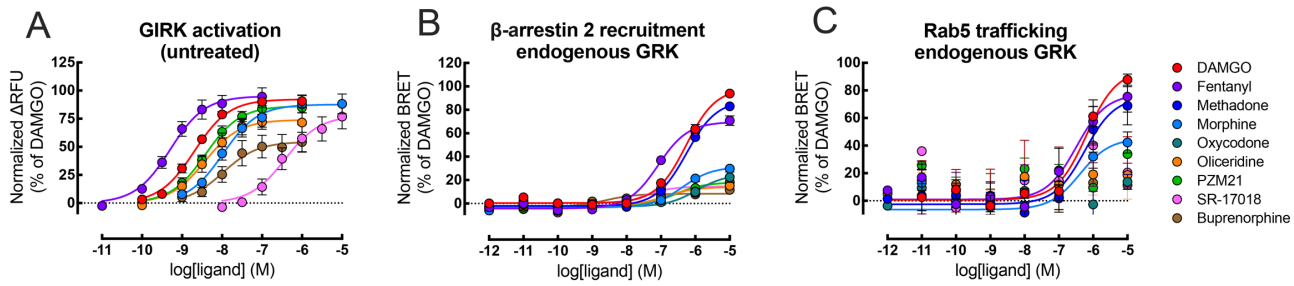

Fig. S1. Additional concentration-response curves. Concentration-response curves for DAMGO, fentanyl, methadone, morphine, oxycodone, oliceridine, PZM21, SR-17018, and buprenorphine were constructed for **(A)** GIRK activation in the absence of β-CNA treatment, **(B)** β-arrestin 2 recruitment, and **(C)** trafficking of MOR to Rab5a-positive endosomes in the absence of GRK2 overexpression. Data are means ± SEM of 3 to 10 experiments, each performed in duplicate (GIRK activation) or triplicate (β-arrestin 2 recruitment and Rab5 trafficking).

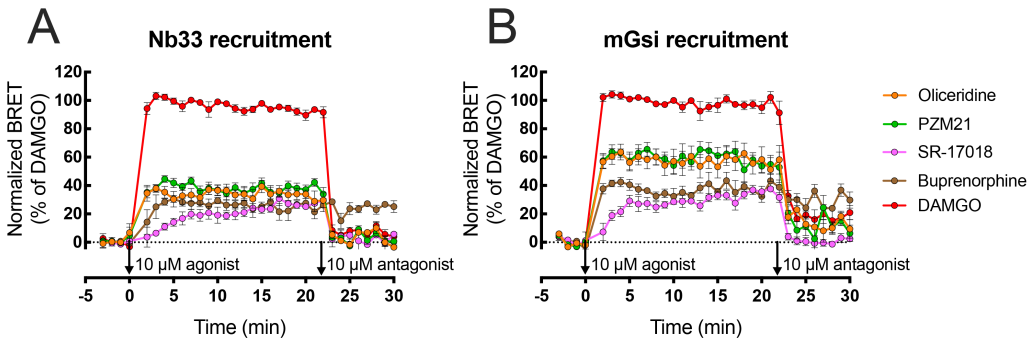

Fig. S2. Kinetics of Nb33 and mGsi recruitment and naloxone antagonism. Kinetics of **(A)** Nb33 and **(B)** mGsi recruitment to MOR induced by DAMGO, oliceridine, PZM21, SR-17018, and buprenorphine (all at 10 µM) and reversed by injection of 10 µM Naloxone. Data are means ± SEM of 3 experiments, each performed in triplicate.

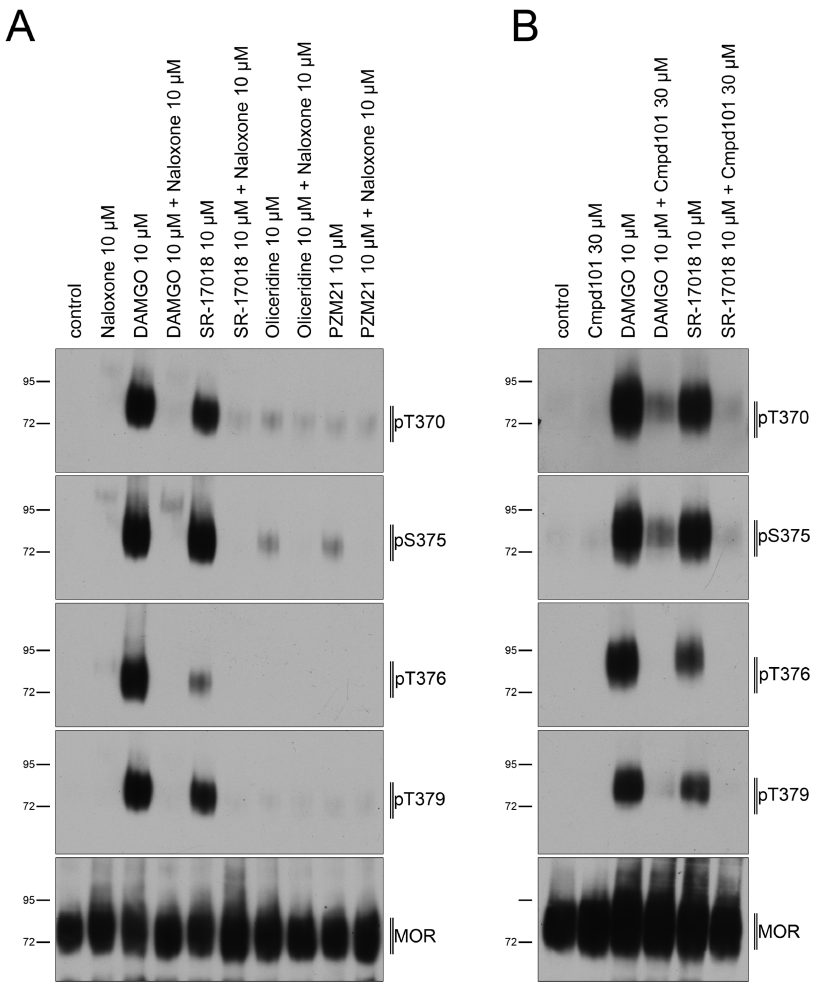

Fig. S3. Sensitivity of MOR phosphorylation to naloxone and Cmpd101. Effect of **(A)** 10 µM Naloxone or **(B)** 30 µM Cmpd101 on MOR phosphorylation induced by 10 µM SR-17018, PZM21, or oliceridine (TRV130). Blot is representative of 3 independent experiments.

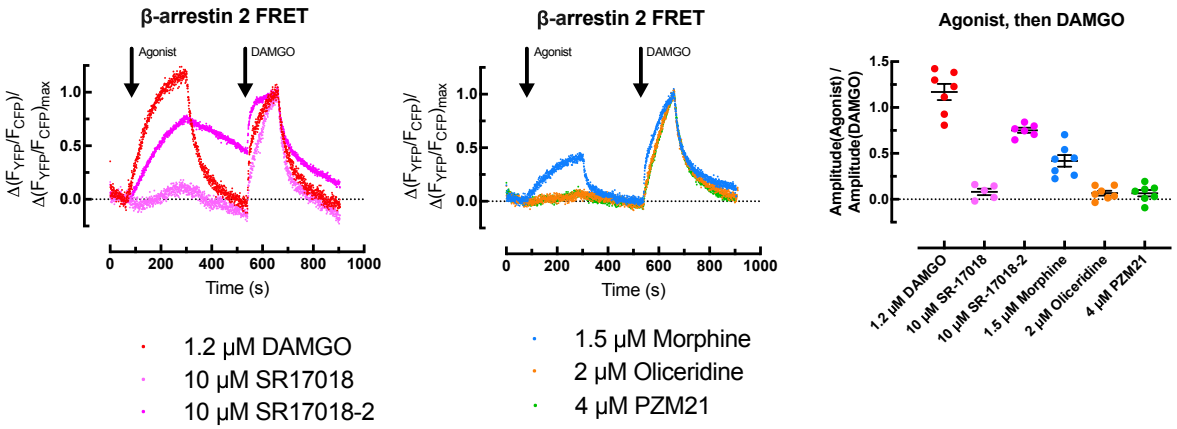

Fig. S4. Kinetics of interaction of MOR with ß-arrestin 2 by FRET. Arrestin recruitment induced by DAMGO, morphine, SR-17018, PZM21, or oliceridine measured using FRET. In all conditions, DAMGO was added at t=600 for normalization. Traces are representative of 5 to 7 independent experiments.

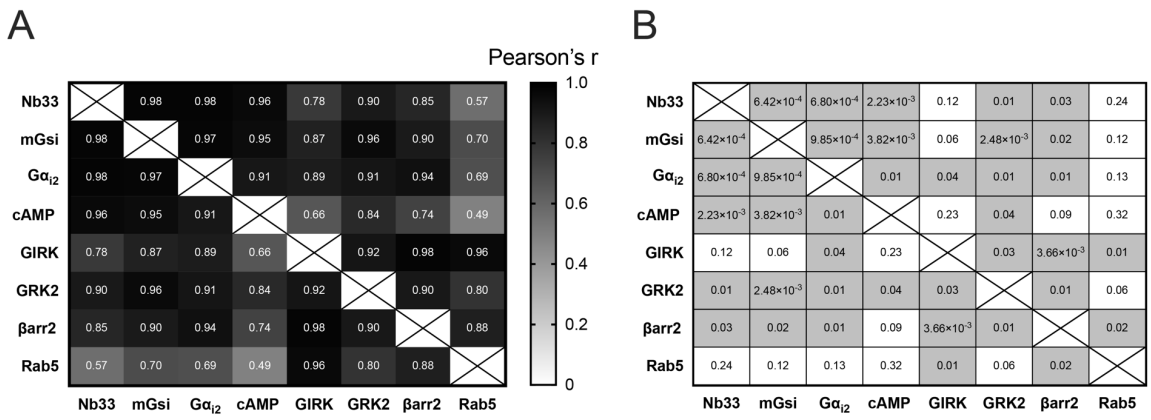

Fig. S5. Correlation matrix and statistical testing of correlation between log(τ) values. (A) Pearson r and **(B)** SEM of log(τ) correlations shown in Fig. 4.

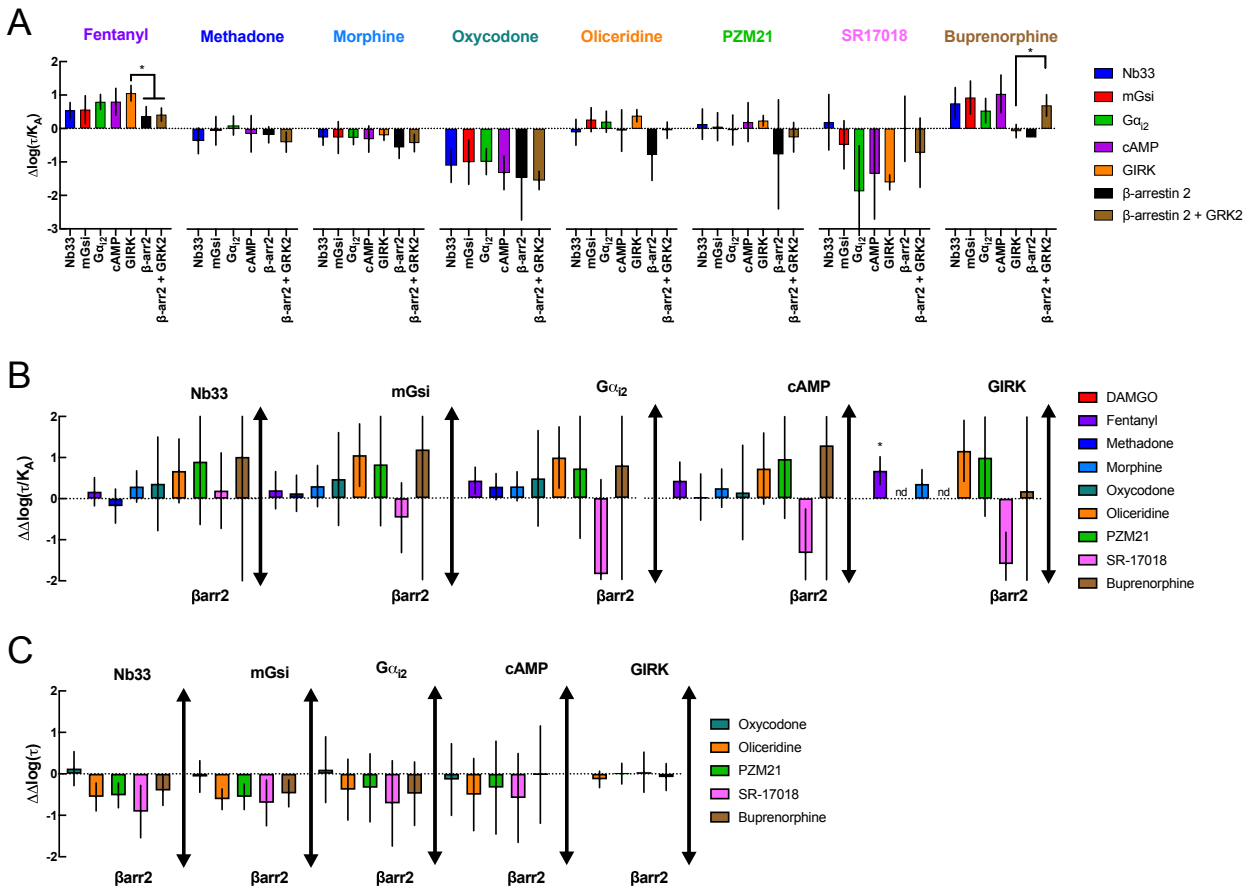

Fig. S6. Additional bias calculations. (A) $\Delta Log(\tau/K_A)$ for the indicated agonists across the different signaling pathways and **(B)** bias factors $\Delta\Delta Log(\tau/K_A)$ in the absence of GRK2 overexpression. **(C)** Determination of biased agonism using an alternative, efficacy-driven approach ($\Delta\Delta log(\tau)$). The system response in each pathway was defined by the maximal response to DAMGO, and the $\Delta log(\tau)$ was calculated by normalizing to morphine within each pathway as in Burgueño *et al.* (2017). In all cases, error was propagated using standard rules, and error bars represent 95% confidence intervals. Each set of $\Delta Log(\tau/K_A)$ values for a given agonist was statistically tested using a Brown-Forsythe unequal variance ANOVA, with Welch's t-tests conducted comparing agonists between each pathway multiplicity corrected using the Holm-Sidak method for the total of 76 comparisons made.

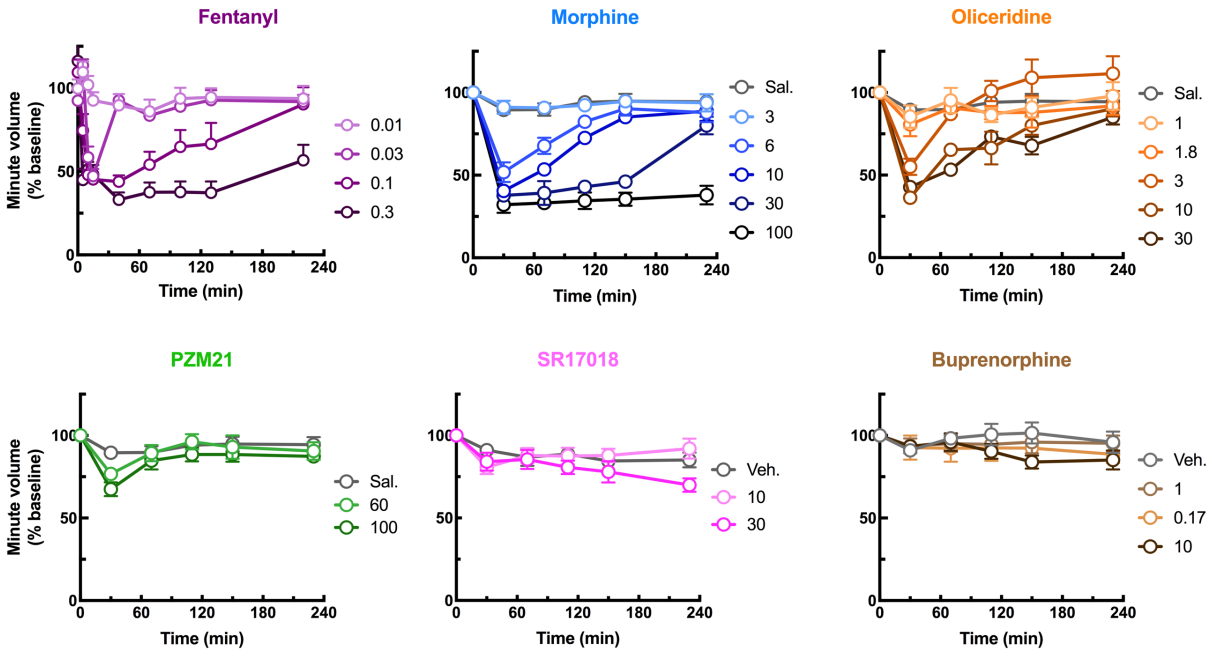

Fig. S7. Opioid-induced respiratory depression analyzed as minute volume. Respiratory depression induced by fentanyl, morphine, oliceridine, PZM21, SR-17018, or buprenorphine shown as minute volume. Data are means ± SEM of 5 to 9 animals.

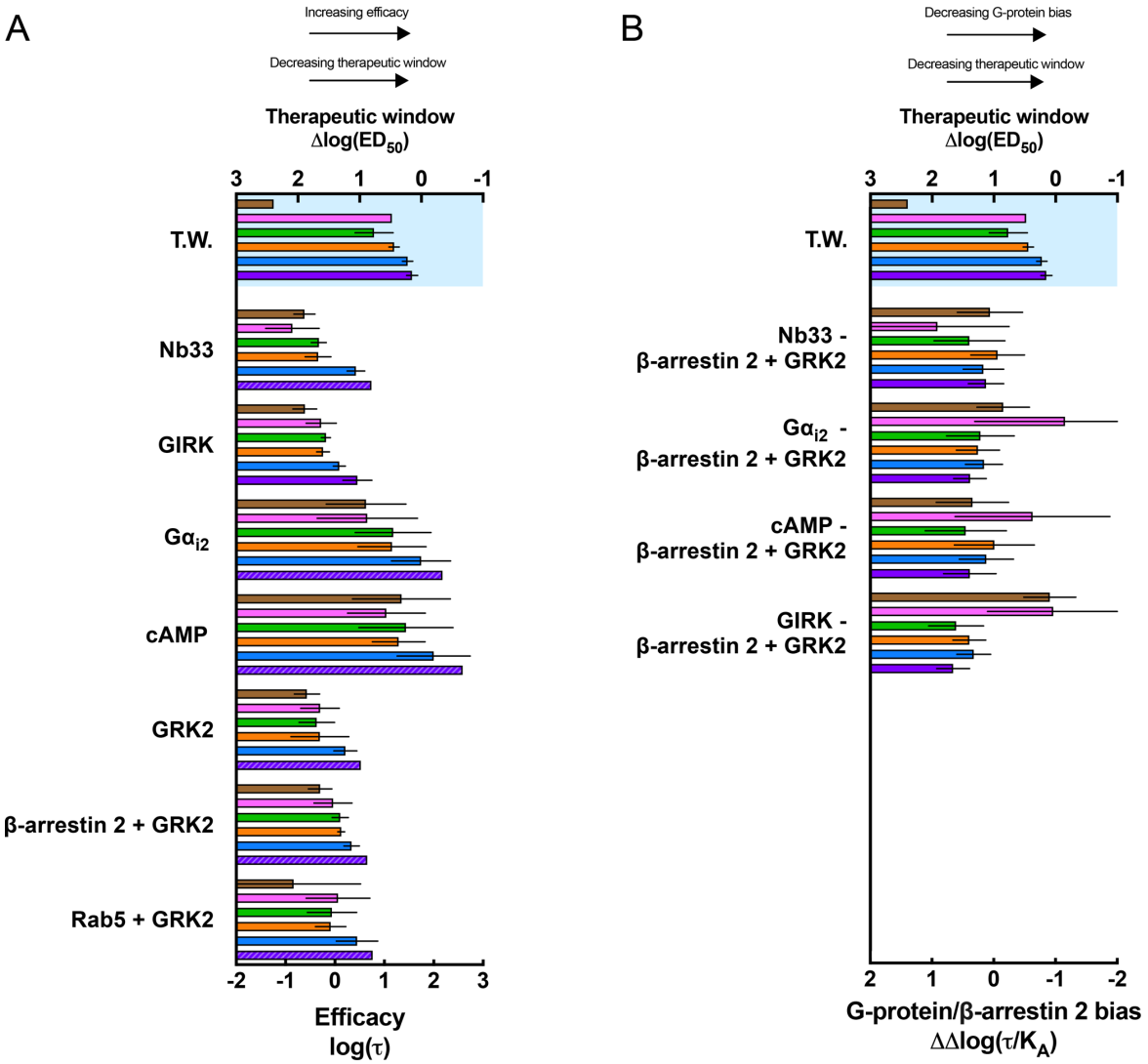

Fig. S8. Therapeutic window compared to efficacy and G-protein or ß-arrestin 2 bias for additional signaling assays. Relationships of the therapeutic windows of fentanyl, morphine, oliceridine, PZM21, SR-17018, and buprenorphine with the **(A)** operational efficacies or **(B)** bias factors at the indicated signaling endpoints.

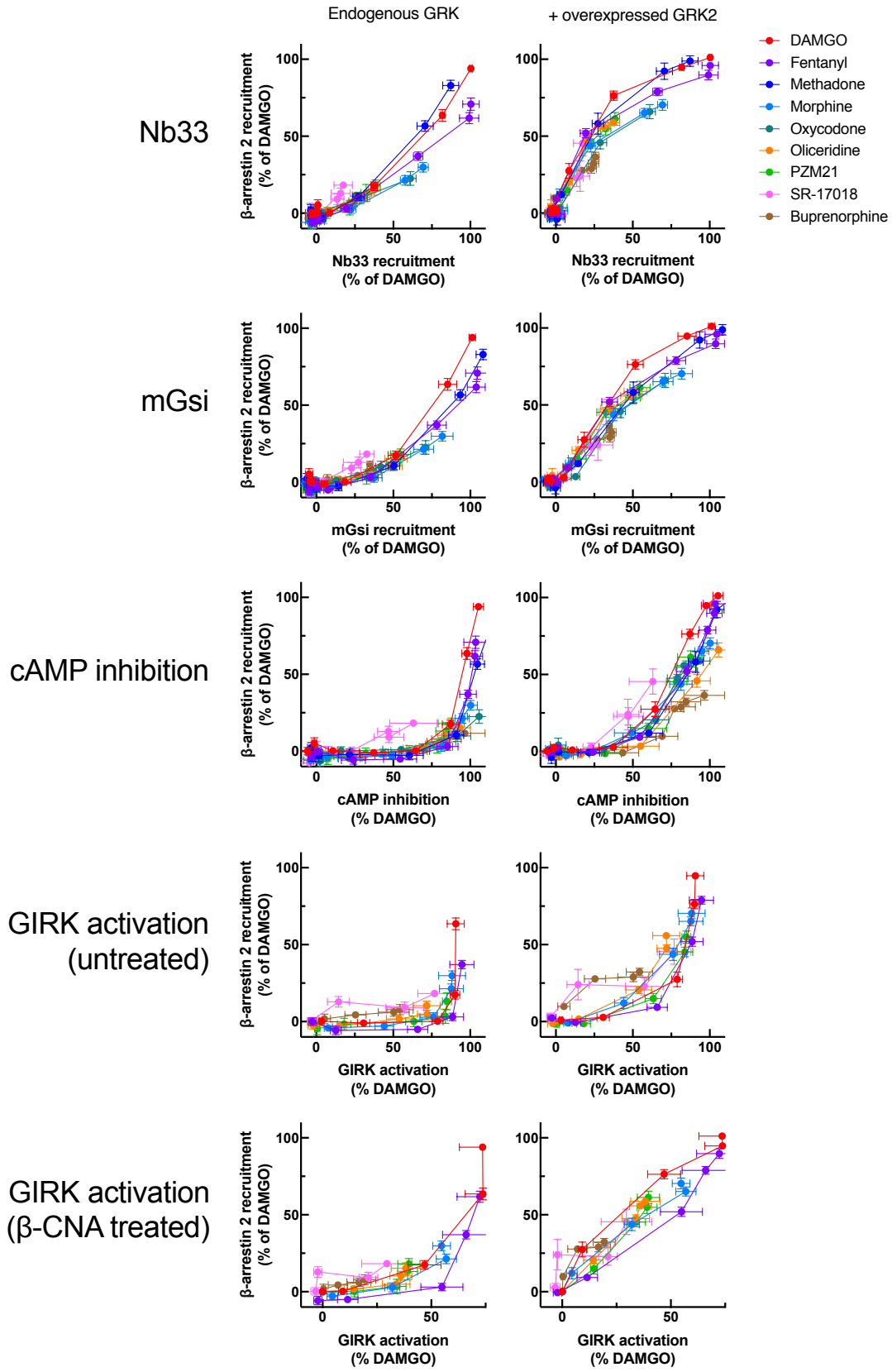

Fig. S9. Bias plots for all additional assays. Bias plots of β-arrestin 2 recruitment and Nb33, mGsi, cAMP inhibition, and GIRK channel activation in the absence or presence of overexpressed GRK2.

Table S1. **Calculation of transduction coefficients.** Transduction coefficient, log(τ/K_A), values used in generation of $\Delta\Delta\log(\tau/K_A)$ estimates. Mean ± S.E.M. from 3 to 14 experiments.

log(τ/K_A)	Nb33	mGsi	GPA	cAMP	GIRK
DAMGO	6.8 ± 0.08	7.1 ± 0.11	8.0 ± 0.14	8.0 ± 0.14	7.1 ± 0.06
Fentanyl	7.4 ± 0.08	7.7 ± 0.11	8.8 ± 0.16	8.8 ± 0.16	8.2 ± 0.08
Methadone	6.5 ± 0.08	7 ± 0.05	8.1 ± 0.16	8.1 ± 0.16	ND
Morphine	6.5 ± 0.04	6.8 ± 0.06	7.8 ± 0.19	7.8 ± 0.19	6.9 ± 0.03
Oxycodone	5.8 ± 0.11	6.1 ± 0.1	7.0 ± 0.24	7.0 ± 0.24	ND
Oliceridine	6.7 ± 0.17	7.4 ± 0.08	8.2 ± 0.24	8.2 ± 0.24	7.5 ± 0.04
PZM21	6.9 ± 0.18	7.2 ± 0.1	8.0 ± 0.27	8.0 ± 0.27	7.3 ± 0.03
SR-17018	7.1 ± 0.37	6.6 ± 0.38	6.1 ± 0.58	6.1 ± 0.58	5.5 ± 0.07
Buprenorphine	7.6 ± 0.2	8.0 ± 0.14	8.6 ± 0.13	8.6 ± 0.13	7.0 ± 0.06

log(τ/K_A)	βArr2	βArr2 (GRK2)	
DAMGO	6.5 ± 0.09	7.6 ± 0.09	
Fentanyl	6.8 ± 0.06	8.0 ± 0.08	
Methadone	6.3 ± 0.05	7.2 ± 0.11	
Morphine	6.0 ± 0.14	7.2 ± 0.14	
Oxycodone	5.2 ± 0.3	6.1 ± 0.07	
Oliceridine	5.7 ± 0.28	7.5 ± 0.07	
PZM21	6.0 ± 0.37	7.3 ± 0.07	
SR-17018	6.7 ± 0.22	6.7 ± 0.5	

Table S2. G protein efficacy estimated from previous publications. G protein efficacy estimates from previous papers describing biased MOR ligands. Efficacy, τ, was estimated from equation described in Black *et al*. [71]:

$$\alpha = \frac{E_m * \tau}{1 + \tau}$$

This relates the asymptote of the concentration-response curve, α, to the efficacy of the test compound, τ, relative to the maximum response of the system, E_m, defined here by the maximal response to DAMGO.

Paper, assay, cell type, receptor	**Compound**	**α ± S.E.M.**	**Efficacy (τ)**
Manglik *et al*. (2016) $G_{i/o}$ GloSensor, HEK293, hMOR	DAMGO	1 ± 0.01	--
	Morphine	0.89 ± 0.02	8.1
	Oliceridine (TRV130)	0.66 ± 0.02	1.9
	PZM21	0.76 ± 0.03	3.2
Schmid *et al*. (2017) GTPγS binding, CHO, mMOR	DAMGO	100	--
	Morphine	82 ± 1	4.6
	SR-17018	72 ± 1	2.6
Present study, mGsi recruitment BRET, HEK293, mMOR	DAMGO	100	--
	Morphine	80 ± 4	4
	Oliceridine (TRV130)	51 ± 4	1
	PZM21	53 ± 3	1.1
	SR-17018	30 ± 4	0.4
Present study, GIRK activation membrane-potential dye, HEK293, mMOR	DAMGO (untreated)	100	--
	DAMGO + β-CNA	76 ± 5	3.2
	Morphine + β-CNA	57 ± 3	1.3
	Oliceridine + β-CNA	39 ± 2	0.6
	PZM21 + β-CNA	40 ± 4	0.7
	SR-17018 + β-CNA	35 ± 7	0.5