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mosGILT controls innate immunity and germ cell development in *Anopheles gambiae*

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Abstract

Gene-edited mosquitoes lacking a gamma-interferon-inducible lysosomal thiol reductase-like protein, namely (*mosGILT^{null}*) have lower *Plasmodium* infection, which is linked to impaired ovarian development and immune activation. The transcriptome of *mosGILT^{null}* *Anopheles gambiae* was therefore compared to wild type (WT) mosquitoes by RNA-sequencing to delineate mosGILT-dependent pathways. Compared to WT mosquitoes, *mosGILT^{null}* *A. gambiae* demonstrated altered expression of genes related to oogenesis, 20-hydroxyecdysone synthesis, as well as immune-related genes. Serendipitously, the zero population growth gene, *zpg*, an essential regulator of germ cell development was found to be one of the most downregulated genes in *mosGILT^{null}* mosquitoes. These results provide a crucial missing link between two previous studies on the role of *zpg* and *mosGILT* in ovarian development. This study further demonstrates that mosGILT has the potential to serve as a target for the biological control of mosquito vectors and to influence the *Plasmodium* life cycle within the vector.

Keywords Anopheles, Mosquito, Immunity, Metabolism, Genomics, Ovarian Development, mosGILT, GILT, ZPG, Mosquito Immunity, Mosquito Reproduction, Fat body, Malaria, 20-Hydroxyecdysone

Introduction

Malaria remains one of the deadliest vector-borne diseases, with over 247 million cases and approximately 619 thousand deaths reported globally in 2021 [1]. It is caused by apicomplexan protozoa of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. During its journey through the vector, *Plasmodium* is profoundly influenced by mosquito factors that either facilitate or suppress parasite development [2–5]. In the mosquito midgut, *Plasmodium* development begins with the differentiation of gametocytes into gametes, followed by the formation of zygotes, ookinetes, and then oocysts. Parasite numbers suffer great losses, especially during traversal of the midgut epithelium [6, 7]. This midgut bottleneck of the *Plasmodium* life cycle is, therefore, an optimal target for blocking malaria transmission [8–13]. Considering the increase of insecticide resistance in *Anopheles* [5, 14–16], a better knowledge of how mosquito-derived factors influence midgut-stage parasites

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is important for developing novel strategies for malaria intervention.

Blood-feeding is important for the female mosquito to obtain the nutrients required for egg development. Studies suggest that *Plasmodium* maturation in the midgut is influenced by concurrent mosquito reproductive processes, yet the underlying mechanisms remain poorly understood [17, 18]. Through CRISPR/Cas9 gene editing, our group demonstrated that disruption of a mosquito gamma-interferon-inducible lysosomal thiol reductase-like protein—*mosGILT*—caused underdeveloped ovaries with a reduction in egg development and the production of ecdysteroid hormone 20-hydroxyecdysone (20E), and vitellogenin (Vg) [19]. Interestingly, *mosGILT*-deficient (*mosGILT*^{null}) *Anopheles gambiae* also showed significant suppression of both *Plasmodium berghei* and *Plasmodium falciparum* infection, as measured by reduced oocyst intensity and prevalence [19]. In parallel, another study showed that *Anopheles* mosquitoes with a disrupted zero-population growth—*zpg*—gene also exhibited underdeveloped ovaries that produced low amounts of 20E and were less permissive to *P. falciparum* infection [20]. Though both *mosGILT*^{null} and *zpg*^{null} mosquitoes exhibited somewhat similar phenotypes, it is unclear whether their mechanisms of action are similar or distinct. In this study, we elucidate the *mosGILT*-dependent global transcriptional responses in *A. gambiae*. The information here may provide novel insights into the *A. gambiae* reproductive system network, as well as innate responses associated with *Plasmodium* infection.

Results

Transcriptome analysis of *mosGILT*^{null} *A. gambiae*

To examine whether the molecular responses to a blood meal were altered in *mosGILT*^{null} *A. gambiae*, RNA sequencing (RNA-seq) was performed. *mosGILT*^{null} and Wild type (WT, control) mosquitoes were fed on naïve mice and then separated into cardboard containers. The whole body, ovaries, and fat bodies were collected from blood-fed mosquitoes 24 h after completion of the meal. RNA-seq was carried out with total RNA extracted from the whole body, ovaries, or fat bodies from *A. gambiae* using Illumina sequencing technology. A total of 16 samples (from control and *mosGILT*^{null} *A. gambiae*) were sequenced (Supplementary Fig. 1). After sequencing, each library generated around 10–28 million reads and approximately 78.0–99.7% of the reads in each library mapped to the *A. gambiae* genome (Supplementary Figs. 2, 3, and 4).

Principal component analysis of mosquito libraries examined the clustering of data/samples after a blood meal. All biological replicates of control and *mosGILT*^{null} samples were distributed into two distinct groups (Fig. 1A). Volcano plots indicated that there were differentially expressed genes (DEGs) in all three groups (Fig. 1B), with whole-body samples having the maximum number of DEGs ($n = 5749$), followed by the ovaries and fat bodies. In all the three samples analyzed, 108 DEGs were common to all three groups (Fig. 2A). Of particular interest were 4991 DEGs in the ovaries of *mosGILT*^{null} mosquitoes which are potentially helpful to elucidate the role of *mosGILT* in *A. gambiae* development and *Plasmodium* infection.

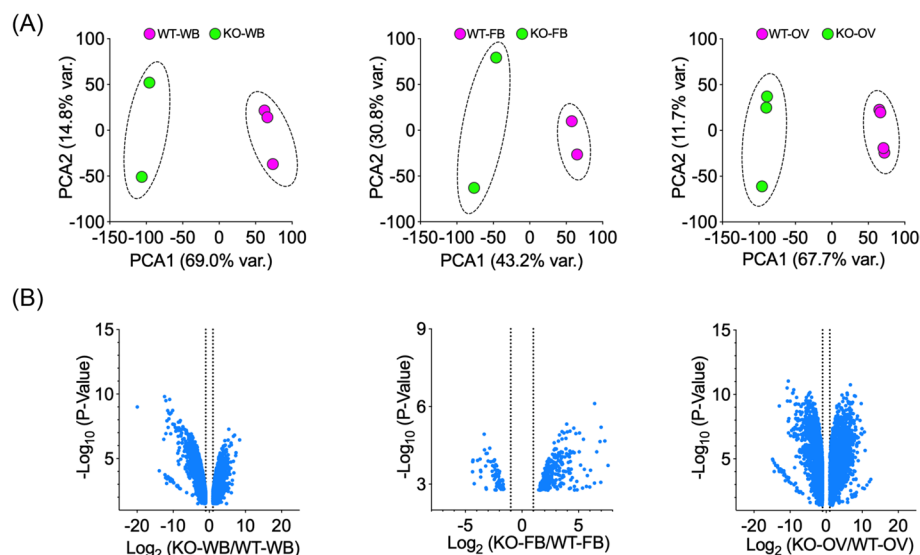


Fig. 1 **A** Principal component analysis of the RNA-Seq data. The whole body (WB), fat body (FB) and ovary (OV) samples were collected at 24 h from blood-fed (BF) wild-type (WT) and *mosGILT*^{null} *Anopheles gambiae*. **B** Volcano plot analysis of differentially expressed genes (DEGs) of whole body (WB), fat body (FB) and ovary (OV) between wild type (control) and *mosGILT*^{null} *Anopheles gambiae*. DEGs with False Discovery Rate (FDR) p -values of < 0.05 and \log_2 fold changes > 1 are presented

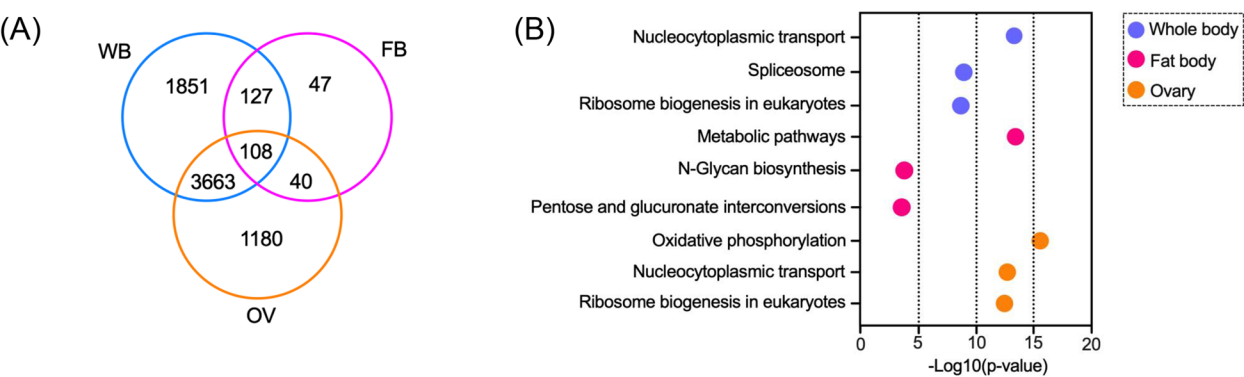


Fig. 2 **A** Venn diagrams showing the number of differentially expressed genes (DEGs) between whole body (WB), fat body (FB) and ovary (OV) tissues of blood-fed *Anopheles gambiae*. The numbers in the overlapping areas indicate genes that were common to both or to all three different time points. The number of DEGs was highest between the whole body and ovary samples. **B** Bubble chart and circle graph showing enriched pathways in the whole body, fat body, and ovary of *mosGILT*^{null} mosquitoes by KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis. Gene ontology annotation and pathway enrichment analysis of the top differentially expressed genes (DEGs) in the whole body, fat body, and ovary are shown. Enriched functional ontology terms are shown on the left. The whole body, fat body, and ovary are represented by blue, red, and orange colors respectively. The Log₁₀ (p-value) is shown on the X-axis

DEG analysis reveals that *mosGILT* has a role in nucleocytoplasmic transport

Since female *mosGILT*^{null} mosquitoes display defects in ovarian development and show refractoriness to *Plasmodium*, we first focused on the nature of genes that showed differential expression patterns in the ovarian tissue. Among the 4991 DEGs, 117 genes had a more than a 100-fold increase in mRNA abundance compared

to the WT controls, and 97 genes showed at least a 100-fold lower mRNA abundance. Several groups of genes showed altered expression patterns in oxidative phosphorylation and nucleocytoplasmic transport based on Gene Ontology (GO) annotation (Figs. 2B, and 3A, B, C, D). Nucleocytoplasmic transport (75 genes, *p*-value 2.60E-13) is a process crucial for cell function (Supplementary Table 1 and Supplementary Fig. 5). Depletion

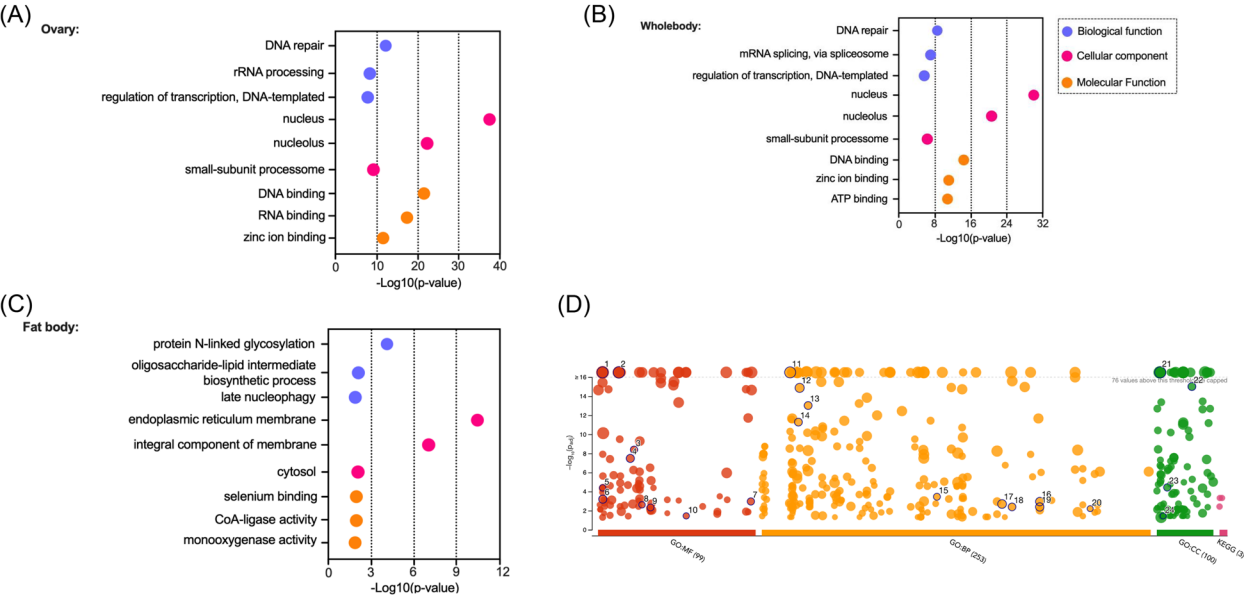


Fig. 3 **A, B and C** Bubble chart showing enrichment of Gene Ontology (GO) terms of the differentially expressed genes in the whole body, fat body, and ovary of *mosGILT*^{null} mosquitoes. The biological processes, cellular components, and molecular functions are shown in blue, magenta and orange respectively. The -Log₁₀ (p-value) for each process is shown on the X-axis. **D** The figure presents the Manhattan plot showcasing enrichment analysis of differentially expressed genes in the ovary of *mosGILT*^{null} mosquitoes performed using g:Profiler. The x-axis represents functional GO terms grouped and color-coded based on data sources. The y-axis displays adjusted enrichment *p*-values on a negative log₁₀ scale

of mammalian GILT (IFI30) in immune cells can inhibit cytokine production and the release of high mobility group box 1 (HMGB1), a nonhistone nuclear protein. The cytosolic translocation of the nuclear HMGB1 protein is then activated and associated with increased autophagy in GILT-depleted fibroblasts [21, 22]. These findings suggest that mammalian GILT is involved in the homeostatic regulation of oxidative stress. In the absence of *mosGILT*, nucleocytoplasmic transport pathways are upregulated which can influence diverse cellular responses, such as autophagy (Fig. 2B). Indeed, autophagy negatively regulates Vg production in gonotrophic cycles in *Aedes* mosquitoes, which is consistent with the changes in Vg production as shown by our group previously [23].

Whole body analysis of *A. gambiae* confirms the role of *mosGILT* in development and metabolism

Our previous studies demonstrated that *mosGILT* is implicated in embryonic and ovarian development and shows refractoriness to *Plasmodium* infection [19]. To fully understand how female *mosGILT^{null}* mosquitoes show resistance to *Plasmodium*, we also examined the genes that showed differential expression patterns in the whole body. Among the 5749 DEGs, 76 genes had a more than a 100-fold decrease in expression compared

to the WT control, and 7 genes showed at least 100-fold upregulation. Similar to the ovaries, nucleocytoplasmic transport pathways were affected in the whole body of *mosGILT^{null}* mosquitoes. The most downregulated proteins included AGAP007823 (meiosis arrest female protein 1, fold change: -1000000, p -value < 0.0001) and AGAP008738 (eukaryotic translation initiation factor 4E transporter, fold change -14625, p -value 0.0001) which is involved in protein translation and oogenesis (Fig. 4A).

The most overexpressed genes included AGAP010658 (fold change 169, p -value < 0.00001) which encodes a homolog of the hexameric 2 beta protein or larval serum protein 1 which serves as a major storage protein. AGAP010658 antagonizes *Plasmodium berghei* oocyst development and silencing of this gene by RNAi results in a significant increase in oocyst infection intensities and melanization of ookinetes [24]. Other highly expressed genes were AGAP000046 (a sugar transporter, fold change 160, p -value < 0.0001) and AGAP002518 (delta 1-pyrroline-5-carboxylate synthetase, fold change 102, p -value < 0.00001). While transcripts of AGAP004203 were decreased (fold change -343), another Vg-like protein was overexpressed (AGAP008369 fold change 102, p -value < 0.00001). Among the major downregulated genes were AGAP002134 (vitelline membrane

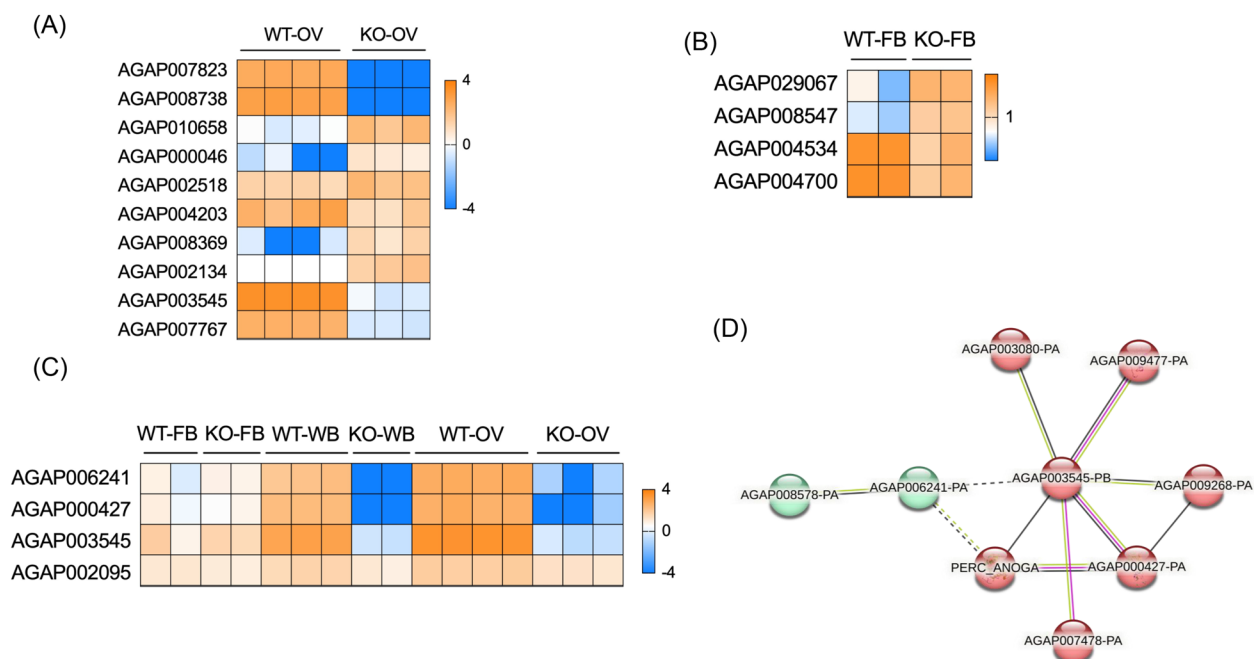


Fig. 4 Heat Map analysis of key differentially expressed genes in the ovary (A), and fat body (B) of *mosGILT^{null}* mosquitoes. C Heat map analysis of key differentially expressed genes that are related to germ cell development in *mosGILT^{null}* mosquitoes. D STRING functional gene analysis of *zpg*-related genes: Each node represents a protein product of an investigated gene. Lines denote protein-protein interactions, with each edge representing an interaction including either physical or functional associations

component which is required for the proper assembly of the eggshell) AGAP003545 (maternal effect protein oskar), and AGAP007767 (mosquito-specific bZIP1 transcription factor) (Fig. 4A). Indeed, silencing of *bZIP1* in the embryos of *Aedes aegypti* resulted in embryonic death [25]. These findings indicate that *mosGILT* is involved in both development and metabolism.

Effect of *mosGILT* on metabolic responses in the *A. gambiae* fat body

Our previous study showed that *mosGILT* is expressed in the ovaries and fat body [19]. In insects, the fat body plays a central role in energy metabolism. After a blood meal, the fat body, which is the mosquito fat storage organ, initiates Vg synthesis. The fat body releases Vg into the hemolymph, and it is then taken up by developing oocytes via receptor-mediated endocytosis. Since *mosGILT^{null}* mosquitoes showed lower Vg mRNA levels, we examined the transcriptional response in the fat body. Our analysis shows that in the fat body, gene transcripts of metabolic pathways (gene count: 93, *p*-value 6.30E-14) and pathways related to carbohydrate metabolism (pentose and glucuronate interconversions, fructose and mannose metabolism, galactose metabolism, biosynthesis of nucleotide sugars, glycolysis/gluconeogenesis and starch, and sucrose metabolism) demonstrated major changes in the *mosGILT^{null}* mosquitoes. Among the most altered DEGs, AGAP029067 (lipid-binding protein) transcripts were enriched by a 194-fold while transcripts of AGAP008547, a gene with a predicted function in carbohydrate metabolism showed nearly 160-fold enrichment in the fat body of *mosGILT^{null}* mosquitoes (Fig. 4A). Blood-meal ingestion, therefore, not only induces the expression of genes related to egg development but also increased the expression of genes related to oxidative stress.

In mammals, GILT is expressed in lysosomes and is required for optimal activity of cysteine proteases, such as cathepsin in macrophages [26, 27]. In mosquitoes, the fat body plays a central role in metabolism and nutrient storage, mirroring the functions of the liver in vertebrates. In response to blood-feeding, the *Aedes* fat body secretes a cathepsin-b-like thiol protease [28]. Interestingly, in our analysis of *mosGILT^{null}* *A. gambiae*, the most down-regulated genes include proteases such as AGAP004534 (cathepsin B-like serine protease, fold change -18, *p*-value 0.0001), and AGAP004700 (trypsin-like serine protease, fold change -20, *p*-value 0.0003) (Fig. 4B).

mosGILT regulates germ cell maintenance by controlling the expression of zero population growth (*zpg*)

A. gambiae females express zero population growth (*zpg*, AGAP006241), an essential gene for germ cell development. Genetic ablation of *zpg* in mosquitoes leads to severely

underdeveloped ovaries and impaired egg production after a blood meal [20, 29, 30] similar to the *mosGILT^{null}* mosquitoes. *zpg* and *mosGILT* are important for *Plasmodium* infection since mosquitoes lacking either gene show fewer oocysts after feeding on *P. falciparum*-infected blood compared to sibling controls. In the ovaries of *mosGILT^{null}* *A. gambiae*, *zpg* was strongly downregulated (fold change -8130, *p*-value 0.0001) which suggests *mosGILT* acts upstream of *zpg* or influences the molecular pathway that regulates *zpg* expression (Fig. 4C). We further performed protein network analysis (Fig. 4D, Supplementary Fig. 6) to determine whether *mosGILT* affected the expression of other genes that are associated with *zpg*. STRING analysis showed the genes that are in the *zpg* network may be involved in germ cell development. Lack of *mosGILT* affected the expression of AGAP000427 (fold change -26814, *p*-value > 0.00001), which is in the *zpg* network and is a putative Vg receptor. In the ovaries of *mosGILT^{null}* mosquitoes, another *zpg*-related protein, *oskar* (AGAP003545) was strongly downregulated (fold change -8162, *p*-value > 0.00001) (Fig. 4C). Oskar is required for the formation of the germline in *Drosophila* [31]. Germline promotes the formation and specification of the primordial germ cells, the first cell lineage to form in the fertilized embryo [32]. Our genetic analyses of *mosGILT^{null}* mosquitoes reveal underdeveloped ovaries and a decrease in 20E production. Here, we have analyzed genes involved in insect hormone biosynthesis and found that five genes (AGAP001038, AGAP001039, AGAP004665, AGAP005992, AGAP000284) involved in 20E biosynthesis were downregulated in *mosGILT^{null}* mosquitoes (Fig. 5). Signaling of 20E is initiated by binding of 20E to the heterodimer ecdysone receptor (EcR)/ultraspiracle (USP) complex, which triggers a transcriptional response that promotes egg development. Our results show that *mosGILT^{null}* also decreases the expression of ultraspiracle (USP, AGAP002095, fold change -4, *p*-value < 0.00001) (Fig. 4C). The complex formed after 20E binding to EcR/USP upregulates the expression of an array of 20E-regulated genes which in turn controls the Vg transcriptional program [33]. These results show that *mosGILT* regulates 20E levels, as well as the downstream transcriptional response.

Immune pathway alteration in *mosGILT^{null}* mosquitoes

GILT was first identified as an IFN γ -inducible, lysosomal thiol reductase in mammals [37]. GILT is also key to antigen processing in mammals but in other organisms it is part of the innate immune response [38]. Thioester-containing protein 1 (TEP1) is an important component of the *A. gambiae* innate immune system and plays a major role against *Plasmodium* parasites [39, 40]. TEP is an antimicrobial protein family that acts like the human complement protein C3 and can damage the cell

Insect Hormone Biosynthesis pathway from KEGG database

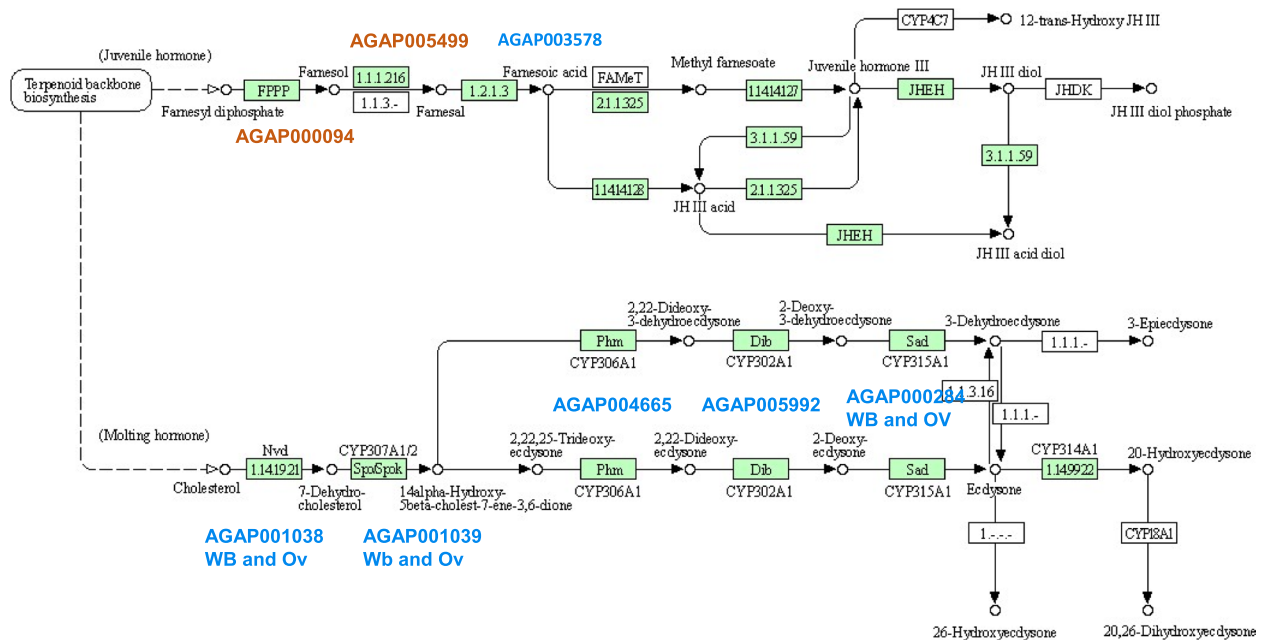


Fig. 5 Insect hormone biosynthesis pathway in *Anopheles gambiae* based on KEGG pathway analysis. Enzymes are indicated in green highlight. The DEGs genes in *mosGILT^{null}* mosquitoes are shown in orange (overexpression) and blue (downregulation) are indicated by the gene ids. KEGG Pathway entry aga00981 framework is used (<https://www.genome.jp/entry/pathway+aga00981>) [34–36]

membranes of pathogens. In our study, we observed an increase in TEP1 activity in *mosGILT^{null}* mosquitoes [19]. We also showed that strong refractoriness to *Plasmodium* infection in *mosGILT^{null}* mosquitoes was related to increased TEP1 activity. Therefore, we examined immune genes that are differentially expressed in *mosGILT^{null}* mosquitoes to better understand how TEP1 activity is influenced.

In our analysis, we found that AGAP028725 (SPCLIP1) was upregulated in the ovary (fold change 73, p -value 0.0001) and whole body (fold change 12, p -value 0.0001) of *mosGILT^{null}* mosquitoes (Fig. 6). Clip domain serine proteases (CLIPs) play an important role in innate immunity in mosquitoes. These proteases are present in hemolymph and are involved in *Plasmodium* immunity, antimicrobial peptide synthesis and melanization. Previously it was shown that complement C3-like protein TEP1 mediated killing of *Plasmodium* is dependent on the CLIP-domain serine protease homolog SPCLIP1 [41, 42]. SPCLIP1 is required for the accumulation of TEP1 on microbial surfaces, a reaction that leads to lysis of malaria parasites [41, 42].

In this study, we also found that *mosGILT^{null}* mosquitoes have increased expression of three TEP proteins—TEP15, TEP14, and TEP2. TEP15 (AGAP008364) expression was increased in the ovary (fold change 230,

p -value < 0.0001) and whole body (fold change 12, p -value 0.0001) (Fig. 6). TEP14 (AGAP008368, fold change 10, p -value 0.0017) and TEP2 (AGAP008366, fold change 9, p -value 0.0001) expression was increased in the whole body. These TEP genes were upregulated significantly upon *P. falciparum* infection in the midgut of *A. gambiae* mosquitoes [42].

mosGILT disruption also increased the expression of another innate immune gene, peptidoglycan recognition protein, PGRPLD (AGAP005552) in the ovary (fold change 125, p -value < 0.0001), whole body (fold change 8, p -value < 0.0001) and fat body (fold change 7, p -value 0.0001). Peptidoglycan recognition proteins (PGRPs) are a family of immune regulators, conserved from insects to mammals. PGRPLD is important for anti-*Plasmodium* immunity and in *PGRPLD*-silenced mosquitoes, *Plasmodium* infection increased significantly [43]. Additional immune-related genes, including 2 serpins, SRPN6 (AGAP009212) and SRPN9 (AGAP003139) was also overexpressed in the ovary (fold change 11, p -value 0.0002, fold change 8, p -value < 0.0001 respectively) and whole body (fold change 3, p -value 0.0135, fold change 3, p -value 0.0002 respectively) and fat body (SRPN9, fold change 5, p -value 0.0007) (Fig. 6). SRPN6 is a component of the midgut epithelial immune-response system which is increased during *Plasmodium* infection and

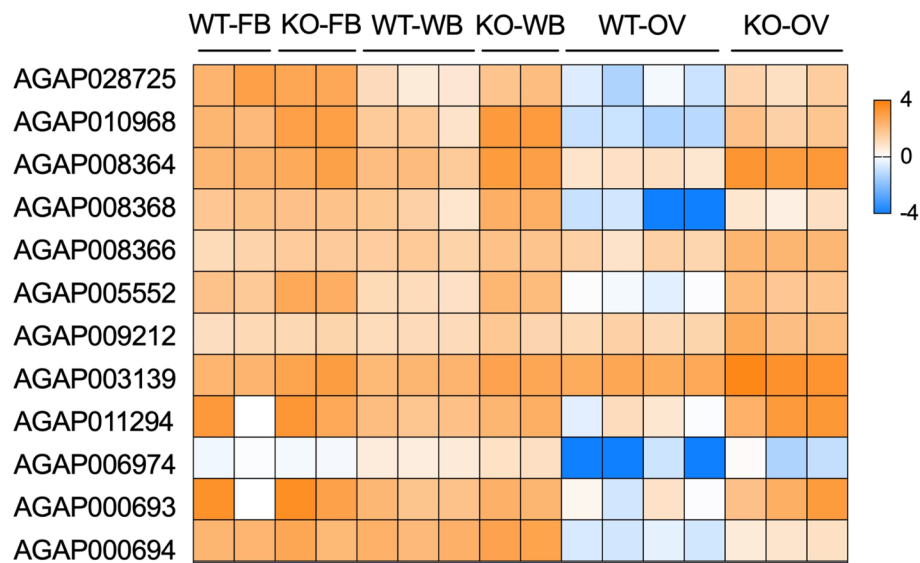


Fig. 6 Heat map analysis of key differentially expressed genes related to immune pathways in the whole body (WB), fat body (FB) and ovary (OV) of *mosGILT^{null}* mosquitoes

knockdown of this gene increased parasite numbers in *A. stephensi* [44].

In addition, we also observed increase in defensin transcripts, an anti-microbial peptide (AGAP011294, DEF1). *Def1* expression was upregulated in the ovary (fold change 255, *p*-value 0.0001) and whole body (fold change 3, *p*-value 0.0029) of *mosGILT^{null}* mosquitoes (Fig. 6). Transcripts of another anti-microbial peptide gene, *cercopin 1* (AGAP000693) was also increased in the ovary (fold change 300, *p*-value 0.0004) and whole body of the *mosGILT^{null}* mosquitoes (fold change 2, *p*-value 0.0235), and *cercopin 3* (AGAP000694) expression was upregulated in the whole body (fold change 3, *p*-value 0.0016). Toll-like receptors (TLRs) are evolutionarily conserved pattern recognition receptor proteins with diverse biological functions. In *Drosophila melanogaster*, Toll plays an important role in pattern formation during embryogenesis and in immunity. In this study we observed that TLR9 expression in the *mosGILT^{null}* mosquito was upregulated in the whole body (AGAP006974, fold change 2, *p*-value 0.0095) Though there is not much information on this gene in *A. gambiae*, in the silk moth, *Bombyx mori*, TLR9 is involved in innate immunity and functions as a pattern recognition receptor that binds lipopolysaccharide.

Discussion

To further understand *Plasmodium* infection of the vector, and develop new mosquito and malaria control strategies, it is important to decipher the molecular mechanisms related to mosquito reproduction and

immunity. Recently our lab uncovered a critical role for *mosGILT* in *A. gambiae* ovary development and *Plasmodium* infection [19]. The *mosGILT^{null}* mosquitoes displayed underdeveloped ovaries and reproductive defects including impaired production of 20E and Vg while showing an increased TEP1-mediated immune response against *Plasmodium* ookinetes [19]. However, there is a lack of knowledge regarding how *mosGILT* functions in *A. gambiae*, its interacting partners, and the implicated signaling pathways. Here we characterize the transcriptional response in *mosGILT^{null}* mosquitoes, including the ovaries, whole body, and fat body, and delineate how *mosGILT* is regulating reproduction and immune responses.

GILT is a thioredoxin-related oxidoreductase which is the only known lysosomal thiol reductase [27]. Mammalian GILT is related to the thioredoxin family of oxidoreductases, characterized by a CXXC motif in the active site and an enzymatic mechanism in which the pair of active site cysteine residues cooperate to reduce substrate disulfide bonds [45–48]. In *Anopheles* mosquitoes and other insect genera, the active motif is CXXS, where the second cysteine is replaced with serine (Fig. 7A and B). This change in the active site of *mosGILT* hinders the thioredoxin reaction of the mixed intermolecular disulfide. The CXXS containing thioredoxins can interact with their substrate proteins in a more stable manner since there is a covalent complex formed between thioredoxins and their target. However, some studies report that at least some CXXS-containing forms of thioredoxin-fold proteins retain catalytic activity albeit at a

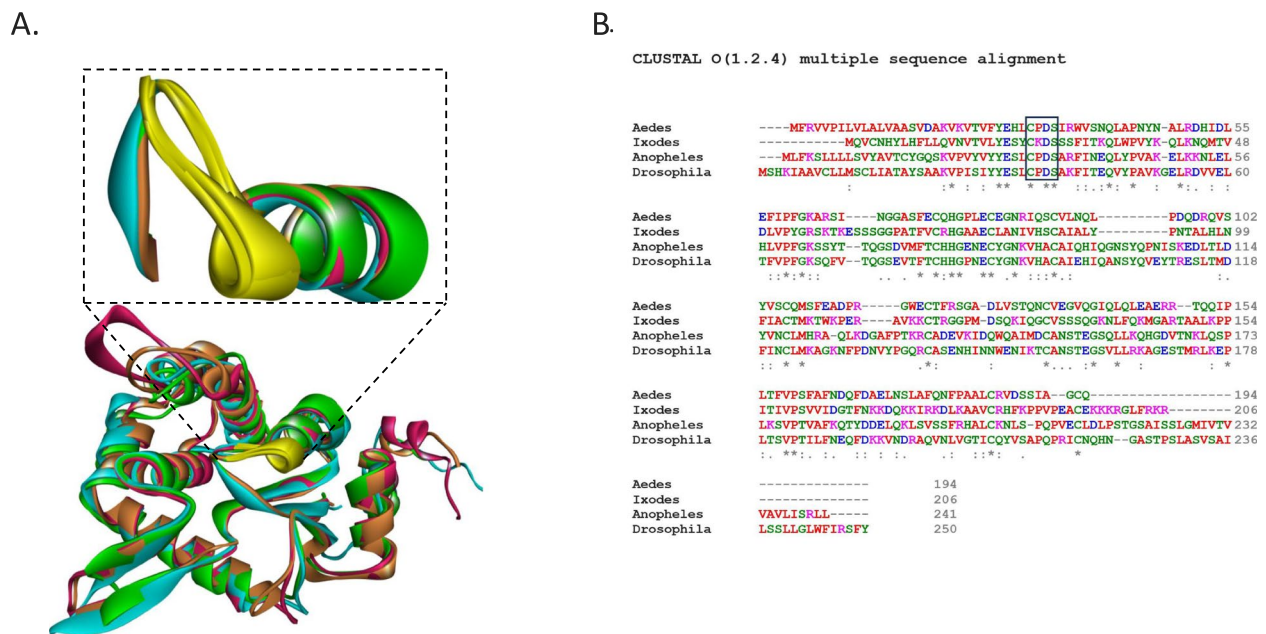


Fig. 7 Structural and sequence analysis of mosGILT **(A)** The mosGILT structural model is created by Alpha Fold. Structural alignment of mosGILT homologs from *Anopheles*, *Aedes*, *Drosophila* and human GILT. The active site motif is shown in the yellow in the inset. **(B)** Sequence alignment of *Anopheles*, *Aedes*, *Drosophila* and *Ixodes* GILT. Protein sequence of mosGILT homologs from *Anopheles gambiae* (AGAP004551), *Aedes aegypti* (XP_021707151.1 GILT-like protein 1), *Drosophila melanogaster* (NP_650287.3 Gamma-interferon-inducible lysosomal thiol reductase 1), *Ixodes scapularis* (XP_029851151.3 GILT-like protein 1). Active site residues are shown in the inset box

lower level [49, 50]. It is therefore possible that mosGILT has partial thioredoxin activity that is critical to the transcriptional response that leads to oogenesis and ovarian development.

Vector reproduction is closely associated with immune defense pathways, as these two processes are simultaneously initiated after a blood meal [51–53]. Identification of mosquito proteins that regulate cross-talk between reproduction and immunity could serve as targets for vector-control strategies. Our previous study demonstrated that mosGILT plays an essential role in the reproduction of *A. gambiae* [19]. In this study we show that mosGILT influences complement activity and upregulates defense-related genes, which are associated with anti-*Plasmodium* responses. In addition, mosGILT regulates TEP1 activity by altering SPCLIP1 expression, which can influence *Plasmodium* survival in mosquitoes. *mosGILT*^{null} mosquito refractoriness to microbes was also suggested by the increase in genes encoding antimicrobial peptides and innate immune molecules. *mosGILT* may therefore be a potential target for new malaria control strategies, such as gene drive -propelled transgenes targeting mosGILT, that would induce mosquito sterility and also reduce the overall malaria parasite burden.

Development and immunity are highly demanding processes and, thus, a balance must be maintained between the two [54]. Previously it was suggested that there is a

trade-off between reproductive fitness and immune defense in mosquitoes including *A. gambiae* and activation of immune pathways can cause a reduction in fecundity by inducing the resorption of ovarian follicles during oocyte development [55–58]. In another example, activation of immune signaling leads to decreased in reproductive capacity of *D. melanogaster* [59]. These observations suggest that in the absence of mosGILT and a reduction in ovary development, there may be an increase in the immune response. This is also supported by a recent study where it was shown that 20E binding to the ecdysone receptor (EcR) directly induces a Pirk-like inhibitor, affecting the immune deficiency (IMD) pathway in *Aedes* mosquitoes [57]. Pirk downregulates the IMD pathway by inhibiting the nuclear translocation of the NF-κB transcriptional factor Rel2 and this downregulation of the immune response is vital for maintaining mosquito fertility. We hypothesize that the effects of downregulating ecdysone on mosquito immunity can be complex. We acknowledge that in some studies 20E is shown to have immunomodulatory effects, and it can enhance the mosquitoes immune response [52, 60–62]. Our results with mosGILT null mosquitoes show that the influence of 20E on immunity may be part of a broader regulatory network. Overall our results suggest that mosGILT may be important for resource allocation in respect to both development and immunity. In the absence of

mosGILT, resources are moved to immune pathways and there is reduction in reproductive capacity. Overall, our results suggest that mosquito immunity is multilayered and hierarchical, where downregulation of the mosGILT in *Anopheles* mosquitoes is sufficient to activate the immune system even in the absence of 20E.

mosGILT mutants have underdeveloped ovaries that produced low amounts of 20E, and were significantly less permissive to *P. falciparum* infection [19]. This is somewhat similar to *Plasmodium* infection in *zpg* mutant mosquitoes, a gene that is essential for germ cell development [20, 30]. Though both mutant mosquitoes provide an association between 20E signaling and intensity of *Plasmodium* infection, it is not known if they act together or independently. Our studies now suggest that mosGILT may affect several genes that are associated with *zpg*. Since *zpg* and other germ cell development genes are considered a prime target for gene drive [63–65], our results suggest mosGILT may be more attractive target for gene drive of *A. gambiae*.

mosGILT plays an important role in reproduction and host defenses in *A. gambiae*. The elucidation of mosGILT as a regulator of *zpg* and involvement in cross-talk between reproduction and immunity could provide new targets for the development of disease-control strategies (Fig. 8). These findings can potentially lead to new preventive strategies for malaria based on *Anopheles* mosGILT and also potentially suggest a role for arthropod GILT-like proteins for other vector-borne diseases.

Materials and methods

Mosquito rearing

All the *A. gambiae* mosquito lines were derived from the G3 strain, including the docking line X1 [66], *vasa2-Cas9* [67],

mosGILT-gRNA₃ [19], *mosGILT* mutant and sibling mosquitoes. They were raised at 27 °C, 80% humidity, under a 12/12 h light/dark cycle and maintained with 10% sucrose. Swiss Webster mice (8-week-old females) were purchased from Charles River Laboratories.

Generation of transgenic *mosGILT* mutant mosaic

Anopheles mosquitoes

CRISPR/Cas9 mediated *A. gambiae* *mosGILT* somatic KO mutants were generated using the *A. gambiae* docking line X1 as described in Yang et al., 2020 [19]. In brief, the *mosGILT*-gRNA female mosquitoes were crossed with the homozygous male *Vasa Cas9* transgenic strain (*vasa2-Cas9*; with green/yellow fluorescence in the eyes;) [67, 68]. The progeny of this cross was screened at the larvae stage with both RFP (red fluorescence marker) and YFP (appeared as green fluorescence through GFP-channel under the fluorescence microscope) signals was gRNA/Cas9 trans-heterozygous. Molecular screening of these trans-heterozygotes by PCR and sequencing was done as described to confirm the *mosGILT*^{null} [19].

RNA isolation

Total RNA was extracted from the whole body, ovaries, or fat bodies from mosquitoes. Trizol was added to the tissues and RNA was isolated using a combination method which utilizes TRIzol (Thermo Fisher Scientific) and RNeasy Mini Kit (QIAGEN) as described before [69].

RNA-seq

RNA was submitted for library preparation using TruSeq (Illumina, San Diego, CA, USA) and sequenced using Illumina HiSeq 2500 by paired-end sequencing at the Yale Center for Genome Analysis (YCGA).

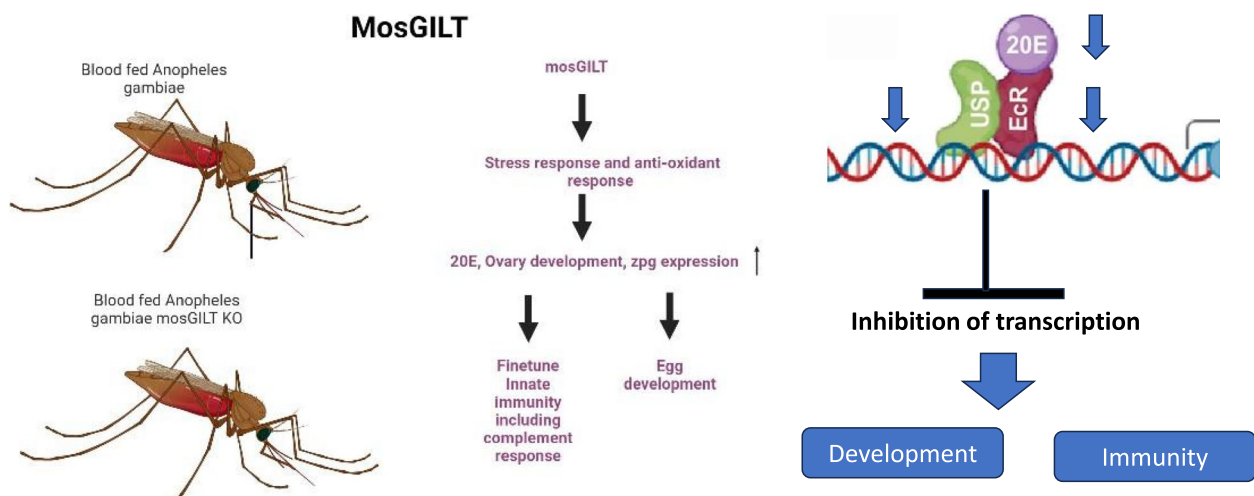


Fig. 8 Diagrammatic representation of mosGILT's role in immunity and development

Differential gene expression analysis

All the RNA-seq analyses including alignment, quantitation, normalization, and differential gene expression analyses were performed using Partek Genomics Flow software (St. Louis, MO, USA). Specifically, RNA-seq data were trimmed and aligned to the *Anopheles* PEST genome, version P4.14, with associated annotation file using STAR (v2.7.3a) [70]. The aligned reads were quantified using the Partek E/M algorithm [71] and the subsequent steps were performed on gene-level annotation followed by total count normalization. The gene-level data were normalized by dividing the gene counts by the total number of reads followed by the addition of a small offset (0.0001).

Principal components analysis

Principal components analysis (PCA) was performed using default parameters for the determination of the component number, with all components contributing equally in Partek Flow. Volcano plot Hierarchical clustering was performed on the genes that were differentially expressed across the conditions ($P < 0.05$, fold change ≥ 2 for each comparison).

Pathway enrichment analysis

Pathway enrichment was also conducted in Partek Flow and the functional annotation tool DAVID (<https://david.ncifcrf.gov/tools.jsp>). The selected genes expression heatmap was further plotted by ggplot2 (R studio) and Prism v8 (Graphpad). The selected immune pathways were further plotted on a bubble diagram by ggplot2 (version 3.3.5) in R Statistical Software (v4.1.2) as described previously [72, 73]. The network analysis was performed in String database [74, 75].

PPI network and Cluster analysis

PPI network was generated by STRING online database and visualized by Cytoscape software [75, 76]. K means clustering with 10 clusters was selected to generate cluster with edges.

Homology modeling and sequence analysis

The mosGILT structural model was created using AlphaFold [77]. The structural model of mosGILT was analyzed as detailed previously [78]. Structural alignment of mosGILT homologs from *Anopheles*, *Aedes*, *Drosophila* and human GILT. (B) Sequence alignment of *Anopheles*, *Aedes*, *Drosophila* and *Ixodes* GILT was performed using Clustal Omega (Version 1.2.4) [79]. Protein sequence of mosGILT homologs from *Anopheles gambiae* (AGAP004551), *Aedes aegypti* (XP_021707151.1 GILT-like protein 1), *Drosophila melanogaster* (NP_650287.3 Gamma-interferon-inducible lysosomal thiol reductase 1),

Ixodes scapularis (XP_029851151.3 GILT-like protein 1) were analyzed.

Statistical analysis

All data analysis, graphing, and statistics were performed in Prism (v8.0, GraphPad Software) and R Statistical Software (v4.1.2) (<https://www.r-project.org/>).

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-023-09887-0>.

Additional file 1: Supplementary Figure 1. Distribution of Raw counts in all *Anopheles* samples. **Supplementary Figure 2.** Key statistics of Ovary samples. **Supplementary Figure 3.** Key statistics of whole-body samples. **Supplementary Figure 4.** Key statistics of Fatbody samples. **Supplementary Figure 5.** Pathway enrichment analysis using the g:Profiler online tool. Table provides comprehensive information related to the selected term, including the data source, term ID, term name, and corresponding p-value. Abbreviations: GO:BP (Gene Ontology: Biological Process); GO:CC (GO: Cellular Component); GO:MF (GO: Molecular Function). **Supplementary Figure 6.** Protein-Protein Interaction (PPI) network: The interaction Network of DEGs in the Ovary of mosGILTnull mosquitoes was created using the STRING database and imported into Cytoscape. The k clustering method was used to identify clusters and key nodes in this network.

Additional file 2: Supplementary Table 1. Fold change data and raw statistics showing the role of mosGILT in *Anopheles* mosquitoes.

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Authors' contributions

Conceived and designed the experiments: GA, XT, EF. Performed the experiments: GA, XT, YC, JY, YMC, AS, YD. Analyzed the data: GA, XT, JJ, EF. Contributed reagents/materials/analysis tools: JY, JJ, YD, PC, GD, EF. Wrote the main manuscript text: GA, XT, EF. All authors reviewed the manuscript.

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Availability of data and materials

RNAseq data are deposited in a public database (Sequence Read Archive (SRA) data. The RNA-seq data are available in the Gene Expression Omnibus (GEO) repository at the National Center for Biotechnology Information under the accession number: GSE239600 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE239600>).

Declarations

Ethics approval and consent to participate

Mice used in the study were housed at the Yale Animal Resource Center of Yale University. Mice were handled in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA. The Institutional Animal Care and Use Committee of Yale University approved the animal experimental protocol (protocol permit no. 2017-07941) and followed the ARRIVE Guidelines (<https://arriveguidelines.org/>).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- World Health Organization. World Malaria report. 2022. <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022>.
- Arora G, Chuang YM, Sinnis P, Dimopoulos G, Fikrig E. Malaria: influence of *Anopheles* mosquito saliva on *Plasmodium* infection. *Trends Immunol.* 2023;44(4):256–65.
- Dong S, Dong Y, Simoes ML, Dimopoulos G. Mosquito transgenesis for malaria control. *Trends Parasitol.* 2022;38(1):54–66.
- Waterhouse RM, Kriventseva EV, Meister S, Xi Z, Alvarez KS, Bartholomay LC, et al. Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. *Science.* 2007;316(5832):1738–43.
- Shaw WR, Catteruccia F. Vector biology meets disease control: using basic research to fight vector-borne diseases. *Nat Microbiol.* 2019;4(1):20–34.
- Volohonsky G, Paul-Gilloteaux P, Stafkova J, Soichot J, Salamero J, Levashina EA. Kinetics of *Plasmodium* midgut invasion in *Anopheles* mosquitoes. *PLoS Pathog.* 2020;16(9):e1008739.
- Baton LA, Ranford-Cartwright LC. How do malaria ookinetes cross the mosquito midgut wall? *Trends Parasitol.* 2005;21(1):22–8.
- Wang S, Jacobs-Lorena M. Genetic approaches to interfere with malaria transmission by vector mosquitoes. *Trends Biotechnol.* 2013;31(3):185–93.
- Smith RC, Vega-Rodriguez J, Jacobs-Lorena M. The *Plasmodium* bottleneck: malaria parasite losses in the mosquito vector. *Mem Inst Oswaldo Cruz.* 2014;109(5):644–61.
- Blandin SA, Marois E, Levashina EA. Antimalarial responses in *Anopheles gambiae*: from a complement-like protein to a complement-like pathway. *Cell Host Microbe.* 2008;3(6):364–74.
- Vijay S, Rawal R, Kadian K, Singh J, Adak T, Sharma A. Proteome-wide analysis of *Anopheles* culicifacies mosquito midgut: new insights into the mechanism of refractoriness. *BMC Genomics.* 2018;19(1):337.
- Bennink S, Kiesow MJ, Pradel G. The development of malaria parasites in the mosquito midgut. *Cell Microbiol.* 2016;18(7):905–18.
- Whitten MM, Shiao SH, Levashina EA. Mosquito midguts and malaria: cell biology, compartmentalization and immunology. *Parasite Immunol.* 2006;28(4):121–30.
- Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet.* 2016;387(10029):1785–8.
- Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol.* 2016;32(3):187–96.
- Benelli G, Beier JC. Current vector control challenges in the fight against malaria. *Acta Trop.* 2017;174:91–6.
- Costa G, Gildenhard M, Elderling M, Lindquist RL, Hauser AE, Sauerwein R, et al. Non-competitive resource exploitation within mosquito shapes within-host malaria infectivity and virulence. *Nat Commun.* 2018;9(1):3474.
- Marcenac P, Shaw WR, Kakani EG, Mitchell SN, South A, Werling K, et al. A mating-induced reproductive gene promotes *Anopheles* tolerance to *Plasmodium falciparum* infection. *PLoS Pathog.* 2020;16(12):e1008908.
- Yang J, Schleicher TR, Dong Y, Park HB, Lan J, Cresswell P, et al. Disruption of *mosGILT* in *Anopheles gambiae* impairs ovarian development and *Plasmodium* infection. *J Exp Med.* 2020;217(1):e20190682.
- Werling K, Shaw WR, Itoe MA, Westervelt KA, Marcenac P, Paton DG, et al. Steroid hormone function controls non-competitive *Plasmodium* development in *Anopheles*. *Cell.* 2019;177(2):315–25.
- Chen R, Kang R, Tang D. The mechanism of HMGB1 secretion and release. *Exp Mol Med.* 2022;54(2):91–102.
- Chiang HS, Maric M. Lysosomal thiol reductase negatively regulates autophagy by altering glutathione synthesis and oxidation. *Free Radic Biol Med.* 2011;51(3):688–99.
- Bryant B, Raikhel AS. Programmed autophagy in the fat body of *Aedes aegypti* is required to maintain egg maturation cycles. *PLoS One.* 2011;6(11):e25502.
- Lombardo F, Christophides GK. Novel factors of *Anopheles gambiae* haemocyte immune response to *Plasmodium berghei* infection. *Parasit Vectors.* 2016;9:78.
- Criscione F. A mosquito-specific bZIP transcription factor and the influence of a Y-specific gene on sex determination in *Anopheles stephensi*. Virginia Polytechnic Institute and State University. 2013. <https://vtechworks.lib.vt.edu/server/api/core/bitstreams/9a507e7f-fd95-47b2-97f4-773435537f71/content>.
- Ewanchuk BW, Arnold CR, Balce DR, Premnath P, Orsetti TL, Warren AL, et al. A non-immunological role for gamma-interferon-inducible lysosomal thiol reductase (GILT) in osteoclastic bone resorption. *Sci Adv.* 2021;7(17):eabd3684.
- Balce DR, Allan ERO, McKenna N, Yates RM. gamma-Interferon-inducible lysosomal thiol reductase (GILT) maintains phagosomal proteolysis in alternatively activated macrophages. *J Biol Chem.* 2014;289(46):31891–904.
- Cho WL, Tsao SM, Hays AR, Walter R, Chen JS, Snigirevskaya ES, et al. Mosquito cathepsin B-like protease involved in embryonic degradation of vitellin is produced as a latent extraovarian precursor. *J Biol Chem.* 1999;274(19):13311–21.
- Magnusson K, Mendes AM, Windbichler N, Papathanos PA, Nolan T, Dottorini T, et al. Transcription regulation of sex-biased genes during ontogeny in the malaria vector *Anopheles gambiae*. *PLoS One.* 2011;6(6):e21572.
- Tazuke SI, Schulz C, Gilboa L, Fogarty M, Mahowald AP, Guichet A, et al. A germline-specific gap junction protein required for survival of differentiating early germ cells. *Development.* 2002;129(10):2529–39.
- Ephrussi A, Dickinson LK, Lehmann R. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell.* 1991;66(1):37–50.
- Kistler KE, Trcek T, Hurd TR, Chen R, Liang FX, Sall J, et al. Phase transitioned nuclear Oskar promotes cell division of *Drosophila* primordial germ cells. *Elife.* 2018;7:e37949.
- Ferracchiato S, Catteruccia F, Peirce MJ. 20E-dependent tyrosine phosphorylation of phospholipase C gamma underpins egg development in the malaria vector *Anopheles gambiae*. *bioRxiv.* 2022:2022.09.01.506175. <https://www.biorxiv.org/content/10.1101/2022.09.01.506175v1>.
- Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res.* 2023;51(D1):D587–92.
- Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* 2019;28(11):1947–51.
- Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 2000;28(1):27–30.
- Arunachalam B, Pan M, Cresswell P. Intracellular formation and cell surface expression of a complex of an intact lysosomal protein and MHC class II molecules. *J Immunol.* 1998;160(12):5797–806.
- Liu M, Liu H, Guan X, Ai H, Wu H, Liu P, et al. Characterization and expression of gamma-interferon-inducible lysosomal thiol reductase (GILT) gene in rainbow trout (*Oncorhynchus mykiss*) with implications for GILT in innate immune response. *Immunogenetics.* 2013;65(12):873–82.
- Blandin S, Shiao SH, Moita LF, Janse CJ, Waters AP, Kafatos FC, et al. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell.* 2004;116(5):661–70.
- Levashina EA, Moita LF, Blandin S, Vriend G, Lagueux M, Kafatos FC. Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito. *Anopheles gambiae* Cell. 2001;104(5):709–18.
- Povelones M, Bhagavatula L, Yassine H, Tan LA, Upton LM, Osta MA, et al. The CLIP-domain serine protease homolog SPCLIP1 regulates complement recruitment to microbial surfaces in the malaria mosquito *Anopheles gambiae*. *PLoS Pathog.* 2013;9(9):e1003623.
- Dong Y, Aguilar R, Xi Z, Warr E, Mongin E, Dimopoulos G. *Anopheles gambiae* immune responses to human and rodent *Plasmodium* parasite species. *PLoS Pathog.* 2006;2(6):e52.

43. Gendrin M, Turlure F, Rodgers FH, Cohuet A, Morlais I, Christophides GK. The peptidoglycan recognition proteins PGRPLA and PGRPLB regulate anopheles immunity to bacteria and affect infection by plasmodium. *J Innate Immun.* 2017;9(4):333–42.
44. Abraham EG, Pinto SB, Ghosh A, Vanlandingham DL, Budd A, Higgs S, et al. An immune-responsive serpin, SRPN6, mediates mosquito defense against malaria parasites. *Proc Natl Acad Sci U S A.* 2005;102(45):16327–32.
45. Kongton K, McCall K, Phongdara A. Identification of gamma-interferon-inducible lysosomal thiol reductase (GILT) homologues in the fruit fly *Drosophila melanogaster*. *Dev Comp Immunol.* 2014;44(2):389–96.
46. Fomenko DE, Gladyshev VN. CxS: fold-independent redox motif revealed by genome-wide searches for thiol/disulfide oxidoreductase function. *Protein Sci.* 2002;11(10):2285–96.
47. Hastings KT, Cresswell P. Disulfide reduction in the endocytic pathway: immunological functions of gamma-interferon-inducible lysosomal thiol reductase. *Antioxid Redox Signal.* 2011;15(3):657–68.
48. Schleicher TR, Yang J, Freudzon M, Rembisz A, Craft S, Hamilton M, et al. A mosquito salivary gland protein partially inhibits *Plasmodium* sporozoite cell traversal and transmission. *Nat Commun.* 2018;9(1):2908.
49. Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A, et al. ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. *EMBO J.* 2002;21(4):835–44.
50. Norgaard P, Winther JR. Mutation of yeast Eug1p CXXS active sites to CXXC results in a dramatic increase in protein disulphide isomerase activity. *Biochem J.* 2001;358(Pt 1):269–74.
51. Clayton AM, Dong Y, Dimopoulos G. The *Anopheles* innate immune system in the defense against malaria infection. *J Innate Immun.* 2014;6(2):169–81.
52. Reynolds RA, Kwon H, Smith RC. 20-Hydroxyecdysone primes innate immune responses that limit bacterial and malarial parasite survival in *Anopheles gambiae*. *mSphere.* 2020;5(2):e00983–19.
53. Upton LM, Povelones M, Christophides GK. *Anopheles gambiae* blood feeding initiates an anticipatory defense response to *Plasmodium berghei*. *J Innate Immun.* 2015;7(1):74–86.
54. Schwenke RA, Lazzaro BP, Wolfner MF. Reproduction-immunity trade-offs in insects. *Annu Rev Entomol.* 2016;61:239–56.
55. Critchlow JT, Prakash A, Zhong KY, Tate AT. Mapping the functional form of the trade-off between infection resistance and reproductive fitness under dysregulated immune signaling. *bioRxiv.* 2023. <https://www.biorxiv.org/content/10.1101/2023.08.10.552815v1>. <https://pubmed.ncbi.nlm.nih.gov/37645726/>.
56. Ahmed AM, Hurd H. Immune stimulation and malaria infection impose reproductive costs in *Anopheles gambiae* via follicular apoptosis. *Microbes Infect.* 2006;8(2):308–15.
57. Wang M, Wang Y, Chang M, Wang X, Shi Z, Raikhel AS, et al. Ecdysone signaling mediates the trade-off between immunity and reproduction via suppression of amyloids in the mosquito *Aedes aegypti*. *PLoS Pathog.* 2022;18(9):e1010837.
58. Shaw WR, Marcenac P, Catteruccia F. *Plasmodium* development in *Anopheles*: a tale of shared resources. *Trends Parasitol.* 2022;38(2):124–35.
59. Nystrand M, Dowling DK. Dose-dependent effects of an immune challenge at both ultimate and proximate levels in *Drosophila melanogaster*. *J Evol Biol.* 2014;27(5):876–88.
60. Reynolds RA, Kwon H, Alves ESTL, Olivas J, Vega-Rodriguez J, Smith RC. The 20-hydroxyecdysone agonist, halofenozide, promotes anti-*Plasmodium* immunity in *Anopheles gambiae* via the ecdysone receptor. *Sci Rep.* 2020;10(1):21084.
61. Keith SA. Steroid hormone regulation of innate immunity in *Drosophila melanogaster*. *PLoS Genet.* 2023;19(6):e1010782.
62. Rus F, Flatt T, Tong M, Aggarwal K, Okuda K, Kleino A, et al. Ecdysone triggered PGRP-LC expression controls *Drosophila* innate immunity. *EMBO J.* 2013;32(11):1626–38.
63. Terradas G, Hermann A, James AA, McGinnis W, Bier E. High-resolution in situ analysis of Cas9 germline transcript distributions in gene-drive *Anopheles* mosquitoes. *G3 (Bethesda).* 2022;12(1):jkab369.
64. Hammond A, Karlsson X, Morianou I, Kyrou K, Beaghton A, Gribble M, et al. Regulating the expression of gene drives is key to increasing their invasive potential and the mitigation of resistance. *PLoS Genet.* 2021;17(1):e1009321.
65. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, et al. A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nat Biotechnol.* 2018;36(11):1062–6.
66. Volohonsky G, Terenzi O, Soichot J, Naujoks DA, Nolan T, Windbichler N, et al. Tools for *Anopheles gambiae* Transgenesis. *G3 (Bethesda).* 2015;5(6):1151–63.
67. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nat Biotechnol.* 2016;34(1):78–83.
68. Dong Y, Simoes ML, Marois E, Dimopoulos G. CRISPR/Cas9-mediated gene knockout of *Anopheles gambiae* FREP1 suppresses malaria parasite infection. *PLoS Pathog.* 2018;14(3):e1006898.
69. Untergasser. RNAprep: Trizol combined with columns. 2008.
70. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29(1):15–21.
71. Xing Y, Yu T, Wu YN, Roy M, Kim J, Lee C. An expectation-maximization algorithm for probabilistic reconstructions of full-length isoforms from splice graphs. *Nucleic Acids Res.* 2006;34(10):3150–60.
72. Wickham H. ggplot2. *WIREs Comput Stat.* 2011;3(2):180–5.
73. Arora G, Lynn GE, Tang X, Rosen CE, Hoornstra D, Sajid A, et al. CD55 facilitates immune evasion by *Borrelia crocidurae*, an agent of relapsing fever. *mBio.* 2022;13(5):6122.
74. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryar F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* 2023;51(D1):D638–46.
75. Snel B, Lehmann G, Bork P, Huynen MA. STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. *Nucleic Acids Res.* 2000;28(18):3442–4.
76. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003;13(11):2498–504.
77. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. *Nature.* 2021;596(7873):583–9.
78. Arora G, Sajid A, Singhal A, Joshi J, Virmani R, Gupta M, et al. Identification of Ser/Thr kinase and forkhead associated domains in *Mycobacterium ulcerans*: characterization of novel association between protein kinase Q and MupFHA. *PLoS Negl Trop Dis.* 2014;8(11):e3315.
79. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol.* 2011;7:539.

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