Supporting Information

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SI Experimental Procedures

***P. falciparum* Gametocyte Culture and Ngousso Colony Mosquito Infection.** Parasite culture and experimental feedings with the *P. falciparum* isolate NF54 were done as previously described (1). Briefly, *P. falciparum* NF54 was cultured using the automated tipper-table system of Ponnudurai et al. (2) implemented at the Center for Production and Infection of Anopheles mosquito facility of the Institut Pasteur. Fourteen days after initiating the parasite subculture and before each infection experiment, gametocyte maturity was assessed by testing exflagellation of male microgametes.

For an infectious blood meal, 10 mL of the gametocyte culture was then centrifuged at 500 × *g*, and the cell pellet was resuspended in an equal volume of normal type AB human serum. The infected erythrocytes were added to fresh erythrocytes in AB human serum and transferred into a membrane feeder warmed to 37 °C. Antibiotic-treated or untreated female mosquitoes (4–5 d old) were allowed to feed for 15 min, unfed mosquitoes were removed, and only fully engorged females were maintained on 10% sucrose with or without antibiotic solution for further analysis.

Analysis of Infection Phenotypes. As infection phenotypes, we analyzed oocyst prevalence and intensity. Infection prevalence is the fraction of mosquitoes carrying at least one oocyst, and intensity is the number of oocysts per mosquito midgut.

To determine infection phenotype, midguts of blood-fed females were dissected 7–8 d postinfection and stained in 1× PBS buffer with 0.4% mercury dibromofluorescein (Sigma), and the number of oocysts per midgut was determined using a light microscope.

1. Mitri C, et al. (2009) Fine pathogen discrimination within the APL1 gene family protects Anopheles gambiae against human and rodent malaria species. *PLoS Pathog* 5(9): e1000576.
2. Ponnudurai T, Lensen AH, Leeuwenberg AD, Meuwissen JH (1982) Cultivation of fertile Plasmodium falciparum gametocytes in semi-automated systems. 1. Static cultures. *Trans R Soc Trop Med Hyg* 76(6):812–818.

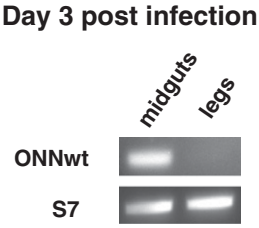

Fig. S1. ONNwt has not crossed the midgut escape barrier 3 d after an infectious blood meal. RNA was extracted in TRIzol from pooled midguts and legs 3 d after an infectious blood meal with ONNwt. RT was performed with Superscript II (Invitrogen) and a random hexamer, and PCR was performed with DreamTaq (Thermo Scientific) at 56 °C, annealing with primers ONN-8961F (forward) and ONN-9425R (reverse) on the ONN genome (Table S2). S7, transcripts of control, ribosomal protein S7 transcript.

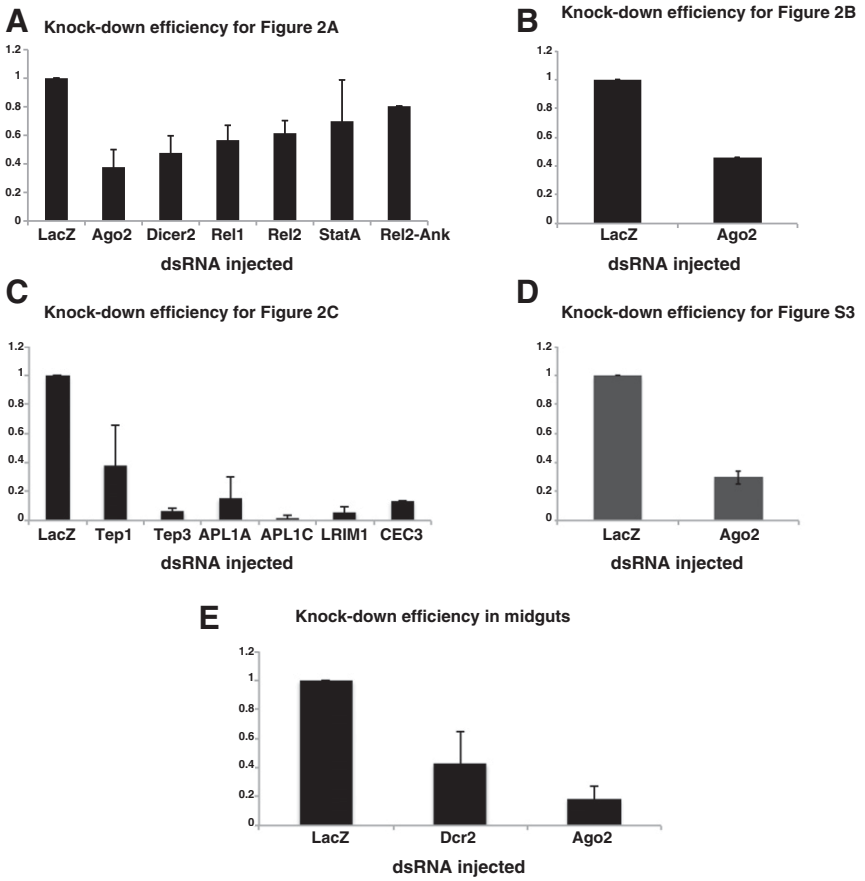

Fig. S2. Gene silencing efficiency is equivalent in whole mosquitoes and midguts. (*A–D*) Transcript levels of genes targeted by dsRNA injection were assessed in pools of five mosquitoes at the time of ONNV infection. Transcripts were quantified using the $2^{-\Delta\Delta Ct}$ (cycle threshold) method utilizing ribosomal protein S7 (rpS7) control RNA. (*E*) Transcript levels of genes targeted by dsRNA injection were assessed in pools of five dissected midguts 2–3 d postinjection of dsRNA, indicating that gene silencing was efficient in the midgut. LacZ, irrelevant control dsRNA targeting the *E. coli* lacZ gene encoding β-galactosidase.

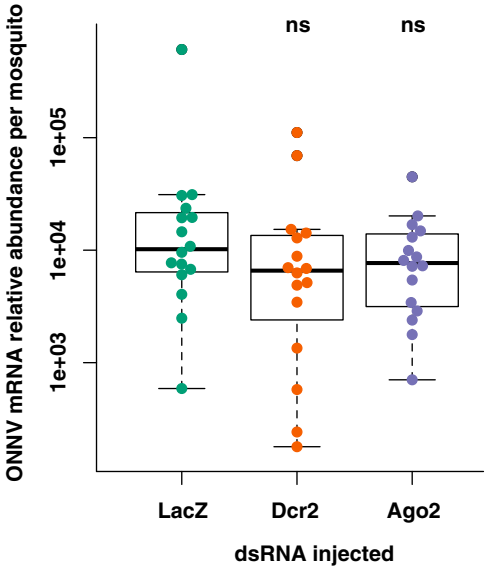

Fig. S3. Silencing of the siRNA pathway does not increase ONNV mRNA quantity. All mosquitoes that took blood are represented in the figure. Viral RNA relative abundance was quantified using the $2^{-\Delta\Delta Ct}$ method utilizing rpS7 control RNA. Total RNA from individual mosquitoes was extracted from the samples in one of the replicates of the experiment shown in Fig. 2, which showed that silencing of siRNA components also did not lead to increased viral titers. LacZ, irrelevant control dsRNA targeting the *E. coli* lacZ gene encoding β-galactosidase; ns, not significant.

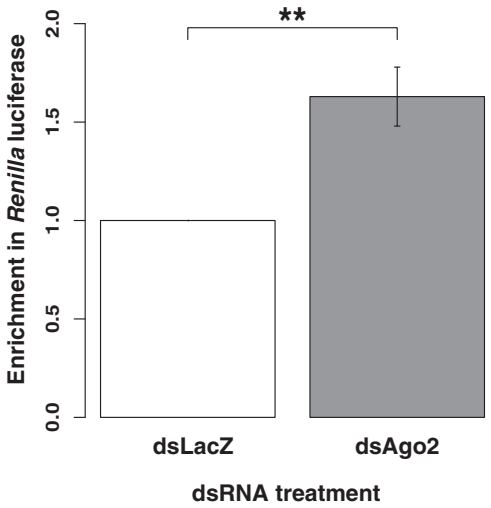

Fig. S4. Exogenous siRNA pathway displays antiviral activity in cultured anopheline 4A3A cells. Cells treated with dsRNA control or directed against Ago2 were infected with ONN-*RLuc* at a multiplicity of infection of 1. Viral replication was assessed by *RLuc* activity quantification. $^{**}P < 0.005$. dsLacZ, irrelevant control dsRNA targeting the *E. coli* lacZ gene encoding β-galactosidase.

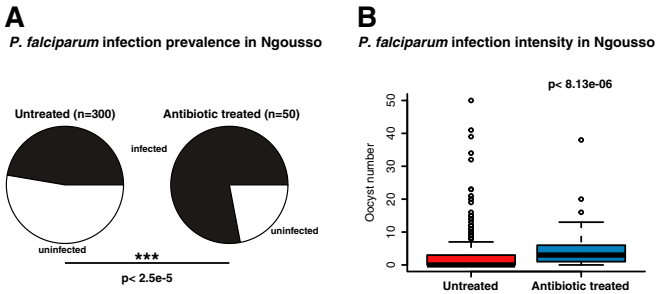

Fig. S5. Antibiotic treatment increases susceptibility of the *An. gambiae* Ngousso strain to *P. falciparum* infection (NF54 strain). Mosquitoes from the same colony used for all ONNV experiments throughout this work were treated or not treated with antibiotics from adult emergence. (*A*) Oocyst prevalence 7 d after *P. falciparum* infection in *An. gambiae* Ngousso mosquitoes. (*B*) Oocyst intensity in the same mosquitoes.

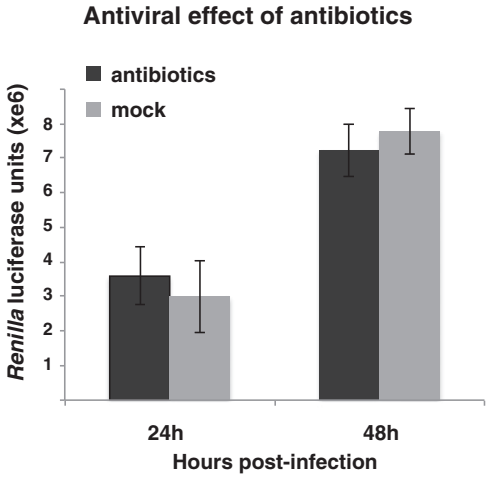

Fig. S6. Antibiotic treatment has no direct antiviral activity. 4A3A cells were plated with or without antibiotics (penicillin, streptomycin, and gentamicin at the concentrations used for mosquito treatment) for 24 h, and cells were then infected with ONN-*RLuc* directly in the supernatant (in contact or not in contact with antibiotics). A luciferase assay was performed in triplicate at 24 h and 48 h postinfection using the passive lysis buffer and the *Renilla*-Glo Luciferase Assay System (Promega).

Table S1. Primers used for synthesis of dsRNAs (prefix T7, T7 RNA polymerase promoter underlined) of target genes

T7-GFP-F	GAATTGTAATACGACTCACTATAGGGCATGGTGAGCAAGGGCGAG
T7-GFP-R	GAATTGTAATACGACTCACTATAGGGCTTACTTGTACAGCTCGTC
T7-βGal-F	TAATACGACTCACTATAGGGGTCGCCAGCGGCACCGCGCCTTTC
T7-βGal-R	TAATACGACTCACTATAGGGCCGGTAGCCAGCGCGGATCATCGG
T7-APL1A-F	TAATACGACTCACTATAGGACTACCACCAGCCGAAAGATG
T7-APL1A-R	TAATACGACTCACTATAGGATCTGGTCTTGTATAGTACAATGG
T7-APL1C-F	TAATACGACTCACTATAGGAGGCCAAGAAGAACCGCAATCC
T7-APL1C-R	TAATACGACTCACTATAGGATCACAGTGATTTCAGGGTGTGC
T7-Ago2-F	TAATACGACTCACTATAGGGGTTCGCGCCCATACCTAAA
T7-Ago2-R	TAATACGACTCACTATAGGTTGTTTTTGTTCAGCGCCTG
T7-Dcr2-F	TAATACGACTCACTATAGGAGGTGCTGAACCAAATCCAC
T7-Dcr2-R	TAATACGACTCACTATAGGGTACACCGAGACGGCAAACT
T7-Rel1-F	TAATACGACTCACTATAGGGCAACAGAACCCGTTCAACTTGC
T7-Rel1-R	TAATACGACTCACTATAGGGAATGGATGCTTACGGGCTAACG
T7-Rel2-F	TAATACGACTCACTATAGGGCAACAGCAGCAACAACATC
T7-Rel2-R	TAATACGACTCACTATAGGGCACAGGCACACCTGATTGA
T7-Rel2(F)-F	TAATACGACTCACTATAGGAATCCGACGCAACGATACG
T7-Rel2(F)-R	TAATACGACTCACTATAGGGACCGCAATGTGAAGGATG
T7-StatA-F	TAATACGACTCACTATAGGCCGGAGAGCAACTTCACGAT
T7-StatA-R	TAATACGACTCACTATAGGGATGAACGTGTTGTAATGAGC
T7-LRIM1-F	TAATACGACTCACTATAGGGCTGGAACGTAAAGGAGCTTG
T7-LRIM1-R	TAATACGACTCACTATAGGGCGCTCGGCAAAGTTCACCGT
T7-Tep1-F	GAATTGTAATACGACTCACTATAGGGCGTTTGTGGGCCTTAAAGCGCTG
T7-Tep1-R	GAATTGTAATACGACTCACTATAGGGCGACCACGTAACCGCTCGGTAAG
T7-Tep3-F	TAATACGACTCACTATAGGGCACCTCGACTGAGAAAGGTTTG
T7-Tep3-R	TAATACGACTCACTATAGGGCTGATTATTTATATAGTTTTAC
T7-CEC3-F	GAATTGTAATACGACTCACTATAGGGAGAGAGATCTCTCCCGTGTGGA
T7-CEC3-R	GAATTGTAATACGACTCACTATAGGGAGAGCGGTGACCTCTTTCAGTCT

Final suffix in column 1 indicates the sense of the primers. F, forward; R, reverse. dsRNA synthesized by T7-βGal-F and T7-βGal-R is a control dsRNA targeting the irrelevant *E. coli* lacZ gene encoding β-galactosidase.

Table S2. Primers used for verification of target gene silencing or quantification of viral RNA by RT-PCR or one step quantitative RT-PCR

qS7-F	AGAACCAGCAGACCACCATC
qS7-R	GCTGCAAACTTCGGCTATTC
qAgo2-F	CCAAGCCGACCAAGTACG
qAgo2-R	GCAAACAGGTGGCACAGATT
qDcr2-F	CCCACTTTGACGCCAACAC
qDcr2-R	GTCAGGATGCCGAAATTGTT
qRel1-F	TCAACAGATGCCAAAAGAGGAAAT
qRel1-R	CTGGTTGGAGGGATTGTG
qRel2-F	CGGGCAGAGGGAAGCAT
qRel-2-R	AGGCCCGCTCACCGTT
qStatA-F	TACAACGAAACGACCAAGCA
qStatA-R	GGTCCATACCGAAAAGACGA
qAPL1A-F	GACTGCAAGCCGAGATCGATACC
qAPL1A-R	CATCCATCTGGTCCTTGAGCTTA
qAPL1C-F	AAGCAGGCTGAGTTGAGACAGG
qAPL1C-R	GCCCAAGTAACATCATACACAC
qLRIM1-F	GTGCCAAGTCGTCCTATTGCTC
qLRIM1-R	TGCTGTCCGTTACCTTCTCGAT
qTep1-F	CAGATGGTTCGTTTGGTGTG
qTep1-R	GCAATGCCGTCAACACATAC
qTep3-F	ACCGCCAGGCGTACGTGATGG
qTep3-R	CAAACCTTTCTCAGTCGAGGT
qCEC3-F	AACCACCTGCGCGTTAGTAG
qCEC3-R	GCCGATGAACTTGAAGGTTTT
qONN-F	ACTCCAGGAGAGTGCTTCCA
qONN-R	CCTGCAACCCTCTTTCAGTA
S7-F	AGGCGATCATCATCTACG
S7-R	GTAGCTGCTGCAAACTTC
EGFP 135F	GAAGTTCATCTGCACCACC
EGFP 439R	TTGTACTCCAGCTTGTGCC
ONN-8961F	AAGGAGAGACGCTGACGGTA
ONN-9425R	CCAGTGGAAATGGGATATGG