Figure S1.

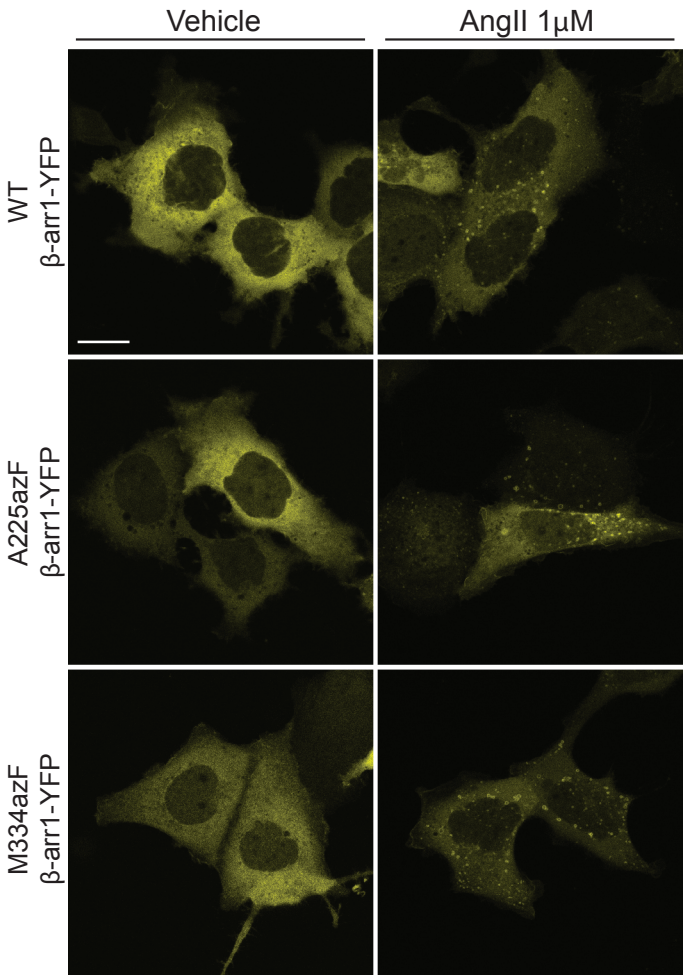

Figure S1. Assessment of β-arrestin internalization with azF-incorporated mutants following AngII stimulation. Confocal images of HEK293SL cells expressing β-arr1-YFP along with WT-AT1R or amber mutants, in the presence of 0.5 mM azF. Representative images before and after AngII stimulation of three independent experiments are shown. Scale bar, 20 μm.

Figure S2.

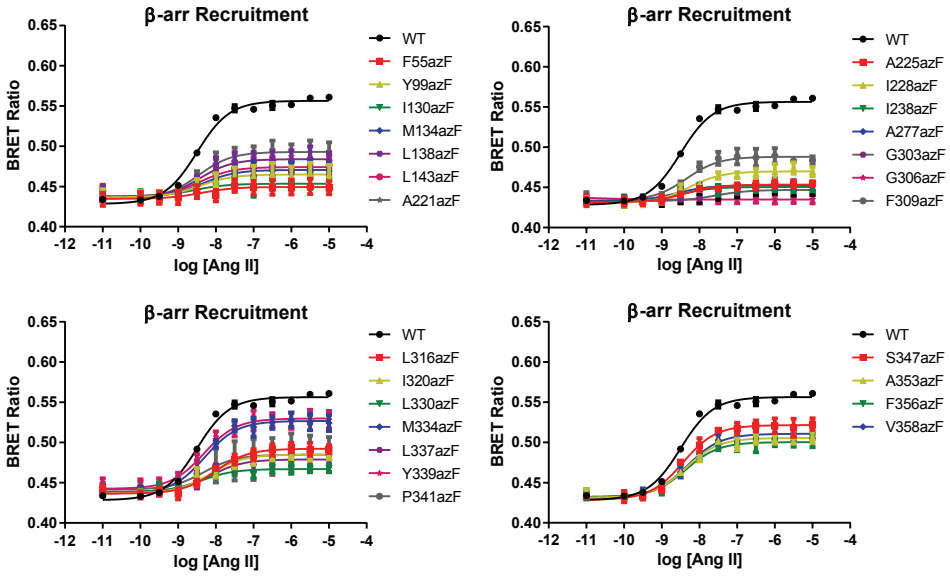

Figure S2. BRET concentration-response curves for AngII-mediated β-arrestin recruitment to azF-AT1R mutants. HEK293T cells transiently expressing RlucII-tagged receptor (WT-AT1R or amber mutants) along with β-arr1-YFP in the presence of 0.5 mM azF were stimulated with increasing concentrations of AngII. BRET measurements were recorded and expressed as BRET ratio. Data are means ± SEM of three independent experiments.

Figure S3.

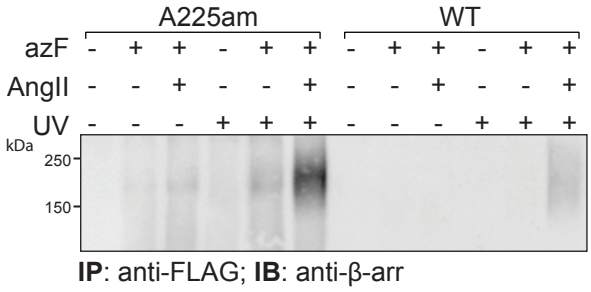

Figure S3. Comparison of β-arrestin photocross-linking to WT-AT1R and A225amb-AT1R. HEK293T cells transiently expressing WT-AT1R and mutant A225amb (internal control) in the absence (–) and presence (+) of 0.5 mM azF were incubated with vehicle (-) or 1 μM AngII (+), followed by exposure (+) or not (-) to UV light for 20 min at 4 °C. Total cell lysates were then immunoprecipitated (IP) using an anti-FLAG antibody to isolate AT1Rs, and products were resolved by SDS-PAGE. Cross-linked complexes were detected with an anti-β-arr1 antibody (immunoblot, IB). Shown are representative blots from two independent experiments.

Figure S4.

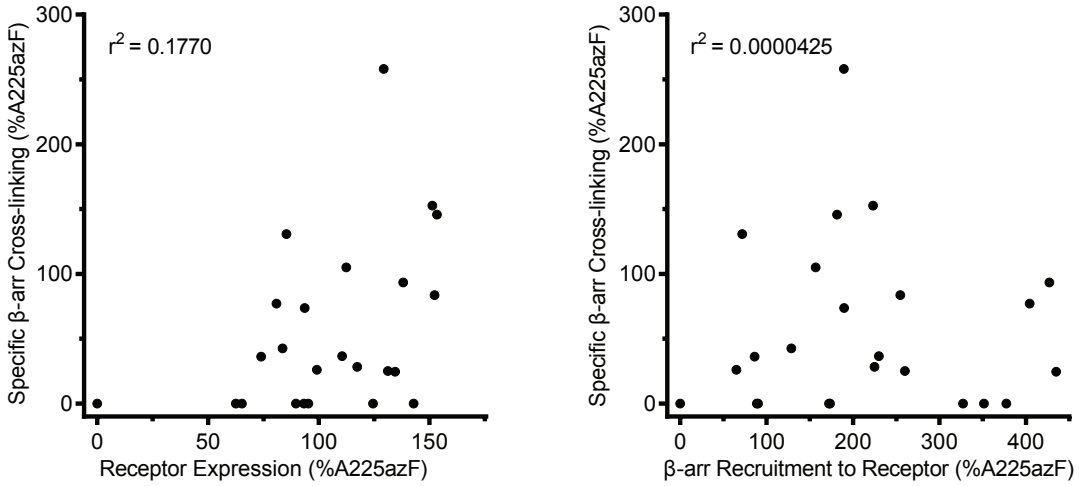

Figure S4. **Correlation analyses between cross-linked AT1R-β-arrestin complexes vs. the expression of azF-incorporated mutant receptors, and their ability to bind β-arrestin**. Data from Fig. 2C were plotted as scatter plot graphs, and linear regression analysis was used to determine the correlation (r2).