

Supplementary Information

Desat1-mediated lipid homeostasis mitigates 20E-induced lipotoxicity in blood-fed mosquitoes

Peilu Sun^{1,2,†}, Guandong Wang^{1,2,†}, Bingchen Yang^{1,2}, Yiguan Wang^{1,2}, Chunlai Cui^{1,2,3}, Ling Dong^{1,2}, Yifei Li^{1,2}, Tao Zhou^{1,2}, Fang Li^{1,2}, Sibao Wang^{1,2*}

¹ New Cornerstone Science Laboratory, Key Laboratory of Insect Developmental and Evolutionary Biology, State Key Laboratory of Plant Trait Design, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China.

² CAS Center for Excellence in Biotic Interactions, University of Chinese Academy of Sciences, Beijing, China.

³ Shanghai Institute of Wildlife Epidemics, School of Life Sciences, East China Normal University, Shanghai, China.

*** To whom corresponding may be addressed:**

Wang, S., CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences

300 Feng Lin Road, Xuhui District, Shanghai, China

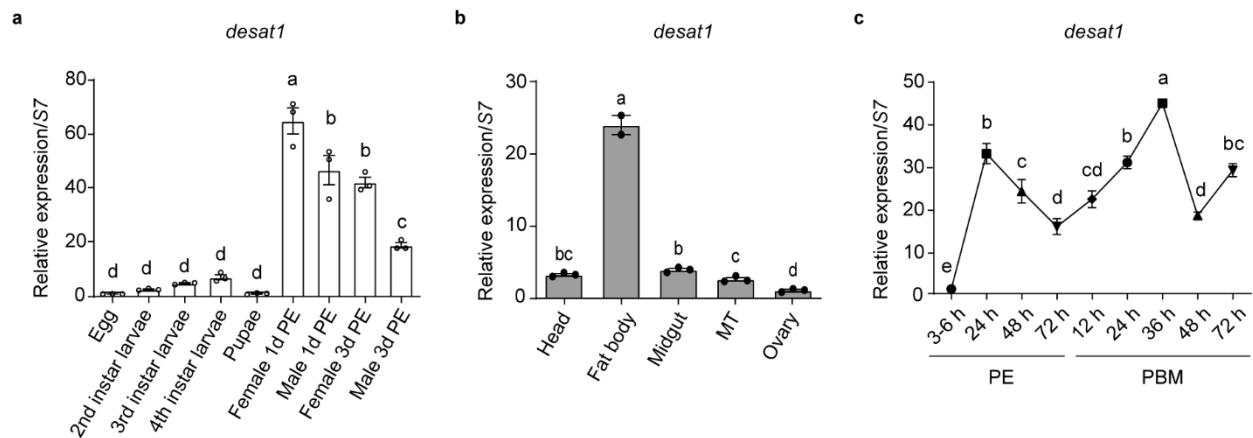
E-mail: sbwang@cemps.ac.cn

Phone: +86 (0)21-54924339; FAX: +86 (0)21-54924339

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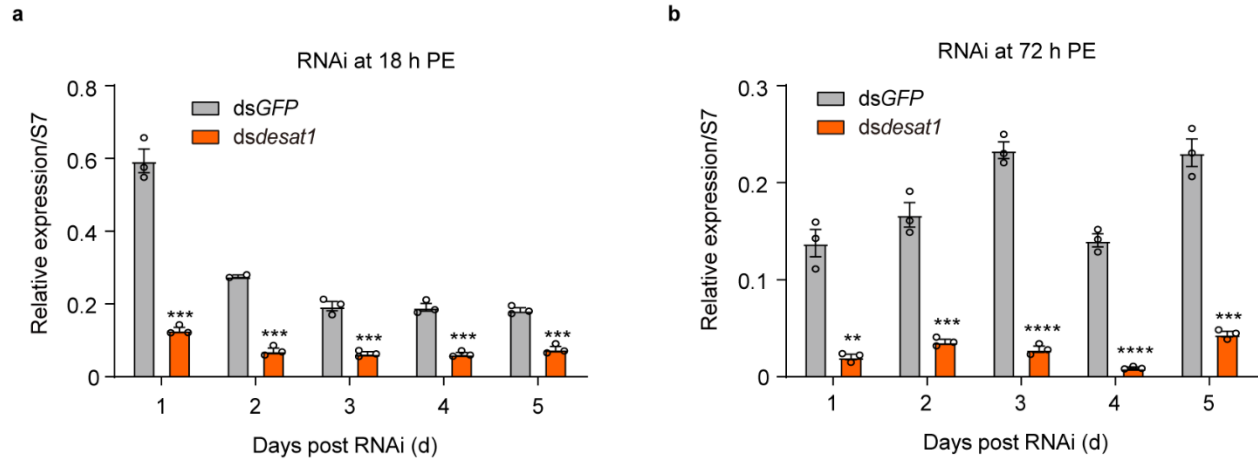
Supplementary Figures 1 to 10

Supplementary Table 1



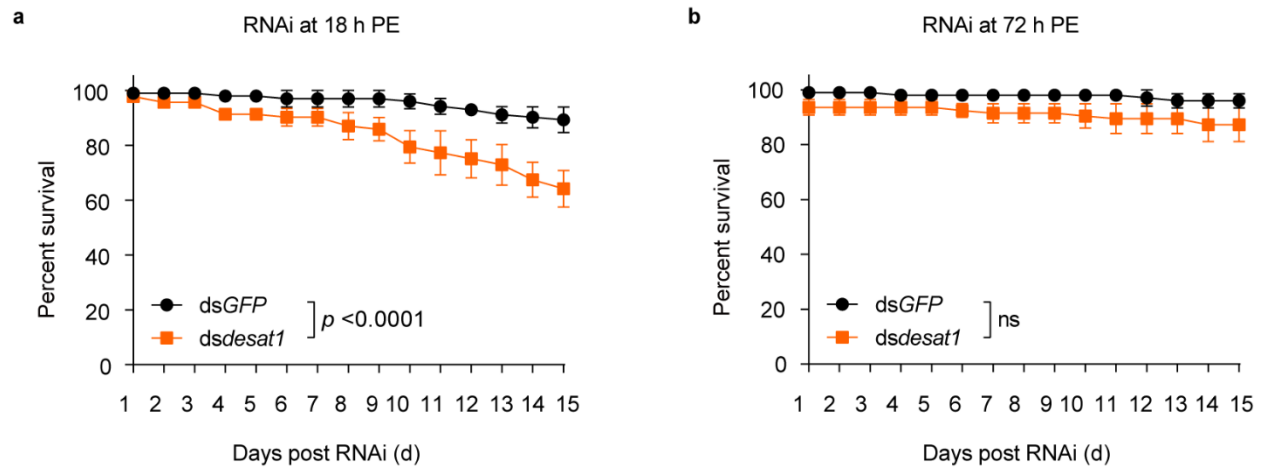
Supplementary Figure 1 | *An. stephensi desat1* expression at different developmental stages and tissues.

a-c qPCR analysis of *An. stephensi desat1* transcription at different developmental stages (**a**), in different adult female tissues (**b**), and in the female fat body (**c**). MT, Malpighian tubules; PE, post-eclosion; PBM, post-blood meal. *S7* was used as a reference gene. Error bars indicate SEM. Statistical analyses were performed using one-way ANOVA followed by Turkey's HSD multiple comparison test. Significant differences are marked with letters. Same letters indicate no significant difference and different letters represent significant difference ($p < 0.05$). Source data are provided as a Source Data File.



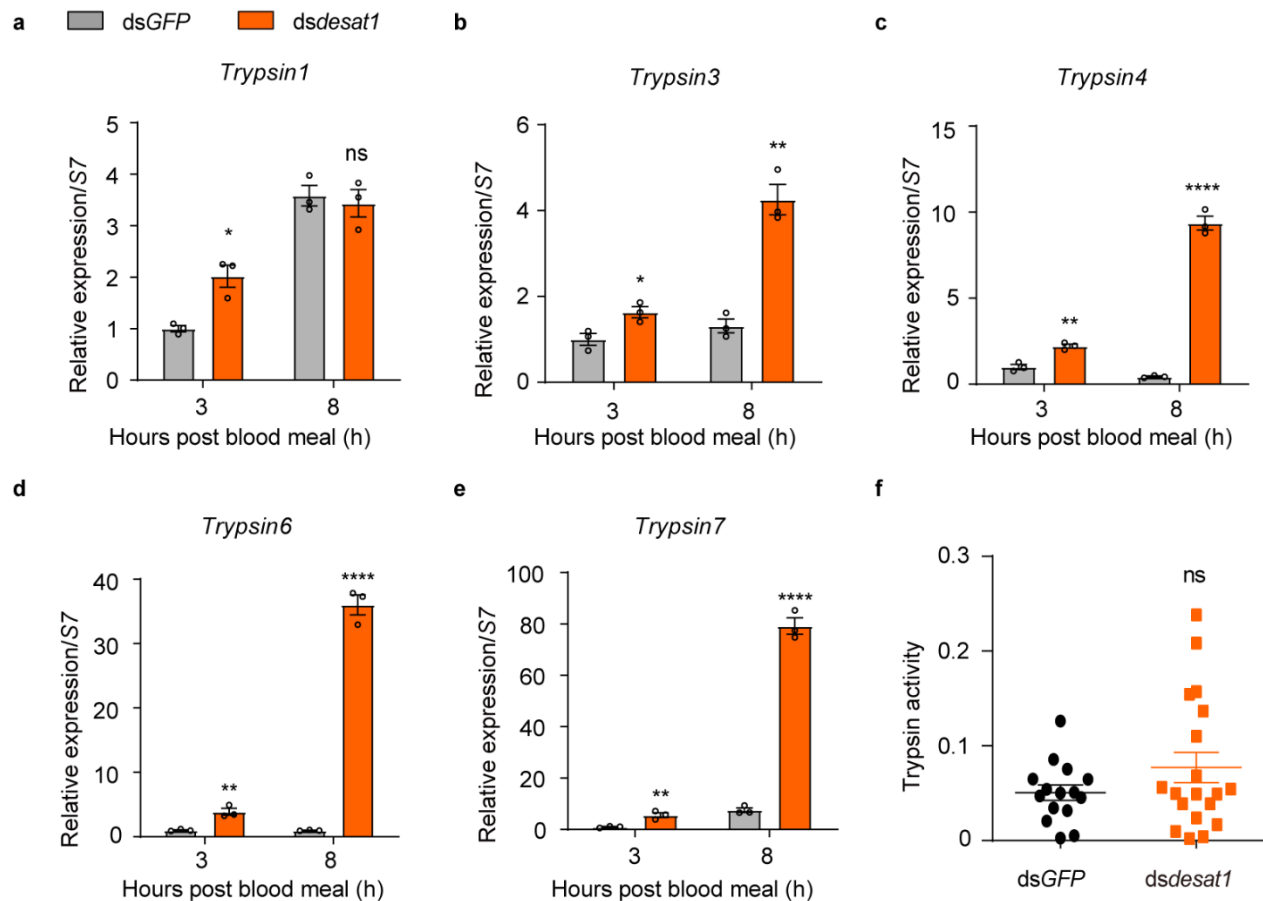
Supplementary Figure 2 | Efficiency of *desat1* gene silencing at different times of female post-eclosion.

a, b qPCR analysis of *desat1* silencing efficiency in female mosquitoes at 18 h post-eclosion (PE) (**a**) and 72 h PE (**b**). dsRNA encoding the green fluorescent protein gene (*dsGFP*) served as controls. *S7* was used as the reference gene. Error bars indicate SEM. Statistics were determined using Student's *t* test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Source data are provided as a Source Data File.



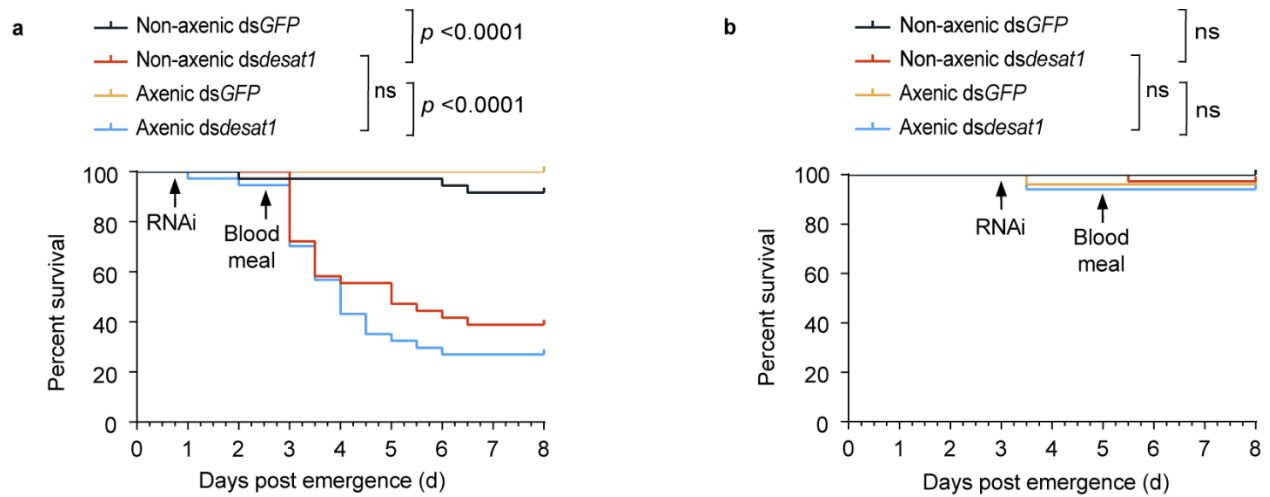
Supplementary Figure 3 | Survival rates of sugar-fed female mosquitoes injected with *dsdesat1* at two different times post-eclosion.

a,b Survival curves of sugar-fed female mosquitoes injected with *dsdesat1* at 18 h PE (**a**) or at 72 h PE (**b**). Each treatment was replicated three times, with 30-36 female mosquitoes per replicate. Error bars represent SEM. Statistics were performed using the Log-rank (Mantel-Cox) test. Source data are provided as a Source Data File.



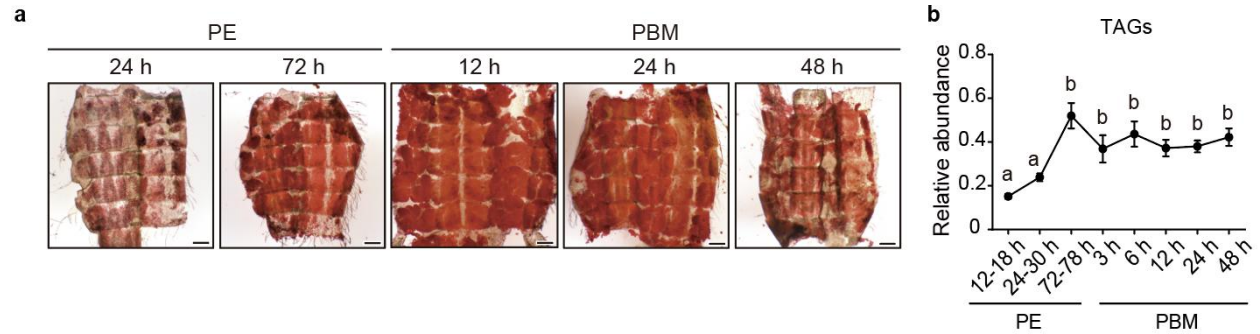
Supplementary Figure 4 | Trypsin gene expression and activity in midguts of female mosquitoes silenced for *desat1* expression early after emergence.

a-e Expression at 3 h and 8 h PBM of five trypsin genes in midguts of early *desat1*-silenced female mosquitoes. *S7* served as a reference gene. Error bars indicate SEM. Statistics were performed with Student's *t* test. **f** Midgut trypsin activity at 12 h after blood feeding of early *desat1*-silenced female mosquitoes (n=20). The horizontal lines represent the median, and error bars represent SEM. Significance was determined using the Mann-Whitney test and indicated as follows: **, $p < 0.01$; ****, $p < 0.0001$; ns: not significant. Similar results were obtained from two biological repeats. Source data are provided as a Source Data File.



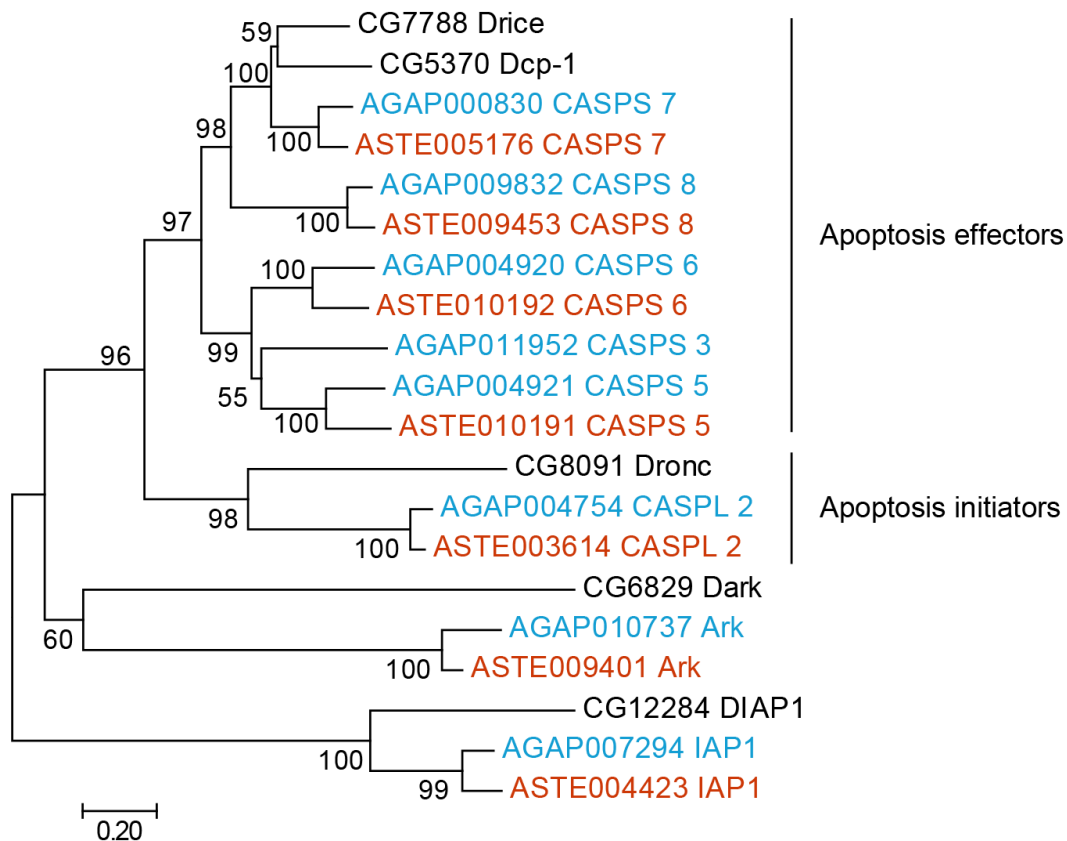
Supplementary Figure 5 | Midgut bacteria are not responsible for post-blood meal death of early *desat1*-silenced female mosquitoes.

a, b Female mosquitoes ($n = 40$) injected with *dsGFP* or *dsdesat1* at 18 h PE (**a**) or at 72 h PE (**b**) were treated with oral antibiotics to eliminate gut bacteria, while a normal sugar solution was used as controls. Survival rates were recorded after adult emergence. Statistical analyses were performed using the Log-rank (Mantel-Cox) test. Similar results were obtained from three biological repeats. Source data are provided as a Source Data File.



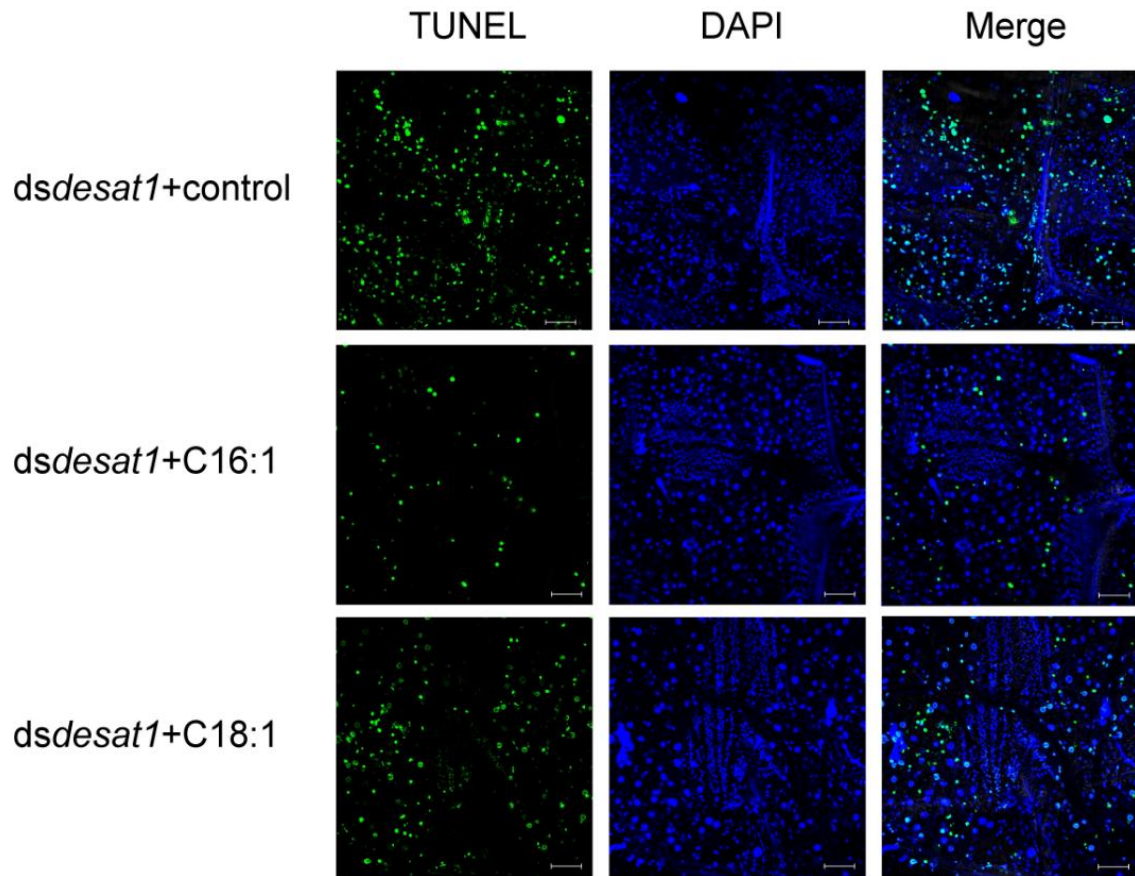
Supplementary Figure 6 | TAG content of female *An. stephensi* across different developmental times post emergence (PE) and post blood meal (PBM).

a Oil Red O staining of fat bodies of female adult mosquitoes at different times PE and PBM. Scale bars, 200 μ m. **b** Relative female fat body TAG abundance at different times PE and PBM. Error bars indicate SEM. Statistics were performed with one-way ANOVA followed by Turkey's HSD test. Different letters above bars denote significant differences ($p < 0.05$), whereas the same letter indicates no significant difference between groups. Source data are provided as a Source Data File.



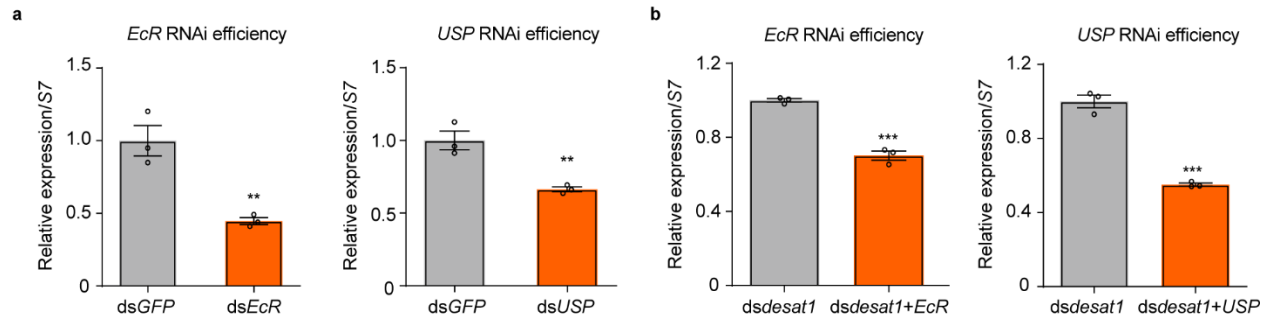
Supplementary Figure 7 | Phylogenetic relationships among apoptosis-related proteins.

By comparing the genomes of *Drosophila melanogaster* (GCA_000001215.4) from GenBank and *Anopheles gambiae* (AgambiaePEST) from VectorBase, homologous *An. stephensi* caspase genes were identified. Based on their prodomain sequences, these genes are classified into initiator caspase CASPL2 and effector caspases (CASPS5, CASPS6, CASPS7, and CASPS8). Amino acid sequences were aligned, and a neighbor-joining tree was generated using MEGA7 software. Bootstrap values are shown.



Supplementary Figure 8 | Supplementation of palmitoleic acid or oleic acid alleviates blood meal-induced apoptosis in *desat1*-silenced mosquitoes.

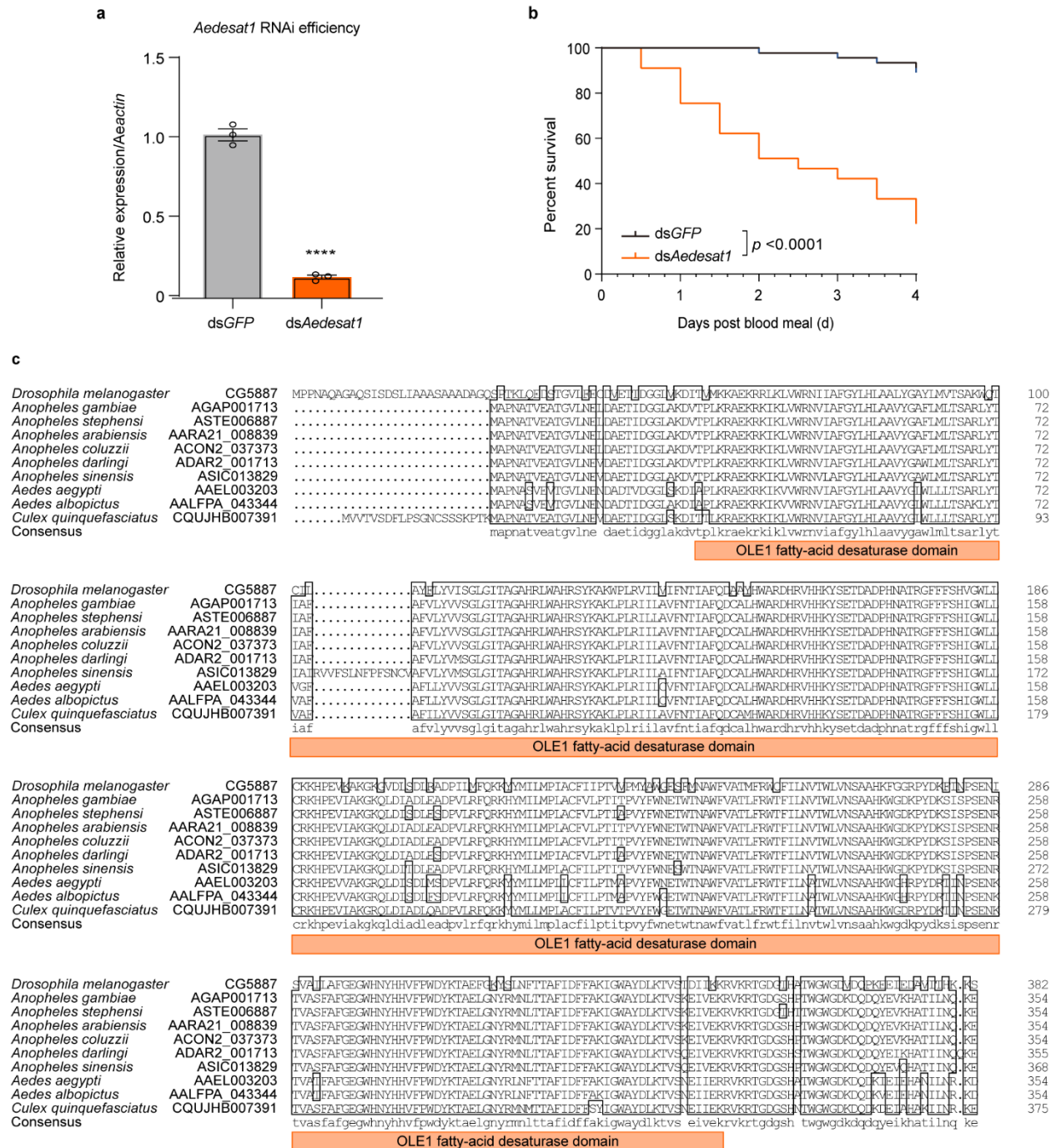
Fat bodies of *desat1*-silenced females supplemented with palmitoleic acid (C16:1) or oleic acid (C18:1) (200 μ M each) were stained with TUNEL to assess apoptosis at 12 h PBM (n=15). An equivalent volume of ethanol as USFA stock solution was used for controls. Scale bar: 100 μ m.



Supplementary Figure 9 | Silencing efficiency of *EcR* and *USP* genes.

a qPCR analysis of *EcR* and *USP* silencing efficiency in female adults. Female adults injected with dsGFP served as controls. **b** qPCR analysis of *EcR* and *USP* silencing efficiency in *desat1*-silenced female adults. Female mosquitoes injected with ds*desat1* served as a reference. Expression shown relative to the *S7* gene. Error bars indicate SEM. Statistics were performed using Student's *t* test.

, $p < 0.01$; *, $p < 0.001$. Source data are provided as a Source Data File.



Supplementary Figure 10 | Silencing *desat1* gene in newly emerged female *Ae. aegypti* mosquitoes induces post-blood meal mortality.

a qPCR analysis of *Aedes desat1* silencing efficiency in *Ae. aegypti* female adults at 3 days post-dsRNA injection. Female adults injected with ds*GFP* served as controls. Expression levels are

shown relative to the *Ae. aegypti* *Actin* gene. Error bars indicate SEM. Statistics were performed using Student's *t* test. ****, $p < 0.0001$. **b** Post-blood-feeding survival of *Ae. aegypti* female mosquitoes injected with *dsGFP* or *dsAedesat1* at 18 h PE (n = 45). Statistical analyses were performed using the Log-rank (Mantel-Cox) test. **c** The *Desat1* proteins are highly conserved across different mosquito species. Multiple sequence alignment of *Desat1* orthologs from *Drosophila melanogaster* (CG5887), *Anopheles gambiae* (AGAP001713), *Anopheles stephensi* (ASTE006887), *Anopheles arabiensis* (AARA21_008839), *Anopheles coluzzii* (ACON2_037373), *Anopheles darlingi* (ADAR2_001713), *Anopheles sinensis* (ASIC013829), *Aedes aegypti* (AAEL003203), *Aedes albopictus* (AALFPA_043344), and *Culex quinquefasciatus* (CQUJHB007391) generated by DNAMAN 9.0.1. Conserved catalytic residues are boxed in black. OLE1 fatty-acid desaturase domains are highlighted in orange. Source data are provided as a Source Data File.

Supplementary Table 1.

Primers used in this study

Primers	5'-3' sequences	Application
qdesat1-F	ACGTGATTGCCTTCGGTTACC	RT-qPCR
qdesat1-R	AGCCCGGATACAACGTACAG	
qDUOX-F	GGAAGTGTCTCTCGGCTGTCA	
qDUOX-R	TGATGTCCCAGAGCGTGAAC	
qNOX5-F	GTCGCACAGCCGCAGGAT	
qNOX5-R	GGTTTGCCAACGGGGTAGTTAA	
qHPX2-F	GATCTGATCGTCGGTGCCAT	
qHPX2-R	ACCTTGCTGCACTGTACTCC	
qNOS-F	ACATCAAGACGGAAATGGTTG	
qNOS-R	ACAGACGTAGATGTGGGCCTT	
qCatalase-F	AGCTTCATCCACACGCAGAA	
qCatalase-R	AGTTCTTGATGCCCTGGTCG	
qCASPS7-F	CACACAGAAGGGTTCCTGGT	
qCASPS7-R	ATCATCGGTATGTCCGGTGT	
qCASPS5-F	AGGATCATTCGGATGCTGAC	
qCASPS5-R	AACTTTGGCTTGCCGAGTAA	
qCASPS6-F	GATGTGCTGTACGCGAAAGA	
qCASPS6-R	GTCGCCTGTTTGTTGGATTT	
qCASPS8-F	ATCTGGAGTCACTTCACCGC	
qCASPS8-R	CCATGATCCTTCGGTGGTGT	
qCASPL2-F	ACATGCTCGTGTGCTATGCT	
qCASPL2-R	CAGCATATTCGCCAACCAGC	
qIAP1-F	TCTGTCCGCTGCTGAAGAAG	
qIAP1-R	CTCCACTCCACGAATCTCCG	
qArk-F	CTGGGAAGTGTGGACGTTTT	
qArk-R	GGAAGCGATCGTAAAGCAAG	
qEcR-F	CAGGATGGGTACGAGCAAC	
qEcR-R	AAATGCTGGCAGTCCCTTCG	
qUSP-F	GATCAGGTGACCTTGTTGCG	
qUSP-R	GGTGAAGTTCGGTCCCAAAC	
qCPT1-F	CCGAACGGTATCAATGGCCT	
qCPT1-R	CCATCTGTATGTCCGAGGCC	
qCPT2-F	GGAACATTGAACAACCGGCC	
qCPT2-R	ACAGAGACAGAACAGTGCCG	

qACD-F	ACCAATCCCGACCCCAAATG	
qACD-R	TTAAAACCGGCTCCTTCCCC	
qECH-F	CGGACTGGTTAGCAAGGTGT	
qECH-R	GCTTCTCGACAAATGCCGTC	
q3HCD-F	AAATTCGGTCGGCTGGATGT	
q3HCD-R	ATTCACGATGACACCACGCT	
qTrypsin1-F	AGTTCTAGTTGCGGTGGTGG	
qTrypsin1-R	GCAGTTGTGACGCTTGTTGT	
qTrypsin3-F	TCGCATCGTAGGTGGATTCTG	
qTrypsin3-R	GGAAGATCCCAGGCGTACAG	
qTrypsin4-F	GGAGAATGCGGTCACCTTCA	
qTrypsin4-R	TCGGTAAGCCTTGTCGCATT	
qTrypsin6-F	ATCGCACTGCTGGAAGTAA	
qTrypsin6-R	CACTCCTGCTGATTCACGGT	
qTrypsin7-F	GAGGATCGGTGCTGAACAGT	
qTrypsin7-R	CTCGTCTTCCACTTCCAGCA	
16S rRNA-F	TCCTACGGGAGGCAGCAGT	
16S rRNA-R	GGACTACCAGGGTATCTAATCCTGTT	
qRPS7-F	TCGGTTCCAAGGTGATCAAAGC	
qRPS7-R	AGCGCGGTCTCTTCTGCTTGT	
qAedesat1-F	CTGTTCCGCTGGACCTTCAT	
qAedesat1-R	CGGCAGTCTTGTAGTCCCAG	
qAeActin-F	GAACACCCAGTCCTGCTGACA	
qAeActin-R	TGCGTCATCTTCTCACGGTTAG	
dsdesat1-F	taatacgactcactatagGTTCCAACGCAAGCATTACATGATT	
dsdesat1-R	taatacgactcactatagGACTGGTCCTGATCCTTGTCGC	
dsGFP-F	taatacgactcactatagGAGATGGTGAGCAAGGGCGAGGAGCTGT	
dsGFP-R	taatacgactcactatagGAGTTACTTGTACAGCTCGTCCATGCCG	
dsEcR-F	taatacgactcactatagGAACACGATCATGAACACG	
dsEcR-R	taatacgactcactatagTGGTGATTTCCGTTATGTGC	
dsUSP-F	taatacgactcactatagCCCAACACACCCTCCAACAT	
dsUSP-R	taatacgactcactatagCGACACAGCACCCCTTGTA	
dsAedesat1-F	taatacgactcactatagGGCCTCTGGCTGTTGTTGACCT	
dsAedesat1-R	taatacgactcactatagCCATGCGTTAGTCCAGGTTT	

dsRNA
synthesis