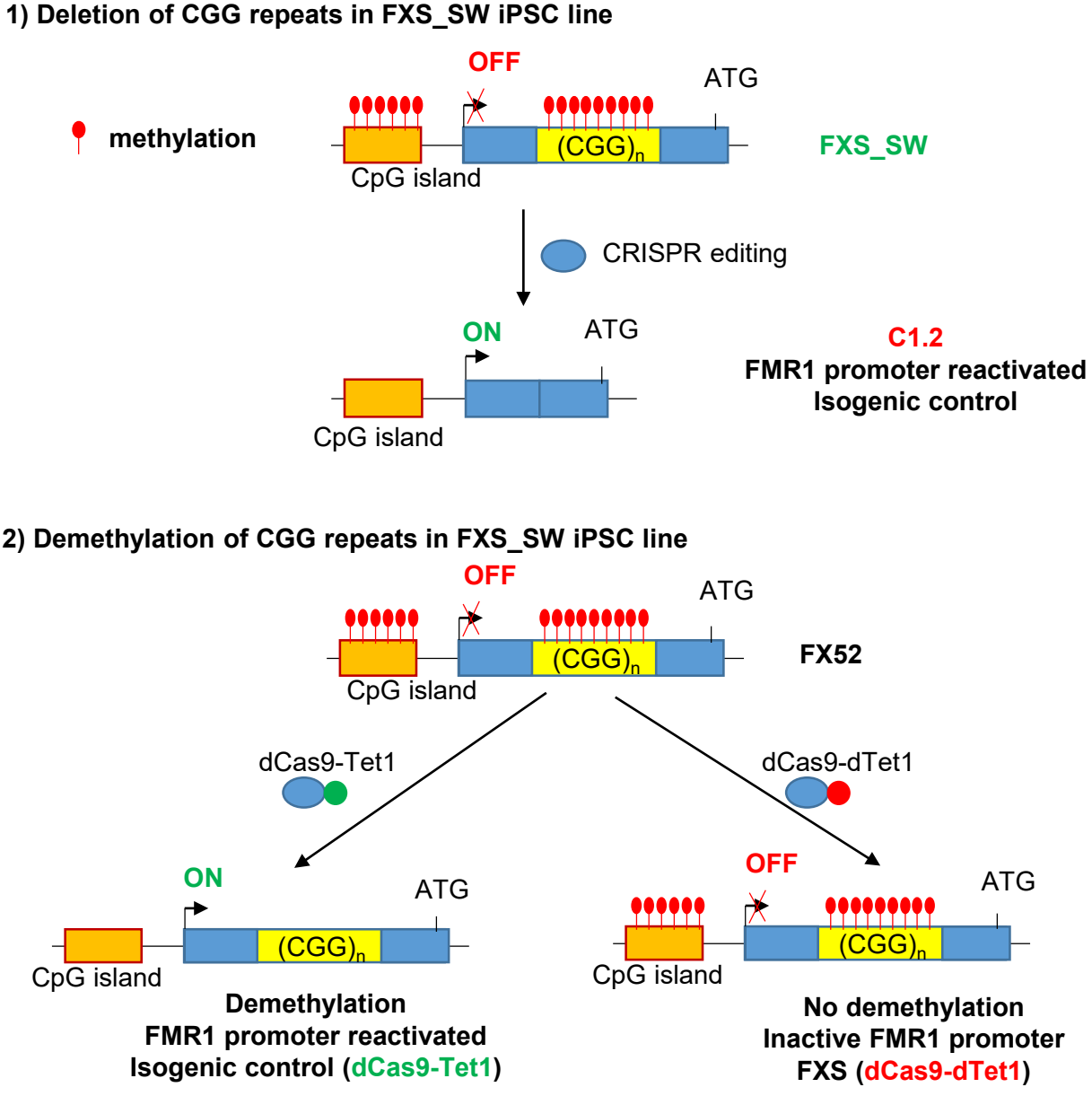
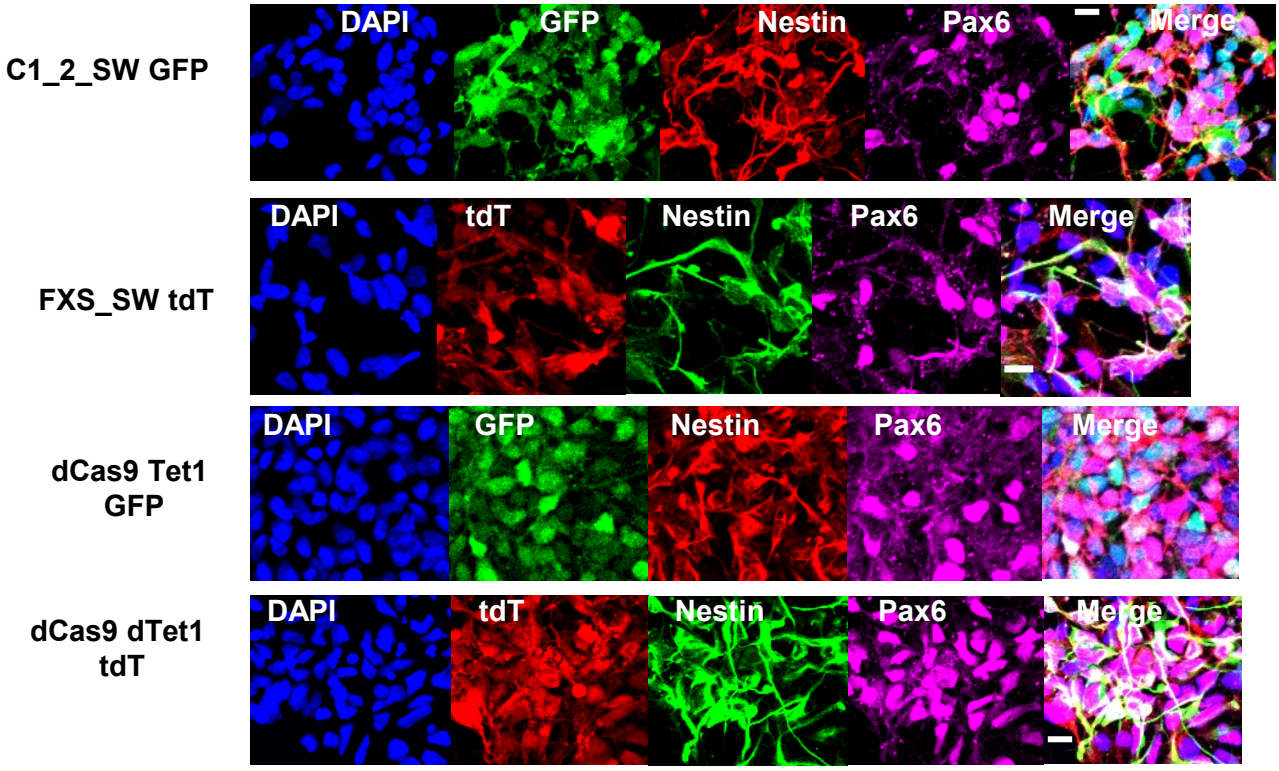
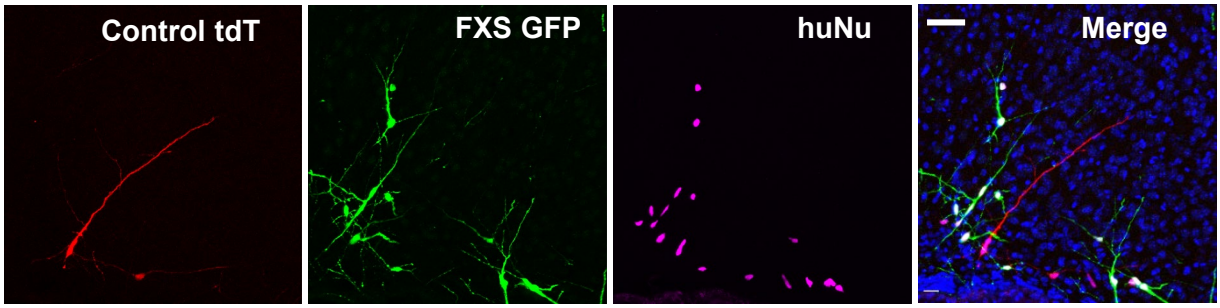
Supplementary material



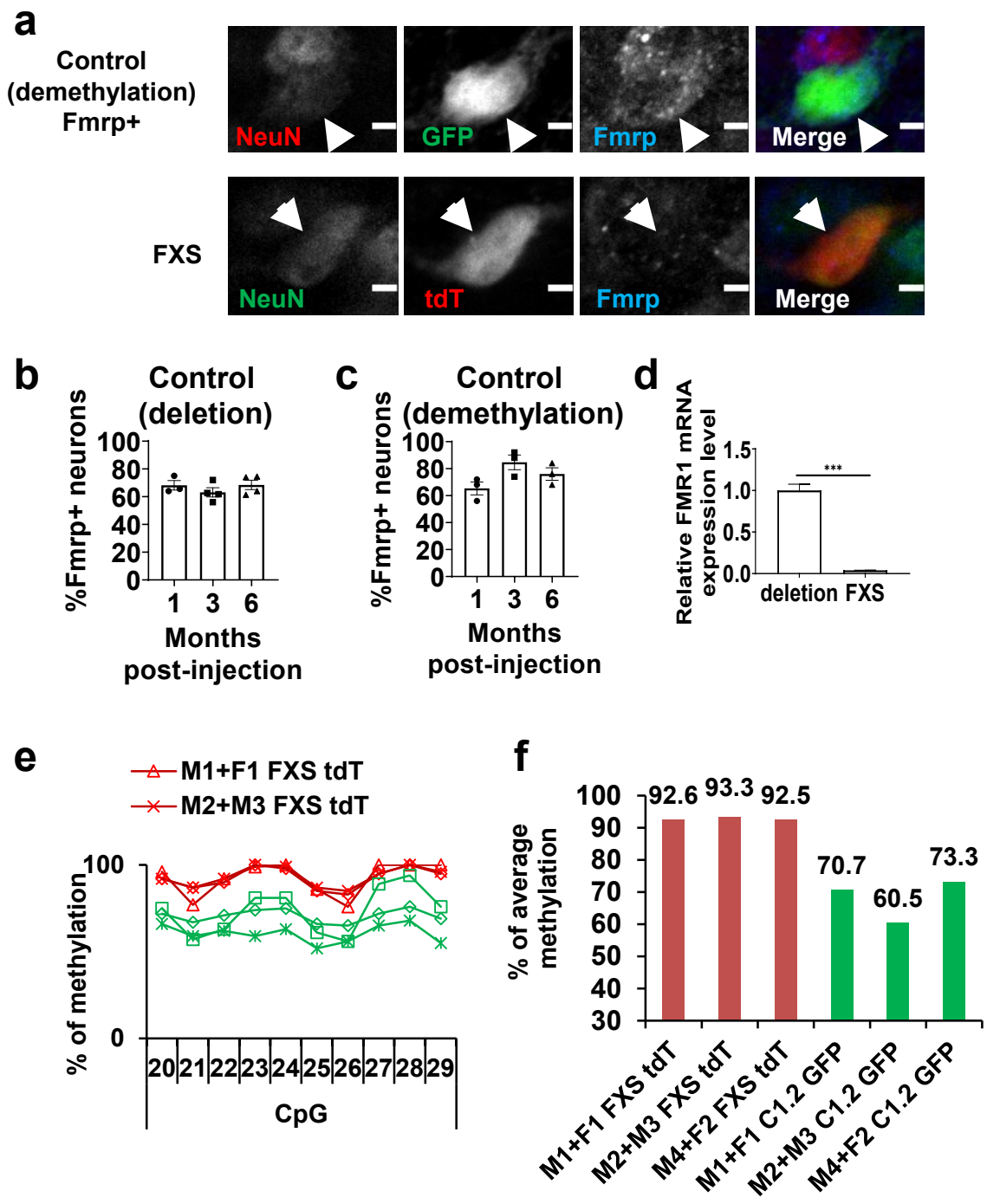
Suppl. Figure 1: **Isogenic pairs used in this study.** Two different male FXS cell lines were used. In FXS_SW, CGG repeats were deleted using CRISPR editing, and in FXS2, CGG repeats were demethylated using dCas9-Tet1/single guide RNA. Both approaches led to the reactivation of the Fmr1 promoter.



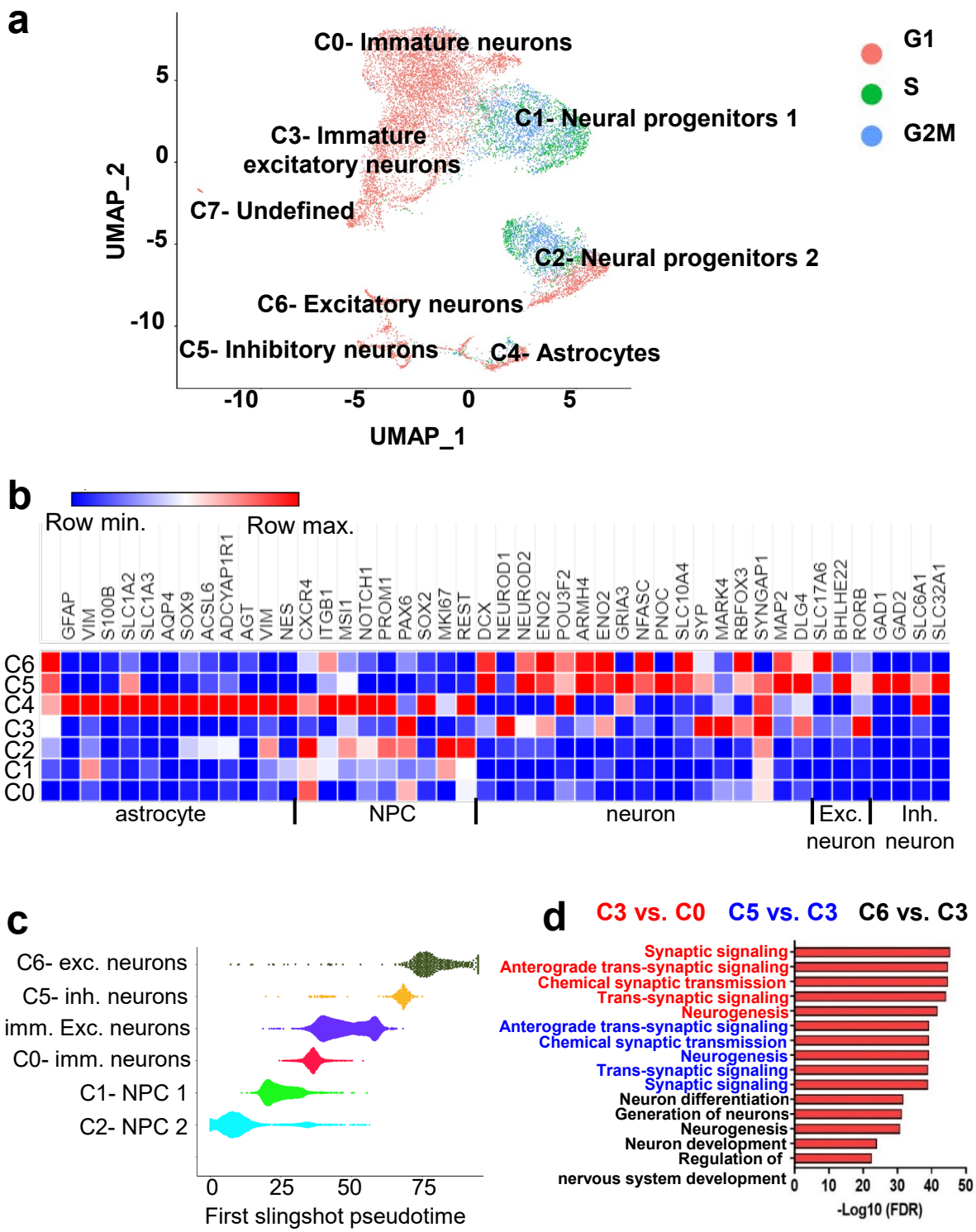
Suppl. Figure 2: hNPCs derived from isogenic control and FXS iPSC express neural progenitor markers before transplantation. Confocal maximum intensity projections of immunostainings against Nestin and Pax6 on cultured hNPCs from the different isogenic pairs. Scale bars represent 5 µm.



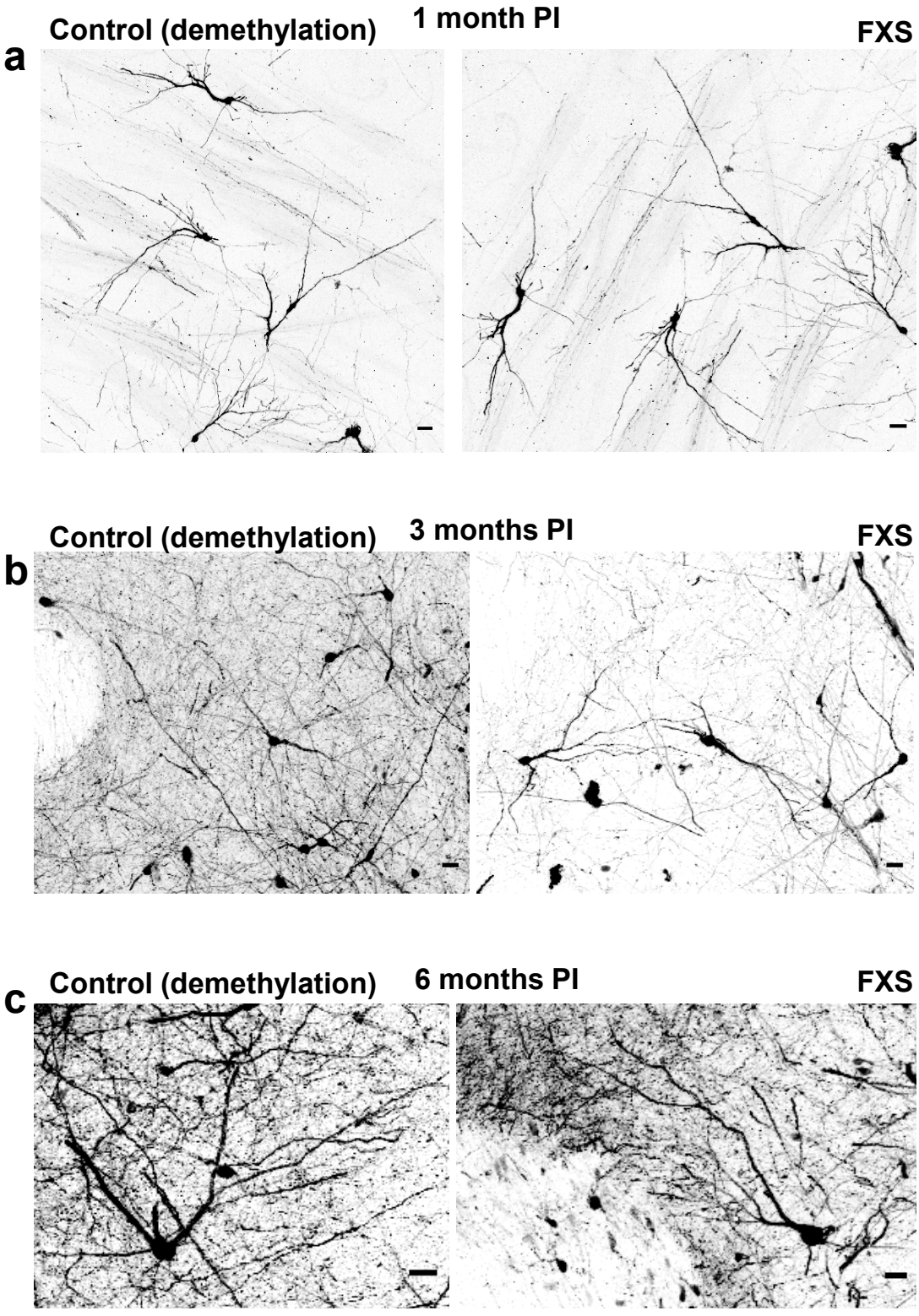
Suppl. Figure 3: Fluorescent cells in the chimeric brains are all of human origin. Representative confocal maximum intensity projections of the cortical region of a mouse brain at 1 month post-injection engrafted with tdTomato-labeled C1_2_SW and GFP-labeled FXS_SW isogenic NPCs at P1. All the engrafted cells were stained for human nuclei antigen (HuNu) and GFP+ and tdT+ cells were mutually exclusive. Stained in green, GFP; red, tdT; magenta, huNu; blue, DAPI.



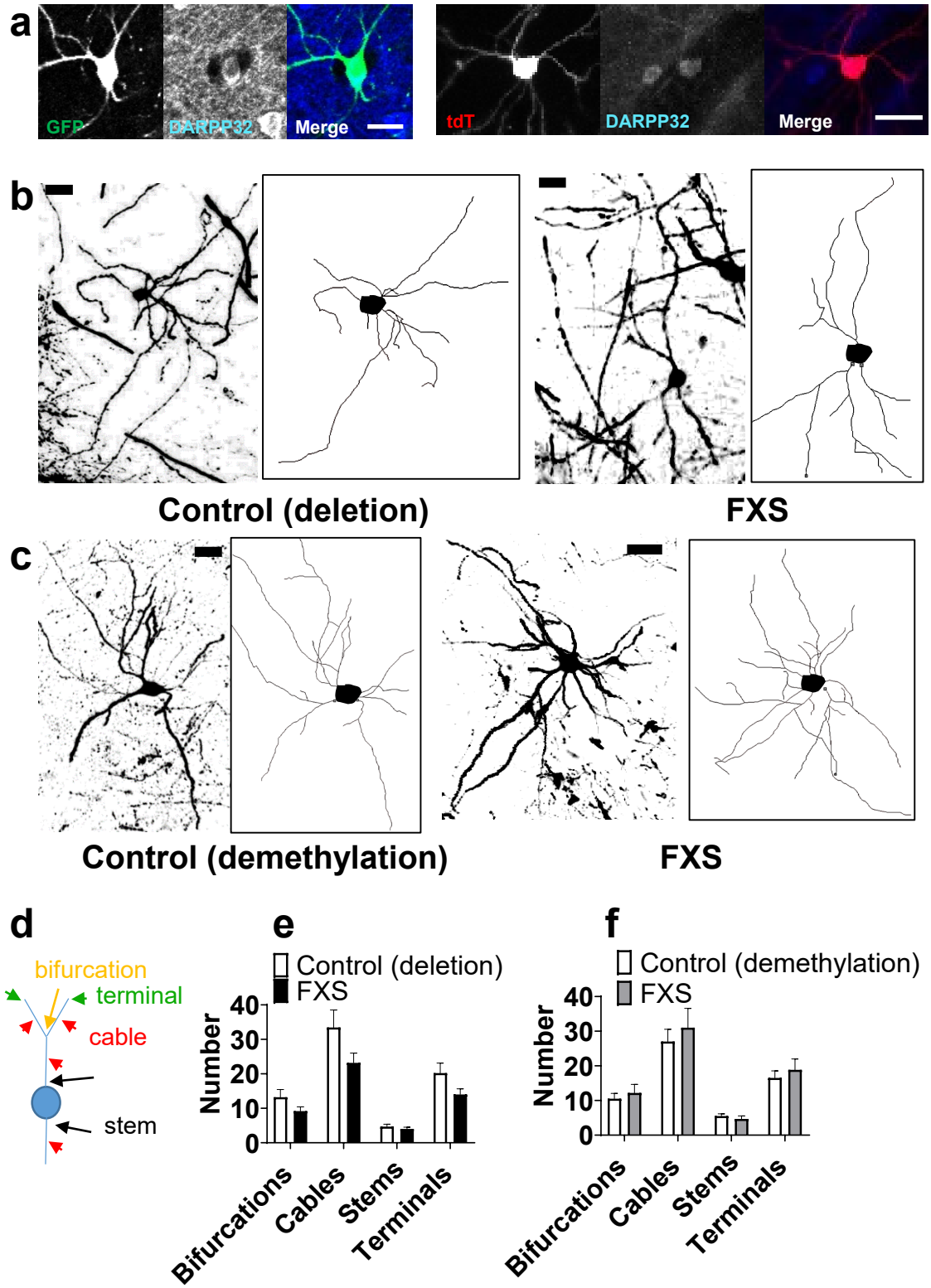
Suppl. Figure 4: Isogenic control cells transplanted in the mouse brain express Fmrp. a. Confocal maximum intensity projections of cell bodies of control (demethylation) GFP Fmrp-positive transplanted NeuN-positive neurons (green, upper panel) and FXS tdT transplanted NeuN-positive neurons (red, lower panel) at 3 months PI. Scale bars represent 5 µm. **b.** Percentage of isogenic control (deletion) neurons expressing Fmrp as assessed by immunofluorescence against Fmrp at 1, 3 and 6 months PI. Neurons were defined as doublecortin-positive cells at 1 month PI and NeuN-positive cells at 3 and 6 months PI. One-way ANOVA; N=3 to 4 animals, 25 to 59 neurons analyzed per animal per group. **c.** Percentage of isogenic control (demethylation) neurons reexpressing Fmrp as assessed by immunofluorescence against Fmrp at 1, 3 and 6 months PI. Neurons were defined as doublecortin-positive cells at 1 month PI and NeuN-positive cells at 3 and 6 months PI. One-way ANOVA; N=3 to 4 animals, 50 neurons analyzed per animal per group. **d.** *FMR1* expression by RT-qPCR on total mRNA of isogenic control (deletion) and FXS cells extracted from the mouse brain at 1 month PI. Unpaired t-test, N= 3 technical replicates. **e.** DNA methylation level of 10 CpGs in the promoter of FMR1 measured by Pyro-sequencing of transplanted cells extracted from the mouse brain at one-month PI. **f.** Average DNA methylation levels of the 10 CpGs in the promoter of FMR1 presented in **g.** Bar heights and whiskers represent the mean +/- SEM. ***: p<0.001



Suppl. figure 5: Characterization of UMAP clusters from transplanted cells extracted at 1 month post-injection. a. Cell cycle markers in the different UMAP clusters **b.** Heatmap of canonical marker genes used to determine cell types in the different UMAP clusters. Red indicates higher expression compared to values within the row. Blue indicates lower expression compared to values within the row. **c.** Slingshot pseudotime analysis of the UMAP clusters with NPCs (C2-NPC 2) as the starting point. Since C4-Astrocytes were assigned to the 2nd lineage, and all the cells were displayed along the 2nd pseudotime, the cells in C4-Astrocytes are absent along the first pseudotime in this figure. **d.** Five top upregulated gene pathways in cluster C3-Immature excitatory neurons compared to C0- Immature neurons (red font), five top upregulated gene pathways in C5- Inhibitory neurons compared to C3- Immature excitatory neurons (blue font), five top upregulated gene pathways in C6- Excitatory neurons compared to C3- Immature excitatory neurons (black font). Up- or downregulation of pathways was considered significant when FDR<0.05.



Suppl. figure 6: The morphology of transplanted neurons evolves over time. Dendrites of transplanted neurons become thicker, and the cell bodies become less oblong. **a.** Confocal maximum intensity projections of isogenic control (demethylation) (left panel) and FXS (right panel) doublecortin-positive neurons (right panel) in the striatum at 1 month post-injection (PI). **b.** Confocal maximum intensity projections of isogenic control (demethylation) (left panel) and FXS (right panel) NeuN-positive neurons (right panel) in the striatum at 3 months PI. **c.** Confocal maximum intensity projections of isogenic control (demethylation) (left panel) and FXS (right panel) NeuN-positive neurons (right panel) in the striatum at 6 months PI. Scale bars represent 20 µm.



Suppl. figure 7: Control and FXS striatal medium spiny neurons (MSNs) do not display significant changes in neuronal arbor complexity at 6-7 months post-transplantation. a. Single confocal planes of FXS (tdT) and isogenic control (GFP) neurons stained with DARPP32. **b.** Representative 3D reconstructions and corresponding tracings of transplanted control (left panel) and FXS (right panel) DARPP32-positive neurons from the deletion pair. **c.** Representative 3D reconstructions and corresponding tracings of transplanted control (left panel) and FXS (right panel) DARPP32-positive neurons from the demethylation pair. **d.** Scheme illustrating bifurcations (nodes), terminals, cables (branches) and stems of a neuron. **e.** Number of bifurcations, cables, stems and terminals of FXS and isogenic control (deletion) DARPP32-positive control neurons. Mixed-effects analysis; N=6 to 11 neurons per group. **f.** Number of bifurcations, cables, stems and terminals of FXS and isogenic control (demethylation) DARPP32-positive control neurons. Mixed-effects analysis; N=6 neurons per group. Scale bars represent 20 µm.

Table S1: Most strongly downregulated genes in FXS neurons as assessed by single cell RNA seq analysis. The threshold was set at log2FC< -2.

Gene symbol	Gene name	Function	Log_2(FC)
FMR1	FMRP translational regulator 1	Translational regulator	-7.0
CA9	Carbonic anhydrase 9	Carbonic anhydrase	-6.3
HHEX	Hematopoietically expressed homeobox	Transcription factor	-6.2
ZNF736	Zinc finger protein 736	Involved in transcriptional regulation?	-3.0
HES5	Hes family bHLH transcription factor 5	Transcription factor	-2.5
OTX1	Orthodenticle homeobox 1	Transcription factor	-2.1
CDCA2	Cell division cycle associated 2	Regulation of DNA damage response	-2.1

Table S2: Most strongly upregulated genes in FXS neurons as assessed by single cell RNA seq analysis. The threshold was set as log2FC>2.

Gene symbol	Gene name	Function	Log_2(FC)
HOXC10	Homeobox C10	Transcription factor	8.9
TAF9B	TATA-box binding protein associated factor 9b	Transcription factor subunit	6.8
NKX2-2	NK2 homeobox 2	Transcription factor	6.4
TBX1	T-box transcription factor 1	Transcription factor	5.8
HOXA10	Homeobox A10	Transcription factor	4.3
CCDC125	Coiled-coil domain containing 125	Regulation of cell migration?	4.1
SPINK5	Serine peptidase inhibitor Kazal type 5	Serine protease inhibitor	4.0
C9orf64	Chromosome 9 open reading frame 64	Unknown	3.8
HMX1	H6 family homeobox 1	Transcription factor	3.5
TRIM61	Tripartite motif containing 61	unknown	3.2
SP140L	SP140 nuclear body protein like	unknown	3.1
HOXD3	Homeobox D3	Transcription factor	3.1

Table S3: FXS patient-derived iPSC lines and isogenic control cell lines used in this study

Induced pluripotent stem cell lines	Reference
FXS_SW (from Steven Warren's group)	Xie et al., 2016
C1_2_SW (from Steven Warren's group)	Xie et al., 2016
FXS2	Park et al., 2015
FXS2 dCT	Liu et al., 2018
FXS2 dCdT	Liu et al., 2018

Table S4: Antibodies used in this study

Reagent	Source	Identifier
Primary antibodies		
Guinea pig anti-Arc	Synaptic Systems	156 004
Guinea pig anti-doublecortin	MilliporeSigma	AB2253MI
Rabbit anti-doublecortin	Cell Signaling Technology	4604
Rabbit anti-FMRP	Cell Signaling Technology	4317S
Rabbit anti-FMRP	Cell Signaling Technology	7104
Chicken anti-GFP	AVES Labs	GFP-1020
Mouse anti-human nuclei	MilliporeSigma	MAB1281
Guinea pig anti-NeuN	Life Technologies	ABN90MI
Mouse anti-NeuN	MilliporeSigma	MAB377
Goat anti-tdTomato	SICGEN	AB8181-200
Mouse anti-Tuj1	Biolegend	801201
Rat anti-DARPP32	Lifespan Biosciences	LS-C36138
Secondary antibodies		
Donkey Alexa 488 fluor anti-chicken	Jackson ImmunoResearch	703-545-155
Donkey Alexa 555 fluor anti-goat	Life Technologies	A21432
Donkey IRDye 680LT anti-guinea pig	LI-COR Biosciences	925-68030
Donkey Alexa fluor 405 anti-mouse	Abcam	ab175659
Donkey Alexa fluor 647 anti-mouse	Life Technologies	A31571
Donkey Alexa fluor 405 anti-rabbit	Fisher Scientific	NC0192764
Donkey Alexa fluor 488 anti-rabbit	Life Technologies	A21206
Donkey Alexa fluor 594 anti-rabbit	Life Technologies	A21207
Donkey Alexa fluor 647 anti-rabbit	Life Technologies	A31573
Donkey Alexa fluor 647 anti-rat	Jackson ImmunoResearch	712-605-153