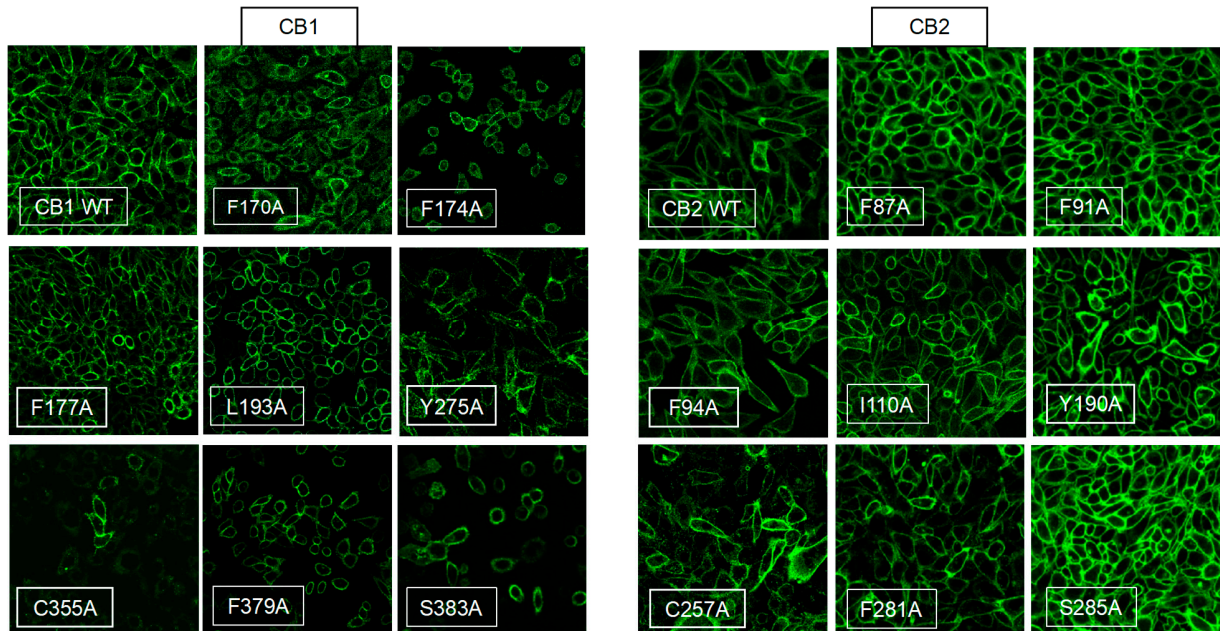


**Table S1. Primers**

			<b>Forward</b>	<b>Reverse</b>
<b>CB1</b>	F170A	Sigma	5' – GTGTCATTGCTGTCTACAGC – 3'	5' – TCCCCAGGAGGTCTG – 3'
	F174A	Sigma	5' – CTACAGCGCCATTGACTTC – 3'	5' – ACAAAAATGACTCTCCCAG – 3'
	F177A	Sigma	5' – CTTTCATTGACGCCACGTGTT – 3'	5' – CTGTAGACAAAAATGACTCTCCC – 3'
	L193A	Sigma	5' – TCTGTTCAAAGCGGGTGGGGT – 3'	5' – AACACGTTGCGGCTATCTTTGC – 3'
	Y275A	Sigma	5' – GATGAAACCGCCCTGATGTTC – 3'	5' – AATGTGTGGGAAAATGTCTG – 3'
	C355A	Sigma	5' – GTTGATCATCGCCTGGGGCCCTCTG – 3'	5' – ACCACCAGGATCAGGACC – 3'
	S383A	Sigma	5' – TGCGCTATGCTCTGCCT – 3'	5' – GAATGCAAACACCGTCTTAATGAGC – 3'
	F379A	Sigma	5' – GTGGCTGCATTCTGC – 3'	5' – CGTCTTAATGAGCTTGTTTCATC – 3'
<b>CB2</b>	F87A	Sigma	5' – GTCGCTGCATGCAGC – 3'	5' – CACTGGCCAGGAAGTC – 3'
	F91A	Sigma	5' – CAGCGCTGTGAATTTCCATG – 3'	5' – CATGCAAAGACCACACTGGC – 3'
	F94A	Sigma	5' – GAATGCCCATGTTTTCCATGG – 3'	5' – ACAAAGCTGCATGCAAAGACC – 3'
	I110A	GeneScript		
	Y190A	Sigma	5' – GACGCCCTGCTGAGCT – 3'	5' – ATTGGGGATCAGTGGGAAAAG – 3'
	C257A	Sigma	5' – GCTCCTCATCgcTTGGTTCCCAGTGCTG – 3'	5' – ACAGCCAACACTAGCCCT – 3'
	F281A	GeneScript		
	S285A	GeneScript		

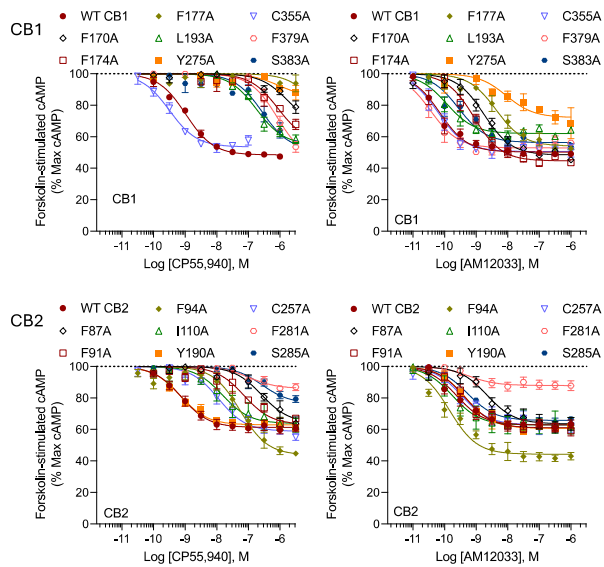
**Supplemental Figure S1.**



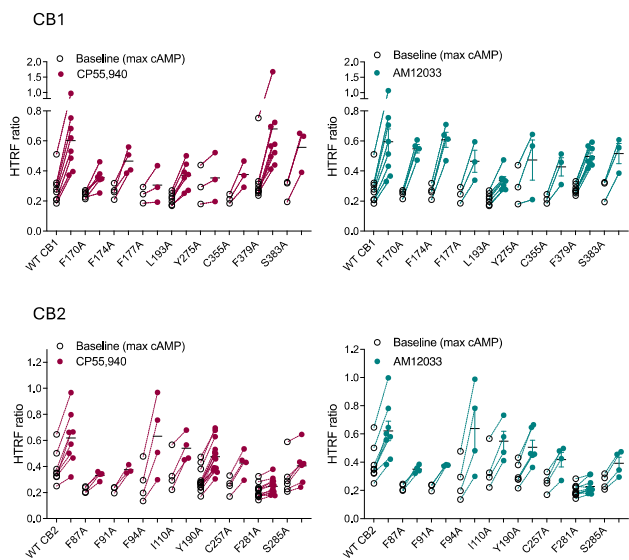
**Supplemental Figure S1. Nonpermeabilized CHO-K1 cells stained with DyLight 488 HA-antibody.** Confocal images were taken at 40x or 60x and were imaged following flow cytometry isolation of surface receptor expressing cells (N-terminally HA tagged receptors).

## Supplemental Figure S2.

A. Data presented as % maximum cAMP produced

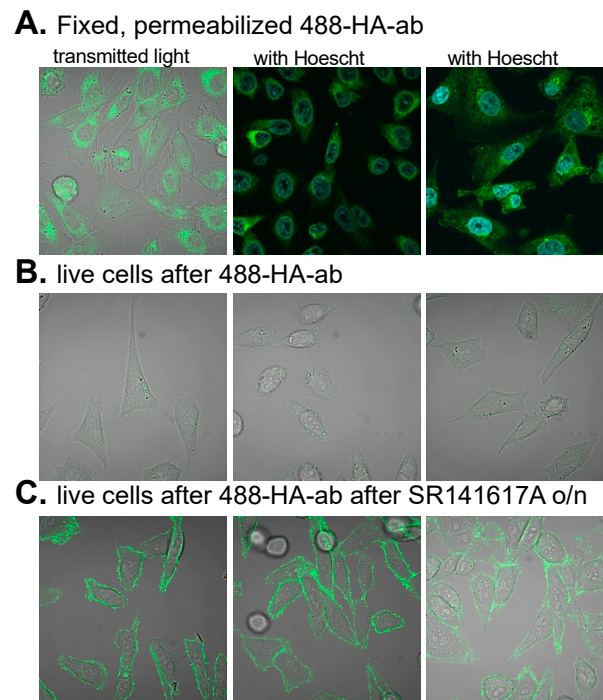


B. HTRF ratios for vehicle + max compound concentration



**Supplemental Figure S2 to accompany Figures 1-3 and 5.** Data in the manuscript presented as **A**). % of maximal cAMP stimulated in the transfected CHO-K1 cells (mean with SEM). For the WT CB1, CP55,940 and AM12033 decrease forskolin-stimulated cAMP levels by ~50% (%cAMP remaining: CP:  $48.4 \pm 0.9$ ; AM12033:  $50.2 \pm 1.0$  % mean with SEM). For the WT CB2, CP55,940 and AM12033 decrease forskolin-stimulated cAMP levels by ~40 % (% cAMP remaining: CP:  $61.3 \pm 1.01$ ; AM12033:  $62.9 \pm 1.1$  % mean with SEM); the F94A2.64 decreases cAMP levels by ~60% (% cAMP remaining: CP:  $43.7 \pm 2.2$ ; AM12033:  $44.2 \pm 1.4$  % mean with SEM, three parameter nonlinear regression with top constrained to 100%, GraphPad Prism). **B**). Raw HTRF ratios showing the values of vehicle treatment (maximum forskolin stimulated cAMP levels) and the maximum dose tested for each compound in (A). Ordinary one-way ANOVA comparing Baselines (max cAMP) between WT and each mutant indicate that for CB1, none of the mutants differed from WT. For CB2, F87A ( $p < 0.01$ ) and F281A ( $p < 0.001$ ) had higher forskolin-stimulated cAMP levels than WT (lower HTRF values).

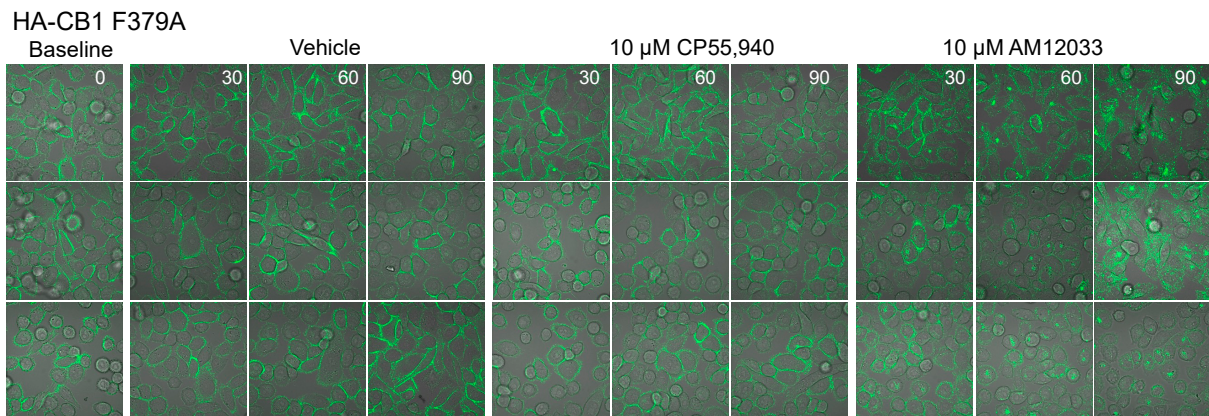
### Supplemental Figure S3.



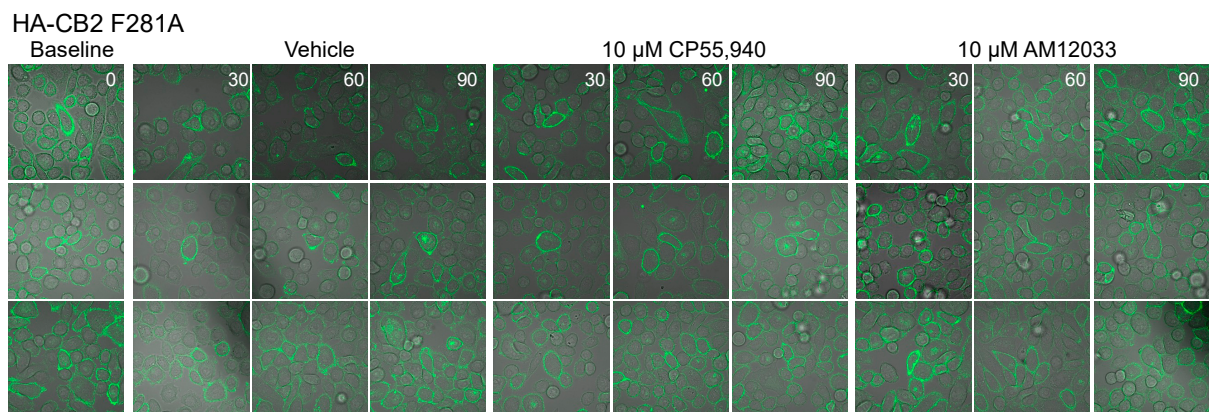
**Supplemental Figure S3 to accompany Figure 4.** CHO-K1 cell surface expression profile of N-terminally tagged HA-CB1 Y275A<sup>5,39</sup> in representative images of 3 independent studies. After several passages, 488-HA antibody was observed only upon fixation and permeabilization of cells (**A**) shown with transmitted light and following Hoescht staining of nuclei. No cell surface staining of live cells were observed with the 488-HA antibody (**B**). Overnight treatment with 10  $\mu$ M SR141617A restores surface labeling of HA-CB1 Y275A<sup>5,39</sup> in live cells (**C**).

## Supplemental Figure S4.

**A.**



**B.**



**Supplemental Figure S4 to accompany Figure 5.** Internalization profiles of the N-terminally tagged F7.35A mutant CB receptors in response to Vehicle, 10  $\mu$ M CP55940 or 10  $\mu$ M AM12033 over time in live CHO-K1 cells. Cell surface labeling is evident under all conditions for **(A)** CB1 F379A<sup>7.35</sup> while AM12033 leads to a loss of surface labeling for **(B)** CB2 F281A<sup>7.35</sup>. Baseline images were taken after fluorescent 488-HA antibody staining of live cells and prior to addition of indicated treatment. Replicate data from 3 separate transfections representing baseline, Vehicle, CP55,940 or AM12033 treatment over time (indicated in minutes as 0, 30, 60, or 90 min; 100X objective for all images).