



**Supplementary information, Fig. S7. Overexpression of the Rett syndrome-related MBD mutants or benign variants in NIH 3T3 cells.**

**a** Localizations of overexpressed mCherry-MeCP2 MBD missense mutants in NIH 3T3 cells. All these mutant proteins are dispersed within the nucleus. Scale bars, 5  $\mu\text{m}$ .

**b** Similar to WT MeCP2, benign variants are mainly localized to heterochromatic chromocenters marked by DAPI. Scale bars, 5  $\mu\text{m}$ .

**c** Co-transfection of NIH 3T3 cells with EGFP-MeCP2 and mCherry-MeCP2 R111G. WT protein (green) colocalizes with heterochromatin, while the R111G mutant (red) is dispersed in nuclei but shows some heterochromatin localization and is enriched in the nucleolus. Scale bar, 5  $\mu\text{m}$ .

**d** Co-expression of WT MeCP2 and the neutral variant MeCP2 T197M in NIH 3T3 cells.

They both localize to heterochromatin. Scale bar, 5  $\mu\text{m}$ .