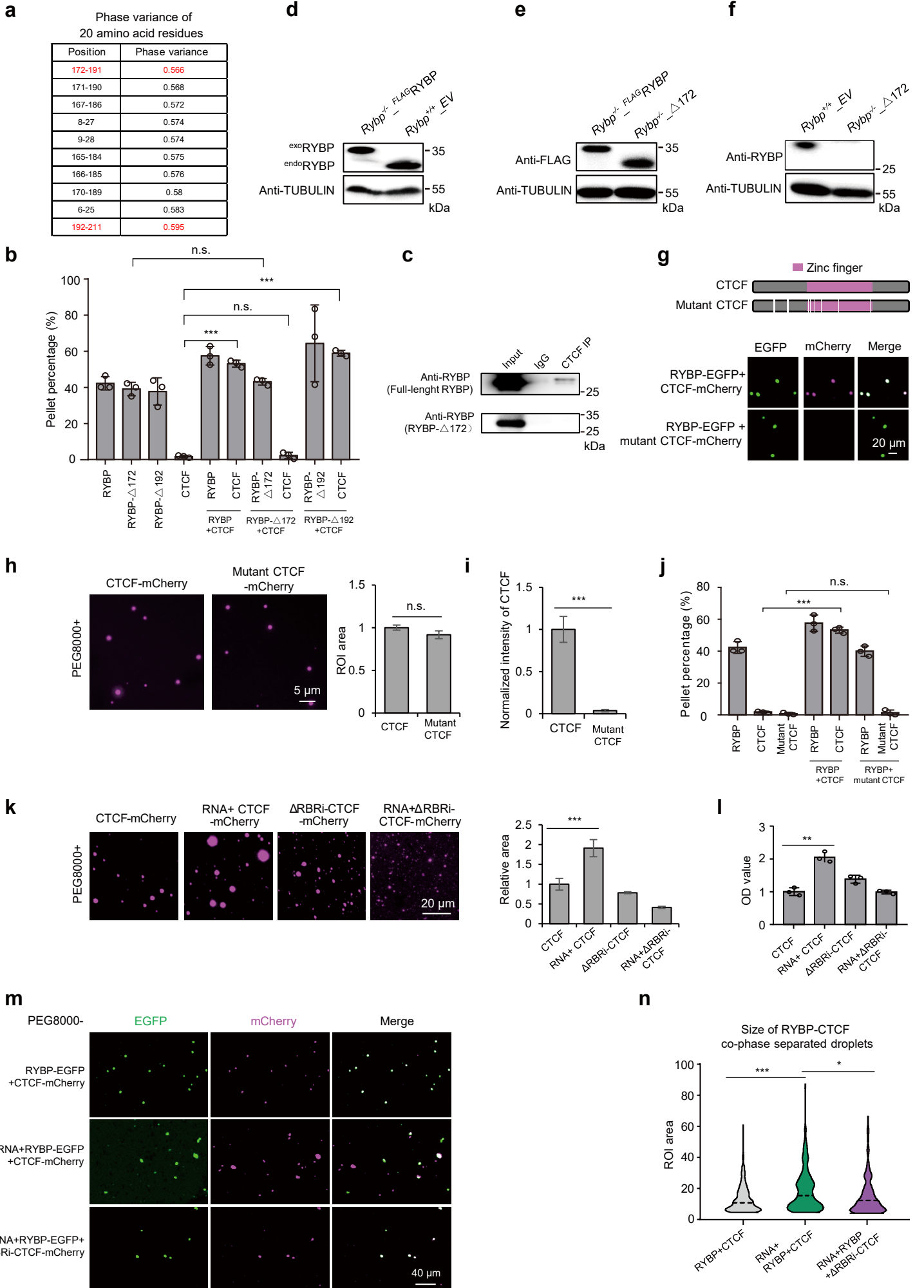


# Supplementary information, Fig.S5



**Supplementary information, Fig. S5 The molecular basis for the co-phase separation between RYBP and CTCF.** **a** PSPHunter predicted the continuous region of RYBP that might be important for the phase separation behavior of RYBP, the lower score of phase variance denotes the higher influence of the unit on phase separation. **b** Quantification of sedimentation assay in Supplementary information, Fig.S4e, and showed the percentage of different proteins in pellet upon the mixture of WT or mutant RYBP with CTCF. Welch's *t*-test; all n values are 3; P values are (from left to right): 0.1939, 0.00004787, 0.6325 and 0.0001453. **c** Co-IP experiment showing the interaction between CTCF and full-length RYBP, CTCF and RYBP- $\Delta$ 172. **d** Western blot showing the expression of exogenous RYBP (<sup>exo</sup>RYBP) in *Rybp*<sup>-/-</sup><sub>FLAGRYBP</sub> was similar with endogenous RYBP (<sup>endo</sup>RYBP) in *Rybp*<sup>+/+</sup><sub>EV</sub> cell line. **e** Western blot showing the expression level of exogenous RYBP in *Rybp*<sup>-/-</sup><sub>FLAGRYBP</sub> was similar with exogenous mutant RYBP (RYBP- $\Delta$ 172) in *Rybp*<sup>-/-</sup> <sub>$\Delta$ 172</sub> cell line. **f** Western blot showing the loss of endogenous RYBP in *Rybp*<sup>-/-</sup> <sub>$\Delta$ 172</sub> cell line. **g** Top: Experimental pipeline for the mutational analysis of CTCF. Bottom: RYBP droplets incorporate full-length or mutant CTCF. The concentration of RYBP-EGFP was 40  $\mu$ M, CTCF-mCherry and mutant CTCF-mCherry were 4  $\mu$ M. **h** Representative images (left) and quantification (right) showing the WT CTCF and mutant CTCF droplets, 60  $\mu$ M CTCF were used. Welch's *t*-test; WT CTCF, n = 1455; mutant CTCF, n = 611; P = 0.1312. **i** Normalized intensity of full-length or mutant CTCF in RYBP droplets. Welch's *t*-test; CTCF, n = 25; mutant CTCF, n = 31; P < 2.2e-16. **j** Quantification of sedimentation assay in Supplementary information, Fig.S4e, and showed the percentage of different proteins in pellet upon the mixture of RYBP with WT or mutant CTCF. Welch's *t*-test; all n values are 3; P values are (from left to right): 0.0001453 and 0.6648; **k** Representative images (left) and quantification (right) showing the droplet formation of WT or  $\Delta$ RBRI-CTCF upon RNA addition in the presence of PEG8000, 2  $\mu$ M WT or  $\Delta$ RBRI-CTCF were used. Welch's *t*-test; n values are (from left to right): 999, 1500, 7080 and 2998; P = 0.000491735.

**l** Turbidity assay showing the droplet formation of WT or  $\Delta$ RBRI-CTCF upon RNA addition in the presence of PEG8000, 2  $\mu$ M WT or  $\Delta$ RBRI-CTCF were used. Welch's *t*-test; all *n* values are 3; *P* = 0.0011. **m** Representative images showing the droplet formation of WT or  $\Delta$ RBRI-CTCF upon RNA addition without PEG8000, 10  $\mu$ M WT or  $\Delta$ RBRI-CTCF were used. **n** Size of RYBP-CTCF co-phase separated droplets. *n* values are (from left to right): 418, 519 and 370; *P* values are (from left to right): *P* = 5.3461e-16, *P* = 0.04952.