Supplementary information for: “Agonist binding and desensitization of the mu-opioid receptor is modulated by phosphorylation of the C-terminal tail domain.”
Birdsong W.T., Arttamangkul S., Bunzo W.J.R., Williams J.T., Molecular Pharmacology, 2015

Figure S1

A) DermA594 (100 nM) was applied to either untreated or DAMGO (10μM, 2 hrs) treated HEK293 cells expressing FLAG-MOPr labeled with M1-A488. The intensity of DermA594 (red) and M1-A488 (green) were measured every 2.5 seconds before, during and following a 90 second application of DermA594. The relative non-normalized DermA594: M1-A488 intensity (R/G) is plotted demonstrating more binding of DermA594 following DAMGO treatment.

B) Summarized data showing the raw R/G data for WT FLAG-MOPr treated with ME, DAMGO and morphine and TSST-4A and STANT-3A mutants treated with ME demonstrate that under all conditions there was at least as much binding of DermA594 to MOPr following agonist pretreatment. Raw R/G values represent fluorescence intensity and therefore do not represent an actual ratio of ligand: receptor. The true ratio of DermA594: MOPr is not known.
Figure S2

A) The apparent association rate ($k_{app}$) of DermA594 (100 nM) was measured by fitting the DermA594 : M1-A488 fluorescence intensity during a 90 second application of DermA594 with a single exponential function to get an apparent rate of association ($k_{app}$). Averages from untreated and ME treated (30μM, 2 hrs) cells are plotted (+/- s.e.m.). There was a significant slowing in the $k_{app}$ following ME treatment in WT, TSST, and TSAA mutants (* p<.05, ** p<.01, two-way ANOVA, Tukey’s post hoc). When STANT was mutated, the $k_{app}$ was not significantly changed following ME treatment primarily due to slower $k_{app}$ under untreated conditions.

B) $k_{app}$ was measured for STANT-3A and TSST-4A mutants as described above during 3 minute applications of DermA594 at 30, 100, and 200 nM concentrations. Apparent on rates under each condition were plotted and fit linearly to estimate binding affinity ($k_d$). ME treatment resulted in a significant change in $k_{app}$ in both TSST and STANT (p< 0.001 for TSST vs. TSST+ME and STANT vs. STANT+ME, two way ANOVA, Tukey’s post hoc).

C) Summary of best fit of data from “B” where $k_{on}$ was the slope and $k_{off}$ was the y-intercept. (best fit +/- S.D.;*, p< 0.05 ME vs. untreated, $k_d$ range calculated from S.D. of $k_{off}$ and $k_{on}$)