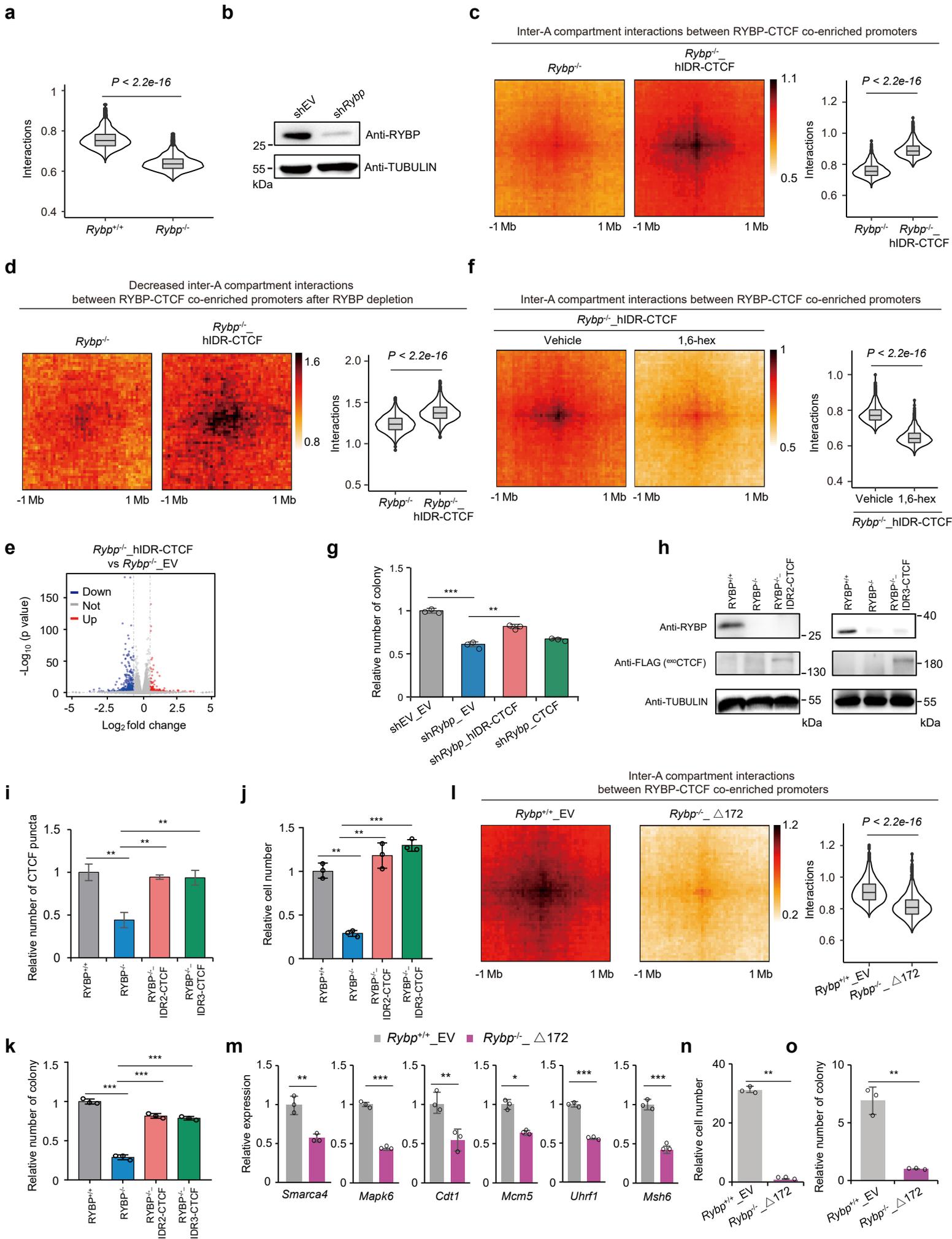


Supplementary information, Fig.S9



Supplementary information, Fig.S9 Induced CTCF phase separation improves self-renewal of ESCs. **a** Quantitation of APA showing the genome-wide aggregate strength between RYBP-CTCF co-enriched promoters from different A compartments after RYBP depletion, Wilcoxon rank-sum test. **b** Western blot showing the successful knockdown of RYBP via shRNA for 96h. **c** APA plots (left) and quantitation (right) showing the genome-wide aggregate strength between RYBP-CTCF co-enriched promoters from different A compartments after inducing CTCF phase separation. **d** APA plots (left) and quantitation (right) showing the genome-wide aggregate strength between RYBP-CTCF co-enriched promoters after inducing CTCF phase separation, the most strongly decreased inter-A compartment interactions (top 10%) between RYBP-CTCF co-enriched promoters after RYBP depletion were used for the analysis. **e** Volcano plot showing the expression change of genes after exogenously expressing hIDR-CTCF in *Rybp*^{-/-} mESCs. **f** APA plots (left) and quantitation (right) showing the genome-wide aggregate strength between RYBP-CTCF co-enriched promoters from different A compartments after 1,6-hex treatment in *Rybp*^{-/-}_hIDR-CTCF mESCs, Wilcoxon rank-sum test. **g** Relative number of newly formed ESC colonies in each group. P values are (from left to right): $P = 0.0006$; $P = 0.0053$; $n = 3$, Welch's *t*-test. **h** Western blot showing the successful expression of exogenous RYBP IDR-fused CTCF (*Rybp*^{-/-}-IDR2-CTCF) and FUS IDR-fused CTCF (*Rybp*^{-/-}-IDR3-CTCF) in RYBP-depleted ESCs. **i** Relative number of highly concentrated CTCF puncta in different cell lines. n values are (from left to right): 50, 66, 64 and 54 cells; P values are (from left to right): 0.004907, 0.006181 and 0.005171. **j** Relative number of cells for different cell lines after re-seeding with same number of cells for 5 days. All n values are 3; P values are (from left to right): 0.002417, 0.00628 and 0.0001622; **k** Relative number of colonies in different cell lines. All n values are 3; P values are (from left to right): 1.12e-05, 3.09e-05 and 5.22e-05; **l** APA plots (left) and quantitation (right) showing the genome-wide aggregate strength between RYBP-CTCF co-enriched promoters from

different A compartments after RYBP mutation, Wilcoxon rank-sum test. **m** RT-qPCR showing the expression alteration of self-renewal associated genes before and after RYBP mutation. P values are (from left to right): $P = 0.0055$; $P = 0.0004$; $P = 0.0077$; $P = 0.0251$; $P = 0.00074$; $P = 0.00026$, $n = 3$, Welch's t -test. **n**, **o** Relative number of cells (**n**) and newly formed ESC colonies (**o**) in $Rybp^{+/+}_{EV}$ and $Rybp^{-/-}_{\Delta 172}$ groups. P values are (from left to right): $P = 0.001$; $P = 0.003$, $n = 3$, Welch's t -test. *n.s.*, *not significant*, $P > 0.05$; $**P < 0.01$; $***P < 0.001$. qPCR data show means \pm SD.