Supplemental Material

Functional screening of candidate causal genes for insulin resistance in human preadipocytes and adipocytes

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Online Supplemental Methods

SGBS cell culture, adipogenic differentiation and cell treatment

SGBS preadipocytes were obtained as a gift from Dr. Martin Wabitsch's lab, and grown in OF medium (DMEMF12 supplemented with 10% FBS, 33um biotin, 17um panthotenat and 1% Penicillin/Streptomycin)[1, 2]. The preadipocytes were differentiated into adipocytes using Quick-Diff medium (OF medium supplemented with 0.01mg/ml transferrin, 20 nm insulin, 100nm cortisol, 0.2nm T3, 25nm dexamethasone, 250um IBMX and 2um rosiglitazone) for the first three days, followed by maintenance medium (OF medium with 0.01mg/ml transferrin, 20nm insulin, 100nm cortisol and 0.2nm T3) for 12-20 days. Maintenance medium was exchanged every three days [1, 2].

Lentivirus generation and CRISPR/Cas9-based targeting of SGBS preadipocytes

To construct sgRNA lentiviral plasmids, sgRNAs were designed using the on-line tool at http://crispr.mit.edu/ and two oligonucleotides, 5'-CACCG-sgRNA sequence-3' and 5'-CAAA-(reverse compliment of sgRNA)-C-3' were synthesized. Oligonucleotides were annealed and ligated into LentiGuidePuro (Addgene #52963) plasmid after BsmBI digestion (Online Table II). Three sgRNAs were designed for each gene and the oligonucleotides were synthesized at Eurofins Scientific. The Cas9 lentiviral plasmid (lentiCas9-Blast) was purchased at Addgene (#52962). Cas9- and sgRNA-encoding lentivirus were packaged with the second-generation lentivirus system. A Cas9-expressing SGBS-preadipocytes cell line (SGBS-Cas9) was generated by transducing the cells with the lentiCas9-Blast lentivirus followed by 5ug/ml of blasticidin selection three days after transduction. Individual IR-genes were targeted by transduction of SGBS-Cas9 preadipocytes with the corresponding three sgRNA lentiviral particles. Three-day post infection, 3ug/ml puromycin was applied for four days to select for sgRNA-expressing cells.

Polymerase chain reaction (PCR), reverse transcription PCR, quantitative real-time PCR and next generation sequencing

Total mRNA was isolated from SGBS pre- and adipocytes using the trizol protocol (Life Technologies) and 1µg was used for reverse transcription of cDNA using the MaximaTh H Minus cDNA Synthesis Master Mix (Thermo Scientific, #FERM1662). Gene expression was quantified by qRT-PCR using 1µl of the reverse transcription reaction and the Fast SYBR™ Green Master Mix (Thermo Scientific, #4385614). Primer sequences are provided in Online Table III. Genomic DNA of each knockout SGBS line was isolated by DNeasy Blood & Tissue Kit (QIAGEN, #69506). The genomic region of the targeted sites was amplified by PCR of isolated genomic DNA. Amplicons were 200-280 base pairs in length including the target-specific PCR primers (Online Table IV). The sites of interest (i.e. CRISPR cut site) are located within the first 100 base pairs from either the 5'-end or the 3'-end of the amplicon. The CRISPR/Cas9 targeting efficiency was calculated, using the results from pooled next generation sequencing (NGS) of the target-specific amplicons. For the pooled NGS, amplicons from each knockout cell line were barcoded prior to pooling.

Immunofluorescence and high content imaging

For immunofluorescence analyses, SGBS adipocytes were fixed with ice-cold methanol at -20°C for 15 min, and immunolabeled using the primary antibody for cEBPα (1:200, Abcam, #ab40761). Donkey anti-Rabbit Alexa Fluor 488 (1:1000, Life Technologies, #A10040) was used as the secondary antibody. LipidTox Red (1:1000, Life Technologies, #H34476) was applied to stain the lipid droplets. Nuclear DNA was stained by DAPI (1:500, Life Technologies, #62248). The secondary antibody only control was employed to validate its specificity and eliminate the background signal. Images were captured using a high content imaging system (Thermo Fisher Arrayscan XTi). The ratio of cEBPα/DAPI was quantified using an ArrayScan high-content analysis software (ThermoFisher scientific).

Lipid extraction and triglycerides measurement

SGBS adipocytes were washed twice with PBS and detached by scraping. A chloroform:methanol (2:1; v/v) mixture was used to extract lipid from the adipocytes, and evaporated to dry overnight in a fume hood. The dried lipid was suspended in 100 µL of 1%

TritonX100 in absolute ethanol for 1 hour with constant rotation. This was then dried in a speedvac for 30 minutes and suspended in 100 µL PBS with 1% Triton. 3 µL of the suspension was used to measure the lipid levels. Triglyceride was quantified using Infinity Triglycerides Solution (VWR, #46100-346).

Lipolysis

To assist the induction of lipolysis, SGBS adipocytes were starved in serum-free DMEM (with 0.2% BSA) overnight to clear residual insulin and lipid in the differentiation medium. Lipolysis was induced by KRPH buffer (Life Technologies) supplemented with 10 µM forskolin for 4 hours. Glycerol accumulated in the KRPH buffer was measured using the Free Glycerol Reagent (Sigma, #F6428).

Western blot

Adipocytes were harvested in Cell Lysis Buffer (Cell Signaling, #9803) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo, #78440). The cell lysates of SGBS adipocytes were subjected to gel electrophoresis under heat denaturing conditions, using NuPAGE® Novex® 4-12% Bis-Tris Protein Gels (Life Technologies, #NP0323BOX) Primary antibodies for FST (Abcam, #ab157471), PEPD (Thermo, #OTI1B7), PDGFC (Abcam, #ab93899), MAP3K1 (Abcam, #ab212601), PPARG (Cell Signaling, #2443), ARL15 (Abcam, #ab178425) and HSP 90 (Santa Cruz Biotechnology, # sc-13119) were used to detect the protein expression in SCR and KO-adipocytes. Primary antibodies for phospho-AKT2 (Ser474), total AKT2 and β-actin were purchased from Cell Signaling (#8599 and #5239) and Sigma-Aldrich (#A5316), respectively. Anti-rabbit or mouse IgG, HRP-linked Antibody (1: 2000, R&D system, #HAF008 and #HAF018) was used as secondary antibodies for the corresponding species. The ratio of phospho-AKT2 (Ser474) to total AKT was quantified by analyzing the western blots using the NIH ImageJ software and data was normalized to β-actin level. All experiments were performed at least three times and the representative results were presented.

Phospho-AKT2 (Ser474) ELISA

Adipocyte lysate was harvested as above and used for measurement of p-AKT2 (Ser474) and total protein. Phosphorylation of AKT2 (Ser474) was measured by Pathscan® phospho-akt2 (ser474) sandwich ELISA kit (Cell Signaling, #7048) according to the manufacturer's protocol.

LC-MS/MS-based 2-DeoxyGlucose-6-Phosphate detection

To measure the insulin induction of glucose uptake in SGBS adipocytes, cells were starved for 24 hours in glucose- and insulin-free 3FC medium to clear residual insulin and glucose. After starvation, cells were treated with 200 µm 2-DG with or without insulin (10nm) for 30 minutes. To extract metabolites from the treated adipocytes for 2-DG-phospate (2-DG-6P) quantification, the cells were lysed by adding precooled 80% methanol in -80 °C for 15 minutes. Cell were then scraped off the plates and thoroughly lysed by vortexing and trituration. We excluded the cell debris from the lysate by centrifugation and the supernatant was dried by a speedVac. For the detection of 2-DG-6P, dried samples were suspended with 60 µl of acetonitrile / methanol (75:25; v:v) containing 25 µM of deuterated phenylalanine-d8 (Phe-d8, Cambridge Isotope Laboratories) as the internal standard. The suspension was transferred into the 250 µl insert vial (Microsolv Technology) and 20 µl of the sample was loaded onto the HILIC column (Atalantis HILIC 100A, 3um, 2.1mm X 150mm, Waters) using the HTS PAL auto-sampler (LEAP Technologies). Phe-d8 and 2-DG-6P were detected by the HPLC-coupled 4000 Q-TRAP mass spectrometry in MRM-based assay in negative mode. The MRM transition of Phe-d8 was 172.2 to 154 with the DP=-50 V, CE=-20V, CXP=-15V, and EP=-10V. Two different MRM transitions of 2DG-6P were applied, firstly, 243 to 78.9 with the DP=-70V, CE=-60V, CXP=-5V and EP=-10V, and secondly 243 to 96.8 with the DP=-70V, CE=-30V, CXP=-5V and EP=-10V. The source parameters were as follows: curtain gas (20psi), collision gas (4psi), Ionspray voltage (-4500V), temperature (450°C), Ion source gas 1 and 2 (40psi and 50psi, respectively) and the interface heater on. The detected peaks of compounds were integrated using MultiQuant 3.0.3 software (ABSciex) for the quantification.

Sanger sequencing based-off-target analysis

Potential off-target sites were predicted for the sgRNAs targeting *FST*, *PEPD,* and *PDGFC* using the publically accessible web tool, CCTop (Online Table V) [3]. The predicted off-target sites with less than four base-pair mismatches were amplified for off-target analysis. Forward and reverse primers were designed ~150 base pairs upstream and downstream to the predicted site. These loci were amplified from the genomic DNA of SCR and KO-adipocytes and genotyped by Sanger sequencing (Online Table VI). Genome editing events were evaluated by the comparison between the sequence of the predicted site in SCR and KO adipocytes (Online Figure VI).

MRNA transfection

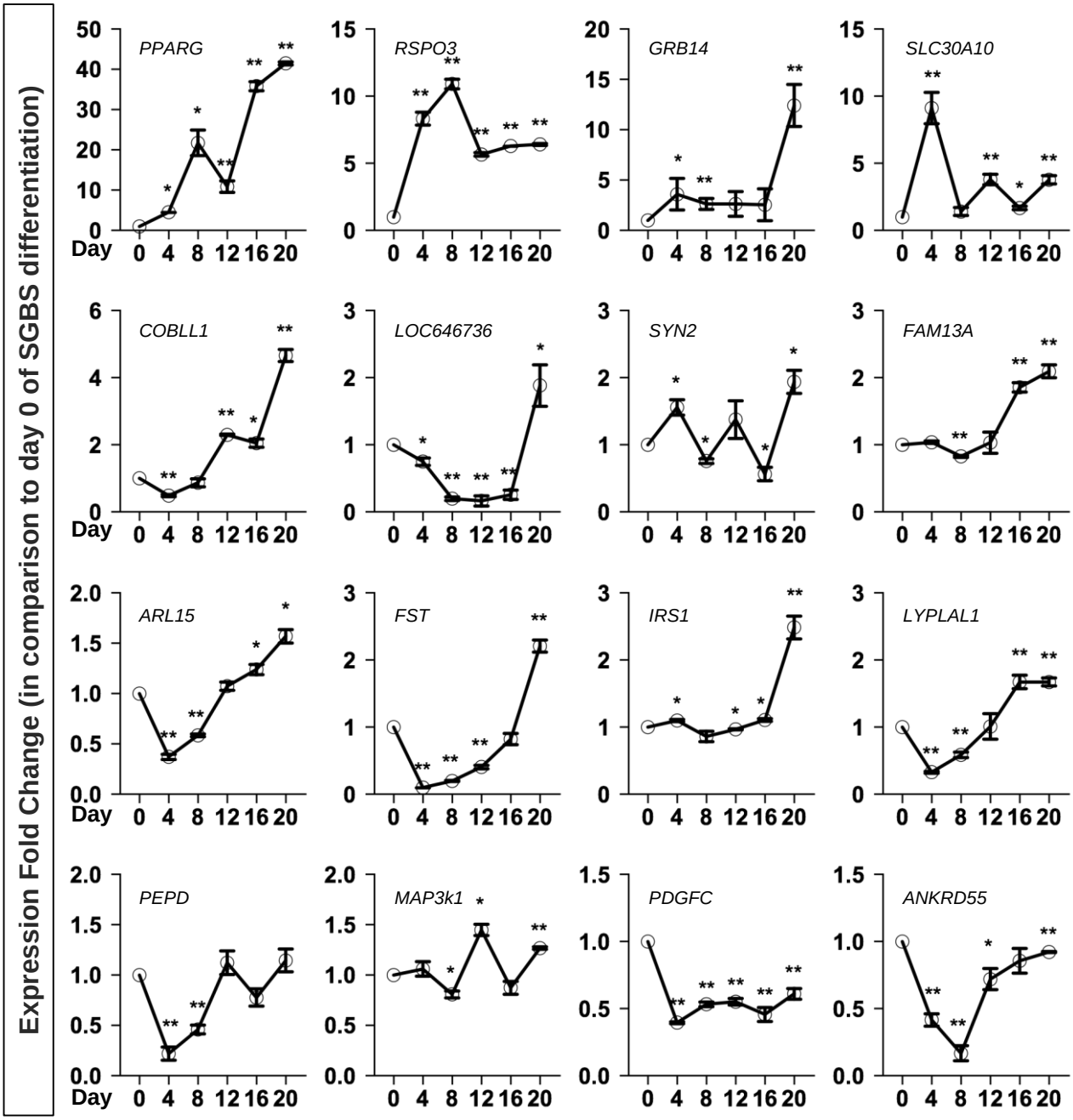
We used the mRNA Synthesis Kit (System Biosciences, # MR-KIT-1) to construct the transcription template plasmids encoding *FST*, *PEPD* and *PDGFC*. We produced the corresponding transcripts using *in vitro* transcription following the manufacturer's manual. Briefly, open reading frames (ORF) of *FST*, *PEPD* and *PDGFC* were amplified from Dharmacon plasmids, # MHS6278-202829884, # OHS6085-213573836 and # MHS1010-202700476 respectively. Restriction enzyme sites (EcoRI at the 5' end and BamHI at the 3' end) were appended using PCR (Online Table VII). The three ORFs were cloned individually into the multiple cloning site (MCS) of pMRNAXP mRNA Synthesis Vector (System Biosciences, # MR000PA-1). The ORFs were cloned into the expression vector using T4 DNA Ligase (NEB, # M0202S). The expression vectors were used as templates for *in vitro* transcription of the mRNA by T7 RNA Polymerase Mix in the mRNA synthesis kit, following the manufacturer's protocols. The *FST*, *PEPD* and *PDGFC* transcripts were delivered into preadipocytes and adipocytes (0.5ug per well of a 24-well plate) by RNAfection (System Biosciences, # MR750A-1).

Reference

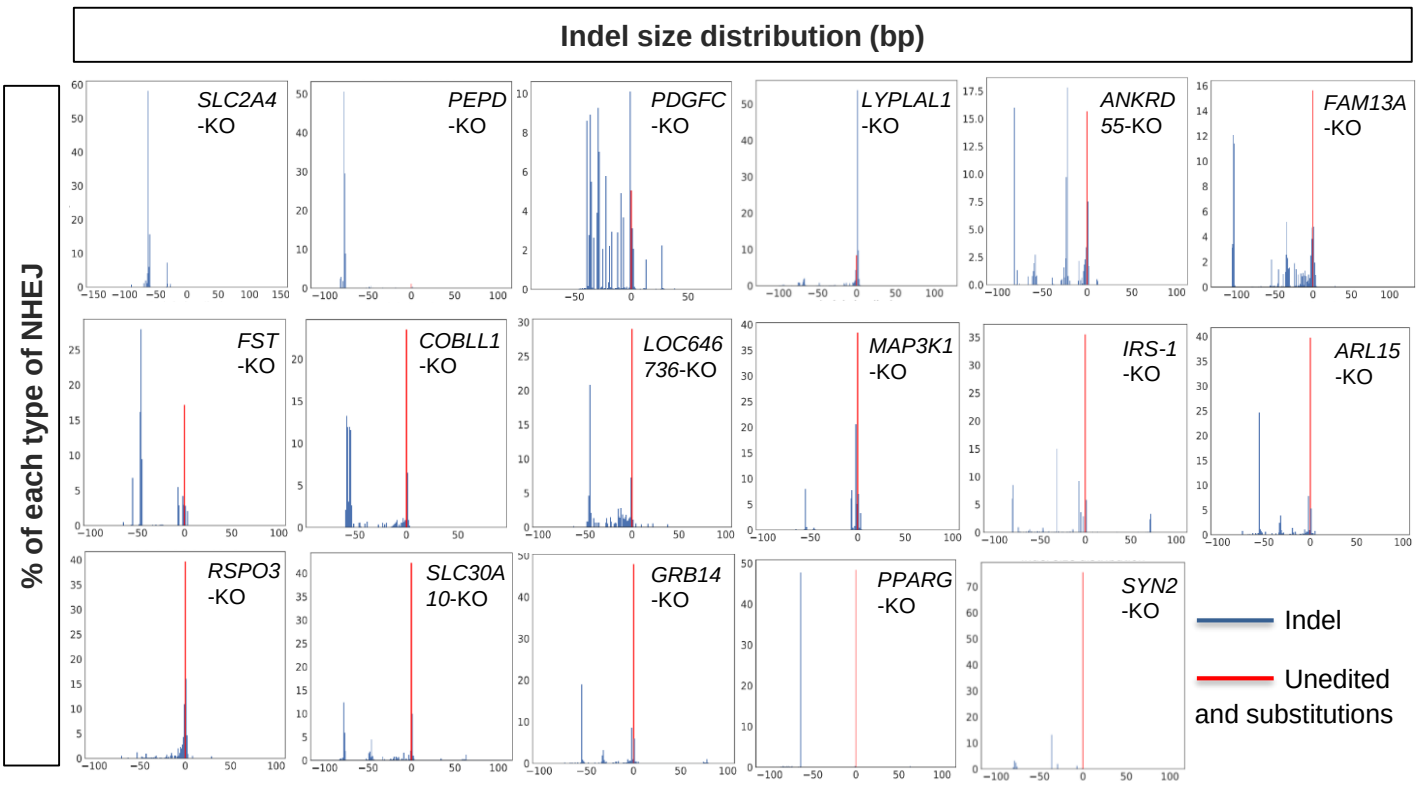
1. Wabitsch M, Brenner RE, Melzner I, Braun M, Möller P, Heinze E, Debatin KM, Hauner H. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. *Int J Obes*. 2001;25:8–15.

2. Fischer-Posovszky P, Newell FS, Wabitsch M, Tornqvist HE. Human SGBS cells - A unique tool for studies of human fat cell biology. *Obes. Facts*. 2008;1:184–189.

3. Stemmer M, Thumberger T, Del Sol Keyer M, Wittbrodt J, Mateo JL. CCTop: An intuitive, flexible and reliable CRISPR/Cas9 target prediction tool. *PLoS One*. 2015;10.

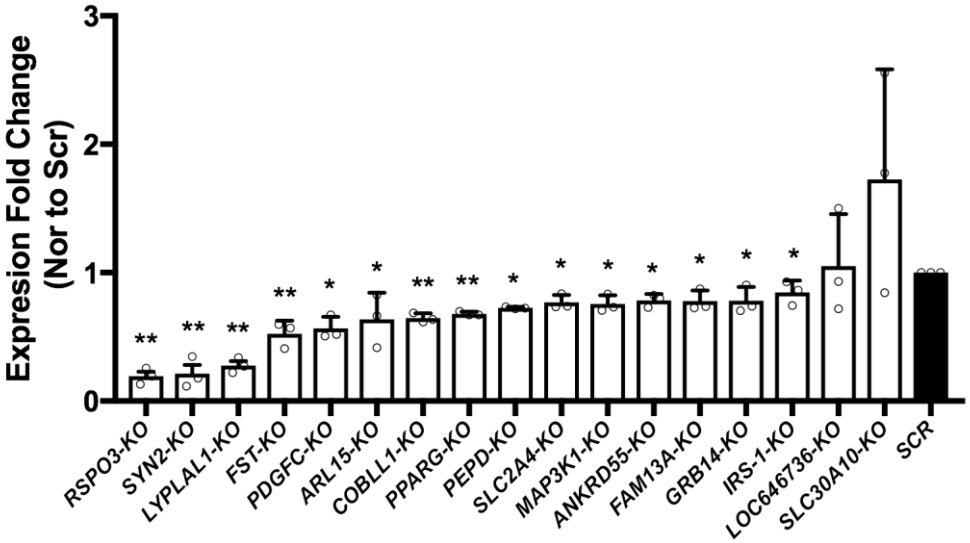


Online Figure I. Evaluation of the IR-gene expression patterns during SGBS preadipocyte differentiation. RT-qPCR was used to analyze mRNA levels at differentiation day 0, 4, 8, 12, 16 and 20. Results were normalized to *RPLP0* mRNA, expression fold change was compared to day 0 and data are presented as line chart, mean ± SD, n=3 (*, $p < 0.05$; **, $p < 0.01$).



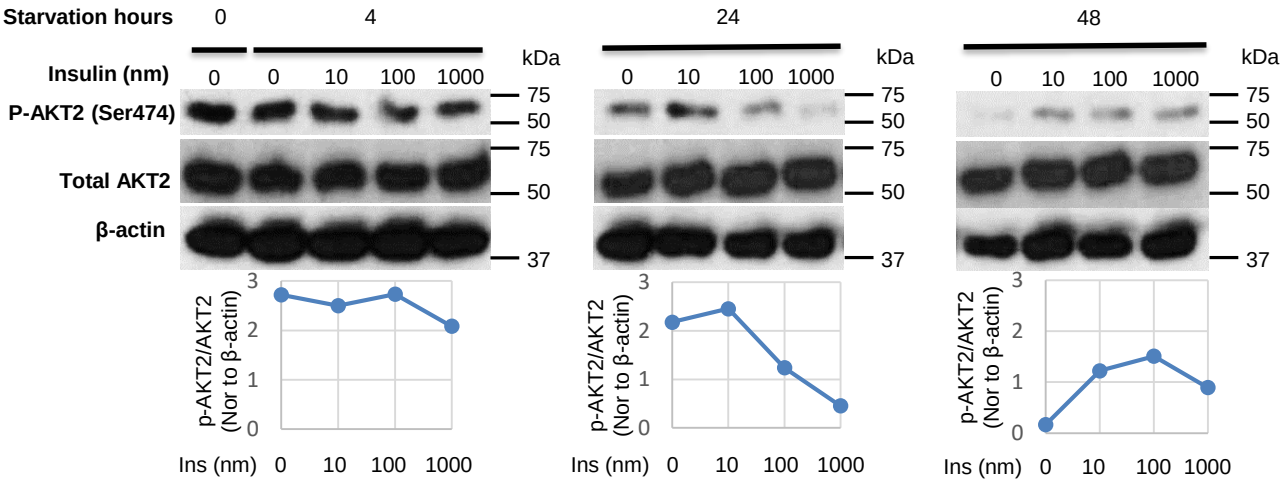
Online Figure II. Knockout efficiency quantified by next generation sequencing (NGS) of the target sites in KO SGBS lines. The square chart for corresponding KO SGBS line displays percentage and size distribution of non-homologous end joining (NHEJ) generated by the three sgRNA targeting system, the size distribution indicated on the x-axis (minus as deletion, plus as insertion and zero as unedited or substitution). Indels are shown in blue and unedited sites or substitutions are shown in red. The total percentage of the three types of NHEJ including insertions, deletions and substitutions is presented in main Figure 1B. Total sequence reads per sample =150,000 – 300,000.

Online Figure III

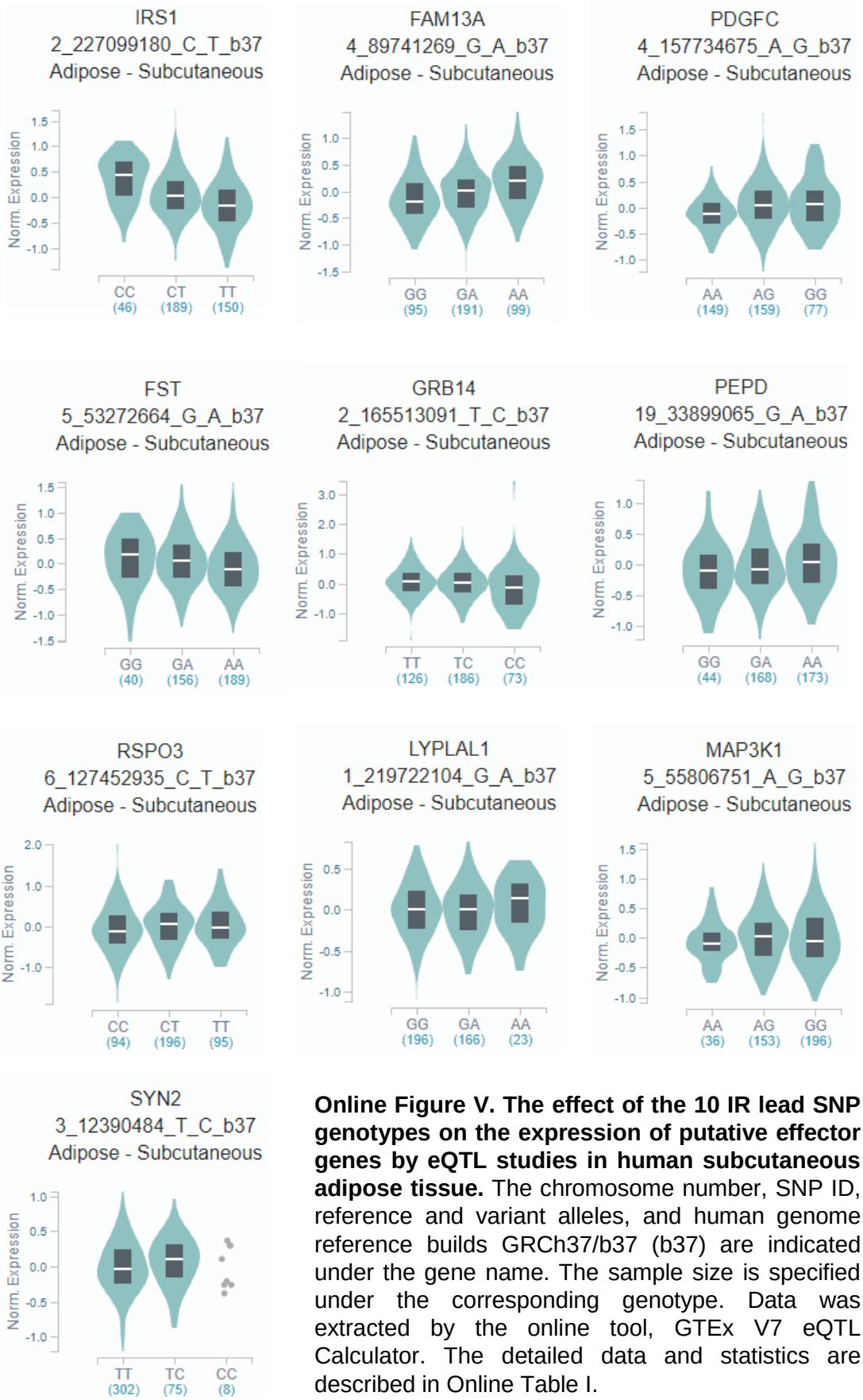


Online Figure III. Evaluation of gene knockout efficiency at the mRNA level. RT-qPCR was used to analyze mRNA levels of each gene in corresponding KO-adipocytes. Results were normalized to *RPLP0* mRNA. Expression fold change was compared to the gene level in SCR adipocytes and data are presented as bar graph, mean ± SD, n=3 (*, $p < 0.05$; **, $p < 0.01$).

Online Figure IV

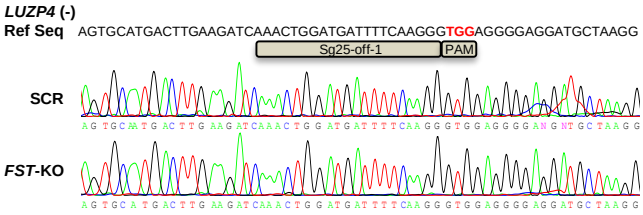


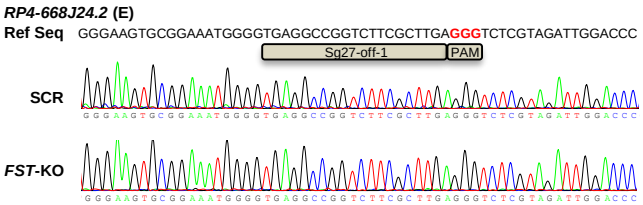
Online Figure IV. Optimization of the condition for insulin treatment. For this purpose, we used the level of AKT2 (Ser474) phosphorylation (p-AKT2) as an indicator. To minimize the basal pAKT2 level, 0, 4, 24 and 48 hours of starvation time were tested. For each starvation time point, we evaluated the levels of phosphor-AKT2 (Ser474), total AKT2 and β-actin of SGBS preadipocyte after the treatment of 0, 10, 100 and 1000nm insulin (Ins). The line charts display the ratio of phospho-AKT2 (Ser474) to total AKT2 and the ratio was normalized to the β-actin level of the same sample.

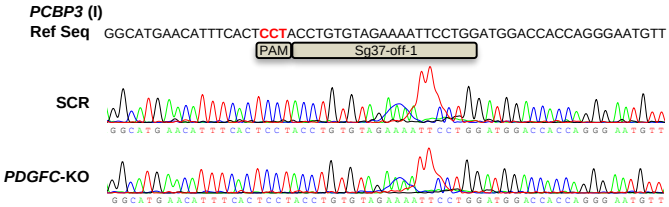


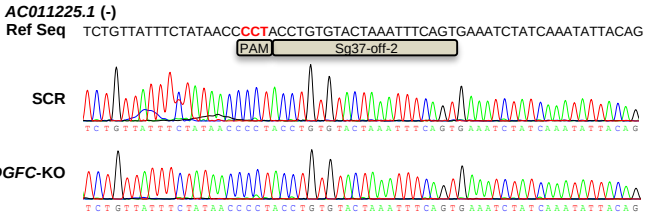
Online Figure V. The effect of the 10 IR lead SNP genotypes on the expression of putative effector genes by eQTL studies in human subcutaneous adipose tissue. The chromosome number, SNP ID, reference and variant alleles, and human genome reference builds GRCh37/b37 (b37) are indicated under the gene name. The sample size is specified under the corresponding genotype. Data was extracted by the online tool, GTEx V7 eQTL Calculator. The detailed data and statistics are described in Online Table I.

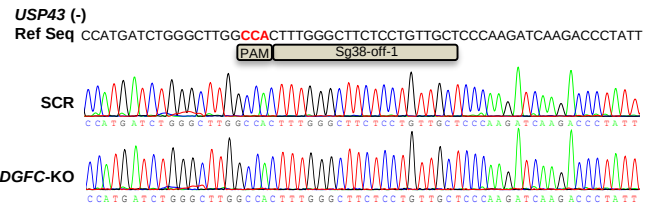
Online Figure VI

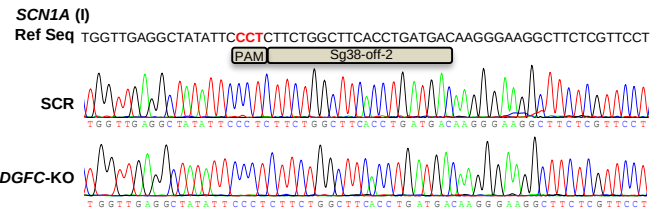


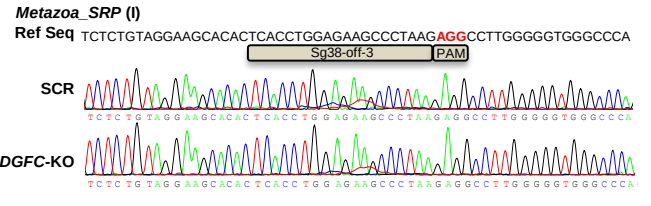


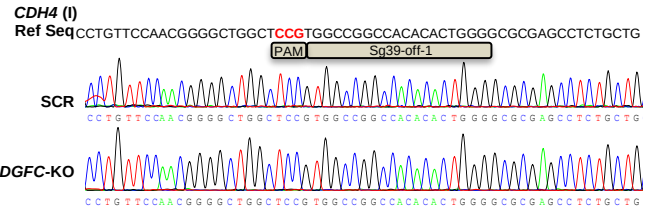


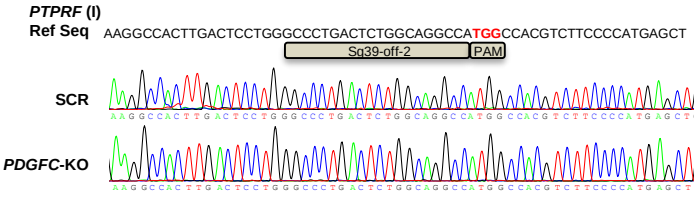


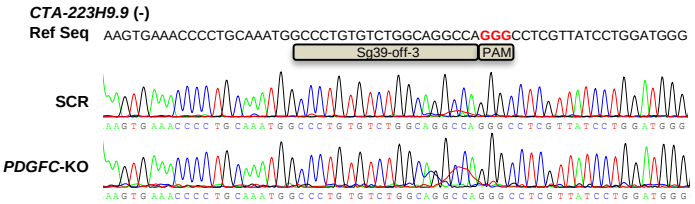


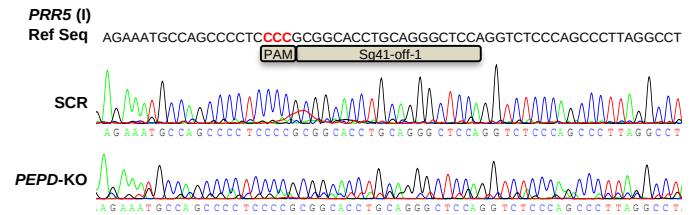


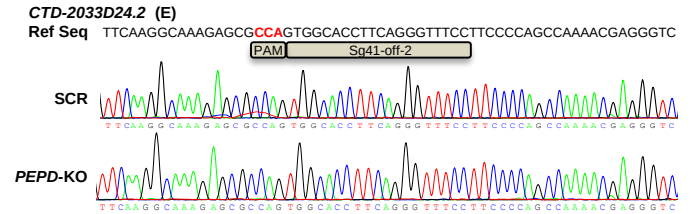


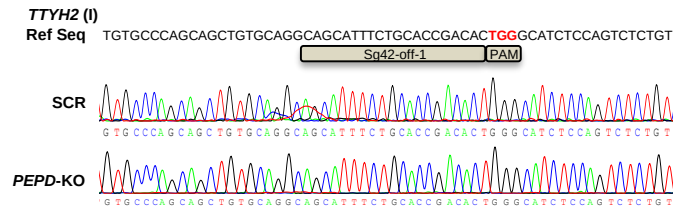


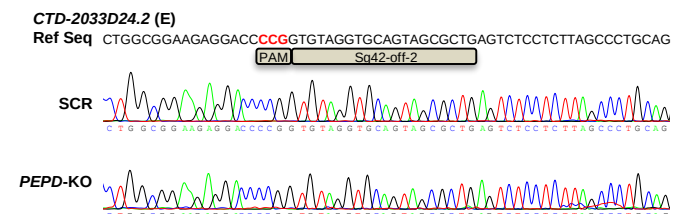


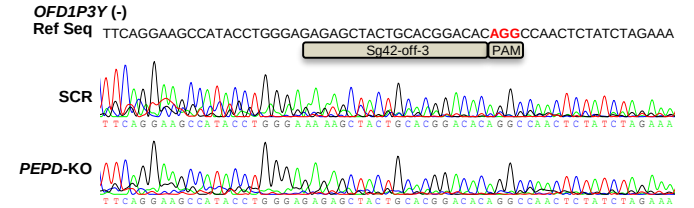




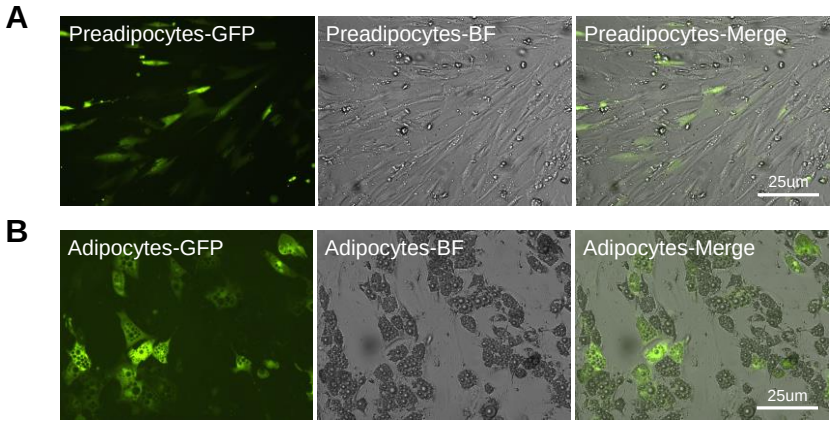








Online Figure VI. Sequencing chromatograms of predicted off-targets sites in SCR and SGBS-KO adipocytes. The reference sequence (Ref Seq, 5'-3') was extracted from hg38. Predicted off-target site positon is indicated with gene name (E = exonic; I = intronic; - = intergenic). Predicted off-target site for corresponding sgRNA shown in the grey box under the Ref Seq.



Online Figure VII. GFP transcript expression in preadipocytes and adipocytes. Representative bright field (BF) and fluorescence images of GFP-expressing preadipocytes and adipocytes.

10 IR loci		eQTL analysis of IR_risk_alleles (RA)				
Transcript	Lead SNP_ref_var	P_var	NES_var	SNP_IR_RA	Action of IR risk allele on gene expression	Tissue
IRS1	rs2943645_C_T	1.70E-10	-0.280	rs2943645_T	Downregulation	Adipose - Subcutaneous
FAM13A	rs3822072_G_A	1.30E-07	0.200	rs3822072_A	Upregulation	Adipose - Subcutaneous
PDGFC	rs6822892_A_G	3.10E-05	0.160	rs6822892_A	Downregulation	Adipose - Subcutaneous
FST	rs4865796_G_A	1.00E-02	-0.120	rs4865796_A	Downregulation	Adipose - Subcutaneous
GRB14	rs10195252_T_C	1.20E-02	-0.140	rs10195252_T	Upregulation	Adipose - Subcutaneous
PEPD	rs731839_G_A	1.60E-02	0.110	rs731839_G	Downregulation	Adipose - Subcutaneous
RSPO3	rs2745353_C_T	2.40E-01	0.055	rs2745353_T	Upregulation	Adipose - Subcutaneous
LYPLAL1	rs4846565_G_A	8.40E-01	-0.007	rs4846565_G	Downregulation	Adipose - Subcutaneous
MAP3K1	rs459193_A_G	6.90E-01	0.017	rs459193_G	Upregulation	Adipose - Subcutaneous
SYN2	rs17036328_T_C	2.00E-01	0.064	rs17036328_T	Downregulation	Adipose - Subcutaneous
10 IR loci		FIadj BMI_ MAGIC_GLYCEMIC_European GWAS[4]		T2Dadj BMI_DIAMANTE (European) T2D GWAS[33]		CVD_CARDIoGRAMplusC4D GWAS[51]
Locus	Lead SNP_ref_var	Effect size _IR _RA	P_IR_RA	OR_var	P_var	OR_var	P_vari
IRS1	rs2943645_C_T	0.019	2.26E-19	1.110	1.60E-39	1.040	6.48E-04
FAM13A	rs3822072_G_A	0.012	1.80E-08	1.040	2.30E-07	1.020	4.20E-02
PDGFC	rs6822892_A_G	0.014	2.60E-10	0.958	7.10E-08	0.991	3.53E-01
FST	rs4865796_G_A	0.015	2.20E-12	1.060	6.70E-14	1.010	2.63E-01
GRB14	rs10195252_T_C	0.017	1.30E-16	0.923	5.70E-26	0.977	2.00E-02
PEPD	rs731839_G_A	0.015	5.10E-12	0.957	7.42E-13	0.973	4.33E-03
RSPO3	rs2745353_C_T	0.014	4.10E-07	0.980	7.50E-03	1.010	1.80E-01
LYPLAL1	rs4846565_G_A	0.013	1.80E-09	0.950	1.40E-10	0.992	3.98E-01
MAP3K1	rs459193_A_G	0.015	1.12E-10	1.080	1.10E-20	1.030	9.43E-03
SYN2	rs17036328_T_C	0.021	3.60E-12	0.896	9.80E-23	1.000	3.16E-01

Ref, reference allele; var, variant allele; NES, normalized effect size; IR, insulin resistance; RA, risk allele; FI, fasting insulin; T2D, type 2 diabetes; CVD,cardiovascular disease; adj BMI, adjusted to BMI; OR, odds ratio.

Online Table I: eQTL genes and GWAS of the 10 IR-SNPs.

Target gene	sgRNA sequence	Primer Forward (5′-3′)	Primer reverse (5′-3′)
PPARG sg1	CCATTCTGGCCCACCAACTT	CACCGCCATTCTGGCCCACCAACTT	AAACAAGTTGGTGGGCCAGAATGGC
PPARG sg2	CTCCGTGGATCTCTCCGTAA	CACCGCTCCGTGGATCTCTCCGTAA	AAACTTACGGAGAGATCCACGGAGC
PPARG sg3	AATGGAATGTCTTCGTAATG	CACCGAATGGAATGTCTTCGTAATG	AAACCATTACGAAGACATTCCATC
IRS1 sg4	ACGCTTCTTCGTACTGCGCG	CACCGACGCTTCTTCGTACTGCGCG	AAACCGC GCA GTA CGA AGA AGC GTC
IRS1 sg5	GCGAGCCCTCCGGAGAGCGA	CACCGGCGAGCCCTCCGGAGAGCGA	AAACTCG CTC TCC GGA GGG CTC GCC
IRS1 sg6	TCGTAGTACTCGAGGCGCGC	CACCGTCGTAGTACTCGAGGCGCGC	AAACGCG CGC CTC GAG TAC TAC GAC
GRB14 sg7	AGCCGCGGGTCCCGTCCGGA	CACCGAGCCGCGGGTCCCGTCCGGA	AAACTCC GGA CGG GAC CCG CGG CTC
GRB14 sg8	ATGGGCAGAGCGCCGCGAGC	CACCGATGGGCAGAGCGCCGCGAGC	AAACGCT CGC GGC GCT CTG CCC ATC
GRB14 sg9	GAGGGGCGACGCCCACGACC	CACCGGAGGGGCGACGCCCACGACC	AAACGGT CGT GGG CGT CGC CCC TCC
GLUT4 sg10	CCCCTCAGCAGCGAGTGACT	CACCGCCCCTCAGCAGCGAGTGACT	AAACAGT CAC TCG CTG CTG AGG GGC
GLUT4 sg11	CTGCAGTTTGGGTACAACAT	CACCGCTGCAGTTTGGGTACAACAT	AAACATG TTG TAC CCA AAC TGC AGC
GLUT4 sg12	GAGCTACAATGAGACGTGGC	CACCGGAGCTACAATGAGACGTGGC	AAACGCC ACG TCT CAT TGT AGC TCC
COBLL1 sg13	GAACTCTCAGTGGTCCTACC	CACCGGAACTCTCAGTGGTCCTACC	AAACGGT AGG ACC ACT GAG AGT TCC
COBLL1 sg14	TCAGTATATTTGGTCTCAGC	CACCGTCAGTATATTTGGTCTCAGC	AAACGCT GAG ACC AAA TAT ACT GAC
COBLL1 sg15	AGAATCCACTGCTTTCATCA	CACCGAGAATCCACTGCTTTCATCA	AAACTGA TGA AAG CAG TGG ATT CTC
ANKRD55 sg16	TGGCCAGGCATAAACTTGTG	CACCGTGGCCAGGCATAAACTTGTG	AAACCAC AAG TTT ATG CCT GGC CAC
ANKRD55 sg17	CCCTTGATGCATGCGGTTTC	CACCGCCCTTGATGCATGCGGTTTC	AAACGAA ACC GCA TGC ATC AAG GGC
ANKRD55 sg18	GGGAGCCAATATTAACATGC	CACCGGGGAGCCAATATTAACATGC	AAACGCA TGT TAA TAT TGG CTC CCC
ARL15 sg19	GCAGAGTTTGGACAACAGAC	CACCGGCAGAGTTTGGACAACAGAC	AAACGTC TGT TGT CCA AAC TCT GCC
ARL15 sg20	TGTGGTCGACACGACGTTAT	CACCGTGTGGTCGACACGACGTTAT	AAACATA ACG TCG TGT CGA CCA CAC
ARL15 sg21	AGGTCATATTCTGGTCGTGC	CACCGAGGTCATATTCTGGTCGTGC	AAACGCA CGA CCA GAA TAT GAC CTC
FAM13A sg22	CTTCGACTGAAGTTCGAGAG	CACCGCTTCGACTGAAGTTCGAGAG	AAACCTC TCG AAC TTC AGT CGA AGC
FAM13A sg23	GAATCGAGGCTGCAACGCTG	CACCGGAATCGAGGCTGCAACGCTG	AAACCAG CGT TGC AGC CTC GAT TCC
FAM13A sg24	TAGGGTGAATGGTAACGTGA	CACCGTAGGGTGAATGGTAACGTGA	AAACTCA CGT TAC CAT TCA CCC TAC
FST sg25	AAGTGGATGATTTTCAACGG	CACCGAAGTGGATGATTTTCAACGG	AAACCCG TTG AAA ATC ATC CAC TTC
FST sg26	GAGCACCTCGTGGACCGAGG	CACCGGAGCACCTCGTGGACCGAGG	AAACCCT CGG TCC ACG AGG TGC TCC
FST sg27	AGCGGCCGTTCTTCGCTTGA	CACCGAGCGGCCGTTCTTCGCTTGA	AAACTCA AGC GAA GAA CGG CCG CTC
LOC646736 sg28	CTCACATGGTTCCGGGATAT	CACCGCTCACATGGTTCCGGGATAT	AAACATA TCC CGG AAC CAT GTG AGC
LOC646736 sg29	ATCTTATTTTTACGCTGGGG	CACCGATCTTATTTTTACGCTGGGG	AAACCCC CAG CGT AAA AAT AAG ATC
LOC646736 sg30	CCAAAGAAGAAGATACCTCC	CACCGCCAAAGAAGAAGATACCTCC	AAACGGA GGT ATC TTC TTC TTT GGC
LYPLAL1 sg31	AAAGTGGCATCAAGAAGAAC	CACCGAAAGTGGCATCAAGAAGAAC	AAACGTT CTT CTT GAT GCC ACT TTC
LYPLAL1 sg32	AAATCTGTCAAACCATACAT	CACCGAAATCTGTCAAACCATACAT	AAACATG TAT GGT TTG ACA GAT TTC
LYPLAL1 sg33	ACATGACATCAATTGATTCA	CACCGACATGACATCAATTGATTCA	AAACTGA ATC AAT TGA TGT CAT GTC
MAP3K1 sg34	GGGGAATCGCGCCTCGTCGT	CACCGGGGGAATCGCGCCTCGTCGT	AAACACG ACG AGG CGC GAT TCC CCC
MAP3K1 sg35	GCCGCCAGTCCGCCCGCTCG	CACCGGCCGCCAGTCCGCCCGCTCG	AAACCGA GCG GGC GGA CTG GCG GCC
MAP3K1 sg36	CAAGGCGAGCAGCGCGCCCG	CACCGCAAGGCGAGCAGCGCGCCCG	AAACCGG GCG CGC TGC TCG CCT TGC
PDGFC sg37	CTGGAATTTACTACTCAGGT	CACCGCTGGAATTTACTACTCAGGT	AAACACC TGA GTA GTA AAT TCC AGC
PDGFC sg38	TCAGCAGGAGAAGCCCGAAG	CACCGTCAGCAGGAGAAGCCCGAAG	AAACCTT CGG GCT TCT CCT GCT GAC
PDGFC sg39	CCCCTGTCTCTGGCCGGCCA	CACCGCCCCTGTCTCTGGCCGGCCA	AAACTGG CCG GCC AGA GAC AGG GGC
PEPD sg40	TGTGCAGGCCGGCTCCATCG	CACCGTGTGCAGGCCGGCTCCATCG	AAACCGA TGG AGC CGG CCT GCA CAC
PEPD sg41	TGAAACCCTGAAGGTGCCGC	CACCGTGAAACCCTGAAGGTGCCGC	AAACGCG GCA CCT TCA GGG TTT CAC
PEPD sg42	CAGCGCTACTGCACCGACAC	CACCGCAGCGCTACTGCACCGACAC	AAACGTG TCG GTG CAG TAG CGC TGC
RSPO3 sg43	TGGCAGCCTTGACTAACGTT	CACCGTGGCAGCCTTGACTAACGTT	AAACAAC GTT AGT CAA GGC TGC CAC
RSPO3 sg44	CGAGTTCCATAATATCCACT	CACCGCGAGTTCCATAATATCCACT	AAACAGT GGA TAT TAT GGA ACT CGC
RSPO3 sg45	GCCCAGACTATTTTTTGCTC	CACCGGCCCAGACTATTTTTTGCTC	AAACGAG CAA AAA ATA GTC TGG GCC
SLC30A10 sg46	TGAGCGCCGGCTACATCGCC	CACCGTGAGCGCCGGCTACATCGCC	AAACGGC GAT GTA GCC GGC GCT CAC
SLC30A10 sg47	CTCGGCGCGGGCGTAGCCGT	CACCGCTCGGCGCGGGCGTAGCCGT	AAACACG GCT ACG CCC GCG CCG AGC
SLC30A10 sg48	GCTACTCTGGCAAGACGTGC	CACCGGCTACTCTGGCAAGACGTGC	AAACGCA CGT CTT GCC AGA GTA GCC
SYN2 sg49	CGGTCATGTAGCCGTTGGGC	CACCGCGGTCATGTAGCCGTTGGGC	AAACGCC CAA CGG CTA CAT GAC CGC
SYN2 sg50	TGATGAACTTCCTGCGGCGC	CACCGTGATGAACTTCCTGCGGCGC	AAACGCG CCG CAG GAA GTT CAT CAC
SYN2 sg51	GCAGCCCGCGCCGACGCCGT	CACCGGCAGCCCGCGCCGACGCCGT	AAACACG GCG TCG GCG CGG GCT GCC

Online Table II: SgRNA sequences and primers for sgRNA plasmid cloning.

Online Table III

Gene	Primer Forward (5'-3')	Primer reverse(5'-3')
PPARG	CGTGGATCTCTCCGTAATGG	GAGATGCAGGCTCCACTTTG
IRS1	GATGGCTTCTCGGACGTGCG	GTTTGGGGGCGCTCGACTTG
GRB14	CACTTCCCTGCAAGATGGGC	GATGGCATTTCCGGAACATC
GLUT4	CGGGCTTCCAACAGATAGGC	GCCACGTCTCATTGTAGCTC
COBLL1	CCGCAGGACGCCCCAGCCAG	ACCACTGAGAGTTCAACGTC
ANKRD55	CAGCACCCCTTCTGTGTTTG	AGGATAGAAGGGTCTTCCCG
ARL15	GAGGCGTTTCTGTACATGG	CGTTATCGGGGCTTTCACTG
FAM13A	CACCGAGAATGGCATTCCAG	AGCAGACATCACCGTCCTTC
FST	TTCATGGAGGACCGCAGTGC	GTGTGTTGTCATTCACGTCC
LOC646736	AATGAACAAGTCCACCCCAG	GGAGGTATCTTCTTCTTTGG
LYPLAL1	ATTTATCCAACAGCTCCTCC	CAGTAAGCACTTGACACATG
MAP3K1	AAGTGCGGAGTGTGGAGCTG	CTGGAAGCCGGTCCCACTC
PDGFC	CTTCTCCTGCTGACATCTGC	CTTGGGCTGTGAATACTTCC
PEPD	TTGAACCGGCAGCGCCTGTG	GTGACACCGAACGCCCAGTG
RSPO3	TATGGAATACATCGGCAGCC	GAGCATGTTGCACAGCCTCC
SLC30A10	GACTCCTTCAACATGCTCTC	AGGAAGACCGCGTTGCTCAG
SYN2	ATGATGAACTTCCTGCGGCG	GGTCGGTCATGTAGCCGTTG

Online Table III: Primers for qRT-PCR.

Online Table IV

Gene	Primer Forward (5'-3')	Primer reverse (5'-3')
PPARG	TTCTCTAGGACTTAACTTCACAGC	TGCAACCACTGGATCTGTTCT
IRS1	CACCCGGTTGTTTTTCGGAG	TTGTGCCGCCACTTCTTCTC
GRB14	ACAATGACCACTTCCCTGCAA	TCCAGGGTTGCCTACCTGT
GLUT4	ATGCTGTGTCTTTGTGTCTGC	ATGGAGCTGGGTCCCTCA
COBLL1	CAAAAGCCAAGGCACCACTT	ACCACTGAGAGTTCAACGTC
ANKRD55	AGGCCAATAAAGCCTGCTCA	TGTGACAGGCACAAGTCAGT
ARL15	CACTTTGCTGCAAGGGACCA	AGGGAGGGGATAAATTGCACA
FAM13A	CCAGGACTTACCCAAGAAGGT	GTGCCCAGCCTAGTAAAGTTGT
FST	CCCACCCTTGTCTCTTCACAG	ACCTTTACAGGGGATGCAGT
LOC646736	TGCAAAACTGGGAGAGCTTCA	CACTAAGGCTCCACTCACCAT
LYPLAL1	CATATTCCCTTTTCCTTTCT	TGAACAGTGATAAATTACCA
MAP3K1	TGTAGCCCGCGAGAGAAAAT	AGCTCCACACTCCGCACTTT
PDGFC	CCAGTCAGCCAAATGAGCCT	ACACACAGCGAGAAACAAGC
PEPD	CTCTGCAGACCCTCGTTTTG	ATCACACACCTGCTCACTTG
RSPO3	TTGTCTCCACAGTGCATCCT	TCGTGTGGGCACTTACTTGT
SLC30A10	ACAATCTGGGAGGCGGGTA	TCCACGAAGATGGTGAAGCA
SYN2	CCAGCCCTTTAAGCCAGA	AGCTGAAGAAGCTGCTGCCCA

Online Table IV: Primers for PCR of targeted genomic site.

FST	**sg25: AAGTGGATGATTTTCAACGG**						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr5:53483023-53483045	+	0	AAGTGGATGATTTTCAACGG	GGG	E	*FST* (on-target)
	chrX:115338062-115338084	+	2	AA**C**TGGATGATTTTCAA**G**GG	TGG	-	*LUZP4*
	sg26: GAGCACCTCGTGGACCGAGG						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr5:53482977-53482999	+	0	GAGCACCTCGTGGACCGAGG	AGG	E	*FST* (on-target)
	sg27: GCGGCCGTTCTTCGCTTGA						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr5:53482896-53482918	-	0	AGCGGCCGTTCTTCGCTTGA	CGG	E	*FST* (on-target)
	chr6:1384949-1384971	+	3	**T**GA**G**GCCG**G**TCTTCGCTTGA	GGG	E	RP4-668J24.2
PDGFC	**sg37: CTGGAATTTACTACTCAGGT**						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr4:156970811-156970833	+	0	CTGGAATTTACTACTCAGGT	TGG	E	*PDGFC* (on-
	chr21:45784281-45784303	-	3	C**A**GGAATTT**T**CTAC**A**CAGGT	AGG	I	*PCBP3*
	chr18:41841371-41841393	-	3	CTG**A**AATTTA**G**TAC**A**CAGGT	AGG	-	*AC011225.1*
	sg38: TCAGCAGGAGAAGCCCGAAG						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr4:156970876-156970898	+	0	TCAGCAGGAGAAGCCCGAAG	AGG	E	*PDGFC* (on-
	chr17:9735253-9735275	-	3	**G**CA**A**CAGGAGAAGCCC**A**AAG	TGG	-	*USP43*
	chr2:165988057-165988079	-	3	TCA**T**CAGG**T**GAAGCC**A**GAAG	AGG	I	*SCN1A*
	chr2:47056412-47056434	+	3	TCA**C**C**T**GGAGAAGCCC**T**AAG	AGG	I	*Metazoa_SRP*
	sg39: CCCCTGTCTCTGGCCGGCCA						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr4:156970847-156970869	+	0	CCCCTGTCTCTGGCCGGCCA	GGG	E	*PDGFC* (on-
	chr20:61504203-61504225	-	3	CCCC**A**GT**G**T**G**TGGCCGGCC	CGG	I	*CDH4*
	chr1:43567087-43567109	+	3	**G**CCCTG**A**CTCTGGC**A**GGCCA	TGG	I	*PTPRF*
	chr22:41431849-41431871	+	3	**G**CCCTGT**G**TCTGGC**A**GGCCA	GGG	-	*CTA-223H9.9*
PEPD	**sg40: TGTGCAGGCCGGCTCCATCG**						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr19:33512655-33512677	-	0	TGTGCAGGCCGGCTCCATCG	TGG	E	*PEPD* (on-target)
	sg41: TGAAACCCTGAAGGTGCCGC						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr19:33512733-33512755	-	0	TGAAACCCTGAAGGTGCCGC	TGG	E	*PEPD* (on-target)
	chr22:44704534-44704556	-	3	TG**G**A**G**CCCTG**C**AGGTGCCG	GGG	I	*PRR5*
	chr17:55561520-55561542	-	2	**G**GAAACCCTGAAGGTGCC**A**C	TGG	E	*CTD-2033D24.2*
	sg42: CAGCGCTACTGCACCGACAC						
	Coordinates (hg38)	**strand**	**MM**	**target_seq**	**PAM**	**Position**	**gene name**
	chr19:33512609-33512631	-	0	CAGCGCTACTGCACCGACAC	CGG	E	*PEPD* (on-target)
	chr17:74222922-74222944	+	3	CAGC**AT**T**T**CTGCACCGACAC	TGG	I	*TTYH2*
	chr17:55561396-55561418	-	1	CAGCGCTACTGCACC**T**ACAC	CGG	E	*CTD-2033D24.2*
	chrY:9015703-9015725	+	3	**G**AG**A**GCTACTGCAC**G**GACAC	AGG	-	*OFD1P3Y*

Mismatched(MM) bases less than four were considered as potential off-targets, indicated in red and listed in the table following by the on-target site. Legend for off-target site positon: E = exonic; I = intronic; - = intergenic.

Online Table V. Predicted off-target sites of the nine sgRNAs by CCTop.

Online Table VI

Position of the off-site	Name of the off-site	Primer Forward (5'-3')	Primer reverse (5'-3')
LUZP4 (-)	sg25-0ff-1	GGCTTGTGTGGGTGTAGGTT	GGACAACCGTAAGTTGGGGA
RP4-668J24.2 (E)	sg27-0ff-1	CAACTCGCCACCCCTAAACG	CCAGGTCTGAGGGTGCTTGA
PCBP3 (I)	sg37-off-1	GTAGCTCTGAATGTGCTGATTC	GAG GTA GGA GGT AGG GGT AG
AC011225.1 (-)	sg37-off-2	AGCTTCTCCCAGGGTATCAA	GCT GAA ATT GAG TAC TGT ATG ATG
USP43 (-)	sg38-off-1	TTCTGGAGCACCAAGTCACG	GCACTTCATGTCTGTACTCAAGTT
SCN1A (I)	sg38-off-2	AGGCAAAGTCAGTCCATTGT	CACAGTGAAGTCCAGTGCAGA
Metazoa_SRP (I)	sg38-off-3	AGCCATGGTGAATTCTTTTGGG	GCCCACTTTCCATGGGCTA
CDH4 (I)	sg39-off-1	CACGCCTGTCTTGGGAACAA	ACCACAATCCGAACATCTGAGT
PTPRF (I)	sg39-off-2	TCCCAAAGACCTTCCTCGTGT	CCAACACCTGGGGACACTCG
CTA-223H9.9 (-)	sg39-off-3	CCCAAGGTTGGGTGGTCAG	ACTTCTGGACAGGTTGCAGG
PRR5 (I)	sg41-off-1	TCTTCTCAGAGTTCAGGGAGGC	CAGTTAAGGGCAGTGAACGGG
CTD-2033D24.2 (E)	sg41-off-2	GTAGCGCTGAGTCTCCTCTT	CCCTTTCTGAGCTTTAATTTTCTCA
TTYH2 (I)	sg42-off-1	GGAATCAGATGCCTGGGGTG	TGGAATTGATTTGTGCTGGAGTC
CTD-2033D24.2 (E)	sg42-off-2	GATGAAGCCGTAGCAGCCT	AAGGAAACCCTGAAGGTGCC
OFD1P3Y (-)	sg42-off-3	AGCACTCACAAACACACAATGC	GCCTGCCTAGTTTTGTAGGG
Predicted off-target site positon is indicated with gene name (E = exonic; I = intronic; - = intergenic).			

Online Table VI. Primers for PCR of predicted off-target genomic sites.

Online Table VII

Gene name	Primer Forward (5'-3')	Primer reverse (5'-3')
FST	GAAGAAATATAAGAGAATTCGCCACCATGGTCCGCGCGAGGCACCAG	CCGCAGAAGGCAGCGGATCCCTACCACTCTAGAATAGAAGA
PEPD	GAAGAAATATAAGAGAATTCGCCACCATGGCGGCGGCCACCGGACCC	CCGCAGAAGGCAGCGGATCCCTACTTGGGGCCAGAGAAGGG
PDGFC	GAAGAAATATAAGAGAATTCGCCACCATGAGCCTCTTCGGGCTTCTC	CCGCAGAAGGCAGCGGATCCCTATCCTCCTGTGCTCCCTCT

Online Table VII. Primers for cloning of mRNA expression constructs.