Supplemental Figures

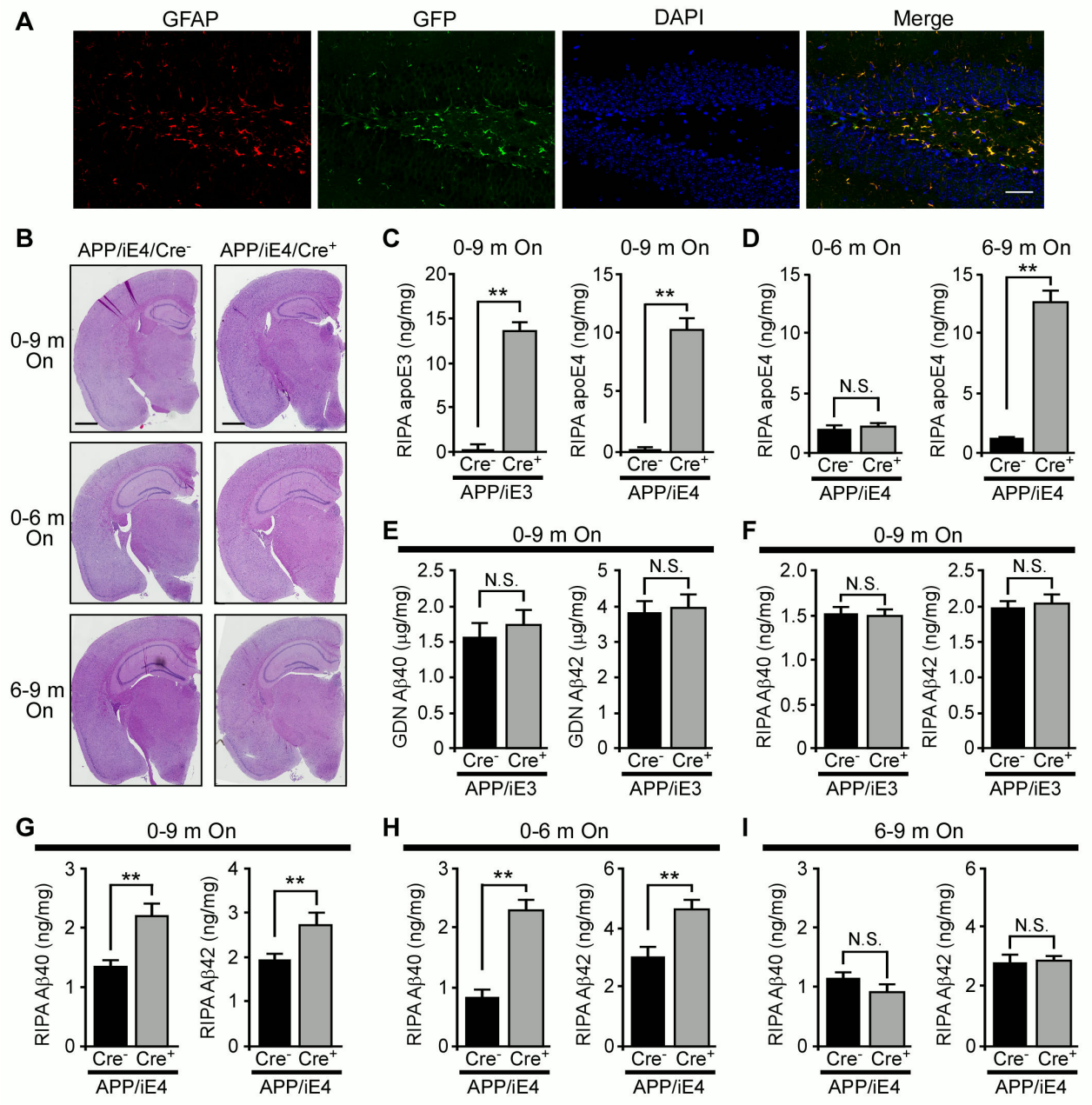

Figure S1. Expression of Astrocytic ApoE4 during the Amyloid Seeding Stage Increases the Levels of Soluble Aβ. Related to Figure 1 and 2.

(A) Subgranular zone in the hippocampus from apoE3 inducible (Cre^+) amyloid model mice were co-immunostained for astrocyte-specific marker (anti-GFAP; red) and GFP which represents apoE distribution (green). Scale bar, 50 μm.

(B) H&E staining of brain sections of APP/PS1 mice expressing apoE4 throughout the entire 9 months (0-9 m On), during 0-6 months (0-6 m On) or during 6-9 months (6-9 m On) (n=8-10/group) are shown. Scale bar, 1 mm.

(C) The cortical brain tissues of mice at 9 months of age were fractionated into RIPA-soluble and -insoluble (guanidine-HCl, GDN) fractions. RIPA-soluble apoE levels in APP/PS1 mice expressing apoE3 (APP/iE3; n=16-18/group) or apoE4 (APP/iE4; n=13-16/group) in the astrocytes throughout the entire 9 months (0-9 m On) were examined by ELISA. Data represent mean ± SEM. **, $p<0.01$.

(D) RIPA-soluble apoE in the cortex of APP/PS1 mice expressing apoE4 during 0-6 months (0-6 m On; n=6-8/group) or during 6-9 months (6-9 m On; n=8-10/group) were examined by ELISA. Data represent mean ± SEM. **, $p<0.01$; N.S., not significant.

(E, F) RIPA-soluble and -insoluble Aβ40 and Aβ42 in the cortex of 9-month-old APP/PS1 mice expressing apoE3 (APP/iE3; n=17-18/group) throughout the entire 9 months (0-9 m On) were examined by specific Aβ ELISA. Data represent mean ± SEM. N.S., not significant.

(G-I) Soluble Aβ40 and Aβ42 levels in the cortex of APP/PS1 mice expressing apoE4 (APP/iE4) throughout the entire 9 months (0-9 m On; n=13-16/group), during 0-6 months (0-6 m On; n=6-8/group) or during 6-9 months (6-9 m On; n=7-9/group) were examined by specific Aβ ELISA. Data represent mean ± SEM. **, $p<0.01$; N.S., not significant.

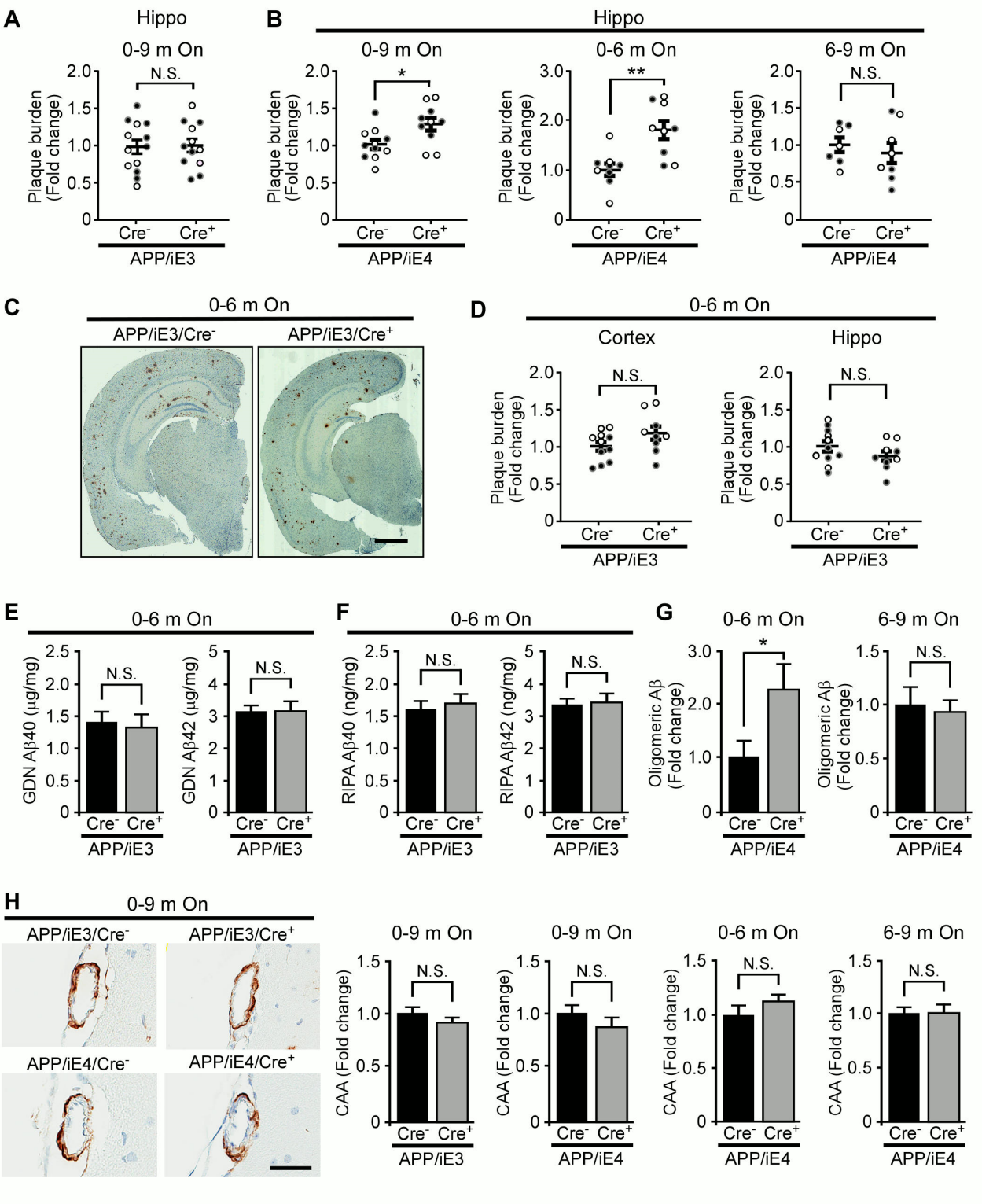
A
Hippo
0-9 m On
Plaque burden (Fold change)
N.S.
Cre- Cre+
APP/iE3
B
Hippo
0-9 m On
*
APP/iE4
0-6 m On
**
APP/iE4
6-9 m On
N.S.
APP/iE4
C
0-6 m On
APP/iE3/Cre-
APP/iE3/Cre+
D
0-6 m On
Cortex
Hippo
N.S.
APP/iE3
E
0-6 m On
GDN Aβ40 (μg/mg)
GDN Aβ42 (μg/mg)
N.S.
APP/iE3
F
0-6 m On
RIPA Aβ40 (ng/mg)
RIPA Aβ42 (ng/mg)
N.S.
APP/iE3
G
0-6 m On
6-9 m On
Oligomeric Aβ (Fold change)
*
N.S.
APP/iE4
H
0-9 m On
APP/iE3/Cre-
APP/iE3/Cre+
APP/iE4/Cre-
APP/iE4/Cre+
CAA (Fold change)
N.S.
APP/iE3
APP/iE4

Figure S2. Effects of Astrocytic ApoE Expression on Amyloid Pathology and Cerebral Amyloid Angiopathy (CAA) Formation. Related to Figure 2.

(A, B) Brain sections from APP/PS1 mice expressing apoE3 (APP/iE3; n=12-13/group), or apoE4 (APP/iE4; n=10-11/group) throughout entire 9 months, during 0-6 months (n=9/group) or 6-9 months (n=7-8/group) were immunostained with a pan-Aβ antibody. The plaque burden in the hippocampus was normalized to that of APP/PS1 mice. Open circles are females and closed circles are males. Data represent mean ± SEM. *, $p<0.05$; **, $p<0.01$; N.S., not significant.

(C, D) Brain sections from 9-month-old APP/PS1 mice expressing apoE3 (APP/iE3) during seeding stage (0-6 m On; n=9-11/group) were immunostained with a pan-Aβ antibody. The plaque burden in the cortex and hippocampus was quantified and normalized to that of APP/PS1 mice. Data represent mean ± SEM. N.S., not significant.

(E, F) The levels of Aβ40 and Aβ42 in RIPA fraction and guanidine (GDN) fraction in the cortex of APP/PS1 mice expressing apoE3 during seeding stage (0-6 m On; n=9-11/group) were examined by specific Aβ ELISA. Data represent mean ± SEM. N.S., not significant.

(G) The levels of Aβ oligomers in the cortex of APP/PS1 mice expressing apoE4 during seeding stage (0-6 m On; n=8-9/group) or rapid growing period (6-9 m On; n=8/group) were examined by oligomeric Aβ ELISA. Data represent mean ± SEM. *, $p<0.05$; N.S., not significant.

(H) The representative images of leptomeningeal CAA are shown. Scale bar, 50 μm. Quantification of amyloid deposition in cerebrovasculature of 9-month-old APP/PS1 mice expressing apoE3 for the entire 9 months (0-9 m On; n=11-12/group), or apoE4 for 9 months (0-9 m On; n=13/group), during seeding stage (0-6 m On; n=7-8/group) or rapid growing period (6-9 m On; n=7-8/group). The burden of CAA in leptomeningeal arteries (13-15 arteries/mouse) was normalized to that of APP/PS1 mice. Data represent mean ± SEM. N.S., not significant.

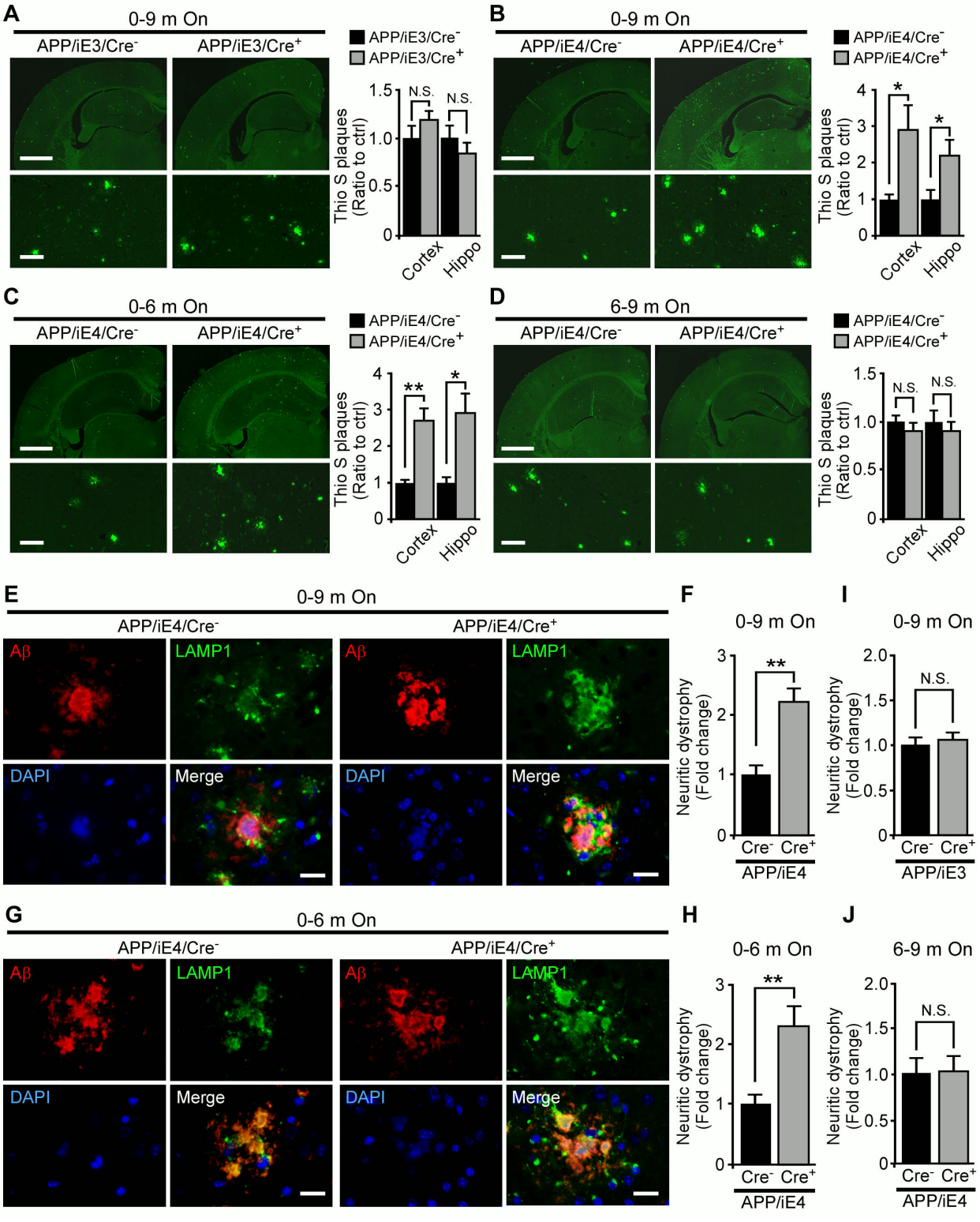
A
0-9 m On
APP/iE3/Cre-
APP/iE3/Cre+
APP/iE3/Cre-
APP/iE3/Cre+
Thio S plaques (Ratio to ctrl)
N.S.
N.S.
Cortex
Hippo
B
0-9 m On
APP/iE4/Cre-
APP/iE4/Cre+
APP/iE4/Cre-
APP/iE4/Cre+
Thio S plaques (Ratio to ctrl)
*
*
Cortex
Hippo
C
0-6 m On
APP/iE4/Cre-
APP/iE4/Cre+
APP/iE4/Cre-
APP/iE4/Cre+
Thio S plaques (Ratio to ctrl)
**
*
Cortex
Hippo
D
6-9 m On
APP/iE4/Cre-
APP/iE4/Cre+
APP/iE4/Cre-
APP/iE4/Cre+
Thio S plaques (Ratio to ctrl)
N.S.
N.S.
Cortex
Hippo
E
0-9 m On
APP/iE4/Cre-
APP/iE4/Cre+
Aβ
LAMP1
DAPI
Merge
F
0-9 m On
Neuritic dystrophy (Fold change)
**
Cre-
Cre+
APP/iE4
I
0-9 m On
Neuritic dystrophy (Fold change)
N.S.
Cre-
Cre+
APP/iE3
G
0-6 m On
APP/iE4/Cre-
APP/iE4/Cre+
Aβ
LAMP1
DAPI
Merge
H
0-6 m On
Neuritic dystrophy (Fold change)
**
Cre-
Cre+
APP/iE4
J
6-9 m On
Neuritic dystrophy (Fold change)
N.S.
Cre-
Cre+
APP/iE4

Figure S3. Overexpression of Astrocytic ApoE4 for the Entire 9 Months and during the Amyloid Seeding Stage Enhances Fibrillar Aβ Deposition and Neuritic Dystrophy. Related to Figure 2.

(A, B) Brain sections from 9-month-old APP/PS1 mice expressing apoE3 (APP/iE3) or apoE4 (APP/iE4) throughout the entire 9 months (0-9 m On; n=5-8/group) were labeled for fibrillar Aβ using Thioflavin S (Thio S).

(C, D) Brain sections from 9-month-old APP/PS1 mice expressing apoE4 during 0-6 months (0-6 m On; n=4-7/group) or during 6-9 months (6-9 m On; n=7/group) were labeled for fibrillar Aβ using Thio S. Representative images are shown. Scale bar (upper panels), 1 mm; Scale bar (bottom panels), 100 μm. The percentage of area covered by Thio S-positive plaques in the cortex and hippocampus of experimental mice was quantified, and was normalized to that of APP/PS1 mice. Values represent mean ± SEM. *, $p<0.05$; **, $p<0.01$; N.S., not significant.

(E-J) Brain sections from 9-month-old APP/PS1 mice expressing apoE3 (APP/iE3) or apoE4 (APP/iE4) throughout the entire 9 months (0-9 m On), during seeding stage (0-6 m On) or rapid growing period (6-9 m On) were co-immunostained with Aβ and LAMP1 antibodies to examine plaque-associated neuritic dystrophy. DAPI staining was used for visualization of nuclei. (E, G) Images from mice expressing apoE4 for entire 9 months (0-9 m On) and 0-6 months (0-6 m On) are shown. Scale bar, 20 μm.

(F, H, I, J) Quantification of LAMP1 intensity within 15 μm of plaque in brain sections of experimental mice expressing astrocytic apoE isoforms (n=6 mice/group; 8 plaques/mouse). Values represent mean ± SEM. **, $p<0.01$; N.S., not significant.

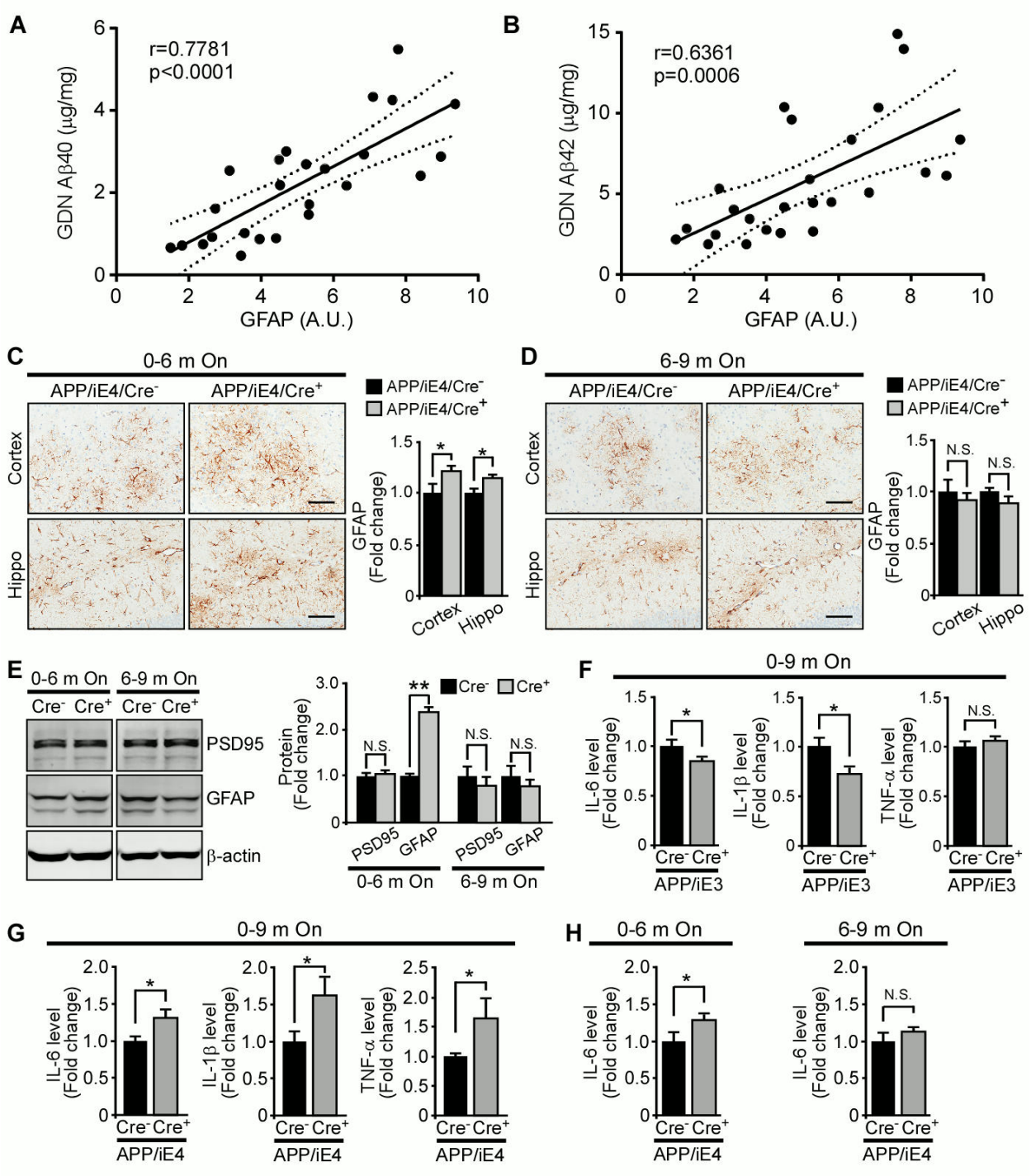
A
r=0.7781
p<0.0001
GDN Aβ40 (μg/mg)
GFAP (A.U.)
B
r=0.6361
p=0.0006
GDN Aβ42 (μg/mg)
GFAP (A.U.)
C
0-6 m On
APP/iE4/Cre-
APP/iE4/Cre+
Cortex
Hippo
GFAP (Fold change)
D
6-9 m On
N.S.
E
0-6 m On
6-9 m On
PSD95
GFAP
β-actin
Protein (Fold change)
F
0-9 m On
IL-6 level (Fold change)
IL-1β level (Fold change)
TNF-α level (Fold change)
APP/iE3
G
APP/iE4
H

Figure S4. Astrocytic ApoE4 Expression during the Amyloid Seeding Stage Increases Aβ-associated Neuroinflammation. Related to Figure 4.

(A, B) The correlation between the levels of insoluble Aβ40 or Aβ42 and GFAP in the cortex of 9-month-old APP/PS1 mice expressing apoE4 for the entire 9 months (0-9 m On) was analyzed and plotted. The Aβ levels in the cortex were analyzed by specific Aβ ELISAs, and the GFAP level (Arbitrary Unit) was examined by Western blotting. Correlation coefficient (r) and p-value were acquired by Pearson correlation test. The line of best fit (black line) and 95% confidence intervals (dashed lines) are shown. The levels of GFAP are positively correlated with those of insoluble Aβ40 and Aβ42.

(C, D) Brain sections from 9-month-old APP/PS1 mice expressing apoE4 during 0-6 months (0-6 m On; n=5-7 mice/group) or during 6-9 months (6-9 m On; n=7-8 mice/group) were immunostained with GFAP antibody. Representative images of GFAP staining in the cortical and hippocampal regions are shown. Scale bar, 100 μm. The immunoreactivity of GFAP staining in cortex and hippocampus was quantified. Data represent mean ± SEM. *, $p<0.05$; N.S., not significant.

(E) The levels of GFAP and postsynaptic marker PSD-95 in the cortex of 9-month-old APP/PS1 mice expressing apoE4 during 0-6 months (0-6 m On; n=4-6 mice/group) or during 6-9 months (6-9 m On; n=7-9 mice/group) were examined by Western blot and quantified. Data represent mean ± SEM. **, $p<0.01$; N.S., not significant.

(F, G) The levels of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α in the brains of 9-month-old APP/PS1 mice expressing apoE3 or apoE4 for the entire 9 months (0-9 m On) were quantified by ELISAs. Values represent mean ± SEM. *, $p<0.05$; N.S., not significant.

(H) The levels of IL-6 in the brains of 9-month-old APP/PS1 mice expressing apoE4 during seeding stage (0-6 m On) or rapid growing period (6-9 m On) were quantified by ELISA. Values represent mean ± SEM. *, $p<0.05$; N.S., not significant.

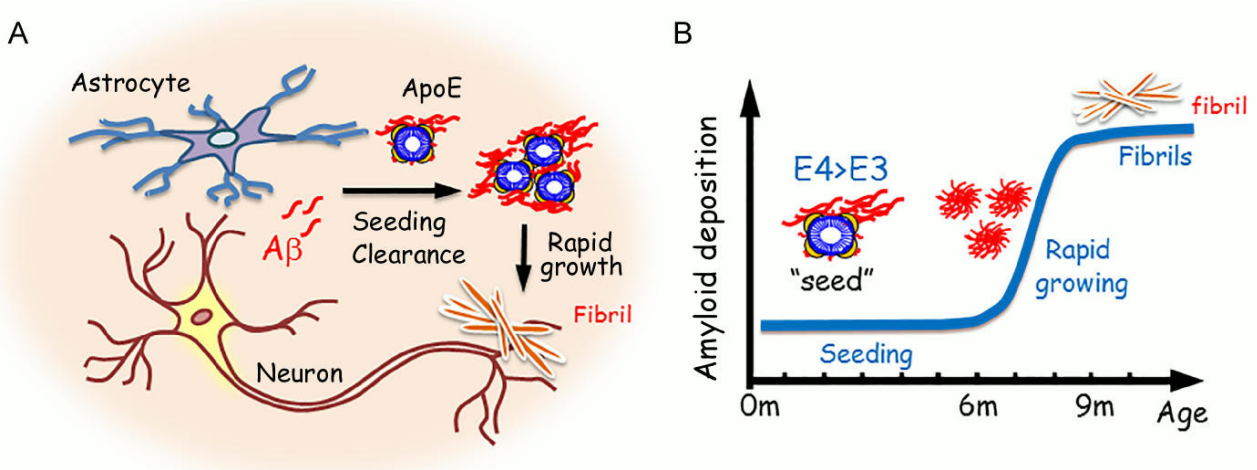

Figure S5. ApoE4 Accelerates Amyloid Pathology During the Seeding Stage. Related to Figure 1-4.

(A) Amyloid development and progression consist of two stages: 1) the initial formation of aggregation nuclei during the lag phase, leading to the development of small amount of Aβ aggregates, and 2) the subsequent extension of these Aβ seeds during the rapid growth phase including fibril formation (Burgold et al., 2014). ApoE4 secreted from astrocytes impairs Aβ clearance and accelerates Aβ aggregation in the seeding stage of amyloid development. ApoE itself forms aggregates at different rates depending on the isoform (apoE4 > apoE3 > apoE2) (Hatters et al., 2006). It is possible that apoE4 accelerates Aβ aggregation by co-aggregating with Aβ partially through its self-aggregating propensity.

(B) Schematic diagram of amyloid development and progression at different stages in APP/PS1 mice. Note that apoE4 promotes amyloid seeding during the Aβ nucleation stage (0-6 m), but not during the rapid growing stage (6-9 m), leading to enhanced amyloid deposition.