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Supplemental information

Identification of a population of peripheral sensory neurons that regulates blood pressure

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Supplemental Information

This file includes:

Supplemental Figures S1 to S7

Other Supplemental Materials for this manuscript include the following:

Videos S1 to S7

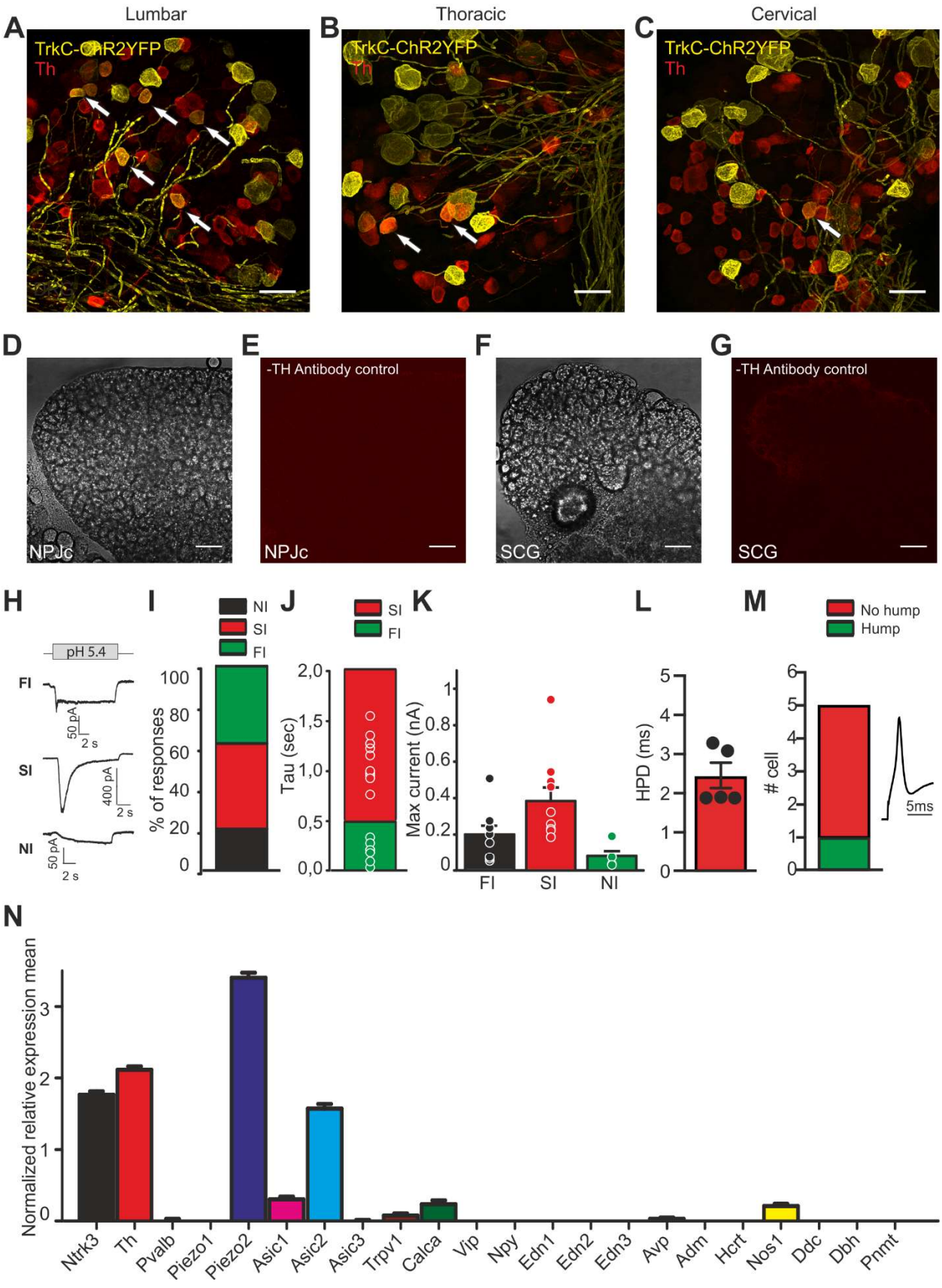
A
Lumbar
TrkC-ChR2YFP
Th
B
Thoracic
TrkC-ChR2YFP
Th
C
Cervical
TrkC-ChR2YFP
Th
D
NPJc
E
-TH Antibody control
NPJc
F
SCG
G
-TH Antibody control
SCG
H
pH 5.4
FI
50 pA
2 s
SI
400 pA
2 s
NI
50 pA
2 s
I
NI
SI
FI
% of responses
J
SI
FI
Tau (sec)
K
Max current (nA)
FI
SI
NI
L
HPD (ms)
M
No hump
Hump
cell
5ms
N
Normalized relative expression mean
Ntrk3
Th
Pvalb
Piezo1
Piezo2
Asic1
Asic2
Asic3
Trpv1
Calca
Vip
Npy
Edn1
Edn2
Edn3
Avp
Adm
Hcrt
Nos1
Ddc
Dbh
Pnmt

Figure S1. Characterization of TrkC^{+} neurons. Related to Figure 1

(A-C) Immunofluorescent staining of DRG sections from TrkC^{CreERT2}::Rosa26$^{ChR2\text{-}YFP}$ mice labelled with anti-Th antibodies. TrkC expression was investigated in lumbar DRG (A), thoracic DRG (B) and cervical DRG (C). Double positive neurons are indicated by arrows. Scale bars, 50 µm. (D-G) Antibody controls for Th showing phase contrast (D and F) and red channel images(E and G) in the NPJc and SCG. (H) Example traces of pH activated currents recorded in small TrkC^{+} neurons (24 cells from 3 mice). (I) Distribution of neurons according to the pH current type: fast inactivating (FI), slowly inactivating (SI) and non-inactivating (NI). (J) Distribution of the inactivation constants of the pH elicited currents. (K) Comparison of the maximal current amplitude in the different pH elicited current types. (L) Action potential Half Peak Duration (HPD) in small TrkC^{+} neurons (n=5). (M) Proportion of TrkC^{+} neurons with a hump on the falling phase of the action potential (left) and action potential example (right). (N) Gene expression panel of TrkC^{+} Th^{+} positive neurons (n=197) (data taken from (Zeisel et al., 2018)).

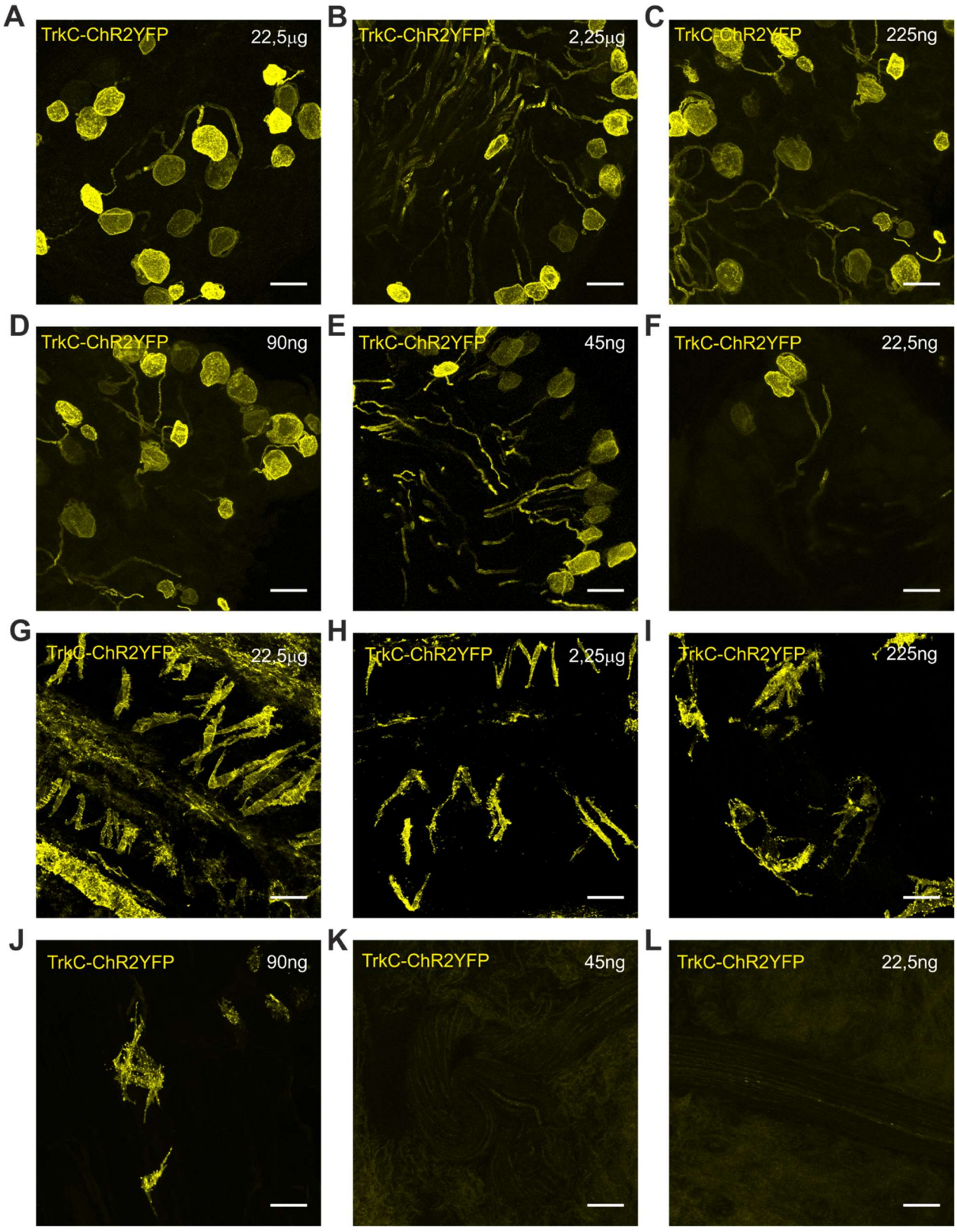
A
TrkC-ChR2YFP
22,5µg
B
TrkC-ChR2YFP
2,25µg
C
TrkC-ChR2YFP
225ng
D
TrkC-ChR2YFP
90ng
E
TrkC-ChR2YFP
45ng
F
TrkC-ChR2YFP
22,5ng
G
TrkC-ChR2YFP
22,5µg
H
TrkC-ChR2YFP
2,25µg
I
TrkC-ChR2YFP
225ng
J
TrkC-ChR2YFP
90ng
K
TrkC-ChR2YFP
45ng
L
TrkC-ChR2YFP
22,5ng

Figure S2. Concentration dependent recombination upon intrathecal injection of 4-OH tamoxifen. Related to Figure 2.

(A-F) Cre-driven recombination and YFP expression in DRG neurons and vSMC. (G-L) At lower concentrations, YFP is evident only in DRG and not in vSMC. Scale bars 50 μm.

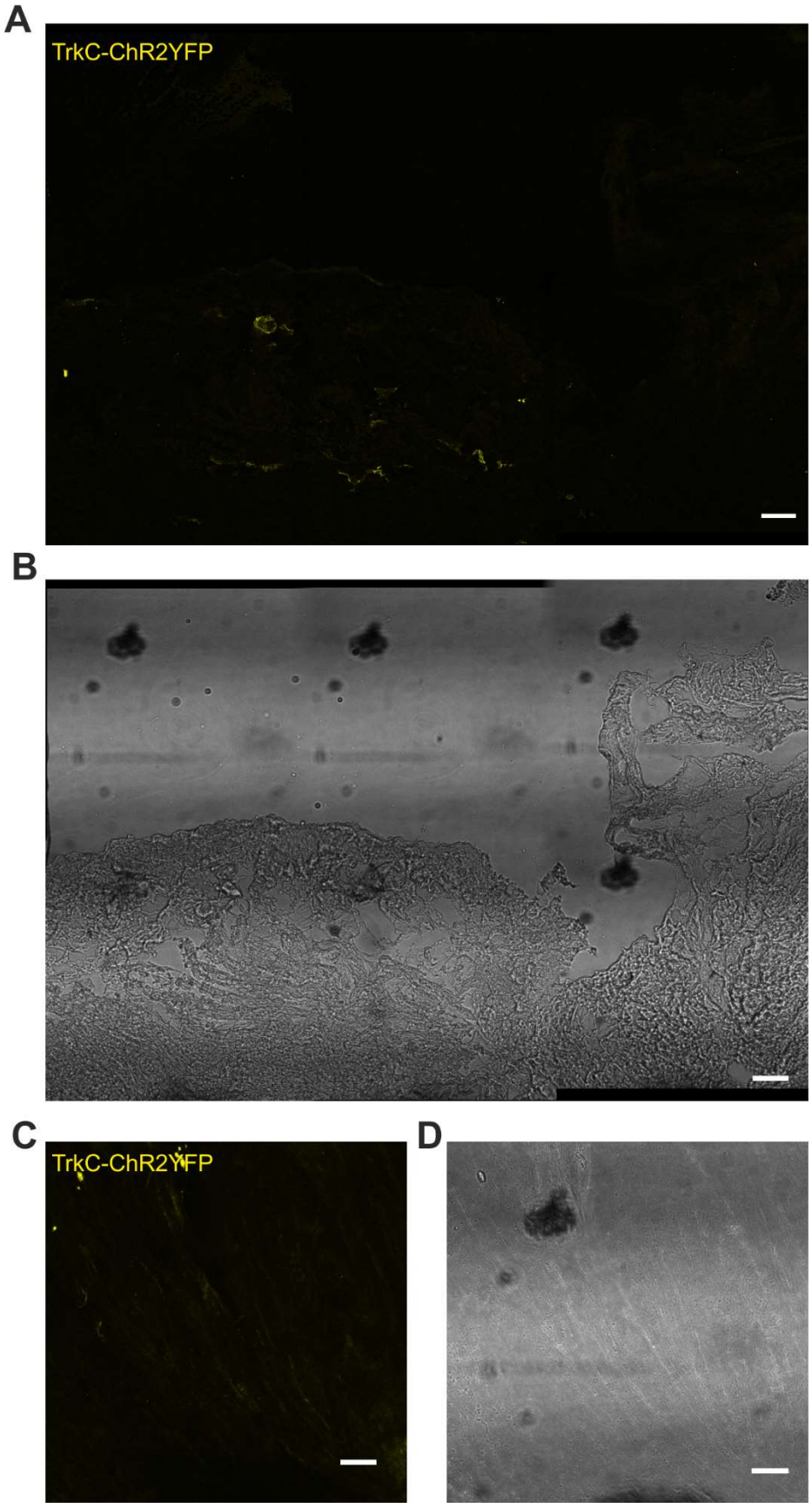
A
TrkC-ChR2YFP
B
C
TrkC-ChR2YFP
D

Figure S3. TrKC^{CreERT2} mediated recombination in the heart. Related to Figure 2.

(A and B) Large scale mosaic image of a section of heart tissue from TrkC^{CreERT2}::Rosa26$^{ChR2\text{-}YFP}$ mice. (A) YFP channel, (B) phase contrast. (C and D) Higher magnification image from the ventricles. (C) YFP channel, (D), phase contrast. Note the absence of TrkC+ neurons. Scale bars 50µm.

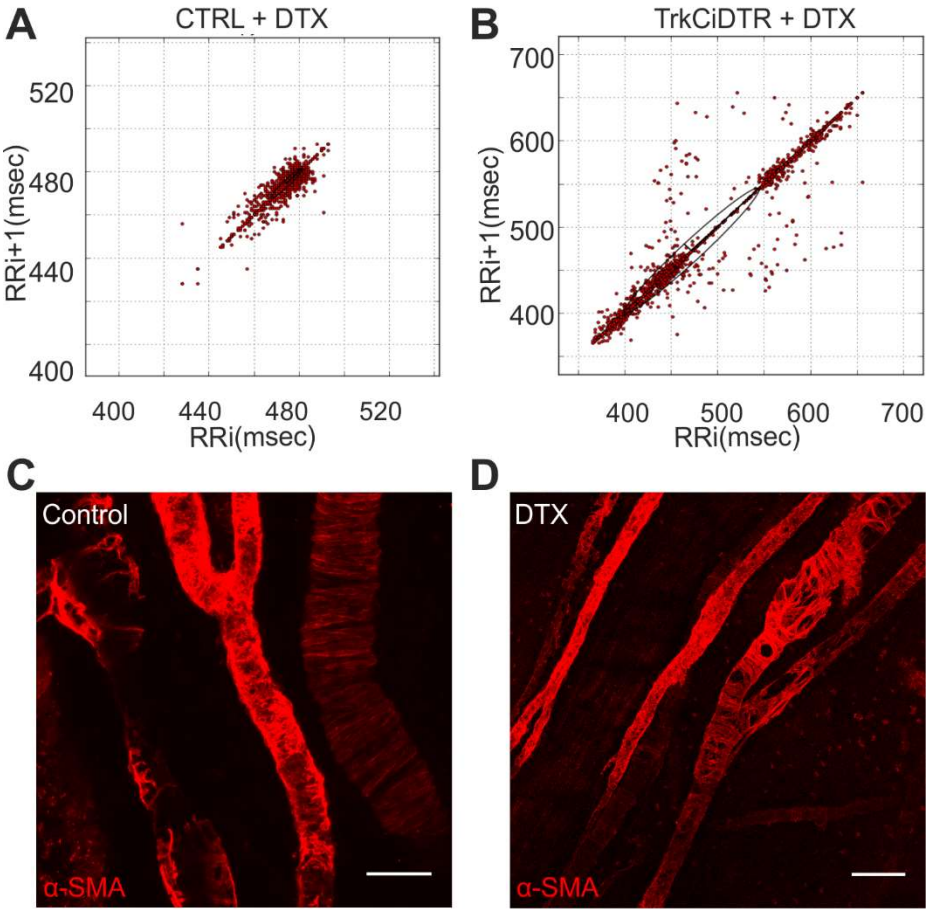

Figure S4. Ablation of TrkC^{+} neurons. Related to Figure 4.

(A and B) Representative Poincaré plots of a control (A) and TrkC^{CreERT2}::Avil^{iDTR} (B) mouse treated with DTX. (C and D) Representative images of vSMC labelled with an α-SMA antibody in control (C) and TrkC^{CreERT2}::Avil^{iDTR} (D) mouse treated with DTX.

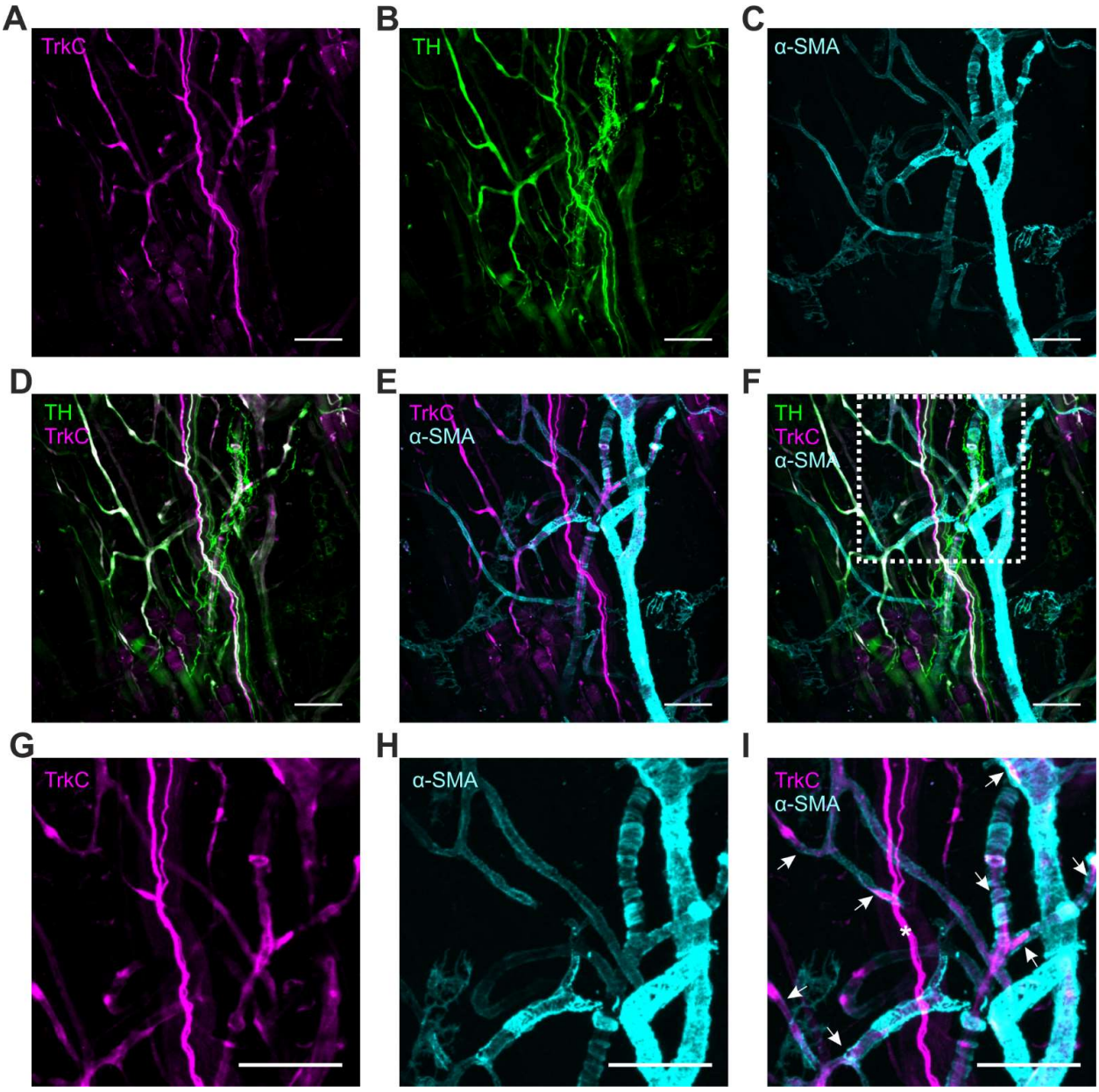

Figure S5. Imaging of TrkC$^+$ neurons in whole mount ear preparations. Related to Figure 5.
(A and F) Representative low magnification images of TrkC$^+$ neurons in whole mount ear preparations. (A) TrkC$^+$ neurons (magenta). (B) TH$^+$ neurons (green). (C) α-SMA labelled vSMC (cyan). (D) TrkC$^+$ neurons coexpress TH$^+$ (E) TrkC$^+$ neurons were often closely associated α-SMA labelled vSMC. (F) Composite image of TrkC, TH and α-SMA labelling. (G-I) High magnification images of area indicated by dotted box in (F). (G) TrkC$^+$ neurons. (H) α-SMA labelled vSMC. (I) Association of TrkC$^+$ neurons with blood vessels (arrows). The larger TrkC$^+$ fiber which runs vertically through the center of the image is part of a nerve fascicle (asterisk). All scale bars 100μm.

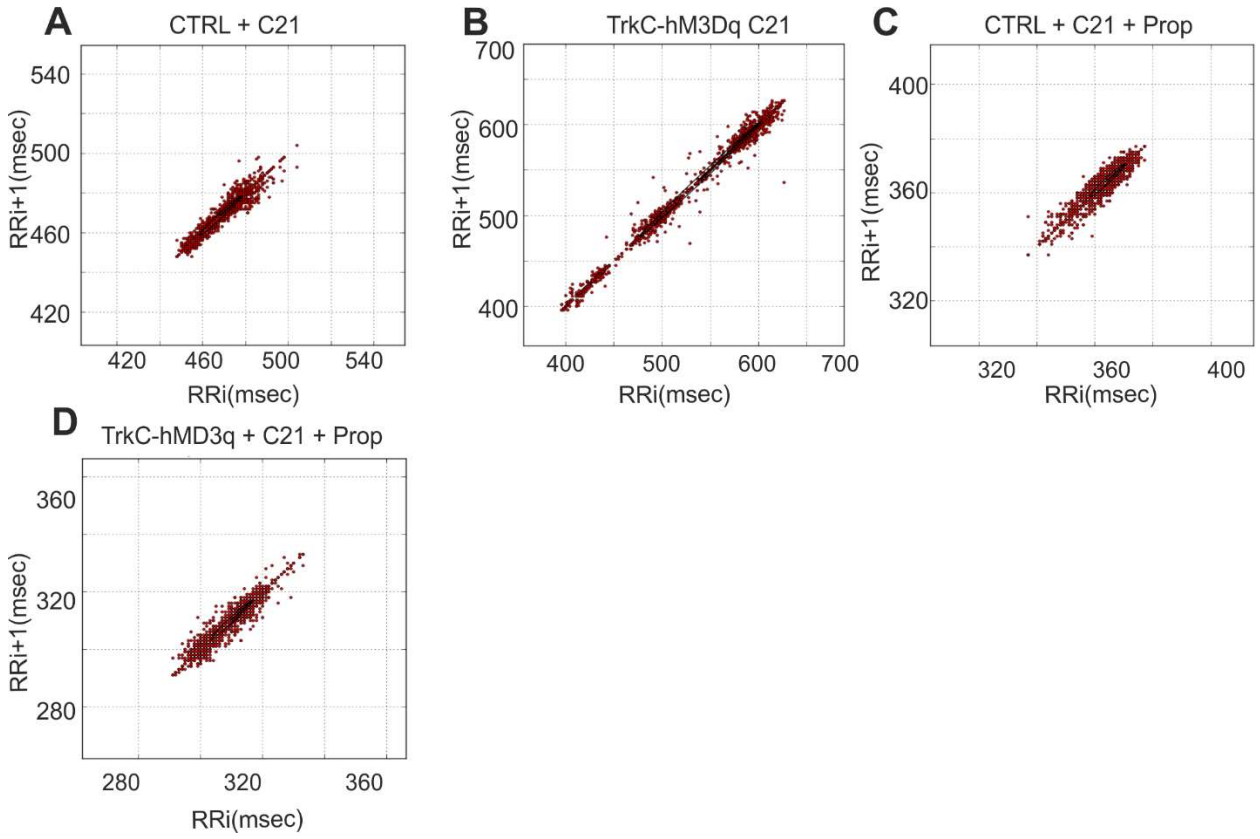

Figure S6. Activation of TrkC^{+} neurons. Related to Figure 6.
(A and B) Poincaré plot of a control (A) and a TrkC^{CreERT2}::Avil^{hM3Dq} (B) mouse treated with C21. (C and D) Representative Poincaré plot of a control mouse treated with C21 and Propranolol (C) and a TrkC^{CreERT2}::Avil^{hM3Dq} mouse treated with C21 and Propranolol (D).

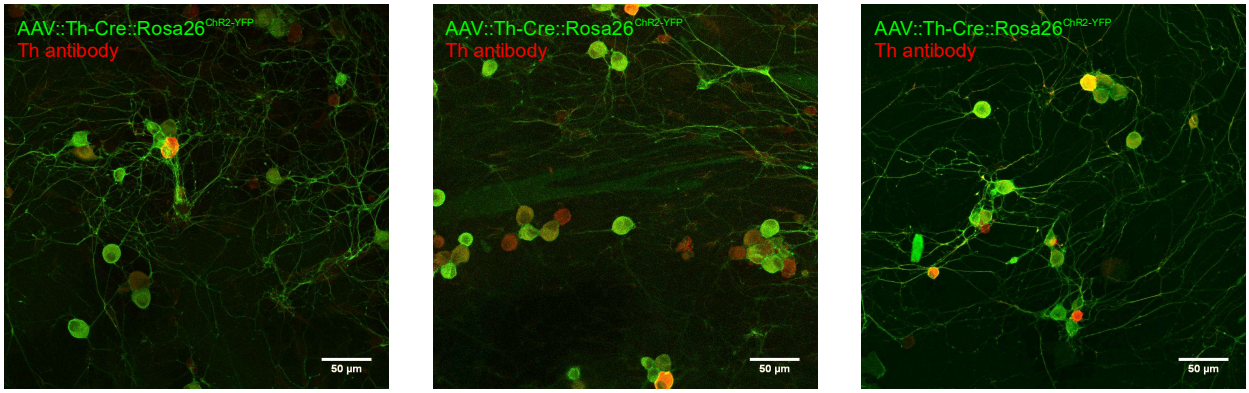

Figure S7. PHP.S AAV Th promoter driven Cre recombination. Related to STAR Methods.
Examples of Th promoter driven Cre recombination and Th antibody staining in DRG neurons