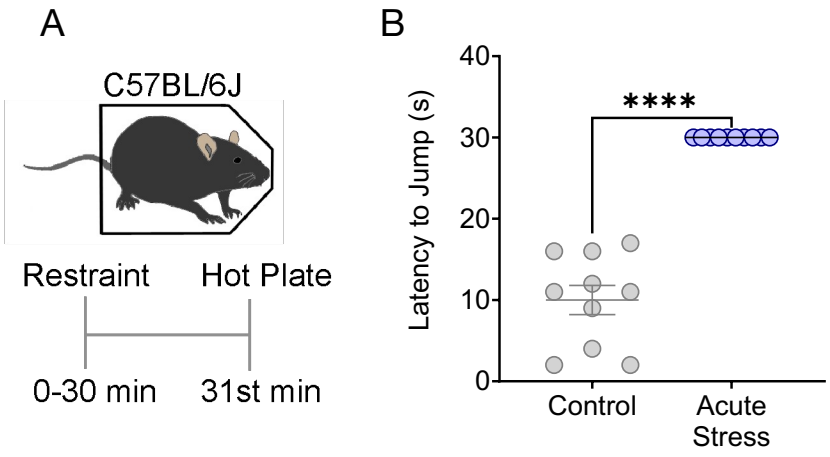

A
C57BL/6J
Restraint
Hot Plate
0-30 min
31st min
B
Latency to Jump (s)
40
30
20
10
0
****
Control
Acute
Stress

Figure S1

Supplementary Figure 1. Acute restraint stress causes antinociception. (**A**) Cartoon and experimental timeline for restraint stress-induced antinociception. (**B**) 30 minutes of restraint stress drives thermal antinociception as delayed nocifensive responses on a 55ºC hot plate with 30 second cutoff. Student's t-test, t = 11.1, ****p<0.0001. Data represented as mean ± SEM.

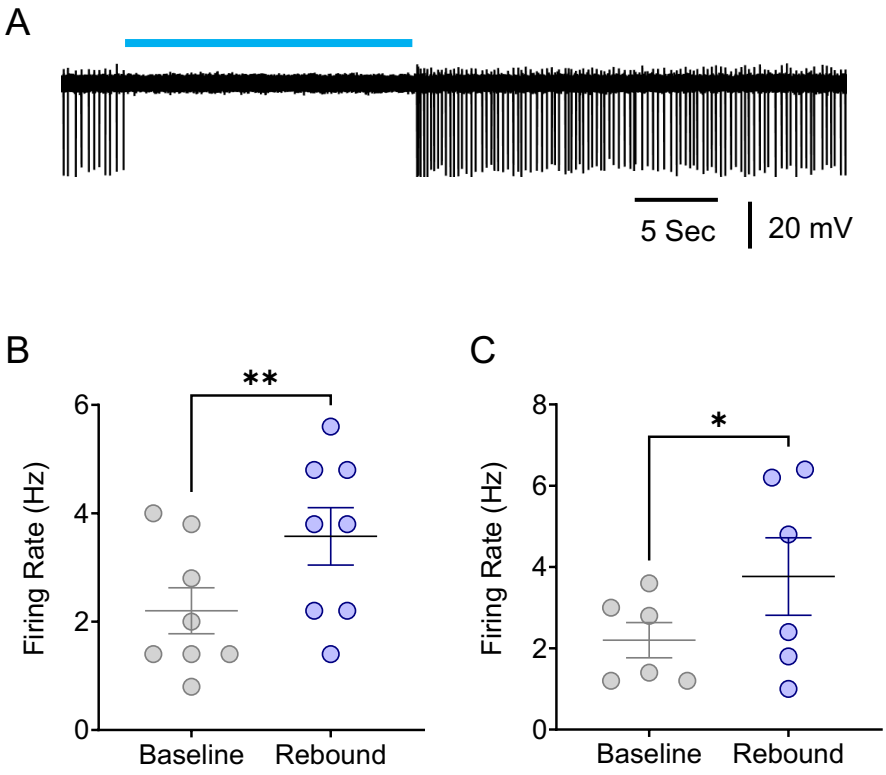

A
5 Sec
20 mV
B
**
Firing Rate (Hz)
6
4
2
0
Baseline
Rebound
C
*
Firing Rate (Hz)
8
6
4
2
0
Baseline
Rebound

Figure S2

Supplemental Figure 2. Rebound of neural activity after cessation of stGtACR2-mediated optical inhibition. (**A**) Representative cell-attached recording showing stGtACR2-mediated inhibition in spontaneous firing rate from an LC neuron. (**B&C**) Rebound neural activity following cessation of optical inhibition in either whole-cell (**B**) or cell-attached (**C**) recordings. Paired-t tests, $t = 4.604$ (whole-cell); 2.731 (cell-attached), $^{*}p<0.05$; $^{**}p<0.01$. Data represented as mean ± SEM.

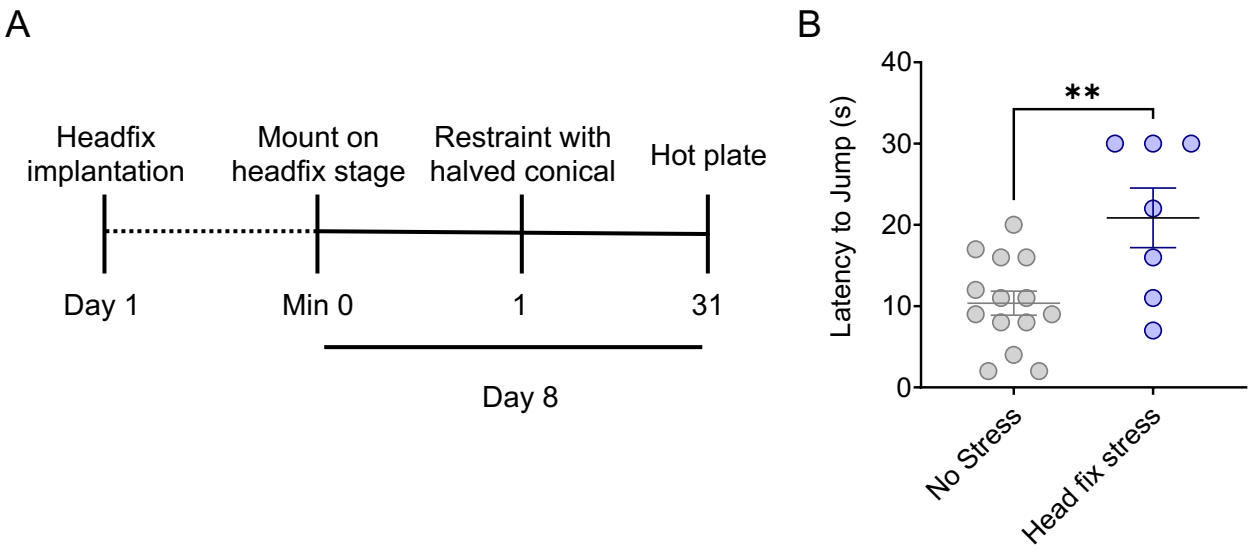

A
Headfix implantation
Mount on headfix stage
Restraint with halved conical
Hot plate
Day 1
Min 0
1
31
Day 8
B
Latency to Jump (s)
40
30
20
10
0
**
No Stress
Head fix stress

Figure S3

Supplementary Figure 3: Modified acute restraint stress in concert with head-fixation causes acute antinociception. (**A**) Experimental timeline of modified restraint stress-induced antinociceptive testing in the hot plate test. (**B**) 30 minutes of head-fixed restraint stress drives thermal antinociception as delayed nocifensive responses on a 55^{o}C hot plate with 30 second cutoff. Student's t-test, t = 3.184, **p<0.01. Data represented as mean ± SEM.

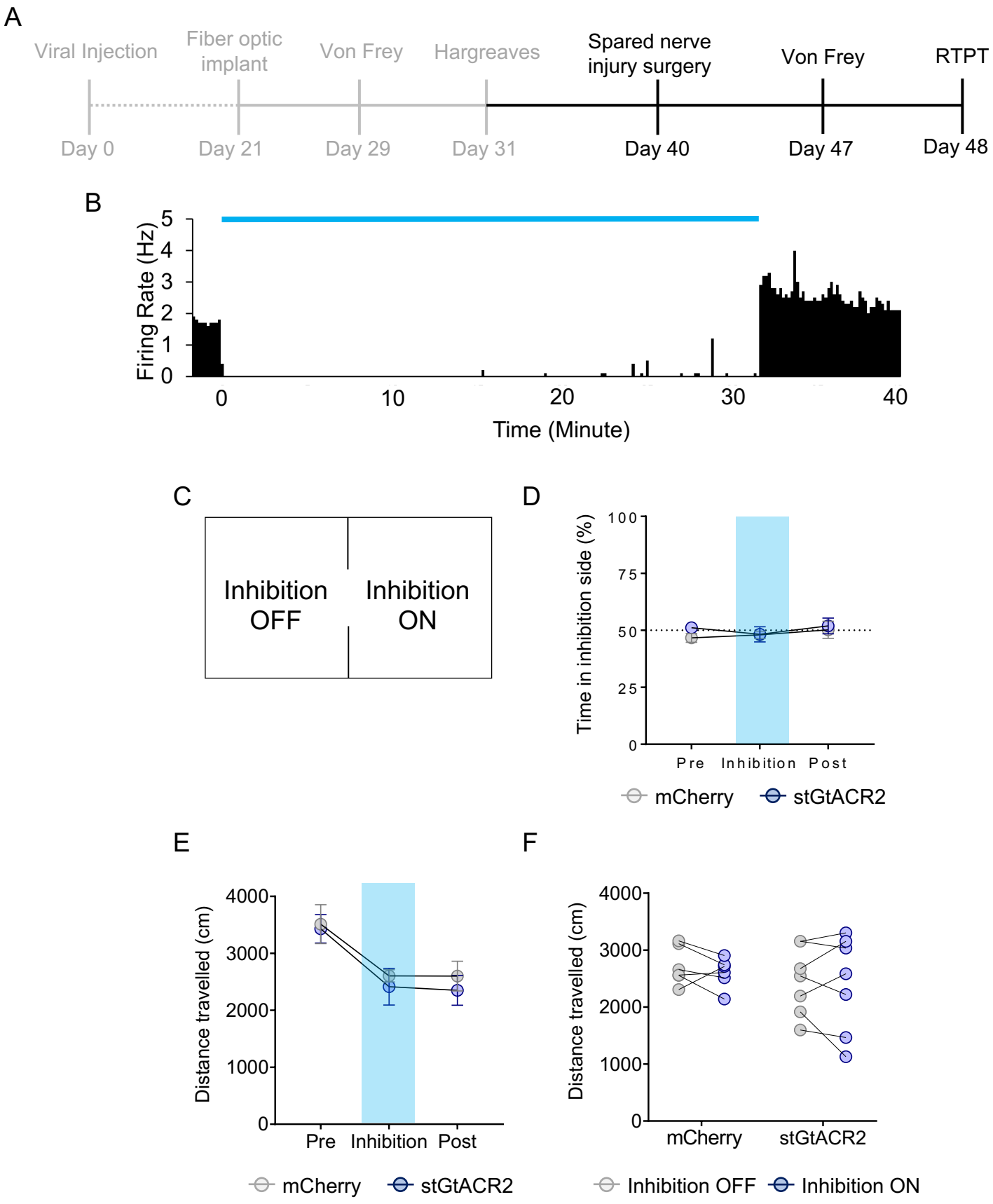

A
Viral Injection
Fiber optic implant
Von Frey
Hargreaves
Spared nerve injury surgery
Von Frey
RTPT
Day 0
Day 21
Day 29
Day 31
Day 40
Day 47
Day 48
B
Firing Rate (Hz)
Time (Minute)
C
Inhibition OFF
Inhibition ON
D
Time in inhibition side (%)
Pre
Inhibition
Post
mCherry
stGtACR2
E
Distance travelled (cm)
Pre
Inhibition
Post
mCherry
stGtACR2
F
Distance travelled (cm)
mCherry
stGtACR2
Inhibition OFF
Inhibition ON

Figure S4

Supplement Figure 4. LC optical inhibition does not induce real-time preference four weeks after neuropathic injury (**A**) Experimental timeline of behavioral testing. (**B**) Histogram showing prolonged LC optical inhibition for 30 minutes using 100 Hz, 0.5 ms pulse width, 470 nm illumination. (**C**) Diagram of real-time place testing apparatus. (**D**) LC inhibition does not induce real-time place preference four weeks post-SNI. Repeated measures two-way ANOVA followed by Tukey's posthoc test, F = 0.604 (Inhibition epoch); 0.773 (photo-inhibition); 0.302 (interaction); 1.152 (animal). (**E&F**) LC inhibition does not alter distance travelled in real-time place test. Repeated measures two-way ANOVA. For (E): F = 14.41 (inhibition epoch); 0.346 (photo-inhibition); 0.082 (interaction); 2.965 (animal). For (F): F = 0.559 (inhibition On/Off); 0.643 (photo-inhibition); 0.119 (interaction); 8.129 (animal). Data represented as mean ± SEM, no significant difference was found.

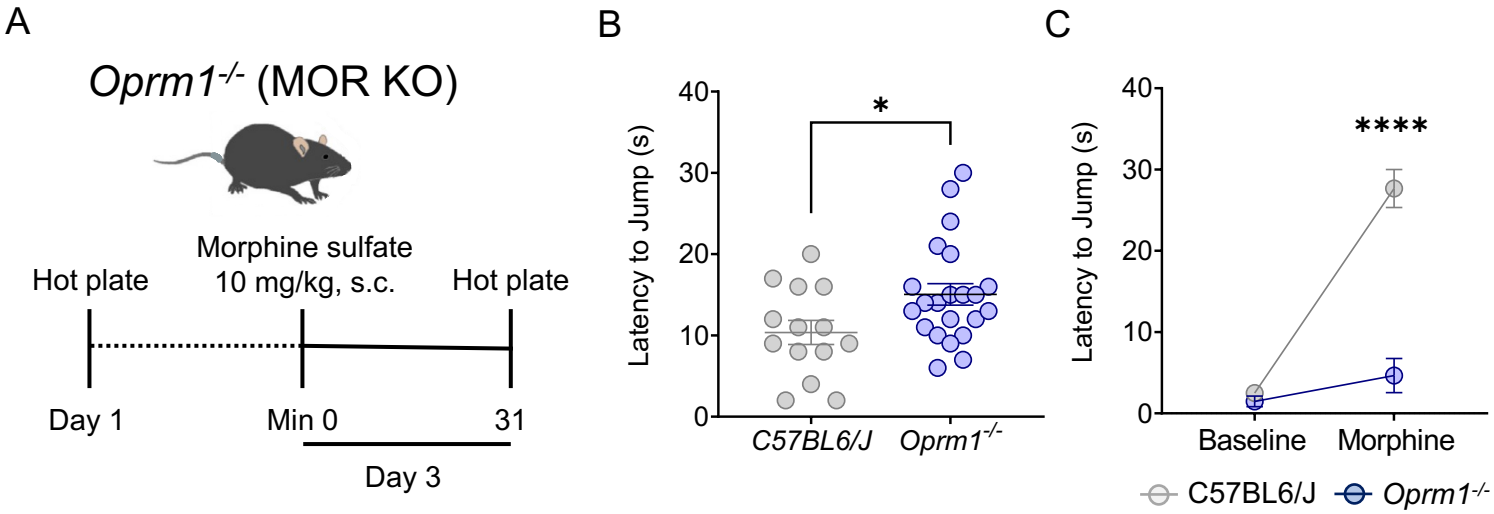

A
Oprm1-/- (MOR KO)
Morphine sulfate
10 mg/kg, s.c.
Hot plate
Hot plate
Day 1
Min 0
31
Day 3
B
Latency to Jump (s)
40
30
20
10
0
*
C57BL6/J
Oprm1-/-
C
Latency to Jump (s)
40
30
20
10
0
****
Baseline
Morphine
C57BL6/J
Oprm1-/-

Figure S5

Supplement Figure 5. Global *Oprm1* knockout influences nociceptive responses (**A**) Experimental timeline for behavioral tests. (**B**) Naïve *oprm1*$^{-/-}$ mice have increased nociceptive thresholds on the hot plate. Student's T-test, t = 2.297, *p<0.05. (**C**) *Oprm1*$^{-/-}$ mice do not have morphine analgesia. Repeated measures two-way ANOVA followed by Tukey's posthoc test, F = 48.41 (MOR KO); 37.60 (Morphine application); 29.14 (interaction); 0.927 (animal), ****p<0.0001. Data represented as mean ± SEM.

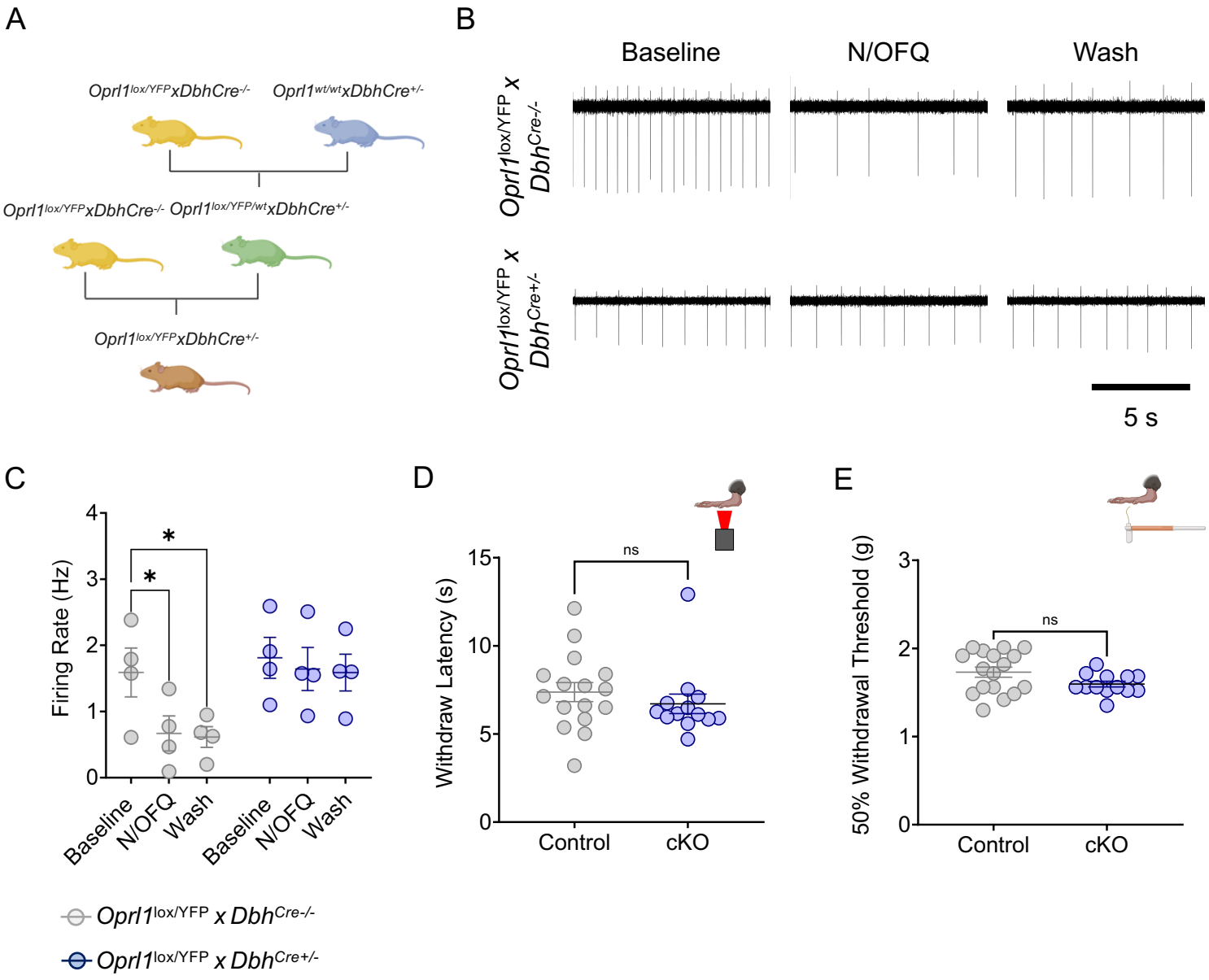
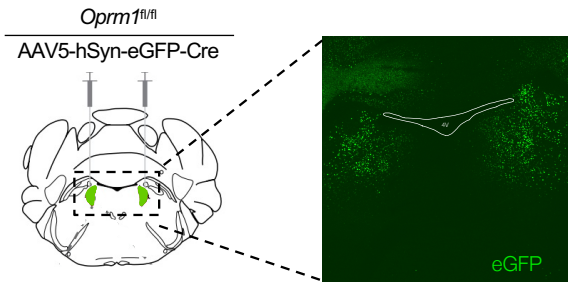

A
Oprl1lox/YFPxDbhCre-/-
Oprl1wt/wtxDbhCre+/-
Oprl1lox/YFPxDbhCre-/-
Oprl1lox/YFP/wtxDbhCre+/-
Oprl1lox/YFPxDbhCre+/-
B
Baseline
N/OFQ
Wash
Oprl1lox/YFP x DbhCre-/-
Oprl1lox/YFP x DbhCre+/-
5 s
C
Firing Rate (Hz)
Baseline
N/OFQ
Wash
Baseline
N/OFQ
Wash
Oprl1lox/YFP x DbhCre-/-
Oprl1lox/YFP x DbhCre+/-
D
Withdraw Latency (s)
ns
Control
cKO
E
50% Withdrawal Threshold (g)
ns
Control
cKO

Figure S6

Supplement Figure 6. Noradrenergic NOP is not required for noradrenergic-modulated nociception. (**A**) Schematic describing the breeding strategy of *Oprl1*$^{lox/YFP}$x*Dbh*$^{Cre+/-}$ conditional knockout mouse line. (**B**) Representative traces of cell-attached LC recordings with NOP cKO (*Oprl1*$^{lox/YFP}$x*Dbh*$^{Cre+/-}$) losing N/OFQ-mediated LC inhibition (bottom) compared to Control mice (*Oprl1*$^{lox/YFP}$x*Dbh*$^{Cre-/-}$). (**C**) N/OFQ-mediated inhibition of LC neurons is lost in *Oprl1*$^{lox/YFP}$x*Dbh*$^{Cre+/-}$ mice. Repeated-measures two-way ANOVA, F = 24.86 (cKO); 3.306 (pharmacology); 10.68 (interaction); 26.80 (cell), *p<0.05. (**D&E**) Hargreaves (Mann-Whitney, U = 69, ns = not significant) (**D**) and von Frey (Student's t-test, t = 1.886, ns = not significant) (**E**) tests showing that the conditional exclusion of NOP in noradrenergic neurons does not alter baseline nociception. Data represented as mean ± SEM.

A


Oprm1fl/fl
AAV5-hSyn-eGFP-Cre
eGFP

B

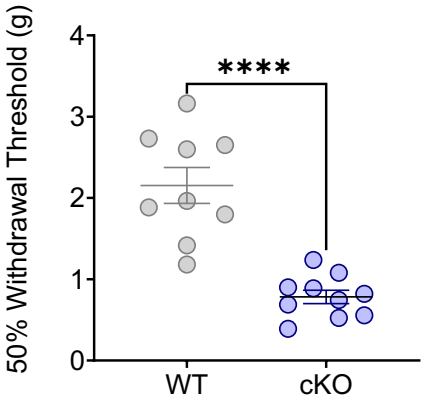

50% Withdrawal Threshold (g)
4
3
2
1
0
****
WT
cKO

Figure S7

Supplementary Figure 7. Spatial expression of Cre recombinase in the adult LC of O*prm1*$^{fl/fl}$ mice causes mechanical hypersensitivity. (**A**) Schematic and fluorescent image of the bilateral viral Cre expression in the LC. (**B**) *oprm1* deletion in the LC decreases baseline 50% mechanical withdrawal threshold. Student's t-test, t = 6.06, ****p<0.0001. Data represented as mean ± SEM.

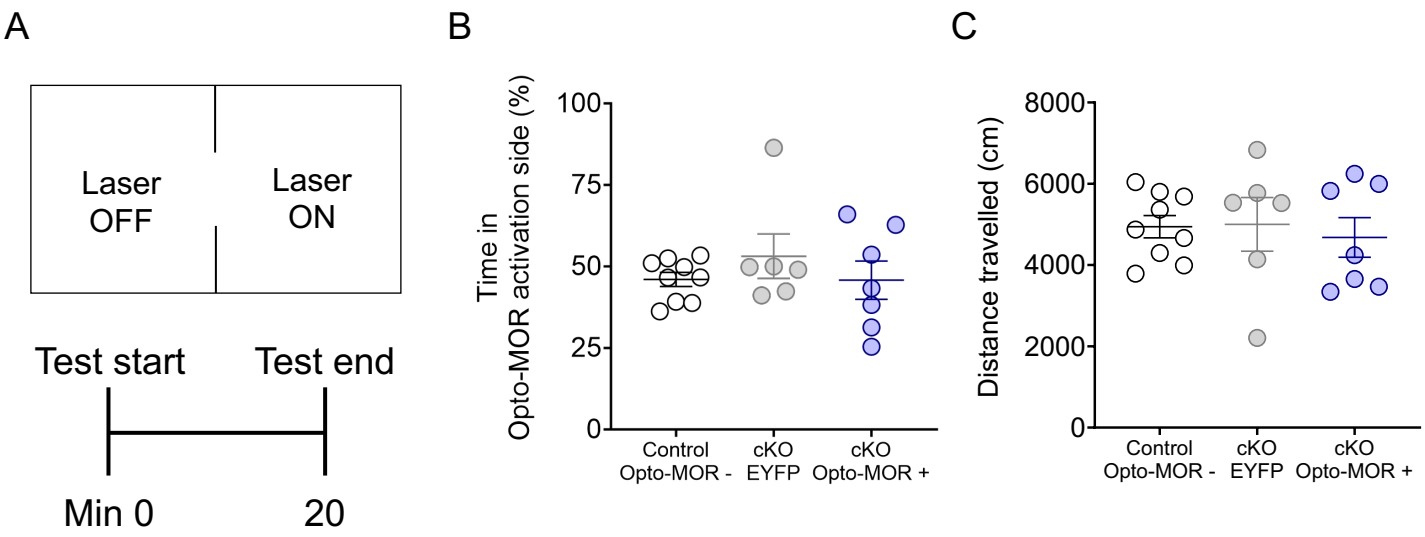

A
Laser
OFF
Laser
ON
Test start
Test end
Min 0
20
B
Time in
Opto-MOR activation side (%)
100
75
50
25
0
Control
Opto-MOR -
cKO
EYFP
cKO
Opto-MOR +
C
Distance travelled (cm)
8000
6000
4000
2000
0
Control
Opto-MOR -
cKO
EYFP
cKO
Opto-MOR +

Figure S8

Supplementary Figure 8. Optical activation of Opto-MOR signaling in LC of *Oprm1*$^{fl/fl}$x*Dbh*$^{Cre+/-}$ mice does not alter real-time place preference or locomotor behavior. (**A**) Schematics illustrating the experimental arrangement of real-time place preference test. (**B**) % time spent in Opto-MOR-paired side of real-time place testing apparatus shows no significant difference between groups. Kruskal-Wallis test, Kruskal-Wallis statistic = 0.4421. (**C**) Distance travelled during real-time place testing shows no significant effect of opto-MOR activation. One-way ANOVA, F = 0.1352. Data represented as mean ± SEM, no significant difference was found.