SUPPLEMENTARY FIGURE LEGENDS

Table 1. Ectopic expression of green fluorescent protein (GFP)-tagged Gpc-1:
Primers used for amplication of cDNA for introduction into pEGFP C1

1.	hGPC 1-92	5′-TAAT *GC TAGC* GCC GCC ACC ATG GAG CTC CGG GCC CGA-3′ 5′-TAA *ACC GGT* CTC CGG CTC TTG CTG GC-3′
2.	hGPC 91-1677	5′-TAA T*AA GCT T*CA AGC TGC GGC GAG GTC CGC CAG-3′ 5'-TT *GAA TTC* TTA CCG CCA CCG GGG CCT GGC TAC-3'

Restriction enzyme cleavage sites are marked in italics.

Mutagenic primer to disrupt Kozak sequence in EGFP

5′-CACCGGTCGCCACTGTAGTGAGCAAGGGCGAG-3′

Fig. S1. Schematic structures of amyloid precursor protein (APP), glypican-1 (Gpc-1), their degradation products and possible HS-Aβ conjugates. (**a**) APP is a type I transmembrane protein with a large N-terminal ectodomain, a short C-terminal cytosolic portion and an Aβ segment (black box) that is partially embedded in the cell membrane (grey box). β-Secretase cleavage generates (**b**) a C-terminal fragment (expanded) containing closely clustered γ-secretase cleavage sites. Subsequent cleavage at these sites generates (**c**) Aβ peptides (mainly Aβ40/42). Antibodies used to detect the C-terminus of APP and the Aβ region are indicated. **(d)** Gpc-1 is lipid-anchored (oval with two short rods), carries three heparan sulfate (HS) chains and has a large globular N-terminal domain (grey) (Svensson et al., 2009). Conserved Cys residues in the globular part are S-nitrosylated (S*NO*) by endogenously formed NO in a Cu(II)-dependent redox reaction. Cu(II)-loaded APP supports this reaction (Cappai et al., 2005). Gpc-1 can be endocytosed and recycled. An endogenous reducing agent present in endosomes (Fivaz et al., 2002; Mani et al., 2006), or exogenously supplied ascorbate, releases NO from Cys (SH). NO cleaves heparan sulfate at $GlcNH_3^+$ units (GN, see left blow-up), generating anhydromannose-containing oligosaccharides (AM) which contain a free aldehyde (see right blow-up). (**e**) The free aldehyde of the reducing terminal anhydromannose (anMan) in the released HS degradation products can form a Schiff base with amino groups in a reversible reaction. Stable HS-Aβ conjugates may be formed by reduction or via various rearrangements. The free aldehyde can also be reduced, generating a terminal anhydromannitol (anManOH) residue (containing $-CH_2OH$). The anMan-specific mAb AM was raised against partially deaminatively cleaved heparin ($Fragmin^R$). The principal epitope should be a tetrasaccharide sequence IdoA(2-OSO_3)-$GlcNSO_3$(6-OSO_3)-IdoA(2-OSO_3)-anMan(6-OSO_3), where $GlcNSO_3$ is N-sulfated glucosamine and IdoA is L-iduronic acid (Pejler et al., 1988). Similar sequences have been found in HS associated with amyloid deposits (Smits et al., 2010).

Fig. S2. Expression of APP in mouse embryonic fibroblasts (MEF) from Tg2576 (Tg) and wild-type (non Tg) mice as demonstrated by SDS-PAGE and western blotting using mAb WO2.

Fig. S3. Localization of anMan-immunoreactivity in Tg2576 fibroblasts. The immunofluorescence microscopy images were obtained after staining cultures of Tg2576 fibroblasts with the anMan-specific mAb (AM, green) and **(a)** a polyclonal anti-Rab7 (red) or **(b)** LysoTrackerRed (LTR, red). Scale bar: 20 μm.

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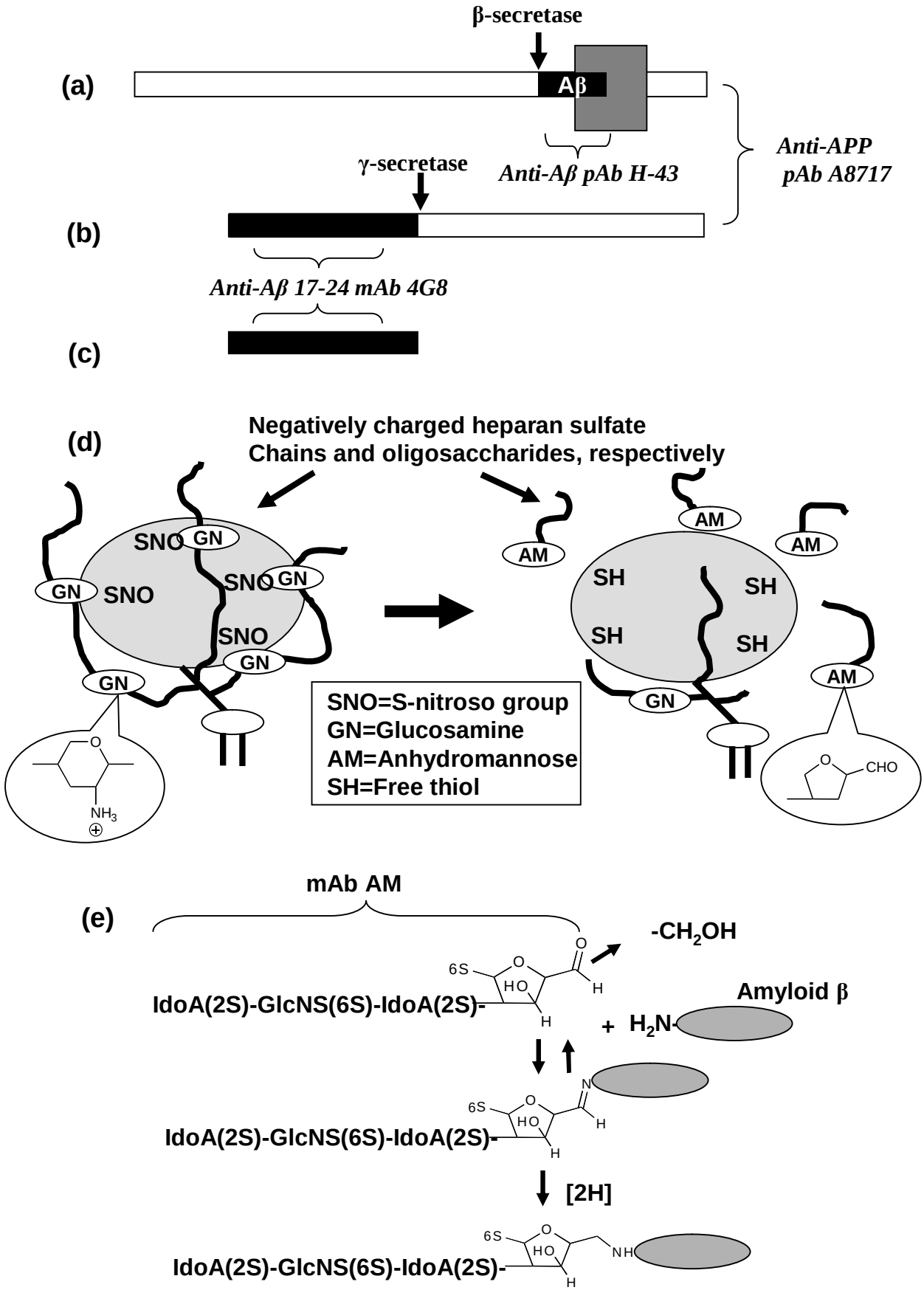
β-secretase
(a)
Aβ
Anti-APP
pAb A8717
γ-secretase
Anti-Aβ pAb H-43
(b)
Anti-Aβ 17-24 mAb 4G8
(c)
(d)
Negatively charged heparan sulfate
Chains and oligosaccharides, respectively
SNO
GN
SH
AM
SNO=S-nitroso group
GN=Glucosamine
AM=Anhydromannose
SH=Free thiol
(e)
mAb AM
-CH2OH
IdoA(2S)-GlcNS(6S)-IdoA(2S)-
Amyloid β
+ H2N-
[2H]

Fig.S1

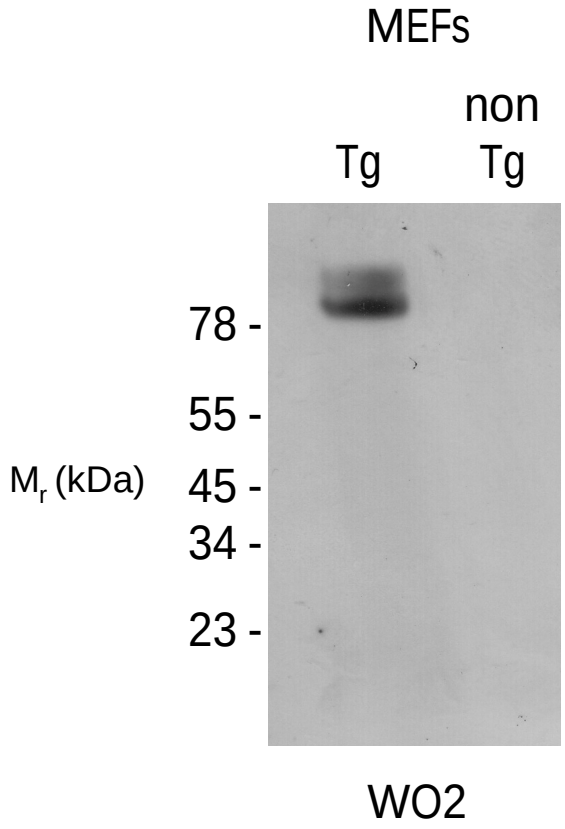

Fig. S2

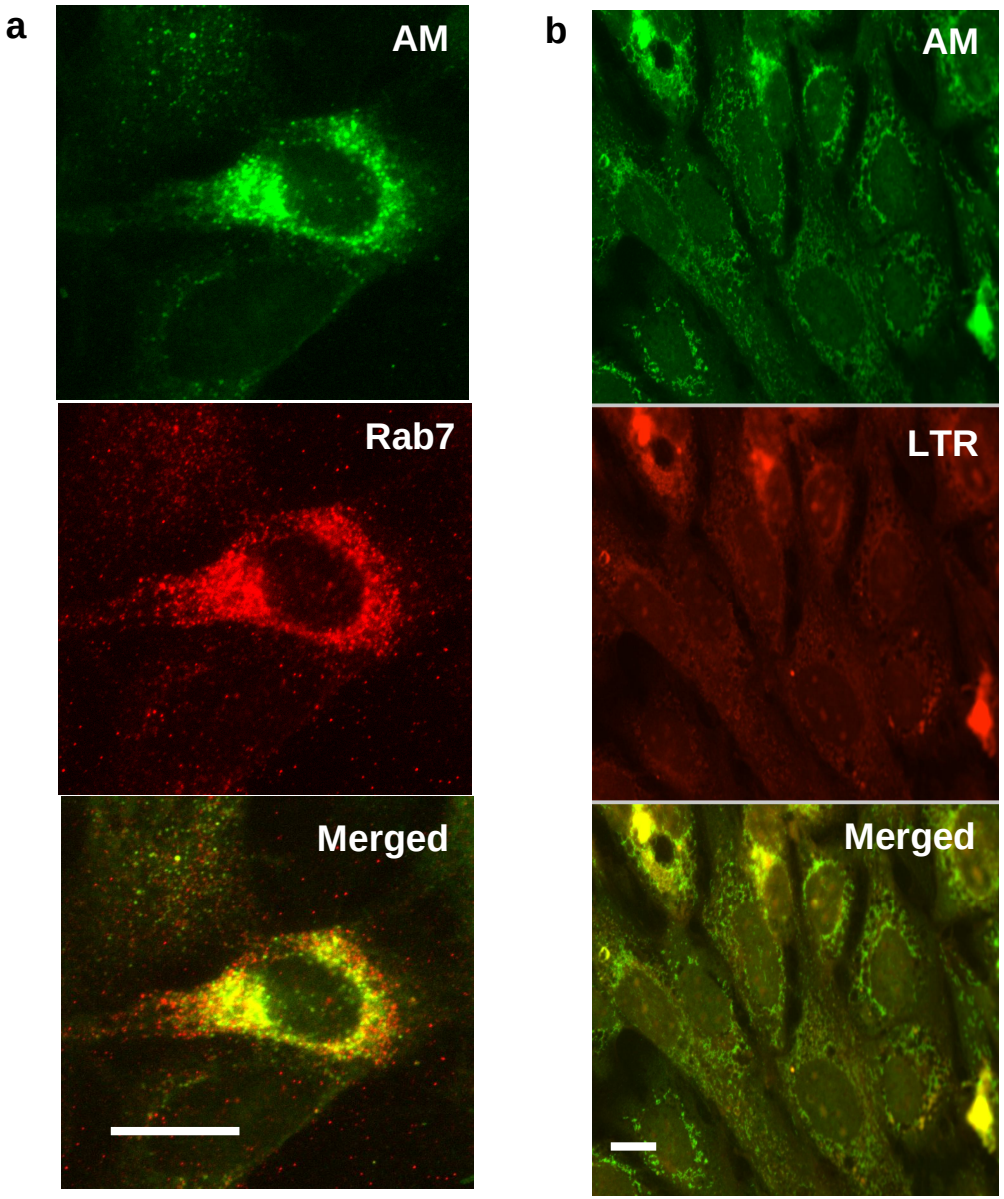

Fig. S3