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SUPPLEMENTARY MATERIAL

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Immunofluorescence characterization of innervation and nerve-immune cell interactions in mouse lymph nodes

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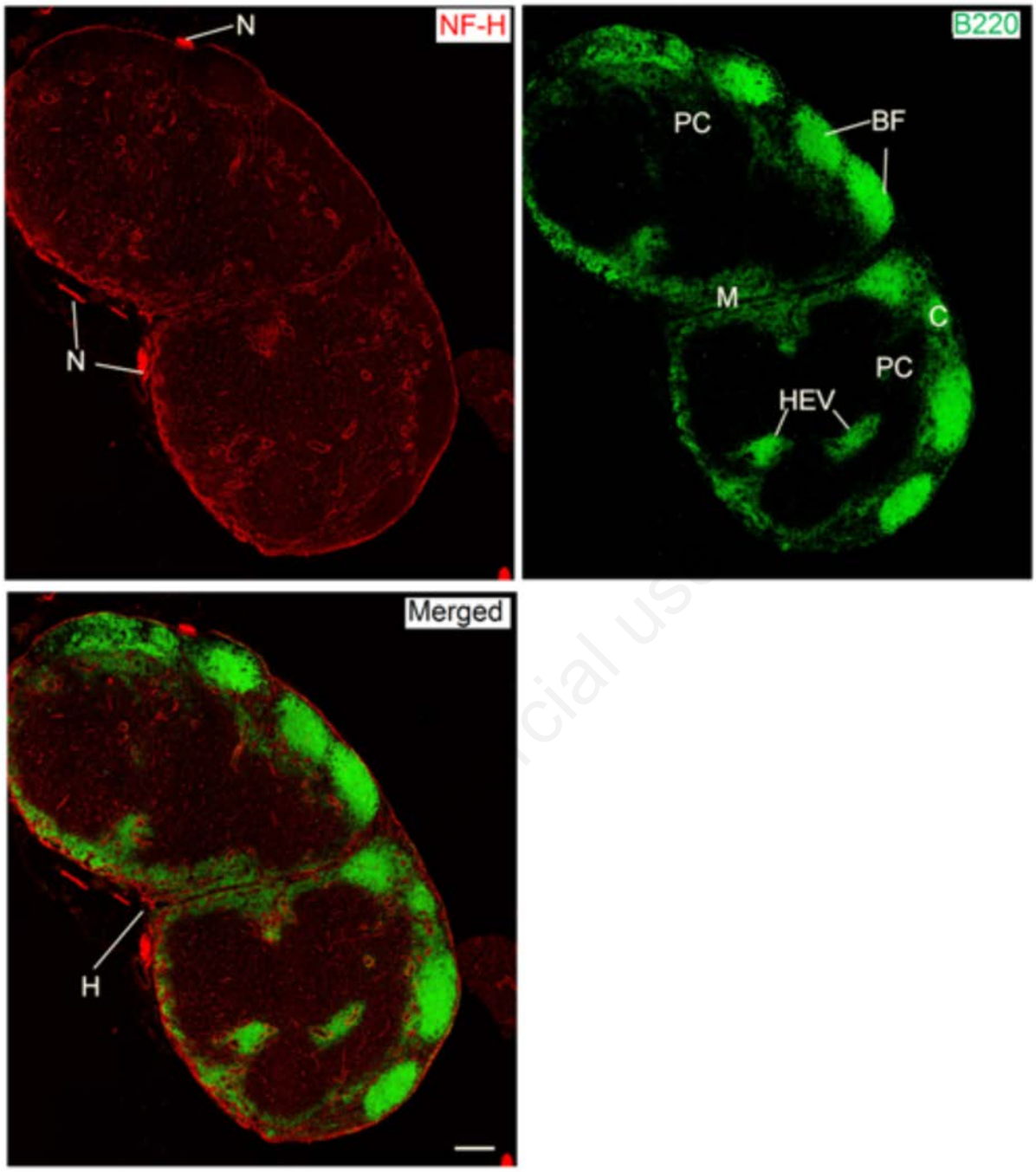
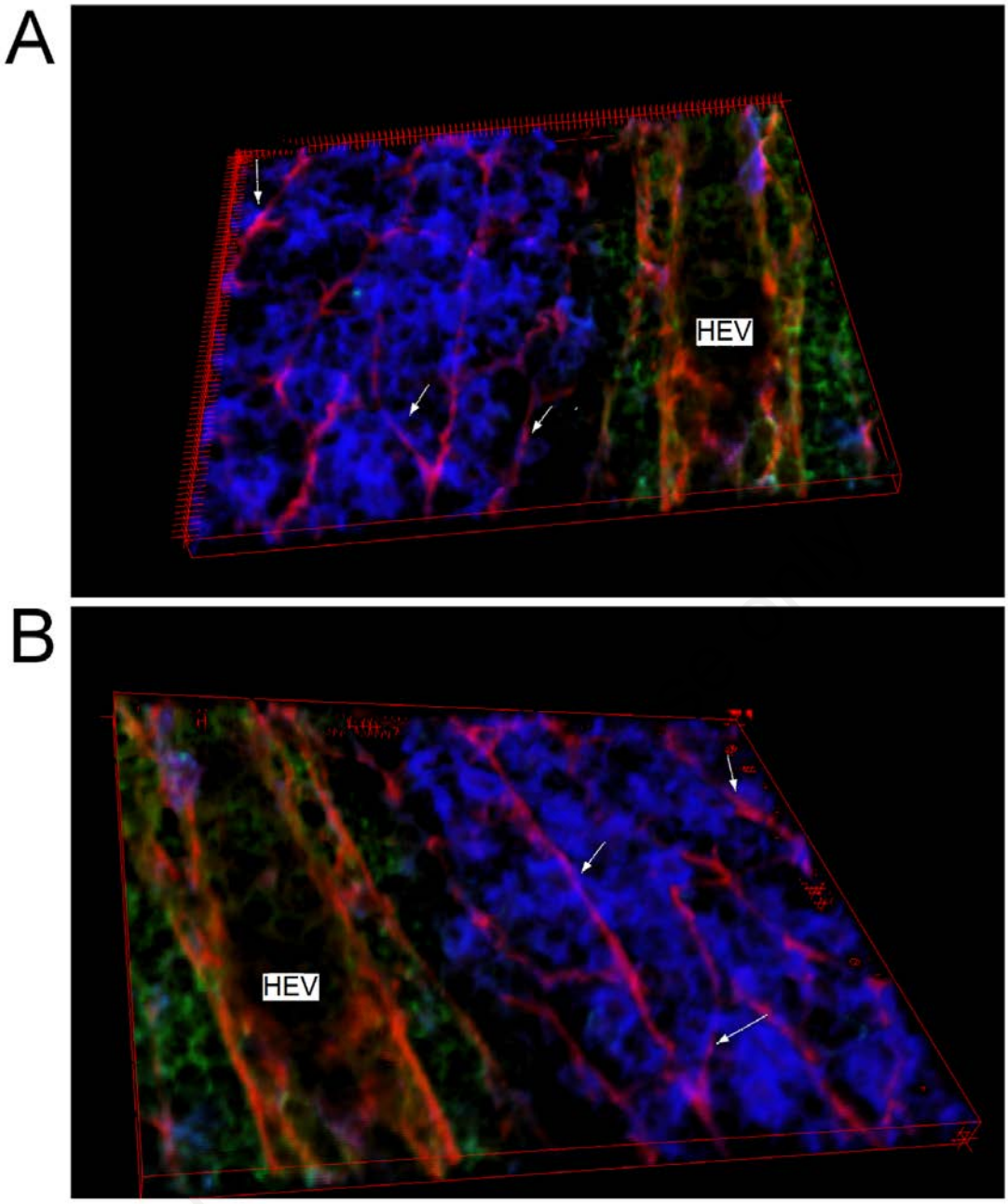
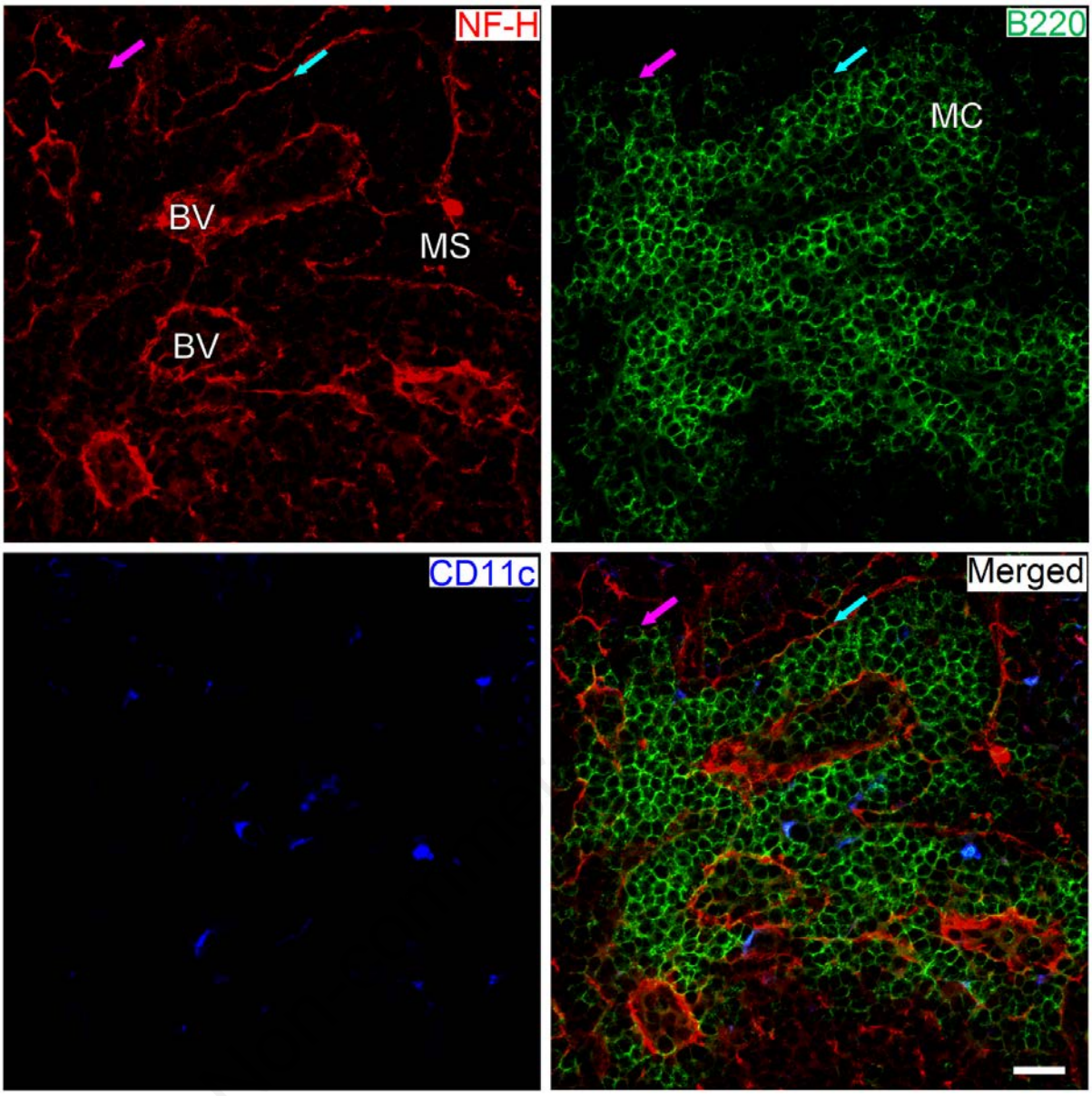
Key words: Innervation; lymph node; dendritic cell; lymphocytes; neurofilament; immunofluorescence staining.



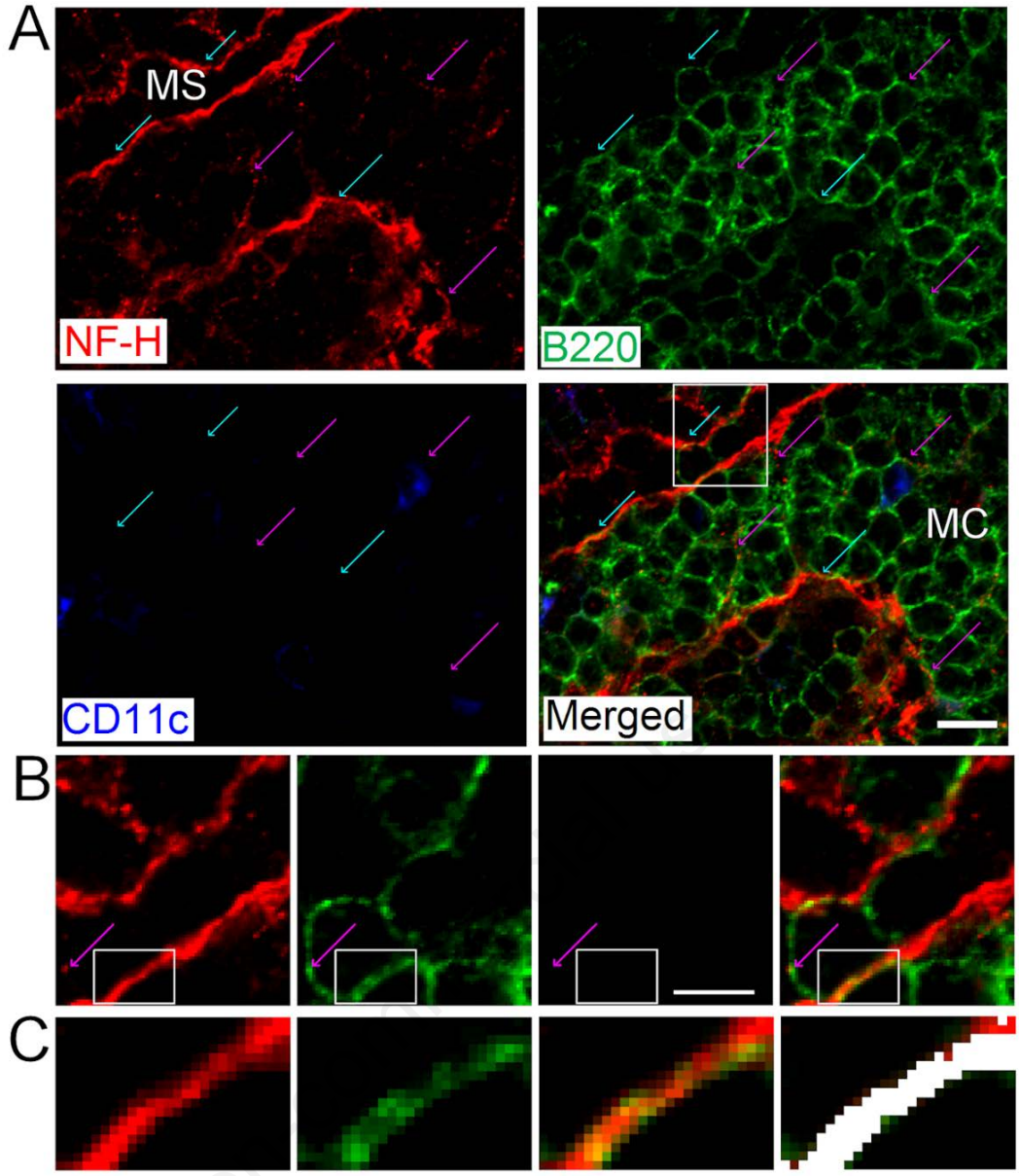
Supplementary Figure 1.
Innervation of a mesenteric lymph node from a C57BL/6 mouse. Antibodies against NF-H (red) and B220 (green) label mainly nerve fibers and B cells inside lymph node, respectively. BF, B cell follicle; C, cortex; PC, paracortex; HEV, high endothelial venules; M, medulla; H, hilum; N, nerves. Objective lens: 40x; Scanning mode: tile scan; scale bar: 200 µm.



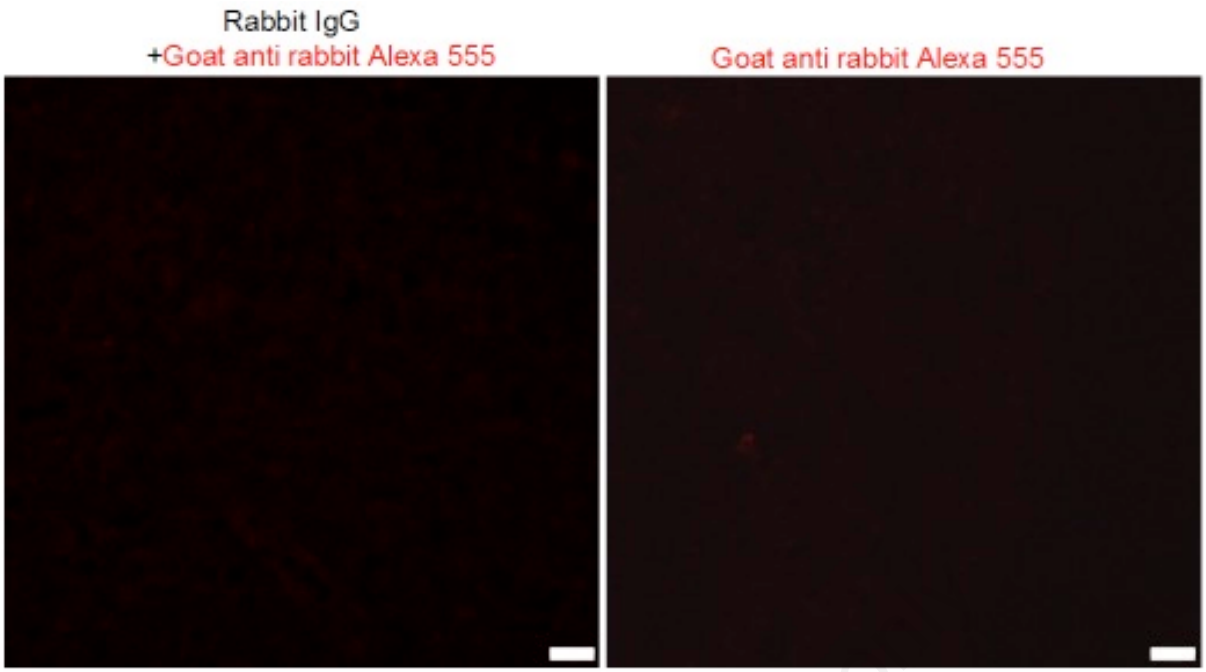
Supplementary Figure 2.
3D view (two snapshots from volume rendering by using 3D viewer in ImageJ) of paracortex from a mesenteric lymph node of a C57BL/6 mouse. Antibodies against NF-H (red), B220 (green), and CD11c (blue) label mainly nerve fibers, B cells, and DCs, respectively. A) Top view. B) Bottom view. The white arrows show $B220^{-}CD11c^{+}$ DCs that have close associations with the nerve fibers. HEV, high endothelial venules. Objective lens: 40x; stack size: 6.17 µm; optical slice interval: 0.47 µm; threshold: 0; resampling factor: 2.



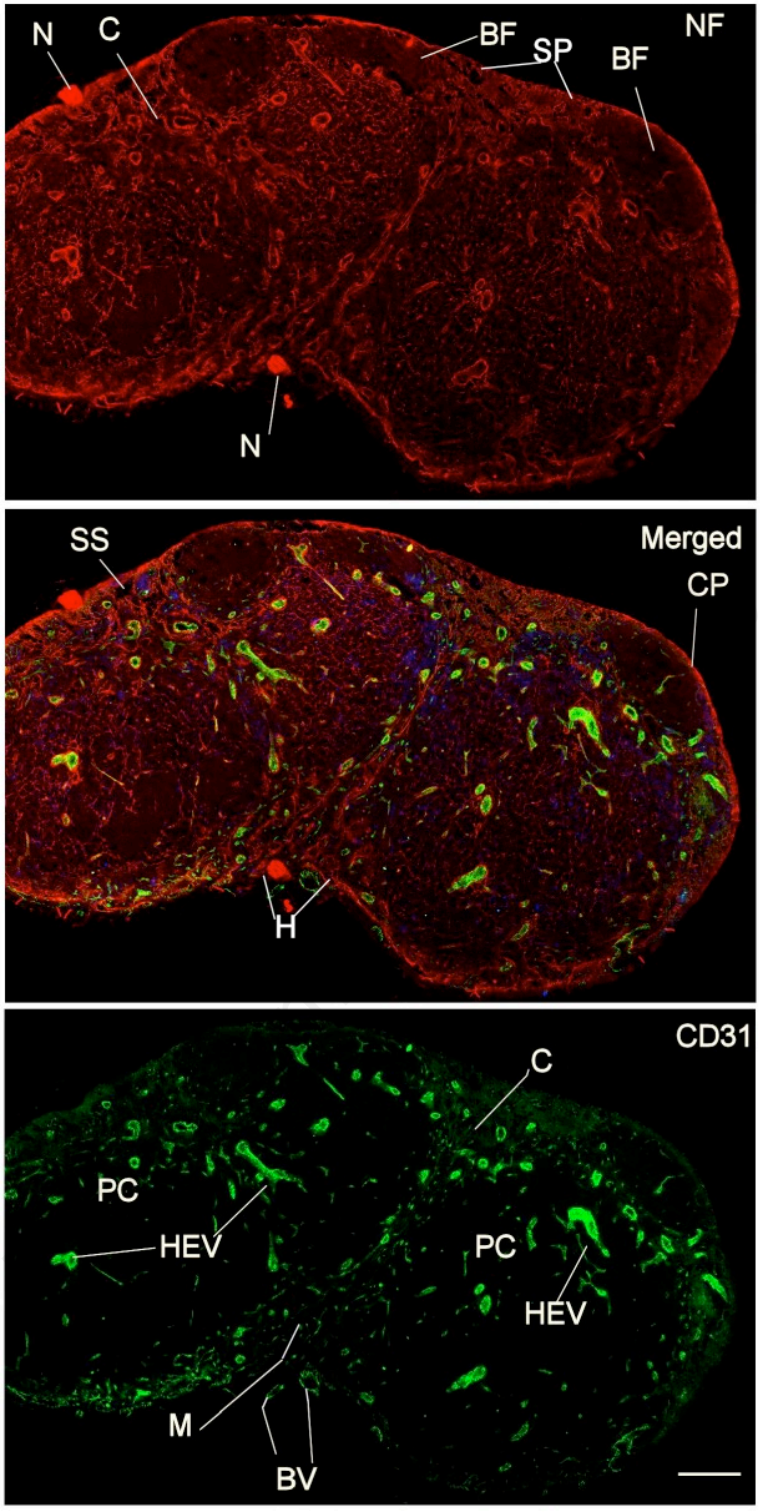
Supplementary Figure 3.
Higher-magnification view of innervation of the medulla of a mesenteric lymph node from a C57BL/6 mouse. Antibodies against NF-H (red), B220 (green), and CD11c (blue) label mainly nerve fibers, B cells, and DCs, respectively. The cyan arrows show B cells that have a close association with the nerve fibers; the magenta arrows indicate two B cells ($B220^{+}CD11c^{-}$) that have a close association with the nerve endings (appearing as red dots around B cells). MC, medullary cord; MS, medullary sinus; BV, blood vessel. Objective lens: 60x; scale bar: 20 µm.



Supplementary Figure 4.
Higher-magnification images (cropped from Supplementary Figure 3) show cell-cell contact of nerve fibers with immune cells in the medulla of a mesenteric lymph node. Antibodies against NF-H (red), B220 (green), and CD11c (blue) label mainly nerve fibers, B cells, and DCs, respectively. The cyan arrows show B cells ($B220^{+}CD11c^{-}$) that show membrane-membrane contact with the nerve fibers. The magenta arrows indicate a few B cells ($B220^{+}CD11c^{-}$) that have a close association with the nerve endings (appearing as red dots around B cells); MC, medullary cord; MS, medullary sinus; objective lens: 60x. A) Scale bar: 20 µm. B) High-resolution view of region cropped from (A). Colocalized pixels appear yellow in the merged image; scale bar: 5.0 µm. C) Images from the cropped region from B (marked with a white square) show colocalization of nerve fibers with part of B cells; single pixel (zoomed in, not according to the scale) can be observed as small squares. In the fourth panel (extreme right), colocalized pixels are marked with white after colocalization analysis of red (nerve fiber) and green (B cell) channels. The length of the image is 5.46 µm (26 pixels). % intensity above threshold colocalized for Channel 1 (red): 84.21%; % intensity above threshold colocalized for Channel 2 (green): 84.96%.



Supplementary Figure 5.
Confocal images of the lymph node (from a C57BL/6 mouse) taken from the isotype control (a rabbit IgG was applied) and negative staining control (primary antibodies were omitted). The images were acquired by using the same camera and device settings for the acquisition of other images in experimental groups. Objective lens: 40x; scale bar: 20 µm.



Supplementary Figure 6.
Overview of innervation and blood vessel distribution in an inguinal lymph node of a C57BL/6 mouse. Antibodies against NF-H (red), CD31 (green) label nerve fibers, and blood vessel endothelial cells, respectively. The merged image is shown in the middle. CP, capsule; BF, B cell follicle; BV, blood vessel; C, cortex; PC, paracortex; SS, subcapsular sinus; SP, subcapsular plexus; HEV, high endothelial venules; M, medulla; N, nerves; H, hilum. Objective lens: 40x; scanning mode: tile scan; scale bar: 200 µm.