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Supplemental Information

A Neurotrophic Mechanism Directs Sensory Nerve Transit in Cranial Bone

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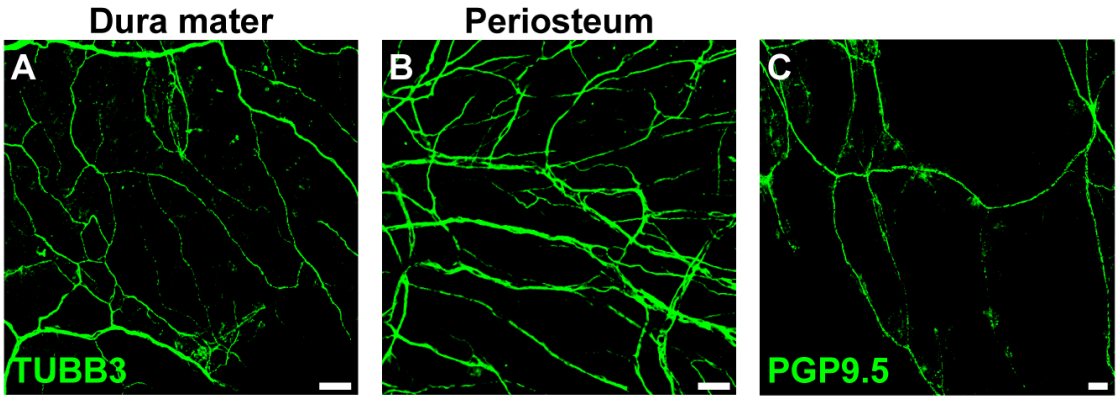
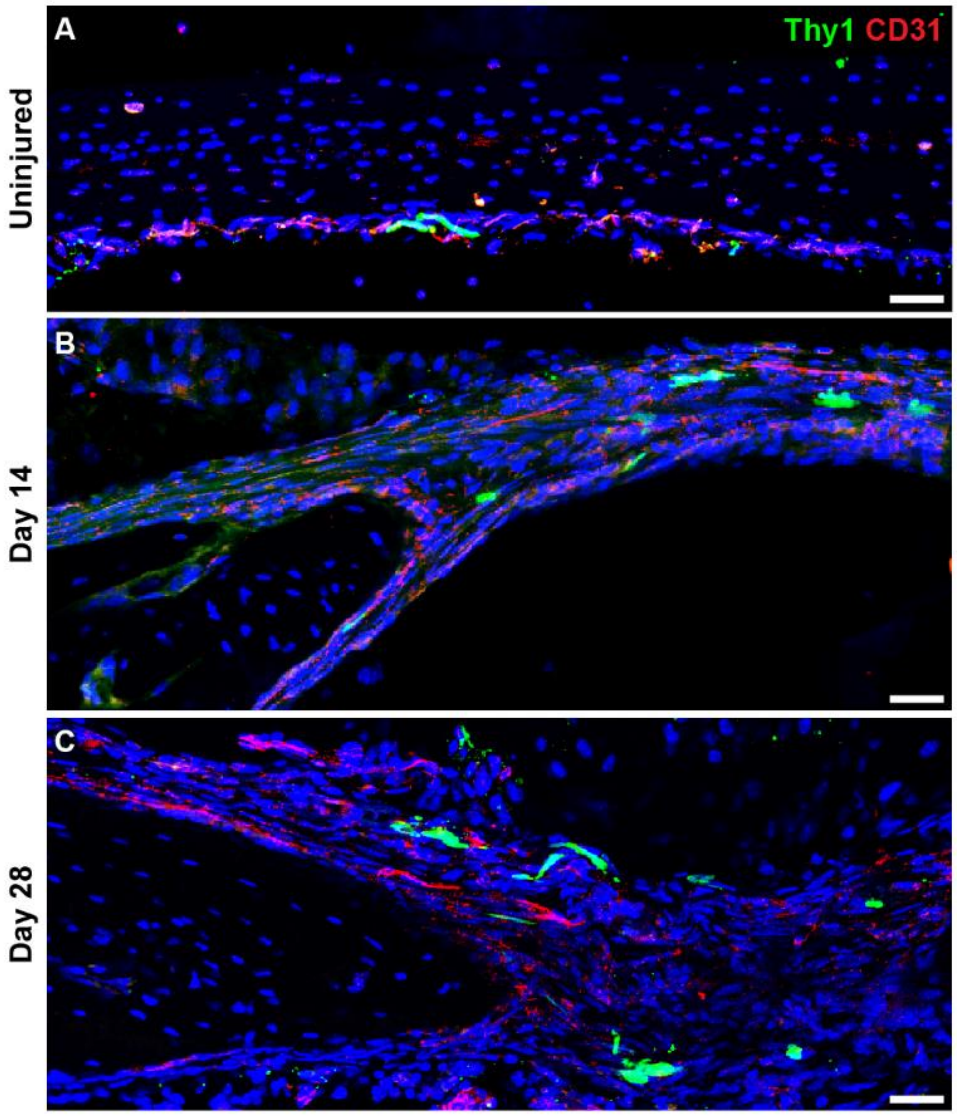
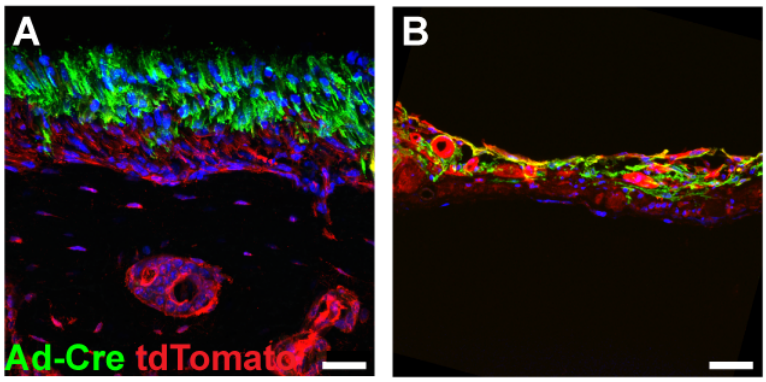
Supplemental Items



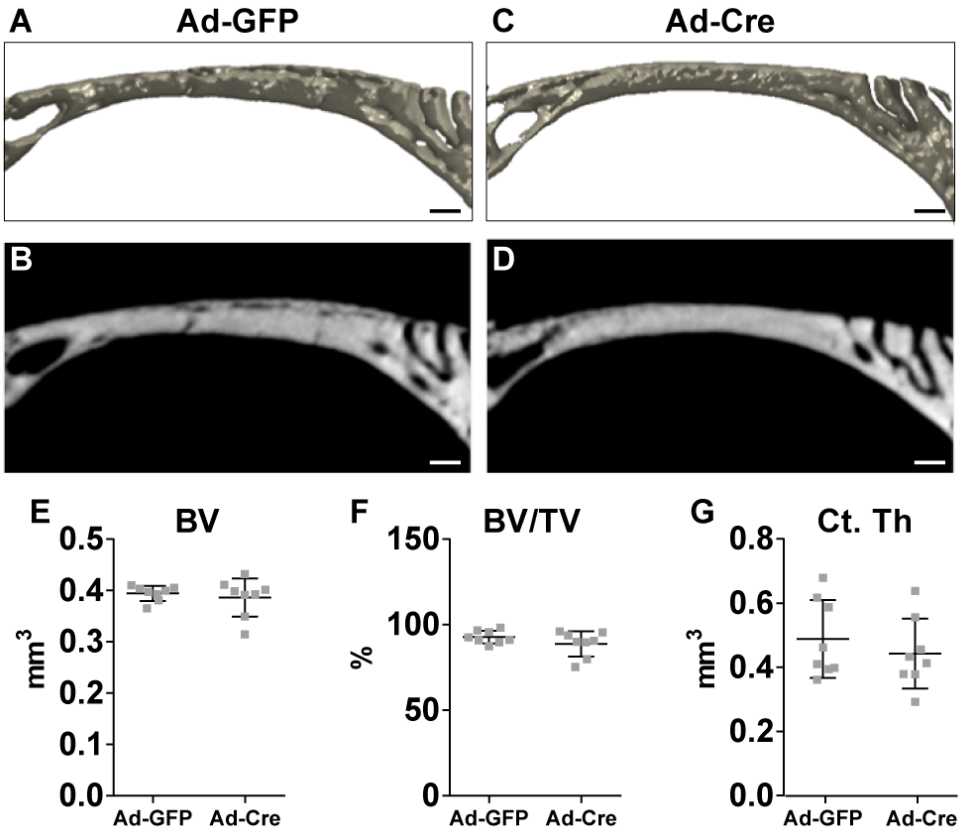
Supplemental Figure S1. Presence of TUBB3^{+}PGP9.5^{+} nerves within the dura mater and periosteum of the uninjured calvaria, related to Figure 1. Whole mount anti-TUBB3 immunofluorescence of the uninjured frontal bone visualized from below and above. TUBB3^{+} nerves are present in the intact coverings of the frontal bone, including **(A)** dura mater and **(B)** periosteum lining the uninjured bone. **(C)** Whole mount anti-PGP9.5 immunofluorescence of uninjured frontal bone visualized from below. White scale bar: 50 μm.



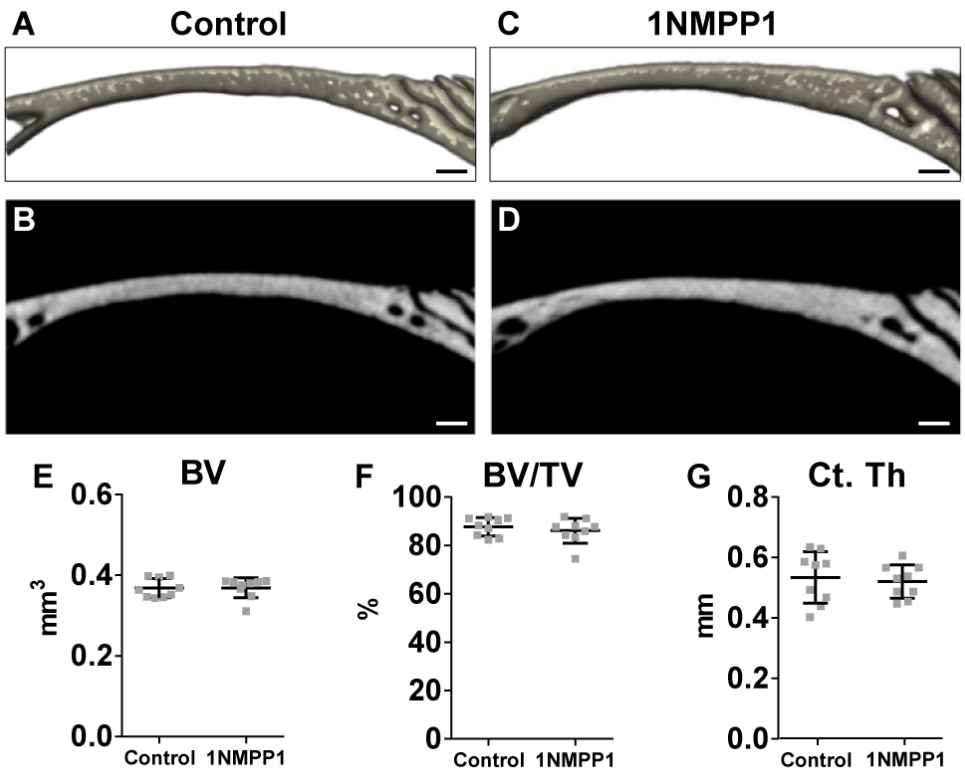
Supplemental Figure S2. Nerve fibers closely associate with blood vessels before and after calvarial injury, related to Figure 4. Immunohistochemical staining for CD31 (appearing red) was performed on calvarial sections of Thy1-YFP pan-neuronal reporter animals, before and after bone defects. $CD31^+$ vessels appear red, while YFP^+ nerve fibers appear green. **(A)** Uninjured frontal bone, focusing on the periosteum. **(B)** Appearance 14 d post-injury, focusing on the bone defect edge. **(C)** Appearance 28 d post-injury, focusing on the bone defect edge. White scale bar: 50 μm.



Supplemental Figure S3. Validation of Cre-recombination in calvarial defect following injection of Ad-Cre, related to Figure 5. High magnification images following percutaneous Ad-Cre viral injection within the **(A)** uninjured calvaria and **(B)** 48 h after bone defect creation in an mT/mG mouse. Cells with Cre-mediated recombination appear green, while all other cells appear red. White scale bar: 50 μm.



Supplemental Figure S4. Lack of uninjured calvarial bone phenotype in Ad-Cre treated NGF $^{fl/fl}$ animals, related to Figure 5. Analysis of uninjured frontal bones among NGF $^{fl/fl}$ mice treated with Ad-Cre or Ad-GFP control. Analysis performed after 28 d. **(A-D)** μCT images of uninjured frontal bone among **(A,B)** Ad-GFP or **(C,D)** Ad-Cre injected animals, including three dimensional μCT coronal reconstruction (above) and coronal cross-section (below). **(E-G)** Quantitative μCT analysis of the frontal bone among Ad-GFP or Ad-Cre treated animals, including **(E)** Bone Volume (BV), **(F)** Bone Volume/ Tissue Volume (BV/TV) and **(G)** Cortical Thickness (Ct. Th). For all graphs, each dot represents a single animal; N=8 per group. Black and white scale bars: 200 μm.



Supplemental Figure S5. Lack of uninjured calvarial bone phenotype in 1NMPP1-treated TrkA^{F592A} animals, related to Figure 7. Analysis of uninjured frontal bones among TrkA^{F592A} mice treated with 1NMPP1 or vehicle control for 28 d. **(A-D)** μCT images of uninjured frontal bone among TrkA^{F592A} mice treated with **(A,B)** vehicle control or **(C,D)** 1NMPP1, including three dimensional coronal μCT reconstruction (above) and coronal cross-section (below). **(E-G)** Quantitative μCT analysis of the frontal bone among TrkA^{F592A} mice treated with 1NMPP1 or vehicle control, including (**E)** Bone Volume (BV), **(F)** Bone Volume/ Tissue Volume (BV/TV) and **(G)** Cortical Thickness (Ct. Th). For all graphs, each dot represents a single animal; N=9 per group. Black and white scale bars: 200 μm.

Supplemental Table S1: Antibodies used, related to STAR Methods.

Name	Vendor	Catalog No.	Concentration	Use
Anti-Axin2	Abcam	ab109307	1:200	IF
Anti-CD31	Abcam	ab28364	1:100	IF
Anti-CD45	BioLegend	103144	1:200	IF
Anti-CGRP	Sigma Aldrich	C8198	1:200	IF
Anti-F4/80	Abcam	ab204467	1:200	IF
Anti-Gli1	Abcam	ab49314	1:200	IF
Anti-IκBα	Cell Signaling	9936T	1:1000	WB
Anti-IKKα	Cell Signaling	9936T	1:1000	WB
Anti-IKKβ	Cell Signaling	9936T	1:1000	WB
Anti-IL1β	Abcam	ab9722	1:100	IF
Anti-NF-κB p65	Cell Signaling	9936T	1:1000	WB
Anti-NGF	Abcam	ab6199	1:100	IF
Anti-Osteocalcin	Abcam	ab93876	1:200	IF
Anti-PDGFRa	Abcam	ab15501	N/A	IF
Anti-PGP 9.5	Agilent Tech.	Z511601-2	1:200	IF
Anti-Phospho- IκBα	Cell Signaling	9936T	1:1000	WB
Anti-Phospho-IKKα/β	Cell Signaling	9936T	1:1000	WB
Anti-Phospho-NF-κB p65	Cell Signaling	9936T	1:1000	WB
Anti-TH	EMD Millipore	AB152	1:200	IF
Anti-TNFa	Abcam	ab6671	1:100	IF
Anti-TUBB3	Abcam	ab18207	1:1500	IF
Goat Anti-Mouse IgG	Abcam	ab150119	1:200	IF
Goat-Anti-Rabbit IgG	Cell Signaling	9936T	1:1500	WB
Goat Anti-Rabbit IgG	Vector	DI-1594	1:200	IF
Horse Anti-Mouse IgG	Cell Signaling	9936T	1:1500	WB