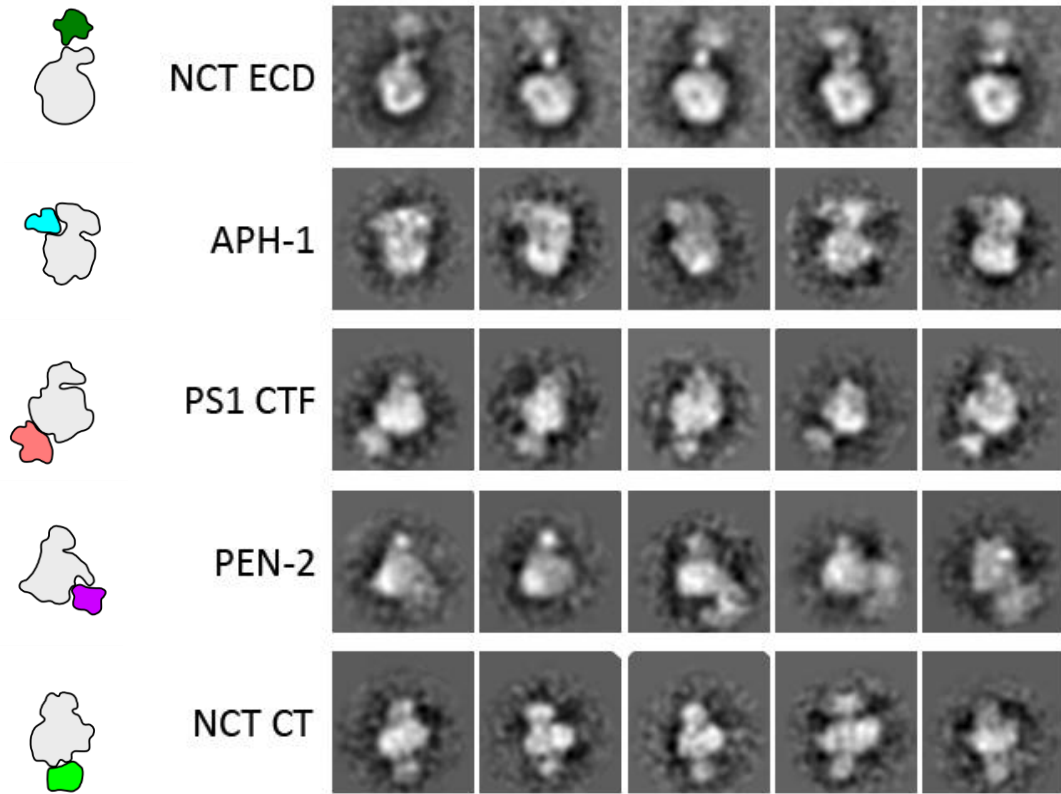
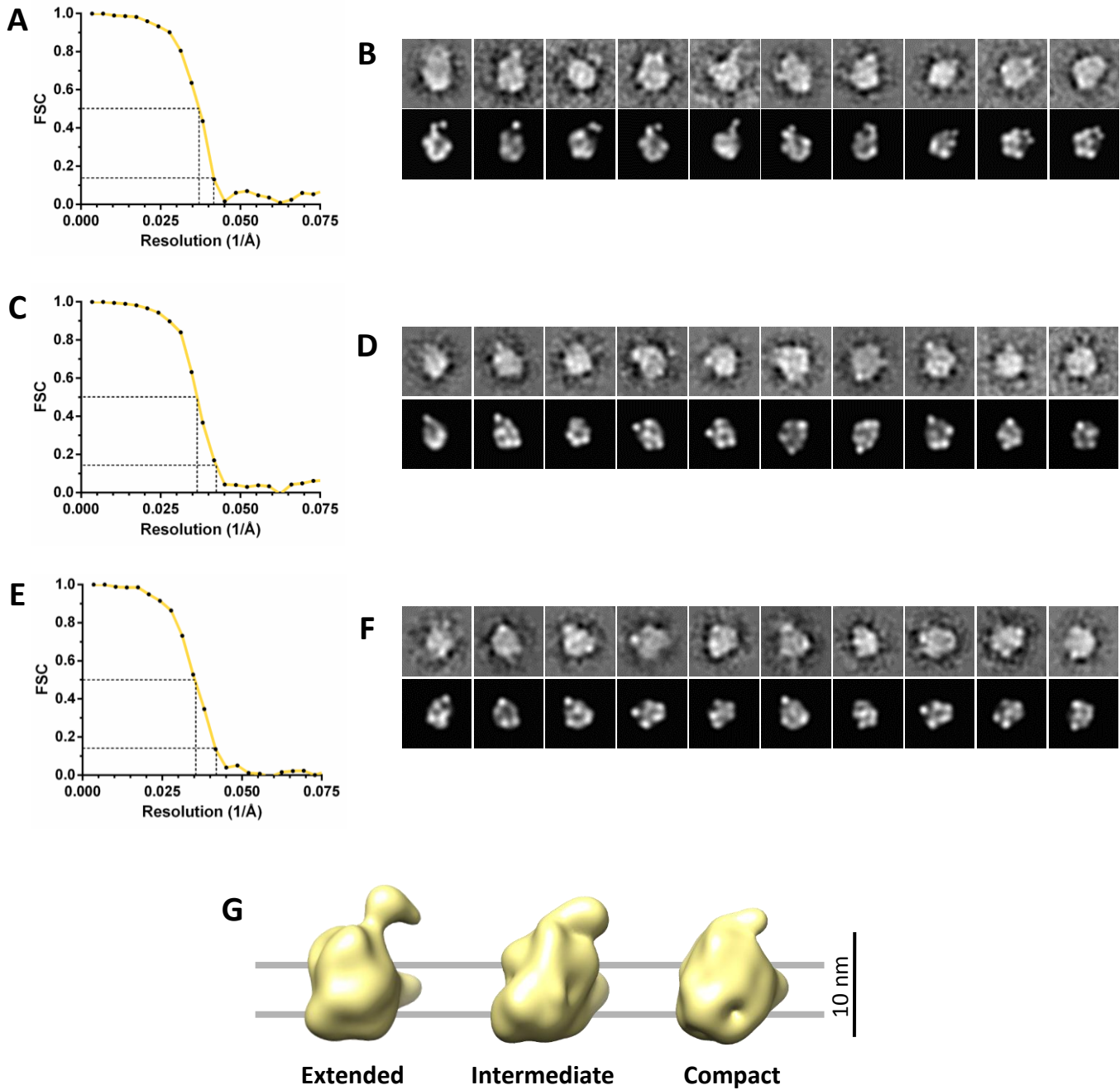


# Figure S1



Antibody labeling in 2D class averages as presented in Fig. 2F-H. For each labeled subunit, the outline (first column) and class average (second column) from Fig. 2H and G respectively are presented along with 4 additional class averages, showing antibody density at the same position with respect to the  $\gamma$ -secretase.

# Figure S2

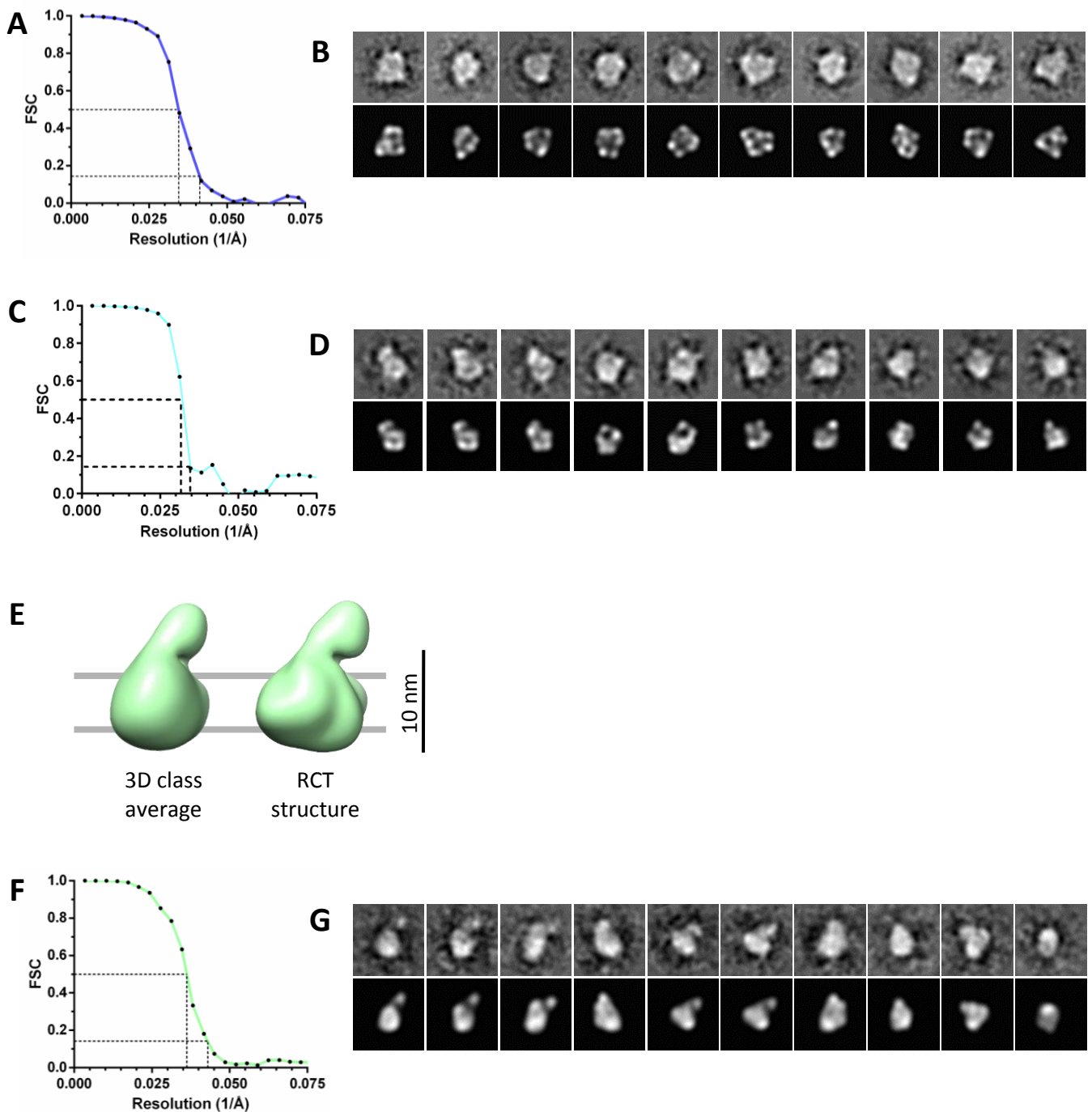


(A, C, E) FSCs using 'gold standard' (Scheres and Chen 2010) generated for the extended, intermediate and compact wt  $\gamma$ -secretase structures in Fig. 3B-D respectively. Dotted lines in the FSC curves are 0.5 and 0.143 cutoffs.

(B, D, F) Class averages and matching re-projections for each structure.

(G) 3D reconstruction and unsupervised 3D classification performed independently using RELION (Scheres, 2012)

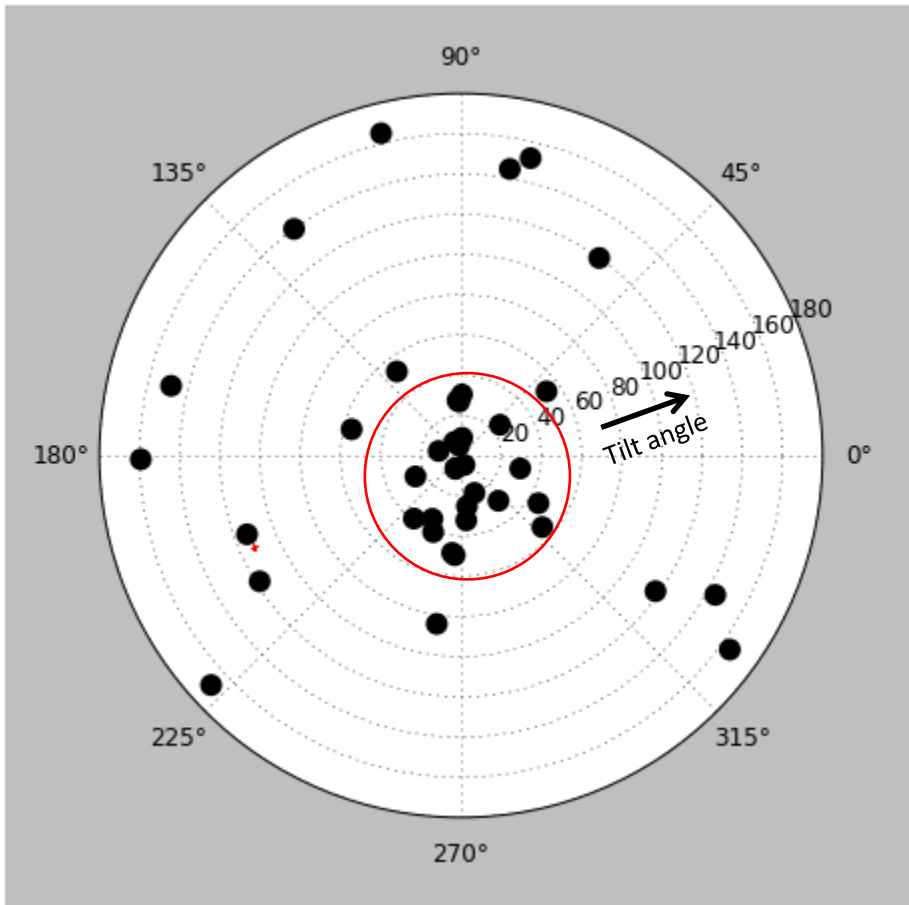
# Figure S3



FSCs from 'gold standard' refinement of half data sets (Scheres and Chen 2010), class averages and matching re-projections shown for the representative structures of the  $\gamma$ -secretase-inhX (A,B)  $\gamma$ -secretase-pep15 (C,D)  $\gamma$ -secretase- $\Delta 9$  (F,G).

(E) 3D average and representative RCT structure from the  $\gamma$ -secretase- $\Delta 9$  data set.

Figure S4



Tilt-pair validation plot (Roenthal and Henderson, 2003) for the  $\gamma$ -secretase-inhX structure. Micrograph was collected at 0° and 25°. The average tilt angle of the clustered tilt-pairs surrounded by a red circle is 24.8°, confirming the overall correctness of the structure.