Supplementary Information

Functional selectivity of GPCR-directed drug action through location bias

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Supplementary Results

Supplementary Figures

a

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β1AR   29   AARLLVPASPPASLLPPASESPEPLSQQWTAGMGLLMALIVLLIVAGNVLVIVAIAKTPR  88
β2AR   8    SAFLLAPNGSHA----PDHDVTQERDEVWVVGMGIVMSLIVLAIVFGNVLVITAIAKFER  63
β1AR   89   LQTLTNLFIMSLASADLVMGLLVVPFGATIVVWGRWEYGSFFCELWTSVDVLCVTASIET  148
β2AR   64   LQTVTNYFITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIET  123
β1AR   149  LCVIALDRYLAITSPFRYQSLLTRARARGLVCTVWAISALVSFLPILMHWWRAESDEARR  208
β2AR   124  LCVIAVDRYFAITSPFKYQSLLTKNKARVIILMVWIVSGLISFLPIQMHWYRATHQEAIN  183
β1AR   209  CYNDPKCCDFVTNRAYAIASSVVSFYVPLCIMAFVYLRVFREAQKQVKKIDSCERRFLGG  268
β2AR   184  CYANETCCDFFTNQAYAIASSIVSFYVPLVIMVFVYSRVFQEAKRQLQKIDKSEGRF---  240
β1AR   269  PARPPSPSPSPVPAPAPPPGPPRPAAAAATAPLANGRAGK--RRPSRLVALREQKALKTL  326
β2AR   241  ----HVQNLSQVE--------------------QDGRTGHGLRRSSKF-CLKEHKALKTL  275
β1AR   327  GIIMGVFTLCWLPFFLANVVKAFHRELVPDRLFVFFNWLGYANSAFNPIIYCRSPDFRKA  386
β2AR   276  GIIMGTFTLCWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIA  335
β1AR   387  FQGLLCCAR---RAARRRHATHGDRPRASG  413
β2AR   336  FQELLCLRRSSLKAYGNGYSSNGNTGEQSG  365
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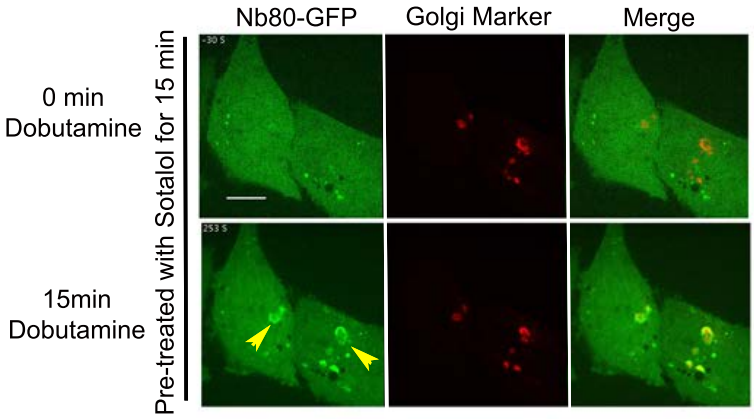
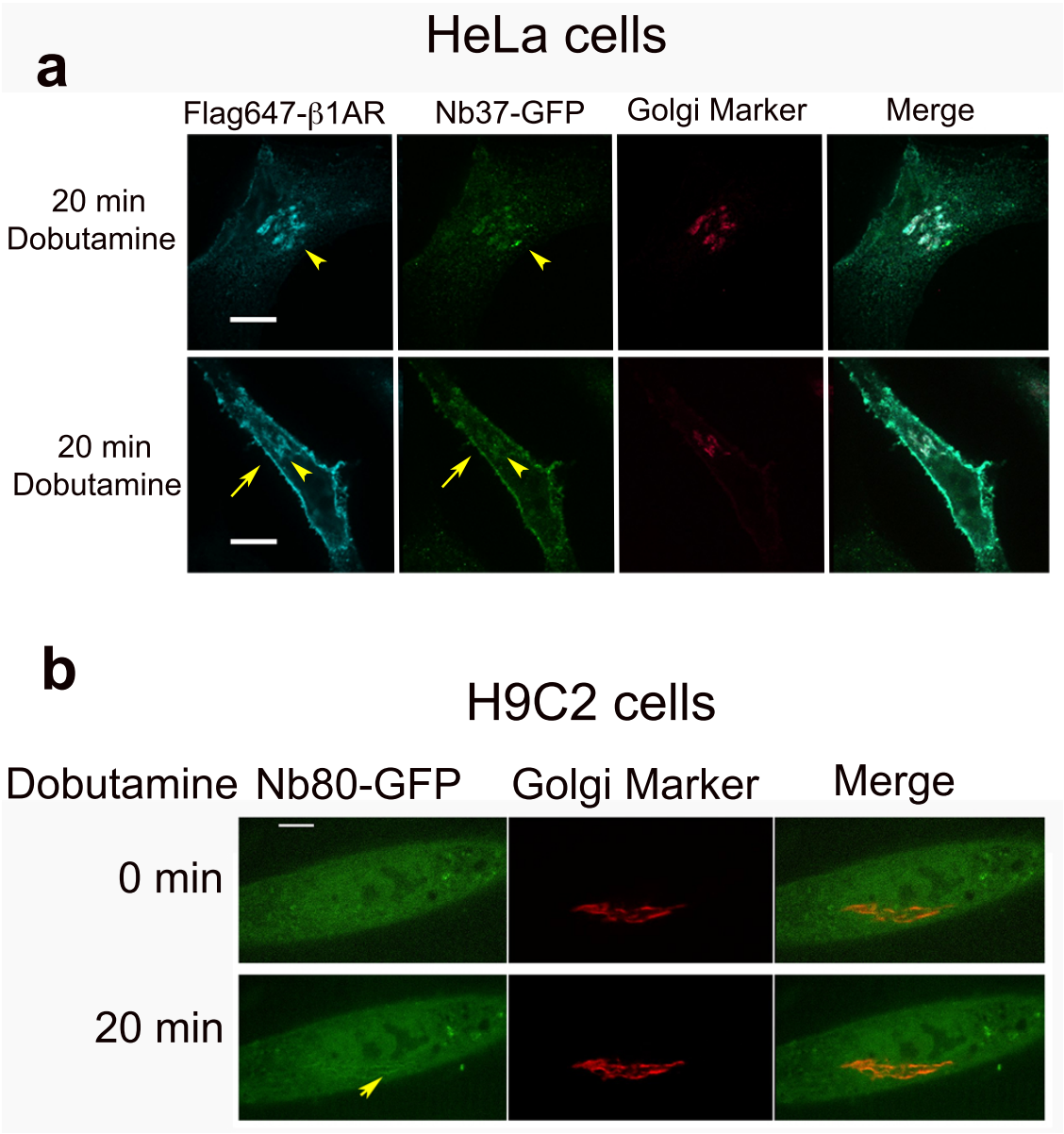
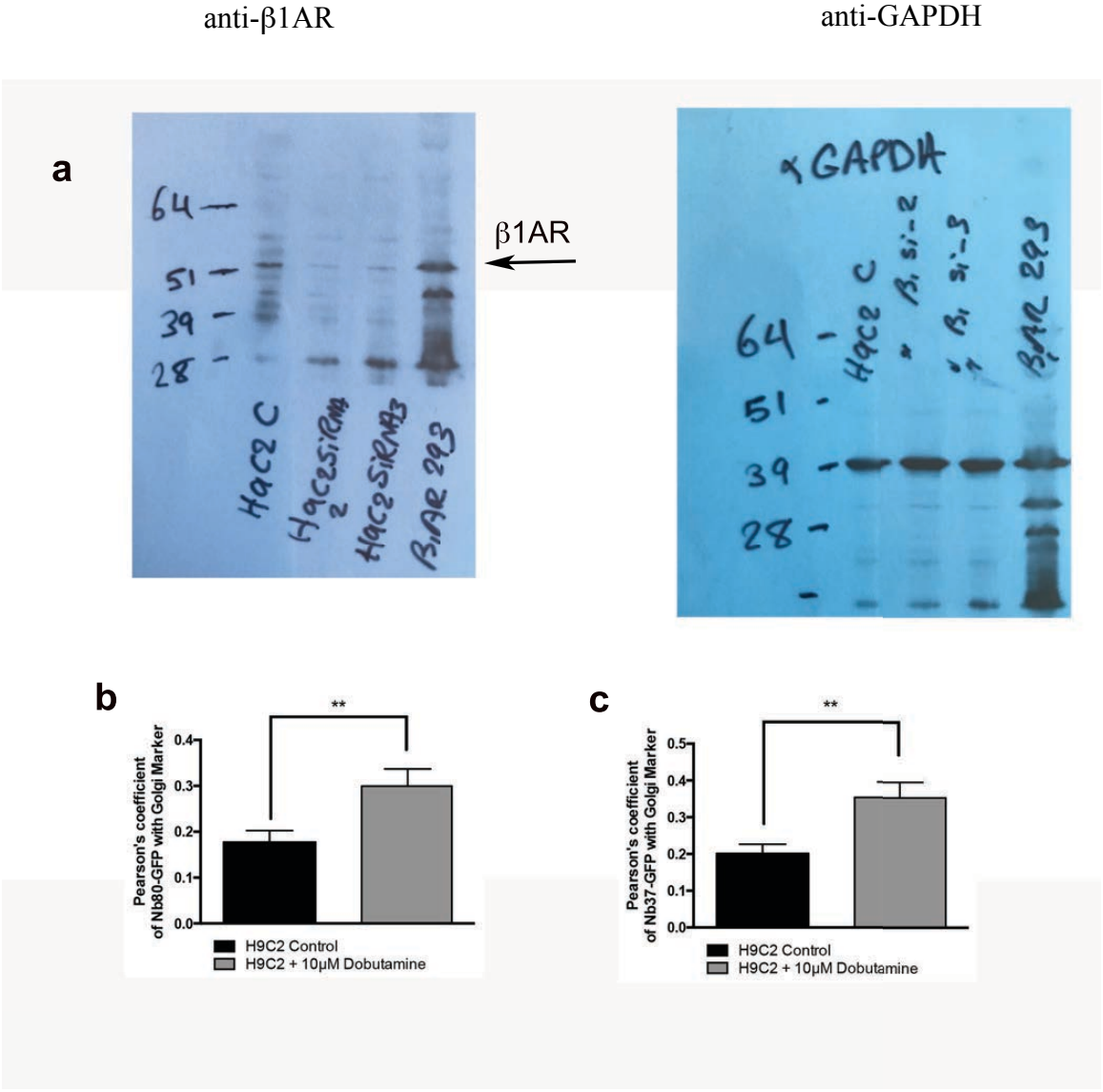
b



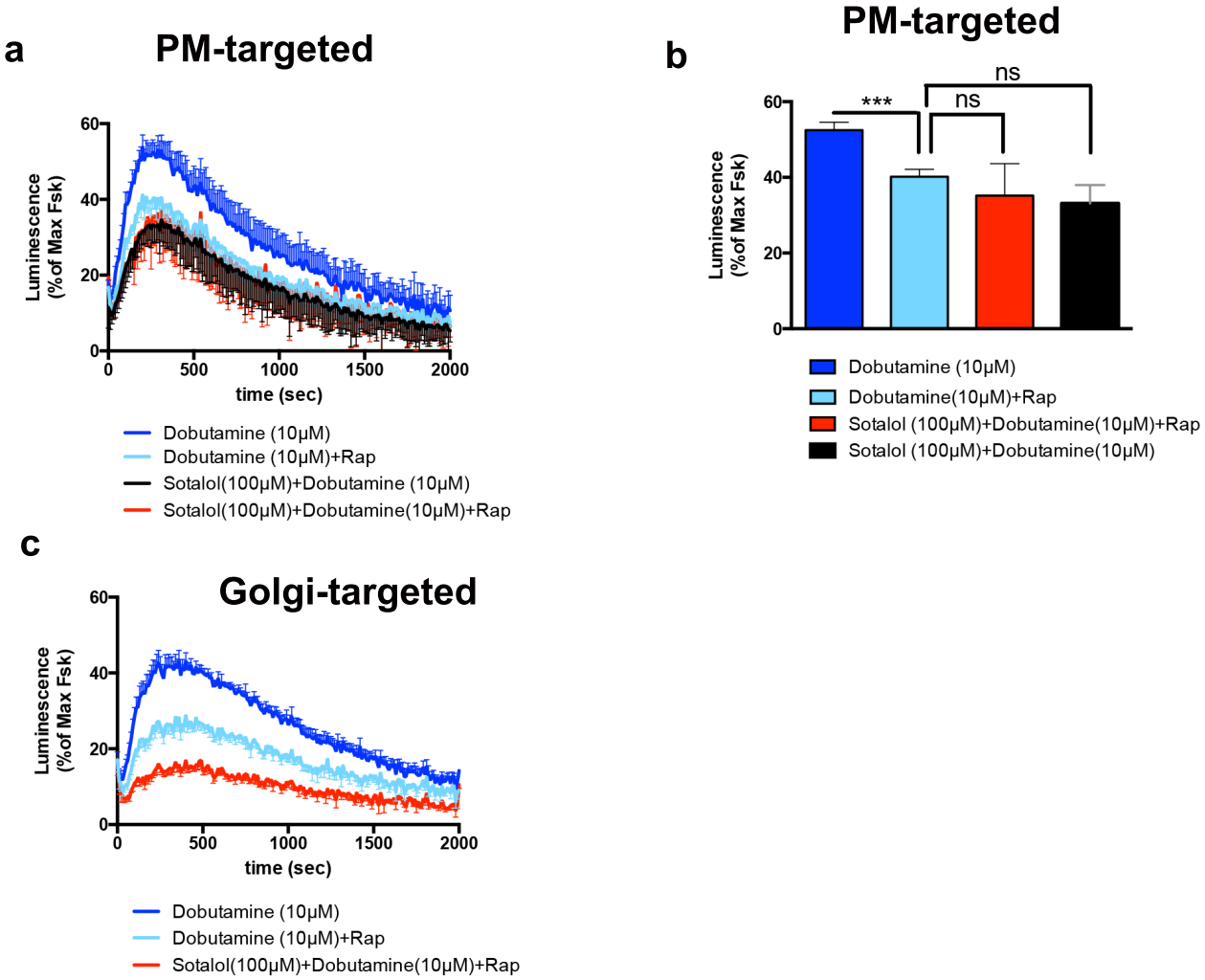
Supplementary Figure 1. a. Sequence alignment of human β_1AR and β_2AR (conserved Nb80-binding sites are highlighted in yellow). **b.** Confocal image frames of β_1AR expressing HeLa cells pre-treated with 100μM Sotalol for 15 min to block plasma membrane activation of β_1AR at indicated time points after 10μM dobutaine addition (n = 5 cells, Pearson's coefficient=0.46, 2 biological replicates, arrowheads indicate Golgi recruitment of Nb80-GFP). Scale bars, 10 μm.



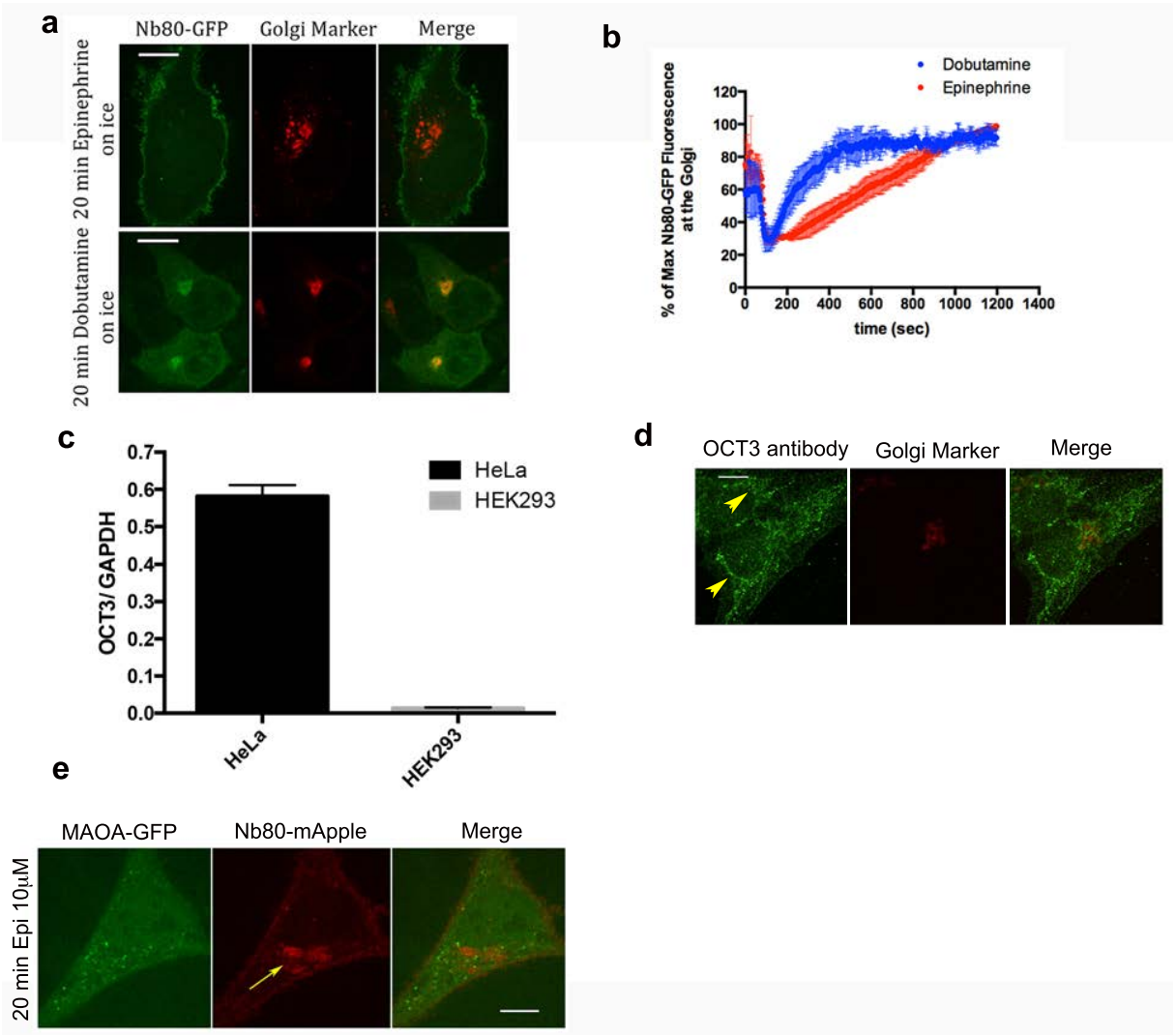
Supplementary Figure 2. a. Representative confocal images of HeLa cells expressing β_1AR, Nb37-GFP and Golgi marker (GalT-mRFP) (red) after 10μM dobutamine addition for 20min. Cells were treated with 0.05% saponin to reduce the cytoplasmic background and stained with M1 anti-Flag and anti-GFP antibodies (*n* = 12 cells, Pearson's coefficient=0.6, 3 biological replicates, arrow and arrowheads indicate plasma membrane and Golgi localization, respectively). **b.** Representative confocal image frames of H9C2 cells expressing Nb80-GFP and Golgi marker (GalT-mRFP) (red) before and after 10μM dobutamine addition (*n*= 8 cells, 3 biological replicates; arrowhead indicates Nb80 recruitment to the Golgi upon activation of endogenous β_1AR on the Golgi membrane). Scale bars, 10 μm.



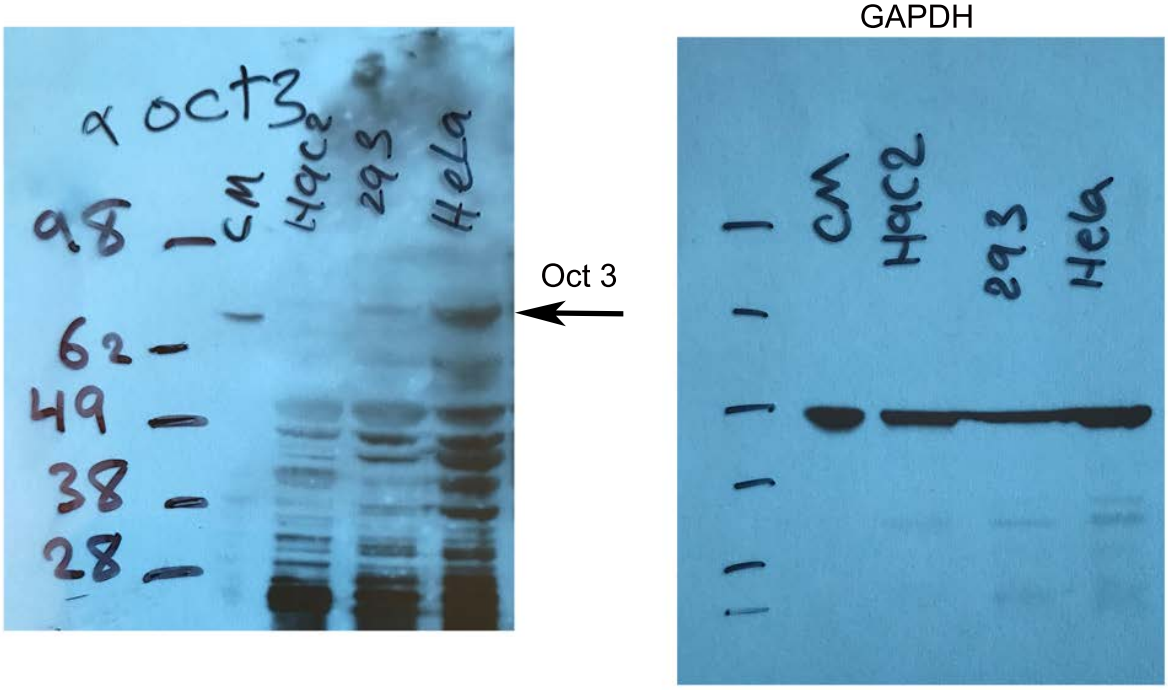
Supplementary Figure 3. a. Full images of Western blots shown in Figure 2c. **b.** Average Pearson's coefficient of Nb80-GFP (green) and the Golgi marker (red) before and after 10μM dobutamine treatment. **c.** Average Pearson's coefficient of Nb37-GFP (green) and the Golgi marker (red) before and after 10μM dobutamine treatment. (bars represents mean ± s.e.m., statistical analysis performed using two-tailed t-test p=0.0077 and p=0.0035, respectively).



Supplementary Figure 4. a. Time course and **b.** maximum forskolin-normalized β_1AR-mediated cAMP response in the absence (dark blue bar) or presence (light blue bar) of 15 min pre-treatment of 1 μM rapamycin, rapamycin + 100μM sotalol (red bar) or 100μM sotalol alone (black bar), in the plasma membrane-targeted Nb80 cells (bars represents mean ± s.e.m., $n = 4$ biological replicates, statistical analysis performed by two-tailed unpaired t-test, p=0.0008, 0.3477 and 0.0571, respectively). **c.** Time course of forskolin-normalized β_1AR-mediated cAMP response in the absence (dark blue bar) or presence (light blue bar) of 15 min pre-treatment of 1 μM rapamycin or rapamycin + 100μM sotalol (red bar) in the Golgi-targeted Nb80 cells ($n = 7$ biological replicates).



Supplementary Figure 5. a, Confocal image frames of β_1AR expressing HeLa cells upon 20 min incubation on ice showing plasma membrane only recruitment of Nb80–GFP (green) after 10 μM epinephrine (top) and plasma membrane and Golgi recruitment of Nb80–GFP after 10 μM dobutamine (bottom) addition (representative of $n = 15$ and 10 cells respectively, 4 biological replicates). **b**, Kinetics of Nb80-GFP recruitment to the Golgi membrane at 37°C degree after dobutamine (blue) or epinephrine (red) addition (n= 4cells each, 2 biological replicates), the initial 60-80% fluorescent is due to Nb80-GFP cytoplasmic background (see methods). **c.** RNA levels of EMT/OCT3 were analysed by reverse transcription and qPCR. Data normalized to total GAPDH (bars represents mean ± s.e.m., $n = 3$ biological replicates). **d.** Representative image of HeLa cells treated with 0.05% saponin and stained with anti-SLC22A3 antibody (anti-EMT/OCT3) (yellow arrowheads indicate peri-nuclear localization) ($n = 12$ cells, 2 biological replicates). e**.** Representative confocal image of HeLa cells co-expressing MAO-A-GFP, β_1AR and Nb80-mApple after 20 min epinephrine (10μM) treatment (arrow indicates Golgi localization) ($n = 12$ cells, 2 biological replicates). Scale bars, 10 μm.



anti-OCT3

anti-GAPDH

Supplementary Figure 6. Full images of Western blots shown in Figure 4c

Supplementary Video 1- Confocal image series of β_1AR expressing HeLa cells with Nb80-GFP (green) and the Golgi marker (red), incubated with 10μM epinephrine. The time between each frame is 3 sec (t=0 corresponds to the time of agonist addition).

Supplementary Video 2- Confocal image series of β_1AR expressing HeLa cells with Nb80-GFP (green) and Golgi marker (red), incubated with 10μM dobutamine. The time between each frame is 3 sec (t=0 corresponds to the time of agonist addition).

Supplementary Video 3 and 4- Confocal image series of β_1AR (cyan), Nb80-GFP (green) and the Golgi marker (red) expressing HeLa cells incubated with 10μM isoproterenol. The time between each frame is 3 sec (t=0 corresponds to the time of agonist addition).

Supplementary Video 5- Reversal of Nb80-GFP plasma membrane and the Golgi recruitment after addition of 10μM metoprolol. The time between each frame is 3 sec (t=0 corresponds to the time of antagonist addition).

Supplementary Video 6- Reversal of Nb80-GFP plasma membrane recruitment after addition of 100μM sotalol. The time between each frame is 3 sec (t=0 corresponds to the time of antagonist addition).

Supplementary Video 7- PKA Cα-YFP translocation from the Golgi membrane to the cytoplasm in β_1AR expressing HeLa cells upon addition of 10μM dobutamine. The time between each frame is 3 sec (t=0 corresponds to the time of dobutamine addition).

Supplementary Video 8- Inhibition of PKA Cα-YFP translocation from the Golgi membrane to the cytoplasm in β_1AR expressing HeLa cells pre-treated with 10μM metoprolol for 15 min and upon addition of 10μM dobutamine. The time between each frame is 3 sec (t=0 corresponds to the time of dobutamine addition).

Supplementary Video 9- Delayed PKA Cα-YFP translocation from the Golgi membrane to the cytoplasm in β_1AR expressing HeLa cells pre-treated with 5mM sotalol for 15 min and upon addition of 10μM dobutamine. The time between each frame is 3 sec (t=0 corresponds to the time of dobutamine addition).