

Supplementary Information

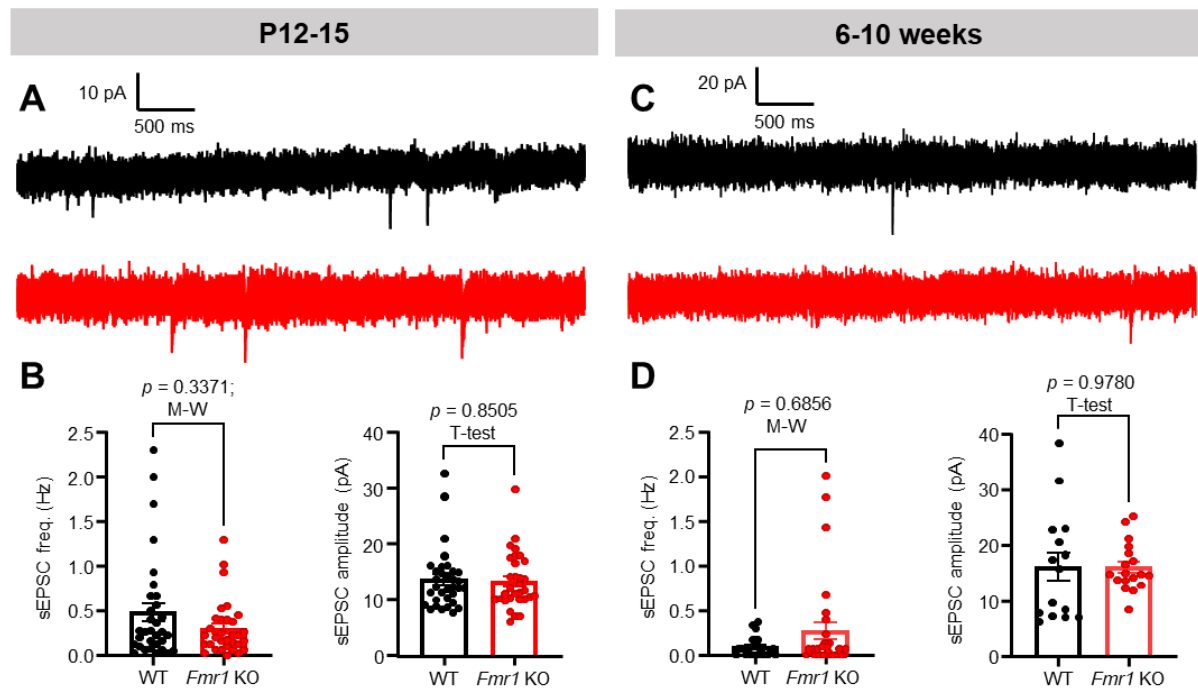


Figure S1: Spontaneous excitatory synaptic drive onto CA1 pyramidal neurons in male *Fmr1* KO rats is unaffected throughout development. (A) Example traces of spontaneous excitatory postsynaptic currents (sEPSCs) recorded in CA1 pyramidal neurons in acute slices from P12-15 *Fmr1* KO vs WT rats. (B) Comparison plots of sEPSC frequency and amplitude in both conditions. Frequency = 32 neurons from $n = 11$ biologically independent WT rats vs 32 neurons from $n = 13$ biologically independent KO rats; amplitude = 32 neurons from $n = 11$ biologically independent WT rats vs 32 neurons from $n = 13$ biologically independent KO rats. (C) and (D) As for (A) and (B), in 6-10-week old rats. Frequency = 24 neurons from $n = 11$ biologically independent WT rats vs 30 neurons from $n = 8$ biologically independent KO rats; amplitude = 15 neurons from $n = 11$ biologically independent WT rats vs 18 neurons from $n = 13$ biologically independent KO rats. All summary data are mean $\pm$ SEM.

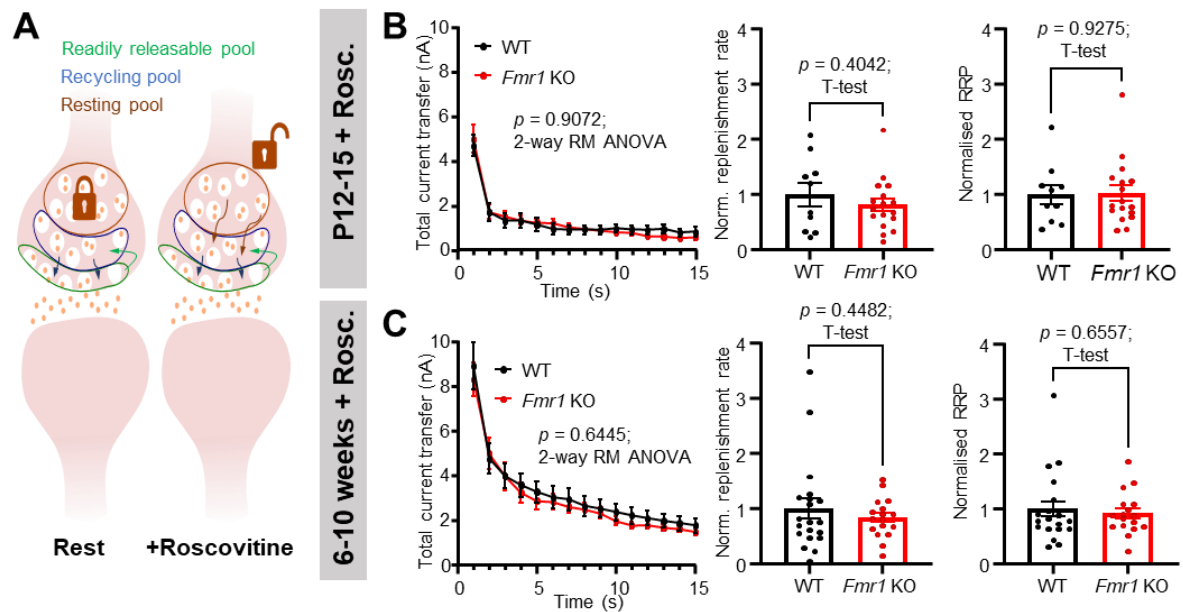


Figure S2. *Fmr1* KO does not alter the static size of the RRP: (A) Schematic showing mechanism of action of the Cdk5 inhibitor roscovitrine. Roscovitine unlocks access to the resting vesicle pool, thus allowing a greater number of vesicles to be recruited during neuronal activity. (B) Left: Plot of total current transfer binned per second during a presynaptic SC 40 Hz stimulation train of 600 action potentials recorded in CA1 pyramidal neurons from slices pretreated with 100 μ M roscovitrine (Rosc.) in P12-15 *Fmr1* KO vs WT rats. Middle: Comparison of vesicle replenishment rates and right; Readily Releasable Pool (RRP) estimates from the same experiments. 10 neurons from $n = 5$ biologically independent WT rats vs 17 neurons from $n = 9$ biologically independent KO rats for all comparisons. (C) As for (B), in 6–10-week-old rats. 20 neurons from $n = 8$ biologically independent WT rats vs 17 neurons from $n = 6$ biologically independent rats for all comparisons. All summary data are mean $\pm$ SEM.

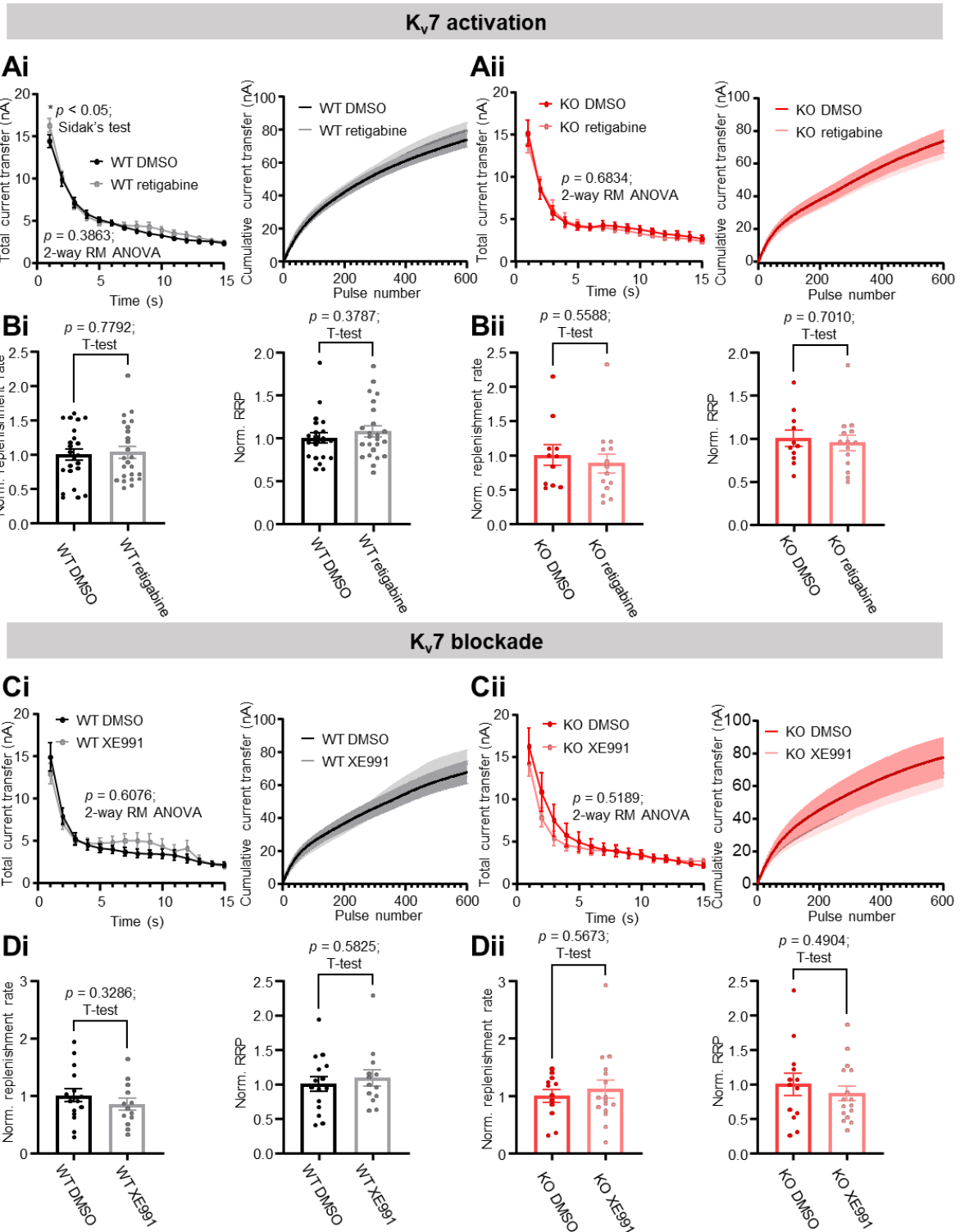


Figure S3. Effect of K_v7 channel modulation on SV pool replenishment in P12-15 *Fmr1* KO rats. (Ai) Left: Total current transfer measured during a presynaptic 40Hz stimulation train of 600 action potentials binned as total current per second and right: cumulative current transfer over the course of stimulation recorded in acute slices from WT rats pretreated with either 0.1 % DMSO or 2.5 μ M retigabine. 23 DMSO-treated neurons vs 24 retigabine-treated neurons from $n = 13$ biologically independent rats in both treatment conditions. **(Bi)** Left: comparison of vesicle replenishment rates and right: Readily Releasable Pool (RRP) estimates extrapolated from graphs in **(Ai)**. Details on calculations for these measurements

can be found in methods. **(Aii & Bii)** As for **(Ai)** and **(Bi)** in *Fmr1* KO rats. 11 DMSO-treated neurons vs 14 retigabine-treated neurons from n = 7 biologically independent rats in both treatment conditions. **(C-D)** As for **(A-B)**, treated with 5 μ M XE991 or 0.1 % DMSO. WT : 16 DMSO-treated neurons from n = 10 biologically independent rats vs 13 XE991-treated neurons from n = 9 biologically independent; KO : 13 DMSO-treated neurons from n = 10 biologically independent rats vs 13 XE991-treated neurons from n = 9 biologically independent rats. All summary data are mean $\pm$ SEM.

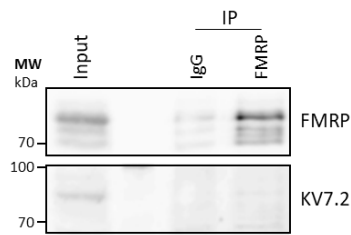


Figure S4. FMRP does not directly interact with Kv7.2. Western blot of immunoprecipitation assay showing a lack of interaction between FMRP and Kv7.2 in P14 WT rat brain lysates. Either FMRP or control IgG immunoprecipitates were probed for both FMRP and Kv7.2. Immunoprecipitate input is also displayed (10 % of total). Western blots are representative of experiments with $n = 3$ biologically independent rats.

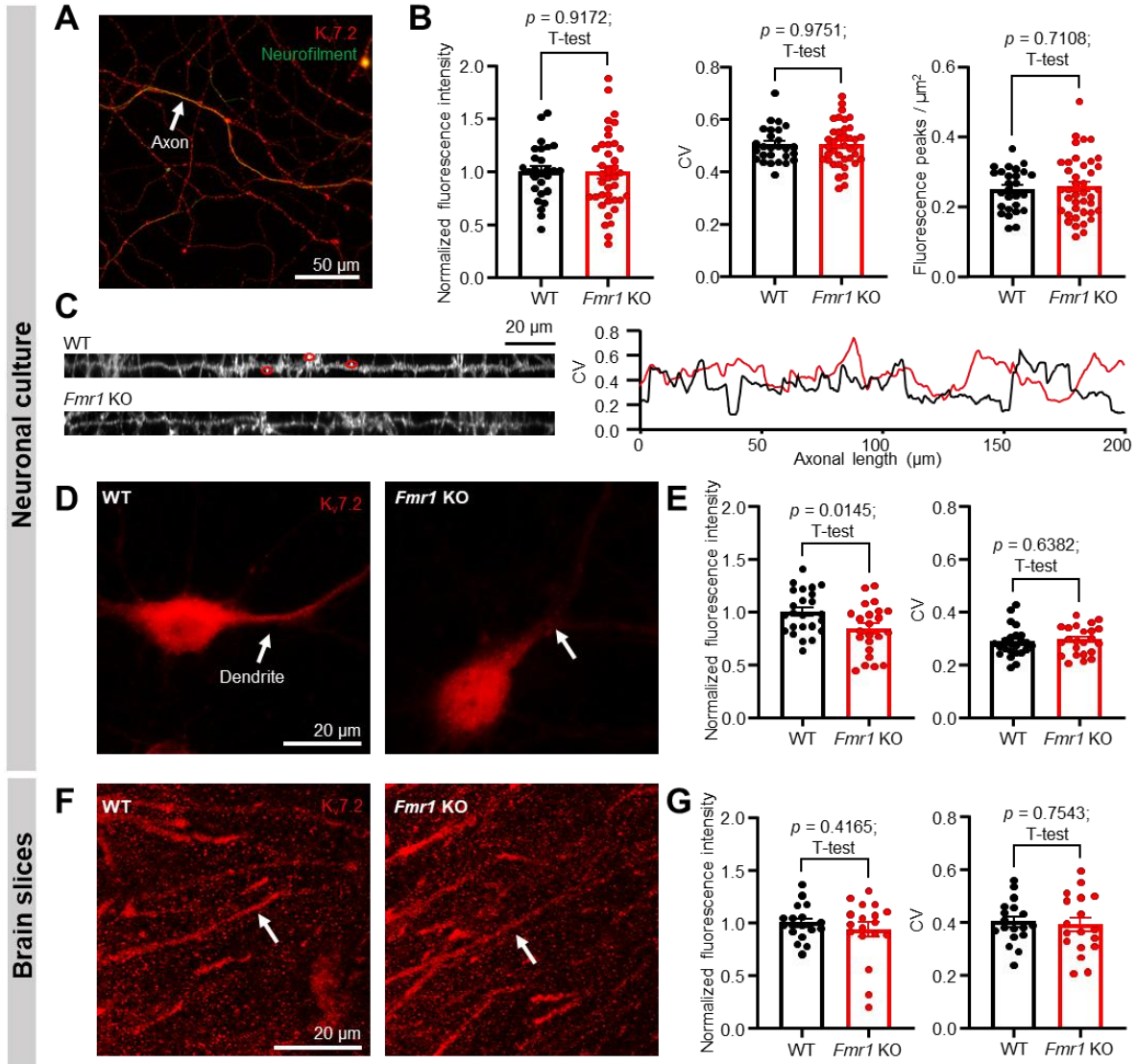


Figure S5: K_v7.2 gross expression and clustering is broadly unaltered in *Fmr1* KO rat neurites. (A) Example images depicting the K_v7.2 staining profiles along the axon of cultured WT hippocampal neurons. (B) Comparison plots of normalised fluorescence intensity, CV, and detected fluorescence peaks in axons of WT vs *Fmr1* KO neurons. (C) Left: example outputs of background-corrected axonal K_v7.2 intensity profiles in WT vs *Fmr1* KO neurons. Red circles indicate fluorescence peaks. Right: example coefficient of variation (CV) profile comparison derived from the two above images; an indication of channel clustering. (D) Example images of K_v7.2 staining profiles in the dendrites of cultured WT vs *Fmr1* KO neurons. (E) Comparison plots of normalised fluorescence intensity and CV in dendrites of WT vs *Fmr1* KO neurons. Arrows indicate portions of dendrites analysed. 27 coverslips from $n = 7$ biologically independent WT embryos vs 39 coverslips from $n = 11$ biologically independent KO embryos for all primary neuronal culture comparisons. (F) and (G) As for (D) and (E), in fixed brain slices. 18 slices from $n = 6$ biologically independent WT rats vs 18 slices from $n = 6$ biologically independent KO rats for all brain slice comparisons.

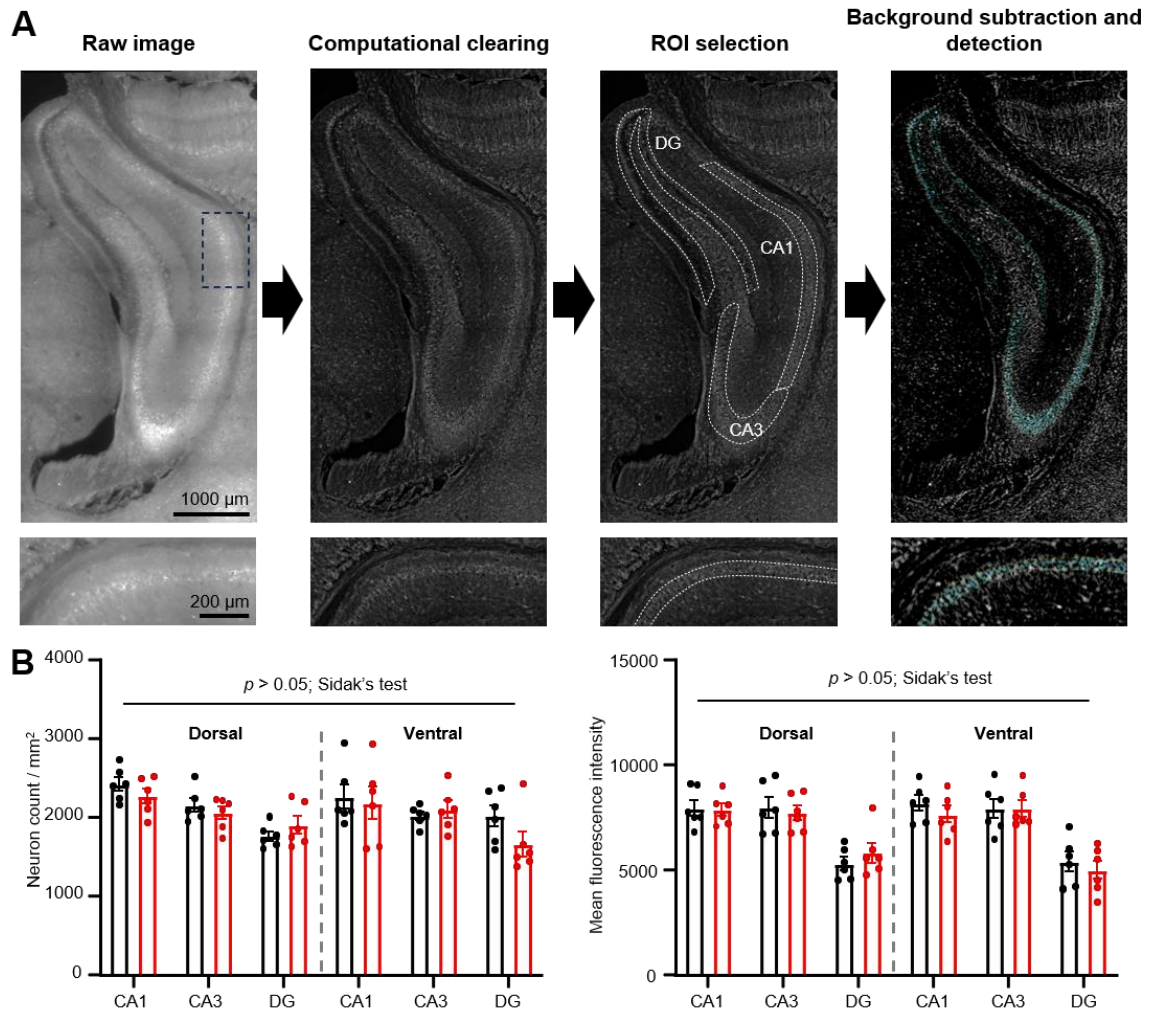


Figure S6: Automated regional detection of $K_v7.2$ -positive neurons. (A) Top: example images of $K_v7.2$ expression throughout the hippocampal formation outlining workflow. Raw tile scans (left) were computationally cleared using THUNDER software (middle left). Regions of interest (ROI's) are annotated (middle right), and then custom software performs background subtraction on the image, and automatically detects $K_v7.2$ -positive neurons within each ROI (right; cyan markers depict detected neurons). Bottom: zoom insets of the boxed CA1 region highlighted on the top leftmost image. (B) Quantification of total positive neuron counts (left) and mean fluorescence intensity of detected neurons (right) in each hippocampal sub-region in *Fmr1* KO slices vs WT littermate controls. CA = cornu ammonis; DG = dentate gyrus. $n = 6$ biologically independent rats for both WT and KO conditions.

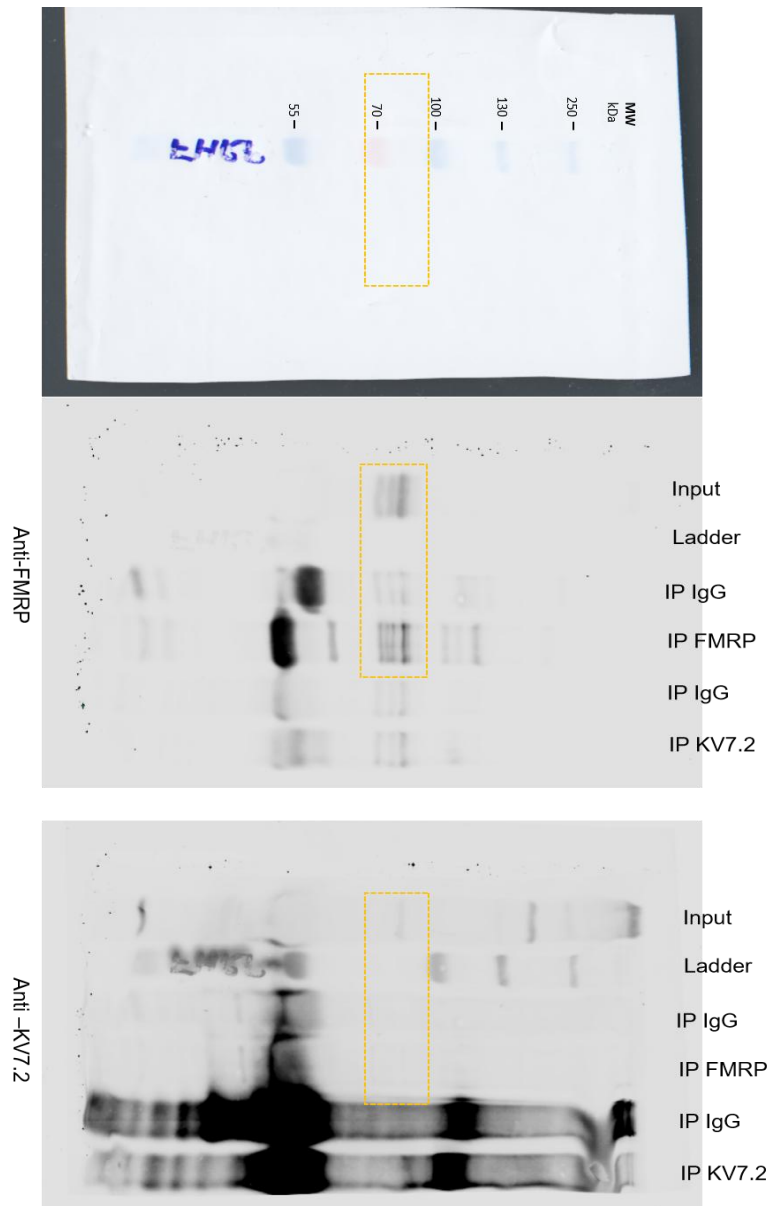


Figure S7: Raw uncropped immunoprecipitation blots used to create Figure S4.

	WT		KO			
Property	Mean / median	SEM ($\pm$)	Mean / median	SEM ($\pm$)	<i>p</i> value	Statistical test
RMP (mV)	-60.35	0.82	-62.31	0.75	0.0846	T-test
R _{in} (M Ω)	159.50	6.66	170.70	7.99	0.2883	T-test
Rheobase (pA)	69.85	3.91	68.57	3.45	0.8066	T-test
Sag (mV)	19.67	0.75	21.55	0.70	0.0728	T-test
AP threshold (mV)	-45.30	0.71	-43.53	0.96	0.1483	T-test
AP amplitude (mV)	107.40	0.93	105.10	1.25	0.1495	T-test
AP RT (ms)	0.37	0.27	0.38	1.25	0.5844	T-test
AP HW (ms)	1.99	0.04	2.04	0.05	0.5070	T-test
AP latency (ms)	71.15	9.57	83.05	15.74	0.0600	Mann-Whitney
fAHP (mV)	11.25	0.4456	10.99	0.5722	0.8534	Mann-Whitney
mAHP (mV)	12.74	0.5083	13.24	0.5784	0.5186	T-test

Table S1: Intrinsic properties of CA pyramidal neurons recorded acute slices from P12-15 *Fmr1* KO vs WT rats. Note that mean value is given where a T-test was used, and median value is given where a Mann-Whitney test was used (methods). All action potential property values are derived from the rheobase action potential. Abbreviations: RMP = resting membrane potential; R_{in} = input resistance; AP = action potential; RT = rise time; HW = half-width; fAHP = fast afterhyperpolarisation; mAHP = medium afterhyperpolarisation.

	WT		KO			
Property	Mean / median	SEM ($\pm$)	Mean / median	SEM ($\pm$)	<i>p</i> value	Statistical test
RMP (mV)	-67.05	1.09	-64.65	1.26	0.1566	Mann-Whitney
R _{in} (M Ω)	74.30	3.95	82.59	6.88	0.0940	Mann-Whitney
Rheobase (pA)	125.00	9.46	125.00	6.85	0.3282	Mann-Whitney
Sag (mV)	10.32	0.65	10.31	0.73	0.9908	T-test
AP threshold (mV)	-44.27	0.76	-45.25	0.52	0.2967	T-test
AP amplitude (mV)	107.70	0.96	107.90	1.45	0.8926	Mann-Whitney
AP RT (ms)	0.36	0.02	0.35	0.01	0.6837	T-test
AP HW (ms)	2.01	0.03	2.01	0.04	0.9355	T-test
AP latency (ms)	50.45	10.00	51.70	17.29	0.5966	Mann-Whitney
fAHP (mV)	18.77	0.5042	18.07	0.5658	0.3710	T-test
mAHP (mV)	16.94	0.4972	16.48	0.8094	0.6327	T-test

Table S2: Intrinsic properties of CA pyramidal neurons recorded acute slices from 6 to 10-week old *Fmr1* KO vs WT rats. Note that mean value is given where a T-test was used, and median value is given where a Mann-Whitney test was used (methods). All action potential property values are derived from the rheobase action potential. Abbreviations: RMP = resting membrane potential; R_{in} = input resistance; AP = action potential; RT = rise time; HW = half-width; fAHP = fast afterhyperpolarisation; mAHP = medium afterhyperpolarisation.

	WT DMSO		WT Retigabine			
Property	Mean / median	SEM ($\pm$)	Mean / median	SEM ($\pm$)	<i>p</i> value	Statistical test
RMP (mV)	-60.61	0.6107	-60.26	0.8753	0.7376	T-test
R _{in} (M Ω)	168.3	4.591	162.3	6.482	0.4532	T-test
AP threshold (mV)	-43.95	0.9276	-43.97	0.6797	0.9851	T-test
Rheobase fAHP (mV)	12.61	0.4285	12.80	0.4599	0.7624	T-test
Rheobase mAHP (mV)	13.92	0.5114	13.92	0.4638	0.9899	T-test
SFA index slope	0.0577	0.0093	0.0721	0.0100	0.2974	T-test

Table S3: Intrinsic properties of CA1 pyramidal neurons recorded acute slices from P12-15 WT rats pretreated with either 0.1 % DMSO or 2.5 μ M retigabine. Note that mean value is given where a T-test was used, and median value is given where a Mann-Whitney test was used (methods). Abbreviations: RMP = resting membrane potential; R_{in} = input resistance; AP = action potential; fAHP = fast afterhyperpolarisation; mAHP = medium afterhyperpolarisation; SFA = spike frequency adaptation.

	KO DMSO		KO Retigabine			
Property	Mean / median	SEM ($\pm$)	Mean / median	SEM ($\pm$)	<i>p</i> value	Statistical test
RMP (mV)	-59.59	0.9081	-60.49	0.6047	0.3965	T-test
R _{in} (M Ω)	186.1	14.38	190.5	11.02	0.6565	Mann-Whitney
AP threshold (mV)	-45.67	0.8878	-45.75	0.8922	0.4925	Mann-Whitney
Rheobase fAHP (mV)	11.22	0.5427	11.88	0.5221	0.4708	Mann-Whitney
Rheobase mAHP (mV)	13.39	0.5944	13.60	0.5467	0.7950	T-test
SFA index slope	0.05181	0.0100	0.05737	0.0093	0.3667	Mann-Whitney

Table S4: Intrinsic properties of CA1 pyramidal neurons recorded acute slices from P12-15 *Fmr1* KO rats pretreated with either 0.1 % DMSO or 2.5 μ M retigabine. Note that mean value is given where a T-test was used, and median value is given where a Mann-Whitney test was used (methods). Abbreviations: RMP = resting membrane potential; R_{in} = input resistance; AP = action potential; fAHP = fast afterhyperpolarisation; mAHP = medium afterhyperpolarisation; SFA = spike frequency adaptation.

	WT DMSO		WT XE991			
Property	Mean / median	SEM ($\pm$)	Mean / median	SEM ($\pm$)	<i>p</i> value	Statistical test
RMP (mV)	-63.55	0.8854	-58.30	1.2590	0.0011	T-test
R _{in} (M Ω)	147.6	6.342	157.8	7.465	0.2986	T-test
AP threshold (mV)	-46.09	0.7114	-44.77	0.9300	0.2605	T-test
Rheobase fAHP (mV)	10.71	0.4905	10.49	0.5637	0.7610	Mann-Whitney
Rheobase mAHP (mV)	12.93	0.6365	12.57	0.5114	0.5807	Mann-Whitney
SFA index slope	0.0665	0.0207	0.0609	0.0185	0.8436	T-test

Table S5: Intrinsic properties of CA1 pyramidal neurons recorded acute slices from P12-15 WT rats pretreated with either 0.1 % DMSO or 5 μ M XE991. Note that mean value is given where a T-test was used, and median value is given where a Mann-Whitney test was used (methods). Abbreviations: RMP = resting membrane potential; R_{in} = input resistance; AP = action potential; fAHP = fast afterhyperpolarisation; mAHP = medium afterhyperpolarisation; SFA = spike frequency adaptation.

	KO DMSO		KO XE991			
Property	Mean / median	SEM ($\pm$)	Mean / median	SEM ($\pm$)	<i>p</i> value	Statistical test
RMP (mV)	-59.56	0.9062	-58.96	0.9130	0.6513	T-test
R _{in} (M Ω)	152.4	6.485	153.7	4.050	0.8650	T-test
AP threshold (mV)	-45.65	0.7031	-44.43	0.7579	0.2460	T-test
Rheobase fAHP (mV)	12.00	0.4457	10.31	0.5496	0.0202	T-test
Rheobase mAHP (mV)	13.54	0.4522	12.46	0.5350	0.1280	T-test
SFA index slope	0.0609	0.0149	0.0610	0.0164	0.9960	T-test

Table S6: Intrinsic properties of CA1 pyramidal neurons recorded acute slices from P12-15 *Fmr1* KO rats pretreated with either 0.1 % DMSO or 5 μ M XE991. Note that mean value is given where a T-test was used, and median value is given where a Mann-Whitney test was used (methods). Abbreviations: RMP = resting membrane potential; R_{in} = input resistance; AP = action potential; fAHP = fast afterhyperpolarisation; mAHP = medium afterhyperpolarisation; SFA = spike frequency adaptation.