

MLL3/MLL4 are required for CBP/p300 binding on enhancers and super-enhancer formation in brown adipogenesis

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SUPPLEMENTAL TABLES

Table S1. Datasets used in this study.

Adipogenesis of <i>Pparγ^{ff}</i> brown preadipocytes		
ChIP-Seq	Transcription factor	EBF2 (D-3, D0, D2, D7) C/EBPβ (D-3, D0, D2, D7) C/EBPα (D-3, D0, D2, D7) PPARγ (D-3, D0, D2, D7) CTCF (D-3, D0, D2, D7)
	Epigenomic writer	CBP (D-3, D0, D2, D7)
	Other factor	Pol II (D-3, D0, D2, D7) Med1 (D-3, D0, D2, D7)
	Histone mark	H3K4me1/2/3 (D-3, D0, D2, D7) H3K27ac (D-3, D0, D2, D7) H3K36me3 (D-3, D0, D2, D7) H3K9me2 (D-3, D0, D2, D7) H3K27me3 (D-3, D0, D2, D7)
FAIRE-Seq		FAIRE (D-3, D0, D2, D7)
RNA-Seq		RNA (D-3, D0, D2, D7)

Adipogenesis of <i>MII3^{-/-}MII4^{ff}</i> brown preadipocytes		
ChIP-Seq	Epigenomic writer	MLL4 (D-3, D0 ¹ , D2 ¹ , D7 ¹) CBP (D2) p300 (D2)
Adipogenesis of <i>MII3^{-/-}MII4^{-/-}</i> brown preadipocytes		
ChIP-Seq	Epigenomic writer	CBP (D2) p300 (D2)

Adipogenesis of 3T3L1 white preadipocytes		
ChIP-Seq	Epigenomic writer	MLL4 (D7)
RNA-Seq		RNA (D0 ² , D7 ²)

Brown adipose tissue (adult mice, 24-week-old)		
ChIP-Seq of histone mark		H3K4me1 ³ H3K27ac ³
RNA-Seq		RNA

1. From Ref.1 (GEO: GSE50466). 2. From Ref. 3 (GEO: GSE87113). 3. From Ref. 4 (GEO: GSM929703; GSM1000071)

Table S2. Pearson correlations between biological replicates of RNA-Seq at each time point.

Experiments	Samples	Pearson correlations
RNA-Seq (D-3)	<i>Pparγ</i> ^{ff} preadipocytes	0.962
	<i>Brd4</i> ^{ff} preadipocytes	
RNA-Seq (D0)	<i>Pparγ</i> ^{ff} preadipocytes	0.966
	<i>Brd4</i> ^{ff} preadipocytes	
RNA-Seq (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.967
	<i>Brd4</i> ^{ff} preadipocytes	
RNA-Seq (D7)	<i>Pparγ</i> ^{ff} preadipocytes	0.964
	<i>Brd4</i> ^{ff} preadipocytes	

Note: all the p values of the correlations are smaller than 1E-300.

Table S3. Pearson correlations between biological replicates of ChIP-Seq.

Experiments	Samples	Pearson correlations
C/EBP β (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.867
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
C/EBP α (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.848
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
PPAR γ (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.954
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
CBP (D0)	<i>Pparγ</i> ^{ff} preadipocytes	0.943
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes	
CBP (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.935
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes	
CBP (D7)	<i>Pparγ</i> ^{ff} preadipocytes	0.868
	<i>Brd4</i> ^{ff} preadipocytes	
Pol II (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.966
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
MED1 (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.867
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
H3K4me1 (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.841
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
H3K4me2 (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.914
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
H3K4me3 (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.949
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
H3K27ac (D2)	<i>Pparγ</i> ^{ff} preadipocytes	0.831
	<i>Mll3</i> ^{-/-} <i>Mll4</i> ^{ff} preadipocytes ¹	
H3K9me2 (D0)	<i>Pparγ</i> ^{ff} preadipocytes	0.835
	3T3L1 preadipocytes ²	
H3K27me3 (D0)	<i>Pparγ</i> ^{ff} preadipocytes	0.841
	3T3L1 preadipocytes ²	

1. From Ref.1 (GEO: GSE50466). 2. From Ref. 2 (GEO: GSE41455).

Note: all the p values of the correlations are smaller than 1E-300.

Table S4. Brown-specific super-enhancer-associated genes.

Gene	Brown SEs	Gene expression (rpkm)							
		<i>Pparγ</i> ^{+/+} brown preadipocytes		GR ^{+/+} brown preadipocytes ¹		3T3L1 white preadipocytes ²		BAT	WAT ³
		D0	D7	D0	D7	D0	D7		
<i>Abi3</i>	chr11:95634650-95662299	1.34	82.59	2.57	30.42	1.34	12.27	91.12	2.72
<i>Acacb</i>	chr5:114593300-114613499	0.88	27.42	0.93	17.65	2.32	6.31	164.67	39.95
<i>Acsm3</i>	chr7:127102550-127109849	2.62	8.03	1.49	2.39	1.13	2.21	48.96	1.16
<i>Adrb1</i>	chr19:56750950-56754799	1.21	2.54	0.46	2.51	0.24	0.49	5.93	0.03
<i>Adrb2</i>	chr18:62243900-62256399	0.77	17.05	1.99	3.93	0.02	1.16	5.67	1.25
<i>Aspg</i>	chr12:113332850-113335649	0.35	65.71	0.08	12.57	0.00	0.16	58.92	3.05
<i>C130074G19Rik</i>	chr1:186693500-186696599	0.11	59.83	0.30	60.35	0.00	0.08	29.95	4.40
<i>Ccdc69</i>	chr11:54694550-54727849	0.99	6.96	0.57	4.78	0.00	0.42	14.10	0.14
<i>Chchd10</i>	chr10:75360650-75367499	3.57	138.82	1.26	45.80	1.07	2.16	537.18	46.33
<i>Cidea</i>	chr18:67486050-67492249	0.00	6.65	0.00	7.36	0.00	2.98	1163.94	87.86
<i>Col27a1</i>	chr4:62859950-62866899	1.16	4.50	1.43	6.99	5.81	2.60	6.42	0.09
<i>Ebf2</i>	chr14:68033900-68037449	4.57	20.01	8.23	12.28	1.44	2.96	10.98	0.15
<i>Gngt2</i>	chr11:95634650-95662299	2.43	14.29	1.61	6.53	0.00	0.43	17.18	0.36
<i>Gpd2</i>	chr2:57104500-57109299	11.06	40.53	11.83	27.84	9.36	14.69	197.89	2.68
<i>Gpx3</i>	chr11:54694550-54727849	0.27	3.06	50.48	223.16	0.26	0.16	619.38	11.14
<i>Id2</i>	chr12:25835600-25865449	3.65	39.27	13.99	46.52	2.40	0.33	13.96	7.02
<i>Ifi2712a</i>	chr12:104715600-104725949	7.17	54.83	67.23	797.21	161.44	14.40	96.21	12.42
<i>Irs3</i>	chr5:137922350-137935649	1.16	7.17	0.25	5.44	0.01	0.63	12.08	0.05
<i>Lctl</i>	chr9:63806150-63837999	2.74	20.85	2.10	22.85	0.37	8.95	14.90	1.76
<i>Lym7</i>	chr11:54694550-54727849	1.07	4.13	0.70	2.12	0.47	1.29	7.38	1.81
<i>Mb</i>	chr15:76895650-76911399	0.11	55.25	0.04	27.23	0.04	5.08	157.40	0.94
<i>Phospho1</i>	chr11:95634650-95662299	2.47	160.55	5.41	60.47	2.73	24.90	178.83	5.03
<i>Pim1</i>	chr17:29521550-29526649	5.60	35.85	5.80	15.75	2.23	3.10	51.51	5.57
<i>Ppp1r3b</i>	chr8:36256700-36279749	2.02	79.60	2.61	24.11	2.43	15.28	87.69	0.66

<i>Rbp4</i>	chr19:38157550-38162149	0.18	75.17	0.24	36.36	0.00	0.00	45.57	0.19
<i>Trib1</i>	chr15:59604200-59618049	3.20	11.93	3.10	13.52	2.82	1.70	10.87	3.27
<i>Tspan18</i>	chr2:93159650-93169799	0.04	12.00	1.19	16.09	0.00	2.93	53.03	1.53
<i>Ucp1</i>	chr8:85800650-85809849	0.00	1.07	0.00	0.88	0.00	0.00	1015.15	0.10

1. From Ref. 5 (GEO: GSE76619).
2. From Ref. 3 (GEO: GSE87113).
3. From Ref. 4 (GEO: GSM970858).

SUPPLEMENTAL REFERENCES

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4. Yue, F., Cheng, Y., Breschi, A., Vierstra, J., Wu, W., Ryba, T., Sandstrom, R., Ma, Z., Daves, C., Pope, B.D. *et al.* (2014) A comparative encyclopedia of DNA elements in the mouse genome. *Nature*, **515**, 355-364.
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SUPPLEMENTAL FIGURES

Figure S1. Genome browser view of the *Ucp1* locus before and after brown adipogenesis (Related to Figure 2). ChIP-Seq, FAIRE-Seq, and RNA-Seq data at the *Ucp1* locus before (D0) and after (D7) adipogenesis of brown preadipocytes are shown.

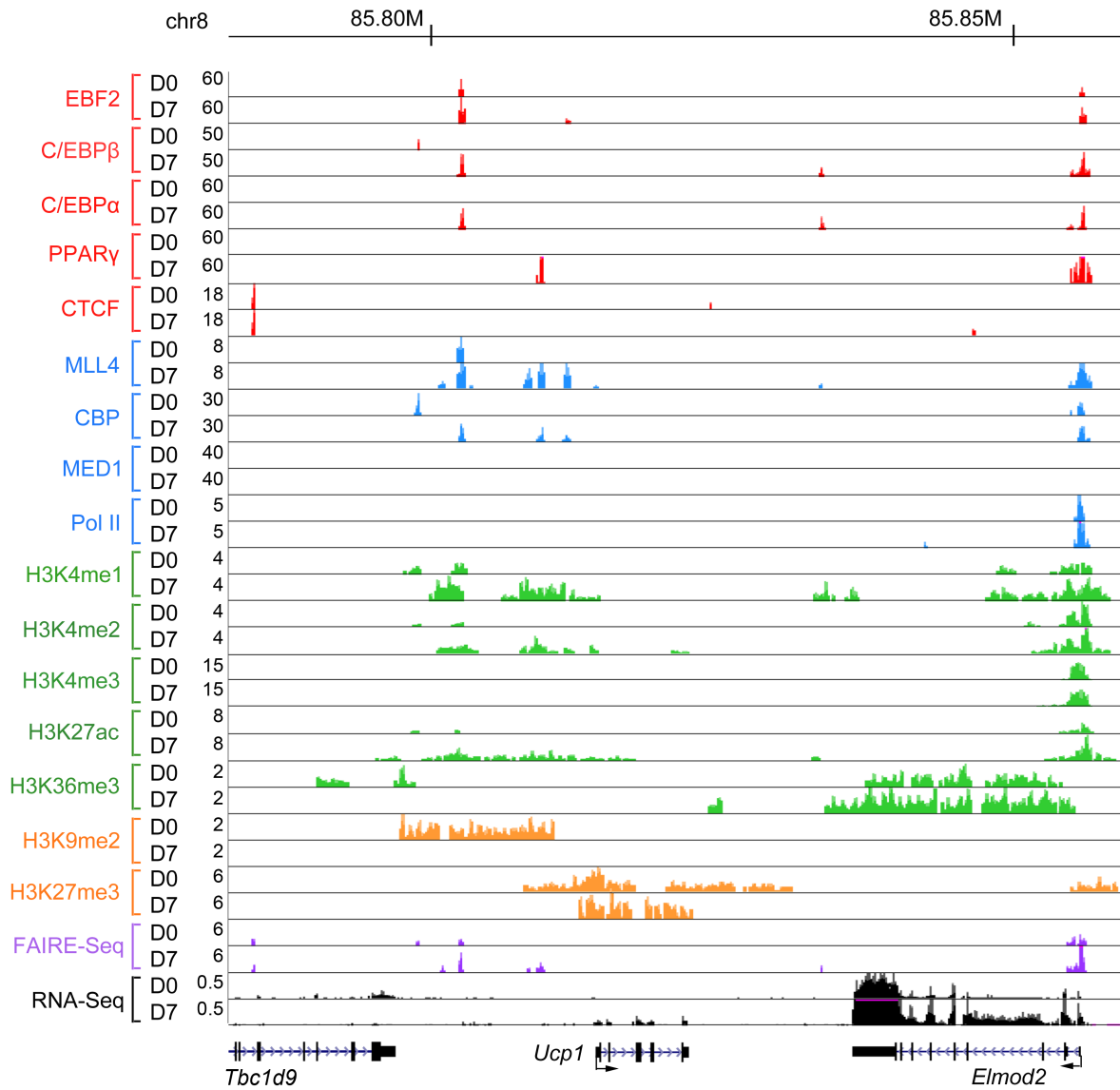


Figure S2. Dynamic MLL4 and CBP binding on enhancers in adipogenesis (Related to Figure 4).

(A) Changes in MLL4 and CBP binding on enhancers between two successive time points. The most dramatic changes occur between D0 and D2.

(B) Enrichment analysis of temporal patterns of MLL4 (left panel) and CBP (right panel) binding on enhancers. We classified MLL4 and CBP binding patterns into four types depending on which time point MLL4/CBP binding intensity levels are the highest at: D-3 (a), D0 (b), D2 (c), or D7 (d). For each gene group, we determined whether each MLL4/CBP binding pattern was enriched (over-represented) or depleted (under-represented). Using the hypergeometric test, we calculated the p value to determine the significance of enrichment or depletion for each pattern and generated a heat map to visualize the significance. For each group, the most enriched pattern correlates with the pattern of gene expression changes while the depleted patterns anti-correlates.

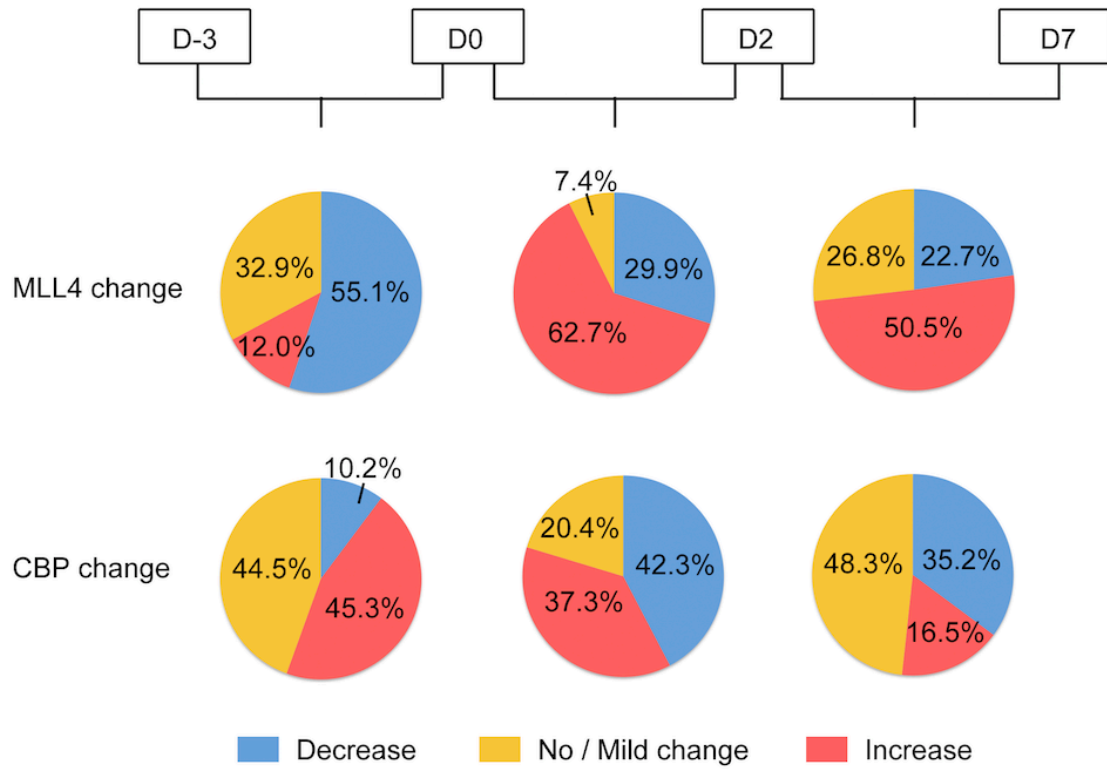
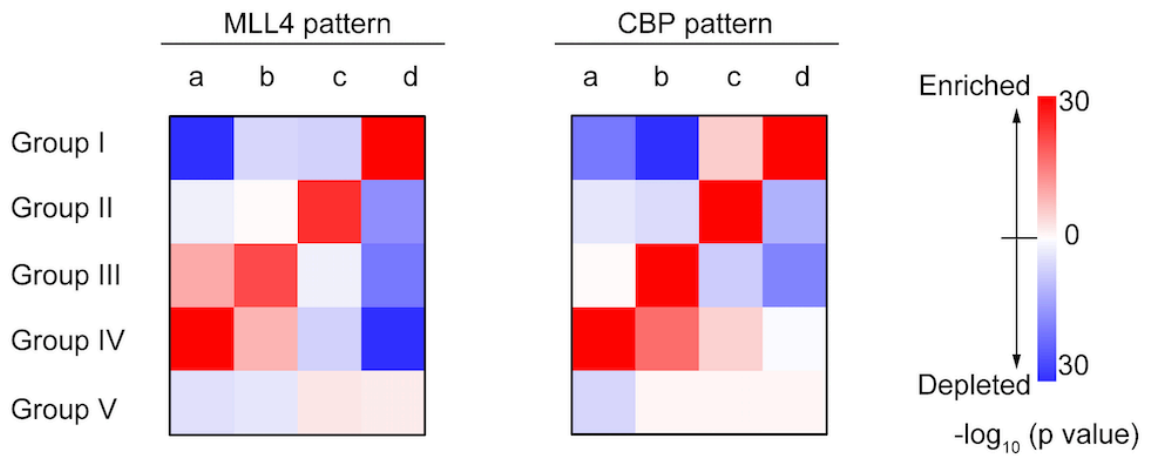
A**B**

Figure S3. Profiles of MLL4 and CBP binding as well as H3K4me1 and H3K27ac levels on enhancers of *Cebpa* and *Wnt10b* gene loci in adipogenesis (Related to Figure 4).

(A) The enhancer region (highlighted in gray) around the *Cebpa* locus exhibits increased MLL4 and CBP binding as well as increased H3K4me1 and H3K27ac levels during adipogenesis.

(B) The enhancer region (highlighted in gray) around the *Wnt10b* locus exhibits decreased MLL4 and CBP binding as well as decreased H3K4me1 and H3K27ac levels during adipogenesis.

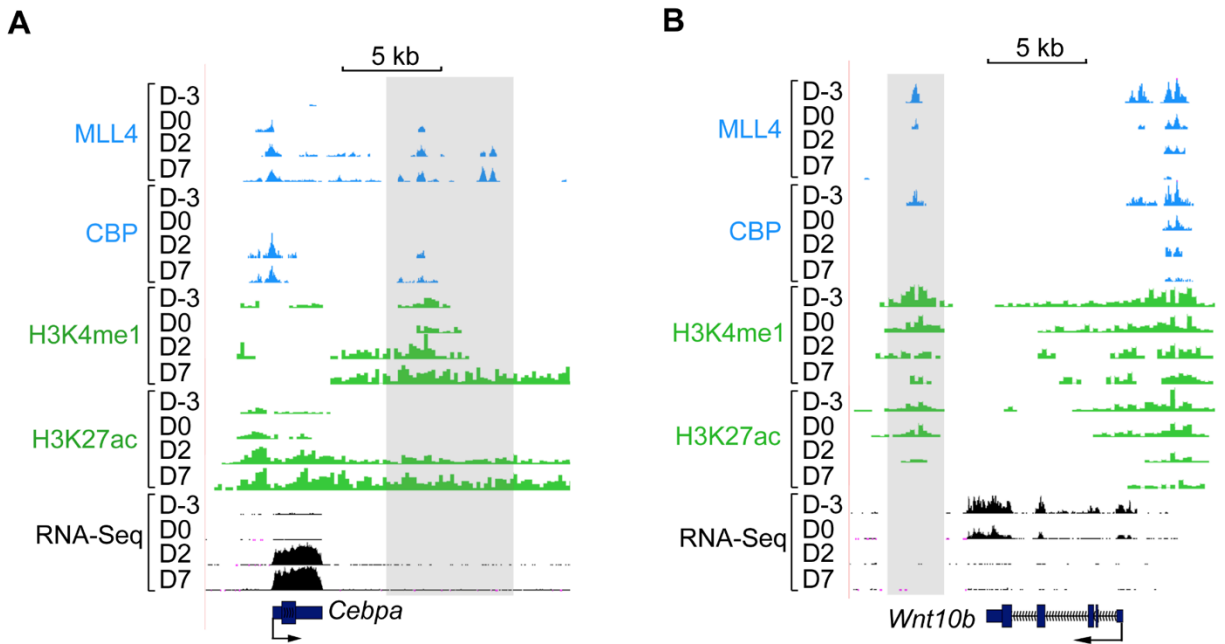


Figure S4. GO analysis of super-enhancers defined by three methods (Related to Figure 6).

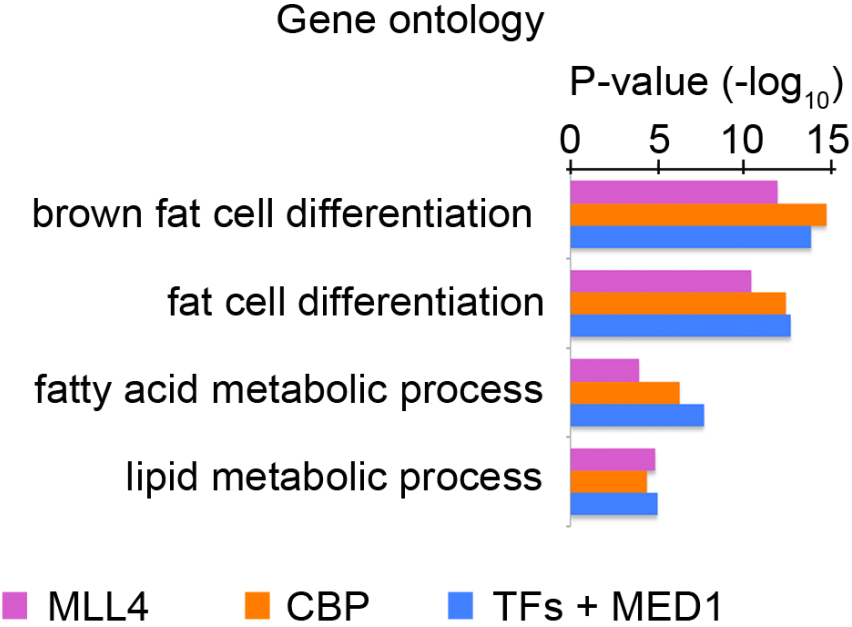
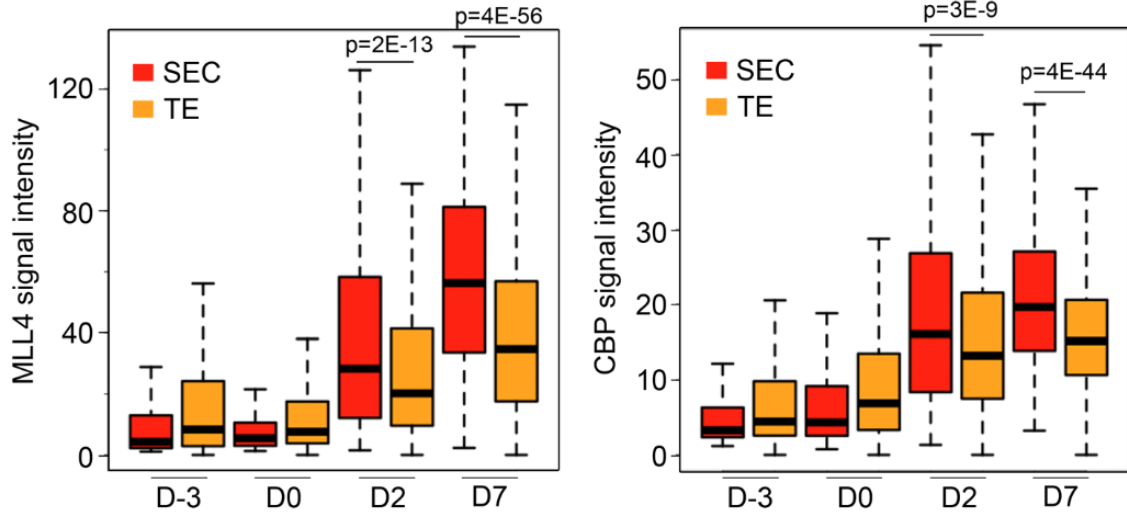


Figure S5. Higher levels of MLL4 and CBP at individual super-enhancer constituents than at typical enhancers (Related to Figure 6).

MLL4 and CBP signal levels are shown for both super-enhancer constituents (SECs) and typical enhancers (TEs) from D-3 to D7. Super-enhancers (SEs) are identified using CBP or TFs + MED1.

A

CBP defined SEs (D7)



B

TFs + MED1 defined SEs (D7)

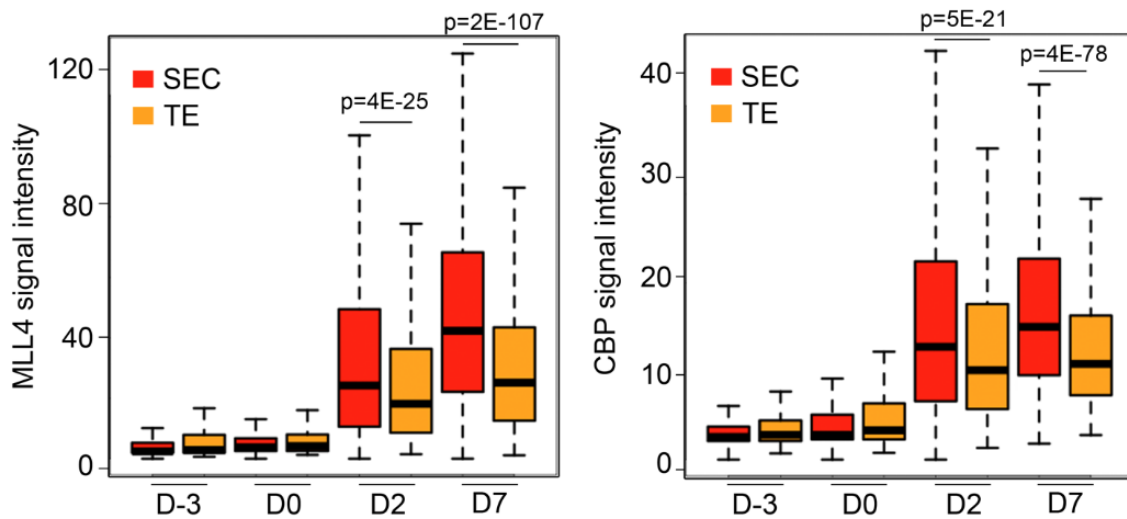


Figure S6. MLL4 pre-marks a subset of super-enhancer constituents before adipogenesis (Related to Figure 6).

D7 super-enhancers (SEs) are defined by MLL4 binding.

(A) MLL4 pre-marks 374 D7 super-enhancer constituents (SECs), which belong to 215 out of 438 D7 SEs.

(B) D7 SECs pre-marked by MLL4 at D0 have higher levels of FAIRE signals, H3K4me1, and H3K27ac.

(C) MLL4 pre-marks D7 SECs associated with adipogenic genes *Pparg*, *Cebpa* and *Ucp1* at D0.

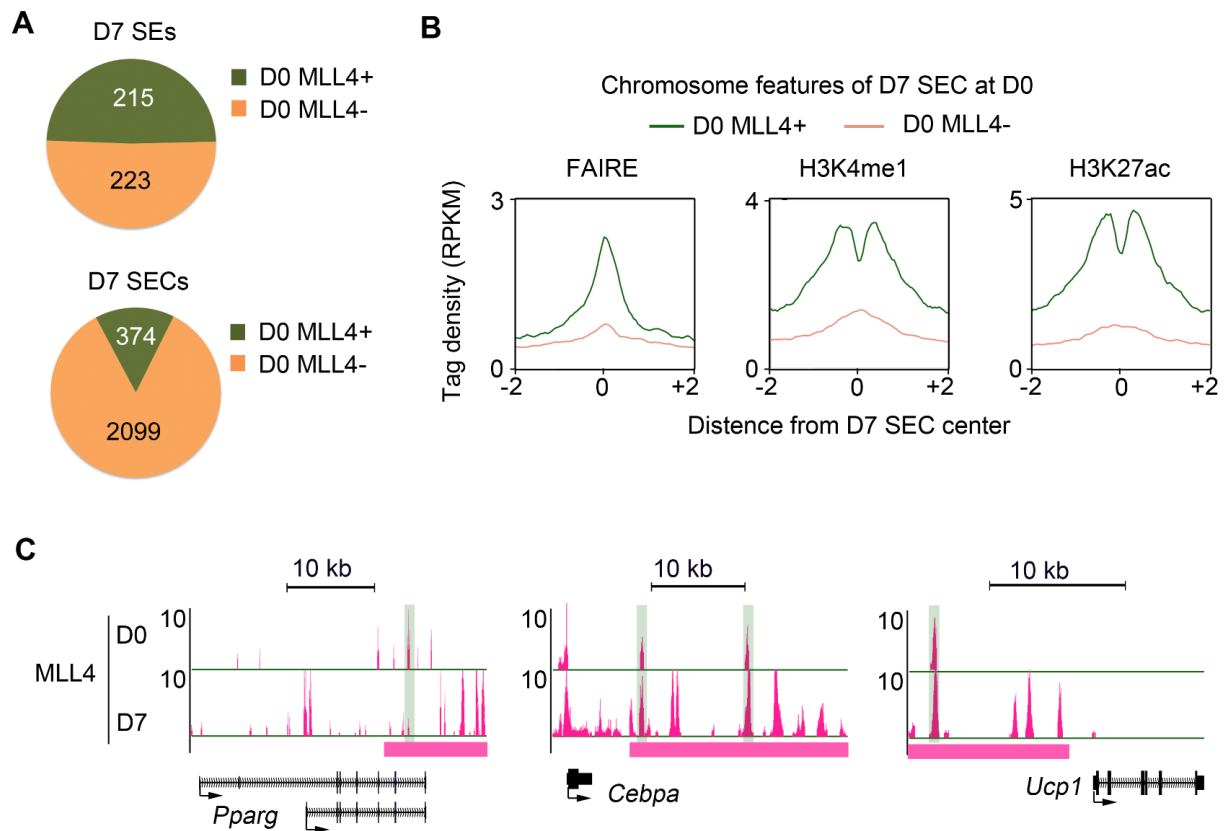


Figure S7. Super-enhancers identified by MLL4 at D2 of adipogenesis (Related to Figure 6).

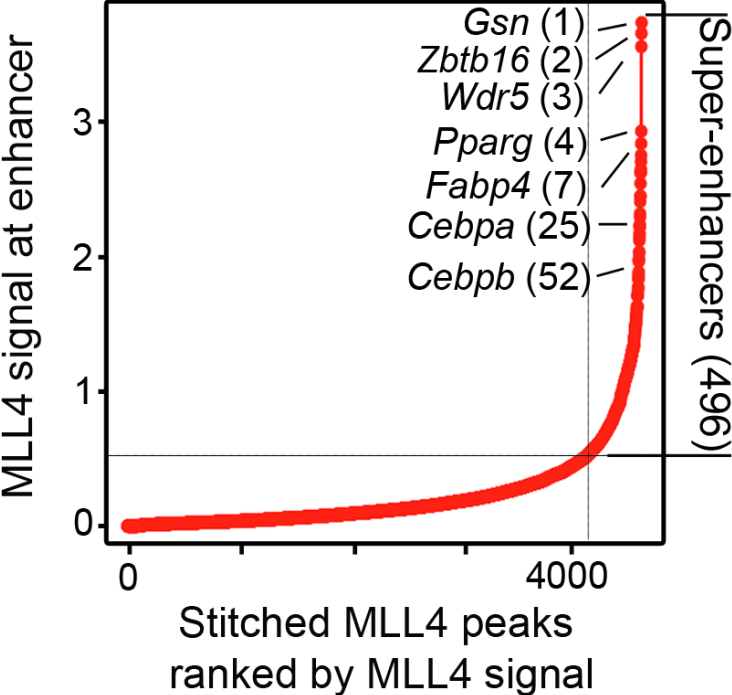


Figure S8. A subset of MLL4-specific super-enhancers is primed in brown adipocytes differentiated in culture but activated in BAT (Related to Figure 7).

(A) The list of MLL4-specific SE-associated genes expressed at 5-fold higher level in BAT than in D7 brown adipocytes. BAT-selective genes are indicated.

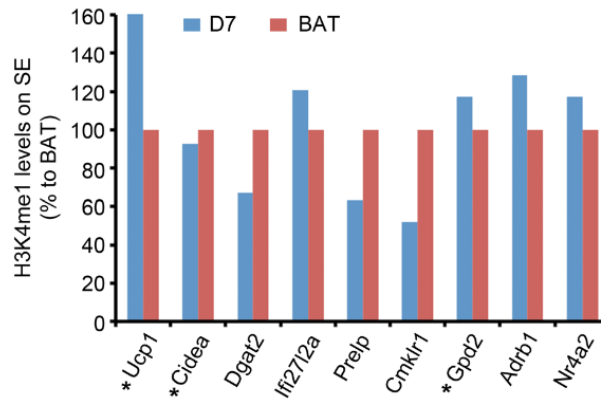
(B-C) Comparison of (B) H3K4me1 and (C) H3K27ac levels on SEs in brown adipocytes (D7) and in BAT for genes shown in (A). Stars indicate BAT-selective genes.

A List of MLL4-specific SE-associated genes expressed at higher level (>5-fold) in BAT than in brown adipocytes (D7) differentiated in culture

	BAT/D7 fold change	FPKM at D7	FPKM in BAT
* Ucp1	2157.3	1.0	2178.6
* Cidea	344.3	7.3	2498.0
Dgat2	16.4	69.6	1141.6
Ifi2712a	15.7	6.9	108.3
Prelp	13.6	8.8	119.7
Cmklr1	11.5	0.4	4.7
* Gpd2	11.2	38.0	424.7
Adrb1	7.4	1.7	12.7
Nr4a2	6.9	1.7	11.5

* BAT-selective genes reported in Seale et al, 2007

B



C

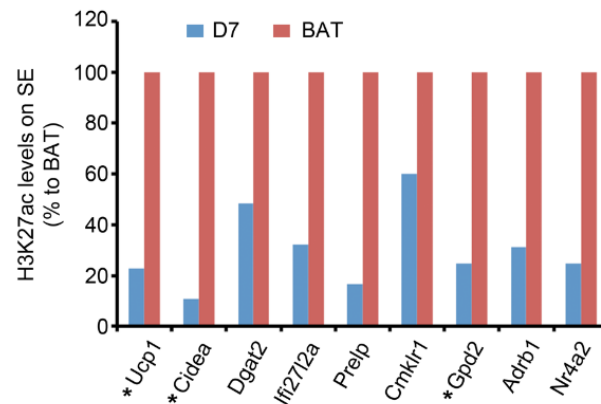


Figure S9. Comparative epigenomic profiling of brown and white adipogenesis at *Ucp1* locus.

ChIP-Seq and RNA-Seq data at the *Ucp1* locus before (D0) and after (D7) adipogenesis of brown preadipocytes (left) and 3T3L1 white preadipocytes (right) are shown.

