

Supplementary Methods

CD31 Alexa-647 antibody and FITC-albumin hydrogel co-perfusion

For perfusion with the CD31 Alexa-647 antibody (BioLegend Clone: MEC13.3, Cat.-No.: 102516) the mouse were not narcotized but put in a restrainer and 10µg/mouse (20µl CD31 antibody + 130µl PBS) was administered intravenously into the tail vein. After 60 minutes the mice were then perfused with PBS/heparin, 4% PFA in PBS and the FITC-albumin hydrogel, as described in the in the main methods text.

Co-labeled CD31 Alexa-647 antibody and FITC-albumin image acquisition and analysis

For the co-labelled cleared brain, an image stack on the lightsheet microscope at 1.6X magnification was acquired of the whole mouse brain in a ventral dorsal direction starting at bregma -6.24mm. A 647nm diode laser (50mW) was used to excite the CD31 Alexa-647 and the resulting fluorescence imaged through a 680/30 bandpass filter, and a 488nm diode laser (50mW) was used for the FITC-albumin excitation with the resultant fluorescence imaged through a 525/50 bandpass filter. From the whole brain image, regions in the ipsi and contralateral portions of the brain were selected as previously described in the main methods. For quantification, the vessel length density was estimated from four equidistant positions within the 1.5mm length z-stacks for the ipsi- and contralateral regions. At each position 10 images were extracted, corresponding to a sub-stack slice of 40 microns. A sum projection of each sub-stack was taken and subsequent background subtraction performed. It should be noted that the intensity of the FITC-albumin hydrogel scales with the volume of the filled vessel, whereas the CD31 being a surface marker does not, consequently the signal intensities between the two images will not show a linear correlation. Therefore, in order to better compare and quantify the two labels we performed a cubed root operation on the FITC-albumin images and a subsequent histogram intensity normalization was then performed on both, after which a 2D vessel enhancement filter was applied. The total length of the vessels in each of the sub-volumes was then estimated by performing an intensity threshold to segment the vessels and a skeletonisation of the resulting mask. Although we calculate the length of the 'projected' vessels onto a plane, the calculated vessel length density in the slices (Supplementary Table 1) showed

good agreement with the results we obtained from the 3D filament models. This is probably as a result of the fact that the vast majority of the small vessels are lying in the imaging plane.

All image processing was performed using the open source software ImageJ (National Institutes of Health, Bethesda, MD, U.S.A.)

Table 1. Average vessel length density for the CD31 Alexa-647 and FITC-albumin co-labels, taken from four sampled sections in the non-ischemic and ischemic regions of a cleared mouse brain. Data are mean \pm SD values (n=4).

	Non-ischemic		Ischemic	
	CD31-Alexa-647	FITC-albumin	CD31-Alexa-647	FITC-albumin
Average vessel length density (mm/mm³)	853.01 \pm 54.97	872.65 \pm 25.82	460.36 \pm 140.67	422.66 \pm 136.46

Supplementary Figures

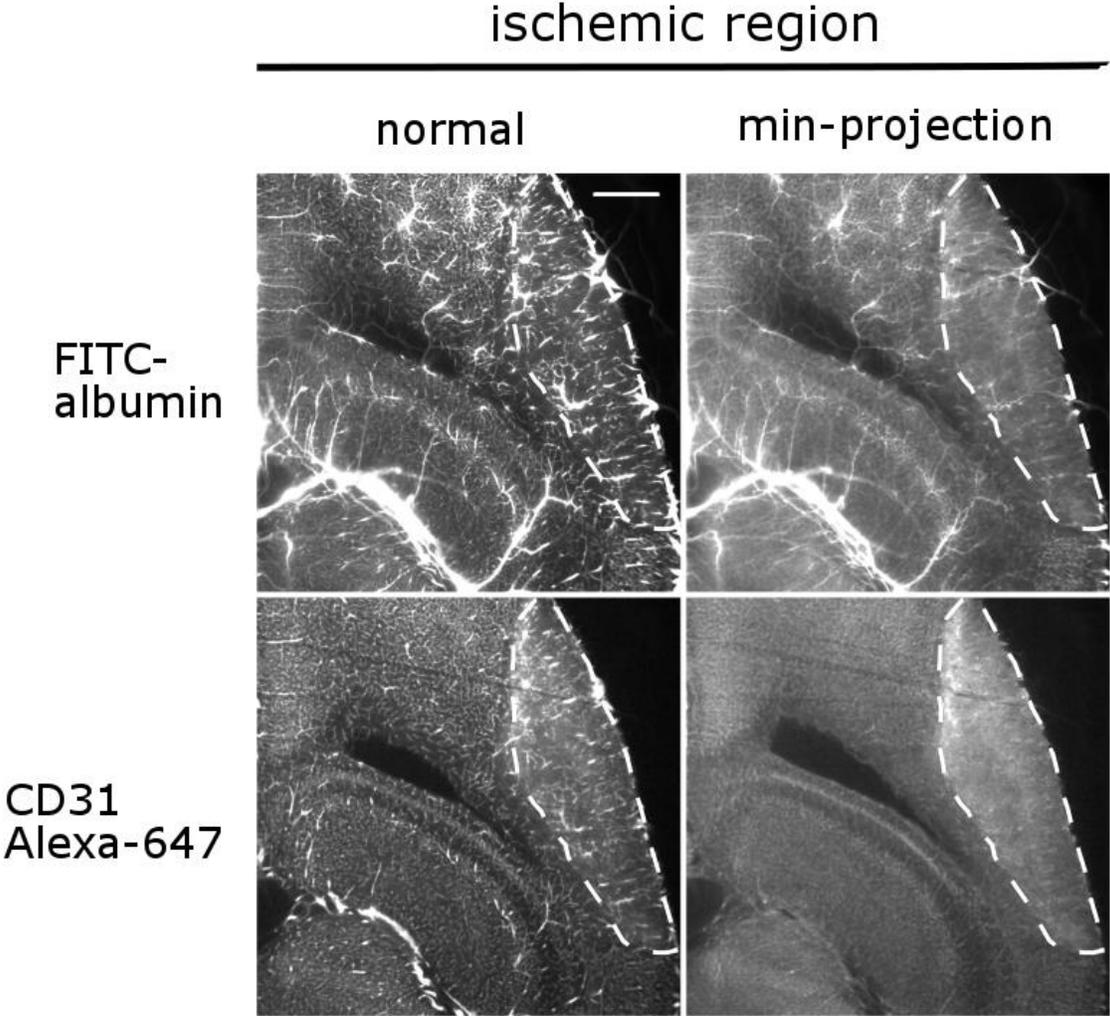


Figure 1: Ischemic region (dotted white border) of a cleared mouse brain which was pre-perfused with both an Alexa-647 labeled CD31 antibody and FITC-albumin hydrogel. Upper row shows the FITC-Albumin label and the lower row the CD31. In order to better visualize the background signal a minimum projection was taken of a ten image section (corresponding to a 40 μm z-slice) centered around the image shown in the first column. A clear and distinct background staining is visible in the CD31 image, which is absent from the FITC-albumin image. Scale bar represents 500 μm .

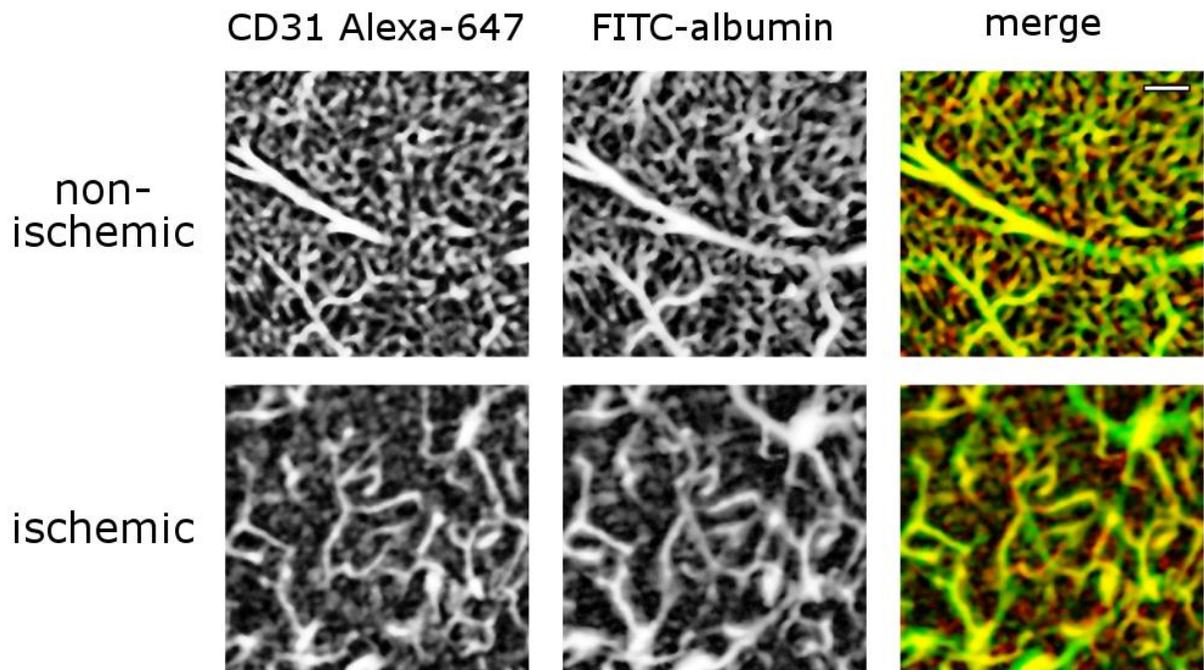


Figure 2: Detailed images from the non-ischemic (first row, contralateral) and ischemic (second row, ipsilateral) regions of a cleared mouse brain comparing the co-labeled signal from the CD31 Alexa-647 antibody (first column) and FITC-albumin (second column), taken from a mouse pre-perfused with both. The third column shows a merge of the two labels, with CD31 in red and FITC-albumin in green. The images show a sum-projection of 10 z-slices (corresponding to a 40 micron z-slice) and the intensities of the two images were subsequently ‘equalized’ in order to better compare the co-labeling of structures. Scale bar represents 50 μm .