1) Supplemental Figures

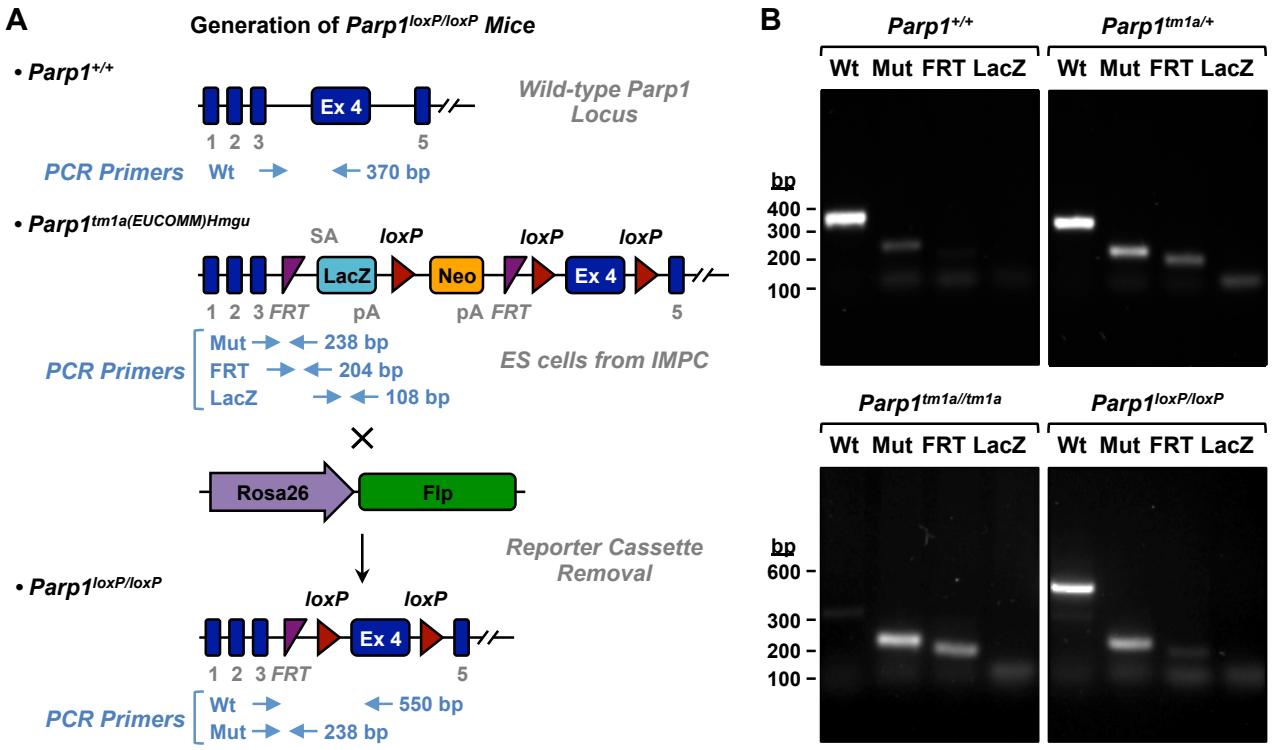


Figure S1. Generation of *Parp1*$^{loxP/loxP}$ mice, related to Figure 1.
(A) Schematic diagram showing a flow chart for the generation of *Parp1*$^{loxP/loxP}$ mice on a C57BL/6N background. The PCR primer pairs used for genotyping at each step are indicated.
(B) Ethidium bromide-stained agarose gels showing the diagnostic PCR products used for genotyping at each step in the generation of *Parp1*$^{loxP/loxP}$ mice. The PCR products are indicated by the PCR primer sets shown in (A). Size markers in base pairs (bp) are shown.

[Figure S2 is shown on the next page]

Figure S2. Enhanced differentiation of 3T3-L1 cells upon chemical inhibition or knockdown of PARP-1, with rescue by re-expression of RNAi-resistant PARP-1, related to Figure 2.

(A) Western blot showing the inhibition of PARylation at different doses of BYK204165 in 3T3-L1 cells. PAR detection was with a custom recombinant antibody-like anti-pan-ADP-ribose binding reagent (anti-panADPR). snRNP70 was used as a nuclear loading control.

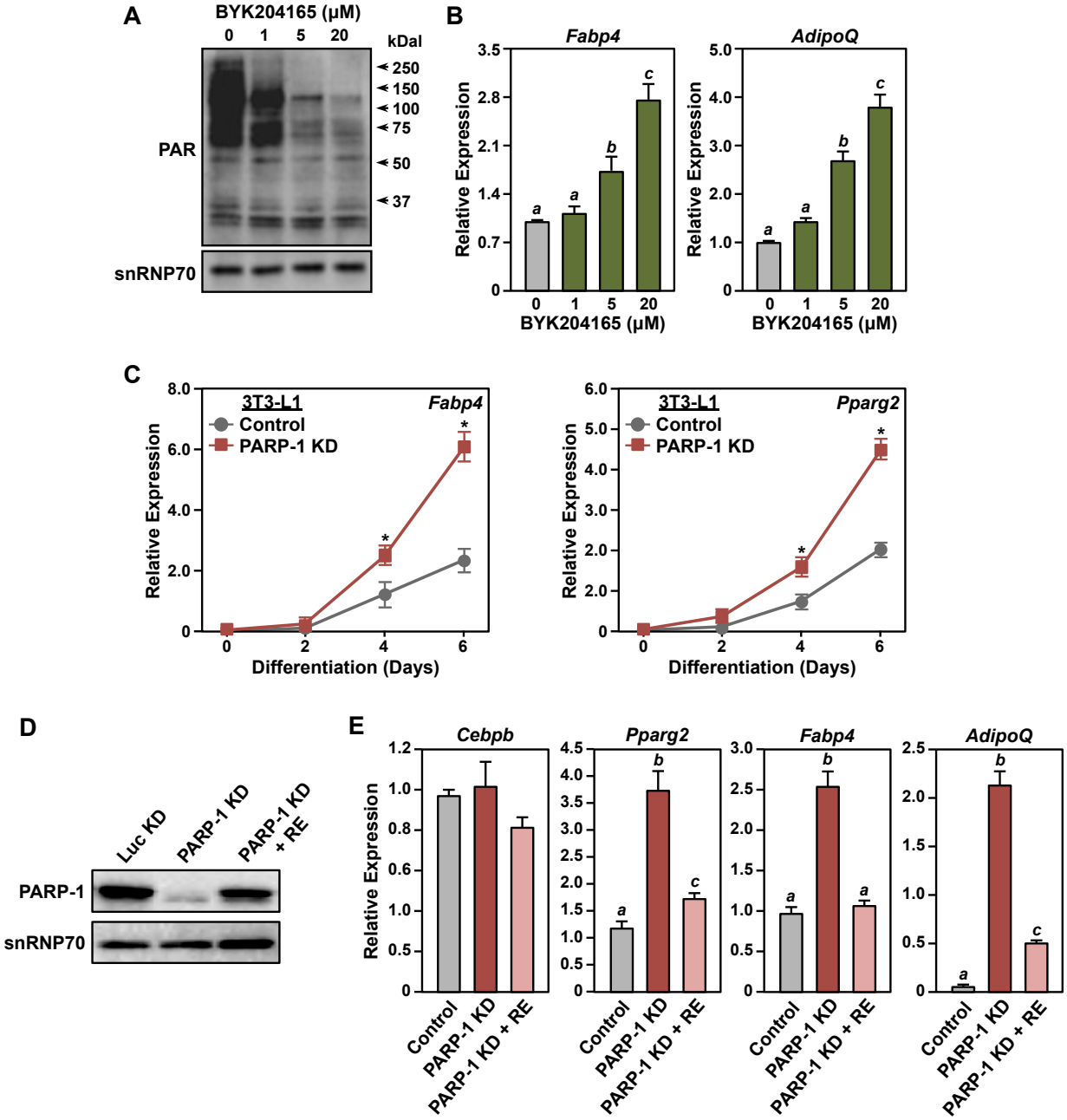
(B) The PARP-1-selective inhibitor BYK204165 exhibits dose-dependent inhibition of PARylation and promotion of adipogenesis in 3T3-L1 cells. Results from RT-qPCR assays showing the dose-dependent effects of PARP-1 inhibition with BYK204165 on the expression of genes encoding markers of adipocyte differentiation (*Fabp4* and *AdipoQ*) in 3T3-L1 cells. Each bar represents the mean + SEM for three independent biological replicates. Bars marked with different letters are statistically different from each other (ANOVA; p-value < 0.05).

(C) Results from RT-qPCR assays showing the effects of PARP-1 knockdown (KD) on the expression of genes encoding a late adipogenic transcription factor (*Pparg2*) or a marker of adipocyte differentiation (*Fabp4*) in 3T3-L1 cells over a six-day time course of differentiation. Each point represents the mean ± SEM for three independent biological replicates. Points marked with an asterisk are statistically different from the vehicle-treated control (Student's t-test; p-value < 0.05).

(D) Western blot showing shRNA-mediated depletion of PARP-1 (knockdown; KD) in 3T3-L1 cells with or without re-expression of RNAi-resistant PARP-1 (re-expression; RE). snRNP70 was used as a loading control.

(E) The effects of PARP-1 knockdown using PARP-1 shRNA are specific and can be rescued by re-expression of RNAi-resistant PARP-1. Results from RT-qPCR assays showing that the effects of PARP-1 knockdown (KD) on the expression of genes encoding late adipogenic transcription factors (*Pparg2*) or markers of adipocyte differentiation (*Fabp4, AdipoQ*) are abrogated by re-expression (RE) of RNAi-resistant PARP-1 in 3T3-L1 cells. The expression of the gene encoding the early adipogenic transcription factor *Cebpb*, however, is not affected by PARP-1 knockdown or re-expression, as suggested by the results shown in Figure 3C. Each bar represents the mean + SEM for three independent biological replicates. Bars marked with different letters are statistically different from each other (ANOVA; p-value < 0.05).

Figure S2.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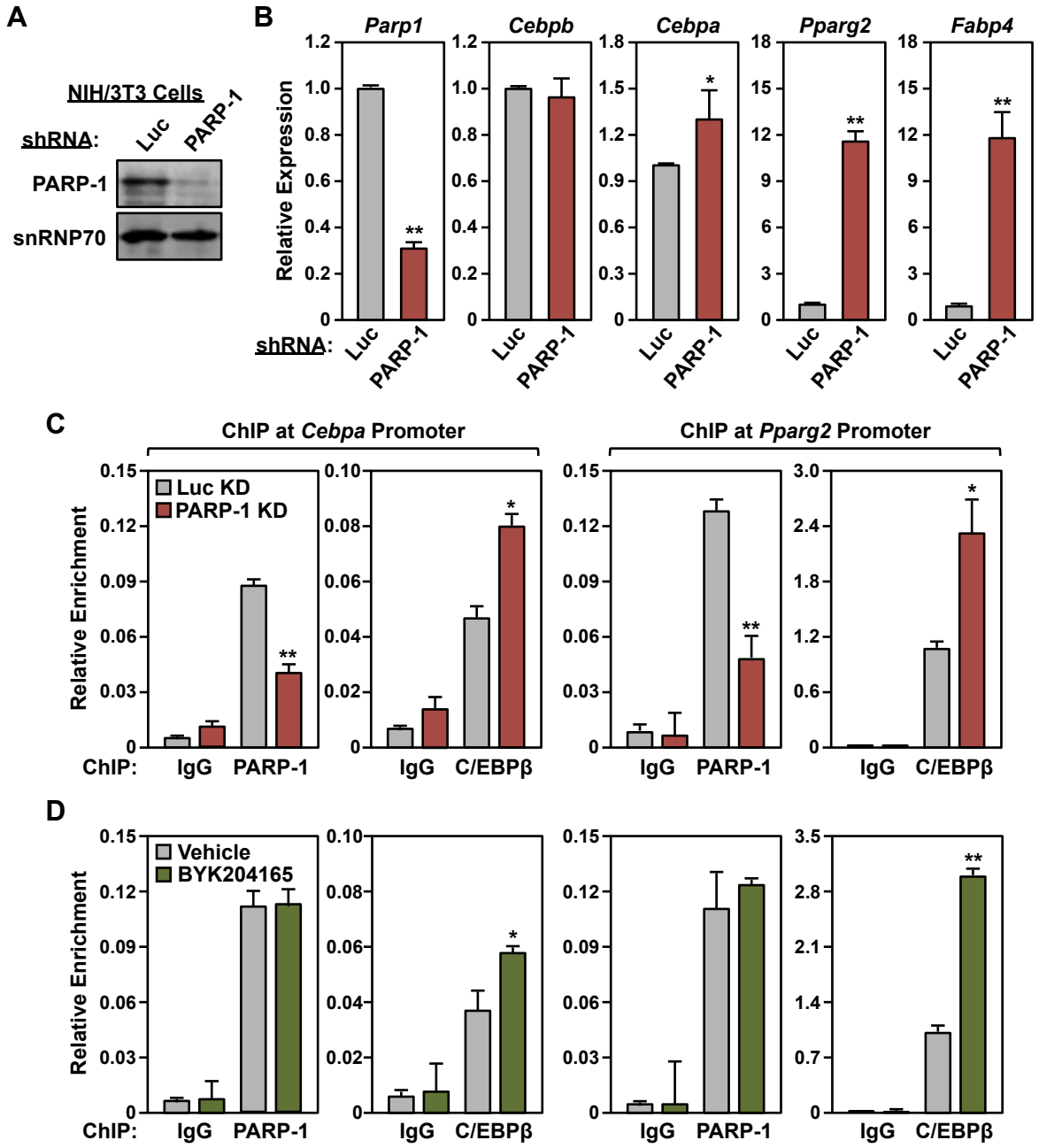
kDal
250
150
100
75
50
37
PAR
snRNP70
B
Fabp4
AdipoQ
Relative Expression
BYK204165 (μM)
C
3T3-L1
Control
PARP-1 KD
Fabp4
Pparg2
Relative Expression
Differentiation (Days)
D
Luc KD
PARP-1 KD
PARP-1 KD + RE
PARP-1
snRNP70
E
Cebpb
Pparg2
Fabp4
AdipoQ
Relative Expression
Control
PARP-1 KD
PARP-1 KD + RE

[Figure S3 is shown on the next page]

Figure S3. PARP-1 and PARylation modulate C/EBPβ binding to chromatin and the expression of C/EBPβ target genes encoding late transcription factors in NIH/3T3 cells, related to Figures 3 and 4.
(A) Knockdown of PARP-1 in NIH/3T3 cells. Western blot showing the levels of PARP-1 after knockdown using a retroviral vector driving expression of shRNAs targeting PARP-1 or luciferase (Luc, as a control). snRNP70 was used as an internal loading control.
(B) Knockdown of PARP-1 selectively affects the expression of adipogenic transcription factors. RT-qPCR analysis showing the expression of early transcription factors (2 hours post MDI treatment; *Cebpb*, *Cebpd*), and late transcription factors (4 days post MDI treatment; *Cebpa*, *Pparg2*) in NIH/3T3 cells + knockdown using shRNAs targeting PARP-1 or luciferase (Luc, as a control). Results for *Parp1* are shown to confirm knockdown. Each bar represents the mean + SEM for three independent biological replicates. Bars marked with asterisks are statistically different from the control (Student's t-test; ** p-value < 0.01 or * p-value < 0.05).
(C and D) Knockdown of PARP-1 or inhibition of PARP-1 catalytic activity enhances the binding of C/EBPβ to the *Cebpa* (*left*) and *Pparg2* (*right*) gene promoters. Results from ChIP-qPCR assays for PARP-1 or C/EBPβ (as indicated) performed in NIH/3T3 cells 24 hours after MDI-induced differentiation. The assays were performed (C) with shRNA-mediated knockdown of PARP-1 or luciferase (Luc, as a control), or (D) ± treatment with 20 μM BYK204165 (1 hour pretreatment, followed by 24 hours of treatment after adding MDI). Each bar represents the mean + SEM for three independent biological replicates. Bars marked with asterisks are statistically different from the control (Student's t-test; ** p-value < 0.01 or * p-value < 0.05).

Figure S3.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NIH/3T3 Cells
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PARP-1
snRNP70
B
Parp1
Cebpb
Cebpa
Pparg2
Fabp4
Relative Expression
shRNA: Luc PARP-1
C
ChIP at Cebpa Promoter
ChIP at Pparg2 Promoter
Luc KD
PARP-1 KD
Relative Enrichment
ChIP: IgG PARP-1 IgG C/EBPβ
D
Vehicle
BYK204165
Relative Enrichment
ChIP: IgG PARP-1 IgG C/EBPβ

[Figure S4 is shown on the next page]

Figure S4. PARylation and inducible expression of C/EBPβ in 3T3-L1 cells, related to Figure 5.

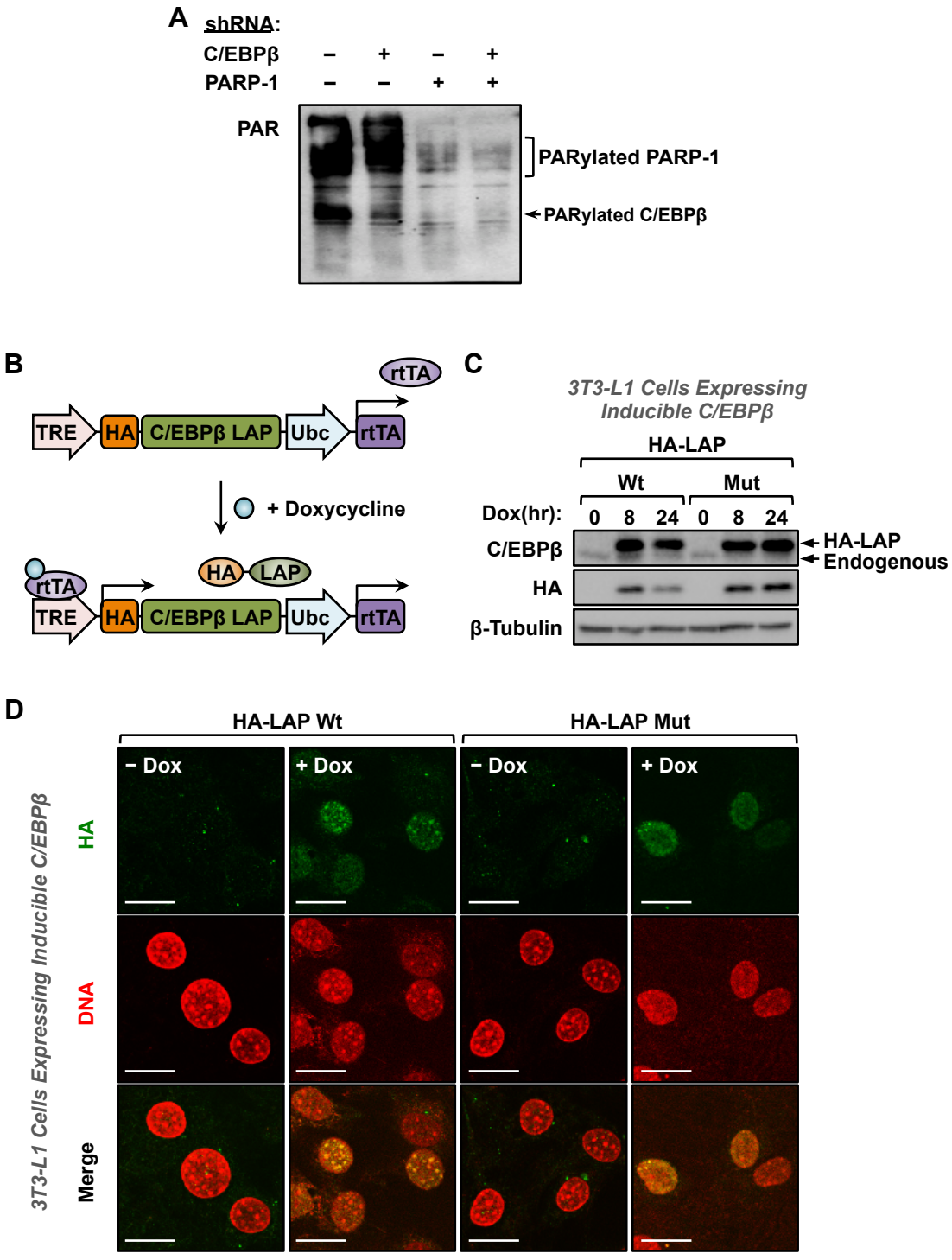
(A) PARP-1-dependent PARylation of C/EBPβ. Western blots showing the levels of PARylated and total C/EBPβ and PARP-1 in control, C/EBPβ, or PARP-1 depleted 3T3-L1 cells. Knockdown was achieved by using retroviral vectors driving expression of shRNAs targeting C/EBPβ, PARP-1, or luciferase (Luc, as a control). snRNP70 was used as an internal loading control. This is the entire blot of PARylated C/EBPβ shown in Figure 5A.

(B) Schematic of the system used for doxycycline (Dox)-inducible expression of HA-tagged full-length mouse C/EBPβ in 3T3-L1 cells. The system contains the following: (1) the Tet response element (TRE) linked to the Cytomegalovirus (CMV) promoter, (2) a cDNA encoding HA-tagged full-length mouse C/EBPβ (LAP), and (3) the *Ubc* promoter driving expression of a cDNA encoding the reverse tetracycline-controlled transactivator (rtTA).

(C) Western blots showing Dox-inducible expression of HA-tagged wild-type or ADP-ribosylation site mutant (K133A, E135A, E139A) mouse C/EBPβ in 3T3-L1 cells using the expression system described in (B). Expression was induced for 8 hours (used for the immunoprecipitation assays in Figure 5 and the ChIP-seq assays in Figure 6) or 24 hours, as indicated. Cell extracts were blotted for C/EBPβ or the HA tag. β-tubulin was used as an internal loading control.

(D) Immunofluorescent staining showing Dox-inducible expression of HA-tagged wild-type or ADP-ribosylation site mutant (K133A, E135A, E139A) mouse C/EBPβ in 3T3-L1 cells using the expression system described in (B). Expression was induced for 8 hours. The signals for the HA tag and DNA (TO-PRO-3) are shown, along with the merged image.

Figure S4.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shRNA:
C/EBPβ – + – +
PARP-1 – – + +
PAR
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PARylated C/EBPβ
B
rtTA
TRE
HA
C/EBPβ LAP
Ubc
rtTA
+ Doxycycline
HA
LAP
C
3T3-L1 Cells Expressing Inducible C/EBPβ
HA-LAP
Wt
Mut
Dox(hr): 0 8 24 0 8 24
C/EBPβ
HA
β-Tubulin
HA-LAP
Endogenous
D
HA-LAP Wt
HA-LAP Mut
– Dox
+ Dox
– Dox
+ Dox
3T3-L1 Cells Expressing Inducible C/EBPβ
HA
DNA
Merge

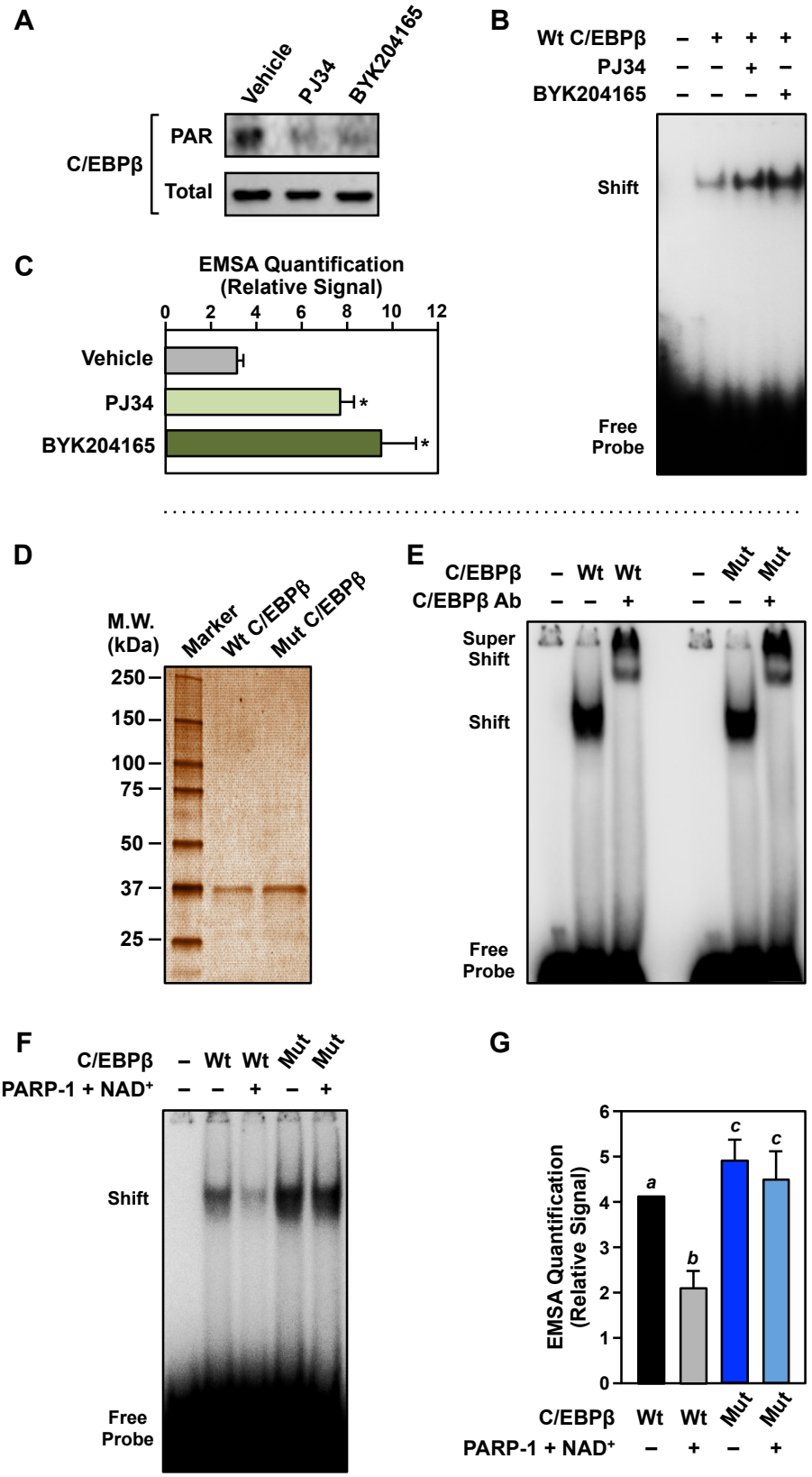
[Figure S5 is shown on the next page]

Figure S5. PARylation of C/EBPβ by PARP-1 at specific sites inhibits C/EBPβ binding to DNA, related to Figure 6.

(A through C) Chemical inhibition of PARP enzymatic activity increases DNA binding by C/EBPβ. HA-tagged wild-type mouse C/EBPβ (LAP) was expressed in 293T cells, and the cells were then treated with vehicle, 5 μM PJ34, or 20 μM BYK204165 for 1 hour prior to collection, nuclei isolation, and nuclear extract preparation. (A) Western blots showing the levels of PARylated (*top*) and total (*bottom*) C/EBPβ in nuclear extracts prepared from the transfected 293T cells. (B) Representative EMSA experiment performed using 4 μg of 293T cell nuclear extract per reaction and a labeled double stranded oligonucleotide probe corresponding to the C/EBP regulatory element in the *Cebpa* promoter (probe). (C) Quantification of the results from experiments like those shown in (B). Each bar represents the mean + SEM for three independent experiments. Bars marked with an asterisk are statistically different from the control (Student's t-test; p-value < 0.05).

(D through G) PARylation of purified recombinant C/EBPβ in vitro reduces its DNA binding. HA-tagged wild-type (Wt) or K133A/E135A/E139A triple point mutant (Mut) C/EBPβ proteins were expressed in 293T cells and then purified using HA affinity chromatography. The purified proteins were subjected to in vitro PARylation with purified PARP-1 + NAD^+ as indicated, and were then used in EMSA experiments. (D) Silver stained SDS-PAGE analysis of purified recombinant Wt or Mut C/EBPβ. (E) Representative EMSA experiment performed using Wt or Mut C/EBPβ and a labeled double stranded oligonucleotide probe corresponding to the C/EBP regulatory element in the *Cebpa* promoter (probe). The supershift was performed using a C/EBPβ antibody. (F) EMSA experiment performed using Wt or Mut C/EBPβ as in (E), with or without in vitro PARylation with purified PARP-1 + NAD^+ as indicated. (G) Quantification of the results from experiments like those shown in (F). Each bar represents the mean + SEM for six independent experiments. Bars marked with different letters are statistically different from each other (ANOVA; p-value < 0.05).

Figure S5.


A
Vehicle
PJ34
BYK204165
C/EBPβ
PAR
Total
B
Wt C/EBPβ – + + +
PJ34 – – + –
BYK204165 – – – +
Shift
Free Probe
C
EMSA Quantification (Relative Signal)
0 2 4 6 8 10 12
Vehicle
PJ34
BYK204165
*
*
D
M.W. (kDa)
Marker
Wt C/EBPβ
Mut C/EBPβ
250
150
100
75
50
37
25
E
C/EBPβ – Wt Wt – Mut Mut
C/EBPβ Ab – – + – – +
Super Shift
Shift
Free Probe
F
C/EBPβ – Wt Wt Mut Mut
PARP-1 + NAD+ – – + – +
Shift
Free Probe
G
EMSA Quantification (Relative Signal)
0 1 2 3 4 5 6
a
b
c
c
C/EBPβ Wt Wt Mut Mut
PARP-1 + NAD+ – + – +

2) Supplemental Experimental Procedures

Generation of *Parp1* conditional knockout mice

Parp1$^{tm1a(EUCOMM)Hmgu}$ embryonic stem (ES) cells on a C57BL/6N background were obtained from the International Mouse Phenotyping Consortium (IMPC; colony name G4786, colony number HEPD0555_6_C04). Chimeric mice were generated from the ES cells at UT Southwestern's Transgenic Core Facility. All mice were housed and maintained at UT Southwestern's Animal Resource Center. The chimeric mice were bred to C57BL/6 (*Parp1*$^{+/+}$) mice to generate *Parp1*$^{tm1a/+}$ heterozygous progeny (see Figure S1A). The presence of the *Parp1*^{tm1a} cassette was determined using the short-range PCR primers listed below (see Figure S1B). The *Parp1*$^{tm1a/+}$ mice were self-crossed to generate homozygous *Parp1*$^{tm1a/tm1a}$ progeny. The reporter cassette was excised by crossing the *Parp1*$^{tm1a/tm1a}$ mice with FLP recombinase-expressing B6.129S4-*Gt(ROSA)26Sor*$^{tm2(FLP*)Sor}$/J mice (from The Jackson Laboratory, stock no. 012930) (see Figure S1A). Removal of the reporter cassette was verified by short-range qPCR using the *FRT* and *LacZ* primer pairs listed below (see Figure S1B). Homozygous *Parp1*$^{loxP/loxP}$ mice were subsequently generated by self-crossing. To produce mice with a Tamoxifen-inducible conditional allele of *Parp1* (*Parp1*$^{loxP/loxP}$*;Pdgfra-cre/ERT*), *Parp1*$^{loxP/loxP}$ mice were crossed with transgenic mice containing a *Pdgfra-cre/ERT* cassette [B6N.Cg-*Tg(Pdgfra-cre/ERT)467Dbe/J*; The Jackson Laboratory, stock no. 018280]. The presence of the *Pdgfra-cre/ERT* allele was determined using the short-range *Pdgfra* and *Cre* PCR primers listed below (see Figure S1B).

The following short-range PCR primers used for genotyping:

Wild-type (Wt):
Forward: 5'-CTGTGGTCCTCTTGCCTCTG-3'
Reverse: 5'-ACTTCCCCAGGGATGGGTTA-3'

Mutant (Mut):
Forward: 5'-CTGTGGTCCTCTTGCCTCTG-3'
Reverse: 5'-TCGTGGTATCGTTATGCGCC-3'

FRT:
Forward: 5'-AGGCGCATAACGATACCACGAT-3'
Reverse: 5'- CCACAACGGGTTCTTCTGTT-3'

LacZ:
Forward: 5'-ATCACGACGCGCTGTATC-3'
Reverse: 5'-ACATCGGGCAAATAATATCG-3'

Pdgfra:
Forward: 5'- TCAGCCTTAAGCTGGGACAT-3'
Reverse 5'-ATGTTTAGCTGGCCCAAATG-3'

Cre:
Forward: 5'-GGACATGTTCAGGGATCGCCAGGCG-3'
Reverse: 5'- CCATGAGTGAACGAACCTGG-3'

Isolation and differentiation of stromal vascular fraction (SVF) cells

Isolation of SVF cells was performed as essentially as described previously (Gupta et al., 2012). Briefly, two 6-week-old male C57BL/6 mice were sacrificed and four pads of inguinal

white adipose tissue were dissected and placed in sterile 1x PBS. The adipose tissue was minced with scissors until homogeneous, added to 10 mL of digestion solution [100 mM HEPES pH 7.4, 120 mM NaCl, 50 mM KCl, 5 mM glucose, 1 mM $CaCl_2$, 1 mg/mL collagenase D (Roche, 11088858001), and 1.5% BSA], and incubated at 37°C with shaking for 2 hours, with gentle vortexing every 30 min. The digested tissue was mixed by pipetting up and down and then passed through a 100 μm cell strainer into a new 50 mL conical tube containing 30 mL of SVF cells culture medium [10% FBS in DMEM/F-12, GlutaMAX™ (Life Technologies, 10565-018)] to dilute the digestion buffer. The cells were collected by centrifugation at 600 x g for 5 min, resuspended in 10 mL of SVF culture medium, and passed through a 40 μm cell strainer. The cells were collected again by centrifugation at 600 x g for 5 min, resuspended in 5 mL of SVF culture medium, and plated onto a 6 cm diameter collagen-coated plate until well attached. The cells were grown to ~80 to 90% confluence, split and expanded, and frozen in aliquots for later use.

To test the differentiation potential of the SVF cells, they were seeded at ~5 x 10^4 cells per well in 12-well plates, grown to confluence, and then grown for another two days under contact inhibition. Induction of adipogenesis was achieved by the addition of a cocktail of differentiation agents (MDI), including 0.5 mM IBMX (3-isobutyl-1-methylxanthine; Calbiochem, 410957), 1 μM dexamethasone (Dex; Sigma, D4902), and 5 μg/mL insulin (Sigma, I-5500) for two days. Subsequently, the cells were grown in culture medium with 5 μg/mL insulin (Sigma, I-5500) for the indicated times before collection. In some cases, the cells were treated with vehicle, 20 μM BYK204165, or 5 μM PJ34 for 2 days or as indicated. To assess adipogenesis, we performed RT-qPCR on total RNA isolated from the cells and staining of lipid droplets in intact cells using BODIPY 493/503 NHS Ester (Life Technologies, D2191).

Generation of *Parp1* knockout SVF cells

SVF cells were isolated from *Parp1*$^{loxP/loxP}$ or *Parp1*$^{loxP/loxP}$*;Pdgfra-cre/ERT* mice as described above for wild-type mice. For adenovirus-Cre mediated *Parp1* deletion, *Parp1*$^{loxP/loxP}$ SVF cells were grown to ~80 to 90% confluence, infected with adenovirus expressing GFP (ad-GFP; Vector Biolabs, 1060) or Cre-GFP (AdVCre-GFP; Vector Biolabs, 1700) with a MOI of ~50 for two days. The cells were then cultured in fresh medium for two more days under contact inhibition before the induction of adipogenesis. For Tamoxifen-inducible Cre-mediated *Parp1* deletion, *Parp1*$^{loxP/loxP}$*;Pdgfra-Cre/ERT* SVF cells were cultured to ~80% confluence and then treated with 5 μM 4-Hydroxytamoxifen (4-OHT; Sigma, H7904) for four days before the induction of adipogenesis, as described above.

Cell culture and treatments

3T3-L1 cells (Green and Kehinde, 1975) were obtained from the American Type Cell Culture (ATCC, CL-173TM) and NIH/3T3 cells (Todaro and Green, 1963) were kindly provided by Dr. Rana Gupta at UT Southwestern Medical Center. The cells were maintained in DMEM (Cellgro, 10-017-CM) supplemented with 10% fetal bovine serum (Atlanta Biologicals, S11550). For induction of adipogenesis in 3T3-L1 cells, the cells were plated in 6-well plates, grown to confluence, and then grown for another two days under contact inhibition. Induction of adipogenesis was achieved by the addition of a cocktail of differentiation agents (MDI), including 0.25 mM IBMX (3-isobutyl-1-methylxanthine; Calbiochem, 410957), 1 μM dexamethasone (Dex; Sigma, D4902), and 10 μg/mL insulin (Sigma, I-5500) for two days. Subsequently, the cells were grown in culture medium with 10 μg/mL insulin (Sigma, I-5500)

for the indicated times before collection. The induction of adipogenesis in NIH/3T3 cells was performed in a similar fashion by the addition of 5 μM of Rosiglitazone (Sigma, R2408) together with the MDI cocktail. For experiments with the PARP inhibitors BYK204165 (Eltze et al., 2008) (Tocris Bioscience, 1104546-89-5) and PJ34 (Jagtap et al., 2002) (Enzo Life Sciences, ALX-270-289**)**, the cells were pretreated with the inhibitor at the indicated concentrations, or with DMSO vehicle, for 1 hour prior to the addition of the MDI cocktail and during the first two days of differentiation after adding the MDI cocktail. To assess adipogenesis, intact cells were stained with 5% Oil Red O (Sigma, O0625).

293T cells were purchased from the ATCC (CRL-3216) and maintained in DMEM (Cellgro, 10-017-CM) supplemented with 10% fetal bovine serum (Atlanta Biologicals, S11550).

Antibodies

The custom rabbit polyclonal antiserum against PARP-1 used for Western blotting and ChIP assays was generated by using an antigen comprising the amino-terminal half of PARP-1 (Kim et al., 2004) (now available from Active Motif; cat. no. 39559). The custom recombinant antibody-like anti-poly-ADP-ribose binding reagent (anti-PAR) was generated and purified in-house (now available from EMD Millipore; cat. no. MABE1031). The other antibodies used are as follows: rabbit polyclonal ChIP-grade C/EBPβ antibody (Santa Cruz, sc-150X), mouse monoclonal ChIP-grade HA antibody (Abcam, ab9110), rabbit polyclonal PPARγ antibody (Santa Cruz, sc-7196), rabbit polyclonal SNRP70 antibody (Abcam, ab51266), rabbit polyclonal β-tubulin antibody (Abcam, ab6046), and rabbit IgG (Invitrogen, 10500C).

Cloning, mutagenesis, and generation of retroviral vectors for expression and knockdown

The following cloning strategies were used to make retroviral and lentiviral vectors for expressing C/EBPβ and knocking down C/EBPβ or PARP-1.

Generation of wild-type and PARylation mutant C/EBPβ cDNAs. pcDNA 3.1(-) mouse C/EBPβ (LAP isoform) and pcDNA 3.1(-) mouse C/EBPβ (LIP isoform) were purchased from Addgene (plasmids 12557 and 12561). A C/EBPβ-based DNA sequence encoding an HA tag was generated by PCR using the following primers:
Forward: CEBPB_F 5'-acgcggccgcgaattcatgTACCCATACGATGTTCCAGATTACGCTgaagtg-gccaacttctactacgag-3'
Reverse: CEBPB_R 5'-gcaagcttggatccctagcagtggcccgccgaggccag-3'

The PCR product with sequences encoding an HA tag at the 5' end of the cDNA (for an amino-terminal tag) were cloned into pcDNA 3.1 (-) using digestion with *NotI* and *HindIII*.

Mutant C/EBPβ (LAP) cDNAs encoding single, double, and triple point mutants of C/EBPβ were generated with the QuickChange Site-Directed Mutagenesis Kit from Stratagene using the following primers:

• Mutant 1 - C/EBPβ K133A (AAG to GCC)
Forward: 5'-CTCCCGCCGCGCTCgccGCGGAGCCGGGCTTC-3'
Reverse: 5'-GAAGCCCGGCTCCGcggCGAGCGCGGCGGGAG-3'

• Mutant 2 - C/EBPβ E135A (GAG to GCC)
Forward: 5'-CGCTCAAGGCGGccCCGGGCTTCGAAC-3'
Reverse: 5'-GTTCGAAGCCCGGggCCGCCTTGAGCG-3'

• Mutant 3 - C/EBPβ K133A (AAG to GCC) / E135A (GAG to GCC)
Forward: 5'-CGCTCgccGCGGccCCGGGCTTCGAAC-3'
Reverse: 5'-GTTCGAAGCCCGGggCCGCggcGAGCG-3'

• Mutant 4 - C/EBPβ E139A (GAA to GCC)
Forward: 5'-GGAGCCGGGCTTCGccCCCGCGGACTGCAAG-3'
Reverse: 5'-CTTGCAGTCCGCGGGggCGAAGCCCGGCTCC-3'

• Mutant 5 - C/EBPβ E135A (GAG to GCC) / E139A (GAA to GCC)
Forward: 5'-GGccCCGGGCTTCGccCCCGCGGACTGCAAG-3'
Reverse: 5'-CTTGCAGTCCGCGGGggCGAAGCCCGGGggCC-3'

• Mutant 6 - C/EBPβ K133A (AAG to GCC) / E135A (GAG to GCC) / E139A (GAA to GCC)
Mutant 6 was generated with the Mutant 5 primers using the Mutant 3 cDNA as the template.

Generation of retroviral expression vectors for C/EBPβ and PARP-1. The wild-type and mutant HA-tagged mouse C/EBPβ cDNAs were cloned from the pcDNA 3.1(-) vectors described above into the pQCXIP retroviral expression vector (BD Biosciences; puromycin resistant) using *NotI* and *BamHI*. Flag-tagged human PARP-1 cDNA was cloned into pQCXIP as described previously (Frizzell et al., 2009).

Generation of retroviral expression vectors for shRNAs targeting C/EBPβ and PARP-1. Vectors for expression of shRNAs were generated by cloning double-stranded oligonucleotides containing shRNA sequences targeting luciferase (Luc, as a control), PARP-1, or C/EBPβ into the pSUPER.retro vector (OligoEngine; puromycin or neomycin resistant). The oligonucleotide sequences are as follows:

Luc:	5' - gatatgggctgaatacaaa - 3'	(Reynolds et al., 2004)
PARP-1:	5' - gggcaagcacagtgtcaaa - 3'	(Frizzell et al., 2009)
C/EBPβ:	5' - ggccctgagtaatcacttaaa - 3'	Dharmacon siDESIGN Center

Generation of doxycycline (Dox)-inducible lentiviral expression vectors for C/EBPβ. The wild-type and mutant HA-tagged mouse C/EBPβ cDNAs were cloned from the pcDNA 3.1(-) vectors described above into modified pINDUCER20 lentiviral expression vector (Addgene, plasmid no. 44012) (Meerbrey et al., 2011) using *XhoI*.

Generation of stable knockdown and ectopic expression cell lines

The retroviral and lentiviral expression vectors were used to generate stable cell lines, as described below.

Generation of stable cell lines using retroviral expression vectors. Retroviruses were generated by transfection of the pSUPER.retro or pQCXIP constructs described above, together with an expression vector for the VSV-G envelope protein, into Phoenix Ampho cells using GeneJuice transfection reagent (Novagen, 70967) according to the manufacturer's protocol. The resulting viruses were used to infect 3T3-L1 or NIH/3T3 cells. Stably transduced cells were selected with puromycin (Sigma, P9620; 2 μg/mL) or G418 sulfate (Sigma, A1720; 1 mg/mL).

Generation of stable cell lines using lentiviral expression vectors. The pINDUCER20 vectors described above were transfected into 293T cells using GeneJuice transfection reagent

(Novagen, 70967), together with an expression vector for the GAG-Pol-Rev, VSV-G envelope protein, and pAdvantage according to the manufacturer's protocol. The resulting viruses were then concentrated by using a Lenti-X concentrator (Clonetech, 631231) and used to infect 3T3-L1 cells. Stably transduced cells were selected with G418 sulfate (Sigma, A1720; 1 mg/mL). For experiments, the expression of C/EBPβ was achieved by treating the cells with 1 μg/mL Dox for 8 or 24 hours, as specified below.

Preparation of nuclear extracts and Western blotting

293T cells were seeded at ~1.5 x 10^6 cells per 10 cm diameter plate and transfected with the pcDNA 3.1(-)-based wild-type or mutant mouse C/EBPβ expression vectors described above using GeneJuice transfection reagent (Novagen, 70967) for 24 hours. The 3T3-L1 stable cell lines described above were seeded at ~1.5 x 10^6 cells per 10 cm diameter plate and treated as described above. After collecting the cells, extracts of the cytoplasmic and nuclear fractions were made using the Sigma CelLytic NuCLEAR Extraction Kit according to the manufacturer's protocol. Specifically, the cells were incubated in isotonic buffer [10 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.3 M sucrose, 1 mM DTT, 250 nM ADP-HPD (Sigma, A0627; a PARG inhibitor to prevent PAR chain cleavage during extraction), 10 μM PJ34 (a PARP inhibitor to prevent new PAR chain synthesis during extraction), and 1x complete protease inhibitor cocktail (Roche, 11697498001)] on ice for 15 minutes and lysed by the addition of 0.6% IGEPAL CA-630 detergent with vortexing. The lysates were centrifuged and the supernatants were collected as the cytoplasmic fraction. The crude nuclear pellet was washed once with isotonic buffer, resuspended in extraction buffer C (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, 25% v/v glycerol, 1 mM DTT, 250 nM ADP-HPD, 10 μM PJ34, and 1x complete protease inhibitor cocktail), and incubated for 20 minutes at 4°C with intermittent vigorous vortexing. The resuspended nuclear material was then clarified by centrifugation and the supernatant was collect as the nuclear extract. For each fraction under the indicated conditions, 20 μg of total protein were separated on an 8% polyacrylamide-SDS gel and transferred to a nitrocellulose membrane. Western blotting was performed with antibodies to the following: panADPR (1:3000), PARP-1 (1:2000), C/EBPβ (1:1000), PPARγ (1:1000), and SNRP70 (1:1000).

Immunofluorescent staining of cells

3T3-L1 cells were seeded at a density of ~3 x 10^4 per well on sterile cover slips in 24-well plates and differentiated as described above. The cells were rinsed twice with ice cold 1x PBS and fixed with ice cold methanol/acetone (7:3 ratio). The fixed cells were washed three times with ice cold 1x PBS and blocked with blocking solution (5% non-fat milk or 5% BSA, plus 0.05% Tween-20 in 1x PBS) for 30 minutes at room temperature. After blocking, the cells were incubated with anti-poly(ADP-ribose) binding reagent (anti-PAR) or anti-HA antibody (Abcam, ab9110) overnight at 4°C. The cells were washed three times with ice cold 1x PBS and incubated with secondary antibody (Alexa fluor 488 goat anti-rabbit IgG (H+L) at 1:1000; Life Technologies, A-11034) for 30 minutes at room temperature in the dark. The cells were then washed three times with cold 1x PBS and stained with 1 μM TO-PRO-3 (Life Technologies, T3605) for 2 minutes at room temperature, followed by three washes with ice cold 1x PBS. The cover slips were then mounted onto glass slides with Vectashield HardSet Mounting Medium (Vector Laboratories, H-1400). The slides were imaged using a Leica SP2 confocal microscope.

Immunoprecipitation and detection of PARylated proteins and HA-tagged C/EBPβ

The following procedures were used to immunoprecipitation and detect PARylated proteins and HA-tagged C/EBPβ.

Assays in 293T cells. 293T cells were seeded at ~3 x 10^6 cells per 10 cm diameter plate and transfected with the pcDNA 3.1(-)-based CEBPβ (LAP isoform) expression vectors described above using GeneJuice transfection reagent (Novagen, 70967) for 24 hours. The cells were collected and nuclear extracts were prepared as described above. The nuclear extracts were mixed with an equal volume of buffer B (20 mM HEPES pH 7.6, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 250 nM ADP-HPD, 10 μM PJ34, and 1x complete protease inhibitor cocktail (Roche, 11697498001)]. Aliquots of nuclear extract containing 800 μg of total protein were used for each IP condition. The nuclear extracts were pre-cleared with 5 μg of rabbit IgG antibody for 1 hour at 4°C and then with 100 μL of a 50% slurry of protein A-agarose beads for another 30 minutes at 4°C with constant mixing. After centrifugation at 1000 x g for 2 minutes, the supernatants were collected and incubated with 8 μL of anti-poly-ADP-ribose binding reagent (anti-PAR) or rabbit IgG (as a control) at 4°C overnight with constant mixing. A 100 μL of a 50% slurry of protein A-agarose beads was then added to the incubated sample for an extra 2 hour at 4°C with constant mixing. The beads were then washed three times in wash buffer (20 mM HEPES pH 7.6, 1.5 mM $MgCl_2$, 0.2 mM EDTA) for 10 minutes at 4°C with constant mixing. The beads were then heated to 100°C for 5 minutes in SDS-PAGE loading solution to release the bound proteins. The immunoprecipitated material was subjected to Western blotting using a mouse monoclonal HA antibody (1:1000) or the anti-pan-ADP-ribose binding reagent (anti-panADPR) as described above. The immunoprecipitation assays were performed a minimum of three times to ensure reproducibility.

Assays in 3T3-L1 cells. 3T3-L1 cells expressing inducible HA-tagged wild-type or triple mutant (K133A, E135A, E139A) C/EBPβ were seeded at ~5 x 10^6 cells per 15 cm diameter plate and cultured, differentiated, and treated as described above. For the induction of HA-tagged C/EBPβ expression, the cells were treated with 1 μg/mL of doxycycline (Dox) for 8 hours before collection. The cells were collected and the nuclei were isolated as described above. The nuclear pellet was resuspended in nuclear extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 % IGEPAL CA-630, 250 nM ADP-HPD, 10 μM PJ34, 1 mM NaF, mM $NaVO_4$, and 1x complete protease inhibitor cocktail), incubated for 30 minutes at 4°C, and clarified by centrifugation. The supernatant was collected as the nuclear extract. Aliquots of nuclear extract containing 1 mg of total protein were used for each IP condition. The nuclear extracts were incubated with 30 μL of anti-HA magnetic beads (Thermo Fisher, 88836) at 4°C for 4 hours and then washed five times in nuclear extraction buffer for 10 minutes at 4°C with constant mixing. The beads were then heated to 100°C for 10 minutes in SDS-PAGE loading solution to release the bound proteins. The immunoprecipitated material was subjected to Western blotting as described above.

RNA isolation and RT-qPCR

RNA isolation and RT-qPCR were performed as described previously (Luo et al., 2014). Briefly, 3T3-L1 cells, NIH/3T3 cells, or SVF cells were seeded at ~2 x 10^5 cells per well in 6-well plates and treated as described above. After collecting the cells, total RNA was isolated using Trizol Reagent (Invitrogen) according to the manufacturer's protocol. Total RNA was

reverse transcribed using oligo (dT) primers and MMLV reverse transcriptase (Promega) and subjected to quantitative real-time PCR (qPCR) using gene-specific primers as described below. All target gene expression was normalized to the expression of the gene encoding TBP.

Chromatin immunoprecipitation-qPCR (ChIP-qPCR)

3T3-L1 cells were seeded at ~5 x 10^6 cells per 15 cm diameter plate and treated as described above. ChIP was performed as described previously (Kininis et al., 2007; Krishnakumar et al., 2008), with a few modifications. The cells were cross-linked with 1% formaldehyde in PBS for 10 minutes at 37°C and quenched in 125 mM glycine in PBS for 5 minutes at 4°C. The cells were then collected and lysed in Farnham lysis buffer [5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40, 1 mM DTT, and 1x complete protease inhibitor cocktail (Roche)]. A crude nuclear pellet was collected by centrifugation, resuspended in lysis buffer (50 mM Tris-HCl pH 7.9, 1% SDS, 10 mM EDTA, 1 mM DTT, and 1x complete protease inhibitor cocktail), and incubated on ice for 10 minutes. The chromatin was sheared at 4°C by sonication using a Bioruptor UC200 at the highest setting for four 5-minute cycles of 30 seconds on and 60 seconds off to generate chromatin fragments of ~300 bp in length. The soluble chromatin was diluted 1:10 with dilution buffer (20 mM Tris-HCl pH 7.9, 0.5% Triton X-100, 2 mM EDTA, 150 mM NaCl, 1 mM DTT, and 1x complete protease inhibitor cocktail) and pre-cleared with protein A agarose beads. The pre-cleared supernatant was used in immunoprecipitation reactions with antibodies against the factor of interest (PARP-1 or C/EBPβ) or with rabbit IgG as a control. The immunoprecipitated material was washed once with low salt wash buffer (20 mM Tris-HCl pH 7.9, 2 mM EDTA, 125 mM NaCl, 0.05% SDS, 1% Triton X-100, 1 μM aprotinin, and 1 μM leupetin), once with high-salt wash buffer (20 mM Tris-HCl pH 7.9, 2 mM EDTA, 500 mM NaCl, 0.05% SDS, 1% Triton X-100, 1 μM aprotinin, and 1 μM leupetin), once with LiCl wash buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 250 mM LiCl, 1% NP-40, 1% sodium deoxycholate, 1 μM aprotinin, and 1 μM leupetin), and once with 1x Tris-EDTA (TE). The immunoprecipitated material was eluted in elution buffer (100 mM $NaHCO_3$, 1% SDS) and was then digested with proteinase K and RNase H to remove protein and RNA, respectively. The immunoprecipitated genomic DNA was then extracted with phenol:chloroform:isoamyl alcohol and precipitated with ethanol. The ChIPed genomic DNA was subjected to qPCR using gene-specific primers as described below.

Quantitative real-time PCR (qPCR) for gene expression and ChIP analyses

Reverse transcribed cDNA and ChIPed genomic DNA were analyzed by quantitative PCR (qPCR) as described previously (Luo et al., 2014). Briefly, cDNA or ChIPed genomic DNA, 1x SYBR Green PCR master mix, and forward and reverse primers (250 nM) were mixed and subjected to 45 cycles of amplification (95°C for 10 second, 60°C for 10 second, 72°C for 1 second) following an initial 5 minute incubation at 95°C using a Roche LightCycler 480 384-well detection system. Melting curve analyses were performed to ensure that only the targeted amplicon was amplified. All experiments were performed on at least three separate biological replicates to ensure reproducibility. The sequences of the primers used for RT-qPCR and ChIP-qPCR are listed below.

• mRNA expression primers

AdipoQ forward: 5'-GACAAGGCCGTTCTCTTCAC-3'
AdipoQ reverse: 5'-CAGACTTGGTCTCCCACCTC-3'
Cebpa forward: 5'-GAACAGCAACGAGTACCGGGTA-3'

Cebpa reverse: 5'-GCCATGGCCTTGACCAAGGAG-3'
Cebpb forward: 5'-CAAGCTGAGCGACGAGTACA-3'
Cebpb reverse: 5'-CAGCTGCTCCACCTTCTTCT-3'
Cebpd forward: 5'-TGCCCACCCTAGAGCTGTG-3'
Cebpd reverse: 5'-CGCTTTGTGGTTGCTGTTGA-3'
Fabp4 forward: 5'-AAGTGGGAGTGGGCTTTGC-3'
Fabp4 reverse: 5'-CCGGATGGTGACCAAATCC-3'
Parp1 forward: 5'-TGGTTTCAAGTCCCTTGTCC-3'
Parp1 reverse: 5'-TGCTGTCTATGGAGCTGTGG-3'
Pparg1 forward: 5'-CTGTGAGACCAACAGCCTGA-3'
Pparg1 reverse: 5'-CAGTGGTTCACCGCTTCTTT-3'
Pparg2 forward: 5'-TGCTGTTATGGGTGAAACTCT-3'
Pparg2 reverse: 5'-CGCTTGATGTCAAAGGAATGC-3'
Tbp forward: 5'-TGCTGTTGGTGATTGTTGGT-3'
Tbp reverse: 5'-CTGGCTTGTGTGGGAAAGAT-3'

• ***ChIP primers:***

Cebpa promoter forward: 5'-CTGGAAGTGGGTGACTTAGAGG-3'
Cebpa promoter reverse: 5'-GAGTGGGGAGCATAGTGCTAG-3'
Pparg promoter forward: 5'-GGCCAAATACGTTTATCTGGTG-3'
Pparg promoter reverse: 5'-GTGAGGGGCGTGAACTGTA-3'

C/EBPβ ChIP-seq

ChIP-seq for C/EBPβ in 3T3-L1 cells was performed as described below.

Generation of C/EBPβ ChIP-seq libraries. 3T3-L1 cells expressing inducible HA-tagged wild-type or triple mutant (K133A, E135A, E139A) C/EBPβ were seeded at ~5 x 10^6 cells per 15 cm plate and treated as described above. Inducible expression of C/EBPβ was achieved by treating the cells with 1 μg/mL of doxycycline for 8 hours. For some experiments, the cells were treated with the PARP inhibitor BYK204165 during induction of C/EBPβ. ChIP was performed as described above using a C/EBPβ antibody or rabbit IgG, and the resulting immunoprecipitated genomic DNA was purified using AMPure XP beads (Beckman Coulter, A63881). After purification, 5 to 10 ng of ChIPed genomic DNA per condition were used to generate ChIP-seq libraries. For the spike-in control, 20 pg of phiX DNA was added to each condition. ChIP-seq libraries were generated as described previously (Franco et al., 2015) with some modifications. Briefly, the ChIPed DNA was end-repaired using an end repair kit (Enzymatics, Y9140-LC-L) and a single "A"-base overhang was added using the Klenow DNA polymerase (Enzymatics, P7010-HC-L). The A-modified DNA was ligated to Illumina sequencing adaptors using T4 DNA ligase (Enzymatics, L6030-HC-L). The ligated DNA was amplified using Kapa HiFi Hot Start Ready Mix (KAPA Biosystems, KK2612), size-selected (250 - 300 bp) by agarose gel electrophoresis, and purified using a QIAquick Gel Extraction Kit (Qiagen, 28706). The size-selected fragments were further amplified using Illumina TruSeq P5 and P7 PCR primers and subjected to another round of agarose gel electrophoresis. Quality control was performed to determine the size, concentration, and purity of the final libraries. The libraries were sequenced using an Illumina Next seq 500 per the manufacturer's instructions.

ChIP-seq data analyses. ChIP-seq data analyses were performed largely as described previously (Franco et al., 2015). The ChIP-seq libraries containing phiX spike-in controls were first aligned to the mouse genome (mm10) using Bowtie ver. 2 with default parameters (Langmead and Salzberg, 2012). The libraries were then aligned to phiX DNA to estimate the spike in levels in each individual condition. Uniquely mapped reads from the alignment were converted to bigWig files using BEDTools for visualization in the UCSC genome browser (Quinlan and Hall, 2010). The inputs for each condition were used as controls to call peaks using MACS software (Zhang et al., 2008).

Venn diagrams: We used the mergePeaks function in the HOMER software suite (Heinz et al., 2010) to determine the overlap of peaks between conditions.

Metagene analyses: We used metagene representations to illustrate the distribution of reads near the C/EBPβ binding sites. To compare different conditions by metagene analyses, the total reads in each condition were scaled to 62 million reads and then divided by the individual read depth for each condition. The spike in reads were also used to normalize the final read counts. The signals from the corresponding conditions without doxycycline were then subtracted to remove the background signal from endogenous C/EBPβ. For some analyses, we calculated the fold change between the wild-type and mutant C/EBPβ with doxycycline treatment, and then extracted the top 50 percent of C/EBPβ peaks for representation.

Box plots: For quantitatively assessing the read distribution in a fixed window around each binding site under various conditions, we generated box plots for the data. The read distribution surrounding the C/EBPβ peaks was calculated and plotted using the box plot function in R (R Development Core Team, 2006). The reads were normalized in a similar manner as described for the metagene analyses. Wilcoxon rank sum tests were performed to determine the statistical significance of all comparisons. For some analyses, we used the reads around the top 50 percent of C/EBPβ peaks extracted from the metagene analysis, which were then normalized and plotted using the box plot function in R (R Development Core Team, 2006).

Data accession. The ChIP-seq data can be accessed from the NCBI's Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) using the series accession number GSE85100.

In nuclei PARylation reactions

293T cells were seeded at ~1.5 x 10^6 cells per 10 cm diameter plate and transfected with the pcDNA 3.1(-)-based wild-type or mutant mouse C/EBPβ expression vectors described above using GeneJuice transfection reagent (Novagen, 70967) for 24 hours. Luc, PARP-1 or C/EBPβ knockdown 3T3-L1 cells were seeded at ~1.5 x 10^6 cells per 10 cm diameter plate and differentiated for 4 hours as described above. The cells were collected and nuclei were isolated as previously described (Dignam et al., 1983). Briefly, the cells were suspended in 1 mL of buffer A (10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, and 1x complete protease inhibitor cocktail) for 10 minutes on ice and collected by centrifugation at 1000 x g for 2 minutes at 4°C. The cell pellets were resuspended in 1 mL buffer A and then lysed by dounce homogenization (10 strokes with a Wheaton Dounce tissue grinder, loose pestle; Fisher Scientific, 357538). After centrifugation, the nuclei were resuspended in 100 μL of freezing buffer (50 mM Tris-HCl pH 8.3, 5 mM $MgCl_2$, 40 % glycerol, 0.1 mM EDTA, 1 mM DTT, and 1x complete protease inhibitor cocktail).

For in nuclei PARylation reactions, a 50 μL volume containing suspended nuclei was incubated with an equal volume of 2x reaction buffer (10 mM Tris-HCl pH 8.5, 20 mM KCl, 2

mM DTT, and 2x complete protease inhibitor cocktail) with (3T3-L1 nuclei) or without (293T cells nuclei) 200 μM NAD^+ for 30 minutes at room temperature. The reactions were stopped by adding 33 μL of 4x stop buffer (4% SDS and 20 mM EDTA), followed by heating to 100°C for 5 minutes in SDS-PAGE loading buffer. A 20 μL aliquot of each reaction from the 293T nuclei (without NAD^+) was analyzed by Western blotting as described above to assess the PARylation levels of the ectopically expressed wild-type and mutant C/EBPβ proteins. A 5 μL aliquot of each reaction from the Luc, PARP-1 or C/EBPβ knockdown 3T3-L1 nuclei (with NAD^+) was analyzed by Western blotting as described above to assess the PARylation levels of endogenous C/EBPβ (NAD^+ was added to the reactions containing 3T3-L1 nuclei to enhance the PARylation of endogenous C/EBPβ since the levels of endogenous C/EBPβ are low).

Electrophoretic mobility shift assays (EMSAs)

We performed EMSAs to determine the effect of PARylation on C/EBPβ binding to DNA using C/EBPβ PARylated in nuclei or in vitro.

EMSAs with PARylation of C/EBPβ in nuclei. 293T cells were seeded at ~1.5 x 10^6 cells per 10 cm diameter plate and transfected with the pcDNA 3.1(-)-based wild-type or mutant (K133A/E135A/E139A) mouse C/EBPβ expression vectors described above using GeneJuice transfection reagent (Novagen, 70967) for 24 hours. In some cases, the cells were treated with the PARP inhibitors (5 μM PJ34 or 20 μM BYK204165) for 1 hour before transfection. The nuclei were isolated as described above. For in nuclei PARylation reactions, a 50 μL volume containing suspended nuclei was incubated with an equal volume of 2x reaction buffer (10 mM Tris-HCl pH 8.5, 20 mM KCl, 2 mM DTT, and 2x complete protease inhibitor cocktail) containing 200 μM NAD^+ for 30 minutes at room temperature. The nuclei were then washed with buffer A and nuclear extracts were prepared with extraction buffer C as described above. DNA binding reactions were set up in a 25 μL in reaction buffer (20 mM HEPES pH 7.9, 50 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 8% glycerol, and 1 mM β-mercaptoethanol) containing: 4 μg of nuclear extract, 2.5 pmol of a ^{32}P-end-labeled double-stranded oligonucleotide probe corresponding to the C/EBP regulatory element in the *Cebpa* promoter (-191 to -171, 5'-GCGTTGCGCCACGATCTCTC-3'; (Christy et al., 1991; Tang et al., 1999)), and 1 μg poly[d(I-C)] for 20 minutes at room temperature. For supershifting, 1 μg of C/EBPβ antibody (Santa Cruz, sc-150X) was added to the reaction mix before the probe was added. The reactions were run on 6% acrylamide/25% glycerol/0.5x TBE gels, which were dried and then analyzed by autoradiography with Kodak BioMax XAR film or by phosphorimager analysis.

EMSAs with PARylation of C/EBPβ in vitro. 293T cells were seeded at ~2 x 10^6 cells per 15 cm diameter plate and transfected at ~50% confluence with the pcDNA 3.1(-)-based wild-type or mutant (K133A/E135A/E139A) mouse C/EBPβ expression vectors as described above using Lipofectamine LTX Reagent (Invitrogen) for 48 hours. The cells were then treated with 20 mM PJ34 for 1 hour to inhibit cellular ADP-ribosylation, collected in ice cold PBS, and pelleted by centrifugation. The cells were then resuspended in ice-cold isotonic buffer (10 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.3 M sucrose, and 1x complete protease inhibitor cocktail) and allowed to swell for 15 minutes on ice. The nuclei were released from the cells by the addition of NP-40 to 0.65% v/v with 10 seconds of vortexing, followed immediately by centrifugation to collect the nuclei. The supernatant was removed and the nuclei were subjected

to extraction on ice for 30 minutes in C/EBPβ immunoaffinity purification buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 1 mM EDTA, and 1x complete protease inhibitor cocktail containing 20 mM PJ34) with occasional mixing. The resulting nuclear extract was clarified by two rounds of centrifugation and incubated with equilibrated anti-HA agarose resin (Thermo) for 3 hours at 4°C with gentile mixing. The resin was washed thoroughly with immunoaffinity purification wash buffer (25 mM Tris-HCl pH 7.5, 250 mM NaCl, 1% NP-40, 1 mM EDTA, and 1x complete protease inhibitor cocktail). The immunoaffinity purified C/EBPβ was eluted from the agarose resin by the addition of elution buffer [25 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.25% NP-40, 10% glycerol, 1 mM EDTA, and 1x complete protease inhibitor cocktail containing 2 mg/mL HA peptide (Sigma)].

The purified C/EBPβ was PARylated or mock-PARylated by PARP-1 in vitro and analyzed in EMSA assays as follows. Ten nM of recombinant PARP-1 was incubated with 100 nM of wild-type or mutant C/EBPβ in PARylation/EMSA buffer [50 mM Tris-HCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 1.5 mg/mL BSA, 5% glycerol, 5 mM DTT, 0.1 mg/mL poly(dI:dC)] with or without 25 mM NAD^+ in the presence of 2.5 pmol of a ^{32}P-end-labeled double-stranded oligonucleotide probe corresponding to the C/EBP regulatory element in the *Cebpa* promoter (described above). The reactions were incubated for 30 minutes at room temperature and run on 5% acrylamide/0.5x TBE gels for 1 hour at 4°C, which were then dried and analyzed by autoradiography with Kodak BioMax XAR film or by phosphorimager analysis.

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