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Supplementary Information for

p53-Inducible DPYSL4 Associates with Mitochondrial Supercomplexes and Regulates Energy Metabolism in Adipocytes and Cancer Cells

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Supplementary Information Text
Supplementary Material and Methods.

RNA-sequence Analysis and Assessment of Differentially Expressed Genes

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H1299 cells were infected with adenoviruses expressing either LacZ or p53 (p53WT) for 24 h. Human preadipocytes were either untreated (Control) or treated with doxorubicin (ADR; 1 μM) for 24 h. Developing H1299 cells and preadipocytes were harvested, and total RNA was extracted using an RNeasy kit (Qiagen). Total RNA (200 μg) was prepared using the mRNA-seq Sample Preparation Kit according to the manufacturer's instructions (Illumina). A total of 36 bp single-end-read RNA-seq tags were generated using a Hiseq 2000 sequencer according to the standard protocol. The generated sequence tags were mapped onto the human genomic sequence (hg19 from the University of California Santa Clara Genome Browser) using the Eland program (Illumina). Differential expression was estimated and tested with the TCC software package in R-bioconductor 3.1 for each library with reference to the control (1). The sequences reported in this paper have been deposited in the DNA Data Bank of Japan, https://www.ddbj.nig.ac.jp/index-e.html (accession no. DRA007067).

Transcriptional Start Site (TSS) Sequence Analysis

Total RNA (200 μg) was subjected to oligo-capping as published previously with some modifications (2). Approximately 1 ng of size-fractionated cDNA was used for sequencing using an Illumina GAIIx. The generated sequence tags were mapped onto the hg19 human genomic sequence using the Eland program. Unmapped or redundantly mapped sequences were removed from the dataset, and only uniquely mapped sequences without any mismatches were used for analyses.

ChIP-sequence Analysis

ChIP was performed using anti-p53 DO1 (Santa Cruz Biotechnology), anti-p53 18O1 (Santa Cruz Biotechnology), anti-acetylhistone H3 (Millipore), and anti-trimethylhistone H3-K4 (Millipore) antibodies. For ChIP-seq analysis, the immunoprecipitate and input samples were prepared using a ChIP-seq Sample Prep kit (Illumina). Adaptor-ligated DNA fragments were size-fractionated on 12% acrylamide gels, and the 150-250-bp fraction was recovered. This DNA was then amplified by 18 cycles of PCR. A total of 1 ng of DNA was used for the sequencing reaction (GAIIx, Illumina) according to the manufacturer's instructions. An estimated 150,000-250,000 clusters were generated per tile, and 36 cycles were performed for the sequencing reactions. Short-read sequences were aligned to the hg19 human genome sequence using the Eland program. Sequences with no more than two mismatches were used for analysis.

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells, A549 cells, MCF7 cells, and preadipocytes were exposed to the DNA-damaging agents doxorubicin and daunorubicin at the concentration of 0.5 and 1 μM. After 24 h, total RNA was extracted from the cells using an RNeasy kit (Qiagen). The RNA (4 μg) was then reverse-transcribed using the Prime Script first-strand cDNA system (TaKaRa), and the cDNA products were subjected to real-time RT-PCR using the 7500 Fast Real-Time PCR system (Applied Biosystems) with the following primer pairs: DPYSL4, 5′-AGAAAACCTCATCGTCCCTG-3′ and 5′-AGATCATGGTGGTTCCTCCT-3′; GAPDH, 5′-CAACTACATGGTTTACATGTTC-3′ and 5′-GCCAGTGGACTCCACGAC-3′; GLS2, 5′-TGCCTATAGTGGCGATGTCTCA-3′ and 5′-GTTCCATATCCATGGCTGACAA-3′; L32, 5′-

TTCCTGGTCCACAACGTCAAG-3′ and 5′-TGTGAGCGATCTCGGCAC-3′; p21/CDKN1A, 5′-GTTCCTTGTGGAGCCGGAGC-3′ and 5′-GGTACAAGACAGTGACAGGTC-3′; MCP1, 5′-ATCAATGCCCAGTCACCT-3′ and 5′-CTTTGGGACACTTGCTGCT-3′; IFNg, 5′-GGAGACCATCAAGGAAGACA-3′ and 5′-TGCTTTGCGTTGGACATTCA-3′. All gene-specific mRNA expression values were normalized against the internal housekeeping genes L32 or glyceraldehyde phosphate dehydrogenase.

Western Blot Analysis and Antibodies

Human p53, actin, and mitochondrial proteins were detected using the following antibodies: p53 DO1 (Santa Cruz Biotechnology), actin (Sigma), NDUFV1 (Abnova), NDUFA9 (abcam), Complex II 70-kDa Fp Subunit (Invitrogen), SDHB (abcam), UQCRFS1 (Abcam), COX4 (CST), and ATP5O (Abcam). A polyclonal antibody, hDPYSL4, was raised against human DPYSL4 protein using a synthetic peptide corresponding to a sequence within the DPYSL4 C terminus (QADDHIARRTAQKIMAPC) conjugated to keyhole limpet hemocyanin (Cyclex). Human DPYSL4 was also detected using anti-CRMP3 (abcam).

RNA Interference

The siRNA oligonucleotide against *DPYSL4*, *p53*, or *luciferase* mRNA were synthesized by Qiagen to the following target sequences: si-DPYSL4, 5′-CGTGGAAGATGGCTTGATAAA-3′; si-p53, 5′-AACTACTTCCTGAAAACAACG-3′; and si-luciferase, 5′-AACTTACGCTGAGTACTTCGA-3′. HCT116 cells, A549 cells and preadipocytes were transfected with indicated siRNA oligonucleotide using Dharma-fect 1 (Dharmacon) or RNAi MAX (Invitrogen) for 48 h, and then cells were analyzed as described.

Plasmid Construction, Recombinant Virus Production, and Transduction

We used the following PCR primer pair to amplify full-length DPYSL4 from cDNA derived from IMR32 neuroblastoma cells: forward, 5′-TCGGATCCGCCACCATGTCCTCCCAGGGCAAGAAAAGCATCC-3′ and reverse, 5′-CCTCGAGGGAGAGAGAGGTGATGTTGGAGCGGCC -3′. The primer pair contains sequences encoding a BamHI restriction site at the 5′ end of the forward primer and a XhoI restriction site at the 5′ end of the reverse primer for subcloning. We used Gateway Cloning technology (Invitrogen) to subclone the amplified fragments into pcDNA3 vector or CSII-EF-RfA-IRES2-Venus (RDB4389, Riken BioResource Center) expressing the DPYSL4 coding sequence with a FLAG tag. We used PCR to generate dihydropyrimidinase domain (D domain)-deleted mutant, in which 50 amino acids are deleted (LAQVHAENGDIVEEEQKRLLELGITGPEGHVLSHPEEVEAEAVYRAVTIA), from the full-length DPYSL4. We produced recombinant lentiviruses as described previously (3).

Generation of p53 or DPYSL4 knockout preadipocytes

The protocol used for the CRISPR/Cas9 system was consistent with that reported by Cong et al. (4). The backbone vectors pX458 pSpCas9(BB)-2A-GFP was obtained from

Addgene (Cambridge, MA, USA). The target guide RNA sequences were designed at the p53 genome (5'-CGTCGAGCCCCCTCTGAGTC-3') and DPYSL4 genome (5'-CCGGCTGACGACTTCTGTCA-3'). These oligos were annealed and phosphorylated using T4 DNA Ligase Reaction Buffer and T4 Polynucleotide Kinase (New England Biolabs, Ipswich, MA, USA) at 37℃ for 30 min and 95℃ for 5 min. pX458 was digested using BbsI (Thermo Scientific, Yokohama, Japan) at 37℃ for 30 min, and gel purification was performed using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). The ligation reactions of pX458 and the annealed oligos were performed at room temperature for 10 min using a Quick Ligation Kit (New England Biolabs, Ipswich, MA, USA). Then, the ligated oligos were purified using PlasmidSafe exonuclease (Cambio, Cambridge, UK) at 37℃ for 30 min. The plasmids were transfected into Stbl3, and the appropriate transfectants were amplified and collected using NucleoBond Xtra Midi (Takara, Kusatsu, Japan). Each plasmid was transfected into preadipocytes using Neon Transfection System (Thermo Scientific, Yokohama, Japan) according to the manufacturer's protocol. The plasmid-expressing preadipocytes were selected by GFP soating using BD FACS Aria Ⅲ ether to establish the monoclonal cell lines or to directly subject to each experiments including mitochondrial fractionation and flux-analyzer.

Preparation of Mitochondrial Fractions

H1299 cells and preadipocytes were collected on ice and then centrifuged at 1,500 rpm for 5 min at 4°C. Cell pellets were washed in ice-cold phosphate-buffered saline (PBS) solution without Ca^{2+} or Mg^{2+}, gently resuspended in 500 µL sucrose buffer (0.07 M sucrose; 10 mM Hepes, pH 7.4; 0.1 mM EDTA; 0.22 M mannitol), and then homogenized in a Dounce tissue homogenizer on ice. The resulting homogenate was centrifuged at 500 x *g* for 15 min at 4°C to remove unlysed cells and nuclei. The supernatant was then centrifuged at 10,000 x *g* for 15 min at 4°C to collect the mitochondrial fraction, which was then resuspended in 500 µL sucrose buffer for storage at -80 °C. We determined the protein concentration of the mitochondrial fraction using the BCA Protein Assay (Thermo).

Blue Native Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The mitochondrial pellet (50 µg) was suspended in 10 µL of buffer containing 50 mM bisTris (pH 7.0), 1 M 6-aminocaproic acid, and 2% dodecyl-β-D-maltoside. After 20 min at 4ºC, solubilized proteins were obtained in the supernatant fraction centrifugation at 22,000 x *g*. Solubilized proteins were supplemented with 0.5 µL sample buffer (5% G-250 in 0.5 M 6-aminocaproic acid). We performed 2D BN/SDS-PAGE that involves first dimensional BN-PAGE (8–12%) followed by second dimensional SDS-PAGE (10%). The cathode buffer consisted of 50 mM Tricine, 15 mM bisTris (pH 7.0), and 0.02% G-250 in the inner buffer chamber. The anode buffer consisted of 50 mM bisTris (pH 7.0) in the outer buffer chamber. We performed the first-dimension electrophoresis on ice (100 V for 10 min, followed by 200 V for 20 min and 500 V for 60 min). For the second-dimension SDS-PAGE, we sliced strips from the first-dimension gel and performed the second electrophoresis on ice as previously described (5).

Immunostaining

Human dermal fibroblasts (HS68) and preadipocytes were plated on slides in 4-well chambers and cultured in Dulbecco's modified Eagle' medium with 10% FBS and antibiotics. HDF cells were treated with daunorubicin (Dauno, 1 μM) for 24 h and then incubated with Mitotracker for 15 min. The cells were then fixed and stained using anti-DPYSL4 antibody and visualized using Alexa Fluor 488-conjugated secondary antibodies. Nuclei were counterstained with DAPI and then analyzed by confocal microscopy. Preadipocytes were treated with desferrioxamine (DFX, 300μM) for 12 h, then fixed and stained using an anti-DPYSL4 antibody and an anti-NDUFA9 antibody. H1299 cells were either mock-infected or infected with lentivirus expressing DPYSL4 for 3 d and then subjected to mitochondrial fractionation. Immunoblotting was performed to detect DPYSL4-FLAG and the indicated components of mitochondrial complex proteins in the cytosolic and mitochondrial fractions. After 24 h, cells were washed and then fixed with 4% paraformaldehyde. Slides were then re-fixed in 100% ethanol at –20ºC for 10 min, followed by incubation with blocking solution. The slides were incubated primary antibodies against DPYSL4 and Mitotracker (Invitrogen) for HS68, and primary antibodies against DPYSL4 and NDUFA9 for preadipocytes. Signals were detected using labeled rabbit or mouse anti-goat IgG secondary antibodies (Invitrogen). Nuclei were visualized by 4',6-diamidino-2-phenylindole (DAPI) staining. Stained cells were analyzed using a confocal laser microscope (LSM710, Carl Zeiss).

Metabolome Analysis

To extract metabolites, HCT116 cells in a 100-mm dish were incubated with 1 ml methanol containing 25 μM each of methionine sulfone, 2-(N-morpholino)-ethanesulfonic acid, and D-camphor-10-sulfonic acid, washed with 5% mannitol, and then incubated for 10 min at 4°C. The samples were then mixed with deionized water and chloroform at a volumetric ratio of 2:1:2 and centrifuged at 10,000 x *g* for 3 min at 4°C. Then, the aqueous solution was passed through a 5-kDa cutoff filter (Millipore) to remove proteins. The filtrate was then concentrated by centrifugation and dissolved in 25 μl deionized water containing 200 μM each of 3-aminopyrrolidine and 1,3,5-benzenetricarboxylic acid immediately before CE-TOFMS analysis.

Measurement of Mitochondrial Respiration

H1299 cells, HCT116 cells, and preadipocytes were cultured overnight on XF^{e}96 plates, and then medium was replaced for measurement of mitochondrial respiration (Seahorse Bioscience) 1 h before the start of the measurement. For the H1299 cells, oligomycin (2 μM), carbonilcyanide p-triflouromethoxyphenylhydrazone (FCCP, 0.5 μM), antimycin (1 μM), and rotenone (1 μM; Sigma) were added to XF^{e}96 media, and the samples were loaded into the sensor cartridge. For the HCT116 cells, oligomycin (2 μM), FCCP (0.25 μM), antimycin (1 μM), and rotenone (1 μM) were added to the media. For the adipocytes, oligomycin (1 μM), FCCP (1 μM), antimycin (1 μM), and rotenone (1 μM) were added to the media. We injected the components into the wells at the time points specified. We monitored the OCR using a Seahorse Bioscience XF^{e}96 Extracellular Flux Analyzer (Primetech Corporation). The maximal respiration was also calculated using the OCR after FCCP injection subtracted from the OCR after rotenone and antimycin injection. ATP production was measured using the mean OCR measurement prior to the

addition of oligomycin subtracted from the OCR measurement after the addition of oligomycin.

Measurement of Levels of Intracellular NADH
NADH levels were measured using an NAD^+/NADH quantification kit (Biovision) according to the manufacturer's instructions.

Cell Invasion Assay
Cells were mock-infected or infected with lentiviruses expressing either p53, FL-DPYSL4, or DPYSL4Δmid for 72 h. Then, the cells were subjected to a Matrigel invasion assay. The *in vitro* invasive properties of DPYSL4 in H1299 cells were studied using BD BioCoat Tumor Invasion Assay System (BD Bioscience) according to the manufacturer's instructions. Approximately 4×10^4 DPYSL4-overexpressing H1299 cells were seeded in serum-free media in the upper chamber of the system. Bottom wells were filled with complete media. After incubating for 24 h, the cells in the upper chamber were removed, and the cells that had invaded through the Matrigel membrane were stained with crystal violet solution. Invading cells were counted in 4 random fields under a microscope. The invasion index was expressed as the ratio of invading cells relative to the control inserts.

Xenograft Tumorigenesis and Metastasis Analysis
For tumorigenesis analysis, a total volume of 0.1 ml containing 1×10^5 DPYSL4-overexpressing H1299 cells were administrated by subcutaneous injection to six-week-old NOD/SCID male mice (CLEA Japan). Tumor diameters were measured once a week to monitor the growth of primary tumors. Mice were sacrificed 8 weeks after injection, and tumor volume was calculated according to the tumor length and width. For lung metastasis analysis, 1×10^6 DPYSL4-overexpressing H1299 cells were injected into the tail vein. After 10 weeks, lungs were stained with Bouin's solution, and the number of lung metastases was determined.

DPYSL4 Expression and Prognosis Database
The gene expression level of DPYSL4 was analyzed by Oncomine (https://www.oncomine.org/) (6). A database was established using DPYSL4 expression data downloaded from the cancer genome Atlas (TCGA) (http://cancergenome.nih.gov/). The prognostic significance of the mRNA expression was evaluated using the Kaplan-Meier plotter (www.kmplot.com), an online database including gene expression and clinical data of breast cancer (7) and ovarian cancer (8).

Statistical Analysis
Multivariate statistics and associated graphics were performed in R, version 3.1.2. All data are presented as means ± standard derivation. A two-sided Student's *t*-test was used for comparison between two groups. Differences between individual groups were tested using ANOVA. The heat map was drawn using the dendrogram and heatmap.2 function in the gplots package. For all tests, a *p*-value of <0.05 was considered statistically significant.

Human Adipose Tissue

The ethical committee of Chiba University Graduate School of Medicine reviewed and approved the study protocol. A total of 26 subjects who were admitted to Chiba University Hospital and underwent surgery for aldosterone-producing adenoma were enrolled. Perirenal fat from human aldosterone-producing tumors was fixed with formalin, stained with hematoxylin and eosin. and subjected to histological analysis. We obtained informed consent from all subjects. Human p53 and CD68 were detected by immunohistology using anti-p53 DO7 (Nichirei Biosciences) and anti-CD68 PG-M1 (Nichirei Biosciences) antibodies.

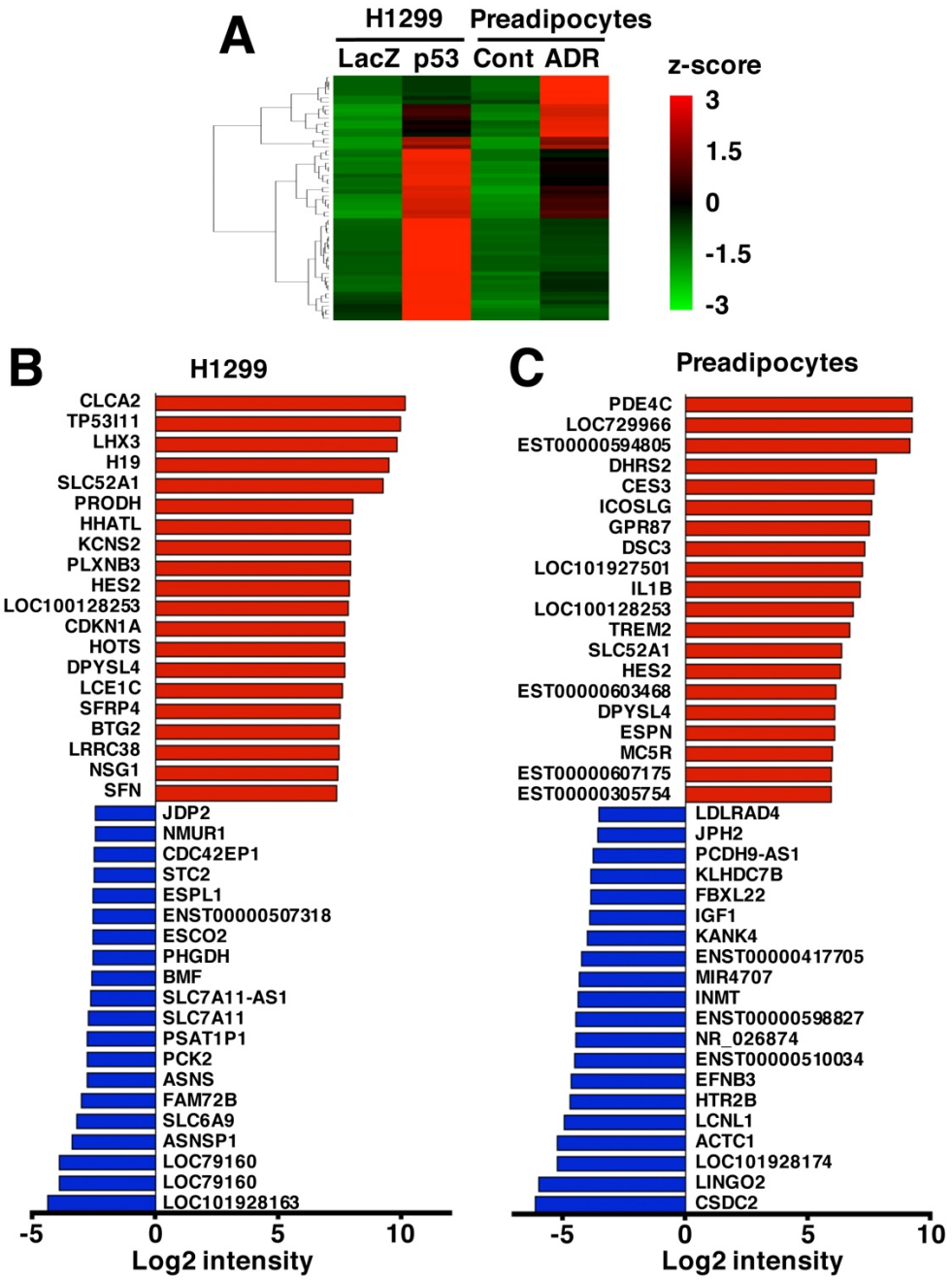

Fig. S1.

Genome map images of p53-inducible genes in H1299 cells and preadipocytes. (A) Heat map with dendrogram (arranged by unsupervised hierarchical clustering) showing z-scores for rpkm of differentially expressed genes from H1299 cells and preadipocytes. (B) p53-dependent induction of proline dehydrogenase (PRODH), acetylcholine esterase (ACHE), glutaminase 2 (GLS2), and phosphodiesterase 4C (PDE4C) in H1299 cells. (C) Doxorubicin-dependent induction of PRODH, ACHE, GLS2, and PDE4C in preadipocytes.

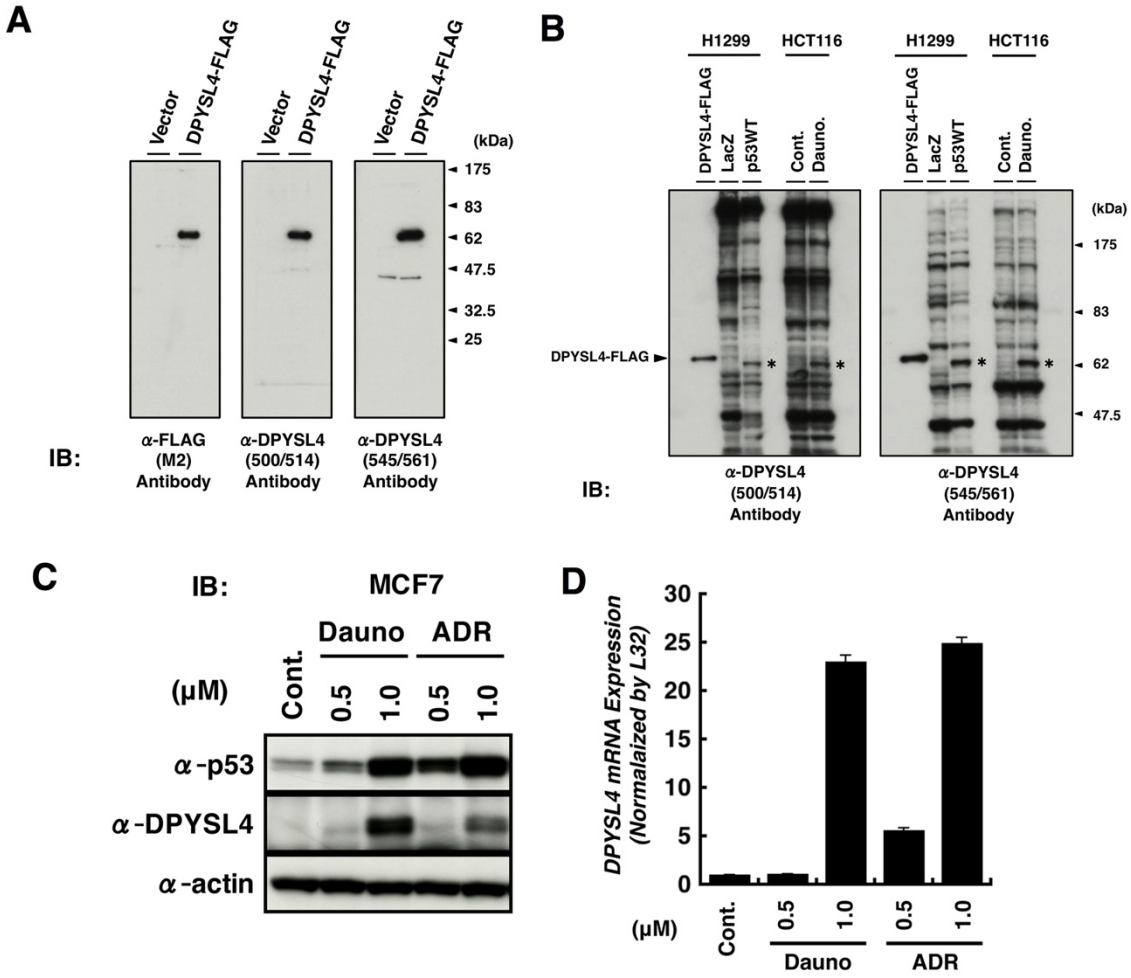

Fig. S2.

Expression of human DPYSL4 and generation and characterization of anti-human DPYSL4 antibodies. (A) Overexpression of human DPYSL4-FLAG in H1299 cells. (B) Immunoblotting of endogenous DPYSL4 in H1299 and HCT116 cells. (C, D) MCF-7 cells were treated with the indicated dose of ADR or Dauno as above. Immunoblotting and real-time RT-PCR analysis to determine the expression levels of *DPYSL4* mRNA were performed.

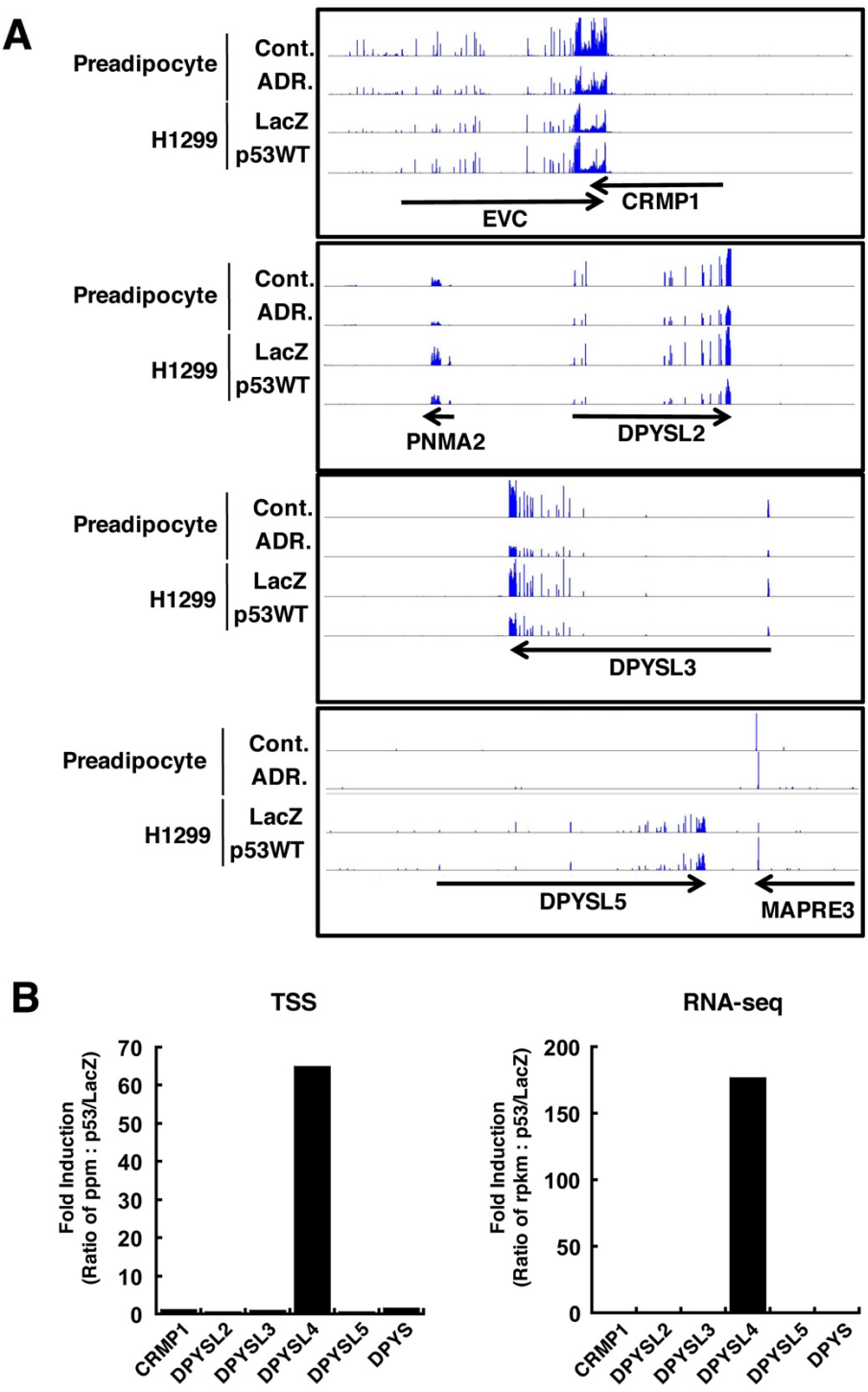

Fig. S3.

DPYSL4 is the only p53-inducible gene in the CRMP family. (A) Expression of CRMP1, DPYSL2, DPYSL4, and DPYSL5 in H1299 cells and preadipocytes following p53 overexpression and doxorubicin (ADR) treatment. (B) Fold induction of the CRMP family and dihydropyrimidinase (DPYS) by TSS and RNA-seq analysis.

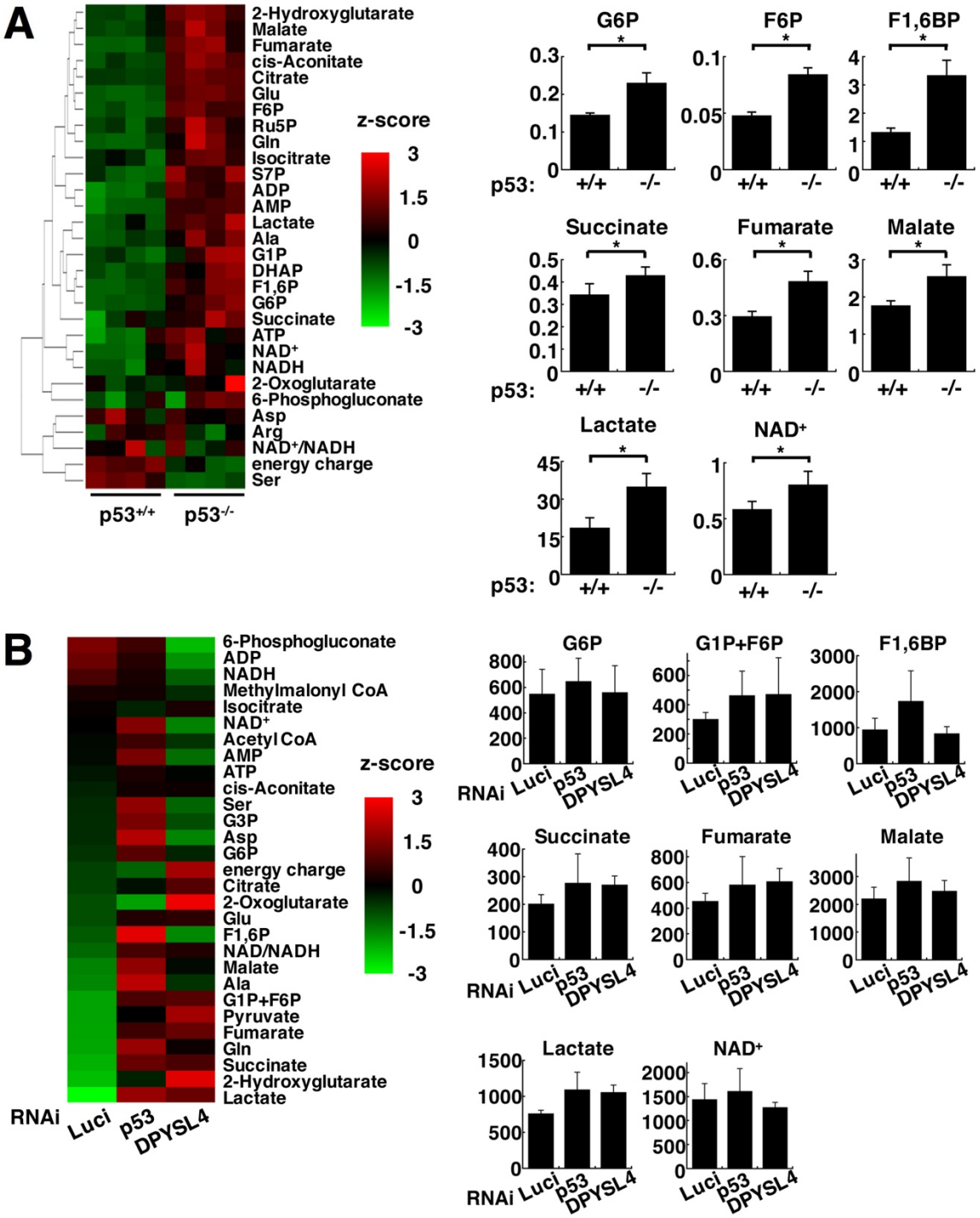

Fig. S4.

DPYSL4 knockdown altered the regulation of central carbon metabolism. (A) Metabolite concentrations in HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells were determined by metabolome analysis. A heat map and dendrogram (arranged by unsupervised hierarchical clustering) show the z-scores for metabolites of glycolysis, the TCA cycle, and the pentose phosphate pathway. Histograms show the average concentration (pmol/1000 cells) of selected

metabolites with error bars in HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells. (B) HCT116 $p53^{+/+}$ cells were treated with Luci, p53, or DPYSL4 RNAi for 48 h and then switched to fresh medium. The cells were then subjected to metabolome analysis. A heat map with dendrogram (arranged by unsupervised hierarchical clustering) shows z-scores for metabolites of glycolysis, the TCA cycle, and the pentose phosphate pathway. Histograms show the average concentration of selected metabolites (pmol/1000 cells) with error bars.

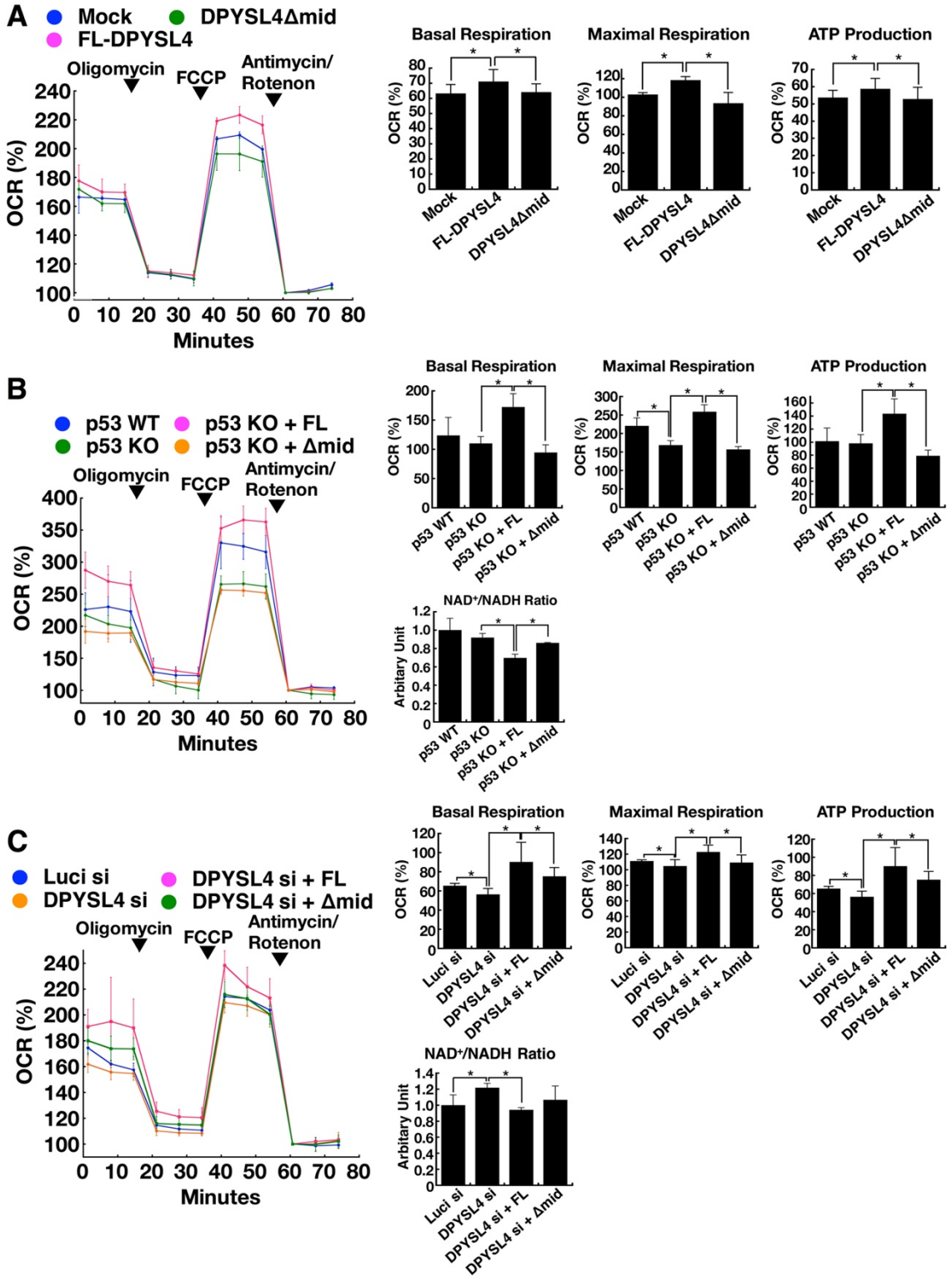

Fig. S5.

DPYSL4 is a potential regulator of ATP production and oxidative phosphorylation in preadipocytes. (A) After preadipocytes overexpressed mock, full-length DPYSL4 (FL), or DPYSL4Δmid (Δmid) for 72 h, the oxygen consumption rate (OCR) was measured at

baseline and after treatment with oligomycin (1 μM), FCCP (1 μM), and a mixture of antimycin (1 μM) and rotenone (1 μM). Basal respiration, maximal respiration, and ATP production were calculated in these cells (*p<0.05). (B) After p53KO-preadipocytes overexpressed FL, or Δmid for 72 h, the OCR was measured at baseline and after treatment with oligomycin (1 μM), FCCP (1 μM), and a mixture of antimycin (1 μM) and rotenone (1 μM). Similarly, NAD^+/NADH was measured. (C) After preadipocytes were treated with Luci, DPYSL4 RNAi, cotransfect DPYSL4 RNAi and FL, or Δmid for 48 h, the OCR was measured at baseline and after treatment with oligomycin (1 μM), FCCP (1 μM), and a mixture of antimycin (1 μM) and rotenone (1 μM). Similarly, NAD^+/NADH was measured.

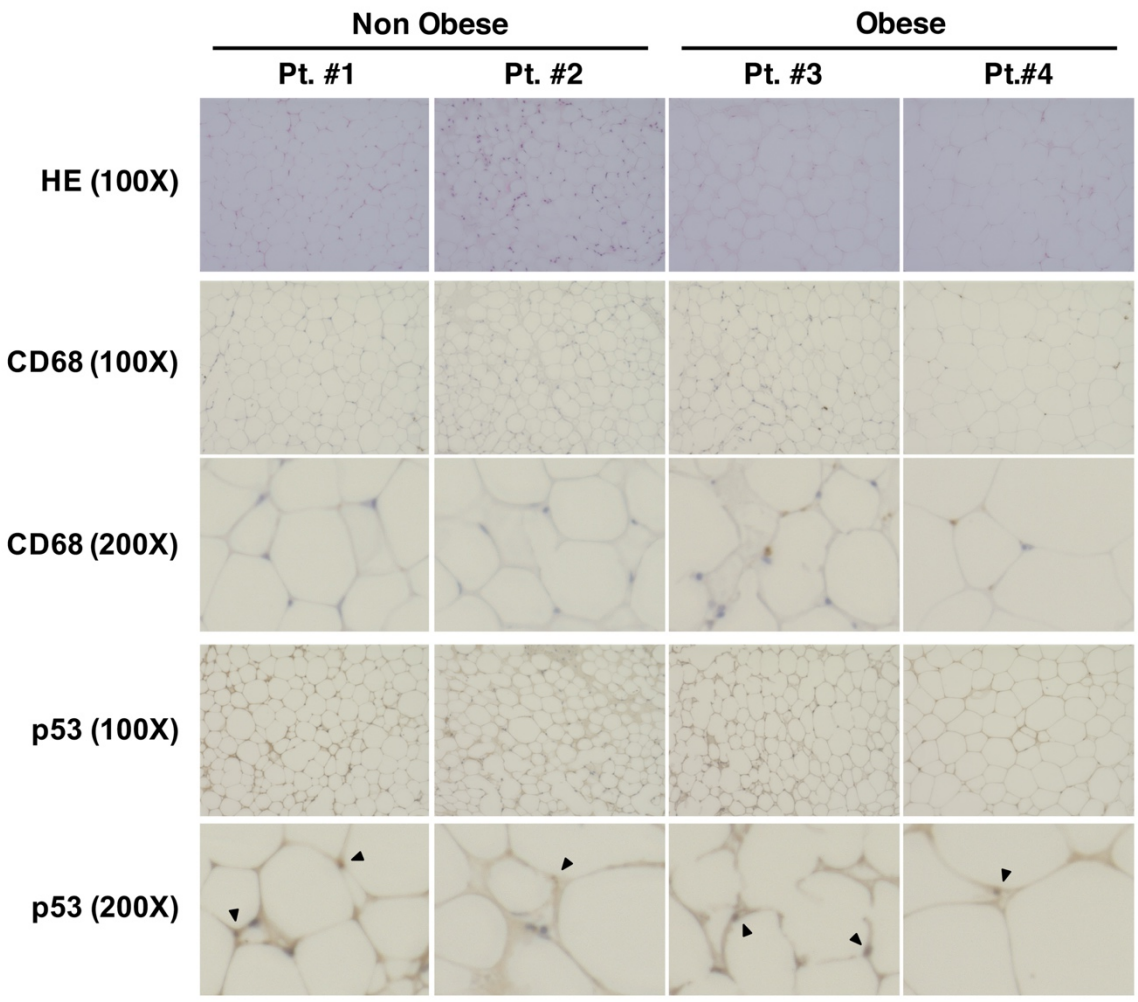

Fig. S6.

Expression of CD68 and p53 in adipose tissue from non-obese and obese patients. Human adipose tissue was stained with hematoxylin and eosin (magnification 100×) and also subjected to immunohistochemistry for p53 and CD68 (magnification 100× and 200×).

Table S1. Sixty genes are common potential p53 targets in both H1299 cells and preadipocytes.

Total RNA derived from H1299 cells and preadipocytes was subjected to RNA-seq. Differentially expressed genes were determined using the TCC software package in R-bioconductor 3.1. q. value <0.05 was considered statistically significant.

	H1299						preadipocyte					
symbol	LacZ rpkm	p53WT rpkm	fold	a.value	m.value	q.value	cont rpkm	ADR rpkm	fold	a.value	m.value	q.value
DPYSL4	0.33	62.8	190.30	8.17	7.70	4.56E-17	0.21	14.98	71.33	7.55	6.08	2.70E-10
CLCA2	0	10.61		4.94	10.16	4.56E-17	0.09	2.9	32.22	6.32	4.93	1.11E-06
LHX3	0	13.17		-0.99	9.83	4.76E-17	0	0.33		-1.00	4.99	3.28E-03
BTG2	0.56	88.79	158.55	8.79	7.45	6.93E-17	4.85	31.05	6.40	10.31	2.65	1.91E-02
SLC52A1	0.03	18.75	625.00	5.48	9.25	1.05E-16	0	0.93		3.24	6.37	2.62E-05
NSG1	0.23	36.73	159.70	7.58	7.43	5.04E-16	0.14	7.3	52.14	6.78	5.64	1.17E-08
HES2	0.05	12.43	248.60	6.39	7.89	8.82E-16	0	0.51		3.21	6.33	3.37E-05
PRODH	0.04	12.22	305.50	5.46	8.04	1.08E-14	0.02	0.71	35.50	3.53	4.96	8.12E-04
SFN	0.12	19.01	158.42	5.55	7.39	3.28E-13	0.03	2.41	80.33	3.98	5.85	8.51E-06
CYFIP2	2.53	155.05	61.28	11.61	6.08	4.48E-13	0.74	12.84	17.35	9.65	4.08	1.24E-05
LOC102724404	2.15	130.47	60.68	10.01	6.06	7.09E-13	1.42	11.4	8.03	8.67	2.97	4.91E-03
LOC100128253	0.03	7.47	249.00	4.78	7.85	4.11E-12	0	1.37		-1.00	6.85	2.27E-07
ACHE	0.95	44.75	47.11	8.87	5.68	1.85E-11	0.07	4.28	61.14	6.09	5.74	1.70E-08
LYNX1	0.16	7.31	45.69	7.13	5.61	2.22E-10	2.49	17.19	6.90	10.42	2.75	1.10E-02
ENST00000517634	0.21	15.32	72.95	5.29	6.23	4.43E-10	0.02	1.71	85.50	2.97	5.83	4.33E-04
KCNS2	0.01	2.87	287.00	3.83	7.93	4.55E-10	0.01	0.47	47.00	3.44	4.78	1.91E-03
FDXR	14.21	435.39	30.64	12.07	5.08	5.40E-10	11.06	69.06	6.24	11.28	2.61	2.16E-02
ESPN	0.13	5.6	43.08	6.18	5.47	2.00E-09	0	0.52		-1.00	6.07	1.96E-05
LINC01021	0	1.97		-0.99	7.13	1.30E-08	0.14	1.36	9.71	5.47	3.23	9.83E-03
VWCE	0.27	7.48	27.70	6.89	4.89	2.27E-08	0.41	4.51	11.00	7.52	3.43	7.31E-04
S1PR3	0.02	1.62	81.00	4.44	6.00	2.96E-08	0.29	2.63	9.07	7.20	3.13	3.42E-03
GPR87	0.24	8.25	34.38	5.64	5.22	5.00E-08	0.01	3.23	323.00	3.79	7.48	3.75E-08
RRAD	13.2	225.04	17.05	10.86	4.23	2.49E-07	3.65	33.57	9.20	9.29	3.17	1.66E-03
WDR63	0.24	5.5	22.92	6.34	4.63	2.92E-07	0.59	3.54	6.00	7.39	2.54	4.27E-02
LOC101927501	0.01	1.95	195.00	3.29	6.87	6.40E-07	0	1.82		3.66	7.21	2.13E-07
PIDD1	9.23	132.49	14.35	11.25	3.99	1.27E-06	4.39	31.15	7.10	10.39	2.79	9.20E-03
GLS2	2.64	37.56	14.23	9.30	3.97	1.91E-06	0.26	3.94	15.15	6.73	3.85	1.40E-04
AKR1B10	0	1.86		-0.99	6.32	2.54E-06	0.75	8.45	11.27	7.27	3.45	7.89E-04

DOK7	0.12	2.51	20.92	5.66	4.43	4.31E-06	0.04	1.34	33.50	5.12	4.97	8.20E-06
DKK1	5.61	71.21	12.69	9.71	3.81	5.03E-06	7.18	43.72	6.09	10.25	2.57	2.61E-02
GDF15	48.74	584.79	12.00	12.19	3.73	6.65E-06	132.48	1023.81	7.73	14.04	2.92	4.87E-03
NTN1	4.64	53.82	11.60	11.09	3.68	1.00E-05	0.31	19.83	63.97	9.16	5.92	1.96E-10
TREM2	0	2.37		-0.99	6.08	1.11E-05	0	2.73		-1.00	6.72	5.32E-07
KITLG	0.68	8.18	12.03	8.22	3.71	1.42E-05	1.3	13.02	10.02	9.74	3.29	8.55E-04
POU3F1	0	0.82		-0.99	6.02	1.48E-05	0.04	2.24	56.00	5.16	5.58	4.15E-07
ACER2	0.72	8.84	12.28	7.38	3.76	1.74E-05	0.23	3.94	17.13	6.71	4.03	5.85E-05
ENST00000563205/ ACER2	1.23	14.25	11.59	7.58	3.67	2.47E-05	0.38	5.4	14.21	6.75	3.79	1.94E-04
DOCK8	0.04	0.74	18.50	4.97	4.23	4.76E-05	0.04	1.48	37.00	6.29	4.86	1.66E-06
ENST00000428151	0.23	4.54	19.74	4.05	4.38	1.97E-04	0.74	14.78	19.97	6.44	4.28	2.58E-05
PPP1R14C	0.16	2.19	13.69	4.96	3.85	3.14E-04	0.18	1.88	10.44	5.68	3.25	6.88E-03
CARNS1	0.31	3.02	9.74	6.50	3.38	3.45E-04	0.02	0.71	35.50	4.40	4.69	1.67E-04
NEFL	0.62	4.98	8.03	7.29	3.15	6.25E-04	1.03	33.92	32.93	9.76	5.00	3.75E-08
PDE4C	0.67	5.15	7.69	7.97	3.08	6.51E-04	0.05	37.71	754.20	8.38	9.26	1.36E-19
PSTPIP2	0.79	6.26	7.92	7.28	3.13	6.85E-04	2.24	12.72	5.68	9.27	2.47	4.04E-02
LOC729966	0.46	3.51	7.63	7.98	3.06	7.14E-04	0.03	25.69	856.33	8.38	9.26	1.36E-19
ENST00000607175	2.31	19.2	8.31	6.45	3.18	1.02E-03	0.03	2.93	97.67	3.01	5.92	2.83E-04
ENST00000339812/ HIST1H2BJ	7.08	49	6.92	8.89	2.93	1.21E-03	0.26	6.46	24.85	5.79	4.56	1.96E-05
LOC102725254	2.95	20.85	7.07	7.78	2.96	1.40E-03	0.26	156.21	600.81	8.22	9.17	2.58E-19
SLC4A11	5.7	37.61	6.60	10.23	2.86	1.56E-03	0.59	3.83	6.49	7.68	2.64	2.65E-02
HOXC13	2.19	14.56	6.65	8.29	2.87	1.97E-03	0.02	0.71	35.50	3.53	4.96	8.12E-04
AK296186	0.65	5.04	7.75	5.98	3.08	2.26E-03	1.34	44.11	32.92	8.78	5.00	5.41E-08
SLC8A3	0.02	0.38	19.00	3.80	3.88	2.82E-03	0.01	0.23	23.00	3.84	3.57	3.04E-02
FAM196A	3.07	18.95	6.17	9.60	2.77	2.83E-03	0.48	7.07	14.73	8.28	3.85	5.87E-05
ENST00000411643	0.12	4.85	40.42	2.55	5.38	3.09E-03	0	1.91		-1.00	4.48	2.55E-02
PADI3	0.21	1.63	7.76	5.47	3.04	5.17E-03	0.01	0.91	91.00	3.92	5.73	1.65E-05
FAM105A	1.8	9.36	5.20	9.39	2.52	1.02E-02	0.02	0.54	27.00	5.12	4.13	3.09E-04
CYGB	0.37	2.45	6.62	5.60	2.83	1.20E-02	2.31	13.92	6.03	8.88	2.55	3.07E-02
HES6	11.25	47.9	4.26	9.61	2.23	3.87E-02	1.56	12.11	7.76	7.92	2.92	7.00E-03
ENST00000420134	0.64	3.05	4.77	7.64	2.27	4.30E-02	0.18	1.6	8.89	4.29	4.48	4.75E-04
HIST1H2BJ	8.56	37.4	4.37	6.10	2.39	4.30E-02	0.2	5.09	25.45	5.48	3.04	2.11E-02

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