C3H/HeJ Tibia
### Introduction

Representative IHC images in the following pages include both male (sections 1-9) and female (sections 10-11) mice; please note that the bone morphology and innervation pattern are relatively conserved, while peripheral fat content is increased in females. As with all immunostaining procedures, staining and sectioning artifacts are possible during processing. These are noted throughout the atlas when present. Click the information buttons for more details about a specific artifact. Nerve tracings rely on the relative strength of immunolabeling and assessment of axon morphology. Axons parallel to the section plane were positively stained, curvilinear structures of 0.2-5 \( \mu m \) in diameter; those perpendicular to the section plane were small punctate structures, also of 1-5 \( \mu m \) diameter, that continued through the entire depth of the 50 \( \mu m \) z-stack. Axons oriented longitudinally, perpendicular to the plane of section, will appear smaller than their actual length when visualized in 2D.

### Methods

The following atlas was constructed from 50 \( \mu m \) frozen serial sections through the tibiae of five male and female C3H/HeJ 12-week-old mice. Calcitonin gene-related peptide (CGRP)-positive peptidergic sensory fibers [Bio-rad, 1720-9007] and tyrosine hydroxylase (TH)-positive sympathetic fibers [Abcam, AB152], as well as perilipin-positive adipocytes [Progen, GP29] were labeled by immunohistochemistry and detected with fluorescent-conjugated secondary antibodies along with DAPI-stained nuclei [Sigma, D9542]. These sections were imaged and analyzed using a Nikon CSU-X1 spinning disk confocal system. Cancellous and cortical bone masks were generated by thresholding max projections in ImageJ. TH+ and CGRP+ axons were traced with the Simple Neurite Tracer plugin. Bone masks and axon traces were overlaid on a grayscale max projection of the FITC and DAPI channels. 3D maps were generated using a 3D model reconstructed from scans of the tibiae of 12-week-old male mice performed on a Scanco \( \mu CT \) 40. 2D schematics showing muscle attachments and muscle labeling were adapted from Charles et. al (1) with muscle placement adjusted based on our serial section analysis. Innervation patterns were illustrated on the bone-tissue interface in our 2D schematics in relation to surrounding muscle attachments and fascial structures. Pacinian corpuscles, specialized nerve endings responsive to mechanical distortion and vibration, were identified by morphology and are also annotated throughout. The final atlas was compiled with Adobe InDesign.

### Notes and Limitations

Representative IHC images in the following pages include both male (sections 1-9) and female (sections 10-11) mice; please note that the bone morphology and innervation pattern are relatively conserved, while peripheral fat content is increased in females. As with all immunostaining procedures, staining and sectioning artifacts are possible during processing. These are noted throughout the atlas when present. Click the information buttons for more details about a specific artifact. Nerve tracings rely on the relative strength of immunolabeling and assessment of axon morphology. Axons parallel to the section plane were positively stained, curvilinear structures of 0.2-5 \( \mu m \) in diameter; those perpendicular to the section plane were small punctate structures, also of 1-5 \( \mu m \) diameter, that continued through the entire depth of the 50 \( \mu m \) z-stack. Axons oriented longitudinally, perpendicular to the plane of section, will appear smaller than their actual length when visualized in 2D.

### Acknowledgements

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### Bibliography

1. Charles JP, Cappellari O, Spence AJ, Hutchinson JR, Wells DJ. Musculoskeletal geometry, muscle architecture and functional specialisations of the mouse hindlimb. PLoS One. 2016 Apr 26;11(4):e0147669.
Guide For Use

CT representation of section placement (black dotted line) and applicable morphology range (gray box) in respect to nerve patterns and interacting tissue groups (striped patterns and colors respectively).

Illustration of 2D section and the associated muscle groups and mechanosensory receptors.

Position of section relative to the knee by the average percentage of full bone length. For best results, measure bone length prior to atlas use.

IHC representative of section.

CLICK channel names to view individual IHC channels.

Image of masked IHC bone overlayed with traced, enlarged TH+ and CGRP+ neurons for easier visualization.

HOVER over abbreviations for full name.

CLICK arrows to show or hide Color Legend for muscle groups and bone.

Quick access instruction key.

CLICK arrows to show or hide Pattern Legend for nerve patterns and symbols.

C3H/HeJ Tibia
Section 1: 2% Site

Proximal Epiphysis

Pattern Legend

Color Legend

Hover over icon for more information

Hover over abbreviations for full definition

Use arrows to access keys and icons

C3H/HeJ Tibia
Section 2: 5% Site

Proximal Epiphysis and Metaphysis

Color Legend
- Hover over icon for more information
- Hover over abbreviations for full definition
- Use arrows to access keys and icons

Pattern Legend

C3H/HeJ Tibia
Section 4: 20% Site

Proximal Diaphysis

Color Legend

Hover over icon for more information
Hover over abbreviations for full definition
Use arrows to access keys and icons

C3H/HeJ Tibia
Section 5: 30% Site

Mid Diaphysis

Pattern Legend

Color Legend

Hover over icon for more information
Hover over abbreviations for full definition
Use arrows to access keys and icons
Section 6: 40% Site

Mid Diaphysis

Pattern Legend

Color Legend

Hover over icon for more information
Hover over abbreviations for full definition
Use arrows to access keys and icons
Section 7: 50% Site

Mid Diaphysis

Color Legend

Pattern Legend

C3H/HeJ Tibia
Section 11: 92% Site

**Distal Metaphysis**

Pattern Legend:
- **DAPI**
- **TH**
- **CGRP**
- **Perilipin**
- **All Channels**

Color Legend:
- **Red**: TIBIA
- **Green**: FOOT
- **Blue**: EDL
- **Orange**: EHL
- **Yellow**: TA
- **Brown**: PB
- **Pink**: PDQ
- **Purple**: MGLG
- **Gray**: SOL
- **Light Blue**: PLT
- **Light Green**: FDL
- **Light Purple**: TP

Legend:
- **LATERAL**
- **POSTERIOR**
- **MEDIAL**
- **ANTERIOR**

Use arrows to access keys and icons.
Hover over icon for more information.
Hover over abbreviations for full definition.