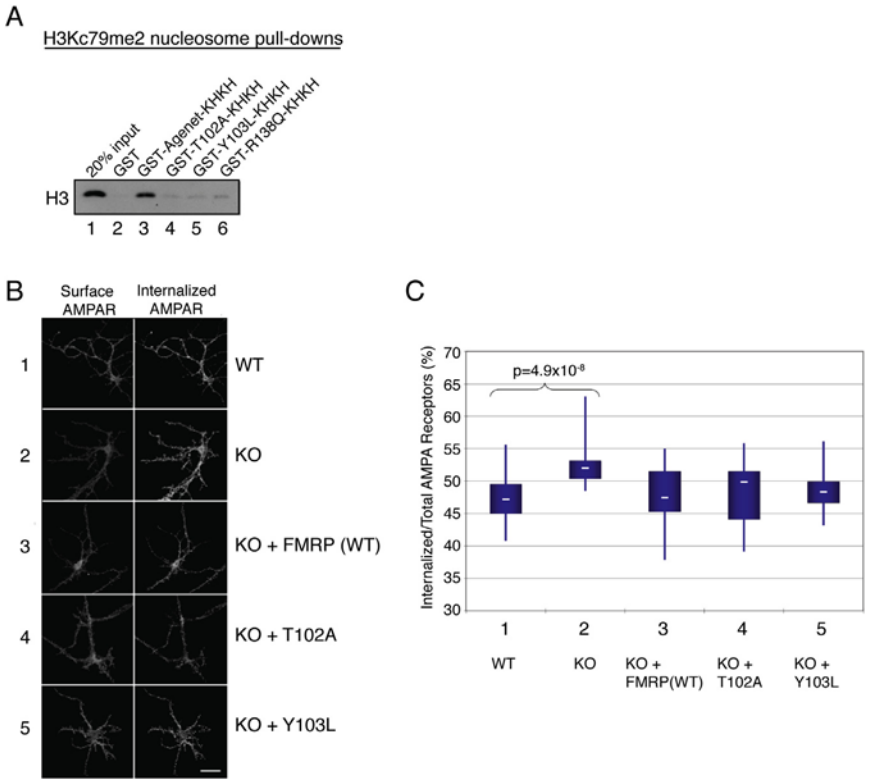

Figure S5. Genetic Complementation Experiments Using Wild-Type FMRP and Its Agenet Domain Mutants in the AMPAR Internalization Assay, Related to Figures 3 and 4

(A) In vitro H3K79me2 MLA nucleosome binding reactions using GST-AgenetKHKH domain. AgenetKHKH$_{FMRP}$ contains 2 additional nucleic acid binding domains adjacent to the double-tudor domain allowing nucleosomal binding. Wild-type FMRP bound H3Kc79me2, whereas Agenet domain mutants T102A, Y103L, and R138Q (patient mutant, see Figure S6A) did not (compare lanes 3 to 4,5, and 6).

(B) Hippocampal primary neurons from a wild-type and an *Fmr1* KO mouse, as well as neurons from *Fmr1* KO mouse reconstituted with lentiviral constructs expressing wild-type FMRP or FMRP mutants T102A and Y103L were immunostained for surface and internalized AMPARs. Internalized AMPAR signal was increased and AMPAR signal remaining on the surface was reduced in FMRP KO neurons as compared to wild-type neurons (panels 1 and 2). FMRP KO neurons infected with lentivirus expressing either wild-type or mutant forms of FMRP displayed reversal of the excessive AMPAR internalization observed in FMRP KO neurons (panels 3–5), indicating that Agenet mutations T102A and Y103L do not affect FMRP function at neuronal dendrites.

(C) Distribution of AMPARs in distal dendrites. Box-and-whisker plot showing that enhanced constitutive endocytosis of AMPARs in FMRP KO neurons (lane 2) is corrected by lentivirus expressing either WT (lane 3), T102A mutant (lane 4), or Y103L mutant (lane 5) FMRP. Median: WT, 47.18; KO, 52.01; KO+FMRP(WT), 47.44; KO+T102A, 49.86; KO+Y103L, 48.30; n = 30 each. p values were determined using one-way ANOVA (α = 0.05) with Bonferroni's post hoc test.