Supporting Information

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SI Materials and Methods

For RNA sequencing analysis, RNA for each sample was quantified on an Agilent BioAnalyzer Eukaryotic Total mRNA Nano chip and then normalized in concentration before library construction. A 75-bp single-direction, stranded library for each sample was then generated using Kapa Biosystems RNA library preparation chemistry that incorporated a unique barcode. The resulting libraries were pooled, divided between two Illumina NextSeq high-throughput chips, and sequenced. A total of 736,933,486 reads were generated, with average total reads per treatment replicate of 33,496,976. Reads were quality-filtered using a FastQ Quality Filter in the FastX Toolkit (hannonlab.cshl.edu/fastx_toolkit/) using the following parameters: -q 30 -p 90. Filtered reads were used as input to HISAT2 (1) with the following parameters: -q --dta. A total of 571,914,475 reads with an average of 25,996,112 reads per treatment replicate were then mapped to the *A. aegypti* genome (assembly AaegL3, genset AaegL3.3) and used in StringTie to assess transcript abundance (2). Read data for *hif-1α* (AAEL001097), *hif-2α* (AAEL015383), *hif-β* (AAEL010343), *phd-1* (AAEL002798), *phd-2* (AAEL002802), *lpp* (AAEL009955), and the *lppR* (AAEL012251) were extracted from the data and statistically analyzed by ANOVA, followed by a post hoc Dunnett's or Tukey–Kramer least significant difference test.

1. Kim D, Langmead B, Salzberg SL (2015) HISAT: A fast spliced aligner with low memory requirements. *Nat Methods* 12:357–360.

2. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* 11: 1650–1667.

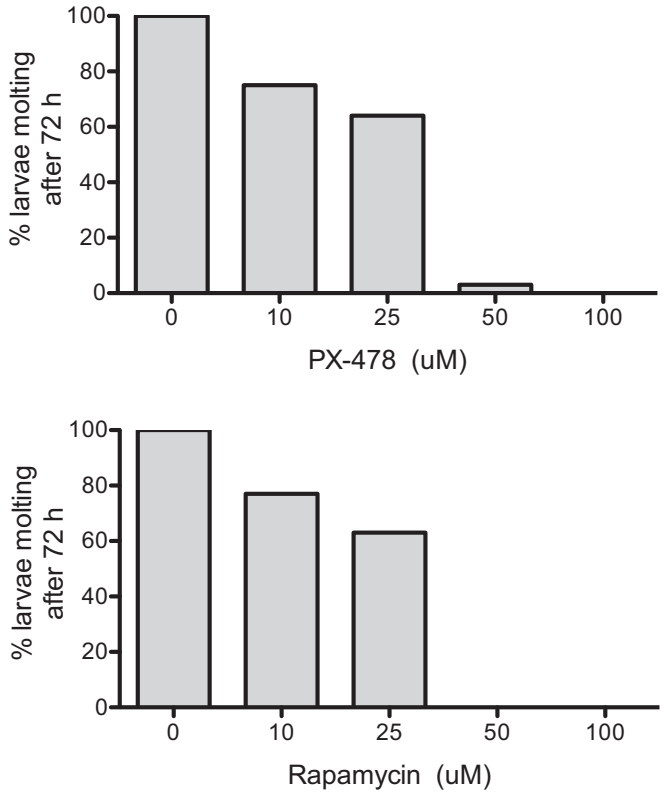

Fig. S1. PX-478 (*Upper*) and rapamycin (*Lower*) dose-dependently inhibit molting of conventional (CN) *A. aegypti* first instars. Larvae were treated by placing four newly hatched individuals per well of a 24-well culture plate containing 1 mL of water plus standard rearing diet per well. From 10 to 100 μM PX-478 or rapamycin was added per well at 12 h posthatching. Untreated larvae served as positive controls. The proportion of larvae that had molted was recorded after 72 h, with a minimum of 40 larvae scored per treatment and dose.

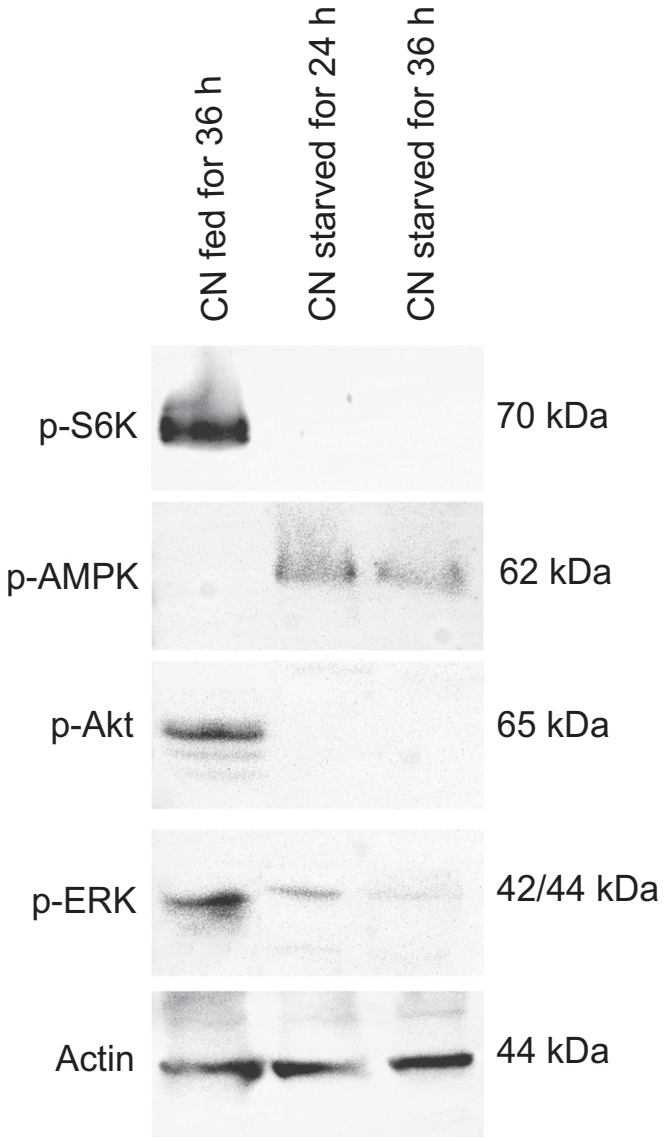

Fig. S2. Starvation of conventional (CN) larvae affects the phosphorylation status of S6K, AMPK, Akt, and ERK. Immunoblots of whole-body extracts collected from fed CN larvae at 36 h posthatching, starved CN larvae at 24 h, or starved CN larvae collected at 36 h. Actin served as a loading control. Molecular masses of each protein are indicated to the right.

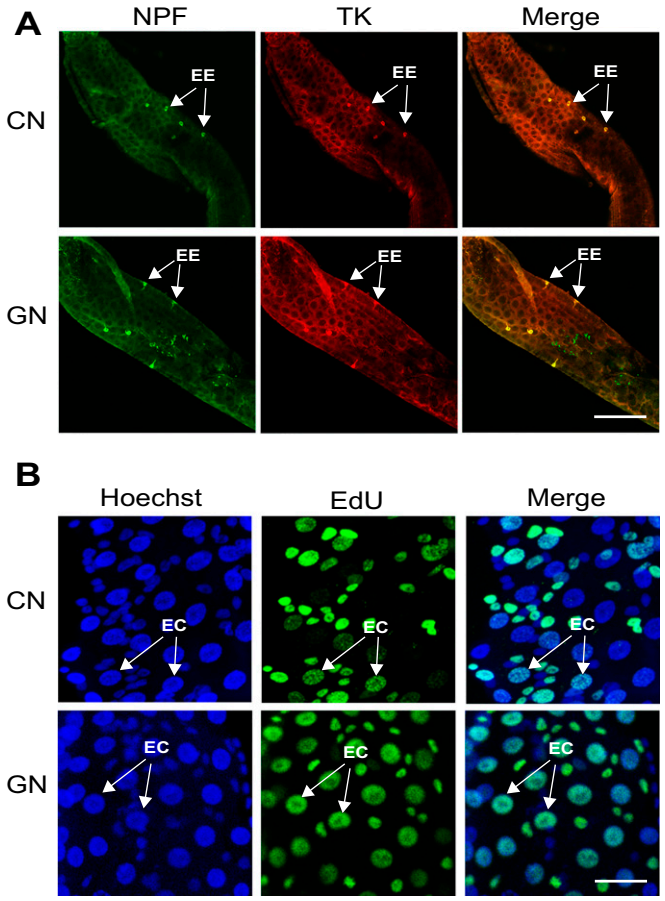

Fig. S3. Conventional (CN) larvae and gnotobiotic larvae with wild-type *E. coli* (GN) contain EEs that express NPF and TK as well as enterocytes (ECs) that incorporate EdU. (*A*) Low-magnification images of midguts from CN larvae and GN at 24 h posthatching showing EEs that are NPF^+ (bright green) and TK^+ (red). The merged panel indicates each EE expresses both peptide hormones (bright yellow). Arrows point to representative EEs. (Scale bar: *Lower Right*, 200 μm.) (*B*) High-magnification images of midgut cell nuclei from CN larvae and GN that are labeled by Hoechst 33342 (blue) and incorporated EdU (green). The merged panel indicates many, but not all, nuclei in the image incorporated EdU (green-aqua). Arrows point to large nuclei of representative ECs that incorporated EdU. Note that several small nuclei that are primarily ISCs/EBs also incorporated EdU. (Scale bar: 10 μm.)

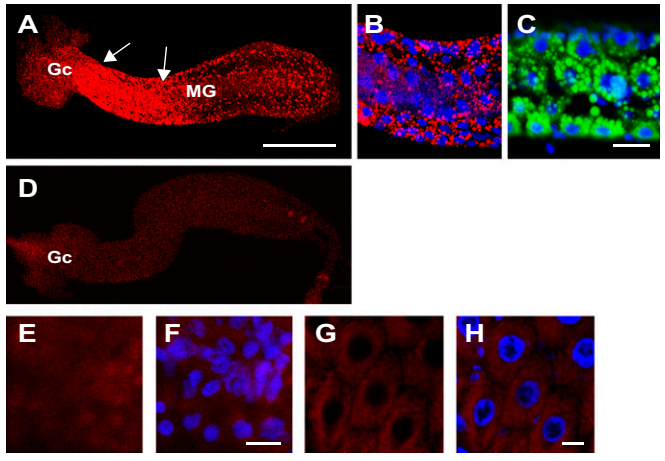

Fig. S4. Gut bacteria and starvation differentially affect lipid accumulation in the midgut and fat body. (*A*) Low-magnification image of the midgut (MG) from an axenic (AX) larva 36 h posthatching stained with Nile red. A larger abundance of lipid droplets is present in the anterior MG distal of the gastric caeca (Gc) (arrows) than in the posterior MG. (Scale bar: 200 μm.) Intermediate-magnification images of the MG from an AX larva stained with Nile red (*B*) or Bodipy (*C*). (Scale bar: *C*, 20 μm.) (*D*) Low-magnification image of the MG from a conventional (CN) larva 36 h posthatching stained with Nile red. No lipid droplets are visible. High-magnification images of fat body adipocytes (*E* and *F*) and midgut ECs (*G* and *H*) from a starved CN larva 36 h posthatching are shown. *E* and *G* are stained with Nile red, while *F* and *H* are stained with Nile red and Hoechst 33342. No lipid droplets are visible in *E*–*H*. (Scale bars: *F*, 20 μm; *H*, 10 μm.)