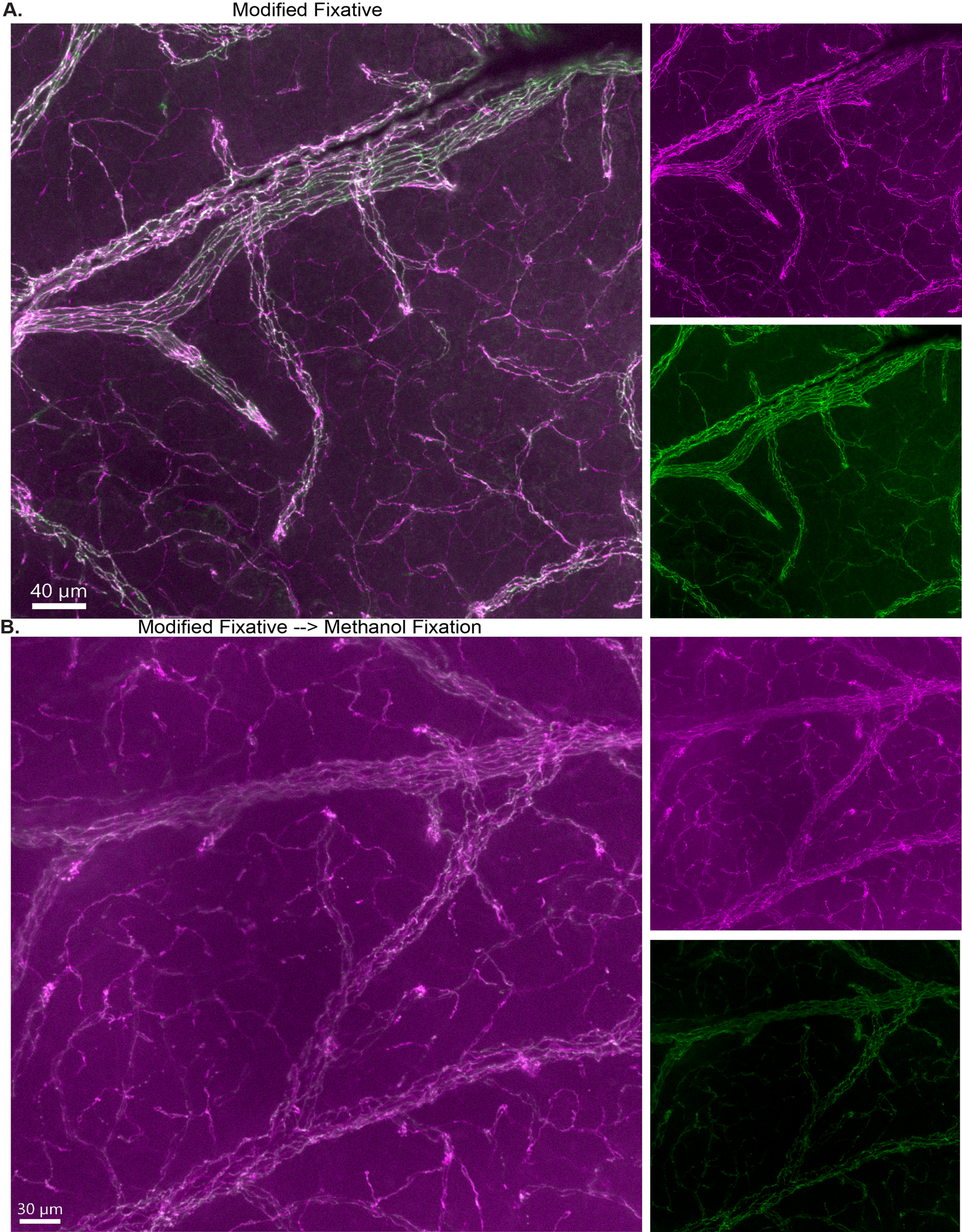
A.
Modified Fixative
40 µm
B.
Modified Fixative --> Methanol Fixation
30 µm

Supplemental Material

Supplemental Figure 1 related to Figure 3. Comparison of ADAPT-3D to retain immunolabeling of tight junctions in leptomeninges. Maximum intensity projections depicting tight junctions in endothelium immunolabeled by occludin (magenta) and claudin-5 (green) using A) modified fixative in ADAPT-3D or B) cardiac perfusion with modified fixative in ADAPT-3D followed by methanol fixation, a standard fixation step for tight junctions.

Video 1 related to Figure 2. 3D visualization of cholinergic nerves using dual illumination light sheet microscopy of whole brain. 3D projection of whole intact brain whose vasculature was labeled by retro-orbital injection of a mixture with Lectin-Dylight649 and CD31-AlexaFluor647 (Fire), in ChAT-TdTomato$^{fl/fl}$ mice (gray).

Video 2 related to Figure 2. 3D visualization of preserved skull-brain interface by dual illumination light sheet microscopy. 3D projection of intact skull with brain whose vasculature was labeled by retro-orbital injection of a mixture with Lectin-Dylight649 and CD31-AlexaFluor647 (gray) in Lyve1CreER-TdTomato$^{fl/fl}$ mice (red).

Video 3 related to Figure 3. 3D visualization using line-scanning confocal microscopy of terminal ileum from mice co-housed with $Tnf^{\Delta ARE/+}$ as littermate controls. Immunolabeling with antibodies against LYVE-1 (magenta) for lymphatics, S100A9 (cyan) for myeloid cells, alpha-smooth muscle actin (α-SMA) for muscle fibers, and DAPI (gray) for nuclei.

Video 4 related to Figure 3. 3D visualization using line-scanning confocal microscopy of terminal ileum from mice co-housed with $Tnf^{\Delta ARE/+}$ as littermate controls. Immunolabeling with antibodies against LYVE-1 (magenta) for lymphatics, S100A9 (cyan) for myeloid cells, alpha-smooth muscle actin (α-SMA) for muscle fibers, and DAPI (gray) for nuclei.

Video 5 related to Figure 3. 3D visualization using line-scanning confocal microscopy of terminal ileum from a non-diseased human. Immunolabeling with antibodies against CD163 (green), IBA1 (red), and DAPI (gray) for nuclei.