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Genetic behavioral screen identifies an orphan anti-opioid system

Dandan Wang[1], **Hannah M. Stoveken**[1], **Stefano Zucca**[1], **Maria Dao**[1], **Cesare Orlandi**[1], **Chenghui Song**[1], **Ikuo Masuho**[1], **Caitlin Johnston**[1], **Karla J. Opperman**[1], **Andrew C. Giles**[1], **Matthew S. Gill**[2], **Erik A. Lundquist**[3], **Brock Grill**[1,*], **Kirill A. Martemyanov**[1,*]

[1]Department of Neuroscience, The Scripps Research Institute, Jupiter, FL, 33458, USA

[2]Department of Molecular Medicine, The Scripps Research Institute, Jupiter, FL, 33458, USA

[3]Department of Molecular Biosciences, The University of Kansas, Lawrence, KS, 66045, USA

Abstract

Opioids target the μ-opioid receptor (MOR) to produce unrivaled pain management but their addictive properties can lead to severe abuse. We developed a whole animal behavioral platform for unbiased discovery of genes influencing opioid responsiveness. Using forward genetics in *C. elegans*, we identified a conserved orphan receptor, GPR139, with anti-opioid activity. GPR139 is coexpressed with MOR in opioid-sensitive brain circuits, binds to MOR and inhibits signaling to G proteins. Deletion of GPR139 in mice enhanced opioid-induced inhibition of neuronal firing to modulate morphine-induced analgesia, reward, and withdrawal. Thus, GPR139 could be a useful target for increasing opioid safety. These results also demonstrate the potential of *C. elegans* as a scalable platform for genetic discovery of GPCR signaling principles.

G protein coupled receptors (GPCRs) constitute the largest class of cell surface receptors and mediate sensory perception and cellular communication via hormones and neurotransmitters (1, 2). GPCRs function in various diseases and are prominent drug targets (3–5). There has been tremendous progress in understanding the molecular mechanisms of GPCR signaling stemming from identification of key components including G protein subunits, β-arrestins, downstream effectors, and regulatory proteins (6, 7). The majority of these components were discovered serendipitously leaving many critical questions about GPCR organization and function open. For one, many receptors are considered ‘orphan’

*Co-corresponding authors: kirill@scripps.edu; bgrill@scripps.edu.

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Competing Interests: B.G. and K.A.M have filed a patent on the utility of GPR139 as a drug target.

Data and Material availability: All data are available in the manuscript or the supplementary material. Reagents developed in this study are freely available upon request.

Supplementary Materials
Figs.S1–S14
Table S1
Movies S1–S10
References (42–45)

with poorly understood biology and unclear roles in cellular signaling (8, 9). Mechanisms that generate diverse physiological effects are not fully understood. Finally, how individual GPCRs adjust signaling in response to changes in the environment or circuit activity remains unclear.

Insufficient understanding of GPCR signaling hampers their targeting by drugs in a safe and effective manner. This is well illustrated by opioid analgesics that act on the μ-opioid receptor (MOR) and offer unsurpassed efficacy for pain management (10, 11). However, opioid drugs have substantial liabilities including dependence, tolerance and somatic side effects (12). Extensive investigation of MOR pharmacology led to the concept that activated MOR triggers distinct signaling events that differentially control various physiological reactions (13). As a result, identification of molecules that control MOR signaling in endogenous neural circuits remains critical, and could provide new pharmacological targets for increasing the efficacy and safety of opioid analgesics.

Opioid stimulation of MOR affects the nervous system and produces effects that are inherently behavioral in nature (14–16). Thus, screens for modulators of opioid signaling that use behavior as an ultimate readout could accelerate the relevance and translatability of discoveries. Fortunately, GPCR signaling is highly conserved and has been studied across mammalian and invertebrate model systems (17–19). Genetic studies in *C. elegans* have allowed discovery and evaluation of many conserved players in GPCR signaling, and elucidated their roles in neural circuits (19, 20). Furthermore, transgenic expression of mammalian GPCRs alters the behavior of *C. elegans,* and these heterologous GPCRs desensitize in response to ligands (21). *C. elegans* also has an opioid-like system that controls feeding behavior and responses to noxious stimuli (22, 23). These considerations prompted us to develop a transgenic *C. elegans* platform we used in an unbiased, forward genetic screen for regulators of MOR-controlled behavior.

Development of *C. elegans* platform for unbiased genetic discovery of opioid modulators

To study MOR signaling using a behavioral platform that can be scaled to cover an entire genome, we generated transgenic *C. elegans* expressing mammalian MOR throughout the nervous system (tgMOR, Fig. 1A, B). Because opioid agonists exert effects on motor activity in mammals, we assessed the effects of MOR activation on *C. elegans* locomotion. Exposure of tgMOR worms to fentanyl, a MOR agonist, reduced their movement (Fig. 1C; Suppl. Movies 1–10). Quantitation showed fentanyl inhibited thrashing of tgMOR animals over time (Fig. 1D). TgMOR animals rapidly recovered from paralysis in the presence of fentanyl indicating conservation of receptor desensitization mechanisms (Fig. 1D). Fentanyl did not affect non-transgenic wt animals, indicating that changes in motor activity result from activation of transgenic MOR (Fig. 1C **and** D; Suppl. Movies 1–10).

Higher concentrations of fentanyl accelerated response onset and subsequent recovery but the duration of paralysis was not affected (Fig. 1E **and** F; Fig. S1A **and** C). Similar behavior was also observed with morphine, a full MOR agonist with distinct pharmacological properties (Fig S1D **and** F) (24, 25). Consistent with morphine having lower potency on

MOR (25), approximately 50-fold higher concentration of morphine was required for maximal effect compared to fentanyl (Fig. 1G **and** H). Although morphine produced a similar magnitude of effect as fentanyl, it had a distinct temporal profile with faster onset and more rapid recovery (Fig. 1G; Fig. S1D). Treatment of tgMOR animals with naloxone, a MOR antagonist, abolished the effect of fentanyl (Fig. 1I). Thus, our tgMOR platform can rapidly evaluate behavioral effects of opioids, and distinguish pharmacological effects and properties of different drugs.

To probe whether conserved molecular mechanisms control opioid signaling, we evaluated opioid-induced behavior in tgMOR animals lacking R7 Binding Protein 1 (RSBP-1). RSBP-1 is orthologous to mammalian Regulator of G protein signaling 7 Binding Protein (R7BP), a subunit of the complex that negatively regulates MOR signaling in mice (Fig. 1A) (26, 27). *rsbp-1* loss-of-function mutants carrying tgMOR reached maximum paralysis and recovered more quickly than tgMOR animals treated with fentanyl (Fig. 1J; Fig. S1B) or morphine (Fig. 1K; Fig. S1E). Dose-response studies with tgMOR; *rsbp-1* mutants showed a prominent left-ward shift in concentration dependence for both fentanyl and morphine (Fig. S1C **and** F). Thus, tgMOR; *rsbp-1* mutants are hypersensitive to opioids, an outcome similar to R7BP deletion in mice (27). Taken together, these observations indicate that opioid signaling via MOR can be effectively modeled in *C. elegans* producing behavioral reactions mediated by conserved GPCR signaling machinery that functions independent of organism-specific neuronal circuitry.

Forward genetic screen identifies genes affecting behavioral sensitivity to opioids

The effects of opioids on tgMOR *C. elegans* and the molecular conservation of regulatory mechanisms prompted us to adopt this platform for an unbiased, forward genetic screen for regulators of opioid signaling (Fig 2A). We focused on identifying mutants with increased opioid sensitivity to uncover negative regulators of MOR signaling.

Key to the design of our screen was the observation that greater opioid response leads to faster paralysis and more rapid recovery. Thus, hypersensitive animals like tgMOR; *rsbp-1* recover faster from the same drug dose than do tgMOR animals (Fig. 1J **and** K). As a result, bulk segregation on plates was used to isolate hypersensitive mutants based on their quicker recovery from opioid-induced paralysis and escape from the starting zone (Fig. 2A). Assay optimization with a mixture of tgMOR animals and hypersensitive tgMOR*; rsbp-1* mutants showed that primary screening with morphine followed by secondary screening with fentanyl minimized false positive rates (Fig. 2A).

For the full-scale screen, we mutagenized ~2,500 tgMOR animals, evaluated ~600,000 progeny, and identified ~900 mutants with abnormal sensitivity to both morphine and fentanyl (Fig. 2B). Secondary evaluation in liquid thrashing assays with fentanyl eliminated false positives, identified mutants that lost opioid sensitivity, and confirmed a small number of hypersensitive mutants (Fig. 2B). We focused our efforts on comprehensive testing of opioid-induced behaviors for two mutants, tgMOR; *bgg8* and tgMOR; *bgg9*. Both mutants had normal overall morphology and behavior in the opioid naïve state, but were paralyzed

by fentanyl significantly faster than tgMOR worms (Fig. 2C **and** D). Additional dose-response studies showed a leftward shift in fentanyl-induced paralysis indicating that tgMOR; *bgg8* and tgMOR; *bgg9* mutants are hypersensitive to opioids (Fig. S2).

We mapped genetic lesions causing hypersensitivity by combining whole-genome sequencing with phenotypic selection (Fig. S3A). This process identified genomic regions of interest (3-5 Mb) that contained approximately 6 to 8 different lesions per mutant. To determine which lesion caused opioid hypersensitivity, we used CRISPR/Cas9 to edit single mutations into candidate genes of tgMOR animals (Fig. S3A).

For tgMOR; *bgg8* animals, we identified a lesion in the calcium channel *egl-19* that introduced a premature stop codon and likely resulted in loss of function (Fig. S3B). CRISPR/Cas9 editing the same *egl-19* mutation into parental tgMOR animals confirmed *egl-19* affects opioid sensitivity (Fig. 2C; Fig. S4A, B). Notably, *egl-19* is homologous to L-type Ca^{2+} channels in mammals and extensive evidence indicates that L-type Ca^{2+} blockers potentiate the nociceptive properties of opioids in a clinical setting (28, 29). These observations demonstrate that our forward genetic screen identified conserved regulators of MOR signaling.

Another hypersensitive mutant, tgMOR; *bgg9*, contained a premature stop in *frpr-13* which encodes an unstudied orphan GPCR (Fig. S3C). CRISPR/Cas9 editing of this lesion into tgMOR increased sensitivity to fentanyl, confirming that *frpr-13* affects opioid sensitivity (Fig. 2D; Fig. S4C **and** D). Because the function of FRPR-13 is unknown, we further validated that it regulates opioid responses by transgenically expressing FRPR-13 in tgMOR; *bgg9* mutants (Fig S3A). FRPR-13 expressed using the native *frpr-13* promoter and Mos single copy insertion (MosSCI) reversed the hypersensitivity of *frpr-13 (bgg8)* mutants back to a normal response (Fig. 2E; Fig. S4E **and** F). Similarly, hypersensitivity of *frpr-13 (bgg8)* mutants was reversed when FRPR-13 was pan-neuronally expressed with MosSCI (Fig. 2E). Collectively, these results indicate that the FRPR-13 receptor alters sensitivity to opioids at a behavioral level.

FRPR-13/GPR139 negatively regulates MOR signaling.

Phylogenetic analysis revealed that FRPR-13 belongs to a large neuropeptide receptor group in *C. elegans* that is similar to two mammalian orphan GPCRs, GPR139 and GPR142 (Fig. S5). GPR139 and GPR142 are in a distinct subfamily of class A orphan receptors (30). Given that nothing is known about FRPR-13 and there is no prior connection between GPR139/142 and opioid signaling, we explored the functional conservation of these receptors. We focused on GPR139 because it is expressed in the central nervous system, whereas GPR142 is predominantly found in the periphery (31, 32). Transgenic expression of human GPR139 in tgMOR; *bgg9* worms with disrupted FRPR-13 significantly reversed hypersensitivity to fentanyl (Fig. 2F). This indicates GPR139 is a functional ortholog of FRPR-13, and GPR139 can inhibit MOR signaling *in vivo*.

We used a panel of assays to evaluate how GPR139 influences MOR signaling in mammalian Human Embryonic Kidney (HEK) 293T cells. MOR activation drove rapid

hyperpolarization of membrane potential upon reconstitution with the G protein-gated Inwardly Rectifying K+ (GIRK) channel (Fig. 3A **and** B). Introduction of GPR139 cDNA in equivalent concentrations to MOR inhibited morphine-induced hyperpolarization, whereas overexpression of GPR139 in high amounts nearly abolished GIRK activation (Fig. 3B **and** C). GPR139 co-immunoprecipitated with MOR indicating that these receptors can interact in a model cellular environment (Fig. 3D; Fig. S6A). The relevance of this interaction in an endogenous context remains to be established. We detected significant reduction of MOR at the cell surface when GPR139 was expressed at high levels suggesting that GPR139 can impede MOR trafficking (Fig. 3E **and** F). Yet, at stoichiometric levels, GPR139 had no effect on surface localization of MOR suggesting that GPR139 has other mechanisms to inhibit MOR (Fig. 3F). Indeed, at these lower stoichiometric levels GPR139 promoted association of the signaling inhibitor β-arrestin with MOR (Fig. 3G **through** I). This suggests that GPR139 has some constitutive activity that is sufficient to trigger β-arrestin recruitment. To further understand the implications of GPR139-MOR heteromerization and ensuing increased β-arrestin recruitment, we tested how GPR139 influenced MOR-mediated activation of G proteins with a Bioluminescence Resonance Energy Transfer (BRET) assay (Fig. 3J) (33). Morphine produced a rapid BRET response reflecting rearrangement in Gαo-Gβγ heterotrimers induced by MOR activation (Fig. 3K **and** L). Coexpression of GPR139 at low amounts inhibited MOR-induced G protein activation (Fig. 3K **and** L; Fig. S6B). This inhibitory effect was more pronounced if GPR139 was expressed at high amounts due to additional loss of MOR from the surface. Together, these results indicate that GPR139 can exert inhibitory effects on MOR in a cell-autonomous manner by affecting both receptor trafficking and signaling properties.

To probe the physiological relevance of inhibitory influences of GPR139 on opioid signaling in the mammalian nervous system, we used mouse models. GPR139 was expressed in brain regions implicated in opioid actions on reward, analgesia and withdrawal (Fig. 4A **and** E; Fig. S7) (31, 32). GPR139 was extensively coexpressed with MOR in a number of neuronal populations in these areas, most prominently in medial habenula (MHb) and locus coeruleus (LC) (Fig. 4A **and** E; Fig. S7; Table S1). To test the role of GPR139 on opioid modulation we obtained *Gpr139* knockout mice (*Gpr139*$^{-/-}$, Fig. S8). We performed patch clamp recordings of MHb neurons in brain slices with drugs that block synaptic communication and circuit activity. In slices from *Gpr139*$^{+/+}$ animals, MOR activation resulted in dose-dependent inhibition of spontaneous firing (Fig. 4B **and** C). Firing of MHb neurons from *Gpr139*$^{-/-}$ mice was significantly reduced by low-level MOR activation that did not cause an effect in *Gpr139*$^{+/+}$ neurons (Fig. 4B **and** C). Furthermore, *Gpr139*$^{-/-}$ neurons showed more pronounced net inhibition by DAMGO, a synthetic enkephalin-mimetic peptide (Fig. 4D; Fig. S9A **and** B). Recovery upon drug washout was delayed in *Gpr139*$^{-/-}$ neurons which indicates greater susceptibility to opioid inhibition (Fig. S9C). Hypersensitivity to morphine-induced inhibition of firing in LC neurons also occurred in *Gpr139*$^{-/-}$ mice (Fig. 4F **and** G). GPR139 ablation resulted in increased basal firing rates selectively in LC but not MHb neurons (Fig. S9D). Taken as a whole, these findings indicate that GPR139 counteracts MOR cell-autonomously in endogenous physiologically relevant neuronal settings as well as reconstituted systems.

GPR139 modulates behavioral responses to opioids

To understand how GPR139 influences opioid actions *in vivo*, we evaluated mouse behavior. Deletion of GPR139 had no overt effects on animal health and body composition (Fig. S10A–C). *Gpr139*$^{-/-}$ mice also had normal baseline learning (Fig. 5A), nociception (Fig. 5B), locomotor activity (Fig. S10D), habituation to a novel environment (Fig. S10E) and motor coordination (Fig S10F). However, responses of *Gpr139*$^{-/-}$ mice to morphine were increased. When tested in a conditioned place preference (CPP) paradigm, *Gpr139*$^{-/-}$ mice showed augmented responses to the rewarding effects of morphine (Fig. 5A) in agreement with increased opioid sensitivity of *Gpr139*$^{-/-}$ MHb neurons (Fig. 4B **through** D), a region involved in drug reward (34). Similarly, *Gpr139*$^{-/-}$ mice exhibited significantly increased morphine analgesia in thermal (Fig. 5B **and** C; Fig. S11A) and mechanical (Fig. S11B) pain paradigms. This augmentation was evident from increases in both maximal response and effect duration across multiple morphine doses (Fig. 5B **and** C; S11A **and** B). Thus, deletion of GPR139 broadly increases sensitivity to the acute effects of morphine. Termination of chronic morphine administration caused lower somatic withdrawal in mice lacking GPR139 across a spectrum of measures (Fig. 5D; Fig. S12). The diminished withdrawal observed in *Gpr139*$^{-/-}$ mice may be related to observed changes in baseline firing rate seen in *Gpr139*$^{-/-}$ LC neurons (Fig. 4F; Fig. S9D), a neuronal population involved in opioid withdrawal (35).

To test the translational relevance of our findings, we examined the effects of JNJ-63533054, a surrogate ligand that facilitates GPR139 actions (32). Administration of JNJ-63533054 dose-dependently diminished morphine analgesia in both thermal and mechanical pain paradigms (Fig 5E). These effects were not observed in *Gpr139*$^{-/-}$ mice, indicating specificity of JNJ-63533054 actions (Fig. S13). To determine if activating GPR139 affects reward, we examined the effects of JNJ-63533054 in a morphine self-administration paradigm. Following escalation of morphine intake, wild-type mice were divided into 2 groups with alternating exposure to JNJ-63533054. Administration of drug suppressed morphine intake (Fig. 5F; Fig. S14). The effect of JNJ-63533054 was dose-dependent and completely reversible upon cessation of exposure (Fig. 5F; Fig. S14). Overall, these *in vivo* results indicate that GPR139 negatively regulates a number of responses to acute opioid exposure, and potentiates withdrawal from chronic opioid administration.

Discussion

We developed a *C. elegans* behavioral platform for the unbiased genetic discovery of GPCR signaling modulators. Transgenic GPCR expression endows animals with the ability to respond to a foreign chemical modality, akin to chemogenetic approaches used to interrogate mammalian circuitry and behavior (36). The platform displays cardinal features of behavioral responses to receptor activation, allowing phenotypic interrogation of signaling pathways using intact neuronal circuitry *in vivo*. Use of behavior as an ultimate readout provides a high degree of relevance and potential translational validity. Characterization of tgMOR *C. elegans* revealed cross-species conservation of critical GPCR signaling elements. This transgenic platform also demonstrated utility in uncovering biology for an opioid receptor signaling network. The scalable nature of our screens may permit further

exploration of signaling mechanisms for GPCRs of interest. Additionally, this approach could be adapted for different neuronal circuitry, behavioral readouts, and other GPCRs thereby expanding potential opportunities for discovery.

Using forward genetic screening we identified an evolutionarily conserved orphan receptor system with anti-opioid activity: FRPR-13 in *C. elegans* and its mammalian ortholog GPR139. Although the full spectrum of GPR139 effects on cellular physiology and mechanisms of suppressing MOR action remain to be elucidated, our examination indicates that some of these actions involve direct inhibitory influences of GPR139 on MOR signaling. Opposing crosstalk between GPCRs is an intriguing concept (37), and our study now adds the poorly understood GPR139 orphan receptor to a growing realm of molecules that oppose MOR (38–40). Notably, α-Melanocyte Stimulating Hormone (a peptide derived from the same precursor as the MOR ligand, β-endorphin) was reported as one endogenous ligand for GPR139 (41). This further argues for the physiological significance of the GPR139-MOR connection and indicates that GPR139 might affect homeostatic control of the endogenous opioid signaling system. Whether GPR139 modulates endogenous opioid function remains to be determined.

Our results suggest that GPR139 could potentially be exploited pharmacologically for increasing safety and efficacy of opioid pharmacotherapy. While our study focused on the anti-opioid effects of GPR139, its widespread expression in the nervous system may indicate that this orphan receptor has additional roles in shaping neuronal physiology independent of MOR.

Materials and Methods

For detailed description of all procedures and methods refer to Supplementary Materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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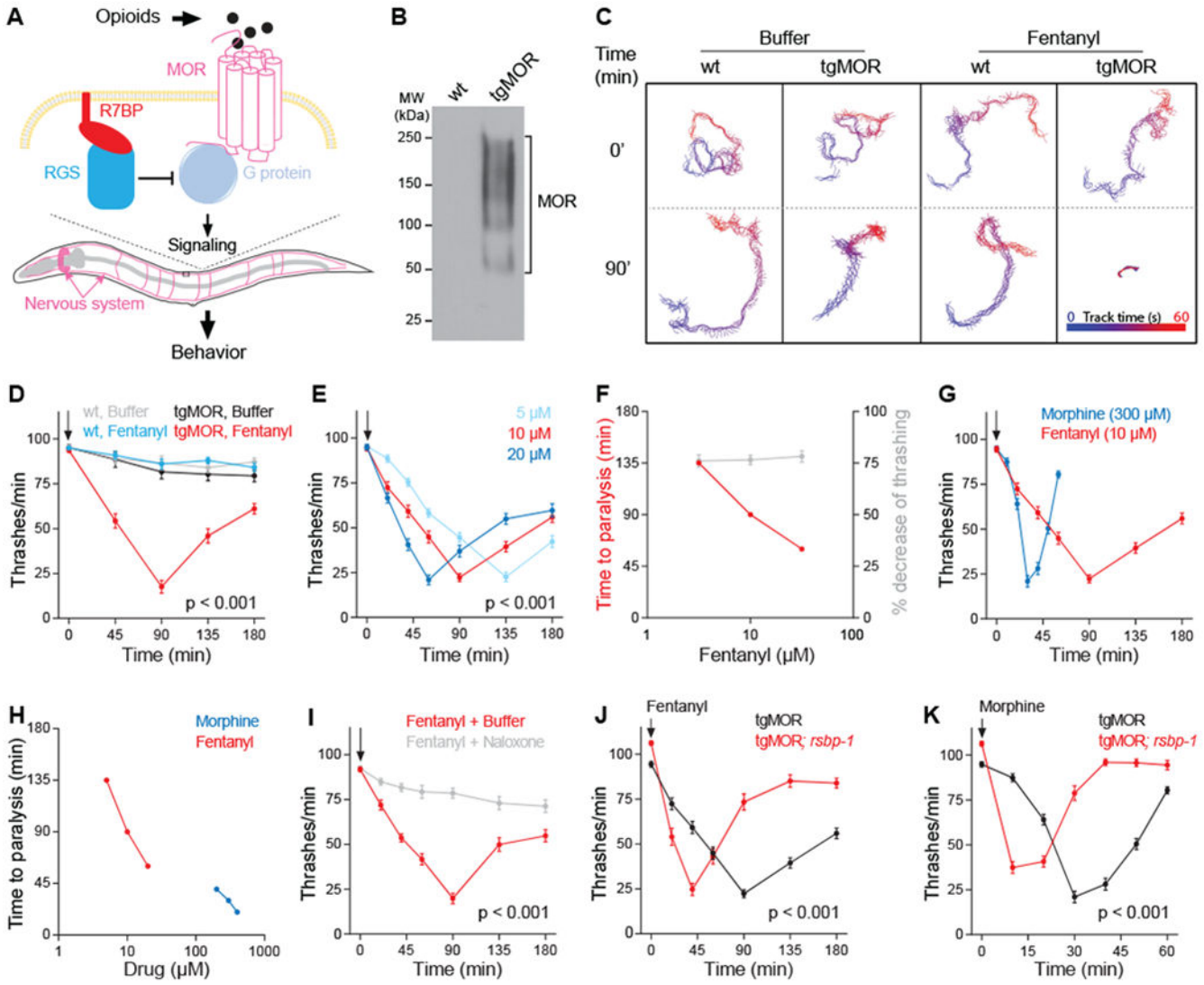

Fig. 1. Transgenic *C. elegans* platform for dissecting opioid signaling mechanisms.

(**A**) Transgenic *C. elegans* model of MOR signaling (tgMOR). (**B**) Western blot showing expression of FLAG::MOR in the nervous system after immunoprecipitation. (**C**) Fentanyl inhibits thrashing of tgMOR. (**D**) Quantitation of fentanyl effects on tgMOR. (**E**) Time course of fentanyl doses on tgMOR. (**F**) Fentanyl dose response for tgMOR. (**G**) Time course for morphine and fentanyl on tgMOR. (**H**) Morphine and fentanyl dose response for tgMOR. (**I**) Naloxone blocks fentanyl effects on tgMOR. (**J, K**) Time courses showing tgMOR; *rsbp-1* mutants are hypersensitive to (**J**) fentanyl and (**K**) morphine. Arrows denote drug application. If not indicated, opioids were used at the following concentrations: fentanyl (10µM), morphine (300µM) and naloxone (20µM). For all genotypes and drug conditions, means are shown from 30 or more animals obtained from three independent experiments. Error bars are S.E.M. Significance tested using two-way ANOVA. P values reported are for genotype/time interactions.

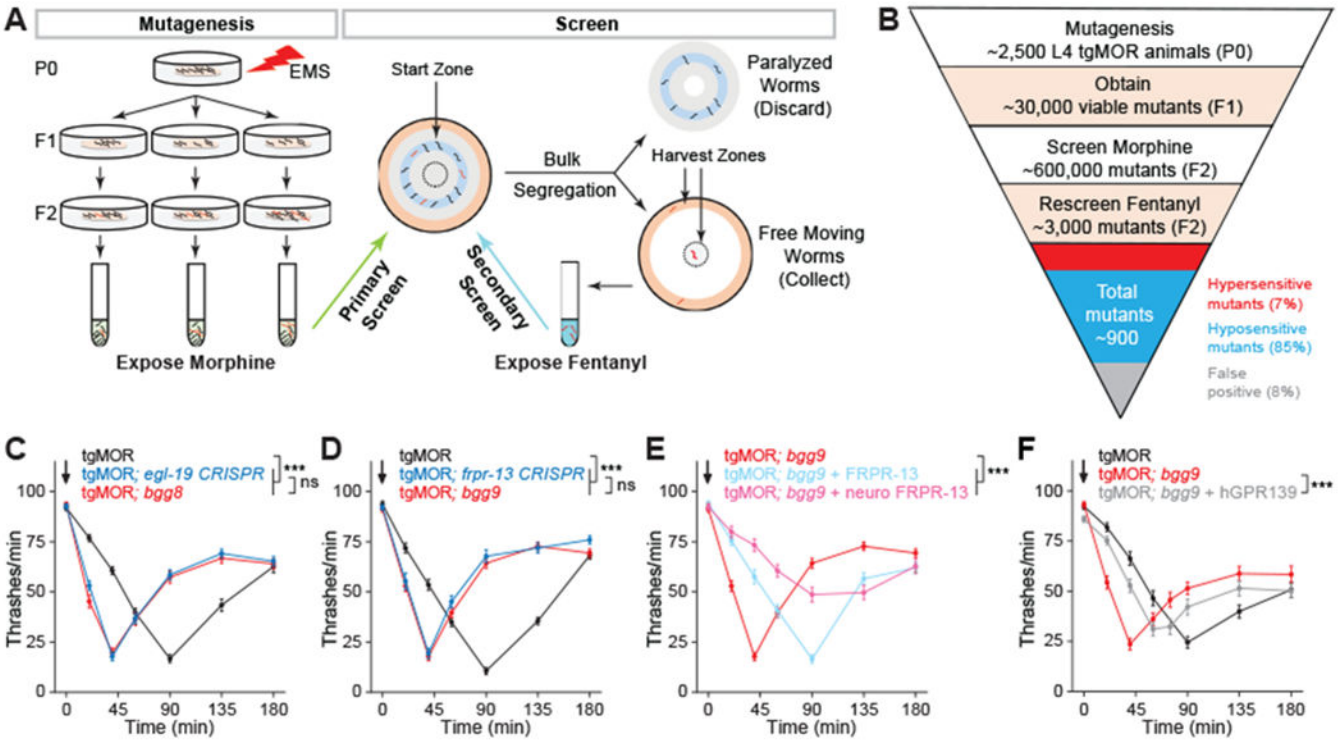

Fig. 2. Forward genetic screen with tgMOR platform identifies orphan receptor FRPR-13 as negative regulator of MOR signaling.

(**A**) Two-step genetic screen for tgMOR mutants with altered opioid sensitivity. (**B**) Outline of steps, generations, number of independent mutants isolated, and phenotypic categories observed for genetic screen with tgMOR. (**C**) tgMOR; *bgg8* mutants are hypersensitive to fentanyl and CRISPR/Cas9 editing validates *egl-19* as gene causing hypersensitivity. (**D**) tgMOR; *bgg9* mutants are hypersensitive to fentanyl and CRISPR/Cas9 editing validates *frpr-13* as gene causing hypersensitivity. (**E**) Transgenic expression of FRPR-13 using native or neuronal promoters reverses fentanyl hypersensitivity in tgMOR; *bgg9* animals. (**F**) Transgenic expression of human GPR139 reverses fentanyl hypersensitivity in tgMOR; *bgg9* animals. Arrows denote fentanyl (10μM) application. For all genotypes and drug conditions, means are shown from 30 or more animals obtained from three independent experiments. Error bars are S.E.M. Significance tested using two-way ANOVA. *** $p<0.001$ and ns = not significant

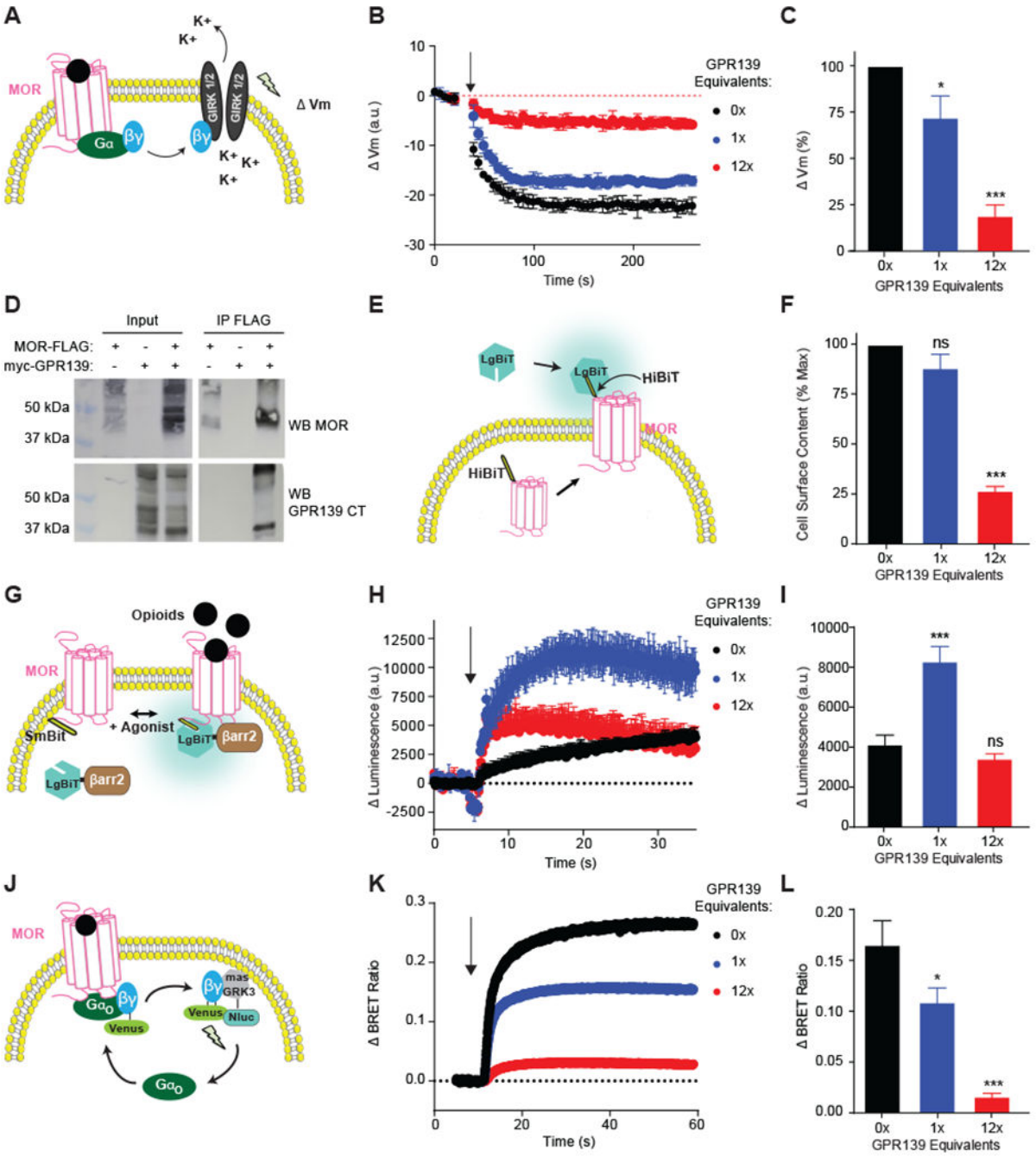

Fig. 3. GPR139 inhibits MOR signaling.

(**A**) Experimental design for evaluating MOR signaling via its effector GIRK. MOR activation leads to Gβγ subunit release, which opens GIRK channels to produce membrane hyperpolarization (Vm) that is measured with voltage sensitive dye. (**B**) Coexpression of GPR139 inhibits MOR-mediated kinetics of membrane potential change in response to morphine (0.1 μM). (**C**) Quantification shows GPR139 reduces morphine effects on Vm amplitude. (**D**) Co-immunoprecipitation of MOR-FLAG and myc-GPR139 following their coexpression. (**E**) Experimental design for evaluating cell surface abundance of MOR.

HiBiT-tagged MOR complements the LargeBiT (LgBiT) nanoluciferase enzyme only at the plasma membrane. (**F**) Quantification of the maximal cell surface content of HiBiT-MOR indicates that GPR139 inhibits MOR surface localization only at high (12X) expression levels. (**G**) Experimental design for evaluating agonist-induced β-arrestin recruitment to MOR. Recruitment of β-arrestin2-LgBiT to SmBiT-MOR generates a functional nanoluciferase enzyme. (**H**) Effect of GPR139 coexpression on the kinetics of β-arrestin2-LgBiT recruitment induced by DAMGO (10 μM). (**I**) Quantification shows that low level GPR139 coexpression increases the extent of β-arrestin2 recruitment to MOR. (**J**) Experimental design for evaluating MOR signaling to G proteins by BRET assay that monitors MOR-mediated release of Gβγ subunits. (**K**) Effect of GPR139 coexpression on the kinetics of G protein activation by MOR in response to morphine (1 μM) application. (**L**) Quantification shows GPR139 coexpression reduces maximal BRET response of MOR-Gαo signaling. All experiments were performed in HEK293T cells. In all panels, means are shown from 3-5 independent experiments with 3-4 replicates each ± S.E.M. Significance tested by one-way ANOVA with Dunnett's *post-hoc* test. *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Arrows denote application of opioids.

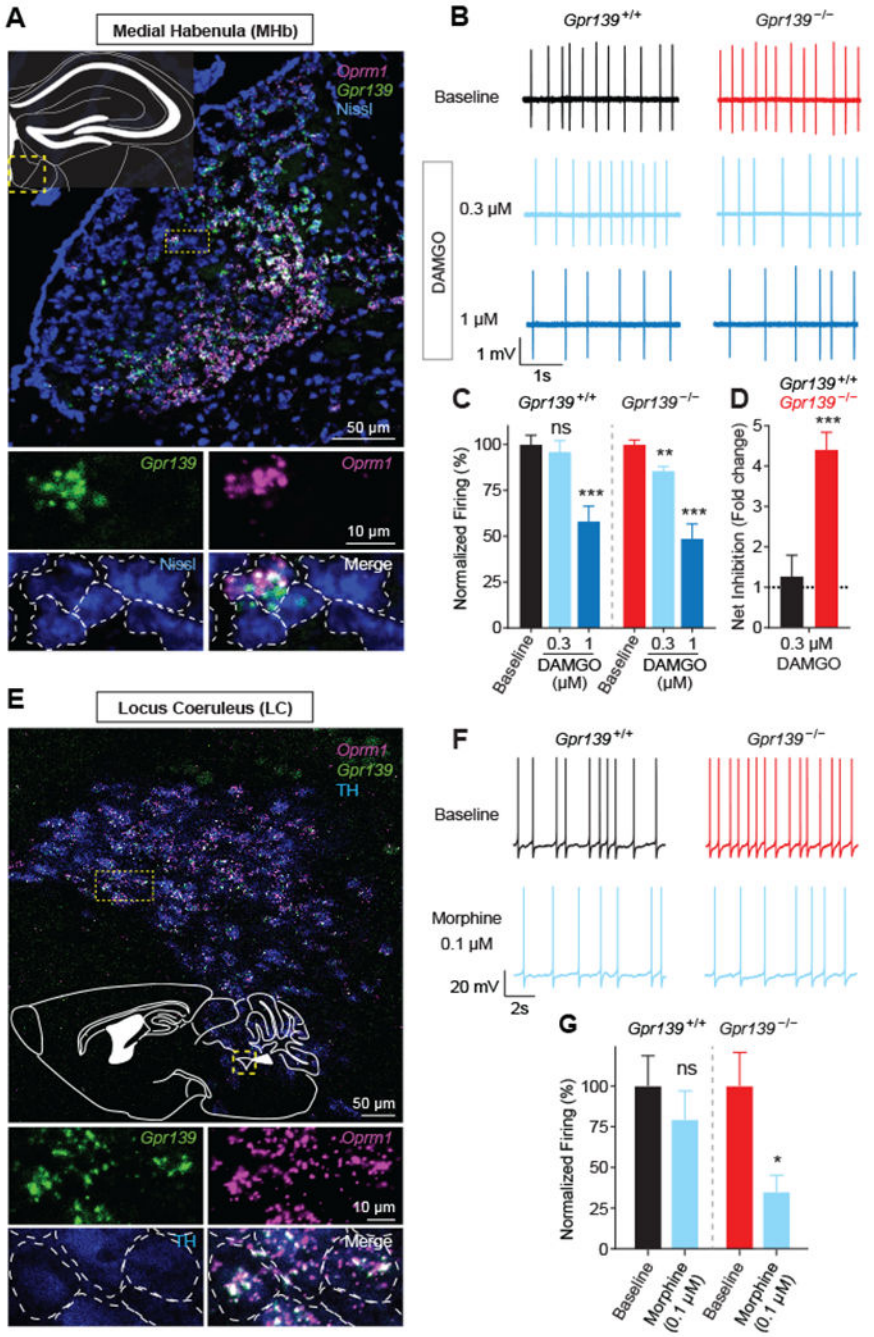

Fig. 4. GPR139 inhibits opioid modulation of neuronal firing.

(**A**) *In situ* hybridization showing extensive coexpression of MOR mRNA (*Oprm1)* and *Gpr139* in medial habenula (MHb) neurons. (**B**) Representative traces showing changes in MHb neuron firing in response to different doses of DAMGO in $Gpr139^{+/+}$ and $Gpr139^{-/-}$ mice. (**C**) Quantification of normalized firing frequency in MHb neurons shows responsiveness to low DAMGO concentration (0.3μM) in $Gpr139^{-/-}$ but not $Gpr139^{+/+}$ (n = 11 cells from 6 mice per genotype). (**D**) Quantification shows MHb neurons from $Gpr139^{-/-}$ animals have increased net inhibition of neuronal firing following DAMGO treatment. (**E**) *In*

situ hybridization showing *Oprm1* and *Gpr139* coexpression in locus coeruleus (LC) neurons. (**F**) Representative traces showing changes in LC neuron firing in response to morphine in $Gpr139^{+/+}$ and $Gpr139^{-/-}$ mice. (**G**) Quantification indicates morphine inhibits firing of LC neurons from $Gpr139^{-/-}$ mice but not $Gpr139^{+/+}$ animals (n = 7-9 cells from 4-6 mice per genotype). All results were reported as mean ± SEM. Significance tested using unpaired Students' *t* test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ns = not significant

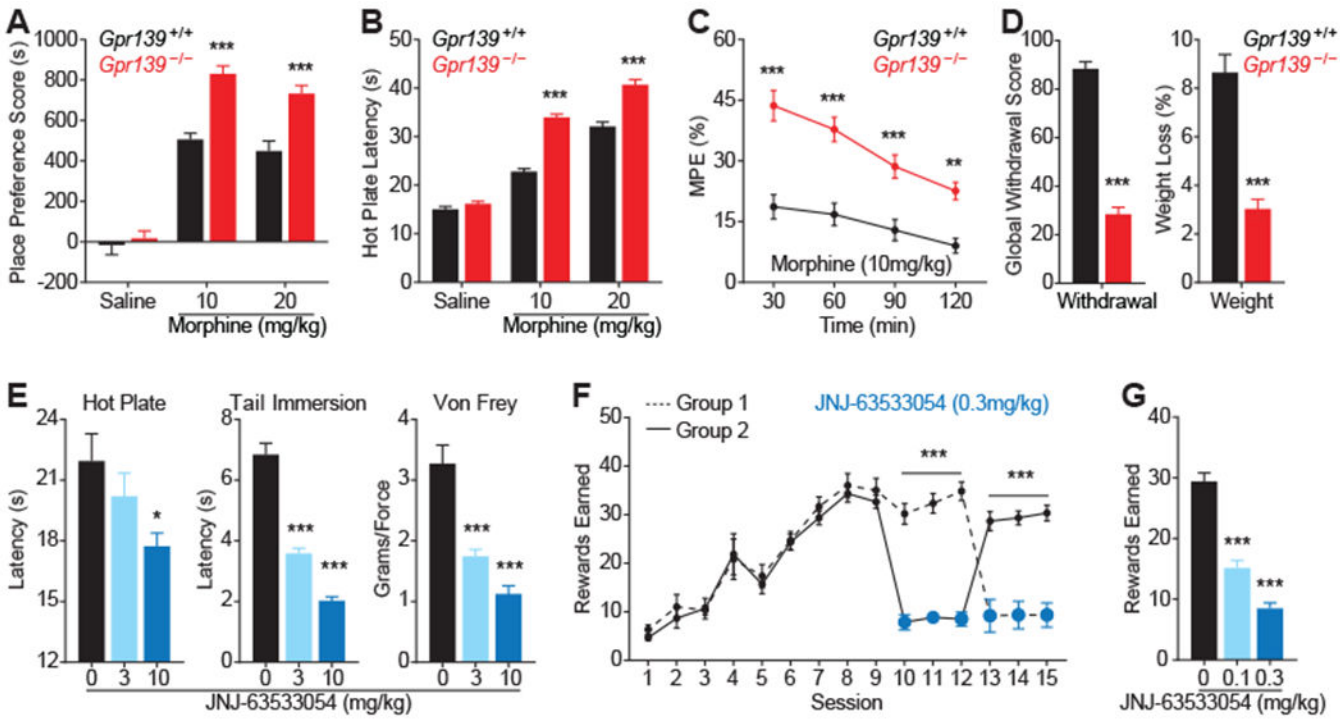

Fig. 5. GPR139 controls behavioral sensitivity of mice to opioid administration.

(A) Conditioned place preference paradigm showing increased reward in *Gpr139*$^{-/-}$ mice. (**B**) Hot plate assay showing increased dose-dependent, anti-nociceptive effects of morphine in *Gpr139*$^{-/-}$ mice. (**C**) *Gpr139*$^{-/-}$ animals have increased duration of morphine analgesia in hot plate assay. (**D**) *Gpr139*$^{-/-}$ *mice* have decreased behavioral responses and weight loss to naloxone-precipitated somatic withdrawal following chronic morphine exposure. Global score reflects aggregate measure of several withdrawal signs (diarrhea, jumps, dog shakes, paw tremor, back walking, tremor and ptosis). (**E**) Augmentation of GPR139 function by JNJ63533054 decreases analgesia induced by morphine (10 mg/kg) across pain models. (**F**) Activation of GPR139 by JNJ63533054 inhibits morphine intake (0.3 mg/kg/infusion) in self-administration task. (**G**) Quantification of JNJ63533054 effects on morphine self-administration. Significance tested using two-way ANOVA or Student's *t*-test. Animal numbers for each test provided in Methods. *** p<0.001, * p<0.05