid molecule of any one of claims 1 to 4 for use in lowering a cholesterol level in a subject, wherein the mRNA-silencing nucleic acid molecule acts as a down-regulator of a heterogeneous nuclear ribonucleoprotein.

9. The use according to claim 8, wherein the down-regulator attenuates or prevents translation of messenger RNA of RNA-binding protein Raly.

10. The use according to claim 8 or 9, wherein the cholesterol level comprises the level of cholesterol in serum LDL.

11. The use according to claim 8 or 9, wherein the cholesterol level comprises the level of cholesterol in hepatocytes of the subject.

12. The use according to any one of claims 8 to 11, wherein the lowering of said cholesterol level is accompanied by a lowering of an inflammation level in cells of the subject.

13. The mRNA-silencing nucleic acid molecule of any one of claims 1 to 4 for use in lowering expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase, wherein the mRNA-silencing nucleic acid molecule acts as a down-regulator of a heterogeneous nuclear ribonucleoprotein.

14. The mRNA-silencing nucleic acid molecule of any one of claims 1 to 4 for use in lowering expression of a gene coding for sterol regulatory element-binding protein 2, wherein the mRNA-silencing nucleic acid molecule acts as a down-regulator of a heterogeneous nuclear ribonucleoprotein.

15. The use according to claim 14, wherein the down-regulator lowers expression of a gene coding for sterol regulatory element-binding protein 2 by reducing amount of nuclear transcription factor Y bound to RNA-binding protein Raly.

16. The use according to any one of claims 5 to 16, wherein the subject is a human.

17. A method of treating a cardiometabolic condition of a subject, the method comprising administering to the subject an effective amount of a down-regulator of a heterogeneous nuclear ribonucleoprotein.

18. The method of claim 17, wherein the heterogeneous nuclear ribonucleoprotein comprises RNA-binding protein Raly.

19. The method of claim 18, wherein the cardiometabolic condition comprises non-alcoholic fatty liver, non-alcoholic steatohepatitis, dyslipidemia, obesity, inflammation, or atherosclerosis.

20. The method of any one of claims 17 to 19, wherein the down-regulator comprises an mRNA-silencing nucleic acid molecule comprising an antisense strand that comprises a nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead.

21. The method of claim 20, wherein the nucleic acid molecule is a single-stranded oligonucleotide, and wherein said antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3.

22. The method of claim 20, wherein the nucleic acid molecule comprises

a small interfering RNA (siRNA) having

a guide strand that comprises said antisense strand, wherein said antisense strand comprises said nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead; and

a passenger strand that is complementary to the guide strand with respect to at least 10 and at most 30 residues;

a short hairpin RNA (shRNA) that is a precursor to said siRNA; or

a double-stranded DNA that is a precursor to said shRNA or to said siRNA.

23. The method of any one of claims 17 to 19, wherein the down-regulator comprises an antibody, an antigen-biding fragment thereof, or a small molecule.

24. The method of any one of claims 17 to 23, wherein the subject is a human.

25. The method of any one of claims 17 to 24, comprising administering the down-regulator to the subject using a mode of administration that delivers the down-regulator to one or more hepatocytes of the subject.

26. A method of lowering a cholesterol level in a subject, the method comprising administering to the subject an effective amount of a down-regulator of RNA-binding protein Raly.

27. The method of claim 26, wherein the down-regulator comprises an mRNA-silencing nucleic acid molecule comprising an antisense strand that comprises a nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead.

28. The method of claim 27, wherein the nucleic acid molecule comprises

a single-stranded oligonucleotide, wherein said antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3;

a small interfering RNA (siRNA) having

a guide strand that comprises said antisense strand, wherein said antisense strand comprises said nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead; and

a passenger strand that is complementary to the guide strand with respect to at least 10 and at most 30 residues;

a short hairpin RNA (shRNA) that is a precursor to said siRNA; or

a double-stranded DNA that is a precursor to said shRNA or to said siRNA.

29. The method of claim 27 or 28, wherein the down-regulator attenuates or prevents translation of messenger RNA of RNA-binding protein Raly.

30. The method of claim 26, wherein the down-regulator comprises an antibody, an antigen-biding fragment thereof, or a small molecule.

31. The method of any one of claims 26 to 30, wherein the cholesterol level comprises the level of cholesterol in serum LDL.

32. The method of any one of claims 26 to 30, wherein the cholesterol level comprises the level of cholesterol in hepatocytes of the subject.

33. The method of any one of claims 26 to 32, wherein the lowering of said cholesterol level is accompanied by a lowering of an inflammation level in cells of the subject.

34. A method of lowering expression of a gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the method comprising administering to the subject an effective amount of a down-regulator of RNA-binding protein Raly.

35. The method of claim 34, wherein the down-regulator comprises an mRNA-silencing nucleic acid molecule comprising an antisense strand that comprises a nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead.

36. The method of claim 35, wherein the nucleic acid molecule comprises

a single-stranded oligonucleotide, wherein said antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3;

a small interfering RNA (siRNA) having

a guide strand that comprises said antisense strand, wherein said antisense strand comprises said nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead; and

a passenger strand that is complementary to the guide strand with respect to at least 10 and at most 30 residues;

a short hairpin RNA (shRNA) that is a precursor to said siRNA; or

a double-stranded DNA that is a precursor to said shRNA or to said siRNA.

37. The method of claim 34, wherein the down-regulator comprises an antibody, an antigen-biding fragment thereof, or a small molecule.

38. A method of lowering expression of a gene coding for sterol regulatory element-binding protein 2, the method comprising administering to the subject an effective amount of a down-regulator of RNA-binding protein Raly.

39. The method of claim 38, wherein the down-regulator comprises an mRNA-silencing nucleic acid molecule comprising an antisense strand that comprises a nucleobase sequence

that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead.

40. The method of claim 39, wherein the nucleic acid molecule comprises

a single-stranded oligonucleotide, wherein said antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3;

a small interfering RNA (siRNA) having

a guide strand that comprises said antisense strand, wherein said antisense strand comprises said nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead; and

a passenger strand that is complementary to the guide strand with respect to at least 10 and at most 30 residues;

a short hairpin RNA (shRNA) that is a precursor to said siRNA; or

a double-stranded DNA that is a precursor to said shRNA or to said siRNA.

41. The method of claim 38, wherein the down-regulator comprises an antibody, an antigen-biding fragment thereof, or a small molecule.

42. The method of any one of claims 38 to 41, wherein the down-regulator lowers expression of a gene coding for sterol regulatory element-binding protein 2 by reducing amount of nuclear transcription factor Y bound to RNA-binding protein Raly.

43. An mRNA-silencing nucleic acid molecule comprising an antisense strand that comprises a nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which none, all, or some but less than all of the thymine nucleobases are uracil nucleobases instead.

44. The mRNA-silencing nucleic acid molecule of claim 43, wherein the nucleic acid molecule is a single-stranded oligonucleotide, and wherein said antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3.

45. The mRNA-silencing nucleic acid molecule of claim 43 or 44, wherein said antisense strand comprises, linking each pair of neighboring sugars, a monothiophosphate group in which the sulfur atom is at a non-bridging position.

46. The mRNA-silencing nucleic acid molecule of any one of claims 43 to 45, wherein said antisense strand comprises 2'-O-(2-methoxyethyl)ribose groups as the sugars for residues 1–5 and 16–20, and 2'-deoxyribose groups as the sugars for residues 6–15 with respect to any one of SEQ ID NOs: 1–3.

47. The mRNA-silencing nucleic acid molecule of any one of claims 43 to 46, wherein the nucleic acid molecule is a gapmer antisense oligonucleotide comprising the sequence of any one of SEQ ID NOs: 7–9.

48. The mRNA-silencing nucleic acid molecule of claim 43, wherein the nucleic acid molecule comprises

a small interfering RNA (siRNA) having

a guide strand that comprises said antisense strand, wherein said antisense strand comprises said nucleobase sequence that is at least 90% identical with the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead; and

a passenger strand that is complementary to the guide strand with respect to at least 10 and at most 30 residues;

a short hairpin RNA (shRNA) that is a precursor to said siRNA; or

a double-stranded DNA that is a precursor to said shRNA or to said siRNA.

49. The mRNA-silencing nucleic acid molecule of claim 48, wherein said guide strand comprises the nucleobase sequence of any one of SEQ ID NOs: 1–3 or 11–69 in which all of the thymine nucleobases are uracil nucleobases instead, and wherein both the guide strand and the passenger strand comprise a 2-nucleotide 3'-overhang.

50. The mRNA-silencing nucleic acid molecule of claim 49, wherein at least one nucleobase, sugar, phosphate group, or a combination thereof is a non-canonical group instead.

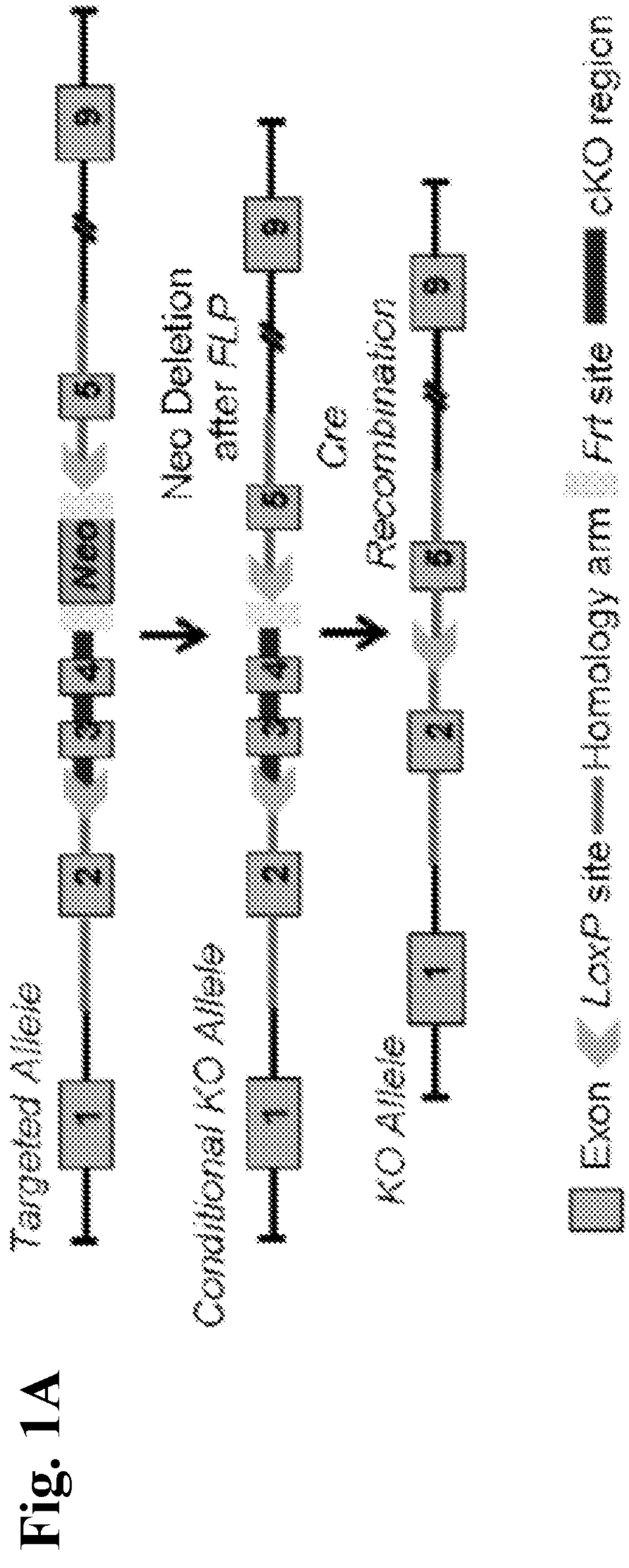


Fig. 1A

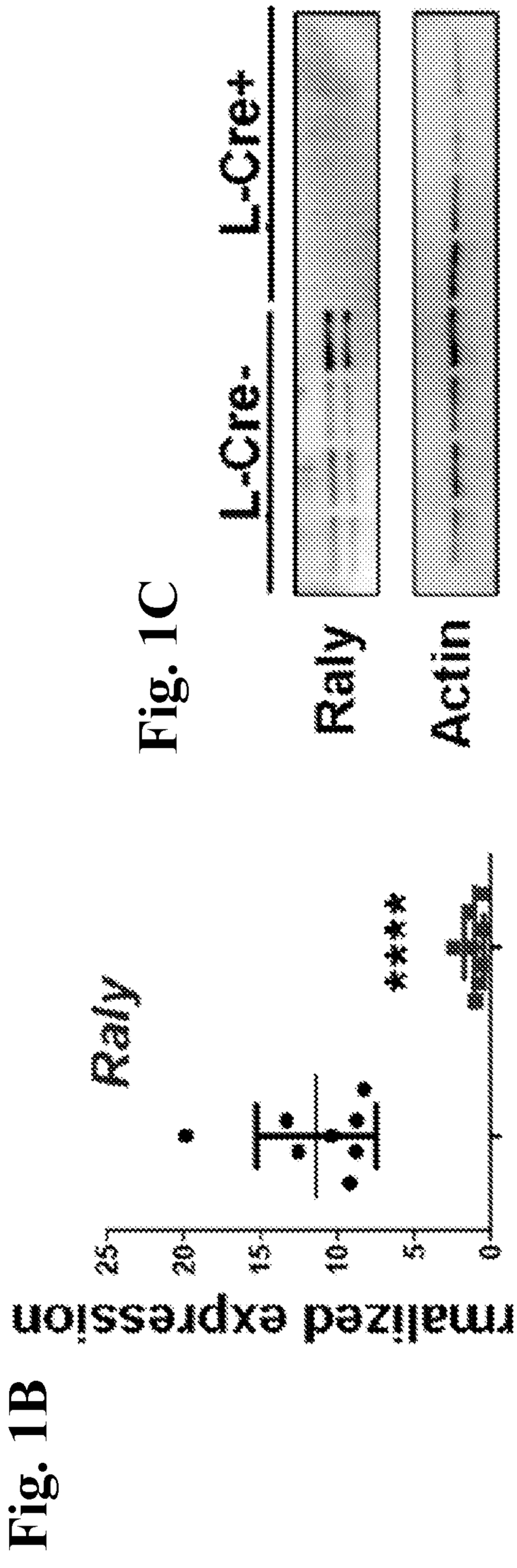


Fig. 1C

Fig. 1B

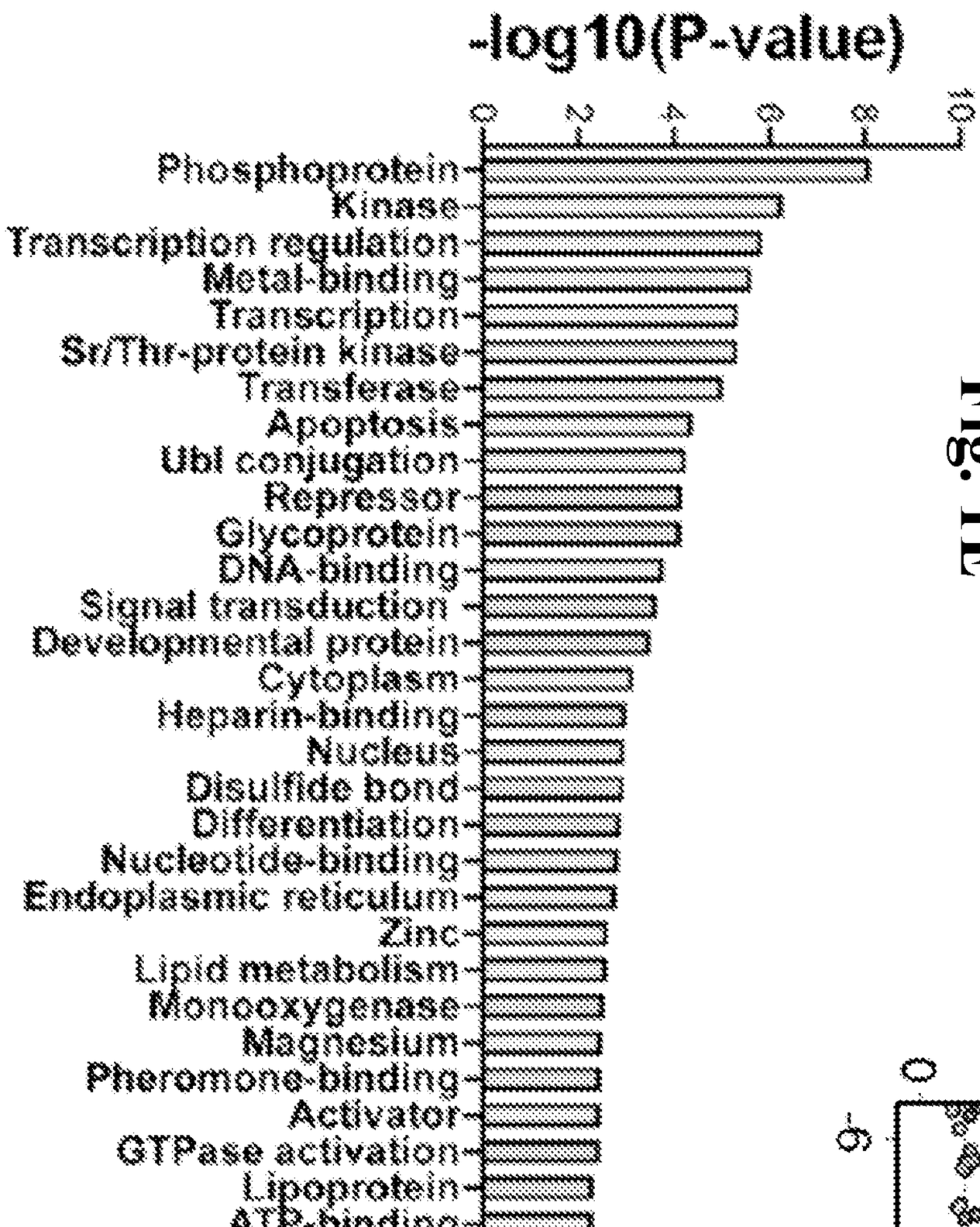

-log10(P-value)
0
2
4
6
8
10
Phosphoprotein
Kinase
Transcription regulation
Metal-binding
Transcription
Sr/Thr-protein kinase
Transferase
Apoptosis
Ubl conjugation
Repressor
Glycoprotein
DNA-binding
Signal transduction
Developmental protein
Cytoplasm
Heparin-binding
Nucleus
Disulfide bond
Differentiation
Nucleotide-binding
Endoplasmic reticulum
Zinc
Lipid metabolism
Monooxygenase
Magnesium
Pheromone-binding
Activator
GTPase activation
Lipoprotein
ATP-binding
Secreted
Unfolded protein response
Neurogenesis
Serine protease inhibitor
Alternative splicing
Cyclin
Heme
Golgi apparatus
Protease inhibitor
Palmitate
Angiogenesis
Gluconeogenesis
Steroid metabolism
Inflammatory response
Microsome
Lipid droplet
Zinc-finger
Signal
Cholesterol metabolism
GPI-anchor
Iron
Antiport
Sterol metabolism

Fig. 1E

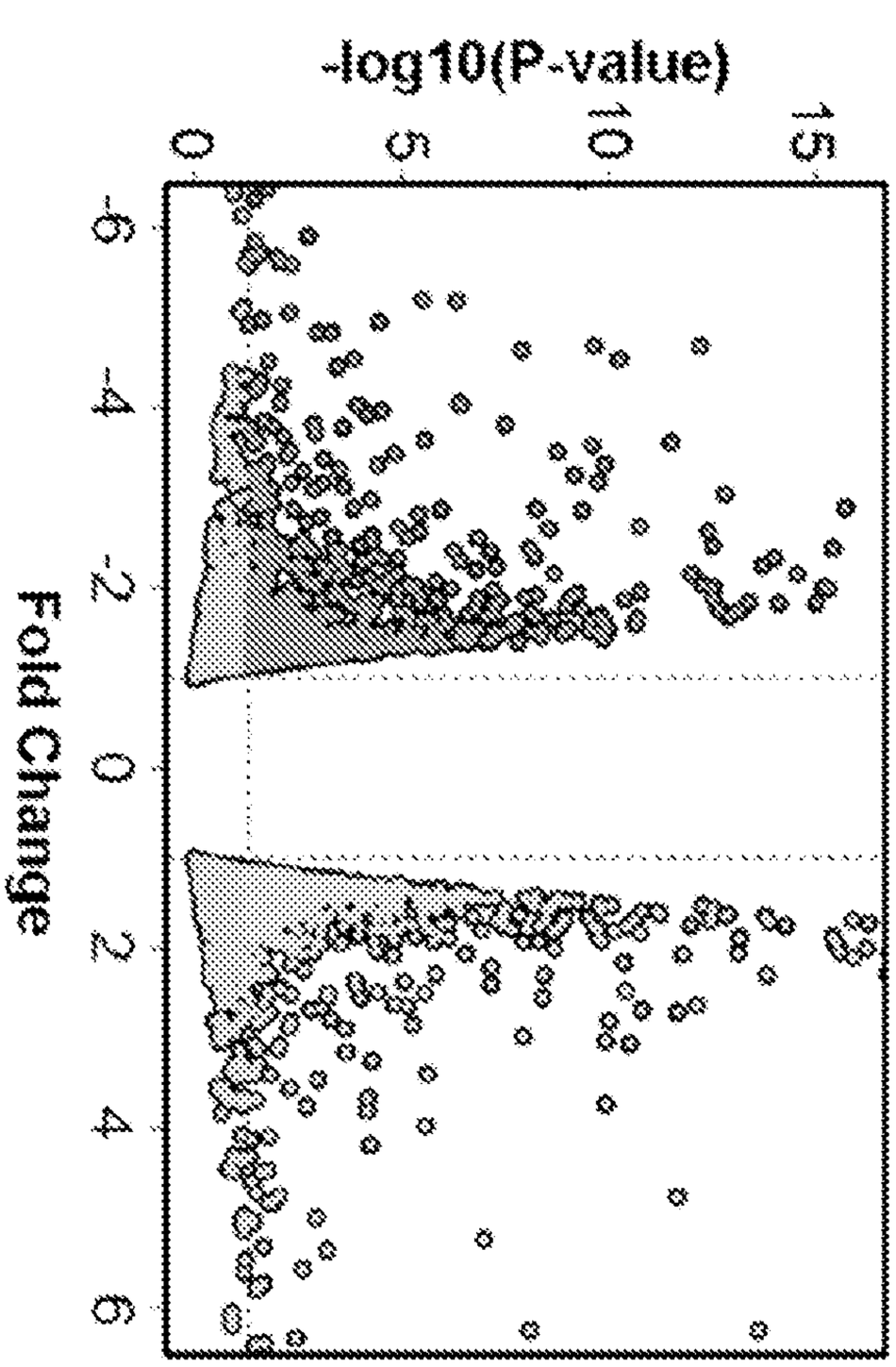

-log10(P-value)
0
5
10
15
Fold Change
6
4
2
0
2
4
6

Fig. 1D

Fig. 1F

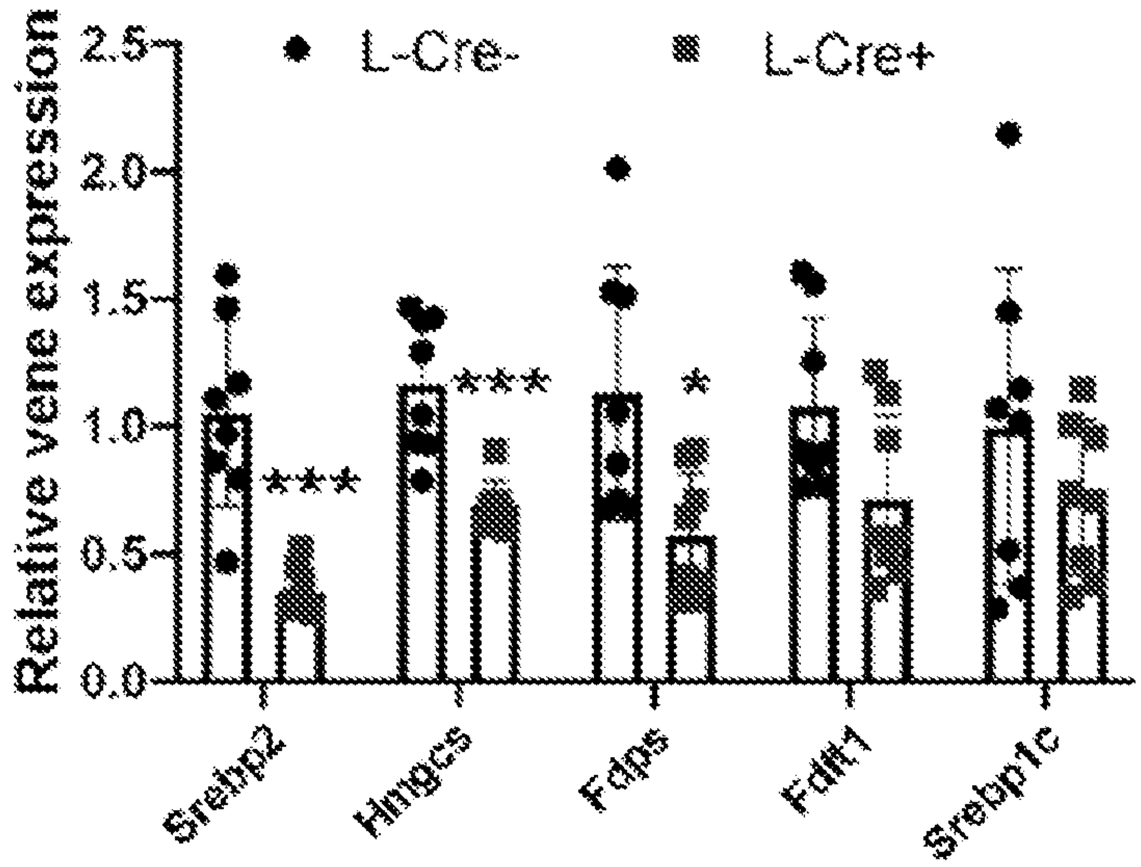


Fig. 1G

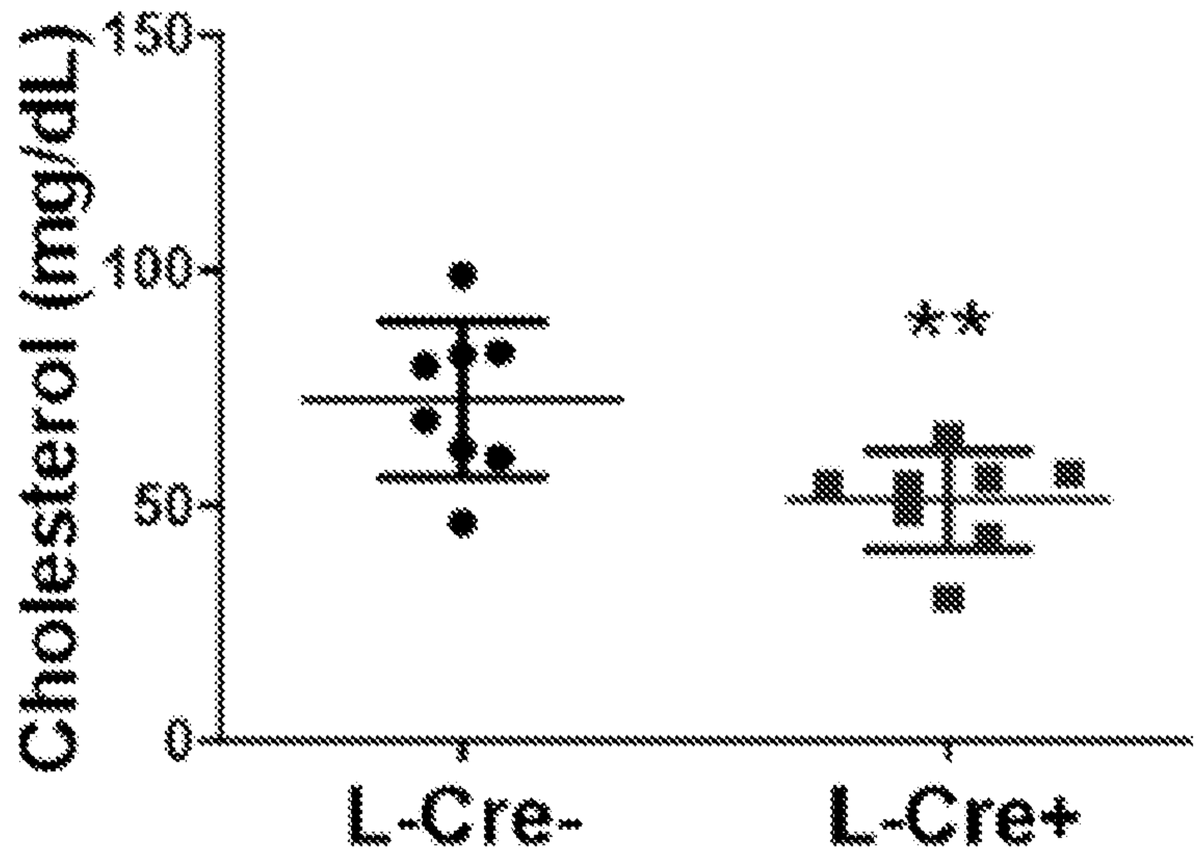
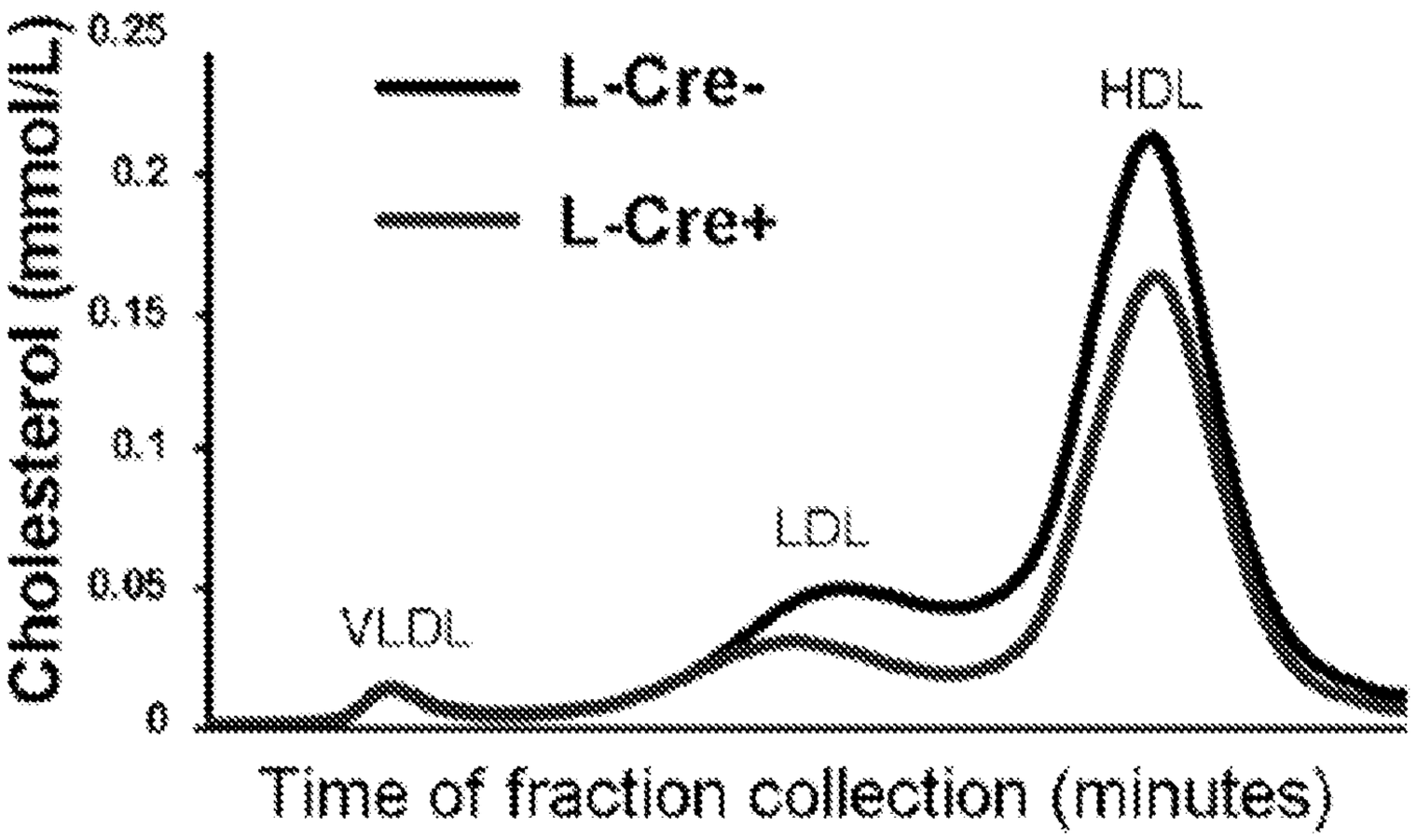


Fig. 1H



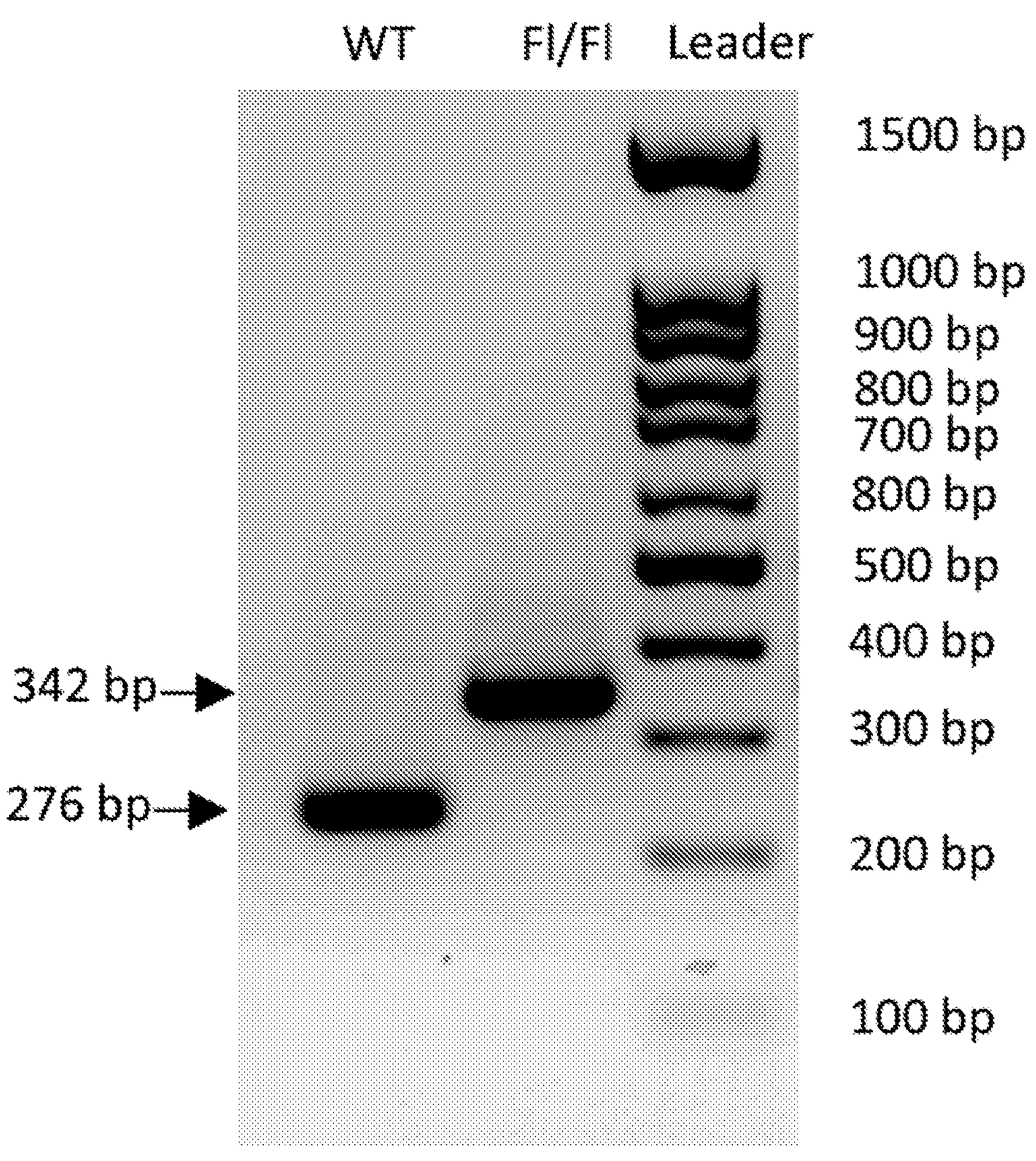

WT
Fl/Fl
Leader
1500 bp
1000 bp
900 bp
800 bp
700 bp
800 bp
500 bp
400 bp
342 bp
300 bp
276 bp
200 bp
100 bp

Fig. 2

Fig. 3A

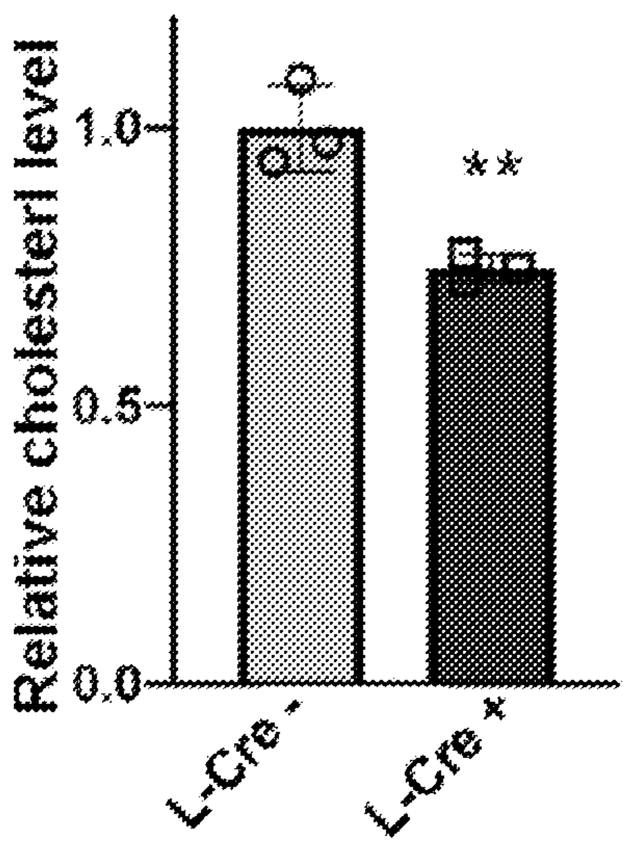


Fig. 3B

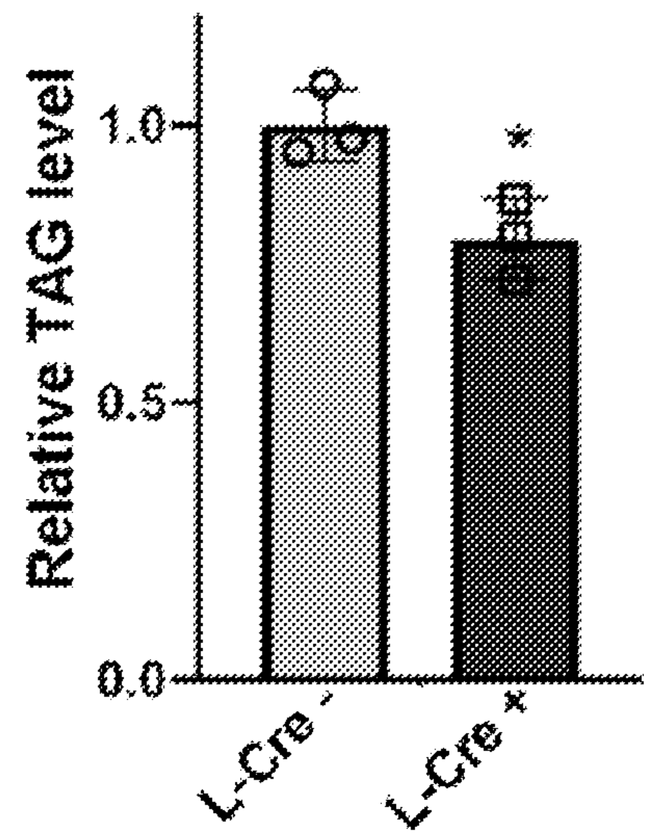


Fig. 3C

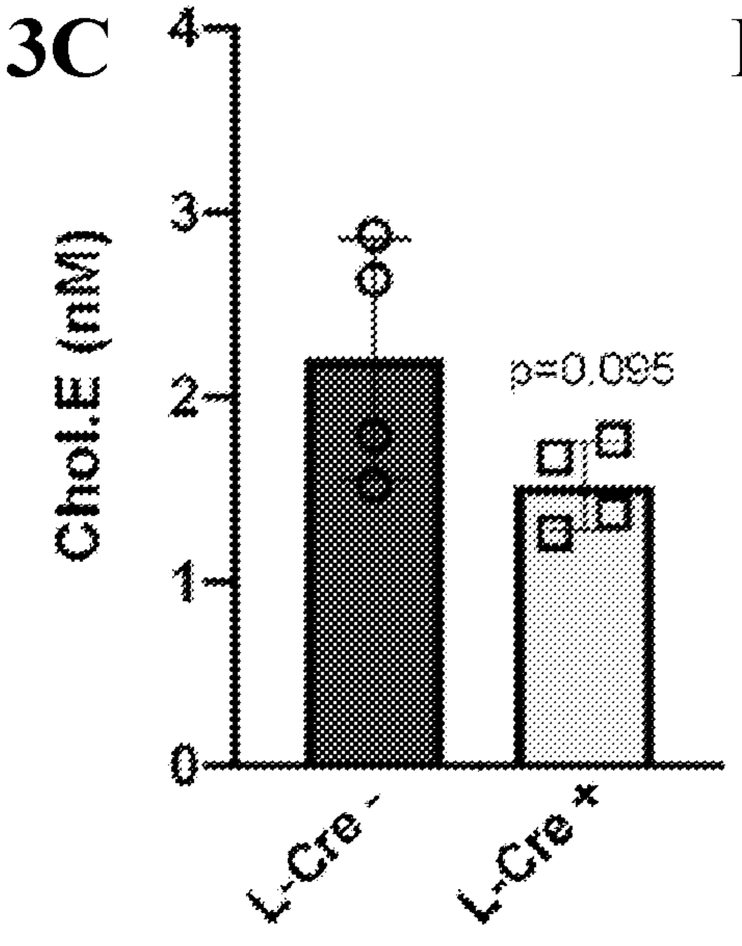


Fig. 3D

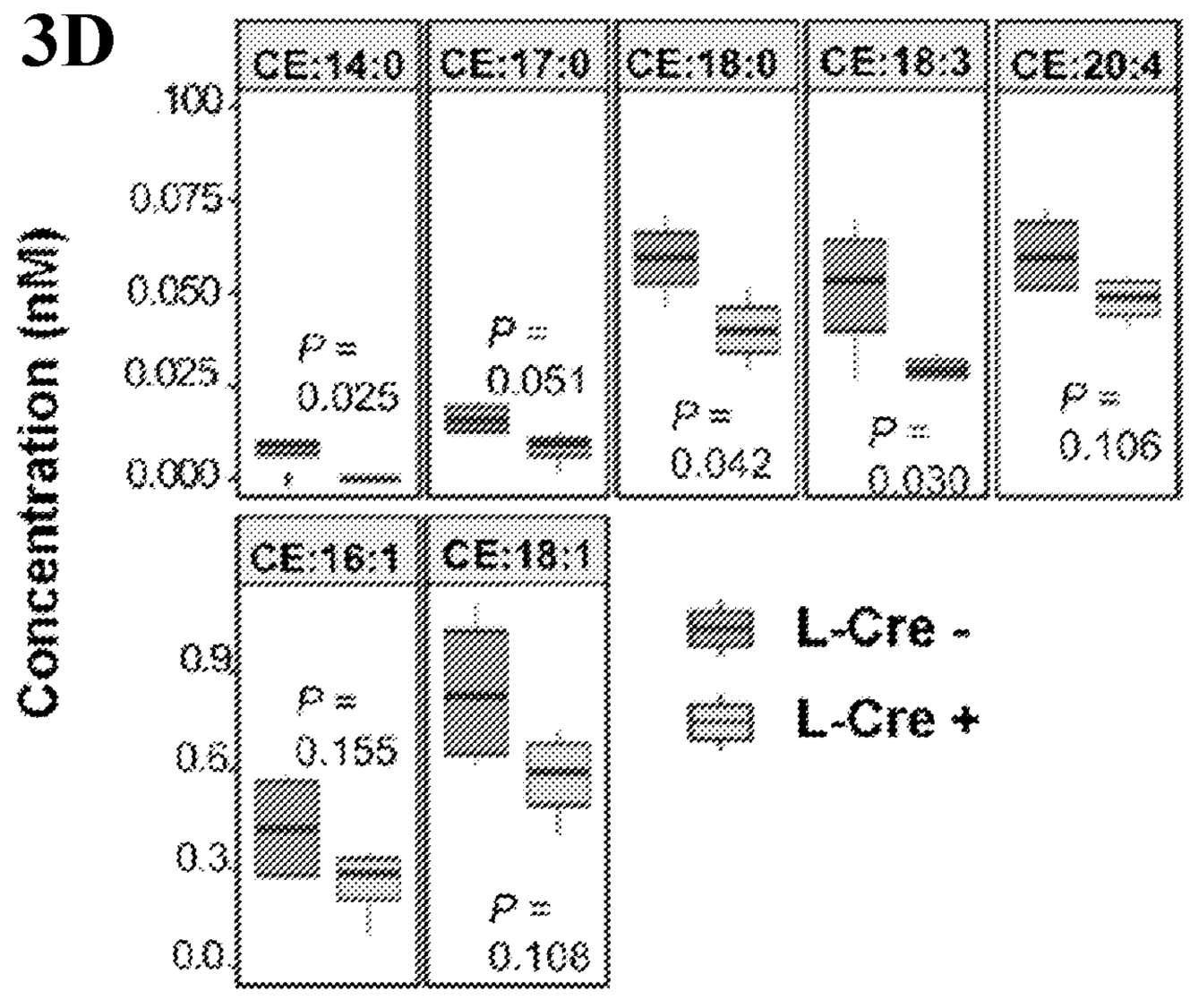


Fig. 3E

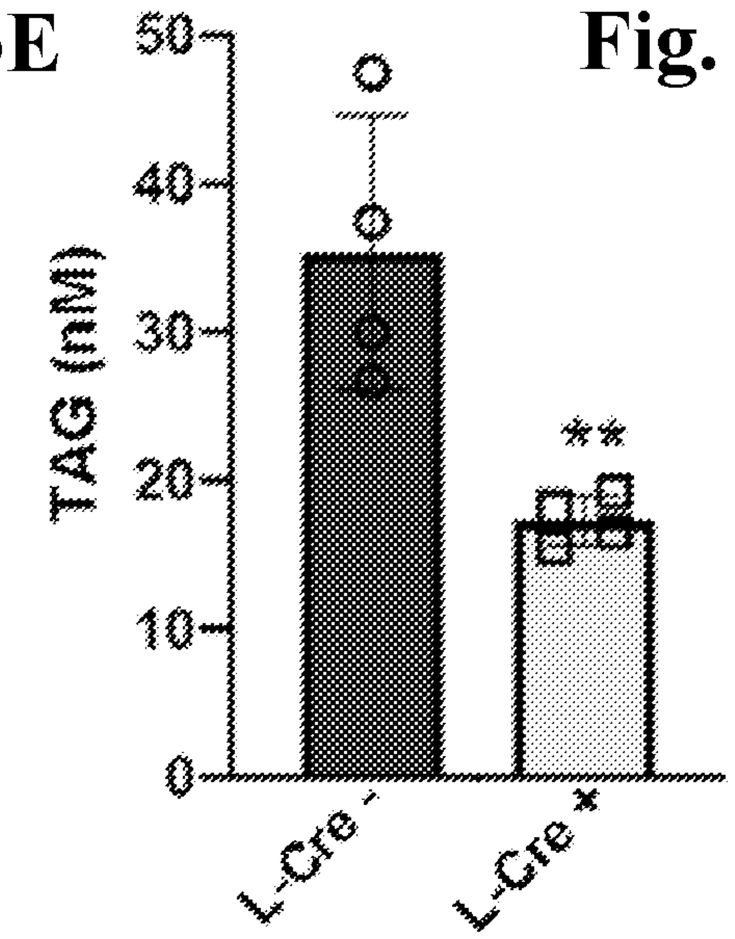


Fig. 3F

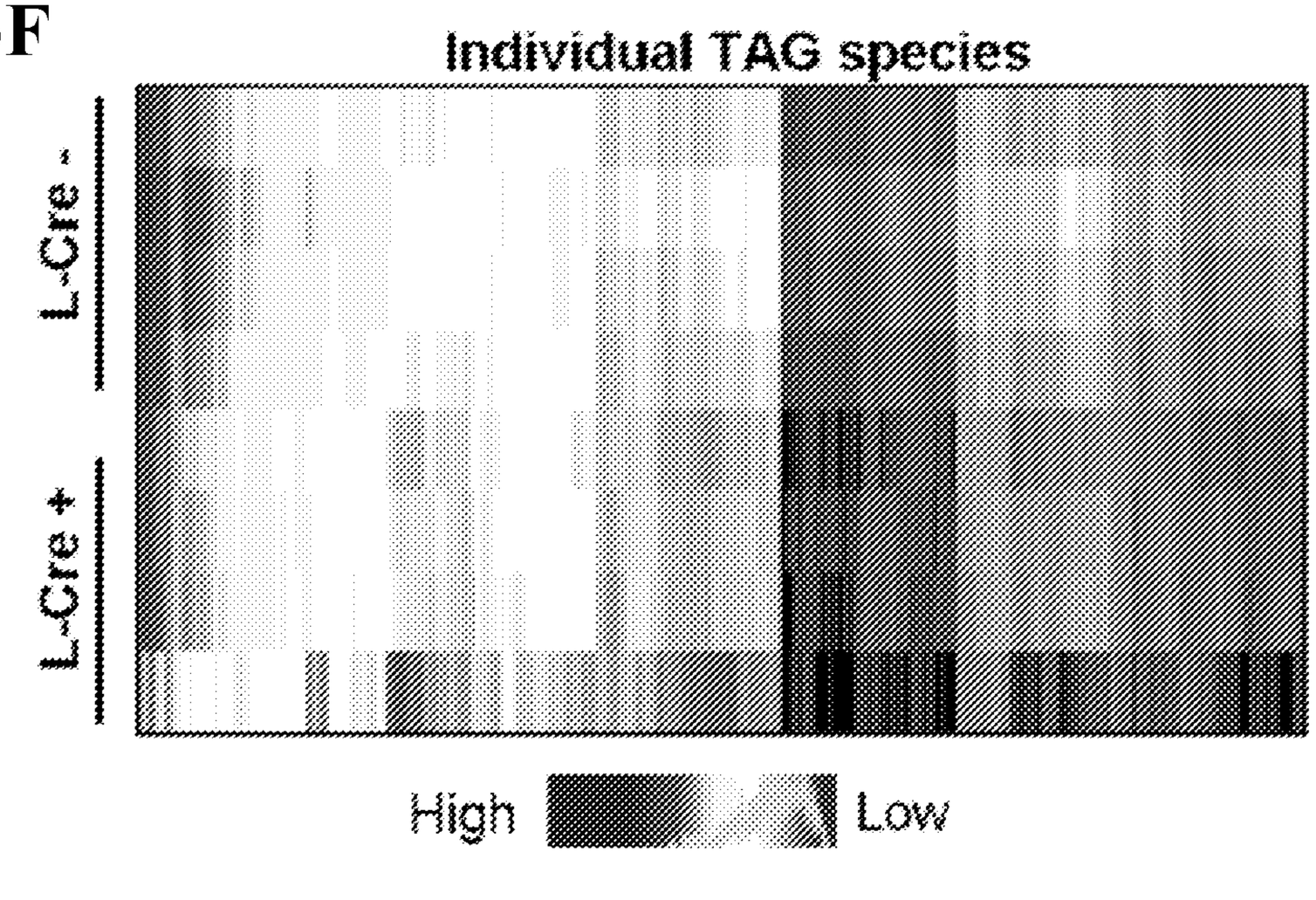


Fig. 3G

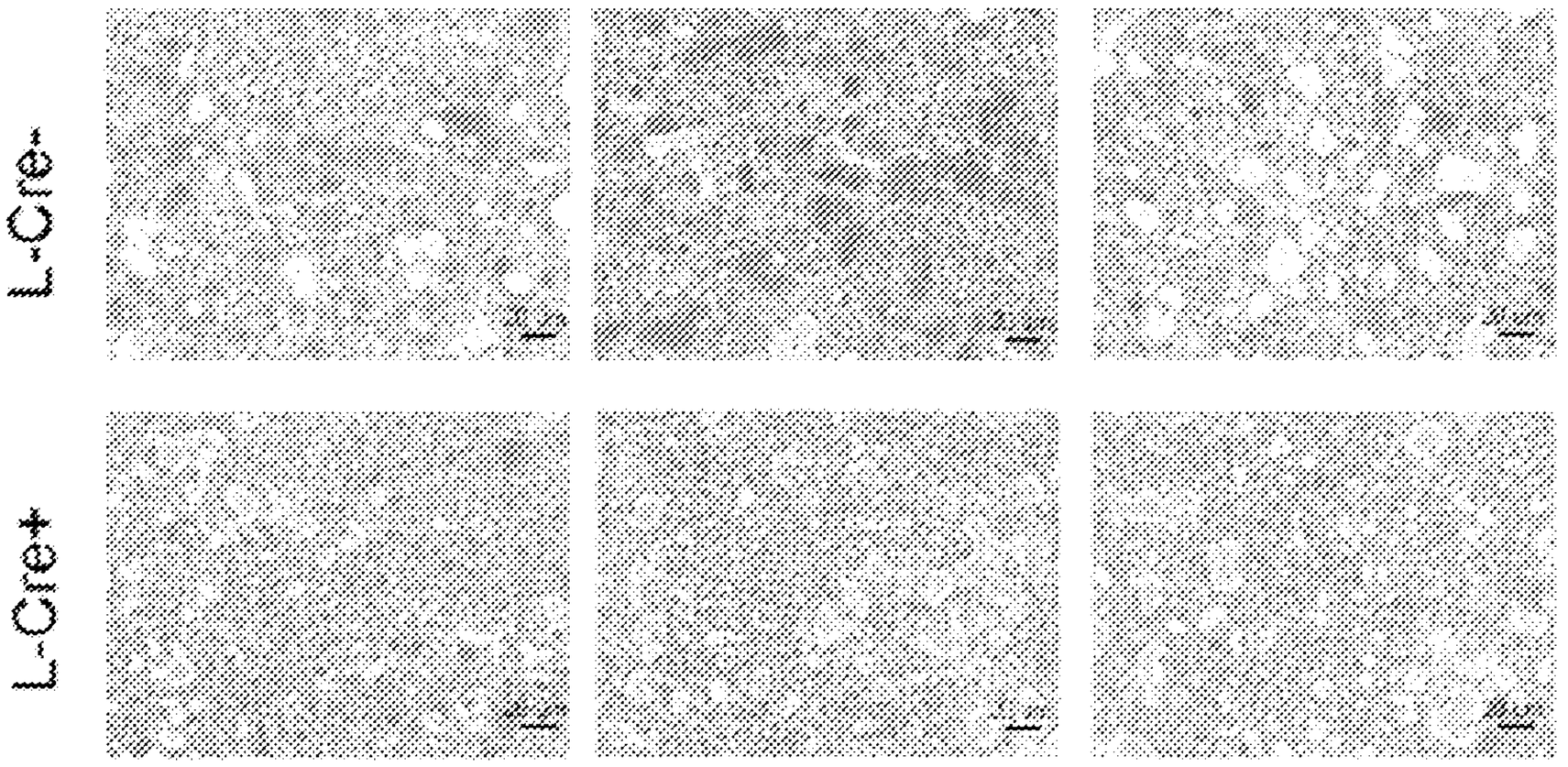


Fig. 3H

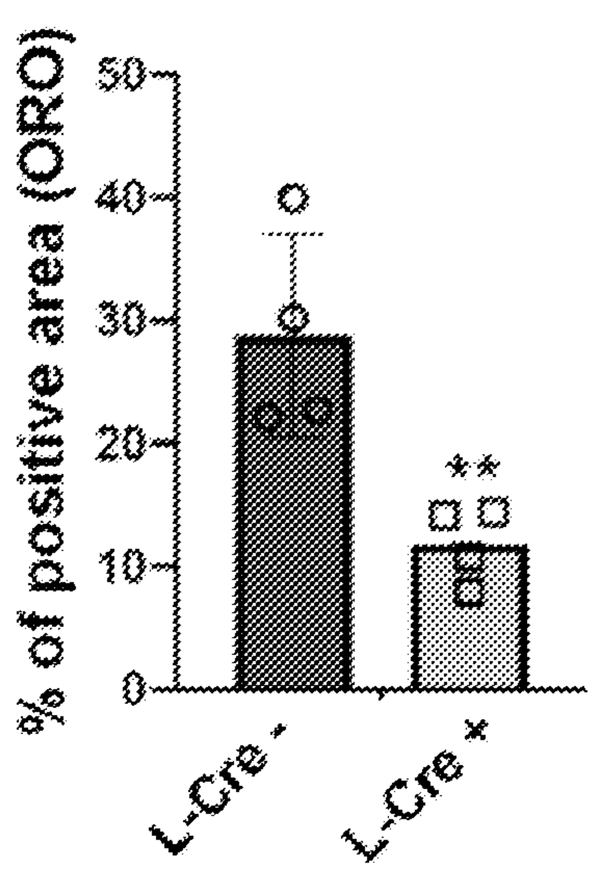


Fig. 4A

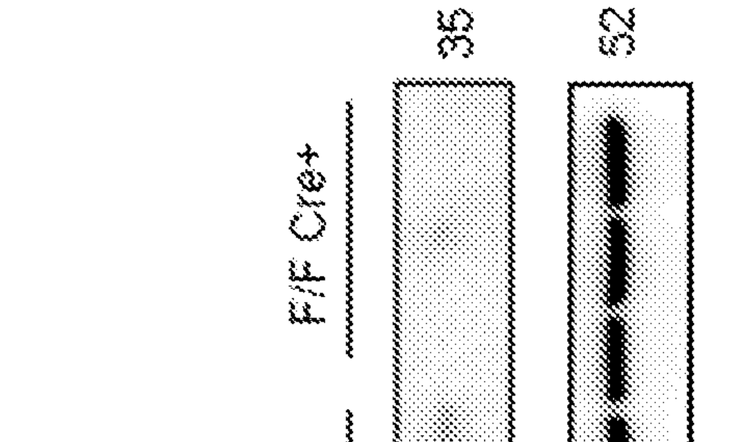


Fig. 4B

Fig. 4C

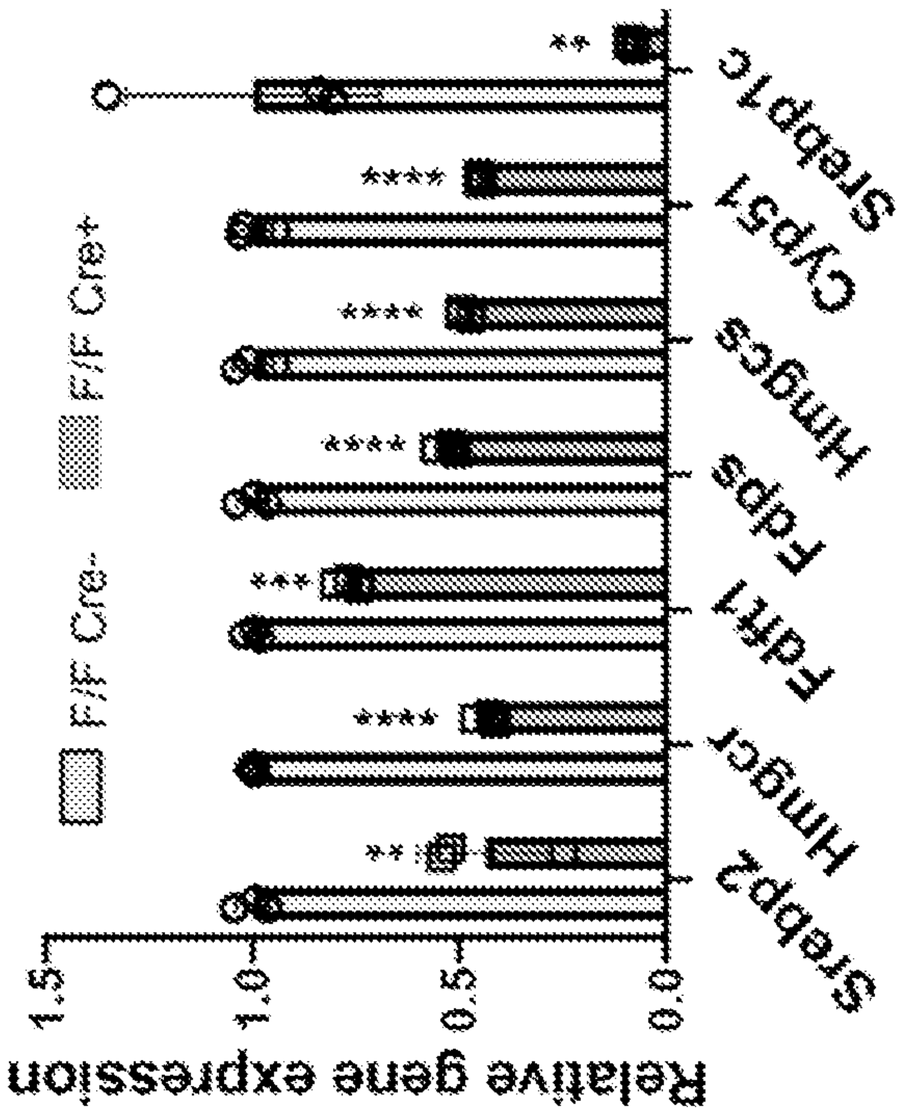


Fig. 4D

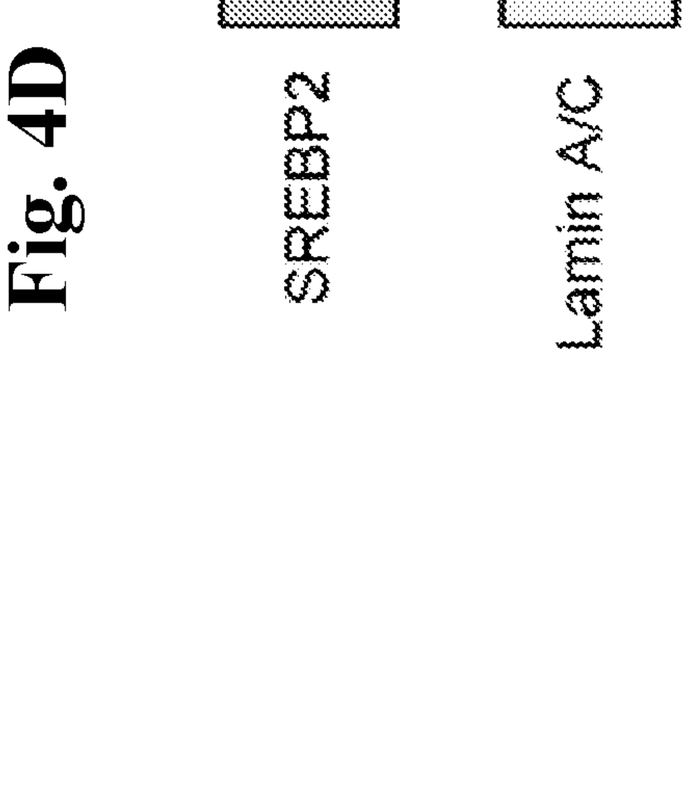
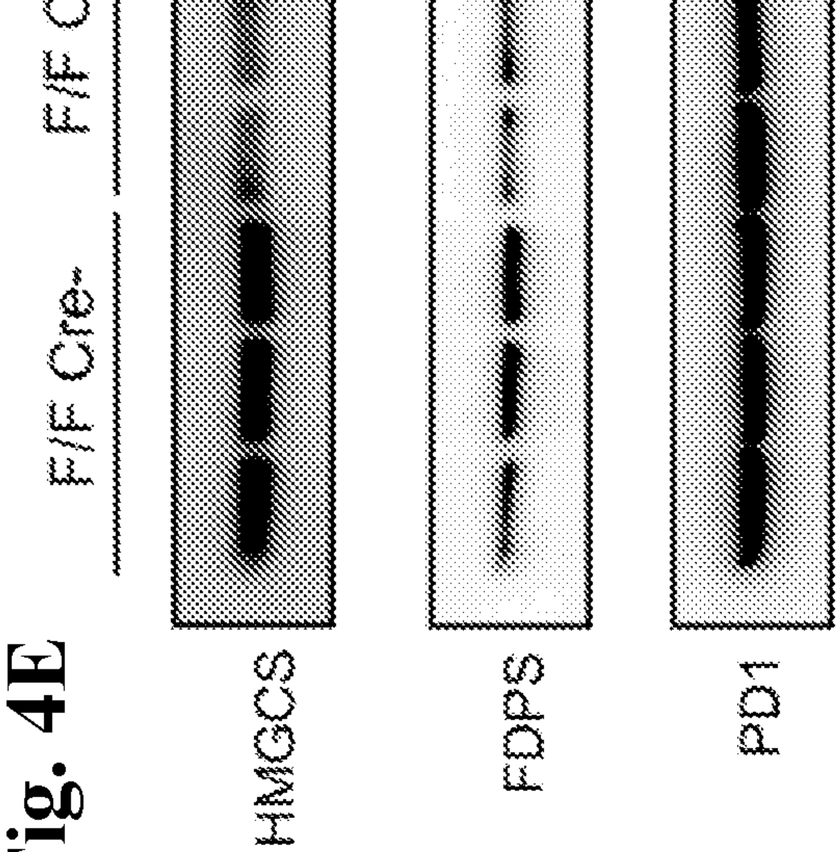


Fig. 4E



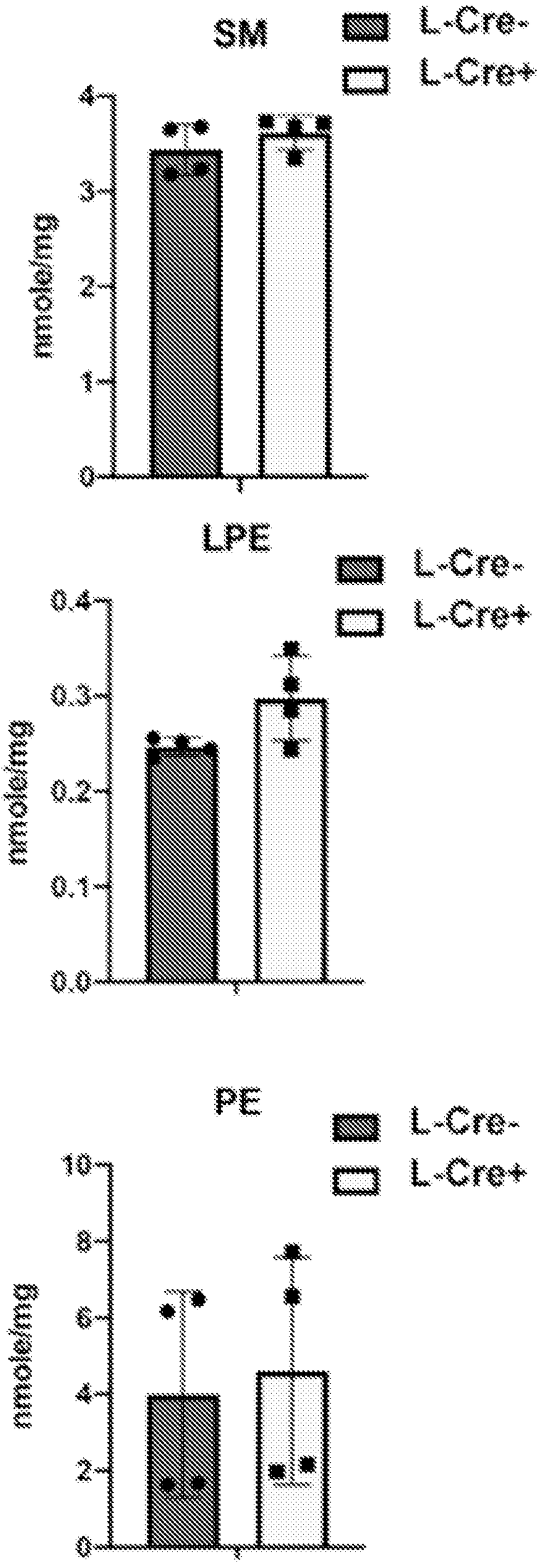

SM
L-Cre-
L-Cre+
nmole/mg
0
1
2
3
4
LPE
PE
nmole/mg
0.0
0.1
0.2
0.3
0.4
PS
nmole/mg
0
2
4
6
8
10
CER
L-Cre-
L-Cre+
nmole/mg
0.00
0.05
0.10
0.15
0.20
0.25
LPC
L-Cre-
L-Cre+
nmole/mg
0.0
0.2
0.4
0.6
0.8
PC
L-Cre-
L-Cre+
nmole/mg
0
10
20
30
PI
nmole/mg
0
2
4
6
8
10

Fig. 5

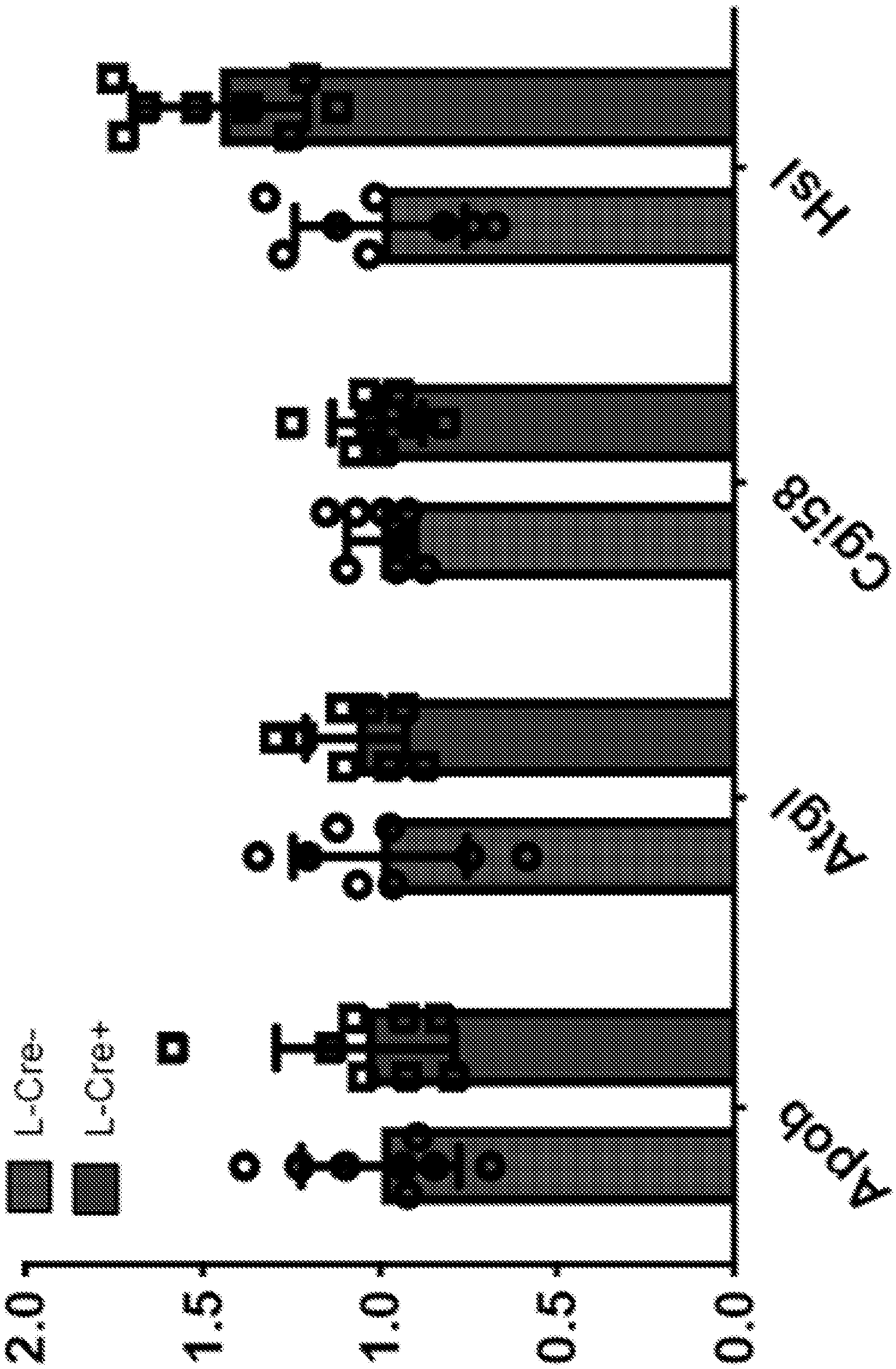

Relative gene expression
2.0
1.5
1.0
0.5
0.0
L-Cre-
L-Cre+
Apob
Atgl
Cpt2
Fasn

Fig. 6

Fig. 7A

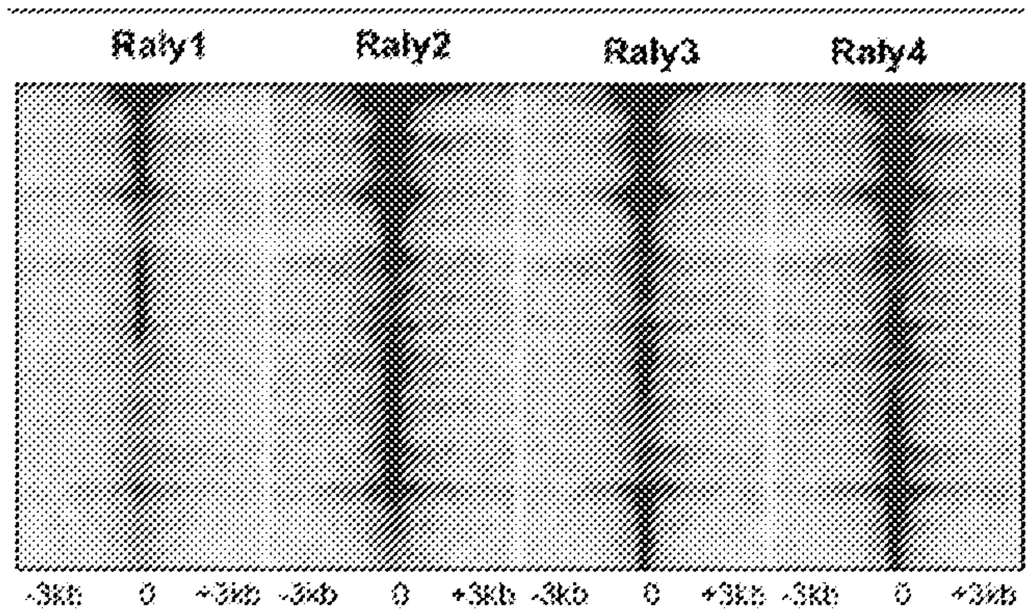


Fig. 7B

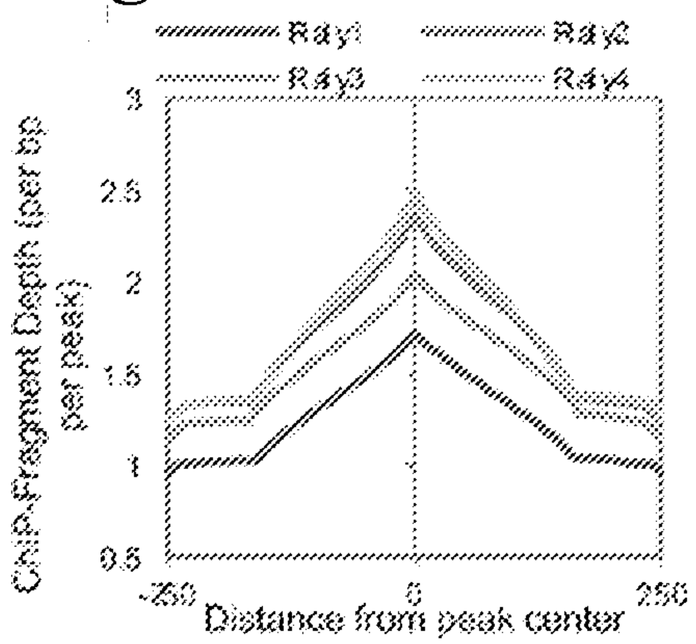
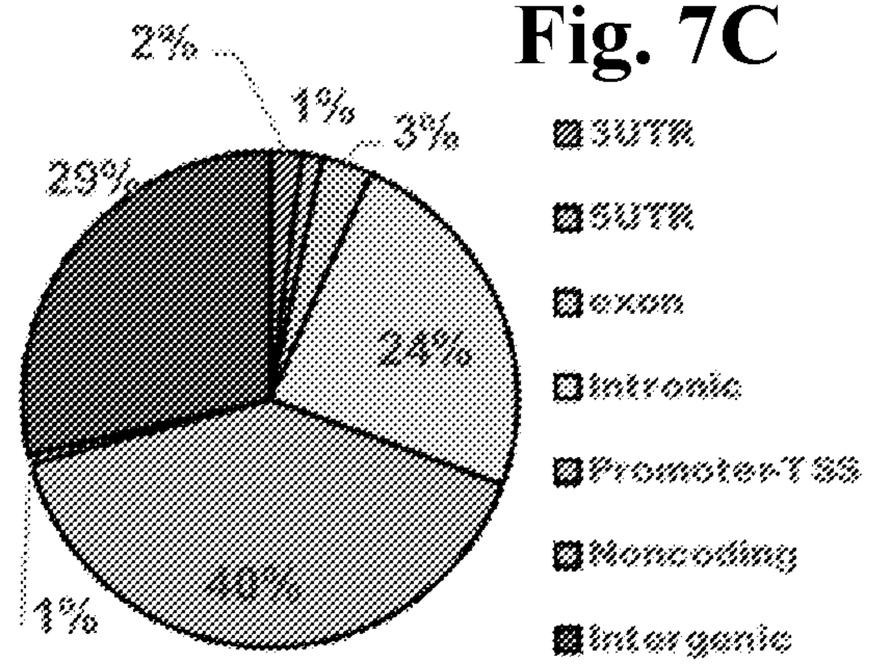


Fig. 7C



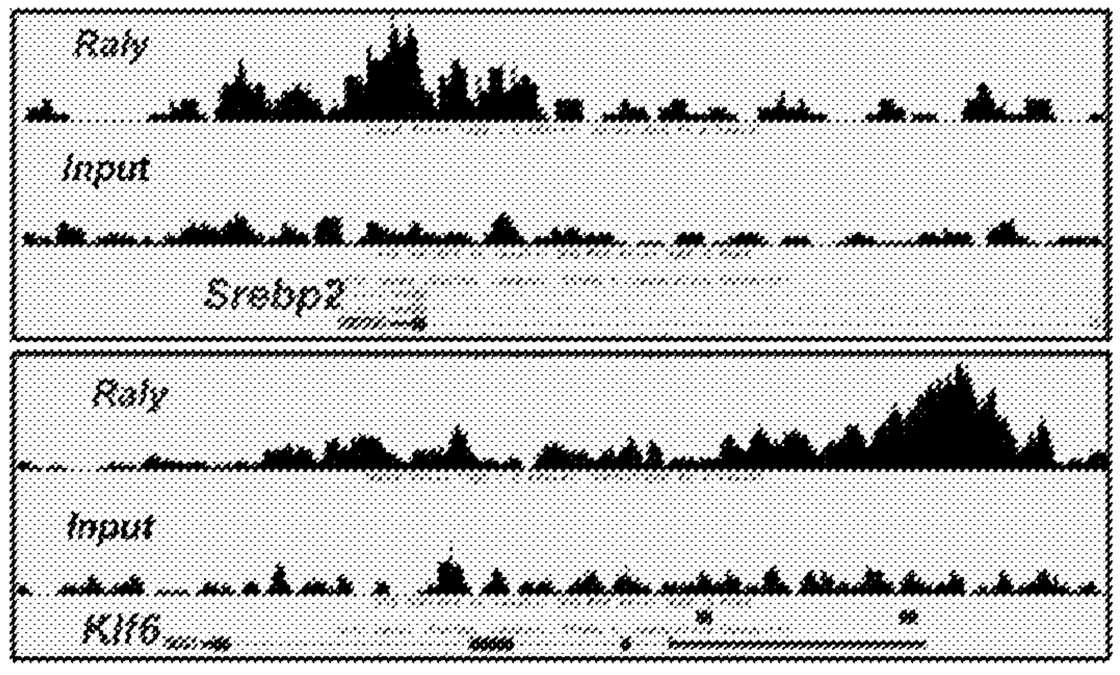


Fig. 7D

Name	Motif ID	P value
AR	MA0007.1	8.12e-33
Pax4	MA0068.1	3.23e-24
NFYA	MA0060.2	4.37e-13

Fig. 7E

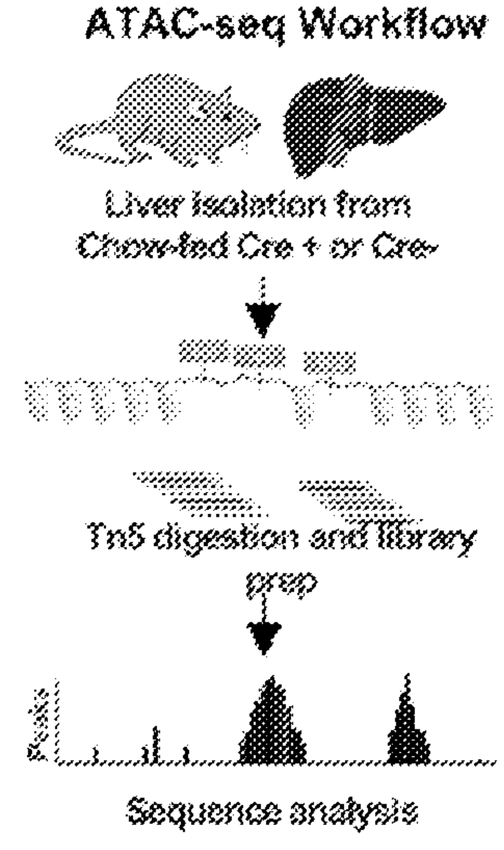


Fig. 7F

Fig. 7G

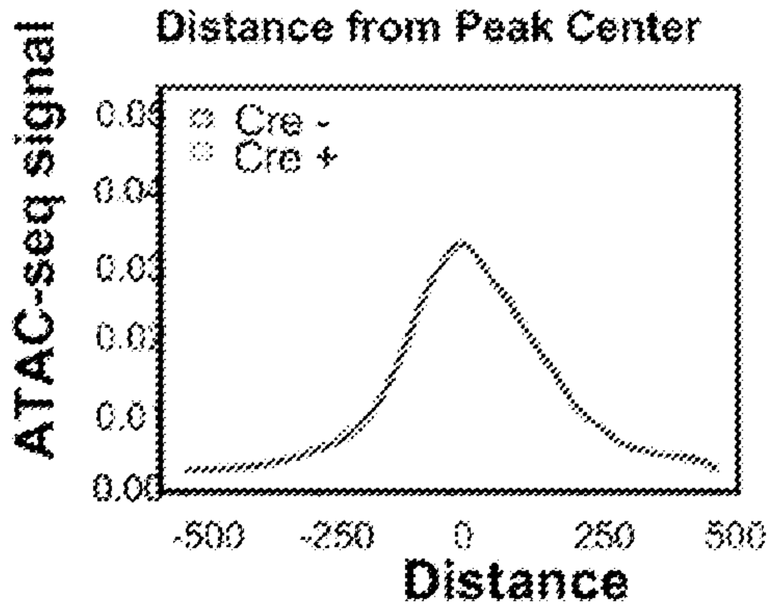


Fig. 7H

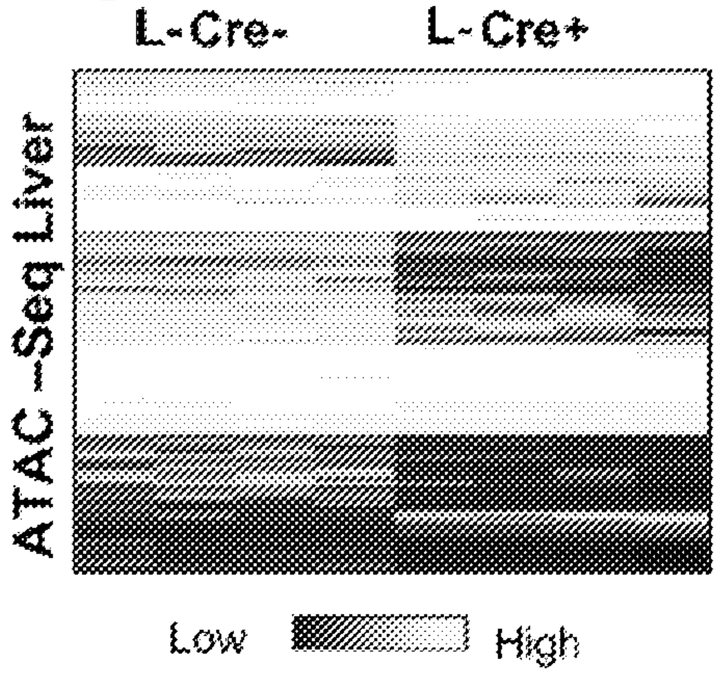
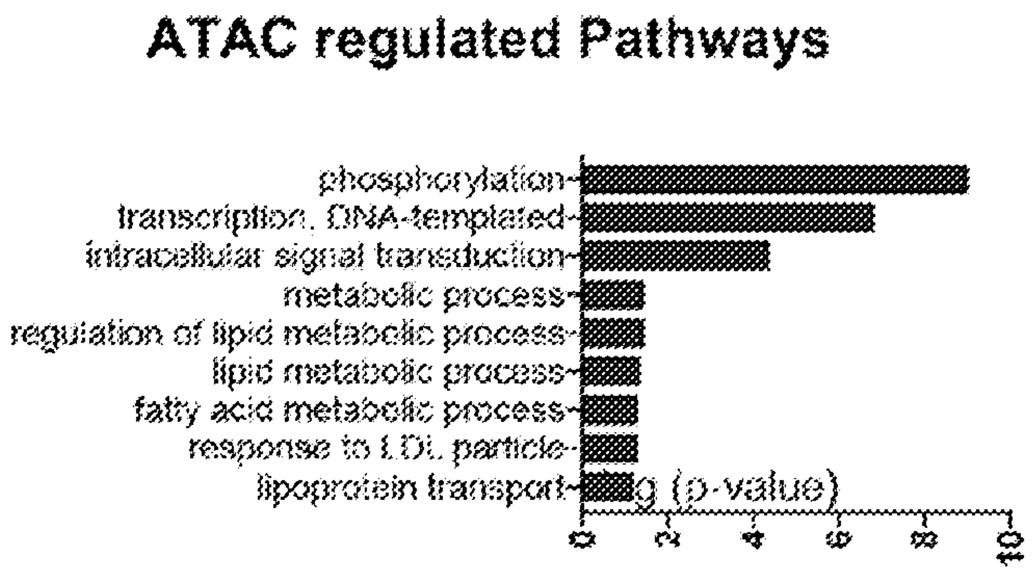


Fig. 7I



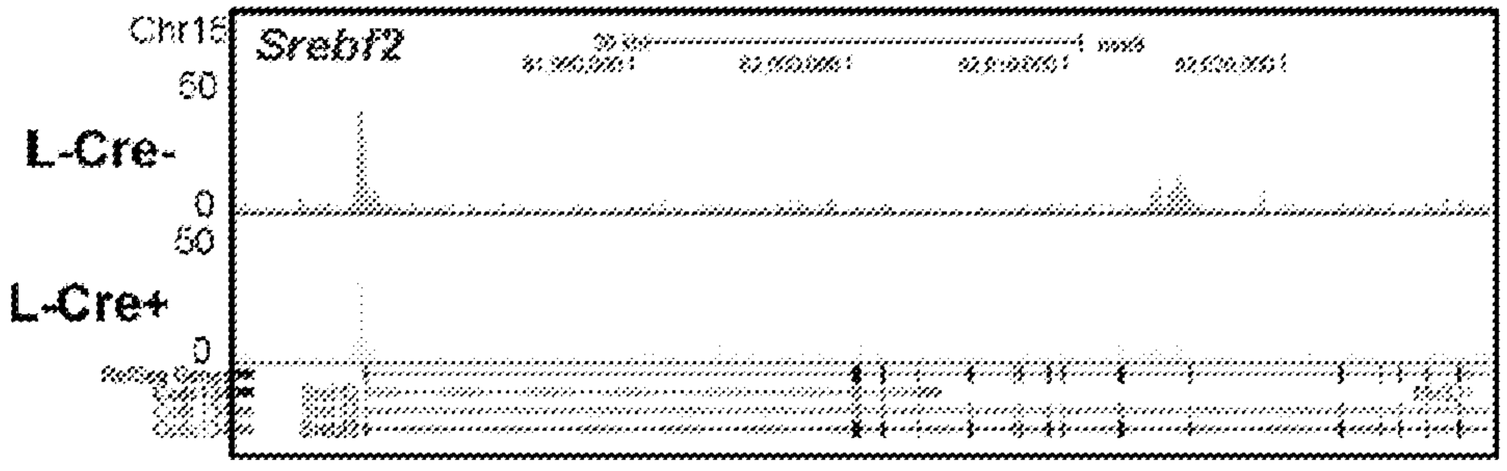


Fig. 7J

Rank	Name	Motif ID
1	bach1	MA0591.1
2	NFYA	MA0060.2
3	RFX2	MA0600.1
4	NFIX	MA0671.1
5	NFYA	MA0060.1
6	RFX5	MA0510.2
7	HNF4A	MA0114.2

Fig. 7K

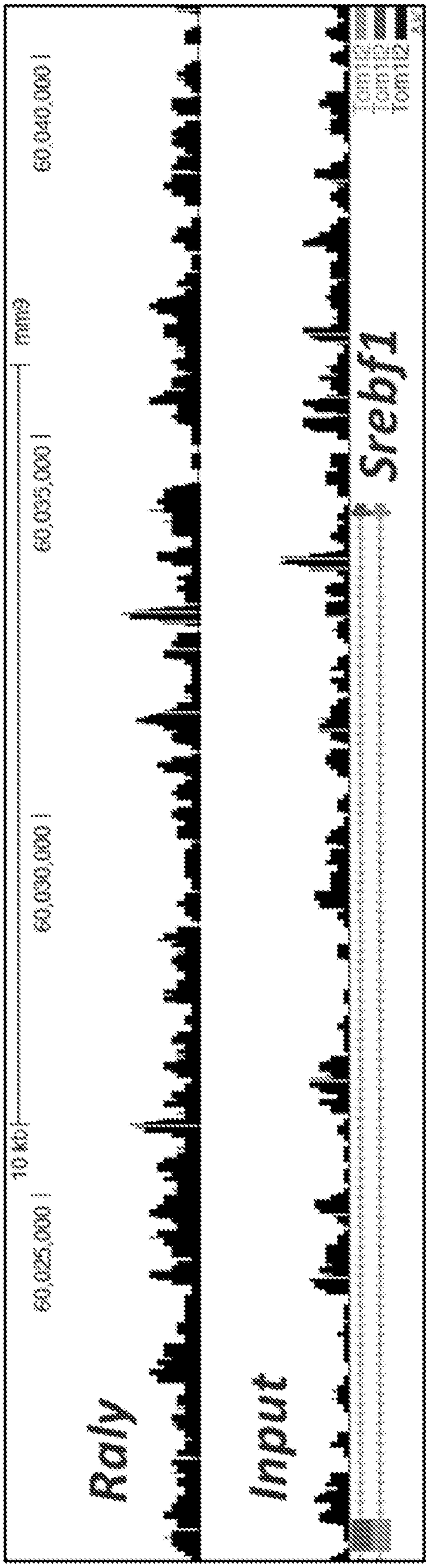

60,025,000
10 kb
60,030,000
60,035,000
mm9
60,040,000
Raly
Input
Srebf1

Fig. 8

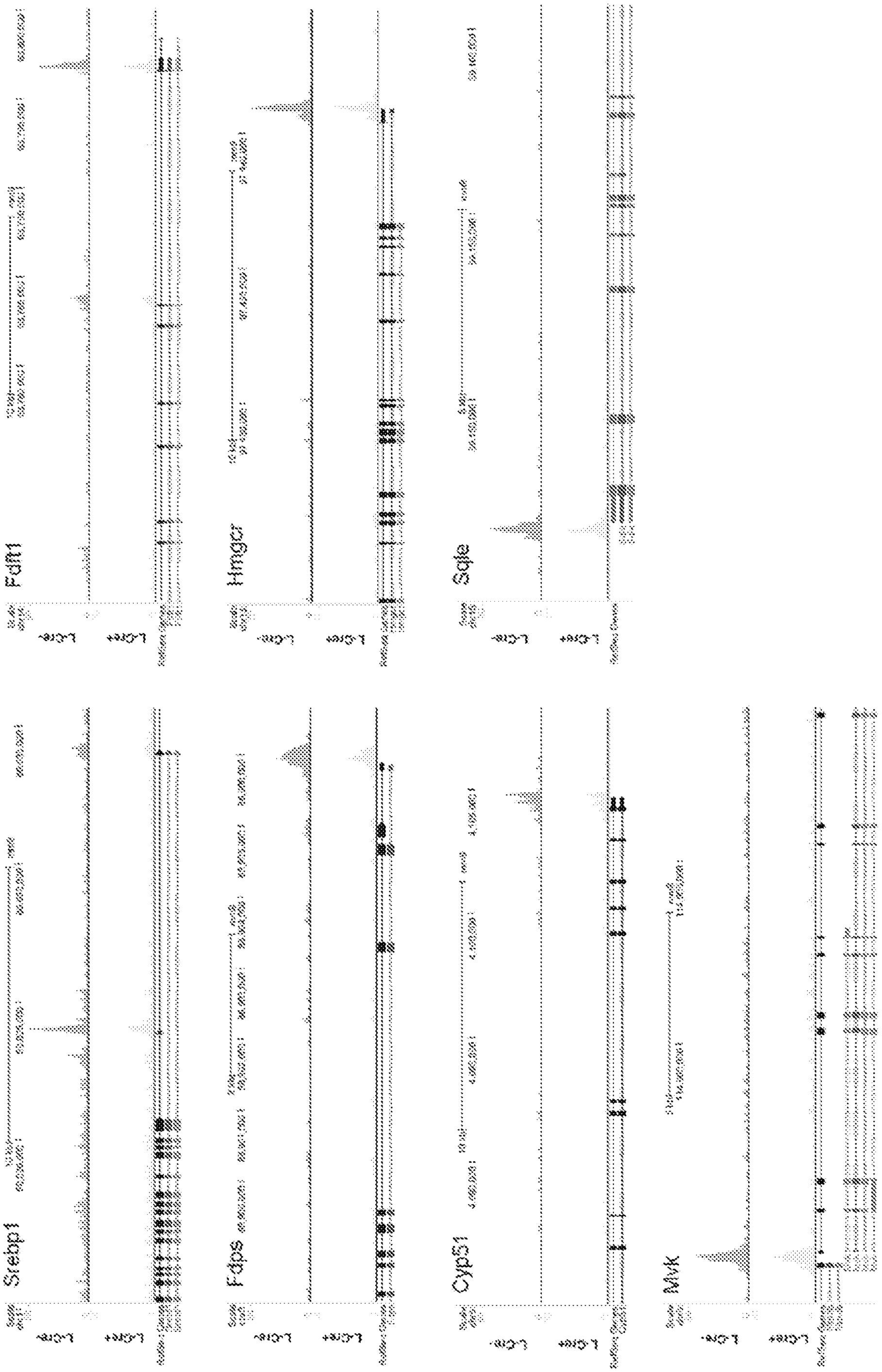


Fig. 6

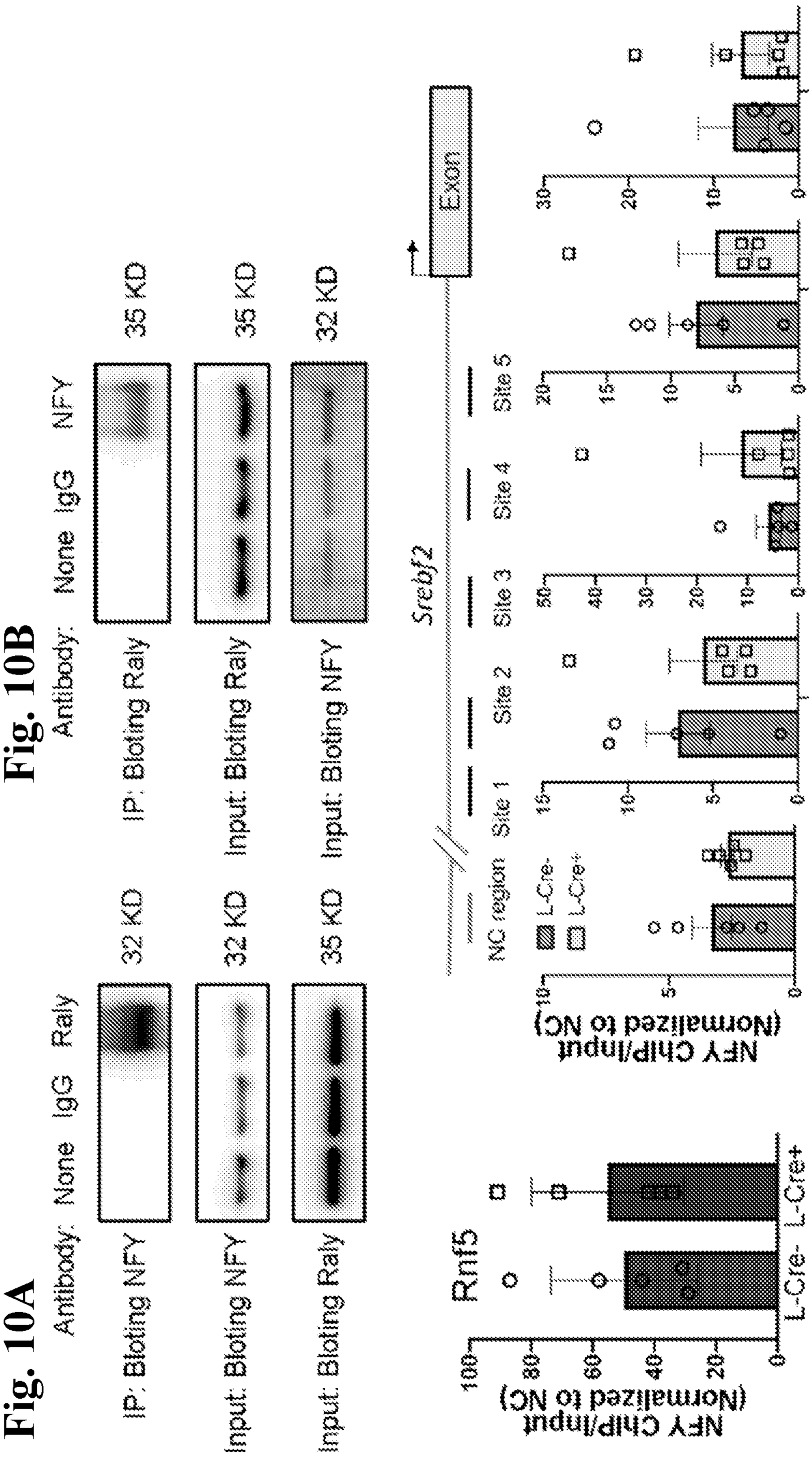


Fig. 10B

Fig. 10D

Fig. 10C

Fig. 10A

Fig. 10E

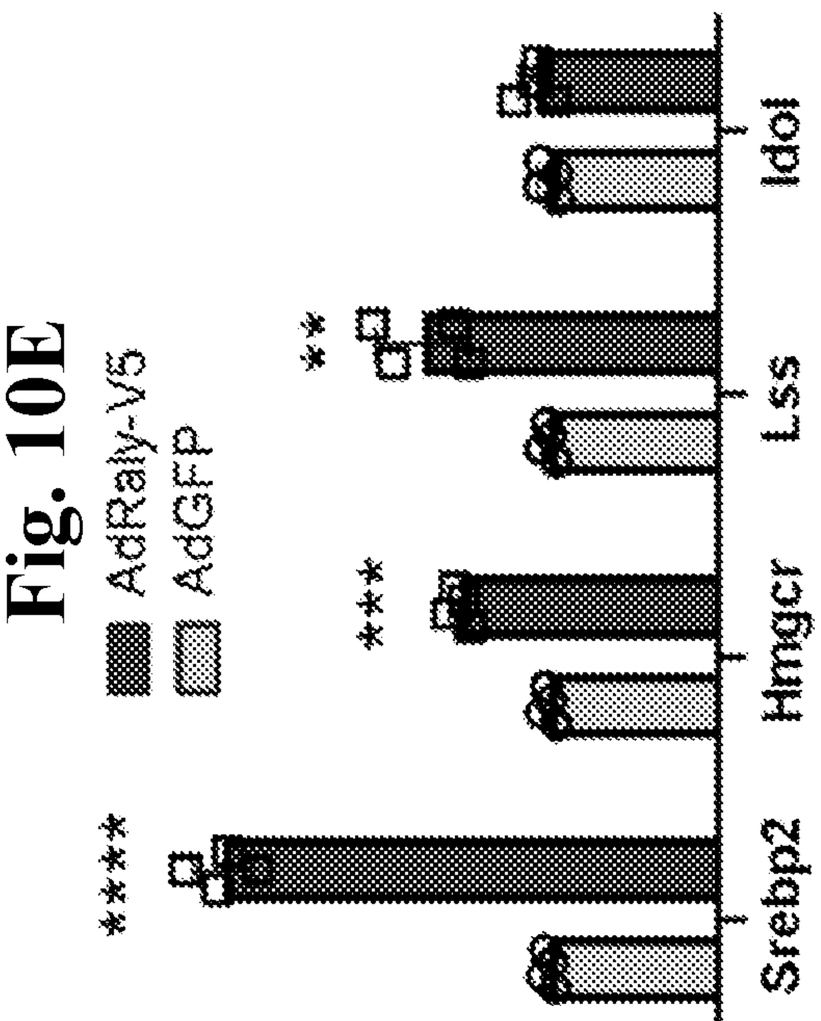


Fig. 10F

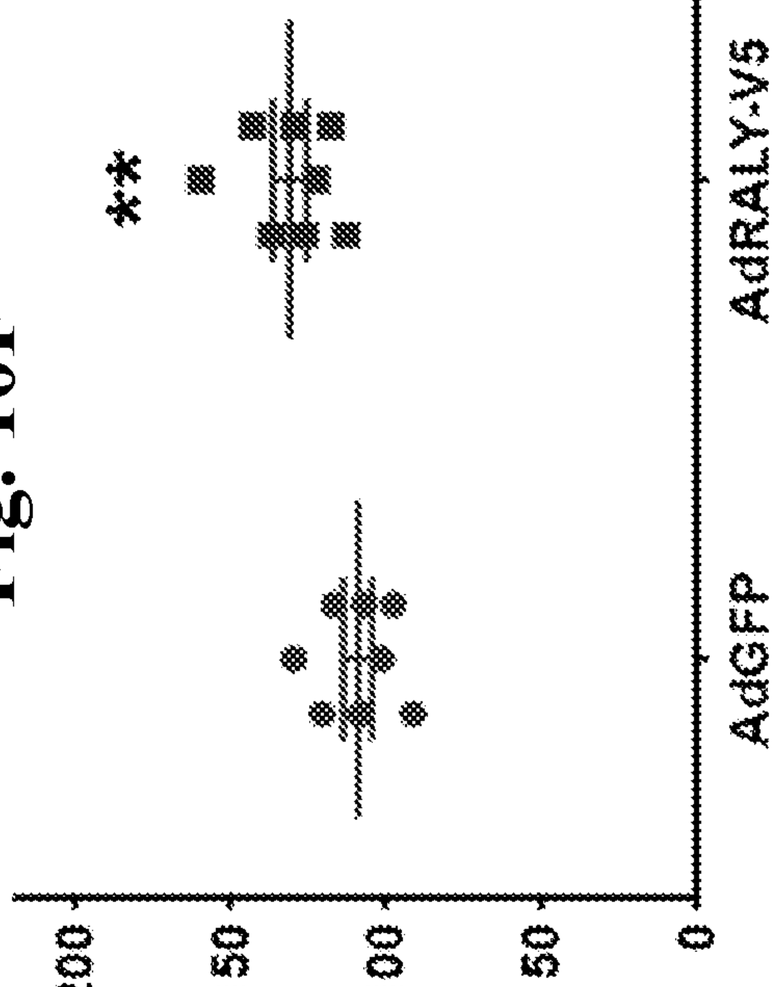


Fig. 10G

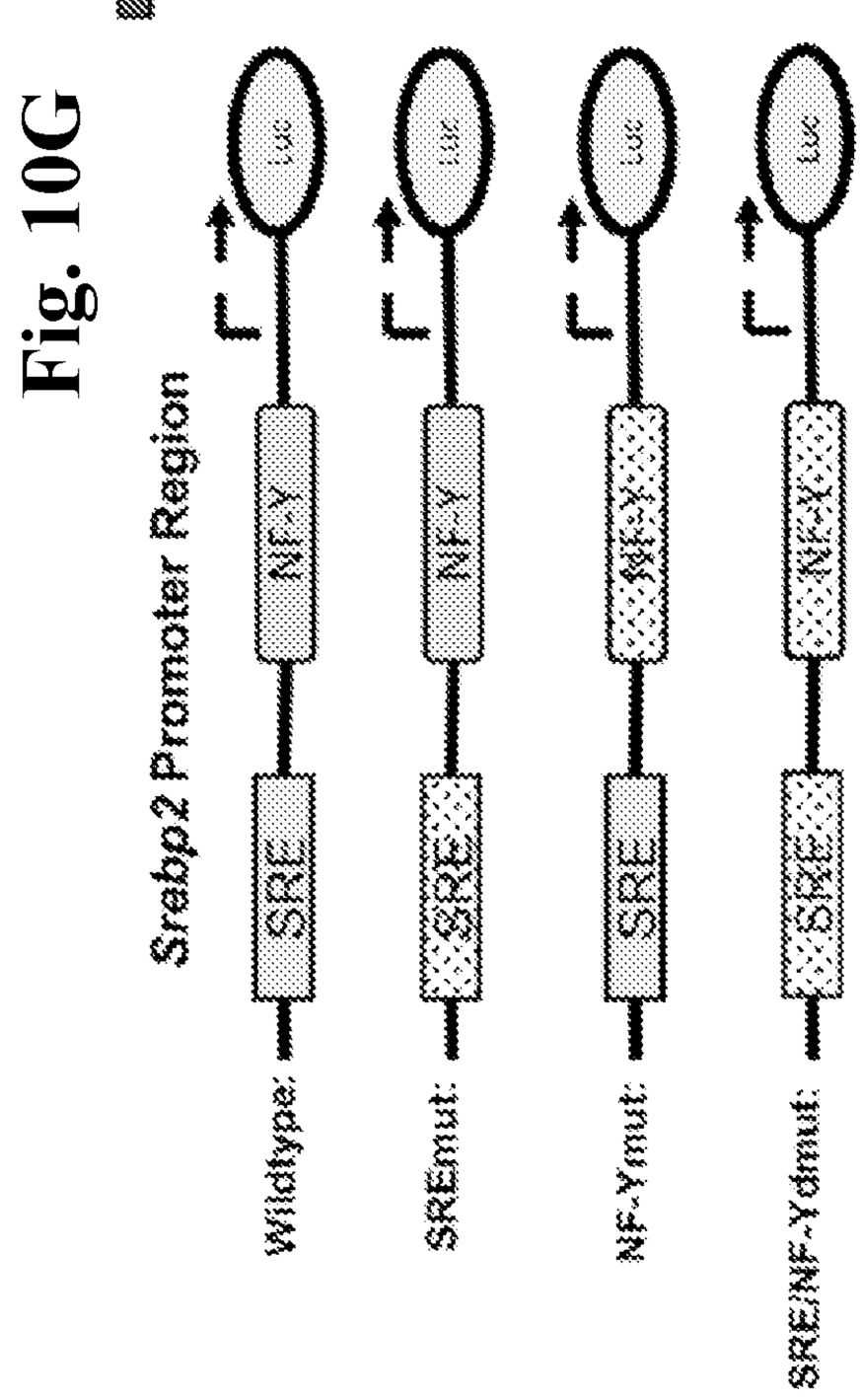
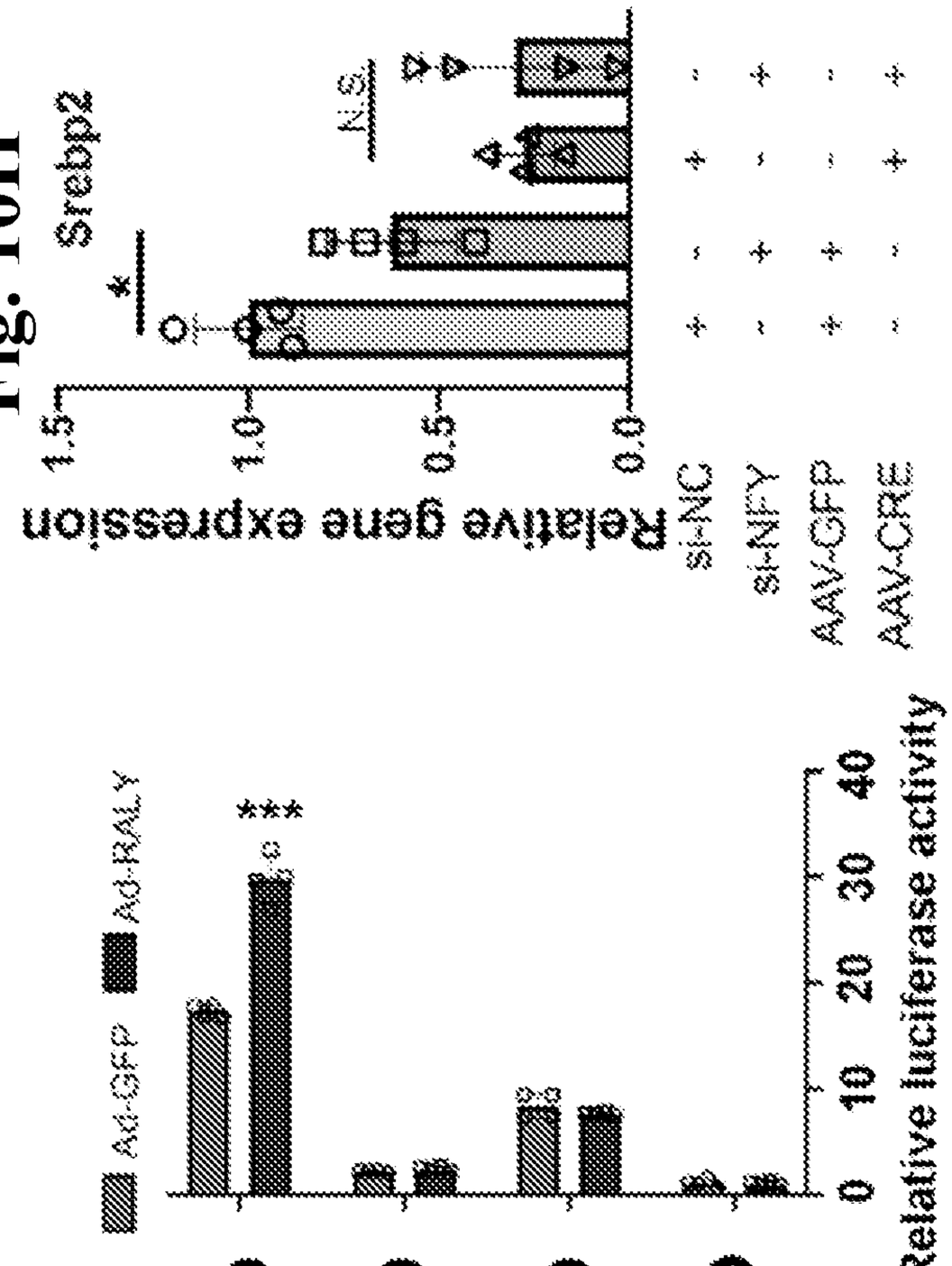


Fig. 10H



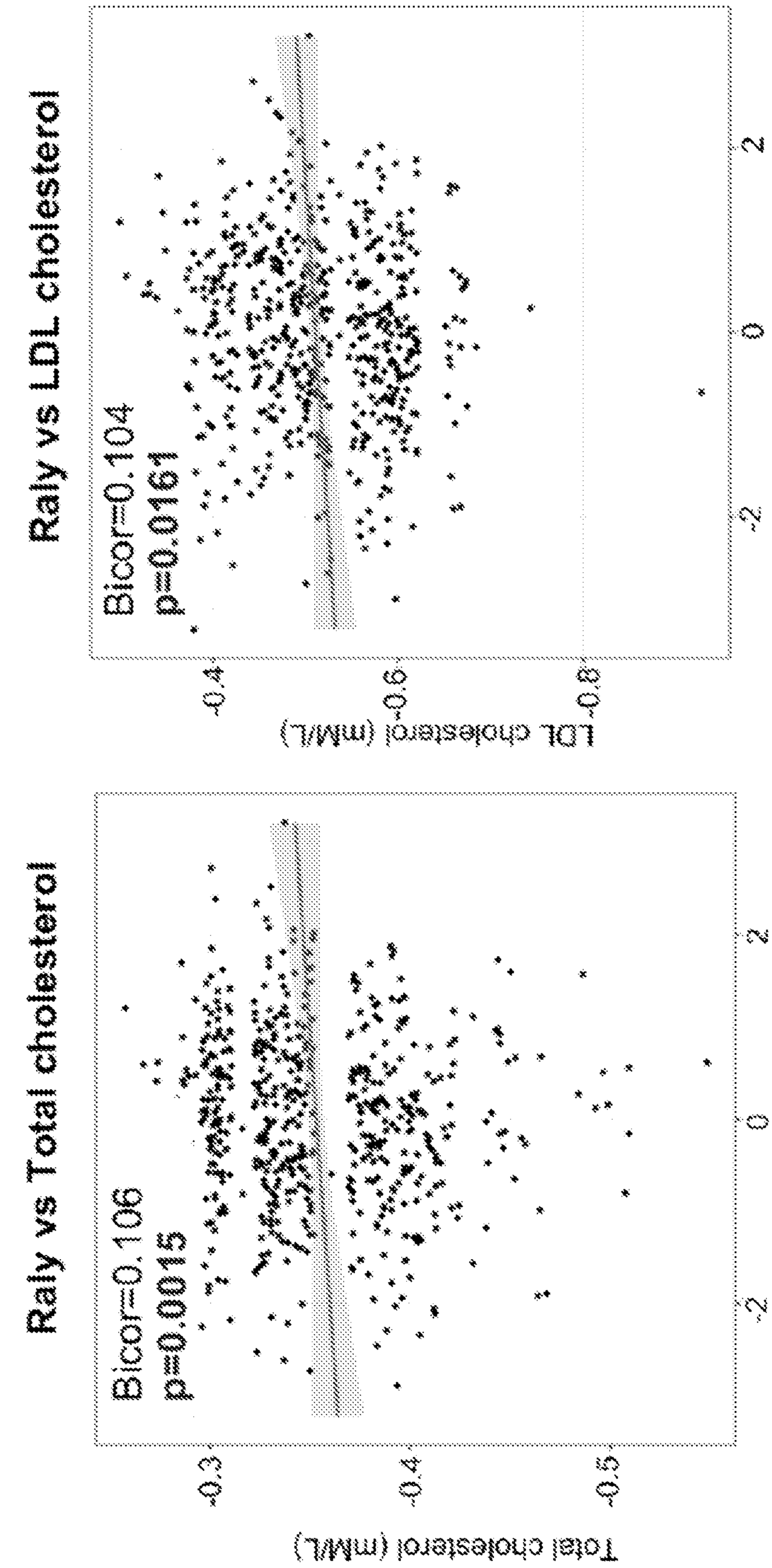

Raly vs LDL cholesterol
Bicor=0.104
p=0.0161
LDL cholesterol (mM/L)
-0.4
-0.6
-0.8
-2
0
2
RALY
Raly vs Total cholesterol
Bicor=0.106
p=0.0015
Total cholesterol (mM/L)
-0.3
-0.4
-0.5
-2
0
2
RALY

Fig. 10I

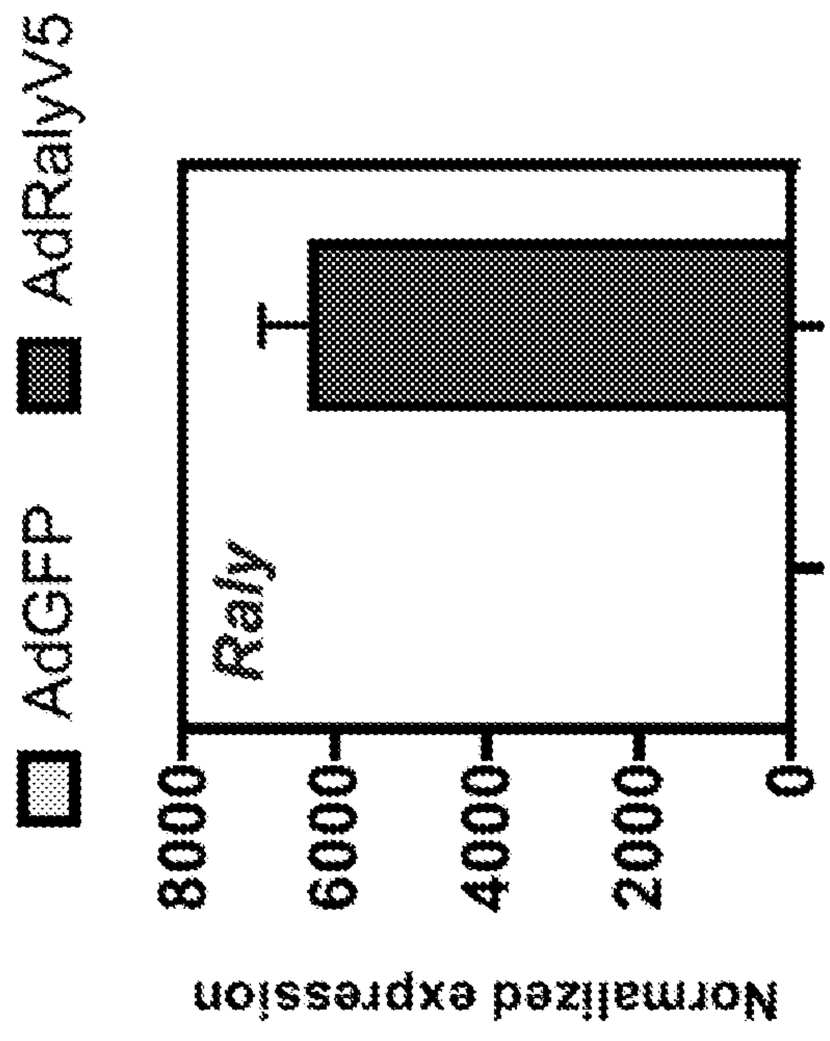


Fig. 11B

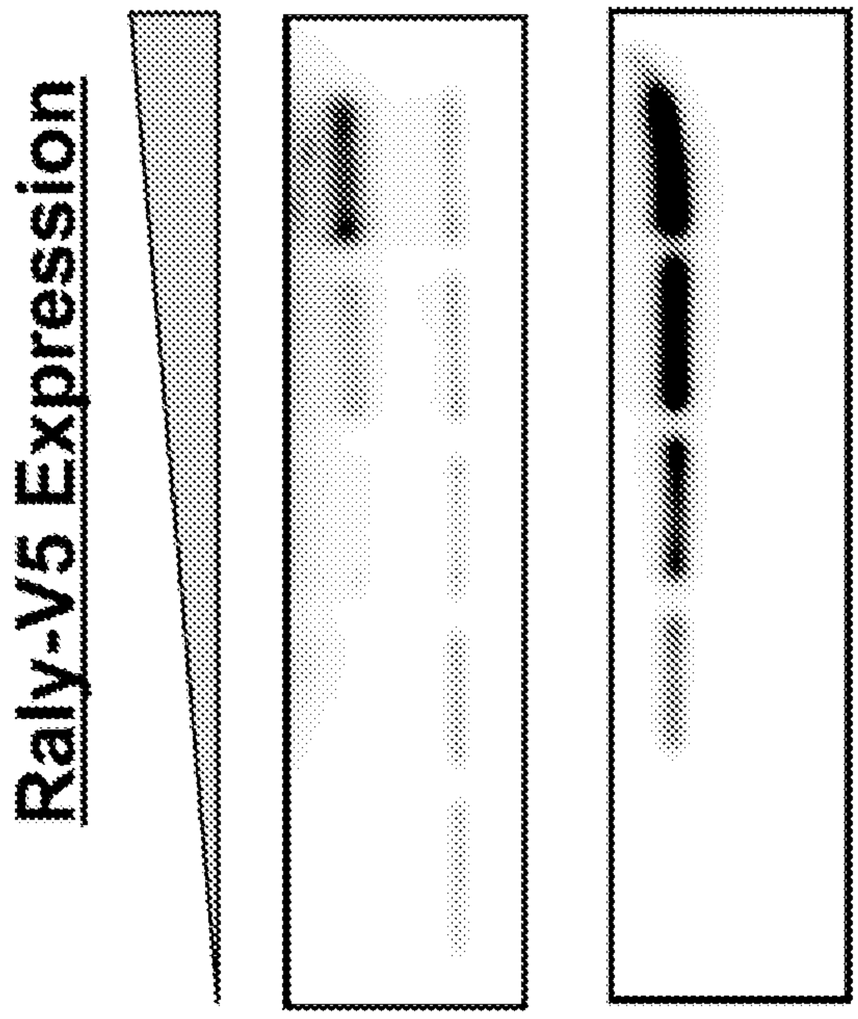


Fig. 11A

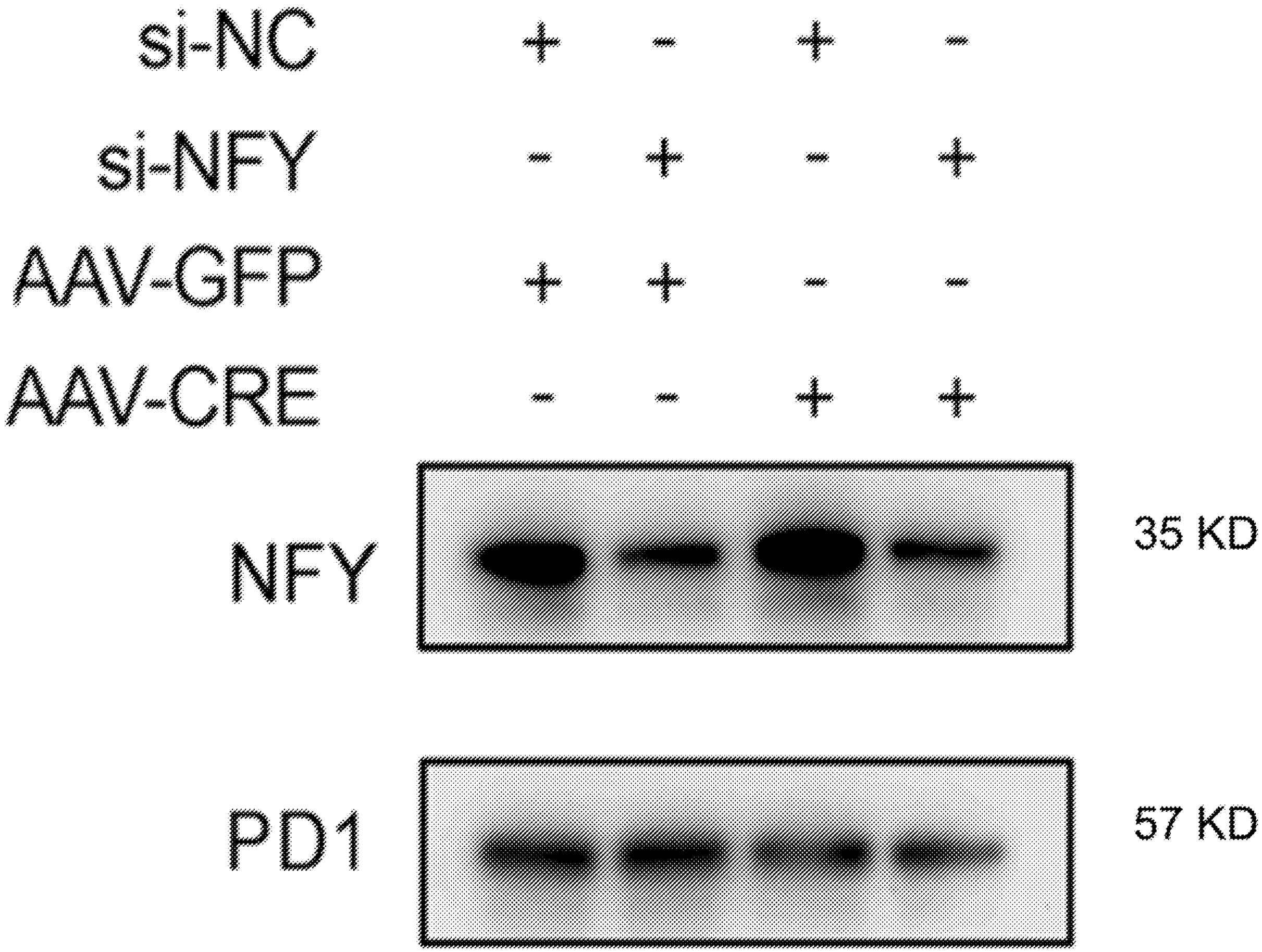
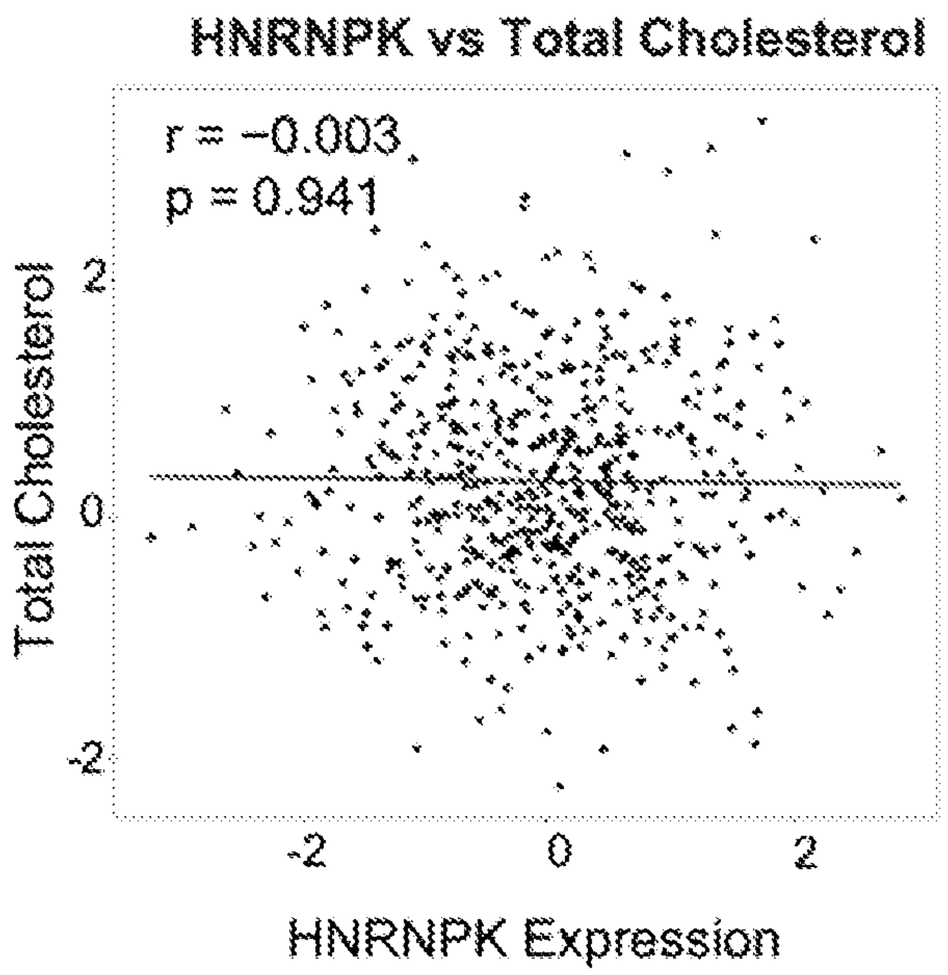
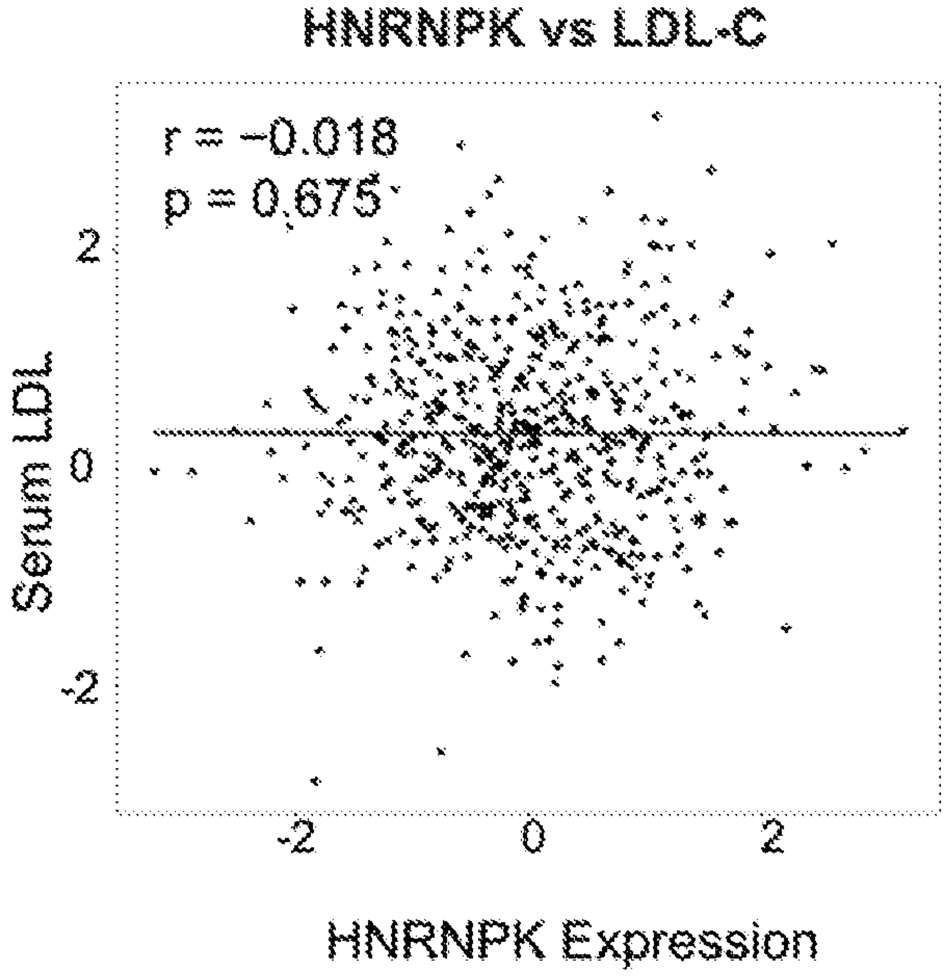
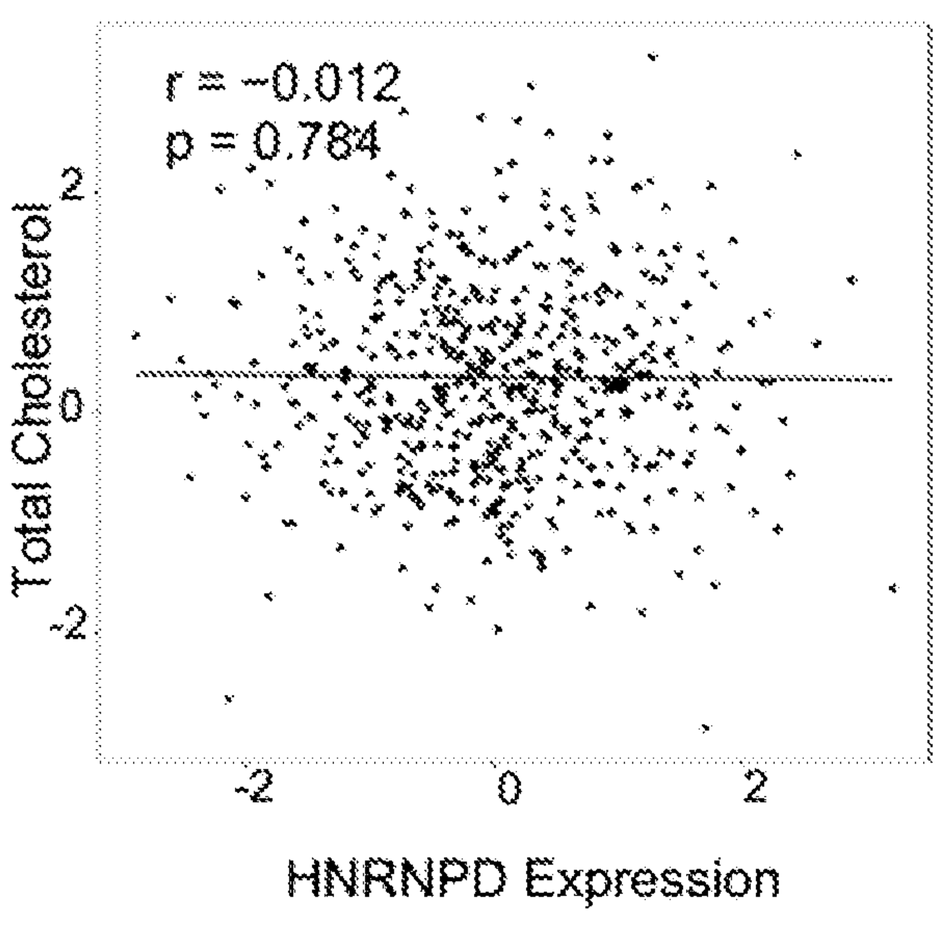


Fig. 12


HNRNPK vs Total Cholesterol
r = −0.003
p = 0.941
Total Cholesterol
2
0
-2
-2
0
2
HNRNPK Expression


HNRNPK vs LDL-C
r = −0.018
p = 0.675
Serum LDL
2
0
-2
-2
0
2
HNRNPK Expression


HNRNPD vs Total Cholesterol
r = −0.012
p = 0.784
Total Cholesterol
2
0
-2
-2
0
2
HNRNPD Expression

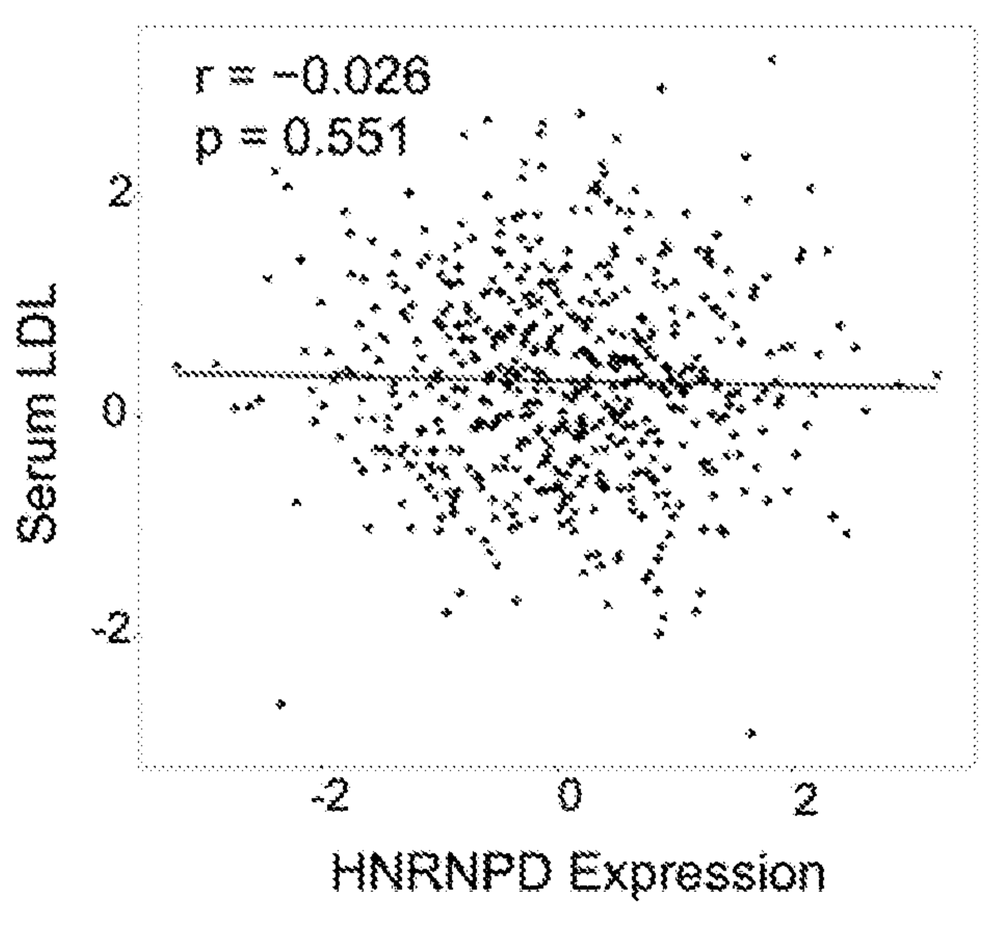
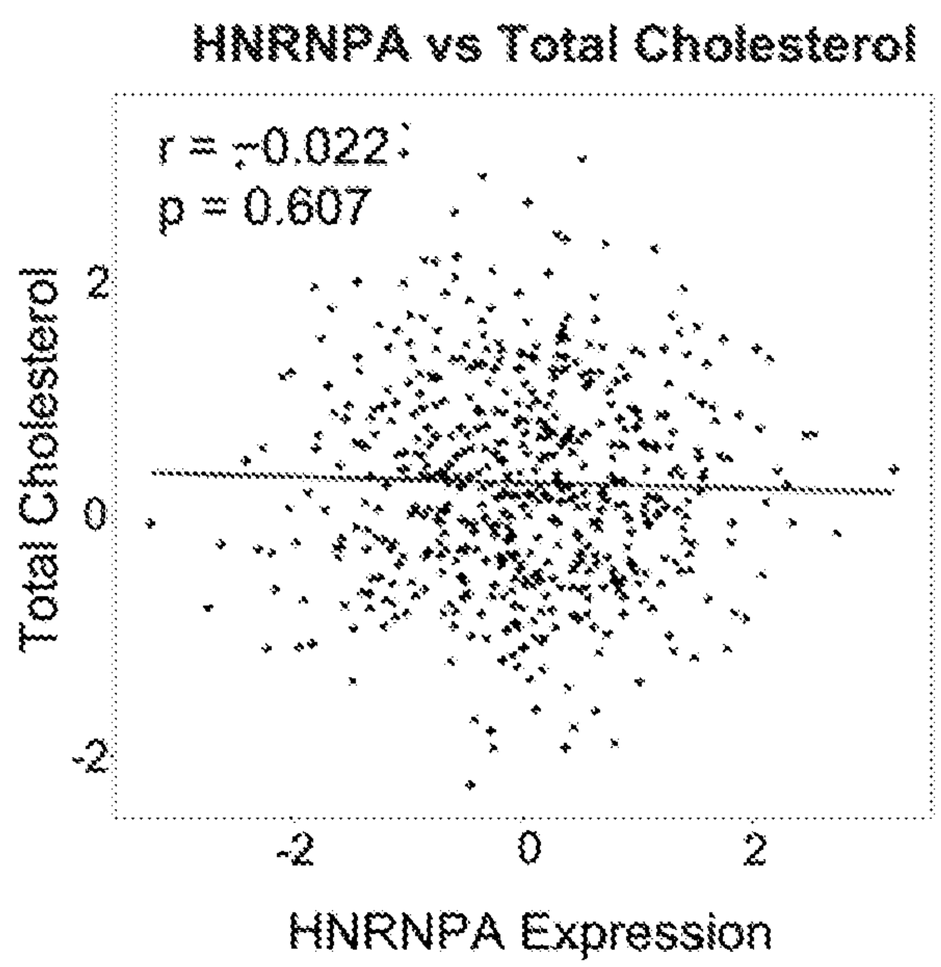
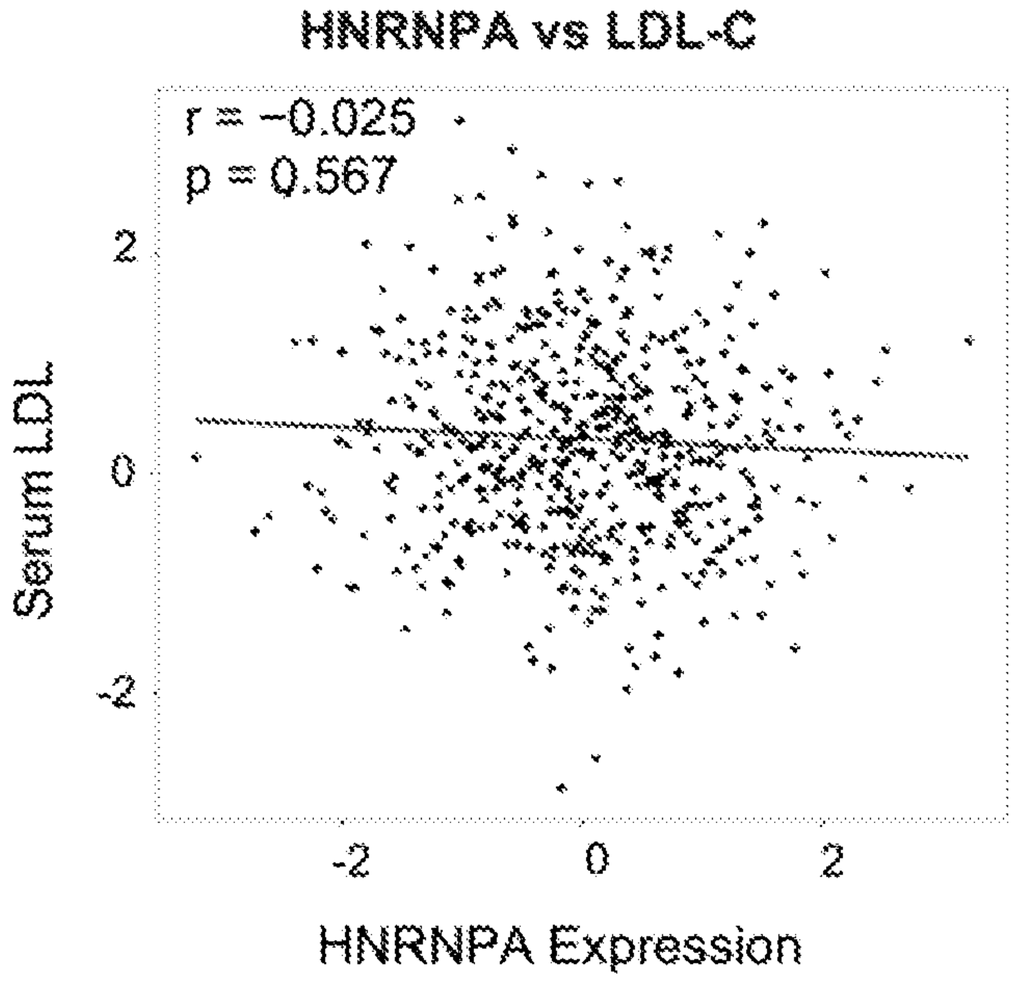
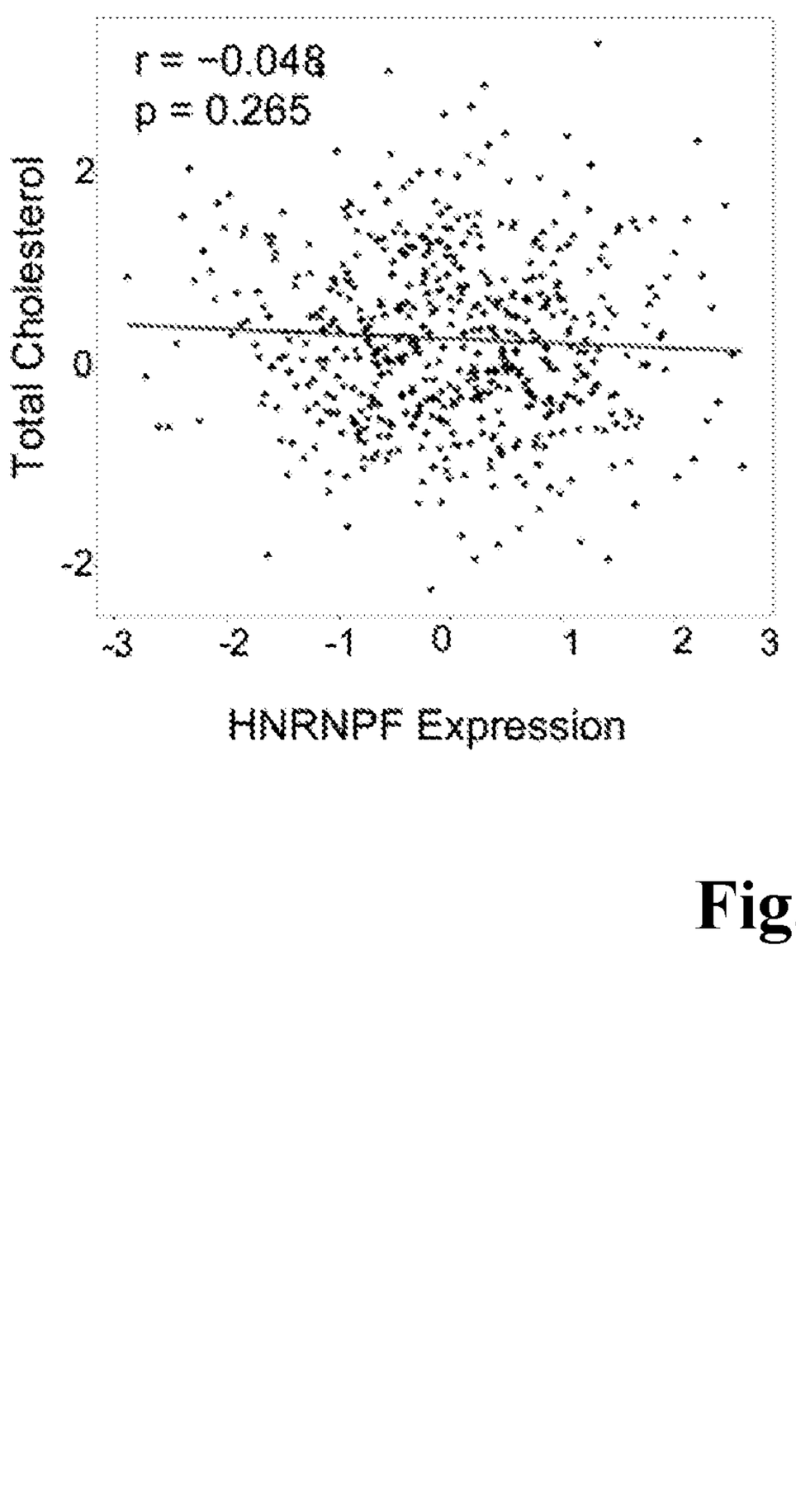

HNRNPD vs LDL-C
r = −0.026
p = 0.551
Serum LDL
2
0
-2
-2
0
2
HNRNPD Expression

Fig. 13A


HNRNPA vs Total Cholesterol
r = -0.022
p = 0.607
Total Cholesterol
HNRNPA Expression
-2
0
2


HNRNPA vs LDL-C
r = −0.025
p = 0.567
Serum LDL
HNRNPA Expression
-2
0
2


HNRNPF vs Total Cholesterol
r = -0.048
p = 0.265
Total Cholesterol
HNRNPF Expression
-3
-2
-1
0
1
2
3

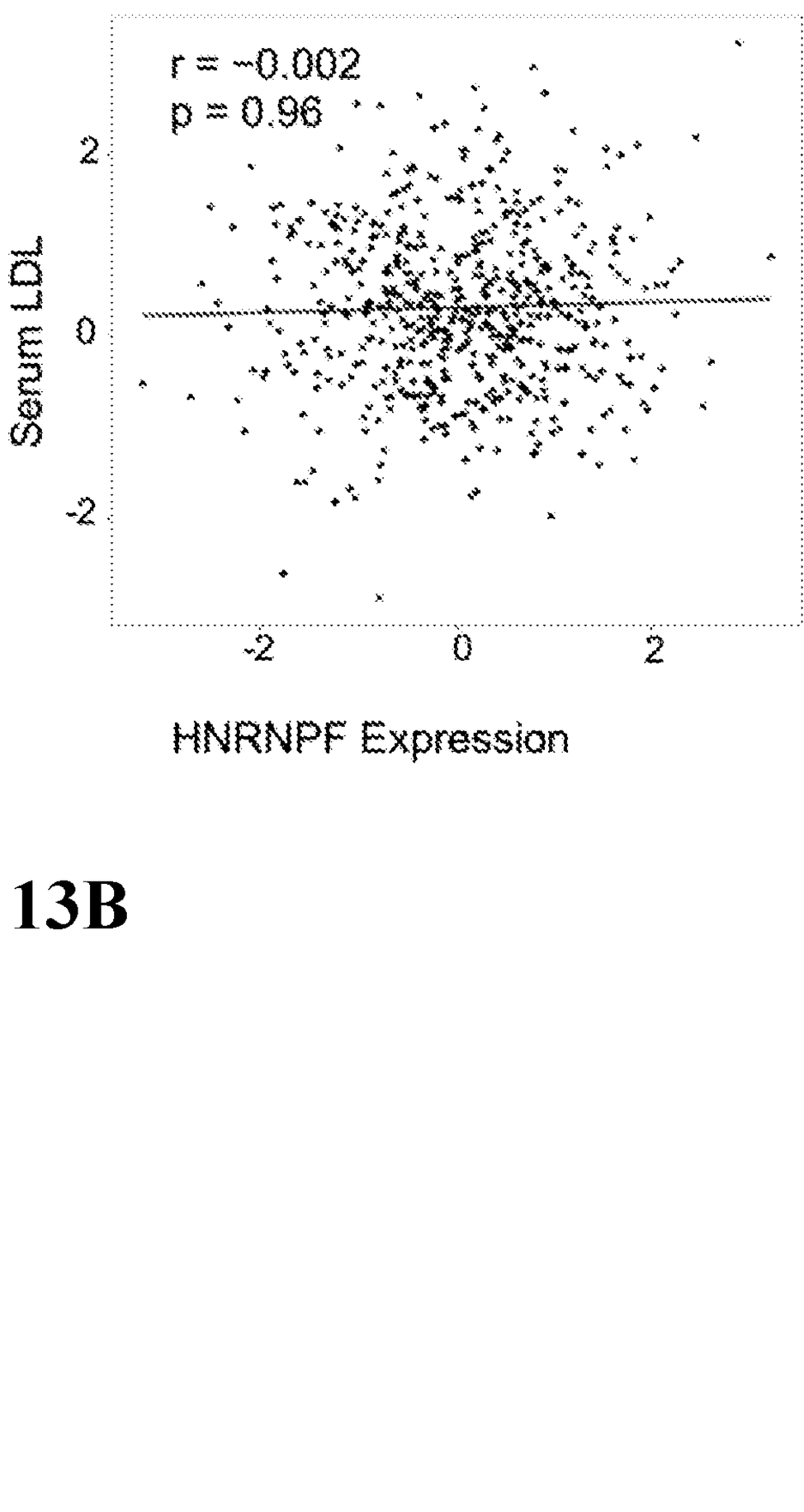

HNRNPF vs LDL-C
r = -0.002
p = 0.96
Serum LDL
HNRNPF Expression
-2
0
2

Fig. 13B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/067347

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/067347

A. CLASSIFICATION OF SUBJECT MATTER
IPC (20210101) C12N 15/113, A61K 31/7105, A61P 3/00, A61P 9/00
CPC (20170101) C12N 15/113, A61K 31/7105, A61P 3/00, A61P 9/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (20210101) C12N 15/113, A61K 31/7105
CPC (20170101) C12N 15/113, A61K 31/7105

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See extra sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Sallam, Tamer, et al. "Feedback modulation of cholesterol metabolism by the lipid-responsive non-coding RNA LeXis." Nature 534.7605 (2016): 124-128. doi: 10.1038/nature17674 11 Nov 2016 (2016/11/11) page 5, Extended data Fig. 7f, 7g, 8a,8b	1-33,38-50
Y		34-37
Y	Wu, Nan, et al. "Activation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase during high fat diet feeding." Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1832.10 (2013): 1560-1568. https://doi.org/10.1016/j.bbadis.2013.04.024 04 May 2013 (2013/05/04) paragraph 3.2, paragraph 3.5, fig 8	34-37

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 Mar 2021	24 Mar 2021
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Email address: pctoffice@justice.gov.il	Authorized officer HOROWITZ Anat Telephone No. 972-73-3927172

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/067347

B. FIELDS SEARCHED:

* Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: CAPLUS, BIOSIS, EMBASE, MEDLINE, REGISTRY, Google Scholar, Derwent Innovation
Search terms used: RNA binding protein RALY, hnRNPCL2, heterogeneous nuclear ribonucleoproteins, cholesterol, SREBP2, Cardiometabolic, Nonalcoholic fatty liver, NAFLD, NASH, Non alcoholic steatohepatitis, Dyslipidemia, HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase