

Figure S1

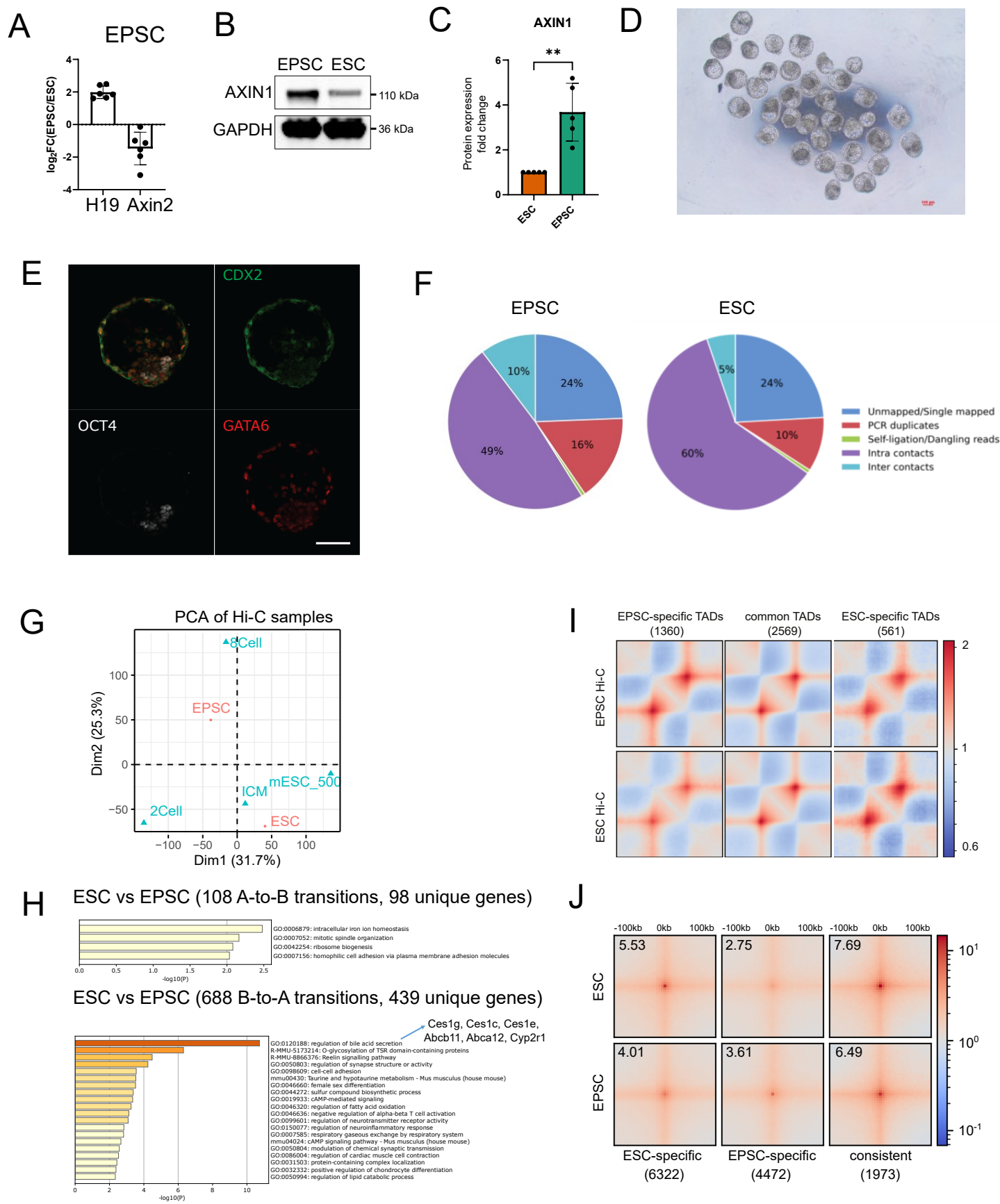


Fig. S1. Characterization of EPSC and quality control of Hi-C data

a, Characterization of EPSC upregulation marker (H19) and downregulation marker (Axin2) genes expression by RT-qPCR. **b**, Western blot showing the expression of AXIN1 (EPSC upregulation marker) protein in EPSC and ESC. **c**, Quantification of AXIN1 protein expression in ESC and EPSC from 3 independent experiments. Unpaired t-test was performed to determine significance. **d**, Representative image of blastoids formed from EPSC. Scalebar, 100 μ m. **e**, Immunofluorescence staining of EPSC-derived blastoid. CDX2, OCT4, and GATA6 were used as trophoctoderm marker, inner cell mass marker, and primitive endoderm marker, respectively. **f**, Percentage of read types from the Hi-C datasets. **g**, PCA analysis of Hi-C data generated from in-vitro cells (EPSC, ESC, mESC) and in-vivo embryos (2-cell, 8-cell, ICM). **h**, GO term enrichment analysis of genes located within A-to-B (top panel) and B-to-A differential compartments (bottom panel). **i**, Local rescaled pile-ups of TADs from 5kb resolution Hi-C data. TADs commonly shared or specific to a certain state were shown. **j**, Pileup plots of state-specific and consistent Hi-C interactions.

Figure S2

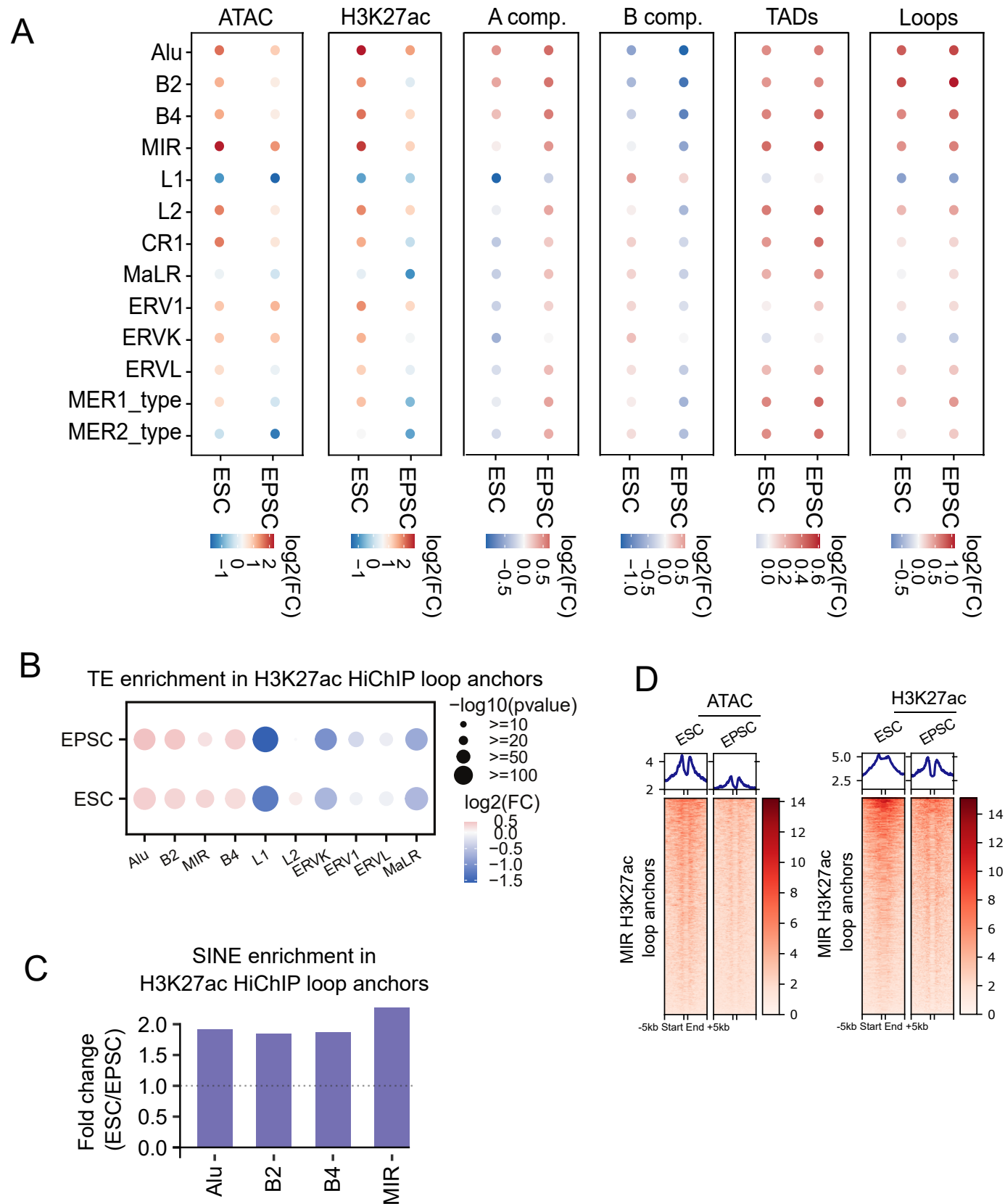


Fig. S2. TE family enrichment analysis in H3K27ac+Hi-C loop anchors and H3K27ac HiChIP loop anchors

a, TE family enrichment analysis in open chromatin regions (ATAC), enhancer regions (H3K27ac), A compartments, B compartments, TADs and loop anchors. Colour indicates the $\log_2$ fold change of enrichment. **b**, TE enrichment value in H3K27ac HiChIP loop anchors. The value represents the $\log_2$ fold change of loop counts with each TE family compared to the average loop counts with randomly sampled sequences with the same number of that TE family. **c**, Fold enrichment of SINE elements in H3K27ac HiChIP loop anchors of ESC compared to EPSC. **d**, Heatmap showing the ATAC-seq and H3K27ac signal on MIR elements located at H3K27ac HiChIP loop anchors.

Figure S3

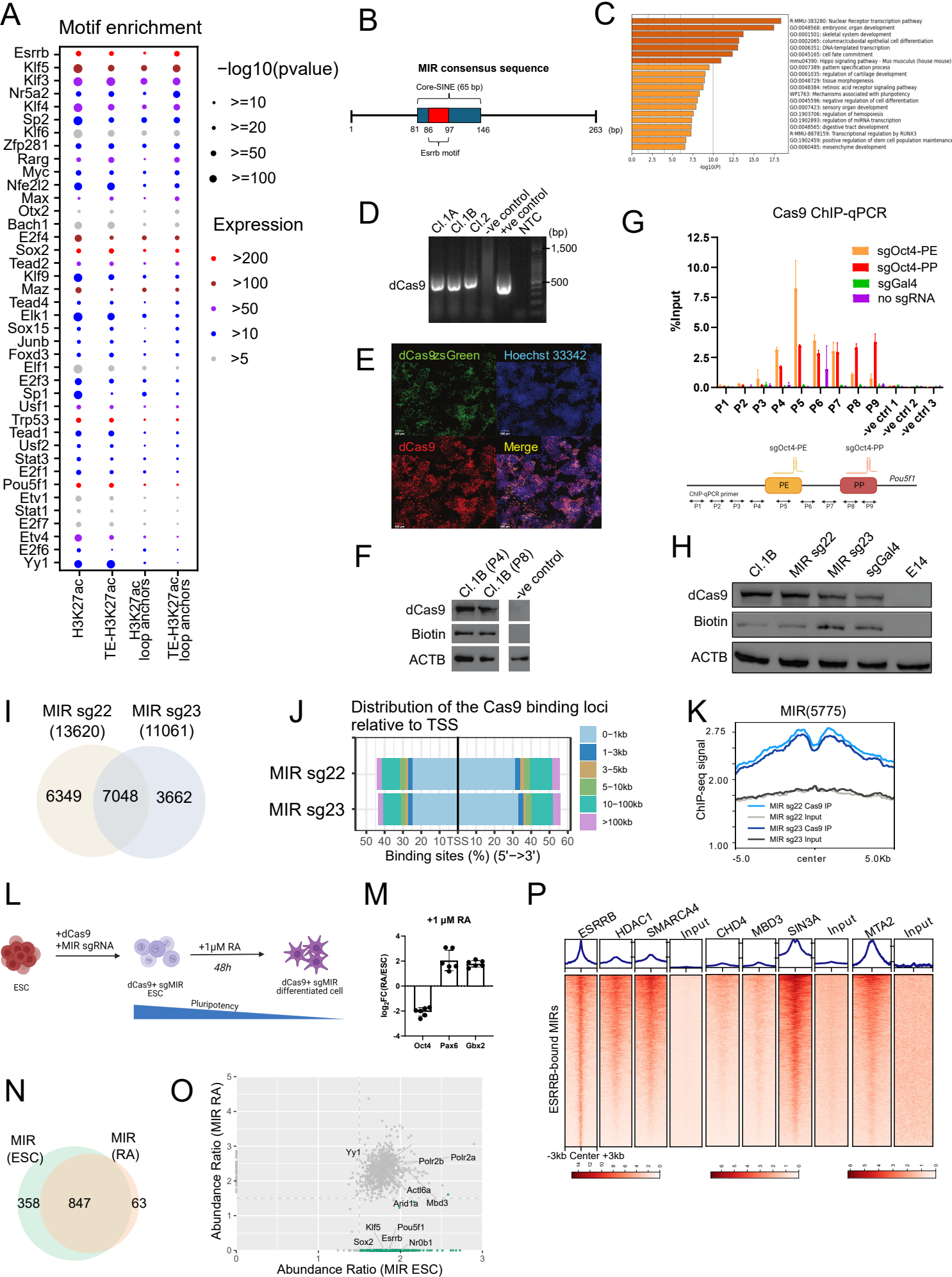


Fig. S3. Motif enrichment analysis in TE-enhancers of ESC and characterization of CAPTURE2.0 system to target MIRs

a, TF motif enrichment analysis in different categories of enhancers and TE-enhancers-derived loop anchors. Size and colour represent the enrichment significance and expression value in ESC. **b**, Schematic depicting Esrrb motif position within the core-SINE region of MIR consensus sequence. **c**, GO-term enrichment of motifs enriched in MIR H3K27ac HiChIP loop anchors. **d**, PCR of genomic DNA from transduced and clonally expanded cell lines (Cl.1A, 1B and 2) detected dCas9 transgene integration in the genomic DNA. Non-transduced E14 was used as negative control. dCas9 plasmid was used as positive control. NTC: no template control. **e**, Immunofluorescence staining of a representative clonal line detected dCas9 protein expression (red). The dCas9-fused zsGreen reporter signal was also detected (green). Nucleus was stained using Hoechst 33342 (blue). **f**, Western blot verified the presence of biotin on dCas9 protein expressed by Cl.1B at earlier (P4) and later (P8) passages. Beta-actin (ACTB) was used as loading control. **g**, Signal enrichment of Cas9-ChIP relative to input in cells expressing Oct4-PE and Oct4-PP sgRNA. The annealing positions of 9 ChIP-qPCR primer pairs along the Pou5f1 (Oct4) regulatory regions were shown at the top. PE: proximal enhancer, PP: proximal promoter. **h**, Western blot identified persistent biotinylated dCas9 protein in the sgRNA-expressing cell lines (MIR sg22, MIR sg23, sgGal4) derived from a parental dCas9-positive clone (Cl.1B). E14 mouse ESC was used as negative control. Beta-actin (ACTB) was used as loading control. **i**, Venn plot showing the number of overlap peaks for MIR sg22 and MIR sg23 Cas9 ChIP-seq called peaks. **j**, The distance distribution of Cas9 binding peaks relative to TSS in MIR sg22 and MIR sg23 sample. **k**, Average ChIP-seq signal profile for MIR sg22 and MIR sg23 Cas9 ChIP-seq sample on 5775 MIRs around called Cas9 binding peaks. **l**, Schematic illustration of the conversion of MIR CAPTURE 2.0 cell line towards differentiated state by retinoic acid (RA). **m**, Gene expression of Oct4 and neuronal marker genes (Pax6 and Gbx2) in ESC treated with 1 μ M RA for 48 hours. RA, retinoic acid. **n**, Venn plot showing the common and state-specific MIR-associated proteins identified by MIR sg22. **o**, Scatterplot of abundance ratio for significantly identified MIR-associated proteins using MIR sg22 in ESC and RA. Proteins that only appeared in ESC sample were labelled in green. **p**, Binding profile of several MIR-associated proteins (HDAC1, CHD4, SMARCA4, MBD3A, SIN3A, AND MTA2) over ESRRB-bound MIR sites based on public ChIP-seq datasets.

Figure S4

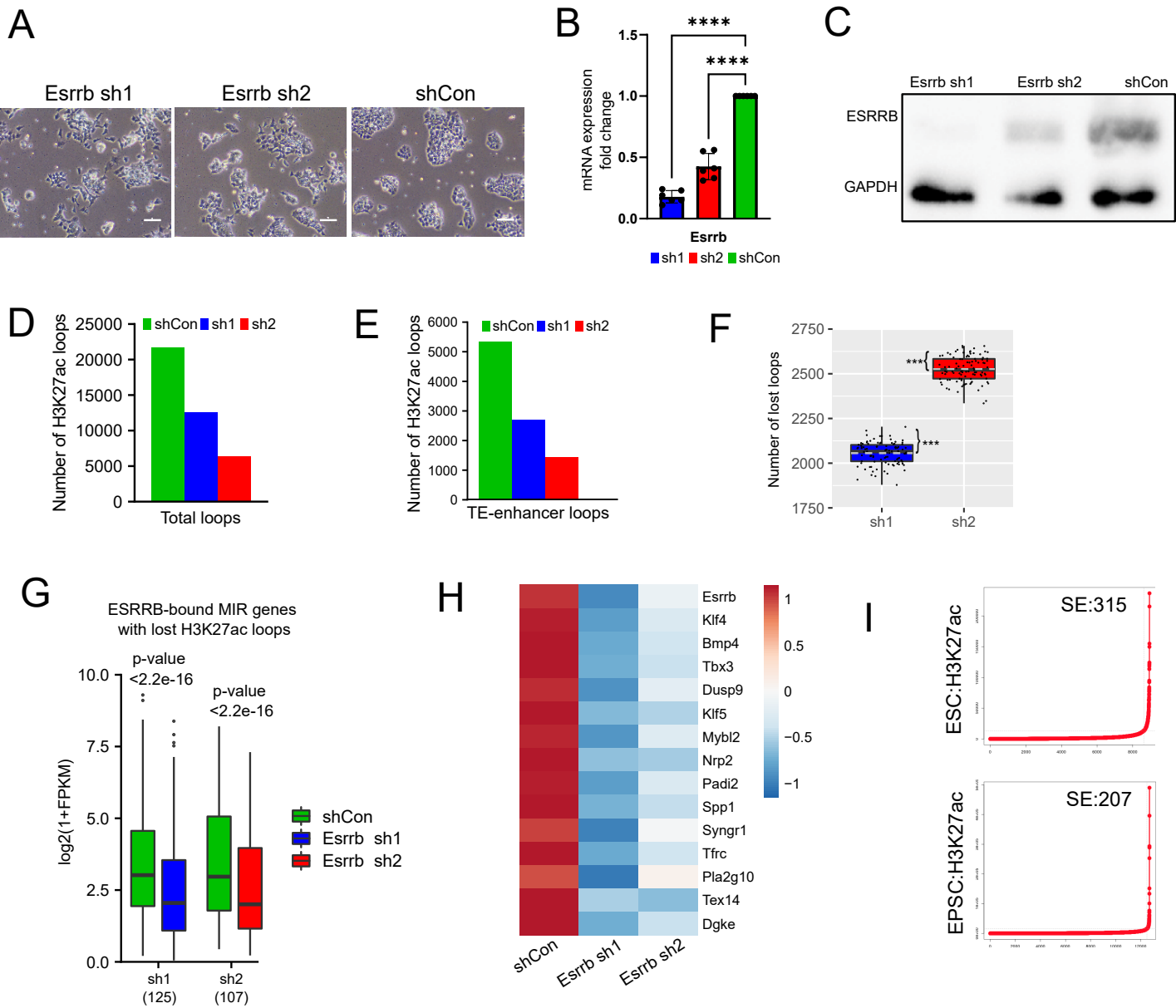


Fig. S4. Effect of Esrrb knockdown on enhancer-gene looping and ESRRB interaction with YY1

a, Cell morphology before and after Esrrb knockdown using two shRNA constructs. **b**, RT-qPCR showing reduced Esrrb gene expression after Esrrb knockdown. One-way ANOVA was used to test for significance. **c**, Western blot showing depleted ESRRB protein level after Esrrb shRNA knockdown relative to control. **d**, Total number of identified H3K27ac loops with and without Esrrb knockdown. **e**, Total number of H3K27ac loops that overlapped with TE-enhancer loops with and without Esrrb knockdown. **f**, Boxplot showing significant difference between MIR and randomly shuffled regions with 100 permutations that fall in the lost H3K27ac loop anchors. Significance levels were computed using Z-test. **g**, Gene expression changes of MIR-interacting genes with lost H3K27ac loops. **h**, Gene expression changes of selected ESRRB-bound MIR interacting genes. Colour indicates the expression fold change. **i**, Number of ROSE-called super-enhancers in ESC and EPSC using H3K27ac ChIP-seq data.

Figure S5

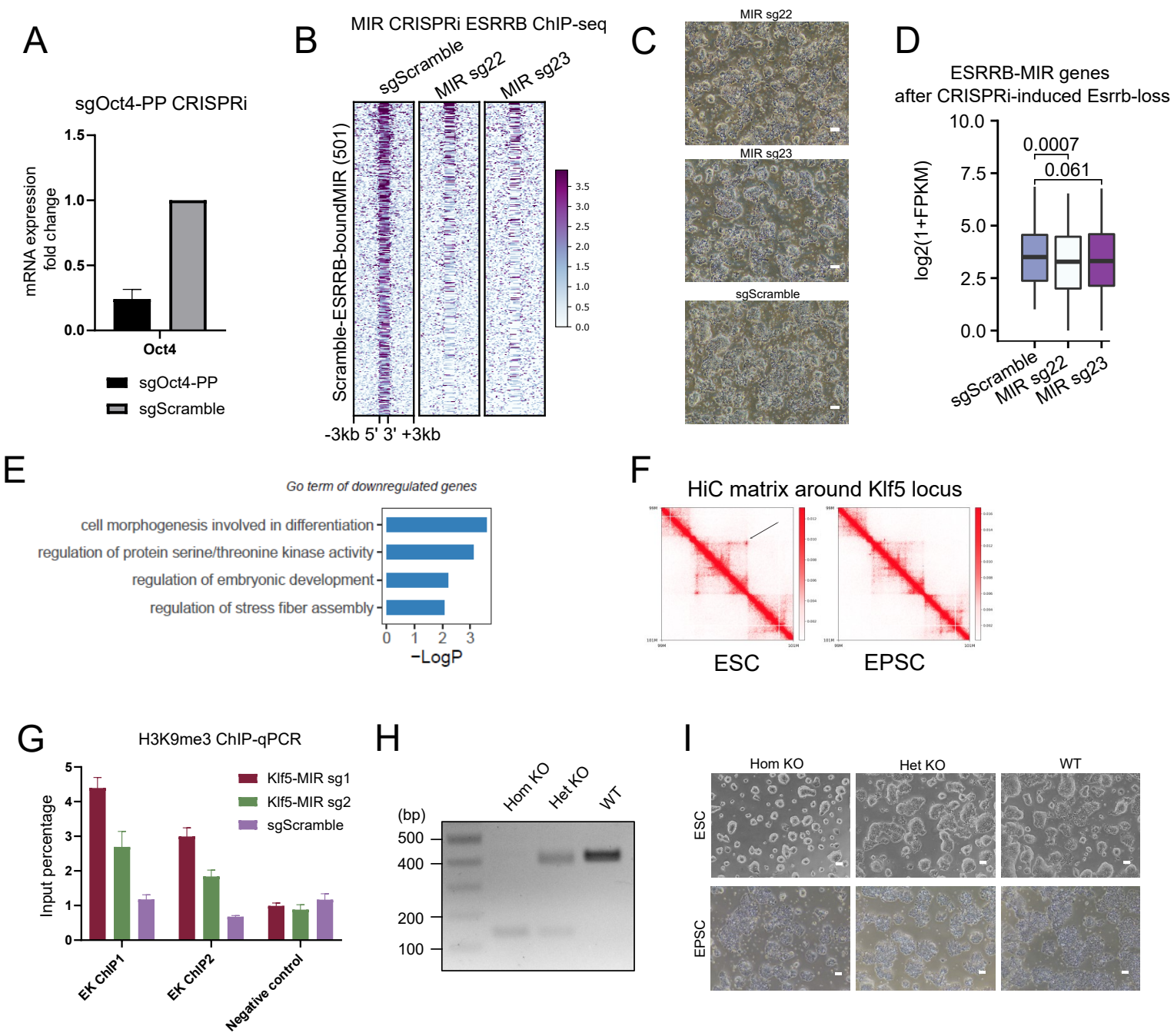


Fig. S5. CRISPR inactivation of MIR and generation of Klf5-MIR CRISPR KO line

a, Validation of CRISPRi capability to suppress Oct4 expression using sgRNA targeted to its promoter (sgOct4 PP). **b**, ESRRB ChIP-seq signal was reduced at control ESRRB-bound MIRs following MIR CRISPRi. **c**, Morphology of ESCs 3 days after MIR CRISPRi. **d**, Expression of genes interacting with ESRRB-bound MIRs with ESRRB binding loss after MIR CRISPRi. Wilcoxon rank-sum test was used to determine *p*-value. **e**, GO-term enrichment of genes downregulated after MIR CRISPRi. **f**, Hi-C chromatin contact heatmap around Klf5 locus. **g**, H3K9me3 ChIP-qPCR of Klf5-MIR regions after CRISPRi. Increased H3K9me3 signal indicated successful deposition of H3K9me3 by KRAB-MeCP2. **h**, Genotyping PCR of clones transfected with Klf5-MIR KO sgRNAs. Homozygous KO was indicated by the presence of amplicon product shorter than 420bp. Heterozygous KO clone produce 2 bands, the 420bp wildtype amplicon product and the <420 bp amplicon product, **i**, Morphology of the Klf5-MIR KO lines in ESC and EPSC states.

Figure S6

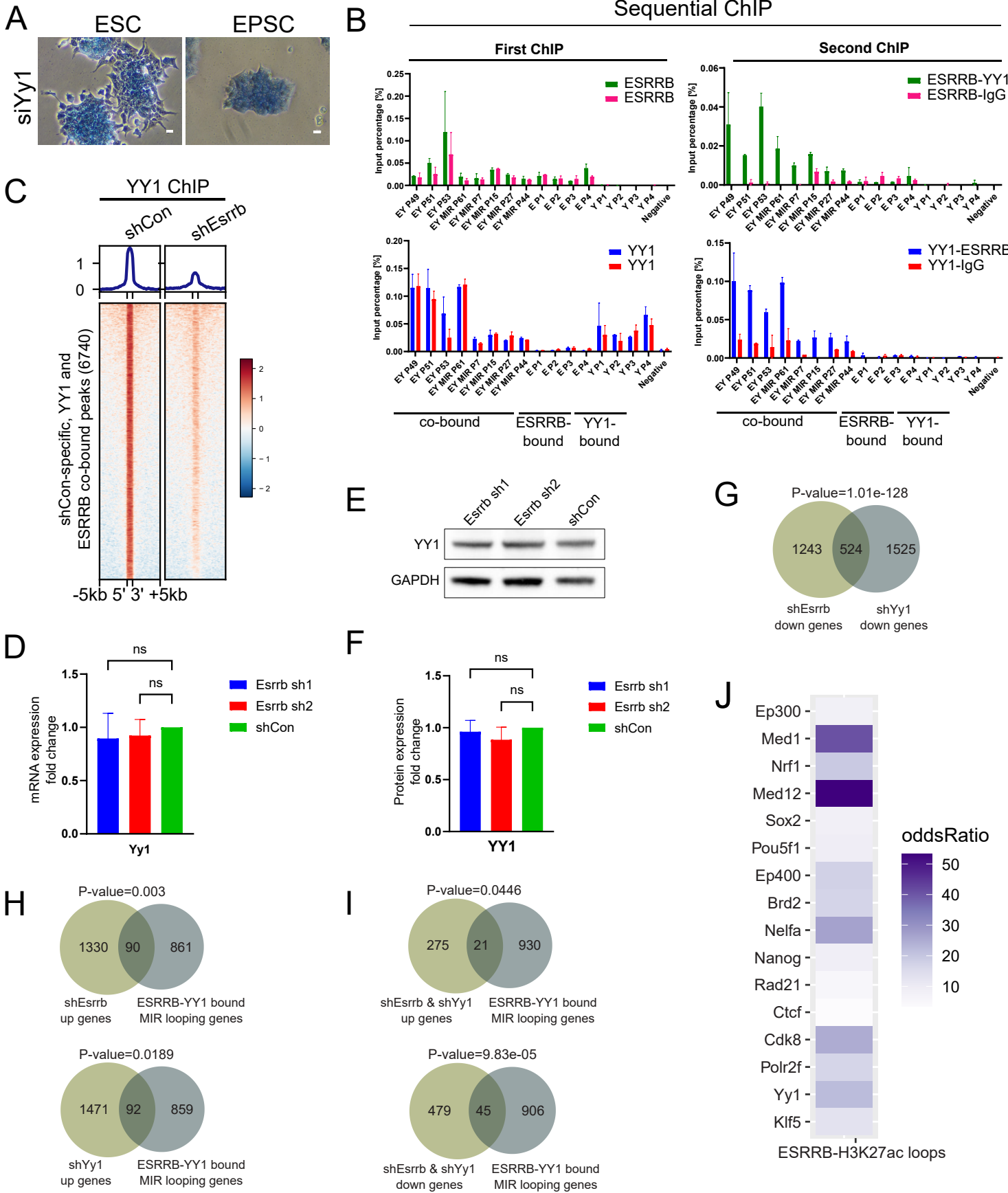


Fig. S6. Yy1 and Esrrb knockdown experiment and sequential ChIP

a, Alkaline phosphatase staining of ESC and EPSC with Yy1 knockdown. **b**, Bidirectional ESRRB-YY1 sequential ChIP. ESRRB or YY1 binding signal after the first and second immunoprecipitation was measured by qPCR at several ESRRB and YY1 co-bound non-MIR (EY), ESRRB and YY1 co-bound MIR (EY MIR), ESRRB-only (E), or YY1-only (Y) sites. **c**, YY1 peak lost at control YY1 and ESRRB co-bound sites after Esrrb knockdown. **d**, RT-qPCR of Yy1 expression in Esrrb knockdown and control samples. **e**, Western blot result of YY1 protein level in Esrrb knockdown and control ESCs. **f**, Western blot quantification of YY1 protein level. One-way ANOVA was used to test for significance. **g**, Venn diagram showing the overlap of downregulated genes in Esrrb and Yy1 knockdown conditions. **h**, Venn diagram of genes regulated by ESRRB-YY1 co-bound MIRs after Esrrb or Yy1 knockdown in ESCs. These MIR-regulated genes were defined by the presence of H3K27ac loops connecting co-bound MIR and gene region (TSS to gene end). **i**, Similar to h, but depicting the overlap of genes regulated under both Esrrb and Yy1 knockdown conditions. **j**, Structural proteins and transcription factors binding peaks enrichment analysis on ESRRB-mediated H3K27ac loop anchors, using public ChIP-seq data. The heatmap shows the significant proteins after Fisher exact test was applied. The color intensity indicates the odds ratio.