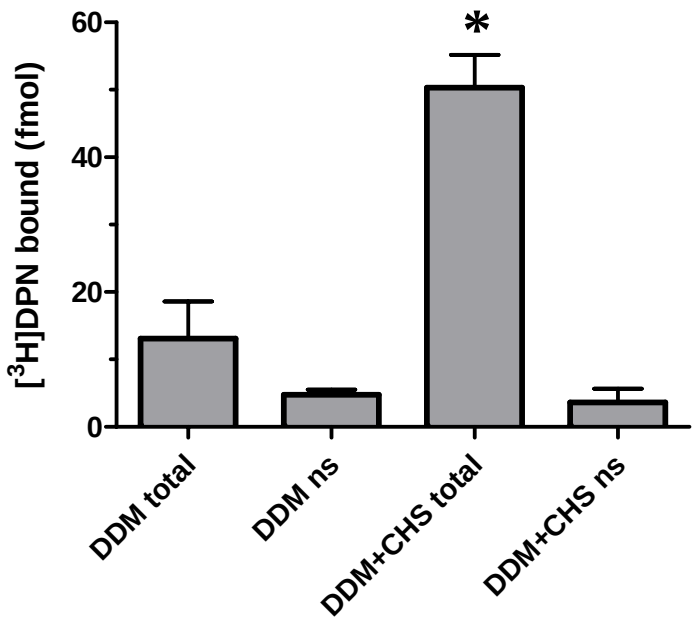
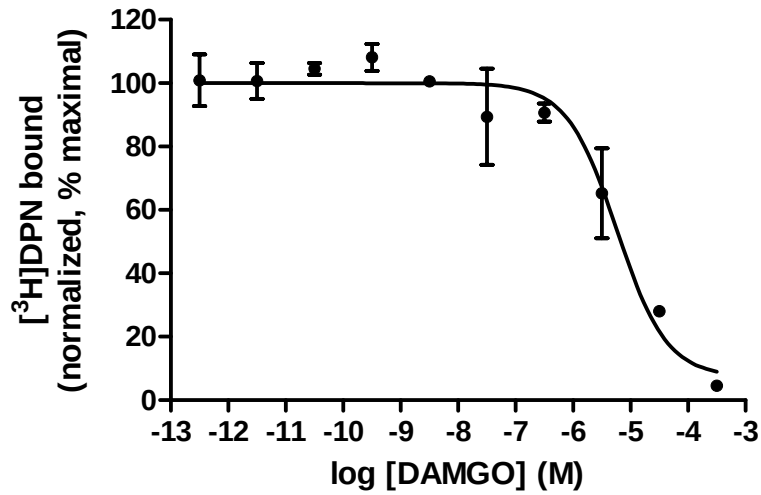
Kuszak et al. Supplemental Figure 1



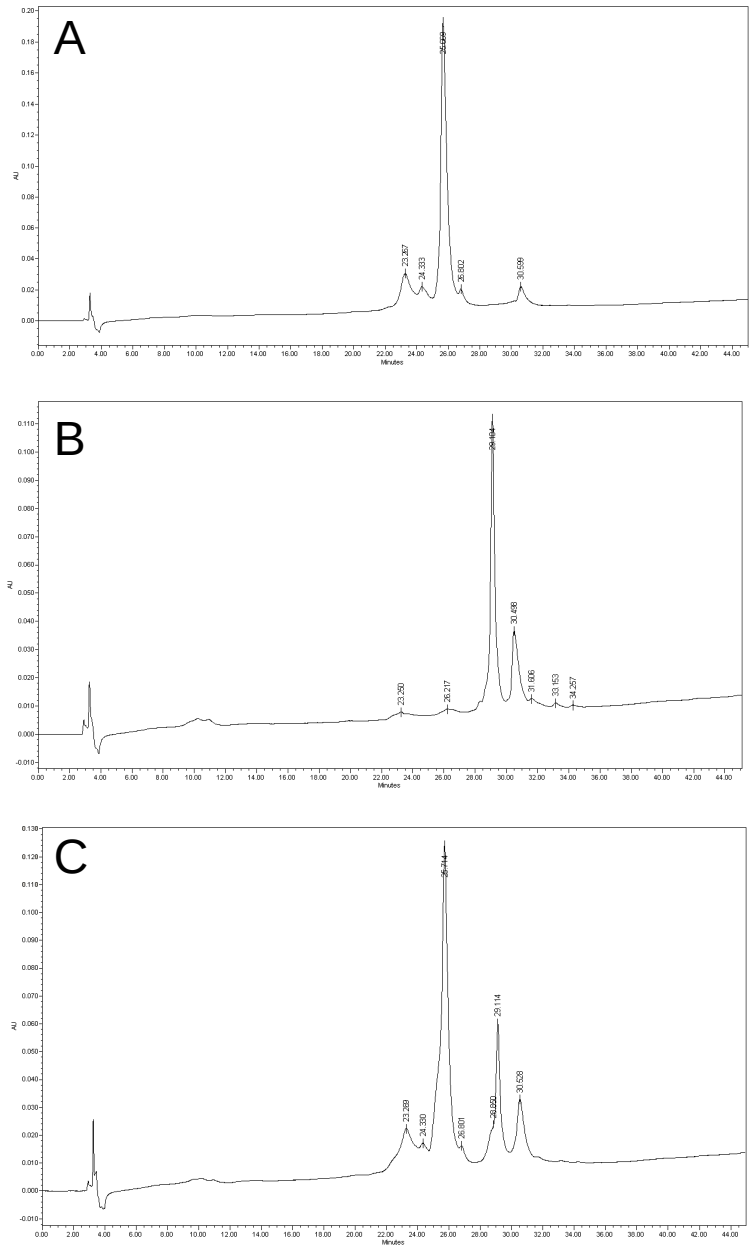
The inclusion of cholesteryl hemisuccinate (CHS) during n-dodecyl-β-D-maltoside (DDM) solubilization stabilizes YMOR. YMOR was solubilized from the plasma membranes of Sf9 cells with 1% DDM in the presence or absence of 0.01% CHS and then enriched on a Talon™ IMAC column in either 0.1% DDM or 0.1% DDM plus 0.01% CHS. [^{3}H]DPN binding to these detergent solubilized YMOR samples was then measured in the absence (total) or presence (NS) of 20 μM naltrexone. Addition of CHS to solubilized YMOR increased [^{3}H]DPN ~3.8-fold, suggesting an improvement in the amount of receptor in an active conformation. Error bars represent the Standard Error of the Mean of two experiments performed in duplicate. *P<0.05, significantly different from DDM total as calculated by a Student's t-test.

Kuszak et al. Supplemental Figure 2



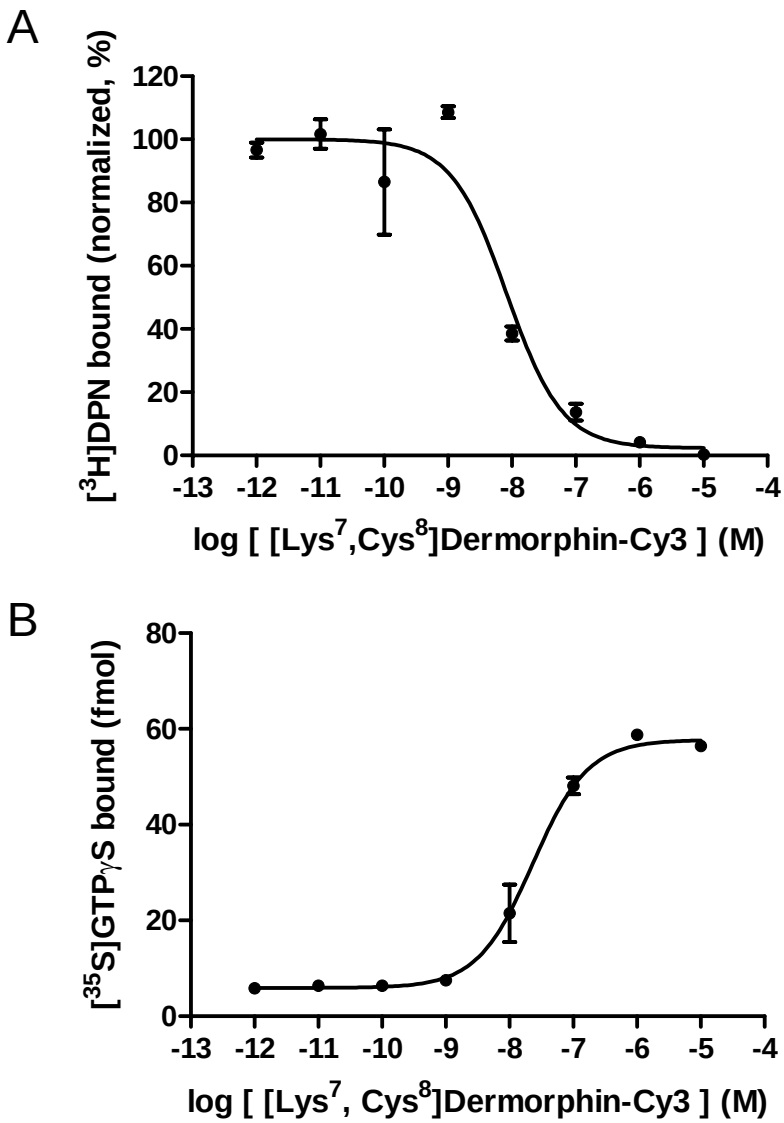
DAMGO displays low affinity binding to rHDL•YMOR. An estimated 15 fmoles of rHDL•YMOR was incubated with 1 nM [^{3}H]DPN and increasing concentrations of DAMGO in 25 mM Tris•HCl, pH 7.7, 100 mM NaCl, 0.1% BSA. DAMGO competed [^{3}H]DPN binding with a K_i of ~1.9 μM, indicating that in the absence of G protein heterotrimer coupling DAMGO binds YMOR with low affinity. These data corroborate those in Figures 1d and 4b, which illustrated that DAMGO binds YMOR with low affinity when G_{i2} heterotrimer is uncoupled from the receptor by the addition of 10 μM GTPγS. Data is representative of three experiments performed in duplicate. Error bars represent the Standard Error of the Mean.

Kuszak et al. Supplemental Figure 3



[Lys^7, Cys^8]dermorphin was synthesized on Rink resin using an Applied Biosystems Peptide Synthesizer and Fmoc chemistry. Samples were resolved on a Waters reverse phase HPLC using a Vydac C18 10 micron column, run on a linear gradient of 0 to 45% acetonitrile containing 0.1% TFA in an aqueous phase containing 0.1% TFA at 35ºC. Samples were monitored at 254 nm. [Lys^7, Cys^8]dermorphin **(A)** has a retention time of 25.9 min and was determined to be 98% pure before labeling. [Lys^7, Cys^8]dermorphin was labeled with Cy3-maleimide dye (GE Healthcare) at a ratio of 1.5:1 peptide:fluorophore. [Lys^7, Cys^8]dermorphin-Cy3 **(B)** has a retention time of 29.2 min and was determined to be 91% pure before use in binding assays and imaging experiments. A mixture and co-elution profile **(C)** shows that the two compounds are well separated.

Kuszak et al. Supplemental Figure 4



A Cy3-labeled [Lys^7, Cys^8]dermorphin retains agonist properties at YMOR. Approximately 50 fmoles of receptor in HDL particles were coupled to purified G_{i2} heterotrimer at a ratio of 30:1 G protein:YMOR. **(A)** [Lys^7, Cys^8]dermorphin-Cy3 binds rHDL•YMOR+G_{i2} with high affinity, displaying a K_i of 3.3 nM in competition assays against 0.75 nM [^{3}H]DPN. The single high affinity binding site suggested by the monophasic competition curve is due to the high molar ratio of G protein addition to YMOR, which results in nearly all of the receptor being coupled to G_{i2} heterotrimer. Data is normalized to the maximal binding level as calculated by a one-site competition curve fit (Prism 5.0, GraphPad). **(B)** [Lys^7, Cys^8]dermorphin-Cy3 stimulated [^{35}S]GTPγS binding to rHDL•YMOR+G_{i2} with an EC_{50} of 23.05 nM. Approximately 52 fmoles of [^{35}S]GTPγS binding was stimulated, suggesting a 1:1 YMOR:G_{i2} coupling, confirming the fact that adding G_{i2} at a 30:1 molar ration results in complete coupling of YMOR. Data are representative of three experiments performed in duplicate are shown. Error bars represent the Standard Error of the Mean.