SUPPLEMENT

The orphan receptor GPR139 signals via Gq/11 to oppose opioid effects

Hannah M Stoveken[1], Stefano Zucca[1], Ikuo Masuho[1], Brock Grill[1] and Kirill A Martemyanov[1]*

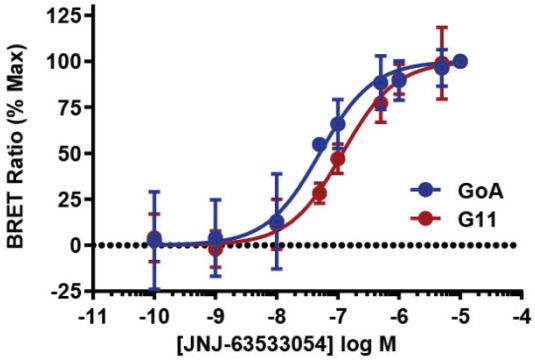

Figure S1. GPR139 activates G11 and GoA with similar potency
Using the G protein BRET fingerprinting assay, the efficacy of activation was measured over multiple doses of JNJ-63533054 and normalized within each experiment to the maximum dose tested. GoA exhibited slightly higher potency compared to G11.

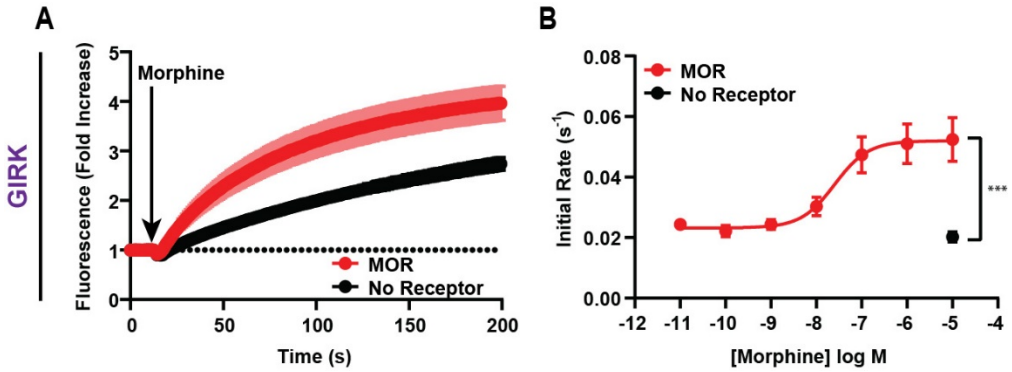

Figure S2. MOR activates GIRK1/2 channels

(A) Thallium flux through GIRK channels in control or MOR-expressing HEK293T cells was measured in response to 10 µM morphine**. (B)** A dose response of morphine was tested and the initial activation rates were calculated in GraphPad Prism8. Data are mean ± S.E.M. of 5 independent experiments. Students unpaired t-test, ***p<0.001.

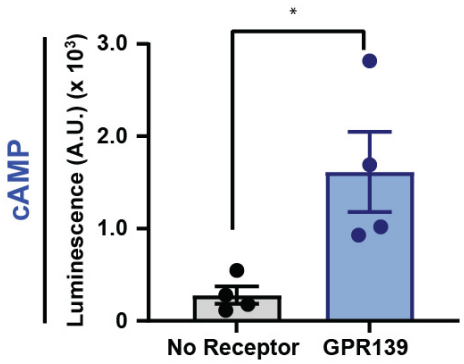

Figure S3. Basal cAMP is increased in GPR139-transfected cells
cAMP was measured in control or GPR139-expressing cells transfected with -22F pGloSensor®. Data are mean ± S.E.M. of 4 independent experiments. Students unpaired t-test, *$p<0.05$.

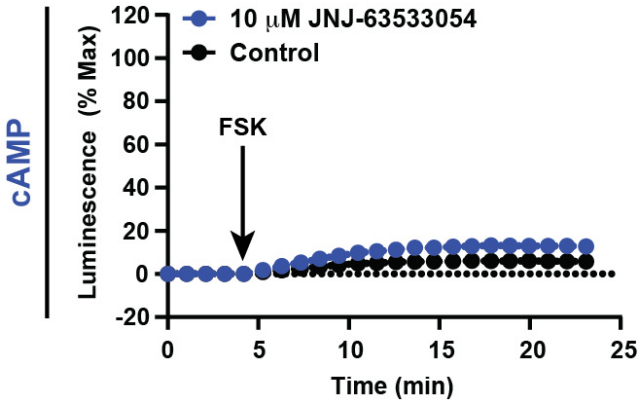

Figure S4. JNJ-63533054 does not enhance cAMP generation in control cells
HEK293T cells transfected with the -22F pGloSensor® were stimulated with 1 μM forskolin and pre-treated with vehicle or 10 μM JNJ-63533054. Data are mean ± S.E.M. of 5 independent experiments and normalized to the maximum response elicited by GPR139-transfected, JNJ-63533054-treated cells (Fig 3A, blue line).

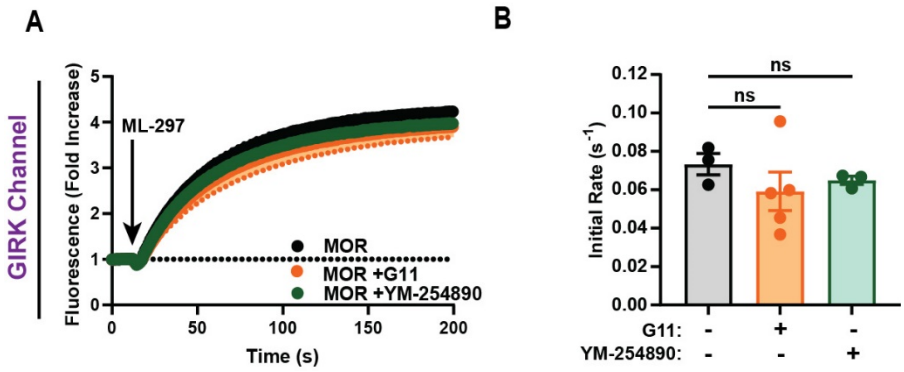

Figure S5. MOR does not affect GIRK opening mediated by ML-297
(A) GIRK channel activity was assessed in MOR-expressing or MOR + G11-expressing HEK293T cells following stimulation with 10 µM ML-297. MOR-expressing cells were additionally treated with vehicle or 10 µM YM-254890. **(B)** Quantification of the initial GIRK channel activation rates. Data are mean ± S.E.M. of 3-5 independent experiments. One-way ANOVA with Tukey's post hoc, ns, not significant.

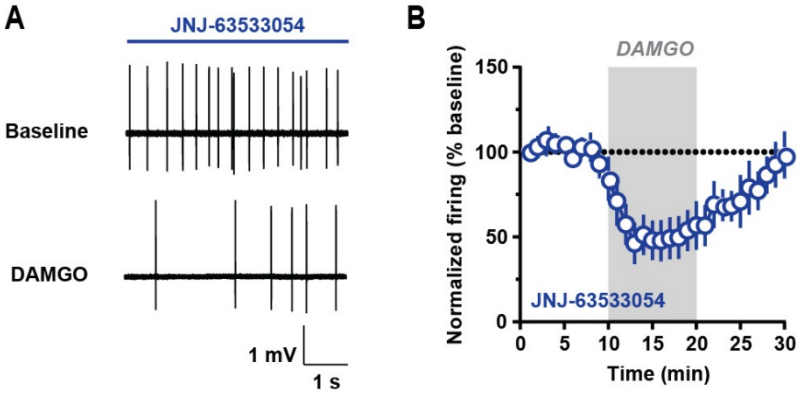

Figure S6. JNJ-63533054 does not affect DAMGO-mediated firing inhibition in *GPR139*$^{-/-}$ mice.

(A) Representative firing traces and **(B)** normalized firing rates recorded from medial habenula neurons in prepared slices from GPR139 knockout mice. Slices were treated with 1 μM DAMGO and pre-treated with 10 μM JNJ-63533.