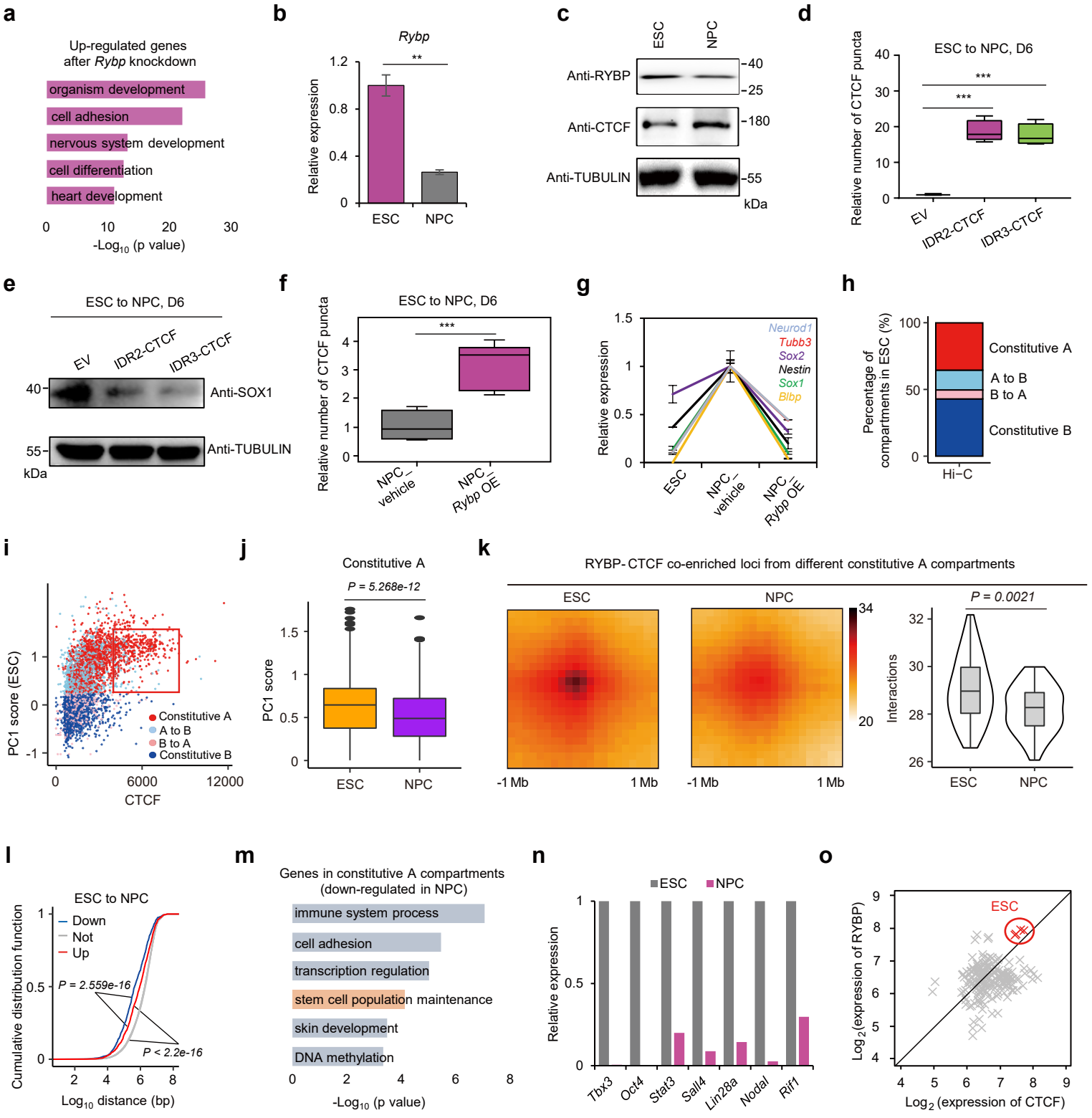
Supplementary information, Fig.S10



Supplementary information, Fig.S10 Induced CTCF phase separation inhibits ESC differentiation toward NPCs. a Gene ontology (biological process) analysis of genes that are up-regulated after *Rybp* knockdown for 96 h. **b** The expression level of *Rybp* mRNA in ESCs and NPCs. Welch's *t*-test; n = 3, *P* = 0.0037. **c** Western blot showing the expression level of RYBP and CTCF in ESCs and NPCs. **d** Boxplot showing the relative number of highly concentrated CTCF puncta in ESC-derived cells. Welch's t-test; EV: n = 59 cells; IDR2-CTCF: n = 94 cells; IDR3-CTCF: n = 67 cells; P values are (from left to right): *P* = 3.302e-06; *P* = 1.753e-05. **e** Western blot showing the expression of SOX1 in ESC-derived cells, which were differentiated from ESCs stably expressing EV, IDR2-CTCF or IDR3-CTCF. **f** Boxplot showing the relative number of highly concentrated CTCF puncta ESC-derived cells, which were induced from ESCs without (NPC_vehicle) or with RYBP overexpression (NPC_*Rybp* OE) to NPCs for 6 days. Welch's *t*-test; NPC_vehicle: n = 169 cells; NPC_*Rybp* OE: n = 149 cells; *P* = 0.0004. **g** Relative mRNA expression of NPC associated genes in ESCs and ESC-derived cells, which were induced from ESCs without (NPC_vehicle) or with RYBP overexpression (NPC_*Rybp* OE) to NPCs for 6 days. **h** Hi-C data showing the percentage of unchanged and changed compartments during the differentiation of NPCs from ESCs. The percentage was calculated in ESCs. **i** The enrichment of CTCF in constitutive A compartments, constitutive B compartments, A to B compartments and B to A compartments in ESCs, highlighting that the constitutive A compartments highly enrich CTCF. **j** Boxplots showing the interaction changes of inter-constitutive A compartments using Hi-C data between ESCs and NPCs, Wilcoxon rank-sum test. **k** In ESCs and NPCs, APA plots (left) and quantitation (right) showing the genome-wide aggregate strength between RYBP-CTCF co-enriched loci from different constitutive A compartments, the highly dynamic inter-A compartment interactions were used for analysis (see methods), Wilcoxon rank-sum test. **l** Cumulative distribution showing the distance in base pair (Log_{10}) between gene promoters and the closest

RYBP-CTCF co-enriched peaks, the RYBP-CTCF co-enriched peaks from the 100 kb bins in Supplementary information, Fig.S10k were used for analysis. As controls, we randomly selected five groups of 1000 not changed (Not) genes each. ‘Up’ represent genes up-regulated from ESCs to NPCs, whereas ‘Down’ represent genes down-regulated from ESCs to NPCs, K-S test. **m** Gene ontology (biological process) analysis of genes in constitutive A compartments, the expression of these genes is down-regulated during the differentiation of ESCs toward NPCs, highlighting the enrichment at the term of stem cell population maintenance. **n** Relative mRNA expression of pluripotency genes in NPCs and ESCs from public RNA-seq data (GSE96107). These genes localize in constitutive A compartment. The difference between the two groups of each gene is significant. **o** Expression of CTCF and RYBP in different types of cells, data were analyzed from BioGPS.