Supporting Information

Sun et al. 10.1073/pnas.1506242112

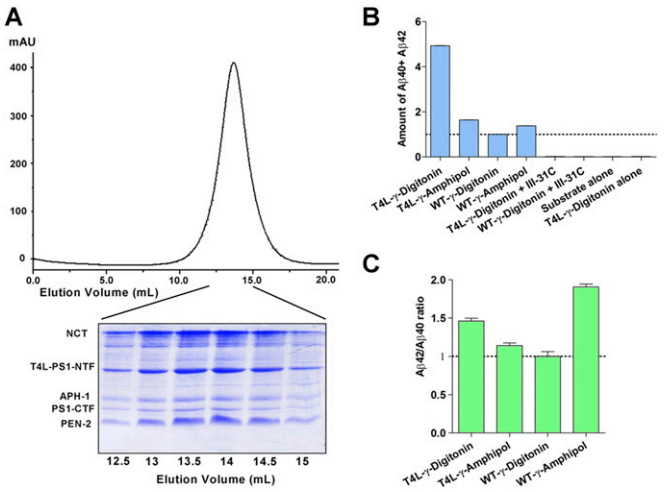

Fig. S1. Preparation of human γ-secretase for cryo-EM analysis. (*A*) A representative gel filtration analysis of recombinant human γ-secretase. The monodisperse peak suggests excellent solution behavior of the sample. The peak fractions from a Sepharose-6 column (10/30, GE Healthcare) were visualized on SDS/PAGE by Coomassie blue staining. (*B*) The purified human γ-secretase exhibited robust protease activity toward the substrate APP C99. Shown here are results of the AlphaLISA cleavage assay. The fusion of T4 lysozyme to the amino terminus of PS1 led to an increased protease activity under the detergent digitonin compared with amphipols. (*C*) The Aβ42/Aβ40 ratios are qualitatively similar for the different detergents. The fusion of T4 lysozyme to the amino terminus of PS1 has little impact on the Aβ42/Aβ40 ratio.

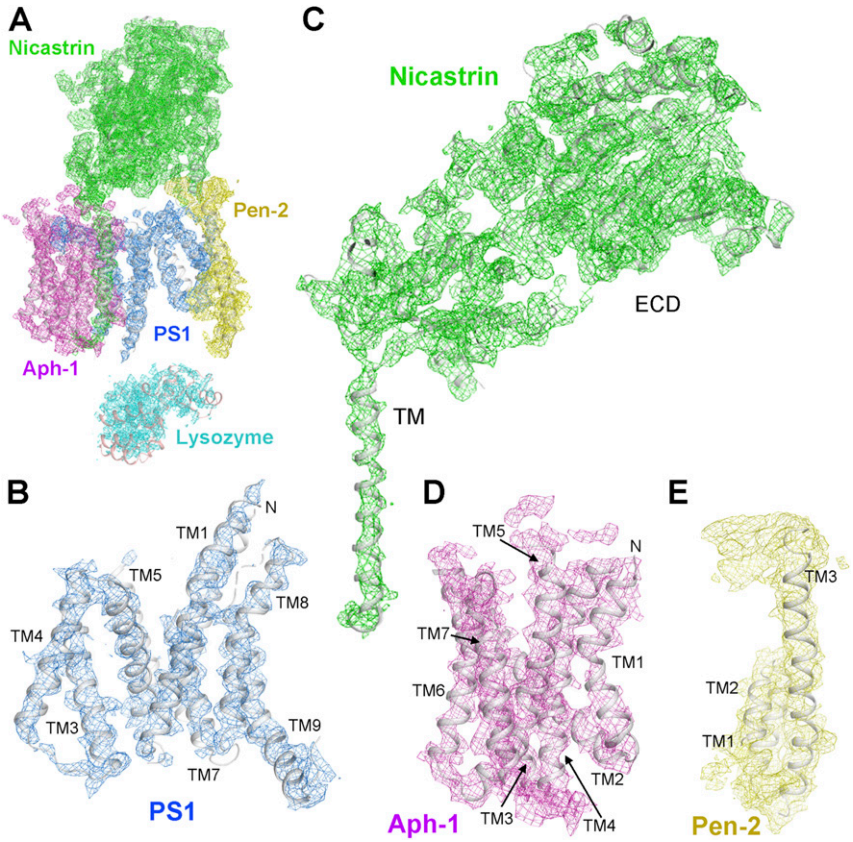

Fig. S2. Identification of the four components of human γ-secretase. (*A*) An overall view of the EM density. The densities are color-coded for PS1 (blue), Pen-2 (yellow), Aph-1 (magenta), nicastrin (green), and T4 lysozyme (cyan). (*B–D*) A step-wise procedure for the sequential identification of PS1, nicastrin, Aph-1, and Pen-2. (*B*) Assignment of PS1. PS1-TM1 was first identified on the basis of its connection to T4 lysozyme. The EM density and the structural homology between PS1 and PSH were used to assign the other TMs of PS1. (*C*) Nicastrin was assigned due to its lone TM connecting to the ECD. (*D*) The seven TMs between nicastrin and PS1 were assigned to Aph-1. The sequential order of these TMs was determined by examination of the inter-TM connectivity in both this study and the published study (1). (*E*) The three TMs on the thin end of the TM horseshow were attributed to Pen-2.

1. Lu P, et al. (2014) Three-dimensional structure of human γ-secretase. *Nature* 512(7513):166–170.

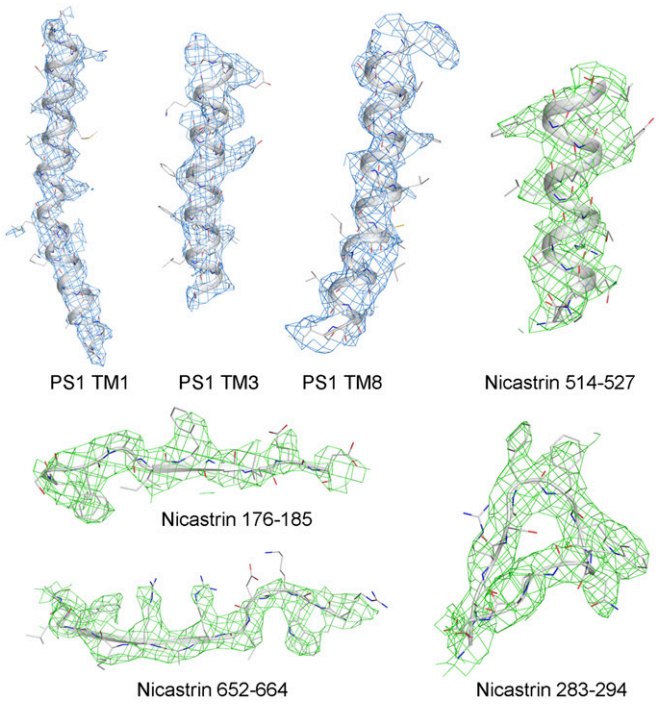

Fig. S3. Representative EM densities for select regions of γ-secretase. Shown here are three TMs from PS1 and four segments from nicastrin.

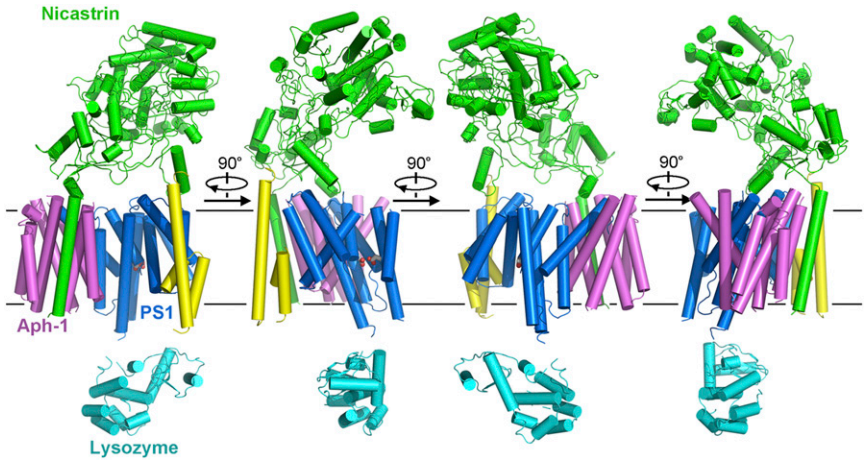

Fig. S4. Overall structure of human γ-secretase is shown in four perpendicular views. The γ-secretase structure is viewed parallel to the lipid membrane. The coloring scheme is the same as in Fig. 2.

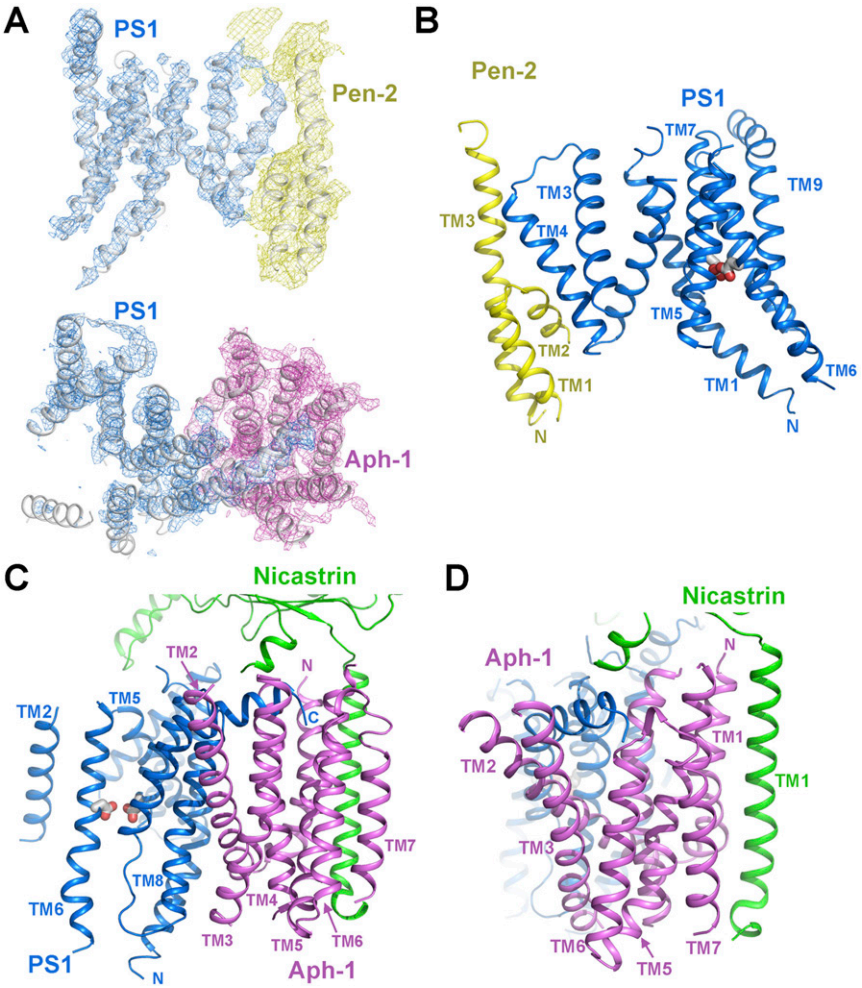

Fig. S5. Assembly interfaces among the four components of γ-secretase in the transmembrane region. (*A*) EM densities for the PS1-Pen-2 interface (*Top*) and the PS1-Aph-1 interface (*Bottom*). (*B*) A cartoon representation of the interface between PS1 and Pen-2. (*C*) A cartoon representation of the interface between PS1 and Aph-1. (*D*) A cartoon representation of the interface between Aph-1 and nicastrin.