# Supporting Information

## Wang and Aksoy 10.1073/pnas.1116431109

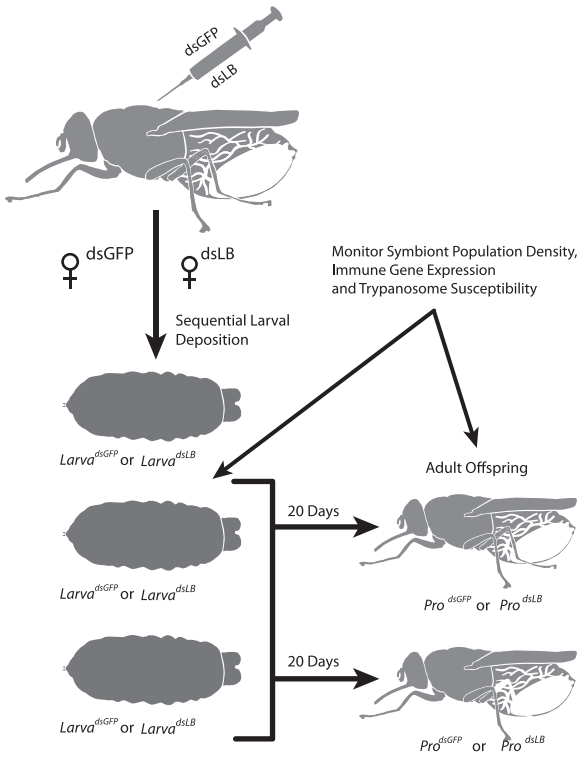


**Fig. S1.** Experimental scheme of dsRNA treatments and sample collection. Eight-day-old mated females were treated with dsGFP ($♀^{dsGFP}$) and dsLB ($♀^{dsLB}$) dsRNA (8 μg per fly), respectively, and monitored for pupal deposition of three gonotrophic cycles (GCs). Pupae from the second and third GCs were allowed to hatch. Larva ($Larva^{dsGFP}$ and $Larva^{dsLB}$) and 20-d-old adult progeny ($Pro^{GFP}$ and $Pro^{dsLB}$) of second and third GCs were collected for symbionts density and immune response test.

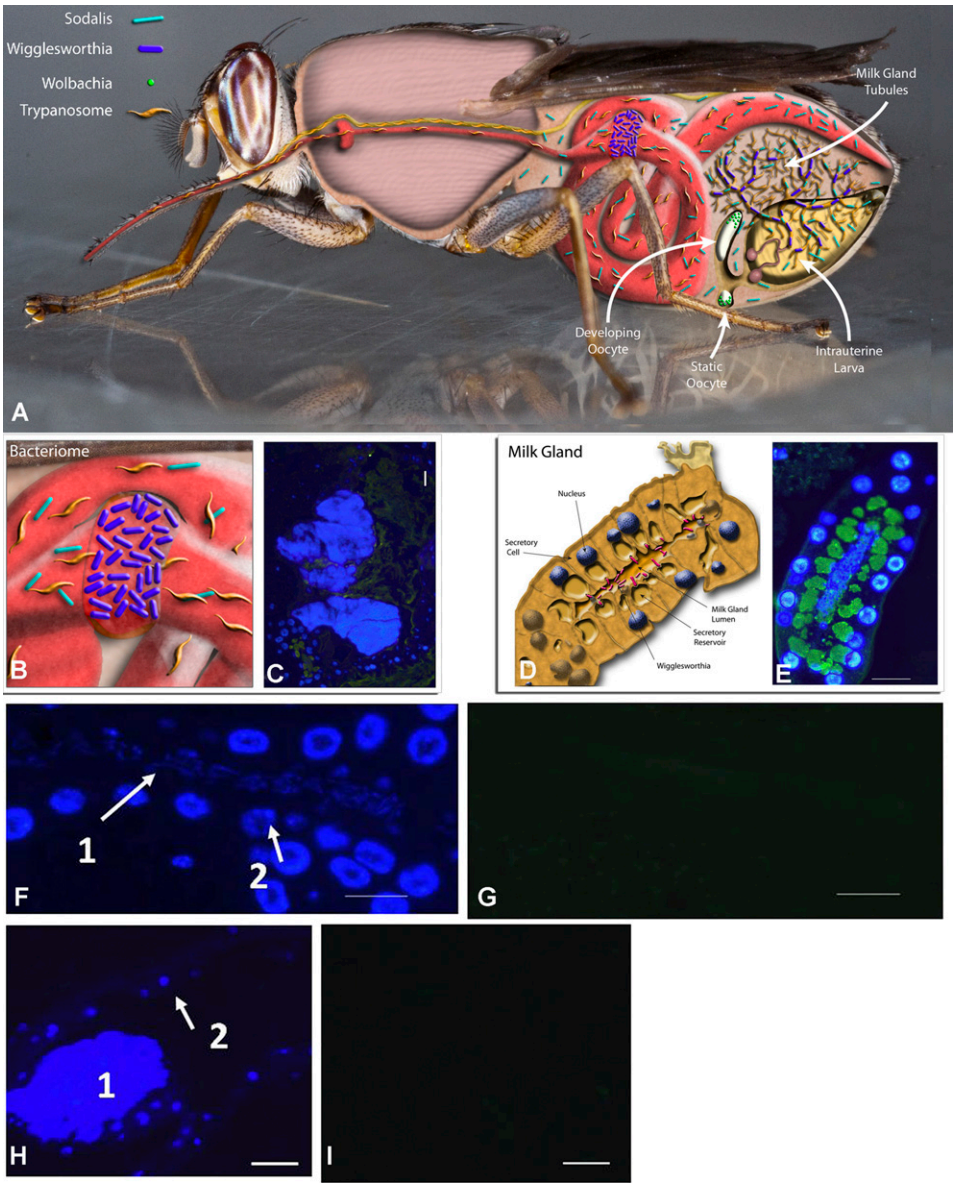


**Fig. S2.** Localization of PGRP-LB and symbionts in tsetse. (*A*) Schematic showing localization of tsetse's symbionts and trypanosomes as well as the intrauterine larva and milk tubules carrying the symbionts. (*B*) Schematic showing localization of *Wigglesworthia* in the gut bacteriome. (*C*) Immunohistochemical analysis showing PGRP-LB protein (in green) in the periphery of bacteriome and in the gut lumen; blue DAPI stains the host cell and bacterial nuclei. (*D*) A section through the milk gland tubule shows milk gland cells and *Wigglesworthia* localization in milk gland lumen. (*E*) PGRP-LB was detected in secretory reservoirs of milk gland cells and lumen. Green Alexa Fluor 488 stained PGRP-LB. Blue DAPI stains host cell nuclei as well as the bacteria in the lumen. (*F–I*) Immunohistochemistry controls. Sections were stained with preimmune sera and DAPI. (*F* and *G*) Milk gland. (*H* and *I*) Midgut sections. (*F*) Blue DAPI-stained nuclei of milk gland cells and bacteria in the lumen. (*H*) A section through the bacteriome organ. 1, symbionts; 2, host cell nuclei. (Scale bar: 25 μm.) (*G* and *I*) Sections stained with preimmune sera. To generate recPGRP-LB rabbit polyclonal sera, recPGRP-LB corresponding to bases 118–567 of *pgrp-lb* CDS expressed in pET-21b and gel-purified protein was used to generate the antibody commercially (Invitrogen).

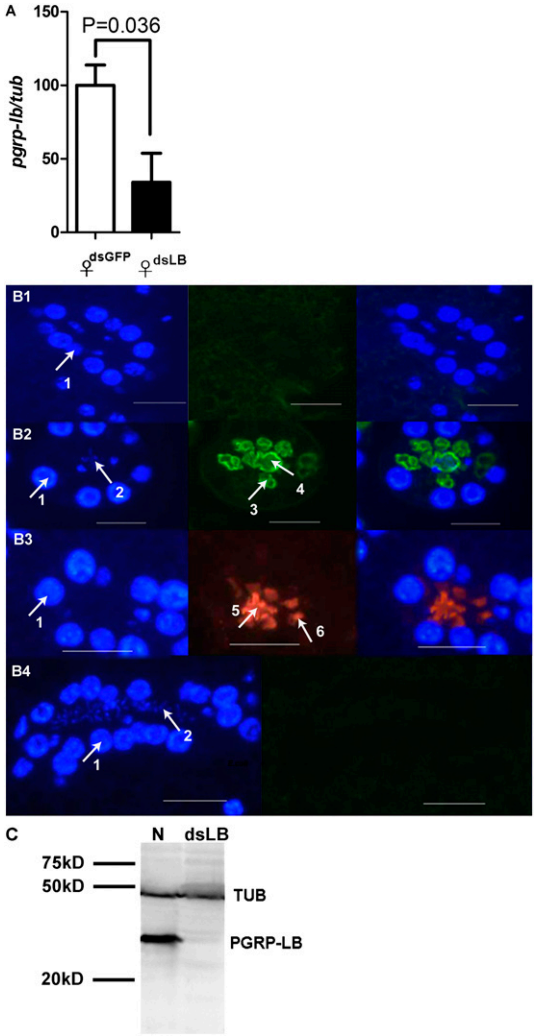


**Fig. S3.** Silencing PGRP-LB RNA and protein expression in milk gland tissue. (*A*) Silencing efficiency of *pgrp-lb* in milk gland tissue. For *pgrp-lb* levels, flies 3 d after eclosion were treated with dsRNAs and analyzed 15 d after treatment. Error bars indicate SEM ($n$ = 5). Around 60% of *pgrp-lb* transcripts were successfully knocked down in comparison with dsGFP controls. (*B*) For immunohistochemical staining of milk gland sections, tissues were prepared from dsLB-treated (*B1*) and dsGFP-treated (*B2*) females, respectively. Tsetse milk gland protein (GmmMGP) (*B3*) and preimmune sera (*B4*) were used as positive and negative controls, respectively. 1, host nuclei; 2, symbionts; 3, PGRP-LB in milk gland storage reservoir; 4, PGRP-LB in milk gland lumen; 5, GmmMGP in milk gland lumen; and 6 in storage reservoir. (Bar: 25 μm.) (*C*) Western analysis shows PGRP-LB from normal and dsLB-treated flies. Tsetse Tubulin antibody was used as control. Gene expression and Western blot analysis confirms the silencing effect we achieve with dsRNA treatments.

**Table S1. Primers used for dsRNA preparation, quantitative PCR, and recombinant protein expression**

| Primer name | Primer pair sequence |
|---|---|
| dsLB | F: 5′ TAATACGACTCACTATAGGGCAACAACAACCCAAAAGG 3′ |
| | R: 5′ TAATACGACTCACTATAGGGGAGTTGGTACTGCCGATGT 3′ |
| dsGFP | F: 5′ TAATACGACTCACTATAGGGTCAGTGGAGAGGGTGAAG 3′ |
| | R: 5′ TAATACGACTCACTATAGGCTAGTTGAACGGATCCATC 3′ |
| qGmmattA* | F: 5′ ATGCCAACCTCTTCAACGAC 3′ |
| | R: 5′ CGTAACCTAAGCCTCCACCA 3′ |
| qGmmtub-β* | F: 5′ CCATTCCCACGTCTTCACTT 3′ |
| | R: 5′ GACCATGACGTGGATCACAG 3′ |
| qPGRP-LB-5* | F: 5′ GATGTAAGCAAACGCCGC 3′ |
| | R: 5′ CAACACAAAAGCACAACATCCA 3′ |
| qthiC *Wigglesworthia** | F: 5′ AAGTTATGATAGAAGGACCAGGAC 3′ |
| | R: 5′ CCCGGAGCAATATCAGTAGTTAG 3′ |
| qfliC *Sodalis** | F: 5′ TGGGGACAGTACGATGGCAGAGC 3′ |
| | R: 5′ TCATAGGCGGTCGGGGATAATTGCG 3′ |
| q*tryptubulin** | F: 5′ GGCTTCAAGTGCGGTATC 3′ |
| | R: 5′ GTGGAGTTGGCGATCATG 3′ |
| LB(EcoRI)[†] | F: 5′GGGAATTCGGAAAAATTTATAGGCCCTTC 3′ |
| LB (XhoI)[†‡] | R: 5′ CGCTCGAGTGGCACGGGAGAAAAATGTGG 3′ |
| LB (BglII) [‡] | F: 5′ GGAGATCT ACGCCACTGCATAGCCGTATAATAG 3′ |
| PGRP-LB | F: 5′ CAACAACAACCCAAAAGG 3′ |
| | R: 5′ GAGTTGGTACTGCCGATGT 3′ |

F, forward; R, reverse.

*Quantitative primer set for gene expression or symbionts and trypanosome.

[†]Primers for protein expression in pET21b.

[‡]Primers for PGRP-LB expression in pMT/Bip/V5-His.