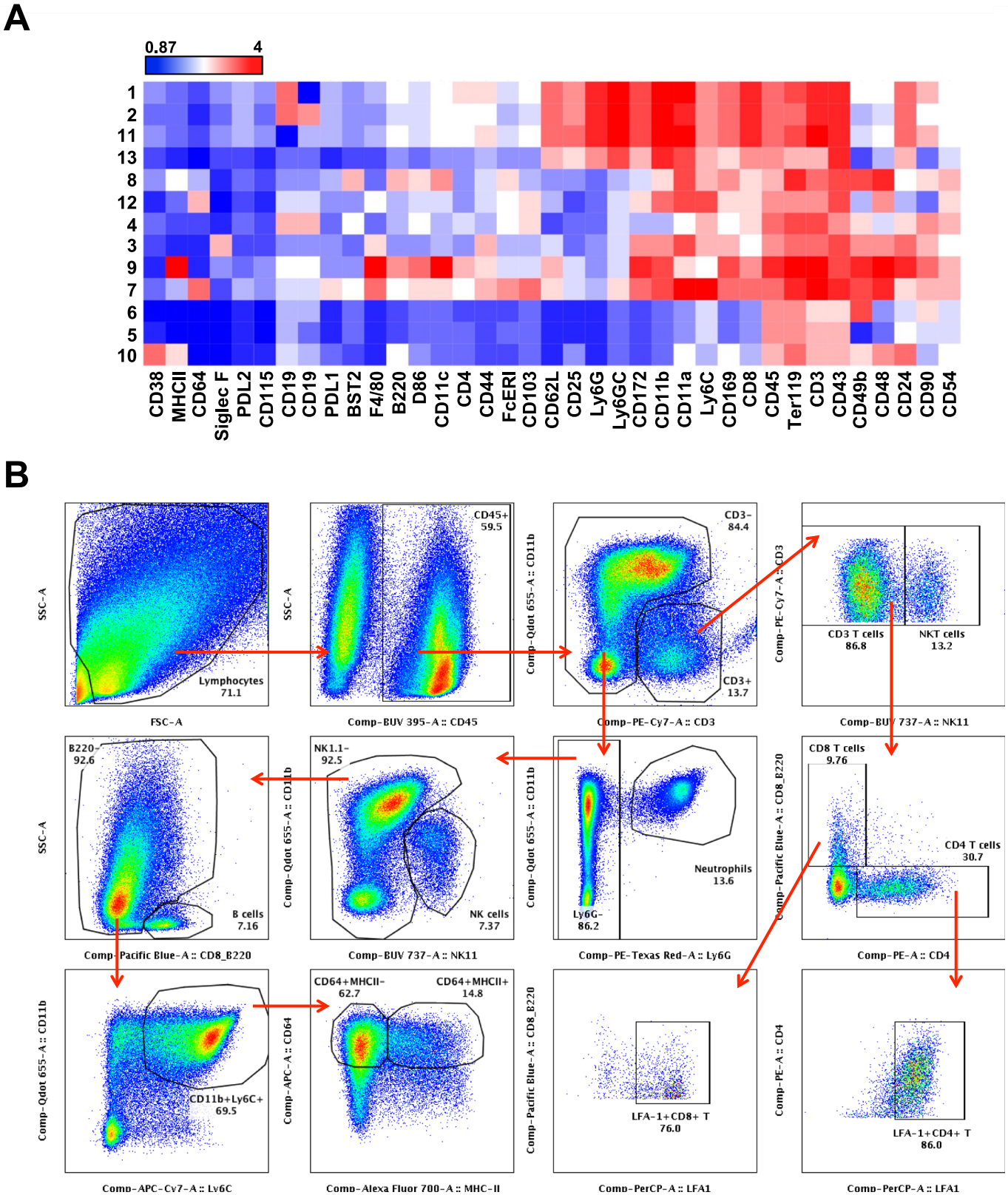
Expanded View Figures

Figure EV1. High-dimensional analysis of mass cytometry data and Immunophenotyping gating strategy.

(**A**) Cluster ID annotation with heatmap. Cluster IDs are indicated in rows, while surface marker expression levels are indicated by the columns. The color represents the relative mean signal intensity of a surface marker. Blue and red represent low and high intensity, respectively. (**B**) Cellular infiltrates in the CHIKV-infected joint-footpad at 6 days post-infection (dpi) were determined with flow cytometry. Briefly, live $CD45^+$ immune cells were gated out before the $CD3^+$ (inclusive of $CD4^+$ and $CD8^+$) T cells, $CD3^+NK1.1^+$ NKT cells, $CD11b^+Ly6G^+$ neutrophils, $NK1.1^+$ NK cells, $CD11b^+Ly6C^+$ monocytes and $CD64^+$ macrophages were identified. Plots shown are representative of a single CHIKV-infected mouse.


A
0.87
4
1
2
11
13
8
12
4
3
9
7
6
5
10
CD38
MHCII
CD64
Siglec F
PDL2
CD115
CD19
CD19
PDL1
BST2
F4/80
B220
D86
CD11c
CD4
CD44
FcERI
CD103
CD62L
CD25
Ly6G
Ly6GC
CD172
CD11b
CD11a
Ly6C
CD169
CD8
CD45
Ter119
CD3
CD43
CD49b
CD48
CD24
CD90
CD54
B
SSC-A
FSC-A
Lymphocytes
71.1
CD45+
59.5
Comp-BUV 395-A :: CD45
Comp-Qdot 655-A :: CD11b
CD3-
84.4
CD3+
13.7
Comp-PE-Cy7-A :: CD3
Comp-PE-Cy7-A :: CD3
CD3 T cells
86.8
NKT cells
13.2
Comp-BUV 737-A :: NK11
B220-
92.6
B cells
7.16
Comp-Pacific Blue-A :: CD8_B220
NK1.1-
92.5
NK cells
7.37
Comp-BUV 737-A :: NK11
Ly6G-
86.2
Neutrophils
13.6
Comp-PE-Texas Red-A :: Ly6G
CD8 T cells
9.76
CD4 T cells
30.7
Comp-PE-A :: CD4
CD11b+Ly6C+
69.5
Comp-APC-Cy7-A :: Ly6C
CD64+MHCII-
62.7
CD64+MHCII+
14.8
Comp-Alexa Fluor 700-A :: MHC-II
LFA-1+CD8+ T
76.0
Comp-PerCP-A :: LFA1
LFA-1+CD4+ T
86.0
Comp-PerCP-A :: LFA1

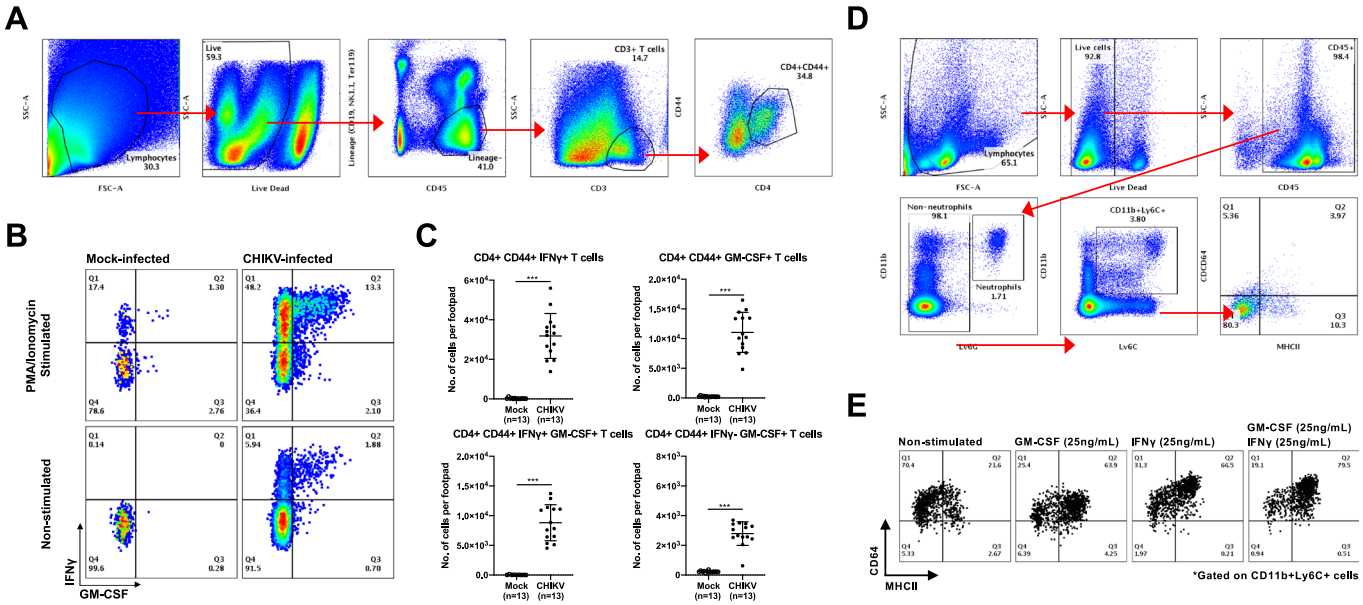


Figure EV2. Intracellular staining reveals presence of IFNγ- and GM-CSF-producing CD4$^+$CD44$^+$ T cells during CHIKV infection.

(**A**) Joint-footpad cells from non-infected and CHIKV-infected animals were harvested at 6 dpi and were subjected to stimulation with ionomycin and Phorbol Myristate Acetate (PMA) for 4 h. Stimulated cells were subsequently stained for the presence of GM-CSF and IFNγ in CD4$^+$CD44$^+$ memory/effector T cells. Representative electronic gating strategy to isolate CD4$^+$CD44$^+$ T cells from joint-footpad cells. Plots shown are concatenated from CHIKV-infected samples. (**B**) Representative flow cytometry plot illustrating the gating strategy in the identification of GM-CSF and IFNγ within the CD4$^+$CD44$^+$ T cells. (**C**) Dotplots showing the numbers of the various identified subsets within the joint-footpads. Data were from biological replicates obtained from two independent experiments. All data are presented as mean ± SD. Data comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed). IFNγ$^+$ T cells, ***$P = 0.0000000499$; GM-CSF$^+$ T cells, ***$P = 0.0000000499$; IFNγ$^+$GM-CSF$^+$ T cells, ***$P = 0.0000000499$; IFNγ$^-$GM-CSF$^+$ T cells, ***$P = 0.0000000499$. (**D**) Fresh blood was obtained from non-infected animals. Red blood cells were subsequently lysed and live cells were stained with a commercially available live/dead dye before being stained with a cocktail of antibodies targeting surface markers CD45, CD11b, Ly6G, Ly6C, CD64, and MHCII. Live CD45$^+$ cells were firstly identified, and non-neutrophils were gated next. CD11b$^+$Ly6C$^+$ monocytes were identified from the non-neutrophils and were shown to be low in CD64 and MHCII expression (Q4). Plots shown are representative of a single non-infected mouse. (**E**) Peripheral whole blood was obtained from non-infected animals and were subjected to GM-CSF and IFNγ stimulation after removal of the red blood cells. Stimulated cells were harvested 24 h later and stained for the identification of monocytes and macrophage subsets. Representative flow cytometry plots showing the gating strategy used to identify CD64$^+$MHCII$^+$ cells among the precursor CD11b$^+$Ly6C$^+$ monocytes.

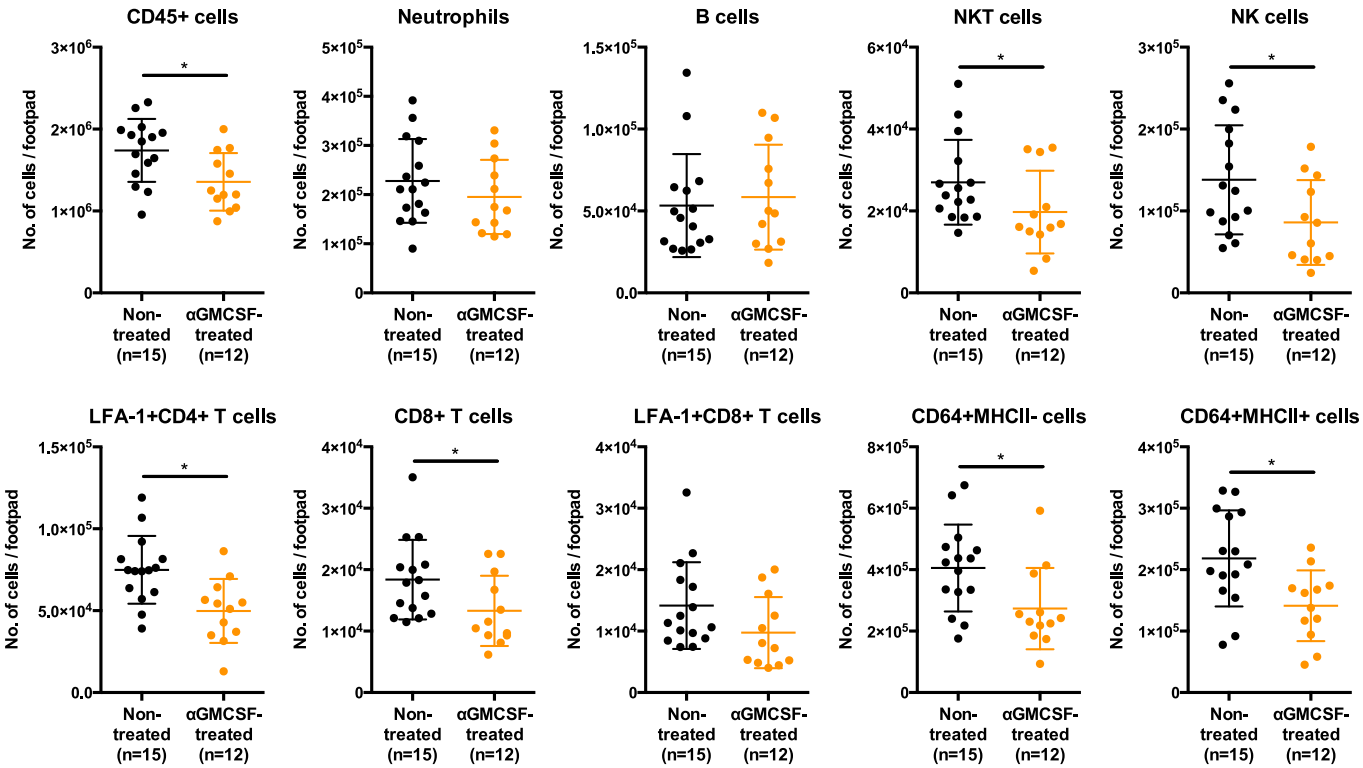


Figure EV3. Depletion of GM-CSF alters the numbers of immune cells infiltrating the joint-footpad.

CHIKV infection (1×10^6 PFU) was performed in the right footpad of wild-type animals. Four days post-infection (dpi), anti-GM-CSF antibodies were given intraperitoneally. Subsequently, numbers of infiltrating $CD45^+$ cells, neutrophils, B cells, NKT cells. NK cells, LFA-1^+CD4^+ T cells, $CD8^+$ T cells, LFA-1^+CD4^+ T cells, $CD11b^+Ly6C^+$ cells, $CD64^+MHCII^-$ cells and $CD64^+MHCII^+$ cells were obtained following immunophenotyping of the joint-footpad at 6 days post-infection (dpi). Data were from biological replicates obtained from two independent experiments. All data are presented as mean ± SD. Data comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed): CD45 +, $^*P = 0.0139$; NKT, $^*P = 0.0469$; NK, $^*P = 0.0214$; LFA-1^+CD4^+ T, $^*P = 0.031$; $CD8^+$ T, $^*P = 0.0281$; $CD64^+MHCII^-$, $^*P = 0.0186$; $CD64^+MHCI^+$, $^*P = 0.0161$.

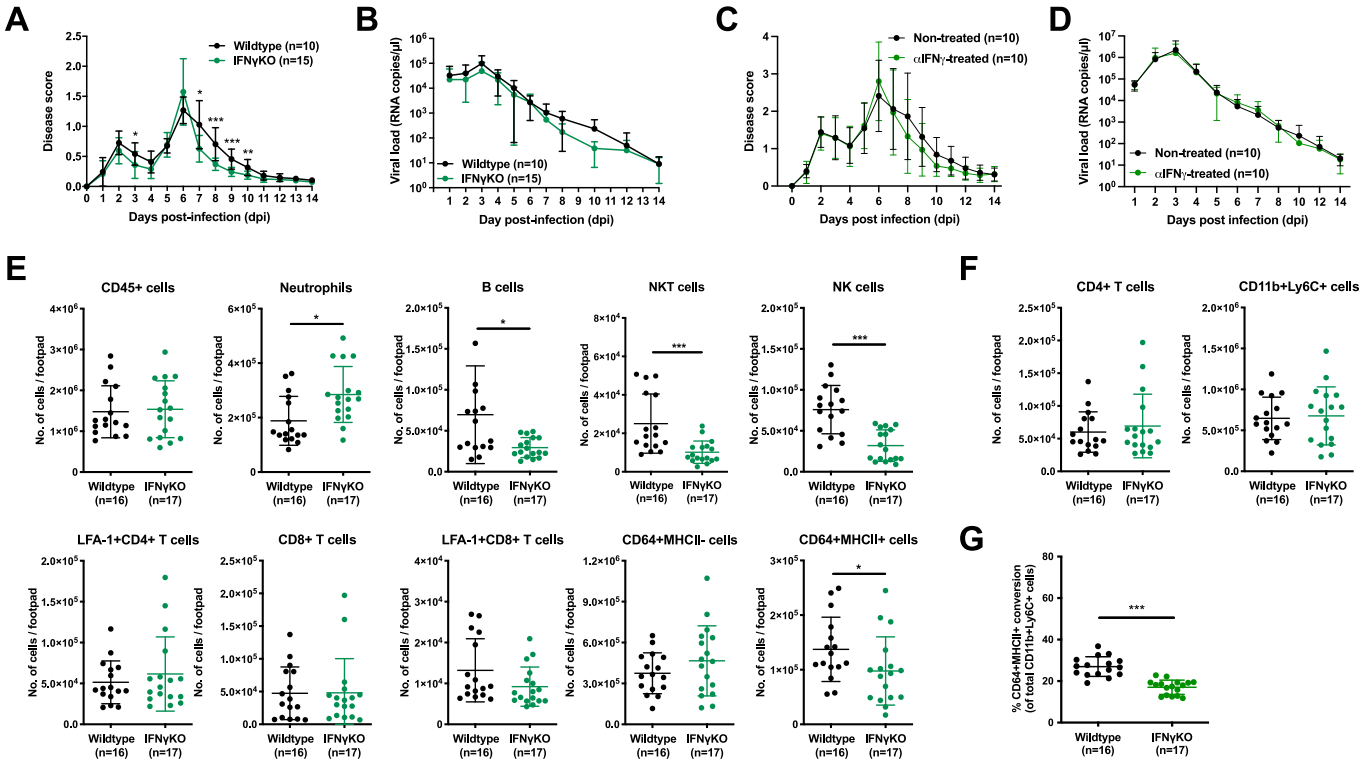


Figure EV4. Absence of IFNγ alters the conversion of CD64$^+$MHCII$^+$ macrophages in the joint-footpad.

(**A**,**B**) IFNγKO animals were infected with 1 × 10^6 PFU of CHIKV in the right footpad. Joint-footpad swelling (**A**) and viral RNA load (**B**) were monitored over 14 days. Data were from biological replicates obtained from two independent experiments and presented as mean ± SD. Comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed). For footpad swelling: 3 dpi, $^*P = 0.0395$; 7 dpi, $^*P = 0.0216$; 8 dpi, $^{***}P = 0.00000214$; 9 dpi, $^{***}P = 0.0003$; 10 dpi, $^{**}P = 0.0051$. (**C**,**D**) Wild-type animals were infected with 1 × 10^6 PFU of CHIKV in the right footpad and were treated with anti-IFNγ antibodies at 0-, 2- and 4-days post-infection (dpi). Joint-footpad swelling (**C**) and viral RNA load (**D**) were monitored over 14 days. Data were from biological replicates obtained from two independent experiments and presented as mean ± SD. (**E**–**G**) Immunophenotyping of IFNγKO joint-footpad was performed at 6 dpi to determine the numbers of infiltrating CD45$^+$ cells, neutrophils, B cells, NKT cells. NK cells, LFA-1$^+$CD4$^+$ T cells, CD8$^+$ T cells, LFA-1$^+$CD8$^+$ T cells, CD64$^+$MHCII$^-$ cells and CD64$^+$MHCII$^+$ cells (**E**). Numbers of joint-footpad infiltrating CD4$^+$ T cells and CD11b$^+$Ly6C$^+$ monocytes are depicted (**F**). Percentage differentiation of CD11b$^+$Ly6C$^+$ monocytes into CD64$^+$MHCII$^+$ macrophages in CHIKV-infected wild type or IFNγKO animals is shown (**G**). Data were from biological replicates obtained from two independent experiments and presented as mean ± SD. Comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed). For neutrophils, $^*P = 0.0136$; B cells, $^*P = 0.0136$; NKT, $^{***}P = 0.0002$; NK, $^{***}P = 0.0001$; CD64$^+$MHCII$^+$, $^*P = 0.0207$; for % CD64$^+$MHCII$^+$ conversion (**G**), $^{***}P = 0.000000105$.

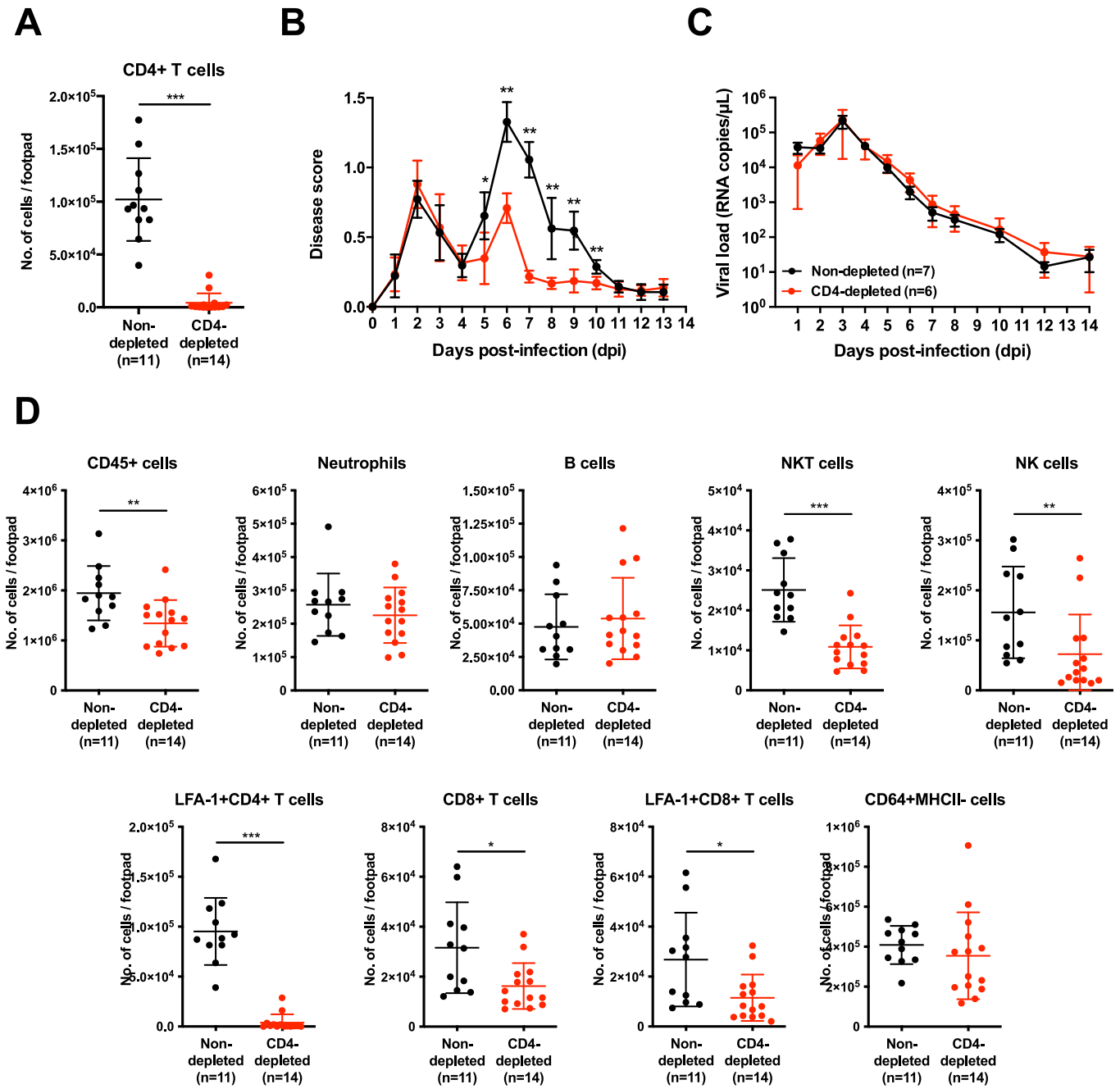


Figure EV5. CD4-depletion reduces CHIKV disease severity.

(**A**) Wild-type animals were infected with 1 × 10^6 PFU of CHIKV in the right footpad and anti-CD4 antibodies were given intraperitoneally at -1 and 4 days post-infection (dpi). Immunophenotyping of joint-footpad was performed at 6 dpi to determine the numbers of infiltrating $CD4^+$ T cells. Data were from biological replicates obtained from two independent experiments and presented as mean ± SD. Data comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed). $CD4^+$ T cells, ***$P = 0.000000449$. (**B**,**C**) Joint-footpad swelling (**B**) and viral RNA load (**C**) of CHIKV-infected animals with or without administration of anti-CD4 antibodies, over a period of 14 days. Data were from biological replicates obtained from two independent experiments and presented as mean ± SD. Data comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed). For footpad swelling: 5 dpi, *$P = 0.0192$; 6 dpi, **$P = 0.0012$; 7 dpi, **$P = 0.0012$; 8 dpi, **$P = 0.0012$; 9 dpi, **$P = 0.0012$; 10 dpi, **$P = 0.0023$. (**D**) Numbers of infiltrating $CD45^+$ cells, neutrophils, B cells, NKT cells. NK cells, LFA-1^+CD4^+ T cells, $CD8^+$ T cells, LFA-1^+CD4^+ T cells, $CD11b^+Ly6C^+$ cells and $CD64^+MHCII^-$ cells were determined following immunophenotyping of the joint-footpad at 6 dpi (**A**). Data were from biological replicates obtained from two independent experiments and presented as mean ± SD. Data comparisons between the groups were performed with non-parametric Mann–Whitney *U* test (two-tailed). $CD45^+$, **$P = 0.0042$; NKT, ***$P = 0.0000306$; NK, **$P = 0.0075$; LFA-1^+CD4^+ T, ***$P = 0.000000449$; $CD8^+$, *$P = 0.0108$; LFA-1^+CD8^+ T, *$P = 0.0179$.