## **Supporting Information**

## Isoe et al. 10.1073/pnas.1102637108

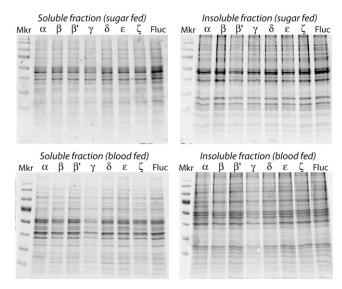


Fig. S1. Midgut protein synthesis is not affected by decreased coatomer protein I (COPI) coatomer protein expression in sugar- and blood-fed mosquitoes. (Upper) Denaturing SDS/PAGE analysis of soluble and insoluble proteins obtained from the midguts of sugar-fed mosquitoes injected 3 d earlier with 400 ng indicated dsRNA. One midgut equivalent of the protein extracts was loaded in each lane, and the gel was stained with Gel Code Blue. (Lower) Denaturing SDS/PAGE analysis of soluble and insoluble proteins in midgut epithelium cell extracts (no food bolus) of blood-fed mosquitoes [24 h postblood meal (PBM)] injected 4 d earlier with 400 ng indicated dsRNA. One midgut equivalent of the protein extracts was loaded in each lane.

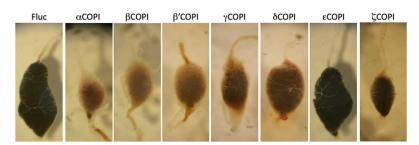


Fig. 52. Midguts from COPI dsRNA-injected mosquitoes contain less blood and lack a defined peritrophic matrix. Photographs of representative midguts removed from dsRNA-injected (400 ng) mosquitoes at 24 h PBM. The decreased blood in the midgut at 24 h PBM in COPI-deficient mosquitoes (except  $\varepsilon$ COPI) is caused by premature defecation. The  $\alpha$ COPI dsRNA midgut is the same as in Fig. 4A.

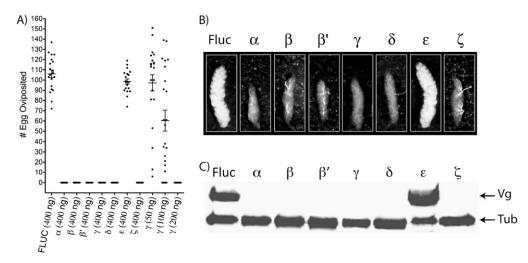


Fig. S3. Impaired blood meal digestion in COPI dsRNA-injected mosquitoes blocks oocyte maturation. (A) Number of eggs oviposited during the first gonotrophic cycle in dsRNA-injected mosquitoes by 5 d PBM. Mosquitoes were kept in separate vials containing egg oviposition paper and water. Each dot represents the number of eggs oviposited by an individual mosquito. Student t test analysis showed that, with the exception of εCOPI and γCOPI at 50 ng dsRNA, all data were statistically significant between COPI and Firefly luciferase (Fluc) dsRNA-injected mosquitoes (P < 0.001). (B) Representative mosquito ovaries removed 24 h PBM from mosquitoes that had been injected with the indicated dsRNA. (C) Western blot analysis showing vitellogenin protein accumulation in ovaries isolated 24 h PBM from mosquitoes that had been injected with the indicated dsRNA. The protein loading control is α-tubulin.

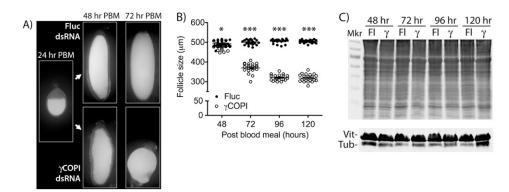


Fig. 54. Injection of γCOPI dsRNA at 24 h PBM blocks follicular development but not global protein expression or vitellogenin accumulation in ovary cells. (A) Representative photos of follicles dissected from uninjected mosquitoes at 24 h PBM or Fluc and γCOPI dsRNA (400 ng) -injected mosquitoes at 48 and 72 h PBM (24 and 48 h postinjection). (B) Follicle length was measured in dissected ovaries obtained from 10 mosquitoes 48–120 h PBM that had been injected 24–96 h earlier with Fluc or γCOPI dsRNA. Follicular length was significantly different between γCOPI and Fluc dsRNA-injected mosquitoes at 48 h PBM (P < 0.05) and 72–120 h PBM (P < 0.001). (C) SDS/PAGE analysis of proteins extracted from ovaries of Fluc or γCOPI dsRNA (400 ng) -injected mosquitoes that were dissected at 48–120 h PBM (Q = 0.05) h postinjection). Western blot of corresponding extracts using vitellogenin and α-tubulin antibodies.

Table S1. Mosquito feeding and RNAi knockdown efficiencies in dsRNA-injected mosquitoes

dsRNA injected	Blood ingested* relative to Fluc (%)	RNAi <sup>†</sup> (100 ng)		RNAi <sup>†</sup> (400 ng)	
		N <sup>‡</sup>	KD (%)	N <sup>‡</sup>	KD (%)
αCOPI	95.8	8/10	86.1	10/10	92.2
βCOPI	95.5	9/10	87.8	10/10	93.8
β'COPI	91.1	9/10	87.4	10/10	92.8
γCOPI	84.0	9/10	88.0	10/10	93.3
δCOPI	88.8	9/10	87.3	10/10	92.8
εCOPI	85.2	9/10	86.2	10/10	91.3
ζCOPI	86.2	8/10	86.7	10/10	93.2

<sup>\*</sup>The relative amount of blood ingested by COPI dsRNA-injected mosquitoes was determined at 30 min PBM using the BSA quantitation assay described in ref. 1. Note that some COPI dsRNA-injected mosquitoes may have initiated premature defecation (Fig. 4B).

Table S2. Statistical analysis of mortality over a 7-d period postinjection using 100 ng COPI dsRNA or 400 ng Fluc dsRNA

dsRNA injected	Blood-fed COPI vs. blood-fed Fluc	Blood-fed COPI vs. sugar-fed COPI	Blood-fed $\beta'$ COPI vs. blood-fed COPI
αCOPI	P < 0.0001	NS	P < 0.0001
βCOPI	P < 0.0001	<i>P</i> < 0.05	<i>P</i> < 0.0001
β'COPI	P < 0.0001	<i>P</i> < 0.0001	_
γCOPI	P < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.01
δCOPI	P < 0.0001	<i>P</i> < 0.001	P < 0.001
εCOPI	NS	NS	P < 0.0001
ζCOPI	<i>P</i> < 0.0001	P < 0.001	<i>P</i> < 0.01

At 400 ng dsRNA injection, all pair-wise comparison showed a significant difference (P < 0.0001) except for  $\varepsilon$ COPI. NS, not significant.

<sup>&</sup>lt;sup>†</sup>Knockdown (KD) efficiency of each COPI subunit was determined compared with Fluc dsRNA-injected mosquitoes.

 $<sup>^{\</sup>ddagger}$ The left number indicates mosquitoes with >80% KD, and the right number shows the total number of injected mosquitoes examined.

<sup>1.</sup> Isoe J, Rascón AA Jr., Kunz S, Miesfeld RL (2009) Molecular genetic analysis of midgut serine proteases in Aedes aegypti mosquitoes. Insect Biochem Mol Biol 39:903–912.

Table S3. Gene-specific primers for dsRNA and quantitative PCR

Primers	Primer sequences (5' to 3')		
αCOPI			
dsRNA-forward	ATGCTGACAAATTTCGAAACCAA		
dsRNA-reverse	TCCGTCGCCGTAGGATTCTT		
qPCR-forward	GTGTCCGCATCGTTGGATCA		
qPCR-reverse	ACAACAGCATCAGCTTGCCCAA		
βCOPI			
dsRNA-forward	ATGTCGCTGTCGGAAGCATC		
dsRNA-reverse	GACATATCCTGCTGGGTATC		
qPCR-forward	GAATGGCTGGTTCCTGACGG		
qPCR-reverse	GTTCAAAGCCCTCTCCTGATC		
β'COPI			
dsRNA-forward	CAGCCACTGCGACTGGACAT		
dsRNA-reverse	TCTACACAGTTGACGCCCTT		
qPCR-forward	GTGTGGCAACTGGGATCGAA		
qPCR-reverse	GATCGTCGGCACCGGATAT		
γCOPI			
dsRNA-forward	CCCTTCACAAACCTGGAGAA		
dsRNA-reverse	CCAATCCATGATACTGCACCAT		
qPCR-forward	AACGGTGGGCAAATGAGGC		
qPCR-reverse	GTCAGCTTGTTCACCAGCTTAGT		
δCOPI			
dsRNA-forward	ATGGTCCTGATAGCGGCCG		
dsRNA-reverse	CCTCCACTGGAGCTACTTCC		
qPCR-forward	CGGCAACGGATGGAGCAGAA		
qPCR-reverse	GACGGTGCCGAGATTGACGA		
εCOPI			
dsRNA-forward	ATGAGCCGTCAAGCGAATGA		
dsRNA-reverse	TGGATGTTCAACCAGGCTTG		
qPCR-forward	TGGCCAGATCGACTCGCG		
qPCR-reverse	GCATCCTGCAGCTTTTCGCC		
ζCOPI			
dsRNA-forward	GTTAGTAATCACGAACCAACCCT		
dsRNA-reverse	AGAACCTGTGCAACAGTTTGCTC		
qPCR-forward	TGTGATGGAGGAATTATCCTGGA		
qPCR-reverse	TTCAACTGTTCTCTAGCGGACTG		

Each dsRNA primer was nested at the 5' end with the T7 promoter sequence (5'-TAATACGACTCACTATAGGGAGA-3'). qPCR, quantitative PCR.