SUPPLEMENTARY METHODS AND FIGURES

SUPPLEMENTARY METHODS

Isolation of mouse preadipocytes and in vitro differentiation- Isolated epididymal fat tissue obtained from 6-wk-old male C57BL/6J mice was rinsed briefly with PBS and minced with a razor blade in collagenase solution (0.2 mg/ml collagenase A in 100 mM HEPES, 120 mM NaCl, 4.8 mM KCl, 1 mM CaCl2 and 4.9 mM glucose, pH 7.4). The mixture was then allowed to digest for 30 min at 37ºC with gentle shaking. The resulting cell suspension was allowed to settle for 5 min to separate into a supernatant containing adipocytes and an inferior layer composed mainly of preadipocytes. The adipocyte-containing supernatant was recovered by pippeting and the infranatant was filtered through a 60 μm cell strainer (BD, Franklin Lakes, NJ) to obtain the preadipocytes. Differentiation was carried out two days after seeding by replacing the media with DMEM supplemented with 10% FBS, 33 μM biotin, 17 μM pantotenate, 100 nM insulin, 1 nM dexamethasone, 0.25 mM IBMX and 1 μM rosiglitazone.

Transfection of D5 adipocytes. For transfection of D5 adipocytes, 3T3-L1 cells were differentiated in 24-well plates as described. At D5, cells were tripsinized, reseeded and transfected while in suspension with the same conditions detailed for D0 fibroblasts. RNA was analyzed 48h after transfection (D7).

SUPPLEMENTARY FIGURES

Suppl Fig.1: *Expression profiles of the studied genes in 3T3-L1 cells throughout adipogenesis.* 3T3-L1 cells were differentiated as usual and RNA was extracted at different days during the process and analyzed by real time RT-PCR. *Cebpa* expression increases over 250 times during differentiation, an order of magnitude above the increases of other adipogenic transcription factors such as *cebpb*, *pparg* or *foxo1*. Adipocytokine *apm1* is even more strongly induced. Anti-adipogenic *dlk1*, on the other hand, must decrease for adipogenesis to proceed. Values are corrected by housekeeping *actb* levels, using specific TaqMan primers and probes. Results are mean ± SD of three experiments performed in duplicate. *<0.05, **<0.005, ***<0.001, ****<0.0005

Suppl Fig.2: *Expression of HMTs and HDMs increase during adipogenesis.* Mouse primary preadipocytes were obtained from male 6wk old mice and differentiated *in vitro*. **A.** Expression of several HDMs and HMTs was analyzed at different time points during the differentiation process. **B**. Expression of several markers of adipocyte differentiation was measured as a control of the efficiency of the process. Results are the mean ± SD of two experiments performed in triplicate. *<0.05, **<0.005

Suppl Fig 3: *Knockdown of lsd1 blocks induction of adipogenesis.* **A.** siLsd1 and siSetdb1 siRNAs specifically affect expression of their cognate RNAs. D0 cells were transfected with the indicated siRNAs and total RNA was extracted 72h after transfection and analyzed by real-time RT-PCR. **B.** siLsd1 transfection decreases *lsd1* expression in mature adipocytes. 3T3-L1 cells were differentiated as usual. At D5, adipocytes were transfected with siC or siLsd1 siRNAs. Total RNA was extracted 48h after transfection (D7) and analyzed by real-time RT-PCR. **C.** Schematic of the experimental design used to analyze the effect of siLsd1 on the induction of adipogenesis. For some experiments, 3T3-L1 cells were transfected as undifferentiated fibroblasts and RNA was extracted 24h, 48h and 72h after trasnfection (arrows). Cells were then stimulated to differentiate and RNA was extracted at D1, D3, D5 and D7. For the study of mature adipocytes, 3T3-L1 were differentiated as usual and RNA was extracted and analyzed 48h after transfection (D7). **D.** Knockdown of *lsd1* affects the expression of key adipogenic transcription factors in 3T3-L1 D0 fibroblasts (left panel) but not mature cells (right panel). RNA was extracted from transfected D0 cells at the indicated time points after transfection and analyzed by real time RT-PCR. In the case of mature adipocytes, cells were transfected at D5 and RNA was analyzed 48h after transfection (D7). Results are the mean ± SD of three experiments performed in triplicate. *<0.05, **<0.005.

Suppl Table 1. Sequences of the different siRNAs used (5' to 3').

Target		
siLsd1 (1)	sense strand	AUAGGUUCCAGAAGAGGAAGAGCUC
	antisense strand	GAGCUCUUCCUCUUCUGGAACCUAU
siLsd1 (2)	sense strand	CAAGGAAAGCUAGAAGAAA
	antisense strand	UUUCUUCUAGCUUUCCUUG
siSetdb1 (1)	sense strand	GGACGAUGCAGGAGAUAGA
	antisense strand	UCUAUCUCCUGCAUCGUCC
siSetdb1 (2)	sense strand	GGACUACAGUAUCAUGACA
	antisense strand	UGUCAUGAUACUGUAGUCC
siC (1)	sense strand	UAAGGCUAUGAAGAGAUACUU
	antisense strand	AAGUAUCUCUUCAUAGCCUUA
siC (2)*	sense strand	-
	antisense strand	-

* Silencer® negative control siRNA #1 (Applied Biosystems)

Suppl Table 2. List of primers and probe sets used for real time RT-PCR analysis

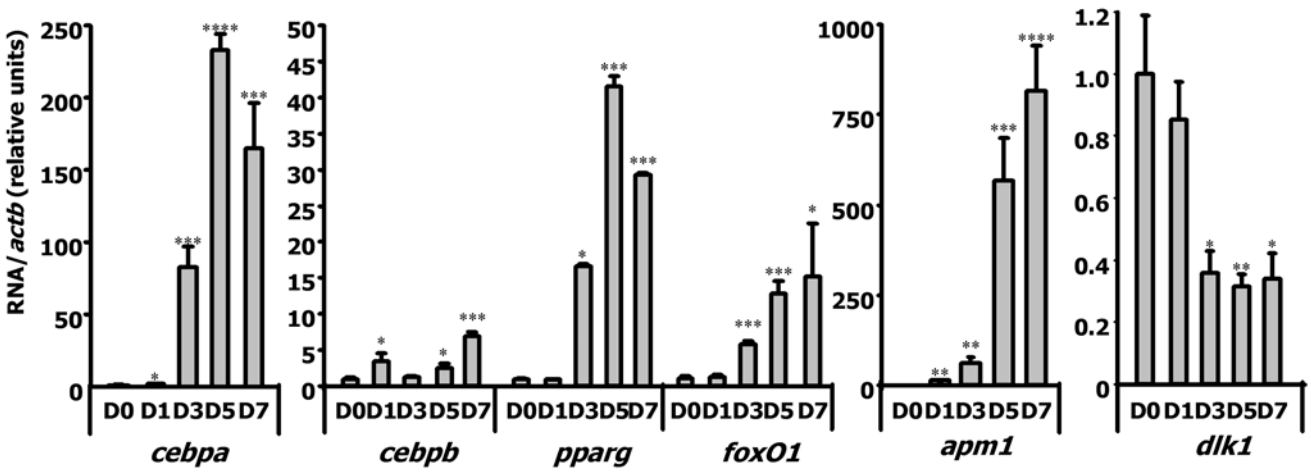
Target	**Reference (ABI)**	**Ref Seq ID**
Actb	Mm00607939_s1	NM_007393
Apm1	Mm01343606_m1	NM_009605
Cebpa	Mm00514283_s1	NM_007678
Cepb	Mm00843434_s1	NM_009883
Dlk1	Mm00494477_m1	NM_010052
Ehmt2	Mm01132261_m1	NM_145830/NM_147151
Fabp4	Mm00445880_m1	NM_024406
FoxO1	Mm00490672_m1	NM_019739
Glut4	Mm01245502_m1	NM_009204
Jarid1a	Mm00524465_m1	NM_145997
Jarid1b	Mm03053411_s1	NM_152895
Jmjd1a	Mm01182127_m1	NM_173001/NM_001038695
Jmjd1b	Mm00804683_m1	NM_001081256
Jmj2da	Mm00805000_m1	NM_172382/NM_001161823
Jmjd2c	Mm00460578_m1	NM_144787
Lsd1	Mm01181029_m1	NM_133872
Leptin	Mm00434759_m1	NM_008493
Ppparg	Mm00440945_m1	NM_001127330/NM_011146
Retn	Mm00445641_m1	NM_022984
Setd1a	Mm00626143_m1	NM_178029
Setd7	Mm00499823_m1	NM_080793
Setdb1	Mm00450791_m1	AK132202.1

Suppl Table 3. List of primers and probe sets used for the analysis of chromatin immunoprecipitations

Target		
actb	Forward Primer (5' to 3')	CCACACCCGCCACCA
	Reverse Primer (5' to 3')	GTGAGGTACTAGCCACGAGAGA
	Reporter (5' to 3')	CCGGCGTCCCTGCTTA
	Position relative to TIS	+55 to +127
apm1	Forward Primer (5' to 3')	AACCAGGTTCCCTAAGGAGTCT
	Reverse Primer (5' to 3')	GAGTGGGCCCCTTGCT
	Reporter (5' to 3')	CCTGGCAGCTGCCTTA
	Position relative to TIS	-164 to -110
cebpa	Forward Primer (5' to 3')	GGGCGCGAGCCAGTT
	Reverse Primer (5' to 3')	GTGGCGCCGCTGTC
	Reporter (5' to 3')	CCACCCAGTGCCCC
	Position relative to TIS	+22 to +72
dlk1	Forward Primer (5' to 3')	GGTCCTCCTGGGCTTCTGA
	Reverse Primer (5' to 3')	GCTCACAGACACAGTAAGACACTT
	Reporter (5' to 3')	TCCCGCGCACACATG
	Position relative to TIS	-150 to -75
myod	Forward Primer (5' to 3')	CTGCCCCAGCACAGAGT
	Reverse Primer (5' to 3')	AGTGTAGTAGGGCGGAGCTT
	Reporter (5' to 3')	CCCCAGTGGCTACCC
	Position relative to TIS	-191 to -107

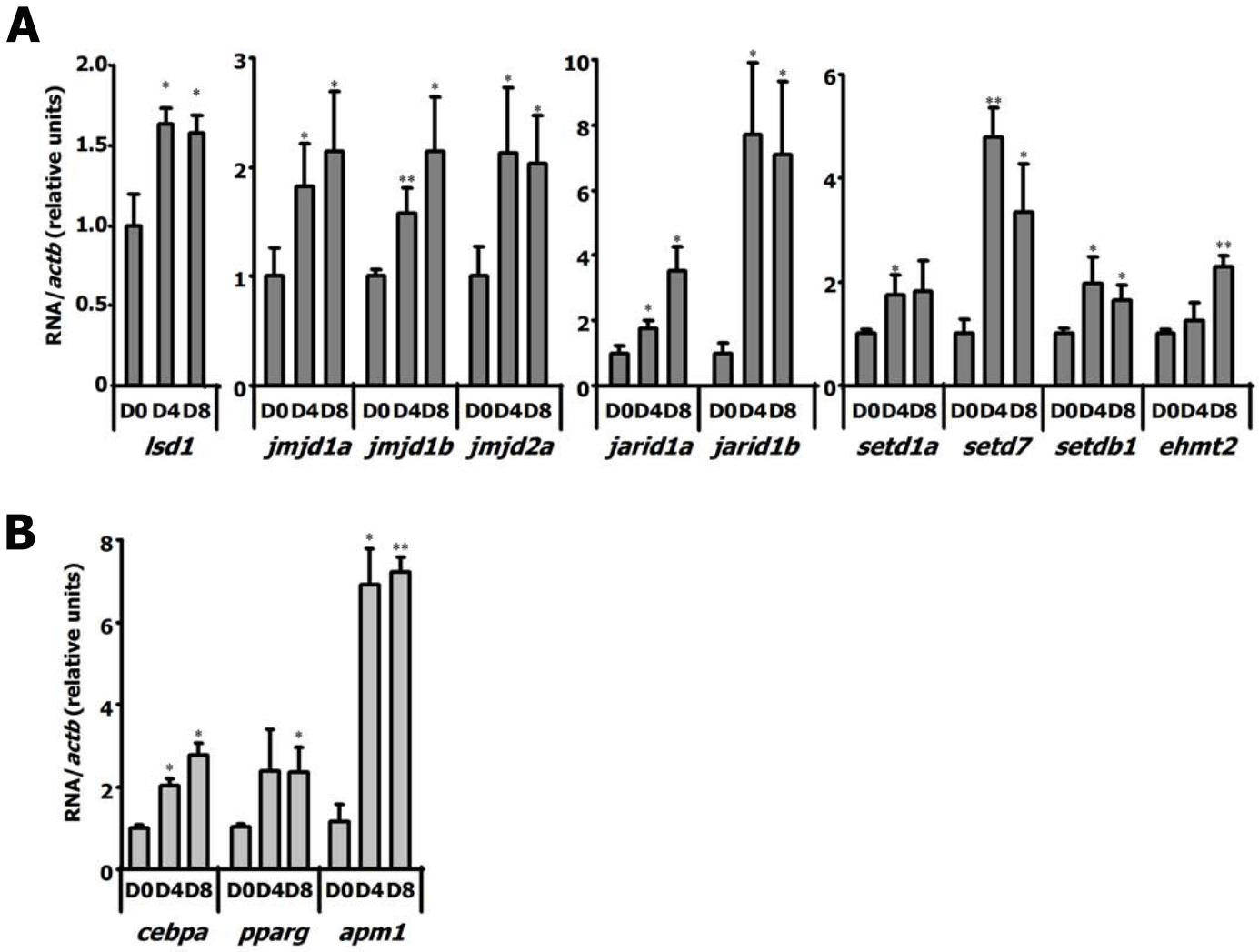
Musri et al.

Suppl Fig 1



Musri et al.

Suppl Fig 2



Musri et al.

Suppl Fig 3