

Supplementary Information for

Thy1-ApoE4/C/EBP β Double Transgenic Mice Act as a Sporadic Model with Alzheimer's Disease

By

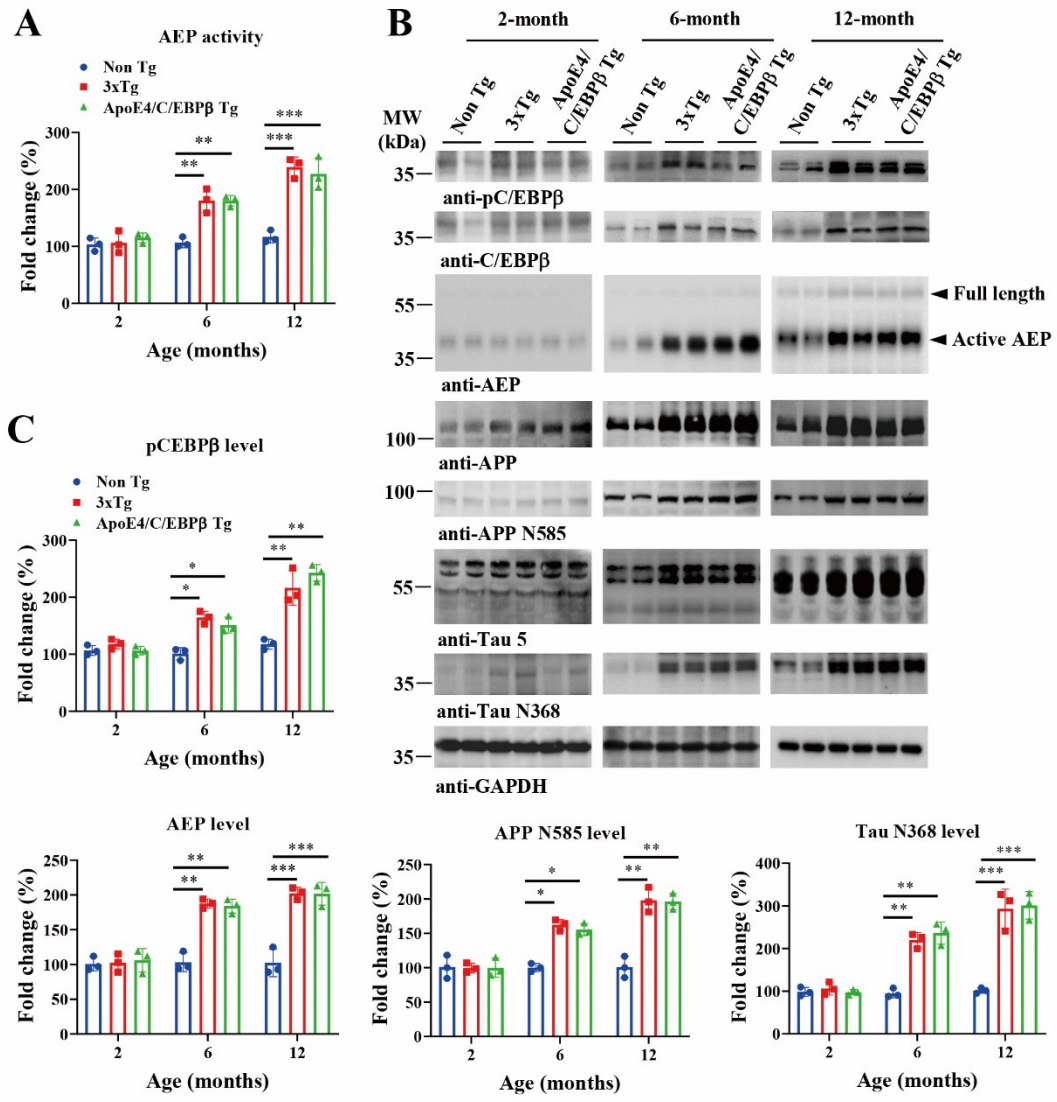
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This PDF file includes:

Supplementary Fig. 1 to Fig. 8

Supplementary Fig. 1



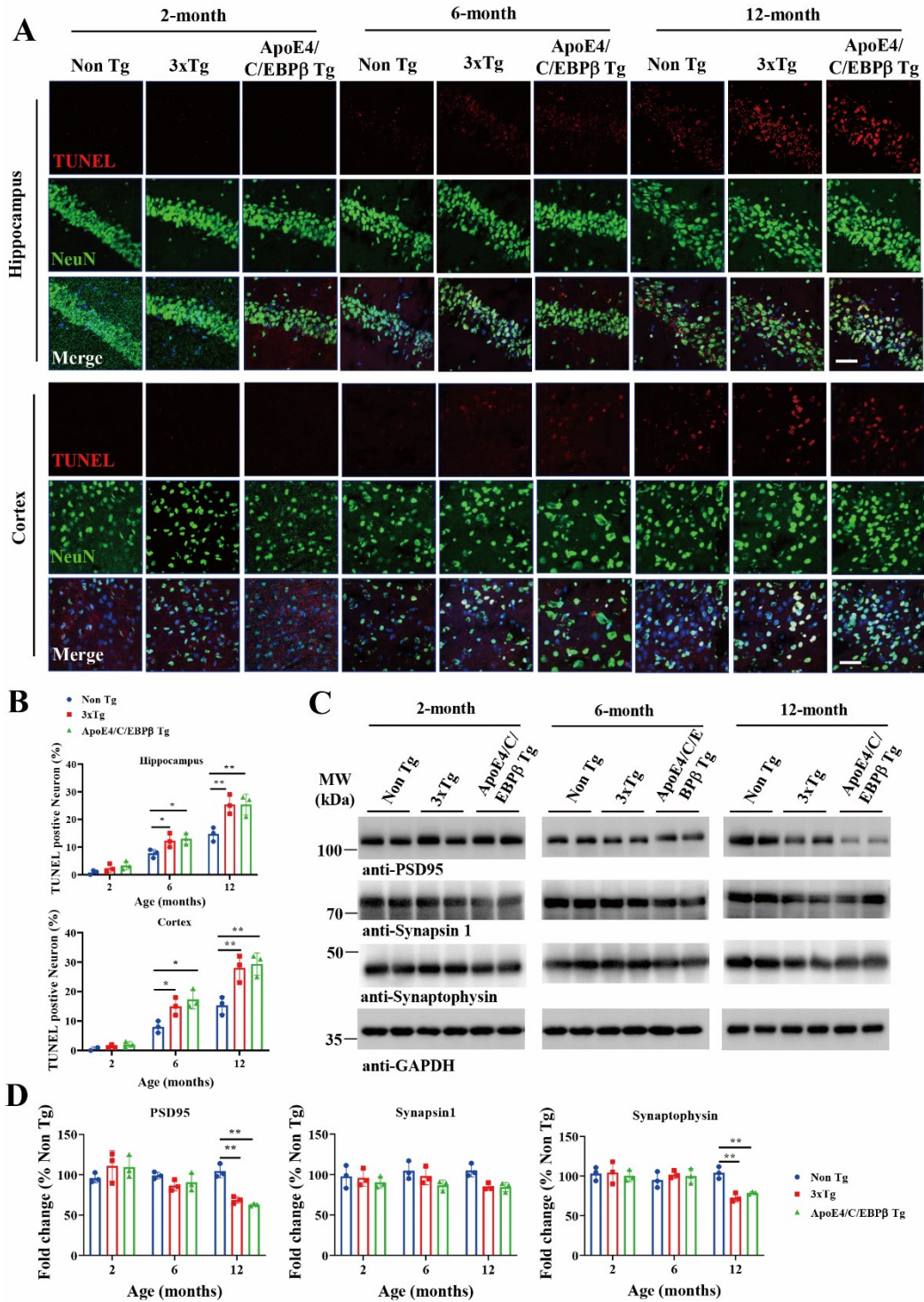
Supplementary Fig. 1 C/EBP β /AEP signaling activation in 3xTg and Thy1-ApoE4/C/EBP β Tg mice

A. AEP enzymatic activity in the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. (n = 3, ** p < 0.01, *** p < 0.001, compared with Non Tg).

B. Western blotting assays of C/EBP β , AEP and its downstream targets in the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age.

C. Relative quantification of protein expression in (B). (n = 3, ** p < 0.01, *** p < 0.001, compared with Non Tg).

Supplementary Fig. 2



Supplementary Fig. 2 Age-dependent neuronal loss in 3xTg and Thy1-ApoE4/C/EBP β Tg mice.

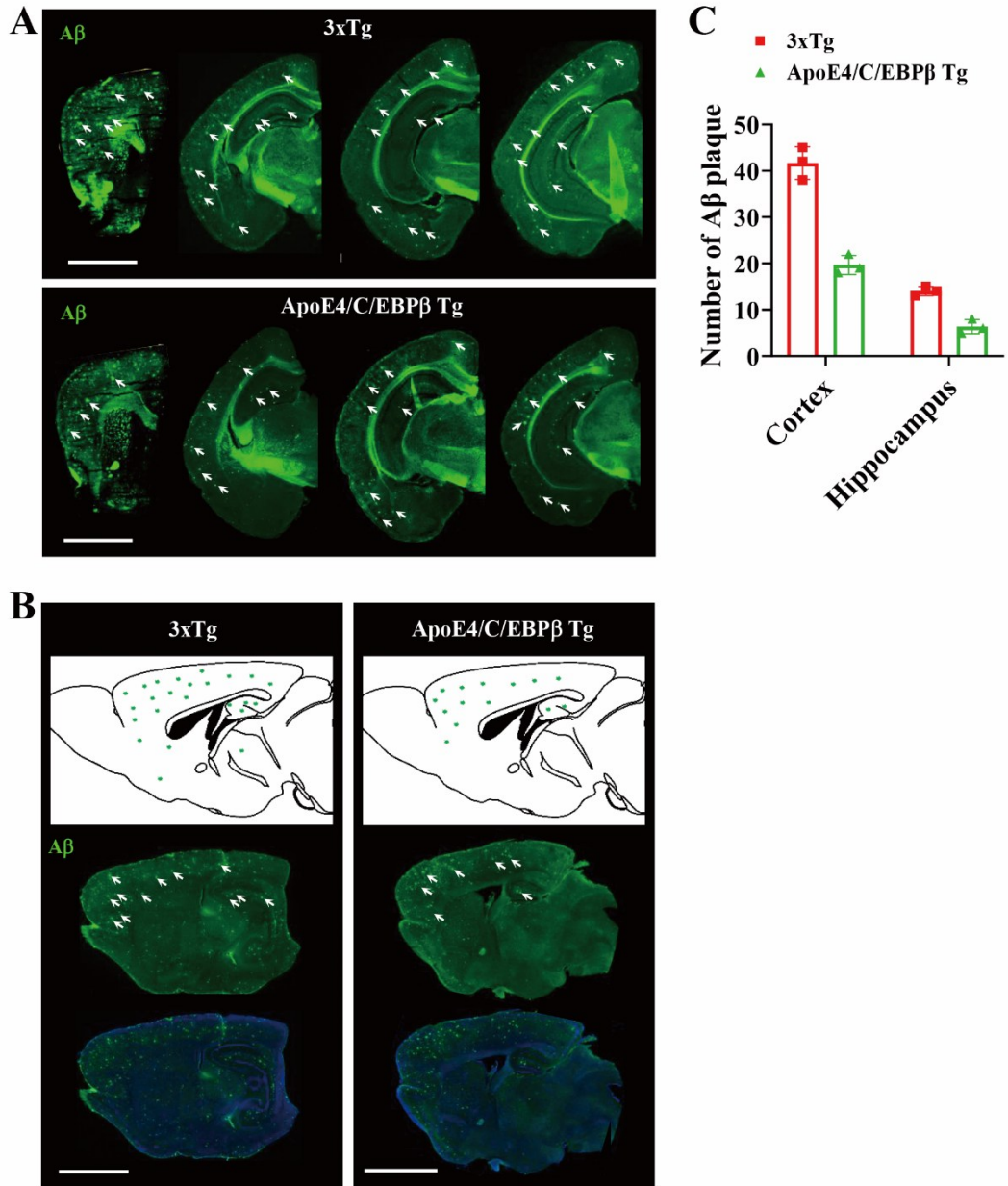
A. Immunostaining of NeuN and TUNEL in hippocampus (top panels) and cortex (bottom panels) of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. Scale bar, 20 μ m.

B. Relative quantification of TUNEL positive NeuN in (A). (n = 6, * p < 0.05, ** p < 0.01, compared with Non Tg).

C. Representative immunoblot images showing the expression of synaptic markers in 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age.

D. Relative quantification of protein levels in (C). (n = 3, ** p < 0.01, compared with Non Tg).

Supplementary Fig. 3



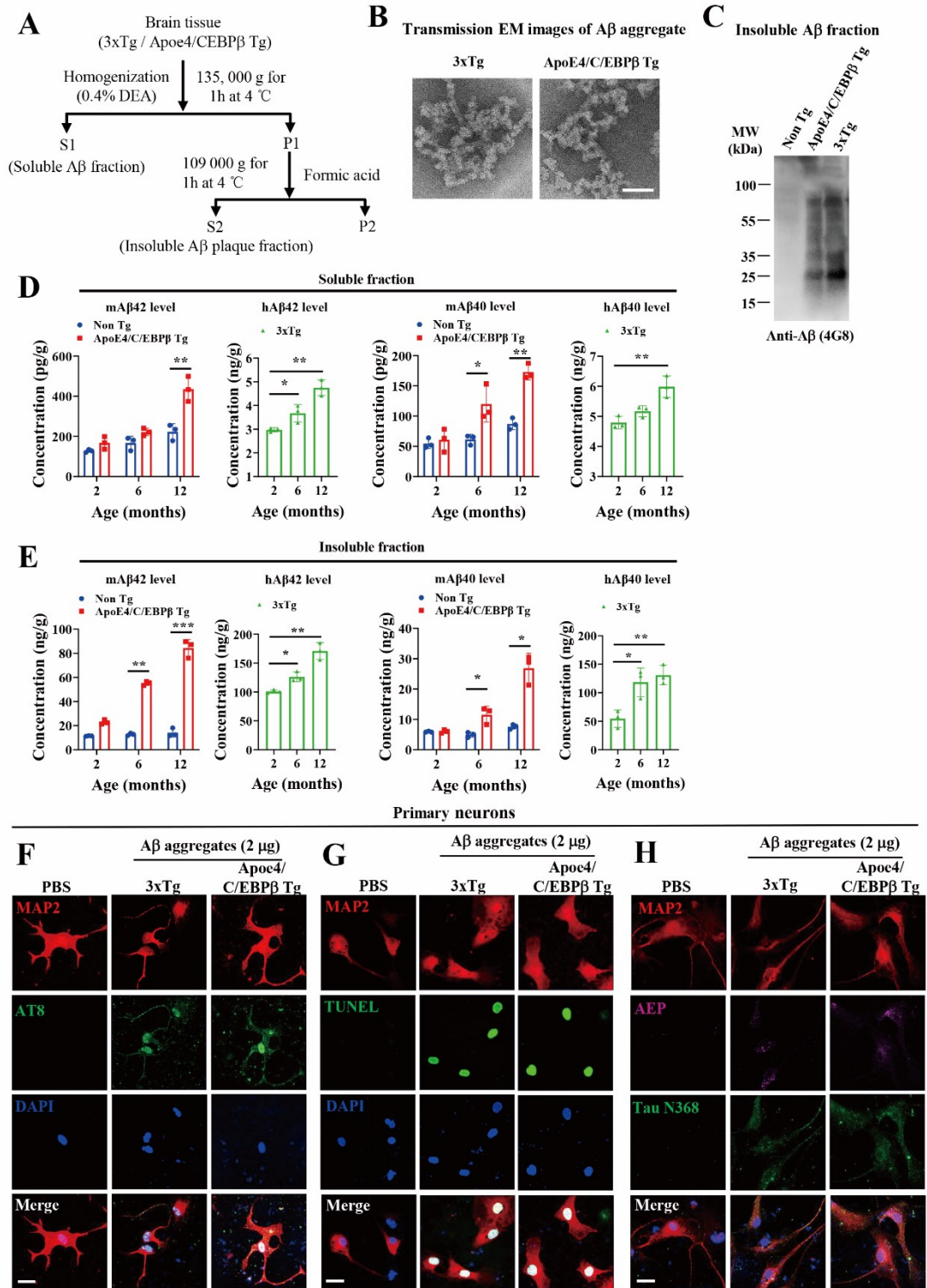
Supplementary Fig. 3 Representative image of distribution of A β pathology in 3xTg and Thy1-ApoE4/C/EBP β Tg mice.

A. Representative coronal section of A β plaque in 3xTg and Thy1/ApoE3/C/EBP β transgenic mice at the age of 12-month. Scale bar 500 μ m

B. Representative sagittal section of A β plaque in 3xTg and Thy1/ApoE3/C/EBP β transgenic mice at the age of 12-month. Scale bar 500 μ m.

C. Relative quantification of A β plaque numbers in cortex and hippocampus of 3xTg and Thy1/ApoE3/C/EBP β transgenic mice at the age of 12-month.

Supplementary Fig. 4



Supplementary Fig. 4 Characterization of A β aggregates in 3xTg and Thy1-ApoE4/C/EBP β Tg mice.

A. Diagram showing the isolation of soluble and insoluble A β in the brain tissues of 3xTg and Thy1-ApoE4/C/EBP β Tg mice.

B. Representative electron microscope images of insoluble A β fraction extracted from the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice. Scale bar 100 nm.

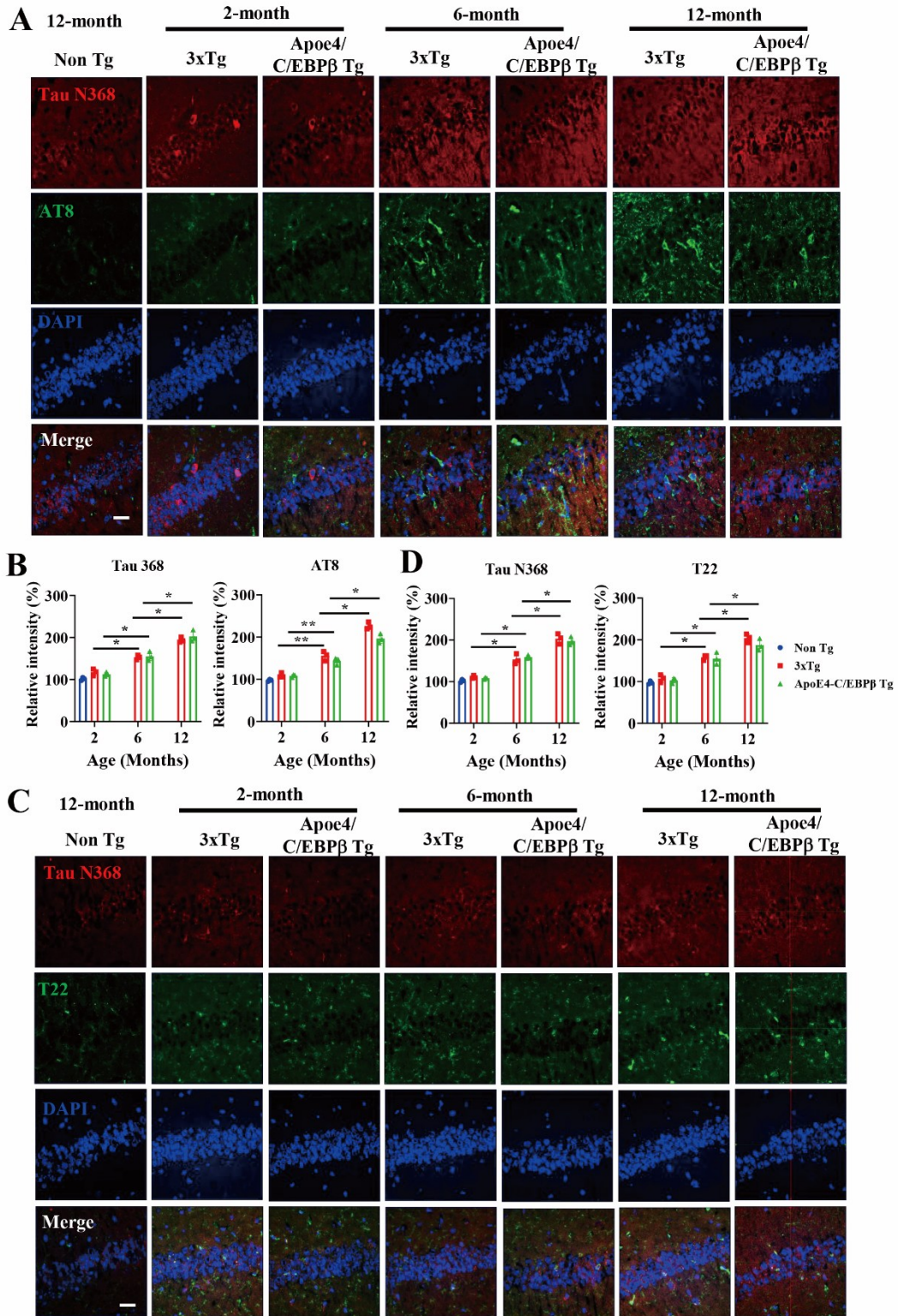
C. Western blotting assay of insoluble A β fraction extracted from the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice.

D. ELISA assay of soluble A β 40 and A β 42 levels in the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at different ages. (n = 3, * p < 0.05, ** p < 0.01).

E. ELISA assay of insoluble A β 40 and A β 42 levels in the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at different ages. (n = 3, * p < 0.05, ** p < 0.01, *** p < 0.001).

F-H. Representative immunostaining images of AT8 (F), TUNEL (G), AEP and Tau N368 (H) in primary rat neurons treated with A β aggregates (2 μ g) extracted from the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice. Scale bar 20 μ m.

Supplementary Fig. 5



Supplementary Fig. 5 Age-dependent increases of Tau pathology in 3xTg and Thy1-ApoE4/C/EBP β Tg mice.

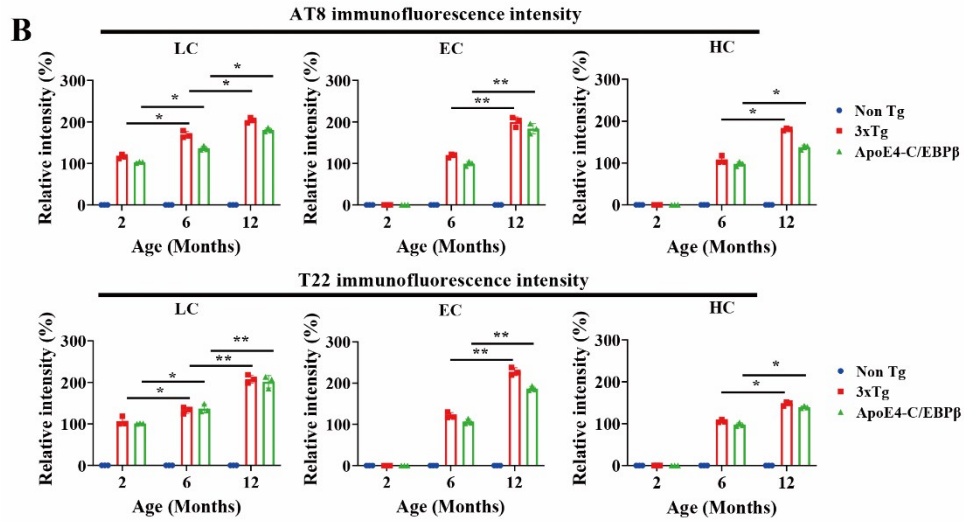
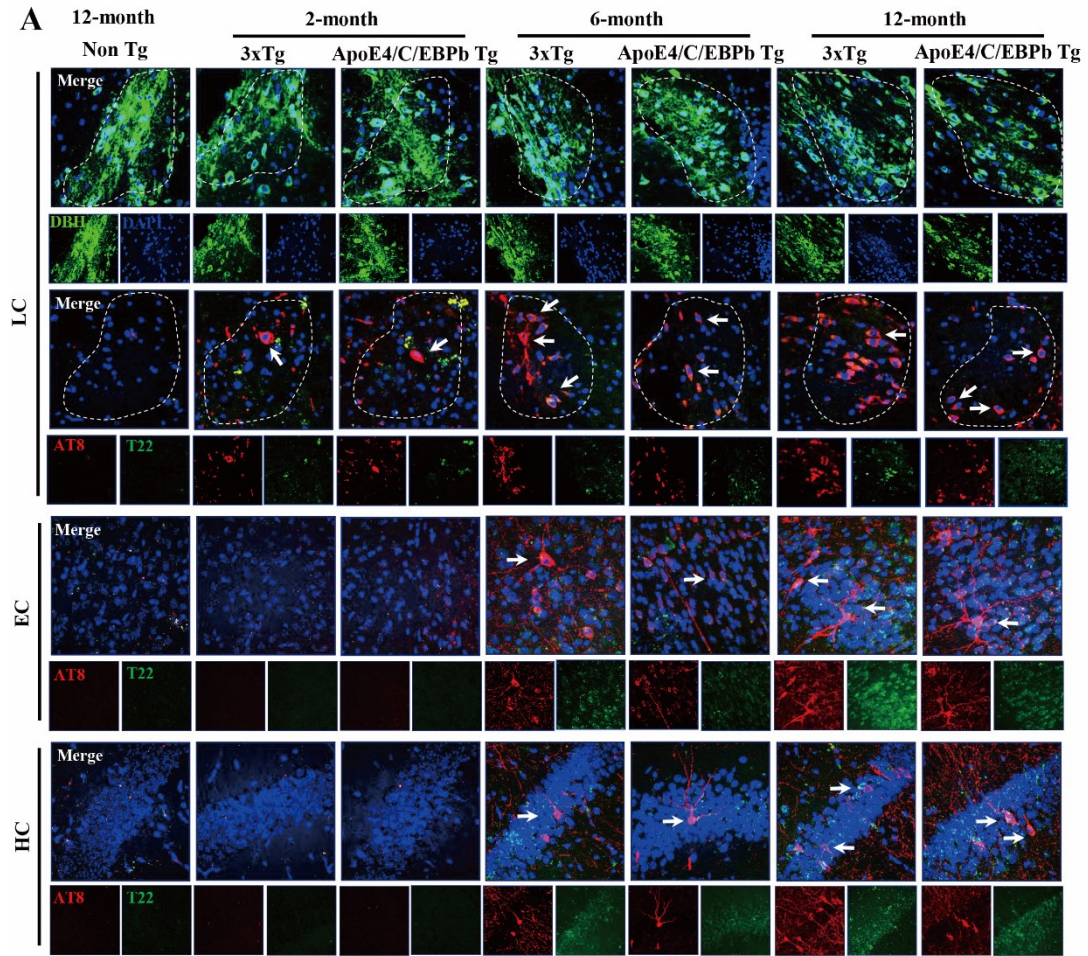
A. Co-staining of Tau N368 and AT8 in the hippocampus of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. Scale bar 20 μ m.

B. Quantification of AT8 and Tau N368 fluorescent signals in (A). (n = 3, * p < 0.05, ** p < 0.01).

C. Co-staining of Tau N368 and AT22 in the hippocampus of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. Scale bar 20 μ m

D. Quantification of T22 and Tau N368 fluorescent signals in (C). (n = 3, * p < 0.05).

Supplementary Fig. 6

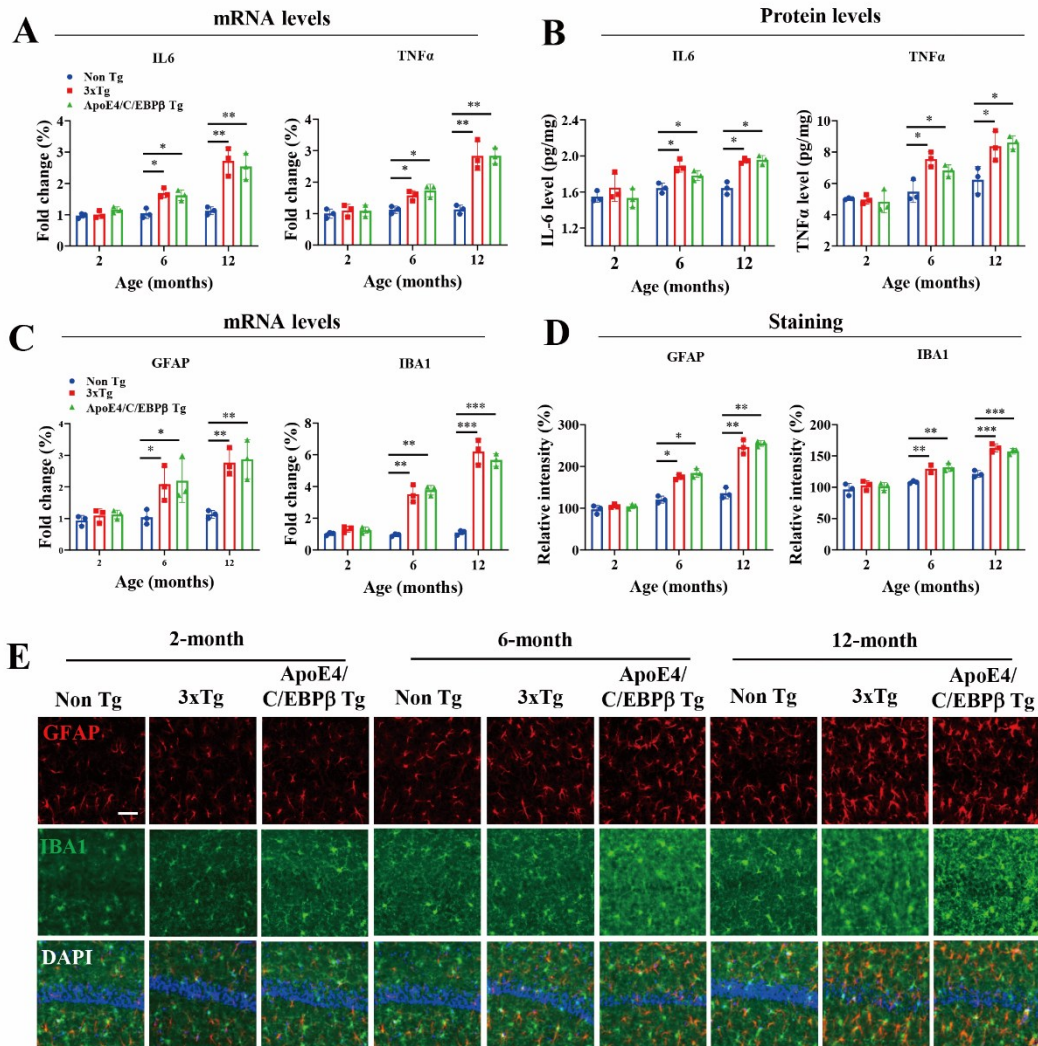


Supplementary Fig. 6 Tau spreading in 3xTg and Thy1-ApoE4/C/EBP β Tg mice

A. Representative AT8 and T22 immunostaining images in LC, EC and HC of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. LC, Locus coeruleus; EC, entorhinal cortex; HC, hippocampus.

B. Quantification of AT8 and T22 fluorescent signals in LC, EC and HC. (n = 3, * p < 0.05, ** p < 0.01)

Supplementary Fig. 7



Supplementary Fig. 7 Neuroinflammation in 3xTg and Thy1-ApoE4/C/EBP β Tg mice

A. The mRNA expression of IL-6 (left panel) and TNF- α (right panel) in the brain of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. (n = 3, * p < 0.05, ** p < 0.01, compared with Non Tg).

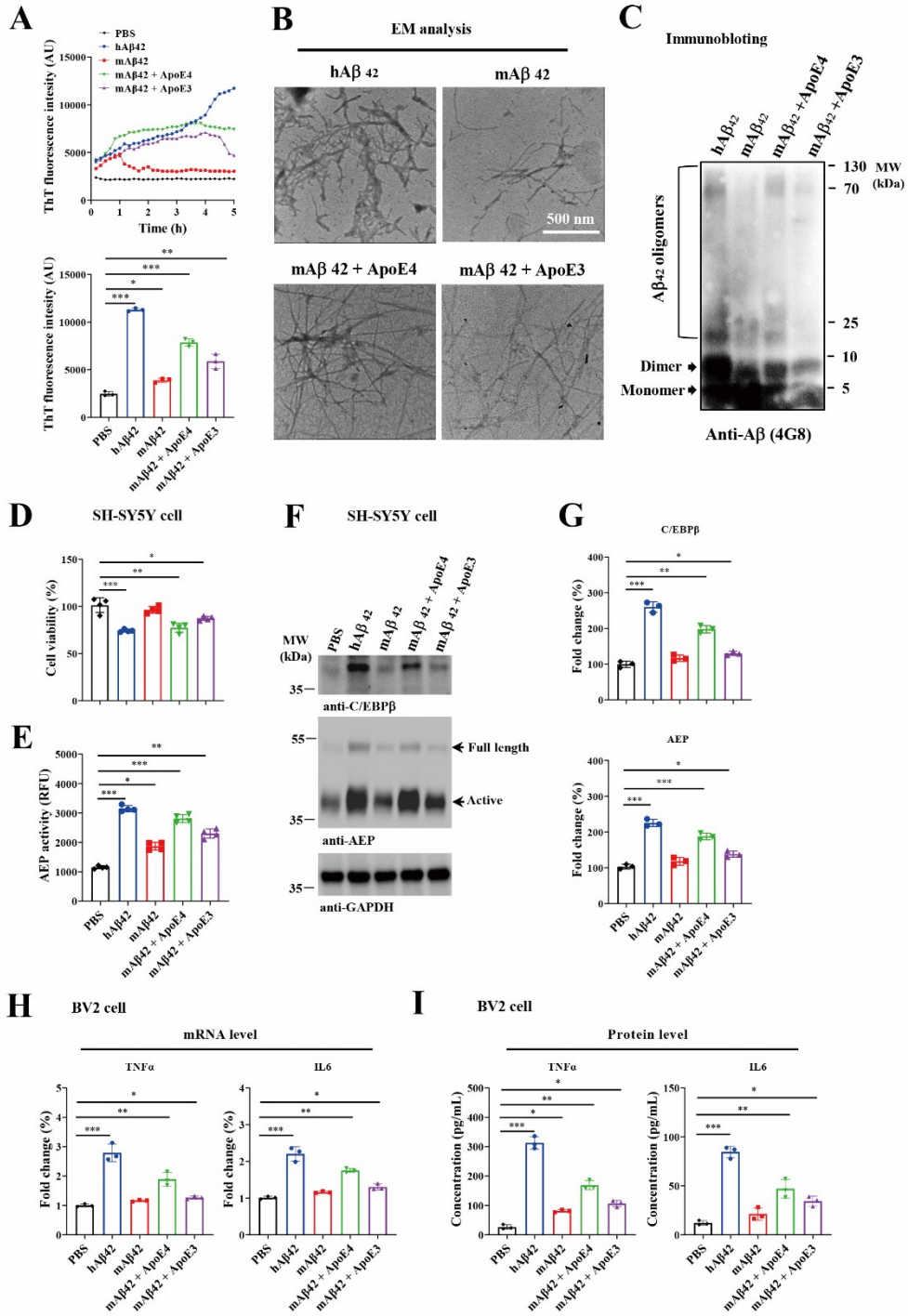
B. ELISA analysis of mouse IL-6 (left panel) and TNF- α (right panel) in the brain tissues of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. (n = 3, * p < 0.05, compared with Non Tg).

C. The mRNA expression of GFAP (left panel) and Iba1 (right panel) in the brains of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month of age. (n = 3, * p < 0.05, ** p < 0.01, *** p < 0.01, compared with Non Tg).

D. Relative quantification of GFAP (left panel) and Iba1 (right panel) fluorescence intensity in (E). (n = 3, * p < 0.05, compared with vehicle).

E. Immunostaining of GFAP and Iba1 in hippocampus of 3xTg and Thy1-ApoE4/C/EBP β Tg mice at 2-, 6- and 12-month age. Scale bar, 100 μ m.

Supplementary Fig. 8



Supplementary Fig. 8 ApoE4 promotes mouse A β aggregation and neurotoxicity

A. Representative curves of the Thioflavin T (ThT) fluorescence assays following ApoE4 and mouse A β 42 (mA β 42) co-incubation. Human A β 42 (hA β 42), mA β 42 alone and ApoE3 / mA β 42 co-incubation were taken as controls. The ThT assay was independently repeated three times.

B. TEM analysis of ApoE4 and mA β 42 incubation at 37 °C for 96 h. Similar results were observed in four independent experiments. Scale bar, 500 nm.

C. Immunoblotting analysis showing ApoE4-mediated enhancement of mA β 42 oligomer formation *in vitro*.

D and E. Effects of ApoE4 / mA β 42 incubation on cell viability (D) and AEP activity in SH-SY5Y cells. (n = 3, * p < 0.05, ** p < 0.01, *** p < 0.01, compared with PBS).

F. Immunoblotting analysis showing ApoE4 / mA β 42 incubation activate C/EBP β and AEP expression in SH-SY5Y cells.

G. Relative quantification of protein expression in (F). (n = 3, * p < 0.05, ** p < 0.01, *** p < 0.001, compared with PBS).

H and I. Effects of ApoE4 / mA β 42 incubation on TNF α and IL-6 at mRNA (H) and protein (I) levels in microglial BV2 cells. (n = 3, * p < 0.05, ** p < 0.01, *** p < 0.001, compared with PBS).