

Figure S1

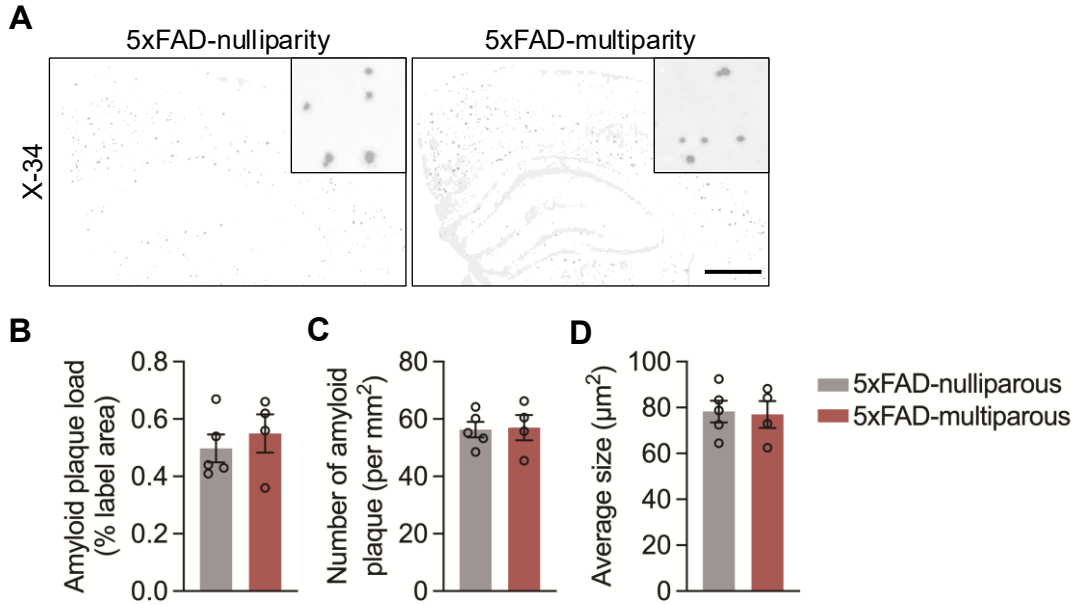


Figure S1. Absence of an effect of multiparity on amyloid pathology in male 5xFAD mice

(A) Images showing no changes in amyloid plaque burden in the hippocampus and cortex of male 5xFAD mice from the control and multiparity-related groups at 6.4 months. Scale bar, 500 μm. (B-D) No changes of amyloid plaque burden (B), $t_{(7)} = 0.6476$, $P = 0.5370$, Cohen's $d = 0.43$, two-tailed unpaired t -test, plaque density (C), $t_{(7)} = 0.1427$, $P = 0.8905$, Cohen's $d = 0.10$, two-tailed unpaired t -test, and average plaque size (D), $t_{(7)} = 0.1773$, $P = 0.8643$, Cohen's $d = 0.12$, two-tailed unpaired t -test, in 5xFAD male mice. $N=4$ mice for multiparity, $N=5$ mice for nulliparity. Data are presented as mean $\pm$ SEM.

Figure S2

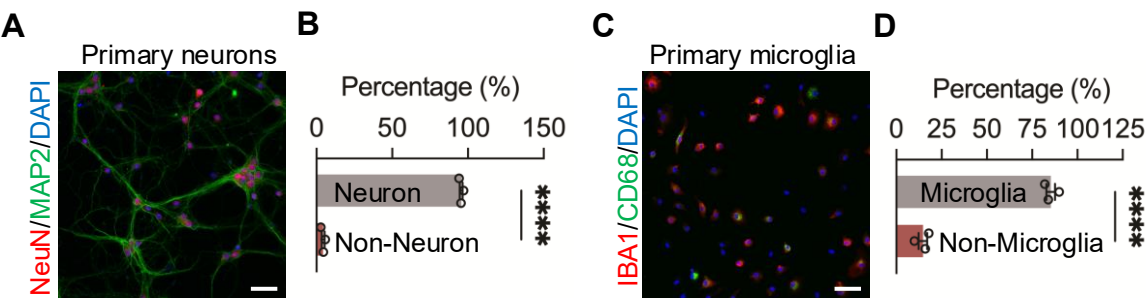


Figure S2. Purity assessment of isolated primary neurons and microglia.

(A) Confocal images of the purified primary neuron culture, immunostained for the neuronal markers NeuN and MAP2. Scale bar: 50 μ m. **(B)** Quantification of NeuN⁺ and MAP2⁺ cells confirms high neuronal purity. N=3 mice; $t_{(4)} = 71.70$, $P < 0.0001$, Cohen's $d = 58.56$, two-tailed unpaired t -test. **(C)** Confocal images of the purified primary microglia culture, immunostaining for the microglial marker IBA1 and CD68. Scale bar: 50 μ m. **(D)** Quantification of IBA1⁺ and CD68⁺ cells confirms high microglial purity. N=3 mice; $t_{(4)} = 20.79$, $P < 0.0001$, Cohen's $d = 16.97$, two-tailed unpaired t -test. Data are presented as mean $\pm$ SEM. **** $P < 0.0001$.

Figure S3

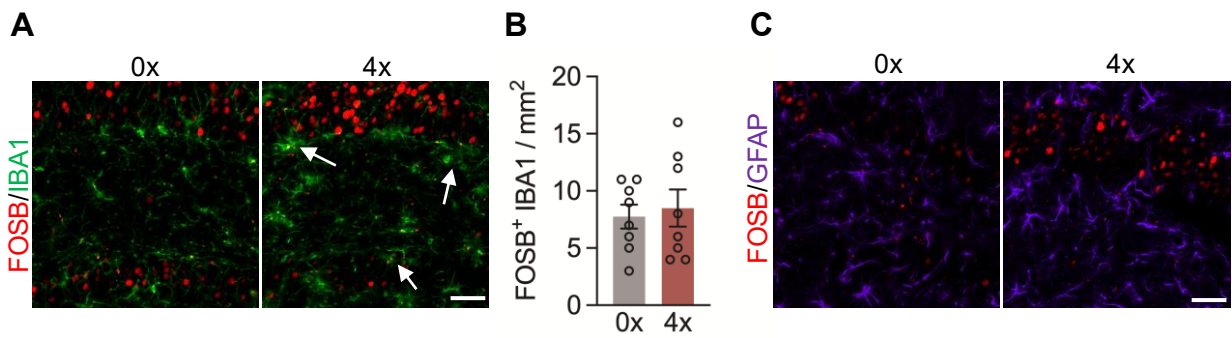


Figure S3. Expression assessment of FOSB in microglia and astrocytes

(A) Confocal images showing limited colocalization between FOSB and IBA1 in the brains of nulliparous and multiparous female 5xFAD mice. Scale bar: 50 μ m. **(B)** Quantification of FOSB⁺IBA1⁺ microglia density, $t_{(14)} = 0.3878$, $P = 0.7040$, Cohen's $d = 0.20$, $N = 8$ mice per group. **(C)** Confocal images showing no colocalization between FOSB and GFAP in the brains of nulliparous and multiparous female 5xFAD mice. Scale bar: 50 μ m. Data are presented as mean $\pm$ SEM.

Figure S4

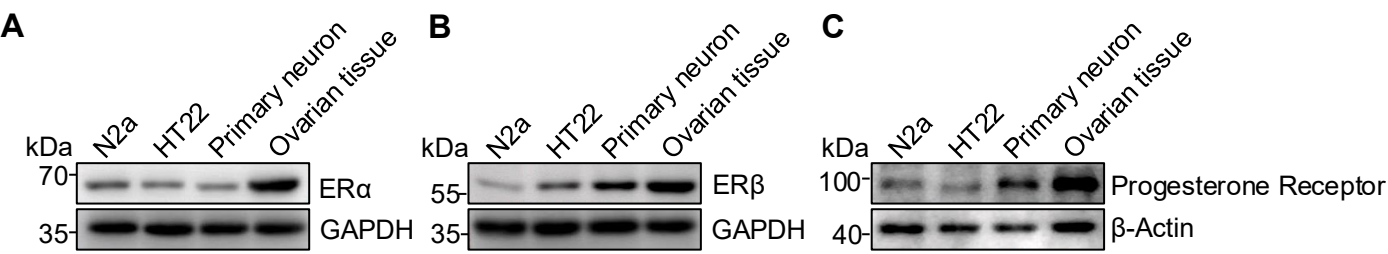


Figure S4. Characterization of hormone receptor expression in neuronal cell models

(A) Western blots showing ERα expression levels in N2a cells, HT22 cells, primary neurons, and ovarian tissue. (B) Western blots showing ERβ expression levels in N2a cells, HT22 cells, primary neurons, and ovarian tissue. (C) Western blots showing progesterone receptor expression levels in N2a cells, HT22 cells, primary neurons, and ovarian tissue.

Figure S5

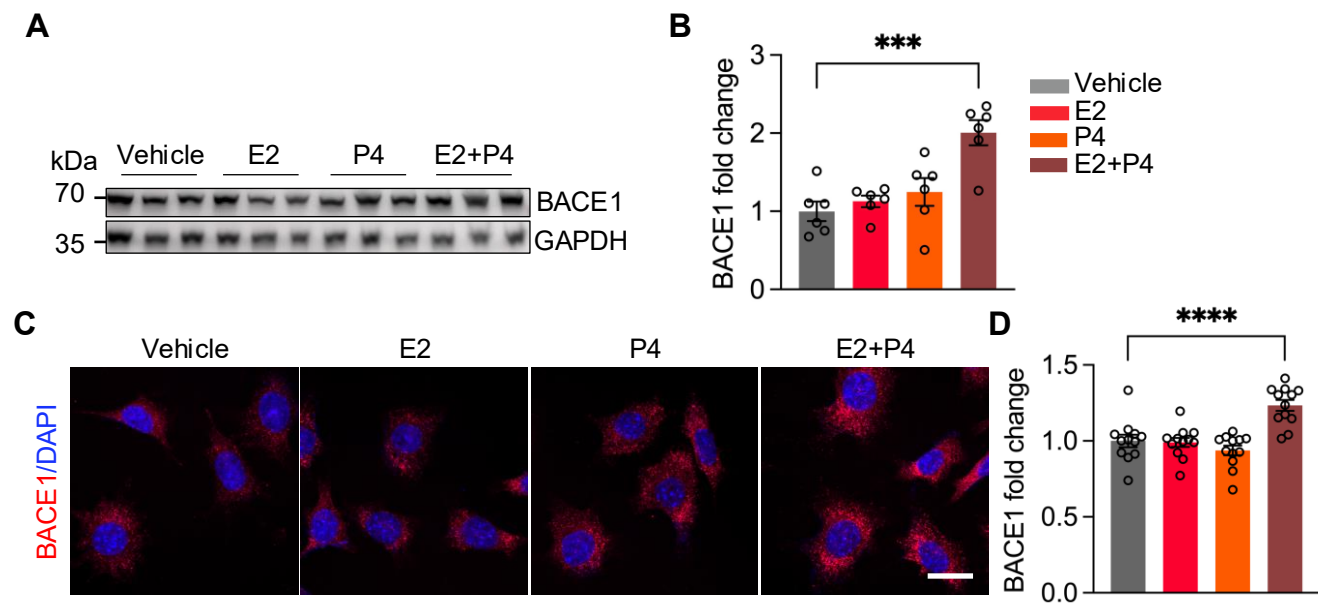


Figure S5. E2+P4 treatment increased BACE1 expression

(A-B) Western blots showing increased BACE1 protein levels in the HT22 cell line treated with 200nM E2 and 200 nM P4. N = 3 per group, from 3 independent experiments. $F_{(3,8)} = 13.96$, $\eta^2 = 0.84$, Vehicle vs E2, $P = 0.9836$; Vehicle vs P4, $P = 0.1758$; Vehicle vs E2+P4, $P = 0.0016$, One-way ANOVA. (C) Confocal images showing increased BACE1 fluorescence intensity in 200 nM E2 combined with 200 nM P4-treated HT22 cells. Scale bar, 20 μ m. (D) Quantification showing BACE1 fluorescence intensity increased in HT22 cells treated with a combination of E2 and P4. N = 6 per group, from 4 independent experiments. $F(3,20) = 10.40$, $\eta^2 = 0.609$, Vehicle vs E2, $P = 0.8541$; Vehicle vs P4, $P = 0.4746$; Vehicle vs E2+P4, $P < 0.0002$, One-way ANOVA. Data are presented as mean $\pm$ SEM. *** $P < 0.001$, **** $P < 0.0001$.

Figure S6

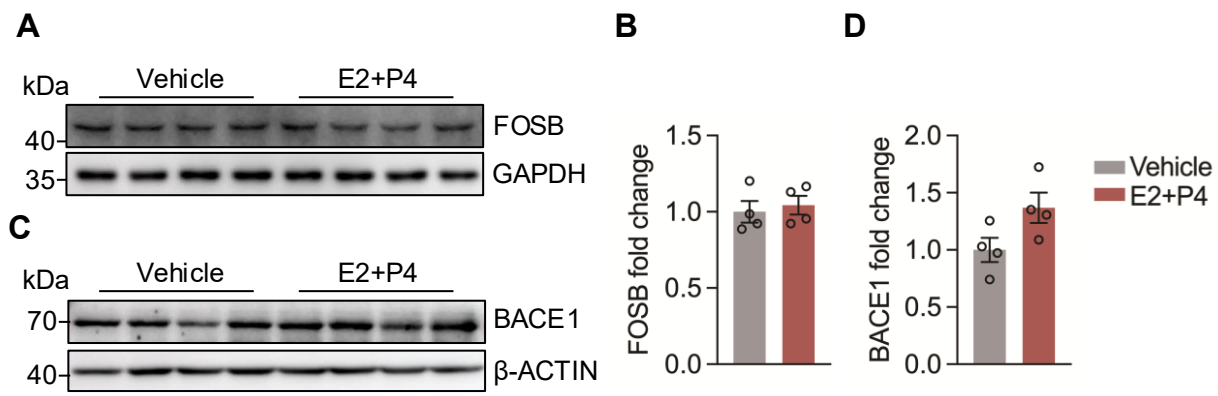


Figure S6. Hormone treatment did not change FOSB and BACE1 expression in microglia

(A-B) Western blots showing no changes of FOSB protein levels in primary microglia treated with vehicle or a combination of E2 and P4, $t_{(6)} = 0.4651$, $P = 0.6582$, Cohen's $d = 0.33$, $N = 4$ per group, from 4 independent experiments. **(C-D)** Western blots showing no change in BACE1 protein levels in primary microglia treated with vehicle or a combination of E2 and P4, $t_{(6)} = 2.175$, $P = 0.0726$, Cohen's $d = 1.51$ ($N = 4$ per group, from 4 independent experiments). Data are presented as mean $\pm$ SEM.

Figure S7

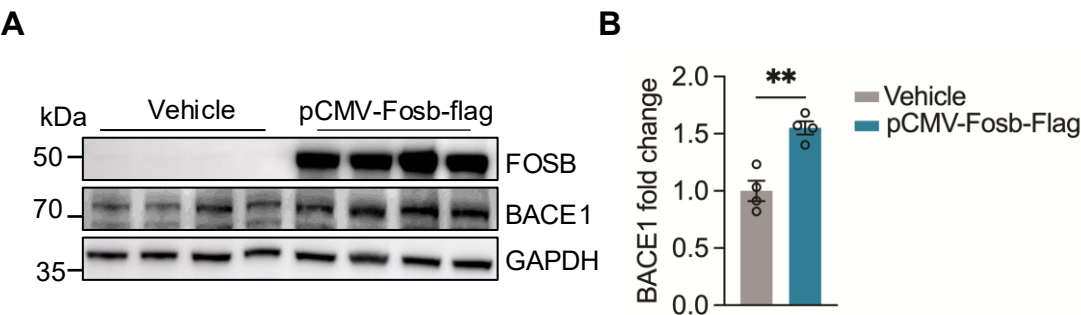


Figure S7. FOSB overexpression upregulated BACE1 expression

(A) Western blot analysis of N2a cells transfected with pCMV-Fosb-flag-plasmid. Validation of successful FOSB overexpression. (B) Consequent upregulation of BACE1 protein levels. N = 4 per group from 3 independent experiments. $t_{(6)} = 5.214$, $P = 0.0020$, Cohen's $d = 3.69$, two-tailed unpaired t test. Data are presented as mean $\pm$ SEM. ** $P < 0.01$.

Figure S8

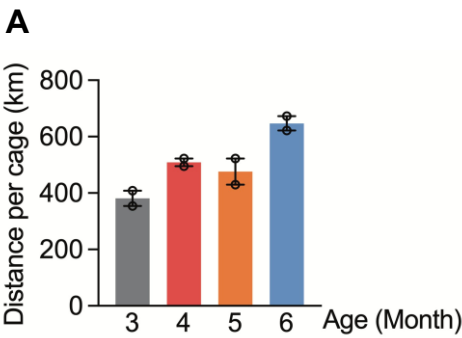


Figure S8. The monthly running distance per cage

(A) The running distance per cage at different month. Data are presented as mean $\pm$ SEM.