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Supplemental Material to:

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Dynamic and distinct histone modifications modulate the expression of key adipogenesis regulatory genes

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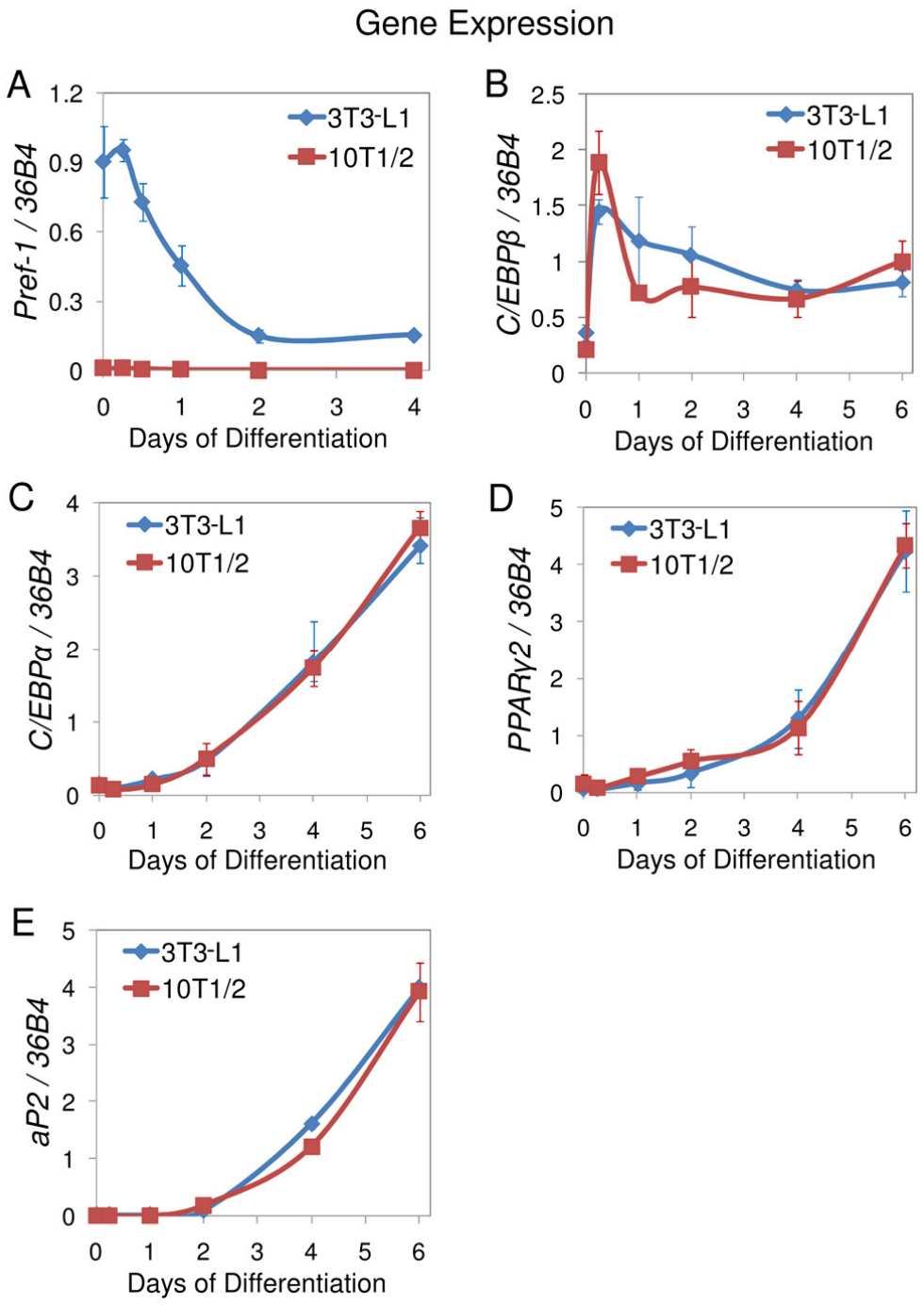

Figure S1. Expression of adipogenic regulatory genes during adipogenesis.

The mRNA levels of five key adipogenic regulatory genes were measured by RT-qPCR at the indicated time points during adipogenesis of 3T3-L1 preadipocytes and C3H 10T1/2 mesenchymal stem cells. The data shown here are the normalized mRNA levels

of the **(A)** *Pref-1*, **(B)** *C/EBPβ*, **(C)** *C/EBPα*, **(D)** *PPARγ2* and **(E)** *aP2* genes. Gene *36B4* was used as an internal control for all gene expression analyses in this study. These results are the averages of three independent RT-qPCR assays, and the error bars indicate standard deviations.

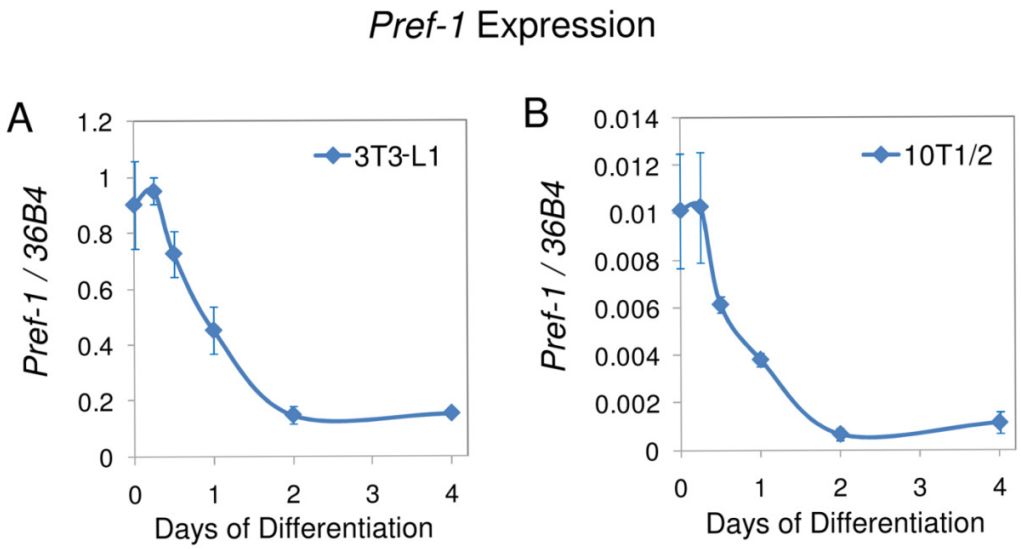

Figure S2. *Pref-1* gene expression is repressed during adipogenesis.

Pref-1 mRNA levels were determined by RT-qPCR at the indicated time points during the adipogenic differentiation of **(A)** 3T3-L1 preadipocytes and **(B)** C3H 10T1/2 mesenchymal stem cells and then normalized to *36B4* levels. These results are the averages of three independent RT-qPCRs, and the error bars indicate standard deviations.

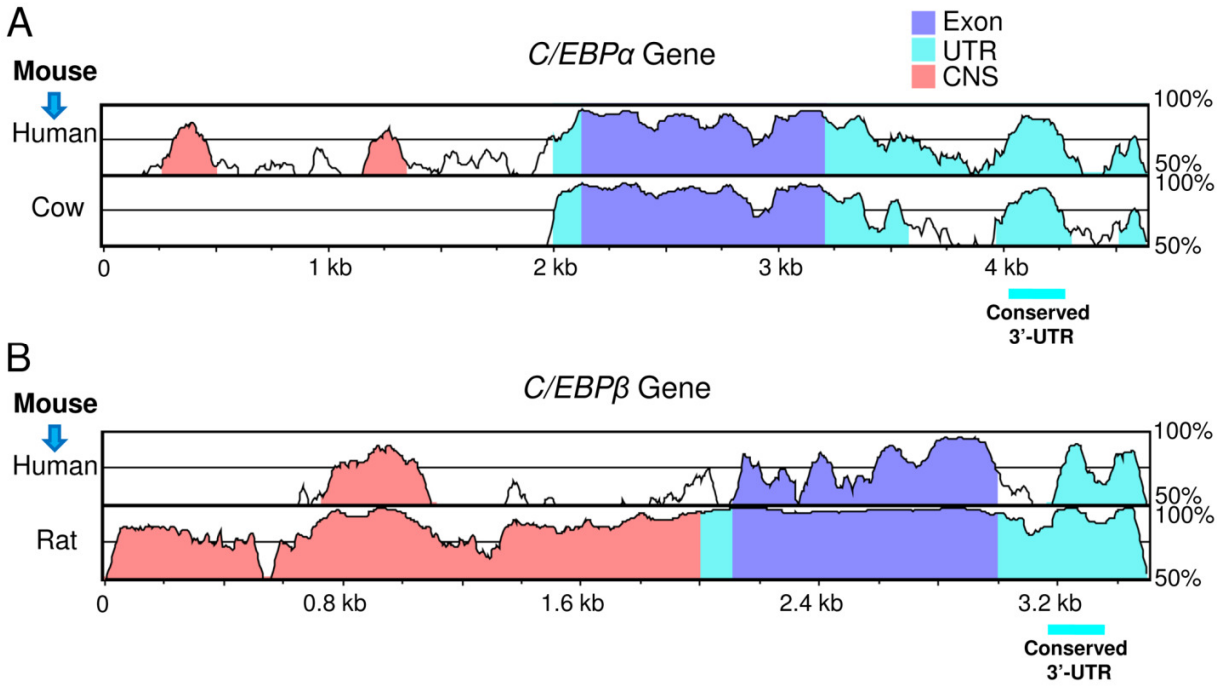

Figure S3. Sequence conservation analysis of the mouse adipogenic genes *C/EBPα* and *C/EBPβ*.

Mouse **(A)** *C/EBPα* and **(B)** *C/EBPβ* genomic sequences were compared to human and cow or human and rat sequences, respectively, using the VISTA algorithm. The highly conserved 3'-UTR sequence for each gene is highlighted by a light blue bar under the graph. (CNS: conserved non-coding sequence)

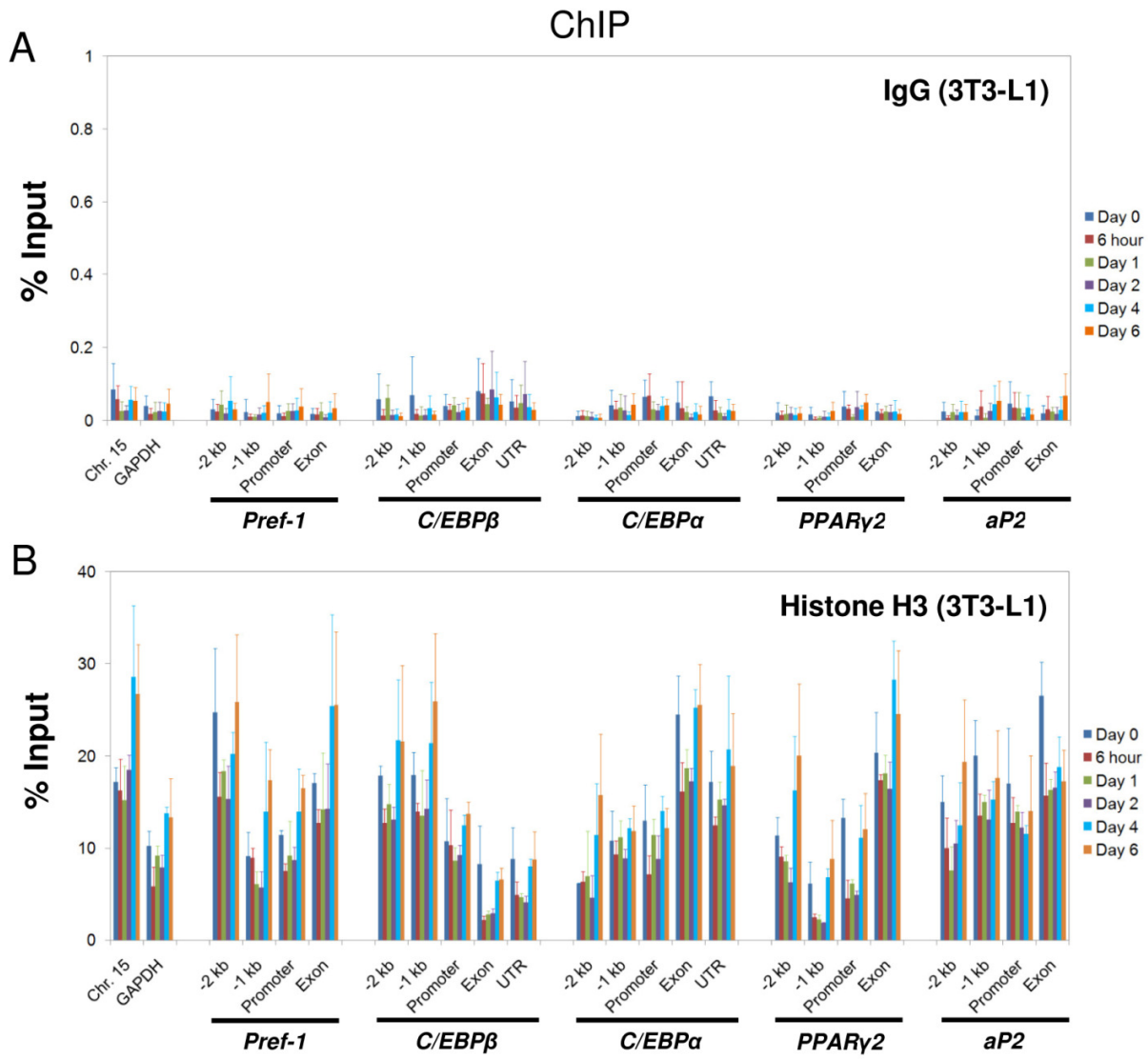

Figure S4. Histone H3 occupancy at the genomic loci of key adipogenic regulators during 3T3-L1 adipogenesis.

(A) Rabbit IgG was used as a negative control for the ChIP assay. **(B)** Histone H3 occupancy was examined by ChIP analysis using an antibody against the histone H3 C-terminus at the genomic loci of key adipogenic regulators, including Pref-1, C/EBPβ, C/EBPα, PPARγ2 and aP2. The primers used in this study are described in Figure 2. ChIP samples were collected at the indicated time points. These results are the averages of three independent ChIP-qPCRs, and the error bars indicate standard deviations.

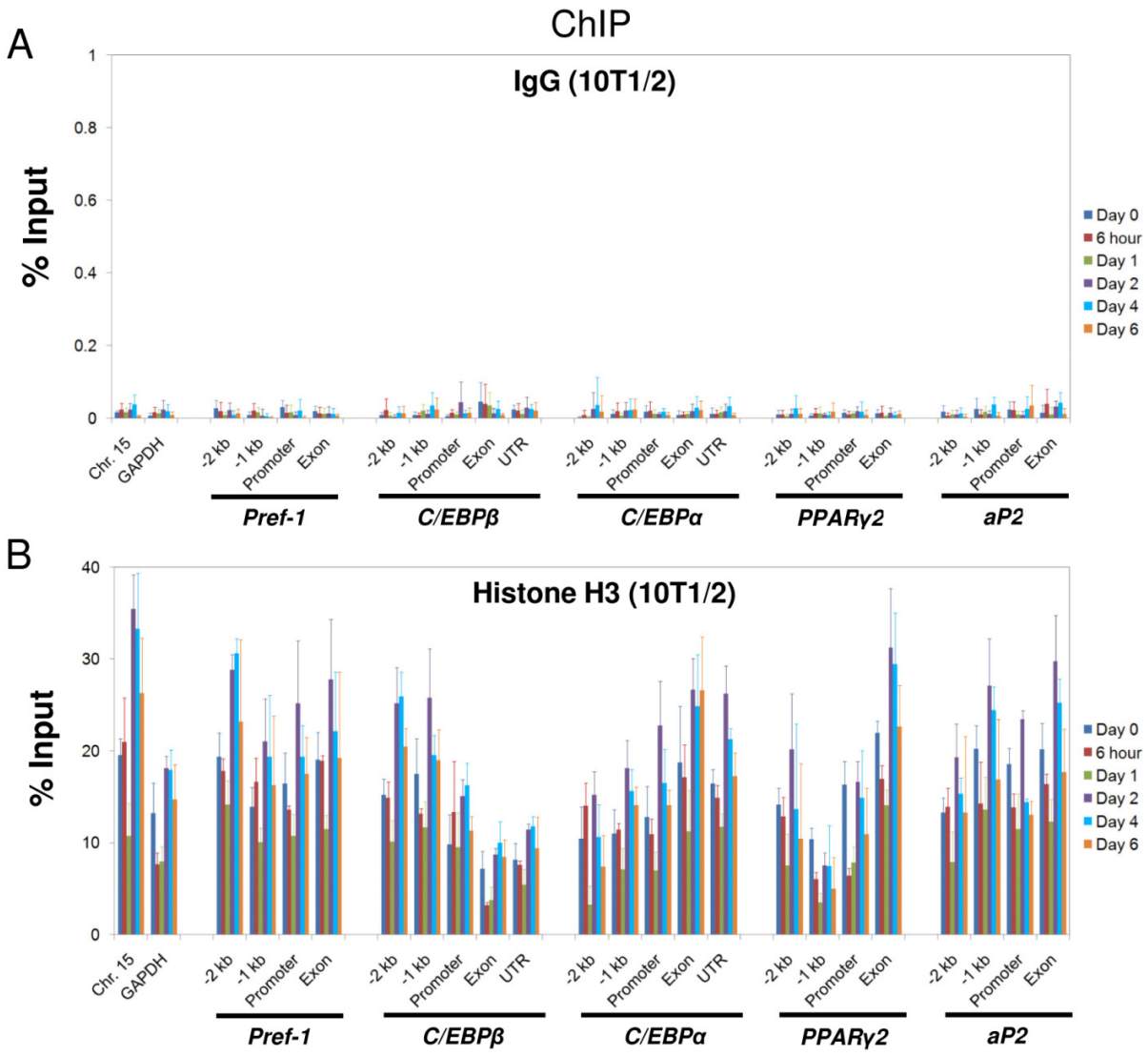

Figure S5. Histone H3 occupancy at the genomic loci of key adipogenic regulators during C3H 10T1/2 adipogenesis.

(A) Rabbit IgG was used as a negative control for the ChIP assay. **(B)** Histone H3 occupancy was examined by ChIP analysis using an antibody against the histone H3 C-terminus at the genomic loci of key adipogenic regulators, including Pref-1, C/EBPβ, C/EBPα, PPARγ2 and aP2. The primers used in this study are described in Figure 2. ChIP samples were collected at the indicated time points. These results are the averages of three independent ChIP-qPCRs, and the error bars indicate standard deviations.

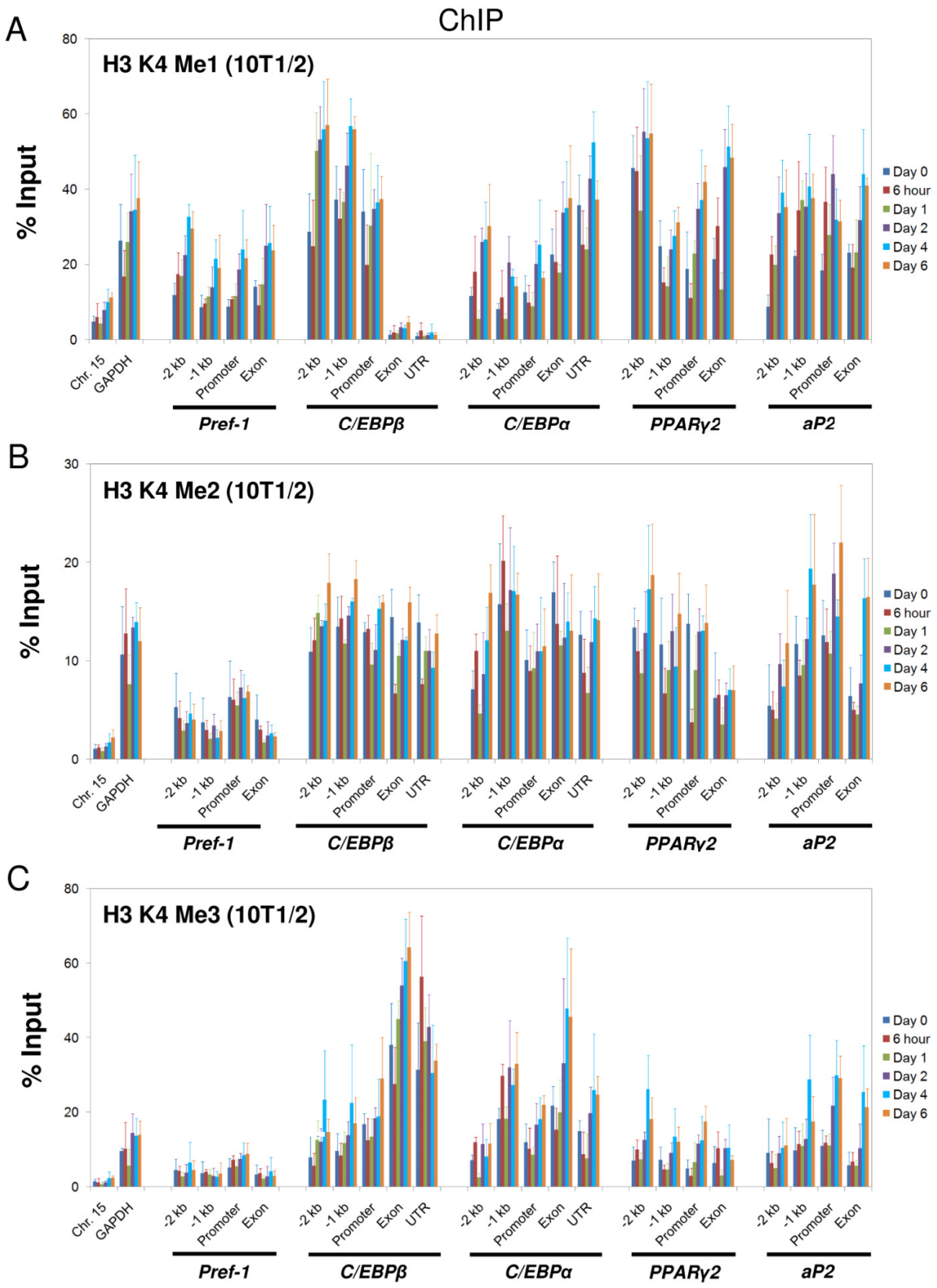
ChIP
A
H3 K4 Me1 (10T1/2)
% Input
80
60
40
20
0
B
H3 K4 Me2 (10T1/2)
% Input
30
20
10
0
C
H3 K4 Me3 (10T1/2)
% Input
80
60
40
20
0
Day 0
6 hour
Day 1
Day 2
Day 4
Day 6
Chr. 15
GAPDH
-2 kb
-1 kb
Promoter
Exon
UTR
Pref-1
C/EBPβ
C/EBPα
PPARγ2
aP2

Figure S6. Histone H3 K4 methylation states at the genomic loci of key adipogenic regulators during C3H 10T1/2 adipogenesis.

Levels of histone H3 K4 **(A)** mono-methylation, **(B)** di-methylation and **(C)** tri-methylation at the genomic loci of key adipogenic regulators, including Pref-1, C/EBPβ, C/EBPα, PPARγ2 and aP2, were examined by ChIP analysis using specific antibodies. The primers used in this study are described in Figure 2. ChIP samples were collected at the indicated time points. These results are the averages of three independent ChIP-qPCRs, and the error bars indicate standard deviations.

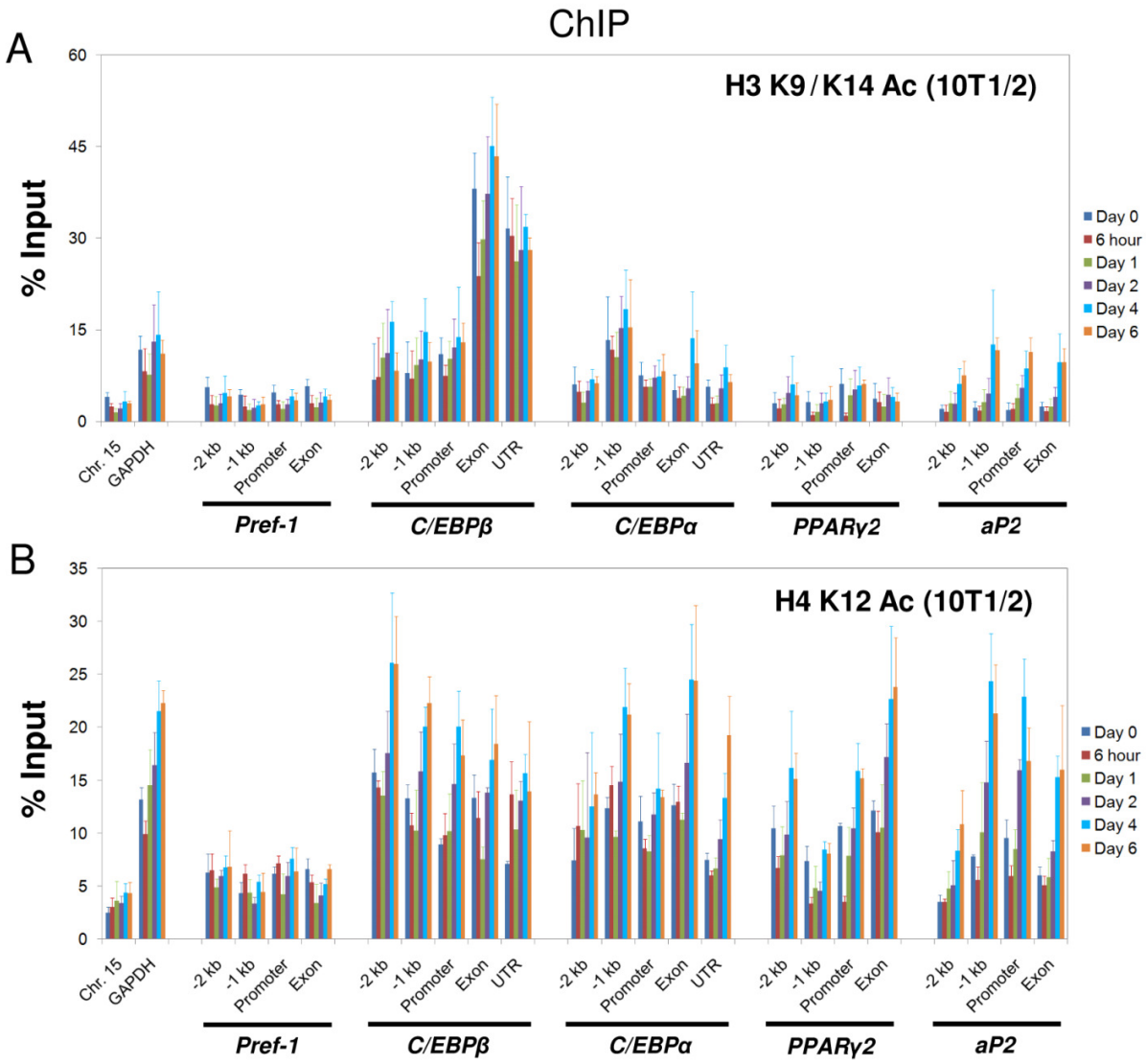

Figure S7. Histone H3 and H4 tails show distinct patterns of acetylation at key adipogenic regulator genes in C3H 10T1/2 cells.

Levels of histone **(A)** H3 K9/K14 acetylation and **(B)** H4 K12 acetylation at the genomic loci of key adipogenic regulators, including Pref-1, C/EBPβ, C/EBPα, PPARγ2 and aP2, were examined by ChIP analysis using specific antibodies. The primers used in this study are described in Figure 2. ChIP samples were collected at the indicated time points. These results are the averages of three independent ChIP-qPCRs, and the error bars indicate standard deviations.

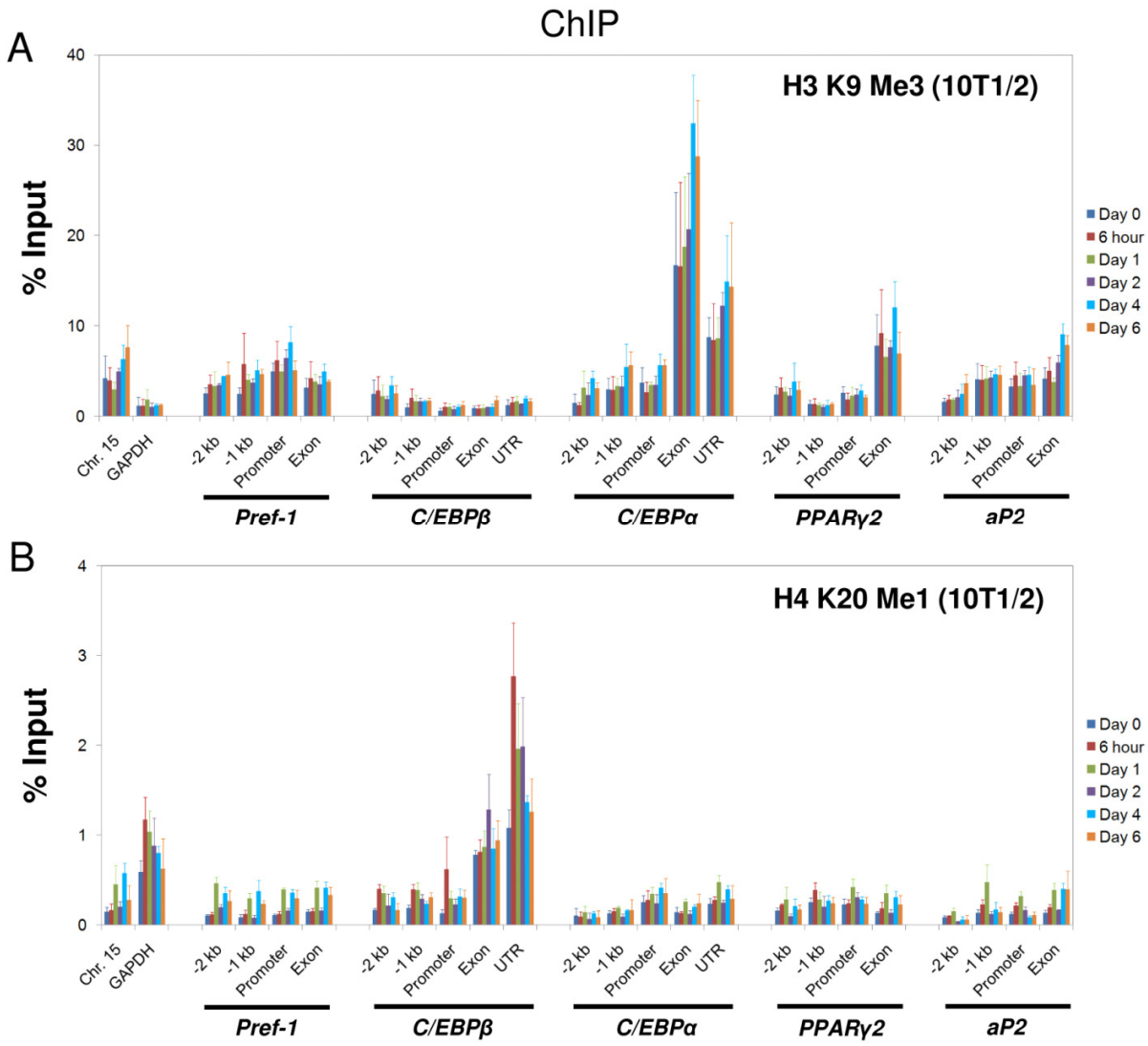

Figure S8. Patterns of histone H3 K9 tri-methylation and H4 K20 mono-methylation at key adipogenic regulator genes in C3H 10T1/2 cells.

Levels of histone **(A)** H3 K9 tri-methylation and **(B)** H4 K20 mono-methylation at the genomic loci of key adipogenic regulators, including Pref-1, C/EBPβ, C/EBPα, PPARγ2 and aP2, were examined by ChIP analysis using specific antibodies. The primers used in this study are described in Figure 2. ChIP samples were collected at the indicated time points. These results are the averages of three independent ChIP-qPCRs, and the error bars indicate standard deviations.

Table S1. Primers for RT-qPCR.

Primer	Sequences (5' - 3')	References
36B4-F	AGATGCAGCAGATCCGCAT	(1)
36B4-R	GTTCTTGCCCATCAGCACC	
Pref1-F	CGTGATCAATGGTTCTCCCT	(2)
Pref1-R	AGGGGTACAGCTGTTGGTTG	
C/EBPβ-F	AAGAGCCGCGACAAGGC	(1)
C/EBPβ-R	GTCAGCTCCAGCACCTTGTG	
C/EBPα-F	GCGGGCAAAGCCAAGAA	(1)
C/EBPα-R	GCGTTCCCGCCGTACC	
PPARγ2-F	AACTCTGGGAGATTCTCCTGTTGA	(1)
PPARγ2-R	TGGTAATTTCTTGTGAAGTGCTCATA	
aP2-F	CACCGCAGACGACAGGAAG	(1)
aP2-R	GCACCTGCACCAGGGC	
EZH2-F	AGCACAAGTCATCCCGTTAAAG	(3)
EZH2-R	AATTCTGTTGTAAGGGCGACC	

Table S2. Primers for ChIP-qPCR.

Primer	Sequences (5' - 3')	Product Size	References
Chr.15-F	AGCGTGGCCTTGGCAGCAAA	138 bp	This study
Chr.15-R	TGCGATTGGCTTCCTCTCCCC		
GAPDH-F	TCCAGCTGGGTGCCGGAAGT	106 bp	This study
GAPDH-R	TCAAGCCCCACCCTCCGCAT		
Pref-1 -2kb-F	TCCCCTGCTTTCTGCCCGAGA	113 bp	This study
Pref-1 -2kb-R	TCAGCCCAAAGAGGGGAGAGCA		
Pref-1 -1kb-F	TGGGCATACGTGTTGCTGCG	91 bp	This study
Pref-1 -1kb-R	CCACAGTCACAGTCTGGGCCT		
Pref-1 Promoter-F	CATGTGTGCGCGGGACTCCA	125 bp	This study
Pref-1 Promoter-R	CAGGCCCGCTTAGCGCAAGT		
Pref-1 Exon-F	TCTGCACCGACATCGGGGGT	136 bp	This study
Pref-1 Exon-R	ACCTGGGTGTGCTGGAGGCA		
C/EBPβ -2kb-F	TTGCTGTGTCTCCCCAGAACCC	153 bp	This study
C/EBPβ -2kb-R	GCTTGGTAGTAACTACCTTAAACC		
C/EBPβ -1kb-F	CAGCTCAGCAGATAACACCGAAG	141 bp	This study
C/EBPβ -1kb-R	ACCCTTCTGCCACTCCTAGGTG		
C/EBPβ Promoter-F	TGGGAGGACATGCACCCCGT	133 bp	This study
C/EBPβ Promoter-R	GCTGTCCCGGACCCCCAACT		
C/EBPβ Exon-F	ACGAGCGCGCCATCGACTTC	152 bp	This study
C/EBPβ Exon-R	ACCGTAGTCGGCCGGCTTCT		
C/EBPβ UTR-F	TGCACAGCGCACCGGGTTTC	62 bp	This study
C/EBPβ UTR-R	ACACGCGCTCAGCCACGTTT		
C/EBPα -2kb-F	GAGAGCGATCCTCTGCTCACACC	198 bp	This study
C/EBPα -2kb-R	CCGACTTCGTCTGAAGGACGCATC		
C/EBPα -1kb-F	ACTAGAGTGCTCCACGCTGG	140 bp	This study
C/EBPα -1kb-R	TAACCCCGGAGCCTGGTG		

C/EBPα Promoter-F	ATCCGGGTGGGAGACAGGCC	142 bp	This study
C/EBPα Promoter-R	CACTAGGGAGCCCGGGAGCA		
C/EBPα Exon-F	CGCTGGTGATCAAACAAGAGCC	160 bp	This study
C/EBPα Exon-R	TGCGCGATCTGGAACTGCAAG		
C/EBPα UTR-F	GCCAGCCGCTGTTGCTGAAG	140 bp	This study
C/EBPα UTR-R	AGGCACCGCTGGGACACAGA		
PPARγ2 -2kb-F	CAAGCAAAAGCTCTACCACAAAGC	139 bp	This study
PPARγ2 -2kb-R	GAATGTAAAACTTCAGCTTCACTTCC		
PPARγ2 -1kb-F	TTATTGCCATCTGATACACTGCCC	154 bp	This study
PPARγ2 -1kb-R	TAAGGCCTTTGCCCTTTTTGGCAG		
PPARγ2 Promoter-F	TGTGTGGGTCACTGGCGAGACA	130 bp	This study
PPARγ2 Promoter-R	TGGCTGGCACTGTCCTGACTGA		
PPARγ2 Exon-F	CAGAGCAAAG AGGTGGCCATCCG	159 bp	This study
PPARγ2 Exon-R	GATCTCATGGACACCATACTTGAG		
aP2 -2kb-F	TCTTGTTATATTAGCCACCTGTCG	69 bp	*
aP2 -2kb-R	GCCAGCCCATGTAAACTTCT		
aP2 -1kb-F	CAGCTAGGTTTCTTTGAGTTAGAG	123 bp	This study
aP2 -1kb-R	TGTTTGGTTTGGGTTGGGTTTTGG		
aP2 Promoter-F	CTGGTCATGAAGGAAATGATCTGG	120 bp	This study
aP2 Promoter-R	GCTGCAGCACAGGAGGGTGCTATG		
aP2 Exon-F	AAGTGGCAGGCATGGCCAAGC	150 bp	This study
aP2 Exon-R	TCACCTTCCTGTCGTCTGCGGT		

* Villanueva, C. and Tontonoz, P. *Personal communication.*

Table S3. Primers for plasmid construction.

Primer	Sequences (5' - 3')	References
pGL3 linker-F	GATCCGACGTCACCGGTGGGCCCGCGCGC ATGCATACTAGTG	This study
pGL3 linker-R	TCGACACTAGTATGCATGCGCGCGGGCCCA CCGGTGACGTCG	
C/EBPβ P3kb-F	TTTTTTGGTACCAGCAACCATCACAGCCACAGCTA	This study
C/EBPβ P3kb-R	TTTTTTTTCTCGAGTGCGTCACGCTGGGGCCCCT	
C/EBPβ UTR-F	AAAAAGACGTCCCTGCACAGCGCACCGGGTT	This study
C/EBPβ UTR-R	AAAAGTCGACGGCTTTTAAACATTCTCCAAAAAAG	

Supplemental References

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