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Mitonuclear protein imbalance as a conserved longevity mechanism

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Abstract

Longevity is regulated by a network of intimately linked metabolic systems. We used a combination of mouse population genetics and RNAi in *C. elegans* to identify mitochondrial ribosomal protein S5 (*Mrps5*) and other mitochondrial ribosomal proteins (MRPs) as metabolic and longevity regulators. MRP knockdown triggers mitonuclear protein imbalance, reducing mitochondrial respiration and activating the mitochondrial unfolded protein response (UPR^{mt}). Specific antibiotics targeting mitochondrial translation and ethidium bromide, which impairs mitochondrial DNA transcription, pharmacologically mimic *mrp* knockdown and extend lifespan by inducing mitonuclear protein imbalance, also in mammalian cells. In addition, resveratrol and rapamycin, longevity compounds acting on different molecular targets, similarly induced mitonuclear protein imbalance, UPR^{mt} and lifespan extention in *C. elegans*. Collectively these data demonstrate that *MRPs* represent an evolutionary conserved protein family that ties the mitochondrial ribosome and mitonuclear protein imbalance to UPR^{mt}, an overarching longevity pathway across multiple species.

Longevity is coordinated by intersecting pathways, often converging on metabolic networks[1–4]. A key player in lifespan regulation is the mitochondrion. Over a thousand proteins encoded by nuclear DNA (nDNA) translocate to and function in mitochondria[5], in synchrony with 13 proteins encoded by the mitochondrial DNA (mtDNA) that require a

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Author Contributions RHH, LM and JA conceived and designed the project. RHH and RWW performed QTL mapping and sequence analyses. RHH, LM, EK, DR, NM, GK performed experiments. RHH and JA wrote the manuscript, with contributions from all other authors.

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separate translation machinery, including mitochondrial ribosomal proteins (MRPs)[6,7]. Many molecular studies of longevity have exploited simple organisms and loss- or gain-of-function mutations, but the complex connectedness of mitochondrial and metabolic longevity networks benefits from an integrative cross-species approach[2].

Here we pioneered such a strategy and used the BXD reference population of mice[2,8–10] to identify mitochondrial ribosomal protein S5 (*Mrps5*) and other members of the *MRP* family as longevity genes. In *C. elegans*, we confirmed this role of MRPs and demonstrated that they induce a stoichiometric imbalance between nDNA- and mtDNA-encoded OXPHOS proteins, hereafter termed “mitonuclear protein imbalance”, which activates the mitochondrial unfolded protein response (UPR^{mt}). Our conclusions were corroborated using specific antibiotics targeting bacterial/mitochondrial translation, and ethidium bromide, which inhibits mtDNA transcription. This mechanism is shared with pathways that induce mitonuclear protein imbalance from a nuclear perspective, such as the UPR^{mt} and lifespan effects of rapamycin and resveratrol. Our data hence tie mitochondrial translation and metabolism to natural lifespan regulation across species.

A QTL for mouse longevity

The BXD family consists of fully inbred progeny of a cross between C57BL/6J and DBA/2J mice, with a complexity that matches many human populations[11]. Both parental strains have been sequenced enabling analysis of sequence variants linked to phenotypes[12]. We used new genomic and genetic resources to reanalyze longevity data for BXD lines[13] using forward and reverse genetic methods[9].

The forward strategy exploits longevity data and updated high-density SNP genotypes[14] archived in www.GeneNetwork.org. As reported[13], lifespan of BXDs varies from ~365d for the shortest lived strain to ~900d for the longest lived (Fig. 1a). We remapped longevity using the new genotypes and detected one genome-wide significant locus on chromosome 2 with a peak at 124–129Mb (Fig. 1b, LOD=4.0). Two additional loci, on chromosomes 4 and 7, were not significant, but suggestive (LOD=2.8 and 3.0, respectively). However, neither was suggestive after controlling for SNP rs6374387 on chromosome 2 using composite interval mapping.

The chromosome 2 locus contains ~70 genes (Supplementary Table 1), none of which previously linked to longevity. To evaluate and rank candidates, we correlated lifespan with multiple gene expression data sets. Only three genes in the locus correlate strongly with lifespan (Fig. 1c, $p<0.01$; Supplementary Fig. 1), i.e. solute carrier family 12 member 1 (*Slc12a1*), mitochondrial ribosomal protein S5 (*Mrps5*), and tubulin-tyrosine ligase (*Ttl*). From the natural variation in expression of these genes we deduced that 50% reduction of expression corresponds to a ~250d lifespan difference.

Conservation of longevity in *C. elegans*

We identified Y37A1C.1/*nkcc-1*, E02A10.1/*mrps-5*, and F25C8.5/*ttll-9* as worm homologs of *Slc12a1*, *Mrps5*, and *Ttl*, respectively. RNAi-mediated knockdown of *nkcc-1* and *mrps-5*, but not of *ttll-9*, extended lifespan (Fig. 2a).

Next, we compared expression of *Mrps5* and other *Mrp* family members in a muscle microarray of aging and CR in C57BL/6J[15]. *Mrp* expression decreased with age, an effect rescued by CR; in contrast, expression of *Slc12a1* and *Ttl* was unaffected (Fig. 2b). Linkage of MRPs with lifespan is strengthened as many other *Mrp* family members also correlate with longevity (Fig. 2c). We extended our analyses to the DNA level using sequence data for *Mrps5* in both parental strains and identified missense variants in exon 3 (rs29667217 and rs13471334; V60A and V67I, respectively). Other sequence variants in *Mrps5* contribute to variation in transcript abundance; *Mrps5* mRNA levels among the BXDs are associated with a strong QTL superimposed over the gene itself—a cis expression QTL.

Using a reverse genetics approach, we studied the *Mrps5*-associated network. *Mrps5* expression covaries with genes involved in oxidative phosphorylation (OXPHOS). Considering that oxidative metabolism is involved in known longevity pathways[2], the set of transcripts that covary with *Mrps5* qualified as an appealing longevity network. OXPHOS was the most enriched network of *Mrps5* covariates in both BXDs[16] and a conventional F2 intercross[17] (p=1.53e-21, p=5.78e-10, respectively). Finally, we generated an interaction network of OXPHOS genes with *Mrps5* (Fig. 2d), in which *Ndufb7* provides the hinge that links *Mrps5* to OXPHOS. Interestingly, knockdown of the worm homologs for the network components *Ndufb7* and *Ndufa6* robustly extended lifespan[18–20]. *Mrps5* hence emerged as a strong longevity candidate, integrating protein synthesis and mitochondrial metabolism—both important longevity modulators.

Mitonuclear protein imbalance and aging

To define causality of the *MRP*s in determining lifespan, we knocked down *mrp* genes during the entire life of the worm and robustly increased lifespan (Fig. 3a, Supplementary Table 2). Similarly to well-characterized mitochondrial mutants that live longer, larval development was delayed (Supplementary Fig. 2a)[21]. Knockdown during development proved crucial and sufficient to extend lifespan, while RNAi during adulthood alone did not (Fig. 3b, Supplementary Fig. 2b and Table 2), as reported in other long-lived mitochondrial mutants[22]. Increased lifespan was not due to effects on feeding, as pharyngeal pumping rates were normal (Supplementary Fig. 2c). *mrps-5* RNAi also delayed physiological decline with age. Even though they moved slightly less in early adulthood (d3), *mrps-5* RNAi worms move twice as much as controls at d13, and this effect becomes more pronounced at d20 (Supplementary Fig. 2d–e and Movies 1–4). This difference was accompanied by a delay in decline of pharyngeal pumping (Supplementary Fig. 2c) and in muscle fiber disorganization (Fig. 3c), hallmarks of fitness of aged *mrps-5* RNAi worms.

In line with the mitochondrial connection of *Mrps5*, basal respiration was reduced upon *mrp* knockdown and unresponsive to the uncoupler FCCP (Fig. 3d). As a consequence, *mrps-5* RNAi worms displayed reduced ATP levels and citrate synthase activity (Fig. 3e–f), indicative for reduced mitochondrial abundance or activity. Consistent with its role in mitochondrial translation, *mrps-5* RNAi induced a stoichiometric imbalance between nDNA- and mtDNA-encoded OXPHOS subunits, termed mitonuclear protein imbalance, visualized by selective reduction in MTCE.26 (MTCO-1 homolog; from mtDNA) relative to H28O16.1 (ATP5A homolog; from nDNA) expression (Fig. 3g). The mitonuclear protein

imbalance and impact on mitochondrial function was similar to the long-lived *cco-1* mutant —deficient for the nDNA-encoded worm homolog of complex IV, subunit Vb/COX4—but not observed in the short-lived complex II SDHC mutant *mev-1* (Fig. 3g). Furthermore, mitochondria had a more punctuate globular pattern instead of the regular reticular/tubular appearance in both muscle (Fig. 3h) and intestine, a finding confirmed by electron microscopy (Supplementary Fig. 3a–b).

To identify which "longevity pathways"—insulin/IGF-1 signaling[23], CR[24], and mitochondrial dysfunction[22]—are required for the lifespan phenotype, we reduced *mrps-5* expression in worms carrying mutations in these pathways. *mrps-5* RNAi increases lifespan by ~40% in wild types (Fig. 3i), similar to the effect in *daf-2*, *daf-16*, *eat-2*, *sir-2.1*, and *aak-2* mutants (Fig. 3j–l, Supplementary Fig. 4a–e) indicating that *mrps-5* regulates longevity independently of insulin/IGF-1 (*daf-16/daf-2*) and CR (*eat-2*/*sir-2.1*) and acts downstream of mitochondrial regulator *aak-2*.

We focused on the mitochondrial pathway, since: (1) it robustly impacts longevity[22]; (2) *MRP*s function in the translation of mtDNA-encoded OXPHOS subunits[6,7]; and (3) in the BXDs, *Mrps5* networked with several OXPHOS components (Fig 2d). Interestingly, *mrps-5* RNAi reverts the short-lived phenotype of *mev-1* mutants, with a dramatic 112% lifespan extension (Fig. 3m, Supplementary Fig. 4f). *mrps-5* RNAi in the *cco-1* mutants did not extend lifespan compared to *mrps-5* RNAi alone, suggesting that *cco-1* and *mrps-5* act in a similar fashion (Fig. 3n, Supplementary Fig. 4g). The same is true for *mrps-5* RNAi in the mitochondrial *clk-1(e2519)* mutant, confirming the link with mitochondrial longevity pathways (Supplementary Fig. 4h).

Mitochondrial unfolded protein response

The mitochondrial unfolded protein response (UPR^{mt}) accounts for longevity upon *cco-1* loss-of-function and is selective for the mitochondrial pathway and not involved in the CR or insulin/IGF-1 pathways[25]. UPR^{mt} is induced by mitochondrial stress, subsequently activating a nuclear transcriptional response, inducing the chaperones HSP-6 (HSP-70 in mammals) and HSP-60 to restore mitochondrial proteostasis[26,27]. We monitored UPR^{mt} using *hsp-6*::GFP and *hsp-60*::GFP reporter worms with reduced *mrp* expression. Similar to the *cco-1* mutant[25], *hsp-6* and *hsp-60* were induced in worms with reduced *mrp* (Fig. 4a–c, Supplementary Fig. 5a–b). This was specific for UPR^{mt}, as *mrps-5* RNAi did not affect UPR in the ER (UPR^{ER}) and cytosolic heat shock response (HSR) (Supplementary Fig. 5c). As for lifespan, UPR^{mt} was not induced when *mrp* expression was only inhibited during adulthood (Supplementary Fig. 5d). We then measured UPR^{mt} upon combined *mrps-5* and *mev-1* inactivation. Whereas *mev-1* RNAi alone did not induce UPR^{mt} (Fig 4c–d), combined inactivation induced mitonuclear protein imbalance (Supplementary Fig. 5e) and synergistically induced UPR^{mt} (Fig. 4d), accounting for the amplified lifespan. Double inactivation of *mrps-5* and *cco-1* did not further enhance UPR^{mt} compared to *mrps-5* alone (Fig. 4d), in line with the similar lifespan.

There are individual differences in the degree of UPR^{mt} within the *mrps-5* RNAi worm population, which tightly correlate with lifespan extension (Supplementary Fig. 5f–h).

Importantly, GFP expression stayed similar throughout life, demonstrating that this is not an artifact of transiently reduced food intake (Supplementary Fig. 5i). To further link the level of UPR^{mt} to longevity, we measured UPR^{mt} in worms treated with RNAi against *mrp* genes[28]. Reduced expression of each *mrp* gene activated UPR^{mt} to a different degree (Supplementary Fig. 6a). The level of UPR^{mt} again correlated significantly with lifespan extension (Fig. 4e, Supplementary Fig. 6a–b and Table 3).

Two downstream effectors of UPR^{mt} are HAF1, a mitochondrial peptide transporter[29], and UBL5, a ubiquitin-like protein that regulates the transcriptional activation of mitochondrial chaperones[30]. Knockdown of *haf-1* in the face of *mrps-5* RNAi reduced lifespan extension, UPR^{mt} and increased respiration (Fig. 4f–h). Similarly, when both *ubl-5* and *mrps-5* were knocked down, lifespan extension, the respiration phenotype and UPR^{mt} were partially lost, in line with double *cco-1* and *ubl-5* RNAi[25] (Supplementary Fig. 7a–f).

This network could be traced back to mice, as *Ubl5* and the most likely mouse *haf-1* homolog—*Abcb10*—correlated tightly with *Mrp* genes, for instance in hippocampus of the BXDs[31] and in adipose tissue of F2-intercrossed mice[17] (Fig. 4i, Supplementary Fig. 7g). Additionally, *Hspd1*—encoding *HSP60*—correlated with multiple *Mrp* genes (Fig. 4i, data not shown). Gene ontology analysis showed strong connectivity between *Ubl5* and OXPHOS genes (p=9e-4 in eye; p=8.62e-10 in hippocampus), the translation process or ribosome (p=6e-4 eye; p=6.03e-10 hippocampus), and the mitochondrial inner membrane (p=1e-4 eye; p=3.31e-27 hippocampus) in the BXDs. Finally, we tied *Hspd1* in a close correlation network with various *Mrp* and OXPHOS genes (Fig. 4j).

Pharmacological mitonuclear protein imbalance

Many mitochondrial functions can be traced back to their endosymbiotic “bacterial” origin. Consequently, antibiotics that target bacterial translation also inhibit mitochondrial translation. We therefore used doxycycline to confirm the role of mitochondrial translation in longevity, while using carbenicillin—targeting the bacterial cell wall—as control. We used heat-killed OP50 or live HT115 bacteria—the latter insensitive to low concentrations of doxycycline (not shown)—to feed worms, to prevent antibiotic effects on bacteria. Doxycycline, given throughout life, dose-dependently extended lifespan, induced UPR^{mt}, not UPR^{ER}, and reduced oxygen consumption, without affecting ATP levels or citrate synthase activity (Fig. 5a–e, Supplementary Fig. 8a–b). Doxycycline at 60μg/mL caused developmental delay, like *mrps-5* RNAi, but no abnormalities were apparent at lower concentrations (not shown). A low concentration of doxycycline (6μg/mL), given only during development, also increased lifespan and UPR^{mt}, and attenuated respiration (Fig. 5f–h). Chloramphenicol—belonging to a different class of antibiotics targeting translation—also increased lifespan and UPR^{mt}, while decreasing respiration (Fig. 5i–k), when administered during development. Similar to *mrps-5* RNAi, doxycycline increased the ratio of nDNA- (ATP5A) over mtDNA-encoded (MTCO1) OXPHOS proteins (Fig. 5l).

Linking back to mammals, doxycycline decreased respiration in a mouse hepatocyte cell line (Fig. 5m). Doxycycline also induced UPR^{mt}, as evidenced by induction of *Hsp60* (Fig. 5n) and the UPR^{mt} protease *ClpP* (Supplementary Fig. 8c), and increased HSP60 protein

expression, in hepatocyte cell lines and primary murine hepatocytes (Fig. 5o–p). Doxycycline induced a striking mitonuclear protein imbalance in hepatocytes (Fig. 5o–p). Finally, feeding mice with doxycycline for 10d lowered oxygen consumption in vivo, indicative for attenuated mitochondrial function (Fig. 5q).

Similar effects on mitonuclear protein imbalance, UPR^{mt}, respiration and lifespan, without affecting mitochondrial morphology, were also observed in worms exposed to low concentrations ethidium bromide, which inhibits mtDNA transcription specifically[32] (Supplementary Fig. 8d–h). This suggests that mitonuclear protein imbalance is the common underlying mechanism that links basic mitochondrial function to lifespan regulation.

A conserved longevity mechanism

To define how intricately mitonuclear protein imbalance and UPR^{mt} are involved in longevity, we analyzed its activation in worms exposed to rapamycin[33,34]. Rapamycin inhibits TOR signaling to alter nDNA translation, inducing mitonuclear protein imbalance[35], and increases lifespan in various species, including mice[33]. Rapamycin also increased mean worm lifespan (by 16%)[34] in a *ubl-5*-dependent manner, induced UPR^{mt}, but not UPR^{ER} or HSR, and increased respiration (Fig. 6a–c, Supplementary Fig. 9a). This was associated with increased ATP levels, equal citrate synthase activity, and altered nDNA/mtDNA OXPHOS protein ratio (Fig. 6d–e). Additionally, rapamycin dose-dependently changed the balance between nDNA and mtDNA-encoded OXPHOS subunits in mouse hepatocytes (Fig. 6f–g). This mitonuclear protein imbalance induced HSP60 and ClpP (Fig. 6f–h). Similarly, the acclaimed lifespan enhancer resveratrol induced mitonuclear protein imbalance in hepatocytes (Fig. 6i) and *ubl-5*-dependently increased worm lifespan and UPR^{mt}, but not UPR^{ER} or HSR, while increasing respiration and maintaining ATP levels and citrate synthase activity (Supplementary Fig. 9b–f). Mitonuclear protein imbalance and UPR^{mt} hence represent an overarching mechanism of longevity that also can be engaged by pathways that signal mainly through the nucleus. Finally, we tested whether reactive oxygen species (ROS) and mitohormesis, which poses that an initial ROS burst (after 24h) induces adaptive long-term protection[36], could explain our worm phenotypes. However, no ROS was produced after 24h *mrps-5* RNAi or doxycycline, rapamycin or ethidium bromide (Supplementary Fig. 10a). In addition, the mitohormesis regulators *daf-16* and *aak-2*[36,37] were not involved in UPR^{mt} induction (Supplementary Fig. 10b) or lifespan extension (Fig. 3k, m) following *mrps-5* RNAi. Finally, the ROS scavenger N-acetylcysteine (NAC) did not abrogate the *mrps-5* RNAi- or rapamycin-induced UPR^{mt}, nor did it suppress longevity (Supplementary Fig. 10c–f), similar to NAC-treated *cco-1* RNAi worms[25]. Together, these data demonstrate that, even though mitohormesis is important for insulin/IGF1-dependent aging[37], UPR^{mt}-mediated longevity is independent of ROS.

Discussion

Using the BXD inbred wild type mouse panel, we identified a chromosome 2 QTL that is responsible for longevity, with *Slc12a1*, *Mrps5* and *Ttl* showing strong correlation with lifespan. A holistic approach, involving bioinformatics, and genetic and pharmacological strategies in both worms and mammals, established that *Mrps5* and the *Mrp* protein family

are the main actors in metabolic lifespan regulation. The MRPs constitute the mitoribosome that regulates translation of 13 mtDNA-encoded proteins, underscoring the vital importance of mitochondrial protein production[6,7].

Inhibiting mitochondrial translation reduced respiration and extended lifespan. There is an apparent dichotomy, however, as rapamycin (this study) as well as NAD^+ boosters—resveratrol (this study), nicotinamide riboside, nicotinamide and PARP inhibitors (LM, RHH, JA, unpublished data)—couple longevity to increased respiration. Abnormal mitochondrial proteostasis could reconcile these disparate observations. Knockdown of *mrps-5* or *cco-1* affect proteostasis and activate UPR^{mt}[25,28]. From the *cco-1* study[25] it was, however, unclear if there was a direct connection between the level of UPR^{mt} and the lifespan extension. Our data unequivocally demonstrate a positive correlation between the level of UPR^{mt} and lifespan. Moreover, UPR^{mt} seems to result from an imbalance between mtDNA- and nDNA-encoded proteins and is a common feature linking mitochondrial longevity pathways. Genetic defects in *mrp*'s or respiratory chain genes, antibiotics that inhibit translation, or moderate mtDNA transcription inhibition, induce such a mitonuclear protein imbalance from within mitochondria. Conversely, resveratrol and rapamycin change the production of nDNA-encoded mitochondrial proteins and if this is not matched with the levels of mtDNA-encoded proteins, mitonuclear protein imbalance and UPR^{mt} will also ensue, which favors longevity (Fig. 6j). The reason why *mev-1* mutants do not display UPR^{mt} is consistent with the fact that complex II is entirely nDNA-encoded and therefore does not require a balanced production of proteins from the nDNA and mtDNA. Additionally, complex II can be bypassed for mitochondrial ATP generation and is not part of OXPHOS supercomplexes[38]. Although further work to validate this hypothesis is warranted, this could explain apparent contradictions such as why mutations that decrease and increase respiration both can induce longevity.

In summary, our data implicate MRPs as a novel longevity protein family, conserved from worms to mammals. The identification of these genes was triggered by analysis of murine reference populations. Hence, it is natural variation in *Mrp* expression, not artificial loss- or gain-of-function, that translates to longevity. In worms, longevity involves enhanced fitness and UPR^{mt}, and correlates tightly with levels of *mrp* knockdown. Our data suggest that stoichiometric imbalance between nDNA- and mtDNA-encoded OXPHOS proteins, or mitonuclear protein imbalance, is at the core of UPR^{mt} activation, both in worms and mammals. The apparent conservation of mitonuclear protein imbalance and UPR^{mt} as a general longevity mechanism should incite studies to explore whether targeting UPR^{mt} can prevent aging-associated functional decline and treat diseases linked with aging.

Methods

Methods and any associated references are available in the online version of the paper at http://www.nature.com/nature/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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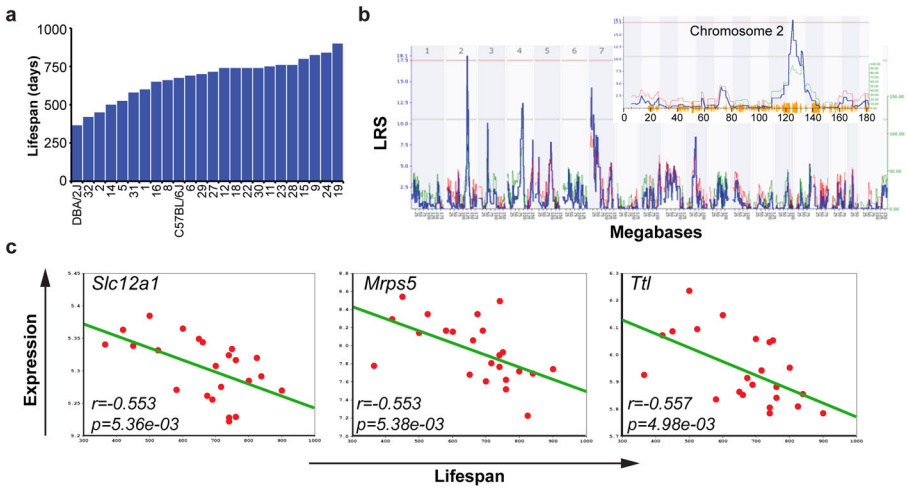

Figure 1. Lifespan regulation in BXD recombinant inbred mice

a, Life span in different BXD strains. **b**, Interval mapping using the BXD lifespan data reveals a strong QTL on chromosome 2, between 124–129 Mb. The red line depicts cutoff for statistical significance (*p* genome-wide <0.05), while the grey line represents the limit for suggestive QTLs. See also Supplementary Table 1. **c**, Pearson's *r* correlation coefficient with corresponding *p* values for the covariation between BXD lifespan (x-axis) and mRNA expression of the indicated gene in the BXD eye microarrays (y-axis). Expression of *Slc12a1*, *Mrps5* and *Ttl* robustly correlates with longevity ($p<0.01$).

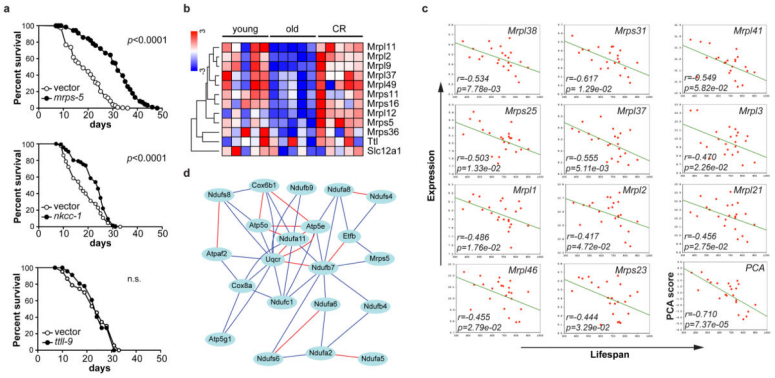

Figure 2. Validation of *Mrps5* as a candidate longevity gene

a, Knockdown during entire life of *mrps-5*, *nkcc-1* or *ttll-9* in *C. elegans* increased lifespan by 60%, 23% or 3%, respectively. See also Supplementary Table 2. **b**, One-way hierarchical clustering showing expression differences in gastrocnemius between young (5mo), old (25mo) and caloric restricted C57BL/6J mice[15]. Expression of mouse *Mrp*'s decreases upon aging, and reverts with CR, whereas *Slc12a1* and *Ttl* do not change. **c**, Pearson's *r* correlation coefficient with corresponding *p* values for covariation between BXD lifespan (x-axis) and mRNA expression of eleven other *Mrp*'s (y-axis) indicates robust correlation. Principle component analysis (PCA) reveals a highly significant correlation between the *Mrp* gene family and BXD lifespan. **d**, *Mrps5* strongly correlates with genes involved in OXPHOS. Red lines indicate a positive Pearson correlation coefficient of 0.7–1.0, and blue lines of 0.5–0.7.

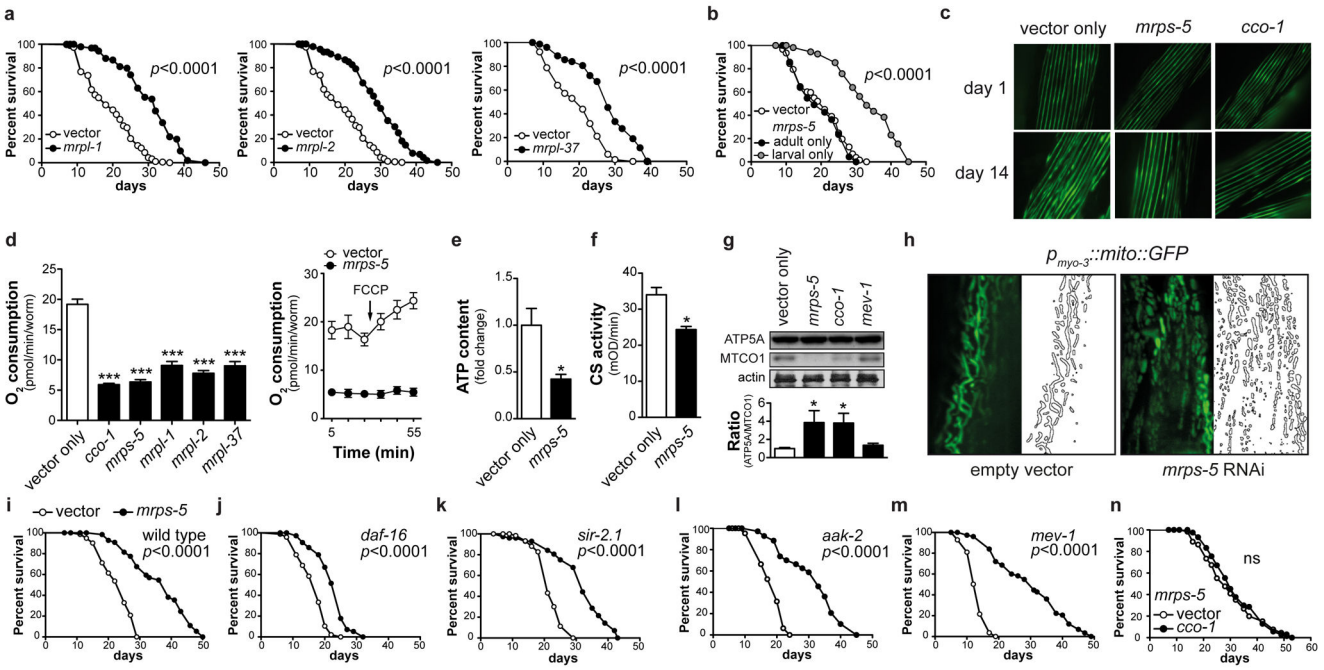

Figure 3. *mrps-5* RNAi prevents aging-associated functional decline and alters mitochondrial function

a, Knockdown of *mrpl-1*, *mrpl-2* or *mrpl-37* increased lifespan by 57%, 54%, or 41%, respectively. **b,** When RNAi of *mrps-5* was performed during the larval stages only, lifespan increased by 48%, while RNAi started from the L4 stage had no effect. $p \leq 0.001$ is for larval-only versus either vector control or adult-only. **c,** *mrps-5* or *cco-1* RNAi prevented age-related changes in muscle morphology as evidenced by a p_{myo-3}MYO-3::GFP reporter worm highlighting myosin heavy chain. **d,** *mrp* RNAi in *C. elegans* decreased respiration. Respiration/worm is shown here, but respiration was similarly decreased when corrected for protein. FCCP was added at the indicated time. Values are mean±SEM (n=10), *** $p \leq 0.001$. **e–g,** *mrps-5* RNAi decreased ATP levels (**e**, n=3), citrate synthase activity (**f**, n=3), and altered the ratio between nDNA (ATP5A) versus mtDNA-encoded (MTCO1) OXPHOS proteins, similar to *cco-1*, but not *mev-1* (**g**, n=4). * $p \leq 0.05$. **h,** *mrps-5* RNAi resulted in fragmented mitochondria, as visualized using the p_{myo-3}::mito::GFP reporter, which expresses mitochondria-targeted GFP driven by the muscle-specific *myo-3* promoter. **i,** *mrps-5* RNAi increased mean lifespan by 40%. **j-m,** *mrps-5* RNAi extends lifespan of *daf-16(mu86)* (**j**), *sir-2.1(ok434)* (**k**), *aak-2(ok524)* (**l**), *mev-1(kn1)* (**m**) mutants by 37%, 40%, 69%, 112%. **n,** Knockdown of *cco-1* does not extend lifespan of *mrps-5* RNAi worms. See Supplementary Table 2 and Fig. 4.

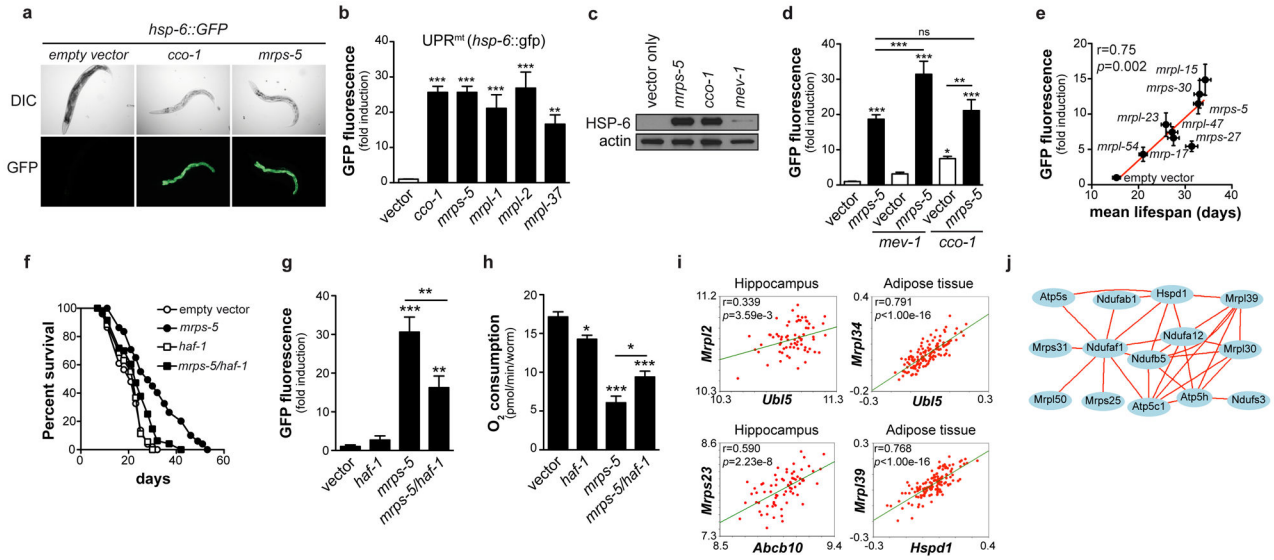

Figure 4. *mrp* genes confer longevity effects through UPR^{mt}

a, RNAi of *mrp* genes induced UPR^{mt} (*hsp-6*::GFP reporter), similar to *cco-1* knockdown. Worms are synchronized at day 1 of adulthood. **b,** Quantification of UPR^{mt} upon knockdown of *mrp* or *cco-1*. (n=4) **c,** *mrps-5* and *cco-1*, but not *mev-1*, RNAi, induce UPR^{mt} as reflected by the induction of HSP-6-GFP protein. **d,** Combined RNAi of *mrps-5* and *mev-1* synergistically increased UPR^{mt}, while combined *cco-1*/*mrps-5* RNAi did not further increase UPR^{mt} (n=6). **e,** Knockdown of different *mrp* genes results in different levels of UPR^{mt}, which correlates with mean lifespan (n=33–61 worms for lifespan, n=3 for GFP). **f–h,** Epistasis with UPR^{mt} regulator *haf-1*. Double RNAi of *mrps-5* and *haf-1* partially prevented lifespan extension (**f**), UPR^{mt} (**g**, n=5), and reduction in respiration (**h**, n=10), compared to *mrps-5* RNAi alone. **i,** In various tissues of mouse crosses, *Ubl5*, *Abcb10* and *Hspd1* expression correlated with *Mrp* expression. **j,** *Hspd1* (HSP60) ties in a correlation network with *Mrp* and OXPHOS genes. Connecting lines indicate a Pearson correlation coefficient of 0.75–1.0. Bar graphs are mean±SEM, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. See also Supplementary Fig. 5–7 and Table 3.

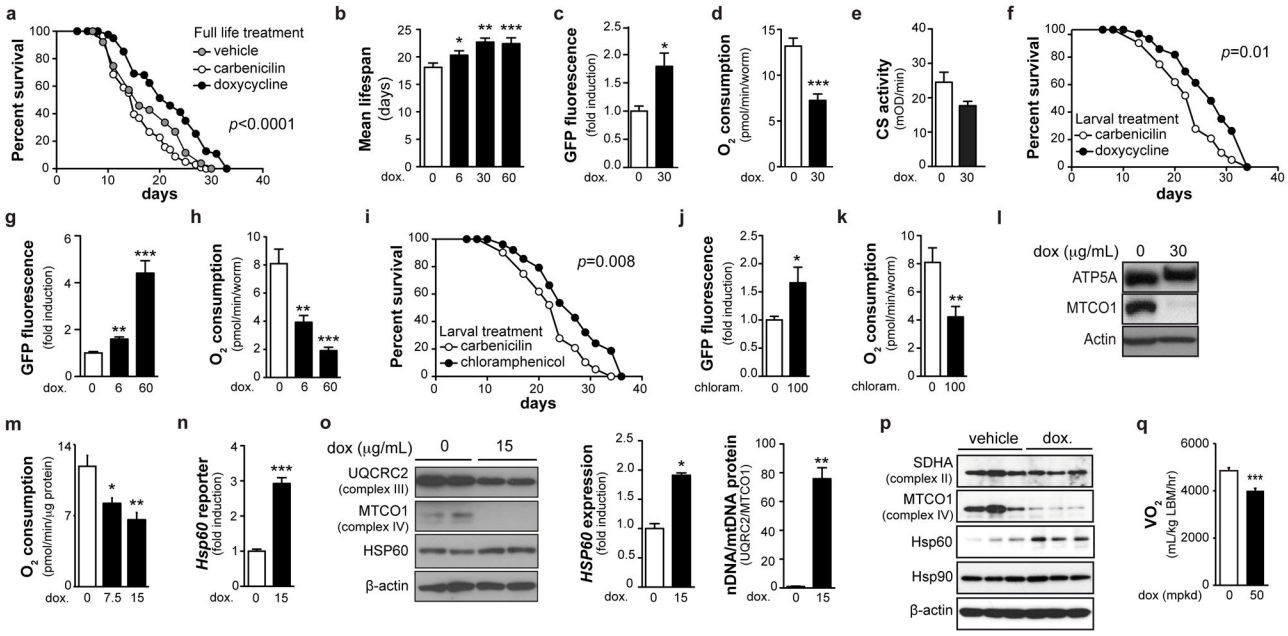

Figure 5. Specific antibiotics extend lifespan by phenocopying *mrps-5* knockdown

a, Effects on worm lifespan of doxycycline (30μg/mL), compared to carbenicilin (30μg/mL) or vehicle. $p \leq 0.001$ refers to statistical significance of doxycycline compared to either vehicle or the carbenicilin control. Antibiotics were given throughout life in panels **a–e. b,** The effects of doxycycline on lifespan are dose-dependent. **c–e**, Doxycycline induced UPR^{mt} (**c**, n=5) and reduced respiration (**d**, n=10), without changing citrate synthase activity (**e**, n=3). **f–k,** When treated only during larval development, doxycycline (6μg/mL; **f–h**) and chloramphenicol (100μg/mL; **i–k**) extend lifespan (**f, i**), induced UPR^{mt} (**g, j**, n=5) and reduced respiration (**h, k**, n=6). **l,** Doxycycline alters the ratio between nDNA- (ATP5A) and mtDNA-encoded (MTCO1) OXPHOS proteins in worms. **m,** Doxycycline decreased respiration in a cultured hepatocyte cell line (n=5), **n,** induced *Hsp60* transcription, as measured using an *Hsp60* promoter reporter (n=8), **o,** increased *HSP60* protein expression and altered the ratio of nDNA- (UQCRC2) versus mtDNA- (MTCOI) encoded proteins (n=2). **p,** Doxycycline increased *HSP60* protein and altered the ratio of nDNA- versus mtDNA-encoded proteins in primary murine hepatocytes. **q,** Doxycycline (50 mpkd) for 10 days in C57BL/6N mice decreased oxygen consumption (n=10). See also Supplementary Table 2.

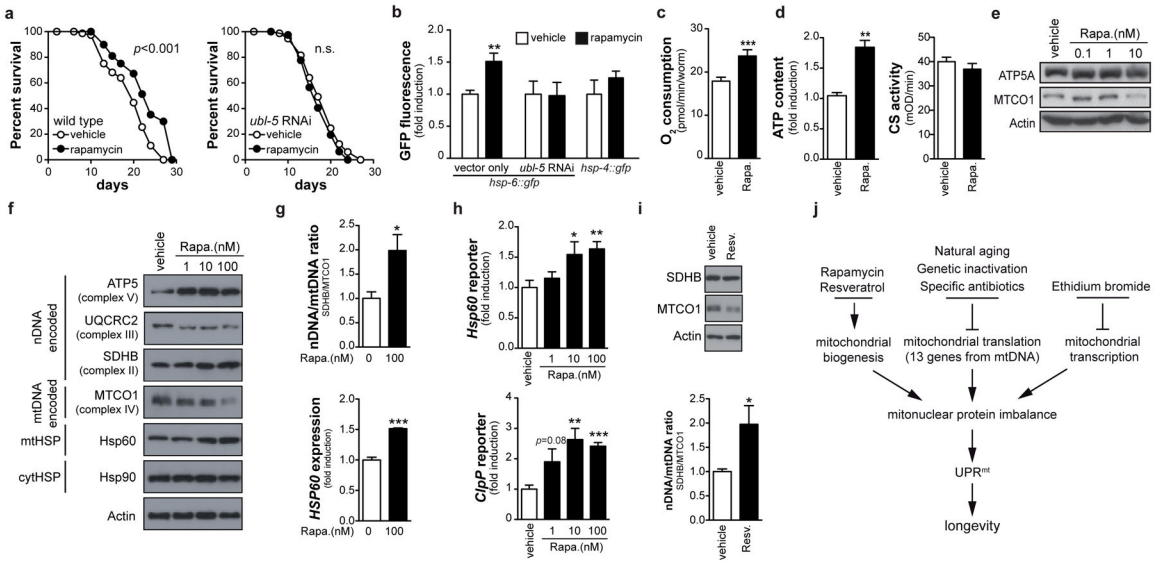

Figure 6. Conserved function of mitonuclear protein imbalance and UPR^{mt} in longevity

a, Rapamycin (1nM) extends worm lifespan in a *ubl-5*-dependent manner, and **b,** *ubl-5*-dependently induced UPR^{mt} (*hsp-6*::GFP) but not UPR^{ER} (*hsp-4*::GFP) (n=4). **c–e,** Rapamycin increased respiration (**c**, n=10) and ATP content but not citrate synthase activity (**d**, n=3) and induced mitonuclear protein imbalance (**e**). **f–h,** In mouse hepatocytes, rapamycin induces mitonuclear protein imbalance (**f–g**) and induces UPR^{mt} as shown at the protein (**f–g**, n=3), and transcriptional (**h**, n=8) level. **i,** Resveratrol (25 μM) induced mitonuclear protein imbalance in mouse hepatocytes (n=4). **j,** Hypothetical scheme of the mechanism by which reduced *Mrp* expression (during aging or genetic inactivation), specific antibiotics, ethidium bromide and rapamycin and resveratrol extend lifespan by inducing UPR^{mt}. Bar graphs are expressed as mean±SEM, * $p \leq 0.05$; *** $p \leq 0.001$. See also Supplementary Table 2.