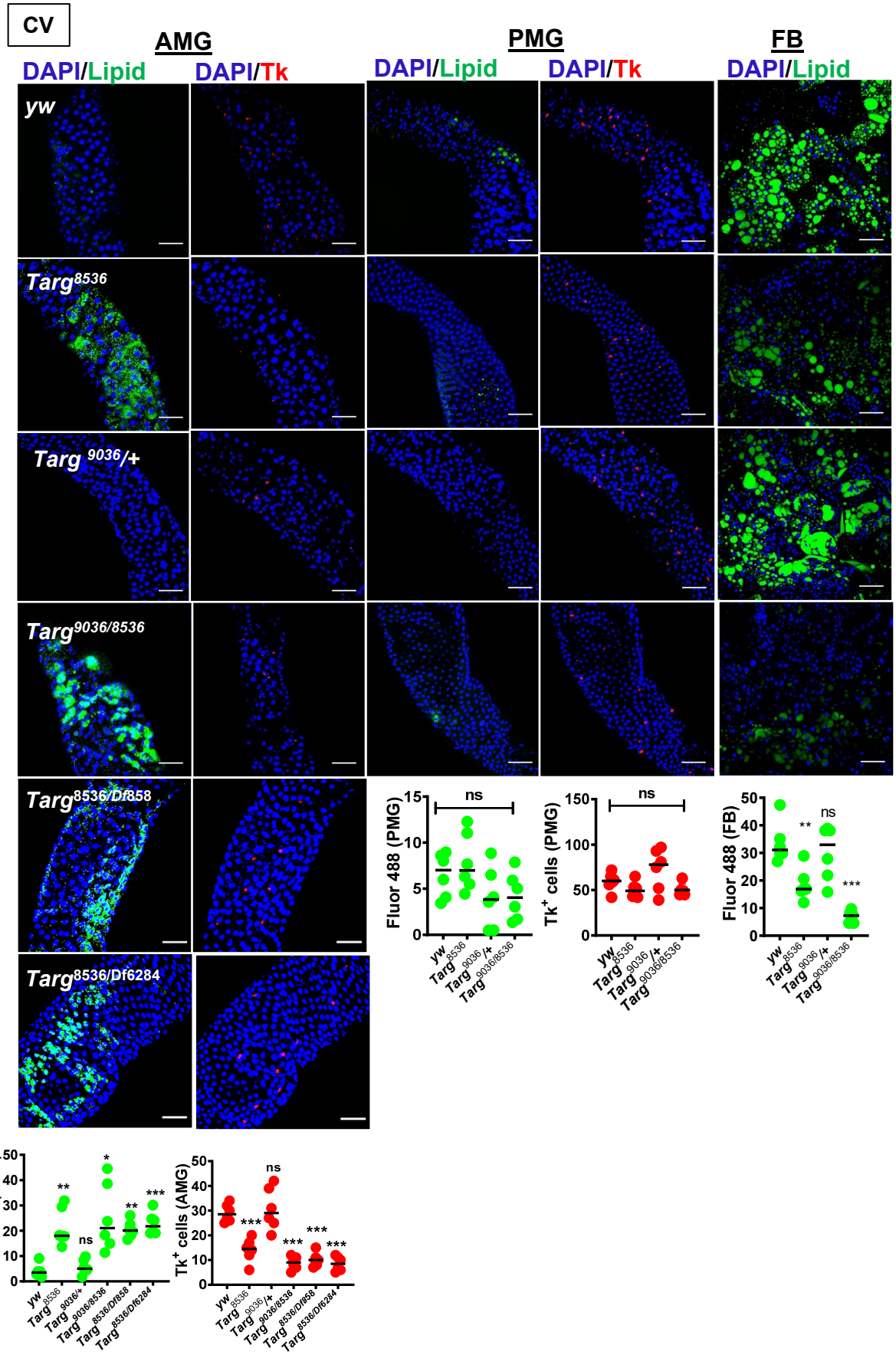

CV
AMG
PMG
FB
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
yw
Targ8536
Targ 9036/+
Targ9036/8536
Targ8536/Df858
Targ8536/Df6284
Fluor 488 (PMG)
Tk+ cells (PMG)
Fluor 488 (FB)
Fluor 488 (AMG)
Tk+ cells (AMG)
ns
**
***
*
yw
Targ8536
Targ 9036/+
Targ9036/8536
Targ8536/Df858
Targ8536/Df6284

Figure S1: *Targ* mutants phenocopy the GF fly phenotype. Representative micrographs (above) and quantification (below) of Bodipy staining (Lipid) and Tk immunofluorescence of CV *yw* flies, Targ ^{MG08536} homozygotes (*Targ*^{0856}), *Targ*^{MI09036}/SM6a (*Targ*^{9036}/+) heterozygotes, and *Targ*$^{MG08536/\ MI09036}$ (*Targ*$^{9036/0856}$), *Targ*$^{MG08536/Df\ (2R)\ BSC858}$ (*Targ*$^{8536/Df858}$) and *Targ*$^{MG08536/Df\ (2R)\ Exel6284}$ (*Targ*$^{8536/Df6284}$) transheterozygotes in the AMG and, where noted, PMG, and FB. Experiments with deficiency transheterozygotes were conducted on a separate day. The mean measurement is indicated. Error bars represent the standard deviation. A Brown-Forsythe ANOVA with a Dunnett's T3 multiple comparisons test was used to evaluate statistical significance. ns not significant, *p<0.05 **p<0.01, ***p<0.001. See also Fig 1.

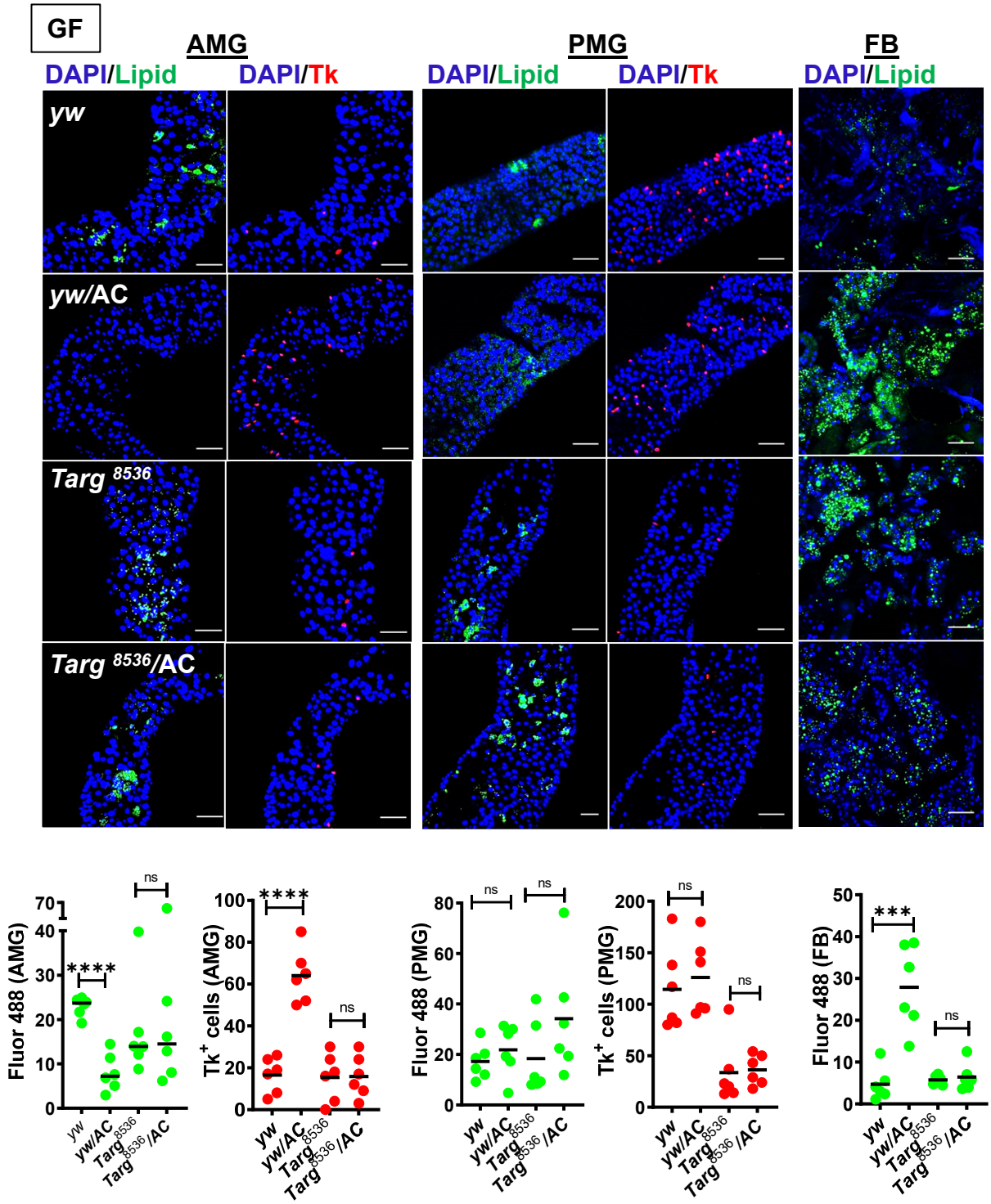
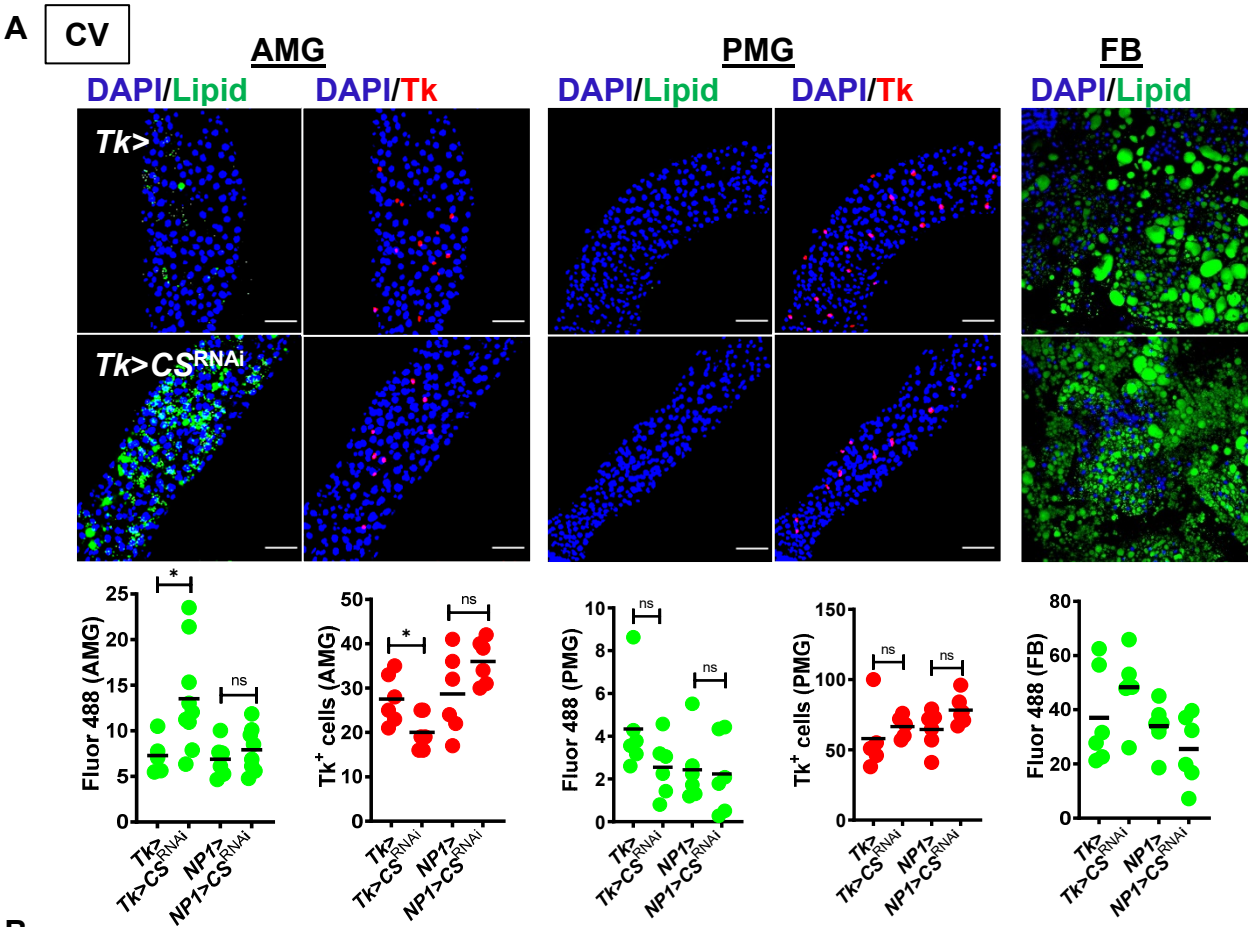


Figure S2: *Targ* mutation blocks acetate rescue of GF flies. Representative micrographs and quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG, PMG, and FB of *yw* flies and Targ ^{MG08536} homozygotes (*Targ*^{8536}) fed LB broth supplemented with antibiotics alone (GF) or with 50 mM acetate (GF/AC). The mean measurement is indicated. Error bars represent the standard deviation. A students t-test was used to evaluate statistical significance. ns not significant, *** p<0.001, ****p<0.0001. See also Fig 1.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CV
AMG
PMG
FB
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
Tk>
Tk>CS^RNAi
Fluor 488 (AMG)
Tk+ cells (AMG)
Fluor 488 (PMG)
Tk+ cells (PMG)
Fluor 488 (FB)
*
ns
*
ns
ns
ns
ns
ns
Tk>
Tk>CS^RNAi
NP1>
NP1>CS^RNAi

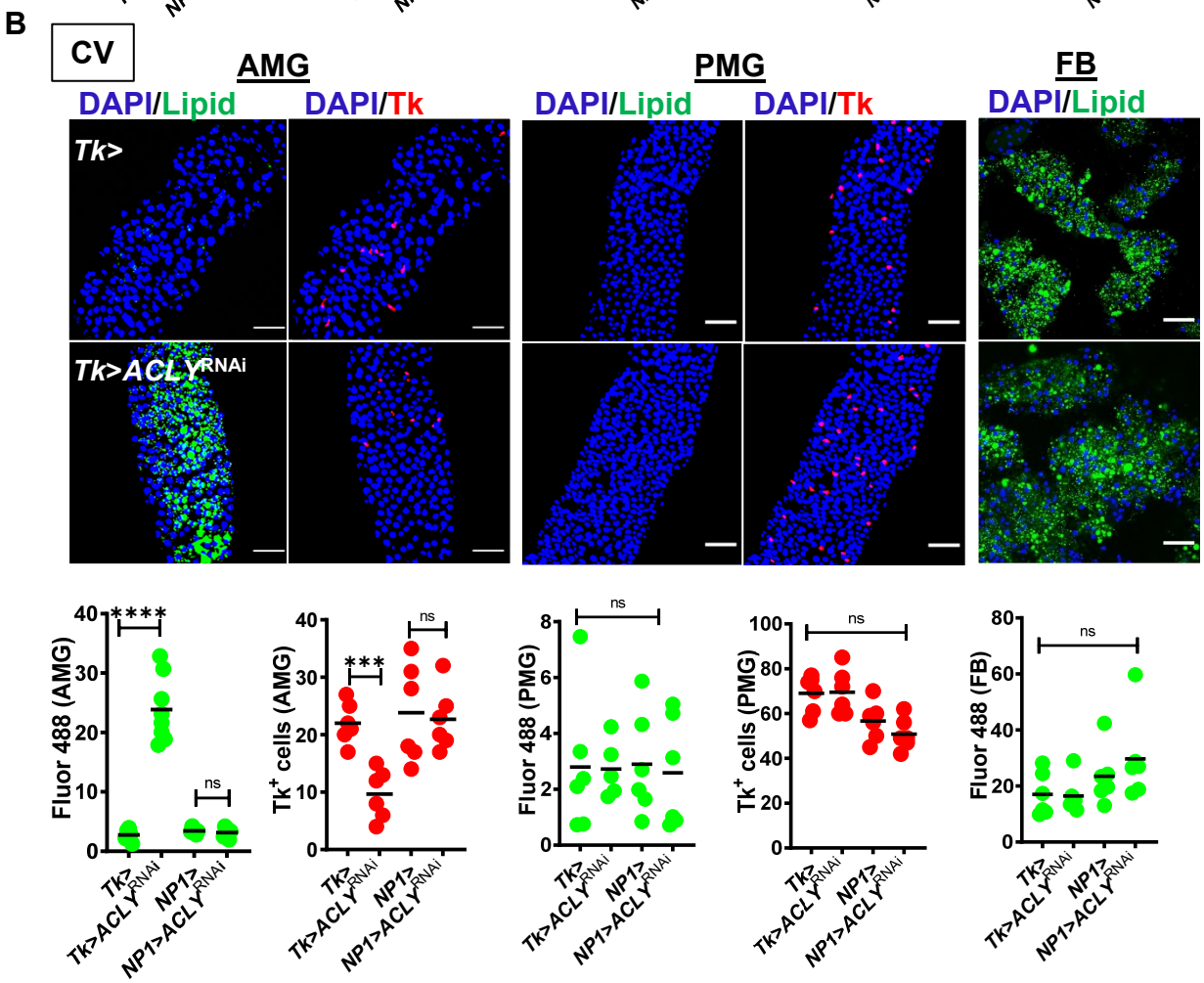
B
CV
AMG
PMG
FB
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
Tk>
Tk>ACLY^RNAi
Fluor 488 (AMG)
Tk+ cells (AMG)
Fluor 488 (PMG)
Tk+ cells (PMG)
Fluor 488 (FB)
****
ns
***
ns
ns
ns
ns
Tk>
Tk>ACLY^RNAi
NP1>
NP1>ACLY^RNAi

Figure S3: *Citrate synthase* (*CS*) and *ATP citrate lyase* (*ACLY*) RNAi in EECs but not ECs reproduces the GF fly phenotype. Representative micrographs and/or quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG, PMG, and FB of CV Tk>, NP1> and **(A)** Tk>*CS*^{RNAi} and NP1>*CS*^{RNAi} or **(B)** Tk>*ACLY*^{RNAi} and NP1>*ACLY*^{RNAi} flies. The mean measurement is indicated. Error bars represent the standard deviation. A student's t-test was used to evaluate statistical significance. ns not significant, *p<0.05. *** p<0.001, **** p<0.0001. See also Fig 3.

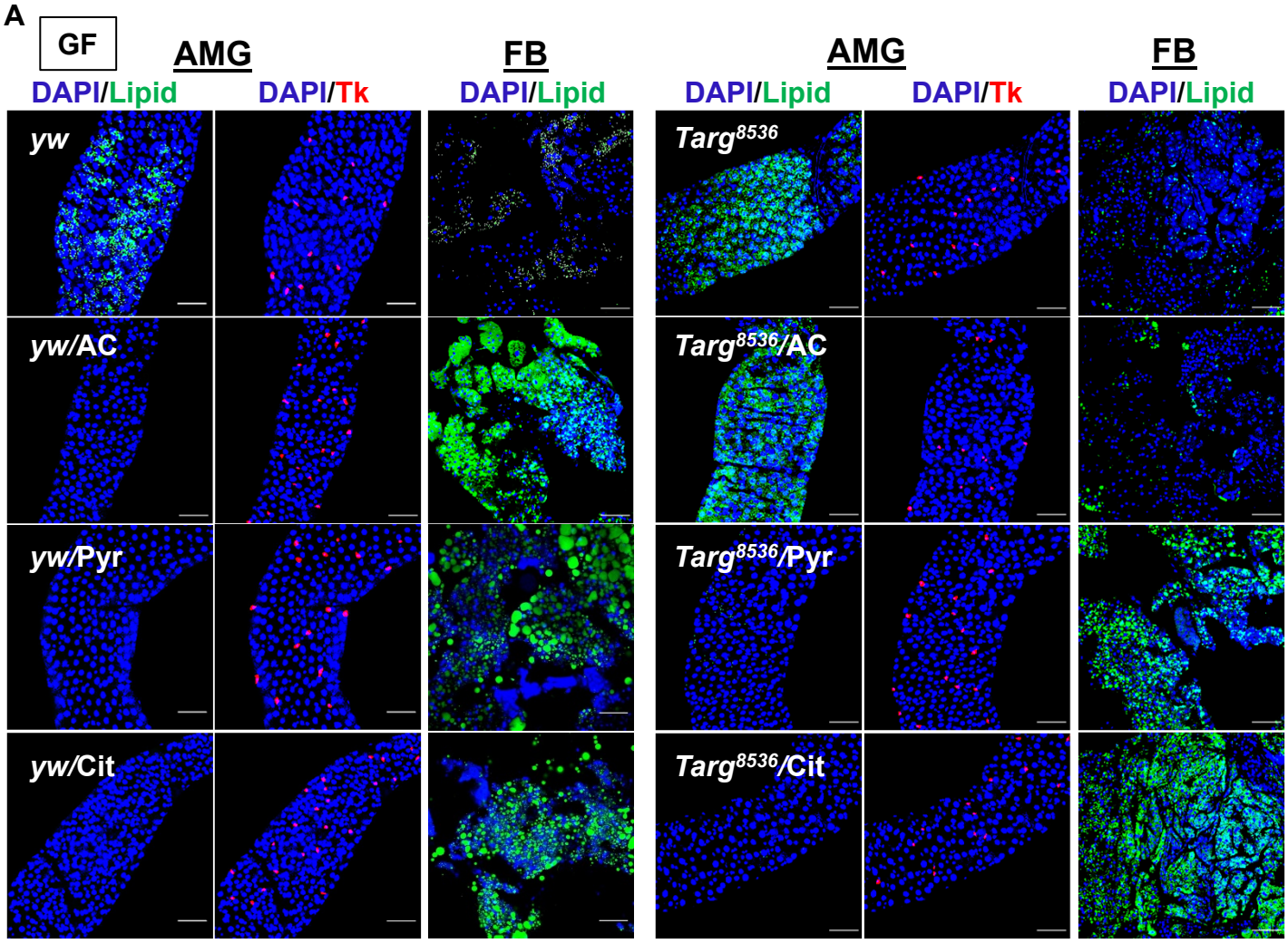
A
GF
AMG
FB
AMG
FB
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
yw
yw/AC
yw/Pyr
yw/Cit
Targ8536
Targ8536/AC
Targ8536/Pyr
Targ8536/Cit
Fluor 488 (AMG)
Tk+ cells (AMG)
Fluor 488 (FB)
yw
yw/Ac
yw/Pyr
yw/Cit
Targ8536
Targ8536/Ac
Targ8536/Pyr
Targ8536/Cit
B
CV
Relative expression
TK>
TK>TargRNAi
TK>ACSRNAi
TK>ACLYRNAi
DptA
PGRP-LC
C
Vc
TK>
TK>ACLYRNAi
Relative expression
DptA
CecA1
D
CV
TK>
TK>TKRNAi
Relative expression
DptA

Figure S4: The cellular acetyl-CoA pool and Tk participate in lipid homeostasis and IMD pathway signaling in GF, CV uninfected, and *V. cholerae*-infected flies. (A) Representative micrographs (above) and quantification (below) of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG and FB of *yw* or*Targ*^{8536} mutant GF flies fed LB broth with or without acetate (AC), pyruvate (Pyr), or citrate (Cit). RT-qPCR analysis of **(B)** *DptA* and *PGRP-LC* expression in CV Tk> and Tk>*Targ*^{RNAi}, Tk>*ACS*^{RNAi}, or Tk>*ACLY*^{RNAi} flies, **(C)** *DptA* and *CecA1* in *V. cholerae*-infected Tk> and Tk>*ACLY*^{RNAi} flies and **(D)** *DptA* expression in CV Tk> or Tk>*Tk*^{RNAi} flies. A subset of the data in S4B is also shown in Fig 1D. Statistical tests take this into account. The mean measurement is indicated. Error bars represent the standard deviation. An ordinary one-way ANOVA with Dunnett's multiple comparisons (A,B) or student's t-test (C-D) was used to evaluate statistical significance. ns not significant, *p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001. See also Fig 3.

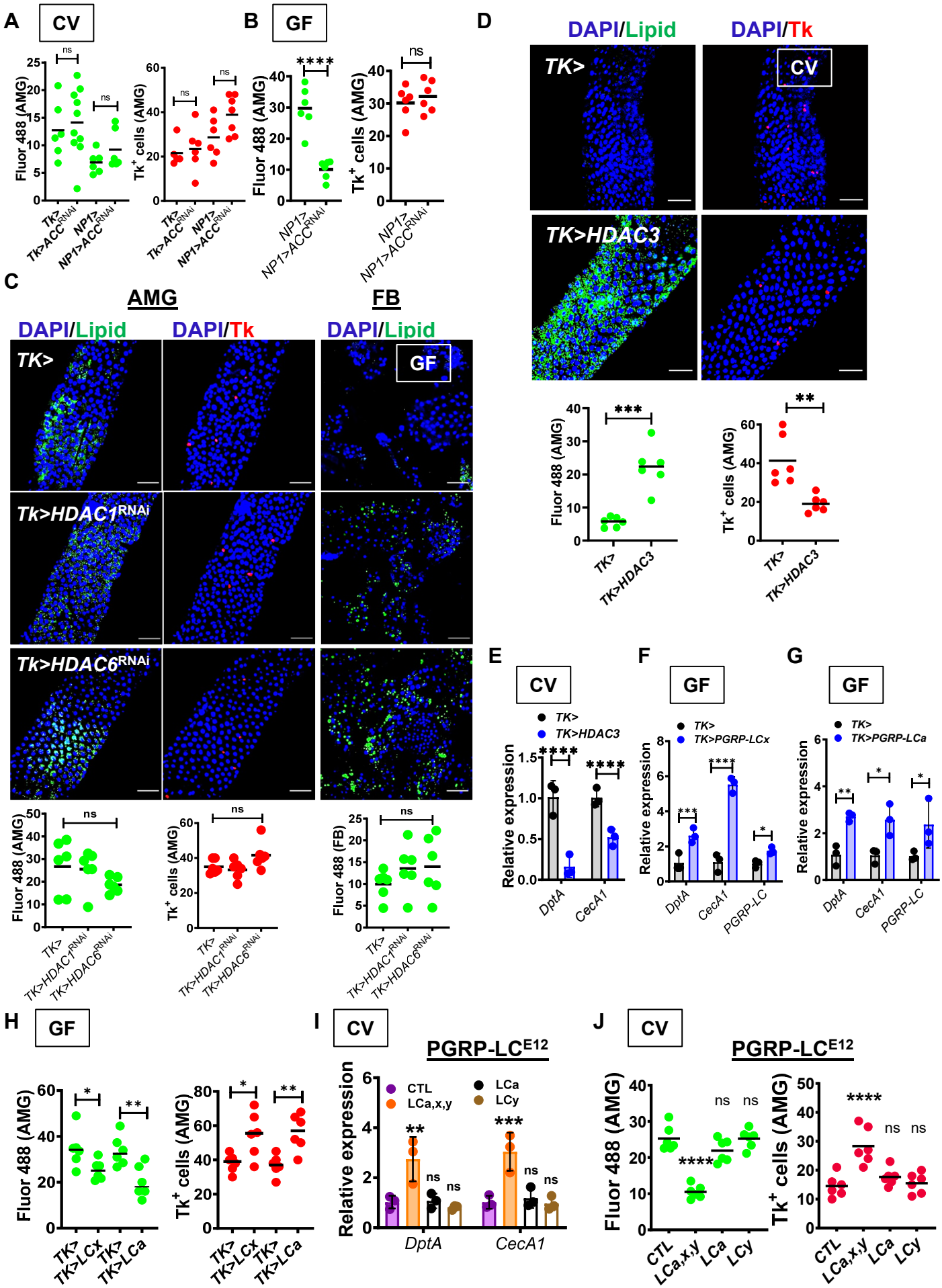
A
CV
B
GF
D
DAPI/Lipid
DAPI/Tk
TK>
CV
TK>HDAC3
Fluor 488 (AMG)
Tk+ cells (AMG)
TK>
TK>HDAC3
C
AMG
FB
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
TK>
GF
Tk>HDAC1RNAi
Tk>HDAC6RNAi
Fluor 488 (AMG)
Tk+ cells (AMG)
Fluor 488 (FB)
TK>
TK>HDAC1RNAi
TK>HDAC6RNAi
E
CV
TK>
TK>HDAC3
Relative expression
DptA
CecA1
F
GF
TK>
TK>PGRP-LCx
Relative expression
DptA
CecA1
PGRP-LC
G
GF
TK>
TK>PGRP-LCa
Relative expression
DptA
CecA1
PGRP-LC
H
GF
Fluor 488 (AMG)
Tk+ cells (AMG)
TK>
TK>LCx
TK>
TK>LCa
I
CV
PGRP-LCE12
CTL
LCa,x,y
LCa
LCy
Relative expression
DptA
CecA1
J
CV
PGRP-LCE12
Fluor 488 (AMG)
Tk+ cells (AMG)
CTL
LCa,x,y
LCa
LCy

Figure S5: *HDAC1* or *6* RNAi does not rescue the antibiotic-treated fly phenotype; overexpression of HDAC3 reproduces the microbe-depleted phenotype. Quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG of **(A)** CV Tk>, NP1> , Tk>*ACC*^{RNAi}, and NP1>*ACC*^{RNAi} flies and **(B)** GF Tk> and Tk>*ACC*^{RNAi} flies. **(C)** Representative micrographs (above) and quantification (below) of Bodipy staining (Lipid) and Tachykinin (Tk) immunofluorescence in the AMG and FB of GF *Tk>*, Tk>*HDAC1*^{RNAi} and Tk>*HDAC6*^{RNAi} flies. This data was acquired with that presented in Fig 4B and shares the driver control. This was taken into account in the statistical analysis. **(D)** Representative micrographs and quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG of CV Tk> and Tk>*HDAC3* flies. This data was acquired with that shown in Fig S7A using the same driver control. This was taken into account in the statistical analysis. RT-qPCR analysis of expression of the indicated genes in the intestines of **(E)** CV Tk> and Tk>*HDAC3* flies, GF Tk> **(F)** Tk>*PGRP-LCx* or **(G)** Tk>*PGRP-LCa* flies. **(H)** Quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG of GF Tk> and Tk>*PGRP-LCx* or Tk>*PGRP-LCa* flies. **(I)** RT-qPCR quantification of *DptA* and *CecA1* and **(J)** quantification of Bodipy staining (Lipid) and Tachykinin (Tk) immunofluorescence in the AMG of CV *PGRP-LC*^{E12} mutant flies expressing PGRP-LCaxy, PGRP-LCa, or PGRP-LCy isoforms. The mean measurement is indicated. Error bars represent the standard deviation. A student's t-test (A,B, D-F, G) or an ordinary one-way ANOVA with Dunnett's multiple comparisons (C, H, I) was used to evaluate statistical significance. ns not significant,* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. See also Fig 4.

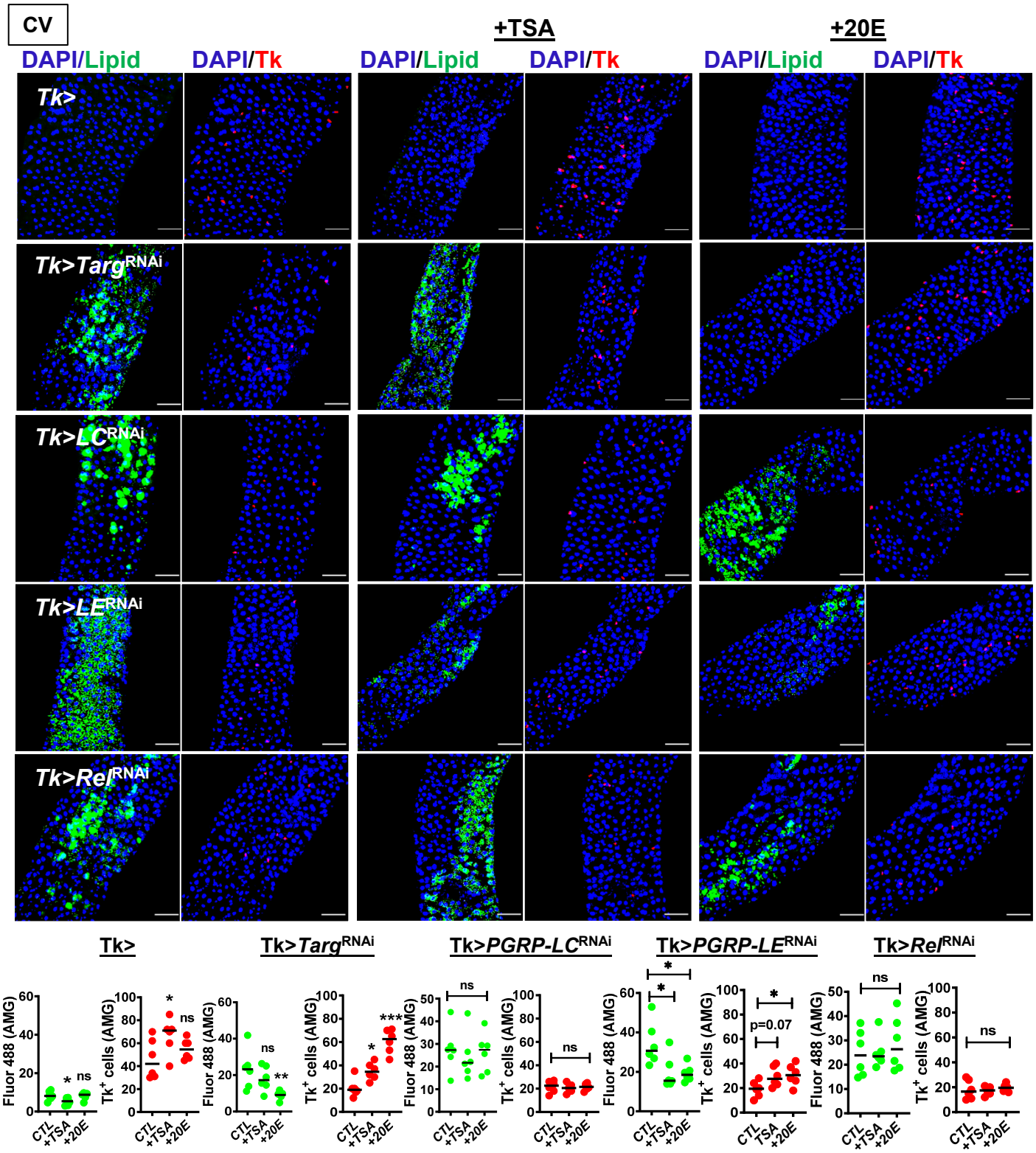

Figure S6: Acetate acts upstream of histone acetylation and 20E, and 20E acts upstream of PGRP-LC. Representative micrographs and quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG of CV Tk> and Tk>*Targ*^{RNAi}, Tk>*LC*^{RNAi}, Tk>*LE*^{RNAi}, or Tk>*Rel*^{RNAi} flies. Flies were fed LB broth alone or supplemented with the histone deacetylase inhibitor trichostatin A (TSA) or 20-hydroxyecdysone (20E) as indicated. An ordinary one-way ANOVA with Dunnett's multiple comparisons test was used to evaluate statistical significance. ns not significant, * p<0.05, ***p<0.001. See also Fig 5.

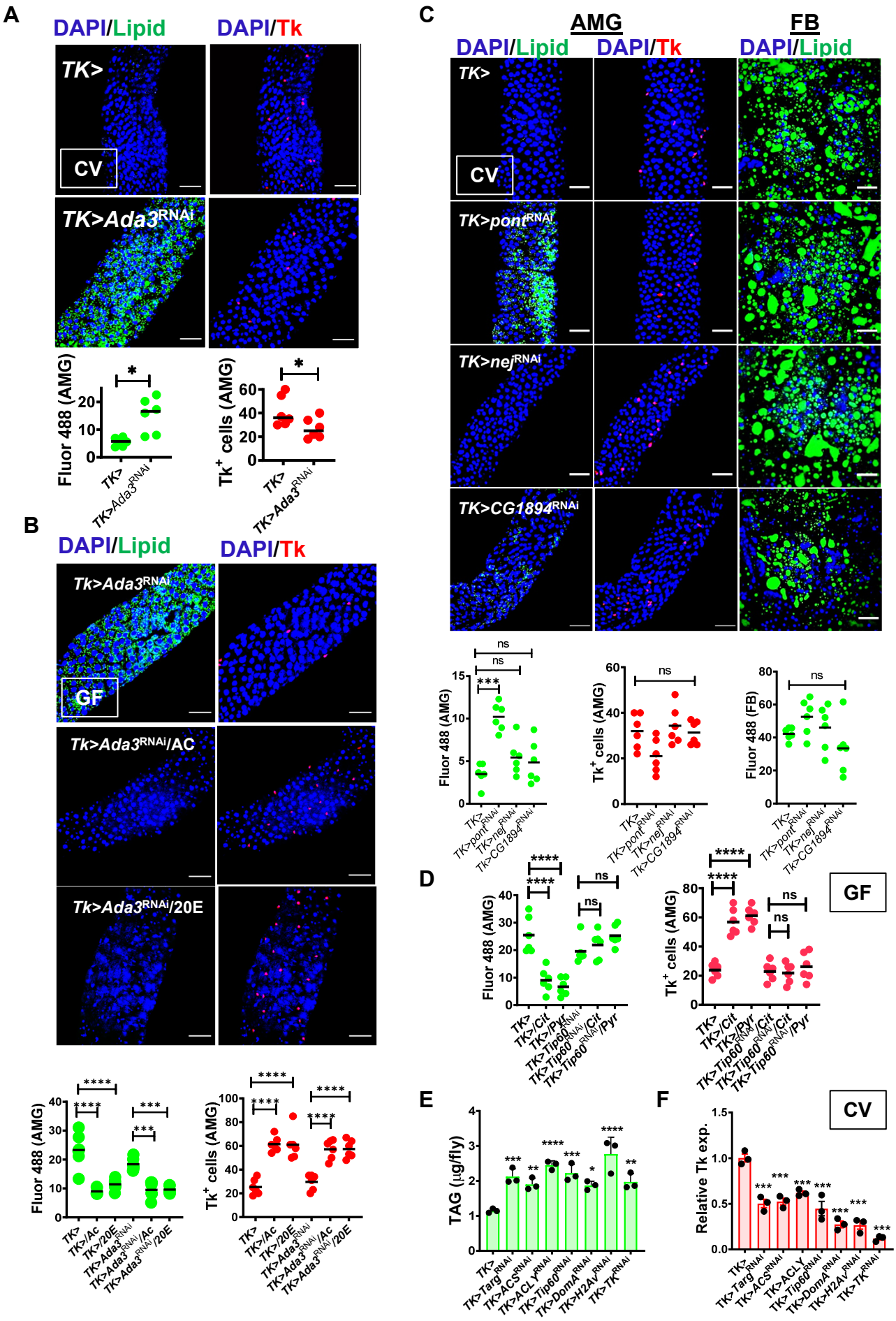
A
DAPI/Lipid
DAPI/Tk
TK>
CV
TK>Ada3RNAi
Fluor 488 (AMG)
Tk+ cells (AMG)
B
DAPI/Lipid
DAPI/Tk
Tk>Ada3RNAi
GF
Tk>Ada3RNAi/AC
Tk>Ada3RNAi/20E
Fluor 488 (AMG)
Tk+ cells (AMG)
C
AMG
FB
DAPI/Lipid
DAPI/Tk
DAPI/Lipid
TK>
CV
TK>pontRNAi
TK>nejRNAi
TK>CG1894RNAi
Fluor 488 (AMG)
Tk+ cells (AMG)
Fluor 488 (FB)
D
GF
Fluor 488 (AMG)
Tk+ cells (AMG)
E
TAG (µg/fly)
F
CV
Relative Tk exp.

Figure S7: RNAi of the genes encoding the Tip60 histone acetylase complex component pontin and the dATAC and dSAGA HAT complex component Ada3 but not those encoding the HATs Nejire and CG1894 recapitulate the microbe-depleted phenotype; *Ada3* RNAi does not block acetate rescue. Representative micrographs and quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG and FB of **(A)** CV Tk> and Tk> *Ada3*^{RNAi} , **(B**) GF Tk> and Tk>*Ada3*^{RNAi} flies with or without 50 µM acetate (AC) or 10 µM 20E supplementation, or **(C)** CV Tk> and Tk>*pont*^{RNAi}, Tk>*nej*^{RNAi}, or Tk>*CG1894*^{RNAi} flies. **(D)** Quantification of Bodipy staining (Lipid) and Tk immunofluorescence in the AMG of the indicated GF flies fed LB alone or supplemented with acetate, pyruvate, or citrate. **(E)** Quantification of the triacylglyceride content (TAG) of whole flies of the indicated genotype. Experimental triplicates consisting of five female flies each were performed. **(F)** RT-qPCR quantification of *Tk* transcription in the intestines of flies with the indicated genotypes. Experimental triplicates were performed. For (A), a student's t-test was used to calculate statistical significance. For (B-F), statistical significance was calculated using a one-way ANOVA with a Dunnett's multiple comparisons test. The data in Fig S7A and S5D and in Fig S7B and 7B were acquired jointly and share the driver-only control. This was taken into account in the statistical analysis. ns not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, See also Fig 7.

Table S1. Percent decrease in gene expression for RNAi and mutant fly lines by RT-qPCR, see also Figures 1, 2, 4, 5, 6, and 7.

RNAi strains	GAL4 strains	% decrease	P value
Targ^{RNAi} (BL 57427)	*Actin-Gal4*	44.4	0.0025
Targ^{8536} (BL 26121)	*NA*	72.8	0.0081
Targ$^{9036/+}$ (BL 51248)	*NA*	33.5	0.0992
Targ$^{8536/9036}$	*NA*	78.0	0.0057
ACS^{RNAi} (BL 41917)	*Tk-Gal4*	61.9	0.0047
ACC^{RNAi} (BL 34885)	*Tk-Gal4*	36.3	0.1041
CS^{RNAi} (BL 36740)	*Tk-Gal4*	46.4	0.0140
ACLY^{RNAi} (BL 65175)	*Tk-Gal4*	32.5	0.0084
HDAC1^{RNAi} (BL 31616)	*Tk-Gal4*	21.5	0.0942
HDAC3^{RNAi} (BL 31633)	*Tk-Gal4*	22.7	0.0347
HDAC4^{RNAi} (BL 28549)	*Tk-Gal4*	31.2	0.0122
HDAC6^{RNAi} (BL 31053)	*Tk-Gal4*	58.7	<0.0001
Tip60^{RNAi} (BL 35243)	*Tk-Gal4*	31.6	0.0912
Pont^{RNAi} (BL 50972)	*Tk-Gal4*	42.5	0.0064
Ada3^{RNAi} (BL 32451)	*Tk-Gal4*	40.6	0.0266
Gcn5^{RNAi} (BL 33981)	*Tk-Gal4*	42.4	0.0009
Nej^{RNAi} (BL 31728)	*Tk-Gal4*	ND	-
HAT (CG1894) ^{RNAi} (BL 34925)	*Tk-Gal4*	ND	-
Shade^{RNAi} (BL 67356)	*Tk-Gal4*	57.2	0.0087
EcR^{RNAi} (BL 58286)	*Tk-Gal4*	17.2	0.1734
Tachykinin^{RNAi} (JF01818)	*Tk-Gal4*	89.8	0.0008
DomA^{RNAi} (BL 65873)	*Tk-Gal4*	51.1	0.0182
DomB^{RNAi} (BL 55917)	*Tk-Gal4*	44.3	0.0231
H2Av^{RNAi} (BL 44056)	*Tk-Gal4*	48.8	0.0358

*ND, not done

Table S2. RT-qPCR primers, see also Figures 1-7.

Primer	Sequence
Targ-F	ACTTGGCTCCGGTATGG
Targ-R	TCGATTATCAGCTGGGCG
ACS-F	CCA TGA TTC TGG AGC TGC CTA
ACS-R	GCC TTC AGG TAC AGG GGT TTC
ACC-F	TAA CAA CGG AGT CAC CCA CA
ACC-R	CAG GTC ACA ACC GAT GTA CG
CS-F	TGC CAA ATG TGG GAG CCT ATG
CS-R	ATG CTG CTT GCG GAA GTT CTT
ACLY-F	TCC GGC AAG GAC ATC CTG A
ACLY-R	GGA ATT TAC TGT GGA AAA ACG GC
HDAC1-F	AAC AAG GCA TCC TCA GAG A
HDAC1-R	CGA TTC CTC GCC ATC AGC TC
HDAC3-F	CTT CCA CAG CGA CGA GTA CA
HDAC3-R	CTT CGT ATA GGC CAC GGA AT
HDAC4-F	GCA CAG GTG GAC CCA CAG AG
HDAC4-R	CAT GAG ATG TTC ACG TTA AAG C
HDAC6-F	ACT GTT GCC TGT GGG ACA AGG
HDAC6-R	TCC GTC AGA TTT AGT TCG
Tip60-F	AAC CAC AAA TAT GAG TTC GAC GA
Tip60-R	TGT GCA TCC TGA CGG GTA AAC
Pont-F	GTG GTG GAC CTC ATC AAA TCG
Pont-R	AGA AGG GCA CTT TGT TAC CCA
Ada3-F	CCT GAA AGC GGA GTA TGA TAG C
Ada3-R	AGA ATG GTC ATC GAA GAG TGC T
Gcn5-F	GGT GGA AAC AAG AGG ACC AGT G
Gcn5-R	CCA AAT TCT CAC TGC TTG GA
TK-F	TACAAGCGTGCAGCTCTCTC
TK-R	CTCCAGATCGCTCTTCTTGC
Dipt-F	AGGTGTGGACCAGCGACAA
Dipt-R	TGCTGTCCATATCCTCCATTCA
PGRP-LC-F	CACGCAGGGTATTGGCAGCATC
PGRP-LC-R	CCTCGCCCCGGTTTCCACTTG
Cecropin-F	CTCTCATTCTGGCCATCACC
Cecropin-R	CTTGTTGAGCGATTCCCAGT
Sad-F	CCGCATTCAGCAGTCAGTGG
Sad-R	ACCTGCCGTGTACAAGGAGAG
Shd-F	CGGGCTACTCGCTTAATGCAG

Shd-R	AGCAGCACCACCTCCATTTC
EcR-F	ACATGAGGCGAAAGTGTCAGGAGT
EcR-R	TTTGTCCTTCTCCTTCTGGGCCTT
Eig75B-F	GCGGTCCAGAATCAGCAG
Eig75B-R	GAGGATGTGGAGGAGGATGA
Eip74EF-F	TTTCATCAAGTGGACGAACCGGGA
Eip74EF-R	CATGTCCGGCTTGTTCTTGTGCAT
DomA-F	AGAGTCCCAAGAAGCAGAAGA
DomA-R	ATCAGGCTCGGAGCACTAAAC
DomB-F	GTCAACGGGGAAGGGAACAGA
DomB-R	CCTCGACGATCAAAGAGGCAT
H2Av-F	CGCACTACGTCACATGGAC
H2Av-R	TGGCGAGGAGTGATACGTTTC
Rp49-F	TACAGGCCCAAGATCGTGAAG
Rp49-R	GACGCACTCTGTTGTCGATACC