

Supplementary Material

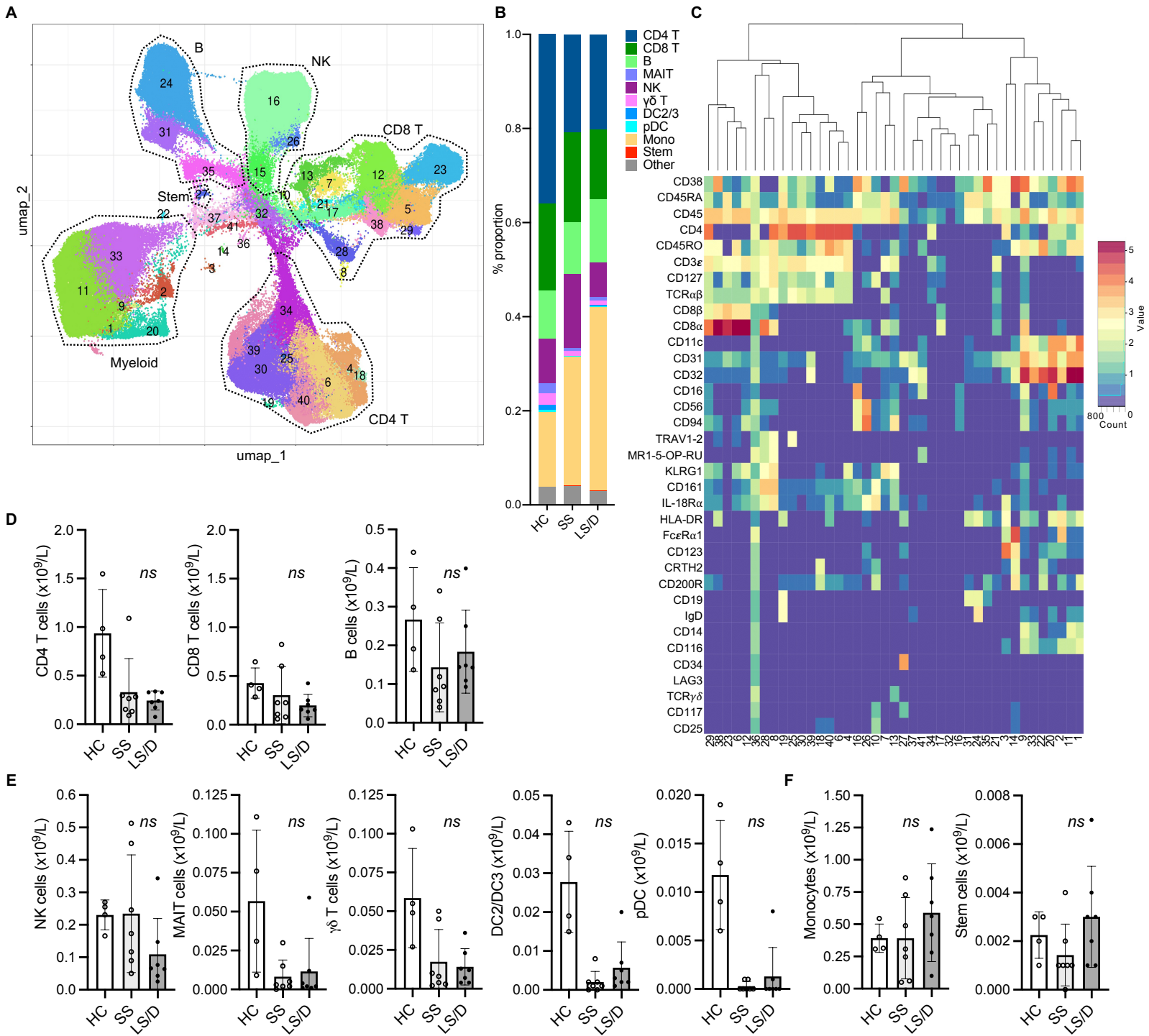


Fig. S1. Major PBMC subsets fail to identify Long-Stay/Died patients (Initial Cohort).

UMAP projection of ungated CyTOF-derived data from the Initial cohort (n=14) (A). Proportion of immune cell subsets in Healthy Controls (HC), Short-Stay (SS) and Long-Stay/Died (LS/D) patient outcome groups (B). Mean marker expression heatmap of clusters shown in a (C). Absolute counts of adaptive PBMC subsets (CD4 T, CD8 T, B) (D). Innate and unconventional subsets (NK, MAIT, $\gamma\delta$ T, pDC, DC2/3) (E). Monocytes and stem cells (F). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p \geq 0.05$ by two-tailed, two-sample unequal variance Student's t-test.

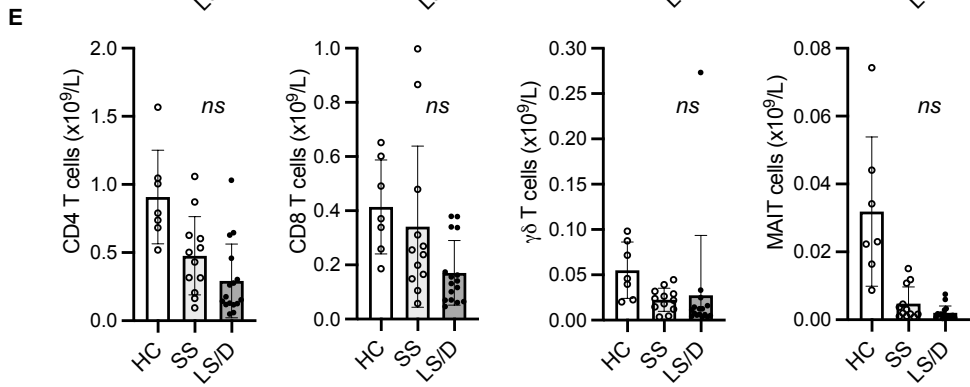
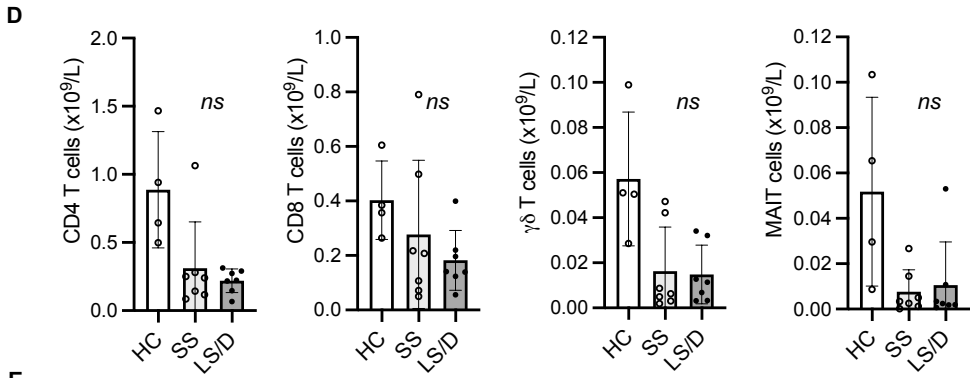
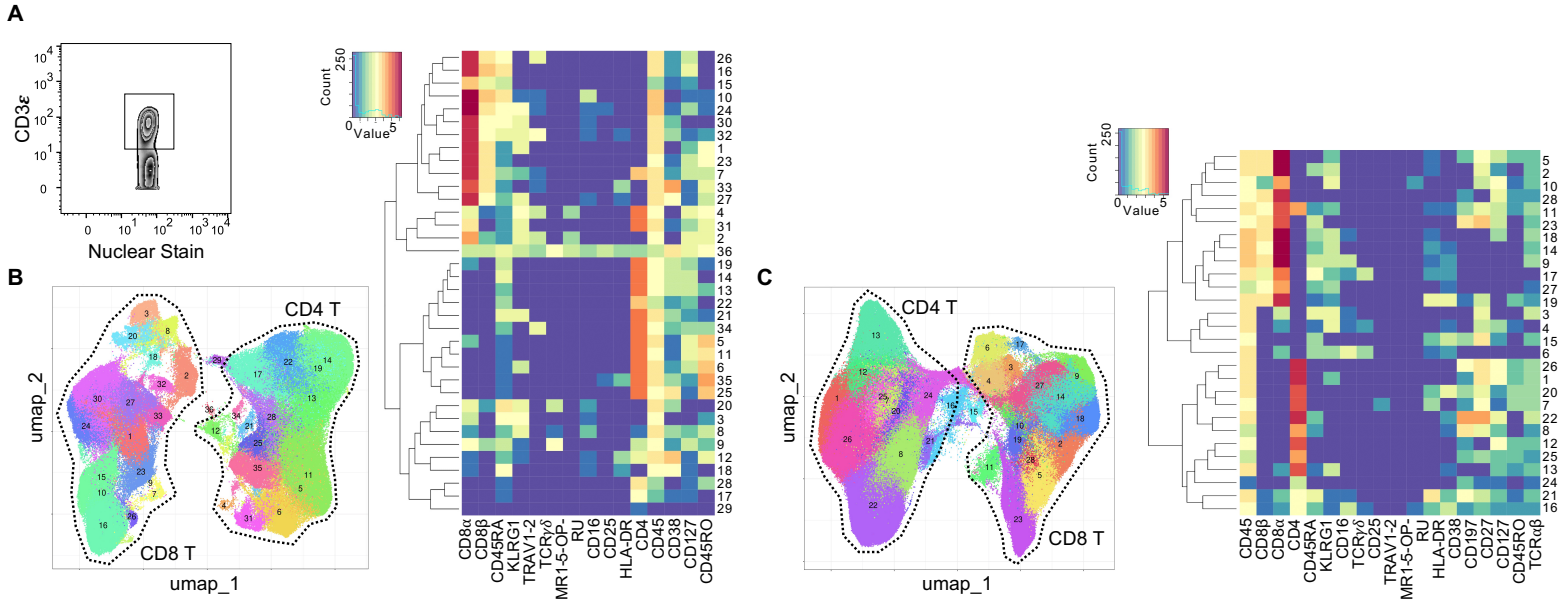


Fig. S2. T cell subsets fail to identify Long-Stay/Died patients. Representative gating of CD3⁺ cells (**A**). Initial Cohort UMAP projections of CD3⁺ gated cells (all samples combined; limited clustering channels) and mean marker expression heatmap (**B**). Same as in B but for the Replication Cohort (**C**). Initial Cohort absolute counts of T cell subsets identified based on gated clustering (**D**). Same as in D but for the Replication Cohort (**E**). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p \geq 0.05$ by two-tailed, two-sample unequal variance Student's t-test.

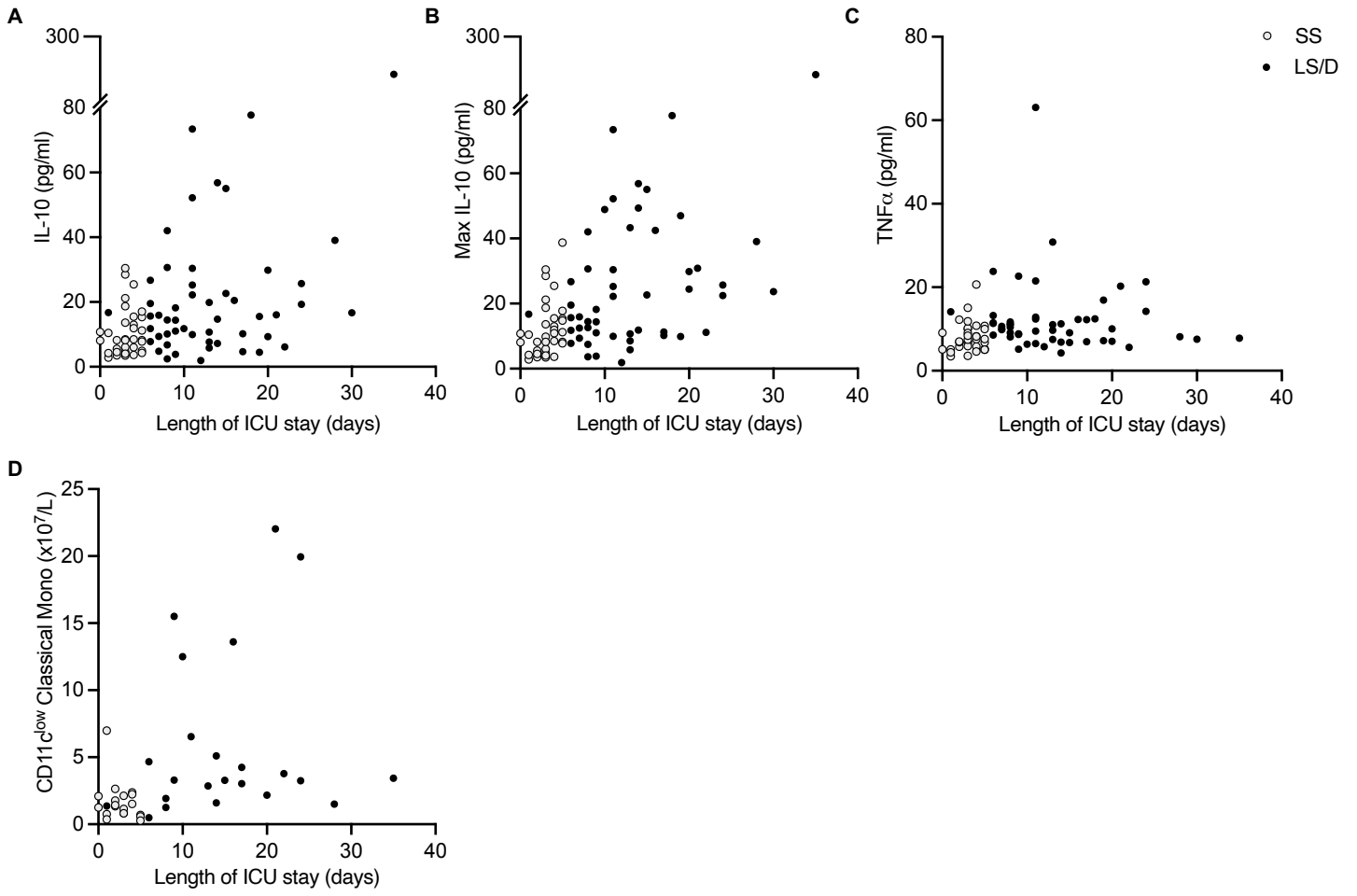


Fig. S3. Cytokine and length of ICU stay correlations. Correlations of length of ICU stay with ICU admission serum IL-10 levels (**A**), maximum serum IL-10 during ICU stay (**B**) serum TNF α ICU admission levels (**C**) and CD11c^{low} Classical Monocytes (**D**). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p \geq 0.05$ by two-tailed, two-sample unequal variance Student's t-test and R^2 by two-tailed Pearson correlation with 95% confidence interval.

Table S1. List of CyTOF antibodies

Antibody/Tetramer	Metal/Fluorophore*	Clone	Company	Used in Cohort
CD1c	151Eu	L161	Biolegend	Replication
CD3 ϵ	143Nd	OKT3	Biolegend	both
CD4	174Yb	SK3	Biolegend	both
CD8 α	168Er	SK1	Biolegend	both
CD8 β	141Pr	SID18BEE	eBioscience	both
CD11c	147Sm	Bu15	Biolegend	both
CD14	153Eu	M5E2	Biolegend	both
CD16	158Gd	3G8	Biolegend	both
CD19	142Nd	HIB19	Biolegend	both
CD25	169Tm	BC96	Biolegend	both
CD27	175Lu	O323	Biolegend	Replication
CD31	145Nd	WM59	Biolegend	both
CD32	160Gd	IV.3	Stemcell Technologies	both
CD34	156Gd	581	Biolegend	both
CD38	106Cd	HIT2	Biolegend	both
CD45	89Y	HI30	Biolegend	both
CD45RA	110Cd	HI100	Biolegend	both
CD45RO	112Cd	UCHL1	Biolegend	both
CD56	148Nd	NCAM16.2	BD Bioscience	both
CD94 (NKG2C)	161Dy	DX22	Biolegend	both
CD116	150Nd	4H1	Biolegend	both
CD117	171Yb	104D2	Biolegend	Initial
CD123	164Dy	6H6	Biolegend	both
CD127	165Ho	A019D5	Biolegend	both
CD161	159Tb	HP-3G10	Biolegend	both
CD197/CCR7	171Dy	G043H7	Biolegend	Replication
CD200R	173Yb	0X-108	BD Bioscience	both
CD294 (CRTH2)	163Dy	BM16	Biolegend	both
CD301 (CLEC10A)	154Sm	H037G3	Biolegend	Replication
CD304 (NRP1)	172Yb	12C2	Biolegend	Replication
Fc ϵ R α 1	176Yb	AER-37	Biolegend	both
HLA-DR	170Er	L243	Biolegend	both
IgD	116Cd	IA6-2	Biolegend	both
IL-18R α	162Dy	H44	Biolegend	both
KLRG1	144Nd	SA231A2	Biolegend	both
LAG3	175Lu	11C3C65	Biolegend	Initial
TCR $\alpha\beta$	155Gd	T10B9.1A-31	BD Bioscience	both
TCR $\gamma\delta$	152Sm	B1	Biolegend	both
TRAV1-2	115Ln	3C10	Biolegend	both
Anti-APC (Secondary)	149Sm	APC003	Biolegend	both
MR1-5-OP-RU (Primary)	APC	NA	NIH Tetramer Facility	both

Table S2. Mean absolute counts and p-values (ungated cluster analyses)

Cohort	Total cell subset	H	SS	LS/D	H vs SS	H vs LS/D	SS vs LS/D
Mean absolute counts (x10 ⁹ /L)					p-values		
Initial	CD4 T cells	0.936	0.330	0.244	0.067	0.053	0.549
	CD8 T cells	0.428	0.304	0.198	0.383	0.049	0.402
	B cells	0.267	0.143	0.184	0.176	0.335	0.509
	Monocytes	0.392	0.392	0.589	0.997	0.237	0.311
	Stem cells	0.002	0.001	0.003	0.247	0.315	0.088
	NK cells	0.230	0.234	0.109	0.961	0.032	0.150
	MAIT cells	0.057	0.008	0.012	0.122	0.140	0.721
	γδ T cells	0.058	0.018	0.014	0.077	0.065	0.705
	pDC	0.012	0.000	0.001	0.029	0.028	0.473
	DC2/3	0.028	0.002	0.006	0.026	0.034	0.205
Replication	CD4 T cells	0.911	0.511	0.309	0.008	<0.001	0.112
	CD8 T cells	0.386	0.374	0.184	0.914	0.005	0.090
	B cells	0.247	0.285	0.237	0.584	0.871	0.565
	Monocytes	0.403	0.657	0.740	0.041	0.028	0.618
	Stem cells	0.002	0.004	0.003	0.223	0.451	0.360
	NK cells	0.259	0.179	0.199	0.108	0.269	0.708
	MAIT cells	0.047	0.007	0.003	0.001	0.000	0.076
	γδ T cells	0.058	0.022	0.028	0.003	0.130	0.732
	pDC	0.009	0.001	0.001	<0.001	<0.001	0.575
	DC2/3	0.037	0.014	0.012	0.003	0.001	0.829

p-values calculated using a two-tailed, two-sample unequal variance Student's t-test.
H = Healthy; SS = Short-Stay; LS/D = Long-Stay/Died

Table S3. Mean absolute counts and p-values (gated monocyte and T cell cluster analyses)

Cohort	Monocyte subset	H	SS	LS/D	H vs SS	H vs LS/D	SS vs LS/D
Mean absolute counts (x10 ⁹ /L)					p-values		
Initial	CD11c ^{low} Classical	0.004	0.019	0.082	0.536*	0.133*	0.388*
	Total Classical	0.108	0.110	0.181	0.620	0.136	0.212
	Total Intermediate	0.218	0.247	0.377	0.939	0.234	0.338
	Total Non-Classical	0.019	0.020	0.011	0.844	0.080	0.462
	CD4 T cells	0.888	0.312	0.219	0.010	0.004	0.506
	CD8 T cells	0.403	0.277	0.182	0.309	0.005	0.415
	MAIT cells	0.052	0.008	0.011	0.014	0.018	0.724
	γδ T cells	0.057	0.016	0.015	0.009	0.006	0.868
Replication	CD11c ^{low} classical	0.001	0.015	0.058	<0.001*	0.010*	0.076*
	Total Classical	0.117	0.193	0.271	0.035	0.010	0.167
	Total Intermediate	0.196	0.412	0.422	0.025	0.021	0.925
	Total Non-Classical	0.022	0.026	0.015	0.890	0.061	0.404
	CD4 T cells	0.899	0.477	0.292	0.004	0.061	0.098
	CD8 T cells	0.412	0.341	0.171	0.489	<0.001	0.083
	MAIT cells	0.035	0.005	0.002	0.001	<0.001	0.093
	γδ T cells	0.055	0.022	0.028	0.003	0.140	0.769

p-values calculated using a two-tailed, two-sample unequal variance Student's t-test.

H = Healthy; SS = Short-Stay; LS/D = Long-Stay/Died

*p-values that have been Bonferroni-adjusted for multiple comparisons

Table S4. Cell surface protein signatures for cluster assignment

Analyses	Cell type	Cell surface protein signatures for cluster assignment
	CD4 T cells	CD3+ TCR $\alpha\beta$ + CD4+ CD8-
	CD8 T cells	CD3+ TCR $\alpha\beta$ + CD4- CD8+
	B cells	HLA-DR+ IgD+ CD19+
	Monocytes	CD116+ CD3- CD19- CD11c+ HLA-DR+ CD32+
Ungated	Stem cells	CD34+
	NK cells	CD94+ CD3- CD116-
	MAIT cells	CD3+ TCR $\alpha\beta$ + IL-18R α + MR1-5-OP-RU+
	$\gamma\delta$ T cells	CD3+ TCR $\alpha\beta$ - TCR $\gamma\delta$ +
	pDC	CD11c- NRP1+ CD123+ Fc ϵ R α 1+ CD116+ HLA-DR+
	DC2/3	CD11c+ CD1c+ NRP1- CD123+ Fc ϵ R α 1+ HLA-DR+
	CD11c ^{low} classical	CD116+ CD14 ^{high} CD16- CD11c ^{low}
Gated on CD3- CD19- CD116+ (Monocytes)	Total Classical	CD116+ HLA-DR+ CD14 ^{high} CD16- CD123-
	Total Intermediate	CD116+ HLA-DR+ CD14 ^{high/int} CD16 ^{low} CD123 ^{low}
	Total Non-Classical	CD116+ HLA-DR+ CD14 ^{low} CD16 ^{high}

Table S5. Sensitivity and specificity calculations for serum IL-6.

	Sensitivity	Specificity	False Positives	False Negatives	LR+
IL-6 screen (Cytokine Cohort; n = 90)	39% (22/56)	76% (22/29)	24% (7/29)	42% (24/56)	1.6