Supporting Information

Mechanistic regulation of γ-secretase by their substrates

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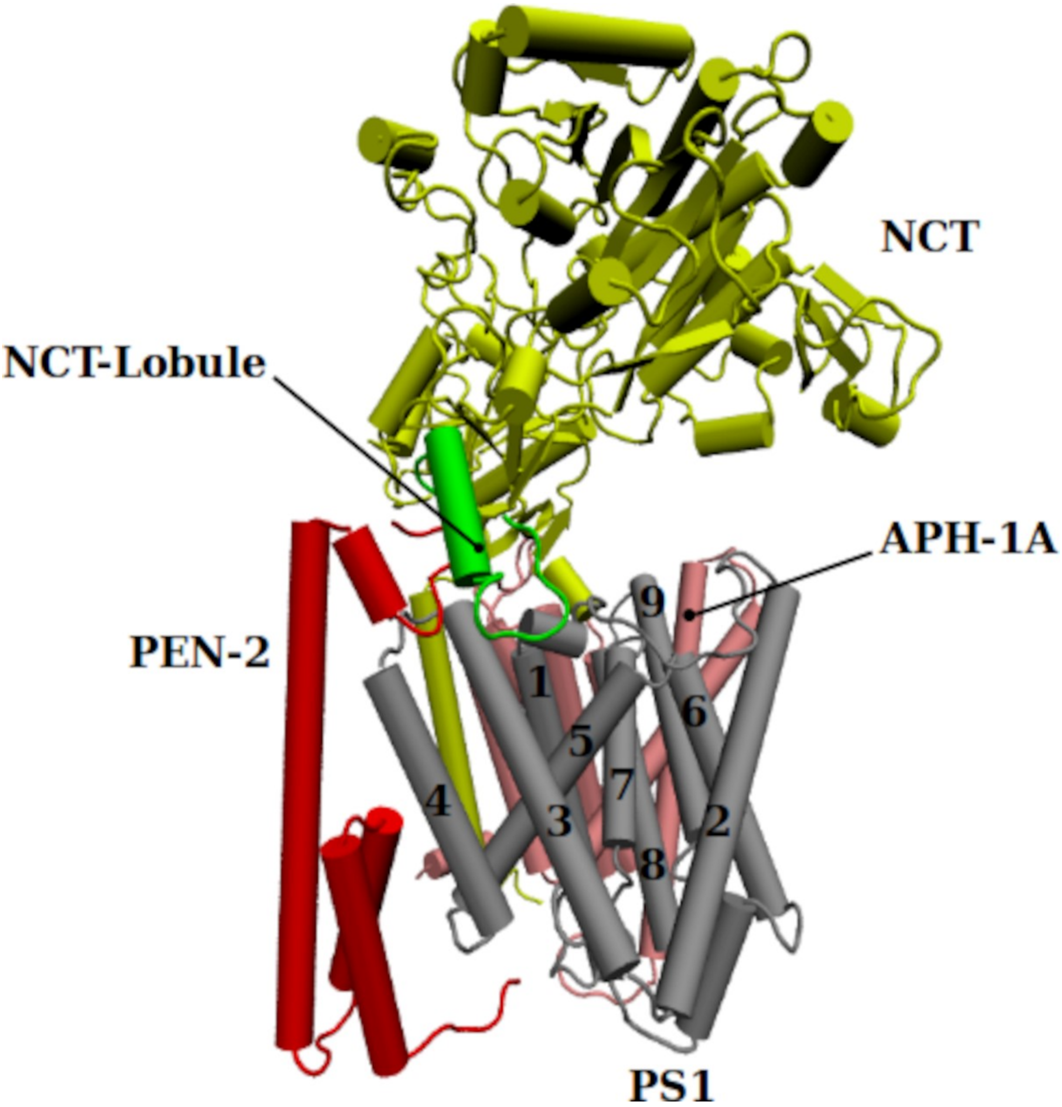

Fig. S1 General depiction of the GS structure. This figure shows the four GS subunits, and the NCT-lobule.

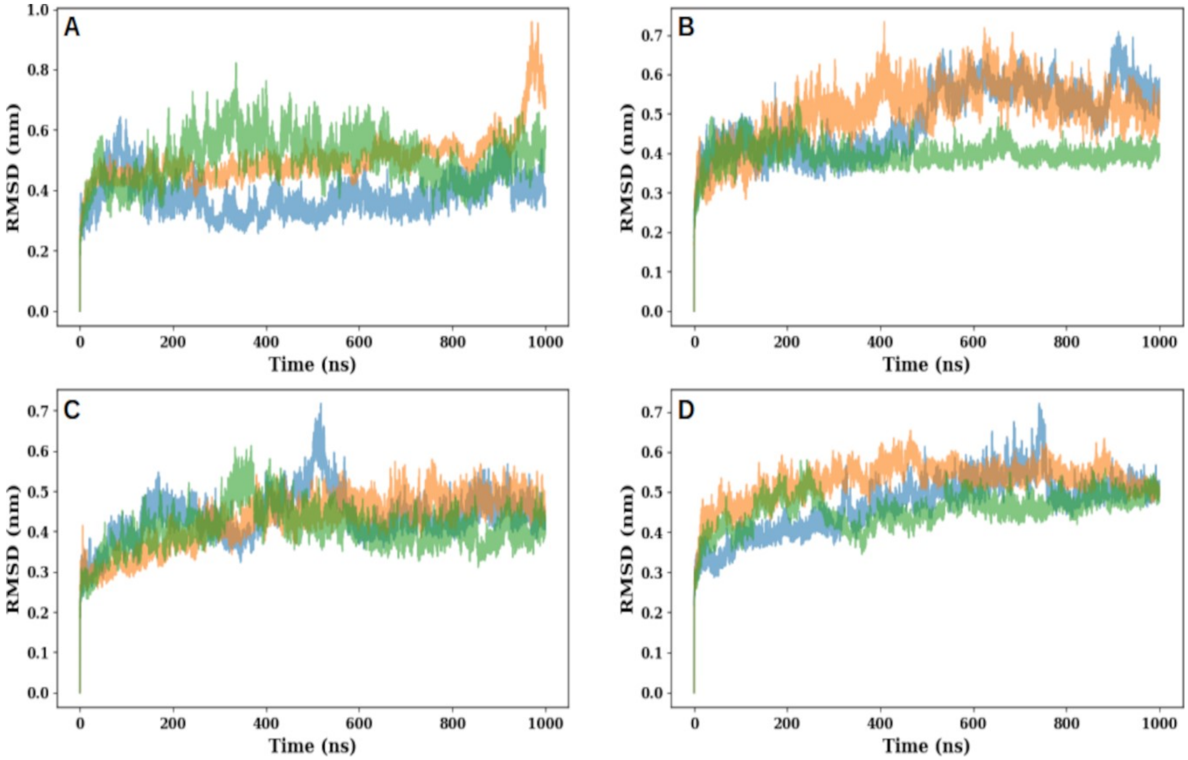

Fig. S2 RMSD of the three replicas of each GS and GS-substrate systems. The RMSD values were computed over 1 µs of molecular dynamic trajectory. (**A**) GS system; (**B**) GS-C99 system; (**C**) GS-C83 system and (**D**) GS-Notch system.

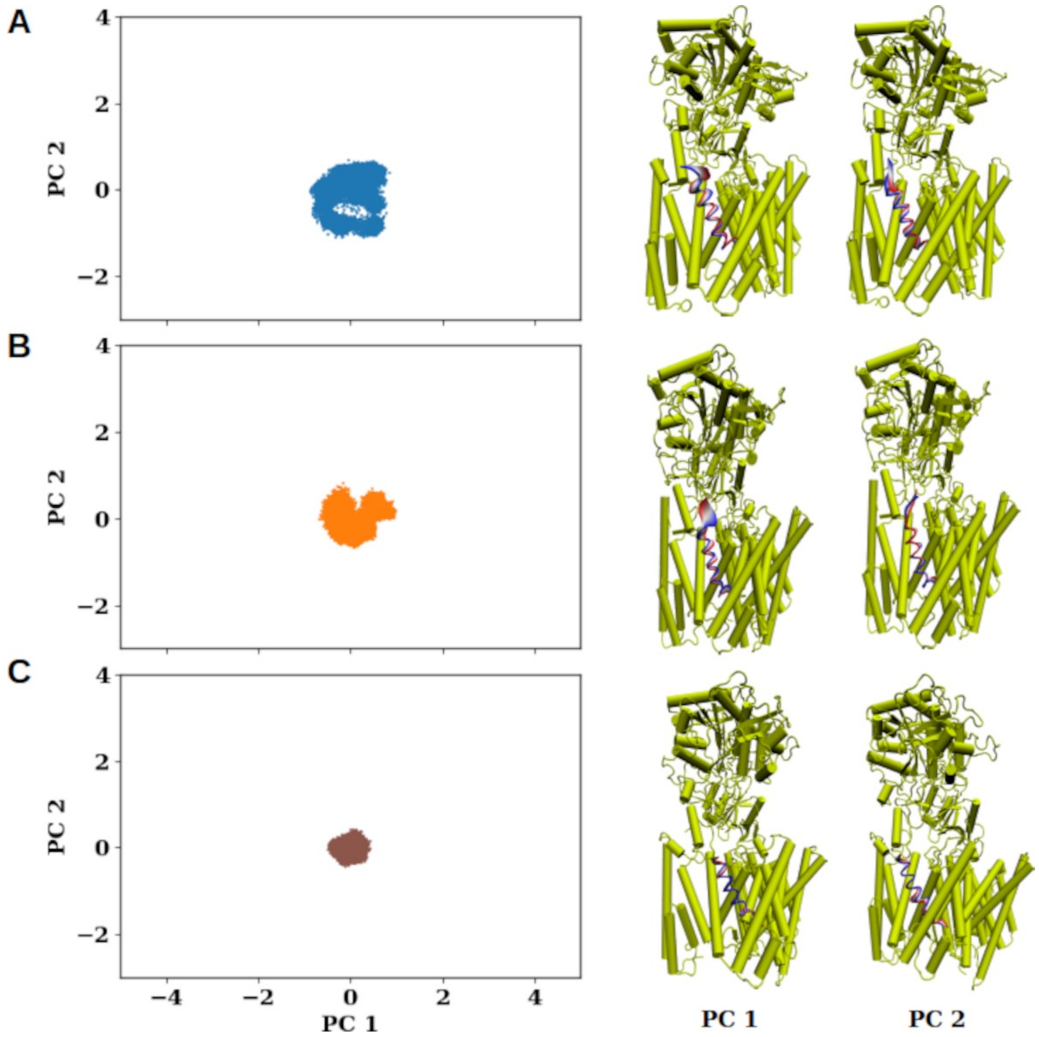

Fig. S3 PCA of the transmembrane fragment of GS substrates. Projection of the first two principal component of the transmembrane fragment (left) computed over 1 μs and the corresponding structures (right). (**A**) GS-C99; (**B**) GS-C83; (**C**) GS-Notch.

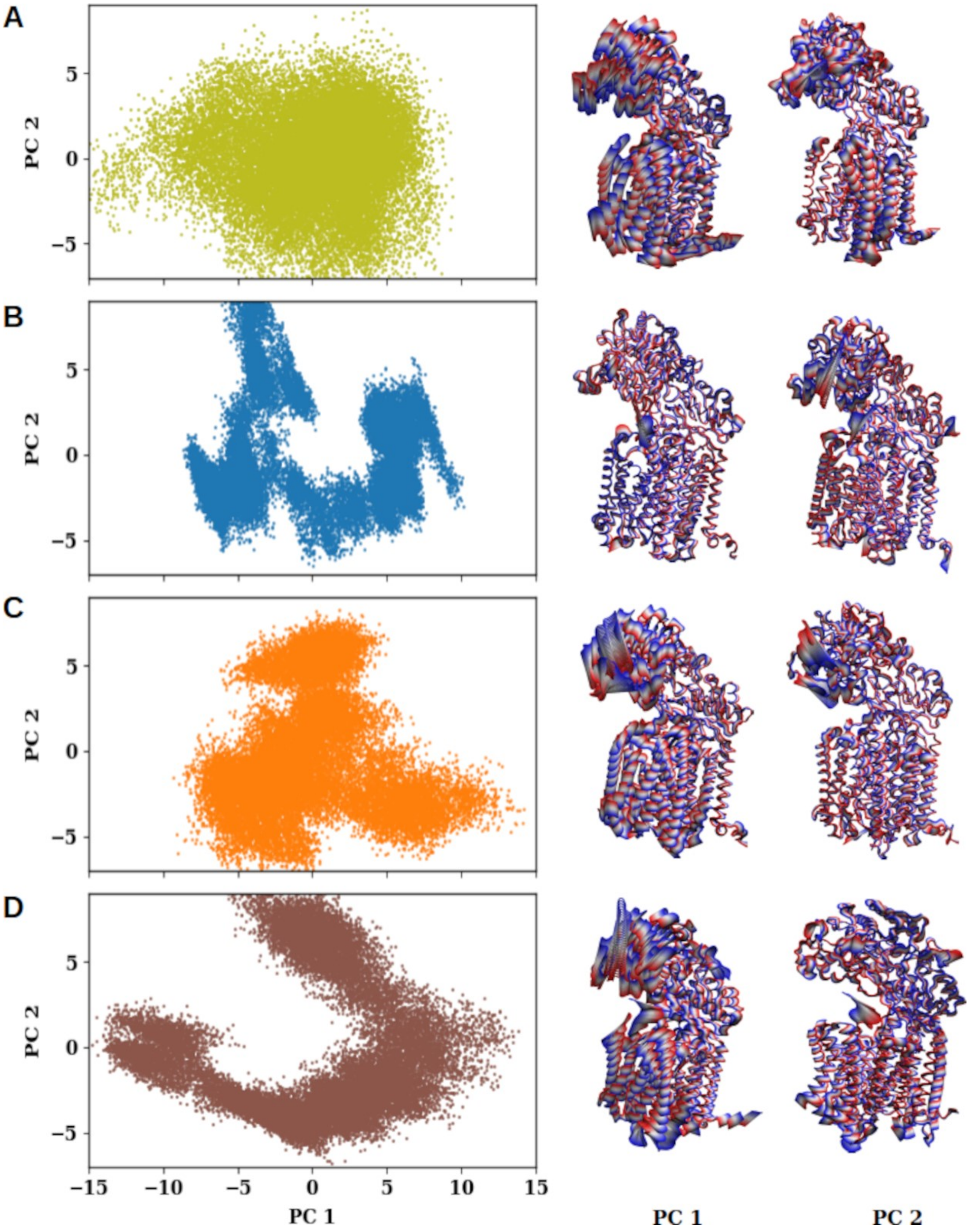

Fig. S4 PCA of whole GS and GS-substrate systems. The first two principal components were computed over 1 μs of molecular dynamic trajectory from a representative replica (left) and their corresponding structures (right). (**A**) GS system; (**B**) GS-C99 system; (**C**) GS-C83 system and (**D**) GS-Notch system.

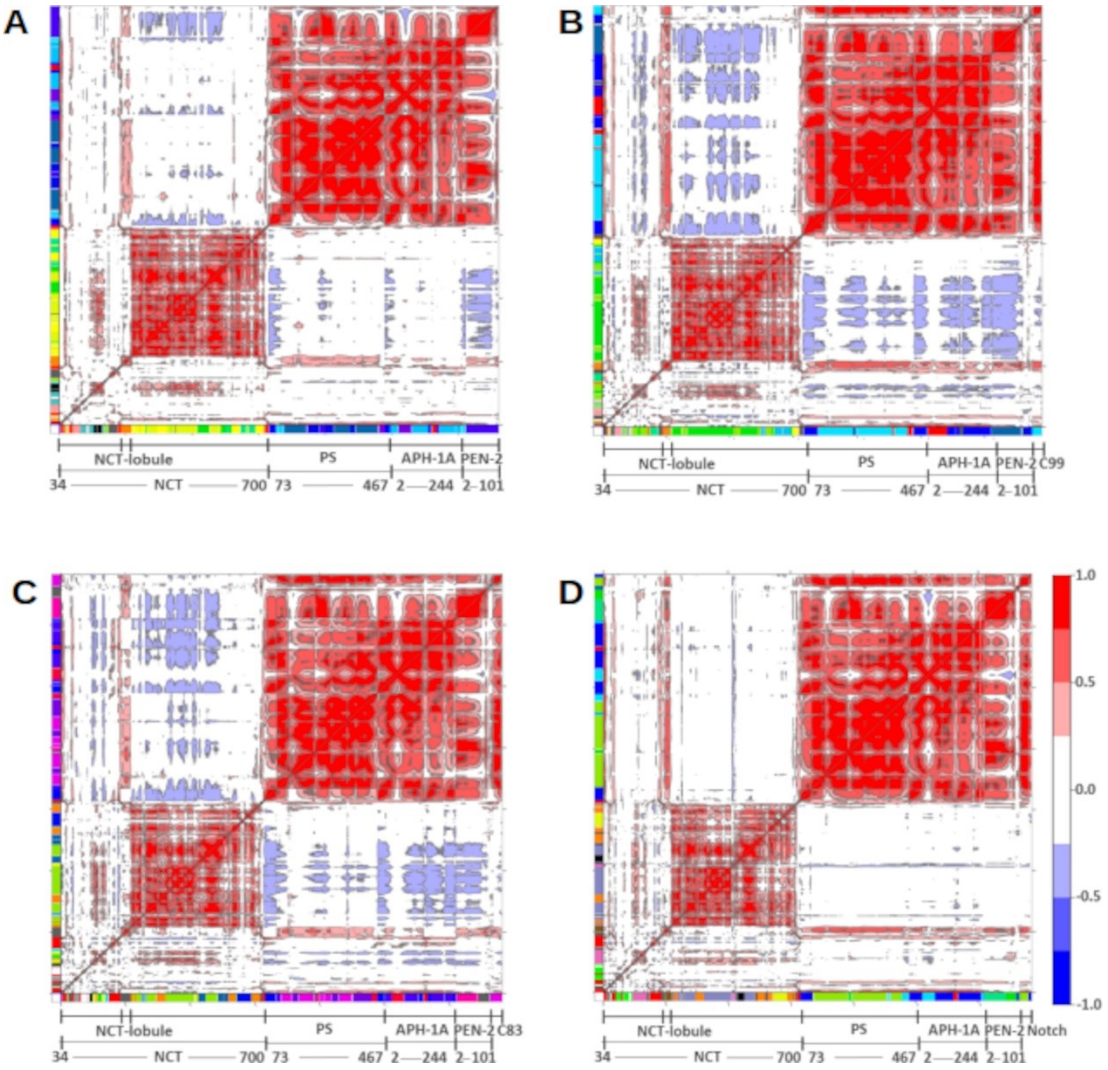

Fig. S5 Residue plot of cross-correlated motions. Each cross-correlated map was computed over 1 μs of molecular dynamic trajectory from a representative replica. A high correlated motion has 1.0 value (red), and high anti-correlated motion has -1.0 value (blue). (**A**) GS system; (**B**) GS-C99 system; (**C**) GS-C83 system and (**D**) GS-Notch system.

Node betweenness analysis

The Figures S6, S7, and S12 show significative differences in node betweenness at the specific components of GS and GS-substrate complexes. NCT in the GS system shows high node betweenness in residues His222, Cys248-Asn255, these residues comprise a short β-sheet that is linked to the NCT-lobule and may act as a hinge in the substrate-free GS simulated system. However, NCT residues with high node betweenness in the GS-C99 system are Met231, Ser235, Ile236, Tyr345, Met347, and Val354. Interestingly, in our GS-C99 system took place a network node connection rearrangement. The Met231, Ser 235, and Ile236, that are in the small α-helix of the NCT-lobule, have a preponderant weight in the network. Therefore, the NCT-lobule begins to act as an important connector between the extracellular and the TM components. Residues Tyr345, Met347, and Val354 of GS-C99 system are located at the NCT extracellular large-lobe region and in close proximity to the PS extracellular loops. On the other hand, NCT residues with high node betweenness in the GS-C83 system, comprise Cys230, Arg233, Cys248, Pro250, Asn255, and Val256. Cys230 and Arg233 are in the NCT-lobule helix, the other residues are in the β-sheet that is linked to the NCT-lobule. Indicating, that both regions have high weight in the whole network and can have a structural hinge function. Interestingly, similarly to the GS system, we did not find residues in the extracellular large-lobe region of NCT with high betweenness values. However, our GS-Notch system displays similar patterns as the GS-C99 system. Residues located in the NCT (Arg232, Arg233, Val275, Val276, Asp346, and Val354) have high betweenness values. Surprisingly, residues Phe94-Leu98 located in the extracellular-short α-helix of PEN-2 also show high node betweenness in all GS and GS-substrate systems.

Node betweenness of the TM components, were also measured. The GS system shows high node betweenness at the residues Ile83, Val87, and Pro88 of TM1; Tyr195, Ala199, Trp203, Val207, and Gly209 of TM4; Tyr820, Leu226, and Ile227 of TM5; Leu30, and Arg31 of APH-1A. The GS-C99 system has the same residues with high node betweenness, adding the residues Leu123 and Ser124 of the APH-1A. Intriguingly, the GS-C83 system changes the arrangement of preponderant node connections, and does not show residues with high node betweenness in TM1 and TM4. Nevertheless, appear the residues Tyr181, Gly183 of TM3; Tyr225, Leu232, Met233, and Ala234 of TM5; Ser390 of TM7; Val412 of TM8; Leu123 and Ser124 of APH-1A. The GS-Notch system shows the same preponderant node connections as GS-C99 in TM1, TM4, and TM5, but does not show residues in APH-1A. The node betweenness analysis shows the impact of the residues positioned at specific regions over the whole of the GS mechanistic function. We observe the importance of the NCT-lobule helix (Fig, S6, S7, and S12) to communicate the extracellular NCT with TM helices. As well as the relevance of TM4 to connect with other TM components of PS1 in GS, GS-C99, and GS-Notch systems. Nevertheless, in the GS-C83 system, the TM4 role is replaced by TM3 and TM5.

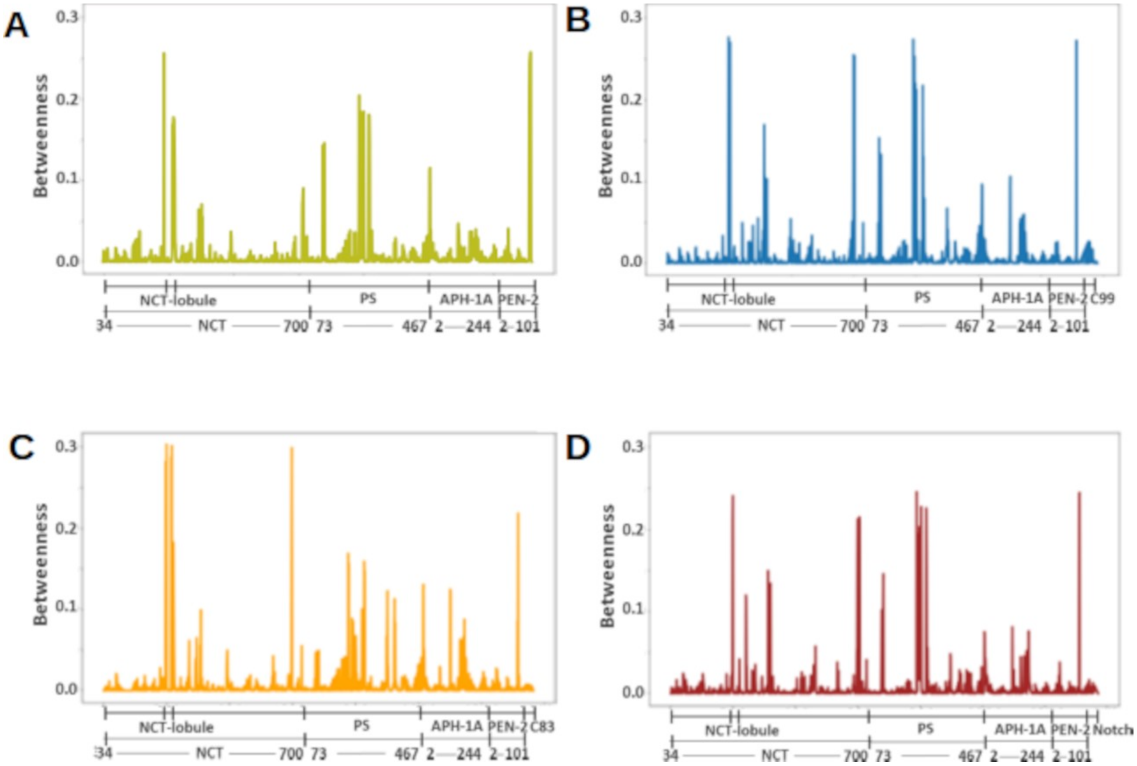

Fig. S6 Residue plot of node betweenness analysis. (**A**) GS system; (**B**) GS-C99 system; (**C**) GS-C83 system and (**D**) GS-Notch system.

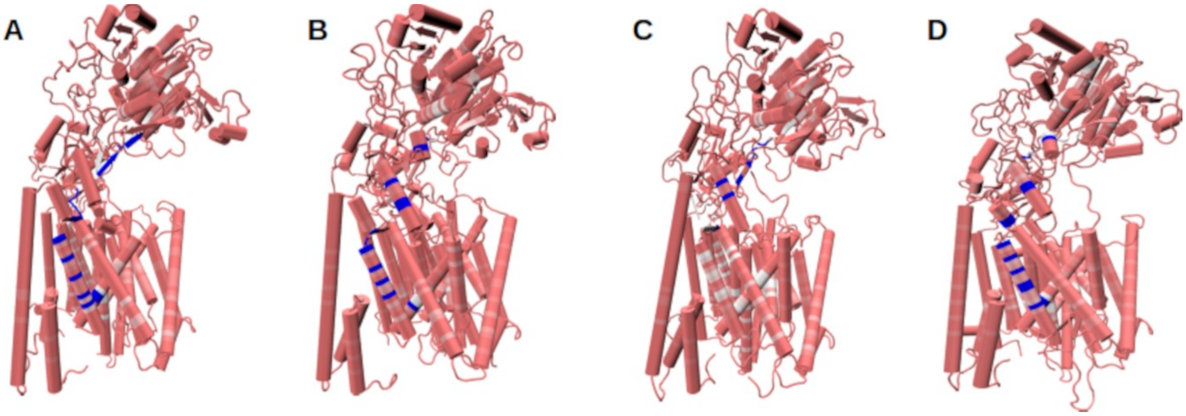

Fig. S7 Node betweenness projected into the GS and GS-substrate structures. (**A**) GS system; (**B**) GS-C99 system; (**C**) GS-C83 system and (**D**) GS-Notch system.

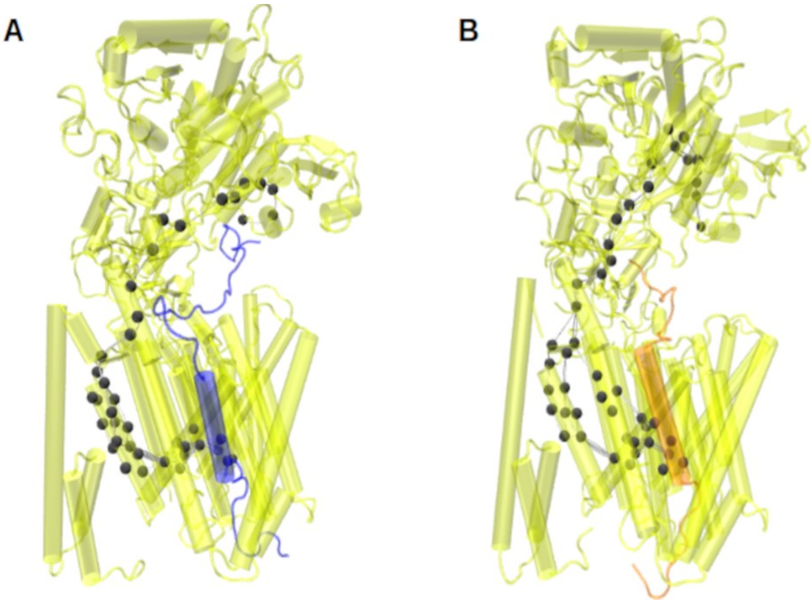

Fig. S8 Suboptimal paths from NCT to catalytic site. The residues participating in the paths are colored in gray, the C99 is colored in blue and C83 in orange. (**A**) GS-C99 system; (**B**) GS-C83 system

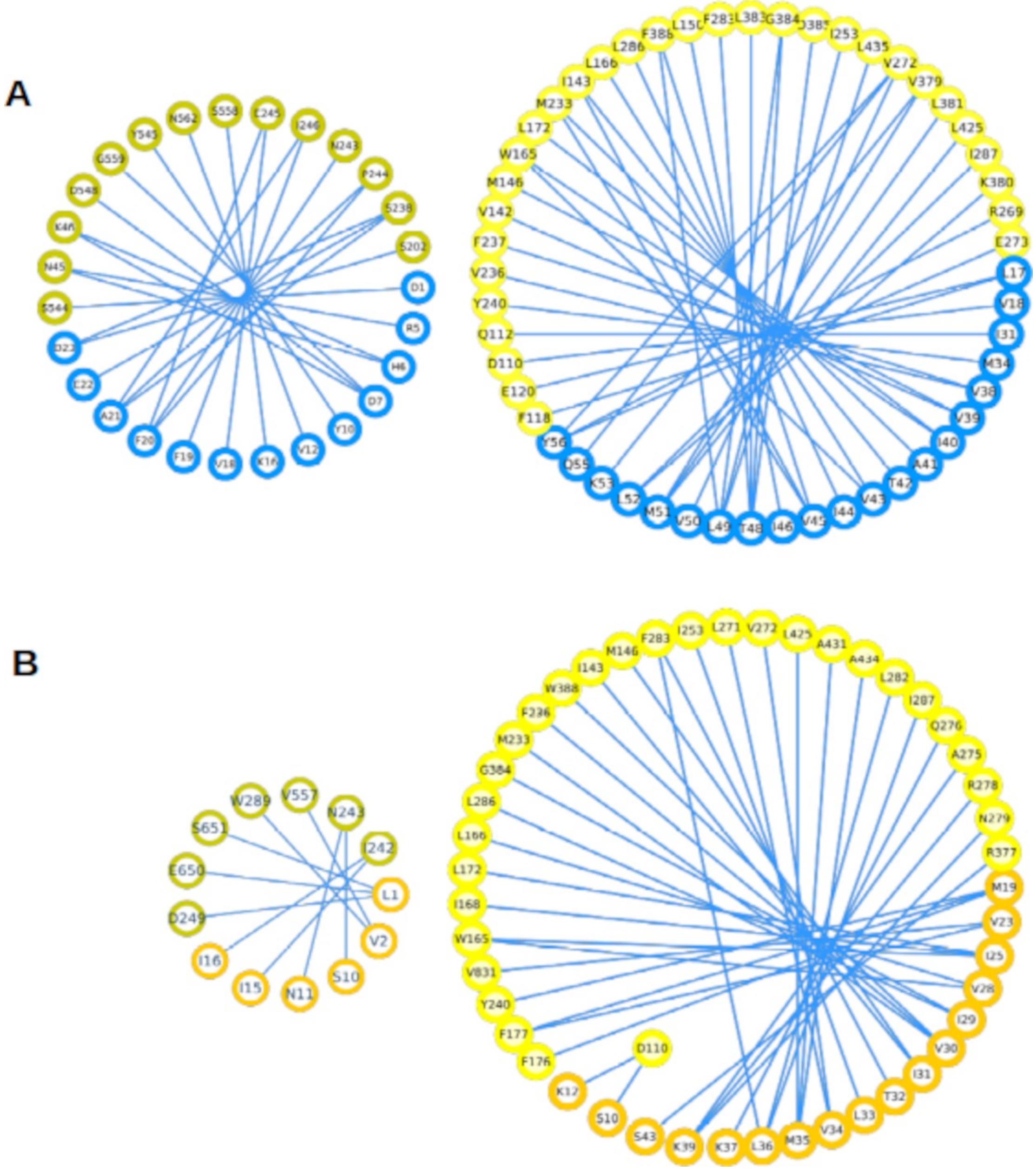

Fig. S9 Interactions of NCT (green circles) and PS (yellow circles) with C99 (blue circles) and C83 (orange circles). (**A**) GS-C99 system; (**B**) GS-C83 system.

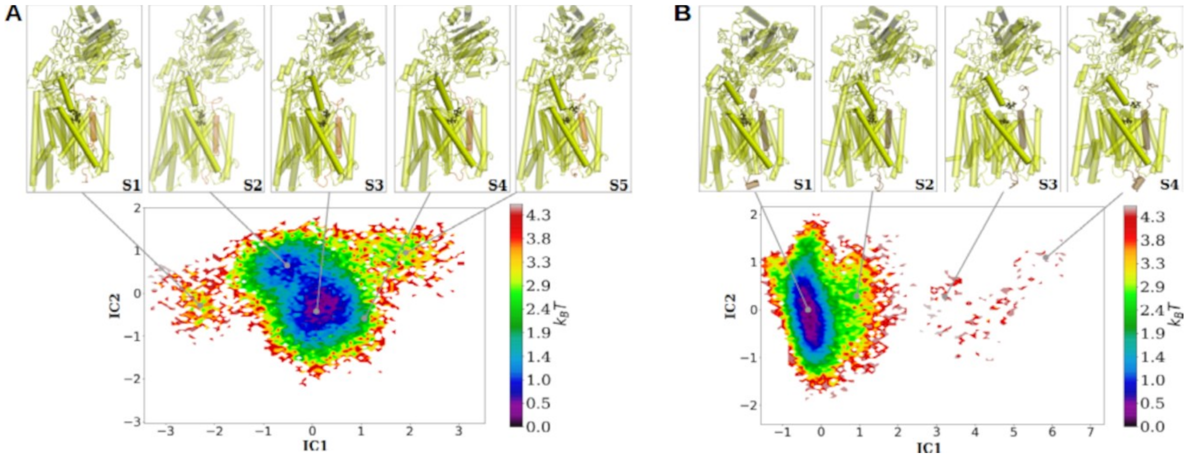

Fig. S10 Free energy surface projected into first two IC. Showing the relevant structures of each macrostate, the Ile242 (NCT-lobule)-Ile180 (TM3) residue-pair is colored in olive, C83 substrate in orange, Notch in brown and GS in green. (A) GS-C83 system; (B) GS-Notch system.

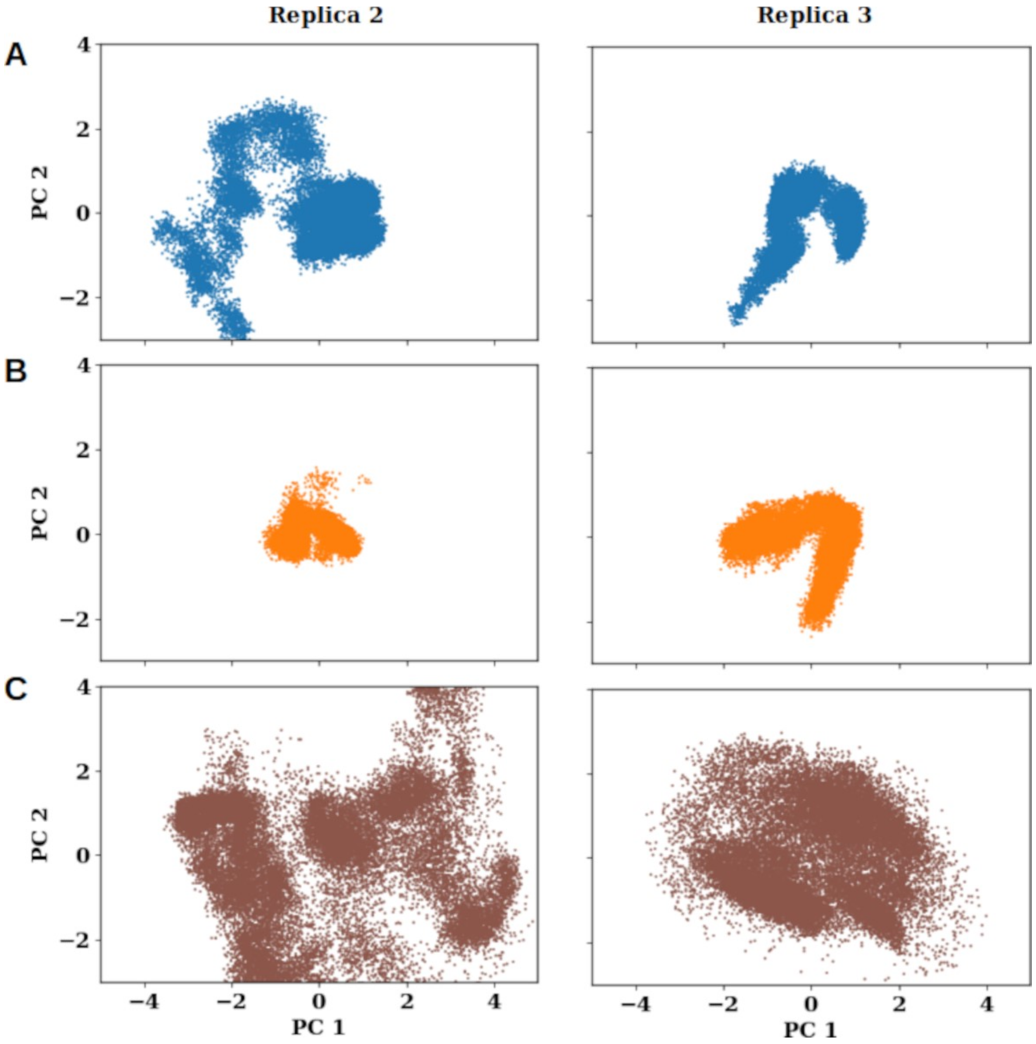

Fig. S11 PCA replicas of GS substrates. Projection of the first two principal component of each GS-substrate systems (replica 2 and replica 3). (**A**) GS-C99; (**B**) GS-C83; (**C**) GS-Notch.

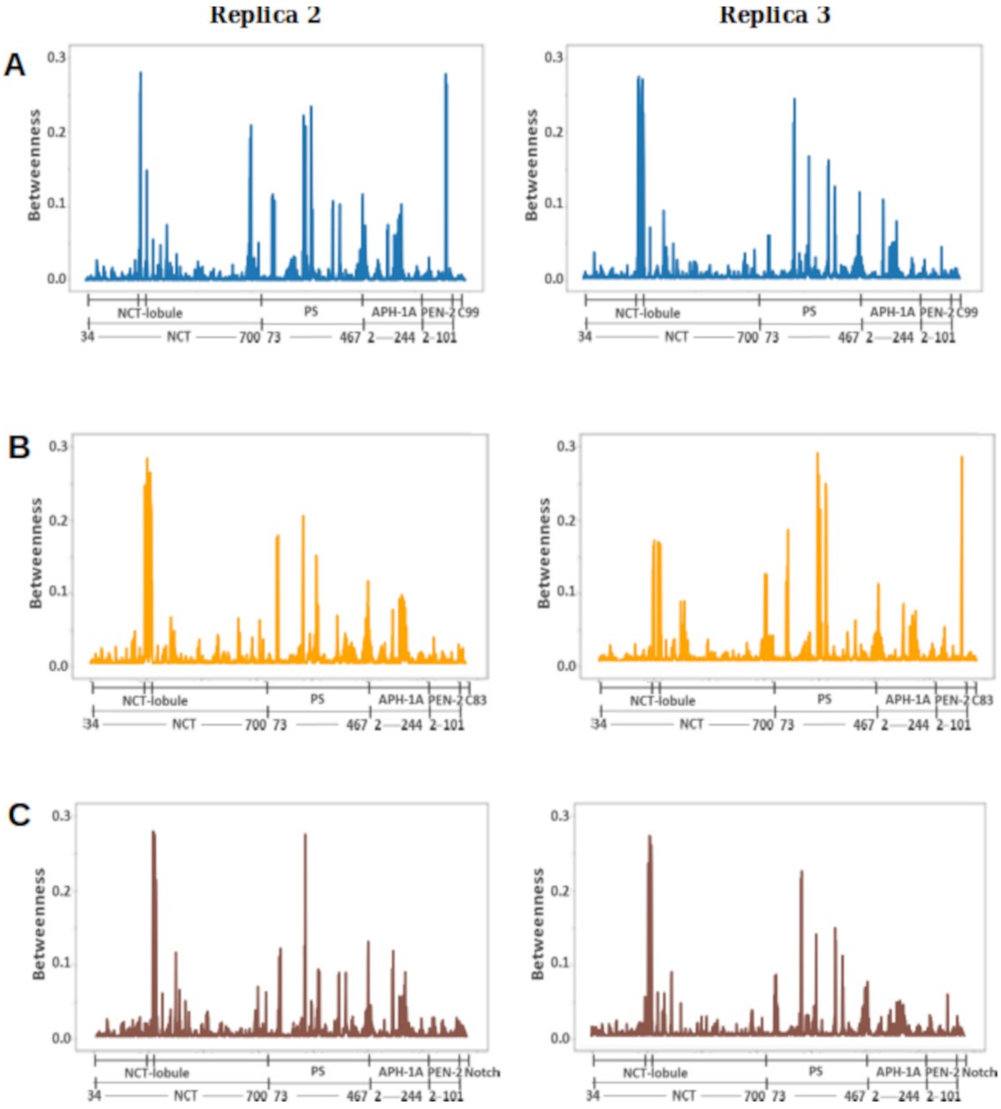

Fig. S12 Residue plot of node betweenness analysis (replica 2 and replica 3). (**A**) GS-C99 system; (**B**) GS-C83 system and (**C**) GS-Notch system.