Supplemental Figures

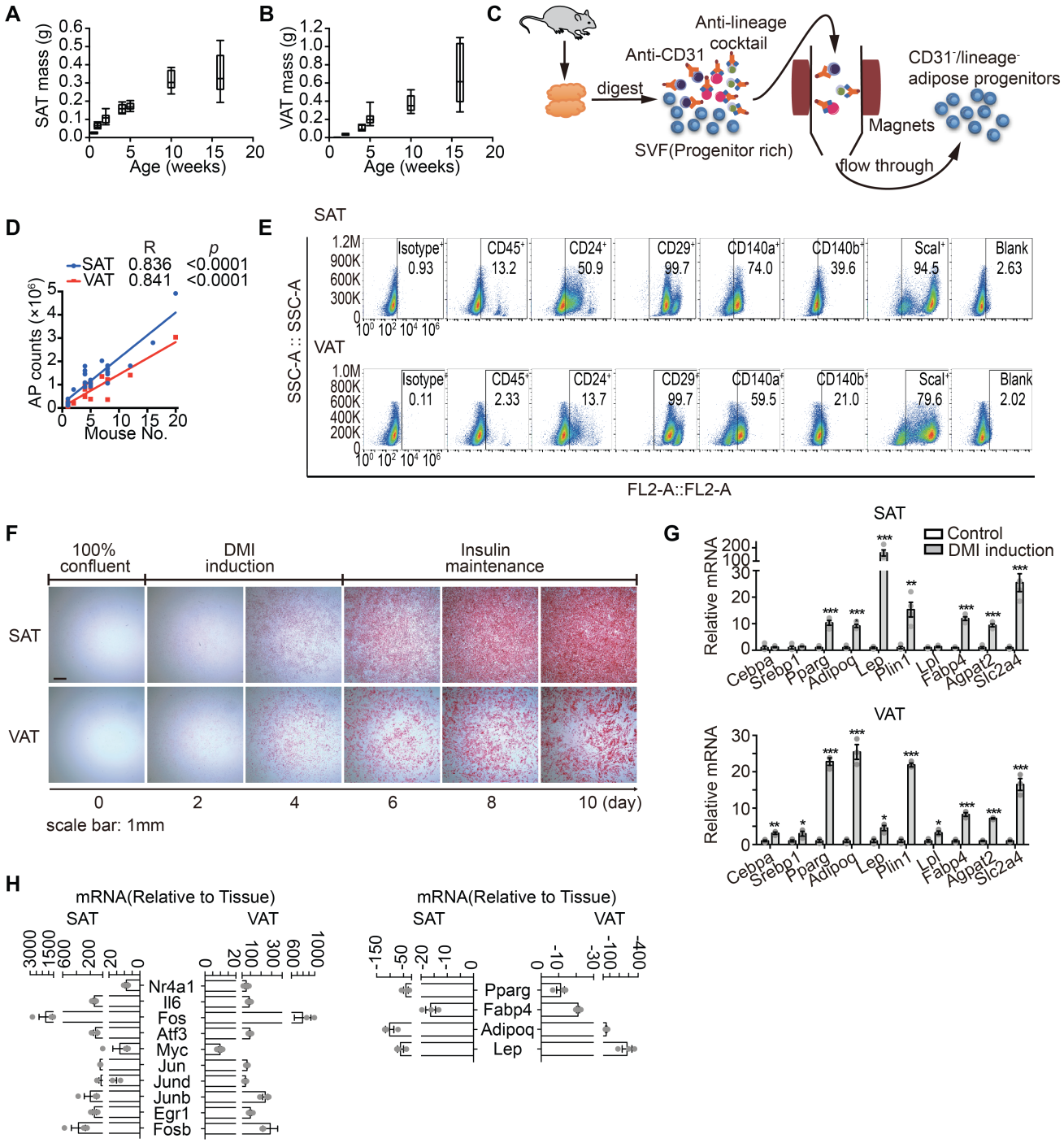

Figure S1. Isolation of adipose progenitors (APs) from mouse adipose tissue (AT).

(A) AT weight from the inguinal (SAT) depots as a function of post-natal age.

(B) AT weight from the perigonadal (VAT) depots as a function of post-natal age. For (A) and (B), data expressed box (line = mean) and whiskers (min to max); n = 5-10 mice per time point.

(C) Schematic diagram of the isolation of APs from mouse adipose tissue by column-based MACS® technology. SVF: stromal vascular fraction.

(D) The number of isolated APs was plotted as a function of the number of mice sacrificed. We obtain approximately 0.2 million and 0.14 million APs for SAT and VAT per mouse respectively.

(E) Flow cytometry analysis of the expression of cell surface markers in freshly isolated APs.
(F) Oil red O staining of primary APs at different days after *ex vivo* induction of adipogenesis by differentiation medium (DMI: dexamethasone 1 μM, isobutyl-1-methyl xanthine 0.5 mM, Insulin 10 μg/ml).
(G) q-PCR analysis of relative expression of late stage adipogenic genes in primary APs with/out DMI induction. Data expressed as mean ± SEM with individual data points displayed as dots, n=4, two-tailed unpaired t-test, **p<0.05, **p<0.01, ***p<0.001*.
(H) q-PCR of freshly isolated APs, in which Actinomycin D(1μg/ml) was added to the buffer during the isolation. The bar graph shows the immediate early genes and terminal adipocyte markers relative to unfractionated AT. Data expressed as mean ± SEM with individual data points displayed as dots, n=3.

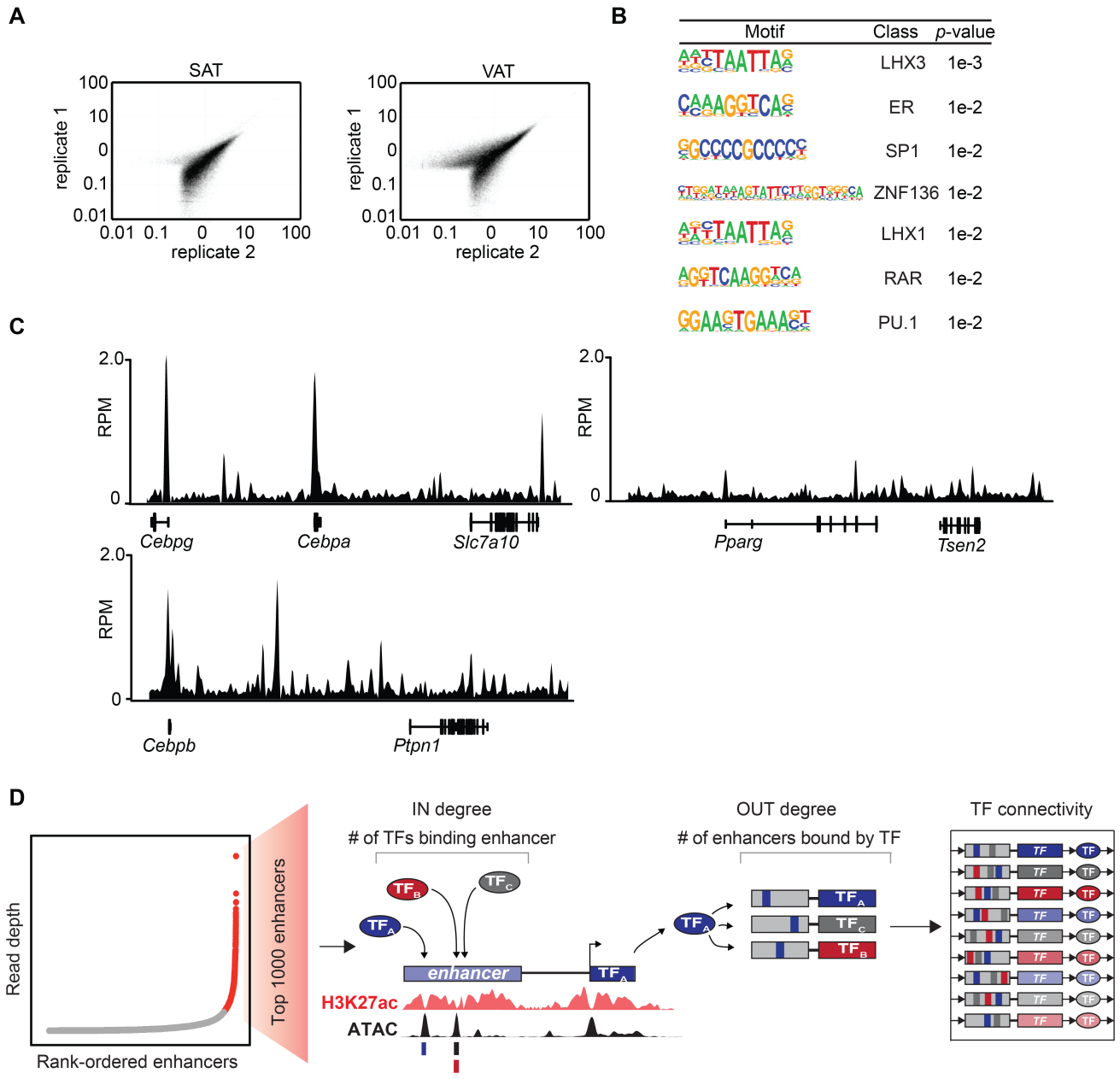

Figure S2. ATAC-seq analyses of APs.

(A) Scatter plot comparing enrichment of ATAC between biological replicates from SAT (left) and VAT (right).

(B) Motifs are enriched in APs relative to whole AT. This list is distinct from the motifs reported in Figure 2C, which were specific to APs (peaks not reported in whole AT).

(C) Gene tracks of ATAC enrichment (in reads per million, RPM) at the *Cebpba*, *Cebpb* and *Pparg* loci. APs isolated from SAT.

(D) Schematic of core circuitry logic.

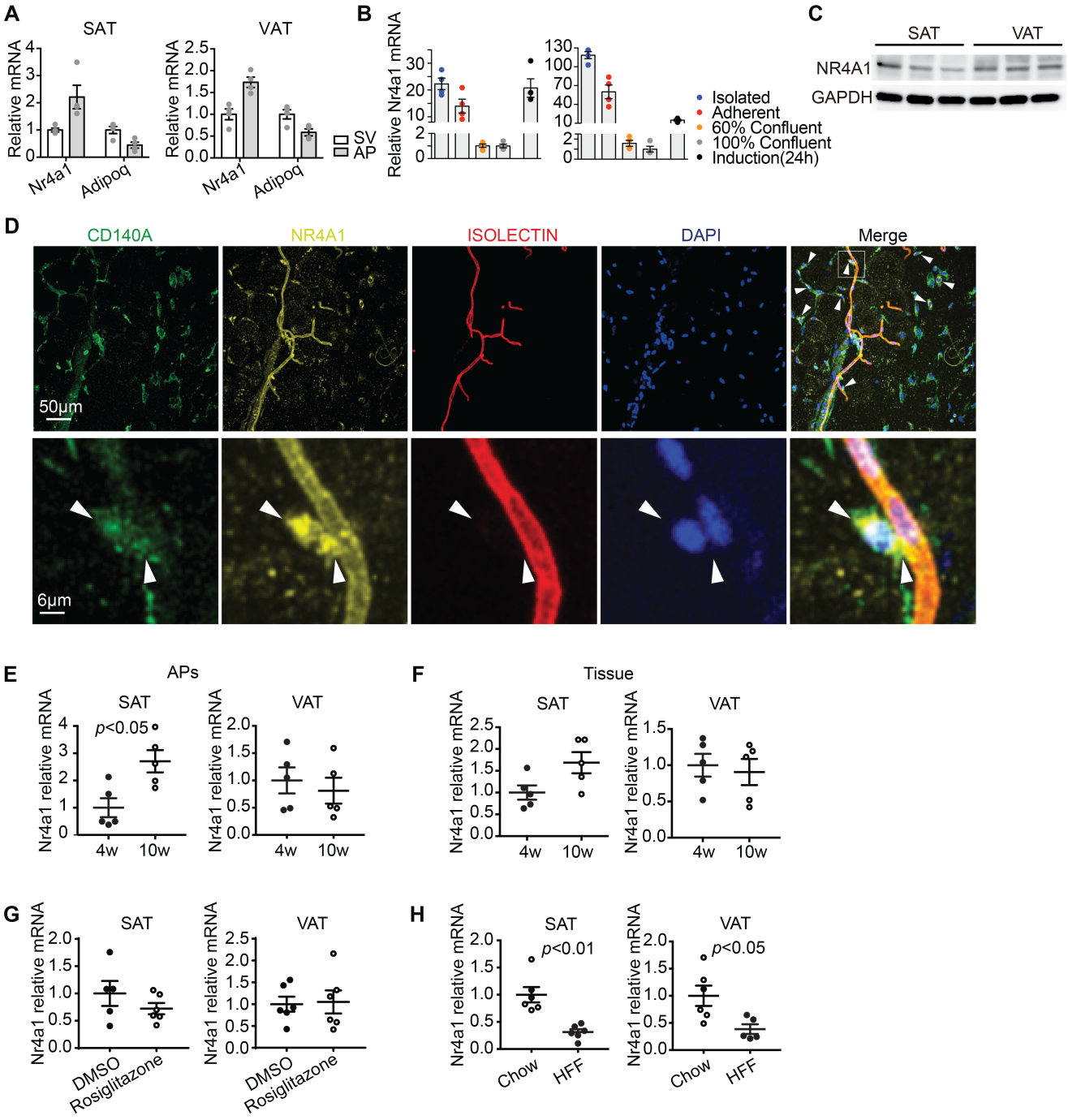

Figure S3. Constitutive and dynamic expression of Nr4a1 in adipose tissue and APs.

(A) q-PCR analysis of *Nr4a1* expression in adipose progenitors and stromal vascular fractions. adipocyte progenitor (AP), and stromal vascular fraction (SV). Data expressed as mean ± SEM with individual data points displayed as dots, normalized to SV, n=4, two-tailed unpaired t-test, **p<0.05*.

(B) q-PCR time-course of *Nr4a1* expression in primary APs during *ex vivo* expansion and after DMI induction, Data expressed as mean ± SEM with individual data points displayed as dots, relative to 100% confluent group, n=4.

(C) Western blot analysis of NR4A1 expression in adipose tissue (n=3 mice).

(D) Co-Immunostaining of CD140a (PDGFRα), NR4A1, Isolectin GS-IB_4 in VAT. White arrows: NR4A1 positive, Isolectin negative cells.
(E) q-PCR analysis of *Nr4a1* expression in APs from 10-week (10w) old mice, relative to 4-week (4w) old mice.
(F) q-PCR analysis of *Nr4a1* expression in AT from 10-week (10w) old mice, relative to 4-week (4w) old mice.
(G) q-PCR analysis of *Nr4a1* expression in adipose tissue from mice treated with rosiglitazone(3mg/kg/day) for 2 weeks relative to vehicle control (DMSO).
(H) q-PCR analysis of *Nr4a1* expression in adipose tissue from mice fed high fat diet for 2 months, relative to control chow.
(E)-(H), data expressed as mean ± SEM with individual data points shown as dots, n=5, two-tailed, unpaired t-test.

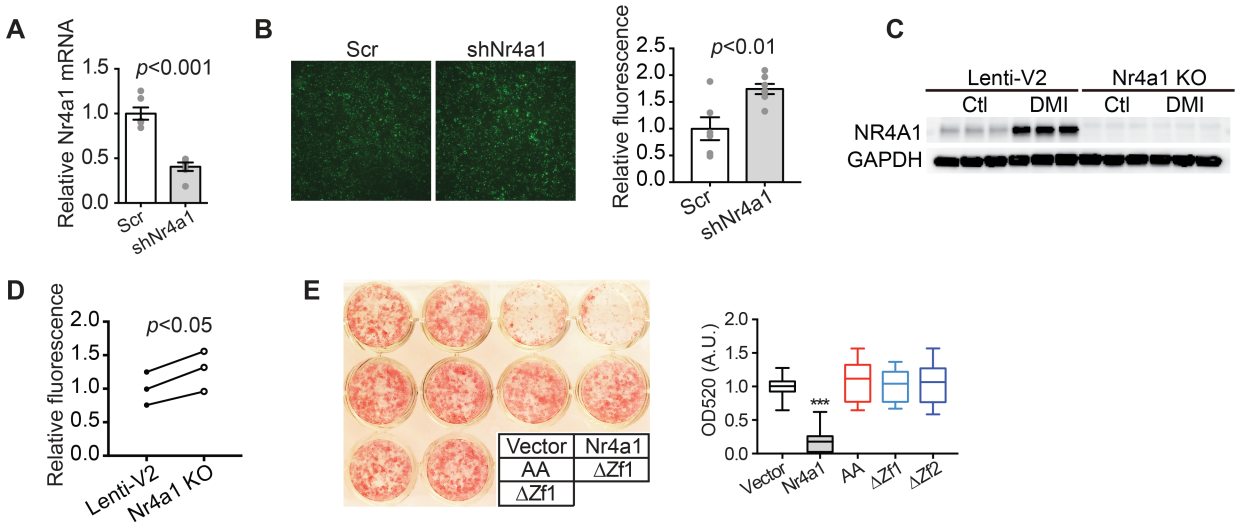

Figure S4. Nr4a1 inhibits adipogenesis in 3T3-L1 cells.

(A) q-PCR analysis of relative expression of Nr4a1 in 3T3-L1 cells after infection with Nr4a1 shRNA retrovirus. Data expressed as mean ± SEM with individual data points shown as dots; n=6 technical replicates, two-tailed unpaired t-test.

(B) Lipid droplet assay by Nile Red staining shown by representative images and relative quantification in 3T3-L1 cells exposed to retroviral mediated knockdown of Nr4a1 followed by adipogenesis induction. Data expressed as mean ± SEM with individual data points shown as dots, n=6-7 technical replicates, two-tailed unpaired t-test.

(C) Western blot validation of NR4A1 expression after 1-hour DMI induction in 3T3-L1 cell line with or without Nr4a1 deletion. The *Nr4a1* null line was generated by CRISPR/Cas9.

(D) Quantification of lipid droplets by Nile Red staining in 3T3-L1 cell line with or without *Nr4a1* deletion. Three independent experiments are shown, significance assessed by two-tailed paired t-test.

(E) Oil Red O staining of 3T3-L1 cells, retrovirally transduced to express wild-type Nr4a1 or DNA-binding-deficient mutants during *in vitro* induction of adipogenesis. Data expressed as box (mean line) and whiskers (min to max), n=4 biological replicates, ****p<0.001*.

A

SAT				VAT			
Leading edge gene sets and signals	NES	Overlap		Leading edge gene sets and signals	NES	Overlap	
GO_ORGANELLE_FISSION	3.4233916	63%	42/67	GO_ORGANELLE_FISSION	2.941127	75%	64/85
GO_CELL_CYCLE	3.3553636	76%	81/107	GO_CHROMOSOME_SEGREGATION	2.919203	80%	51/64
GO_CELL_CYCLE_PROCESS	3.3468845	80%	76/95	GO_MITOTIC_NUCLEAR_DIVISION	2.9181871	76%	58/76
GO_MITOTIC_NUCLEAR_DIVISION	3.2362642	67%	41/61	GO_CELL_DIVISION	2.9050794	71%	60/84
GO_MITOTIC_CELL_CYCLE	3.172809	69%	60/87	GO_SISTER_CHROMATID_SEGREGATION	2.8723314	81%	43/53
GO_CELL_DIVISION	3.0784602	58%	38/65	GO_CELL_CYCLE	2.836243	59%	98/165
GO_CHROMOSOME_SEGREGATION	2.935299	84%	43/51	GO_MITOTIC_CELL_CYCLE	2.801657	64%	80/125
GO_NUCLEAR_CHROMOSOME_SEGREGATION	2.8158023	83%	39/47	GO_CELL_CYCLE_PROCESS	2.7998304	61%	91/150
GO_SISTER_CHROMATID_SEGREGATION	2.7796566	82%	37/45	GO_NUCLEAR_CHROMOSOME_SEGREGATION	2.7876327	79%	45/57
GO_CHROMOSOME_ORGANIZATION	2.6439912	72%	49/68	GO_MICROTUBULE_BASED_PROCESS	2.7813902	82%	37/45

NES: Normalized Enrichment Score . The Nominal p-value and false discovery rate(FDR) in all of the gene set enriched are less than 0.001.

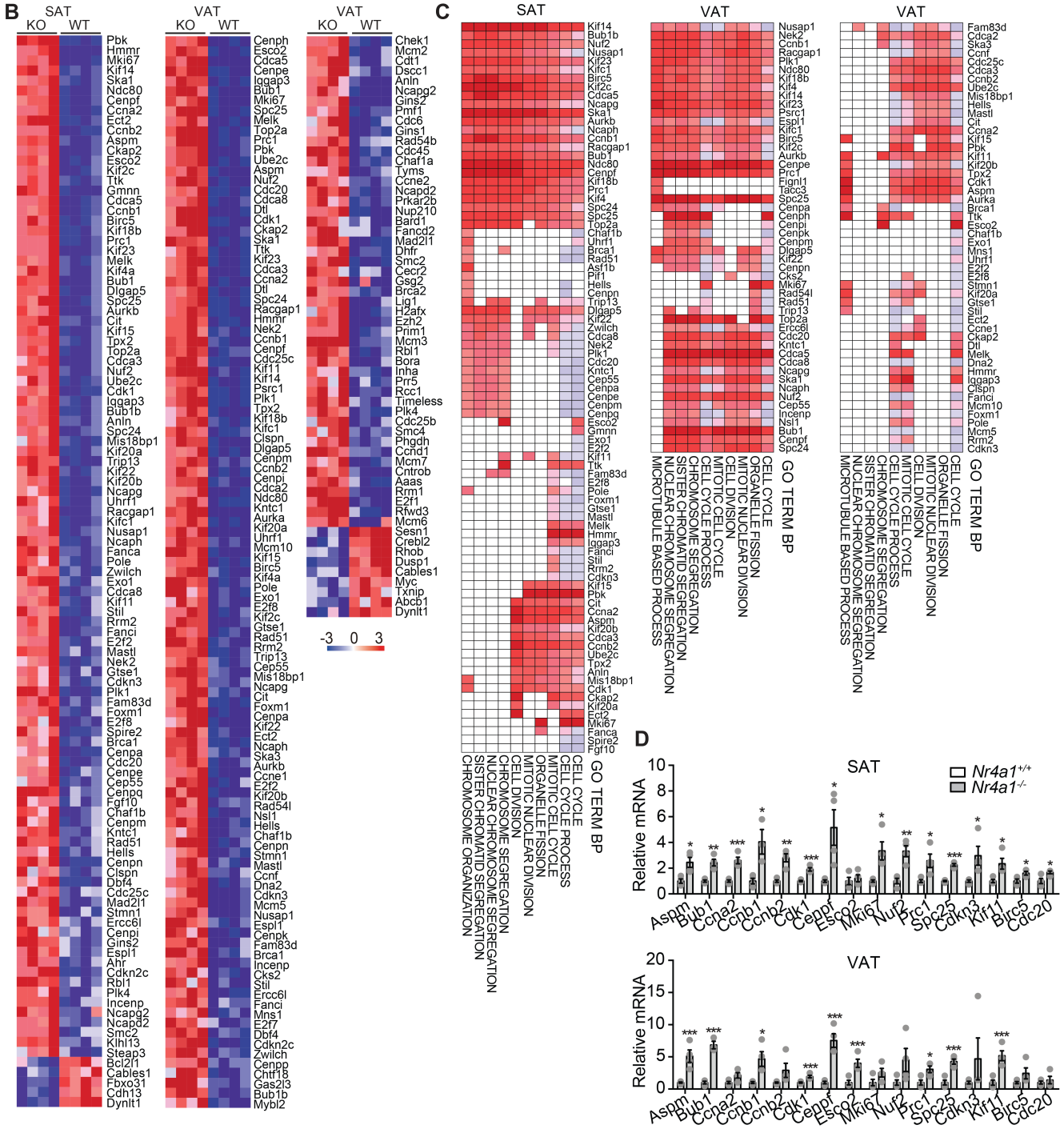

Figure S5. NR4A1 control of AP self-renewal.

(A) Summary of selected leading-edge enriched gene sets and signals in APs isolated from *Nr4a1*$^{-/-}$ (KO) relative to *Nr4a1*$^{+/+}$ (WT) mice based on GSEA (ranked according to decreasing NES). Analysis parameters: Nr4a1 KO versus WT, c5.bp.v6.0.symbols.gmt, permutation type=gene set, number of permutations=1000.
(B) Heat map of the core enrichment genes corresponding to cell cycle signal in *Nr4a1*$^{-/-}$ APs (KO) versus *Nr4a1*$^{+/+}$ APs (WT).
(C) Leading-edge analysis of RNA-seq data sets demonstrating gene expression patterns in the top 10 enriched pathways in APs from Nr4a1 KO relative to WT mice.
(D) q-PCR validation of cell cycle gene expression in APs isolated from SAT and VAT of *Nr4a1*$^{+/+}$ and *Nr4a1*$^{-/-}$ mice, expressed as mean ± SEM with individual data points shown as dots; n=4 mice*;* two-tailed unpaired t-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

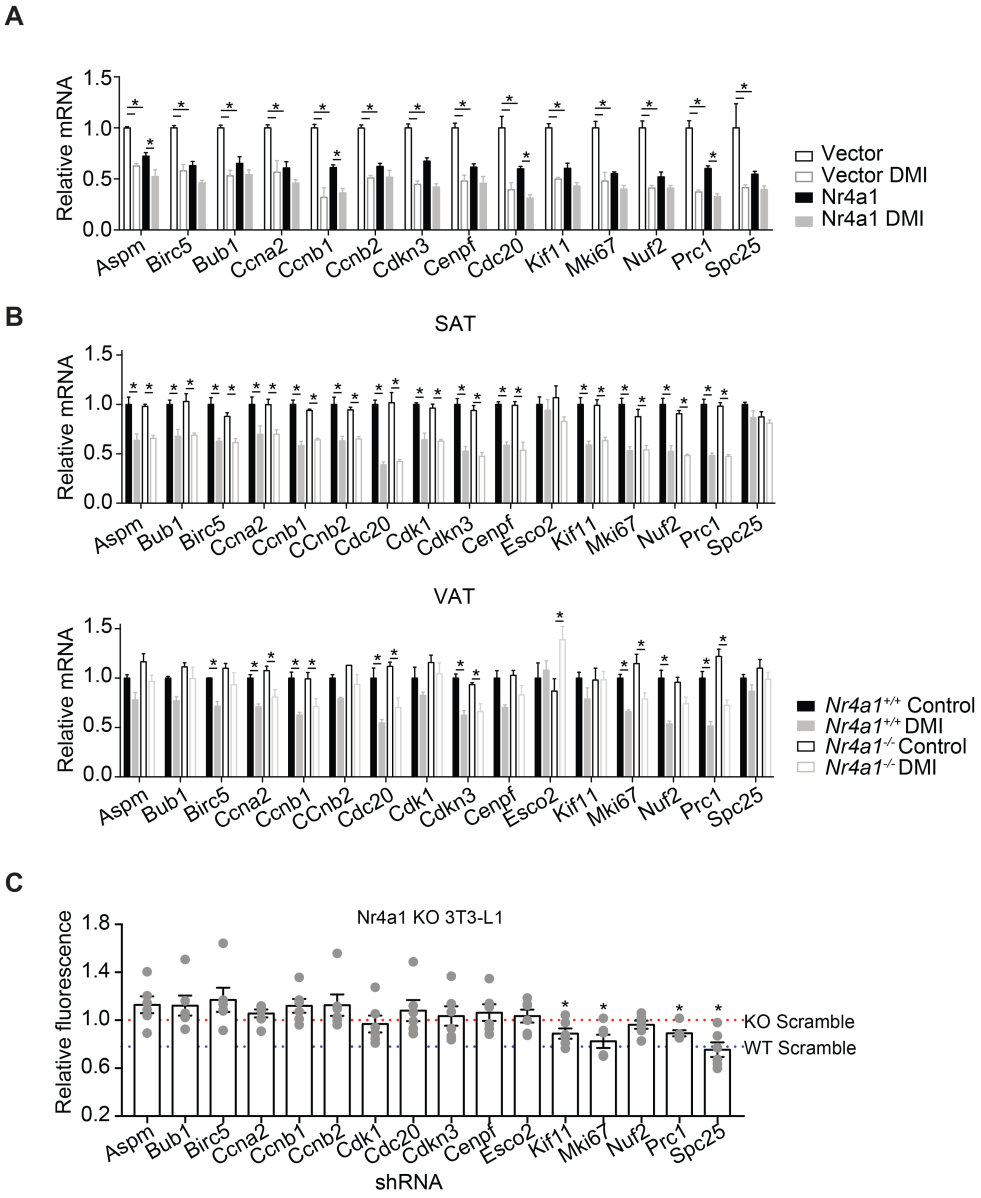

Figure S6. NR4a1 regulates cell cycle gene expression during adipogenesis.

(A) q-PCR analysis of cell cycle genes in APs isolated from SAT and transduced with retroviral vectors driving overexpression of Nr4a1 (MSCV-Nr4a1) or control (MSCV-Vector), expressed as mean ± SEM; n=4 technical replicates; two-tailed unpaired t-test, **p<0.05*.

(B) q-PCR analysis of cell cycle genes in primary cultured APs isolated from SAT and VAT *of* *Nr4a1*$^{+/+}$ and *Nr4a1*$^{-/-}$ mice, expressed as mean ± SEM, n=4 technical replicates.

(C) Quantification of lipid droplets by Nile Red staining in *Nr4a1* knockout 3T3-L1 cell line transduced with retroviral vectors driving knockdown of cell cycle genes, n=6 independent experiments. **p<0.05*. The data were normalized to knockout cells transduced with scramble control shRNA (red dashed line) and expressed as mean ± SEM with individual data points shown as dots. The blue dashed line shows relative lipid staining of wild-type control line transduced with scramble control shRNA.

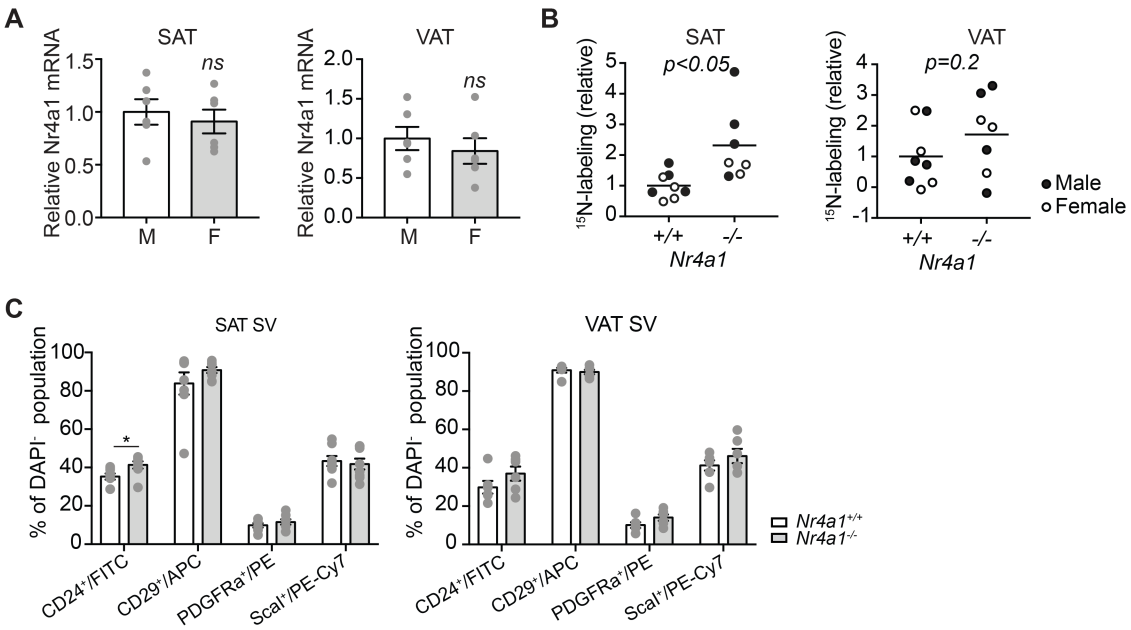

Figure S7. Nr4a1 loss of function *in vivo* and progenitor dynamics.

(A) Expression of Nr4a1 in the mature adipose tissue of male and female mice as assessed by qPCR, n=5 mice per group. Data expressed as mean ± SEM with individual data points shown as dots.

(B) ^{15}N-thymidine 2-week pulse and 4-week chase in mixed gender cohort. Adipocyte labeling measured by IRMS. SAT *p=0.01*; VAT *p=0.25*, two-tailed unpaired t-test.

(C) Flow cytometry for AP cell surface markers in stromal vascular cells isolated from 4-wk old *Nr4a1*$^{+/+}$ and *Nr4a1*$^{-/-}$ mice, n=6~8 mice, two-tailed unpaired t-test, **p<0.05*.

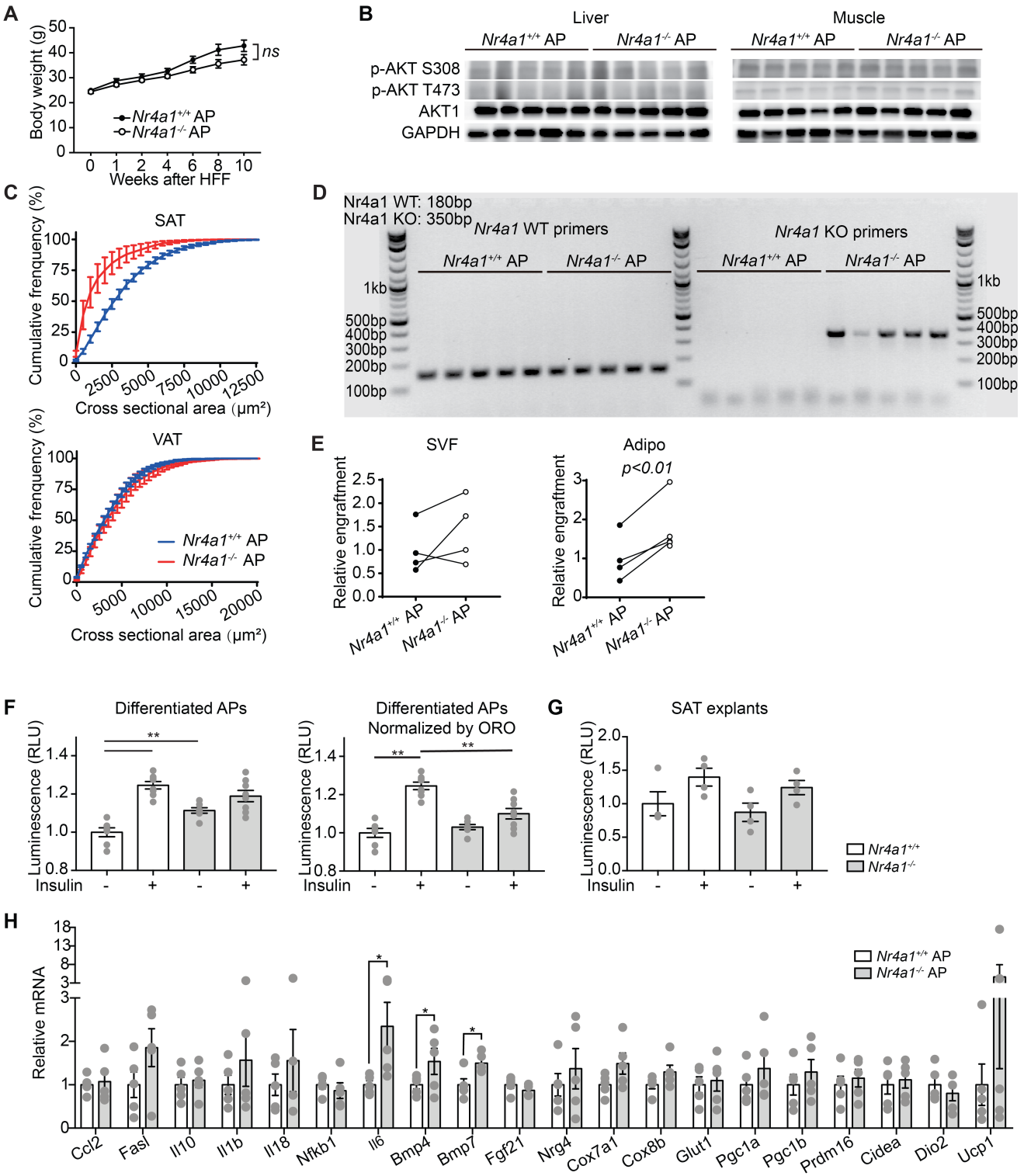

Figure S8. NR4A1 regulates AP function and systemic glucose homeostasis in obesity.
(A) Weekly weight of the mice with *Nr4a1*$^{+/+}$ or *Nr4a1*$^{-/-}$ AP transplantation over 10-weeks of high fat feeding (HFF); n=5, data expressed as mean ± SEM. Two-way ANOVA, Sidak's multiple comparisons correction.
(B) Western blotting for AKT pathway activity in liver and skeletal muscle from *Nr4a1*$^{+/+}$ or *Nr4a1*$^{-/-}$ AP transplanted mice, n=5.

(C). Cumulative frequency distribution of cross section area of adipocytes of SAT and VAT from AP transplanted mice. n=5 mice, data expressed as mean ± SEM.
(D) PCR of genomic DNA isolated from SAT from AP transplanted mice.
(E) Donor APs from male *Nr4a1*$^{+/+}$ or *Nr4a1*$^{-/-}$ were transplanted into the contralateral inguinal depots of female recipients in two weekly doses (1x10^{6} cells/dose). Two weeks after the first injection, the stromal vascular (SVF) or adipocyte fractions (Adipo) were isolated and relative engraftment assayed qPCR in genomic DNA. n=4 mice, significance assessed by two-tailed paired t-test.
(F) Glucose uptake assay in adipocytes differentiated from APs isolated from *Nr4a1*$^{+/+}$ and *Nr4a1*$^{-/-}$ mice. Data expressed as mean ± SEM with individual data points shown as dots. n=7-8 technical replicates. One-way ANOVA, Turkey's multiple comparisons test**.** **p<0.05, **p<0.01.*
(G) Glucose uptake assay in SAT explants from *NR4a1*$^{+/+}$ and *Nr4a1*$^{-/-}$ mice. Data expressed as mean ± SEM with individual data points shown as dots; n=4 mice; one-way ANOVA, Turkey's multiple comparisons test**.**
(H) q-PCR analysis of SAT from AP transplanted mice. Data expressed as mean ± SEM with individual data points shown as dots; n=5 mice; two-tailed unpaired t-test, **p<0.05.*

Table S1. Ct value of TFs for whole AT

Gene	SAT	VAT
Fos	25.900	23.900
Fosb	31.725	28.950
Egr1	24.325	22.175
Nr4a1	27.200	24.225
Nr4a3	23.475	23.525
Junb	24.625	23.325
Atf3	26.825	25.075
Jun	23.475	21.850
Zfp36	22.400	21.325
Myc	34.275	32.400
Klf4	24.825	24.150
Jund	26.575	24.725
Notch3	27.225	25.050
Cebpd	27.550	26.950
Nr4a2	21.775	22.100
Id3	26.160	24.239
Osr2	27.148	26.342
Tnfaip3	27.700	26.125
Twist2	27.275	26.025
Sox9	27.975	22.725
Tcf21	31.125	24.150
Aff2	28.393	26.224
Pparg	24.150	23.575
Cebpa	23.375	22.400
Fabp4	16.475	15.725
Agpat2	22.450	22.375
Adipoq	18.650	18.025
Lept	23.450	21.100
Plin1	20.150	19.375

Table S2

Reference	Comparison	Gene	Reference data	Our data			
			Adipogenic / non-adipogenic	SAT (AP/AT)		VAT (AP/AT)	
				FC	*p*	FC	*p*
Tang et al., 2008 Science	Pparg-GFP^{+} SV vs. Adipocytes (Fresh isolated)	Pref1	+	1.1894	0.0018	1.1351	0.0534
		GATA3	+	1.0173	0.4664	1.1624	0.0041
		Wisp2	+	1.176	0.0014	1.2254	0.0073
		Gli3	+	1.354	0.0011	1.3561	0.0002
		Smo	+	1.1451	0.0105	1.071	0.0036
		Gsc	+	1.0743	0.024	1.0747	0.0981
		Twist2	+	1.3978	0.0164	1.2673	0.004
		Mmp3	+	1.2462	0.0056	1.2664	0.0128
		Egfr	+	1.2369	0.0191	1.2246	0
		Fgf10	+	-1.1543	0.0145	-1.0815	0.0601
		Cebpa	-	-1.4674	0.0001	-1.475	0.0002
		Acc	-	-1.6034	0.0002	-1.6124	0.0001
		Fas	-	-1.0724	0.1177	-1.0698	0.0307
		Adipsin	-	-1.9174	0.0007	-1.821	0.0005
		Lep	-	-2.3089	0.0001	-2.3203	0

FC: fold change; *p*: p value; Yellow shading: directionally concordant genes; Blue shading: non-concordant of genes.

Table S3. q-PCR primers list

Gene	Sense (5' to 3')	Antisense (5' to 3')
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Adipoq	TGTTCCTCTTAATCCTGCCCA	CCAACCTGCACAAGTTCCCTT
Aff2	GCAGCAGTGTCACTATGAACA	TAGGGCTCCCCAAAAAGATCA
Agpat2	CAGCCAGGTTCTACGCCAAG	TGATGCTCATGTTATCCACGGT
Aspm	TGGCTATGAGTGAATGCTCTTCC	TGGCTATGAGTGAATGCTCTTCC
Atf3	GAGGATTTTGCTAACCTGACACC	TTGACGGTAACTGACTCCAGC
Bcl2	AAGCTGTCACAGAGGGGCTA	CAGGCTGGAAGGAGAAGATG
Birc5	GAGGCTGGCTTCATCCACTG	CTTTTTGCTTGTTGTTGGTCTCC
Bmp4	TTCCTGGTAACCGAATGCTGA	CCTGAATCTCGGCGACTTTTT
Bmp7	ACGGACAGGGCTTCTCCTAC	ATGGTGGTATCGAGGGTGGAA
Bub1	AGAATGCTCTGTCAGCTCATCT	TGTCTTCACTAACCCACTGCT
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ccna2	AAGAGAATGTCAACCCCGAAAAA	ACCCGTCGAGTCTTGAGCTT
Ccnb1	AAGGTGCCTGTGTGTGAACC	GTCAGCCCCATCATCTGCG
Ccnb2	GCCAAGAGCCATGTGACTATC	CAGAGCTGGTACTTTGGTGTTC
Cdc20	TTCGTGTTCGAGAGCGATTTG	ACCTTGGAACTAGATTTGCCAG
Cdk1	AGAAGGTACTTACGGTGTGGT	GAGAGATTTCCCGAATTGCAGT
Cdkn3	ACCCTGATACATTGTTACGGAGG	CTCGAAGGCTGTCTATGGCTT
Cebpa	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
Cebpd	CGACTTCAGCGCCTACATTGA	CTAGCGACAGACCCCACAC
Cenpf	GCACAGCACAGTATGACCAGG	CTCTGCGTTCTGTCGGTGAC
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC
Cox7a1	GCTCTGGTCCGGTCTTTTAGC	GTACTGGGAGGTCATTGTCGG
Cox8b	TGTGGGGATCTCAGCCATAGT	AGTGGGCTAAGACCCATCCTG
Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
Egr1	TCGGCTCCTTTCCTCACTCA	CTCATAGGGTTGTTCGCTCGG
Esco2	GACCTATAAGCCAGTTGTGGAC	TCCGCCTTGGAGTGTAACTTG
Fabp4	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Fasl	TCCGTGAGTTCACCAACCAAA	GGGGGTTCCCTGTTAAATGGG
Fgf21	GCCTTGAAGCCGGGAGTTATT	GTGGAGCGATCCATACAGGG
Fos	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
Fosb	TTTTCCCGGAGACTACGACTC	GTGATTGCGGTGACCGTTG
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Glut1	TCAACACGGCCTTCACTG	CACGATGCTCAGATAGGACATC
Id3	CTGTCGGAACGTAGCCTGG	GTGGTTCATGTCGTCCAAGAG
Il-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Il-18	GACTCTTGCGTCAACTTCAAGG	CAGGCTGTCTTTTGTCAACGAn
Il-1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT

Il-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Jun	CCTTCTACGACGATGCCCTC	GGTTCAAGGTCATGCTCTGTTT
Junb	TCACGACGACTCTTACGCAG	CCTTGAGACCCCGATAGGGA
Jund	GAAACGCCCTTCTATGGCGA	CAGCGCGTCTTTCTTCAGC
Kif11	GGCTGGTATAATTCCACGCAC	CCGGGGATCATCAAACATCTG
Klf4	GTGCCCCGACTAACCGTTG	GTCGTTGAACTCCTCGGTCT
Lep	GAGACCCCTGTGTCGGTTC	CTGCGTGTGTGAAATGTCATTG
Lpl	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG
Mki67	ATCATTGACCGCTCCTTTAGGT	GCTCGCCTTGATGGTTCCT
Myc	ATGCCCCTCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
Nfkb1	ATGGCAGACGATGATCCCTAC	TGTTGACAGTGGTATTTCTGGTG
Notch3	TGCCAGAGTTCAGTGGTGG	CACAGGCAAATCGGCCATC
Nr4a1	TTGAGTTCGGCAAGCCTACC	GTGTACCCGTCCATGAAGGTG
Nr4a1 KO	CCACGTCTTCTTCCTCATCC	CACGAGACTAGTGAGACGTG
Nr4a1 WT	CCACGTCTTCTTCCTCATCC	TGAGCAGGGACTGCCATAGT
Nr4a2	GTGTTCAGGCGCAGTATGG	TGGCAGTAATTTCAGTGTTGGT
Nr4a3	AGGATTCACTGATCTCCCCAA	GATGCAGGACAAGTCCATTGC
Nrg4	CACGCTGCGAAGAGGTTTTTC	CGCGATGGTAAGAGTGAGGA
Nuf2	TCCCCAGATACAATGTAGCTGA	CCGGACTCCATACACTAACTGT
Osr2	CTCACCAATTACTCCTTCCTGC	GCACCGCACTGAGACCATAG
Pgc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Pgc1b	GCACACCAGGCAGTAGCTTT	CAGGAGTTGATTCCAGACAGGTA
Plin1	GGGACCTGTGAGTGCTTCC	GTATTGAAGAGCCGGGATCTTTT
Pparg	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
Prc1	CAGATGAGTCTATCACATGCCTG	CCTCGGTTCTTTGTAGCCTCT
Prdm16	CCACCAGCGAGGACTTCAC	GGAGGACTCTCGTAGCTCGAA
Slc2a4	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
Sox9	AGTACCCGCATCTGCACAAC	ACGAAGGGTCTCTTCTCGCT
Spc25	ATTGCTGAAGTAAAAGGCAAGGA	TGGGCTCTCAGGATTCTTAGG
Srebp1	GCAGCCACCATCTAGCCTG	CAGCAGTGAGTCTGCCTTGAT
Tcf21	CCCACTAAGAAAAGCCCGCTC	CCGTTCTCGTACTTGTCGTTG
Tnfaip3	GAACAGCGATCAGGCCAGG	GGACAGTTGGGTGTCTCACATT
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Zfp36	CCACCTCCTCTCGATACAAGA	GCTTGGCGAAGTTCACCCA
Zfy1	TGGAGAGCCACAAGCTAACCA	CCCAGCATGAGAAAGATTCTTC