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Supplementary Materials for

Lysosomal cystine mobilization shapes the response of TORC1 and tissue growth to fasting

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Text

Supplementary Text 1: An amino acid supplementation screen reveals cysteine as a growth suppressor

To evaluate the effects of individual amino acids on growth, we developed an amino acid supplementation screen on developing *Drosophila* larvae and measured their development rate (Supplementary Materials). The screen identified cysteine as a strong growth suppressor (Fig. 2A), an effect that could be due to the cytotoxicity of cysteine previously reported in cell culture, yeast, and chicks (47-*49*). However, we found that the effect of cysteine supplementation was diet-dependent, with cysteine strongly suppressing growth upon starvation while having weaker effect in fed animals (Fig. S4A-D), mitigating toxicity as a unique explanation for this result. In addition, although the effect of cysteine on growth was dose-dependent (Fig. S4A), variation in cysteine intake between fed and starved conditions was not sufficient to explain the diet-dependent toxicity (Fig. S4D). Therefore, we conclude that the growth-suppressive effect of cysteine was multifactorial and decided to analyze the endogenous role of intracellular cysteine.

Supplementary Text 2: Developmental delay versus starvation sensitivity

Gain and loss of function of *dCTNS* have opposite effect on TORC1 but showed similar phenotype in term of larval development: an increase in the time to pupariation. To avoid confusion between opposite processes that lead to similar phenotypes in appearance, we adapted our nomenclature accordingly. Because the loss of *dCTNS* caused reduced cellular cysteine, upregulation of TORC1, inhibition of autophagy, and that cysteine and rapamycin treatments rescued/accelerated the time to pupariation (Fig. 3), we termed $dCTNS^{-/-}$ developmental phenotypes “starvation sensitivity”. Accordingly, $dCTNS^{-/-}$ or depletion of dCTNS in fat body did

not affect development of fed larvae. By contrast, *dCTNS* overexpression increased cellular cysteine, downregulated TORC1 and induced autophagy. In agreement with TORC1 loss of function delaying larval growth and development, we termed the developmental phenotype of *dCTNS* overexpression "developmental delay". Consistently, *dCTNS* overexpression retarded development in both fed and starved conditions.

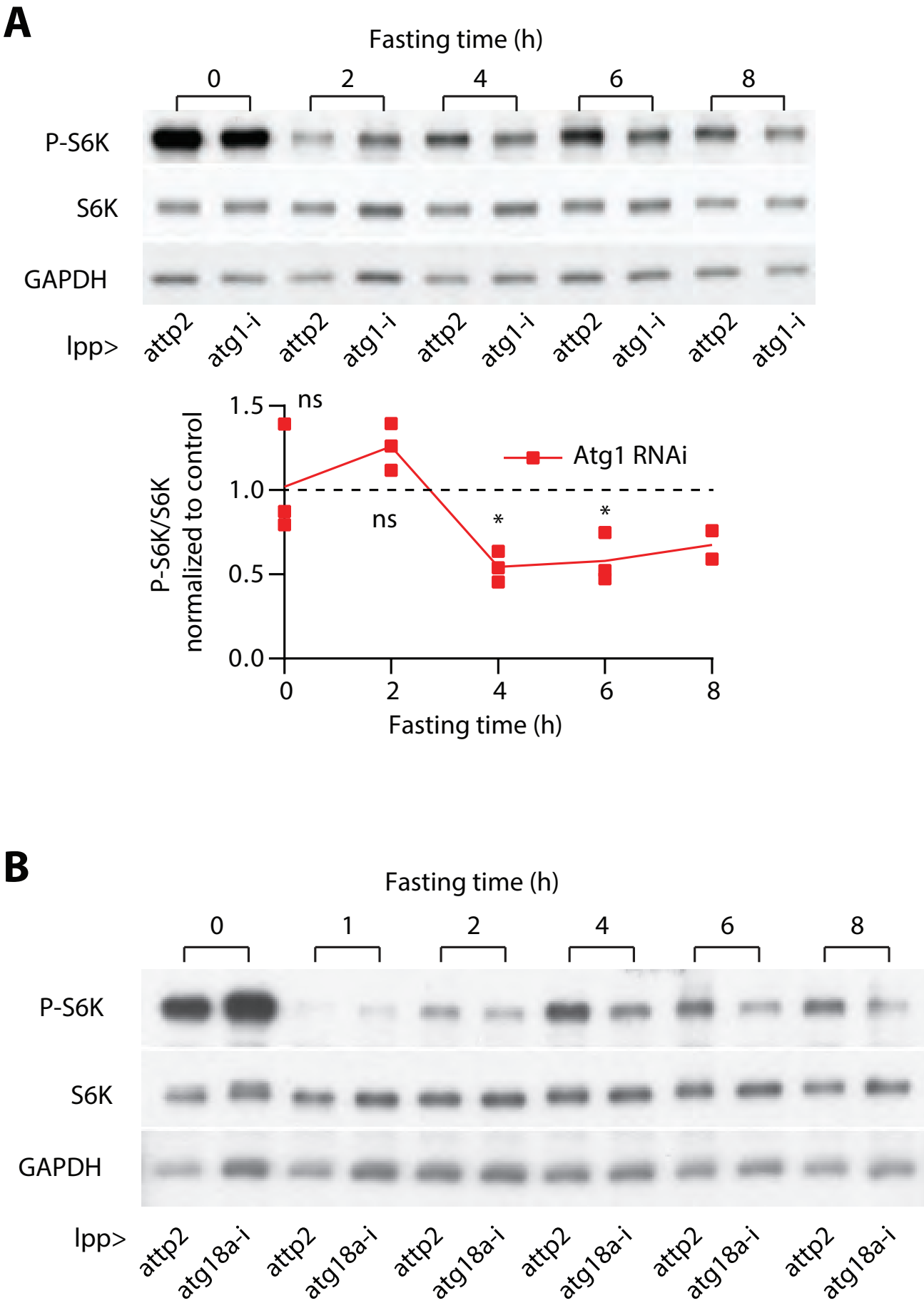
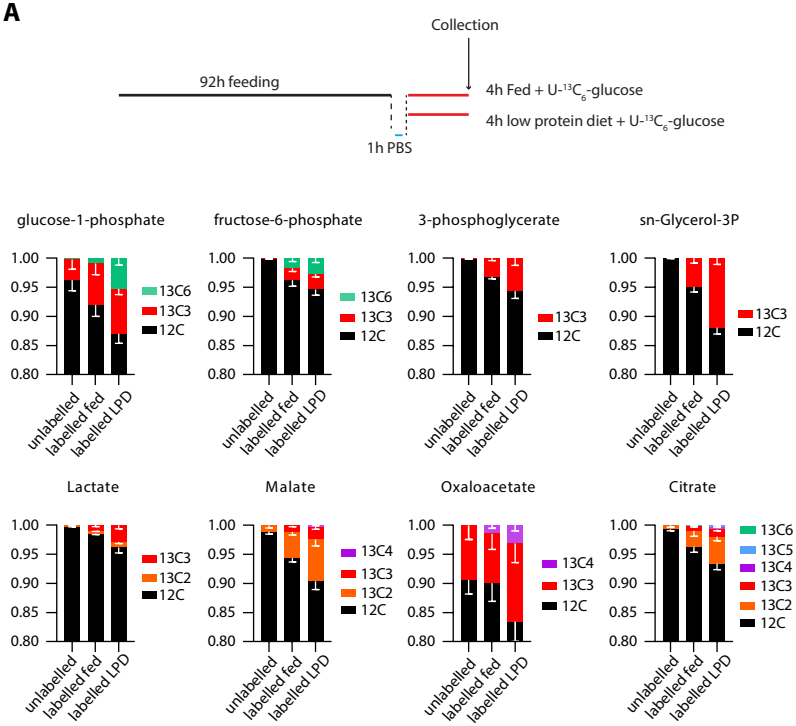

A
Fasting time (h)
0
2
4
6
8
P-S6K
S6K
GAPDH
lpp>
attp2
atg1-i
P-S6K/S6K
normalized to control
1.5
1.0
0.5
0.0
ns
ns
*
*
Atg1 RNAi
Fasting time (h)
B
Fasting time (h)
0
1
2
4
6
8
P-S6K
S6K
GAPDH
lpp>
attp2
atg18a-i

Fig. S1

Fig. S1: TORC1 reactivation upon fasting requires autophagy. A and B) P-S6K levels in dissected fat body from larvae fasted for the indicated time, control or expressing *Atg1 RNAi* (A) or *Atg18a RNAi* (B) in the fat body.


A
Collection
92h feeding
4h Fed + U-13C6-glucose
4h low protein diet + U-13C6-glucose
1h PBS
glucose-1-phosphate
fructose-6-phosphate
3-phosphoglycerate
sn-Glycerol-3P
Lactate
Malate
Oxaloacetate
Citrate
unlabelled
labelled fed
labelled LPD
13C6
13C5
13C4
13C3
13C2
12C

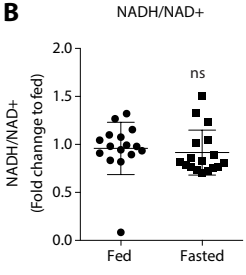

B
NADH/NAD+
NADH/NAD+
(Fold channge to fed)
ns
Fed
Fasted

Fig. S2

Fig. S2: The TCA cycle is functional during fasting. A) ^{13}C fractional enrichment for metabolite isotopologues (different number of ^{13}C atoms) and isotopomers (^{13}C at different atomic position) measured in whole larvae. Animals placed on a low protein diet for 4 hours were supplemented with 25 mM U-$^{13}C_6$-glucose. B) Relative NADH and NAD+ ratios measured by LC-MS/MS in larvae fed and fasted during 6 hours.

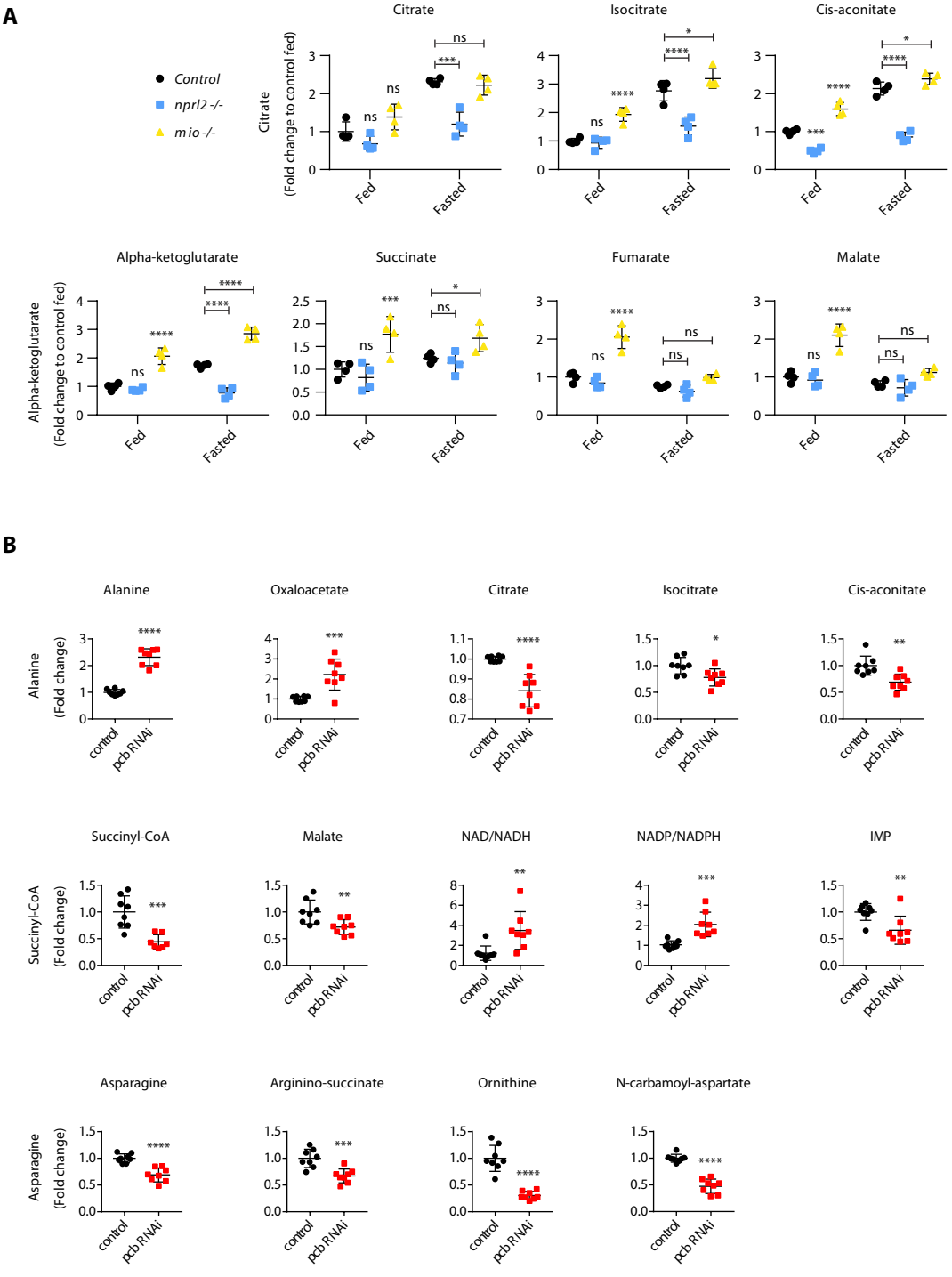

A
Control
nprl2 -/-
mio -/-
Citrate
Isocitrate
Cis-aconitate
Alpha-ketoglutarate
Succinate
Fumarate
Malate
Citrate (Fold change to control fed)
Alpha-ketoglutarate (Fold change to control fed)
Fed
Fasted
ns
B
Alanine
Oxaloacetate
Citrate
Isocitrate
Cis-aconitate
Succinyl-CoA
Malate
NAD/NADH
NADP/NADPH
IMP
Asparagine
Arginino-succinate
Ornithine
N-carbamoyl-aspartate
control
pcb RNAi
(Fold change)

Fig. S3

Fig. S3: mTOR and Pyruvate Carboxylase affect the concentration of the TCA cycle intermediates. A) Relative metabolites levels +/- SD measured by LC-MS/MS in whole larvae control (w^{1118}), Gator1 mutant (*nprl2*) and Gator2 mutant (*mio*) fed and fasted during 6 hours. B) Relative metabolites levels +/- SD measured by LC-MS/MS in fat bodies following depletion of pcb/PC in the fat body. Control is *attp40*. ^{ns}, $P \geq 0.05$; **, $P \leq 0.01$, ***, $P \leq 0.005$; ****$p \leq 0.0001$ (for further details about statistics see Methods).

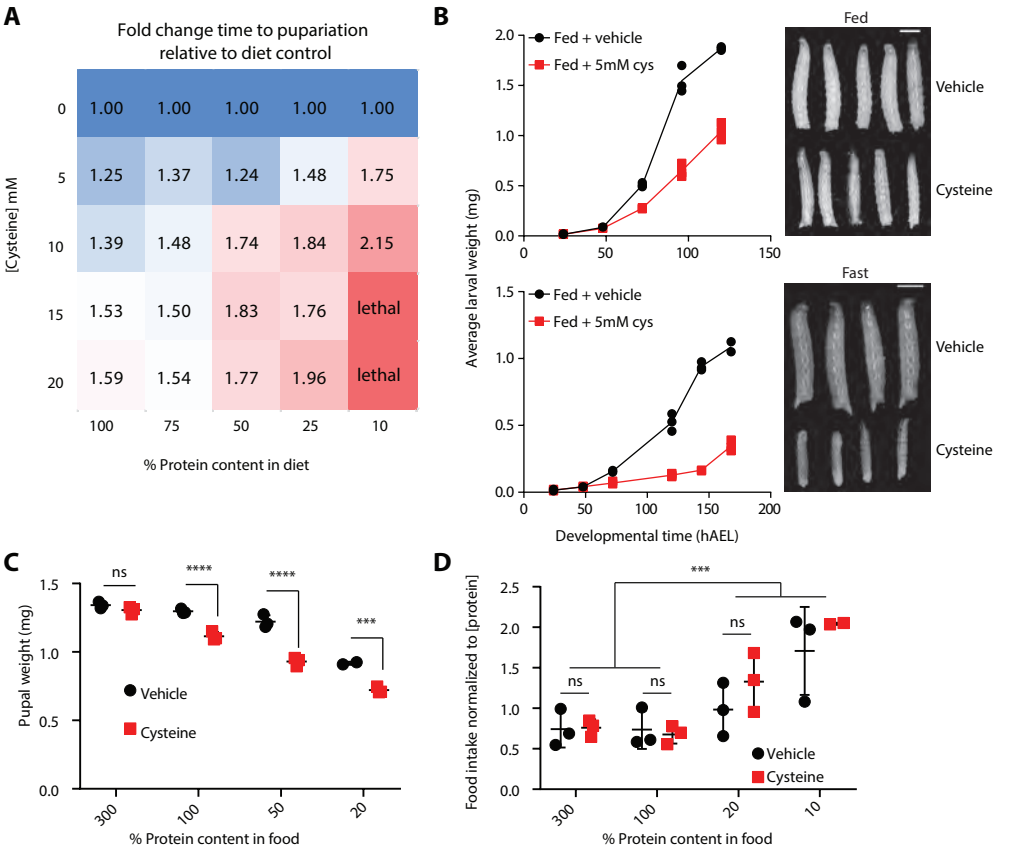

A
Fold change time to pupariation relative to diet control
[Cysteine] mM
0
5
10
15
20
1.00 1.00 1.00 1.00 1.00
1.25 1.37 1.24 1.48 1.75
1.39 1.48 1.74 1.84 2.15
1.53 1.50 1.83 1.76 lethal
1.59 1.54 1.77 1.96 lethal
100 75 50 25 10
% Protein content in diet
B
Fed + vehicle
Fed + 5mM cys
Average larval weight (mg)
Developmental time (hAEL)
Fed
Fast
Vehicle
Cysteine
C
Pupal weight (mg)
ns
****
****
***
Vehicle
Cysteine
300 100 50 20
% Protein content in food
D
Food intake normalized to [protein]
***
ns
ns
ns
Vehicle
Cysteine
300 100 20 10
% Protein content in food

Fig. S4

Fig. S4: Cysteine suppresses growth in a diet-dependent manner. A) Cysteine suppresses growth in a dose and diet dependent manner. Mean fold change time to pupariation (w^{1118} larvae) as a function of total protein content and cysteine concentration supplemented in the food. Data are normalized to control for each diet. B) Cysteine supplementation affects larval growth all along development. Growth curves (mean larval weight (mg) as a function of time (hours AEL)) for w^{1118} larvae fed *ad libitum* a 100% [AA] (top) or 10% [AA] (bottom) diet with 5 mM cysteine or vehicle. N=3. Pictures show age-matched larvae at 96 and 144 hours AEL in fed and fast conditions, respectively. Scale bar 1 mm. C) Animals fed cysteine are reduced in adult size and this process is diet dependent. Pupal weight +/- SEM (mg) of w^{1118} larvae fed *ad libitum* 300, 100, 50 and 20% total protein (supplied as yeast extract) diet with 10 mM cysteine or vehicle. 300% protein indicates three times the amount of yeast that we use in our fed control. D) Diet affects food intake whereas cysteine supplementation does not. Food intake normalized +/- SD to protein content of larvae raised on the indicated diet with 5 mM cysteine or vehicle. ^{ns}, $P \geq 0.05$; ***, $P \leq 0.005$; ****$p \leq 0.0001$ (for further details about statistics see Methods)

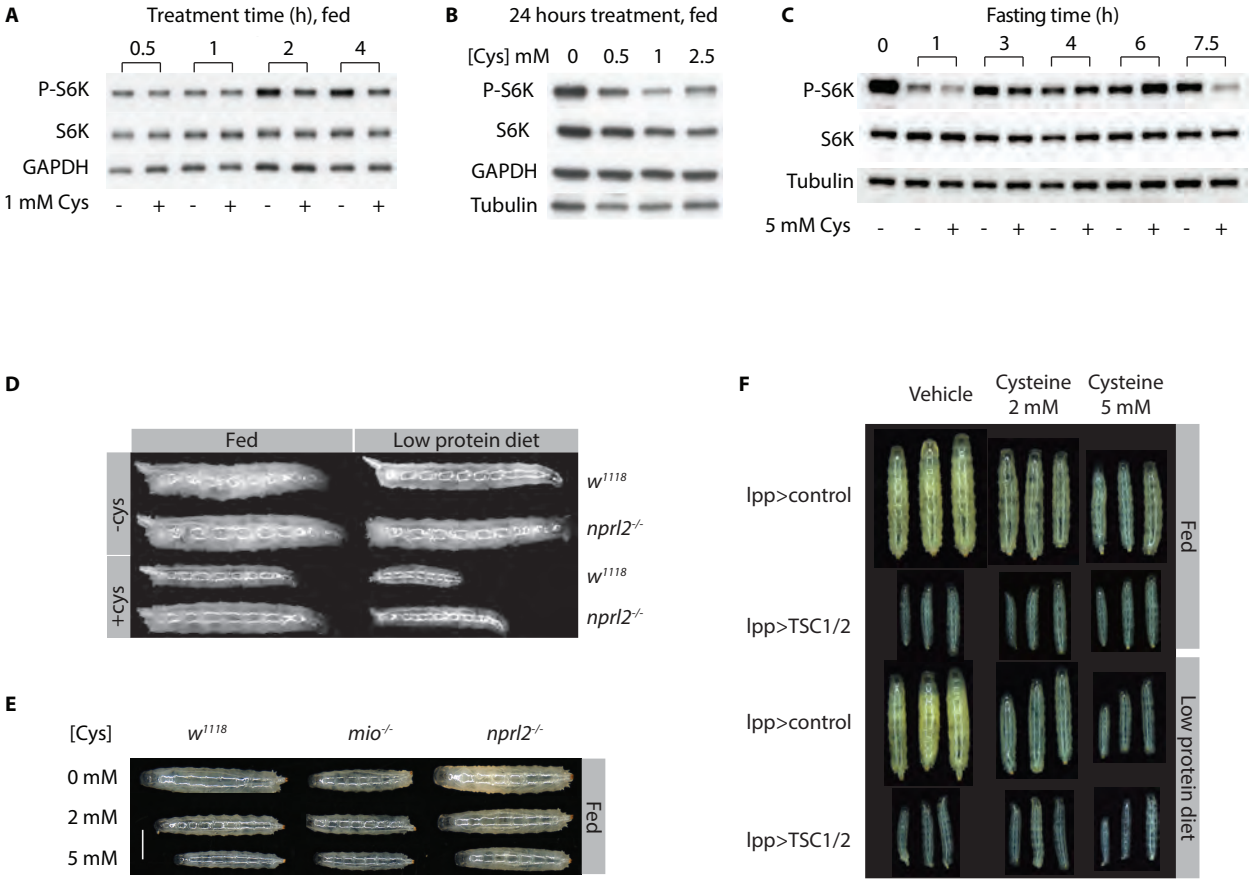

A
Treatment time (h), fed
0.5
1
2
4
P-S6K
S6K
GAPDH
1 mM Cys
- + - + - + - +
B
24 hours treatment, fed
[Cys] mM 0 0.5 1 2.5
P-S6K
S6K
GAPDH
Tubulin
C
Fasting time (h)
0 1 3 4 6 7.5
P-S6K
S6K
Tubulin
5 mM Cys
- - + - + - + - + - +
D
Fed
Low protein diet
-cys
+cys
w1118
nprl2-/-
w1118
nprl2-/-
E
[Cys]
w1118
mio-/-
nprl2-/-
0 mM
2 mM
5 mM
Fed
F
Vehicle
Cysteine 2 mM
Cysteine 5 mM
lpp>control
lpp>TSC1/2
lpp>control
lpp>TSC1/2
Fed
Low protein diet

Fig. S5

Fig. S5: Suppression of growth by dietary cysteine involves downregulation of TORC1 in the fat body. A and B) P-S6K levels in dissected fat body from larvae fed a full diet with the indicated concentrations of cysteine or vehicle for the indicated time. C) Prolonged cysteine treatment during fasting causes downregulation of S6K levels. D) Fed and fasted, control and GATOR1 mutant (*nprl2*$^{-/-}$) larvae supplemented with 5 mM cysteine or vehicle all along development. E) Fed, control, GATOR1 mutant (*nprl2*$^{-/-}$) and GATOR2 mutant (*mio*$^{-/-}$) larvae supplemented with 2 and 5 mM cysteine or vehicle all along development. F) Control larvae or larvae overexpressing TSC1 and TSC2 in the fat body raised with 2 and 5 mM cysteine or vehicle all along development in a control or low protein diet.

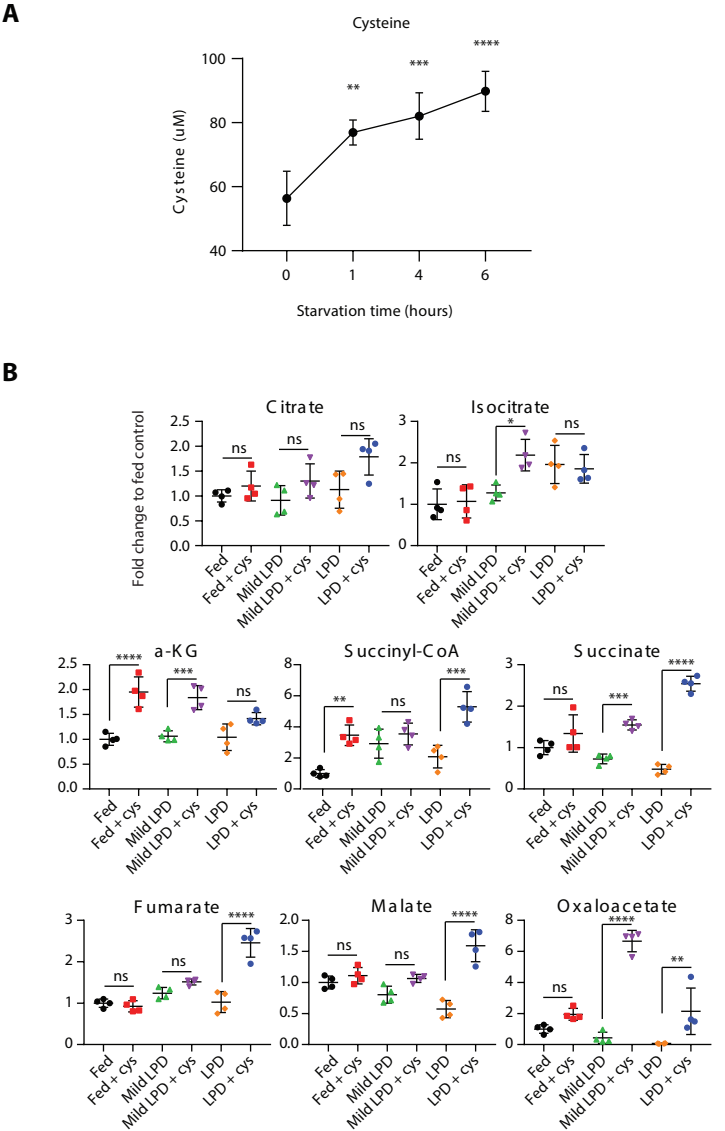

A
Cysteine
Cysteine (uM)
Starvation time (hours)
B
Citrate
Isocitrate
a-KG
Succinyl-CoA
Succinate
Fumarate
Malate
Oxaloacetate
Fold change to fed control
Fed
Fed + cys
Mild LPD
Mild LPD + cys
LPD
LPD + cys
ns

Fig. S6

Fig. S6: Cysteine increases the level of TCA cycle intermediates during fasting. A) Cysteine concentration in whole control larvae (w^{1118}). N=4. B) Cysteine increases the level of TCA cycle intermediates. Relative metabolites levels +/- SD measured by LC-MS/MS in whole mid-second instar w^{1118} larvae fed the indicated diet all along development (LPD, low protein diet), with 10 mM cysteine or vehicle. ^{ns}, P≥0.05; **, P≤0.01, ***, P≤0.005; ****p≤0.0001 (see Methods for further details).

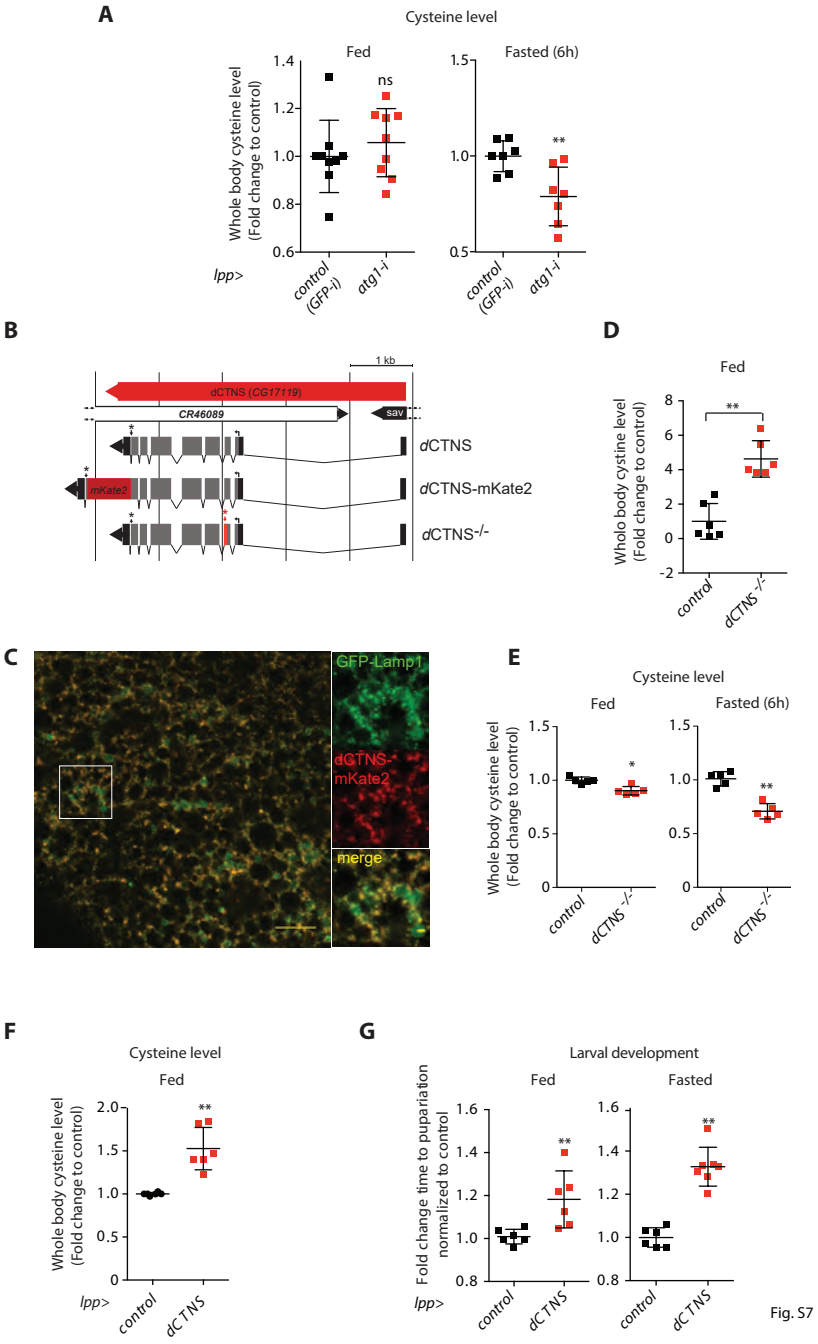

A
Cysteine level
Fed
Fasted (6h)
Whole body cysteine level
(Fold change to control)
ns
**
**
lpp>
control
(GFP-i)
atg1-i
B
1 kb
dCTNS (CG17119)
CR46089
sav
mKate2
dCTNS
dCTNS-mKate2
dCTNS-/-
C
GFP-Lamp1
dCTNS-
mKate2
merge
D
Fed
Whole body cystine level
(Fold change to control)
**
control
dCTNS-/-
E
Cysteine level
Fed
Fasted (6h)
Whole body cysteine level
(Fold change to control)
*
**
control
dCTNS-/-
F
Cysteine level
Fed
Whole body cysteine level
(Fold change to control)
**
lpp>
control
dCTNS
G
Larval development
Fed
Fasted
Fold change time to pupariation
normalized to control
**
**
lpp>
control
dCTNS
control
dCTNS

Fig. S7

Fig. S7: Lysosomal cystine efflux through *dCTNS* regulates cysteine concentration during fasting. A) Autophagy controls cysteine levels upon fasting. Relative cysteine levels measured from whole larvae. GFP-i, control background. B) Schematic of the *dCTNS* locus and alleles generated by CRISPR/Cas9. *dCTNS-mKate2*, C-terminal *mKate2* insertion at the endogenous locus; $dCTNS^{-/-}$, frameshift mutant causing a premature stop codon. C) dCTNS is targeted to the lysosomal membrane. Co-localization of dCTNS-mKate2 with lysosomal Lamp1-GFP in the fat body. Scale bar, 20 μm and 2 μm. D) $dCTNS^{-/-}$ larvae accumulate cystine. Relative cystine levels in whole third instar larvae fed a standard diet. E) dCTNS controls cysteine levels upon fasting. Relative cysteine levels in whole larvae. Controls are heterozygote animals ($dCTNS^{+/-}$). F) *dCTNS* overexpression in larval fat body (*lpp>dCTNS*) increases cysteine levels. Whole body cysteine levels from fed larvae. Control is GFP-i. G) *dCTNS* overexpression in larval fat body causes a developmental delay. Fold change time to pupariation (hAEL).

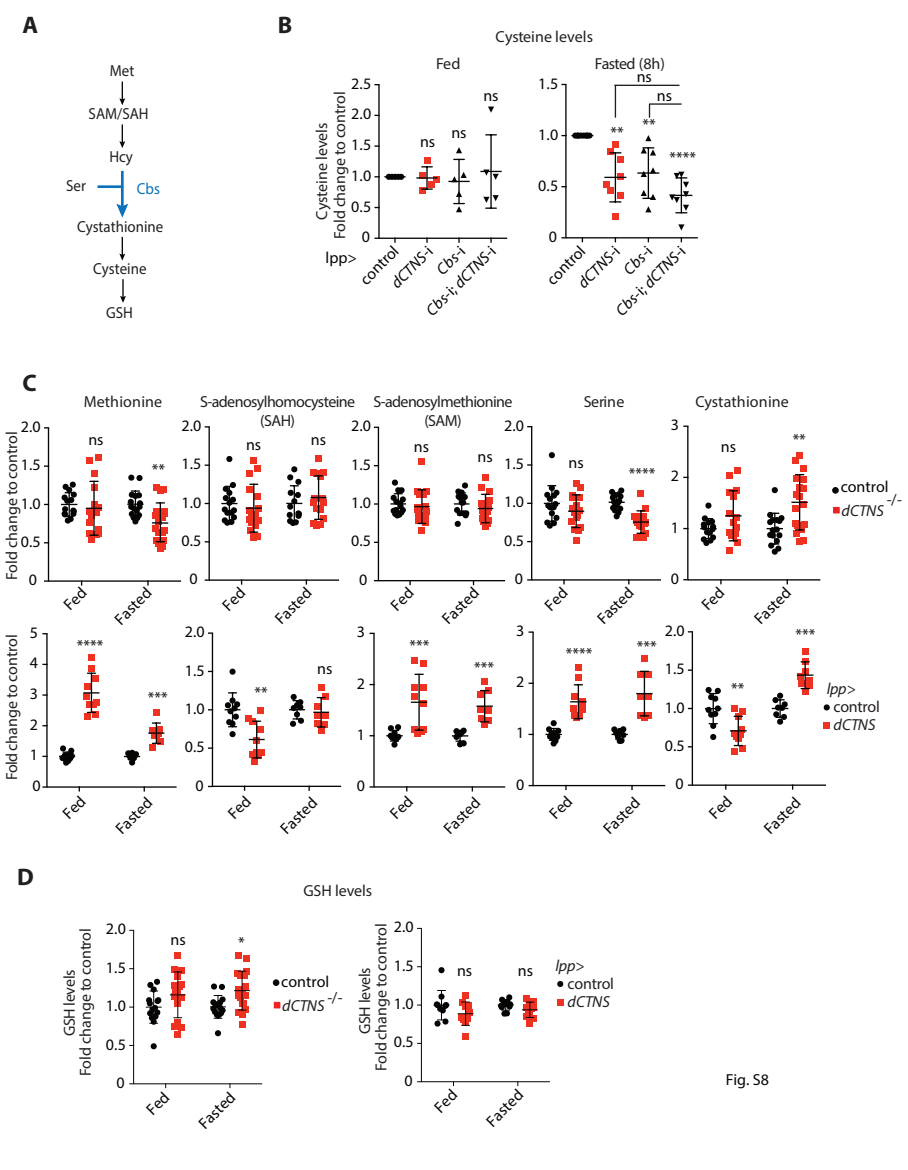

A
Met
SAM/SAH
Hcy
Ser
Cbs
Cystathionine
Cysteine
GSH
B
Cysteine levels
Fed
Fasted (8h)
Cysteine levels
Fold change to control
lpp>
control
dCTNS-i
Cbs-i
Cbs-i; dCTNS-i
ns
**
****
C
Methionine
S-adenosylhomocysteine (SAH)
S-adenosylmethionine (SAM)
Serine
Cystathionine
Fold change to control
Fed
Fasted
control
dCTNS -/-
lpp>
control
dCTNS
D
GSH levels
GSH levels
Fold change to control
control
dCTNS -/-
lpp>
control
dCTNS

Fig. S8

Fig. S8: The transsulfuration pathway and dCTNS show compensatory effects for the regulation of cysteine concentration during fasting. A) Schematic of the transsulfuration pathway. B) dCTNS and Cbs mutually compensate cysteine levels during fasting. Relative cysteine levels in whole larvae with fat body-specific (*lpp>*) knockdown of *dCTNS* (*dCTNS*-i) and *Cbs* (*Cbs*-i), and *GFP* (*GFP*-i, control). C) Relative metabolites levels +/- SD measured by LC-MS/MS in whole larvae fed or fasted for 8 hours. D) Relative glutathione (GSH) levels +/- SD measured by LC-MS/MS in whole larvae fed or fasted for 8h. ^{ns}, P≥0.05; *, P≤0.05; **, P≤0.01, ***, P≤0.005; ****p≤0.0001 (for further details see Methods).

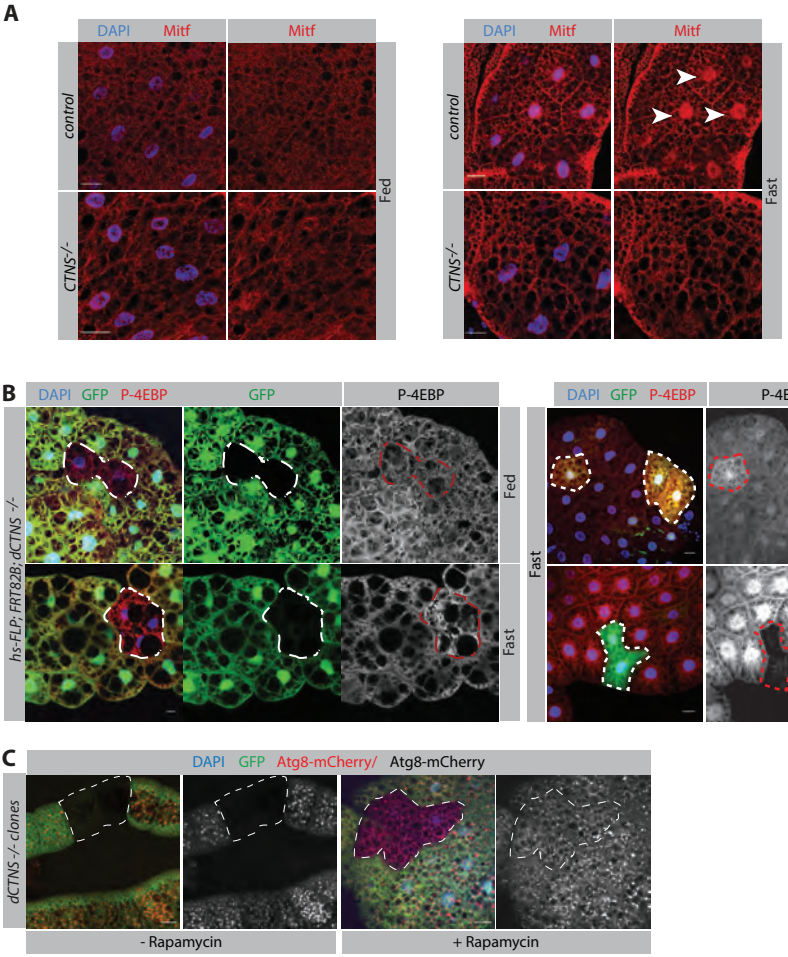

A
DAPI
Mitf
Mitf
control
CTNS-/-
Fed
DAPI
Mitf
Mitf
control
CTNS-/-
Fast
B
DAPI GFP P-4EBP
GFP
P-4EBP
hs-FLP; FRT82B; dCTNS -/-
Fed
Fast
DAPI GFP P-4EBP
P-4EBP
Fast
rheb4W4
tsc1, tsc2
C
DAPI GFP Atg8-mCherry/
Atg8-mCherry
dCTNS -/- clones
- Rapamycin
+ Rapamycin
D
Cysteine levels
Low protein diet
lpp>
control-i
dCTNS-i
Cysteine levels
Fold change to "untreated"
2.0
1.5
1.0
0.5
0
ns
ns
ns
ns
Rapamycin (uM) 0 0.01 0.1 0 0.01 0.1

Fig. S9

Fig. S9: dCTNS regulates TORC1 reactivation and autophagy during fasting. A) Fasted *dCTNS*$^{-/-}$ fat body cells suppress Mitf translocation to the nucleus. Fat body of 80 hAEL old larvae control and *dCTNS*$^{-/-}$ fasted overnight, and stained for the TORC1 substrate Mitf/TFEB. B) dCTNS limits TORC1 reactivation upon fasting. *dCTNS*$^{-/-}$ fat body clones (non-GFP, outlined) and Rheb and TSC1, TCS2 overexpression clones (GFP, outlined) in 80h AEL animals starved for 24h and stained for P-4EBP. DAPI, blue; GFP, green; P-4EBP, red or white. Scale bar 10 μm. C) The TORC1 inhibitor rapamycin restores autophagy in fasted dCTNS$^{-/-}$ cells. Fat body *dCTNS*$^{-/-}$ clones (non-GFP, outlined) in 80h AEL larvae with fat body-specific expression of mCherry-Atg8a starved for 8h. DAPI, blue; GFP, green; mCherry-Atg8a, red or white. Scale bar 10 μm. D) Rapamycin treatment does not affect cysteine concentration. Relative levels of cysteine in whole larvae with fat body-specific (*lpp*>) knockdown of *dCTNS* (*dCTNS*-i) and *GFP* (*GFP*-i, control). 72 hAEL old larvae were fed a low protein diet overnight supplemented with two concentrations of rapamycin (0.01 and 0.1 μM) or vehicle. ^{ns}, $P \geq 0.05$ (see Methods for further details).

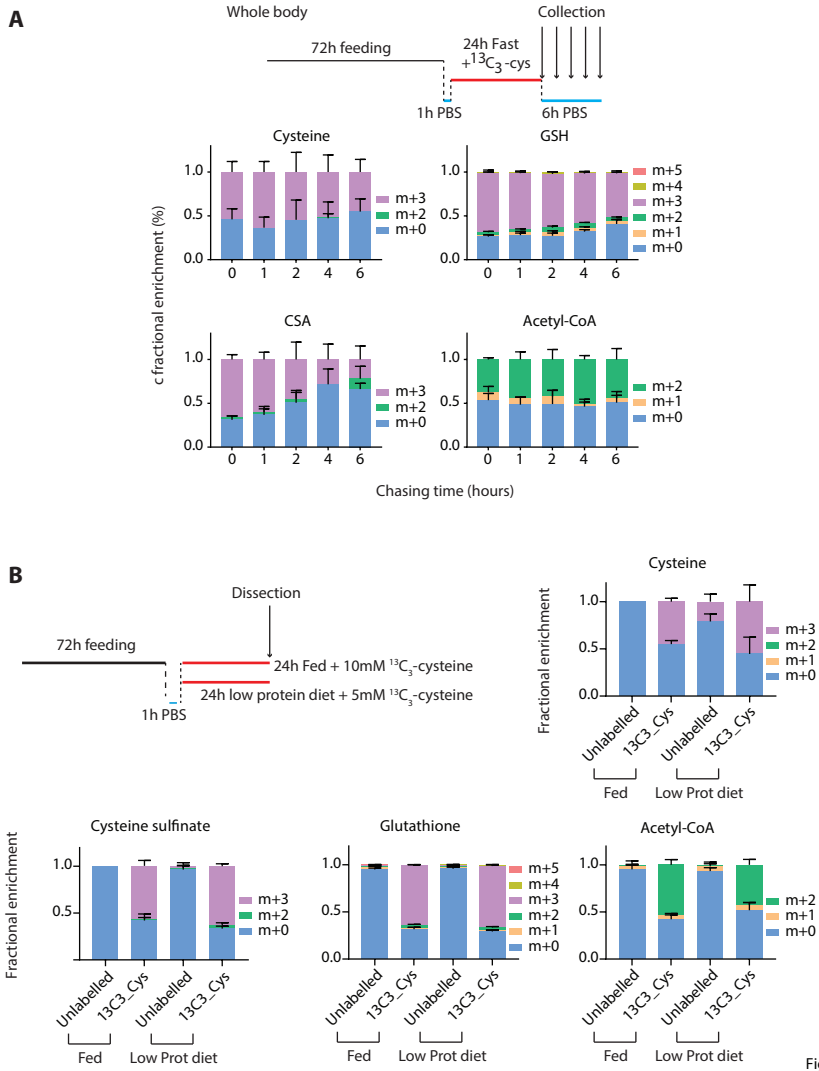
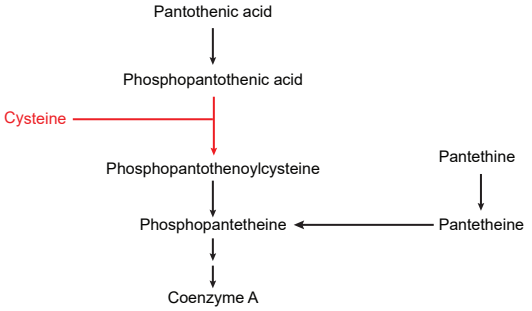

A
Whole body
72h feeding
24h Fast
+13C3-cys
Collection
1h PBS
6h PBS
Cysteine
GSH
CSA
Acetyl-CoA
c fractional enrichment (%)
m+5
m+4
m+3
m+2
m+1
m+0
Chasing time (hours)
B
Dissection
72h feeding
24h Fed + 10mM 13C3-cysteine
24h low protein diet + 5mM 13C3-cysteine
1h PBS
Cysteine
Fractional enrichment
Unlabelled
13C3_Cys
Fed
Low Prot diet
Cysteine sulfinate
Glutathione
Acetyl-CoA

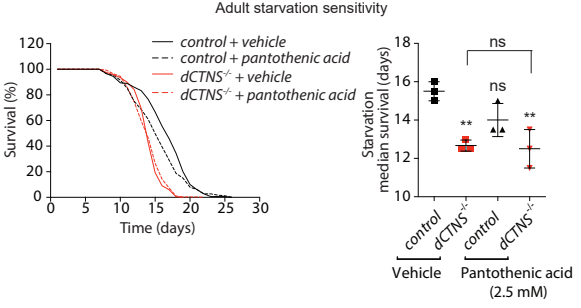
Fig. S10

Fig. S10: Cysteine metabolism fuels acetyl-CoA synthesis. Mean +/- SD ^{13}C-fractional enrichment for metabolite isotopologues and isotopomers measured in whole larvae (A) or dissected fat bodies (B). Fed and fasted animals were supplemented for the indicated time with 10 mM and 5 mM $^{13}C_3$-cysteine, respectively.

A


Pantothenic acid
Phosphopantothenic acid
Cysteine
Phosphopantothenoylcysteine
Pantethine
Phosphopantetheine
Pantetheine
Coenzyme A

B


Adult starvation sensitivity
control + vehicle
control + pantothenic acid
dCTNS-/- + vehicle
dCTNS-/- + pantothenic acid
Survival (%)
Time (days)
Starvation median survival (days)
ns
ns
**
**
control
dCTNS-/-
control
dCTNS-/-
Vehicle
Pantothenic acid (2.5 mM)

C

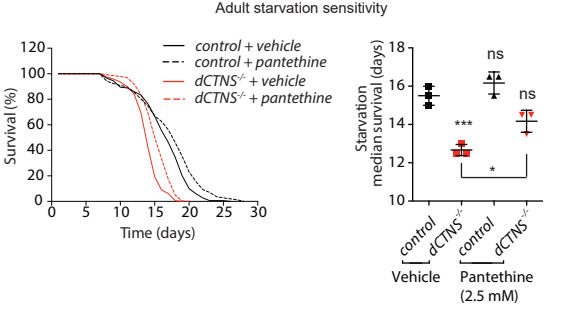

Adult starvation sensitivity
control + vehicle
control + pantethine
dCTNS-/- + vehicle
dCTNS-/- + pantethine
Survival (%)
Time (days)
Starvation median survival (days)
ns
ns
***
*
control
dCTNS-/-
control
dCTNS-/-
Vehicle
Pantethine (2.5 mM)

Fig. S11

Fig. S11: Pantethine suppresses starvation sensitivity of *dCTNS*$^{-/-}$ animals. A) Schematic of CoA biosynthesis. B-C) Pantethine (C) but not pantothenic acid (vitamin B5) (B) treatment partially restores starvation resistance of *dCTNS*$^{-/-}$ animals. Survival of control (w^{1118}) and *dCTNS*$^{-/-}$ animals fed a chemically defined starved diet. Mean +/- SEM; ^{ns}, P≥0.05; *, P≤0.05; **, P≤0.01; ***, P≤0.005 (see statistic details in Methods).

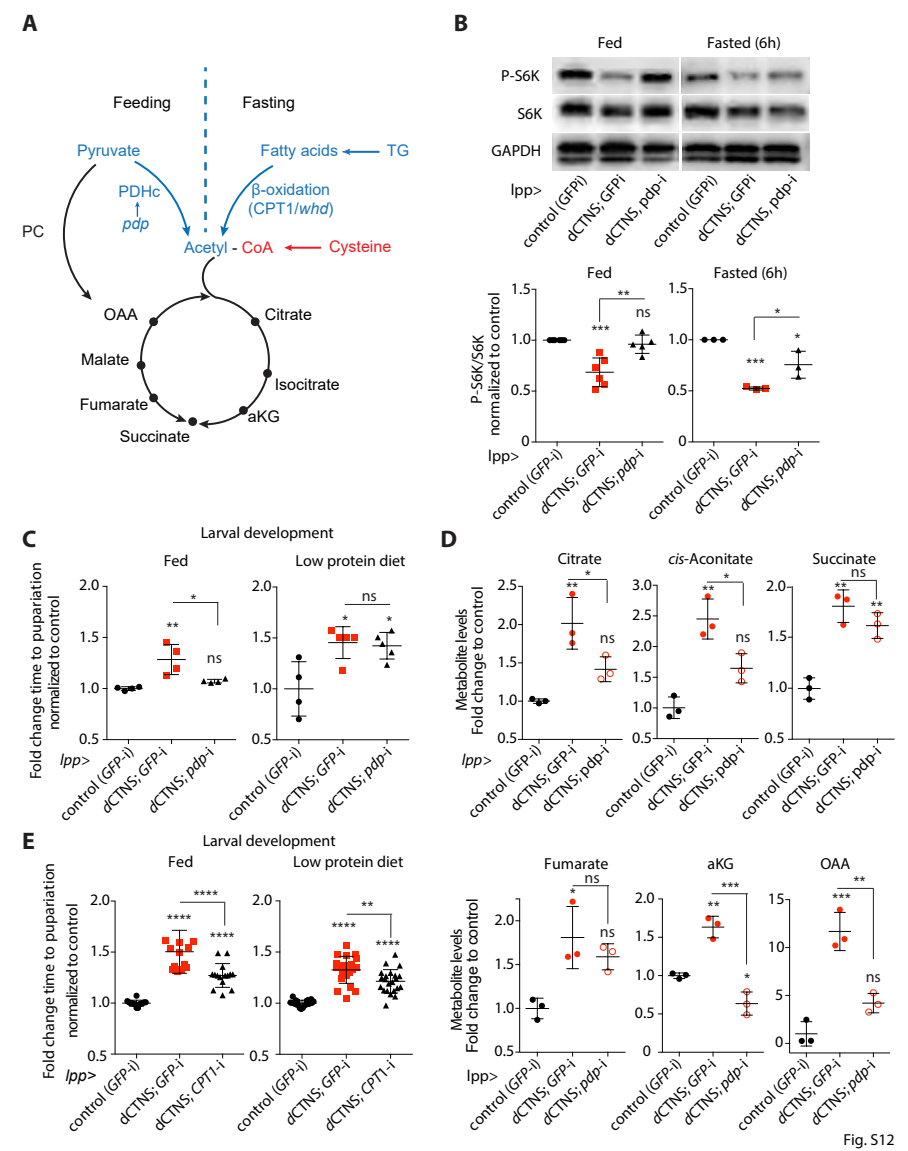

A
Feeding
Fasting
Pyruvate
Fatty acids
TG
PDHc
pdp
β-oxidation
(CPT1/whd)
PC
Acetyl - CoA
Cysteine
OAA
Citrate
Malate
Isocitrate
Fumarate
aKG
Succinate
B
Fed
Fasted (6h)
P-S6K
S6K
GAPDH
lpp>
control (GFPi)
dCTNS; GFPi
dCTNS; pdp-i
P-S6K/S6K normalized to control
control (GFP-i)
dCTNS; GFP-i
dCTNS; pdp-i
C
Larval development
Fed
Low protein diet
Fold change time to pupariation normalized to control
dCTNS; pdp-i
D
Citrate
cis-Aconitate
Succinate
Metabolite levels Fold change to control
E
dCTNS; CPT1-i
Fumarate
aKG
OAA

Fig. S12

Fig. S12: Cysteine suppresses growth through acetyl-CoA metabolism. A) Schematic of acetyl-CoA synthesis in fed and fast conditions. B) P-S6K levels in fat body from fed and fasted (6h on PBS) larvae of indicated genotypes. C, E, F) Fold change time to pupariation (hours AEL) normalized to control for larvae of indicated genotypes fed a control or low protein diet. D) Relative metabolites levels +/- SD measured by LC-MS/MS in whole fed larvae of indicated genotypes. ^{ns}, P≥0.05; *, P≤0.05; **, P≤0.01, ***, P≤0.005; ****p≤0.0001 (for further details see Methods).

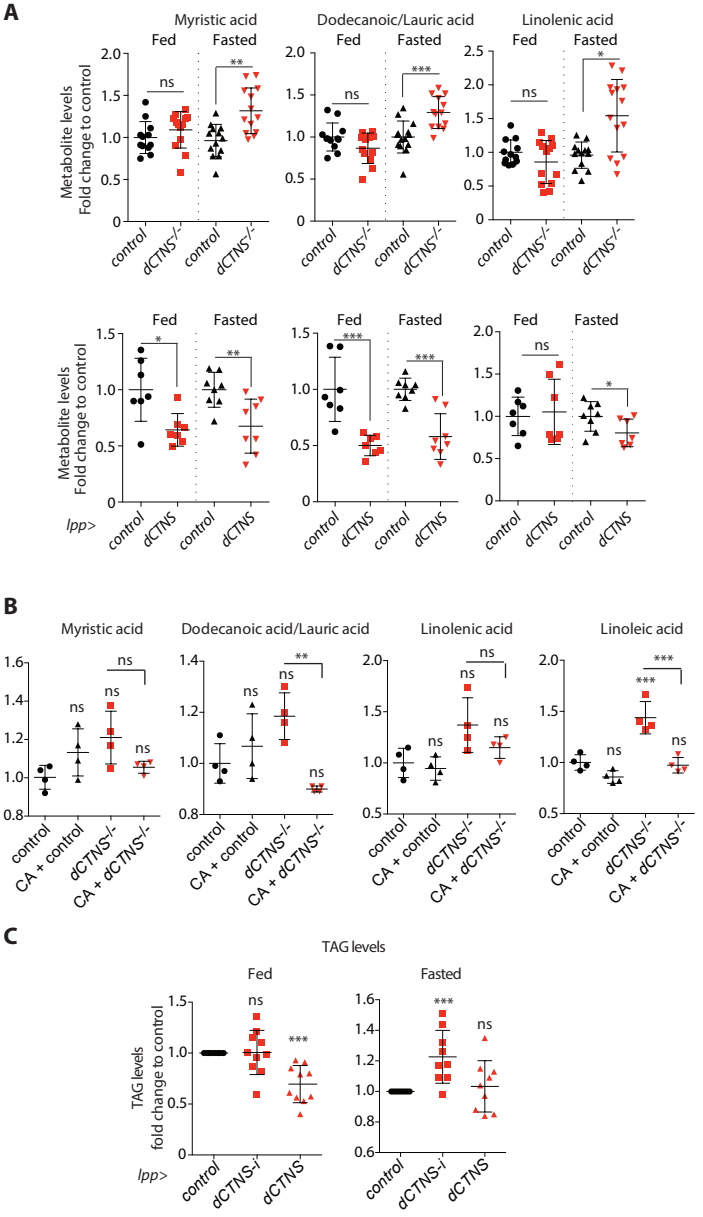

A
Myristic acid
Dodecanoic/Lauric acid
Linolenic acid
Fed
Fasted
Metabolite levels
Fold change to control
ns
**
***
*
control
dCTNS-/-
lpp>
dCTNS
B
Myristic acid
Dodecanoic acid/Lauric acid
Linolenic acid
Linoleic acid
Metabolite levels
Fold change to control
CA + control
CA + dCTNS-/-
C
TAG levels
Fed
Fasted
TAG levels
fold change to control
dCTNS-/-

Fig. S13

Fig. S13: dCTNS affects the levels of fatty acids. A) Relative fatty acid levels +/- SD measured by LC-MS/MS in control (*w*^{1118} or *lpp>GFP RNAi*), *dCTNS*$^{-/-}$ and *lpp>UAS-dCTNS* whole 3rd instar animals fed or fasted for 8 h. B) Relative fatty acid levels +/- SD measured by LC-MS/MS in whole 3rd instar control and *dCTNS*$^{-/-}$ animals after 8 h on low protein diet supplemented with cysteamine (CA, 1 mM) or vehicle. C) Whole body triacylglycerol (TAG) levels in 3rd instar larvae fed or fasted for 8 h following *dCTNS* knockdown (*dCTNS*-i) or overexpression (*dCTNS*) in the fat body (lpp>). Control is *GFP* RNAi. ^{ns}, P≥0.05; *, P≤0.05; **, P≤0.01, ***, P≤0.005; ****p≤0.0001 (See details in Methods).

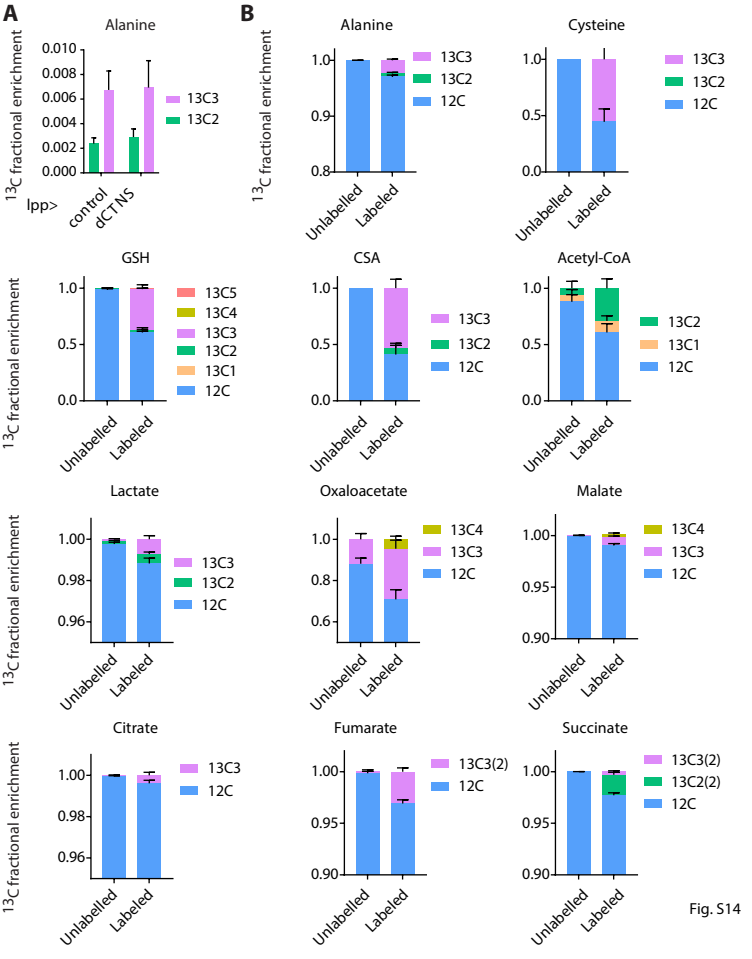

A
Alanine
13C fractional enrichment
0.010
0.008
0.006
0.004
0.002
0.000
13C3
13C2
lpp>
control
dCTNS
B
Alanine
Cysteine
GSH
CSA
Acetyl-CoA
Lactate
Oxaloacetate
Malate
Citrate
Fumarate
Succinate
Unlabelled
Labeled
13C5
13C4
13C1
12C
13C3(2)
13C2(2)

Fig. S14

Fig. S14: Alanine does not impair cysteine metabolism to acetyl-CoA. A) Mean +/- SD ^{13}C-fractional enrichment in alanine for larvae fed 25 mM [U-^{13}C]alanine for 6 hours on a low protein diet. B) Mean +/- SD ^{13}C-fractional enrichment for metabolite isotopologues and isotopomers measured in whole larvae supplemented for 6 hours with 5 mM [U-^{13}C]cysteine and 25 mM [U-^{13}C]alanine simultaneously.

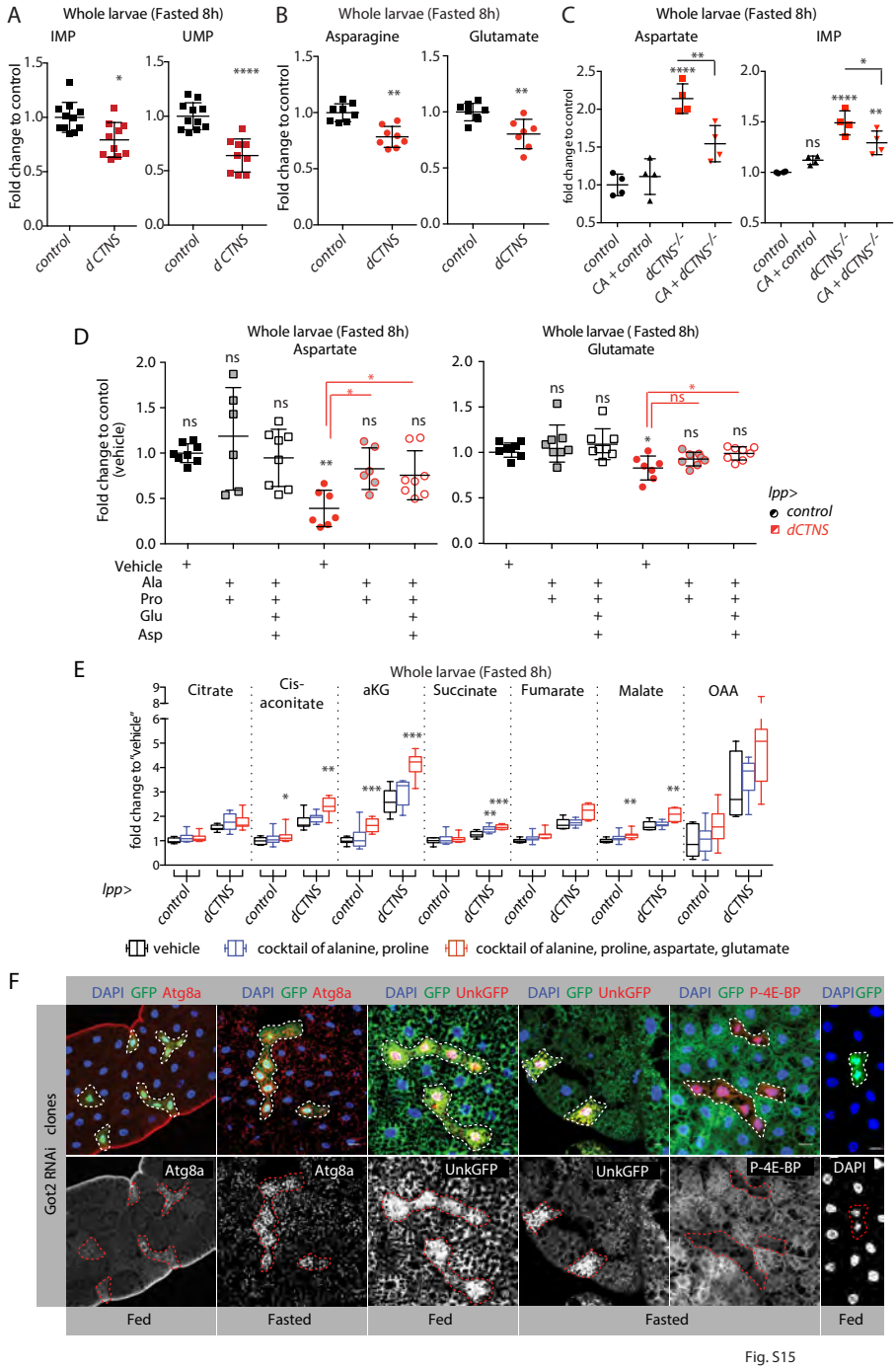

A
Whole larvae (Fasted 8h)
IMP
UMP
Fold change to control
control
d CTNS
B
Whole larvae (Fasted 8h)
Asparagine
Glutamate
dCTNS
C
Whole larvae (Fasted 8h)
Aspartate
IMP
fold change to control
ns
control
CA + control
dCTNS-/-
CA + dCTNS-/-
D
Whole larvae (Fasted 8h)
Aspartate
Whole larvae (Fasted 8h)
Glutamate
Fold change to contol (vehicle)
lpp>
control
dCTNS
Vehicle
Ala
Pro
Glu
Asp
E
Whole larvae (Fasted 8h)
Citrate
Cis-aconitate
aKG
Succinate
Fumarate
Malate
OAA
fold change to "vehicle"
lpp>
control
dCTNS
vehicle
cocktail of alanine, proline
cocktail of alanine, proline, aspartate, glutamate
F
DAPI GFP Atg8a
DAPI GFP UnkGFP
DAPI GFP P-4E-BP
DAPIGFP
clones
Got2 RNAi
Atg8a
UnkGFP
P-4E-BP
DAPI
Fed
Fasted
Fed
Fasted
Fed

Fig. S15

Fig. S15: Cysteine metabolism regulates growth through aspartate levels during fasting. A, B) Whole body metabolite levels (inosine monophosphate, IMP; uridine monophosphate, UMP) in 3rd instar animals following *dCTNS* overexpression in the fat body. C) Relative aspartate and IMP levels +/- SD measured by LC-MS/MS in whole 3rd instar control (w^{1118}) and $dCTNS^{-/-}$ animals after 8 h on low protein diet supplemented with cysteamine (CA, 1 mM) or vehicle. D) Relative levels of aspartate and glutamate in 85 hAEL larvae fed a minimal diet all along development, with or without supplementation with the indicates amino acid cocktails for 8h before collection (Ala, Pro: 20 mM, Asp, Glu: 10 mM). Controls are GFP RNAi (GFP-i). D) Relative levels of TCA cycle intermediates in 85 hAEL larvae fed a minimal diet with or without supplementation with amino acid cocktails for 8 h (amino acid concentrations: Ala, Pro: 20 mM, Asp, Glu: 10 mM). Controls are GFP-i. E) Fat body flip-out clones of *Got2-RNAi* (GFP or RFP, outlined) in larvae fed or fasted for 6 hours stained with GFP or P-4E-BP (independent experiments and confocal settings). Atg8a, mCherryATG8a. Unk-GFP is a reporter induced by TORC1 inhibition (*50*). Scale bar 20 μm. A-D) Mean +/- SD; ns, P≥0.05; *, P≤0.05; **, P≤0.01; ***, P≤0.005; ****, P≤0.0001 (Details in Methods).

	50x MEM [AA]mM	AA Screen [AA]mM
Glycine	5	10
L-Arginine hydrochloride	30	30
L-Aspartic acid	5	5
L-Cysteine hydrochloride		5
L-Cystine dihydrochloride	5	
L-Glutamic acid	5	5
L-Glutamine	5	5
L-Histidine hydrochloride monohydrate	10	10
L-Isoleucine	20	20
L-Leucine	20	20
L-Lysine hydrochloride	20	20
L-Methionine	5	5
L-Phenylalanine	10	10
L-Proline	5	5
L-Serine	5	5
L-Threonine	20	5
L-Tryptophan	2.5	2.5
L-Tyrosine disodium salt	10	10
L-Valine	20	20
Alanine	5	5
Asparagine	5	5

Table S1: Amino acid concentrations used in the screen. Comparative table of concentration of each amino acid (mM) used in Minimum Essential Media (MEM) amino acid supplementation for cell culture, and in our amino acid add-back screen (Fig.2A).

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Materials Design Analysis Reporting (MDAR) Checklist for Authors

The MDAR framework establishes a minimum set of requirements in transparent reporting applicable to studies in the life sciences (see Statement of Task: doi:10.31222/osf.io/9sm4x.). The MDAR checklist is a tool for authors, editors and others seeking to adopt the MDAR framework for transparent reporting in manuscripts and other outputs. Please refer to the MDAR Elaboration Document for additional context for the MDAR framework.

Materials

Antibodies	Yes (indicate where provided: page no/section/legend)	n/a
For commercial reagents, provide supplier name, catalogue number and RRID, if available.	Materials and Methods, Immunostaining page 9, western blots page 10	

Cell materials	Yes (indicate where provided: page no/section/legend)	n/a
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID		n/a
Primary cultures: Provide species, strain, sex of origin, genetic modification status.		n/a

Experimental animals	Yes (indicate where provided: page no/section/legend)	n/a
Laboratory animals: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID		n/a
Animal observed in or captured from the field: Provide species, sex and age where possible		n/a
Model organisms: Provide Accession number in repository (where relevant) **OR** RRID	Materials and Methods, Fly stocks and maintenance, page 2	

Plants and microbes	Yes (indicate where provided: page no/section/legend)	n/a
Plants: provide species and strain, unique accession number if available, and source (including location for collected wild specimens)		n/a
Microbes: provide species and strain, unique accession number if available, and source		n/a

Human research participants	Yes (indicate where provided: page no/section/legend)	n/a
Identify authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		n/a
Provide statement confirming informed consent obtained from study participants.		n/a
Report on age and sex for all study participants.		n/a

Design

Study protocol	Yes (indicate where provided: page no/section/legend)	n/a
For clinical trials, provide the trial registration number **OR** cite DOI in manuscript.		n/a

Laboratory protocol	Yes (indicate where provided: page no/section/legend)	n/a
Provide DOI or other citation details if detailed step-by-step protocols are available.		n/a

Experimental study design (statistics details)	Yes (indicate where provided: page no/section/legend)	n/a
State whether and how the following have been done**, or** if they were not carried out.		
Sample size determination	Determined based on previously standardized protocols	
Randomisation	Animals were randomly allocated to experimental replicate groups.	
Blinding	Only mass spectrometry experiments were blinded	
Inclusion/exclusion criteria	No data were excluded	

Sample definition and in-laboratory replication	Yes (indicate where provided: page no/section/legend)	n/a
State number of times the experiment was replicated in laboratory	Indicated in legends, or Material and Methods, section Statistics.	
Define whether data describe technical or biological replicates	All data describe biological replicates.	

Ethics	Yes (indicate where provided: page no/section/legend)	n/a
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		n/a
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		n/a
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.		n/a

Dual Use Research of Concern (DURC)	Yes (indicate where provided: page no/section/legend)	n/a
If study is subject to dual use research of concern, state the authority granting approval and reference number for the regulatory approval		n/a

Analysis

Attrition	Yes (indicate where provided: page no/section/legend)	n/a
State if sample or data point from the analysis is excluded, and whether the criteria for exclusion were determined and specified in advance.	No data were excluded	

Statistics	Yes (indicate where provided: page no/section/legend)	n/a
Describe statistical tests used and justify choice of tests.		n/a

Data Availability	Yes (indicate where provided: page no/section/legend)	n/a
State whether newly created datasets are available, including protocols for access or restriction on access.		n/a
If data are publicly available, provide accession number in repository or DOI or URL.		n/a
If publicly available data are reused, provide accession number in repository or DOI or URL, where possible.		n/a

Code Availability	Yes (indicate where provided: page no/section/legend)	n/a
For all newly generated code and software essential for replicating the main findings of the study:		n/a
State whether the code or software is available.		n/a
If code is publicly available, provide accession number in repository, or DOI or URL.		n/a

Reporting

Adherence to community standards	Yes (indicate where provided: page no/section/legend)	n/a
MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.		n/a
State if relevant guidelines (eg., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (eg., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.		n/a