Synchronized cluster firing, a distinct form of sensory neuron activation, drives spontaneous pain

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SUPPLEMENTARY FIGURE LEGENDS, TABLES, AND MOIVES

Figure S1.

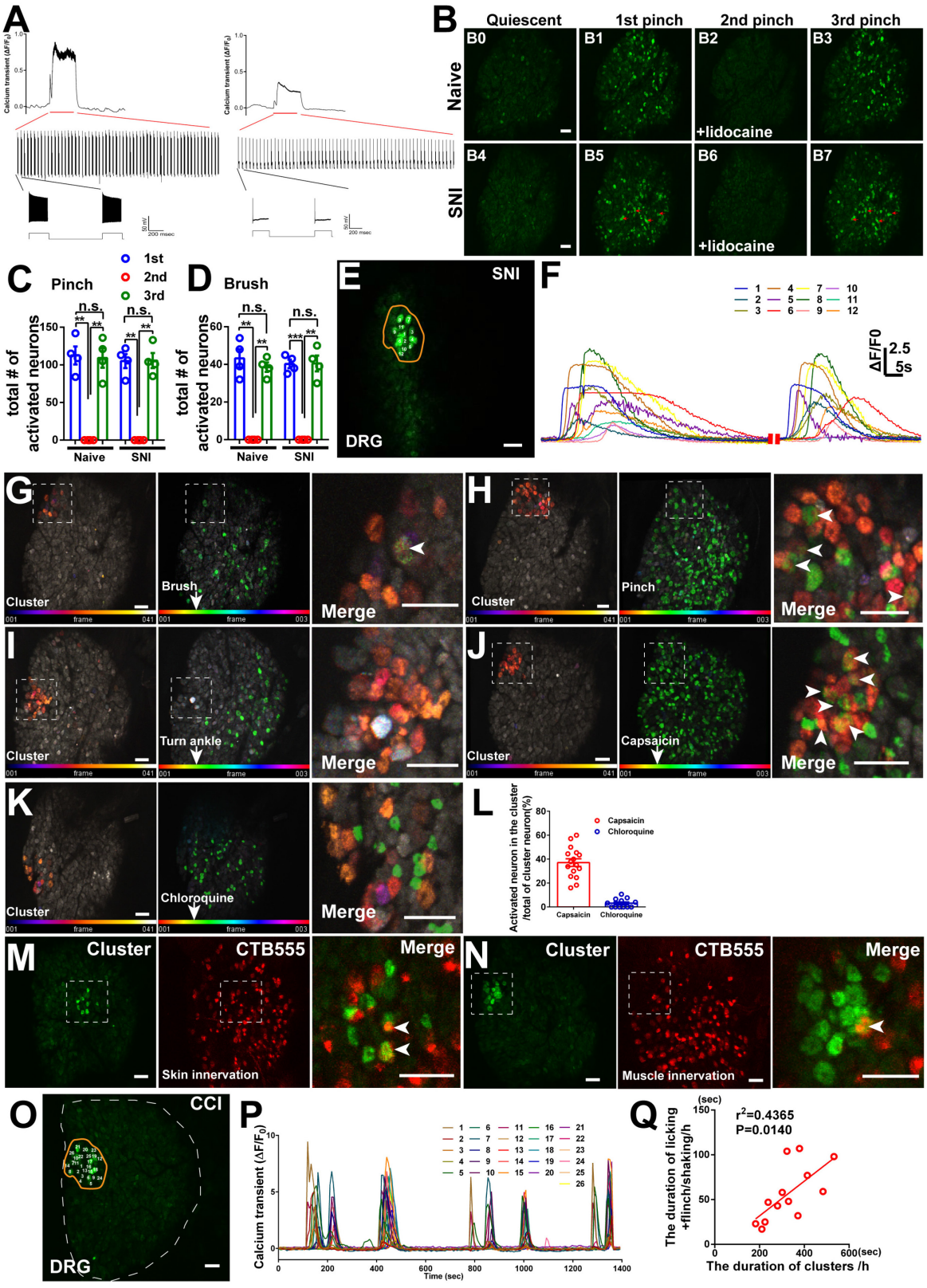

Figure S1. GCaMP6s signal's relationship to DRG neuron firing and characterization of neurons in the clusters. Related to Figure 1.

(A) Relationship between GCaMP6 signal and neuron firing. Examples of simultaneous calcium imaging recording (top) and intracellular microelectrode recording (middle, bottom) of single DRG neurons in an ex vivo, whole DRG preparation from a naïve mouse. Cells were classified as firing a single action potential (right) or multiple action potentials (left) in response to a 270 msec suprathreshold stimulus sufficient to evoke the maximum number of action potentials (stimulus timing indicated by bottom traces in expanded graph). These suprathreshold stimuli were applied at a rate of 1 Hz for 1 minute (red line). In both cell types, the calcium response was predominantly a single waveform with an exponential decay with time constants of 2 – 4 seconds after the stimulus ceased. The entire stimulation period is shown in the inset, with expansion of the first 2 stimuli below from which the stimulation artifact has been removed.

(B) An example of 1.5% lidocaine application onto the DRG. Recordings of neurons activated by pinching the hind paw before (1st) and after (2nd) application of lidocaine. After 3 hours of washing out the lidocaine, the paw was pinched again (3rd). Red arrows show coupled activation by pinch, as described in our previous study (Kim et al., 2016). SNI data are from day 21.

(C-D) Summary of the number of neurons activated by pinching (C) and brushing (D) before and after application and washing out of lidocaine.

(E) Representative images of one cluster from an SNI mouse (Day 21), observed with high time resolution (238ms/frame); (F) time traces of GCaMP6s signal from individual neurons in (E).

(G-K) Representative images of the neurons in a cluster overlapped with images of the neurons activated by paw brushing (G), paw pinching (H), turning ankle (I) in an SNI animal (Day 21).

(J-L) Representative images of the neurons in a cluster in an SNI animal (Day 21), overlapped with the neurons activated by 1uM capsaicin (J) and (in a different animal) 1.5 mM chloroquine (K) applied to the DRG. (L) summary data.

(M-N) Representative images of the neurons in the cluster overlap with the neurons innervating skin (M) and muscle (N) in an animal after CCI (Day 20).

(O) Representative images of one cluster (orange circle) in vivo calcium imaging of L4 DRG (white outline) in CCI mouse.

(P) Time course of calcium transient in individual neurons from (O), showing several instances of neurons in the cluster firing synchronously.

(Q) Pearson correlation between the duration of cluster activity and the duration of spontaneous pain behaviors (from SNI mice in (Figure 1E) that showed cluster firing.

Scale bar, 100μm. Each open circle in (C) to (D) represents an individual mouse; Each open circle in (L) represents a cluster. Data are represented as mean ± SEM. **$p < 0.01$;***$p < 0.001$; n.s., not significant; by one-way ANOVA test with Tukey's posttest.

Figure S2.

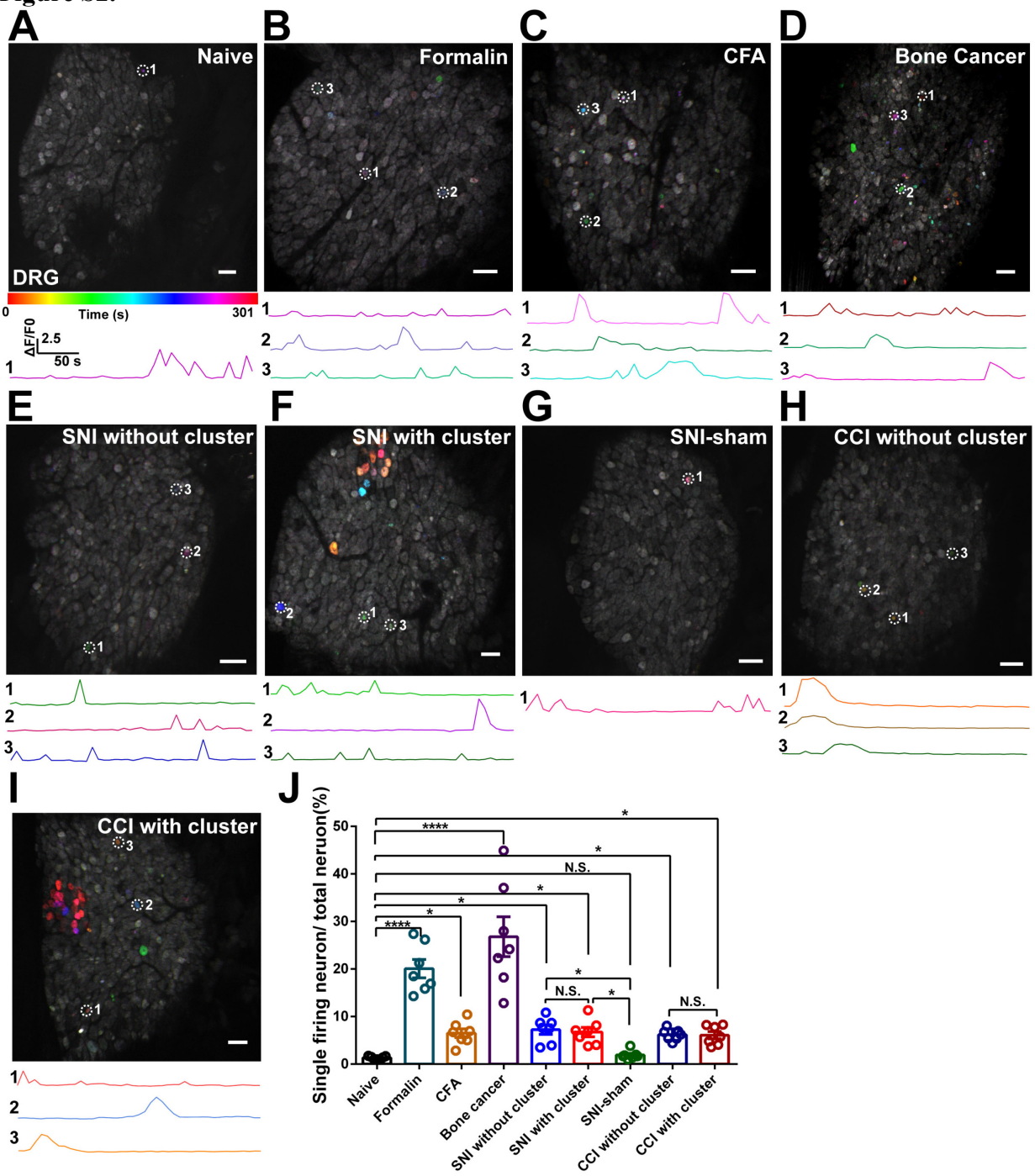

Figure S2. Scattered spontaneous single neuron firing in vivo DRG calcium imaging in different pain models. Related to Figure 1 and Table S1.

(A-I) Overlapping frames of images (301s, 7s/frame), and time-lapse color-coded images (temporal code, color indicates 0-301s) of spontaneous activity of DRG neurons in naïve (A),

Formalin 1h post injection (B), CFA at POD3 day (C), tibial bone cancer at POD14 day (D), SNI without (E) and with (F) cluster at POD21 day, SNI-sham (G) at POD21 day and CCI without (H) and with (I) cluster at POD21 day mice. Time course of the calcium signal in the traced and numbered neurons is shown under the images.

(J) Summary of the percentage of singly firing neuron in different pain models.

Scale bar, 100μm. Each open circle represents an individual mouse. Data are represented as mean ± SEM. *, $p<0.05$; ****, $p<0.0001$; n.s., not significant; by one ANOVA with Fisher's LSD posttest.

Figure S3.

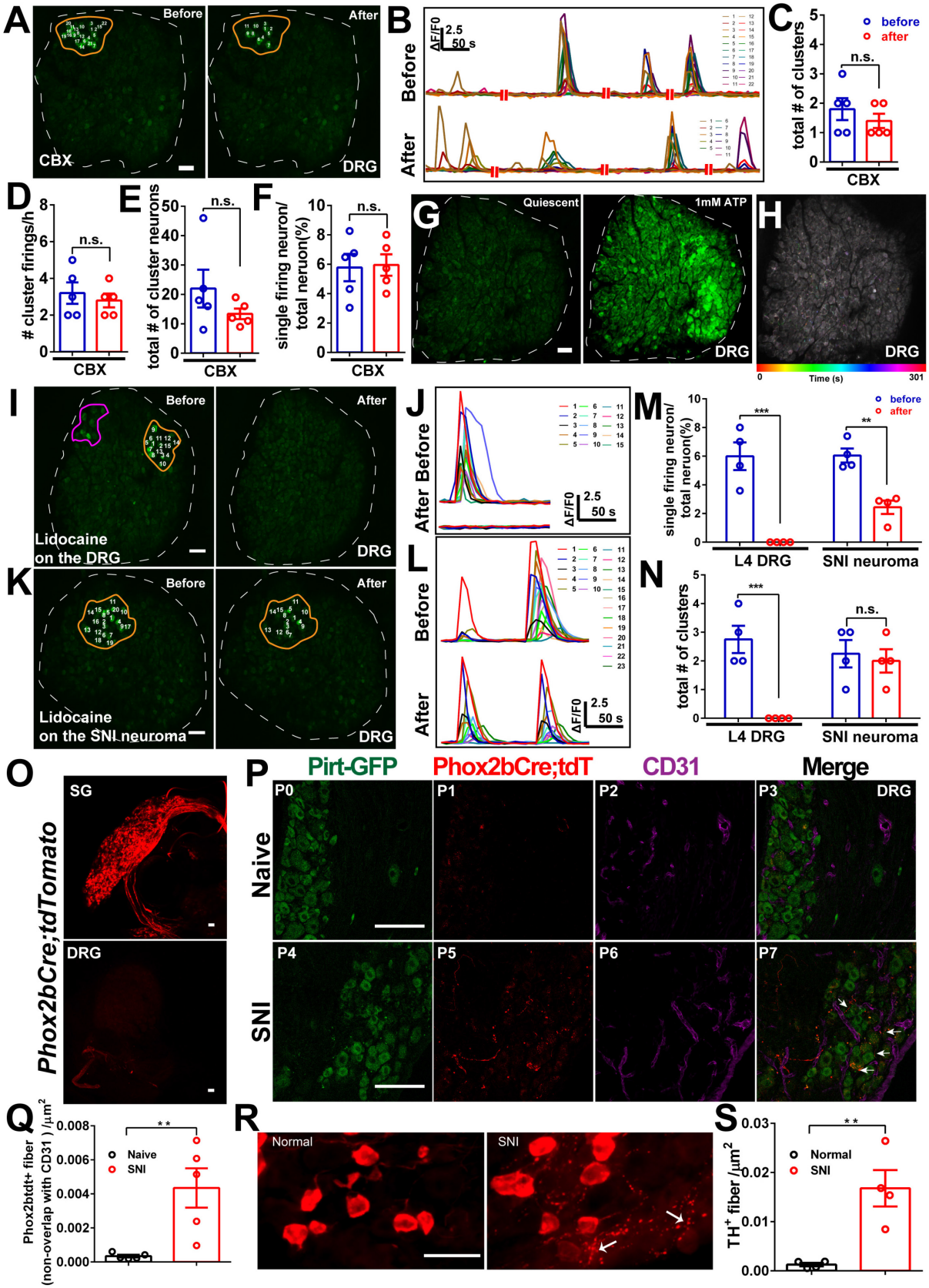
A
Before
After
CBX
DRG
B
ΔF/F0
2.5
50 s
Before
After
C
before
after
n.s.
total # of clusters
CBX
D
n.s.
cluster firings/h
CBX
E
n.s.
total # of cluster neurons
CBX
F
n.s.
single firing neuron/ total neruon(%)
CBX
G
Quiescent
1mM ATP
DRG
H
DRG
0
Time (s)
301
I
Before
After
Lidocaine on the DRG
DRG
J
After Before
ΔF/F0
2.5
50 s
K
Before
After
Lidocaine on the SNI neuroma
DRG
L
Before
After
ΔF/F0
2.5
50 s
M
before
after
***
**
single firing neuron/ total neruon(%)
L4 DRG
SNI neuroma
N
***
n.s.
total # of clusters
L4 DRG
SNI neuroma
O
Phox2bCre;tdTomato
SG
DRG
P
Pirt-GFP
Phox2bCre;tdT
CD31
Merge
Naive
SNI
P0
P1
P2
P3
DRG
P4
P5
P6
P7
Q
**
Naive
SNI
Phox2btdt+ fiber (non-overlap with CD31) /μm2
R
Normal
SNI
S
**
Normal
SNI
TH+ fiber /μm2

Figure S3. Cluster firing is gap junction independent and uncoupled from satellite glial cell activity; cluster firing can be inhibited by lidocaine application onto DRG; and the density of sympathetic fibers in DRG increases after nerve injury. Related to Figure 1 and Figure 2.

(A) An example of cluster firing from an SNI mouse (Day 21-28) that is unchanged after using CBX. Left is before using CBX; Right is after.

(B) Representative traces of neurons in the cluster which are numbered in (A) before and after addition of CBX. Upper is before; lower is after.

(C-F) The total number of clusters (C), frequency of cluster firing (D), total number of cluster firing neurons (E) and singly firing neurons (F) are not changed after addition of 50μM CBX to DRG.

(G) Representative images of L4 DRG of *GFAP-Cre; GCaMP6s* mice at day 21 after SNI surgery. Left is quiescent without any stimuli; Right is after dropping 1mM ATP onto the DRG after 2h of recording.

(H) Overlapping frames of images (301s, 7s/frame, taken at various time points during the 2 hour recording), and time-lapse color-coded images (temporal code, 0-301s) of spontaneous activity of satellite glial cells in mice (G) that appears sporadically). The calcium transients in satellite glial cells are frequent and scattered, and do not synchronously coordinate across long distances or in clusters.

(I-J) An example of cluster firing that is inhibited after application with 1.5% lidocaine onto the L4 DRG of an SNI mouse (Day 21-28). Left is before; Right is after. (J) Representative traces of neurons in the cluster which are numbered in (I) before and after application.

(K-L) An example of cluster firing that is unchanged after application of 1.5% lidocaine on the neuroma in an SNI mouse (Day 21-28). Left is before; Right is after. (L) Representative traces of

neurons in the cluster which are numbered in (K) before and after application. (M-N) Summary of singly firing neurons (M) and CFEs (N) changes before and after using lidocaine at the two different sites.

(O) tdTomato signals in the L3 Sympathetic ganglion (upper) and the L4 DRG (lower) whole mount images of naive Phox2bCre;tdTomato mice.

(P) Section staining of CD31 in L4 DRG of *Phox2bCre;tdTomato; Pirt-GFP* mice with and without SNI (Day 21-28). GFP is expressed in DRG neurons, tdTomato is expressed in the sympathetic axons. CD31 is a blood vessel marker. Arrows indicate examples of increased fibers seen after SNI.

(Q) Quantification of sympathetic fibers (tdT) which are non-overlapped with CD31 in in the cellular area of the DRGs.

(R) Section staining of TH in mice with and without SNI (Day 28). Arrows indicate examples of increased fibers seen after SNI. TH-positive sensory neurons are also labeled but were not included in the quantification, which was done by tracing fibers in the cellular area of the DRGs.

(S) Summary of total length of TH fibers in cellular area of the DRG normalized to the area measured.

Scale bar, 100μm. Each open circle in (C) to (F), (M), (N), (Q), (S) represents an individual mouse. Data are represented as mean ± SEM. **, $p<0.01$; ***$p < 0.001$; n.s., not significant; (C) to (F) by two-way repeated measures ANOVA with Sidak's posttest, compared to saline group in Figure 3E-3G and Figure S4F; (M) and (N) by two-way repeated measures ANOVA with Sidak's posttest, (Q) and (S) by unpaired t-test.

Figure S4.

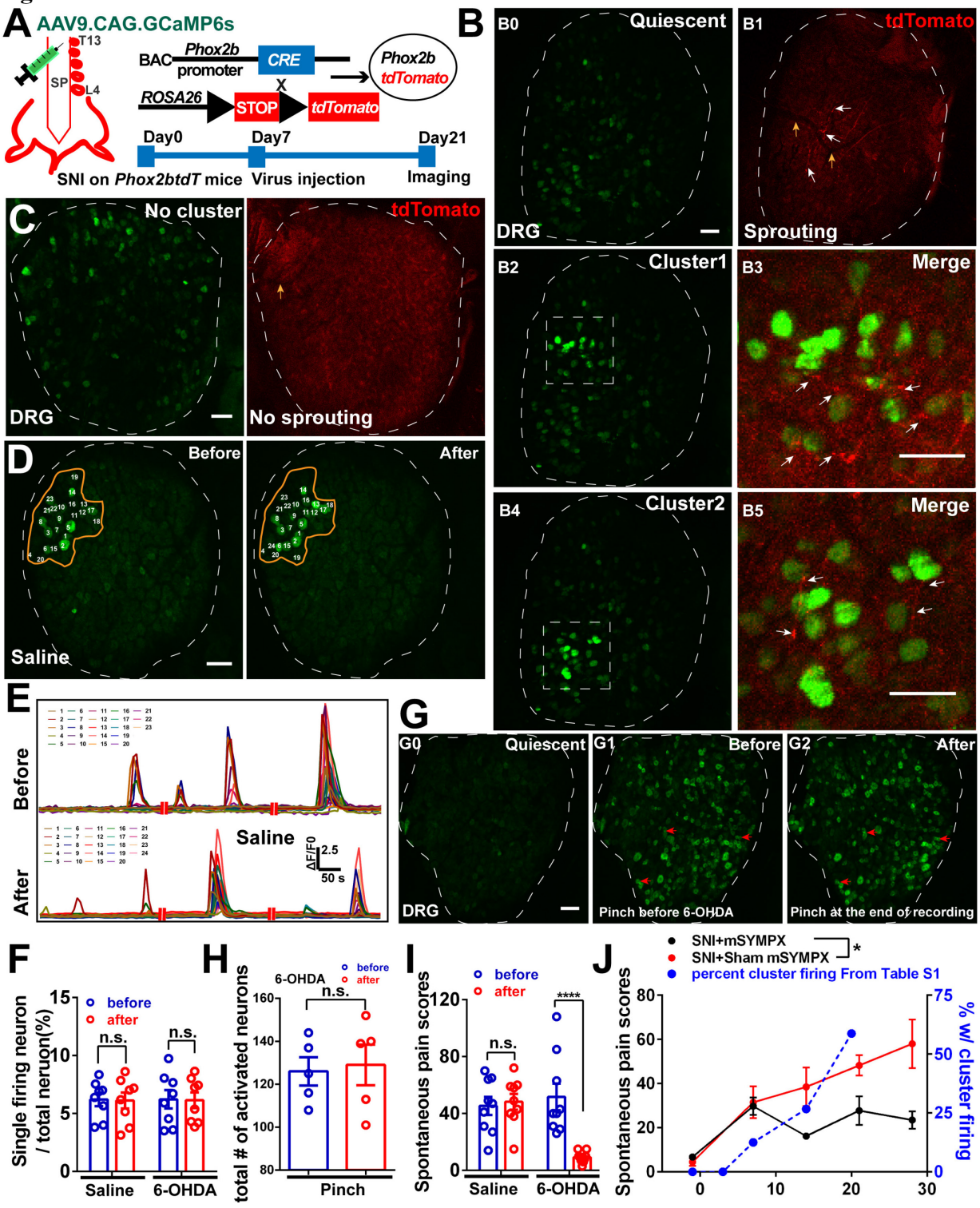
A
AAV9.CAG.GCaMP6s
T13
SP
L4
BAC
Phox2b promoter
CRE
Phox2b tdTomato
ROSA26
STOP
tdTomato
Day0
Day7
Day21
SNI on Phox2btdT mice
Virus injection
Imaging
B
B0 Quiescent
DRG
B1 tdTomato
Sprouting
B2 Cluster1
B3 Merge
B4 Cluster2
B5 Merge
C
No cluster
DRG
tdTomato
No sprouting
D
Before
Saline
After
E
Before
After
Saline
ΔF/F0
2.5
50 s
G
G0 Quiescent
DRG
G1 Before
Pinch before 6-OHDA
G2 After
Pinch at the end of recording
F
Single firing neuron / total neruon(%)
before
after
n.s.
n.s.
Saline
6-OHDA
H
6-OHDA
before
after
n.s.
total # of activated neurons
Pinch
I
Spontaneous pain scores
before
after
n.s.
****
Saline
6-OHDA
J
SNI+mSYMPX
SNI+Sham mSYMPX
*
percent cluster firing From Table S1
Spontaneous pain scores
% w/ cluster firing

Figure S4. Cluster firing only occurs in the SNI mice with sympathetic nerve sprouting, and no change of CFEs is observed with addition of saline. Related to Figure 2, Figure 3, Figure 7 and Table S2

(A) Diagram showing the mating strategy and intrathecal injection with AAV9.CAG.GCaMP6s in *Phox2bCre; tdTomato* mice and diagram showing the experimental procedure.

(B) An example of cluster firing occurring at the site of sympathetic fiber sprouting. (B0) is quiescent. (B1) is sympathetic nerves which are indicated by white arrows. The blood vessels are black which are indicated by yellow arrows. (B2) one of the clusters is labeled in white dashed square. (B3) Zoomed-in the white dashed square in (B2), overlapped with fiber images from (B1). (B4) Another cluster is labeled in white dashed square. (B5) Zoomed-in the white dashed square in (B4), overlapped with fiber images from (B1).

(C) An example of SNI mice without cluster firings. Left shows no cluster firing, right shows no sympathetic sprouting.

(D) An example of cluster firing before (left) and after (right) addition of saline onto the DRG of SNI mice with cluster firing.

(E) Representative traces of neurons in the clusters which are numbered in (D) before and after addition of saline. Upper is before; lower is after.

(F) The percentage of singly firing neurons is unchanged after addition of either saline or 6-OHDA to the DRG.

(G-H) Addition of 6-OHDA does not affect the viability of DRG neurons. (G) is example of before paw pinching (G0), pinching before 6-OHDA addition (G1) and pinching and at the end of experiment after 6-OHDA addition. Red arrows show examples of coupled activation by pinch. (H) is summary data.

(I) Spontaneous pain scores significantly decreased after i.t. administration of 5uL, 5uM 6-OHDA in SNI mice (Day 20). No change was observed with administration of saline.

(J) Effect of mSYMPX on spontaneous pain scores. Two way repeated measures ANOVA indicated a significant effect of the mSYMPX factor (*, P = 0.011) and of the time-mSYMPX interaction (p = 0.02). N = 4 mice/group. For comparison, the time course of cluster firing from Table S1 is also plotted (blue).

Scale bar, 100μm. Each open circle in (F) to (I) represents an individual mouse. Data are represented as mean ± SEM. ****p < 0.0001; n.s., not significant; (F) and (I) by two-way repeated measures ANOVA with Sidak's posttest; (H) by paired t-test..

Figure S5.

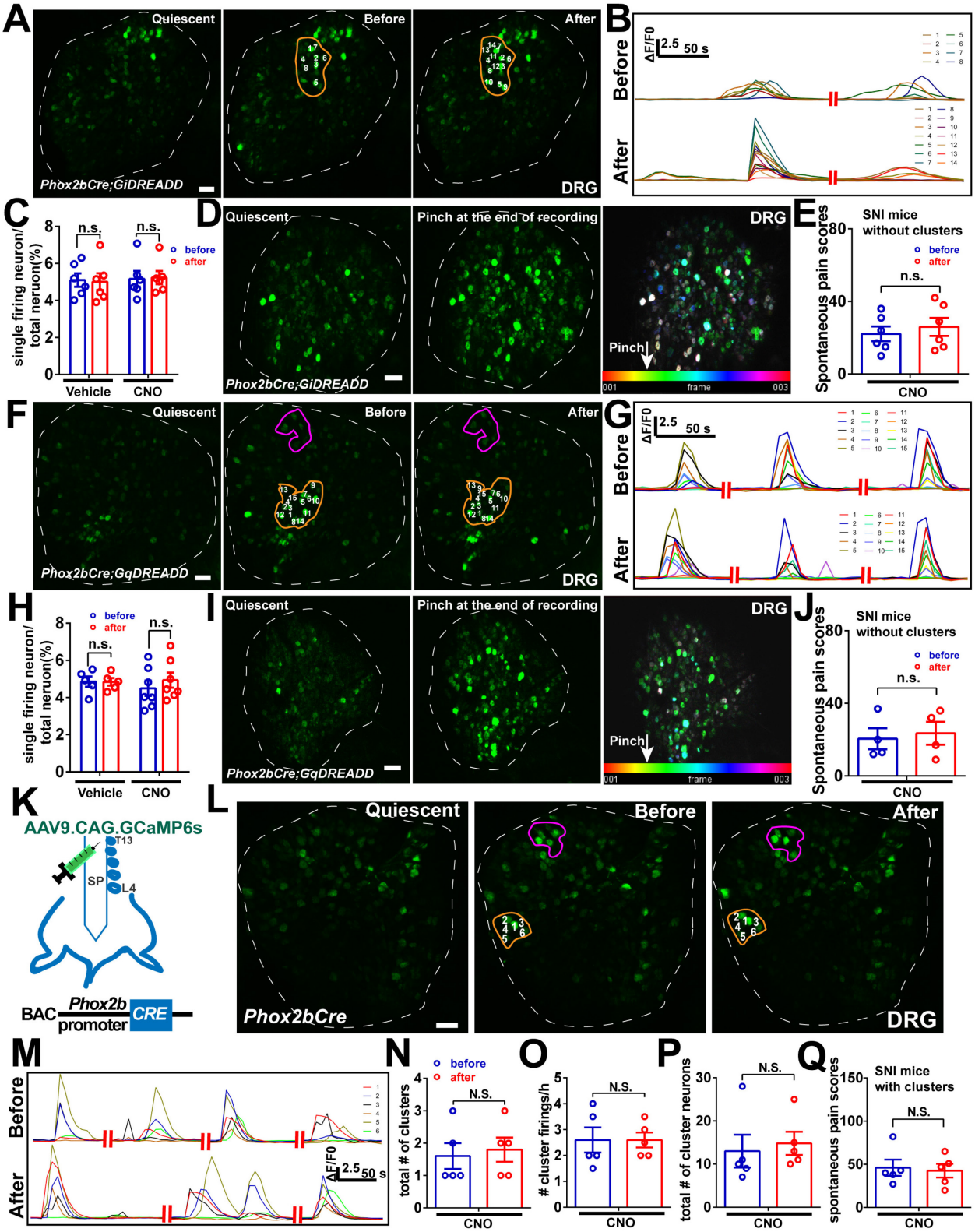
A
Quiescent
Before
After
Phox2bCre;GiDREADD
DRG
B
Before
After
ΔF/F0
2.5
50 s
C
single firing neuron/ total neruon(%)
n.s.
n.s.
before
after
Vehicle
CNO
D
Quiescent
Pinch at the end of recording
DRG
Pinch
frame
Phox2bCre;GiDREADD
E
SNI mice without clusters
Spontaneous pain scores
before
after
n.s.
CNO
F
Quiescent
Before
After
Phox2bCre;GqDREADD
DRG
G
Before
After
ΔF/F0
2.5
50 s
H
single firing neuron/ total neruon(%)
before
after
n.s.
n.s.
Vehicle
CNO
I
Quiescent
Pinch at the end of recording
DRG
Pinch
frame
Phox2bCre;GqDREADD
J
SNI mice without clusters
Spontaneous pain scores
before
after
n.s.
CNO
K
AAV9.CAG.GCaMP6s
T13
SP
L4
BAC
Phox2b promoter
CRE
L
Quiescent
Before
After
Phox2bCre
DRG
M
Before
After
ΔF/F0
2.5
50 s
N
total # of clusters
before
after
N.S.
CNO
O
cluster firings/h
N.S.
CNO
P
total # of cluster neurons
N.S.
CNO
Q
SNI mice with clusters
spontaneous pain scores
N.S.
CNO

Figure S5. No change of CFEs is observed with addition of vehicle in Phox2bCre; DREADD mice, and no change of CFEs or spontaneous pain score is observed with addition of CNO in Phox2bCre mice. Related to Figure 4 and Figure 5.

(A) An example of cluster firing in *Phox2bCre; GiDREADD* mouse 21 days after SNI, that had no change after addition of vehicle (0.1% DMSO in saline). Left is quiescent (i.e. period with no cluster firing); Middle is example of cluster firing before addition of vehicle, and there is one cluster which contains 8 neurons; Right is after addition of vehicle, and there is one cluster which contains 14 neurons.

(B) Representative traces of neurons in the clusters which are numbered in (A) before and after administration of vehicle.

(C) The singly firing neurons have no change after addition of either vehicle or CNO to DRG of SNI *Phox2bCre; GiDREADD* mice with cluster firing.

(D) The viability of DRG neurons in an SNI (day 21) *Phox2bCre; GiDREADD* mouse cannot be affected by addition of CNO. Left is quiescent; Middle is pinching at the end of recording; Right is the time-lapse color-coded images (temporal code, 3 frames, one frame is 7 seconds) of neurons activated by pinching.

(E) Spontaneous pain scores are not affected by i.t. administration of CNO in SNI (Day 20) *Phox2bCre; GiDREADD* mice without cluster firing.

(F) An example of cluster firing 21 days after SNI in a *Phox2bCre; GqDREADD* mouse that had no change after addition of vehicle (0.1% DMSO in saline). Left is quiescent (i.e. period with no cluster firing); Middle is example of cluster firing before addition of vehicle, and there are two clusters. Cluster1 (orange circle) contains 5 neurons, Cluster2 (purple circle) contains 15 neurons;

Right is after addition of vehicle, and there are 2 clusters. Cluster1 contains 5 neurons, Cluster2 contains 15 neurons. Scale bar, 100μm.

(G) Representative traces of neurons in the clusters which are numbered in (F) before and after administration of vehicle.

(H) The incidence of singly firing neurons has no change after addition of either vehicle or CNO to DRG of SNI (Day 21) *Phox2bCre; GqDREADD* mice with cluster firing.

(I) The viability of DRG neurons in SNI (Day 21) *Phox2bCre; GqDREADD* mouse is not affected by addition of CNO. Left is quiescent; Middle is pinching at the end of recording after CNO application; right is the time-lapse color-coded images (temporal code, 3 frames, one frame is 7 seconds) of neurons activated by pinching.

(J) Spontaneous pain scores are not affected by i.t. administration of CNO in SNI (Day 20) *Phox2bCre; GqDREADD* mice without cluster firing.

(K) Diagram showing the mating strategy, intrathecal injection with AAV9.CAG.GCaMP6s in *Phox2bCre* mice.

(L) An example of cluster firing in an SNI mouse (Day 21) that had no change after addition of CNO if no DREADDS are expressed. Left is quiescent (i.e. period with no cluster firing); Middle is example of cluster firing before addition of CNO, and there are 2 clusters. Cluster1 (orange circle) contains 5 neurons, Cluster2 (purple circle) contains 6 neurons; Right is after addition of CNO, and there are 2 clusters. Cluster1 contains 5 neurons, Cluster2 contains 6 neurons.

(M) Representative traces of neurons in the clusters which are numbered in (L) before and after administration of CNO.

(N-P) The total number of clusters (N), frequency of cluster firing (O), and total number of cluster firing neurons (P) had no change after addition of 10μM CNO to DRG.

(Q) Spontaneous pain scores had no significant change after i.t. administration of 5μL, 10μM CNO to mice without cluster firing at day 20 after SNI.

Scale bar, 100μm. Each pair of open circles (before and after) represents an individual mouse. Data are represented as mean ± SEM. N.S., not significant; (C) and (H) by two-way repeated measures ANOVA with Sidak's posttest; (E) by two-way repeated measures ANOVA with Sidak's posttest, compared to SNI mice with clusters in Figure 4H; (J) by two-way repeated measures ANOVA with Sidak's posttest, compared to SNI mice with clusters in Figure 5H, (N) to (Q) by paired t-test.

Figure S6.

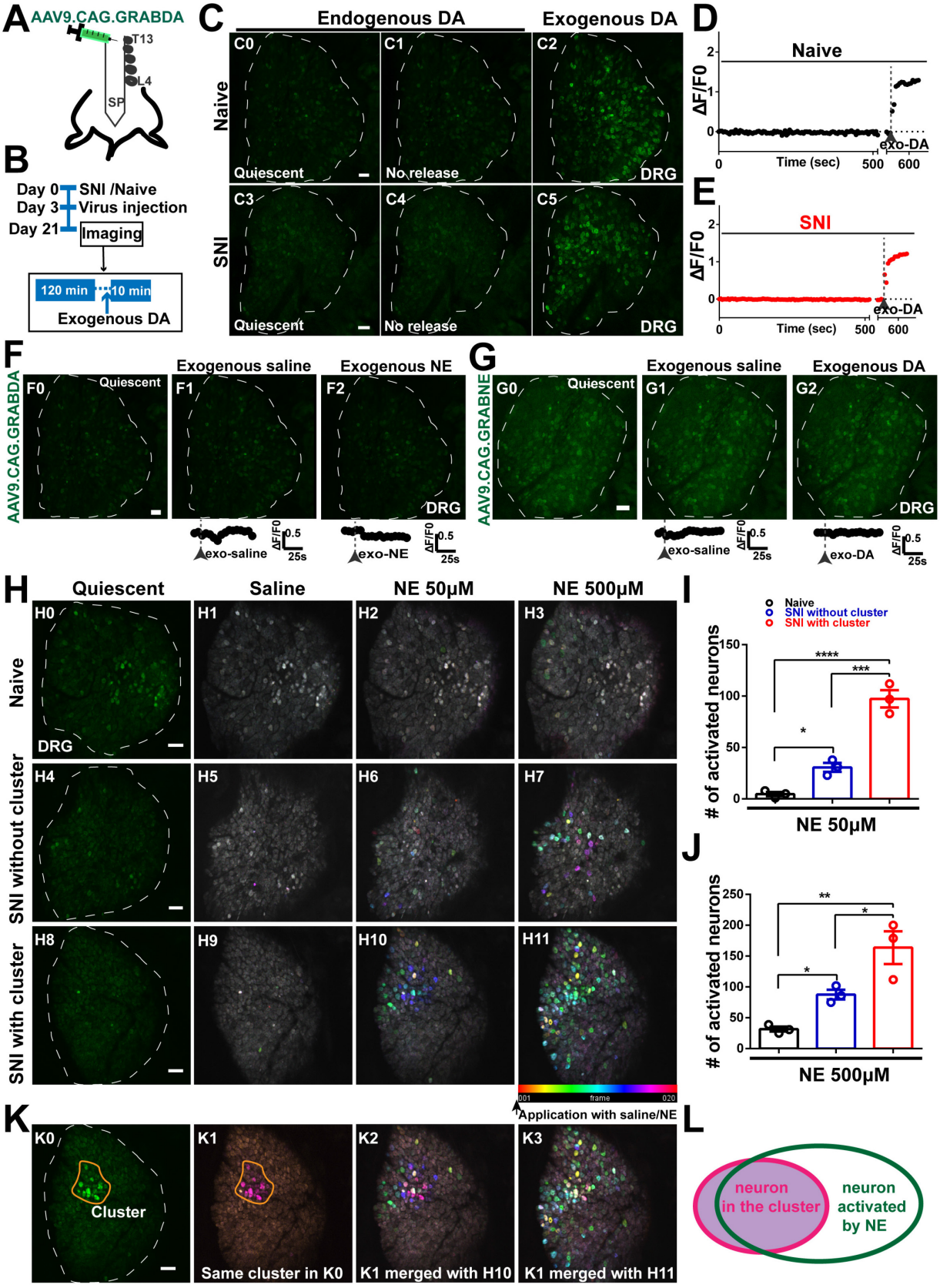
A
AAV9.CAG.GRABDA
T13
L4
SP
B
Day 0 SNI /Naive
Day 3 Virus injection
Day 21 Imaging
120 min
10 min
Exogenous DA
C
Endogenous DA
Exogenous DA
Naive
SNI
C0
C1
C2
C3
C4
C5
Quiescent
No release
DRG
D
Naive
ΔF/F0
Time (sec)
exo-DA
E
SNI
F
AAV9.CAG.GRABDA
Exogenous saline
Exogenous NE
F0
F1
F2
exo-saline
exo-NE
G
AAV9.CAG.GRABNE
G0
G1
G2
exo-DA
H
Quiescent
Saline
NE 50μM
NE 500μM
Naive
SNI without cluster
SNI with cluster
H0
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
Application with saline/NE
I
Naive
SNI without cluster
SNI with cluster
# of activated neurons
NE 50μM
J
NE 500μM
K
K0
K1
K2
K3
Cluster
Same cluster in K0
K1 merged with H10
K1 merged with H11
L
neuron in the cluster
neuron activated by NE

Figure S6. SNI does not evoke endogenous DA release in DRG; GRAB sensors are specific, and DRG neurons response to NE increases after nerve injury. Related to Figure 6.

(A-B) Diagram showing the intrathecal injection with AAV9.CAG.GRABDA in WT mice (A) and the experimental procedure (B).

(C) Representative images of DA sensor signals in naive and SNI mice. Neither naive mice nor mice with SNI have endogenous DA release. At the end of the recording session exogenous DA was dropped onto the DRG. Both naive and SNI mice showed DA sensor activation.

(D-E) Averaged DA signal traces for the whole ganglion in naive mouse (D) and mouse with SNI (E), no spontaneous release events were found.

(F) Representative images of DA sensor signals after addition of saline (middle) and 1μM exogenous NE (right). Neither saline nor NE can induce fluorescence changes of the DA sensor. Lower: Averaged DA signal traces for the whole ganglion in naive mouse with addition of saline (middle) and NE (right).

(G) Representative images of NE sensor signals after addition of saline (middle) and 1μM exogenous DA (right) in naive mice. Injection of AAV-GRABNE followed the same time course as in (A). Neither saline nor DA can induce fluorescence changes of the NE sensor. Lower: Averaged NE signal traces for the whole ganglion in naive mouse with addition of saline (middle) and DA (right).

(H) Representative images of DRG neurons in naïve or SNI mice (Day 21) with and without cluster firing showing responses to low concentration (50 μM) and high concentration (500 μM) NE. Saline and NE images are the time-lapse color-coded images (temporal code, 20 frames, one frame is 7 seconds) of activated neurons by saline or NE.

(I-J) Summary of neuron responses to NE. SNI mice with cluster firing show higher response to both 50 and 500 μM NE, compared to naïve and SNI mice without cluster firing.

(K) Representative images of one cluster in an SNI mouse (same mouse as in H8-H11). (K0) is original picture of one cluster. (K1) is converted to rose in order to be merged with images in (H10) and (H11). (K1) and (K3) are merged images, which show a lot overlap between NE-sensitive and cluster neurons.

(L) Diagram showing the degree of overlap between cluster neurons and neurons responding to NE.

Scale bar, 100μm. Each pair of open circles represents an individual mouse. Data are represented as mean ± SEM. *, $p<0.05$;**, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$; (I) and (J) by one way ANOVA with Holm-Sidak's posttest.

Figure S7.

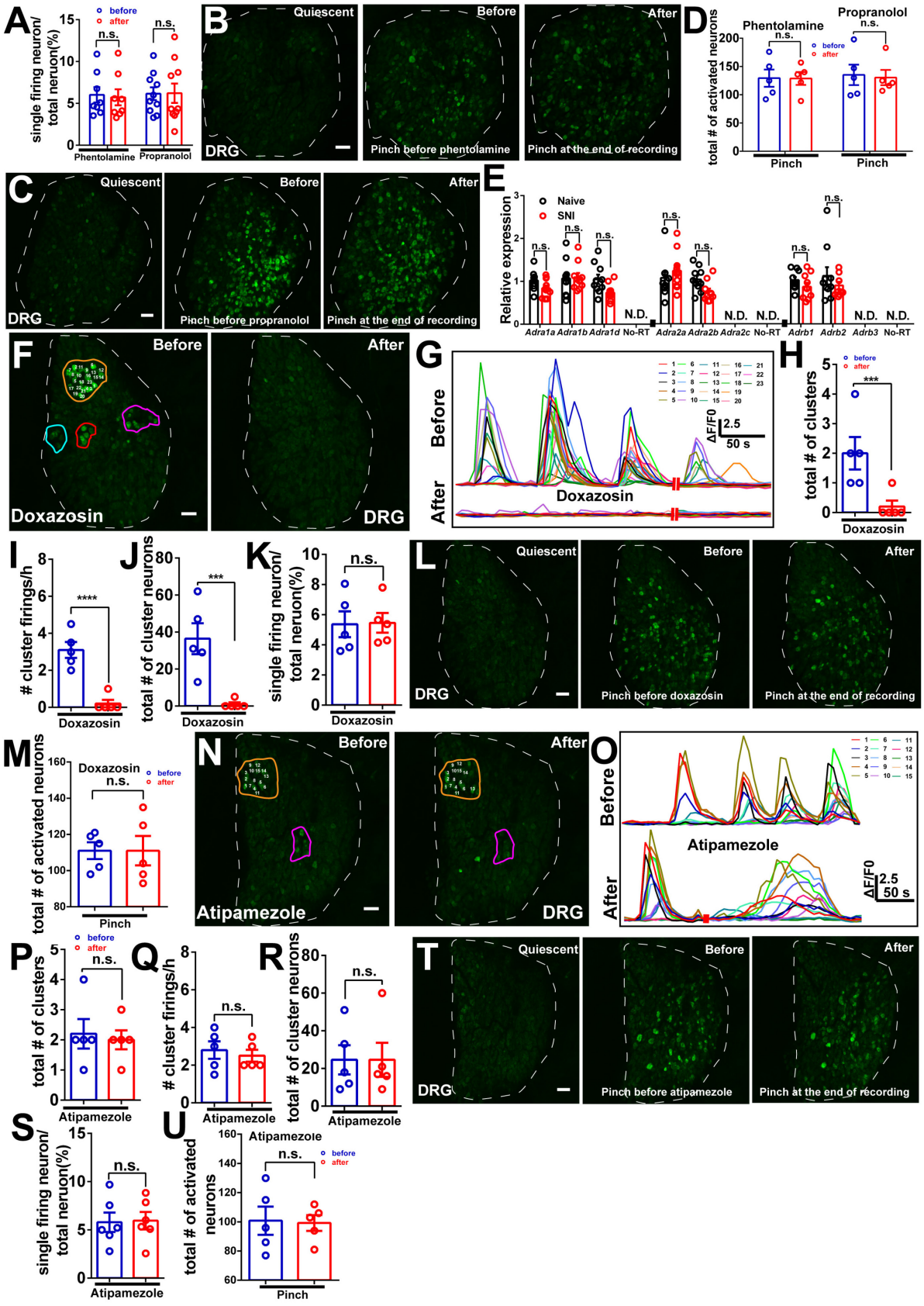
A
single firing neuron/ total neruon(%)
before
after
n.s.
Phentolamine
Propranolol
B
Quiescent
Before
After
DRG
Pinch before phentolamine
Pinch at the end of recording
D
Phentolamine
Propranolol
total # of activated neurons
Pinch
C
Quiescent
Before
After
DRG
Pinch before propranolol
Pinch at the end of recording
E
Naive
SNI
Relative expression
Adra1a Adra1b Adra1d No-RT Adra2a Adra2b Adra2c No-RT Adrb1 Adrb2 Adrb3 No-RT
N.D.
F
Before
After
Doxazosin
DRG
G
Before
After
Doxazosin
ΔF/F0
2.5
50 s
H
total # of clusters
Doxazosin
I
cluster firings/h
****
Doxazosin
J
total # of cluster neurons
***
Doxazosin
K
single firing neuron/ total neruon(%)
n.s.
Doxazosin
L
Quiescent
Before
After
DRG
Pinch before doxazosin
Pinch at the end of recording
M
Doxazosin
total # of activated neurons
Pinch
N
Before
After
Atipamezole
DRG
O
Before
After
Atipamezole
ΔF/F0
2.5
50 s
P
total # of clusters
Atipamezole
Q
cluster firings/h
Atipamezole
R
total # of cluster neurons
Atipamezole
T
Quiescent
Before
After
DRG
Pinch before atipamezole
Pinch at the end of recording
S
single firing neuron/ total neruon(%)
Atipamezole
U
Atipamezole
total # of activated neurons
Pinch

Figure S7. Block of some subfamilies of adrenergic receptors in DRG reduces incidence of CFEs and relieves spontaneous pain. Related to Figure 6 and Figure 7.

(A) The singly firing neurons have no change after addition of either phentolamine or propranolol to DRG in SNI mice (Day 21-28) with cluster firing.

(B-D) Addition of phentolamine or propranolol does not affect the viability of DRG neurons. (B-C) are examples of pinch responses before drug addition and at the end of the experiment after drug exposure. (D) is summary data of neurons activated by pinching.

(E) qRT-PCR indicates that both α and β receptors can be detected in the DRG of both naïve and SNI (Day 21) mice.

(F-G) An example of cluster firing in an SNI mouse (Day 21) that decreased after using 10 μM doxazosin (antagonist of α1-receptor). There are 4 clusters before addition of doxazosin. All clusters are inhibited after drug addition (F). (G) Representative traces of neurons in the orange cluster which are numbered in (F) before and after addition of doxazosin.

(H-K) The total number of clusters (H), frequency of cluster firing (I), and total number of cluster firing neurons (J) significantly decreased after addition of doxazosin on DRG, but singly firing neurons have no change (K).

(L-M) Addition of doxazosin does not affect the viability of DRG neuron. (L) is examples of paw pinch responses before drug addition and at the end of experiment after drug addition. (M) is summary data of neurons activated by pinching. From an SNI mouse (Day 21).

(N-O) An example of cluster firing in an SNI mouse (Day 21) that was unchanged after addition of 0.25 μM atipamezole (antagonist of α2-receptor). There are 2 clusters before drug addition. All clusters are still there after (N). (O) Representative traces of neurons in the orange cluster which are numbered in (N) before and after addition of atipamezole.

(P-S) The total number of clusters (P), frequency of cluster firing (Q), and total number of cluster firing neurons (R) and singly firing neurons (S) are unchanged by addition of atipamezole on DRG. (T-U) Addition of atipamezole cannot affect the viability of DRG neurons. (T) is example of paw pinch responses before drug addition and at the end of experiment after drug addition. (U) is summary data of neurons activated by pinching.

Scale bar, 100 μm. each pair of open circles represents an individual mouse. Data are represented as mean ± SEM. ***$p < 0.001$; ****$p < 0.0001$; N.S., not significant; (A), (K) and (S) by two-way repeated measures ANOVA with Sidak's posttest, compared to saline in Figure S4F, (H-J) and (P-R) by two-way repeated measures ANOVA with Sidak's posttest, compared to saline in Figure 3E-3G, (D), (M) and (U) by paired t test; (E) by unpaired t test.

Table S1. Cluster firing occurs in different pain models, related to Figure 1. and Figure S1.

Table S1.

Pain model	The number of mice having cluster firing	The total number of tested mice	Percentage
Naive	0	7	0.0
CFA(day 2-3)	0	10	0.0
Formalin induced	0	10	0.0
B16F10 bone cancer	1	10	10.0
CCI(day 7)	3	8	37.5
CCI(day 14-20)	12	23	52.2
SNI(day 3)	0	7	0
SNI(day 7)	2	16	12.5
SNI(day 14)	4	15	26.7
SNI(day 21)	20	34	58.8

Table S2. Sympathetic sprouting and cluster firing occur in *Phox2bcre; tdTomato* mice with SNI, Related to Figure S4

Table S2.

Mouse number	Sympathetic sprouting	Cluster firing
No1.	**(+)**	**(+)**
No2.	**(+)**	**(-)**
No3.	**(-)**	**(-)**
No4.	**(+)**	**(+)**
No5.	**(-)**	**(-)**
No6.	**(-)**	**(-)**
No7.	**(+)**	**(+)**
No8.	**(-)**	**(-)**
No9.	**(+)**	**(+)**
No10.	**(-)**	**(-)**
No11.	**(-)**	**(-)**
No12.	**(-)**	**(-)**

(+): occur; (-): not occur.