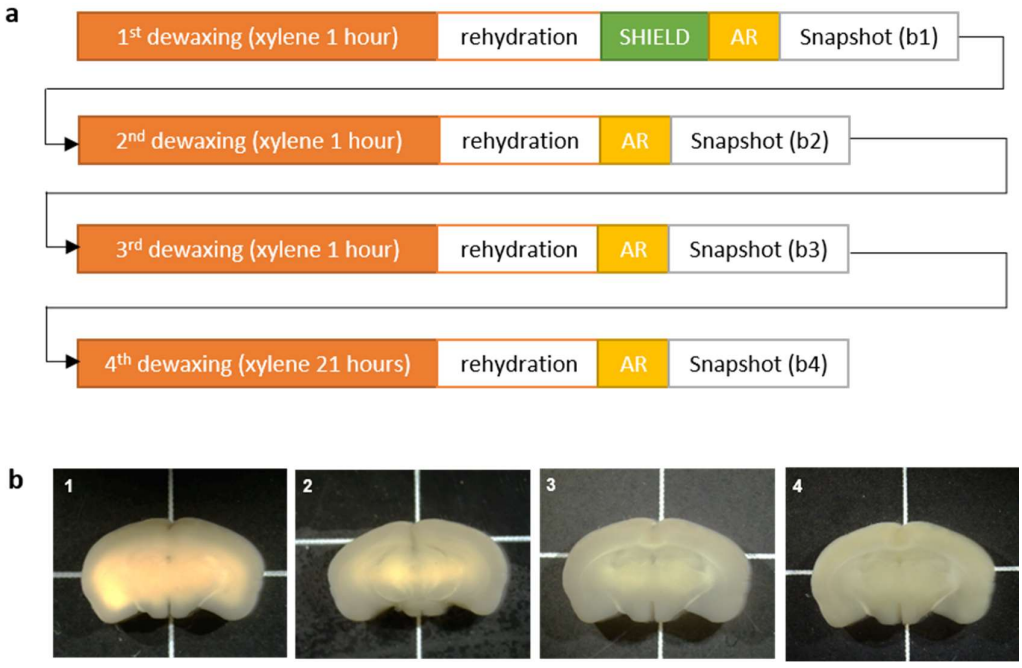
Supplementary table 1: The antibodies used in the study.

Primary antibodies

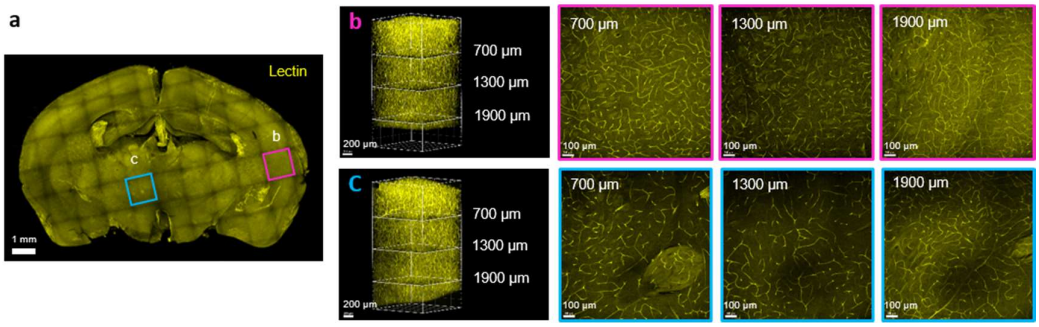
Target	Brand	Catalog number	Amount (for a whole mouse brain)
Tyrosine hydroxylase (TH)	Biolegend	818001	6 µg
Tryptophen hydroxylase (TPH2)	Invitrogen	PA1778	6 µg
Calbindin	Cell signaling technology	13176S	10 µg
Parvabumin	Invitrogen	PA1933	10 µg
Choline Acetyltransferase (ChAT)	Millipore	AB144P	40 µl
Pan-axonal filaments (SMI312)	Biolegend	837904	6 µg
GFP-tag	Abclonal	AE012	6 µg
Glial fibrillary acidic protein (GFAP)	abcam	ab7260	6 µg
Microtubule-associated protein 2 (MAP2)	Cell signaling technology	#8707	20 µg

Secondary antibodies and lectin

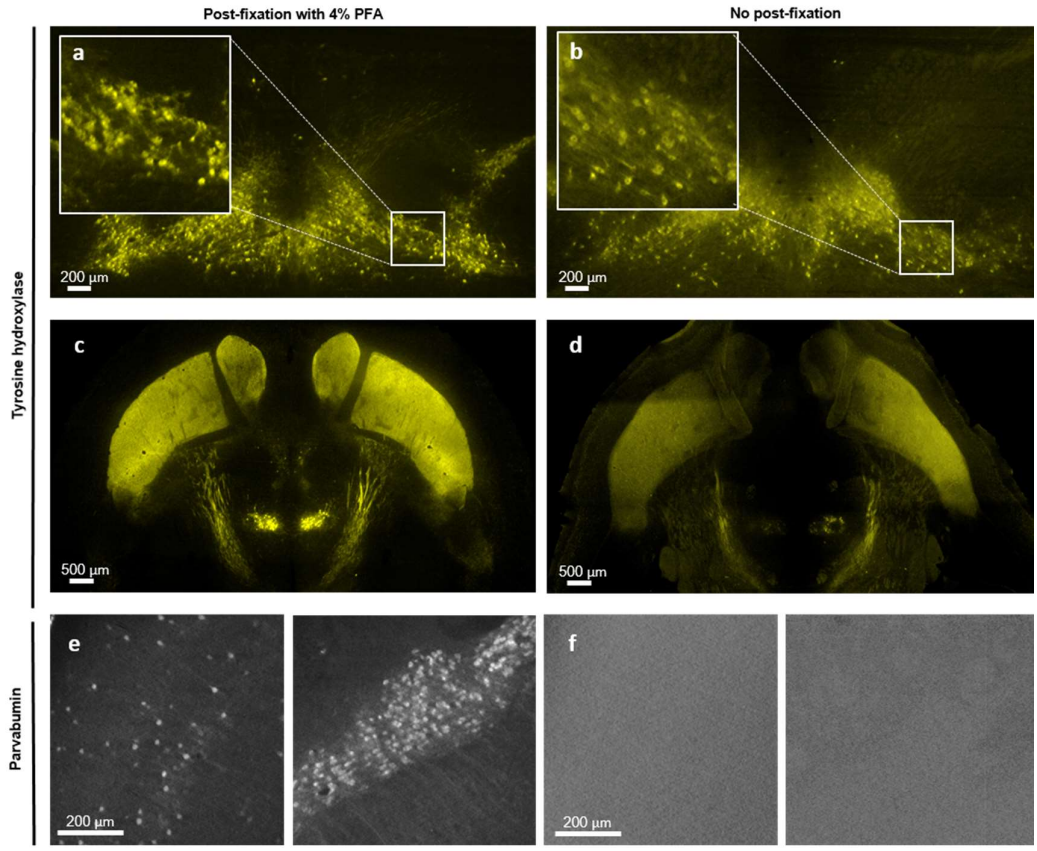
Item	Brand	Catalog number	Amount (for a whole mouse brain)
Alexa Fluor® 647 Fab Fragment Donkey Anti-Goat IgG	Jackson ImmunoResearch	705-607-003	1.3 µg
Alexa Fluor® 647 Fab Fragment Donkey Anti-Rabbit IgG	Jackson ImmunoResearch	711-607-003	molar ratio of 1^{o}ab : 2^{o}ab = 1:2
Alexa Fluor® 647 Fab Fragment Donkey Anti-Mouse IgG	Jackson ImmunoResearch	715-607-003	molar ratio of 1^{o}ab : 2^{o}ab = 1:2
Rhodamine Red X Fab Fragment Donkey Anti-Rabbit IgG	Jackson ImmunoResearch	711-297-003	molar ratio of 1^{o}ab : 2^{o}ab = 1:2
Rhodamine Red X Fab Fragment Donkey Anti-Mouse IgG	Jackson ImmunoResearch	715-297-003	molar ratio of 1^{o}ab : 2^{o}ab = 1:2
Lectin-Dylight 488	Vector Labs	DL-1178	40 µl



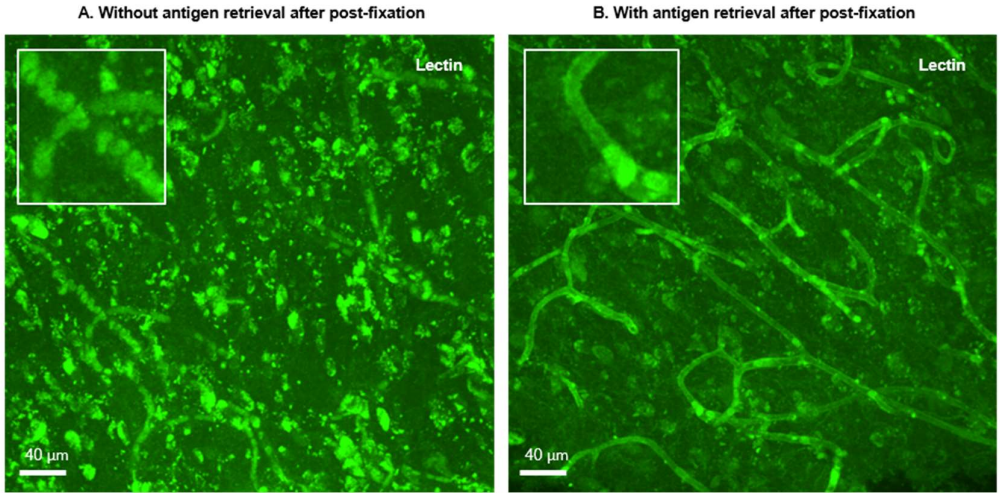
Supplementary Figure 1: Repeated dewaxing test. The test is to mimic the situation in which the sample is large and the optimal dewaxing time needs further test. (**a**) The procedure of test. A 2-mm thick mouse brain slice was fixed with 4% PFA for 24 hours and then embedded in paraffin wax. The FFPE brain slice was incubated at 65°C to melt paraffin, and then immersed in xylene for 1 hour ("The 1st dewaxing"). The dewaxed brain slice was rehydrated, SHIELD-processed and antigen-retrieved as described in Methods (see the MOCAT session in Methods). The sufficiently dewaxed region would turn translucent (b-1, surrounding area of the brain) while the region with residual wax remained opaque (b-1, central area of the brain. The insufficiently dewaxed brain slice was dehydrated and immersed in xylene for another hour ("The 2nd dewaxing"). The twice dewaxed sample was rehydrated, antigen-retrieved again and snapped a photo. The process was repeated, and at the end of each cycle, photo was snapped. (**b**) The snapshots of the brain slice after each dewaxing. Notice the translucent bordering area increases while the opaque central area decreases with the addition of dewaxing time.



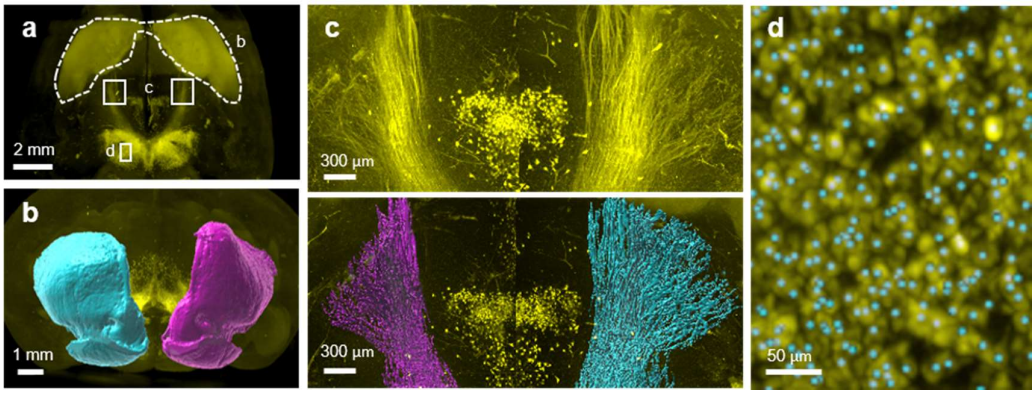
Supplementary Figure 2: Repeated dewaxing does not influence antigenicity. The brain slice going through 4 times of dewaxing (shown in Supplementary Fig. 1b-4) are labeled with lectin to examine antigenicity. (**a**) Projection image. Scale bar: 1 mm. (**b**) The 3D image and the optical sections of the magenta-lined box in **a**. (**c**) The 3D image and the optical sections of the cyan-lined box in **a**. Scale bar in **b** and **c**: 200 µm, 100 µm, 100 µm, 100 µm (left to right).



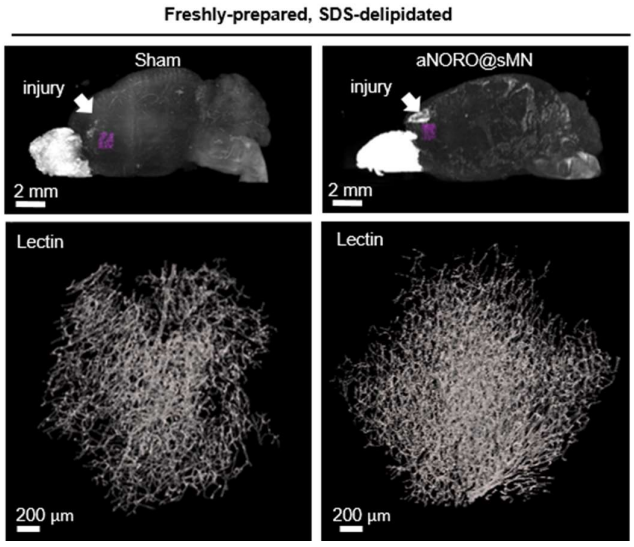
Supplementary Figure 3: Immuno-labeled whole mouse brain without post-fixation has faint or negative staining. (**a**)(**c**)(**e**) The mouse brain immuno-labeled with tyrosine hydroxylase (TH) and parvabumin (PV) with post-fixation. (**b**)(**d**)(**f**) The mouse brain immuno-labeled with tyrosine hydroxylase (TH) and parvabumin without post-fixation. Without post-fixation, the signal of TH is fainter (**b**) and dimmer (**d**), and the signal of PV is completely lost (**f**).



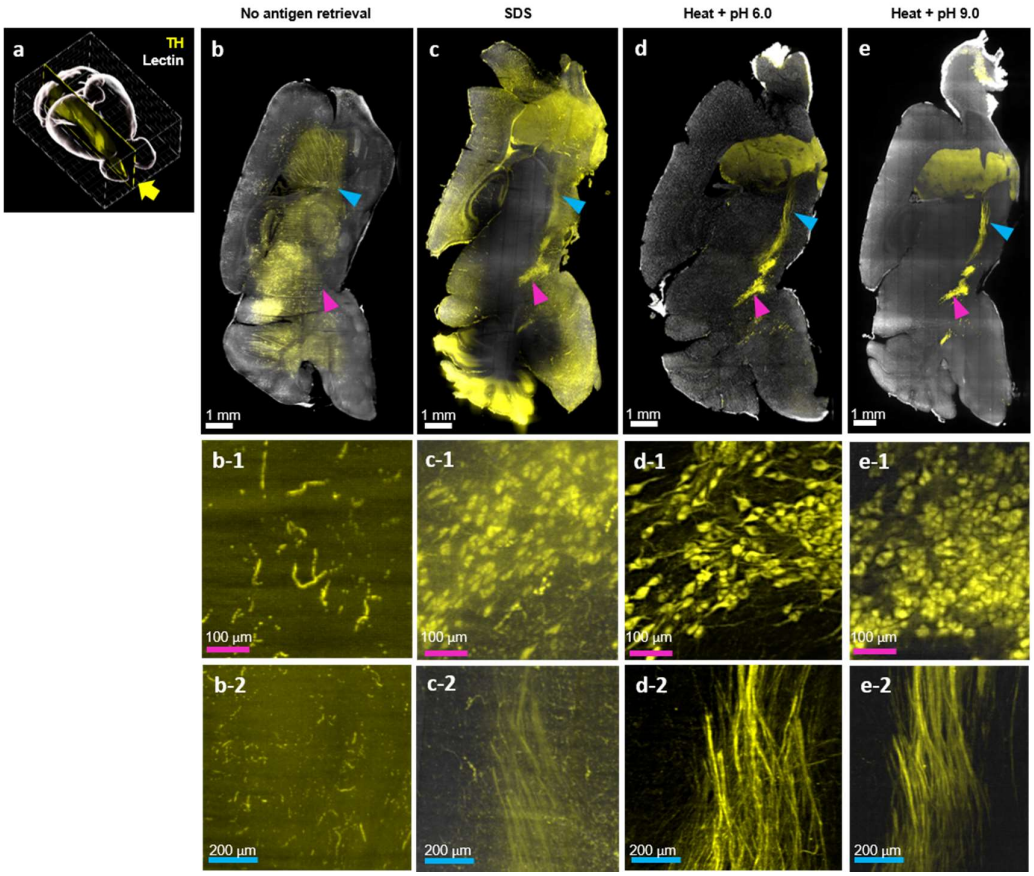
Supplementary Figure 4: Antigen retrieval allows re-immuno-labeling after post-fixation. The second round of staining (using lectin to label blood vessels) are performed on the human brain tissues that went through the 1st immuno-labeling and post-fixation. Only autofluoresence of blood cells could be seen in the sample without antigen retrieval before the lectin staining (**a**). In contrast, on the sample with antigen retrieval before the lectin staining, the endothelium of blood vessels is clearly labeled (**b**).



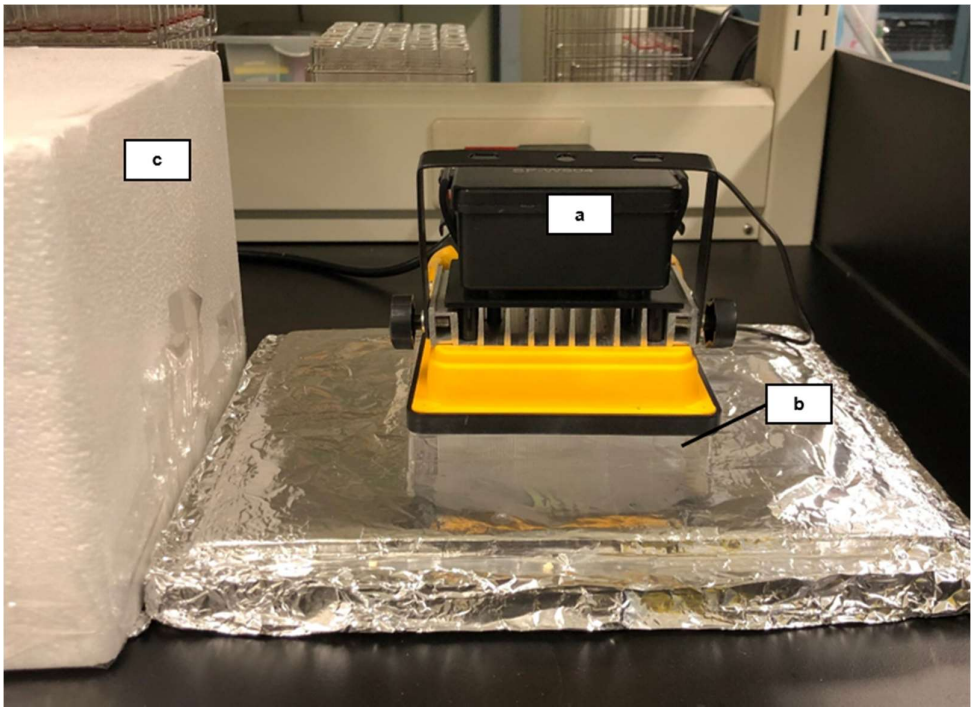
Supplementary Figure 5: Segmentation of dopaminergic structure in a freshly-prepared SDS-delipidated TBI brain. (**a**) Fluorescent image of TH immunolabeling. (**b**) Frontal view of segmented striatum (dot line circled in **a**). Magenta: the injury side; cyan: the contralateral side. (**c**) The horizontal views of the nigrostriatal fiber tract (marked in **a**); fluorescent and segmented images are shown (upper and bottom in order). Magenta: the injury side; cyan: the contralateral side. (**d**) Detection of dopaminergic cells in substantia nigra (SN). Magnification of the marked area in **g**, including the original TH fluorescent signal (yellow) and segmented cell spots (cyan dots), is shown.



Supplementary Figure 6: Reconstruction of blood vessels in freshly-prepared SDS-delipided TBI brains with and without treatments. The regions of interested (ROIs) selected for assessment of angiogenesis were indicated in the upper row (magenta boxes, right below the injury site). The 3D reconstructions of blood vessels were shown in the bottom row.



Supplementary Figure 7: Effect of antigen retrieval on FFPE mouse brains. Immunolabeling of TH (yellow) and lectin (white) are used to evaluate the effect of different antigen retrieval methods. (**a**) The position of Y-Z plane shown in **b, c, d, e** is indicated (arrow). (**b**) (**c**) (**d**) (**e**) The Y-Z plane of a whole mouse brain treated without antigen retrieval or with various antigen retrieval methods (see Methods for details). Substantia nigra (magenta arrow head) and nigrostriatal fiber tract (cyan arrow head) are indicated. In the mouse brain without antigen retrieval (shown in **b**), the pattern of TH could not be observed. The location where substantia nigra and nigrostriatal fiber tract are supposed to be are indicated. **b**-1, **c**-1, **d**-1 and **e**-1 show the magnification of substantia nigra indicated by magenta arrow heads in **b**, **c**, **d** and **e**. **b**-2, **c**-2, **d**-2 and **e**-2 show the magnification of nigrostriatal fiber tract indicated by cyan arrow heads. Scale: white, 1 mm; magenta, 100 um; cyan, 200 um.



Supplementary Figure 8: A representative photobleaching apparatus. (**a**) The 100W projection lamp with an LED array. (**b**) The multi-well plate containing RI-matching solution and the sample. (**c**) The cover for the apparatus while the lamp turned on.

Supplementary video 1: Spatial distribution of the 6 biological markers shown in Fig. 2c.

Supplementary video 2: Spatial relationship of the tumor and surrounding astrocytes in Fig. 3a-e. The segmented astrocytes are colored in cell-to-tumor distance-code.

Supplementary video 3: 3D visualization of the segmented blood vessels shown in Fig. 4f.