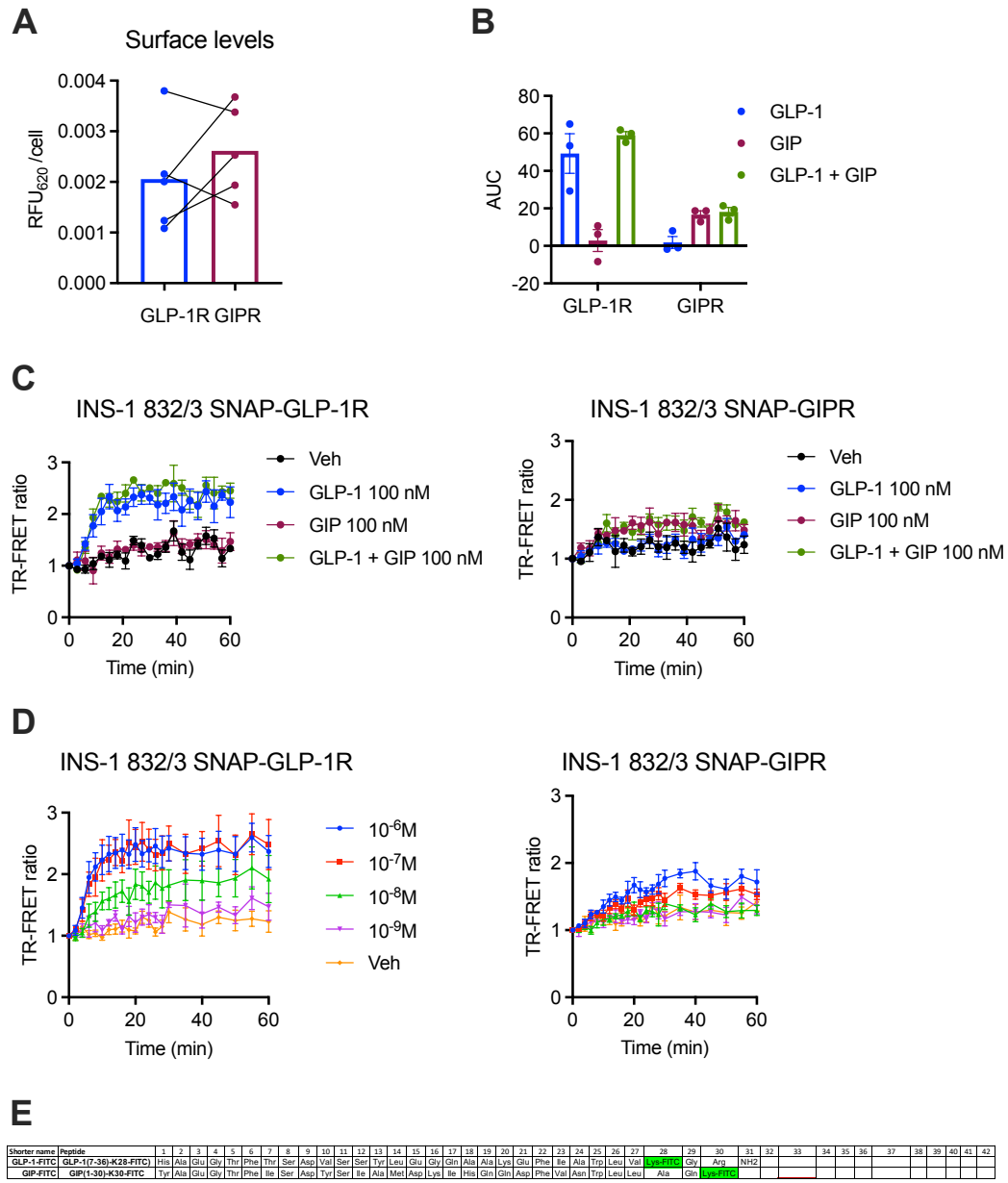
## Supplemental Figures

### Figure S1




| Shorter name | Peptide | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GLP-1-FITC | GLP-1(7-36)-K28-FITC) | His | Ala | Glu | Gly | Thr | Phe | Thr | Ser | Asp | Val | Ser | Ser | Tyr | Leu | Glu | Gly | Gln | Ala | Ala | Lys | Glu | Phe | Ile | Ala | Trp | Leu | Val | Lys-FITC | Gly | Arg | NH2 | | | | | | | | | | | |
| GIP-FITC | GIP(1-30)-K30-FITC | Tyr | Ala | Glu | Gly | Thr | Phe | Ile | Ser | Asp | Tyr | Ser | Ile | Ala | Met | Asp | Lys | Ile | His | Gln | Gln | Asp | Phe | Val | Asn | Trp | Leu | Leu | Ala | Gln | Lys-FITC | | | | | | | | | | | | |

**Figure S1. Beta cell GLP-1R *versus* GIPR trafficking – extra data.** (**A**) SNAP-GLP-1R *vs* SNAP-GIPR cell surface levels in in INS-1 832/3 SNAP-GLP-1R *vs* SNAP-GIPR cells. (**B**) Level of internalization of GLP-1R compared to GIPR, measured by DERET assay in INS-1 832/3 SNAP-GLP-1R *vs* SNAP-GIPR cells following 1 hr stimulation with 100 nM GLP-1, GIP or a combination of GLP-1 and GIP and expressed as response AUC; *n*=3. (**C**) TR-FRET traces from (B). (**D**) TR-FRET traces from Figure 1A. (**E**) Amino acid sequences indicating the position of the modifications in the FITC-labelled agonists.

## Figure S2

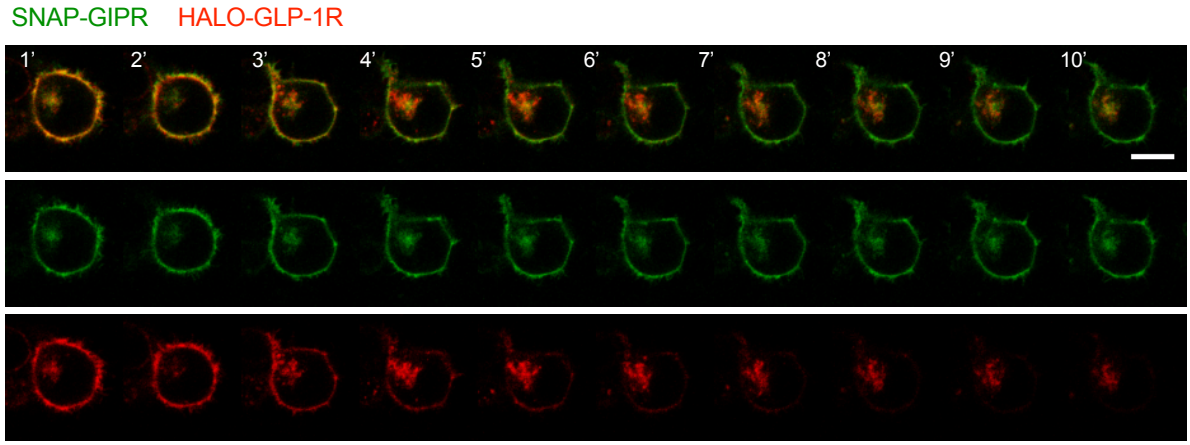


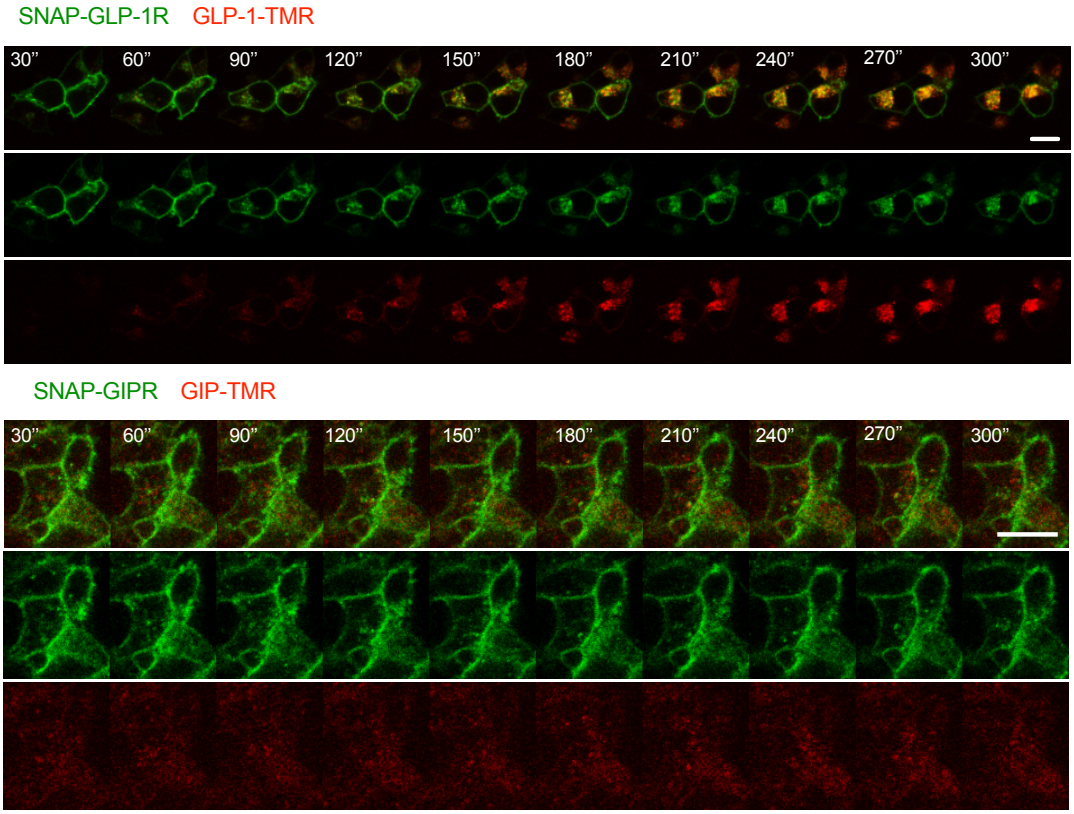

**Figure S2. SNAP-GIPR / HALO-GLP-1R internalization in beta cells.** Time frame montage with the indicated time points of an INS-1 832/3 WT cell co-expressing SNAP-GIPR + HALO-GLP-1R prior to labeling with SNAP-Surface 549 + HaloTag AlexaFluor 660 impermeable probes and imaging at 10 sec/frame for 10 min post-stimulation with a mixture of 100 nM GLP-1 + GIP; size bar: 10 µm.

# Figure S3

## A

| Shorter name | Peptide | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GLP-1-TMR | GLP-1(7-37)-GGK-TMR | His | Ala | Glu | Gly | Thr | Phe | Thr | Ser | Asp | Val | Ser | Ser | Tyr | Leu | Glu | Gly | Gln | Ala | Ala | Lys | Glu | Phe | Ile | Ala | Trp | Leu | Val | Lys | Gly | Arg | Gly | Gly | Lys-TMR | | | | | | | | | |
| GIP-TMR | GIP(1-42)-K37-TMR | Tyr | Ala | Glu | Gly | Thr | Phe | Ile | Ser | Asp | Tyr | Ser | Ile | Ala | Met | Asp | Lys | Ile | His | Gln | Gln | Asp | Phe | Val | Asn | Trp | Leu | Leu | Ala | Gln | Lys | Gly | Lys | Lys | Asn | Asp | Trp | Lys-TMR | His | Asn | Ile | Thr | Gln |

## B




## C

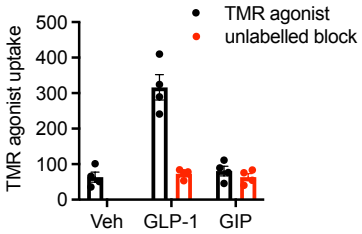



**Figure S3. GLP-1-** ***versus*** **GIP-TMR uptake in beta cells.** (**A**) Amino acid sequences indicating the position of the modifications in the TMR-labelled agonists. (**B**) Time frame montage with the indicated time points from INS-1 832/3 SNAP-GLP-1R (top) *vs* -GIPR (bottom) cells labeled with SNAP-Surface 649 and stimulated with 100 nM GLP-1- or GIP-TMR for 5 min; size bars: 10 µm. (**C**) Quantification of TMR uptake in INS-1 832/3 WT cells treated with vehicle or 100 nM GLP-1- *vs* GIP-TMR +/- blocking with the corresponding unlabeled agonist at 10 µM.

# Figure S4

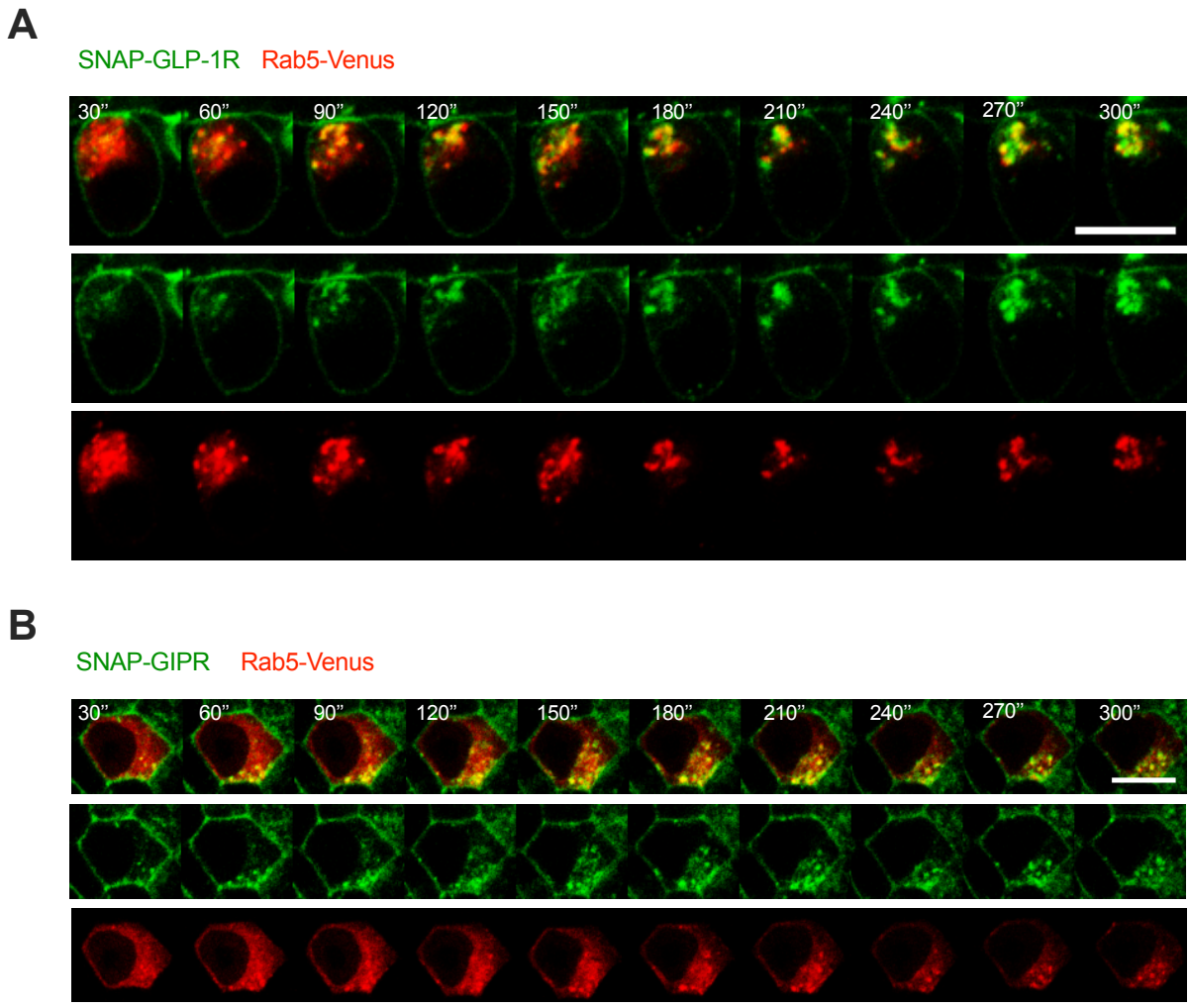
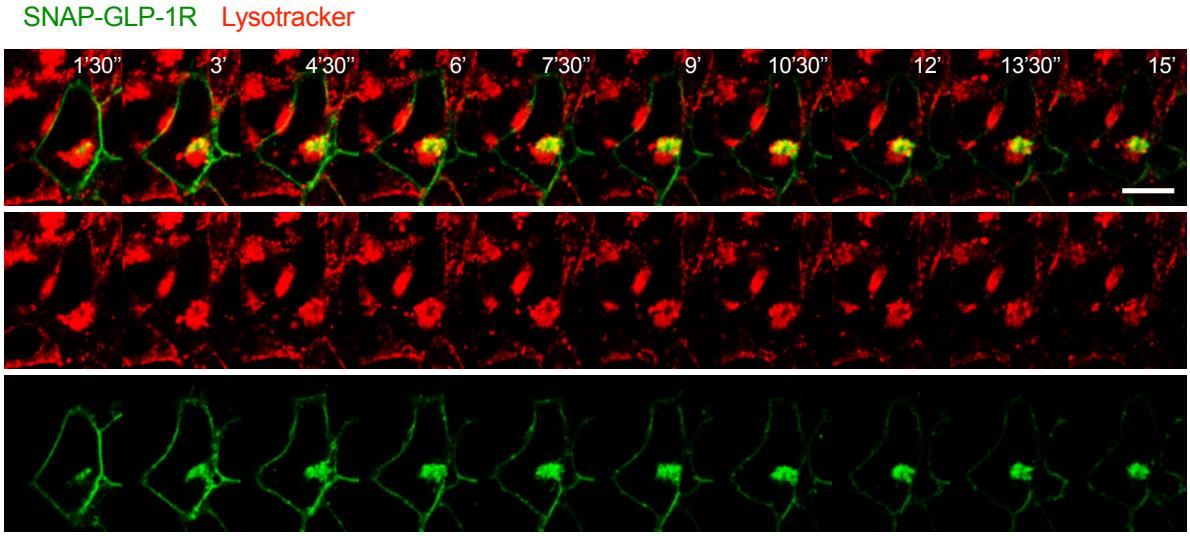



**Figure S4. GLP-1R *versus* GIPR localization to early endosomes in beta cells.** (**A**) Time frame montage with the indicated time points from INS-1 832/3 SNAP-GLP-1R cells transfected with Rab5-Venus prior to labeling with SNAP-Surface 549 and stimulation with 100 nM GLP-1 for 5 min. (**B**) As in (A) but from INS-1 832/3 SNAP-GIPR cells transfected with Rab5-Venus prior to labeling with SNAP-Surface 549 and stimulation with 100 nM GIP for 5 min. Size bars: 10 µm.

# Figure S5

## A




## B

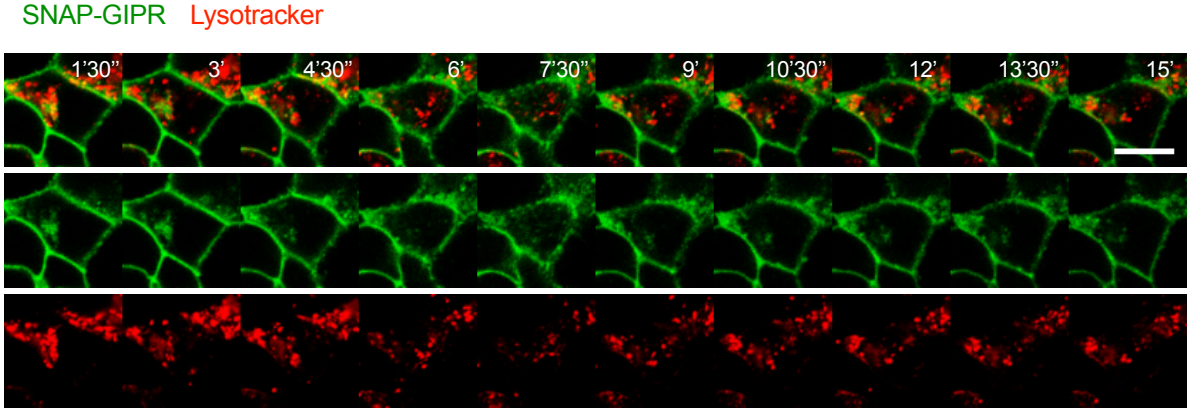



**Figure S5. GLP-1R *versus* GIPR localization to lysosomes in beta cells.** (**A**) Time frame montage with the indicated time points from INS-1 832/3 SNAP-GLP-1R cells labeled with Lysotracker Green + SNAP-Surface 649 and stimulation with 100 nM GLP-1 for 10 min. (**B**) As in (A) but from INS-1 832/3 SNAP-GIPR cells labeled with Lysotracker Green + SNAP-Surface 649 and stimulation with 100 nM GIP for 15 min. Size bars: 10 µm.

# Figure S6

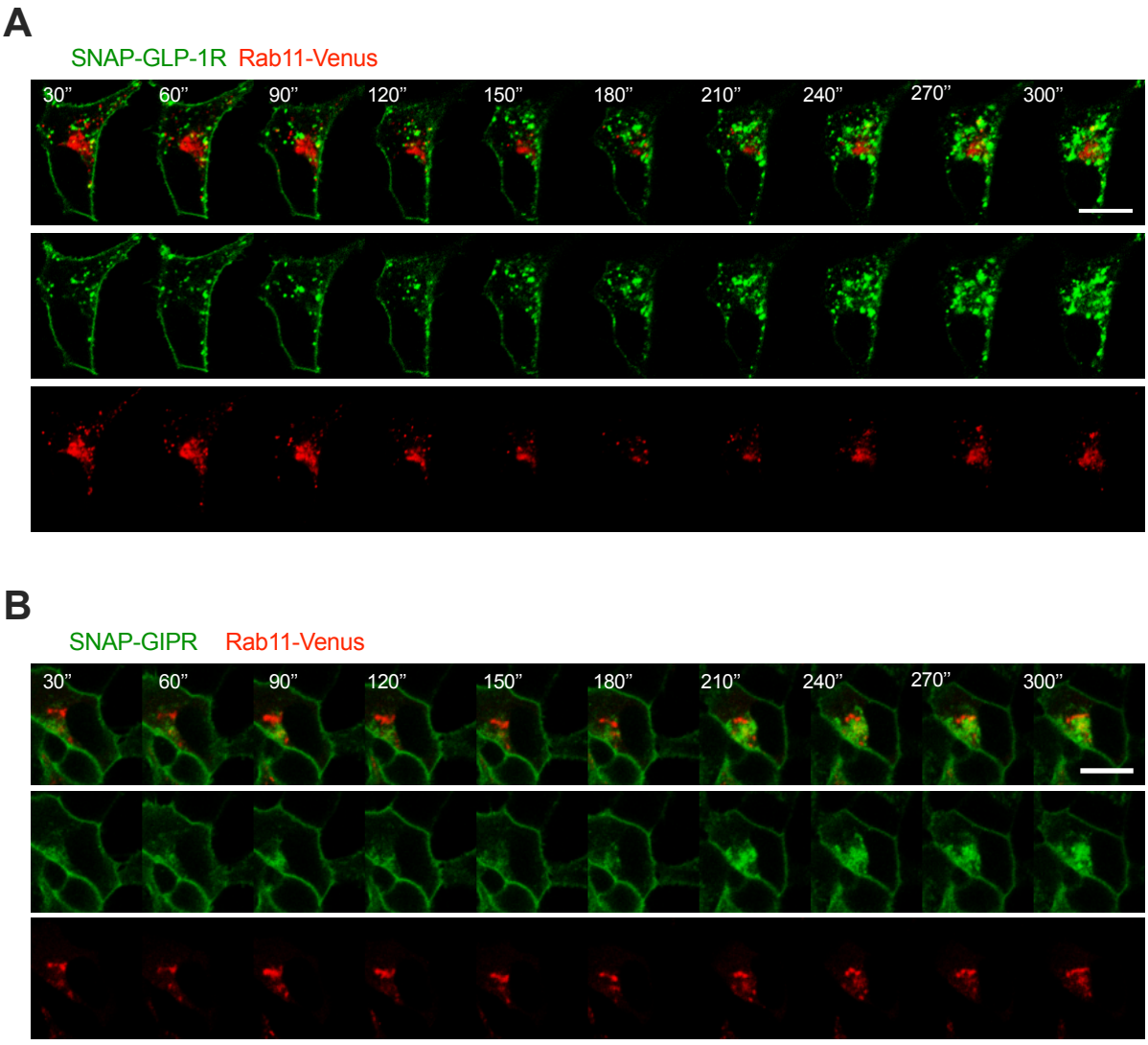



**Figure S6. GLP-1R *versus* GIPR localization to recycling endosomes in beta cells.** (**A**) Time frame montage with the indicated time points from INS-1 832/3 SNAP-GLP-1R cells transfected with Rab11-Venus prior to labeling with SNAP-Surface 549 and stimulation with 100 nM GLP-1 for 5 min. (**B**) As in (A) but from INS-1 832/3 SNAP-GIPR cells transfected with Rab11-Venus prior to labeling with SNAP-Surface 549 and stimulation with 100 nM GIP for 5 min. Size bars: 10 µm.

# Figure S7

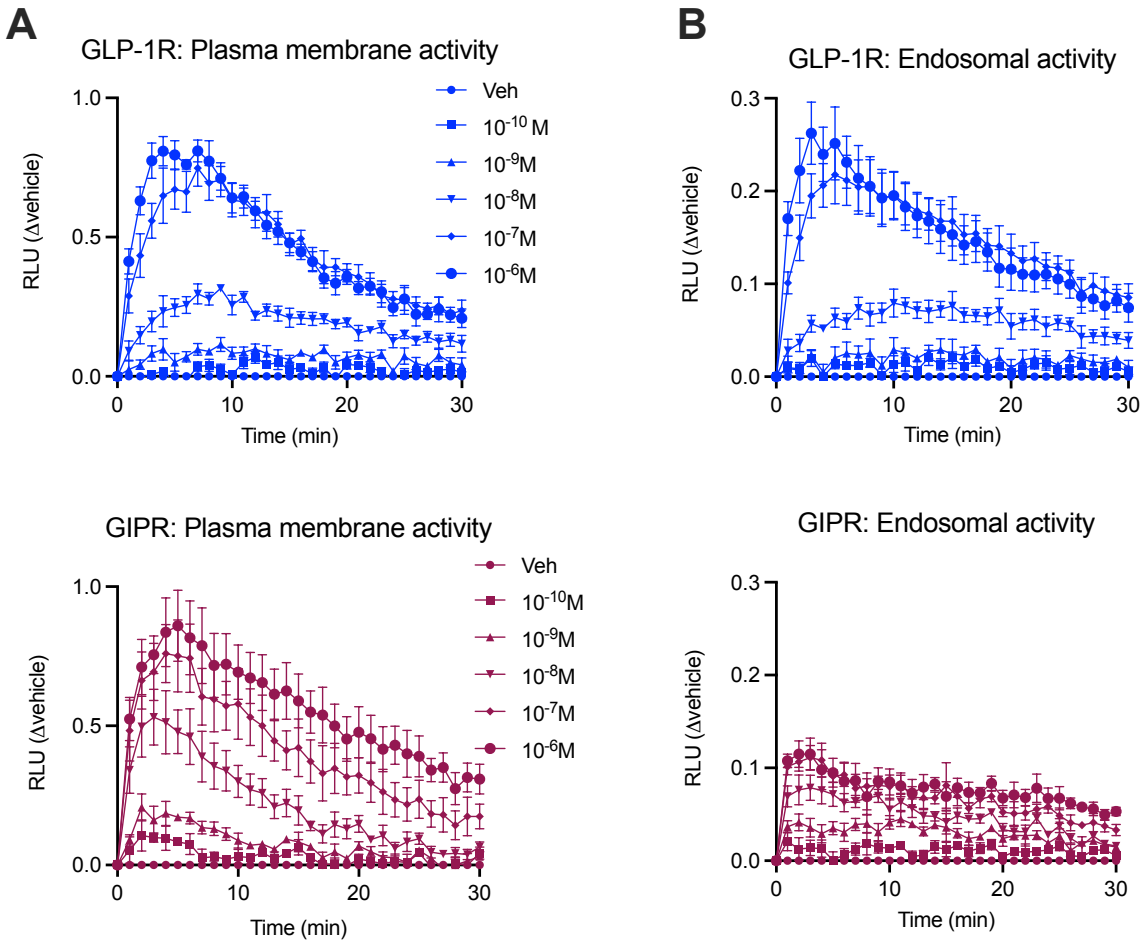



**Figure S7. GLP-1R *versus* GIPR endosomal *versus* plasma membrane activity in beta cells – extra data.** (**A**) Bystander NanoBiT plasma membrane response curves for each agonist concentration from dose response curves shown in Figure 6A. (**B**) Bystander NanoBiT endosomal response curves for each agonist concentration from dose response curves shown in Figure 6B. Data are mean ± SEM.