JEM

Supplemental material

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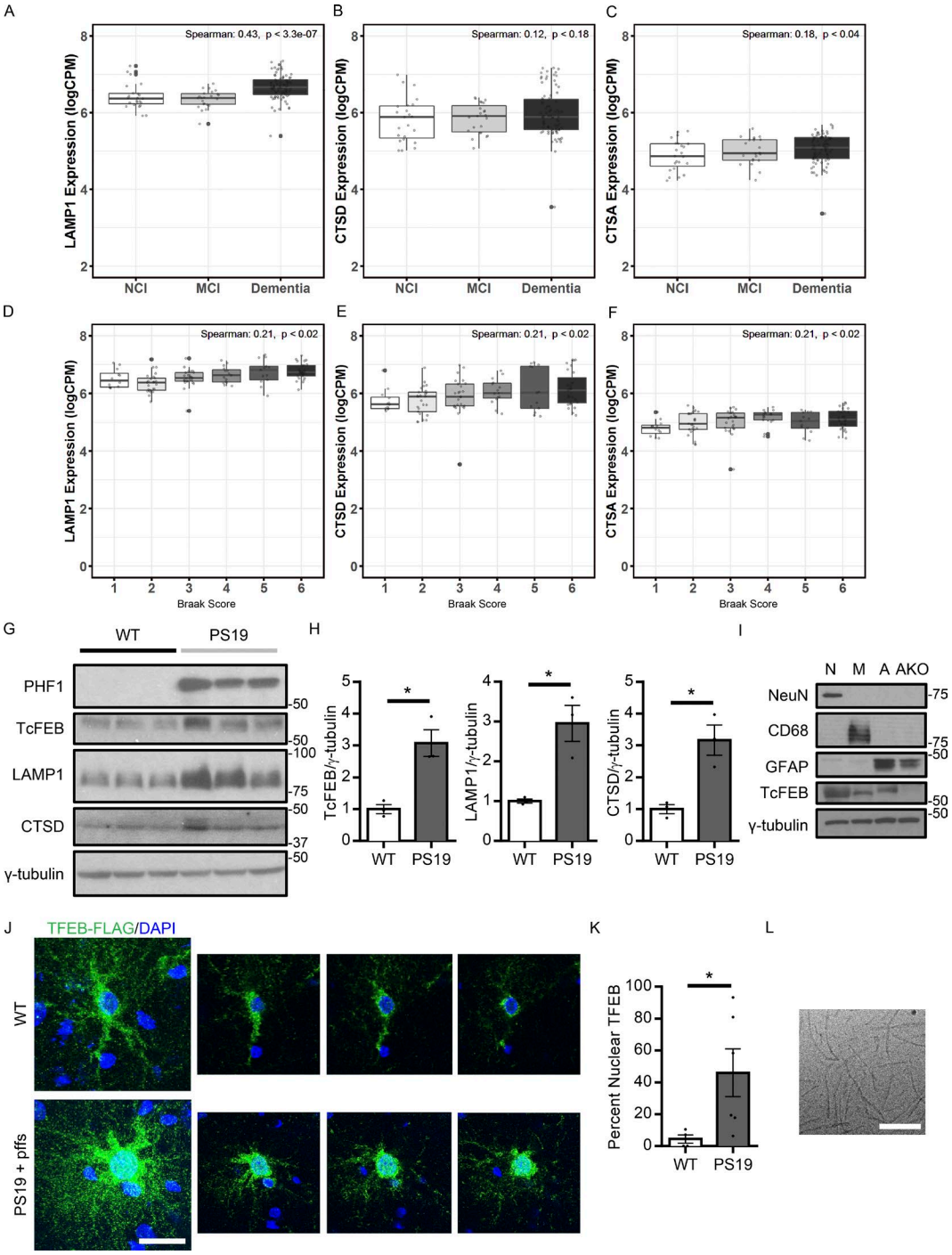

Figure S1. **Lysosomal TFEB targets are positively correlated with cognitive decline. (A–C)** MSSM cohort human brain RNA-seq showing correlation of LAMP1 (A), CTSD (B), and CTSA (C) transcript levels with cognitive status (parahippocampal gyrus; *n* = 130). The three dementia groups are defined based on CERAD score (CDR): CDR = 0 is NCI, CDR = 0.5 is MCI, CDR ≥1 is Dementia. **(D–F)** LAMP1 (D), CTSD (E), and CTSA (F) transcript levels stratified by Braak score (1–6) from the MSSM cohorts in AMP-AD reprocessed RNA-seq project (parahippocampal gyrus, *n* = 130). Spearman's correlation coefficient is computed between the expression and dementia group status (A–C) or Braak score (D–F) and P value show the significance of the observed correlation computed using the asymptotic t approximation algorithm. Performed once. **(G and H)** Western blot with quantification (H) of phospho-tau (PHF1) along with endogenous TFEB (TcFEB) and TFEB targets (LAMP1 and CTSD) in hippocampal lysates of 10-mo-old PS19 or wild-type littermates. *n* = 3/group; Student's *t* test, representative of two independently performed experiments. **(I)** Western blot of endogenous mouse TFEB (TcFEB) using lysates from primary culture neurons (N), microglia (M), astrocytes (A), and TFEB KO astrocytes (AKO). Antibodies specific for neurons (NeuN), microglia (CD68), and astrocytes (GFAP) were also used. Representative of three independently performed experiments. **(J and K)** Representative confocal images of FLAG (green) and DAPI (blue) staining in the hippocampus of P3 AAV-GFAP-TFEB–injected wild-type or tau spreading mice at 1 mo after tau fibril injection with quantification (K). The tau spreading mice are PS19 tau transgenic mice injected with pffs in the left hippocampus at 3 mo of age (described in Fig. 6 A and Iba et al., 2013). Bar, 10 µm. (*n* = 5/group with 5 images/animal; Student's *t* test, representative of two independently performed experiments). **(L)** Cryo-electron microscopy image of pffs. Bar, 200 nm. Representative of two independently performed experiments. Molecular mass indicated in kilodaltons. Error bars designate SEM. *, P < 0.05.

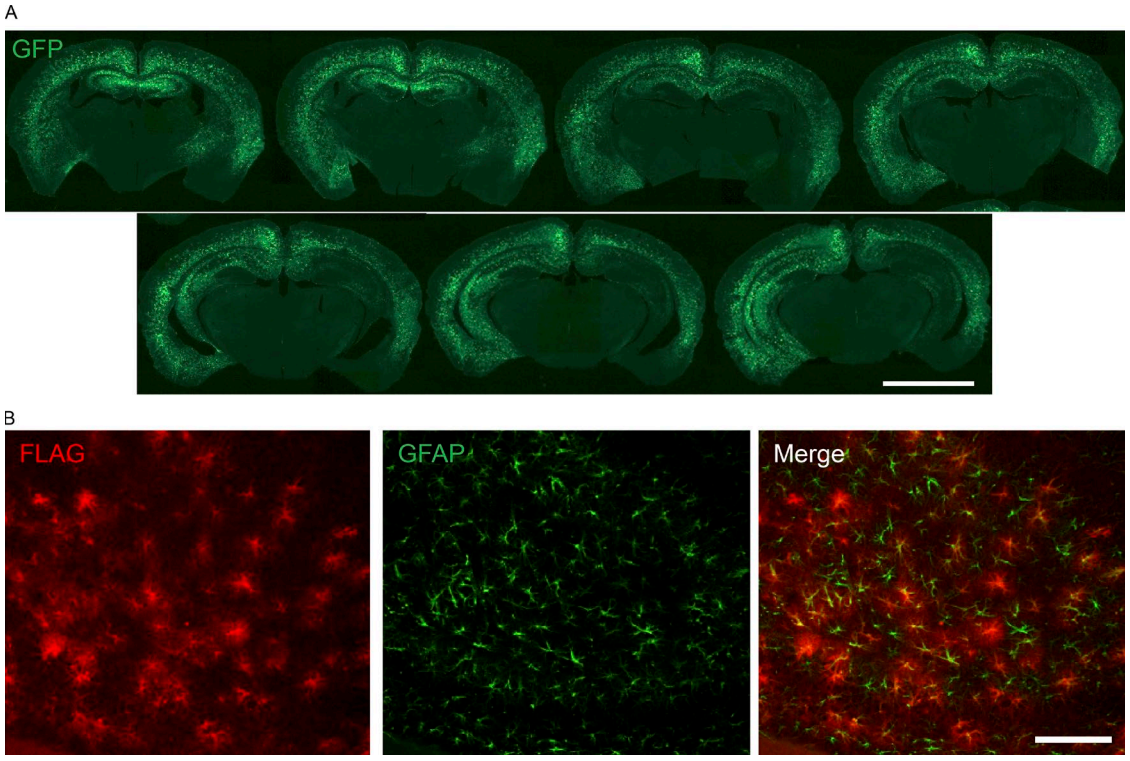

Figure S2. **P3 AAV-GFAP ICV injections achieve widespread astroglial expression in the mouse brain. (A)** Fluorescence images of successive coronal sections of a wild-type mouse injected ICV with AAV-GFAP-EGFP at P3. Bar, 5 mm. Representative image from *n* = 9. **(B)** Representative confocal images of FLAG (red) and GFAP (green) immunostaining of a wild-type mouse injected ICV with AAV-GFAP-TFEB3XFLAG at P3. *n* = 4; representative of two independently performed experiments. Bar, 100 µm.

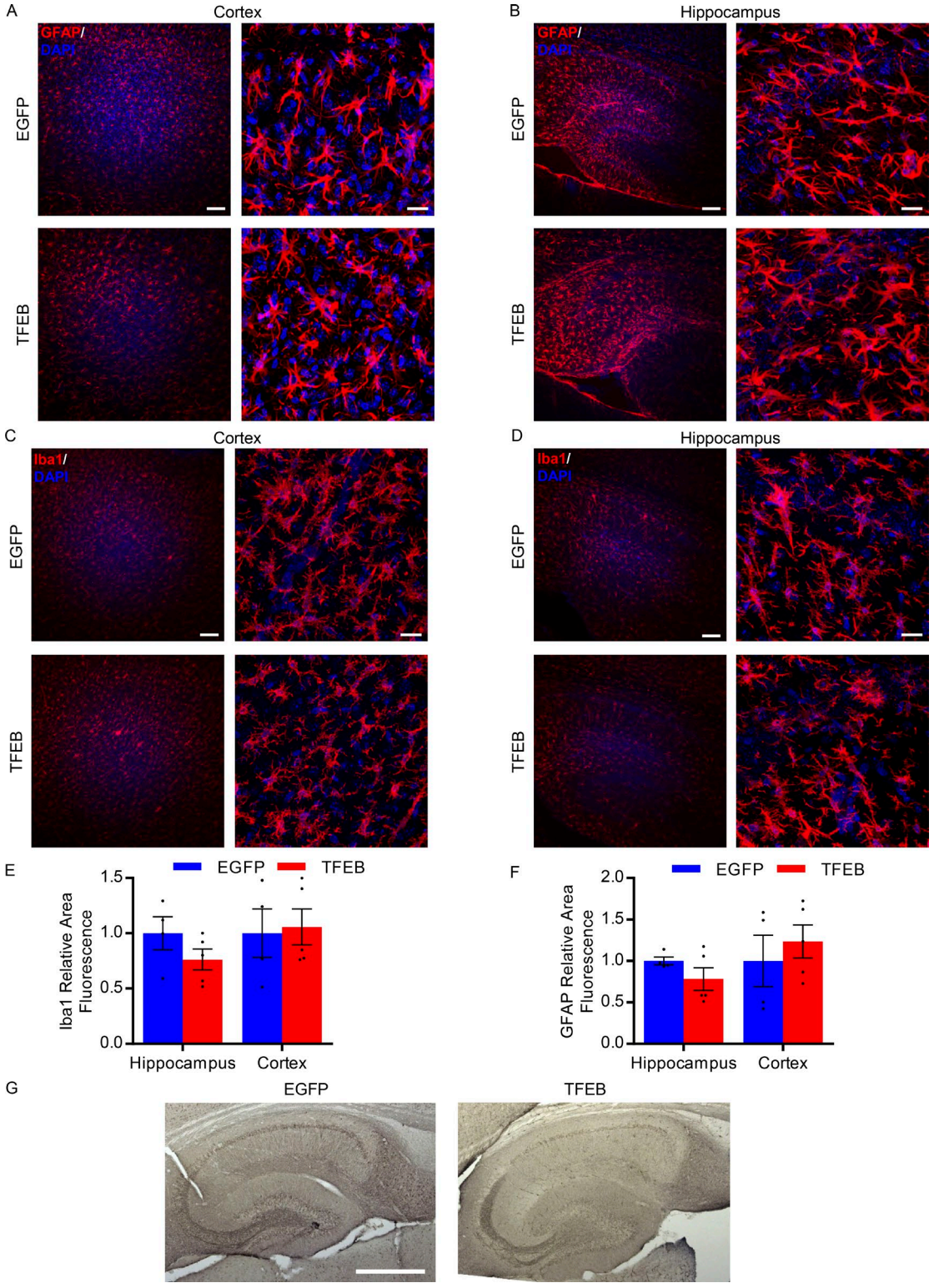

Figure S3. **Astroglial TFEB does not impact gliosis in rTg4510 mice but reduces pathology in PS19 mice. (A and B)** Representative fluorescent confocal images of GFAP immunostaining (red) in the cortex (A) and hippocampus (B) of AAV-GFAP-EGFP– or AAV-GFAP-TFEB–injected rTg4510 male mice at 4 mo of age at low (left) and high (right) magnification. Bars, 100 µm and 20 µm, respectively. *n* = 5/group; representative of two independently performed experiments. **(C and D)** Representative fluorescent confocal images of Iba1 immunostaining (red) in the cortex (C) and hippocampus (D) of AAV-GFAP-EGFP– or AAV-GFAP-TFEB–injected rTg4510 male mice at 4 mo of age at low (left) and high (right) magnification. Bars, 100 µm and 20 µm, respectively. (*n* = 5/group; representative of two independently performed experiments). **(E and F)** Area fluorescence quantitation of GFAP (E) and Iba1 (F) immunostaining of 4-mo-old male rTg4510 mice. *n* = 5/group; Student's *t* test; representative of two independently performed experiments. **(G)** Representative images of CP13 immunostaining in the hippocampus of 9-mo-old PS19 mice injected with AAV-GFAP-EGFP or AAV-GFAP-TFEB. Bar, 500 µm. Error bars designate SEM (*n* = 5/group; representative of two independently performed experiments).

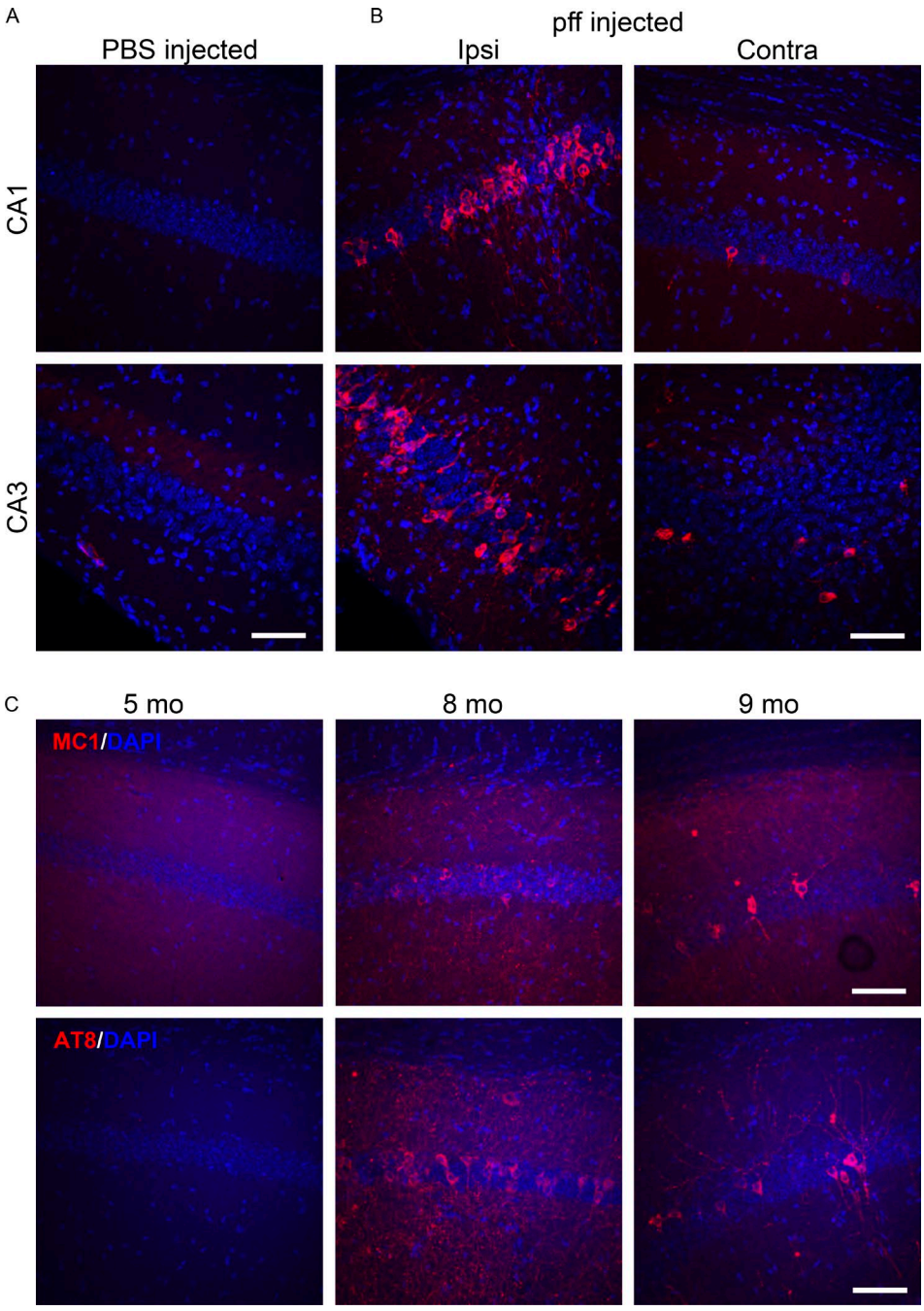

Figure S4. **Unilateral hippocampal injection of pffs results in reliable tau spreading model. (A and B)** Representative fluorescent images of MC1 immunostaining (red) of the CA1 and CA3 hippocampus in PS19 mice 3 wk after stereotaxic injection of PBS or pffs (B) in the left hippocampus. *n* = 4/group; representative of two independently performed experiments. **(C)** Representative fluorescent images of MC1 and AT8 immunostaining (red) of CA1 hippocampus of PS19 mice at 5, 8, and 9 mo of age. *n* = 3/group; representative of two independently performed experiments. Bars, 40 µm.

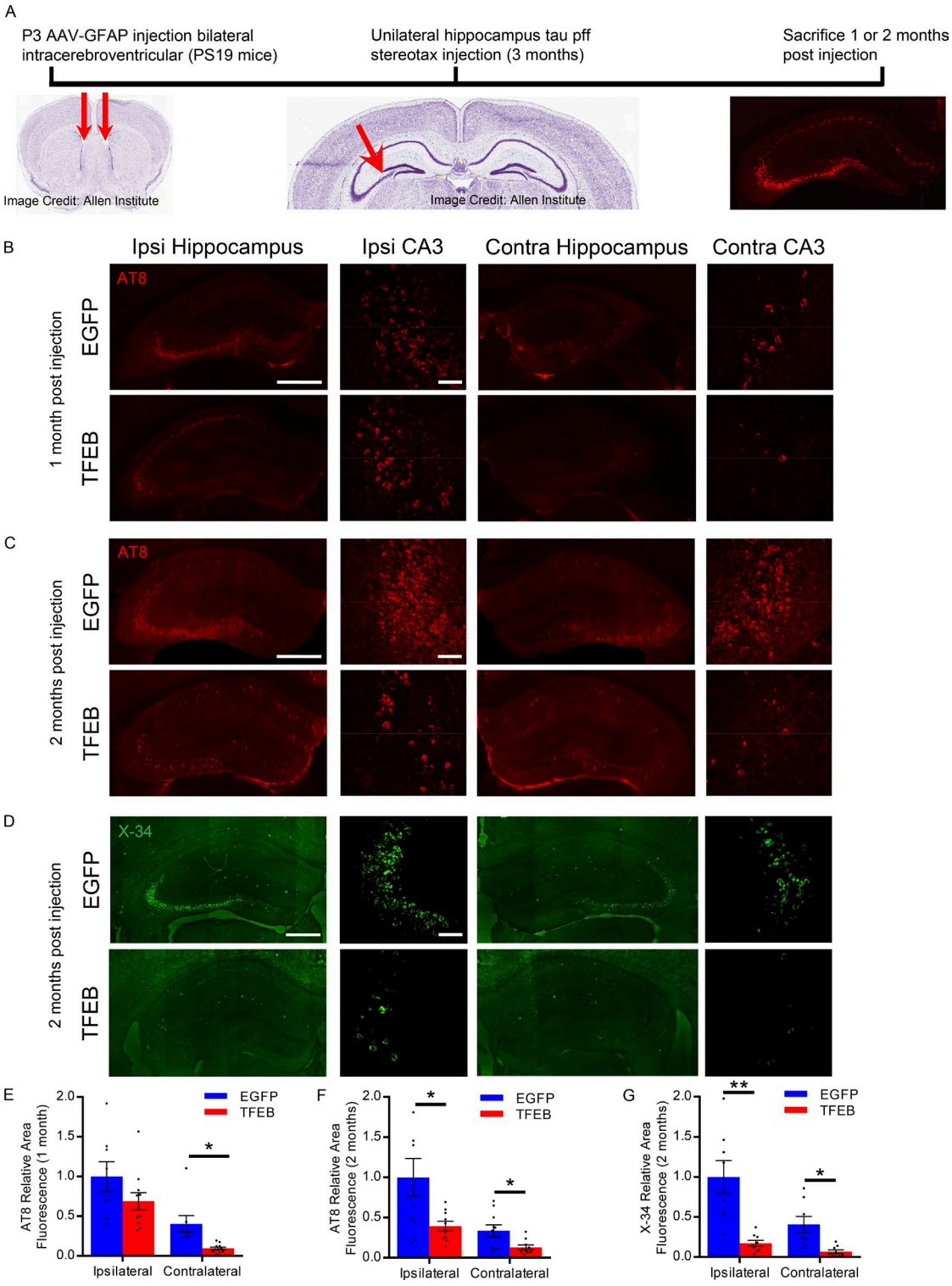

Figure S5. **Astroglial TFEB reduces tau spreading in PS19 mice. (A)** Experimental design for testing the impact of astroglial TFEB on tau spreading (http://atlas.brain-map.org; accessed November 17, 2016). **(B and C)** Representative fluorescent images of AT8 immunostaining (red) of the ipsilateral and contralateral hippocampus of PS19 mice with P3 ICV injection of AAV-GFAP-EGFP or AAV-GFAP-TFEB at 1 mo (B) and 2 mo (C) after unilateral tau fibril injection. Bars: 500 µm for whole hippocampus; 40 µm for CA3. *n* = 8–11/group; representative of two independently performed experiments for 1 mo; 2 mo technically replicated twice. **(D)** Representative fluorescent images of X-34 staining (green) of the ipsilateral and contralateral hippocampus of PS19 mice with P3 ICV injection of AAV-GFAP-EGFP or AAV-GFAP-TFEB at 2 mo after unilateral tau fibril injection. Bars: 500 µm for whole hippocampus, 40 µm for CA3. (*n* = 10/group; technically replicated twice). **(E and F)** Area fluorescence quantitation of AT8 immunostaining of the ipsilateral and contralateral hippocampus of PS19 mice 1 mo (E) and 2 mo (F) after tau fibril injection. *n* = 10/group; Student's *t* test, representative of two independently performed experiments for 1 mo; 2 mo technically replicated twice. **(G)** Area fluorescence quantitation of X-34 staining of the ipsilateral and contralateral hippocampus of PS19 mice 2 mo after tau fibril injection. *n* = 10/group; Student's *t* test, technically replicated twice. Error bars indicate SEM. *, P < 0.05; **, P < 0.01.

Tables S1 and S2 are included are as Excel files and list human brain demographic data and antibodies used in experiments, respectively.

Reference

Iba, M., J.L. Guo, J.D. McBride, B. Zhang, J.Q. Trojanowski, and V.M. Lee. 2013. Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. *J. Neurosci.* 33:1024–1037. https://doi.org/10.1523/JNEUROSCI.2642-12.2013