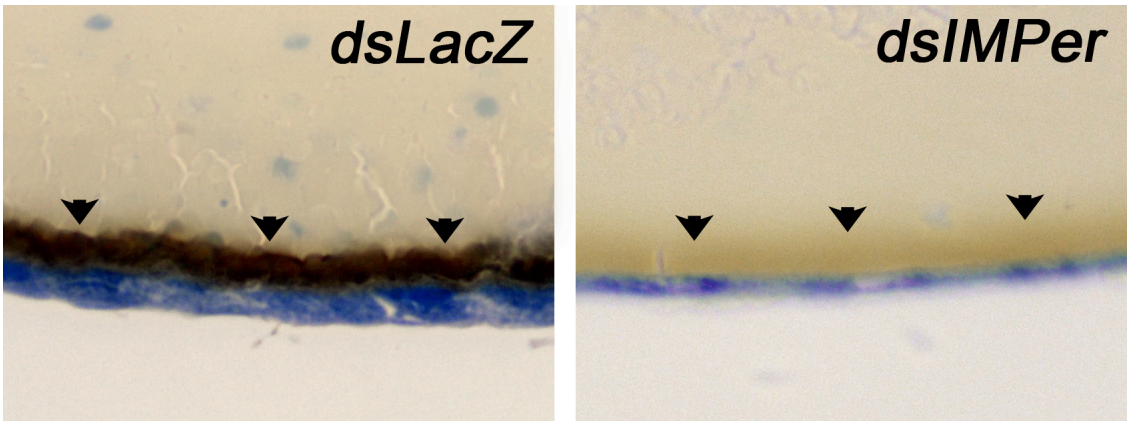

Fig S1. Effect of IMPer silencing on midgut peroxidase activity detected using 3,3'-diaminobenzidine (DAB) staining in midgut sections 12 h after feeding. Arrowheads indicate DAB staining**.** Epithelial cells were counterstained with toluidine blue.

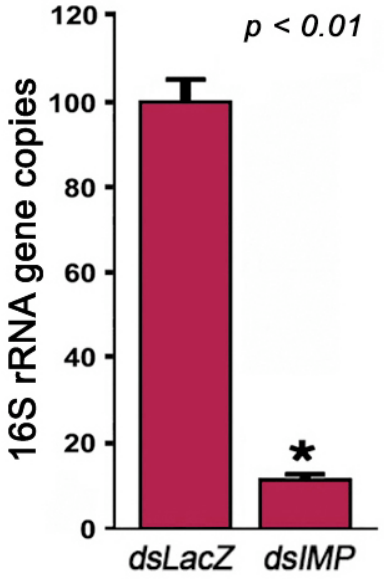

Fig S2. Effect of IMPer silencing on bacterial 16S rRNA gene copies in groups of midguts collected 24 h after feeding (mean ± SEM).

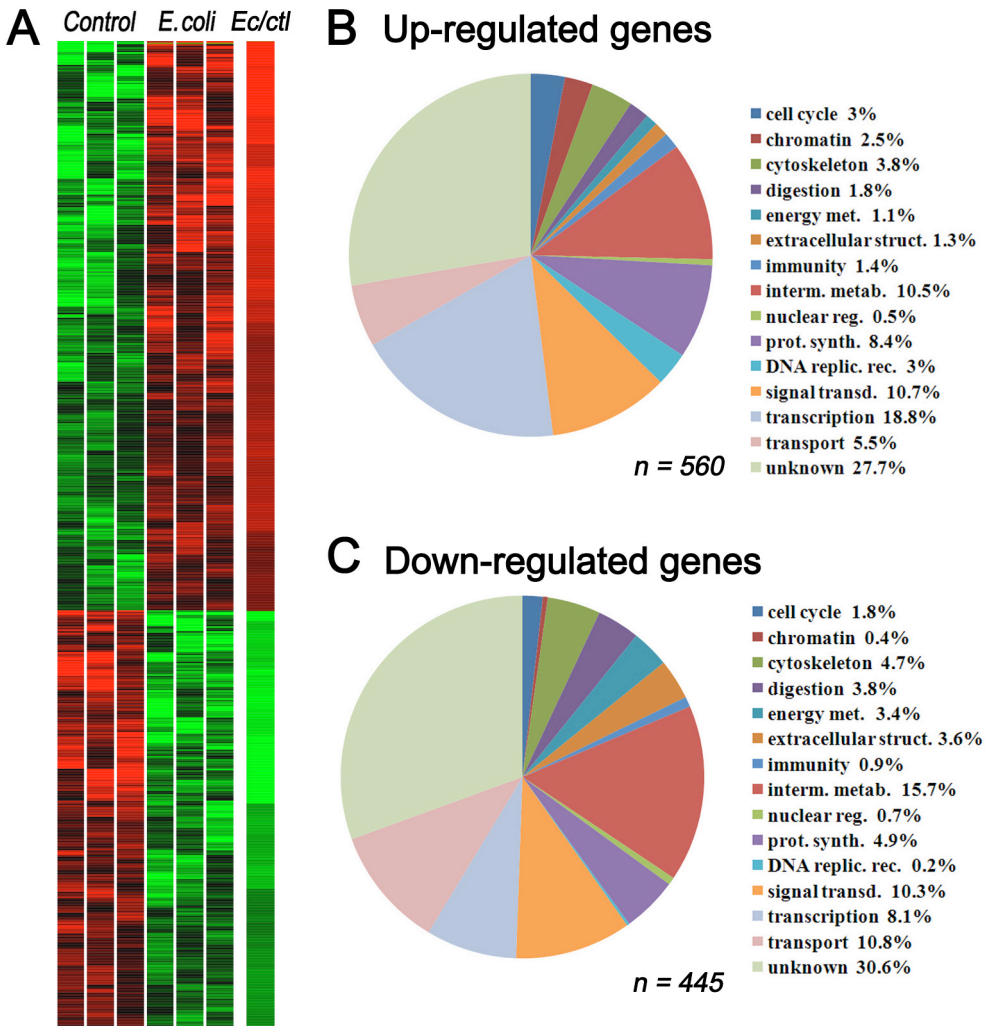

Fig. S3. Microarray Analysis of midgut mRNA expression in response to an artificial meal containing *in vitro* cultured bacteria. *Anopheles gambiae* G3 mosquitoes (5-6 day-old) were fed a BSA solution with or without (Control) heat-killed *Escherichia coli*. Mosquito midguts were dissected 3h post-feeding. (**A**) Hierarchical clustering based on the signal intensity of three biological replicates for Control and *E. coli*-fed mosquitoes and ratio of *E. coli* fed/Control (*Ec/ctl*) expression. Upregulated genes appear in red and those downregulated in green. (**B**) Graphic representation of gene categories that are upregulated in response to *E.* coli. (**C**) Graphic representation of gene categories that are downregulated in response to *E. coli.*

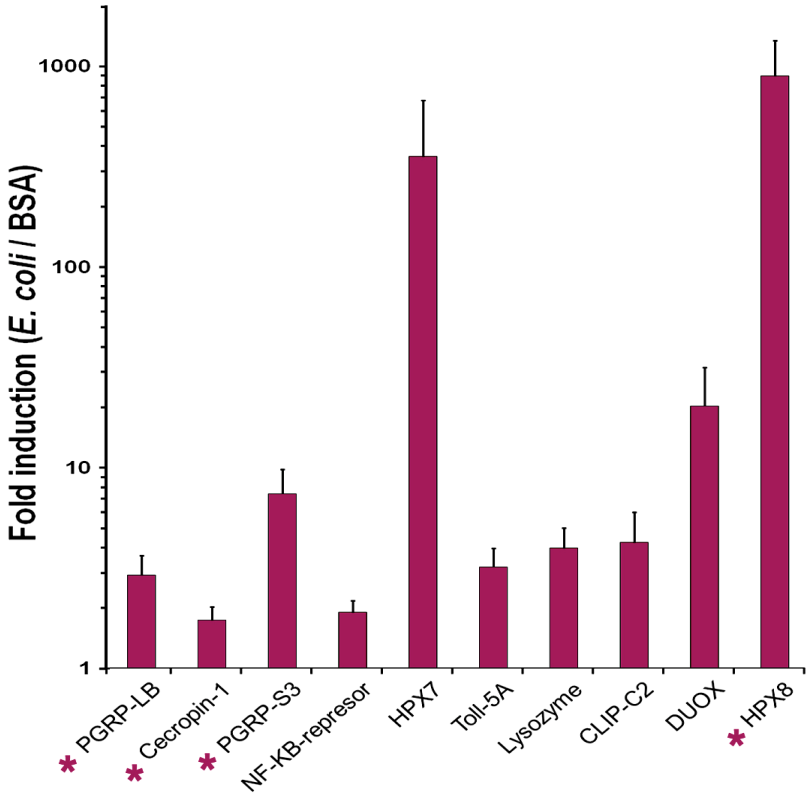

Fig. S4. Transcriptional activation of midgut immune markers in response to an a protein meal containing *in vitro* cultured bacteria. *Anopheles gambiae* G3 mosquitoes (5–6 day-old) were fed a BSA solution with or without heat-killed *Escherichia coli*. Mosquito midguts were dissected 3 h post feeding. Total RNA was extracted from dissected midguts and mRNA expression quantified by real-time PCR. Data are shown as the fold induction in mRNA levels in midguts of females fed a meal containing bacteria relative to those that received a BSA solution without bacteria. All qRT-PCR data were normalized using ribosomal protein S7 as internal standard. Accession numbers and primer sequences can be found in Table S2. HPX8 induction was only detected by qRT-PCR. The asterisks indicate genes that are also induced when IMPer is silenced.

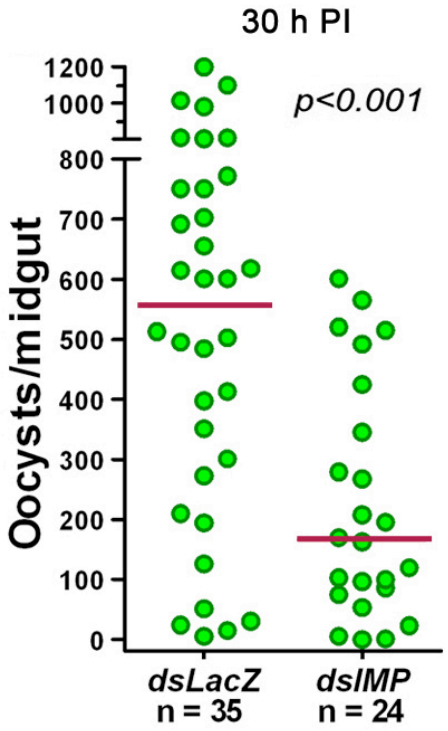

Fig. S5. Effect of IMPer silencing on the number of *Plasmodium berghei* ookinetes 30 h PI. Each circle represents the number of parasites on an individual midgut, and the line indicates the median number of oocysts. Distributions were compared using the Kolmogorov-Smirnov and Mann-Whitney tests.

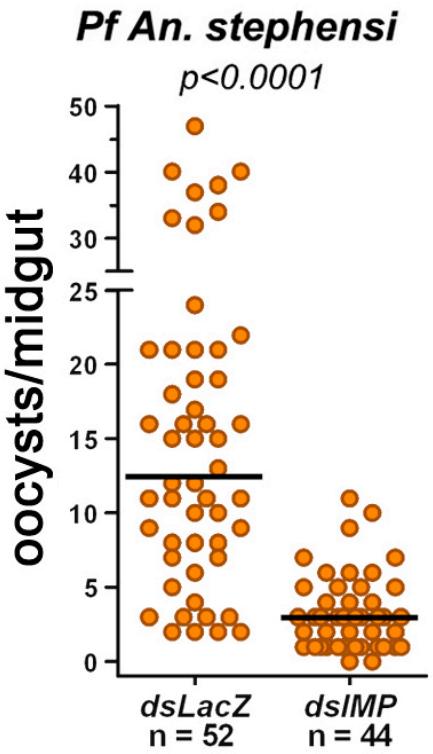

Fig. S6. Effect of IMPer silencing on the number of *Plasmodium falciparum* oocysts present in *Anopheles stephensi* mosquitoes 8 days PI. Each circle represents the number of parasites on an individual midgut, and the line indicates the median number of oocysts. Distributions were compared using the Kolmogorov-Smirnov and Mann-Whitney tests.

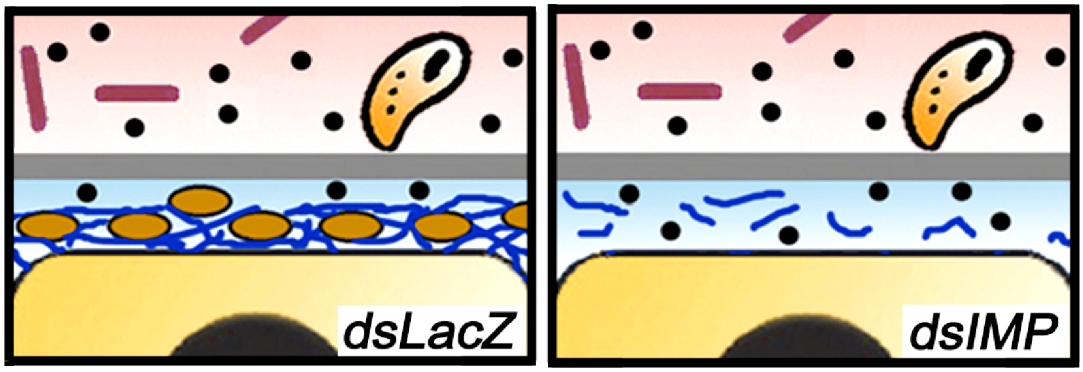

Fig. S7. Model of the proposed mechanism of action of the IMPer/Duox system. When the IMPer/Duox system is intact (left panel), Duox activation generates hydrogen peroxide on the epithelial surface, which serves as substrate for IMPer. IMPer (brown ovals) crosslinks proteins (blue lines) by forming dityrosine bonds. This network reduces the permeability to immune elicitors (black dots), decreasing their interaction with the epithelial surface. IMPer (or Duox) silencing (right panel) would disrupt the formation of this barrier and immune elicitor (and other molecules) in the midgut lumen would have increased access to the surface of epithelial cells.

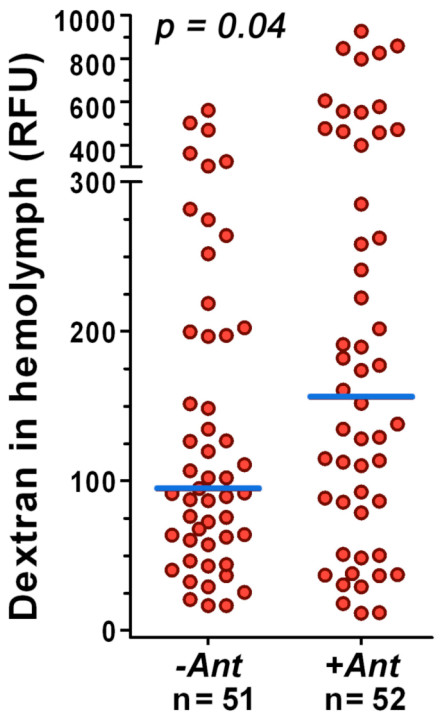

Fig. S8. Effect of oral antibiotics on gut permeability to fluorescent dextran (4 kDa). Each circle represents fluorescence in the hemolymph of an individual mosquito 16–18 h after feeding (line indicates the median). Distributions were compared using the Mann-Whitney test. Ant, antibiotics.

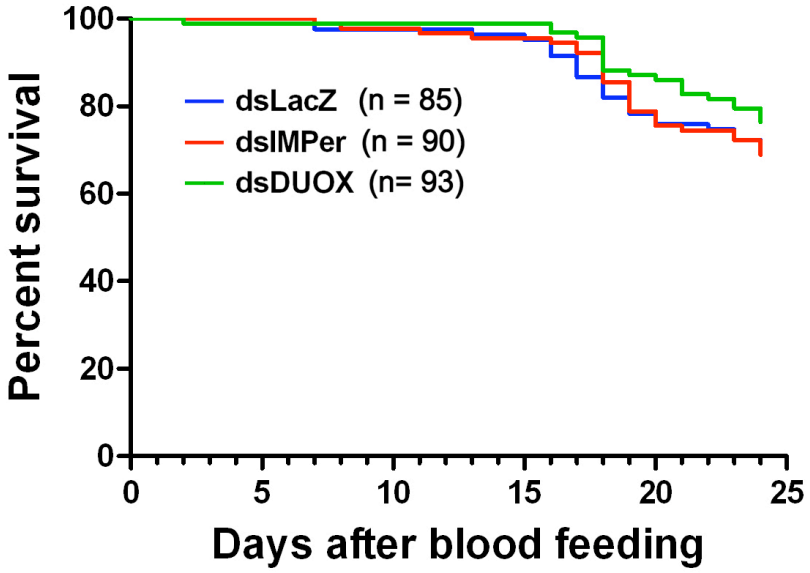

Fig. S9. Effect of IMPer and Duox silencing or LacZ dsRNA injection (control) on survival of blood-fed females. Mosquitoes were blood fed 5 days after emergence, and survival was recorded daily for 24 days. Three independent sets of 25-39 mosquitoes were used in each experiment. The survival curves of the three treatments are not significantly different (Log-rank Mantel-Cox test). The graph represents the survival curve of the three sets pooled together.