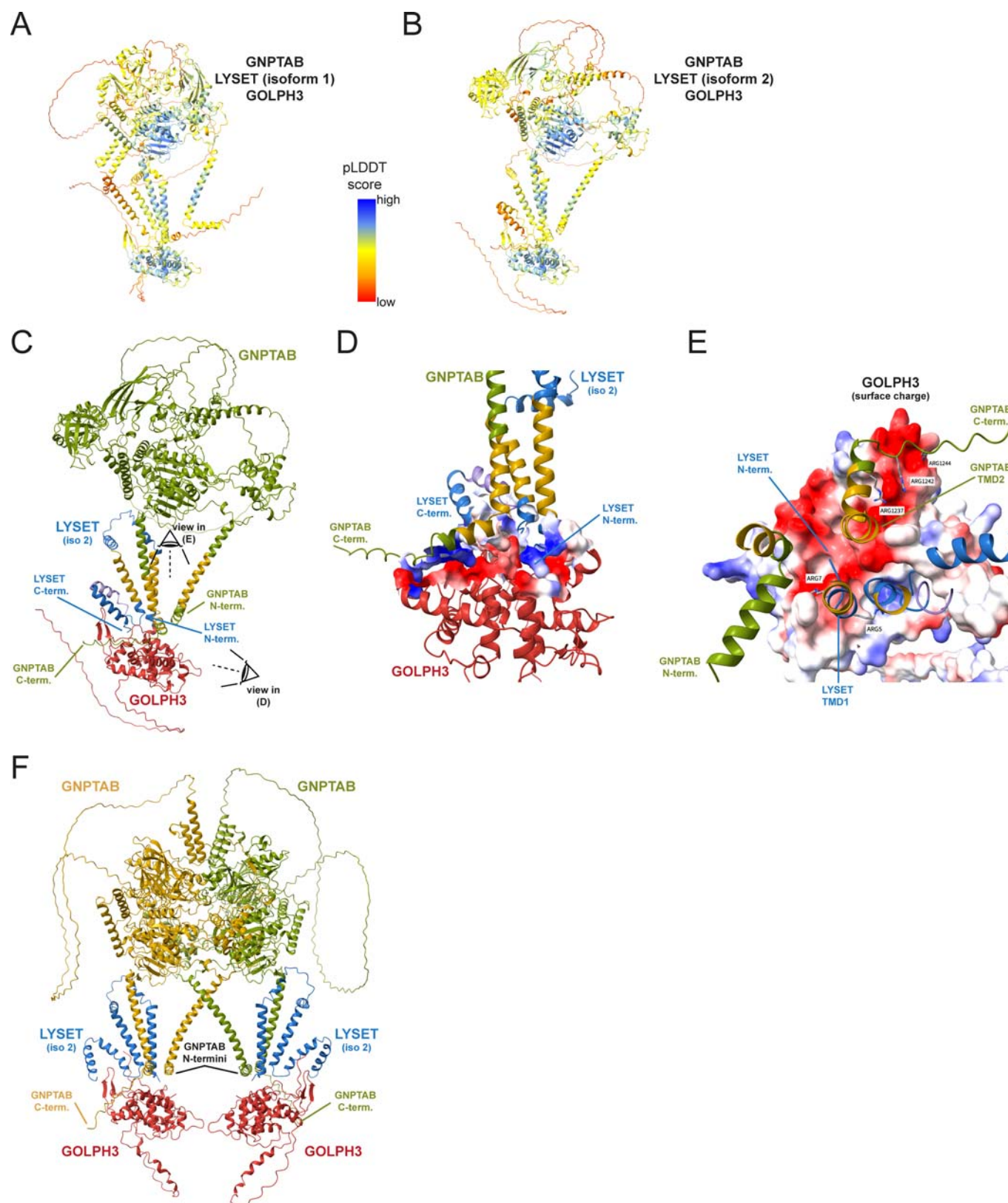


Expanded View Figures

Figure EV1. Alphafold2 Multimer prediction of a potential interaction of LYSET and GOLPH3.

(A) Schematic overview of the topology of LYSET. Based on Uniprot annotations, LYSET is a double-pass membrane protein with cytosolic N- and C-termini. Putative initiator methionines for the long and short isoform are highlighted in magenta and positively charged lysine and arginine residues in green. The sketch was generated using Protter (Omasits et al, 2014). (B) Alphafold2 Multimer prediction of a potential interaction of LYSET and GOLPH3 with coloring based on the prediction confidence score. (C) LYSET:GOLPH3 complex as depicted in (B) with GOLPH3 colored in red and LYSET in blue. Annotated TMD1 and TMD1 along with a putative TMD predicted by DeepTMHMM (Hallgren et al, 2022) in LYSET are colored in yellow. Insert, magnification of the LYSET:GOLPH3 interaction interface with side chains of select positively charged residues in LYSET and negatively charged residues in GOLPH3 shown. (D) Face-down view from the membrane plane on the predicted interaction of the LYSET N-terminus (blue) and GOLPH3 (surface charge shown). Side chains of positively charged amino acid residues in the LYSET N-terminus are shown. A similar putative cargo binding site is conserved in GOLPH3L (right). (E) Co-immunoprecipitation of LYSET and GOLPH3 in HEK293 transfectants. WT HEK293 cells were transfected as indicated and GOLPH3 was immunoprecipitated with anti-GOLPH3 (ab98023) from obtained cell lysates. Immunoprecipitates were analyzed by immunoblotting. HC, antibody heavy chain. (F) Transient overexpression of C-terminally myc-tagged LYSET (isoform 1) variants, that carry point mutations in select arginine residues in the N-terminal cytosolic region, in LYSET KO HEK293FT cells. LYSET protein levels were analyzed by immunoblotting with equal proteins amounts loaded and actin serving as a loading control. Source data are available online for this figure.





◀ **Figure EV2. AlphaFold 3 predictions of a ternary interaction of GOLPH3, LYSET and GNPTAB.**

(A) Structure of the predicted GOLPH3:LYSET (isoform 1):GNPTAB complex shown in Fig. 6A with coloring reflecting the prediction score. (B) Structure of the predicted GOLPH3:LYSET (isoform 2):GNPTAB complex with prediction score coloring. (C) Structure of the complex shown in (B). Individual subunits are given in different colors. Annotated TMD regions are colored in yellow. In case of LYSET, a putative TMD region predicted by DeepTMHMM (Hallgren et al, 2022) is shown in purple. (D) Side view the complex containing LYSET isoform 2 shown in (B, C), highlighting close proximity of GOLPH3 and the LYSET N-terminus as well as the GNPTAB C-terminus. Surface electrostatic potential is shown for selected amino acid side chains. (E) Same complex as shown in (B, C), but top-down view from the membrane plane onto the GOLPH3 surface. Positively charged amino acid side chains in LYSET and GNPTAB are shown as stick representation. (F) AlphaFold 3 prediction of a complex containing a GNPTAB dimer (yellow and green) as well two LYSET (isoform 2) and two GOLPH3 subunits.