Supporting Information

Watts et al. 10.1073/pnas.1408900111

SI Materials and Methods

Mouse Inoculations. Brain tissue samples from human control or Alzheimer's disease cases were homogenized to 10% (wt/vol) in calcium- and magnesium-free PBS (Life Technologies) using a Precellys 24 bead beater (Bertin Technologies) and then diluted to 1% using 5% BSA before inoculation. Extracts were not sonicated before inoculation. Weanling (~2-mo-old) Tg(APP23:*Gfap*-luc) mice were anesthetized with isoflurane and then inoculated in the right parietal lobe with 30 μL 1% brain homogenate using a 27-gauge syringe. At the completion of an experiment (typically 330 d postinoculation), brains were removed and either snap-frozen on dry ice for biochemical analyses (male animals) or fixed in formalin for neuropathology (female animals).

Bioluminescence Imaging. Bioluminescence imaging on the brains of bigenic Tg(APP23:*Gfap*-luc) mice was performed essentially as previously described (1). Briefly, mice were imaged after receiving an i.p. injection of 50 μL 30 mg/mL D-luciferin potassium salt solution (Gold Biotechnology) that was prepared in PBS, pH 7.4 (a dose of ~60 mg/kg). After luciferin injection, mice were placed in an anesthetization chamber and exposed to an isoflurane/oxygen gas mix for 10 min. During this time, the heads of the mice where shaved bald to enhance the bioluminescence signal. After anesthetization, mice were placed in an IVIS Lumina II imaging system (PerkinElmer) and kept under constant anesthesia. Black construction paper cutouts were placed over the ears to prevent extraneous signals. Mice were imaged for 60 s and then allowed to recover in their home cages. Brain bioluminescence values were calculated from images displaying surface radiance using circular regions of interest and then converted to total photon flux (photons per second) using Living Image software version 4.3 (PerkinElmer). All experiments used an equal number of male and female mice, except where noted. The incubation period for individual mice was calculated as the time from inoculation to when two successive scans revealed bioluminescence values of greater than 1×10^6 photons/s.

Conformational Stability Assays. Ten percent (wt/vol) brain homogenates were prepared in calcium- and magnesium-free PBS using an Omni Tip (Omni International) with a PowerGen homogenizer (Fisher Scientific). Nine volumes 10% (wt/vol) brain homogenate were added to one volume 10× detergent buffer [5% (vol/vol) Nonidet P-40, 5% (wt/vol) sodium deoxycholate in PBS] and then incubated on ice for 20 min. Samples were centrifuged at 1,000 × *g* for 5 min, and the supernatants were removed; 20 μL detergent-extracted brain homogenate was mixed with 2× stocks of guanidine hydrochloride (GdnHCl) to give final concentrations of 1, 2, 2.5, 3, 3.5, or 4 M GdnHCl. For the 4.5, 5, 5.5, and 6 M samples, only 10 μL brain homogenate was used in a final reaction volume of 40 μL. Samples were incubated at 22 °C with shaking (800 rpm) for 2 h and then diluted to 0.4 M GdnHCl in 1× detergent buffer (final volume of 500 μL). Proteinase K (PK) was added to a final concentration of 20 μg/mL, and the samples were digested at 37 °C with shaking for 1 h. Digestions were then terminated by adding PMSF to a final concentration of 2 mM. Sarkosyl solution [100 μL 12% (vol/vol)] was then added to give a final concentration of 2%. Samples were then ultracentrifuged at 100,000 × *g* for 1 h at 4 °C, and the supernatants were removed by gentle aspiration. Pellets were resuspended in 1× NuPAGE loading buffer by vortexing, boiled for 10 min, and then analyzed by immunoblotting as described above. Films were scanned using a CCD camera (FluorChem 880; Alpha Innotech), and then, densitometry was performed using Image J. $[GdnHCl]_{1/2}$ values were calculated using the variable slope (four-parameter) function in Prism 5.

Immunoblotting and PK Digestions. Ten percent (wt/vol) brain homogenates were prepared in calcium- and magnesium-free PBS using an Omni Tip (Omni International) with a PowerGen homogenizer (Fisher Scientific) or a Precellys 24 bead beater. Nine volumes 10% (wt/vol) brain homogenate were added to one volume 10× detergent buffer [5% (vol/vol) Nonidet P-40, 5% (wt/vol) sodium deoxycholate in PBS] and then incubated on ice for 20 min. Samples were centrifuged at 1,000 × *g* for 5 min, and the supernatants were removed. Protein concentrations in the supernatant fraction were determined using the BCA protein assay (Thermo Scientific). One milligram detergent-extracted protein was diluted to a final volume of 398 μL 1× detergent buffer [0.5% (vol/vol) Nonidet P-40, 0.5% (wt/vol) sodium deoxycholate in PBS]; 2 μL 10 mg/mL PK stock solution (Fermentas) was then added to the samples, resulting in a final PK concentration of 50 μg/mL (a PK:protein ratio of 1:50). Samples were then incubated at 37 °C with shaking for 1 h. PK digestions were terminated by the addition of PMSF to a final concentration of 2 mM, and then, sarkosyl was added to a final concentration of 2% (vol/vol). Samples were then ultracentrifuged at 100,000 × *g* for 1 h at 4 °C, and the supernatants were removed by aspiration. Pellets were resuspended in 1× NuPAGE loading buffer by vortexing and boiled, and then, 200–400 μg PK-digested total protein was loaded onto 10% or 4–12% NuPAGE gels (Life Technologies). SDS/PAGE was then performed using the MES buffer system, and gels were subsequently transferred to PVDF membranes using a wet blotting system. Membranes were blocked for 2 h at room temperature using blocking buffer [5% (wt/vol) nonfat milk in Tris-buffered saline containing 0.05% (vol/vol) Tween-20 (TBST)] and then incubated with primary antibody overnight at 4 °C. Blots were washed three times with TBST, incubated with HRP-conjugated secondary antibodies (Bio-Rad) or streptavidin (Pierce) diluted in blocking buffer for 2 h at room temperature, and then washed three times with TBST. Blots were developed using the enhanced chemiluminescent detection system (GE Healthcare) and then exposed to X-ray film. The following primary antibodies were used: biotinylated anti-human amyloid-β (Aβ) antibody 6E10 (1:4,000 dilution; Covance) and anti-Aβ38 antibody 7-14-4 (1:1,000 dilution; Covance).

Quantification of Aβ Peptide Levels by ELISA. Ten percent (wt/vol) brain homogenates were normalized for protein concentration by the BCA assay. Two volumes cold formic acid were added to one volume brain homogenate followed by a 5-min sonication using a water bath sonicator. Samples were then centrifuged at 100,000 × *g* for 1 h at 4 °C, and the supernatants were neutralized by the addition of 19 volumes 1 M Tris·HCl and 500 mM sodium phosphate (pH unadjusted). After additional dilution, Aβ peptide levels were quantified using sandwich ELISAs specific for each peptide according to the manufacturer's instructions. Aβ(x–40) and Aβ(x–42) ELISA kits were purchased from Life Technologies, and Aβ(1–38) ELISA kits were obtained from IBL International.

Thioflavin Staining. For Thioflavin S (Thio S) staining, slides were baked at 50 °C overnight, deparaffinized with xylenes, and rehydrated with alcohols. Sections were stained with 0.05% (wt/vol) Thio S (Sigma-Aldrich) in 50% (vol/vol) ethanol in the

dark for 8 min, and residual Thio S was removed by a rapid rinse in distilled water. Slides were mounted with Vectashield with DAPI (Vector Laboratories) aqueous mount and viewed with a Leica DM-RB microscope outfitted with an Olympus DP-70 camera.

Quantification of Aβ Staining in Blood Vessels. The number of Aβ38-positive blood vessels in inoculated mice was determined by inspection of whole-brain sections stained with the antibody BA1-13 and visualized using 3–3′-diaminobenzidine. Vessel counts were conducted in the thalamus and leptomeninges (anterior/posterior = −1.8 mm) as well as the frontal cortex (anterior/posterior = 0.8 mm). The number of furry Aβ cerebral amyloid angiopathy deposits in the thalamus of inoculated mice was quantified using 4G8-stained whole-brain sections. Sections from three to six mice per inoculum were quantified.

For quantification of the relative amounts of various Aβ peptides in cerebral amyloid angiopathy deposits, Aβ40 (11A50-B10 antibody) and Aβ38 (7-14-4 antibody) fluorescence intensities of individual blood vessels were imaged using a Leica SP8 confocal microscope with a 40× water immersion lens (1.1 N.A.) in sequential scan mode at 1,024 × 1,024-pixel resolution and then analyzed with National Institutes of Health ImageJ software. A *z* projection of three confocal slices was prepared from each image stack and used for analysis. A standard region of interest was used to measure the intensities of Aβ40 and Aβ38 fluorescence along the length of the vessel walls. Multiple regions of interest were acquired per individual vessel segment and used to calculate the average fluorescence for both Aβ40 and Aβ38. Brain slices from three animals per experimental group were used for quantification.

Statistical Analysis. All statistical analyses were performed using Prism 5 software (GraphPad Software). Statistical differences between groups of mice were assessed using unpaired, two-tailed *t* tests or by performing one-way ANOVA with Tukey multiple comparison posttest. A significance threshold of $P = 0.05$ was used for all experiments.

1. Watts JC, et al. (2011) Bioluminescence imaging of Abeta deposition in bigenic mouse models of Alzheimer's disease. *Proc Natl Acad Sci USA* 108(6):2528–2533.

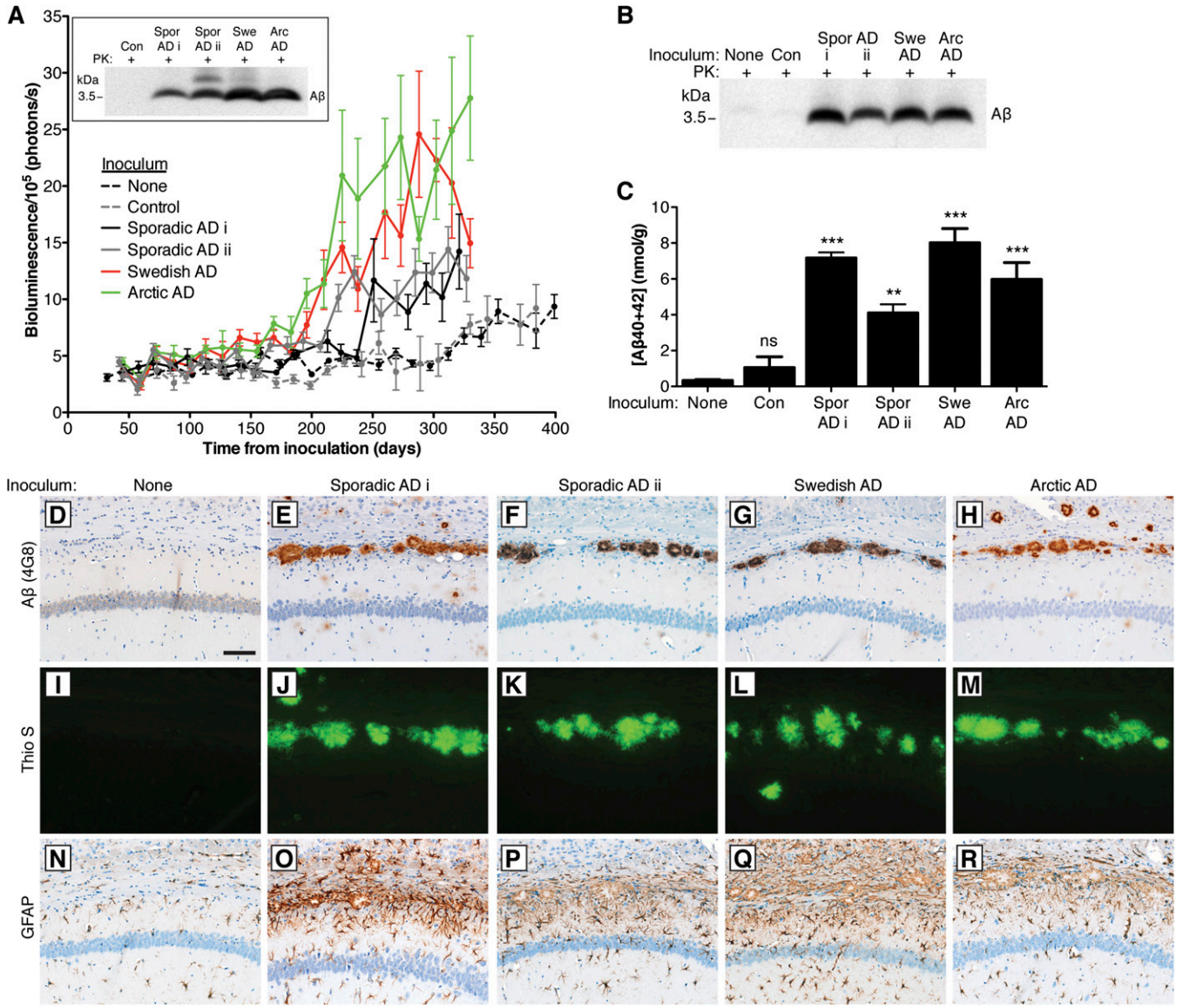

Fig. S1. Induction of Aβ deposition and astrocytic gliosis in Tg(APP23:*Gfap*-luc) mice inoculated with various AD brain homogenates. (*A*) Bioluminescence imaging curves for uninoculated mice (dashed black line; $n = 12$), mice inoculated with control (dashed gray line; $n = 6$), Swedish AD (solid red line; $n = 12$), Arctic AD (solid green line; $n = 12$), sporadic AD case i (solid black line; $n = 8$), and sporadic AD case ii (solid gray line; $n = 10$) brain homogenate. *Inset* shows an immunoblot of the relative amounts of PK-resistant total Aβ (6E10 antibody) in the various inocula. (*B*) Immunoblot of PK-resistant Aβ levels in the brains of inoculated male Tg(APP23:*Gfap*-luc) mice at 330 dpi. Higher levels of aggregated, PK-resistant Aβ were observed in the mice inoculated with the various AD brain homogenates compared with uninoculated mice and control-inoculated mice. Aβ peptide was detected with the antibody 6E10. (*C*) Quantification of formic acid-extractable Aβ40 and Aβ42 levels in the brains of inoculated male mice (330 dpi) by ELISA. Significantly higher levels of Aβ were observed in the mice inoculated with the various AD brain homogenates compared with control-inoculated mice. (*D*–*R*) Neuropathology of female Tg(APP23:*Gfap*-luc) mice at 330 dpi with (*E*, *J*, and *O*) sporadic AD case i, (*F*, *K*, and *P*) sporadic AD case ii, (*G*, *L*, and *Q*) Swedish AD, or (*H*, *M*, and *R*) Arctic AD brain homogenate. (*D*, *I*, and *N*) Sections from an age-matched, uninoculated mouse at 390 d of age are shown as a control. Tissue sections were stained with (*D*–*H*) anti-Aβ antibody 4G8, (*I*–*M*) Thio S, or (*N*–*R*) anti-GFAP antibody. The hippocampus and corpus callosum are shown in all panels. An increase in GFAP immunostaining and Thio S-positive Aβ deposits was observed in the AD-inoculated animals. (Scale bar: *D*–*R*, 100 μm.) AD, Alzheimer's disease; Arc, Arctic; Con, control; dpi, days postinoculation; ns, not significant; Spor, sporadic; Swe, Swedish. $^{**}P < 0.01$; $^{***}P < 0.001$.

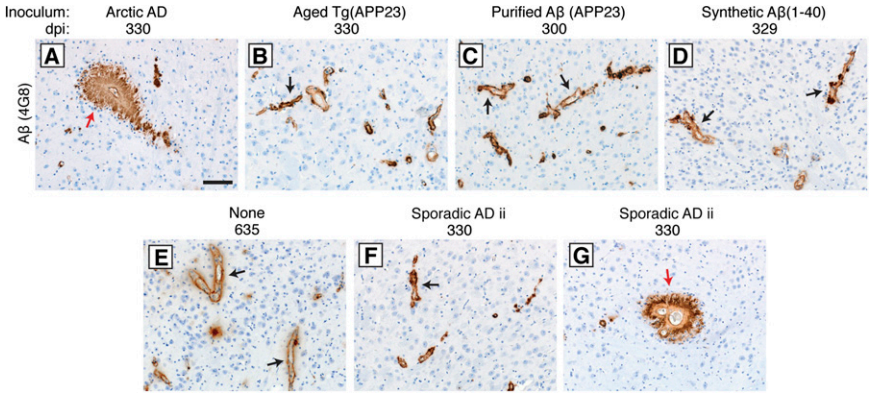

Fig. S2. CAA morphology in Tg(APP23:*Gfap*-luc) mice inoculated with various Aβ aggregate-containing preparations. CAA in the thalamus of female Tg (APP23:*Gfap*-luc) mice inoculated with (*A*) Arctic AD brain homogenate, (*B*) aged Tg(APP23) brain homogenate, (*C*) purified Aβ fibrils from the brains of aged Tg(APP23) mice, (*D*) Aβ aggregates composed of synthetic Aβ(1–40) peptide, or (*F* and *G*) sporadic AD (case ii) brain homogenate. Slides were prepared from mice euthanized at the indicated dpi. (*E*) An uninoculated mouse at 635 d of age is shown as a control. A thin, compact layer of Aβ deposition surrounding blood vessels in the thalamus (black arrows) was observed in all mice except the Arctic AD-inoculated animals, which exhibited furry CAA deposits (red arrows). (*G*) Rare furry blood vessels were also found in mice inoculated with sporadic AD case ii. Aβ deposits were stained with the antibody 4G8. (Scale bar: 100 μm.) AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; dpi, days postinoculation.

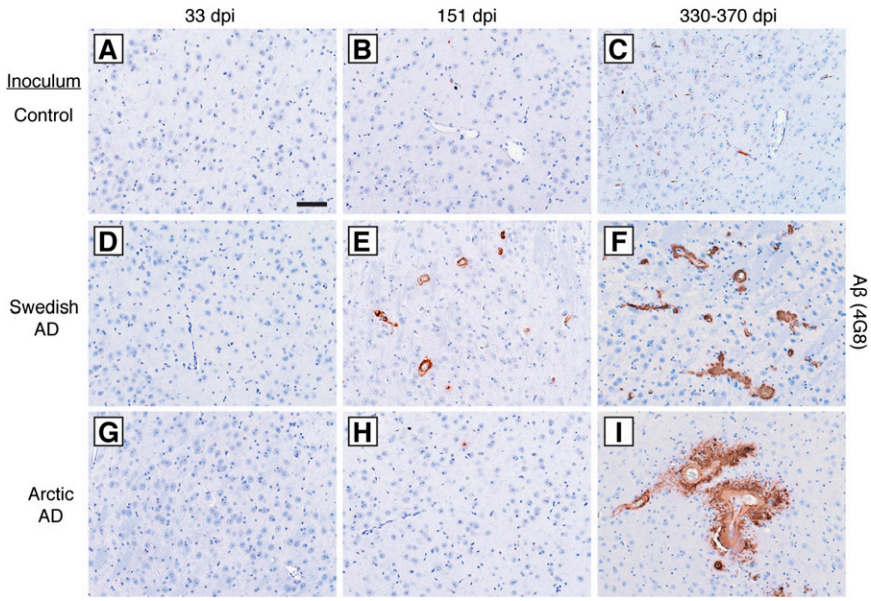

Fig. S3. Time course of induced thalamic CAA in Tg(APP23:*Gfap*-luc) mice inoculated with AD and control brain homogenates. Aβ deposition in the thalamus of female Tg(APP23:*Gfap*-luc) mice at (*A*, *D*, and *G*) 33, (*B*, *E*, and *H*) 151, (*F* and *I*) 330, or (*C*) 370 dpi with (*A*–*C*) control, (*D*–*F*) Swedish AD, or (*G*–*I*) Arctic AD brain homogenate. No Aβ deposits were observed at 33 dpi, indicating that the Aβ deposits observed at later time points are not composed of residual inoculum. Aβ deposits were stained with the antibody 4G8. (Scale bar: 100 μm.) AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; dpi, days postinoculation.

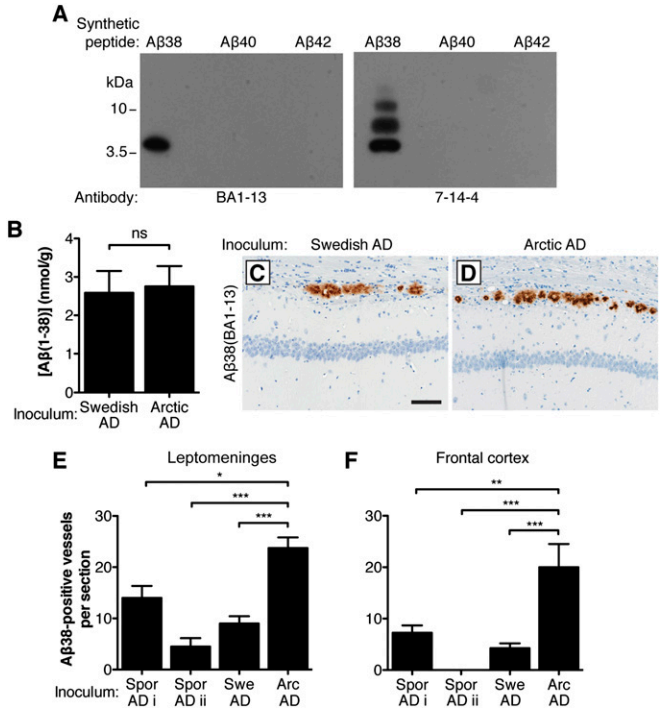

Fig. S4. Increased vascular, but not plaque-associated, Aβ38 deposition in Tg(APP23:*Gfap*-luc) mice inoculated with Arctic AD brain homogenate. (*A*) An immunoblot of synthetic Aβ38, Aβ40, and Aβ42 peptides reveals that both the BA1-13 and 7-14-4 antibodies are specific for the Aβ38 peptide. (*B*) Quantification of formic acid-extractable Aβ38 peptide levels in the brains of male Tg(APP23:*Gfap*-luc) mice at 330 dpi with Swedish or Arctic AD brain homogenate ($n = 6$ each). No significant difference in Aβ38 levels was observed. (*C* and *D*) Aβ38 immunostaining on brain sections from female Tg(APP23:*Gfap*-luc) mice at 330 dpi with (*C*) Swedish AD or (*D*) Arctic AD brain homogenate. The hippocampus and corpus callosum are shown in *C* and *D*. The induced Aβ deposits in the corpus callosum were immunopositive for Aβ38 in both the Swedish AD- and Arctic AD-inoculated animals. Aβ38 deposition was detected using the antibody BA1-13. (Scale bar: *C* and *D*, 100 μm.) (*E* and *F*) Quantification of Aβ38-positive blood vessels in the (*E*) leptomeninges or (*F*) frontal cortex of female Tg(APP23:*Gfap*-luc) mice at 330 dpi with the indicated brain homogenates ($n = 4$ each). Significantly more Aβ38 deposits were observed in the Arctic AD-inoculated animals. AD, Alzheimer's disease; Arc, Arctic; dpi, days postinoculation; ns, not significant; Spor, sporadic; Swe, Swedish. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

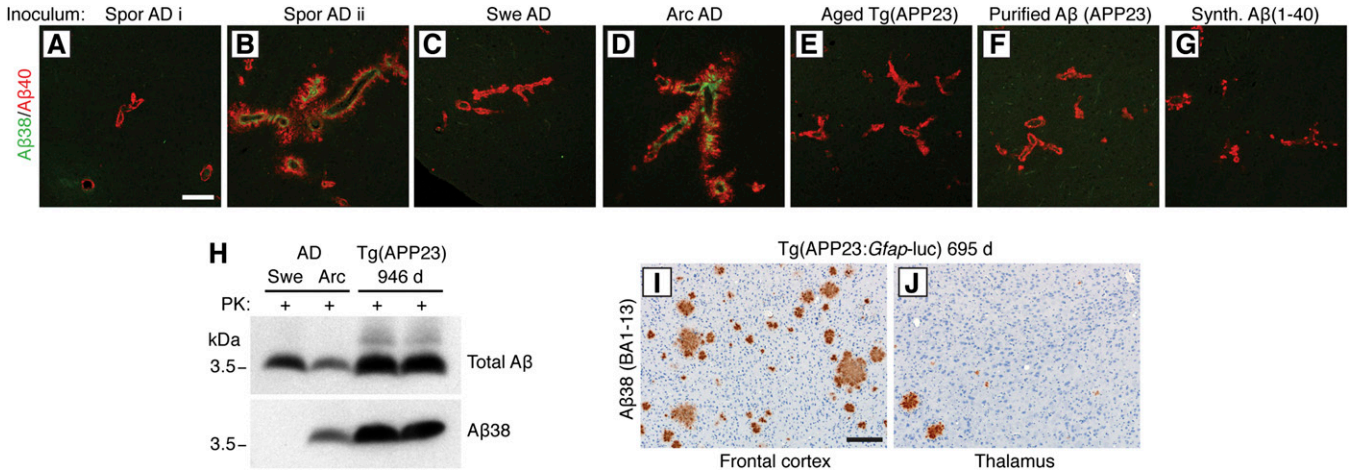

Fig. S5. Spontaneous Aβ38 deposition in aged Tg(APP23) mice and lack of thalamic Aβ38 deposition in Tg(APP23:*Gfap*-luc) mice inoculated with various Aβ aggregate-containing preparations. (*A*–*G*) Double labeling of Aβ38 (green) and Aβ40 (red) in the thalamus of female Tg(APP23:*Gfap*-luc) mice at 330 dpi with brain homogenate from (*A*) sporadic AD case i, (*B*) sporadic AD case ii, (*C*) Swedish AD, (*D*) Arctic AD, or (*E*) an aged Tg(APP23) mouse or with (*F*) purified Aβ fibrils from the brains of aged Tg(APP23) mice or (*G*) Aβ aggregates composed of synthetic Aβ(1–40) peptide. Codeposition of Aβ38 and Aβ40 peptides was observed in the mice inoculated with Arctic AD and sporadic AD case ii. Aβ38 deposition was detected using the antibody BA1-13; Aβ40 was detected with antibody 11A50-B10. (Scale bar: *A*–*G*, 100 μm.) (*H*) Immunoblot of PK-resistant (*Upper*) total Aβ and (*Lower*) Aβ38 levels in human Arctic and Swedish AD brains as well as the brains from two 946-d-old Tg(APP23) mice. High levels of aggregated, PK-resistant Aβ38 were observed in the aged Tg(APP23) brains. Total Aβ was probed with antibody 6E10; Aβ38 was detected using the antibody 7-14-4. (*I* and *J*) Aβ38 immunostaining on brain sections from a 695-d-old Tg(APP23:*Gfap*-luc) mouse. (*I*) Abundant Aβ38-containing plaques were observed in the frontal cortex, and (*J*) moderate numbers were observed in the thalamus. Notably, no Aβ38-positive CAA was observed in the thalamus. Aβ38 deposition was detected using the antibody BA1-13. (Scale bar: *I* and *J*, 100 μm.) AD, Alzheimer's disease; Arc, Artic; CAA, cerebral amyloid angiopathy; dpi, days postinoculation; Spor, sporadic; Swe, Swedish; Synth, synthetic.

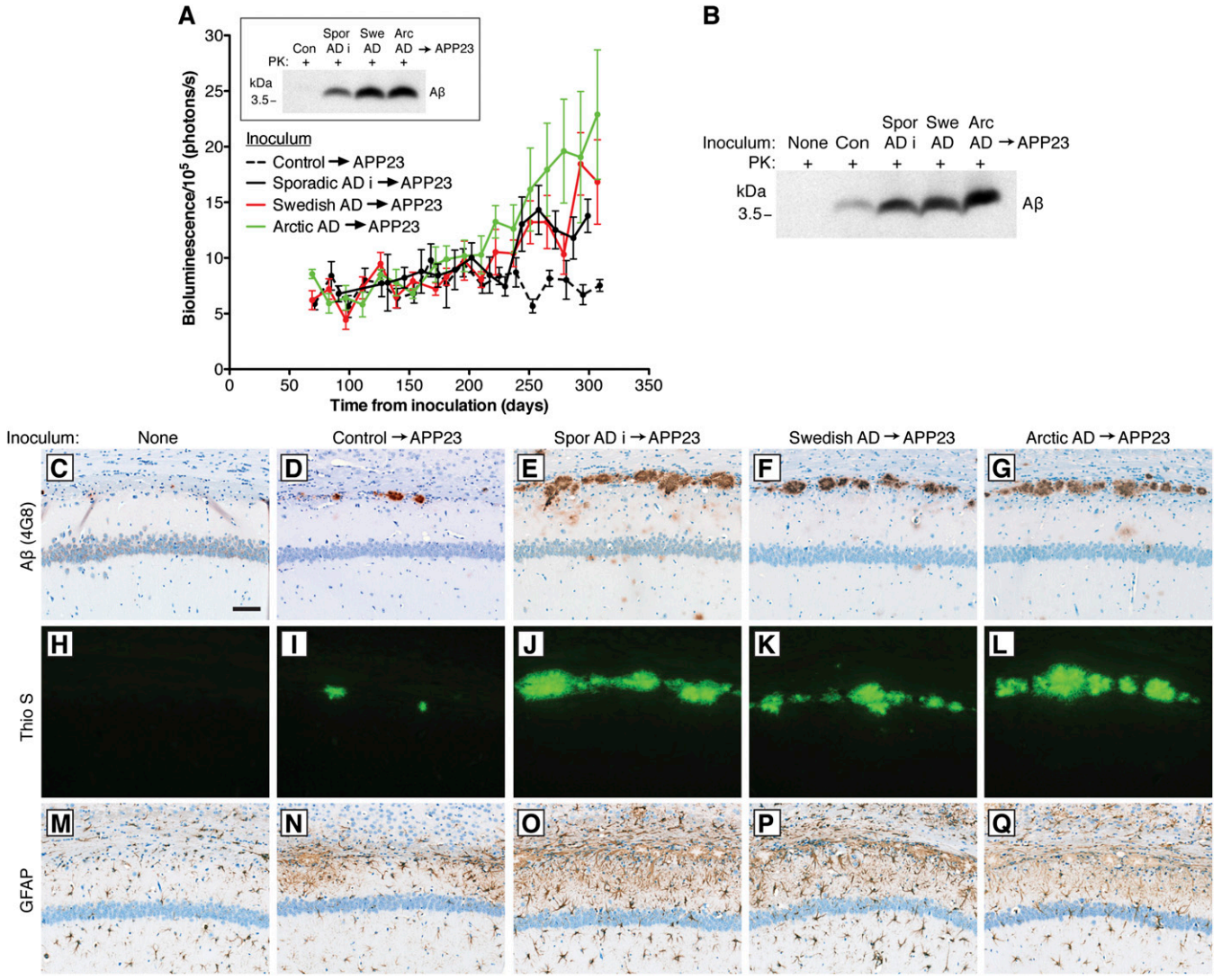

Fig. S6. Induction of Aβ deposition and astrocytic gliosis in Tg(APP23:*Gfap*-luc) mice on serial passage of AD brain homogenates. (*A*) Bioluminescence imaging curves for the second passage of control (dashed black line; $n = 10$), Swedish AD (solid red line; $n = 8$), Arctic AD (solid green line; $n = 6$), and sporadic AD case i (solid black line; $n = 10$) samples. *Inset* shows an immunoblot of the relative amounts of PK-resistant total Aβ (6E10 antibody) in the various inocula. (*B*) Immunoblot of PK-resistant Aβ levels in the brains of male Tg(APP23:*Gfap*-luc) mice at 330 dpi with the indicated passaged samples. An uninoculated mouse (None) at 390 d of age is also shown. Higher levels of aggregated, PK-resistant Aβ were observed in the mice inoculated with the various passaged AD brain homogenates, although some PK-resistant Aβ was also observed in the mice inoculated with the passaged control sample. (*C*–*Q*) Neuropathology of female Tg (APP23:*Gfap*-luc) mice at 330 dpi with (*D*, *I*, and *N*) passaged control, (*E*, *J*, and *O*) sporadic AD case i, (*F*, *K*, and *P*) Swedish AD, or (*G*, *L*, and *Q*) Arctic AD brain homogenate. (*C*, *H*, and *M*) Sections from an uninoculated mouse at 390 d of age are also shown. Tissue sections were stained with (*C*–*G*) anti-Aβ 4G8 antibody, (*H*–*L*) Thio S, or (*M*–*Q*) anti-GFAP antibody. The hippocampus and corpus callosum are shown in all panels. A pronounced increase in GFAP immunostaining and Thio S-positive Aβ deposits was observed in the animals inoculated with the passaged AD samples compared with uninoculated mice. Small amounts of Aβ deposition and a mild increase in GFAP staining were observed in mice inoculated with the passaged control sample. (Scale bar: *D*–*Q*, 100 μm.) AD, Alzheimer's disease; Arc, Artic; Con, control; dpi, days postinoculation; Spor, sporadic; Swe, Swedish.

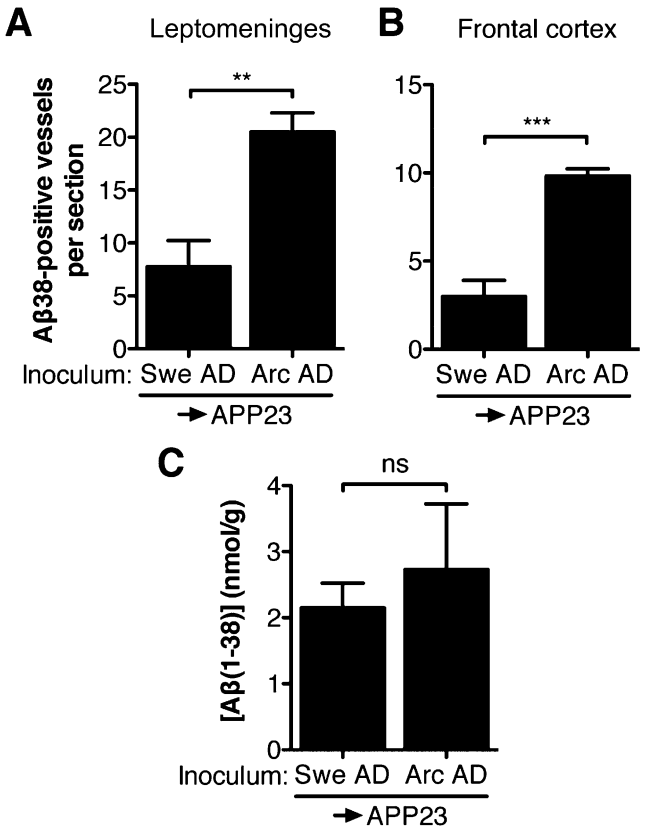

Fig. S7. Increased vascular Aβ38 deposition in Tg(APP23:*Gfap*-luc) mice on serial passage of Arctic AD brain homogenate. Quantification of Aβ38-positive blood vessels in the (*A*) leptomeninges or (*B*) frontal cortex of female Tg(APP23:*Gfap*-luc) mice inoculated with the indicated passaged brain homogenates ($n = 4$–6 each). Significantly more Aβ38 deposits were observed in animals inoculated with the passaged Arctic AD samples. (*C*) Quantification of formic acid-extractable Aβ38 peptide levels in the brains of male Tg(APP23:*Gfap*-luc) mice at 330 dpi with passaged Swedish ($n = 6$) or Arctic ($n = 3$) AD brain homogenate. No significant difference in Aβ38 levels was observed. AD, Alzheimer's disease; Arc, Arctic; dpi, days postinoculation; ns, not significant; Swe, Swedish. $^{**}P < 0.01$; $^{***}P < 0.001$.

Table S1. Samples used for inoculation experiments on first passage

Sample	Age (y)	Sex	[Aβ40] (nmol/g)	[Aβ42] (nmol/g)	[Aβ38] (nmol/g)
Control	79	Male	<0.005	<0.004	<0.007
Sporadic AD case i	62	Male	6.57	0.70	0.14
Sporadic AD case ii	85	Female	4.78	1.15	3.09
Swedish AD	61	Female	4.55	2.20	0.46
Arctic AD	64	Male	0.91	0.48	1.17

AD, Alzheimer's disease.