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Supplemental information

Vaccinia virus E3 prevents sensing of Z-RNA to block ZBP1-dependent necroptosis

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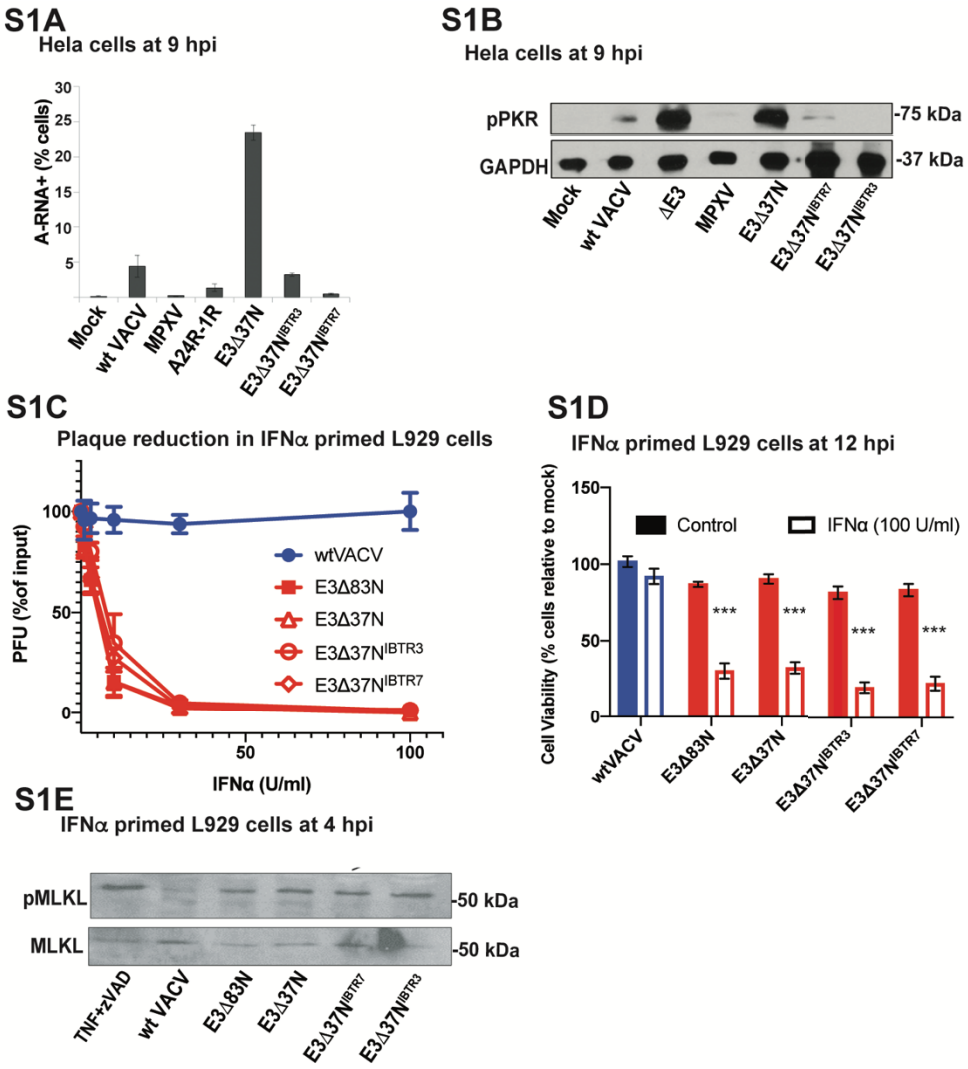

Figure S1, Additional data on dsRNA, related to Figure 4.

(S1A) Flow cytometric evaluation of free A-RNA in Hela cells. Cells were either left uninfected (Mock) or infected with wt VACV, MPXV, A24R-R1, E3Δ37N, E3Δ37N^{IBTR3} or E3Δ37N^{IBTR7} (MOI of 5). Following fixation and permeabilization at 9 hpi, the frequency of A-RNA^{+} cells was determined by flow cytometry with the J2 antibody. Single cell suspensions were generated and gated to exclude doublets.

(S1B) IB of Hela cells for activated PKR that were either left uninfected (Mock) or infected with wt VACV, MPXV, E3Δ37N, E3Δ37N^{IBTR3} or E3Δ37N^{IBTR7} (MOI of 5). Lysates were harvested at 9 hpi, denatured and separated by SDS-PAGE prior to transfer and detection of phospho-PKR (pPKR).

(S1C) Plaque reduction assays were carried out on L929 cells as described in Figure 1.

(S1D) L929 cells either left untreated or IFNα-pretreated as described in Figure 1 and infected (MOI of 5) with the indicated viruses. Cell viability was determined by Sytox dye exclusion as described in Figure 1.

(S1E) IB of L929 cell lysates as described in Figure 1. Error bars represent the SD. Statistical significance was determined as described in Figure 1. Each set of data is representative of three replicates except for panel A, C and D which compiles the results of the replicates. Statistical significance was determined as described in Figure 1.

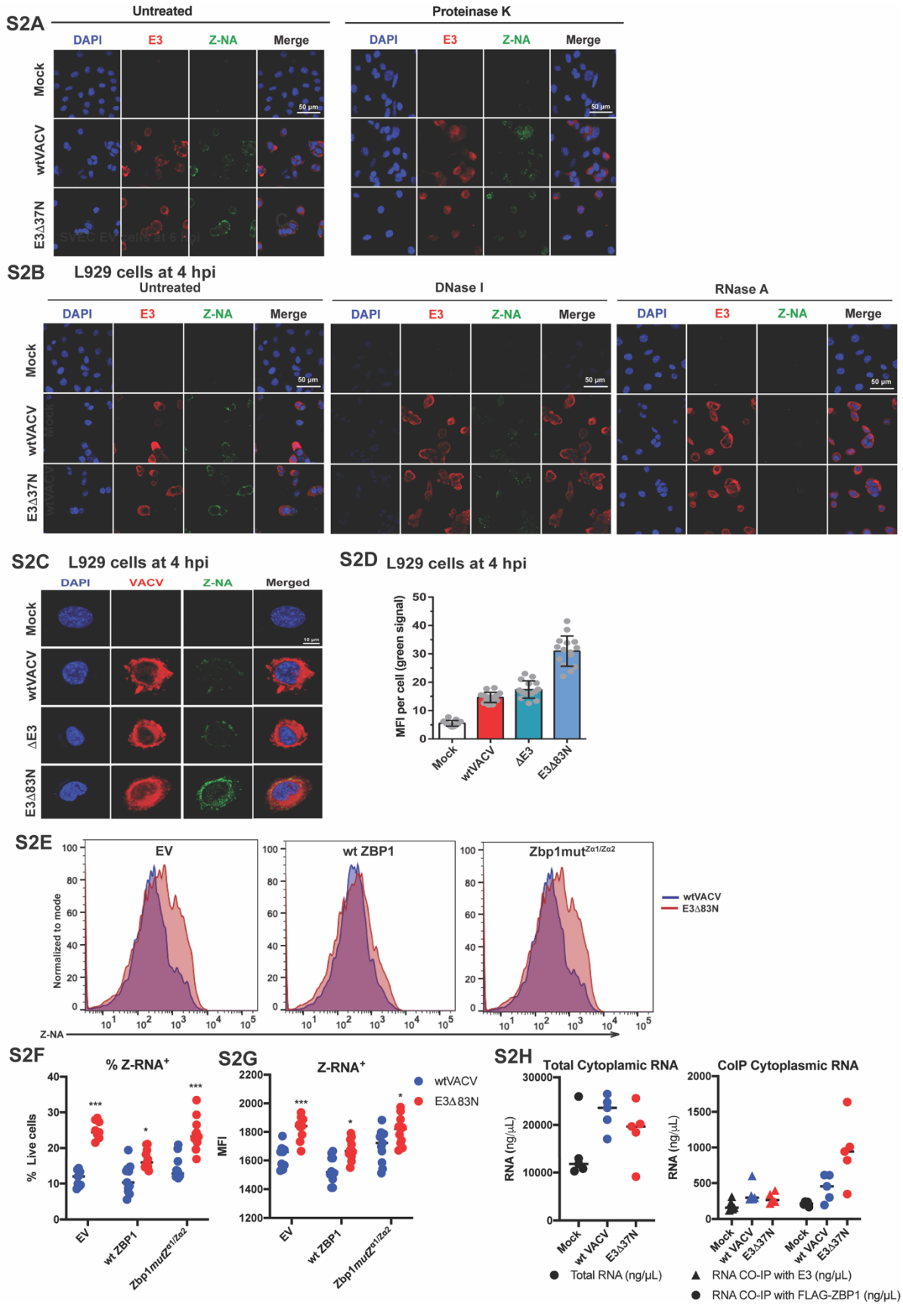
S2A
Untreated
Proteinase K
DAPI
E3
Z-NA
Merge
Mock
wtVACV
E3Δ37N
50 μm
S2B
L929 cells at 4 hpi
Untreated
DNase I
RNase A
S2C
L929 cells at 4 hpi
DAPI
VACV
Z-NA
Merged
ΔE3
E3Δ83N
10 μm
S2D
L929 cells at 4 hpi
MFI per cell (green signal)
S2E
EV
wt ZBP1
Zbp1mut Zα1/Zα2
Normalized to mode
Z-NA
wtVACV
E3Δ83N
S2F
% Z-RNA+
% Live cells
S2G
Z-RNA+
MFI
S2H
Total Cytoplamic RNA
CoIP Cytoplasmic RNA
RNA (ng/μL)
Mock
wt VACV
E3Δ37N
Total RNA (ng/μL)
RNA CO-IP with E3 (ng/μL)
RNA CO-IP with FLAG-ZBP1 (ng/μL)

Figure S2, Detection of Z-RNA, related to Figure 5.
(S2A) Confocal immunofluorescent micrographs showing a field of L929 cells evaluated as single cells in Figure 5A.
(S2B) Confocal immunofluorescent micrographs showing a field of L929 cells evaluated as single cells in Figure 5C.
(S2C) Confocal immunofluorescent micrographs evaluating single L929 cells infected with additional VACV mutants, E3Δ83N and ΔE3.
(S2D) Quantification of the median fluorescence intensity of Z-NA signal intensity of 20 individual cells from S2C analyzed as described in Figure 5B.
(S2E) Flow cytometric evaluation of Z-NA, showing representative flow cytometric histograms employed for evaluation shown in S2F and S2G. SVEC-derived EV, wt ZBP1 and Zbp1*mut*$^{Z\alpha1/Z\alpha2}$ cells were infected with wt VACV or E3Δ83N (MOI of 5), UV-crosslinked at 6 hpi, fixed and permeabilized, DNase I treated and evaluated for the presence of Z-NA by flow cytometry with anti-Z-NA antibody. Single cell suspensions were evaluated by employing gating to exclude doublets and dead cells.
(S2F) Proportion of Z-NA positive cells from 10 to 12 replicate flow cytometric histograms infected and treated as described in S2E.
(S2G) MFI of Z-NA+ SVEC-derived EV, wt ZBP1 and ZBP1*mut*$^{Z\alpha1/Z\alpha2}$ cells following infection at a MOI of 5 with wt VACV or E3Δ83N. Cells were infected, treated and harvested as described in Panel S2E.
(S2H) Quantification of total RNA (left panel) or co-IP (right panel) of either ZBP1- or E3-associated RNA extracted from Mock or infected SVEC-derived wt ZBP1 cells and used to calculate proportions depicted in Figure 5D.
Error bars represent the SD. Each set of data is representative of three replicates which compiles the results of the replicates. Statistical significance was determined as described in Figure 1.