



Supplementary Information for

G protein-signaling biased mu opioid receptor agonists that produce sustained G protein activation are noncompetitive agonists

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Figures S1 to S3

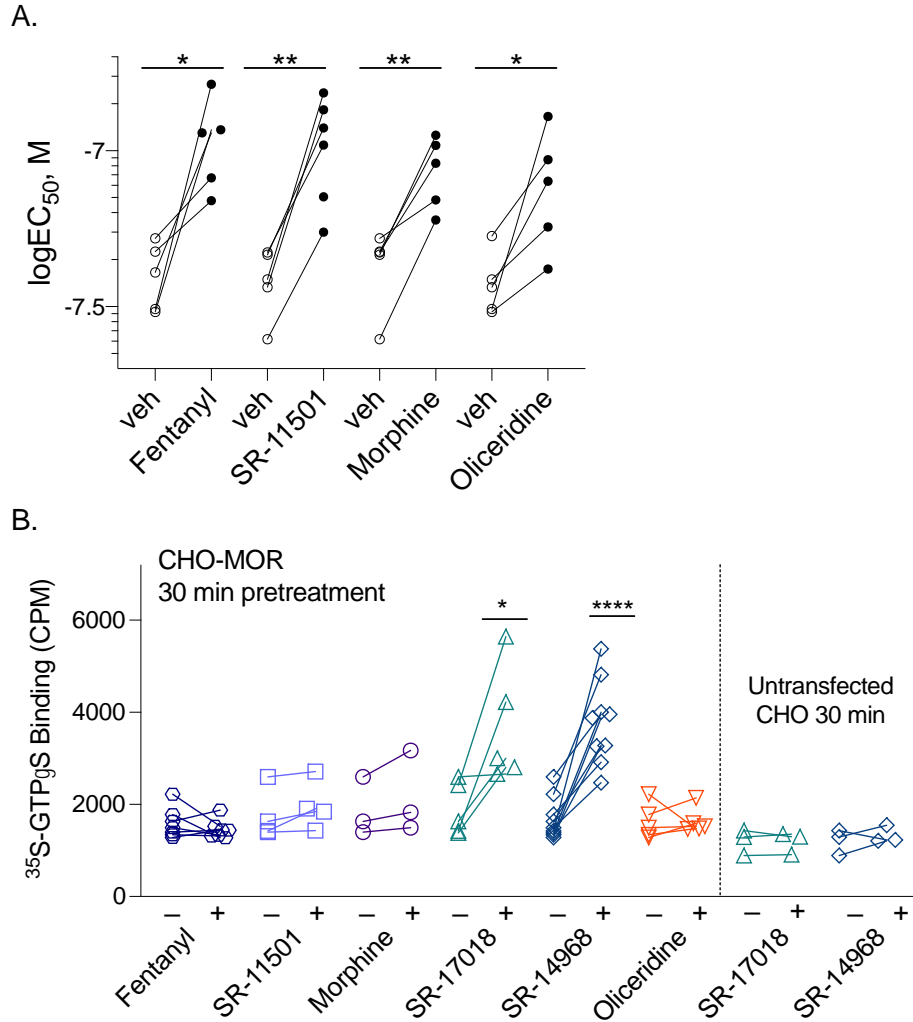


Figure S1. ^{35}S -GTP γ S binding in membranes prepared following 10 μM pretreatment of live CHO-hMOR cells with the indicated agonists. **A**). Comparison of DAMGO potency values following 2 hour pre-treatment. (Accompanies Figure 1, Table 1; paired Student's t test $*p < 0.05$, $**p < 0.01$). **B**). Changes in baseline after 30 minutes pretreatment of live cells. Untransfected cells are shown for comparison (same treatment conditions). $*p < 0.05$; $****p < 0.0001$, paired Student's t -test. (Accompanies Figure 1).

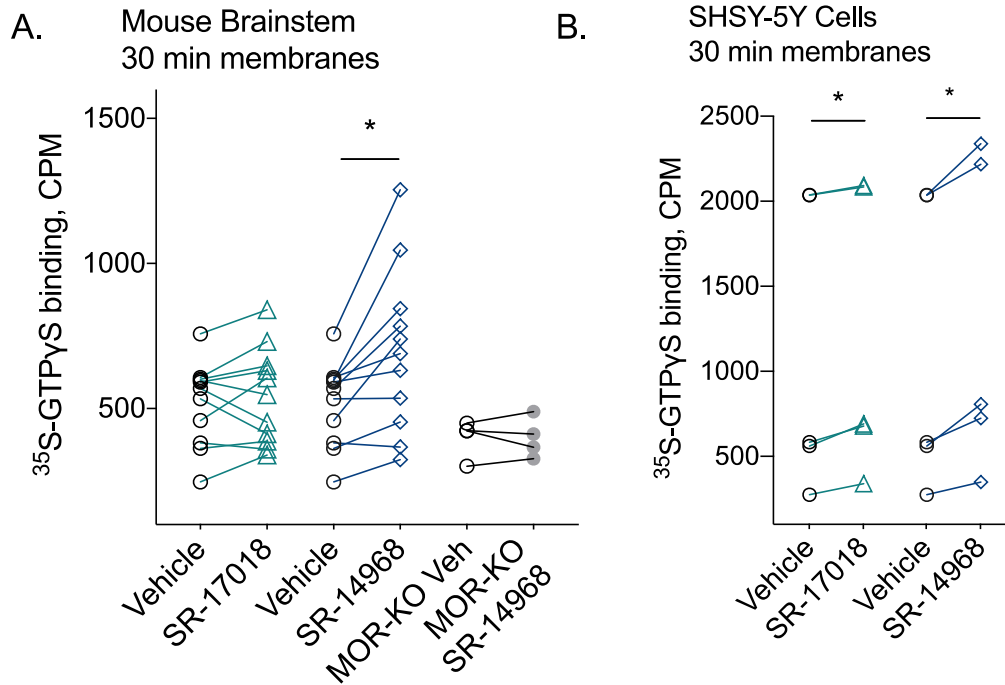


Figure S2. $^{35}\text{S-GTP}\gamma\text{S}$ baseline binding following SR-17018 or SR14968 (10 μM , 30 min) pretreatment in **A**). mouse brainstem membranes and **B**). SH-SY5Y cell membranes. The normalized means data are presented in Figures 2H and I, respectively. Since the vehicle treated group was matched for each replicate of each drug treatment, a one-way RM-ANOVA followed by a Dunnett's post-hoc test for variance was applied, $*p < 0.05$. **A**). No stimulation was observed in the MOR-KO (paired t -test, $p > 0.05$).

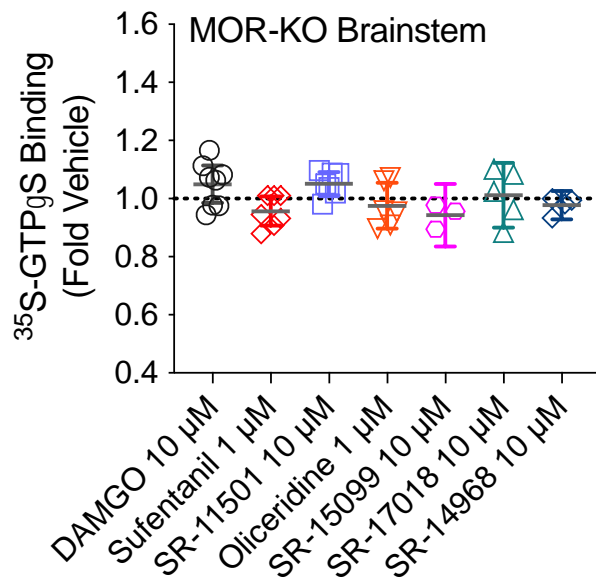


Figure S3. Lack of MOR agonist-stimulated ³⁵S-GTP_γS binding in membranes prepared from MOR-KO mouse brainstem (mean with 95% CI). (Accompanies Figure 4)