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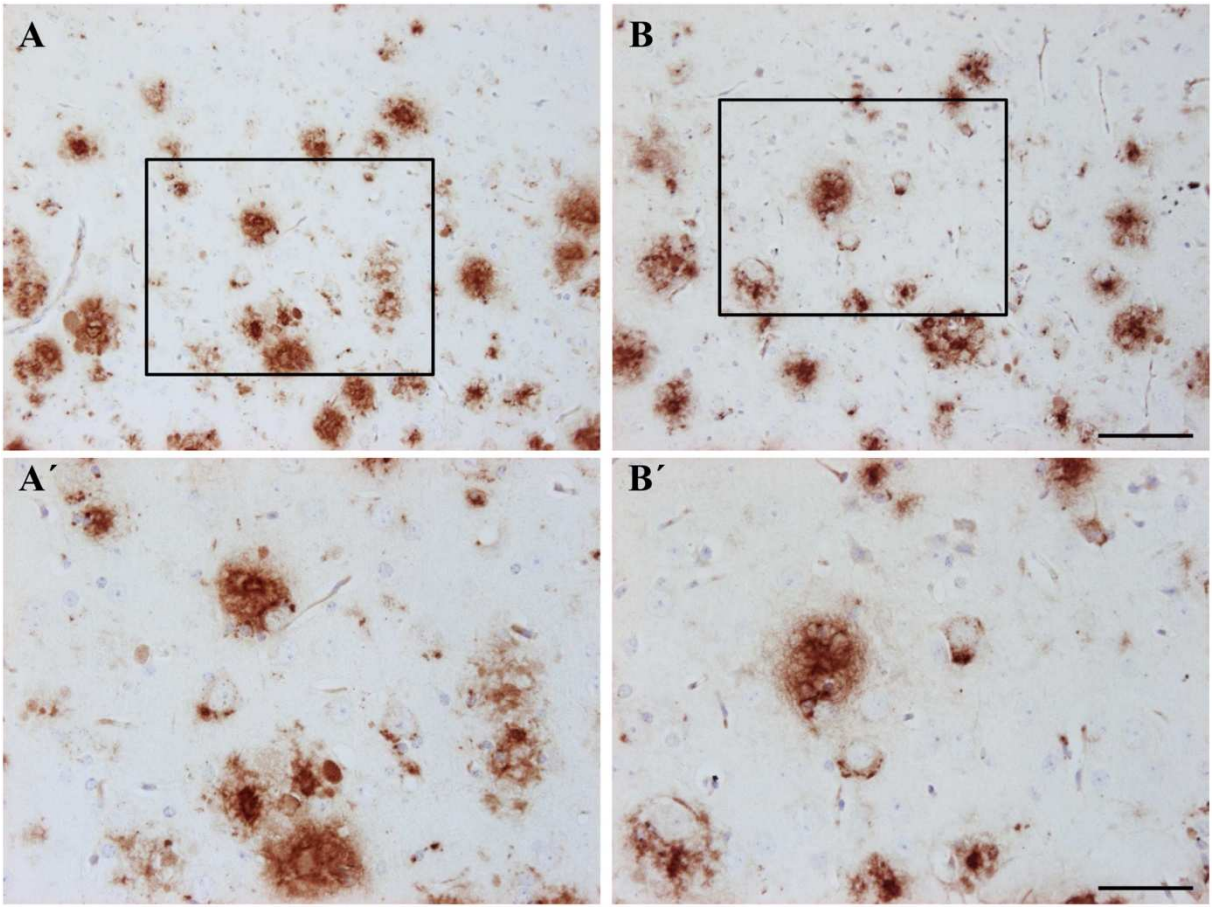
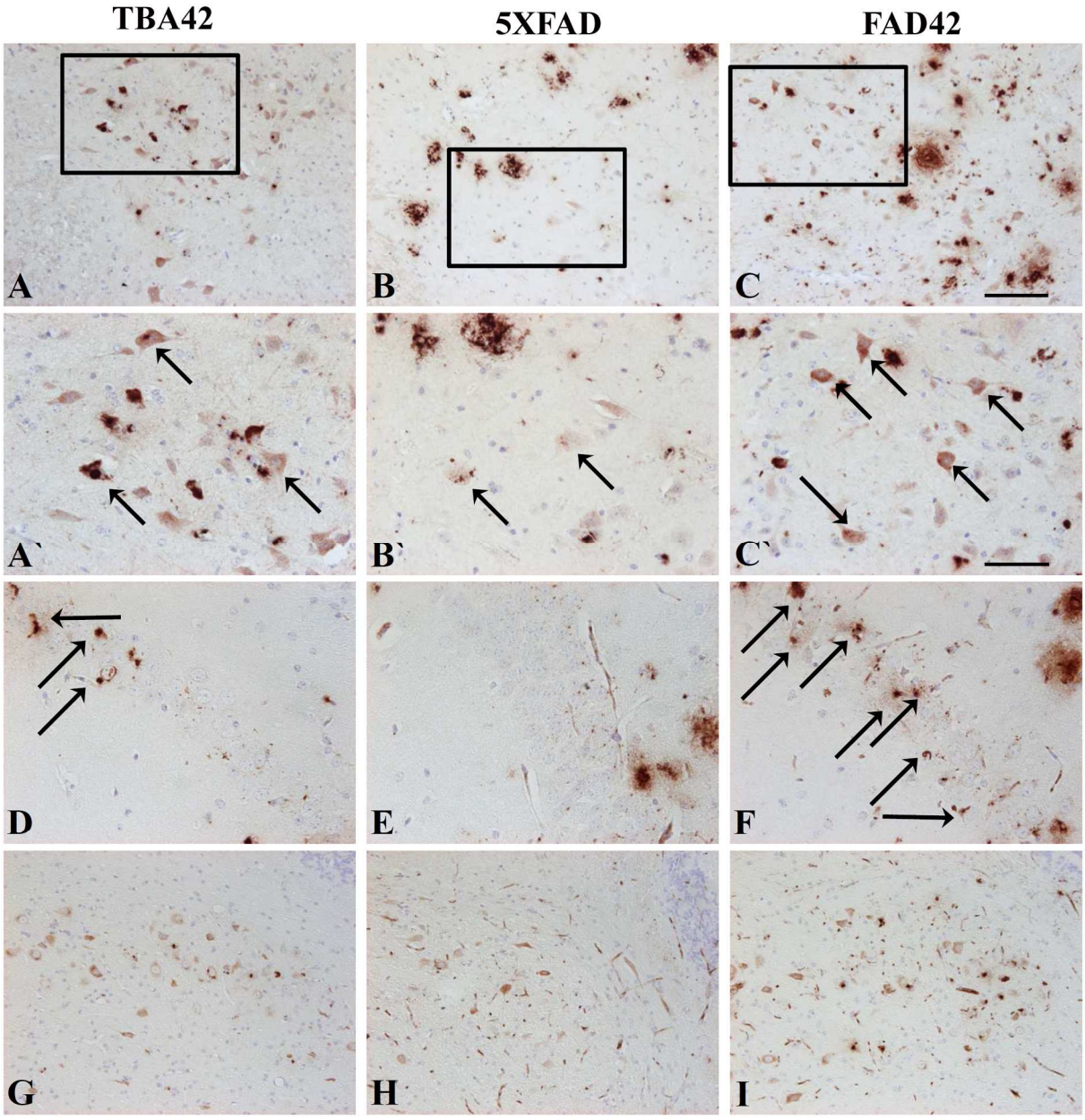
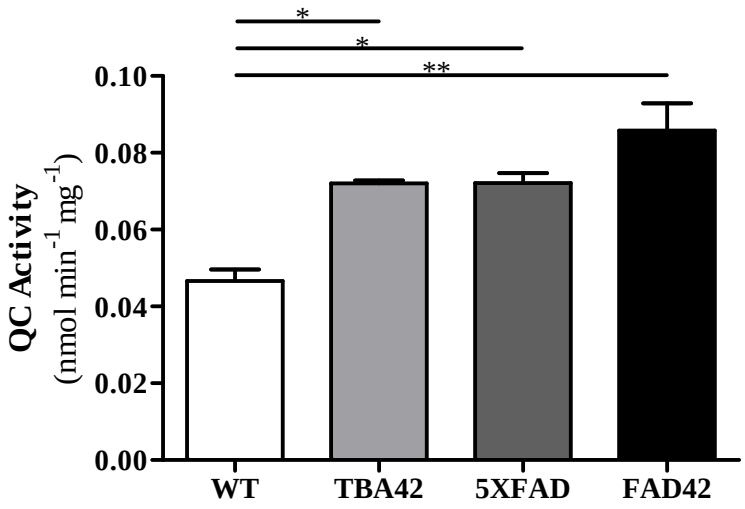
Supplemental Figure 1. FAD42 mice did not show evidence for elevated intraneuronal Aβ accumulation compared to 5XFAD mice at 6 months of age in the primary motor cortex. Immunostaining against Aβ (4G8) in (**A**) 5XFAD compared to (**B**) FAD42 mice. (**A`**) und (**B`**) represent magnifications of A and B. Scale Bar: A and B: 100 µm; A` and B`, 50 µm.



Supplemental Figure 2. Pathology in the spinal cord, hippocampus and cerebellum in TBA42, 5XFAD and FAD42 mice at the age of 6 months. (**A-C**) Immunostaining against pan-Aβ revealed that TBA42 mice developed mostly intraneuronal Aβ accumulation, 5XFAD mice showed intra- and extracellular Aβ deposition, whereas FAD42 mice revealed an increased intraneuronal signal compared to TBA42 and 5XFAD mice. (**A'-C'**) High power view of figures A-C. (**D-F**) Whilst immunostaining against pan-Aβ demonstrated intraneuronal accumulation in TBA42, the staining was more abundant in FAD42 with no reactivity in 5XFAD. The lack of intraneuronal Aβ has been previously demonstrated in this model. (**G-I**) In the white matter of the cerebellum, 5XFAD elicited only few positive neurons, increased numbers in TBA42 and highest in FAD42. Arrows point to intraneuronal Aβ accumulation. Scale bar A-C; D-I: 100 µm; A'-C': 50 µm.



Supplemental Figure 3. Activity assay for glutaminyl cyclase (QC) in brain lysates of WT, TBA42, 5XFAD and FAD42 transgenic mice. Significantly increased QC activity was observed in TBA42, 5XFAD and FAD42 mice compared to WT controls. There was an insignificant trend toward higher activity in FAD42 mice relative to TBA42 and 5XFAD. Abbreviation: WT, wildtype mice. One-way ANOVA with Bonferroni post-hoc tests; * p <0.05; ** p < 0.01.



Supplemental Experimental Procedure

QC activity was determined using a previously published protocol (1). Samples from mouse brains were homogenized (10 mm Tris, 100 mm NaCl, 5 mm EDTA, 0.5% Triton X-100, and 10% glycerol, pH 7.5, using a Precellys homogenizer (Peqlab)). The homogenate was further sonicated and centrifuged at 16,000 × g for 30 min and 4 °C. Reaction was carried out at 37 °C in 25 mm MOPS (pH 7.0, 0.1 mm N-ethylmaleinimide). After centrifugation at 16,000 × g for 10 min, the supernatant was applied to HPLC analyses using a RP18 LiChroCART HPLC Cartridge and the HPLC system D-7000 (Merck-Hitachi). The samples were injected and separated by increasing the concentration of solvent A (acetonitrile containing 0.1% TFA) from 8 to 20% in solvent B (H_2O containing 0.1% TFA). QC activity was quantified from a standard curve of pGlu-naphthylamide (Bachem) determined under assay conditions.

Supplemental Table 1. Amount of $A\beta_{pE}$ levels in the brain of Alzheimer mouse models and Alzheimer patients measured by ELISA.

Pyroglutamate Aβ levels in mouse models			
Mouse model	*TBS Fraction*	*SDS/FA Fraction*	**Citation**
TBA42	0.05 ng/g (6 mon)	~4 ng/g (SDS; 6 mon)	current work
5XFAD	0.14 ng/g (6 mon)	16 ng/g (SDS; 6 mon)	current work
FAD42	0.24 ng/g (6 mon)	29 ng/g (SDS; 6 mon)	current work
TBA2.1	NA	60 ng/g (SDS + FA; 1 mon) 30 ng/g (SDS + FA; 7 mon)	(2)
APP/PS1 KI	NA	~2 pg/mg (SDS; 2 mon) 91 pg/mg (SDS;6 mon)	(3)
TBA2	NA	53 pg/mg (SDS; 2 mon)	(3)
Tg2576	NA	~1 ng/g (SDS + FA; 10 mon) 25 ng/g (SDS + FA; 16 mon)	(4)
TASD - 41	NA	14 ng/g (SDS + FA; 7 mon)	(4)
hAPP[V717I]	NA	~3 ng/g (SDS; 12 mon) 20 ng/g (SDS; 15 mon)	(5)
5XFAD/hQC	0.12 ng/g (6 mon)	115 ng/g (SDS + FA; 6 mon)	(6)
5XFAD/QC-KO	undetectable	39 ng/g (SDS + FAD; 6 mon)	(6)

Pyroglutamate Aβ levels in Alzheimer brain				
AD Stage	**Brain region**	*TBS Fraction*	*SDS/FA Fraction*	**Citation**
Braak Stage I-II	Neocortex	NA	25 ng/g (SDS + FA)	(4)
Braak Stage V-VI	Neocortex	NA	45 ng/g (SDS + FA)	(4)

Abbreviations: mon, months of age; NA, not examined; FA, formic acid.

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