EXTENDED EXPERIMENTAL PROCEDURES

Chemicals
Aphidicolin (Sigma), hydroxyurea (Sigma), leptomycin B (Sigma).

Cells and Cell Culture
Cells were grown in DMEM media (GIBCO) supplemented with 10% FBS, 2 mM L-Glutamine, 100 units/ml Penicillin, and 100 μg/ml Streptomycin.

MEFs
FMRF KO MEFs were created with an insertion in exon 5 resulting in FMRP protein null MEFs with low level of FMRP mRNA (The Dutch-Belgian Fragile X Consortium, 1994). DOT1L mutant MEFs, which have a significant reduction in H3K79 methylation levels, were created using a gene trap approach (Steger et al., 2008).

Antibodies
Mouse anti-FMRP (Chemicon, MAB2160), rabbit anti-FMRP (Abcam, ab17722), rabbit anti-H3 (Abcam), mouse anti-H2A.X phosphorylated on Ser 139 (anti-γH2A.X) (Abcam), mouse anti-H2A.X phosphorylated on Ser 139 (anti-γH2A.X) (Millipore), rabbit anti-H2A.X (Abcam), mouse anti-Actin (Abcam), goat anti-ATR (Santa Cruz), rabbit anti-BRCA1 (Bethyl), rabbit anti-mouse BRCA1 (gift of S. Namekawa), rabbit anti-phospho-BRCA1(Ser1423) (Abcam), rabbit anti-phospho-BRCA1(Ser1423) (Mybiosource) (recognizes mouse epitope), rabbit anti-RPA32 (Bethyl), mouse anti-CENTB (Santa Cruz), mouse anti-SYCP3 (Santa Cruz), rabbit anti-SYCP1 (Abcam), goat anti-SYCP1 (Santa Cruz), rabbit anti-DMC1 (Santa Cruz), mouse anti-MLH1 (Millipore), mouse anti-SPO11. Anti-SPO11 hybridoma cell line 180 was generated by M.P. Theelen at Lawrence Livermore National Laboratory, California; the purified monoclonal antibody was provided by S. Keeney, Memorial Sloan-Kettering Cancer Center, New York.

Western Blot Band Intensity Quantification
For western blot quantification we measured band intensity using ImageJ software. In the case of BRCA1 phosphorylation we used Photoshop software. For γH2A.X induction we quantified the γH2A.X band in the “treatment” lane and then compared the value to control treatment, e.g., DMSO or the 0 time point.

Histone MLA Binding Reactions
The binding reactions were performed in binding buffer (50 mM Tris pH 7.5, 1 M NaCl, 2 mM MgCl₂, 0.5% Triton X-100) at 4°C for 1 hr with rotation. After incubation with glutathione agarose beads (GE Healthcare) the reactions were washed 5 times with binding buffer.

Calculation of Chromatin-Associated FMRP
In quantification of FMRP chromatin recruitment, the y axis represents the ratio of chromatin-associated FMRP to total FMRP. It was calculated based on the level of FMRP on chromatin divided by total FMRP. The data are from 3 independent experiments. The relative fold increase in FMRP loading on chromatin in response to aphidicolin (APH) is compared to the levels of chromatin-associated FMRP upon DMSO treatment, which was designated as 1 (DMSO).

Aphidicolin Treatment
APH treatment was performed for 24 hr.

γH2A.X Analysis in MEF Cells after Replication Inhibition by Aphidicolin
MEFs were treated with DMSO or APH (0.5μM) for 24 hr. Cells were then lysed in SDS loading buffer and samples were subjected to western analysis for γH2A.X and total H2A.X. Three independent experiments were performed.

pS1423 BRCA1 Analysis after Replication Inhibition by Aphidicolin
For HeLa cells, the APH treatment was 0.5μM for 24 hr. For 293 and MEF cells, the treatment was 5μM for 24 hr. Cells were then lysed in SDS loading buffer and samples were subjected to western analysis for pS1423 BRCA1 and total BRCA1. The quantification was performed as a ratio of phospho-BRCA1 to total BRCA1. Three independent experiments were performed.

FMRP RNAi Analysis in HeLa Cells and Treatment with Aphidicolin for γH2A.X Induction
HeLa cells were transfected with two different FMRP shRNA constructs (FMRP shRNA1 and FMRP shRNA2) or control scramble shRNA. After 3 days cells were treated with DMSO or aphidicolin (0.5μM) for 24 hr. Cells were then lysed in SDS sample buffer and subjected to western blot using FMRP, actin, γH2A.X, and H2A.X antibodies. Three independent experiments were performed.
Hydroxyurea Treatment Experiments
MEFs were treated with DMSO or hydroxyurea at 2 mM final concentration and incubated for 0, 30, 60, and 120 min. Cells were then lysed in SDS sample buffer and samples were analyzed by western blotting using γH2A.X antibody and H2A.X antibody. Three independent experiments were performed.

UV Irradiation
MEF cells were UV irradiated with a dose of 50 J/m² and cell samples were collected 0, 30, 60, and 120 min postirradiation in SDS sample buffer. Samples were then western blotted for γH2A.X and H2A.X. Three independent experiments were performed.

Ionizing Radiation Treatment
MEFs were treated with 5 Gy of ionizing radiation and samples were collected in SDS loading buffer 30 min postirradiation. Western blots were performed using γH2A.X and H2A.X antibodies. Experiments were performed 3 times.

Colony Survival Assay
For the colony survival assays we used HeLa cells stably expressing FMRP RNAI vector, which were complemented with stably expressing rescue vector, either wild-type FMRP-Flag-HA or Flag-HA alone. Cells were seeded at 200 cells/dish density and allowed to attach. The media was then changed to aphidicolin (APH) containing media (0.05 µM, 0.1 µM, and 0.3 µM) or control media containing DMSO. Cells were incubated for 2 weeks until the appearance of colonies. The colonies were then rinsed in PBS, fixed in methanol and stained with Coomassie stain. Survival was determined by comparing the number of colonies in APH containing media to the number of colonies in control media. Statistical analysis of data from 3 independent experiments was done using 2-way ANOVA. For MEF cells, the protocol was the same except that the cells were treated with 0.01 µM or 0.05 µM of APH, or 0.01 or 0.03 mM of hydroxyurea (HU).

Lentiviral Production
Lentiviral particles were produced as previously described (Lois et al., 2002). Briefly, HEK293FT cells were transfected using the calcium phosphate method with FIV-CMV-pEGFP-FMRP (gift, G.J. Bassel, Emory University) and packaging vectors. The supernatants containing the viral particles were collected 48 hr after transfection and concentrated using ultracentrifugation. Viral titers were determined by serial dilution of HEK293FT cells as 10³ per ml. Primary cell cultures were transduced by adding concentrated lentivirus to the growth media.

Hippocampal Neuron Cell Culture and Infection
Hippocampal primary neurons were dissected from WT and Fmr1 KO mice at embryonic day 16.5 and cultured as described previously (Bassell and Warren, 2008) in accordance with the Institutional Animal Care and Use Committee guidelines. Cells were plated (2000 cells/cm²) on poly-1-lysine-coated Biotech coverslips (1 mg/ml) in MEM with FBS (10%) for 2 hr, supplemented with B-27 and Gluta MAX-1 (Invitrogen, San Diego, CA). Neurons were transduced with lentivirus at 14 days in vitro (DIV). Six hours posttransduction, live neurons were processed for the AMPAR internalization assay. FMRP KO MEFs were reconstituted with FMRP-Flag-HA constructs using pMSCV-based viral vector system.

Constitutive AMPAR Internalization Assay
The assay was performed as described previously for rat neurons (Nakamoto et al., 2007) with modifications (Bhattacharya et al., 2009). Surface AMPARs in live Fmr1 knockout mouse neurons were labeled with a rabbit polyclonal antibody against the N terminus of the GluR1 subunit (Calbiochem, 1:5 in conditioned media) for 15 min at 37°C, 0.5% CO₂. After incubation with antagonists (1 µM TTX, 10 µM NBOX, and 50 µM APV; Tocris) in conditioned media for 15 min at 37°C, 0.5% CO₂, cells were fixed in 4% FA for 15 min on ice, and labeled surface GluR1s were visualized with saturating with Alexa 546 conjugated goat-anti-rabbit secondary antibody (Invitrogen). After permeabilization with 0.1% Triton X-100 for 30 min at room temperature, internalized GluR1s were saturated with Alexa 633-conjugated donkey anti-rabbit secondary antibody. Raw images were taken with a Zeiss confocal microscope and LSM software, and were compared between samples in the same culture preparation. Signal in identical areas of thick distal dendrites (>20 µm from the soma) were measured, p values were determined using one-way ANOVA (α = 0.05) with Bonferroni’s post hoc test.

Microscale Thermophoresis from Nanotemper Technologies
The process involves measurement of the fluorescence distribution of fluorescently labeled molecules inside a capillary upon laser irradiation. A temperature gradient is generated by an IR-Laser focused into the capillary. Binding affinities are calculating measuring a temperature jump in the initial stage of irradiation, thermophoretic movement of the molecules within the gradient at later stages, or both (Jäbake-Willemansen et al., 2011; Wienken et al., 2010). Independent experiments were performed a minimum of 3 times for each histone modification. In MST experiments using MLA histones, the Agenet domain was labeled, Histone MLAs were prepared as described (Simon et al., 2007) and purchased from Active Motif. Histone binding reactions with the Agenet domain were performed in binding buffer (50 mM Tris pH 7.5, 1 mM NaCl, 2 mM MgCl₂, 0.5% Triton X-100) at room temperature for 30 min. In the case of the nucleosome MST reaction recombinant H3K79me2 nucleosome was labeled. Mononucleosomes were prepared as described (Lu
et al., 2008), and incubation in binding buffer (50 mM Tris pH 7.5, 150 mM NaCl, 2 mM MgCl₂, 0.5% Triton X-100) was for 3 hr at room temperature. Data were plotted using GraphPad Prizm software.

**Fmr1 KO Mice**

Fmr1 KO mice (allele tm) were congenic on a C57BL/6J background; same mutation as used for Fmr1 KO MEFs) were a kind gift from Mark Bear. Mice were kept under standard conditions and all experiments were conducted in compliance with the Animal Welfare act and approved by the Animal Care and Use Committee at the appropriate institution.

**Dot1L cKO Mice**

Dot1L conditional KO mice were a kind gift from Scott Armstrong. To generate a germ-cell-specific Dot1L deletion, a germ-line-specific Cre transgene (Mvh-Cre, [Hu et al., 2013]) was crossed into the Dot1L background. Mvh-Cre;Dot1L cKO male mice (germ cell genotype Dot1L cKO) were mated to Dot1L females, so that F1 males were either Dot1L cKO or Dot1L cKO.Conditional KO (cKO) animals were Cre-positive Dot1L cKO males (germ cell genotype Dot1L cKO), and Cre-negative Dot1L cKO males were used as littermate controls.

**SUPPLEMENTAL REFERENCES**


