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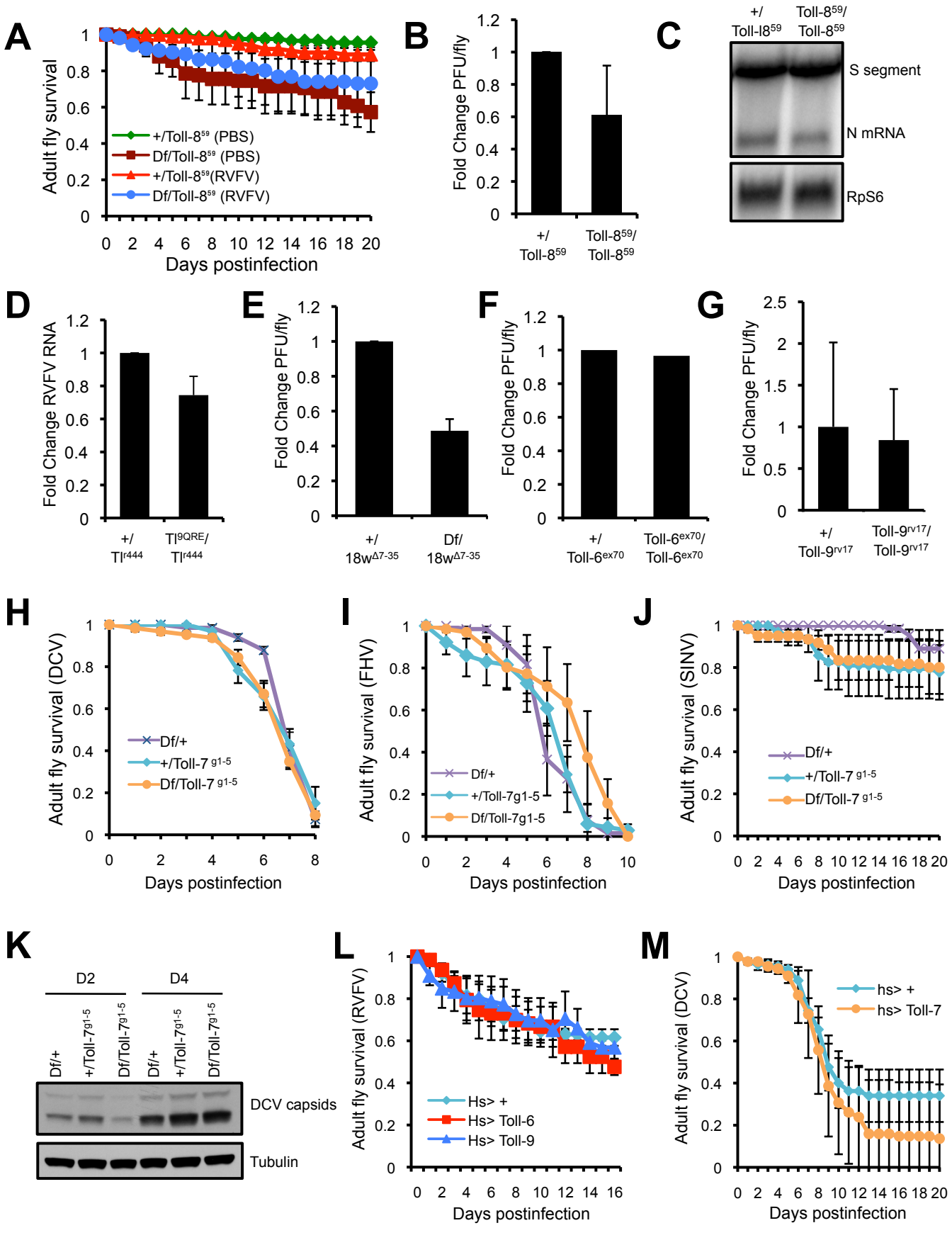

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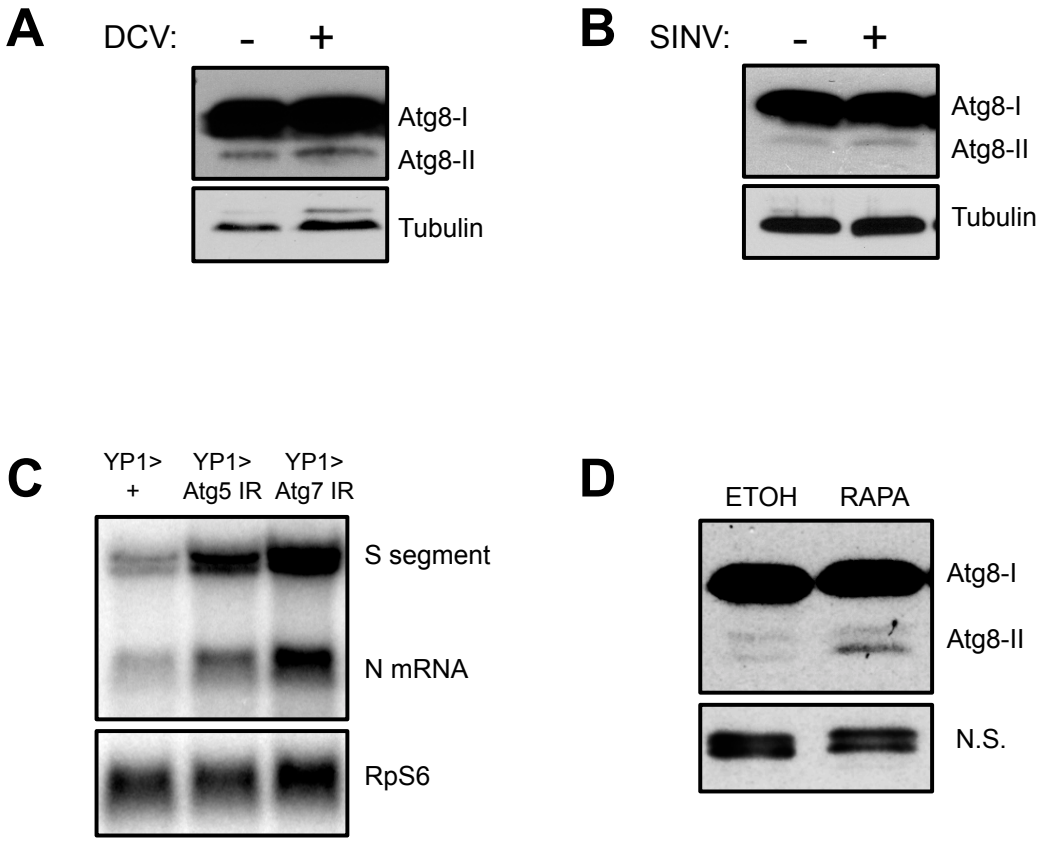

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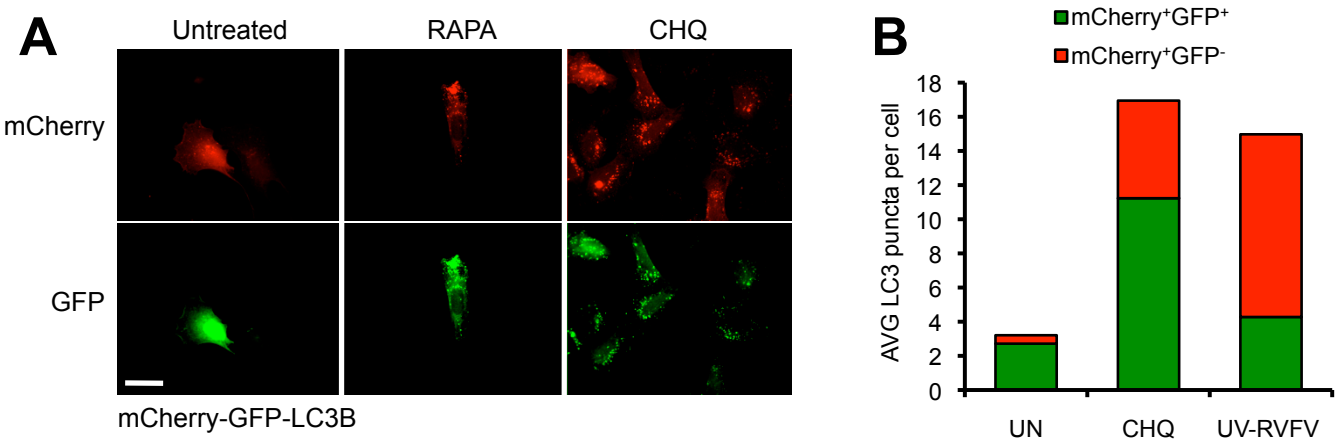

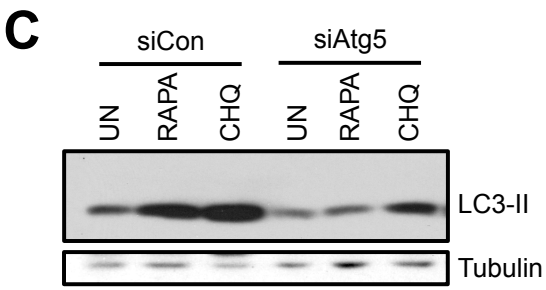

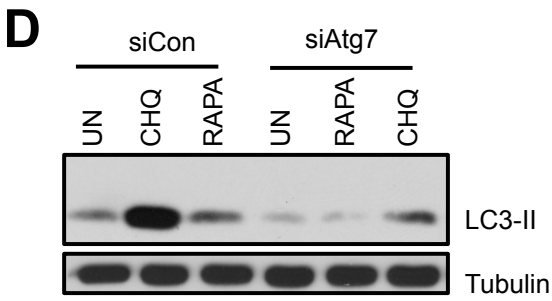

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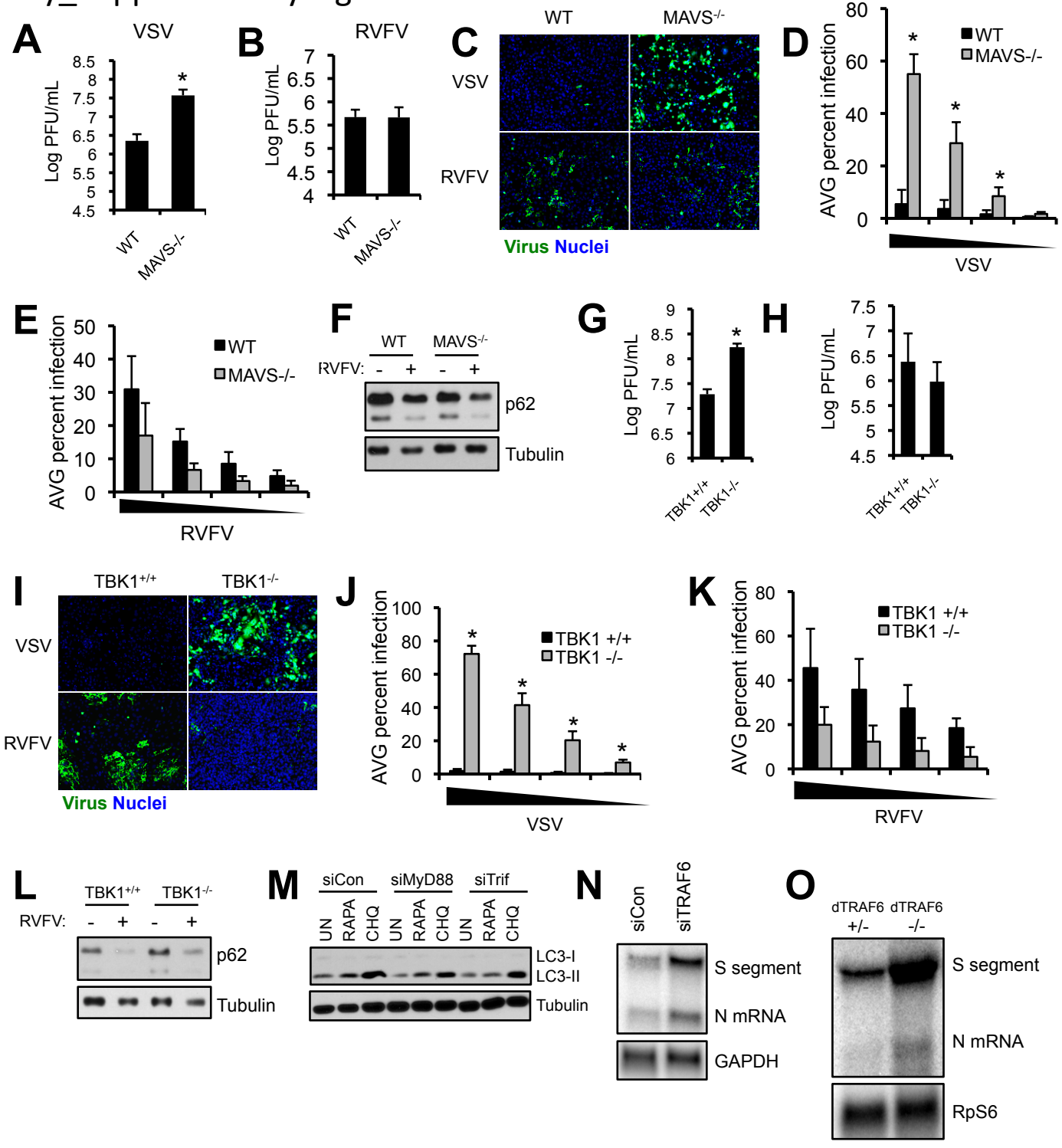

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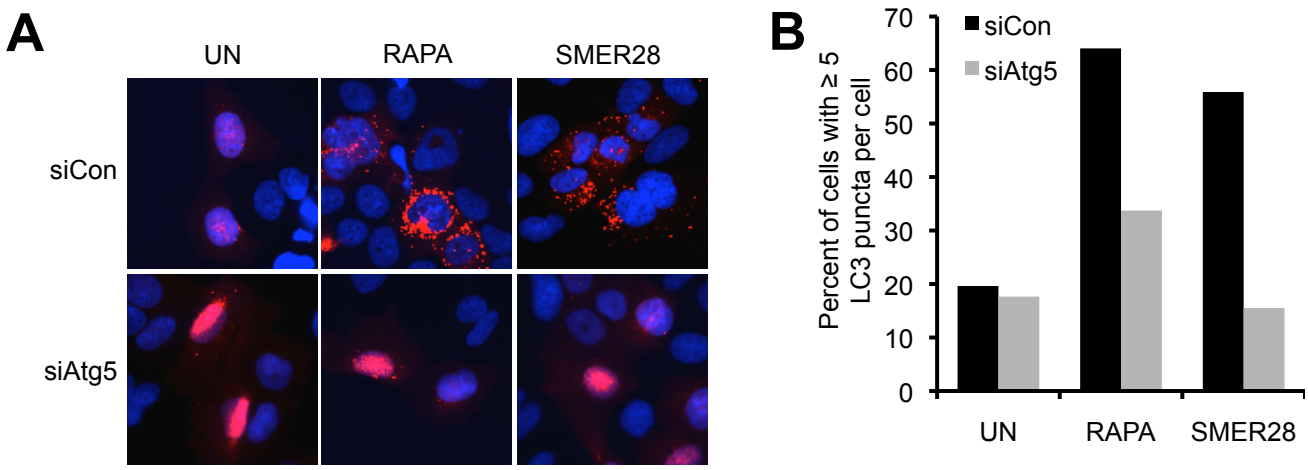

Supplemental Figure Legends:

Figure S1: Toll-7 antiviral activity is specific for RVFV *in vivo*. **A.** Survival of Toll-8 mutant flies (Df(3L)Brd15/Toll-8^{59}) or sibling control flies either uninfected or infected with RVFV. **B.** RVFV titers from Toll-8 mutant or control flies as measured by plaque assay 6 dpi. **C.** Representative RNA blot for RVFV RNA from infected Toll-8 mutant or control flies 6 dpi. **D.** Northern blot quantification of RVFV RNA from infected Toll mutant flies (Tl^{9QRE}/Tl^{r444}) or control flies 6 dpi for three independent experiments; Mean±SE. **E.** Viral titers from RVFV-infected Toll-2 mutant flies (Df/18w $^{\Delta 7\text{-}35}$) or control flies. **F.** Viral titers from RVFV-infected Toll-6 mutant (Toll-7^{ex70}/Toll-6^{ex70}) or sibling control flies 6 dpi for two independent experiments. **G.** RVFV titers from Toll-9 mutant flies (Toll-9^{rv17}/Toll-9^{rv17}) or sibling control flies. **H.** Survival of Toll-7 mutant flies (Df(2R)BSC22/Toll-$7^{g1\text{-}5}$) or sibling control flies infected with DCV. **I.** Survival of FHV-infected Toll-7 mutant or control flies. **J.** Survival of SINV-infected Toll-7 mutant or control flies. **K.** Immunoblot for DCV capsids from infected Toll-7 mutant or control flies at the indicated time points post-infection. Data are representative of two independent experiments. **L.** Survival of RVFV-infected transgenic flies overexpressing the indicated Toll receptors or control flies. **M.** Survival of Toll-7-overexpressing or control flies after DCV infection. Data are representative of at least three independent experiments unless otherwise noted. Figure S1, related to Figure 1.

Figure S2: Autophagy genes restrict RVFV infection in adult flies. **A.** Wild-type flies were infected with DCV and Atg8 expression was monitored by immunoblot 2 dpi. **B.**

Wild-type flies were infected with SINV and Atg8 expression was assessed by immunoblot 2 dpi. **C.** RNA blot for RVFV RNA from control flies or flies depleted of the indicated autophagy genes using a fat body specific driver (YP1-Gal4) day six post-infection. **D.** Rapamycin feeding activates autophagy in adult flies as assessed by Atg8 immunoblot. Figure S2, related to Figure 2.

Figure S3: Functional validation of mCherry-GFP-LC3 reporter and autophagy gene siRNA silencing. **A.** Representative images of U2OS cells stably expressing mCherry-GFP-LC3 treated with rapamycin (RAPA) or chloroquine (CHQ) for six hours. **B.** Quantification of $mCherry^{+}GFP^{+}$ (green) and $mCherry^{+}GFP^{-}$ (red) puncta from and CHQ-treated and UV-RVFV treated cells. A representative experiment of >3 independent experiment is shown. **C,D.** LC3 immunoblot from U2OS cells transfected with control siRNA or siRNAs targeting Atg5 **(C)** or Atg7 **(D)** after treatment with rapamycin or chloroquine. Figure S3, related to Figure 4.

Figure S4: TRAF6 but not TBK1 or MAVS restricts RVFV infection. **A,B.** Plaque assays were performed on WT MEFs or *MAVS*$^{-/-}$ MEFs to assess viral titers with VSV **(A)** and RVFV **(B).** Mean±SE **C.** Representative immunofluorescence images of WT or *MAVS*$^{-/-}$ MEFs infected with VSV or RVFV for 12 hours or 16 hours, respectively. **D,E.** Quantification of the percentage of infected WT MEFs or *MAVS*$^{-/-}$ MEFs challenged with either VSV **(D)** or RVFV **(E)** at varying MOI. Mean±SE **F.** Immunoblot for p62 expression 12 hours post-infection with RVFV. **G,H.** Viral titers with VSV **(G)** or RVFV **(H)** from infected *TBK1*$^{+/+}$ or *TBK1*$^{-/-}$ MEFs as assessed by plaque assay. Mean±SE **I.**

Representative immunofluorescence images of VSV or RVFV-infected *TBK1*$^{+/+}$ or *TBK1*$^{-/-}$ MEFs. **J,K.** Quantification of **(I)** for VSV infection **(J)** or RVFV infection **(K)**. Mean±SE **L.** p62 immunoblot from uninfected or RVFV-infected *TBK1*$^{+/+}$ and *TBK1*$^{-/-}$ MEFs at 12 hpi. **M.** LC3 immunoblot from U2OS cells transfected with the indicated siRNAs and left untreated or treated with rapamycin (1 μM) or chloroquine (20μM) for six hours. **N.** Representative RNA blot from RVFV-infected U2OS transfected with control or TRAF6-specific siRNA and infected with RVFV 16 hpi. **O.** Representative RNA blot form RVFV-infected *Traf6*$^{-/-}$ flies (dTraf6^{Ex1}/dTraf6^{Ex1}) or sibling control flies 6 dpi. Figure S4, related to Figure 5.

Figure S5: Rapamycin and SMER28 activate autophagy in an Atg5-dependent manner. **A.** Representative images of control siRNA- or Atg5 siRNA-transfected U2OS cells expressing an mCherry-GFP-LC3 reporter left untreated or treated with rapamycin (1 μM) or SMER28 (50 μM) for 16 hours. mCherry represents total LC3 puncta. **B.** Percentage of cells containing at least 5 mCherry-positive LC3 puncta with the indicated treatment conditions. Quantification for one representative experiment from two independent experiments is shown. Figure S5, related to Figure 6.

Supplemental Experimental Procedures

Cells and viruses

MEFs, U2OS and BHK-21 cells were maintained at 37°C in DMEM supplemented with 10% FBS (Sigma), 100 μg/mL penicillin/streptomycin and 2mM L-glutamine. *Drosophila* DL1 cells were maintained at 25°C in complete *Drosophila* media (Schneider's

Drosophila medium (Gibco) containing 10% FBS, L-glutamine (Gibco) and penicillin/streptomycin (Gibco)). Primary rat neuroglial cells were isolated from embryonic day 17 Sprague Dawley rat pups as previously described (Soldan et al., 2010) and plated on plates pre-coated with poly-L-lysine (Sigma). Neuronal cells were cultured in neurobasal media supplemented with B27 (Invitrogen). For primary hepatocyte isolation, livers of C57Bl/6 mice were perfused with 1X HBBS solution followed by 1X HBSS containing 0.1 M $CaCl_2$ and 5mg/ml Liberase (Fisher Scientific). The digested liver and cells were further agitated by pipetting and treated with ACK lysis buffer (Lonza) and collected through a 70-µm cell strainer. Digested cells were centrifuged at slow speed (50 g) for 5 minutes and the pellet (hepatocytes) was resuspended in collection media (DMEM + 10% FBS). MP12 strain of RVFV was grown in BHK-21 cells supplemented with 10% FBS (Filone et al., 2010). VSV-GFP was grown in BHK cells as described (Ramsburg et al., 2005). SINV-GFP (gift from R. Hardy) was grown in C636 cells as described (Burnham et al., 2007). FHV (gift from P. Ahlquist) was grown in DL1 cells. DCV was grown and purified as described (Cherry and Perrimon, 2004). RVFV was inactivated with UV light using a UV Stratalinker 2400 (Stratagene) and tested for infectivity.

Reagents

The following primary antibodies were used: anti-tubulin (Sigma), anti-actin (Santa Cruz), anti-LC3B (Sigma), anti-p62 (Sigma), anti-Atg5 (Sigma), anti-Atg7 (Sigma), anti-Beclin 1 (Cell Signaling), anti-MyD88 (Abcam), anti-TRIF (Cell Signaling), anti-TRAF6 (Santa Cruz), anti-phospho-*Drosophila* Akt (Ser505) (Cell Signaling), anti-phospho-Akt

(Ser473) (Cell Signaling), anti-Akt (Cell Signaling), anti-Toll-7 (Nakamoto et al., 2012), anti-RVFV ID8 (gift from R. Doms), anti-Atg8 (Shelly et al., 2009) and anti-DCV (Cherry and Perrimon, 2004). Fluorescently labeled secondary antibodies were from Invitrogen. HRP-conjugated secondary antibodies were obtained from Amersham. Drugs were obtained from the following sources: rapamycin (Sigma), SMER28 (EMD Millipore), chloroquine (Sigma).

siRNA transfections

U2OS cells were reverse transfected with siRNAs at a final concentration of 10 nM using HiPerFect transfection reagent (Qiagen). siRNAs were obtained from the following sources: non-targeting control, Atg5, Atg7, Fip200, Atg13 (Ambion); MyD88, TRIF (Dharmacon); Beclin 1 (Cell Signaling); TRAF6 (Qiagen). Cells were processed for immunoblot or infected on day three post-transfection.

Immunoblotting and streptavidin precipitation

Cells or flies were collected and lysed in radioimmunoprecipitation (RIPA) buffer supplemented with a protease inhibitor cocktail (Boehringer). Samples were processed for immunoblot as described previously (Shelly et al., 2009). Relative protein levels were determined using ImageJ software. Purified RVFV was biotinylated using Sulfo-NHS-LC-Biotin (Thermo) following the manufacturer's protocol and incubated with *Drosophila* cells at 4ºC. For immunoprecipitations, samples were lysed in lysis buffer (20mM Tris at pH 7.6, 150 mM NaCl, 2mM EDTA, 10% glycerol, 1% Triton X-100, 1mM

DTT, and protease inhibitors) (Aggarwal et al., 2008). Protein lysates were precipitated with streptavidin-agarose (Pierce) and immunoblotted.

Northern blotting and quantitative RT-PCR

Total RNA was isolated from cells or flies using Trizol (Invitrogen) according to the manufacturer's instructions. For Northern blots, RNA was separated using a 1% agarose/formaldehyde gel and blotted as previously described (Cherry et al., 2005). RNA levels were analyzed by normalizing to the indicated loading control using ImageQuant software. For quantitative RT-PCR, cDNA was first prepared from total RNA using random primers and M-MLV reverse transcriptase (Invitrogen). PCR was then performed on cDNA, and results were analyzed by relative quantitation with normalization to GAPDH.

***Drosophila melanogaster* Strains and Maintenance**

Flies were maintained on standard medium at room temperature. Toll-2 mutants were generated by crossing $18w^{\Delta 7\text{-}35}$/Cyo to Df(2R)017/SM1 (Ligoxygakis et al., 2002). Toll-$7^{g1\text{-}5}$/CyO were crossed to Df(2R)BSC22/CyO to generate Toll-7 mutants (Yagi et al., 2010). Toll-8^{59} was crossed to Df(3L)Brd15 to generate Toll-8 mutants. Tl^{r444}/TM3 Sb to Tl^{9QRE}/TM3 Ser to generate Toll mutants. Homozygous Toll-6 mutants (Toll-6^{ex70}) and Toll-9 mutants (Toll-9^{rv17}) were compared to heterozygous sibling controls (Yagi et al., 2010). Toll receptor overexpressing flies were generated by crossing heat-shock Gal4 to UAS-Toll-3, UAS-Toll-4, UAS-Toll-5, UAS-Toll-6, UAS-Toll-7, UAS-Toll-8 and UAS-Toll-9 (Yagi et al., 2010). UAS-Atg18 IR (VDRC), UAS-Atg5 IR, UAS-Atg7 IR and UAS-

GFP-Atg8a were crossed to Actin-Gal4 or YP1-Gal4 (Hu et al., 2004). Homozygous Traf6 mutant flies ($dTraf6^{Ex1}/dTraf6^{Ex1}$) were compared to heterozygous sibling controls (Cha et al., 2003).

Plaque assay

Viral titers were measured by plaquing virus on the indicated MEFs as described previously (Moser et al., 2012).

Primers

qRT-PCR primers are as follows:

mouse ISG56 forward 5'-CCAAGTGTTCCAATGCTCCT-3';

mouse ISG56 reverse 5'-GGATGGAATTGCCTGCTAGA-3';

mouse GAPDH forward 5'-ACCCTGTTGCTGTAGCCGTATTCA-3';

mouse GAPDH reverse 5'-TCAACAGCAACTCCCACTCTTCCA-3';

rat GAPDH forward 5'-GACATGCCGCCTGGAGAAAC-3';

rat GAPDH reverse 5'-AGCCCAGGATCGCCTTTACGT-3';

RVFV forward 5'-CAAGCAGTGGACCGCAATGAGA-3';

RVFV reverse 5'-GGGCTTGTTGCCACGAGTTAGA-3';

Supplemental References

Aggarwal, K., Rus, F., Vriesema-Magnuson, C., Erturk-Hasdemir, D., Paquette, N., and Silverman, N. (2008). Rudra interrupts receptor signaling complexes to negatively regulate the IMD pathway. PLoS Pathog *4*, e1000120.
Cha, G.H., Cho, K.S., Lee, J.H., Kim, M., Kim, E., Park, J., Lee, S.B., and Chung, J. (2003). Discrete functions of TRAF1 and TRAF2 in Drosophila melanogaster mediated by c-Jun N-terminal kinase and NF-kappaB-dependent signaling pathways. Mol. Cell. Biol. *23*, 7982-7991.
Cherry, S., Doukas, T., Armknecht, S., Whelan, S., Wang, H., Sarnow, P., and Perrimon, N. (2005). Genome-wide RNAi screen reveals a specific sensitivity of IRES-containing RNA viruses to host translation inhibition. Genes Dev. *19*, 445-452.
Cherry, S., and Perrimon, N. (2004). Entry is a rate-limiting step for viral infection in a Drosophila melanogaster model of pathogenesis. Nature immunology *5*, 81-87.
Hu, X., Yagi, Y., Tanji, T., Zhou, S., and Ip, Y.T. (2004). Multimerization and interaction of Toll and Spatzle in Drosophila. Proc. Natl. Acad. Sci. U. S. A. *101*, 9369-9374.
Ligoxygakis, P., Bulet, P., and Reichhart, J.M. (2002). Critical evaluation of the role of the Toll-like receptor 18-Wheeler in the host defense of Drosophila. EMBO reports *3*, 666-673.
Moser, T.S., Schieffer, D., and Cherry, S. (2012). AMP-activated kinase restricts Rift Valley fever virus infection by inhibiting fatty acid synthesis. PLoS pathogens *8*, e1002661.
Nakamoto, M., Moy, R.H., Xu, J., Bambina, S., Yasunaga, A., Shelly, S.S., Gold, B., and Cherry, S. (2012). Virus recognition by Toll-7 activates antiviral autophagy in Drosophila. Immunity *36*, 658-667.
Shelly, S., Lukinova, N., Bambina, S., Berman, A., and Cherry, S. (2009). Autophagy is an essential component of Drosophila immunity against vesicular stomatitis virus. Immunity *30*, 588-598.
Yagi, Y., Nishida, Y., and Ip, Y.T. (2010). Functional analysis of Toll-related genes in Drosophila. Dev. Growth Differ. *52*, 771-783.