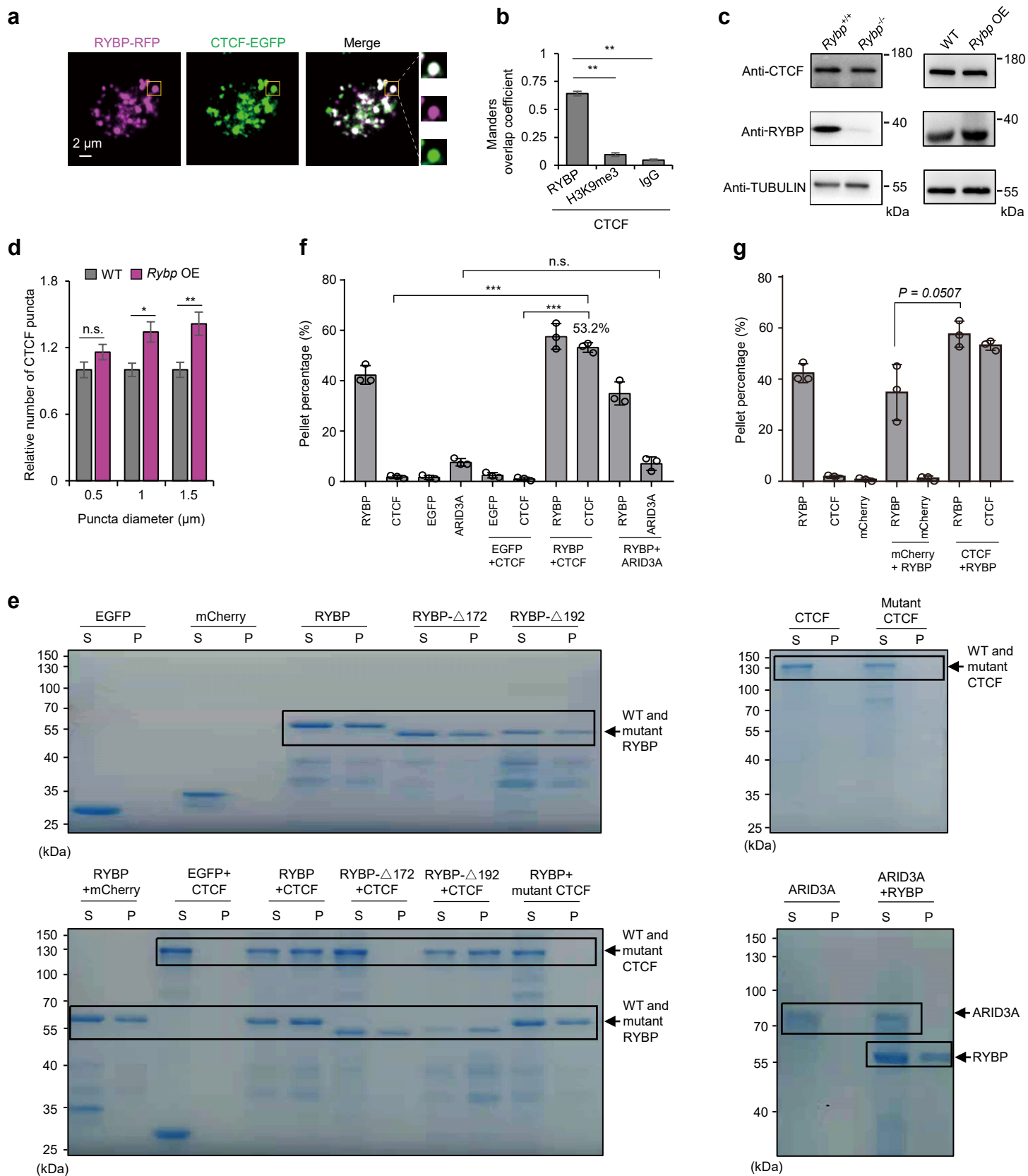
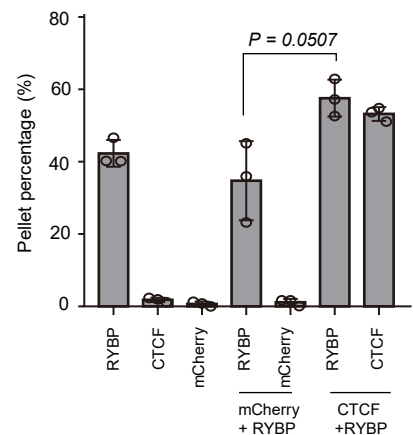


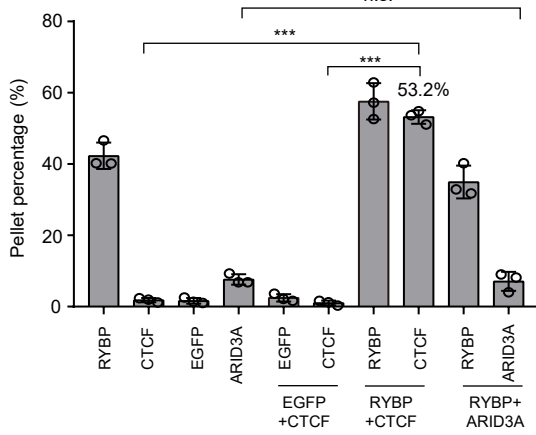
Supplementary information, Fig.S4

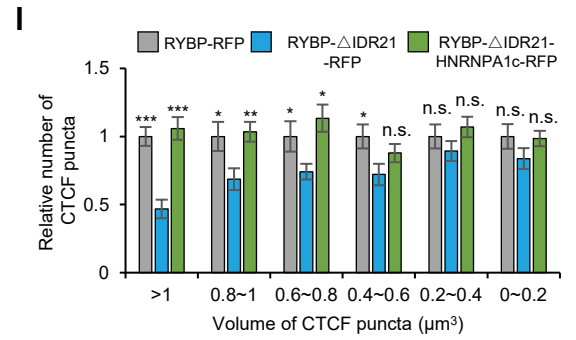
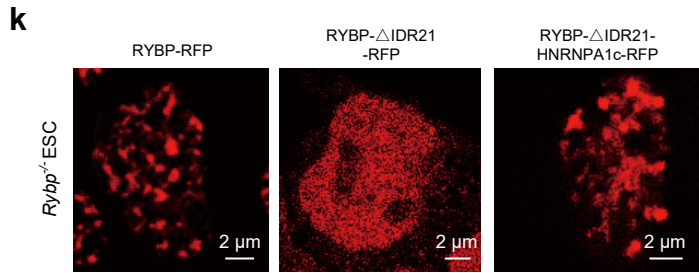
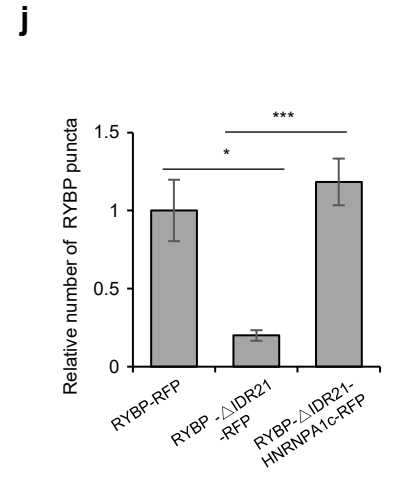
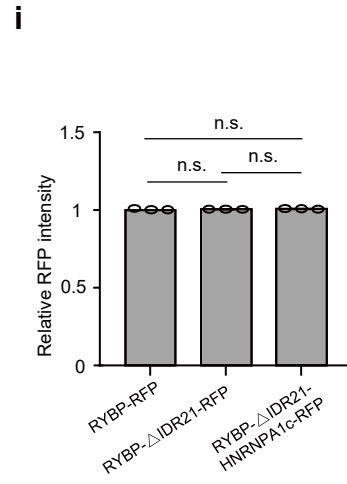
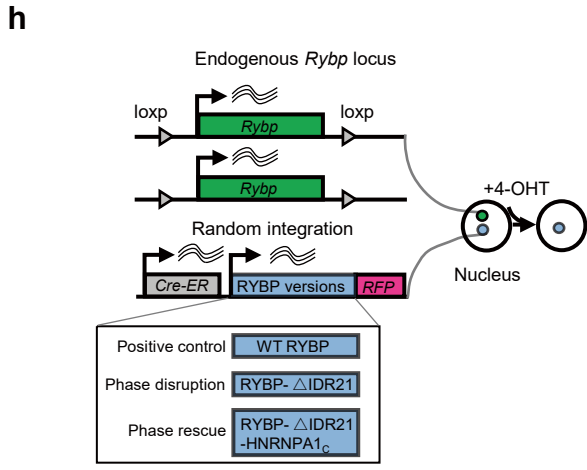


g



f





Supplementary information, Fig. S4 RYBP facilitates CTCF to undergo phase separation. a

Live cell images showing the co-localization between RYBP and CTCF, both RFP-tagged RYBP (RYBP-RFP) and EGFP-tagged CTCF (CTCF-EGFP) were exogenously expressed in ESCs. **b** Manders overlap coefficient showing the co-localization efficient between different groups. The histone modification in B compartment (H3K9me3) and IgG were used as controls. Welch's *t*-test; RYBP, n = 127 cells; H3K9me3, n = 112 cells; IgG, n = 129 cells, P values are (from left to right): $P = 0.0011$; $P = 0.0011$. **c** Western blot displaying the expression of CTCF and RYBP in ESCs after RYBP depletion or overexpression (*Rybp* OE). **d** Relative number of CTCF puncta with various diameters before and after RYBP overexpression. Welch's *t*-test; WT, n = 54; *Rybp* OE, n = 40; P values are (from left to right): $P = 0.1894$; $P = 0.034$; $P = 0.0034$. **e-g** Representative SDS-PAGE analysis (**e**) and quantification (**f, g**) data showing the distribution of proteins between aqueous-solution/supernatant (S) and condensed liquid phase/pellet (P) fractions for various protein mixtures in the absence of PEG8000. The concentration of each protein is 50 μ M. Welch's *t*-test; all n values are 3; P values are (Supplementary information, Fig.S4f, from left to right): 0.0001453, 0.0001039, 0.7733; **h** Experimental pipeline for the construction of different versions of RYBP cell lines. WT RYBP, RYBP- Δ IDR21 and RYBP- Δ IDR21-HNRNPA1c were exogenously expressed in ESCs, respectively. Endogenous *Rybp* was depleted by 4-OHT treatment. **i** Flow cytometry showing the exogenously expression level of different versions of RYBP cell lines. Welch's *t*-test; all n values are 3; P values are: 0.0505, 0.0767, 0.134; **j** Relative number of RYBP puncta in different versions of RYBP cell lines. Welch's *t*-test; RYBP-RFP, n = 86 cells; RYBP- Δ IDR21-RFP, n = 196 cells; RYBP- Δ IDR21-HNRNPA1c-RFP, n = 134 cells; P values are (from left to right): 0.0142, 0.0007. **k, l** Representative images (**k**) and quantification (**l**) showing the relative number of CTCF puncta with different sizes from different versions of RYBP cell lines. Welch's *t*-test; RYBP-RFP, n = 109 cells; RYBP- Δ IDR21-RFP, n = 118 cells; RYBP- Δ IDR21-HNRNPA1c-

RFP, n = 117 cells; Compared to the RYBP- Δ IDR21-RFP group in each size, P values are (from left to right): 0.0001, 0.0002, 0.0337, 0.0058, 0.0417, 0.0417, 0.0165, 0.0814, 0.2024, 0.0592, 0.1058 and 0.0718.