Figure S1

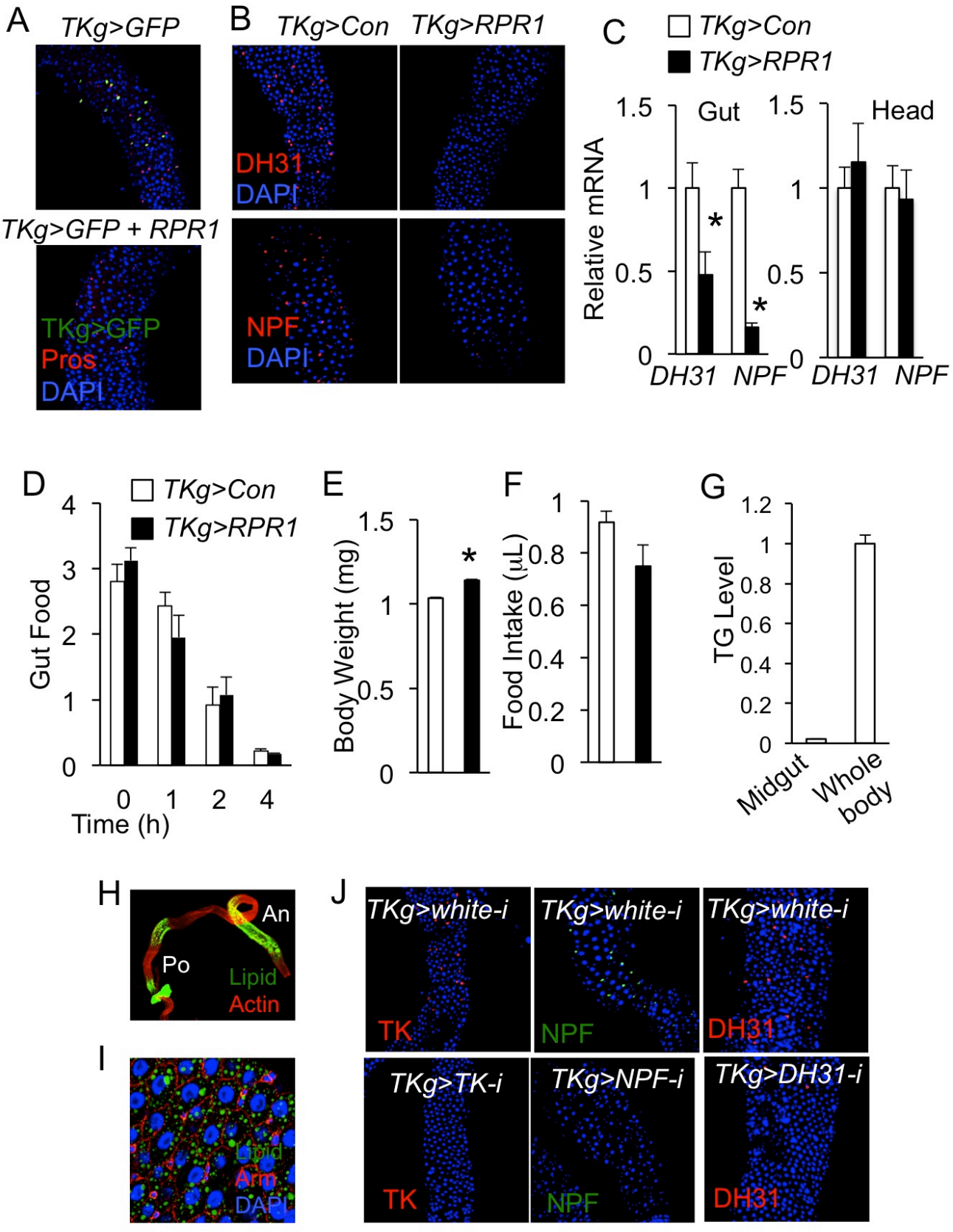

A
TKg>GFP
TKg>GFP + RPR1
TKg>GFP
Pros
DAPI
B
TKg>Con
TKg>RPR1
DH31
DAPI
NPF
DAPI
C
TKg>Con
TKg>RPR1
Relative mRNA
Gut
Head
DH31
NPF
D
TKg>Con
TKg>RPR1
Gut Food
Time (h)
E
Body Weight (mg)
F
Food Intake (μL)
G
TG Level
Midgut
Whole body
H
An
Po
Lipid
Actin
I
Lipid
Arm
DAPI
J
TKg>white-i
TK
NPF
DH31
TKg>TK-i
TKg>NPF-i
TKg>DH31-i

Figure S1. Ablation of TK EEs in midgut, related to Figure 1 and 2

A, TK EEs ablation (*TKg>GFP + RPR1*) decreased EEs (anti-Pros, red; TKg>GFP, green) number and impaired EEs paired appearance compared to Control (*TKg>GFP*). **B,** Expression of DH31 (anti-DH31, red, top panels) and NPF (anti-NPF, red, bottom panels) were dramatically decreased in the gut of TK EEs ablated flies (*TKg>RPR1*). **C**, qPCR results indicate that mRNA levels of *DH31* and *NPF* are decreased only in the gut, but not brain, of *TKg>RPR1* (n=3, 30 guts per group). **D,** Gut emptiness of control (*TKg>Con*) and ablated flies (*TKg>RPR1*), as determined by the blue dye containing food that is remained in the gut. **E, F,** body weight (**E**) (n=3, 60 flies per group) was increased slightly, whereas food intake (**F**) (n=3, 30 flies per group) was slightly decreased in *TKg>RPR1*. **G,** TG level in gut and whole body of wild type flies. **H,** Neutral lipid accumulates in the anterior (An) and posterior (Po) region of the adult midgut as indicated by Bodipy staining. **I,** most lipid droplets (Bodipy, green) are located in ECs with large nuclei (DAPI, blue). Outline of the cells are labeled with membrane-enriched Armadillo (anti-Arm, red). **J,** Specific knockdown of TK (anti-TK, red, left), NPF (anti-NPF, green, middle), or DH31 (anti-DH31, red, right) expression in the midgut.

Figure S2

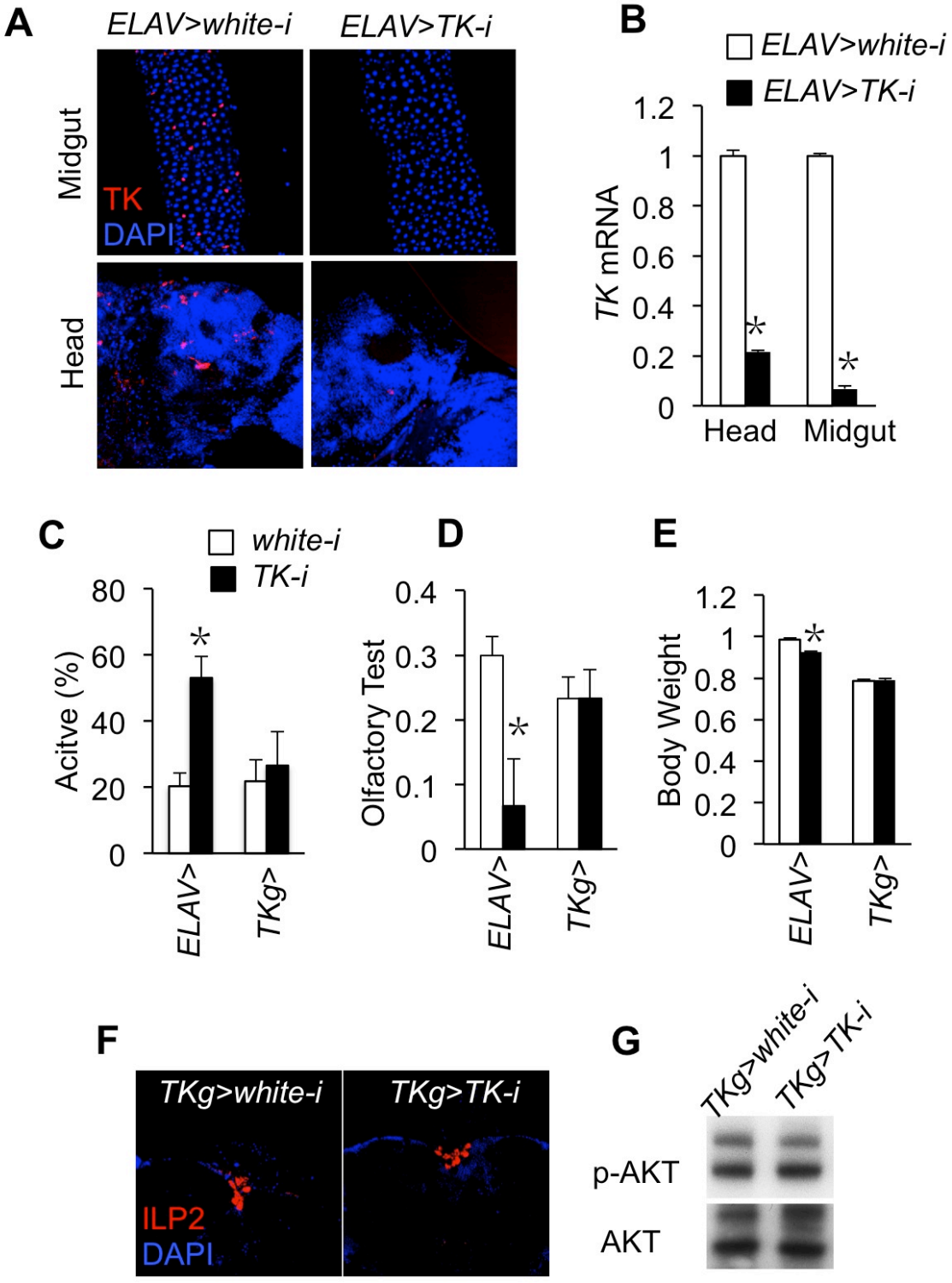
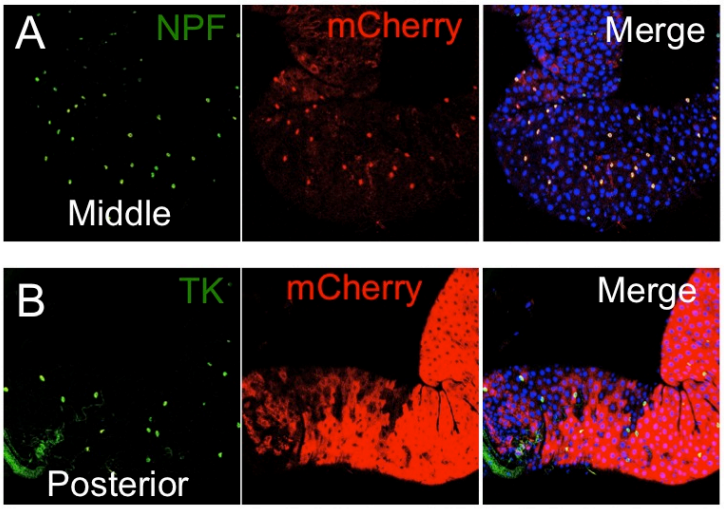
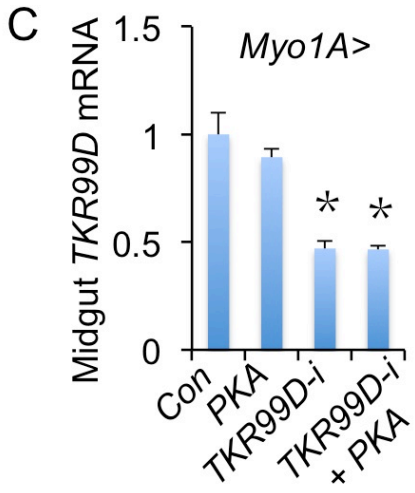

A
ELAV>white-i
ELAV>TK-i
Midgut
Head
TK
DAPI
B
ELAV>white-i
ELAV>TK-i
TK mRNA
1.2
1
0.8
0.6
0.4
0.2
0
Head
Midgut
C
white-i
TK-i
Acitve (%)
80
60
40
20
0
ELAV>
TKg>
D
Olfactory Test
0.4
0.3
0.2
0.1
0
ELAV>
TKg>
E
Body Weight
1.2
1
0.8
0.6
0.4
0.2
0
ELAV>
TKg>
F
TKg>white-i
TKg>TK-i
ILP2
DAPI
G
TKg>white-i
TKg>TK-i
p-AKT
AKT

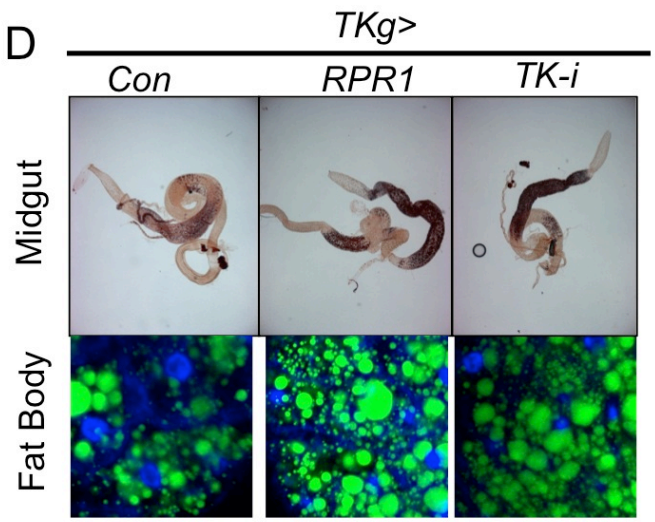
Figure S2. TKs from brain and EEs have different physiological roles, related to Figure 2

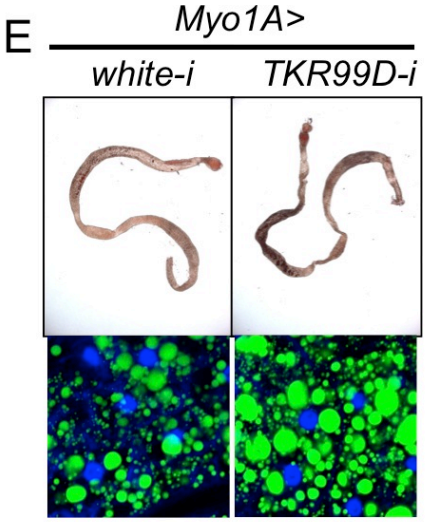
A, TK expression (anti-TK, 1:500, red) is absent in both the midgut (upper) and brain (lower) in *ELAV>TK-i* (*ELAV-Gal4/+; UAS-TK-RNAi/+*) animals, compared to control *ELAV>white-i (ELAV-Gal4/+; UAS-white-RNAi/+)*. **B**, qPCR analysis of *TK* mRNA expression in both the midgut and brain revealed a significant decrease in *TK* knockdown (*ELAV>TK-i*) flies (n=3, 60 head or 30 guts per group). **C-E**, Locomotor activity (**C**) (n=3, 45 flies per group), olfactory response to butanol (**D**) (n=3, 60 flies per group), and body weight (**E**) (n=3, 60 flies per group) are affected by TK knockdown in both the brain and midgut (*ELAV>TK-i*), but not affected when TK is knocked down in the midgut only (*TKg>TK-i*). **F, G,** Either brain dILP2 level (**F**) or whole body phosphorylated AKT (**G**) was not affected in *TKg>TK-i* compared to *TKg>white-i* (control) flies.

Figure S3


A
NPF
mCherry
Merge
Middle
B
TK
mCherry
Merge
Posterior


C
Myo1A>
Midgut TKR99D mRNA
1.5
1
0.5
0
*
*
Con
PKA
TKR99D-i
TKR99D-i + PKA


D
TKg>
Con
RPR1
TK-i
Midgut
Fat Body


E
Myo1A>
white-i
TKR99D-i

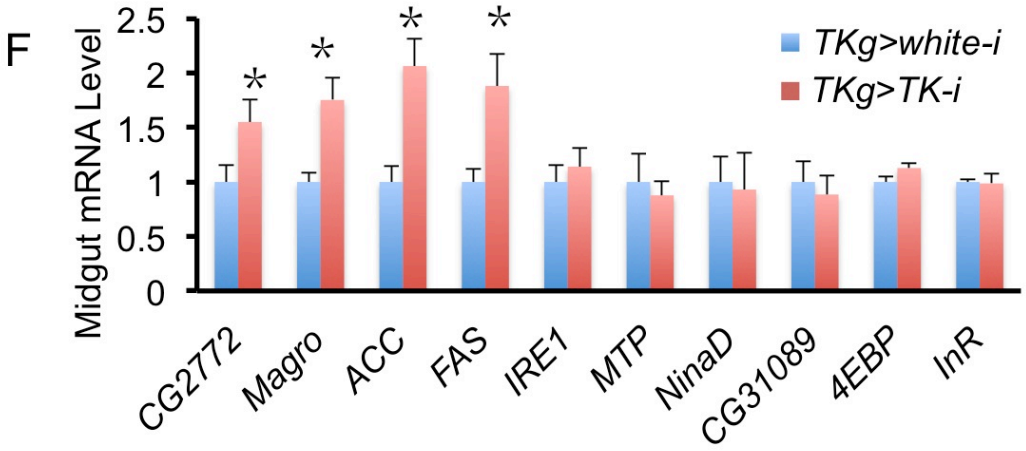

F
Midgut mRNA Level
2.5
2
1.5
1
0.5
0
*
*
*
*
TKg>white-i
TKg>TK-i
CG2772
Magro
ACC
FAS
IRE1
MTP
NinaD
CG31089
4EBP
InR

Figure S3. TK/TKR99D signaling affects lipid metabolism in ECs, related to Figure 2, 3, and 4

A, B, mCherry expression in *TKR99D>mCherry* co-localizes with NPF/TK EEs in the mid-midgut (**A,** anti-NPF, green) but not in posterior midgut TK EEs (**B,** anti-TK, 1:500, green). **C,** Knockdown of *TKR99D* mRNA expression in midgut (n=3, 30 guts per group). **D, E,** Lipid levels in adult midgut (upper, indicated by Oil Red staining) and fat body (lower, indicated by Bodipy staining). **F,** qPCR results in midguts of *TKg>white-i* and *TKg>TK-i* flies (n=3, 30 guts per group).

Figure S4

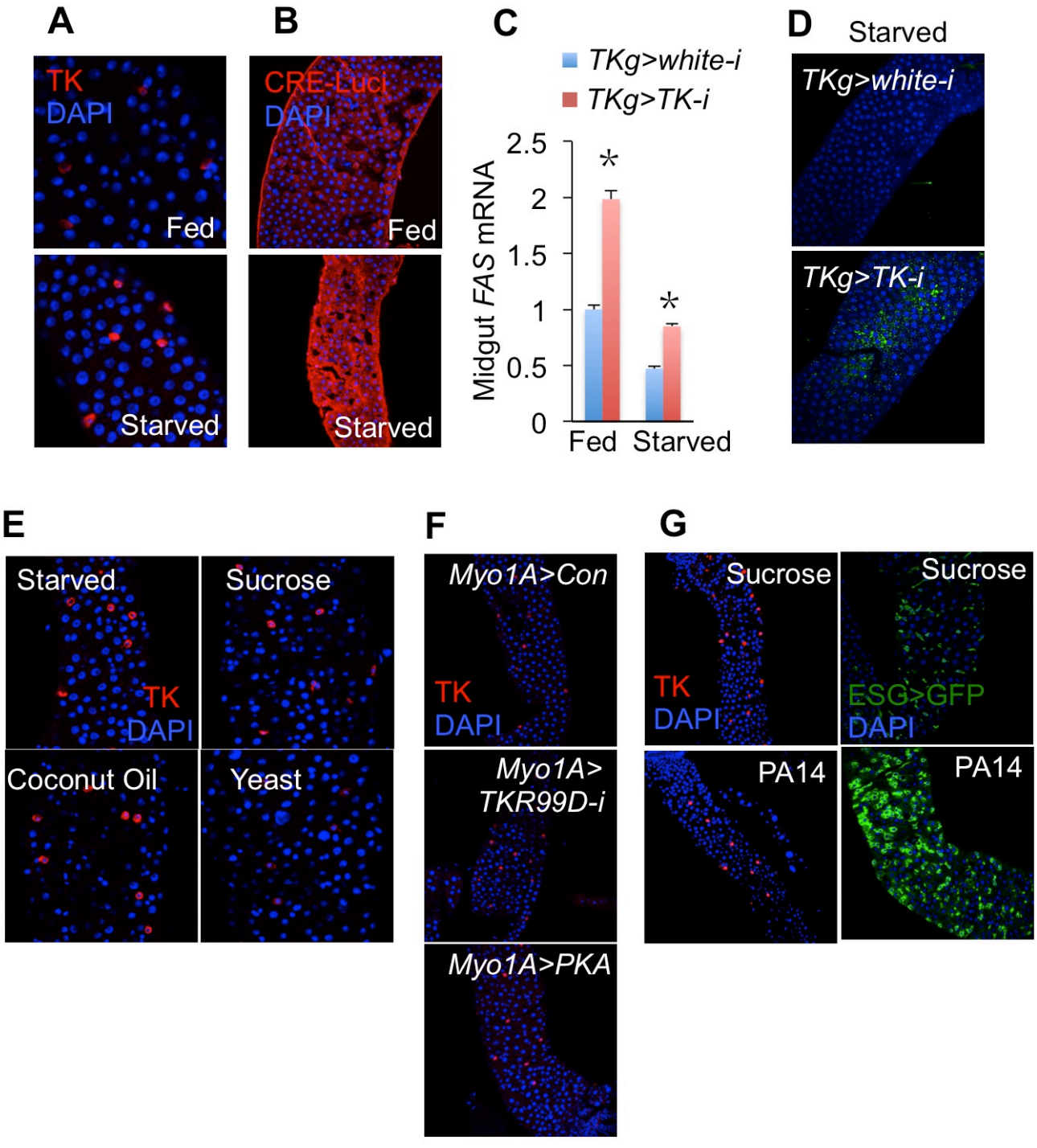

A
TK
DAPI
Fed
Starved
B
CRE-Luci
DAPI
Fed
Starved
C
TKg>white-i
TKg>TK-i
Midgut FAS mRNA
2.5
2
1.5
1
0.5
0
*
*
Fed
Starved
D
Starved
TKg>white-i
TKg>TK-i
E
Starved
TK
DAPI
Sucrose
Coconut Oil
Yeast
F
Myo1A>Con
TK
DAPI
Myo1A>
TKR99D-i
Myo1A>PKA
G
Sucrose
TK
DAPI
Sucrose
ESG>GFP
DAPI
PA14
PA14

Figure S4. Nutrient deprivation enhances TKs production in TK EEs, related to Figure 2 and 4

A, B, Intracellular TK levels (anti-TK, 1:5000, red) in TK EEs (**A**) and enteric CRE-Luciferase (CRE-Luci) levels (**B**) in wild type fly guts under fed or starved condition. **C, D**, *FAS* mRNA expression levels (**C**) (n=3, 30 guts per group) and lipid levels (**D**) in *TKg>white-i* or *TKg>TK-i* guts under 24h fed or starved condition. **E,** Intracellular TK levels (anti-TK, 1:5000, red) in TK EEs when flies were starved for 24h or starved for 12h and refed with 10% sucrose, 25% coconut oil or yeast paste for 12h. **F,** Intracellular TK levels in TK EEs (anti-TK, 1:5000, red) of *Myo1A>Con*, *Myo1A>TKR99D-i*, and *Myo1A>PKA* flies. **G,** Intracellular TK levels in TK EEs (left, anti-TK, 1:5000, red) and intestinal stem cell (ISC) proliferation (right, esg>GFP, green) of flies treated with 5% sucrose (control) or PA14 (pathogen) for 48h.

Supplemental Experimental Procedures

***Drosophila* Strains.** *UAS-RPR1* (BL 5823)*, UAS-AMON-RNAi* (BL 29010)*, UAS-PKA* (BL 35555)*, ELAV-Gal4* (BL 8765)*, UAS-mCherry* (BL 27392)*, UAS-srcGFP* (BL 5432)*, UAS-SREBP (BL 8244), UAS-SREBP-RNAi (BL25975)* and w^{1118} were obtained from the Bloomington *Drosophila* Stock Center (http://flystocks.bio.indiana.edu/). RNAi lines against *white* (JF01545), *TK* (JF01818)*, NPF* (JF02555)*, and TKR99D* (JF02663) were obtained from the TRiP at Harvard Medical School (http://www.flyrnai.org/TRiP-HOME.html). RNAi line against *DH31* (VDRC50295) was obtained from VDRC (http://stockcenter.vdrc.at/). Other stocks used in this study are: *esg-Gal4* and *myo1A-Gal4* (Karpowicz et al., 2010), *CRE-Luci* (Belvin et al., 1999).

Immunostaining, lipid staining, microscopy and western blot. Guts and brains were dissected in PBS and fixed for 15 min in 4% formaldehyde/PBS. After fixation, the samples were washed with 0.2% Triton/PBS and incubated in primary antibodies overnight at 4°C. Secondary antibody stainings were incubated for 1h at room temperature. Tissues were incubated in DAPI for 10 min (1:1000, Invitrogen), washed and mounted in Vectashield (Vector). Primary antibodies used in this study are: rabbit anti-Tachykinin (Veenstra, 2009; Veenstra et al., 2008) (1:500 to detect TK EEs ablation or 1:5000 to detect TKs production), rabbit anti-NPF (a gift from Dr. Ping Shen) (1:1000), rabbit anti-DH31 (Veenstra, 2009; Veenstra et al., 2008) (1:1000), mouse anti-Prospero (Karpowicz et al., 2010) (1:100), mouse anti-Armadillo (Karpowicz et al., 2010) (1:50), rabbit anti-dILP2 (1:5000, gift from Dr. Linda Partridge), rabbit anti-Firefly Luciferase (1:100, Abcam). F-Actin was visualized with Alexa 555-Phallodin (1:1000, Invitrogen). Bodipy 493/503 (1mg/mL, Invitrogen) and Oil Red (0.5%, Sigma) were used for neutral lipid staining for 30 min at room temperature. Regular microscopy was performed on a Zeiss Axioskop 2motplus upright and confocal images were obtained using a Leica TCS SP2 AOBS system. For

Western blot, 8 whole flies were lysed in buffer (50 mM Tris-HCl [pH 7.5], 5 mM EDTA, 10 mM Na4P2O7, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1% Nonidet P-40). Extracts were immunoblotted with indicated antibodies: rabbit anti-AKT (1:1000, Cell Signaling) and rabbit anti-phospho-AKT (S473, 1:1000, Cell Signaling).

TG measurement. TG measurement and starvation resistance were performed as previously described (Song et al., 2010). Briefly, 6 flies were anesthetized and homogenized in 500μL 0.1% Triton/PBS, heated at 70°C for 5 min, and centrifuged at 14,000 rpm for 10 min. 10μl supernatant was used to measure TG using Serum TG determination kits (Sigma). Protein amounts were measured using Bradford Reagent (Sigma). TG storage was normalized to protein amount.

RT-qPCR. 10 midguts or 20 heads from each genotype were collected on ice. RNA was isolated using Trizol (Invitrogen) and cDNA was transcribed using the iScript cDNA Synthesis Kit (Biorad). qPCR was then performed using iQ SYBR Green Supermix on a CFX96 Real-Time System/C1000 Thermal Cycler (Biorad). Gene expression was normalized to *RPL32*. qPCR primers used are:

RPL32-F: *gctaagctgtcgcacaaatg*
RPL32-R: *gttcgatccgtaaccgatgt*
TK-F: *tacaagcgtgcagctctctc*
TK-R: *ctccagatcgctcttcttgc*
NPF-F: *gaggcgtccaactccagac*
NPF-R: *gctctgtcgccgtagtaggt*
DH31-F: *gccaatccaatggaggatac*
DH31-R: *gtatgatggtgcgtccaaag*
TKR99D-F: *tacttcctgcccatcgtctc*
TKR99D-R: *atcatcttcaccacccttcg*
CG2772-F: *ggaagtttagctggcacgag*
CG2772-R: *gaacccatcacgaagaagga*
Margo-F: *acaccgaactgattccgaac*

Margo-R: *atccaccattggcaaacatt*
ACC-F: *taacaacggagtcacccaca*
ACC-R: *caggtcacaaccgatgtacg*
FAS-F: *cgtacgacccctctgttgat*
FAS-R: *agtgcaagttaccgggaatg*
IRE1-F: *cagcctggctaagaacaagg*
IRE1-R: *attggtggtggtggtcagat*
MTP-F: *gtggatctcaatggcaaggt*
MTP-R: *gtgggtgatgctgaaatcct*
NinaD-F: *accaaatgcggaatagcaac*
NinaD-R: *ggcgtaatgcaaaaattcgt*
CG31809-F: *gtcgagcaaatagccgaaag*
CG31809-R: *tacgaacctcccagttcagg*
4EBP-F: *ctcctggaggcaccaaacttatc*
4EBP-R: *ttcccctcagcaagcaactg*
InR-F: *acaaaatgtaaaaccttgcaaatcc*
InR-R: *gcaggaagccctcgatga*

Behavior Assays. The olfactory choice assay has been previously described(Winther et al., 2006). Briefly, 20 flies were introduced in a small chamber where they were allowed to choose between Butanol (1/10, vol/vol, dissolved in water containing 1% Triton-X) and control (water containing 1% Triton-X). The olfactory response index was calculated after 22h as (C-T)/(T+C+NR-D). T is the number of flies in the test vial, C is the number of flies in the control vial, NR is the number of flies remaining in arena, and D is the number of dead flies. For locomotor activity, 20 flies were individually allowed to walk in a narrow transparent tube, and the number of active flies was recorded within 5 min.

Supplemental References

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