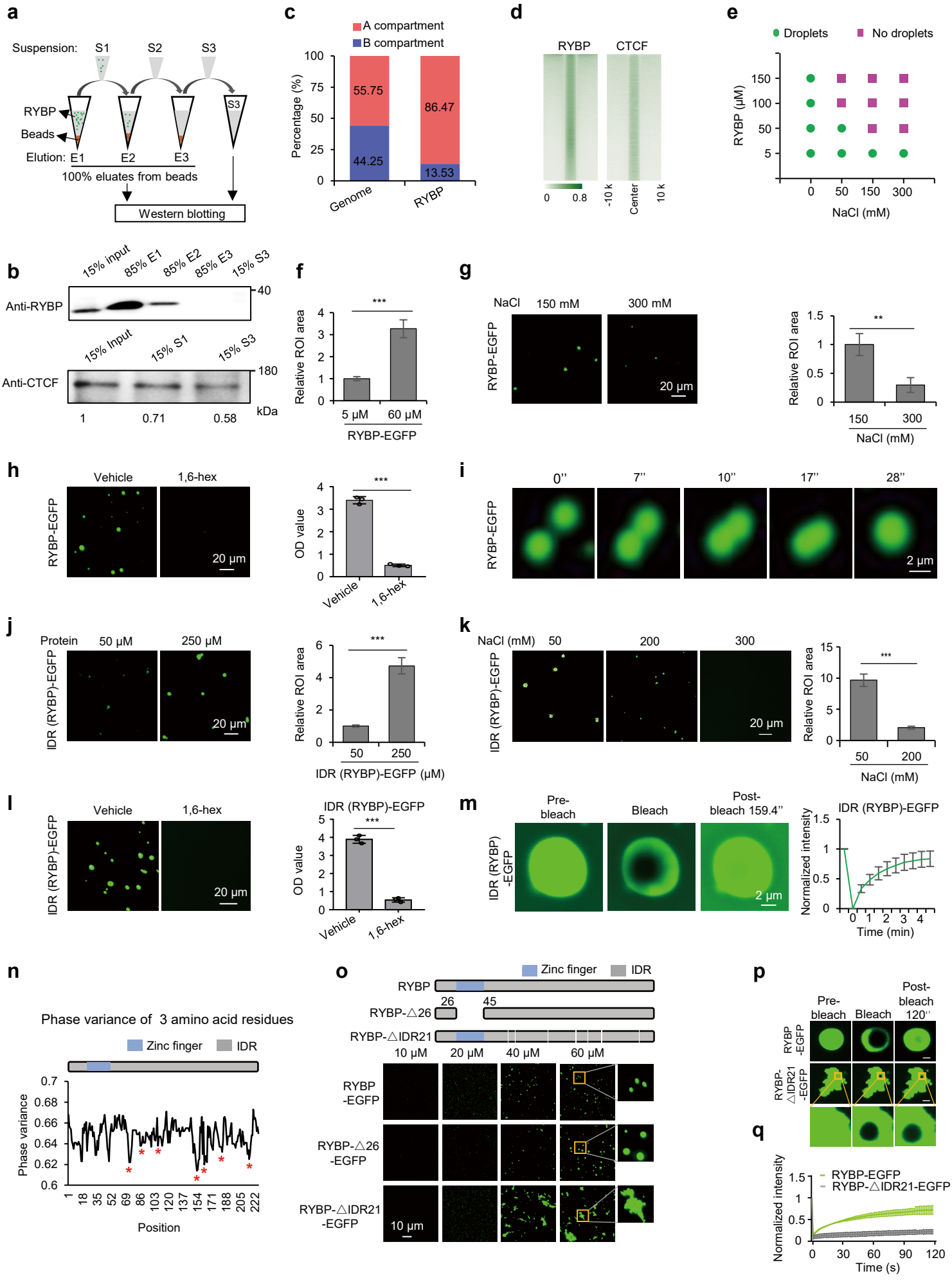
Supplementary information, Fig.S3



Supplementary information, Fig. S3 RYBP undergoes phase separation. a Experimental schematic of immunodepletion. **b** Western blot showing amount of RYBP among the three rounds of immunoprecipitation (top) and the amount of CTCF in suspension after RYBP immunodepletion (bottom). **c** Distribution of RYBP ChIP-seq peaks (GSM4052120) in different compartments of mESCs. **d** Distribution of CTCF ChIP-seq peaks (GSE29218) at the center of RYBP ChIP-seq peaks (GSM4052120). **e** Phase diagrams showed RYBP droplet formation under different protein and salt ion concentrations. **f** Relative ROI area of droplets in the buffer with 5 and 60 μM RYBP-EGFP recombinant protein. Welch's *t*-test; 5 μM, n = 108; 60 μM, n = 60; *P* = 7.136e-08. **g** Representative images (left) and relative ROI area (right) of droplets in the buffer with 150 or 300 mM NaCl, the protein concentration of RYBP-EGFP was 5 μM. Welch's *t*-test; 150 mM, n = 19; 300 mM, n = 30; *P* = 0.00425. **h** Representative images (left) and turbidity assay (right) of RYBP-EGFP formed droplets before and after treatment with 10% 1,6-hex *in vitro*, the protein concentration of RYBP-EGFP was 40 μM. Welch's *t*-test; n = 3; *P* = 0.0003651. **i** Droplet fusion behavior of RYBP-EGFP recombinant protein (40 μM). **j** Representative images (left) and statistics (right) of droplet formation in the buffer with 50, and 250 μM IDR (RYBP) recombinant protein. Welch's *t*-test; 50 μM, n = 368; 250 μM, n = 137; *P* = 1.844e-11. **k** Representative images (left) and statistics (right) of droplet formation in the buffer with 50, 200 and 300 mM NaCl. The protein concentration of IDR (RYBP) was 100 μM. Welch's *t*-test; 50 mM, n = 60; 200 mM, n = 112; *P* = 3.52e-10. **l** Representative images (left) and turbidity assay (right) of IDR (RYBP) formed droplets before and after treatment with 10% 1,6-hex *in vitro*, the protein concentration of IDR (RYBP)-EGFP was 200 μM. Welch's *t*-test; n = 3; *P* = 0.0001356. **m** Representative images (left) and statistics (right) of FRAP data for IDR (RYBP)-EGFP droplets *in vitro*. The protein concentration of IDR (RYBP)-EGFP was 300 μM, n = 3. **n** PSPhunter predicted the key amino acid residues of RYBP that might be important for the phase separation of RYBP. The lower score

denotes the higher influence of the unit (3 amino acid residues per unit) on phase separation, 21 candidate amino acid residues (seven red stars, each star indicates 3 amino acid residues) in RYBP IDR were deleted. **o** Experimental pipeline for the mutational analysis of RYBP (top); The droplet formation assay of different versions RYBP *in vitro* (bottom). **p, q** Representative images (**p**) and quantification (**q**) of FRAP data for RYBP-EGFP and RYBP-△IDR21-EGFP droplets *in vitro*. The scale bar of images on the top and middle row denotes 2 μm and 5 μm, respectively. The concentration of both proteins was 100 μM.