Supporting Information

Fig. S1

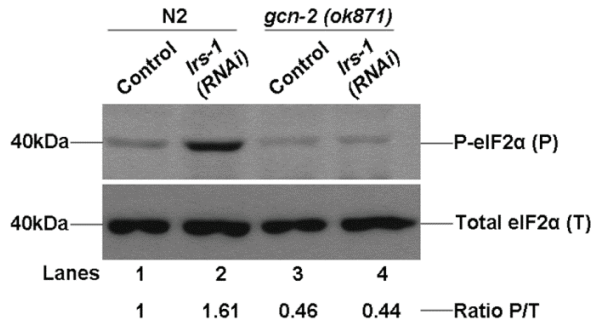

Fig. S1: Inactivation of leucyl-tRNA synthetase *lrs-1* induces phospho-eIF2α levels in a *gcn-2*-dependent manner. Western blot analysis showing the levels of phosphorylated eIF2α (P-eIF2α), normalized by the total amount of eIF2α, in whole extracts of 1-day N2 and *gcn-2(ok871)* worms, fed RNAi bacteria harboring the empty vector (Control) or expressing dsRNA for *lrs-1*.

Fig. S2

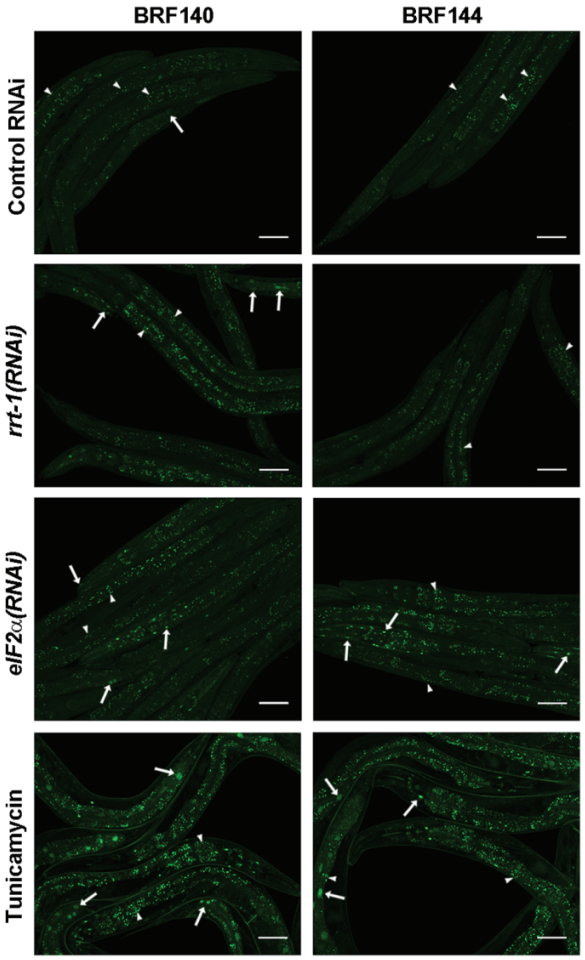

Fig. S2: Induction of *atf-5::gfp* transgene by *eIF2α(RNAi)* or tunicamycin does not require GCN-2 activity. Confocal images of adult transgenic worms BRF140 (N2Ex[ATF-5::GFP; pRF4]) and BRF144 (*gcn-2(ok871)*Ex[ATF-5::GFP; pRF4]) that were fed Control, *rrt-1(RNAi)* or *eIF2α(RNAi)* bacteria, or were treated with tunicamycin (5μg/ml for 24h). White arrows indicate fluorescent nuclei and white arrowheads show regions of autofluorescence. All images were taken at 20x magnification (scale bar: 50μm).

Fig. S3

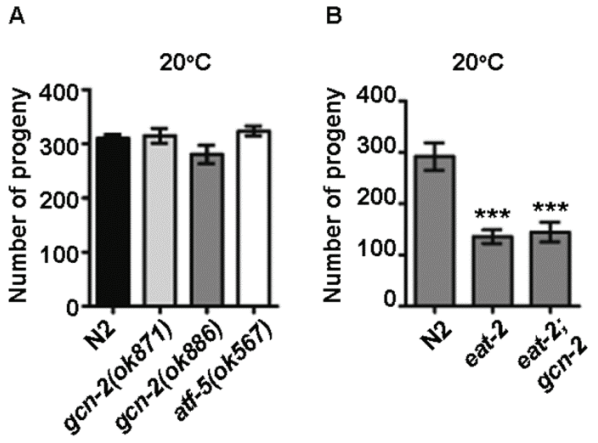

Fig. S3: *gcn-2* deletion does not alter fertility in wild-type or *eat-2* mutants. Brood size (mean ± SD) of 5-10 individuals of the indicated strains at 20°C: **(A)** N2, *gcn-2(ok871)*, *gcn-2(ok886)* and *atf-5(ok567)*. **(B)** N2, *eat-2* and *eat-2;gcn-2* mutant animals.The asterisks represent statistical significant difference from N2 (***p<0.001 in unpaired t-test).

Fig. S4

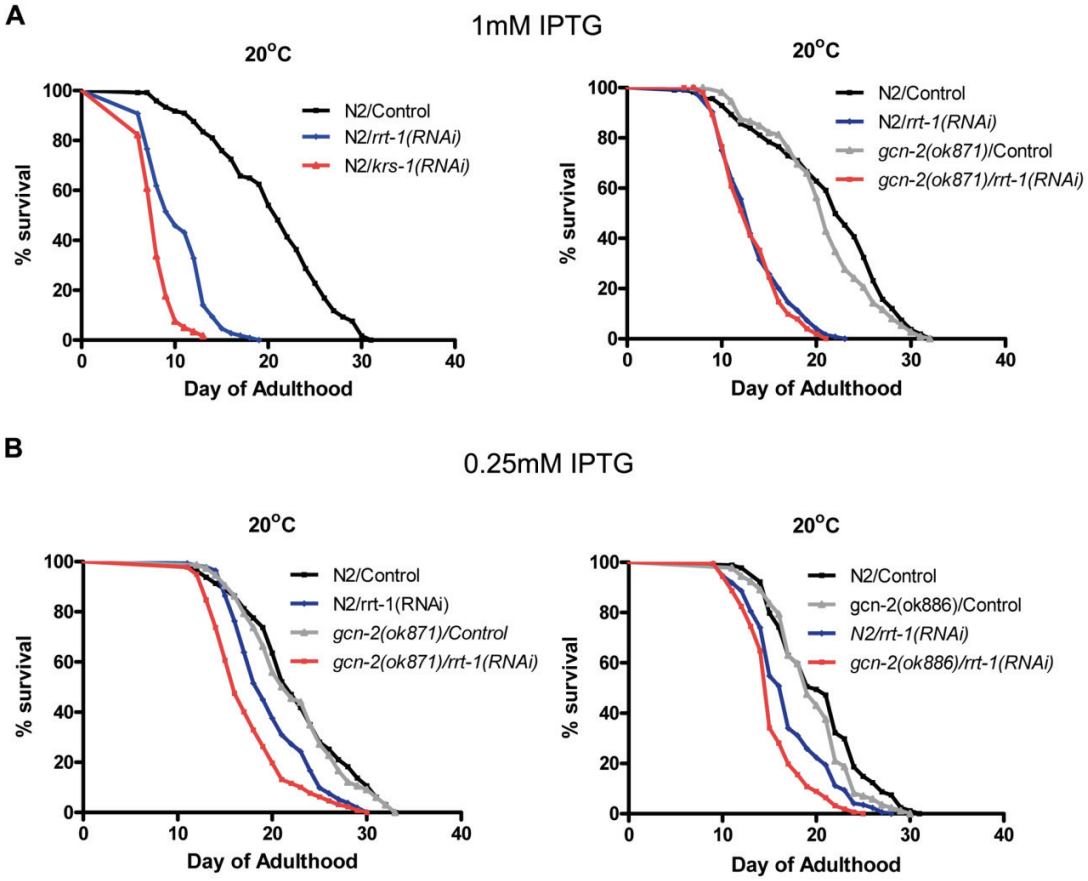

Fig. S4: Loss of GCN-2 sensitizes animals to amino acid limitation. **(A)** Survival curves of N2 and *gcn-2* mutant worms subjected to strong RNAi conditions (1mM IPTG) to inactivate *rrt-1* or *krs-1*, compared to empty vector (Control), at 20°C. **(B)** Survival curves of N2 and *gcn-2* mutant worms subjected to weak RNAi conditions (0.25mM IPTG) to inactivate *rrt-1* gene, compared to empty vector (Control), at 20°C. See Table 2 for additional data.

Fig. S5

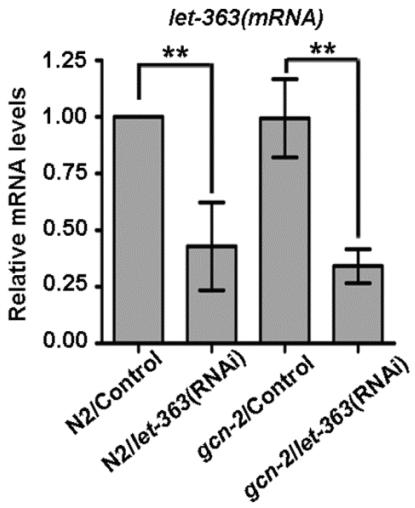

Fig. S5: RNAi efficiency is not affected in *gcn-2* mutant worms. qRT-PCR of *let-363* transcript on N2 and *gcn-2* worms, subjected to Control RNAi or *let-363(RNAi)* expressing bacteria for 4 days. The asterisks represent statistical significant difference (*p<0.05, **p<0.01, ***p<0.001 in unpaired t-test).

Fig. S6

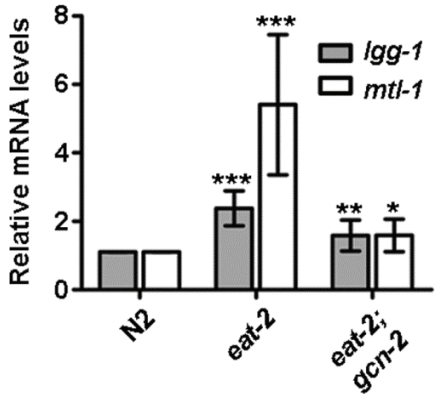

Fig. S6: GCN-2 regulates the induction of PHA-4 target genes in *eat-2* mutants. qRT-PCR of *lgg-1* and *mtl-1* transcript levels in 1-day adults of N2, *eat-2* and *eat-2;gcn-2* raised on OP-50 bacteria at 20^{o}C. The asterisks represent statistical significant difference from N2 (*p<0.05, **p<0.01, ***p<0.001 in unpaired t-test)

Fig. S7

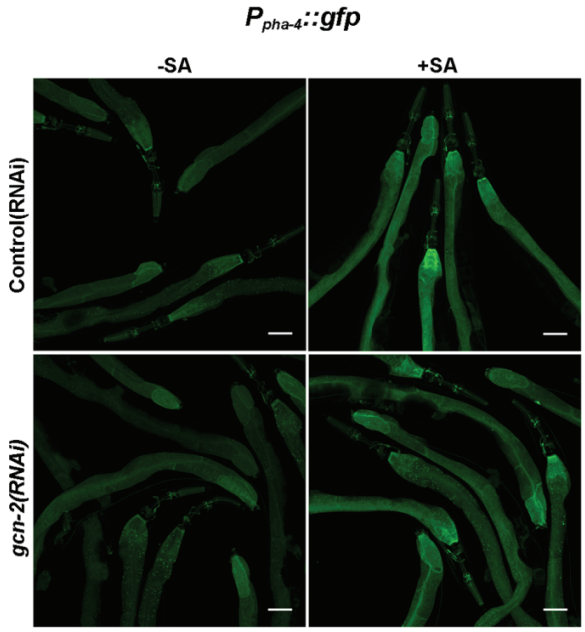

Fig. S7: Inactivation of *gcn-2* affects the induction of a P_{pha-4}*::gfp* reporter under oxidative stress. Confocal images of 1-day adults expressing a membrane-bound GFP under the *pha-4* promoter (SM481strain), fed for two generations either Control RNAi or *gcn-2(RNAi)* expressing bacteria and treated (+SA) or not (-SA) with sodium arsenite (15mM for 3h before observation). All images were taken at 20x magnification under the same microscopy settings (scale bar: 50µm).

Fig.S8

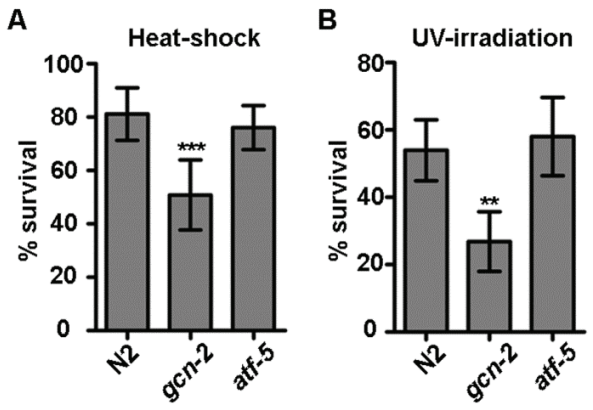

Fig. S8: Loss of *gcn-2* but not *atf-5* increases the stress sensitivity of worms. **(A)** Survival of 1-day old N2, *gcn-2* and *atf-5* adults subjected to heat-shock (35^{o}C for 6h). **(B)** Survival of 5-day old N2, *gcn-2* and *atf-5* adults after UV-irradiation (0.2J/cm^{2}).

Table S1: List of the strains used in this study

Strain	Genotype	Information	Reference
N2		Wild-type	
BRF162	*gcn-2(ok871 II)*	Backcrossed to N2 4x	This study
BRF163	*gcn-2(ok881 II)*	Backcrossed to N2 4x	This study
BRF172	*atf-5(ok576 X)*	Backcrossed to N2 4x	This study
DA465	*eat-2(ad465 II)*	Point mutation of Y48B6A.4. Backcrossed to N2 1x	CGC
BRF178	*gcn-2(ok871I);eat-2(ad465 II)*	From crossing of BRF162 with DA465	This study
BRF140	N2 Ex[ATF-5::GFP;pRF4 *rol-6(su1006)*]	Translational fusion of the intact gene T04C10.4 under its promoter	This study
BRF144	*gcn-2(ok871)*Ex[ATF-5::GFP;pRF4 *rol-6(su1006)*]	From crossing of BRF140 with BRF162	This study
BRF152	N2 Ex[$_{\text{-uORFs}}$ATF-5::GFP;pRF4 *rol-6(su1006)*]	Translational fusion of the uORF-less gene T04C10.4 under its promoter	This study
SM481	pxIs10[$P_{pha\text{-}4}$::GFP::CAAX ; pRF4 *rol-6(su1006)*]	Membrane-bound GFP under the *pha-4* promoter	CGC
KX15	*ife-2(ok306 X)*	Deletion of R04A9.4 (*ife-2*)	CGC

Table S2: List of primers used in this study

Primer	Sequence (5' to 3')	Used for
GCN-2/1 (G1)	GCGATTGATGTTGTTCCAG	*gcn-2(ok871* and *ok886)* genotyping
GCN-2/2 (G2)	GAGACCACATCCATCGC	>>
GCN-2/3 (G3)	GTGAGTAGACTCGTCCG	>>
ATF-5/1 (A1)	ACATGGCATGCATGATTTATAACGGAAGTTCAG	*atf-5(ok576)* genotyping
ATF-5/2 (A2)	GGTCTAGAAATTTTCAGATGAGATGTTTCTGCG	>>
Eat-2/1 (E1)	GCTAGTCGATTTTCATCATCG	*eat-2(ad465)* genotyping
Eat-2/2 (E2)	GGCTAACCTTCAAATAGCAAAC	>>
GCN-2/4 (G4)	CTACCTACTCTCGAGTTCC	*gcn-2(RNAi)* construct
GCN-2/6 (G6)	AACTGCAGCTCATTGCTTCCAGCG	>>
ATF-5/3 (A3)	AACTGCAGAGTCGTCTCCCTTTCCTC	*atf-5(RNAi)* construct
ATF-5/4 (A4)	CGGGATCCGTCGGTGACAGTTTTCATTC	>>
RRT-1/1 (R1)	GGCAGATCTGGATACTCTGACTACTCAG	*rrt-1(RNAi)* construct
RRT-1/2 (R2)	GGCCTGCAGCAGCTTCACAACATACTCG	>>
KRS-1/1 (K1)	GCGCCATGGCAAGCCAAGAAGGAACAAG	*krs-1(RNAi)* construct
KRS-1/2 (K2)	GGGCTGCAGCGAGTGGTGACATGATTTG	>>
ATF-5/1 (A1)	ACATGGCATGCATGATTTATAACGGAAGTTCAG	$P_{atf\text{-}5}$*::atf-5::gfp* construct
ATF-5/4 (A4)	CGGGATCCGTCGGTGACAGTTTTCATTC	>>
ATF-5/2(A2)	GGTCTAGAAATTTTCAGATGAGATGTTTCTGCG	$P_{atf\text{-}5}$*::gfp* construct
ATF-5/5 (A5)	GGTCTAGAATGGCTTATGTAAATGAACAAAATCC	$_{\text{-}uORF}$*::atf-5::gfp* construct
LRS-1/1 (L1)	GGCAAGCTTCAAGGATGATAAGGGAACTGG	*lrs-1(RNAi)* construct
LRS-1/2 (L2)	GGCCTCGAGCACGAAGCATCTGTCATCTG	>>
PHA-4/1 (P1)	CCCAAGCTTGCGGTCATCGGAAGAAGC	*pha-4(RNAi)* construct
PHA-4/2 (P2)	CCGCTCGAGCTGGTATACTCCGTTGGTG	>>

Table S3: Summary of Data from independent repeats of lifespan assays

	Strain/RNAi	Treatment (Temp/IPTG)	Median/Max Lifespan (days)[b]	Mean lifespan ± s.e.m (days)[c]	Number (T/C)[d]	p-value against N2[e]	p-value against specific control[f]
Fig. 4A	N2	20°C	20/29.5	20.5±0.5	64/2		
	gcn-2(ok871)	>>	21/31.4	20.67±0.33	116/4	0.7592	
	gcn-2(ok886)	>>	20/29.3	20.33±1.2	101/2	0.5729	
	atf-5(ok576)	>>	19/26.6	18.17±0.6	100/2	0.0117	
	N2	20°C	24/32.4	24.17±0.44	85/5		
	gcn-2(ok871)	>>	23/31.9	23±0.57	78/9	0.4991	
	gcn-2(ok886)	>>	23/29.8	23.17±0.61	77/2	0.0219	
	atf-5(ok576)	>>	22/28.4	22.33±0.33	109/5	0.0001	
	N2	20°C	21/28.6	21±0.67	82/12		
	gcn-2(ok871)	>>	22/27.9	22±1.00	83/13	0.9383	
Fig. 4C	*eat-2(ad465)*	20°C	24/33.3	23.5±0.5	71/3		
	gcn-2(ok871);eat-2(ad465)	>>	19/29.3	19.33±0.33	117/4	<0.0001	
Fig. 4B	N2/Control	20°C	24/32.2	24±1	80/4		
	N2/*gcn-2*(RNAi)	>>	25/32.8	25.33±0.33	92/2	0.1738	
	N2/*atf-5*(RNAi)	>>	23/30.7	23.25±0.75	84/1	0.0536	
Fig. S4 A	N2/Control	20°C/1mM	21/28.7	20.83±0.6	120/4		
	N2/*rrt-1*(RNAi)	>>	13/19.5	12.67±0.33	112/9	<0.0001	
	N2/*krs-1*(RNAi)	>>	12/14.5	11.67±0.33	114/2	<0.0001	
	gcn-2(ok871) /Control	>>	18/28.5	19±0.57	113/6	0.1614	
	gcn-2(ok871)/*rrt-1*(RNAi)	>>	12/19.3	12.67±0.33	122/12	<0.0001	0.1534
	gcn-2(ok871)/*krs-1*(RNAi)	>>	11/14.7	10.67±0.33	123/3	<0.0001	0.0009
	gcn-2(ok886)/Control	>>	17/29.4	17.67±0.88	114/7	0.2635	
	gcn-2(ok886)/*rrt-*	>>	13/20.1	12.5±0.28	120/6	<0.0001	0.6139

	1(RNAi)						
	gcn-2(ok880)/*krs-1*(RNAi)	>>	12/14.6	11.67±0.33	114/3	<0.0001	0.8192
Fig. S4 A	N2/Control	20°C/1mM	21/28.6	21.33±0.67	82/12		
	N2/*krs-1*(RNAi)	>>	12/14.4	12±0.0	96/9	<0.0001	
	gcn-2(ok871) /Control	>>	22/27.9	22±1	83/13	0.9383	
	gcn-2(ok871)/*krs-1*(RNAi)	>>	11/12.3	11±0.0	79/9	<0.0001	<0.0001
Fig. S4 B	N2/Control	20°C/0.25mM	19/26	19.83±0.44	61/4		
	N2/*rrt-1*(RNAi)	>>	16/25.3	15.67±0.33	131/4	<0.0001	
	gcn-2(ok871) /Control	>>	18/24.1	18.33±0.33	82/5	0.0510	
	gcn-2(ok871)/*rrt-1*(RNAi)	>>	14/23.2	13.83±0.16	135/3	<0.0001	0.0005
	gcn-2(ok886)/Control	>>	18/23.4	17.83±0.44	56/3	0.0673	
	gcn-2(ok886)/*rrt-1*(RNAi)	>>	14/22.8	14.33±0.33	135/3	<0.0001	0.0006
Fig. S4 B	N2/Control	20°C/0.25mM	20/32.2	20.33±0.67	89/4		
	N2/*rrt-1*(RNAi)	>>	16/25.3	16.33±0.33	117/7		<0.0001
	gcn-2(ok871) /Control	>>	21/30.8	20.75±0.43	129/1	0.6456	
	gcn-2(ok871)/*rrt-1*(RNAi)	>>	15/21.8	15±0.57	99/19		<0.0001
	atf-5(ok576)/Control	>>	21/26.2	21.5±0.29	142/2	0.3275	
	atf-5(ok576)/rrt-1(RNAi)	>>	17/26	17.67±0.33	132/12		<0.0001

a: Data sets within each panel were done in parallel and statistical analysis were performed within the data set.
b: Max lifespan is the mean of the last 10% surviving worms
c: Mean lifespan and standart error of the mean (s.e.m.) of 2-4 plates
d:Total number (T) of dead and censored (C) worms/censored (C)
e: *p*-value from log rank test comparing a strain to N2 or N2/Control(RNAi)(<0.05 is considered statistically significant)
f: *p*-value from log rank test comparing a strain to a specific control stain (e.g. *gcn-2;pek-1* vs *gcn-2* or *gcn-2/rrt-1(RNAi)* vs N2/*rrt-1(RNAi*)