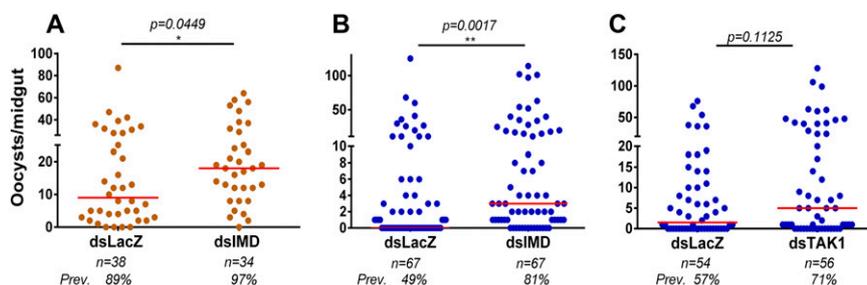
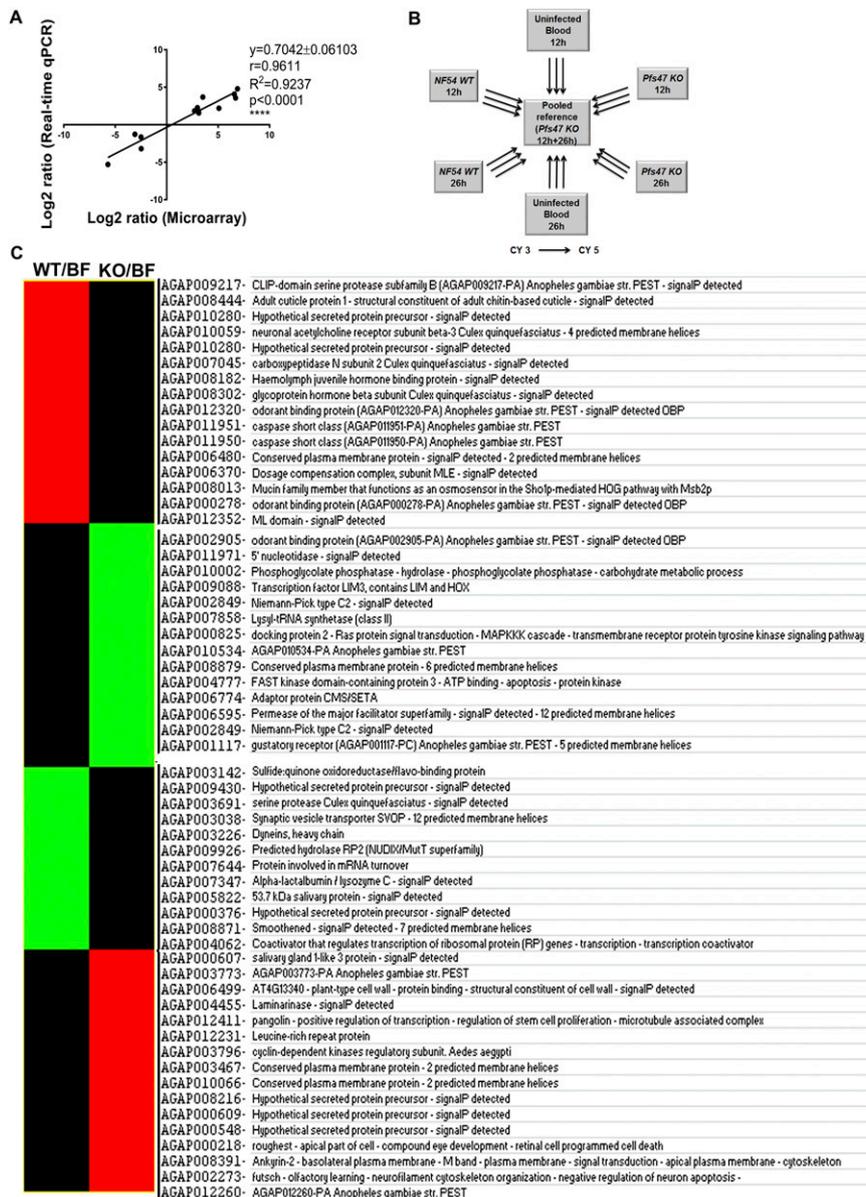


# Supporting Information

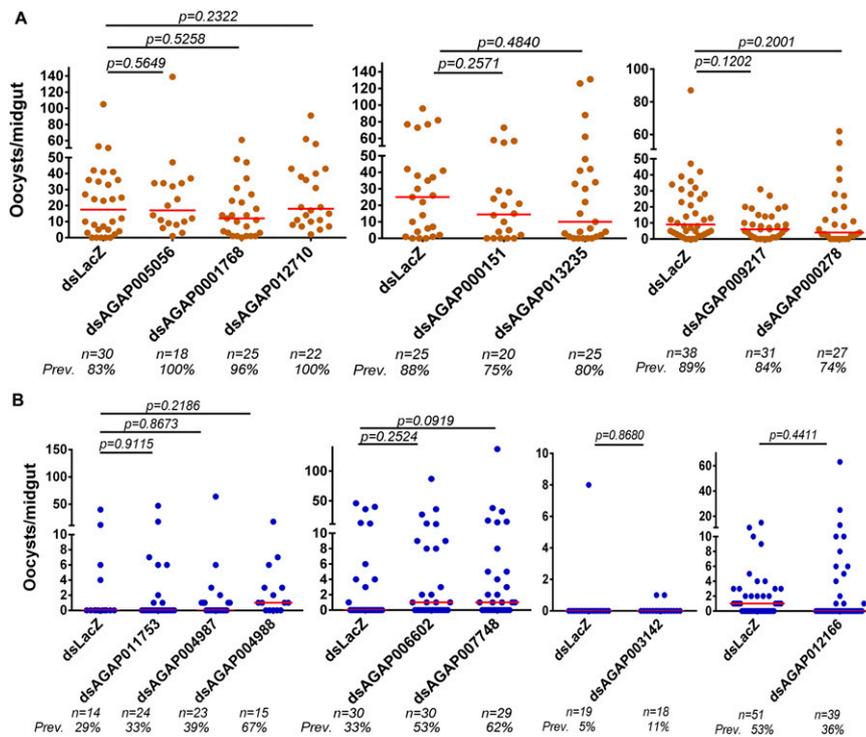
Ramphul et al. 10.1073/pnas.1423586112



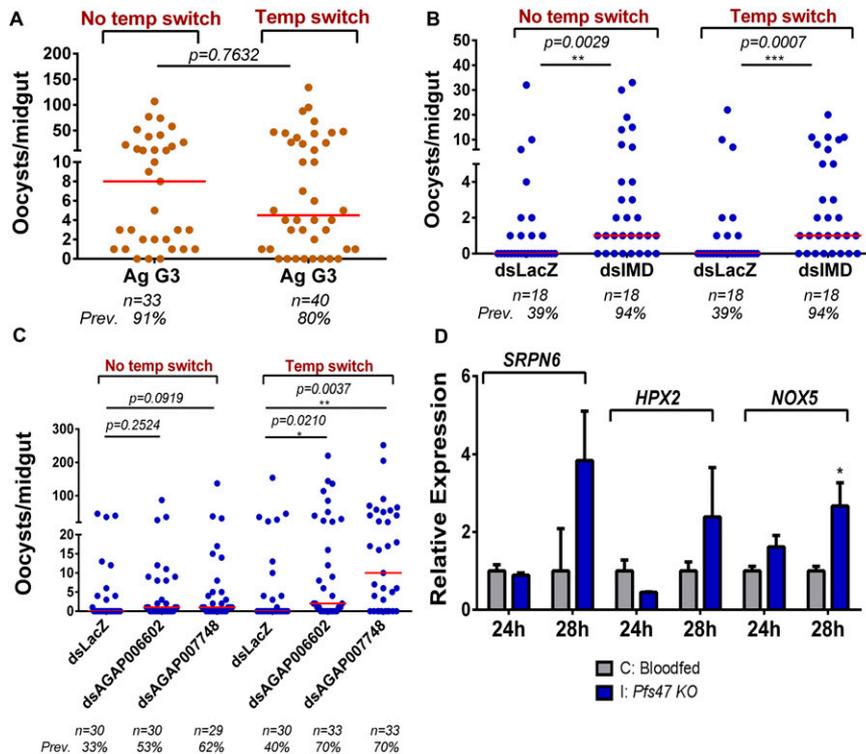
**Fig. S1.** Effects of silencing IMD and TAK1 on *Plasmodium falciparum* infections. (A) Effect of silencing IMD on *NF54* WT (orange) and (B) *Pfs47* KO (blue) infection as determined by oocysts per midgut. (C) Effect of silencing TAK1 on *Pfs47* KO infection. Dots represent oocyst counts from individual mosquito midguts and medians are represented by red lines.



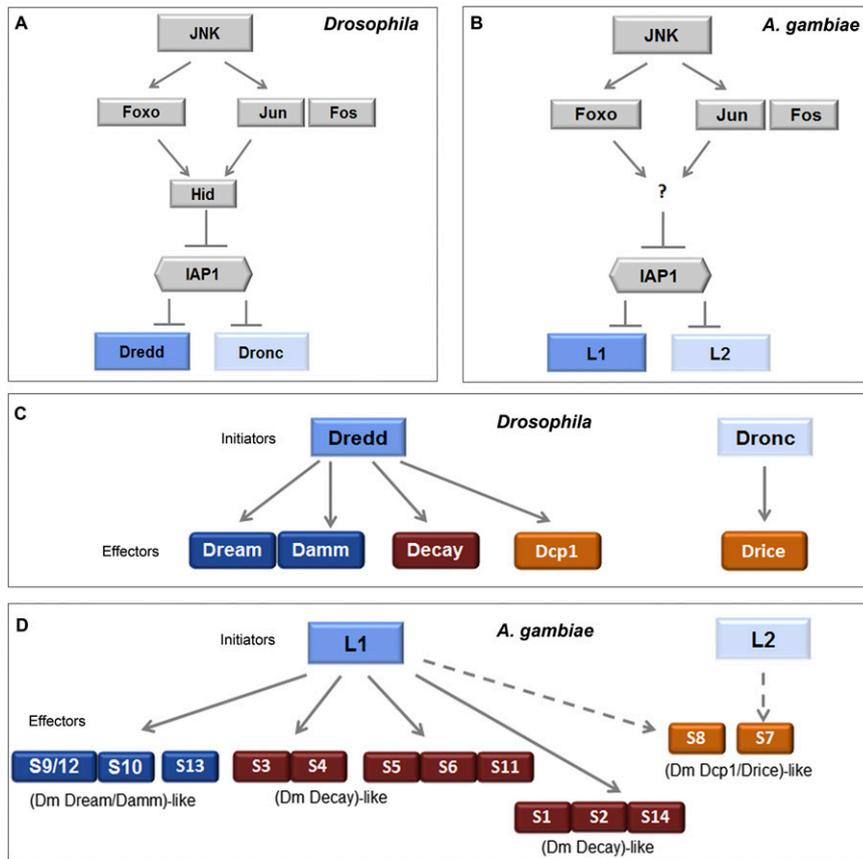
**Fig. S2.** Microarray validation, experimental design and heatmap of genes induced by *Plasmodium* infection, relative to mosquitoes fed uninfected blood. (A) Microarray validation with real-time qPCR. The expression data of 12 randomly selected genes determined by qPCR are plotted against their corresponding expression values obtained from the microarray analysis. The Pearson correlation coefficient ( $P < 0.0001$ ) and regression analysis ( $R^2 = 0.9237$ ) exhibit a high level of reliability. (B) Experimental design of microarray. Arrows represent three replicates. (C) Heatmap of *NF54 WT* relative to bloodfed control (BF) and *Pfs47 KO* relative to BF control at 12 h postinfection of a subset of genes with drastic differences in expression in response to infection with the two lines (fold-differences from 3.02- to 91.5-fold).



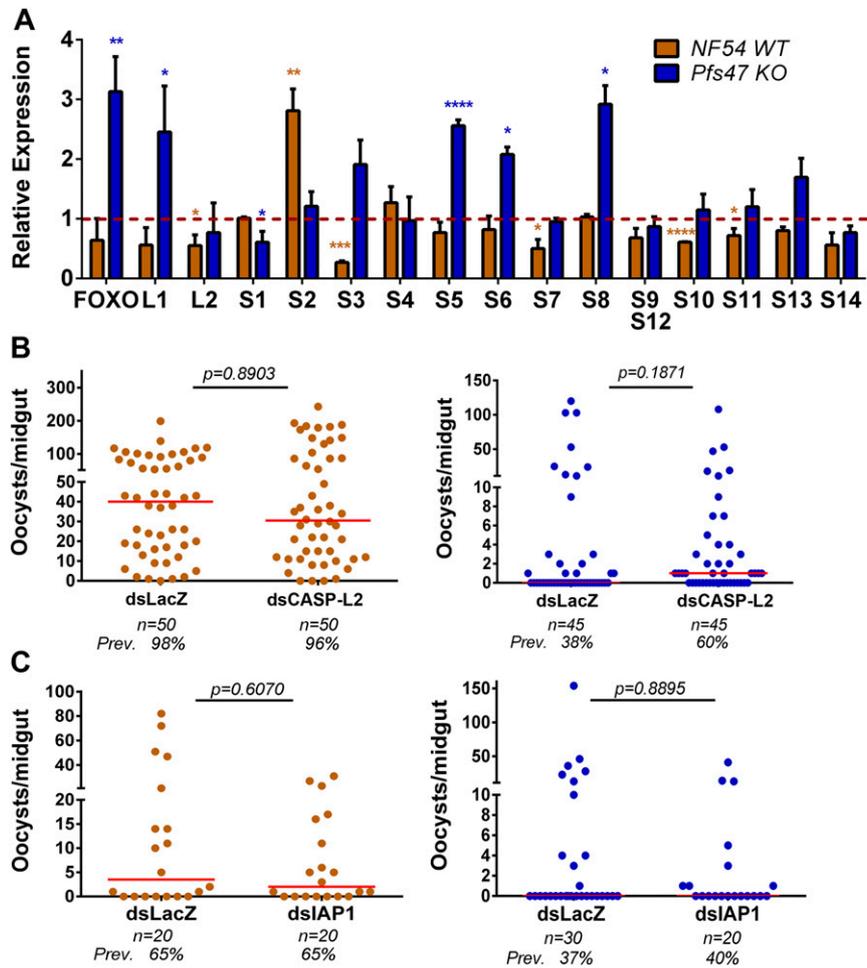
**Fig. 53.** Phenotypes of microarray candidates upon *P. falciparum* infections. (A) Infection with *NF54* WT parasites (orange) (B) infection with *Pfs47* KO parasites (blue). Dots represent oocyst counts from individual mosquito midguts and medians are represented by red lines.



**Fig. 54.** Effect of slowing down the invasion process by adopting a temperature-switch protocol. Mosquitoes were transferred to an incubator kept at 22.5 °C 6 h after infection. Mosquitoes were transferred back to 26 °C after 36 h. (A) *Pfs47* KO infection intensity in *Anopheles gambiae* G3 mosquitoes with and without a temperature switch. (B) Enhanced IMD silencing efficiency shown with and without a temperature switch. (C) Enhanced silencing efficiency of microarray candidates after a temperature switch. Dots represent oocyst counts from individual mosquito midguts and medians are represented by red lines. (D) Mean midgut mRNA expression of *SRPN6*, *HPX2*, and *NOX5* 24 and 28 h PF on *Pfs47* KO parasites from two independent experiments. Mosquitoes in this experiment were subjected to the temperature-switch protocol. C, control mosquitoes fed on uninfected human blood (gray bars); I, infected mosquitoes fed on *Pfs47* KO parasites (blue bars); \* $P < 0.05$ .



**Fig. S5.** Diagram representing initiator and effector caspases in *Drosophila* and the putative *A. gambiae* orthologs. (A) Diagram representing the regulation of apoptosis in *Drosophila* by the JNK pathway and initiator caspases (blue box) and (B) *A. gambiae* orthologs. JNK activates FOXO and Jun and Fos transcription factors, which induce Hid. Hid can inhibit IAP1 in *Drosophila*. (C) Diagram representing initiator and effector caspases in *Drosophila* and (D) their putative *A. gambiae* orthologs. The colors highlighting the caspases (blue, red, and orange) indicate three homologous groups.



**Fig. S6.** Expression of caspases and effect of gene silencing on *Plasmodium* infection. Expression of *A. gambiae* caspases 28 h postinfection from two to three independent replicates relative to control mosquitoes fed on uninfected human blood (dotted red line). Mosquitoes in this experiment were subjected to the temperature-switch protocol. Infected mosquitoes fed on (A) *NF54 WT* (orange bars) or *Pfs47 KO* parasites (blue bars). Colored stars represent significance of *NF54 WT* relative to BF control (orange stars) or significance of *Pfs47 KO* relative to BF control (blue stars). (B) Effect of silencing initiator caspase CASP-L2 or (C) IAP1 on *NF54 WT* (orange) or *Pfs47 KO* (blue) infections. Mosquitoes in this experiment were subjected to the temperature-switch protocol. Dots represent oocyst counts from individual mosquito midguts and medians are represented by red lines. All gene-silencing phenotype graphs represent two to three independent experiments; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

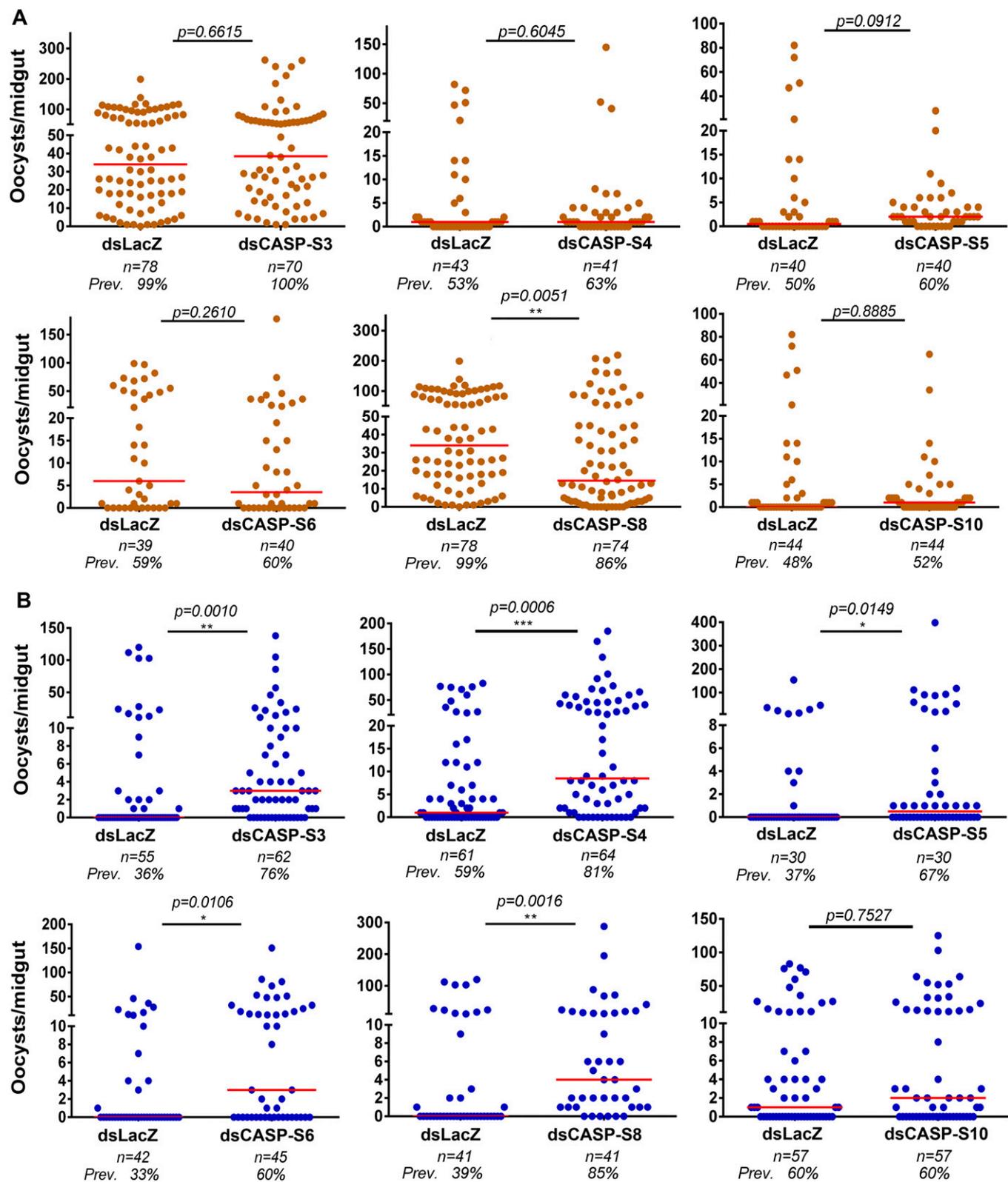


Fig. S7. Phenotypic analysis of effector caspases following infections with *Plasmodium*. (A) *NF54* WT (orange) or (B) *Pf54* KO (blue). Mosquitoes in this experiment were subjected to the temperature-switch protocol. All gene-silencing phenotype graphs represent two to three independent experiments. Dots represent oocyst counts from individual mosquito midguts and medians are represented by red lines.

## Other Supporting Information Files

[Table S1 \(DOCX\)](#)

[Table S2 \(DOCX\)](#)

[Table S3 \(DOCX\)](#)