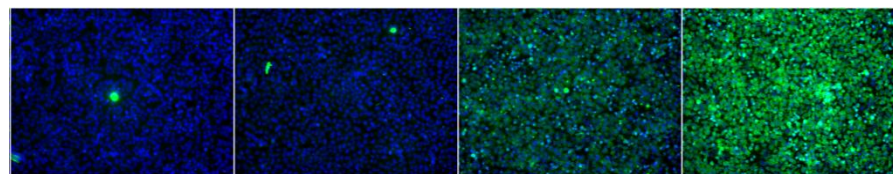


Ahuman
(U20S)

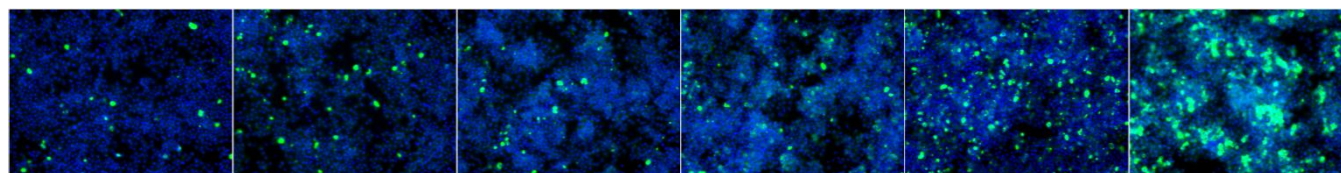
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B*Drosophila*
(DL-1)

h.p.i.

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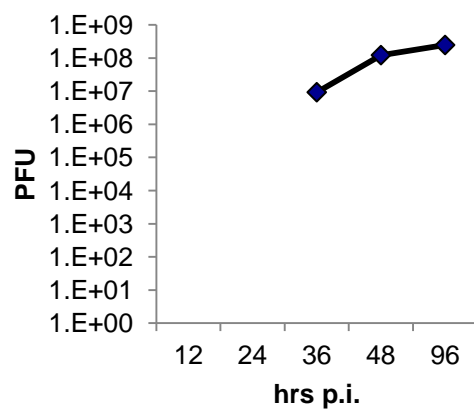
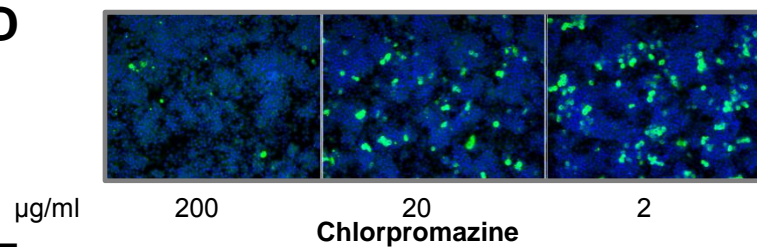
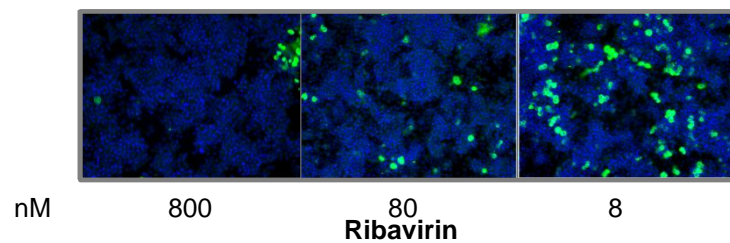
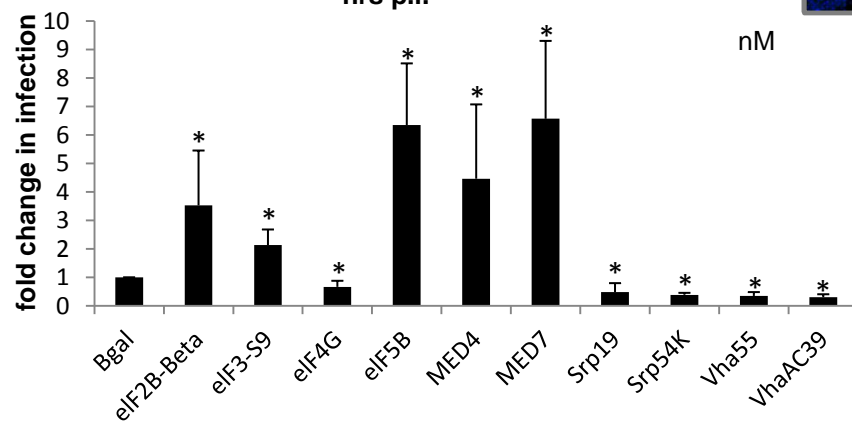
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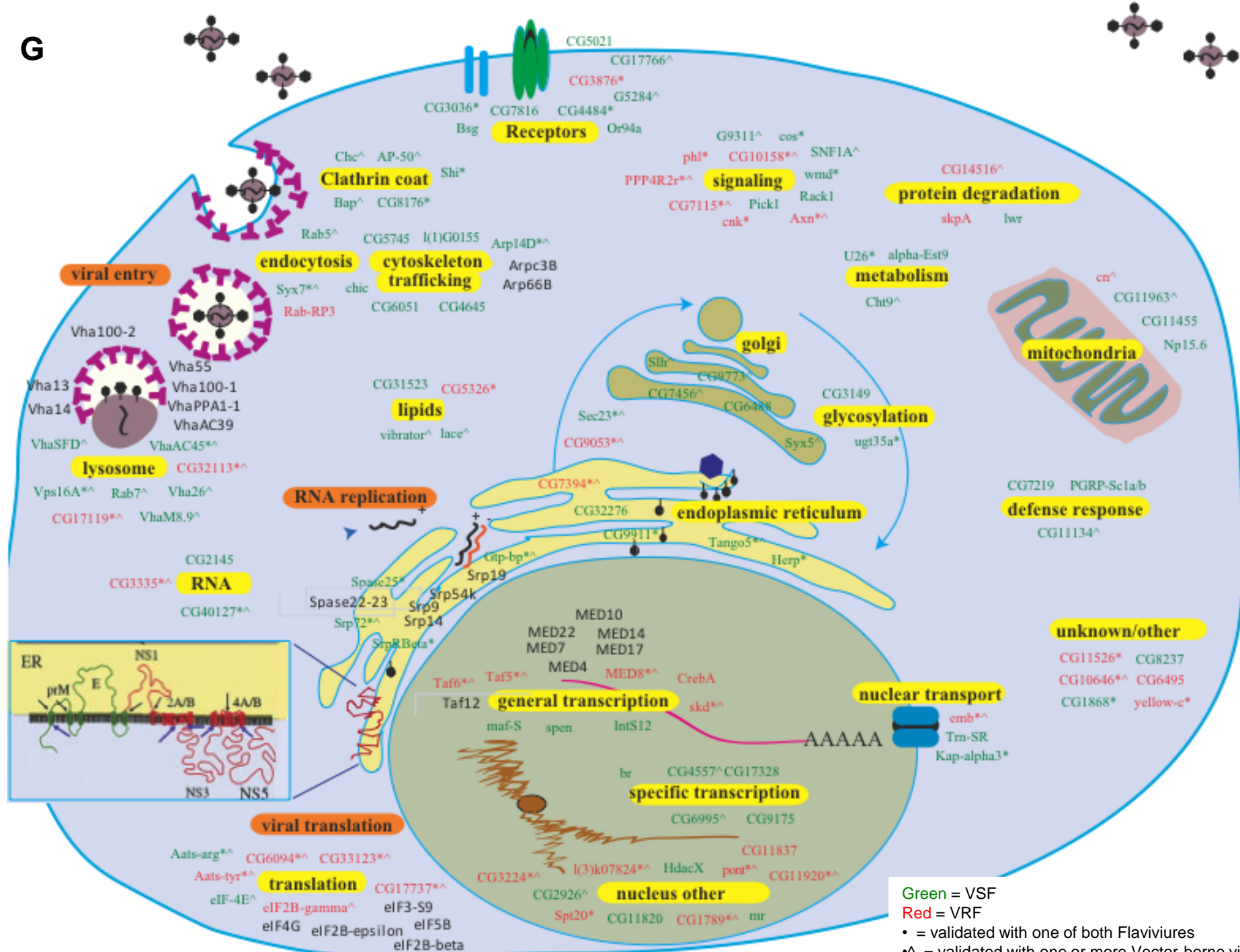
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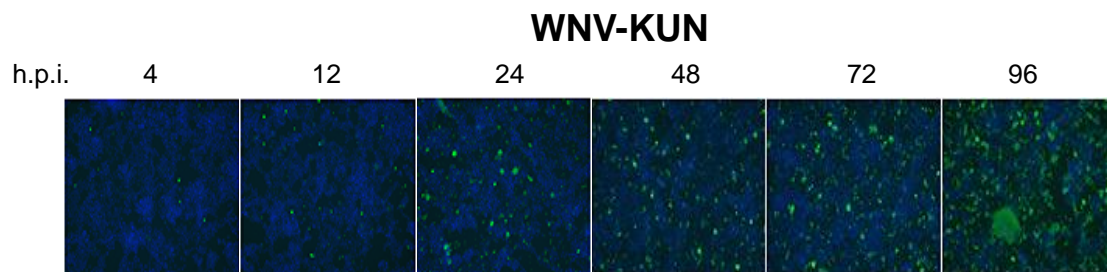
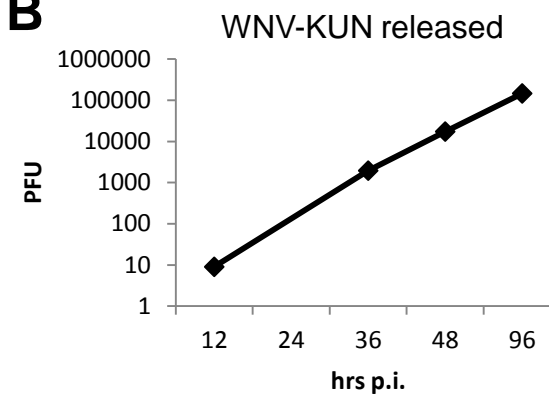
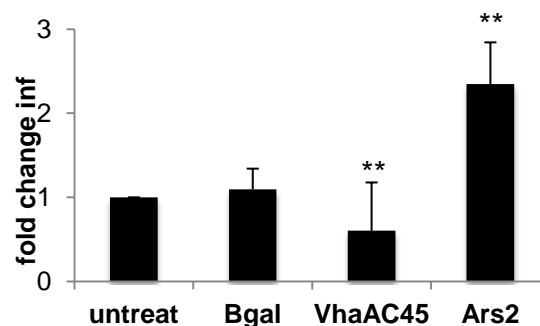
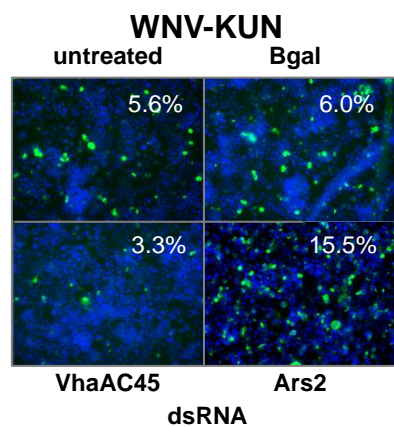
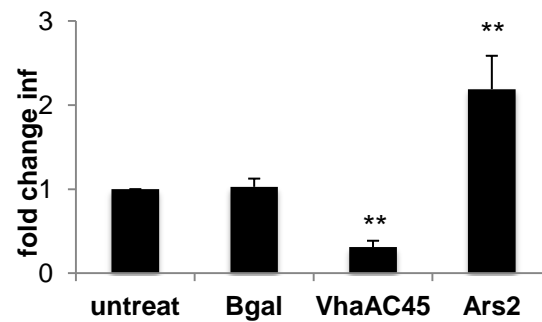
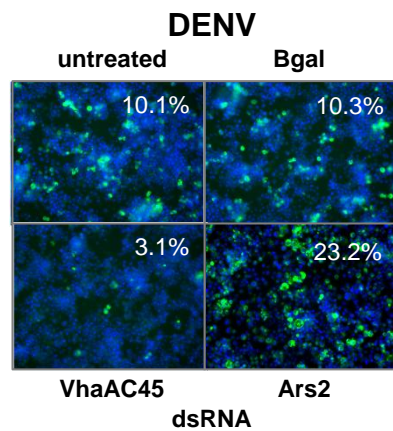
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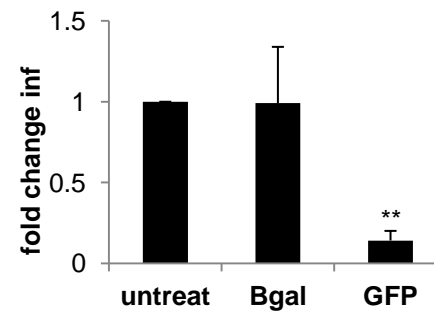
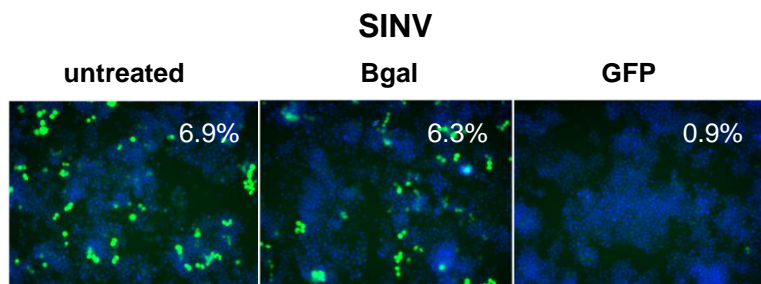
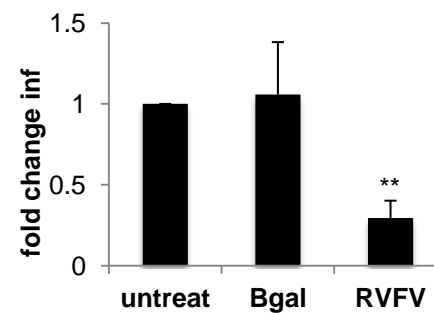
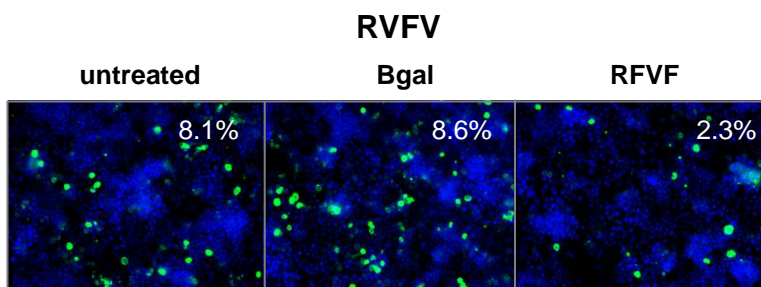
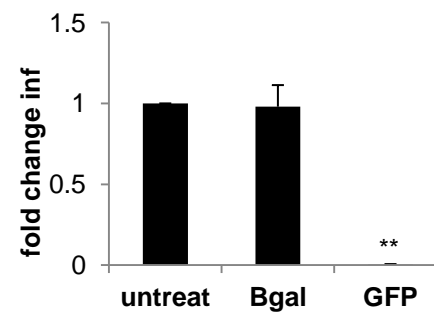
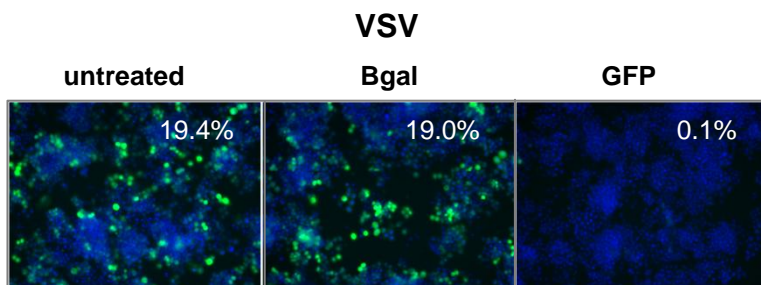
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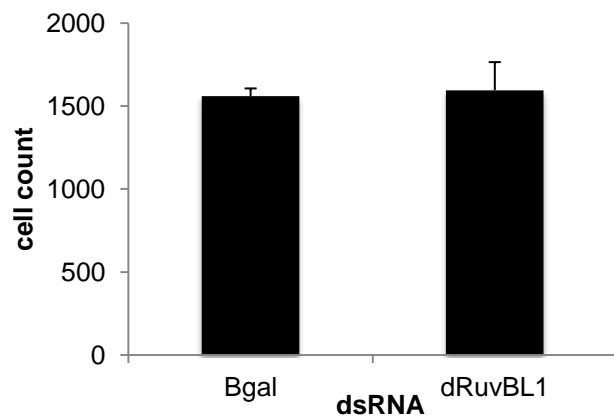
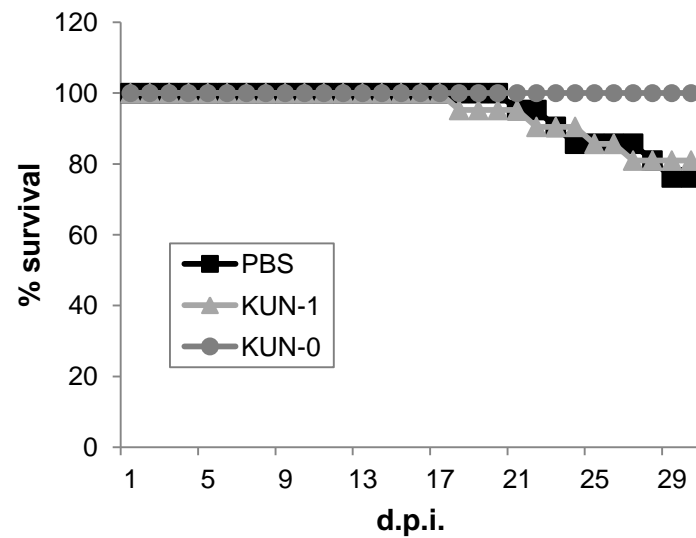
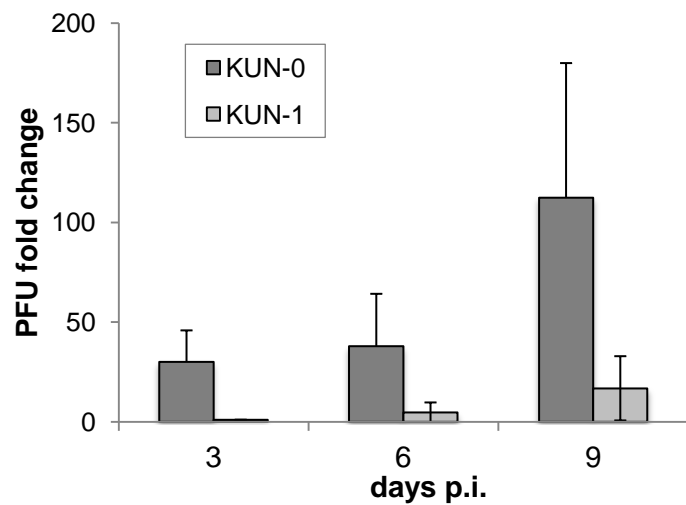
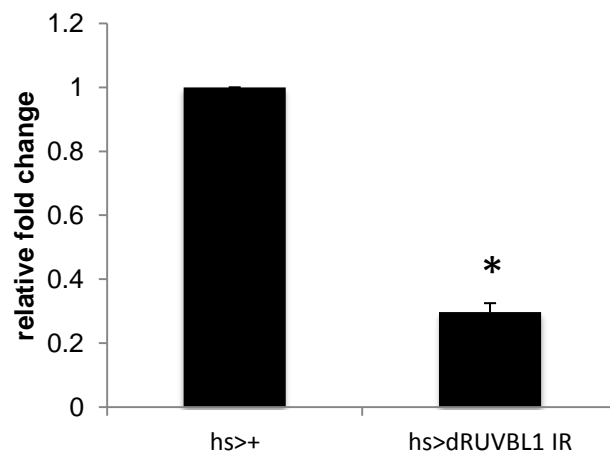
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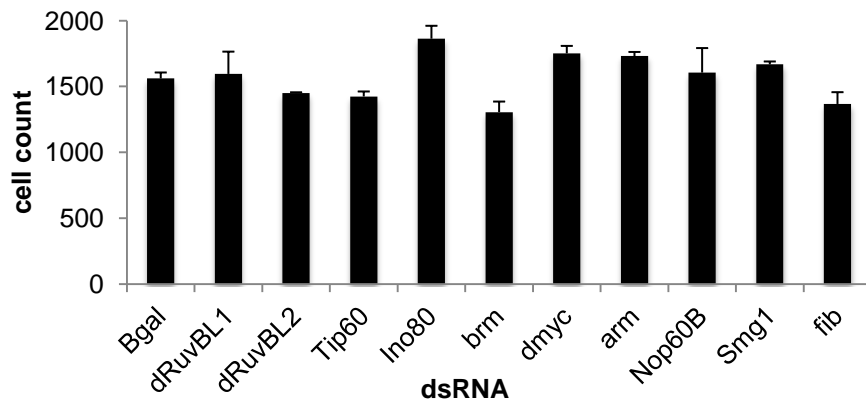
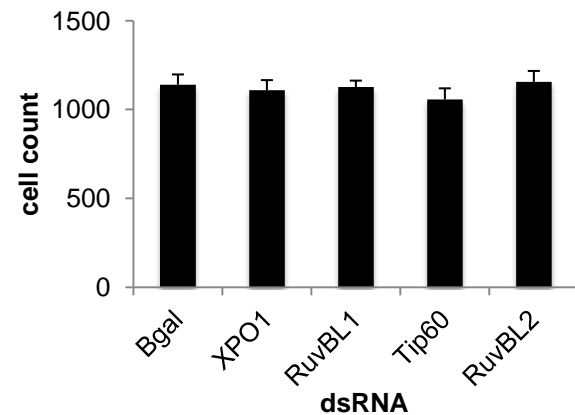
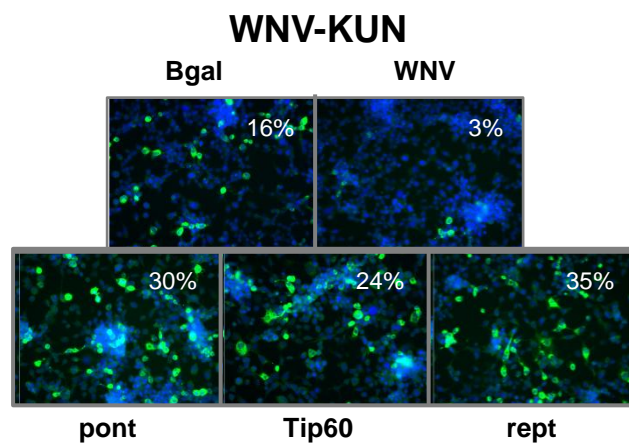
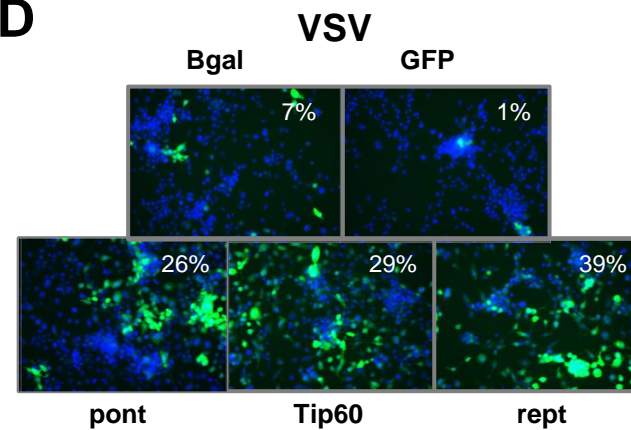
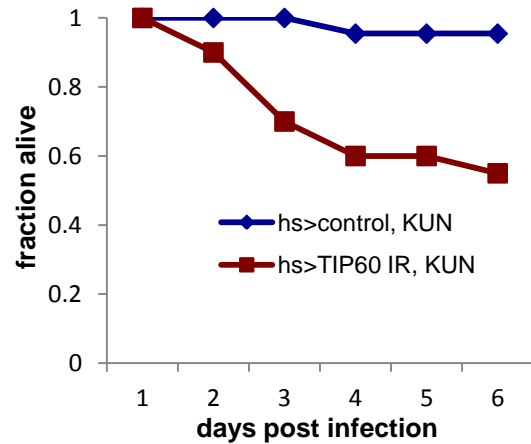
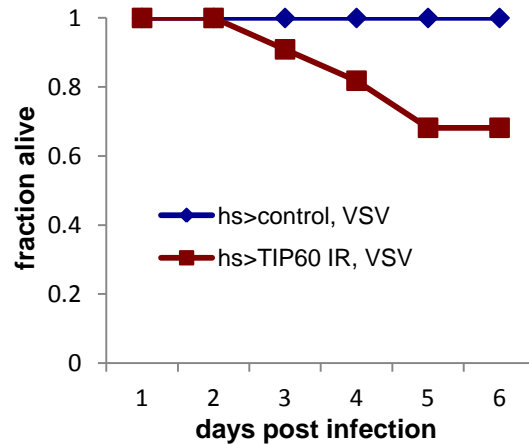
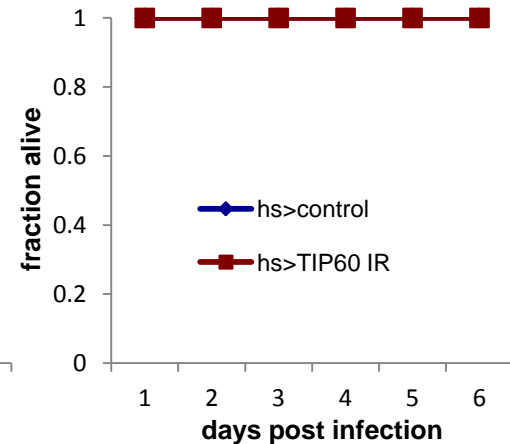
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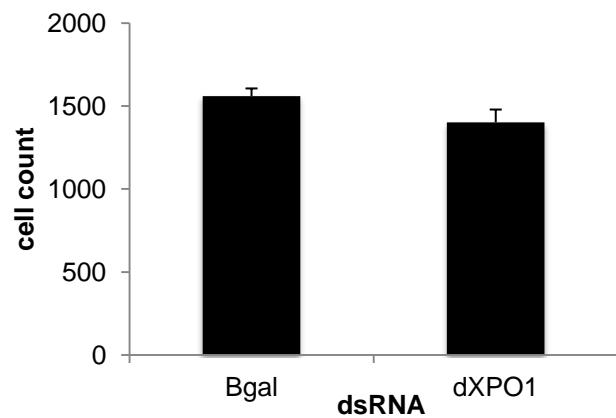
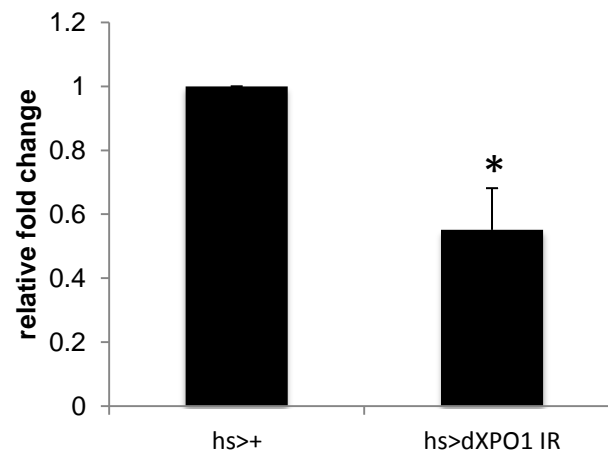
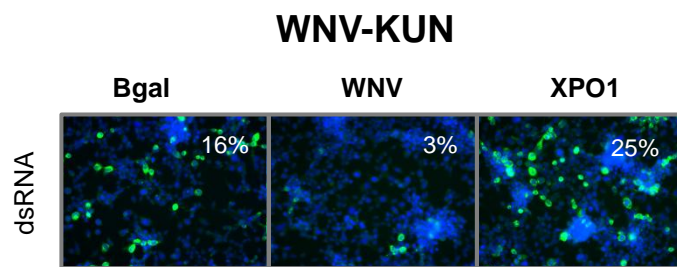
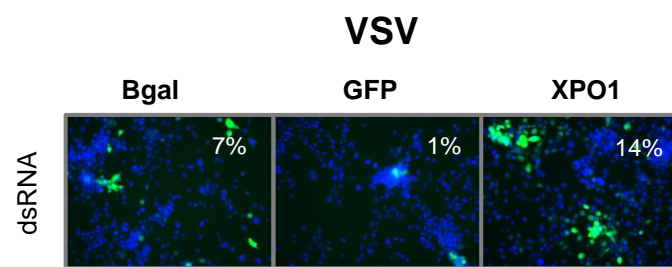


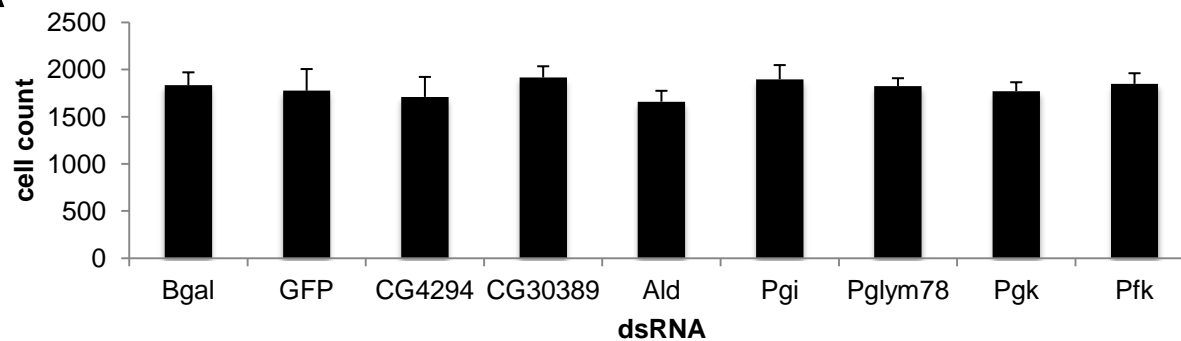
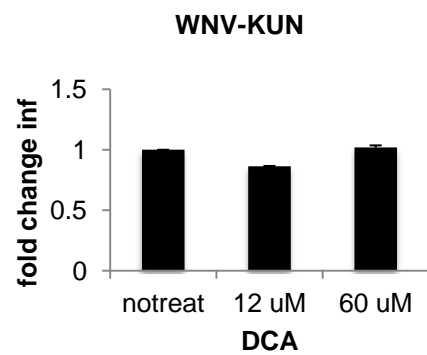
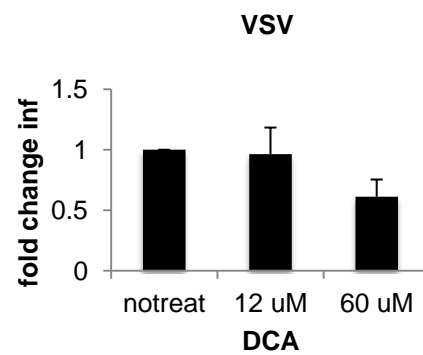
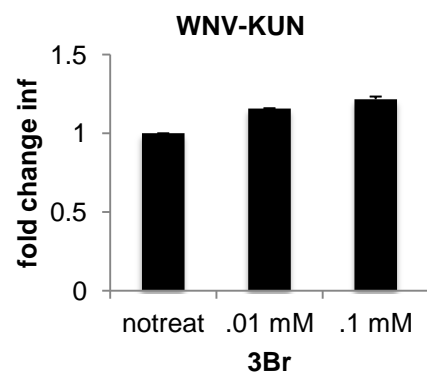
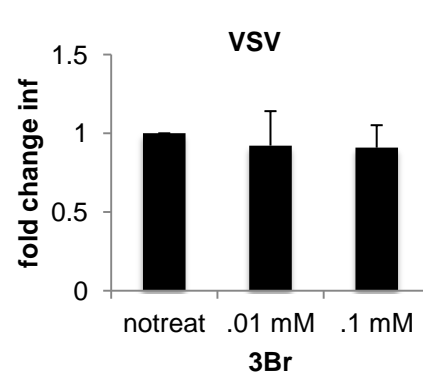
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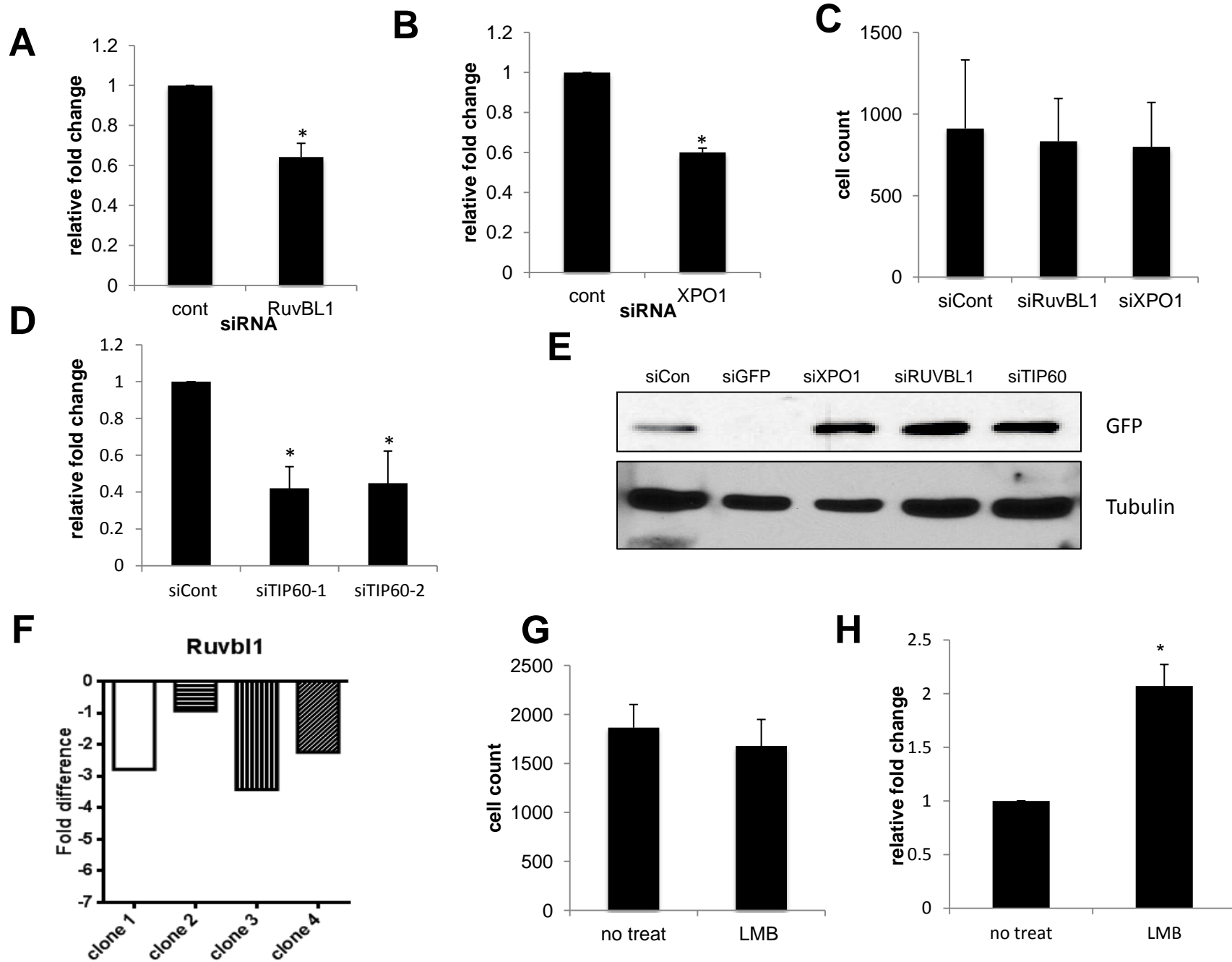
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A**B****C****D**

A**B****C****D****E****F****G**

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Supplemental Figures.

Figure S1. Characterization of WNV infection and schematic of validated screening results.

A-B. Images of WNV-infected (**A**) human U20S cells (MOI 0.5) or (**B**) *Drosophila* cells (MOI 10) that were fixed at indicated time points post infection ((blue, nuclei; green, virus). **C.** Supernatant from WNV-infected DL1 cells collected at designated time points post infection was titered on BHK cells to determine infectious viral release. Initial viral inoculum was removed after 12 hrs, cells washed of residual virus, and fresh media replaced. **D-E.** Representative images of DL1 cells pretreated with (**D**) chlorpromazine or (**E**) ribavirin at designated concentrations for 30 minutes prior to infection with WNV (MOI 10) (blue, nuclei; green, virus). **F.** *Drosophila* cells were treated with the indicated dsRNAs for three days and infected with WNV-KUN (MOI of 10) for 48 hours and processed for microscopy. Quantification of the fold change in infection is presented as mean \pm SD for three independent experiments; * $p < 0.05$. **G.** Pictorial schematic of a cell detailing the predominant cellular function of validated genes identified in the WNV genome-wide RNAi screen. VSFs and VRFs confirmed in the secondary screen are identified in green and red respectively. Those genes included as part of a validated complex are in black. * identifies genes also validated with one or more of the other vector-borne viruses tested. Underlined genes impacted infection with all the viruses tested.

Figure S2. Optimization of arbovirus screening assay.

A. Representative images of WNV-KUN-infected DL1 cells (MOI 10) that were fixed at 4, 12, 24, 48 and 96 hrs post infection (blue, nuclei; green, virus). **B.** Supernatant from WNV-KUN-infected DL1 cells collected at designated time points post infection and titered on BHK cells to determine infectious virus release. Initial viral inoculum was removed after 12 hrs, cells washed of residual virus, and fresh media replaced. **C-G,** Left, representative images of dsRNA-treated DL1 cells infected with (**C**) WNV-KUN, (**D**) DENV, (**E**) SINV, (**F**) RVFV, or (**G**) VSV. Right, Mean \pm SD of fold change in infection normalized to untreated for 3 independent experiments; ** $p < 0.01$.

Figure S3. Characterization of WNV-KUN infection of flies.

A. Gene silencing of dRUVBL1 does not impact cell viability as measured by nuclei number. Mean \pm SD for 3 independent experiments. **B-C.** Adult wild-type flies (w1118) were challenged with WNV-KUN at two different dilutions (100 PFU (KUN-0) or 10 PFU (KUN-1)) and **(B)** mortality was monitored with a representative experiment shown; similar findings were made in at least three experiments. **(C)** Viral titer was monitored at the indicated day post infection. Results of at least 3 experiments are plotted as Mean \pm SD in the fold change in PFU setting D3 KUN-1 to one. **D.** RT-qPCR was used to confirm knockdown of dRUVBL1 in hs>dRUVBL1 IR heat shocked flies. Mean \pm SD of fold change normalized to control for 3 independent experiments; * $p < 0.05$.

Figure S4. Tip60 complex is antiviral in insects.

A-B. Silencing of indicated genes in **(A)** DL1 or **(B)** Aag2 cells does not impact cell number. Mean \pm SD of cell number normalized to control dsRNA (bgal) for 3 independent experiments. **C-D.** Representative images of dsRNA-treated Aag2 cells infected with **(C)** WNV-KUN, **(D)** VSV. **E-G.** Adult flies of the indicated genotypes were challenged with WNV-KUN **(E)** VSV **(F)** or vehicle **(G)**. Mortality was monitored as a function of time post-infection.

Figure S5. XPO1 is antiviral in insects

A. Silencing of dXPO1 does not impact cell viability as measured by nuclei number. Mean \pm SD of cell number normalized to control dsRNA (bgal) for 3 independent experiments. **B.** qRT-PCR was used to confirm knockdown of dXPO1 in hs>dXPO1 IR heat shocked flies. Mean \pm SD of fold change normalized to control for 3 independent experiments; * $p < 0.05$. **C-D.** Representative images of dsRNA-treated Aag2 cells infected with **(C)** WNV-KUN or **(D)** VSV.

Figure S6. Glycolysis does not control infection.

A. Silencing of indicated genes does not impact cell viability as measured by nuclei number. Mean \pm SD of cell number normalized to control dsRNA (bgal) for 3 independent experiments. **B-E.** DL1 cells pretreated with **(B-C)** DCA or **(D-E)** 3Br at indicated concentrations 30 minutes prior to infection compared to untreated cells. Mean \pm SD for fold

change in infection normalized to control (notreat) shown for three independent experiments. **B,D**, WNV-KUN infected. **C,E**, VSV infected.

Figure S7. RUVBL1 and XPO1 are antiviral in human cells.

A-C, Human U2OS cells were transfected with indicated siRNAs. 3 days post transfection qRT-PCR was used to confirm knockdown with **(A)** hRUVBL1 siRNA or **(B)** hXPO1 siRNA. **C**. Cell numbers were not affected. Mean \pm SD for 3 independent experiments; * $p < 0.05$. **D**. Human 293T cells were transfected with siRNAs against TIP60 and monitored for TIP60 levels by RT-qPCR. Mean \pm SD for 3 independent experiments; * $p < 0.05$. **E**. 293T cells expressing a subgenomic WNV replicon (expressing GFP) were transfected with the indicated siRNAs for four days and analyzed by immunoblot. A representative blot is shown. **F**. Mouse neurons were infected with the indicated lentiviruses expressing one of four shRNAs against RUVBL1 and qRT-PCR was used to confirm knockdown. **G**. There was no decrease in viability upon treatment with LMB in U2OS cells as measured by cell number. Mean \pm SD shown for three independent experiments. **H**. 293T cells expressing a subgenomic WNV replicon (expressing GFP) were treated with LMB and analyzed by microscopy. Mean \pm SD of GFP shown normalized to untreated cells; * $p < 0.05$.

Supplementary Methods

Gene silencing validation:

To determine efficiency of gene silencing in flies, total RNA was isolated from 15 flies of the indicated genotypes. cDNA was generated using random hexamers and MLV-RT (Invitrogen). Transcripts were quantified using Sybrgreen with the primers (dRUVBL1: Forward CTTACATTTCTCTCCCTCG; Reverse GCCTCTAAATCCGTGTCCTG and dXPO1: Forward TGCTGGAGAACTCGATGAATG; Reverse GGAACATGGGTACGCTAAGG). Cellular levels of rp49 were quantified in parallel and used for normalization.

To determine efficiency of gene silencing in neurons, total RNA was isolated from shRNA-transduced cerebellar granule cell neurons. cDNA was generated from RNA using random hexamers and Multiscribe reverse transcriptase (Applied Biosystems). Transcripts were quantified on a 7500 Fast Real-time PCR system (Applied Biosystems) with Taqman primers and probe sets (Forward

ATGACCTGGATGTGGCTAATG; Reverse CTCCCCTCGAAGTTTATCTGTG; and Probe CCTCAGGGTGGGCAAGATATTCTGTC). Cellular levels of 18S ribosomal RNA were quantified in parallel by TaqMan analysis and used for normalization.