Figure S1

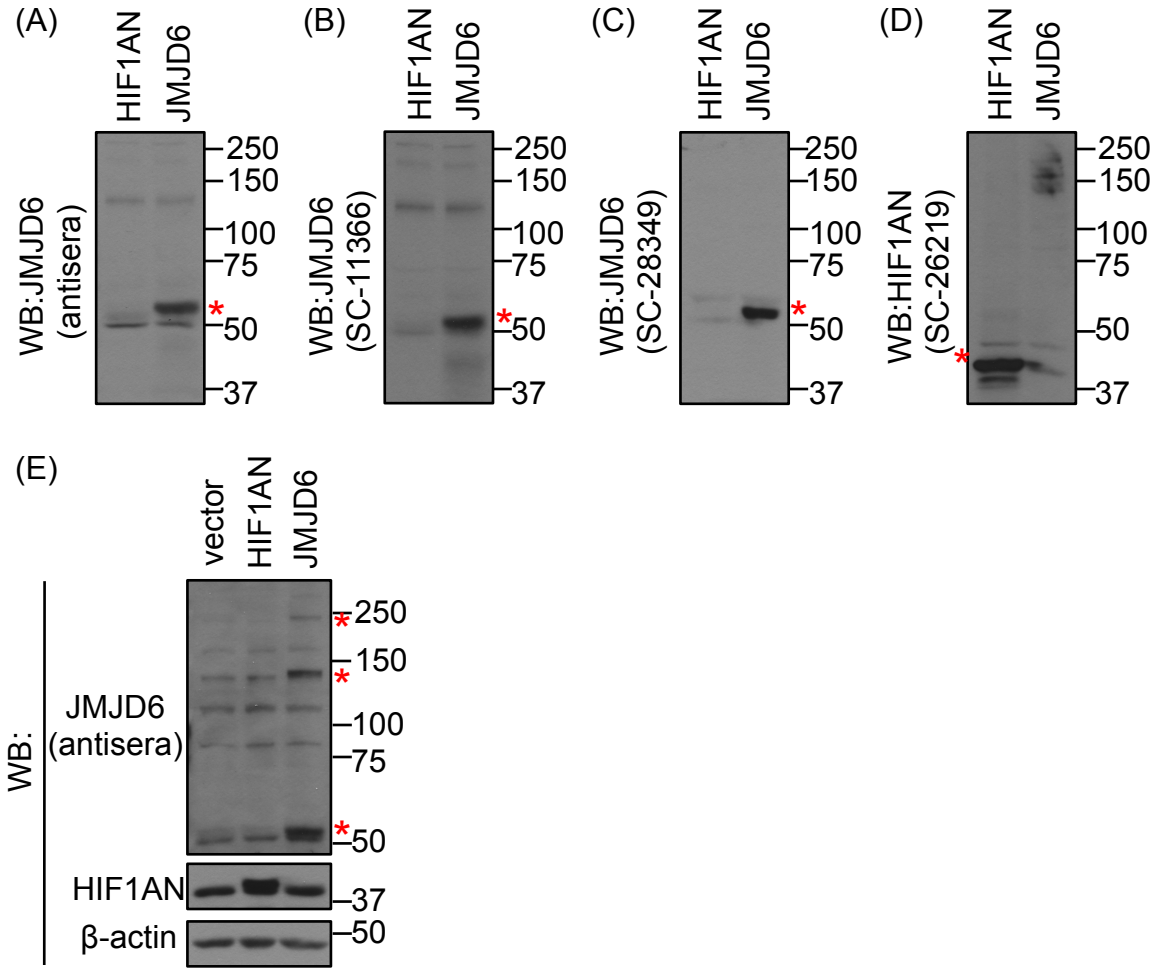

(A)
HIF1AN
JMJD6
WB:JMJD6 (antisera)
250
150
100
75
50
37
(B)
HIF1AN
JMJD6
WB:JMJD6 (SC-11366)
250
150
100
75
50
37
(C)
HIF1AN
JMJD6
WB:JMJD6 (SC-28349)
250
150
100
75
50
37
(D)
HIF1AN
JMJD6
WB:HIF1AN (SC-26219)
250
150
100
75
50
37
(E)
vector
HIF1AN
JMJD6
WB:
JMJD6 (antisera)
HIF1AN
β-actin
250
150
100
75
50
37
50

Figure S2

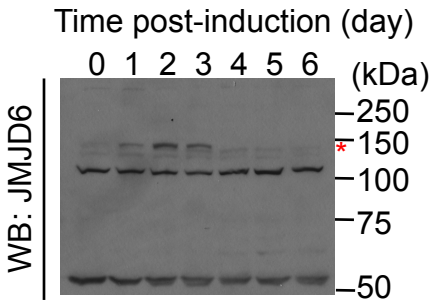

Time post-induction (day)
0 1 2 3 4 5 6
(kDa)
250
150
100
75
50
*
WB: JMJD6

Figure S3

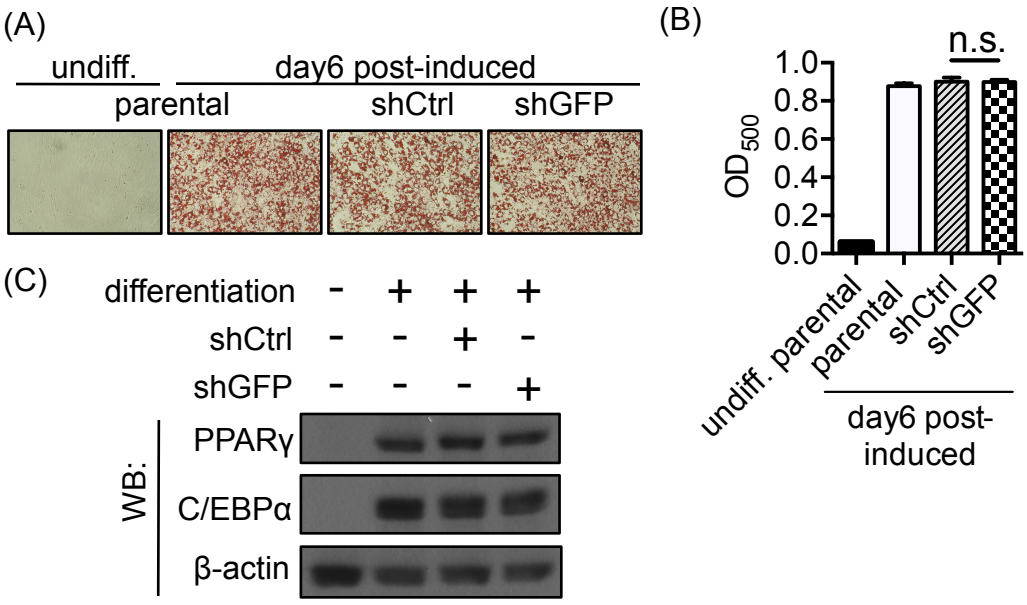

(A)
undiff.
day6 post-induced
parental
shCtrl
shGFP
(B)
n.s.
1.0
0.8
0.6
0.4
0.2
0.0
OD500
undiff. parental
parental
shCtrl
shGFP
day6 post-induced
(C)
differentiation - + + +
shCtrl - - + -
shGFP - - - +
WB:
PPARγ
C/EBPα
β-actin

Figure S4

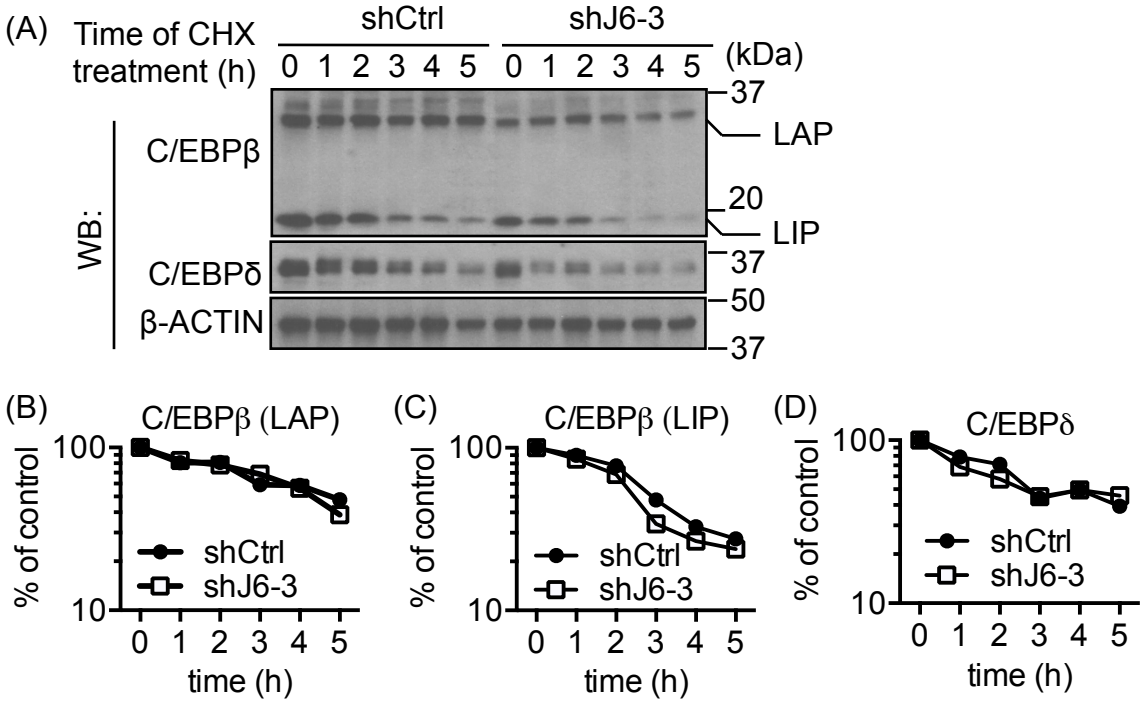

(A)
Time of CHX treatment (h)
shCtrl
shJ6-3
0 1 2 3 4 5 0 1 2 3 4 5
(kDa)
37
LAP
20
LIP
37
50
37
WB:
C/EBPβ
C/EBPδ
β-ACTIN
(B)
C/EBPβ (LAP)
(C)
C/EBPβ (LIP)
(D)
C/EBPδ
% of control
100
10
shCtrl
shJ6-3
0 1 2 3 4 5
time (h)

Figure S5

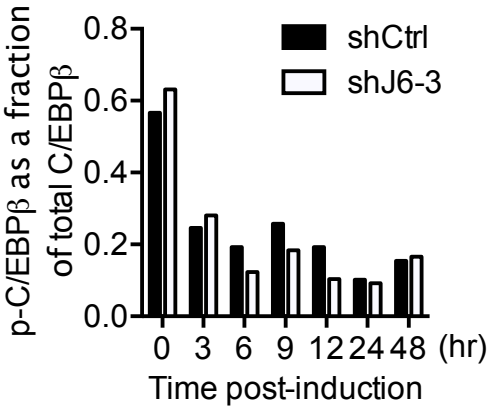
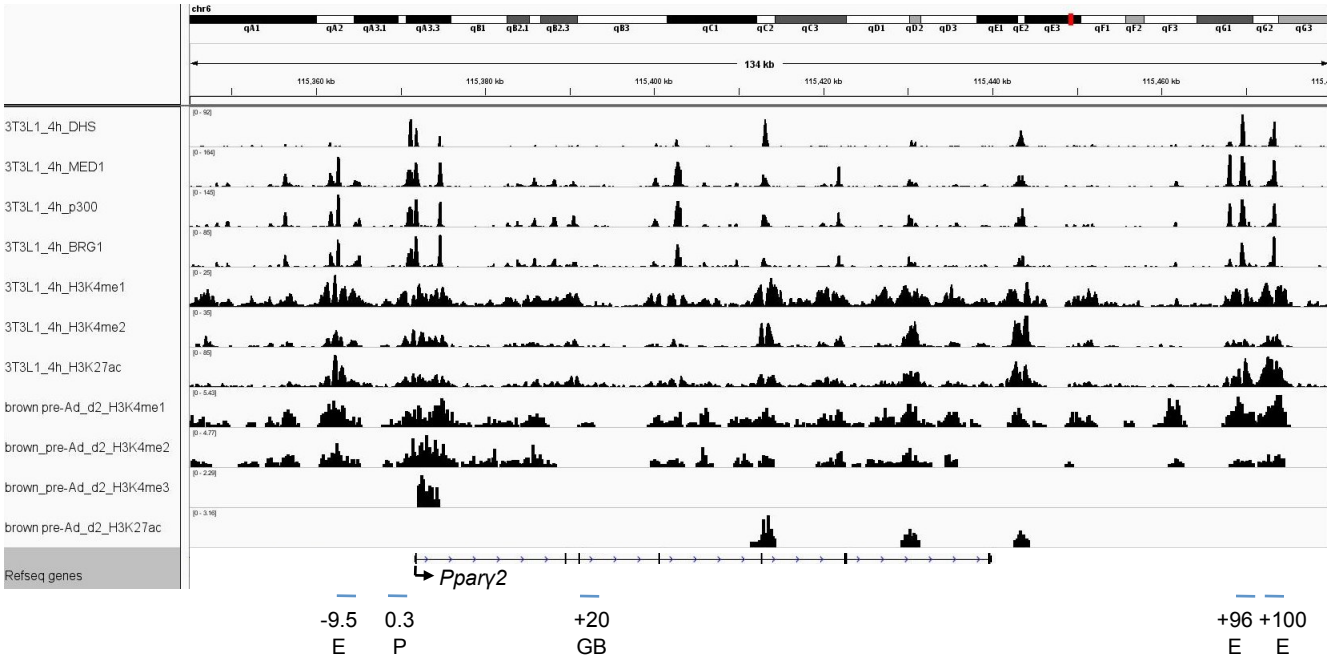

p-C/EBPβ as a fraction of total C/EBPβ
0.8
0.6
0.4
0.2
0.0
shCtrl
shJ6-3
0 3 6 9 12 24 48 (hr)
Time post-induction

Figure S6

(A)


chr6
134 kb
3T3L1_4h_DHS
3T3L1_4h_MED1
3T3L1_4h_p300
3T3L1_4h_BRG1
3T3L1_4h_H3K4me1
3T3L1_4h_H3K4me2
3T3L1_4h_H3K27ac
brown pre-Ad_d2_H3K4me1
brown_pre-Ad_d2_H3K4me2
brown_pre-Ad_d2_H3K4me3
brown pre-Ad_d2_H3K27ac
Refseq genes
Pparγ2
-9.5 E
0.3 P
+20 GB
+96 E
+100 E

(B)

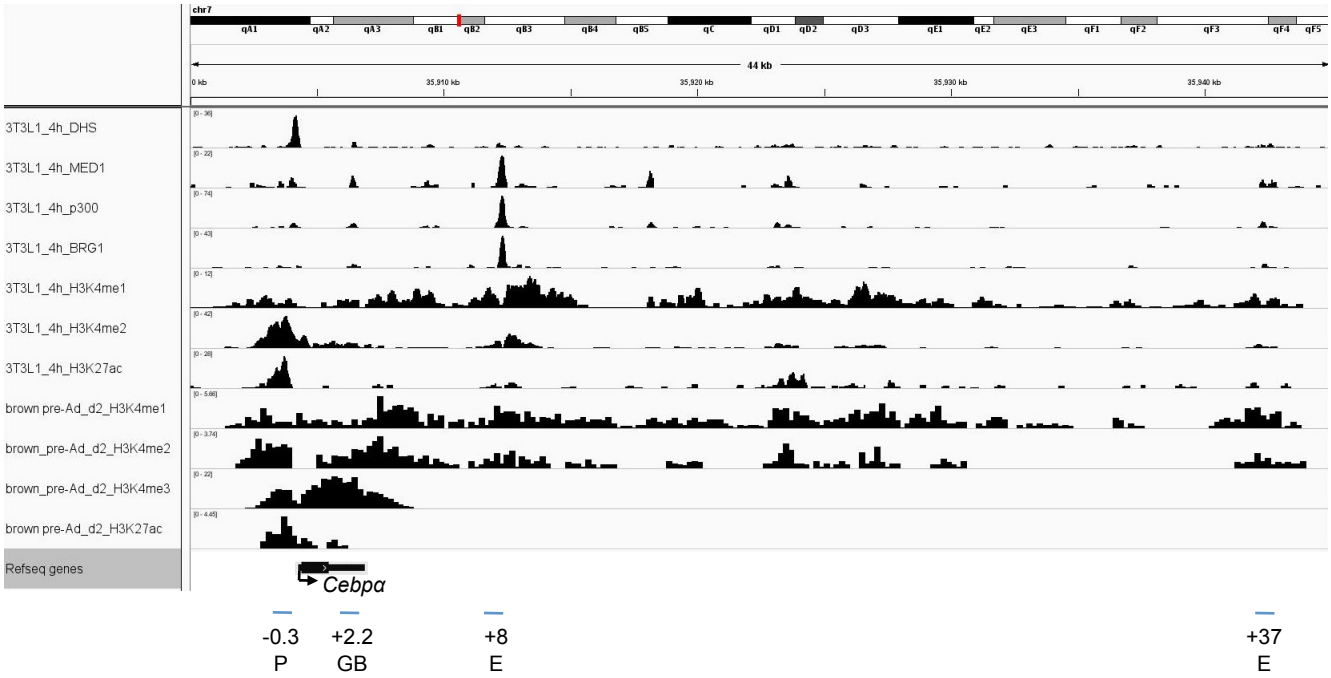

chr7
44 kb
3T3L1_4h_DHS
3T3L1_4h_MED1
3T3L1_4h_p300
3T3L1_4h_BRG1
3T3L1_4h_H3K4me1
3T3L1_4h_H3K4me2
3T3L1_4h_H3K27ac
brown pre-Ad_d2_H3K4me1
brown_pre-Ad_d2_H3K4me2
brown_pre-Ad_d2_H3K4me3
brown pre-Ad_d2_H3K27ac
Refseq genes
Cebpα
-0.3 P
+2.2 GB
+8 E
+37 E

Figure S7

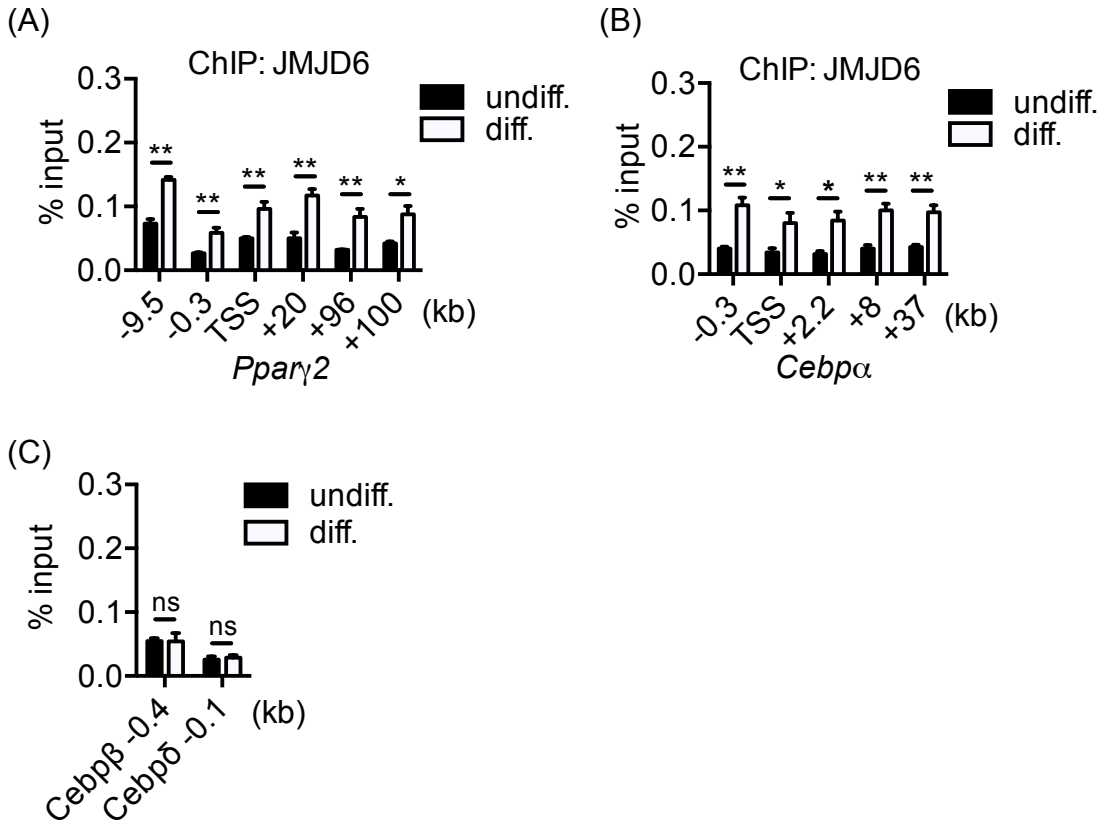

(A)
ChIP: JMJD6
undiff.
diff.
% input
0.3
0.2
0.1
0.0
**
**
**
**
**
*
-9.5
-0.3
TSS
+20
+96
+100
(kb)
Pparγ2
(B)
ChIP: JMJD6
undiff.
diff.
% input
0.3
0.2
0.1
0.0
**
*
*
**
**
-0.3
TSS
+2.2
+8
+37
(kb)
Cebpα
(C)
undiff.
diff.
% input
0.3
0.2
0.1
0.0
ns
ns
Cebpβ -0.4
Cebpδ -0.1
(kb)

Figure S8

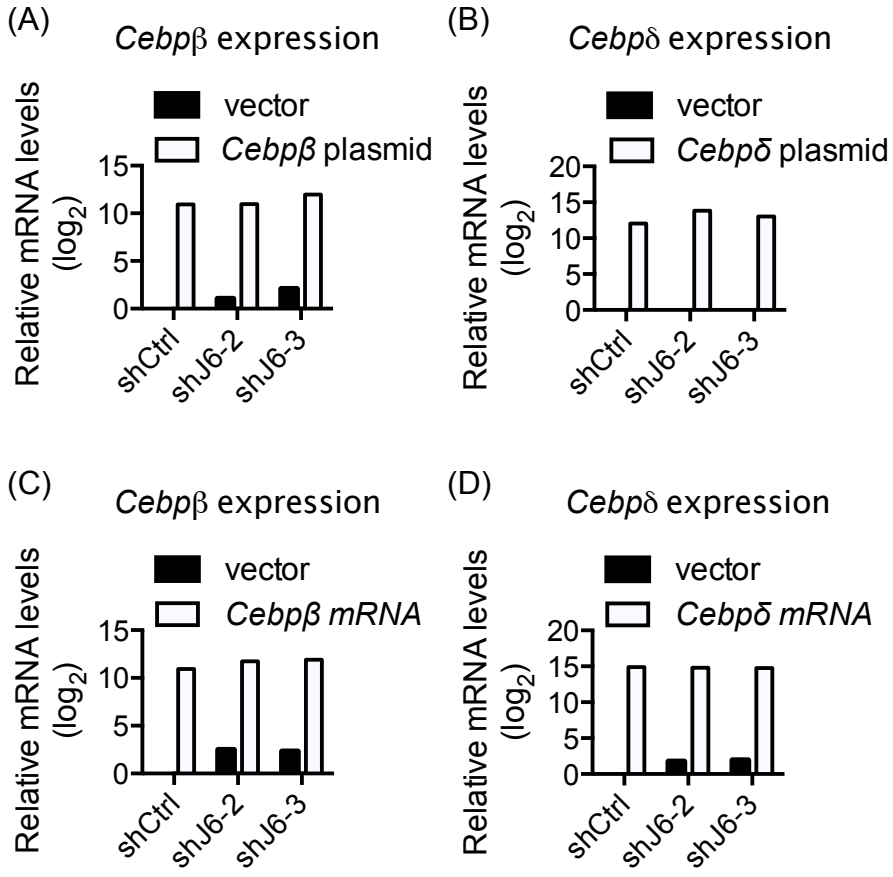

(A)
Cebpβ expression
vector
Cebpβ plasmid
Relative mRNA levels (log2)
15
10
5
0
shCtrl
shJ6-2
shJ6-3
(B)
Cebpδ expression
vector
Cebpδ plasmid
Relative mRNA levels (log2)
20
15
10
5
0
shCtrl
shJ6-2
shJ6-3
(C)
Cebpβ expression
vector
Cebpβ mRNA
Relative mRNA levels (log2)
15
10
5
0
shCtrl
shJ6-2
shJ6-3
(D)
Cebpδ expression
vector
Cebpδ mRNA
Relative mRNA levels (log2)
20
15
10
5
0
shCtrl
shJ6-2
shJ6-3

Figure S9

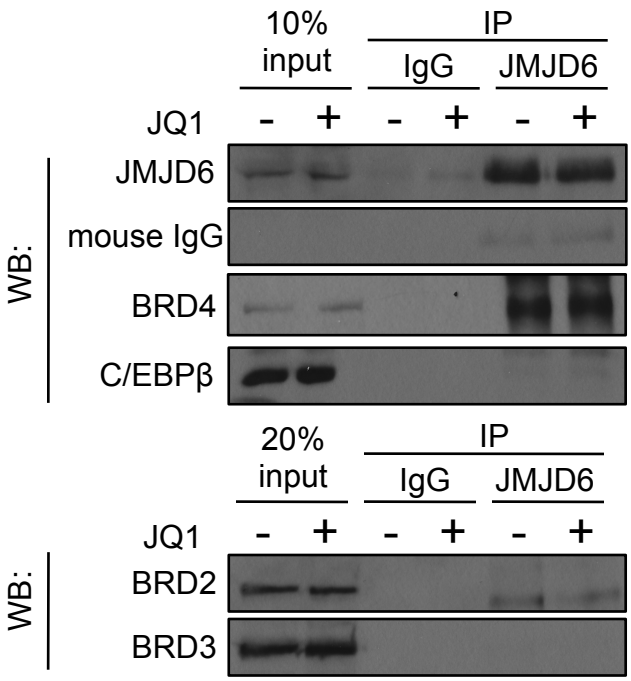

10% input
IP
IgG
JMJD6
JQ1
- + - + - +
WB:
JMJD6
mouse IgG
BRD4
C/EBPβ
20% input
IP
IgG
JMJD6
JQ1
- + - + - +
WB:
BRD2
BRD3

Figure S10

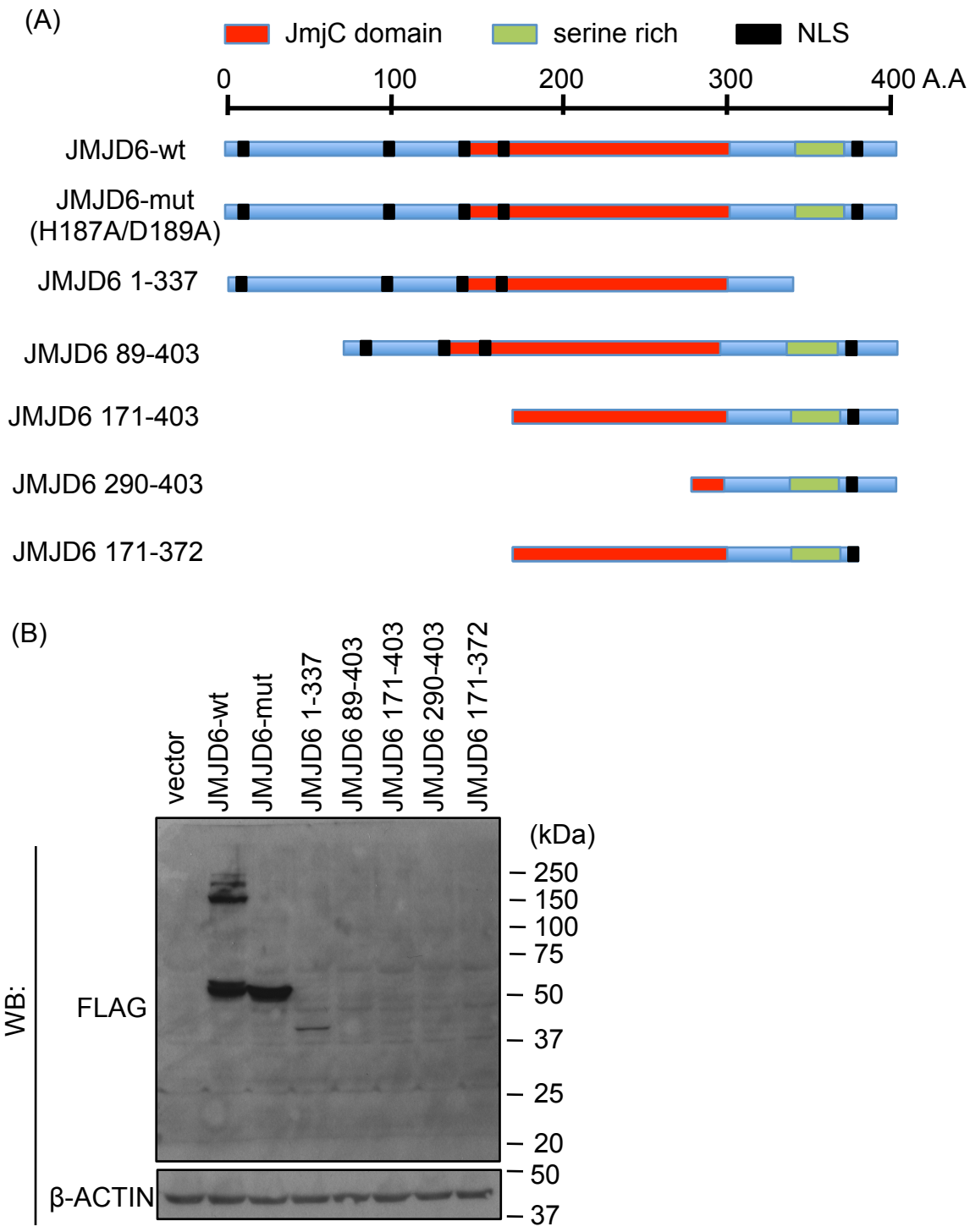

(A)
JmjC domain
serine rich
NLS
0
100
200
300
400 A.A
JMJD6-wt
JMJD6-mut
(H187A/D189A)
JMJD6 1-337
JMJD6 89-403
JMJD6 171-403
JMJD6 290-403
JMJD6 171-372
(B)
vector
JMJD6-wt
JMJD6-mut
JMJD6 1-337
JMJD6 89-403
JMJD6 171-403
JMJD6 290-403
JMJD6 171-372
(kDa)
250
150
100
75
50
37
25
20
50
37
WB:
FLAG
β-ACTIN

Table S1. The oligonucleotides and primers for plasmid construction

Primer name	Sequence 5' to 3'
shCtrl forward	GATCCTGGCGGCGAGTGAAGTACGTGATAAGTGTGCTG TCCTTATCACGTACTTCACTCGCCGCCATTTTTGGAAA
shCtrl reverse	AGCTTTTCCAAAAATGGCGGCGAGTGAAGTACGTGATA AGGACAGCACACTTATCACGTACTTCACTCGCCGCCAGG
shGFP forward	GATCCCGAAGCAGCACGACTTCTTCGTGTGCTGTCCGA AGTCGTGCTGCTTCTTTTTGGAAA
shGFP reverse	AGCTTTTCCAAAAAGAAGCAGCACGACTTCTTCGGACA GCACACGAAGAAGTCGTGCTGCTTCGG
shJ6-2 forward	GATCCCCGATGAACCACAAGAGCAAGTGTGCTGTCCTT GCTCTTGTGGTTCATCGTTTTTGGAAA
shJ6-2 reverse	AGCTTTTCCAAAAACGATGAACCACAAGAGCAAGGAC AGCACACTTGCTCTTGTGGTTCATCGGG
shJ6-3 forward	GATCCCGCTGACACCCAGAGAACAAGTGTGCTGTCCTT GTTCTCTGGGTGTCAGCTTTTTGGAAA
shJ6-3 reverse	AGCTTTTCCAAAAAGCTGACACCCAGAGAACAAGGAC AGCACACTTGTTCTCTGGGTGTCAGCGG
pBABE-JMJD6 forward	CCCGGATCCCTCACGATGAACCACAAGAGCAAG
pBABE-JMJD6 reverse	CGGTCGACTTATCATTTGTCATCGTCGTCCTTGTAGTCC CTGGAGGAGCTGCGCTC
pcDNA3.1-JMJD6 forward	same as pBABE-JMJD6
pcDNA3.1-JMJD6 reverse	CGAAGCTTTTATCATTTGTCATCGTCGTCCTTGTAGTCC CTGGAGGAGCTGCGCTC
pcDNA3.1-HIF1AN forward	CCCGAATTCGTAGAGATGGCGGCGACGGCAGCC
pcDNA3.1-HIF1AN reverse	CGAAGCTTTTATTATTTGTCATCGTCGTCCTTGTAGTCG TTGTAACGGCCTTTAAT
JMJD6 H187AD189A forward	GCGTTCTGGAACTGGGATTGCCATCGCCCCTCTGGGG
JMJD6 H187AD189A reverse	CCCCAGAGGGGCGATGGCAATCCCAGTTCCAGAACGC
GST-JMJD6 2-414 forward	GCGGATCCAACCACAAGAGCAAGAAGCGCATC
GST-JMJD6 2-414 reverse	CCCAAGCTTTTATTTGTCATCGTCGTCCTTGTAGTCCCT GGAGGAGCTGCGCTCTTTGCT

Table S2. The primers for gene expression analysis

Primer name	Sequence 5’ to 3’
Jmjd6 forward	GAGCGCCTCAAAAGGAAATA
Jmjd6 reverse	TGGGCACCTTGTAGTCTTCC
Pparγ2 forward	GCATGGTGCCTTCGCTGATGC
Pparγ2 reverse	AGGCCTGTTGTAGAGCTGGGT
Cebpα foward	CCGGCCGCCTTCAACGAC
Cebpα reverse	CTCCTCGCGGGGCTCTTGTTT
Cebpβ forward	TGGACAAGCTGAGCGACGAG
Cebpβ reverse	TGTGCTGCGTCTCCAGGTTG
Cebpδ forward	TTCAGCGCCTACATTGACTC
Cebpδ reverse	TGTGGTTGCTGTTGAAGAGG
Srebp1c forward	AGCTGTCGGGGTAGCGTCTG
Srebp1c reverse	GAGAGTTGGCACCTGGGCTG
Fasn forward	CGTGTTGGCCTACACCCAGAGCT
Fasn reverse	GGCAGCAGGGCCTCCAGCACCTT
Fabp4 forward	GCGTGGAATTCGATGAAATCA
Fabp4 reverse	CCCGCCATCTAGGGTTATGA
AdipoQ forward	CAGTGGATCTGACGACACCA
AdipoQ reverse	CGAATGGGTACATTGGGAAC
5S rRNA forward	GTCTACGGACATACCACCCTG
5S rRNA reverse	TACAGCACCCGGTATTCCCAG

Table S3. The primers for ChIP assay

Name	**Sequence 5' to 3'**
Pparγ2 -9.5kb forward	TTCTTCCCAGTAGGAACTGCAT
Pparγ2 -9.5kb reverse	GATCACTCAGTTGGCATTTCTC
Pparγ2 -0.3kb forward	TGGCCAAATACGTTTATCTGG
Pparγ2 -0.3kb reverse	CCAGTGACCCACACATTCACTG
Pparγ2 TSS forward	ATTCCCACCTCTCCCAAATA
Pparγ2 TSS reverse	GCTCTGGGTCAACAGGAGAA
Pparγ2 +20kb forward	CGAGTCTGTGGGGATAAAGC
Pparγ2 +20kb reverse	CCAAAACAACTCCCCACAAC
Pparγ2 +96kb forward	CTTGGGAGCTACAGCCTTGTG
Pparγ2 +96kb reverse	GCTGTGGTGAAACGACAGTTATTA
Pparγ2 +100kb forward	CCGGGCTCCCTAGATGTAGAC
Pparγ2 +100kb reverse	ACCCTTTCTGAGCGACAACCT
Cebpα -0.3kb forward	CTCCCTAGTGTTGGCTGGAA
Cebpα -0.3kb reverse	GGTGAGTGGGGAGCATAGTG
Cebpα TSS forward	GCCCGACCCTCTATAAAAGC
Cebpα TSS reverse	GGCTCCACCTCGTAGAAGTC
Cebpα +2.2kb forward	GCAGTGTGCACGTCTATGCT
Cebpα +2.2kb reverse	AGCCCACTTCATTTCATTGG
Cebpα +8kb forward	CACAGTGTCTGCTCGCTGTT
Cebpα +8kb reverse	ATTACAGTGCCGCCAGGTAG
Cebpα +37kb forward	CCCCAATCTTCCCTCAAATGA
Cebpα +37kb reverse	GGAGCCCGGAACCAGAA
Cebpβ -0.4kb *forward*	CGTGTAGCTGGAGGAACGAT
Cebpβ -0.4kb *reverse*	CTCGGGAACACGGAGGAG
Cebpδ -0.1kb *forward*	AGGAGGGAAGGCAAGGAGT
Cebpδ -0.1kb *reverse*	CTTTTCTAGCCCCAGCTGAC

Supplemental Figure and Table Legends

Figure S1. JMJD6 antisera and antibodies specifically recognize *in vitro* translated and *in vivo* JMJD6 protein. Representative western blots probed with **(A)** the JMJD6 antisera, **(B and C)** the commercial antibodies for JMJD6, and **(D)** the commercial antibody for HIF1AN, a different JmjC protein. JMJD6 and HIF1AN proteins were *in vitro* translated from 1 μg of the pcDNA3.1(-) plasmids encoding either FLAG-tagged mouse JMJD6 or HIF1AN using a TNT Quick coupled Transcription/Translation Systems (Promega) according to the manufacturer's instructions. 15 μl of the in vitro translated mixture was run on 10% SDS-PAGE and Western blotting was performed. **(E)** Representative western blots for JMJD6 and HIF1AN expression in the 24 h post-transfected C3H10T1/2 cells. 2 μg of the pcDNA3.1(-) plasmids encoding either FLAG-tagged mouse JMJD6 or HIF1AN, or the empty vector were transiently transfected into C3H10T1/2 cells using Lipofectamine 2000 according to the manufacturer's instructions. 20 μg of the total cell lysate was run on 10% SDS-PAGE for electrophoresis and Western blotting. β-ACTIN was probed as a loading control. Red asterisks indicate the bands corresponding to the target monomer and oligomeric proteins.

Figure S2. JMJD6 oligomerization in the C3H10T1/2 cells during adipogenic differentiation. A representative western blot for JMJD6 levels in undifferentiated (day 0) and in differentiating (day 1 to 6) C3H10T1/2 cells. The oligomeric JMJD6 protein bands (~130kDa) are indicated by the red asterisk.

Figure S3. Two distinct control shRNAs, shCtrl and shGFP, did not affect the adipogenic differentiation of C3H10T1/2 cells. **(A)** Representative Oil Red O staining images, **(B)** the levels of Oil-Red O staining, and **(C)** the protein levels of PPARγ and C/EBPα from the cells as indicated.

Figure S4. JMJD6 knockdown had no effect on C/EBPβ and C/EBPδ protein stability. (A) Representative western blots for C/EBPβ and C/EBPδ protein levels upon cycloheximide treatment. The 3 h post-induction cells were treated with 100 μg/ml cycloheximide. The total cell lysates were harvested every hour for 5 additional hours in the presence of cycloheximide. β-ACTIN was probed as a loading control. **(B-D)** C/EBPβ(LAP), C/EBPβ(LIP) and C/EBPδ protein levels in two independent experiments (n=2) were quantified by ImageJ. The protein levels were normalized to β-ACTIN loading control and were presented as the relative expression levels to the 0 h sample (mean, n=2).

Figure S5. The level of C/EBPβ phosphorylation is proportional to total C/EBPβ levels both in the control (shCtrl) and in the JMJD6 knockdown cells (shJ6-3) throughout the early phase of differentiation. The levels of C/EBPβ and phosphorylated C/EBPβ in Figure 4A were quantified by ImageJ.

Figure S6. Calling the putative enhancers using the published DHS-seq and ChIP-seq datasets. The DHS-seq datasets (GSE27826) and ChIP-seq datasets (GSE56872 and GSE50466) were uploaded to Integrative Genomics Viewer. (A) Screenshot of *Pparγ2*

locus. (B) Screenshot of *Cebpα* locus. Blue bars indicate the regions for primer design. P: promoter; E: putative enhancer; GB: gene body.

Figure S7. Inducible binding of JMJD6 at the *Pparγ2* and *Cepbα* loci during differentiation. (A-B) ChIP of JMJD6 at the *Pparγ2* and *Cebpα* loci in the undifferentiated (0h post-induced) and in the differentiating (24h post-induced) C3H10T1/2 cells. The values are presented as the average % input from three independent experiments (S.E.M, n=3, **p*<0.05, ***p*<0.01). **(C)** The binding of JMJD6 at the *Cebpβ* and *Cebpδ* promoters was examined as negative controls. The values are presented as the average % input from three independent experiments (S.E.M, n=3, ns: not significant)

Figure S8. Ectopic expression of *Cebpβ* and *Cebpδ* by introduction of plasmid or *in vitro* synthesized RNA. (A-B) *Cebpβ* and *Cebpδ* mRNA levels in the plasmid-transfected cells. The cells were transfected with the plasmids encoding either C/EBPβ or C/EBPδ or empty vector for 24 h and then harvested for gene expression analysis. **(C-D)** *Cebpβ* and *Cebpδ* mRNA levels in the RNA-transfected cells. The cells were transfected with the *in vitro* synthesis mRNA encoding either C/EBPβ or C/EBPδ for 24 h and harvested for gene expression analysis. The values presented are the average relative expression levels from technical replicates of the matching RNA samples from the western blot experiment presented in Figs. 5A-B.

Figure S9. BRD4, but not BRD2, BRD3 or C/EBPβ, is immunoprecipitated with JMJD6 regardless of JQ1 treatment. Endogenous JMJD6 proteins in day1 post-induced C3H10T1/2 cells with and without JQ1 treatment were immunoprecipitated. The eluted samples were run on 8% SDS-PAGE for western blotting. The blots were probed with antibodies against JMJD6 (sc-28349), mouse IgG (NA9310), BRD4 (A301-985A50), C/EBPβ (sc-150), BRD2 (A302-583A), and BRD3 (A302-367A).

Figure S10. Ectopically expression of JMJD6 protein in C3H10T1/2 cells.

(A) Scheme of the full-length wild type, catalytic-inactive and truncated JMJD6 mutants. The DNA sequences encoding the truncated proteins were PCR amplified and cloned into a pBABE vector as the same as for the wild type and catalytic-inactive JMJD6 mutant. **(B)** A representative western blot for the expression of JMJD6 proteins in C3H10T1/2 cells. The blot was probed with a serum recognizing FLAG sequence and re-probed with an antibody for β-ACTIN as a loading control.

Table S1. The oligonucleotides for plasmid construction

Table S2. The primers for gene expression analysis

Table S3. The primers for ChIP assay