Supplemental material

Croft et al., https://doi.org/10.1084/jem.20182184

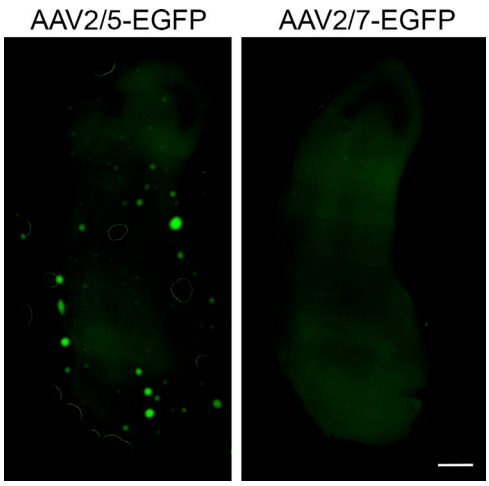

Figure S1. **rAAV2/5 and rAAV2/7 do not efficiently transduce organotypic BSCs.** Organotypic BSCs were prepared and transduced at 0 DIV with rAAVs (1–2 × 10^{10} VGs per well) packaged in capsid serotype 2/5 and 2/7 to express an EGFP transgene driven by the hCBA promoter. BSCs were maintained in culture until DIV 28, and then slices were fixed, and fluorescence of EGFP throughout the BSC was imaged. Bar, 100 µm. *n* = 9 slices from *n* = 3 wells.

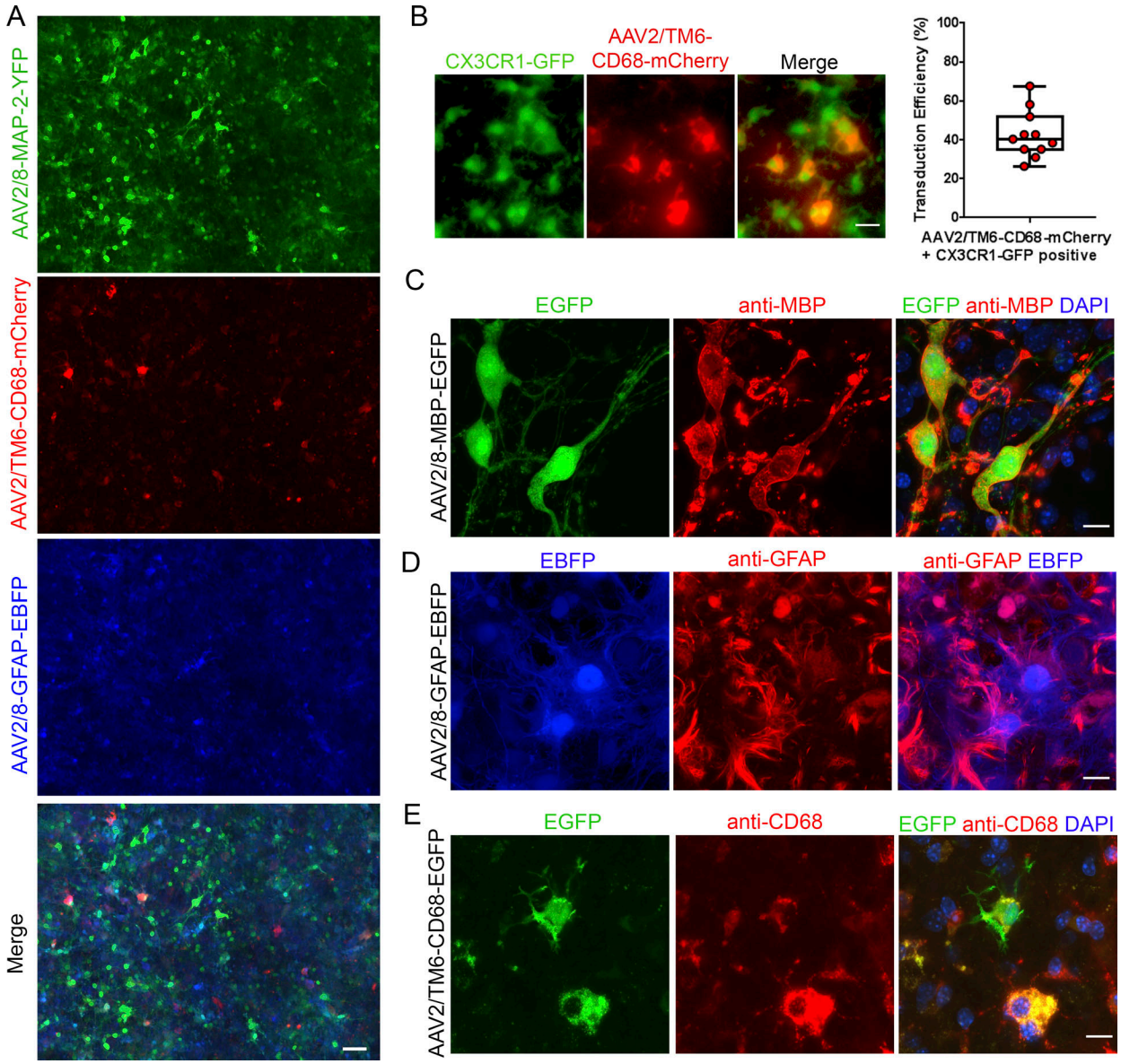

Figure S2. **Multiple CNS cell types can be transduced in the same organotypic BSC. (A)** Organotypic BSCs were prepared and transduced at 0 DIV with three rAAVs (1–2 × 10^{10} VGs per well); a MAP-2 promoter to express YFP packaged in rAAV2/8 (green), a CD68 promoter to express mCherry packaged in rAAV2/6 with three mutations—Y731F/Y705F/T492V (TM6; red), and a GFAP promoter to express EBFP packaged in rAAV2/8 (blue) and fixed at 14 DIV, and then fluorescence was imaged. Bar, 50 µm. **(B)** BSCs were prepared from CX3CR1-GFP transgenic mice and transduced with rAAV2/TM6-CD68-mCherry, and fluorescence was imaged. Bar, 10 µm. Colocalization of mCherry as a proportion of EGFP expressing cells was quantified as percent transduction efficiency and is plotted on the graph. Data are mean ± SEM. **(C–E)** BSCs were prepared and transduced with rAAV2/8-MBP-EGFP (C), rAAV2/8-GFAP-EBFP (D), or rAAVTM6-CD68-GFP (E) at 0 DIV, maintained until 14 DIV, fixed, and then immunostained with anti-MBP, anti-GFAP, or anti-CD68 (red), respectively, to examine colocalization between transduced cells and cell markers. DAPI is also shown to mark cell nuclei. Bars, 10 µm.

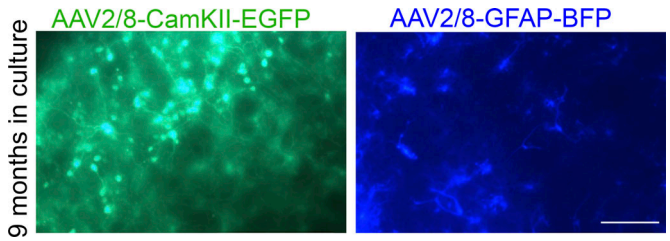

Figure S3. **rAAV expression is maintained in neurons and astrocytes until at least 9 mo in culture.** Organotypic BSCs were prepared and transduced at 0 DIV with rAAVs (1–2 × 10^{10} VGs per well) with a CamKII promoter to express EGFP packaged in rAAV2/8, or a GFAP promoter to express EBFP packaged in rAAV2/8. BSCs were maintained in culture until 9 mo in vitro. Slices were then fixed, and fluorescence of EGFP/EBFP of CNS cells at this time point in the BSCs were imaged. Bar, 400 µm.

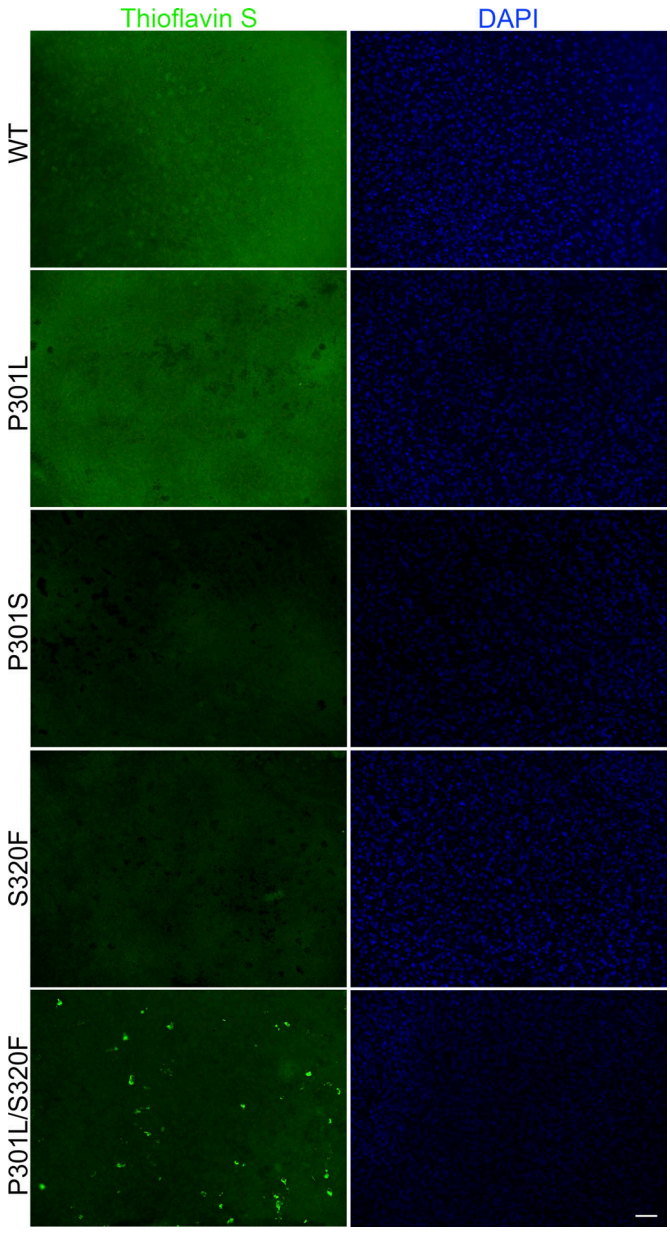

Figure S4. **WT or single mutations in MAPT do not induce Thioflavin S–positive tau inclusions in BSCs by 28 DIV.** Organotypic BSCs were prepared and transduced with rAAV2/1-WT-htau, rAAV2/1-P301L-htau, rAAV2/1-P301S-htau, rAAV2/1-S320F-htau, or rAAV2/1-P301L/S320F-htau (1–2 × 10^{10} VGs per well) at 0 DIV and then maintained in culture until 28 DIV. BSCs were fixed, stained with 0.0125% Thioflavin S, and imaged to identify any β-sheet structures in these sections. A DAPI counterstain is also shown. Bar, 50 µm. *n* = 9.