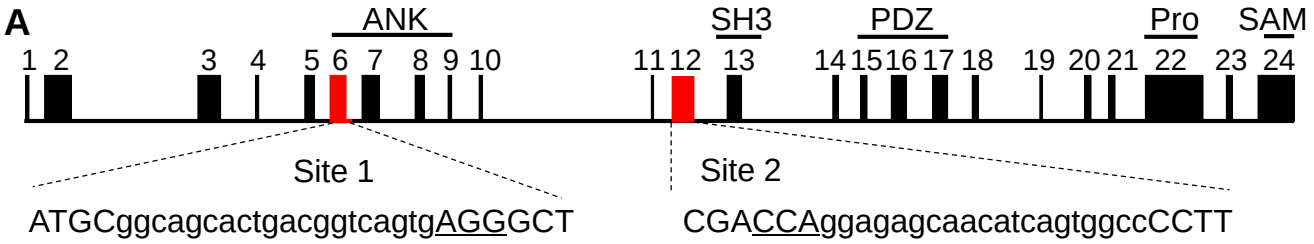
Supplementary Information

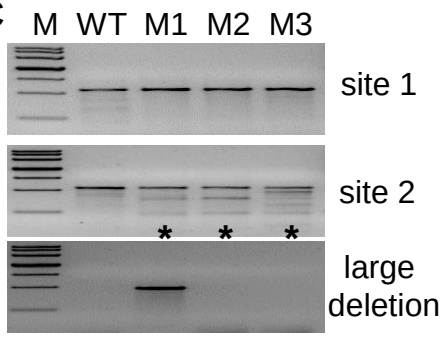
Figures

Figure S1



B

code	Gender	Mutation site	Note	pregnancy period
M1	F	sites 1 and 2	aborted	125 days
M2	M	site 2	dystocia	169 days
M3	M	site 2	live birth	163 days



D Mosaic analysis of varies tissues of M1 and M2 by T7EN1 cleavage assay

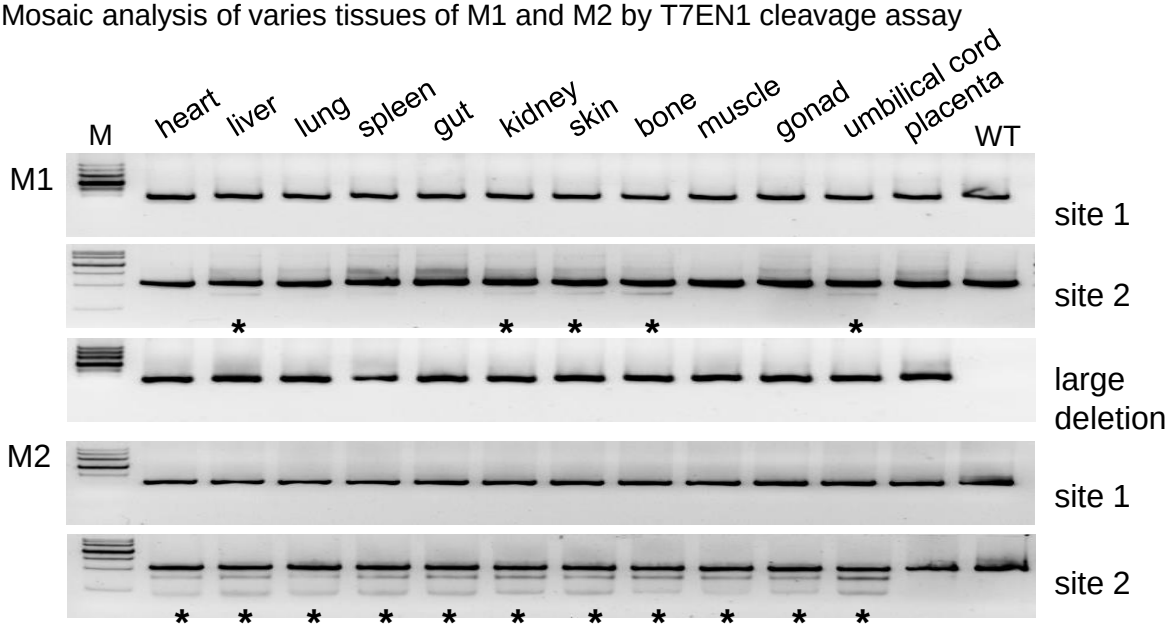


Figure S1-continued

E DNA sequence of mutant alleles at site 2 induced by sgRNA

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        R   D   A   D   Q   E   S   N   I   S   G   P
        CGG GAT GCC GAC CAg gag agc aac atc agt ggc cCC  (site 2, WT)
M1      CGG GAT GCC GA-  - - -  - - -  -gc aac atc agt ggc cCC  (-8 bp, 0.8%)
        CGG GA-  - - -  - - -  - - -  - - -  - - - - - - - -agt ggc cCC  (-22 bp, 2.2%)
        CAG CGG AAT GC- - - - - - - //- - - -aac atc agt ggc cCC  (-11 456 bp, 70%)

M2      CGG GAT GCC GAC CAg - - -  - -c aac atc agt ggc cCC  (-5 bp, 36.4%)
        CGG GAT GCC GAC CAg gag agc aac atc agt ggc cCC  (+1 bp, 8.4%)
                           ^
                           t
M3      CGG GAT GCC GAC CAg ga-  -gc aac atc agt ggc cCC  (-2 bp, 8.1%)
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F Deep amplicon sequencing analysis of different tissue of M1, M2 and M3 mutants

		WT reads	-22 reads	-8 reads	-5 reads	-2 reads	+1 reads	Mutation rate %
M1	PFC	4686	374	144				9.95%
	striatum	148681	10853	3044				8.55%
	liver	76736	6213	1251				8.86%
	kidney	53252	5344	1877				11.94%
	gonad	101564	7528	5049				11.02%
M2	PFC	3366			2223		510	44.81%
	striatum	101045			61455		14335	42.86%
	liver	42379			32005		5082	46.67%
	kidney	41220			20833		6159	39.57%
	gonad	70680			39074		12142	42.02%
M3	blood	298858				19304		6.07%
	hair	10779				947		8.08%
	placenta	108484				41099		27.48%

Figure S1-continued

G Off target analysis

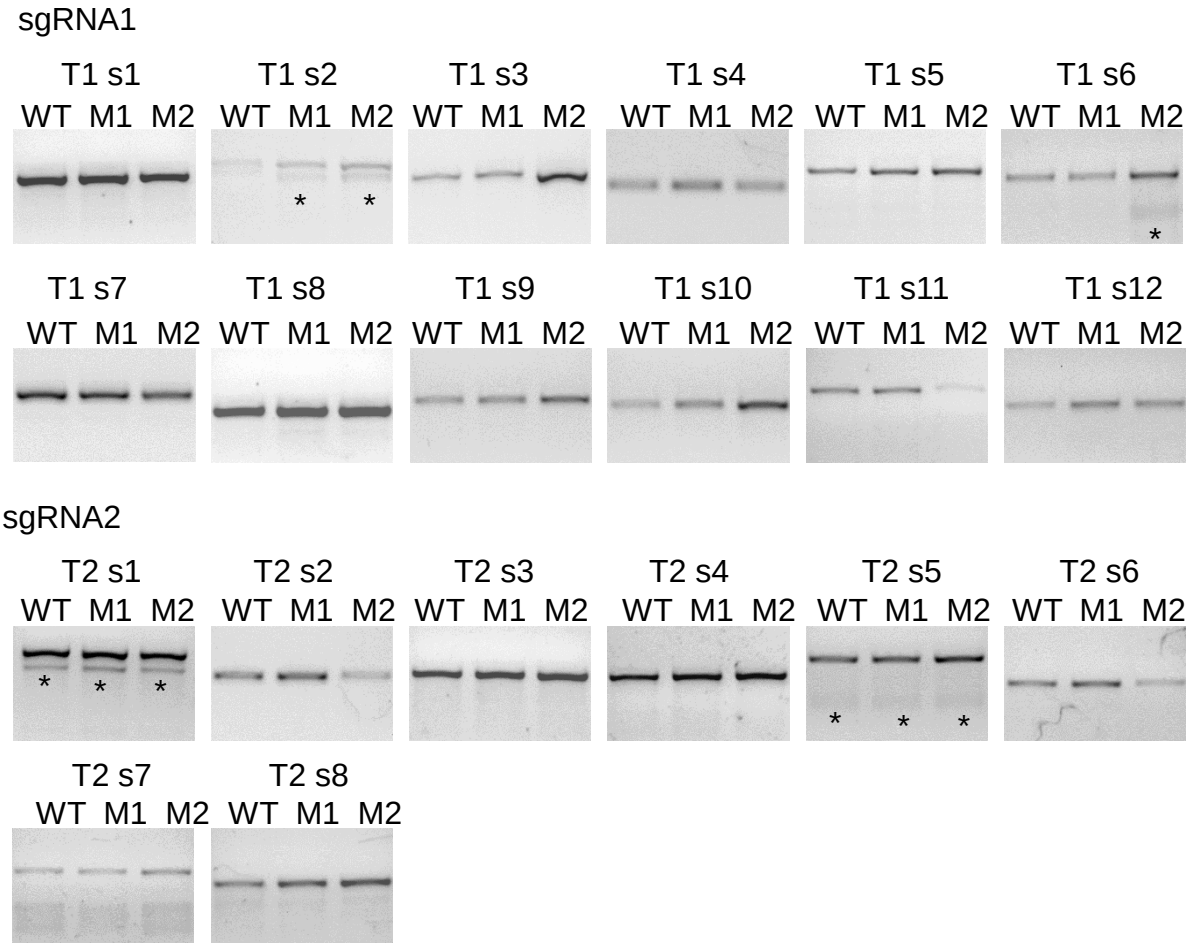

Figure S1 Generation of *SHANK3* gene (NCBI Reference, NW_012011200.1) mutant monkeys. **(A)** *SHANK3* gene structure and protein domain, and CRISPR/Cas9 sgRNA targeting sites**. (B)** Summary of three gene-edited offspring. **(C)** T7 endonuclease I (T7EN1) cleavage assay of PCR products amplified from brain tissues of *SHANK3*^{M1} and *SHANK3*^{M2}, and the umbilical cord of *SHANK3*^{M3}. * indicates samples with cleaved bands. M, DNA marker III; WT, wild-type brain tissues. **(D)** Mosaic analysis of various tissues of *SHANK3*^{M1} and *SHANK3*^{M2} by T7EN1 cleavage assay. * indicates samples with cleaved bands. M, DNA marker III; WT, wild-type brain tissues. **(E)** DNA sequences of *SHANK3* mutations at site 2 in three offspring. *SHANK3*^{M1} has a large deletion of 11 456 bp as a result of targeting both sites. **(F)** Deep amplicon sequencing of different tissues from three offspring. The reads number for each allele is presented. **(G)** Off-target analysis of two sgRNAs in WT, *SHANK3*^{M1} and *SHANK3*^{M2} brain tissues. Sequencing of the PCR products with cleavage (*) did not reveal authentic mutations in any clone but showed polymorphism. * denotes cleaved band.

Figure S2

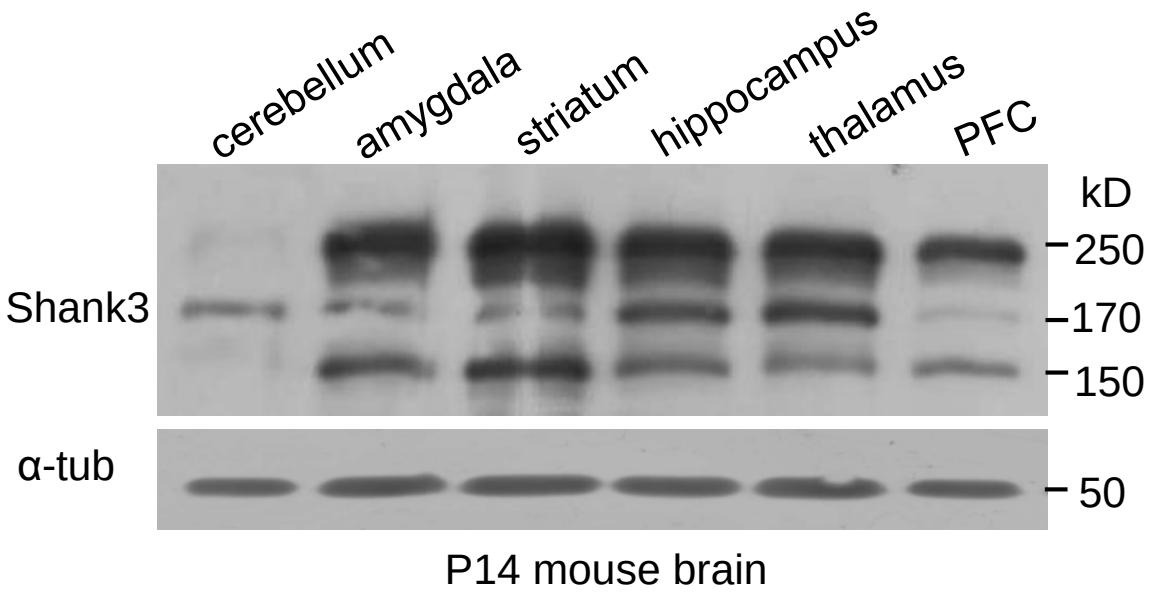

Figure S2 Brain region specific expression profiles of Shank3 protein in different regions of P14 mouse brain. Shank3 is expressed at the highest level in the striatum.

Figure S3

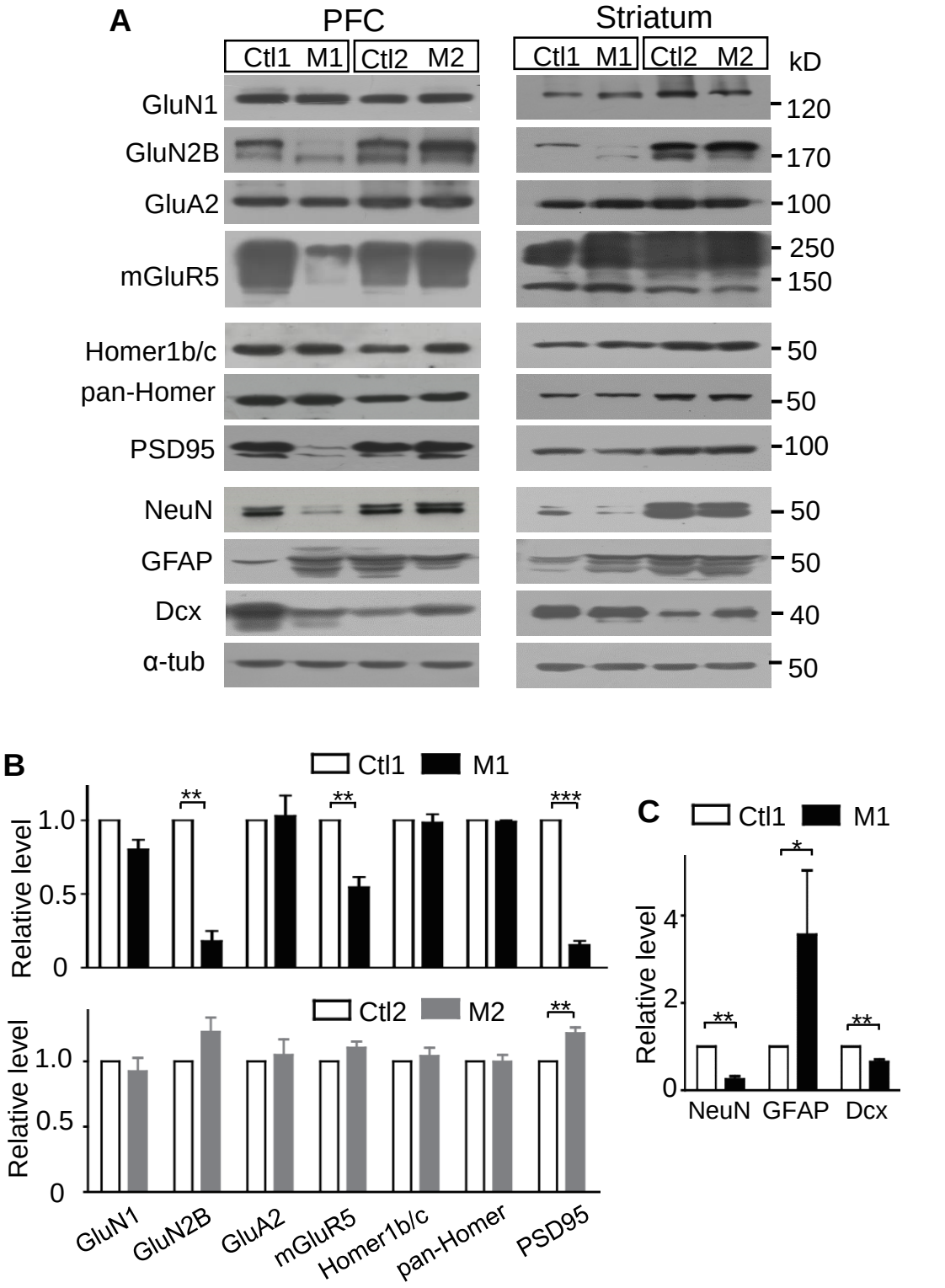

Figure S3 Western analysis of postsynaptic proteins and neuronal cell markers. **(A)** Altered expression of postsynaptic proteins and neuron/glia markers in PFC and striatum of mutants. **(B)** Relative levels of receptors and postsynaptic proteins normalized to α-tubulin in PFC of *SHANK3*^{M1} and *SHANK3*^{M2} fetuses. **(C)** Relative levels of NeuN, GFAP and DCX normalized to α-tubulin in the PFC. Western blot analysis was repeated 3-6 times for each protein. Data are presented as mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by Student's t test.

Figure S4

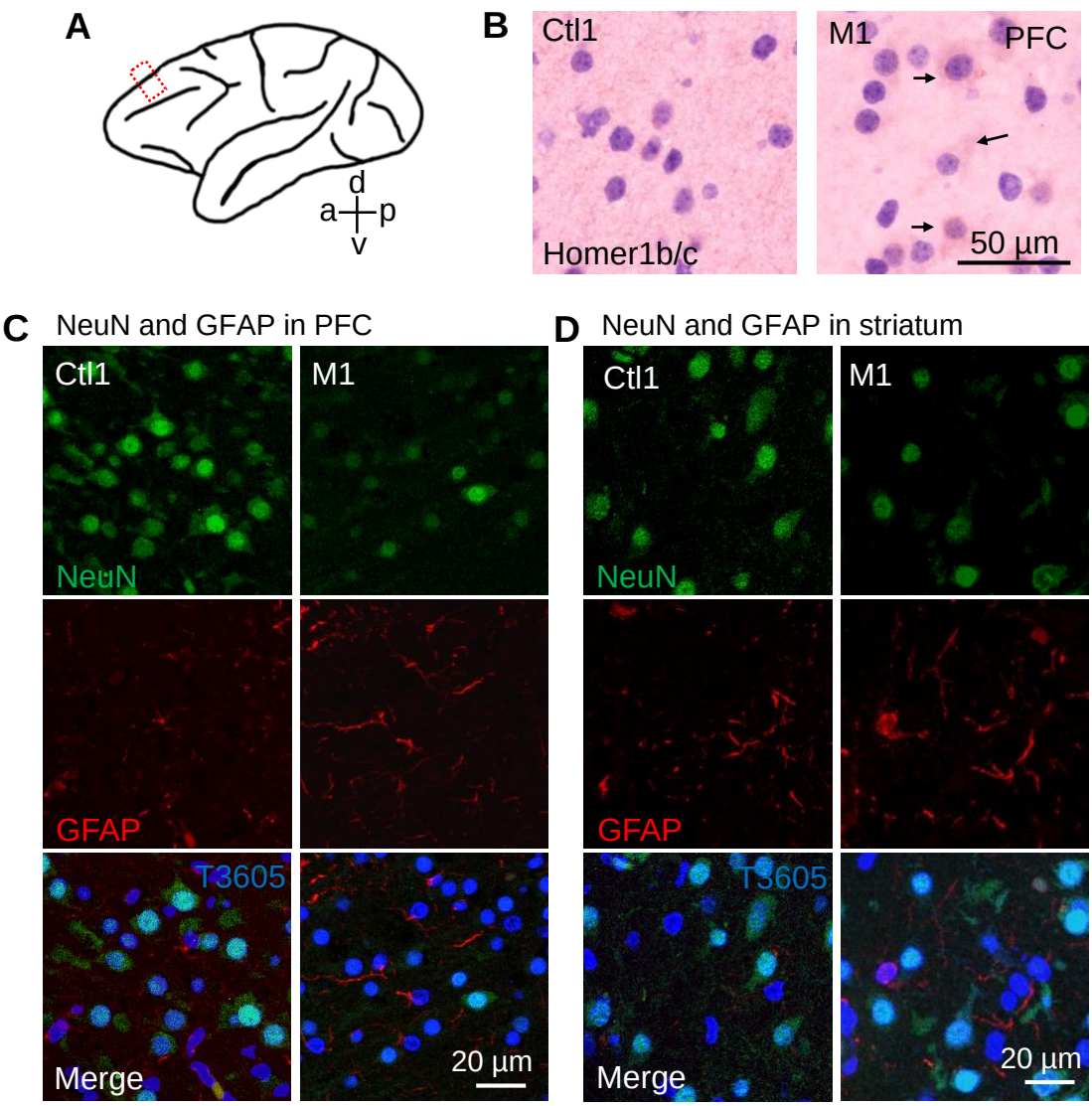

Figure S4 Altered localization of Homer1b/c and expression levels of NeuN and GFAP in the PFC and striatum. **(A)** Diagram of the PFC region used for immunostaining analysis. **(B)** Homer1b/c staining (DAB, brown) in the PFC of Ctl1 and *SHANK3*^{M1}. Nuclei were stained with hematoxylin (blue). Arrowheads point to accumulated Homer1b/c in the cytoplasm. Scale bar, 50 µm. **(C**, **D)** Immunofluorescent staining of NeuN (green) and GFAP (red) in the PFC **(C)** and striatum **(D)**. Nuclei were stained with T3605 (blue). Scale bar, 20 µm.

Figure S5

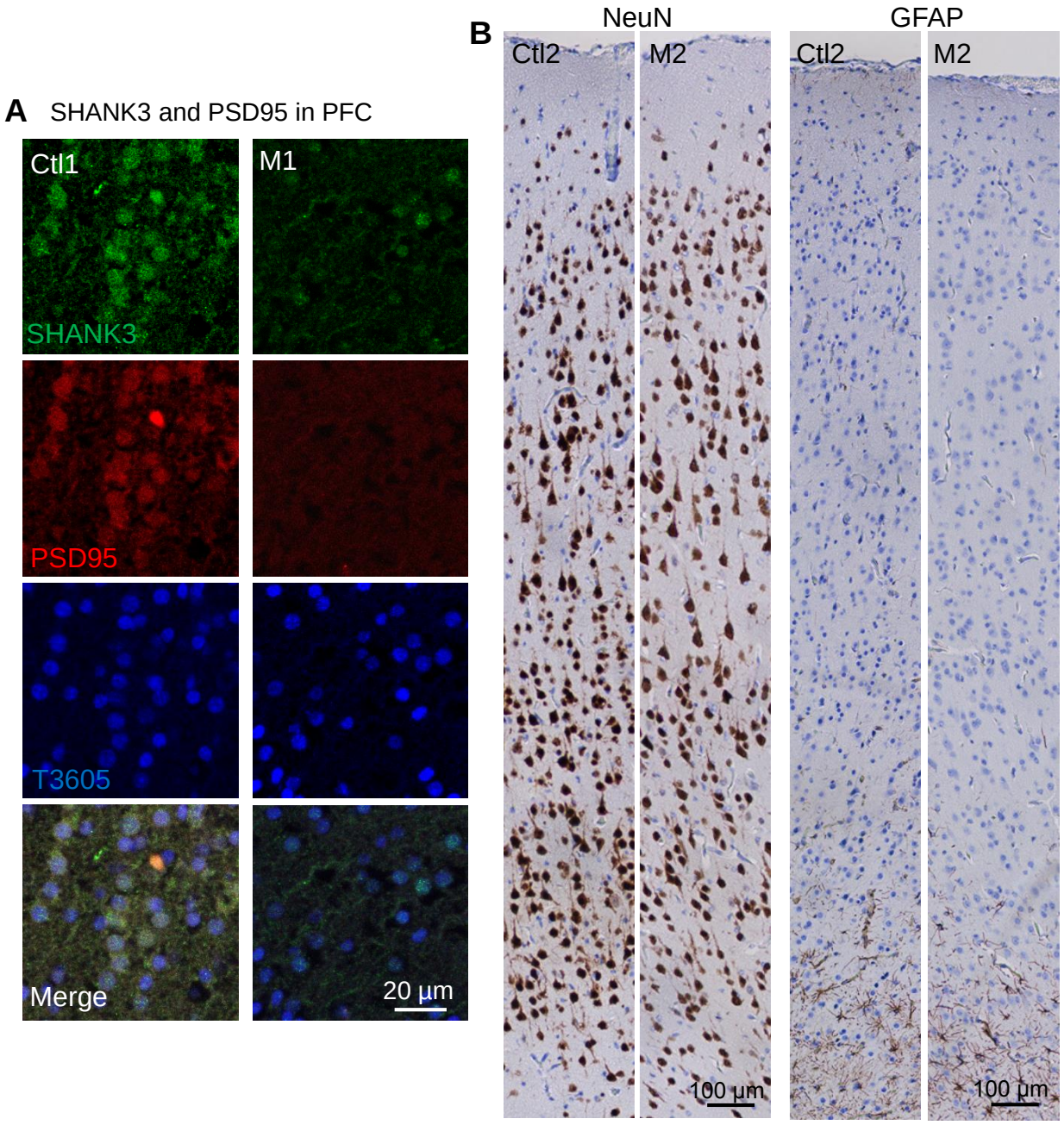

Figure S5 Decreased SHANK3-PSD95 staining in *SHANK3*^{M1} PFC and normal NeuN and GFAP staining in *SHANK3*^{M2} PFC. **(A)** Immuno-staining of SHANK3 (green) and PSD95 (red) in PFC. Nuclei were stained with T3605 (blue). Scale bar, 20 µm. **(B)** NeuN and GFAP staining (brown) in layers I-VI of PFC. Nuclei were stained with hematoxylin (blue). Scale bar, 100 µm.

Figure S6

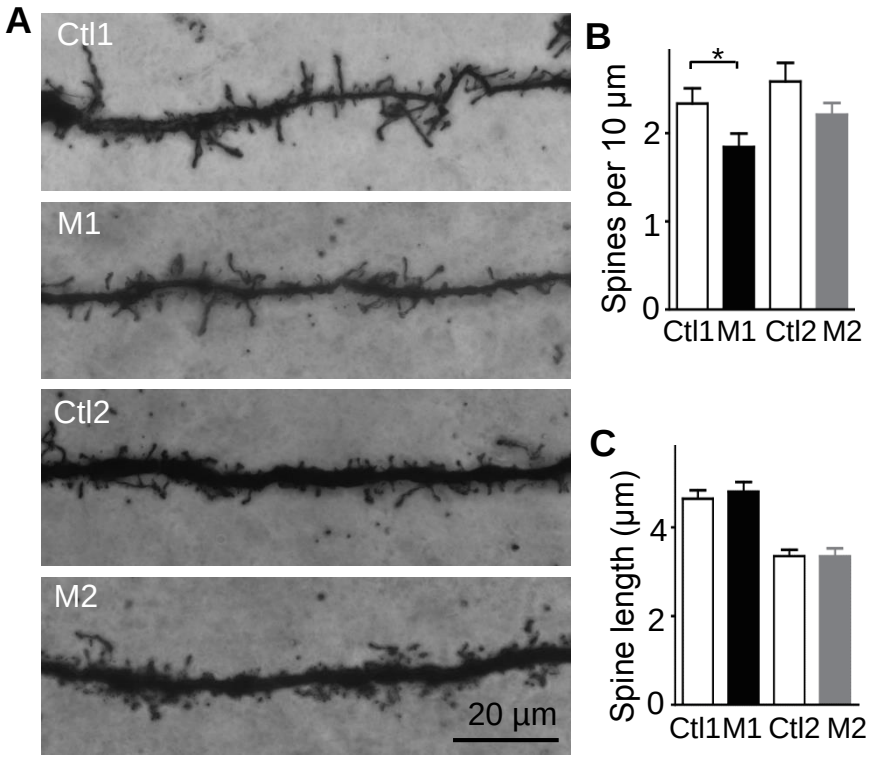

Figure S6 Reduced density of dendritic spines in the PFC of *SHANK3*^{M1} animal. **(A)** Representative images of Golgi impregnation analysis of the PFC of different animals. Scale bar, 20 µm. Reduced spine density (number of spines/10 µm dendrite) in the PFC of *SHANK3*^{M1} **(B)** but normal length **(C)** of dendritic spines in the PFC of *SHANK3*^{M1} and *SHANK3*^{M2} brains. *n* = 10-15 neurons/animal, **P* < 0.05.