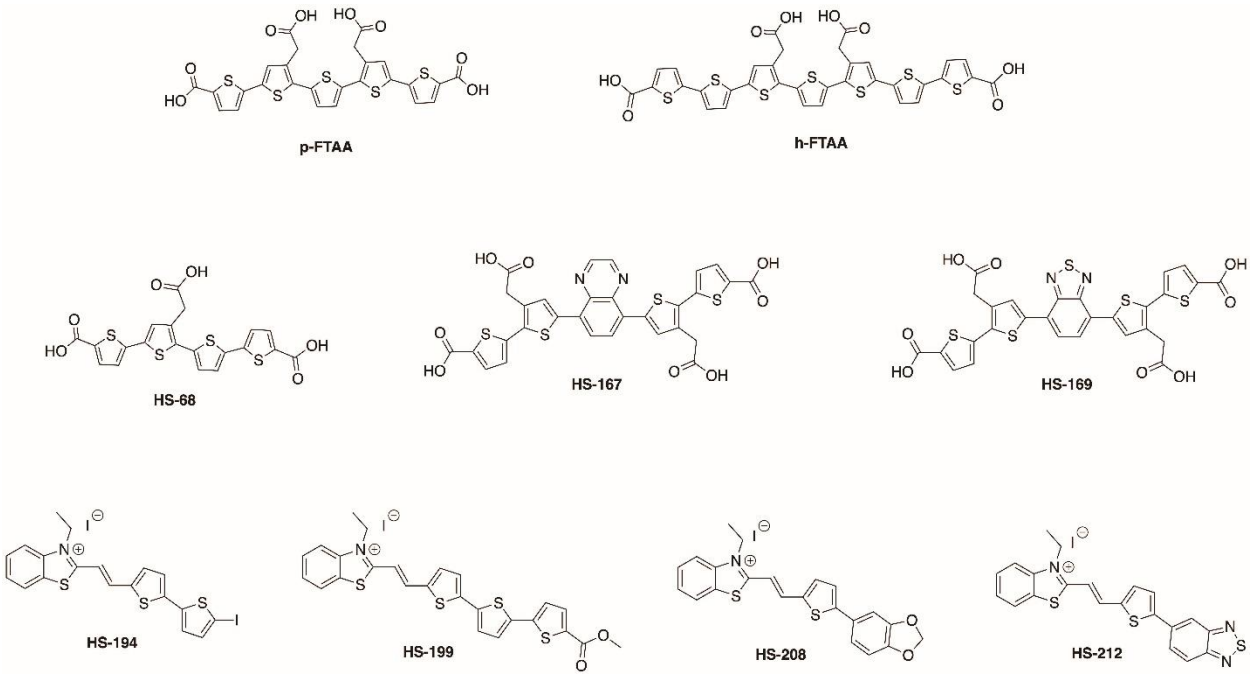
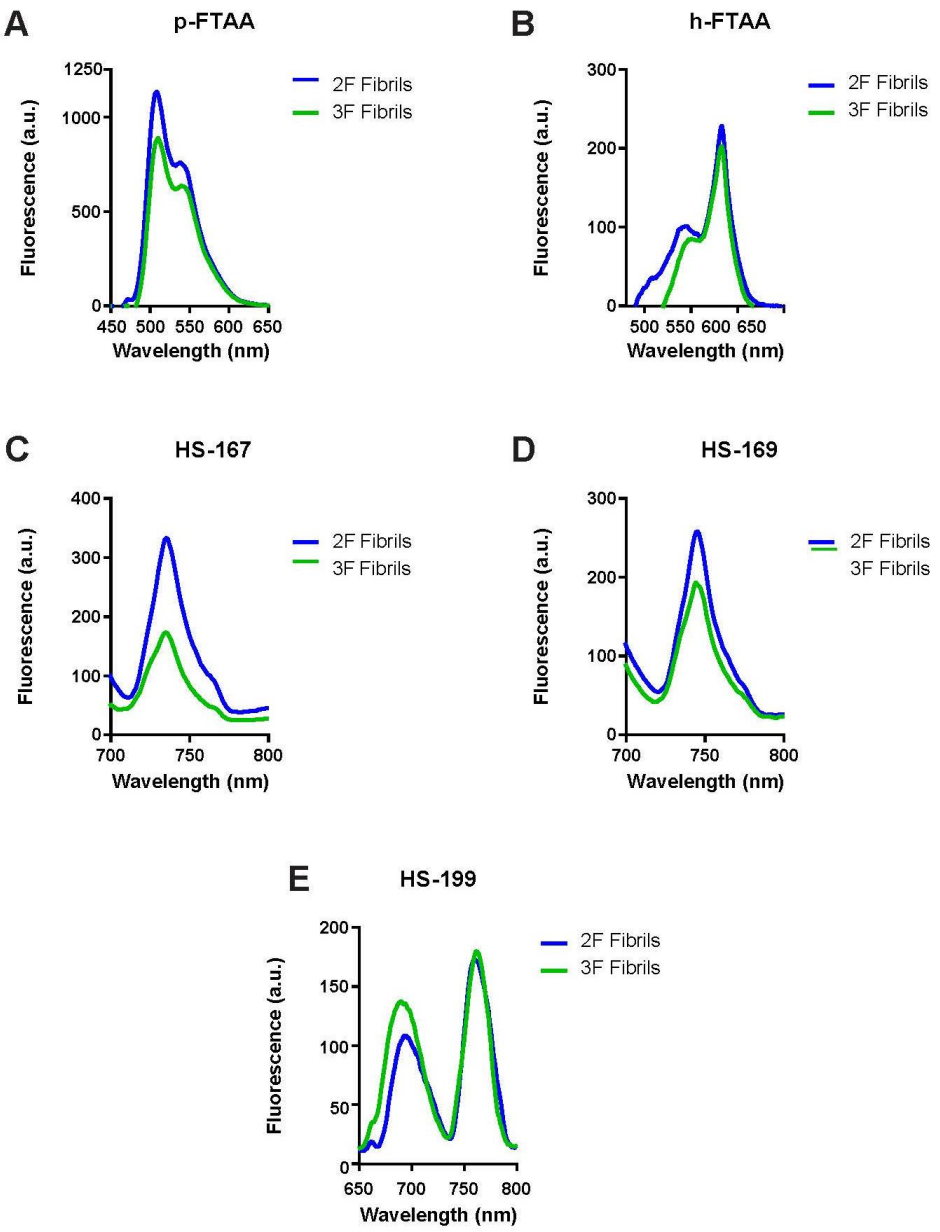
Appendix to:

Two structurally defined Aβ polymorphs promote different pathological changes in susceptible mice

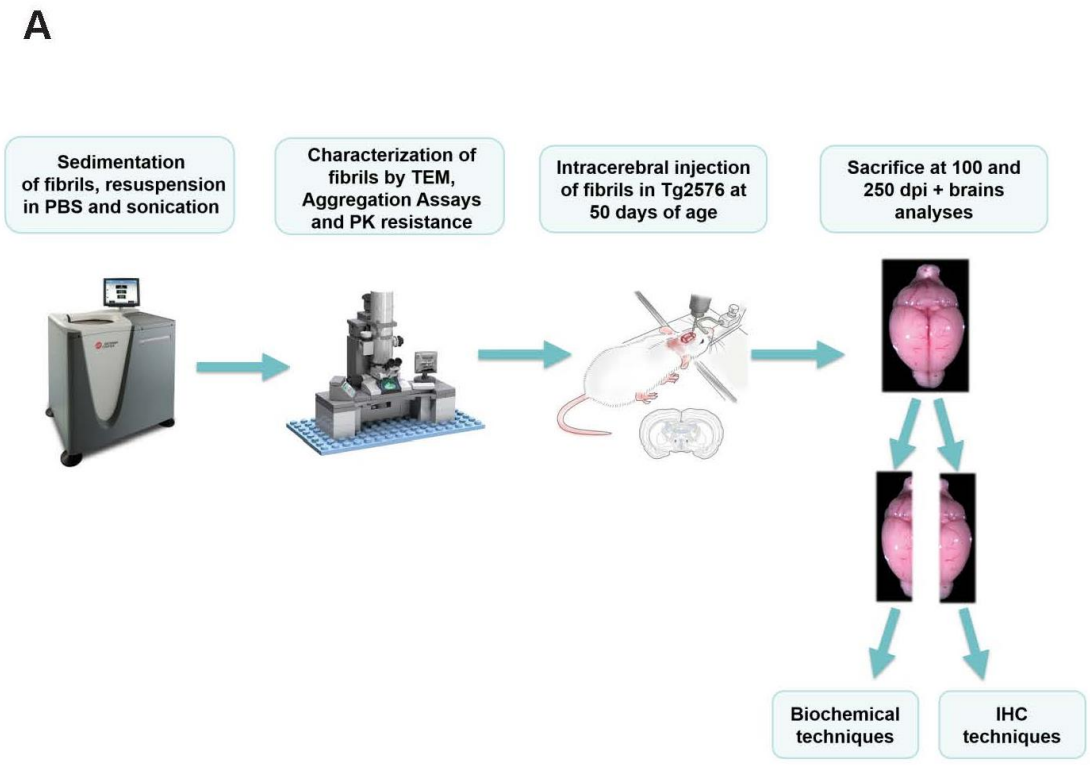
TABLE OF CONTENTS



<u>Appendix Figure S1.</u> Chemical structures of the luminescent dyes used in this study.

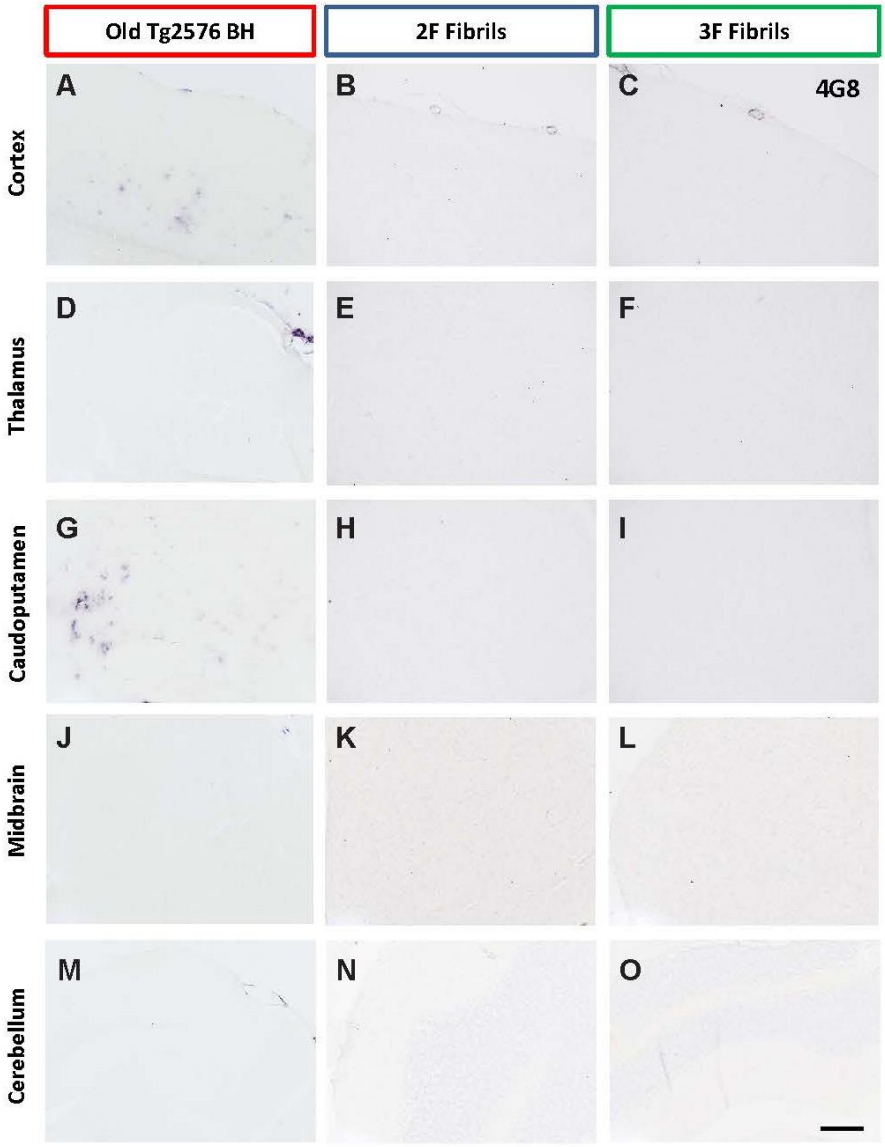


<u>Appendix Figure S2.</u> 2F and 3F fibrils testing against additional luminescent conjugated oligothiophenes. As in figure 1G-J, 2F and 3F fibrils **(A-E)** were tested for their reactivity against additional LCOs able to discriminate among conformational variants of misfolded proteins. p-FTAA **(A)**, h-FTAA **(B)**, HS-169 **(D)** and HS-199 **(E)** shows none or slightly differences in their emission spectra when tested in combination with either of the two synthetic fibrils. HS-167 **(C)** discriminate among conformational variants of misfolded proteins. One tissue slice per animal was used in this study.

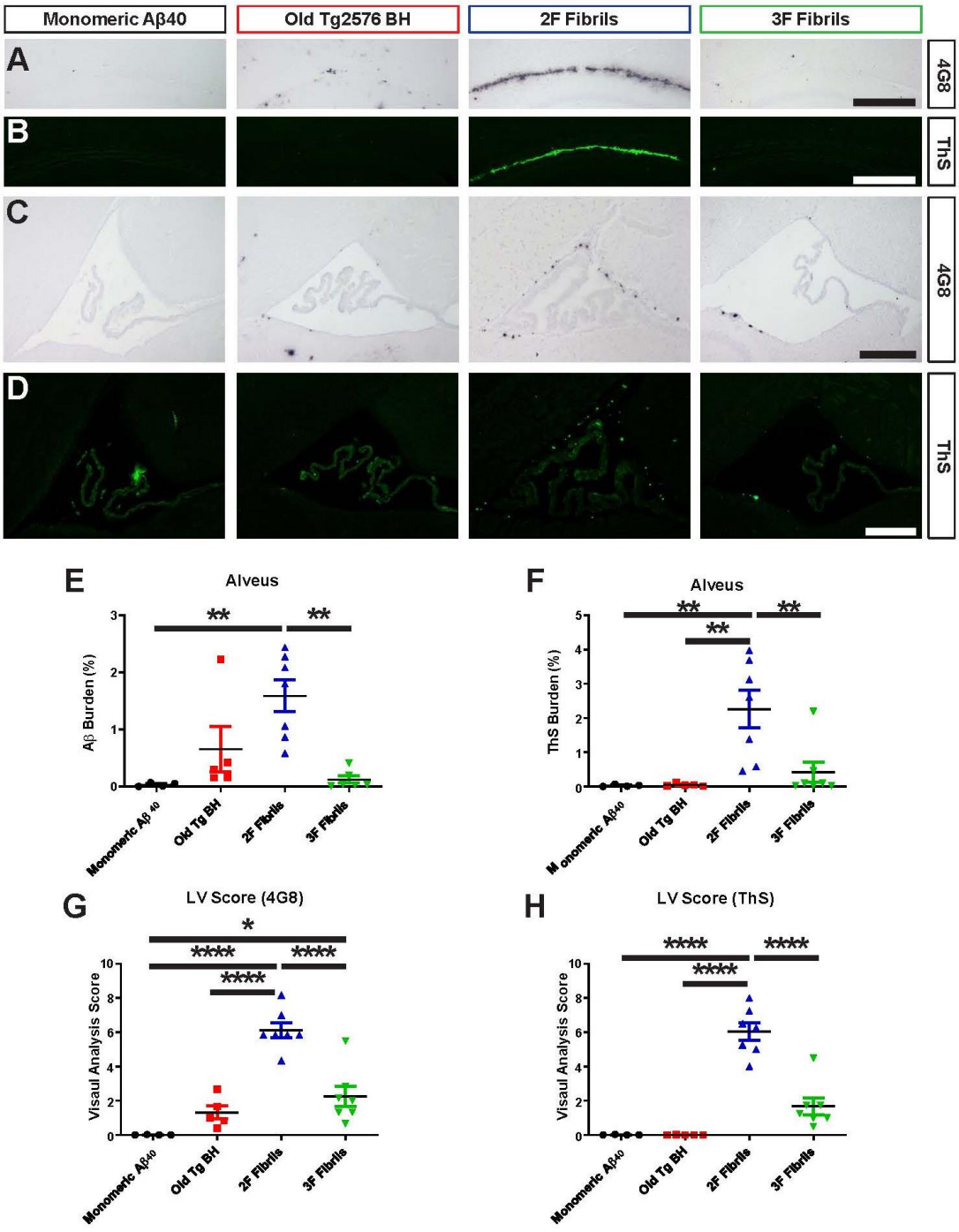


<u>Appendix Figure S3.</u> Project workflow to assess the *in vivo* prion-like propagation of 2F and 3F seeds. (A) For this project, both 2F and 3F fibrils were pelleted by ultracentrifugation at 100,000 x g and resuspended in PBS at a final concentration of 1 mg/mL. These fibrils were later characterized by Transmission Electron Microscopy (TEM), *in vitro* aggregation assays, PK resistance and LCOs co-incubation and spectra analysis. The PBS resuspended Aβ fibrils were stored at -80°C until used for animal injection. Prior to use, inocula (Synthetic 2F/3F Fibrils and

brain homogenates) were sonicated for 30 seconds in an ice-cold water bath and injected the hippocampus (both hemispheres) of Tg2576 animals at 50 days of age. Mice were euthanized at 100- or 250-days post-injection (dpi), and brains were quickly removed and hemi-dissected. Left brain halves were frozen to be analyzed by protein-based multiplex immunoassay for their PBS-insoluble $A\beta_{40}$/$A\beta_{42}$ contents, and cytokines and chemokines levels using commercially available multiplex kits. Opposite brain halves were fixed by immersion in formalin and saved for further immunohistochemical (IHC) studies. **(B)** Timeline of this study as described in **(A).**

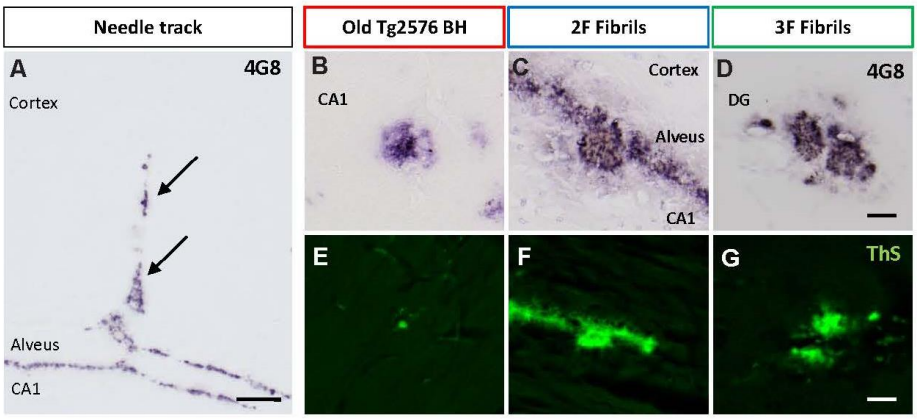


<u>Appendix Figure S4.</u> Distribution of seeded Aβ deposition across different brain regions. Different brain regions including cortex (A-C), thalamus (D-F), caudate nucleus/putamen (G-I), midbrain (J-L) and cerebellum (M-O) were analyzed for mice treated with Old Tg BH (left column), 2F (middle column) and 3F (right column) fibrils. Scale bar in (O) represents 200 µm and applies to all micrographs. One tissue slice per animal was used for this study.

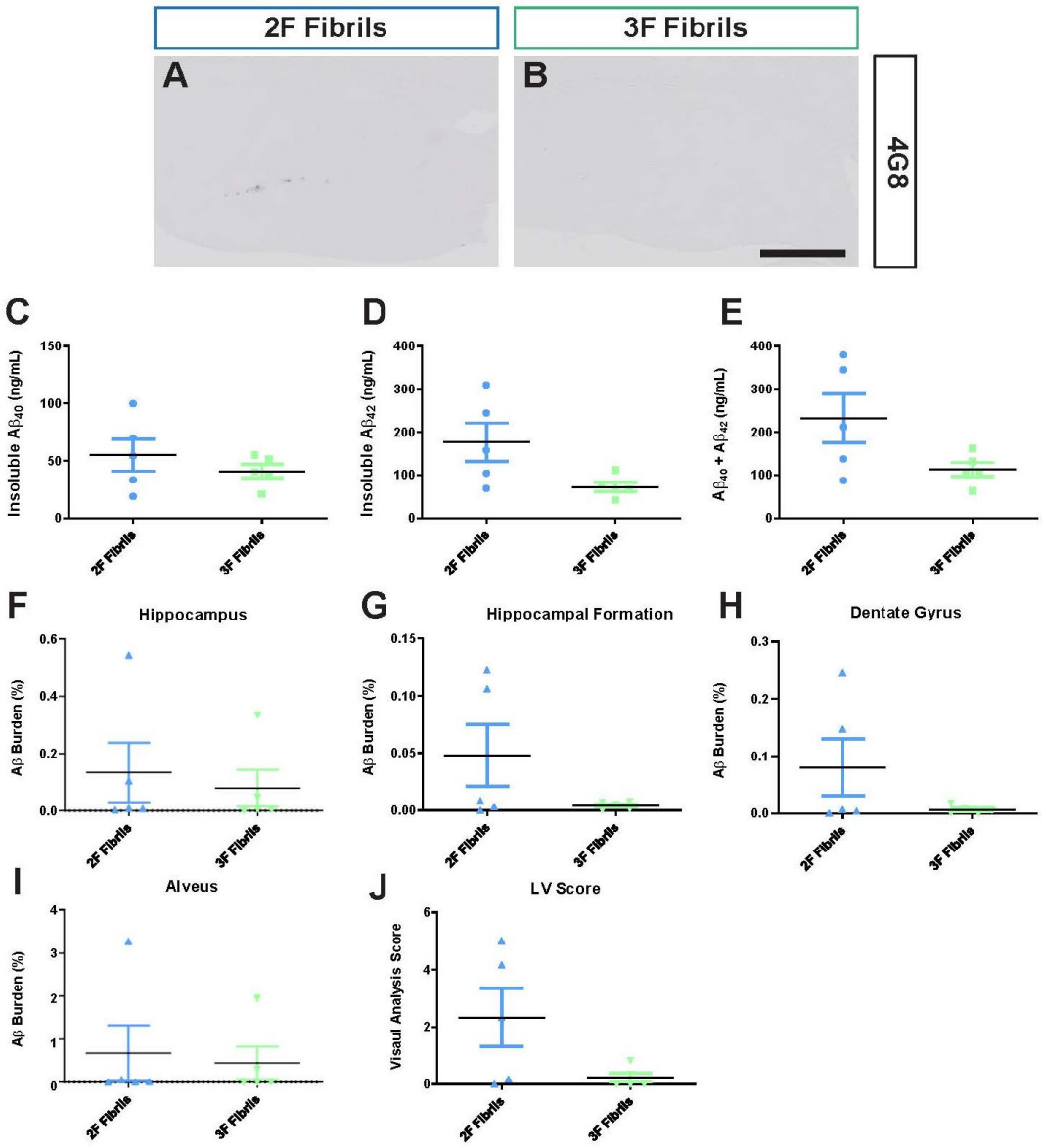


<u>Appendix Figure S5.</u> Region-specific (alveus and lateral ventricles) analyses of amyloid pathology in animals seeded with 2F and 3F fibrils and a brain extract from an old Tg2576 mouse. Representative pictures of the alveus of mice treated with 2F-, 3F- and Tg2576- derived

seeds after visualization for Aβ deposits using the 4G8 antibody **(A)** or ThS staining **(B)**. Representative pictures of the lateral ventricles from mice treated with 2F-, 3F- and Tg2576-derived seeds after visualization for Aβ deposits using the 4G8 antibody **(C)** or ThS staining **(D)**. Scale bars at the right panels represent 500 µm and are applicable to all pictures. Aβ **(E)** and ThS **(F)** burden quantification in alveus. Aβ **(G)** and ThS **(H)** visual score analysis in the lateral ventricles. Statistical analyses were performed by One-way ANOVA (* $p<0.05$, ** $p<0.01$ and **** $p<0.0001$). One tissue slice per animal was used in this study.

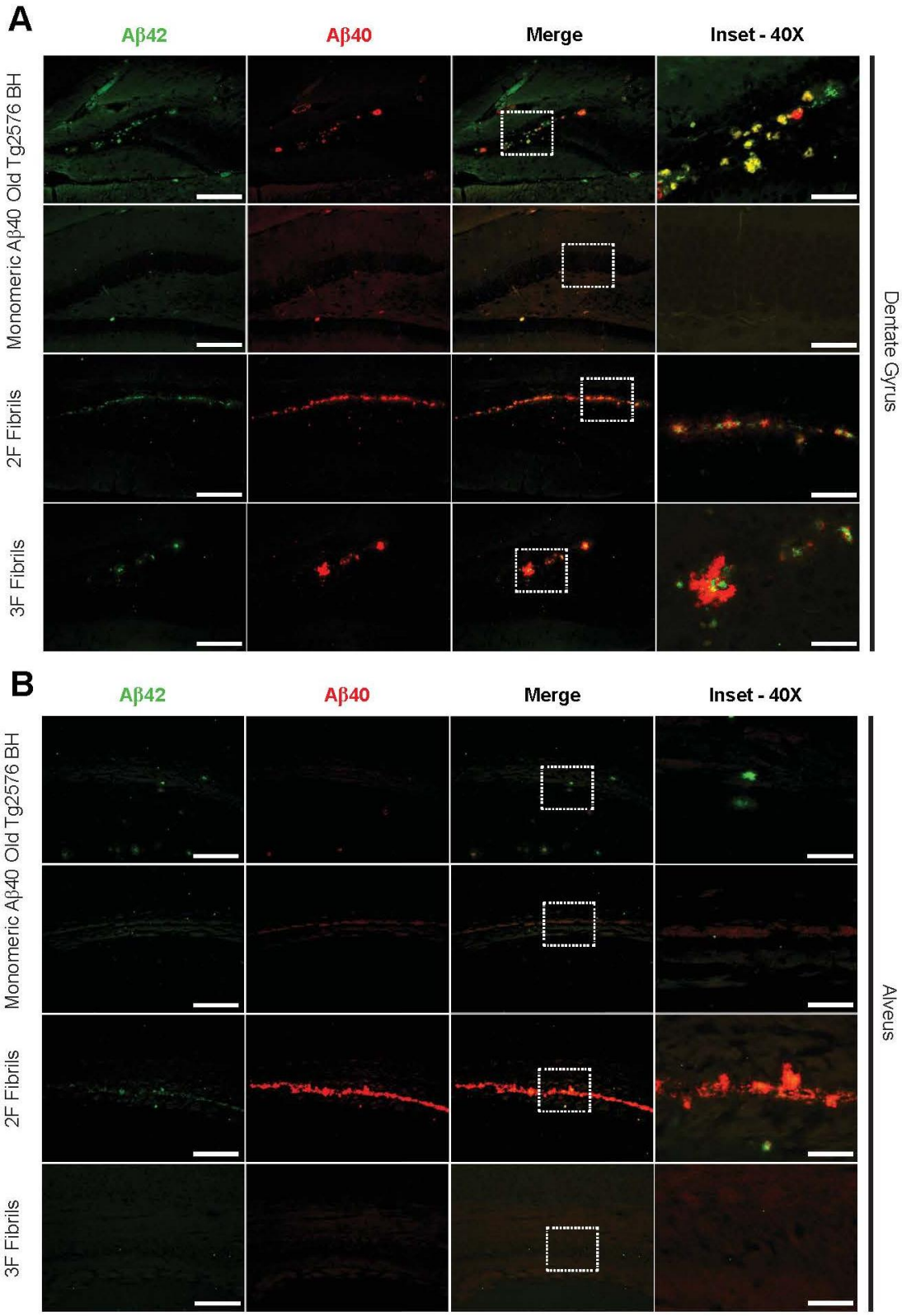


<u>Appendix Figure S6.</u> Needle track lesion and ThS reactivity of Aβ deposits seeded by OldTg2576 BH, and 2F and 3F seeds. A) Needle track lesion and amyloid deposition observed in Tg2576 mice treated with 2F fibrils (100 days post-injection). Details of Aβ composition (B-D) and ThS reactivity (E-G) of amyloid plaques generated by the inoculation of OldTg2576 BH (B, E), or 2F(C, F) or 3F (D, G) fibrils (300 days old group; details of representative images in Figure 2). Scale bar in A) represents 100 µm, and scale bar in D) represents 25 µm and applies to all micrographs from B-G. One tissue slice per animal was used in this study.

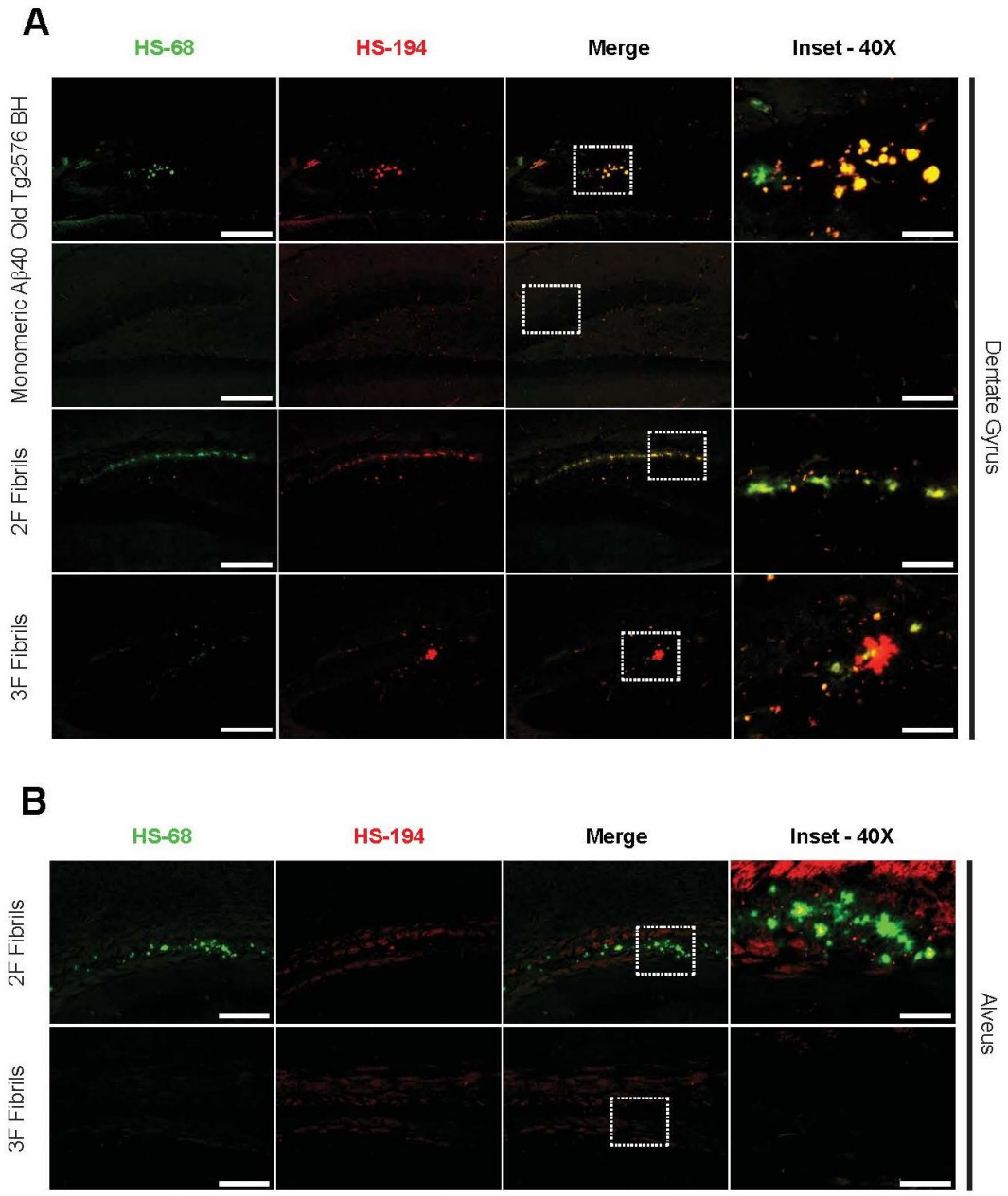


<u>Appendix Figure S7.</u> Amyloid pathology in Tg2576 mice 100 days after treatment with 2F- and 3F-derived aggregates. Representative pictures of hippocampi from mice treated with 2F- and 3F-derived seeds after visualization for Aβ deposits using the 4G8 antibody **(A-B).** Scale bar in (A) and (B) represents 500 µm and applies to all panels. Levels of insoluble $A\beta_{40}$ **(C)** and $A\beta_{42}$ **(D)** were measured using a commercially available multiplex immunoassay. Levels of both proteins were also added to quantify the total amount of both proteins **(E)**. Region-specific Aβ

burden quantification in the hippocampus **(F)**, hippocampal formation **(G)**, dentate gyrus **(H)** and alveus **(I)** of 2F- and 3F-fibrils treated mice. **(J)** Aβ visual score analysis in the lateral ventricles. Five tissue slices per animal were used in this study

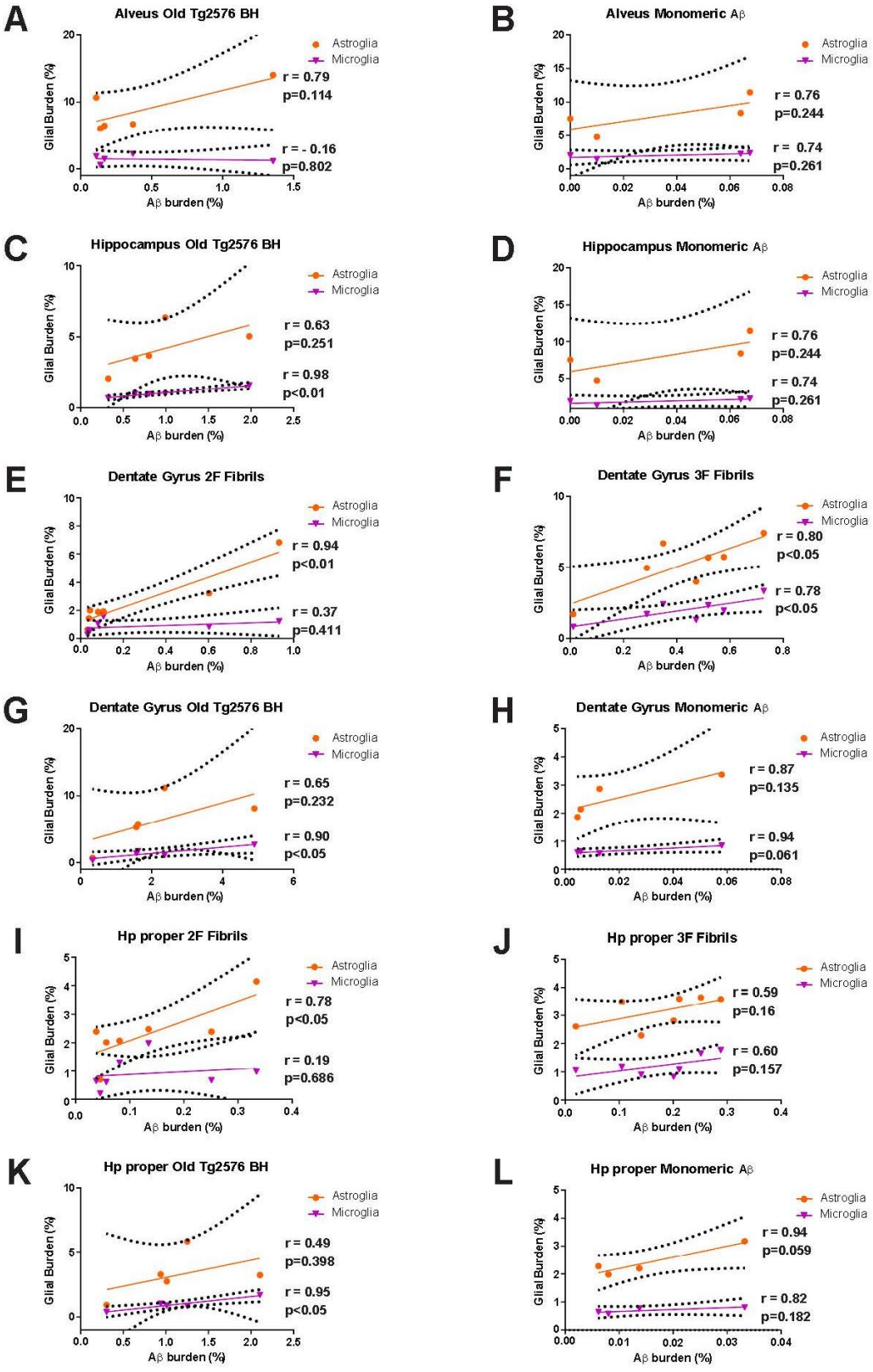

A
Aβ42
Aβ40
Merge
Inset - 40X
Old Tg2576 BH
Monomeric Aβ40
2F Fibrils
3F Fibrils
Dentate Gyrus
B
Aβ42
Aβ40
Merge
Inset - 40X
Old Tg2576 BH
Monomeric Aβ40
2F Fibrils
3F Fibrils
Alveus

<u>Appendix Figure S8.</u> $A\beta_{40}$- and $A\beta_{42}$-specific amyloid pathology in the dentate gyrus and alveus of experimental and control mice. Representative pictures of the dentate gyrus from mice treated with Tg2576-derived, monomeric $A\beta_{40}$ and 2F- and 3F-seeds after visualization by immunofluorescence of Aβ deposits using the $A\beta_{42}$- (green) and $A\beta_{40}$- (red) specific antibodies **(A)**. Representative pictures of the alveus from mice treated with Tg2576-derived, monomeric $A\beta_{40}$, 2F- and 3F-seeds after visualization by immunofluorescence of Aβ deposits using the $A\beta_{42}$- (green) and $A\beta_{40}$- (red) specific antibodies **(B)**. The third column of panels from left to right depict merged images. Right panels are insets obtained from the merged images (white punctuated squares). Scale bars at the left panels represent 200 µm and are applicable to the pictures labeled as "$A\beta_{42}$", "$A\beta_{40}$" and "Merge". Scale bars on the right images (Inset) represent 50 µm. One tissue slice per animal was used in this study.

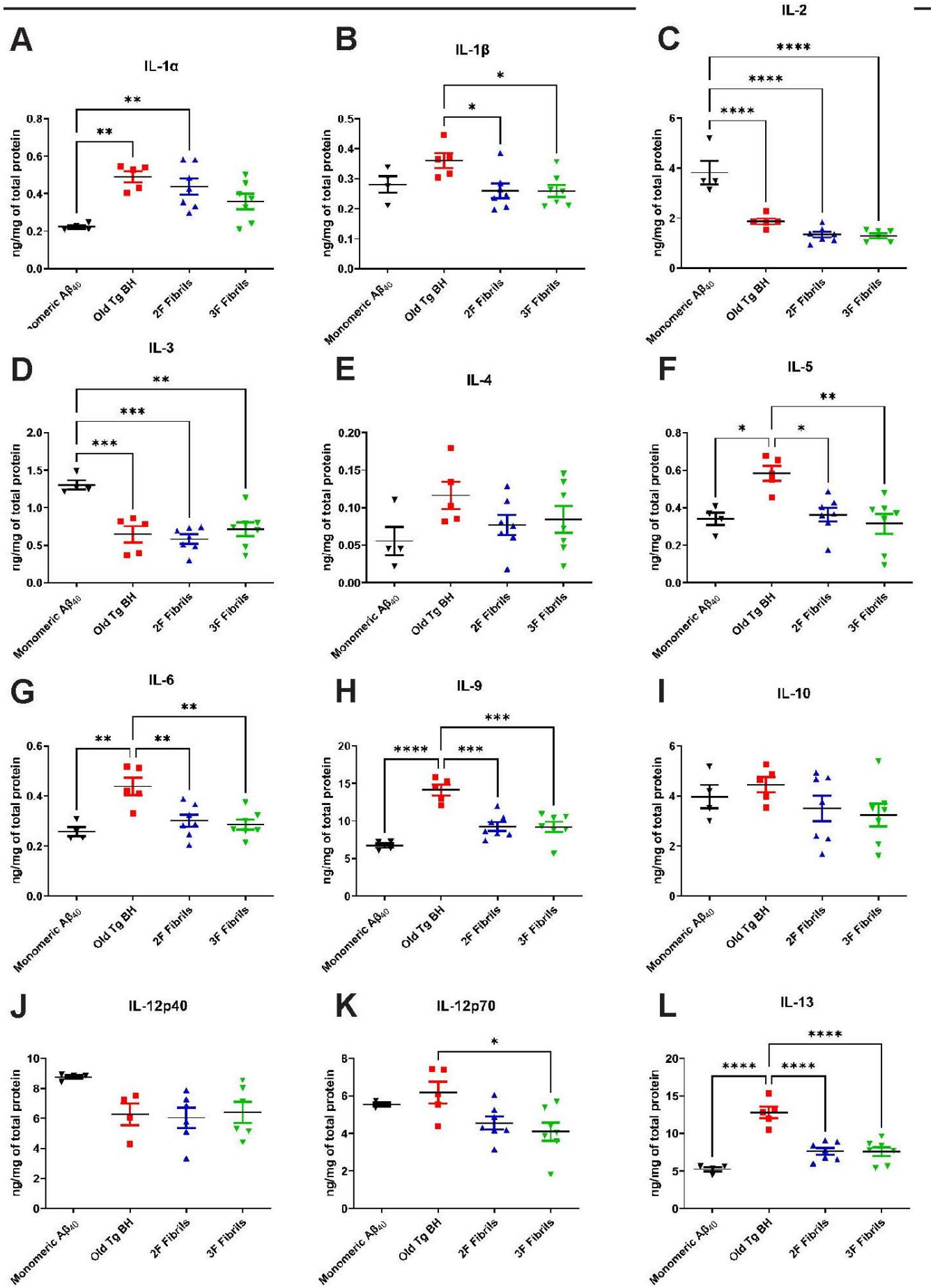


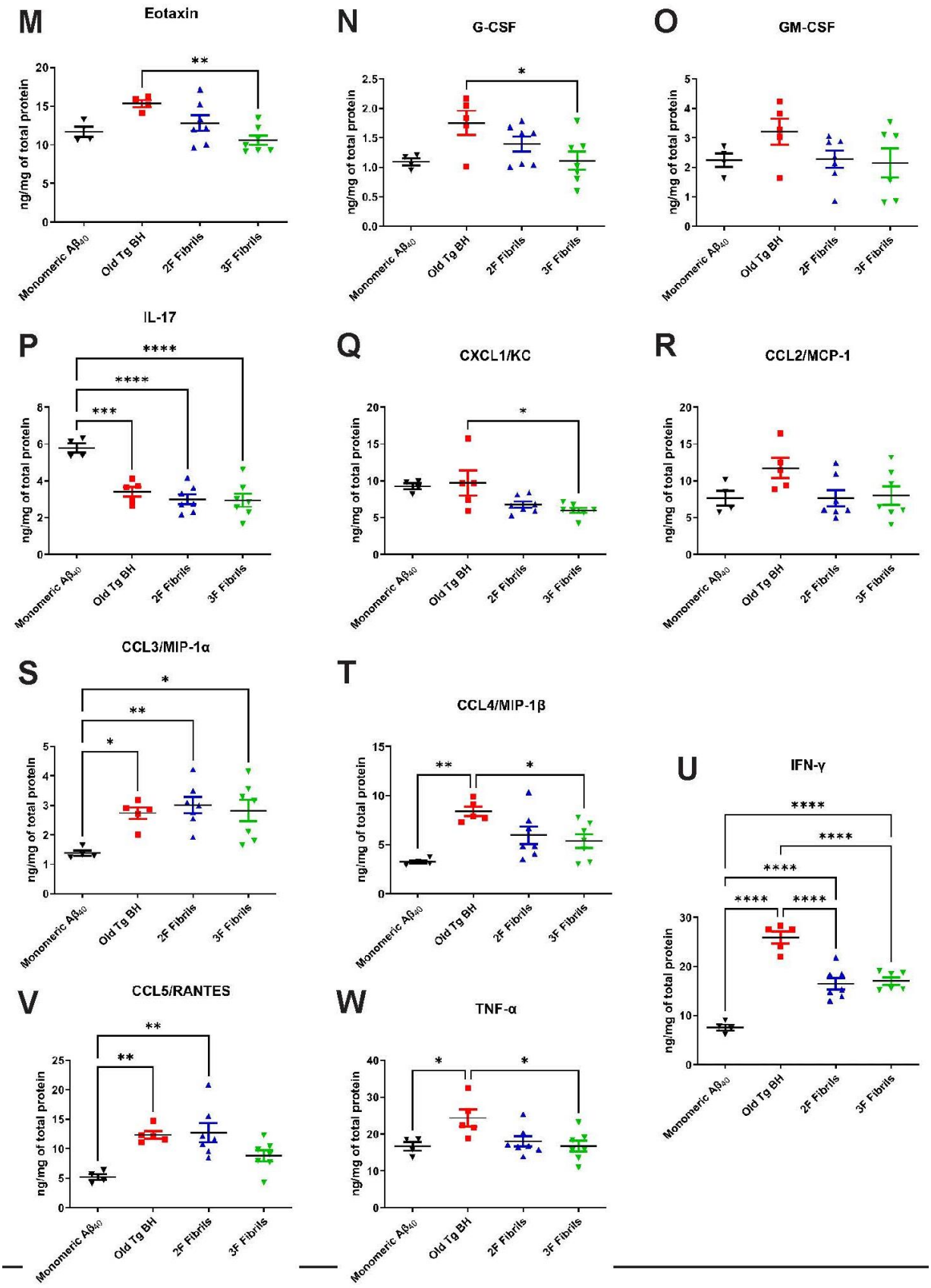
Appendix Figure S9. Luminescent conjugated oligothiophenes (LCOs) staining in the dentate gyrus and alveus of control and experimental mice. Representative pictures of the dentate gyrus **(A)** and alveus **(B)** of mice treated with Tg2576-derived, monomeric $A\beta_{40}$, 2F- and 3F-seeds after visualization by fluorescence of Aβ deposits using the HS-68 (left, green) and HS-194 (right, red) LCOs. The third column of panels from left to right depict merged images. Right

panels are insets obtained from the merged images (white punctuated squares). Scale bars at the left panels represent 200 μm and are applicable to the pictures labeled as “HS-68”, “HS-194” and “Merge”. Scale bars on the right images (Inset) represent 50 μm. One tissue slice per animal was used in this study.

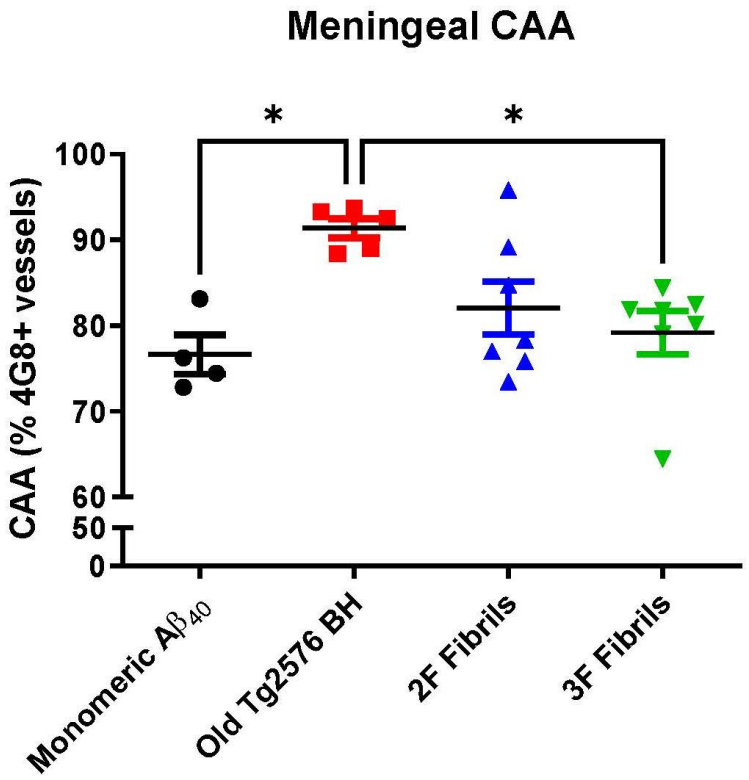

A
Alveus Old Tg2576 BH
Astroglia
Microglia
r = 0.79
p=0.114
r = - 0.16
p=0.802
Glial Burden (%)
Aβ burden (%)
B
Alveus Monomeric Aβ
r = 0.76
p=0.244
r = 0.74
p=0.261
C
Hippocampus Old Tg2576 BH
r = 0.63
p=0.251
r = 0.98
p<0.01
D
Hippocampus Monomeric Aβ
r = 0.76
p=0.244
r = 0.74
p=0.261
E
Dentate Gyrus 2F Fibrils
r = 0.94
p<0.01
r = 0.37
p=0.411
F
Dentate Gyrus 3F Fibrils
r = 0.80
p<0.05
r = 0.78
p<0.05
G
Dentate Gyrus Old Tg2576 BH
r = 0.65
p=0.232
r = 0.90
p<0.05
H
Dentate Gyrus Monomeric Aβ
r = 0.87
p=0.135
r = 0.94
p=0.061
I
Hp proper 2F Fibrils
r = 0.78
p<0.05
r = 0.19
p=0.686
J
Hp proper 3F Fibrils
r = 0.59
p=0.16
r = 0.60
p=0.157
K
Hp proper Old Tg2576 BH
r = 0.49
p=0.398
r = 0.95
p<0.05
L
Hp proper Monomeric Aβ
r = 0.94
p=0.059
r = 0.82
p=0.182

<u>Appendix Figure S10.</u> Glial burden and Aβ burden correlation in alveus, hippocampus, dentate gyrus and hippocampus proper of mice treated with Tg2576-derived, monomeric $A\beta_{40}$, 2F- and 3F-derived Aβ seeds. Correlations between Aβ burden and glial burden in the alveus **(A-B)**, hippocampus **(C-D)**, dentate gyrus **(G-H)** and hippocampus proper **(K-L)** were calculated for mice injected with Tg2576-derived brain homogenate **(A, C, G and K)**, monomeric $A\beta_{40}$ **(B, D, H and L)**. In the same way, the relationship between glial activation and Aβ burden in the dentate gyrus **(E-F)** and hippocampus proper **(I-J)** was calculated for mice treated 2F **(E and I)** and 3F (**F and J)** fibrils. Glial burden/Aβ burden correlation was statistically analyzed by using Pearson's correlation analysis and expressed as Pearson's Correlation coefficient (r). Significance of these correlations was also added to the graphs (p).

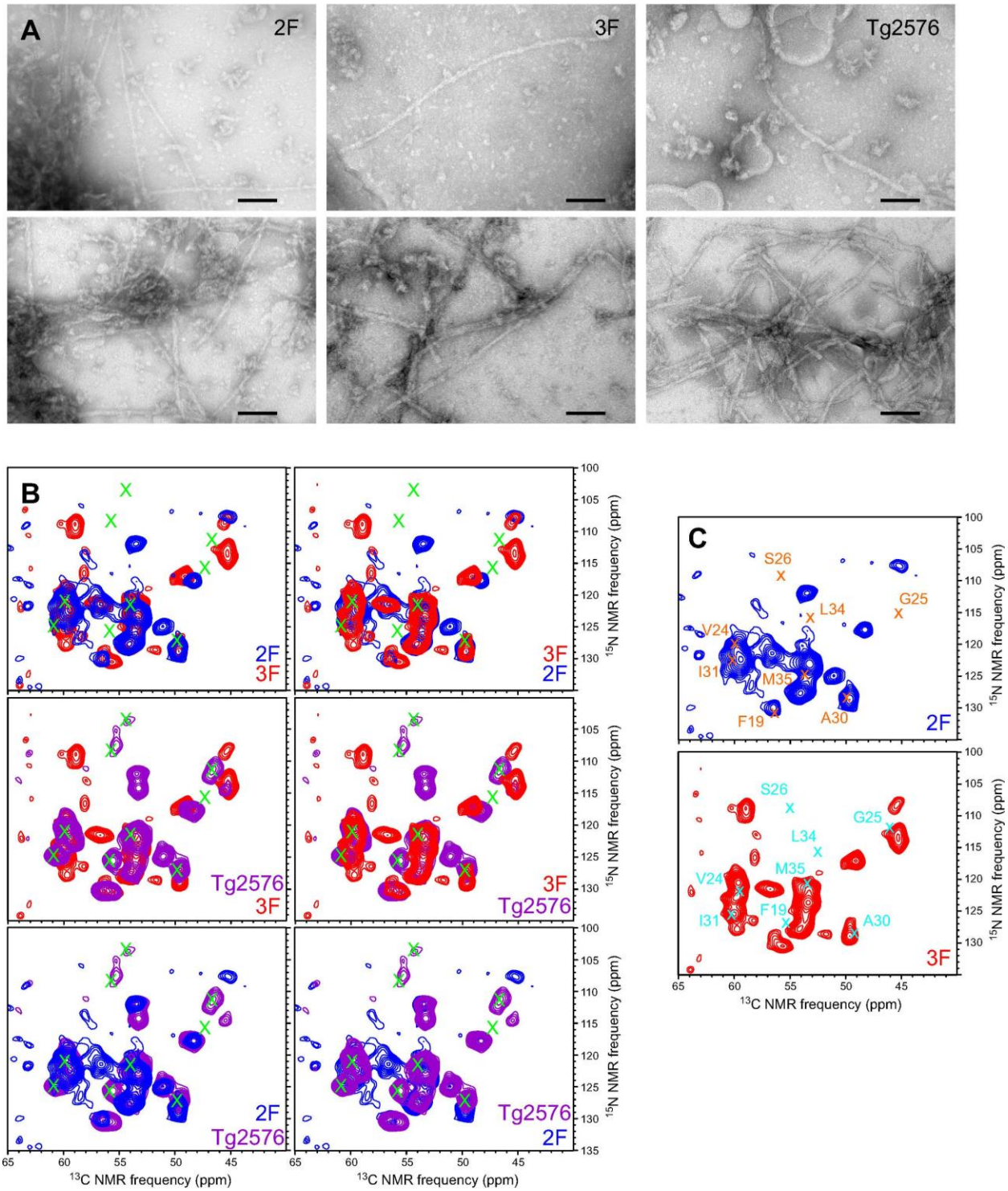

A
IL-1α
ng/mg of total protein
Monomeric Aβ40
Old Tg BH
2F Fibrils
3F Fibrils
B
IL-1β
C
IL-2
D
IL-3
E
IL-4
F
IL-5
G
IL-6
H
IL-9
I
IL-10
J
IL-12p40
K
IL-12p70
L
IL-13


M
Eotaxin
ng/mg of total protein
Monomeric Aβ40
Old Tg BH
2F Fibrils
3F Fibrils
N
G-CSF
O
GM-CSF
P
IL-17
Q
CXCL1/KC
R
CCL2/MCP-1
S
CCL3/MIP-1α
T
CCL4/MIP-1β
U
IFN-γ
V
CCL5/RANTES
W
TNF-α

<u>Appendix Figure S11.</u> 23-plex mouse cytokine/chemokine analyses in the brain of experimental and control mice. A panel of 23 cytokines/chemokines were measured in pbs-soluble (S1) brain fractions samples from mice treated with Tg2576-derived, monomeric $A\beta_{40}$, 2F- and 3F-seeds using the Bio-Plex Pro Mouse Cytokine 23-plex Assay. Concentrations of cytokines/chemokines, normalized to the total amount of protein in the S1 fraction (ng/mL), were significantly different among the groups for IL-1α **(A)**, IL-1β **(B)**, IL-2 **(C)**, IL-3 **(D)**, IL-5 **(F)**, IL-6 **(G)**, IL-9 **(H)**, IL-12p70 **(K)**, IL-13 **(L)**, Eotaxin **(M)**, G-CSF **(N)**, IL-17 **(P)**,CXCL1/KC **(Q)**, CCL3/MIP-1α **(S)**, CCL4/MIP-1β **(T)**, IFN-γ **(U)**, CCL5/RANTES **(V)** and TNF- α **(W)**. Statistical analyses were performed using One-way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Two brain aliquots per mice were used for cytokine measurements.

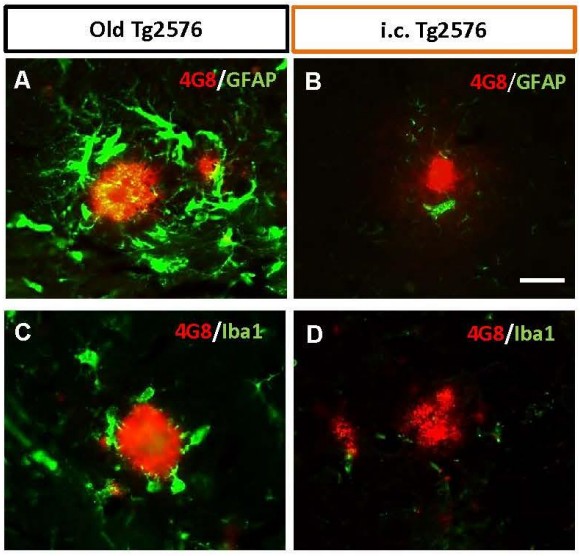


Appendix Figure S12. Quantification of meningeal cerebral amyloid angiopathy (CAA) in animals injected with 2F and 3F fibrils, monomeric $A\beta_{40}$ and brains extracts from old Tg2576 mice. Quantification of the percentage of total meningeal vessels that are 4G8-positive in the different animal groups. Statistical analyses were performed using One-way ANOVA (*$p<0.05$). Five tissue slices per animal were used in this study.

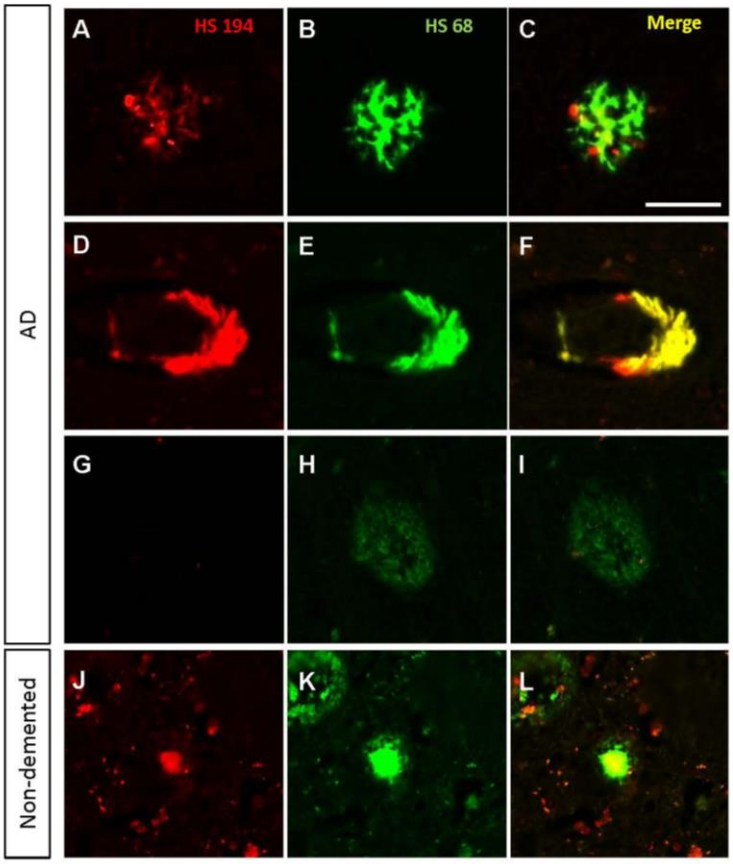


<u>Appendix Figure S13.</u> Additional characterization of brain-seeded, isotopically labeled Aβ$_{40}$ fibrils and ssNMR data. A) Negative stain TEM images of fibrils that were prepared *in vitro* by seeded growth from amyloid-containing extracts of mouse brain homogenates. Images of the three samples were acquired after 4 h incubation (top row) and after 4-5 days incubation (bottom

row). Bars represent 100 nm. **B)** Superpositions of the 2D ^{15}N-^{13}C ssNMR spectra of samples that were derived from mice that had been treated with 2F-, 3F-, or Tg2576-derived seeds (blue, red, or purple contours, respectively). Two superpositions are shown for each pair of spectra, with each of the spectra in the foreground. As an additional comparison, green X's indicate the positions of crosspeaks in spectra of $A\beta_{40}$ fibrils with the same isotopic labeling pattern that were prepared from cortical tissue extracts of typical Alzheimer's disease patients, as reported by Qiang *et al.* (Qiang et al., 2017b). **C)** Comparison of 2D ^{15}N-^{13}C ssNMR spectra of $A\beta_{40}$ fibrils that were derived from 2F- and 3F-treated mice with crosspeak positions in spectra of 2F and 3F fibrils with the same isotopic labeling pattern (orange and cyan X's, respectively). One technical replicate per group was used in this study.



Appendix Figure S14. Naturally occurring Aβ aggregates in Tg2576 mice induce a stronger glial response compared with Old Tg2576-seeded deposits. Representative Aβ deposits found in aged Tg2576 mice (A, C) and Tg2576 mice i.c. inoculated with Old Tg2576 brain extracts (B, D). Amyloid deposits are depicted in red, and either GFAP (A, B) or Iba-1 (C, D) signals are depicted in green. The bar in panel (B) represents 25 µm and applies to all micrographs. One tissue slice per animal was used in this study.



<u>Appendix Figure S15.</u> Reactivity of human Aβ deposits to HS-194 and HS-68 LCOs. Different brain amyloid deposits in the temporal cortex of an AD patient and a non-demented individual (AD patient, panels A-I; non-demented individual, panels J-L) were tested for their LCOs binding. Plaques shown included parenchymal deposits with different morphologies (panels A-C, G-I, J-L) and one vascular deposit (D-F). Scale bar in panel (C) represents 50 µm and applies to all micrographs. One tissue slice per individual was used in this study.