

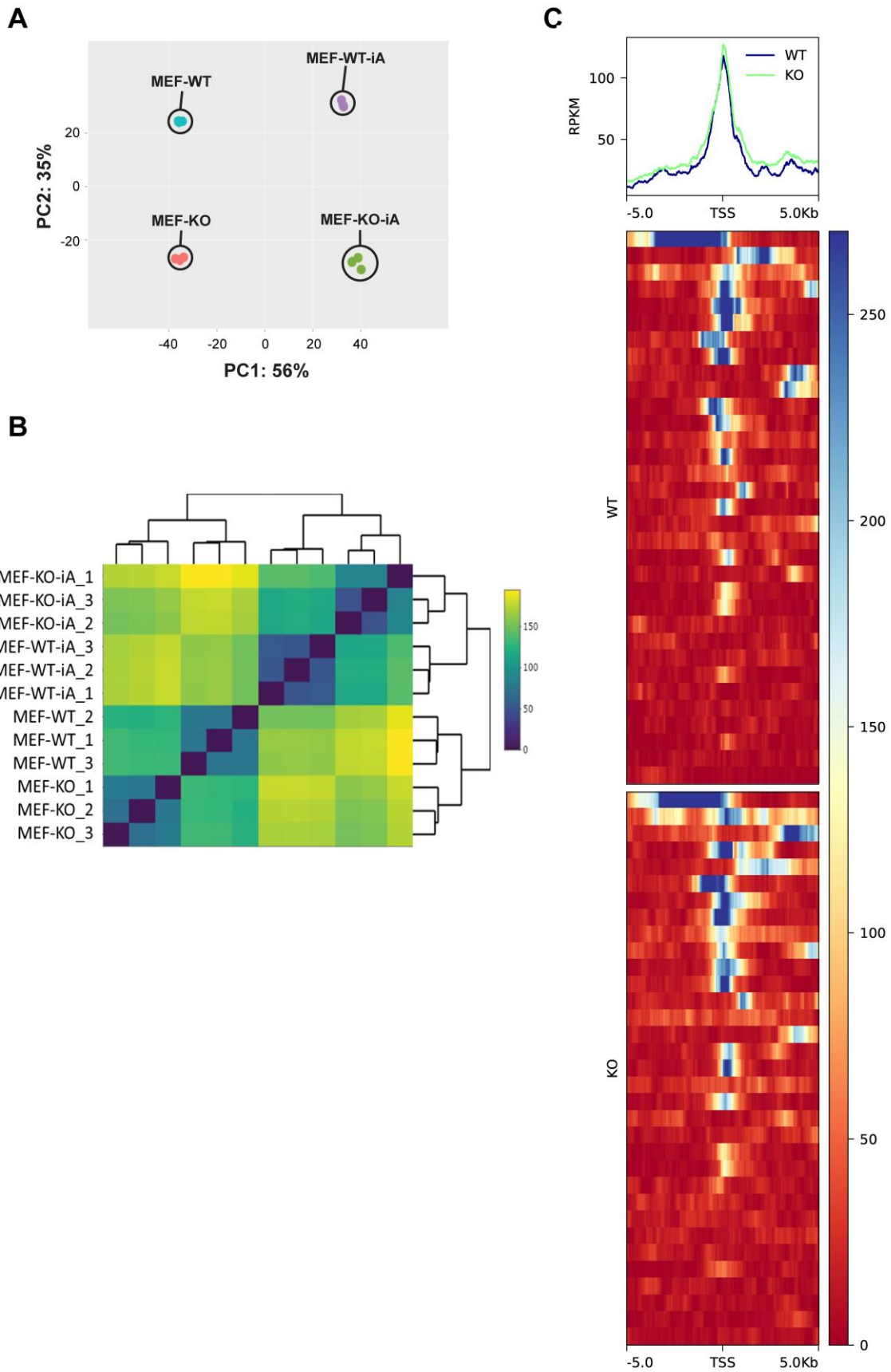
# Supplemental Materials

*Molecular Biology of the Cell*

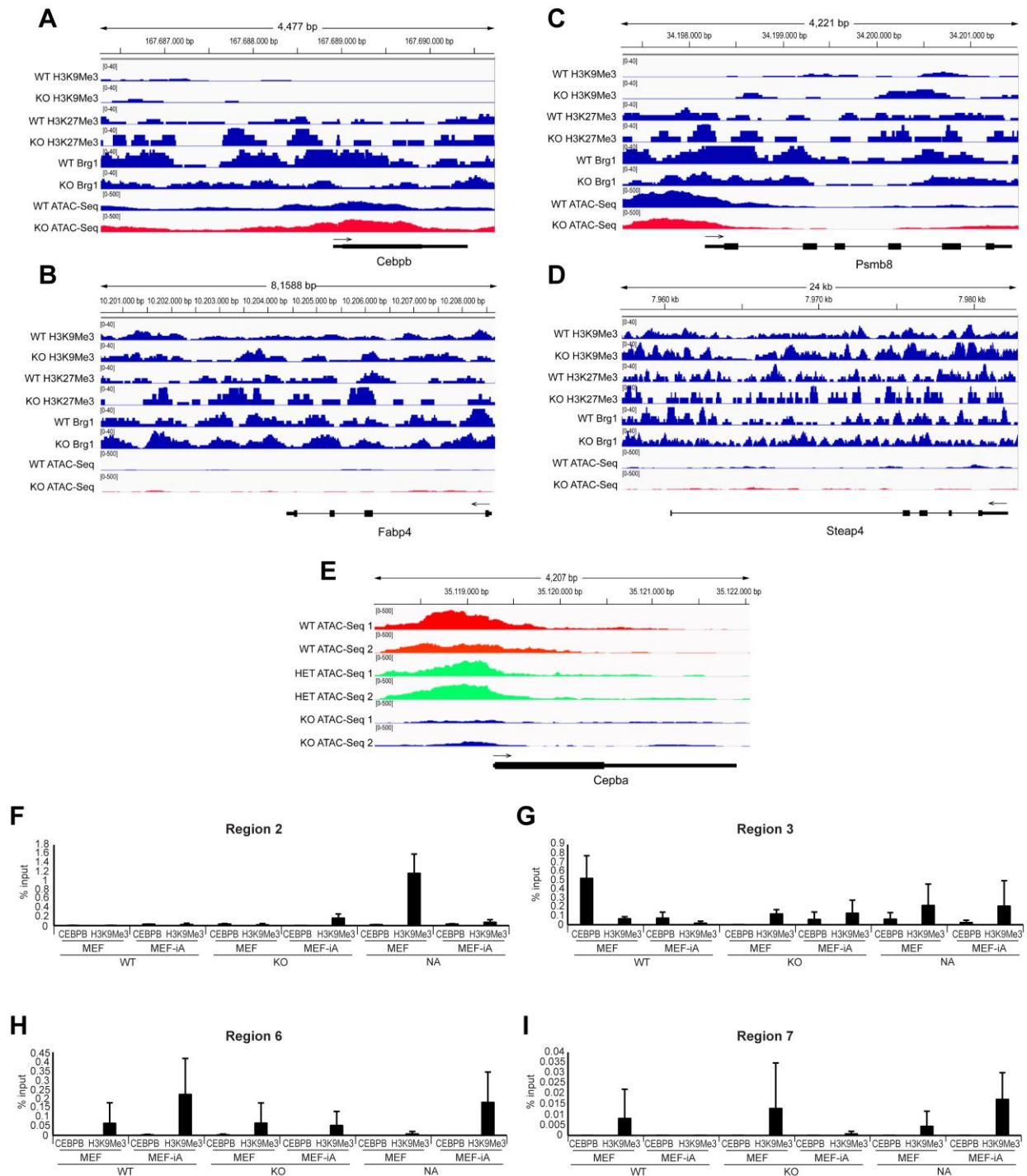
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## **SUPPLEMENTAL INFORMATION**

### **Legends to supplemental figures and tables**



**Figure S1. Correlation analysis on the effect of differentiation in WT and KO conditions and analysis of chromatin accessibility on differentially expressed genes involved in adipocyte differentiation.** (A) Principal component analysis (PCA) illustrating total transformed variances including PC1 (48%) and PC2 (21%) for WT and KO MEF and MEF-iA condition. (B) A complementary distance heatmap representation of PCA illustrating high level of clustering based mainly on differentiation treatment. Clustering is based on CV of gene expression count levels of three biological replicates (n=3). (C) Transcription start sites of differentially expressed genes do not show significant difference in average chromatin accessibility between WT and KO cells. Density map of ATAC-seq reads  $\pm 5$  kb of TSSs of all 33 genes involved in fat cell differentiation. Top: Mean ATAC-Seq signal  $\pm 5$  kb around TSSs. TSS: Transcription start site. Scale bar: normalized RPKM. ATAC-seq profile data for each assay are presented as the mean of two biologically independent replicates (n=2).



**Figure S2.  $\beta$ -actin dependent changes in chromatin accessibility are *Cebpa* specific and not observed in *Cebpb*, *Fabp4* and in the downregulated genes *Psmb8* or *Steap4*.** ChIP-seq profiles of H3K9Me3, H3K27Me3, Brg1 and ATAC-Seq profiles of WT and KO cells around

the *Cebpb* (A) and *Fabp4* (B). ChIP-seq profiles of H3K9Me3, H3K27Me3, Brg1 and ATAC-Seq profiles of WT and KO cells around the *Psmb8* (A) and *Steap4* (B) genes. The y-axis data range represents RPKM (Reads Per Kilobase of sequence range per Million mapped reads) per bin. The y-axis of tracks in the same image were set to the same range. Gene body position (exon: box, intron: line) shown below the tracks. (E) ATAC-Seq profiles of WT (WT ATAC-Seq 1 and WT ATAC-Seq 2) and KO MEF (KO ATAC-Seq 1 and KO ATAC-Seq 2) biological replicas around the *Cebpa* gene. The y-axis data range represents RPKM (Reads Per Kilobase of sequence range per Million mapped reads) per bin with scale range shown in parentheses. Gene body position (exon: box, intron: line) shown below the tracks. ATAC-seq profile data for each assay are presented as individual points consisting of two biologically independent replicates (n=2). (F-I) Results from ChIP experiments on MEFs and corresponding induced adipocytes (MEF-iA) in the wild type and knockout condition as well as in knockout cells expressing  $\beta$ -actin in the cell nucleus. For these experiments, antibodies against CEBPB and H3K9Me3 were used and analysis was performed by qPCR with primers amplifying regions R2 and R3 within the regulatory region of the *Cebpa* gene, R6 located within the *Cebpa* gene and R7 located further upstream the regulatory region of the gene within the *Cebpa* gene regulatory. Results from these experiments show that CEBPB does not bind to these regions during adipogenesis. See main Figure 4, panel A, for the location of the R1-R7 regions amplified by qPCR. The analysis was normalized against % input (n=3; \*P-Value<0.05, \*\*P-Value<0.01). CV for all three replicates are presented as mean value with error bars representing SD. Significance t-test (P-Value) were performed based a one-tailed hypothesis.

**Table 1S.** Differentially expressed genes in MEF-KO/MEF-WT under GO:0045600~positive regulation of fat cell differentiation and GO:0060612~adipose tissue development. Data presented are based on three biological replicates (n=3).

**Table 2S.** Differentially expressed genes in MEF-WT-iA/MEF-WT and MEF-KO-iA/MEF-KO under GO:0045444~fat cell differentiation. Data presented are based on three biological replicates (n=3).

**Table 1.S Differentially expressed genes in MEF-KO/MEF-WT**

(A) GO:0045600~positive regulation of fat cell differentiation

<b><u>Ensemble ID</u></b>	<b><u>Gene ID</u></b>	<b><u>log2FoldChange</u></b> <b><u>(MEF-KO/MEF-WT)</u></b>	<b><u>padi</u></b> <b><u>(MEF-KO/MEF-WT)</u></b>
ENSMUSG00000000440	<i>Pparg</i>	1.34868566	1.19E-11
ENSMUSG00000042677	<i>Zc3h12a</i>	1.5549223	0.00000092
ENSMUSG00000021127	<i>Zfp361l</i>	1.14045465	0.0000839
ENSMUSG00000027358	<i>Bmp2</i>	-3.4668085	8.97E-20
ENSMUSG00000034997	<i>Htr2a</i>	6.43577755	0.00000124
ENSMUSG00000020053	<i>Igf1</i>	-1.4945989	4.33E-12
ENSMUSG00000018209	<i>Stk4</i>	-0.5643366	0.00000572
ENSMUSG00000020218	<i>Wif1</i>	-3.9588552	0.00034309
ENSMUSG00000021067	<i>Sav1</i>	0.44691109	0.00206445
ENSMUSG00000040711	<i>Sh3pxd2b</i>	0.68186279	6.70E-12
ENSMUSG00000030170	<i>Wnt5b</i>	-4.649544	3.38E-09
ENSMUSG00000031548	<i>Sfrp1</i>	-2.6152183	1.90E-24
ENSMUSG00000022111	<i>Uchl3</i>	-0.9220723	2.24E-09
ENSMUSG00000027004	<i>Frzb</i>	-4.7464187	0.00696555
ENSMUSG00000024913	<i>Lrp5</i>	-0.9176541	0.0000326
ENSMUSG00000001729	<i>Akt1</i>	0.74545534	0.0008639
ENSMUSG00000035642	<i>Aamd</i>	-0.9538121	0.00248618
ENSMUSG00000042190	<i>Cmklr1</i>	1.40311632	0.00179365
ENSMUSG00000020644	<i>Id2</i>	1.25934194	9.45E-18
ENSMUSG00000043252	<i>Tmem64</i>	1.09027204	4.39E-14
ENSMUSG00000029659	<i>Medag</i>	3.17432793	2.07E-24

(B) GO:0060612~adipose tissue development

<b><u>Ensemble ID</u></b>	<b><u>Gene ID</u></b>	<b><u>log2FoldChange</u></b> <b><u>(MEF-KO/MEF-WT)</u></b>	<b><u>padi</u></b> <b><u>(MEF-KO/MEF-WT)</u></b>
ENSMUSG00000029167	<i>Ppargc1a</i>	3.27872573	2.05E-17
ENSMUSG00000058881	<i>Zfp516</i>	0.67114812	0.00051125
ENSMUSG00000040711	<i>Sh3pxd2b</i>	0.68186279	6.70E-12
ENSMUSG00000028150	<i>Rorc</i>	3.15213294	1.55E-10
ENSMUSG00000006728	<i>Cdk4</i>	0.45344491	0.00010043
ENSMUSG00000019947	<i>Arid5b</i>	0.87606858	4.03E-10
ENSMUSG00000024913	<i>Lrp5</i>	-0.9176541	0.0000326
ENSMUSG00000056758	<i>Hmga2</i>	2.21831473	2.06E-59
ENSMUSG00000019505	<i>Ubb</i>	-1.0181737	2.73E-13
ENSMUSG00000022053	<i>Ebf2</i>	-4.0170838	5.31E-08
ENSMUSG00000022186	<i>Oxct1</i>	0.4873298	0.0000596
ENSMUSG00000020644	<i>Id2</i>	1.25934194	9.45E-18



ENSMUSG00000037270	<i>4932438A13Rik</i>	-0.5678336	0.00041071
ENSMUSG00000032047	<i>Acat1</i>	-0.5525831	0.0000609

**Table 2. S Differentially expressed genes in MEF-WT-iA/MEF-WT and MEF-KO-iA/MEF-KO under GO:0045444~fat cell differentiation**

EsembleID	GeneID	log2FoldChange (MEF-WT-iA/MEF-WT)	padj (MEF-WT-iA/MEF-WT)	log2FoldChange (MEF-KO-iA/MEF-KO)	padj (MEF-KO-iA/MEF-KO)
ENSMUSG00000000440	<i>Pparg</i>	0.51786289	0.00942606	-0.076585	0.79773526
ENSMUSG00000003228	<i>Grk5</i>	0.6613539	0.0000679	1.91050874	9.58E-10
ENSMUSG000000038539	<i>Atf5</i>	2.65873477	2.4E-52	0.68590505	0.00274475
ENSMUSG000000089665	<i>Fcor</i>	2.23656316	0.0000295	2.96478959	0.00016277
ENSMUSG000000024338	<i>Psmb8</i>	1.01433206	6.94E-09	3.54056955	3.18E-13
ENSMUSG000000003032	<i>Klf4</i>	2.10870806	1.42E-12	-0.4916305	0.03141769
ENSMUSG000000063810	<i>Alms1</i>	0.52772706	0.00568059	-0.0212573	0.94802479
ENSMUSG000000056492	<i>Adgrf5</i>	-2.0346365	0.00055821	2.98809205	0.0000605
ENSMUSG000000031755	<i>Bbs2</i>	0.30561865	0.06190752	1.10772403	0.0000471
ENSMUSG000000023034	<i>Nr4a1</i>	1.74224129	4.33E-11	1.94871999	0.00216802
ENSMUSG000000051675	<i>Trim32</i>	0.60606065	0.0000156	0.3730978	0.09127414
ENSMUSG000000021379	<i>Id4</i>	1.87359404	0.0000496	2.46133238	0.00000133
ENSMUSG000000034957	<i>Cebpa</i>	3.36178146	4.64E-15	0.35130934	0.83710212
ENSMUSG000000038037	<i>Socs1</i>	2.3520802	1.14E-08	2.79174612	0.000000687
ENSMUSG000000032405	<i>Pias1</i>	-0.0257479	0.87261186	0.70991335	0.00451748
ENSMUSG000000071637	<i>Cebpd</i>	5.34031462	1.16E-18	4.51279253	8.29E-18
ENSMUSG000000037868	<i>Egr2</i>	2.35876057	0.00057074	0.59950705	0.39259965
ENSMUSG000000024176	<i>Sox8</i>	1.35441719	0.01113285	4.94458324	0.001218
ENSMUSG000000022428	<i>Cbyl</i>	1.02171115	4.10E-12	0.74077583	0.0000388
ENSMUSG000000014763	<i>Fam120b</i>	0.48596971	0.0000139	0.0679004	0.86116408
ENSMUSG000000036867	<i>Smad6</i>	2.43417555	1.1E-20	1.44037089	0.00000121
ENSMUSG000000020892	<i>Aloxe3</i>	1.0574964	0.00543788	1.03145657	0.16016254
ENSMUSG000000029076	<i>Sdf4</i>	0.04750651	0.7278131	0.50183989	0.0000254
ENSMUSG000000056501	<i>Cebpb</i>	4.65172726	7.58E-98	4.4640503	4.48E-53
ENSMUSG000000071657	<i>Bscl2</i>	1.73134079	1.18E-17	-0.0439241	0.88174368
ENSMUSG000000027274	<i>Mkks</i>	0.27857214	0.21307337	0.50051426	0.00262598
ENSMUSG000000048040	<i>Arxes2</i>	-1.1563253	0.00141289	1.05430579	0.00040725
ENSMUSG000000037270	<i>4932438A13Rik</i>	-0.6755517	0.0014751	0.63768847	0.00030112

ENSMUSG00000054434	<i>Tmem120b</i>	0.4223289	0.034537	1.03916588	0.00236029
ENSMUSG00000051177	<i>Plcb1</i>	2.42149652	1.83E-16	0.95891963	0.00000408
ENSMUSG00000039886	<i>Tmem120a</i>	0.69272148	1.08E-08	0.16022916	0.36102691
ENSMUSG00000012428	<i>Steap4</i>	4.1155451	3.59E-34	2.71783163	0.01874944
ENSMUSG00000006464	<i>Bbs1</i>	0.80670504	0.00020617	0.20377535	0.64370011