Capillary, venous, and arterial vasculature 3D light sheet imaging and AI analysis in intact mouse organs

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Background & Aim

Vascular changes, from rarefaction to malformation, are key features of diseases like cancer and diabetes. Distinguishing vascular segments and determining their spatial distribution in a 3D tissue environment has remained challenging. Here we aimed at utilizing light sheet fluorescence microscopy (LSFM) and developing methods that enable accurate detection of vascular cells at the mRNA and protein level in entire mouse organs.

Methods

+ Whole organ processing using different labelling methods:
  + (2) Injectable dyes
  + (3) mRNA reporter (*in situ*)
  + (4) Antibodies
+ Tissue clearing for high-throughput and fully automated light-sheet fluorescence microscopy (LSFM)
+ AI-assisted segmentation to distinguish capillaries, veins and arteries and quantification of vasculature parameters (length, volume, branching, etc.).

Conclusion

+ Whole-organ LSFM imaging of blood vessel systems requires tissue specific processing with the optimal choice of endothelial-specific markers.
+ Understanding blood vessel complexity in different and diseased organs, e.g. vascular complications in peripheral artery or venous diseases, are pivotal for the effective translation of novel therapeutics.

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1 Methods

(2) *In vivo labelling* Whole organ staining or (3) *In situ* hybridization (4) Immunostaining Clearing and 3D imaging AI-assisted segmentation

>cm-mm (Arteries, Veins) / mm-µm / µm (Capillaries)

Scan the QR code for video:

2 *In vivo* Dye Perfusion

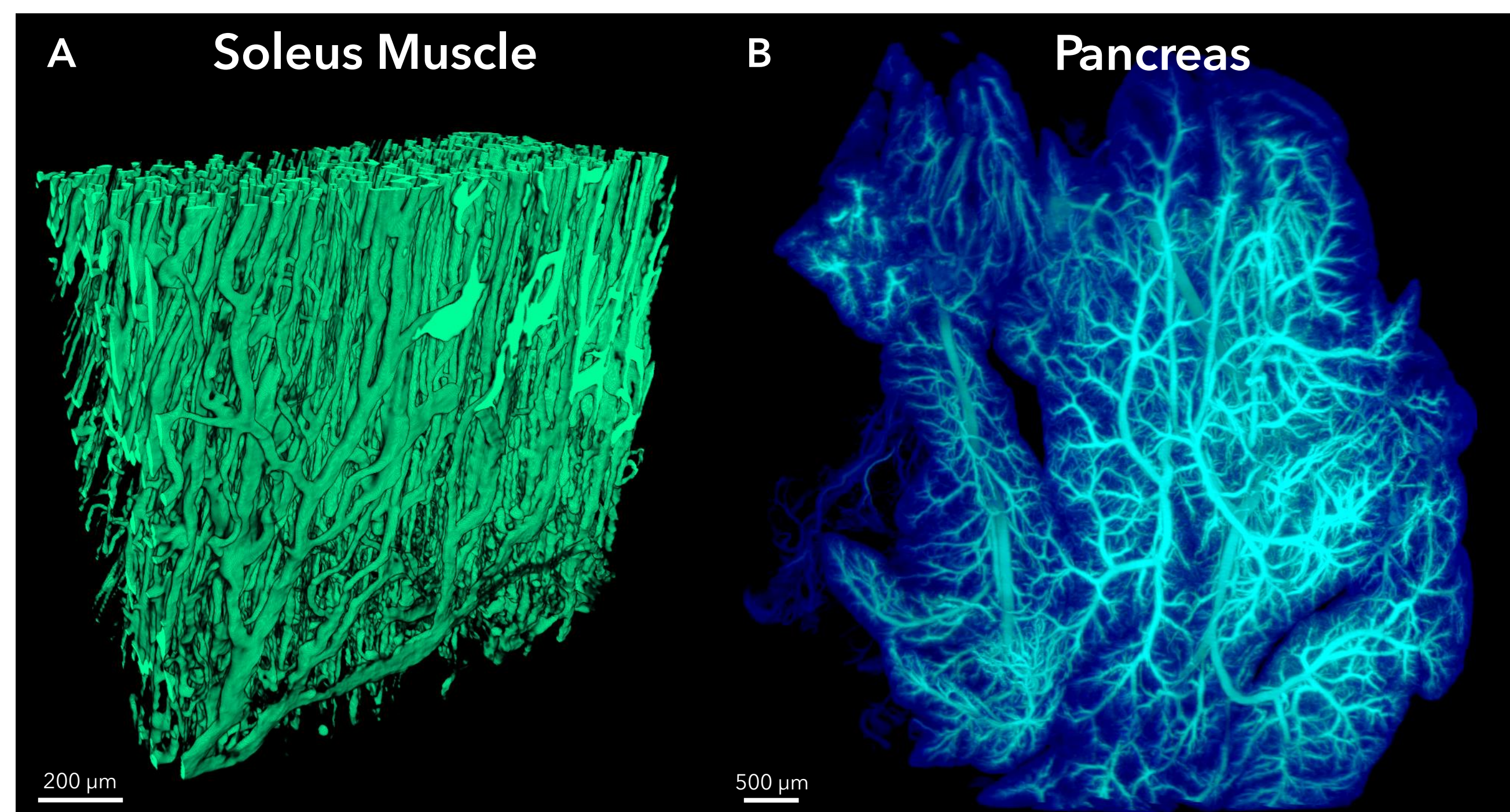**A Soleus Muscle**   **B Pancreas**
200 µm   500 µm

Figure 1. Injectable dyes allow for rapid and reliable vasculature labelling in dense organs or tissues that are difficult to stain for LSFM with conventional Immunohistochemical methods. (A) Image from a rat soleus muscle (hind leg) stained by intravenously injected fluorescent tomato Lectin *(Lycopersicon Esculentum; DyLight 594)*, here shown as a green surface render depicts the highly dense 3D vasculature. **(B)** Image of the pancreatic blood vessel system from a healthy mouse (C57B/6j) labelled with a directly conjugated CD31 antibody (AlexaFluor 647) was imaged in 3D using LSFM. The branching vasculature is shown in teal and anatomical outline in blue. Scale bars are 200µm and 500µm in (A) and (B), respectively.

3 *In situ* Hybridization
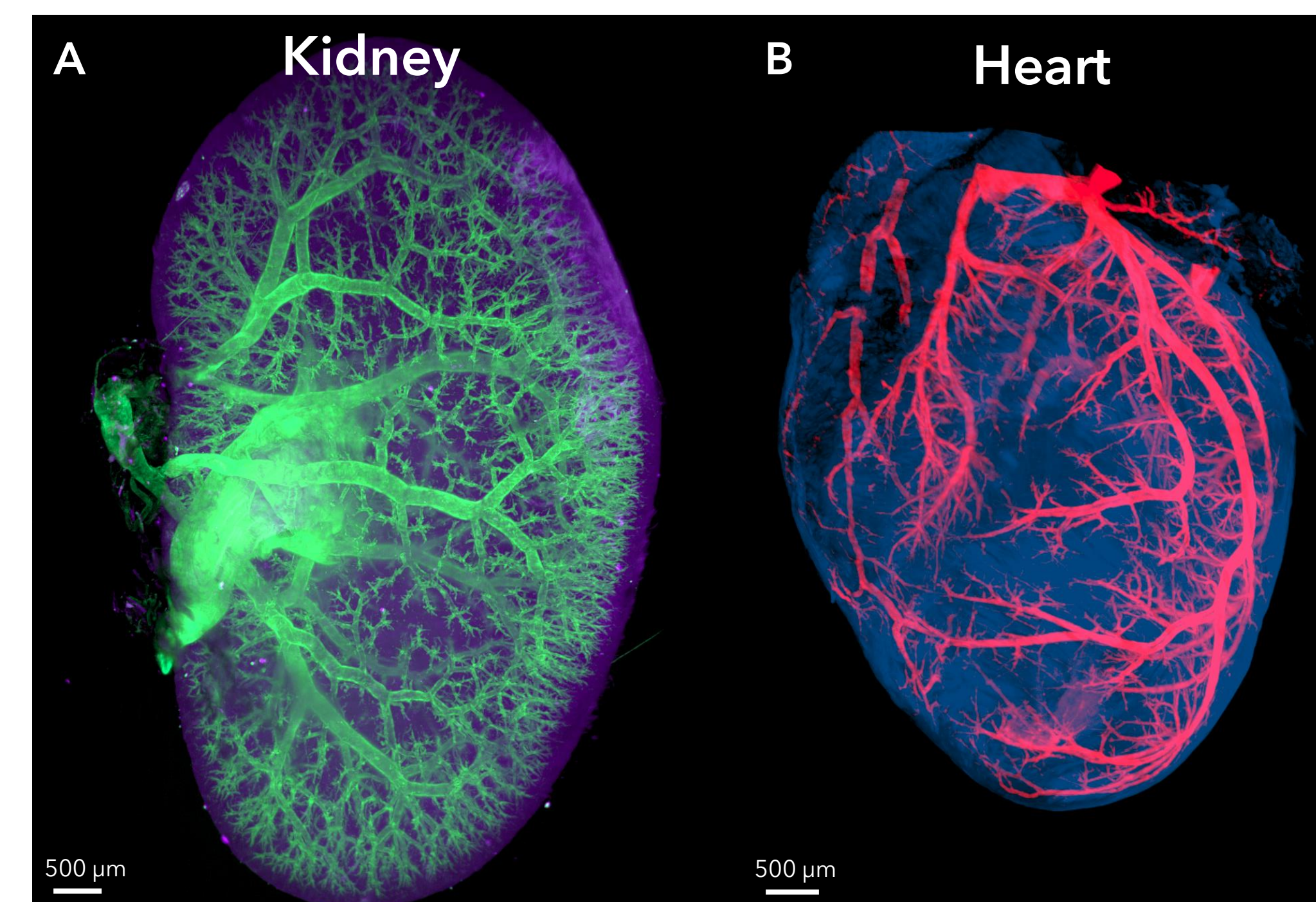

A Kidney   **B Heart**
500 µm   500 µm

Figure 2. Whole-organ fluorescent *in situ* hybridization allows for spatial RNA visualization of vasculature gene expression. (A) Images of 3D maximum intensity projections of a whole mouse kidney and **(B)** a whole mouse heart, both labelled fluorescently for transcribed *MHY11* mRNA (smooth muscle myosin heavy chain 11) is show in green and pink in the kidney and heart, respectively. The auto-fluorescent anatomy is depicted in purple in the kidney and in blue in the heart. Scale bars are 500µm.

4 Multiplex Whole Organ Immunostaining

A Liver
500 µm

B Vasculature volume density in whole organs
vasculature vol. / anatomy vol. in %
(16, 14, 12, 10, 8, 6, 4, 2, 0)
Soleus Muscle | Heart | Liver | Kidney | Pancreas (healthy) | Panc. cancer (PDAC)

C PDAC
1500 µm
C'   C''

D SM22 (arteries)   **E** vWF (veins)   **F** CD31 (pan-vascular)   **G** overlay
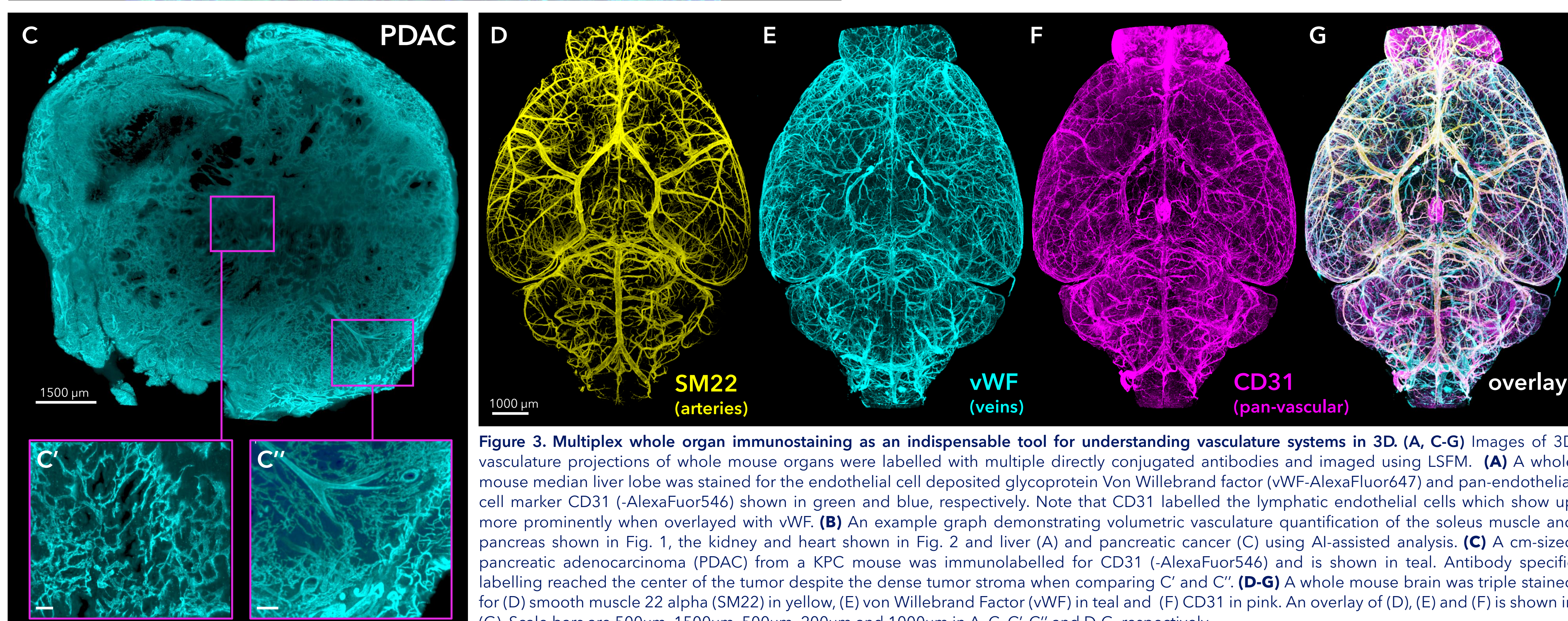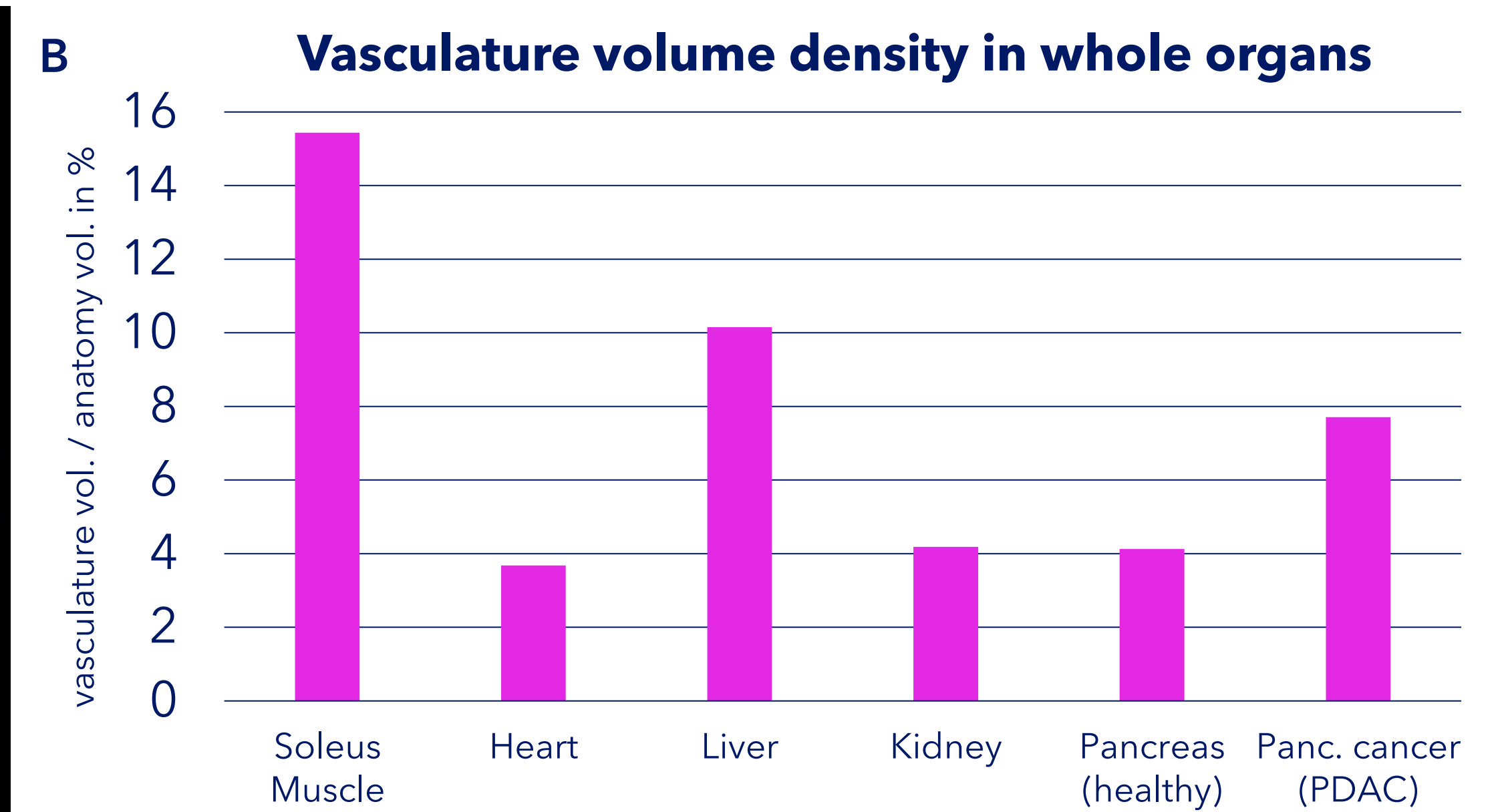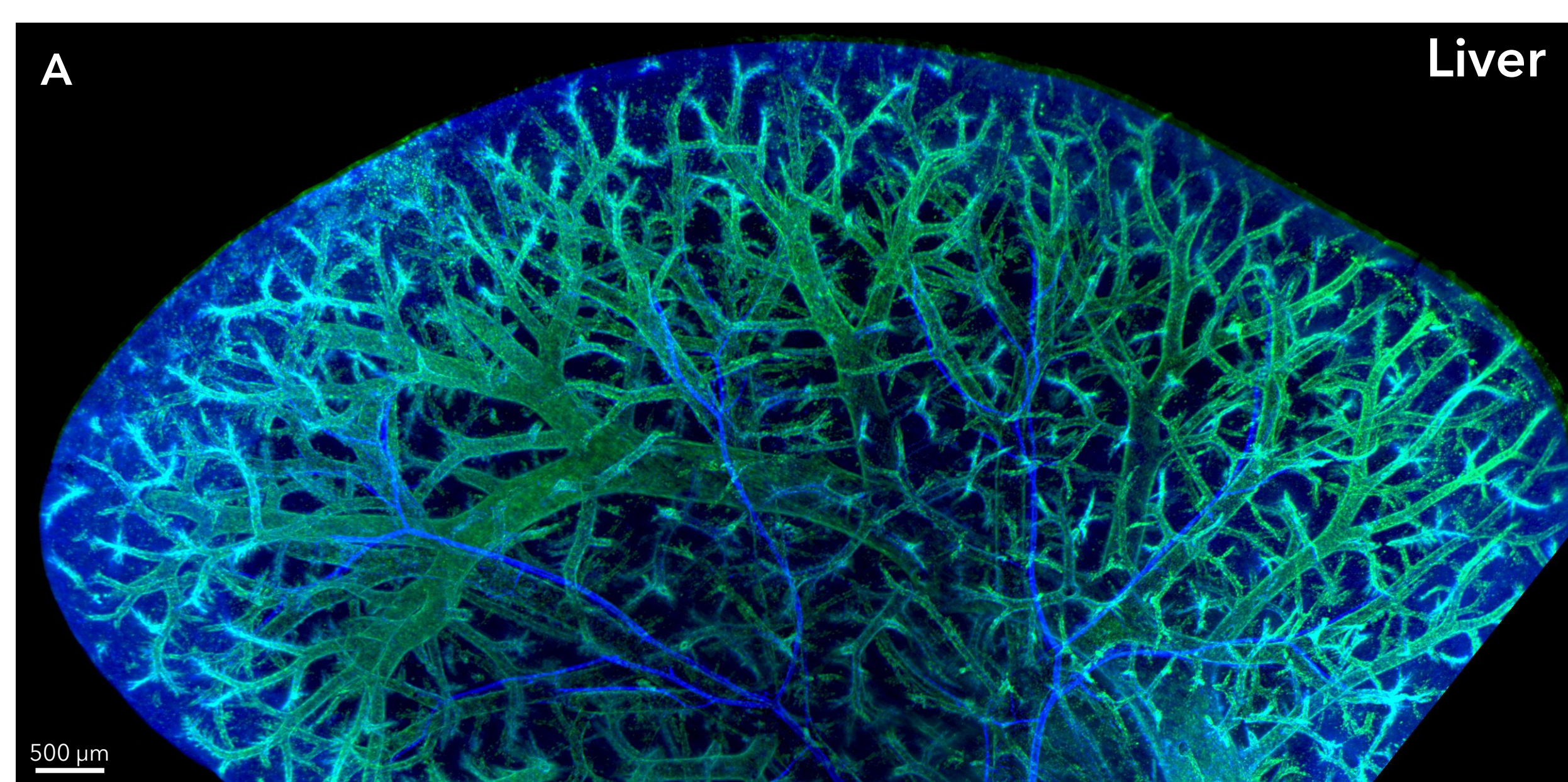1000 µm

Figure 3. Multiplex whole organ immunostaining as an indispensable tool for understanding vasculature systems in 3D. (A, C-G) Images of 3D vasculature projections of whole mouse organs were labelled with multiple directly conjugated antibodies and imaged using LSFM. **(A)** A whole mouse median liver lobe was stained for the endothelial cell deposited glycoprotein Von Willebrand factor (vWF-AlexaFluor647) and pan-endothelial cell marker CD31 (-AlexaFuor546) shown in green and blue, respectively. Note that CD31 labelled the lymphatic endothelial cells which show up more prominently when overlayed with vWF. **(B)** An example graph demonstrating volumetric vasculature quantification of the soleus muscle and pancreas shown in Fig. 1, the kidney and heart shown in Fig. 2 and liver (A) and pancreatic cancer (C) using AI-assisted analysis. **(C)** A cm-sized pancreatic adenocarcinoma (PDAC) from a KPC mouse was immunolabelled for CD31 (-AlexaFuor546) and is shown in teal. Antibody specific labelling reached the center of the tumor despite the dense tumor stroma when comparing C' and C''. **(D-G)** A whole mouse brain was triple stained for (D) smooth muscle 22 alpha (SM22) in yellow, (E) von Willebrand Factor (vWF) in teal and (F) CD31 in pink. An overlay of (D), (E) and (F) is shown in (G). Scale bars are 500µm, 1500µm, 500µm, 200µm and 1000µm in A, C, C', C'' and D-G, respectively.