Supplementary Figures
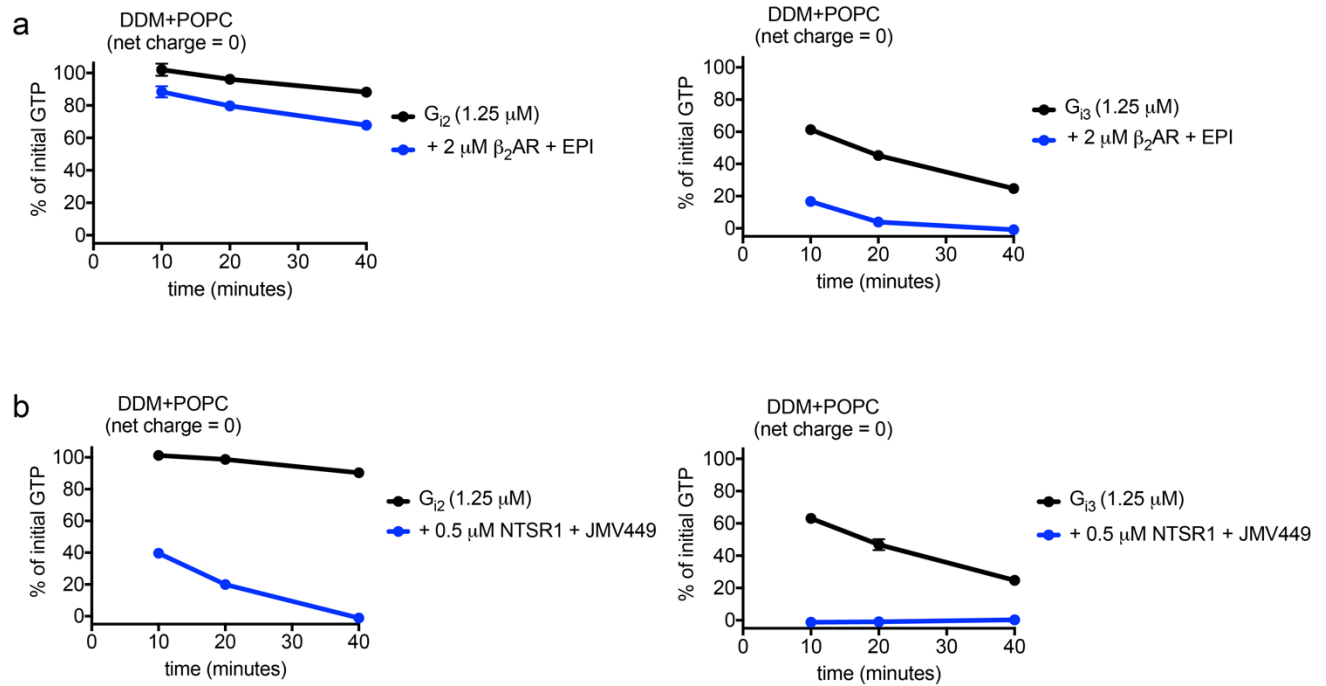
Local membrane charge regulates β_2 adrenergic receptor coupling to G_{i3}
Strohman et al.

**Supplementary figure 1. Simulated mB-β₂AR populations**

This figure shows simulations to establish that, under the particular conditions of the experiments reported here, fluorescence emission maxima can be used to measure the fraction of G protein coupled β₂AR. (**a**) Left: Representative raw spectra from Figure 1d (DDM+CHS+POPC condition). Spectra are labeled as followed: **A** represents Apo mB-β₂AR, **E** represents mB-β₂AR bound to 100 μM epinephrine and **G** represents the mB-β₂AR-G$_s$ complex. Right: Spectrum **E** relative to a simulated spectral population calculated as the weighted average intensity of spectra **A** and **G** (Merged$_{AG}$=0.75*Intensity$_A$+0.25*Intensity$_G$). (**b**) Left: Spectra **A** and **G** from panel A, with smoothed spectra underlaid in black. Middle: Simulated spectra with varying fractions of **A** and **G**. The simulated spectra were calculated as in panel A and were subsequently smoothed. Right: The λ$_{max}$ has a linear correlation with the fraction of spectrum **G**. Data represent mean +/- s.e.m from three simulations based on spectra from three independent experiments. (**c**) Left: Spectra **E** and **G** from panel A, with smoothed spectra underlaid in black. Middle: Simulated spectra with varying fractions of **E** and **G**. The simulated spectra were calculated as in panel A and were subsequently smoothed. Right: The λ$_{max}$ has a linear correlation with the fraction of spectrum **G**. Data represent mean +/- s.e.m from three simulations based on spectra from three independent experiments. Source data are provided in the Source Data File.

Supplementary figure 2. Effect of PKA phosphorylation on mB-β₂AR interaction with Gᵢ (a) Phosphorylation status of mB-β₂AR before and every 0.5 hours after the addition of protein kinase A (PKA) and excess ATP (2 mM). -P indicates the unphosphorylated mβ-β₂AR used in Panel B; +P indicates the phosphorylated mβ-β₂AR used in Panel B (b) Effect of PKA phosphorylation on G_{i1}, G_{i2}, G_{i3}, and G_s interaction with mB-β₂AR in the presence of epinephrine. Measurements were performed in DDM+CHS+POPC mixtures (5:1:1 DDM:CHS:POPC mole ratio). mB-β₂AR concentration is 300 nM. Data are mean +/- s.e.m of three independent experiments. Source data, including uncropped images, are provided in the Source Data File.

Supplementary figure 3. GTP turnover activity of G_{i2} (left) and G_{i3} (right) in DDM+POPC (5:1 DDM:POPC mole ratio) mixed micelles, in the absence or presence of ligand-stimulated $\beta_2 AR$ (panel A) and NTSR1 (panel B), using the ligand epinephrine (EPI) and JMV 449, respectively. Data are mean +/- s.e.m of three independent experiments. Source data are provided in the Source Data File.
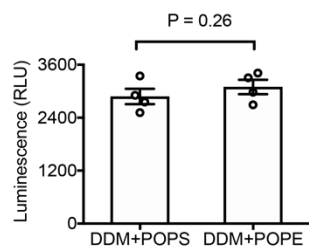
Supplementary figure 4. Luminescence signals from the Fig. 1f condition with G_{i3} alone (0.25 µM). Data are mean +/- s.e.m of four independent experiments. Statistical significance was determined using a two-sided Student's *t*-test. Source data are provided in the Source Data File.
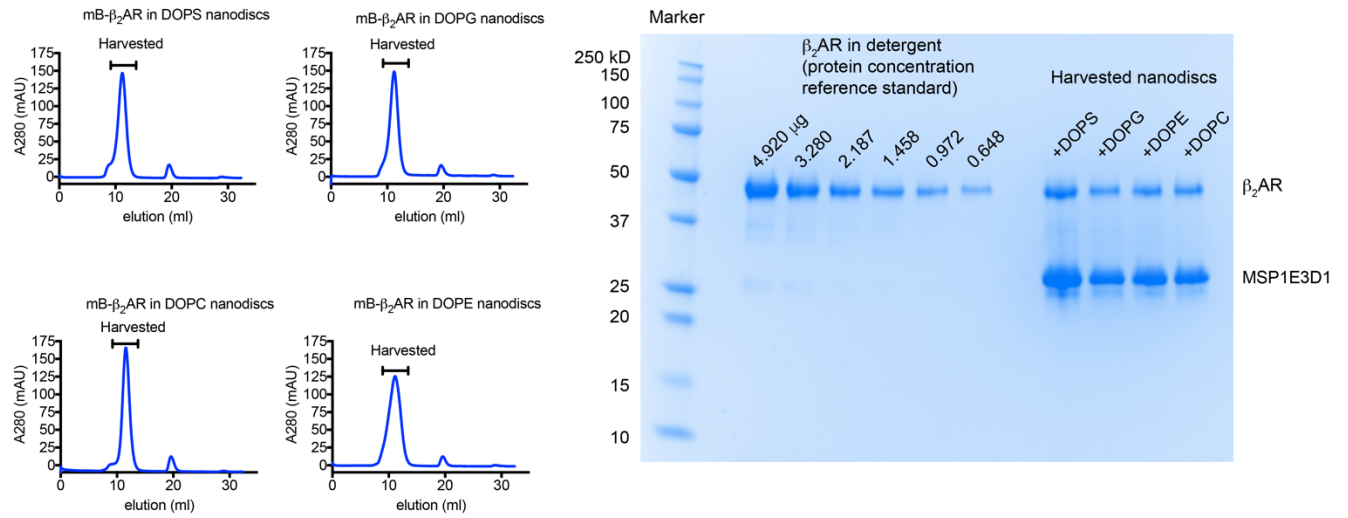
Supplementary figure 5. Left: Size exclusion profiles of nanodisc mB-β₂AR; Right: SDS-PAGE of harvested nanodisc mB-β₂AR after concentration
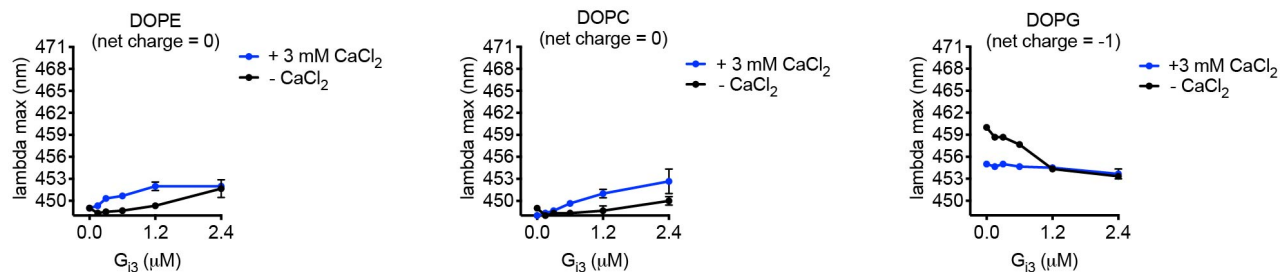
Supplementary figure 6. Effect of Ca^{2+} on basal mB-β$_2$AR-G$_{i3}$ interaction in bilayers of varying phospholipid composition The effect of G$_{i3}$ concentration on mB-β$_2$AR fluorescence (+/- 3 mM CaCl$_2$) was examined in DOPE, DOPC, and DOPG nanodisc bilayers in the absence of epinephrine. mB-β$_2$AR concentration is 100 nM. Data are mean +/- s.e.m of three independent experiments. The net charge of the phospholipid is indicated in parentheses. Source data are provided in the Source Data File.

Adenosine A1 Receptor

Gαi2

Amino acids in Gαi2 that differ from Gαi1 and Gαi3

Gβ1

Gγ2
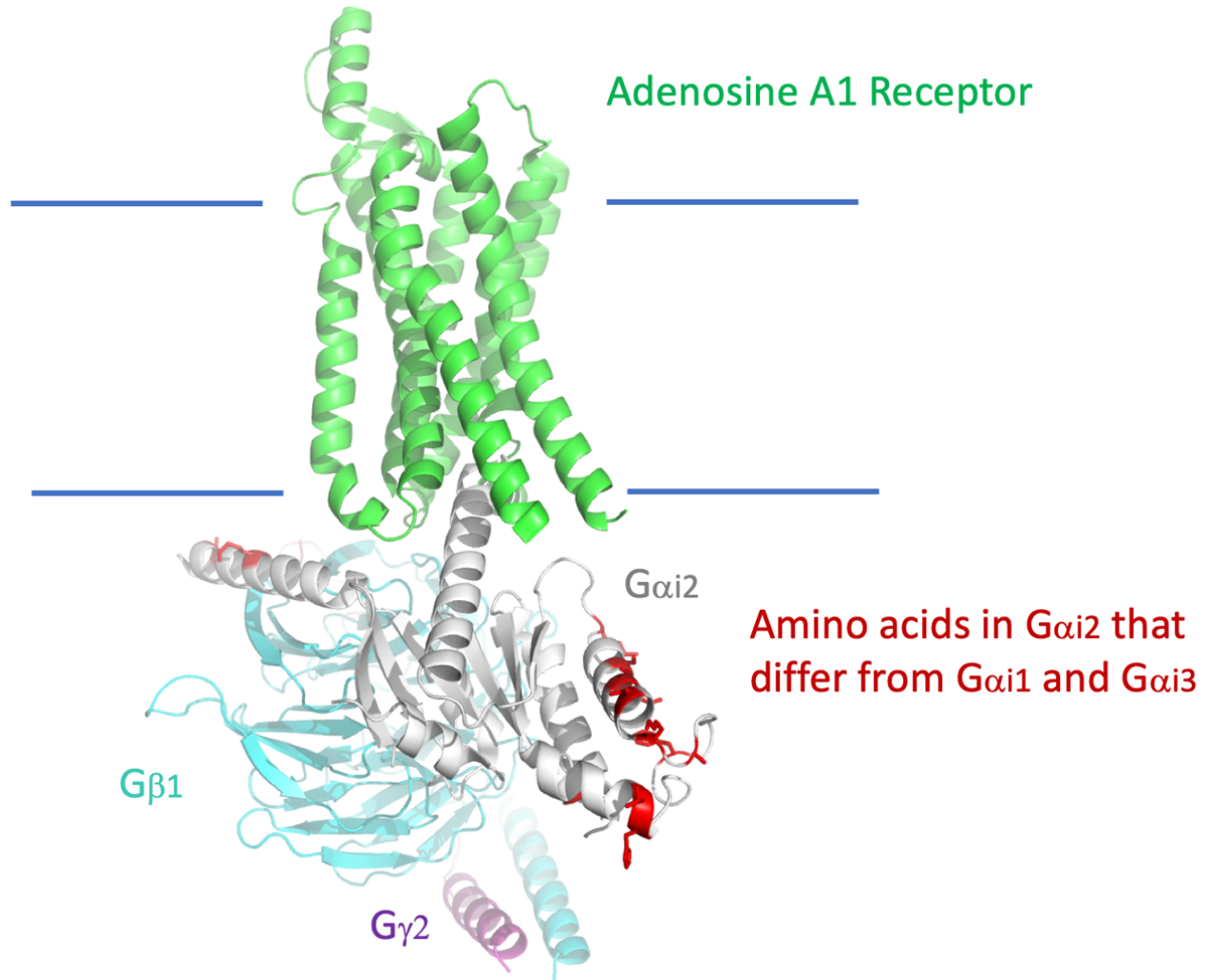
Supplementary figure 7. Cryo-electron microscopy structure of the adenosine A1-G_{i2} complex (PDB: 6D9H). Highlighted in red are amino acids in the Ras domain of $Gα_{i2}$ that differ from $Gα_{i1}$ and $Gα_{i3}$.