Figure S1. Binding prediction of single-pass receptor ligand complexes of AMH-AMHR2 using full or truncated sequences. (A-E) Structural binding prediction and corresponding contact maps for, light green: pdb database AMH, dark green: AF2 predicted AMH, light blue: PDB database AMHR2, dark blue: AF2 predicted AMHR2, purple: contacts (A) PDB entry, (B)

secreted ligand and extracellular domain (ECD) of receptor, (**C)** secreted AMH and full receptor including intra (ICD), transmembrane (TCD) and ECD, (D) full ligand and ECD and (E) full ligand and full receptor. Tick labels indicate residue position in full length canonical protein (AMH accession= P03971, AMHR2 accession=Q16671).

Figure S2. Composition of the single pass transmembrane receptor library. (A) Pie graph of tissue distribution of the receptors in the library according to the human protein atlas (HPA). (B) Receptor expression (mean nTPM) relative to the number of receptors (# Receptors) across tissues. (C) Receptor expression as mean normalized transcript per million (nTPM)

relative to the number of tissues each receptor is expressed in. Each dot represents a receptor. Colors denote tissue distribution according to HPA. (D) Pie graph of the tissue expression of receptors in the library. (E) Receptor expression (mean nTPM) relative the number of receptors (# receptors) across cell types. (F) Classification of molecular functions for receptors in the library.
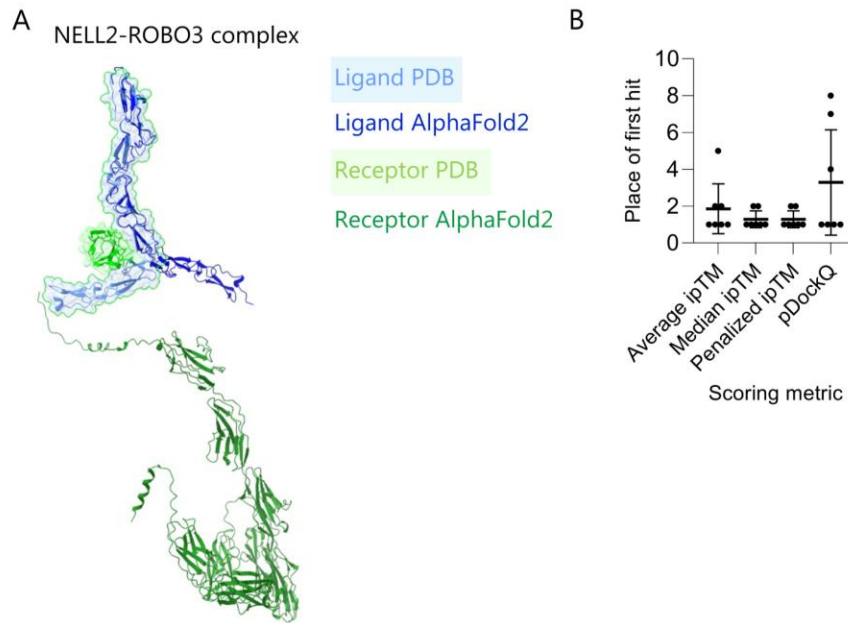
Figure S3. AF2 accurately predicts single-pass receptors for secreted ligands. (A) predicted structure of the NELL2-ROBO3 complex. Annotation as follows: light green cartoon and surface: PDB database receptor, dark green cartoon: AF2 predicted receptor, light blue cartoon and surface: PDB database ligand, dark blue cartoon: AF2 predicted ligand. (B) ranking of first hit when using the scoring metrics average ipTM, median ipTM, or penalized ipTM and pDockQ. Mean and 95% CI indicated.
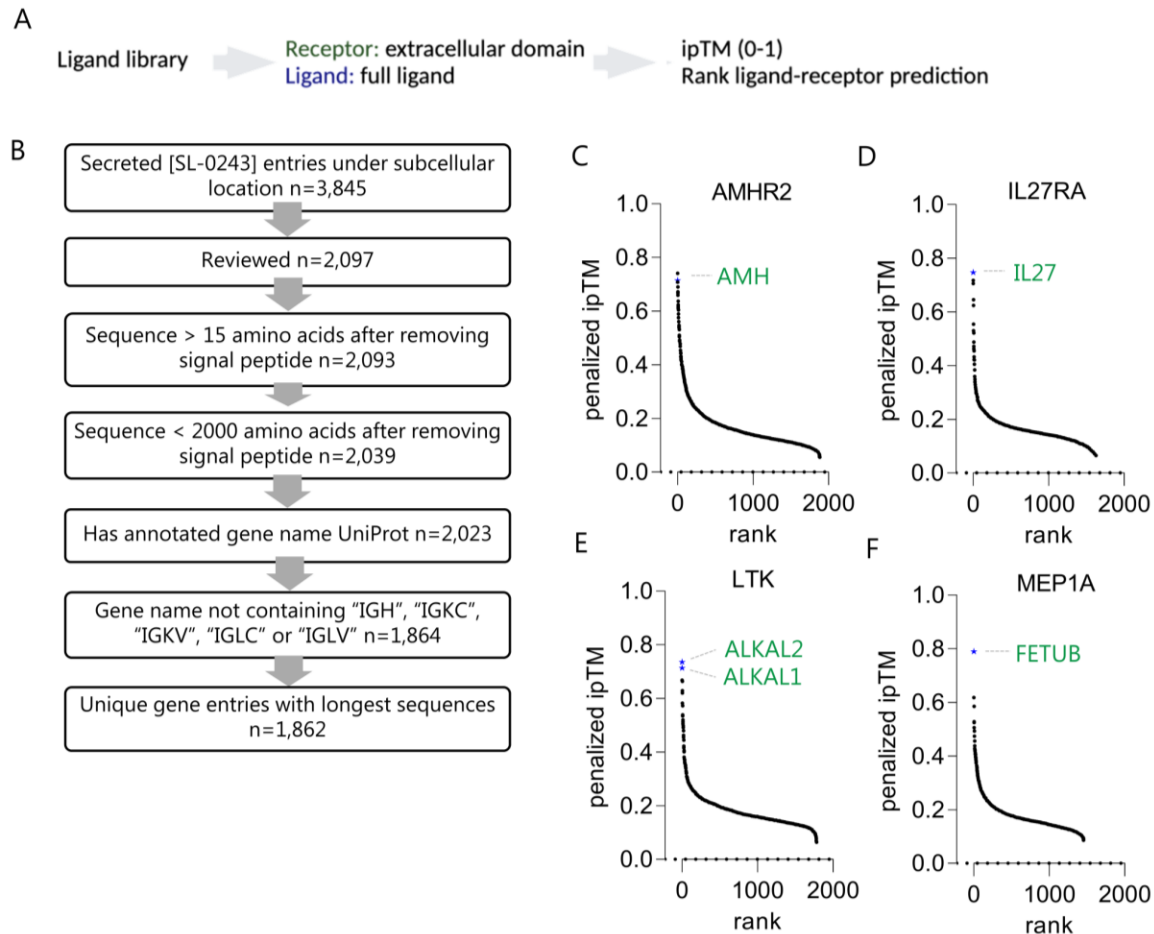
Figure S4. AF2 accurately predicts single-pass receptors for secreted ligands. (A) Approach and metric for scoring binding of single-pass transmembrane receptors against a ligand library. (B) Schematic of ligand library construction. 1: Extract human entries annotated as secreted [SL-0243] under subcellular location n=3,845. 2: Retain reviewed entries n=2,097. 3: Exclude secreted peptides/proteins with an extracellular domain shorter than 16 amino acids n=2,093 and 4: longer than 2,000 amino acids n=2,039. 5: Keep proteins with annotated gene names n=2,023. 6: Remove immunoglobulins with gene names either including "IGH, "IGKC", "IGKV", "IGLC" or "IGLV" n=1,864. 6: exclude duplicated gene names retaining entry with the longest sequence, n=1,862. Binding prediction of (C) AMHR2, (D) IL27RA, (E) LTK, and (F) MEP1A to the ligand library. Values are expressed as ranked penalized ipTM. The predictions are the median minus median absolute deviation of five independent predictions for each ligand-receptor.
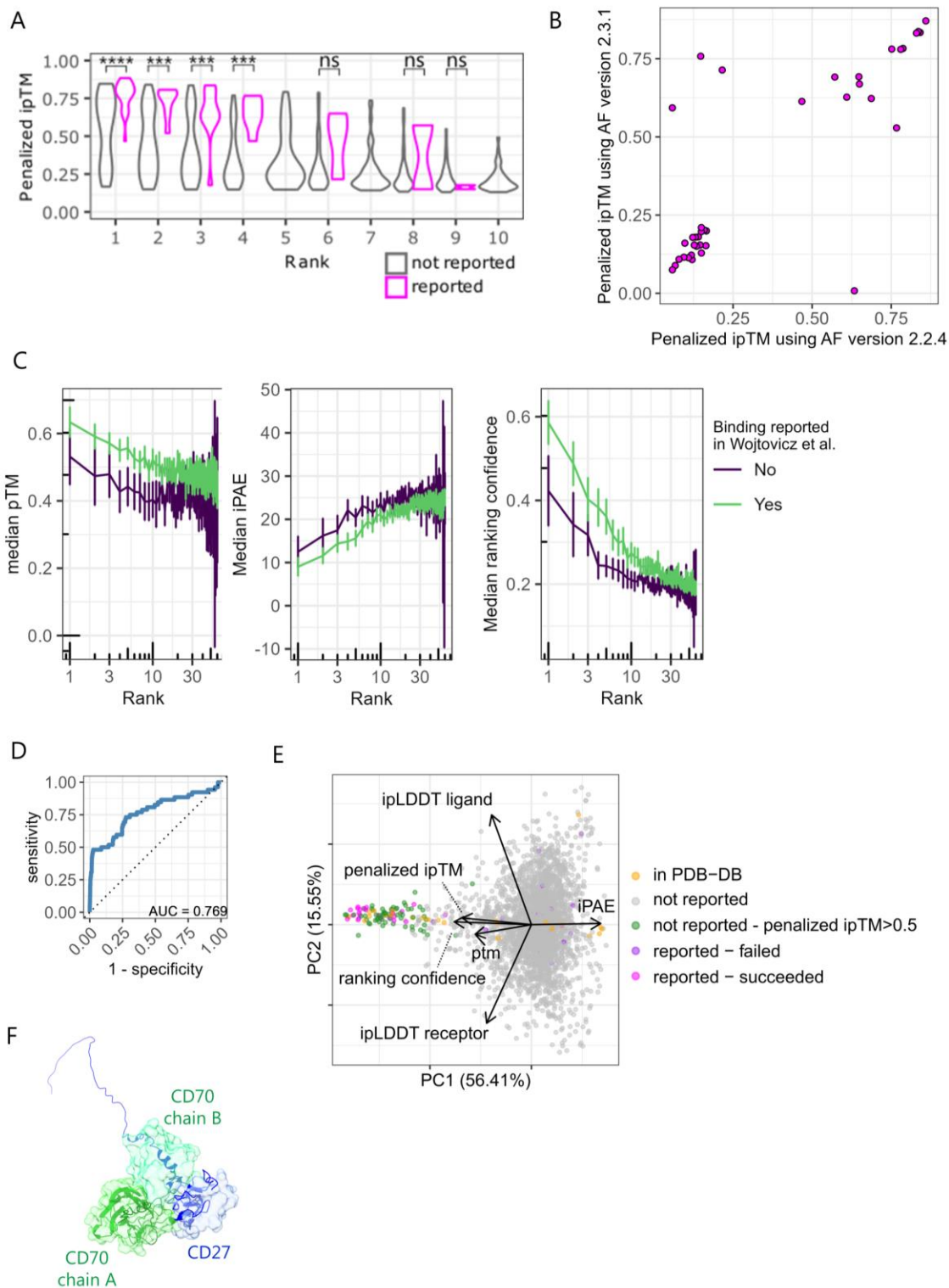
Figure S5. Ligands that fail prediction may be inferred from AF metrics. (A) Violin plot of relationship between rank and ipTM value for binding pairs in *Wojtowicz et al.* (n=2-79). One reported binding ranked fifth, seventh and tenth not shown. **(B)** correlation between penalized ipTM predicted using AlphaFold 2.2.4 and 2.3.1 of 40 ligand-receptor pairs that did not have released structures at the training date cutoff for AlphaFold version 2.3.0. **(C)** Median pTM,

iPAE and ranking confidence as a function of rank for non-binding ligand-receptor pairs grouped by whether the ligand was successfully predicted (ipTM > 0.5) as binding to a receptor also determined to bind in Wojtowicz *et al*. (n=919-2430). Values are presented as mean ± 95% CI. (D) ROC curve using pairs identified in Wojtowicz *et al.* as ground truth (AUC=0.769). (E) principal component analysis (PCA) including indicated metrics from predictions in Fig. 4A. (F) best prediction of the CD27-CD70 heterodimer overlaid with partial crystal structure (7KX0) displaying compound interface, (green cartoon: predicted structure of CD70, blue cartoon predicted structure of CD27, green surface: CD70 crystal structure chain A, turquoise surface: CD70 crystal structure chain B, blue surface: crystal structure of CD27).
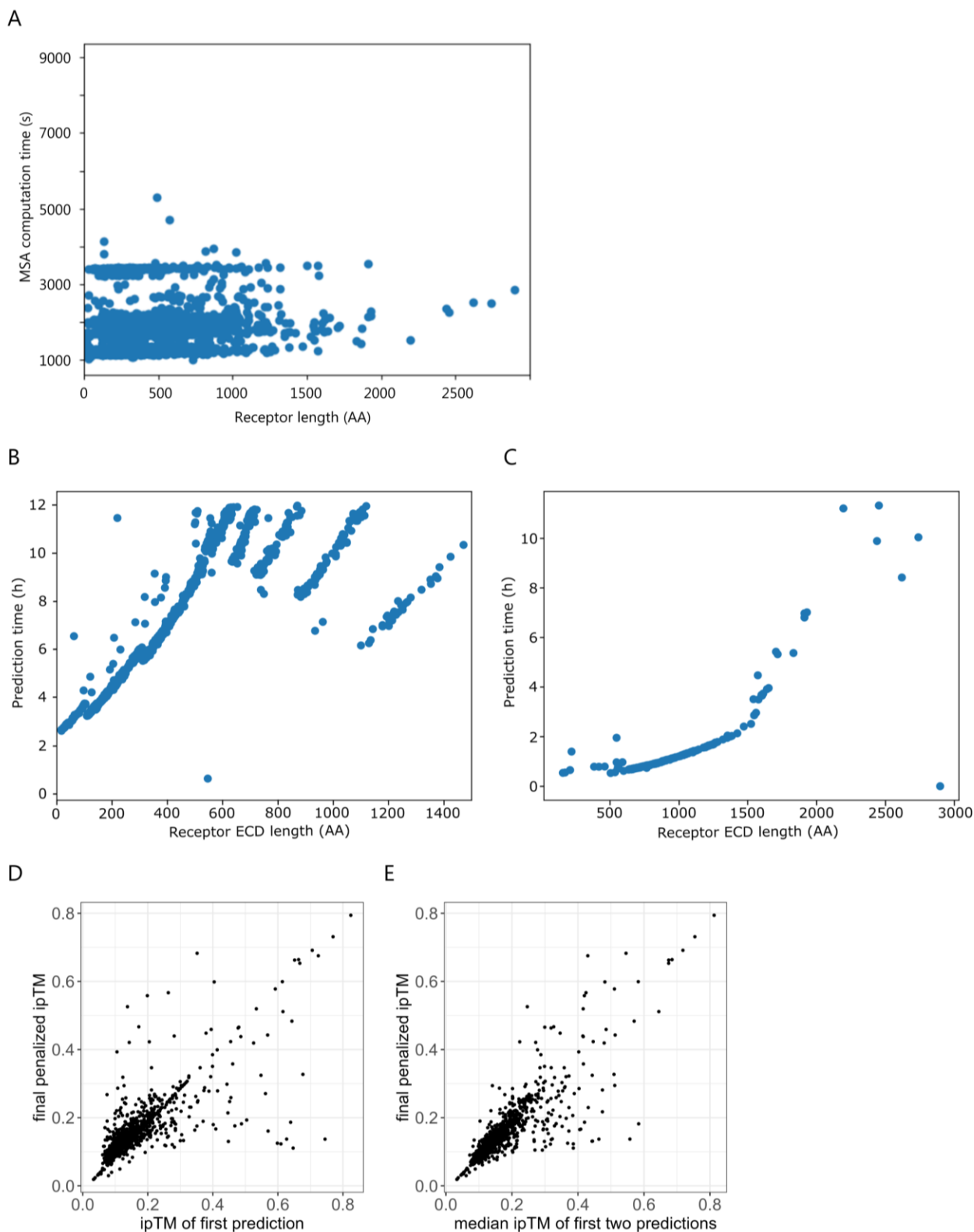
Figure S6. Computational cost and mitigation. (A) computation time needed for multiple sequence alignments (MSAs) by receptor length in amino acids (AA). (B) CPU time spent per receptor in library predicted with BMP10. (C) GPU time spent per receptor in library predicted with BMP10. (D) relationship between ipTM value in first prediction and final penalized ipTM

value after five predictions for IL27. (E) relationship between median ipTM value in the first two predictions and final penalized ipTM value after five predictions for IL27.

Supplementary Data Tables

Table S1. Information on the test set of ligand-receptor pairs with release dates after AlphaFold2 training date cut-off.

Table S2. Library of single-pass receptors included in receptor screen and secreted proteins in ligand screen.

Table S3. List of orphan ligands tested against the receptor screen. This panel was used for Fig. 5.

Table S4. Top-ranking receptor hits for 45 orphan ligands with at least one receptor with a penalized ipTM value > 0.5. List of orphan ligand-receptor pairs with missing predictions.

Table S5. List and data origin of ligand-receptor pairs either binding single-pass or multi-pass receptors used for Fig. 2d.

Table S6. List of genes in the IgSF superfamily used to test the performance of AlphaFold as screen related to Fig. 4.