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Supplemental information

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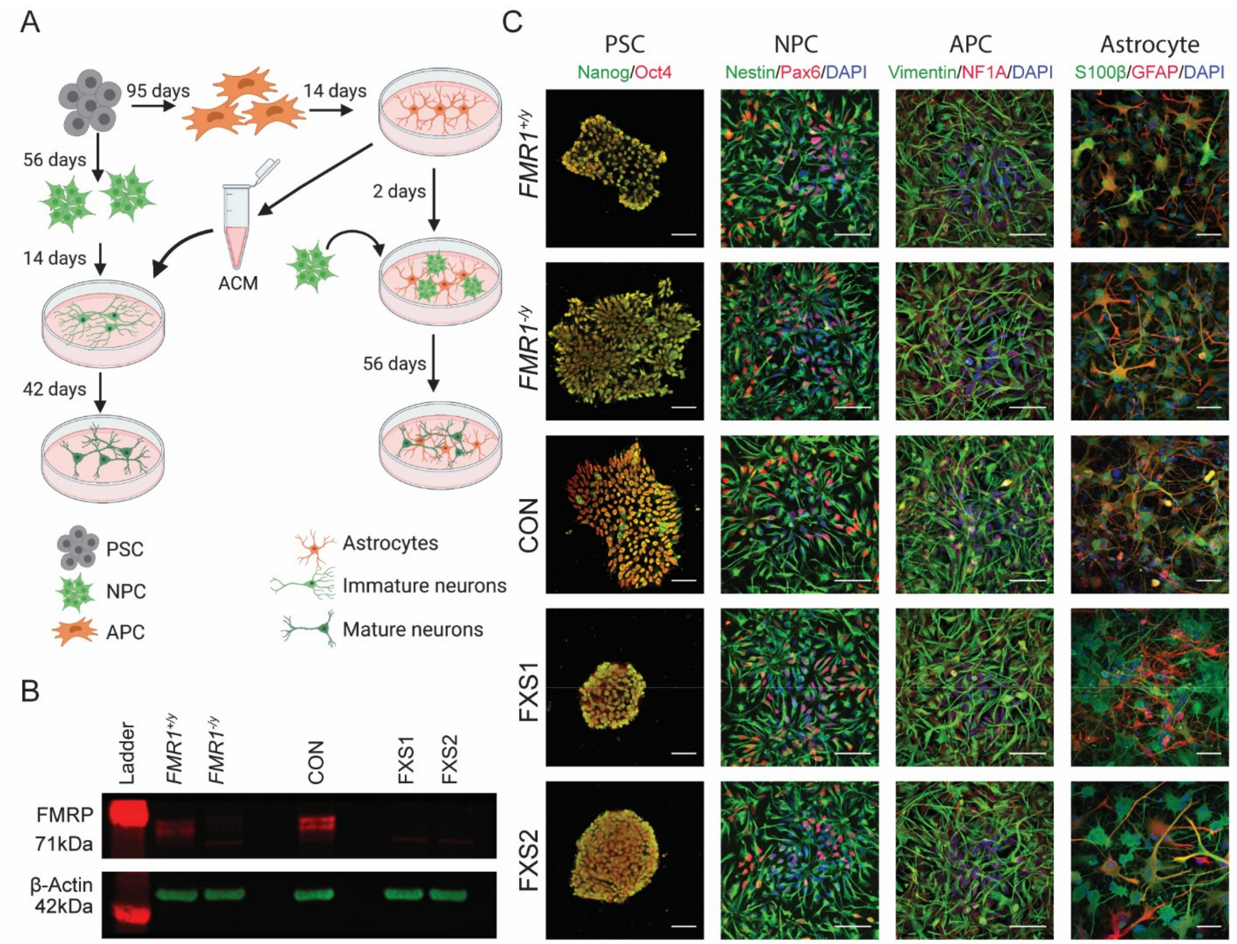
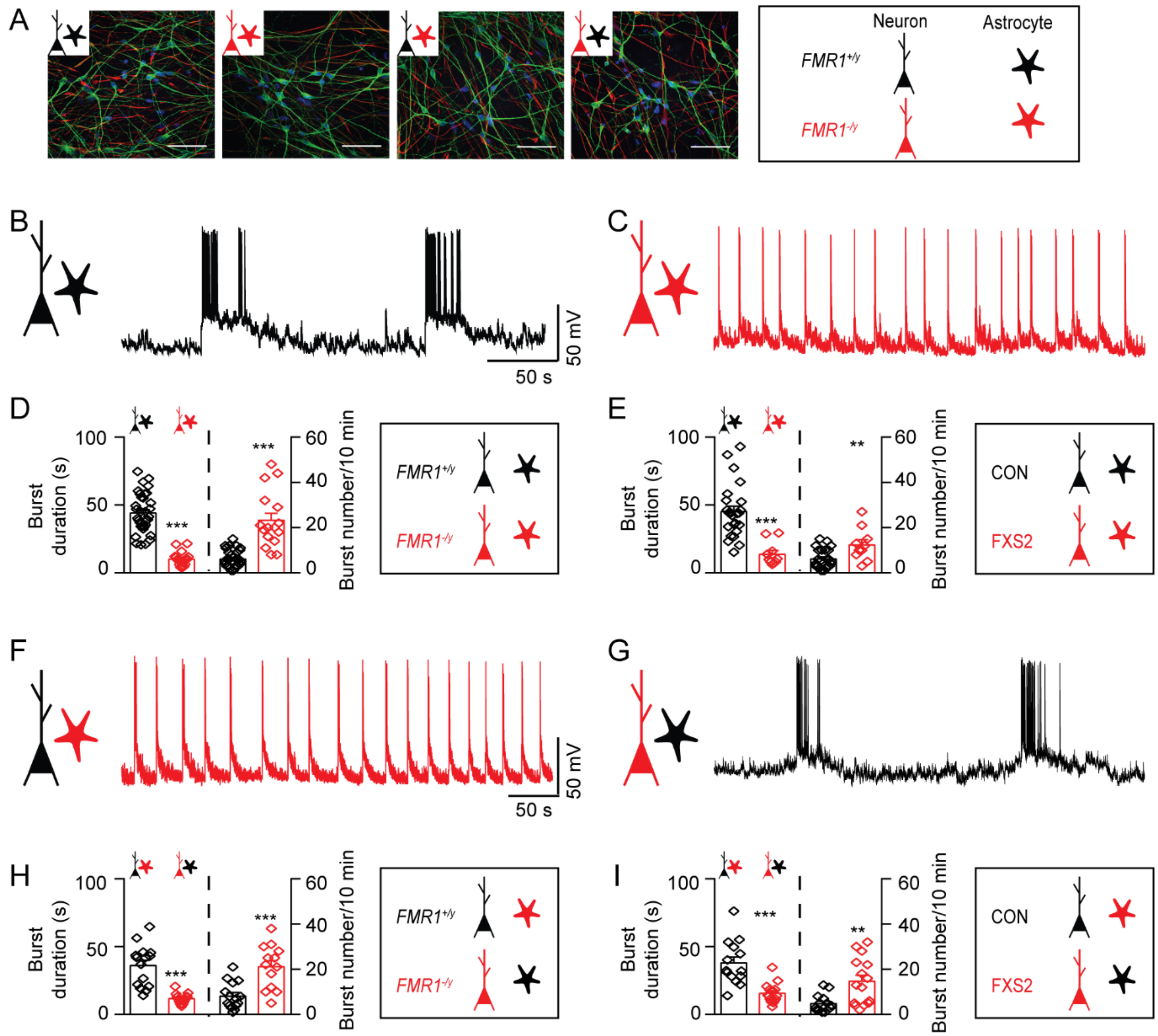
Figure S1



Derivation of neurons and astrocytes from human pluripotent stem cells. (A) A Schematic protocol (described in 'Methods') for differentiation and co-culture of neurons and astrocytes from human pluripotent stem cells. **(B)** Western blot analysis of FMRP expression from pluripotent stem cell lines illustrates *FMR1*$^{+/y}$ and CON expressing FMRP and absence of FMRP in *FMR1*$^{-/y}$, FXS1, and FXS2 lines. **(C)** Left to right panel: Representative immunofluorescent images expressing pluripotent markers Nanog and Oct4. Immunofluorescent staining of neural precursor cells (NPC) expressing Nestin and Pax6. Representative images of astrocyte precursor cells (APC) expressing Vimentin and NFIA. Right panel illustrates pluripotent stem cell-derived astrocytes expressing S100β and GFAP. Cells were co-labeled with nuclear marker DAPI. Scale bar 50 µm.

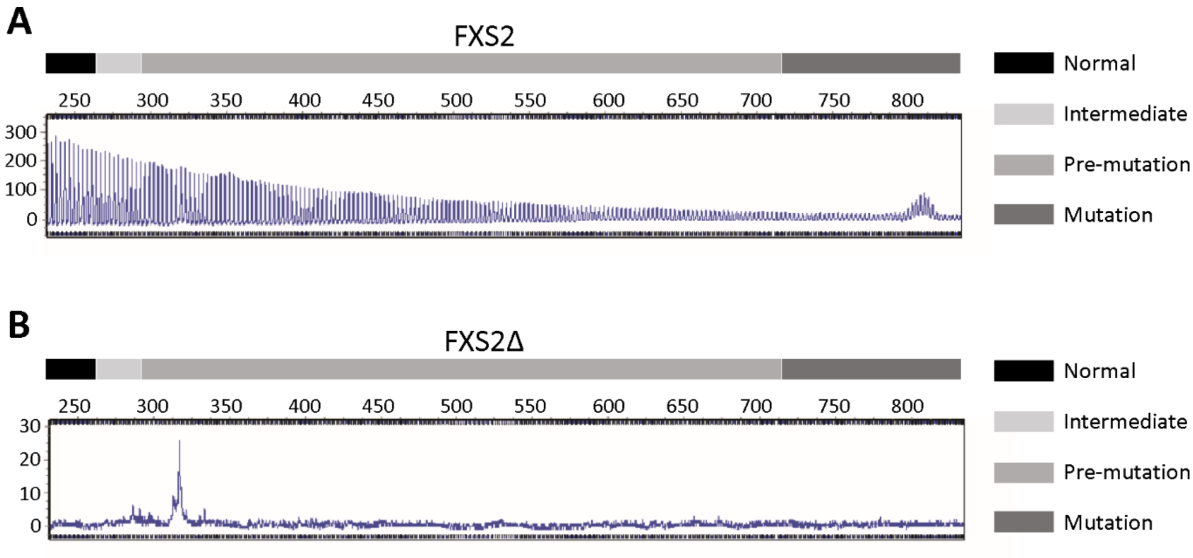
Figure S2



For both iPSC and ESC derived lines distinct patterns of bursting activity of hPSC-derived cortical neurons is determined by the genotype of the astrocyte (A) Representative confocal images of hESC-derived cortical neurons *(FMR1$^{+/y}$* and *FMR1$^{-/y}$*) expressing Map2ab co-cultured with hESC derived astrocytes expressing GFAP. *Left to right*, neurons were co-cultured in four combinations: *FMR1$^{+/y}$* neurons with *FMR1$^{-/y}$* astrocytes, *FMR1$^{-/y}$* neurons with *FMR1$^{-/y}$* astrocytes, *FMR1$^{-/y}$* neurons with *FMR1$^{+/y}$* astrocytes, and *FMR1$^{+/y}$* neurons with *FMR1$^{-/y}$* astrocytes. *Right*, legend for icons. Scale bar = 50 µm. **(B)** Representative current-clamp recording (V_{HOLD} = -70 mV) from a *FMR1$^{+/y}$* neuron, co-cultured with *FMR1$^{+/y}$* astrocyte, firing spontaneous bursts of action potentials occurring at low frequencies but with longer durations. **(C)** Representative current-clamp recording of aberrant spontaneous activity in a *FMR1$^{-/y}$* neuron, co-cultured with *FMR1$^{-/y}$* astrocyte, containing a significantly higher number of bursts, but of shorter duration. **(D)** Comparison of mean burst duration of neurons and astrocytes co-cultures derived from ESC (*FMR1$^{+/y}$* neurons with *FMR1$^{+/y}$* astrocytes, 44.1 ± 2.58 s, n = 33, N = 4; *FMR1$^{-/y}$* neurons with *FMR1$^{-/y}$* astrocytes, 10.13 ± 1.4 s, n = 16, N = 3) and mean burst number per 10 min of recording (*FMR1$^{+/y}$* neurons with *FMR1$^{+/y}$* astrocytes, 6.03 ± 0.65; *FMR1$^{-/y}$* neurons with *FMR1$^{-/y}$* astrocytes, 23.25 ± 3.116). **(E)**

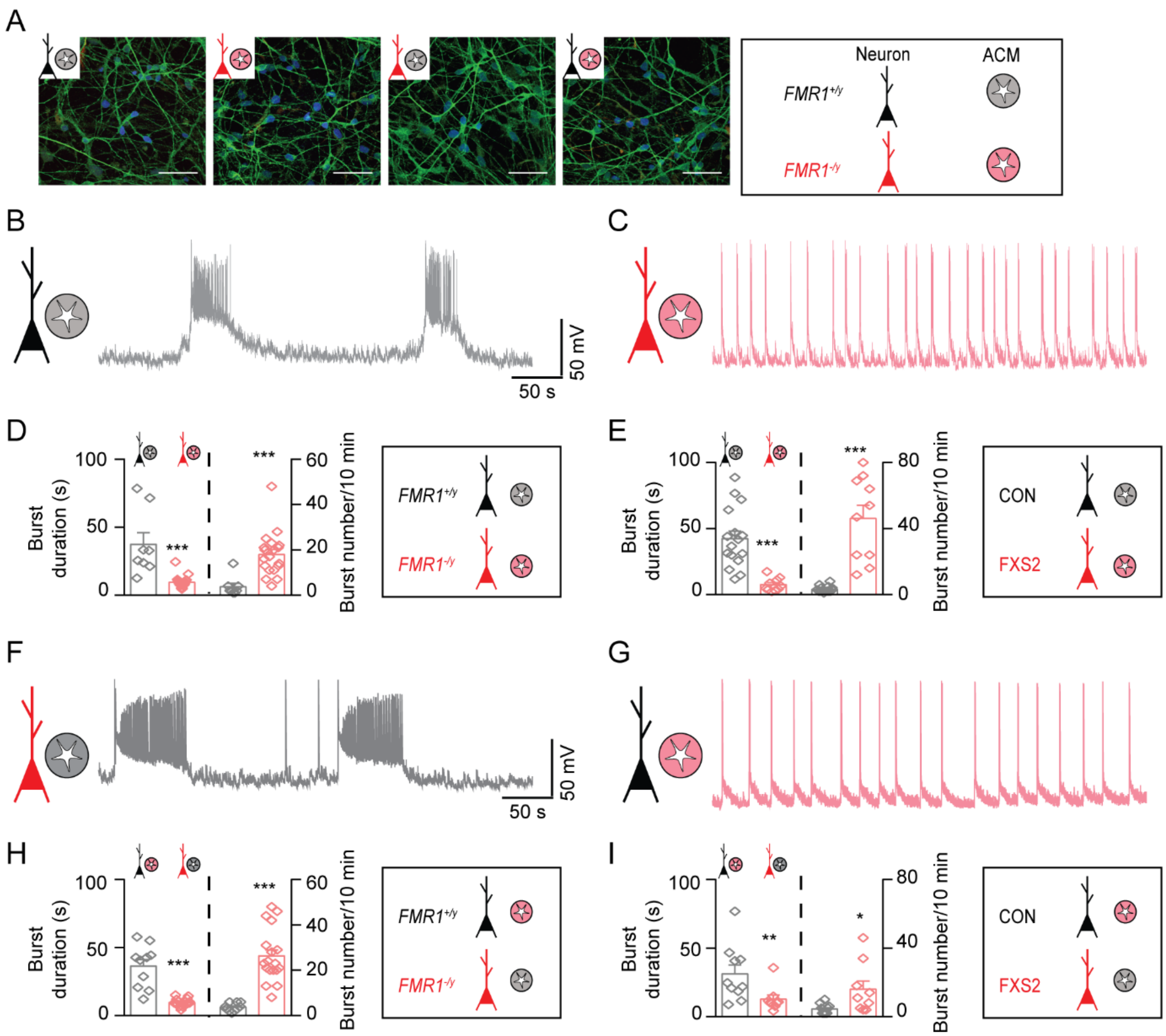
Comparison of mean burst duration of neurons and astrocytes cocultures derived from iPSC (CON neurons with CON astrocytes, 45.19 ± 4.065 s, n = 24, N = 4; FXS2 neurons with FXS2 astrocytes, 13.81 ± 2.71 s, n = 10, N = 3) and mean burst number per 10 min of recording (CON neurons with CON astrocytes, 6.167 ± 0.89; FXS2 neurons with FXS2 astrocytes, 12.4 ± 2.28). **(F)** Representative trace from a *FMR1*$^{-/y}$ neuron, co-cultured with *FMR1*$^{+/y}$ astrocyte, exhibits a normal activity with low burst number and longer burst duration. **(G)** Conversely, a *FMR1*$^{+/y}$ neuron, co-cultured with *FMR1*$^{-/y}$ astrocyte, shows aberrant activity containing higher number of bursts of duration. **(H)** Comparison of mean burst duration (*FMR1*$^{-/y}$ neurons with *FMR1*$^{+/y}$ astrocytes, 36.02 ± 3.93 s, n = 15, N = 3; *FMR1*$^{+/y}$ neurons with *FMR1*$^{-/y}$ astrocytes, 11.81 ± 1.108 s, n = 13, N = 3) and mean burst number per 10 min of recording (*FMR1*$^{-/y}$ neurons with *FMR1*$^{+/y}$ astrocytes, 8.2 ± 1.51; *FMR1*$^{+/y}$ neurons with *FMR1*$^{-/y}$ astrocytes, 21.15 ± 2.7). **(I)** Comparison of mean burst duration of neurons and astrocytes cocultures derived from iPSC (FXS2 neurons with CON astrocytes, 38.03 ± 4.39 s, n = 14, N = 3; CON neurons with FXS2 astrocytes, 15.62 ± 1.89 s, n = 15, N = 3) and mean burst number per 10 min of recording (FXS2 neurons with CON astrocytes, 4.86 ± 1.032; CON neurons with FXS2 astrocytes, 14.67 ± 2.68). ***$p < 0.001$, **$p < 0.01$, unpaired *t*-test, Mann-Whitney test. All values are mean ± SEM.

Figure S3



The CGG repeats are absent after gene correction. (**A, B**) Triplet repeat prime PCR in FXS2 line and its edit (FXS2Δ) shows the absence of the 5'UTR repeat in the edited line.

Figure S4



For both iPSC and ESC derived lines the bursting activity of hPSC-derived cortical neurons is determined by the genotype of the astrocyte secretome. (A) Representative confocal images of Map2ab-expressing hESC-derived cortical neurons *(FMR1$^{+/y}$* and *FMR1$^{-/y}$*) grown in astrocytic conditioned media (ACM). *Left to right*, neurons were grown in ACM in four combinations: *FMR1$^{+/y}$* neurons with *FMR1$^{-/y}$* ACM, *FMR1$^{-/y}$* neurons with *FMR1$^{-/y}$* ACM, *FMR1$^{-/y}$* neurons with *FMR1$^{+/y}$* ACM, and *FMR1$^{+/y}$* neurons with *FMR1$^{-/y}$* ACM. *Right*, legend for icons. Scale bar = 50 µm. **(B)** Recording from a *FMR1$^{+/y}$* neuron, grown in *FMR1$^{+/y}$* ACM, exhibits normal firing consisting of a low number of longer bursts. **(C)** Recording from *FMR1$^{-/y}$* neuron grown in *FMR1$^{-/y}$* ACM shows a high number of bursts of shorter duration. **(D)** Comparison of mean burst duration (*FMR1$^{+/y}$* neurons in *FMR1$^{+/y}$* ACM, 37.47 ± 8.55 s, n = 8, N = 2; *FMR1$^{-/y}$* neurons in *FMR1$^{-/y}$* ACM, 9.602 ± 1.033 s, n = 19, N = 3) and mean burst number per 10 min of recording (*FMR1$^{+/y}$* neurons in *FMR1$^{+/y}$* ACM, 3.75 ± 1.52; *FMR1$^{-/y}$* neurons in *FMR1$^{-/y}$* ACM, 17.95 ± 2.245). **(E)** Comparison of mean burst duration of neurons and astrocytes cocultures derived from iPSC (CON neurons with CON ACM, 42.49 ± 5.65 s, n = 16, N = 3; FXS2 neurons with FXS2 ACM, 7.52 ± 1.56 s, n = 10, N = 3) and mean burst number per 10 min of recording (CON neurons with CON ACM, 3.2 ± 0.46; FXS2 neurons with FXS2 ACM, 46.2 ± 8.009). **(F)** Representative recording from a *FMR1*$^{-/y}$ neuron, grown in *FMR1*$^{+/y}$ ACM, exhibiting control burst firing, i.e., less frequent bursts of longer duration. Thus, control ACM exerts a non-cell autonomous effect in reversing aberrant FXS bursting. **(G)** Representative recording from a *FMR1*$^{+/y}$ ACM neuron, grown in *FMR1*$^{-/y}$ ACM, shows the aberrant FXS bursting. **(H)** Comparison of mean burst duration (*FMR1*$^{-/y}$ neurons in *FMR1*$^{+/y}$ ACM, 36.37 ± 4.94 s, n = 10, N = 3; *FMR1*$^{+/y}$ neurons in *FMR1*$^{-/y}$ ACM, 9.55 ± 0.64 s, n = 18, N = 3) and mean burst number per 10 min of recording (*FMR1*$^{-/y}$ neurons in *FMR1*$^{+/y}$ ACM, 3.7 ±0.59; *FMR1*$^{+/y}$ neurons in *FMR1*$^{-/y}$ ACM, 26.33 ± 2.77). **(I)** Comparison of mean burst duration of neurons with ACM cultures derived from iPSC (FXS2 neurons with CON ACM, 31.29 ± 6.5 s, n = 10, N = 3; CON neurons with FXS2 ACM, 12.76 ± 3.02 s, n = 9, N = 2) and mean burst number per 10 min of recording (FXS2 neurons with CON ACM, 4.4 ± 0.92; CON neurons with FXS2 ACM, 16 ± 4.9). ****p* < 0.001, ***p* < 0.01, **p* < 0.01, unpaired *t*-test, Mann-Whitney test. All values are mean ± SEM.

Table S1. Cell line details

ID in manuscript	ID at source	Age (years)	Sex	Reprogrammed cell line name	Reprogramming method	Starting cell type	G band karyotype
CON	ND30625	76	M	CS25iCTR-18n6	Episomal vectors	Fibroblast	Normal
FXS1	GM05848	4	M	CS848iFXS-n5	Episomal vectors	Fibroblast	Normal
FXS2	GM07072	22	M	CS072iFXS-n4	Episomal vectors	Fibroblast	Normal
FMR1$^{+/y}$	Shef 4	n/a	M	Shef 4	n/a	Embryonic stem cell	Normal
FMR1$^{-/y}$	Shef 4-*FMR1* null	n/a	M	Shef 4-*FMR1* null	n/a	Embryonic stem cell	Normal
FXS2Δ	n/a	22	M	CS072iFXS-n4 (Parental line)	Episomal vectors	Fibroblast	Normal

Table S2. Intrinsic electrophysiological properties: CON, FXS1

	CON neurons CON astrocytes	FXS1 neurons FXS1 astrocytes	CON neurons FXS1 astrocytes	FXS1 neurons CON astrocytes
Number of cells	23	12	11	12
Resting membrane potential (mV)	-52.23 ± 1.13	-54.08 ± 1.24	-56.96 ± 2.08	-57.09 ± 2.21
Input Resistance (GΩ)	1.04 ± 0.09	0.97 ± 0.17	1.3 ± 0.13	1.06 ± 0.14
Capacitance (pF)	31.19 ± 1.68	42.9 ± 6.01	33.12 ± 3.23	33.86 ± 3.95
Rheobase (pA)	18.04 ± 2.28	20 ± 3.05	17.86 ± 2.44	20 ± 3.01
Spike amplitude (mV)	68.03 ± 3.18	66.22 ± 5.3	64.95 ± 3.10	62.74 ± 4.21
Spike half-width (ms)	3.11 ± 0.19	3.1 ± 0.26	2.74 ± 0.25	3.26 ± 0.34
Max no. of spikes fired	5.87 ± 0.6	5.16 ± 0.92	4.61 ± 0.84	4.93 ± 1

Table S3. Intrinsic electrophysiological properties: CON, FXS2

	CON neurons CON astrocytes	FXS2 neurons FXS2 astrocytes	CON neurons FXS2 astrocytes	FXS2 neurons CON astrocytes
Number of cells	23	12	15	14
Resting membrane potential (mV)	-52.23 ± 1.13	-54.85 ± 2.11	-52.66 ± 1.84	-52.17 ± 2.31
Input Resistance (GΩ)	1.04 ± 0.09	1.13 ± 0.15	1.16 ± 0.14	1.16 ± 0.11
Capacitance (pF)	31.12 ± 1.68	27.55 ± 2.42	30.23 ± 3.44	32.5 ± 3.86
Rheobase (pA)	18.04 ± 2.28	20 ± 3.43	21.67 ± 3.54	17.86 ± 2.8
Spike amplitude (mV)	68.03 ± 3.18	61.7 ± 4.9	66.08 ± 2.35	59.42 ± 3.5
Spike half-width (ms)	3.11 ± 0.19	3.36 ± 0.27	3.17 ± 0.18	2.99 ± 0.22
Max no. of spikes fired	5.87 ± 0.6	4.5 ± 0.84	5.8 ± 0.75	6.5 ± 0.66

Table S4. Intrinsic electrophysiological properties: *FMR1*$^{+/y}$, *FMR1*$^{-/y}$

	FMR1$^{+/y}$ **neurons** ***FMR1***$^{+/y}$ **astrocytes**	***FMR1***$^{-/y}$ **neurons** ***FMR1***$^{-/y}$ **astrocytes**	***FMR1***$^{+/y}$ **neurons** ***FMR1***$^{-/y}$ **astrocytes**	***FMR1***$^{-/y}$ **neurons** ***FMR1***$^{+/y}$ **astrocytes**
Number of cells	23	11	10	12
Resting membrane potential (mV)	-50.2 ± 1.61	-51.66 ± 2.47	-50.66 ± 3.13	-56.11 ± 1.34
Input Resistance (GΩ)	1.06 ± 0.07	1.5 ± 0.16	1.34 ± 0.2	1.35 ± 0.17
Capacitance (pF)	37.33 ± 1.98	30.6 ± 3.22	25.94 ± 2.08	33.9 ± 2.77
Rheobase (pA)	17.61 ± 1.93	16.82 ± 2.72	13 ± 3	20 ± 3.53
Spike amplitude (mV)	65.11 ± 2.26	52.86 ± 3.43	62.21 ± 3.35	64.14 ± 3.13
Spike half-width (ms)	3.24 ± 0.2	3.84 ± 0.28	2.97 ± 0.22	3.66 ± 0.16
Max no. of spikes fired	7.09 ± 0.78	5.9 ± 0.84	5.9 ± 1.17	7.5 ± 1.01

Table S5. Intrinsic electrophysiological properties when cultured in presence of ACM: CON, FXS1

	CON neurons CON ACM	**FXS neurons FXS1 ACM**	**CON neurons FXS1 astrocytes**	**FXS1 neurons CON astrocytes**
Number of cells	11	11	11	10
Resting membrane potential (mV)	-53.07 ± 3.12	-58.02 ± 2.3	-55 ± 2.32	-58 ± 2.06
Input Resistance (GΩ)	1.37 ± 0.13	1.56 ± 0.19	1.44 ± 0.25	1.21 ± 0.16
Capacitance (pF)	27.54 ± 2.51	25.39 ± 2.96	41.16 ± 4.6	36.86 ± 7.9
Rheobase (pA)	20 ± 1.9	17.27 ± 1.95	18.18 ± 2.8	21.5 ± 3.42
Spike amplitude (mV)	57.38 ± 3.8	64.23 ± 5.97	58.95 ± 2.74	65.35 ± 4.39
Spike half-width (ms)	3.91 ± 0.5	3.86 ± 0.38	3.42 ± 0.15	3.61 ± 0.27
Max no. of spikes fired	5.5 ± 0.95	6.3 ± 1.06	5.18 ± 0.76	5.3 ± 0.85

Table S6. Intrinsic electrophysiological properties when cultured in presence of ACM: CON, FXS2

	CON neurons CON ACM	**FXS2 neurons FXS2 ACM**	**CON neurons FXS2 astrocytes**	**FXS2 neurons CON astrocytes**
Number of cells	11	11	11	10
Resting membrane potential (mV)	-53.07 ± 3.12	-53.83 ± 2.36	-54.5 ± 2.83	-52.97 ± 2.63
Input Resistance (GΩ)	1.37 ± 0.13	1.07 ± 0.17	1.3 ± 0.16	1.35 ± 0.15
Capacitance (pF)	27.54 ± 2.51	38.99 ± 5.28	33.13 ± 2.32	33.7 ± 4.9
Rheobase (pA)	20 ± 1.9	22.5 ± 4.23	25 ± 3.09	15.91 ± 2.76
Spike amplitude (mV)	57.38 ± 3.8	67.35 ± 5.05	60.88 ± 3.5	64.67 ± 3.83
Spike half-width (ms)	3.91 ± 0.5	3.13 ± 0.31	3.72 ± 0.3	3.39 ± 0.31
Max no. of spikes fired	5.5 ± 0.95	6.18 ± 0.97	4.45 ± 0.47	5.36 ± 0.78

Table S7. Intrinsic electrophysiological properties when cultured in presence of ACM: *FMR1*$^{+/y}$, *FMR1*$^{-/y}$

	FMR1$^{+/y}$ **neurons** ***FMR1***$^{+/y}$ **ACM**	***FMR1***$^{-/y}$ **neurons** ***FMR1***$^{-/y}$ **ACM**	***FMR1***$^{+/y}$ **neurons** ***FMR1***$^{-/y}$ **astrocytes**	***FMR1***$^{-/y}$ **neurons** ***FMR1***$^{+/y}$ **astrocytes**
Number of cells	12	18	17	10
Resting membrane potential (mV)	-52.43 ± 2.35	-57.99 ± 1.73	-54.51 ± 1.21	-50.81 ± 2.96
Input Resistance (GΩ)	1.36 ± 0.19	1.35 ± 0.13	1.19 ± 0.13	1.48 ± 0.21
Capacitance (pF)	32.96 ± 3.34	35.07 ± 1.76	33.38 ± 2.32	32.54 ± 2.76
Rheobase (pA)	23.75 ± 2.47	26.4 ± 2.41	20.6 ± 1.7	23.5 ± 4.89
Spike amplitude (mV)	63.37 ± 3.16	63.84 ± 3.04	66.83 ± 2.7	72.07 ± 5.03
Spike half-width (ms)	3.31 ± 0.27	3.24 ± 0.23	3.45 ± 0.16	3.35 ± 0.27
Max no. of spikes fired	5.83 ± 0.75	6.72 ± 0.67	5 ± 0.41	5.3 ± 1.2

All values are given as mean ± SEM