Supplementary figure 1:

Control experiments. (A) GIRK channel activation kinetics mediated via α_{2A}-YFP-AR were not significantly different from kinetics obtained using wild-type receptors (n=4-5, sample traces shown). (B) Colocalization of a membrane-anchored YFP (mYFP, left) with $G\alpha i_1\beta_1$ CFP-γ_2 (right) at the cell membrane (scale bar: 5μm). (C) Upon agonist stimulation of cells transfected with mYFP and $G\alpha i_1\beta_1$ CFP-γ_2, no change in FRET was detectable. (D) Averaged FRET traces±s.e.m of cells transfected with constitutive active $G\alpha i_1$ (Q204L, open circles, n=15) upon stimulation with 100μM NE. Averaged wild-type data from Figure 1D (filled circles, n=8) are shown again for comparison.

Supplementary figure 2:

Inactivated Gi proteins interact with receptors. After cotransfection of PTX-sensitive $G\alpha i_1$ subunits with α_{2A}-YFP-AR and $G\beta_1$ CFP-γ_2, cells were treated with 50ng/ml PTX for 4h. An agonist-induced increase in FRET was detected, which was not significantly different from non-PTX-treated cells (representative experiment out of 10).

Supplementary figure 3:

(A) Agonist-dependent FRET signal of cells transfected with α_{2A}-YFP and $G\alpha i_1$-CFP $\beta_1\gamma_2$. (B) Agonist-dependent FRET signal of cells transfected with α_{2A}-YFP and $G\alpha i_1$Cerulean –β_1 γ_2.

Supplementary frigure 4:

Average±s.e.m. of FRET traces of cells transfected with α_{2A}-YFP, $G\beta_1$ CFP-γ_2 and $G\alpha i_1$ ND (dark red; n=6) upon stimulation with 100μM NE. Averaged wild-type data from Figure 1D (light red, n=8) are shown again for comparison.