SUPPLEMENTAL DATA

Figure S1. Brefeldin A treatment (BFA) destroys the struction of the trans-Golgi network. HA-β1AR cells were surface-labeled with HA mAb were incubated with ISO and BFA (5 μg/ml) at 37 °C, fixed at 4h and immunostained for β1AR (red) and TGN46 (green).

Figure S2. Brefeldin A treatment does not influence the endocytosis of β1AR. Surface-labeled HA-β1AR cells were incubated at 37 °C with ISO and BFA or vehicle and fixed at 20 min. Surface and endocytosed receptors were visualized with goat anti-rabbit Alexa-594 (red). The nuclei were detected with DAPI (blue).

Figure S3. Brefeldin A treatment neither impacts the distribution of EEA-1 nor alters the colocalization of endocytosis of β1AR with EEA-1. Surface-labeled HA-β1AR cells were incubated at 37 °C with ISO and BFA or vehicle, fixed at 4h and immunostained for β1AR (red) and EEA-1 (green). Colocalization of HA-β1AR with EEA-1 was shown in yellow in the merged images. The bottom panels show quantitative colocalization analysis using linescan.

Figure S4. Brefeldin A treatment blocks the entry of endocytosed β1AR into the late endosomes. Surface-labeled HA-β1AR cells were incubated at 37 °C with ISO and BFA or vehicle, fixed at 4h and immunostained for β1AR (red) and Rab-7 (green). Colocalization of HA-β1AR with Rab-7 was shown in yellow in the merged images. The bottom panels show quantitative colocalization analysis using linescan.

Figure S5. Endocytosed β1AR does not accumulate in the endoplasmic reticulum after Brefeldin A treatment. Surface-labeled HA-β1AR cells were incubated at 37 °C with ISO and BFA or vehicle, fixed at 4h and immunostained for β1AR (red) and calnexin (green). Colocalization of HA-β1AR with Rab-7 was shown in yellow in the merged images. The bottom panels show quantitative colocalization analysis using linescan.

Figure S6. Co-localization of β1AR with GFP-arrestin-3 in response to ISO stimulation. HA-β1AR cells were transfected with cDNA encoding GFP-arrestin-3 and surface-labeled with rabbit HA antibody. Cells were incubated with ISO at 37 °C, fixed at indicated time points and immunostained for surface and endocytosed β1AR (red). Note: ISO stimulation triggers GFP-arrestin-3 forming punctures before β1AR endocytosis at 2 min. Images were selected from 120 cells in 3 independent experiments.

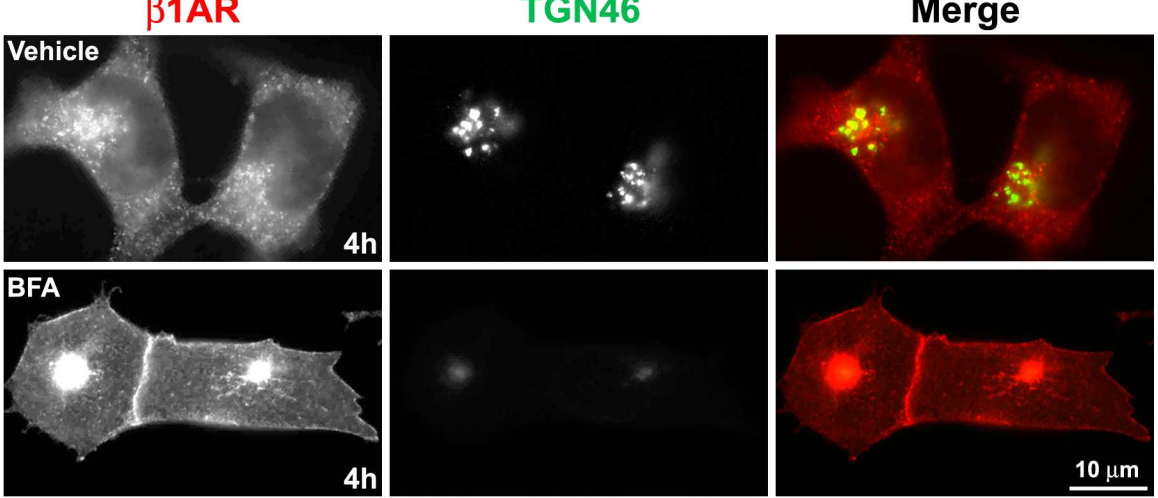

Fig. S1

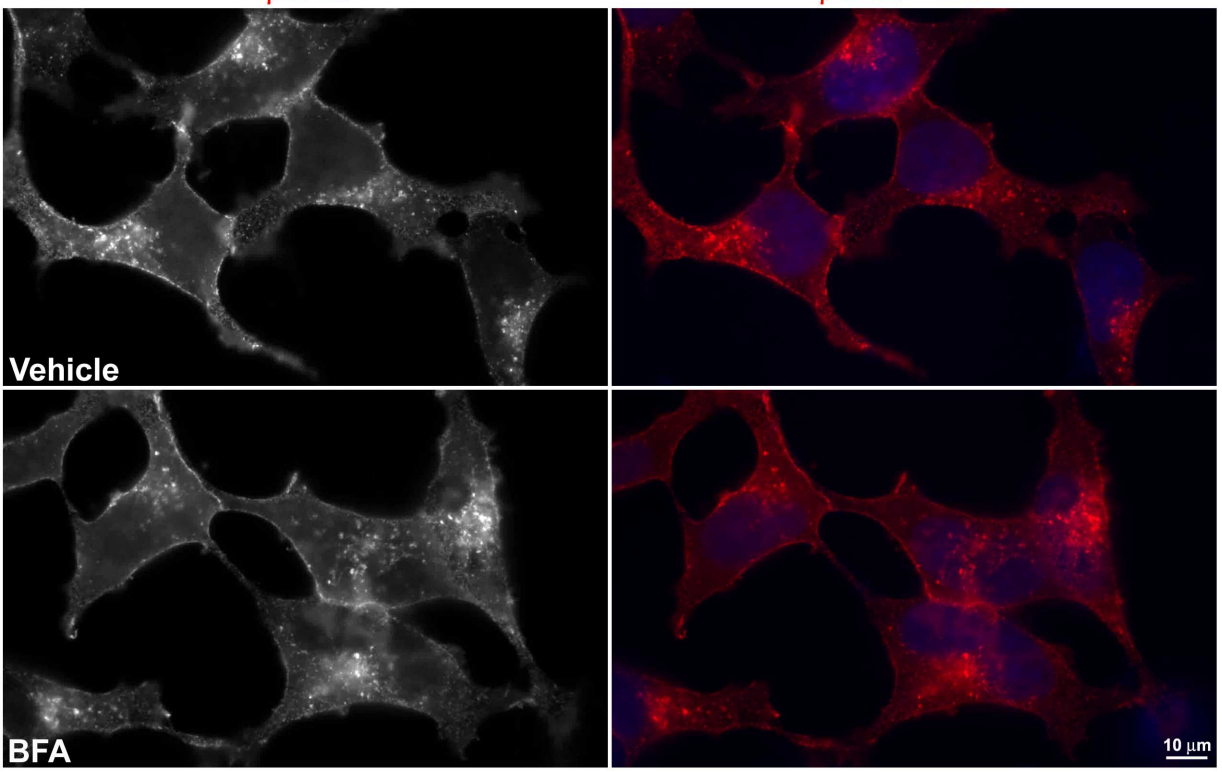

Fig. S2

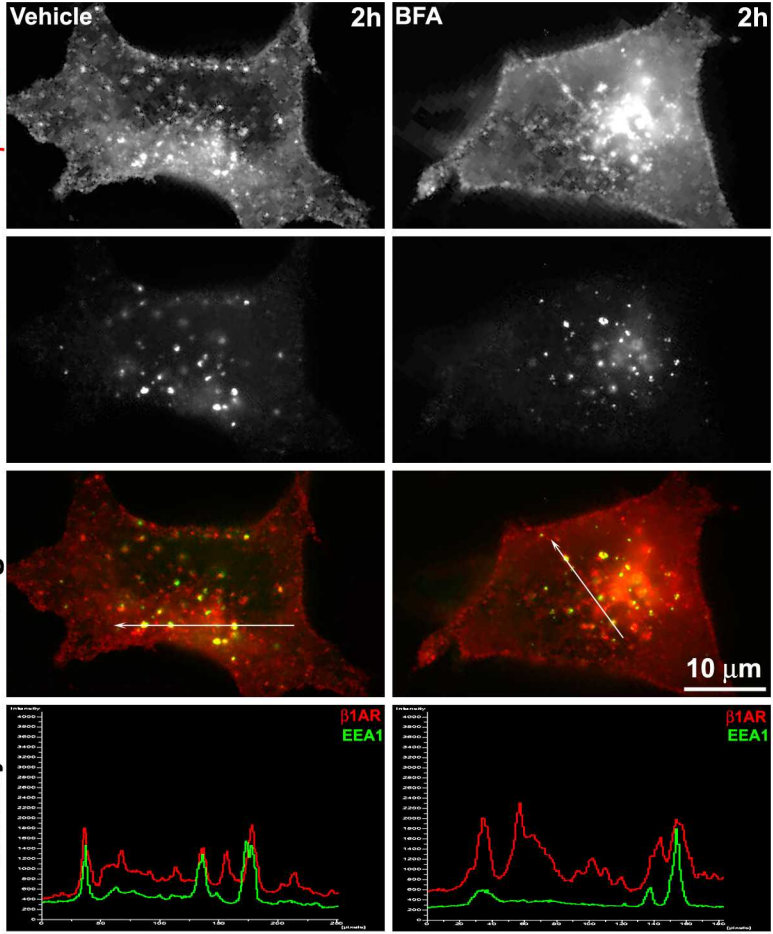
Vehicle
2h
BFA
2h
β1AR
EEA1
Merge
10 μm
Intensity
β1AR
EEA1
Distance

Fig. S3

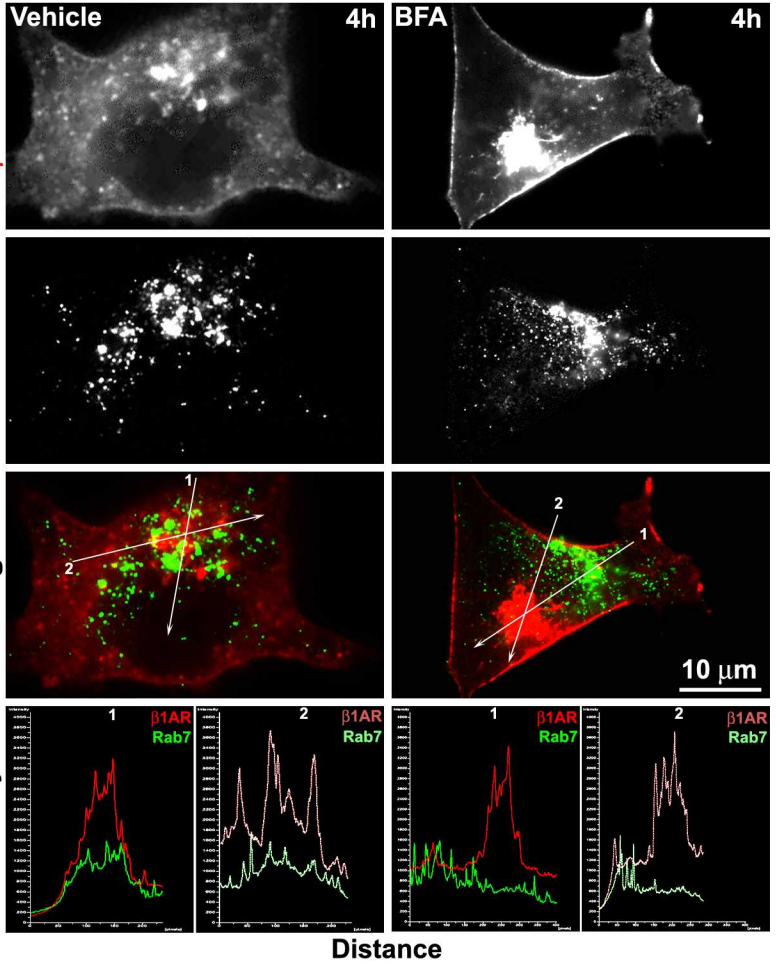
Vehicle
4h
BFA
4h
β1AR
Rab7
Merge
1
2
10 μm
Intensity
β1AR
Rab7
Distance

Fig. S4

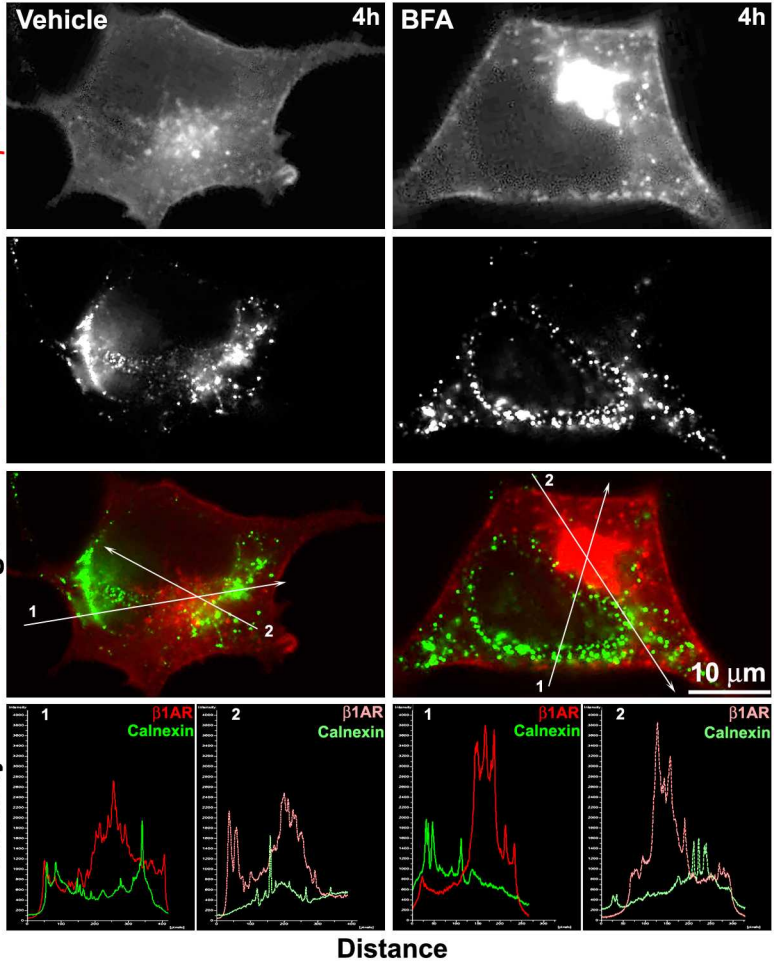
Vehicle
4h
BFA
4h
β1AR
Calnexin
Merge
1
2
2
1
10 μm
Intensity
1
β1AR
Calnexin
2
β1AR
Calnexin
1
β1AR
Calnexin
2
β1AR
Calnexin
Distance

Fig. S5

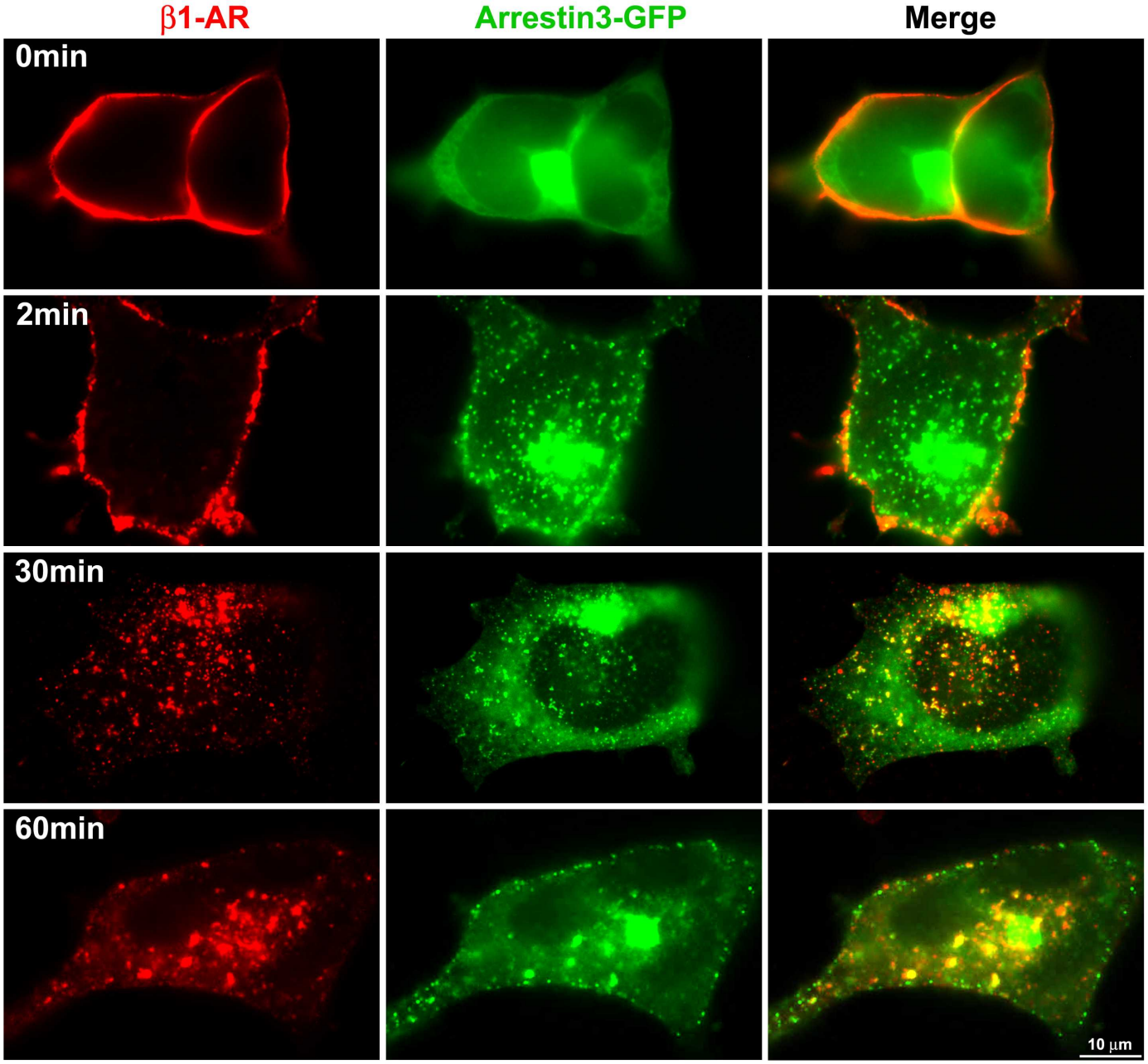

Fig. S6