Supporting Information

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SI Materials and Methods

Experimental Animals. *Fmr1* KO mice (FVB) and YFP-H (C57BL/6J) used at New York University were obtained from the Jackson Laboratory. FVB *Fmr1* KO and C57BL/6J YFP-H mice were first crossed to generate F_1 female mice ($Fmr1^{+/-}$), which were backcrossed with the YFP-H C57BL/6J males. From this breeding paradigm, both WT and KO littermates could be obtained. Only male mice were used in this study. Some of the heterozygous F_2 female mice were used as breeders to get subsequent WT and KO littermates. Genotyping was performed using the same primers and protocol (version 2.1) as the ones used in the Jackson Laboratory. In the second breeding paradigm used at University of Illinois, either C57BL/6J WT or *Fmr1* KO (from lines maintained at Dr. Greenough's laboratory and recrossed every three to four generations) were bred with YFP-H C57BL/6J mice to obtain either WT or *Fmr1* KO F_1 litters. The significant differences between the two breeding paradigms include (*i*) different background strains, (*ii*) different recent origin of the *Fmr1* KO gene segment from FVB versus C57BL/6J (thus including any piggy-backing genes that could inadvertently differ between WT and KO mice), and (*iii*) Use of littermate controls (New York University) vs. F_1 crosses of YFP-H mice to separately maintained WT/KO lines (University of Illinois).

In Vivo Transcranial Imaging and Data Quantification. A transcranial two-photon imaging technique was used to follow identified spines of layer 5 pyramidal neurons in the primary somatosensory barrel cortex of living transgenic mice expressing YFP. The Ti-sapphire laser was tuned to the excitation wavelength for YFP (920 nm) at a low laser power (20 mW on the sample) to minimize the possibility of phototoxicity. A stack of image planes was acquired by using a water immersion objective lens (60×, 1.1 numerical aperture; Olympus), an external detector, and a digital zoom of 3.0×. The imaging depth was between 15 and 150 μm from the pial surface, and the step size was 0.75 μm; 2D projections of 3D image stacks containing dendritic segments of interest were used for all figures. Filopodia turnover rates are calculated as $(F_{\text{formed}} + F_{\text{eliminated}})/2F_{\text{total}}$, where F_{formed} is the number of newly formed filopodia, $F_{\text{eliminated}}$ is the number of eliminated filopodia, and F_{total} is the total number of filopodia at the first view. Data throughout the text is presented as mean ± SEM. *P* values were calculated using the Student's *t* test, except where otherwise noted.

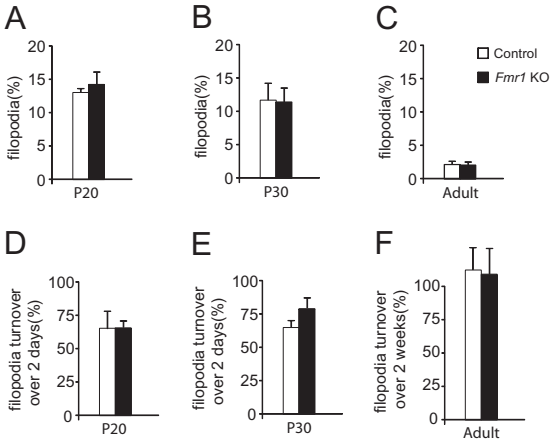

Fig. S1. Filopodia percentage and their turnover rates were similar between WT and *Fmr1* KO animals. (*A*–*C*) The percentages of dendritic filopodia among all protrusions were not significantly different between WT control and *Fmr1* KO mice at postnatal day 20 (*A*), postnatal day 30 (*B*), and in adulthood (*C*). (*D*–*F*) The filopodia turnover rates were similar between WT and *Fmr1* KO animals over 2 d at postnatal day 20 (*D*), postnatal day 30 (*E*), and over 2 wk in adults (*F*).

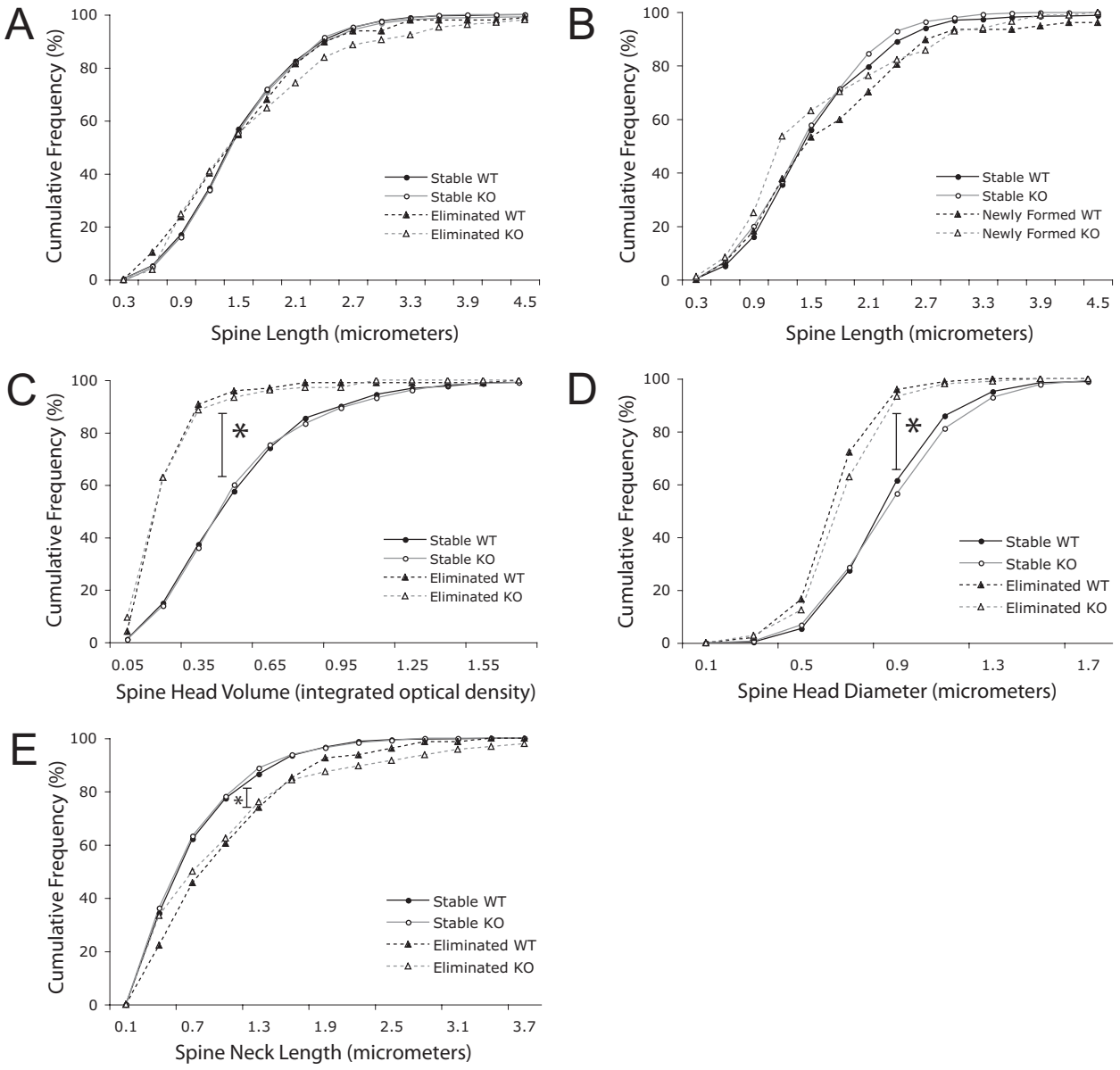

Fig. S2. Measures of spine morphology in stable and unstable spines. Spine size distribution was examined by plotting the cumulative frequency of sizes of all examined spines and comparing distributions using the Kolmogorov–Smirnov test $*P < 0.05$. (*A*) Total spine length was not significantly different between stable and eliminated spines or (*B*) stable and newly formed spines. (*C*) Spines that were eliminated over 2 d in 1-mo-old animals had a smaller head size compared with spines that persisted during both imaging sessions (stable spines), using a measure of spine volume (integrated optical density), and (*D*) spine head diameter. (*E*) Spine neck length, excluding neckless spines, was significantly longer in eliminated spines compared with stable spines.

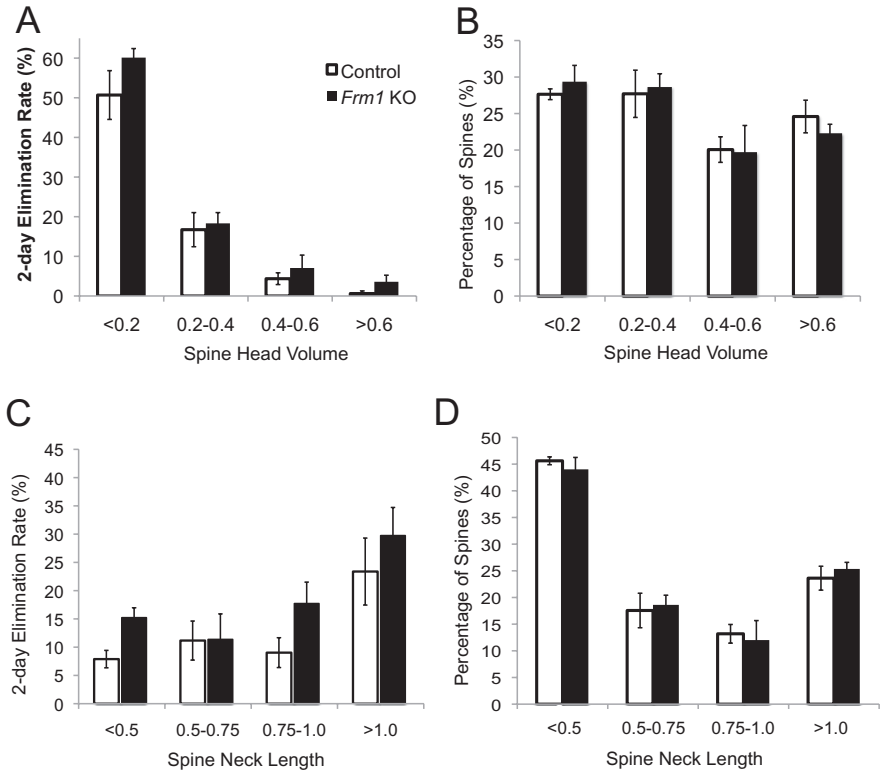

Fig. S3. The 2-d spine elimination rate for all spines (including filopodia) grouped by measures of morphology in 1-mo-old WT and *Fmr1* KO mice. Spine head volume (integrated optical density) (*A*) and spine neck length (μm) (*C*) were found to have a significant effect on the probability of elimination (repeated measures ANOVA; $P < 0.0001$ and $P < 0.01$, respectively), whereas there was no significant effect of genotype ($P > 0.05$). The percentage of spines in each category, as grouped by head volume (*B*) or neck length (*D*), was not different between the genotypes in these animals ($P > 0.05$).