SUPPLEMENTAL MATERIAL

Ising et al., https://doi.org/10.1084/jem.20162125

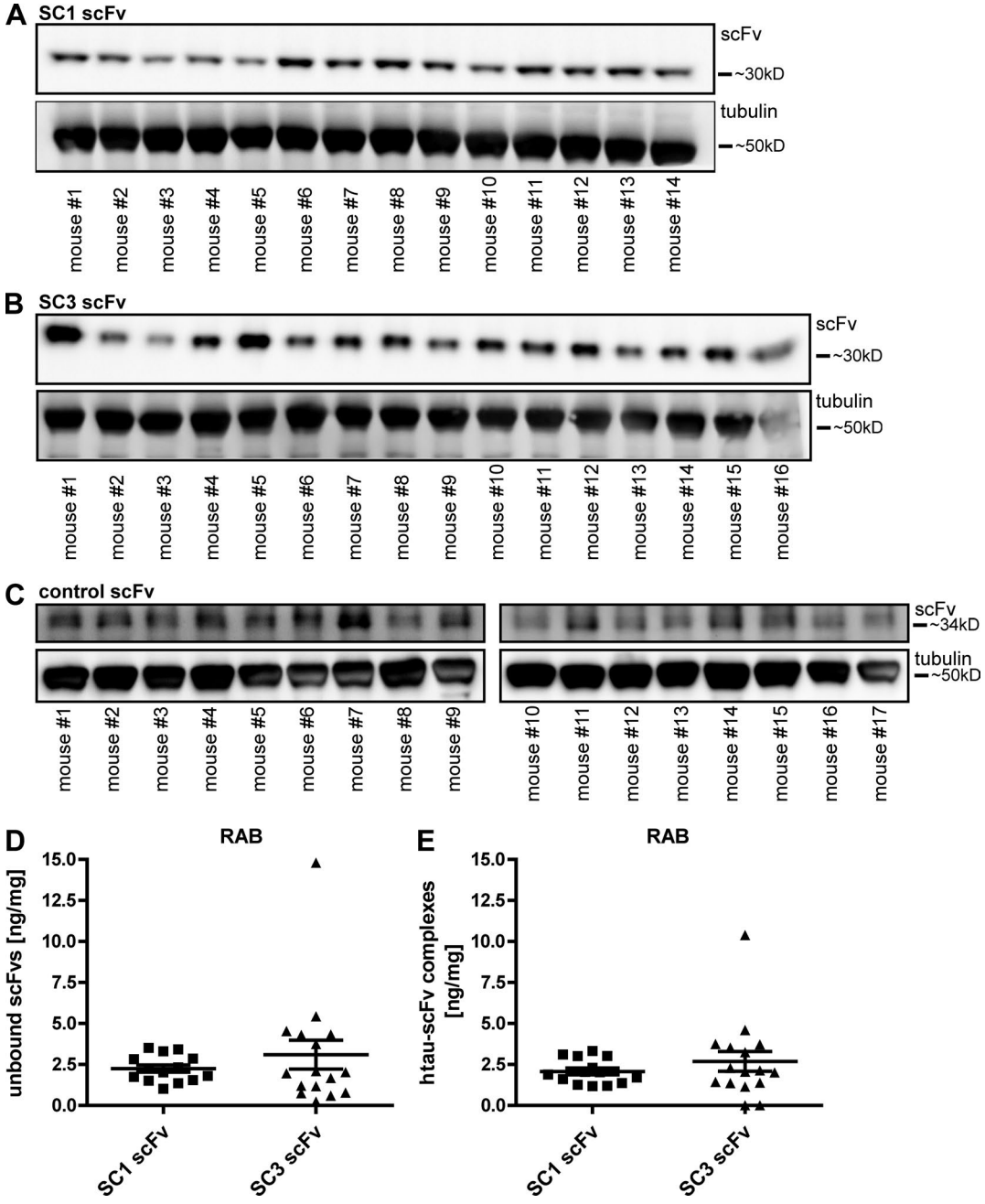

Figure S1. **All 9-mo-old mice express the scFvs.** (A–C) Immunoblot analysis shows expression of SC1 (A; $n = 14$), SC3 (B; $n = 16$), and control (C; $n = 17$) scFv in cortex samples of all 9-mo-old P301S-tg mice analyzed in the study. (D) ELISA for unbound scFvs revealed similar scFv levels in hippocampal RAB fractions ($n = 14$ for SC1 scFv and $n = 16$ for SC3 scFv). (E) ELISA for tau-scFv complexes detected complexes in hippocampal RAB fractions for all 9-mo-old P301S-tg mice except for lysates from two AAV2/8 SC3 scFv–injected mice that were below the detection limit of the assay ($n = 14$ for SC1 scFv and $n = 16$ for SC3 scFv). All graphs represent means ± SEM.

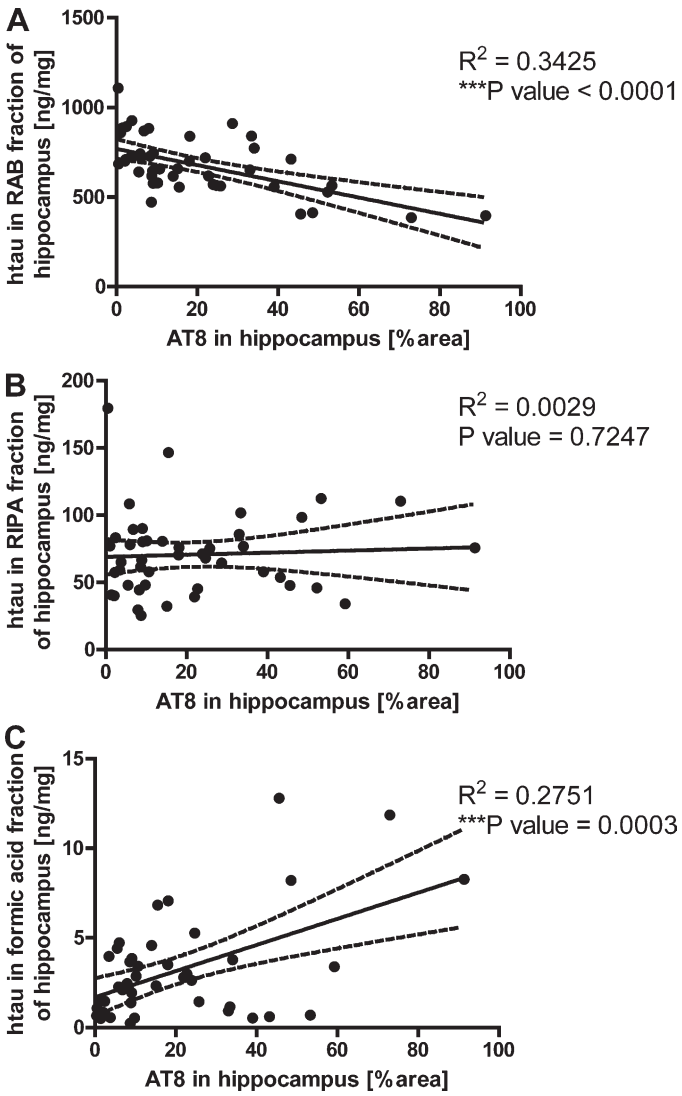

Figure S2. **AT8 staining correlates with salt-soluble and insoluble tau levels.** (A–C) Correlation analysis across all groups showed a highly significant negative correlation between htau levels in RAB fractions and positive AT8 staining in the hippocampus (A), whereas there was a positive correlation for htau levels in FA fractions (C) and no correlation at all for htau levels in RIPA fractions (B; $n = 45$ in each analysis; 9-mo-old P301S-tg mice). Dashed lines represent 95% confidence intervals.

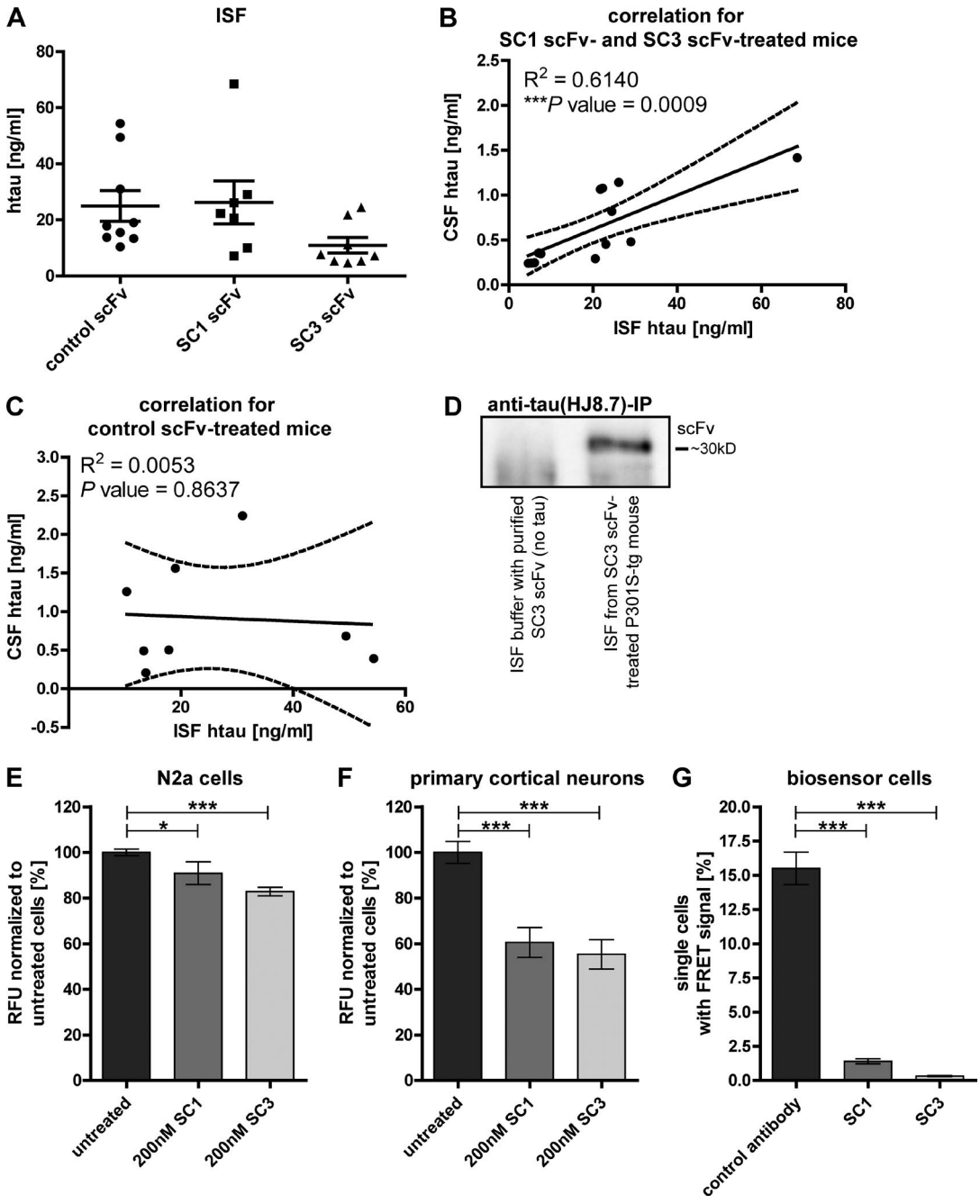

Figure S3. **ScFvs bind to extracellular htau and inhibit tau uptake and seeding.** (A) In vivo brain microdialysis followed by ELISA showed no significant differences (measured by one-way ANOVA) of extracellular htau levels between treatment groups in young (3–4.5 mo old) P301S-tg mice despite a trend to lower tau in SC3 scFv–treated mice (n = 9 for control scFv, n = 7 for SC1 scFv, and n = 8 for SC3 scFv; ISF collected at a flow rate of 1 µl/min). (B) Analysis of SC1 scFv– and SC3 scFv–treated mice showed a significant correlation between CSF and ISF htau levels in young P301S-tg mice (n = 6 for SC1 scFv and n = 8 for SC3 scFv). (C) No correlation between CSF and ISF htau levels was observed in young control scFv–treated P301S-tg mice (n = 8 for control scFv). (D) Coimmunoprecipitation from ISF samples (collected at a flow rate of 0.5 µl/min) with the anti-tau antibody HJ8.7 revealed coprecipitation of SC3 scFv with tau when analyzed by immunoblot. As a control, ISF sample buffer was mixed with the same concentration of purified SC3 scFv (n = 3). (E) Fluorescently labeled tau uptake by N2a cells measured by flow cytometry revealed the potential of SC1 and SC3 scFv to block cellular uptake (n = 7). (F) Fluorescently labeled tau uptake by primary cortical neuronal cultures measured by flow cytometry showed a significant inhibition of tau uptake in the presence of SC1 or SC3 scFvs (n = 4). (G) Immunodepletion with SC1 or SC3 scFvs inhibits seeding activity of P301S-tg brain lysate when analyzed by measuring the FRET signal in the biosensor cells (n = 3). Dashed lines in B and C represent 95% confidence intervals. All graphs are means ± SEM. A and E–G were analyzed by one-way ANOVA, followed by Dunnett's multiple comparison post hoc test comparing every column to control scFv/untreated: *, $P < 0.05$; ***, $P < 0.001$.