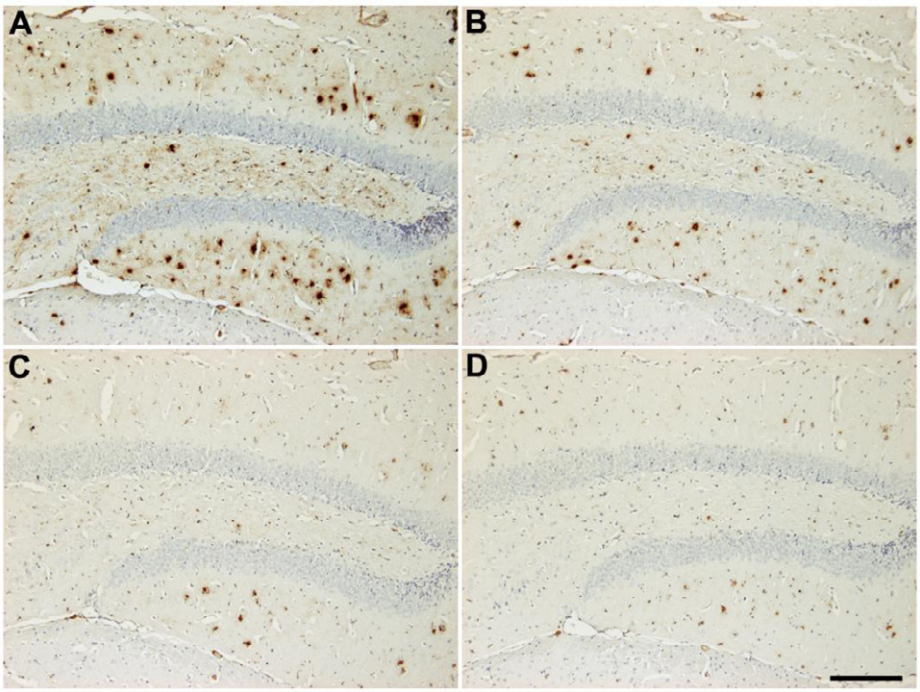
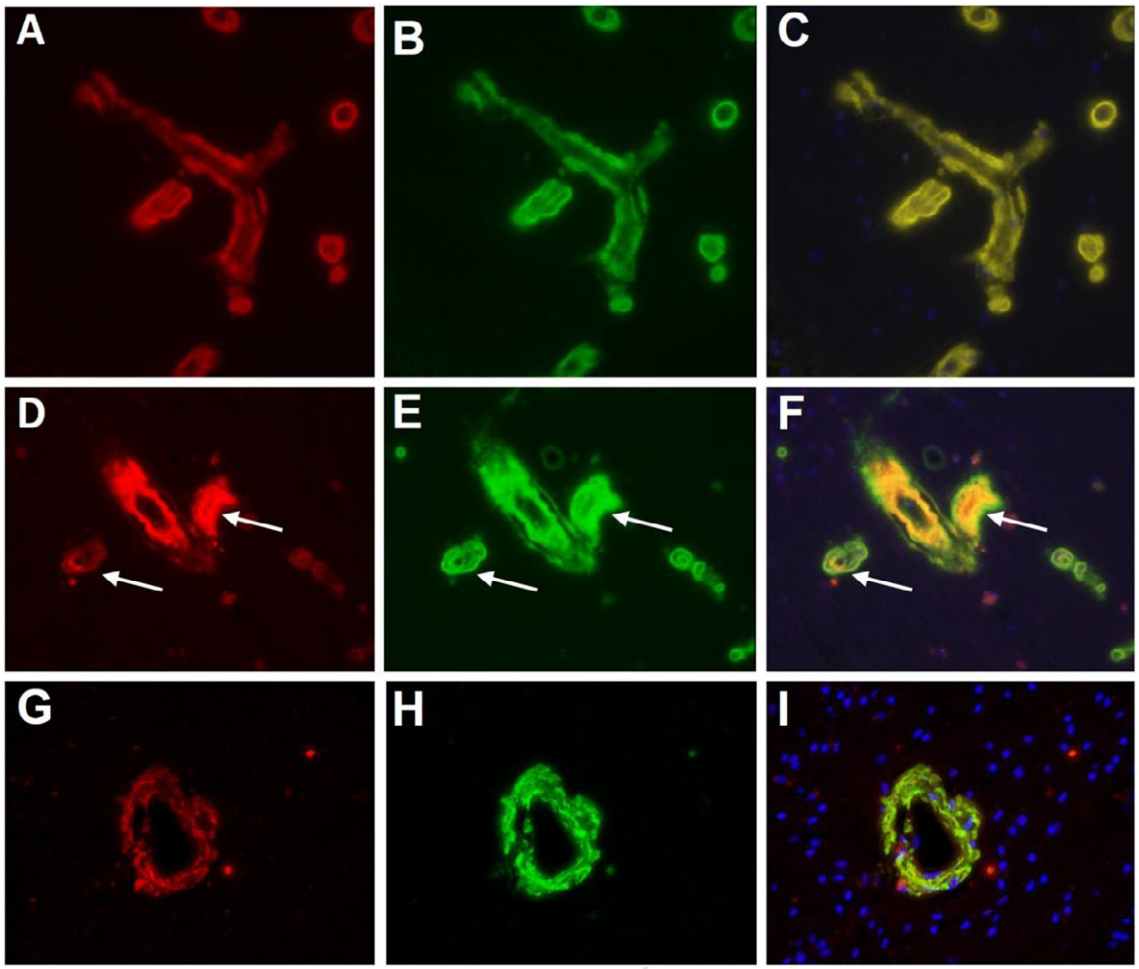
Supplemental information: Saul et al.

Supplemental methods:

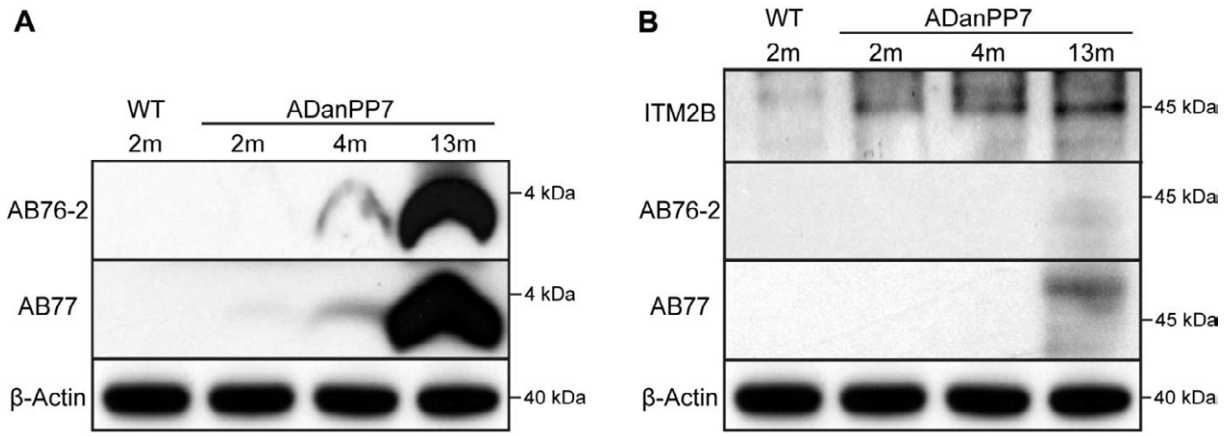
Immunoadsorption of AB77 was carried out using full-length non-modified and pGlu-modified synthetic ADan peptides (5 µg of peptide per 20 µl of antibody), incubated overnight at 4°C with continuous agitation and followed by centrifugation at 14.000 x g for 5 min as published previously [1].



Supplemental Fig. 1. Immunoadsorption using non-modified and pGlu-modified ADan peptides. Staining of a 13-month-old ADanPP7 mouse using AB77 with either (A) no blocking peptide, (B) 5 µg non-modified ADan, (C) 5 µg pGlu-modified ADan or (D) both peptides. Most of the AB77 immunoreactivity could be blocked by pGlu-ADan preincubation, suggesting that AB77 preferentially detects pGlu-modified ADan peptides in this mose model. Scale bar: 200 µm



Supplemental Fig. 2: AB77 (A) and AB76-2 (D) were combined with an ADan C-terminal antibody (antibody 5282, B and E) in FDD tissue. In addition, AB76-2 (G) was combined with Thioflavin-S (H). A comparable staining pattern was observed with both AB76-2 and AB77 when combined with the ADan C-terminal antibody. However, whereas AB77 reveals a complete co-localization (C), AB76-2 showed only a partial co-localization especially in the vessel periphery (F, arrow). Interestingly, AB76-2 and Thioflavin-S showed a complete overlap (G-I). Original magnification: 400x



Supplemental Fig. 3. (A) Western blot analysis using SDS-soluble fractions from brains of ADanPP7 mice revealed a faint band using AB77 already at 2 months of age, with strongly increased signal intensity at 4 and 13 months of age. Using AB76-2, a clear signal could be detected at 4 months of age with markedly enhanced signal intensity at 13 months of age.
(B) There is no evidence for a cross-reactivity of AB77 or AB76-2 with the parental full-length Bri protein (ITM2B) which migrates at ~45 kDa.

Supplemental References:

1 Lashley T, Revesz T, Plant Get al. (2008) Expression of BRI2 mRNA and protein in normal human brain and familial British dementia: its relevance to the pathogenesis of disease. Neuropathol Appl Neurobiol 34: 492-505