



**Figure 4—figure supplement 1. Cryo-EM analysis of AP2 in the presence of an anionic polymer (DNA).**

**A.** 2D class averages for WT AP2 + DNA are shown. Averages corresponding to the closed conformation (red inset) and open conformation (blue inset) are shown next to their respective molecular models (PDB 2VGL; PDB 2XA7).

**B.** 3D classification shows that the dataset contains a mixture of open and closed conformations. Classification was performed in Relion 3. Resolution is limited for all open structures because of an extreme preferred orientation in the cryo-EM grids.

**C.** Quantification of 3D classification. Four datasets were analyzed and the percentage of particles that classified into open or closed 3D classes were quantified and plotted.

**D.** Representative 2D class averages from AP2 samples. Mutations and the presence of DNA are labeled. DNA is in a 5-fold molar excess. Closed 2D classes are boxed in red, open 2D classes are boxed in blue.

**E.** Binding curve of phosphoAP2(mu E302K)-NECAP and a 47 bp ssDNA oligo. 20 nM DNA was incubated with increasing amounts of phosphoAP2-NECAP. A native gel shift assay was used to measure binding. Data was plotted and analyzed using the Prism software package.