Supplementary Information

Exploring pharmacological inhibition of $G_{q/11}$ as an analgesic strategy.

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Running Title: Antinociceptive effect of $G_{q/11}$ inhibition in mice.

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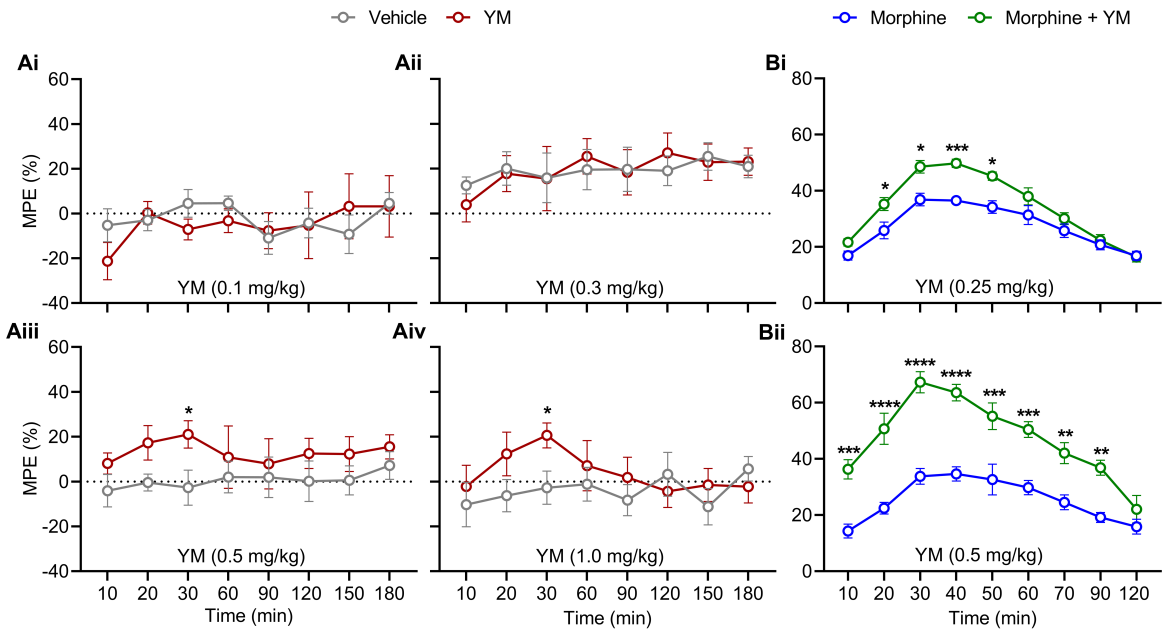
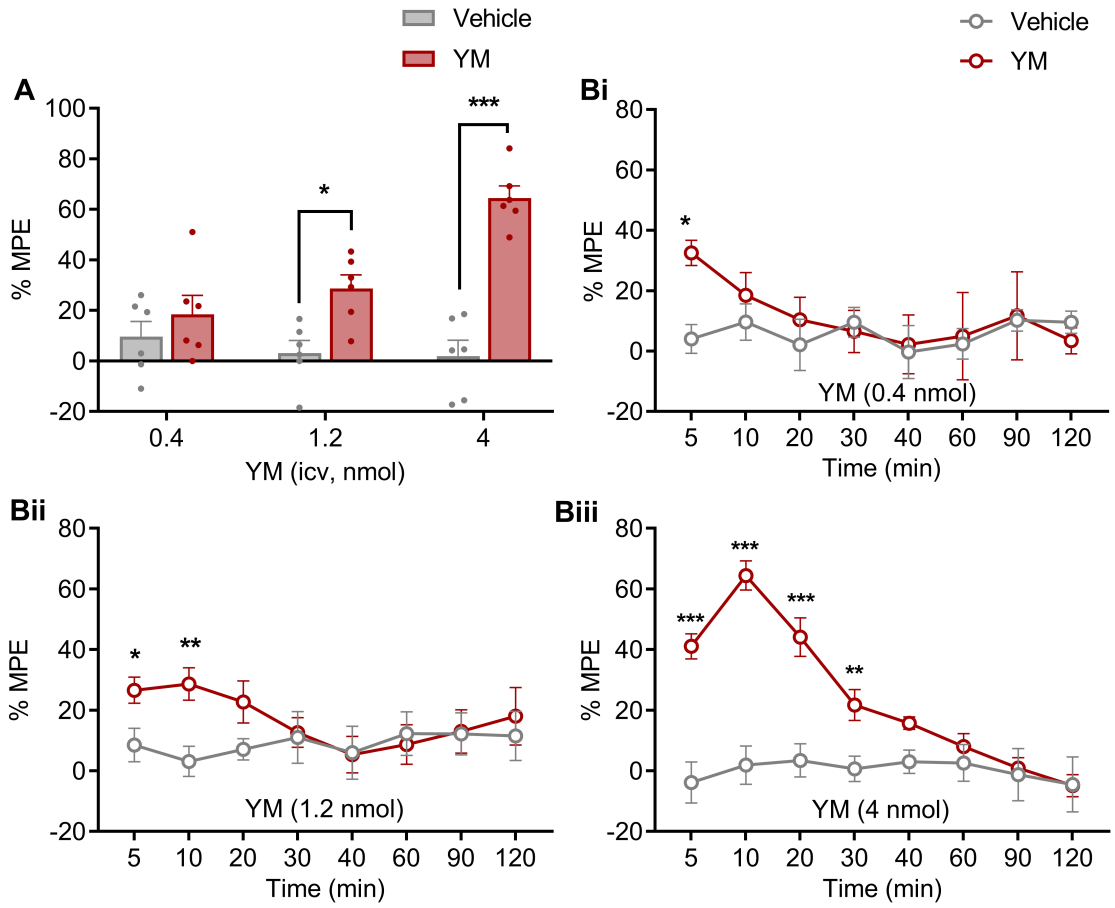


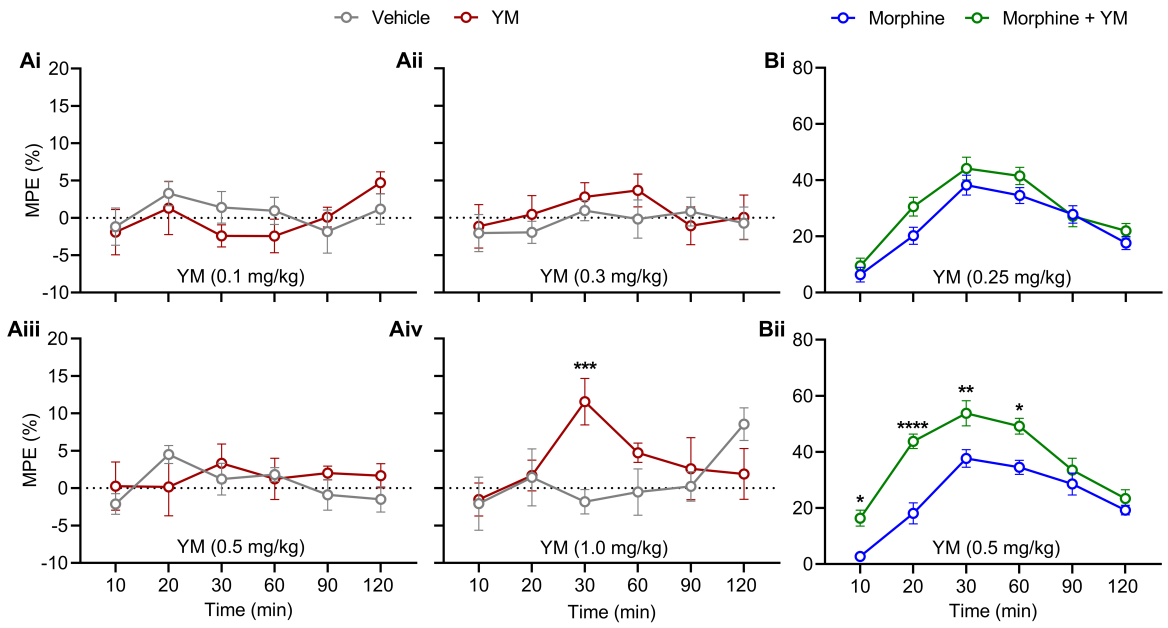
Supplementary Figure S1. The effect of systemic subcutaneous administration of YM on nociception.

A. Dose-response effect of different concentration of subcutaneous YM and vehicle were tested on hot plate test for 3 h. **Ai.** YM 0.1 mg/kg: Treatment $F_{(1, 80)} = 0.31$, time $F_{(7, 80)} = 0.84$, interaction $F_{(7, 80)} = 0.59$. **Aii.** YM 0.3 mg/kg: Treatment $F_{(1, 80)} = 0.001$, time $F_{(7, 80)} = 0.76$, interaction $F_{(7, 80)} = 0.20$. **Aiii.** YM 0.5 mg/kg: Treatment $F_{(1, 80)} = 10.27$, time $F_{(7, 80)} = 0.26$, interaction $F_{(7, 80)} = 0.26$. **Aiv.** YM 1 mg/kg: Treatment $F_{(1, 80)} = 3.62$, time $F_{(7, 80)} = 0.81$, interaction $F_{(7, 80)} = 0.90$. Two-way ANOVA with Bonferroni's post hoc test. **B.** Time course effect of combined administration of subcutaneous YM (0.25 and 0.5 mg/kg) with single dose of subcutaneous morphine (5 mg/kg) on hot plate test for 2 h. YM was administered 10 min before the administration of morphine. **Bi.** YM 0.25 mg/kg: Treatment $F_{(1, 90)} = 46.32$, time $F_{(8, 90)} = 44.07$, interaction $F_{(8, 90)} = 2.44$. **Bii.** YM 0.5 mg/kg: Treatment $F_{(1, 90)} = 177.8$, time $F_{(8, 90)} = 19.30$, interaction $F_{(8, 90)} = 2.62$. Two-way ANOVA with Bonferroni's post hoc test. N = 6 mice/group. In all panels statistical analysis was performed combining both sexes and significance was $^{*}p < 0.05$ and $^{**}p < 0.001$, data sets (mean ± SEM) as analyzed using two-way ANOVA with Bonferroni's post hoc tests.



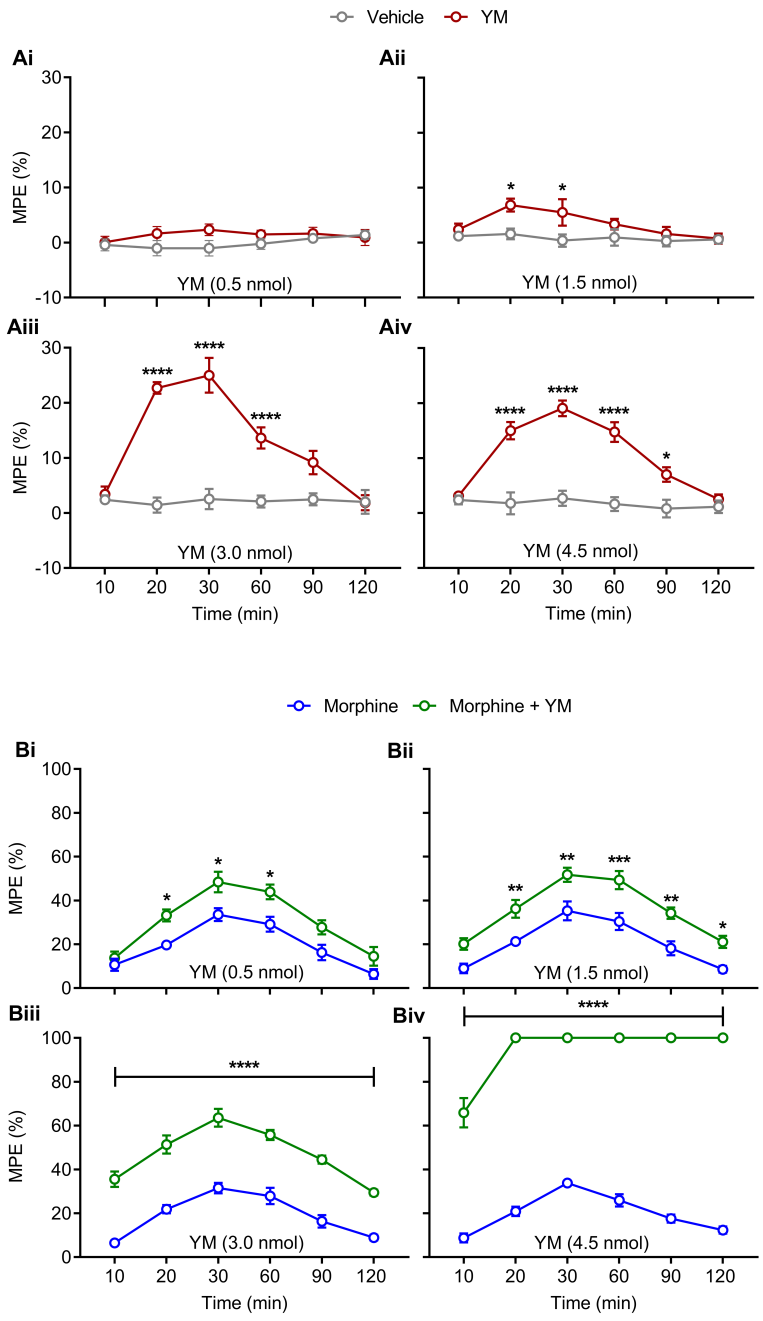
Supplementary Figure S2. The effect of intracerebroventricular injection of YM on nociception

A. Dose-response effect of different concentration of i.c.v. YM (0.4, 1.2, 4 nmol) and vehicle were tested on hot plate test after 10 min of administration. Treatment $F_{(1, 30)} = 45.04$, dose $F_{(2, 30)} = 6.40$, interaction $F_{(2, 30)} = 10.86$. Two-way ANOVA with Bonferroni's post hoc test. **B.** Time course effect of different concentration of i.c.v. YM and vehicle were tested on hot plate test for 2 h. **Bi.** YM 0.4 nmol: Treatment $F_{(1, 5)} = 0.62$, time $F_{(7, 35)} = 1.57$, interaction $F_{(7, 35)} = 1.55$. **Bii.** YM 1.2 nmol: Treatment $F_{(1, 5)} = 2.28$, time $F_{(7, 35)} = 0.97$, interaction $F_{(7, 35)} = 3.45$. **Biii.** YM 4 nmol: Treatment $F_{(1, 5)} = 17.86$, time $F_{(7, 35)} = 13.54$, interaction $F_{(7, 35)} = 15.30$. Two-way ANOVA with Bonferroni's post hoc test. N = 6 mice/group. In all panels statistical analysis was performed combining both sexes and significance was $^{*}p < 0.05$ and $^{**}p < 0.001$, data sets (mean ± SEM) as analyzed using two-way ANOVA with Bonferroni's post hoc tests.



Supplementary Figure S3. The effect of systemic subcutaneous administration of YM on spinal analgesia.

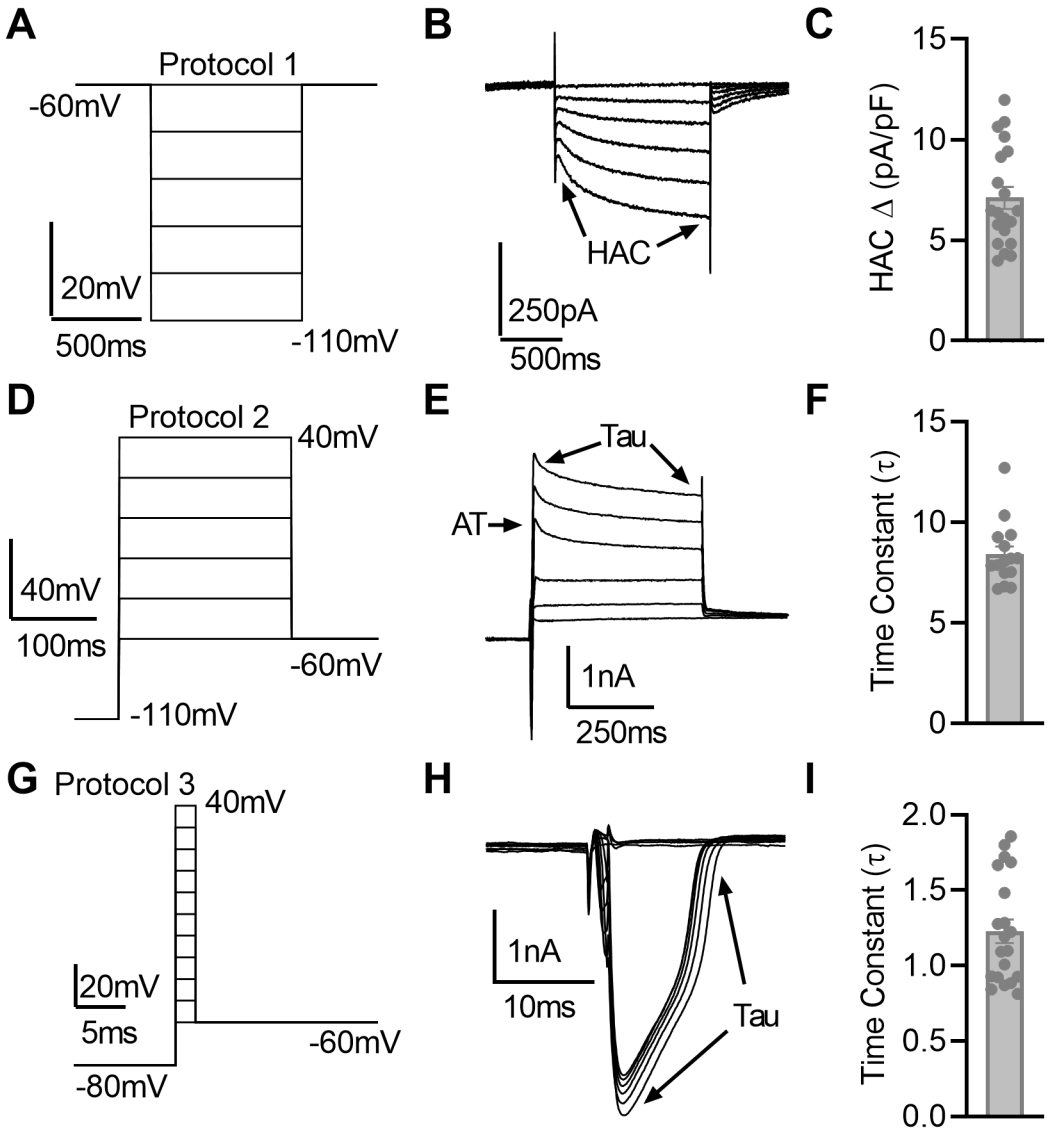
A. Dose-response effect of different concentration of subcutaneous YM and vehicle were tested on tail immersion test for 2 h. **Ai.** YM 0.1 mg/kg: Treatment $F_{(1, 60)} = 0.32$, time $F_{(5, 60)} = 1.34$, interaction $F_{(5, 60)} = 0.84$. **Aii.** YM 0.3 mg/kg: Treatment $F_{(1, 60)} = 0.96$, time $F_{(5, 60)} = 0.73$, interaction $F_{(5, 60)} = 0.35$. **Aiii.** YM 0.5 mg/kg: Treatment $F_{(1, 60)} = 0.55$, time $F_{(5, 60)} = 0.69$, interaction $F_{(5, 60)} = 0.86$. **Aiv.** YM 1 mg/kg: Treatment $F_{(1, 60)} = 2.37$, time $F_{(5, 60)} = 1.65$, interaction $F_{(5, 60)} = 2.70$. Two-way ANOVA with Bonferroni's post hoc test. **B.** Time course effect of combined administration of subcutaneous YM (0.25 and 0.5 mg/kg) with single dose of subcutaneous morphine (5 mg/kg) on tail immersion test for 2 h. YM was administered 10 min before the administration of morphine. **Bi.** YM 0.25 mg/kg: Treatment $F_{(1, 60)} = 7.28$, time $F_{(5, 60)} = 30.48$, interaction $F_{(5, 60)} = 0.71$. **Bii.** YM 0.5 mg/kg: Treatment $F_{(1, 60)} = 52.40$, time $F_{(5, 60)} = 35.48$, interaction $F_{(5, 50)} = 3.17$. Two-way ANOVA with Bonferroni's post hoc test. N = 6 mice/group. In all panels statistical analysis was performed combining both sexes and significance was $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$ and $^{****}p < 0.00001$, data sets (mean ± SEM) as analyzed using two-way ANOVA with Bonferroni's post hoc tests.



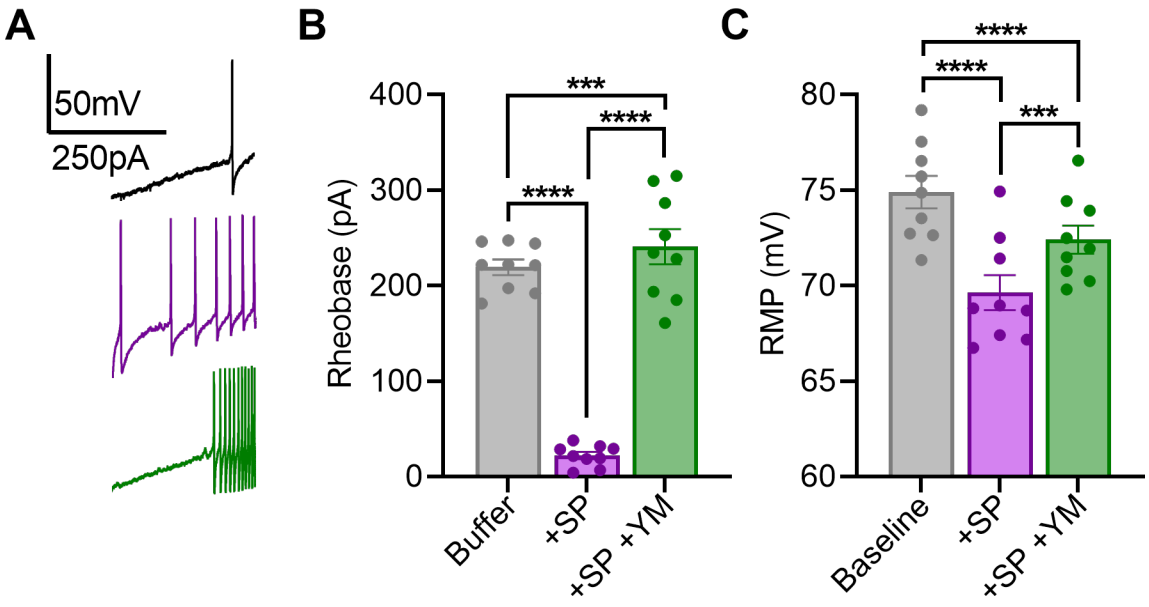
Supplementary Figure S4: The effect of local intrathecal administration of YM on spinal analgesia.

A. Dose-response effect of different concentration of intrathecal YM and vehicle were tested on tail immersion test for 2 h. **Ai.** YM 0.5 nmol: Treatment $F_{(1, 60)}$ = 4.92, time $F_{(5, 60)}$ = 0.44, interaction $F_{(5, 60)}$ = 0.80. **Aii.** YM 1.5 nmol: Treatment $F_{(1, 60)}$ = 13.63, time $F_{(5, 60)}$ = 2.38, interaction $F_{(5, 60)}$ = 1.57. **Aiii.** YM 3.0 nmol: Treatment $F_{(1, 60)}$ = 109.7, time $F_{(5, 60)}$ = 15.41, interaction $F_{(5, 60)}$ = 16.01. **Aiv.** YM 4.5 nmol: Treatment $F_{(1, 60)}$ = 116.1, time $F_{(5, 60)}$ = 14.21, interaction $F_{(5, 60)}$ = 11.88. Two-way ANOVA with Bonferroni's post hoc test. **B.** Time course

effect of combined administration of intrathecal YM with single dose of subcutaneous morphine (2.5 mg/kg) on tail immersion test for 2 h. YM was administered 10 min before the administration of morphine. **Bi.** YM 0.5 nmol: Treatment $F_{(1, 10)} = 15.40$, time $F_{(5, 50)} = 39.79$, interaction $F_{(5, 50)} = 1.38$. **Bii.** YM 1.5 nmol: Treatment $F_{(1, 60)} = 68.29$, time $F_{(5, 60)} = 29.80$, interaction $F_{(5, 60)} = 0.40$. **Biii.** YM 3.0 nmol: Treatment $F_{(1, 60)} = 306.3$, time $F_{(5, 60)} = 33.79$, interaction $F_{(5, 60)} = 0.99$. **Biv.** YM 4.5 nmol: Treatment $F_{(1, 48)} = 3103$, time $F_{(5, 48)} = 40.06$, interaction $F_{(5, 48)} = 11.70$. Two-way ANOVA with Bonferroni's post hoc test. N = 6 mice/group. In all panels statistical analysis was performed combining both sexes and significance was $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$, and $^{****}p < 0.00001$ data sets (mean ± SEM) as analyzed using two-way ANOVA with Bonferroni's post hoc tests.



Supplementary Figure S5. Physiological parameters of morphine-responsive DRG neurons
A. Illustration of Protocol 1, used to characterize hyperpolarization-activated current. **B.** Representative trace of DRG produced by Protocol 1. Hyperpolarization-activated current delta is indicated. **C.** Quantification of hyperpolarization-activated current of nociceptors. **D.** Illustration of Protocol 2. **E.** Representative trace produced by Protocol 2, used to characterize activation threshold and A-current inactivation rate Tau as indicated. **F.** Quantification of nociceptor A-current tau. **G.** Illustration of Protocol 3. **H.** Representative trace produced by Protocol 3, used to determine the inactivation decay constant of the first inward current response as indicated, and the response amplitude. **I.** Quantification of the inactivation decay constant.



Supplementary Figure S6. The effect of YM on Substance P induced excitation of DRG nociceptors.

A. Representative voltage traces from a continuous 0-2 nA ramp stimulation protocol illustrating excitability of cultured DRG neurons at baseline (black), and after bath application of 10 nM Substance P (SP, purple) followed by 10 nM SP and 100nM YM (green). **B.** Quantification of rheobase from DRG recordings illustrated in A. Treatment: $F_{(8, 24)} = 5.812$. One-way repeated-measures ANOVA with Tukey's multiple comparisons test. **C.** Quantification of resting membrane potential from DRG recordings illustrated in A. Treamtent: $F_{(8, 24)} = 23.16$. One-way repeated-measures ANOVA with Tukey's multiple comparisons test. Statistical analysis was performed combining both sexes, and significance was ***$p < 0.01$, and ****$p < 0.0001$.