Supplementary Materials

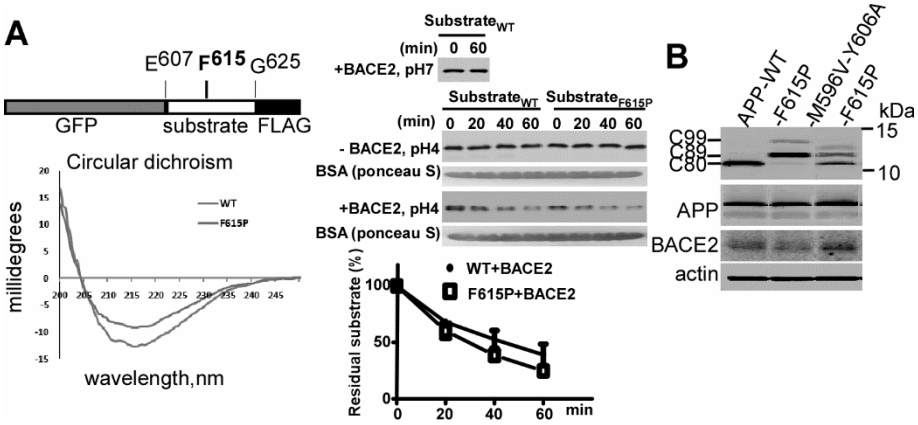

Figure S1. F615P mutation does not destroy the θ-site where BACE2 cleaves APP_{WT}. (**A**) The fragment E^{607}-G^{625} of $APP^{695}$with or without the F615P substitution was C-terminally fused to GFP and N-terminally fused to FLAG-His tag, and the recombinant proteins, as BACE2 substrates, were purified. Circular dichroism (CD) revealed a reduction of the α-helix feature bands at~210 and ~220nm by the F615P substitution, suggesting a structural perturbation. The substrates were incubated with or without recombinant BACE2 in either 25mM sodium acetate buffer (pH4) or PBS (pH7.3) for indicated times, and the residual substrates were blotted with anti-FLAG antibody (mean±SEM, n=3 repeats). GFP antibody failed to detect a lower protein band after BACE2 cleavage, presumably because the cleavage products were instable in acidic buffer or further degraded by BACE2. After blotting, BSA, supplemented to the sodium acetate reaction buffer to reduce non-specific interaction, was stained with Ponceau S as equal loading control. (**B**) APP_{WT}, APP_{F615P} and $APP_{M596V\text{-}Y606A\text{-}F615P}$wereco-expressed with BACE2-Myc-His in HEK293 cells. Cell lysates were blotted for APP, CTFs and BACE2.

BACE2 and AD

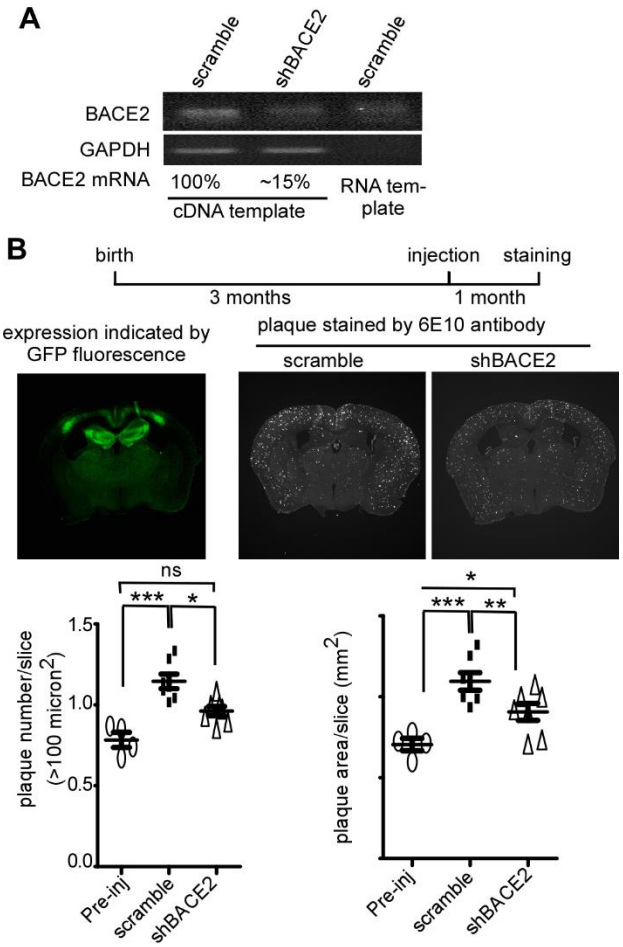

Figure S2.BACE2 suppression delayed neuritic plaque formation in APP$^{\text{Swedish-Arctic-Iberian/ Swedish –Arctic-Iberian}}$ knock-in mice. Primary neurons from E18 APP$^{\text{Swedish-Arctic-Iberian/ Swedish-Arctic-Iberian}}$ knock-in mice (3KI) or APP$^{\text{Swedish-Iberian/ Swedish -Iberian}}$ knock-in mice (2KI) were transduced with Adeno-Associated Virus-9 (AAV9) carrying scrambled shRNA or shRNA against mouse BACE2 (shBACE2). Cells were harvested 7 days later and subjected to RT-PCR for BACE2 mRNA quantification (**A**). (**B**) AAV9 containing scramble shRNA or shBACE2 was intra-ventricularly injected into the brains of 3KImice at 3 months old, the age when neuritic plaques (NPs) were readily detectable (pre-inj). One month after injection, brain slices were stained with 6E10 antibody against Aβ (epitope N-terminal to the Arctic site) for NPs. As the diffusion of virus in adult brains tends to be locally restricted, only slices within 1mm range flanking the injection sites were stained and quantified. Areas covered by NPs and numbers of NPs were quantified with Image-J software (NP size cut-off: 100 μm^2). No clear difference was found between genders. N=4 for pre-inj, 6 for scramble and 7 for both shBACE2 *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ns: non-significant. (unpaired t-tests). All bars represent mean±SEM.

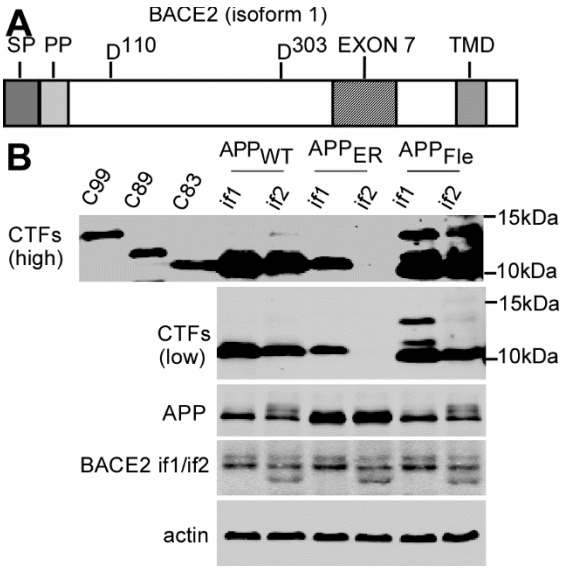

Figure S3. BACE2 isoform 1 and isoform 2 function differently in APP processing. (**A**) Schematic diagram indicating the position of the sequence encoded by exon 7. SP: signal peptide; PP: propeptide; D^{110} and D^{303}: activity sites of BACE2; EXON 7: the 50 amino acids sequence encoded by exon 7; TMD: transmembrane domain. (**B**) BACE2 (isoform 1, if1) and BACE2 isoform 2 (if2) without the sequence encoded by exon 7 were co-expressed in HEK293 cells with APP_{WT}, APP_{ER} or APP Flemish mutant (APP_{Fle}), and cell lysates were blotted for CTFs, APP and BACE2. CTF markers including C99, C89, and C83 were loaded side by side with lysates on the CTF gel.

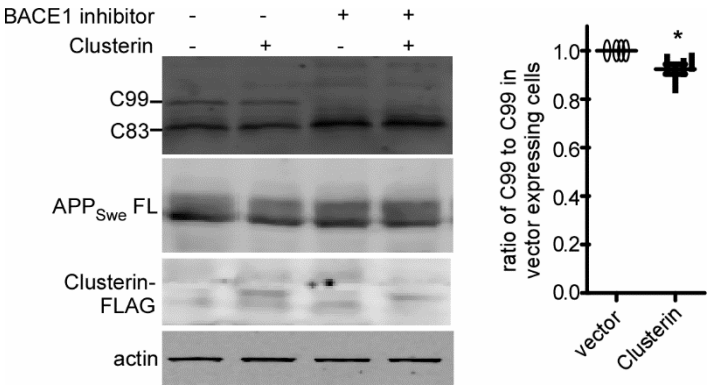

Figure S4. Clusterin does not induce β-secretase activity of endogenous BACE1 or α-secretases. Cluterin was overexpressed in a stable cell line expressing human APP_{Swe} mutant, and cells were treated with or without a BACE1 inhibitor (LY2886721, MCE) to indicate the cleavage by endogenous BACE1. Cell lysates were blotted for CTFs, full-length APP_{Swe}, and clusterin. N=4 repeats, *:$p<0.05$ (Paired t-tests). Values are expressed as mean±SEM.