



**Supplementary information, Fig. S10. A Rett syndrome-related missense mutation in the TRD (P302R) affects MeCP2-driven chromatin LLPS.**

**a** Phase diagram of the MeCP2 TRD missense mutant P302R. Scale bar, 20  $\mu\text{m}$ .

**b** Cross-linking result of the MeCP2 mutant P302R alone. Each intra- or inter-molecular crosslink of MeCP2 is indicated by a different line. The intra- or inter-molecular crosslinks between the MeCP2 K223 residue and other sites are highlighted.

**c** Localization of overexpressed mCherry-MeCP2 P302R in NIH 3T3 cells. Scale bar, 5  $\mu\text{m}$ . **d** Colocalization of EGFP-MeCP2 with the TRD mutant mCherry-MeCP2 P302R in NIH 3T3 cells. Scale bar, 5  $\mu\text{m}$ .

**e** Left panels, snapshots of puncta formed by the TRD mutants mCherry-MeCP2 P302R or R306C in Supplementary information,

Fig. S10c and 5B. Right panel, average fluorescence recovery traces for the FRAP experiments (n=6 puncta per mutant). All data are presented as mean  $\pm$  SD. **f** Left panels, snapshots of puncta formed by WT EGFP-MeCP2 and the TRD mutant mCherry-MeCP2 P302R in Supplementary information, Fig. S10d. Right panel, average fluorescence recovery traces for the FRAP experiments (n=6 puncta). All data are presented as mean  $\pm$  SD. **g** Left panels, snapshots of puncta formed by WT EGFP-MeCP2 and the TRD mutant mCherry-MeCP2 R306C in Fig. 5c. Right panel, average fluorescence recovery traces (n=6 puncta). All data are presented as mean  $\pm$  SD.