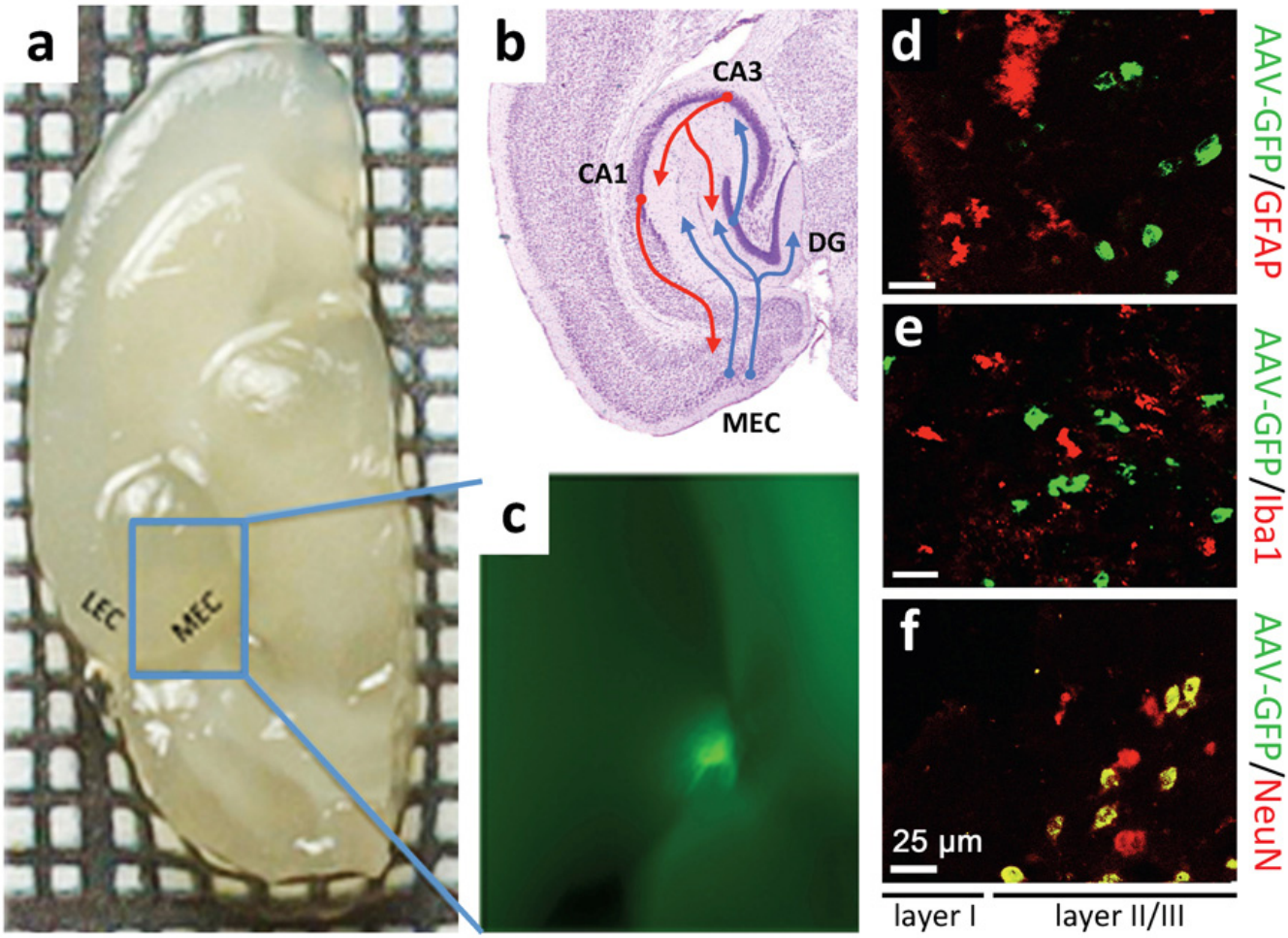
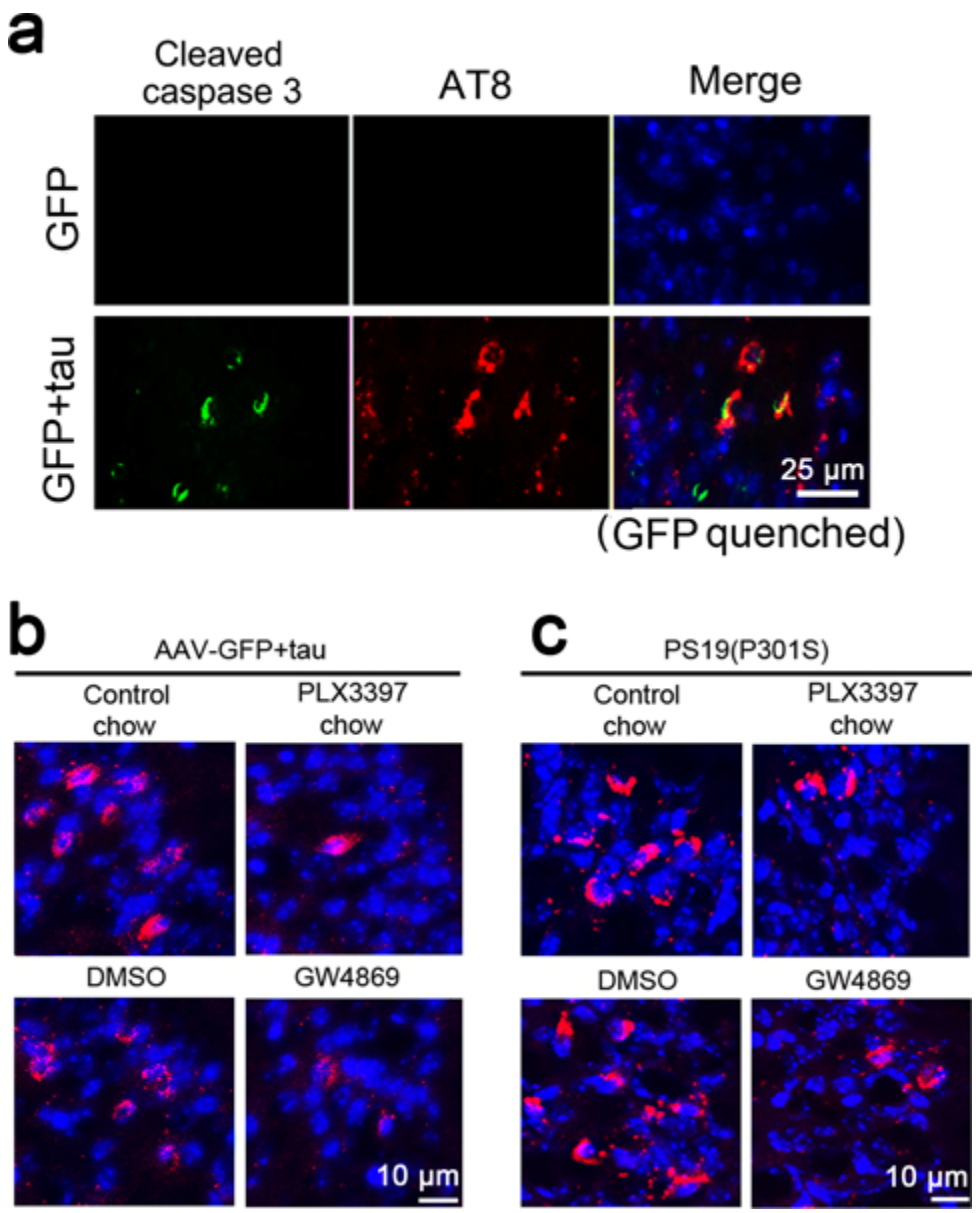


Supplementary Figure 1

AAV-GFP injection in the MEC of the mouse brain

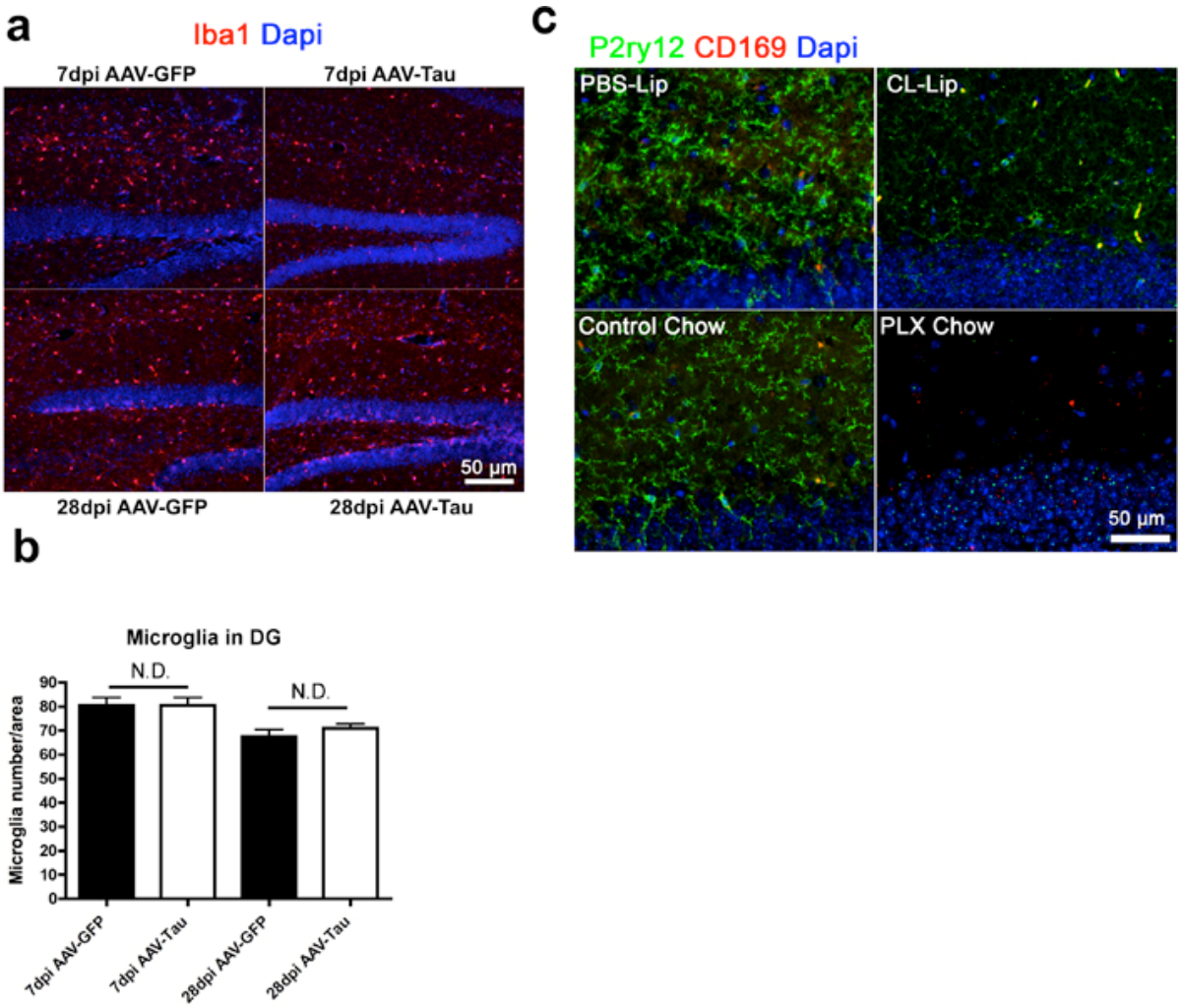
C57Bl/6 mice at 4 months of age were injected with AAV-GFP into the MEC and sacrificed at 7 days post injection (dpi). (**a**) Brains were sectioned with 0.5-mm thickness, followed by 4M urea containing *Scale* solution treatment to make brain transparent. (**c**) Green fluorescence protein (GFP) was detected in MEC injected site. (**d**) Immunofluorescence of GFP (green) with GFAP (astrocytic marker, red) (**e**) Iba1 (mononuclear phagocyte maker, red) and (**f**) NeuN (neuron marker, red) in the MEC of injected mouse brain; Scale bars: 25 µm. (Scheme in b adapted from The Mouse Brain in Stereotaxic Coordinates 2nd ed (2001); p.333)



Supplementary Figure 2

Cytotoxic changes of tau-bearing neurons in the DG and reduction of changes by microglial depletion or nSMase2 inhibition in two different tau mouse models

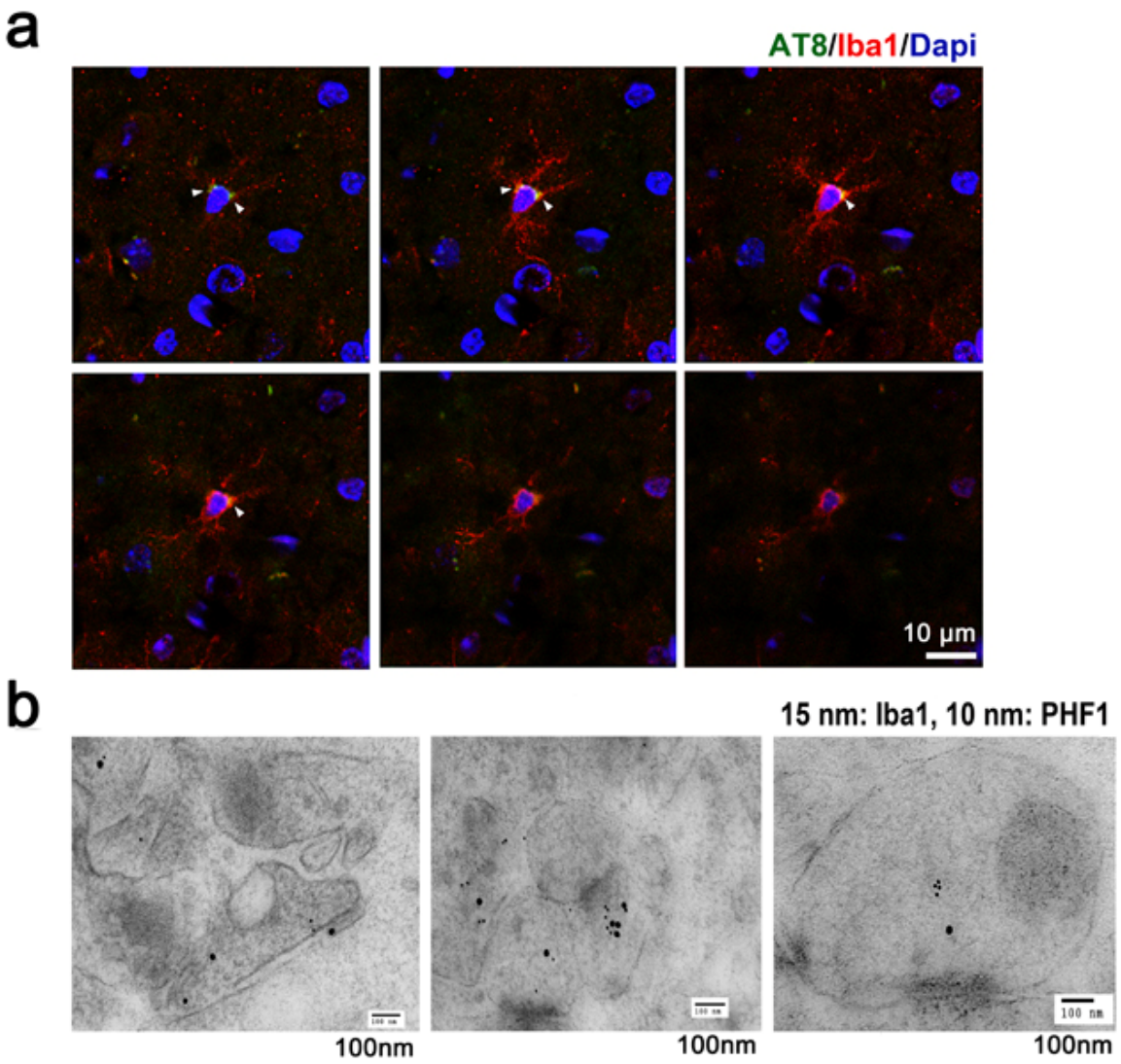
(**a**) Cleaved caspase-3 (green), AT8 (pTau, red), and Dapi (blue) staining in the dentate granule cell layer (GCL) of AAV-GFP/tau mice at 28 dpi. Scale bar: 20 μm. GFP signal was quenched by methanol/acetone treatment of tissue sections prior to the immunostaining. (**b,c**) AAV-GFP/tau mice or PS19 mice were treated for microglial depletion by feeding mice with PLX3397 chow (upper panel) or nSMase2 inhibition by ip injection of GW4869 (lower panel). The frozen tissue sections were immunostained for cleaved caspase-3 (red) and Dapi (blue) in the GCL of AAV-GFP/tau mice at 28 dpi (A) or PS19 tau mice at 3.5 months of age (B); scale bars: 10 μm.



Supplementary Figure 3

Microglial density after AAV-GFP/tau injection into the MEC and depletion of microglia by clodronate liposome or PLX3397

(**a,b**) AAV-GFP or AAV-GFP/tau mice are subjected to immunofluorescence of Iba1 (mononuclear phagocyte marker, red) and Dapi (blue) in the DG at 7 or 28 dpi. (**b**) Quantification of microglial density in the DG. N.D.: No statistical difference between AAV-GFP and AAV-GFP/tau mice at 7 or 28 dpi as determined by one-way ANOVA ($p>0.999$, $t(32)=0.0$, $n=3$, 17 sections per group for 7 dpi; $p=0.3231$, $t(42)=0.999$, $n=3$, 22 sections mice per group for 28 dpi). Scale bar: 50 μm (**c**) C57BL/6 mice are pre-treated with clodronate liposome (CL-Lip) or PBS liposome (PBS-Lip) through a cannula implanted into the lateral ventricle at the time of AAV-GFP/tau injection until 28 dpi (a total of 4 week treatment, see **Supplementary Figure 6** for details). PBS-Lip was injected in the control group. For PLX3397 study, C57BL/6 mice are pre-fed with the control or PLX-containing chow for 4 weeks prior to the AAV-GFP/tau injection, and maintained in the same chow until 28 dpi (a total of 8 week treatment). immunofluorescence of P2ry12 (microglia-specific marker, green) and CD169 (infiltrating monocytes marker, red) in the DG of AAV-GFP/tau mice at 28 dpi. Scale bar: 50 μm.

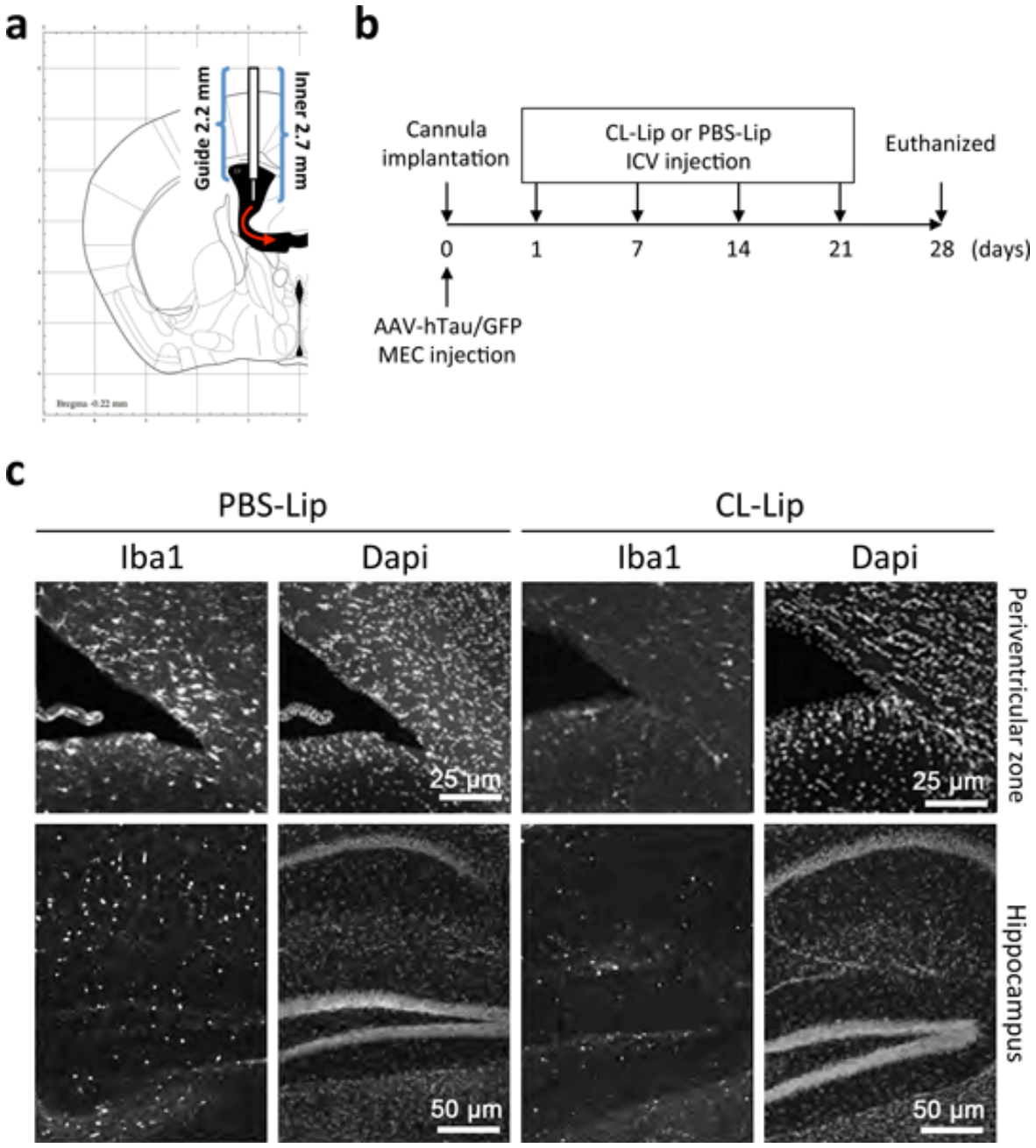


Supplementary Figure 4

Colocalization of pTau within Iba1-positive microglia

(a) Sequential imaging of confocal microscopy in the hippocampal area of the 6 month-old PS19 mouse brain. Green; AT8 (red), Iba1 (green). Scale bar: 10 μm (**b**) Double-immunogold labeling and electron microscopy in the hippocampal area of the 9-month-old PS19 mouse brain. 15-nm gold particles bind to Iba1 and 10-nm gold particles bind to PHF1 (pTau at pSer396/Ser404). Scale bars: 100 nm.

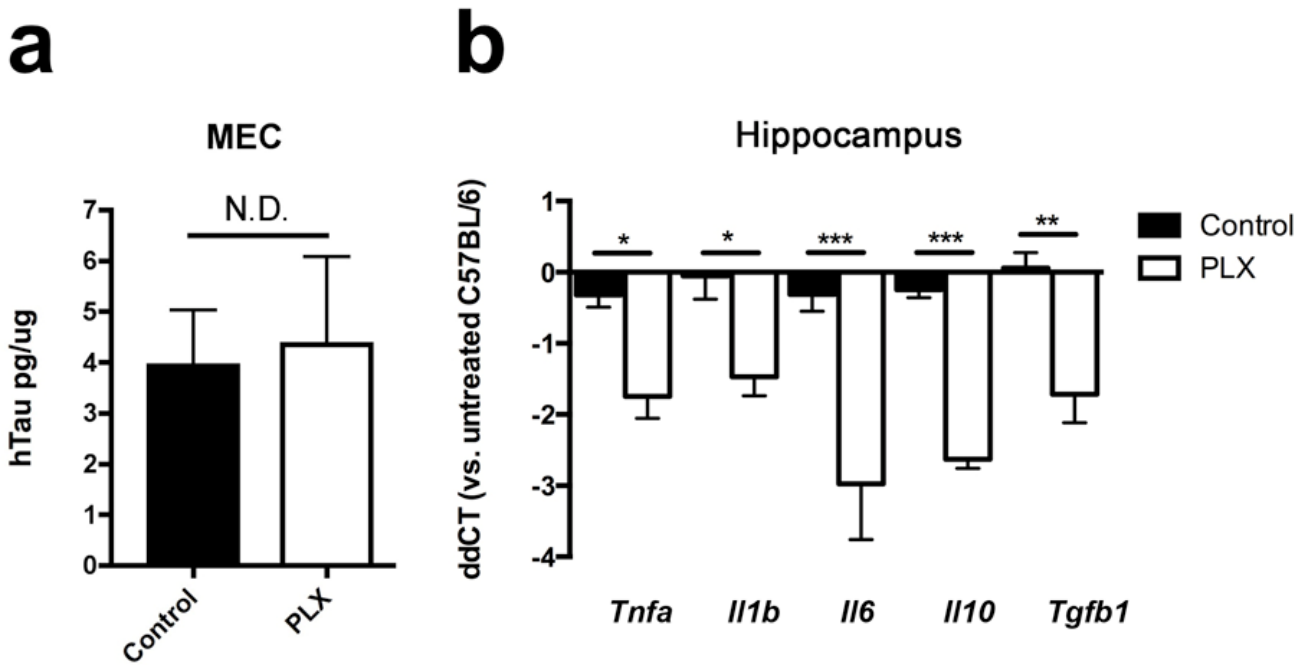
Nature Neuroscience: doi:10.1038/nn.4132



Supplementary Figure 5

Microglial depletion by clodronate liposome

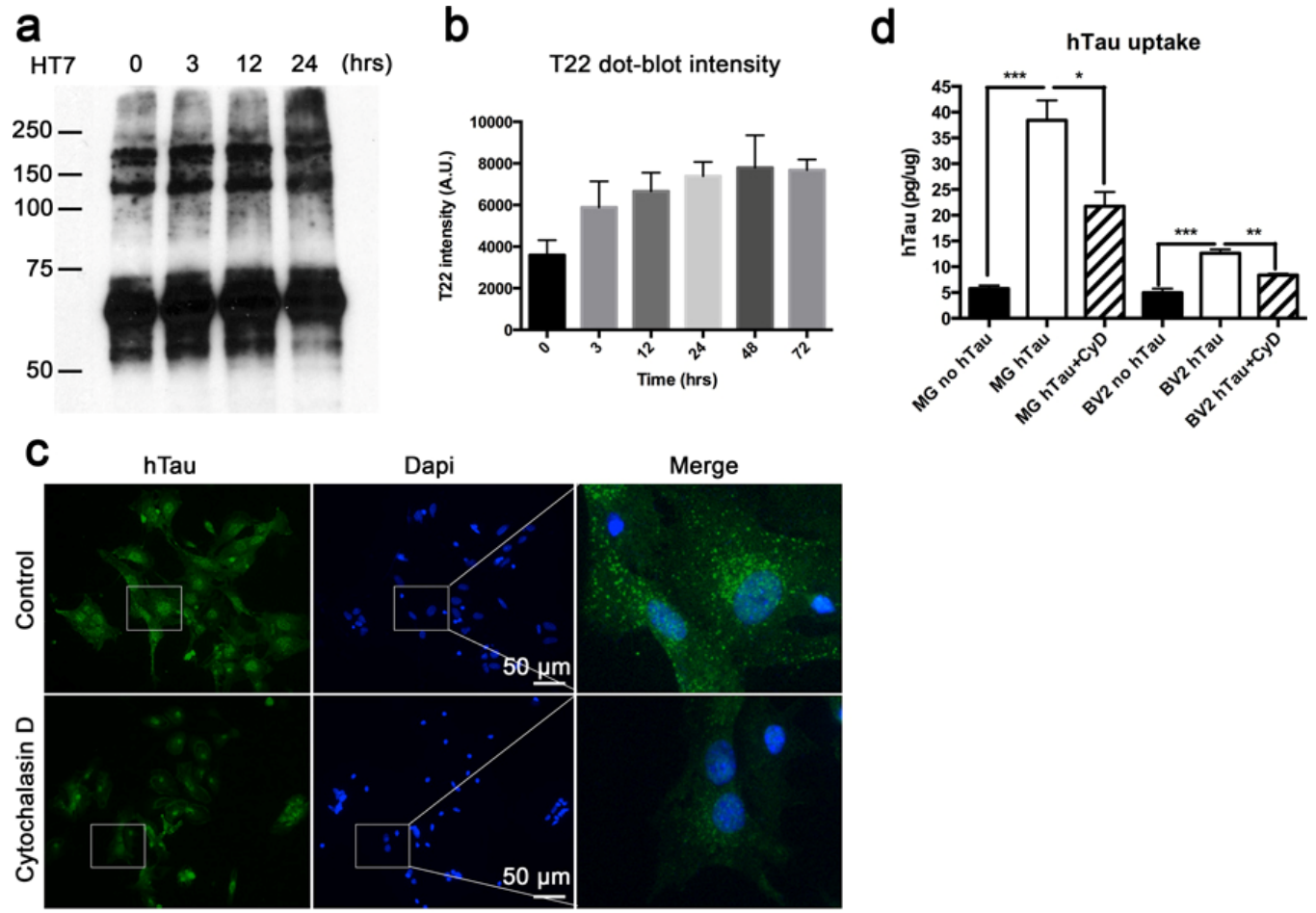
(**a**) Cannula coordinate and time point for the CL-liposome ICV injection. (**b**) CL or PBS-Lip was ICV injected once a week for 4 weeks through the implanted cannula for one month after the AAV-GFP/tau injection. (**c**) Iba1 and Dapi staining in periventricular zone (top panels) and hippocampus (bottom panels). Scale bars: top panels: 25 µm; bottom panels: 50 µm (Scheme in **a** adapted from The Mouse Brain in Stereotaxic Coordinates second edition (2001), p. 83)



Supplementary Figure 6

Quantification of hTau in the MEC of AAV-GFP/tau-injected mice and PLX treatment and gene expression profile of immune molecules in the hippocampus

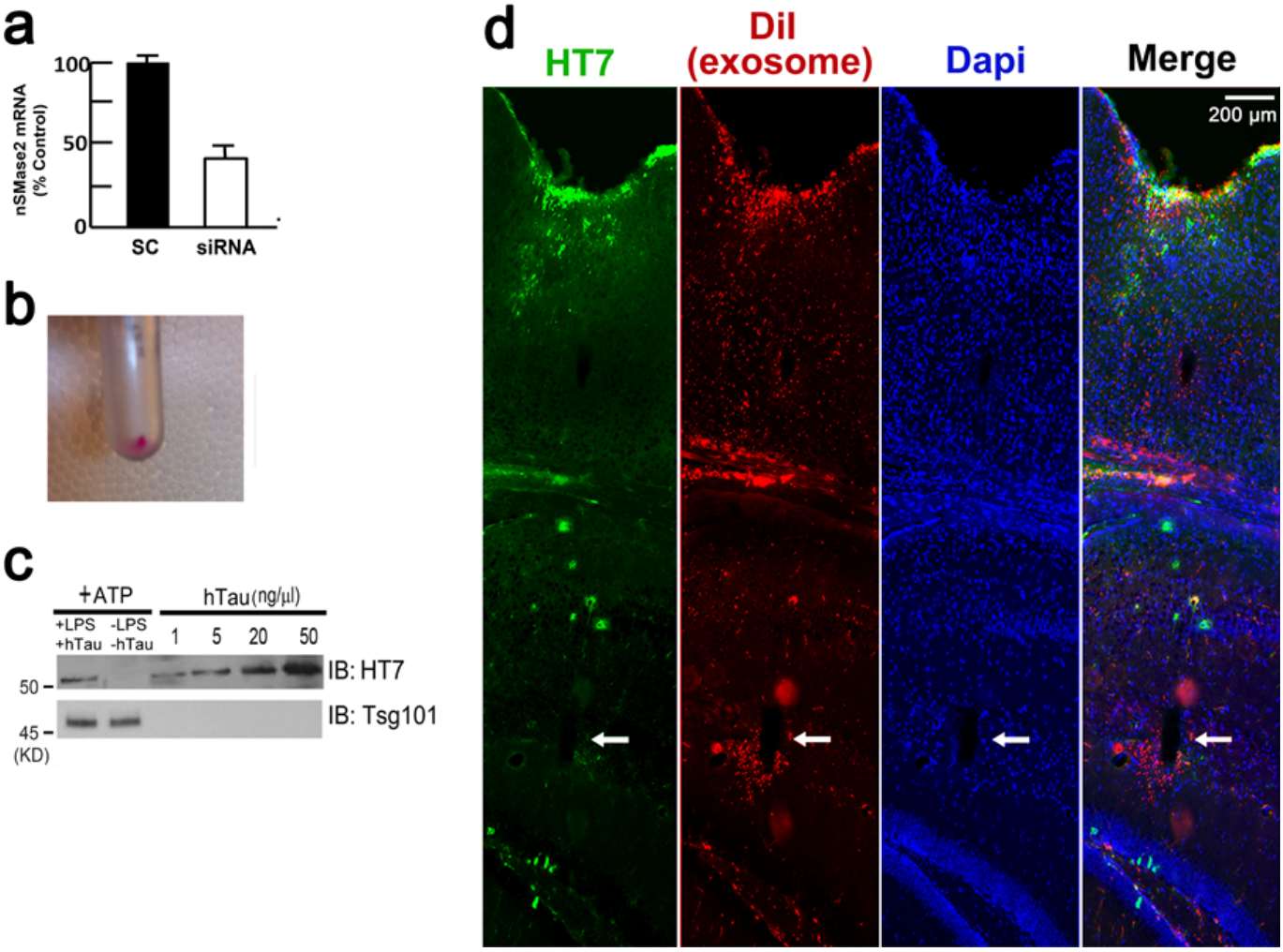
PLX3397 (or control) chow-fed C57BL/6 mice were injected with AAV-GFP/tau into the MEC and hippocampal regions were isolated at 28 dpi. (**a**) Quantification of hTau in the MEC using hTau specific ELISA. No statistical difference between control- and PLX-chow fed groups (p=0.8515, t(4)=0.1996, n=3 per group). (**b**) Gene expression profiles of pro-inflammatory cytokines (*Tnfa, Il1b*, and *Il6*) and anti-inflammatory cytokines (*Il10* and *Tgfb1)* in the hippocampus of control or PLX chow-fed mice with AAV-GFP/tau injection. Each column represents ΔΔCT value vs. age-matched untreated C57BL/6 mice. p<0.0001, F(1,20)=77.29, n= 3 per group as determined by two-way ANOVA and multiple comparison tests. t(20)=2.987 (*Tnfa*), 2.877 (*Il1b*), 5.422 (*Il6*), 4.852 (*Il10*), and 3.610 (*Tgfb1*). **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 between control and PLX group as determined by two-way ANOVA and multiple comparison tests (n=3 per group).



Supplementary Figure 7

Aggregation of purified hTau and microglial phagocytosis *in vitro*.

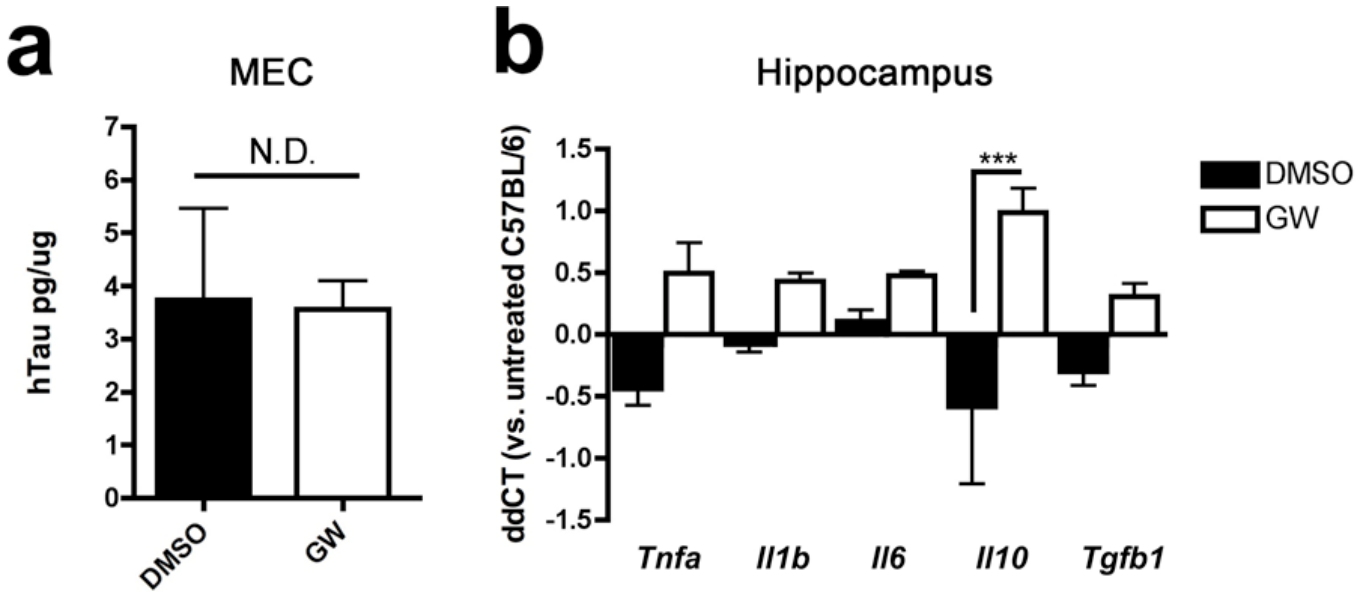
(**a**) Recombinant hTau protein was aggregated in the presence of heparin for 0-24 hrs as described in methods, and subjected to western blotting using HT7 (anti-hTau mAb). High molecular weight tau bands (120kDa and above) are visible even at 0 time point. (**b**) Quantification of tau oligomers by dot-blot analysis of aggregated tau protein for 0-72 hours. Tau oligomerization is increased after 3 hr incubation and is saturated by 24 hrs. (**c**) Murine primary microglia (MG) are treated with aggregated tau ± 1µM cytochalasin D (CyD, phagocytosis inhibitor) for 30 min, and the cells were washed with PBS and fixed with 4% paraformaldehyde for immunofluorescence using HT7 (green) and Dapi (blue); scale bar: 50µm. (**d**) After the phagocytosis assay of MG or BV-2 cells (murine microglial cell line), the cells were trypsinized to remove extracellular tau, and subjected to hTau ELISA. MG: p=0.0005, F(2,6)=34.93, q(6)=11.82 (MG no hTau vs. MG hTau), 5.781 (MG no hTau vs. MG hTau+CyD), and 6.039 (MG hTau vs. MG hTau+CyD). BV2: p=0.0005, F(2,6)=34.94, q(6)=11.80 (BV2 no hTau vs. BV2 hTau), 5.285 (BV2 no hTau vs. BV2 hTau+CyD), 6.517 (BV2 hTau vs. BV2+CyD). **P* < 0.05, **P < 0.01 and ****P* < 0.001 as determined by one-way ANOVA and Tukey post hoc.



Supplementary Figure 8

nSMase2 knockdown and purified tau-containing exosomes injected into the OML of mouse brain

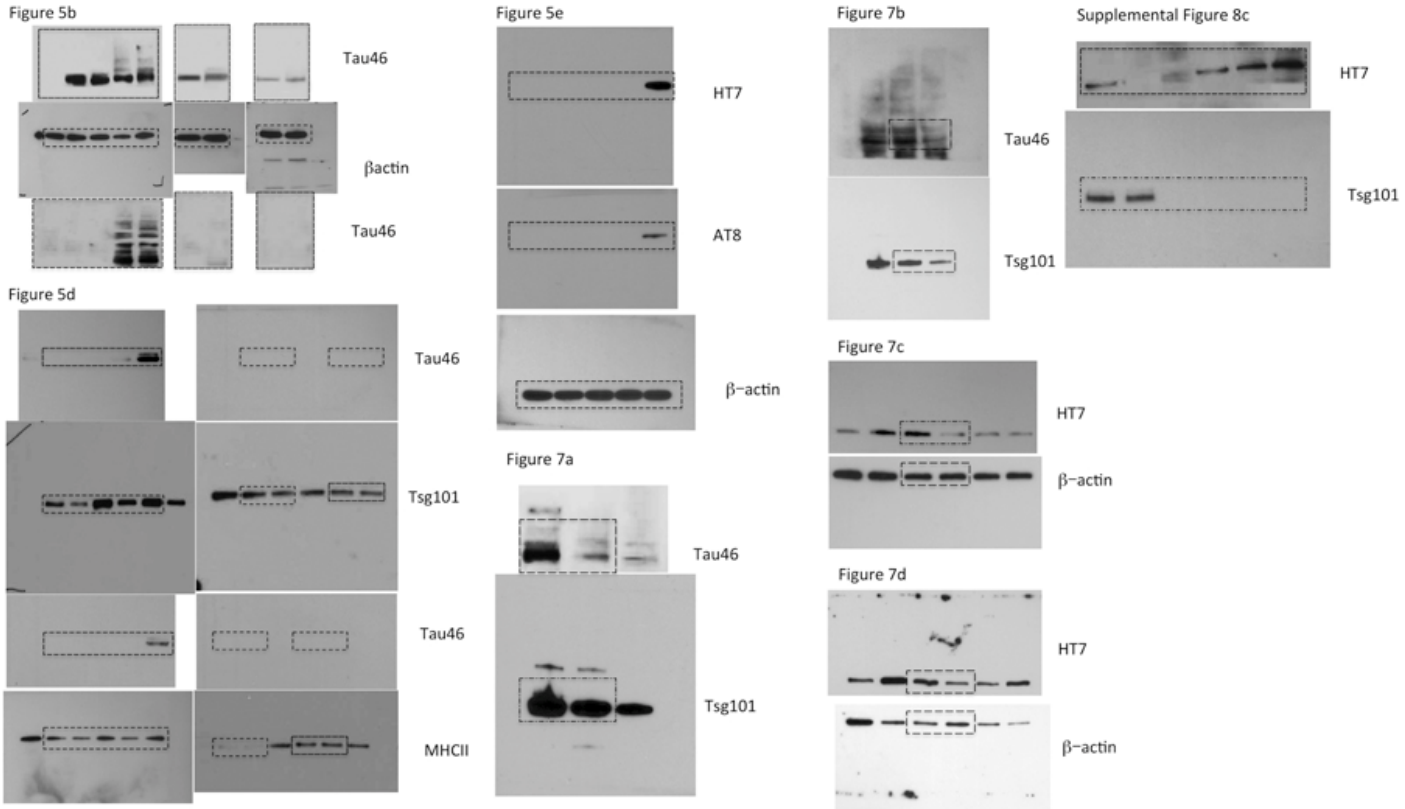
(**a**) Neutral sphingomyeliase-2 (*nSMase2*) mRNA expression levels in mouse primary microglia treated with siRNA against murine *nSMase2* or scramble siRNA. (**b**) Purified tau-containing exosomes from hTau-phagocytosed microglia was DiI-labeled. (**c**) One µl of the exosomal fraction from microglia with $hTau^{+}LPS^{+}ATP^{+}$ or $hTau\text{-}LPS^{-}ATP^{+}$ treatment, and 1 to 50 ng/µl of aggregated recombinant hTau were blotted with HT7 or Tsg101. Full-length blots/gels are presented in Supplementary Figure 10. (**d**) Immunofluorescence of of HT7 (green), DiI (exosome, red), Dapi (blue) of mouse brain sections after injection of tau containing exosomes to the OML of the DG of C57BL/6 mice. Scale bar: 200 µm



Supplementary Figure 9

Quantification of hTau in the MEC of AAV-GFP/tau injected mice and GW4869 treatment and gene expression profile of immune molecules in the hippocampus

C57BL/6 mice were injected with AAV-GFP/tau into the MEC, had daily ip injection of 1.25 mg/kg of GW4869 or 200µl of 5% DMSO in saline (control vehicle) from 0 to 28 dpi, and the MEC and hippocampal regions were isolated at 28 dpi. (**a**) Quantification of hTau in the MEC using hTau specific ELISA. No statistical difference between DMSO- and GW4869-treated groups (p=0.9295, t(4)=0.0942, n=3 per group). (**b**) Gene expression profiles of pro-inflammatory cytokines (*Tnfa, Il1b*, and *Il6*) and anti-inflammatory cytokines (*Il10* and *Tgfb1)* in the hippocampus of DMSO or GW4869-treated AAV-GFP/tau mice. Each column represents ΔΔCT value vs. age-matched untreated C57BL/6 mice. p<0.0001, F(1,20)=28.89, t(20)=2.819 (*Tnfa*), 1.535 (*Il1b*), 1.114 (*Il6*), 4.725 (*Il10*), and 1.826 (*Tgfb1*) groups as determined by two-way ANOVA and multiple comparison tests (n=3 per group). *** *P* < 0.001 between DMSO and GW4869.



Supplementary Figure 10

Full-length blots for Figures 5 and 7 and Supplementary Figure 8c.