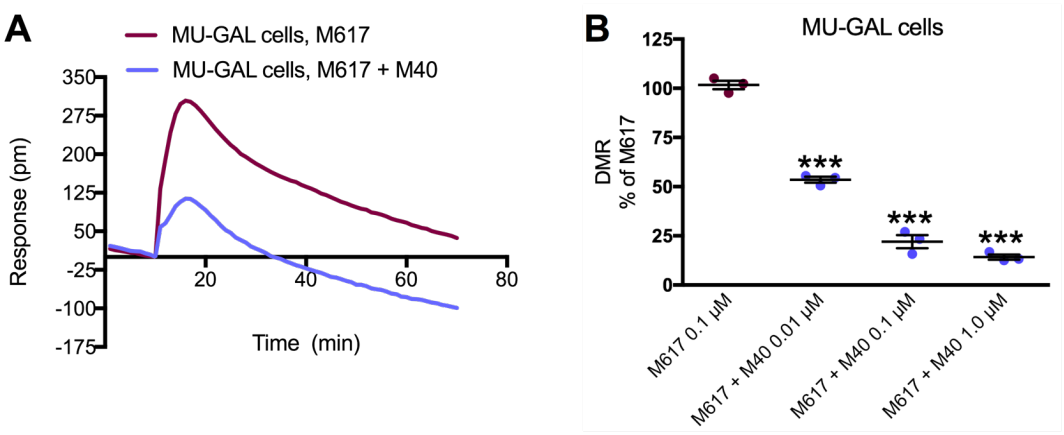
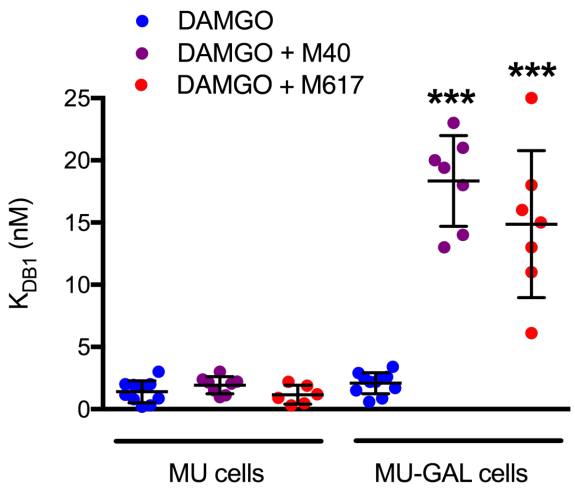
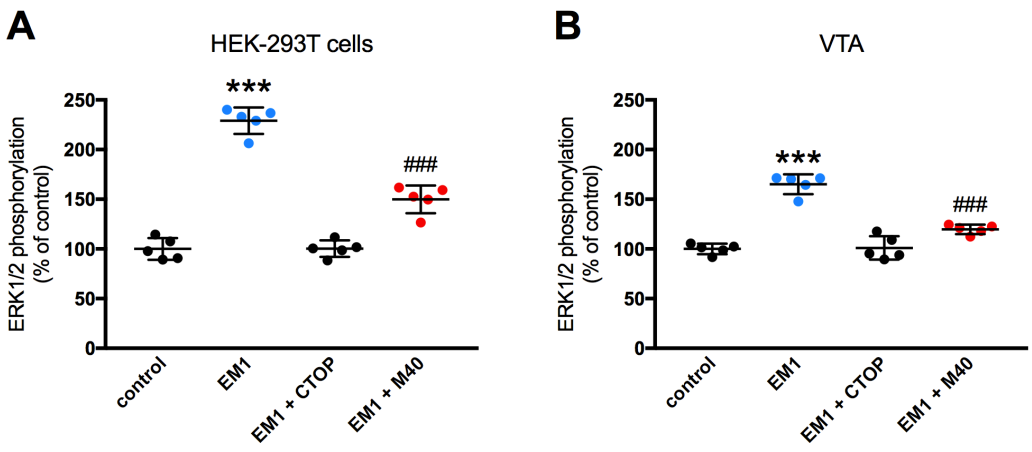
Supplemental data



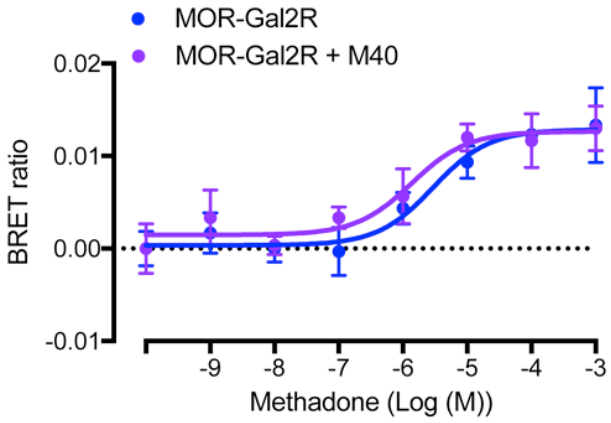
Supplemental Figure 1. Antagonism of Gal1R agonist-induced signaling by M40. (**A** and **B**) Dynamic mass redistribution (DMR) experiments in FLP-FRT-HEK cells stably transfected with MOR and Gal1R (MU-GAL cells); the Gal1R/Gal2R antagonist M40 counteracts the ability of the Gal1R agonist M617 to induce DMR. (**A**) Representative picometer shifts of reflected light wavelength (picometers) versus time induced by M617 (0.1 μM) alone or in the presence of M40 (1 μM). (**B**) Dose dependent counteraction by M40 (0.01-1 μM) on DMR induced by M617 (0.1 μM); values are shown as dots and in means (horizontal lines) ± SEM; n = 3 triplicates/group; ***: $P < 0.001$, versus M617 alone; one-way ANOVA with Dunnetts's multiple comparisons.



Supplemental Figure 2. Decreased affinity of DAMGO by Gal1R ligands in MU-GAL cells. Affinity of DAMGO obtained from competitive inhibition experiments of [^{3}H]DAMGO versus DAMGO in membrane preparations from MU and MU-GAL cells; values are shown as dots and in means (horizontal lines) ± SEM of K_{DB1} values (see Materials and Methods), n = 6-11 triplicates/group; both the Gal1R agonist M617 (1 μM) and the Gal1R/Gal2R antagonist M40 (1 μM) produce a significant increase in K_{DB1} values in MU-GAL cells but not in MU cells; ***: $P < 0.001$ *versus* DAMGO; one-way ANOVA with Dunnett's multiple comparisons.



Supplemental Figure 3. Cross-antagonism of EM1-induced MAPK activation by M40. (**A**) Counteraction of ERK1/2 phosphorylation induced by the MOR agonist EM1 (0.1 μM) by the MOR antagonist CTOP (1 μM) and the Gal1R/Gal2R antagonist M40 (1 μM) in HEK-293T cells transiently transfected with MOR and Gal1R. (**B**) Counteraction of ERK1/2 phosphorylation induced by EM1 (1 μM) by CTOP (10 μM) and M40 (10 μM) in rat VTA slices. Values are shown as dots and in means (horizontal lines) ± SEM;,n = 5 triplicates/group; *** and ###: $P < 0.001$ *versus* control and *versus* EM1, respectively; one-way ANOVA with Tukey's multiple comparisons.



Supplemental Figure 4. Lack of effect of M40 on methadone-induced signaling in cells transfected with Gal2R. Representative concentration-response of three experiments of methadone induced BRET changes in HEK-293T cells, transiently transfected with MOR fused to Rluc, the alpha subunit of the Gi1 protein fused to the YFP (Venus variant) and Gal2R, in the presence and absence of the Gal1R/Gal2R antagonist M40 (1 μM); values are expressed in means ± SEM of triplicates; the curves do not show shift to the right with Gal2R transfection or flattening with M40, as shown in cells transfected with Gal1R (see Figure 2).

Supplemental Table 1. Characteristics of study participants in clinical trial studies

RLS Study	**N=226**
Mean (and range) age in years	65.0 (33-89)
Gender:	
Male	96 (42.5%)
Female	130 (57.5)
Self-identified race:	
African-American	0
White	221 (97.8%)
Other	5 (2.2%)
OUD Three-Month Study	**N=30**
Mean (and range) age in years	51.4 (31-67)
Gender:	
Male	19 (36.7%)
Female	11 (63.3%)
Self-identified race:	
African-American	21 (70.0%)
White	9 (30.0%)
Other	0
Mean (+SD) years of heroin use	25.6 (+11.2)
Mean (+SD) 14-day methadone dose, mg	56.2 (+10.4)
Mean (+SD) 84-day methadone dose, mg	74.5 (+20.0)***
OUD Drug Use Assessment Study	**N= 49**
Mean (range) age in years	47.3 (19-67)
Gender:	
Male	28 (57.1%)
Female	21 (42.9%)
Self-identified race:	
African-American	31 (63.3%)
White	18 (36.7%)
Other	0
Mean (+SD) years of opioid use	21.1 (+11.4)

***: significantly different dose as compared with mean of the 14-day dose; $P < 0.001$; paired *t* test.