Figure S1

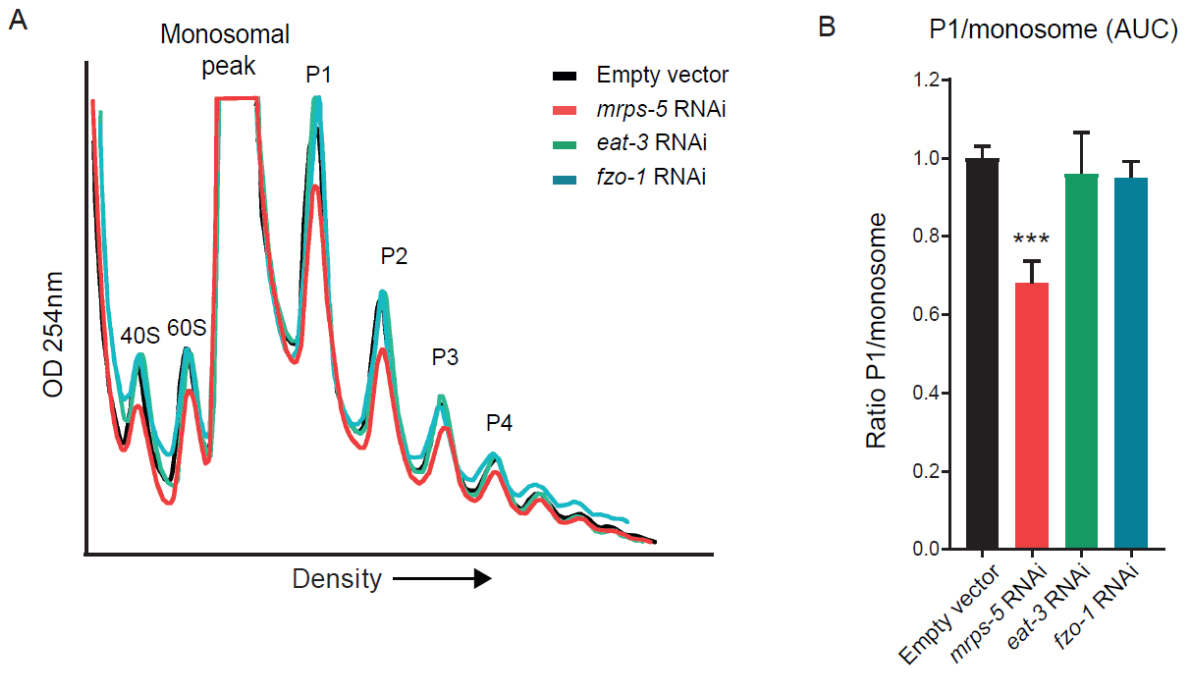

Figure S1. Cytosolic translation repression is not a universal response to mitochondrial stress, related to Figure 1.

(A) Representative polysome profiles showing decreased cytosolic polysome abundances in worms with impaired mitochondrial ribosomal biogenesis (*mrps-5* RNAi) but not with mitochondrial stress induced by inhibition of mitochondrial fusion through *eat-3* RNAi (OPA1 homolog) or *fzo-1* RNAi (mitofusin homolog). Lysate is normalized to protein levels. The subunits (40S and 60S), monosomal peak, and polysomal peak numbers are indicated (P1–P4).

(B) In *mrps-5* RNAi treated worms (red) the ratio between the AUC of the first polysomal peak and the monosome is decreased compared to control worms (black), but RNAi of either *eat-3* (green) or *fzo-1* (blue) does not change polysomal peaks.

Figure S2

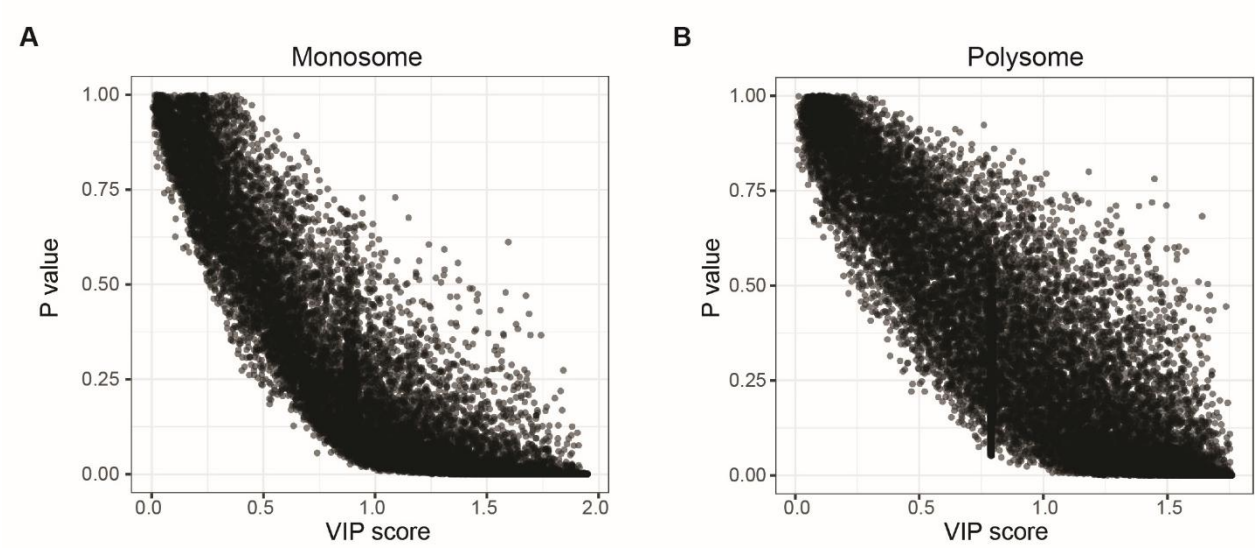

Figure S2. VIP score correlates highly to traditional *p*-value ranking, related to Figure 2 and Table S2.

(A-B) Partial least squares discriminant analysis (PLS-DA) derived a variable of importance (VIP) score for each gene. Bayes moderated *t*-test used to derive *p* values. Performed for both (A) monosomal and (B) polysomal RNAseq results.

Figure S3

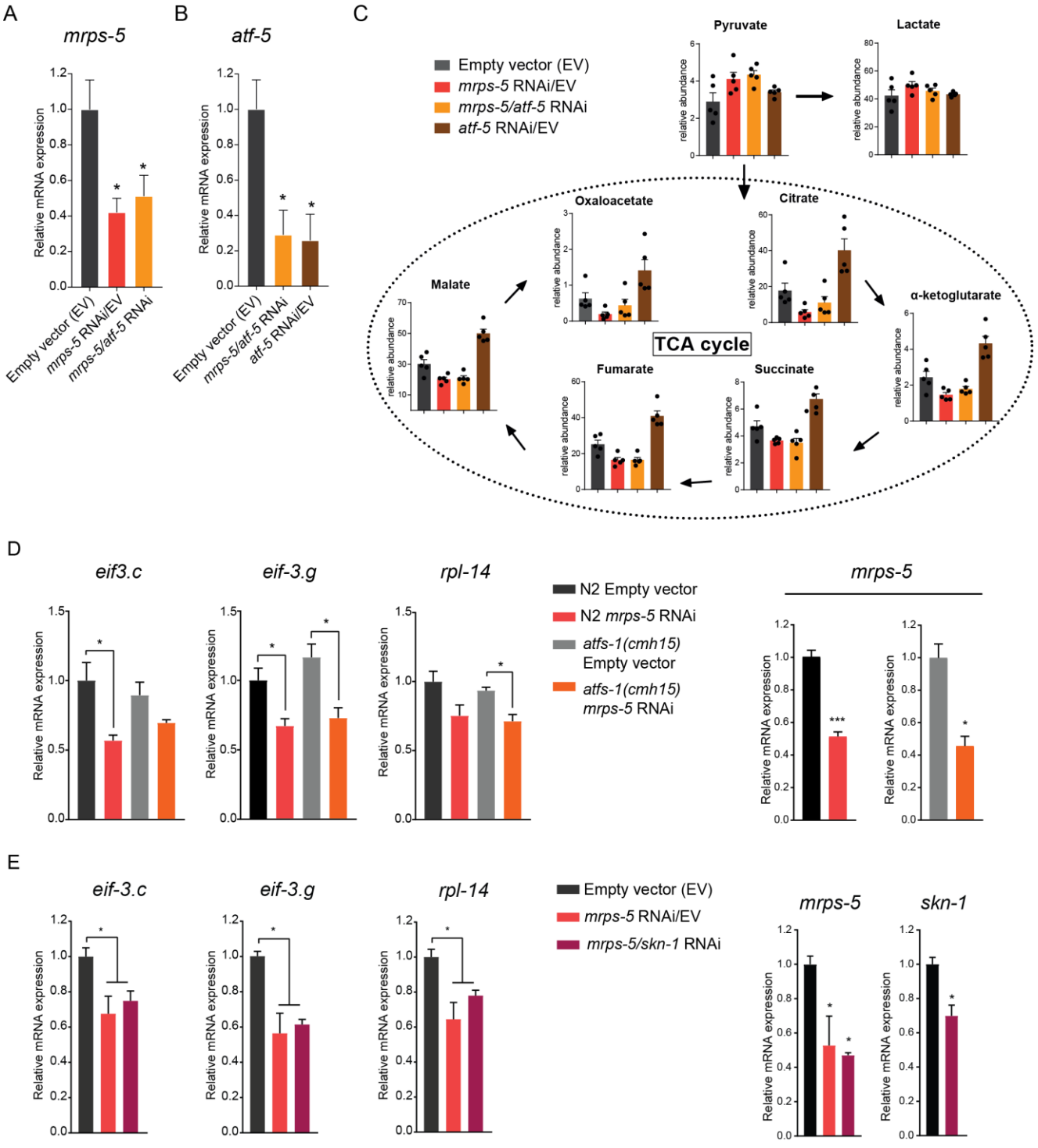
A
mrps-5
Relative mRNA expression
Empty vector (EV)
mrps-5 RNAi/EV
mrps-5/atf-5 RNAi
B
atf-5
Relative mRNA expression
Empty vector (EV)
mrps-5/atf-5 RNAi
atf-5 RNAi/EV
C
Empty vector (EV)
mrps-5 RNAi/EV
mrps-5/atf-5 RNAi
atf-5 RNAi/EV
Pyruvate
Lactate
Oxaloacetate
Citrate
Malate
α-ketoglutarate
TCA cycle
Fumarate
Succinate
relative abundance
D
eif3.c
eif-3.g
rpl-14
Relative mRNA expression
N2 Empty vector
N2 mrps-5 RNAi
atfs-1(cmh15) Empty vector
atfs-1(cmh15) mrps-5 RNAi
mrps-5
E
eif-3.c
eif-3.g
rpl-14
Relative mRNA expression
Empty vector (EV)
mrps-5 RNAi/EV
mrps-5/skn-1 RNAi
mrps-5
skn-1

Figure S3. Role for *atf-5* (but not *atfs-1* or *skn-1*) in regulating the mito-cytosolic translational balance, related to Figure 4

(A) Expression of *mrps-5* was downregulated in the *mrps-5*/EV-treated worms (red) and in the *mrps-5/atf-5*-treated worms (yellow), with N=4 per condition.

(B) Expression of *atf-5* was downregulated in the *mrps-5/atf-5* RNAi treated worms (yellow) and in the *atf-5*/EV-treated worms (brown), N=4 per condition.

(C) Lower levels of TCA cycle intermediates (citrate, α-ketoglutarate, succinate, fumarate, and malate) and increased pyruvate levels in the *mrps-5/EV* and *mrps-5/atf-5* worms compared to control worms (N=5).

(D) Mutant *atfs-1(cmh15)* worms exposed to *mrps-5* RNAi show downregulation of translational genes (*eif-3.c, eif-3.g and rpl-14*) similar to wildtype N2 worms treated with *mrps-5* RNAi. Similar *mrps-5* knockdown efficiencies were observed in the N2 and *atfs-1(cmh15)* worms.

(E) Double knockdown with both *mrps-5* and *skn-1* still repressed translational genes *eif-3.c, eif-3.g* and *rpl-14* upon *mrps-5* RNAi similar to *mrps-5* RNAi alone, suggesting that this repression is independent of *skn-1*. Similar knockdown efficiencies were observed in the double RNAi treated worms as the RNAi mixed with EV treated worms

Figure S4

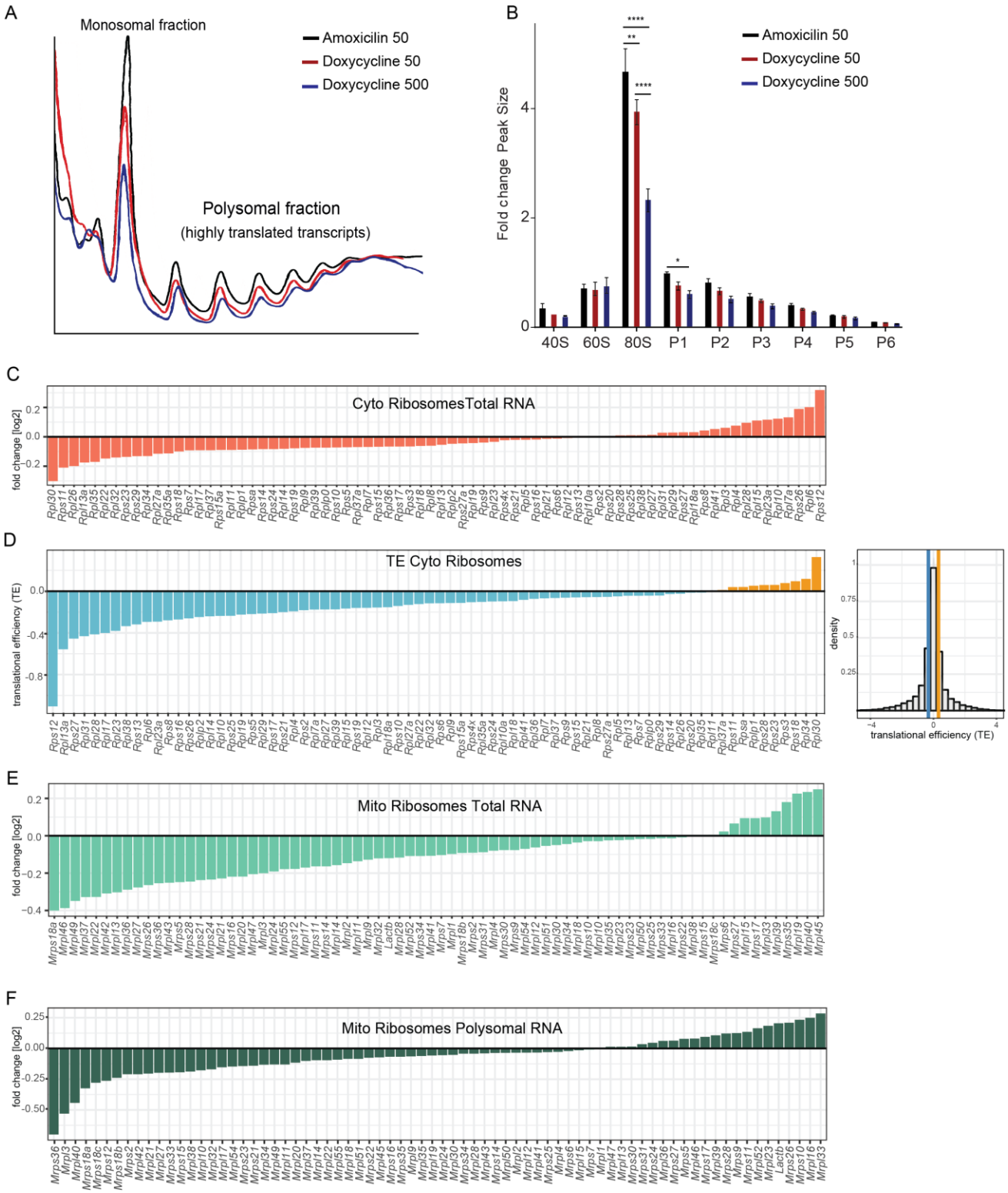
A
Monosomal fraction
Amoxicilin 50
Doxycycline 50
Doxycycline 500
Polysomal fraction
(highly translated transcripts)
B
Amoxicilin 50
Doxycycline 50
Doxycycline 500
Fold change Peak Size
40S
60S
80S
P1
P2
P3
P4
P5
P6
C
Cyto RibosomesTotal RNA
fold change [log2]
D
TE Cyto Ribosomes
translational efficiency (TE)
density
translational efficiency (TE)
E
Mito Ribosomes Total RNA
fold change [log2]
F
Mito Ribosomes Polysomal RNA
fold change [log2]

Figure S4. Doxycycline treatment resulted in suppression of global translation in liver in a dose-dependent manner, related to figure 6 and Table S7.

(A) Representative polysome profiles showing decreased cytosolic polysome abundances in the liver of mice treated with either a low dose of doxycycline (50 mg/kg/d) or a high dose of doxycycline (500 mg/kg/d) compared to control amoxicillin-treated mice (50 mg/kg/d). Lysate is normalized to protein levels.

(B) Quantification of polysome peak sizes with N=3 mice per condition normalized to P1 peak of amoxicillin-treated mice. Bars represent mean ± SEM and significance was tested with Student's t-test, and *p*-values were adjusted to correct for multiple testing using the Holm- Šídák method, with $\alpha = 0.05$.

(C) Fold changes of individual cytosolic ribosomal proteins in the total RNA in doxycycline-treated mouse livers.

(D) Left panel: Individual cytosolic ribosomal proteins have a low TE in doxycycline-treated mice (50 mg/kg/d) compared to control amoxicillin-treated mice. TE of transcripts of polysomal vs monosomal differences between doxycycline-treated (50 mg/kg/d) and amoxicillin-treated mice gene expression. Right panel: Translational efficiencies (TE) of transcripts, defined as the log2 ratio of polysomal vs total differences between doxycycline-treated mice (50 mg/kg/d) and control amoxicillin-treated mice (50 mg/kg/d) gene expression, shows shifts in transcripts from either the monosome to the polysome (high TE, yellow) or the polysome to the monosome (low TE, blue).

(E) Fold changes of individual mitochondrial ribosomal proteins in the total RNA in doxycycline-treated mouse livers.

(F) Fold changes of individual mitochondrial ribosomal proteins in the polysomal RNA in doxycycline-treated mouse livers.

Table S6. Lifespan information, related to Figure 4

RNAi culture	mean lifespan	p-values against control	p-values against *mrps-5* RNAi	N (trials)
HT115 (EV)	21			205 (2)
mrps-5 RNAi/EV	35	<0.0001		165 (2)
mrps-5 + *atf-5* RNAi	25	<0.0001	<0.0001	177 (2)
atf-5 RNAi/EV	23	0.3894		200 (2)