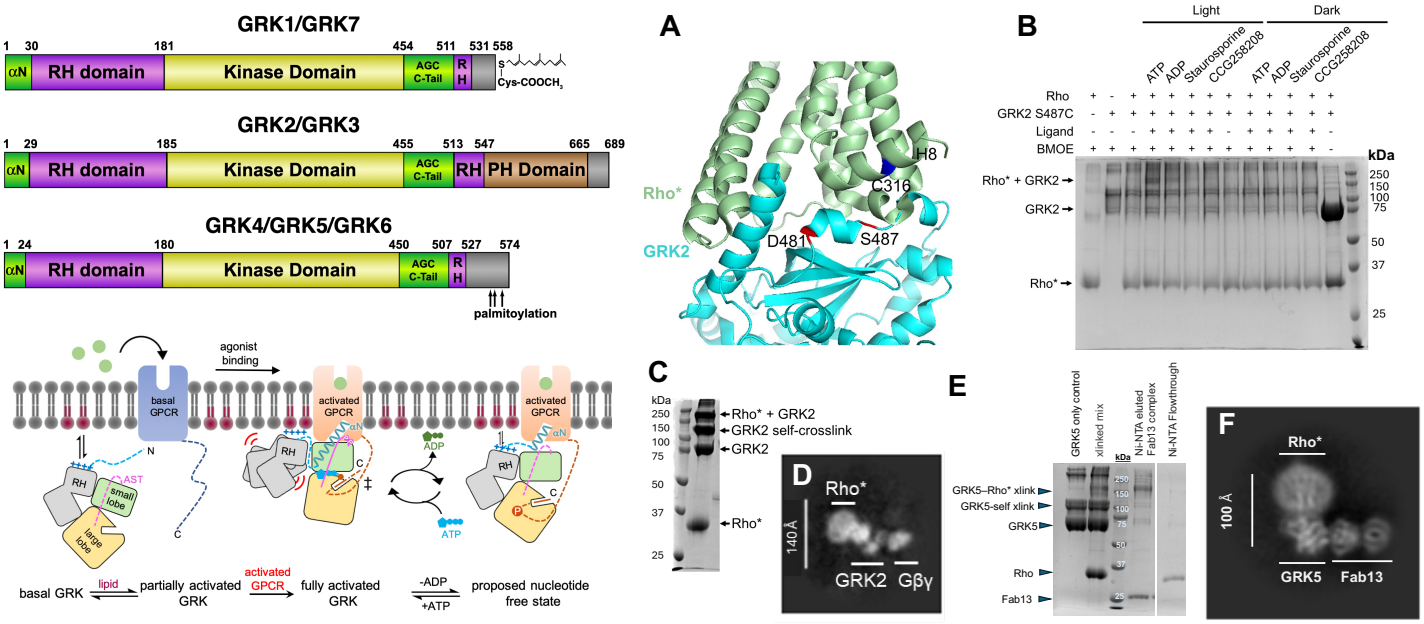

Figure 1: GRK domain structure and function. Top, domain diagram of GRK subfamilies. Green regions are implicated in receptor binding. Bottom: Model of GRK action at an activated GPCR. In its basal state, the GRK kinase domain is in an open, inactive conformation. Upon encounter of an activated receptor, the disordered GRK N terminus forms a helix (αN) that docks into the cytoplasmic cleft of the activated GPCR and also packs against the hinge and AST of the kinase domain to trigger domain closure to an active state. GRK1 may need to at least partially dissociate from the receptor to exchange adenine nucleotides, and in this state αN and the large lobe could remain bound to the cytoplasmic cleft and the phosphorylated tail of the receptor, respectively, to facilitate additional rounds of phosphorylation. Pink circles indicate autophosphorylation sites, and the red Rho* phosphosites.

Figure 2: **Workflows for trapping and imaging GRK–Rho* complexes.** **A)** AlphaFold2 model of GRK2–Rho* and the position of two residues in the GRK2 AST mutated to Cys (D481C and S487C) to enable a chemical crosslink to Rho*. Only GRK2-S487C formed a crosslink to Rho* using BMOE. **B)** The Rho*–GRK2 complex only forms in light and with the ligands ATP and ADP, but not with active site inhibitors staurosporine and CCG258208. **C)** Optimization generates higher yields of the Rho*–GRK2 complex. **D)** 2D class average of the Rho*–GRK2–Gβγ complex using an LMNG micelle to solubilize Rho*. **E)** Auto-phosphorylated GRK5·ADP crosslinks using BM(PEG)$_3$ to Rho* in a light dependent manner (data not shown) and can be separated from unreacted and self-crosslinked GRK5 by passage through tandem Q and SP Sepharose columns, and then from unreacted Rho* by Ni^{2+}·NTA pulldown in the presence of Fab13, which co-elutes with the complex. **F)** 2D class average of the Rho*–GRK5–Fab13 complex using an LMNG micelle to solubilize Rho*.

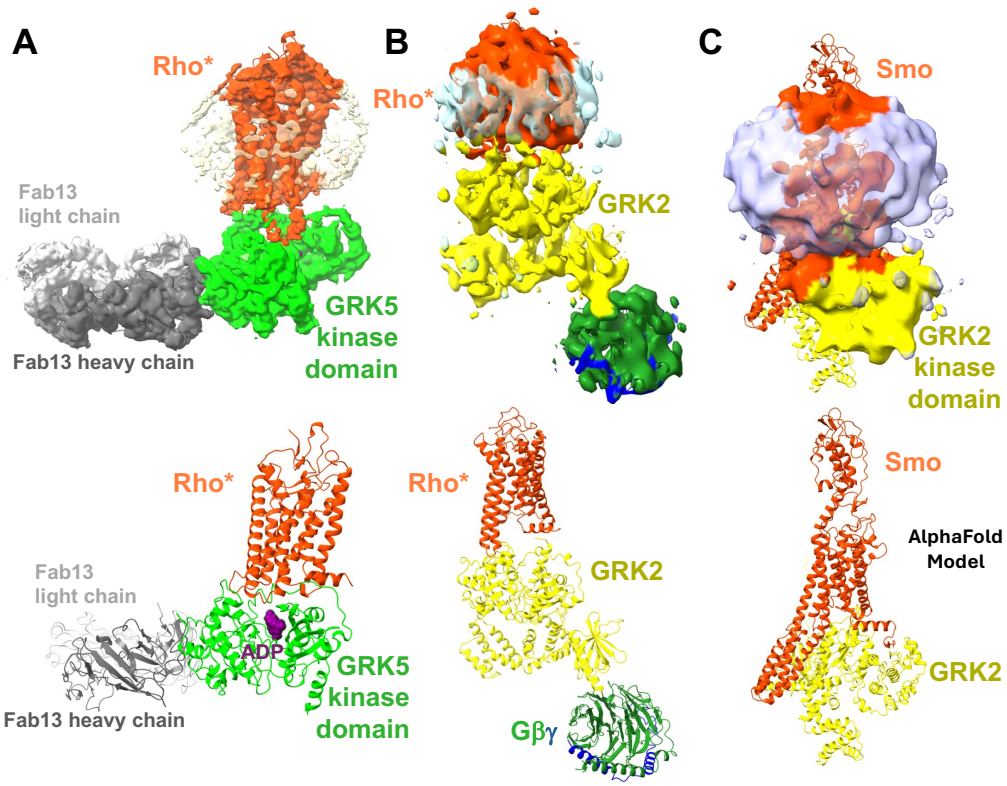

Figure 3: **Preliminary reconstructions and models of GRK–GPCR complexes. A)** Rho*–GRK5–Fab13. **B)** Rho*–GRK2–Gβγ. **C)** Smo–GRK2.