Supplementary materials

Pharmacological ischemic conditioning with roxadustat
does not affect pain-like behaviors but mitigates sudomotor impairment
in a murine model of deep hind paw incision

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Supplementary Table 1 Full details of antibodies used in immunohistochemistry and immunocytochemistry

Antibody	Species	Manufacturer	Catalog RRID	Dilution
Anti-pimonidazole antibody	Rabbit	Cosmobio	HP3-100 /	1:100
Anti-prolyl hydroxylase 2 (PHD2) antibody	Rabbit	Novus Biologicals	NB100-2219SS AB_791552	1:500
Anti-neurofilament heavy (NF-H) antibody	Chicken	Neuromics	CH22104 AB_2737101	1:5000
Anti-tyrosine hydroxylase (TH) antibody	Chicken	Abcam	Ab76442 AB_1524535	1:100
Anti-tyrosine hydroxylase (TH) antibody	Rabbit	Merck	657012 AB_2201407	1:500 1:100
Anti-lymphocyte antigen 6 complex, locus G + locus C (Ly6G+Ly6C) antibody	Rat	Arigo Biolaboratories	ARG22104 /	1:100
Anti-aquaporin (AQP) 5 antibody, Alexa Fluor 488	Rabbit	Bioss Antibodies Inc.	bs-1554R-A488 /	1:100
Anti-hypoxia inducible factor (HIF) 1 alpha antibody	Rabbit	GeneTex	GTX127309 AB_2616089	1:100
Anti-rabbit Alexa Fluor 594	Goat	Thermo Fisher Scientific	A11037 AB_2534095	1:500
Anti-chicken Alexa Fluor 594	Goat	Jackson ImmunoResearch Laboratories, Inc.	103-545-155 AB_2337390	1:500
Anti-rat Alexa Fluor 488	Donkey	Jackson ImmunoResearch Laboratories, Inc.	712-545-153 AB_2340684	1:500
Anti-rabbit Alexa Fluor 594	Donkey	Jackson ImmunoResearch Laboratories, Inc.	711-585-152 AB_2340621	1:500
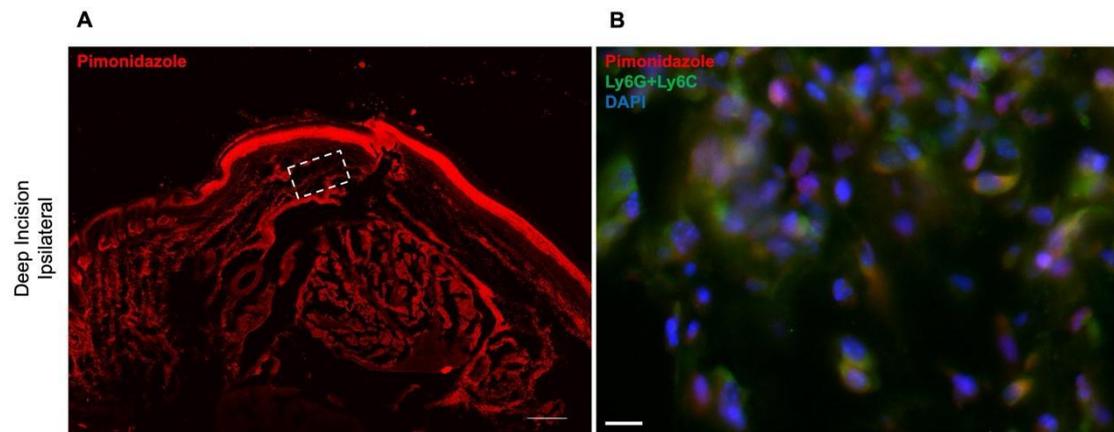
Supplementary Figure 1 Tissue hypoxia was detected in infiltrated polymorphonuclear neutrophils in the surgical wound.

Note: (A) Representative images of deep-incised paw sections stained with pimonidazole on POD 1, indicating the location of infiltrating immune cells (dashed boxes) in the wound. Scale bar = 200 μm. (B) Representative confocal microscopy images displaying the colocalization of pimonidazole/Ly6G+Ly6C/DAPI in the polymorphonuclear neutrophils on POD1. Scale bar: 10 μm.

Abbreviations: Ly6G+Ly6C, lymphocyte antigen 6 complex, locus G + locus C; POD, postoperative day.
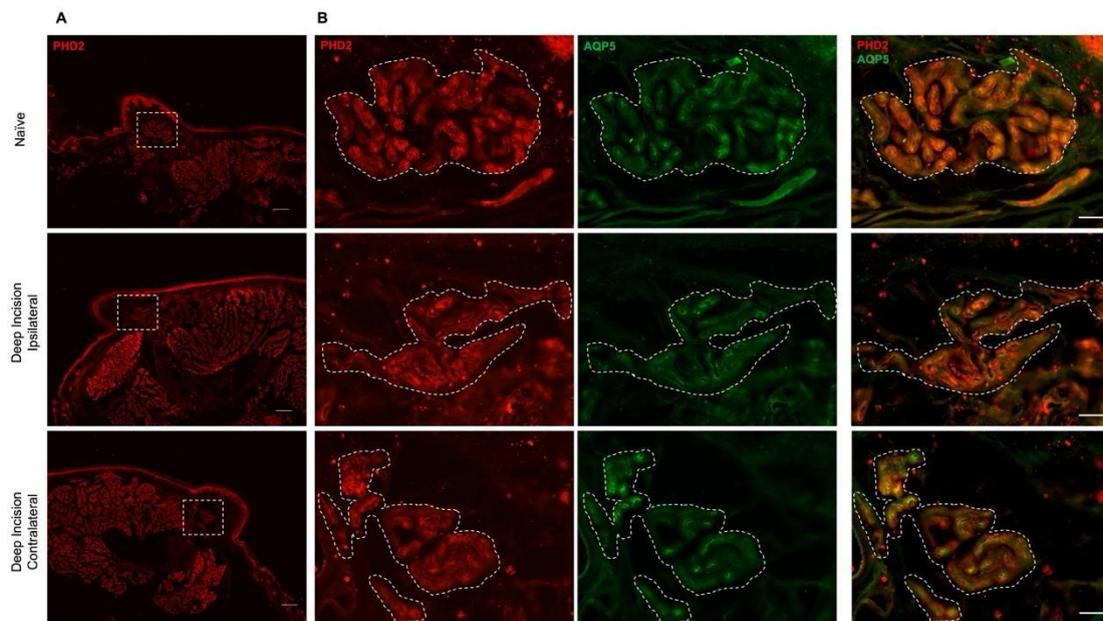
Supplementary Figure 2 PHD2 was observed in eccrine sweat gland cells of both naïve and operated mice.

Note: (A) Representative images of paw sections stained with PHD2 on POD 1 indicating the location of eccrine glands (dashed boxes). Scale bar = 200 μm. (B) Representative images of eccrine glands (dashed lines) stained for PHD2 and AQP5 on POD1. Scale bar = 50 μm.

Abbreviations: PHD2, prolyl hydroxylase domain protein 2; AQP5, aquaporin 5; POD, postoperative day.
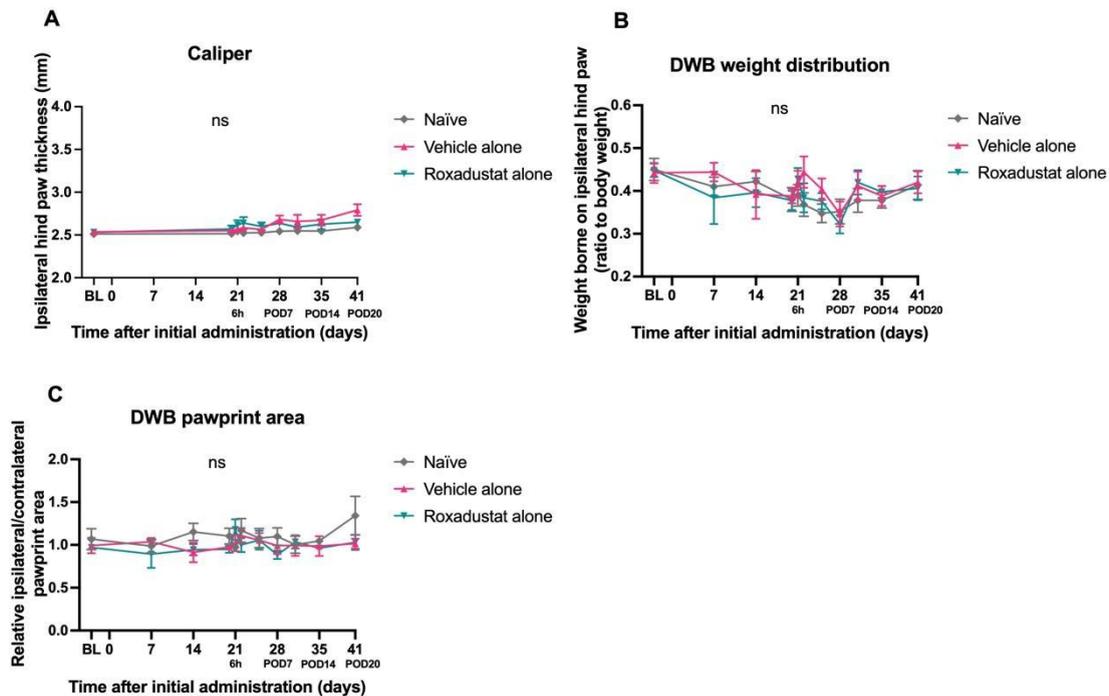
Supplementary Figure 3 PIC alone without surgery did not affect ipsilateral hind paw thickness and spontaneous pain-like behaviors.

Note: Changes in ipsilateral hind paw thickness (A), weight distribution ratio of the ipsilateral paw to the total body weight (B) and paw print area ratio of the ipsilateral paw to the contralateral paw (C) of naïve and vehicle- or roxadustat-treated mice from BL to day 41 (n = 19 per group). Data are expressed as mean ± SEM, assessed by two-way ANOVA with Šídák's multiple comparisons test.

Abbreviations: BL, baseline; POD, postoperative day; ns, not significant; DWB, dynamic weight-bearing; PIC, pharmacological ischemic conditioning.
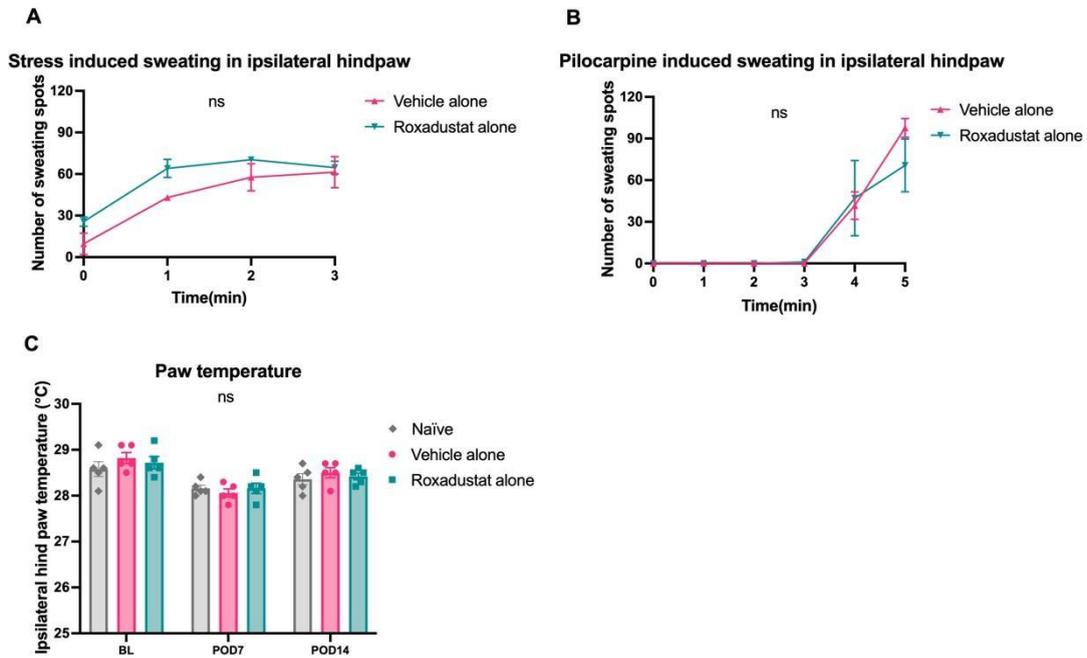
Supplementary Figure 4 Pharmacological ischemic conditioning (PIC) alone without surgery did not affect sudomotor function and paw temperature.

Note: (A) Quantification of restraint stress-induced sweating of vehicle- or roxadustat-treated mice on day 41(n = 5 per group). (B) Quantification of pilocarpine-induced sweating of vehicle- or roxadustat-treated mice on day 42 (n = 5 per group). (C) Ipsilateral hind paw temperature of naïve and vehicle- or roxadustat-treated mice at BL, on POD 7 and on POD14 (n = 5 per group). Data are expressed as mean ± SEM, assessed by two-way ANOVA with repeated measures followed by Šídák's multiple comparisons test.

Abbreviations: BL, baseline; POD, postoperative day; ns, not significant; PIC, pharmacological ischemic conditioning

A

TH in sweat gland

B

TH⁺ surrounding nerves

C

TH⁺ whole nerves

D

| Naïve | Vehicle alone | Roxadustat alone |
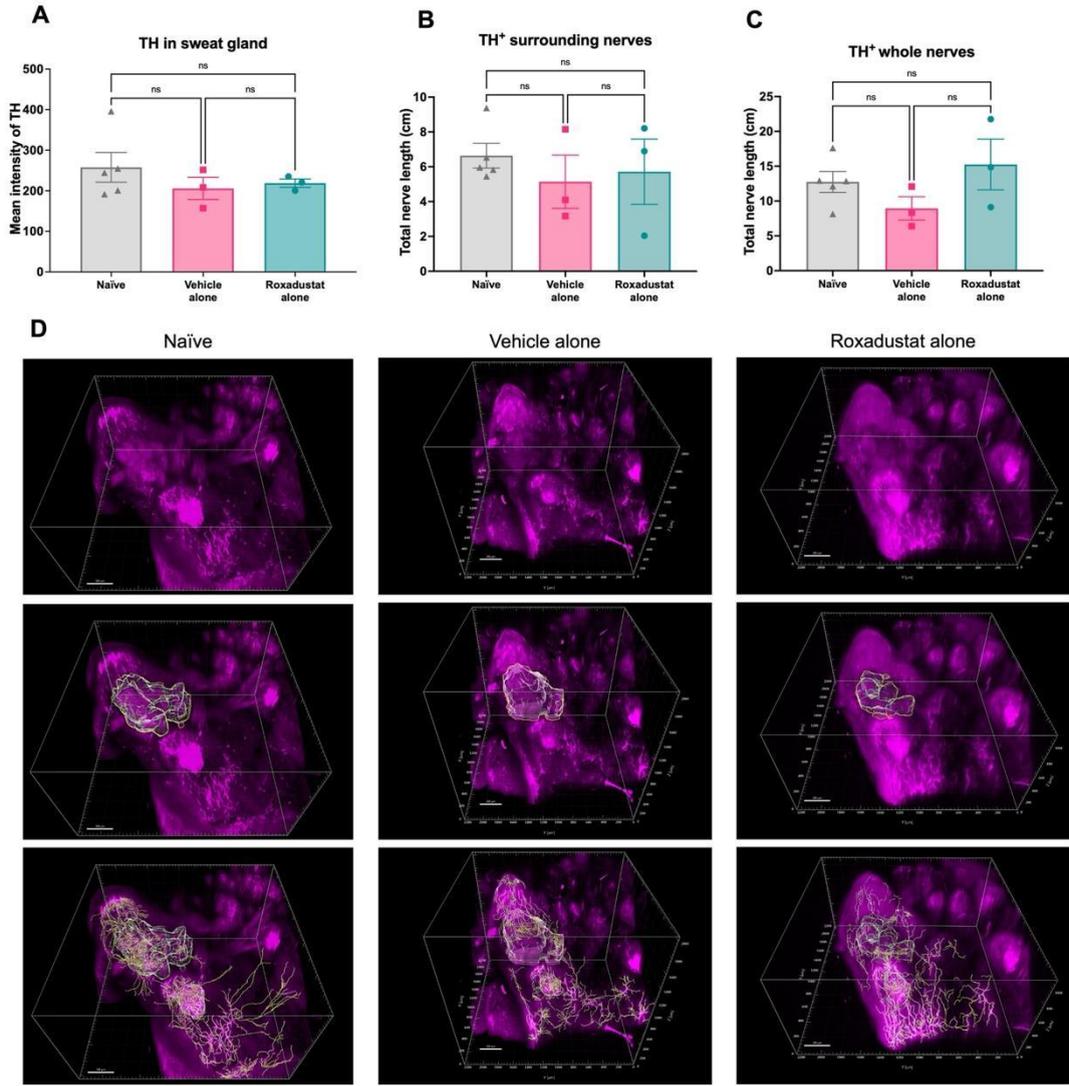
Supplementary Figure 5 PIC alone without surgery did not affect TH-positive sympathetic innervation around the eccrine glands.

Note: (A) Mean intensity of TH immunoreactivity in the eccrine glands in the ipsilateral hind paws (n = 3–5 per group). Total length of TH-positive nerve fibres surrounding (B) and within and around (C) the eccrine glands in the ipsilateral hind paws of naïve and vehicle- or roxadustat-treated mice (n = 3–5 per group) on day 42. (D) Representative images of skin samples adjacent to the surgical wound from ipsilateral hind paws with AbSca*l*e labeling of TH. Upper panels display stacked images without 3D rendering. Middle panels display stacked images with 3D rendering of the main eccrine glands. Bottom panels display stacked images with 3D rendering of the main eccrine glands and TH-positive nerve fibres within and around the eccrine glands. Scale bar = 300 µm. Data are expressed as mean ± SEM, assessed by one-way ANOVA with Tukey's multiple comparisons test.

Abbreviations: TH, tyrosine hydroxylase; ns, not significant; PIC, pharmacological ischemic conditioning.

Supplementary Videos

3D reconstruction of TH-positive nerve fibres within and around the eccrine glands in the ipsilateral hind paws of naïve (Supplementary Video 1), deep incision + vehicle (Supplementary Video 2), deep incision + roxadustat (Supplementary Video 3), roxadustat alone (Supplementary Video 4), and vehicle alone (Supplementary Video 5) mice on day 42 (POD21). Scale bar = 300 µm.