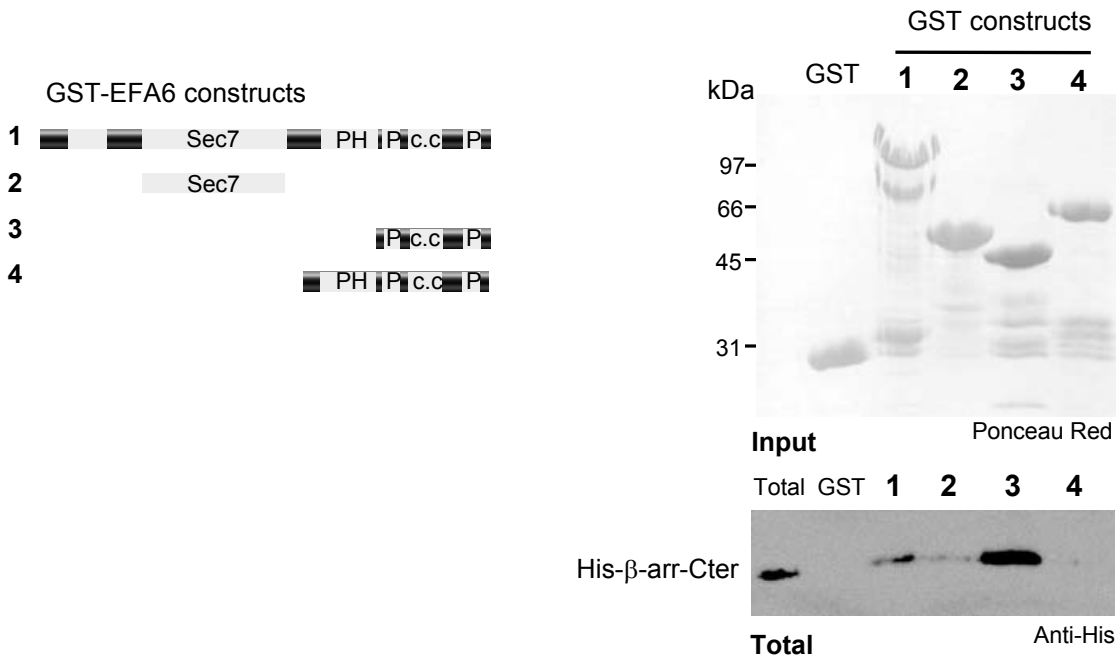

Fig. S1. The C-terminal domain of EFA6 is responsible for β-arrestin interaction. GST pull-down of purified His-tagged C-terminal domain of β-arrestin1 (1 μM) with different constructs of EFA6 fused to GST protein (2 μM).

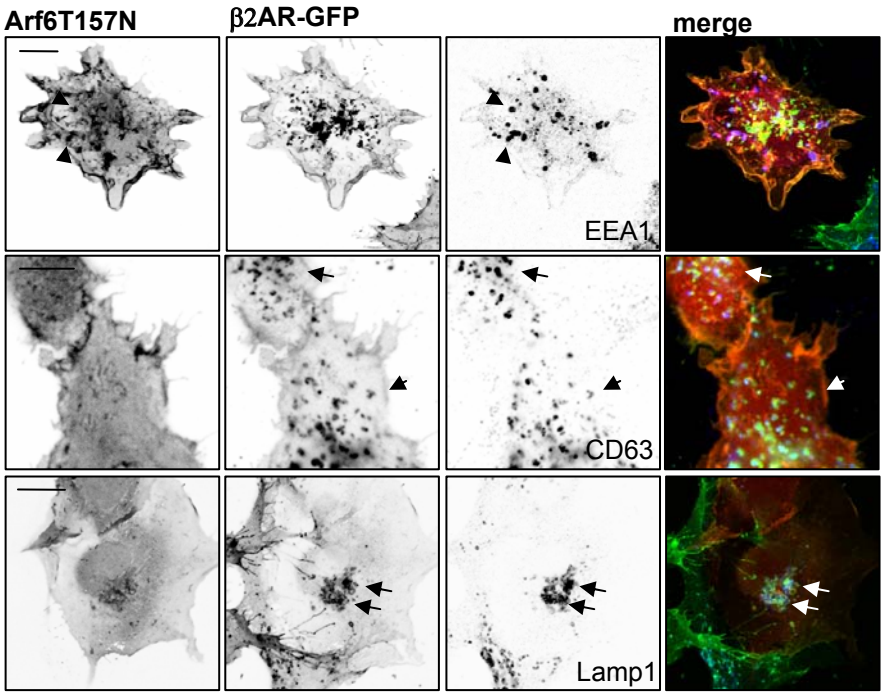

Fig. S2. Activated Arf6 induces the accumulation of the β2AR into the degradative pathway. HEK293 cells stably expressing β2AR-GFP were transiently transfected with plasmid encoding mcherry-tagged Arf6T157N. After 24 h, cells were fixed and stained with anti-EEA1 (as a marker for early endosomes), anti-CD63 (as a marker for both late endosomes and lysosomes) and anti-Lamp2 (as a marker for lysosomes) antibodies. Scale bars, 10 μm.

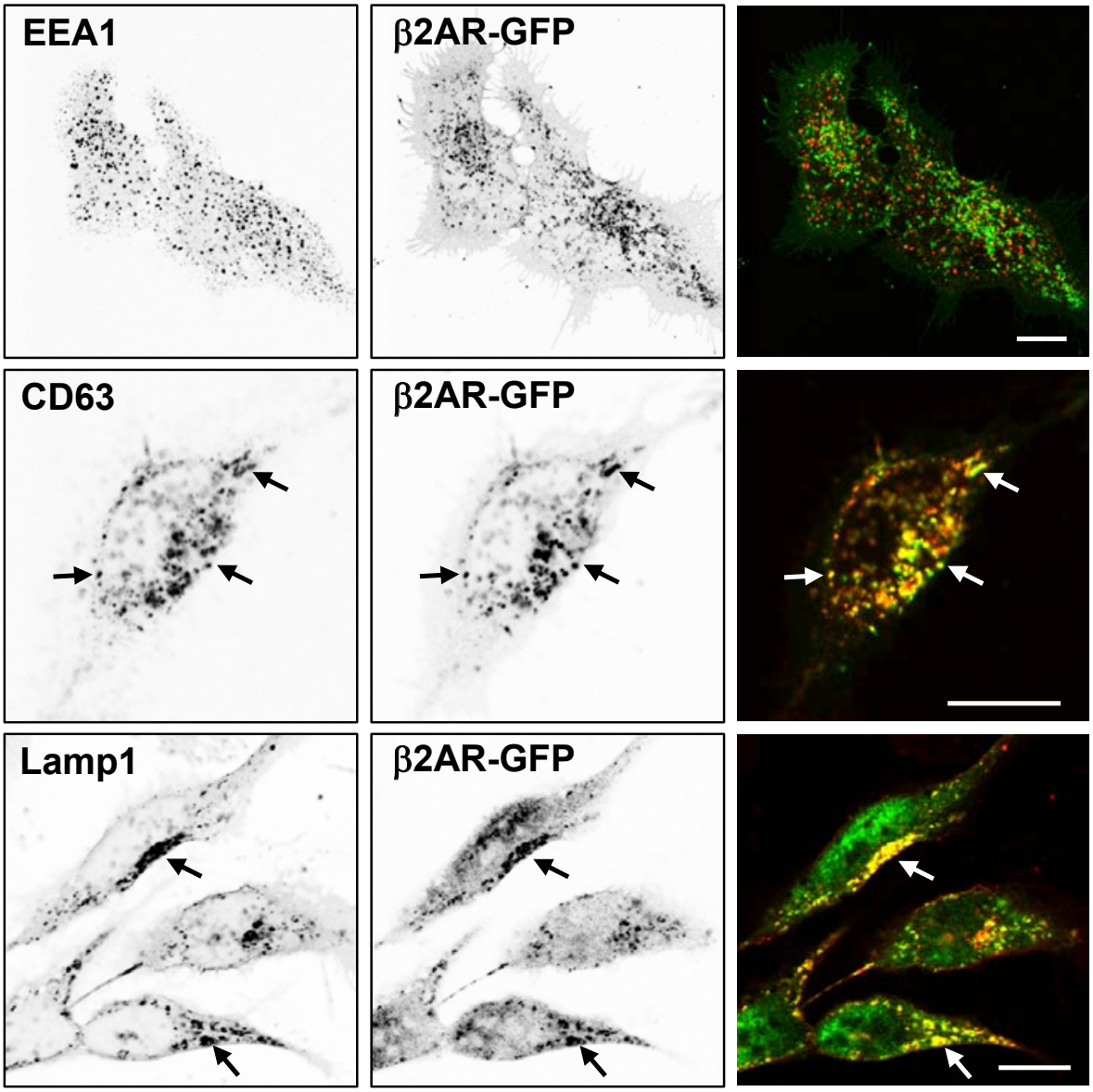

Fig. S3. Prolonged isoproterenol treatment induces the accumulation of β2AR into the degradative pathway. HEK293 cells stably expressing β2AR-GFP were treated with isoproterenol for 3h. Cells were fixed and stained with anti-EEA1 (as a marker for early endosomes), anti-CD63 (as a marker for both late endosomes and lysosomes) and anti-Lamp2 (as a marker for lysosomes) antibodies. Scale bars, 10 μm.