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., BunzoW J.R., Williams J.T., Molecular Pharmacology, 2015

Figure S1

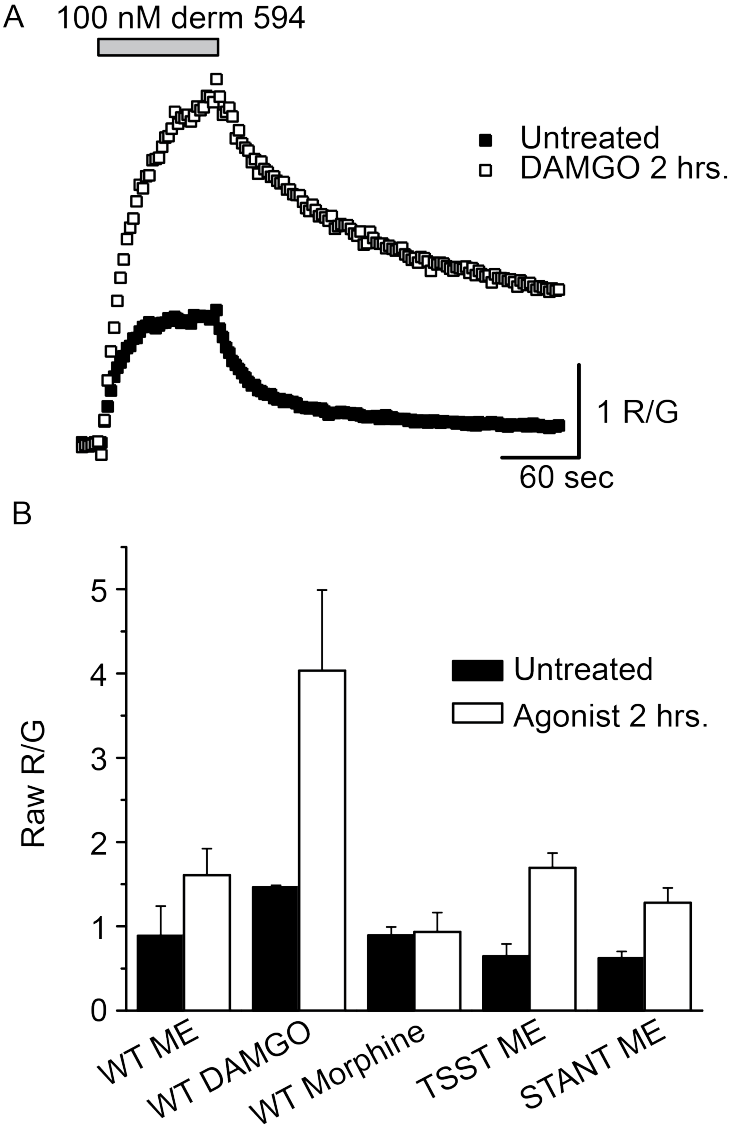
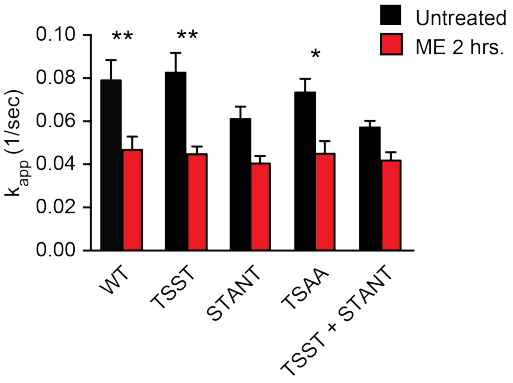


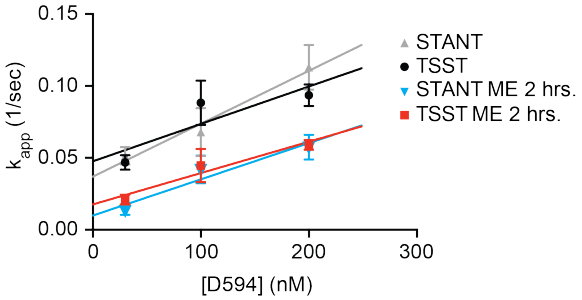
Figure S1: A) DermA594 (100 nM) was applied to either untreated or DAMGO (10μM, 2 hrs) treated HEK293 cells expressing FLAG-MOPr labeled with M1-A488. The intensity of DermA594 (red) and M1-A488 (green) were measured every 2.5 seconds before, during and following a 90 second application of DermA594. 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



B



C

Condition	k_{on} (1/mol/sec)	k_{off} (1/sec)	K_d (nM) range
TSST-Untreated	260000 ± 150000	0.0476 ± 0.0195	183 (61-600)
TSST-ME	218000 ± 53000	0.0176 ± 0.0069 *	81 (39-148)
STANT-Untreated	368000 ± 59000	0.0369 ± 0.0077	101 (69-148)
STANT-ME	252000 ± 70000	0.0099 ± 0.0092 *	39 (2.3-105)

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})