

# Supporting Information

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## SI Text

**Fluorescent Marker Measurements** Upon DAPI and/or antibody staining, dissected intestines were mounted on microscope slides and confocal microscopy images of the posterior part of the midgut were acquired using 40× lenses plus 2× digital zoom. At least four intestines, corresponding to a total of >700 cells, were measured for each condition. All cells were manually enumerated using Microsoft Photoshop and the percentage of GFP, LacZ or antibody positive cells were calculated as the number of dye positive cells per intestine, multiplied by 100 and divided by the number of DAPI positive nuclei per intestine.

**Bacterial Clearance** Infected flies were shifted to conventional fly food containing the antimicrobials methyl paraben (Tegosept) and propionic acid for 5 days at 25 °C. Bacteria were completely absent from the gut within 2 days following transfer to normal food.

**Statistical Analysis** For the statistical evaluation of pairwise differences in intestinal width and percentage of cells expressing molecular markers, the two-sided Student's *t* test was used. Variation error is shown by the standard deviation from the mean. For all fly survival assays comparisons were made between isogenic flies, and curve differences were evaluated using the Kaplan-Meier method using the long-rank test (MedCalc). Tests were repeated at least twice with similar results.

**Bacteria Culture and Secreted Factor Preparation** *P. aeruginosa* strains PA14 and CF5 and *E. coli* strain DH5 $\alpha$  were grown on Luria Bertani (LB) media to OD<sub>600 nm</sub> 3, which corresponds to a concentration of  $5 \times 10^9$  bacteria/mL. Cell-free supernatants of strain PA14 (1) were prepared by further centrifugation and filtering of the supernatant with 0.2- $\mu$ m filters. Pyocyanin was prepared by dilution of stock solution (Cayman Chemicals) in water at a concentration of 50 mg/mL. Fresh cell-free supernatants and pyocyanin solution were prepared daily for 5 days and were added in rayon balls. Strain CF5 was originally described in Wolfgang et al. (2).

**Infection Assay** Flies were transferred to vials containing a cotton ball impregnated with 5 mL of a solution composed of 10% LB medium, 4% sucrose, with or without bacteria ( $5 \times 10^8$ /mL). Bacterial loads in the cotton ball remained constant over time. Fly mortality was assessed daily until all flies died ( $\approx$ 30 days).

**Bacterial Counts** Bacterial counts in different parts of the intestine were assessed as follows: flies were decapitated, briefly soaked in pure ethanol, dried, and the guts were dissected out on silicon

plates. Anterior and posterior midgut, as well as hindgut, were split with forceps and the different parts were ground separately in tubes with a pestle. Serial dilutions of the lysate were plated on LB media and plates were incubated at 37 °C and colonies were counted 14 h later.

Systemic presence of bacteria in ethanol-washed flies was assessed via either the aspiration of hemolymph using a Pasteur pulled-glass pipet or the dissection of the thorax in a drop of PBS solution. The hemolymph sample or the PBS eluate were plated on LB and cultured. We did not detect systemic bacteria 5 days after infection with either of these two methods.

**Pyocyanin Measurements** PA14 and CF5 5 mL cultures were spun down and the supernatants were mixed with equal volumes of chloroform. The lower blue organic phase was collected and mixed with 5 mL of HCl (0.2 N). The upper reddish phase was collected and its OD<sub>520 nm</sub> was measured. The concentration of pyocyanin was determined by the formula: mg/L = OD<sub>520nm</sub>  $\times$  17.072 normalized to cell counts and the statistical significance was assessed using the Student's two-tailed *t* test assuming equal variance (3). The experiments were carried out in duplicate.

**Measurements of Gut Diameter** To minimize random differences in gut diameter, the gut of female flies of similar size, grown in similar culture densities, in parallel, were fixed for 20 min in 4% formaldehyde, stained with DAPI and mounted on a microscope slides using 35  $\mu$ L of either 80% glycerol in PBS or vectashield (Vector Labs). The maximal diameter of the most posterior part of the midgut (magnification,  $\times$ 40 frame) was calculated by conversion of the gut diameter in the image using the magnification bar assigned by the microscope camera.

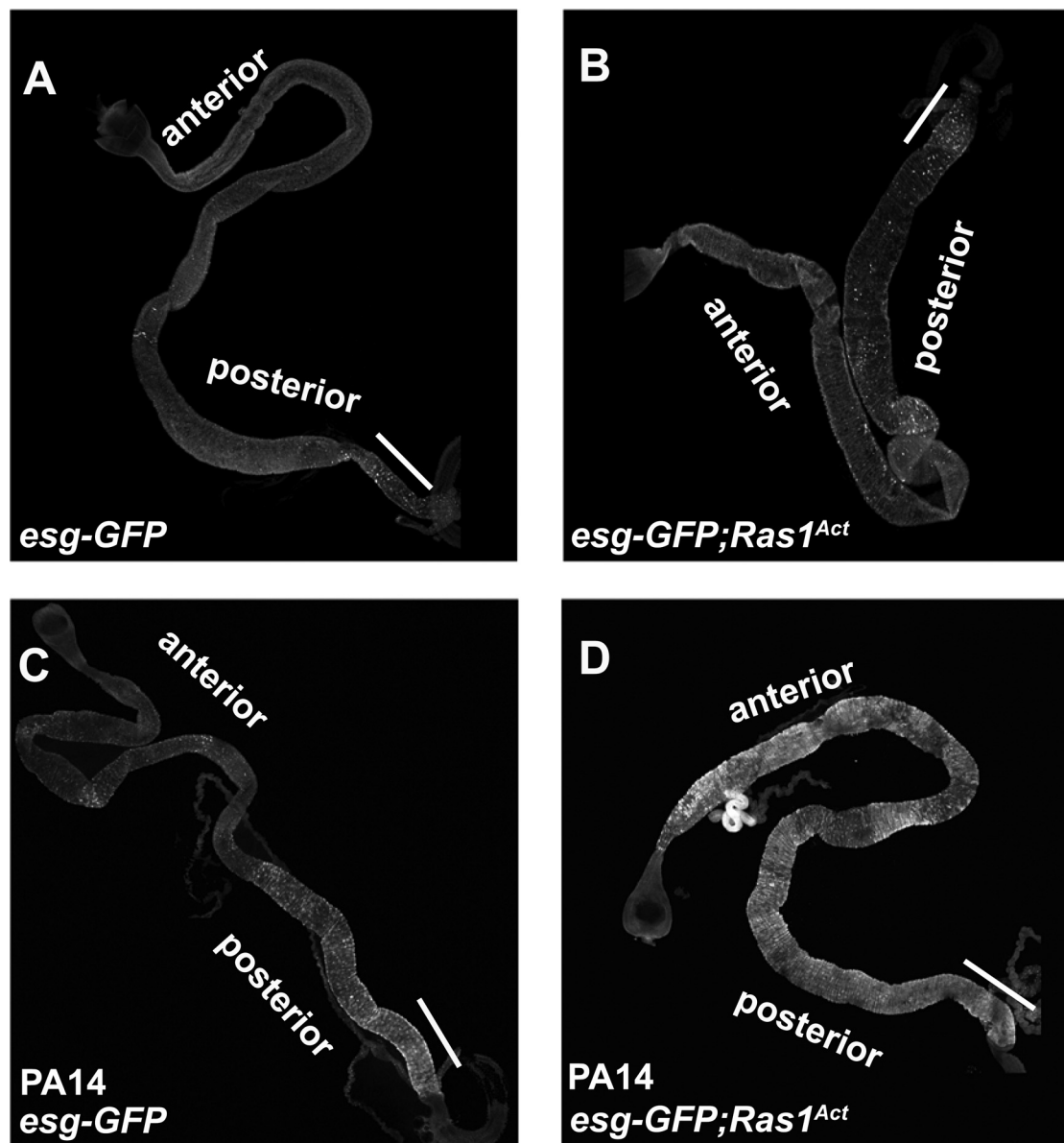
**Defecation Rate and Measurement of Inner Gut Diameter** Five days after infection, flies were transferred to freshly prepared cornmeal containing 0.5% of Brilliant Blue FCF (Arioglaucine, Sigma) for 8 h, then transferred to fresh vials containing 1 mL of the same blue food. Blue spots on the vial wall produced within 24 h at 25 °C were divided by the number of flies in the vial (four to five flies). For inner diameter measurements, flies were fed on blue food for 24 h and their guts subsequently dissected in PBS under a stereoscope. Pictures were taken immediately following dissection to minimize dye diffusion.

**Acridine Orange Staining** Dissected guts were incubated for 5 min in a freshly diluted solution (5  $\mu$ g/mL in PBS solution), washed rapidly in PBS, mounted in a microscope slide, and monitored immediately via confocal microscopy (488 nm excitation and 500–530 nm detection).

1. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM (1995) Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268:1899–1902.  
2. Wolfgang MC, et al. (2003) Conservation of genome content and virulence determinants among clinical and environmental isolates of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 100:8484–8489.

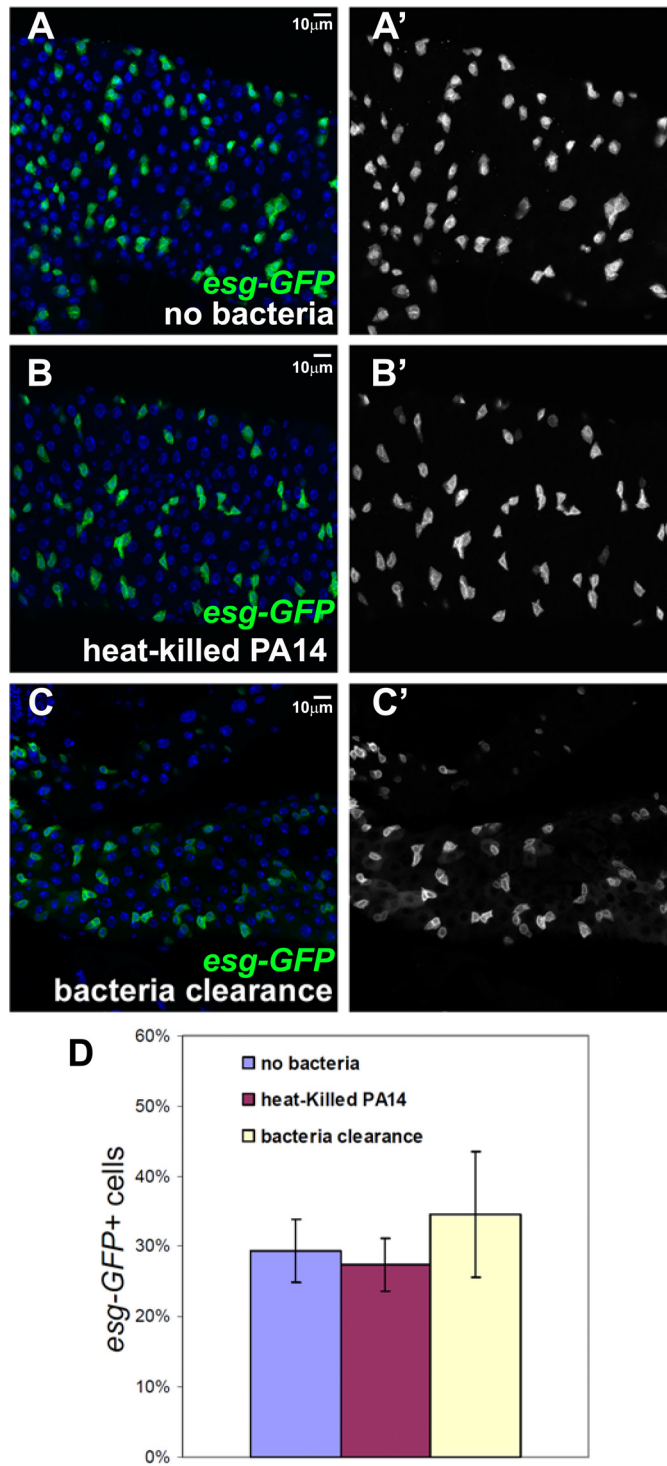
3. Essar DW, Eberly L, Hadero A, Crawford IP (1990) Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: Interchangeability of the two anthranilate synthases and evolutionary implications. *J Bacteriol* 172: 884–900.



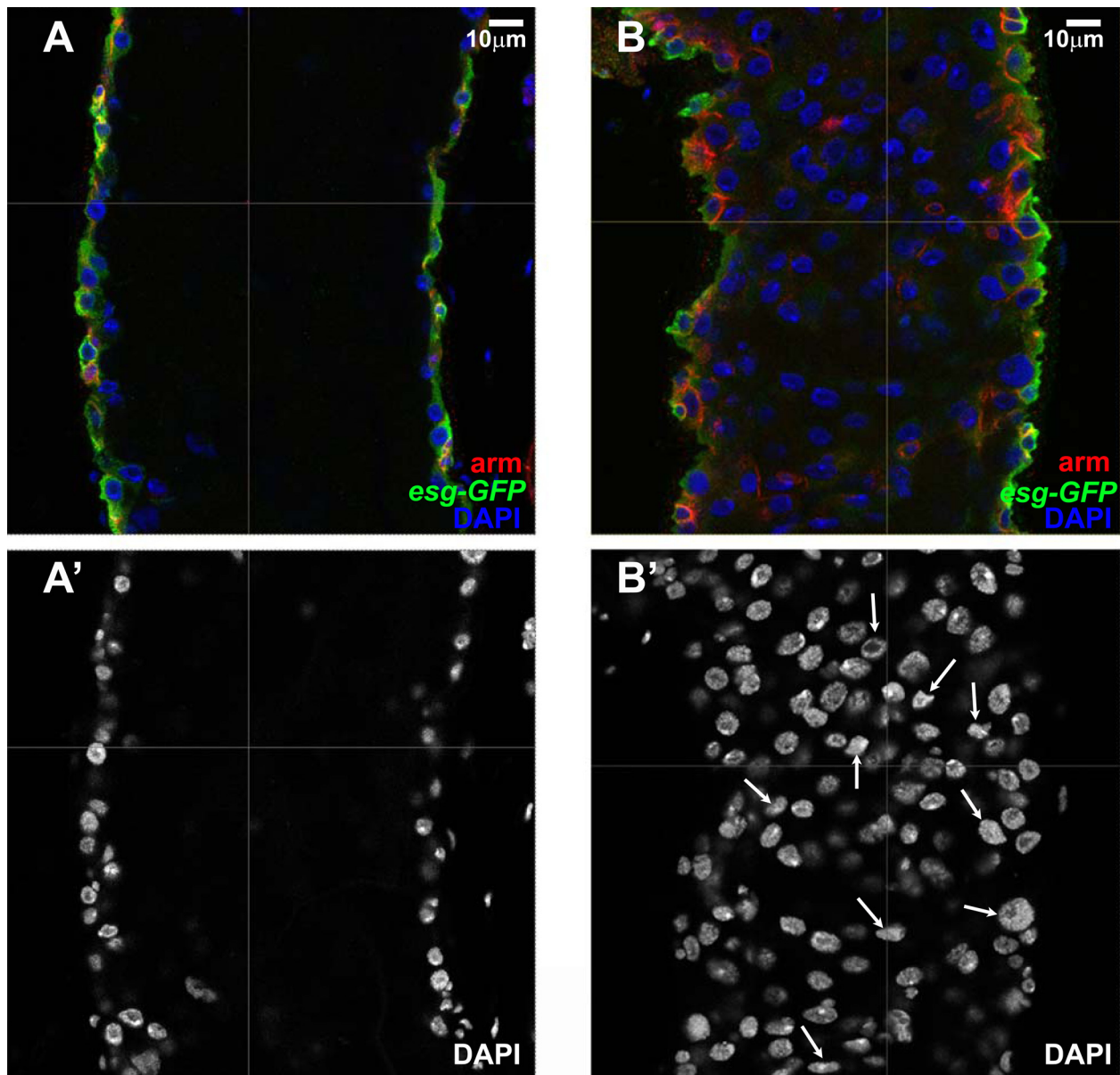


**Fig. S2.** Progenitor cell population is increased by infection and is further enhanced in a *Ras1<sup>Act</sup>* background. Control *tub-GAL80<sup>ts</sup> esg-GAL4 UAS-GFP* gut (A and C) and *tub-GAL80<sup>ts</sup> esg-GAL4 UAS-GFP UAS-Ras1<sup>Act</sup>* (B and D) flies, uninfected (A and B) or PA14-fed for 5 days (C and D). The GFP signal (white), while predominantly restricted to the posterior midgut in (C), extends anteriorly in (D). Posterior-most midgut selectively shown in all other figures is delineated by white lines.





**Fig. S4.** Flies feeding on heat-killed PA14, or cleared from PA14 infection, exhibit normal SC numbers. (A–C) Posterior midgut cell nuclei marked with DAPI [blue in (A–C)] and SCs and progenitors marked with GFP [green in (A–C), white in (A'–C')] from uninfected *esg-GAL4 UAS-srcGFP* flies (A) or flies fed with heat-killed PA14 cells for 5 days (B) or cleared following PA14 infection by transfer of flies to food containing antimicrobials (C). (D) Percentage of *esg*<sup>+</sup> cells is comparable for all three experimental conditions.



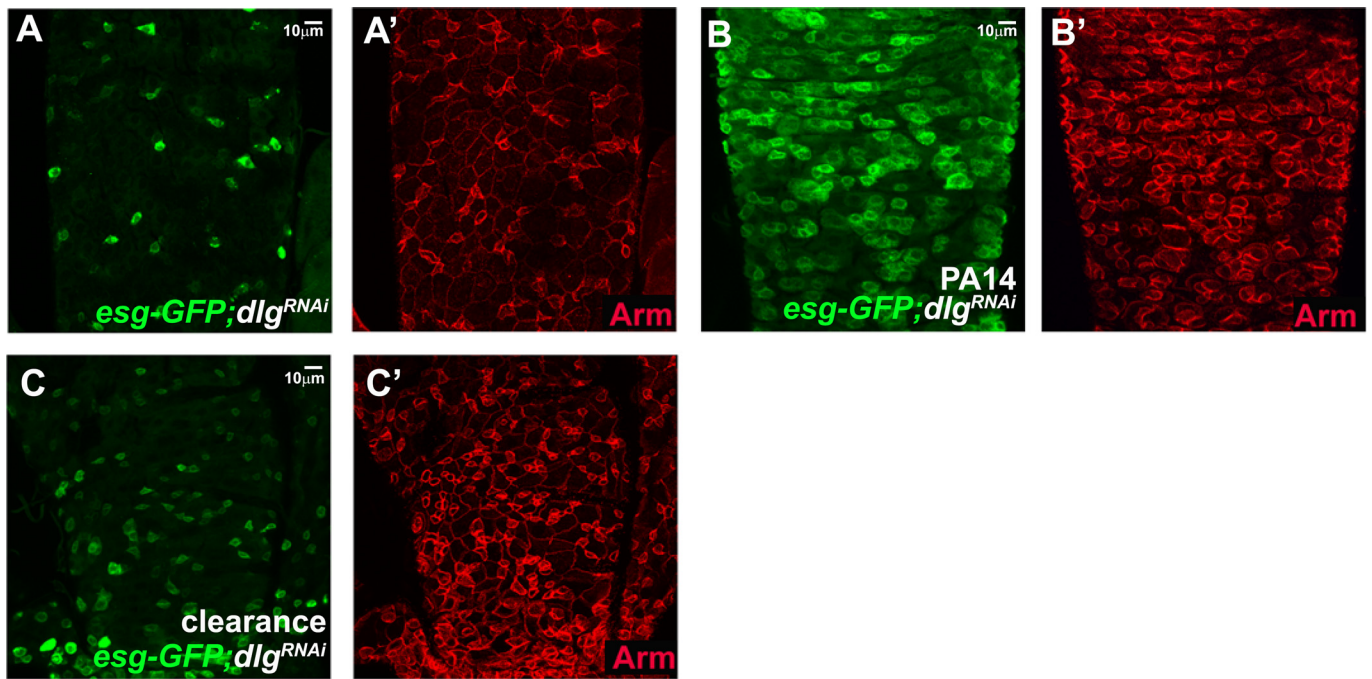
**Fig. S5.** Multilayer epithelium formation via cooperation of infection and *Ras1<sup>Acct</sup>* oncogene expression. Single confocal surface section in the middle plane of the posterior midgut of *tub-GAL80<sup>ts</sup> esg-GAL4 UAS-GFP* (A) and *tub-GAL80<sup>ts</sup> esg-GAL4 UAS-GFP UAS-Ras1<sup>Acct</sup>* (B) flies feeding on PA14 for 5 days. Green is GFP, red is Arm staining and blue is DAPI [white in (A' and B')]. The midgut of *Ras1<sup>Acct</sup>* infected flies becomes multilayered, while infected wt flies have a normal epithelium. Arrows in (B') indicate nuclei in the inner layers of cells with aberrant morphology. Horizontal and vertical gray lines demarcate respectively the regions from which the cross-sections (Fig. 4 G and H) and sagittal sections (Fig. 4 I and J) were acquired.











**Fig. S8.** Infection cooperates with *dlG<sup>RNAi</sup>* expression to induce SC and progenitor hyperplasia. (A–C) Posterior midguts of *tub-GAL80<sup>ts</sup> esg-GAL4 UAS-GFP UAS-dlG<sup>RNAi</sup>* feeding for 5 days on sucrose (A), PA14 (B), and after bacterial clearance for 5 days (C). *esg<sup>+</sup>* cells increase in number in the infected midguts and do not return to their original number after retraction. Green is GFP in (A–C) and red is Arm in (A'–C').