Supporting Information

Hughes et al. 10.1073/pnas.1408888111

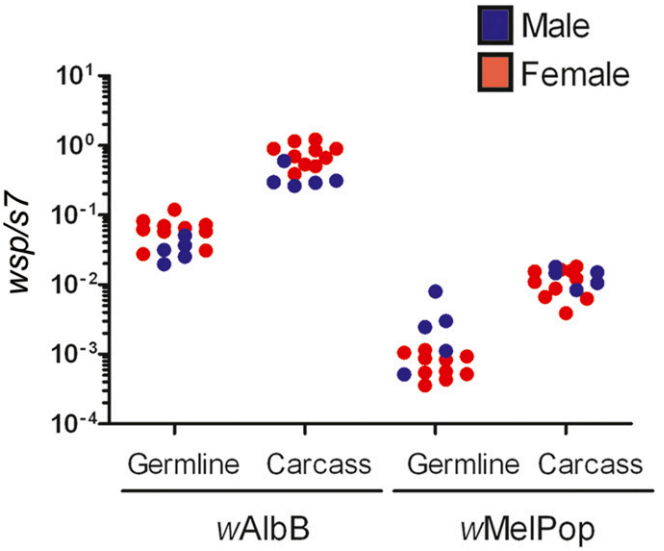

Fig. S1. Comparison of *Wolbachia* titers after intrathoracic microinjection into *Anopheles gambiae* males and females. *w*AlbB infected both ovaries and testes better than *w*MelPop ($P < 0.05$), and higher titers were observed in the carcass vs. the gonads for both strains ($P < 0.05$). No significant difference was observed between males and females in either the gonads or carcass within treatments. Data were analyzed by Kruskal–Wallis test using the Dwass procedure for multiple comparisons.

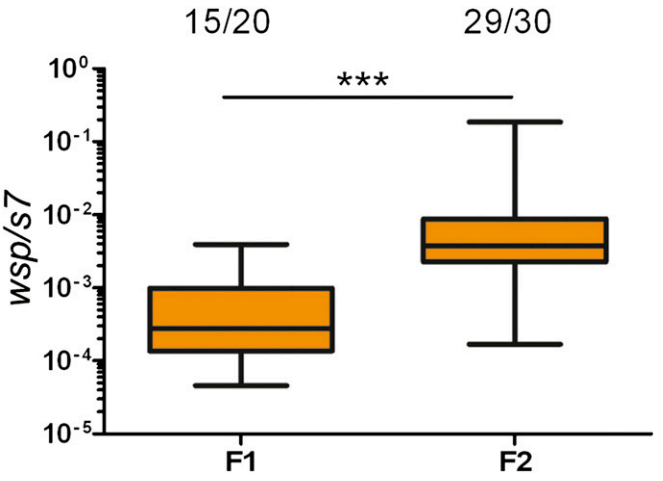

Fig. S2. Injection of *w*AlbB over multiple generations in antibiotic-treated *A. stephensi* significantly boosts both *Wolbachia* infection frequency (Fisher's exact test, $P = 0.03$) and titer (Mann–Whitney *U* test, $^{***}P < 0.0001$). Fractions represent the number of infected offspring over the total number. Box and whiskers represent data quartiles and range, respectively.

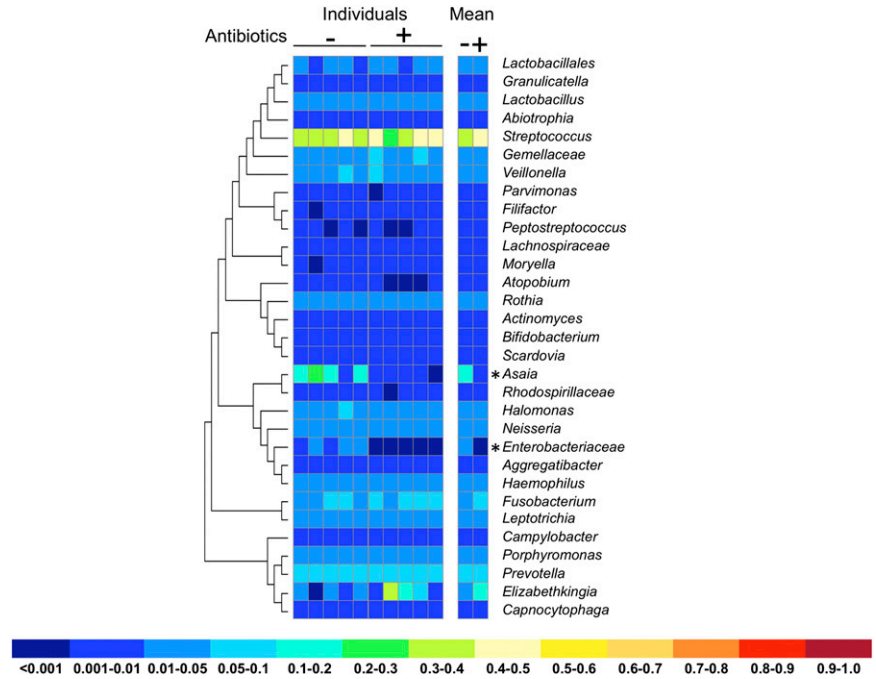

Fig. S3. Microbiome analysis of *A. gambiae* mosquitoes reared on conventional sugar (–) compared with those on an antibiotic mixture (+). Operational taxonomic units (OTUs) were grouped by genus (where possible) or higher rank, and relative abundance of bacterial taxa in individual samples calculated. The mean relative abundance per treatment is also shown. Asterisks denote the presence of OTUs within that taxon that significantly change in frequency between treatments (nonparametric *t* test with Monte Carlo simulation, $P < 0.01$). A maximum likelihood phylogenetic tree was constructed based on the alignment of representative nucleotide OTU sequences for each taxonomic group. A significant decrease in *Asaia* and members of the family *Enterobacteriaceae* following antibiotic treatment is observed.

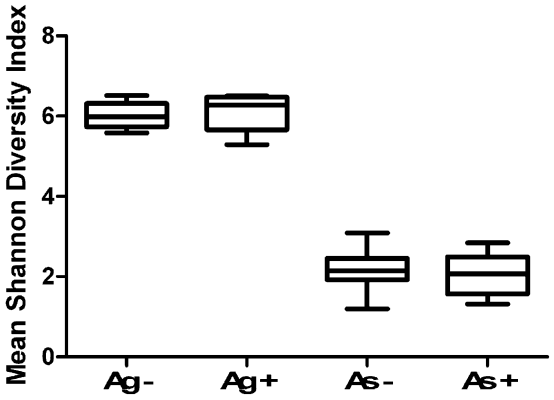

Fig. S4. Microbial diversity within treatments. Box plot of mean Shannon Diversity Index values by treatment for *A. gambiae* (Ag) and *Anopheles stephensi* (As). There was no significant difference in overall microbial diversity between antibiotic (+) and conventionally (–) reared *A. gambiae* (Mann–Whitney *U* test, $P = 0.69$) or *A. stephensi* (Mann–Whitney *U* test, $P = 0.60$). Box and whiskers represent data quartiles and range, respectively.

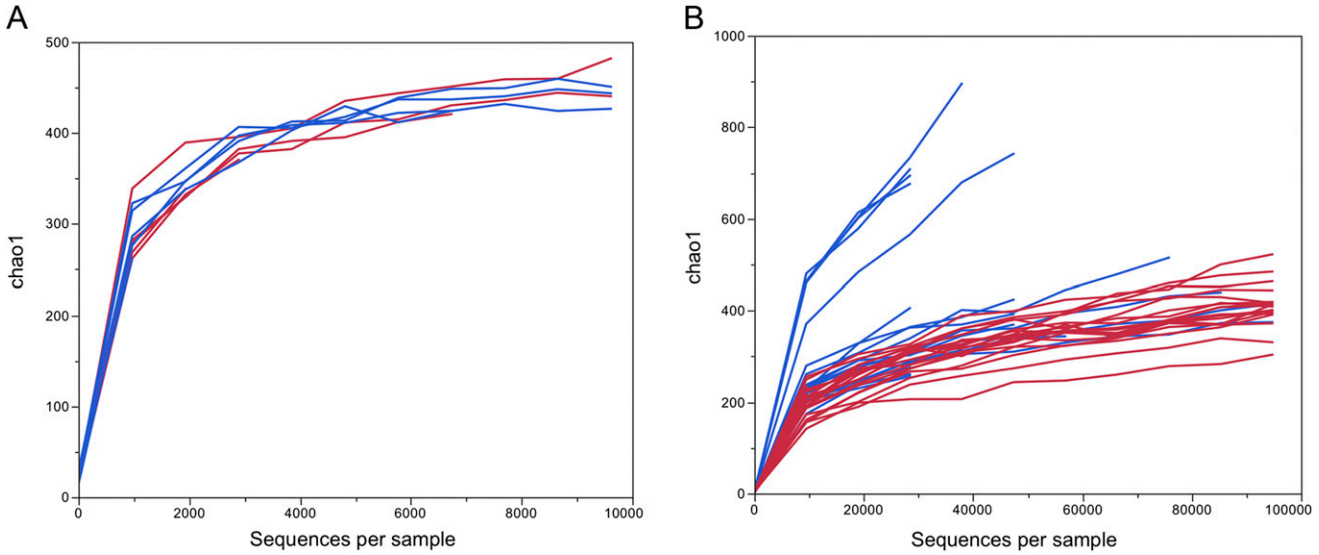

Fig. S5. Rarefaction curves showing within-sample OTU diversity (chao1) were generated for conventionally reared (red) and antibiotic-treated (blue) whole mosquitoes for (*A*) *A. gambiae* and (*B*) *A. stephensi*, with respect to sequencing depth. Note differing scale on the *y* axis. Only OTUs with more than five reads were retained for analysis.

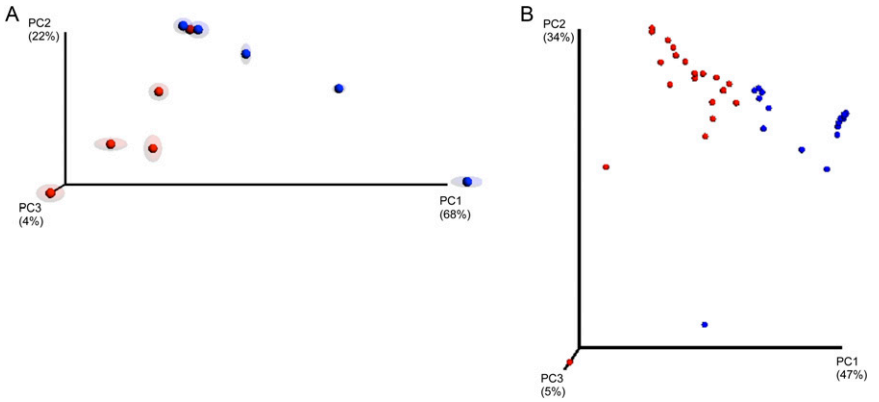

Fig. S6. Principle coordinate analysis (PCoA) plots. Three-dimensional PCoA plots to compare samples based on weighted Unifrac distance with jackknifed support for (*A*) *A. gambiae* and (*B*) *A. stephensi*. Samples are grouped by color, with those treated with antibiotics shown in blue and those without antibiotics in red.

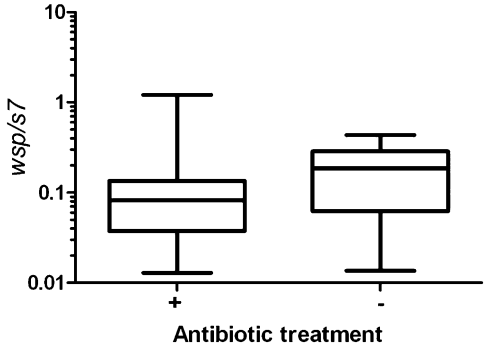

Fig. S7. Comparison of *w*AlbB density in the ovaries of antibiotic-treated (+) and conventionally reared (–) *A. stephensi* mosquitoes 12 d postinjection. No significant difference was observed (Mann–Whitney *U* test, $P = 0.1$). Box and whiskers represent data quartiles and range, respectively.

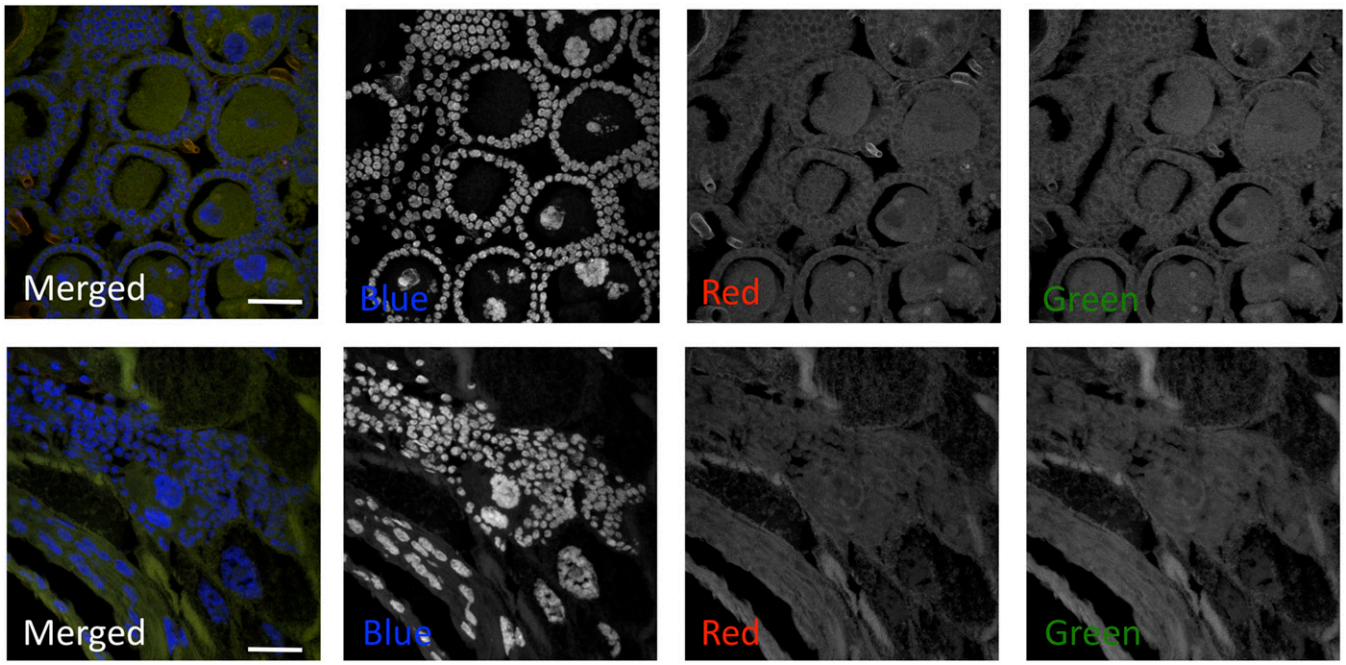

Fig. S8. No-probe (*Upper*) and uninfected mosquito (*Lower*) controls for FISH showing the merged image and individual channel (blue, red, and green). Blue denotes mosquito DNA. In the no-probe control, trachea surrounding the ovary display autofluorescence in the red channel. (Scale bars: 60 μm.)

Table S1. Mean OTU frequency (read count) for conventionally reared vs. antibiotic-treated *A. gambiae* and *A. stephensi* mosquitoes

Table S1

For each species, *16S* sequencing reads were clustered at the 97% similarity level against the 13_8 release of the Greengenes *16S* database (1) into OTUs (*Materials and Methods*). OTUs that significantly ($P < 0.05$) changed in frequency between treatments are highlighted in bold. Significance was calculated by a nonparametric *t* test with Monte Carlo simulation (implemented in Quantitative Insights into Microbial Ecology) (2).

1. DeSantis TZ, et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72(7):5069–5072.
2. Caporaso JG, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7(5):335–336.