

Supplementary information for

Demethylase-independent roles of LSD1 in regulating enhancers and cell fate transition

Cheng Zeng, Jiwei Chen, Emmalee W. Cooke, Arijita Subuddhi, Eliana T. Roodman, Fei Xavier Chen, and

Kaixiang Cao

Correspondence should be sent to the following address:

Kaixiang Cao, PhD

Assistant Professor

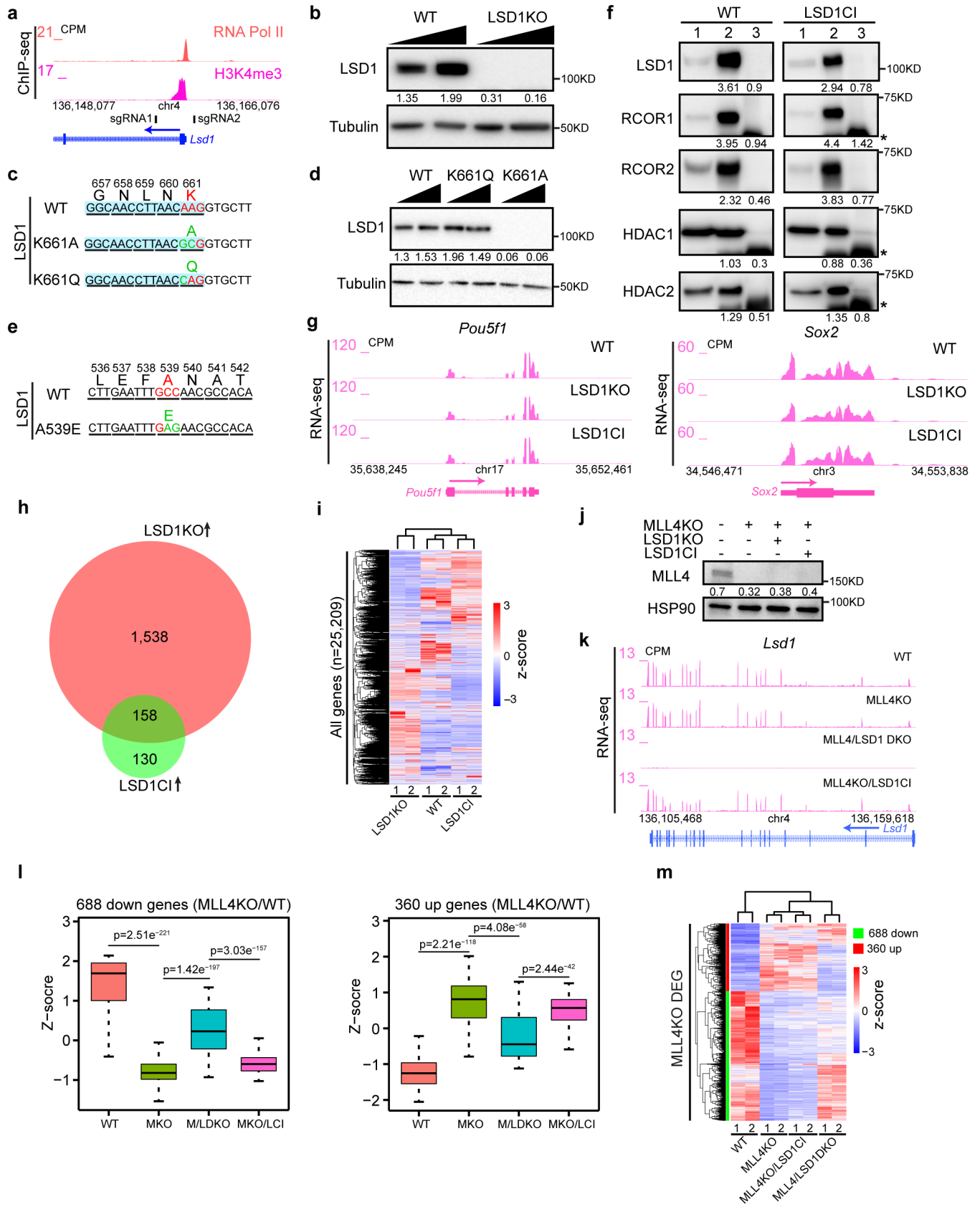
Department of Biochemistry, Case Western Reserve University

10900 Euclid Avenue, Cleveland, OH 44106, USA

Office: (216) 368-1218

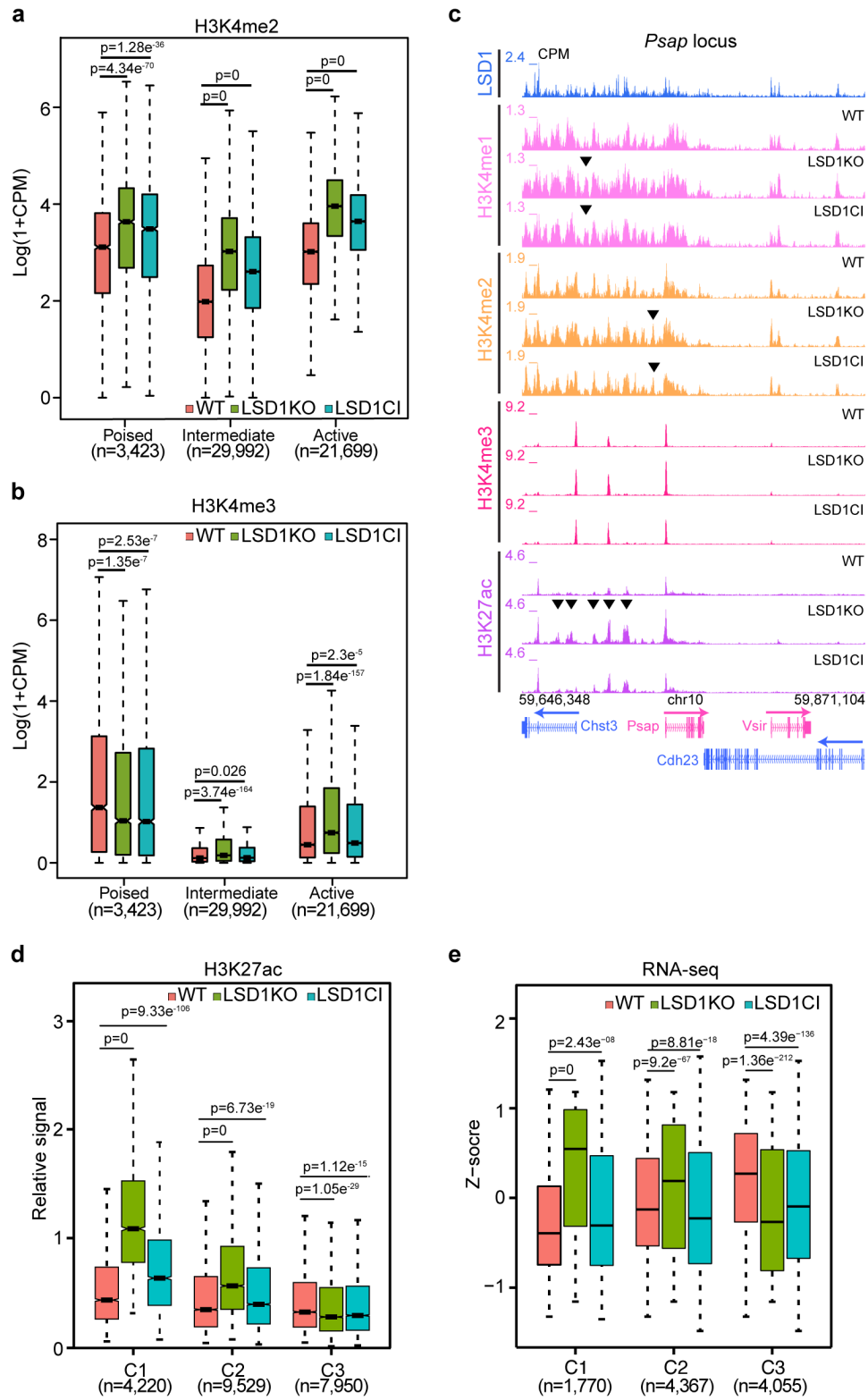
Email: kxc725@case.edu

Supplementary information includes 8 Supplementary Figures.



Supplementary Figure 1. Generation and characterization of LSD1 KO and LSD1 CI ESCs.

- (a) The genome location of sgRNAs used to delete LSD1.
- (b) Western blotting showing the loss of LSD1 in LSD1 KO ESCs. Experiments were repeated three times independently with similar results observed. Source data are provided as a Source Data file.
- (c) Sequences of the WT, LSD1 K661A, and K661Q mutant alleles. The exonic region is highlighted in blue.
- (d) Western blotting indicating LSD1 levels in WT, K661Q, and K661A ESCs. Experiments were repeated three times independently with similar results observed. Source data are provided as a Source Data file.
- (e) Sequences of the WT and LSD1 A539E alleles.
- (f) Western blotting indicating WT and K661Q/A539E mutant LSD1 co-immunoprecipitate (co-IP) with CoREST components RCOR1, RCOR2, HDAC1, and HDAC2. Lane 1: 4% input; lane 2: LSD1 IP; lane 3: IgG IP. *: IgG heavy chain. LSD1 IP and IgG IP lanes were quantified by normalizing with the corresponding input lanes. Experiments were repeated three times independently with similar results observed. Source data are provided as a Source Data file.
- (g) Genome browser view of RNA-seq signals at *Pou5f1* and *Sox2* genes in WT, LSD1 KO and LSD1 CI ESCs.
- (h) Venn diagram indicating the overlap between genes upregulated in LSD1 KO and CI ESCs.
- (i) Hierarchical clustering analysis of expression of all 25,209 genes comparing WT, LSD1 KO, and LSD1 CI ESCs. Numbers below the heatmap denote the 2 biological replicates of each genotype.
- (j) Western blotting indicating the loss of MLL4 in MLL4KO, LSD1/MLL4 DKO, and LSD1 CI/MLL4 KO ESCs. Experiments were repeated three times independently with similar results observed. Source data are provided as a Source Data file.
- (k) Genome browser view of RNA-seq signals at LSD1 gene in cells mentioned in (j).
- (l) Box plots quantifying the levels of genes downregulated (left) and upregulated (right) by MLL4 deletion in labelled cells. MKO: MLL4KO, M/LDKO: MLL4/LSD1 DKO, MKO/LCI: MLL4KO/LSD1CI. n=2 biologically independent experiments. P-values were calculated using two-sided Wilcoxon signed-rank tests. Center line: median; top and bottom hinges of box: the third and first quartiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range.
- (m) Hierarchical clustering analysis of expression levels of the differentially regulated genes in MLL4 KO cells comparing WT, MLL4 KO, MLL4/LSD1 DKO, and MLL4KO/LSD1CI ESCs. Numbers below the heatmap denote the 2 biological replicates of each genotype.



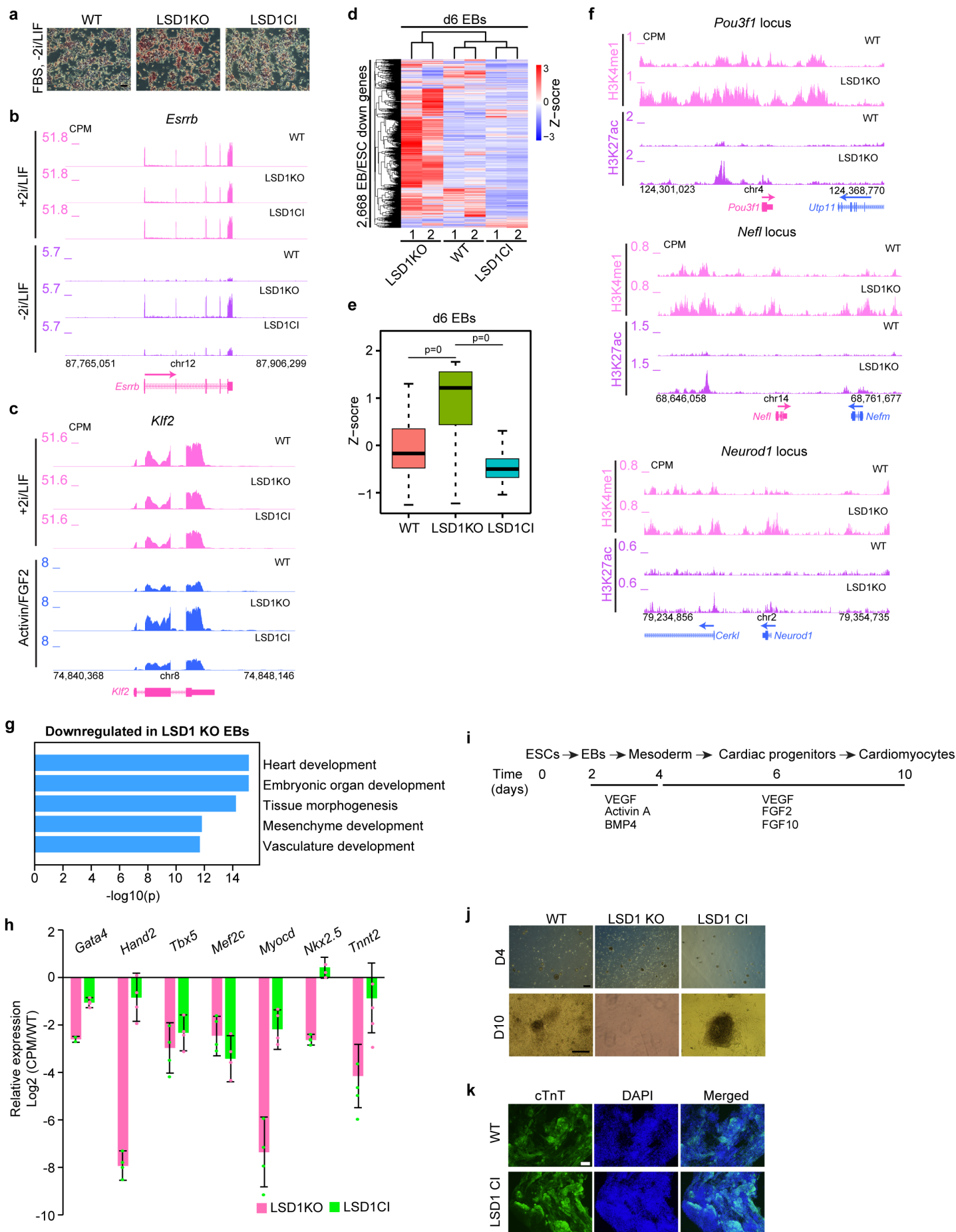
Supplementary Figure 2. The impact of LSD1 deletion and catalytic inactivation on enhancer histone modifications.

(a-b) Box plots of H3K4me2 (a) and H3K4me3 (b) levels at LSD1 bound enhancers in WT, LSD1 KO, and LSD1 CI cells. 3,423 poised, 29,992 intermediate, and 21,699 active enhancers were called based on H3K4me1, H3K27ac, H3K27me3, and LSD1 ChIP-Rx data. n=2 biologically independent experiments. P-values (p) from two-sided Wilcoxon signed-rank tests on $\log_2(\text{LSD1KO/WT})$ and $\log_2(\text{LSD1CI/WT})$ are denoted in each panel. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range.

(c) Genome browser view of H3K4me1/2/3 and H3K27ac ChIP-Rx signals at the *Psap* locus in WT and LSD1 KO, and LSD1 CI ESCs. Black arrows indicate changes between LSD1 mutant and WT cells.

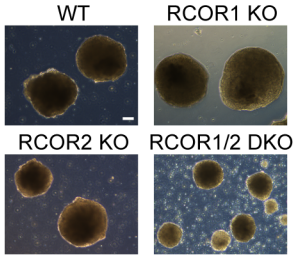
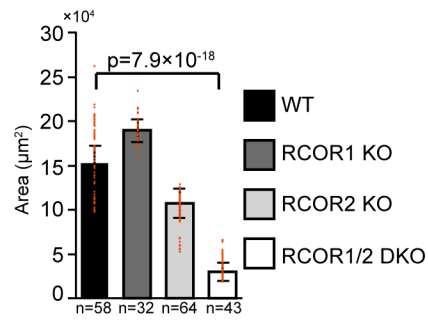
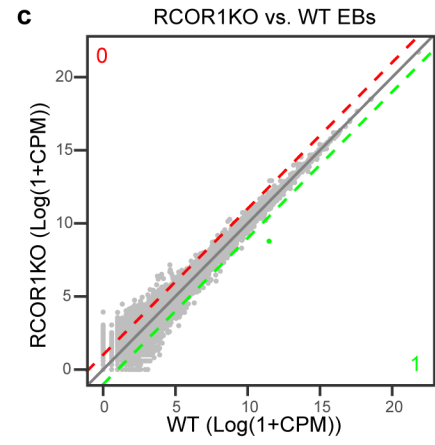
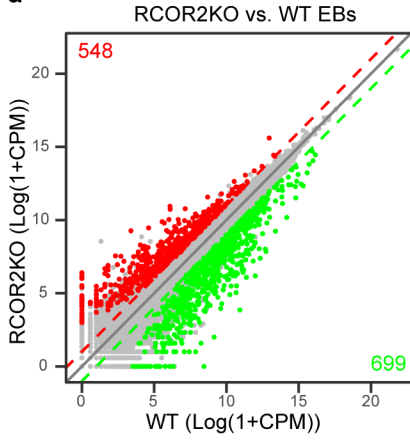
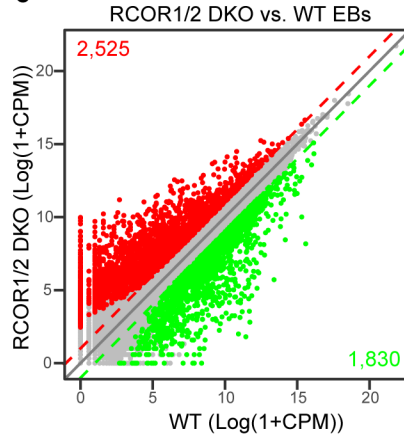
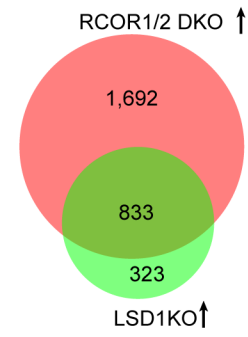
(d) Box plots showing H3K27ac levels of WT, LSD1 KO, and LSD1 CI ESCs at the three clusters of LSD1 bound active enhancers in Fig. 2e. P-values were calculated using two-sided Wilcoxon signed-rank tests. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range.

(e) Box plots showing RNA-seq signals in WT, LSD1 KO, and LSD1 CI ESCs of nearest gene to the three clusters of LSD1 bound active enhancers in Fig. 2e. Z-scores were used to generate the heat map. P-values were calculated using two-sided Wilcoxon signed-rank tests. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range.



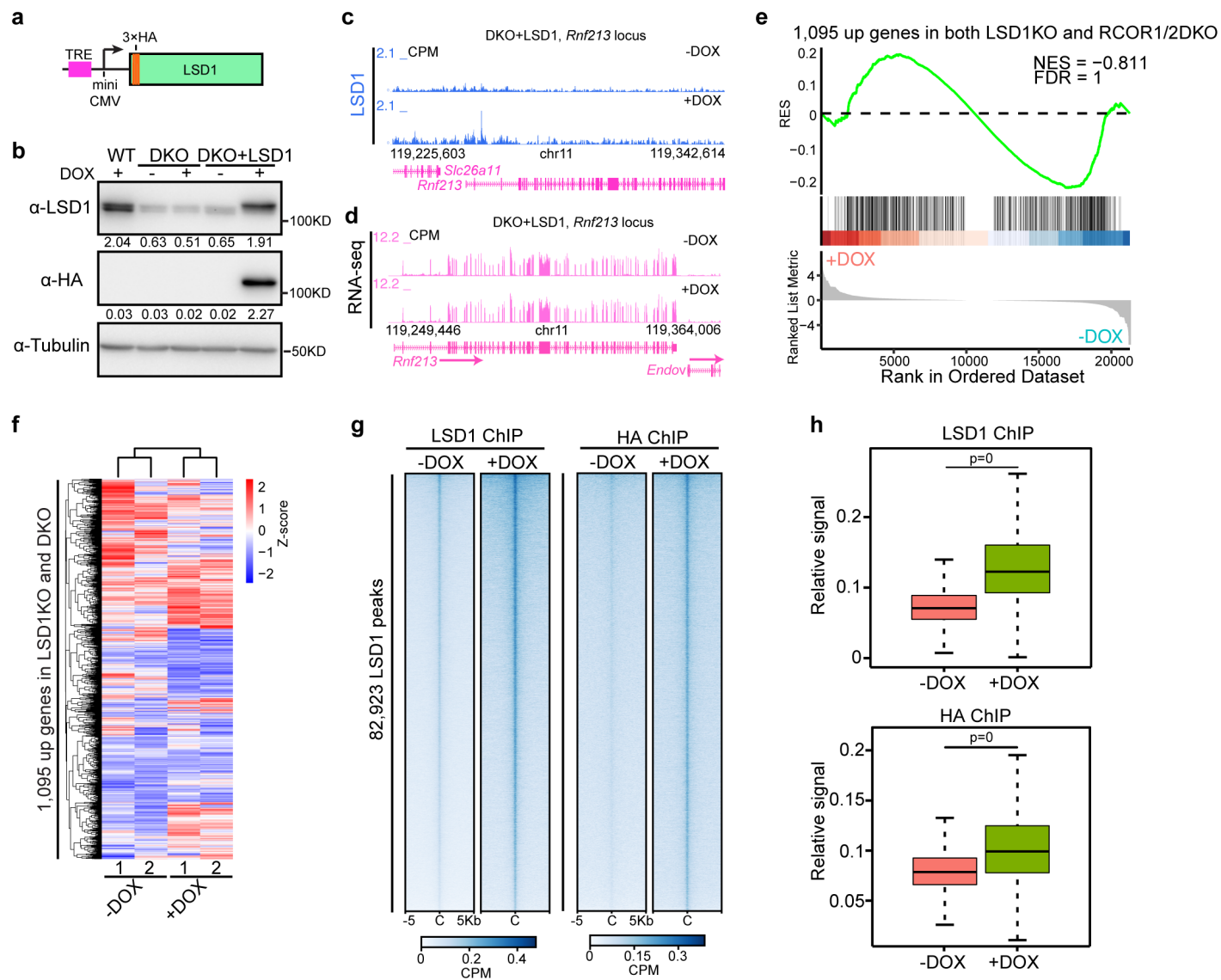
Supplementary Figure 3. The impact of LSD1 deletion and inactivation on ESC differentiation.

- (a) AP staining of WT, LSD1 KO, and LSD1 CI cells two passages after 2i/LIF withdrawal. Experiments were repeated three times independently with similar results observed. Scale bar: 100µm.
- (b) Genome browser view of RNA-seq signals at *Esrrb* gene in WT and LSD1 mutants under naive state (top) and spontaneous differentiation (bottom).
- (c) Genome browser view of RNA-seq signals at *Klf2* gene in WT and LSD1 mutants under naive (top) and primed (bottom) states.
- (d-e) Hierarchical clustering (d) and box plot (e) analyses of the expression levels of 2,668 genes downregulated during EB differentiation in day 6 WT, LSD1 KO, and LSD1 CI EBs. P-values in (e) were calculated using two-sided Wilcoxon signed-rank tests. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range.
- (f) Genome browser view of H3K4me1 and H3K27ac levels in WT and LSD1 KO EBs at neural marker gene loci.
- (g) GO analysis of genes downregulated in LSD1 KO day 6 EBs vs. WT EBs.
- (h) Fold change of RNA-seq signals (CPM) in LSD1 KO or LSD1 CI over WT EBs is shown for cardiac marker genes. n=2 biologically independent experiments. Data are presented as mean values $\pm$ SD. Source data are provided as a Source Data file.
- (i) The cardiac differentiation strategy.
- (j) Phase contrast images of day 4 (top) and day 10 (bottom) cardiac differentiation cultures of WT, LSD1 KO, and LSD1 CI cells. Experiments were repeated three times independently with similar results observed. Scale bar: 250µm.
- (k) Immunostaining images of WT and LSD1 CI cardiac differentiation cultures at day 10. Cells were stained with anti-cTnT antibodies and DAPI. Experiments were repeated three times independently with similar results observed. Scale bar: 100µm.

a**b****c****d****e****f**

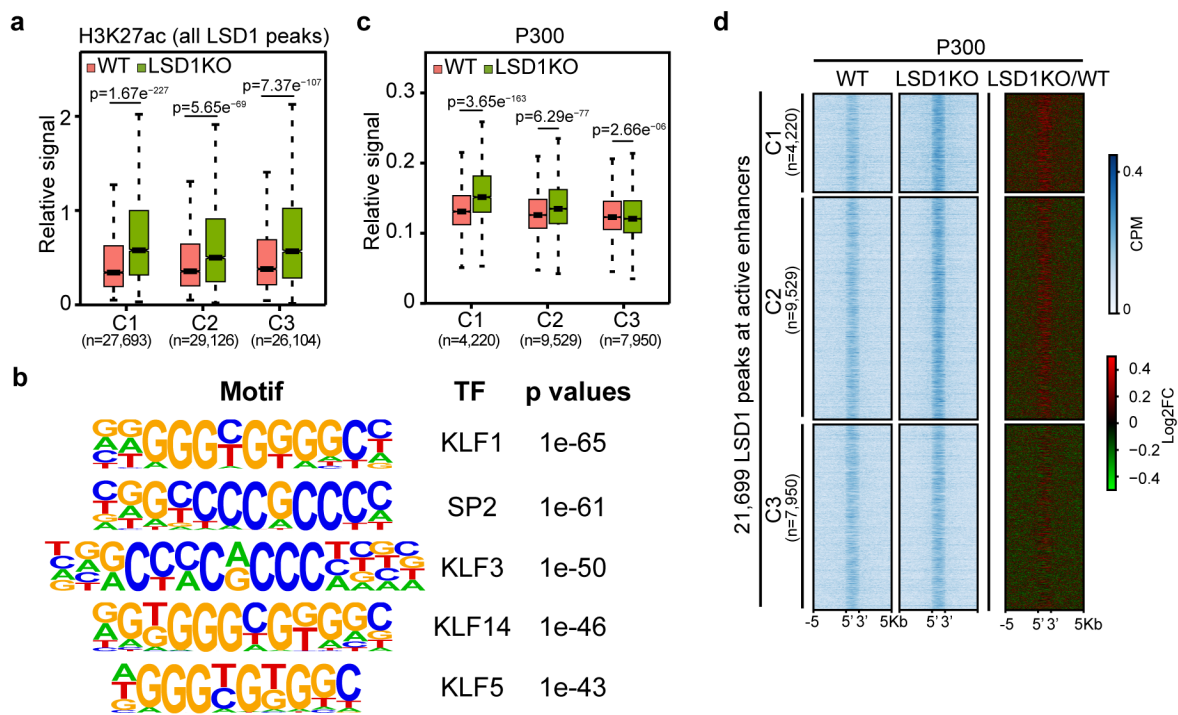
Supplementary Figure 4. RCOR1/2 deletion impairs EB differentiation.

- (a) Phase contrast images of day 6 EBs generated from WT, RCOR1 KO, RCOR2 KO, and RCOR1/2 DKO ESCs. Experiments were repeated three times independently with similar results observed. Scale bar: 100 μ m.
- (b) Quantification of EB sizes in (a). Data are presented as mean values $\pm$ SD. n=3 biologically independent experiments. P-values were calculated using two-sided student's t-test. Source data are provided as a Source Data file.
- (c-e) Correlation plots of RNA-seq data between RCOR1 KO (c), RCOR2 KO (d), and RCOR1/2 DKO (e) EBs and WT EBs. Statistical significance was determined by two-sided Wald test and p-values were corrected for multiple testing using the Benjamini-Hochberg method. Significantly up- and downregulated genes are labelled in red and green with numbers of genes noted, respectively.
- (f) Venn diagram showing the overlap of upregulated genes between LSD1 KO and RCOR1/2 DKO EBs compared with WT EBs.



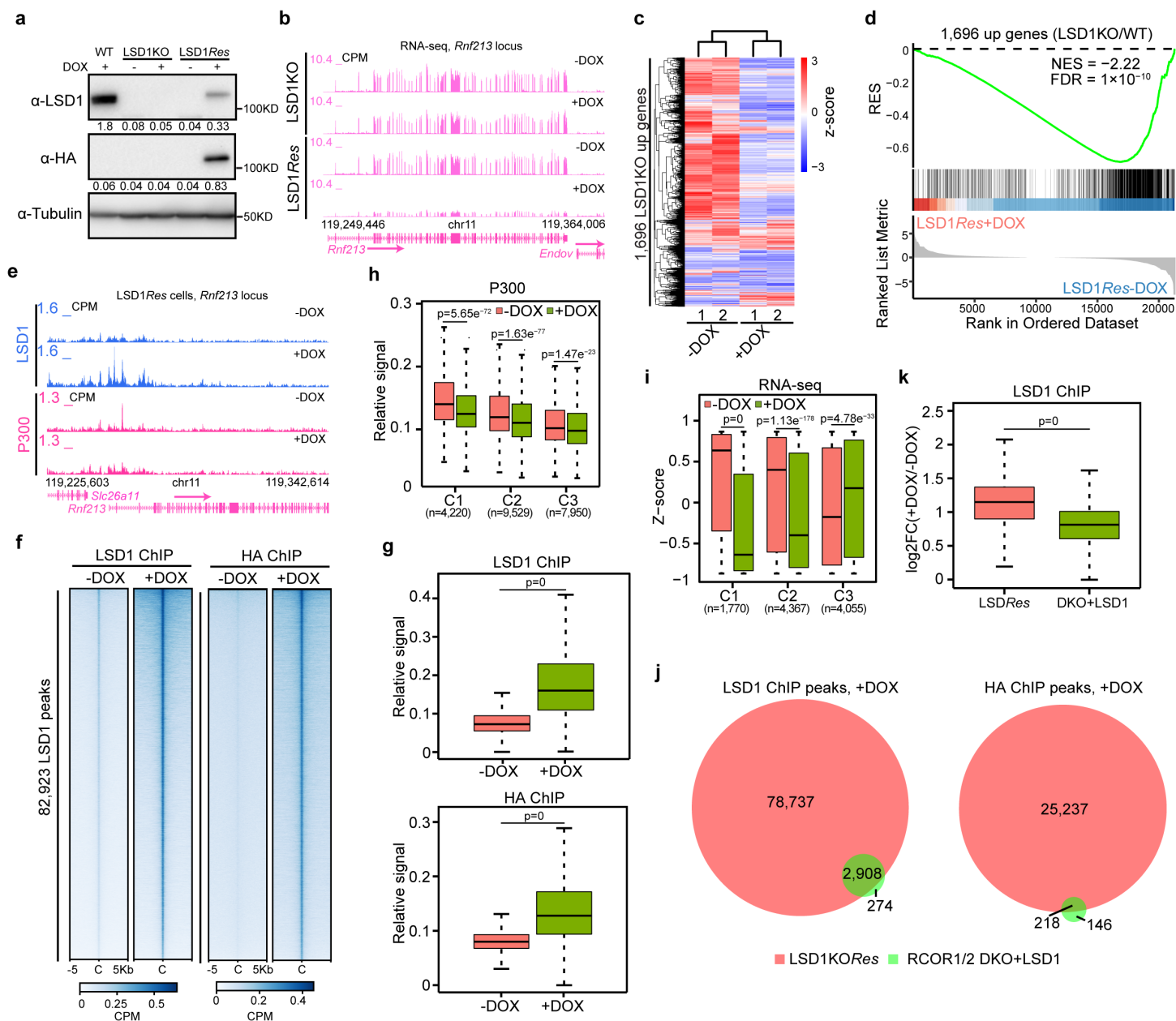
Supplementary Figure 5. LSD1 restoration has no significant impact on rescuing de-repressed genes in CoREST null cells.

- (a) Schematic representation of the doxycycline (DOX) inducible LSD1 overexpressing system. TRE: Tetracycline response element.
- (b) Western blotting analysis of DKO+LSD1 cells with and without DOX induction. WT and RCOR1/2 DKO cell lysates served as controls. Levels of LSD1 and HA-LSD1 in each lane were quantified by normalizing with Tubulin signals of the corresponding lane. Normalized ratios were provided under each blot. Experiments were repeated three times independently with similar results observed. Source data are provided as a Source Data file.
- (c) Genome browser view of LSD1 ChIP-Rx signals at the *Rnf213* locus in DKO+LSD1 cells with and without DOX induction.
- (d) Genome browser view of RNA-seq signals of *Rnf213* gene in DKO+LSD1 cells with and without DOX induction.
- (e) GSEA analysis of 1,095 genes upregulated by both LSD1 and RCOR1/2 loss in the right panel of Fig. 4f comparing DKO+LSD1 cells with and without DOX induction.
- (f) Hierarchical analysis of 1,095 genes upregulated by both LSD1 and RCOR1/2 loss in DKO+LSD1 cells with and without DOX induction. Numbers below the heatmap denote the 2 biological replicates of each condition.
- (g) Heatmap analysis showing LSD1 ChIP-Rx (left) and HA ChIP-seq (right) signals in DKO+LSD1 cells with and without DOX induction. Signals were centered on total LSD1 peaks and the profiles were sorted in a descending order of LSD1 occupancy in WT ESCs.
- (h) Box plot analysis of LSD1 ChIP-Rx (top) and HA ChIP-seq (bottom) signals in DKO+LSD1 cells with and without DOX induction at 82,923 LSD1 peaks. n=2 biologically independent experiments. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated with two-sided Wilcoxon signed-rank tests.



Supplementary Figure 6. LSD1 deletion leads to increased P300 recruitment to enhancers.

- (a) Box plot analysis of H3K27ac levels in WT and LSD1 KO ESCs at all 82,923 LSD1 peaks according to the three clusters in Fig. 5b. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated with two-sided Wilcoxon signed-rank tests.
- (b) Top transcription factors motifs enriched in Cluster 1 of all 82,923 LSD1-enriched regions in Fig. 5b. Motif analysis was performed using Cluster 2 and 3 regions as controls. P-values were calculated using an one-tailed hypergeometric test.
- (c) Box plot analysis of P300 levels in WT and LSD1 KO ESCs at 21,699 LSD1 enriched active enhancers according to the three clusters in Fig. 2e. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated with two-sided Wilcoxon signed-rank tests.
- (d) Heatmap analysis indicating P300 occupancy (left) and log 2 fold change (right) at 21,699 LSD1 enriched active enhancers. Clusters are the same as in Fig. 2e.



Supplementary Figure 7. LSD1 re-introduction rescues gene de-repression caused by LSD1 loss in ESCs.

(a) Western blotting analysis of LSD1*Res* cells with and without DOX induction. WT and LSD1 KO cell lysates served as controls. Levels of LSD1 and HA-LSD1 in each lane were quantified by normalizing with Tubulin signals of the corresponding lane. Normalized ratios were provided under each blot. Experiments were repeated three times independently with similar results observed. Source data are provided as a Source Data file.

(b) Genome browser view of RNA-seq signals of *Rnf213* gene in LSD1 KO ESCs and LSD1*Res* cells with and without DOX treatment.

(c) Hierarchical clustering analysis of RNA-seq signals of 1,696 genes upregulated by LSD1 deletion in LSD1*Res* cells with and without DOX induction. Numbers below the heatmap denote the 2 biological replicates of each condition.

(d) GSEA analysis of 1,696 genes upregulated by LSD1 deletion comparing LSD1*Res* cells with and without DOX induction.

(e) Genome browser view of LSD1 (top) and P300 (bottom) ChIP-Rx signals at the *Rnf213* locus in LSD1*Res* cells with and without DOX induction.

(f) Heatmap analysis showing LSD1 ChIP-Rx (left) and HA ChIP-seq (right) signals in LSD1*Res* cells with and without DOX induction. Signals were centered on total LSD1 peaks and the profiles are sorted in a descending order of LSD1 occupancy in WT ESCs.

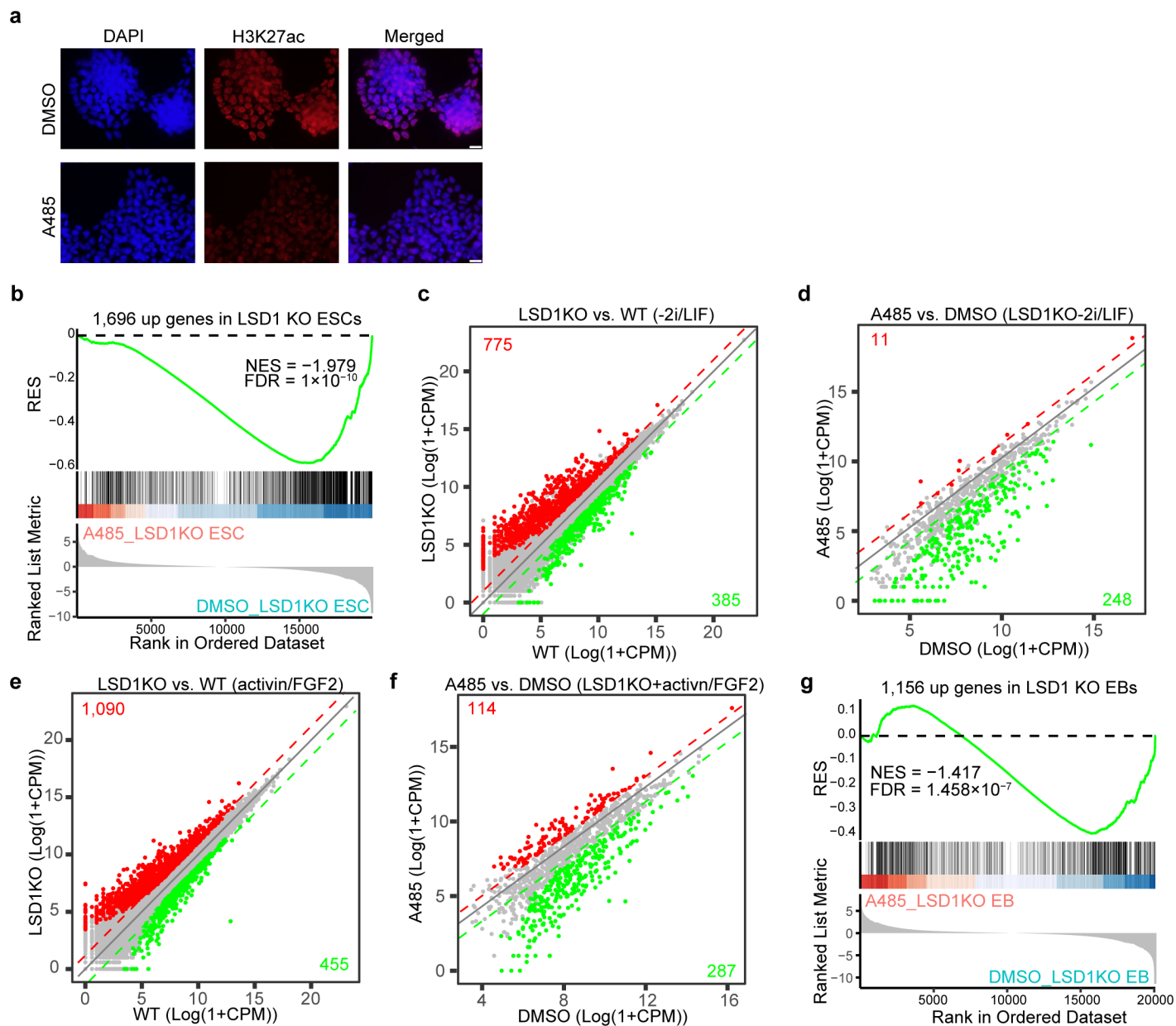
(g) Box plot analysis of LSD1 ChIP-Rx (top) and HA ChIP-seq (bottom) signals in LSD1*Res* cells with and without DOX induction at 82,923 LSD1 peaks. n=2 biologically independent experiments. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated using two-sided Wilcoxon signed-rank tests.

(h) Box plot analysis of P300 occupancy at 21,699 LSD1 enriched active enhancers in LSD1*Res* cells with and without DOX induction. n=2 biologically independent experiments. The three clusters are as in Fig. 2e with number of peaks in each cluster denoted. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated using two-sided Wilcoxon signed-rank tests.

(i) Box plot analysis of RNA-seq signals of nearest genes to LSD1 peaks at the three clusters in Fig. 2e in LSD1*Res* cells with and without DOX induction. n=2 biologically independent experiments. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated using two-sided Wilcoxon signed-rank tests.

(j) Venn diagrams indicating the overlap of LSD1 (left) and HA (right) ChIP-Rx peaks in LSD1*Res* and RCOR1/2 DKO+LSD1 cells upon DOX induction.

(k) Box plot analysis indicating the Log 2 fold change of LSD1 ChIP-Rx signals with and without DOX induction in LSD1*Res* and RCOR1/2 DKO+LSD1 cells. n=2 biologically independent experiments. Center line: median; top and bottom hinges of box: the third and first quantiles; whiskers: quartiles $\pm 1.5 \times$ interquartile range. P-values were calculated using two-sided Wilcoxon signed-rank tests.



Supplementary Figure 8. P300/CBP inhibition rescues gene misregulation caused by LSD1 loss.

- (a) Immunostaining of H3K27ac in ESCs treated with DMSO or 10 μ M A485 for 24 hours. Scale bar: 100 μ m. Experiments were repeated three times independently with similar results observed.
- (b) GSEA analysis of 1,696 genes upregulated by LSD1 deletion in ESCs comparing LSD1 KO ESCs treated with 10 μ M A485 and DMSO.
- (c) Correlation plots of RNA-seq data between LSD1 KO and WT ESCs under spontaneous differentiation conditions for two generations. Statistical significance was determined by two-sided Wald test and p-values were corrected for multiple testing using the Benjamini-Hochberg method. Significantly up- and downregulated genes are labelled in red and green with numbers of genes noted, respectively.
- (d) Correlation analysis of upregulated genes in LSD1 null ESCs upon spontaneous differentiation (775 red genes in c) treated with respective A485 and DMSO. Statistical significance was determined by two-sided Wald test and p-values were corrected for multiple testing using the Benjamini-Hochberg method. Significantly up- and downregulated genes are labelled in red and green with numbers of genes noted, respectively.
- (e) Correlation plots of RNA-seq data between LSD1 KO and WT ESCs under EpiLC differentiation. Statistical significance was determined by two-sided Wald test and p-values were corrected for multiple testing using the Benjamini-Hochberg method.
- (f) Correlation analysis of upregulated genes in LSD1 null ESCs upon EpiLC differentiation (1,090 red genes in e) treated with respective A485 and DMSO. Statistical significance was determined by two-sided Wald test and p-values were corrected for multiple testing using the Benjamini-Hochberg method. Significantly up- and downregulated genes are labelled in red and green with numbers of genes noted, respectively.
- (g) GSEA analysis of 1,156 genes upregulated by LSD1 deletion in EBs comparing LSD1 KO EBs treated with 0.4 μ M A485 and DMSO during differentiation.