Supporting Information

Targeted nanocarriers co-opting pulmonary intravascular leukocytes for drug delivery to the injured brain

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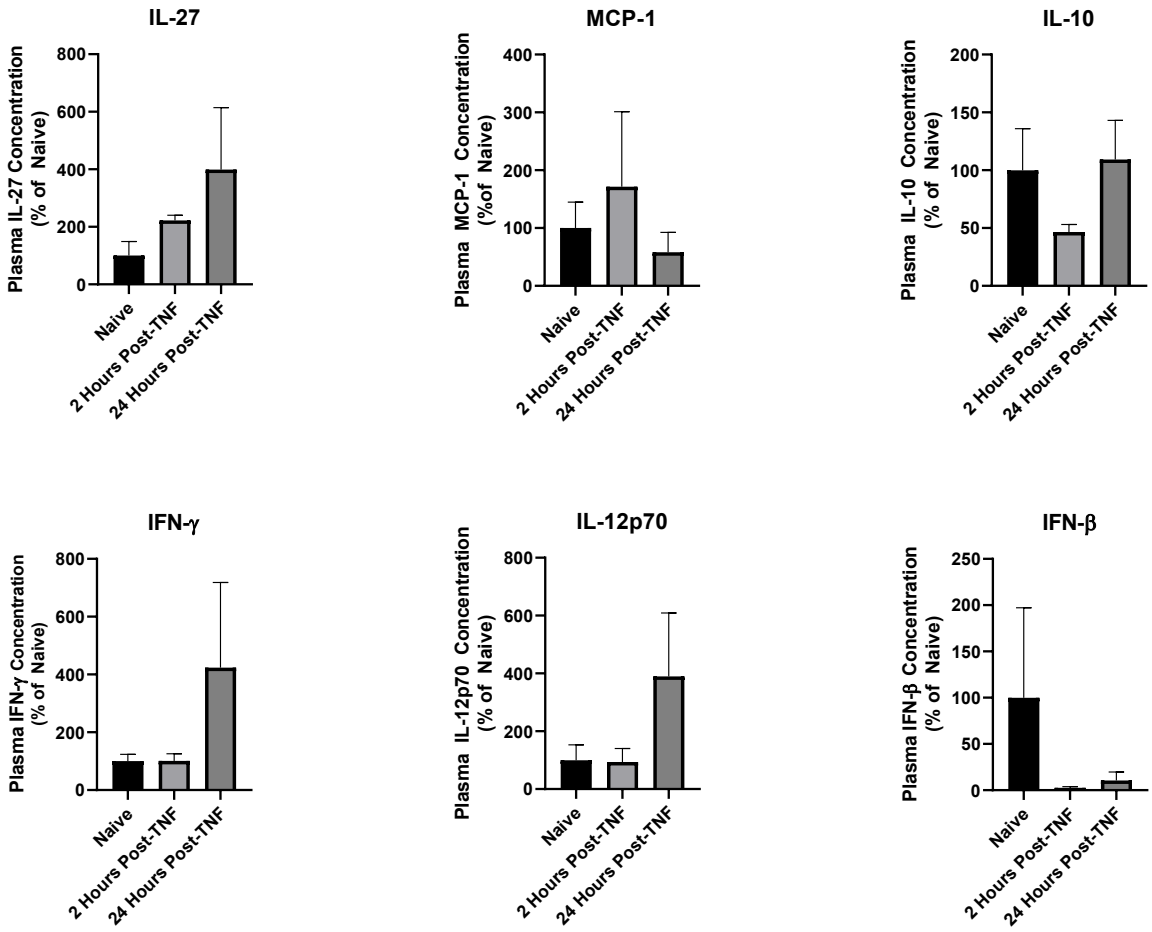
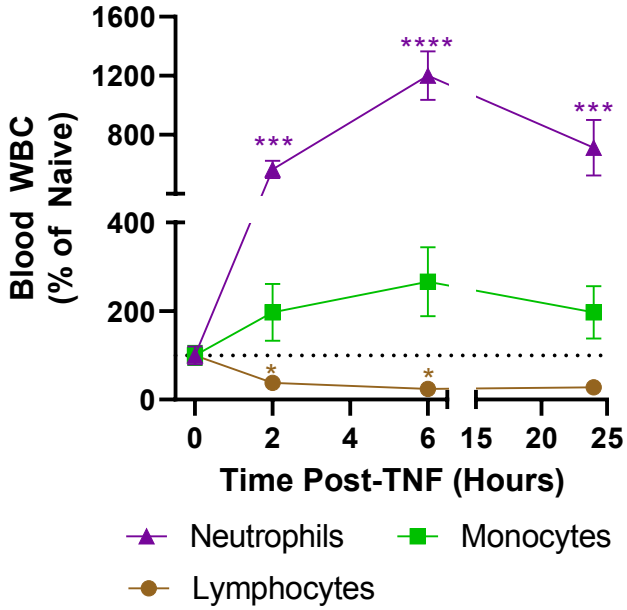
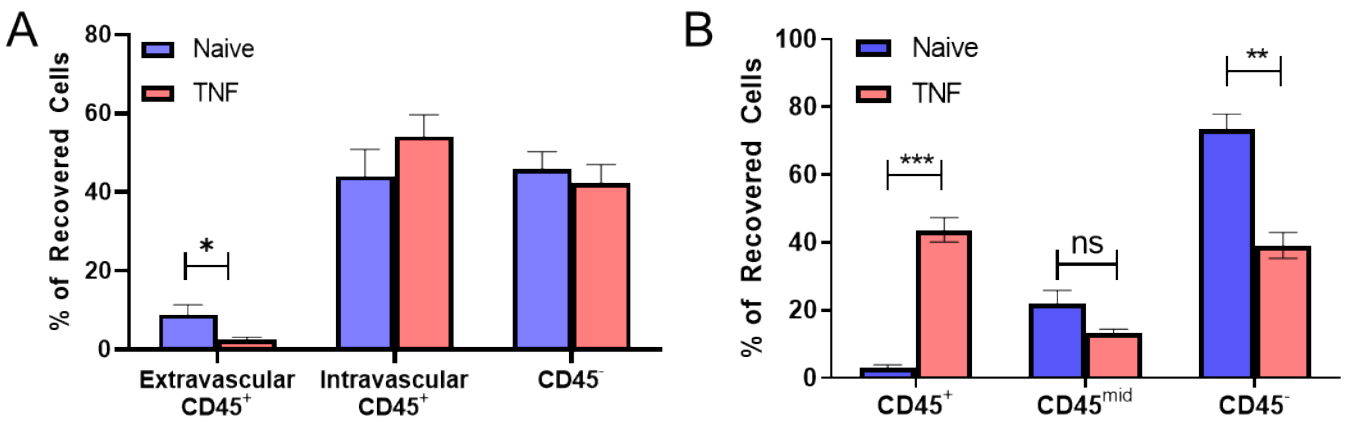


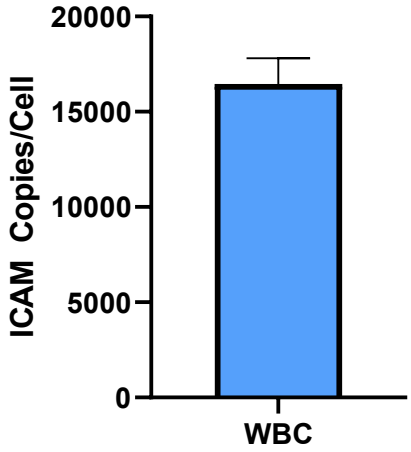
Supplemental Figure 1. Kinetics of plasma cytokine concentrations following TNF-α injury as measured by multiplex assay. Abbreviations: IL-27: Interleukin-27; MCP-1: Monocyte Chemoattractant Protein-1; IL-10: Interleukin-10; IFN-γ: Interferon-gamma; IL-12p70: Interleukin-12p70; IFN-β: Interferon-beta. N=3 mice/group.



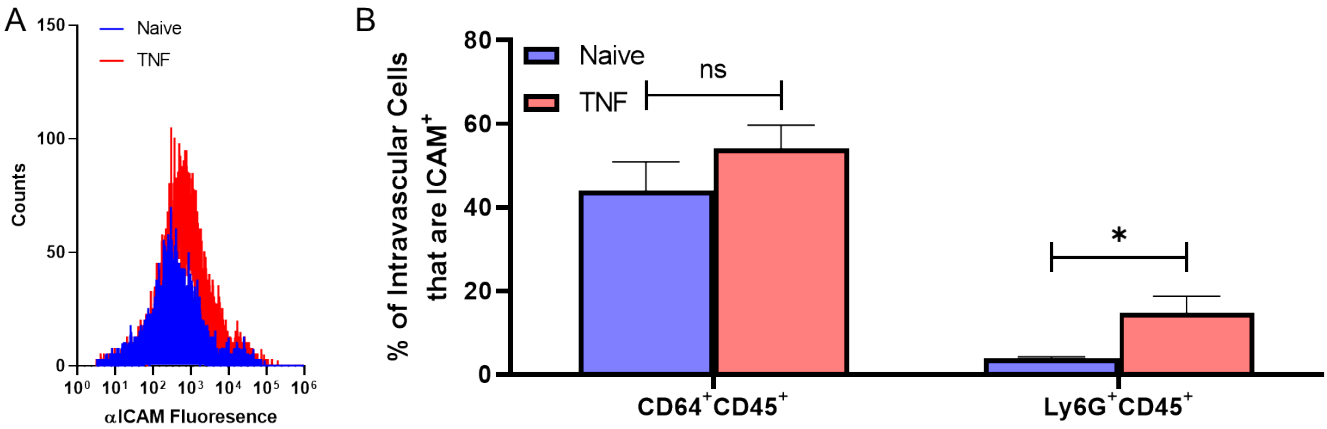
Supplemental Figure 2. Dynamics of white blood cells as measured by complete blood counts following TNF-α injury. Counts were normalized to pre-injury values for each individual animal. N=3/group. Comparisons were made via 1-way ANOVA with Dunnett's post-hoc test vs. naïve.



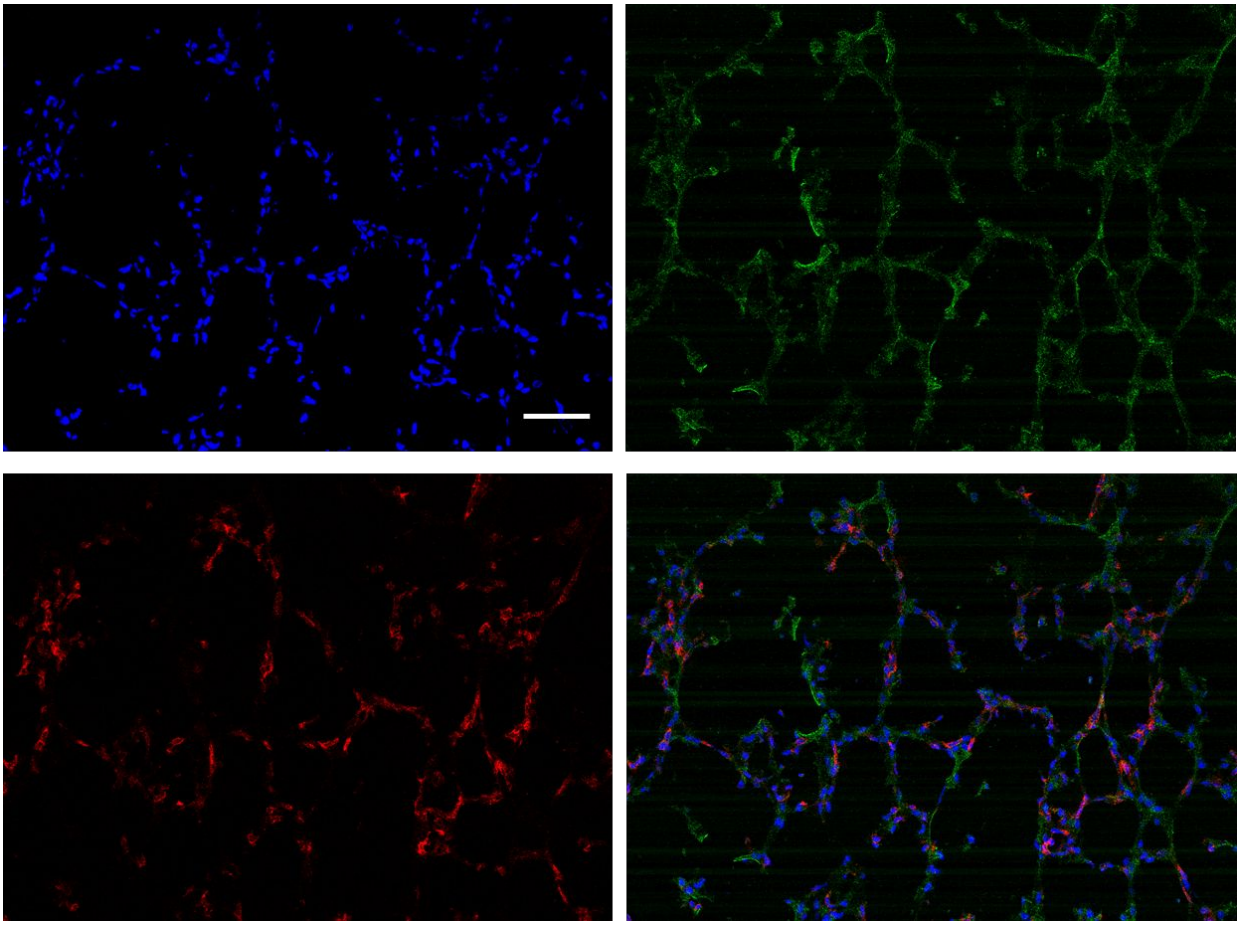
Supplemental Figure 3. Relative abundance of leukocytes in A) lungs 2 hours post-TNF-α injection and B) brain 24 hours post-TNF-α injection as measured by flow cytometry of single cell homogenates. N=3-4 mice/group. Comparisons made by unpaired t-test.



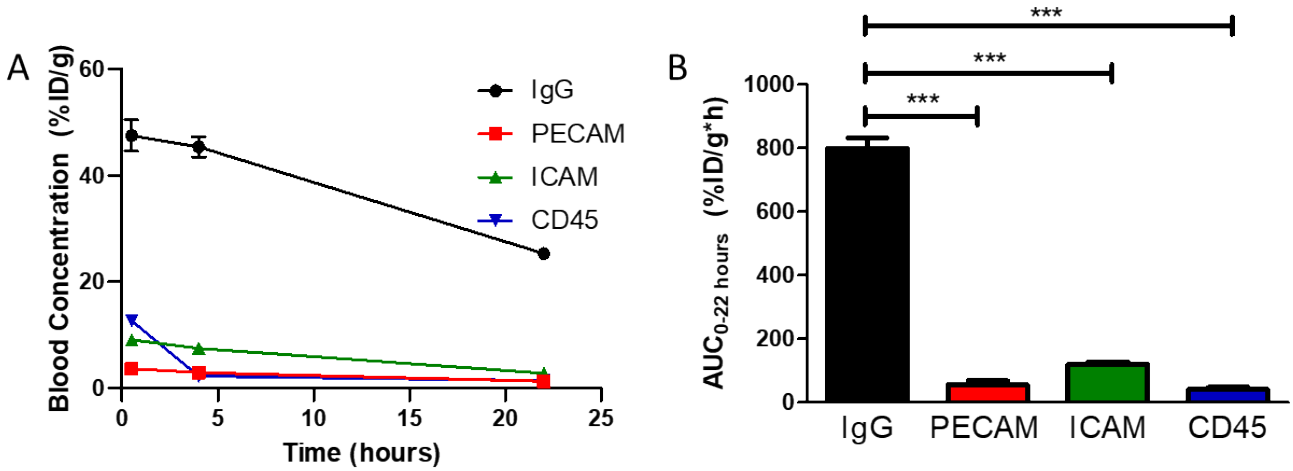
Supplemental Figure 4. Surface expression of ICAM on peripheral blood leukocytes extracted from mice. Expression was measured by determining the saturation level of αICAM mAb on cells in a radioimmunoassay.



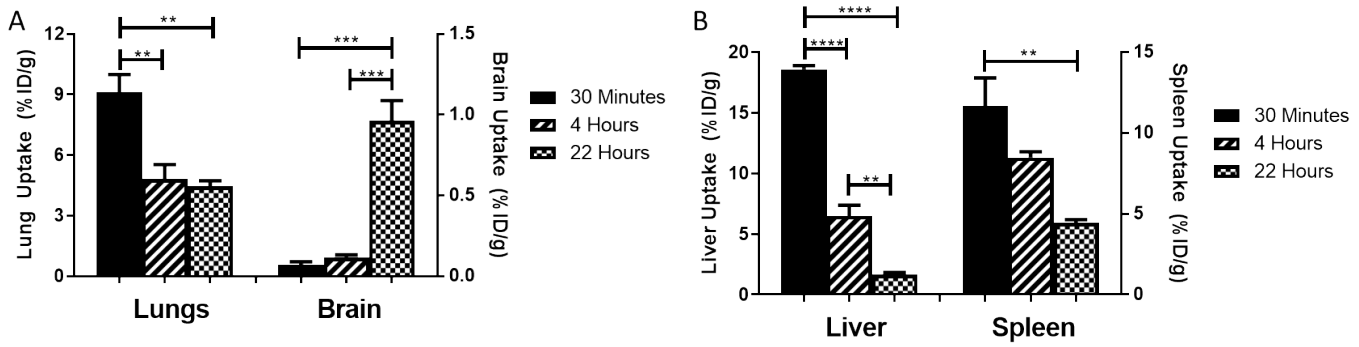
Supplemental Figure 5. Flow cytometry of lungs 2 hours after TNF-α injection. A) Histogram analysis of ICAM expression on $CD45^+$ cells showed a significant increase in the number of $ICAM^+CD45^+$ cells after injury. B) Subtyping of leukocytes demonstrated high levels of ICAM-expressing monocyte/macrophages ($CD64^+CD45^+$) and neutrophils ($Ly6G^+CD45^+$). N=3 mice/group. Comparisons made by unpaired t-test.



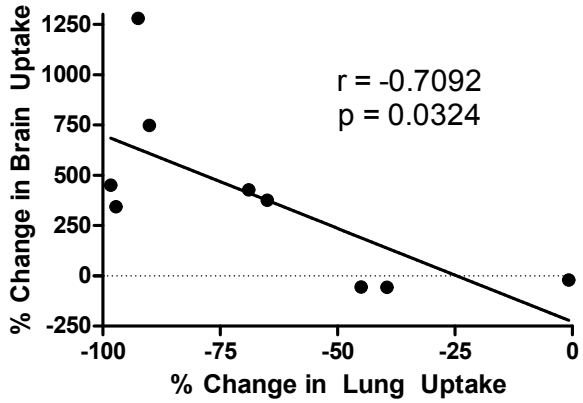
Supplemental Figure 6. Histological analysis of mouse lungs 2 hours after TNF-α injection into the brain. Blue: DAPI (nucleus); Green: Tissue autofluorescence (anatomical structure); Red: Leukocytes (CD45).



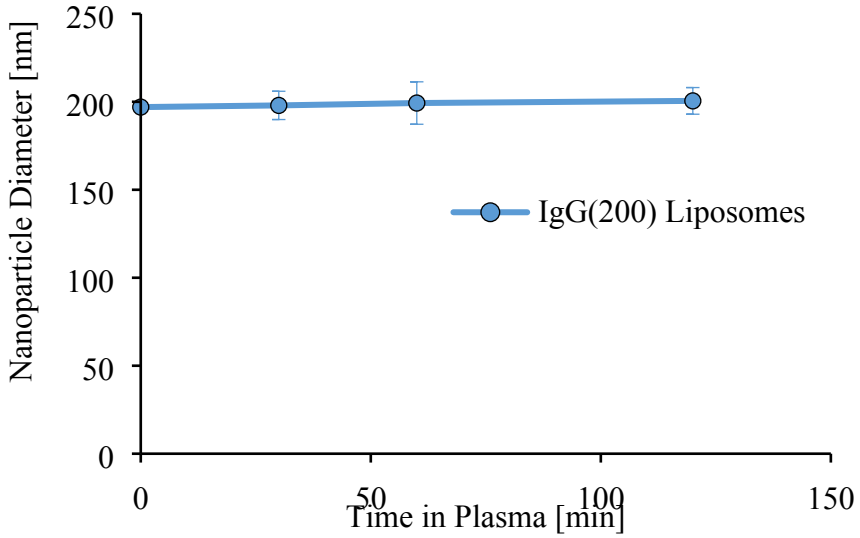
Supplemental Figure 7. Blood pharmacokinetics of mAbs against distinct vascular accessible epitopes following IV injection 2 hours post-TNF-α injury. A) Blood concentration vs. time data. B) Area under the concentration vs. time curve from 0 – 22 hours ($AUC_{0\text{-}22\,h}$). Data represented as mean ± SEM. *** denotes $p<0.001$ by 1-way ANOVA with Dunnett's post-hoc test. N = 3 mice/group.



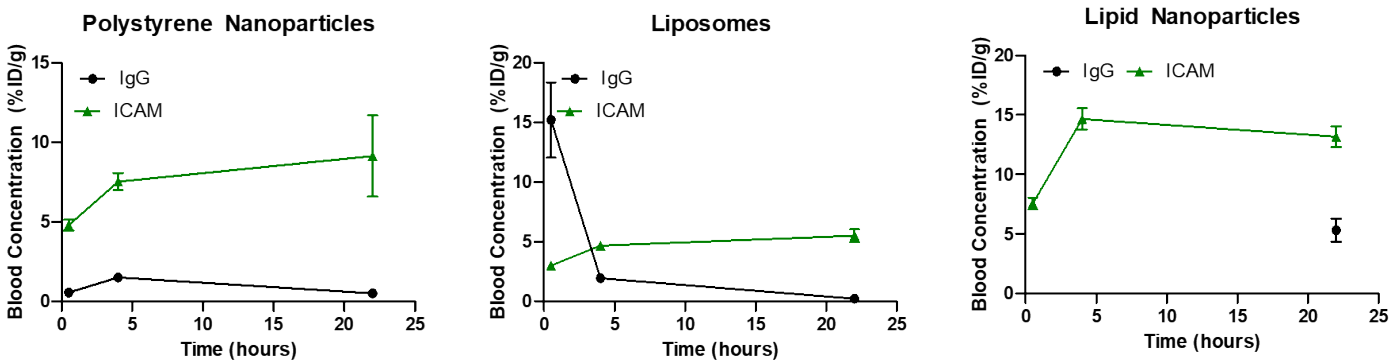
Supplemental Figure 8. Organ pharmacokinetics of control IgG injected 2 hours post-TNF-α injury. Experimental timeline as in Figure 2b. A) Lung and brain pharmacokinetics, B) Pharmacokinetics in clearance organs (liver and spleen). Comparisons made by 1-way ANOVA with Tukey's post-hoc test. Data represented as mean ± SEM. N = 3 mice/group.



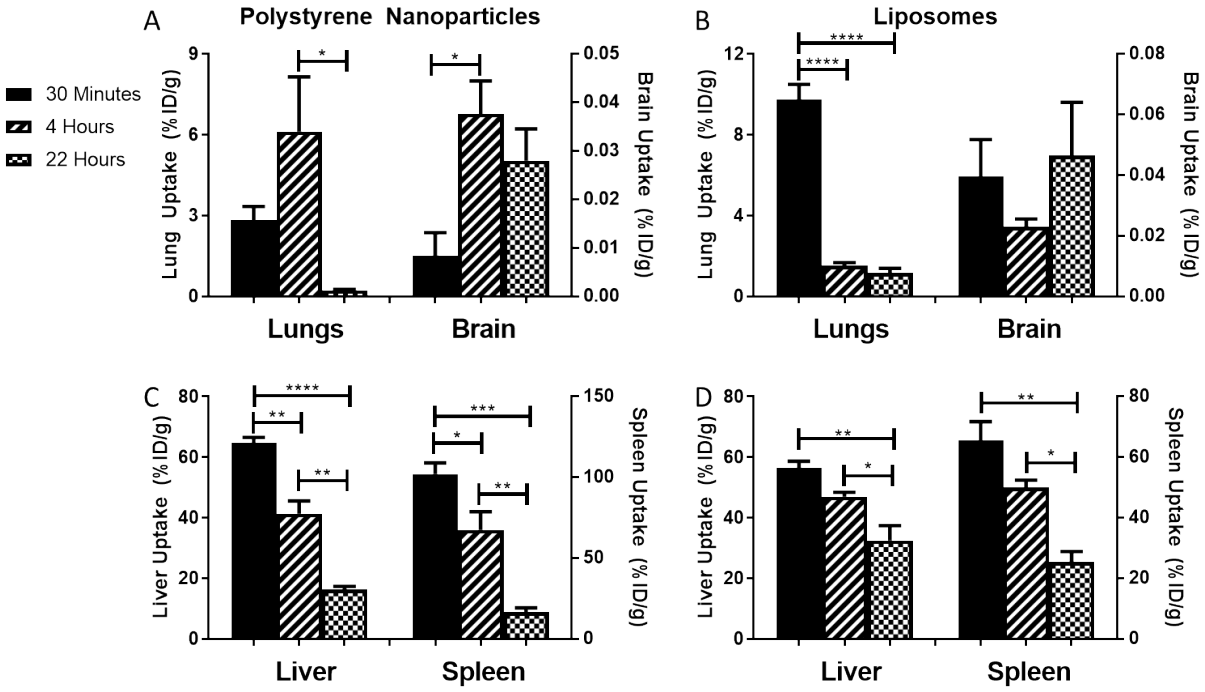
Supplemental Figure 9. Changes in brain uptake correlate with clearance from the lung. Data from Figure 2 displayed as individual animals. Pearson's correlation analysis was used to derive the correlation coefficient (r).



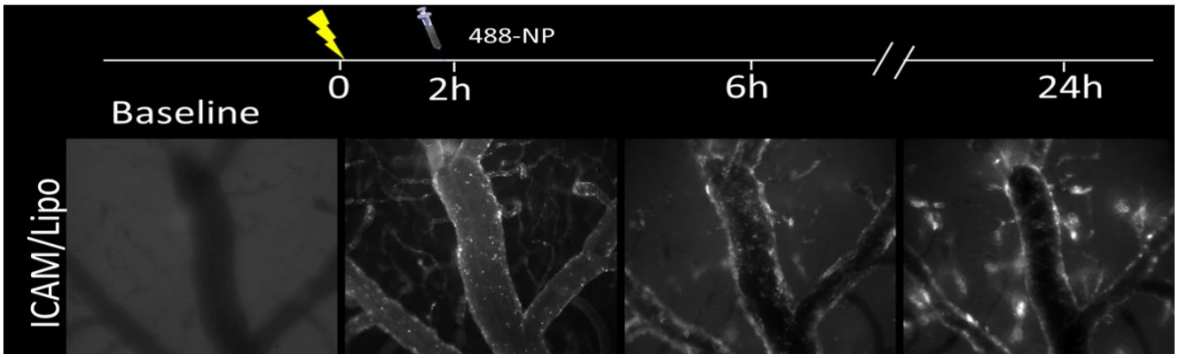
Supplemental Figure 10. Stability of IgG-coated liposomes (200 IgG molecules per liposomes, same coating density as ICAM liposomes) in mouse plasma as measured by nanoparticle tracking analysis.



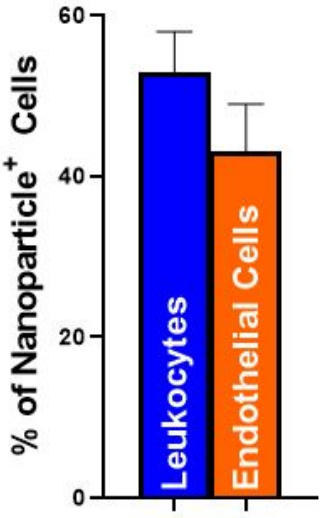
Supplemental Figure 11. Blood pharmacokinetics of nanoparticles injected intravenously 2 hours post-TNF-α injury. N=3 mice/group.



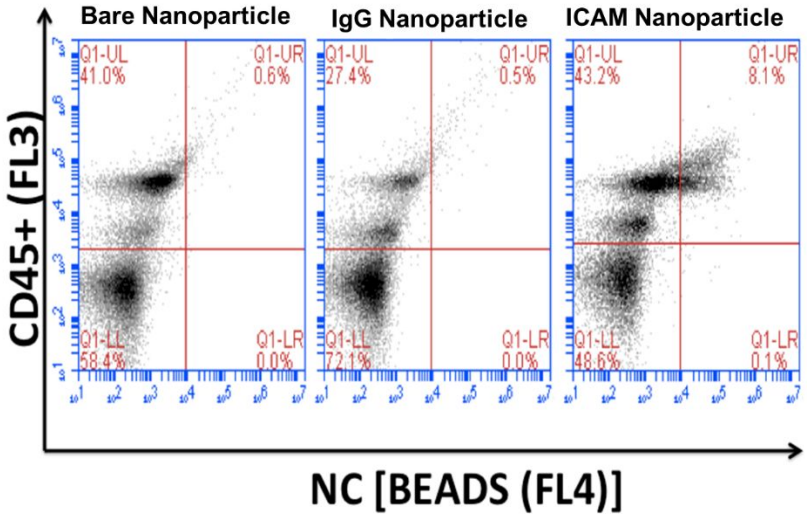
Supplemental Figure 12. Pharmacokinetics of control IgG-coated nanoparticles injected 2 hours post-TNF-α injury. Experimental timeline as in Figure 3a. Pharmacokinetics in lungs and brain for A) polystyrene nanoparticles and B) liposomes. Pharmacokinetics in clearance organs for C) polystyrene nanoparticles and D) liposomes. Comparisons made by 1-way ANOVA with Tukey's post-hoc test. Data represented as mean ± SEM. N = 3 mice/group.



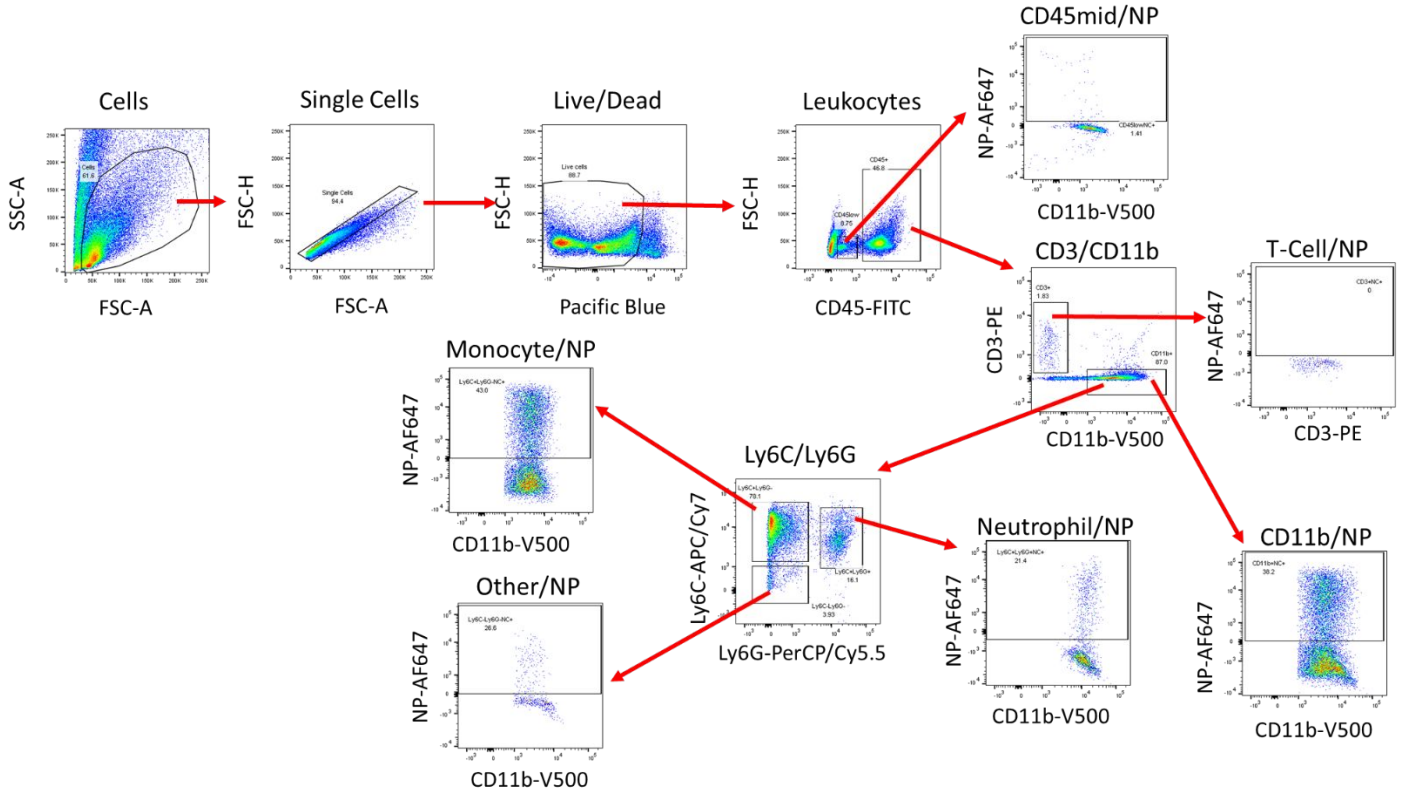
Supplemental Figure 13. Cranial window intravital microscopy of ICAM-targeted liposomes in the brain at designated time points after TNF-α injury.



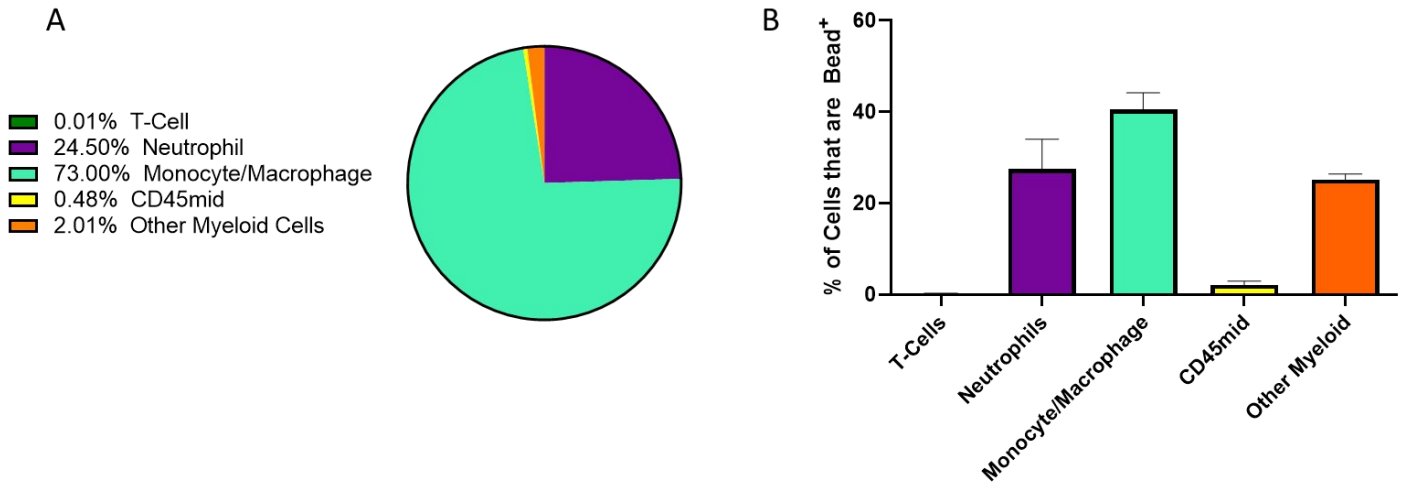
Supplemental Figure 14. Cellular specificity of ICAM-targeted polystyrene beads 30 minutes after intravenous injection in the lungs of naïve mice. Data represents the percentage of total nanoparticle-positive cells that are the given cell type. N=3 mice/group.



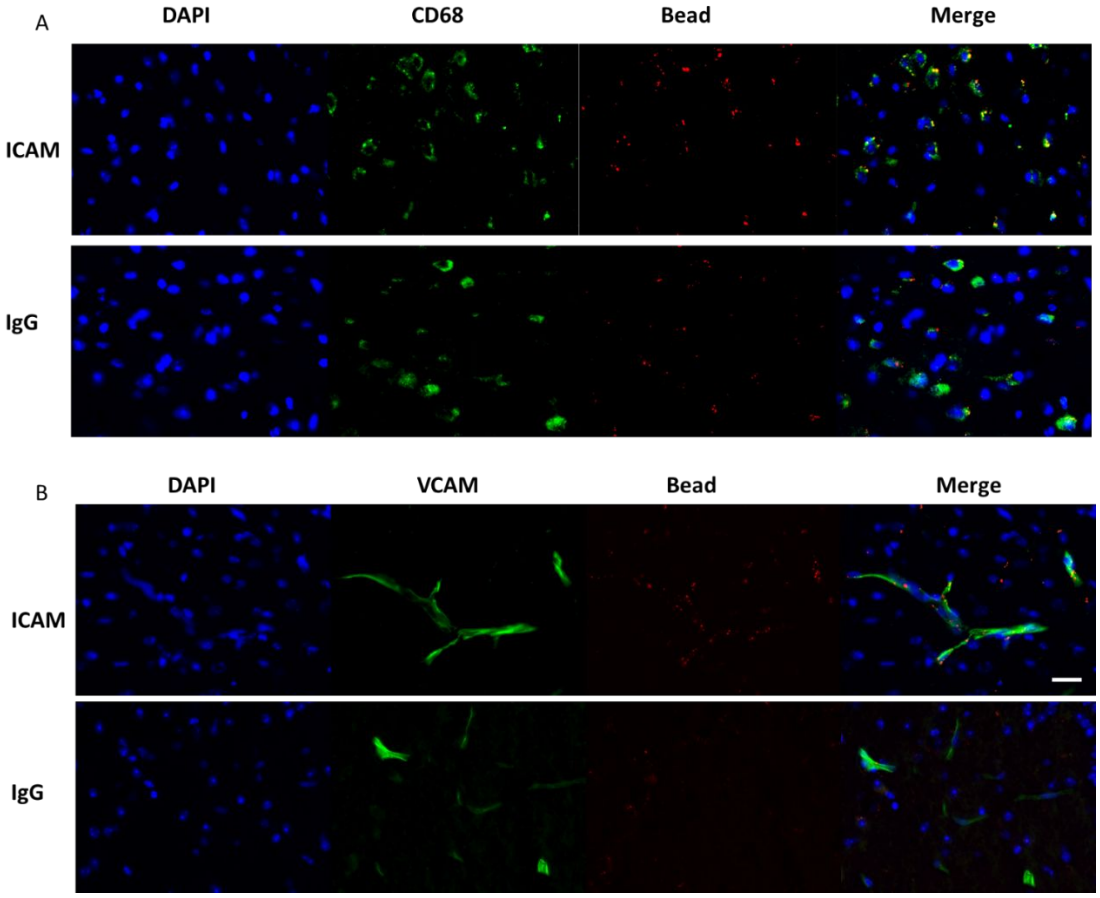
Supplemental Figure 15. Representative flow cytometry dot-plots of polystyrene bead (bare, IgG, ICAM) cellular uptake in the brain 22 hours post-injection (24 hours post-TNF).



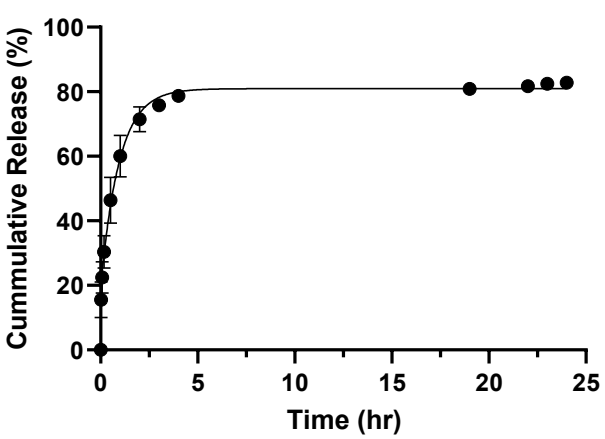
Supplemental Figure 16. Gating scheme for detailed flow cytometry of leukocyte sub-types in brain single cell suspensions.



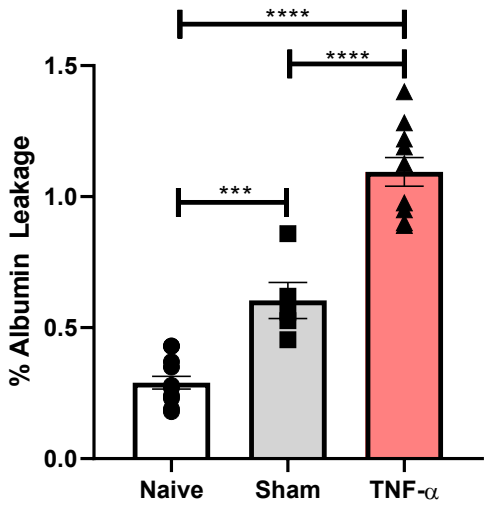
Supplemental Figure 17. Flow cytometry of ICAM-targeted polystyrene nanoparticle distribution in leukocytes in the brain 22 hours post-injection (24 hours post-TNF). A) Fraction of nanoparticle-positive leukocytes for each sub-type. B) Fraction of recovered cells that were nanoparticle^{+}. T-Cell: CD45^{+}CD11b^{-}CD3^{+}, Neutrophil: CD45^{+}CD11b^{+}Ly6G^{+}, Monocyte: CD45^{+}CD11b^{+}Ly6C^{+}Ly6G^{-}, Microglia: CD45^{mid}, Other Myeloid: CD45^{+}CD11b^{+}Ly6C^{-}Ly6G^{-}. Data represented as mean ± SEM. N = 3 mice/group



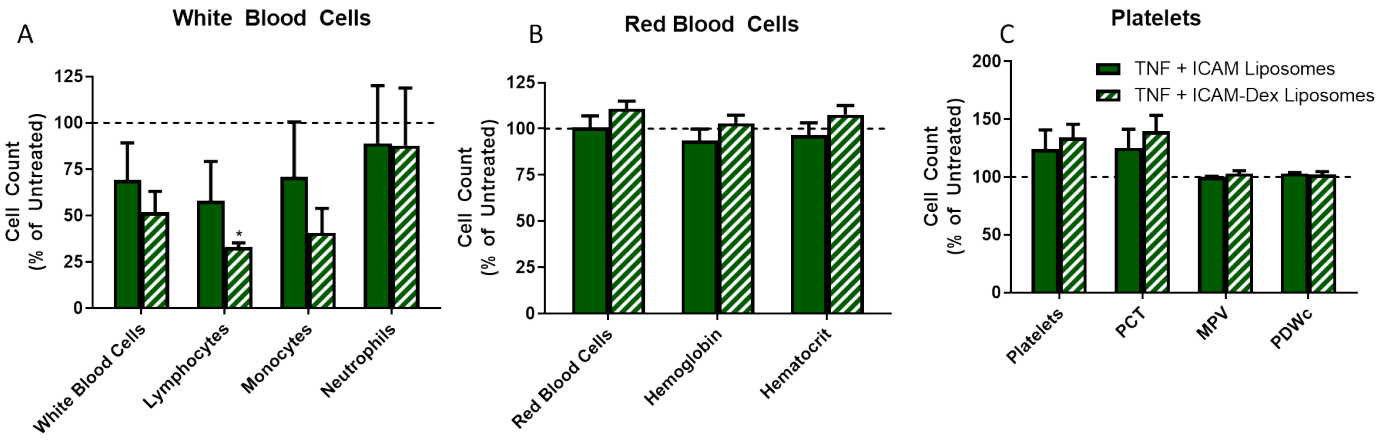
Supplemental Figure 18. Histology of brain slices taken 22 hours post-injection of ICAM-targeted or IgG polystyrene nanoparticles (24 hours post TNF-α injection). Staining was performed to determine co-localization with A) monocytes/macrophages (CD68) and B) endothelial cells (VCAM). Scale bar: 50 μm.



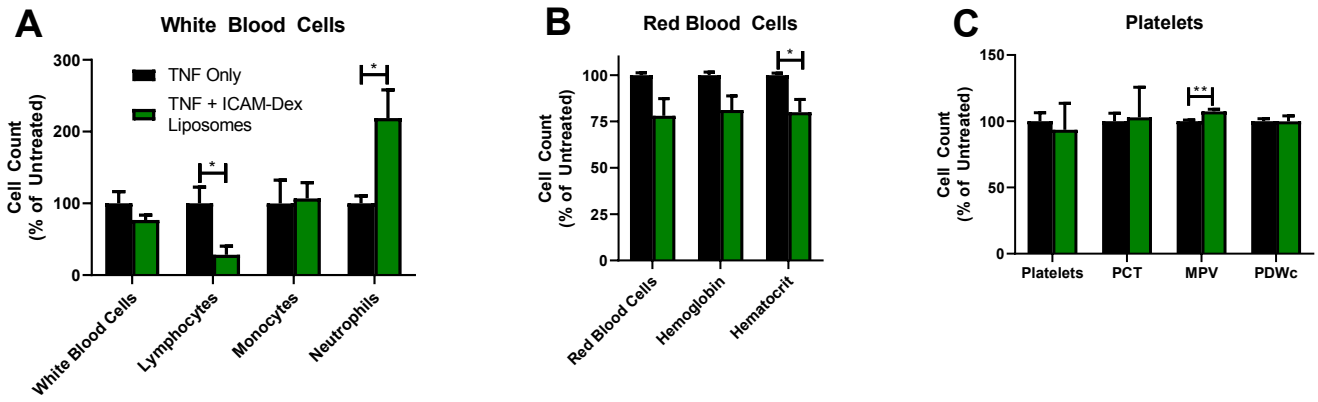
Supplemental Figure 19. Dexamethasone release from liposomes incubated in PBS at 37°C.



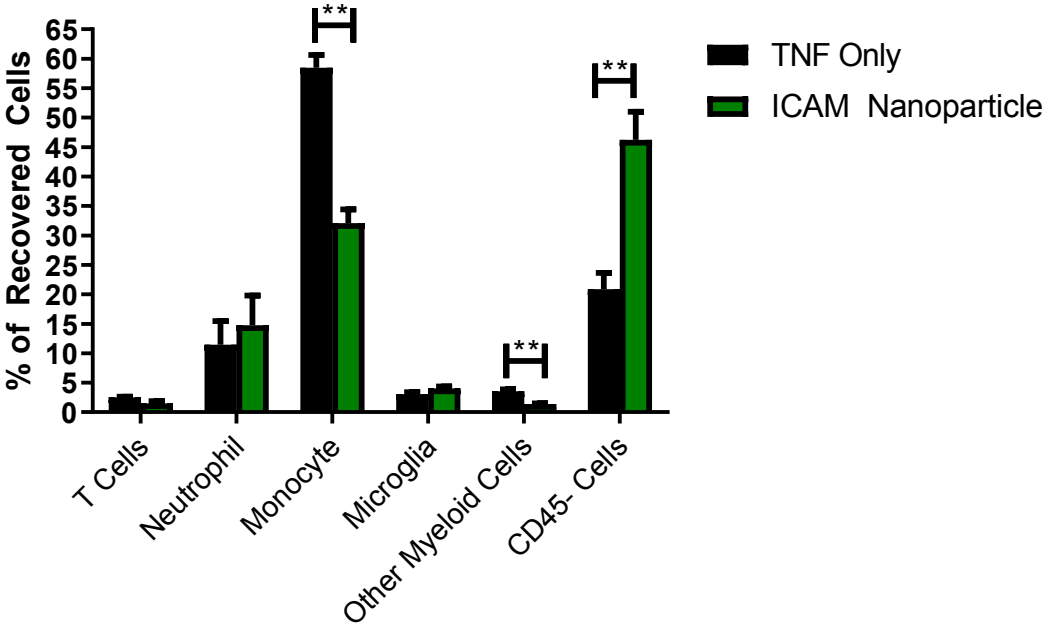
Supplemental Figure 20. TNF-α injury induces albumin leak into the brain. 20 hours after injection of TNF-α mice were IV injected with radiolabeled albumin. Following perfusion, the degree of albumin leak was calculated as the percentage of brain albumin concentrations vs. blood. Data represented as mean ± SEM. Comparisons made by 1-way ANOVA with Tukey's post-hoc test. N = 5-12 mice/group.



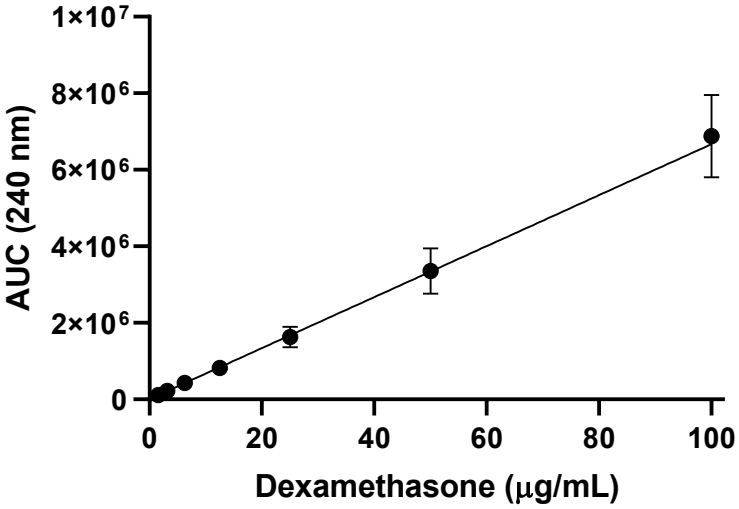
Supplemental Figure 21. Complete blood counts 22 hours post-injection of ICAM-targeted liposomes and dexamethasone-loaded ICAM-targeted liposomes (24 hours post-TNF-α). A) White blood cells, B) Red blood cells, C) Platelets. Data represented as mean ± SEM. Dashed line represents values for untreated mice. Comparisons made by 1-way ANOVA with Dunnett's post-hoc test vs. untreated. N ≥ 3 mice/group.



Supplemental Figure 22. Complete blood counts 30 minutes post-injection of dexamethasone-loaded ICAM-targeted liposomes (2.5 hours post-TNF-α). A) White blood cells, B) Red blood cells, C) Platelets. Data represented as mean ± SEM. Comparisons made by unpaired t-test. N = 4 mice/group.



Supplemental Figure 23. Impact of ICAM-targeted polystyrene nanoparticles on immune cell infiltration into the brain, as measured by flow cytometry. N=3 mice/group. Comparisons made by unpaired t-test.



Supplemental Figure 24. Standard curve for dexamethasone HPLC assay. Linear range: 1.56 – 100 μg/mL. Data represents mean ± SD of 15 independently run standard curves.

αICAM	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
Naïve	4.69±1.59	106±13	19.6±2.1	9.51±0.90	3.53±0.29	47.8±4.1	0.347±0.049
1 hour	6.04±0.21	216±8**	17.5±1.0	29.2±1.3***	10.2±0.3***	24.3±2.4**	0.731±0.049
2 hours	7.23±0.22	258±16***	33.2±4.8**	19.3±0.8***	6.40±0.61**	44.5±4.2	0.655±0.162
3 hours	5.99±0.59	194±16**	18.7±0.6	31.0±1.3***	11.9±0.5***	31.6±1.0*	0.882±0.028
6 hours	3.19±0.42	115±12	16.8±0.6	11.1±1.0	5.86±0.40*	22.7±1.8***	1.31±0.16**
24 hours	5.02±0.13	108±24	14.2±1.0	7.74±1.97	4.79±0.86	27.2±4.6**	2.43±0.24***
αCD45	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
Naïve	12.7±0.7	13.2±2.2	27.8±2.8	5.01±0.13	1.77±0.22	189±16	0.034±0.005
2 hours	12.7±0.6	60.5±10.2**	33.5±0.3	5.68±0.37	0.946±0.110	136±17	0.061±0.010
24 hours	12.6±0.6	35.9±7.3	27.4±1.2	7.30±0.70*	1.56±0.29	215±16	0.536±0.042****
IgG	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
Naïve	30.4±0.9	0.931±0.526	3.69±0.52	4.29±0.98	1.28±0.17	2.36±0.01	0.077±0.022
1 hour	50.2±1.9**	1.33±0.42	15.8±2.4***	11.7±2.2	1.26±0.25	9.75±0.51	0.073±0.007
2 hours	41.5±2.6	6.88±2.90*	11.2±1.3**	2.11±0.09	1.58±0.31	7.33±0.62	0.033±0.006
3 hours	49.0±0.5**	6.30±1.31	16.2±1.0***	16.4±5.7*	1.26±0.16	9.90±1.85	0.074±0.025
6 hours	31.0±5.6	0.862±0.060	4.12±0.86	4.30±0.35	1.33±0.26	3.70±0.84	0.069±0.021
24 hours	43.2±2.1*	0.917±0.293	4.65±0.02	4.09±0.11	1.37±0.21	4.33±0.21	0.109±0.021

Supplemental Table 1. Biodistribution of αICAM, αCD45, and IgG injected at different times post-TNF-α injury. Values reported as percent of injected dose/g organ (%ID/g). Samples were collected 30 minutes after IV injection of mAb. Data reported as mean ± SEM. Comparisons made by 1-way ANOVA with Dunnett's post-hoc test vs. naïve. * denotes $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$, **** denotes $p<0.0001$. N = 3/group.

αPECAM	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	3.64±0.73	133±22	22.6±4.2	26.0±5.0	19.6±4.7	32.3±6.5	3.99±0.61
4 hours	2.91±0.44	143±16	26.0±4.9	30.7±4.3	22.4±4.5	37.2±9.1	3.42±0.47
22 hours	1.27±0.22	90.4±5.1	19.8±0.4	20.6±1.3	15.0±1.0	18.8±2.2	2.27±0.08
αICAM	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	9.08±0.29	223±28	23.1±2.6	30.4±2.0	11.2±1.4	38.1±9.4	0.605±0.053
4 hours	7.49±0.35	119±10	36.8±1.3	18.1±1.7	7.14±0.38	73.8±2.9	0.868±0.035
22 hours	2.77±0.37	59.3±6.2	16.2±0.8	7.25±0.32	3.26±0.20	29.3±0.4	2.52±0.14
αCD45	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	12.7 ± 0.6	60.5 ± 10.2	35.5 ± 1.7	5.68 ± 0.37	0.946 ± 0.110	136 ± 17	0.0610 ± 0.010
4 hours	2.22±0.18	8.45±0.69	23.8±3.7	2.00±0.49	0.243±0.023	153±36	0.146±0.040
22 hours	1.42±0.45	3.70±0.88	3.74±0.49	0.974±0.242	0.268±0.084	89.6±29.2	0.437±0.116
IgG	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	47.5±3.0	9.12±0.88	18.6±0.3	2.67±0.26	3.46±0.52	11.6±1.7	0.071±0.018
4 hours	45.4±1.9	4.80±0.73	6.49±0.90	3.88±0.28	2.86±0.11	8.49±0.36	0.114±0.017
22 hours	25.4±0.9	4.45±0.28	1.65±0.19	1.95±0.35	2.44±0.06	4.46±0.18	0.960±0.125

Supplemental Table 2. Pharmacokinetics and biodistribution of mAb against endothelial and leukocyte epitopes. Data represented as mean ± SEM. N = 3/group

αICAM	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	1.91±0.14	147±1	25.9±0.5	2.78±0.20	0.578±0.110	21.4±4.4	0.051±0.005
4 hours	2.64±0.53	8.82±2.75	14.1±1.1	1.23±0.10	0.341±0.067	29.2±5.1	0.086±0.009
22 hours	3.65±1.02	20.2±5.9	8.86±0.94	2.14±0.49	0.726±0.288	25.1±2.6	0.254±0.029
IgG	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	0.545±0.066	2.83±0.51	64.7±1.8	0.282±0.025	0.062±0.017	102±7	0.008±0.005
4 hours	1.49±0.07	6.12±2.03	41.3±4.3	0.816±0.065	0.215±0.014	67.4±11.2	0.038±0.007
22 hours	0.499±0.081	0.222±0.037	16.2±1.0	0.273±0.055	0.053±0.006	16.6±2.7	0.028±0.007

Supplemental Table 3. Pharmacokinetics and biodistribution of ICAM-targeted and IgG polystyrene nanoparticles injected 2 hours after intrastriatal TNF-α. Experimental design as in **Figure 3a**. Values reported as percent of injected dose/g organ (%ID/g). Samples were collected 30 minutes after IV injection of polystyrene nanoparticles. Data reported as mean ± SEM. N = 3/group.

αICAM	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	2.99±0.05	174±6	36.7±0.4	22.1±1.0	7.99±0.40	40.8±6.7	0.498±0.054
4 hours	4.65±0.25	131±9	30.4±1.1	18.3±1.9	6.79±0.39	38.6±6.3	0.702±0.085
22 hours	5.49±0.53	50.2±6.0	14.3±1.4	13.2±0.6	4.44±0.17	10.6±0.6	0.287±0.006
IgG	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	15.2±3.2	9.76±0.74	56.1±2.4	0.547±0.020	0.367±0.068	65.5±6.2	0.040±0.012
4 hours	1.92±0.26	1.52±0.15	46.9±1.5	1.47±0.11	0.101±0.010	49.9±2.5	0.023±0.003
22 hours	0.206±0.091	1.16±0.22	32.5±4.9	1.41±0.34	0.090±0.033	25.4±3.4	0.046±0.018

Supplemental Table 4. Pharmacokinetics and biodistribution of ICAM-targeted and IgG liposomes injected 2 hours after intrastriatal TNF-α =. Experimental design as in **Figure 3a**. Values reported as percent of injected dose/g organ (%ID/g). Samples were collected 30 minutes after IV injection of liposomes. Data reported as mean ± SEM. N = 3/group.

αICAM	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
30 minutes	7.55±0.49	123±9	39.2±1.6	10.7±0.6	5.11±0.32	56.9±7.0	0.281±0.005
4 hours	14.7±0.9	53.2±1.4	22.1±0.7	9.89±0.65	2.52±0.25	24.2±3.4	0.313±0.019
22 hours	13.2±0.9	14.4±1.3	7.72±0.70	5.66±0.61	2.20±0.36	9.96±0.73	0.344±0.004
IgG	Blood	Lung	Liver	Kidney	Heart	Spleen	Brain
22 hours	5.31±0.98	0.738±0.243	1.73±0.20	1.81±0.46	0.373±0.053	6.81±0.21	0.0632±0.0459

Supplemental Table 5. Pharmacokinetics and biodistribution of ICAM-targeted and IgG lipid nanoparticles (LNP) injected 2 hours after intrastriatal TNF-α. Experimental design as in **Figure 3a**. Values reported as percent of injected dose/g organ (%ID/g). Samples were collected 30 minutes after IV injection of LNP. Data reported as mean ± SEM. N = 3/group.

Surface Marker	**Clone**	**Fluorophore**
CD3ϵ	145-2C11	PE
CD11b	M1/70	V500
CD45.2	104	FITC
Ly6G	1A8	PerCP-Cy5.5
Ly6C	AL-21	APC-Cy7
iNOS	CXNFT	APC
Arginase 1	A1exF5	PE

Supplemental Table 6. Antibodies used for flow cytometry

Surface Marker	**Working concentration**	**Manufacturer**
CD68	10 µg/ml	Bio-Rad
VCAM (MK2.7)	5 µg/ml	In house production

Supplemental Table 7. Antibodies used for histology