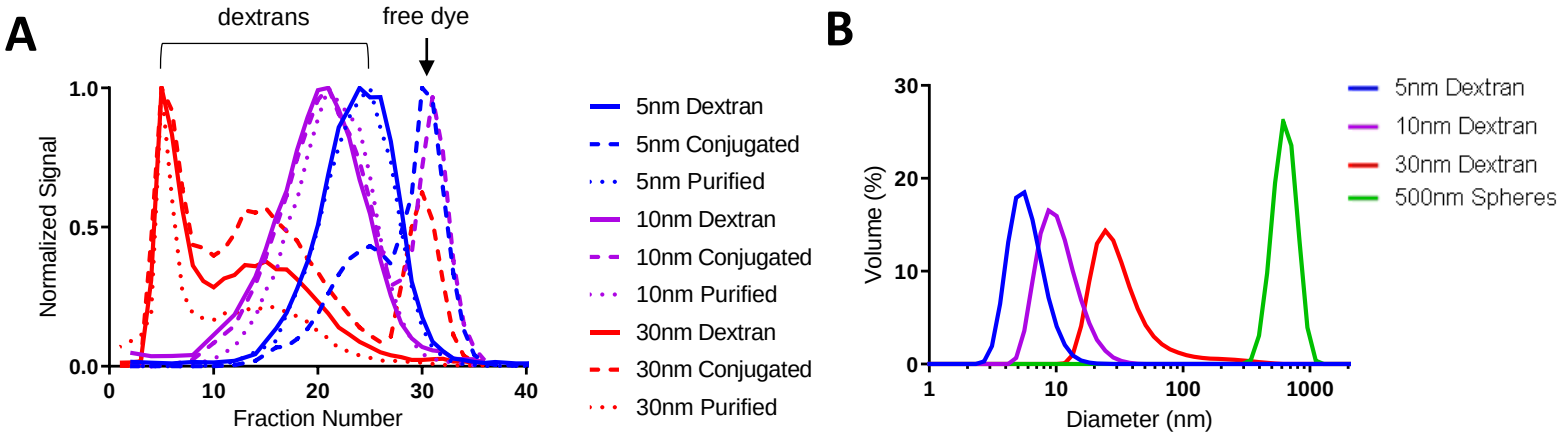
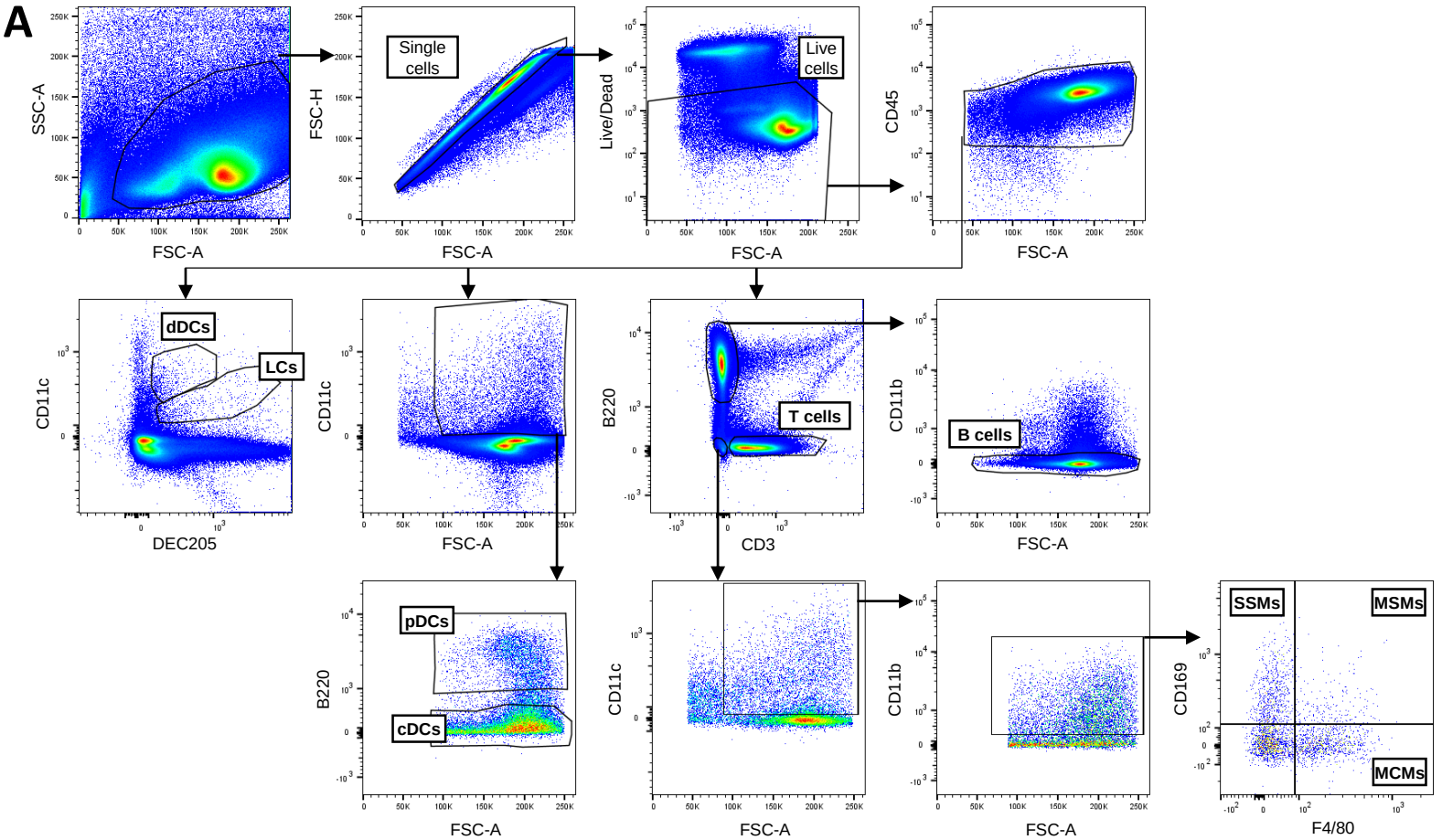
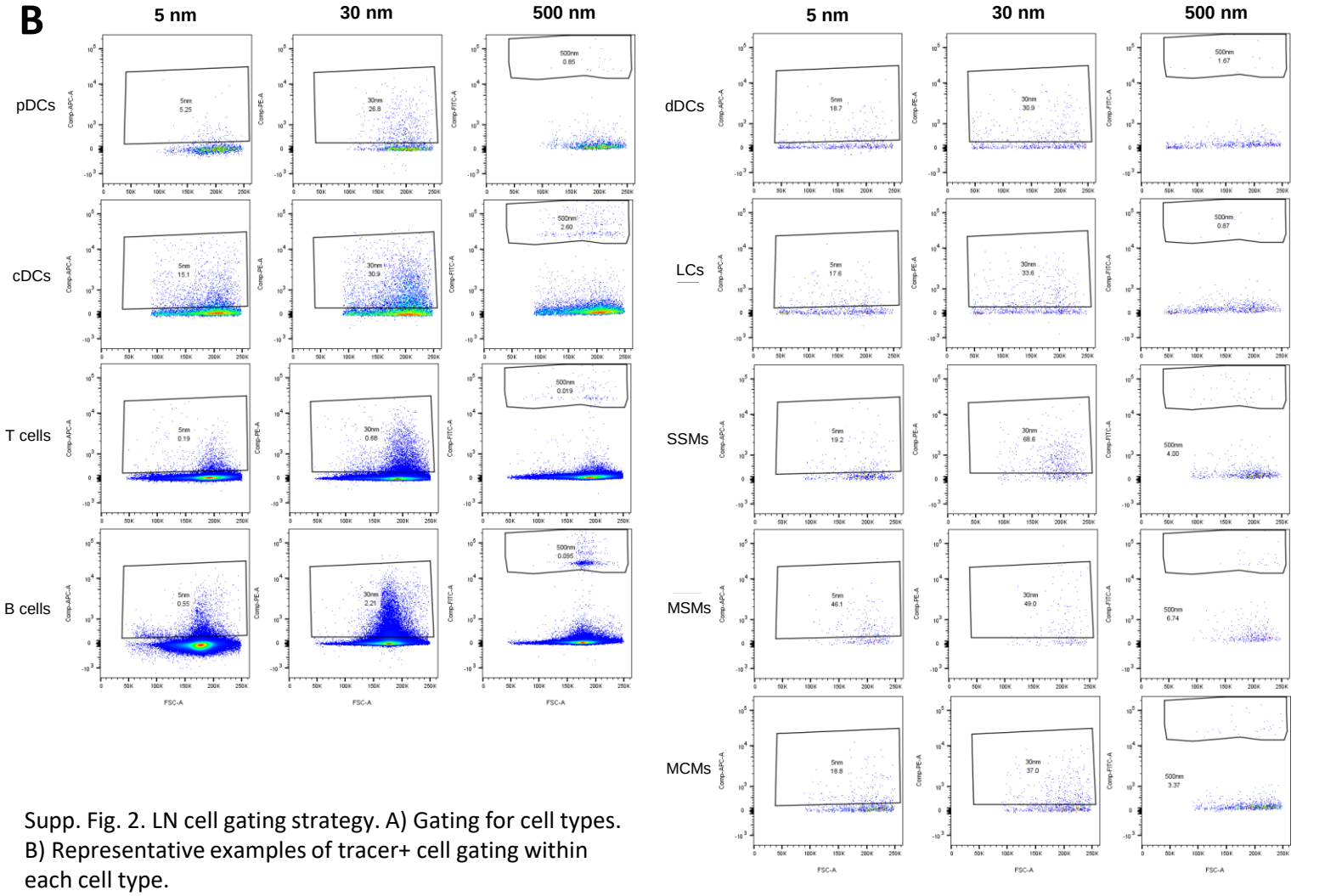


C

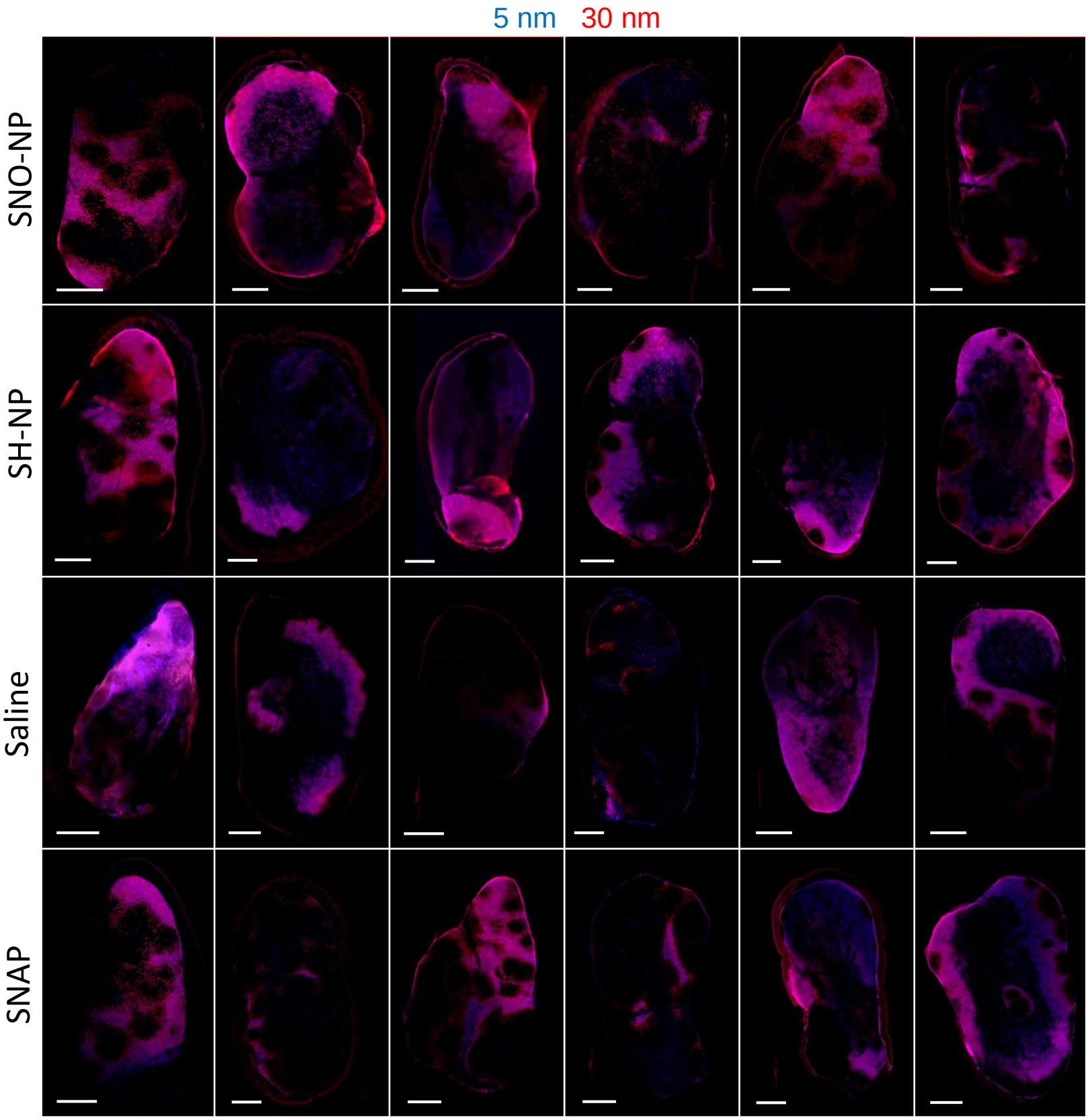
LOD (pM) in:	610-conjugated 10 kDa dextran (603/623)	647-conjugated 500 kDa dextran (650/670)	500nm red sphere (580/610)
Lymph Node	1200	47.6	1.99E-04
Spleen	1200	95.2	1.59E-03
Lung	1800	190.4	1.59E-03
Liver	4500	714	2.99E-03
Kidney	6000	47.6	3.99E-03
Heart	2400	190.4	2.19E-03
Skin	1500	95.2	7.97E-04

Supp Fig 1. Tracer synthesis and characterization. A) Synthesis and purification of fluorescent dextran tracers by SEC. For each dextran tracer, two peaks are observed – early peaks represent the dextran molecule, and late peaks are residual, unconjugated free dye. After purification, the free dye peak is removed, leaving only dextran-conjugated dyes ready for injection. B) 10 kDa dextran (blue), 40 kDa dextran (purple), 500 kDa dextran (red), and 500 nm polystyrene FluoSpheres (green) show approximate hydrodynamic diameters of 5, 10, 30, and 500 nm as measured by DLS. C) Bulk tissue homogenate measurements are very sensitive, and tracers can be detected in tissue homogenate at pM concentrations.

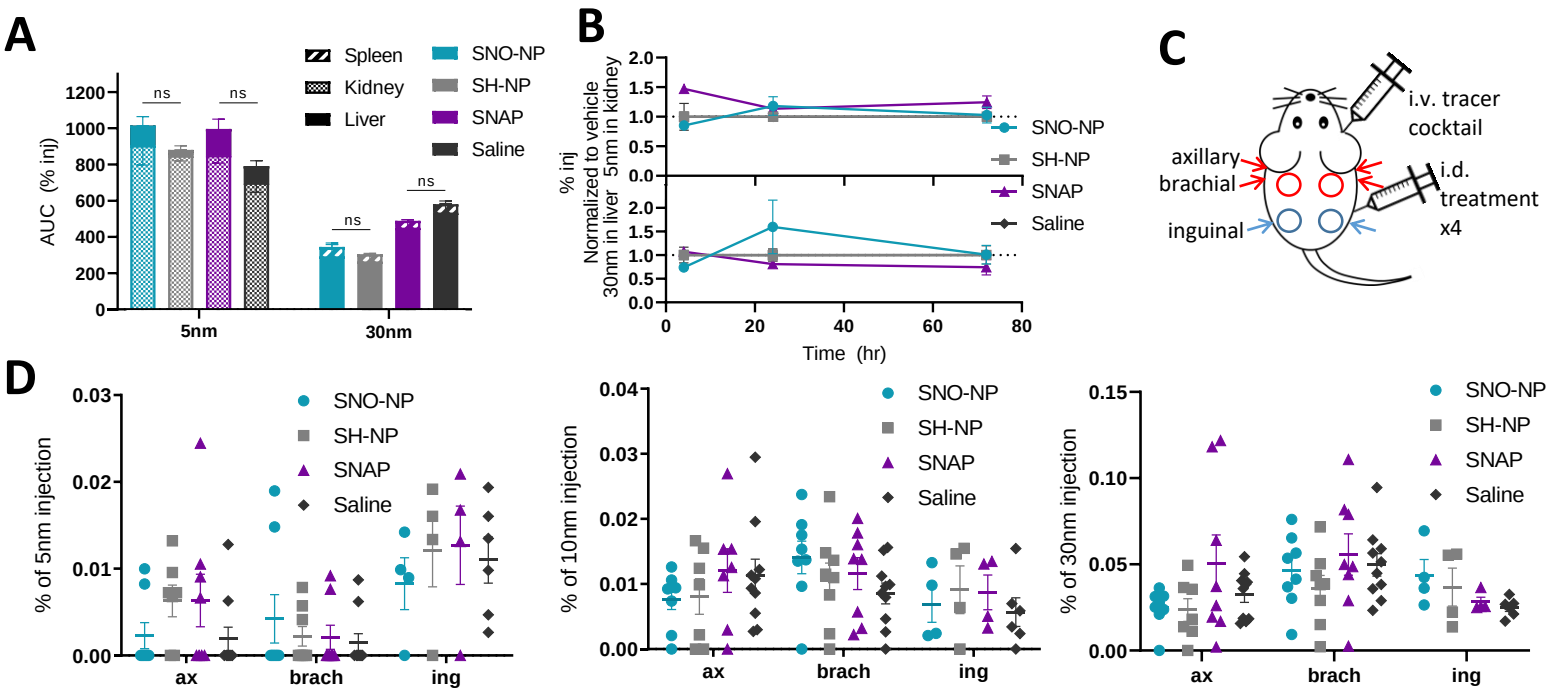




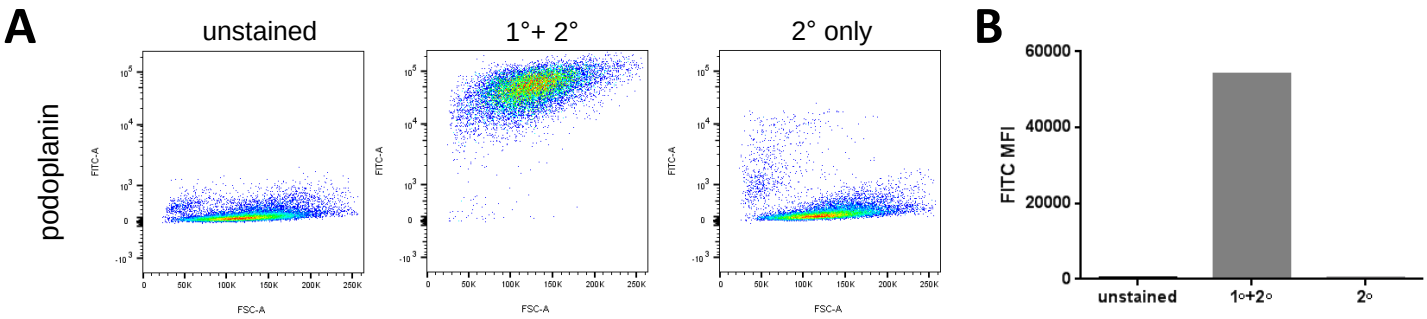
Supp. Fig. 2. LN cell gating strategy. A) Gating for cell types. B) Representative examples of tracer+ cell gating within each cell type.



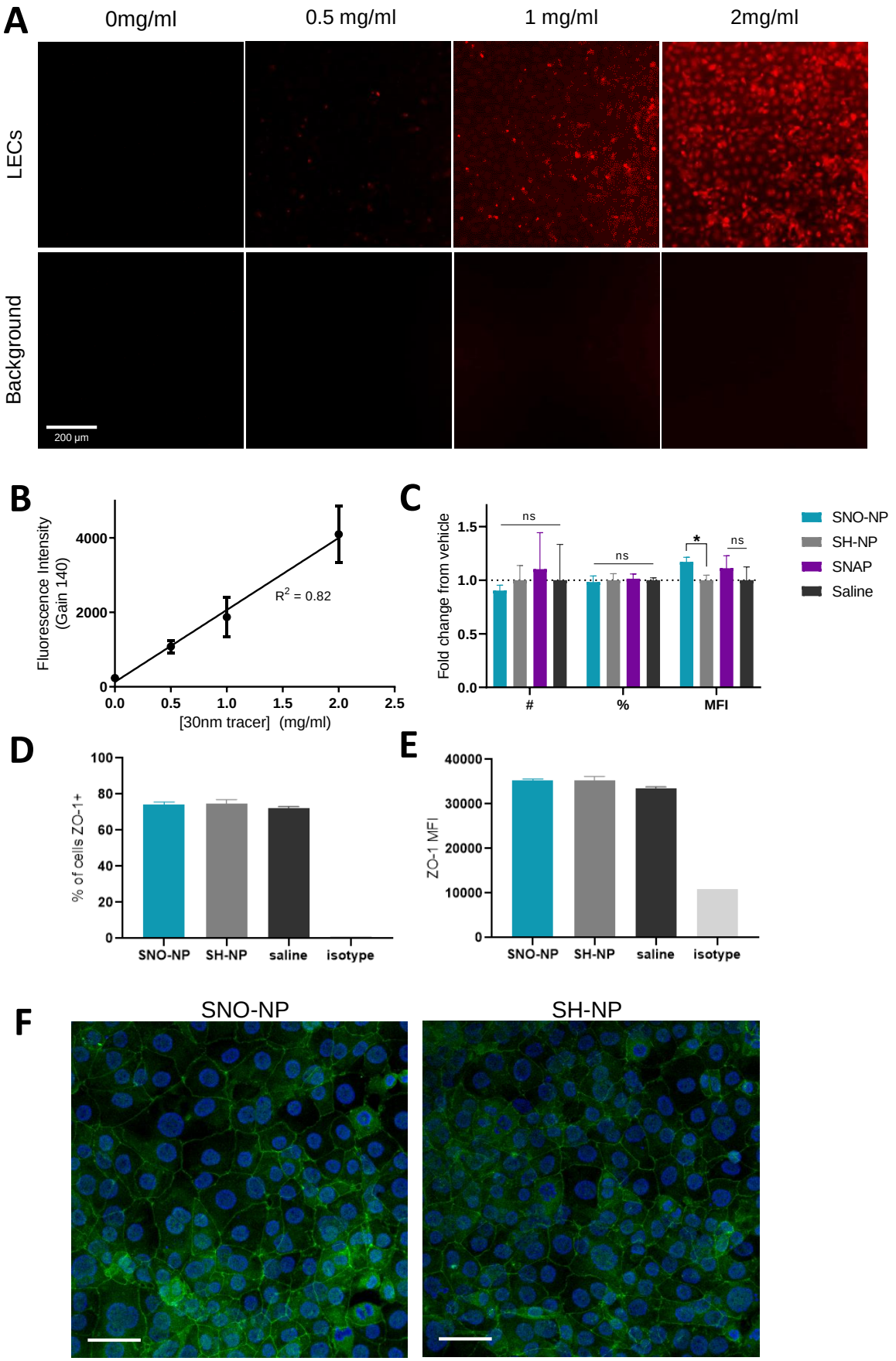
Supp. Fig. 3. Confocal images of LN draining forelimb tracer injections. 5 nm tracer is shown in blue, and 30 nm tracer is shown in red. All images are maximum intensity projections at γ = 0.45. Scale bar = 500 µm.



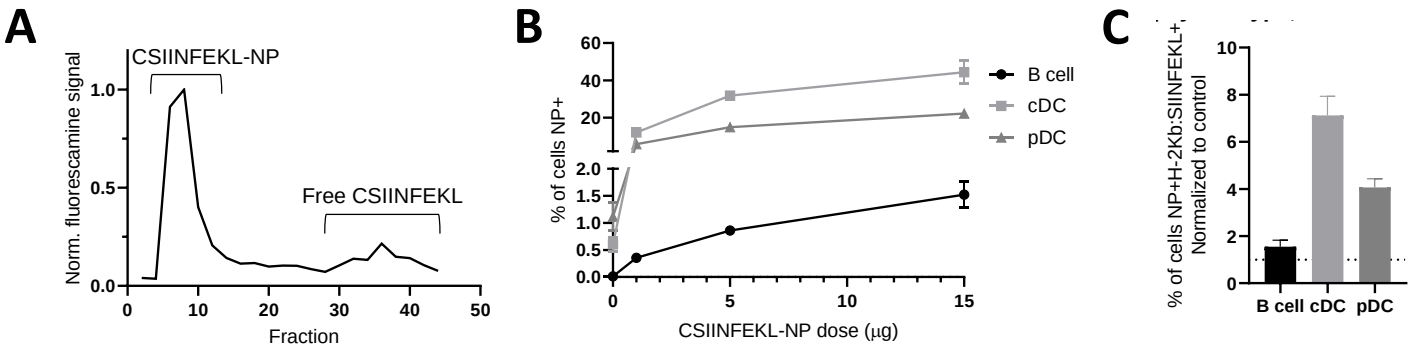
Supp. Fig. 4. NO effects on vasculature. A) Systemic distribution of passively draining tracers is unaffected by NO treatment. ns indicates no significant difference between liver, kidney, or spleen AUC values. B) NO treatment does not alter the accumulation of 5 (top) or 30nm tracer (bottom) in their primary clearance organs. Presented as SNO-NP and SNAP normalized to vehicle-treated controls (SH-NP and saline, respectively). C) Injection schematic for evaluating LN accumulation of i.v. tracers. Mice were given four i.d. injections draining to either axillary/brachial or inguinal LN, followed by an i.v. tracer injection. Circles indicate i.d. injection sites, and arrows indicate dLN. Red i.d. injection site drains to red LN (axillary and brachial), while blue i.d. injection site drains to blue LN (inguinal). D) 4 h after i.v. tracer injection, no differences in LN accumulation of tracers of any size were observed with any treatment.



Supp. Fig 5. SV-LEC characterization. A) Dot plots of LECs after staining with no antibody, primary and secondary antibodies, or secondary antibody only for LEC marker podoplanin. B) MFI of cells in A, showing more intense signal in the primary antibody stain compared to the unstained or secondary antibody controls.



Supp. Fig 6. Effect of SNO-NP treatment on 30 nm tracer uptake and ZO-1 expression by LECs *in vitro*. A) Representative images of TRITC-30nm dextran uptake in confluent LECs after 4 h of incubation at 37°C. Background images are empty wells (no cells) incubated with tracer and washed using an identical protocol. B) Fluorescence of LEC suspension 4 h after incubation with TRITC-30nm dextran, measured using a plate reader. Fluorescence signal increases linearly as tracer concentration increases, with a significantly nonzero slope (****). C) The number, frequency, and MFI of 30nm+ LECs after 4 h tracer incubation, measured by flow cytometry. Data are presented as normalized to vehicle control (SNO-NP/SH-NP, SNAP/saline), and statistical significance was determined by t-tests comparing NO treatment to the corresponding vehicle control. D) The percent of LECs ZO-1+ and E) their ZO-1 MFI after 6 h of treatment with SNO-NP or controls. F) Representative confocal images of ZO-1 stained LECs after SNO-NP (left) or SH-NP (right) treatment. Scale bar = 50 µm.



Supp Fig 7. NP-S-S-CSIINFEKL characterization. A) NP-S-S-CSIINFEKL are cleaned of excess free CSIINFEKL by SEC, and the presence of peptide in each fraction evaluated by fluorescamine signal. B) The percent of B cells, cDCs, and pDCs that are NP+ 72 h after NP-S-S-CSIINFEKL injection increases with increasing NP-S-S-CSIINFEKL dose. Administration of 5 μg of NP-S-S-CSIINFEKL results in significant NP uptake without saturating uptake capabilities of any cell type of interest. C) B cells, cDCs, and pDCs all show increased NP+H-2KB:SIINFEKL+ frequencies compared to saline controls 72 h after injection.