Cell Host & Microbe, Volume *12*

Supplemental Information

Degradation of Host MicroRNAs by Poxvirus Poly(A) Polymerase Reveals Terminal RNA Methylation as a Protective Antiviral Mechanism

Simone Backes, Jillian S. Shapiro, Leah R. Sabin, Alissa M. Pham, Ismarc Reyes, Bernard Moss, Sara Cherry, and Benjamin R. tenOever

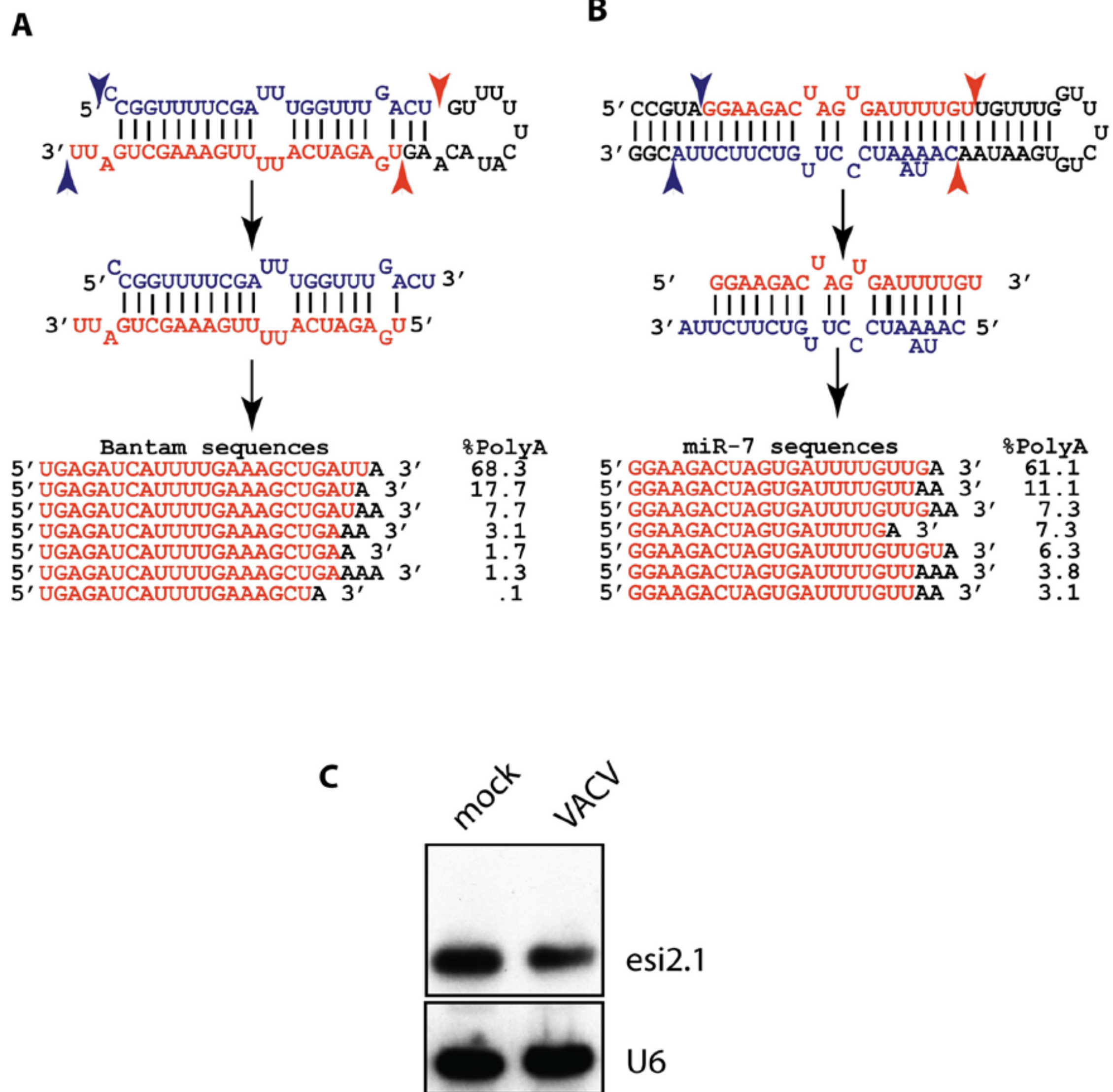
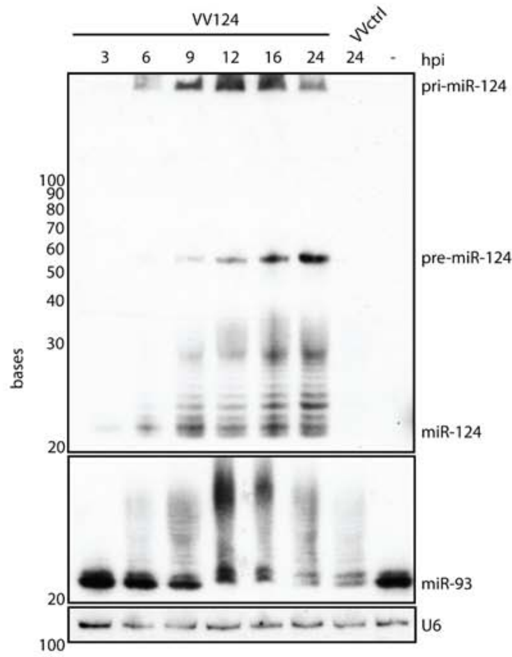
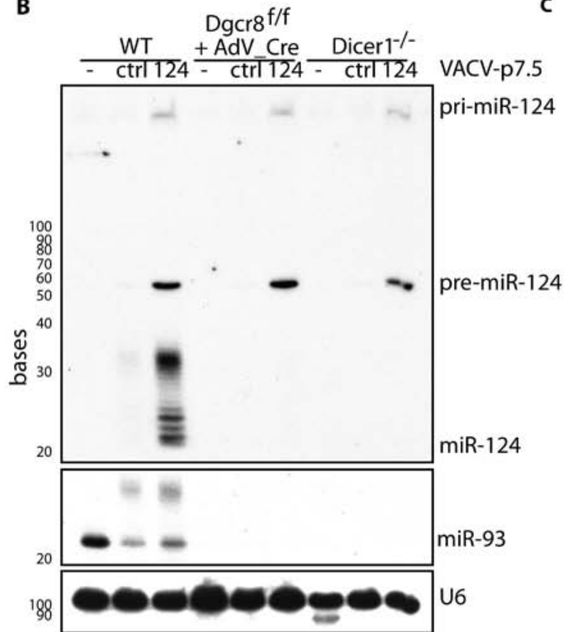


Figure S1. Poxvirus Infection Induces Tailing of *Drosophila* miRNAs (Related to Figure 1)

(A and B) Schematic of bantam and miR-7 processing and corresponding miRNA duplex (middle) and mature miRNAs (bottom). The mature miRNA sequence-specific reads were determined by deep sequencing of the 15-29 nt fraction of VACV-infected cells. Adenosines (A) in black depict non-templated bases and % representation reflects the portion of the corresponding sequence in the total miRNA-specific tailed fraction.

(C) Northern blot of mock or VACV-infected *Drosophila* cells (MOI of 300) probed for esi2.1 and U6 at 72 hpi.


A
VV124
VVctrl
3
6
9
12
16
24
24
-
hpi
pri-miR-124
pre-miR-124
miR-124
miR-93
U6
bases


B
WT
Dgcr8 f/f + AdV_Cre
Dicer1-/-
- ctrl 124
VACV-p7.5
pri-miR-124
pre-miR-124
miR-124
miR-93
U6
bases

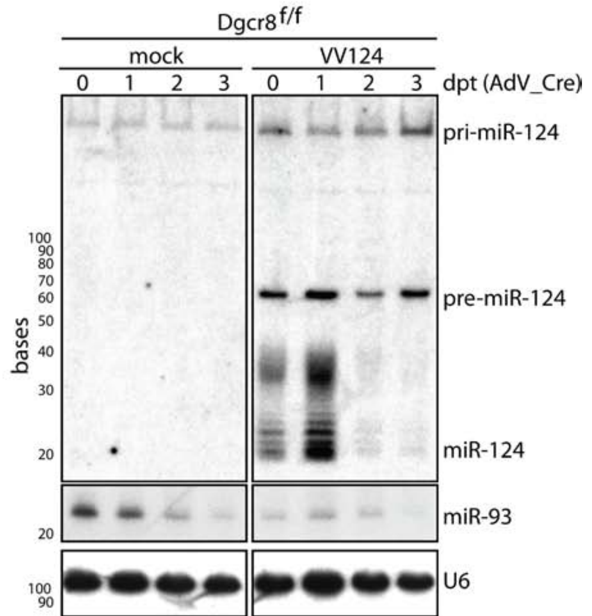

C
Dgcr8 f/f
mock
VV124
0 1 2 3
dpt (AdV_Cre)
pri-miR-124
pre-miR-124
miR-124
miR-93
U6
bases

Figure S2. Vaccinia Virus Mediated miRNA Production Is Dicer-Dependent (Related to Figure 2)

(A) Northern blot of RNA derived from BHK cells infected with VV124 or VVctrl and analyzed for miR-124, miR-93 and U6 at the indicated time points.

(B) Northern blot of RNA derived from wildtype (wt) or Dicer-deficient (Dicer $^{-/-}$) murine embryonic fibroblasts (MEFs) or from condition DGCR8 knockout cells (DGCR8$^{f/f}$) treated with an Adenovirus vector expressing Cre (AdV_Cre) for 4 days and subsequently infected with VV124 or VVctrl for 18 h (MOI of 10). Northern blots were probed for miR-124, miR-93 and U6.

(C) VV124 infected DGCR8 knockout cells (DGCR8$^{f/f}$+ AdV_Cre) as described in (B), comparing days post AdV transduction (dpt) in response to mock treatment or infection with VV124 (18 h, MOI of 10).

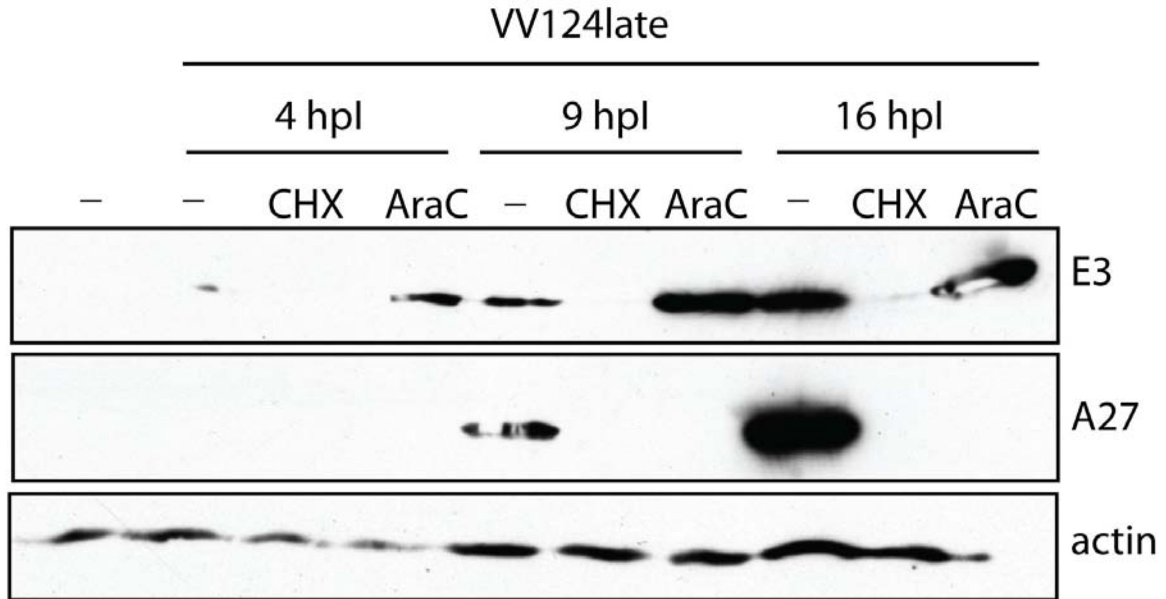


Figure S3. CHX and AraC Inhibit VACV Early Protein Synthesis and Postreplicative Gene Expression, Respectively (Related to Figure 3)
Western blot of whole cell extract derived from BHK cells mock-treated or infected with VV124late (MOI of 10) in presence or absence of AraC or CHX and harvested at the indicated hours post infection (hpi). Immunoblots probed for VACV E3, VACV A27 and actin.

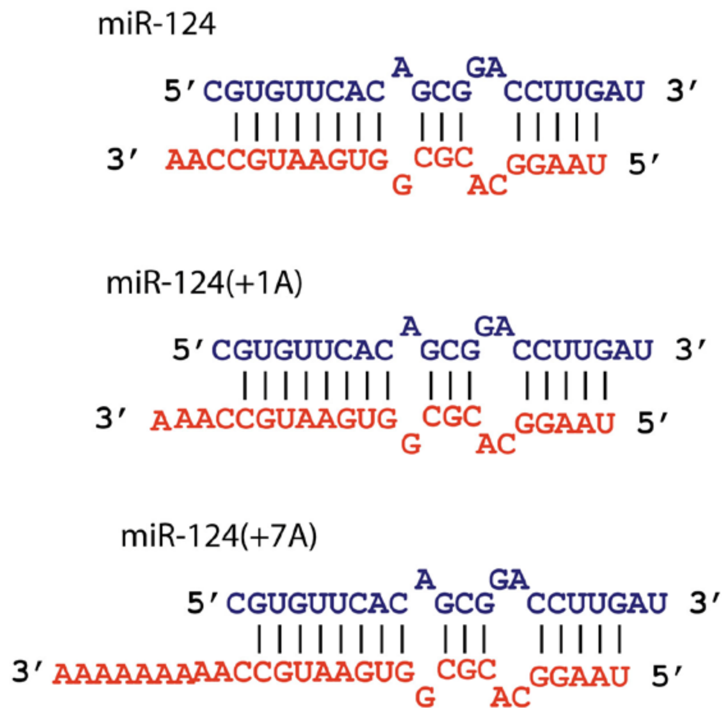
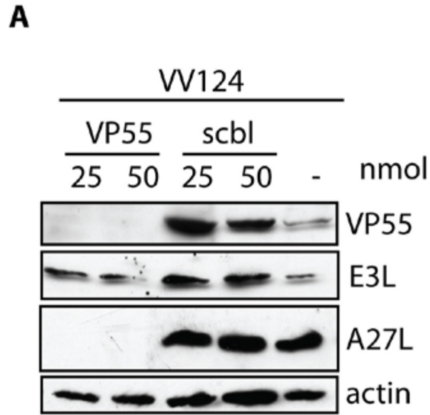
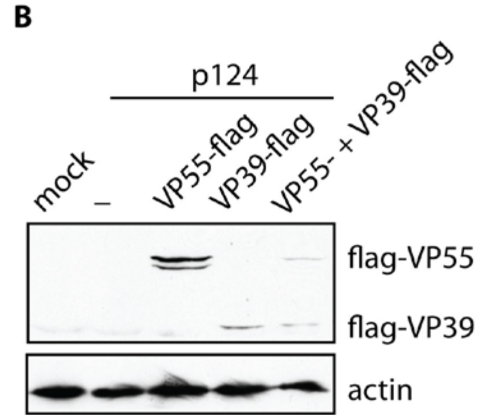


Figure S4. VACV-mediated miRNA Degradation Is Unbiased (Related to Figure 5)
Schematic of synthetic miR-124-duplexed miRNAs, including unmodified miR-124 (top) or miR-124 containing one (miR-124(+1A)) or seven (miR-124(+7A) non-templated adenosines on the mature strand (middle and bottom, repectively).

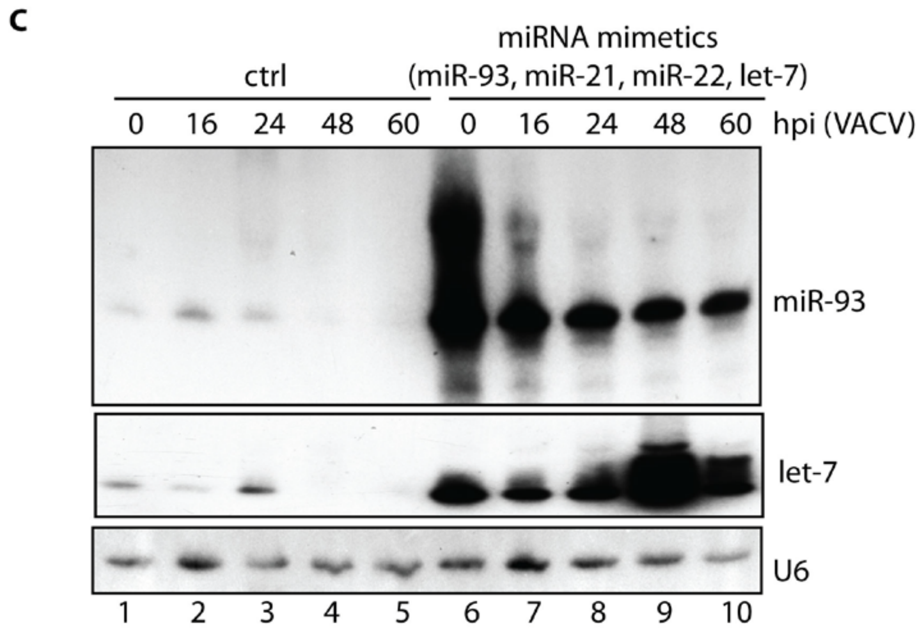
A



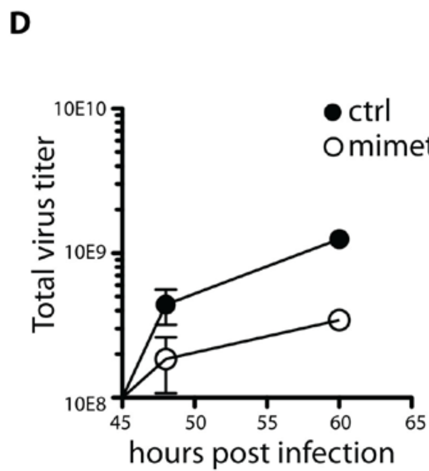
B



C



D



E

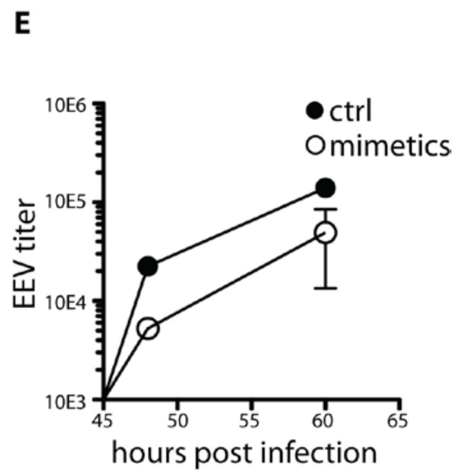


Figure S5. VACV VP55-Mediated Tailing Inhibits miRNA Function (Related to Figure 6)

(A) Western blot of mammalian cells transfected with scrambled (scbl) or VP55-specific siRNAs and mock-treated or infected with VV124 for 16 h probed for actin and VACV proteins VP55, E3 and A27.

(B) Western blot of mammalian cells mock-treated or transfected with plasmids expressing miR-124 (p124) and flag-tagged VP55 and/or VP39 for 36 h probed for flag and actin.

(C) Northern blot of RNA derived from BHK cells transfected with control small RNA mimetics (ctrl) or a pool of miR-93, miR-21, miR-22 and let-7 mimetics (mimetics). Six hours post transfection, cells were infected with VVctrl (MOI of 0.05) and harvested at the indicated hours post infection (hpi). Northern blots were probed for miR-93, let-7 and U6.

(D) Cells treated as described in (C). Six hours post transfection, cells were infected with VVctrl (MOI of 0.05) and total titers or (E) extracellular enveloped virus (EEV) titers in the supernatants were determined at 48 h and 60 h post infection.

Supplemental Experimental Procedures

Cells and transfections

Dicer deficient fibroblasts were a kind gift from A. Tarakhovsky (Rockefeller University, NYC) and DGCR8-deficient fibroblasts were a kind gift from Robert Blelloch (UCSF, CA). They were grown at 37°C in DMEM media supplemented with 10% Fetal Bovine Serum and 1% penicillin-streptomycin.
Mimetics for miR-21, miR-22, miR-93 and let-7 (Thermo Scientific Dharmacon) were transfected into BHK cells in suspension using RNAiMAX (Invitrogen). Cells were infected with VACV six hours post transfection (MOI of 0.05) and harvested at the indicated time points.

Determination of VACV growth

Extracellular enveloped virus (EEV) and total VACV titers were determined using standard methodology. EEV titers were quantified from fresh virus supernatants collected at the indicated time post infection. Supernatants were centrifuged at low speed and incubated with L1R antibody (*bei*resources NR-417) (1:1000) for 90 minutes at 37°C to neutralize contaminating Intracellular mature virus (IMV). Infected cells were washed after 1h of adsorption.