Quantification of GLP-1R trafficking in primary β-cells in response to different ligands

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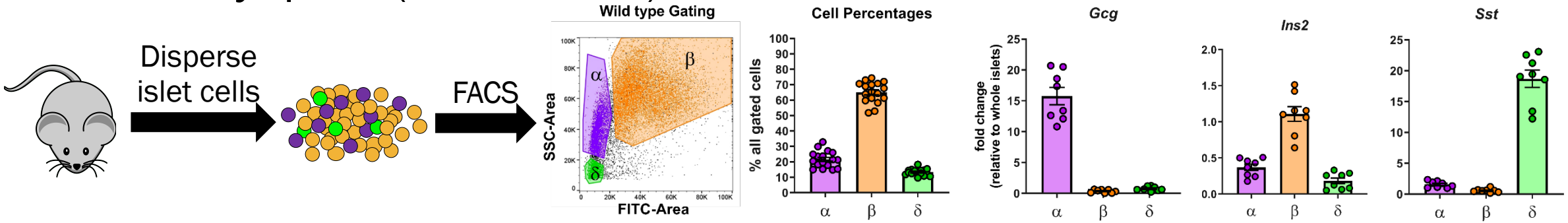
Introduction

- The glucagon-like peptide 1 receptor (GLP-1R) is a class B G-protein coupled receptor expressed in islet β-cells. Activation of the GLP-1R is insulinotropic.
- Multiple endogenous and synthetic ligands exist for the GLP-1R. Emerging evidence suggests that each ligand has a unique interaction with GLP-1R, causing distinct responses of receptor physiology.
- In these studies, we test the hypothesis that specific GLP-1R ligands differentially affect GLP-1R trafficking and internalization in primary β-cells.

Methodology

- Enriched populations of β-cells were generated by flow cytometry/FACS and validated by qPCR (see 2113-P)



- Glp1r0017, an antibody for GLP-1R (Biggs et al., *Diabetologia* 2018), was conjugated to APC (GLP1R-APC) and validated for use in flow cytometry applications (see 2113-P)

- Dispersed islet cells were treated with GLP-1R ligands, incubated with GLP1R-APC, and β-cell staining was analyzed on a flow cytometer (Attune NxT Analyzer) or imaged by ImageStream X

Ligand exposure decreases GLP1R-APC Staining in β-cells and suggests internalization

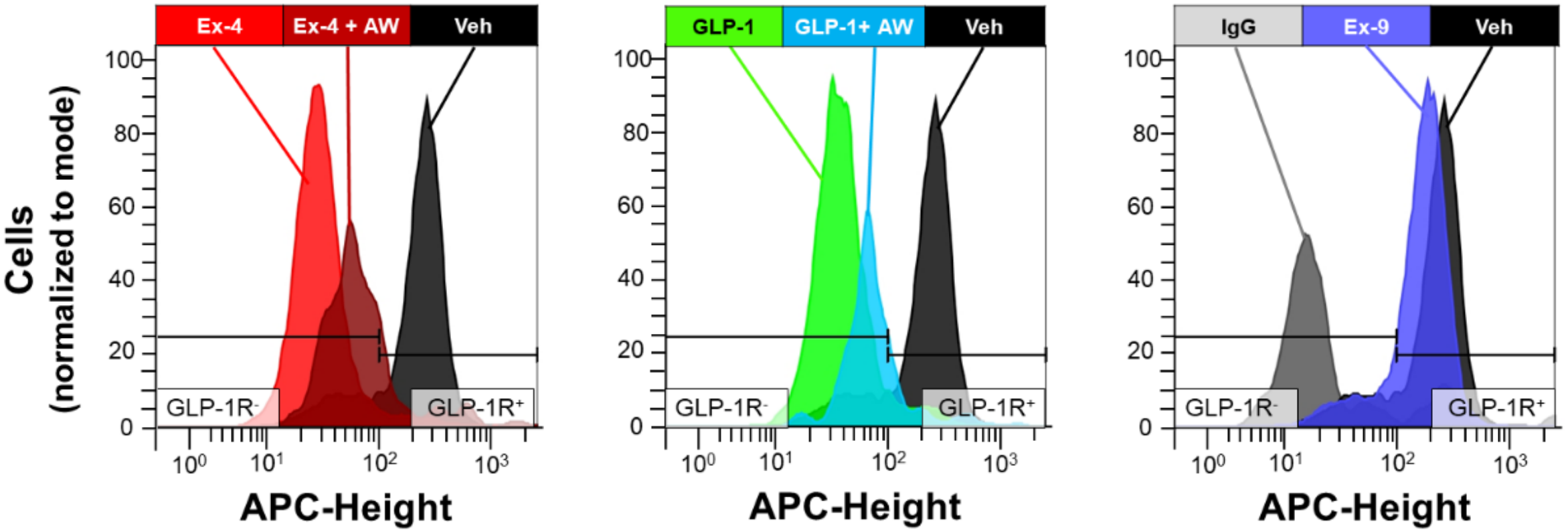


Figure 1. Following ligand treatment, GLP-1R is internalized and there is a left-shift in GLP1R-APC staining. Acid wash does not significantly alter this, suggesting ligands do not interfere with GLP1R-APC Staining. Histogram of β-cells stained with GLP1R-APC following **A)** exendin-4 alone (Ex-4) or followed by acid wash (Ex-4 + AW), or vehicle (Veh), **B)** GLP-1 alone (GLP-1) or GLP-1 followed by acid wash, or vehicle, **C)** exendin 9-39 (Ex-9) or vehicle, with IgG-APC (IgG) shown. All ligands were given at 100 nM for 30 min at 37°C. Acid wash conditions followed ligand treatment (2x wash with 0.5M NaCl, 0.2 M acetic acid, pH 2.5). Following treatment, reactions were stopped on ice and incubated with GLP1R-APC or IgG-APC.

Ligand exposure reduces GLP1R-APC Staining

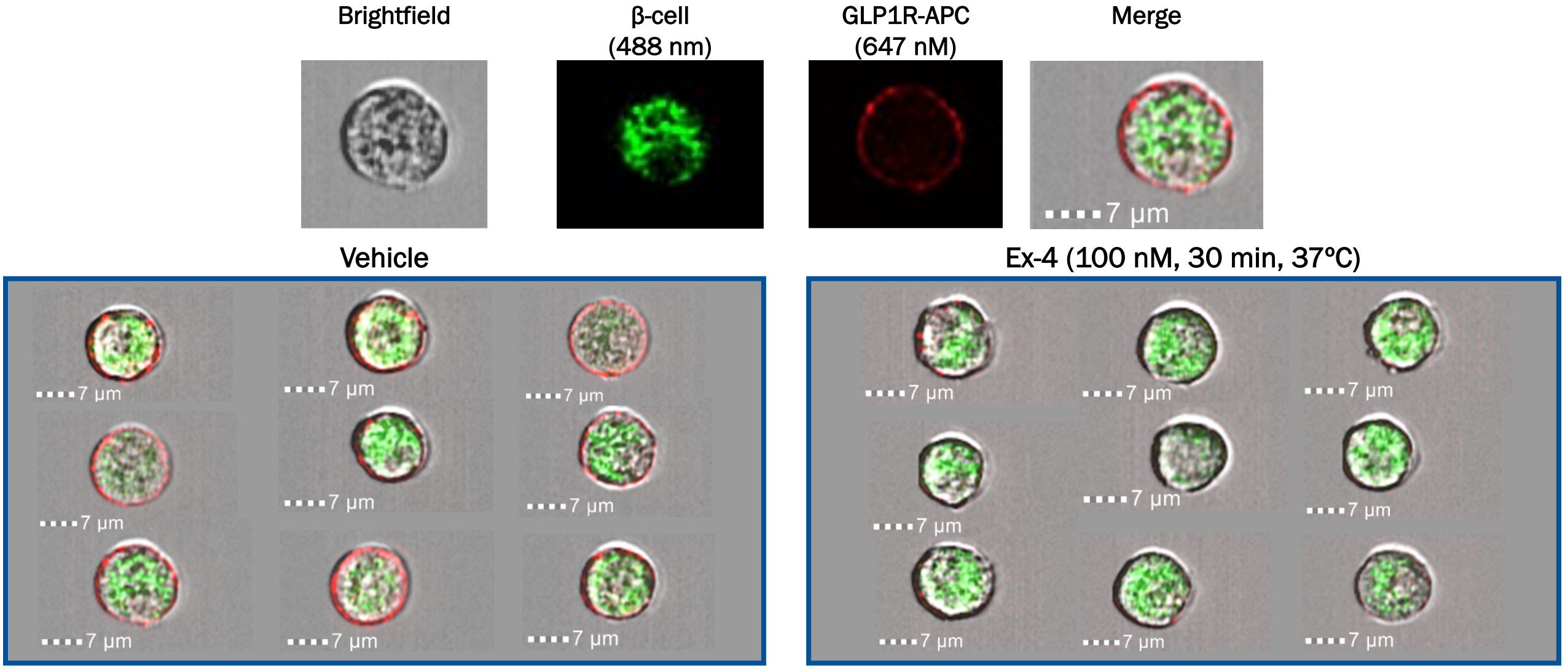


Figure 2. Imagestream allows for combined microscopy and flow cytometry and demonstrates decreased GLP1R-APC staining in β-cells following Ex-4 treatment. Top: Representative images from brightfield, 488 nm, and 647 nm channels with merged image for a single β-cell. **Bottom:** GLP-1R staining in β-cells treated with vehicle or exendin-4 (Ex-4). Autofluorescence (488, green) was used to select β-cells and APC (red) was used to visualize GLP-1R.

Exposure to ligands causes a dose-dependent reduction of GLP-1R on β-cell plasma membrane

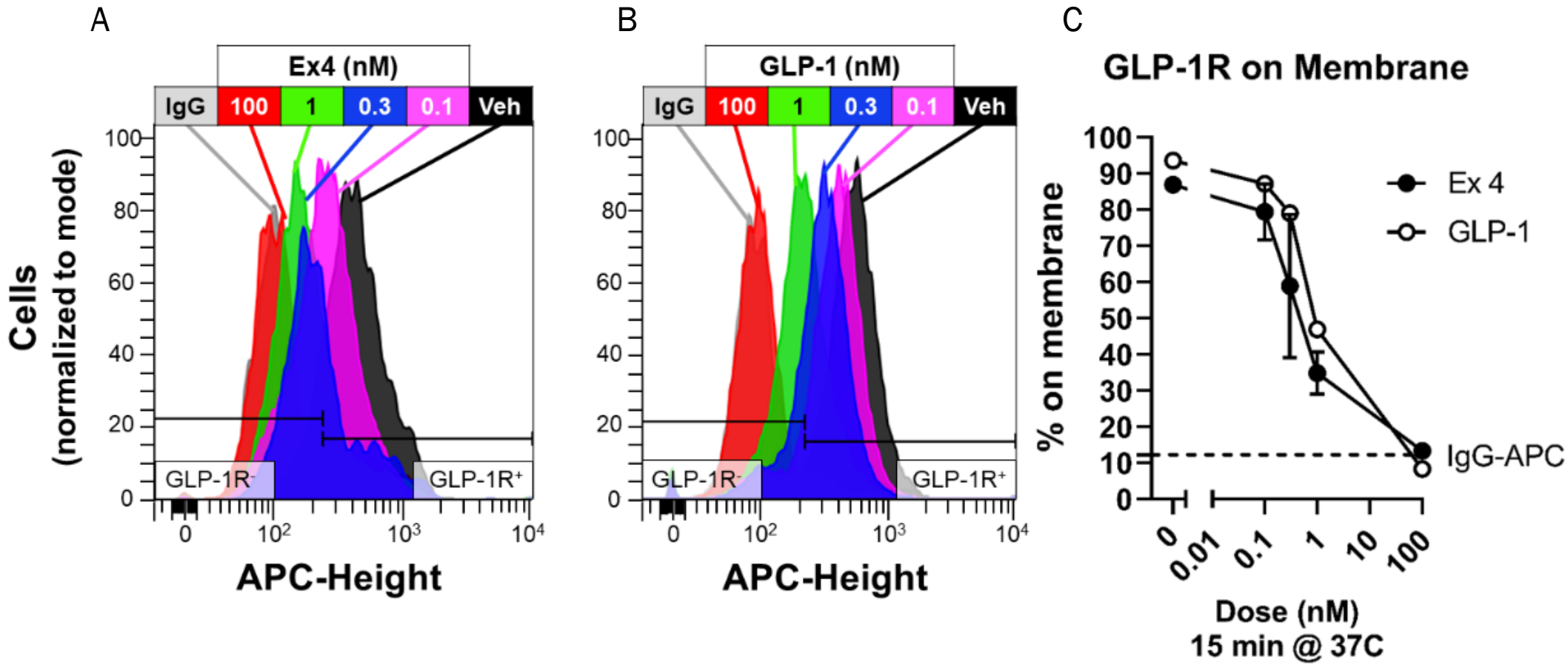


Figure 3. Increasing doses of GLP-1R ligand caused a shift in GLP1R-APC staining from normal (Veh) to absent (IgG), suggesting progressive internalization in response to ligand exposure. Histograms of GLP1R-APC staining following increasing doses of **A)** exendin-4 (Ex4) and **B)** GLP-1 with **C)** quantification. Dispersed cells were treated for 15 min at 37°C with ligand or vehicle prior to GLP1R-APC staining. N=1-2 replicates per ligand.

Exendin-4 promotes GLP-1R internalization in proportion to time of exposure

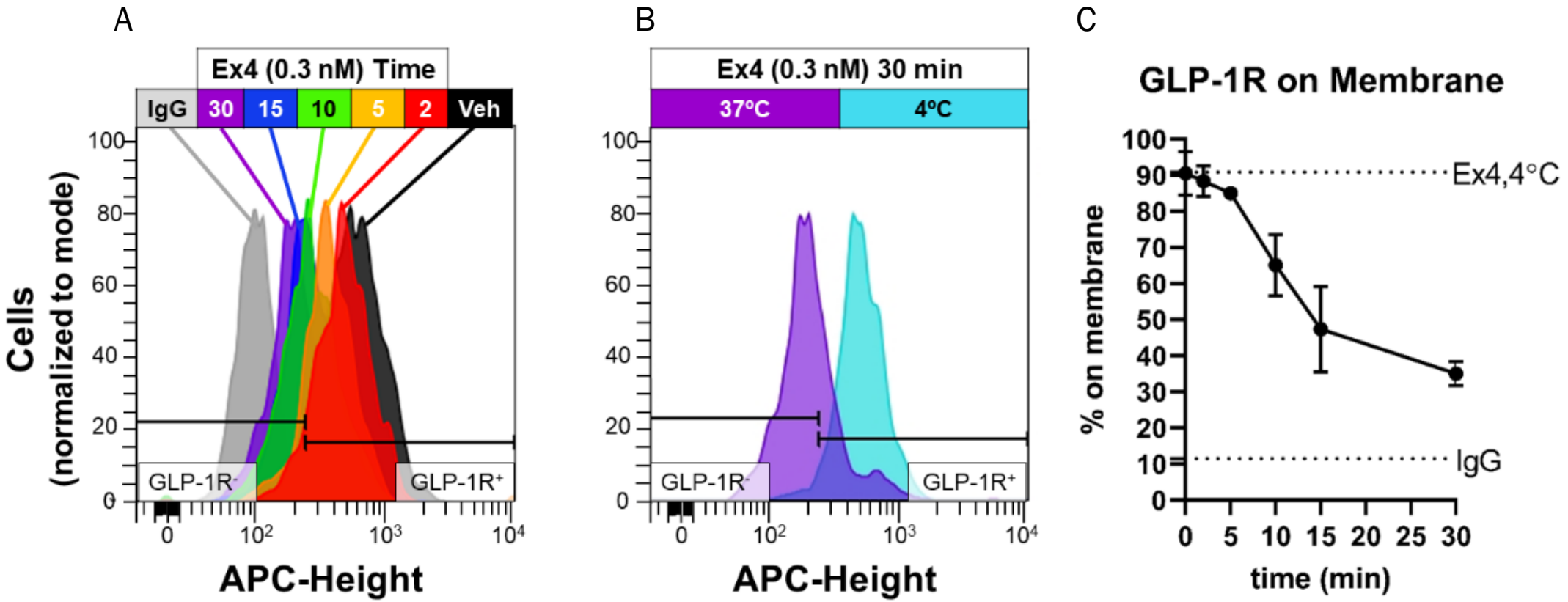


Figure 4. Increased time of exposure to Ex-4 shifted GLP1R-APC staining from normal (veh) toward absent (IgG) and this response was muted at 4°C. Histograms of GLP1R-APC staining following **A)** Time course of exendin-4 (Ex4, 0.3 nM) and **B)** 30 min at 37°C or 4°C with **C)** quantification**.** Dispersed cells were treated for with 0.3 nM Ex4 at 37°C or 4°C with Ex4 or vehicle prior to GLP1R-APC staining. N=2.

GLP-1R recycles slowly after maximal Exendin-4 dose

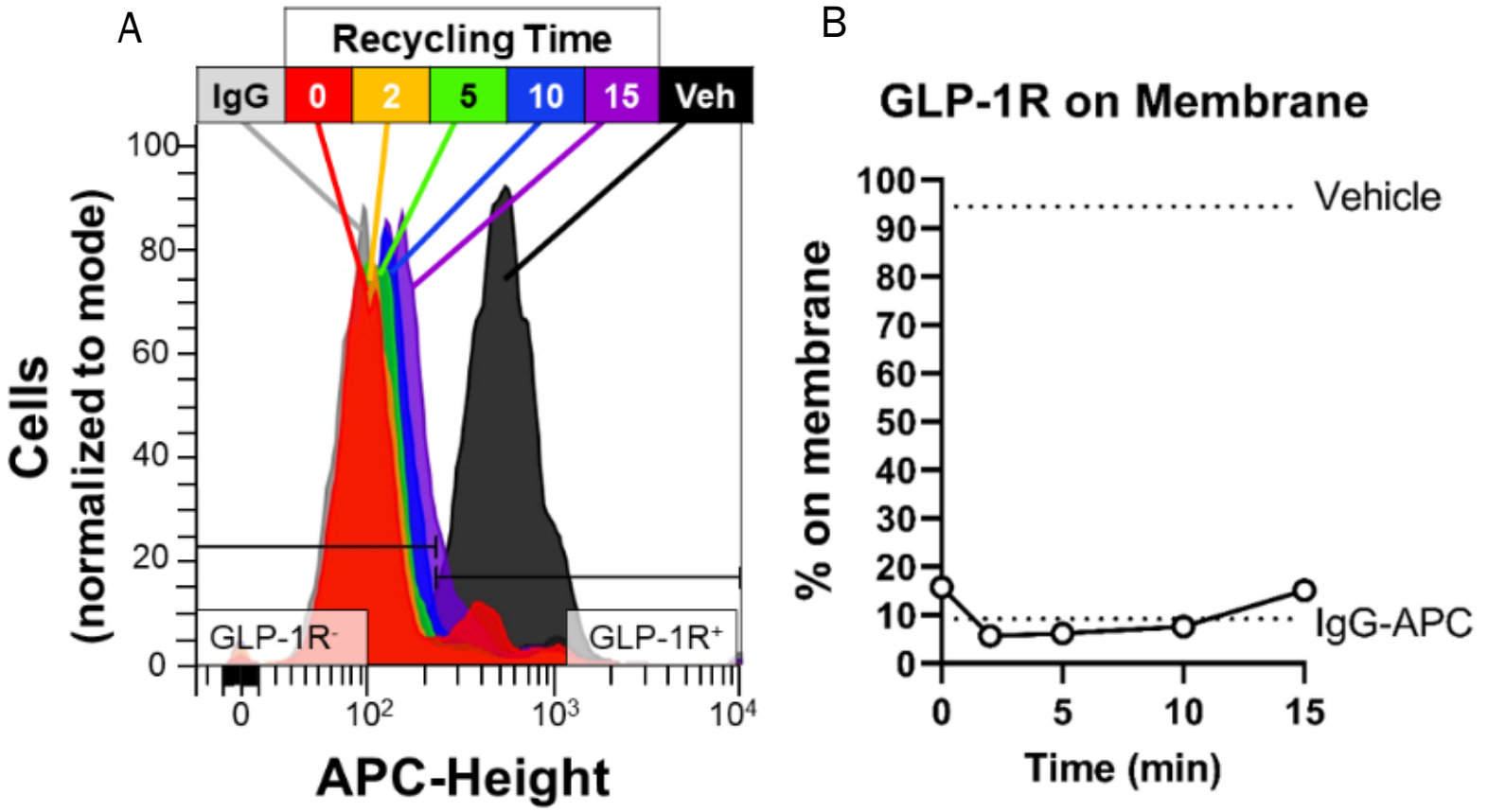


Figure 5. Following exposure to Ex-4, there was a modest right-shift of GLP1R-APC staining for 15 minutes after exposure, consistent with slow recycling of GLP-1R after maximal ligand exposure and internalization. Dispersed islet cells were treated with Ex4 at 100 nM for 30 min at 37°C before ligand was washed out and cells were incubated in buffer at 37°C for specified amount of time to allow GLP-1R to recycle to plasma membrane. **A)** Time course of GLP-1R recycling after Ex4 and **B)** quantification. Dispersed cells were treated for with 0.3 nM Ex4 at 37°C or 4°C with Ex4 or vehicle prior to GLP1R-APC staining. N=1.

Conclusions

- Conjugation of Glp1r0017 to a fluorophore (GLP1R-APC) allows for imaging of the GLP-1R during flow cytometry applications in live cells
- GLP1R-APC binding allows for inferences about GLP-1R internalization
- GLP1R-APC binding is not prevented by ligand treatment
- Following maximal Ex4 treatment, GLP-1R on plasma membrane is undetectable and slow to recycle back to the plasma membrane
- Differences in dose- and time-dependent movement of GLP-1R in response to GLP-1R ligands requires further investigation

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