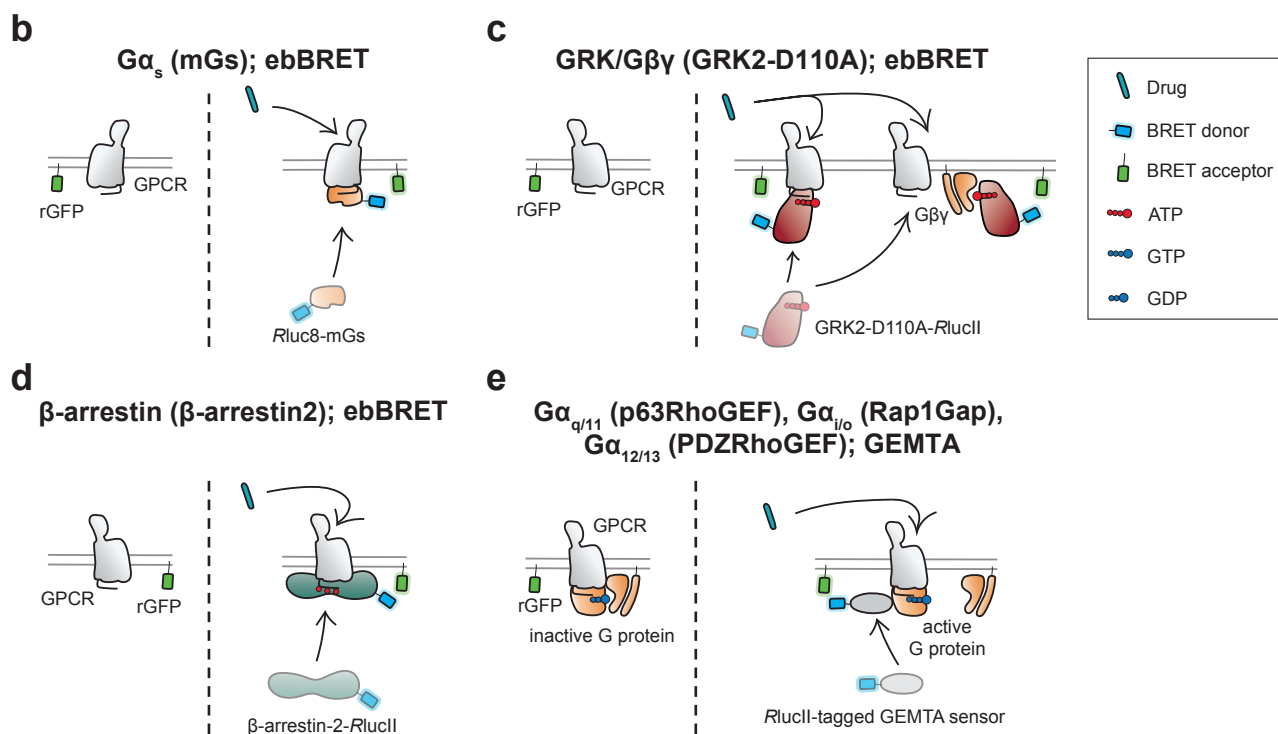
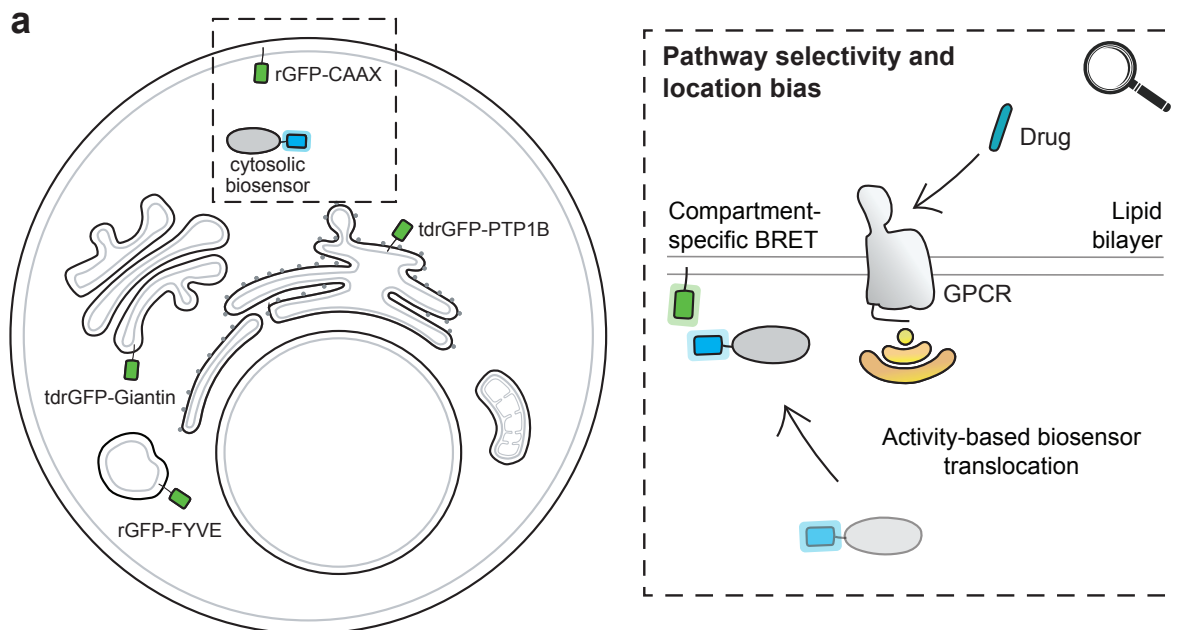


Supplementary information

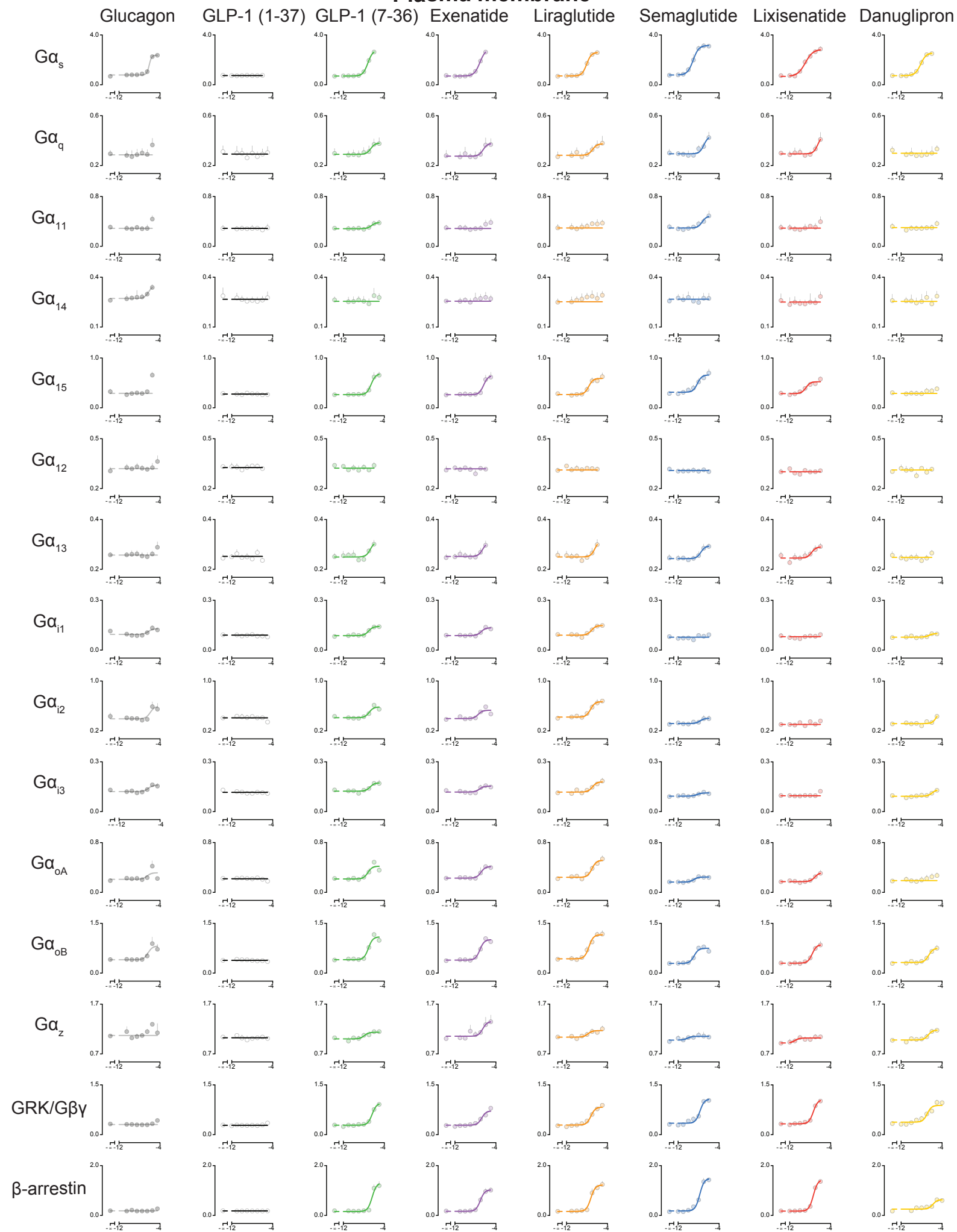
GLP-1R signaling neighborhoods associate with the susceptibility to adverse drug reactions of incretin mimetics

Shane C. Wright, Aikaterini Motso, Stefania Koutsilieri, Christian M. Beusch, Pierre Sabatier, Alessandro Berghella, Élodie Blondel-Tepaz, Kimberley Mangenot, Ioannis Pittarokoilis, Despoina-Christina Sismanoglou, Christian Le Gouill, Jesper V. Olsen, Roman A. Zubarev, Nevin A. Lambert, Alexander S. Hauser, Michel Bouvier and Volker M. Lauschke



Supplementary Fig. 1. Illustrations depicting the ebBRET-based biosensor platform for investigating GPCR signaling neighborhoods. **a**, ebBRET monitors *Rluc*-tagged biosensor translocation with subcellular resolution through co-expression of organelle-specific markers tagged with rGFP. In this study, signaling neighborhoods were investigated at the (1) plasma membrane – rGFP-CAAX; (2) early endosomes – rGFP-FYVE; (3) Golgi apparatus – tdrGFP-Giantin and (4) endoplasmic reticulum – tdrGFP-PTP1B. **b-e**, Schematics depicting the various ebBRET sensors employed in this study. mGs was used as a proxy for measuring G_s pathway activation by detecting the active receptor conformation that is competent for engaging $G\alpha_s$ (**b**). GRK2-D110A acted as a tool for probing receptor binding or free $G\beta\gamma$ (**c**). β -arrestin2 provided information about either receptor engagement or ligand-directed arrestin trafficking (**d**). GEMTA biosensors measured effector recruitment following G protein activation for the following G protein families: (1) $G_{q/11}$ – p63RhoGEF; (2) $G_{i/o}$ – Rap1Gap or (3) $G_{12/13}$ – PDZRhoGEF (**e**).

Plasma membrane

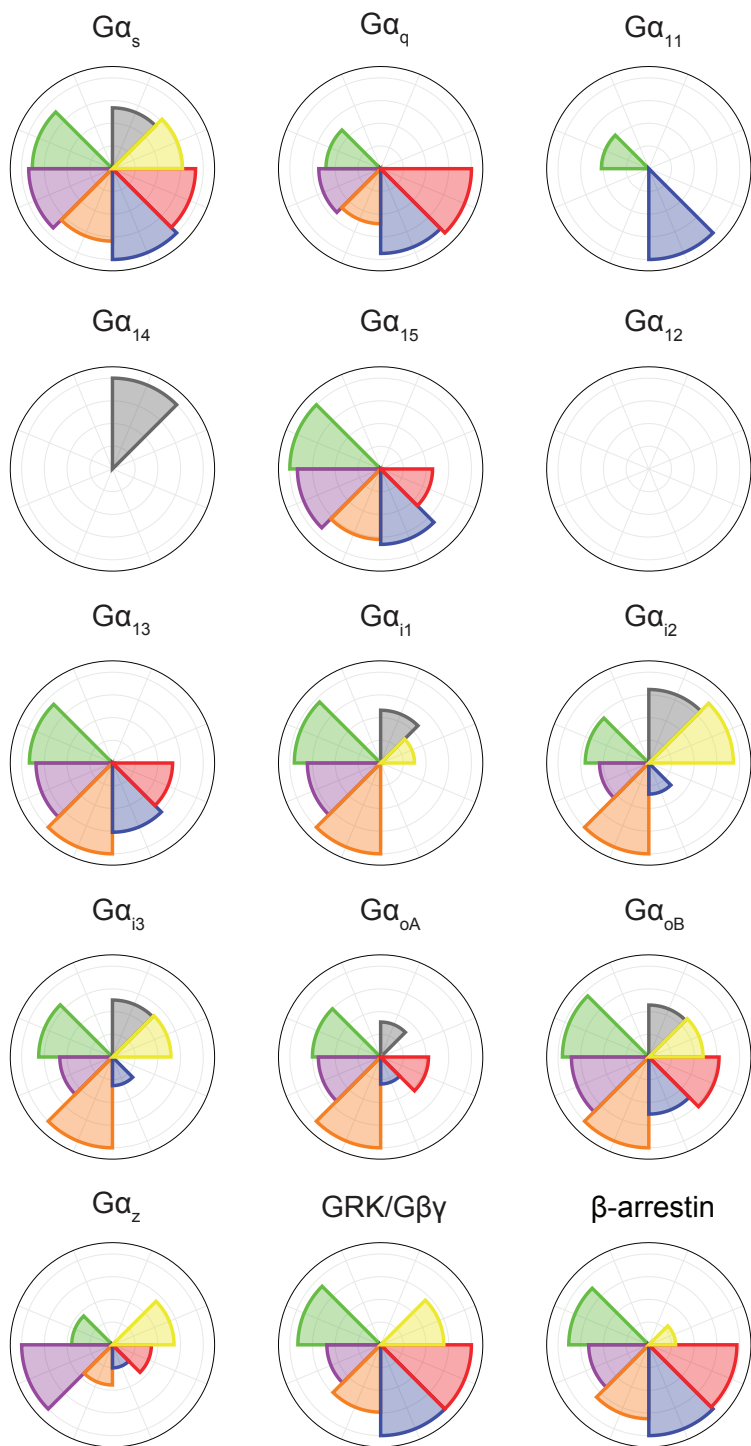


Supplementary Fig. 2. Drug-induced transducer engagement by GLP-1R at the plasma membrane. Agonist concentration response curves for 15 transducers at the plasma membrane. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Data are represented as the mean $\pm$ SEM ($n=3-6$ biologically independent experiments). Sigmoidal dose-response curves are present for responsive pathways and flat lines indicate non-responding pathways.

Plasma membrane

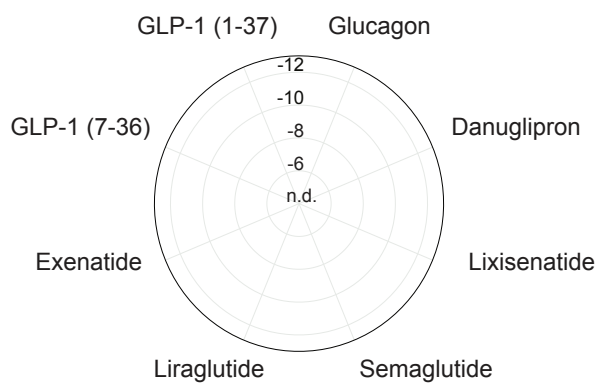
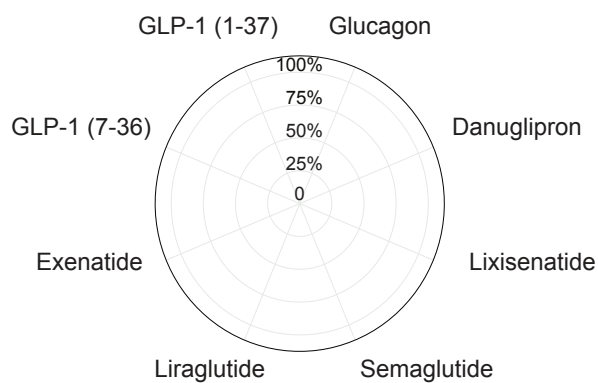
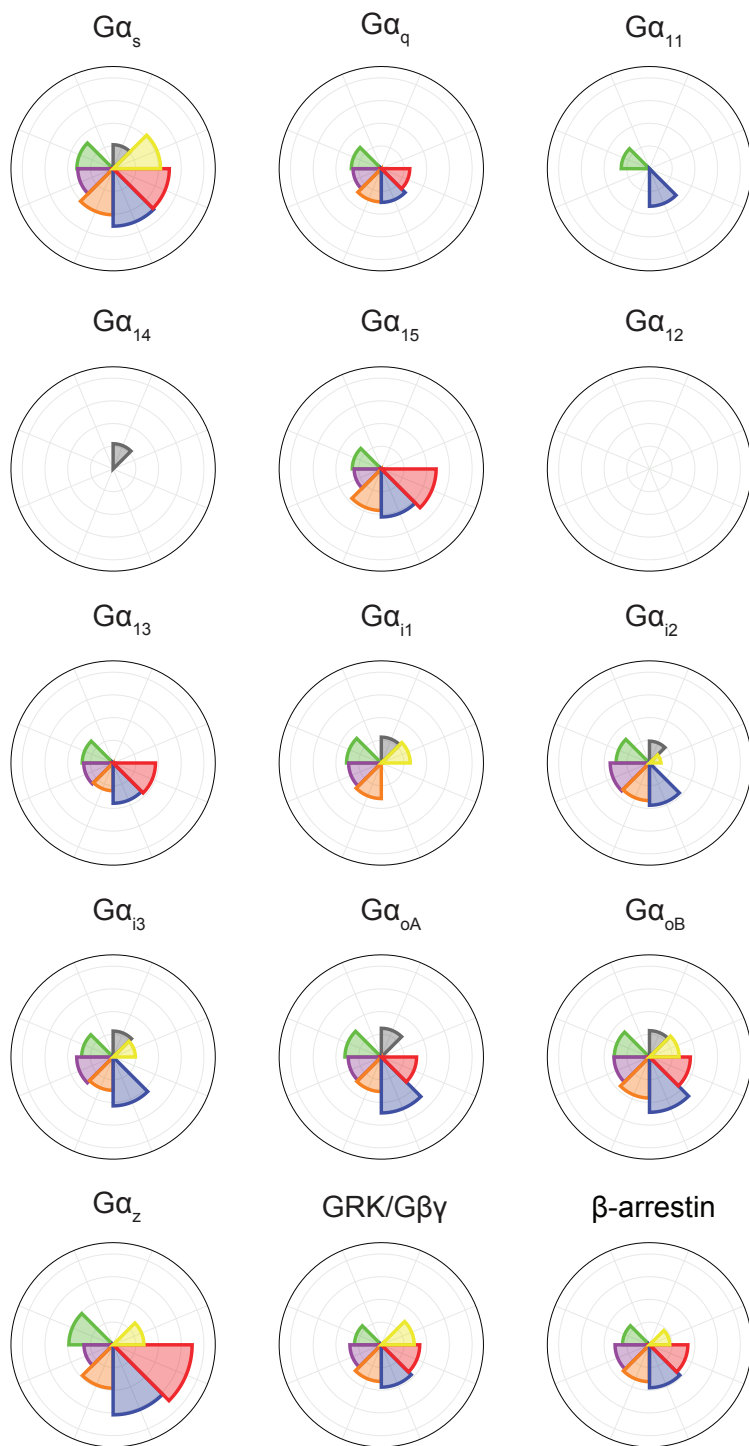
a

Efficacy (normalized)



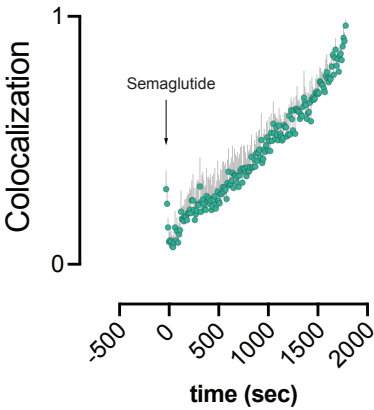
b

Potency (logEC₅₀)

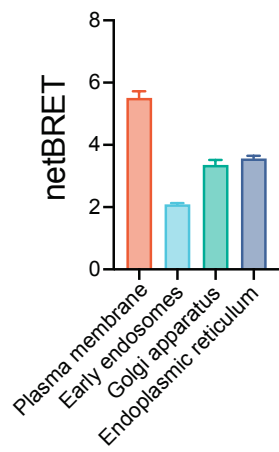
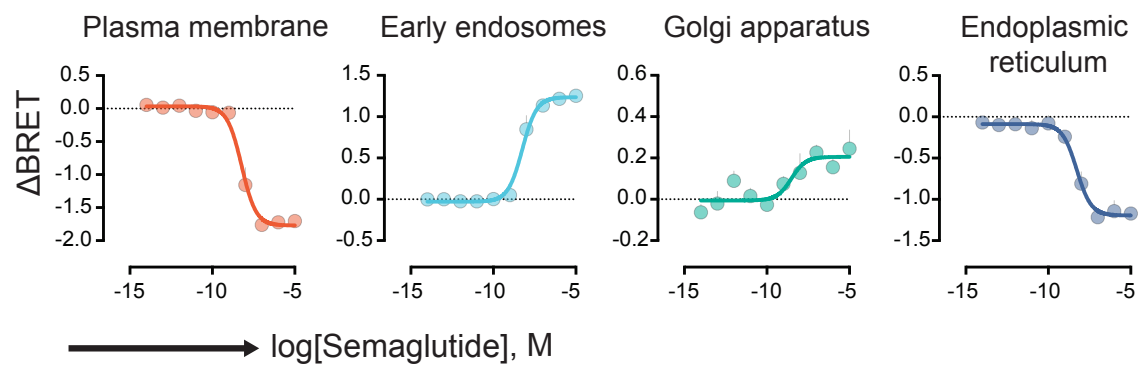


Supplementary Fig. 3. Polar area diagrams depict pathway activation at the plasma membrane. Pathway-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**a**) and potency ($\log EC_{50}$) (**b**) of 8 drugs at the plasma membrane. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$).

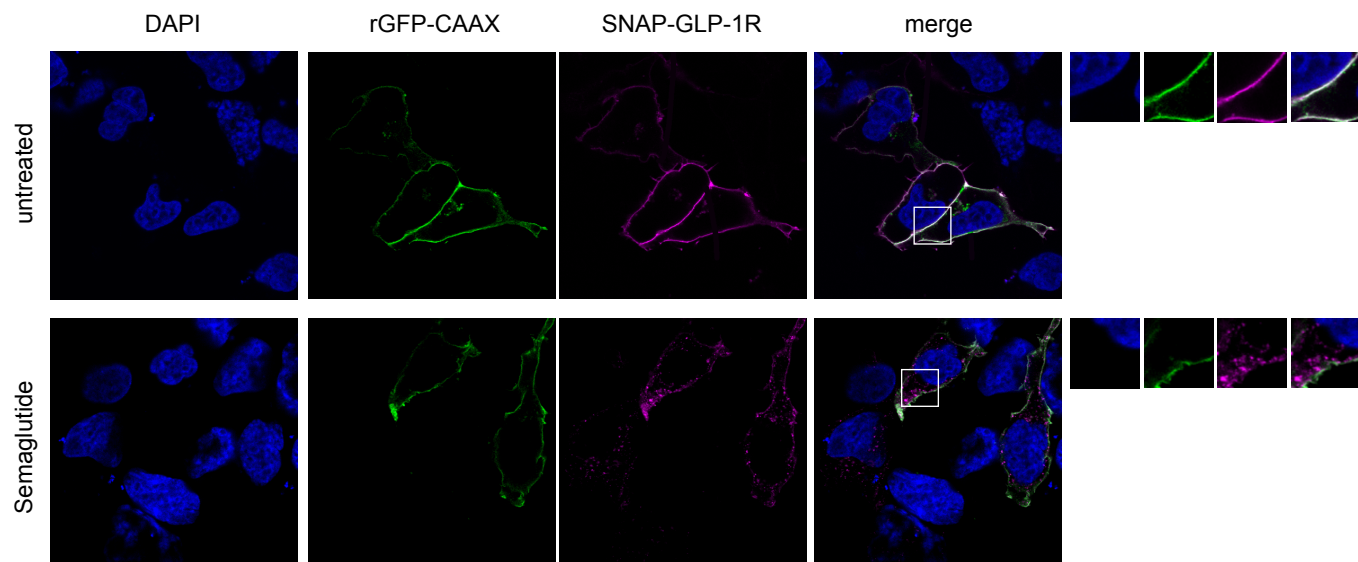
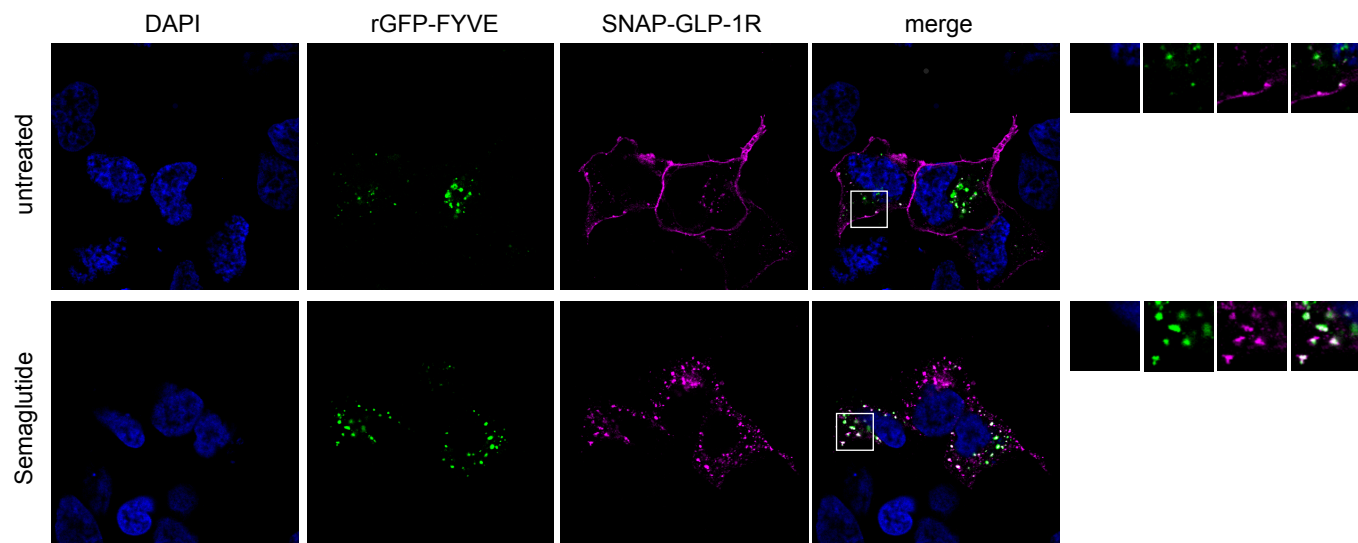
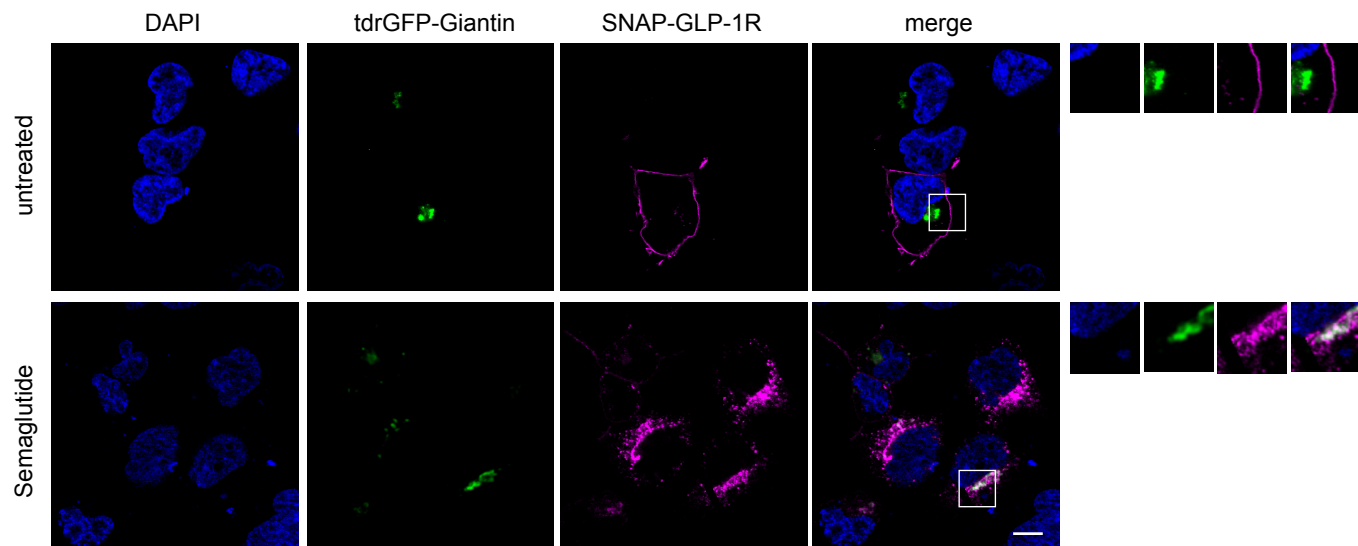
mGs translocation to Golgi



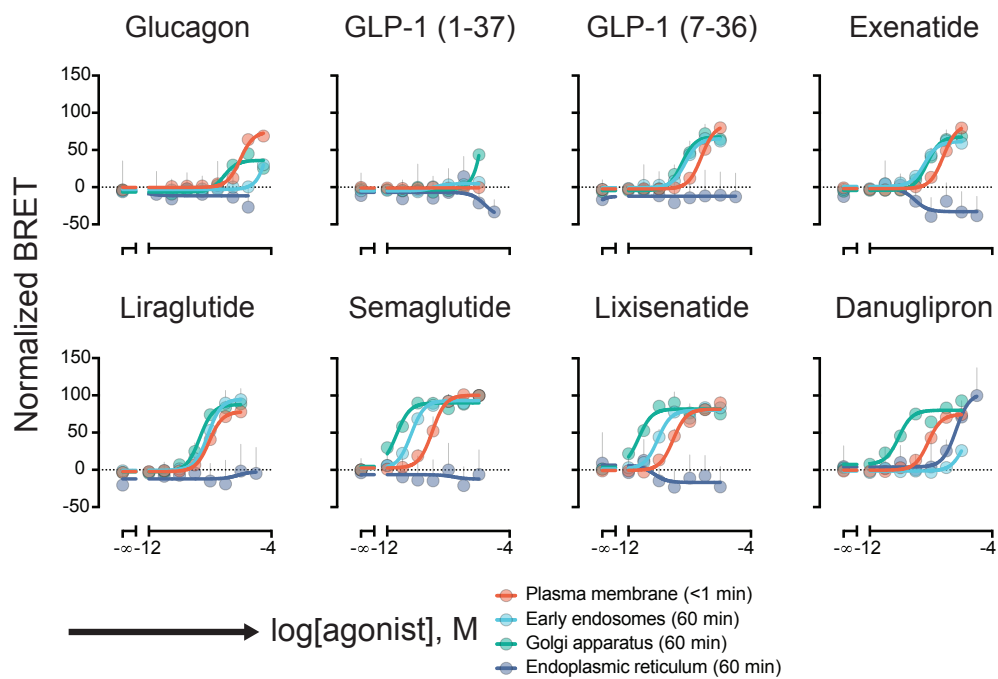
Supplementary Fig. 4. Quantification of colocalization between mGs and Giantin following agonist stimulation. Colocalization between mRuby2-mGs and tdrGFP-Giantin was quantified from timelapse confocal images of HEK293 cells expressing GLP-1R and exposed to semaglutide (1 μ M) for 30 min. Data are normalized and represented as the mean $\pm$ SEM ($n=6$ cells examined over 3 independent experiments).

a Basal localization**b****Agonist-induced trafficking**

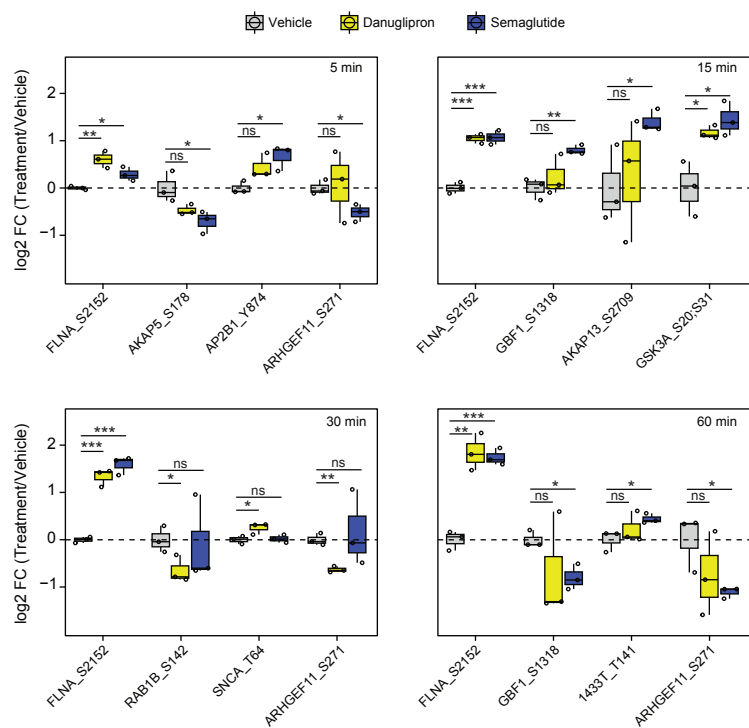
Supplementary Fig. 5. Basal localization and agonist-directed trafficking of GLP-1R by BRET. **a**, Basal localization of GLP-1R was measured in cells expressing *Rluc8*-tagged GLP-1R and rGFP-tagged compartment markers [rGFP-CAAX (PM), rGFP-FYVE (EE), tdrGFP-Giantin (GA) or tdrGFP-PTP1B (ER)]. Data are represented as the mean $\pm$ SEM ($n=3$ biologically independent experiments). **b**, Cells expressing *RlucII*-tagged GLP-1R together with rGFP-tagged compartment markers were stimulated with increasing concentrations of semaglutide for the indicated incubation times [PM–15 min; EE–25 min; GA–15 min; ER–25 min]. Data are represented as the mean $\pm$ SEM ($n=3$ biologically independent experiments).

a**b****c**

Supplementary Fig. 6. Translocation of activated GLP-1R from plasma membrane to early endosomes and Golgi apparatus after semaglutide stimulation. a-c, Confocal images of HEK293 cells expressing SNAP-GLP-1R and rGFP-CAAX (**a**), rGFP-FYVE (**b**) or tdrGFP-Giantin (**c**). Cells were treated with vehicle or semaglutide (1 μ M) for 10 min (PM) – 20 min (EE) or 30 min (GA). The presence or absence of colocalization is shown in the inset. Images are representative of 3 independent experiments. Scale bar = 10 μ m.



Supplementary Fig. 7. Analysis of G_s pathway engagement by GLP-1R agonists in different compartments. GLP-1R agonist-specific concentration-response curves for G_s pathway activation at the plasma membrane, early endosomes, Golgi apparatus and endoplasmic reticulum. Data are represented as the mean $\pm$ SEM ($n=3$ biologically independent experiments).

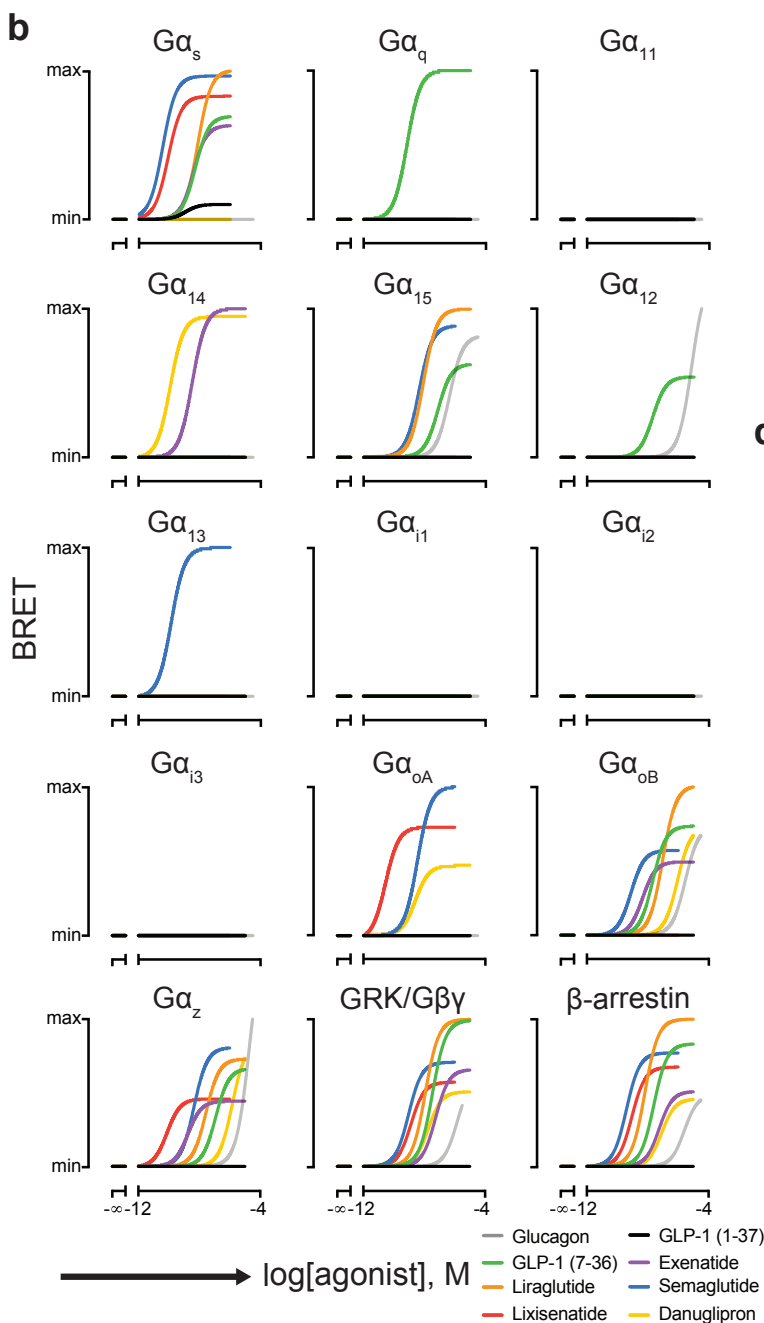
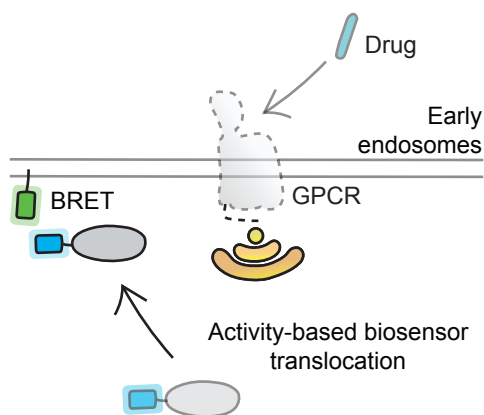


Supplementary Fig. 8. Phosphoregulation of proteins downstream of GPCR activation.

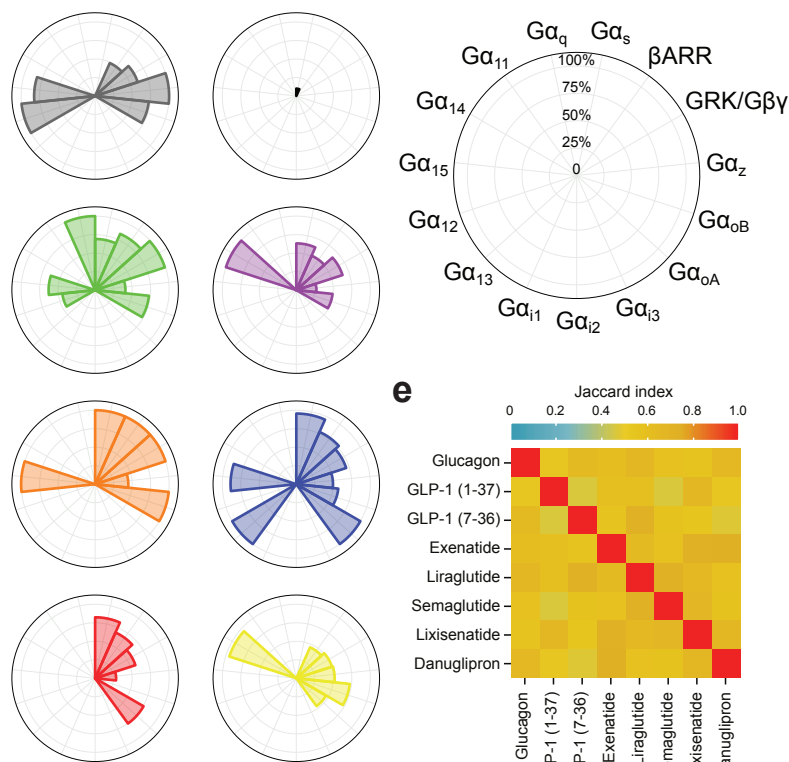
Box plots of phosphopeptides that are derived from proteins downstream of GPCR activation (UniProt annotation) following exposure to danuglipron or semaglutide at each time point. Phosphorylation sites are annotated beside the protein name (UniProt). Horizontal line in the box plots represents the median, 25th and 75th percentiles and whiskers represent measurements to the 5th and 95th percentiles. $n=3$ biologically independent samples. P -values were calculated using the two-sided Student t -test with equal variance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Early endosomes

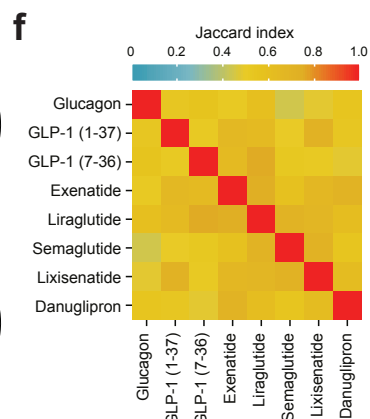
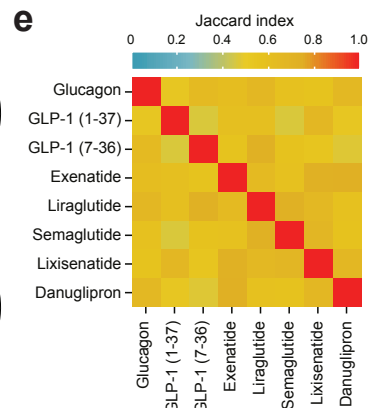
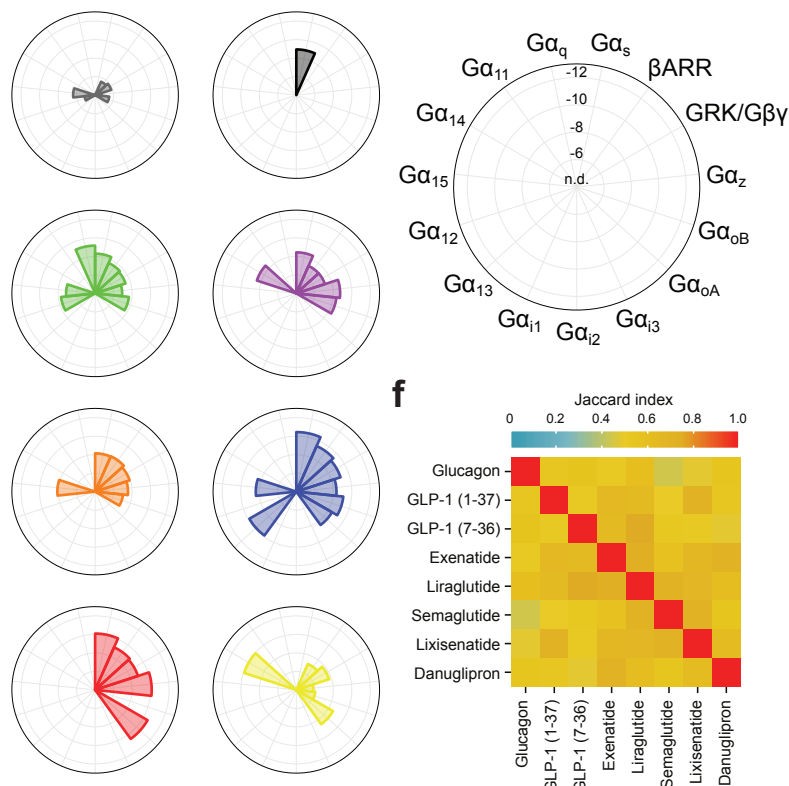
a Ligand-directed pathway selectivity



c Efficacy (normalized)



d Potency (logEC₅₀)



Supplementary Fig. 9. Pharmacological characterization of GLP-1R agonist signaling profiles at early endosomes. **a**, Illustration depicting the ebBRET approach to monitoring signaling at early endosomes. **b**, Concentration-response curves of GLP-1R agonists across 15 pathways at early endosomes using rGFP-FYVE. Data are represented by the non-linear fit and scaled according to the highest responding drug ($n=3$ biologically independent samples). Drug-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**c**) and potency ($\log EC_{50}$) (**d**) of 15 pathways at early endosomes. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Jaccard similarity index for efficacy (**e**) and potency (**f**) quantifies similarities and differences across drug responses.

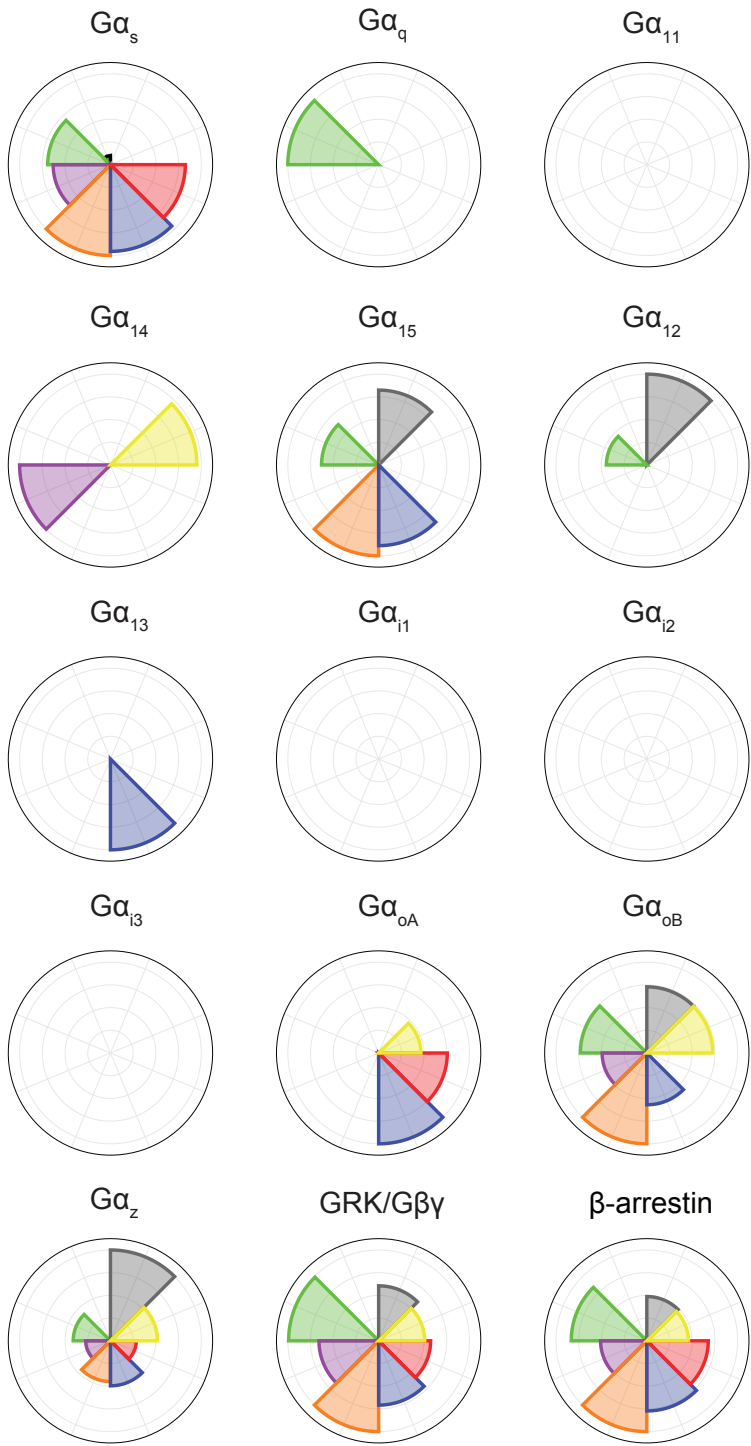
Early endosomes



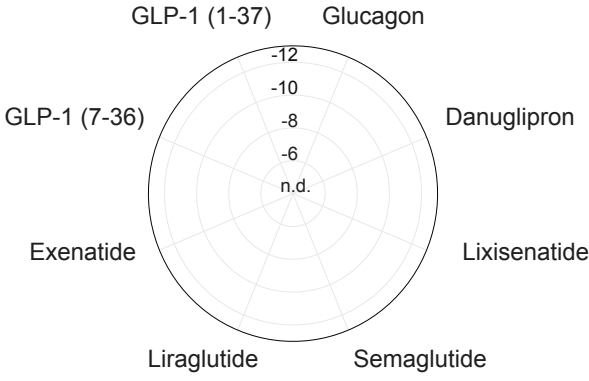
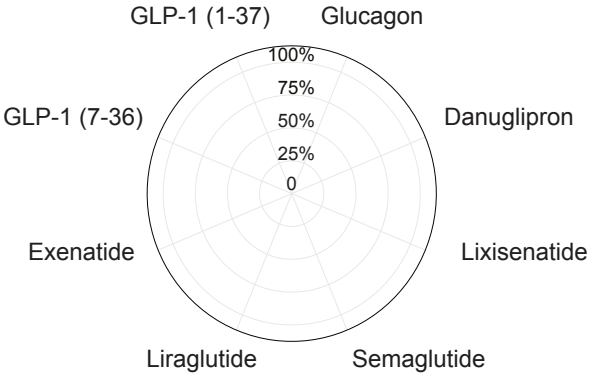
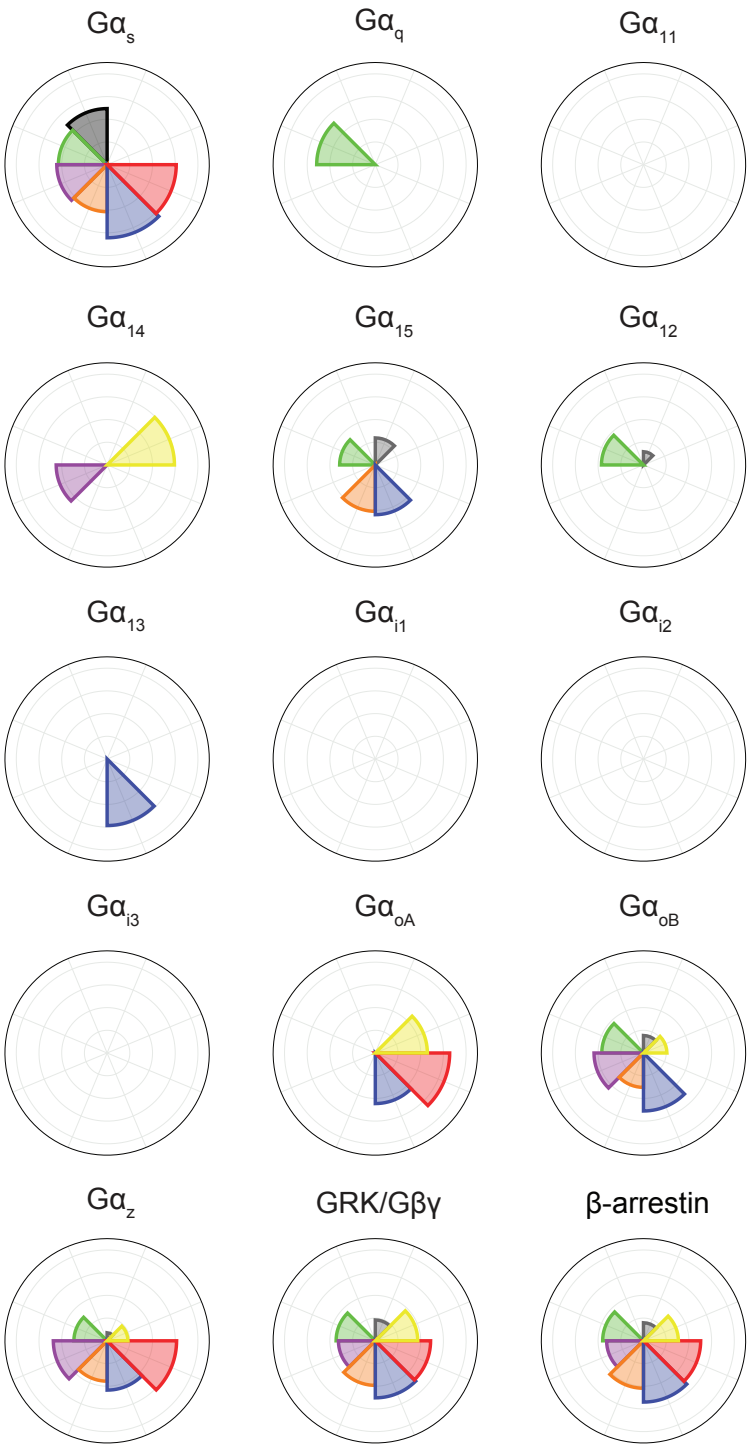
Supplementary Fig. 10. Drug-induced transducer engagement by GLP-1R at early endosomes. Agonist concentration response curves for 15 transducers at early endosomes. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Data are represented as the mean $\pm$ SEM ($n=3$ biologically independent samples). Sigmoidal dose-response curves are present for responsive pathways and flat lines indicate non-responding pathways.

Early endosomes

a Efficacy (normalized)



b Potency ($\log EC_{50}$)

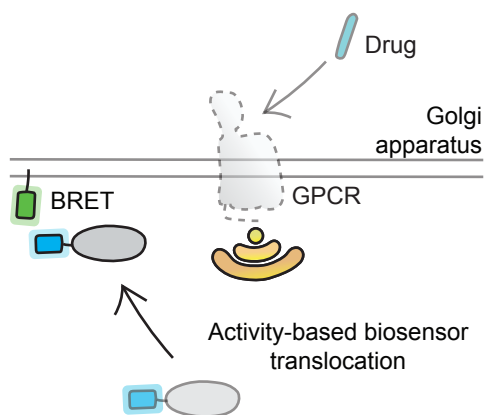


Supplementary Fig. 11. Polar area diagrams depict pathway activation at early endosomes.

Pathway-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**a**) and potency ($\log EC_{50}$) (**b**) of 8 drugs at early endosomes. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$).

Golgi apparatus

a Ligand-directed pathway selectivity



b

max

min

$G\alpha_s$

$G\alpha_q$

$G\alpha_{11}$

$G\alpha_{14}$

$G\alpha_{15}$

$G\alpha_{12}$

BRET

max

min

$G\alpha_{13}$

$G\alpha_{i1}$

$G\alpha_{i2}$

max

min

$G\alpha_{i3}$

$G\alpha_{oA}$

$G\alpha_{oB}$

max

min

$G\alpha_z$

GRK/G $\beta\gamma$

β -arrestin

$-\infty$ -12 -4

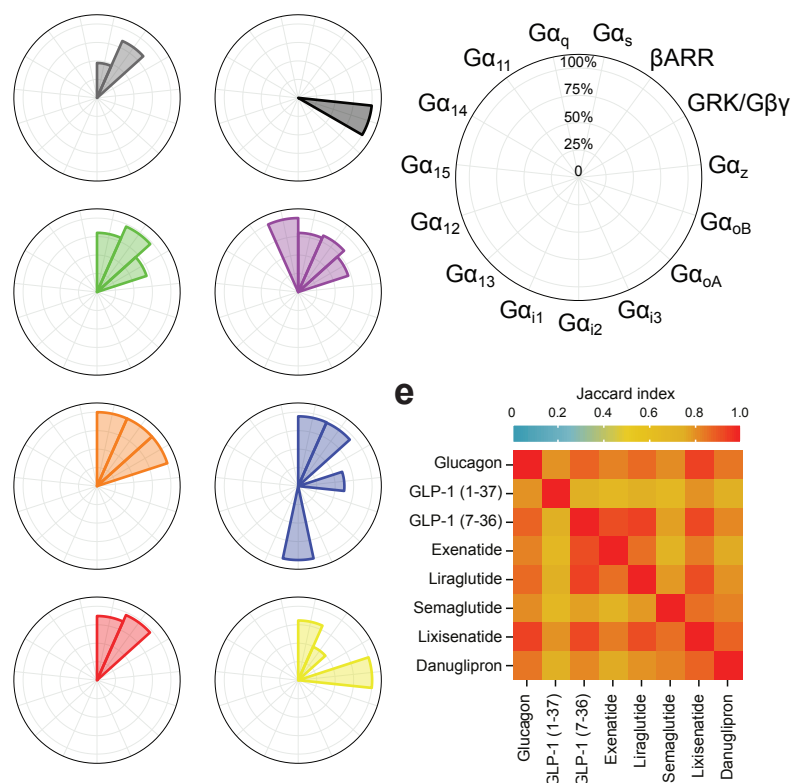
$-\infty$ -12 -4

$-\infty$ -12 -4

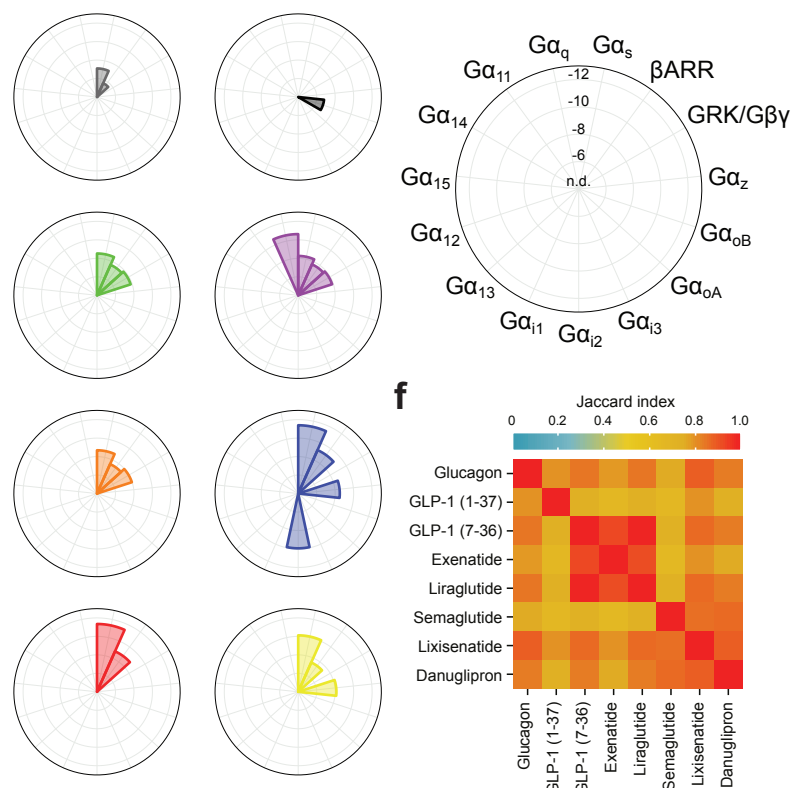
→ log[agonist], M

— Glucagon — GLP-1 (1-37)
 — GLP-1 (7-36) — Exenatide
 — Liraglutide — Semaglutide
 — Lixisenatide — Danuglipron

C Efficacy (normalized)

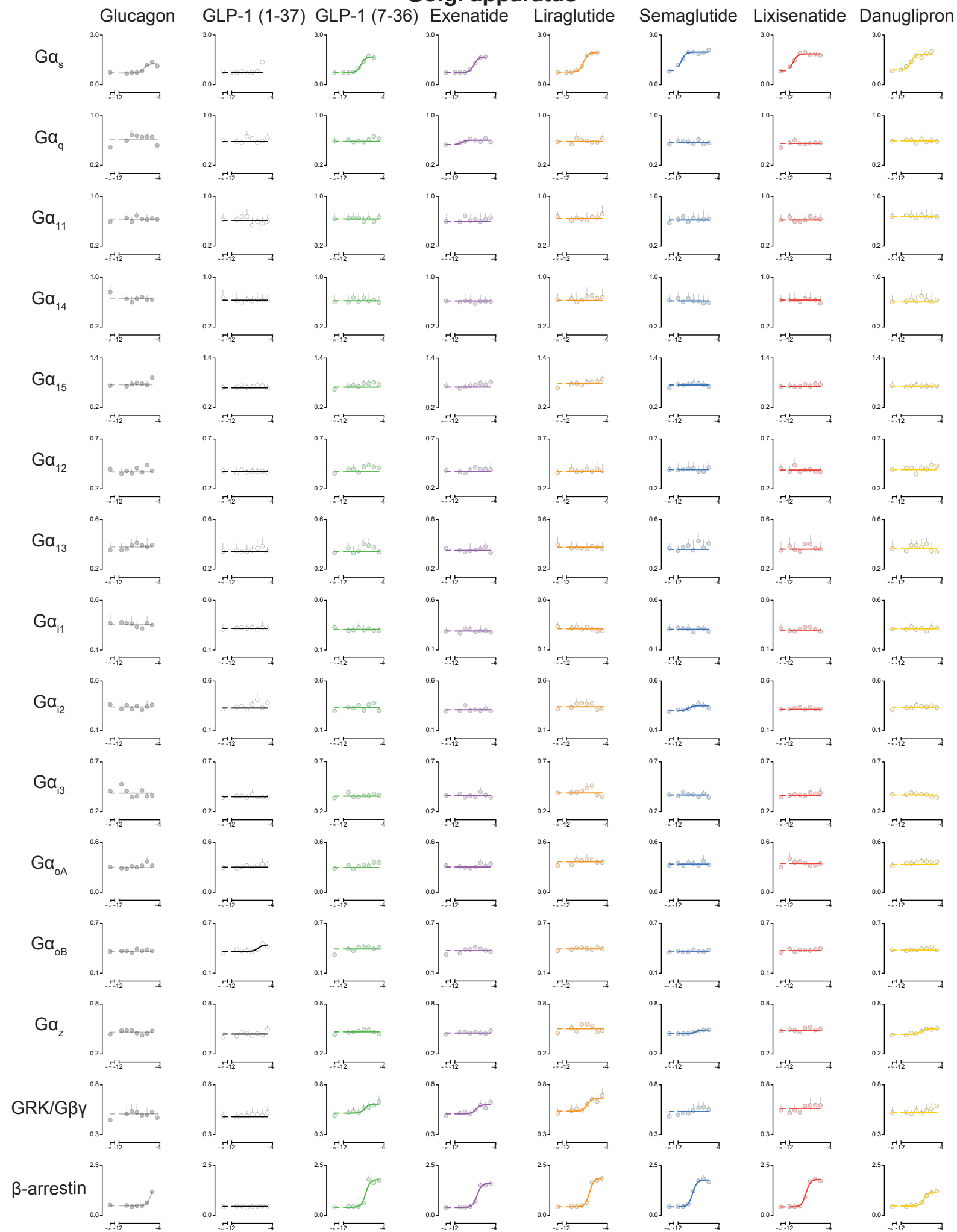


d Potency (logEC₅₀)



Supplementary Fig. 12. Pharmacological characterization of GLP-1R agonist signaling profiles at the Golgi apparatus. **a**, Illustration depicting the ebBRET approach to monitoring signaling at the Golgi apparatus. **b**, Concentration-response curves of GLP-1R agonists across 15 pathways at the Golgi apparatus using tdrGFP-Giantin. Data are represented by the non-linear fit and scaled according to the highest responding drug ($n=3-5$ biologically independent samples). Drug-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**c**) and potency ($\log EC_{50}$) (**d**) of 15 pathways at the Golgi apparatus. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Jaccard similarity index for efficacy (**e**) and potency (**f**) quantifies similarities and differences across drug responses.

Golgi apparatus

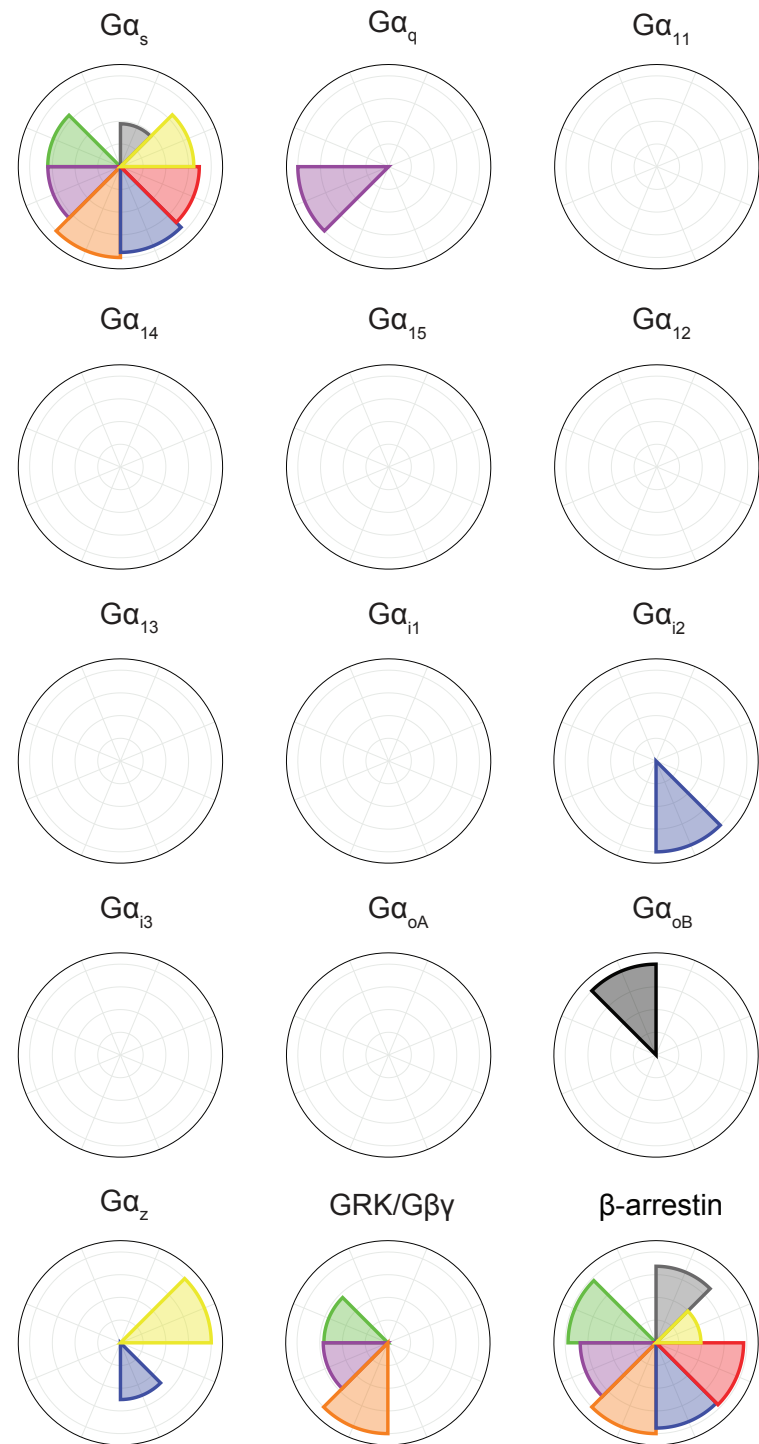


Supplementary Fig. 13. Drug-induced transducer engagement by GLP-1R at the Golgi apparatus. Agonist concentration response curves for 15 transducers at the Golgi apparatus. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Data are represented as the mean $\pm$ SEM ($n=3-5$ biologically independent samples). Sigmoidal dose-response curves are present for responsive pathways and flat lines indicate non-responding pathways.

Golgi apparatus

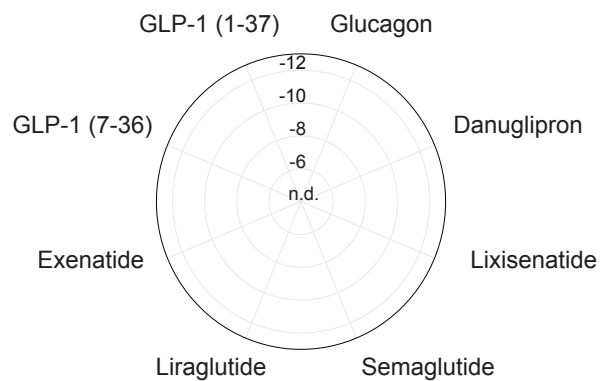
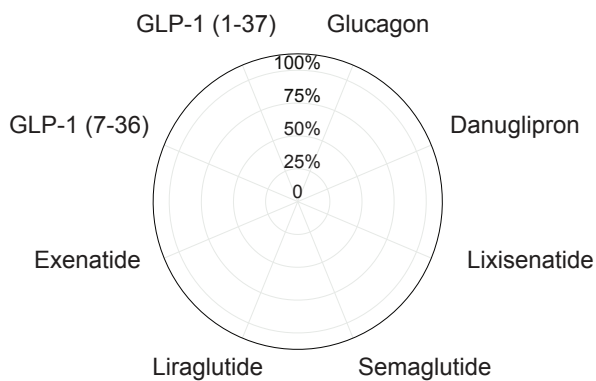
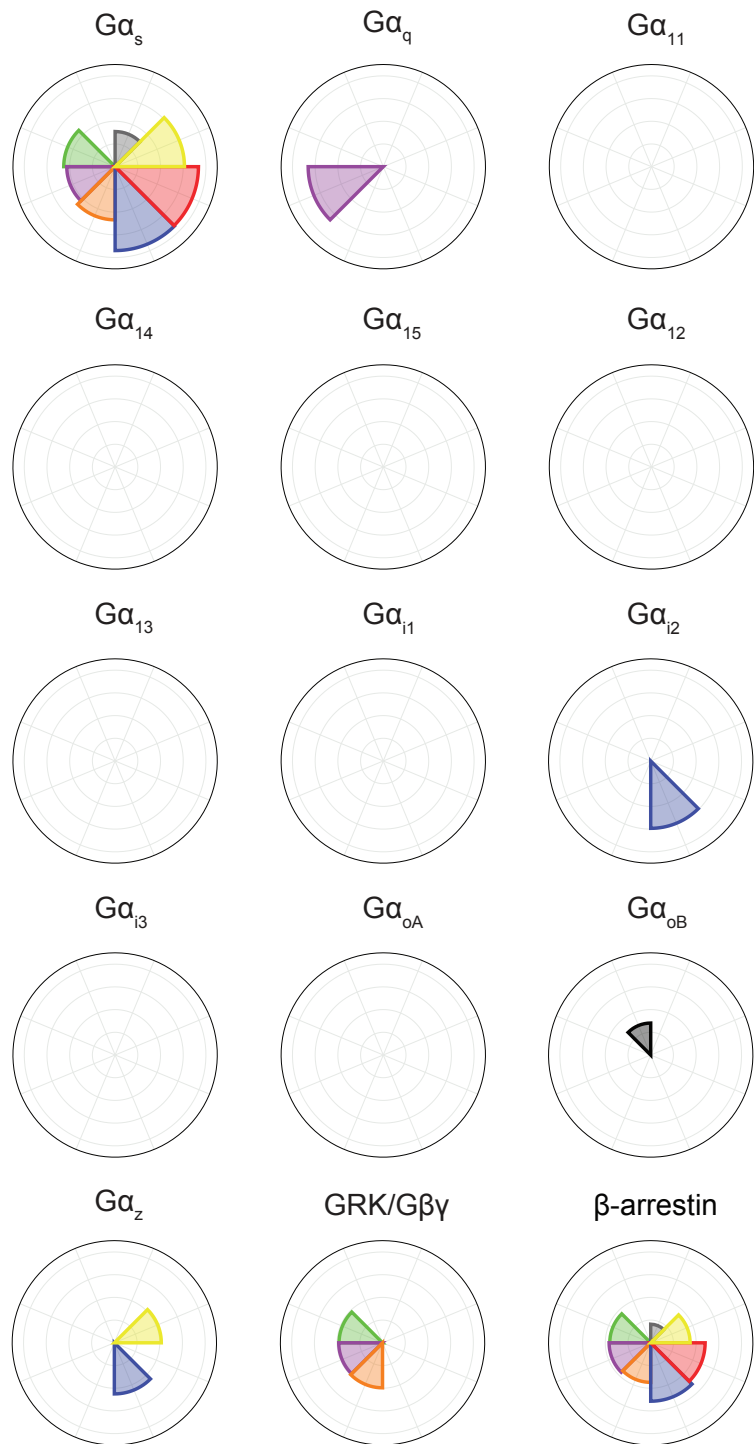
a

Efficacy (normalized)



b

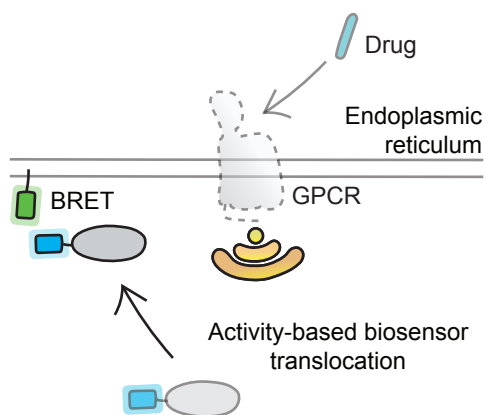
Potency (logEC₅₀)



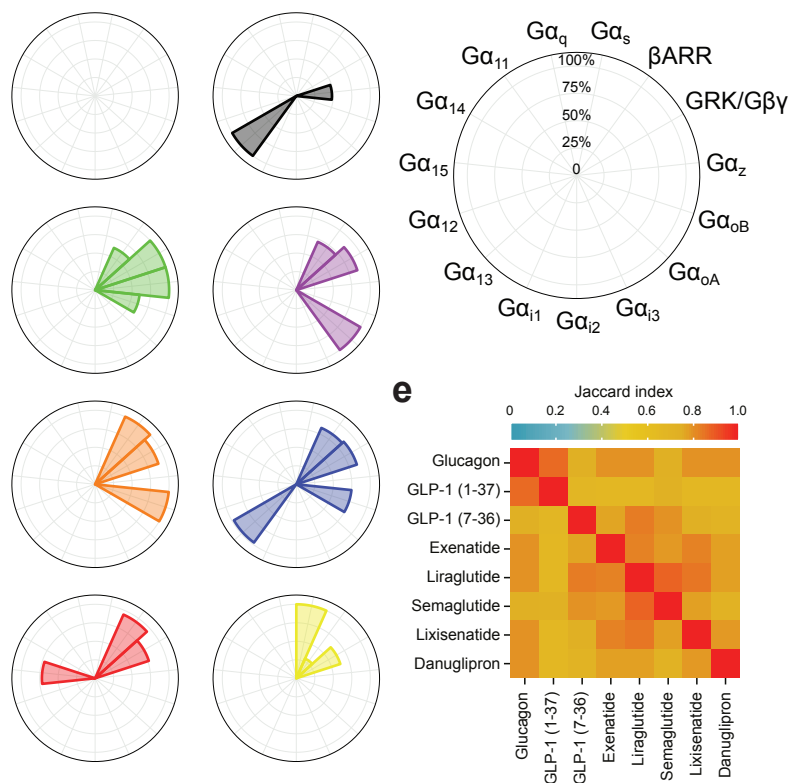
Supplementary Fig. 14. Polar area diagrams depict pathway activation at the Golgi apparatus. Pathway-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**a**) and potency ($\log EC_{50}$) (**b**) of 8 drugs at the Golgi apparatus. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$).

Endoplasmic reticulum

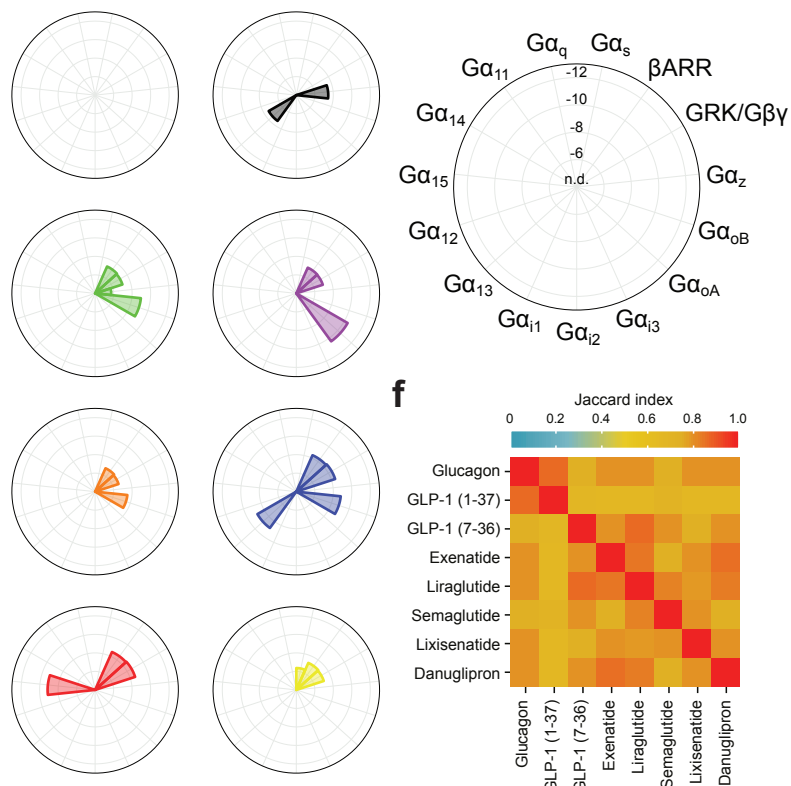
a Ligand-directed pathway selectivity



C Efficacy (normalized)



d Potency (logEC₅₀)



Supplementary Fig. 15. Pharmacological characterization of GLP-1R agonist signaling profiles at the endoplasmic reticulum. **a**, Illustration depicting the ebBRET approach to monitoring signaling at the endoplasmic reticulum. **b**, Concentration-response curves of GLP-1R agonists across 15 pathways at the endoplasmic reticulum using tdrGFP-PTP1B. Data are represented by the non-linear fit and scaled according to the highest responding drug ($n=3$ biologically independent samples). Drug-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**c**) and potency ($\log EC_{50}$) (**d**) of 15 pathways at the endoplasmic reticulum. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Jaccard similarity index for efficacy (**e**) and potency (**f**) quantifies similarities and differences across drug responses.

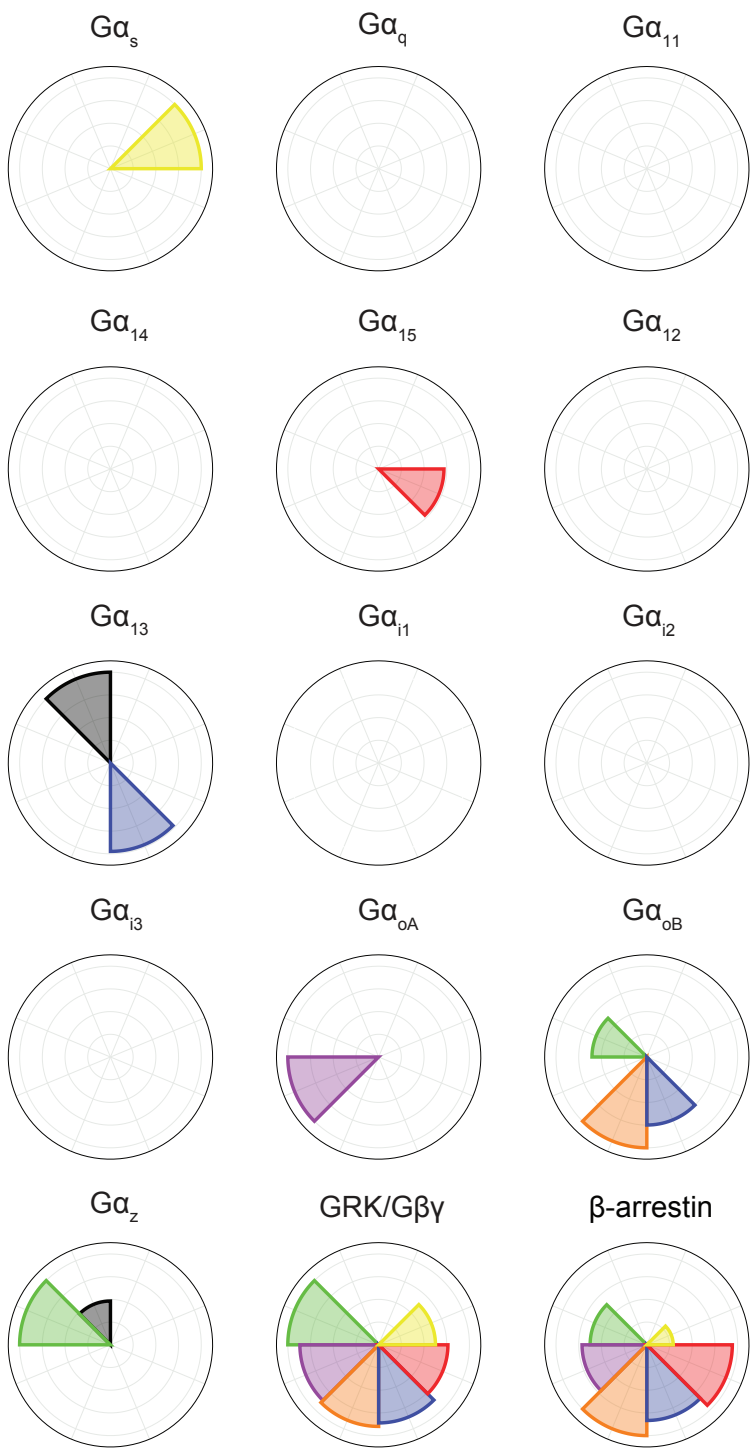
Endoplasmic reticulum



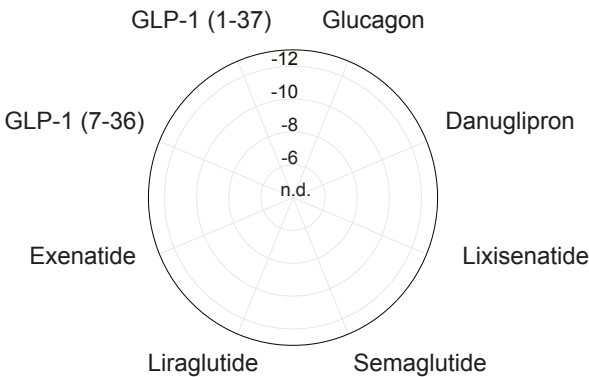
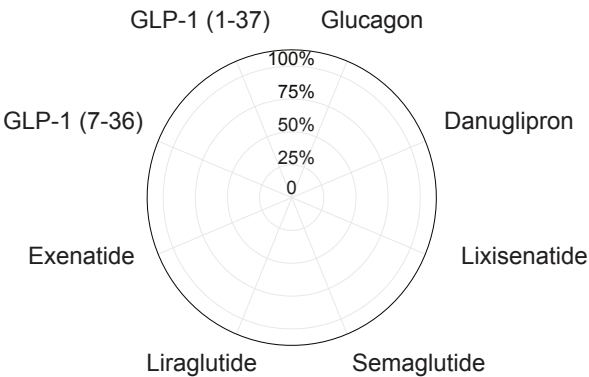
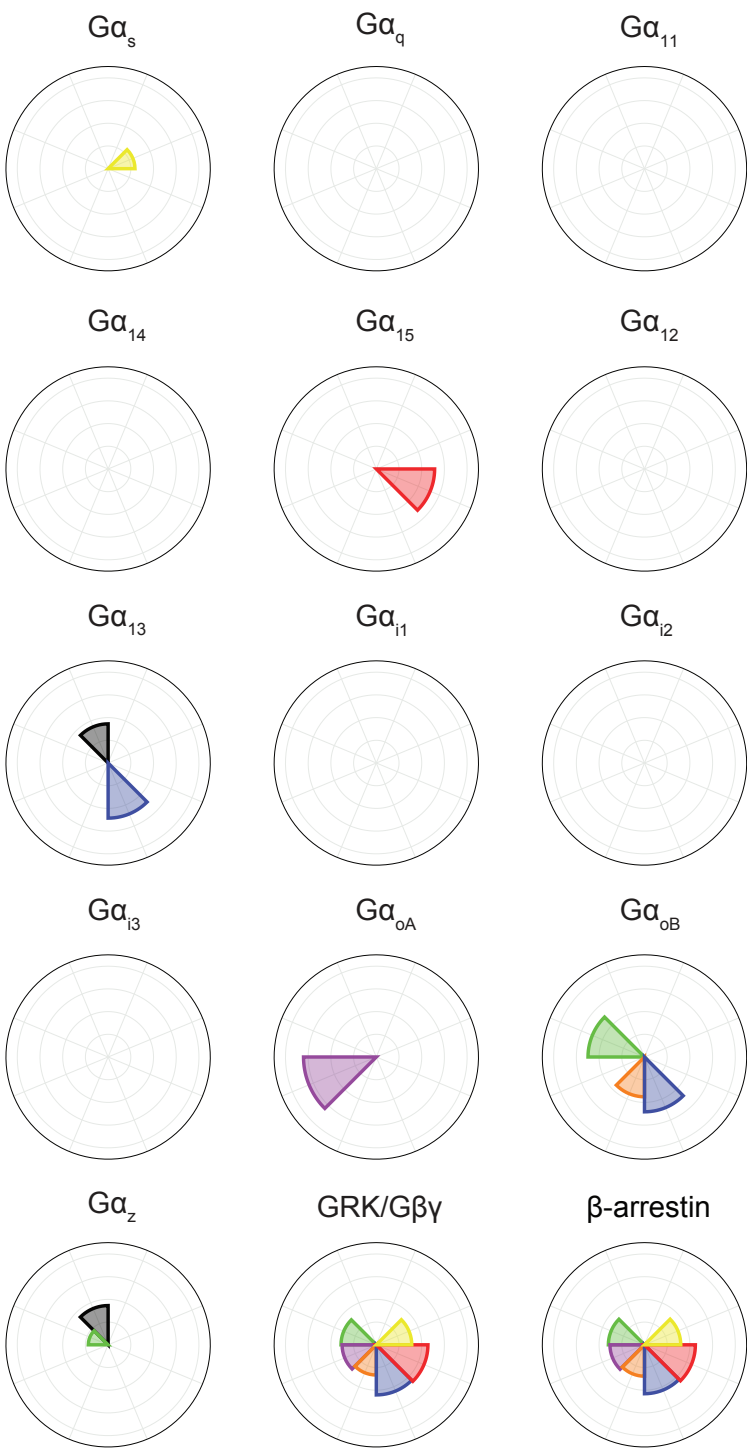
Supplementary Fig. 16. Drug-induced transducer engagement by GLP-1R at the endoplasmic reticulum. Agonist concentration response curves for 15 transducers at the endoplasmic reticulum. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$). Data are represented as the mean $\pm$ SEM ($n=3$ biologically independent samples). Sigmoidal dose-response curves are present for responsive pathways and flat lines indicate non-responding pathways.

Endoplasmic reticulum

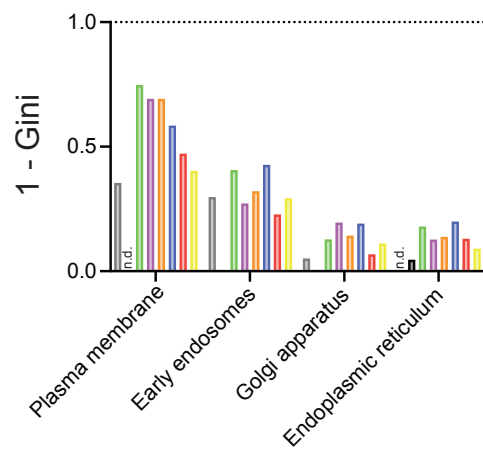
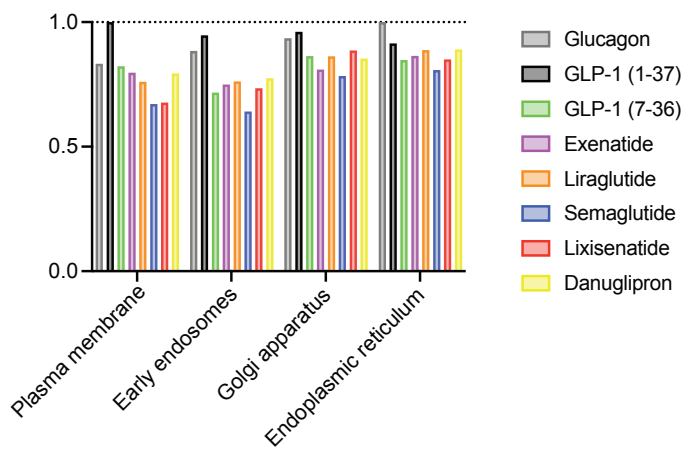
a Efficacy (normalized)



b Potency ($\log EC_{50}$)

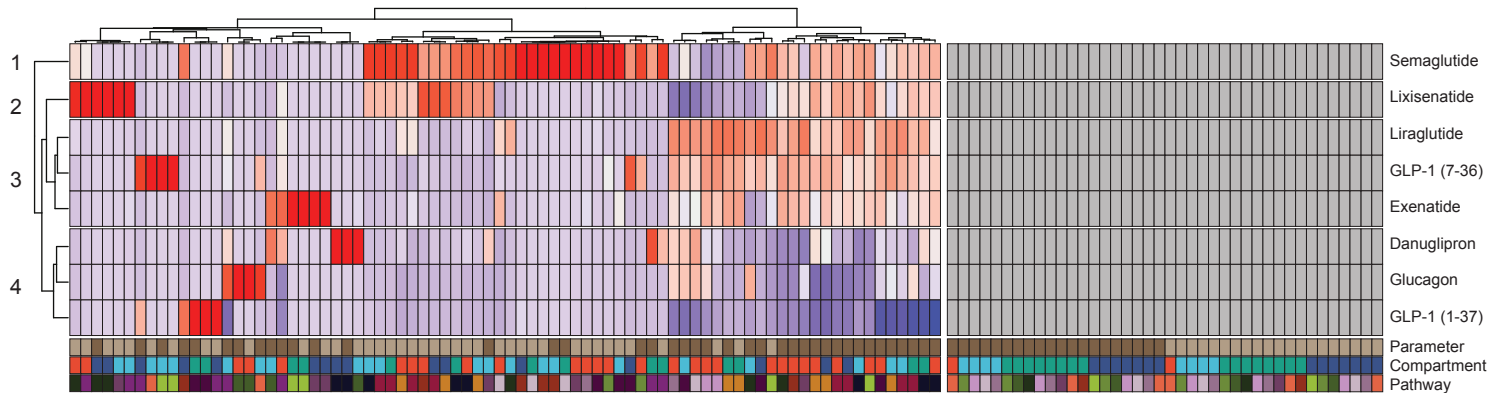
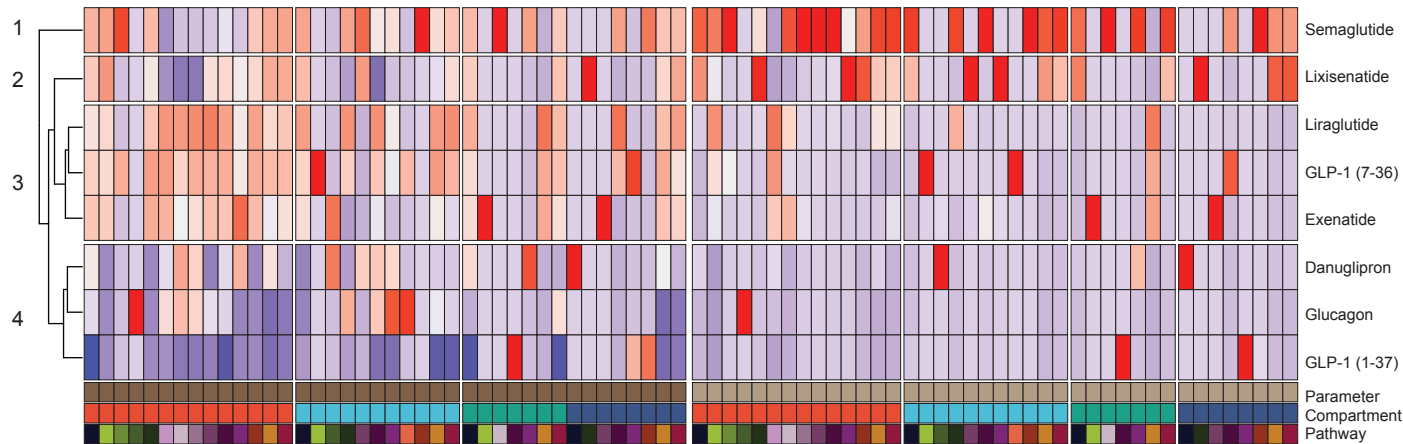


Supplementary Fig. 17. Polar area diagrams depict pathway activation at the endoplasmic reticulum. Pathway-specific polar area diagrams for efficacy (normalized to the highest responding drug) (**a**) and potency ($\log EC_{50}$) (**b**) of 8 drugs at the endoplasmic reticulum. Drugs were deemed to activate a given pathway after comparing the top and bottom parameters from non-linear regression by one-sided extra sum-of-squares F-test followed by Bonferroni correction for 8 compounds ($P < 0.00625$).

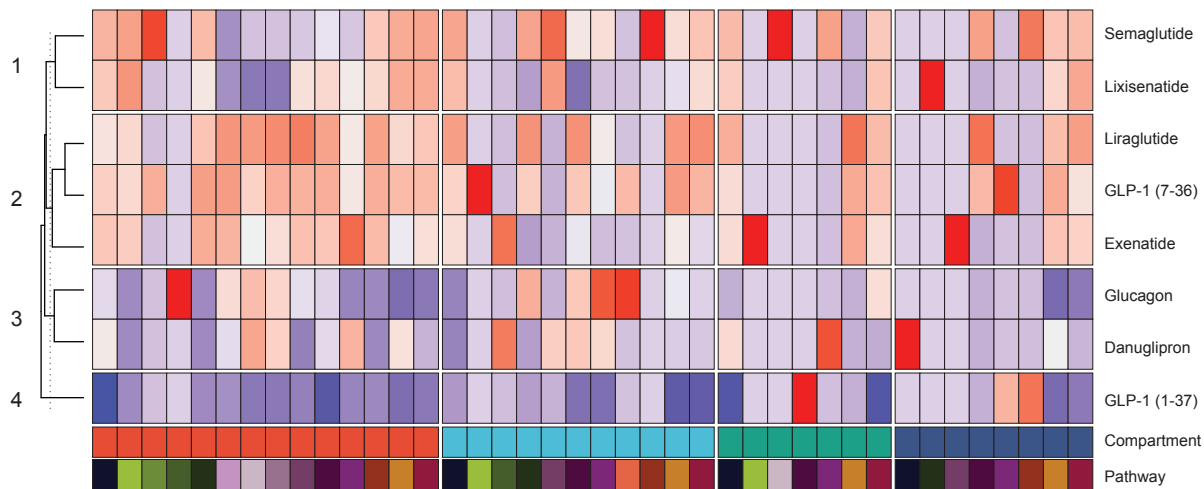
a**Efficacy****b****Potency**

Supplementary Fig. 18. Inverted Gini coefficients depict differences in drug-induced pathway selectivity for efficacy and potency. Inverted Gini coefficients were calculated to compare the similarities among the 15 tested pathways (G_s , G_q , G_{11} , G_{14} , G_{15} , G_{12} , G_{13} , G_{i1} , G_{i2} , G_{i3} , G_{oA} , G_{oB} , G_z , GRK/ $G\beta\gamma$ and β ARR) for efficacy (**a**) and potency (**b**) across the four compartments included in this study (PM, EE, GA, ER) where 1 represents equality and 0 represents dissimilarity.

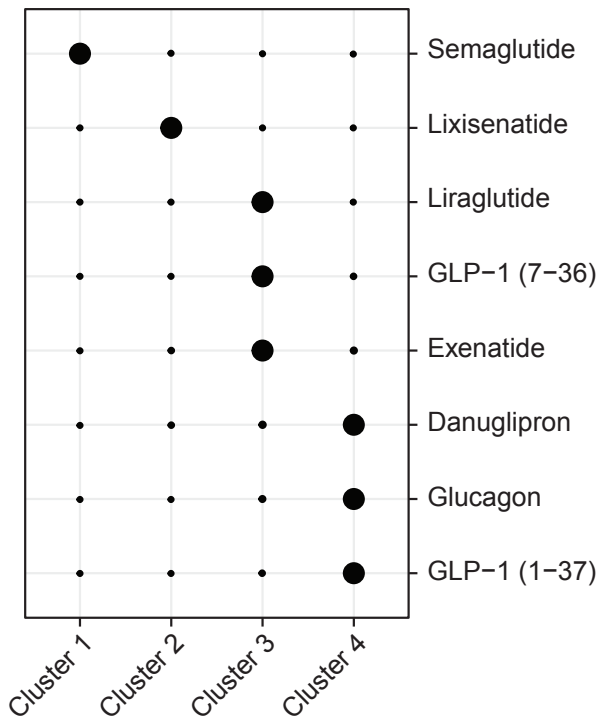
a

**b**

C



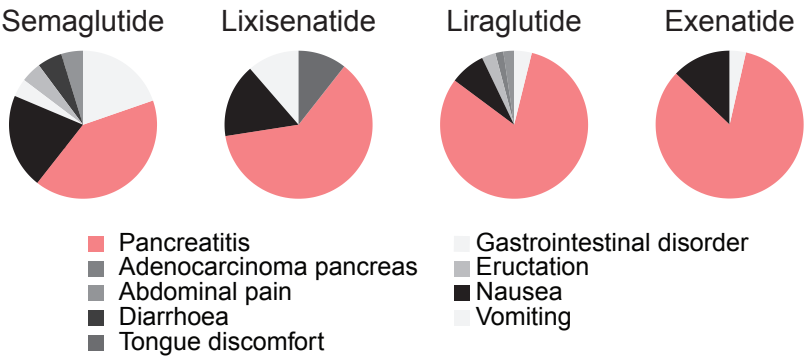
Supplementary Fig. 19. Hierarchical clustering of GLP-1R agonist profiles. **a**, Heatmap of efficacy and potency for 8 drugs in 15 pathways and 4 cellular compartments. Data are represented as z-scores. Pathways that were not activated by GLP-1R upon exposure to ligand are included to the right of the heatmap. Hierarchical clustering was applied to pathways with at least one measured value. **b**, Reductionist representation of heatmap in (a) that is sorted according to efficacy or potency, compartments, and pathways. Pathways that were not activated by any drug were removed for clarity. **c**, Heatmap and hierarchical clustering of efficacy for 8 drugs in 15 pathways and 4 cellular compartments. Data are represented as z-scores.



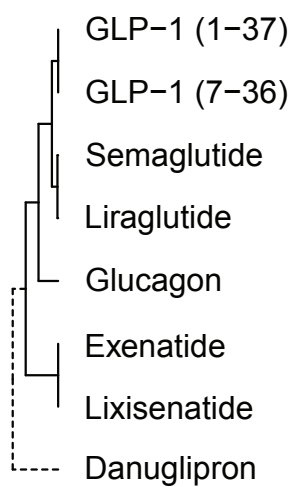
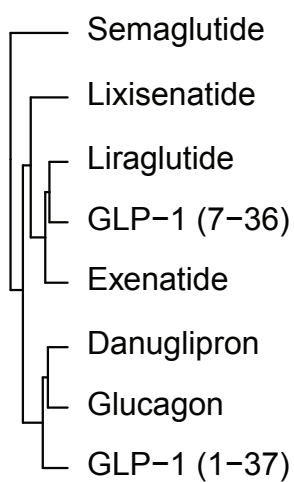
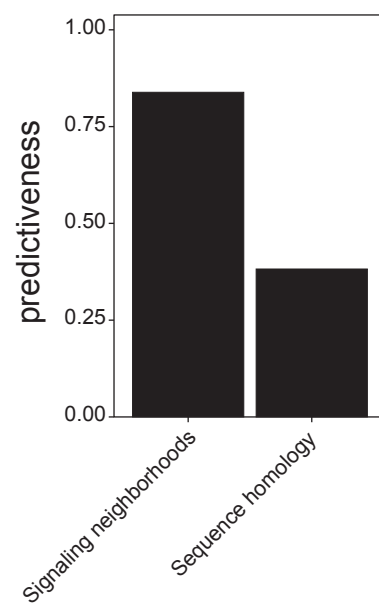
Fuzzy membership probability

• 0 ● 0.25 ● 0.50 ● 0.75 ● 1

Supplementary Fig. 20. Fuzzy clustering of GLP-1R agonists. Cluster membership probabilities for the GLP-1R agonists were determined based on Fuzzy clustering.



Supplementary Fig. 21. Pie chart of gastrointestinal disorder likelihood for GLP-1R agonists. The proportion of different ADR gastrointestinal disorder subgroups is shown relative to the overall likelihood. The number underneath each pie chart represents the overall number of events.

a**Sequence homology****Signaling neighborhoods****b**

Supplementary Fig. 22. Dendrograms of GLP-1R agonists based on sequence homology identity and signaling neighborhood profiles. **a**, GLP-1R agonists were clustered according to their sequence homology or their experimentally determined signaling neighborhoods. Note that danuglipron is a small molecule and is displayed as an outgroup (dotted line) in the sequence homology dendrogram. **b**, Predictiveness of ADRs based on the entanglement score for signaling neighborhoods and sequence homology data.