SUPPLEMENTARY DATA

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Primers used in H3K27ac ChIP-qPCR in three condition MEFs

Name	Primer sequence
SAEs-e-1	For: 5'-TTCTTGCTGATTCAGAGGCATGGTCAA-3' Rev: 5'-AAGTTGATTGCACGGACAGAGGAGTTT-3'
SAEs-e-2	For: 5'-TCCTTGGGAGGAGGCAAGCATTGGTGA-3' Rev: 5'-TGAGAATCGGGCATGGCTGACAGGTAC
SAEs-e-3	For: 5'-CTCCTTTGCAGCAAGCGAAATCCCACC-3' Rev: 5'-CAGCCACATCCATCATCAATCATCAGTCTTT-3'
SAEs-e-4	For: 5'-GAAGGTCCAAACCCTATCCTCGTAATCT-3' Rev: 5'-GAAAGGCTCGGTCTTGTTTGCTTCATC-3'
SAEs-e-5	For: 5'-TAGCAAGGTGAGGACAAGCAGGCAGAG-3' Rev: 5'-GTCTCATTGTAGCCCATCCTGACCTGA-3'
SIAEs-e-1	For: 5'-TTGCTCCATCCATTATGCCTTTGTCTT-3' Rev: 5'-TGGGAACCATGTGACACCGCTAACGAG-3'
SIAEs-e-2	For: 5'-TTGACTCTTACCACAACCCTACAGCAT-3' Rev: 5'-GGAGTGGAATGTCTGACCGAGCC-3'
SIAEs-e-3	For: 5'-ATTCCTTCCCTGTATTCTCACATCCTT-3' Rev: 5'-GATGTGCTCTAAAGGCTATTCCGTGCT-3'
SIAEs-e-4	For: 5'-TCAGAGCAGTTTGGAGAAGGTTCAGGTGGAG-3' Rev: 5'-ACATAGAGCCGCTTGAGCCGCCTTGGA-3'
SIAEs-e-5	For: 5'-TCTTCTCCCATTAGACAGGTGGTGATT-3' Rev: 5'-AATACGGTGACCAAGGGTAACATACAA-3'
CEs-e-1	For: 5'-ATGTTTCCTCCTCTGCTGTTGAAGGTC-3' GCAACTGAATCAAATCATTGTTGTGCC-3'
CEs-e-2	For: 5'-GTGCGATAGATTGGGAAGCCTGAAATG-3' Rev: 5'-AGAAAGACAAAGATGGGTGATGGAAAG-3'
CEs-e-3	For: 5'-AAGAGCTTTATGGAAGATTTGGGTGTA-3' Rev: 5'-TTGTGACGGACTGGCAAGATAGTTTAG-3'
CEs-e-4	For: 5'-GAGGCAGGAGGACTGGATAAGTTGGAG-3' Rev: 5'-GTGTCTGAAGAAAGGAAGGGAGGGAGG-3'
CEs-e-5	For: 5'-CTCCCACTGAGACCCAACTAAGCAATC-3' Rev: 5'-AGTAGACAATCCTAAGGCAGCAAGCAC-3'
Cdkn2a-e	For: 5'-GAGAAAGCGGGAAGTCAAGCCCAGGAT-3' Rev: 5'-ACCCTGCTGTGGATTGCTACTTTACTG-3'
Lmnb1-e	For: 5'-GGGGCAACTACTTAAATCCAACT-3' Rev: 5'-CCCTTTACCTGCTGATGCGTCCC-3'
Rbp4-e	For: 5'-AAAGTTGGTAGCCATCTGGACAT-3' Rev: 5'-GCTGCTGGCTTCTACCTTCTTTA-3'

Primers used in C/EBPα and C/EBPβ ChIP-qPCR in MEFs

Name	Primer sequence
Mamstr-e (1) (Negative control)	For: 5'-GGGACCAAAGCGATGCTCCT-3'
	Rev: 5'-CTGGGCTTCGGCCGTTCTC-3'
Nab1-e (2) (Negative control)	For: 5'-CTGTCACATCGGGAGATTCC-3'
	Rev: 5'-CGGCGAATGGAGCAAGAGC-3'
Pck1-e (2) (Positive control)	For: 5'-TGGGAGTGACACCTCACAGC-3'
	Rev: 5'-ATTGTCAGGTGGGGGTCAAG-3'
p21-e (2) (Positive control)	For: 5'-TCAGTCTGGTTTCCCAACATAGG-3'
	Rev: 5'-CACGATGAGCCCAGGCTTC-3'
Cmbl-e (2) (Positive control)	For: 5'-CCCATGGATCTGAATTTTGCAATTC-3'
	Rev: 5'-TCCTAACCAATAACCCGTGACC-3'
Cdk19-e	For: 5'-TCGACCTCAAATCCGCAACG-3'
	Rev: 5'-TAGCCAGGTCTAGTGGTACATG-3'
Angpt1-e	For: 5'-GATTTCATTCGTGTCCAGCTACT-3'
	Rev: 5'-ATTCCCAAAGGGTACAAAGTCAT-3'
Angpt2-e	For: 5'-TCTCGGTTAGAGGTCTCAGTTCA-3'
	Rev: 5'-ATCATTATGCTTTGGTGGTAGGG-3'
Ccl11-e	For: 5'-AACTTCAGCCGTCTACCTACAAC-3'
	Rev: 5'-GCAGAGTCAAGGGATTCCTATTT-3'
Cxcl1-e	For: 5'-ATGGGTTGCCAGCGAGTA-3'
	Rev: 5'-TTTGCTGCGTTCTACAT-3'
Cxcl5-e	For: 5'-GACCCACTGGTTTCTGA-3'
	Rev: 5'-GAGGCAAGATTGGACTA-3'
Cxcl15-1-e	For: 5'-GTTTGTTGAAGGTGGTA-3'
	Rev: 5'-GAGTTGGGAGATAGGAG-3'
Cxcl15-2-e	For: 5'-AAGGCAATAGCAATCCA-3'
	Rev: 5'-TACGAGGTAGTCAAATCAGC-3'
Hgf-e	For: 5'-ATGGAAAGCATGTAGCGTGGGTG-3'
	Rev: 5'-CAACGGTAGGAACCTGCTAGTGC-3'
Igfbp2-e	For: 5'-GGCAAATACAGAAGTGG-3'
	Rev: 5'-CTTTGATTGGTGGTTTA-3'
Igfbp6-e	For: 5'-GGCAGAACAGGAGCAGCCAACTA-3'
	Rev: 5'-CCCACGGAGGGTAAGAAACAGAC-3'
Il15-e	For: 5'-TCAGTCACTTCCCGTGGCTATGT-3'
	Rev: 5'-GACCTGCTCTACTTCCAGTTTACCTTC-3'
Gas6-e	For: 5'-ACTGCCTAACTCCTACAA-3'
	Rev: 5'-CACTCCCTGATGACAAA-3'

Cst3-e	For: 5’-TACTCCTCCCTACTCCACAATCCC-3’ Rev: 5’-TTCTTCTCCGCCGCTGTTTACTT-3’
Ptx3-e	For: 5’-TATAGTTCCCTCAGATGC-3’ Rev: 5’-TAGCCAGATGTAGTTGC-3’
Il1rn-e	For: 5’-ACAAGCAGCAAAGACCG-3’ Rev: 5’-GGCAACCATCAATCCCT-3’
Spp1-e	For: 5’-TTGCTTAGGCGGGCTCTATCTGG-3’ Rev: 5’-AACCTCGTGAACCTTGTTGCTCTGT-3’

Primers used in RT-qPCR (*Mus musculus*)

Name	Primer sequence
Cebpa	For: 5'-GCGGGAACGCAACAACATC-3' Rev: 5'-GTCACTGGTCAACTCCAGCAC-3'
Mki67	For: 5'-ATCATTGACCGCTCCTTTAGGT-3' Rev: 5'-GCTCGCCTTGATGGTTCCT-3'
p16	For: 5'-CGCAGGTTCTTGGTCACTGT-3' Rev: 5'-TGTTCACGAAAGCCAGAGCG-3'
Gapdh	For: 5'-GTGTTCCTACCCCCAATGTGT-3' Rev: 5'-ATTGTCATACCAGGAAATGAGCTT-3'
Angpt1	For: 5'-CACATAGGGTGCAGCAACCA-3' Rev: 5'-CGTCGTGTTCTGGAAGAATGA-3'
Angpt2	For: 5'-CCTCGACTACGACGACTCAGT-3' Rev: 5'-TCTGCACCACATTCTGTTGGA-3'
Ccl11	For: 5'-GAATCACCAACAACAGATGCAC-3' Rev: 5'-ATCCTGGACCCACTTCTTCTT-3'
Cxcl1	For: 5'-CTGGGATTCACCTCAAGAACATC-3' Rev: 5'-CAGGGTCAAGGCAAGCCTC-3'
Cxcl5	For: 5'-TCCAGCTCGCCATTCATGC-3' Rev: 5'-TTGCGGCTATGACTGAGGAAG-3'
Cxcl15	For: 5'-CAAGGCTGGTCCATGCTCC-3' Rev: 5'-TGCTATCACTTCCTTTCTGTTGC-3'
Hgf	For: 5'-GGGTCCTTTGAAACCATGTAGGC-3' Rev: 5'-TACACTCAGGAAACGCAGTCATT-3'
Igfbp2	For: 5'-CAGACGCTACGCTGCTATCC-3' Rev: 5'-CCCTCAGAGTGGTCGTCATCA-3'
Igfbp6	For: 5'-GCTGCTAATGCTGTTGTTCGC-3' Rev: 5'-GCACTTAGGGCTGTAGACCC-3'
Il15	For: 5'-ACATCCATCTCGTGCTACTTGT-3' Rev: 5'-GCCTCTGTTTTAGGGAGACCT-3'
Il1rn	For: 5'-GCTCATTGCTGGGTACTTACAA-3' Rev: 5'-CCAGACTTGGCACAAGACAGG-3'
Gas6	For: 5'-TGCTGGCTTCCGAGTCTTC-3' Rev: 5'-CGGGGTCGTTCTCGAACAC-3'
Cst3	For: 5'-AGGAGGCAGATGCCAATGAG-3' Rev: 5'-GGGCTGGTCATGGAAAGGA-3'
Ptx3	For: 5'-CCTGCGATCCTGCTTTGTG-3' Rev: 5'-GGTGGGATGAAGTCCATTGTC-3'
Spp1	For: 5'-AGCAAGAAACTCTTCCAAGCAA-3' Rev: 5'-GTGAGATTCGTCAGATTCATCCG-3'

Cebpb	For: 5’-CAACCTGGAGACGCAGCACAAG-3’
	Rev: 5’-GCTTGAACAAGTTCCGCAGGGT-3’
Nfkb1	For: 5’-ATGGCAGACGATGATCCCTAC-3’
	Rev: 5’-TGTTGACAGTGGTATTTCTGGTG-3’
Hoxc4	For: 5’-CTACCCTGAGCGTCAGTATAGC-3’
	Rev: 5’-CGCAGAGCGACTGTGATTTCT-3’
Hoxc6	For: 5’-TTACCCCTGGATGCAGCGAATG-3’
	Rev: 5’-CCGAGTTAGGTAGCGGTTGAAG-3’
Hoxc9	For: 5’-CAGCAAGCACAAAGAGGAGAAGG-3’
	Rev: 5’-AGTTCCAGCGTCTGGTACTTGG-3’
Hoxc10	For: 5’-CAGTCCAGACACCTCGGATAAC-3’
	Rev: 5’-TCTCCAATTCCAGCGTCTGGTG-3’
Hoxc13	For: 5’-CCTGTTGAAGGCTACCAGCACT-3’
	Rev: 5’-CTCACTTCGGGCTGTAGAGGAA-3’

Primers used in RT-qPCR (*Rattus norvegicus*)

Name	Primer sequence
Cebpa	For: 5'-AGACATCAGCGCCTACATCG-3' Rev: 5'-CCGGTACTCGTTGCTGTTCT-3'
Gapdh	For: 5'-GTGTTCCTACCCCCAATGTGT-3' Rev: 5'-ATTGTCATACCAGGAAATGAGCTT-3'
Angpt1	For: 5'-TGGCTTGGATGTGCAACCTT-3' Rev: 5'-TGTAACCGTTCAGCGTGGAG-3'
Angpt2	For: 5'-GCACCGCTAACCAACCAAAG-3' Rev: 5'-AATGCATGCTGTCCCTGTGA-3'
Ccl11	For: 5'- GCACCTGGACCAAAAACTCC-3' Rev: 5'-AAGCCAAGTCCTTGGGGGAT-3'
Cxcl1	For: 5'-CAGACAGTGGCAGGGATTCA-3' Rev: 5'-CCTCGCGACCATTCTTGAGT-3'
Cxcl6	For: 5'-TCCCAACTCAAAGGTGCGAG-3' Rev: 5'-CCAGTGCAAGTGCATTCCG-3'
Hgf	For: 5'-ACAGCTTTTTGCCTTCGAGC-3' Rev: 5'-GCAAGAATTTGTGCCGGTGT-3'
Igfbp2	For: 5'-AGAACCATGTGGACGGAACC-3' Rev: 5'-TTCCAGAGGACCCCGATCAT-3'
Igfbp6	For: 5'-GACCGGCAAAAGAATCCACG-3' Rev: 5'-CAGAGCACTGAGAGCTTCCC-3'
Il15	For: 5'-TGTAGGAACCCATTGCAGGT-3' Rev: 5'-CTTCGCAGACAAACTGTGGC-3'
Il1rn	For: 5'- GGAAATCTGCAGGGGACCTTA-3' Rev: 5'-AGTGATCAGGCAGTTGGTGG-3'
Gas6	For: 5'- CCCCCGTGATTAGACTACGC-3' Rev: 5'-TTCTCAACTGCCAGGACCAC-3'
Cst3	For: 5'-CTTCGCCGTAAGCGAGTACA-3' Rev: 5'-GTAGTTCGGCCCATCTCCAC-3'
Ptx3	For: 5'-TGGGGGCTTTGACGAAACAT-3' Rev: 5'-CCCCACTTGCCCTTATCTCC-3'

siRNA sequence used in knock down expriments

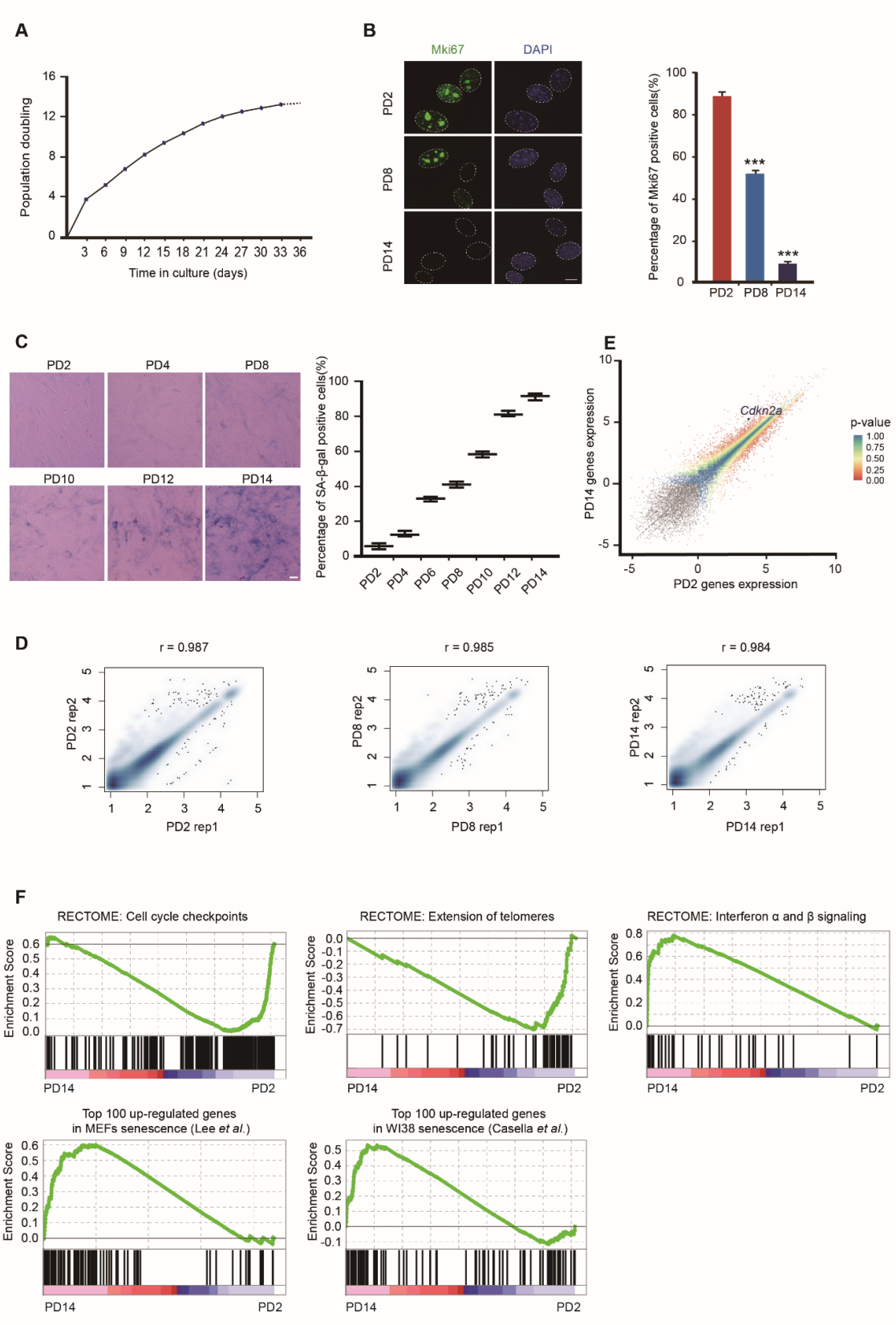
Name	Primer sequence
si*Cebpa* 1# (*Mus musculus& Rattus norvegicus*)	For: 5'-TTATTTCAAGTGTTATCCCCCTCTC-3' Rev: 5'-ACUUCUUGGCUUUGCCCGCTT-3'
si*Cebpa* 2# (*Mus musculus& Rattus norvegicus*)	For: 5'-GCGCAAGAGCCGAGAUAAATT-3' Rev: 5'-UUUAUCUCGGCUCUUGCGCTT-3'
si*Cebpb* 1# (*Mus musculus*)	For: 5'-ACAAGGCCAAGAUGCGCAATT-3' Rev: 5'-UUGCGCAUCUUGGCCUUGUTT-3'
si*Cebpb* 2# (*Mus musculus*)	For: 5'-GGAACUUGUUCAAGCAGCUTT-3' Rev: 5'-AGCUGCUUGAACAAGUUCCTT-3'

Top 100 up-regulated genes in WI38 and MEFs

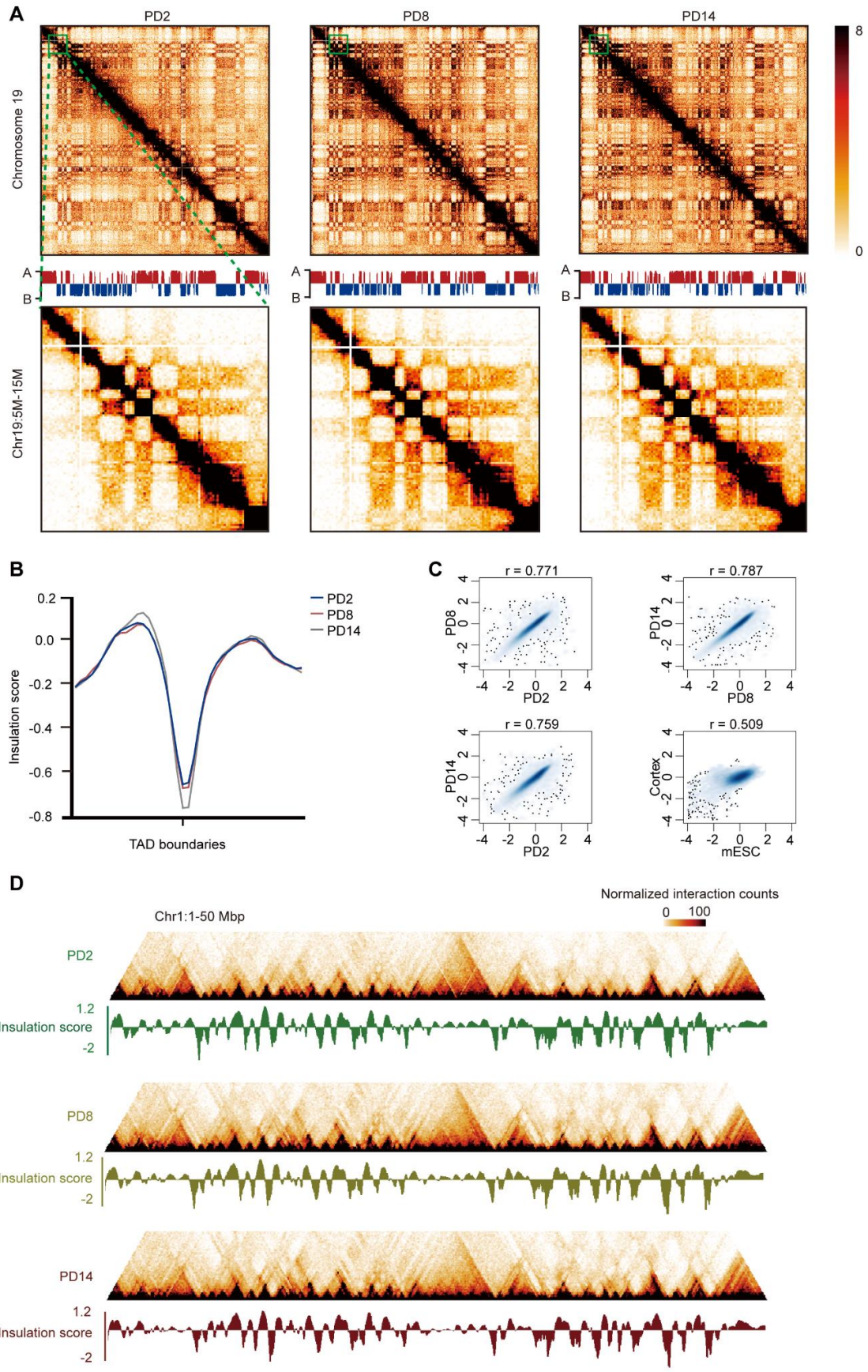
WI38_cells (3)	MEF_cells (4)
MMP3	*Gbp6*
CST1	*1300002K09Rik*
CST4	*Sema5a*
LEPREL1	*Igfbp3*
IFI6	*Col6a2*
DSG2	*Lama4*
OAS2	*Col6a3*
GPX3	*Pi15*
MAP2	*Col6a1*
ADAMTS19	*Ctsk*
LAMA3	*P4ha3*
PNMA2	*Ptafr*
CCDC3	*Plxdc1*
SLCO2B1	*Penk*
SERPINE2	*Agtr2*
SCUBE3	*Il21r*
ANK1	*Gm11428*
BMP2	*Podn*
WFDC1	*Fat3*
FILIP1L	*Cd68*
CCND2	*Apoe*
IL13RA2	*Apobec1*
MCAM	*Prelp*
ITGA8	*Ccl11*
MMP10	*Cybb*
ROBO2	*Mrc1*
PITPNM3	*Lyz2*

CST3	*C1qb*
HAPLN1	*Laptm5*
RAB27B	*Csf1r*
CYTL1	*C3ar1*
HERC6	*Cd300lf*
ACKR4	*Chl1*
NALCN	*Thbs2*
TMEM229B	*Lgals3bp*
ARRDC4	*Fcer1g*
MGAT3	*Cd180*
AREG	*Msr1*
TMEM176B	*Heg1*
AKR1C2	*Gas6*
IFI27	*Cd84*
ANGPTL4	*Spp1*
AREGB	*Figf*
KIAA1324	*5430435G22Rik*
ATP6V0D2	*Mfap5*
KCNQ3	*Matn2*
HIST2H2AA4	*C1qtnf3*
RSAD2	*Fam105a*
HIST2H2AA3	*H2-D1*
KCND3	*Abca1*

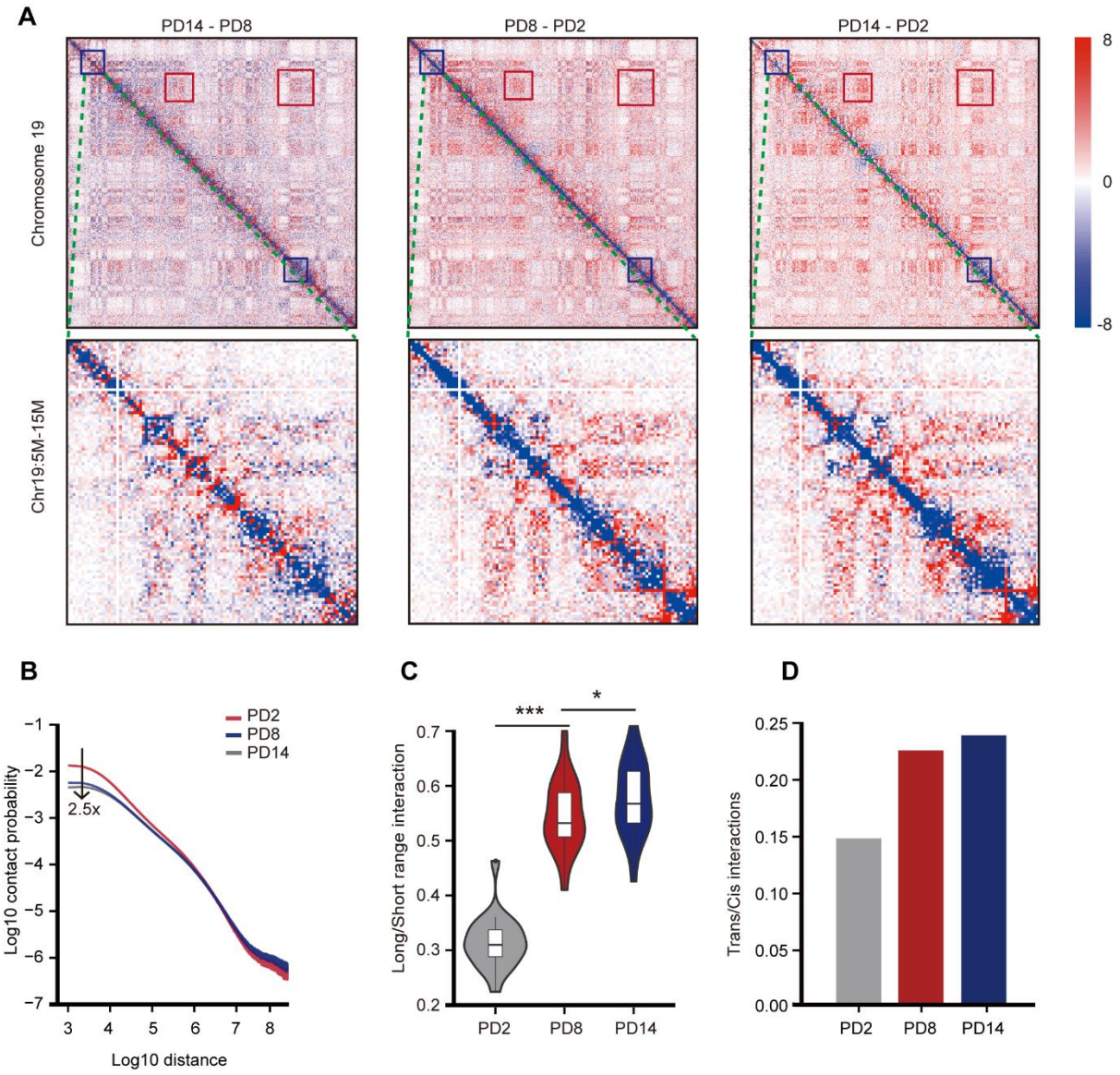
SUPPLEMENTARY FIGURES AND LEGENDS



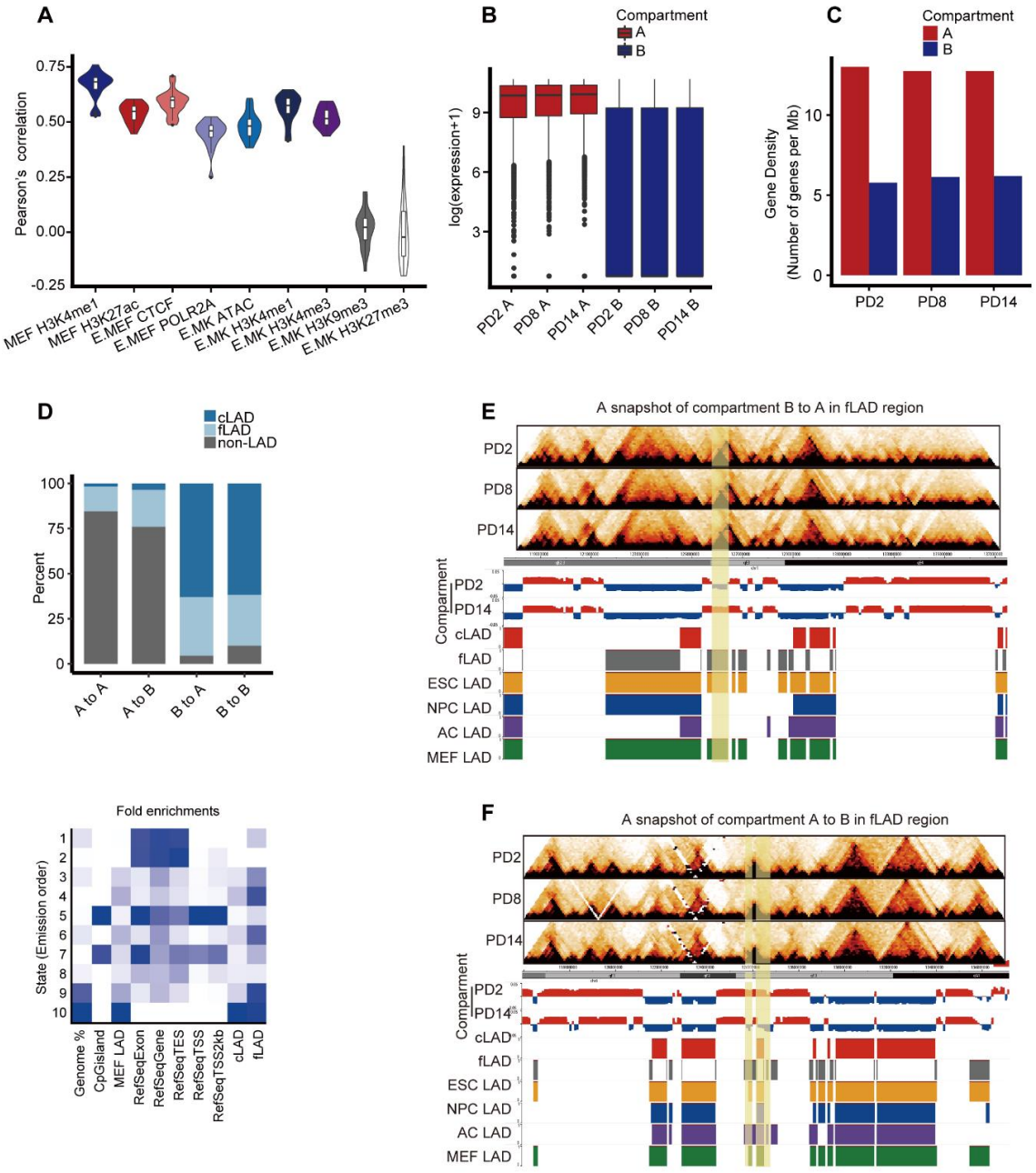
Supplementary Figure S1. Establishment of a senescence model system *in vitro*. (**A**) Limited growth curve revealing the serial propagation of mouse embryonic fibroblasts (MEFs). (**B**) Immunofluorescence staining and statistical diagrams of Mki67 in MEFs. DAPI staining was used to show the position of the nucleolus. Scale bar, 10 μm. Error bars represent the S.D. obtained from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (**C**) SA-β-gal staining and statistical diagrams of MEFs during senescence. Scale bar, 50 μm. (**D**) Pearson's correlation of the two-comparison Hi-C matrix in the three PDs. (**E**) RNA-Seq scatter diagram showing the gene expression profiles of PD2 versus PD14 cells. Representative *p16* gene is labeled. (**F**) GSEA showing the enrichment of cell cycle checkpoint genes, extension of telomeres genes and SASP-related interferon factor genes of PD2 versus PD14 cells，and the enrichment of top 100 up-regulated genes in WI38 senescence (3) and MEFs senescence (4) from the public RNA-seq data.


A
PD2
PD8
PD14
Chromosome 19
Chr19:5M-15M
A
B
8
0
B
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Insulation score
TAD boundaries
PD2
PD8
PD14
C
r = 0.771
r = 0.787
r = 0.759
r = 0.509
PD8
PD14
PD14
Cortex
PD2
PD8
mESC
-4
-2
0
2
4
D
Chr1:1-50 Mbp
Normalized interaction counts
0
100
PD2
PD8
PD14
1.2
Insulation score
-2

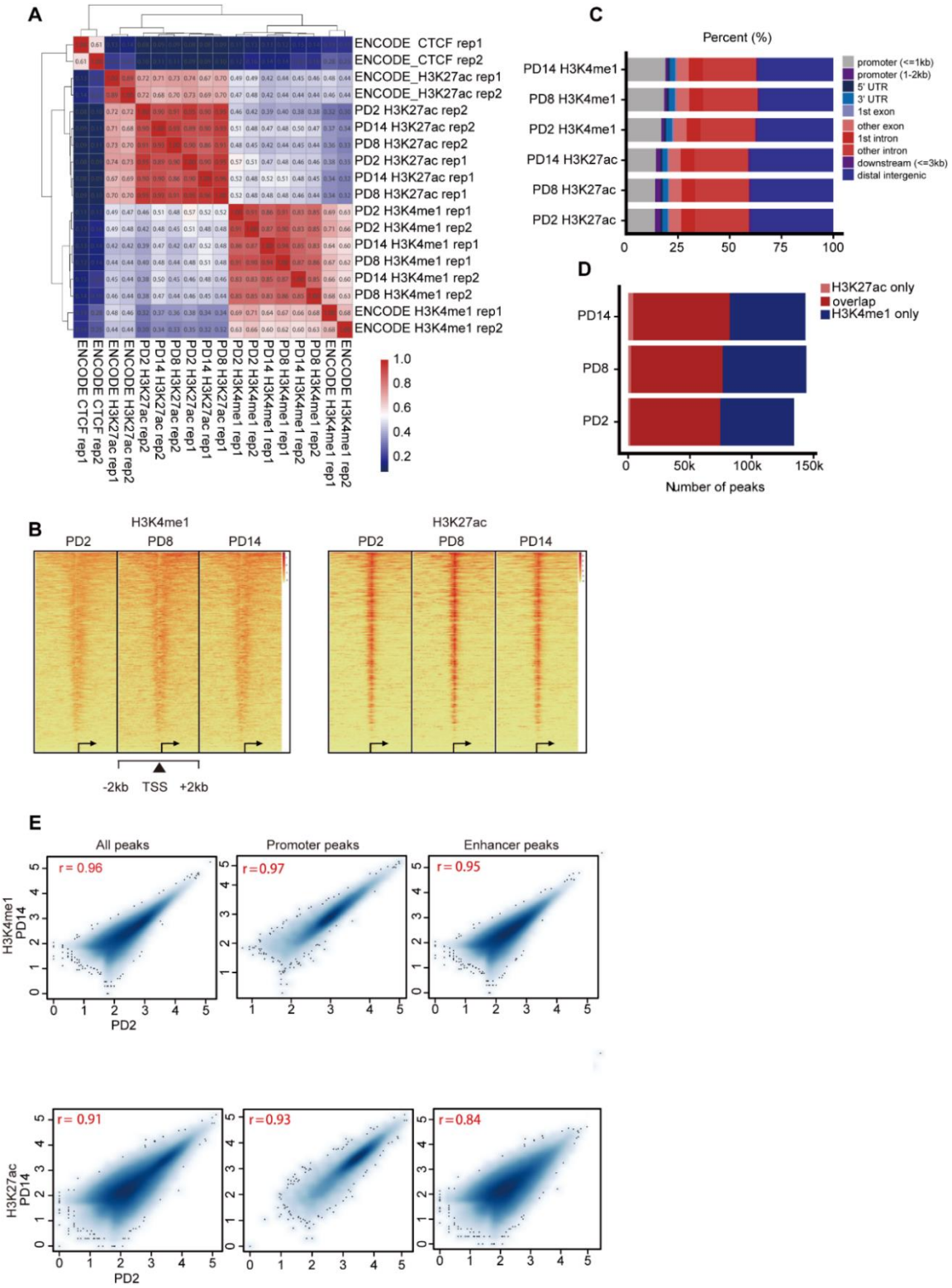
Supplementary Figure S2. TADs and TAD boundaries are conserved in MEFs during senescence. (**A**) Normalized Hi-C contact frequency along the arm of chromosome 19 among PD2, PD8, and PD14 cells at 100-kb resolution (top) and 40-kb resolution (bottom). Compartments were defined by principal components 1 (PC1) values; the positive PC1 values marked in red represent the A compartment and the negative PC1 values marked in blue represent the B compartment. (**B**) The average insulation scores for PD2, PD8, and PD14 at TAD boundaries. (**C**) Pearson's correlation of the insulation scores among the three PDs, as well as mESC and cortex cells. (**D**) A snapshot of chromosome 1 with respect to the interaction matrix (top) and insulation score (bottom) in MEFs of different passage numbers.



Supplementary Figure S3. Chromatin interaction reorganization during senescence. (**A**) Subtraction Hi-C heatmaps showing the interaction frequencies along the arm of chromosome 19 among the three PDs at 100-kb resolution (top) and 40-kb resolution (bottom). Typical long- and short-range interaction regions are labeled with red and blue boxes, respectively. (**B**) The contact probability was calculated as a function of genomic distance for all interactions within the same chromosome arms across the genome. (**C**) The ratios of long-range (≥ 2 Mb) to short-range (< 2 Mb) interactions in the three PDs. **p* < 0.05, ***p* <0.01, ****p* < 0.001. (**D**) The ratios of *trans*- (interchromosomal) to *cis*- (intrachromosomal) interactions in the three PDs.

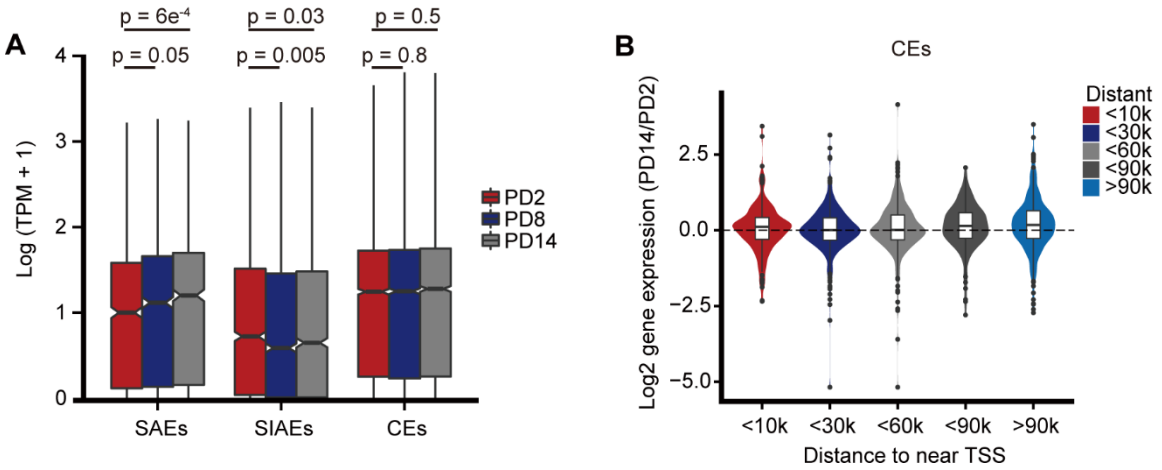


Supplementary Figure S4. Definition and characterization of A and B compartments. (**A**) Violin plot showing the Pearson's correlation between PC1 and epigenetic markers in chromosomes. These epigenetic modification data were obtained in primary MEFs or primary megakaryocyte cells from the ENCODE project. (**B, C**) The distribution of gene expression and gene density within A/B compartments during senescence. (**D**) The occupancy of LAD regions at A/B compartment switching loci. Analysis of the constitutive LADs (cLADs), facultative LADs (fLADs), and regions outside of LADs in MEFs, embryonic stem cells (ESCs), neural precursor cells (NPCs), and astrocytes (ACs). (**E, F**) Snapshots of B to A compartment switching (**E**) and A to B compartment switching (**F**) in the LAD regions during senescence.

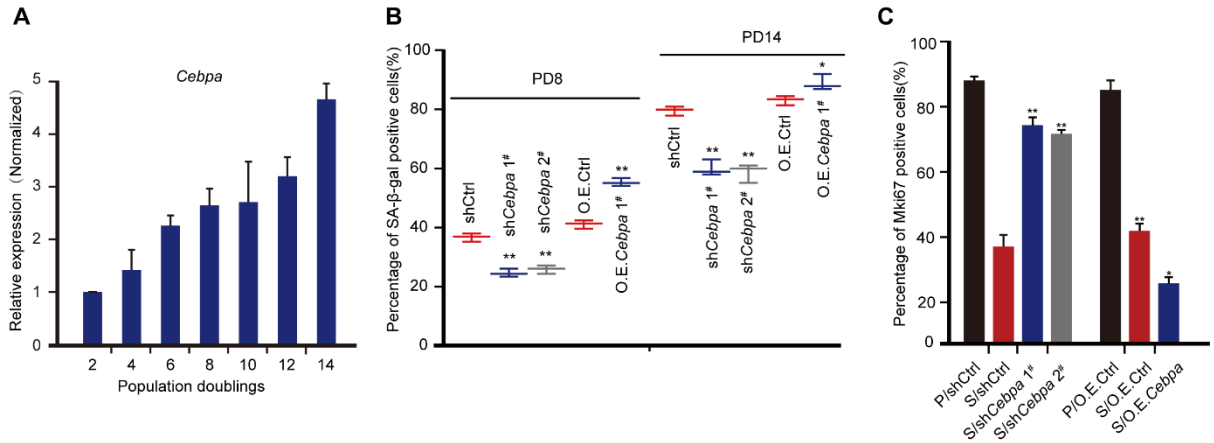


Supplementary Figure S5. H3K27ac distinguishes active enhancers from inactive/poised enhancer elements marked by H3K4me1. (**A**) The correlation heatmap of ChIP-seq data between two independent biological replicates and ENCODE. The ENCODE data were obtained in primary MEFs. Pearson's correlation was applied. (**B**) Enrichment of H3K4me1 and H3K27ac ChIP-seq signals in the TSS regions of genes.

(**C**) The genetic annotations of H3K4me1 and H3K27ac ChIP-seq peaks. (**D**) Bar chart showing the overlap of H3K4me1 and H3K27ac peaks in PD2, PD8, and PD14 cells. (**E**) Scatter diagrams displaying the Pearson's correlation of H3K4me1 and H3K27ac signals at the promoter (< 2 kb around TSS), enhancer (≥ 2 kb away from TSS), and all peaks.

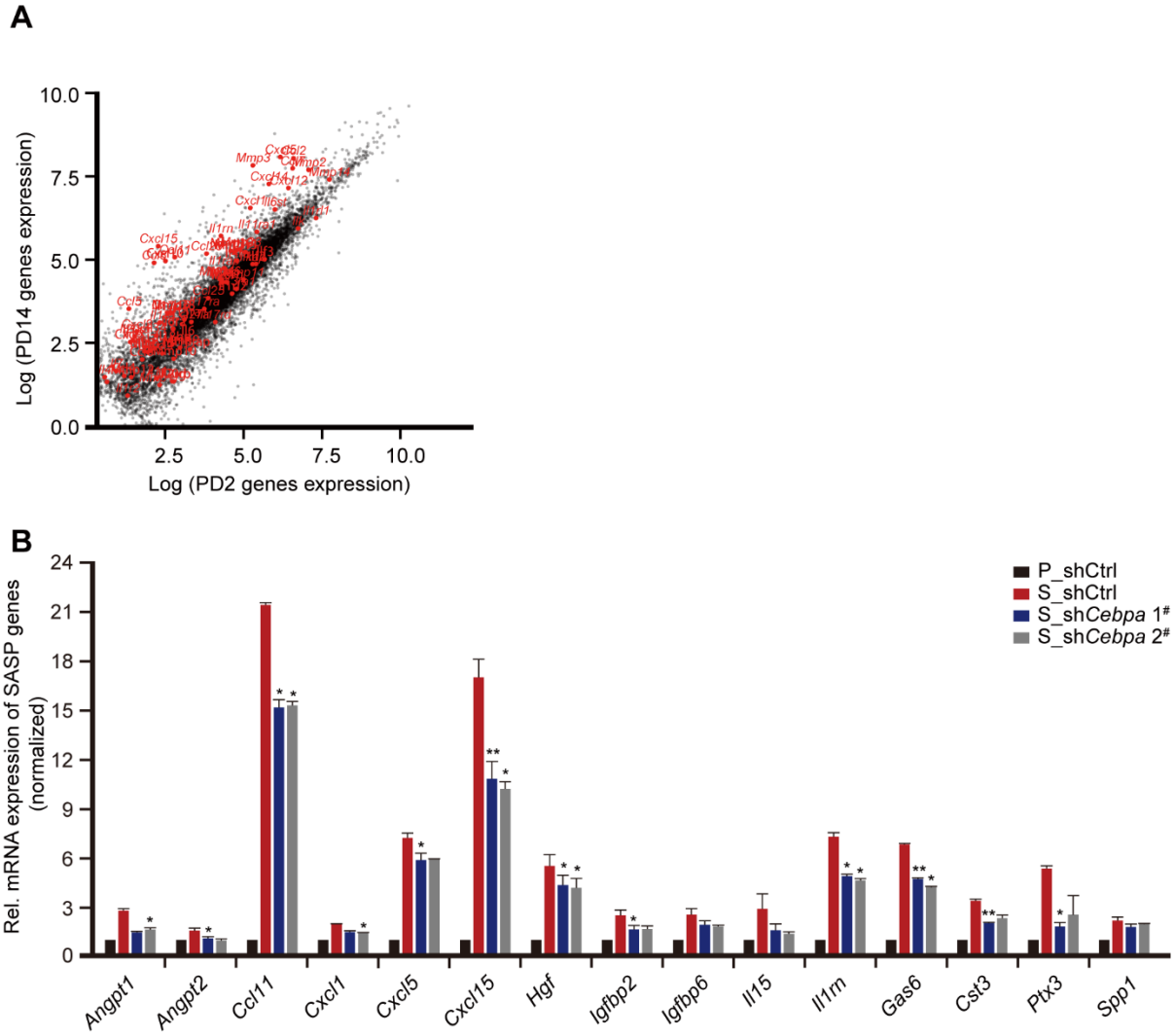


Supplementary Figure S6. Enhancers during senescence are linked to distinct gene expression. (**A**) The expression of genes adjacent to SAEs, SIAEs, and CEs. The Wilcoxon test was performed to calculate the *P* value. (**B**) Violin plots showing the correlation between CE-adjacent gene expression and the distance to TSS during senescence.



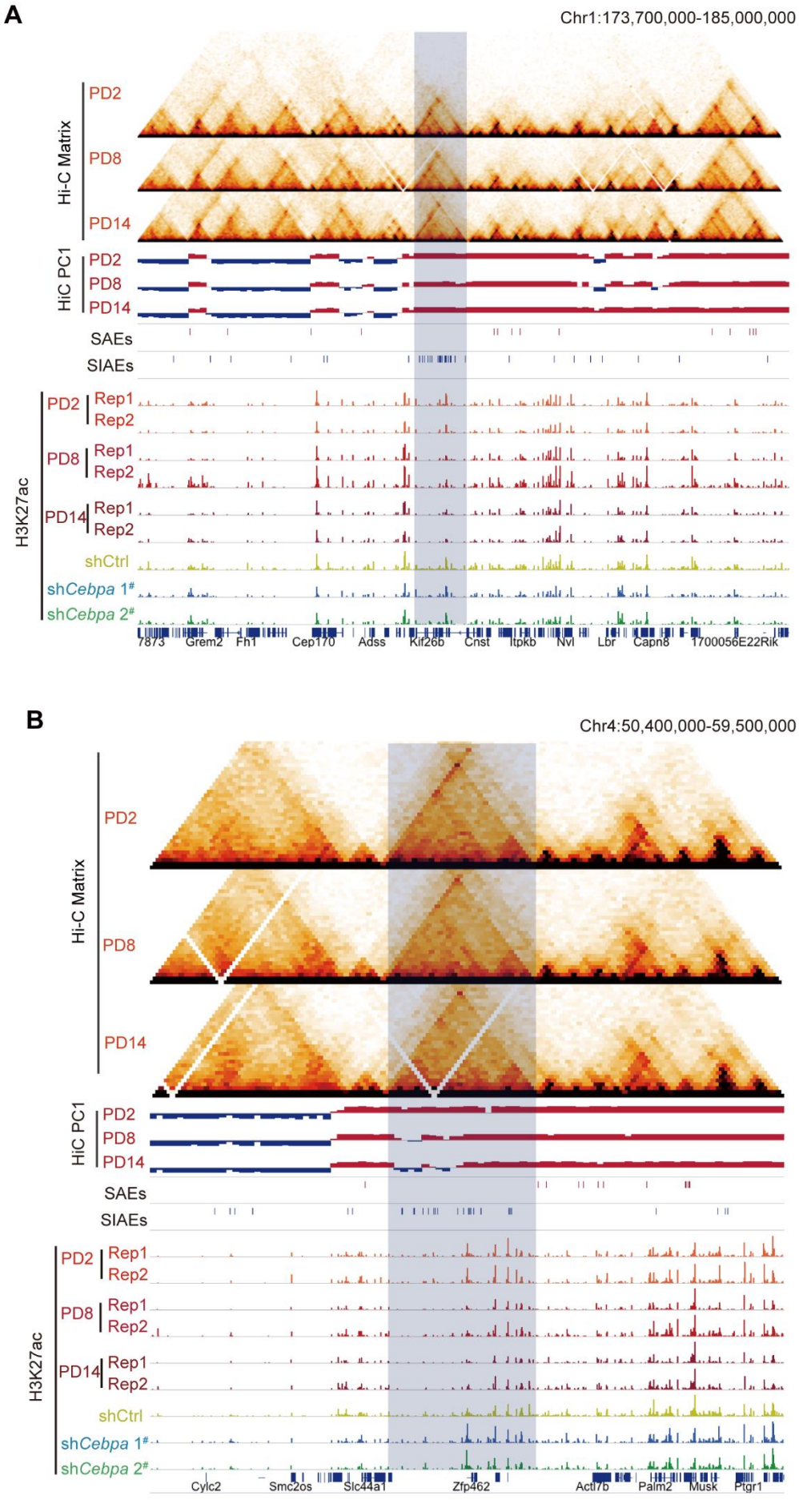
Supplementary Figure S7. C/EBPα binds to SAEs and induces senescence. (**A**) Real-time qPCR analysis of *Cebpa* mRNA expression in MEFs of different passage numbers

normalized to that of *Gapdh*. Error bars represent the S.D. obtained from three independent experiments. (**B**) SA-β-gal staining after down- or upregulation of C/EBPα expression via transfection of lentiviral particles was analyzed using the Image J software. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison test. (**C**) Changes in Mki67 immunofluorescence after down- or upregulation of C/EBPα expression. P represents proliferating cells, and S represents senescent cells. One-way ANOVA with Dunnett's multiple comparison test was performed. Error bars represent the S.D. obtained from three independent experiments. $*p < 0.05$, $**p < 0.01$.

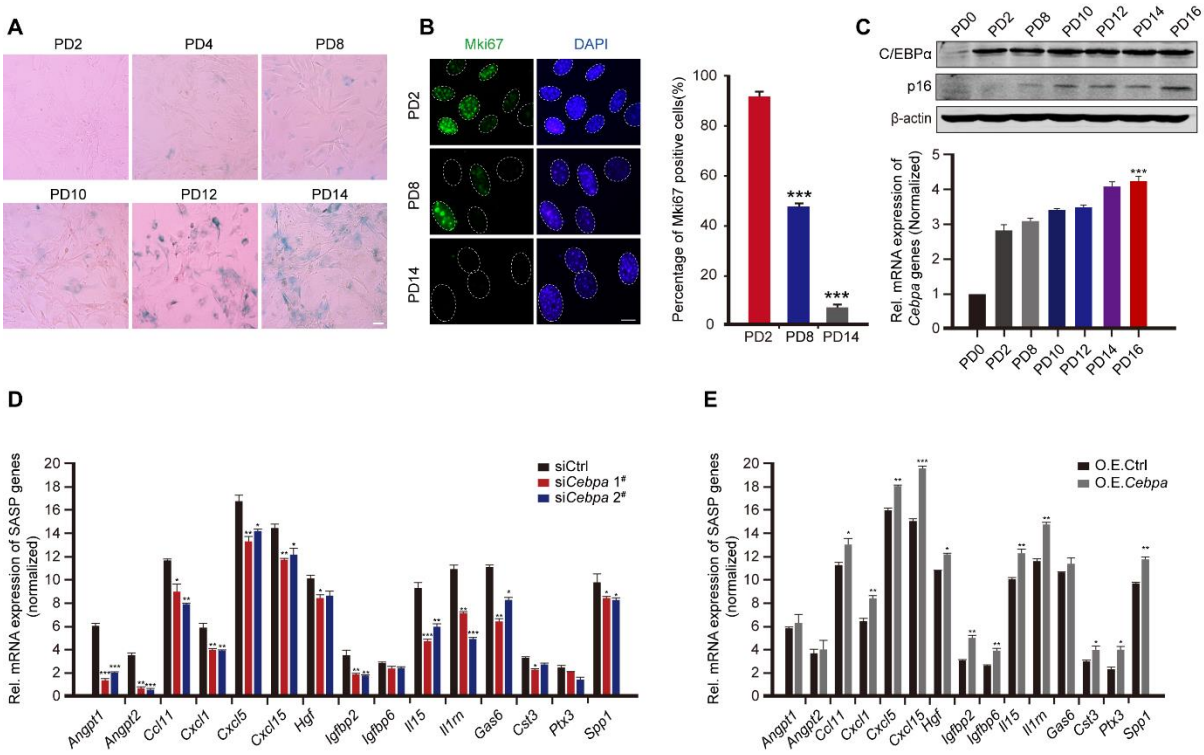


Supplementary Figure S8. C/EBPα facilitates SASP gene expression. (**A**) RNA-Seq scatter diagram showing the gene expression profiles of PD2 versus PD14 cells. Representative SASP genes are labeled. (**B**) The relative expression of SASP genes

after downregulation of C/EBPα expression was measured by RT-qPCR. P represents proliferating cells, and S represents senescent cells. Error bars represent the S.D. obtained from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. One-way ANOVA with Dunnett's multiple comparison test was performed.

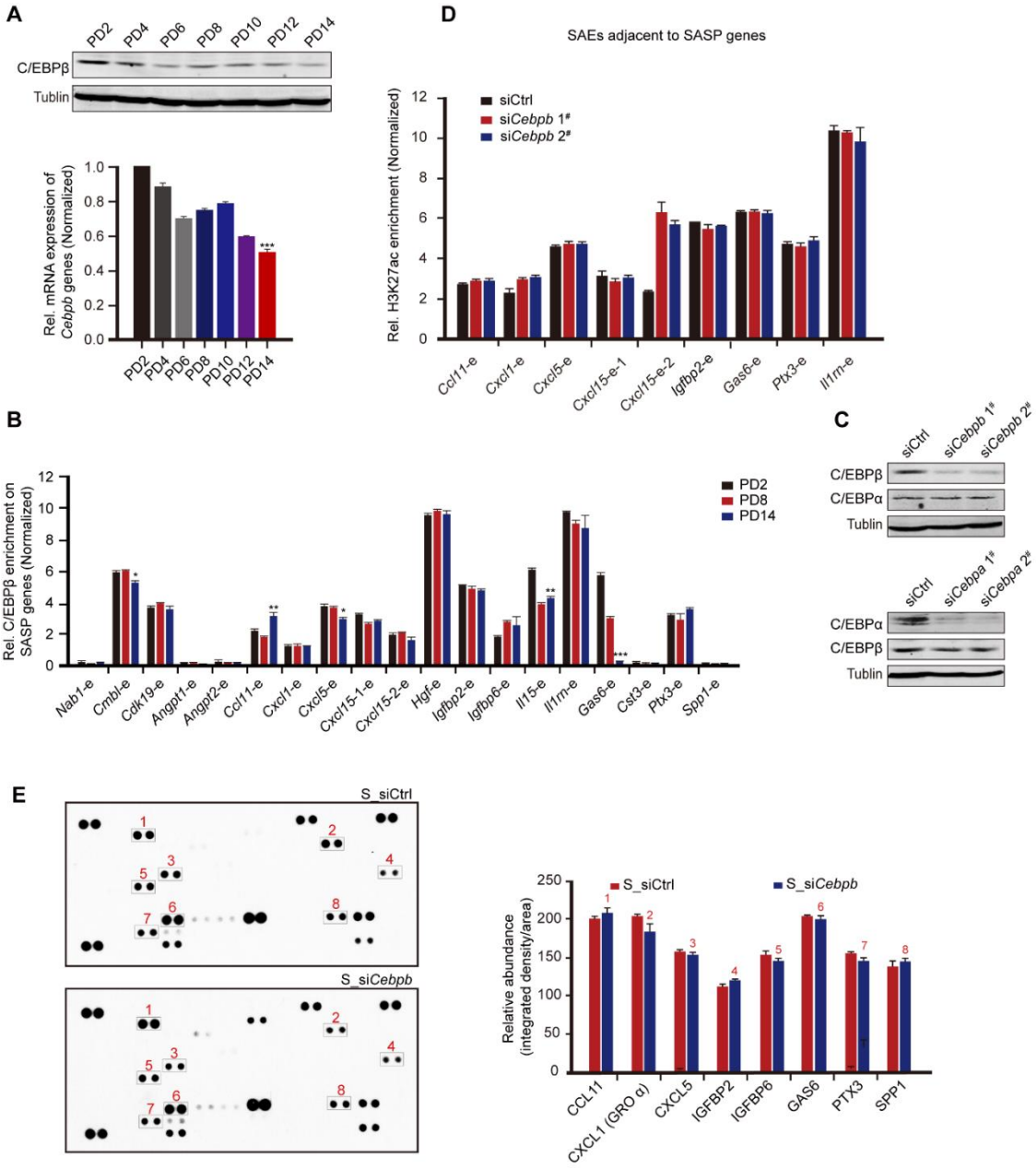

A
Chr1:173,700,000-185,000,000
Hi-C Matrix
PD2
PD8
PD14
HiC PC1
PD2
PD8
PD14
SAEs
SIAEs
H3K27ac
PD2
Rep1
Rep2
PD8
Rep1
Rep2
PD14
Rep1
Rep2
shCtrl
shCebpa 1#
shCebpa 2#
7873
Grem2
Fh1
Cep170
Adss
Klf26b
Cnst
Itpkb
Nvl
Lbr
Capn8
1700056E22Rik
B
Chr4:50,400,000-59,500,000
Hi-C Matrix
PD2
PD8
PD14
HiC PC1
PD2
PD8
PD14
SAEs
SIAEs
H3K27ac
PD2
Rep1
Rep2
PD8
Rep1
Rep2
PD14
Rep1
Rep2
shCtrl
shCebpa 1#
shCebpa 2#
Cylc2
Smc2os
Slc44a1
Zfp462
Actl7b
Palm2
Musk
Ptgr1

Supplementary Figure S9. (**A, B**) Visualization of the TAD structure, A/B compartments, and H3K27ac signals at representative loci. TADs are marked by dashed triangles. A compartments are colored red, and B compartments are colored blue. H3K27ac signals are shown as red bars during senescence and as blue bars after downregulation of C/EBPα expression.



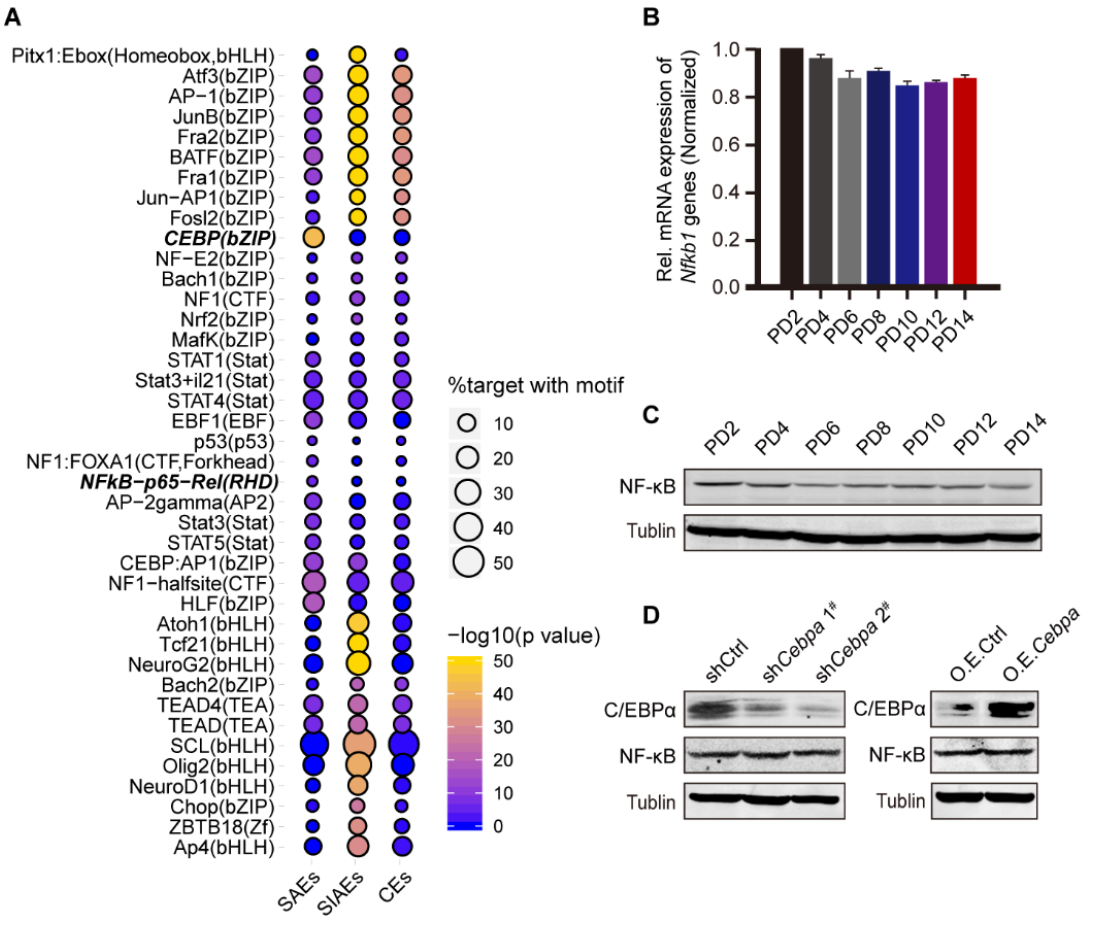
Supplementary Figure S10. C/EBPα facilitates the expression of SAEs flanking SASP genes in serially passaged REFs. (**A**) SA-β-gal staining and statistical diagrams of REFs during senescence. Scale bar, 50 μm. (**B**) Immunofluorescence staining and statistical diagrams of Mki67 in REFs. DAPI staining shows the position of the nucleolus. Scale bar, 10 μm. Error bars represent the S.D. obtained from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (**C**) Top: Western blotting analysis of total C/EBPα and p16 protein expression in serially passaged REFs. Tubulin served as a loading control. Bottom: RT-qPCR analysis of *Cebpa* mRNA expression in REFs of different passaged-cells, normalized to that of *Gapdh*. Error bars represent the S.D. obtained from three independent experiments. (**D, E**) The relative expression of SASP genes in REFs during senescence after down- (**D**) or upregulation (**E**) of C/EBPα expression was measured by RT-qPCR. Error bars represent the S.D. obtained from three independent

experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure S11. C/EBPβ is not involved in the regulation of C/EBPα-mediated SAEs flanking SASP genes. (**A**) Top: Western blotting analysis of total C/EBPβ protein expression in serially passaged MEFs. Tubulin served as a loading control. Bottom: RT-qPCR analysis of *Cebpb* mRNA expression in MEFs of different passage numbers, normalized to that of *Gapdh*. Error bars represent the S.D. obtained from three independent experiments. (**B**) The relative levels of C/EBPβ at SAEs flanking SASP genes in MEFs of different passage numbers were measured by ChIP-

qPCR. Specifically, C/EBPβ enrichment for *Nab1* served as a negative control, and C/EBPβ enrichment for *Cmbl* and *Cdk19* served as positive controls, as previously described (1,2). The enrichment of C/EBPα was normalized to 10% input. Error bars represent the S.D. obtained from three independent experiments. One-way ANOVA with Dunnett's multiple comparison test was performed.**$p < 0.01$, ***$p < 0.001$. (**C**) Western blotting showing the expression of C/EBPα and C/EBPβ after downregulation of C/EBPα and C/EBPβ expression, respectively, via siRNA. Tubulin served as a loading control. (**D**) ChIP-qPCR analysis of H3K27ac measuring the SAE activity adjacent to SASP genes after downregulation of C/EBPα expression. The enrichment of H3K27ac was normalized to 10% input, and IgG was used as a negative control. (**E**) Cytokine array analysis of secreted proteins and the relative quantitation of SASP factors in senescent (S) control, and C/EBPβ-knockdown cells. Error bars represent the S.D. obtained from three independent experiments. Statistical analysis was performed using a Student's t-Test.



Supplementary Figure S12. C/EBPα does not impact the expression of NF-κb. (**A**)

The top 20-ranked enriched motif families among SAEs, SIAEs, and CEs (regions defined by Figure 2A–C) determined via Homer2 algorithms are listed. The color of the circles denotes the enriched *p*-values, and the size of the circles denotes the percentage of motifs in target regions. (**B**) Western blotting analysis of NF-κB protein expression in serially passaged MEFs. Tubulin served as a loading control. (**C**) RT-qPCR analysis of *nfkb1* mRNA expression in MEFs of different passage numbers, normalized to that of *Gapdh*. Error bars represent the S.D. obtained from three independent experiments. (**D**) Western blotting showing the expression of NF-κB after down- or upregulation of C/EBPα via siRNA. Tubulin served as a loading control.

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