Supplementary Information

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Acylation of the incretin peptide exendin-4 directly impacts GLP-1 receptor signalling and trafficking

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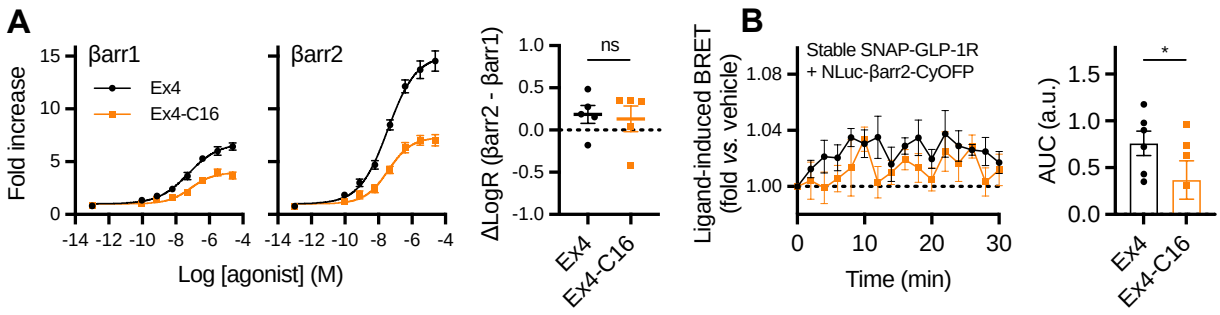
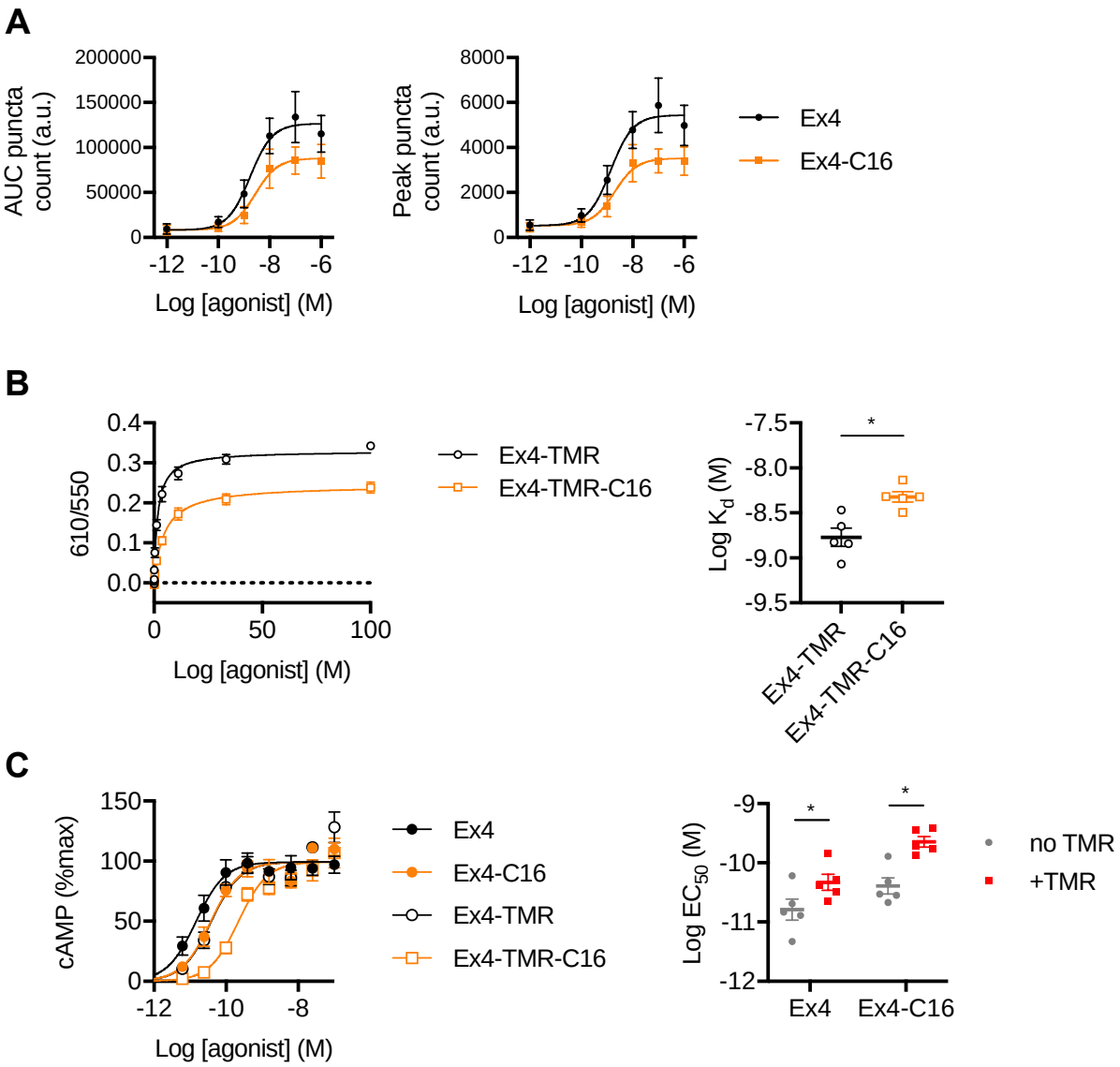
Supplementary Figure 1



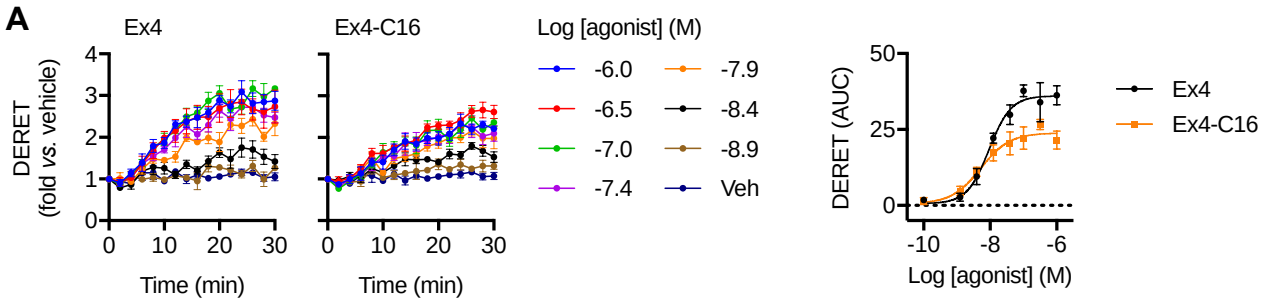
Supplementary Figure 1. Pharmacological responses to exendin-4 and exendin-4-C16. (**A**) β-arrestin-1 and β-arrestin-2 responses measured in PathHunter GLP-1R cells, *n=5*, with bias quantification showing selectivity for β-arrestin-2 *versus* β-arrestin-1 with comparison by paired t-test. (**B**) Measurement of β-arrestin-2 activation in HEK293-SNAP-GLP-1R cells transiently transfected with NLuc-4myc-βarr2-CYOFP1 and stimulated with 100 nM agonist or vehicle, *n*=6, with AUCs compared by paired t-test. Data are shown as mean ± SEM with individual replicates shown for AUC graph. * p<0.05 by statistical test indicated.

Supplementary Figure 2



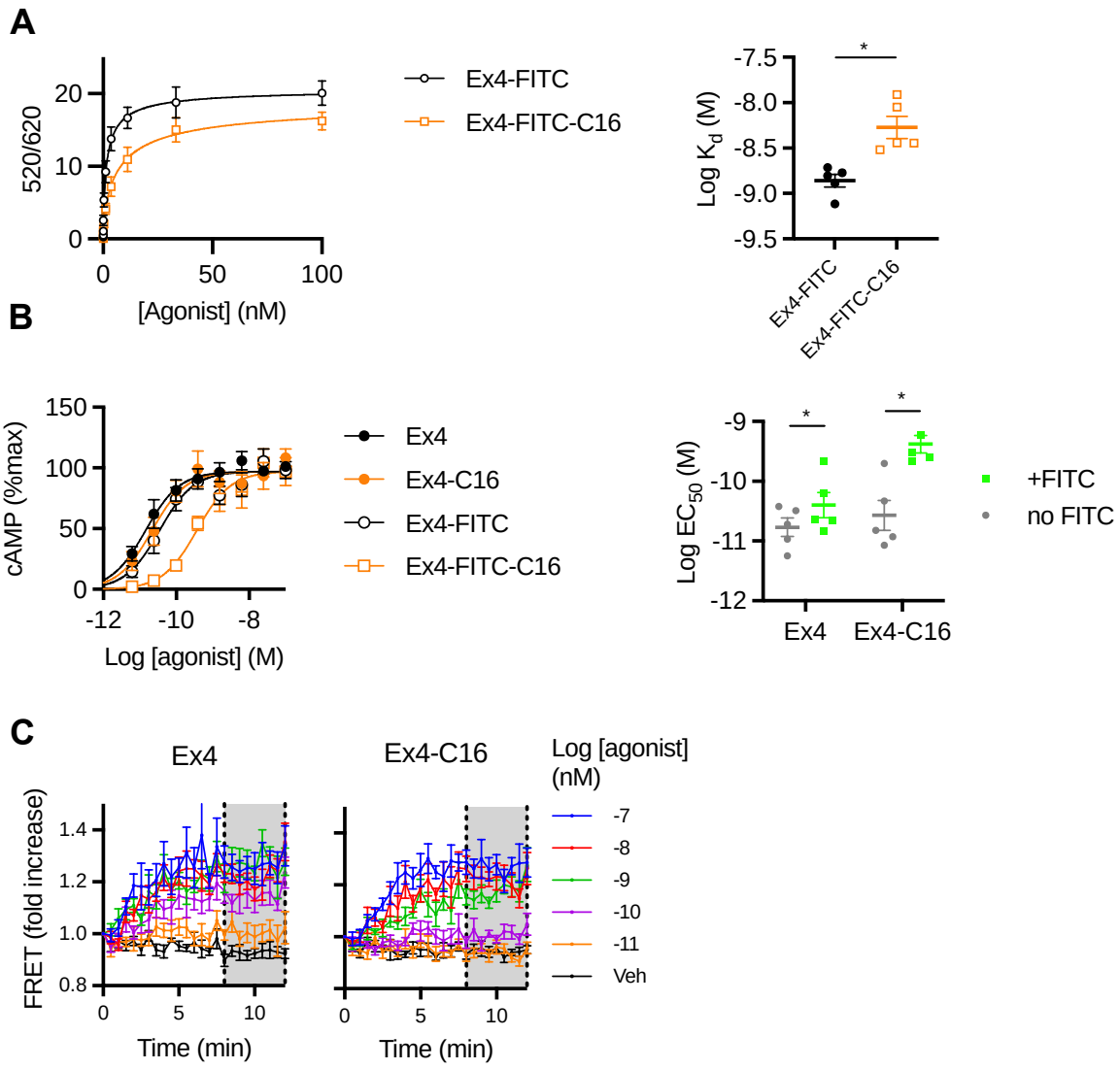
Supplementary Figure 2. Trafficking responses to exendin-4, exendin-4-C16, and functional characterization of TMR-conjugates. (**A**) Alternative quantification of endosomal puncta formation from Figure 2B, with concentration-dependent peak number of spots, or AUC across the entire 30-min stimulation period indicated with 3-parameter fit shown. (**B**) TR-FRET binding data for exendin-4-TMR and exendin-4-TMR-C16 in HEK293-SNAP-GLP-1R cells, *n*=5, with log K_d compared by paired t-test. (**C**) cAMP data for TMR-modified or not exendin-4 / exendin-4-C16, *n*=5, with 3-parameter fits shown. LogEC_{50} values are compared by 2-way randomised block ANOVA with Sidak's test. Data are shown as mean ± SEM with individual replicates shown where possible. * p<0.05 by statistical test indicated

Supplementary Figure 3



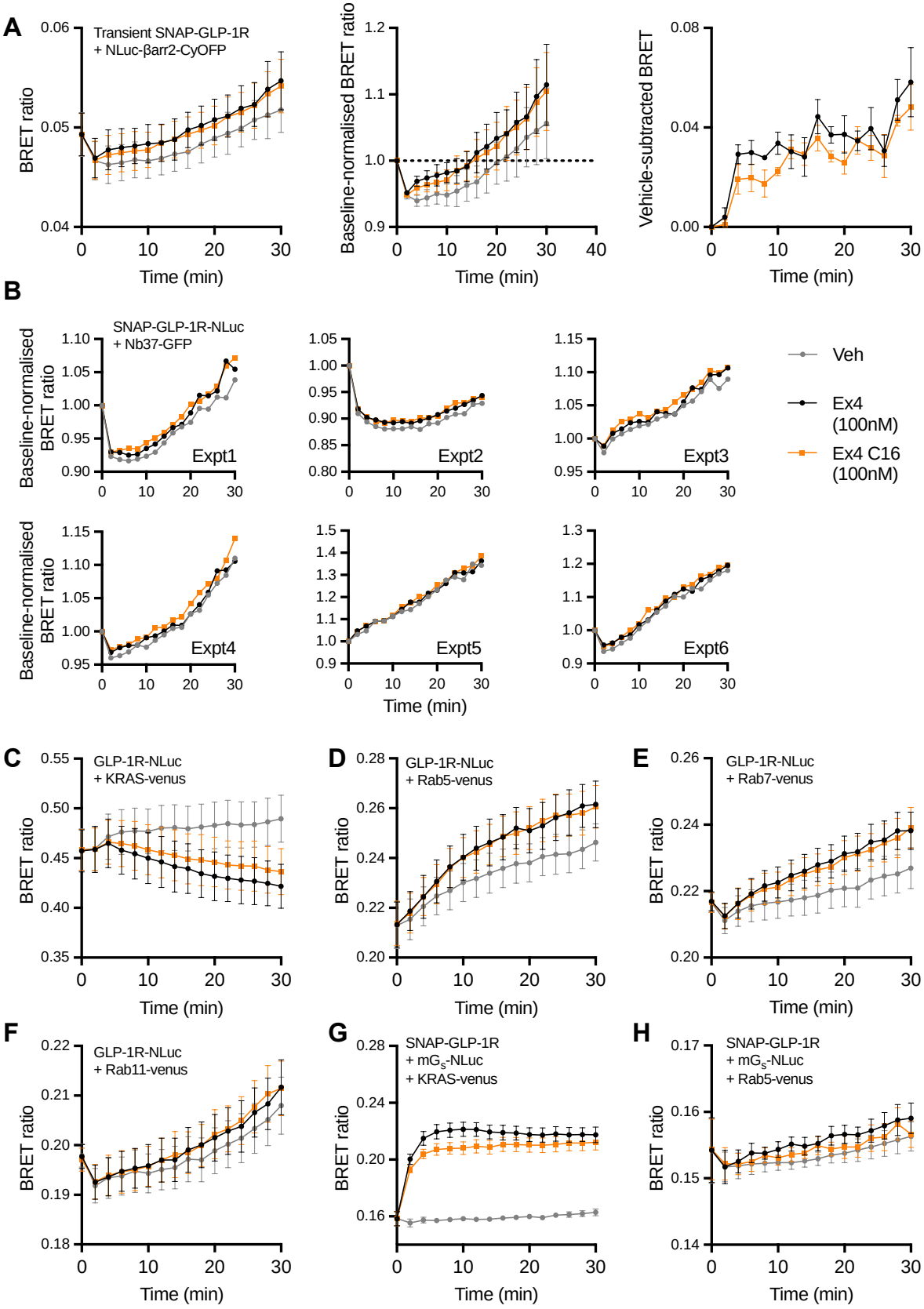
Supplementary Figure 3. GLP-1R trafficking responses by DERET. (**A**) GLP-1R endocytosis with exendin-4 and exendin-4-C16 measured by DERET in HEK293-SNAP-GLP-1R cells, *n*=5. Concentration response is quantified from AUC with 3-parameter fit shown. Data are shown as mean ± SEM.

Supplementary Figure 4



Supplementary Figure 4. Exendin-4-FITC and exendin-4-FITC-C16 functional characterisation. (**A**) TR-FRET binding data for exendin-4-FITC and exendin-4-FITC-C16 in HEK293-SNAP-GLP-1R cells, *n*=5, with log K_d compared by paired t-test. (**B**) cAMP data for FITC-modified or not exendin-4 / exendin-4-C16, *n*=5, with 3-parameter fits shown. LogEC_{50} values are compared by 2-way randomised block ANOVA with Sidak's test. (**C**) FRET responses for T-REx-SNAP-GLP-1R cells expressing AKAP79-CUTie stimulated with each ligand, *n*=6. The Shaded area indicates the time-points from which the average response wa calculated in Figure 4D. Data are shown as mean ± SEM with individual replicates where possible. * p<0.05 by statistical test indicated.

Supplementary Figure 5.



Supplementary Figure 5. BRET analytical approach and raw BRET ratios. (**A**) β-arrestin-2 activation assay (refers to Figure 1F). The raw BRET ratios, baseline-normalised, and baseline-normalised and vehicle-subtracted traces are shown to indicate how the specific ligand-induced BRET signal presented in Figure 1F and corresponding AUC values are derived. The BRET signal drift is substantial but agonist-induced BRET ratios are consistently higher at each time-point. (**B**) Nb37 G protein activation assay (refers to Figure 1E). Due to the low dynamic range of this assay, individual experimental repeats have been presented as baseline-normalised BRET ratios. It can be seen that, in spite of significant drift in all traces over time, agonist-induced BRET values are consistently subtly increased compared to vehicle throughout each experiment. Moreover, on each occasion, exendin-4-C16 responses are slightly greater than those of exendin-4. (**C**), (**D**), (**E**) and (**F**) GLP-1R-Nluc redistribution assays (refers to Figure 3F-I). (**G**) and (**H**) Mini-G_s redistribution assay (refers to Figure 3K). Mean ± SEM is shown.