Molecular Pharmacology

Supplemental Data

Segregation of family A GPCR protomers in the plasma membrane

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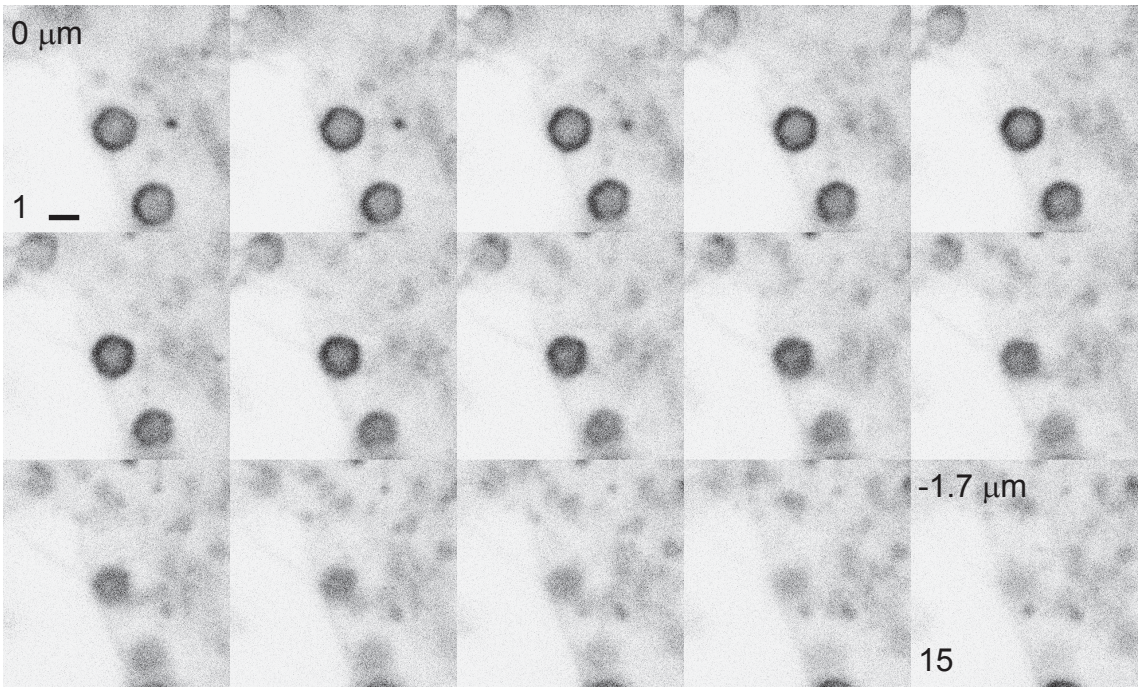
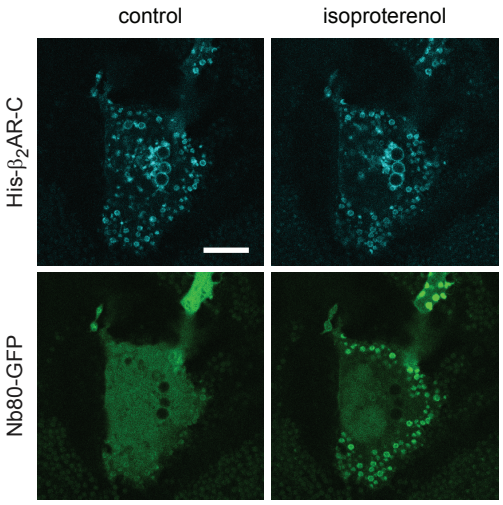
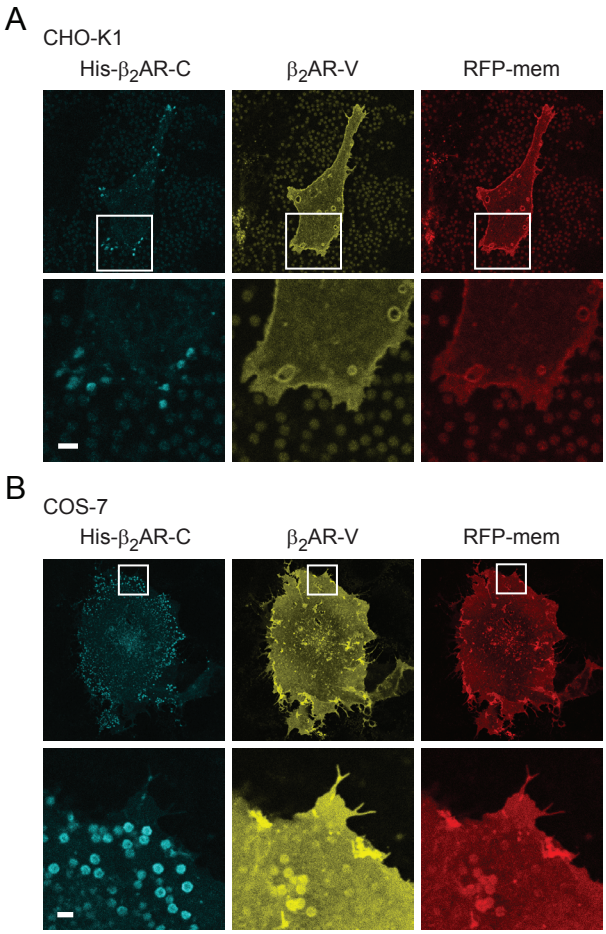


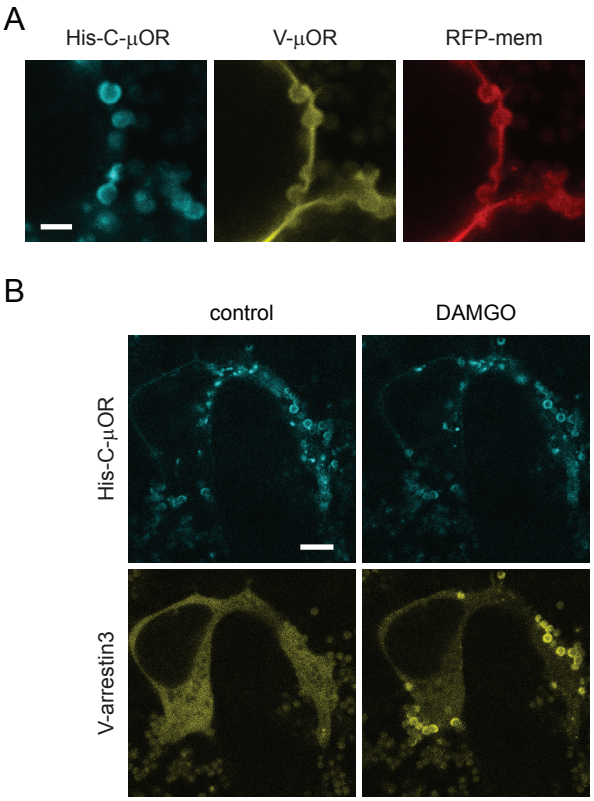
Supplemental Figure 1. A montage (z-stack) of images of bead-induced domains of His-β_2AR-venus. Profiles of bead-induced domains appear smaller as the image plane moves towards the bottom of each bead. Image number 1 and image number 15 are labeled, and the depth of the image in the z-axis is indicated. Scale bar in image 1, 1 μm.



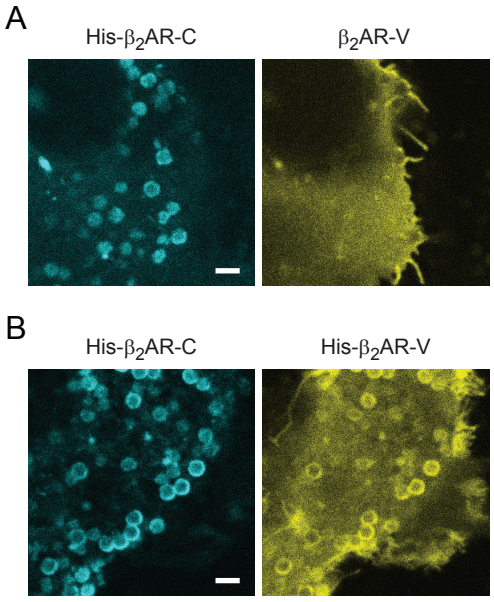
Supplemental Figure 2. Recruitment of cytosolic Nb80-GFP to bead-attached His-β_2AR-cerulean. Confocal images showing bead-associated His-β_2AR-cerulean and Nb80-GFP before and after agonist stimulation (10 μM isoproterenol). Scale bar, 10 μm.



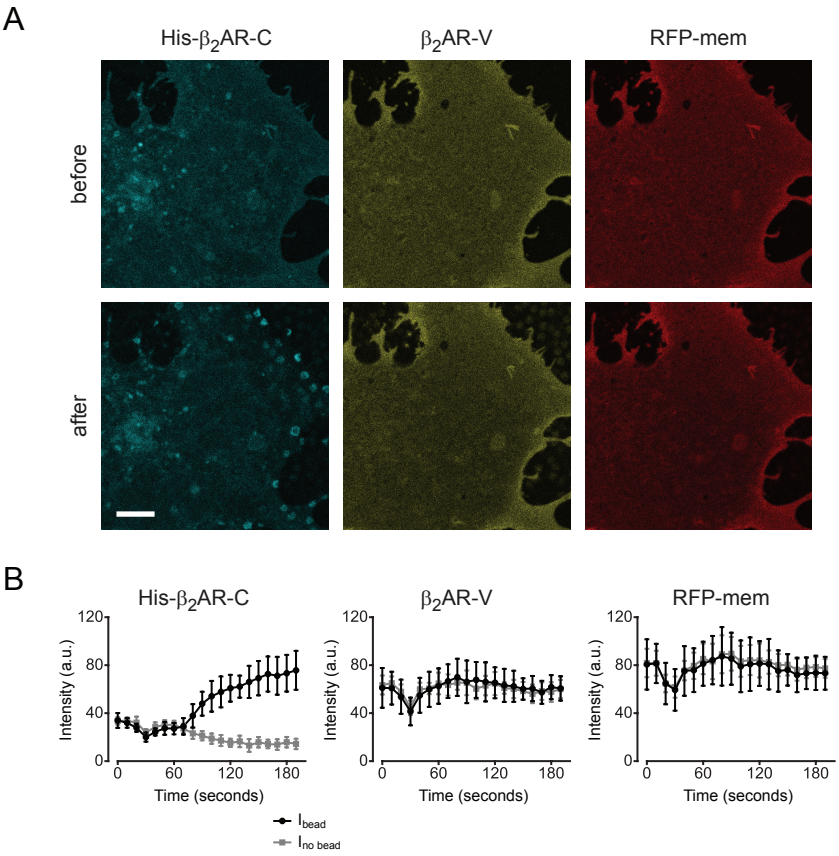
Supplemental Figure 3. Recruitment of His-β_2AR-cerulean to IMAC beads without corecruitment of β_2AR-venus in (A) CHO-K1 and (B) COS-7 cells. Scale bars, 2 μm.



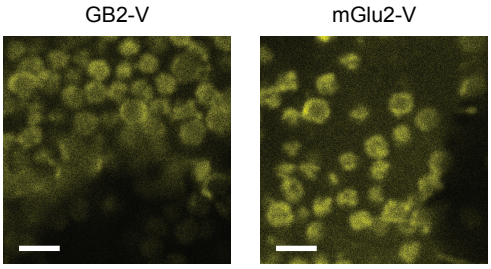
Supplemental Figure 4. Recruitment of His-C-μ-OR to IMAC beads. (A) His-C-μ-OR protomers do not corecruit untagged V-μ-OR protomers to IMAC beads. Scale bar, 2 μm. (B) His-C-μ-OR protomers recruit V-arrestin3 from the cytosol to the bead-associated plasma membrane after agonist stimulation (10 μM DAMGO). Scale bar, 5 μm.



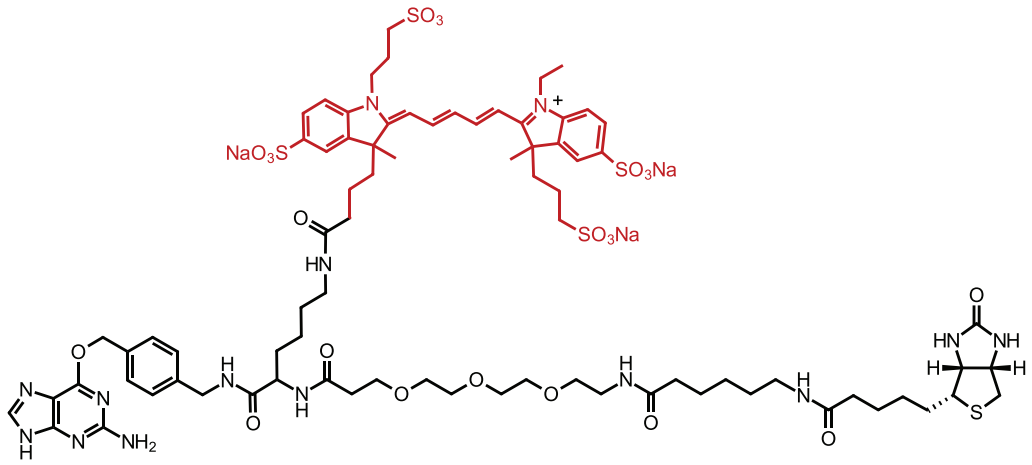
Supplemental Figure 5. IMAC beads that recruit His-β_2AR-cerulean have capacity to recruit additional (His-β_2AR-venus) protomers. Cells expressed either His-β_2AR-C and β_2AR-V (A), or His-β_2AR-C and His-β_2AR-V (B). RFP-mem (not shown) was also expressed in both cases. Scale bars, 2 μm.



Supplemental Figure 6. β_2AR-venus fluorescence in bead-apposed (I_{bead}) and surrounding ($I_{no\ bead}$) regions of the plasma membrane is unchanged as His-β_2AR-cerulean fluorescence increases. (A) Confocal images of His-β_2AR-C, β_2AR-V, and RFP-mem fluorescence before and after formation of bead-induced domains. Scale bar, 5 μm. (B) Time course data from the same cell shown in A. Data points represent the mean ± S.D. of six bead-apposed and six surrounding ROIs, each 1 μm in diameter. Beads are added 30 seconds after the start of the experiment.



Supplemental Figure 7. Recruitment of untagged GABA(B) and metabotropic glutamate receptors to IMAC beads. Scale bars, 2 μm.



Supplemental Figure 8. The structure of BG-649-PEG-biotin.

Supplemental Movie Legends

Supplemental Movie 1. IMAC beads recruit His-β_2AR-venus. RFP-mem is used to normalize changes due to bead-induced membrane deformations. Frames were acquired every 10 seconds. The same cell is illustrated in Figure 1B.

Supplemental Movie 2. Recruitment of cytosolic Venus-arrestin3 to bead-associated His-β_2AR-cerulean after stimulation with 10 μM isoproterenol. Frames were acquired every 10 seconds. The same cell is illustrated in Figure 1C.

Supplemental Movie 3. Recruitment of cytosolic Nb80-GFP to bead-associated His-β_2AR-cerulean after stimulation with 10 μM isoproterenol. Frames were acquired every 5 seconds. The same cell is illustrated in Supplemental Figure 2.

Supplemental Movie 4. IMAC beads recruit His-β_2AR-cerulean but do not corecruit β_2AR-venus. Frames were acquired every 10 seconds. The same cell is illustrated in Supplemental Figure 6.

Supplemental Movie 5. Segregation of His-β_2AR-cerulean and SNAP-β_2AR-venus by mixed IMAC and sAV beads. Frames were acquired every 10 seconds. The same cell is illustrated in Figure 4A.