Supplemental Information

Increased excitation-inhibition ratio stabilizes synapse and circuit excitability in four autism mouse models

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Supplemental Information contains:

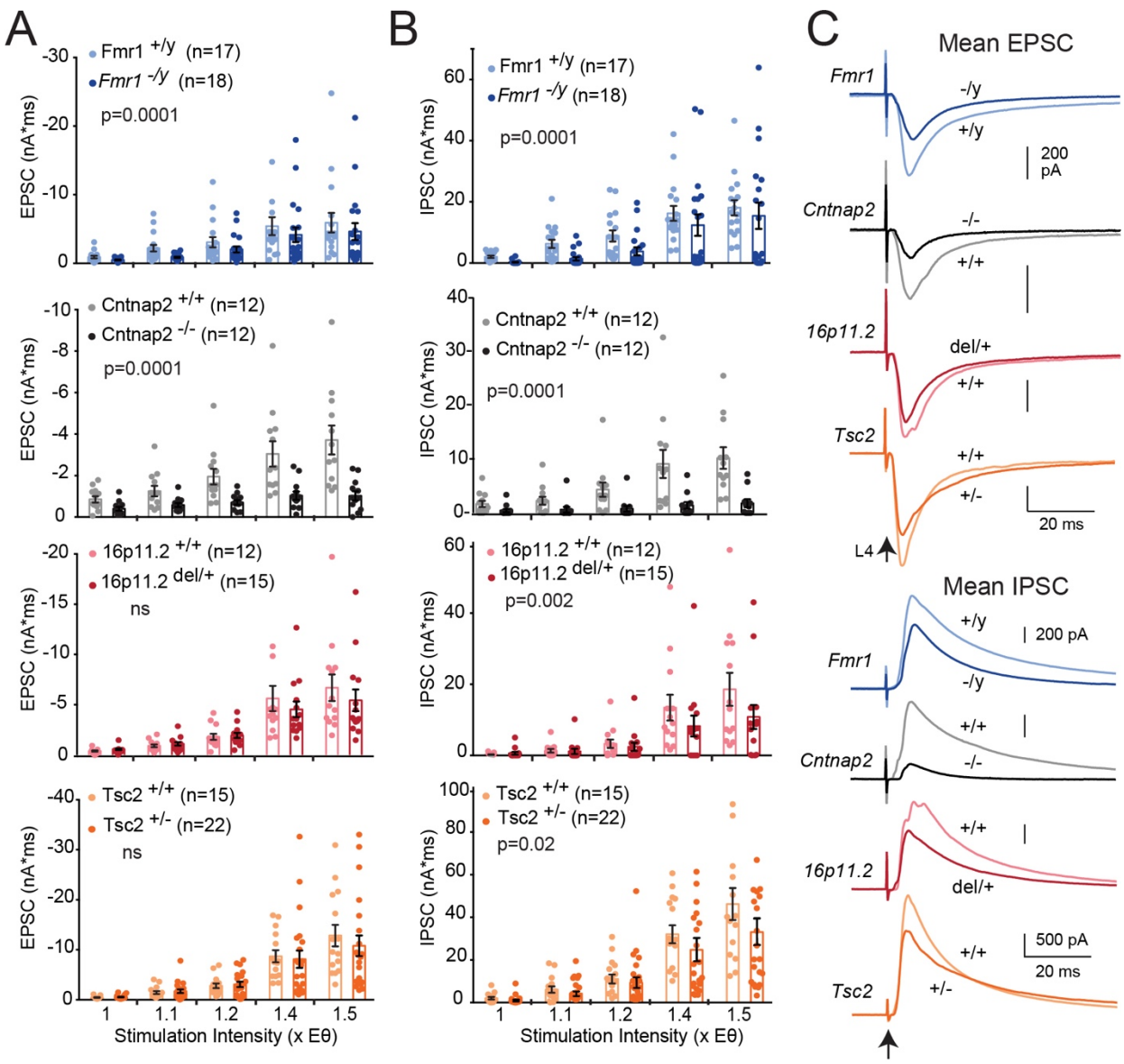

Figure S1. Individual cell data points and mean EPSC and IPSC waveforms for the input-output curve experiment in Figure 1. Related to Figure 1.

(A) Input-output curves for EPSCs. Each point is a cell. Bars show mean ± SEM. P-values are for genotype factor in a 2-way ANOVA on log-transformed data. N are number of cells.

(B) Input-output curves for IPSCs.

(C) Mean EPSC and IPC waveforms at 1.4x Eθ, across all cells in (A) and (B).

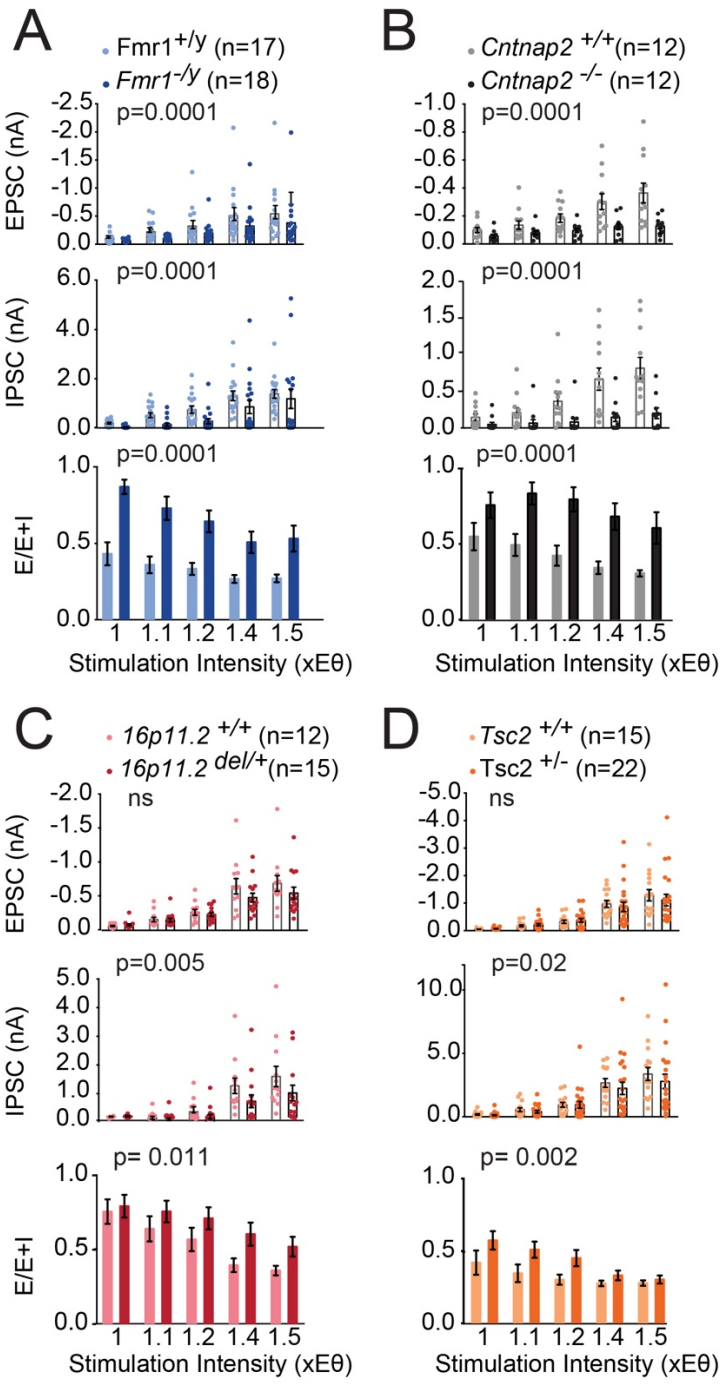

Figure S2. EPSC, IPSC and E-I ratio for the cells in Figure 1 analyzed by peak current amplitude. Related to Figure 1.

(A) Reanalysis of Figure 1 input-output curves for L4-evoked EPSCs, IPSCs, and E-I ratio in *Fmr1*$^{-/y}$ and *Fmr1*$^{+/y}$ mice, analyzed by peak current amplitude. Conventions as in Figure S1. P-values are for genotype factor in 2-way ANOVA on log-transformed data.

(B-D) Same analysis for *Cntnap2*$^{-/-}$, *16p11.2*$^{del/+}$ and *Tsc2*$^{+/-}$ and corresponding wild types.

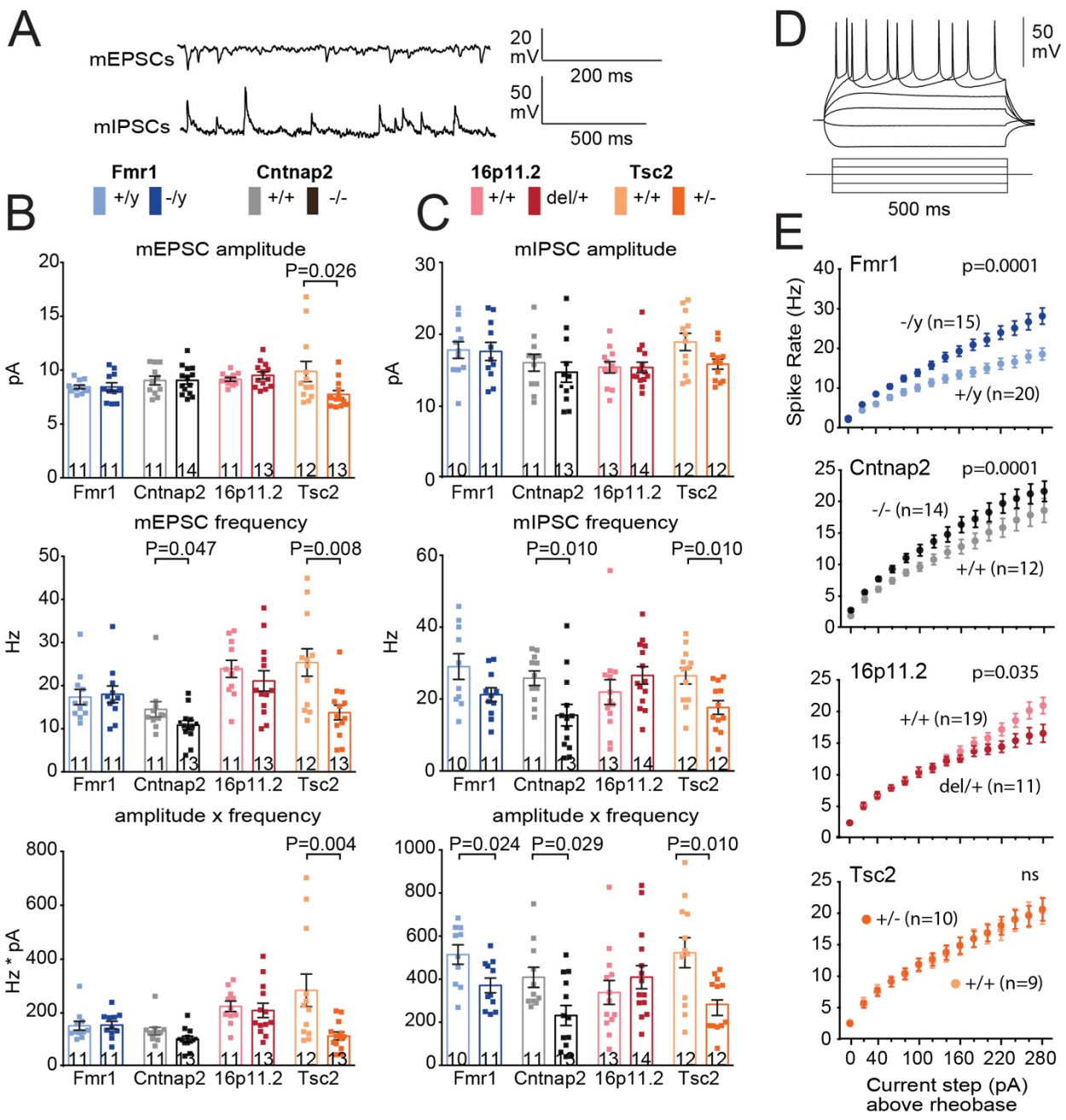

Figure S3. Miniature synaptic currents and intrinsic excitability in L2/3 PYR cells. Related to Figure 1.

(A) Example mEPSCs and mIPSCs traces.

(B) mEPSC amplitude, frequency and overall activity (product of amplitude x frequency within each cell). Each dot is one cell. Bars, mean ± SEM. Numbers are cell n.

(C) mIPSC amplitude, frequency and overall activity. P-values from Mann-Whitney test between ASD mutant and corresponding wild type. Overall mIPSC activity was reduced 3 strains (*Fmr1*$^{-/y}$, *Cntnap2*$^{-/-}$ and *Tsc2*$^{+/-}$) relative to wild type. mEPSC activity was normal in 3 strains, but was reduced in *Tsc2*$^{+/-}$.

(D) F-I curve protocol showing current injection (500 ms) and evoked spiking in one L2/3 PYR cell. **(E)** Quantification of evoked spiking for F-I curves across all cells (9-20 cells per genotype). Points show mean ± SEM. p-values report genotype effect in 2-factor ANOVA on log-transformed data. *Fmr1*$^{-/y}$ and *Cntnap2*$^{-/-}$ exhibited increased spiking relative to wild type, while *16p11.2*$^{del/+}$ showed reduced spiking,

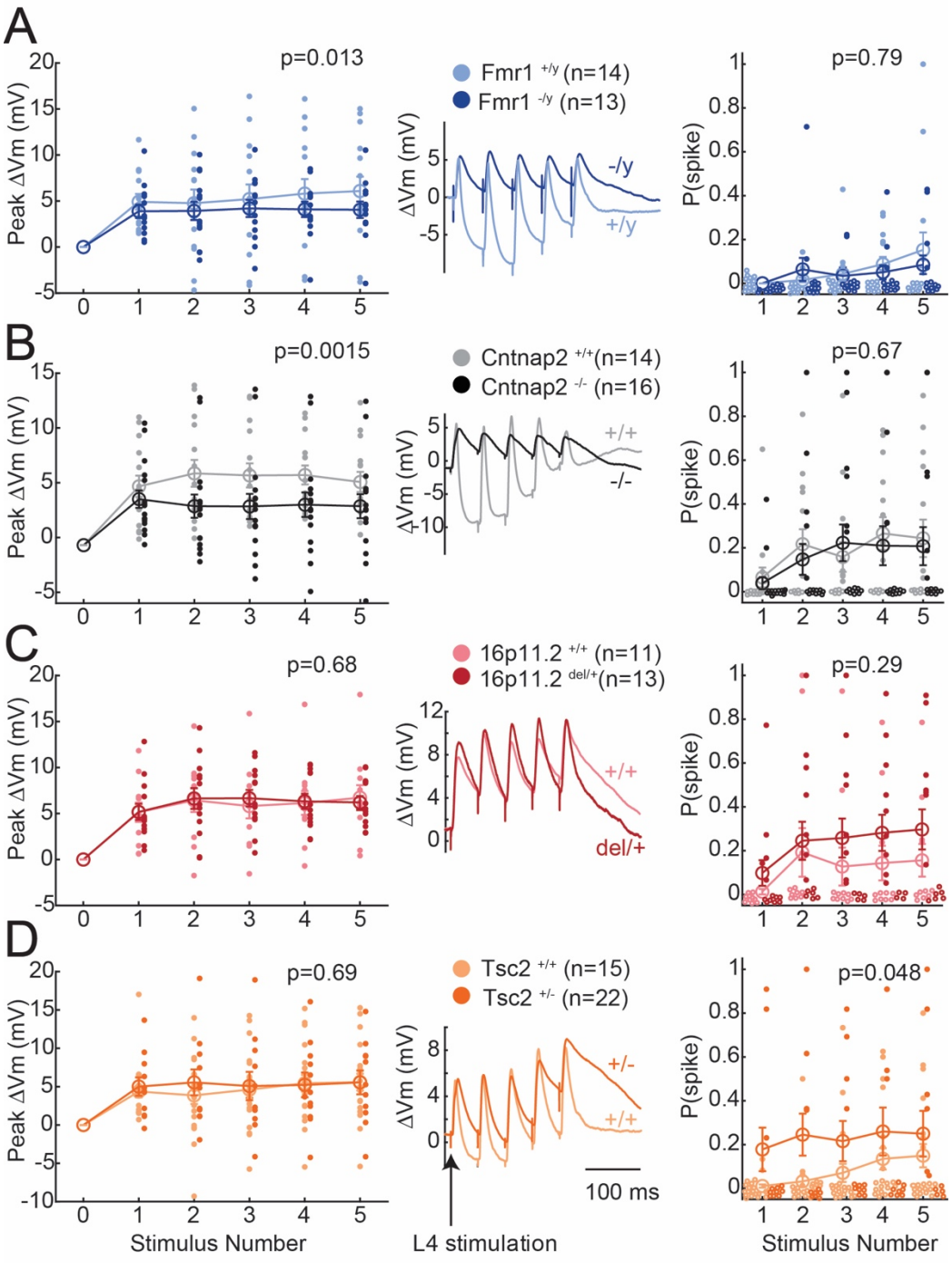

Figure S4. L4 train-evoked PSPs and spikes in L2/3 PYR cells. Related to Figure 2.

(A) Amplitude of PSPs evoked by L4 stimulus trains (5 pulses at 20 Hz) from baseline Vm of -50 mV. L4 stimulation was at 1.4x Eθ. Amplitude of each PSP was calculated relative to pre-train baseline Vm. Each small point is one cell, open points and error bars are mean ± SEM for the cell population. p-values are for genotype factor within a 2-factor ANOVA.

(B) PSP waveforms for example cells for the experiment in (A). In the y-axis, Vm is relative to pre-stimulus baseline (mV).

(C) Train-evoked spike probability for the same experiment. P-values are from permutation test on total number of evoked spikes in the train (α=0.05). Points at y=0 are spread out to allow visualization.

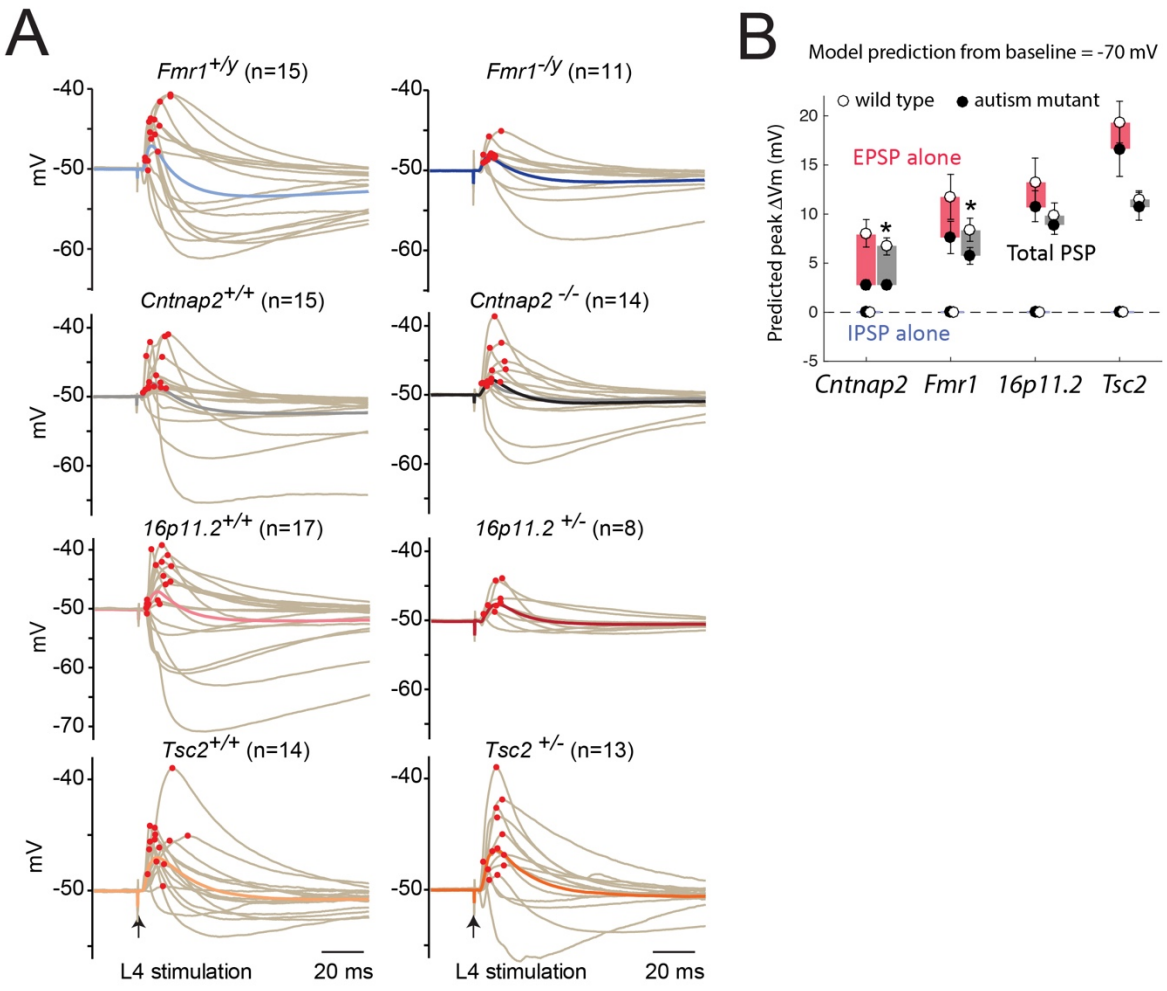

Figure S5. Validation of model results by measuring L4-evoked PSPs with NMDA currents blocked, and model results from baseline Vm of -70 mV. Related to Figure 4.

(A) Validation of model results by measuring L4-evoked PSPs with NMDA currents blocked. Measurements were from baseline Vm of -50 mV, with L4 stimulation at 1.4x Eθ. Each trace is one cell (mean of 16 sweeps). APV was present to block NMDA currents, in order to better match the conditions of parallel conductance model, which lacks voltage-activated conductances. Dots show the peak depolarization for each cell. Bold traces show mean PSP.

(B) Model predictions of PSPs from baseline Vm of -70 mV. Circles show mean predicted EPSP, IPSP, and total PSP peak for each genotype. Results show a predicted decrease in evoked PSP peak. Stars, $p<0.05$, KS test.

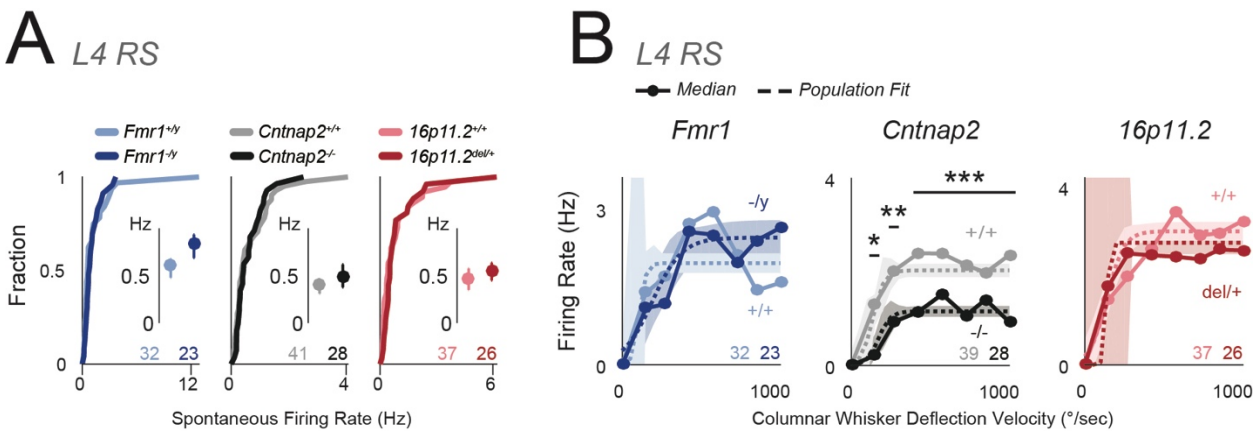

Figure S6. Firing of L4 RS units is largely normal in anesthetized mice *in vivo*. Related to Figure 6.

(A) Spontaneous firing rate for L4 RS units, shown as cumulative distributions. Insets: Bootstrapped medians with 68% CI. In all panels, numbers are units per genotype.

(B) Velocity response curves. Conventions as in Figures 5-6. * p=0.04, ** p=0.004, *** p<0.0001 t-test. CI for *Fmr1*$^{+/y}$ and *16p11.2*$^{+/+}$ are truncated at axis limit.

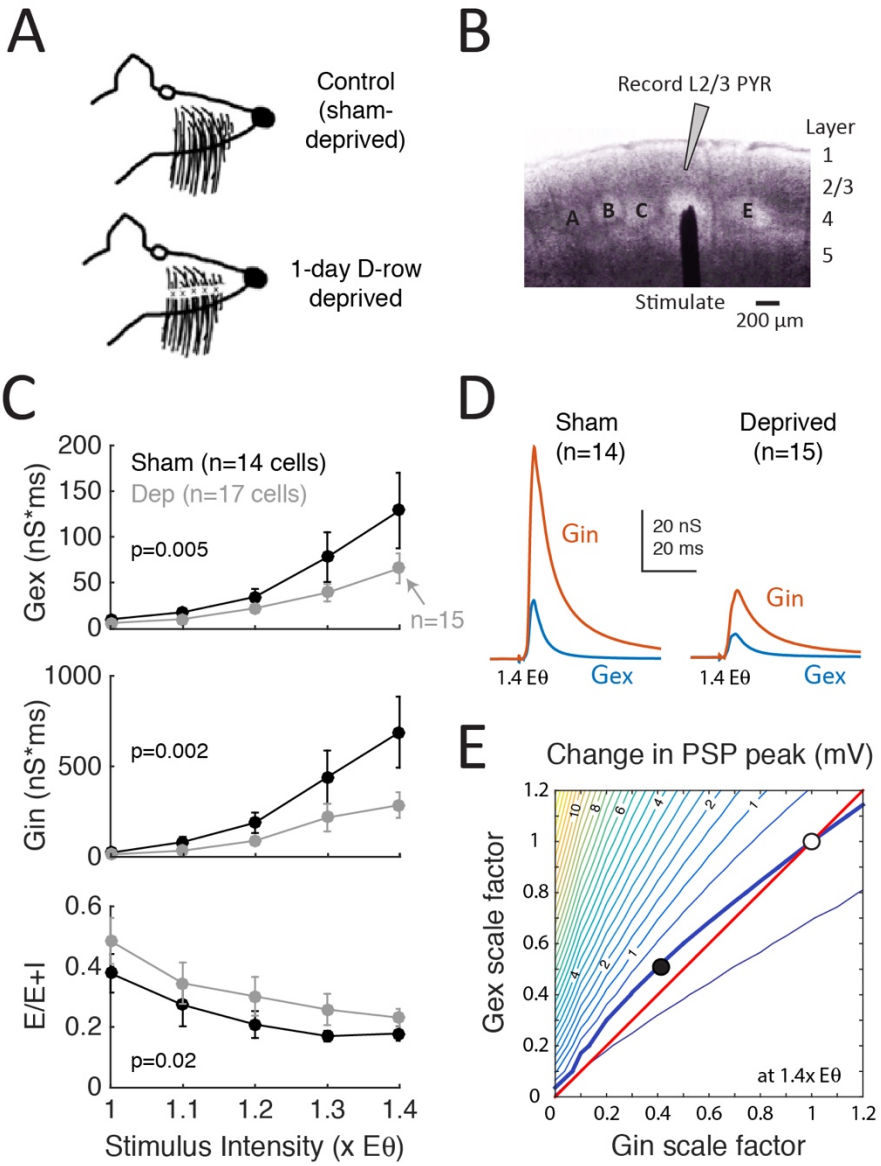

Figure S7. Brief whisker deprivation induces E-I homeostasis, including increased E-I ratio, to stabilize PSP peak in L2/3 PYR cells. Related to Figure 3.

This figure is based on data from (Gainey et al., 2018), who studied the effect of 1-day whisker deprivation on Gex, Gin and E-I conductance ratio in the L4-L2/3 feedforward projection in S1 in wild type C57BL/6 mice. Recording and analysis methods were identical to the current study.

(A) Mice had D-row whiskers trimmed at P17-20 for 24 hrs. Age-matched, sham-deprived littermates were used as controls. After 24 hr, S1 slices were prepared and used to assay for changes in L4-L2/3 feedforward circuit physiology.

(B) Recordings were made in the D column of S1 slices. L4-evoked Gex and Gin were measured in L2/3 PYR cells, using identical methods to the current study. Input-output curves were characterized in response to L4 stimulation at 1.0-1.4x Eθ.

(C) Input-output curves for Gex, Gin and E-I ratio. These show that deprivation reduced Gex and Gin, and increased E-I ratio. Points are mean ± SEM across cells. p-values represent deprived vs. sham factor in 2-factor ANOVA. The increase in E-I ratio indicates that deprivation weakened Gin more than Gex in individual cells.

(D) Mean Gex and Gin waveforms across cells, in response to L4 stimulation at 1.4x Eθ. On average, deprivation reduced Gex to 0.51 of control levels, and reduced Gin to 0.41 of control levels.

(E) Contour plot showing predicted change in evoked PSP peak for control (non-deprived cells) in response to scaling Gex and Gin by different amounts. Contour labels are in mV. The thick contour is the PSP stability contour. Red line, stable E-I ratio. This is the same method as in Figure 3D. ❍, average Gex and Gin of control cells [(1,1) by definition]. ●, average Gex and Gin measured after 1 day deprivation, as fraction of control. The substantial changes in Gex and Gin offset each other, so that no change in PSP peak is predicted. This is a new simulation based on data in Gainey et al. (Gainey et al., 2018). Direct measurement of L4-evoked PSPs in L2/3 PYR cells confirmed that deprivation does not alter PSP peak (Gainey et al., 2018).

Supplemental Tables

		Fmr1		*Cntnap2*		*16p11.2*		*Tsc2*	
Figure		N mice	Age (days)	N mice	Age (days)	N mice	Age (days)	N mice	Age (days)
1	wt	5	19.3±0.3	4	17.9±0.2	5	19.7±0.5	3	18.5±0.4
	mut	5	19.6±0.4	5	18.8±0.4	5	19.3±0.7	5	18.3±0.4
2C	wt	5	20.8±0.8	4	21.0±1.4	5	19.4±0.7	6	18.7±0.7
	mut	5	19.6±0.7	4	22.2±0.5	5	20.2±0.9	5	19.0±0.7
2E-F	wt	5	21.2±0.3	3	19.9±0.3	3	19.4±0.2	8	19.2±0.3
	mut	5	20.4±0.4	3	21.4±0.4	4	21.4±0.3	3	22.2±0.3
4E-F	wt	3	20.3±0.1	3	22.5±0.1	2	18.2±0.3	4	20.6±0.4
	mut	3	21.5±0.1	3	21.4±0.5	4	20.0±0.0	3	20.8±0.6
S3A-C	wt	3	21.1±0.3	5	20.3±0.5	3	19.8±0.4	3	22.4±0.1
	mut	3	21.0±0.3	4	18.9±0.5	3	20.6±0.4	4	21.8±0.6
S4	wt	5	20.9±0.3	6	19.8±0.3	6	18.8±0.3	12	19.3±0.3
	mut	7	19.1±0.5	6	20.1±0.4	7	20.1±0.6	4	21.4±0.7
S3D	wt	4	21.7±0.5	3	22.3±0.2	6	20.3±0.5	3	18.3±0.2
	mut	4	19.7±0.3	3	21.9±0.2	3	20.2±0.6	5	21.5±0.4

Table S1: Number and age of mice for *in vitro* experiments. Related to Figures 1, 2 and 4.

Number of mice and age (mean±SEM) for each experiment. wt, wild type. mut, mutant.

	Fmr1$^{+/y}$ Fmr1$^{-/y}$	*Cntnap2*$^{+/+}$ *Cntnap2*$^{-/-}$	*16p11.2*$^{+/+}$ *16p11.2*$^{del/+}$	*Tsc2*$^{+/+}$ *Tsc2*$^{+/-}$
EPSC amplitude at Eθ (nA)	-0.098 ± 0.02 -0.064 ± 0.01	-0.099 ± 0.02 -0.054 ± 0.01	-0.054 ± 0.01 -0.070 ± 0.01	-0.050 ± 0.01 -0.070 ± 0.01
Stimulus intensity at Eθ (μA)	6.6 ± 0.9 5.4 ± 0.6	6.5 ± 0.6 5.5 ± 0.8	5.8 ± 0.9 4.1 ± 0.2	3.5 ± 0.1 4.4 ± 0.5
V_{rest} (mV)	-78.0 ± 1.1 -78.6 ± 1.0	-76.7 ± 1.0 -77.9 ± 1.0	-79.7 ± 1.6 -79.5 ± 1.3	-82.3 ± 1.2 -81.6 ± 1.1
C_m (pF)	171.1 ± 17.3 183.6 ± 18.3	149.6 ± 17.3 115.9 ± 21.6	215.7 ± 12.5 209.7 ± 17.3	192.6 ± 11.4 213.1 ± 12.4
R_{input} (MΩ)	325.3 ± 37.7 316.9 ± 18.8	298.2 ± 41.6 324.2 ± 38.1	250.2 ± 40.6 243.9 ± 37.3	349.5 ± 29.0 267.6 ± 23.6*
EPSC onset latency (ms)	2.6 ± 0.1 2.9 ± 0.2	2.6 ± 0.2 2.5 ± 0.1	2.8 ± 0.1 2.4 ± 0.2	2.2 ± 0.1 2.4 ± 0.1
IPSC onset latency (ms)	4.2 ± 0.3 4.3 ± 0.3	5.0 ± 0.5 5.3 ± 0.3	4.0 ± 0.1 4.1 ± 0.3	3.3 ± 0.3 3.4 ± 0.1
EPSC peak latency (ms)	7.6 ± 0.3 6.6 ± 0.4	8.0 ± 0.3 7.0 ± 0.5	6.8 ± 0.4 6.3 ± 0.5	5.7 ± 0.2 6.3 ± 0.4
IPSC peak latency (ms)	9.5 ± 0.7 8.1 ± 0.5	9.4 ± 0.9 7.5 ± 0.5	7.8 ± 0.7 8.3 ± 0.8	6.5 ± 0.2 7.6 ± 0.8
IPSC-EPSC peak latency (ms)	1.9 ± 0.6 1.0 ± 0.5	1.5 ± 0.7 0.8 ± 0.7	0.9 ± 0.5 1.8 ± 0.7	0.8 ± 0.3 1.2 ± 0.5

Table S2: Cellular properties of L2/3 PYR neurons for Figure 1 experiments. Related to Figure 1.
Entries are mean ± SEM for each ASD mutant genotype (bottom) and corresponding wild-type control (top). Asterisk shows significant difference ($p<0.05$, Mann-Whitney) between mutant and wild type comparison. No other measures were significantly different.

	Fmr1$^{+/y}$ *Fmr1*$^{-/y}$	*Cntnap2*$^{+/+}$ *Cntnap2*$^{-/-}$	*16p11.2*$^{+/+}$ *16p11.2*$^{del/+}$	*Tsc2*$^{+/+}$ *Tsc2*$^{+/-}$
General passive and spiking properties				
Membrane time constant (τ_{mem}) (ms)	32.4 ± 2.9 34.1 ± 4.0	35.9 ± 3.5 40.7 ± 4.4	37.5 ± 2.0 41.7 ± 3.3	35.3 ± 4.8 35.0 ± 5.6
R_{input} (MΩ)	92.8 ± 6.6 98.2 ± 5.8	117.0 ± 9.8 102.6 ± 11.3	99.9 ± 9.0 111.3 ± 20	126.1 ± 16.8 112.0 ± 14.5
V_{rest} (mV)	-81.6 ± 1.0 -82.6 ± 0.8	-83.3 ± 1.1 -83.6 ± 1.5	-85.1 ± 1.0 -81.3 ± 1.8	-82.0 ± 1.2 -75.0 ± 1.3
Spike threshold (mV)	-43.4 ± 1.5 -46.9 ± 1.3	-44.5 ± 1.2 -45.1 ± 1.0	-45.2 ± 1.0 -43.9 ± 1.2	-47.2 ± 1.1 -45.6 ± 1.5
Rheobase (pA)	248.6 ± 17.1 222.4 ± 17.9	19.4 ± 17.8 220.3 ± 15.9	244.7 ± 16.4 211.0 ± 21.7	205.0±29.9 227.4±28.8
Numeric values for L4-evoked spiking (Panel 2F)				
Num. evoked spikes (mean ± SEM)	0.41 ± 0.3 0.27 ± 0.2	1.46 ± 0.6 0.23 ± 0.2	0.23 ± 0.2 1.67 ± 0.9	0.53 ± 0.2 2.20 ± 1.5
Mann-Whitney test	p=0.94	p=0.09	p=0.22	p=0.64
% of cells with L4-evoked spiking	2/17 (11.8%) 2/11 (18.2%)	6/13 (46.1%) 2/13 (15.4%)	2/13 (15.4%) 6/18 (33.3%)	7/19 (36.8%) 2/10 (20.0%)
Fisher's exact test	p=0.99	p=0.20	p=0.41	p=0.43

Table S3: Cellular properties of L2/3 PYR neurons for Figure 2 experiments. Related to Figure 2. Conventions as for Table S2. No measures were significantly different between mutant and corresponding wild-type genotype.

	Fmr1 +/y	*Fmr1* -/y	*Cntnap2* +/+	*Cntnap2* -/-	*16p11.2* +/+	*16p11.2* del/+
L2/3 recordings, anesthetized mice (Figures 5 & 6)						
Total mice	11	9	7	9	12	6
Age (Mean±SEM)	69.2±1.1	65.5±1.3	64.4±1.3	61.2±1.4	52.2±0.6	48.8±1.1
L2/3 FS units, anesthetized mice (Figure 5)						
Total FS units	17	32	18	25	30	19
Spontaneous firing Units (mice)	17 (6)	32 (8)	18 (7)	25 (9)	30 (10)	19 (5)
VRC[1] Units (mice)	17 (6)	32 (8)	17 (6)	25 (9)	30 (10)	19 (5)
L2/3 RS units, anesthetized mice (Figure 6)						
Total RS Units	49	86	35	39	85	32
Spontaneous firing Units (mice)	49 (11)	86 (9)	35 (7)	39 (9)	85 (12)	32 (6)
VRC[1] Units (mice)	48 (10)	86 (9)	31 (6)	39 (9)	80 (11)	32 (6)
Best whisker firing[2] Units (mice)	31 (8)	75 (7)	29 (5)	24 (7)	75 (10)	32 (6)
Jitter, CW tuning, tuning sharpness[3] Units (mice)	19 (10)	46 (8)	9 (6)	15 (6)	38 (9)	19 (6)
Correlations and synchrony[4]. Pairs (units, mice)	95 (42,7)	303 (82,8)	44 (30,7)	62 (32,6)	290 (78,10)	46 (28,5)
L4 RS units, anesthetized mice (Figure S6)						
Total mice	8	5	8	9	9	6
Total RS units	32	23	41	28	37	26
Spontaneous firing Units (mice)	32 (8)	23 (5)	41 (8)	28 (9)	37 (9)	26 (6)
VRC[1] Units (mice)	32 (8)	23 (5)	39 (7)	28 (9)	37 (9)	26 (6)

[1] Velocity response curve (VRC) was quantified for all units for which the VRC stimulus protocol was applied to the unit's columnar whisker.

[2] Best whisker (BW)-evoked firing rate was quantified for all units whose columnar whisker was at the center of the 3x3 piezo array. This criterion ensures the greatest accuracy for identifying the BW.

[3] Jitter, columnar whisker (CW) tuning, and tuning sharpness were quantified for all whisker-responsive units.

[4] Signal and noise correlations and firing synchrony were calculated across all pairs of simultaneously recorded L2/3 RS units located < 0.2 mm apart.

Table S4: Sample sizes for *in vivo* anesthetized experiments. Related to Figures 5-6.

Number of units and mice in each analysis for *in vivo* anesthetized experiments.