



Figure S6. Analysis of the R138Q FMRP Patient Mutant in the Chromatin Interaction, DDR, and AMPAR Internalization Assays, Related to Figure 4

(A) Diagram of the FMRP Agenet double-tudor domain showing the location of the patient mutation R138Q.

(B) Coomassie gel showing GST-AgenetKHKH and GST-R138QKHKH proteins used in MST experiments for Kd determination.

(C) Western blot showing the levels of expression of exogenous Flag-HA-FMRP (lane 1) and FMRP (R138Q) Agenet domain patient mutant (lane 2) in FMRP KO MEFs, using anti-Flag antibody.

(D) Western blot showing the levels of expression of exogenous wild-type FMRP (lane 2) and R138Q FMRP patient mutant (lane 3) rescue constructs in FMRP KO MEFs using anti-FMRP antibody. Lane 1 contains FMRP KO MEFs without the rescue for comparison.

(E) Colony survival assay in the presence of hydroxyurea (HU) using FMRP KO MEFs reconstituted with wild-type FMRP or R138Q FMRP patient mutant. R138Q-reconstituted MEFs were more sensitive to the increased doses of HU as compared to wild-type FMRP reconstituted MEFs ($p = 0.0045$, 2-way ANOVA).

(F) Images of hippocampal neurons immunostained for surface (S) and internalized (I) AMPARs. Panel 1: wild-type neurons, panel 2: FMRP KO neurons, panel 3: FMRP KO neurons rescued with wild-type FMRP, panel 4: FMRP KO neurons rescued with FMRP R138Q mutant.

(G) Quantification of results in (F). Both rescue proteins functioned similarly in AMPARs internalization experiments, reversing AMPAR constitutive endocytosis in FMRP KO neurons. Compare lanes 3 and 4 (y axis is the ratio of internalized to total AMPARs). Median: WT, 52.60; KO, 74.20; KO+FMRP (WT), 51.57; KO+R138Q, 51.44; $n = 30$ each. p values were determined using one-way ANOVA ($\alpha = 0.05$) with Bonferroni's post hoc test.