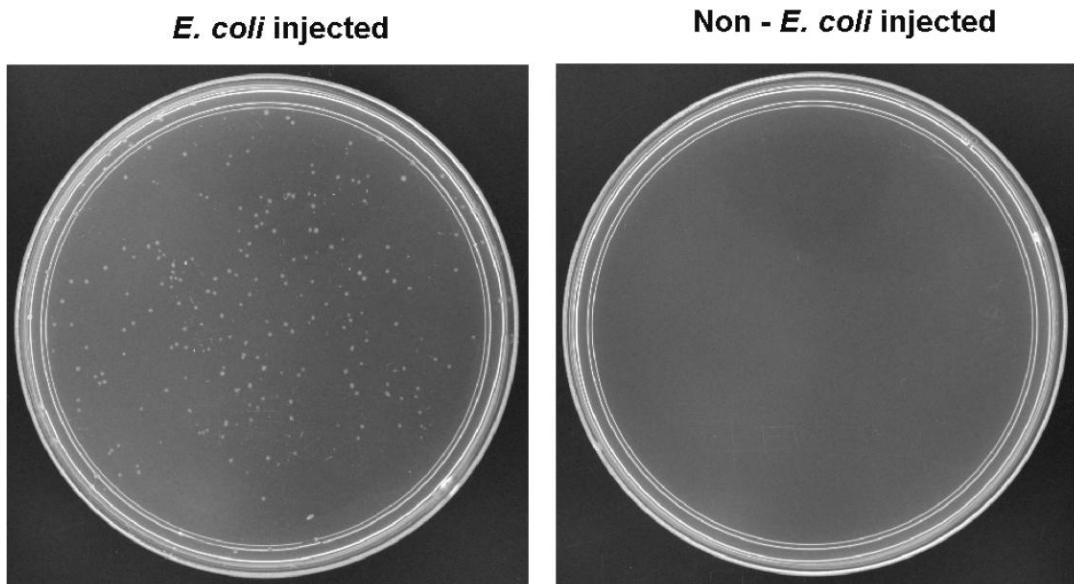
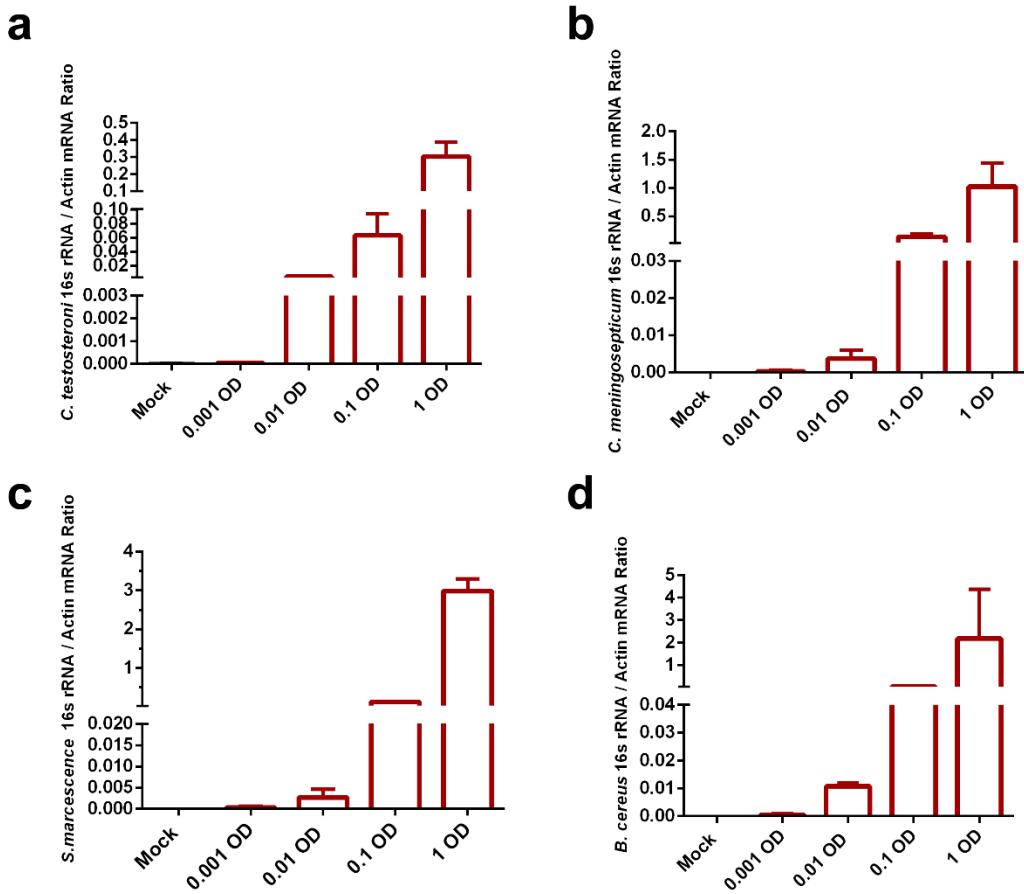


Mosquito C-type lectins maintain gut microbiome homeostasis

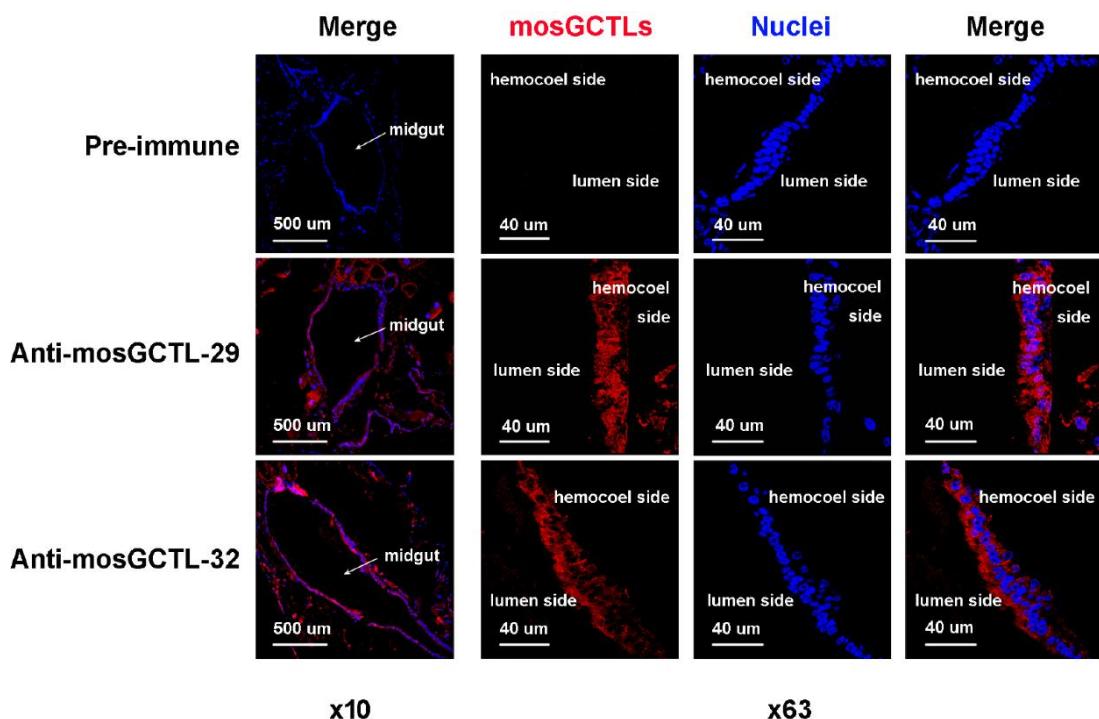
Xiaojing Pang, Xiaoping Xiao, Yang Liu, Radian Zhang, Jianying Liu, Qiyong Liu,
Penghua Wang and Gong Cheng



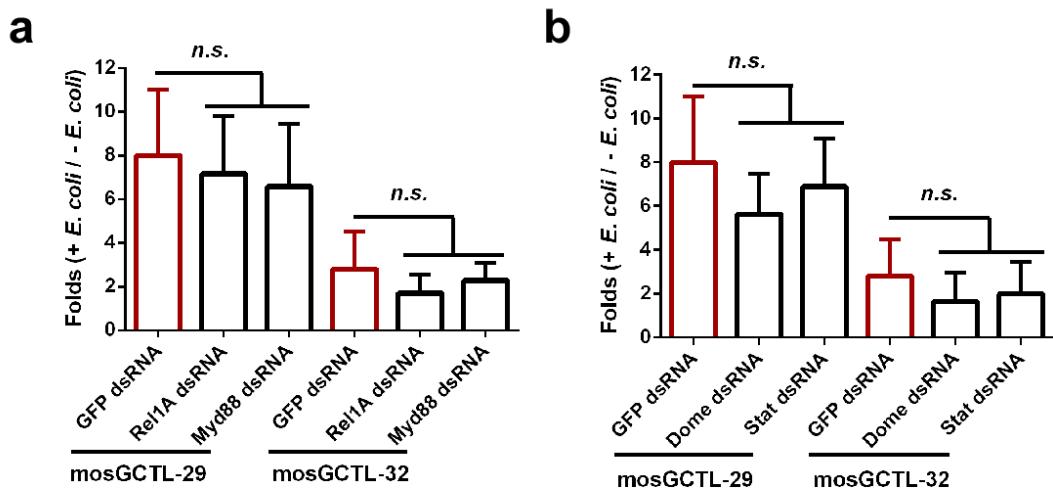
Supplementary Figure 1. Detection of the spectinomycin-resistant commensal microbiome in *A. aegypti*. Untreated mosquitoes were ground in PBS buffer. Then, bacterial viability was assessed by the CFU assay on LB plates with 100 µg/ml spectinomycin. Mosquitoes inoculated with *E. coli* (ST515 strain) cells were used as the positive controls.



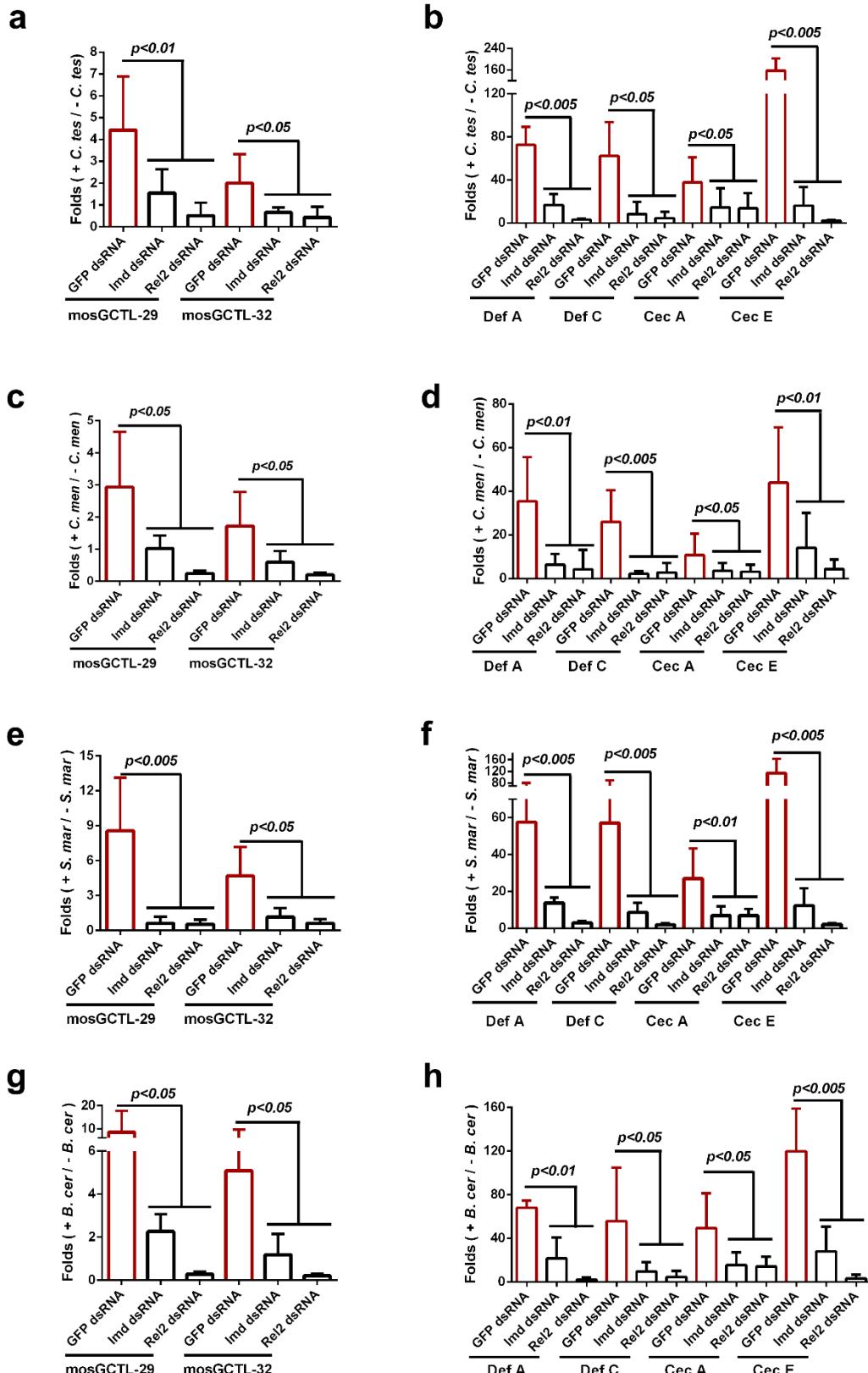
Supplementary Figure 2. Validation of the specificity of qPCR primers for the 16S gene of gut bacteria. *C. testosteroni* (a), *C. meningosepticum* (b), *S. marcescens* (c) and *B. cereus* (d), which were serially diluted by PBS buffer, were inoculated into antibiotic-treated mosquitoes, respectively. The total RNA from the antibiotic-treated and mock mosquitoes was isolated to synthesize cDNA, and subsequently the cDNA was used for the detection of the 16S gene of these bacteria. The primers for qPCR detection are described in Supplementary Table 5. Data are represented as mean \pm SD in each group.



Supplementary Figure 3. Detection of mosGCTLs in the mosquito midgut. The female *A. aegypti* were cryo-sectioned, and slides were used for *in situ* staining. The anti-mosGCTL mouse polyclonal antibody and anti-mouse IgG Alexa-546 antibody were used for surface staining of the mosGCTL proteins (Red). Nuclei were stained blue with To-Pro-3 iodide. Images were examined using the 10 \times and 63 \times objective lens of a Zeiss LSM 780 meta confocal microscope.

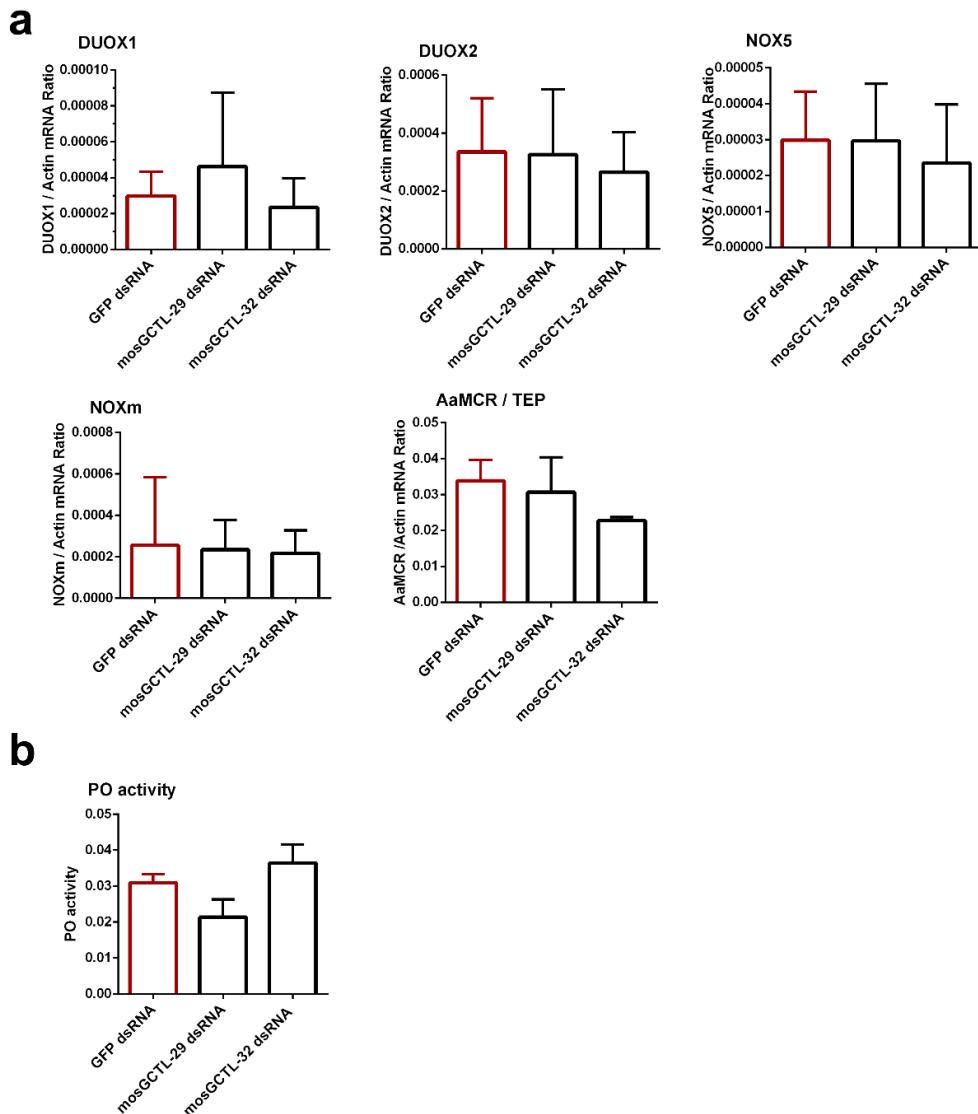


Supplementary Figure 4. The role of the Toll and JAK-STAT signaling pathways in *mosGCTL* induction. The key factors in the Toll (a, *RelIA* and *Myd88*) and JAK-STAT (b, *Dome* and *Stat*) pathways were silenced in *A. aegypti*. 0.005 O.D. *E. coli* cells in PBS was sequentially microinjected 3 days later, and the *mosGCTL* induction was assessed 4 hr after bacterial inoculation. Mosquitoes inoculated with *GFP* dsRNA and subsequently infected with *E. coli* were included as controls. The induction is presented as the fold change relative to that in the uninfected mosquitoes. Data are represented as mean \pm SD in each group and analyzed using the non-parametric *Mann-Whitney* test.

**Supplementary Figure 5. The role of the Imd pathway in *mosGCTLs*/AMPs induction.**

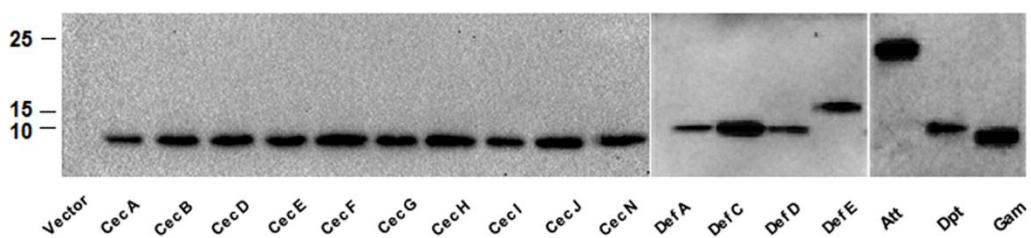
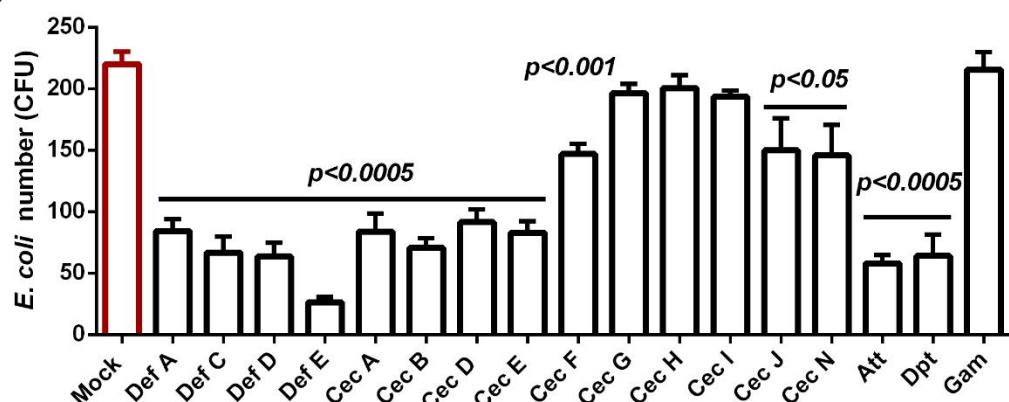
The key factors in the Imd pathway (*Imd* and *Rel2*) were silenced in *A. aegypti*. 0.05 O.D. of

C. testosteroni (*C. tes*) (a, b), *C. meningosepticum* (*C. men*) (c, d), *S. marcescens* (*S. mar*) (e, f) and *B. cereus* (*B. cer*) (g, h) in PBS was sequentially inoculated 3 days later, respectively, and the expression of inducible *mosGCTLs* (a, c, e, g) and *AMPs* (b, d, f, h) was assessed 4 hr after bacterial inoculation. Mosquitoes inoculated with *GFP* dsRNA and subsequently infected with bacteria were included as controls. qPCR analysis was normalized to *A. aegypti actin* (AAEL011197). The induction is presented as the fold change relative to that in the uninfected mosquitoes. The experiment was repeated 2 times with similar results. Data are represented as mean \pm SD in each group and analyzed using the non-parametric *Mann-Whitney* test.

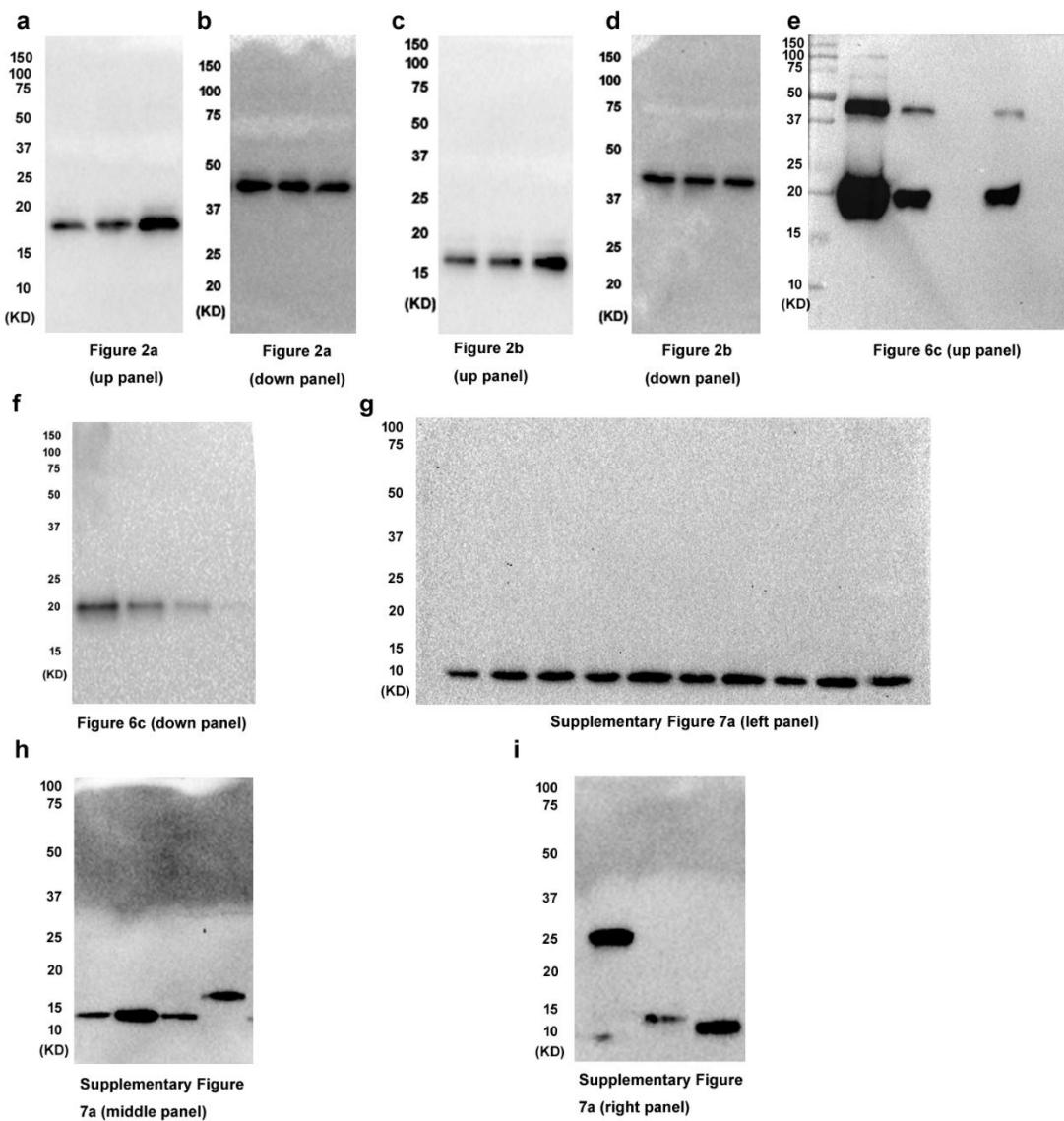


Supplementary Figure 6. The role of *mosGCTLs* in the regulation of immune responses.

a, Knockdown of *mosGCTL-29* and *mosGCTL-32* did not regulate the expression of the ROS-related NADPH oxidase and *TEP* genes. Gene expression was determined by SYBR Green® qPCR and normalized to *A. aegypti* actin. The qPCR primers are shown in Supplementary Table 5. **b**, Silencing *mosGCTL-29* and *mosGCTL-32* did not regulate the melanization activity in the mosquitoes. The mosquito lysates were collected by centrifugation at 3,000 rpm for 10 min at 4 °C to remove debris. The PO activity assays were performed in 96-well plates. Subsequently, 100 µl of 50 mM sodium phosphate buffer (pH 6.5) containing 2 mM dopamine was added to 20 µl of cell culture medium. PO activity was monitored over 30 min by measuring the absorbance at 490 nm using a plate reader. Data are represented as mean ± SD in each group.

a**b**

Supplementary Figure 7. The role of AMPs in the elimination of *E. coli* cells. **a**, The 17 *A. aegypti* AMP genes were cloned into the pMT/Bip/V5-His A vector and individually expressed in *Drosophila* S2 cells. The recombinant AMPs in the supernatant were detected via western blotting with an anti-V5 antibody. **b**, Detection of the anti-bacterial activity of AMPs. The supernatants with AMPs were individually mixed with the *E. coli* cells. After 1 hr of incubation, bacterial viability was assessed by a CFU assay. The experiment was repeated 3 times with similar results. Data are represented as mean \pm SD in each group and analyzed using the non-parametric *Mann-Whitney* test.



Supplementary Figure 8. Full-length blots from Figures 2, 6 and Supplementary Figure 7. **a-d**, The abundance of mosGCTL-29 and mosGCTL-32 in *A. aegypti*. **e**, The interaction between mosGCTL-32 and bacterial cells by elution assay. **f**, The interaction between mosGCTL-32 and bacterial cells by competitive assay. **g-i**, Expression of AMPs in *Drosophila* S2 cell supernatant. Molecular weight standards are shown on the left.

Supplementary Table 1. The 16S rDNA-based phylogenetic affiliation of culturable microorganisms from the *A. aegypti* midgut

Bacterium	Gram staining	Percent in total sequenced microorganisms (%)
<i>Alcaligenes</i> sp.	-	2.5
<i>Acinetobacter</i> sp.	-	2.5
<i>Achromobacter</i> sp.	-	2.5
<i>Bacillus simplex</i>	+	2.5
<i>Bacillus cereus</i>	+	7.5
<i>Comamonas testosteroni</i>	-	17.5
<i>Chromobacterium aquaticum</i>	-	5.0
<i>Chryseobacterium meningosepticum</i>	-	12.5
<i>Chryseobacterium</i> sp.	-	7.5
<i>Leucobacter</i> sp.	+	2.5
<i>Pseudomonas protegens</i>	-	5.0
<i>Serratia marcescens</i>	-	25.0
<i>Sphingobium</i> sp.	-	2.5
<i>Staphylococcus hominis</i>	+	5.0

Supplementary Table 2. *mosGCTL* orthologs in *Culex quinquefasciatus*

mosGCTL-29 (183AA) orthologs in <i>Culex quinquefasciatus</i>				
Gene Number	Name	Lengh(AA)	Query cover	Identity
CPIJ000449	C-type lectin	160	69%	62%
CPIJ015401	C-type lectin	116	61%	63%
CPIJ006802	C-type lectin	186	72%	51%
CPIJ006813	C-type lectin	172	92%	42%
CPIJ006812	C-type lectin	172	92%	41%

mosGCTL-32 (159AA) orthologs in <i>Culex quinquefasciatus</i>				
Gene Number	Name	Lengh(AA)	Query cover	Identity
CPIJ007869	C-type lectin	307	77%	55%
CPIJ014105	C-type lectin	156	88%	37%
CPIJ015095	C-type lectin	162	90%	34%
CPIJ001323	C-type lectin	136	72%	38%
CPIJ009922	C-type lectin	173	77%	32%

Supplementary Table 3. *mosGCTLs* expression after systemic bacterial challenge in *A. aegypti*

Gene Symbol	Gene Number	<i>mosGCTLs</i> Regulation (Fold/Mock) post <i>E. coli</i> challenge	<i>mosGCTLs</i> Regulation (Fold/Mock) post <i>S. marcescens</i> challenge	<i>mosGCTLs</i> Regulation (Fold/Mock) post <i>C. testosteroni</i> challenge	<i>mosGCTLs</i> Regulation (Fold/Mock) post <i>C. meningosepticum</i> challenge	<i>mosGCTLs</i> Regulation (Fold/Mock) post <i>B. cereus</i> challenge
mosGCTL-1	AAEL000563	0.94	1.98	0.59	2.04	0.62
mosGCTL-2	AAEL000533	1.11	1.15	16.11	7.06	2.08
mosGCTL-3	AAEL000535	0.69	1.97	0.96	10.43	0.90
mosGCTL-4	AAEL000543	1.14	1.17	0.96	1.18	1.21
mosGCTL-5	AAEL000556	0.62	0.92	0.67	0.62	0.84
mosGCTL-6	AAEL000283	N/A*	N/A	N/A	N/A	N/A
mosGCTL-7	AAEL002524	1.93	2.67	2.33	1.86	3.78
mosGCTL-8	AAEL004679	1.44	2.99	0.52	3.76	0.64
mosGCTL-9	AAEL005482	0.63	0.76	0.58	1.08	1.31
mosGCTL-10	AAEL005641	4.64	12.51	16.56	11.39	67.65
mosGCTL-11	AAEL006456	N/A	N/A	N/A	N/A	N/A
mosGCTL-12	AAEL008299	1.04	1.24	0.52	4.56	1.27
mosGCTL-13	AAEL008681	N/A	N/A	N/A	N/A	N/A
mosGCTL-14	AAEL009209	1.46	1.59	0.51	2.87	0.54
mosGCTL-15	AAEL010992	0.96	1.27	0.70	3.53	0.52
mosGCTL-16	AAEL011070	0.81	0.96	1.42	1.11	1.15
mosGCTL-17	AAEL011079	N/A	N/A	N/A	N/A	N/A
mosGCTL-18	AAEL011402	1.30	0.49	0.72	3.78	0.58

mosGCTL-19	AAEL011404	0.79	1.35	1.04	3.20	0.58
90.8% Nucleotide identity to AAEL011404						
mosGCTL-20	AAEL011408	1.72	1.19	1.19	1.39	1.17
mosGCTL-21	AAEL011446	N/A	N/A	N/A	N/A	N/A
mosGCTL-22	AAEL011453	0.70	2.12	1.83	2.03	4.92
mosGCTL-23	AAEL011455	0.67	0.46	1.28	0.69	1.78
mosGCTL-24	AAEL011607	3.05	5.05	3.51	4.53	3.56
mosGCTL-25	AAEL011609	N/A	N/A	N/A	N/A	N/A
mosGCTL-26	AAEL017265	2.26	5.80	1.82	3.01	2.69
mosGCTL-27	AAEL011612	0.72	0.79	0.85	1.55	0.53
mosGCTL-28	AAEL011616	2.79	2.37	0.61	3.23	0.75
mosGCTL-29	AAEL011619	5.76	9.48	5.78	6.96	13.83
mosGCTL-30	AAEL011621	2.01	2.07	0.80	2.91	0.82
mosGCTL-31	AAEL011622	4.89	7.97	3.39	3.20	3.36
mosGCTL-32	AAEL012353	2.05	2.89	2.98	3.78	2.93
98.3% Nucleotide identity to AAEL011607						
mosGCTL-33	AAEL014385	1.61	4.1	1.03	2.35	0.65
98.9% Nucleotide identity to AAEL011619						

* N/A, gene not analyzed.

Red number represents the increased expression by more than 2 folds.

Supplementary Table 4. AMPs regulation by knockdown of *mosGCTL-29* and *mosGCTL-32* in *A. aegypti*

Gene Symbol	Gene Number	Function	Fold Regulation (Mean) in whole body		Fold Regulation (Mean) in midgut	
			mosGCTL-29 dsRNA	mosGCTL-32 dsRNA	mosGCTL-29 dsRNA	mosGCTL-32 dsRNA
Def-A	AAEL003841	Defensin	1.25	0.81	1.44	0.65
Def-C	AAEL003832	Defensin	1.00	0.64	1.18	1.15
Def-D	AAEL003857	Defensin	1.23	0.87	1.13	1.25
Def-E	AAEL003849	Defensin	1.48	1.38	0.90	0.97
Cec-A	AAEL000627	Cecropin	1.01	0.63	1.74	1.28
Cec-B	AAEL004223	Cecropin	1.20	0.85	0.90	1.14
Cec-D	AAEL000598	Cecropin	1.22	1.15	0.89	0.84
Cec-E	AAEL000611	Cecropin	1.37	1.27	0.83	1.05
Cec-F	AAEL000625	Cecropin	1.29	0.72	0.76	1.13
Cec-G	AAEL015515	Cecropin	0.92	1.29	0.91	1.61
Cec-H	AAEL017211	Cecropin	1.13	1.36	1.22	1.35
Cec-I	AAEL000775	Cecropin	0.86	1.21	0.68	0.81
Cec-J	AAEL000777	Cecropin	0.98	1.28	1.18	1.07
Cec-N	AAEL000621	Cecropin	1.06	1.14	1.33	1.27
Dpt	AAEL004833	Diptericin	0.68	0.87	0.83	1.03
Gam	AAEL004522	Gambicin	0.98	1.23	0.86	1.44
Att	AAEL003389	Attacin	1.33	0.74	1.05	1.47

Supplementary Table 5. Primers for qPCR, dsRNA synthesis and genes cloning

<i>Cecropin F</i>	GATCTGGTGGCCCTGAAGAAGCTGGGAAAGAAATTGGAAGGGTCGGCAA GGGAGTGTCAAGCATCGGAAAAGGCCCTCCAGTTAACGGGATAC AAGGC1GTGAAAGT	CTAGACTTCCAACAGCCTTGATCCGTTATAACGGAAAGGGCTTTTC CGATGCTTGAACACTCGCTGCCACTCTCCAAATTCTTCCCAGC TTC1CAGGCCACCA
<i>Cecropin G</i>	GATCTGGTGGCCCTCAAGAAGCTGGGAAAGAAATTGGGAGGTGAGGAAA ACGAGTITTCATGCTGCCAGAAGGCTCTTCTGTGTTAGCTGGGCT AAAGCCTAGGAAAGT	CTAGACTTCCAACAGCCTTGATCCGTTATAACGGAAAGGGCTTTTC CGGAGCATTTGAAAGACTCGTTTCCGACCTCCCAATTCTTCCCAGC CTCTTGAGGGCCACA
<i>Cecropin H</i>	GATCTGGAAAGCTGAAGAAAGTGGCAAAAAGATGAAAGAGCTGGGAAA CACGTCGCCATGCAAGCTCAAAGGCTGAGCTGCTGCTGGCT GGGGCTCTGGTT	CTAGAAACAGAGCCGCCAGCCAGCAGCACAGGTCAGGCCCTTTGA GCTGCAATTGGCAGTGTGTTGCCCTTTCACCTTTGCAACTTTC TCTTCAGCTTCCA
<i>Cecropin I</i>	GATCTGGAAAGCTGAAGAAAGTGGCAAAAAGATGAAAGAGCTGGGAAA AAAGTAGTCTCATGCTGATAGGTGATACCACCTGCTTCAAGGTTA	CTAGAGTTTATATCTTGTGTTCTGTTTCAAGGATTGTTGTAACCC TAGGACAGTTGATCACCTTATGCGACAGCATGGACTACGTTTGGCC
<i>Cecropin J</i>	GATCTGGAAAGCTGAAGAAAGCTGGCAAAAAGATGAAAGAGCTGGGAAA CACGTCGCCACCGCTGCGATAAGGTGATACCAACTGTCTGGGATTA	CTAGAGTTTATATCTTGTGTTTCAAGGATTGTTGTAACCC AGGACAGTTGATCACCTTATGCGACAGCTGGACAGCTGTTGGCC
<i>Cecropin N</i>	GATCTCAGCAACAGTCGGGCCGTGGAAACGGGGCCAGGTGAAATTGCA GCAAGAAATTGGAAAAGATGCTGGAAAATGTGTTAACGCTGCTAAAGAA GCACTGCCAGTGTGCCGGTACAAGGCCCTAGGAC	TGAGACTCTAGGGCTGTACCCGCCAGGACTGCCAGTGGCTTTTA GGAGCTTAAACACATTTCGCCACTTTTCAAATTCTTGGCAATT CCACCTGGGCCGCCCTTCAGGCCGACTGTGCTGA
<i>Attacin</i>	CCCTCAAGATCTCACTCATGGTCGTCCTCAGCTAAC	CCCTCATCTAGAGAAGCGATGACTCAGTCCAACCCGGC
<i>Diptericin</i>	GCCACCAAGATCTGCGATGGCCAATTCTAG	GCCACCTCTAGAGTAGTTGTGTAAGTGT
<i>Gambicin</i>	CCCTCAGAATTGCCACCATGGCTGGTTGTTATGT	CCCTCAGGGCCCGGCCCTGATCCGATGTAGCATTCGTTGA
The primers for cloning into pET28	Upper primer	Lower primer
<i>mosGCTL-29</i> (<i>His tag in both N and C-terminal</i>)	CGCCTAGCTAGCCCCAATTGTCGAG	TATCCGCTCGAGCGACCAGATGGACTGAC
<i>mosGCTL-32</i> (<i>His tag in both N and C-terminal</i>)	CGCCTAGCTAGCCAATCTCAAGTATCAAAT	TATCCGCTCGAGAAACTCTTGGATGC
The primers for PCR	Upper primer	Lower primer
<i>Bacteria Universal 16s rDNA</i>	AGAGTTTGATCTGGCTCAG	TACGGYTAGCCTGTTACGACTT