Supplementary Information

Lung type II alveolar epithelial cells collaborate with CCR2^{+} inflammatory monocytes in host defense against poxvirus infection

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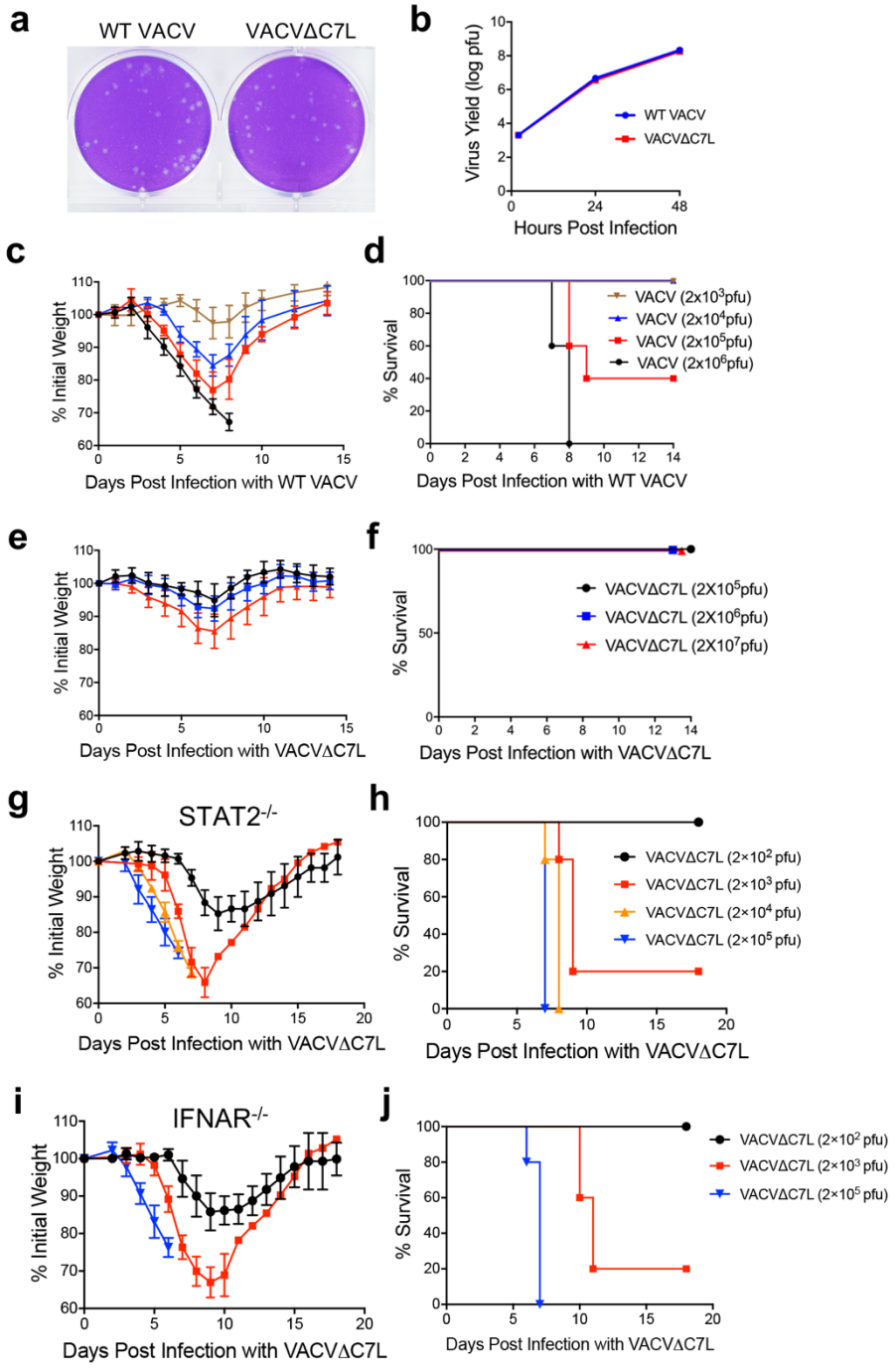
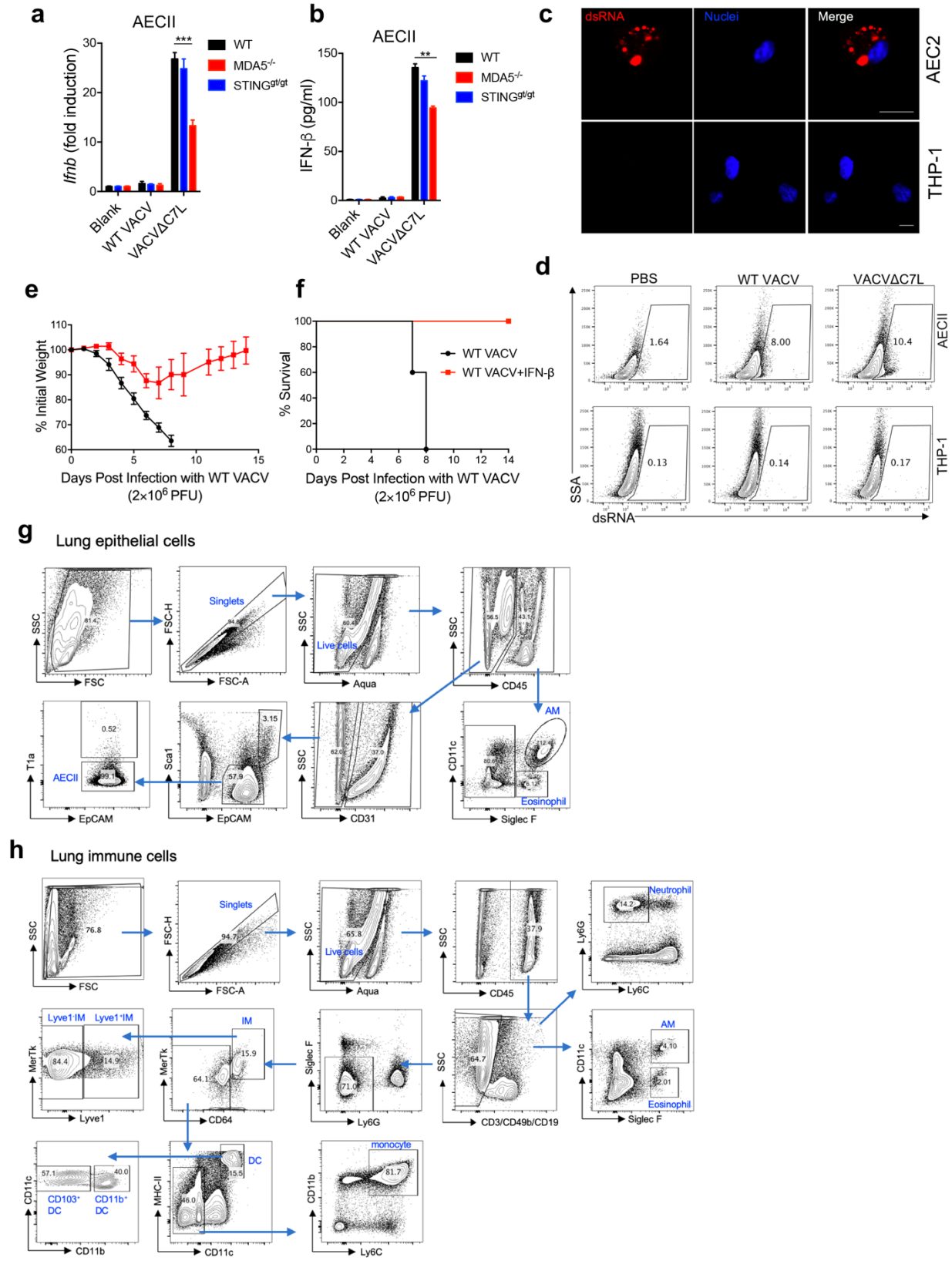
Figure S1.


a
WT VACV
VACVΔC7L
b
Virus Yield (log pfu)
Hours Post Infection
WT VACV
VACVΔC7L
c
% Initial Weight
Days Post Infection with WT VACV
d
% Survival
VACV (2x10³pfu)
VACV (2x10⁴pfu)
VACV (2x10⁵pfu)
VACV (2x10⁶pfu)
Days Post Infection with WT VACV
e
% Initial Weight
Days Post Infection with VACVΔC7L
f
% Survival
VACVΔC7L (2X10⁵pfu)
VACVΔC7L (2X10⁶pfu)
VACVΔC7L (2X10⁷pfu)
Days Post Infection with VACVΔC7L
g
STAT2-/-
% Initial Weight
Days Post Infection with VACVΔC7L
h
% Survival
VACVΔC7L (2×10² pfu)
VACVΔC7L (2×10³ pfu)
VACVΔC7L (2×10⁴ pfu)
VACVΔC7L (2×10⁵ pfu)
Days Post Infection with VACVΔC7L
i
IFNAR-/-
% Initial Weight
Days Post Infection with VACVΔC7L
j
% Survival
VACVΔC7L (2×10² pfu)
VACVΔC7L (2×10³ pfu)
VACVΔC7L (2×10⁵ pfu)
Days Post Infection with VACVΔC7L

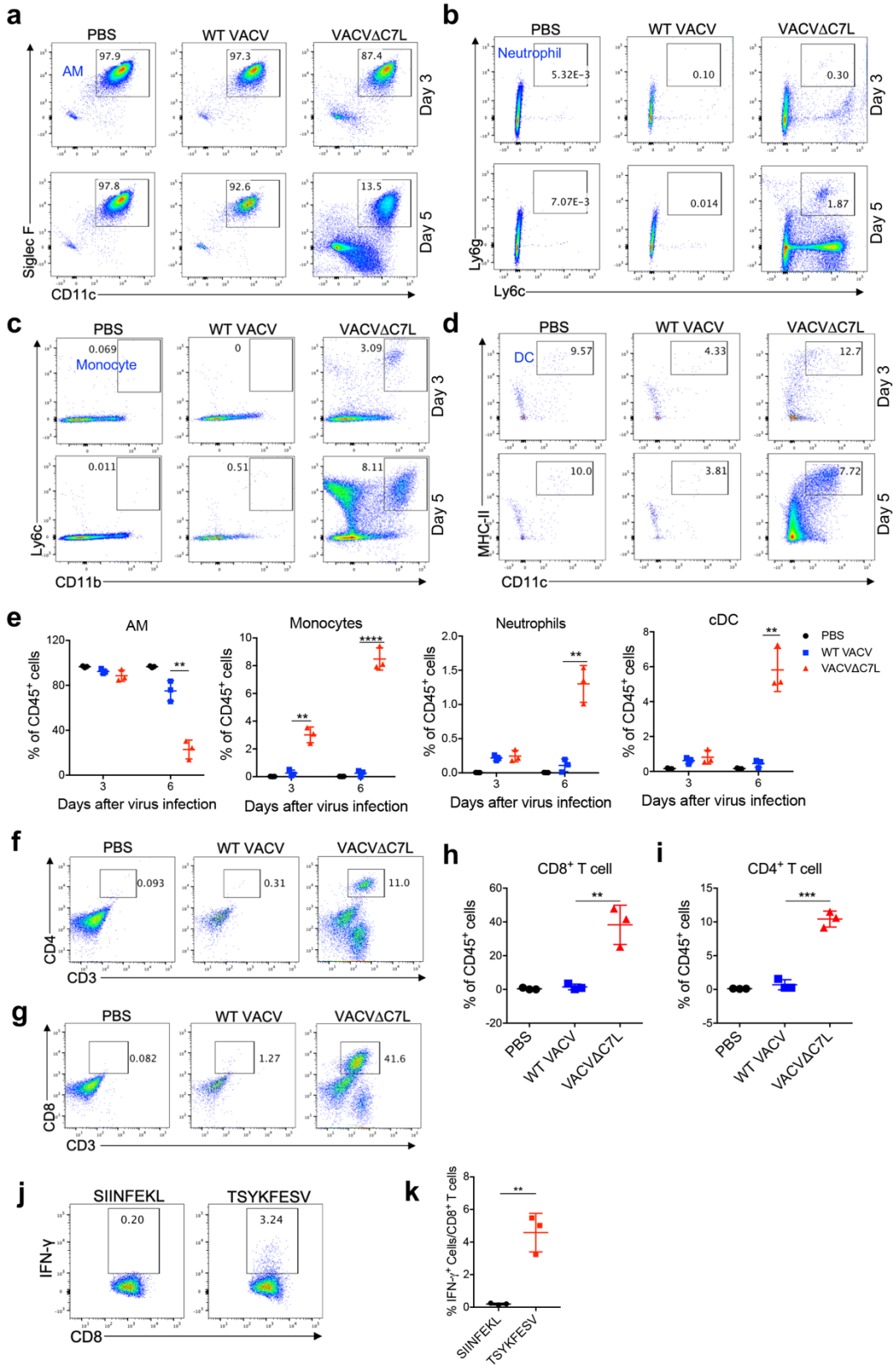
Supplementary Fig. 1 Determination of LD50 of WT VACV or VACVΔC7L intranasal infection in WT, STAT2$^{-/-}$ or IFNAR$^{-/-}$ mice. a Plaque sizes of WT VACV and VACVΔC7L at day 2 post-infection in BSC40 cells. **b** Viral growth curve in BSC40 cells at an initial MOI of 0.05. **c-d** Percentages of initial weight (**c**) and Kaplan-Meier survival curve (**d**) of WT C57BL/6J control mice (n=5 in each group) over days post intranasal infection with WT VACV at different doses. **e-f** Percentages of initial weight (**e**) and Kaplan-Meier survival curve (**f**) of WT C57BL/6J control mice (n=5 in each group) over days post intranasal infection with VACVΔC7L at different doses. **g-j** STAT2$^{-/-}$ or IFNAR1$^{-/-}$ mice were infected with VACVΔC7L intranasally at different doses. Mice were monitored for weight daily. **g, i** Percentages of initial weight over days in STAT2$^{-/-}$ (**g**) or IFNAR1$^{-/-}$ mice (**i**) post intranasal infection with VACVΔC7L at increasing doses. **h**, **j** Kaplan-Meier survival curve of STAT2$^{-/-}$ (**h**) or IFNAR1$^{-/-}$ mice (**j**) infected with VACVΔC7L at increasing doses (n=5 in each group). Data are representative of two independent experiments.

Figure S2.


a
AECII
Ifnb (fold induction)
WT
MDA5-/-
STINGgt/gt
Blank
WT VACV
VACVΔC7L
b
AECII
IFN-β (pg/ml)
c
dsRNA
Nuclei
Merge
AEC2
THP-1
d
PBS
WT VACV
VACVΔC7L
AECII
THP-1
SSA
dsRNA
e
% Initial Weight
Days Post Infection with WT VACV
(2×10^6 PFU)
f
% Survival
WT VACV
WT VACV+IFN-β
Days Post Infection with WT VACV
(2×10^6 PFU)
g
Lung epithelial cells
Singlets
Live cells
AECII
AM
Eosinophil
h
Lung immune cells
Singlets
Live cells
Neutrophil
Lyve1-IM
Lyve1+IM
IM
AM
Eosinophil
CD103+ DC
CD11b+ DC
DC
monocyte

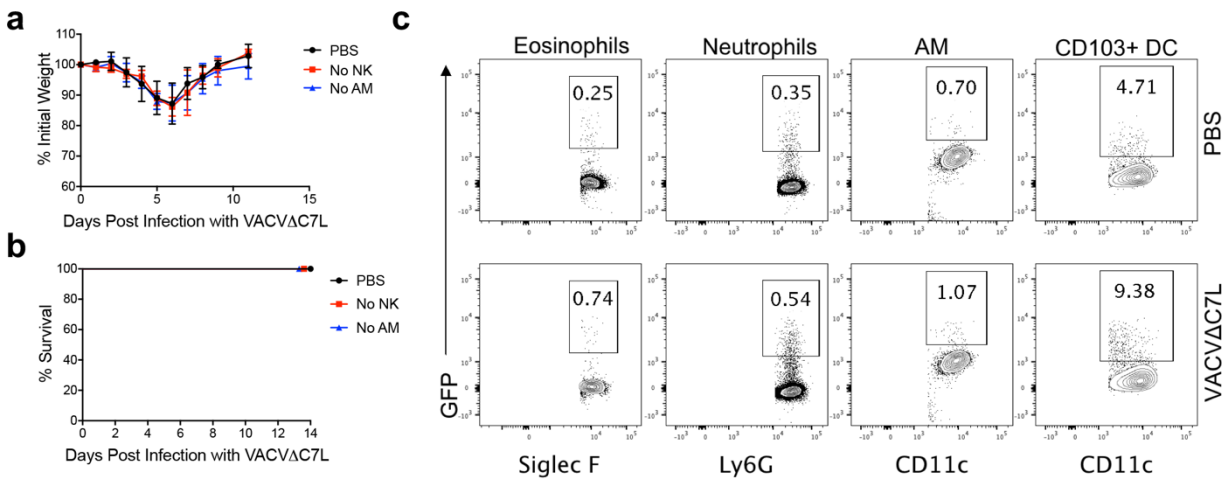
Supplementary Fig. 2. DsRNA was produced in AECIIs after VACVΔC7L infection and intranasal administration of IFN-β rescues mice from lethal WT VACV infection. a AECII were generated through culturing lineage negative epithelial progenitor cells isolated from WT, $STING^{Gt/Gt}$, or $MDA5^{-/-}$ mice as described in Fig. 2. RT-PCR analyses of Ifnb gene expression of AECIIs infected with either WT VACV or VACVΔC7L at a MOI of 5 for 12 h. PBS was used as a mock infection control. **b** ELISA analyses of IFN-β levels in the supernatants of AECIIs infected with either WT VACV or VACVΔC7L at a MOI of 5 for 24 h. PBS was used as a mock infection control. **c** Representative confocal images showing dsRNA staining in AECIIs or THP-1 cells infected with VACVΔC7L at a MOI of 5 for 16 h. Scale bar, 10 μm. **d** FACS analysis of dsRNA from AECIIs or THP-1 cells infected with VACVΔC7L at a MOI of 5 for 16 h. **e-f** C57BL/6J mice were infected with WT VACV at 2 x 10^6 pfu. They were either treated with intranasal administration of IFN-β (1 μg/mouse) or PBS at day one post infection, shown are percentages of initial weight (**e**) and Kaplan-Meier survival curve (**f**) over days. (n=5 in each group). ** $p<0.01$, and *** $p<0.001$ (unpaired t test). Data are representative of two independent experiments and represented as mean ± SD. **g-h** Gating strategy to define epithelial cells and myeloid cell populations in the lung. Within the single cell suspension, doublets and dead cells were excluded from analysis. **g** Among $CD45^-$ cells, $CD31^+$ cells represent endothelial cells. Among $CD31^-$ cells, after excluding bronchioalveolar stem cells (BASCs), $EpCAM^+Sca1^-$ cells represent lung epithelial cells and AECIIs are further defined as $EpCAM^+T1a^-$. **h** Among $CD45^+$ cells, Siglec F^+ $CD11c^+$ cells represent alveolar macrophages (AMs). Siglec F^+ $CD11c^-$ cells represent eosinophils. Neutrophils are defined as $Ly6G^+$ $Ly6C^-$ cells. After excluding Siglec F^+, $Ly6G^+$, $CD3^+$, $CD49b^+$, $CD19^+$ cells, $CD64^+$ $MerTk^+$ cells represent interstitial macrophages (IMs), which can be further divided into $Lyve1^+$ IMs and $Lyve1^-$ IMs. Among $CD64^-MerTk^-$ cells, DCs are defined as MHC-II^+ $CD11c^+$ cells. Monocytes are defined as $Ly6C^+$ $CD11b^+$ cells.

Figure S3.


a
PBS
WT VACV
VACVΔC7L
AM
97.9
97.3
87.4
97.8
92.6
13.5
Day 3
Day 5
Siglec F
CD11c
b
PBS
WT VACV
VACVΔC7L
Neutrophil
5.32E-3
0.10
0.30
7.07E-3
0.014
1.87
Day 3
Day 5
Ly6g
Ly6c
c
PBS
WT VACV
VACVΔC7L
Monocyte
0.069
0
3.09
0.011
0.51
8.11
Day 3
Day 5
Ly6c
CD11b
d
PBS
WT VACV
VACVΔC7L
DC
9.57
4.33
12.7
10.0
3.81
7.72
Day 3
Day 5
MHC-II
CD11c
e
AM
Monocytes
Neutrophils
cDC
% of CD45+ cells
Days after virus infection
PBS
WT VACV
VACVΔC7L
f
PBS
WT VACV
VACVΔC7L
0.093
0.31
11.0
CD4
CD3
g
PBS
WT VACV
VACVΔC7L
0.082
1.27
41.6
CD8
CD3
h
CD8+ T cell
% of CD45+ cells
PBS
WT VACV
VACVΔC7L
i
CD4+ T cell
% of CD45+ cells
PBS
WT VACV
VACVΔC7L
j
SIINFEKL
TSYKFESV
0.20
3.24
IFN-γ
CD8
k
% IFN-γ+ Cells/CD8+ T cells
SIINFEKL
TSYKFESV

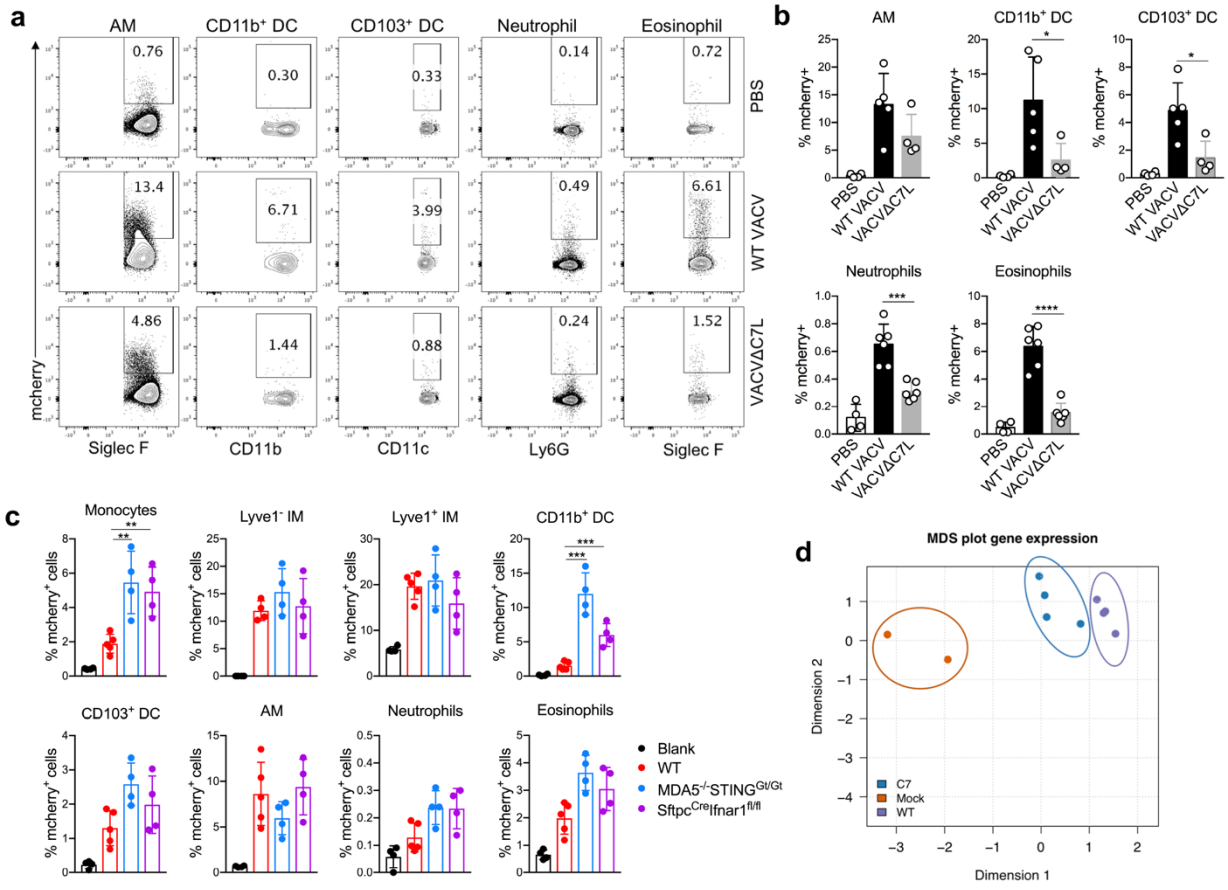
Supplementary Fig. 3 Intranasal infection of VACVΔC7L results in the influx of dendritic cells (DCs), monocytes, neutrophils, $CD8^+$, and $CD4^+$ T cells into bronchoalveolar space of the infected lungs. WT C57BL/6J mice were infected with either WT VACV at 2 x 10^5 pfu or with VACVΔC7L at 2 x 10^7 pfu, or mock-infected with PBS. BAL was collected at day 3 and day 5 post infection or PBS treatment. Myeloid cell populations in the BAL were analyzed by FACS. **a** Dot plots of Siglec F^+CD11c^+ lung alveolar macrophages in the BAL from mice infected with either WT VACV, VACVΔC7L, or mock infected. **b** Dot plots of $Ly6G^+Ly6C^+$ neutrophils in the BAL from mice infected with either WT VACV, VACVΔC7L, or mock infected. **c** Dot plots of $Ly6C^+CD11b^+$ inflammatory monocytes in the BAL from mice infected with either WT VACV, VACVΔC7L, or mock infected. **d** Dot plots of $MHCII^+CD11c^+$ DCs in the BAL from mice infected with either WT VACV, VACVΔC7L, or mock infected. **e** Quantification of data from (**a-d**) with independent experimental replicates. **f-g** Dot plots of $CD4^+$ or $CD8^+$ T cells in the BAL from mice at day 5 post infected with either WT VACV, VACVΔC7L, or mock infected. **h-i** Quantification of data from (**f-g**) with independent experimental replicates. **J** Dot plots of B8R specific $CD8^+$ T cells in BAL from mice at day 5 post infected with VACVΔC7L. SIINFEKL as non-specific peptide control. **k** Quantification of data from **J**. ** $p<0.01$, *** $p<0.01$, and **** $p<0.0001$ (unpaired t test). Data are representative of two (**f-k**) or three (**a-e**) independent experiments.

Figure S4.



Supplementary Fig. 4 Alveolar macrophages and NK cells are not required for host defense against VACVΔC7L infection. **a-b** Percentages of initial weight (**a**) and Kaplan-Meier survival curve (**b**) over days in WT C57BL/6J mice, NK cell depletion mice or AM depletion mice infected with VACVΔC7L at 2 x 10^7 pfu. (n=5 in each group). **c** Dot plots showing GFP^+ eosinophils, GFP^+ neutrophils, and GFP^+ AM and GFP^+ $CD103^+$ DCs in the lungs of CCR2-GFP mice at day 3 post infection with VACVΔC7L at 2 x 10^7 pfu compared with PBS-mock infected mice. Data are representative of two independent experiments.

Figure S5.


a
AM
CD11b⁺ DC
CD103⁺ DC
Neutrophil
Eosinophil
PBS
WT VACV
VACVΔC7L
mcherry
Siglec F
CD11b
CD11c
Ly6G
Siglec F
b
AM
CD11b⁺ DC
CD103⁺ DC
Neutrophils
Eosinophils
% mcherry+
PBS
WT VACV
VACVΔC7L
c
Monocytes
Lyve1⁻ IM
Lyve1⁺ IM
CD11b⁺ DC
CD103⁺ DC
AM
Neutrophils
Eosinophils
% mcherry⁺ cells
Blank
WT
MDA5-/-STINGGt/Gt
SftpcCreIfnar1fl/fl
d
MDS plot gene expression
Dimension 1
Dimension 2
C7
Mock
WT

Supplementary Fig. 5 CCR2^{+} inflammatory monocytes and IMs are the main cell populations infected by vaccinia virus. a-b Representative flow cytometry dot plots (**a**) and bar graph (**b**) showing mcherry^{+} of different cell populations in the lungs of C57BL/6J mice at day 3 post infection with WT VACV-mcherry, VACVΔC7L-mcherry at 2 x 10^{7} pfu or PBS-mock infected mice. **c** bar graph showing mcherry^{+} of different cell populations in the lungs of C57BL/6J, MDA5$^{-/-}$STING$^{Gt/Gt}$ or Sftpc^{cre}Ifnar1$^{fl/fl}$ mice at day 3 post infection with VACVΔC7L-mcherry at 2 x 10^{7} pfu. PBS-mock infected C57BL/6J mice as blank control. **d** Multidimensional scaling (MDS) plot of RNAseq results in monocytes. ∗ $p<0.05$, ∗∗ $p<0.01$, ∗∗∗ $p<0.001$ and ∗∗∗∗ $p<0.0001$ (unpaired t test). Data are representative of two (**a-d**) independent experiments and presented as mean ± SD.

Supplementary Table 1. List of primers used in this paper for qRT-PCR.

Primer sequence	SOURCE
qPCR *Ifnb* For: TGGAGATGACGGAGAAGATG	Integrated DNA technologies IDT
qPCR *Ifnb* Rev: TTGGATGGCAAAGGCAGT	
qPCR *Ccl4* For: GCCCTCTCTCTCCTCTTGCT	
qPCR *Ccl4* Rev: CTGGTCTCATAGTAATCCATC	
qPCR *Gapdh* For: AGGTCGGTGTGAACGGATTTG	
qPCR *Gapdh* Rev: TGTAGACCATGTAGTTGAGGTCA	
qPCR *Ccl5* For: GCCCACGTCAAGGAGTATTTCTA	
qPCR *Ccl5* Rev: ACACACTTGGCGGTTCCTTC	
qPCR *E5* For: TCTCGGACATTTCAGCCATC	
qPCR *E5* Rev: GGAAACATGTAAAGCAGCAGAG	
qPCR *A34* For: GGCATAGGAACATTTCTGCATTAC	
qPCR *A34* Rev: TACGACACTGATAAACCGCATT	
qPCR *A27* For: CCGTCCAGTCTGAACATCAAT	
qPCR *A27* Rev: GTGTTGTAAACGCAACGATGAA	