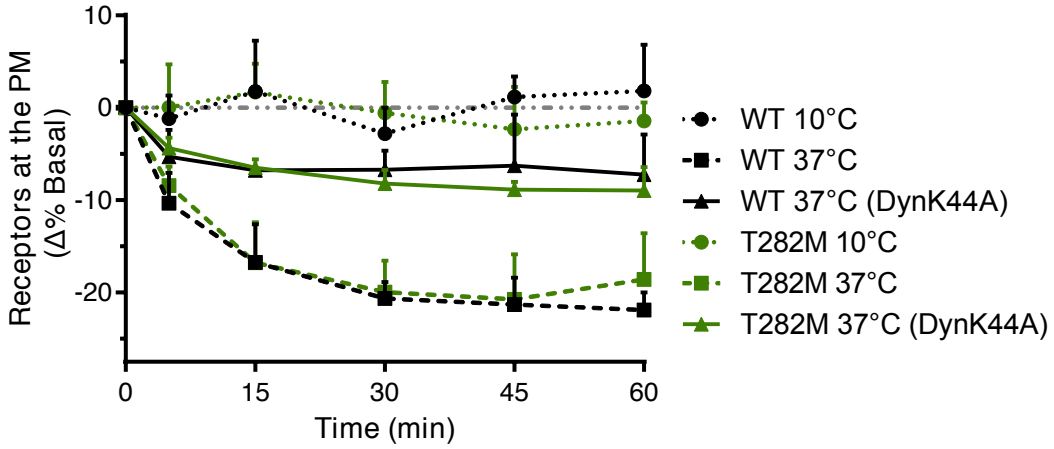
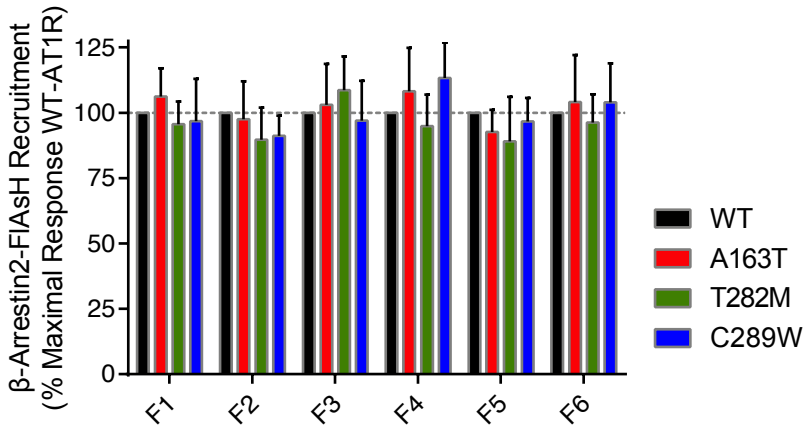
Figure S1



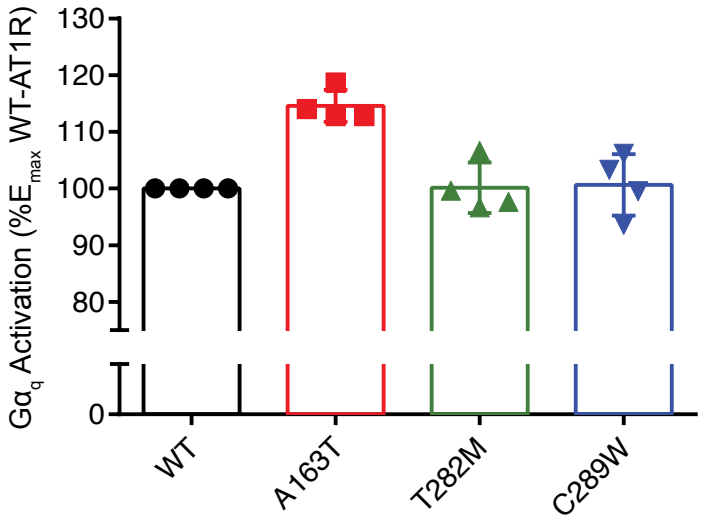
Supplemental Figure 1. Effects of temperature and inhibition of CCV internalization on WT and T282M removal from the PM. HEK293 cells were transfected with RlucII-tagged WT or mutant AT1R, PM-anchored rGFP-CAAX, and with or without dominant-negative inhibitor of dynamin-mediated internalization, DynK44A. Cells were stimulated with 1 μM AngII concentrations for up to 1 h at 10°C or 37°C and receptor removal from the PM was assessed by BRET as described in the Material and Methods. The percentage change in basal BRET is reported, and data are represented as means ± s.d. of triplicates from 3–6 independent experiments.

Figure S2



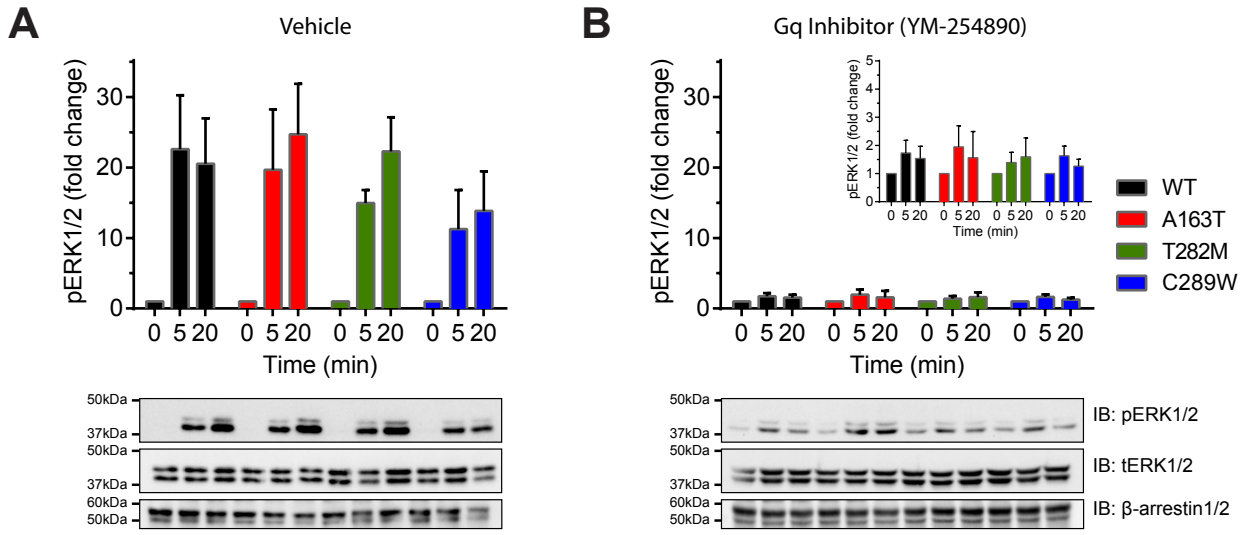
Supplemental Figure 2. AngII-induced β-arrestin2-FlAsH reporter recruitment to receptors at the PM. HEK293 cells were transfected with WT or mutant AT1R, rGFP-CAAX, and Rluc-β-arrestin2-FlAsH reporters (F1–F6). Cells were stimulated with 1 μM AngII for 2 min, and the BRET change was normalized and reported as percentage of WT maximal response for each sensor. Data are represented as means ± s.d. of triplicates from 4 independent experiments.

Figure S3



Supplemental Figure 3. AngII-mediated $G\alpha_q$ activation by WT and mutant AT1R. HEK293 cells were transfected with WT or mutant AT1R and $G\alpha_q$ BRET biosensor. Cells were stimulated with 1 μM AngII and measurements were taken at 2 min. BRET change was normalized to that of WT and reported as percentage of WT response. Data are represented as means ± s.d. of triplicates from 4 independent experiments.

Figure S4



Supplemental Figure 4. Role of $G\alpha_{q/11}$ in ERK1/2 activation by WT and mutant receptors. A and B, HEK293 cells were transfected with WT or mutant AT1R and pretreated with vehicle (A) or a selective $G\alpha_{q/11}$ inhibitor YM-254890 (B) to determine the contribution of $G\alpha_{q/11}$- vs. β-arrestin-mediated ERK1/2 activation (as compared to Fig. 6). Cells were stimulated with 1 μM AngII for 0, 5, or 20 min and then lysed and analyzed via western blotting with antibodies specific for phospho-ERK, total-ERK, and β-arrestin1/2. Quantifications are represented as means ± s.d. from 4–6 independent experiments, and presented on the same scales (A and B). Top inset (B) represents data of the main body of the figure, but on a reduced y-axis scale.