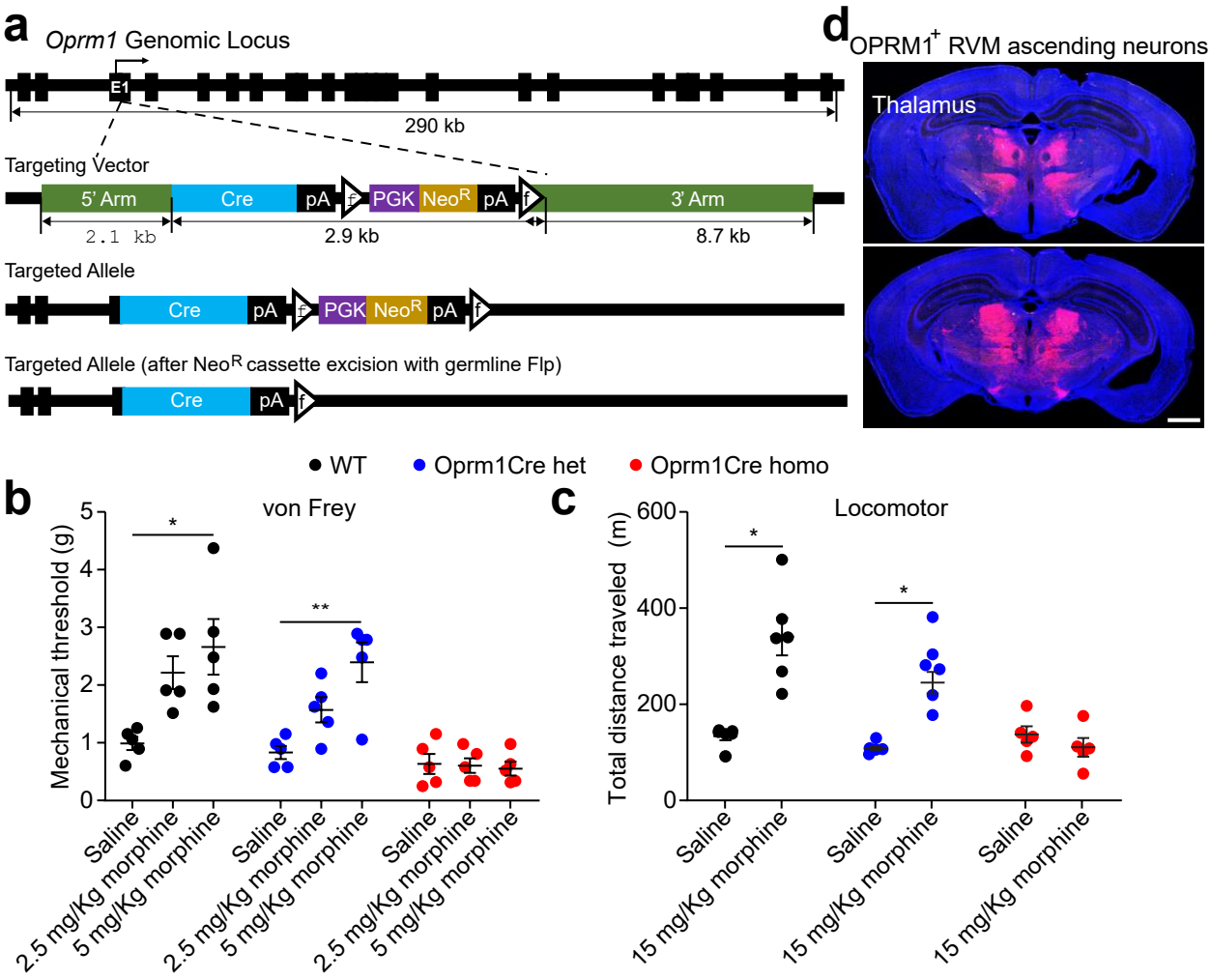
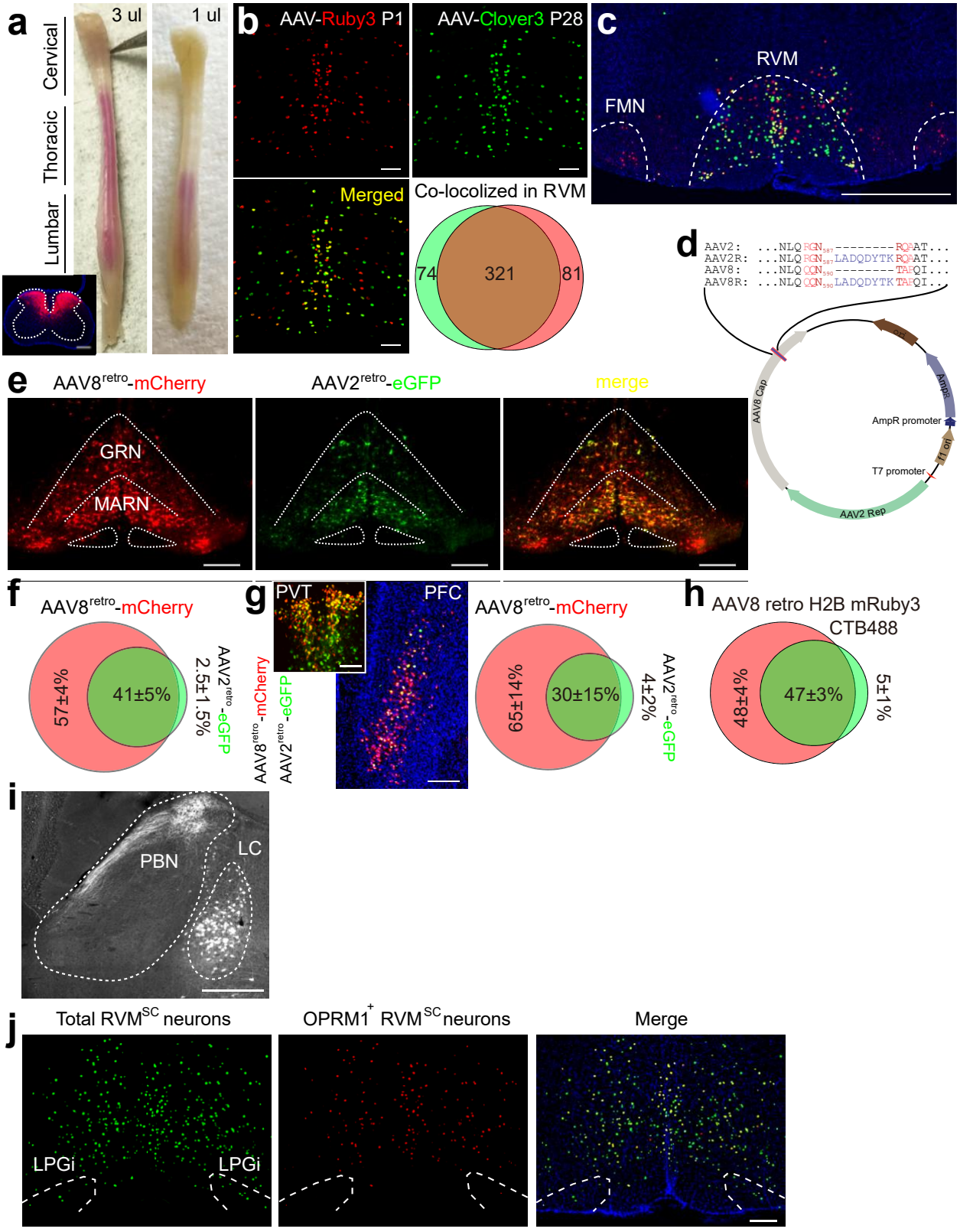
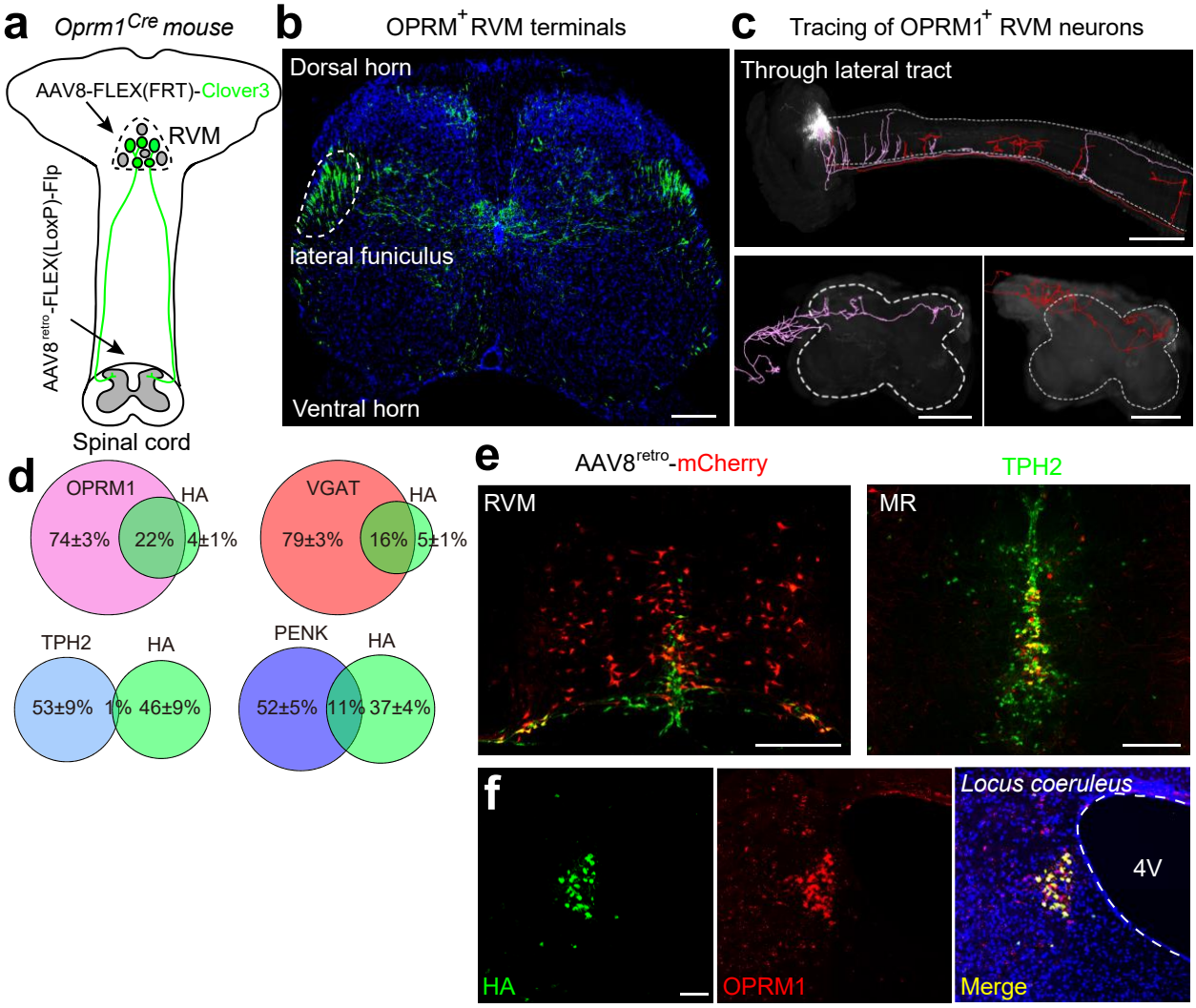


Extended Data Fig. 1 Generation and characterization of *Oprm1-Cre* knockin mouse line. (**a**) Gene targeting strategy used to generate *Oprm1-Cre* knockin mouse line. (**b**) Morphine produced similar analgesic effect in WT (black) and *Oprm1*$^{Cre/+}$ (blue), but not in *Oprm1*$^{Cre/Cre}$ (red) mice (Friedman test, post-hoc pairwise comparisons were conducted using Dunn's multiple comparison test, saline vs. 5 mg morphine. WT saline vs. 5 mg morphine, * $P < 0.05$; *Oprm1*$^{Cre/+}$ saline vs. *Oprm1*$^{Cre/+}$ 5 mg Kg^{-1} morphine, ** $P < 0.01$. 5 mice for each genotype). (**c**) Morphine increased locomotion in WT (black) and *Oprm1*$^{Cre/+}$(blue), but not in *Oprm1*$^{Cre/Cre}$ (red) mice (WT saline vs. WT 15 mg Kg^{-1} morphine, 6 mice, * $P < 0.05$; *Oprm1*$^{Cre/+}$ saline vs. *Oprm1*$^{Cre/+}$ 15 mg Kg^{-1} morphine, 6 mice, * $P < 0.05$; *Oprm1*$^{Cre/Cre}$ saline vs. *Oprm1*$^{Cre/Cre}$ 15 mg Kg^{-1} morphine, 5 mice, $P > 0.05$. Wilcoxon matched-pairs signed rank test). (**d**) Representative image shows the Ruby3-expressing terminal in the thalamus from OPRM1^{+} ascending RVM neurons after injection of AAV9-FLEX(LoxP)-Ruby3 into the RVM of the *Oprm1-Cre* mice. Scale Bar: 1mm.

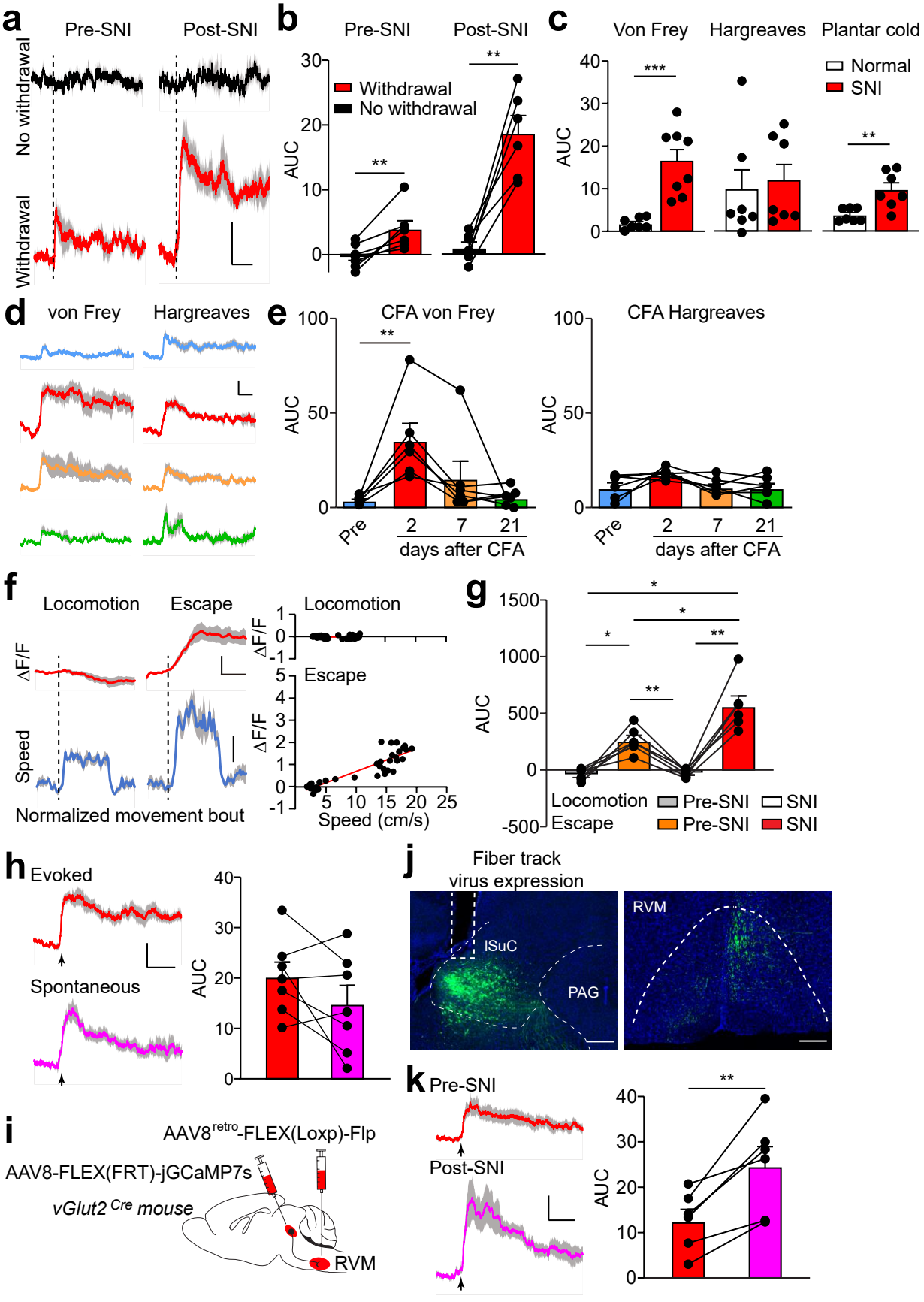

a
3 ul
1 ul
Cervical
Thoracic
Lumbar
b
AAV-Ruby3 P1
AAV-Clover3 P28
Merged
Co-locolized in RVM
74
321
81
c
RVM
FMN
d
AAV2: ...NLQRGN587--------RQAAT...
AAV2R: ...NLQRGN587LADQDYTKRQAAT...
AAV8: ...NLQQQN590--------TAAQI...
AAV8R: ...NLQQQN590LADQDYTKTAAQI...
AAV8 Cap
AmpR
AmpR promoter
T7 promoter
11 aa
AAV2 Rep
e
AAV8retro-mCherry
AAV2retro-eGFP
merge
GRN
MARN
f
AAV8retro-mCherry
57±4%
41±5%
AAV2retro-eGFP
2.5±1.5%
g
PVT
PFC
AAV8retro-mCherry
AAV2retro-eGFP
AAV8retro-mCherry
65±14%
30±15%
AAV2retro-eGFP
4±2%
h
AAV8 retro H2B mRuby3
CTB488
48±4%
47±3%
5±1%
i
PBN
LC
j
Total RVMSC neurons
OPRM1+ RVMSC neurons
Merge
LPGi
LPGi

Extended Data Fig. 2 Developing and characterization of neonatal intraspinal injection strategy and AAV8^{retro}. (**a**) Images of dissected spinal cord from mice injected with 3 μl (left) and 1 μl (right) of AAV8^{retro}-mCherry. Neonatal injection of 1 μl or 3 μl of AAV8^{retro}-mCherry led the virus infection of the entire lumbar region or both lumber and thoracic regions of the spinal cord, respectively. Inset shows the expression of mCherry was restricted largely in the dorsal horn. Scale Bar: 1 mm. (**b**) Representative images of RVM section following spinal cord injection of AAV8^{retro} H2B-Ruby3 at Postnatal day 1(neonatal, P1) and injection of AAV8^{retro} H2B-Clover3 at P28 (adult), and Venn diagram illustrated the number of Ruby3-labeled, Clover3-labeled, and colocalized neurons in the RVM. Scale Bar: 100 μm. (**c**) Representative medulla section following neonatal and adult spinal cord injection of AAV8^{retro}-H2B-Ruby3 and AAV8^{retro}-H2B-Clover3, respectively. Retrogradely labeled neurons in RVM and facial motor nucleus (FMN). Scale Bar: 1 mm. (**d**) Plasmid map shows the site for inserting the decapeptide LADQDYTKTA between N590 and T591 of the AAV8 capsid (AAV8^{retro}). (**e**) Representative images of retrogradely labeled RVM neurons by AAV8^{retro}-mCherry (red), AAV2^{retro}-eGFP (green), and merge (yellow) after co-injection of AAV8^{retro}-mCherry and AAV2^{retro}-eGFP into the spinal cord of P1.5 wild type mice. Scale Bar: 200 μm. GRN: gigantocellular reticular nucleus; MARN: magnocellular reticular nucleus. (**f**) Quantification of (**e**) (n = 3). (**g**) Representative images (left) and quantification (right) of retrogradely labeled neurons in the prefrontal cortex (PFC) when co-inject AAV8^{retro}-mCherry (red) and AAV2^{retro}-eGFP (green) into the paraventricular nucleus of the thalamus (PVT) (inset). Scale Bar: PVT: 100 μm; PFC: 200 μm. (**h**) Quantification of retrogradely labeled neurons in the RVM when co-inject AAV8^{retro}-mCherry (red) and CTB488 (green) into the adult spinal cord. (**i**) Representative image of retrogradely labeled neurons in the LC and axon terminals in parabrachial nucleus (PBN) when inject AAV8^{retro}-Cre in the spinal cord of Ai14 mice. Scale Bar: 500 μm. (**j**) Representative images of retrogradely labeled neurons in the RVM when inject AAV8^{retro}-H2BClover3-FLEX(LoxP)-H2BRuby3 in the spinal cord of *Oprm1*$^{Cre/+}$ mice. Majority of OPRM1^{+} RVM neurons were located outside the lateral paragigantocellular nucleus (LPGi). Scale Bar: 200 μm.

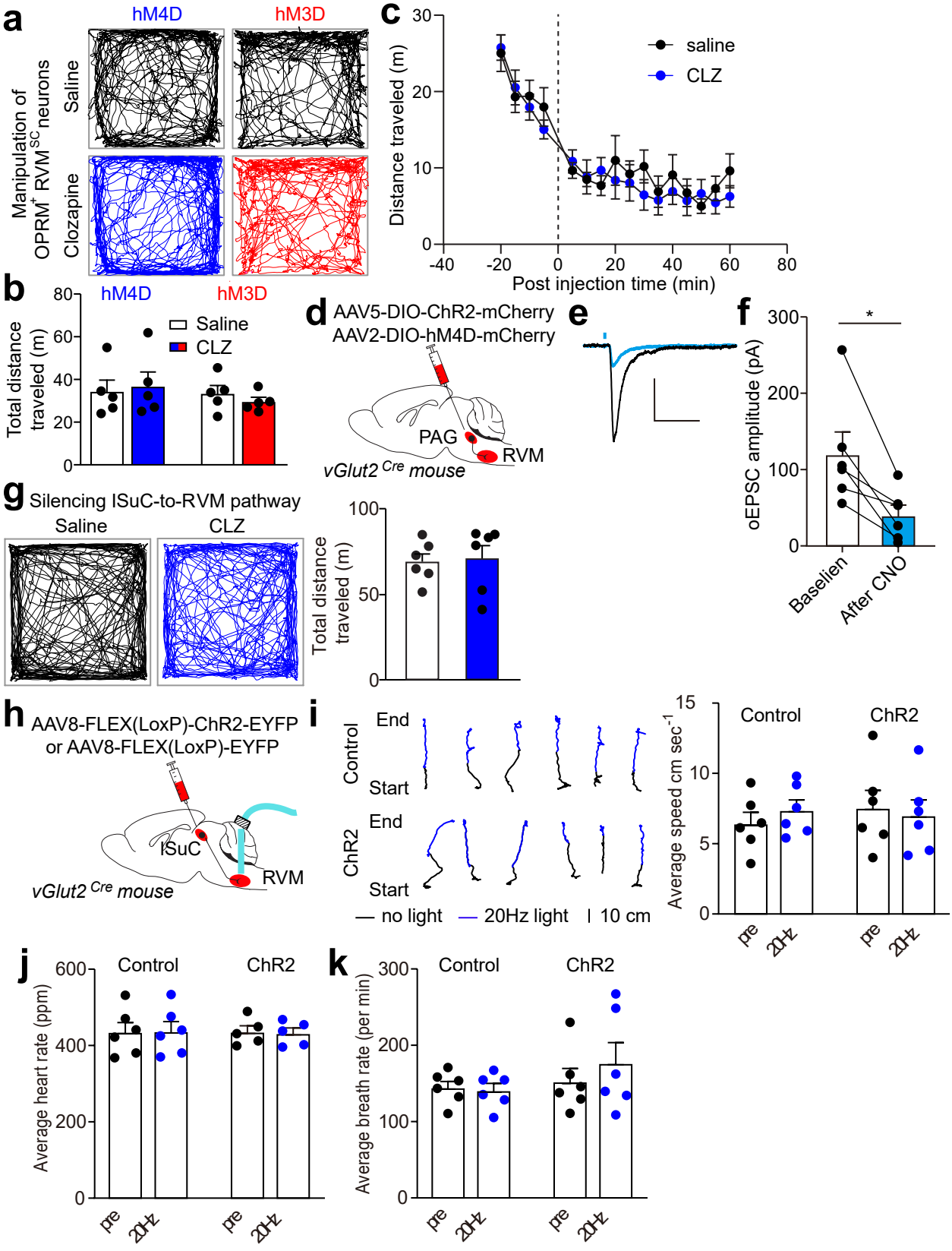


Extended Data Fig. 3 Characterization of OPRM1⁺ RVM^{SC} neurons. (**a**) Schematic shows spinal injection of AAV8-retro-FLEX(LoxP)-Flp at P1.5 in Oprm1-Cre mice, then four weeks later, RVM injection of AAV8-FLEX(FRT)-Clover3. (**b**) Representative images show the terminals of descending OPRM1⁺ RVM^{SC} neurons in the spinal cord. Notably, axons of OPRM1⁺ RVM^{SC} neurons are clustered in lateral funiculus (dash line) before gets into the spinal cord, and its terminals are enriched in the dorsal horn but not in the motor area in the spinal cord. Scale Bar: 200 μm. (**c**) TESOS imaging and single-cell tracing of OPRM1⁺ RVM^{SC} neurons. Axons of OPRM1⁺ RVM^{SC} neurons of both red and pink neurons are traveled along the left lateral funiculus, then enter the spinal cord to innervate both sides of the dorsal horn. Scale Bar: 500 μm. (**d**) Quantification of molecular markers expressed in the OPRM1⁺ RVM^{SC} neurons. Related to Fig. 1e. (**e**) Representative images of retrogradely labeled spinal cord projecting Tph2⁺ neurons in the RVM (left) and median raphe (MR, right) after spinal injection of AAV8-retro-mCherry. More Tph2⁺ neurons were labeled in MR than in RVM. mCherry labeled neurons are in red, Tph2⁺

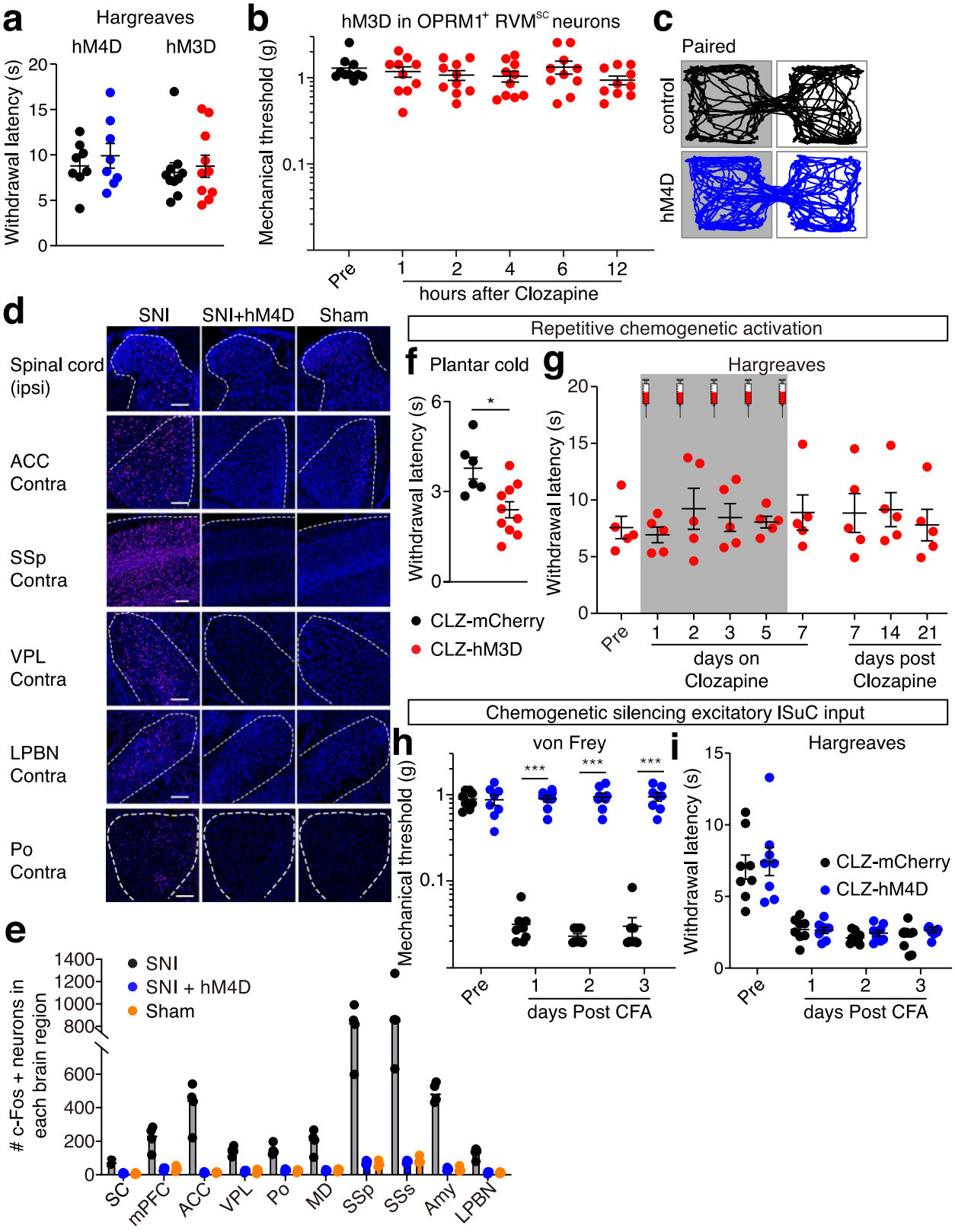
neurons are visualized by immunostaining in green. Scale bar: 500 μm. (**f**) Representative image shows OPRM1^{+} LC^{SC} neurons labeled by intraspinal injection of AAV8-retro-FLEX(LoxP)-Rpl22-3XHA. RNAscope probes were used to visualize Oprm1 (red), HA tag (green) was visualized by immunostaining. Scale Bar: 100 μm.


a
Pre-SNI
Post-SNI
No withdrawal
Withdrawal
b
Pre-SNI
Post-SNI
Withdrawal
No withdrawal
AUC
c
Von Frey
Hargreaves
Plantar cold
Normal
SNI
AUC
d
von Frey
Hargreaves
e
CFA von Frey
CFA Hargreaves
AUC
Pre
2
7
21
days after CFA
f
Locomotion
Escape
ΔF/F
Speed
Normalized movement bout
Speed (cm/s)
g
AUC
Locomotion
Escape
Pre-SNI
SNI
h
Evoked
Spontaneous
AUC
i
AAV8retro-FLEX(Loxp)-Flp
AAV8-FLEX(FRT)-jGCaMP7s
vGlut2 Cre mouse
RVM
j
Fiber track
virus expression
lSuC
PAG
RVM
k
Pre-SNI
Post-SNI
AUC

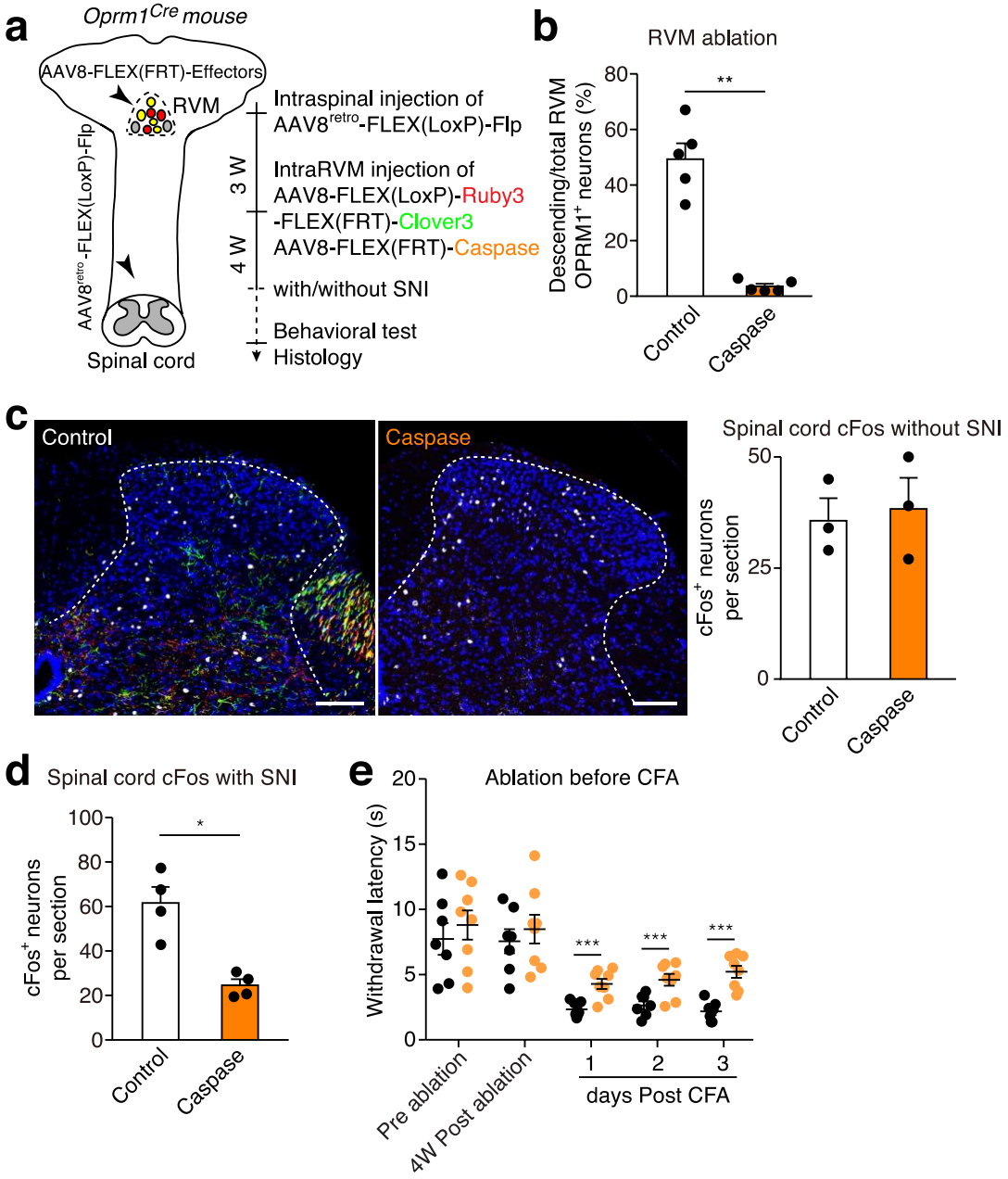
Extended Data Fig. 4 Fiber photometry recording. (**a**) Representative average traces of non-withdrawal and withdrawal trails Pre-(left) and Post-SNI (right) in OPRM1^{+} RVM^{SC} neurons. Scale bar: 1% △F/F, 3 sec. (**b**) Quantification of AUC of (**a**). n = 6, Paired t test, ** $P < 0.01$. (**c**) Quantification of OPRM1^{+} RVM^{SC} neurons respond to mechanical (von Frey) and thermal (Hargreaves and Plantar cold) stimuli in normal and SNI mice (n = 7-8). Mann-Whitney test, ** $P < 0.01$, *** $P < 0.001$. Related to Fig. 1h. (**d, e**) Representative traces (**d**) and quantification (**e**) of mechanical (von Frey) and thermal (Hargreaves) stimulation evoked calcium response in OPRM1^{+} RVM^{SC} neurons of before, 2, 7, and 21 days after CFA injection. n = 6, Friedman test, post-hoc pairwise comparisons were conducted using Dunn's multiple comparison test, **$P < 0.01$. Scale bar: 1% △F/F, 2 sec. (**f**) Representative traces of calcium response (left, upper panel) and movement speed (left, lower panel), and correlation of calcium response vs. movement speed (right) in normal mice. Dash line indicates the time of movement start. Dot indicates the average speed calcium signal during each movement event. Red line: linear fitting of dots. R^2= 0.021 or 0.842 for locomotion and escape, respectively. Scale bar: 50 % of normalized movement bout, 1% ΔF/F, 5 cm/s. Related to Fig. 1j. Locomotion: spontaneous movement without stimulus; Escape: escape response after von Frey stimulus. Mean ± SEM. (**g**) Quantification of (**f**) and Fig.1j. n=6, one-way ANOVA, post-hoc pairwise comparisons were conducted using Tukey's multiple comparisons test, * $P < 0.05$, ** $P < 0.01$. (**h**) Representative traces (left) and quantification (right, n = 7) of calcium response in OPRM1^{+} RVM^{SC} neurons evoked by von Frey stimulation or occurring spontaneously following SNI. Scale bar: 1% △F/F, 3 sec. (**i**) Experimental paradigm for (**j**) and (**k**). (**j**) Representative image shows fiber track and jGCaMP7s expression in RVM-projecting vGlut2^{+} lSuC neurons. Scale bar: 500 μm (lSuC), 200 μm (RVM). (**k**) Representative traces (left) and quantification (right) of calcium response in RVM-projecting vGlut2^{+} lSuC neurons evoked by von Frey stimulation before and after SNI. Scale bar: 1% △F/F, 3 sec. n = 6, Paired t test, ** $P < 0.01$.


a
hM4D
hM3D
Manipulation of OPRM+ RVMSC neurons
Saline
Clozapine
c
Distance traveled (m)
Post injection time (min)
saline
CLZ
b
Total distance traveled (m)
hM4D
hM3D
Saline
CLZ
d
AAV5-DIO-ChR2-mCherry
AAV2-DIO-hM4D-mCherry
PAG
RVM
vGlut2 Cre mouse
e
f
oEPSC amplitude (pA)
*
Baselien
After CNO
g
Silencing ISuC-to-RVM pathway
Saline
CLZ
Total distance traveled (m)
h
AAV8-FLEX(LoxP)-ChR2-EYFP
or AAV8-FLEX(LoxP)-EYFP
ISuC
RVM
vGlut2 Cre mouse
i
Control
ChR2
End
Start
no light
20Hz light
10 cm
Average speed cm sec-1
pre
20Hz
j
Average heart rate (ppm)
k
Average breath rate (per min)

Extended Data Fig. 5 Activity of OPRM1^{+} RVM^{SC} neurons or the lSuC→RVM pathway has no effect on locomotion. (**a,b**) Example traces (**a**) and quantification (**b**) of total distance traveled in open field for 10mins after saline or clozapine (CLZ, 0.1 mg/Kg) injection for chemogenetic manipulation of OPRM1^{+} RVM^{SC} neurons (n = 5). (**c**) Locomotion dynamics of WT mice after i.p. injection of clozapine (CLZ, 0.1 mg/Kg) or saline (n = 6). (**d**) Electrophysiological validation of terminal silencing of the vlPAG→RVM pathway. AAV5-DIO-ChR2 and AAV2-DIO-hM4D were co-injected into the vlPAG of vGlut2-Cre mice. Representative optogenetic stimulation evoked EPSC (oEPSC) traces (**e**) and quantification (**f**) recorded on the RVM slices containing ChR2-expressing terminals from the vlPAG before (baseline, black) and after infusion of CNO (5 μM). Scale bar: 50 pA, 25 ms. n = 6 cells from 3 mice. Wilcoxon matched-pairs signed rank test, * $P < 0.05$. (**g**) Example traces (left) and quantification (right) of total distance traveled in open field for 10mins after saline or clozapine (0.1 mg/Kg) injection for chemogenetic inhibition of the lSC→RVM pathway (n = 6). (**h,i**) Unilateral optogenetic activation of lSuC→RVM pathway (**h**) did not alter the locomotion trajectory and average speed of the ChR2-expressed mice (**i**). Control n = 6, ChR2 n = 6. (**j,k**) Unilateral Optogenetic activation of lSuC→RVM pathway did not alter the heart rate (**j,** control n = 6, ChR2 n = 5) and breath rate of the ChR2-expressed mice (**k,** control n = 6, ChR2 n = 6).

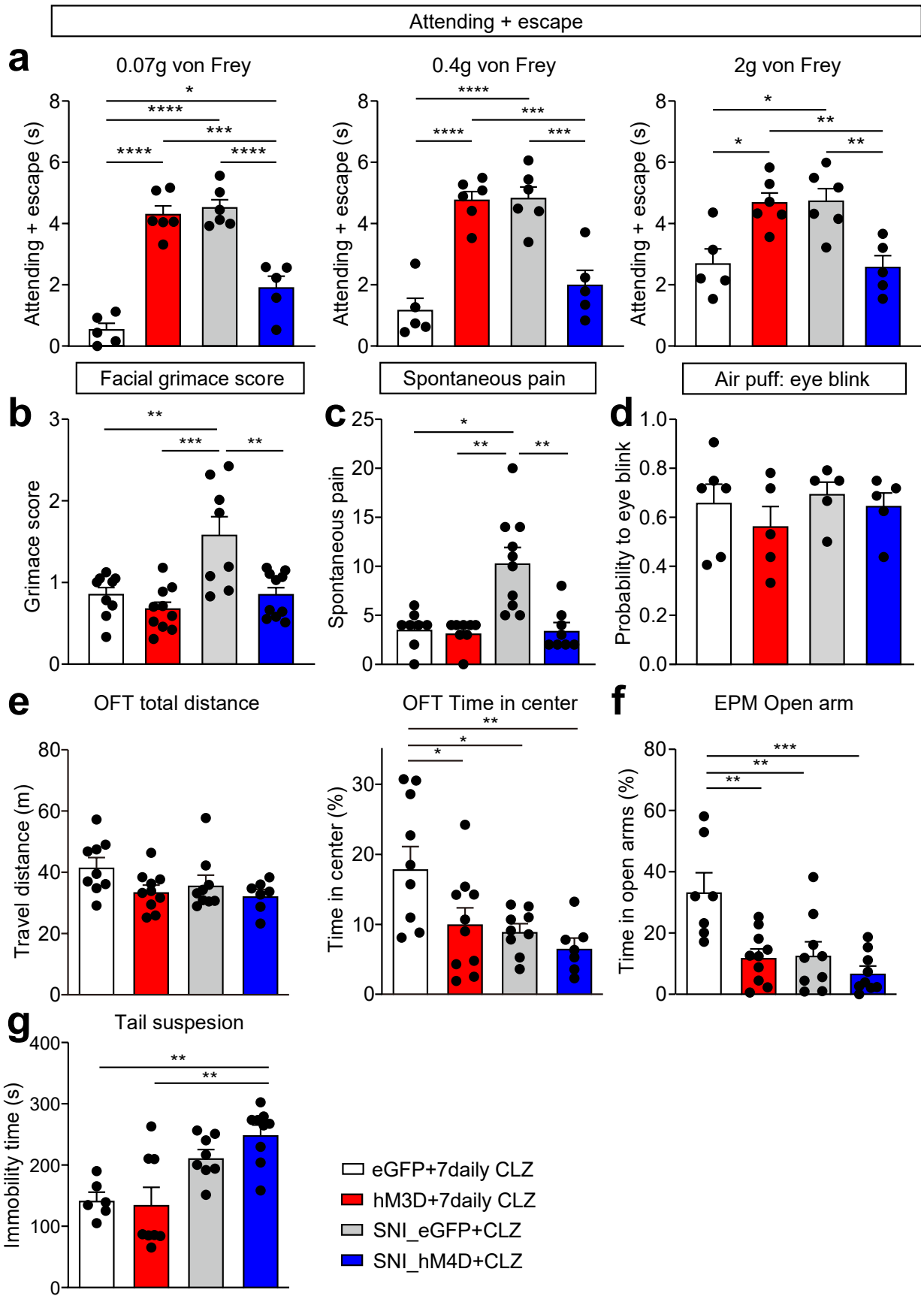

a
Hargreaves
hM4D
hM3D
Withdrawal latency (s)
b
hM3D in OPRM1+ RVMSC neurons
Mechanical threshold (g)
Pre
hours after Clozapine
c
Paired
control
hM4D
d
SNI
SNI+hM4D
Sham
Spinal cord (ipsi)
ACC Contra
SSp Contra
VPL Contra
LPBN Contra
Po Contra
e
SNI
SNI + hM4D
Sham
c-Fos + neurons in each brain region
SC
mPFC
ACC
VPL
Po
MD
SSp
SSs
Amy
LPBN
Repetitive chemogenetic activation
f
Plantar cold
CLZ-mCherry
CLZ-hM3D
g
Hargreaves
days on Clozapine
days post Clozapine
Chemogenetic silencing excitatory ISuC input
h
von Frey
days Post CFA
i
Hargreaves
CLZ-mCherry
CLZ-hM4D
days Post CFA

Extended Data Fig. 6 Chemogenetic manipulation of OPRM1^{+} RVM^{SC} neurons or vGlut2^{+} RVM-projecting lSuC neurons. (**a**) Quantification of Hargreaves withdrawal latency in healthy mice expressing hM4D (blue, n = 8) or hM3D (red, n = 10) in OPRM1^{+} RVM^{SC} neurons, following saline (black) or clozapine (0.1 mg/Kg) treatment. (**b**) Temporal dynamics of mechanical thresholds in mice expressing hM3D in OPRM1^{+} RVM^{SC} neurons from 1 to 12 hours after a single clozapine injection (0.1 mg/kg, i.p.) (n = 10). (**c**) Example CPA trace after SNI in mice expressing mCherry- or hM4D- in OPRM1^{+} RVM^{SC} neurons. Related to Fig. 2h. (**d,e**) Representative images (**d**) and quantification (**e**) of von Frey fiber (0.16 g) induced cFos-expressing neurons in the spinal cord of SNI (black, n = 4), SNI+hM4D (blue, n = 4) expressed in OPRM1^{+} RVM^{SC} neurons, and sham control mice (orange, n = 4). Scale Bar: 100 μm. (SC: spinal cord; mPFC: medial prefrontal cortex; ACC: anterior cingulate cortex; VPL: ventral posterolateral nucleus of thalamus; Po: posterior complex of the thalamus; MD: medial dorsal nucleus of thalamus; SSp: primary somatosensory cortex; SSs: secondary somatosensory cortex; Amy: amygdala; LPBN: lateral parabrachial nucleus). (**f**) Quantification of plantar cold withdrawal latency in mice expressing mCherry- (black, n = 6) or hM3D- (red, n = 10) in OPRM1^{+} RVM^{SC} neurons following repetitive clozapine administration (7 daily injections, 0.1 mg/kg). Mann-Whitney test, * $P < 0.05$. (**g**) Quantification of thermal withdrawal latency during and after repetitive daily injection of clozapine in mice expressing hM3D in OPRM1^{+} RVM^{SC} neurons (n = 5). Clozapine was injected 23 hours before each von Frey test for 7 consecutive days (shade area), related to Fig.2i. (**h**) Quantification of mechanical thresholds (left) and Hargreaves withdrawal latency (right) following clozapine infusion in terminals of vGlut2^{+} RVM-projecting lSuC neurons expressing hM4D (blue, n = 8) or mCherry (black, n = 8) pre and post CFA injection. Mann-Whitney test, *** $P < 0.001$.

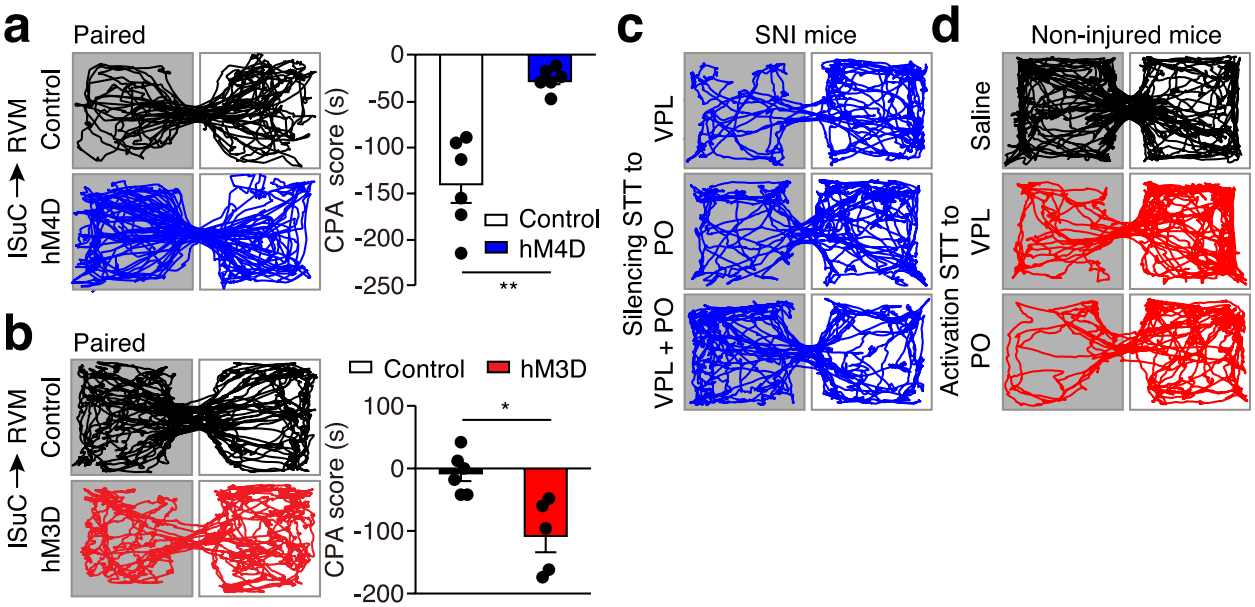


Extended Data Fig. 7 Quantification of cFos expression and withdrawal latency after ablation of OPRM1⁺ RVM^{SC} neurons. (**a**) Experiment timeline for **(b,c,d,e)**. (**b**) Quantification of the percentage of OPRM1⁺ descending neurons in total OPRM1⁺ RVM neurons (left) of control- (white, n = 5) and Caspase- (orange, n = 5) expressing mice with SNI. Mann-Whitney test, ** $P <$ 0.01. Related to Fig.2c, d. (**c**) Representative images (left) and quantification (right) of von Frey fiber (1.4 g) induced cFos-expressing neurons in the spinal cord of control- (white, n = 3) and Caspase- (orange, n = 3) expressing mice without injury. Dash line indicates the dorsal horn. (**d**) Quantification of the von Frey fiber (0.16 g) induced cFos-expressing neurons in the spinal cord

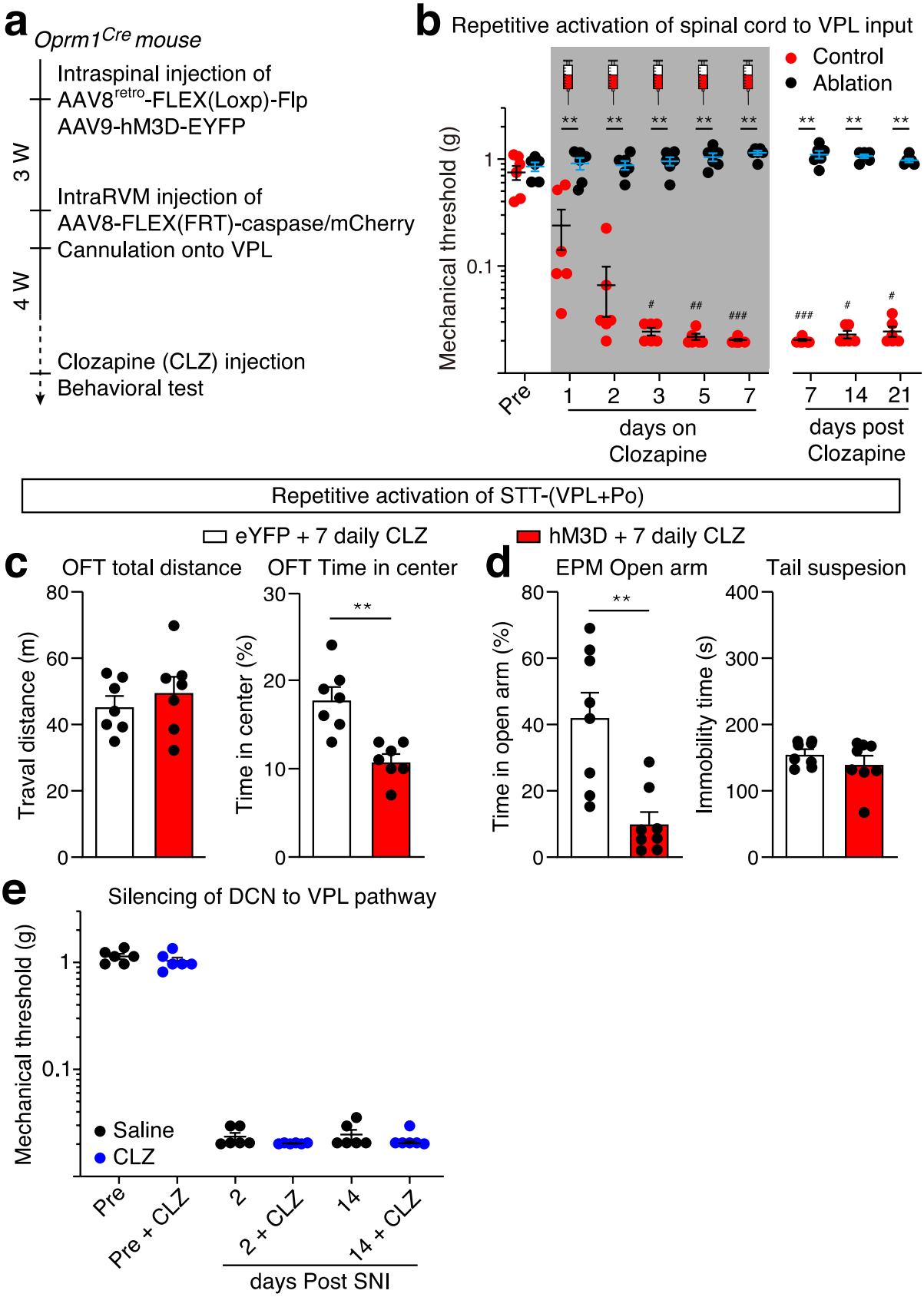
of control- (white, n = 4) and Caspase- (orange, n = 4) expressing mice with SNI. Mann-Whitney test, * $P < 0.05$. Related to Fig.2c, d. (**e**) Quantification of thermal withdrawal latency of control- (black, n = 7) and Caspase3- (orange, n = 8) expressing mice after CFA injection. Mann-Whitney test, *** $P < 0.001$. Related to Fig.2e.


Attending + escape
a
0.07g von Frey
0.4g von Frey
2g von Frey
Attending + escape (s)
Facial grimace score
Spontaneous pain
Air puff: eye blink
b
Grimace score
c
Spontaneous pain
d
Probability to eye blink
e
OFT total distance
OFT Time in center
f
EPM Open arm
Travel distance (m)
Time in center (%)
Time in open arms (%)
g
Tail suspesion
Immobility time (s)
eGFP+7daily CLZ
hM3D+7daily CLZ
SNI_eGFP+CLZ
SNI_hM4D+CLZ

Extended Data Fig. 8 Affective pain, spontaneous pain and anxiety-like behaviors after manipulation of OPRM1^{+} RVM^{SC} neurons. (**a**) Attending and escape behaviors assessed with three different von Frey fibers stimuli. Groups: eGFP + 7 daily CLZ (white, n = 5), hM3D + 7 daily CLZ (red, n = 6), SNI + eGFP + CLZ (gray, n = 6), SNI + hM4D + CLZ (blue, n = 5). One-way ANOVA test, * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. (**b**) Histogram shows facial grimace score in different groups: eGFP + CLZ (white, n = 9), hM3D + CLZ (red, n = 10), SNI + eGFP + CLZ (gray, n = 8), SNI + hM4D + CLZ (blue, n = 10). One-way ANOVA test, Turkey's multiple comparison. ** $P < 0.01$, *** $P < 0.001$. (**c**) Histogram shows spontaneous pain in different groups: eGFP + CLZ (white, n = 8), hM3D + CLZ (red, n = 8), SNI + eGFP + CLZ (gray, n = 10), SNI + hM4D + CLZ (blue, n = 8). Nonparametric ANOVA (Kruskal–Wallis test), followed by post-hoc pairwise comparisons using Dunn's multiple comparison test, * $P < 0.05$, ** $P < 0.01$. (**d**) Eye blink responses to 20 PSI air puff directed at the cornea show no difference between each group: eGFP + CLZ (white, n = 6), hM3D + CLZ (red, n = 5), SNI + eGFP + CLZ (gray, n = 5), SNI + hM4D + CLZ (blue, n = 5). (**e**) Quantification of total distance traveled (left) and time in center (right) in open field test. Groups: Groups: eGFP + 7 daily CLZ (white, n = 9), hM3D + 7 daily CLZ (red, n = 10), SNI + eGFP + CLZ (gray, n = 9), SNI + hM4D + CLZ (blue, n = 7). Nonparametric ANOVA (Kruskal–Wallis test), followed by followed by post-hoc pairwise comparisons using Dunn's multiple comparison test, * $P < 0.05$, ** $P < 0.01$. (**f**) Quantification of time in open arm in elevated plus maze test. Groups: eGFP + 7 daily CLZ (white, n = 7), hM3D + 7 daily CLZ (red, n = 10), SNI + eGFP + CLZ (gray, n = 9), SNI + hM4D + CLZ (blue, n = 9).One-way ANOVA, post-hoc pairwise comparisons were conducted using Tukey's multiple comparisons test, ** $P <0.01$, *** $P <0.001$. (**g**) Quantification of immobility time in tail suspension test. Groups: eGFP + 7 daily CLZ (white, n = 6), hM3D + 7 daily CLZ (red, n = 8), SNI + eGFP + CLZ (gray, n = 8), SNI + hM4D + CLZ (blue, n = 9). Nonparametric ANOVA (Kruskal–Wallis test), followed by post-hoc pairwise comparisons using Dunn's multiple comparison test, ** $P <0.01$.

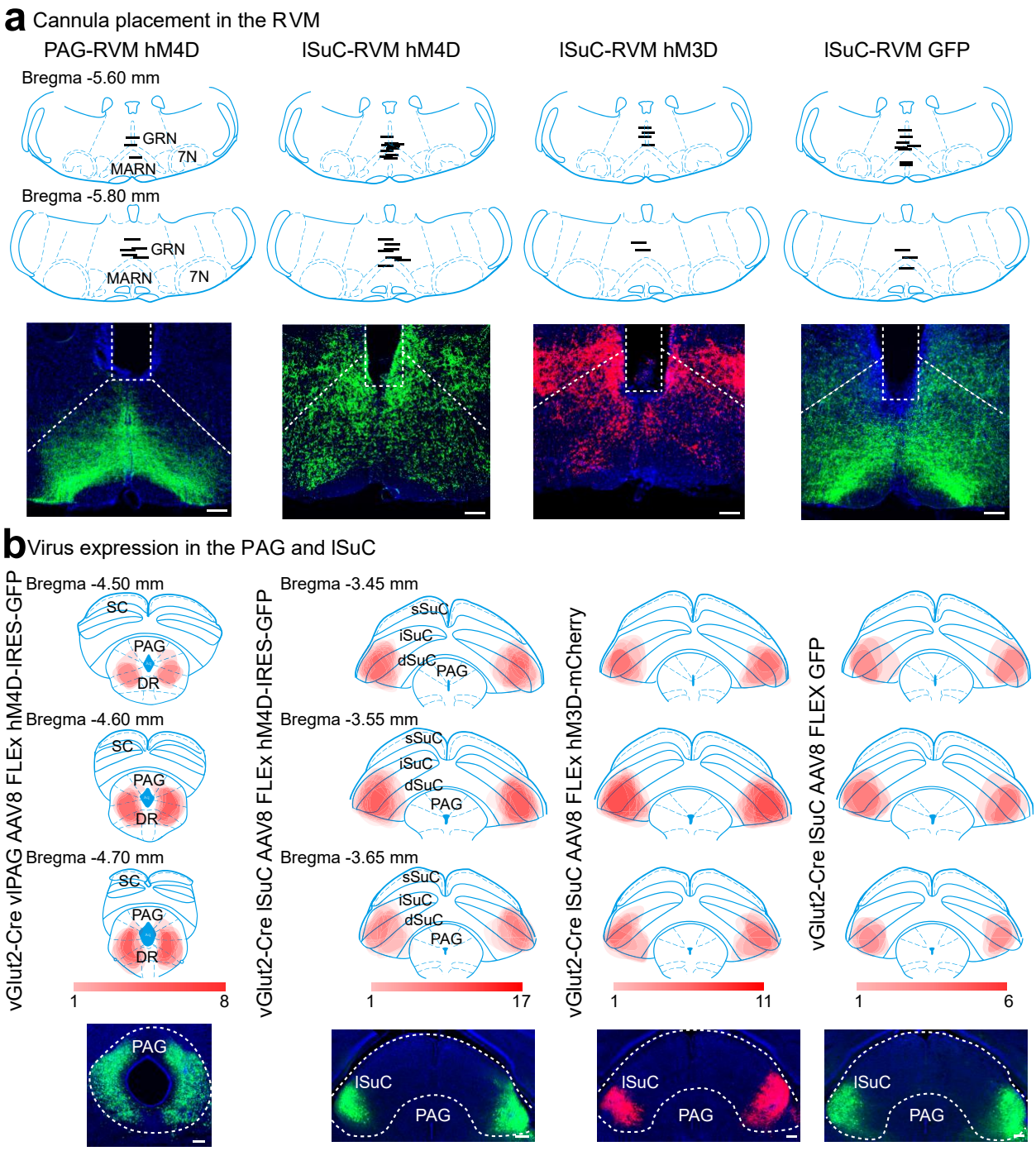


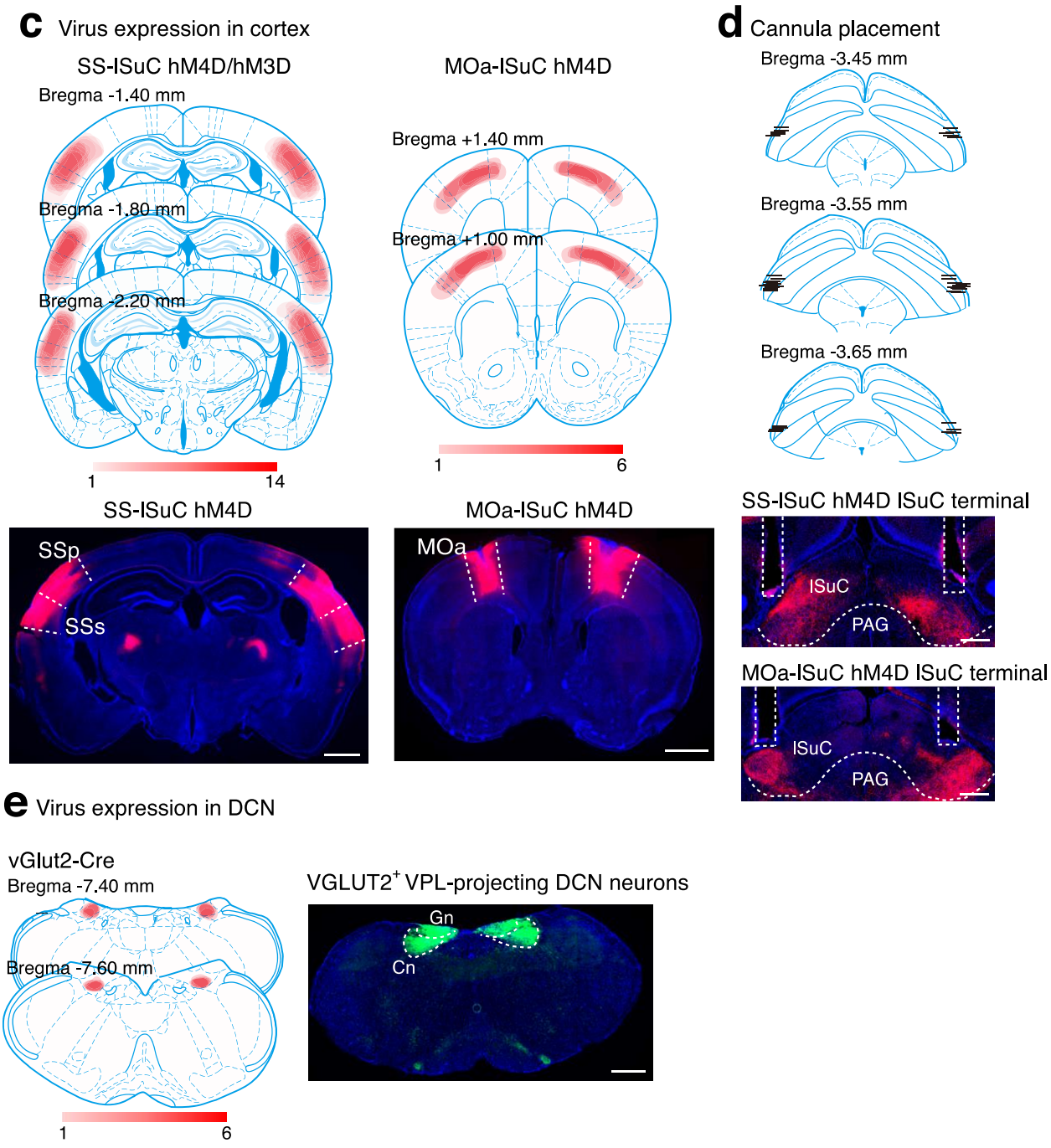
Extended Data Fig. 9 Impact of manipulating the activity of the excitatory lSuC→RVM pathway, or SS→lSuC pathway on pain-induced CPA. (**a**) Example traces (left) and quantification (right) of mechanical stimuli (0.16 g von Frey filament) evoked CPA in control mice (black, n = 6) and mice with the lSuC→RVM pathway silenced (blue, n = 6). Related to Fig. 3g. Mann-Whitney test, ** $P < 0.01$. (**b**) Example traces (left) and quantification (right) of mechanical stimuli (0.16g von Frey filament) evoked CPA in control mice (black, n = 6) and mice after repetitive activation of the excitatory lSuC→RVM pathway (red, n = 5). Related to Fig. 3h. Mann-Whitney test, * $P < 0.05$. (**c**) Example traces of mechanical stimuli (0.16 g von Frey filament) evoked CPA for Fig. 5e. (**d**) Example traces of mechanical stimuli (0.16 g von Frey filament) evoked CPA for Fig. 5g. Mean ± SEM.


a
Oprm1Cre mouse
Intraspinal injection of
AAV8retro-FLEX(Loxp)-Flp
AAV9-hM3D-EYFP
3 W
IntraRVM injection of
AAV8-FLEX(FRT)-caspase/mCherry
Cannulation onto VPL
4 W
Clozapine (CLZ) injection
Behavioral test
b
Repetitive activation of spinal cord to VPL input
Control
Ablation
Mechanical threshold (g)
Pre
days on Clozapine
days post Clozapine
Repetitive activation of STT-(VPL+Po)
eYFP + 7 daily CLZ
hM3D + 7 daily CLZ
c
OFT total distance
OFT Time in center
Traval distance (m)
Time in center (%)
d
EPM Open arm
Tail suspesion
Time in open arm (%)
Immobility time (s)
e
Silencing of DCN to VPL pathway
Mechanical threshold (g)
Saline
CLZ
Pre
Pre + CLZ
2
2 + CLZ
14
14 + CLZ
days Post SNI

Extended Data Fig. 10 Anxiety-like behaviors after repetitive activation of the STT, and chemogenetic silencing of the VPL-projecting DCN neurons.

(**a**) Experimental time line for (**b**). (**b**) Quantification of mechanical thresholds after repetitive chemogenetic activation of the spinal cord to VPL pathway in mCherry expressing control mice (Control, red, n = 6) but not in OPRM1^{+} RVM^{SC} neurons ablation mice (Ablation, black, n = 6). CLZ was infused 23 hours before each von Frey test for 7 consecutive days (shade area). Pre vs. post clozapine infusion: nonparametric ANOVA (Friedman test), followed by Dunn's multiple comparison test, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$. Control vs. ablation: Mann-Whitney test, ** $P < 0.01$. (**c**) Quantification of total distance traveled and time in center in open field test. eYFP + 7daily CLZ (white, n = 7), hM3D + 7daily DCLZ (red, n = 7). Mann-Whitney test, ** $P < 0.05$. (**d**) Quantification of time in open arm in elevated plus maze test (left) and immobility time in tail suspension test (right). eYFP + 7daily CLZ (white, n = 8), hM3D + 7daily CLZ (red, n = 8). (**e**) Chemogenetic silencing of the vGLut2^{+} VPL-projecting DCN neurons did not affect animals' mechanical threshold under normal or SNI conditions (n = 6).


a Cannula placement in the RVM
PAG-RVM hM4D
lSuC-RVM hM4D
lSuC-RVM hM3D
lSuC-RVM GFP
Bregma -5.60 mm
GRN
7N
MARN
Bregma -5.80 mm
GRN
MARN
7N
b Virus expression in the PAG and lSuC
vGlut2-Cre vlPAG AAV8 FLEx hM4D-IRES-GFP
Bregma -4.50 mm
SC
PAG
DR
Bregma -4.60 mm
SC
PAG
DR
Bregma -4.70 mm
SC
PAG
DR
1
8
PAG
vGlut2-Cre lSuC AAV8 FLEx hM4D-IRES-GFP
Bregma -3.45 mm
sSuC
iSuC
dSuC
PAG
Bregma -3.55 mm
sSuC
iSuC
dSuC
PAG
Bregma -3.65 mm
sSuC
iSuC
dSuC
PAG
1
17
lSuC
PAG
vGlut2-Cre lSuC AAV8 FLEx hM3D-mCherry
1
11
lSuC
PAG
vGlut2-Cre lSuC AAV8 FLEX GFP
1
6
lSuC
PAG



Extended Data Fig. 11 Canula placement in the RVM or lSuC and virus expression in the PAG, lSuC, SS, MO, and DCN for chemogenetic manipulation of the PAG-RVM, lSuC→RVM, SS→lSuC, MO→lSuC pathways, and VPL-projecting DCN neurons. (**a**) Canula placement in the RVM. Scale bar: 200 μm. GRN: gigantocellular reticular nucleus; MARN: magnocellular reticular nucleus, 7N: 7th nucleus. (**b**) Virus expression in the PAG and lSuC. Scale bar: 200 μm. SuC: superior colliculus; sSuC: superficial SuC; iSuC: intermediate

SuC; dSuC: deep SuC; PAG: periaqueductal gray; DR: dorsal raphe. Related to experiments in Fig. 3. (**c**) Virus expression and (**d**) cannula placement for manipulating the SS→lSuC or MO→lSuC pathway. Scale bar: 1 mm (**c**), 500 μm (**d**). SS: somatosensory cortex; MO: motor cortex. Related to experiments in Fig. 4. (**e**) Virus expression for manipulating the DCN→VPL pathway. Scale bar: 500 μm. Related to experiments in Figs. 3-5. Gn: Gracile nucleus; Cn: Cuneate nucleus.