

Supplementary Material

Immunohistochemical visualization of lymphatic vessels in human dura mater: Methodological perspectives

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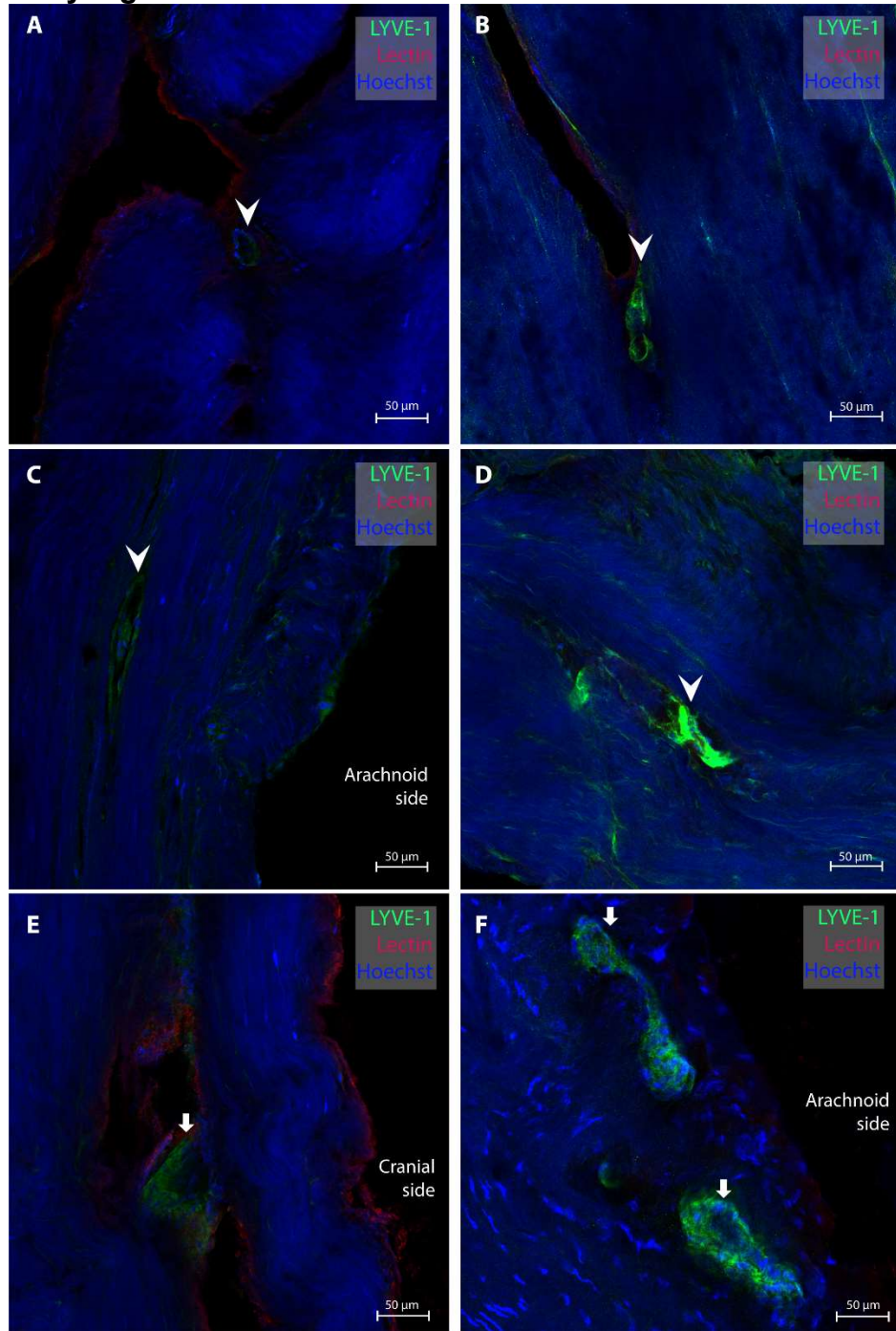
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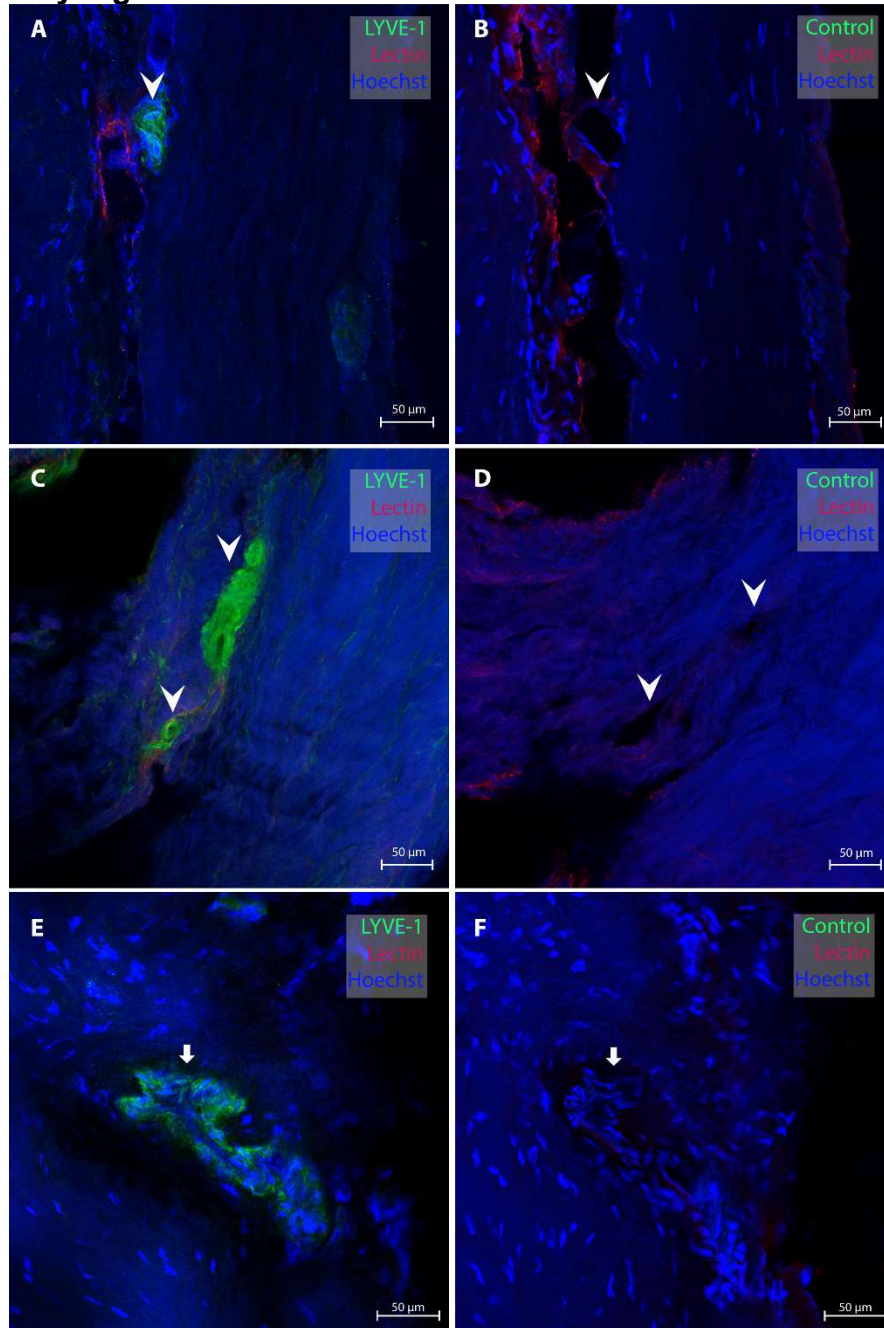
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Supplementary Figure 1



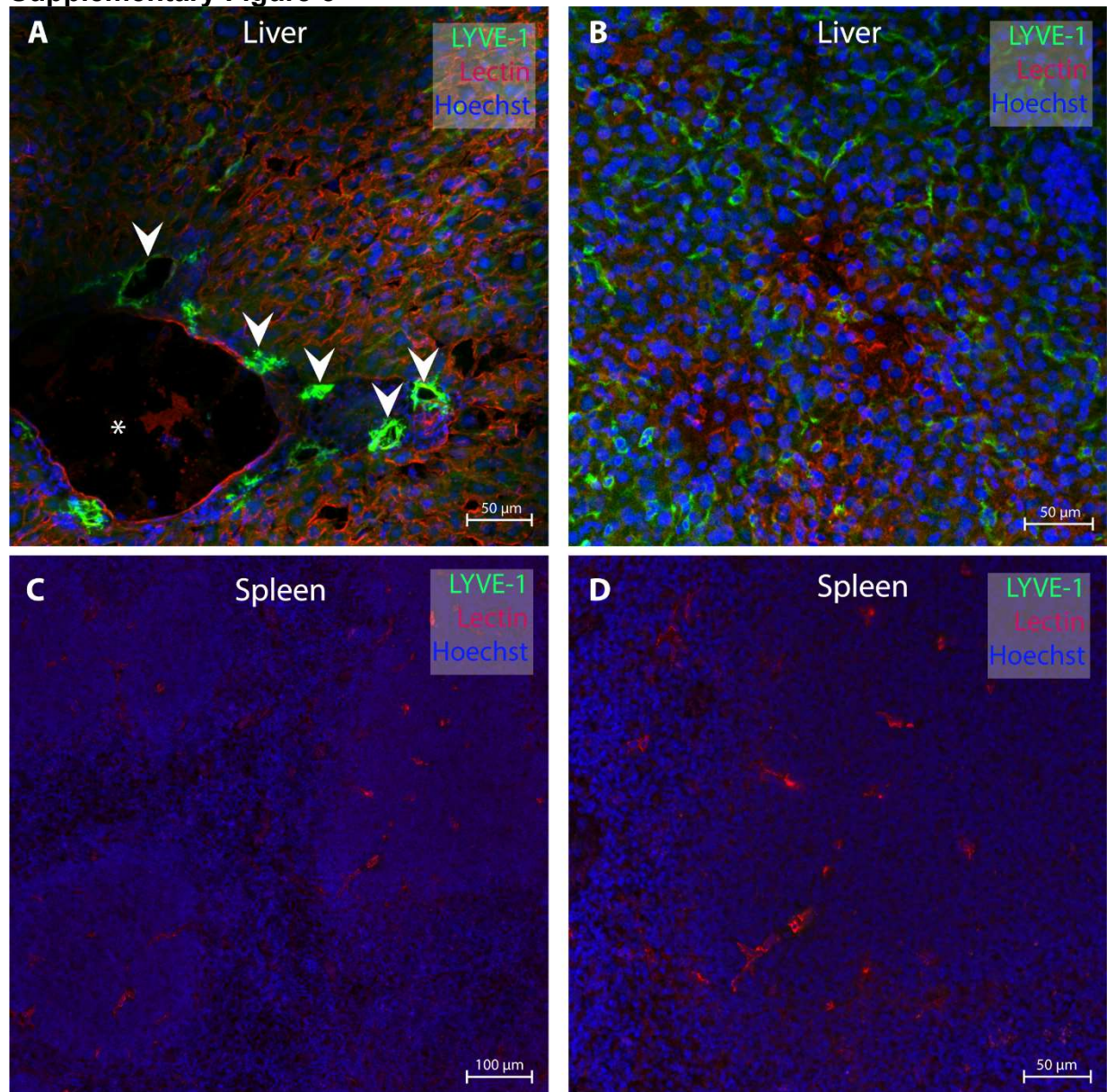
Comparison between fixation with 0.5%PFA and freezing method. The sections on the left column (**A**, **C**, **E**) were fixed in 0.5% PFA overnight while the sections on the right column (**B**, **D**, **F**) were frozen immediately after dissection and fixed 10 minutes in 0.5% PFA before IHC. Note the difference in signal intensity between fixation protocols. Top panels (**A** patient #27, **B** patient #21) are lymphatic vessels (white arrowheads) in proximity to blood vessels, middle panels (**C** patient #28, **D** patient #22) are lymphatic vessels (white arrowheads) in distance to blood vessels and bottom panel (**E** patient #26, **F** patient #13) are "clusters" (white arrows) of lymphatic tissue, some of which may be collapsed lymphatic vessels.

Supplementary Figure 2



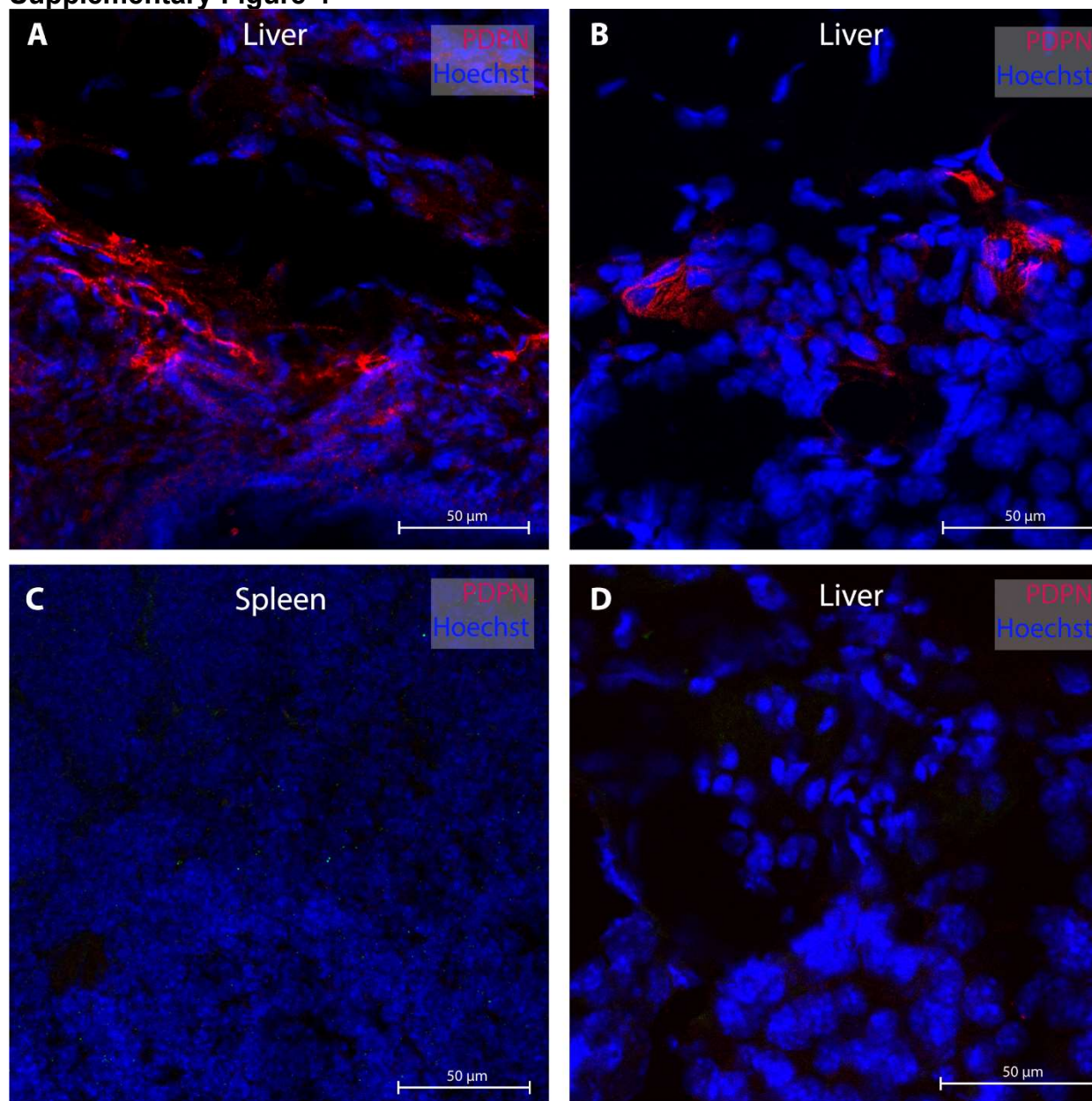
Control experiments showing specific antibody binding to lymphatic structures. For sections on the left column (**A, C, E**) primary anti-LYVE-1 antibody and secondary CY3 antibody was used for staining. Sections on the right column (**B, D, F**) were immediately adjacent to sections on the left used as controls where no primary antibody against LYVE-1 was used. Top panels (**A, B** from patient #8) are lymphatic vessels (white arrowheads) in proximity to blood vessels, middle panels (**C, D** from patient #9) are lymphatic vessels (white arrowheads) in distance to blood vessels and bottom panel (**E, F** from patient #13) are “clusters” (white arrows) of lymphatic tissue.

Supplementary Figure 3



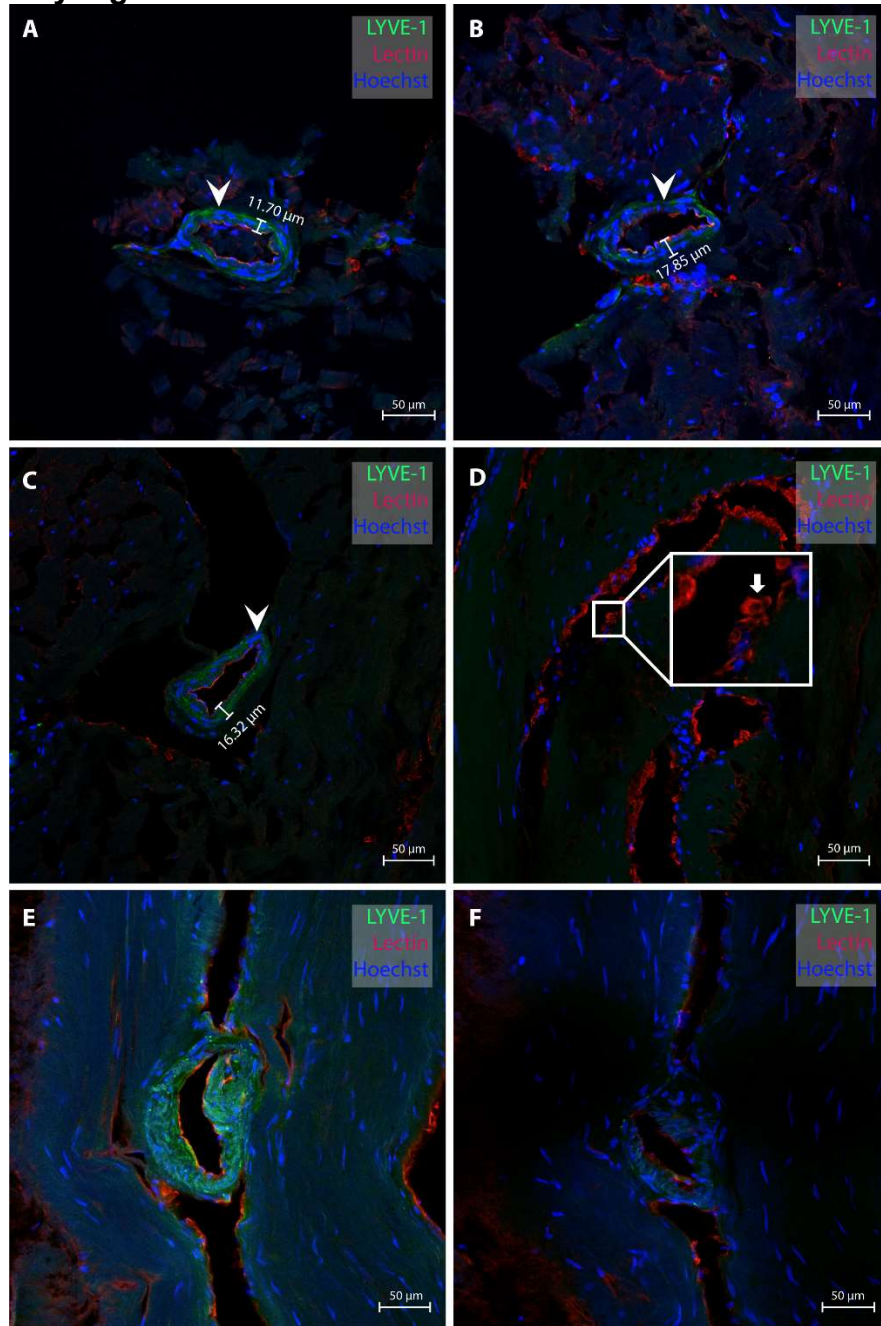
Positive and negative controls of LYVE-1 staining in mouse tissue. Top panels (A, B) are mouse liver sections (positive control) showing stained lymphatic vessels (white arrowheads) and blood vessels (asterisk) using antibodies against LYVE-1 and Lectin. Bottom panels (C, D) are mouse spleen sections showing absence of LYVE-1 as expected (negative controls).

Supplementary Figure 4



Positive and negative controls of PDPN staining in mouse tissue. Top panels (A, B) are mouse liver sections (positive control) showing stained lymphatic vessels using antibodies against PDPN. Bottom panel C is a mouse spleen section showing absence of PDPN as negative control while panel D is a serial section of panel B used as negative control where no primary antibody was added.

Supplementary Figure 5



Separating lymphatic vessels from blood vessels. A-C (patient #6). Blood vessels (white arrowheads) express LYVE-1 in their tunica media, but with vascular endothelial cells expressing Lectin (red). Blood vessel walls are generally thicker (A: 11.70 μm , B: 17.85 μm , C: 16.32 μm) compared to lymphatic vessels. Panel **D** (patient #5) shows labelled erythrocytes (white arrow) within lumen of blood vessel. Panel **E** (patient #2) shows positive LYVE staining with lectin stained endothelium. Panel **F** shows the same vessel in another section as negative control, as illustrated in Figure 8, where no primary anti-LYVE-1 antibody was added during immunohistochemistry disproving background staining.