Supplementary Material (ESI) for Chemical Communications

Supporting Information

Human Serum Albumin Coated Iron Oxide Nanoparticles for Efficient Cell Labeling

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1. EXPERIMENTAL

1.1 Preparation of HSA-IONPs

Oleate coated iron oxide nanoparticles (15 nm) were obtained from Ocean NanoTech (Springdale, AR). For surface modification, about 5 mg of oleate coated nanoparticles were dispersed in 5 ml of chloroform, and into the solution, 20 mg dopamine in 2 ml DMSO was added, forming a homogeneous solution. The mixture was heated at 70 ºC for 1 hour, and was

cooled down to r.t. Hexane was added as poor solvent to precipitate the nanoparticles, and the nanoparticles were collected by centrifugation at 15,000 g for 15 minutes. The raw products were blown dry with nitrogen, and were redispersed in DMSO with the aid of sonication. On the other hand, 50 mg HSA was dissolved in borate buffer (50 nM, pH 8.5). With sonication, the nanoparticles in DMSO were added drop-wise to the HSA solution. The nanoparticles were centrifuged at 30,000 g for 20 minutes (3x) to remove the free HSA, and were redipersed in PBS buffer. The particles were dialyzed (MWCO = 10,000) against PBS to remove the residual DMSO, afterwards, small aggregates was removed by passing the particles through syringe filter (0.22 μm).

1.2 Small animal MR imaging

All animal experiments were approved by the Stanford University Administrative Panel on Laboratory Animal Care. The T2-weighted MRI scans were performed on a 7.0-T small animal system (GE Healthcare) with the following parameters: matrix 128×128; FOV=3.0×3.0; TR/TE=4000 ms/40 ms; NEX=3.0; slice thickness=1.00; spacing=0.0. Signal intensities were measured in defined ROIs by imaging analysis software Image J (National Institute of Health).

The Xenograft U87MG model preparation Human glioblastoma cancer cell line U87MG was purchased from American Type Culture Collection (ATCC) and was cultured with ATCC-formulated Eagle's Minimum Essential Medium in cell culture incubator. Athymic Nude mice were purchased from Harland Laboratories. Approximately 5×10^6 U87MG cells were subcutaneously inoculated on the front flank of the mice, and the in vivo imaging was carried out 4 weeks after the inoculation when the tumor size reached 350 mm^3.

The focal cerebral ischemia model preparation The stroke model was induced on adult male Sprague Dawley rats (Charles River, Wilmington, DE, U.S.A.). Briefly, a small skin incision

was made midway between the left eye and ear. The temporalis muscle was bisected and a 2 mm burr hole was drilled at the junction of the zygomatic arch and squamous bone. The distal middle cerebral artery (dMCA) was exposed, cauterized, and cut permanently just above the rhinal fissure. The bilateral common carotid arteries CCAs were isolated by means of a ventral midline incision and occluded with small aneurysm clips for 30 min for stroke onset, and then aneurysm clips were released, the wound was closed, and the animals were allowed to recover.

1.3 Histology studies

Prussian blue staining: The sample tumors/brains were acquired after imaging and were kept in o.c.t. compound under -80 ºC. Later on, the tissue samples were cut into 40 μm thick slices. Before staining, the tissue slides were warmed up for 20 min at room temperature and were fixed with ice-cold acetone for 5 min. The slides were then dried for 20 min and were immersed in staining solution (20% hydrochloric acid and 10% potassium ferrocyanide solution mixture, 1:1 volume ratio) for 40 min., following counterstaining with eosin for 5 min. Afterwards, the slides were dehydrated consecutively with 90%, 95% and 100% EtOH (3 min. each), cleaned with xylene, and were mounted with Permount medium.

Prussian blue and CD31/F4/80 double staining: Frozen U87MG tumor tissues were sectioned into 40 μm thick slices and were stored at -80 ºC. Before examination, the specimens were taken out of the freezer, warmed for 20 min at room temperature and were fixed with ice-cold acetone for 5 min. After fixation, slides were incubated with 0.3% H_2O_2 solution in PBS for 10 min to block endogenous peroxidase activity, and were rinsed 3 times with PBS (2 min each). Primary rat anti-mouse CD31 antibody diluent (1:50) was subsequently applied to the tissue sections, and the incubation was allowed for 1 h in a humid chamber. After rinsing with PBS (3×2 min), a biotinylated anti-rat IgG secondary antibody solution (1:50) was applied, and the incubation was

proceeded for 30 min. The slides were rinsed again with PBS and were incubated with streptavidin-HRP solution for 30 min. After another washing cycle, the slides were developed with DAB substrate solution until the desired color intensity was reached. The resulting slides were subjected to Prussian blue staining with the procedure described above. Double staining of Prussian blue and F4/80 was conducted with the same protocol except changing the primary antibody (for F4/80 staining, primary rat anti-mouse F4/80 was used).

2. SUPPORTING FIGURES

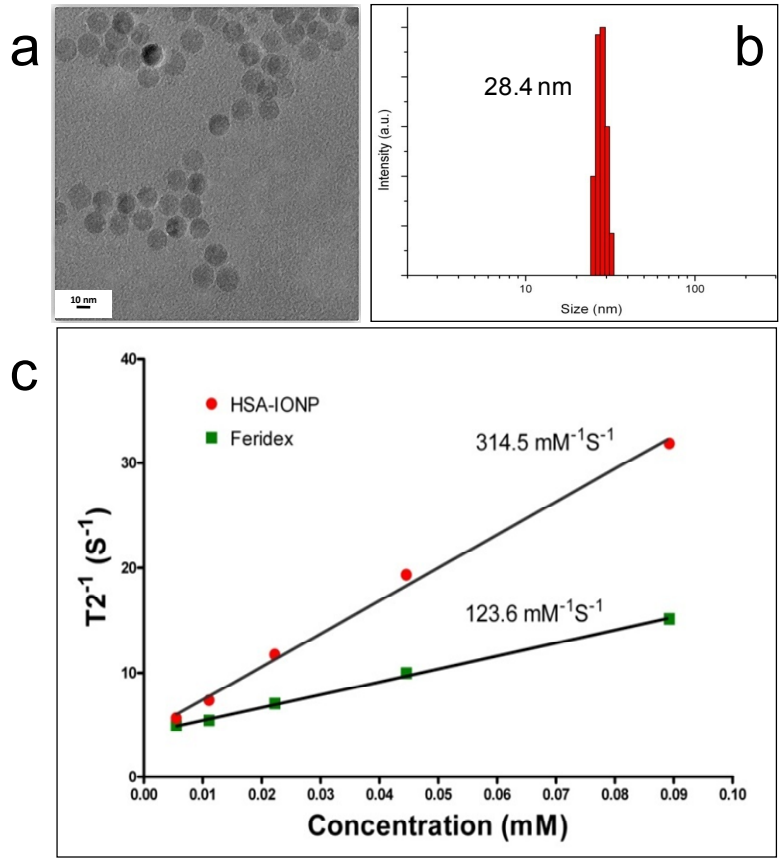


Fig. S1 Physical characterizations of the HSA-IONPs. a) TEM analysis of HSA-IONPs. b) Dynamic lighter scattering result of the HSA-IONPs. c) r2 relaxivities of HSA-IONPs and Feridex.

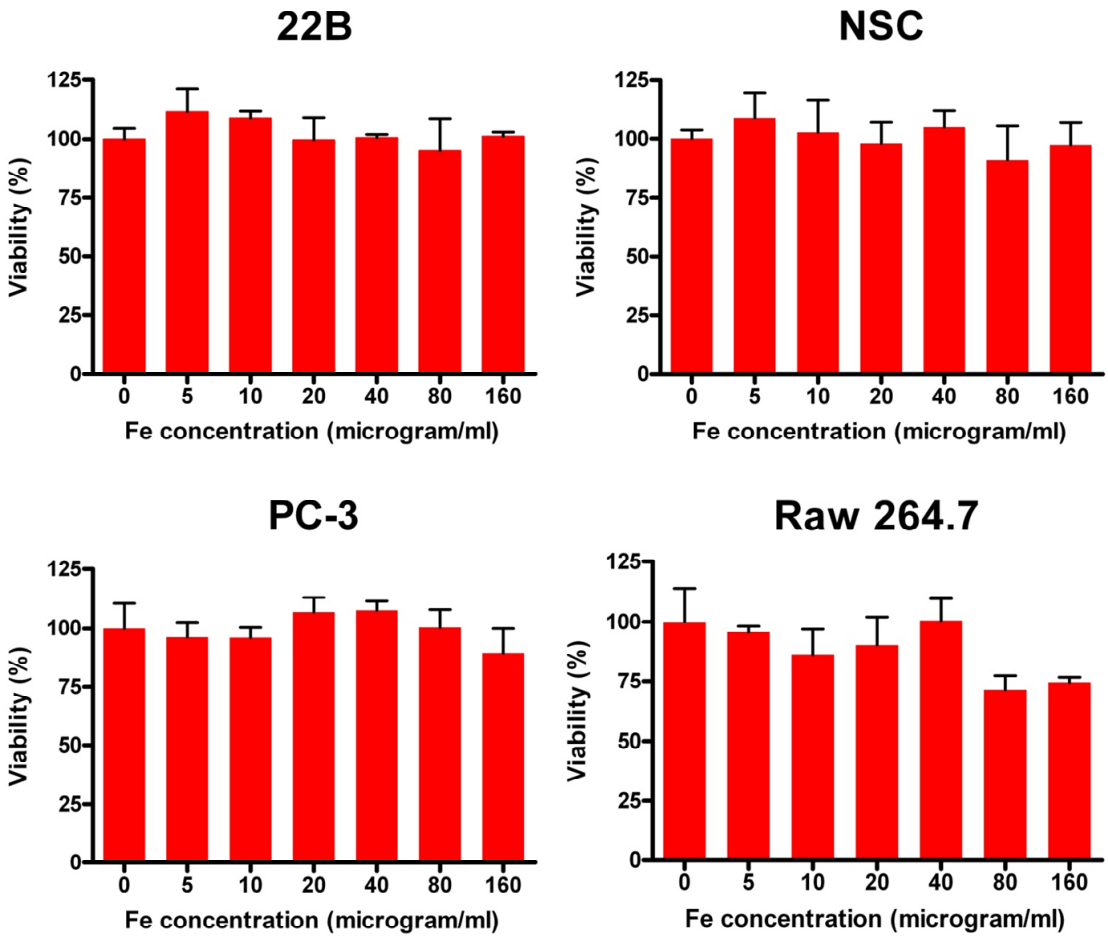


Fig. S2 Cytotoxcity evolution of HSA-IONPs with raw 264.7 (mouse leukaemic monocyte macrophage cell line), neural stem cells (NSCs), 22B head and neck squamous cell carcinoma cells (22B) and PC-3 prostate cancer cells (PC-3).

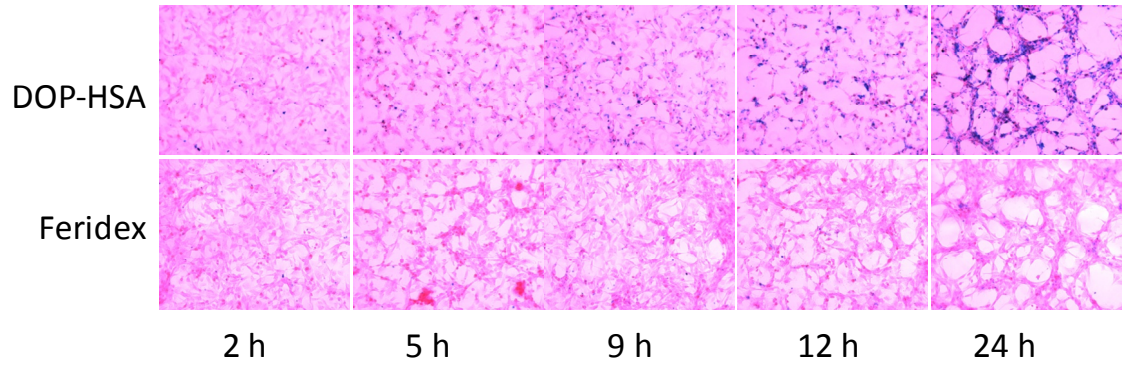


Fig. S3 22B tumor cell uptake of the HSA-IONPs.

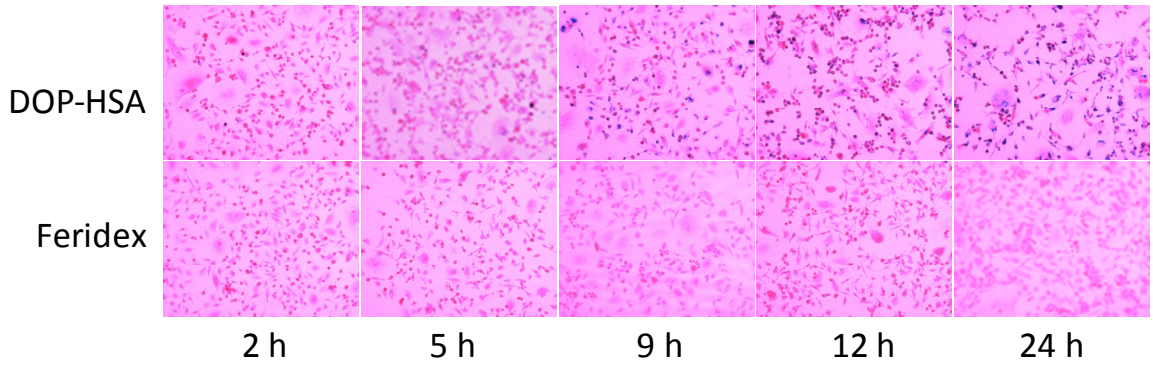


Fig. S4 PC-3 cell uptake of the HSA-IONPs.

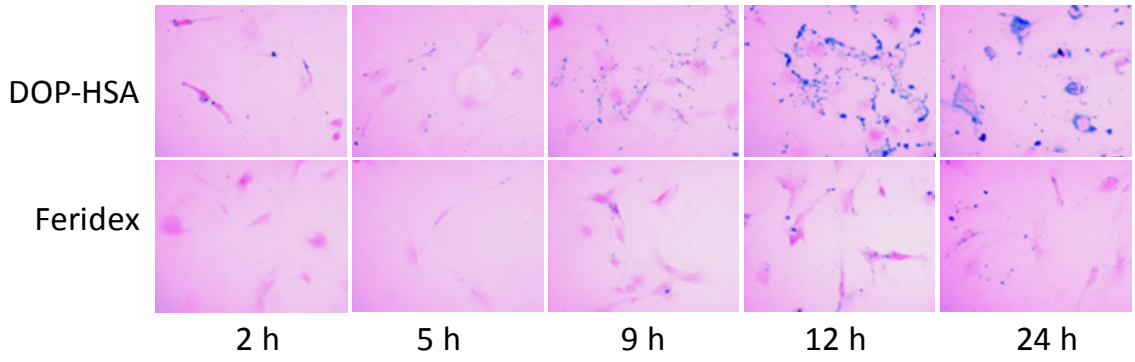


Fig. S5 Mesenchymal stem cell (MSC) uptake of the HSA-IONPs.

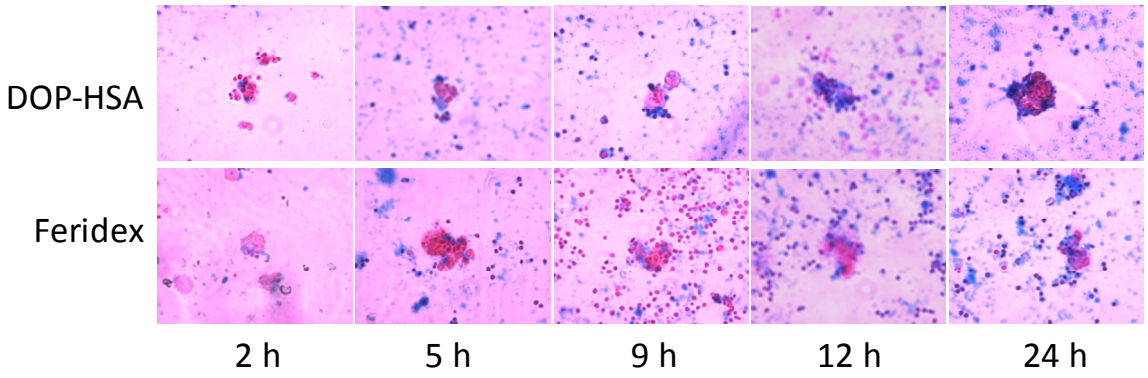


Fig. S6 Embryonic stem cell (ESC) uptake of the HSA-IONPs.

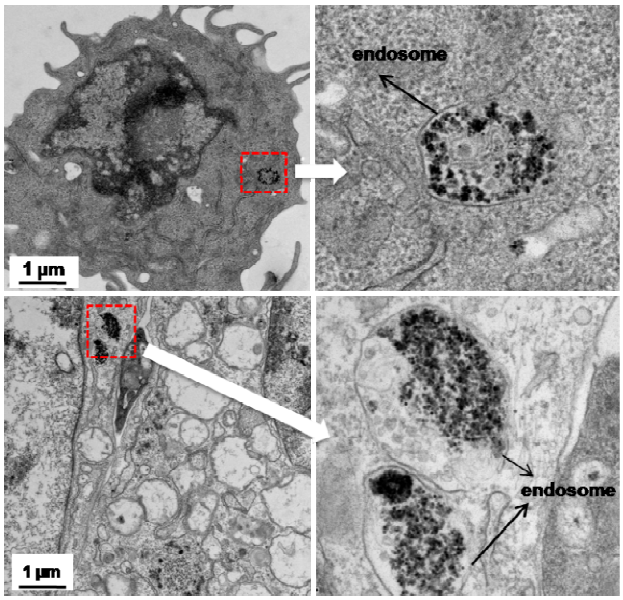
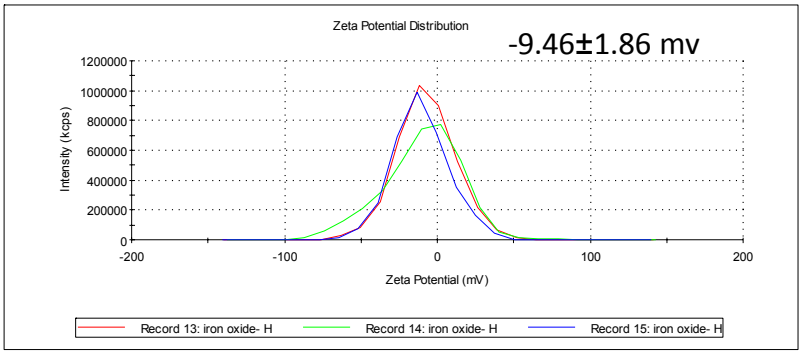


Fig. S7 Top row: TEM of the representative HSA-IONP loaded macrophages. Bottom row: TEM of the representative HSA-IONPs loaded NSCs. In both cases, populations of particles were found within endosomes/lysosomes, suggesting an endocytosis/phagocytosis mediated particle uptake.

HSA-IONPs



Feridex

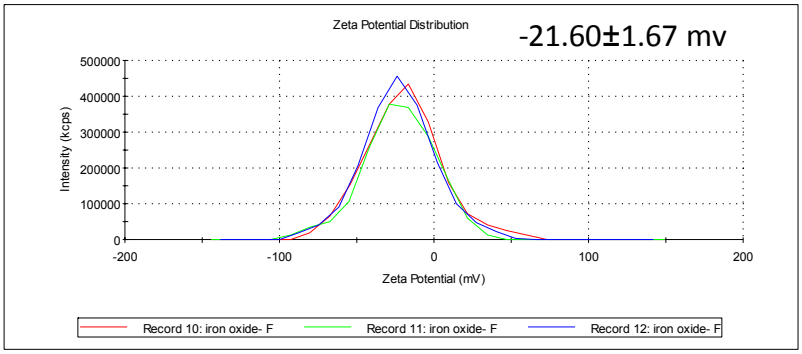


Fig. S8 Zeta potentials of HSA-IONPs and Feridex in PBS.

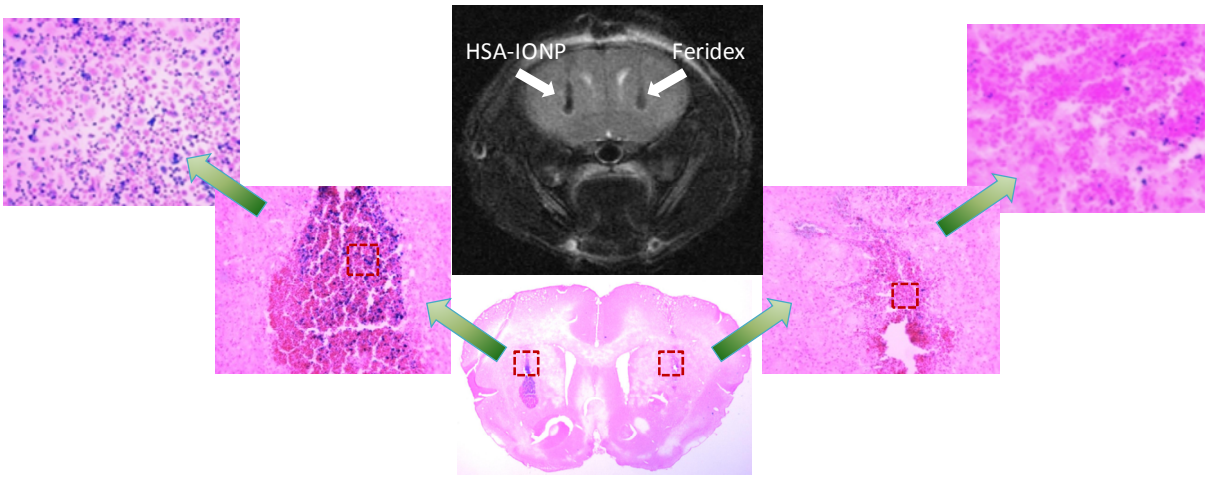


Fig. S9 Direct implantation of HSA-IONP/Feridex labeled NCSs into mouse left and right strata. The cell implanted regions were visualized as hypointensities on T2 weighted images, with a signal drop percentage being 44.7% (HSA-IONP labeled) vs. 24.2% (Feridex labeled) compared to the normal tissue. Positive Prussian blue staining, which reports iron particle whereabouts, was spatially well correlated with the hypointensities, validating that the signal loss was indeed caused by iron-laden cells.

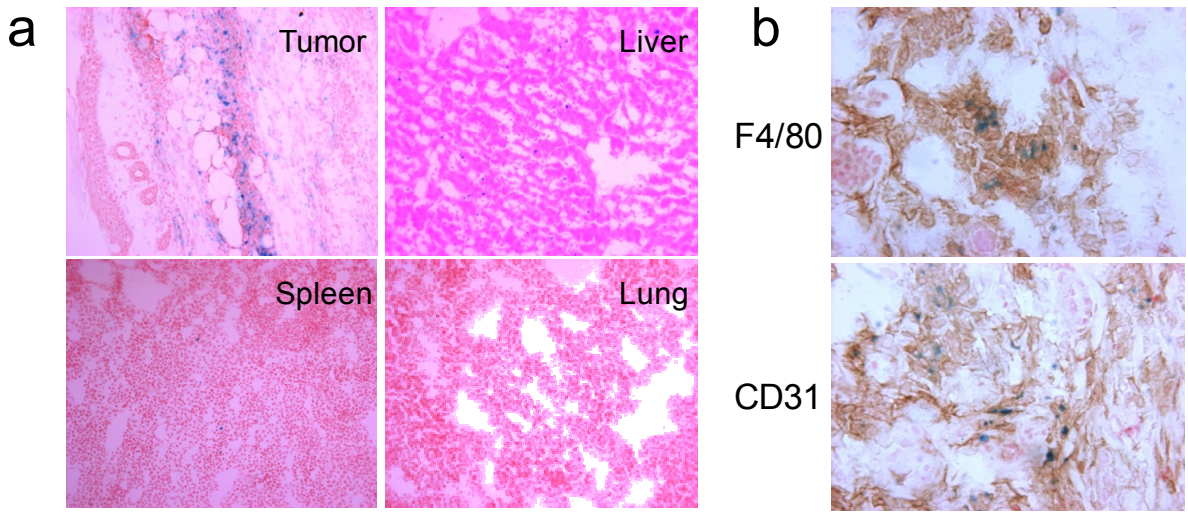


Fig. S10 a) Prussian blue staining of tumor and major organ sections after 7d imaging. A lot positive staining spots were found in tumor; on the contrary, few or none positive staining was found in liver, spleen and lung. b) F4/80/Prussian blue and CD31/Prussian blue double staining on the tumor section. Good co-localization was found between particles and F4/80 positive macrophages, suggesting that the particles stayed within the macrophages at day 7. On the other hand, positive Prussian blue staining was found in the leading edge of CD31-positive vessel lumen, indicating that the macrophages might've actively involved in tumor neovascularization.