Supplemental Information

NF-κB Shapes Metabolic Adaptation by Attenuating Foxo-mediated Lipolysis in *Drosophila*

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Figure S1

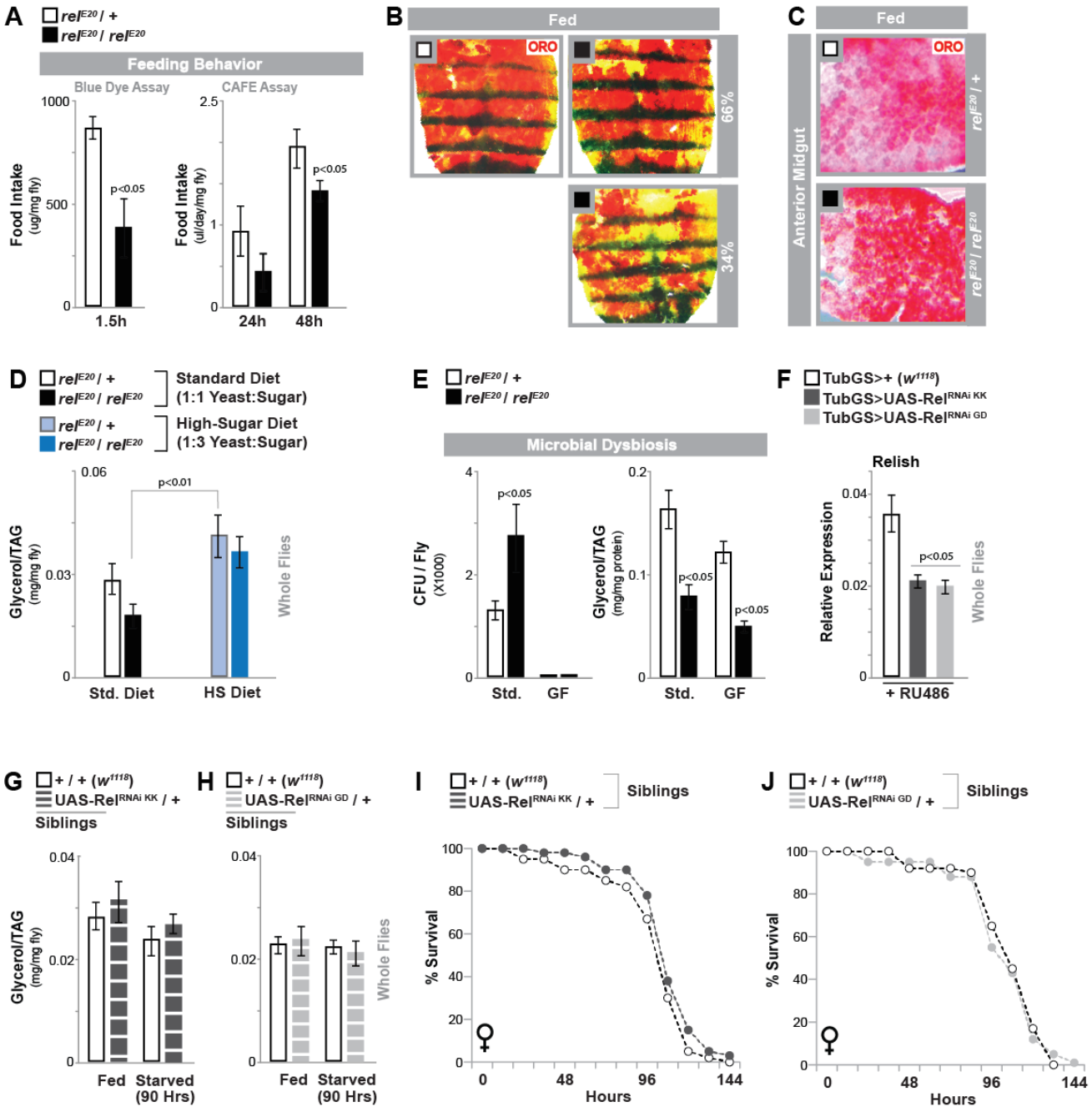
A
rel^E20 / +
rel^E20 / rel^E20
Feeding Behavior
Blue Dye Assay
CAFE Assay
Food Intake (ug/mg fly)
Food Intake (ul/day/mg fly)
p<0.05
p<0.05
1.5h
24h
48h
B
Fed
ORO
66%
34%
C
Fed
ORO
Anterior Midgut
rel^E20 / +
rel^E20 / rel^E20
D
rel^E20 / +
rel^E20 / rel^E20
Standard Diet (1:1 Yeast:Sugar)
High-Sugar Diet (1:3 Yeast:Sugar)
Glycerol/TAG (mg/mg fly)
p<0.01
Whole Flies
Std. Diet
HS Diet
E
rel^E20 / +
rel^E20 / rel^E20
Microbial Dysbiosis
CFU / Fly (X1000)
Glycerol/TAG (mg/mg protein)
p<0.05
p<0.05
p<0.05
Std.
GF
Std.
GF
F
TubGS>+ (w^1118)
TubGS>UAS-Rel^RNAi KK
TubGS>UAS-Rel^RNAi GD
Relish
Relative Expression
p<0.05
Whole Flies
+ RU486
G
+ / + (w^1118)
UAS-Rel^RNAi KK / +
Siblings
Glycerol/TAG (mg/mg fly)
Fed
Starved (90 Hrs)
H
+ / + (w^1118)
UAS-Rel^RNAi GD / +
Siblings
Whole Flies
Fed
Starved (90 Hrs)
I
+ / + (w^1118)
UAS-Rel^RNAi KK / +
Siblings
% Survival
Hours
J
+ / + (w^1118)
UAS-Rel^RNAi GD / +
Siblings
% Survival
Hours

Figure S2

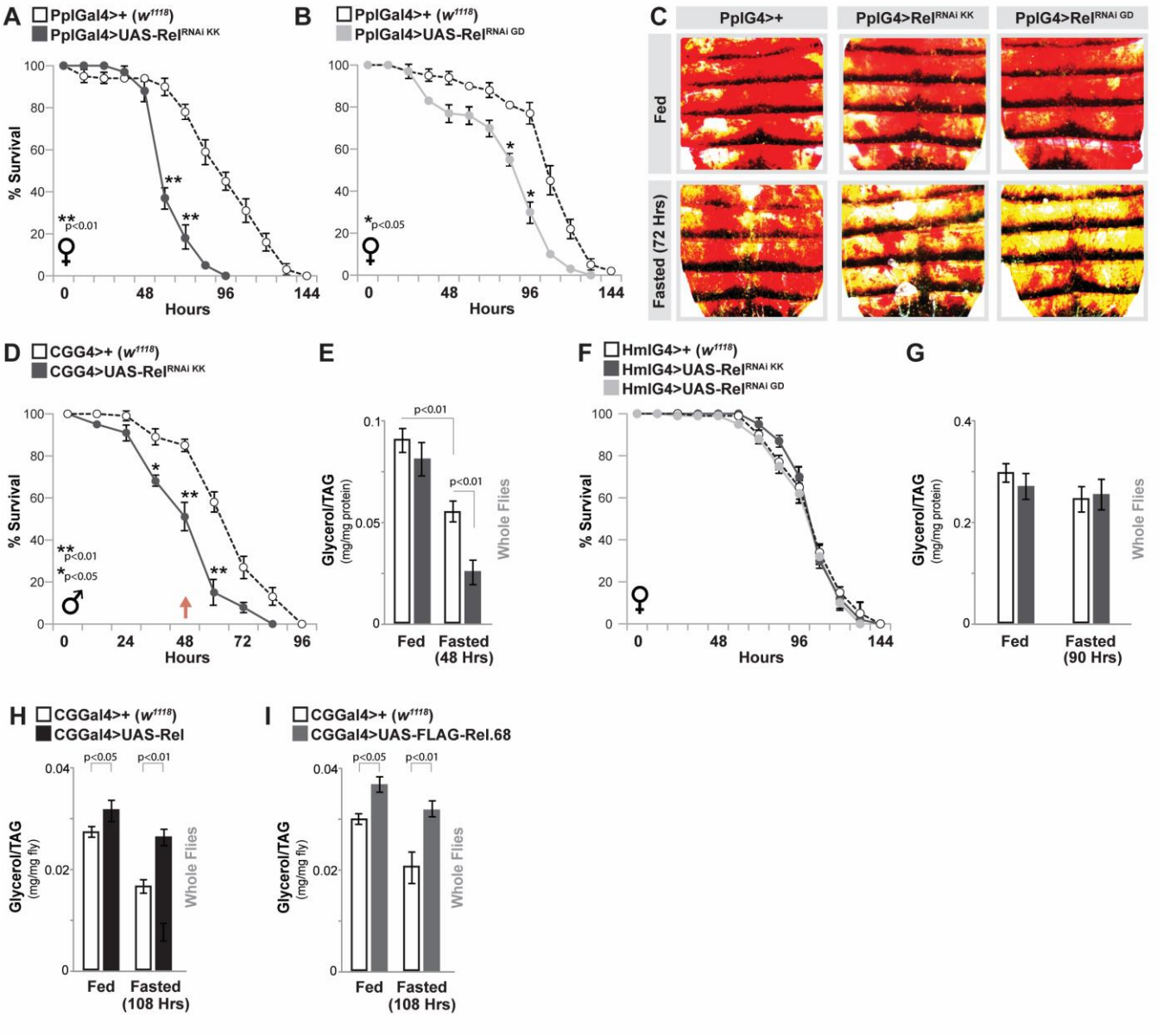
A
PplGal4>+ (w1118)
PplGal4>UAS-RelRNAi KK
% Survival
Hours
**p<0.01
B
PplGal4>+ (w1118)
PplGal4>UAS-RelRNAi GD
*p<0.05
C
PplG4>+
PplG4>RelRNAi KK
PplG4>RelRNAi GD
Fed
Fasted (72 Hrs)
D
CGG4>+ (w1118)
CGG4>UAS-RelRNAi KK
**p<0.01
*p<0.05
E
Glycerol/TAG
(mg/mg protein)
Whole Flies
p<0.01
Fed
Fasted
(48 Hrs)
F
HmlG4>+ (w1118)
HmlG4>UAS-RelRNAi KK
HmlG4>UAS-RelRNAi GD
G
Fasted
(90 Hrs)
H
CGGal4>+ (w1118)
CGGal4>UAS-Rel
p<0.05
Glycerol/TAG
(mg/mg fly)
Fasted
(108 Hrs)
I
CGGal4>+ (w1118)
CGGal4>UAS-FLAG-Rel.68

Figure S3

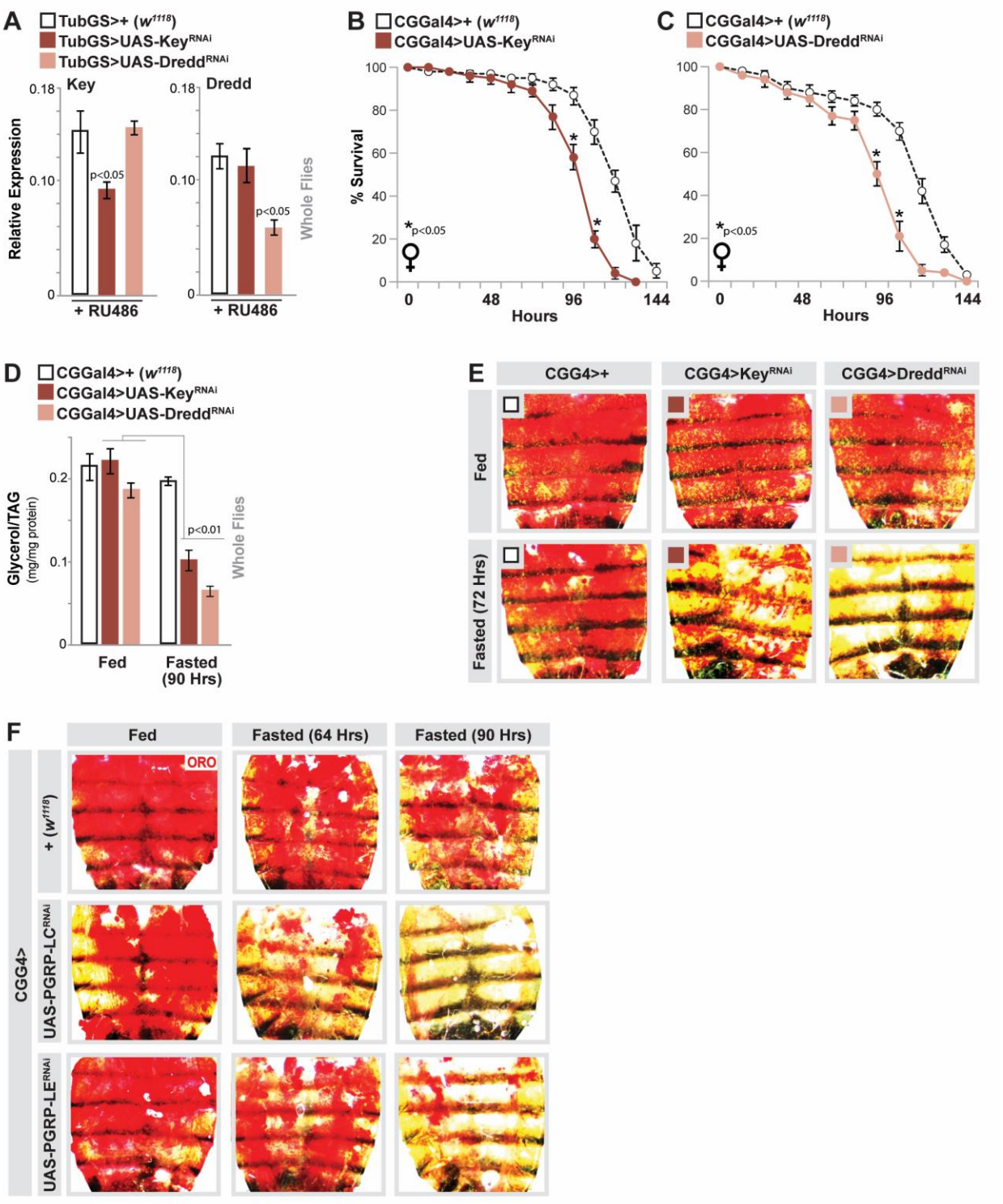
A
TubGS>+ (w1118)
TubGS>UAS-KeyRNAi
TubGS>UAS-DreddRNAi
Key
Dredd
Relative Expression
0.18
0.10
0
p<0.05
p<0.05
+ RU486
+ RU486
Whole Flies
B
CGGal4>+ (w1118)
CGGal4>UAS-KeyRNAi
% Survival
100
80
60
40
20
0
*p<0.05
0
48
96
144
Hours
C
CGGal4>+ (w1118)
CGGal4>UAS-DreddRNAi
100
80
60
40
20
0
*p<0.05
0
48
96
144
Hours
D
CGGal4>+ (w1118)
CGGal4>UAS-KeyRNAi
CGGal4>UAS-DreddRNAi
Glycerol/TAG
(mg/mg protein)
0.2
0.1
0
p<0.01
Whole Flies
Fed
Fasted
(90 Hrs)
E
CGG4>+
CGG4>KeyRNAi
CGG4>DreddRNAi
Fed
Fasted (72 Hrs)
F
Fed
Fasted (64 Hrs)
Fasted (90 Hrs)
CGG4>
+ (w1118)
UAS-PGRP-LCRNAi
UAS-PGRP-LERNAi
ORO

Figure S4

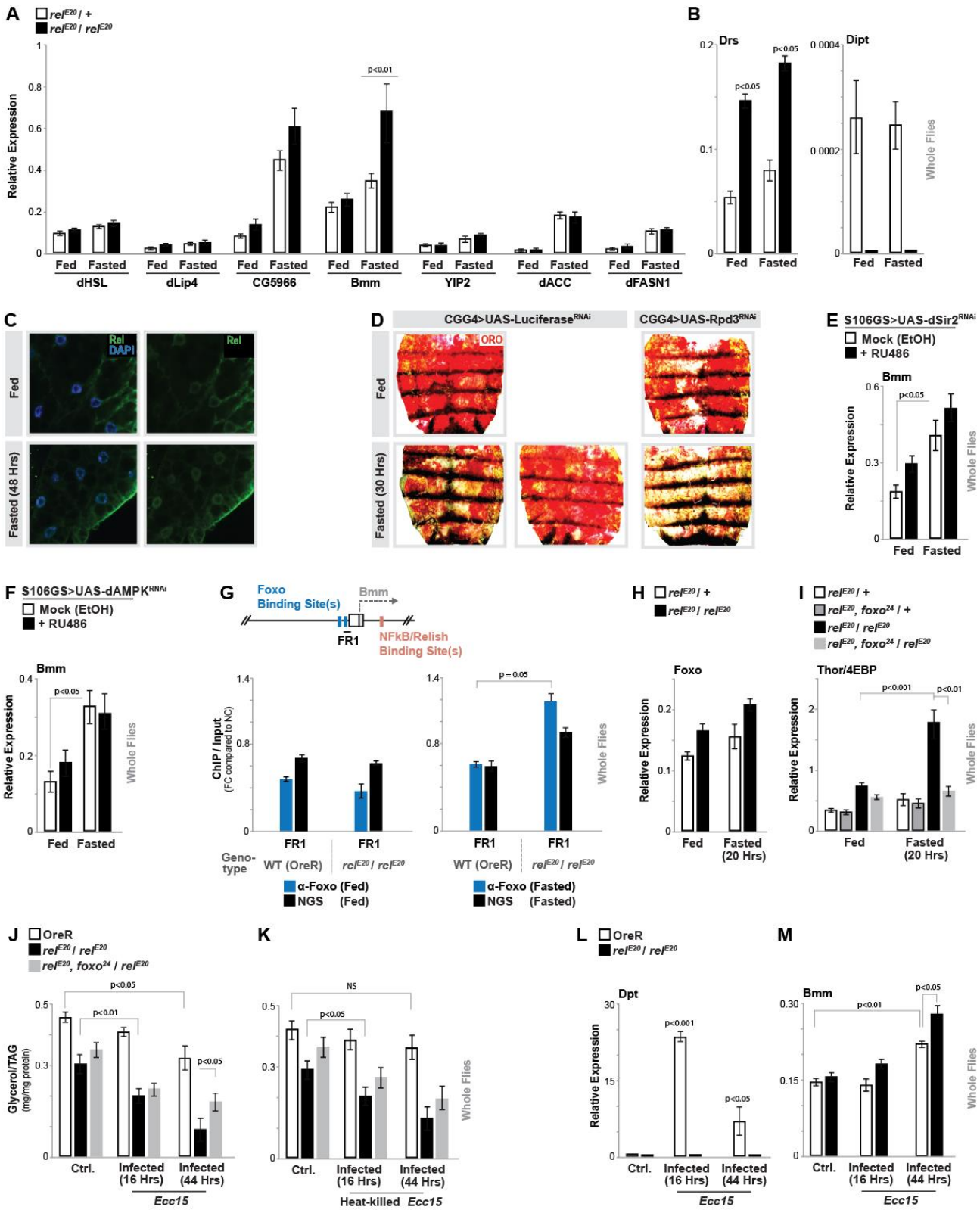
A
relE20 / +
relE20 / relE20
Relative Expression
p<0.01
Fed Fasted
dHSL
dLip4
CG5966
Bmm
YIP2
dACC
dFASN1
B
Drs
Dipt
p<0.05
Whole Flies
C
Rel
DAPI
Fed
Fasted (48 Hrs)
D
CGG4>UAS-LuciferaseRNAi
CGG4>UAS-Rpd3RNAi
ORO
Fed
Fasted (30 Hrs)
E
S106GS>UAS-dSir2RNAi
Mock (EtOH)
+ RU486
Bmm
F
S106GS>UAS-dAMPKRNAi
G
Foxo Binding Site(s)
Bmm
FR1
NFkB/Relish Binding Site(s)
ChIP / Input (FC compared to NC)
p = 0.05
Geno-type
WT (OreR)
α-Foxo (Fed)
NGS (Fed)
α-Foxo (Fasted)
NGS (Fasted)
H
Foxo
Fasted (20 Hrs)
I
relE20, foxo24 / +
relE20, foxo24 / relE20
Thor/4EBP
p<0.001
J
OreR
Glycerol/TAG (mg/mg protein)
Ctrl.
Infected (16 Hrs)
Infected (44 Hrs)
Ecc15
K
NS
Heat-killed Ecc15
L
Dpt
M
Bmm

Figure S1: NF-κB /Relish-dependent changes in organismal lipid homeostasis. Related to Figure 1.

(A) Relish-dependent changes in feeding behavior. *relE20*/*relE20* (mutant) female flies display decreases in food intake using both the Blue Dye feeding assay (n=3-6 cohorts of 5 flies, measured ZT(8-10) after 1.5 hours feeding) and the CAFE assay (n=7 samples measured after 24 hours and 48 hours); compared to *relE20*/+ controls. Note: *relE20*/*relE20* mutant show stronger decreases in acute food intake (Blue Dye, 1.5 hours) as opposed to chronic (CAFE, 48 hours).

(B-C) Oil red O (ORO) neutral lipid stain in dissected (B) fat body/carcass and (C) intestine from *relE20*/*relE20* (mutant) female flies compared to *relE20*/+ control flies. Images from intestine taken from anterior midgut. Note: *relE20*/*relE20* mutant show variable decreases in lipid storage in fat body, but not in the intestine. Percentages represent relative contribution of mild (top panel) or strong (bottom panel) lipid storage defects from 45 unique fat body/carcass dissections from multiple, independent experiments.

(D) Relish-dependent (*relE20*/*relE20* mutant) changes in total triglyceride (TAG) levels (whole flies) during *ad libitum* feeding (compared to *relE20*/+ controls) can be minimized by feeding a high calorie/high sugar diet (n = 5 samples).

(E) Relish-dependent changes in triglyceride levels during *ad libitum* feeding are not caused by microbial dysbiosis. Colony forming units (CFU per fly, n = 4) and total triglyceride (TAG) levels of whole flies (n = 4 samples) in standard rearing conditions (Std.) or germ-free rearing conditions (GF); genotypes *relE20*/+ (control) or *relE20*/*relE20* (mutant).

(F) UAS-Rel RNAi efficiency. Changes in *relish* transcription (measured by qRT-PCR in whole flies) upon ubiquitous Relish depletion (RNAi line v108469-KK and v49413-GD) using Tubulin-GeneSwitch-Gal4 (TubGS) after 5 days feeding RU486; compared to controls (TubGS>+(w^{1118})). n = 4 samples.

(G-J) UAS-Rel RNAi transgenes (RNAi lines v108469-KK and v49413-GD) alone do not affect fasting-induced triglyceride metabolism or survival during metabolic adaptation. (G-H) Total TAG levels of whole female flies (n=5 samples, before and after fasting (90 hours)) and (I-J) starvation resistance of female flies (n = 5 cohorts, total 89-96 flies) from UAS-Rel RNAi/+(w^{1118}) and +/+(w^{1118}) female siblings.

All bars and line graph markers represent mean±SE. All flies were 7 days old post-eclosion.

Figure S2: NF-κB/Relish-dependent changes in lipid metabolism in response to metabolic adaptation. Related to Figure 1.

(A-C) Changes in lipid metabolism and survival upon Relish depletion (RNAi lines v108469-KK and v49413-GD) in fat body (PplGal4). (A-B) Starvation resistance of female flies. n = 6 cohorts (total 110-141 flies). (C) Oil Red O (ORO) neutral lipid stain of dissected carcass/ fat body before and after fasting (72 hours).

(D-E) Changes in lipid metabolism and survival upon Relish depletion (RNAi lines v108469-KK) in fat body (CGGal4) of male flies. (D) Starvation resistance of male flies. n = 5 cohorts (total 90-95 flies). (E) Total triglyceride (TAG) levels of whole male flies (n=5 samples, before and after fasting (48 hours)).

(F-G) Absence of changes in lipid metabolism and survival upon Relish depletion (RNAi lines v108469-KK and v49413-GD) in hemocytes (HmlGal4) of female flies. (F) Starvation resistance of female flies. n = 4 cohorts (total 77-80 flies). (G) Total triglyceride (TAG) levels of whole male flies (n=3 samples, before and after fasting (90 hours)).

(H-I) Changes in fasting-induced lipid metabolism upon Relish over-expression (both (H) full-length Relish (UAS-Rel) and (I) the tagged N-terminal active fragment (UAS-FLAG-Rel.68)) in fat body (CGGal4). Total TAG levels of whole female flies (n=5 samples, before and after fasting (108 hours)). Control genotype CGGal4/+ (w^{1118}).

Bars and line graph markers represent mean ± SE. All flies were 7 days old post-eclosion.

Figure S3: Kenny and Dredd-dependent changes in lipid metabolism in response to metabolic adaptation. Related to Figure 1 and 3.

(A) RNAi efficiency. Changes in *kenny or dredd* transcription (measured by qRT-PCR in whole flies) upon ubiquitous depletion (Kenny (Key) RNAi line v7723-GD and Dredd RNAi line v104725-KK) using Tubulin-GeneSwitch-Gal4 (TubGS) after 5 days feeding RU486; compared to controls (TubGS>+(w^{1118})). n = 4 samples.

(B-E) Changes in lipid metabolism and survival upon Kenny or Dredd depletion (RNAi lines v7723-GD (Key) and v104726-KK (Dredd)) in fat body (CGGal4) of female flies. (B-C) Starvation resistance of female flies. n = 7 cohorts (total 140-149 flies). (D) Total triglyceride (TAG) levels of whole female flies (n=5 samples, before and after fasting (90 hours)) and (E) Oil Red O (ORO) neutral lipid stain of dissected carcass/ fat body before and after fasting (72 hours). Control genotype CGGal4/+ (w^{1118}).

(F) Changes in lipid metabolism upon PGRP-LC or PGRP-LE depletion (RNAi lines v101636-KK (LC) and v23664-GD (LE)) in fat body (CGGal4) of female flies. Oil Red O (ORO) neutral lipid stain of dissected carcass/ fat body before and after fasting (64 and 90 hours). Control genotype CGGal4/+ (w^{1118}).

Bars and line graph markers represent mean ± SE. All flies were 7 days old post-eclosion.

Figure S4: Fasting-induced and NF-κB/Relish-dependent changes in gene expression and transcriptional regulation. Related to Figure 3-4.

(A) *Drosophila HSL*, *lip4*, *CG5966*, *bmm*, *YIP2* (yippee interacting protein 2/thiolase), *ACC* (acetyl-CoA carboxylase), and *FASN1* (fatty acid synthase) transcription (measured by qRT-PCR in whole flies) before and after fasting (20 hours). *relE20*/+ (heterozygote control), or *relE20*/*relE20* (mutant) genotypes. n = 3 samples.

(B) *Drosophila drs* (Drosomycin) and *dipt* (Diptericin) transcription (measured by qRT-PCR in whole flies) before and after fasting (20 hours). *relE20*/+ (heterozygote control), or *relE20*/*relE20* (mutant) genotypes. n = 4 samples.

(C) Relish immunostaining in carcass/fat body before and after fasting (20 hours; OreR female flies). Stained with anti-Rel and nuclei visualized with DAPI (blue). Weak Rel signal is detected in the nucleus during fed and fasted conditions, however, the staining is slightly more perinuclear during *ad libitum* feeding (upper right panel).

(D) Changes in lipid metabolism upon Rpd3 depletion (RNAi TRiP 36800) in fat body (CGGal4) of female flies. Oil Red O (ORO) neutral lipid stain of dissected carcass/ fat body before and after fasting (30 hours). Control genotype CGGal4>UAS-Luciferase RNAi (slight variation in control phenotype after fasting is highlighted with two independent images).

(E-F) Changes in *bmm* transcription (measured by qRT-PCR in whole flies) upon fat body-specific depletion of *Drosophila* (E) AMPKα (RNAi line v1827-GD) and (F) Sir2 (homolog of SIRT1; dSir2 RNAi line v23201-GD) using S106-GeneSwitch-Gal4 after 5 days feeding RU486; compared to controls (mock (EtOH) treated siblings) before and after fasting (90 hours). n = 4-5 samples.

(G) Schematic shows Bmm locus (focusing on the upstream promoter and first intron proximal to transcription start site), as well as Foxo and NF-κB/Rel binding motifs. FR1 represents regional target site of Foxo binding (and corresponding primer set) tested in ChIP-qPCR analysis. The histogram represents ChIP-qPCR analysis of Foxo binding to the Bmm promoter/locus in fed (left panel) and fasted (20 hours; right panel) conditions. ChIP-qPCR

analysis with normal goat serum (NGS) is included as a control. Plotted as fold change (FC) of indicated PCR primer sets compared to a negative control (NC) primer set. n = 3 samples.

(H) *Drosophila foxo* transcription (measured by qRT-PCR in whole flies) before and after fasting (20 hours). *relE20*/+ (heterozygote control), or *relE20*/*relE20* (mutant) genotypes. n = 3 samples.

(I) Relish-Foxo antagonism controls Thor/4EBP expression during fasting. *Drosophila thor* (homolog of 4EBP) transcription (measured by qRT-PCR in whole flies) before and after fasting (20 hours) in controls (*relE20*/+ and *relE20, foxo24*/+), mutant (*relE20*/*relE20*) and mutant with reduction in Foxo gene dose (*relE20, foxo24*/*relE20*) genotypes. n = 3 samples.

(J-K) Changes in lipid metabolism (total triglyceride (TAG) levels of whole female flies) after systemic *Ecc15* infection with (J) live or (K) dead bacteria (16 and 44 hours post infection) in controls (OreR*),* mutant (*relE20*/*relE20*), and mutant with reduction in Foxo gene dose (*relE20, foxo24*/*relE20*) genotypes. n=3-5 samples. TAG levels decline after acute (16 hours) and chronic (44 hours) infection with live bacteria in Relish mutant flies, while slight TAG level changes in controls are observed only after 44 hours. Reducing gene dose of Foxo in Relish mutant flies partially rescues the accelerated loss of TAG levels only 44 hours post-infection. Treatment with dead bacteria drive similar, but slightly attenuated, changes in TAG levels, suggesting that the presence of live bacteria may impinge different energy demands on the host that shape metabolic adaptation in response to infection.

(L-M) Changes in (L) *Dpt* and (M) *bmm* transcription (measured by qRT-PCR in whole flies) before and after systemic *Ecc15* infection (16 and 44 hours post infection) in controls (OreR*)* and mutant (*relE20*/*relE20*) genotypes. n = 3-4 samples. *bmm* transcription is slightly elevated after chronic systemic infection in controls (44 hrs, as *Dpt* transcription begins to subside) and is further enhanced at this time-point in Relish mutant flies.

Bars represent mean ± SE. All flies were 7 days old post-eclosion.

	Forward	Reverse
qRT-PCR Primers		
Relish	CATCAGGAGACAGAGCGTGA	CCGACTTGCGGTTATTGATT
Kenny	TGACAAGGTCAACCAAACCA	CCTGCTCCTTTAGCCTGATG
Dredd	CAGGAGATCCACTTCGCTTC	CGACTGCTGGTTATCCGATT
Brummer	CAATAAGGGTCTGGCCAACTGGAT	TAAGTCCTCCACCATTACTCTGGC
dHSL	ATGAGTGGCTTTCCCAACTG	CATGGCTTCGTTGGATAACA
dLip4	TGGATAGCTCAGCCACTT	GCGGGTATATCATGCTTTCC
CG5966	CTGCAATCACATTCGCAGTC	TGCTCCTGGTAATCCTCCTG
YIP2	CGGTCTTAAGGGTGAGCAA	ACATTACGGGCAATGAAAGG
dACC	CTATCGCTATGGTTACCTGCCGTA	AACATGATCTGTGTGCCACCCAA
dFASN1	TGATGGCCGGTATTCTGGAAGAGA	ATTGCTCATCAGCTCAGCGAACCT
Dipt	TTCATTGGACTGGCTTGTGCCTTC	TGAGGCTCAGATCGAATCCTTGCT
Drs	AAGTACTTGGCCCTCTTCGCT	TCCTTCGCACCAGCACTTCAGACT
Foxo	TCTCGCCGAACTCAGTAACC	CCTCCAGGCATTGTCCTATC
Thor	CACTTGCGGAAGGGAGTACG	TAGCGAACAGCCAACGGTG
Actin5c	CTCGCCACTTGCGTTTACAGT	TCCATATCGTCCCAGTTGGTC
ChIP-qRT-PCR Primers		
R1 (Bmm locus)	GCTTGTTTGCGTTTGTAGGTC	TTCGAAATTGGACAAACACG
R2 (Bmm locus)	TGTCGCTGACAATCAAAAGC	TTCTGGGTGGAGTTTGGAAC
Act5c^{P}	AACCCCCAAATTGAATCACA	GAGAATTTCCTCCGCAACTG
Dipt^{P}	AAGAAAGATCCCCTGGTGGT	TTTTATAGGCCGCTTTCCAA
FR1 (Bmm locus)	CACCGCGCCGCAATGAATGTATAA	TTCAATCACTGTTTGTCGGTCGGC

Table S1: Primer sequences used for qRT-PCR (related to Figures 2, 3, 4, S1, S3, and S4)