Supporting Information

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SI Materials and Methods

DNA Constructs. The GFP-MS2-nls (nuclear localization signal) and MS2 binding site (MS2bs)-CaMKIIα 3′UTR constructs were kindly provided by Dr. Kenneth S. Kosik. MS2bs vector was generated from MS2bs-CaMKIIα 3′UTR by removing CaMKIIα 3′UTR using BglII and NotI. MS2bs-*Fmr1* was constructed by insertion of the *Fmr1* ORF and 3′UTR PCR fragment into an MS2bs vector. The *Fmr1* fragment was generated from Mc2.17 by PCR and digestion with BamHI and NotI. All plasmids were sequenced to verify their composition.

Colocalization Between Fragile X Mental Retardation Protein and Surface Receptor. Primary hippocampal neurons were transfected with FLAG-mGluR1a, FLAG-mGluR5, or FLAG-β2AR (surface β2 adrenergic receptor), which were kindly provided by Dr. Stephan Ferguson, at 7 or 8 days in vitro (DIV). Cultured neurons were treated with (S)-3,5-dihydroxyphenylglycine (DHPG; Tocris) for 5 min and left for the indicated duration after DHPG was removed. In cycloheximide-treatment groups, 60 μM cycloheximide was included in medium 30 min before and during experiment periods. Then neurons were subjected to surface receptor labeling and immunocytochemistry. Images were taken by a Leica SP2 with a 63× (NA 1.4) objective as Z stacks with 0.3-μm interval.

Imaging Analysis of Colocalization. Neuronal dendrites were deconvolved and 3D reconstructed by using Autoquant (Media Cybernetics) and Imaris (Bitplane). Manders's coefficient of fragile X mental retardation protein (FMRP) = sum of colocalized (intensity of surface receptor) × (intensity of FMRP)/sum of intensity of FMRP in one voxel, where (intensity of surface receptor) = 1 when the voxel contains surface receptor staining, otherwise (intensity of surface receptor) = 0. Manders's coefficient varies from 0 to 1, corresponding to nonoverlapping images and 100% colocalization.

Fluorescence in Situ Hybridization. A cRNA probe to CaMKIIα was generated from a restriction-digested fragment corresponding to nucleotides 1014–1332 of CaMKIIα cDNA. The plasmid pBS-CaMKIIα was kindly provided by Dr. Oswald Steward (1). A cRNA probe to *Fmr1* was generated from a restriction-digested fragment corresponding to nucleotides 263–313 of *Fmr1* cDNA. The *Fmr1* cDNA fragment was kindly provided by Dr. Jim Eberwine and inserted into pBS. CaMKIIα and *Fmr1* probes were labeled by in vitro transcription of the cloned insert in the presence of digoxigenin-dUTP (Roche). To detect total polyadenylated mRNA, a synthetic 50-mer nucleotide oligo-dT-labeled with Cy3 at the 5′ end was used. Hybridization buffer: 40% formamide, 10% dextran sulfate, 1× Denhardt's solution, 4× SSC, 10 mM DDT, 1 mg/mL tRNA, and 1 mg/mL denatured salmon sperm DNA.

Sources of Antibodies. The sources of the antibodies are as follows: rabbit anti-mGluR5 (Millipore), rabbit anti-mGluR1a (Millipore), mouse anti-MAP2 (Sigma), rabbit anti-MAP2 (Millipore), chicken anti-MAP2 (EnCor), rabbit anti-GFP (AbCam), chicken anti-GFP (AbCam), rabbit anti-FLAG (Sigma), mouse anti-FLAG (Sigma), mouse anti-FMRP (1C3, a gift of J.-L. Mandel, Collège de France, France), and mouse anti-CaMKIIα (Millipore).

1. Paradies MA, Steward O (1997) Multiple subcellular mRNA distribution patterns in neurons: A nonisotopic in situ hybridization analysis. *J Neurobiol* 33:473–493.

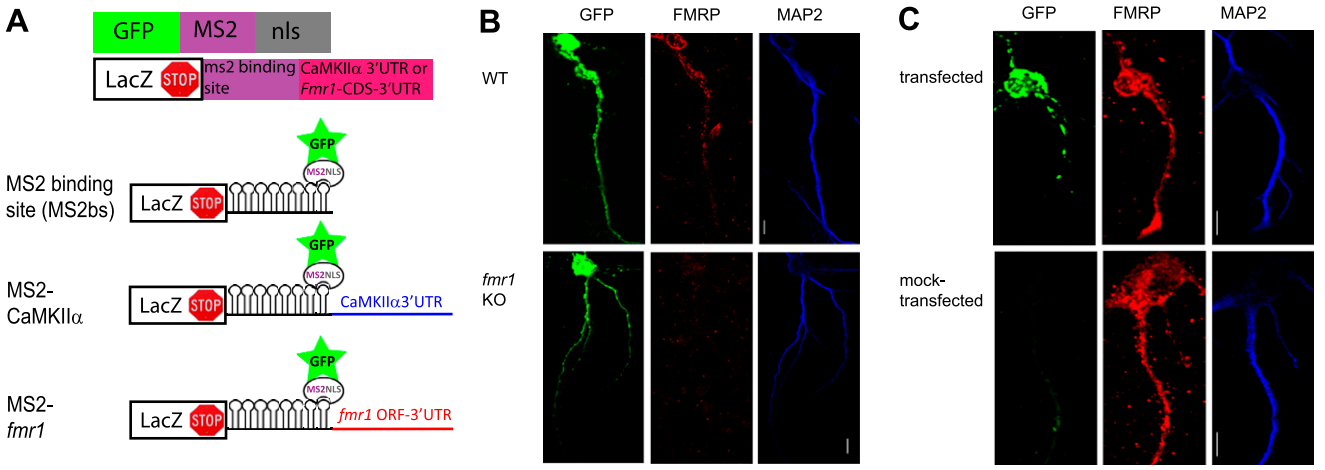


Fig. S1. MS2 labeling strategy and its controls. (*A*) Dual constructs for GFP labeling of mRNA were shown previously (1). Briefly, CaMKIIα 3′UTR was linked with eight copies of MS2 binding site RNA (MS2bs-CaMKIIα), which has strong affinity to MS2 protein. *Fmr1* ORF-3′UTR was subcloned to replace CaMKIIα (MS2bs-*Fmr1*) to label *Fmr1* mRNA. MS2 binding site RNA alone (MS2bs) was used as a negative control. GFP-tagged MS2 with nuclear localization signal (GFP-MS2-nls)-labeled CaMKIIα or *Fmr1* mRNA via MS2-MS2bs interaction in live neurons. (*B*) WT or *fmr1* KO neurons were transfected with GFP-MS2-nls and MS2bs-*Fmr1*. GFP-labeled *Fmr1* form puncta (green) in dendrite. FMRP (red) appeared only in WT neurons by immunostaining, demonstrating that FMRP cannot be translated from MS2bs-*Fmr1* construct. (Scale bars, 10 μm.) (*C*) There is similar subcellular distribution of FMRP (red) in mock-transfected (no plasmid, transfection reagents only) or dual *Fmr1*-labeling constructs-transfected WT neurons; therefore, nuclear localization signal on GFP-MS2-nls does not trap FMRP in the nucleus. (Scale bars, 10 μm.)

1. Rook MS, Lu M, Kosik KS (2000) CaMKIIα 3′ untranslated region-directed mRNA translocation in living neurons: Visualization by GFP linkage. *J Neurosci* 20:6385–6393.

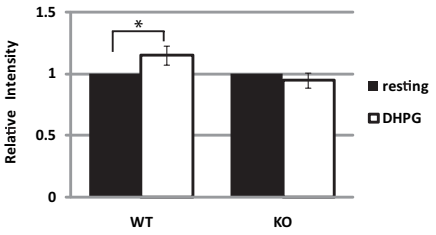


Fig. S2. The intensity of GFP-labeled *fmr1* mRNA was elevated after stimulation in WT neurons but not in *fmr1* KO neurons. The intensity of GFP-labeled *fmr1* mRNA in time-lapse imaging was measured before or after DHPG treatment. The pairwise comparison of granule intensity was only applied to the time series with exact imaging parameters before and after stimulation. Statistical analysis by two-tailed *t* test. Error bars denote SEM.

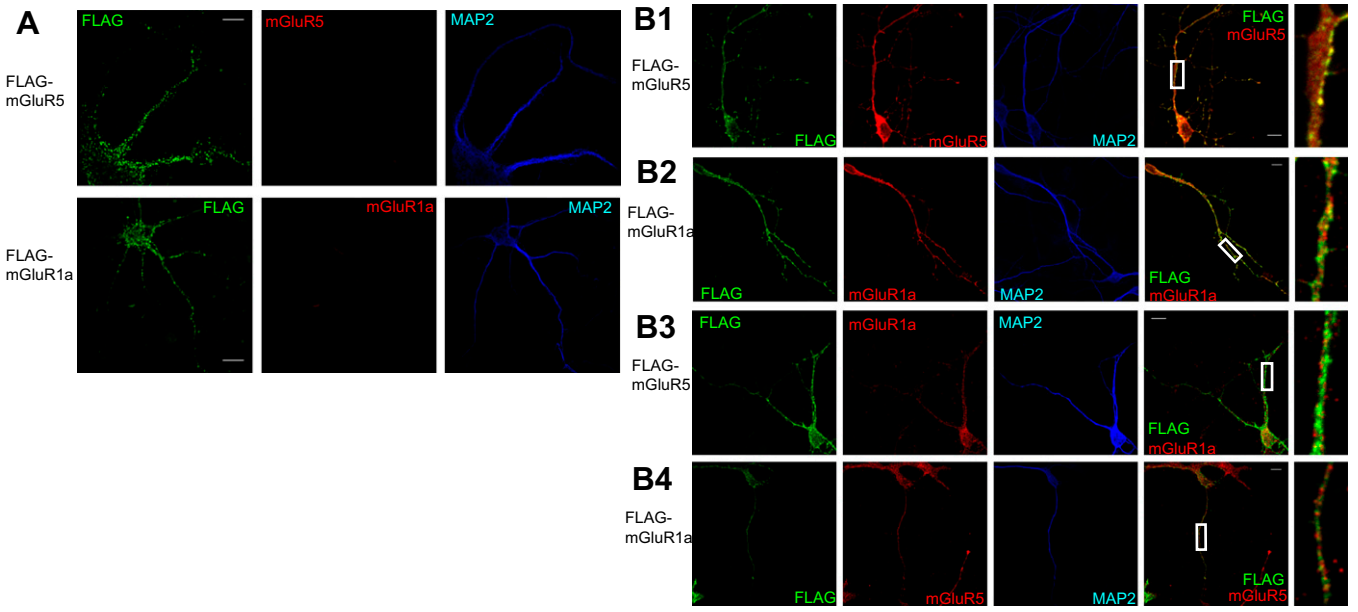


Fig. S3. Controls for surface staining of FLAG-tagged receptor and relative distribution of surface FLAG receptor and endogenous receptors. (*A*) Immunofluorescence of anti-FLAG (green) and anti-mGluR1a or anti-mGluR5 (red), which recognizes intracellular epitope of the receptor, was performed under nonpermeant conditions in FLAG-mGluR1a- or FLAG-mGluR5-transfected hippocampal neurons. Only FLAG on the cell surface can be stained but not the intracellular portion of endogenous mGluR1a or mGluR5. This shows that only the surface portion of receptors was labeled by this method. (*B*) Immunofluorescence of surface group I mGluR was compared with the staining pattern of endogenous receptors. In *B*1, almost all surface staining of mGluR5 (green) corresponds to the staining of endogenous mGluR5 (red). However, in *B*2, the staining of surface mGluR1a (green) does not fully colocalize with endogenous mGluR1a (red). The relative distribution of surface mGluR5 versus endogenous mGluR1a (*B*3) and surface mGluR1a versus endogenous mGluR5 (*B*4), as negative controls, showed the degree of overlapping of two different receptors.

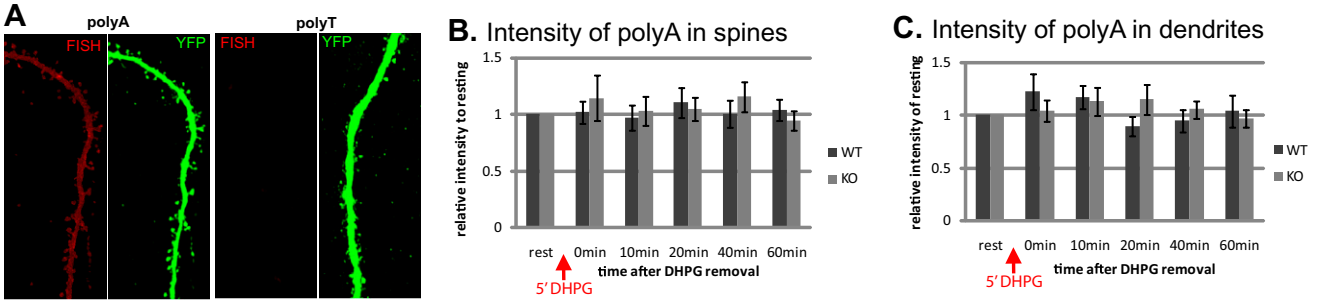
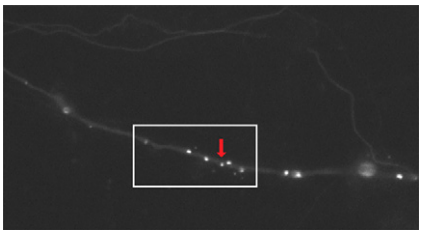
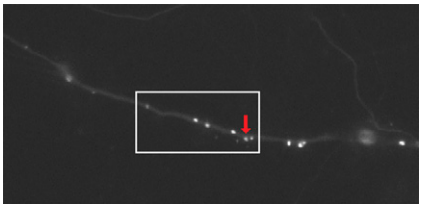


Fig. S4. The distribution of polyA mRNA in neuronal dendrites or spines. (*A*) Representative figures showing fluorescence in situ hybridization of polyA mRNA in YFP neurons. The negative control, a probe to recognize polyT, did not show clear staining in neuronal dendrites. (*B* and *C*) The intensity of polyA FISH was measured in spines (*B*) or adjacent dendrites (*C*) of YFP neurons. Statistical analysis by two-way ANOVA. Error bars denote SEM.



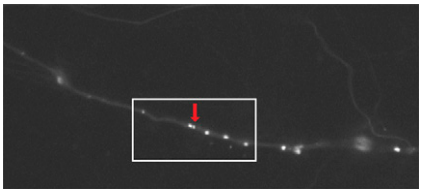
Movie S1. The dynamic movement of *fmr1* granule in one WT neuron before DHPG treatment. The movie corresponds to the granules in the first WT kymograph of Fig. 2*A*. One *Fmr1* RNA particle oscillated before DHPG treatment. Movie S1, S2, S3, S4, S5, and S6 were imaged 5 s per frame for 25 frames (2 min in total).

Movie S1



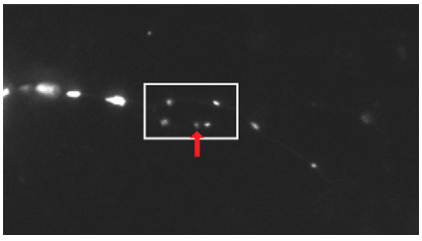
Movie S2. The dynamic movement of *fmr1* granule in one WT neuron at 28 min after DHPG removal. The movie corresponds to the granules in the second WT kymograph of Fig. 2*A*. All RNA particles remained stationary.

Movie S2



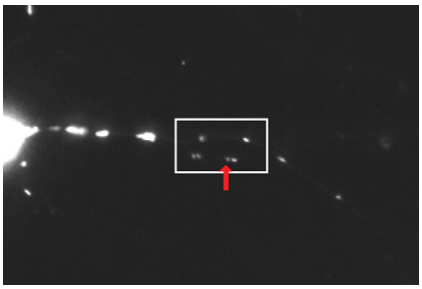
Movie S3. The dynamic movement of *fmr1* granule in one WT neuron at 58 min after DHPG removal. The movie corresponds to the granules in the third WT kymograph of Fig. 2*A*. One RNA particle moved toward distal dendrite.

Movie S3



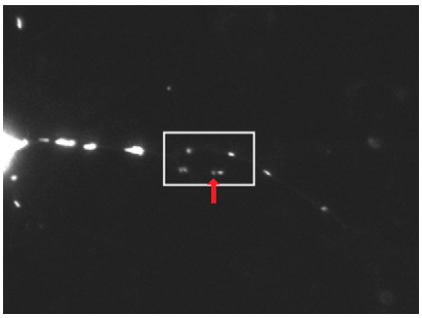
Movie S4. The dynamic movement of *Fmr1* granule in one *fmr1* KO neuron before DHPG treatment. The movie corresponds to the granules in the first *fmr1* KO kymograph of Fig. 2*A*. All RNA particles remain stationary before DHPG treatment.

Movie S4



Movie S5. The dynamic movement of *Fmr1* granule in one *fmr1* KO neuron at 27 min after DHPG removal. The movie corresponds to the granules in the second *fmr1* KO kymograph of Fig. 2*A*. One *Fmr1* RNA particle was oscillating.

Movie S5



Movie S6. The dynamic movement of *Fmr1* granule in one *fmr1* KO neuron at 57 min after DHPG removal. The movie corresponds to the granules in the third *fmr1* KO kymograph of Fig. 2*A*.Most RNA granules remain static again.

Movie S6