Supporting Information

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SI Materials and Methods

Quantitative and Semiquantitative RT-PCR. Primers used for quantitative real-time PCR were as follows: RpL32 forward, 5′-GACGCTTCAAGGGACAGTATCTG-3′, and reverse, 5′-AAACGCGGTTCTGCATGAG-3′; FHV1 forward, 5′-TTTAGAGCACATGCGTCCAG-3′, and reverse, 5′-CGCTCACTTTCTTCGGGATA-3′; FHV2 forward, 5′-CAACGTCGAACTTGATGCAG-3′, and reverse, 5′-GCTATACAGGGCATTTCCAA-3′; DCV forward, 5′-TCATCGGTATGCACATTGCT-3′, and reverse, 5′-CGCATAACCATGCTCTTCTG-3′. Gene expression was normalized to the expression of RNA encoding the "housekeeping" ribosomal protein L32 (RpL32), used as a loading control, and the data represented as the ratio $2^{CT(RpL32)}/2^{CT(FHV)}$.

For *dSUR*, *Ir*, and *Irk2* expression studies in *Drosophila*, single-step semiquantitative RT-PCR was performed with the Brilliant II RT-PCR Core Reagent Kit (Stratagene) as described previously (1). Sequences of primers used for semiquantitative RT-PCR were as follows: *dSUR* forward 5′- TCGCCAGCTATCCGTATTTC-3′, and reverse, 5′- TGCCACCGTACTGATCACAT-3′; *Ir* forward 5′- ACGCACACAATGATCTGGAG-3′, and reverse, 5′- ATGGTAGTGGGCCAGATGAA-3′; *Irk2* forward 5′- GGAGTGTCCACCTGAGTGGT-3′, and reverse, 5′- TCATGATGTGCTTCCAGTCC-3′. Amplifications were performed under the following cycling conditions: 45 °C for 30 min; 95 °C for 10 min; 25 cycles of 95 °C for 30 s, 64 °C for 1 min, and 72 °C for 30 s. PCR control reactions for RpL32 were performed at 20 cycles. PCR controls without template or reverse transcriptase were also included.

Median Tissue Culture Infective Dose Assays. Infected flies were homogenized in Schneider's *Drosophila* media (BioWest), and following centrifugation of fly parts, supernatants were filtered and dilutions of virus suspensions were used to infect *D. melanogaster* Kc167 cells plated out in wells of a 96-well plate (1.2×10^5 cells/well). Following incubation of infected cells at 23 °C for 1 d, cells were fixed in 8% formaldehyde solution for 10 min at room temperature, washed twice in PBS and 0.1% Triton X-100 (PBT) and blocked in blocking solution (PBS, 10% FBS; Thermo Scientific HyClone) for 30 min at room temperature. Cells were incubated with polyclonal antibodies to FHV capsid (1:500 dilution in blocking solution) for 2 h at room temperature, washed twice in PBT solution, and stained with goat anti-rabbit IgG FITC secondary antibodies (Invitrogen; 1:500 dilution in blocking solution) for 1 h at room temperature. Finally, cells were dried following washing in PBT solution and FHV titer was determined by fluorescence microscopy (Zeiss Axioscope 2).

1. Croker B, et al. (2007) ATP-sensitive potassium channels mediate survival during infection in mammals and insects. *Nat Genet* 39:1453–1460.

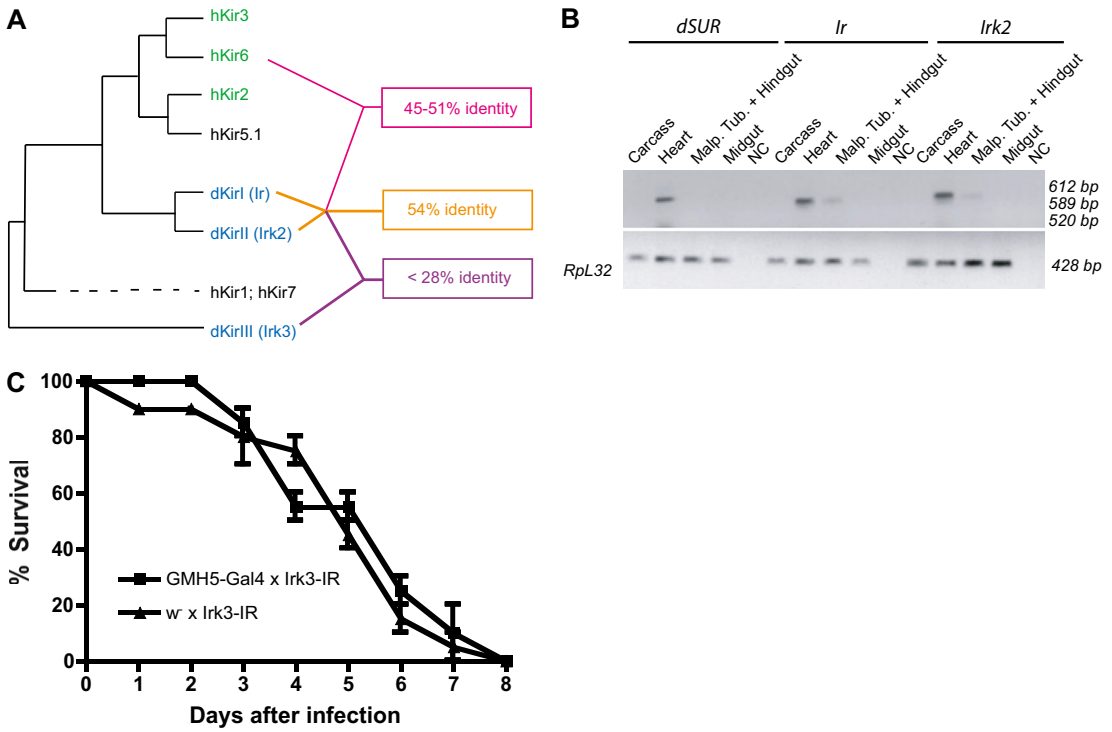

Fig. S1. *Drosophila Ir* and *Irk2* genes are orthologs to human *Kir6.x* genes, and are expressed in the heart. (*A*) Phylogenetic comparison of *Kir* genes in *Drosophila* and human. (*B*) Semiquantitative RT-PCR was used to analyze the expression of *dSUR*, *Ir*, and *Irk2* genes in different tissues dissected from wild-type flies. The sizes of the amplicons are 612 bp (*dSUR*), 589 bp (*Irk2*), and 520 bp (*Ir*). Note that *Ir* and *Irk2* are highly expressed in the *Drosophila* heart, like *dSUR*. The gene encoding the ribosomal protein RpL32 was used as a control. For *B*, data represent three independent experiments involving dissection of tissues from at least 50 flies. (*C*) Survival curves of flies obtained from crosses between UAS-Irk3 RNAi line (IR, inverted repeat) with the heart-specific GMH5-Gal4 driver and control flies (UAS-Irk3 RNAi line crossed with w^- flies) after infection with FHV. Data represent the mean ± SD of two independent experiments, each involving two groups of 10 flies.

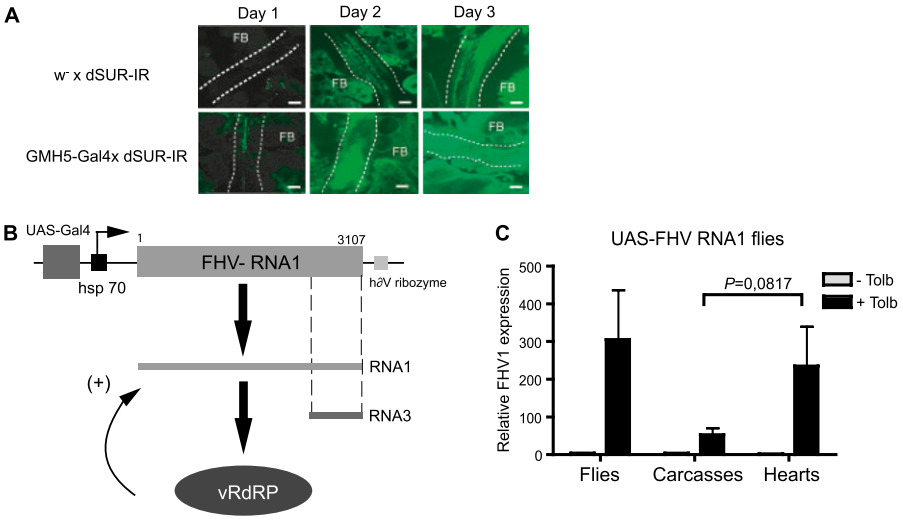

Fig. S2. Potassium channels regulate antiviral RNA interference in the *Drosophila* heart. (*A*) Immunostaining of heart and fat body tissue from dSUR-silenced flies and control flies with antibody to FHV capsid protein (green; Alexa Fluor 488) at different times after virus infection. Data are from one experiment representative of three with at least 10 flies for each treatment. (Scale bar: 35 μm.) FB, fat body. (*B*) RNA1 construct used for generating transgenic flies. RNA1- and subgenomic RNA3- encoded transcripts are shown. RNA1 encodes the viral RNA-dependent RNA polymerase (vRdRP). (*C*) Quantitative RT-PCR of FHV RNA1 levels in whole flies, carcasses, and hearts from 1-wk-old UAS-RNA1 transgenic flies treated or not with tolbutamide. For *C*, data represent the mean ± SD of three independent experiments each involving at least 30 flies.

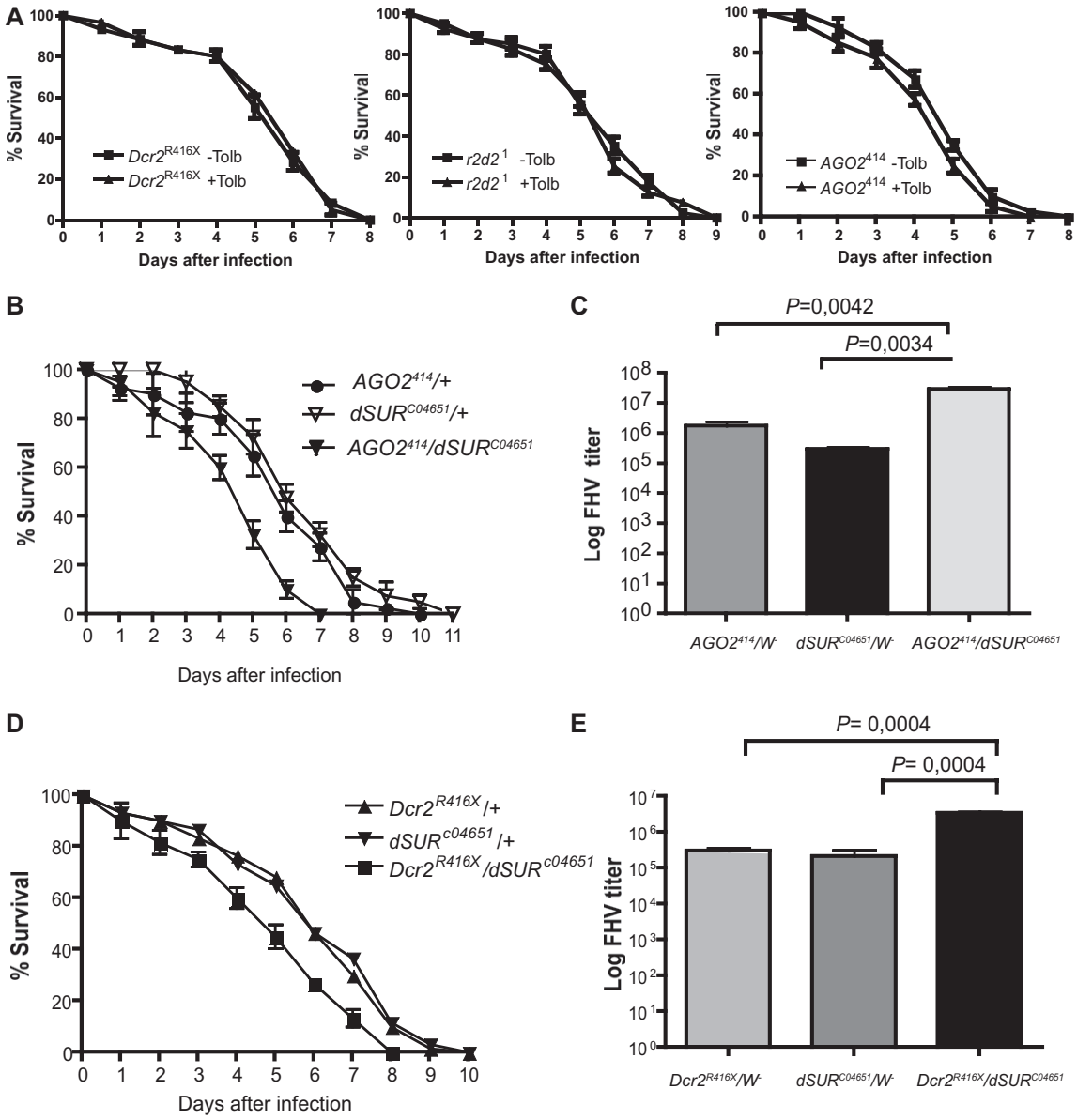

Fig. S3. Genetic interaction between *dSUR* and the antiviral RNAi pathway genes. (*A*) Survival curves of 1-wk-old *Dcr-2*, *r2d2*, and *AGO2* mutant flies with or without treatment with tolbutamide and following infection with FHV. (*B*) Survival curves of *AGO2*;*dSUR* double-heterozygote flies and control single-heterozygous flies after FHV infection. (*C*) FHV titers in *AGO2*;*dSUR* double-heterozygote flies compared with control single-heterozygous flies 3 d after FHV infection. (*D*) Survival curves of *Dcr-2*;*dSUR* double-heterozygote flies and control single-heterozygous flies after FHV infection. (*E*) FHV titers in *Dcr-2*;*dSUR* double-heterozygote flies compared with control single-heterozygous flies 3 d after FHV infection. Data represent the mean ± SD of three independent experiments, each involving at least two groups of 10 flies (survival) or 30 flies (viral load) per treatment.

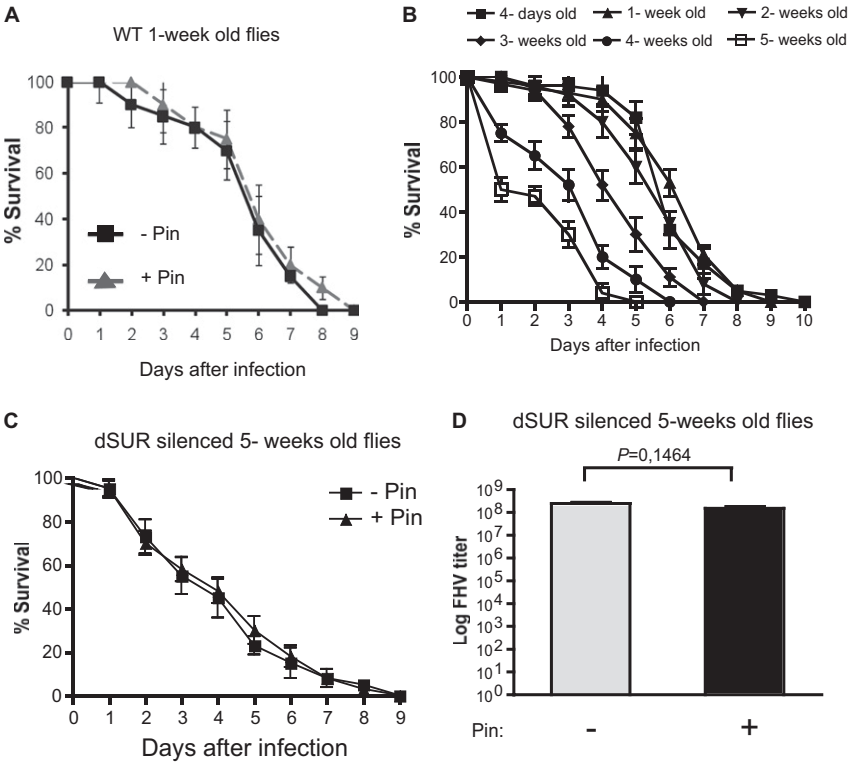

Fig. S4. The K_{ATP} channel agonist drug pinacidil does not protect old *dSUR* mutant flies against FHV infection. (*A*) Survival curves of 1-wk-old flies treated or not with pinacidil after FHV infection. (*B*) Survival curves of aging wild-type flies were determined after infection with FHV. Note the increased lethality in aged flies. (*C* and *D*) Survival curves (*C*) and viral load (*D*) of *dSUR*-silenced 5-wk-old flies fed on the K_{ATP} agonist pinacidil or on sucrose solution alone after infection with FHV. FHV titers in infected flies were estimated at 3 d following virus infection. Data represent the mean ± SD of three independent experiments, each containing two groups of 10 flies.

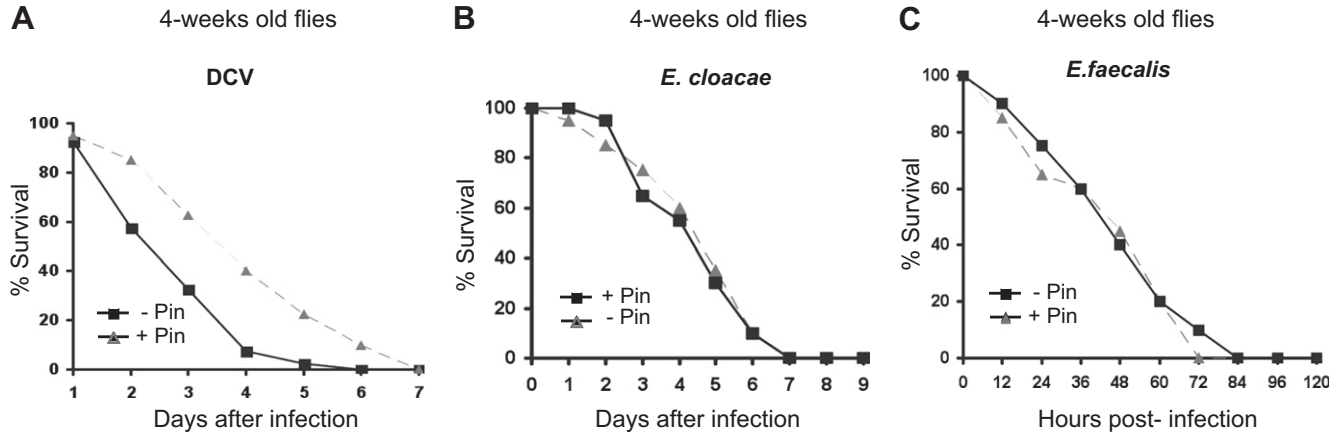

Fig. S5. The K_{ATP} channel agonist drug pinacidil does not significantly improve resistance of old flies to other infections. Survival curves of w^{-} wild-type 4-wk-old flies fed on the K_{ATP} agonist pinacidil or on sucrose solution alone after infection with DCV (*A*), the Gram-negative bacteria *Enterobacter cloacae* (*B*), and the Gram-positive bacteria *Enterococcus faecalis* (*C*). A representative experiment involving two groups of 10 flies per treatment is shown.

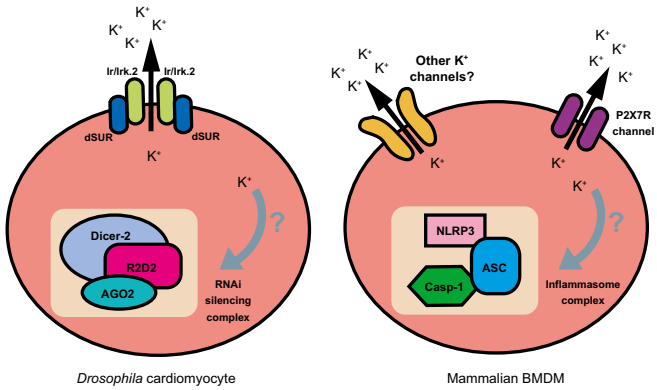

Fig. S6. A model for the evolutionary conserved role of K^+ ion efflux in the regulation of innate immunity in flies and mammals. See *Discussion* for more details. BMDM, bone-marrow derived macrophage.