Supplemental Materials:

Temporal Immune Profiling in the CSF and Blood of Patients with Aneurysmal Subarachnoid Hemorrhage

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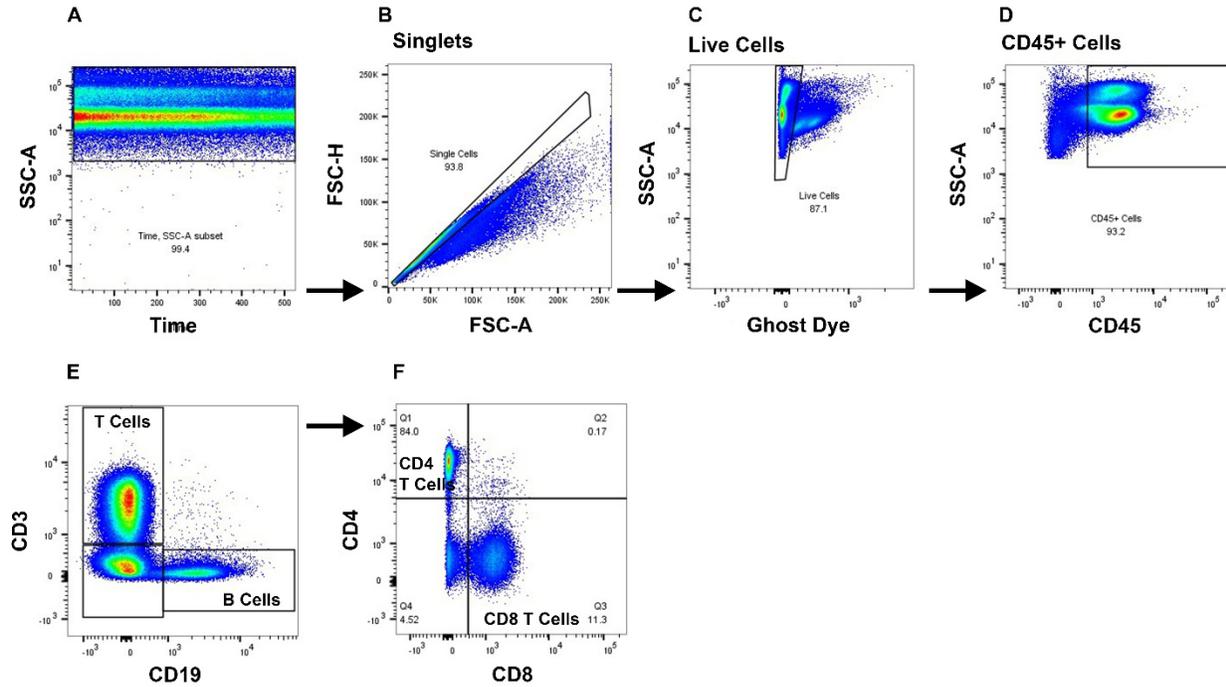
Supplemental Figure 1: Flow cytometry gating strategy. Samples were gated as shown in Supplemental Figure 1A-D, with numeric tags assigned for later unblinding. Live CD45+ cells were concatenated for high-dimensional analysis. UMAP was used to reduce high-dimensional data to two dimensions, followed by FlowSOM for unbiased clustering on the dimensionality reduction map. This approach yielded automatic cluster gates revealing unique populations, as shown in Figure 1. Cell populations from FlowSOM-driven meta clustering were identified based on markers listed in Figure 1's legend. Supplemental Figure 1E-F display traditional gating of CD4 and CD8 T cells for verification of FlowSOM meta clusters.
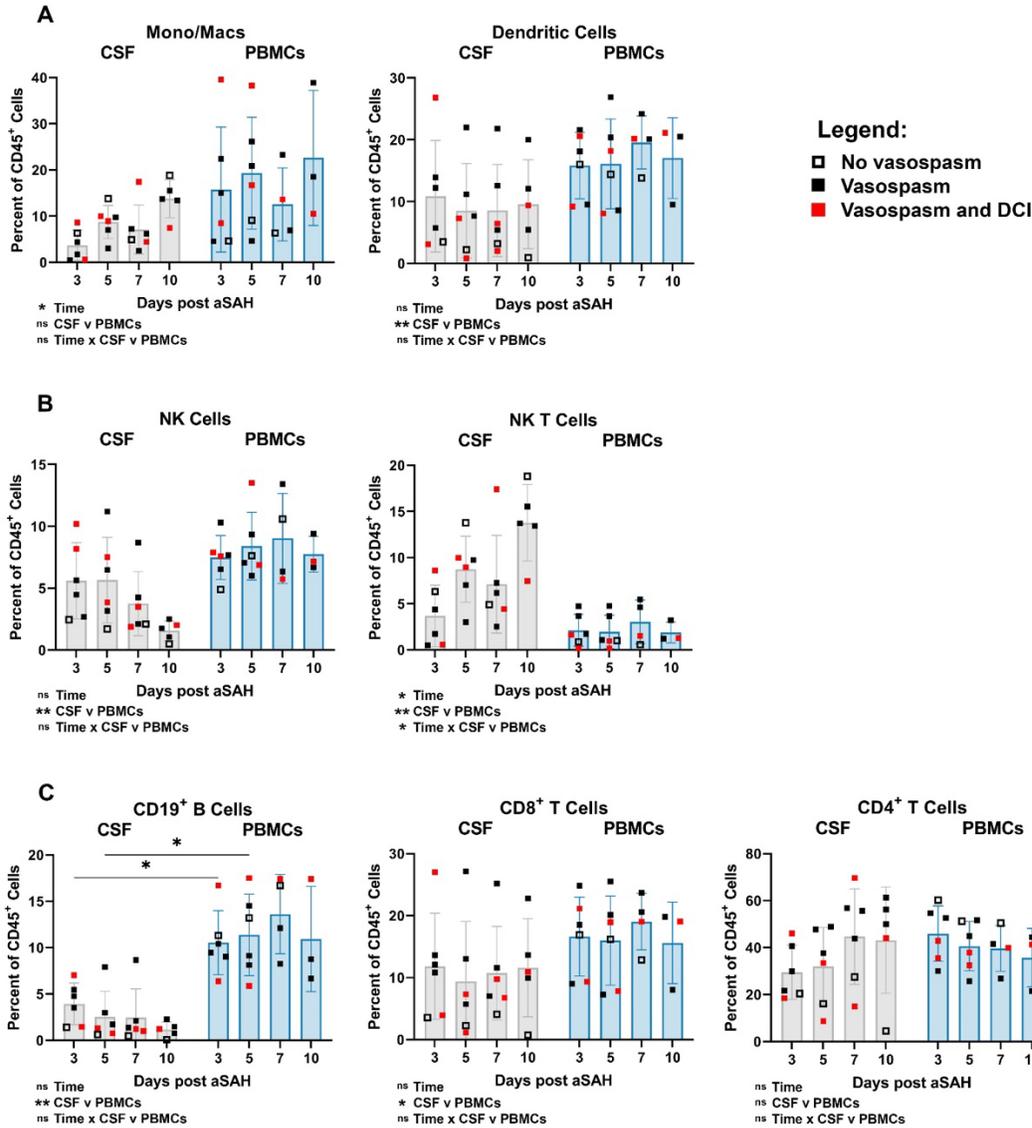
Supplemental Figure 2: Comparison of immune cell populations in CSF and PBMCs from aSAH patients.
A) Innate immune cells. B) NK cells and NK T cells. C) Lymphocytes. Bar graphs represent mean values; error bars indicate SD. Cell populations are shown as a percentage of live CD45+ cells. Hollow squares indicate patients without vasospasm, filled squares indicate patients with vasospasm, and red squares indicate patients with vasospasm and delayed cerebral ischemia (DCI). A 2-way ANOVA (mixed-effects model REML) with Geisser-Greenhouse correction was performed, followed by Tukey's multiple comparisons test for each comparison. Fixed effects (type III) are listed under each graph. Multiple comparisons were only conducted for corresponding days between CSF and PBMCs. Day 14 PBMC data is not shown due to fitting a full model for column effect, row effect, and column/row interaction effect. *$P<0.05$; **$P<0.01$; ns = not significant. n = 6 patients with matched CSF and PBMC samples. Total samples: CSF, n = 23; PBMC, n = 19
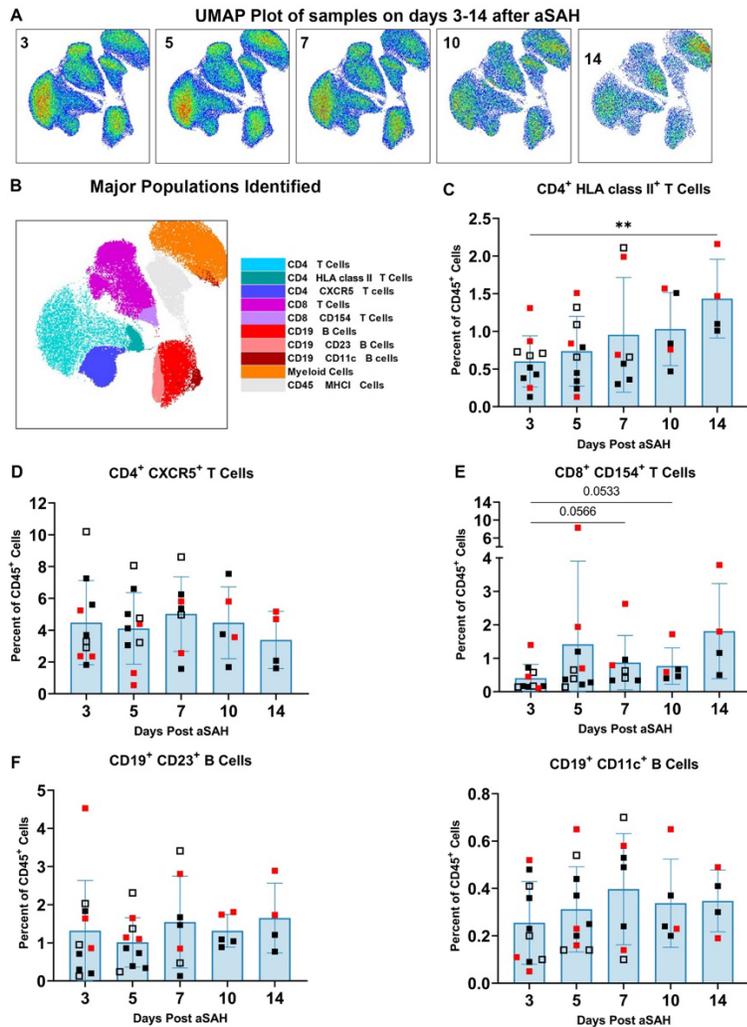
Supplemental Figure 3: Specific T and B cell subpopulations from aSAH patient PBMC samples over 14 days. A) Flow cytometry UMAP plot of all samples across a 14-day time span. B) Major populations identified. C) CD4+ MHCII+ T cells. D) CD4+ CXCR5+ T cells. E) CD8+ CD154+ T cells. F) CD19+ CD23+ B cells and CD19+ CD11c+ B cells. Bar graphs represent mean values; error bars indicate SD. Cell populations are shown as a percentage of live CD45+ cells. Hollow squares indicate patients without vasospasm, filled squares indicate patients with vasospasm, and red squares indicate patients with vasospasm and delayed cerebral ischemia (DCI). Statistical analysis: A one-way ANOVA (mixed-effects model REML) with Geisser-Greenhouse correction was performed, followed by Dunnett's multiple comparisons test comparing the means of each day to the mean from day 3. *P<0.05; **P<0.01. Data from 10 patients, n = 36 samples.
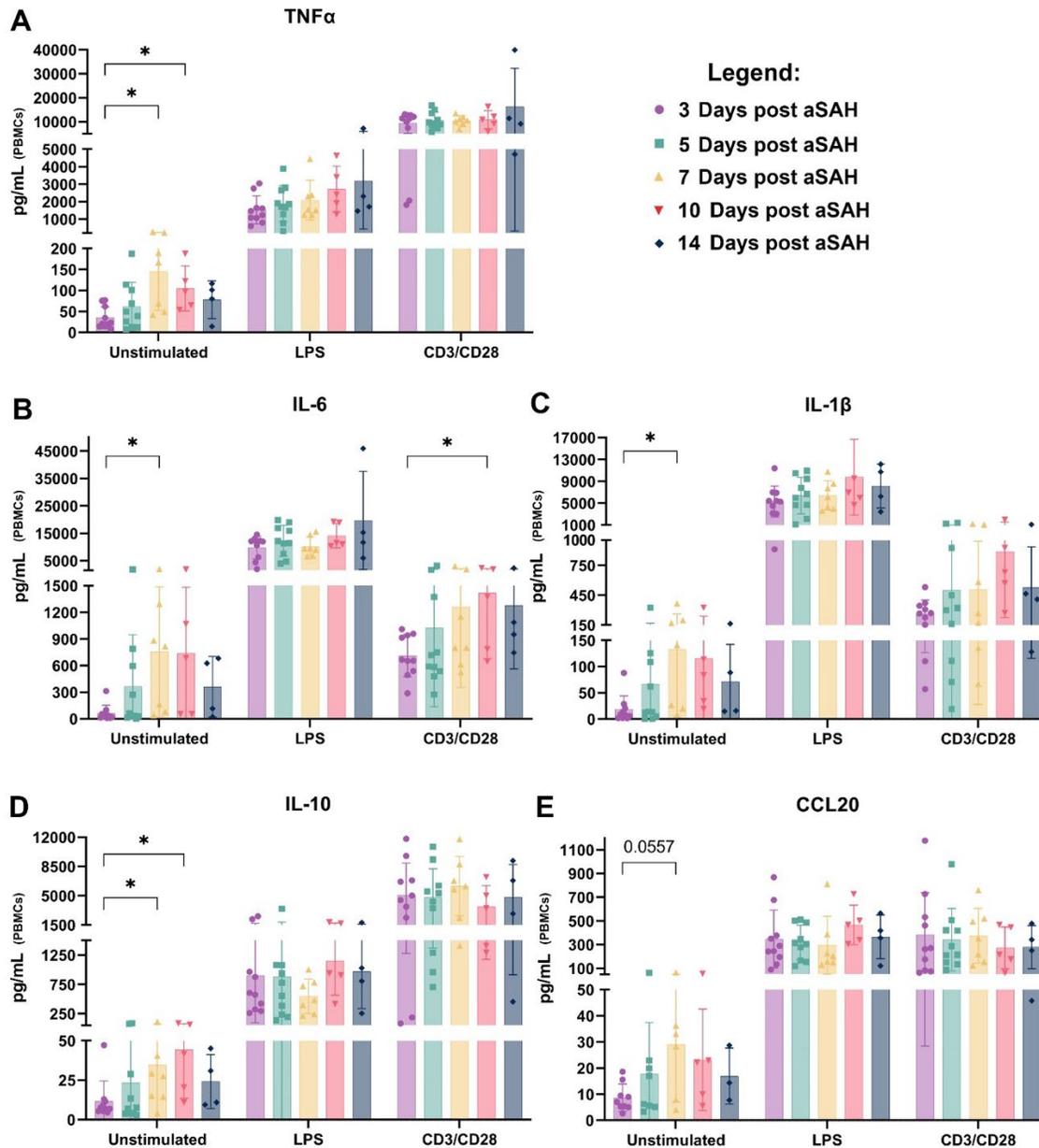
Supplemental Figure 4: Select PBMC cytokines over days 3-14 comparing cytokine production in unstimulated, LPS, and CD3/CD28 stimulated conditions. A) TNFα B) IL-6 C) IL-1β D) IL-10 E) CCL20. We conducted a 2-way ANOVA or mixed-effects model (REML). We tested fixed effects (type III) for stimulation, days post-aSAH, and Stimulation x days post-aSAH. Stimulation was significant to at least $P<0.01$ in all cases, while days post-aSAH, and Stimulation x days post-aSAH did not yield significance. Additionally, we performed Fisher's LSD for a planned comparison of cytokine levels at different days against the first collection time point. *$P<0.05$; **$P<0.01$. Data shown is from 10 patients, with $n = 108$ samples or $n = 36$ per condition (Unstimulated, LPS, CD3/CD28).

Supplemental Table 1: Flow Cytometry Key Resource Table, Extracellular Panel

Antibody	Fluorophore	Isotype/Clone	Vendor	Catalog number
Fixable Viability Dye 780	APC-Cy7	N/A	BD	565388
CD45	BUV805	HI30	BD Horizon	612891
CD19	PE-Cy7	HIB19	TONBO	60-0199-T100
CD3	BV480	UCHT1	BD Horizon	566105
CD4	BUV396	M-T477	BD Biosciences	742738
CD8	BV650	RPA-T8	BD Biosciences	563821
CD11b	BB515	ICRF44	BD Horizon	564517
CD11c	BUV661	B-ly6	BD Horizon	612967
CD14	BUV737	M5E2	BD Horizon	612763
CD66b	BV421	G10F5	BD Horizon	562940
CD138	BV711	MI15	BD Horizon	583184
CXCR3 (CD183)	BUV496	1C6/CXCR3	BD Biosciences	741178
CD161	**PE**	**HP-3G10**	**BD Biosciences**	**566843**

Supplemental Table 2: Flow Cytometry Key Resource Table, Extracellular Panel II

Antibody	Fluorophore	Isotype/Clone	Vendor	Catalog number
Fixable Viability Dye 780	APC-Cy7	N/A	BD	565388
CD45	BUV805	HI30	BD Horizon	612891
CD19	BUV563	SJ25C1	BD	612916
CD3	BV480	UCHT1	BD Horizon	566105
CD4	BUV396	SK3	BD	563550
CD8b	BV650	RPA-T8	BD	742393
CD11b	BV711	ICRF44	BD	740771
CD11c	PE-Cy7	B-ly6	BD	561356
CD25	BV421	BC96	BD	567485
CD23	BV605	M-L233	BD	740414
CCR7	BUV496	2-LI-A	BD	749827
CXCR5	R718	RF8B2	BD	752012
CCR3 (CD193)	APC	5 E8	BD	558208
CD154 (CD40L)	PE-CF594	TRAP1	BD Horizon	563589
IL-21R	BB700	2SX21R	Invitrogen	46-3601-42
CD69	BV786	FH50	BD Horizon	563834
MHC I (HLA I)	BB515	W6/32	Biolegend	311404
MHC II (HLA DR DQ DP)	**PE**	**TU39**	**Biolegend**	**361716**