Science Advances
AAAS

Supplementary Materials for

CD38 reduces mitochondrial fitness and cytotoxic T cell response against viral infection in lupus patients by suppressing mitophagy

Ping-Min Chen *et al.*

Corresponding author: George C. Tsokos, gtsokos@bidmc.harvard.edu

Sci. Adv. **8**, eabo4271 (2022)
DOI: 10.1126/sciadv.abo4271

This PDF file includes:

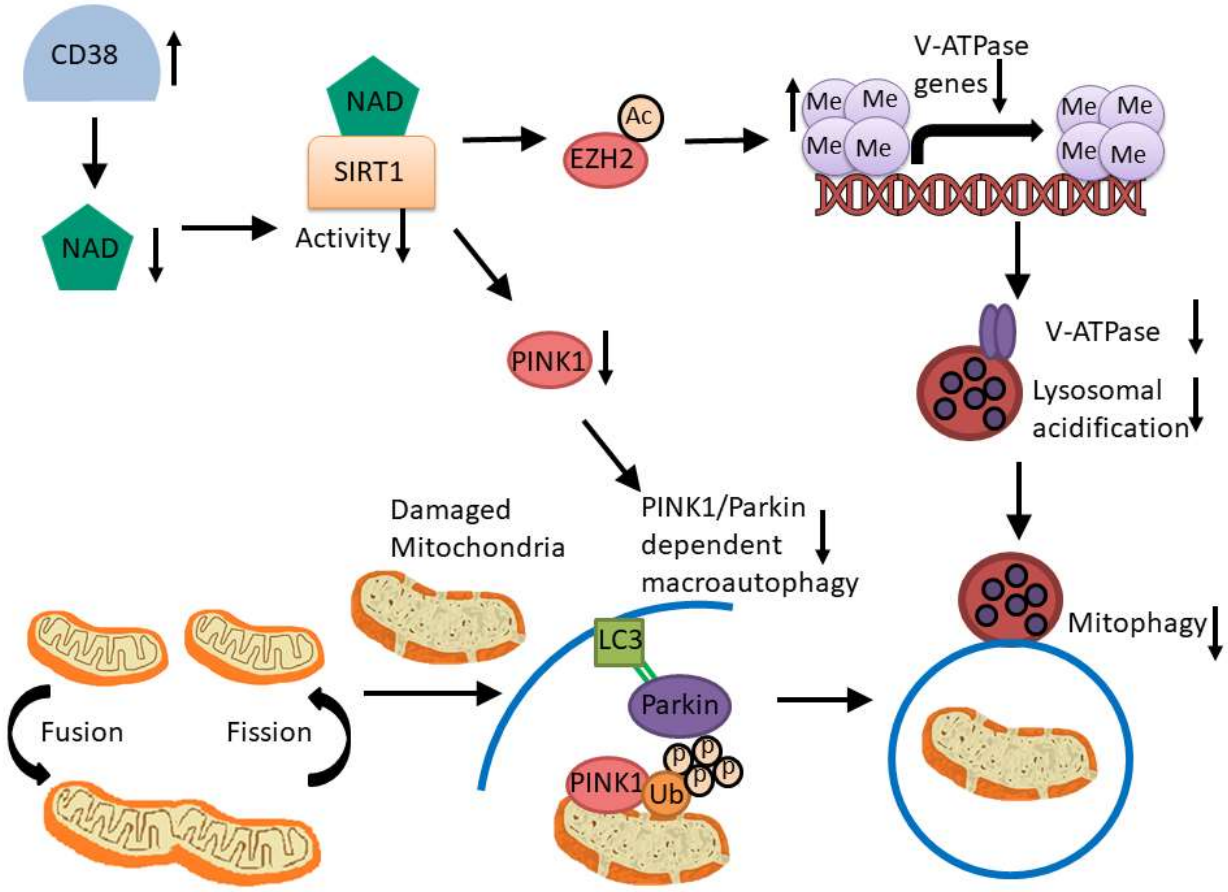


Figure S1. Graphical abstract of events related to the CD38-mediated regulation of mitophagy in CD8$^+$ T cells. NAD is depleted due to upregulation of CD38, which further reduces Sirtuin 1 (SIRT1) activity. The reduction of SIRT1 activity reduces expression of PINK1, which negatively impacts the PINK1-Parkin dependent macroautophagy. Meanwhile, reduced SIRT1 activity also increases acetylation of EZH2, which increases methylation in numerous loci, including promoter regions of V-ATPase subunit genes, and results in reduction in lysosomal acidification.

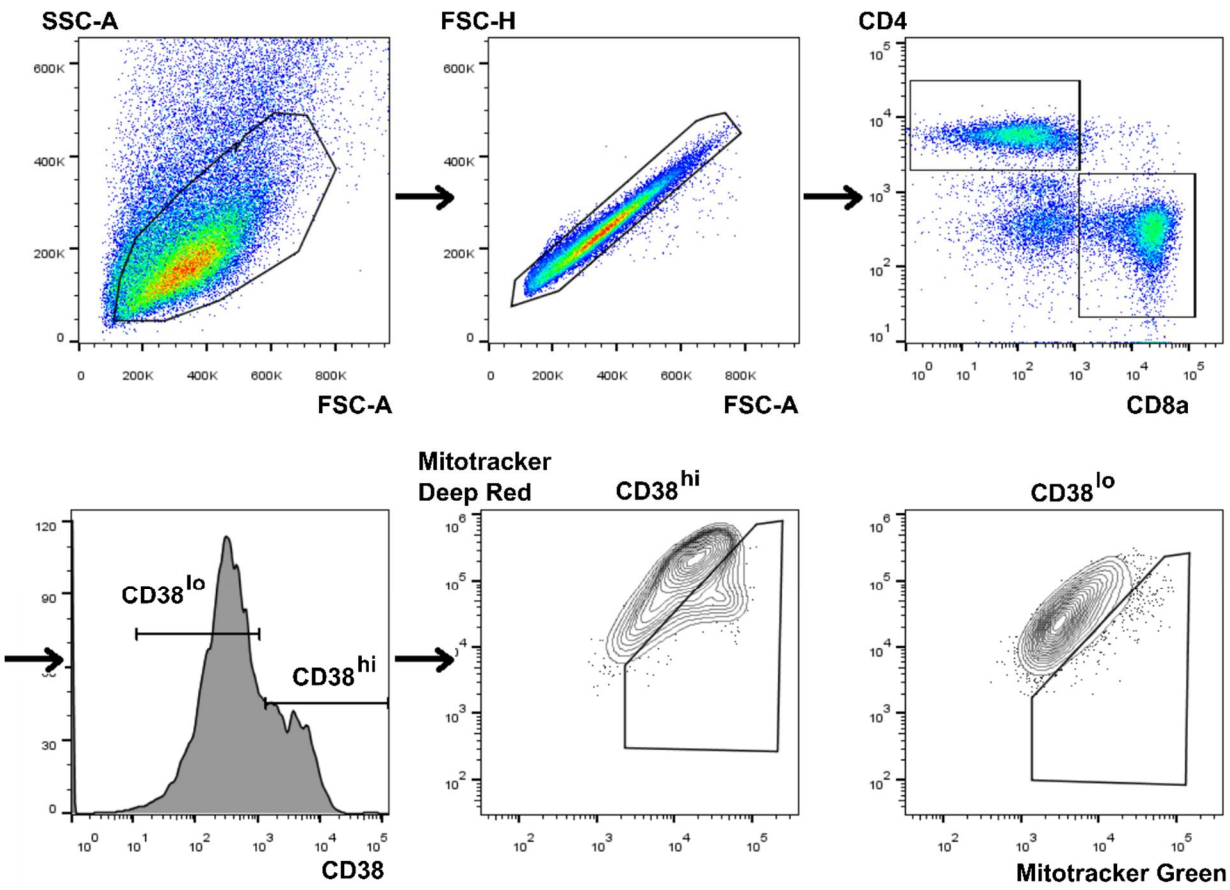


Figure S2. Gating strategy for CD38^{hi} CD8^{+} T cells. After defining single cell lymphocyte gates by forward and side scatter, CD8^{+} T cell populations were determined using the CD8a and CD4 staining. Within the CD8^{+} T cell, two separate populations of CD38^{hi} and CD38^{lo} can be determined based on surface CD38 staining, and these two populations have different Mitotracker green and Mitotracker deep red staining properties.

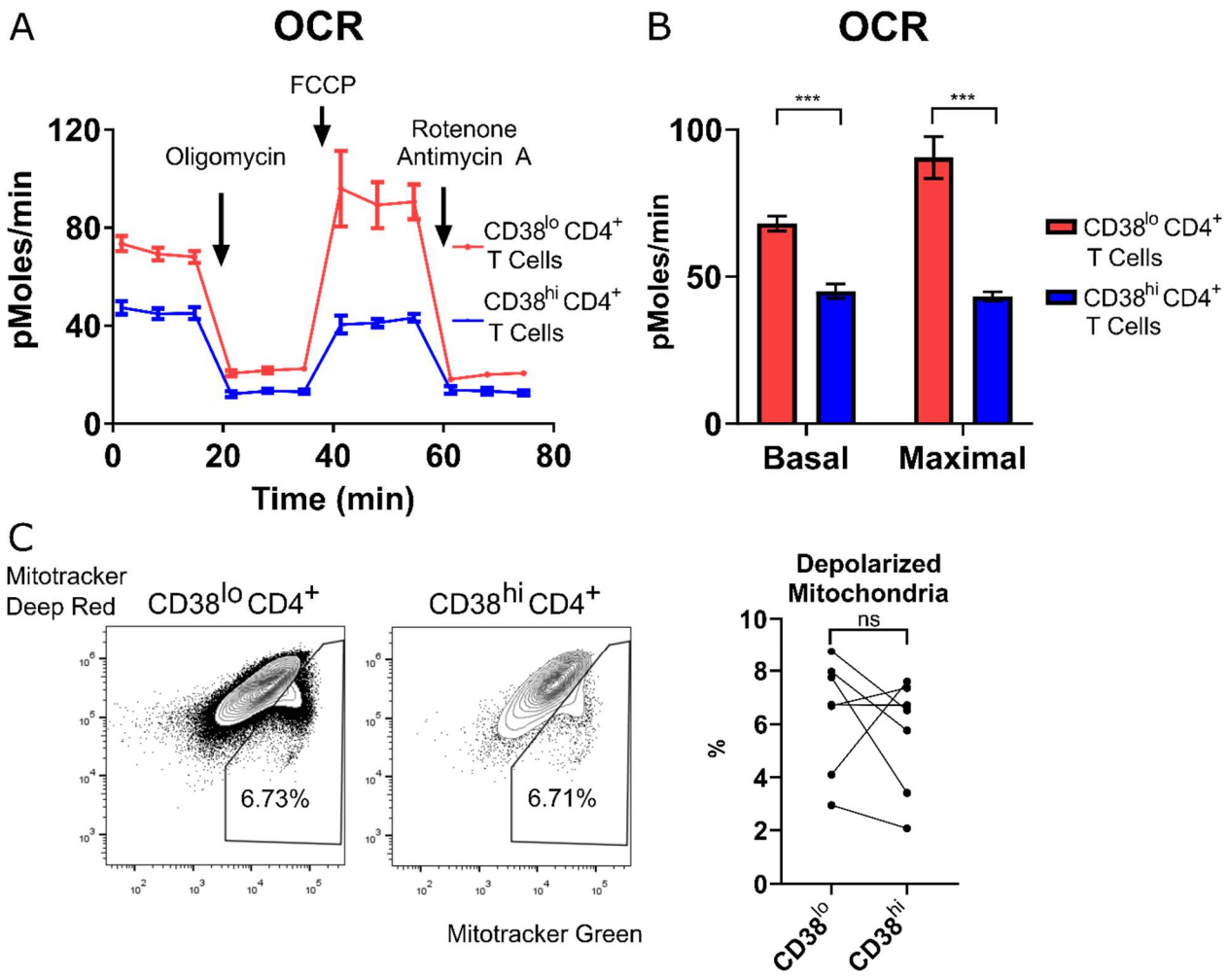


Figure S3. CD38^{hi} CD4^{+} T cells display limited mitochondrial respiration. **A.** Oxygen consumption rate (OCR) of CD38^{hi} and CD38^{lo} CD4^{+} T cells sorted from healthy donors at baseline and after addition of oligomycin, FCCP, and rotenone/Antimycin A. **B.** Basal and maximal OCR of CD38^{hi} and CD38^{lo} CD4^{+} T cells sorted from healthy donor in A. **C.** Representative flow cytometry plot of Mitotracker green and Mitotracker deep red staining in CD38^{hi} and CD38^{lo} CD4^{+} T cells from the peripheral blood of SLE patients. Percentage of depolarized mitochondria in CD38^{hi} and CD38^{lo} CD4^{+} T cells. Data shown are mean ± s.d.; statistical analysis by two-tailed t-test (B), paired t-test (C), ns = $p > 0.05$, ***$p < 0.001$

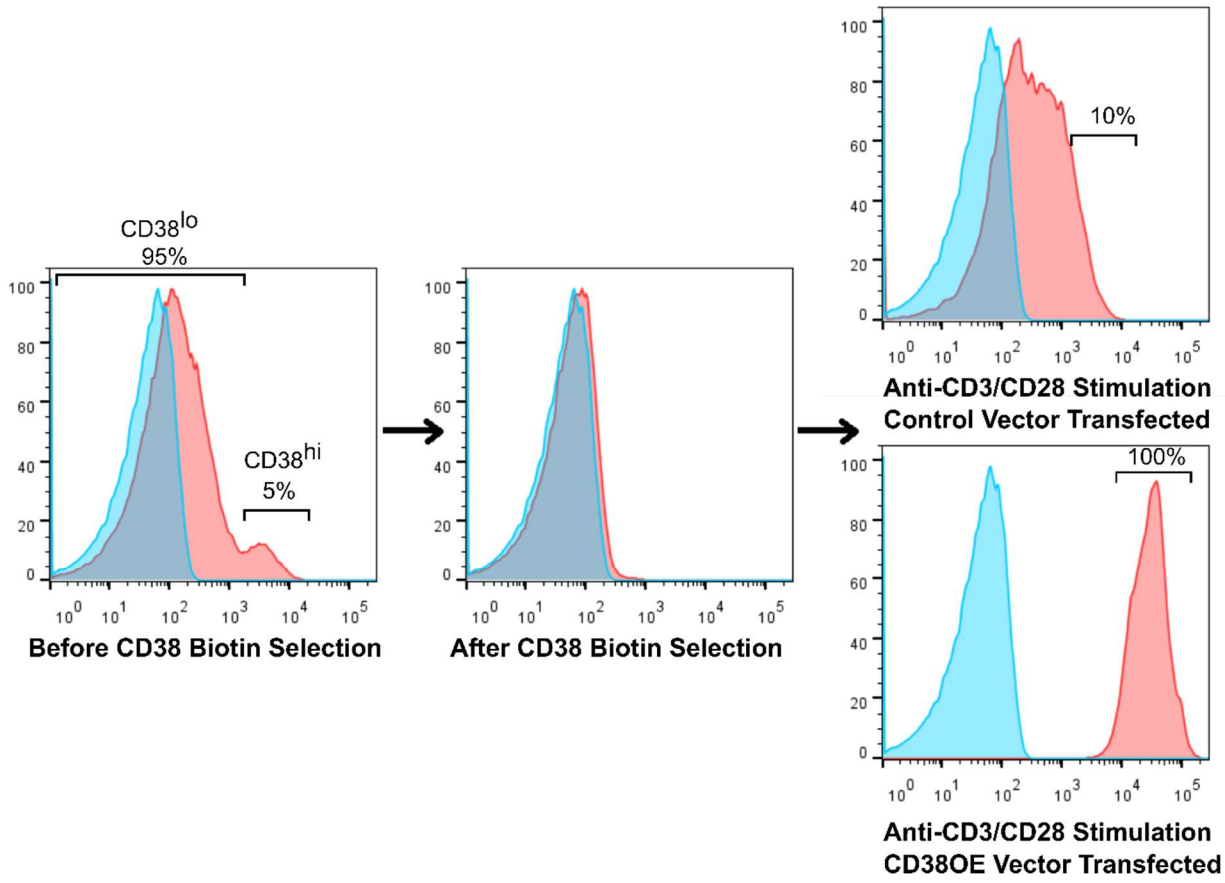


Figure S4. Representative CD38 expression of freshly isolated, control vector and CD38 overexpression vector transfected CD8$^+$ T cells After isolating CD8$^+$ T cell from apheresis leukoreduction collars, CD38$^+$ cells, including all CD38^{hi} and part of CD38^{lo} population, were removed using CD38 biotin magnet selection. Isolated CD8$^+$ T cells were transfected after overnight stimulation with anti-CD3 and anti-CD28 coated plate on day 1 with either control vector or CD38 overexpression (CD38OE) vector. The transfected cells were further cultured overnight with anti-CD3 and anti-CD28 coated plate before analysis of their phenotype on day 2. Red histogram showed CD38 expression of the cells of interest, while the blue histogram indicated the staining for isotype control.

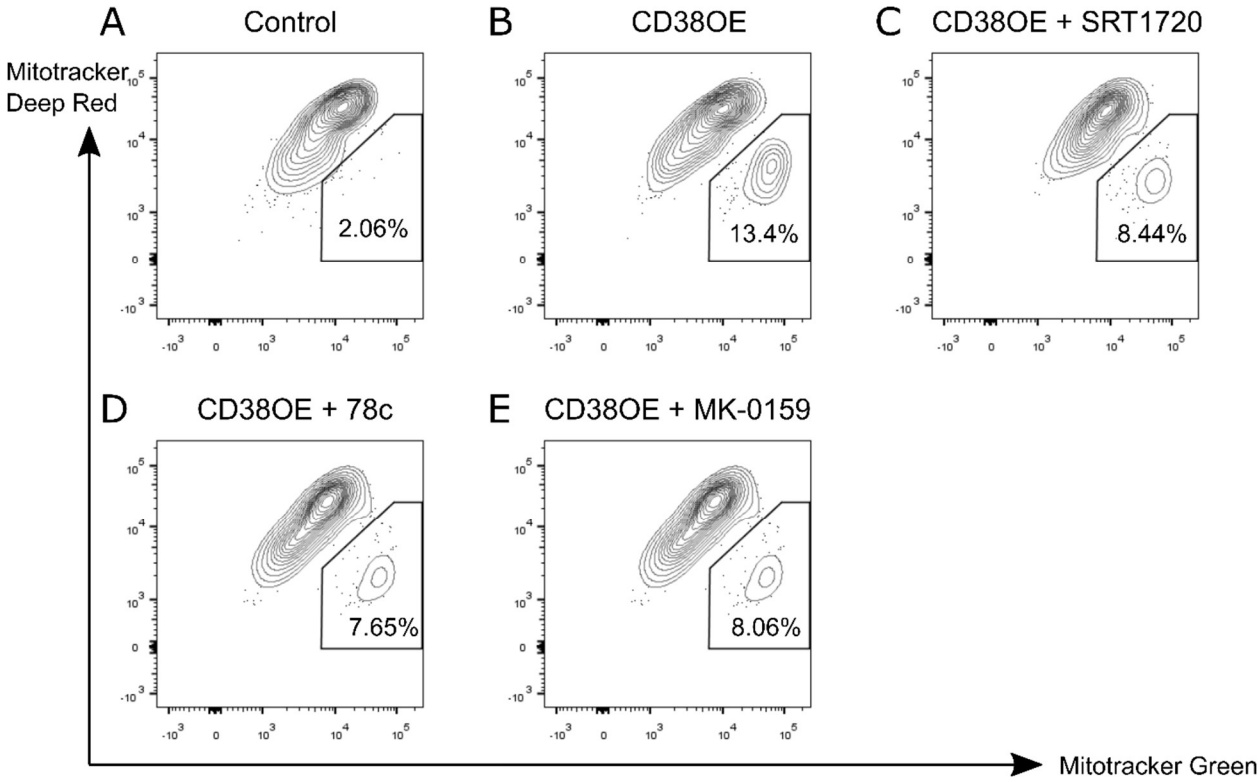
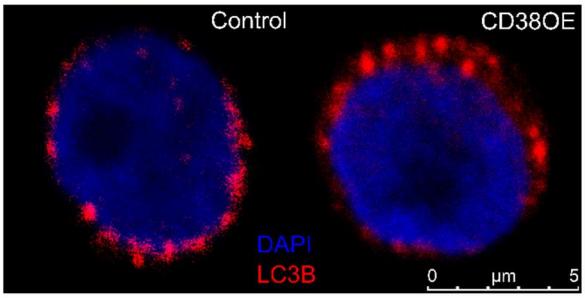


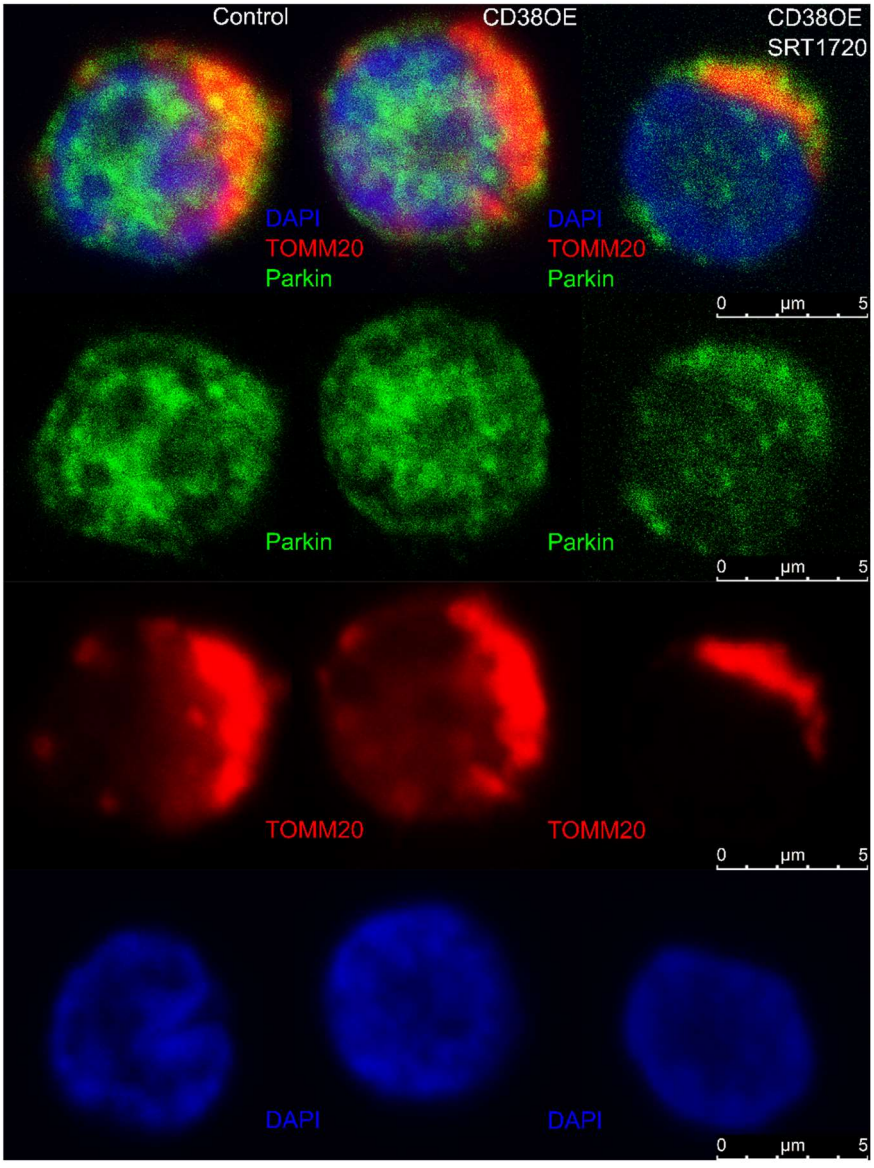
Figure S5. CD38 leads to increased numbers of depolarized mitochondria in CD8$^+$ T cells.

A-E. Representative flow plot of CD8$^+$ T cells transfected with control, CD38 overexpression vector, or CD38 overexpression vector with addition of SIRT1 activator SRT1720 or either one of CD38 inhibitor 78c or MK-0159 after overnight stimulation and culture after transfection. The cell population with depolarized mitochondria (Mitotracker green^{hi} Mitotracker deep red^{lo}) is highlighted in all the singlet transfected cells.

A


Control
CD38OE
DAPI
LC3B
0 µm 5

B


Control
CD38OE
CD38OE
SRT1720
DAPI
TOMM20
Parkin
DAPI
TOMM20
Parkin
0 µm 5
Parkin
Parkin
0 µm 5
TOMM20
TOMM20
0 µm 5
DAPI
DAPI
0 µm 5

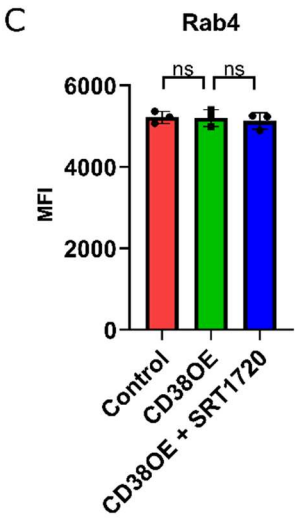


Figure S6. The effect of CD38 on autophagy pathways. A. Representative confocal immunofluorescence images of LC3B(Red) and DAPI(Blue) staining and counts of LC3B positive dots per cell of control or CD38 overexpressed $CD8^+$ T cells **B.** Representative confocal immunofluorescence images of Parkin (Green), mitochondrial marker TOMM20 (Red) and DAPI (Blue) staining of control, CD38 overexpressing, or CD38 overexpressing treated with SRT1720 $CD8^+$ T cells. **C.** Mean fluorescence intensity of Rab4 staining in control, CD38 overexpressing, or CD38 overexpressing treated with SRT1720 $CD8^+$ T cells. Data shown are mean ± s.d.; statistical analysis by two-tailed t-test. ns = $p > 0.05$

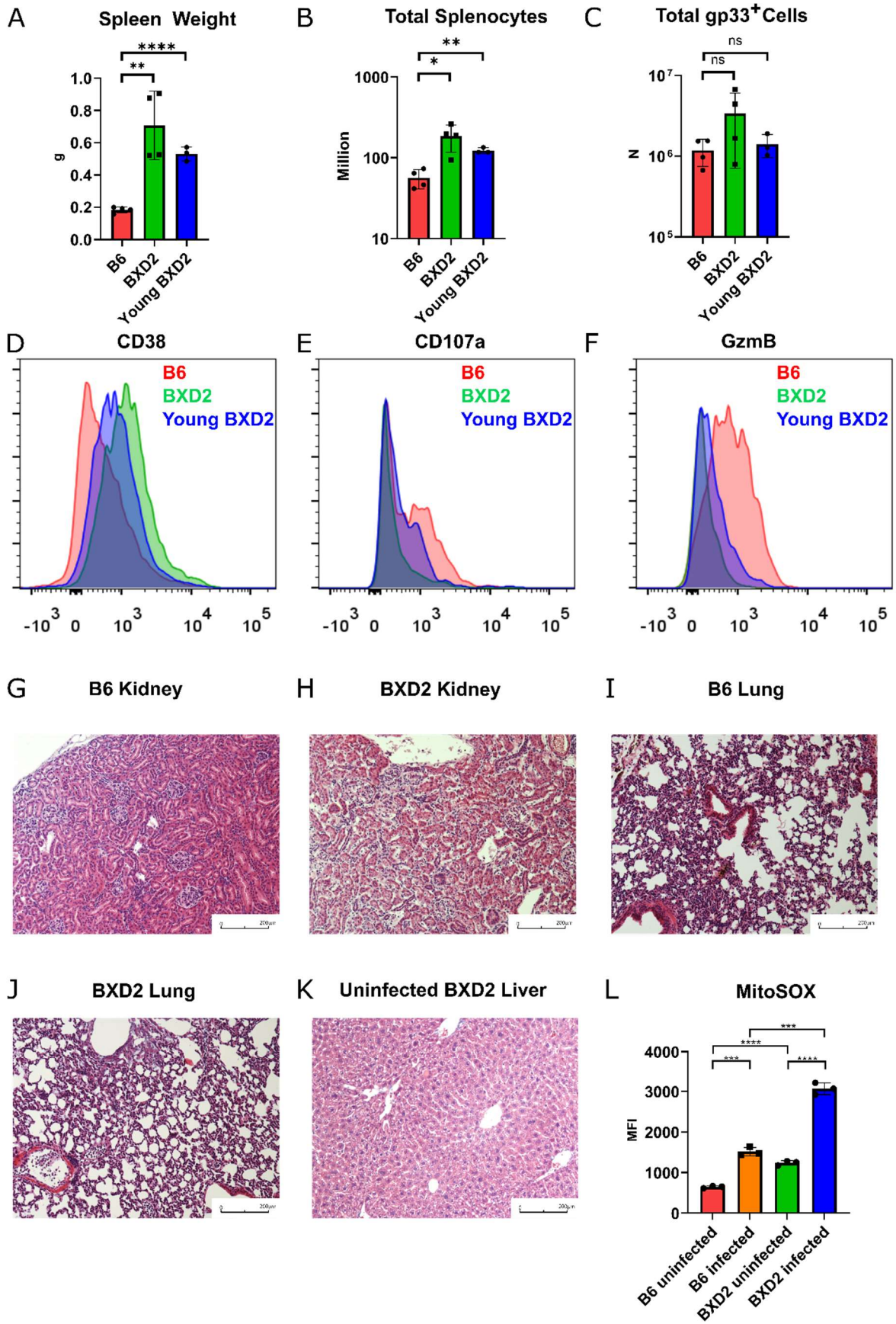
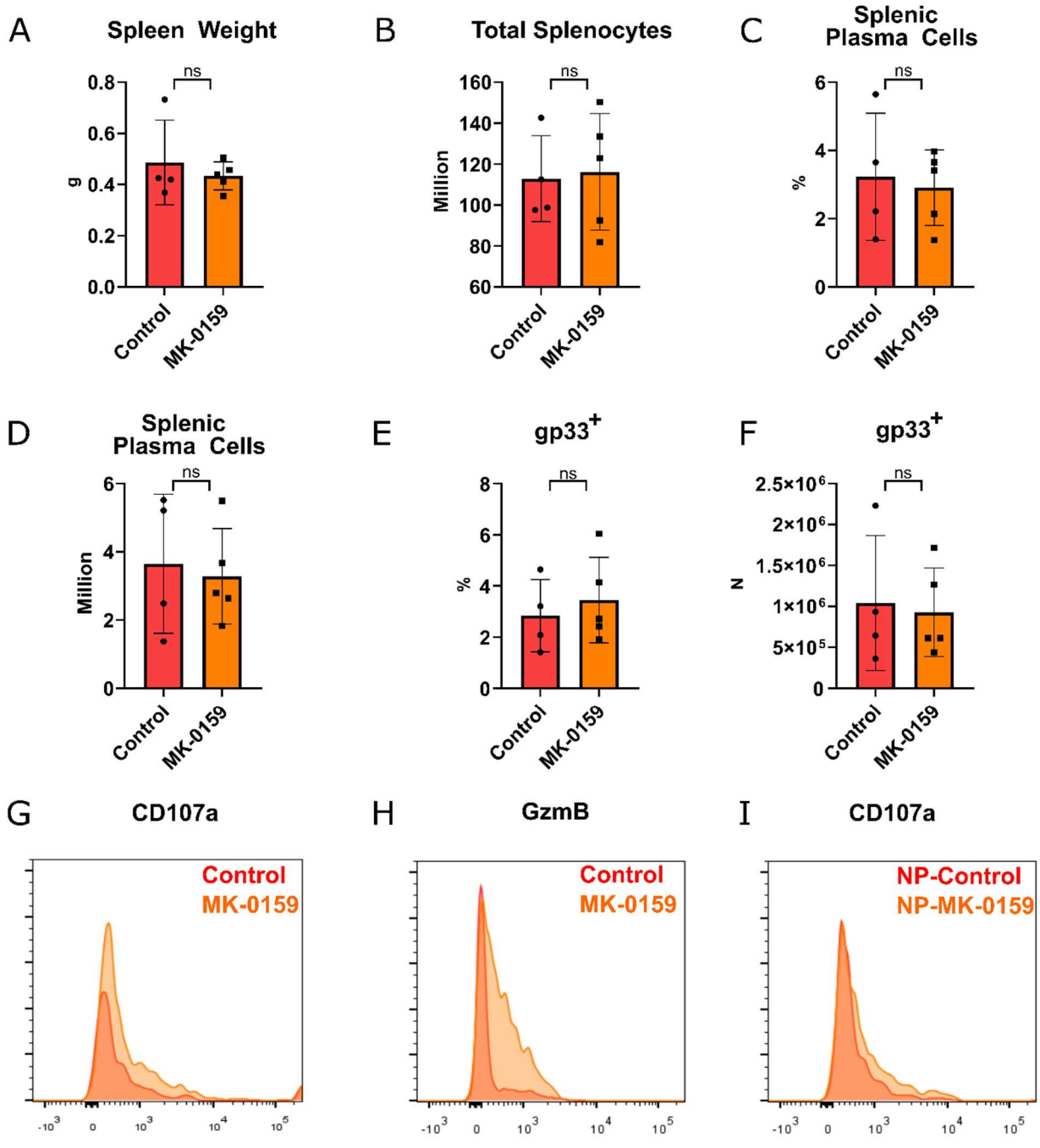

A
Spleen Weight
g
B6
BXD2
Young BXD2
B
Total Splenocytes
Million
C
Total gp33+Cells
N
ns
D
CD38
E
CD107a
F
GzmB
B6
BXD2
Young BXD2
G
B6 Kidney
H
BXD2 Kidney
I
B6 Lung
J
BXD2 Lung
K
Uninfected BXD2 Liver
L
MitoSOX
MFI
B6 uninfected
B6 infected
BXD2 uninfected
BXD2 infected

Figure S7. Features of LCMV infection in B6 and BXD2 lupus mice. A-C. Spleen weight (A), total splenocytes (B) and gp33^{+} CD8^{+} T cells(C) of B6, diseased BXD2, or young non-diseased BXD2 mice 8 days after LCMV Armstrong infection. **D-F**. Representative flow cytometry plots of CD38 (D), CD107a (E), and granzyme B (F) of gp33^{+} CD8^{+} T cells of B6, diseased BXD2, or young non-diseased BXD2 mice 8 days after LCMV Armstrong infection. **G-J**. Representative histology pictures of B6 kidney (G), diseased BXD2 kidney (H), B6 lung (I), and diseased BXD2 kidney (J) mice 8 days after LCMV Armstrong infection. **K.** Representative histology pictures of diseased BXD2 liver without LCMV infection. **L.** Mean fluorescence intensity of MitoSOX staining of freshly isolated hepatocytes from either B6 or diseased BXD2 mice with or without LCMV infection. Data shown are mean ± s.d. statistical analysis by two-tailed t-test. ns = $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$


A
Spleen Weight
ns
0.8
0.6
0.4
0.2
0.0
g
Control
MK-0159
B
Total Splenocytes
ns
160
140
120
100
80
60
Million
Control
MK-0159
C
Splenic
Plasma Cells
ns
6
4
2
0
%
Control
MK-0159
D
Splenic
Plasma Cells
ns
6
4
2
0
Million
Control
MK-0159
E
gp33+
ns
8
6
4
2
0
%
Control
MK-0159
F
gp33+
ns
2.5×10⁶
2×10⁶
1.5×10⁶
1×10⁶
5×10⁵
0
N
Control
MK-0159
G
CD107a
Control
MK-0159
-10³
0
10³
10⁴
10⁵
H
GzmB
Control
MK-0159
-10³
0
10³
10⁴
10⁵
I
CD107a
NP-Control
NP-MK-0159
-10³
0
10³
10⁴
10⁵

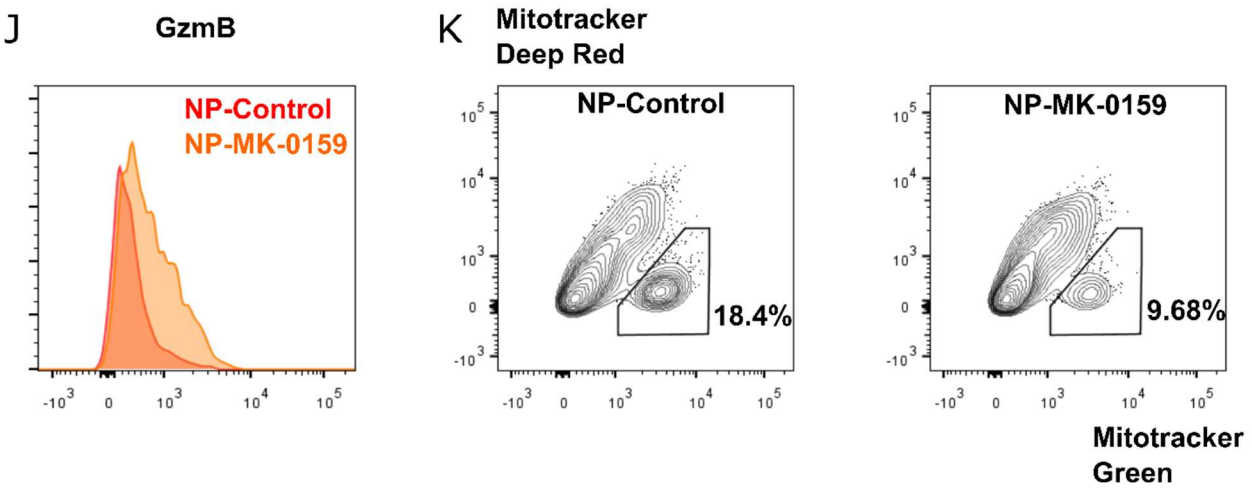


Figure S8. Features of LCMV infection in BXD2 lupus mice with or without CD38 inhibition. **A-F.** Spleen weight (A), total splenocytes (B), percentage (C) and total numbers (D) of spleen plasma cells, percentage of $gp33^+$ out of total $CD8^+$ T cells (E), and total numbers of $gp33^+$ $CD8^+$ T cells (F) of diseased BXD2 treated with control water or MK-0159. **G-H**. Representative flow cytometry plots of CD107a (G), and granzyme B (H) of $gp33^+$ $CD8^+$ T cells of diseased BXD2 treated with control water or MK-0159. **I-J**. Representative flow cytometry plots of CD107a (I), and granzyme B (J) of $gp33^+$ $CD8^+$ T cells of diseased BXD2 treated with NP-Control or NP-MK-0159. **K**. Representative flow cytometry plots of mitotracker green and mitotracker deep red staining of $gp33^+$ $CD8^+$ T cells of diseased BXD2 treated with NP-Control or NP-MK-0159. Data shown are mean ± s.d. statistical analysis by two-tailed t-test. ns = $p > 0.05$

ATAC region	Quantitative PCR Primers
ATP6V1B2	Forward 5‘-GCCGCCTTGGTATATCTGC -3’ Reverse 5‘-CGCCATCTTGTCTCCTCTGT -3’
ATP6V1D	Forward 5‘-CTAAGAGGCAGCAGGGACAC -3’ Reverse 5‘-AGGCGAACCGGAATTGA -3’
KIF26B	Forward 5‘-AAGCTCGGTGAAGGAGACAA -3’ Reverse 5‘-ACGAGGAAAGCGAGGGATAC -3’

Table S1. Primers for ATAC quantitative PCR.

Transcripts	Quantitative PCR Primers
LCMV GP gene	Forward 5‘-CATTCACCTGGACTTTGTCAGACTC -3’ Reverse 5‘-GCAACTGCTGTGTTCCCGAAAC -3’
Actb (Mouse)	Forward 5‘- AGAGGGAAATCGTGCGTGAC -3’ Reverse 5‘- CAATAGTGATGACCTGGCCGT -3’

Table S2. Primers for quantitative PCR.