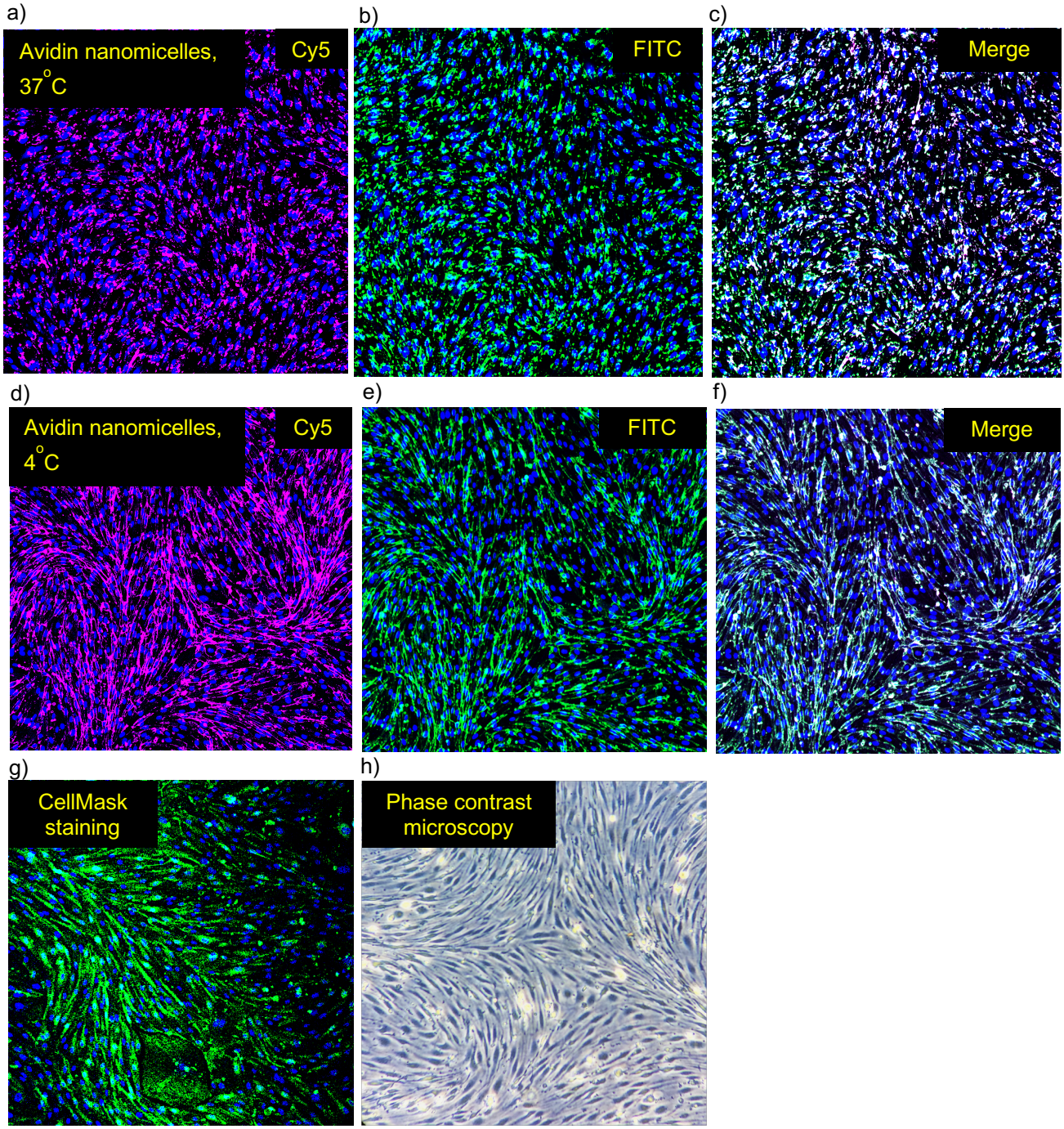

Figure S1. Energy-dependent internalization of avidin-NM into biotin-labelled brain endothelial cells. The cell surface of primary rat brain endothelial cells was biotin labelled through biotin-NHS binding onto primary amines of cell surface proteins. Endothelial cells were treated with avidin-NM (4 hr) at 37°C (a-c) or 4°C (d-f). Nanomicelle localization was visualized by confocal laser scanning microscopy. Colocalization of the nanomicelle (Cy5) and avidin (FITC) fluorescence signals was employed to examine retention of the protein-nanoparticle complex during internalization. Cell bodies were imaged through fluorescent CellMask staining (g) or phase contrast microscopy (h).

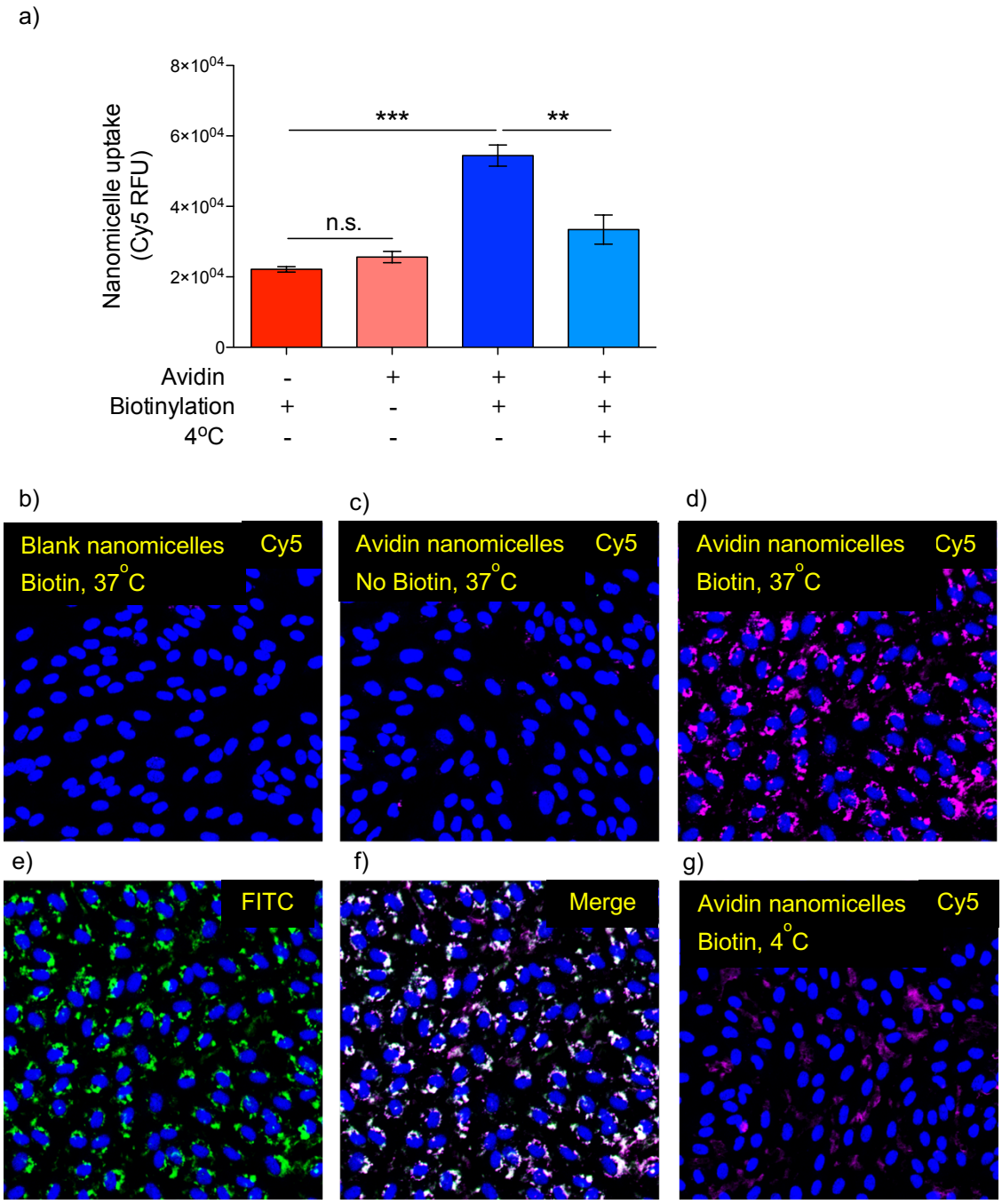

Figure S2. Avidin-NM entry into biotin-labelled peripheral endothelial cells. The cell surface of HUVEC cells was biotinylated with a biotin-NHS reagent and the cells were treated with avidin-nanomicelles or blank-nanomicelles (120 min) at 37ºC or 4ºC. Nanomicelle uptake was assessed through Cy5 fluorescence quantification (a). Nanomicelle cellular internalization was corroborated through confocal laser scanning microscopy detection of nanomicelle (Cy5) or avidin (FITC) fluorescence signal (b-g). Results are displayed as mean ± SEM of independent triplicate measurements. **, *** denote $p < 0.01$, 0.001 *vs.* respective column, as determined by a one-way ANOVA with Tukey's *post-hoc* test.

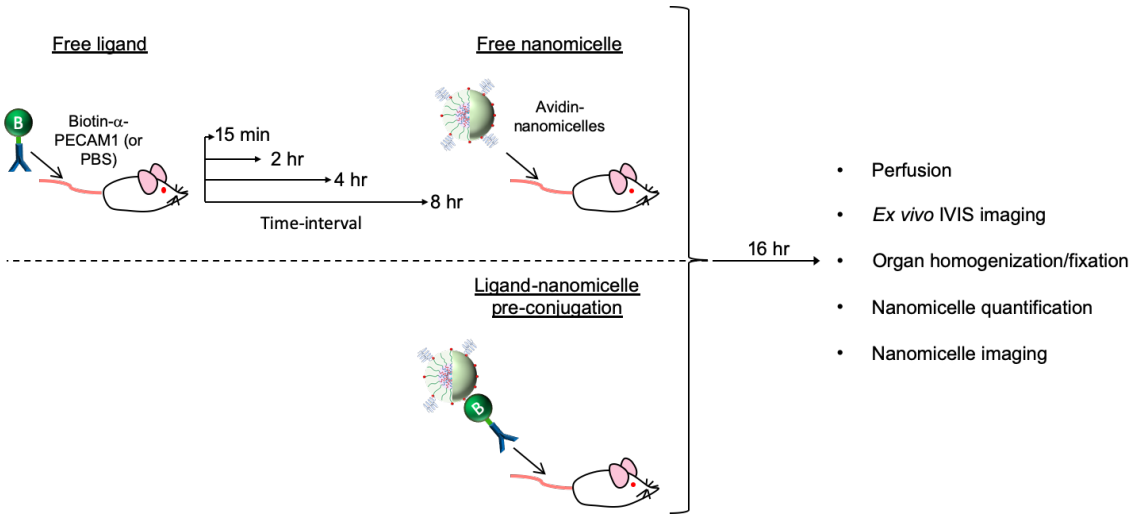

Figure S3. Experimental protocol to examine *in vivo* generation of brain targeting through selective labelling of the brain microvasculature. Balb/c mice were intravenously (tail vein) injected with free (*i.e.* unconjugated) biotinylated anti-PECAM1 (biotin-α-PECAM1) antibody (25 μg) (or PBS) followed by intravenous injection of avidin-functionalized nanomicelles (avidin-NM, 200 μg) at increasing time-intervals (top panel). Alternatively, avidin-NM were pre-conjugated with biotin-α-PECAM1 before administration into mice (i.v.; equal antibody and nanomicelle dose as above) (bottom panel). Mice were trans-hepatically and trans-cardially perfused (PBS) 16 hr following nanomicelle injection and nanomicelle organ accumulation assessed through Cy5 fluorescence quantification in whole organs (through IVIS) and tissue homogenates, and nanomicelle localization visualized through confocal laser scanning microscopy of immunostained fixed (PFa) tissue.

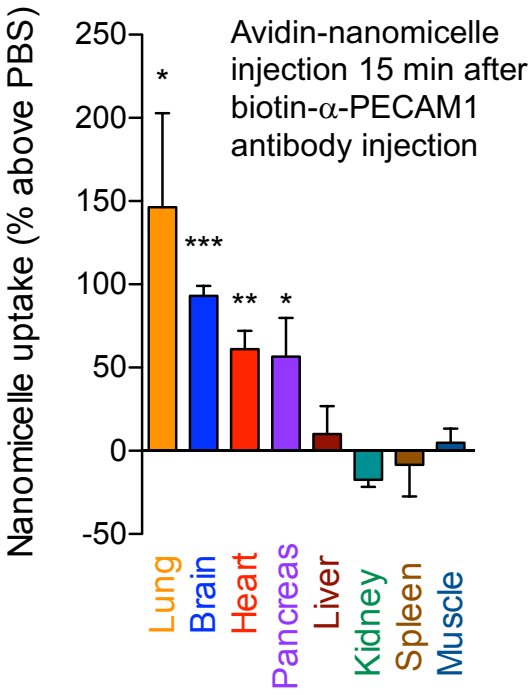

Figure S4. Biodistribution of avidin-NM injected 15 min after intravenous injection of biotinylated anti-PECAM1 (biotin-α-PECAM1) antibody. Balb/c mice were intravenously injected (tail-vein) with biotin-α-PECAM1 (25 μg) (or PBS) followed by avidin-NM intravenous injection (200 μg) 15 min after antibody injection. Mice were trans-hepatically/cardially perfused 16 hr following nanomicelle injection and nanomicelle accumulation assessed through nanomicelle fluorescence (Cy5) quantification in organ homogenates. Results are displayed as mean ± SEM of 5 mice. *, **, *** denote $p < 0.05$, 0.01, 0.001 *vs.* respective PBS value, as determined by a student's t-test.

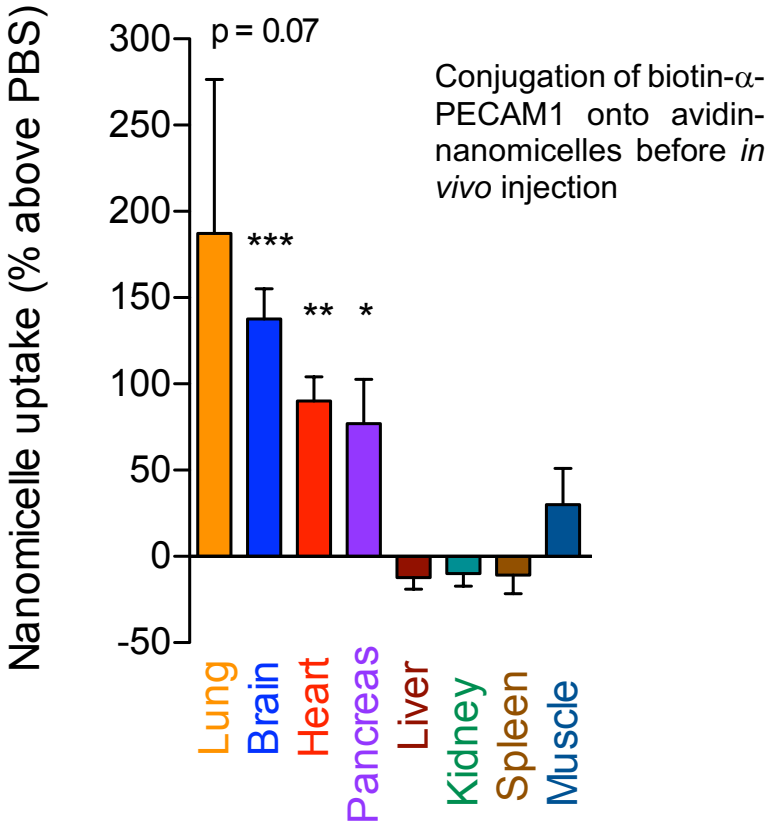

Figure S5. Biodistribution of avidin-NM pre-conjugated with biotin-α-PECAM1 before intravenous injection. Balb/c mice were intravenously injected (tail-vein) with avidin-NM pre-conjugated with biotin-α-PECAM1 (200 μg nanomicelles) (or PBS) followed by trans-hepatical/cardial perfusion 16 hr following nanomicelle injection. Nanomicelle accumulation was assessed through nanomicelle fluorescence (Cy5) quantification in organ homogenates. Results are displayed as mean ± SEM of 3 or 4 mice. *, **, *** denote $p < 0.05, 0.01, 0.001$ *vs*. respective PBS value, as determined by a student's t-test.

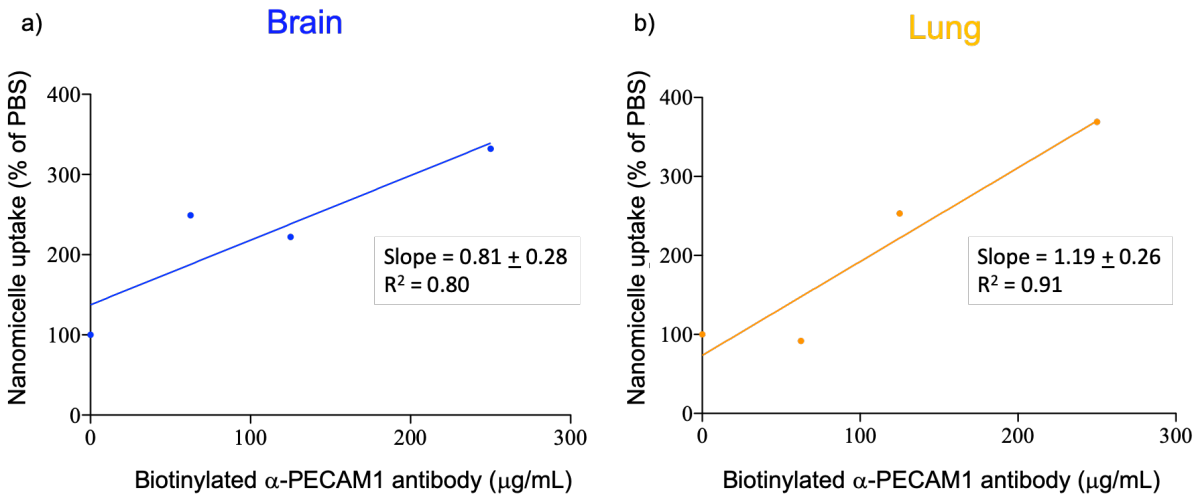

Figure S6. Dose-dependent organ targeting of avidin-nanomicelles by biotinylated anti-PECAM1 antibody ligand (biotin-α-PECAM1). Balb/c mice were intravenously injected with increasing concentrations of biotin-α-PECAM1 15 mins before intravenous injection of avidin-nanomicelles (1 mg/mL, 200 μL). Thirty minutes following nanomicelle injection, mice were trans-hepatically/cardially perfused and the Cy5 fluorescence in the brain and lung homogenates quantified to assess nanoparticle accumulation. (n = 1).

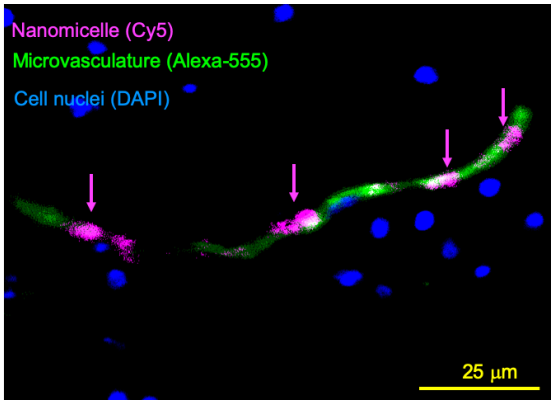

Figure S7. Avidin-NM localization in brain microvasculature labelled with biotin-α-PECAM1 antibody ligands. Balb/c mice were intravenously (i.v.) injected with biotin-α-PECAM1 (25 μg) (or PBS) followed by i.v. avidin-NM injection after indicated time intervals. Nanomicelle localization (magenta signal) in brain tissue of perfused animals was visualized through immunohistochemichal CLSM imaging. The microvasculature was visualized by staining with an independent non-biotinylated α-PECAM1 antibody (green signal). Cell nuclei were visualized through DAPI staining (blue signal).

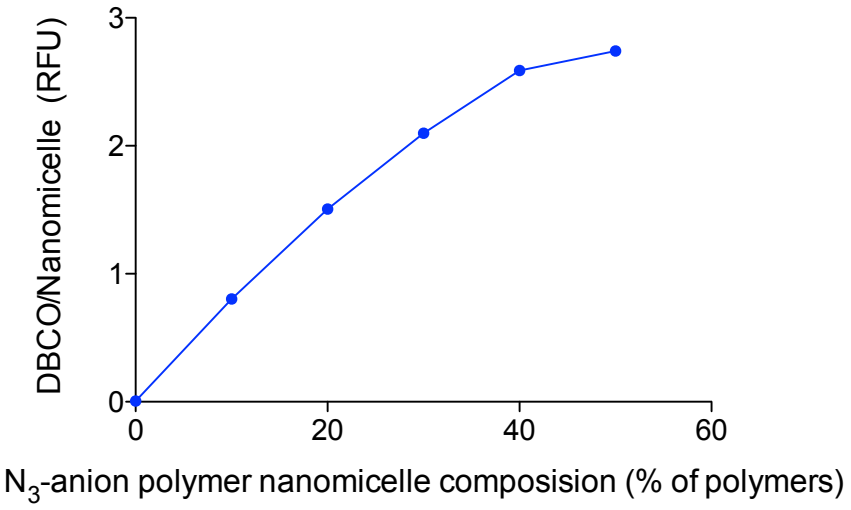

Figure S8. DBCO binding by polymeric nanomicelles as a function of N_3 decoration. Polymeric nanomicelles with increasing degree of N_3-group decoration on their surface were created by varying the ratio of N_3-capped anionic polymers to uncapped (*i.e.* MeO-terminated) anionic polymers during nanomicelle assembly. Nanomicelles were then reacted with fluorescently labelled DBCO molecules and DBCO incorporation calculated through fluorescence quantification.

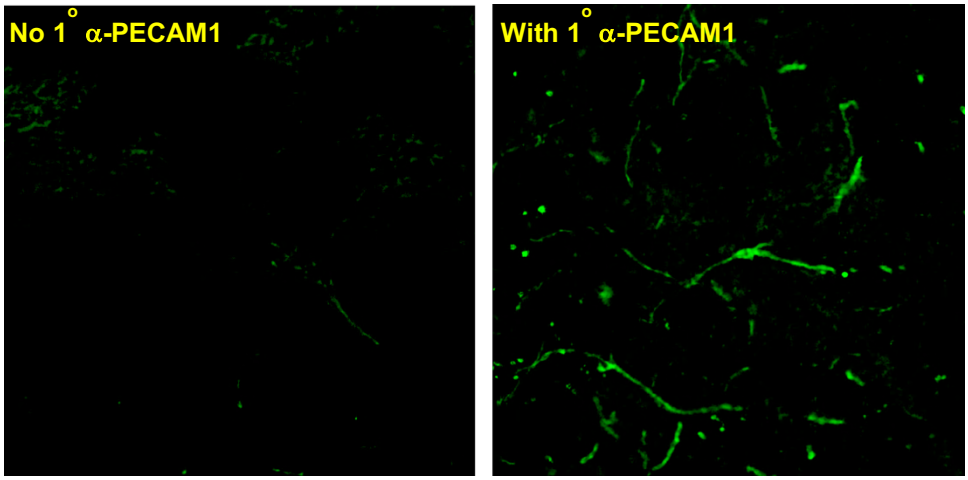

Figure S9. Specific vascular staining by non-biotinylated 1° α-PECAM1 antibody. To ensure vascular visualization was due to the primary α–PECAM1 antibody employed during immunostaining and not cross reactivity with the biotinylated α-PECAM1 antibody used for nanomicelle targeting, immunostaining was carried out in the absence (a) or presence (b) of primary α–PECAM1 antibody.