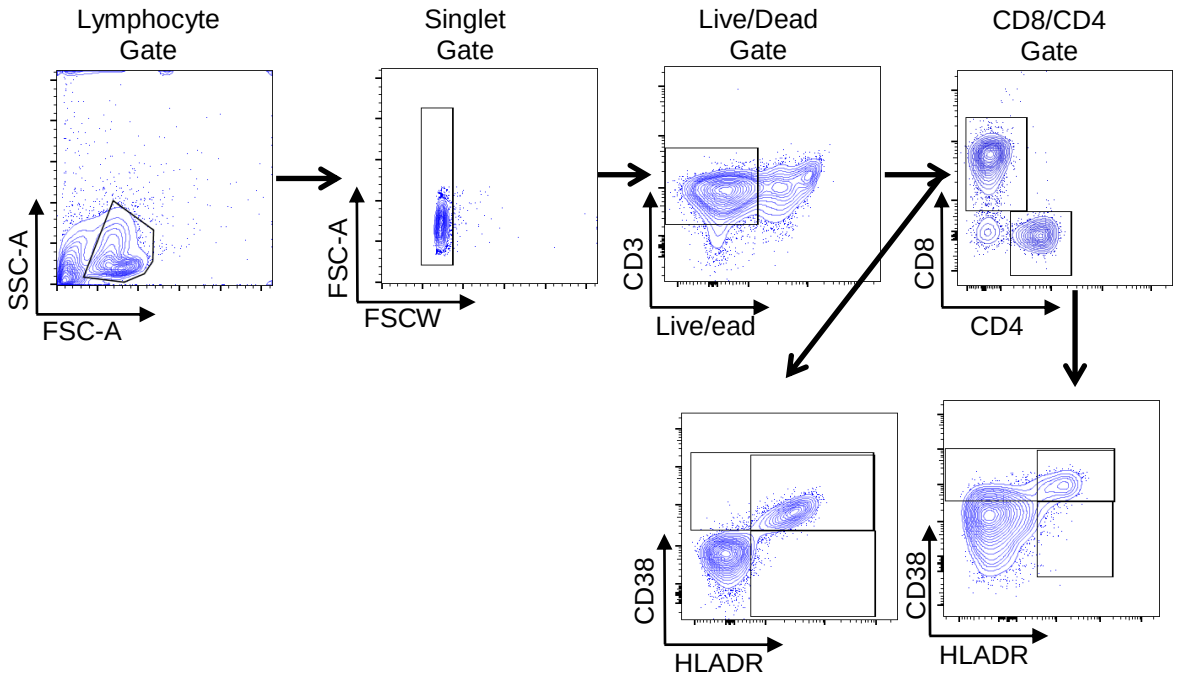
A

HLH associated genes	N	Disease causing (biallelic/hemizygous)	Single allele (ambiguous)
PRF1	6	5	1
UNC13A	9	8	1
STXBP2	6	4	2
SH2D1A	3	3	0
RAB27A	1	1	0

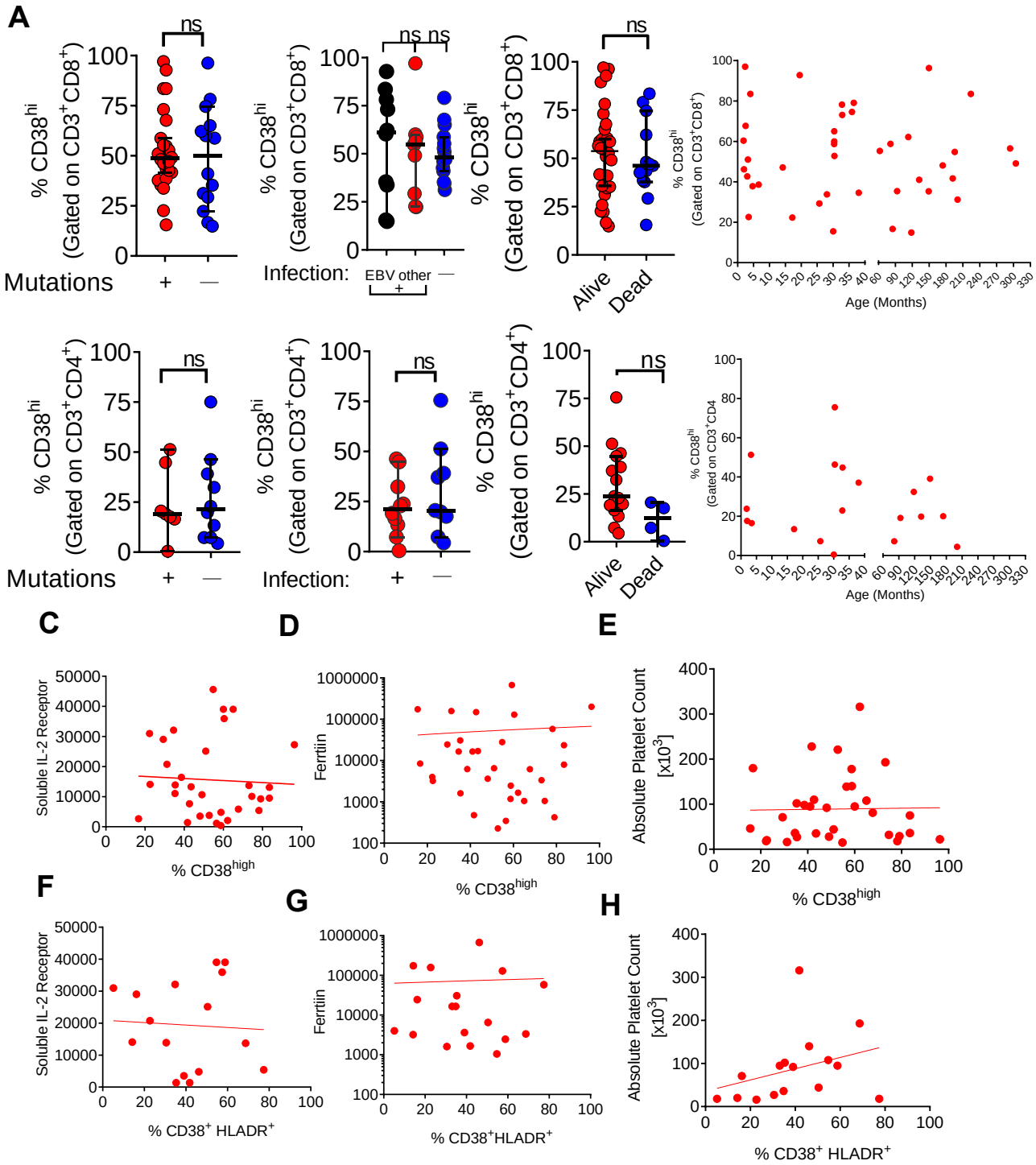
B

Age (Years)	HLH (N) (% of total)	Sepsis (N) (% of total)
0-1	13 (30%)	4 (22%)
>1-4	15 (35%)	8 (44%)
>4- 16	10 (23%)	5 (28%)
>16-25	15 (12%)	1 (6%)

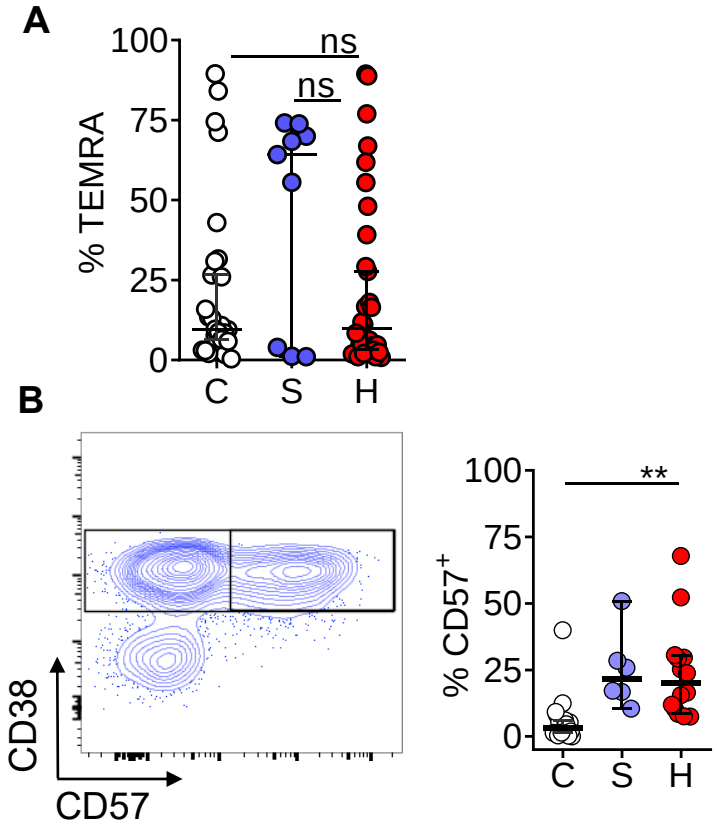
C



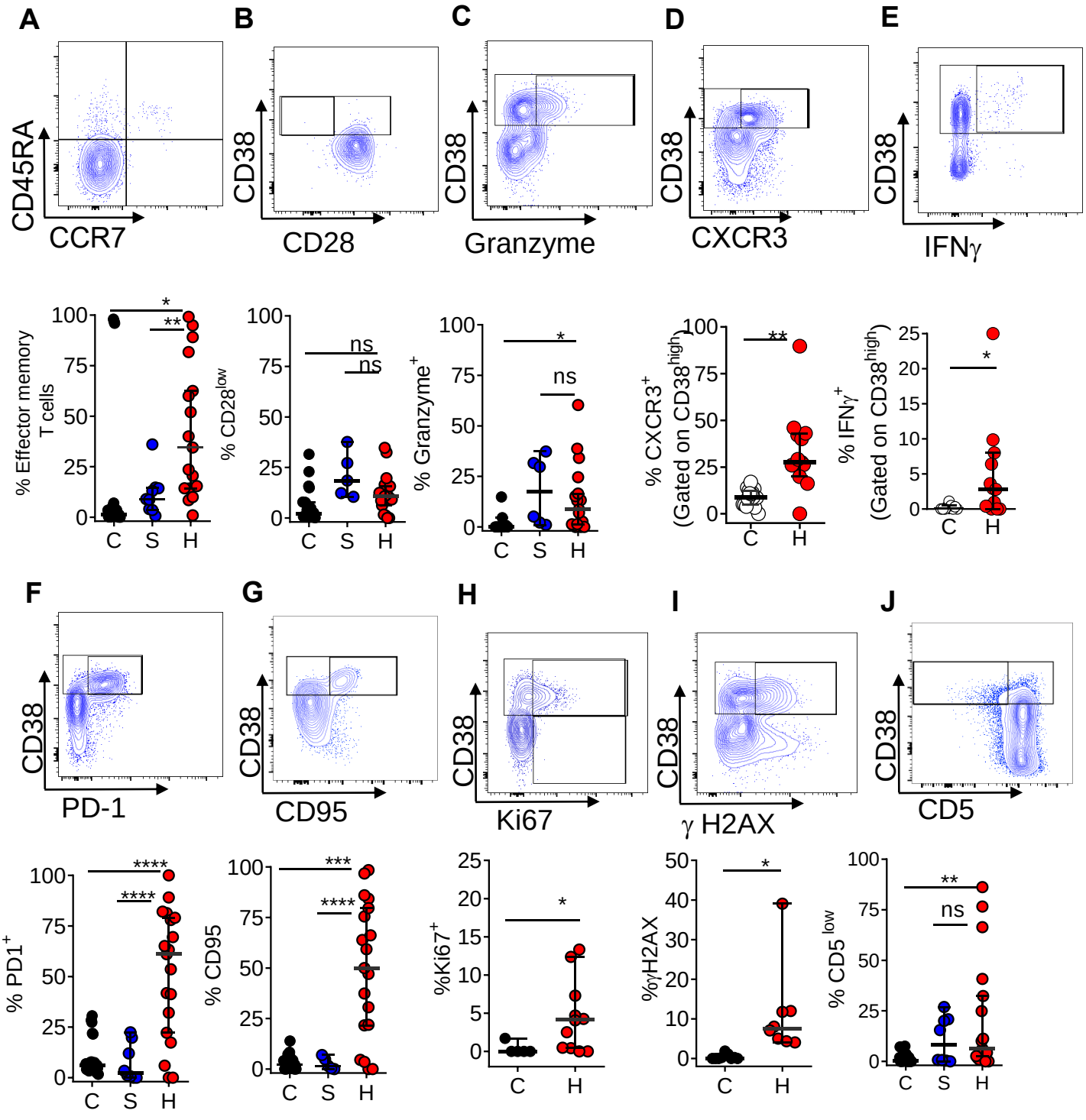
Supplemental Figure 1. Additional subject information and gating strategy for analysis of CD4+ and CD8+ T cells. (A) Additional details of HLH associated genetic mutations in the HLH cohort. (**B**) Age distribution of our cohorts with HLH or sepsis. **(C**) Gating strategy for analysis of CD4+ and CD8+ T cells.



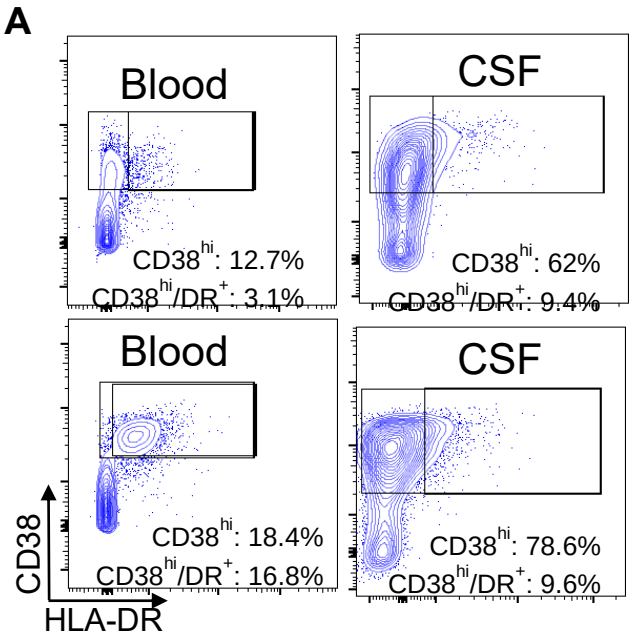
Supplemental Figure 2. Additional comparisons of T cell populations and clinical features in patients with HLH. (A-B) Frequency of CD38^{high} CD8^{+} or CD4^{+} T cells in patients with HLH is plotted, comparing presence of HLH-associated mutation, identifiable infection at diagnosis, survival, or age. (C-E) Frequency of CD38^{high} CD8^{+} T cells as a percentage of CD8+ T cells in patients with HLH are plotted against the indicated clinical indices. (F-H) Frequency of CD38^{high}/HLA-DR^{+} (double positive) CD8^{+} T cells as a percentage of CD8+ T cells in patients with HLH are plotted against the indicated clinical indices.

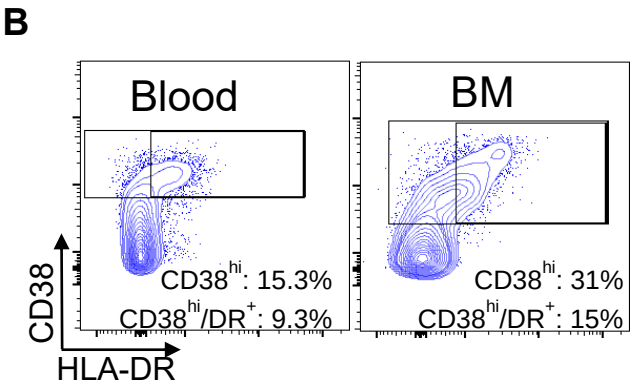


Supplemental Figure 3. Additional phenotyping of CD8+ T cells in patients with HLH. A. Frequency of CD45RA+/ CCR7- (TEMRA) cells among $CD38^{high}$/$HLA\text{-}DR^{+}$ (double positive) $CD8^{+}$ T cells in patients with HLH. **B.** Frequency of CD57+ cells among $CD38^{high}$/$HLA\text{-}DR^{+}$ (double positive) $CD8^{+}$ T cells in patients with HLH

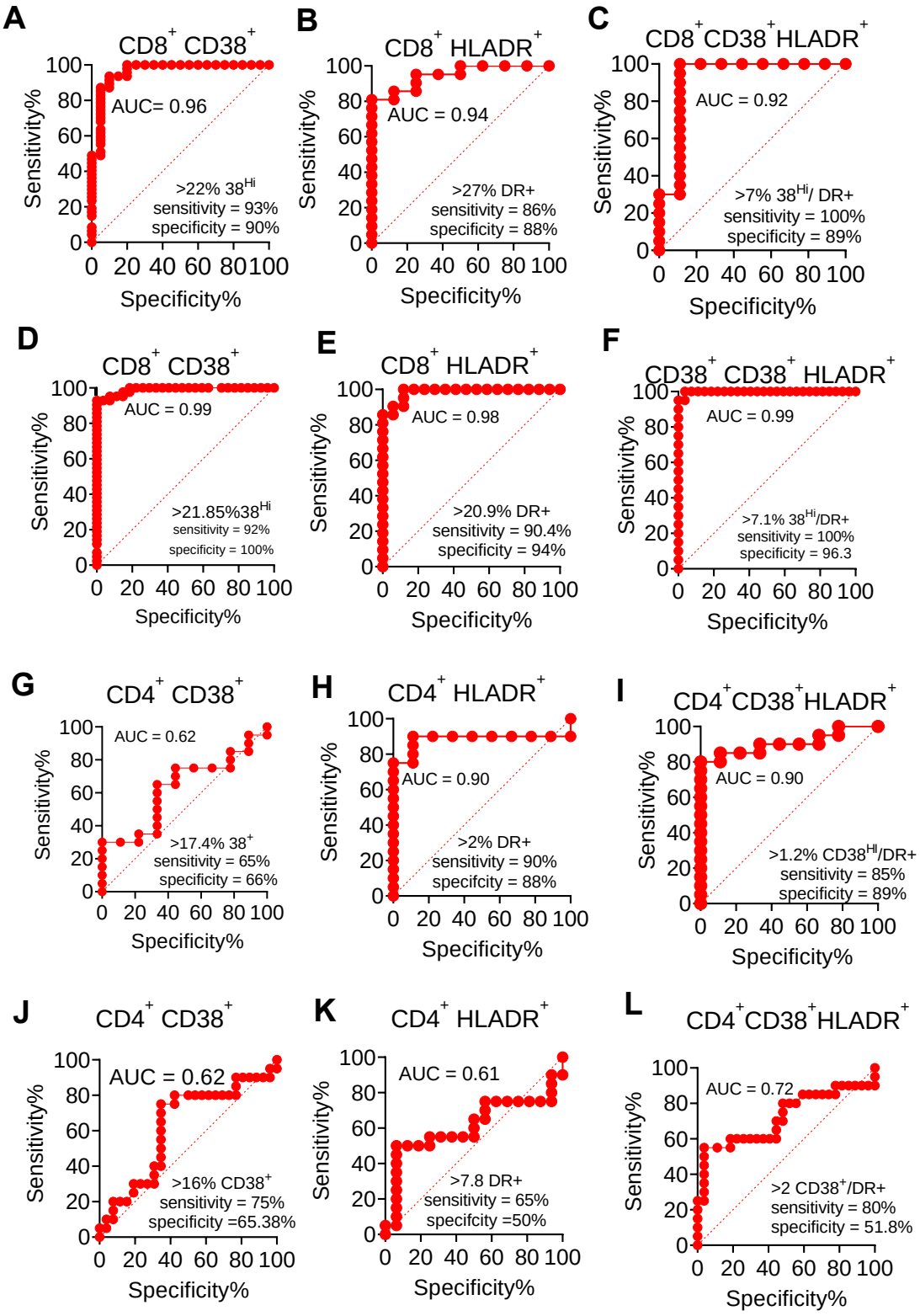


Supplemental Figure 4. Extended phenotype of CD38^{high} CD4^{+} T cells in patients with HLH. A-J Representative FACS plots and cumulative data comparing the frequency of the indicated markers within CD38^{high} CD4^{+} T cells in pediatric controls (C), patients with sepsis (S), or patients with HLH (H). For controls and patients with sepsis, frequency shown is derived from gating on the top 10% of CD38 expressing CD4^{+} T cells.





Supplemental Figure 5. Tissue infiltrating CD4+ T cells in patients with HLH are more activated than concurrently circulating T cells in blood. (A) Representative FACS plots of CD4+ T cells from patients with HLH, comparing blood and cerebrospinal fluid (CSF) or (B) blood and bone marrow (BM) in samples obtained concurrently from three different patients.



Supplemental Figure 6. ROC analyses comparing indicated populations in patients with HLH to those with sepsis or healthy controls. A-C ROC analysis of indicated CD8+ cell populations, comparing HLH and sepsis. **D-F** ROC analysis of indicated CD8+ cell populations, comparing HLH and control. **G-I** ROC analysis of indicated CD4+ cell populations, comparing HLH and sepsis. **J-L** ROC analysis of indicated CD4+ cell populations, comparing HLH and control

Low false positive Activation Marker	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR
$CD8^+$ $CD38^+$ >22%	0.93 (0.81 -0.97)	0.96 (0.82-1.0)	0.97 (0.87-1)	0.9 (0.74-0.96)	26.0
$CD8^+$ $HLADR^+$ >21%	0.90 (0.72-0.98)	0.96 (0.80-1.00)	0.95 (0.77-1.0)	0.92 (0.75-0.98)	22.7
$CD8^+$ $CD38^+$/ $HLADR^+$ >7%	**1.0 (0.85-1)**	**0.96 (0.81-1.0)**	**0.95 (0.79-1)**	**1 (0.87-1)**	**27**
$CD4^+$ $CD38^+$ >16%	0.75 (0.53-0.88)	0.66 (0.48-0.81)	0.62 (0.42-0.79)	0.78 (0.58-0.90)	2.2
$CD4^+$ $HLADR^+$ >7.8%	0.73 (0.51-0.88)	0.43 (0.25-0.63)	0.51 (0.34-0.69)	0.66 (0.41-0.85)	1.3
$CD4^+$ $CD38^+$/ $HLADR^+$ >2%	0.80 (0.58-0.92)	0.51 (0.34-0.69)	0.55 (0.37-0.71)	0.77 (0.54-0.91)	1.67

Supplemental Table 1. T cell activation parameters distinguishing patients with HLH from healthy pediatric controls. (PPV = Positive Predictive Value; NPV = Negative Predictive Value, LR= Likelihood Ratio)