

Supplemental Material

Research Articles | Neurobiology of Disease

Astrocytic 4R-tau pathology drives aquaporin-4 (AQP4) mislocalization, glymphatic dysfunction, and neurotransmitter dysregulation in mice

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1 **Supplementary Material**

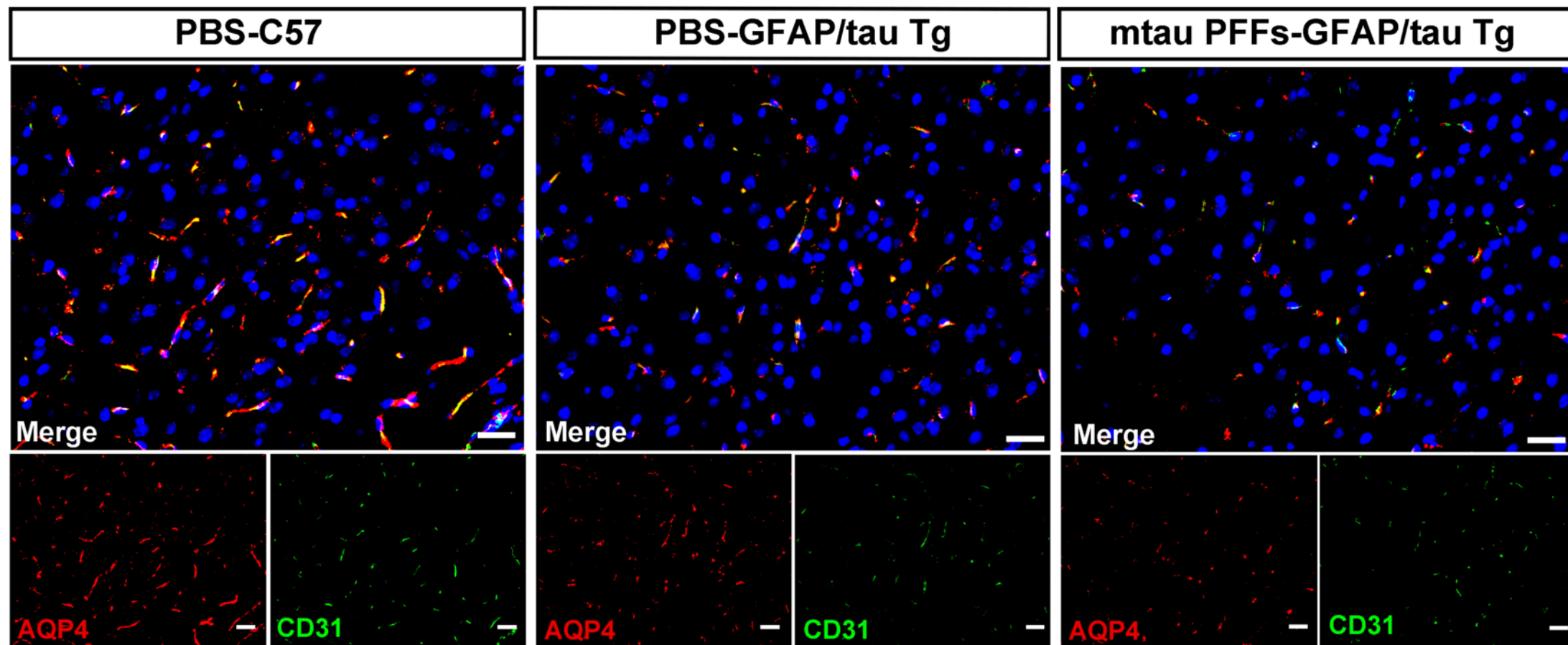
2 **Fig. S1. Astrocytic 4R-tau pathology disrupts perivascular AQP4 polarity in mice.**

3 **a**, Immunofluorescence staining for AQP4 (red) and the vascular endothelial marker
4 CD31 (green) in cortical brain sections from PBS-C57, PBS-GFAP/tau Tg, and mtau
5 PFFs-GFAP/tau Tg mice; merged images show co-localization of AQP4 with CD31-
6 labeled microvessels. Scale bars represent the measurement scale. **b**, Quantification of
7 AQP4 polarity was performed on brain sections from the indicated mouse groups, and
8 *P*-values are annotated to denote statistical significance between groups.

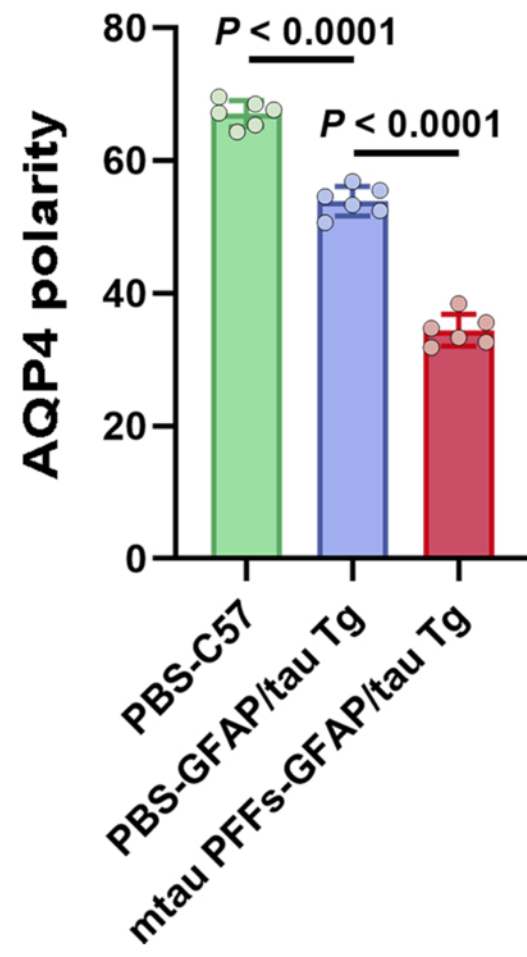
9 **Fig. S2. CSF production, AQP4 expression, ventricular enlargement, cell density,**
10 **and TH-positive neuron changes. a**, CSF production rate in PBS-C57, PBS-GFAP/tau
11 Tg, and mtau PFFs- GFAP/tau Tg mice. **b**, Left: Immunofluorescence images of AQP4
12 (red), GFAP (green) with Hoechst (blue, nuclear stain) and merged channels; Right:
13 Mean fluorescence intensity of AQP4 in different groups. **c**, Left: H&E staining of
14 lateral ventricles in PBS-C57, PBS-GFAP/tau Tg, and mtau PFFs-GFAP/tau
15 Tg mice; Right: Quantification of lateral ventricle area. **d**, Cell density (cell number per
16 field of view) in PBS-C57, PBS-GFAP/tau Tg, and mtau PFFs-GFAP/tau Tg mice. **e**,
17 Left: Tyrosine hydroxylase (TH) immunostaining in striatum and SN of PBS-C57,
18 PBS-GFAP/tau Tg, and mtau PFFs-GFAP/tau Tg mice; Right: Quantification of TH-
19 fiber density (integrated optical density, IOD) in striatum and number of TH-positive
20 neurons in SN.

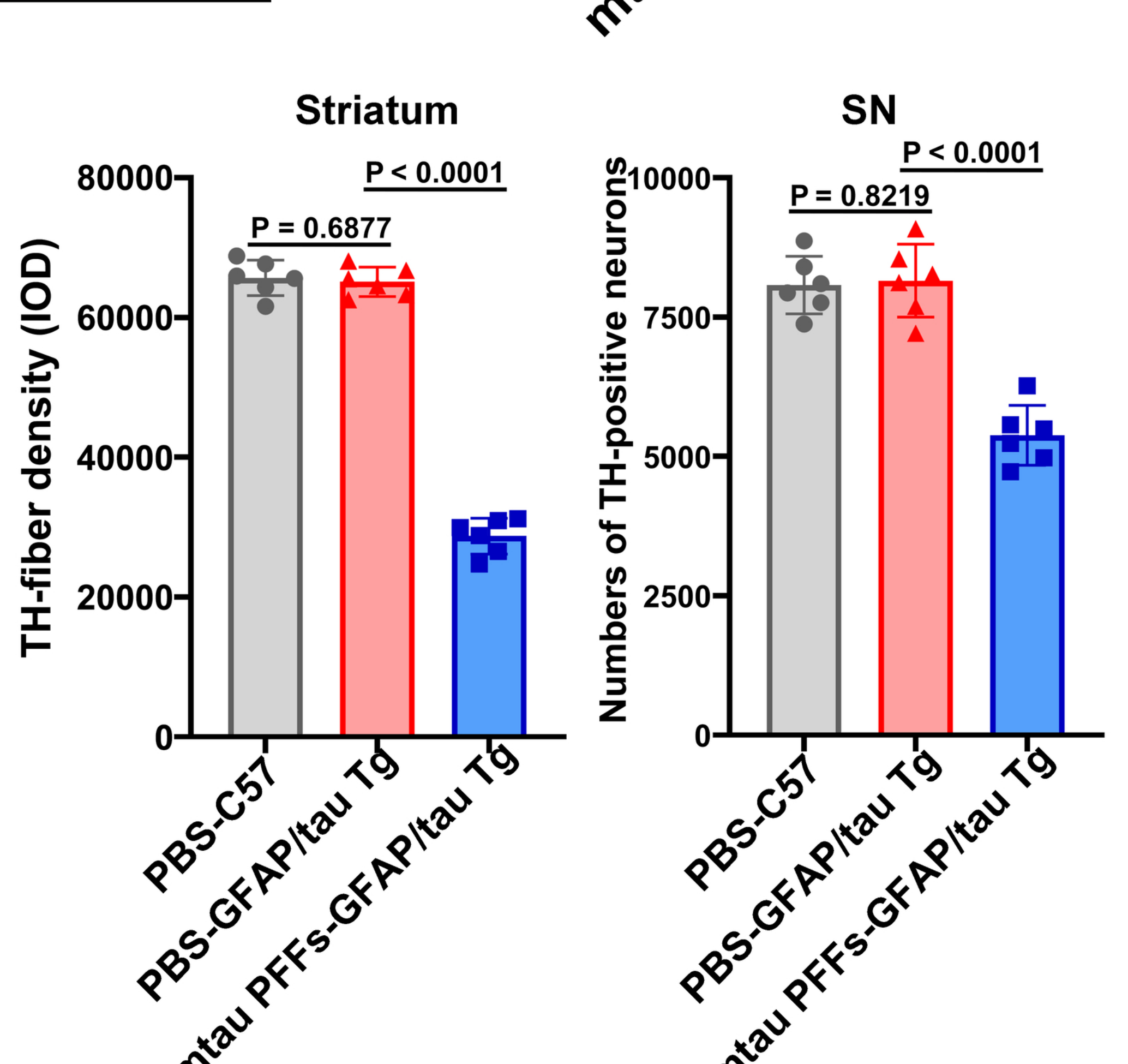
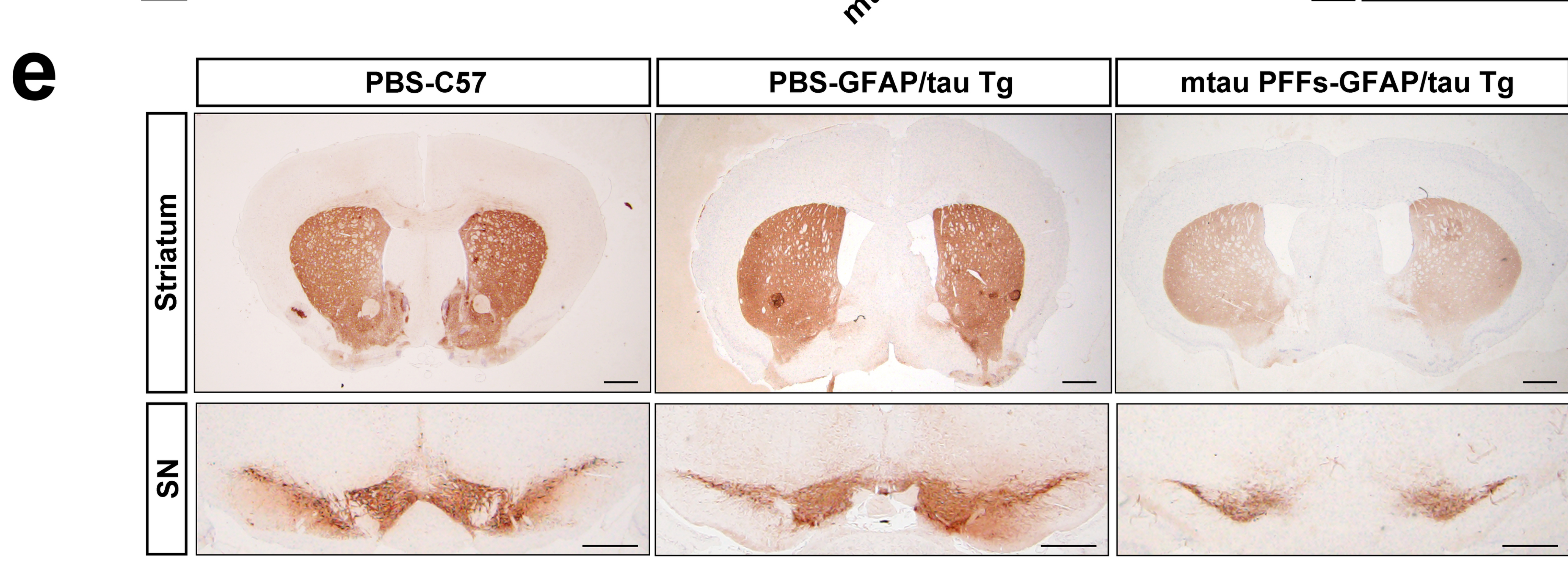
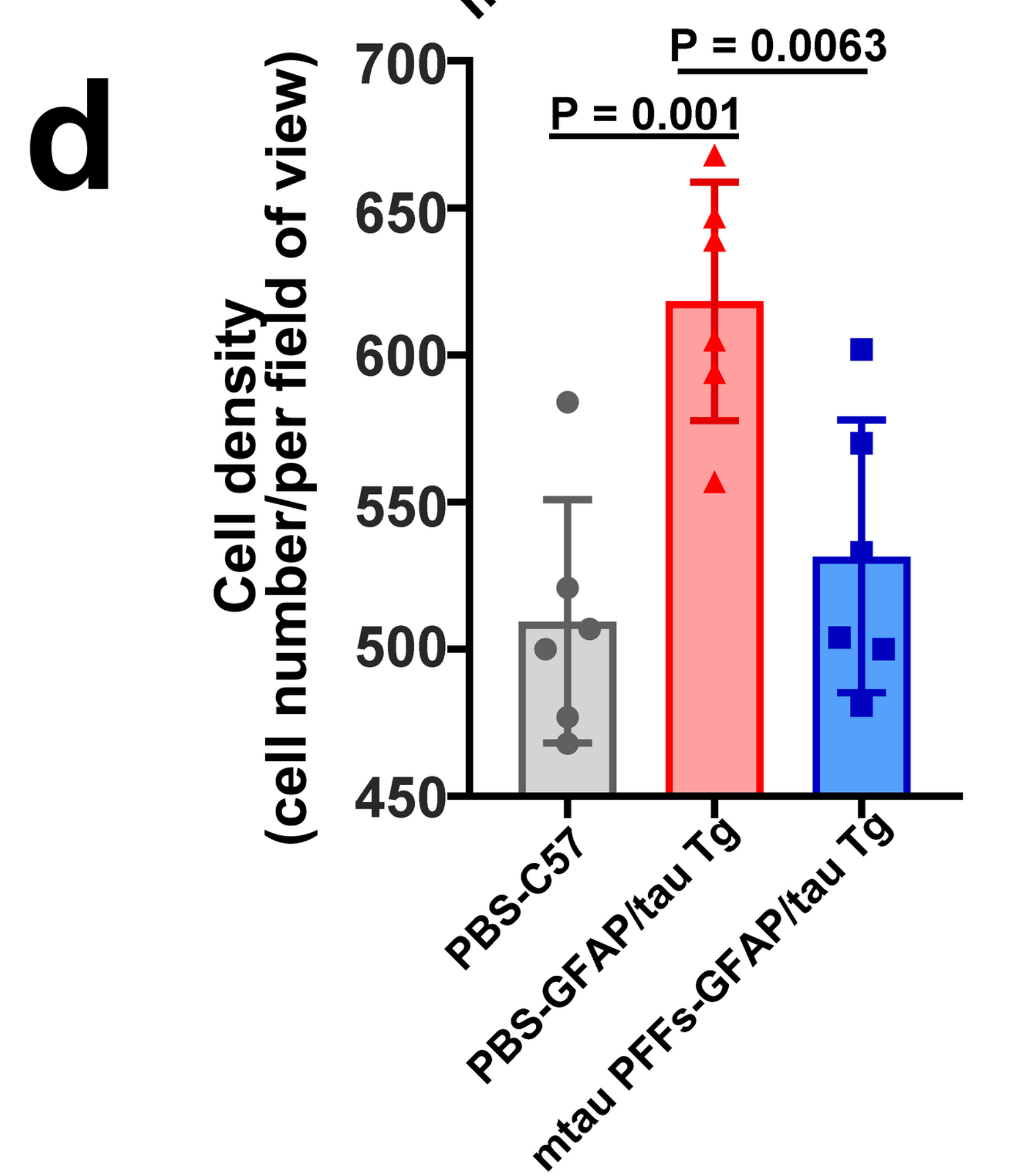
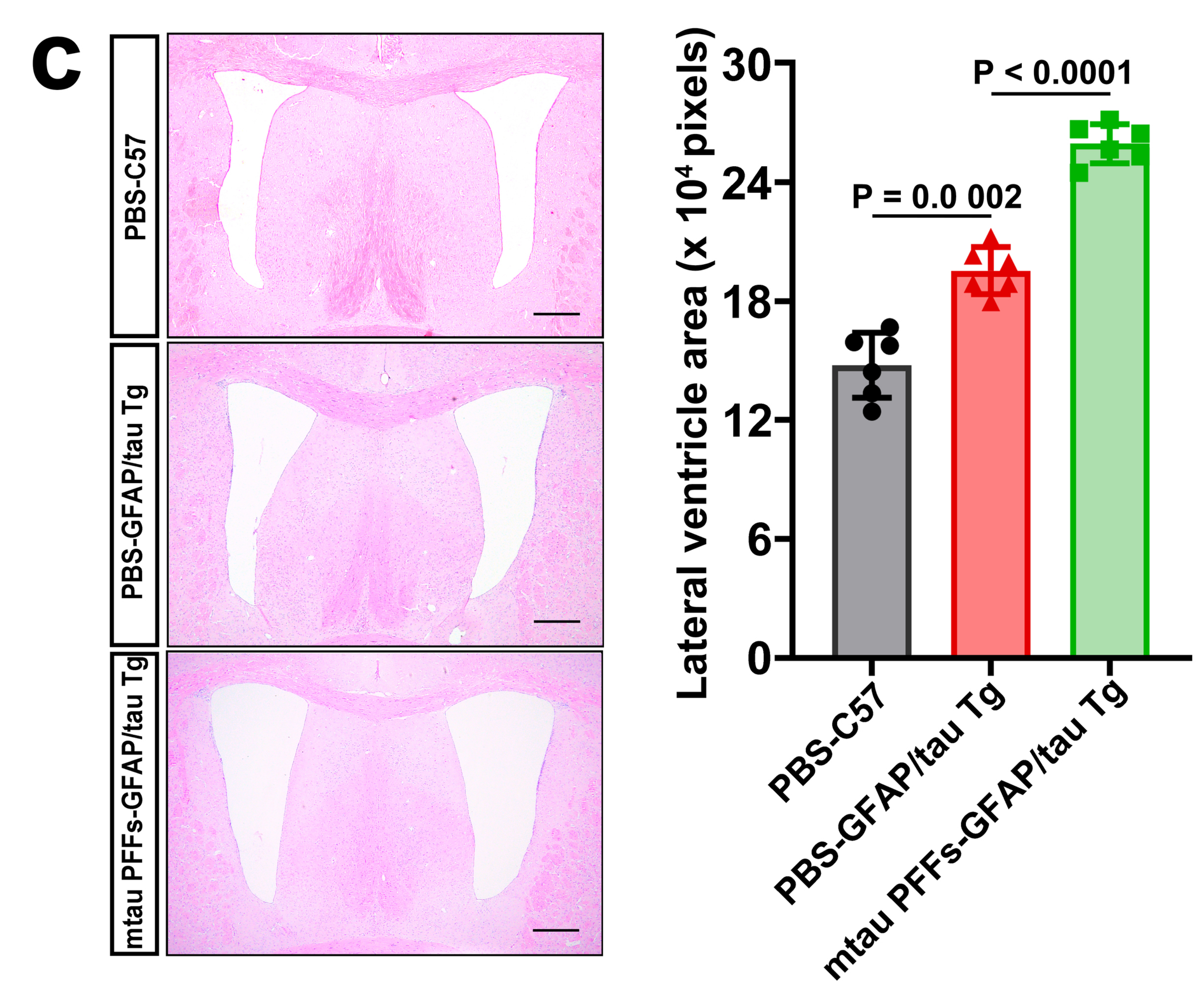
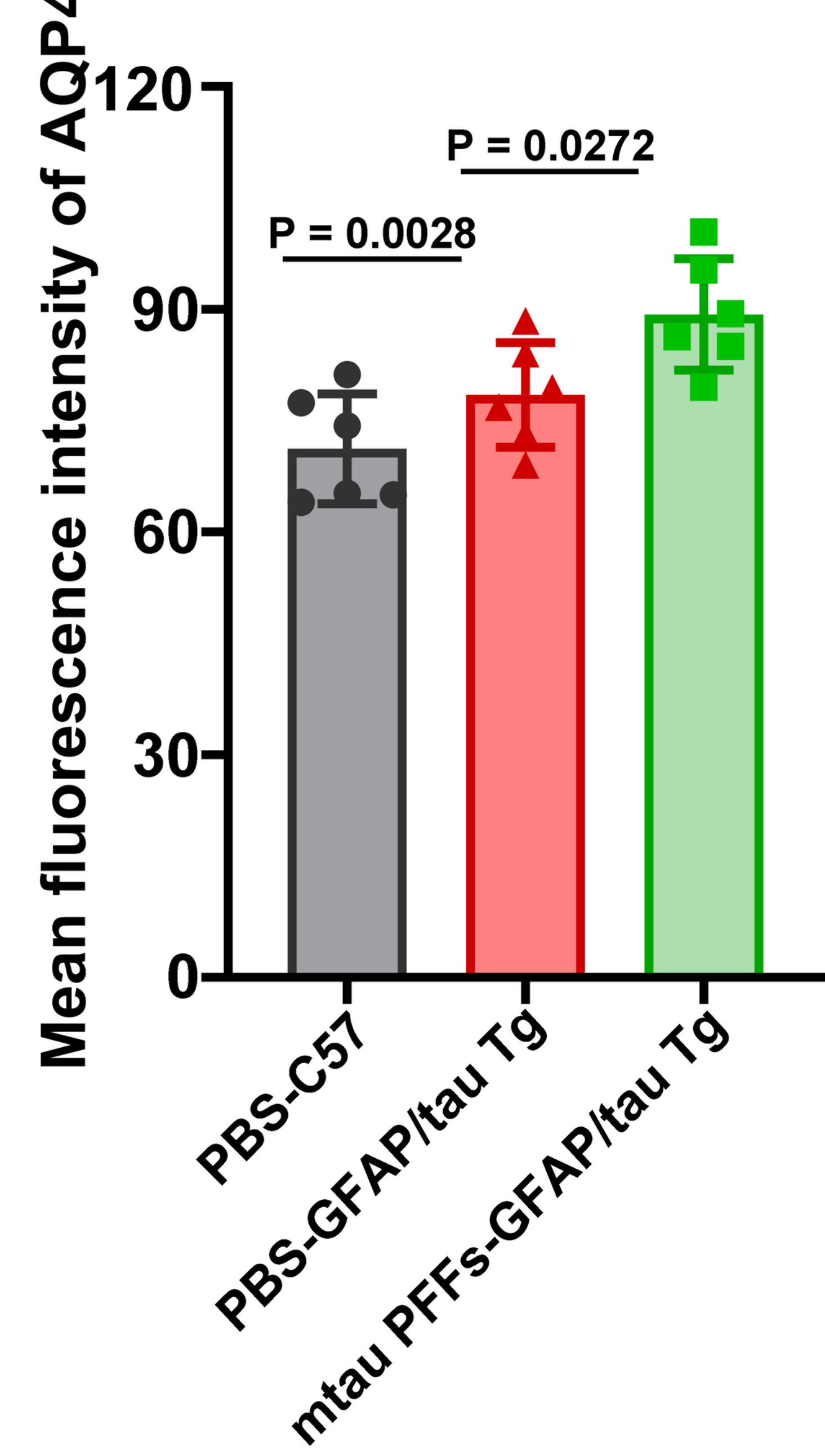
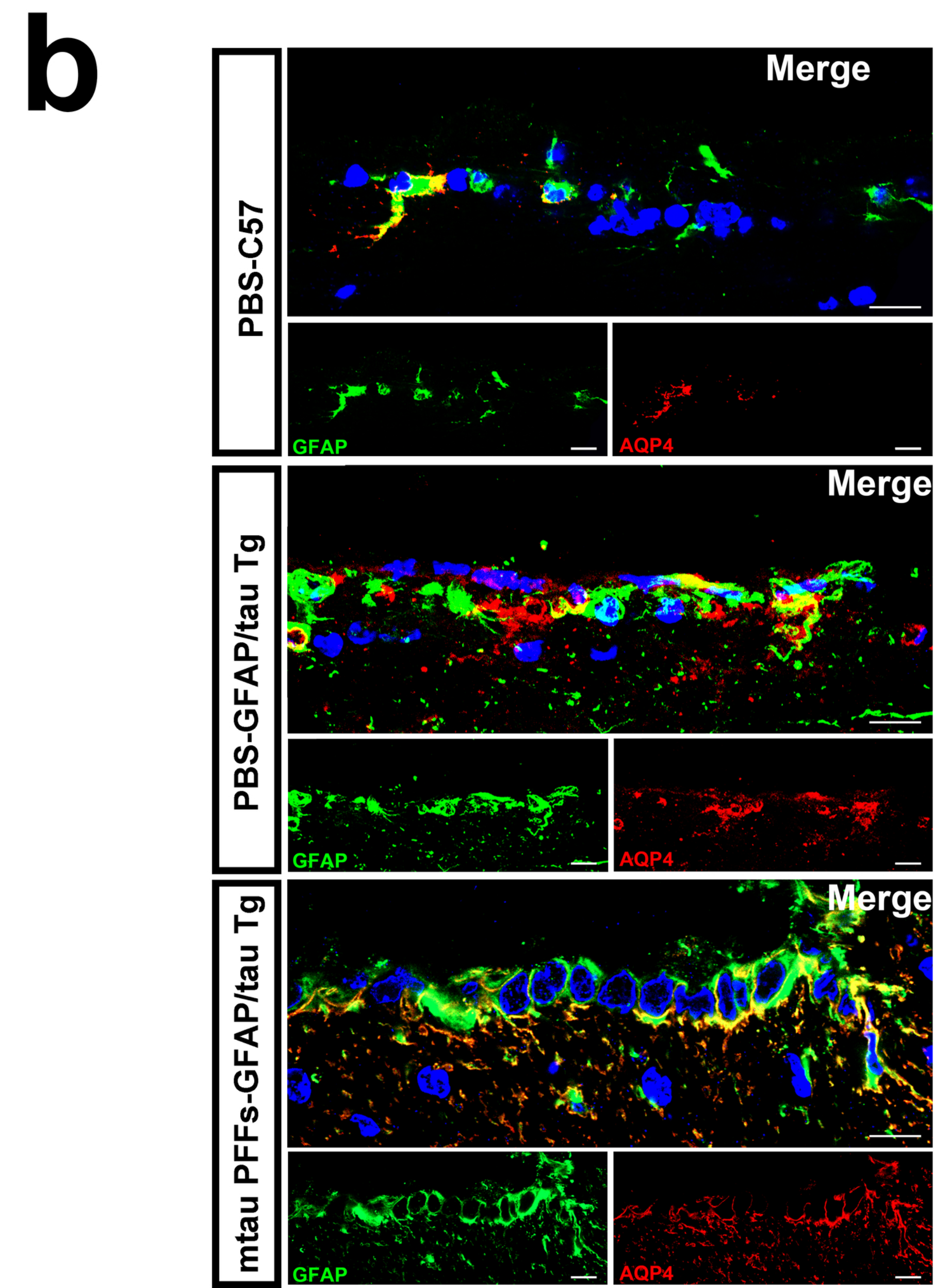
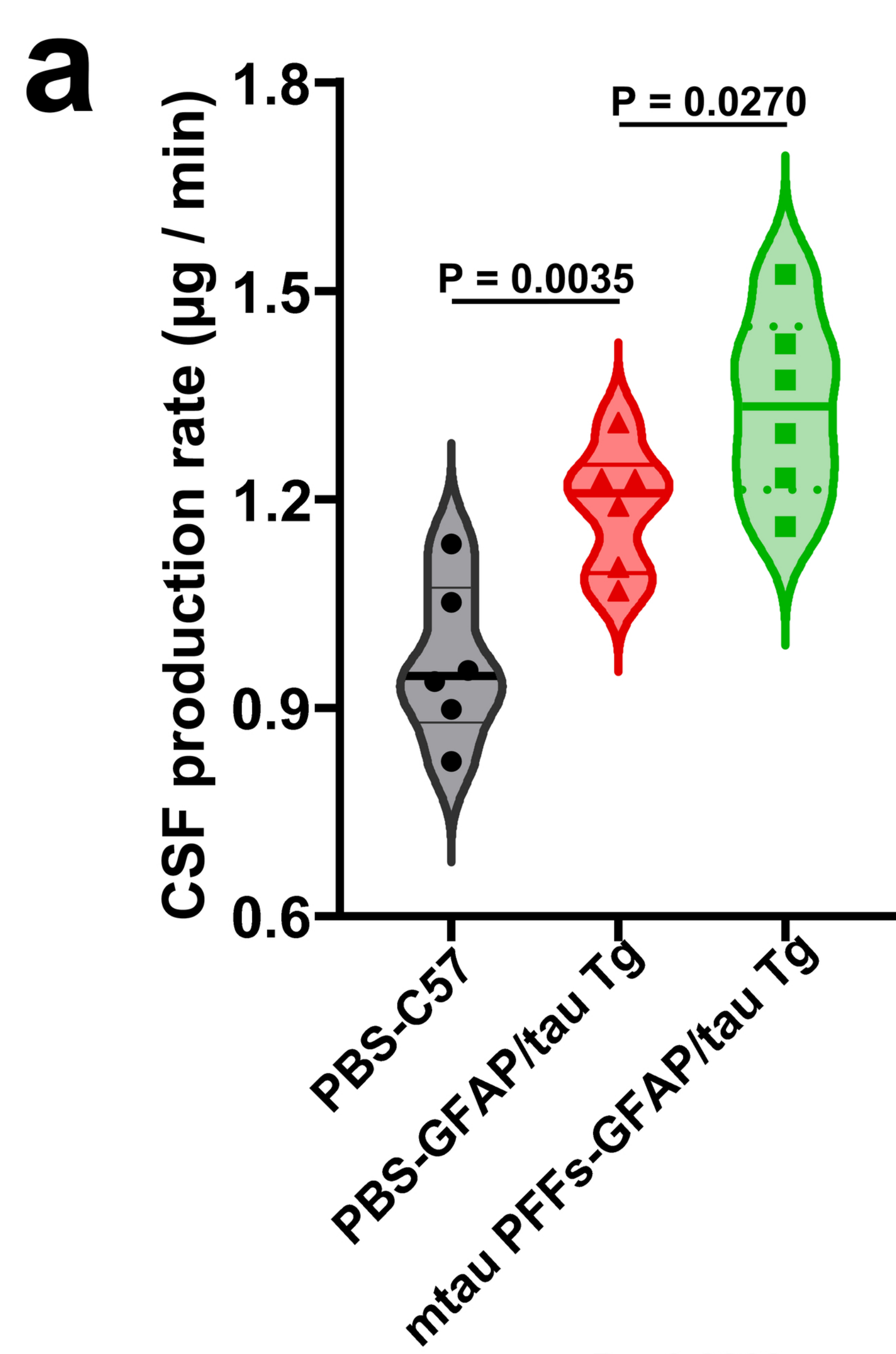
21 **Fig. S3. Assessment of glymphatic and glymphatic transport via fluorescent**
22 **tracers in mouse models. a**, Intracisternal injection (30 minutes post-injection): Left

23 panels: Fluorescent images of tracers (red = Dex-3; green = FITC-OVA) in PBS-C57,
24 PBS-GFAP/tau Tg, and mtau PFFs-GFAP/tau Tg mice under two anesthetic
25 regimens: K/X (ketamine/xylazine) or ISO (isoflurane). Right panels: Quantification
26 of fluorescence fraction (%) for Dex-3 and FITC-OVA, comparing tracer distribution
27 in K/X vs. ISO groups. Statistical P-values indicate significant differences between
28 anesthetic groups. **b**, Intrastriatal injection (2 hours post-injection): Left panels:
29 Fluorescent images of tracers (red = Dex-3; green = FITC-OVA) in PBS-C57, PBS-
30 GFAP/tau Tg, and mtau PFFs-GFAP/tau Tg mice under K/X or ISO anesthesia. Right
31 panels: Quantification of fluorescence fraction (%) for Dex-3 and FITC-OVA, with
32 statistical P-values highlighting differences between K/X and ISO groups.

a**b**

■ PBS-C57
■ PBS-GFAP/tau Tg
■ mtau PFFs-GFAP/tau Tg





a

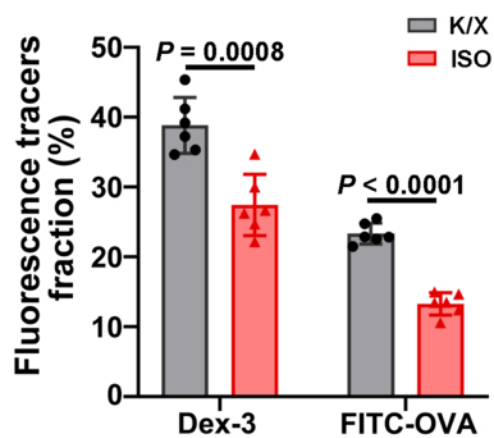
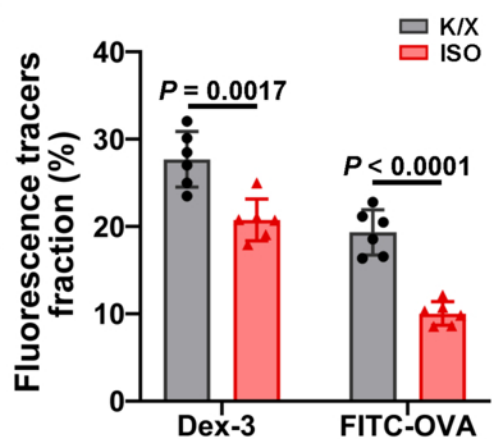
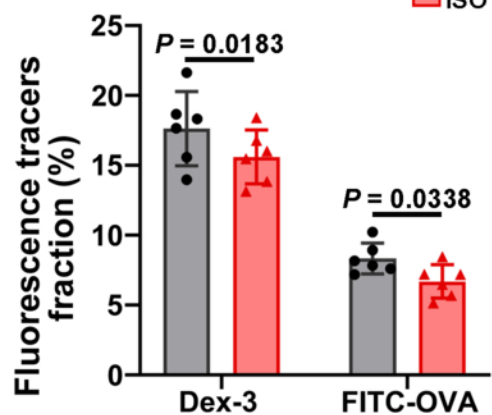
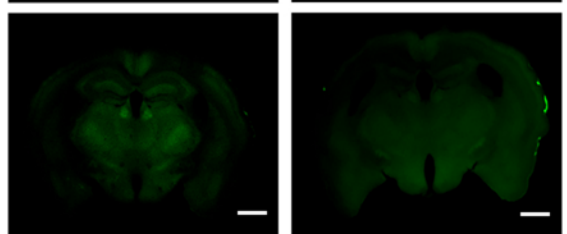
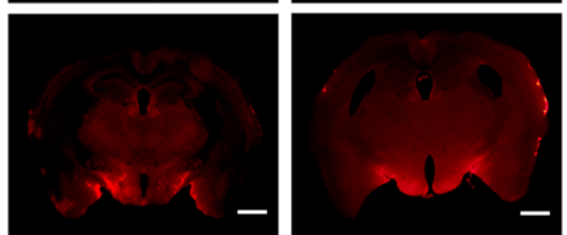
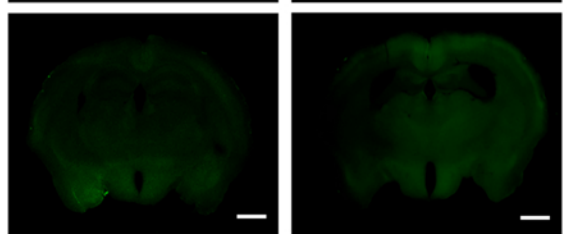
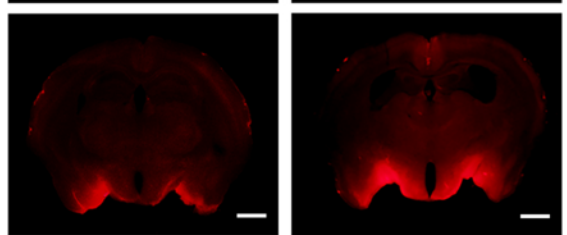
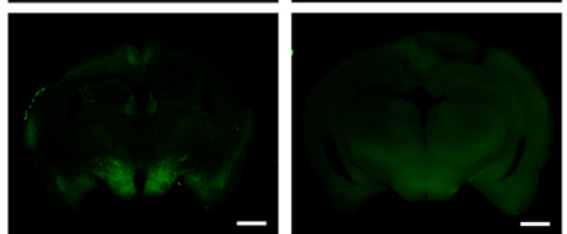
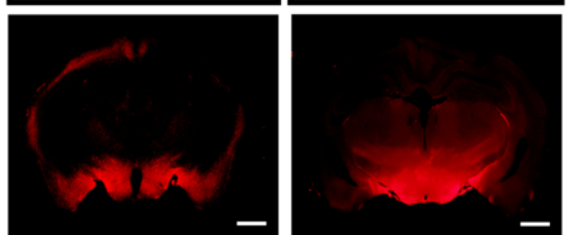
PBS-C57

PBS-GFAP/tau Tg

mtau PFFs-GFAP/tau Tg

Intracisternal injection-30 min

ISO K/X

**b**

Intrastriatal injection-2 h

ISO K/X

