

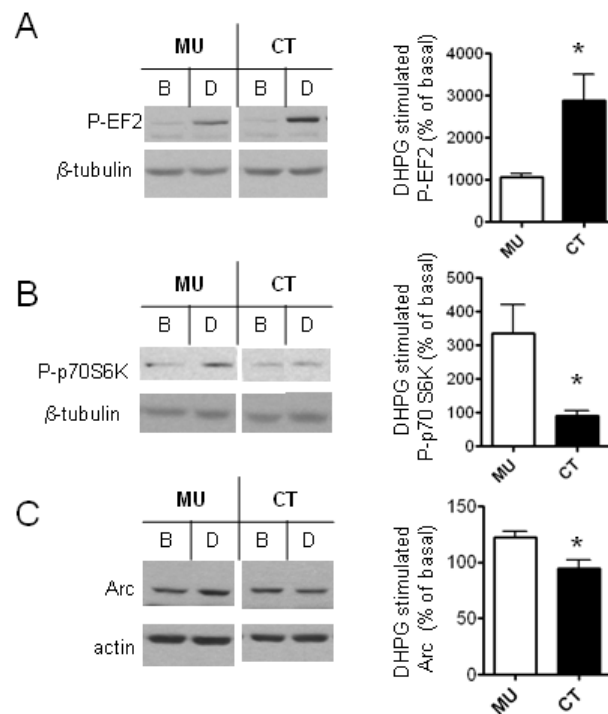
Disrupted mGluR5-Homer scaffolds mediate abnormal mGluR5 signaling, circuit function and behavior in a mouse model of Fragile X Syndrome

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Wildtype mouse slices	MU		CT		n
	Mean	SEM	Mean	SEM	
Basal					
P-mTOR/mTOR	1.39	0.37	2.08	0.64	15
P-p70S6K/p70S6K	1.07	0.4	0.89	0.16	7
P-EF2/EF2	0.97	1.46	0.7	0.69	14
P-ERK/ERK	0.4	0.11	0.48	0.13	4

Rat slices	MU						CT					
	Basal			DHPG			Basal			DHPG		
Basal (% of MU)	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
P-EF2	100	9	13				121	24	13			
Total EF2	100	11	9	116	18	10	131	16	10	98	8	9
Arc	100	5	11				102	10	14			

Supplementary Table 1: mGluR5CT peptide treatment does not affect the basal levels of phosphoproteins examined or total levels of EF2 or Arc in comparison to mGluR5MU. Related to Figure 1. *Upper:* Quantitative western blotting of acute hippocampal slices from WT mice demonstrates that mGluR5CT peptide (CT) treatment has no effect on basal levels P-mTOR, P-p70S6K, P-EF2 and P-ERK in comparison to mGluR5MU (MU) control peptide treated slices. For this analysis phosphoprotein levels were normalized to total protein levels from the same slice homogenates (e.g. P-mTOR/mTOR), each of which was first normalized to tubulin loading control. *Lower:* mGluR5CT peptide treatment of rat hippocampal slices has no effect on basal levels of Phospho-EF2, total EF2, or Arc in comparison to MU treated slices. Previous work demonstrated no effect of the CT peptide basal Phospho or total S6K¹. DHPG treatment does not affect total EF2 levels. Data is normalized to within animal, mGluR5MU peptide treated slices and expressed as the mean \pm SEM; n = # slices.

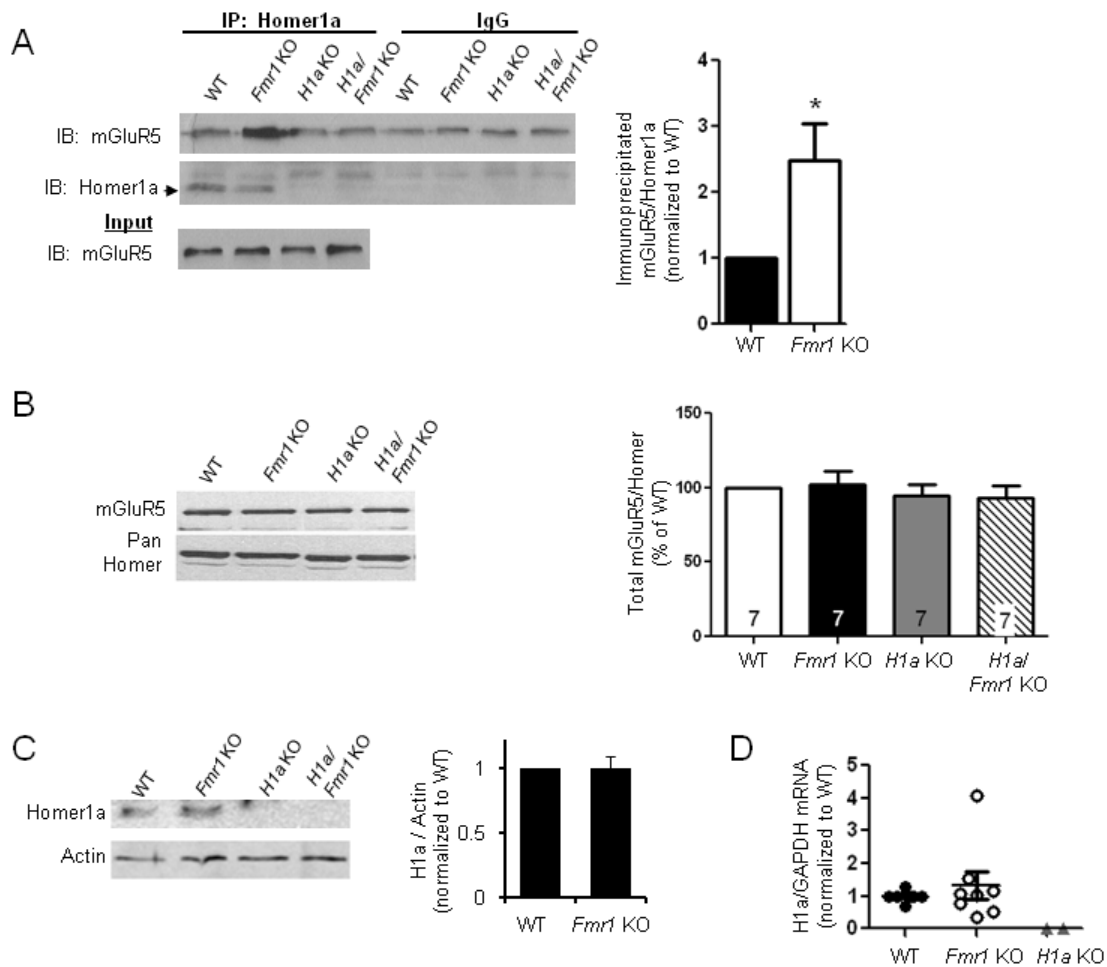


Supplementary Figure 1. Disruption of mGluR5-Homer scaffolds in rat hippocampal slices bidirectionally regulates mGluR signaling to translation initiation and elongation and blocks synthesis of Arc. Related to Fig. 1 **A**. Pretreatment of rat hippocampal slices with a peptide that disrupts mGluR5-Homer interactions (tat-mGluR5CT; CT; 5 hours; 5 μ M) results in enhanced DHPG-induced (100 μ M; 5 min) phosphorylation of EF2 in comparison to slices treated with a control peptide that does not disrupt mGluR5-Homer interactions (tat-mGluR5MU; MU) (CT; 2876 \pm 633% of basal; n = 7 rats; MU; 1060 \pm 87% of basal; n = 6). Left: Representative western blot of Thr56 phosphorylated (P)-EF2 and β -tubulin (loading control) in the basal (B), or untreated, condition and DHPG (D) treated slices. Right: To obtain group data for panels A-C, in each rat, levels of P-EF2 or P-p70S6K or Arc in DHPG treated slices were normalized a loading control (tubulin or actin) and expressed as a percentage of basal levels (as measured in untreated slices) and averaged across animals. **B**. For a subset of the samples analyzed in panel A, DHPG-induced Thr389 phosphorylation of p70S6K was evaluated. MU pretreated slices exhibited robust DHPG-induced phosphorylation of p70S6K, whereas in CT pretreated slices DHPG induced P-p70S6K was blocked (CT; 91 \pm 15% of basal, n=5; MU: 337 \pm 83% of basal, n=4). **C**. In a separate set of animals, DHPG-induced increases in total Arc protein is blocked with CT peptide (CT: 95 \pm 7% of basal, n=7; MU: 122 \pm 5% of basal, n=7). *p < 0.05.

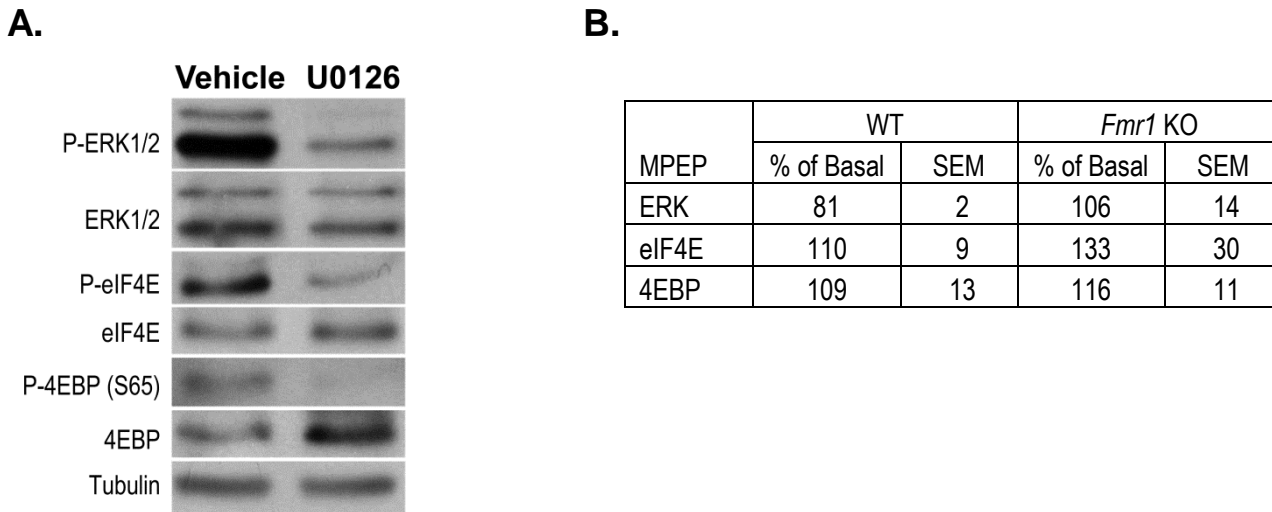
	WT			Fmr1 KO			H1a KO			H1a/Fmr1 KO		
% of WT	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
P-p70S6K	100	9	12	125	23	13	90	7	13	123	25	11
P-EF2	100	11	9	86	16	9	89	25	8	106	16	8
T-p70S6K	100	5	4	101	5	4	102	10	4	102	9	4

	WT						Fmr1 KO						H1a KO						H1a/Fmr1 KO					
% of WT basal	Basal			DHPG			Basal			DHPG			Basal			DHPG			Basal			DHPG		
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
T-EF2	100	7	5	130	15	5	94	19	5	109	31	4	118	29	4	120	24	5	117	13	4	141	22	4

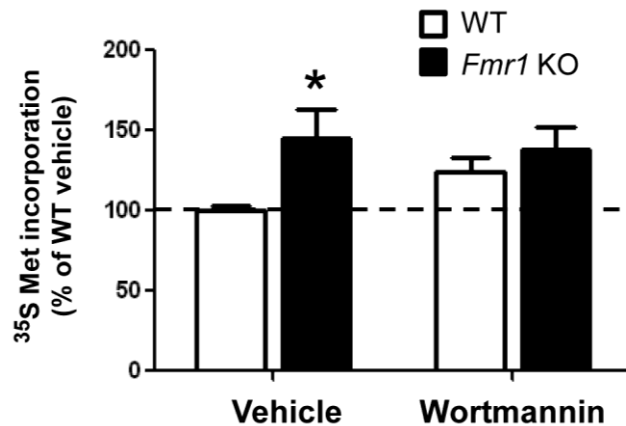
Supplementary Table 2: *H1a* or *Fmr1* genotype does not affect basal levels of P-p70S6K, P-EF2 or total EF2. Upper: Basal levels of phosphorylated or total EF2 or p70S6K are not different in acute hippocampal slices across genotypes (WT, *Fmr1* KO, *H1a* KO, or *H1a/Fmr1* KO). Data is normalized to levels in slices from WT littermates and expressed as the mean \pm SEM; n = # slices. Lower: There is no effect of genotype or DHPG treatment on total EF2 levels in acute hippocampal slices. Data is normalized to levels from untreated slices of WT littermates and expressed as the mean \pm SEM; n = # slices.



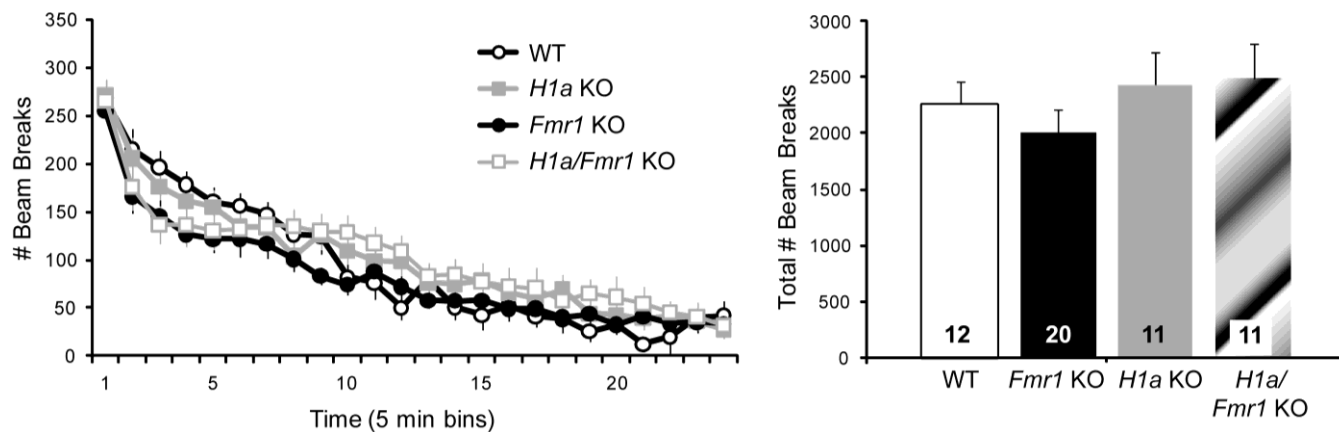
Supplementary Figure 2: H1a-mGluR5 interactions are elevated in *Fmr1* KO mice, but total levels of H1a, long Homers or mGluR5 are unchanged. Related to Figure 2. **A.** mGluR5-Homer1a interactions are increased in hippocampal homogenates of *Fmr1* KO mice. Homer1a was immunoprecipitated from total mouse hippocampal lysate. The level of mGluR5 that coimmunoprecipitated with Homer1a was quantified and normalized to levels of immunoprecipitated Homer1a. Arrowhead indicates specific H1a band that is absent in the H1a KO lysates. There is a low level of mGluR5 that nonspecifically pulls down with goat IgG. Group data from 4 set of littermates reveals an increase in Homer1a/mGluR5 in *Fmr1* KO. * $p < 0.05$. **B.** Total mGluR5 and long Homer (with pan-Homer antibody) is normal in WT, *Fmr1* KO, *H1a* KO and *H1a/Fmr1* KO. **C.** Homer1a protein levels (normalized to actin) are unaffected in total lysates of whole hippocampus of *Fmr1* KO mice. **D.** Quantitative RT-PCR hippocampal H1a and GAPDH mRNA reveal there is no change in H1a mRNA levels in *Fmr1* KO mice. Note: Homogenates prepared from *H1a* KO mice demonstrate the specificity of the H1a RT-PCR primers.



Supplementary Figure. 3. Inhibition of ERK activation in hippocampal slices reduces or abolishes phosphospecific antibody detection of P-ERK, P(S209) eIF4E, and P(65) 4EBP. Related to Fig. 3. Acute hippocampal slices from WT mice were preincubated in the MEK inhibitor U0126 (20 μ M) or vehicle (0.01% DMSO) for 30 min prior to lysis and western blotting. **B. MPEP treatment of hippocampal slices from WT or *Fmr1* KO mice has no effect on total levels of ERK, eIF4E or 4EBP.** Related to Fig. 3F. Acute hippocampal slices were incubated in MPEP (10 μ M) or untreated (basal). Total protein levels for ERK, eIF4E, 4EBP were normalized to a loading control (tubulin) on the same blot each for MPEP and untreated slices. Representative blots of this data are shown in Fig. 3F₁. (n = 2 slices/mouse; 4- 8 mice/condition).



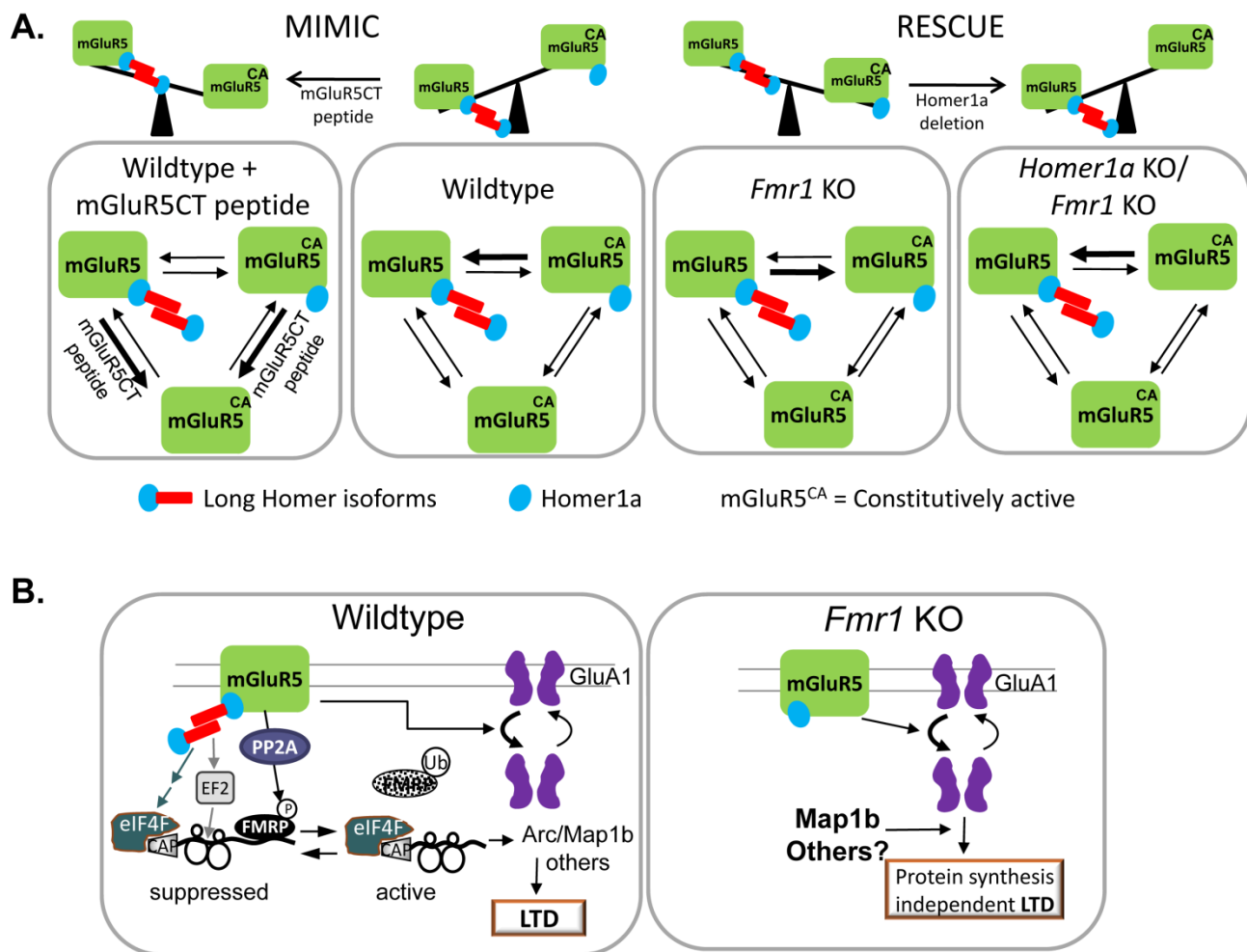
Supplemental Figure 4: Effects of the PI3K inhibitor, wortmannin, on protein synthesis rates in WT and *Fmr1* KO hippocampal slices. Related to Figure 3C. Acute hippocampal slices from *Fmr1* KO mice display elevated protein synthesis rate in comparison to WT littermates as measured by incorporation of ^{35}S Met/Cys into total protein. Slices from WT or *Fmr1* KO mice were preincubated in the PI3K inhibitor wortmannin (100 nM; 30 min) prior to ^{35}S Met incorporation (n = 12 slices/ 6 mice per condition). Inhibition of PI3K using wortmannin (100 nM) pathway equalizes protein synthesis rates between WT and *Fmr1* KO slices, but this may be due to an increase in protein synthesis rates in WT slices ($p < 0.05$; t-test), as opposed to a decrease of protein synthesis rates in *Fmr1* KO slices.



Supplemental Figure 5: *Fmr1*, *H1a*, or *H1a/Fmr1* KO do not affect locomotor activity and therefore do not underlie the rescue of open field activity. Related to Fig. 6. Locomotor activity, as measured by number of beam breaks per unit time, is unaffected by *Fmr1* KO, *H1a* KO or *H1a/Fmr1* KO. N = # of mice is indicated on each bar.

	Wild running	Seizure (clonic/tonic)	Status Epilepticus	% Incidence
WT	1/17	1/17	0/17	5.8%
<i>Fmr1</i> KO	29/41	25/41	14/41	61.0%
<i>H1a</i> KO	1/24	0/24	0/24	0.0%
<i>H1a</i> KO/<i>Fmr1</i> KO	20/38	13/38	8/38	34.2%*

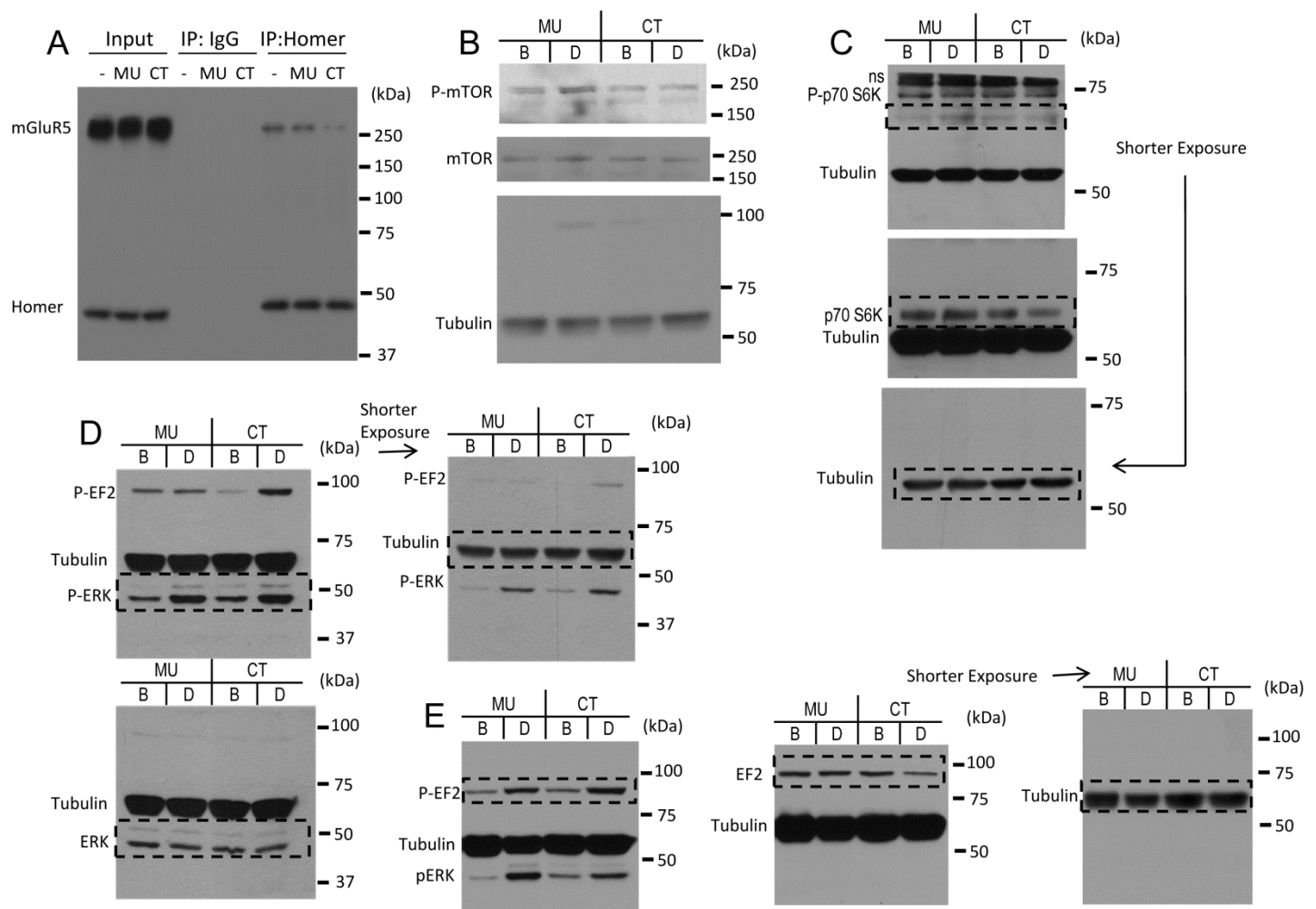
Supplementary Table 3. Seizure occurrence and severity in WT, *Fmr1* KO, *H1a* KO and *H1a/Fmr1* KO genotypes. * $p < 0.05$ Fisher's Exact test. Related to Fig. 6.



Supplementary Figure 6. Model illustrating two mechanisms for mGluR5 dysfunction in Fragile X Syndrome **A.** An altered balance of long and short Homer isoforms bound to mGluR5 accounts for many *Fmr1* KO mouse phenotypes. There is a competition and equilibrium and between long and short Homer (Homer1a) isoforms bound to mGluR5. When bound to the long Homer isoforms, mGluR5 is scaffolded to its effectors (other Homer interacting proteins) and normally gated by glutamate. Free mGluR5 (no Homer bound) or H1a bound mGluR5 is uncoupled from its effectors and constitutively active (mGluR5^{CA})². Therefore, free mGluR5 and H1a bound mGluR5 are functionally equivalent. In WT mice, there is more mGluR5 bound to long Homer isoforms, in comparison to WT mice (Fig. 2A). In contrast, in *Fmr1* KO mice, more mGluR5 is bound to the short Homer isoform, Homer1a (Supplementary Fig. 2). The imbalance in long Homer and H1a bound mGluR5 in *Fmr1* KO mice can be reversed by genetic deletion of H1a which reestablishes the normal levels of long Homer bound mGluR5, mGluR5 signaling (Fig. 2) and many *Fmr1* KO phenotypes. (Figs. 3, 5, 6). In WT mice, a peptide consisting of the C-terminal Homer interacting motif of mGluR5 (mGluR5CT) disrupts both short and long Homer interactions with mGluR5 and shifts the balance from long Homer bound mGluR5 to free mGluR5 which would be expected to mimic H1a bound

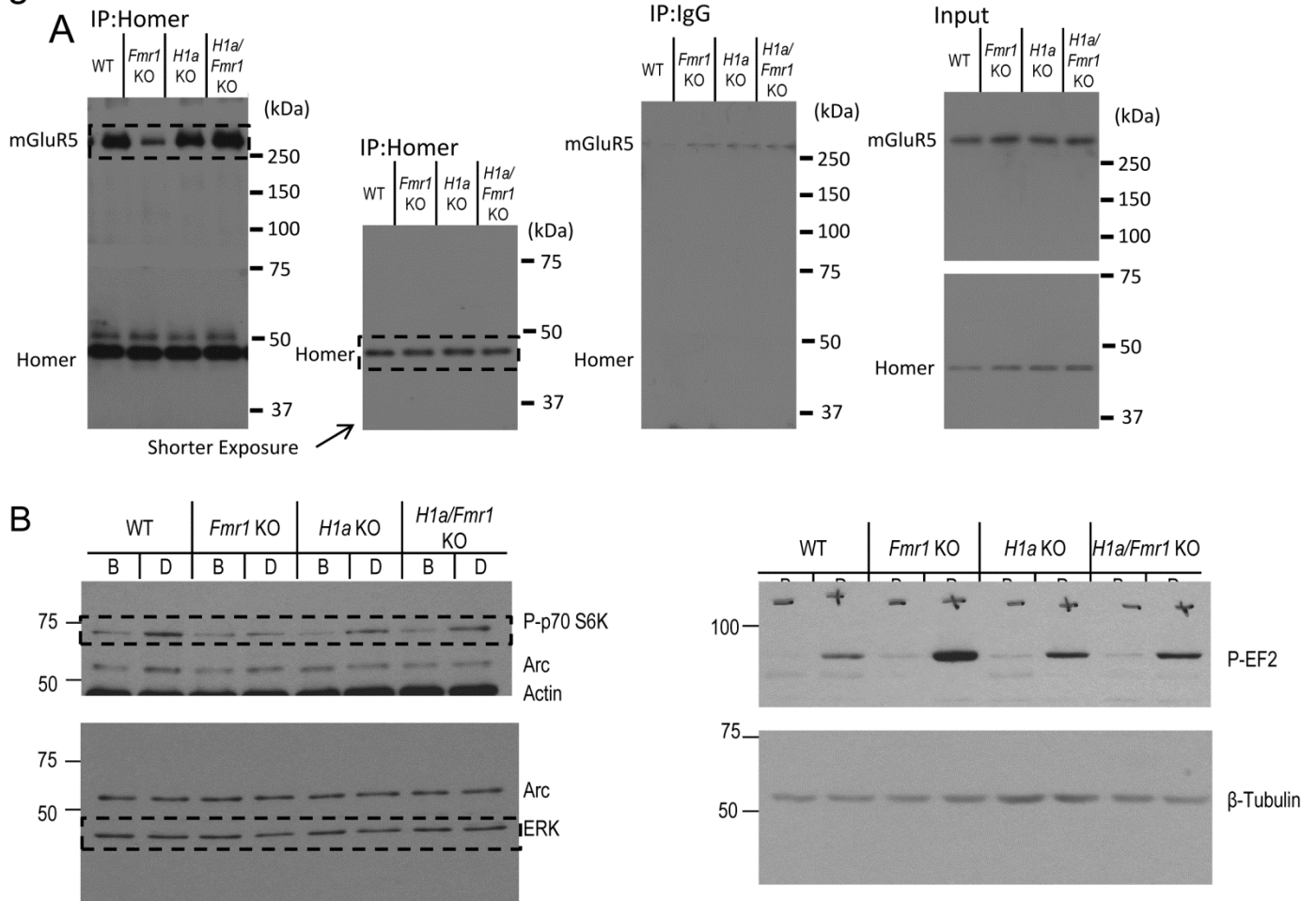
mGluR5 with respect to decreased scaffolding to effectors and constitutive activity. mGluR5CT peptide mimics the altered mGluR5 signaling, enhanced protein synthesis rates and neocortical hyperexcitability observed in *Fmr1* KO mice (Figs. 1, 3, 5). **B. Alterations in mGluR-LTD are hypothesized to be due to loss of FMRP-mediated translational control of dendritic mRNAs such as Arc and Map1b and not altered mGluR5-Homer scaffolds.** In WT mice, brief activation of mGluR5 causes a rapid endocytosis of postsynaptic AMPARs (primarily GluA1) as well translation of Arc and Map1b which are required for LTD and/or the prolonged decrease in surface AMPARs^{3, 4}. FMRP interacts with Arc and Map1b mRNA and is thought to suppress steady state translation of these and perhaps other LTD promoting proteins (reviewed in³). mGluRs rapidly stimulate dephosphorylation of FMRP through PP2A as well as ubiquitination and degradation of FMRP which results in translational activation of FMRP interacting mRNAs⁵ (reviewed in⁶). mGluR5-triggered Arc translation requires both mGluR5-Homer interactions (to trigger translation initiation and regulate EF2; Fig. 1; Supp. Fig. 1), as well as FMRP (Fig. 4) indicating multiple levels of translational control of Arc^{7, 8}. In *Fmr1* KO neurons, loss of steady state translational suppression of Map1b and other mRNAs leads to elevated protein expression in *Fmr1* KO neurons⁷⁻⁹ which may allow mGluR-LTD to persist in the presence of protein synthesis inhibitors. Restoration of mGluR5-Homer scaffolds in *Fmr1* KO mice by deletion of H1a does not rescue elevated MAP1b levels, altered mGluR-LTD or mGluR-stimulated Arc synthesis (Fig. 4).

Fig. 1



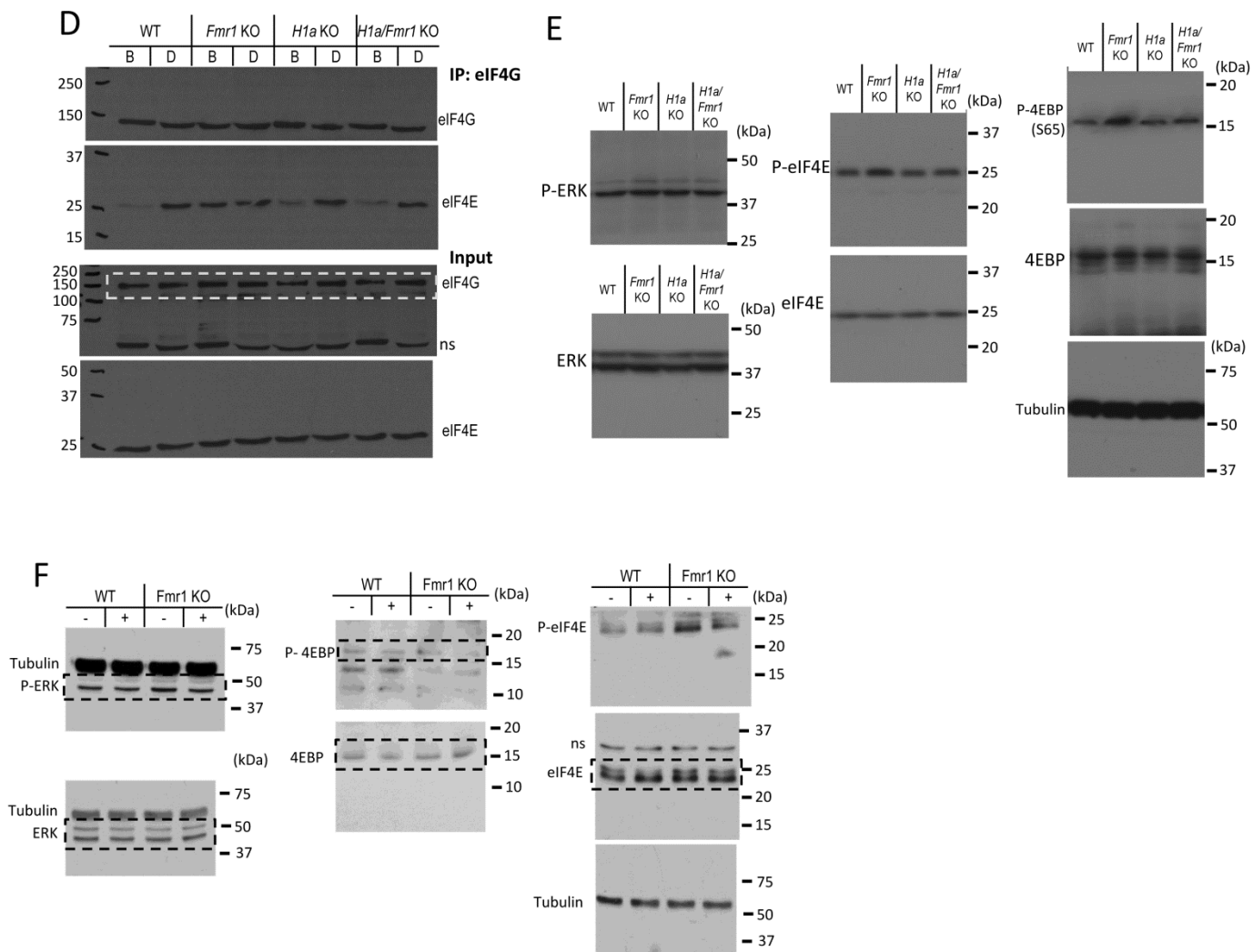
Supplementary Figure 7. Full length western blots for Figure 1. A) Samples were loaded on a 10% SDS gel and blotted for mGluR5 and Homer. B-C) Samples were loaded into two 6% SDS gels. One gel was blotted for P-mTOR, P-S6K, and Tubulin. The other gel was blotted for the total levels of mTOR, S6K, and tubulin. B) The membrane was cut between 100 and 150 kDa. C) A lighter exposure of tubulin was used for quantification. D-E) Samples were loaded into two 8% SDS gels. One gel was blotted for P-ERK, P-EF2, and Tubulin. The other gel was blotted for the total levels of ERK, EF2, and tubulin. A lighter exposure of tubulin was used for quantification. On blots with multiple bands, dotted lines indicate the bands that were used for quantification and inclusion in group data in Figure 1.

Fig.2



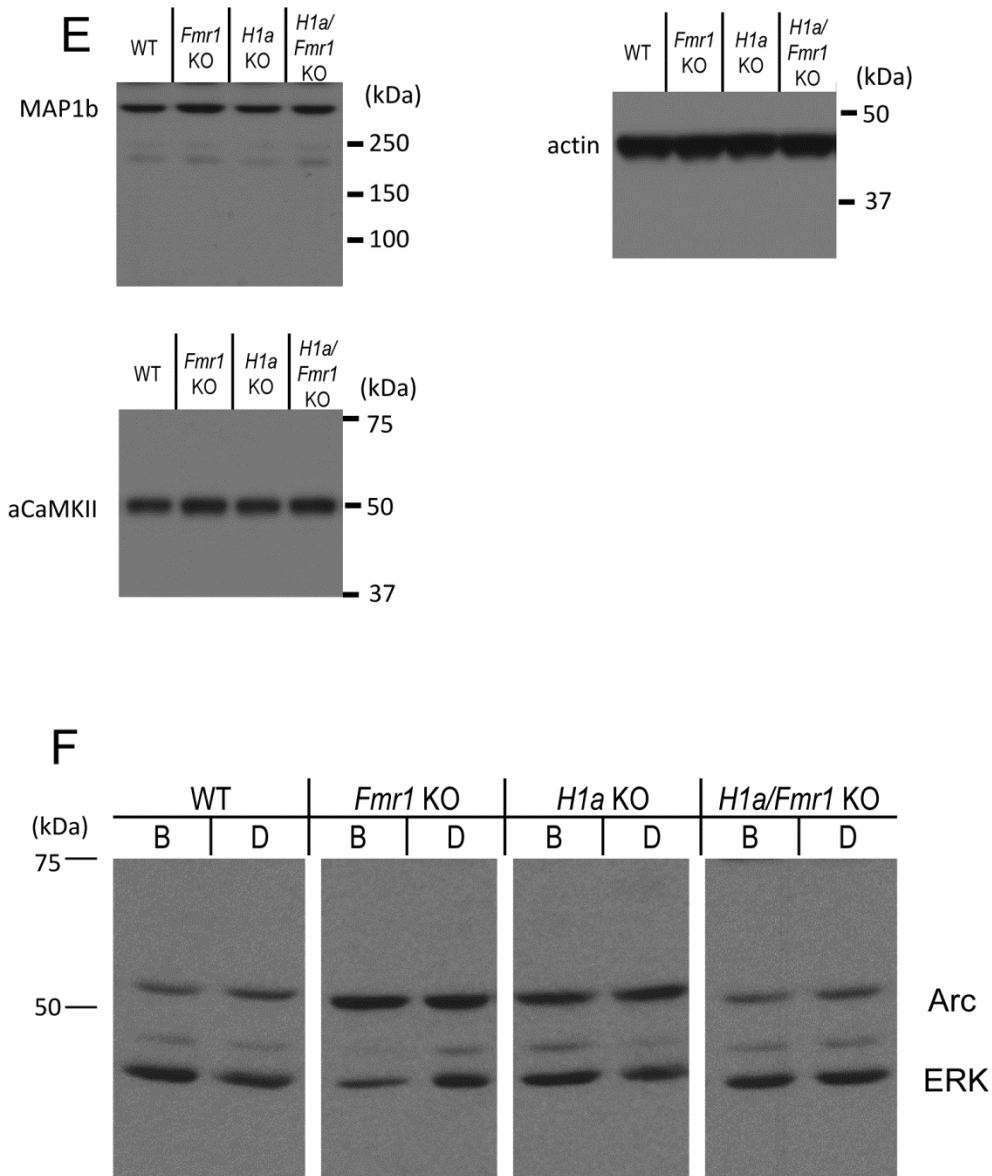
Supplementary Figure 8 Full length western blots for Figure 2. A) Samples were loaded on a 10% SDS gel and blotted for mGluR5 and Homer. The membrane was cut between 75 and 100 kDa. A lighter exposure of the Homer band in the IP was used for quantification B) Membranes were blotted for P-S6K and ERK. C) Membranes were blotted for P-EF2 and Tubulin. The membrane was cut at 75 kDa. On blots with multiple bands, dotted lines indicate the bands that were used for quantification and inclusion in group data in Figure 2.

Fig. 3



Supplementary Figure 9. Full length western blots for Figure 3. D) Samples were loaded on a 10% SDS gel and then cut and blotted for either eIF4G or eIF4E. E-F) Samples were loaded into two 12% SDS gels. One gel was blotted for P-ERK, P-eIF4E, P-4EBP (S65), and Tubulin. The other gel was blotted for the total levels of ERK, eIF4E, 4EBP and tubulin. On blots with multiple bands, dotted lines indicate the bands that were used for quantification and inclusion in group data in Figure 3.

Fig.4



Supplementary Figure 10. Full length western blots for Figure 4. E) Samples were loaded on a 10% SDS gel and blotted for Mab1b, actin and α CamKII. The membrane was cut between 75 and 100 kDa. F) Samples were loaded into a 10% SDS gel and blotted for Arc and ERK.

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