Figure S1

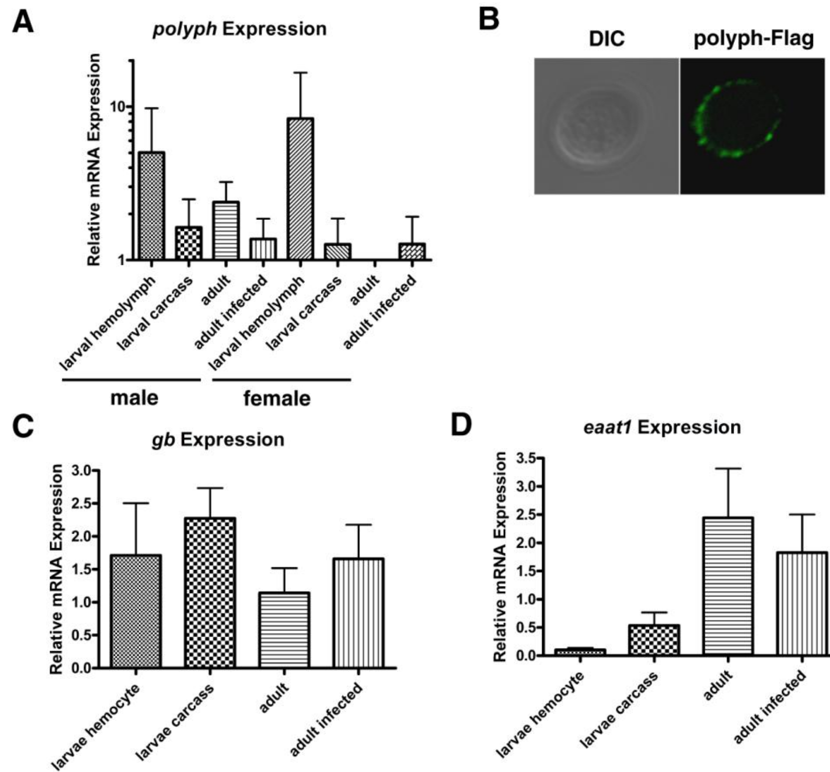

Figure S1: *polyph* and *gb* are expressed in blood cells where *polyph* is required for survival following infection.

(A) Distribution of *polyph* expression in developmental stage, sex, and upon infection via qPCR. Relative expression was measured using *rp49* as an endogenous control. Blood cells were collected by carefully rupturing the anterior end of the larvae into PBS in order to draw out the liquid. Everything that remained in the larvae after 1 minute of bleeding was considered carcass. Infected flies were injected with an overnight culture of *S.aureus* and collected at 6 hours postinfection. (B) Blood cells collected from *hmlΔ*GAL4>UAS *polyph-flag* larvae were immunostained with an anti-Flag primary antibody, and AlexaFluor 488 labeled secondary antibody. *hmlΔ*GAL4 is a blood cell-specific driver. Cells were visualized via confocal microscopy and a representative picture is shown. Left image, DIC. Right image, AlexaFluor 488 derived-fluorescence. n = 20 cells. Expression of *gb* (C), and *eaat1* (D) was measured in larvae, adults, and upon infection via qPCR. Relative expression was measured using *rp49* as an endogenous control. Blood cells were collected as described above. All experiments were performed at least in triplicate. Error bars, ±SE.

Figure S2

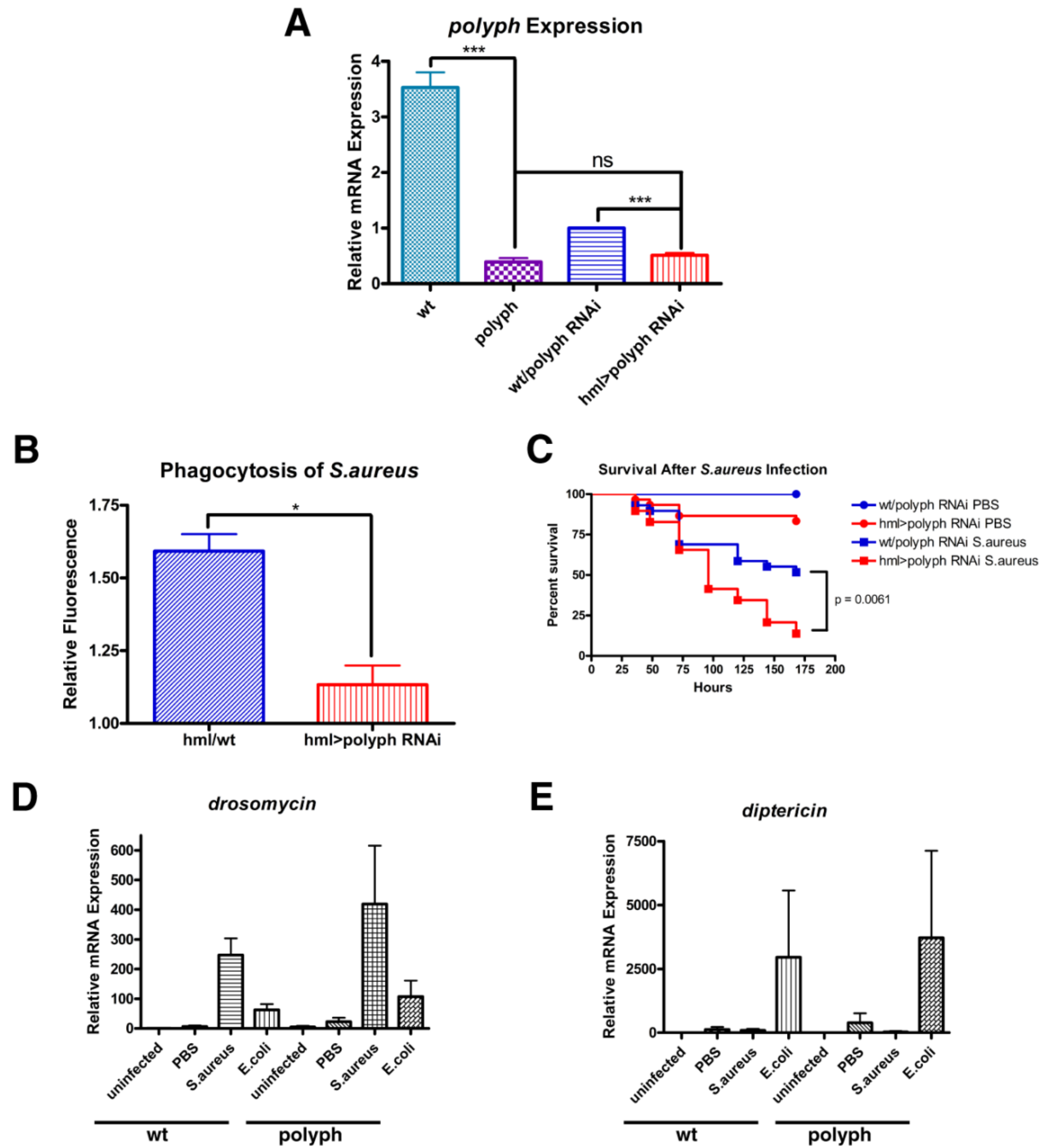

Figure S2: *polyph* is required for phagocytosis and survival following infection but not for induction of *drosomycin* or *diptericin.*

(A) Comparison of *polyph* transcript levels via qPCR in wt, *polyph*, wt/*polyph* RNAi, and *hml>polyph* RNAi flies. Relative expression was measured using *rp49* as an endogenous control. A pool of four female flies per genotype was used in each experiment. (B) Quantification of the phagocytosis of fluorescein-labeled *S.aureus* bioparticles in *hml/*wt and *hml>polyph* RNAi flies. Approximately 6 flies per genotype were used in each experiment(C) Representative survival curve of wt*/polyph RNAi* and *hml>polyph* RNAi flies after injection of either PBS or *S.aureus* (OD 0.5). n = 28-30 flies. The induction of *drosomycin* (D) and *diptericin* (E) was measured in wt and *polyph* flies using qPCR after injection of one of the following: PBS, overnight culture of *S.aureus*, or overnight culture of *E.coli*. *drosomycin* induction was measured 24 hours postinfection. *diptericin* induction was measured 6 hours postinfection. Relative expression was measured using *rp49* as an endogenous control. Pools of 2-10 flies were used in each experiment. Experiments were performed at least in triplicate. Error bars, ±SE. *$p<0.05$.

Figure S3

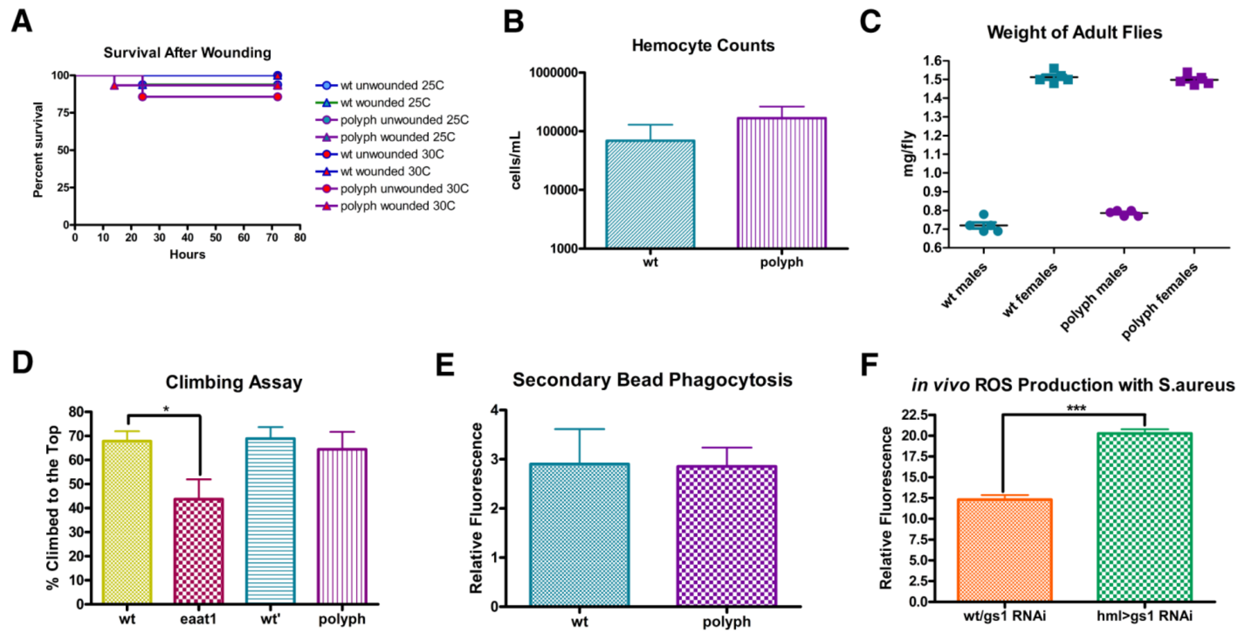

Figure S3: *polyph* flies are robust, normal sized, and phagocytose latex beads normally.

(A) Representative survival curve of wt and *polyph* flies after either no intervention or sterile wounding. Flies were either kept at the normal 25°C or at the stress-inducing temperature 30°C. n = 12-16 flies. Experiment was performed in triplicate. (B) Single wt or *polyph* larvae were bled into 20µL PBS and 10µL of the hemolymph was loaded onto a hemocytometer. n = 20. (C) Flies were collected from crosses of 10 females and 10 males. Five groups of 10 age-matched flies of each sex from each genotype were weighed on weigh paper. Weight was adjusted to mg/fly. (D) A vial containing 30 wt (genetic background control for *eaat1* flies), *eaat1*, wt' (genetic background control for *polyph* flies), or *polyph* flies was gently tapped on the bench, knocking the flies to the bottom of the vial. The number of flies that reached the top inch of the vial within 30 seconds was recorded. Experiment was done in triplicate. (E) Quantification of the phagocytosis of red fluorescently labeled latex beads in wt and *polyph* flies after a 30 minute preinjection of yellow fluorescently labeled latex beads. Approximately 6 flies per genotype were used in each experiment. (F) Quantification of oxidized CM-H2CDFDA-derived fluorescence in wt/*gs1* RNAi and *hml>gs1* RNAi flies after a 30 minute preinjection of overnight culture of *S.aureus*. Approximately 8 flies were used per genotype in each experiment. Error bars, ±SE. *$p<0.05$, ***$p<0.001$

Supplemental Experimental Procedures

Experimental Methods

Fly Stocks

The following stocks were from the Bloomington Drosophila Stock Center: w^{1118}; Mi[ET1]CG12943^{MB02238}, y^{1} w^{67c23}; P{EPgy2}Eaat1^{EY20741}, y^{1} w^{1118}; P{UAS-Eaat1.Exel}3/TM6B, Tb^{1}, y^{1} w^{67c23}; ry^{506} P{SUPor-P}gb^{KG07905}, y^{1}v$^{1;}$ P{TRiP.HMS02002} attp40 (II) RNAi Gs1 . w^{1118} flies were used as background control for *polyph* experiments and y^{1}w^{67c23} flies were used in *eaat1* and *gb* experiments. The Vienna Drosophila RNAi Center provided the polyph RNAi line w^{1118}; P{GD421} and the isogenic control line w^{1118}. The blood cell-specific driver flies, hemlΔGAL4, were received from Bloomington.

The *polyph* rescue construct was made by amplifying *polyph-RA* from the Drosophila Genomics Resource Center plasmid IP11938 using a forward primer containing a BglII site: CCGAGATCTATGGAGCCAAAGTCGCAGGATCAGGCT, and a reverse primer containing a XhoI site: CCGCTCGAGTTACATACTGTTGAGCGTCAACTGAAA. The Flag tag, MDYKDDDDK, was added to the C-terminus of *polyph* by PCR using the above forward primer and the following reverse primer, which also contains a XhoI site: CCGCTCGAGTTATTTATCGTCATCGTCTTTGTAATCCATCATACTGTTGAG. Products were cloned into the pCR 2.1 cloning vector (Invitrogen). Plasmids were transformed, following manufacturer's instructions, into TOP10 Chemically Competent *E.coli* Cells (Invitrogen). Constructs were isolated using the QIAprep Spin Miniprep Kit (Qiagen), according to manufacturer's instructions, sequenced, and then cloned into a pUAST vector. The constructs were transformed as above. The Plasmid Midi Prep Kit (Qiagen) was used according to manufacturer's instructions to isolate and purify the final constructs which were transformed into *Drosophila* embryos (BestGene).

Phagocytosis

To assay *S.aureus* phagocytosis in adults, approximately six flies with an equal distribution of females and males per genotype per experiment were injected with ~0.2µL of 1 mg/mL fluorescein-labeled heat or chemically killed *S.aureus* bioparticles (Invitrogen) using a Pneumatic Picopump Injector (World Precision Instruments). They were then incubated for 30 minutes at room temperature, injected with Trypan Blue, and mounted ventral side down and wings spread to the side onto black electrical tape. Flies were treated as above for the phagocytosis of fluorescein-labeled *E.coli* and zymosan bioparticles (Invitrogen) with the exception that flies were incubated for 10 minutes after the first injection. The 0.2µm Red Fluorescent Carboxylate Modified FluoSpheres (Invitrogen) were injected at a 1:20 dilution. Flies were injected with Trypan Blue after 10 minutes. Fluorescently labeled particles were visualized using either a GFP or Texas Red filter on the Discovery.V8 SteREO Microscope

(Zeiss). AxioVisionLE software was used to quantify the results. Relative fluorescence calculated as: $[\text{fluorescence}]_{\text{dorsal vein area}}/[\text{fluorescence}]_{\text{adjacent area}}$.

To assay secondary phagocytosis of beads, flies were first injected with approximately 0.04µL of one of the following: PBS, overnight culture of *S.aureus* resuspended in PBS, or 1.0µm Yellow/Green Fluorescent Carboxylate Modified FluoSpheres diluted 1:20 (Invitrogen). Flies were incubated at room temperature for 30 minutes, then injected with approximately 0.2µL of 1.0µm Red Fluorescent Carboxylate Modified FluoSpheres diluted 1:20 (Invitrogen), incubated at room temperature for 10 minutes, injected with Trypan blue, and then mounted and visualized as described above.

To assay the effect of THA on phagocytosis, 30 w^{1118} larvae were bled into 400µL of Schneider's media (Gibco). The carcasses were allowed to rest for one minute before they were removed. In microcentrifuge tubes, 90µL of the diluted hemolymph was then supplemented with sterile PBS or THA (Sigma-Aldrich) at a final concentration of 5mM, which is the approximate concentration of glutamate in Schneider's media Fluorescein-conjugated *S.aureus* bioparticles were added at a final concentration of 16µg/mL. Samples were incubated in the dark for 30 minutes before being pipetted onto polylysine coated coverslips (Fisher) where cells were allowed to attach for one minute. Excess liquid was aspirated, and 100µL of Hoescht (10µg/mL) was added for two minutes. Coverslips were then washed twice with PBS, mounted with PBS, and immediately visualized with a Leica DM RB Microscope. The number of bioparticles per cell was recorded for approximately 20 cells per treatment group per experiment. Experiments were performed in triplicate.

To assay the effect of amino acids on phagocytosis, 10 w^{1118} larvae were bled into 100µL minimal media (Gibco) supplemented with nothing, 20mM glutamate (Sigma-Aldrich), 0.417mM cystine (Sigma-Aldrich), or both. Schneider's media contains 0.417mM cystine. Wildtype larval hemolymph contains approximately 2mM glutamate,[23] therefore adding 20mM of glutamate creates a high extracellular glutamate environment. Fluorescein-conjugated *S.aureus* bioparticles were added at a final concentration of 16µg/mL and samples were processed as described above.

Survival Following Infection

S.aureus was cultured in LB broth at 37ºC shaking at 225 rpm. *L.monocytogenes* was cultured in BHI broth statically at 37ºC. Cultures were spun down and cells were resuspended in sterile PBS at the following ODs: *S.aureus,* OD = 0.5; *S.aureus,* OD = 0.05 for *gb* and *eaat1* survival curves; *L.monocytogenes*, OD = 0.1.

Flies were injected with 0.04µL of the bacterial resuspension. Except when specifically noted otherwise, groups consisted of equal numbers of males and females. Flies were kept at 25°C for *S.aureus* infections and 29ºC for *L.monocytogenes* infections, transferred regularly to new food,

and death was monitored daily. None of the fly lines used in survival showed significant death after injection with sterile PBS. All survival curves were done, at minimum, in triplicate.

Reverse Transcriptase Quantitative PCR

To collect RNA from larval blood cells and carcasses, approximately 10 larvae were carefully lacerated with tweezers on their anterior end in 100µL of nuclease-free water. Carcasses were allowed to rest in the water for an additional minute before being homogenized in STAT-60 (Tel-Test, Inc.). To collect RNA from adult flies, 2-10 animals were anesthetized with CO_2 and homogenized in STAT-60. Once samples were in STAT-60, RNA was harvested from all homogenized samples per manufacturer's instructions. Except when specifically noted otherwise, equal number of males and females were used.

The concentration of RNA was measured using the Nanodrop 1000 (Thermoscientific). Between 100 and 500 ng of RNA was then used to make cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas) according to manufacturer's instructions.

To measure AMPs, flies were injected with ~0.04µL of an overnight culture of *S.aureus* or *E.coli* as indicated. Flies were collected at 6 hours postinfection to measure *diptericin* and 24 hours post-infection to measure *drosomycin*. AMPs were measured using LUX-based qPCR and ROX qPCR Mastermix (2X) (Fermentas) on a 7300 Real Time PCR System (Applied Biosystems). Relative quantification was calculated via the $2^{-\Delta\Delta Ct}$ method using RP49 as the endogenous control. Experiments were run at least in triplicate and were analyzed by one-tailed paired t-tests.

To measure *polyph*, *gb*, and *eaat1* expression infected flies were collected 6 hours after injection with 0.04µL of an overnight culture of *S.aureus*. Gene expression was measured using SYBR-based qPCR with the Maxima® SYBR Green/ROX qPCR Mastermix (2X) (Fermentas). Each sample was measured in technical triplicate or quadruplicate.

Immunostaining

Approximately 5 hmlΔGAL4>*polyph-flag* larvae were bled into cold PBS on a polylysine coated coverslip. The carcass was removed after five minutes to allow the blood cells to attach to the coverslip. Excess liquid was removed and cells were fixed in 4% formaldehyde, washed, blocked, and incubated with a 1:500 dilution of a mouse anti-Flag antibody (Sigma) overnight. Cells were then incubated with a 1:200 dilution of the goat α-mouse secondary antibody (Invitrogen) for 2 hours, washed, and mounted in Prolong (Invitrogen). Slides were sealed and incubated at 4°C for at least 24 hours. The LSM 710 confocal microscope (Zeiss) was used for visualizing the cells.

Bacterial Load

S.aureus was grown overnight in a shaking incubator at 37°C, subcultured to an OD of 0.9-1.0, and resuspended in sterile PBS to an OD of 0.5. Approximately 24 flies with equal distribution

of males and females per genotype per experiment were injected with 24nL of the bacterial suspension. Eight flies from each group were then immediately homogenized in individual tubes with 100µL LB + 1% Triton X-100, serially diluted 1:10 (twice) in sterile PBS, and plated in triplicate on LB plates. After 18 and/or 24 hours of infection at 25°C, eight additional flies from each genotype per timepoint were assayed as above with the exception that each sample was serially diluted 1:10 four times. Bacterial colonies were counted after the plates were incubated for 24 hours at 37°C.

In vivo **ROS**

Between 6-8 flies with equal distribution of males and females per genotype per experiment were injected with ~40nL of sterile PBS or *S.aureus* cultured overnight and incubated at room temperature for 30 minutes. Flies were then injected with 0.2µL 1mM CM-H2CDFDA (Invitrogen) diluted in DMSO and incubated in the dark at room temperature for 10 minutes. They were mounted ventral side down and wings spread to the side onto black electrical tape and visualized using the GFP filter on the Discovery.V8 SteREO Microscope (Zeiss). AxioVisionLE software was used to measure fluorescence in a polygon drawn in the dorsal vein area of the injected flies, which was then divided by the autofluorescence measured in the same polygon on an uninjected fly. Experiments were done at least three times.

Ex vivo **ROS**

Thirty larvae of the appropriate genotype were bled into 400µL Schneider's media, carcasses were allowed to rest for one minute before being removed. The diluted hemolymph was divided into two wells in a Costar 96-well black clear bottom plate (Fisher Scientific) with 98µL per well. Wells were treated with 2µL PBS or 1µL *S.aureus* cultured overnight + 1µL 1mM CM-H2CDFDA diluted in DMSO. The SpectraMaxM2 Plate Reader (Molecular Devices) was used with an excitation wavelength of 485 nm and an emission wavelength of 538 nm to measure the fluorescence at 0, 30, and 90 minutes. Relative fluorescence was calculated by first subtracting the fluorescence of the PBS-treated cell from the fluorescence measured in the cell treated with *S.aureus*. Then, to normalize for any difference in cell numbers, the number was divided by the fluorescence of the PBS-treated cells at 0 minutes. Experiments were done in triplicate.

Physiological Experiments

To count blood cells, single larvae of the appropriate genotype were bled into 20µL PBS. The carcasses were removed after 1 minute and 10µL of the diluted hemolymph was loaded onto a hemocytometer. n = 20.

To measure fly weight, flies were collected from crosses of 10 females and 10 males to avoid overcrowding issues affecting growth. Five groups of 10 flies of each sex from each genotype were weighed on weigh paper. The weight was then adjusted to mg/fly.

To measure the activity of the nervous system, thirty flies with equal distribution of males and females of the appropriate genotype were placed in a clean fly vial. The vial was gently tapped on the bench in order to knock all of the flies to the bottom. The number of flies that reached the top inch of the vial within 30 seconds was recorded. The experiment was done in triplicate.

Statistical Tests

Log rank tests were used to determine whether survival curves were significantly different from one another. One-tailed t-tests were performed on all other types of data presented. $p<0.05$ was determined to be statistically significant.