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Supplemental information

Cell-type-specific profiling of human cellular models of fragile X syndrome reveal PI3K-dependent defects in translation and neurogenesis

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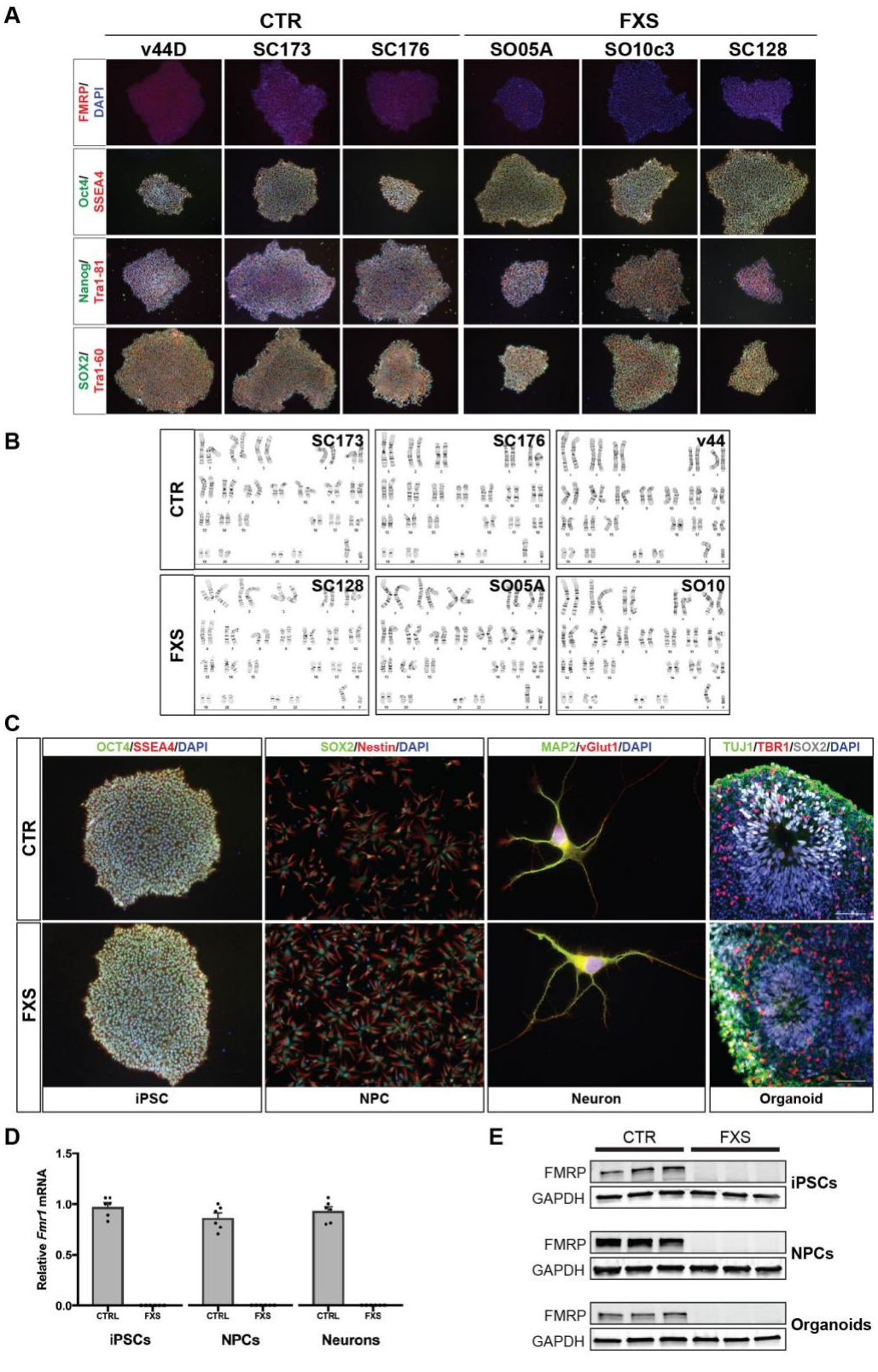

A
CTR
FXS
v44D
SC173
SC176
SO05A
SO10c3
SC128
FMRP/ DAPI
Oct4/ SSEA4
Nanog/ Tra1-81
SOX2/ Tra1-60
B
CTR
FXS
SC173
SC176
v44
SC128
SO05A
SO10
C
OCT4/SSEA4/DAPI
SOX2/Nestin/DAPI
MAP2/vGlut1/DAPI
TUJ1/TBR1/SOX2/DAPI
CTR
FXS
iPSC
NPC
Neuron
Organoid
D
Relative Fmr1 mRNA
1.5
1.0
0.5
0.0
CTRL
FXS
iPSCs
NPCs
Neurons
E
CTR
FXS
FMRP
GAPDH
iPSCs
NPCs
Organoids

Figure S1. Characterization of induced pluripotent stem cell model of FXS. Related to STAR Methods.

(A) Immunofluorescence analysis for markers of pluripotency (OCT4, SOX2, NANOG, SSEA4, TRA1-60, and TRA1-81) and for FMRP in control and FXS iPSC lines used in this study. **(B)** Representative karyograms showing normal karyotypes control and FXS iPSC lines used in this study. **(C)** Representative images of one control and one FXS iPSC line at multiple stages of differentiation. iPSCs express stem cell markers (OCT4, SSEA4), NPCs express proliferative neural markers (Nestin, SOX2), neurons express mature neuronal markers (MAP2, vGlut1) and organoids express immature and mature neural markers (TUJ1, TBR1, SOX2) **(D)** *FMR1* mRNA expression measured by specific taqman assays in iPSCs, NPCs and neurons show loss of expression of *FMR1* in all patient lines (n=6 controls, 6 FXS) **(E)** Representative immunoblots for FMRP showing loss of FMRP protein across FXS patient iPSCs, NPCs and organoids.

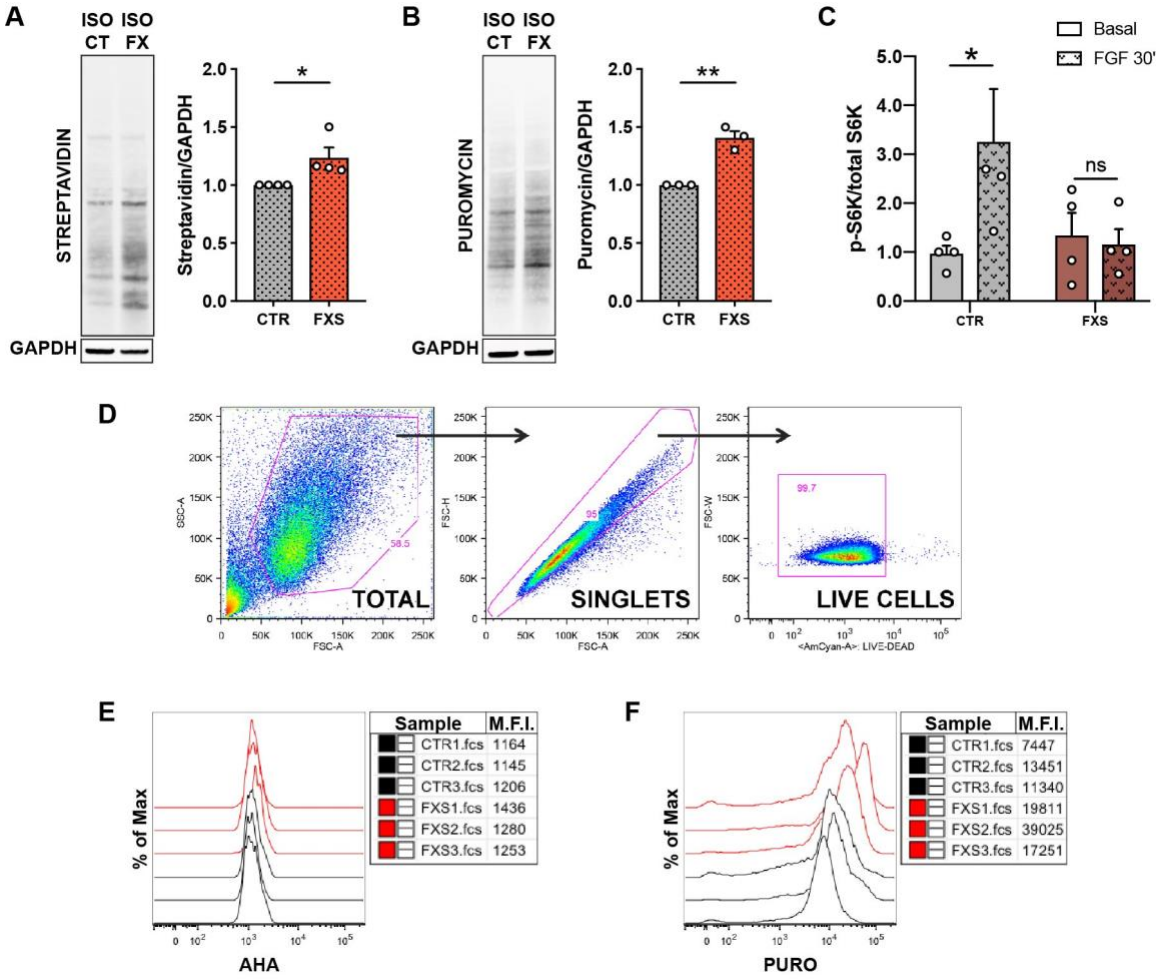
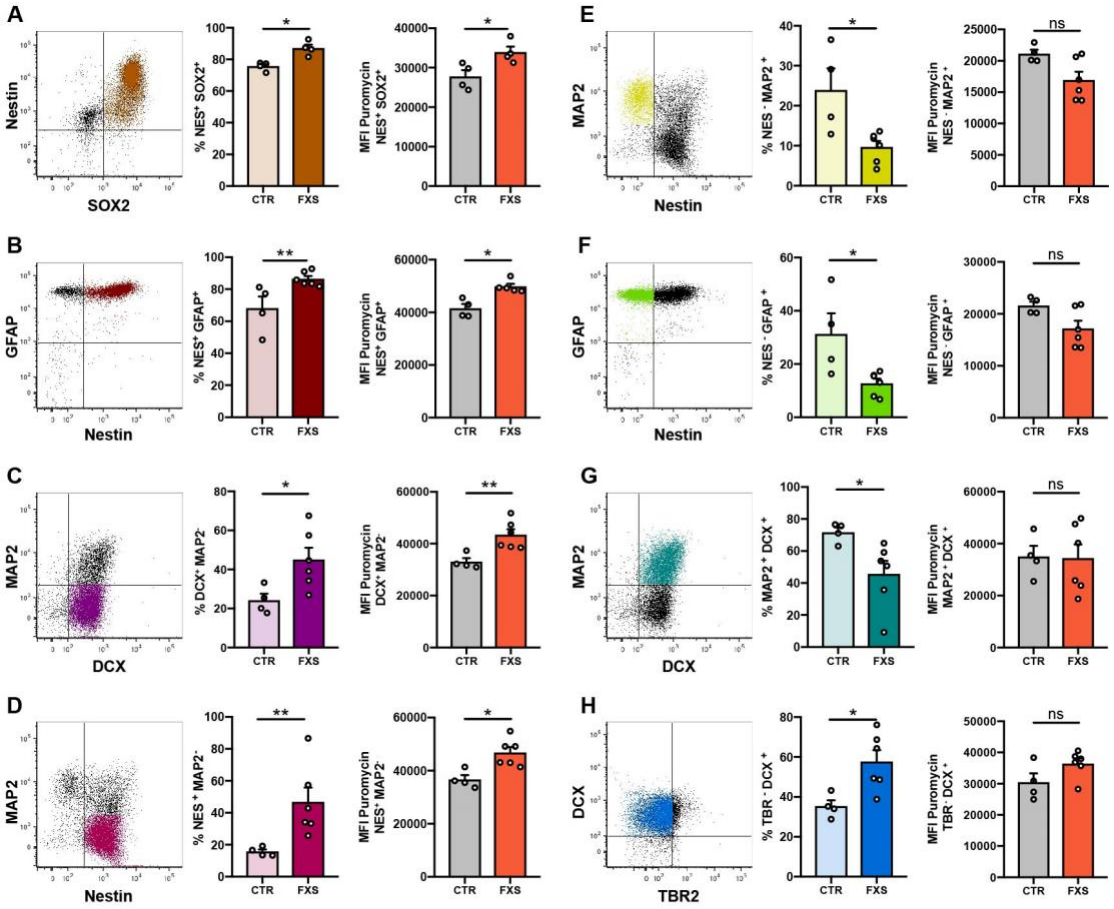


Figure S2: FMRP deficiency results in elevated global protein synthesis. Related to Figure 1.

(A) BONCAT analysis in an isogenic control and FXS line shows increased incorporation of AHA in FMRP-deficient (ISO_KO) NPCs, indicative of higher protein synthesis (Mann-Whitney test, *P<0.05, n= 3 replicates normalized to isogenic control for each replicate. Data are shown as mean ± SEM). **(B)** SUnSET analysis in an isogenic control and FXS line shows increased incorporation of puromycin in FMRP-deficient (ISO_KO) NPCs, indicative of higher protein synthesis (Mann-Whitney test, **P<0.01, n= 3 replicates normalized to isogenic control for each replicate. Data are shown as mean ± SEM). **(C)** Densitometry analysis of NPCs treated with FGF for 30'. FGF stimulation induced phosphorylation of S6K in control NPCs but not in FXS patient NPCs (Two-way ANOVA, Sidak's test, * adj P=0.0416; n=4 CTR 4 FXS patient NPC lines from 2 CTR and 2 FXS iPSC lines) **(D)** Representative flow cytometry gating strategy to isolate single, live cells for analysis. **(E)** Representative histograms showing AHA incorporation is right-shifted in 3 FXS patient lines compared to 3 controls indicative of increased protein synthesis. Median Fluorescent Intensity (MFI) values are shown on the right. **(F)** Representative histograms showing puromycin incorporation is right-shifted in 3 FXS patient lines compared to 3 CTR lines indicative of increased protein synthesis in FXS. Median Fluorescent Intensity (MFI) values are shown on the right.



I

		L/D aqua AmCyan	Nestin V450	Ki67 PE-Cy7	DCX PE	SOX2 PerCP-Cy5.5	Puromycin Alexa 488	MAP2B Alexa 647	GFAP Alexa 700
FMO 1	L/D aqua AmCyan	-	+	+	+	+	+	+	+
FMO 2	Nestin V450	+	-	+	+	+	+	+	+
FMO 3	Ki67 PE-Cy7	+	+	-	+	+	+	+	+
FMO 4	DCX PE	+	+	+	-	+	+	+	+
FMO 5	SOX2 PerCP-Cy5.5	+	+	+	+	-	+	+	+
FMO 6	Puromycin Alexa 488	+	+	+	+	+	-	+	+
FMO 7	MAP2B Alexa 647	+	+	+	+	+	+	-	+
FMO 8	GFAP Alexa 700	+	+	+	+	+	+	+	-

J

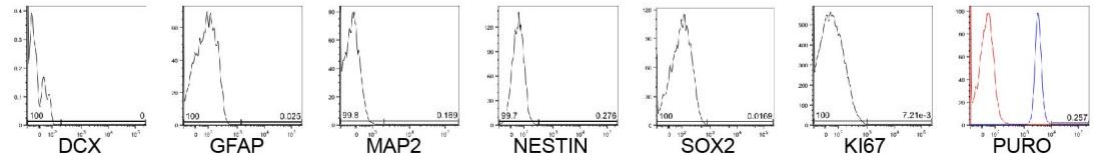


Figure S3: neurOMIP assay reveals cell-type-specific defects in translation. Related to Figure 2.

(A-D) Increased population of proliferative cells with higher protein synthesis in FXS differentiating cultures relative to controls (Mann-Whitney test, n=4 control, 6 FXS, **P<0.01, *P<0.05). Data are shown as Mean±SEM. **(C-H)** Decreased population of non-proliferating cells in FXS differentiating cultures relative to controls. No significant difference in puromycin incorporation between control and FXS cultures (Mann-Whitney test, n=4 control, 6 FXS, **P<0.01, *P<0.05). Data are shown as Mean±SEM. **(I)** Fluorescence-minus-one (FMO) controls setup for each neurOMIP experiment helps to establish gates for each marker. **(J)** Representative histograms of FMO controls showing positive and negative gating for each marker. Puro histogram shows FMO for puro (Red trace) and "no puro" control (Blue trace). "No puro" was used to set positive and negative gates for puromycin.

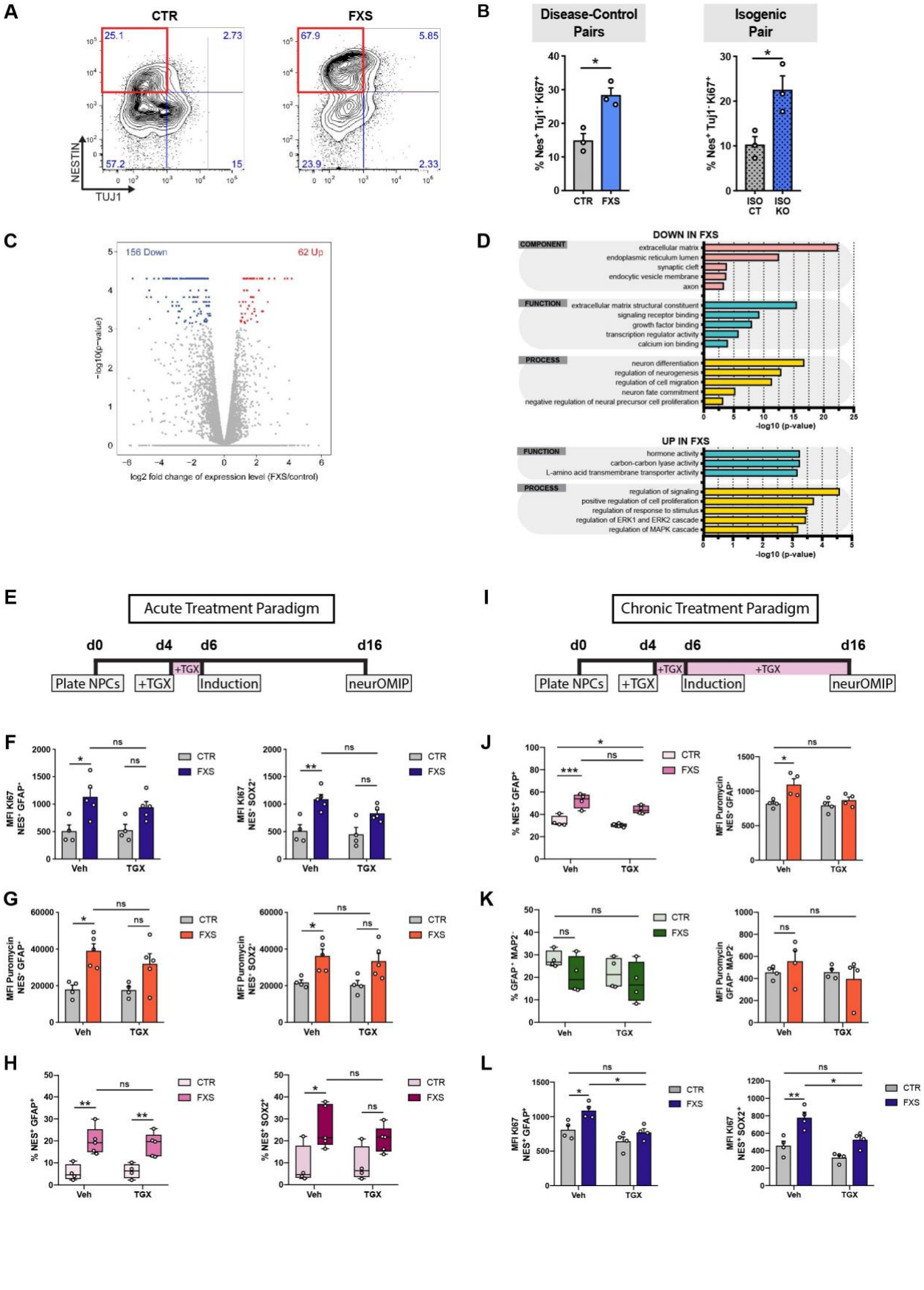

A
CTR
FXS
25.1
2.73
57.2
15
67.9
5.85
23.9
2.33
NESTIN
TUJ1
B
Disease-Control Pairs
Isogenic Pair
% Nes+ Tuj1- Ki67+
CTR
FXS
ISO CT
ISO KO
C
156 Down
62 Up
-log10(p-value)
log2 fold change of expression level (FXS/control)
D
DOWN IN FXS
COMPONENT
extracellular matrix
endoplasmic reticulum lumen
synaptic cleft
endocytic vesicle membrane
axon
FUNCTION
extracellular matrix structural constituent
signaling receptor binding
growth factor binding
transcription regulator activity
calcium ion binding
PROCESS
neuron differentiation
regulation of neurogenesis
regulation of cell migration
neuron fate commitment
negative regulation of neural precursor cell proliferation
-log10 (p-value)
UP IN FXS
FUNCTION
hormone activity
carbon-carbon lyase activity
L-amino acid transmembrane transporter activity
PROCESS
regulation of signaling
positive regulation of cell proliferation
regulation of response to stimulus
regulation of ERK1 and ERK2 cascade
regulation of MAPK cascade
-log10 (p-value)
E
Acute Treatment Paradigm
d0
d4
d6
d16
Plate NPCs
+TGX
Induction
neurOMIP
I
Chronic Treatment Paradigm
d0
d4
d6
d16
Plate NPCs
+TGX
+TGX
Induction
neurOMIP
F
MFI Ki67 NES+ GFAP-
MFI Ki67 NES+ SOX2+
CTR
FXS
Veh
TGX
G
MFI Puromycin NES+ GFAP-
MFI Puromycin NES+ SOX2+
H
% NES+ GFAP+
% NES+ SOX2+
J
% NES+ GFAP+
MFI Puromycin NES+ GFAP+
K
% GFAP+ MAP2-
MFI Puromycin GFAP+ MAP2-
L
MFI Ki67 NES+ GFAP+
MFI Ki67 NES+ SOX2+

Figure S4: Transcriptomic analysis reveals dysregulation of pathways related to neurogenesis, neural cell fate commitment and ERK1/ERK2/MAPK signaling and increased proliferation in FXS can be corrected with PI3K and S6K1 inhibitors. Related to Figure 3 and Figure 4.

(A) Representative contour plots image of single, live proliferating NPCs (Nestin-positive,TUJ1-negative) in one control and one FXS patient line. **(B)** Increased active proliferation in FXS, evidenced by more Ki67+NESTIN+ positive cells in FXS patient NPCs (Mann-Whitney test, **P<0.01, n= 3 CTR, 3 FXS lines) and isogenic engineered FXS line (unpaired t test, *P<0.05, n= 3 replicates). **(C)** Volcano plot of differentially expressed genes in D28 FXS patient organoids versus control organoids. **(D)** Gene ontology (GO) analysis of DEGs in FXS. Downregulated genes in FXS are enriched for GO terms related to processes of neural cell fate commitment and neurogenesis, while upregulated genes in FXS are enriched for GO terms related to regulation of ERK/MAPK signaling and cell proliferation is up in FXS. **(E)** Acute treatment paradigm using TGX-221 followed by neurOMIP analysis **(F,G)** Acute TGX treatment reduces *(F)* Ki67 and *(G)* puromycin signals in proliferative populations. **(H)** Acute treatment paradigm does not correct increased NES+GFAP+ and NES+SOX2+ populations in FXS patient cultures. **(I)** Chronic treatment paradigm using TGX-221 followed by neurOMIP analysis **(J)** Chronic treatment reduces but does not significantly normalize increased NES+GFAP+ population in FXS patient cultures. **(K)** No significant effect of TGX-221 treatment on GFAP+MAP2- cells in FXS cultures. **(L)** Chronic TGX treatment normalizes Ki67 in NES+GFAP+ and NES+SOX2+ proliferative populations in FXS cultures. n= 4 CTR, 4 FXS NPCs; two-way ANOVA, Tukey's test, *** adj P<0.001, ** adj P<0.01, * adj P<0.05. All data are shown as mean ± SEM.

Table S1: Control and Subject patient material used in this study. Related to STAR Methods.

I.D.	Source material	Genotype	Source
SC173	Fibroblast	CTR	P. H. Schwartz (CHOC)
SC176	Fibroblast	CTR	P. H. Schwartz (CHOC)
v36	Fibroblast	CTR	E.M. Berry-Kravis (Rush University)
v44	Fibroblast	CTR	E.M. Berry-Kravis (Rush University)
v48	Fibroblast	CTR	E.M. Berry-Kravis (Rush University)
SC128	Fibroblast	FXS	P.H. Schwartz (CHOC)
CH095	Fibroblast	FXS	C.M. Hales (Emory University)
SO02	Fibroblast	FXS	E.M. Berry-Kravis (Rush University)
SO03	Fibroblast	FXS	E.M. Berry-Kravis (Rush University)
SO04	Fibroblast	FXS	E.M. Berry-Kravis (Rush University)
SO05	Fibroblast	FXS	E.M. Berry-Kravis (Rush University)
SO10	Fibroblast	FXS	E.M. Berry-Kravis (Rush University)
FXSB2	iPSC	FXS	S.T. Warren (Emory University)
C1-2	iPSC (Isogenic 1)	CTR (engineered)	S.T. Warren (Emory University)
FXS	iPSC (Isogenic 1)	FXS	S.T. Warren (Emory University)
F33F7	ESC (Isogenic 2)	CTR	L. Chen, M. Wernig (Stanford)
F33cKO	ESC (Isogenic 2)	FXS (engineered)	L. Chen, M. Wernig (Stanford)
UCD-17-02	Frontal Cortex	Control	V. Martinez-Cerdeño (UC Davis)
UCD-17-07	Frontal Cortex	Control	V. Martinez-Cerdeño (UC Davis)
UCD-18-04	Frontal Cortex	Control	V. Martinez-Cerdeño (UC Davis)
1031-08-GP	Frontal Cortex	Full Mutation	V. Martinez-Cerdeño (UC Davis)
1018-10-RH	Frontal Cortex	Full Mutation	V. Martinez-Cerdeño (UC Davis)
1031-09-LZ	Frontal Cortex	Full Mutation	V. Martinez-Cerdeño (UC Davis)

Table S2: Summary of cell lines/patient tissue used in experiments. Related to STAR Methods.

I.D.	Cells generated			Experiments[a]					
	iPS	NPC	Organoid	BON	SUn	OMIP	EdU	WB	IF
SC173	√	√	√	√	√	√	-	√	-
SC176	√	√	-	√	√	√	√	√	√
v36	√	-	√	-	-	-	-	-	-
v44	√	√	-	-	√	√	-	√	√
v48	√	√	√	√	√	√	√	√	√
SC128	√	√	-	√	√	√	√	√	√
CH095	√	√	-	√	√	√	√	√	√
SO02	√	√	-	√	√	√	-	√	-
SO03	√	√	√	-	√	√	-	-	-
SO04	√	√	√	-	√	√	-	-	-
SO05	√	√		√	√	√	-	√	√
SO10	√	√	√	√	√	√	√	√	√
FXSB2	-	-	√	-	-	-	-	-	-
C1-2	-	√	√	√	√	-	√	√	-
FXS	-	√	√	√	√	-	√	√	-
F33F7	-	√	-	√	√	√	√	√	-
F33cKO	-	√	-	√	√	√	√	√	-
UCD-17-02								√	
UCD-17-07								√	
UCD-18-04								√	
1031-08-GP								√	
1018-10-RH								√	
1031-09-LZ								√	

[a] BON= BONCAT western blots; SUn= SUnSET western blots; EdU= EdU immunofluorescence; OMIP= neurOMIP flow cytometry assay; WB= western blotting, IF= immunofluorescence

Table S3: neurOMIP antibody panel. Related to Figure 2 and Figure S3.

Antibody	uL/test	Fluorophore	Company	Purpose
Ki67	0.25	PE-Cy7	BD Biosciences	Proliferation quantification
Puromycin	0.5	Alexa 488	Millipore	Protein synthesis quantification
Nestin	1	V450	BD Biosciences	Neural Stem Cell/Progenitor
SOX2	1	PerCP-Cy5.5	BD Biosciences	Neural Stem Cell
DCX	1	PE	BD Biosciences	Neuroblast/Immature Neuron
MAP2	1	Alexa 647	BD Biosciences	Mature Neuron
GFAP	0.5	Alexa 700	BD Biosciences	Glial Cell