LIMPID: A Versatile Method for Visualization of Brain Vascular Networks

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Supplementary Information

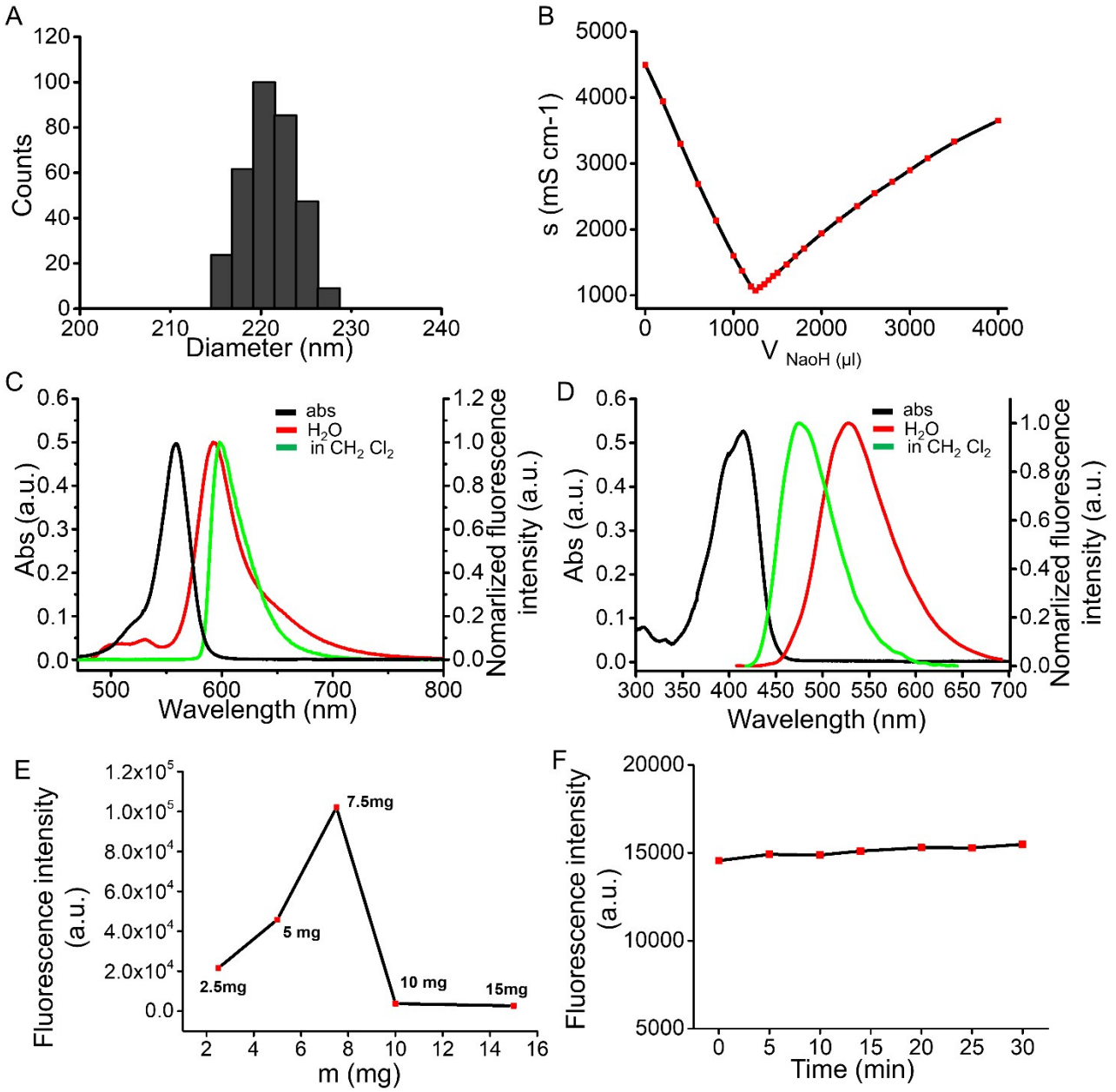

Fig. S1 Fluorescence spectroscopy measurement of fluorescent dyes and fluorescent nanoparticles. (A) Dynamic light scattering (DLS) measurements of SQ7-ANPs. (B) Conductometric titration detection of ANPs. (C) Absorption (black line) and fluorescence spectra (green line) of dye SQ7 in CH2Cl2 solution (1×10^{-5} M, Ex: 450 nm), and fluorescence spectra (red line) of SQ7-ANPs (Ex: 450 nm). (D) Absorption (black line) and fluorescence spectra (green line) of BDT in CH2Cl2 solution (1×10^{-5} M, Ex: 400 nm), and fluorescence spectra (red line) of

BDT-ANPs. (E) Fluorescence intensity of SQ7-ANPs containing different weight of SQ7 in SDS solution (Ex: 557 nm). (F) Fluorescence spectra of SQ7-ANPs in SDS solution (1×10^{-5} M, Ex: 557 nm) exposing to light for different period of time.

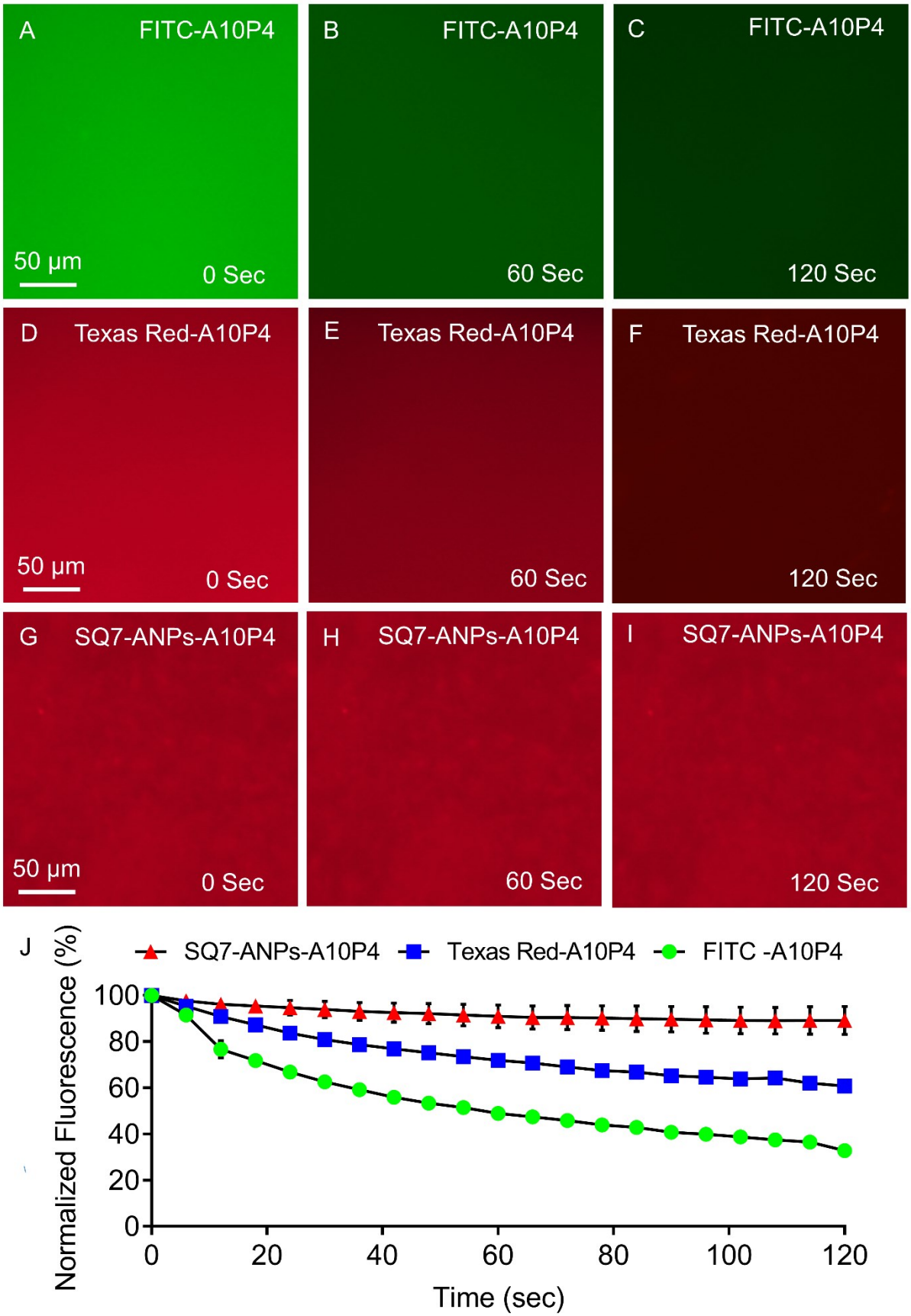

Fig. S2 Measurement of fluorescence loss in photobleaching of fluorescent hydrogels. (A-C) The representative figures of fluorescence intensity change of FITC-A10P4 hydrogel (BP: 470-490 nm, n=3) at different time points

during photobleaching. (D-F) The representative figures of fluorescence intensity change of Texas Red-A10P4 hydrogel (BP: 510-550 nm, n=3) at different time points during photobleaching. (G-I) The representative figures of fluorescence intensity change of SQ7-ANPs-A10P4 hydrogel (BP: 510-550 nm, n=6) at different time points during photobleaching. (J) Statistical diagram showing fluorescence loss of different fluorescent hydrogels at different time points during photobleaching ($n \geq 3$). The fluorescence intensity is normalized to pre-bleach fluorescent intensity.

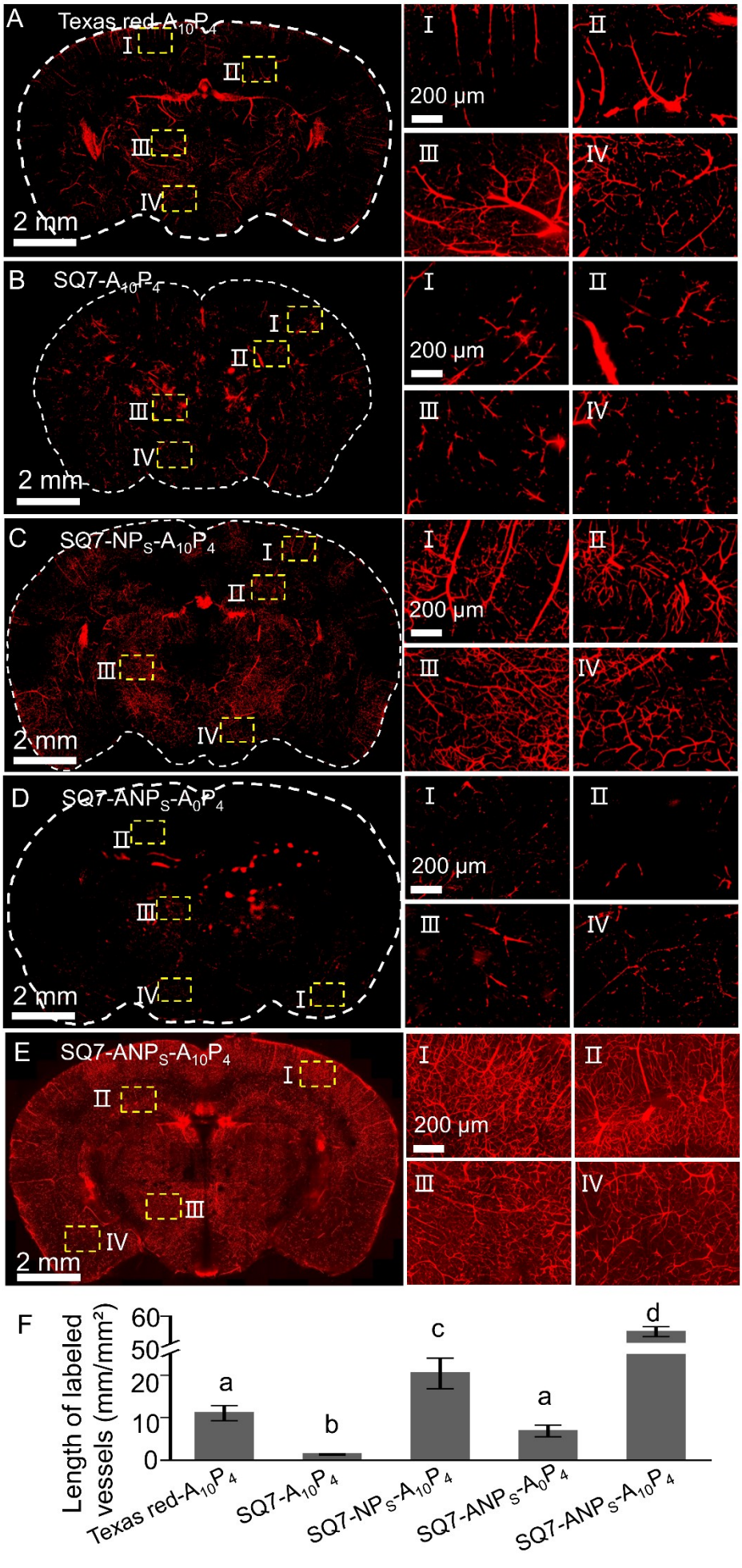
A
Texas red-A10P4
I
II
III
IV
2 mm
200 μm
B
SQ7-A10P4
C
SQ7-NPS-A10P4
D
SQ7-ANPS-A0P4
E
SQ7-ANPS-A10P4
F
Length of labeled vessels (mm/mm²)
60
50
20
10
0
a
b
c
a
d
Texas red-A10P4
SQ7-A10P4
SQ7-NPS-A10P4
SQ7-ANPS-A0P4
SQ7-ANPS-A10P4

Fig. S3 Effect of different fluorescent dyes and fluorescent nanoparticles on labeling vascular networks. (A) Images of labeled blood vessels in the brain perfused with Texas red-A10P4. Higher-magnification images of boxed regions in (A) are shown in (Ⅰ-Ⅳ) (n=4); (B) Blood vessel image in the brain perfused with SQ7-A10P4. Higher-magnification images of boxed regions in (B) are shown in (Ⅰ-Ⅳ) (n=6); (C) Blood vessel image in the brain perfused with SQ7-NPs-A10P4. Higher-magnification images of boxed regions in (C) are shown in (Ⅰ-Ⅳ) (n=6); (D) Blood vessel image in the brain perfused with SQ7-ANPs-A0P4. Higher-magnification images of boxed regions in (D) are shown in (Ⅰ-Ⅳ) (n=5). (E) Blood vessel image in the brain perfused with SQ7-ANPs-A10P4. Higher-magnification images of boxed regions in (E) are shown in (Ⅰ-Ⅳ) (n=6). (F) Statistical analysis of total length of labeled vessels between different labeling dyes or nanoparticles. a-d in (F) indicate significant differences between the two groups [one-way ANOVA $F(4,22)=101.4$, $p<0.0001$].

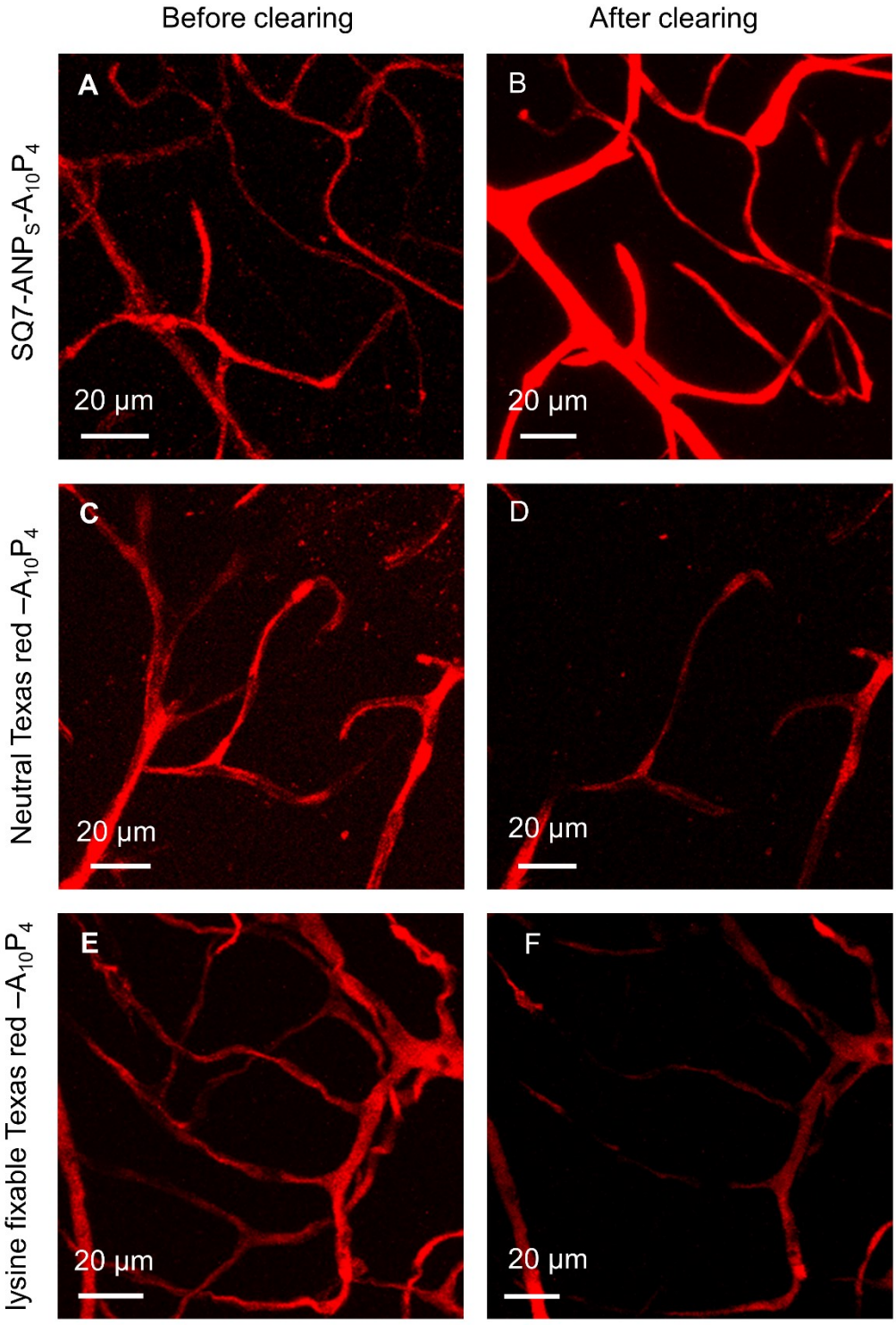

Figure S4. Comparison of the effect of blood vessel labeling using different fluorescent dyes before and after clearing. (A-B) Representative images of SQ7-ANPS-A10P4 labeled blood vessels before and after clearing. SQ7-ANPS-A10P4 can stably labelling blood vessels. (C-D) Representative images of neutral Texas red –A10P4 labeled blood vessels before and after clearing (E-F) Representative images of labeled blood vessels with lysine fixable

Texas red –A10P4 before and after clearing. Scale bar = 20 μm.

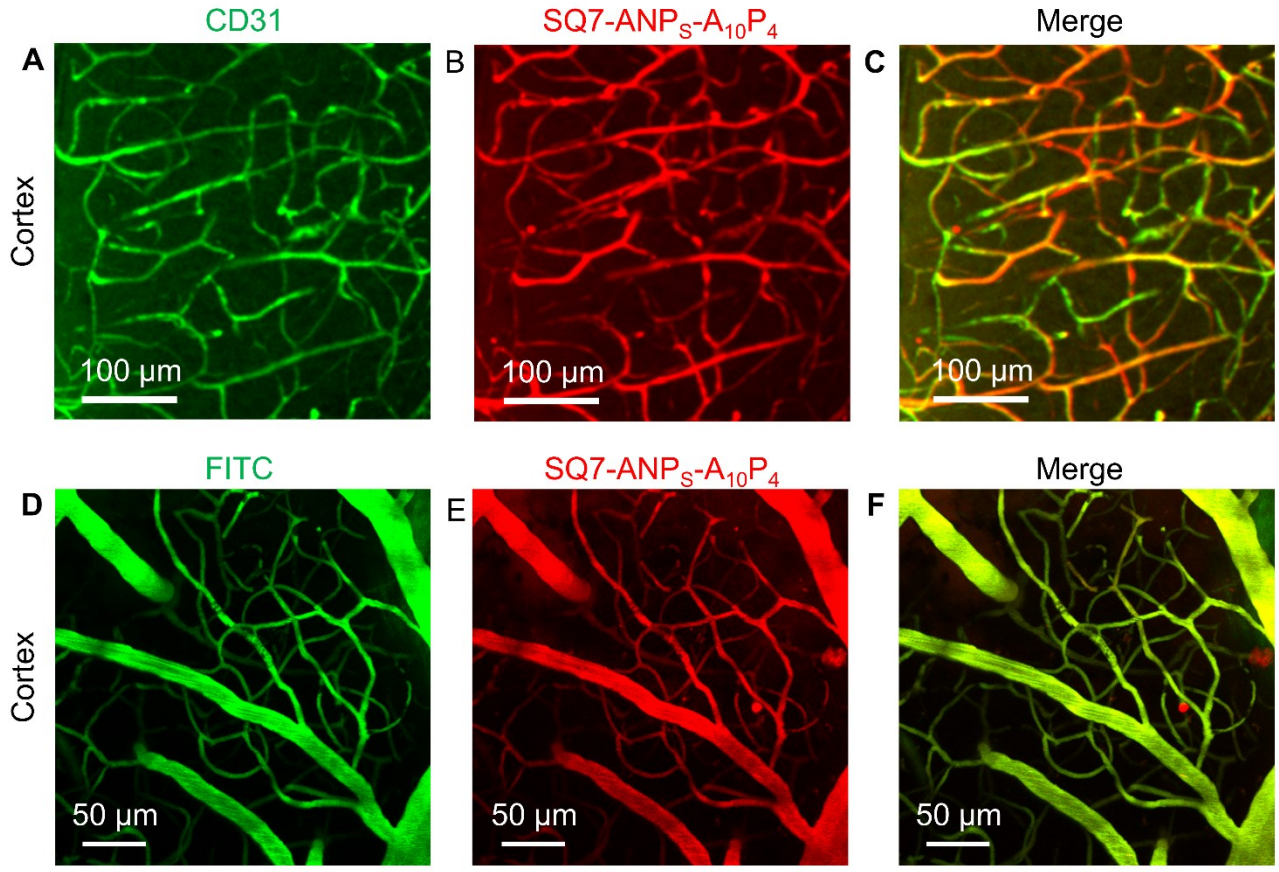

Figure S5. Visualization of cerebral vasculature using LIMPID. (A-B) Representative images showing the overlap of anti-CD31 antibody stained blood vessels (green) and SQ7-ANPS-A10P4 labelled blood vessels (red) in a mouse cortex. (C) Merged image showing the overlaps between anti CD31 antibody stained vessels and SQ7-ANPS-A10P4 labelled vessels. Scale bar = 100 μm. (D) FITC dextran-labelled blood vessels imaged in a live mouse. (E) SQ7-ANPS-A10P4 labelled blood vessels in the same fields as showed in (D). (F) Merged image showing the overlaps between FITC dextran-labelled blood vessels and SQ7-ANPs-A10P4 labelled vessels. Scale bar = 50 μm.

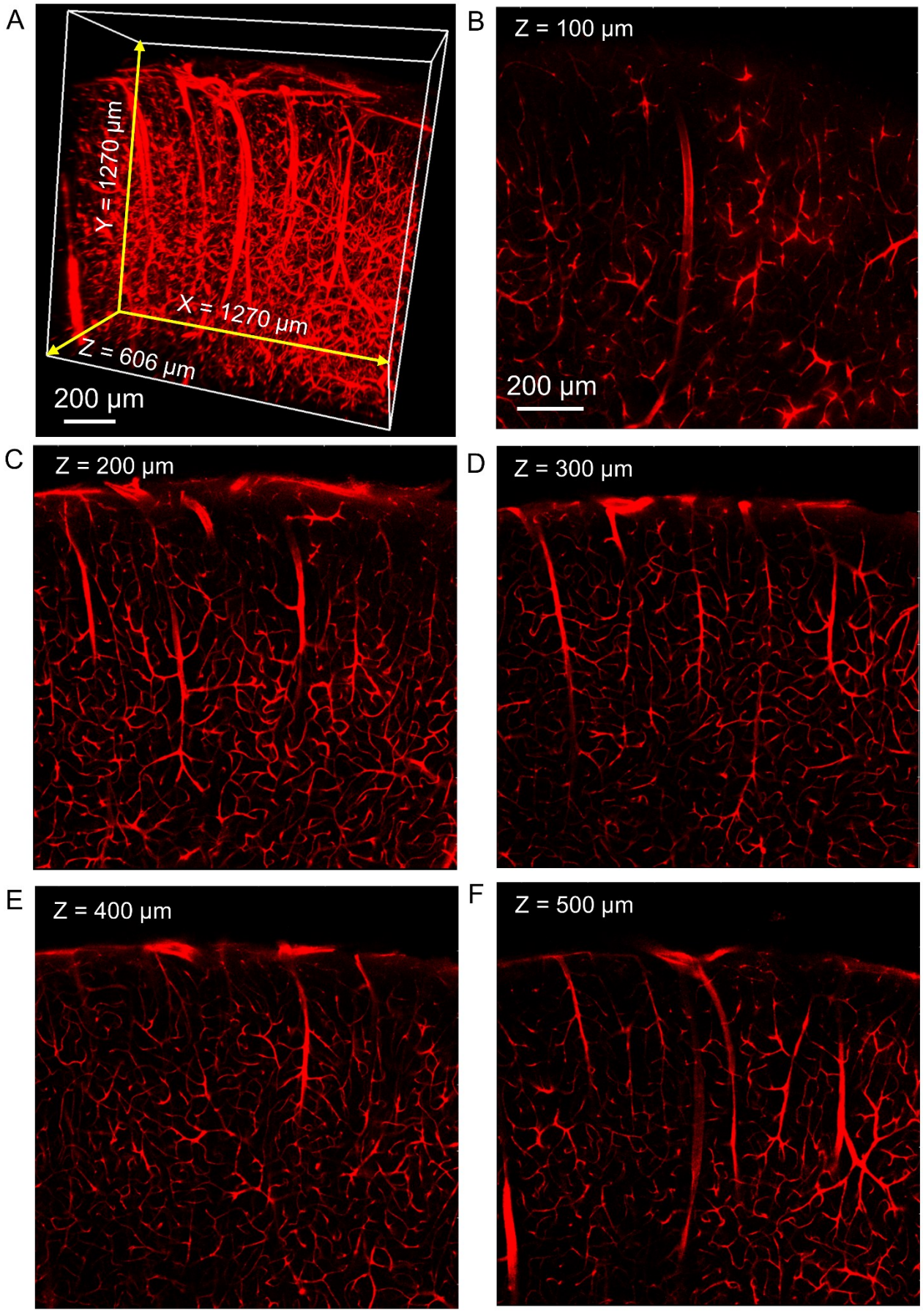

Figure S6. Images of vasculature in a mouse brain slice after LIMPID. (A) Representive 3D raw data view of the

vasculature in a cortical slice. Images were obtained using confocal microscopy and the image volume was 1270 μm×1270 μm×606 μm with a voxel size of 1.242 μm×1.242μm×1.25 μm. (B-F). Representive images at different depths of brain slice (100 μm, 200 μm, 300 μm, 400 μm, and 500 μm relative to the surface of the slice) showing that fluorescent nanoparticles penetrated into both surfaces and the deep tissues. Scale bar = 200 μm.