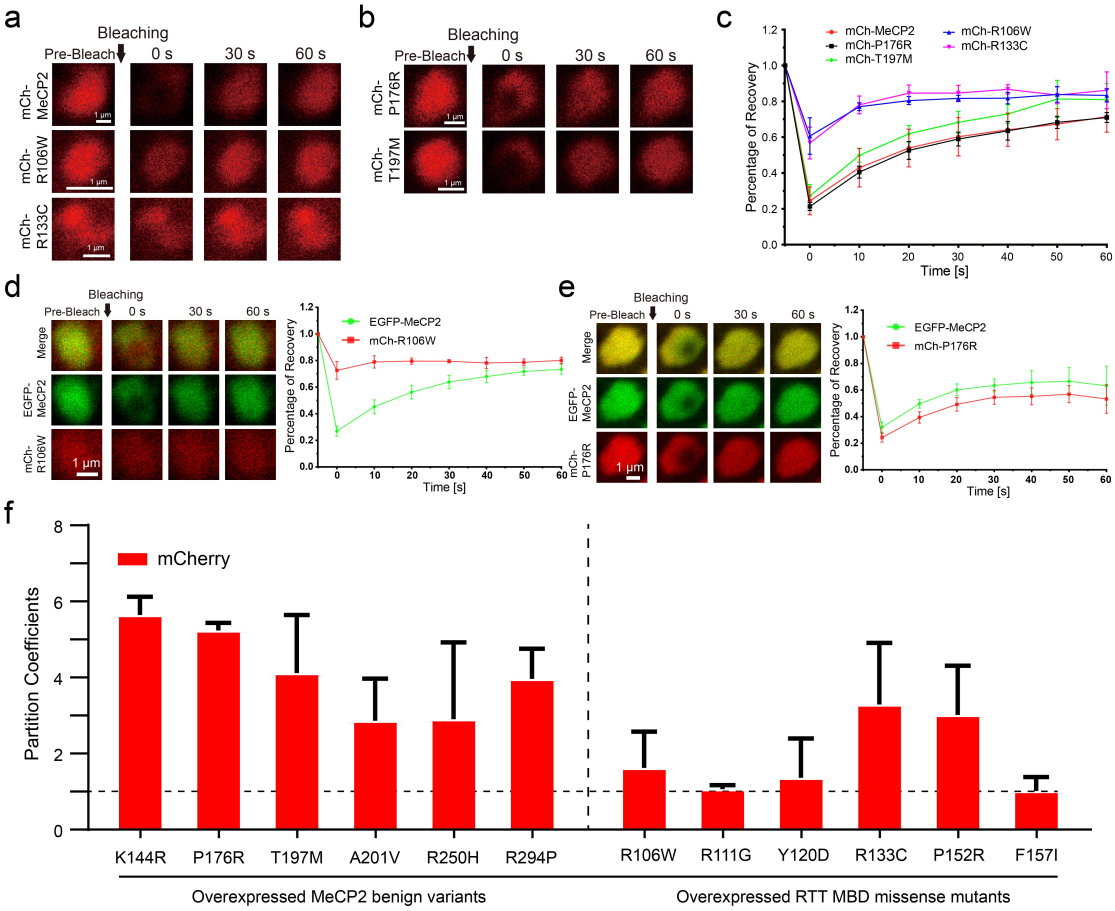

Supplementary information, Fig. S8. Statistical analysis the Rett syndrome-related MBD mutants or benign variants overexpressed in NIH 3T3 cells.

a Snapshots of puncta formed by mCherry-MeCP2 WT and missense mutants in Fig. 3f, 4e (mCherry-MeCP2-R106W and R133C) during FRAP analysis. **b** Snapshots of puncta formed by the neutral variants in Fig. 4g (mCherry-MeCP2-P176R and T197M) during FRAP experiments. Scale bars, 1 μm. **c** Quantitative results for FRAP analyses of the average recovery traces in Supplementary information, Fig. S7e-f (n=6). All data are presented as mean ± SD. **d** Left panels, snapshots of puncta formed by EGFP-MeCP2 WT and the missense mutant mCherry-MeCP2 R106W in Fig. 4i. Right panel, average fluorescence recovery traces from the FRAP experiment (n=6 puncta). All data are presented as mean ± SD. **e** Left panels, snapshots of puncta formed by EGFP-

MeCP2 WT and the polymorphic variant mCherry-MeCP2 P176R in Fig. 4j. Right panel, average fluorescence recovery traces from the FRAP experiment (n=6 puncta). All data are presented as mean ± SD. **f** Statistical analysis the partition coefficients of mCherry-MeCP2 on the heterochromatin in Fig. 4e, 4g and Supplementary information, Fig. S7a-b. The average fluorescence signals for labeled proteins in DAPI-dense regions were analyzed by using the signals in DAPI-dispersed regions as the background.