PNAS
www.pnas.org

Supplementary Information for

Autophagy deficiency modulates microglial lipid homeostasis and aggravates tau pathology and spreading

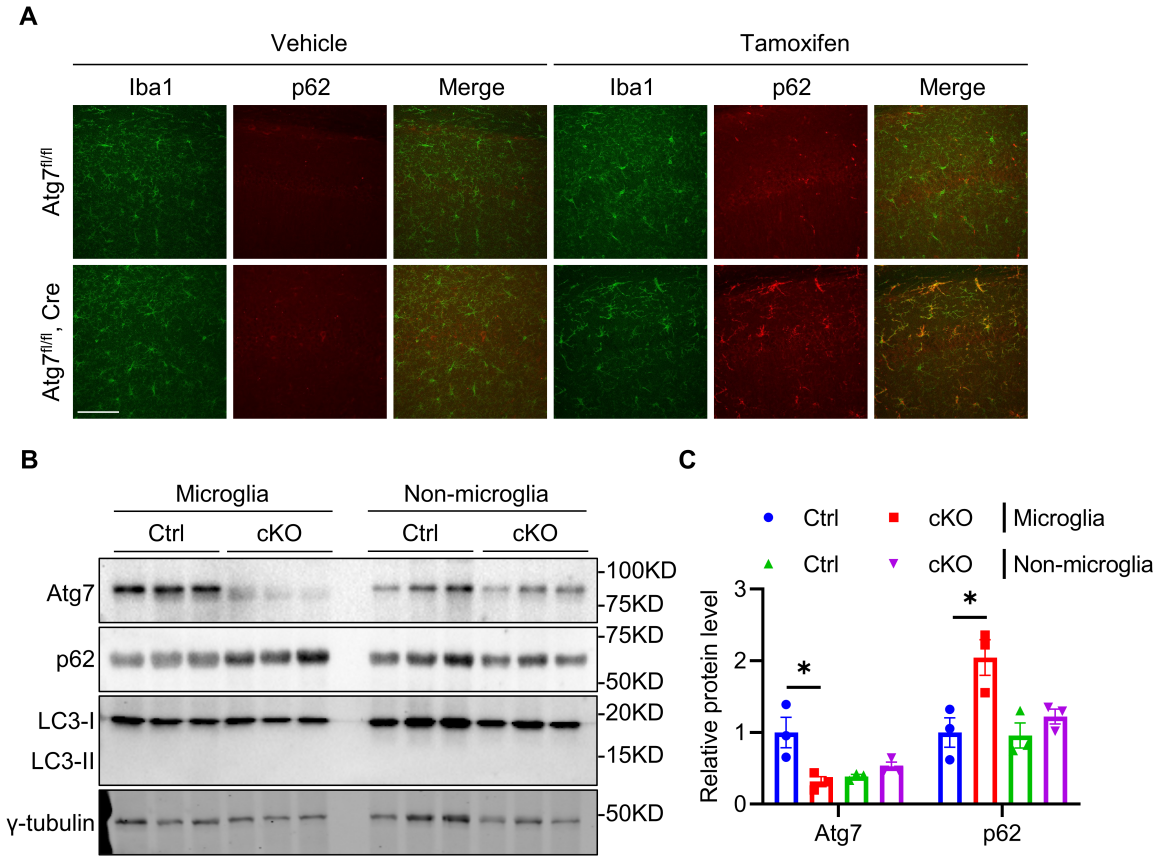
Yin Xu, Nicholas E. Propson, Shuqi Du, Wen Xiong, Hui Zheng

Corresponding authors: Yin Xu, Hui Zheng
Email: Yin.Xu@bcm.edu, huiz@bcm.edu

This PDF file includes:

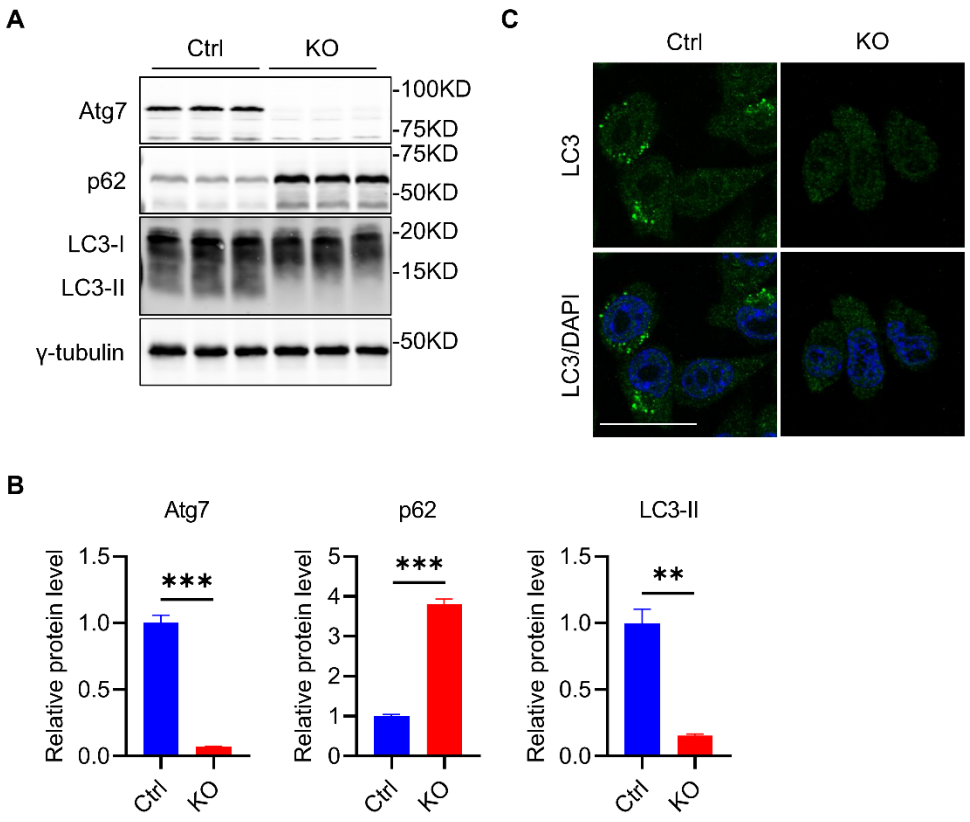
Figures S1 to S8

Fig. S1.



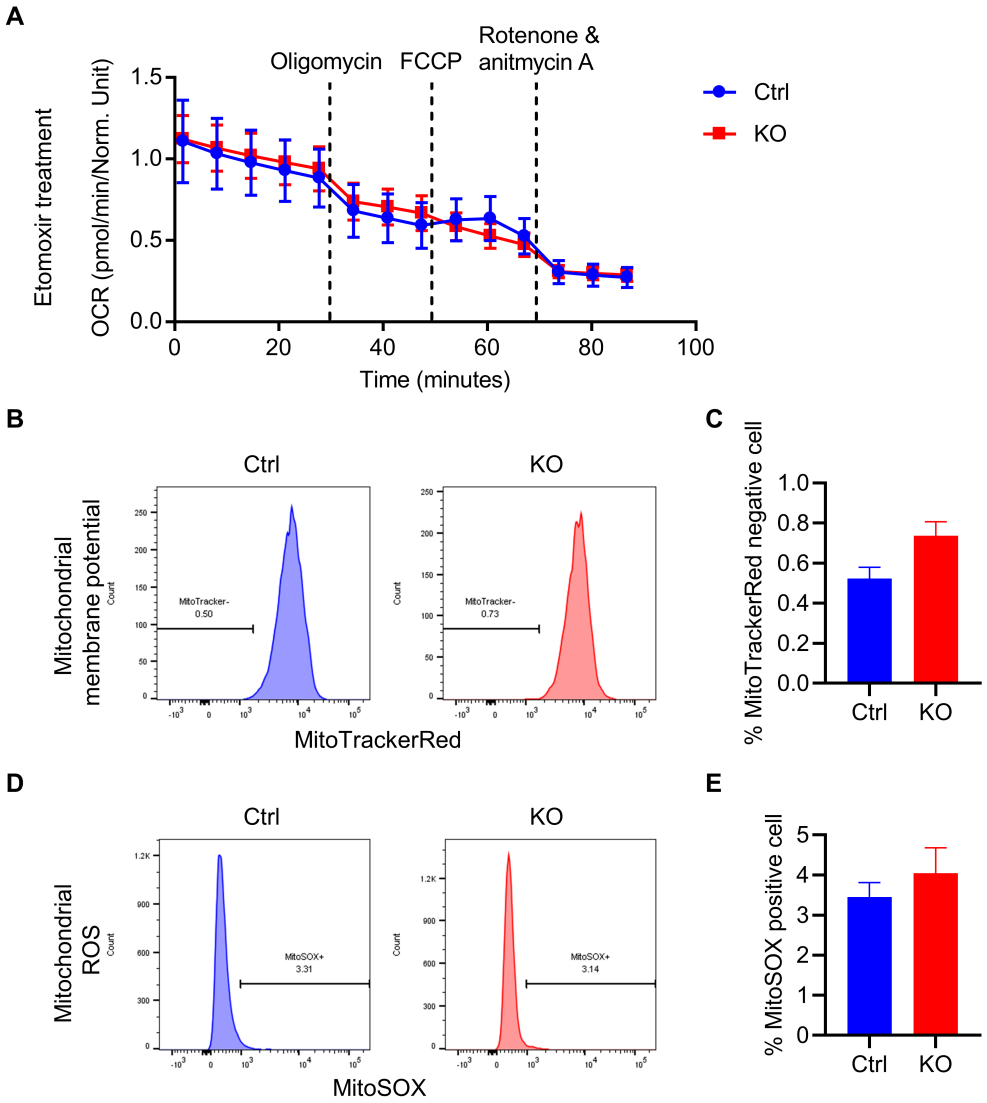
(A) Representative immunofluorescence images of Iba1 and autophagic flux marker (p62) staining in the hippocampus of *Atg7*$^{fl/fl}$ and *Atg7*$^{fl/fl}$, Cre mice with or without tamoxifen injection at 5 months of age. Scale bar: 100 μm. (n=5 for tamoxifen groups; n=3 for Ctrl groups). **(B)** Western blot image of Atg7, p62 and LC3 protein levels in the sorted microglial and non-microglial cell population from the brains of 10-week-old control and *Atg7* cKO mice 3 weeks post-tamoxifen injection. **(C)** Quantification of Atg7 and p62 levels in (B). Two tailed Student's t-test. (n=3/group). Data are presented as mean ± SEM. *P ≤ 0.05.

Fig. S2.



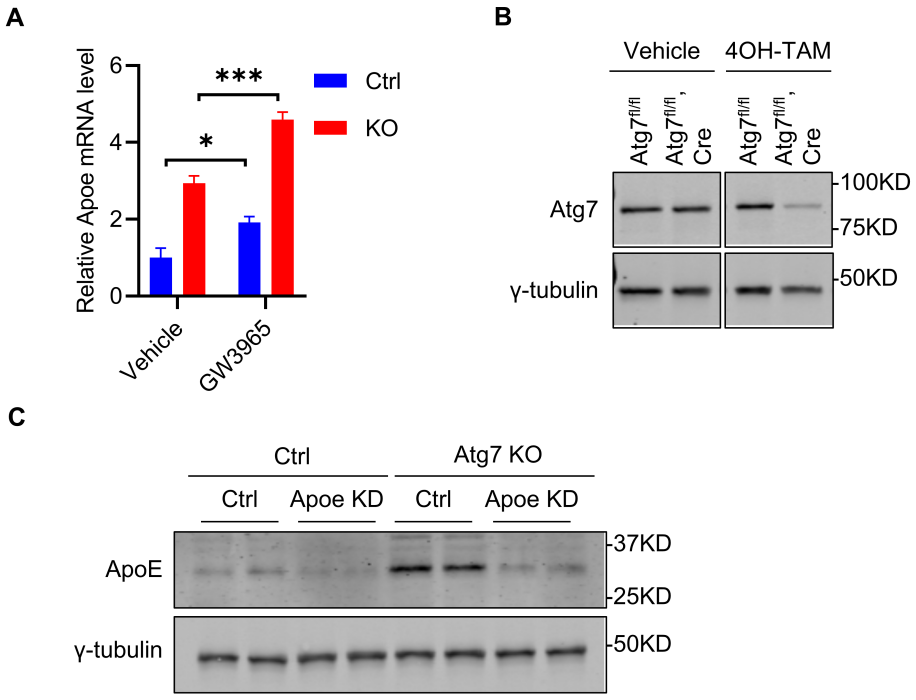
(A) Western blot image of Atg7, p62, and autophagosome marker LC3 protein levels in control and *Atg7* KO BV2 cells. **(B)** Quantification of Atg7, p62, and LC3-II levels in (A). Two tailed Student's t-test. (n=3 of 2 experiments). **(C)** Representative immunofluorescence images of autophagosomes staining in control and *Atg7* KO BV2 cells treated with 10 μg/ml E-64d and 10 μg/ml pepstatin A for 6 h. Scale bar: 20 μm. Data are presented as mean ± SEM. **P ≤ 0.01; ***P ≤ 0.001.

Fig. S3.



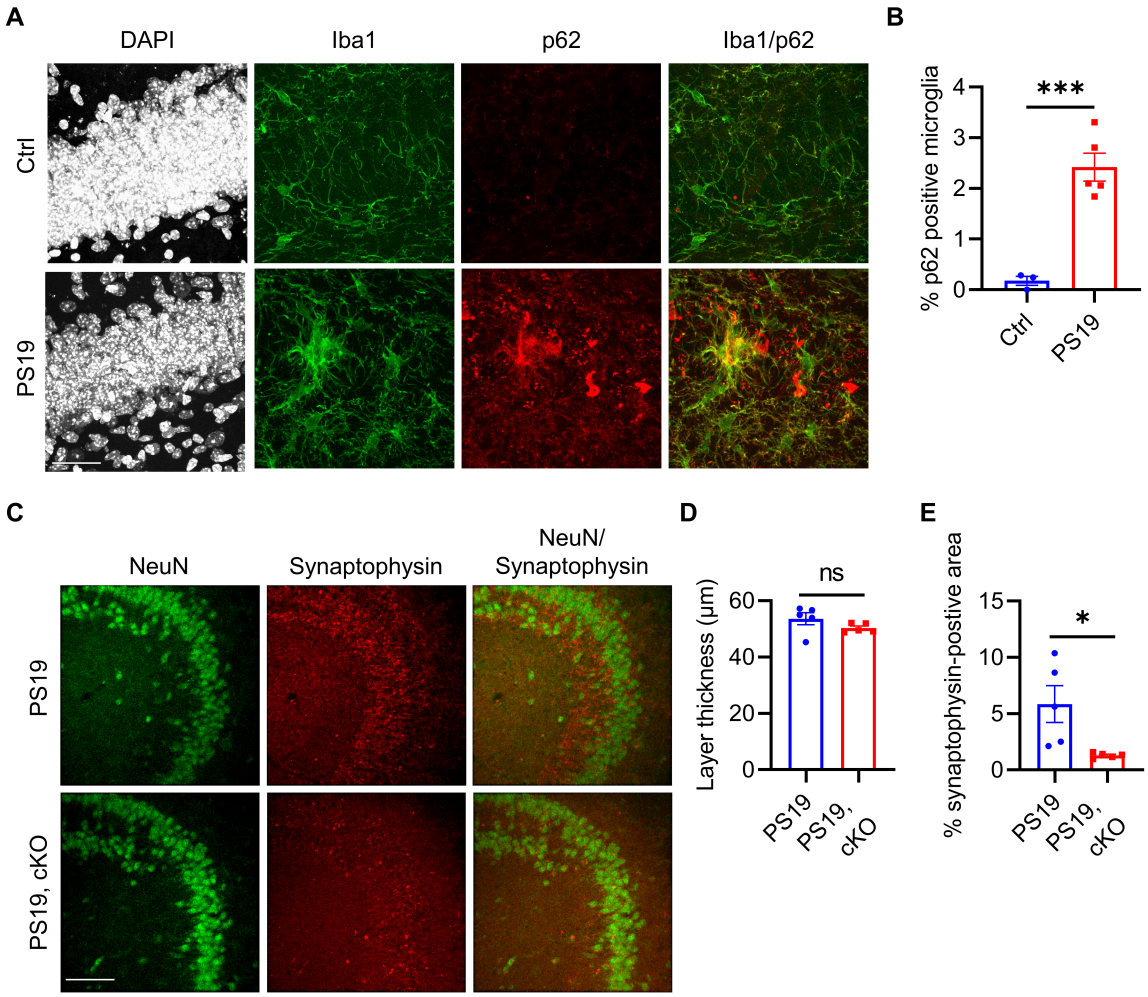
(A) FAO Seahorse assay measurement of OCR levels in control and *Atg7* KO BV2 cells with Etomoxir. **(B)** Flow cytometry analysis of MitoTrackerRed-stained control and *Atg7* KO BV2 cells. **(C)** Quantification of MitoTrackerRed negative cells (n=3 of 2 experiments). **(D)** Flow cytometry analysis of mitochondrial ROS (MitoSox) staining in control and *Atg7* KO BV2 cells. **(E)** Quantification of MitoSox positive cells. (n=3 of 2 experiments). Data are presented as mean ± SEM.

Fig. S4.



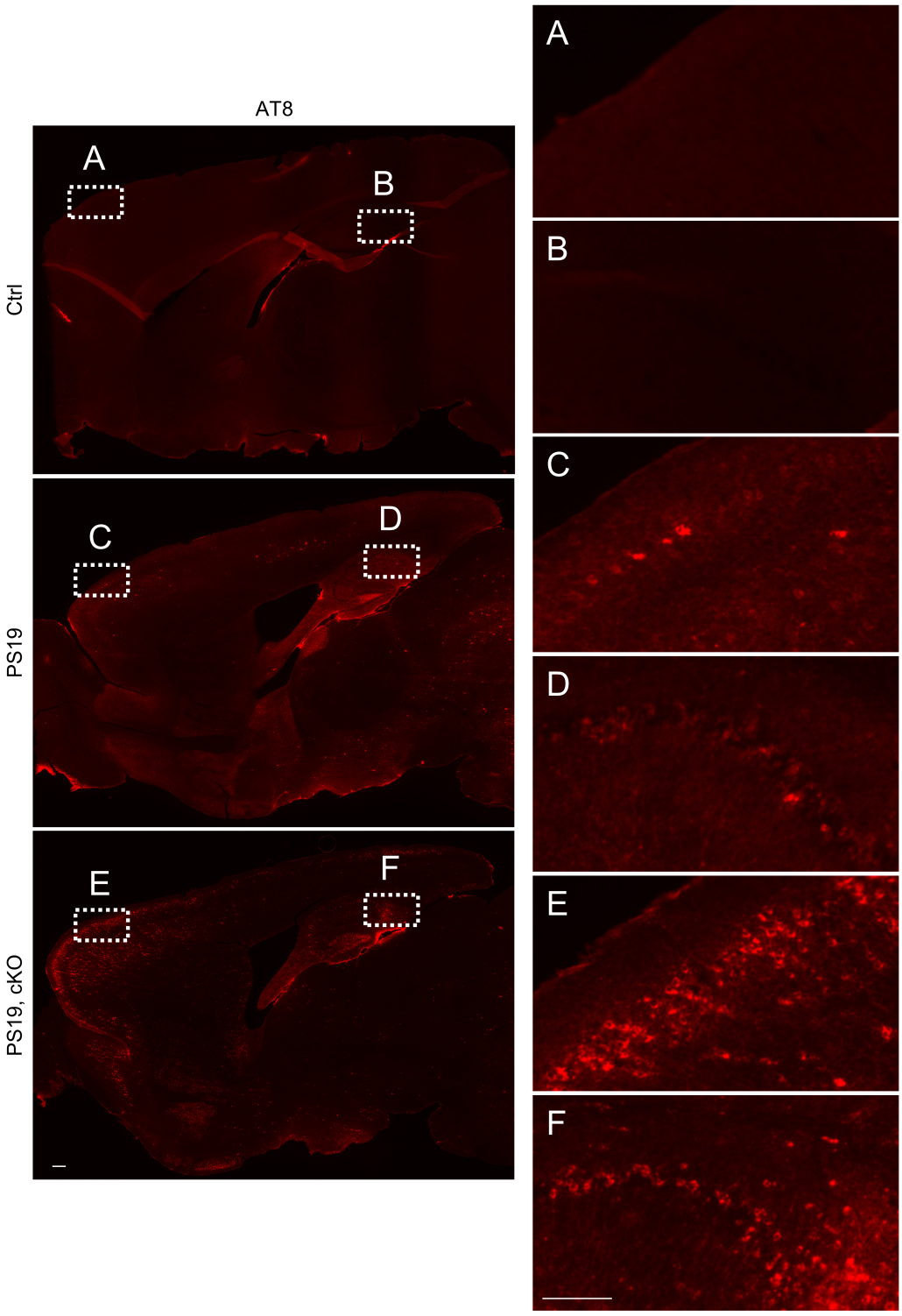
(A) qPCR measurement of mRNA levels of *Apoe* in control and *Atg7* KO BV2 cells treated with vehicle or 10 μm GW3965 for 16 h. Two ways ANOVA followed by Sidak's multiple comparisons test. (n=4 of 2 experiments). **(B)** Western blot image of Atg7 protein level in primary cultured microglia from *Atg7*$^{fl/fl}$ and *Atg7*$^{fl/fl}$, Cre pups treated with vehicle or 4-hydroxy Tamoxifen (100 nM). **(C)** Western blot image of ApoE protein level in control and *Atg7* KO BV2 cells with or without *Apoe* knockdown. Data are presented as mean ± SEM. *P ≤ 0.05; ***P ≤ 0.001.

Fig. S5.



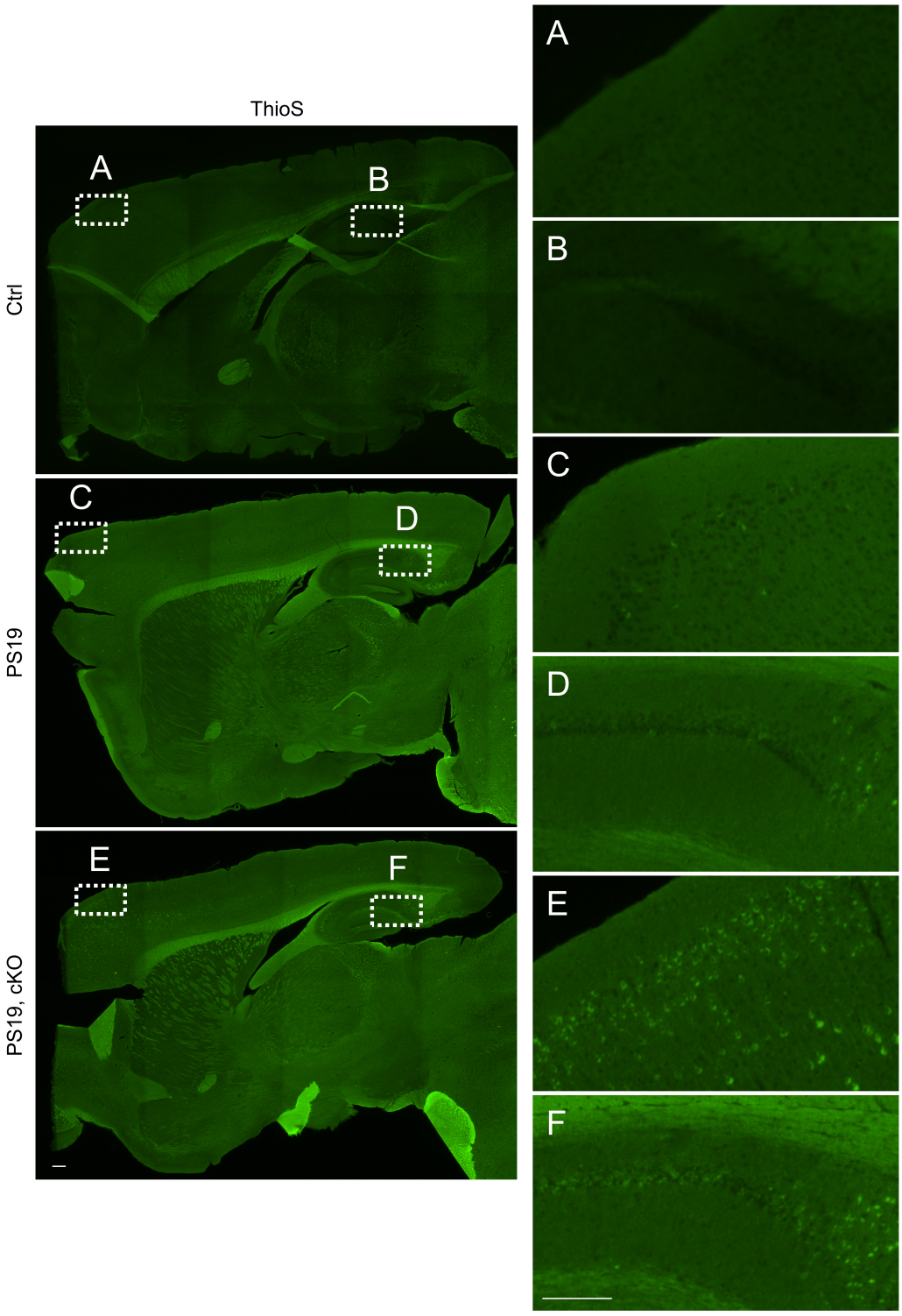
(A) Representative immunofluorescence images of Iba1 and autophagic flux marker (p62) staining in the hippocampus of control and PS19 mice at 12 months of age. Scale bar: 30 μm. **(B)** Quantitative analysis of percentage of p62-positive microglia in (A). Two tailed Student's t-test. (n=4 for control; n=5 for PS19). **(C)** Representative immunofluorescence images of neuronal marker (NeuN) and presynaptic marker (synaptophysin) staining in the hippocampus CA3 of PS19 and PS19, cKO mice at 12 months of age. Scale bar: 100 μm. **(D)** Quantitative analysis of thickness of the CA3 pyramidal layer in (C). Two tailed Student's t-test. (n=5/group). **(E)** Quantitative analysis of synaptophysin-positive areas in (C). Two tailed Student's t-test. (n=5/group). Data are presented as mean ± SEM. ns, not significant; ***P* ≤ 0.05; ****P* ≤ 0.001.

Fig. S6.



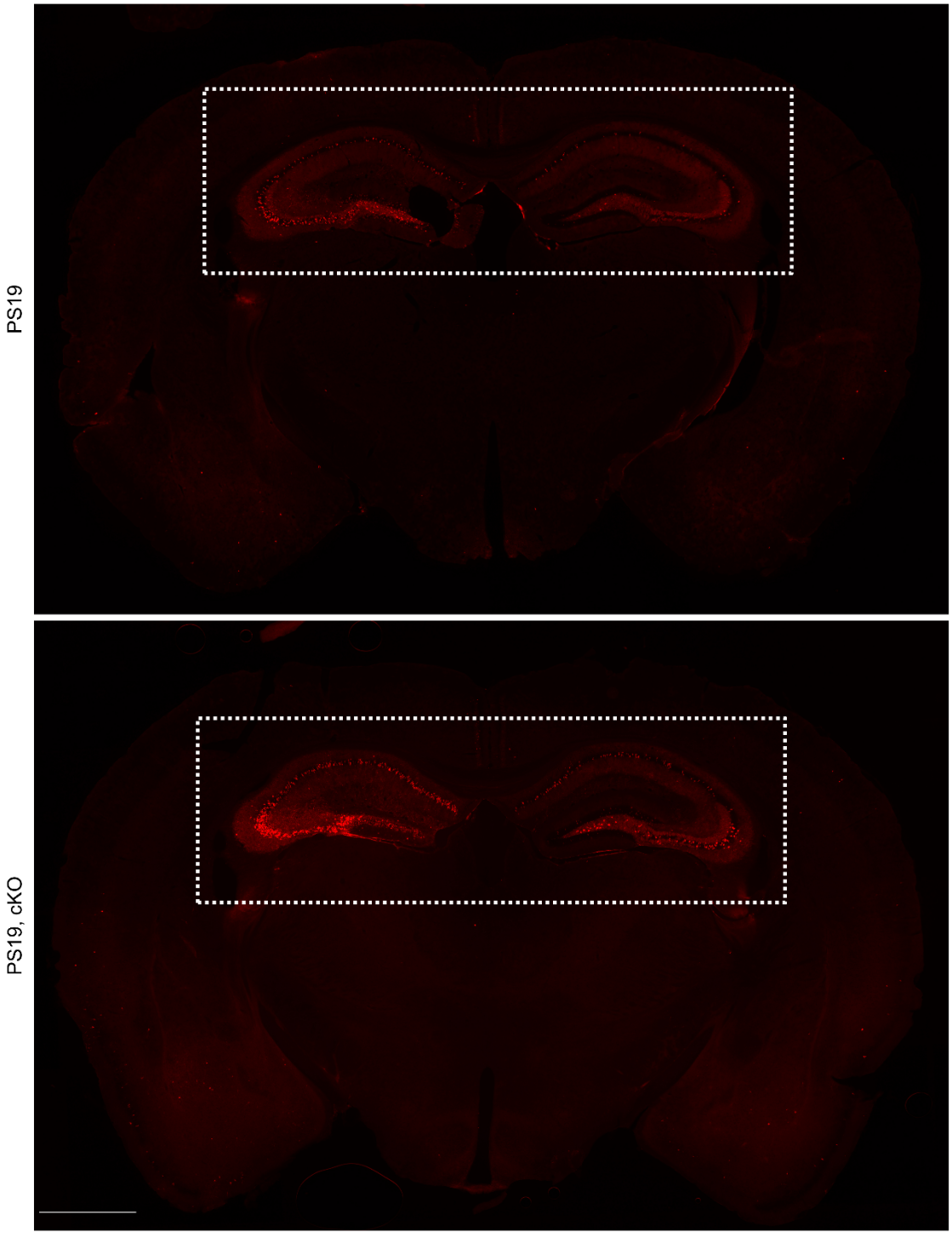
Representative immunofluorescence images of the cortex **(A, C, E)** and hippocampus **(B, D, F)** of control, PS19 and PS19, Atg7 cKO mice at 12 months of age using the AT8 antibody. Right panels are higher magnification images of each of the bracketed areas in left. Scale bar: 200 μm.

Fig. S7.



Representative Thioflavin S (ThioS) staining images of the cortex **(A, C, E)** and hippocampus (**B, D, F)** of control, PS19 and PS19, Atg7 cKO mice at 12 months of age. Right panels are higher magnification images of each of the bracketed areas in left. Scale bar: 200 μm.

Fig. S8.



Whole brain scans of MC1 immunostaining with the white rectangles indicate areas shown in Figure 7. Scale bar: 1000 μm.