Supporting Information

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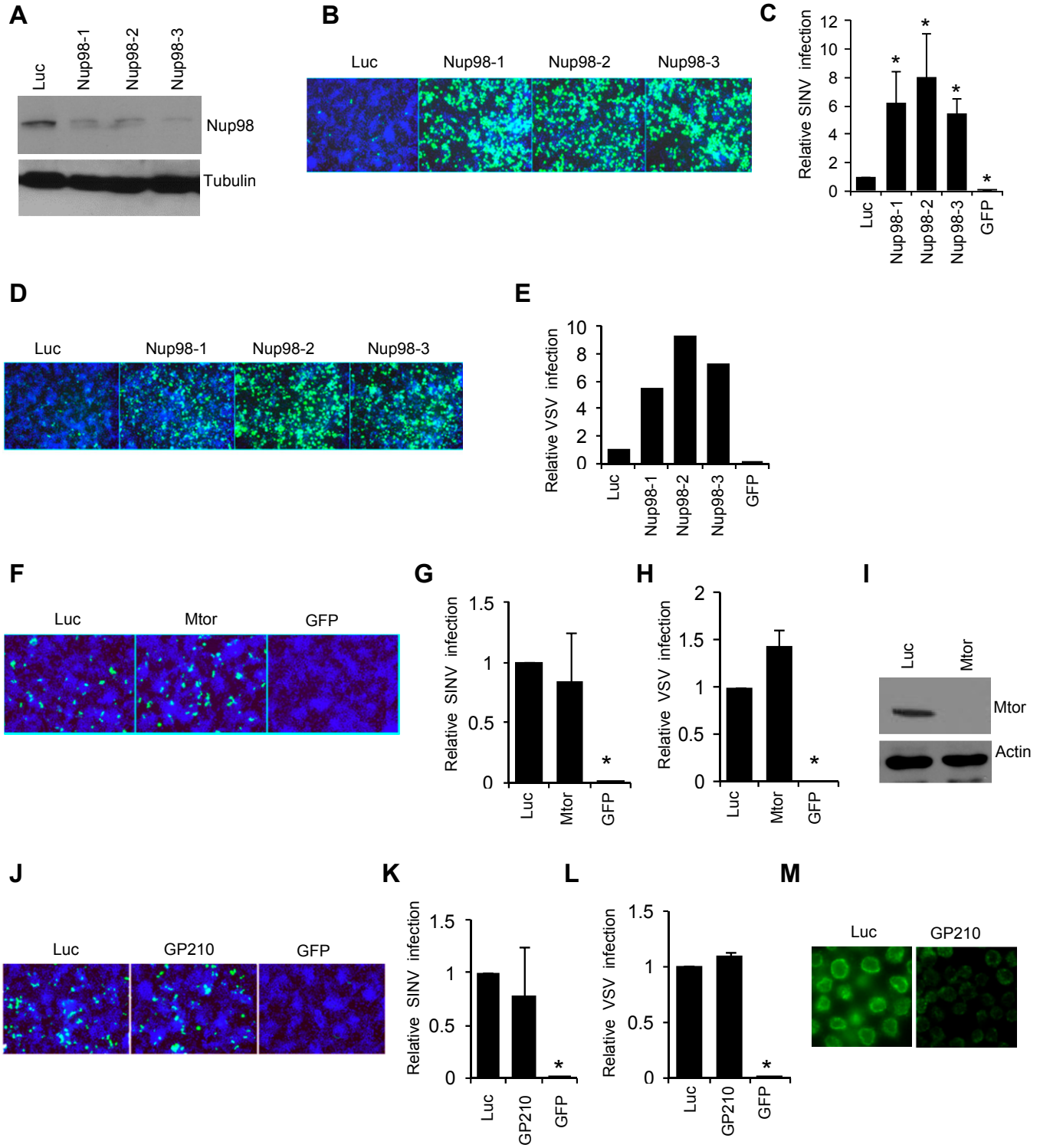

Fig. S1. Nup98 depletion leads to enhanced viral infection. (*A*) Nup98 depletion by dsRNA treatment was examined by immunoblot. (*B* and *C*) *Drosophila* DL1 cells were treated with the indicated dsRNA and 72 h later infected with SINV-GFP at 10 multiplicity of infection (MOI). At 42 hours postinfection (hpi), cells were fixed and processed for microscopy and image analysis. A representative image is shown in *B*, and quantification from three independent experiments is

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shown in *C*. The data represent at least three independent experiments, with mean ± SD shown. **P* < 0.05. (*D* and *E*) DL1 cells were treated with the indicated dsRNA and 72 h later infected with VSV-GFP at 2 MOI. At 42 hpi, cells were fixed and processed for microscopy and image analysis. A representative image is shown in *D*, and quantification from two independent experiments is shown in *E*. (*F*–*H*) DL1 cells were treated with the indicated dsRNA and 72 h later infected with either SINV (*F* and *G*) or VSV (*H*). Cells were fixed and processed for microscopy. A representative image is shown in *F*, and quantification is shown in *G* and *H*. The data represent at least three independent experiments, with mean ± SD. **P* < 0.05. (*I*) RNAi-mediated knockdown of Mtor is shown on immunoblot. (*J*–*L*) DL1 cells were treated with the indicated dsRNA and 72 h later infected with either SINV (*J* and *K*) or VSV (*L*). Cells were fixed and processed for microscopy. A representative image is shown in *J*, and quantification is shown in *K* and *L*. The data represent at least three independent experiments, with mean ± SD. **P* < 0.05. (*M*) Depletion of GP210 by dsRNA treatment was examined by immunofluorescence microscopy.

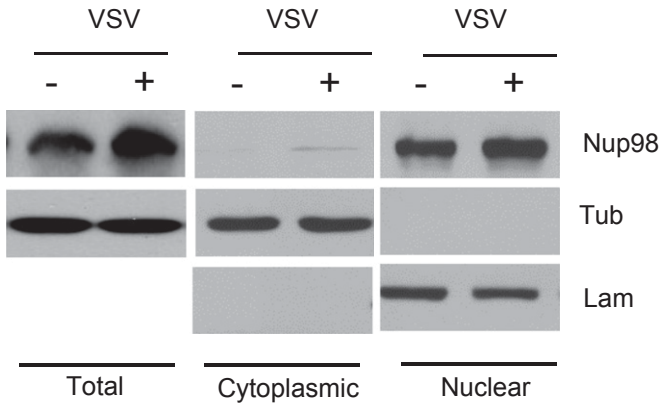

Fig. S2. Nup98 is intranuclear. Control or VSV-infected cells were processed for total, nuclear, and cytoplasmic fractions, and Nup98 expression in each fraction was examined. Tubulin and Lamin expression are shown as controls.

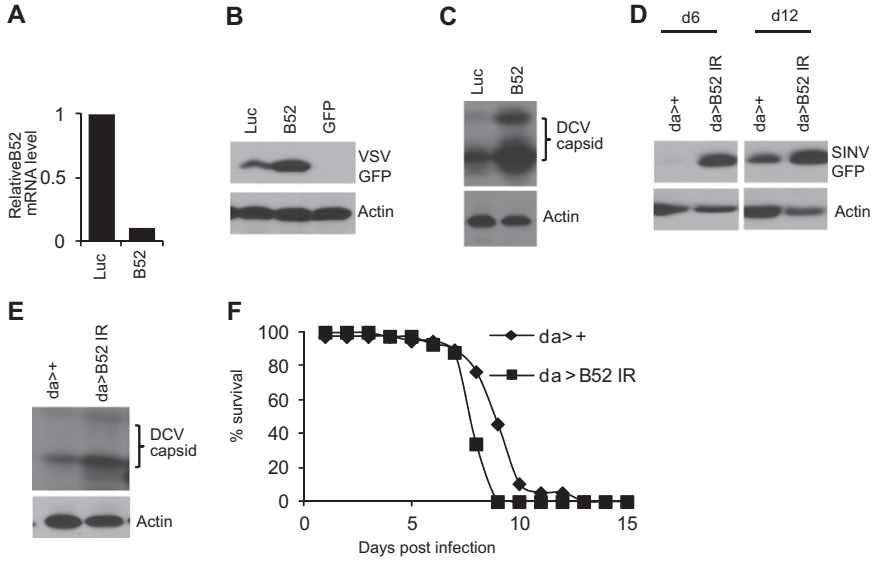

Fig. S3. B52 is broadly antiviral. (*A*) RNAi-mediated depletion of B52 was examined by RT-qPCR. (*B* and *C*) DL1 cells were treated with the indicated dsRNA and 72 h later infected with either VSV (*B*) or DCV (*C*). VSV and DCV gene expression was evaluated by immunoblot analysis. (*D* and *E*) Flies of the indicated genotypes were infected either with SINV-GFP for 6 d or 12 d (*D*) or with DCV for 4 d (*E*). Flies were then crushed and processed for immunoblot analysis. Tubulin or actin served as a loading control. (*F*) Flies of the indicated genotypes were infected with DCV, and percent survival was examined. Survival in B52-depleted flies was significantly different ($P < 0.05$, log-rank test).

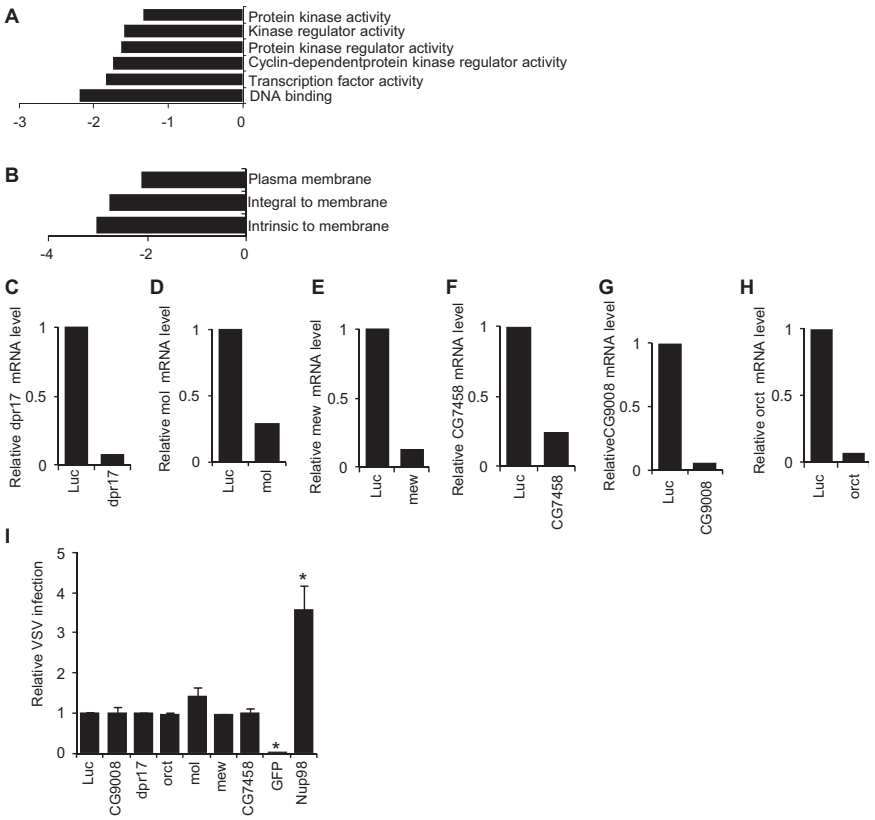

Fig. S4. Nup98 regulates antiviral gene expression. (*A* and *B*) Gene Ontology analysis of SINV induced genes. (*C–H*) RNAi-mediated depletion of Nup98-regulated genes was examined by RT-qPCR, represented as fold change relative to control. Data represent mean from two independent experiments. (*I*) DL1 cells were treated with the indicated dsRNA and infected with VSV at 72 hpi. Cells were processed for microscopy and quantitative image analysis; relative infection from three independent experiments is shown. The data represent mean ± SD. $*P < 0.05$.

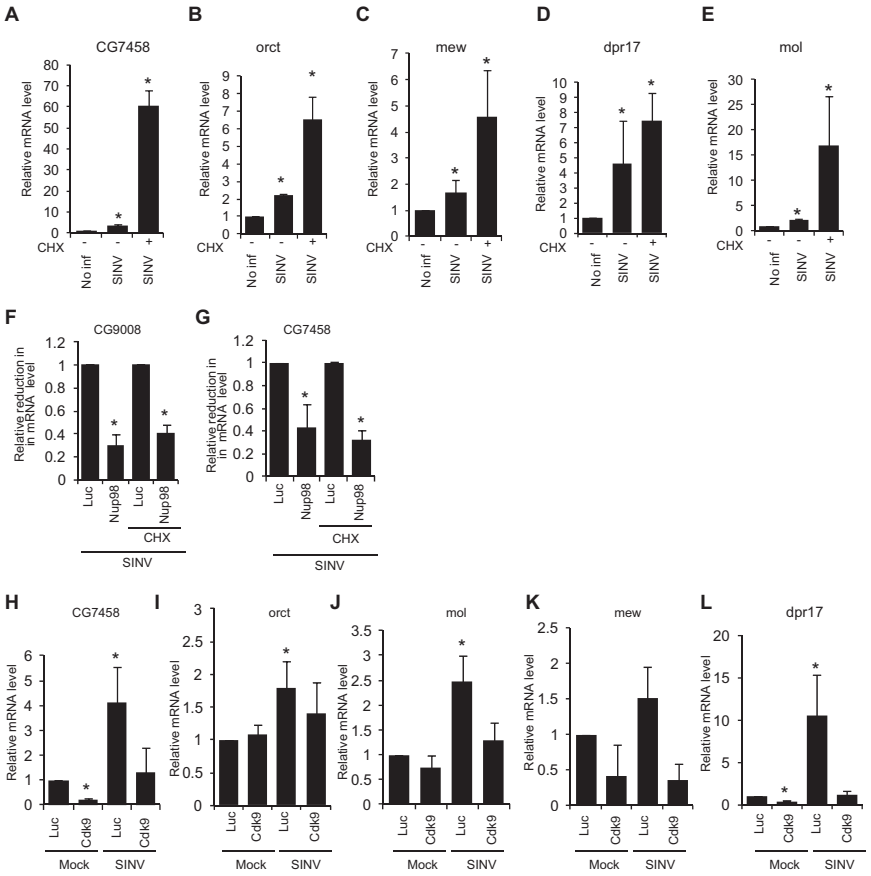

Fig. S5. Nup98 regulates a subset of Cdk9-dependent primary response genes. (*A–E*) DL1 cells were either treated with cycloheximide (CHX) for 1 h or left untreated and then infected with SINV for 2 h. Expression of the indicated genes were examined by RT-qPCR and represented as fold change relative to control. (*F* and *G*) Control or Nup98 depleted DL1 cells were either untreated or treated with CHX and then infected with SINV. CG9008 and CG7458 gene expression was examined by RT-qPCR. Relative expression level compared with control uninfected cells is shown. (*H–L*) Control DL1 cells or cells depleted of Cdk9 were either mock-infected or infected with SINV. Gene expression was examined by RT-qPCR. Relative gene expression compared with control uninfected cells is shown. The data represent mean ± SD from three independent experiments. $*P < 0.05$.

Table S1. List of RT-qPCR primers

Primer	Sequence
RP49-F	AAGAAGCGCACCAAGCACTTCATC
RP49-R	TCTGTTGTCGATACCCTTGGGCTT
B52-F	CTATGCCGATGCCCACAA
B52-R	CTCGGTGTCATCCAACTTCTC
CG9008-F	CCAGCCAGATCCTTCAGATTAT
CG9008-R	CATGGTTGCGAATCGGTTTAC
dpr17-F	GGCCAGAAGAAGATTAGCGATAG
dpr17-R	CTTTCGCACGAGCGGTATAA
orct-F	ATGATGGTGGCCGGAATTAG
orct-R	GGTTCCATACGACGAGGTAATAG
mol-F	TGATGAACCTGCTGCTGATAG
mol-R	TCGAGGTGAATGGACAAAGG
mew-F	GGTGGAAAGGGTCAGGATTT
mew-R	GCGTCTCCACCTCTATGTTATT
CG7458-F	CCAGAAGGAGGGAAATGGATAC
CG7458-R	CATCCAGAGAGTTGTCCTCATC
Nup98-F	GACCAGCAACCAGTACAAGATA
Nup98-R	CAAACTCCTCGAGACCATCAA
B52 ORF +59 F	TGGAGCGCTTTTTCAAAGGC
B52 ORF +135 R	TGTACTCACCACAAAGCCGTAG
CG9008 promoter-388 F	AACTGCGCAGAAGCTTTGTG
CG9008 promoter-468 R	ATGGTTGGCTTTAGGGTGTCC