Isolation and Identification of Leukocyte Populations in Intracranial Blood Collected During Mechanical Thrombectomy

Supplemental Information

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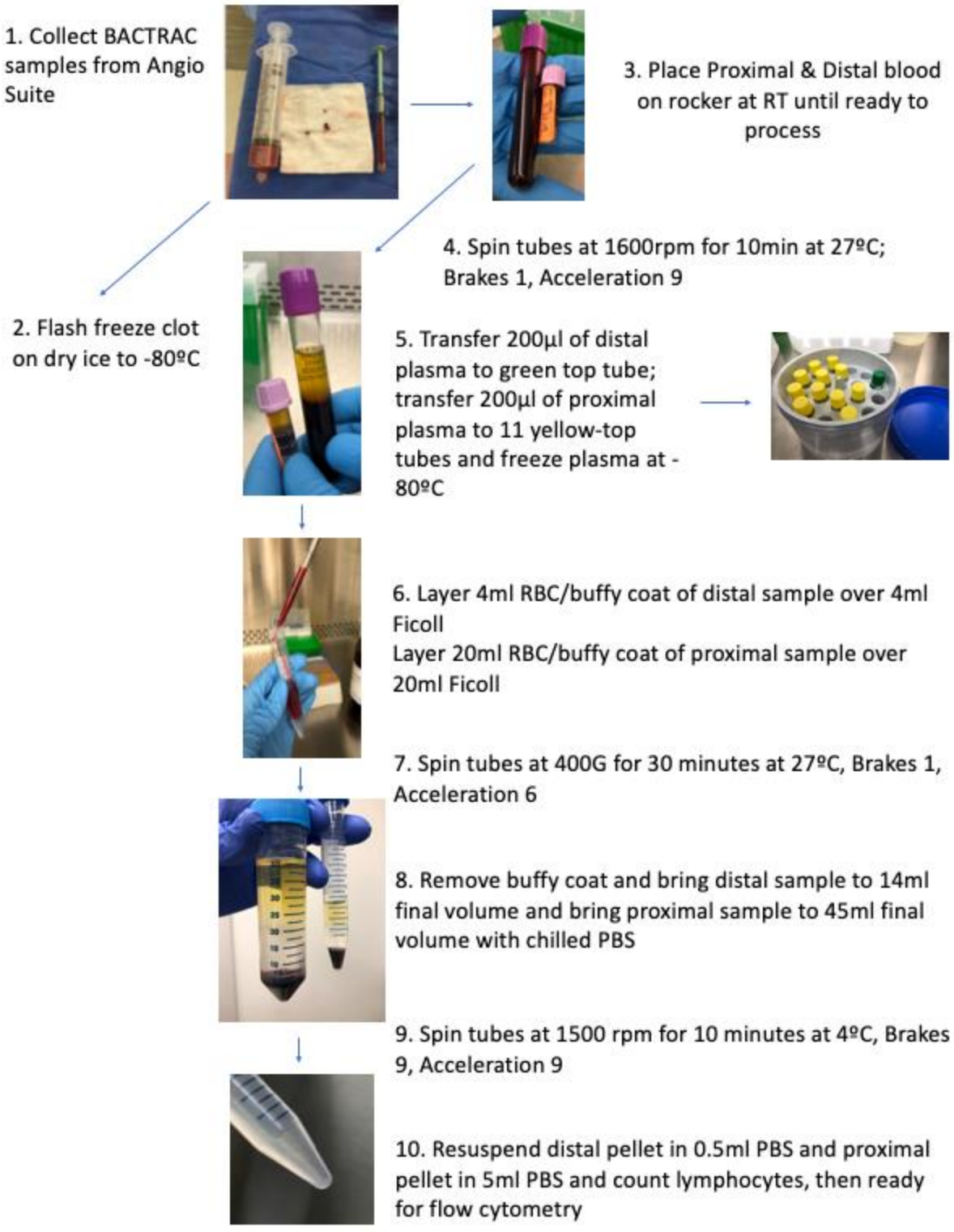
Supplementary Table 1: Freezing medium composition.

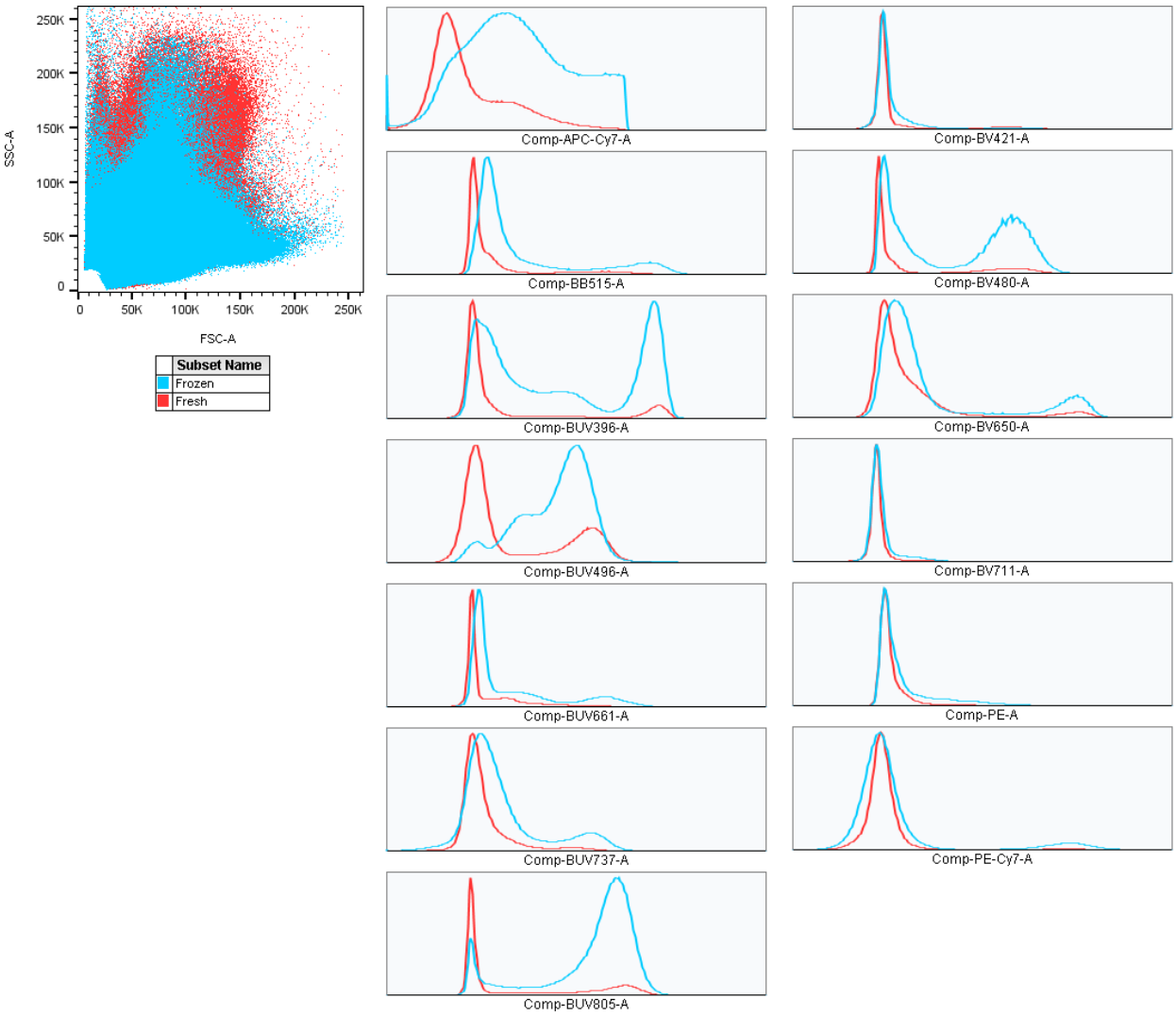
Cryopreservation Medium A (Human Serum)	**Cryopreservation Medium B (DMSO)**
2.5 ml RPMI 1640	10 ml RPMI 1640
10 ml Heat Inactivated Human AB Serum	2.5 ml DMSO
7.5 µl 1M HEPES Buffer	100 µl 1M HEPES Buffer
75 µl L-Glutamine	100 µl L-Glutamine
~12.5 ml	~12.5 ml

Supplementary Table 2: Antibody catalog numbers and conjugates.

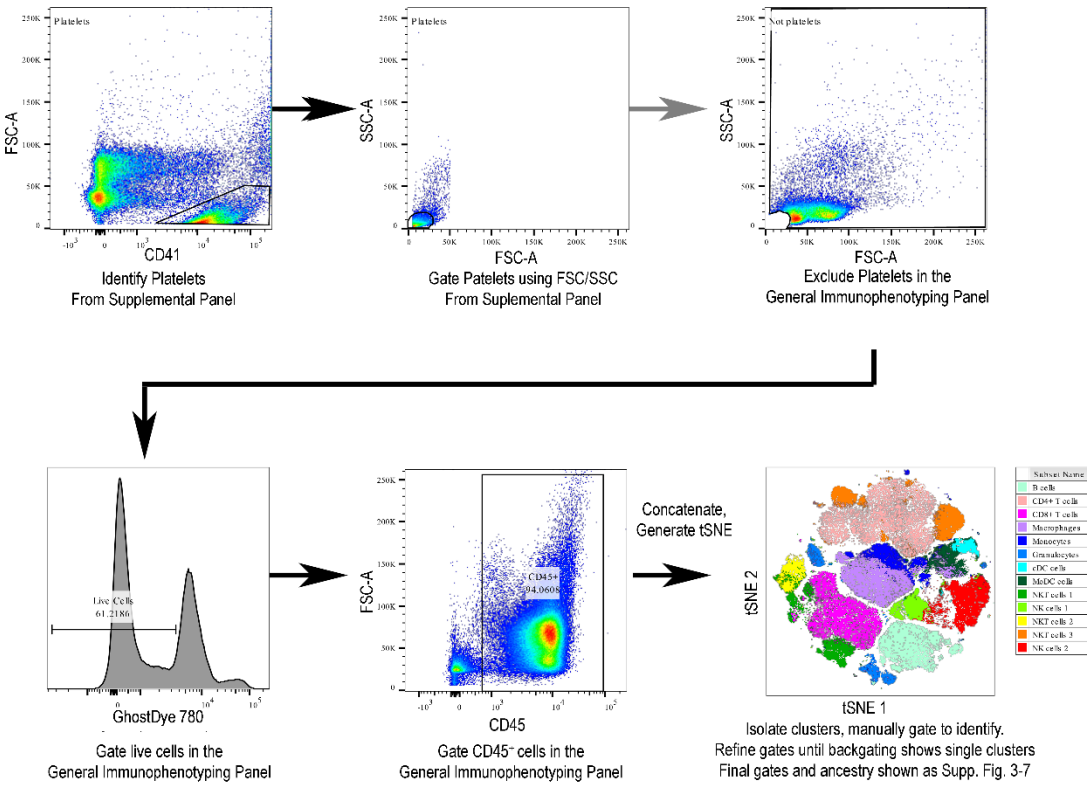
Target	Clone	Conjugate	Catalog number
General Immunophenotyping Panel			
CD3	UCHT1	BV480	BD Horizon #566105
CD4	M-T477	BUV396	BD Biosciences #742738
CD8	RPA-T8	BV650	BD Biosciences #563821
CD11b	ICRF44	BB515	BD Horizon #564517
CD11c	B-ly6	BUV661	BD Horizon #812987
CD14	M5E2	BUV737	BD Horizon #612783
CD19	HIB19	PE-Cy7	TONBO #60-0199-T100
CD45	HI30	BUV805	BD Horizon #612891
CD66b	G10F5	BV421	BD Horizon #562940
CD138	MI15	BV711	BD Horizon #583184
CXCR3	1C6/CXCR3	BUV496	BD Biosciences #741178
CD161	HP-3G10	PE	TONBO #50-1619-T100
Ghost Dye (viability)	N/A	Red 780	TONBO #13-0865-T100
Supplemental Panel			
CD31	MBC78.2	BUV563	BD Biosciences #748916
CD41a	HIP8	APC	BD Pharmingen #559777
CD45	HI30	BUV805	BD Horizon #612891
Ghost Dye (viability)	N/A	Red 780	TONBO #13-0865-T100



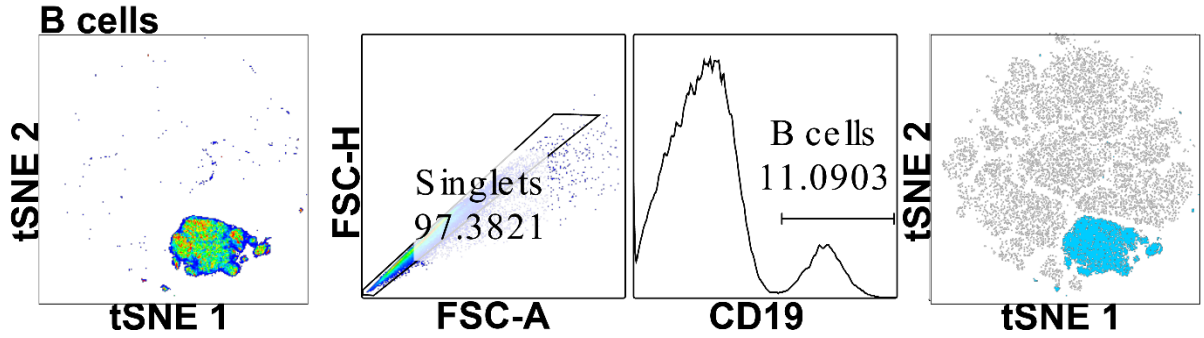
Supplementary Figure 1: BACTRAC sample collection protocol. Flowchart describing the original Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol with our modifications for small sample volume lymphocyte isolation.



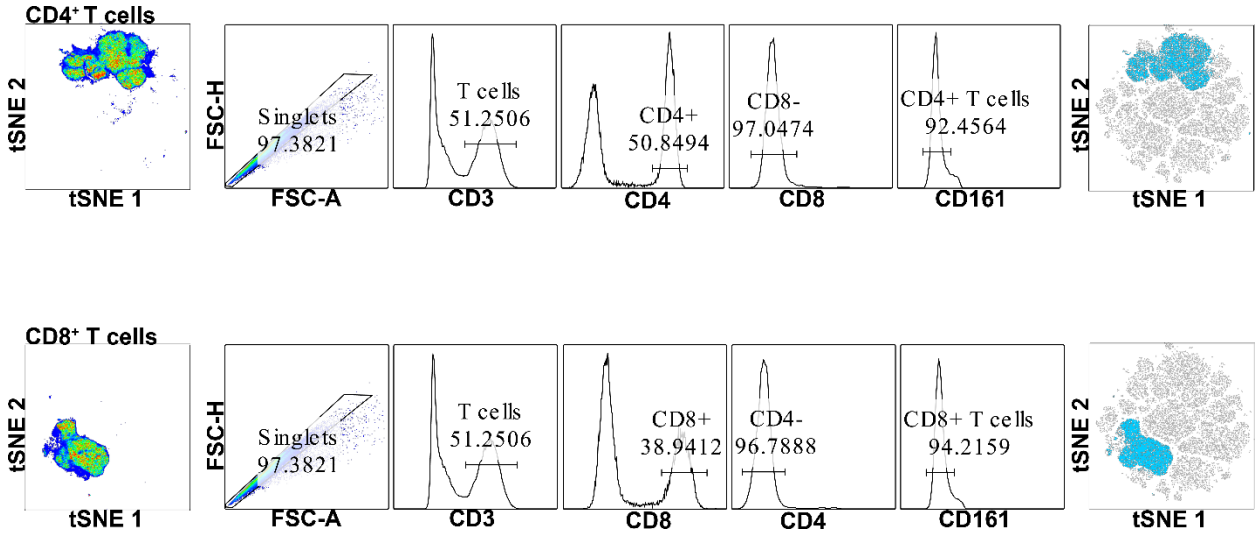
Supplemental Figure 2: Antigen profiles before and after cryopreservation. Left: forward scatter by side scatter profiles before (red) and after (blue) cryopreservation. Right: Histograms of each antigen before (red) and after (blue) cryopreservation. This figure represents all events from 12 arterial samples split into two aliquots; one analyzed at time of collection, and another frozen for at least 1 month before analysis.



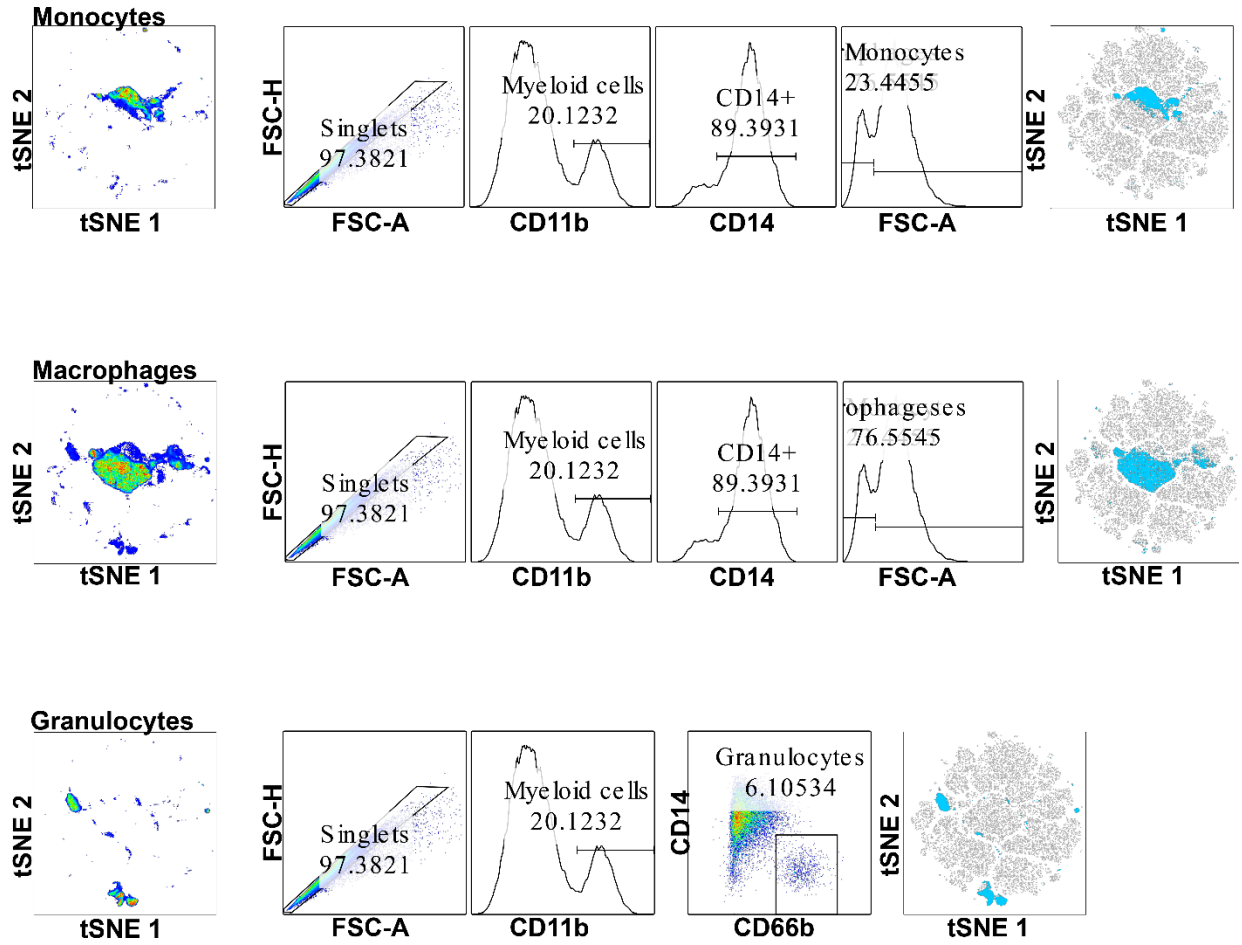
Supplemental Figure 3: General Immunophenotyping Panel gating strategy. Briefly, the supplemental panel was used to identify platelets as CD41^{+} FSC^{low} events, converted to a FSC-A by SSC-A gate, and applied to the full General Immunophenotyping Panel. Dead cells (GhostDye 780^{+} events) were excluded, and live cells were gated on CD45 to include only leukocytes. All collected samples were downsampled to 2000 events and concatenated to a single data file. All fluorescent parameters plus FSC-A and SSC-A were used in the tSNE algorithm to generate the final plot.



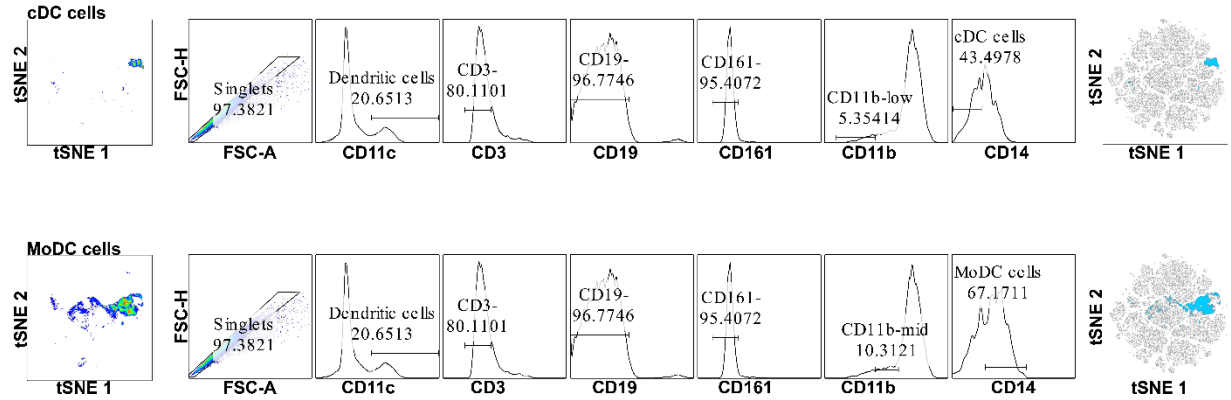
Supplemental Figure 4: B cell gating strategy. B cells were identified as $CD19^{+}$.



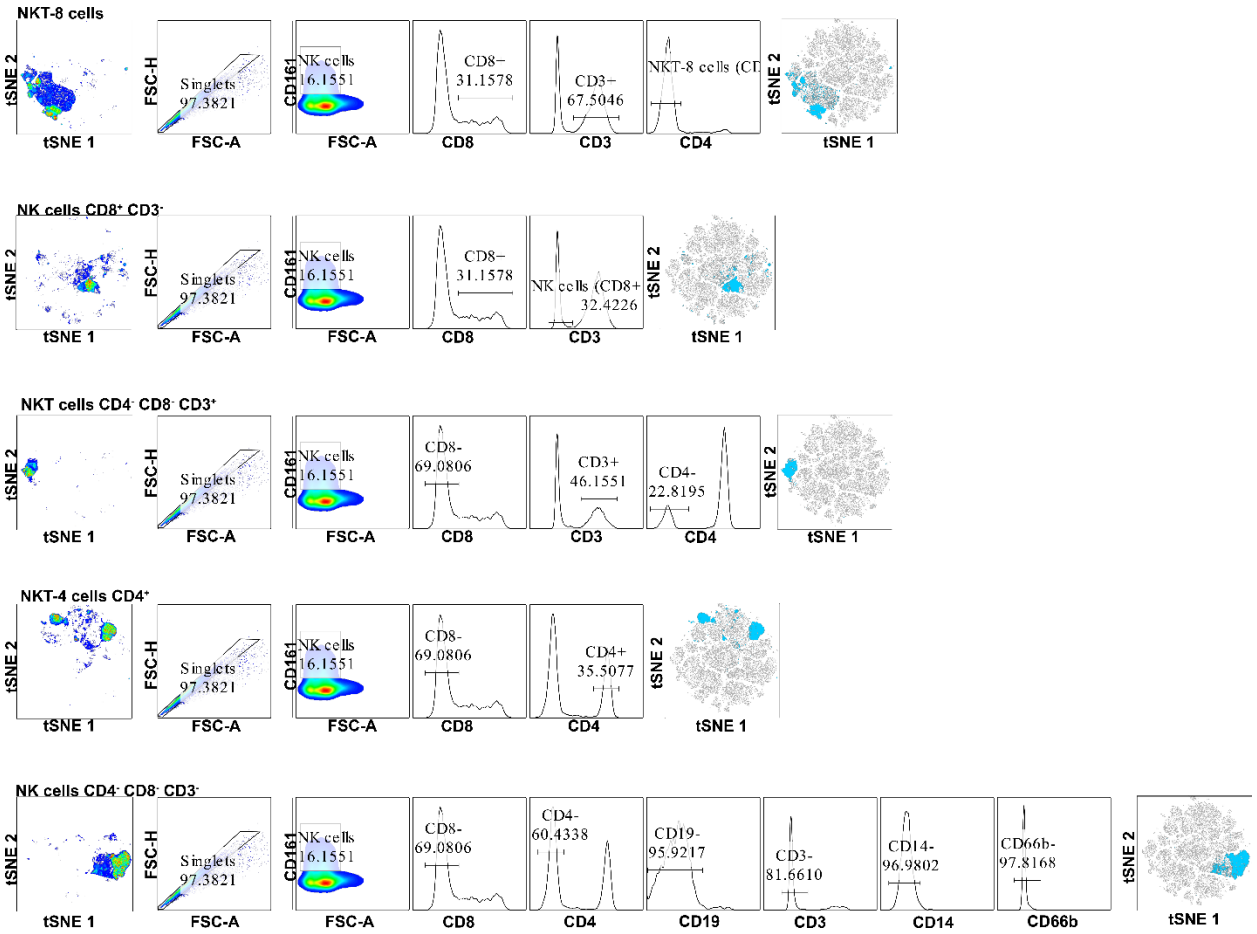
Supplemental Figure 5: T cell gating strategy. T cells were identified first as $CD3^+$, then as either $CD4^+$ or $CD8^+$, confirmed with the complementary gate, and finally gated as $CD161^-$.



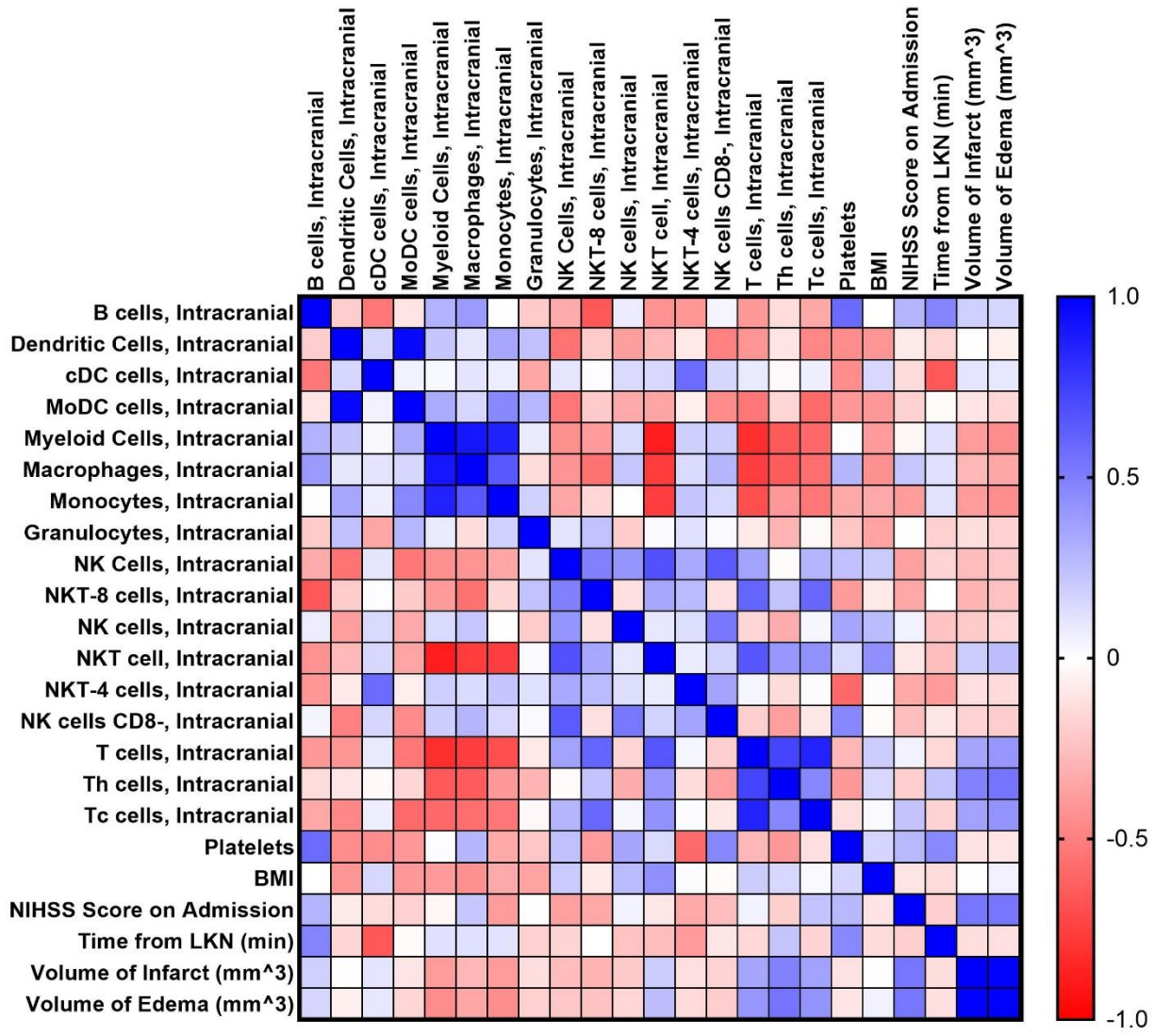
Supplementary Figure 6: Myeloid cell gating strategy. Myeloid cells were first identified as CD11b^{+}. Monocytes and macrophages were further identified as CD14^{+} and distinguished as either FSC-A^{low} or FSC-A^{high}. Granulocytes were identified as CD66b^{+} CD14^{low}.



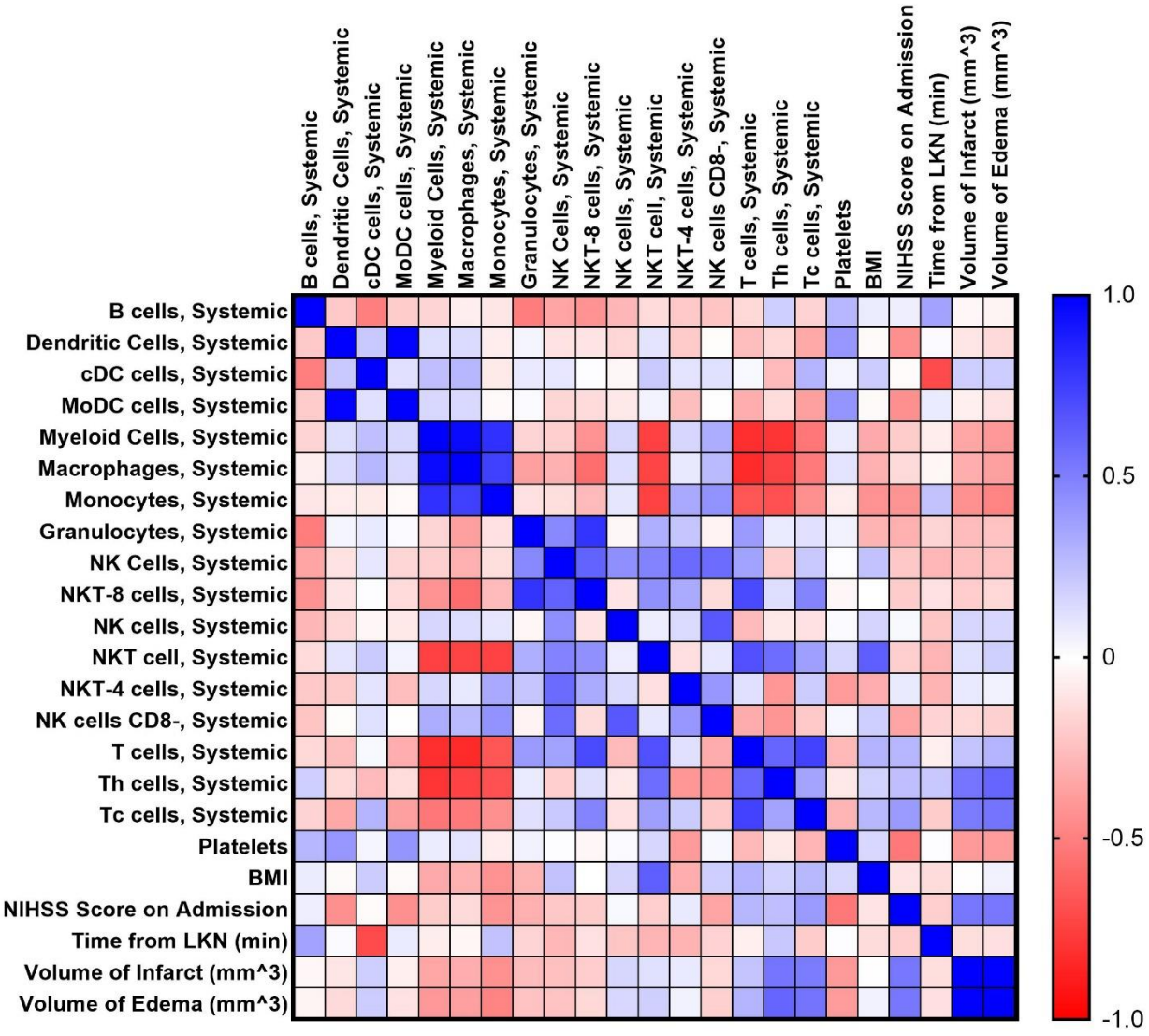
Supplemental Figure 7: Dendritic cell gating strategy. Dendritic cells were first identified as $CD11c^{+}$, then confirmed as $CD3^{-}CD19^{-}CD161^{-}$. Classical dendritic cells (cDCs) were identified as $CD11b^{low}CD14^{-}$. Monocyte-derived dendritic cells (MoDCs) were identified as $CD11b^{mid}CD14^{mid}$.



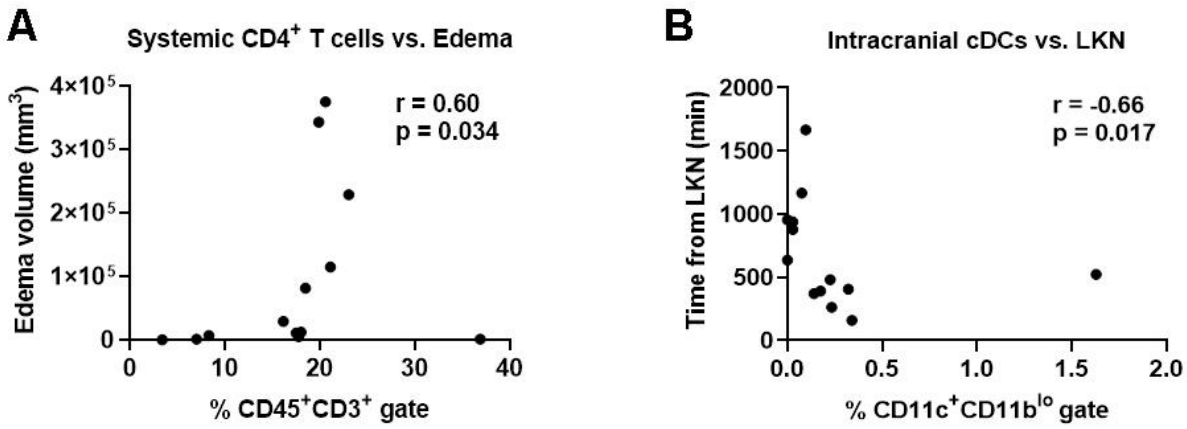
Supplemental Figure 8: NK-like cell gating strategy. NK cells were first identified as CD161^{+}, then using CD8, CD3, CD4, to subset various NK-like populations. Finally, one NK cell population remained which was negative for everything except CD161.



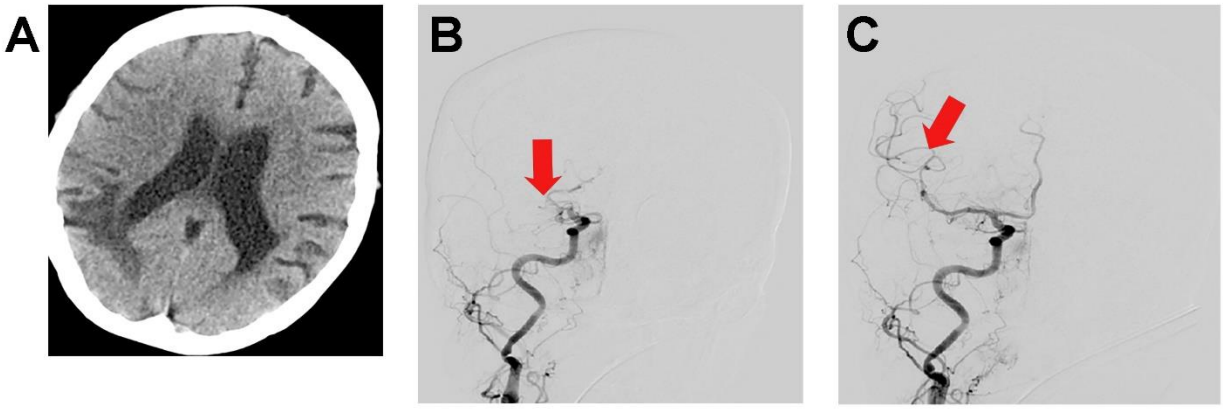
Supplemental Figure 9. Intracranial Blood Correlation Matrix. Correlation matrix shows the intensity of a positive (blue) or negative (red) correlation, as defined by the scale on the right. Leukocyte populations were correlated using non-parametric spearman r values to determine within-patient associations with body mass index (BMI), NIH Stroke Scale (NIHSS), time from last known normal (LKN), and volume of infarct and edema.



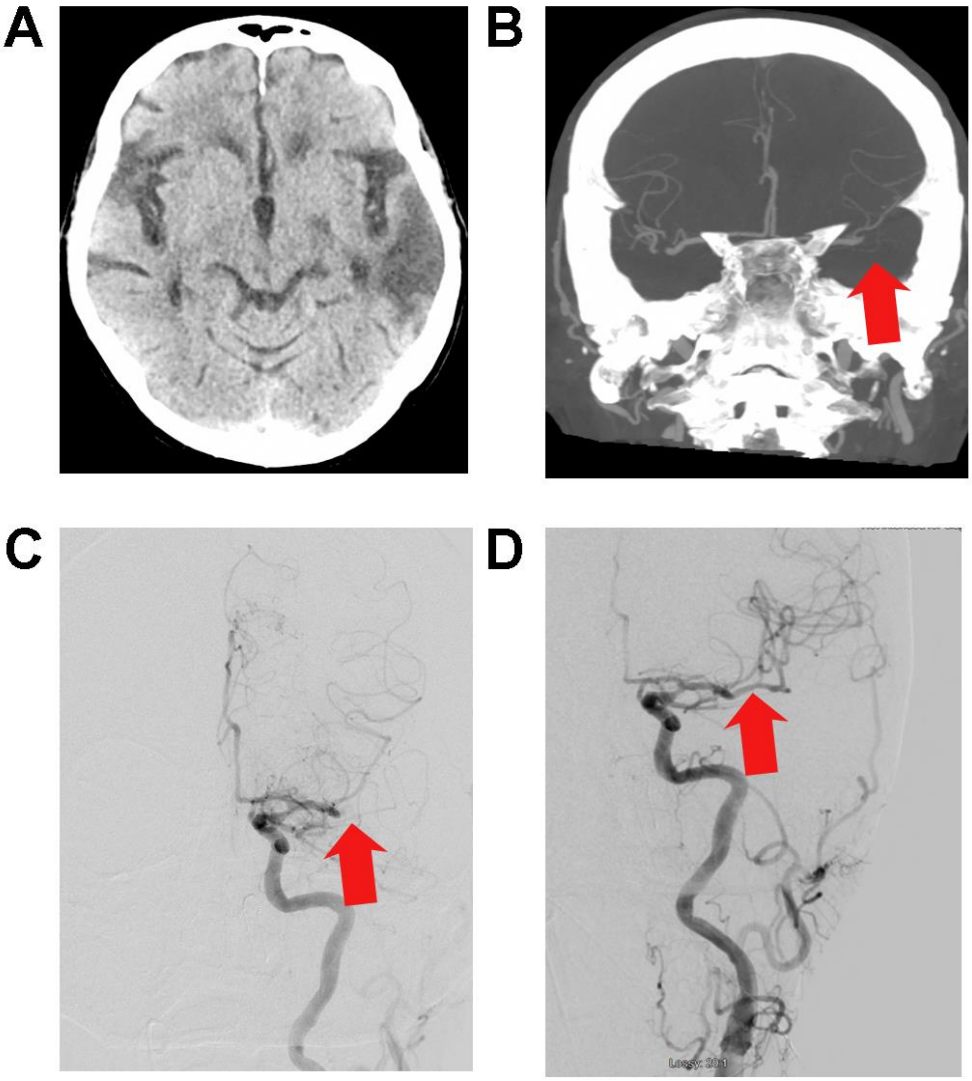
Supplemental Figure 10. Systemic Blood Correlation Matrix. Correlation matrix shows the intensity of a positive (blue) or negative (red) correlation, as defined by the scale on the right. Leukocyte populations were correlated using non-parametric spearman r values to determine within-patient associations with body mass index (BMI), NIH Stroke Scale (NIHSS), time from last known normal (LKN), and volume of infarct and edema.



Supplemental Figure 11. Association of leukocyte subpopulations to within-patient parameters. (A) Spearman non-parametric correlation found significant association with systemic CD4+ T cell populations compared to infarct volume. (B) There was also an association between intracranial classical dendritic cell (cDCs) populations compared to time from last known normal (LKN). r and p values as shown.



Supplemental Figure 12: Patient 112 with hemorrhagic transformation. Patient 112, a 76-year-old woman, presented to a rural hospital as a stroke alert. Patient's primary complaints were left sided weakness, left sided numbness, and speech problems corresponding to NIH Stroke Scale 18. Her medical history included chronic atrial fibrillation with prosthetic mitral valve and warfarin therapy, hypertension, and history of prior stroke with unknown status of residual deficits. Intravenous tissue plasminogen activator (tPA) was given to the patient prior to transfer to the university hospital. Upon arrival, non-contrast computerized tomography (CT) indicated (A) hypodensity in the right middle cerebral artery (rMCA) region indicating an acute infarct. (B, C) The patient immediately underwent mechanical thrombectomy with full reperfusion. Right internal carotid artery (ICA) injection on digitally subtracted angiogram (DSA). Pre-thrombectomy (B) shows an abrupt cut off (red arrow) at the origin in right MCA. Post-thrombectomy (C) shows complete revascularization after mechanical thrombectomy (red arrow). Antiplatelet and atorvastatin therapy was subsequently administered.



Supplemental Figure 13: Patient 103 with new occlusion on day 2 of stroke. Patient 103, an 87-year-old female, also presented to a rural hospital as a stroke alert. Patient's primary complaints were slurred speech, altered mental status, left-sided weakness, and left facial droop corresponding to NIH Stroke Scale 9. Her medical history included atrial fibrillation with rivaroxaban therapy. Intravenous tPA was contraindicated due to rivaroxaban. (A) Initial CT revealed acute hypodensity in the left temporal lobe indicating an acute infarction. angiogram revealed significant atherosclerosis of bilateral carotid arteries. The following day, patient developed new right-sided hemiparesis and facial droop. CT angiogram revealed a new occlusion of the left inferior M2 territory. (B) Cut off in the left M2 inferior division on day 2 (red arrow) led to a (C) pre-thrombectomy left internal carotid injection showing cut off in the left inferior temporal M2 (red arrow). Patient immediately underwent mechanical thrombectomy with complete reperfusion. (D) Post-thrombectomy angiogram shows recanalization of left M2 (red arrow).