Neuronal expression of *Fig4* is necessary and sufficient to prevent spongiform neurodegeneration

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SUPPLEMENTAL FIGURES

Figure S1. Genetic constructs for engineering of new mouse models. (A) NSE transgene. Structure of Tg NSE-Fig4 for expression of FIG4 specifically in neuronal cells. The mouse *Fig4* cDNA was inserted downstream of the 2.8 kb neuron-specific enolase (NSE) promoter fragment. Exons 1 and 2 of *Eno2* do not contain a start codon. Prior to sub-cloning of the mouse Fig4 cDNA, an endogenous HindIII site was removed by synonymous site directed mutagenesis at residue Leu260. **(B) GFAP transgene.** Tg GFAP-Fig4 contains the 2.2 kb *GFAP* promoter fragment driving expression of FIG4 specifically in astrocytes. The GFAP translation initiation codon in exon 1was mutated to TTG to permit translation of the transgene to initiate at the Fig4 initiation codon. Since the Fig4 cDNA contains several BamH1 sites (arrowheads), the cDNA was sub-cloned using BclII, which generates BamH1-compatible overhangs. The 3' segment of GFAP-Fig4 is derived from the *Prm-1* gene and supplies an intron (Lee et al, 2008). Open box, exon; filled box, protein-coding. **(C) *Fig4* floxed allele**. A 2.6 kb fragment ending 70 bp upstream of exon 4, a 400 bp fragment containing exon 4, and a 1.7 kb fragment located 122 bp downstream of exon 4 were amplified separately from 129X1/SvJ DNA (Jackson Laboratory) and sequentially ligated into the SacII/NotI, NotI/BamHI and XhoI sites, respectively, of PL541 (Liu, Jenkins et al. 2003). A loxP site was included in the 5' primer for exon 4 amplification and cloning. The PL451 vector contained the 3' loxP site downstream of the BamHI site 3' of exon 4. The targeting construct was electroporated into R1 ES cells from mouse strain 129 (Nagy, Rossant et al. 1993) by the University of Michigan Transgenic Animal Model Core (http://www.med.umich.edu/tamc) as described (Hughes and Saunders, 2011) and sCreened to detect the targeted allele by Southern blot of BamH1 digested DNA. Chimeric founder mice were generated as described (Howell, de Haan et al. 2008). Mice expressing FLPe recombinase [B6:SJL-Tg(ACTFLPe)9205Dym/J, Jackson Laboratory stock 003800] were used for excision of the neo cassette. The floxed allele was detected by PCR with primers flanking exon 4 and the loxP sites. (D) Correct targeting was evaluated in neomycin-resistant ES cell clones by Southern blot hybridization of BamHI-digested DNA.

Figure S1

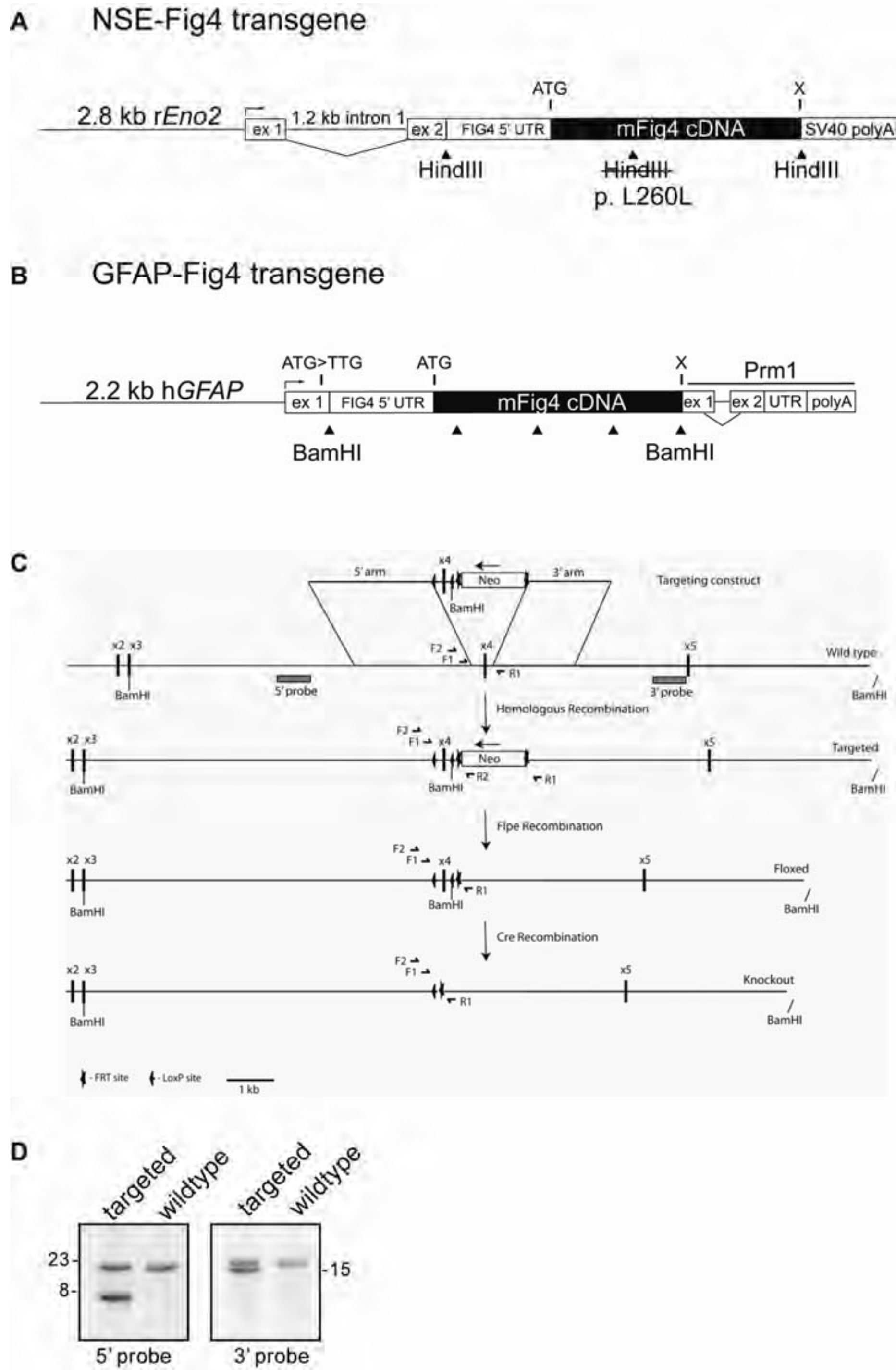
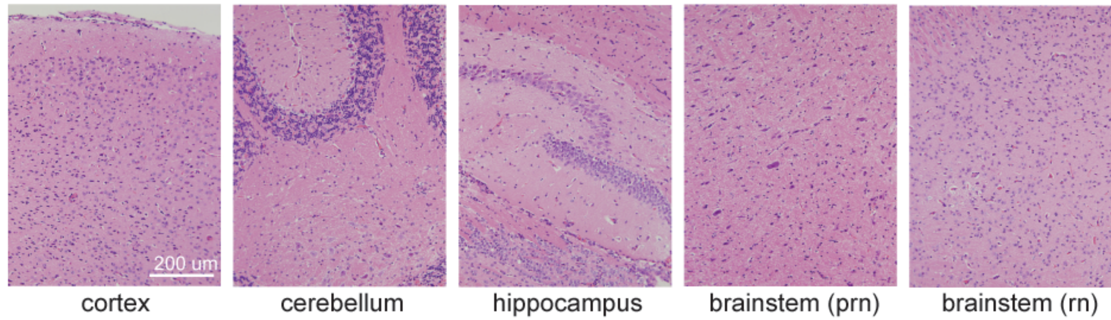
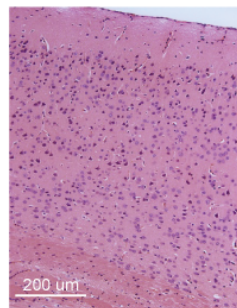


Figure S2. Correction of spongiform degeneration is maintained to 12 months of age in most *Fig4*$^{-/-}$*,NSE-Fig4* mice. A) Brain regions at 9 months of age. B) Neocortex from three Fig4-/-,NSE-Fig4 mice. C) Mild degeneration in one *Fig4*$^{-/-}$*,NSE-Fig4* mouse at 7 months of age. D) Minimal accumulation at 9 months of age of Lamp-1 and GFAP, markers of astrocyte dysfunction in *Fig4* null mice .

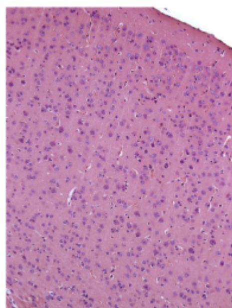
A. 9 months



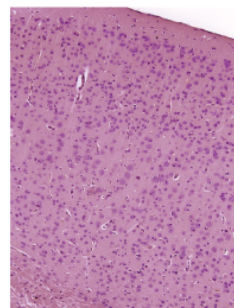
B. 10 months



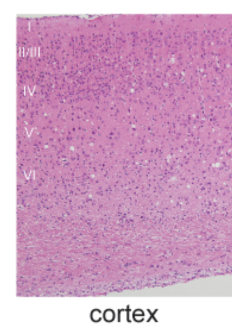
10.5 months



12 months



C. 7 months



D. LAMP1, GFAP, DAPI

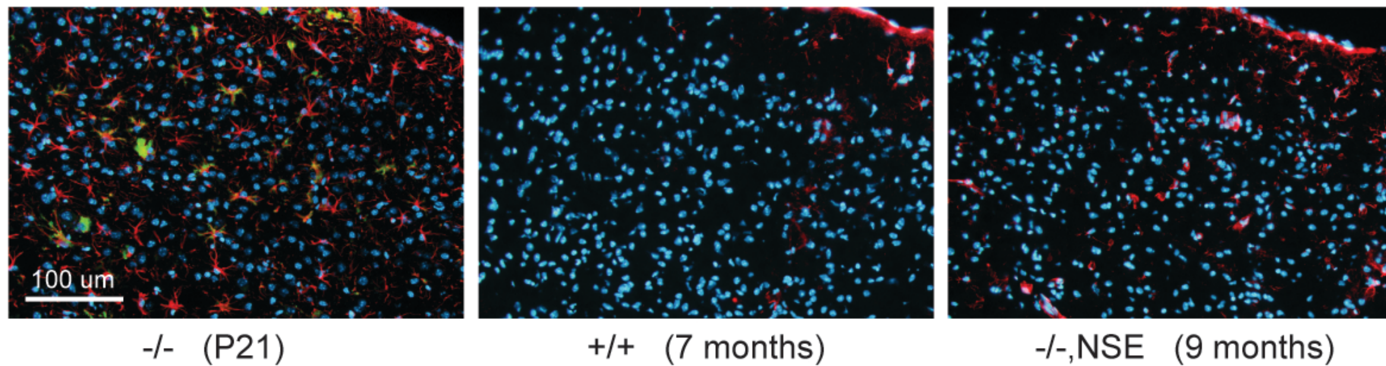


Figure S3. Expression of Fig4 in neurons or astrocytes rescues the accumulation of p62 and LAMP-1 in inclusion bodies. (Immunofluorescence colabeling of LAMP-1 and p62 in Fig4-/-,NSE-Fig4 cortex (A) and cerebellum (B). Immunofluorescence colabeling of LAMP-1 and p62 in Fig4-/-,GFAP-Fig4 cortex (C) and cerebellum (D).

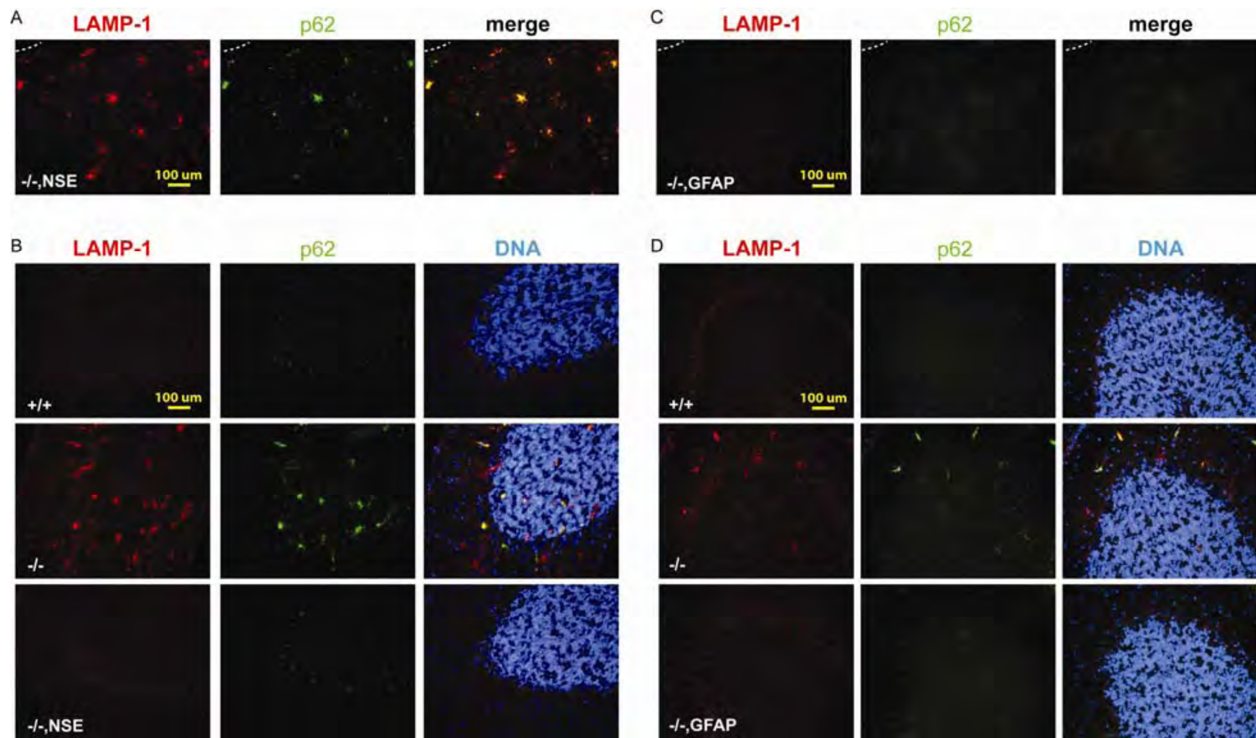
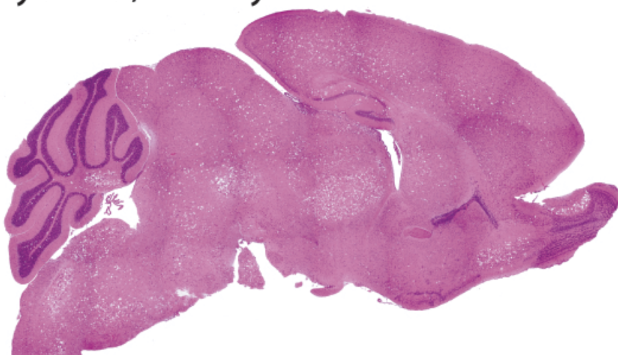
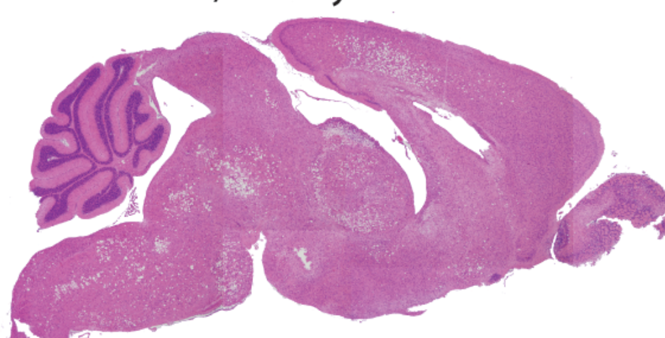


Figure S4. A) Composite saggital sections of Syn-cre mouse and -/- at 30 days demonstrating patterns of spongiform degeneration. B) Syn-cre brain regions at 5 months of age. C) Syn-cre brain cortex at 5 months of age demonstrating no significant accumulation of LAMP-1 and GFAP, markers of astrocyte dysfunction in *Fig4* null mice (Ferguson et al, 2009). See figure 3 and Figure 6 for comparison to +/+ and -/- IHC.

A. Syn-cre, 30 days



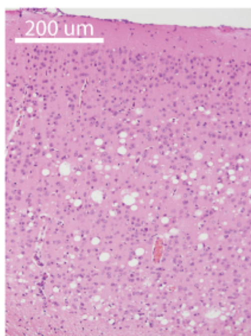
-/-, 30 days



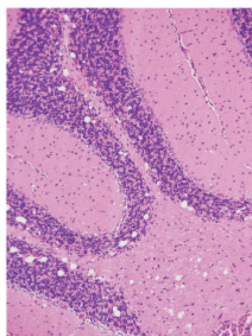
B.

Syn-cre 5 mo

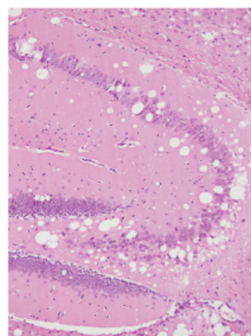
cortex



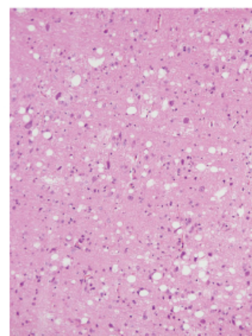
cerebellum



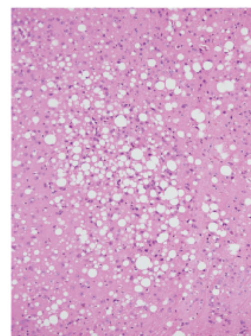
hippocampus



brainstem (prn)

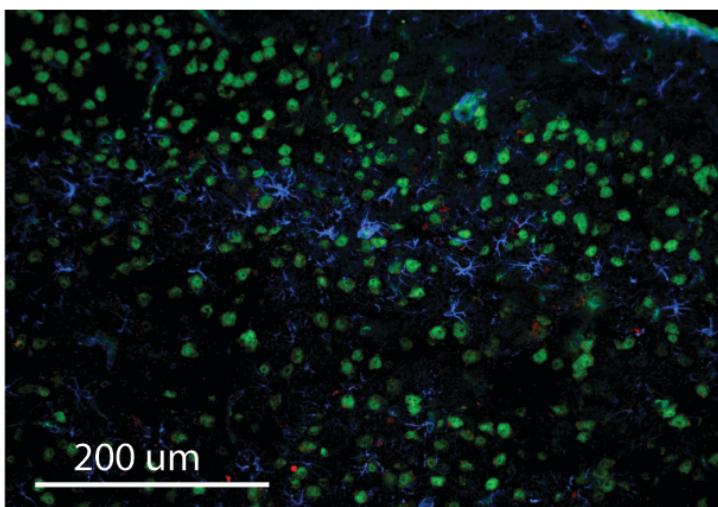


brainstem (rn)



C. LAMP1 GFAP NeuN

Syn-cre 5 mo



SUPPLEMENTAL VIDEOS

Video recording 1. Demonstration of the appearance and motor coordination of $Fig4^{-/-}$ null mice, $Fig4^{-/-}$ null mice expressing the NSE and GFAP transgenes, and *Fig4*$^{flox/flox}$, Synapsin Cre mice.

Video recording 2. Demonstration of the motor coordination and strength of a Fig4-/-,NSE-Fig4 mouse at 4 months of age.

SUPPLEMENTAL REFERENCES

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