

Supplementary method:

Immunohistochemical analyses:

Fixed rat brain tissues obtained from the MRI studies were cut in the sagittal plane at 10-12 μm thickness using a Leica RM2135 microtome (Leica Microsystems Inc., Bannockburn, IL), placed in a flotation water bath at 40°C, and then mounted on Colorfrost/Plus slides (Fisher Scientific, Houston, TX). Antigen retrieval was performed by treating the tissue sections with proteinase K (0.2 mg/ml) for 10 min at 22 °C. Primary antibodies were detected with Alexa Fluor 594-conjugated donkey anti-rabbit or Alexa Fluor 488-conjugated goat anti-mouse secondary antibodies (1:1000). Staining for fibrillar amyloid was performed using thioflavin S. The following antibodies were used for immunohistochemical analysis: mAb66.1 (1:250), which recognizes residues 1-5 of human A β [1] ; rabbit polyclonal antibody to collagen type IV to visualize cerebral microvessels (1:100; ThermoFisher, Rockford, IL); rabbit polyclonal antibodies to GFAP (1:200; Dako, Santa Clara, CA) and Iba-1 (1:200; Fujifilm Wako Pure Chemical, Osaka, Japan) for detection of astrocytes and microglia, respectively. Prussian blue iron staining was performed to detect hemosiderin deposits reflecting signs of previous microhemorrhage. [1] Von Kossa calcium staining was used to detect small vessel occlusion/calcifications in the brain. [2] The percent area amyloid coverage of cerebral microvessels in the different brain regions and the percent area iron staining in the thalamic region were determined using stereological principles as described.[3]

Evaluation of axonal integrity in different brain regions and corpus callosum WM volume and myelin basic protein (MBP) levels in 11-12M old rTg-DI and WT rats were carried out in a separate series of rats not used for MRI. For analysis of axonal integrity rat brain tissue sections were immunolabeled with antibody SM312 pan axonal neurofilament marker (1:250;

BioLegend, San Diego, CA) and nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI, 10236276001, Sigma-Aldrich).

To determine the corpus callosum volume in WT and rTg-DI rats fresh frozen brain sections were cut at 12 μm thickness in the sagittal plane spanning the entire brain hemisphere. Every 4th section was selected and stained for myelin using the Black Gold II Myelin Staining Kit according to manufacturer's protocol (AG105; MilliporeSigma, Burlington, MA). Stitched images of the sagittal brain sections were prepared using a Keyence BZ-X710 microscope. The corpus callosum was outlined and the area was measured and calibrated with BZ-X analyzer software. Then the volume of main corpus callosum (from lateral 0.5 mm to lateral 2.66 mm) was calculated based on the area multiplied by the thickness.

For measurement of MBP levels in the corpus callosum sagittal sections at 25 μM thickness were prepared from fresh frozen WT and rTg-DI rat brains, mounted on Leica Frame Slides (Leica Microsystems, Danvers, MA) and the corpus callosum from each section was identified, excised and captured using a LMD6 laser capture microdissection microscope (Leica Microsystems). Collected tissue samples were lysed in RIPA buffer via sonication (12 x 1 sec bursts) on ice, followed by 1 h incubation on ice. Protein concentrations of each sample were determined by the BCA method and normalized. Sufficient 2 \times SDS sample buffer (2 % (w/v) SDS, 0.01 % (w/v) bromphenol blue, 20 mM DTT, 50 mM Tris-HCl, pH6.8) was added to each sample, resolved via SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane (Imobilon-FL, EMD Millipore, Billerica, MA) for immunoblotting. MBP was detected using affinity-purified goat polyclonal antibody [4] (1:500) and β -actin was detected using a mouse monoclonal anti- β -actin antibody (1:5000; A5441, Sigma). Immunoblots were then probed with species-specific horseradish peroxidase (HRP)-conjugated secondary

antibodies and HRP-catalyzed chemiluminescent signal was revealed using ECL Western Blotting Substrate (32106, ThermoFisher) and detected using an Odyssey Fc imager (LI-COR Biosciences, Lincoln, NE). Signal intensity was measured using ImageJ software (NIH), and the MBP signal in each lane was normalized against its corresponding β -actin signal.

References

- [1] Deane R, Du Yan S, Subramanian RK, LaRue B, Jovanovic S, Hogg E *et al.* RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med* 2003; 9(7): 907-13.
- [2] Rungby J, Kassem M, Eriksen EF, Danscher G. The von Kossa reaction for calcium deposits: silver lactate staining increases sensitivity and reduces background. *Histochem J* 1993; 25(6): 446-51.
- [3] Long JM, Kalehua AN, Muth NJ, Hengemihle JM, Jucker M, Calhoun ME *et al.* Stereological estimation of total microglia number in mouse hippocampus. *J Neurosci Methods* 1998; 84(1-2): 101-8.
- [4] Ou-Yang MH, Xu F, Liao MC, Davis J, Robinson JK, Van Nostrand WE. N-terminal region of myelin basic protein reduces fibrillar amyloid-beta deposition in Tg-5xFAD mice. *Neurobiol Aging* 2015; 36(2): 801-11.