Supplementary Figure 1
Expression of Fmrp in early cortical development.

a) Representative western blot showing Fmrp expression during two developmental stages (E14 and E17) in WT and Fmr1−/− embryonic somatosensory cortex. Ribosomal protein S6 (rpS6) was used as loading control. b) Confocal images showing the distribution of WT and Fmr1−/− neuronal cells, upon IUE with the pCAG-EGFP plasmid at E14.5, in mouse cortices at P0. EGFP fluorescence (green) and DAPI staining (blue). Right histogram shows the frequency distribution and quantification of EGFP positive cells in ten equal bins (VZ 1 to CP 10) in WT (n = 6), Fmr1−/− (n = 5), Two-way ANOVA; F (10, 165) = 51.97, Values: mean ± SEM. Scale bars = 50 µm.
Absence of Fmrp does not affect proliferation of precursor cells at E14.5.

a) Left, confocal images from WT and Fmr1<sup>−/−</sup> E14.5 mouse cortices stained with markers for the intermediate (Tbr2-positive) and radial (Sox2-positive) progenitor populations (anti-Tbr2, green; anti-Sox2, red). Right, the graph represents the ratio of Tbr2-positive to Sox2-positive in WT and Fmr1<sup>−/−</sup> cortices. Scale bars = 20 μm. (Values are mean ± SEM; two tailed unpaired t-test: \( P = 0.1503, \ t=1.776, \ df = 4, \ n = 3 \) WT animals and \( n = 3 \) Fmr1<sup>−/−</sup> animals).

b) Histogram represents the percentage of cells transfected at E14.5 with pCAG-EGFP that are Ki67-positive in the VZ/SVZ/IZ of WT and Fmr1<sup>−/−</sup> cortices. (Values are mean ± SEM; two tailed unpaired t-test: \( P = 0.423, \ t = 0.86, \ df = 6, \ n = 3 \) WT animals and \( n = 3 \) Fmr1<sup>−/−</sup> animals).
Supplementary Figure 3

Morphology and positioning of radial glia cells are not affected in Fmr1<sup>+/−</sup> cortices.

a) Individual radial glia cells in E15.5 WT and Fmr1<sup>+/−</sup> embryos were labeled, by IUE, using a BLBP-EGFP (Brain Lipid Binding Protein promoter specific for radial glia cells) construct. Brain sections were analysed at E16.5. Fmr1<sup>+/−</sup> radial glia cells display normal morphology spanning the entire cortex (green arrow-heads) and have apical (yellow arrow-heads) and basal (red arrow-heads) endfeet as observed in WT. b) Coronal section from E17.5 WT and Fmr1<sup>+/−</sup> littermates labeled with anti β-catenin (upper panel) and γ-tubulin antibodies (lower panels). Scale bars = 50 μm.
Supplementary Figure 4

Migration in vitro of the Fmr1−/− neurons is not affected.

WT and Fmr1−/− cells were isolated from E14.5 embryonic cortices, left to form aggregates and embedded in Matrigel 18 h later. Sequential images show four time points of WT and Fmr1−/− neurons migrating away from the in vitro explants. Both average velocity and directionality (total path length from time 0 to time x/ net distance time 0 to time x) were not different between WT and Fmr1−/− neurons: Average velocity: WT = 0.1537 μm/min ± 0.0226, n = 6 (71 neurons); Fmr1−/− = 0.1609 μm/min ± 0.01687, n = 5 (59 neurons); unpaired two tailed t-test: P = 0.8122, t = 0.2447, df = 9, n = number of explants. Directionality: WT 0.6629 ± 0.03070, arbitrary units; Fmr1−/− 0.6642 ± 0.06615 arbitrary units, P = 0.9857, t=0.01844, df=9. Scale bar = 10 μm.
Absence of Fmrp does not alter cortical layering.

Left, immunohistochemistry showing E17.5 confocal images of coronal sections from WT and Fmr1<sup>−/−</sup> cortices labeled with anti-COUP-TF-interacting protein 2 antibody (Ctip2, transcription factor) for lower layer neurons and anti-Cut homeobox 1 gene antibody (Cux1, transcription factor) for differentiated neurons in the upper layer. Scale bars = 50 µm. Right, quantification of the average line profiles throughout the cortex (n = 3). Normalization was performed taking into account the slice thickness. Displayed is the average plot profile.
Supplementary Figure 6

Activity-driven calcium events were correlated to action potential firing of individual neurons.

a) Cell numbers within networks in WT and postnatal (P0-P7) Fmr1−/− brains (values are mean ± SEM; ANOVA: age 0 (P < 0.0001), genotype (p = 0.64), genotype*age interaction (p = 0.504) F(3,88)=8.618, n = 21 pups and 53 slices from WT and n = 21 pups and 44 slices from Fmr1−/− animals) b) Individual events from sample traces during aCSF baseline and following TTX application. Grey bar indicates events in aCSF condition. c) Cell-attached patch recording showing correlated synchronization of events to AP spiking in cell and neighbor. d) Raster plot for corresponding slice showing network activity across all cells for baseline and TTX perfusion in 4 min recording blocks. e) TTX blockage effect on cell activity across the network.
Supplementary Figure 7

Representative Western blotting.

(a) Representative Western blotting (entire membrane) showing N-cadherin expression in E14.5 embryonic somatosensory cortex lysates from WT and Fmr1<sup>−/−</sup> mice. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been used as the internal control for normalization. This entire gel refers to the experiment described in Figure 4a. 

(b) Representative Western blotting (entire membrane) showing Fmrp immunoprecipitation performed from E17 WT and Fmr1<sup>−/−</sup> cortical extracts. This entire gel refers to the experiment described in Figure 4b.