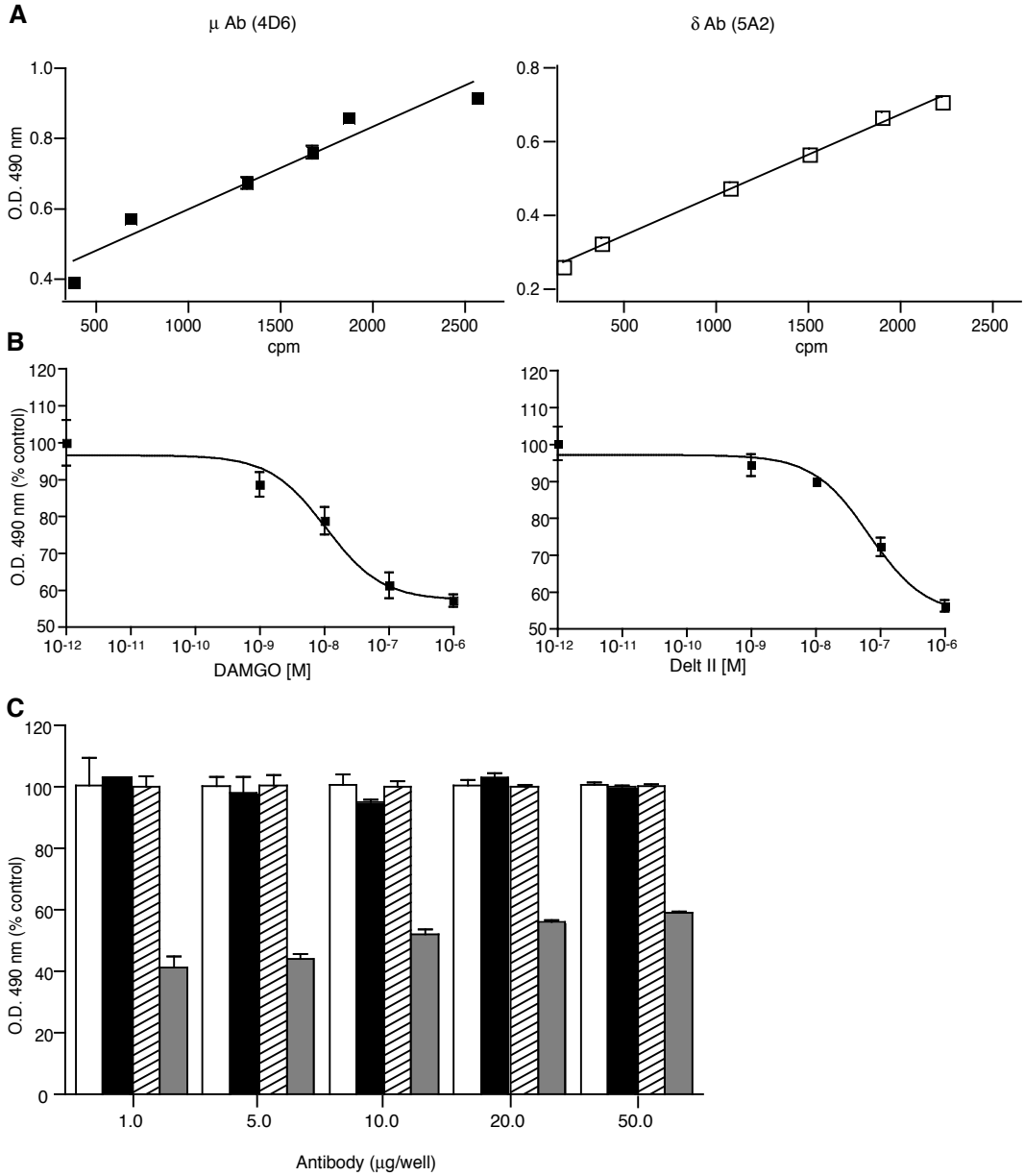
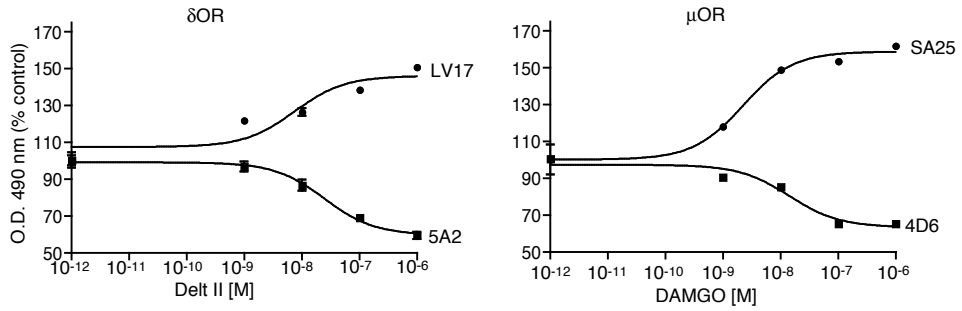
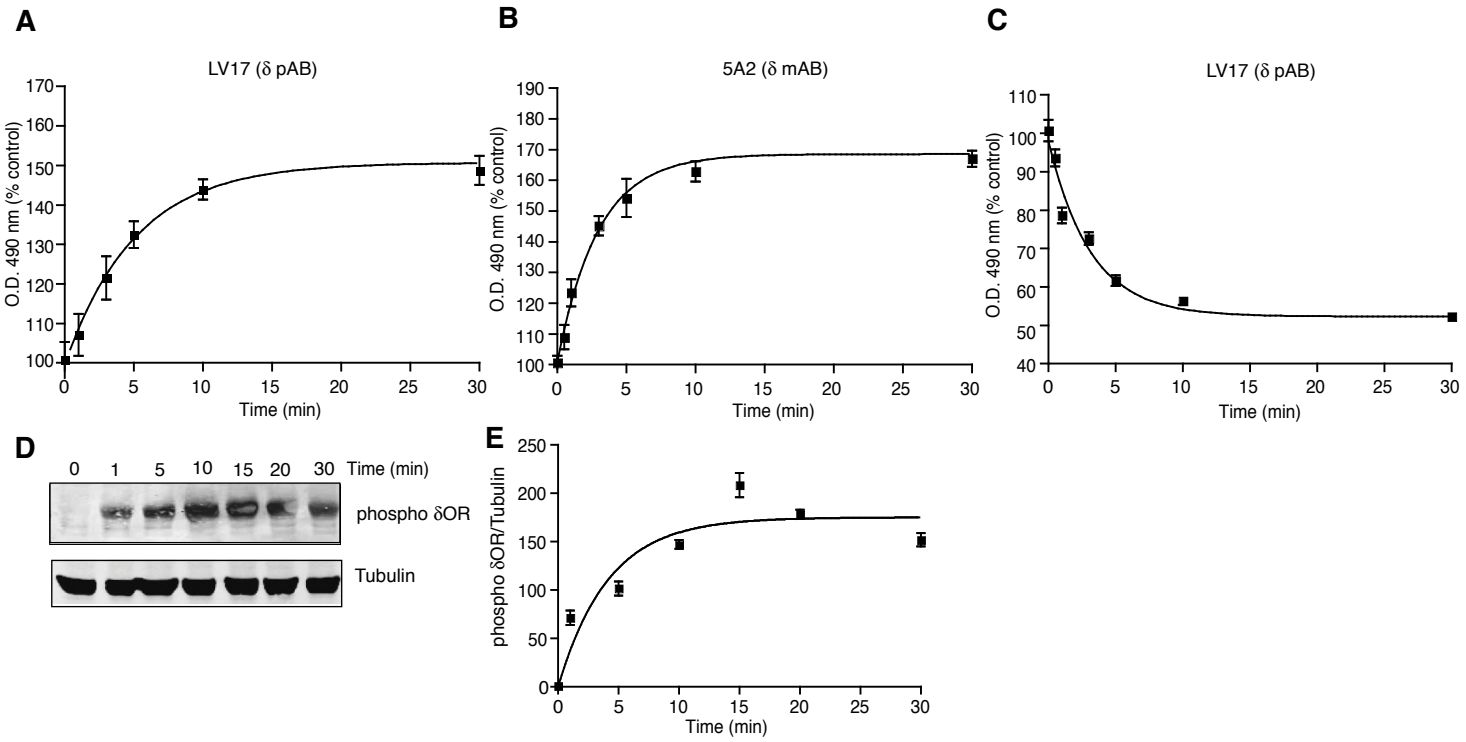


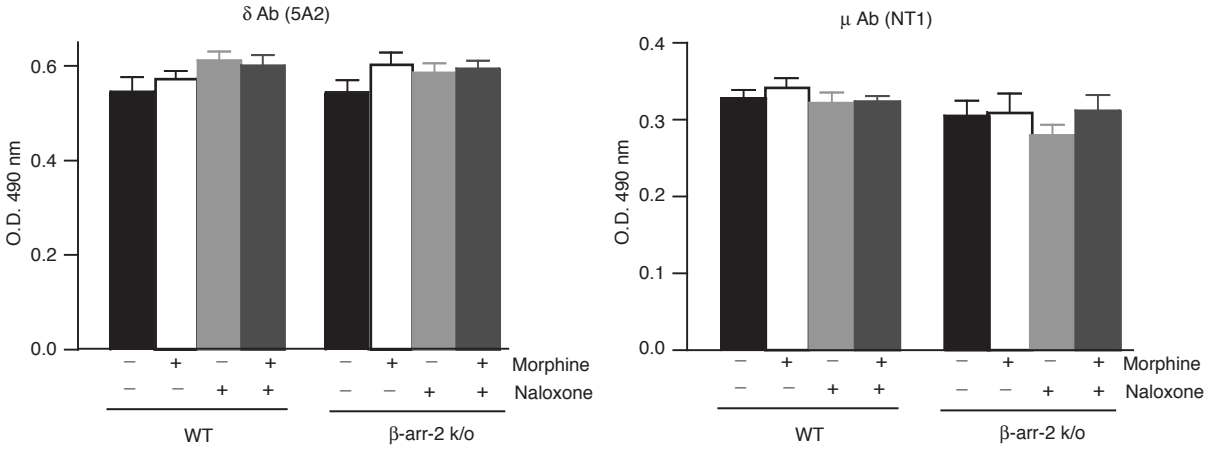
Supplemental Fig. 1. Linear relationship between antibody binding and receptor number (A) CHO cells expressing μOR or δOR (0.5-6 x10^5 cells) were subjected to ELISA using μOR (4D6) or δOR (5A2) mAbs as described in Methods. In a parallel set of plates binding assays were carried out using 10 nM [^{3}H]diprenorphine. Non-specific binding was determined in the presence of 1 μM DAMGO or Deltorphin II. Results are mean ± SEM of 2 experiments in triplicate. **(B) ELISA in unfixed cells.** CHO cells expressing μOR or δOR (2 x10^5 cells/tube) were treated with DAMGO or Deltorphin II (0-1 μM) and subjected to ELISA using μOR (4D6) or δOR (5A2) mAbs as described in Methods. Results are mean ± SEM of 3 experiments in triplicate. **(C) ELISA with different mAb concentrations.** CHO cells expressing Flag tagged μOR (2 x10^5 cells/tube) were treated with (■,▨) or without (□,▨) 1 μM DAMGO and subjected to ELISA using different concentrations (1-50 μg/well) of μOR (4D6;▨ ,▨) or Flag (□,■) mAbs as described in Methods. Results are mean ± SEM of 2 experiments in triplicate.



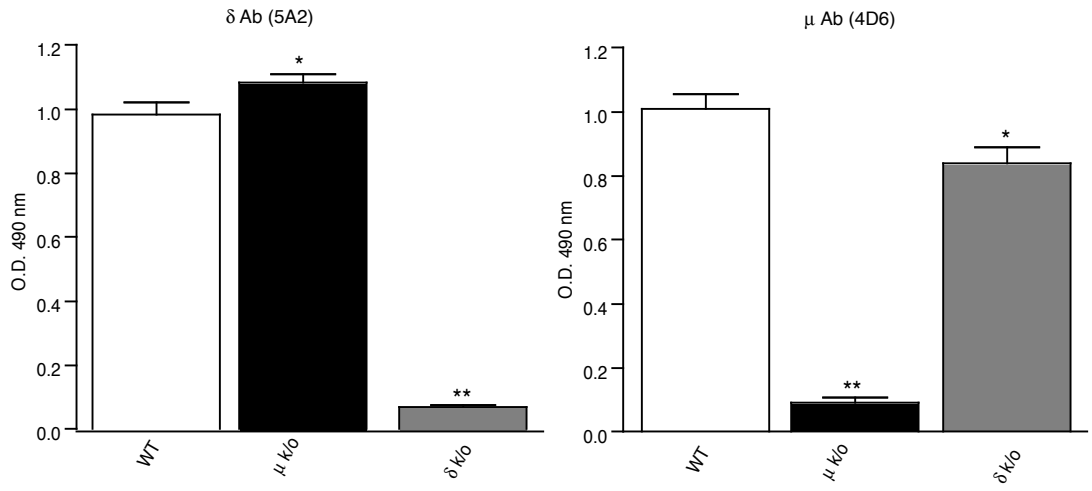
Supplemental Fig. 2. Comparison of the change in receptor recognition following agonist treatment by monoclonal (mAb) and polyclonal (pAb) antibodies. CHO cells expressing μOR or δOR(2 x10^5 cells/well) were treated with indicated doses of DAMGO or Deltorphin II and subjected to ELISA using μOR mAb (4D6), μOR pAb (SA25), δOR mAb (5A2) or δOR pAb (LV17) as described in Methods. Results are mean ± SEM of 3 experiments in triplicate.



Supplemental Fig. 3. Time course of change in receptor recognition following agonist treatment. (A) CHO cells expressing δOR (2 x10^5 cells) were treated with or without 1 μM Deltorphin II (0-60 min) and subjected to ELISA using δOR pAb (LV17) as described in Methods. **(B & C)** CHO cells expressing δOR (2 x10^5 cells) were treated with 1 μM Deltorphin II (60 min) followed by treatment with1 μM $TIPP_{\psi}$ (δOR antagonist) for different time periods (0-60 min) and subjected to ELISA using δOR mAb (5A2) or pAb (LV17) as described in Methods. Results are mean ± SEM of 3 experiments in triplicate. **(D, E)** CHO cells expressing δOR(2 x10^5 cells) were treated with 100 nM Deltorphin II (0-60 min) and subjected to Western blot analysis with rabbit polyclonal anti-phospho δORand mouse monoclonal anti-tubulin antibodies as described in Methods. A representative blot is shown. The data was densitized and represented as a ratio of phospho δOR to tubulin.



Supplemental Fig.4. δOR or μOR levels are not changed in β-arrestin 2 knock-out mice. β-arrestin 2 knock-out mice (β-arr k/o) or their wild-type (WT) littermate controls were injected intraperitoneally with either 10mg/kg morphine, 1mg/kg naloxone, 10mg/kg morphine+1mg/kg naloxone or saline and sacrificed 30 min later. Membranes prepared from the PFC were probed by ELISA with the μOR (NT1) or δOR (5A2) antibodies. Results are mean ± SEM of 3 experiments in triplicate.



Supplemental Fig. 5 Receptor selectivity of μOR and δOR mAbs. Membranes from the brain of wild-type (WT) mice or mice lacking either μOR (μ k/o) or δOR (δ k/o) were subjected to ELISA using mAbs to μOR (4D6) or δOR (5A2) as described in Methods. Results are Mean ± SEM of 3 experiments in triplicate. *p<0.05; ** p<0.001