Cell Reports, Volume *24*

Supplemental Information

Maf1 and Repression of RNA Polymerase III-Mediated Transcription Drive Adipocyte Differentiation

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SUPPLEMENTAL FIGURES

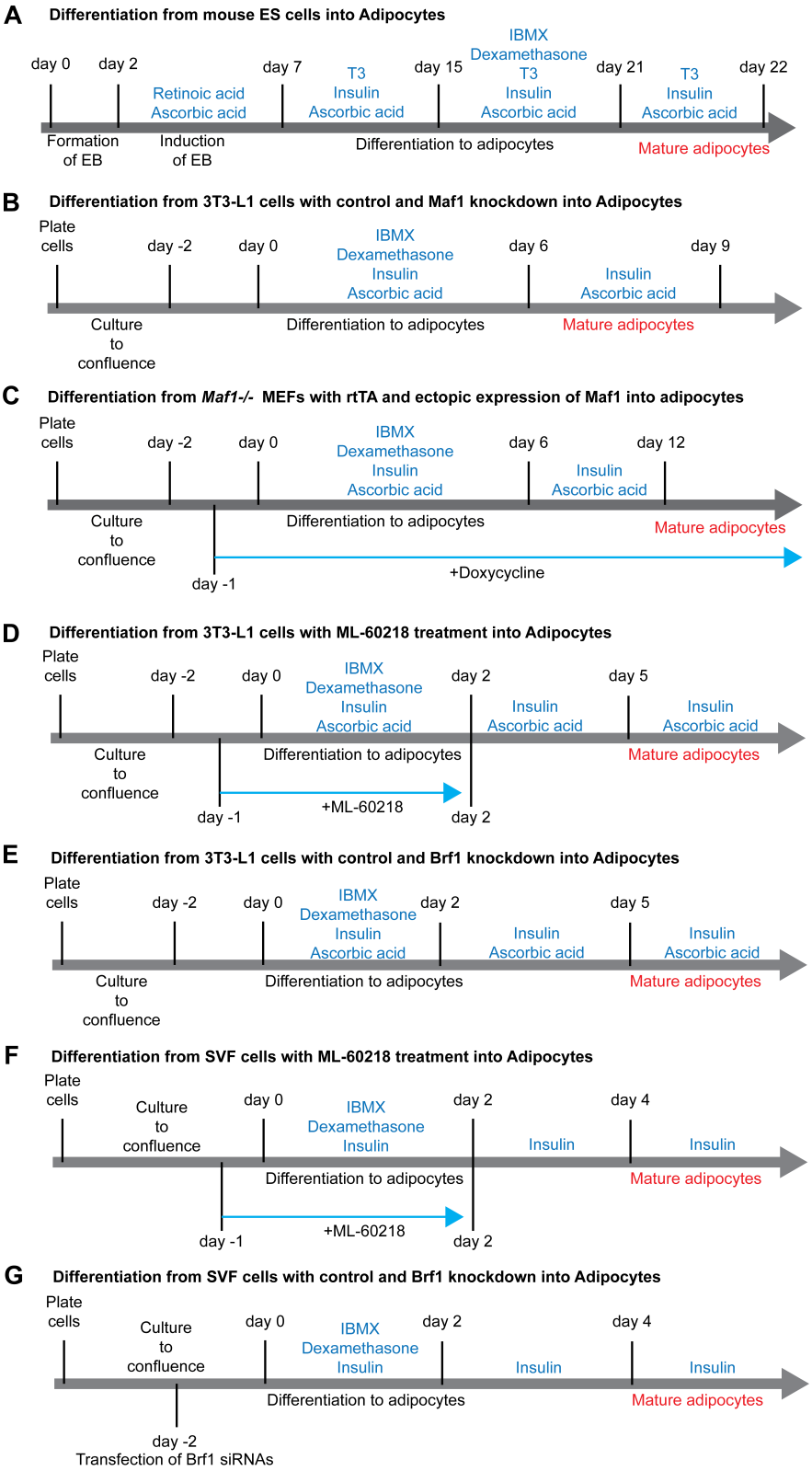


Figure S1: ***Stepwise protocols for the differentiation of mESCs, 3T3-L1 cells, and MEFs into mature adipocytes. Related to Fig. 3-6, S3, and S4.***

The detailed protocols and concentration of each reagent are depicted in the "Methods". **(A)** Differentiation of the control and Maf1 knockdown mESCs into adipocytes. (IBMX, 3-isobutyl-1-methylxanthine; T3, Triiodothyronine) **(B)** Differentiation of the control and Maf1-knockdown 3T3-L1 cells into adipocytes. **(C)** Differentiation of the rtTA control and Maf1-HA expressed *Maf1*-/- MEFs into adipocytes. **(D)** Differentiation of the 3T3-L1 cells with ML-60218 treatment into adipocytes. ML-60218 was added to the cells 24 hours prior the induction of adipogenesis and removed 2 days later. **(E)** Differentiation of the control and Brf1-knockdown 3T3-L1 cells into adipocytes. **(F)** Differentiation of the SVF cells with ML-60218 treatment into adipocytes. ML-60218 was added to the cells 24 hours prior the induction of adipogenesis and removed 2 days later. **(G)** Differentiation of the control and Brf1-knockdown SVF cells into adipocytes.

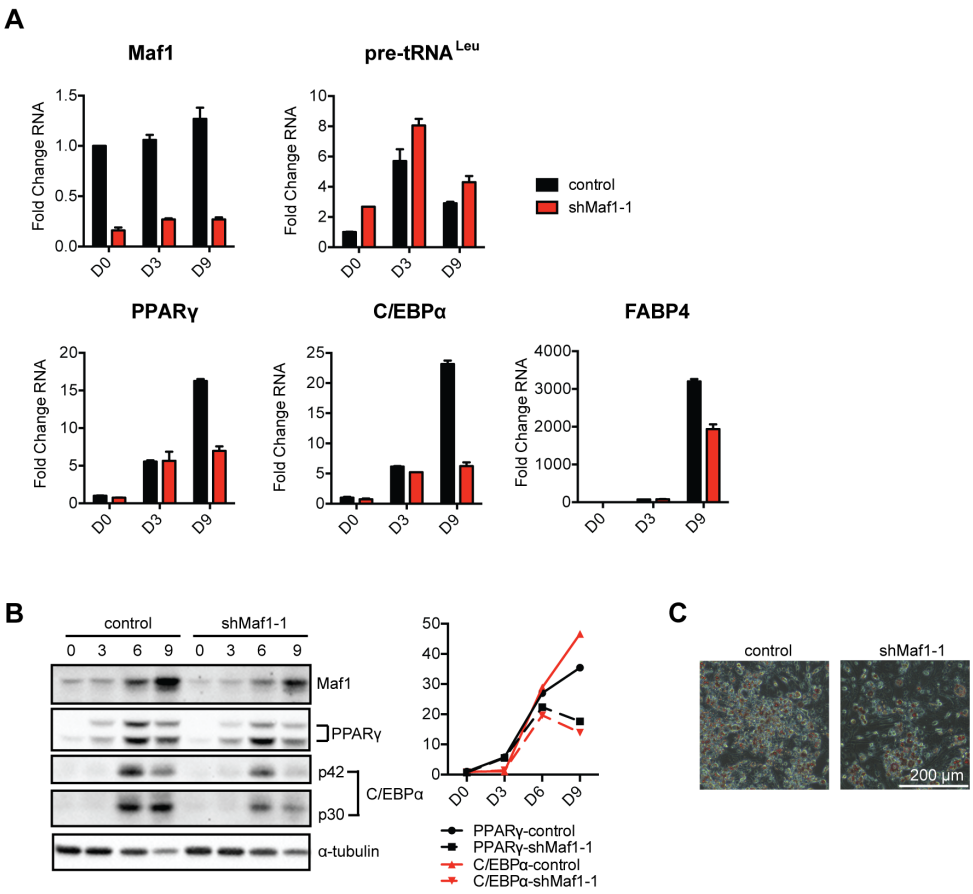
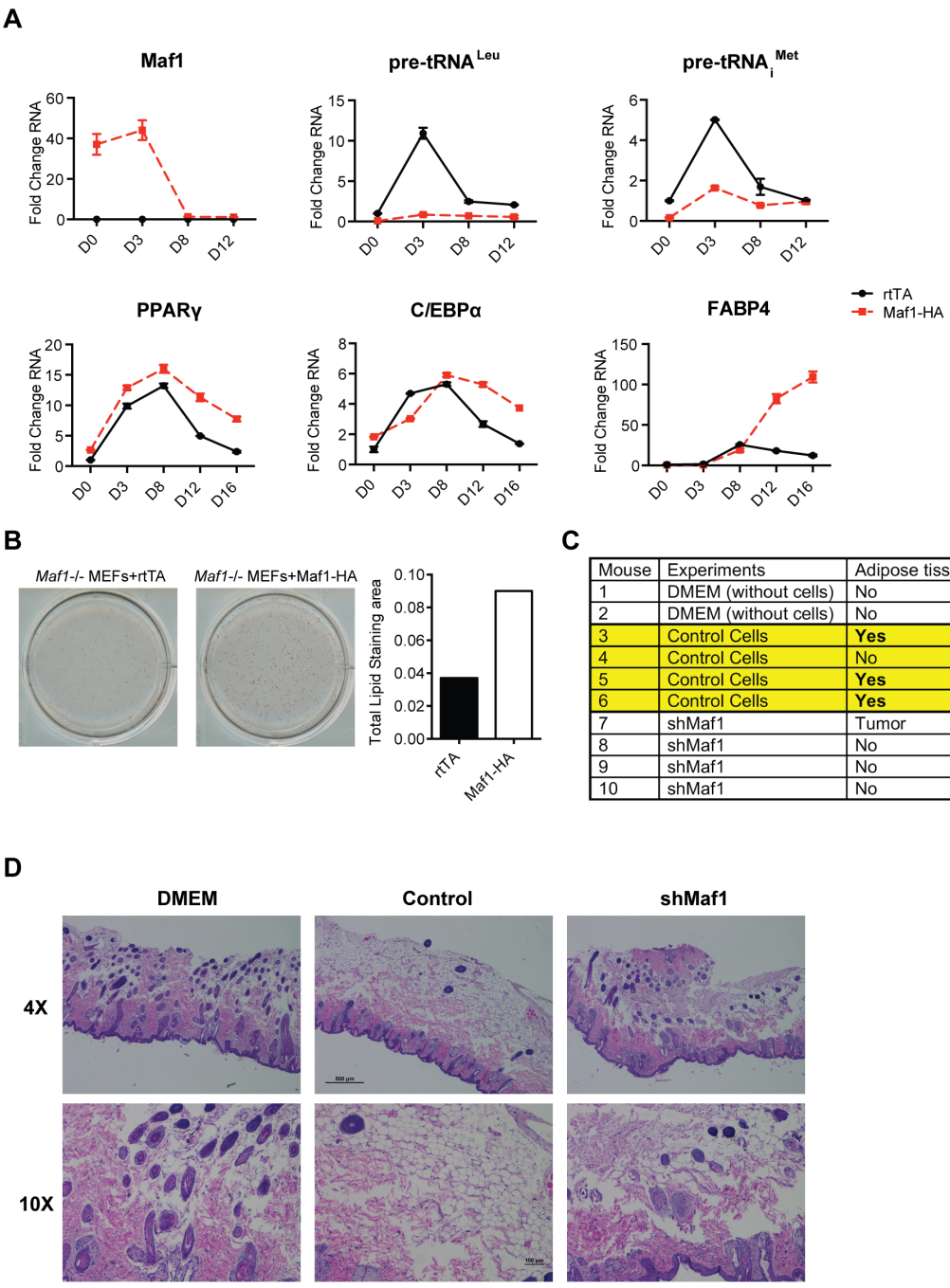


Figure S2: ***Maf1 knockdown reduces adipogenesis in 3T3-L1 cells. Related to Fig. 4.***
Maf1 expression was decreased in 3T3-L1 cells using the lentiviral Maf1 shRNA-1 construct. Lentivirus containing no Maf1 shRNA (control) or Maf1 shRNA-1 (knockdown) was used to infect 3T3-L1 cells, and the cells were terminally differentiated into adipocytes using a standard protocol (Fig. S1B). **(A)** qRT-PCR analysis for expression of Maf1, $tRNA^{Leu}$, PPARγ, C/EBPα, and FABP4. Pre-differentiation at day 0 (D0) and post- differentiation at day 3 (D3) and day 9 (D9). Transcript amounts were normalized to β-actin and the fold change was calculated relative to the amount of transcript in day 0 control cells. **(B)** Immunoblot analysis of Maf1, PPARγ, C/EBPα, and α-tubulin in control and Maf1 knockdown 3T3-L1 cells at the indicated days. Quantification of expression changes for each of the indicated proteins from the immunoblots is shown (right). Protein amounts were normalized to α-tubulin and the fold change was calculated relative to the amount of protein in D0 control 3T3-L1 cells. **(C)** Oil-red O staining of adipocytes differentiated from control and Maf1 knockdown 3T3-L1 cells.



Mouse	Experiments	Adipose tissue observed
1	DMEM (without cells)	No
2	DMEM (without cells)	No
3	Control Cells	**Yes**
4	Control Cells	No
5	Control Cells	**Yes**
6	Control Cells	**Yes**
7	shMaf1	Tumor
8	shMaf1	No
9	shMaf1	No
10	shMaf1	No

Figure S3: ***Maf1 promotes adipogenesis in vitro and in vivo. Related to Fig. 4-5.***
(A, B) Induction of Maf1 at the early stage of adipogenesis is sufficient to promote the adipogenesis in Maf1-/- MEFs. The rtTA control and Maf1-HA expressed *Maf1-/-* MEFs were programmed to differentiate into adipocytes, and treated with 50 ng/ml dox only from day -1 to day 3. **(A)** qRT-PCR analysis of RNA expression of Maf1, $tRNA^{Leu}$, $tRNA_i^{Met}$, PPARγ, C/EBPα, and FABP4 during the differentiation of either control or Maf1-HA expressed *Maf1-/-* MEFs at the indicated days. Transcript amounts were normalized to β-actin and the fold change was calculated relative to the amount of transcript at day 0 (D0) rtTA *Maf1-/-* MEF cells. **(B)** Oil-red O staining of adipocytes that are differentiated from control and Maf1-HA expressed *Maf1 -/-* MEFs (left) and quantification of staining (right). **(C, D)** Reduction of Maf1 compromises the fat pad formation of implanted 3T3-L1 preadipocytes. (C) For the de novo adipogenesis of 3T3-L1 cells in vivo, 10 mice were used. Two mice were injected with plain DMEM medium with no cells as negative controls. Four mice were injected with 3T3-L1 control cells (empty

vector), three out of four developed a visible fat pads in the injected region. The other four mice were injected with Maf1 knockdown 3T3-L1 cells, none of them developed a visible fat pad. **(D)** H&E stained skin cross-sections from the mice that were injected with DMEM, and control and Maf1 knockdown (shMaf1) 3T3-L1 cells. The pictures were taken under microscope with two magnifications, 4X and 10X, of each sample.

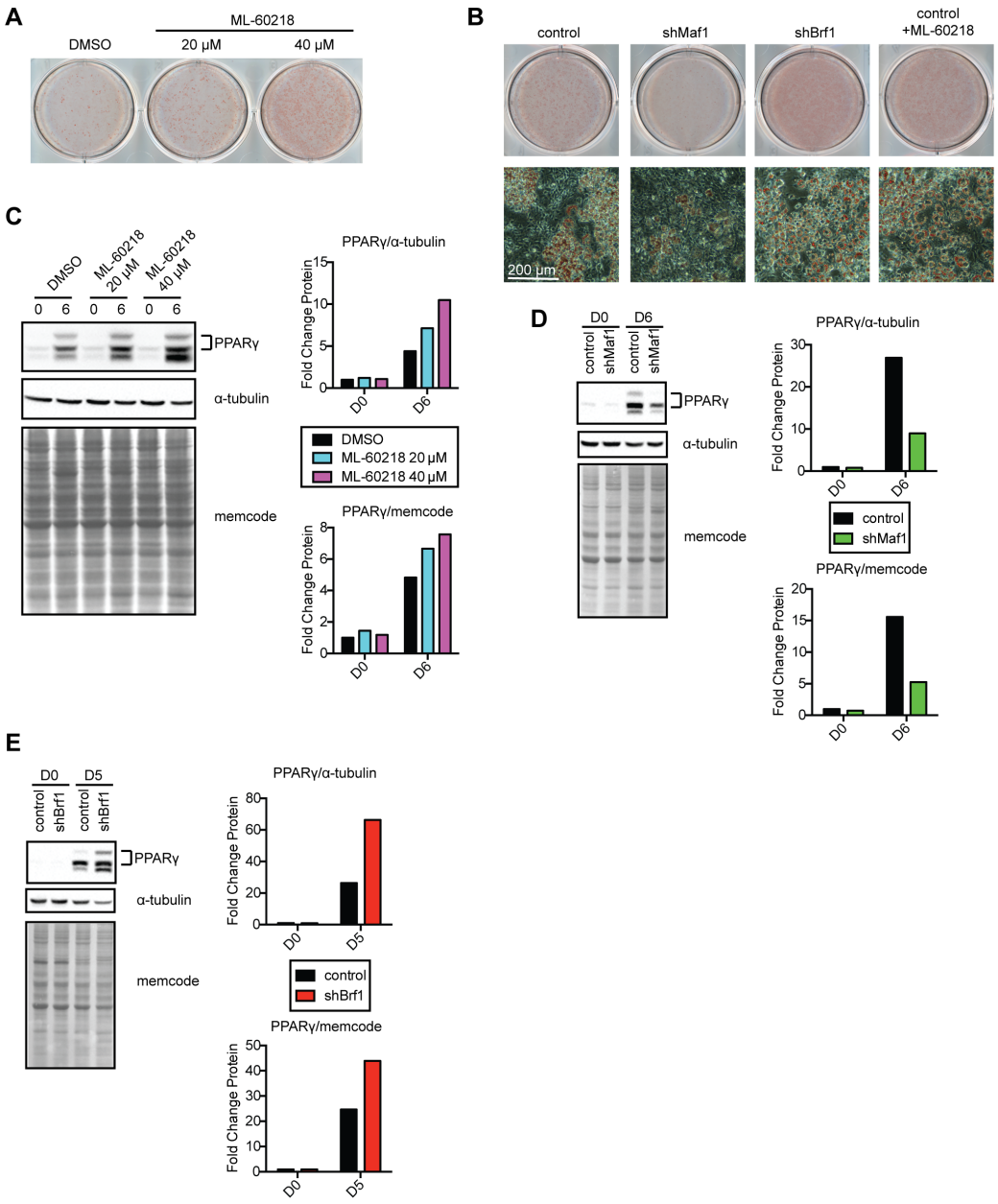


Figure S4: ***Repression of RNA Pol III-dependent transcription by either ML-60218 treatment or Brf1 knockdown promotes adipogenesis in 3T3-L1 cells. Related to Fig. 4 and 6.***

(A) Dose dependent increase of adipogenesis in 3T3-L1 cells with ML-60218 treatment. 3T3-L1 cells were treated with DMSO, and 20 μM or 40 μM of ML-60218 from day -1 to day 2 relative to inclusion of the differentiation cocktailand the cells were fixed at day 6 for Oil-red O staining. **(B)** Maf1 knockdown represses adipogenesis, whereas both Brf1 knockdown and ML-60218 treatment enhances adipogenesis compare to control cells. 40 μM of ML-60218 were treated to the cells from day -1 to day 2, and the all the cells were fixed at day 5 for Oil-red O staining. **(C-E)** Immunoblot analysis of PPARγ and α-tubulin (left) in **(C)** DMSO control and 20 μM or 40 μM ML-60218 treated 3T3-L1 cells; **(D)** control and Maf1-knockdown 3T3-L1 cells; **(E)** control and Brf1-knockdown 3T3-L1 cells, and the quantification of proteins from the immunoblots (right). Protein amounts were normalized to α-tubulin and total protein (memcode), and the fold change was calculated relative to the amount of protein at D0 DMSO control cells.

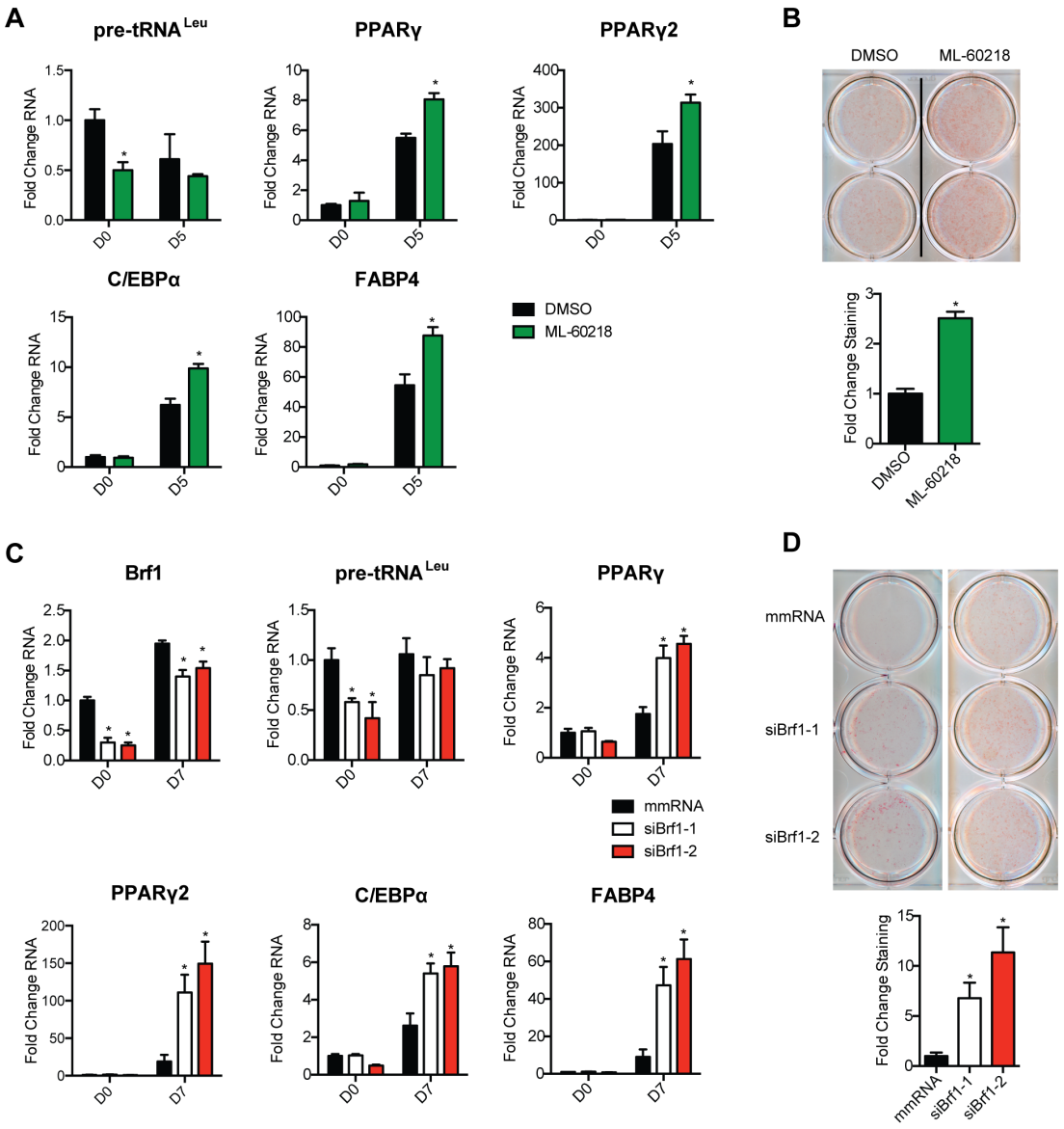


Figure S5: ***Repression of RNA pol III-dependent transcription by either ML-60218 treatment or Brf1 knockdown promotes adipogenesis in primary mouse SVF cells. Related to Fig. 6.***

(A) ML-60218-treated and Brf-knockdown mouse SVF cells were terminally differentiated into adipocytes using a standard protocol (Fig. S1F-G). (A) qRT-PCR analysis of $tRNA^{Leu}$, and PPARγ, C/EBPα, and FABP4 mRNAs during the differentiation of DMSO control and ML-60218-treated SZVF cells at Day 0 and Day 5 (D0 and D5). Cells were treated with 20 μM of ML-60218 from day -1 to day 2. Transcript amounts were normalized to PPia1 and the fold change was calculated relative to the amount of transcript at D0 in control cells. **(B)** Oil-red O staining of adipocytes differentiated from DMSO control and ML-60218 treated SVF cells (Top) and quantification of staining (Bottom). **(C)** qRT-PCR analysis of $tRNA^{Leu}$, and PPARγ, C/EBPα, and FABP4 mRNAs at day 0 (D0) before differentiation and at day 7 (D7) after differentiation of control mismatched RNA (mmRNA) and Brf1-knockdown with two different siRNAs in SVF cells. Transcript amounts were normalized to PPia1 and the fold change was calculated relative to the amount of transcript at D0 for control cells. **(D)** Oil-red O staining of adipocytes differentiated from mmRNA and Brf1-knockdown SVF cells. **(A-D)** Data are mean ± s.d. of n=3 independent experiments. Asterisks represent $p<0.05$ in an unpaired Student's t test.

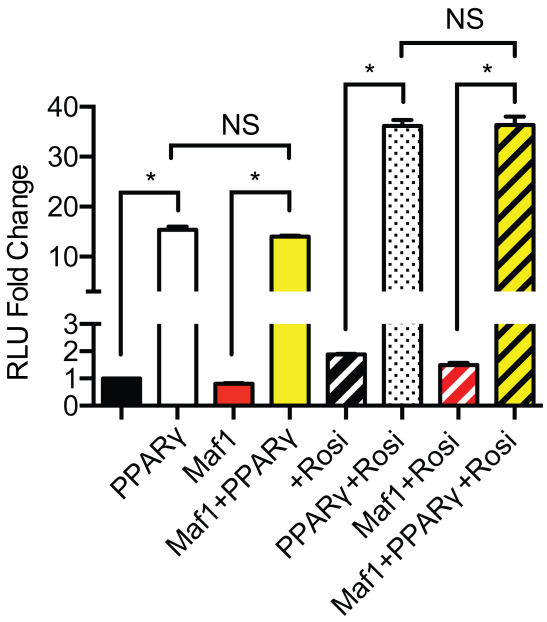


Figure S6: ***Maf1 does not affect the PPARγ transcription activity. Related to Fig 7.***
293T cells were co-transfected with either PPARγ or Maf1-HA or both, and a luciferase reporter containing PPARγ-response element and Renilla, with and without the treatment of rosiglitazone (1μM). Luciferase activity was measured from resulting lysates and normalized to Renilla luciferase activity; fold change was calculated relative to the luciferase activity in control cells without PPARγ and Maf1-HA expression. Values shown are the means ± s.d. of n=4 independent experiments. Asterisks represent $p<0.05$ in an unpaired Student's t test.

SUPPLEMENTAL TABLES

Day0\Day2			Day2\Day0		
Cluster	*DAVID Functional Annotations*	*Score*	*Cluster*	*DAVID Functional Annotations*	*Score*
1	Cell cycle, M phase of mitotic cell cycle, mitosis, cell division	36.62	1	Extracellular region part, extracellular space	13.26
2	Condensed chromosome, centromeric region, kinetochore	18.60	2	Proteinaceous extracellular matrix, extracellular matrix	10.55
3	Chromosomal part, intracellular non-membrane-bounded organelle, cytoskeleton	15.34	3	Extracellular region, transmembrane region, intrinsi/integral to membrane, signal peptide	5.59
4	DNA replication, DNA metabolic process	9.50	4	Cell adhesion, biological adhesion	4.50
5	Spindle, microtubule. intracellular non-membrane-bounded organelle	8.51	5	Defense response, response to wounding, inflammatory response	3.91
6	DNA damage/repair, response to DNA damage cellular response to stress	8.06	6	Response to endogenous stimulus, to peptide hormone stimulus, to hormone stimulus	3.25
7	Microtubule-based process, spindle organization, cytoskeleton organization,	7.79	7	Regulation of cell adhesion, positive regulation of cell-substrate adhesion	3.13
8	Microtubule-based process/movement, Kinesin-motor region, microtubule	6.26	8	GTPase activity, Guanylate-binding protein	2.91
9	Cell cycle, ocyte meiosis, Progesterone-mediated oocyte maturation	5.83	9	Regulation of cytokine production, positive regulation of multicellular organismal process	2.80
10	Meiosis, M phase of meiotic cell cycle	5.77	10	Netrin domain, Netrin module, non-TIMP type	2.77
Cluster	*GSEA Hallmark Gene Set*	*p-val*	*Cluster*	*GSEA Hallmark Gene Set*	*p-val*
1	Genes involved in the G2/M checkpoint, as in progression through the cell division cycle.	1.8E-63	1	Genes up-regulated in response to IFNG [GeneID=3458].	7.1E-58
2	Genes encoding cell cycle related targets of E2F transcription factors.	1.4E-58	2	Genes regulated by NF-kB in response to TNF [GeneID=7124].	5.5E-39
3	Genes important for mitotic spindle assembly.	1.8E-30	3	Genes up-regulated in response to alpha interferon proteins.	1.3E-35
4	Genes encoding proteins involved in glycolysis and gluconeogenesis.	7.5E-07	4	Genes defining epithelial-mesenchymal transition, as in wound healing, fibrosis and metastasis.	1.1E-22
5	Genes defining late response to estrogen.	5.0E-06	5	Genes up-regulated by KRAS activation.	9.5E-21
6	Genes up-regulated during production of male gametes (sperm), as in spermatogenesis.	7.8E-06	6	Genes defining inflammatory response.	8.5E-20
7	Genes defining epithelial-mesenchymal transition, as in wound healing, fibrosis and metastasis.	3.0E-05	7	Genes up-regulated by IL6 [GeneID=3569] via STAT3 [GeneID=6774], e.g., during acute phase response.	1.6E-16
8	Genes encoding components of blood coagulation system; also up-regulated in platelets.	6.7E-05	8	Genes up-regulated during adipocyte differentiation (adipogenesis).	1.4E-13
9	Genes encoding components of apical junction complex.	1.7E-04	9	Genes involved in development of skeletal muscle (myogenesis).	1.4E-13
10	Genes defining inflammatory response.	1.7E-04	10	Genes down-regulated in response to ultraviolet (UV) radiation.	1.6E-13

Table S1: ***Distinctiveness in Maf1 biology prior to differentiation (Day 0) and 2 days after differentiation induction (day 2). Related to Fig. 7.***

Top ten DAVID enrichment clusters and top enriched GSEA Hallmark gene sets for presumtive Maf1 target genes uniquely expressed at Day 0 and Day 2, respectively. Day0\Day2 = {genes in Day0 but not in Day2}, Day2\Day0 = {genes in Day2 but not in Day0}. Only genes with p-val ≤ 0.01 and ≥ 2x Fc normalized RNAseq expression values were used for the analyses. Score: DAVID enrichment score for the annotation cluster.

Cluster	*DAVID Functional Annotations*	*Score*	*Genes*
1	Fat cell differentiation, brown fat cell differentiation, glucose homeostasis	6.54	ADIPOQ, ADRB2, BNIP3, CEBPA, EGR2, FABP4, LPIN1, MRAP, PEX11A, RETN, RGS2, SCD1, SH2B2, SLC2A4
2	Lipid metabolism, phospholipid/neutral lipid/triglyceride/ acylglycerol metabolic/catabolic/biosynthetic processes	3.90	ABHD5, ACSBG1, ACSL1, ADIPOQ, ADIPOR2, AGPAT9, DGAT1, DGAT2, FABP5, FLT1, GPD1, HSD11B1, LIPE, MGST3, PANK3, PIM3, PLIN1, PNPLA2, PNPLA3, SCD1
3	Response to insulin/to peptide hormone/ to endogenous stimulus	3.56	ACSBG1, ADIPOQ, ADIPOR2, EGR2, FABP4, FOXO1, LPIN1, RETN, SH2B2
4	Cell/membrane fraction, microsome, vesicular fraction	2.34	ACSBG1, ACSL1, ADRB2, DGAT1, DGAT2, GPD1, MGST3, PCDHGB8, RHOB, SLC2A4
5	Glucose/hexose/monosaccharide/carbohydrate transport	1.94	FABP5, KLF15, SLC2A4
6	Glucose/hexose/monosaccharide metabolic processes	1.39	ADIPOQ, ALDOA, FABP5, GPD1
7	Palmitate, S-palmitoyl cysteine, lipoprotein, positive regulation of developmental process	1.13	ADRB2, ALPL, CD36, RHOB, RHOV, SLC2A4, TSPAN12
8	Tube development, regulation of cell proliferation	1.01	ADRB2, AGT, CEBPA, FABP4, FLT1, FOXO1, HSD11B1, NOG
9	Identical protein binding, protein homodimerization activity	0.98	ADIPOR2, ADRB2, CEBPA, FLT1, GPD1
10	Positive regulation of protein kinase cascade/ Positive regulation of signal transduction	0.95	AGT, ADIPOQ, ADRB2
Cluster	*KEGG Pathways*	*p-val*	*Genes*
1	mmu03320:PPAR signaling pathway	5.6E-06	SCD1, ACSL1, CD36, PLIN1, FABP4, ADIPOQ, FABP5
2	mmu04920:Adipocytokine signaling pathway	4.0E-05	ACSL1, CD36, SLC2A4, ADIPOR2, ADIPOQ, PPARGC1A
3	mmu00830:Retinol metabolism	0.01	RDH12, BCMO1, DGAT1, DGAT2
4	mmu04910:Insulin signaling pathway	0.01	SLC2A4, FOXO1, SH2B2, PPARGC1A, LIPE
5	mmu00561:Glycerolipid metabolism	0.03	DGAT1, DGAT2, PNPLA3
Cluster	*GSEA Hallmark Gene Set*	*p-val*	*Genes*
1	Genes up-regulated during adipocyte differentiation (adipogenesis).	1.4E-17	ADIPOQ, ADIPOR2, ALDOA, CD36, CMBL, DGAT1, FABP4, LIPE, MGST3, MRAP, ORM1, PIM3, RETN
2	Genes encoding proteins involved in metabolism of fatty acids.	8.1E-09	ACSL1, ADIPOR2, ALDOA, CA2, CD36, G0S2, GPD1
3	Genes involved in metabolism of bile acids and salts.	3.6E-05	ACSL1, BCAR3, LIPE, PEX11A
4	Genes up-regulated in response to ultraviolet (UV) radiation.	1.4E-04	ALDOA, CA2, DGAT1, RHOB
5	Genes up-regulated by KRAS activation.	3.3E-04	CA2, G0S2, HSD11B1, RETN
6	Genes involved in myogenesis.	3.3E-04	ACSL1, BHLHE40, CD36, LPIN1
7	Genes regulated by NF-kB in response to TNF.	3.3E-04	BHLHE40, EGR2, G0S2, RHOB
8	Genes encoding proteins over-represented on the apical surface of epithelial cells	2.4E-03	ADIPOR2, SLC2A4
9	Genes up-regulated in response to TGFB1.	3.6E-03	BCAR3, NOG
10	Genes defining early response to estrogen.	4.4E-03	BHLHE40, ENDOD1, PEX11A

Table S2: ***Alteration of Maf1 expression and RNA pol III-dependent transcription regulates adipogenesis. Related to Fig. 7.***

Top enrichments for DAVID functional annotation clusters, KEGG pathways and GSEA Hallmark gene sets for presumtive Maf1 target genes at Day 2 that were concurrently up-regulated by Brf1 knockdown and ML-60218 treatment. Only genes with p-val $\leq$ 0.01 and $\geq$ 1.7x Fc normalized RNAseq expression values were used for the analyses. Score: DAVID enrichment score for the annotation cluster.

qPCR primers for mouse cell lines		
Targets	**forward**	**Reverse**
Maf1	GACTATGACTTCAGCACAGCC	CTGGGTTATAGCTGTAGATGTCAC
pre-tRNA$_i^{Met}$	CTGGGCCCATAACCCAGAG	TGGTAGCAGAGGATGGTTTC
pre-tRNA^{Leu}	GTCAGGATGGCCGAGTGGTCTAAG	CCACGCCTCCATACGGAGAACCAGAAGACCC
U6 RNA	GGAATCTAGAACATATACTAAAATTGGAAC	GGAACTCGAGTTTGCGTGTCATCCTTGCGC
GATA4	GATGGGACGGGACACTACCTG	TGGCAGTTGGCACAGGAGA
GATA6	GACGGCACCGGTCATTACC	ACAGTTGGCACAGGACAGTCC
T	GCTCTAAGGAACCACCGGTCATC	ATGGGACTGCAGCATGGACAG
Mesp1	GTTCCTGTACGCAGAAACAGCATC	TCAGACAGGGTGACAATCATCCG
Nestin	GCTTAGAGGTGCAGCAGCT	CTGTAGACCCTGCTTCTCCTGCT
Sox1	AGGCAGCTGGGTCTCAGAA	GACTCTGTGGTGGTGAGGTC
Oct4	GTGGAGGAAGCCGACAACAATGA	CAAGCTGATTGGCGATGTGAG
SOX2	CAGGAGAACCCCAAGATGCACAA	AATCCGGGTGCTCCTTCATGTG
Nanog	TGGTCCCCACAGTTTGCCTAGTTC	CAGGTCTTCAGAGGAAGGGCGA
PPARγ	ATCATCTACACGATGCTGGCCT	TGAGGAACTCCCTGGTCATGAATC
C/EBPα	GAACAGCAACGAGTACCGGGTA	CCATGGCCTTGACCAAGGAG
FABP4	TGGGAACCTGGAAGCTTGTCT	TCGAATTCCACGCCCAGTTTGA
Brf1	GGAAAGGAATCAAGAGCACAGACCC	GTCCTCGGGTAAGATGCTTGCTT
β-actin	CGACAACGGCTCCGGCATG	CTGGGGTGTTGAAGGTCTCAAACATG
PPia1	CGAGCTGTTTGCAGACAAAGTTCC	CCCTGGCACATGAATCCTGG
GAPDH	GATGGGTGTGAACCACGAGAA	GGGCCATCCACAGTCTTCTG
H19	AGCAGTGATCGGTGTCTCGAAGA	CCATCACACCGGACCATGTCAT
Wnt6	GCAAGACTGGGGTTCGAGAA	GCCTGACAACCACACTGTAGGAG
qPCR primers for human ES cells		
Targets	forward	Reverse
Maf1	GACTATGACTTCAGCACAGCC	CTGGGTTATAGCTGTAGATGTCAC
pre-tRNA$_i^{Met}$	CTGGGCCCATAACCCAGAG	TGGTAGCAGAGGATGGTTTC
pre-tRNA^{Leu}	GTCAGGATGGCCGAGTGGTCTAAG	CCACGCCTCCATACGGAGAACCAGAAGACCC
U6 RNA	GGAATCTAGAACATATACTAAAATTGGAAC	GGAACTCGAGTTTGCGTGTCATCCTTGCGC
Oct4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC
GAPDH	GATGGGTGTGAACCACGAGAA	GGGCCATCCACAGTCTTCTG

Table S3: ***qPCR primers list. Related to experimental procedures.***
qPCR primers for analyzing the expression of target genes in human and mouse cell lines.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture

46C mESCs were cultured on 0.1% gelatin coated plates. GMEM medium was used to maintain the mESCs (Sigma-Aldrich, G5154) and it was supplemented with 15% ESC-qualified FBS (Life technologies), 0.1 mM MEM non-essential amino acids (NEAA), 2 mM GlutaMax, 1 mM Sodium Pyruvate, 0.1 mM β-mercaptoethanol, 1% penicillin/streptomycin, 100 units/ml LIF. H9 hESCs were maintained on matrigel-coated plates with MEF-conditioned medium consisting of DMEM/F12 supplemented with 20% knockout serum replacement (Life technologies), 0.1 mM MEM NEAA, 0.1 mM β-mercaptoethanol, 1% penicillin/streptomycin, and 4 ng/ml recombinant human FGF2 (Invitrogen). 3T3L1 mouse preadipocyte, MEF, and HEK293T cells were maintained in DMEM-HG supplemented with 10% FBS, 2 mM GlutaMax, and 1% penicillin /streptomycin. The SVF cells were cultured in DMEM-F12 supplemented with 10% FBS, 2 mM GlutaMax, and 1% penicillin /streptomycin. All cells were cultured in incubators with 5% CO_2 at 37ºC.

Isolation of mouse stromal vascular fraction (SVF) cells

The SVF cells were isolated from inguinal white adipose tissue. Male C57 BL/6J mice (6-8 weeks old) were euthanized in isoflurane chamber. The inguinal white adipose tissue from a mouse was washed by PBS and dried quickly on napkins. The tissue was then minced into small pieces and digested in 3 ml Type I Collagenase (3 mg/ml, Gibco) in SVF cell culture medium for 1 hour at 37ºC. After digestion, the mixture was centrifuged at 750 x g for 10 minutes, and the supernatant was carefully removed. The pellet was resuspended in 1 ml SVF culture medium and passed through 40 μm cell strainer. The filtered cell mixture was then centrifuged at 750 x g for 10 minutes, and the supernatant was carefully removed. The pellet was resuspended in SVF culture medium and plated on 6-well plates (The SVF cells from one mouse can be plated into three 6-well plates). 16-20 hours after plating the cells, media was changed.

Production of lentiviral constructs

Non-silencing empty vector control, pLKO.1-mouse Maf1 shRNA (clone IDs: TRCN0000125776 and TRCN0000125778), and pLKO.1-mouse Brf1 shRNA (clone ID: TRCN0000119897) were purchased from Sigma-Aldrich. The inducible pFTREW-Maf1-HA expression construct and FUIPW-rtTA (lentiviral tetracycline transactivator) was previously described (Palian et al., 2014).

Lentiviral particles were produced as previously described. Virus-containing media was collected, and sterile filtered through a 0.45 μm filter. Viruses were then concentrated by Lenti-X concentrator (Clontech), pelleted at 1,500 x g for 45 minutes at 4ºC, and resuspended in DPBS. Cell lines were transduced with the concentrated virus for 16 to 24 hours. Two days after transduction, the infected cells were selected with puromycin.

In vitro differentiation of ESCs

For mouse embryoid body (EB) formation, mESCs were dissociated with Accutase (Life technologies). 150,000 cells/ml mESCs were cultured in mESC medium without LIF on 6-well Ultra-Low attachment plates (Corning). For human EBs formation, 100,000 cells/ml hESCs were cultured in DMEM/F12 supplemented with 10% FBS on 6-well Ultra-Low attachment plates. For both mouse and human EBs, media was changed every other day. EBs were collected at the indicated time points described in the figures.

Differentiation of mESCs, 3T3-L1, MEF, and SVF cells into adipocytes

For the differentiation of mESCs into adipocytes, mESCs were first dissociated to form EBs. Two days after mEBs formation, mEBs were collected, and 10 to 20 mEBs were transferred to a well of gelatin-coated 6-well plates with mESC medium without LIF. On day 3, media containing 1 μM retinoic acid (RA) and 12.5 μg/mL ascorbic acid (AsA) was added, and changed every day until day 7. After day 7, media containing 0.5 μg/mL Insulin, 3nM triiodothyronine (T3), and 12.5 μg/mL AsA was added, and changed daily up to day 11. On day 12, the attached EBs were dissociated by Accutase (Life technologies), and 100,000 cells per well of a 6-well plate were re-plated in differentiation medium with the same hormone cocktail as day 7 to 11. The media was changed daily until day 15. After day 15, the medium was changed every other day and included 0.5 mM 3-isobutyl-1-methyl xanthine (IBMX), 0.1 μM Dexamethasone (Dex), 20 μg/mL Insulin, 0.06 mM indomethacin, and 25 μg/mL AsA until day 21. From day 21 to the end of the differentiation (approximately day 27), the media was changed daily and included 20 μg/mL Insulin, 25 μg/mL AsA, 3 nM and T3.

To differentiate the 3T3-L1 and MEF cells into adipocytes, cells were first grown to confluency. The 2-day post-

confluent cells were induced to differentiate with the differentiation cocktail which contained 10 µg/mL Insulin, 2 µM Dex, 0.5 mM IBMX and 25 µg/mL AsA. The duration of differentiation cocktail treatment varied in different experiments, and was between 2 to 6 days (Figure S1). During the treatment of the differentiation cocktail, the media was changed every 3 days. After the differentiation cocktail was no longer used in the media, the media was changed every other day and contained 10 µg/mL Insulin, and 25 µg/mL AsA until the differentiated cells were collected and analyzed.

To differentiate the SVF cells into adipocytes, cells were first grown to confluency, and were induced to differentiate with the differentiation cocktail which contained 10 µg/mL Insulin, 2 µM Dex, and 0.5 mM IBMX for two days (Figure S1). After incubation with the differentiation cocktail for two days, the media was changed every other day contained 10 µg/mL Insulin until the differentiated cells were collected and analyzed.

To prepare the media for the differentiation of 3T3-L1 cells in Biotin-free condition, the media containing 10% dialyzed FBS (Gibco), 2 mM GlutaMax, and 1% penicillin/streptomycin was incubated with avidin agarose resin (Pierce, 0.25 ml resin for 50 ml medium) at room temperature for 1 hour. The resin was removed by centrifugation at 500 x g, and used for the differentiation of 3T3-L1 cells following the same protocol as above.

RNA Pol III inhibitor treatment

The RNA Pol III chemical inhibitor, ML-60218 (Millipore), was dissolved in DMSO at a final concentration of 25 mM. The inhibitor was added to the cells at 20µM or 40 µM one day before the differentiation cocktail was added, and removed 2 days later. The control cells were treated with an equal volume of DMSO.

RNA isolation and Quantitative Real-Time PCR

Total RNA was isolated from cells using the Zymo Directzol RNA Kit. The RNAs were then reverse-transcribed into cDNA with the Superscript III first strand synthesis Kit (Invitrogen). Real-time quantitative PCR was performed on the Lightcycler 480 (Roche) with SYBR fast qPCR kit (KAPA Biosystems). Relative amounts of transcripts were quantified by comparative threshold cycle method (ΔΔCt) with GAPDH or β-actin as the endogenous reference control. The primers for targets are listed in Table S3. For statistical analysis, unpaired, two-tailed, student's *t*-test was used for all comparisons. All results are from three biological and two technical replicates.

Immunoblot analysis and antibodies

Cells were washed with DPBS twice, then scrapped and pelleted at 400g for 5 minutes. After removing the supernatant, the cells were lysed in triple lysis buffer, (50 mM Tris-Cl pH 8.0, 150 mM sodium chloride, 0.02% w/v sodium azide, 1% w/v SDS, 1 % v/v NP-40, 0.5% w/v sodium deoxycholate, containing protease inhibitor cocktail set III (EMD Millipore)) for 20 minutes on ice, and sonicated for 15 seconds. After sonication, the cells were centrifuged for 20 minutes at 10,000g, and the supernatant was collected. The protein concentration was determined by using the Biorad Protein *Dc* assay. Cell lysates were subjected to immunoblot analysis and transferred onto a nitrocellulose membrane (GE-Healthcare). Membranes were probed using the following antibodies: Maf1 (Abgent), PPARγ, C/EBPα, FABP4, and Perilipin (Cell Signaling), HA (Roche), T (Santa Cruz), Brf1 (Bethyl), β-actin (Sigma Aldrich), α-tubulin (Invitrogen). Protein bands were quantified using Image Lab software (Bio-Rad).

Immunohistochemistry

For Alkaline Phosphatase (AP) staining, 46C mESCs were cultured on 0.1% gelatin coated plates with culture conditions as described above for maintaining the mESCs. After two days, the cells were washed with DPBS twice, and fixed with 4% paraformaldehyde for 10 minutes. The fixed cells were washed with DPBS twice and stained using the Vectastain ABC-AP kit (Vector Laboratories). After staining, the cells were washed three times with DPBS.

For Oil-red O staining, the differentiated adipocytes from mESCs, 3T3-L1, and MEF cells were washed twice with DPBS, and fixed with 4% paraformaldehyde for 10 minutes. The fixed cells were washed with DPBS twice and stained with 0.3% Oil-red O solution (Sigma-Aldrich) for 15 minutes. After staining, the cells were washed three times with DPBS. The pictures were taken using the EVOS XL imaging system at the Human Stem Cell core at Baylor College of Medicine. For quantification, the dye was extracted by 100% isopropanol, and the intensity of Oil-red O extracts was quantified by measuring absorbance at 490 nm.

Immunostaining

Control and Maf1 knockdown mESCs were plated on 12-well plates. 48 hours later, the cells were fixed with 4% paraformaldehyde for 10 minutes, and blocked using 10% normal goat serum for 1 hour at room temperature. After blocking, the cells were incubated with primary SSEA1 antibody (University of Iowa, Developmental Studies Hybridoma Bank) at a dilution of 1:500, overnight at 4^{o} The next day, the cells were incubated with Alexa Fluor 488 (Molecular Probes) secondary antibody at a dilution of 1:250 for 1 hour at room temperature. After washing with PBS, the cells were incubated with DAPI solution (Sigma-Aldrich) at 0.05 μg/ml for 10 minutes at room temperature. The images were captured using a Zeiss Axiovert 200 microscope with a DVC-1310C digital camera (DVC).

Transfection

SVF cells were plated on 6-well plates, and transfected with 100 nM mismatched RNA (mmRNA) and Brf1 siRNAs using Lipofectamine RNAiMax transfection reagent (Invitrogen) according to the manufacturer's instructions. The cells were incubated with the transfection mixture for 48 hours. siRNAs against mouse Brf1 (SASI_Mm01_00136903 (siBrf1-1), SASI_Mm02_00333493(siBrf1-2)) were purchased from Sigma-Aldrich. The transfected SVF cells were then differentiated into adipocytes.

293T cells were plated on 24-well plate, and co-transfected with PPREx3-TK-Luc reporter (0.2 μg) (Addgene) which contains three copies of the PPARγ-respose element (PPRE) upstream of the luciferase gene, and a plasmid expressing Renilla luciferase (0.05 μg), and either PPARγ from pCDH-PPARγ (0.1 μg) (acquired from Sean Hartiq at BCM which is kindly provided by Fred Schaufele at UCSF), and Maf1 from pCDNA3(-)-Maf1-HA (0.1 μg) (previously described (Johnson et al., 2007)) using Fugene 6 transfection reagent (Promega). The transfected 293T cells were then used for luciferase reporter assay.

Luciferase reporter assay

24 hours after transfection of the 293T cells as described above, cells were treated with and without rosiglitazone (1 μM) for another 24 hours. Cells were harvested, and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions with a microplate reader (BioTek Synergy2). The results were normalized to Renilla luciferase activity.

RNAseq and Data Analysis

Purified RNA was processed for RNA-Seq analysis and sent to EA|Q2 Solutions for library preparation and sequencing. Samples were prepared and sequenced according to a standard TS Stranded mRNA sequencing protocol using HiSeq-Sequencing-2x50bp-PE sequencing on an Illumina sequencing platform. RNA samples were converted into cDNA libraries using the Illumina TruSeq Stranded mRNA sample preparation kit (Illumina). After sequencing, initial analysis with a focus on qualtity control and gene quantification was performed using the RNAv9 pipeline developed by EA|Q2 Solutions. Across all samples, the median number of actual reads was 37 million with 36.1 million on-target reads. Mouse mm10 reference includes 31,252 genes of which our samples had a median of 13,814 (44%) genes detected. Clipping percentage was low with less than 1.2% of reads removed due to low quality, and the rank correlation to External RNA Controls Consortium RNA spike-ins (ERCCs) was determined ~94% % and with a slope of a best least-squares line 92% (0% of the reads aligned to the spike-in control sequences). The adjusted read depths ranged from 34.9 to 39 million with a median of 37 million, indicating high sequencing depth, which allows for consistent quantification of the expression levels with relatively high precision. To provide a robust estimate of fold change, the experimental and control values in the ratio are computed using medians. *p*-values were determined using a standard t-test assuming equal variance between groups. A relational database management system (FileMaker Pro 15) was used to filter genes for significance and fold change (all data shown in this manuscript are p-val $\leq$ 0.01 and $\geq$ 2x Fc normalized RNAseq expression values). Database for Annotation, Visualization, and Integrated Discovery Bioinformatics Resources (DAVID) v6.7 (http://david.abcc.ncifcrf.gov/), the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database (http://www.genome.ad.jp/kegg/)) and GSEA (Gene Set Enrichment Analysis) on the Molecular Signatures Databases (MSigDB) collection (gsea@broadinstitute.org) analyses were used on filtered gene groups to identify enriched biological themes. The raw datasets and lists of differentially regulated transcripts were deposited to NCBI GEO (Accession number GSE113324.

SUPPLEMENTAL REFERENCES

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