Cell Reports, Volume *30*

Supplemental Information

The CD38/NAD/SIRTUIN1/EZH2 Axis Mitigates Cytotoxic CD8 T Cell Function and Identifies Patients with SLE Prone to Infections

Eri Katsuyama, Abel Suarez-Fueyo, Sean J. Bradley, Masayuki Mizui, Ana V. Marin, Lama Mulki, Suzanne Krishfield, Fabio Malavasi, Joon Yoon, Shannan J. Ho Sui, Vasileios C. Kyttaris, and George C. Tsokos

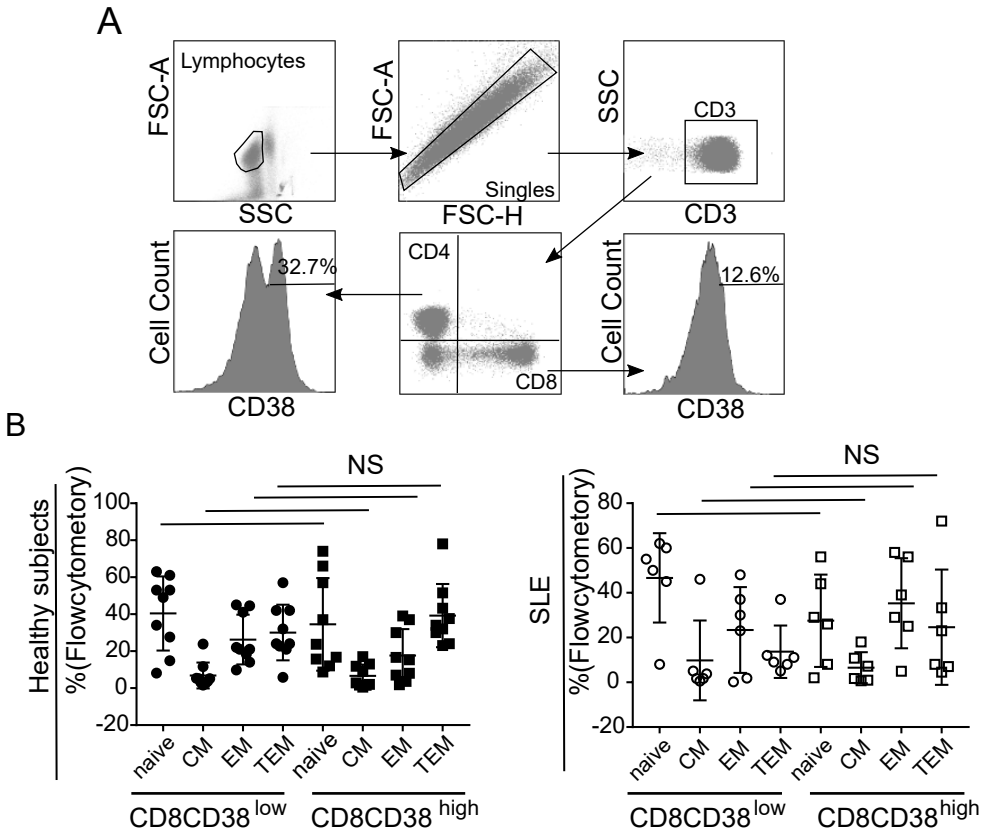
Supplementary table I: Demographic and clinical characteristics of the studied subjects (related to Figure 1).

		Patients with low-CD38 (n=22)	Patients with high-CD38 (n=13)	P value
Age, years		39.7 (±2.9)	41.2 (±3.4)	P=0.73
Sex	Female n, (%)	55.1% (n=16)	41.3% (n=12)	P=0.39
	Male n, (%)	3.4% (n=1)	0% (n=0)	NA
SLEDAI		1.6 (±0.97)	3.2 (±0.94)	P=0.21
Organ damage	Central nervous system lupus	0% (n=0)	0% (n=0)	NA
	Vasculitis	0% (n=0)	0% (n=0)	NA
	Arthritis	3.5% (n=1)	6.9% (n=2)	P=0.35
	Myositis	0% (n=0)	0% (n=0)	NA
	Urinary cast	0% (n=0)	0% (n=0)	NA
	Proteinuria	0% (n=0)	0% (n=0)	NA
	Hematuria	3.5% (n=1)	3.5% (n=1)	NA
	Nephritis	3.5% (n=1)	3.5% (n=1)	NA
	Pyuria	3.5% (n=1)	0% (n=0)	P=0.39
	Rash	3.5% (n=1)	6.9% (n=2)	P=0.35
	Alopecia	0% (n=0)	0% (n=0)	NA
	Mucosal ulcer	3.5% (n=1)	6.9% (n=2)	P=0.35
	Pleurisy	0% (n=0)	0% (n=0)	NA
Flare		0% (n=0)	0% (n=0)	NA
Infection		0% (n=0)	85% (n=11)	P=0.001
Blood test	Low complement (C3 or C4 or CH50)	10.3% (n=3)	20.7% (n=6)	P=0.63
	Positivity of anti-dsDNA	6.9% (n=2)	10.3% (n=3)	P=0.35
	Thrombopenia	0% (n=0)	0% (n=0)	NA
	Leukopenia	3.5% (n=1)	6.9% (n=2)	P=0.35
	Low Hemoglobin	0% (n=0)	0% (n=0)	NA
	High ESR	4.5%(n=1)	22.7%(n=5)	P=0.01*
	Fever	0% (n=0)	0% (n=0)	NA
	Elevated Creatinine	4.5% (n=1)	0% (n=0)	P=0.22
Usage of Treatment	PN	60% (n=6)	30% (n=3)	P=0.49
	HCQ	34.5% (n=10)	27.6% (n=8)	P=0.66
	MMF	20.7% (n=6)	0% (n=0)	P=0.02*
	AZ	3.45% (n=1)	10.3% (n=3)	P=0.14
	Calcineurine inhibitors (TAC+CsA)	3.45% (n=1)	0% (n=0)	P=0.39
	MTX	0% (n=0)	0% (n=0)	NA
	IVCY	0% (n=0)	3.5% (n=1)	P=0.23
	Biologic agents	0% (n=0)	0% (n=0)	NA

PN: prednisolone, HCQ: hydroxycloroquine, MMF: mycophenolic mofetil, AZ: azathioprine, TAC: tacrolimus, CsA: cyclosporin, MTX: methotrexate, IVCY: intravenous cyclophosphamide

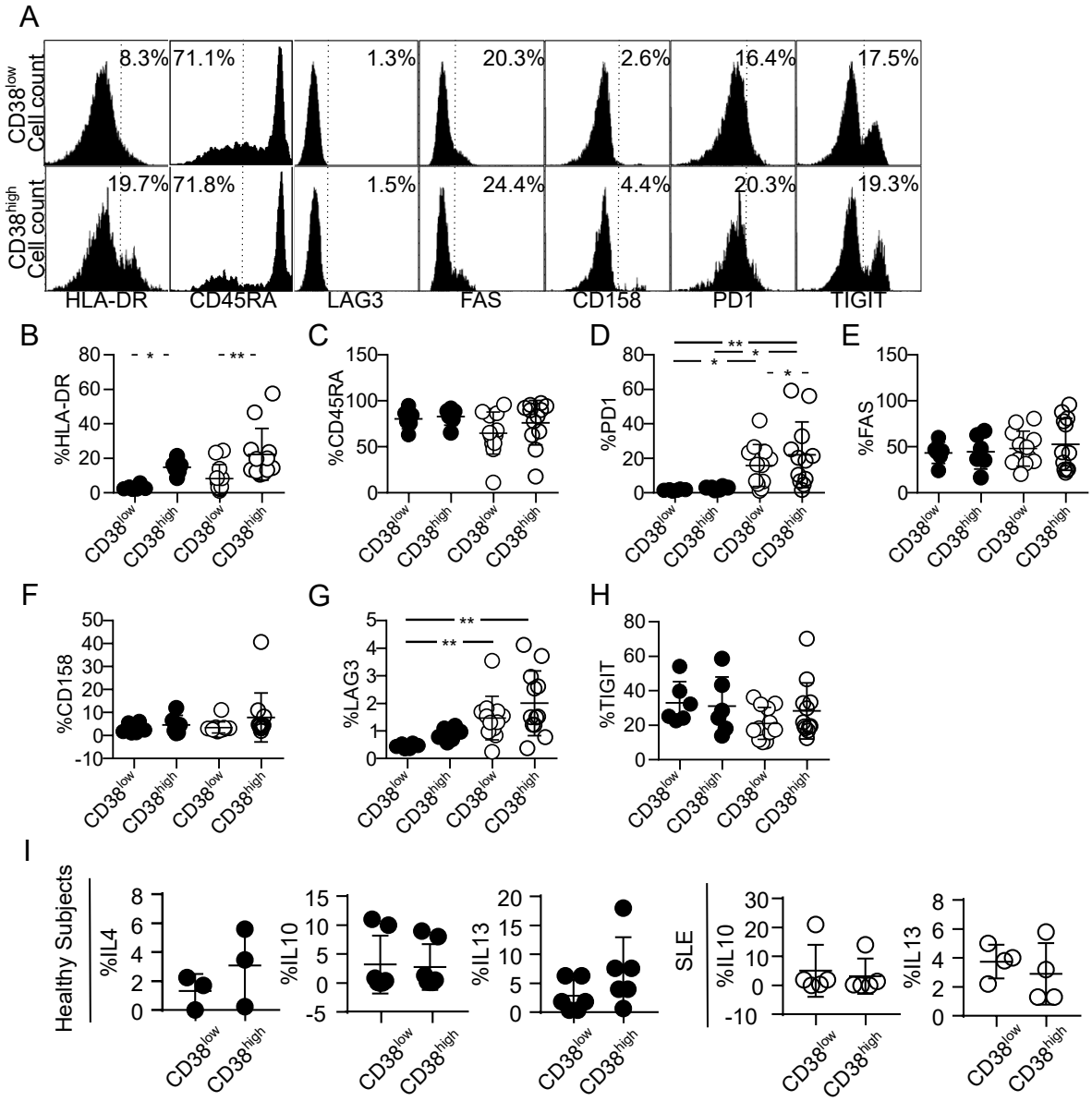
Supplementary Table II: Type of infection in each patient (related to Figure 1).

Patient	CD8CD38(%)	Type of infection	Pathogen
No.1	49.2	viral infection	unknown
No.2	45.6	infection	unknown
No.3	54.3	diverticulitis	unknown
No.4	50.9	small intestinal bacterial overgrowth	unknown
No.5	74	urinary tract infection	unknown
No.6	51.6	urinary tract infection	unknown
No.7	42.9	pneumonia	unknown
No.8	41.8	pneumonia	unknown
No.9	68.5	urinary tract infection	viral
No.10	62.3	infection	unknown
No.11	56.2	urinary tract infection	unknown



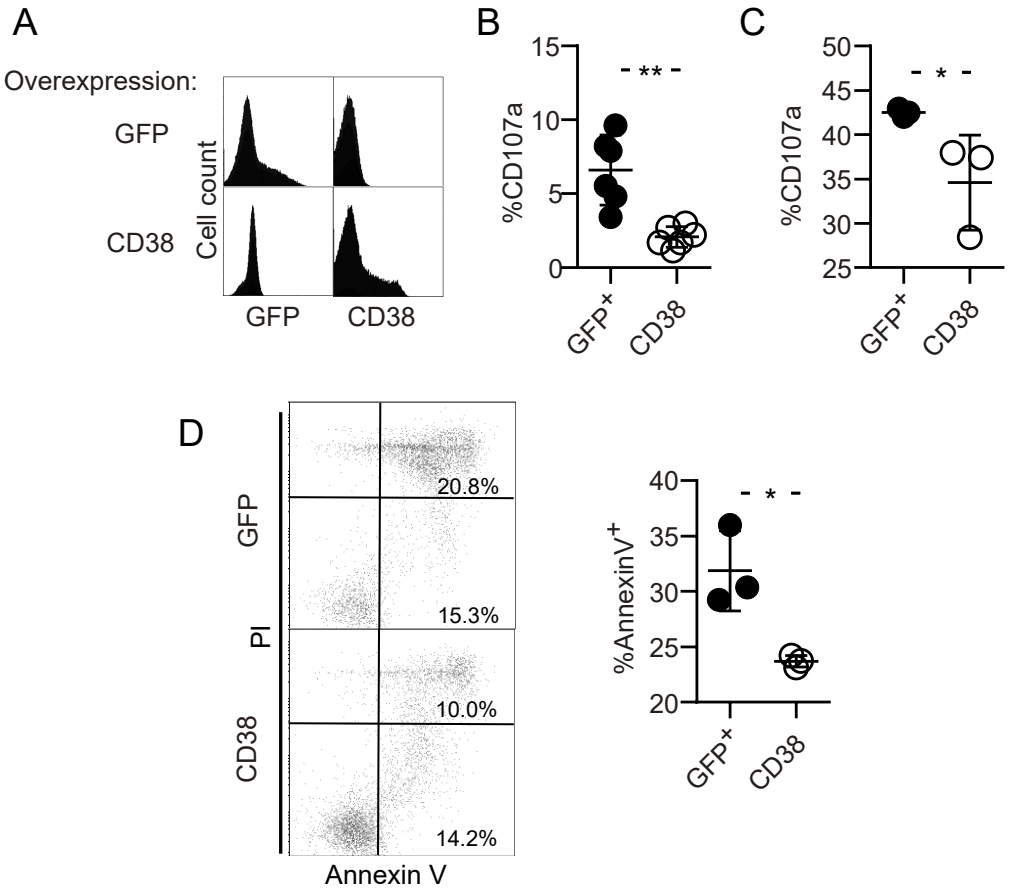
Supplementary Figure 1. Gating strategy for CD8CD38^{low}and CD8CD38^{high} cells (related to Figure 1).

A) Gating Strategy for $CD3^+CD4^+CD38^+$ and $CD3^+CD8^+CD38^+$.
B) Percentage of each subpopulation in CD8CD38^{low} and CD8CD38^{high}T cells from a healthy subjects (left) and a patient with SLE (right) by flow cytometry.
Average data are represented as mean ± SD.



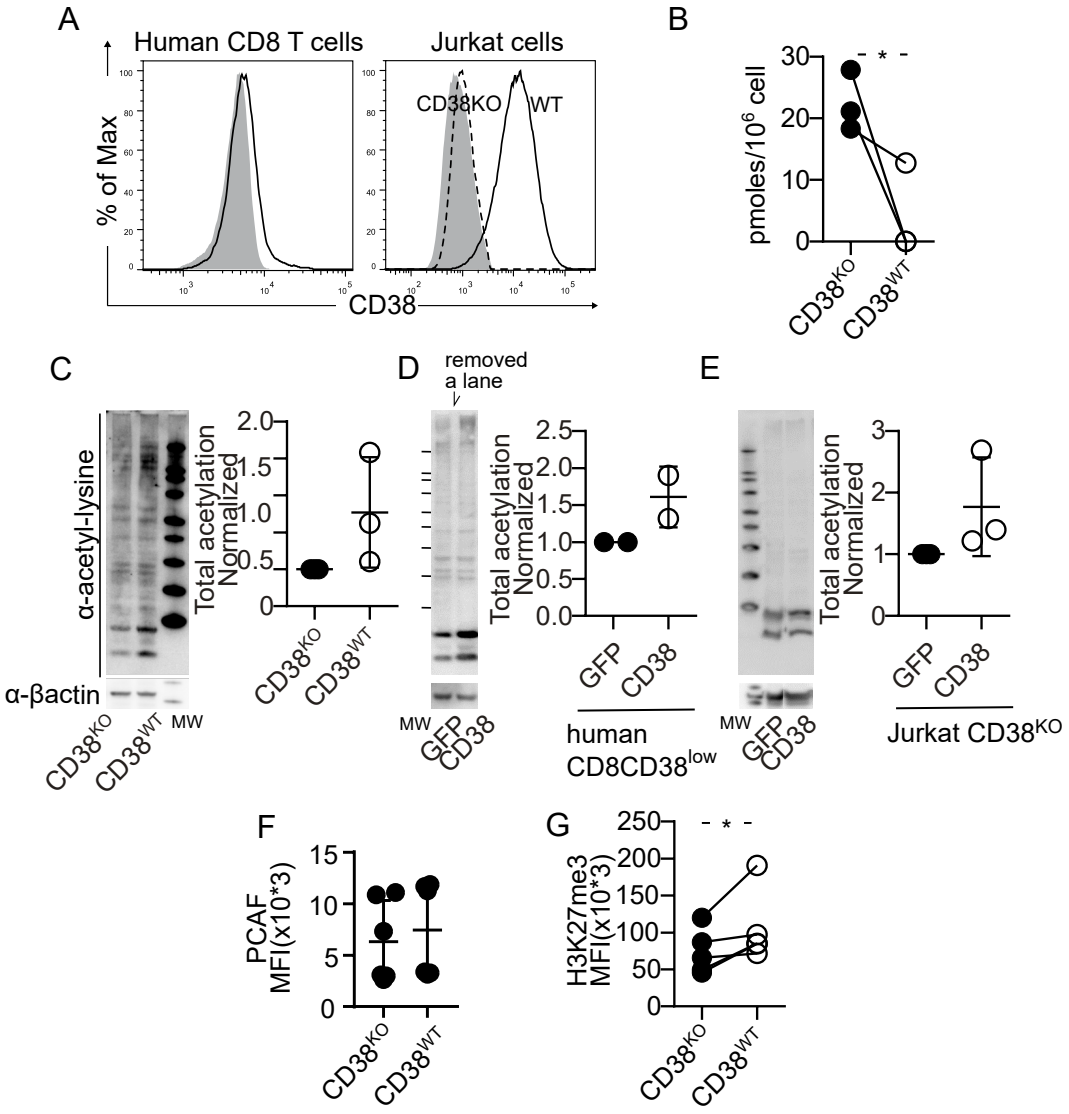
Supplementary Figure 2. The expressions of active and inhibitory markers and cytokines by CD8CD38^{low} and CD8CD38^{high} T cells (related to Figure 1).

A) Representative histograms showing expression of HLA-DR, CD45RA, LAG3, FAS, CD158, PD1 and TIGIT in CD8CD38^{low} and CD38^{high} T cells.
B-H) Percentage of CD8 T cells expressing **B)** HLA-DR, **C)** CD45RA, **D)** PD1, **E)** FAS, **F)** CD158, **G)** LAG3 and **H)** TIGIT in CD8CD38^{low} and CD8CD38^{high} cells from healthy donors and patients with SLE by flow cytometry (Healthy subjects=6, SLE=12; closed circles=Healthy subjects, opened circles=SLE; paired t-test or Wilcoxon test).
I) Percentage of cytokine produced in CD8CD38^{low} and CD8CD38^{high} cells from healthy donors and patients with SLE by flow cytometry (Healthy subjects$\geqq$3, SLE$\geqq$3; paired t-test). Isolated T cells were stimulated with PMA/Ionomycin for 4 hours and stained accordingly.
In all figures, average data are represented as mean ± SD.



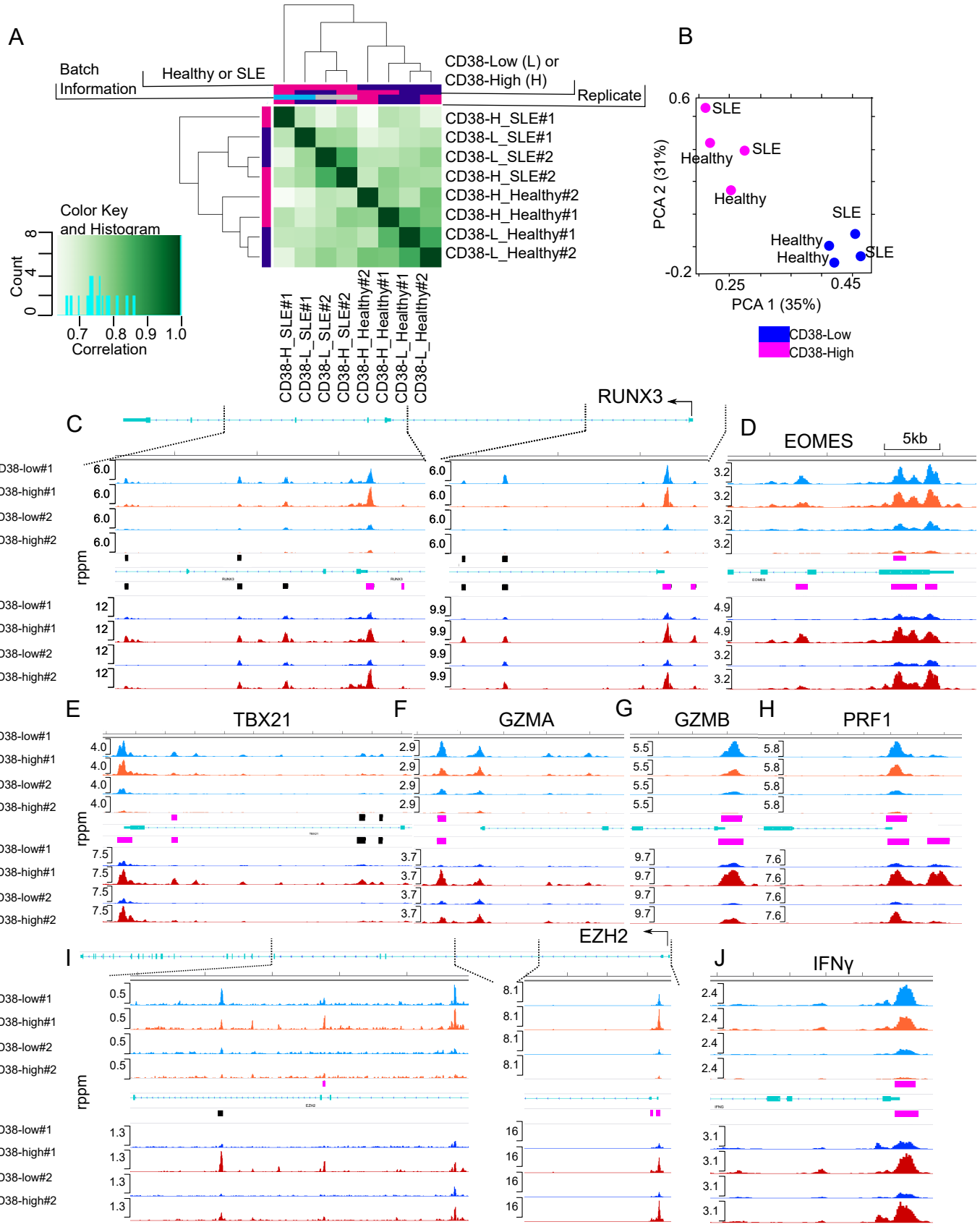
Supplementary Figure 3. The capacity of degranulation and *in vitro* cytotoxicity in CD8 T cells overexpressing CD38 (related to Figure 2).

A) Representative histograms showing GFP and CD38 over-expression in normal CD8CD38^{low} T cells, sorted by Facs Aria.
B) Degranulation (%CD107a) of CD8CD38^{low} T cells from healthy subjects overexpressing GFP or CD38 (Healthy subjects=6, Welch's test).
C) Degranulation (%CD107a) of TALL104 overexpressing GFP or CD38
(3 independent experiment; Welch's test).
D) Percentage of Annexin V^{+} of P815 cells after coculture with TALL104 cells overexpressing GFP or CD38
(3 independent experiment; Welch's test).
In all figures, average data are represented as mean ± SD.



Supplementary Figure 4. The levels of NAD^+ and epigenetic profile of CD38-deficient Jurkat cells (related to Figure 5).

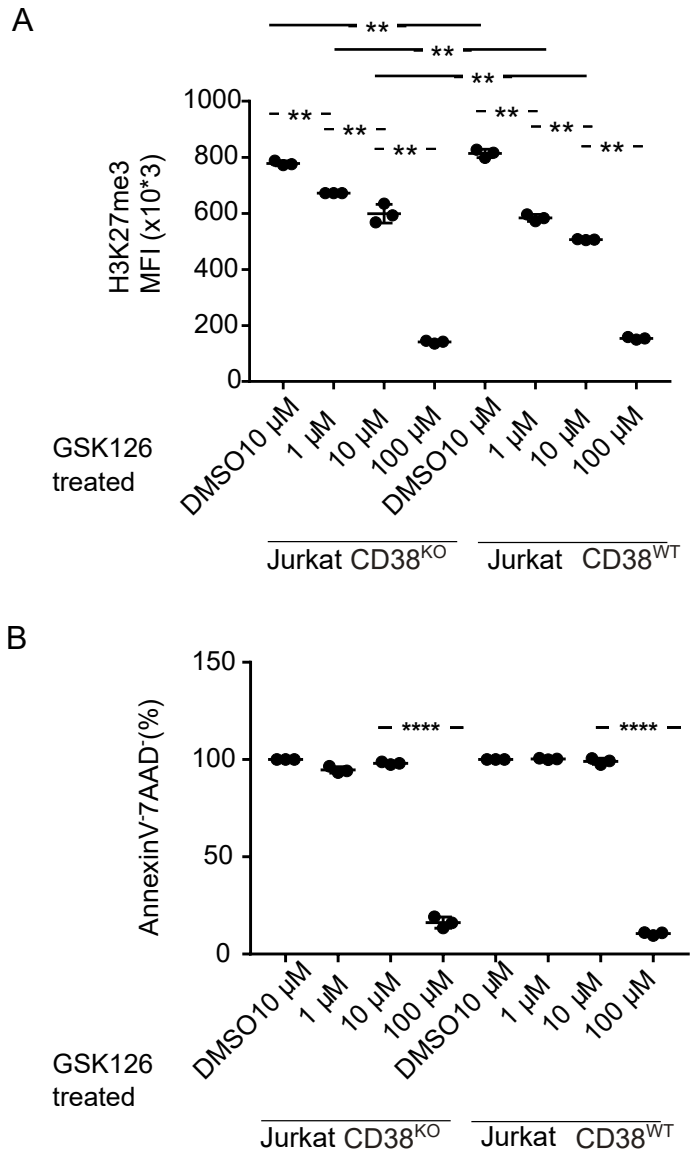
A) Representative histograms comparing CD38 expression on human primary CD8 T cells (solid line, left panel), Jurkat $CD38^{KO}$ (dotted line, right panel) and Jurkat $CD38^{WT}$ cells (solid line, right panel).
Gray shaded represents the isotype control.
B) NAD^+ levels from Jurkat $CD38^{KO}$ and $CD38^{WT}$ cells. NAD^+ levels were quantified by colorimetry.
The most right line shows molecular weight (MW) (3 independent experiments, paired t-test).
C) Western blot and cumulative data representing total acetyl-lysine from lysates from Jurkat $CD38^{KO}$ or $CD38^{WT}$ cells (3 independent experiments, paired t-test).
D) Western blot and cumulative data representing total acetyl-lysine from primary $CD8CD38^{low}$ T cells overexpressing GFP or CD38 (Healthy subjects, 2 independent experiments).
The middle lane between GFP and CD38 was removed because it belonged to an irrelevant sample.
$CD8CD38^{low}$ T cells were sorted by Facs Aria.
E) Same experiment as in D) using Jurkat $CD38^{KO}$ overexpressing GFP or CD38 (3 independent experiments; paired t-test).
F) MFI of PCAF in Jurkat $CD38^{KO}$ and Jurkat $CD38^{WT}$ by flow cytometry (n=5, 3 independent experiments; paired t-test).
G) MFI of H3K27me3 in Jurkat $CD38^{KO}$ and Jurkat $CD38^{WT}$ (n=4, 3 independent experiments; paired t-test).
In all figures, average data are represented as mean ± SD.


A
Batch Information
Healthy or SLE
CD38-Low (L) or CD38-High (H)
Replicate
CD38-H_SLE#1
CD38-L_SLE#1
CD38-L_SLE#2
CD38-H_SLE#2
CD38-H_Healthy#2
CD38-H_Healthy#1
CD38-L_Healthy#1
CD38-L_Healthy#2
Color Key and Histogram
Count
Correlation
B
PCA 2 (31%)
PCA 1 (35%)
SLE
Healthy
CD38-Low
CD38-High
C
RUNX3
D
EOMES
5kb
E
TBX21
F
GZMA
G
GZMB
H
PRF1
I
EZH2
J
IFNγ
Healthy
SLE
CD38-low#1
CD38-high#1
CD38-low#2
CD38-high#2
rppm

Supplementary Figure 5. Chromatin accessibility profiles of genes related to CD8 cytotoxicity shown by ATAC-seq in healthy subjects and patients with SLE (related to Figure 5).

A) Correlation heatmap indicating cross-correlation between each group.
B) Principal component analysis (PCA) plot across all samples.
C-J) Genome plot in the integrative genomics viewer (IGV) focused on the gene body and the regions with significantly different peaks of **C)** RUNX3 and the partial region, **D)** EOMES, **E)** TBX21 **F)** GZMA, **G)** GZMB, **H)** PRF1, **I)** EZH2 and the partial region and **J)** IFNγ.

The black and pink bars in the IGV indicate the peaks with significance in intronic and promoter region, respectively. The bars above the reference sequence are from the differential enrichment analysis from healthy subjects, while the bars under the the reference are from SLE. rppm; reads per peak per million.



Supplementary Figure 6. The basal cell functions affected by GSK126 in Jurkat CD38^{KO} and CD38^{WT} cells (related to Figure 6).

A) MFI of H3K27me3 in Jurkat CD38^{KO} and Jurkat CD38^{WT} pretreated with DMSO as control or GSK126 (EZH2 inhibitor) overnight at the indicated concentration (3 independent experiments; One-way ANOVA with multiple comparisons).
B) Percentage of AnnexinV^{-}7AAD^{-} (non apoptotic population) with same treatment as in A) (3 independent experiments; One-way ANOVA with multiple comparisons).
In all figures, average data are represented as mean ± SD.