

Supporting Information

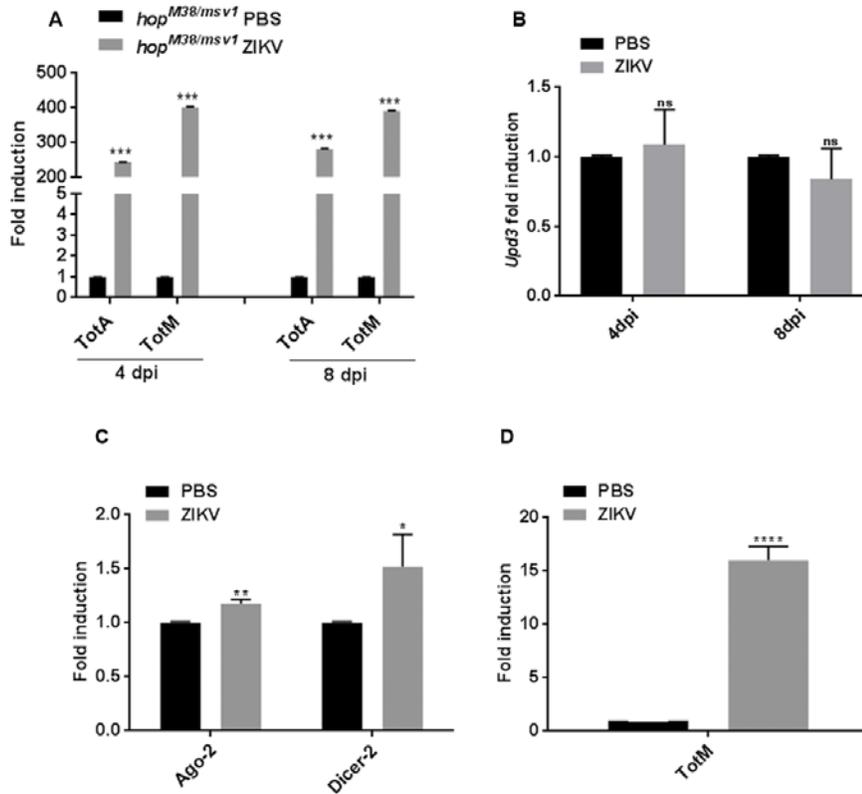


Fig S1. ZIKV infection-induced *Tot* gene expression is independent of JAK/STAT regulation in *Drosophila*. (A) *TotM* and *TotA* mRNA levels in *hop*^{M38/msv1} mutant flies upon infection with ZIKV (strain MR766) compared to controls injected with PBS. (B) mRNA levels of *Upd-3*, regulator of *Tot* gene expression, in wild type *w*¹¹¹⁸ flies injected with ZIKV as compared to controls injected with PBS. Levels of mRNA were normalized with respect to the housekeeping gene *RpL32*. Differential expression of (C) *Ago-2* and *Dicer-2*, and (D) *TotM* in *w*¹¹¹⁸ adult female flies injected with heat-inactivated ZIKV at 8 days post injection (dpi). Gene transcript levels in flies injected with PBS were arbitrarily designated as 1 and the results are shown as relative levels of mRNA, which were normalized with respect to the housekeeping gene *RpL32*. Data were analyzed using Student's test and values represent the mean \pm SD (n=3 experiments, *P<0.05, **P=0.0015, ***P<0.0001 and ****P<0.0001); ns denotes no significant differences between experimental treatments.

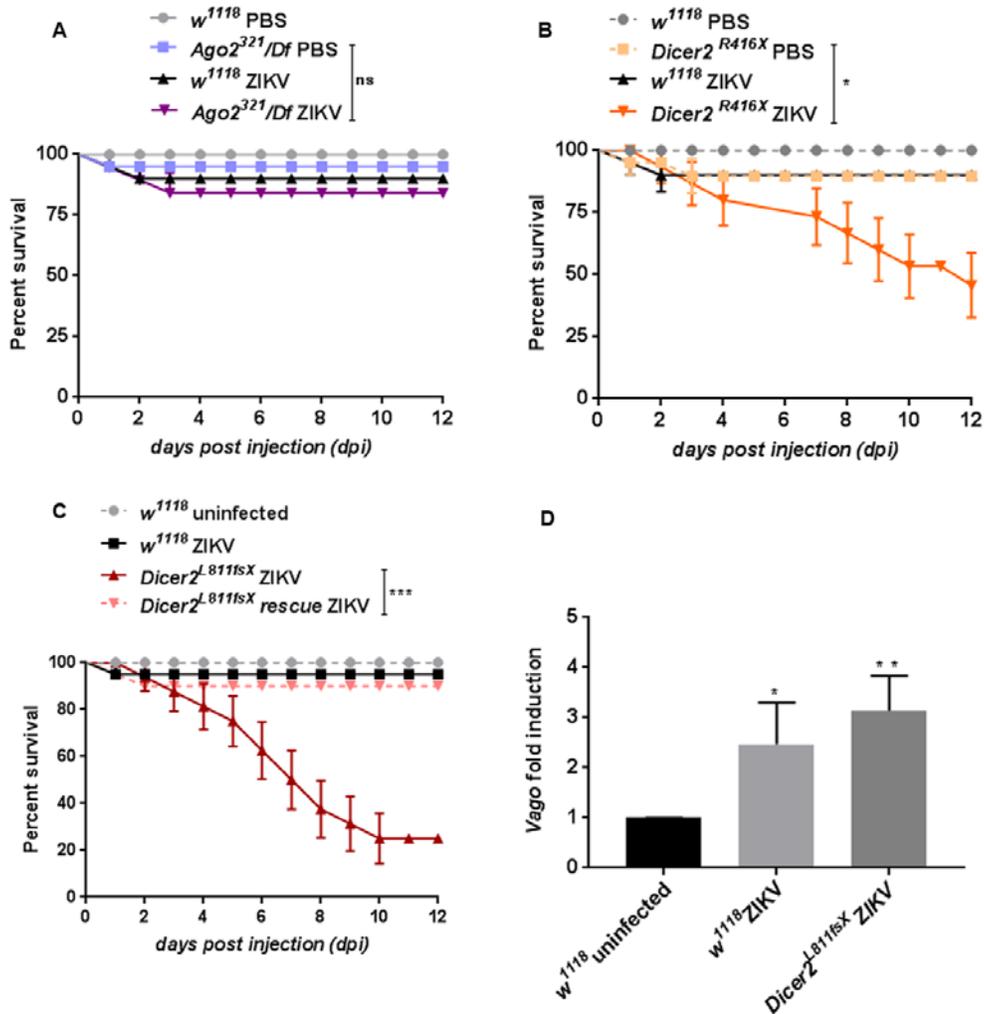


Fig S2. *Dicer-2* mutant flies display enhanced expression of *Vago* upon ZIKV infection. (A) Survival analysis of PBS and ZIKV-injected wild type (w^{1118}) and trans-heterozygous null allelic combination of *Ago2*³²¹/*Df* flies. (B) Survival analysis of PBS and ZIKV injected wild type (w^{1118}) and *Dicer-2* null mutant flies (*Dicer2*^{R416X}). (C) Survival analysis of uninfected wild type control, ZIKV infected wild type flies, flies mutant for *Dicer-2* and those overexpressing a *Dicer-2* rescue construct. For survival experiments, three groups of 20 female flies from each fly strain were injected with ZIKV and fly survival was monitored at 24-hour intervals and up to 12 days post injection (dpi). Log-rank (Mantel-Cox) was used for survival data analysis (*P=0.02 and ***P<0.001), ns denotes no significant differences between experimental treatments. (D) Quantitative RT-PCR analysis of the antiviral gene *Vago* in ZIKV infected w^{1118} flies and *Dicer-2* mutants. Data were analyzed using Student's test and values represent mean ± SD (n=3 experiments, *P=0.03, **P=0.005).

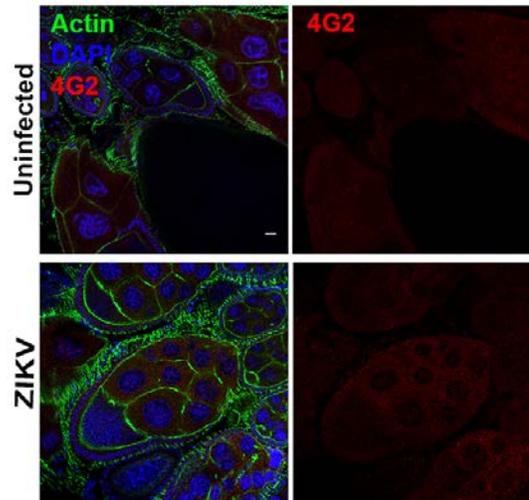


Fig S3. Ovaries of ZIKV infected flies are devoid of flavivirus-specific 4G2 expression. The upper panel shows egg chambers from uninfected wild type w^{1118} adult flies while the lower panel shows egg chambers from ZIKV-infected flies at 8 days post injection (8 dpi). The egg chambers were stained with flavivirus-specific 4G2 antibody. In all images, 4G2 was marked in red, actin in green and nuclei were marked in blue. Scale bars: 100 microns.

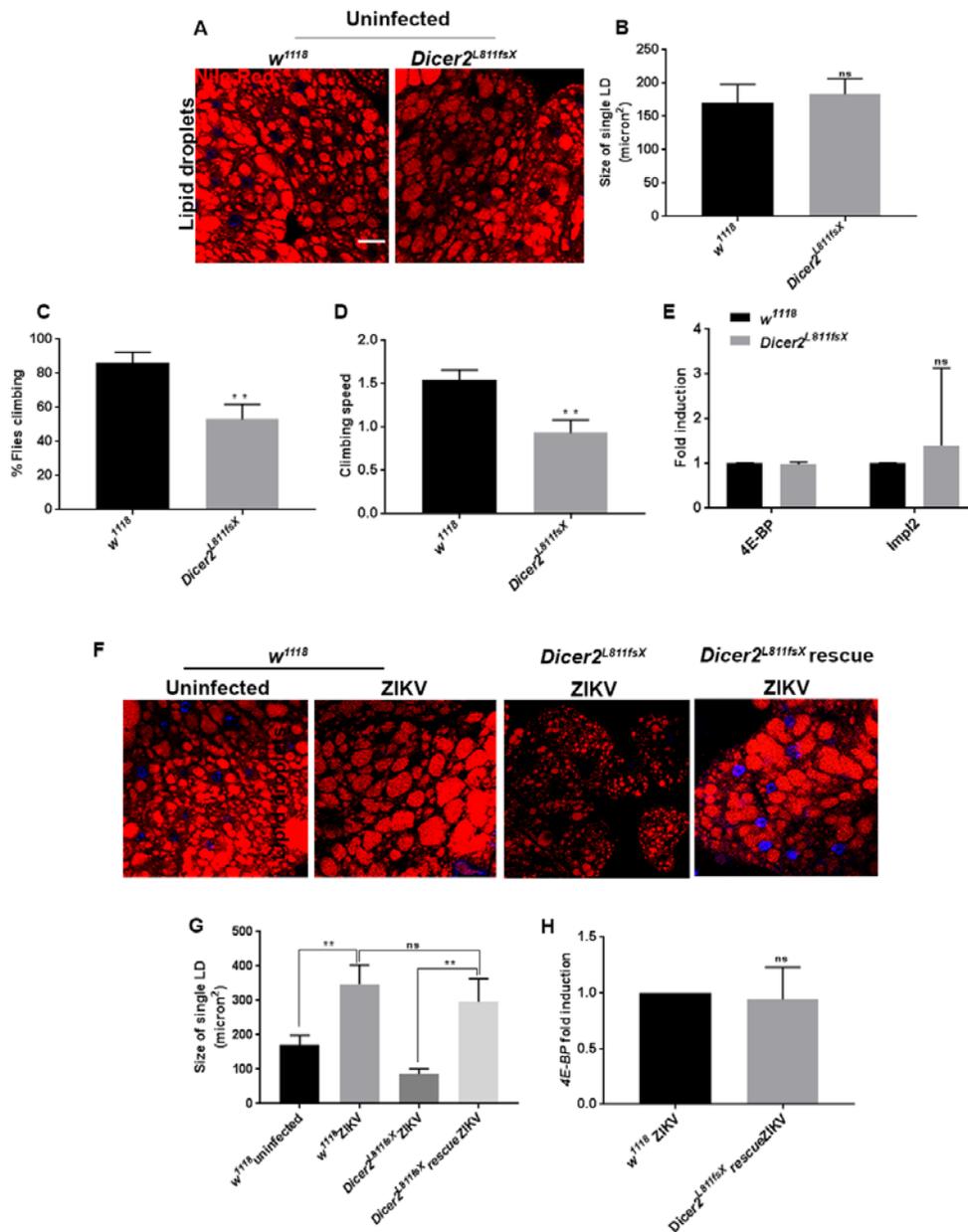


Fig S4. Overexpression of *Dicer-2* rescue construct ameliorates the pathology induced by ZIKV infection. Representative images of fat body lipid droplets in uninfected wild type *w¹¹¹⁸* flies and *Dicer-2* mutants at 8 days post injection (8 dpi). (A) The size of lipid droplets in both fly lines was comparable. The neutral lipids were marked with Nile Red. (B) Quantification of lipid droplet size in uninfected flies. (C, D) Climbing ability and speed of climbing in uninfected *w¹¹¹⁸* and *Dicer-2* mutant flies. (E) Quantitative RT-PCR analysis of the Foxo target gene *4E-BP* and insulin antagonist *Impl2* in uninfected *w¹¹¹⁸* and *Dicer-2* mutant flies. (F) Representative

images of fat body lipid droplets in uninfected and ZIKV-infected wild type controls, *Dicer-2* mutant and *Dicer-2* rescue flies. The neutral lipids were marked with Nile Red. (G) Quantification of lipid droplet size in ZIKV infected wild type, *Dicer-2* mutant and *Dicer-2* rescue flies as well as in uninfected wild type controls. (H) Quantitative RT-PCR analysis of *4E-BP* and *Impl2* mRNA levels in ZIKV-infected flies carrying a *Dicer-2* rescue construct as compared to the ZIKV-infected wild type controls. In all graphs, bars represent mean \pm SD. Statistical analysis was performed using Student's t-test (**p=0.005, **p=0.006, **p=0.007); ns denotes non-significant differences.