Supplementary Methods

Estimation of extravasation volume

Volumes were determined from stacks of *in vivo* TPLSM images. Planes that spanned 5 μm were averaged. The extravasation volume was estimated by counting pixels with intensities greater than 10% of the range of intensities in the spatially averaged image. To ensure similar intensity distributions across image stacks, only image stacks in which vessels outside the extravasation volume equaled the maximum intensity values were used. We only measured the volume of the top half of the extravasation, since many target vessels were near the maximum imaging depth, and doubled this value.

Post mortem analysis

At the end of each experiment, animals were deeply anesthetized and perfused with 100 ml of phosphate buffered saline (PBS), followed by 150 ml of 4 % (w/v) paraformaldehyde in PBS. The glass coverslip and agarose covering the cranial window were removed and electrolytic fiducial marks were made in the corners of the craniotomy by driving a single tungsten electrode into the brain (no. WE3003(2-5)A10; MicroProbe) at a speed of ~ 0.2 mm/s while passing a current of ~ -20 μA. The brains were then removed from the skull, post-fixed in 4 % (w/v) paraformaldehyde in PBS, cryoprotected by equilibration in 30 % (w/v) sucrose in PBS, and cut on a freezing-sliding microtome into 50-μm coronal sections. The sections were incubated with diaminobenzadine (DAB) to stain endogenous peroxidase in RBCs. The sections were wet-mounted on glass slides and photographed with brightfield and epifluorescence microscopy. Vascular disruptions could be identified on the basis of both fluorescein-dextran extravasation[1,2] and DAB stained RBCs and were mapped relative to the location of the electrolytic fiducials.

Hypoxyprobe. Six of the animals received injections of pimonidazole hydrochloride (Hypoxyprobe-1™) (90201; Chemicon) 1 hour before sacrifice. These animals were sacrificed and perfused as above and histological sections were prepared.

Immunohistochemistry. Sections with documented vascular disruption were dried onto glass slides (Superfrost Plus; Fisher) and underwent antigen retrieval in 10 mM citrate buffer, pH 6.0, heated to boiling in a microwave oven (300 s at full power). The slides were incubated overnight in one of four monoclonal antibodies in diluent with PBS and 0.2 % (v/v) Triton X–100 followed by incubation with a biotinylated anti-mouse secondary antibody: (*i*) Hypoxyprobe™ antibody (90204; Chemicon); (*ii*) MAP2 antibody (M1406; Sigma); (*iii*) fibrin antibody (MAB1901; Chemicon); and (*iv*) vimentin antibody (MAB3400; Chemicon). In all cases bound antibody was visualized with the Vector ABC Kit (no. PK6100) using DAB (no. DAB; Vector) as the chromagen. The sections were cover-slipped with Prolong mountant (no. 36930; Molecular Probes).

We checked for nonspecific antibody staining. A photomicrograph, obtained at low magnification, illustrates that the staining with anti-fibrin and visualization with DAB leads to a locally defined dark product and the remainder of the section essentially negative (Fig. SM1).

1. Zhang, Z., Davies, K., Prostak, J., Fenstermacher, J. & Chopp, M. Quantitation of microvascular plasma perfusion and neuronal microtubule-associated protein in ischemic mouse brain by laser-scanning confocal microscopy. *Journal of Cerebral Blood Flow and Metabolism* **19**, 68-78 (1999).
2. Lindsberg, P. J., Siren, A. L. & Hallenbeck, J. M. Microvascular perfusion during focal vasogenic brain edema: A scanning laser fluorescence microscopy study. *Microvascular Research* **53**, 92-103 (1997).

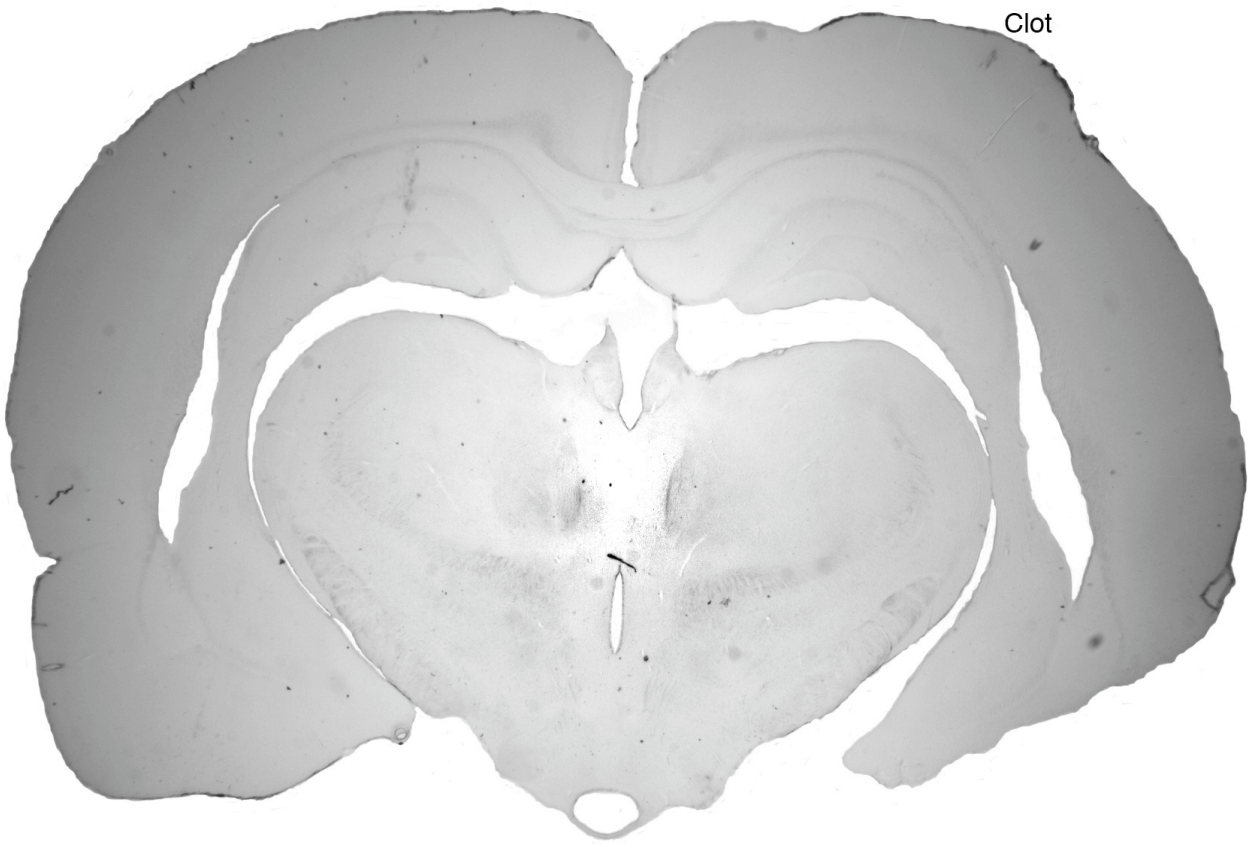

Figure SM1. Anti-fibrin immunostaining indicates localization of fibrin/fibrinogen clot marker (Clot). This is the full section used for figure 5B2. Note that some dirk marks are present in the section.