

Supplementary Information for:

**ENHANCING MICROTUBULE STABILIZATION RESCUES COGNITIVE DEFICITS
AND AMELIORATES PATHOLOGICAL PHENOTYPE IN AN AMYLOIDOGENIC
ALZHEIMER'S DISEASE MODEL**

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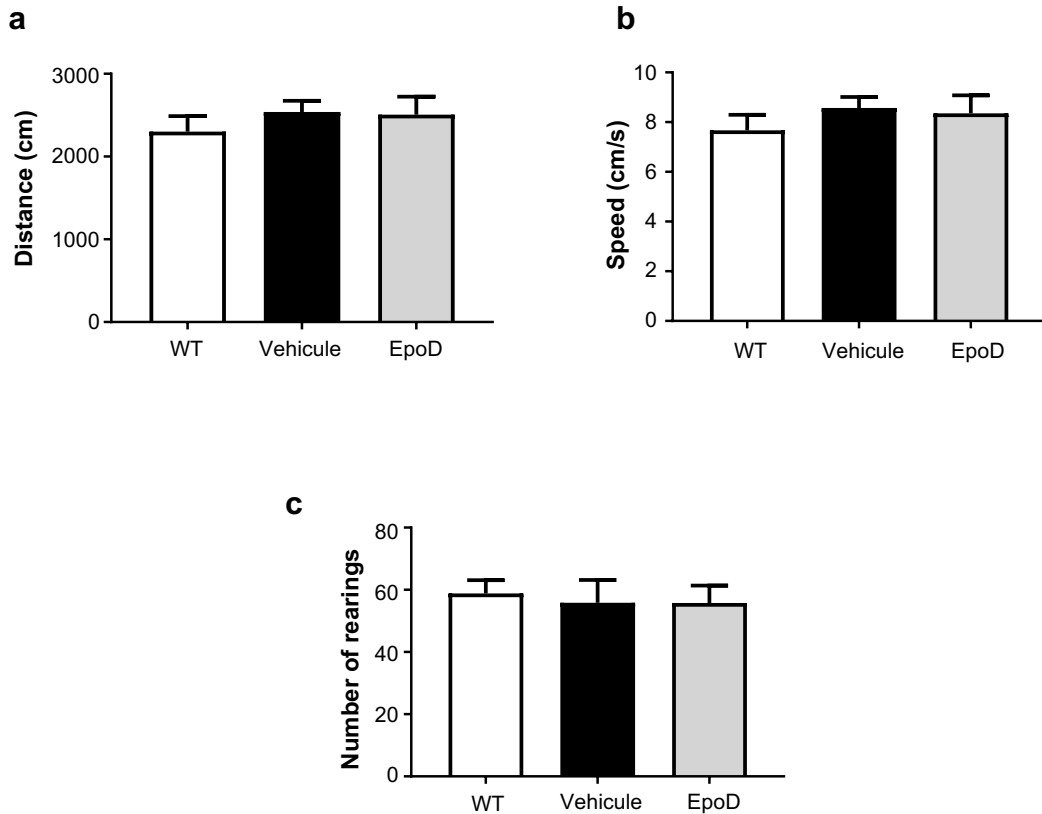
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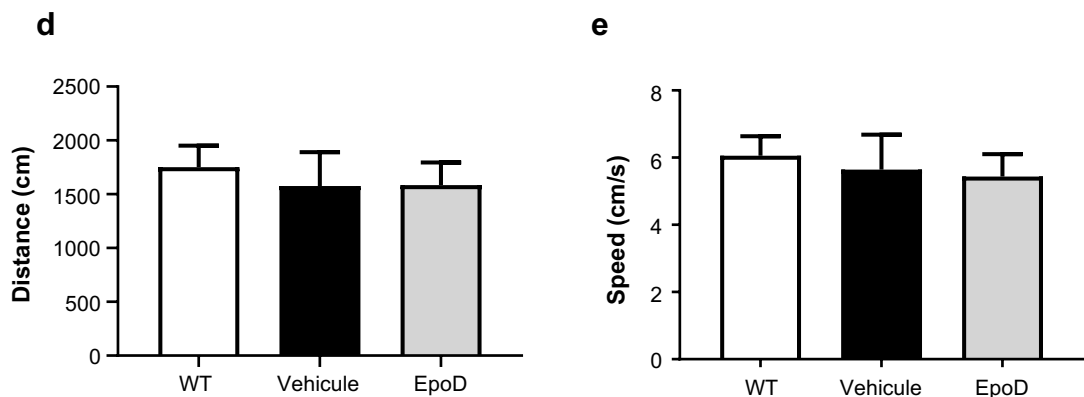
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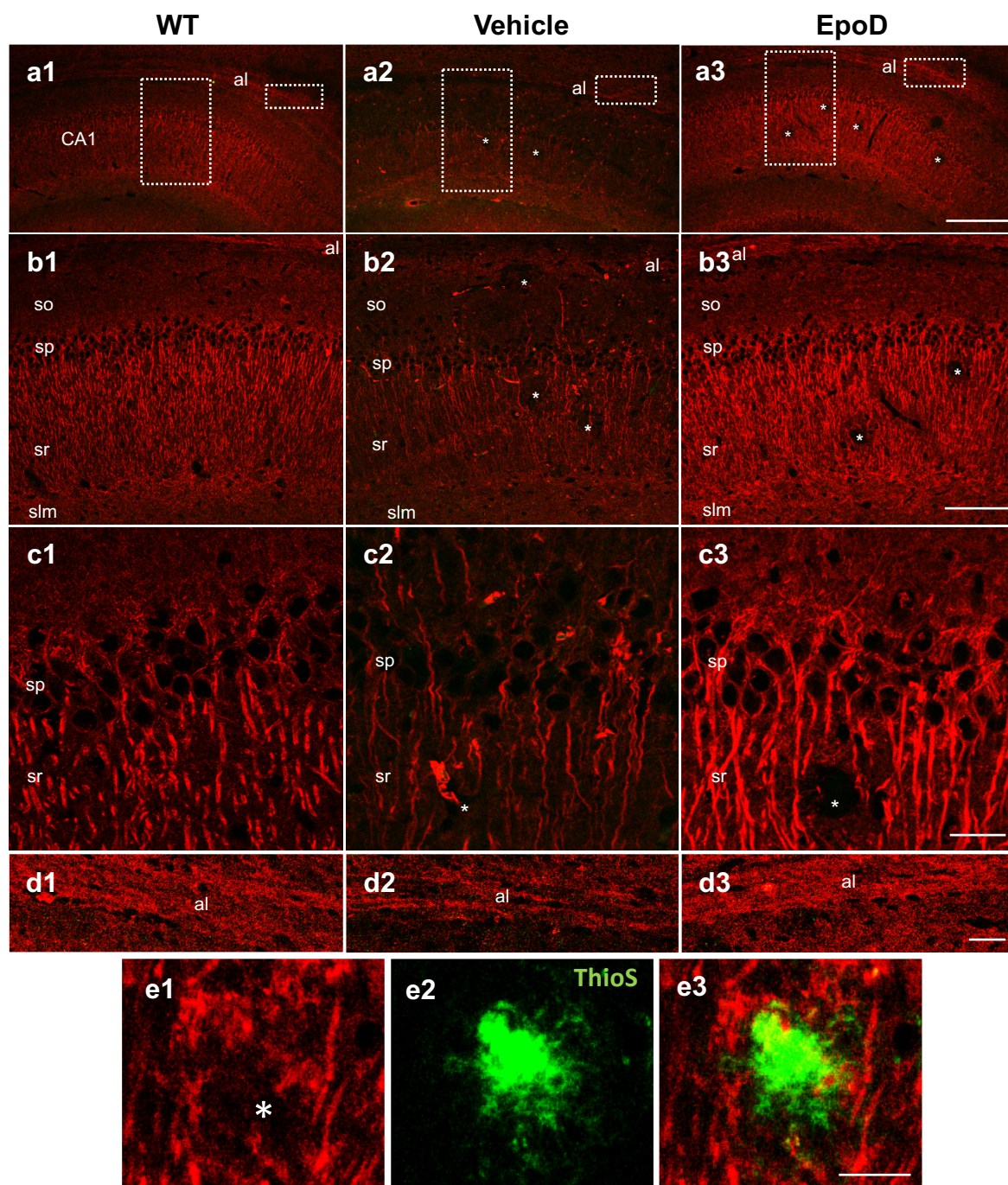
Openfield test



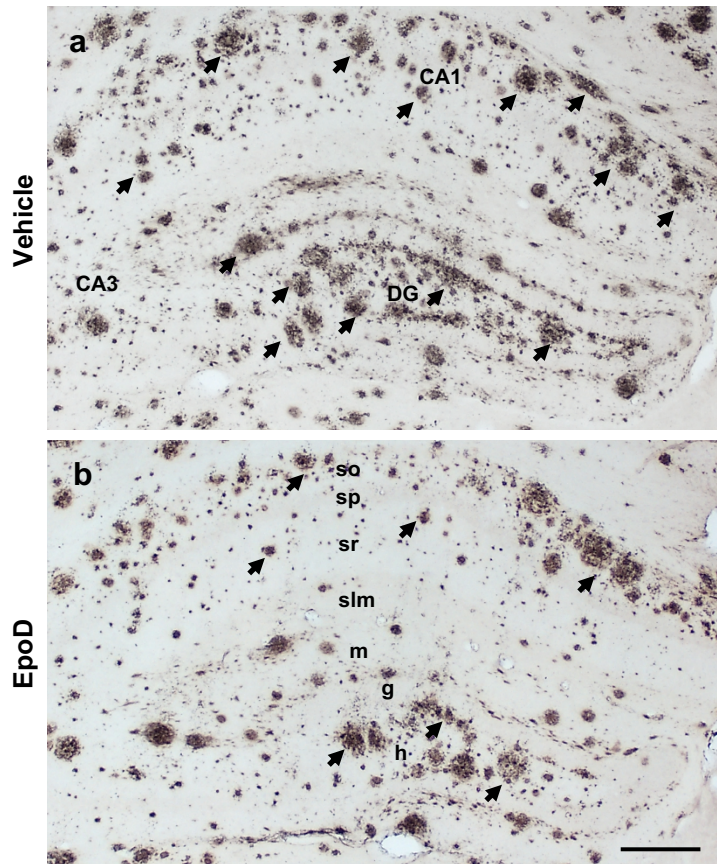
Y-maze specific context test



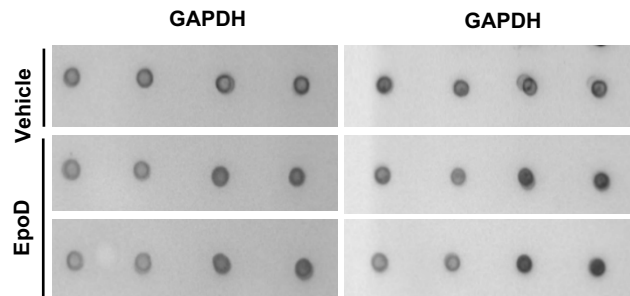
Supplementary Figure S1. EpoD treatment did not affect the locomotive or exploratory behavior of APP/PS1 mice. At openfield test, one-way ANOVA revealed no significant differences between groups in (a) the distance roamed ($F(2,22)=0.491$; $p=0.619$); (b) speed ($F(2,22)=0.576$; $p=0.571$) or (c) the number of rearings (Kruskal-Wallis test, $p = 0.419$). At Y-maze specific context test, one-way ANOVA showed no significant differences between groups in (d) distance roamed ($F(2, 21)=1.529$; $p=0.2398$) nor in (e) speed ($F(2, 21)=1.628$; $p=0.2202$). All data shown correspond to mean $\pm$ SEM.



Supplementary Figure S2. Epothilone D treatment promoted microtubular stability in APP/PS1 hippocampus. Confocal images of acetylated-tubulin (AcTub) immunofluorescence in the hippocampal CA1 region of WT (a1-d1), APP/PS1^{Veh} (a2-d2) and APP/PS1^{EpoD} (a3-d3) mice. Higher magnification images of the alveus are shown (d1-d3). AcTub immunofluorescence combined with thioflavin-S staining in APP/PS1 mice (e1-e3) showing that area labeled with asterisks (in a2-c2 and a3-c3) are occupied by amyloid plaques. Al, alveus; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum. ThioS, thioflavin-S. Scale bars: a, 250 μ m; b-c, 100 μ m; d, 25 μ m; e, 15 μ m.

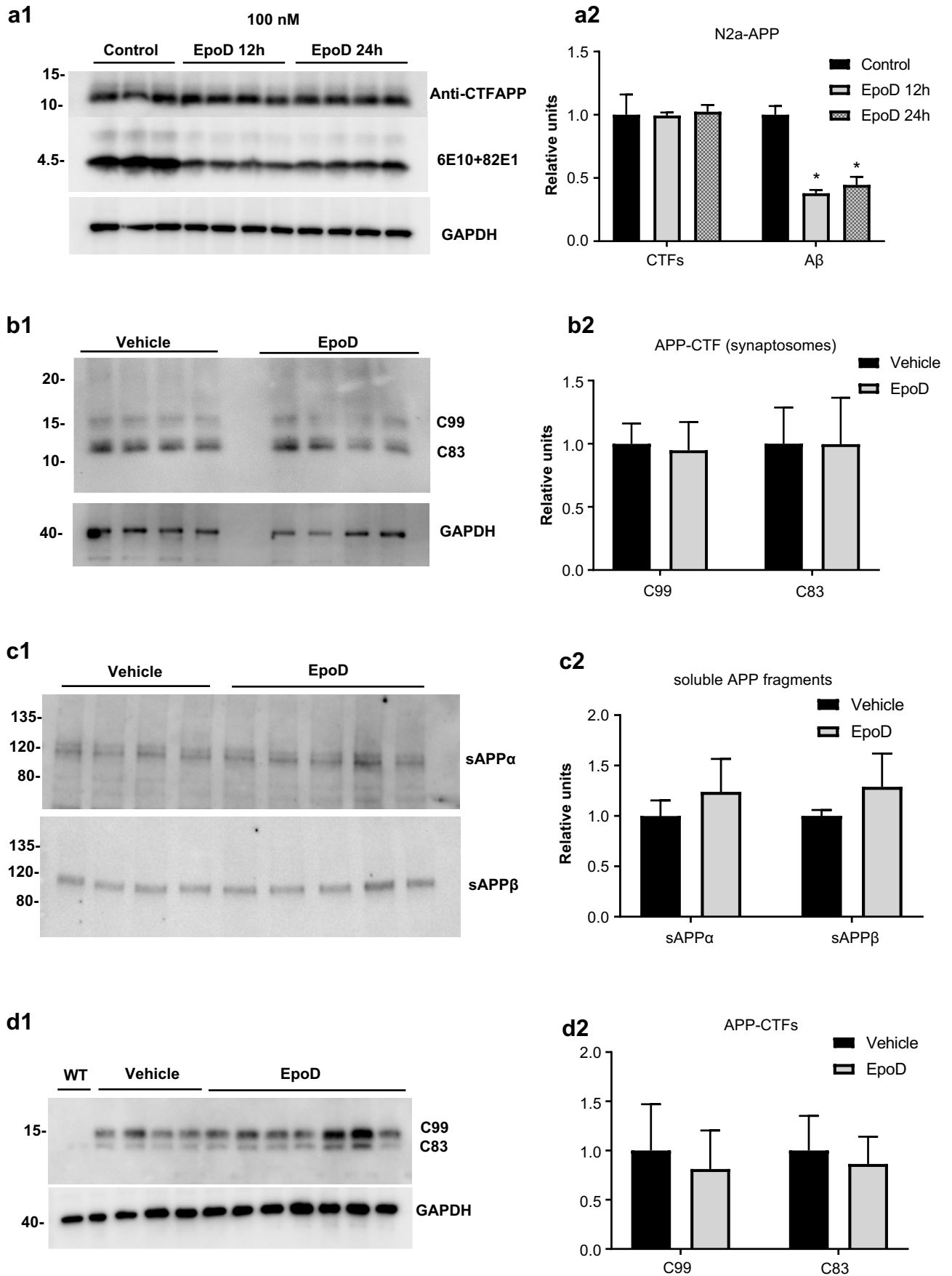


Supplementary Figure S3. Aβ42 immunostaining in the hippocampus of APP/PS1^{Veh} and APP/PS1^{EpoD}. Panoramic views of Aβ42 immunoreactivity in vehicle and EpoD-treated mice hippocampus. Extracellular Aβ42 accumulation was higher in the hippocampus proper and dentate gyrus from vehicle (a) than in the EpoD group (b). CA, Cornu ammonis; DG, dentate gyrus; So, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum; slm, stratum lacunosum-moleculare, m, stratum moleculare, g, granular layer; h, hilus. Scale bar: a-b, 250 μm.



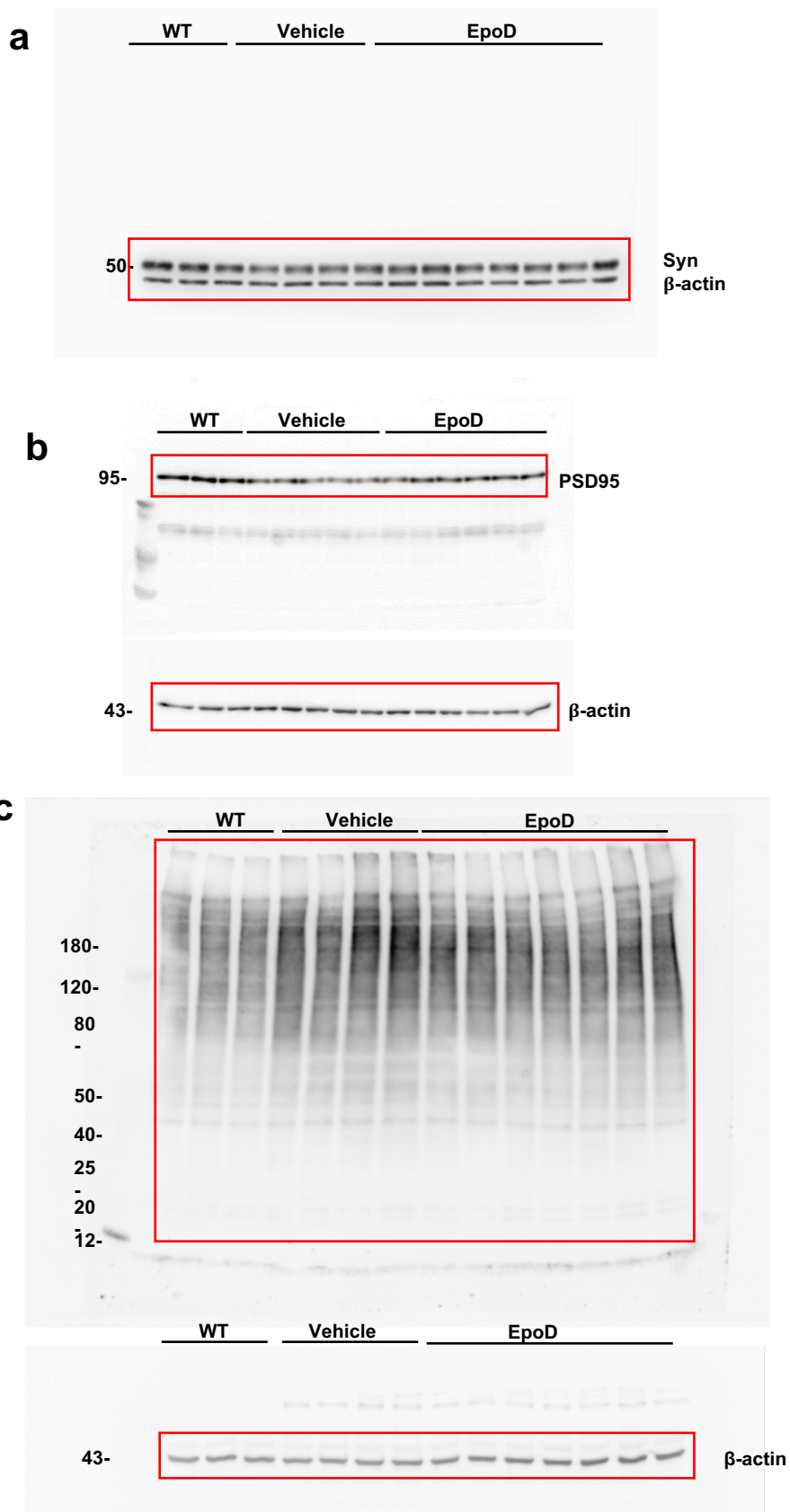
Supplementary Figure S4. Loading controls for S1 dot blot experiments. In parallel experiments to those showed in Fig 6c1, soluble S1 samples from Vehicle (n=4) or EpoD (n=8) treated animals were dotted and developed using anti-GAPDH antibody. These dot blots were quantified and used for OC and A11 normalization.

EpoD reduced Abeta production in N2a-APP cell line

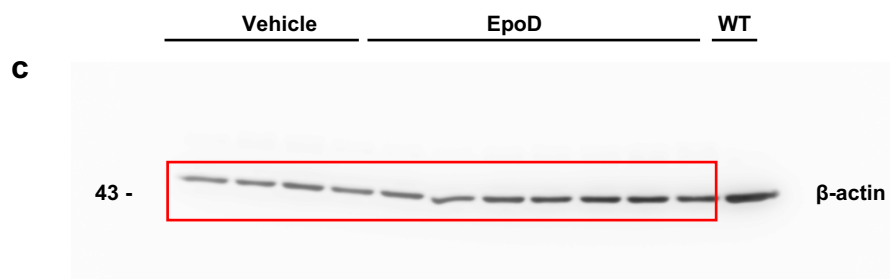
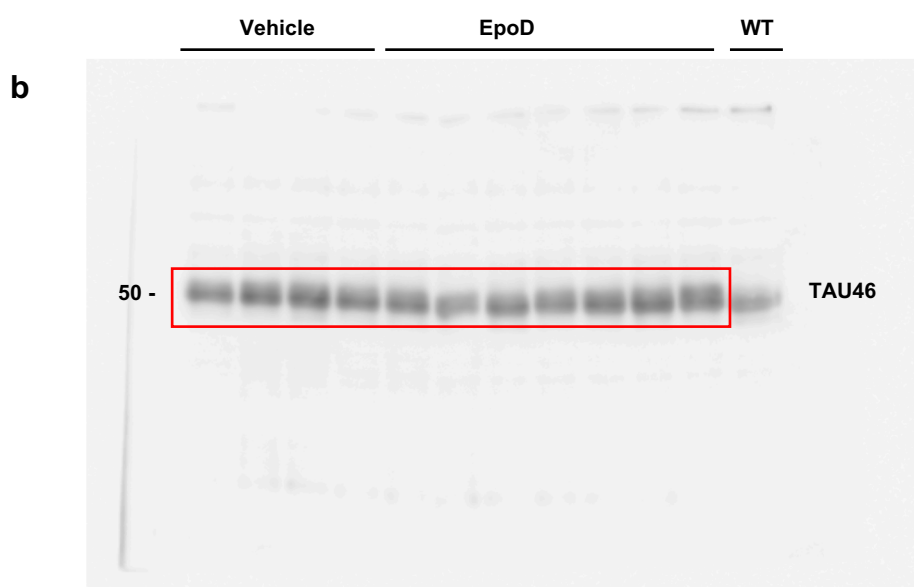
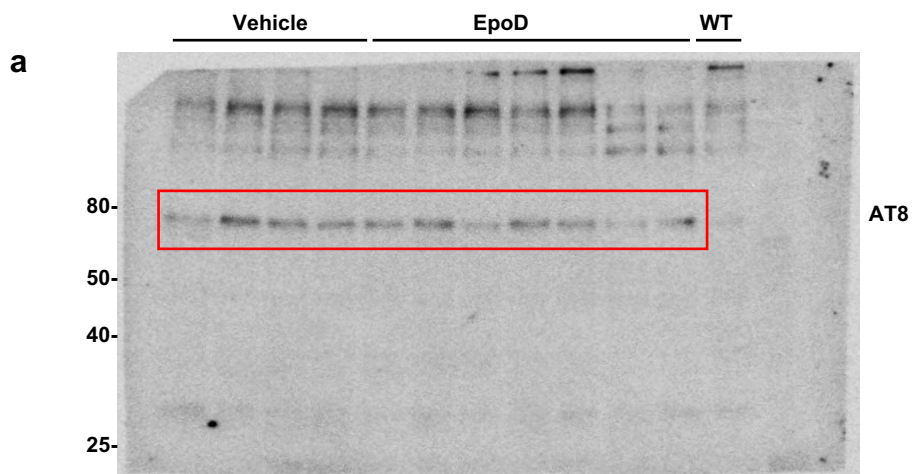


Supplementary Figure S5. EpoD effect on A β production and APP processing. (a1)

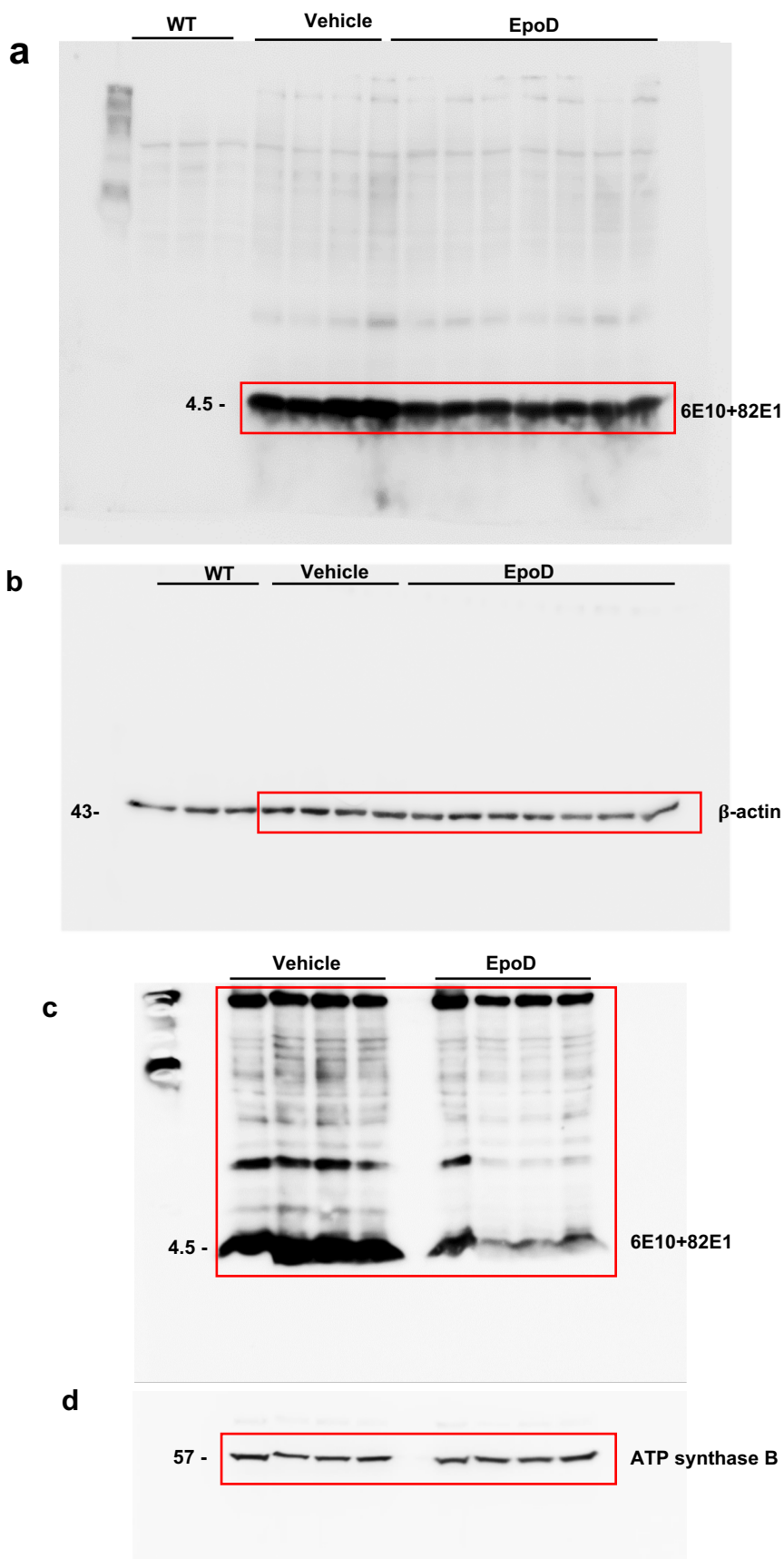
N2a-APP cell line was used to test the effect of EpoD treatment (100nM during 12h or 24h) on APP-derived CTF fragments (upper western) or intracellular A β production (middle western). Parallel cultures were treated with vehicle (controls, n=3) or 100nM EpoD for 12h (n=4) or 24h (n=4). After incubation, cells were washed and proteins extracted and used for western blot analysis. (a2) Right panel showed the quantitative analysis of these experiments. (b1) Synaptosomal APP-CTFs (C99 and C83 fragments) were analyzed (by anti-APP C-terminal antibody) using proteins isolated from synaptosomal fractions obtained from Vehicle (n=4) or EpoD treated APP/PS1 mice. (b2) Right panel showed the quantitative analysis of these experiments. APP-derived soluble (c1) or CTFs (d1) fragments from Vehicle (n=4) or EpoD (n=5 for sAPP or n=7 for C99/C83 CTFs) treated APP/PS1 mice were analyzed using anti-APP α or APP β specific antibodies or anti-APP-C-terminal antibody. (c2-d2) Quantitative analysis were showed in the right panels.



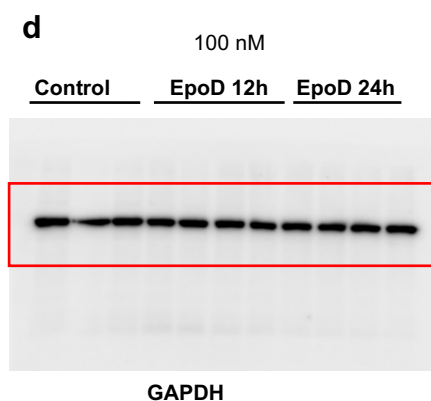
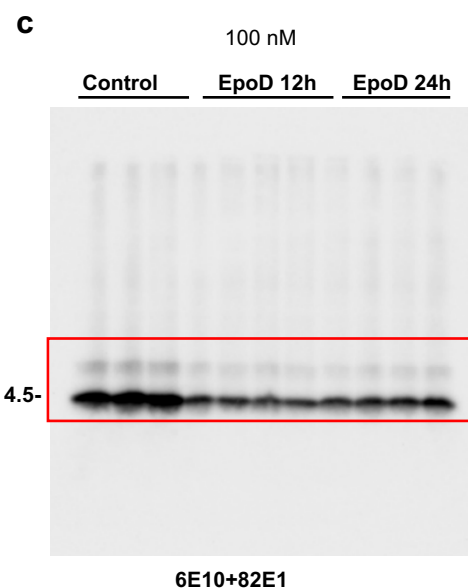
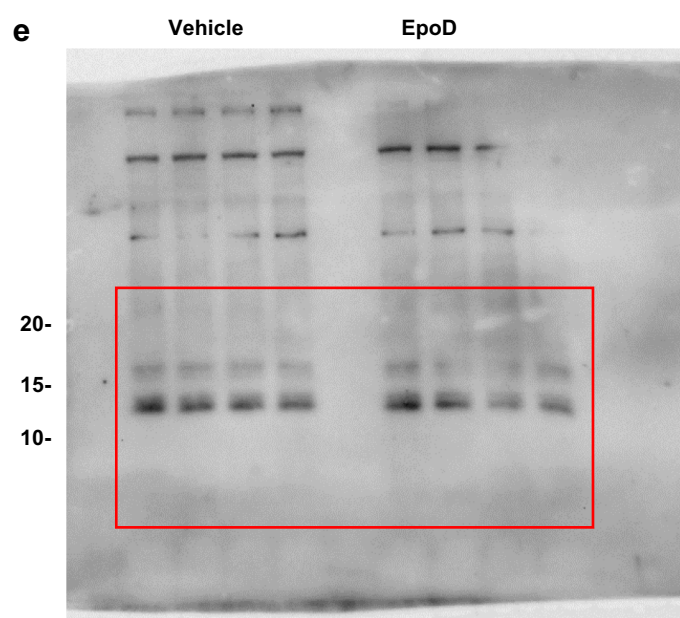
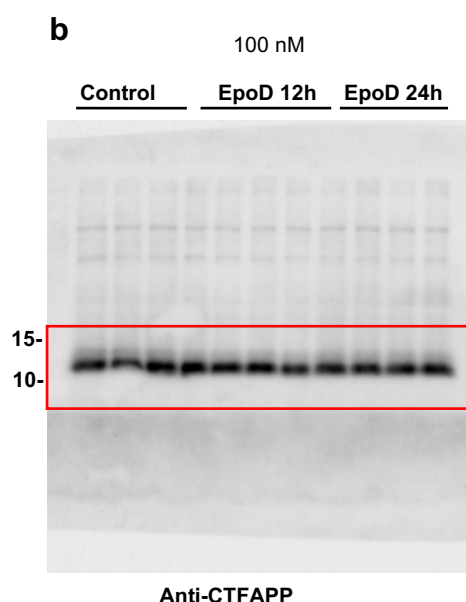
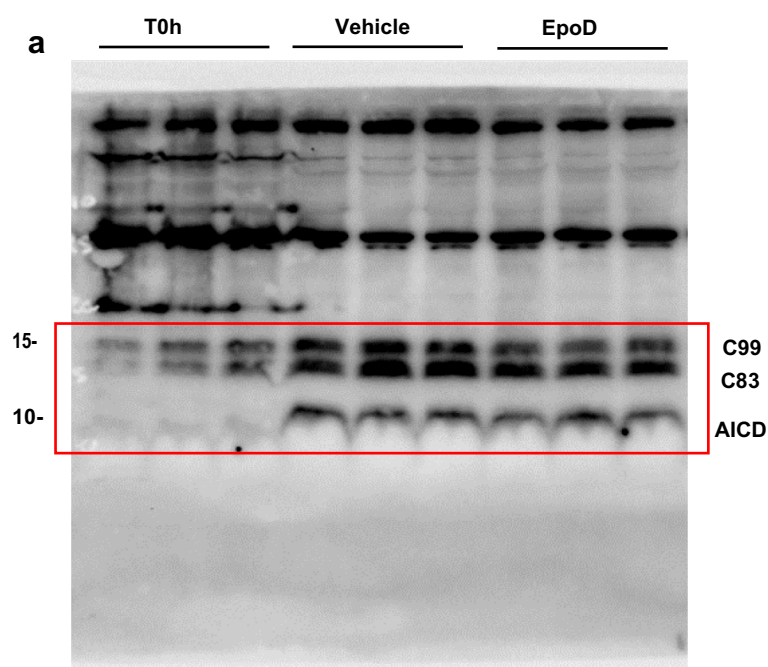
Supplementary Figure S6. Full length blots for Figure 2. (a) Synaptophysin and β -actin. (b) PSD95 and β -actin. (c) Ubiquitin and β -actin. Cropped areas in Fig. 2a, 2e and 2g are shown in red.



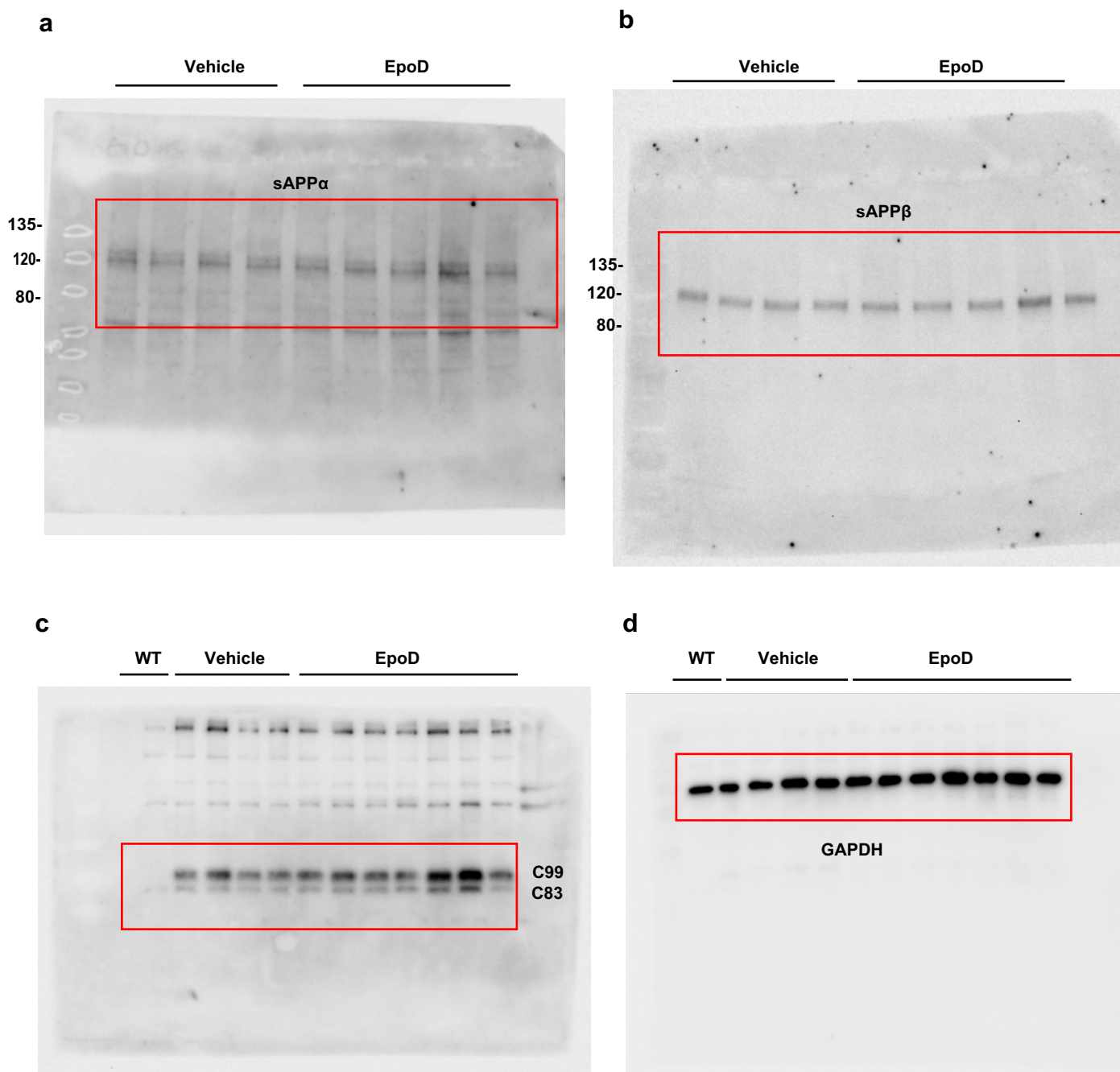
Supplementary Figure S7. Full length blots for Figure 4. (a) AT8 (phospho-tau), (b) TAU46 (tau total) and (c) β -actin. Cropped areas in Fig. 4a are shown in red.



Supplementary Figure S8. Full length blots for Figure 6 a1 and b1. (a) Hippocampal total monomeric A β (6E10 plus 82E1) and β -actin. (b) Synaptosomal monomeric A β (6E10 plus 82E1) and (c) ATP-synthase B. Cropped areas in Fig. 6a1 and 6b1 are show in red.



Supplementary Figure S9. Full length blots for Figure 6e1 and supplemental Fig. S5 a and b. a) Gamma-secretase activity. Cropped area in Fig. S6e1 is shown in red. b-d) Uncropped gels corresponding to Supplemental Fig. S5 a and b.



Supplementary Figure S10. Full length blots for supplemental Fig. 5 c1 and d1. Uncropped western blots corresponding to Fig. S5 c1 and d1. Cropped area are shown in red.