LONP-1 and ATFS-1 sustain deleterious heteroplasmy by promoting mtDNA replication in dysfunctional mitochondria

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The accumulation of deleterious mitochondrial DNA (ΔmtDNA) causes inherited mitochondrial diseases and ageing-associated decline in mitochondrial functions such as oxidative phosphorylation (OXPHOS). Following mitochondrial perturbations, the bZIP protein ATFS-1 induces a transcriptional programme to restore mitochondrial function. Paradoxically, ATFS-1 is also required to maintain ΔmtDNAs in heteroplasmic worms. The mechanism by which ATFS-1 promotes ΔmtDNA accumulation relative to wild-type mtDNAs is unclear. Here we show that ATFS-1 accumulates in dysfunctional mitochondria. ATFS-1 is absent in healthy mitochondria owing to degradation by the mtDNA-bound protease LONP-1, which results in the nearly exclusive association between ATFS-1 and ΔmtDNAs in heteroplasmic worms. Moreover, we demonstrate that mitochondrial ATFS-1 promotes the binding of the mtDNA replicative polymerase (POLG) to ΔmtDNAs. Interestingly, inhibition of the mtDNA-bound protease LONP-1 increased ATFS-1 and POLG binding to wild-type mtDNAs. LONP-1 inhibition in Caenorhabditis elegans and human cybrid cells improved the heteroplasmy ratio and restored oxidative phosphorylation. Our findings suggest that ATFS-1 promotes mtDNA replication in dysfunctional mitochondria by promoting POLG-mtDNA binding, which is antagonized by LONP-1.

Mitochondria provide numerous metabolic functions including being the site of energy production via oxidative phosphorylation (OXPHOS). Most of the ~1,200 proteins that constitute the mitochondrial proteome are encoded by nuclear genes and are imported into each mitochondrion following synthesis on cytosolic ribosomes. However, 13 essential OXPHOS proteins that constitute the mitochondrial proteome are encoded by mitochondrial genomes (mtDNAs), which reside in the mitochondrial matrix. Each mitochondrion harbours at least one mtDNA, and most cells harbour hundreds to thousands of mtDNAs.

Mitochondrial function declines as cells age, which is accelerated in multiple diseases including Parkinson’s disease. A variety of mitochondrial diseases are caused by inherited mutations that impair OXPHOS function. The disease-causing mutations can occur in genes required for OXPHOS encoded by either the nuclear genome or mtDNAs, which acquire mutations at a significantly higher rate. Single nucleotide variants and deletions are associated with inherited mitochondrial diseases that affect approximately 1 in 4,000 individuals. Because of the high number of mtDNAs per cell, a single mutant mtDNA has little impact. To cause the OXPHOS dysfunction that underlies mitochondrial diseases, the mutant mtDNA must accumulate up to ~60% of the total cellular mtDNAs. The mixture of mutant mtDNAs and wild-type mtDNAs is known as heteroplasmy. Studies using mitochondrial-targeted nuclease that specifically cleave mutant mtDNAs suggest that a relatively modest reduction in the percentage of ΔmtDNAs is sufficient to improve mitochondrial function.

The initial mtDNA mutation or deletion probably occurs because of an error in mtDNA replication. Two mechanisms are thought to contribute to the ‘clonal expansion’ of the ΔmtDNA. In dividing cells, nonselective genetic drift can disproportionately increase either genome. An alternative model suggests that large mtDNA deletions enable quicker replication simply because these genomes are smaller. Consistent with both models, inhibition of the replicative mtDNA polymerase POLG causes the preferential depletion of mutant mtDNAs. However, the underlying mechanism that confers a replicative advantage and drives the clonal amplification of ΔmtDNAs to a high enough percentage to cause OXPHOS defects in mitochondrial diseases and ageing and Parkinson’s disease remain unresolved.

Previously, the bZIP protein ATFS-1 was found to be required to maintain deleterious heteroplasmy in C. elegans. ATFS-1 harbours both a mitochondrial targeting sequence (MTS) and a nuclear localization sequence (NLS) (Fig. 1a) and regulates a transcriptional programme known as the mitochondrial unfolded protein response (UPRmt)17. Under basal conditions, the majority of ATFS-1 is imported into mitochondria where it is degraded by the protease LONP-1. Mitochondrial dysfunction reduces mitochondrial import capacity, which results in a percentage of ATFS-1 trafficking to the nucleus where it activates a transcriptional programme to recover mitochondrial function. Importantly, both nuclear and mitochondrial accumulation of ATFS-1 are required for development during mitochondrial dysfunction. However, the function of ATFS-1 within mitochondria is unclear.

Here we report that the maintenance of deleterious heteroplasmy requires the accumulation of ATFS-1 within dysfunctional mitochondria owing to degradation by the mtDNA-bound protease LONP-1.
mitochondria. In a heteroplasmic *C. elegans* strain, ATFS-1 binds predominantly to ΔmtDNAs. Moreover, the replicative polymerase POLG also binds predominantly to ΔmtDNAs. Last, we demonstrate that the mitochondrial protease LONP-1, which degrades ATFS-1 in functional mitochondria, is required to establish the enriched interaction between ΔmtDNAs, ATFS-1 and POLG in heteroplasmic worms. Our findings in *C. elegans* are conserved in cultured human cells, as inhibition of LONP1 by short interfering

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**Fig. 1** | OXPHOS dysfunction increases mtDNA content through ATFS-1. **a.** Schematic of ATFS-1-UPR™ signalling in healthy cells. TIM, translocase of the inner membrane; TOM, translocase of the outer membrane. **b.** Comparison of wild-type (WT) and uaDIS deletion (ΔmtDNA) mtDNAs.

**c.** Photomicrographs of hsp-6_p::gfp and hsp-6_p::gfpuaDIS worms. Representative images from four biological repeats. **d.** Quantification of total mtDNA in homoplasmic WT, uaDIS and uaDIS worms raised on atfs-l(RNAi), n=3, biologically independent samples. *P* = 0.0034, **P** = 0.0017, ***P** = 0.0005.

**e.** Photomicrographs of hsp-6_p::gfp worms raised on control RNAi (Ctrl(RNAi)), cco-1(RNAi) or cyc-1(RNAi). Representative images from four biological repeats.

**f.** Photomicrographs of WT, isp-1(qm150) and clk-1(qm30);hsp-6_p::gfp worms. Representative images from four biological repeats. **g.** Quantification of total mtDNA in homoplasmic WT and atfs-l(RNAi) worms raised on Ctrl(RNAi), cco-1(RNAi) or cyc-1(RNAi). n=3, biologically independent samples. *P* = 0.0128, **P** = 0.0002, ***P** = 0.0005, ****P** = 0.0001.

**h.** Quantification of mtDNA in WT, isp-1(qm150), clk-1(qm30) and nduf-2.1(fc21) mutant worms raised on Ctrl(RNAi) or atfs-l(RNAi). n=3 (WT, isp-1(qm150), isp-1(qm150) atfs-l(RNAi)), biologically independent samples. *****P** = 0.0001 and P = 0.0005 (WT versus isp-1(qm150) and isp-1(qm150) versus isp-1(qm150) atfs-l(RNAi)), n=4 (WT, clk-1(qm30)), n=3 (clk-1(qm30) atfs-l(RNAi)), biologically independent samples. *P* = 0.0443, **P** = 0.0016 (WT, clk-1(qm30) and clk-1(qm30) atfs-l(RNAi)), n=3 (WT, nduf-2.1(fc21) and nduf-2.1(fc21) atfs-l(RNAi)), biologically independent samples. *P* = 0.0151 and P = 0.011 (WT versus nduf-2.1(fc21) and nduf-2.1(fc21) versus nduf-2.1(fc21) atfs-l(RNAi)). Each biologically independent sample contained 40–60 animals and every dot represents the averaged value from 3 technical replicates in **d, g** and **h**, and one-way ANOVA was used; data shown represent the mean ± s.e.m. *P* < 0.05, **P** < 0.01, ***P** < 0.001, ****P** < 0.0001. Scale bars, 0.1 mm (**c, f**).
RNA (siRNA) or the drug CDDO (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid, also known as bardoxolone) improves heteroplasmy and rescues OXPHOS function in heteroplasmic cells.

**Results**

**OXPHOS perturbations increase mtDNA quantity.** OXPHOS proteins are encoded by genes located within the mitochondria and the nucleus. The heteroplasmic *C. elegans* strain *uaDf5* harbours approximately 40% wild-type mtDNAs and 60% AmtDNAs that lack 4 essential OXPHOS protein-coding genes (Fig. 1b). *uaDf5* worms have impaired respiration, constitutive UPRmt activation, as determined by the increased expression of the *hsp-6*::gfp reporter (Fig. 1c), and increased total mtDNA content (Fig. 1d).

To examine the impact of OXPHOS perturbation on mtDNA content, we impaired several OXPHOS components in wild-type homoplasmic worms. As expected, worms raised on agar plates seeded with RNAi specific to *cco-1* (which encodes complex IV; *cco-1*(RNAi)) or *cyc-1* (which encodes cytochrome c; *cyc-1*(RNAi)) had increased *hsp-6*::gfp reporter activity, as did the mutant strains *isp-1(qm150)* (complex III) and *clk-1(qm30)* (ubiquinone biosynthesis) (Fig. 1e,f). Intriguingly, each of the OXPHOS perturbations resulted in increased mtDNA content as determined by quantitative PCR (qPCR) (Fig. 1g,h and Extended Data Fig. 1a), which is consistent with previous reports. Importantly, the increase in mtDNAs caused by OXPHOS perturbation was impaired in *atfs-1*(null) worms, which lack the entire *atfs-1* open reading frame (Fig. 1g), as well as in worms raised on *atfs-1*(RNAi) (Fig. 1h). Similarly, total mtDNA content in heteroplasmic worms was also reduced when raised on *atfs-1*(RNAi) (Fig. 1d). Combined, these findings indicate that the increased mtDNA content in both homoplasmic and heteroplasmic worms caused by OXPHOS perturbation requires *atfs-1*.

**POLG–mtDNA binding during OXPHOS dysfunction requires ATFS-1.** We next sought to gain insight into the mechanism by which mtDNA content is increased during OXPHOS perturbation. We previously found that perturbation of mitochondrial function by inhibiting the mitochondrial protease SPG-7 or by raising worms in the presence of ethionamide, which impairs mtDNA replication, leads to ATFS-1 accumulation within mitochondria. Moreover, chromatin immunoprecipitation followed by sequencing (ChIP–seq) analysis has indicated that ATFS-1 binds mtDNAs during mitochondrial dysfunction within the noncoding region (NCR), which in mammals contains sequence elements that regulate mtDNA replication. Here we found that ATFS-1 also accumulates in the mitochondrial fraction in worms raised on *cco-1*(RNAi) (Fig. 2a). Furthermore, ATFS-1::GFP also accumulates within mitochondria raised on *cco-1*(RNAi), whereas staining with tetramethylrhodamine, ethyl ester (TMRE) was decreased (Fig. 2b), which is indicative of impaired OXPHOS. Last, ATFS-1 ChIP followed by qPCR indicated that ATFS-1 interacts with mtDNA when worms are raised on *cco-1*(RNAi) (Fig. 2c). Combined, these data indicate that ATFS-1 accumulates within mitochondria and interacts with mtDNAs when OXPHOS is impaired.

To further explore the relationship between ATFS-1 accumulation and the mtDNA increase during OXPHOS perturbation, we generated POLG antibodies, which detected an ~120 kDa band that co-fractionated with the OXPHOS protein NDUF53 (Extended Data Fig. 1b) and was depleted by polg(RNAi) (Extended Data Fig. 1c). Similar to ATFS-1, POLG protein levels increased in worms when raised on *cco-1*(RNAi) (Fig. 2d), and POLG interacted with more mtDNAs (Fig. 2e). To determine whether the increased POLG–mtDNA interaction required ATFS-1, we performed POLG ChIP–mtDNA in wild-type and *atfs-1*(null) worms raised on *cco-1*(RNAi). Interestingly, the increased POLG–mtDNA interaction was impaired in *atfs-1*(null) worms, which suggests that ATFS-1 is required for increased POLG–mtDNA binding during OXPHOS dysfunction (Fig. 2f).

As *atfs-1* is required for the increase in polg mRNA during mitochondrial dysfunction, we sought to determine whether the increased POLG–mtDNA binding required nuclear-localized ATFS-1. We previously used genome editing to generate the *atfs-1*^11^ allele strain in which the NLS within ATFS-1 is impaired (Extended Data Fig. 2a,b). Importantly, the *atfs-1*^11^ allele impaired *hsp-6* mRNA accumulation when raised on *spg-7*(RNAi) (Extended Data Fig. 2c). Furthermore, the *atfs-1*^11^ mutation also impaired *hsp-6*::gfp induction in *atfs-1*(cl18) worms, which constitutively activates UPRmt due to a mutation that reduces its import into mitochondria (Extended Data Fig. 2d–f). Induction of *hsp-6* and polg mRNA was also impaired in *atfs-1*^11^ worms when raised on *cco-1*(RNAi) (Extended Data Fig. 2g,h). Unlike in wild-type worms, POLG protein was not increased in *atfs-1*^11^ worms, which indicates that *atfs-1*^11^ worms are unable to regulate nuclear transcription during OXPHOS perturbation (Extended Data Fig. 2i,j). Importantly, ATFS-1^11^ accumulated within mitochondria similar to wild-type ATFS-1 following LONP-1 induction (Extended Data Fig. 2j), which indicates that the protein was expressed and processed similar to wild-type ATFS-1. Last, ATFS-1^11^ bound similar amounts of mtDNA as wild-type ATFS-1 when raised on *cco-1*(RNAi) as determined by ChIP analysis (Fig. 2c,f).

To determine whether the nuclear function of ATFS-1 is required for POLG to bind mtDNAs during OXPHOS perturbation, we examined the POLG–mtDNA interaction in *atfs-1*(null) and *atfs-1*^11^ worms raised on control(RNAi) or *cco-1*(RNAi). Notably, significantly more POLG was bound to mtDNA in *atfs-1*^11^ worms relative to *atfs-1*(null) worms (Fig. 2g), which suggests that the *atfs-1*^11^-dependent increase in POLG–mtDNA binding during OXPHOS perturbation does not require the nuclear activity of ATFS-1. mtDNA content also increased in *atfs-1*^11^ worms.
following OXPHOS perturbation caused by cco-1(RNAi) (Fig. 2h) as well as in clk-1(qm30) worms (Fig. 2i). Combined, these results suggest that the accumulation of ATFS-1 within mitochondria during OXPHOS perturbation is required to increase POLG–mtDNA binding and mtDNA content.

**ATFS-1 degradation by LONP-1 impairs mtDNA propagation.**
We next sought to determine the events that lead to the accumulation of ATFS-1 within mitochondria following OXPHOS perturbation. The ATP-dependent protease LONP-1 degrades the majority of ATFS-1 once the bZIP protein is imported into the mitochondrial matrix19. LONP-1 is an ATP-dependent protease that recognizes and degrades mitochondrial proteins damaged by reactive oxygen species27. LONP-1 can also interact with mtDNA in diverse species28,29 and regulates mtDNA replication30,31.

To further examine the interaction between LONP-1 and mtDNA in *C. elegans*, we used genome editing to generate a strain in which the carboxy terminus of LONP-1 is tagged with the FLAG epitope (Fig. 3a and Extended Data Fig. 3a). Introduction of the FLAG epitope did not impair worm development or cause UPRmt activation,
Fig. 3 | LONP-1 limits ATFS-1 binding to WT mtDNA and impairs replication. a, FLAG immunoblots of LONP-1\textsuperscript{FLAG} worms following fractionation into total lysate, post-mitochondrial supernatant and mitochondrial pellet. Tubulin and the OXPHOS component NDUFS3 were the loading controls. Representative immunoblots from four biological repeats.
b, Quantification of mtDNA from homoplasmic LONP-1\textsuperscript{FLAG} worms following ChIP–mtDNA using FLAG or control (Mock) antibody. \(n = 3\), biologically independent samples. \(* P < 0.05\).
c, LONP-1 consensus binding motif within mtDNA.
d, ChIP–seq profile of mtDNA from homoplasmic LONP-1\textsuperscript{FLAG} worms raised on Ctrl(rNAi) using FLAG antibody (red).
e, Quantification of total mtDNA following ATFS-1 ChIP–mtDNA in WT and atfs-1(null) homoplasmic worms raised on Ctrl(rNAi) or lonp-1(rNAi). \(n = 3\), biologically independent samples. \(* P = 0.0132\).
f, Quantification of mtDNA in homoplasmic WT and atfs-1(null) worms raised on Ctrl(rNAi) or lonp-1(rNAi). \(n = 4\), biologically independent samples. \(* P < 0.05\).
g-h, Representative images. Scale bar, 5 \(\mu m\).
h, Percentage of mitochondria with colocalized TMRE and ATFS-1::GFP signals. \(n = 10\) Ctrl(rNAi) and \(n = 11\) lonp-1(rNAi), biologically independent samples. \(* * * * P < 0.0001\).
i, Quantification of total mtDNA following POLG ChIP–mtDNA in WT homoplasmic worms raised on Ctrl(rNAi) or lonp-1(rNAi). \(n = 3\), biologically independent samples. \(* P = 0.0012\).
j, Schematic of the relationship between LONP-1 activity, mitochondrial ATFS-1 accumulation and mtDNA replication. Each biologically independent sample contained about 150,000 (b,e,i) or 40–60 animals (f), and every dot represents the averaged value from 3 technical replicates (b,e,f and i). Two-tailed Student’s \(t\)-test was used; data shown represent the mean ± s.e.m.
which suggests that it did not adversely affect LONP-1 function (Extended Data Fig. 3b,c). As expected, LONP-1 interacted with mtDNA in *C. elegans* as determined by LONP-1::FLAG ChIP–mtDNA qPCR (Fig. 3b). We next examined the site at which mtDNA binds LONP-1::FLAG in wild-type homoplasmic worms. LONP-1::FLAG ChIP–seq indicated that the protease was enriched at several G-rich sites throughout mtDNA (Fig. 3c), but was especially enriched within the NCR (Fig. 3d). Interestingly, the strongest LONP-1::FLAG peak within the NCR overlapped with the ATFS-1-binding site (Fig. 3d and Extended Data Fig. 3d), which suggests that it has a potential role in mediating ATFS-1–mtDNA and POLG–mtDNA interactions.

We next examined the impact of *lonp-1*(RNAi) on mtDNA accumulation in homoplasmic wild-type worms. When raised on *lonp-1*(RNAi), the binding of ATFS-1 to mtDNA increased by about fivefold (Fig. 3e), which correlated with an increase in mtDNA content (Fig. 3f). ATFS-1::GFP also accumulated within mitochondria raised on *lonp-1*(RNAi) (Fig. 3g,h). Importantly, exposure to *lonp-1*(RNAi) also increased the binding of POLG to mtDNA (Fig. 3i), consistent with increased POLG accumulation (Extended Data Fig. 3e). Moreover, LONP-1 inhibition increased mtDNA content in *atfs-1(mts)(−/−)* worms (Extended Data Fig. 3f), but not in *atfs-1(null)* worms (Fig. 3f).

To examine the role of mitochondrial-localized ATFS-1 in increasing mtDNA quantity, we sought to generate a strain that lacked the MTS. Unfortunately, we were unable to establish a stable *atfs-1(1−/−)* line via genome editing. Thus, we generated *atfs-1(mts)(−/−)* worms. Importantly, mtDNA copy number was not increased in *atfs-1(mts)(−/−)* worms raised on *lonp-1*(RNAi) (Extended Data Fig. 3g). These findings support a role for mitochondrial-localized ATFS-1 in promoting mtDNA replication, which is impaired by LONP-1-dependent degradation (Fig. 3j).

**Nuclear ATFS-1 is not required to maintain heteroplasmacy.** We previously found that ATFS-1 is required to maintain ΔmtDNA levels in *C. elegans* in a heteroplasmic worm strain using *atfs-1*(RNAi) and a hypomorphic *atfs-1* allele. Here we crossed the *atfs-1(null)* allele into *uaDf5* heteroplasmic worms. Notably, *atfs-1(null)* worms were unable to maintain any ΔmtDNAs (Fig. 4a). The loss of ΔmtDNAs in the absence of *atfs-1* may be due to increased mitophagy of mitochondria harbouring ΔmtDNAs, decreased replication of ΔmtDNAs or a combination of both effects. To examine the role of mitophagy, we generated heteroplasmic strains that lack the mitophagy component Parkin (PDR-1 in worms) (Extended Data Fig. 4a). As expected, *pdr-1* defective worms had increased ΔmtDNA levels relative to wild-type worms, a result that is consistent with mitophagy limiting the accumulation of ΔmtDNAs. *atfs-1(null);pdr-1(1tm598) worms also had significantly less ΔmtDNA content than *pdr-1(1tm598) worms, which indicates that ATFS-1 promotes heteroplasmacy via a mechanism independent of mitophagy (Fig. 4a).

As OXPHOS perturbation increased mtDNA content in homoplasmic wild-type worms via ATFS-1, we hypothesized that OXPHOS dysfunction may contribute to the increased mtDNA content in heteroplasmic worms (Fig. 4d). We previously found that heteroplasmic worms consumed less oxygen than wild-type worms. To further evaluate mitochondrial function, we examined the mitochondrial membrane potential by staining with TMRE. As expected, TMRE staining was decreased in heteroplasmic worms relative to wild-type worms, but stronger than in worms raised on *cco-1*(RNAi) or *spg-7*(RNAi), which is consistent with a pattern of intermediate OXPHOS function (Figs. 2b and 4b and Extended Data Fig. 4b). Importantly, ATFS-1 also accumulated within the mitochondrial fraction of heteroplasmic worms similar to worms raised on *spg-7*(RNAi) or *cco-1*(RNAi) (Fig. 4c and compare with Fig. 2a). Similar to homoplasmic worms raised on *cco-1*(RNAi), heteroplasmic worms accumulated ATFS-1::GFP within mitochondria with reduced TMRE staining. However, numerous mitochondria were TMRE positive, but the majority of those mitochondria lacked ATFS-1::GFP, which is consistent with ATFS-1 being degraded in functional mitochondria (Fig. 4d). Combined, these results suggest that ATFS-1 accumulates within dysfunctional mitochondria caused by impairment of either a nuclear-encoded OXPHOS component or a nuclear-encoded mitochondrial protease or in worms harbouring ΔmtDNAs.

We next examined whether maintenance of ΔmtDNAs requires the nuclear activity of ATFS-1. Consistent with impaired nuclear activity, *hsp-6::gfp* was not increased in heteroplasmic *atfs-1(mts)(−/−)* worms (Extended Data Fig. 4c,d). Notably, unlike *atfs-1(null)* worms, *atfs-1(mts)(−/−)* worms were able to maintain ΔmtDNAs, although with fewer ΔmtDNAs than wild-type *atfs-1* worms (Extended Data Fig. 4e). To examine the role of mitochondrial-localized ATFS-1 during heteroplasmacy, we crossed the *atfs-1(mts)(−/−)* allele into heteroplasmic worms. *atfs-1(mts)(−/−)* worms harboured fewer ΔmtDNAs than *atfs-1(mts)(−/−)* worms, which emphasizes the requirement for mitochondrial-localized ATFS-1 to maintain heteroplasmacy (Extended Data Fig. 4f).

Consistent with the accumulation of ATFS-1 in dysfunctional mitochondria (Figs. 2a and 4b–d), ATFS-1 bound more total mtDNA in heteroplasmic worms than in wild-type heteroplasmic worms as determined by ChIP–mtDNA (Fig. 4e). Similarly, POLG also interacted with more total mtDNA in heteroplasmic worms than in wild-type heteroplasmic worms, which suggests that there is increased mtDNA replication (Extended Data Fig. 4g). Combined, these findings suggest a role for mitochondrial-localized ATFS-1 in maintaining deleterious mtDNA heteroplasmacy.

**Fig. 4** | ATFS-1 and POLG primarily interact with ΔmtDNAs in heteroplasmic worms. a, ΔmtDNA quantification as determined by qPCR in *uaDf5*, *atfs-1(null)*;*uaDf5*, *pdr-1(1tm598)*;*uaDf5*, *atfs-1(null);pdr-1(1tm598)* and *atfs-1(mts)(−/−)*;*uaDf5*, *atfs-1(mts)(−/−)* worms. *n* = 4 (*uaDf5* and *atfs-1(null)*;*uaDf5*;*pdr-1(1tm598)*;*uaDf5*) and *n* = 3 (*atfs-1(null)*;*uaDf5* and *pdr-1(1tm598)*;*uaDf5*) biologically independent samples. **p* < 0.01, ***p* < 0.0001, one-way ANOVA. b, Images of TMRE-stained micrographs of heteroplasmic (ΔmtDNA) worms raised on Ctrl (RNAi), or WT worms raised on Ctrl (RNAi) or spg-7 (RNAi) and heteroplasmic (ΔmtDNA) worms raised on Ctrl (RNAi) after fractionation into total lysate, post-mitochondrial supernatant and mitochondrial pellet. Tubulin and the OXPHOS component NDUFS3 were used as the loading controls. The arrowhead indicates mitochondrial-localized ATFS-1. Representative immunoblots from three biological repeats. *atfs-1*, *atfs-1::gfp* and *atfs-1::gfp*;*uaDf5* transgenic animals with TMRE staining. Representative images from six biological repeats. Scale bar, 5 μm. c, Quantification of total mtDNA following ATFS-1 ChIP–mtDNA in homoplasmic WT and ΔmtDNA worms. *n* = 3 WT and *n* = 5 *uaDf5* biologically independent samples. *p* = 0.0226, two-tailed Student’s *t*-test. f, Workflow of ATFS-1 or POLG ChIP–mtDNA and quantification of WT mtDNA and ΔmtDNA in heteroplasmic worms. h, immunoprecipitation. g, Quantification of WT mtDNA and ΔmtDNA by qPCR following ATFS-1 ChIP–mtDNA in heteroplasmic worms. h, Quantification of WT mtDNA and ΔmtDNA following TFAM IP–mtDNA in heteroplasmic worms. The post-lysis/input ΔmtDNA ratio was 60%. *n* = 4, biologically independent samples. h, Quantification of WT mtDNA and ΔmtDNA by qPCR following POLG ChIP–mtDNA in heteroplasmic worms. The post-lysis/input ΔmtDNA ratio was 54%. *n* = 3, biologically independent samples. i, Quantification of WT mtDNA and ΔmtDNA following TFAM IP–mtDNA in heteroplasmic worms. The post-lysis/input ΔmtDNA ratio was 53%. *n* = 4, biologically independent samples. Rep., replicate. Each biologically independent sample contained 40–60 (a) or about 150,000 worms (e.g., i), and every dot represents the averaged value from 3 technical replicates (a.e and g.i); data shown represent the mean ± s.e.m.
ATFS-1 and POLG primarily bind ∆mtDNAs. We next sought to determine whether ATFS-1 differentially interacted with each genome. The interaction between ATFS-1 and wild-type mtDNAs or ∆mtDNAs was evaluated via qPCR or three-dimensional (3D) digital PCR following ATFS-1 ChIP (Fig. 4f). As before, qPCR of mtDNA from heteroplasmic whole worm lysate indicated that the strain harboured ~60% ∆mtDNAs and ~40% wild-type mtDNAs (Fig. 1b and Extended Data Fig. 5a). qPCR following ATFS-1 ChIP showed that of the mtDNAs that interacted with ATFS-1, 90% were ∆mtDNAs and 10% were wild-type mtDNAs, which indicates that ATFS-1 is significantly enriched on ∆mtDNAs (Fig. 4g and Extended Data Fig. 5b,c).
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Previously, we found that inhibition of POLG caused depletion of ∆mtDNAs in heteroplasmic worms relative to wild-type mtDNAs, similar to findings in a Drosophila heteroplasmy model. Both findings suggest that there is increased replication of ∆mtDNAs in heteroplasmic cells. To further explore the relationship between ATFS-1 and mtDNA replication, we performed ChIP–mtDNA using the POLG antibody. POLG ChIP indicated that the replicative polymerase also interacted with ~90% ∆mtDNAs and 10% wild-type

**Fig. 5 | LONP-1 is required to maintain heteroplasmy.** a, ∆mtDNA and WT mtDNA quantification by qPCR following LONP-1 ChIP–mtDNA in heteroplasmic worms. The post-lysis/input ∆mtDNA ratio was 59%. n = 3, biologically independent samples. b, Quantification of WT mtDNA by qPCR in heteroplasmic worms raised on Ctrl(RNAi) or lonp-1(RNAi). n = 3, biologically independent samples. **P = 0.0041. c, Quantification of ∆mtDNA by qPCR in heteroplasmic uaDf5 worms raised on Ctrl(RNAi) or lonp-1(RNAi). n = 3, biologically independent samples. ****P < 0.0001. d, ∆mtDNA and WT mtDNA quantification by qPCR following ATFS-1 ChIP–mtDNA in heteroplasmic worms raised on Ctrl(RNAi) or lonp-1(RNAi). n = 4, biologically independent samples. e–f, atfs-1pr::atfs-1::gfp;uaDf5 transgenic animals were raised on Ctrl(RNAi) or lonp-1(RNAi) with TMRE staining. e, Representative images. Scale bar, 5 µm. f, Percentage of mitochondria with colocalized TMRE and ATFS-1::GFP signals. n = 8 uaDf5 Ctrl(RNAi) and n = 9 uaDf5 lonp-1(RNAi), biologically independent samples. g, ∆mtDNA and WT mtDNA quantification by qPCR following POLG ChIP–mtDNA in heteroplasmic worms raised on lonp-1(RNAi). The post-lysis/input ∆mtDNA ratio was 25%. n = 4, biologically independent samples. Each biologically independent sample contained about 150,000 (a,d,g) or 40–60 animals (b,c), and every dot represents the averaged value from 3 technical replicates (a–d and g); two-tailed Student’s t-test was used in b, c and f; data shown represent the mean ± s.e.m.
mtDNAs (Fig. 4h and Extended Data Fig. 5d,e), similar to ATFS-1 (Fig. 4g). As a control, we generated antibodies against the mtDNA packaging protein HMG-5 (TFAM in mammals) (Extended Data Fig. 5f,g), which interacts with mtDNAs independent of replication\(^1\). In contrast to ATFS-1 and POLG, the percentage of \(\Delta\)mtDNAs bound to HMG-5 reflected the percentage within the whole worm lysate (Fig. 4i). Combined, these data indicate that ATFS-1 and a component of the replisome are enriched on \(\Delta\)mtDNAs in heteroplasmic worms, consistent with the mutant mtDNA having a replicative advantage.

The protease LONP-1 is required to maintain heteroplasmy. Because LONP-1 binds mtDNA and its proteolytic activity limits ATFS-1 accumulation in functional mitochondria\(^3\) (Fig. 3d,e), we examined the role of LONP-1 in heteroplasmy maintenance. We generated antibodies against \(C.\) elegans LONP-1 that recognized an \(~130\)kDa band that was reduced when worms were raised on \(lonp-1\) (RNAi) (Extended Data Fig. 6a). Using ChIP-qPCR, we found that LONP-1 binds \(~60\) fold more mtDNAs than ATFS-1 in wild-type worms (Extended Data Fig. 6b). In heteroplasmic worms, LONP-1 bound similar percentages of wild-type and \(\Delta\)mtDNAs, which suggests that LONP-1 interacts with mtDNAs independent of mitochondrial dysfunction (Fig. 5a and Extended Data Fig. 6c). Combined, these data suggest that LONP-1 is constitutively bound to mtDNAs and that heteroplasmy is not maintained by uneven mtDNA binding by the protease.

We next examined the effect of inhibiting LONP-1 on heteroplasmy. Interestingly, \(lonp-1\) inhibition via RNAi increased wild-type mtDNA (Fig. 5b) and reduced \(\Delta\)mtDNA content, which improved the heteroplasmy ratio from 59% \(\Delta\)mtDNAs to 25% (Fig. 5c). Similar results were obtained in \(atfs-1\) mutant worms after LONP-1 inhibition (Extended Data Fig. 6d). Importantly, \(lonp-1\) (RNAi) did not reduce the brood size of heteroplasmic worms (Extended Data Fig. 6e), which suggests that \(lonp-1\) (RNAi) does not select against embryos with dysfunctional mitochondria due to high levels of \(\Delta\)mtDNAs.

We next examined the interaction between ATFS-1 and mtDNAs in heteroplasmic worms after \(lonp-1\) inhibition. Interestingly, when raised on \(lonp-1\) (RNAi), the percentage of \(\Delta\)mtDNAs and wild-type mtDNAs bound to ATFS-1 nearly reflected the heteroplasmy percentage within the whole worm lysate (Fig. 5d). Consistent with the increased binding of ATFS-1 to wild-type mtDNAs, \(lonp-1\) (RNAi) increased ATFS-1::GFP accumulation within functional mitochondria (Fig. 5e,f). Thus, the mtDNA-bound protease is required to establish the enriched interaction between ATFS-1 and \(\Delta\)mtDNAs.

In addition to increasing the percentage of wild-type mtDNAs bound by ATFS-1, \(lonp-1\) (RNAi) also increased the percentage of POLG that interacted with wild-type mtDNAs (Fig. 5g and compare with Fig. 4h). However, \(lonp-1\) (RNAi) did not alter the percentage of HMG-5 bound to \(\Delta\)mtDNAs, which is consistent with the idea that HMG-5 interacts with all mtDNAs independent of replication (Extended Data Fig. 6f). Consistent with ATFS-1-dependent mtDNA replication occurring in dysfunctional mitochondria, when heteroplasmic worms were raised on \(cco-1\) (RNAi) or crossed into the \(clk-1\) mutant strain, wild-type mtDNAs were also specifically increased (Extended Data Fig. 6g,h).

Combined, these findings suggest that LONP-1-mediated proteolysis antagonizes the ability of mitochondrial ATFS-1 and POLG to stimulate mtDNA replication. LONP-1 activity may be compromised within mitochondrial compartments that harbour \(\Delta\)mtDNAs, which leads to ATFS-1 and POLG accumulation and mtDNA replication. We propose that globally inhibiting LONP-1 promotes ATFS-1-mediated mtDNA replication throughout the cell, not just in compartments enriched in \(\Delta\)mtDNAs, which consequently leads to a recovery of wild-type mtDNAs.

LONP1 inhibition improves heteroplasmy in cybrid cells. Last, we used two human heteroplasmic cybrid cell lines\(^4\) to examine whether the role of LONP1 in maintaining \(\Delta\)mtDNAs is conserved in mammals. While cybrid cells are often used as models of deleterious mtDNA heteroplasy, it is important to note that cybrid cells are cancer cells in which patient-derived heteroplasmic mtDNAs were introduced by cell fusion\(^4\). One of the cybrid lines we used harbours a single nucleotide transition (COXI G6930A) that introduces a premature stop codon in the cytochrome c oxidase subunit I gene, and was isolated from a patient with a multisystem mitochondrial disorder\(^5\) (Fig. 6a). We also examined a cybrid line that harbours a 4,977-base-pair deletion, known as the 'common deletion', which removes multiple OXPHOS genes and is associated with Kearns–Sayre syndrome (KSS), progressive external ophthalmoplegia, cancer and ageing\(^6\) (Fig. 6a). We first examined the impact of LONP1 siRNA on heteroplasmy in the KSS cybrid line. Similar to inhibition of \(C.\) elegans LONP-1, inhibition of human LONP1 by siRNA for 4 days (Fig. 6b) resulted in a 1.5-fold increase in wild-type mtDNAs (Fig. 6c), whereas KSS mtDNAs were decreased \(~2\) fold (Fig. 6d). This resulted in a shift in the heteroplasmy ratio from 57.5% to 25.6% (Fig. 6d).

To further investigate the impact of LONP1 protease activity on heteroplasmy, we used the LONP1 inhibitor CDDO\(^2\) (Fig. 6e). As determined by deep sequencing, the COXI G6930A cybrid line initially harboured \(~90\)% G6930A mutant mtDNAs and \(~10\)% wild-type mtDNAs (Fig. 6f). Incubation with 0.1 \(\mu\)M or 0.25 \(\mu\)M CDDO for 3 weeks resulted in depletion of the COXI G6930A mtDNA from 86% to 68% and 72%, respectively, with a concomitant increase in wild-type mtDNAs (Fig. 6f). Furthermore, continuous incubation with 0.1 \(\mu\)M or 0.25 \(\mu\)M CDDO for 18.5 weeks further decreased the heteroplasmatic ratios from \(~90\)% to \(~47\)% and 62%, respectively (Fig. 6f) for siRNA. Similar results were obtained when the KSS cybrid cells were incubated with CDDO. As determined by qPCR, KSS cells initially harboured \(~50\)% \(\Delta\)mtDNAs. Incubation with 0.1 \(\mu\)M or 0.25 \(\mu\)M CDDO for 4 weeks depleted \(\Delta\)mtDNAs to 19.5% and 20.3%, respectively (Fig. 6g), and incubation for 13 weeks further depleted \(\Delta\)mtDNAs to 18.6% and 10.7%, respectively (Fig. 6g). Importantly, neither 0.1 \(\mu\)M nor 0.25 \(\mu\)M CDDO affected basal respiration in homoplasmic 143b cells (Extended Data Fig. 7b). Furthermore, neither of the concentrations of CDDO used impaired the viability of COXI G6930A or KSS cells (Fig. 6b and Extended Data Fig. 7c), which suggests that the improved heteroplasmy ratio following CDDO exposure was not due to selection against cells with high levels of mutant mtDNAs. However, it remains possible that cell death may occur during longer exposures.

Last, we determined the impact of the CDDO-dependent shifts in heteroplasmy on OXPHOS. Notably, incubation of KSS cells with 0.1 \(\mu\)M or 0.25 \(\mu\)M CDDO for 4 or 13 weeks resulted in significant increases in basal respiration, which suggests that there was improved OXPHOS (Extended Data Fig. 7d). The improved heteroplasmy caused by CDDO in COXI G6930A cells (Fig. 7a) also resulted in increased basal respiration and maximal respiratory capacity (Fig. 7b–d). For example, 3 weeks of exposure to 0.1 \(\mu\)M CDDO increased basal oxygen consumption by \(~2\)-fold, while exposure for 18.5 weeks improved basal oxygen consumption by more than 3-fold (Fig. 7b,c). Taken together, these findings suggest that inhibition of LONP1 improves deleterious heteroplasmy and recovers mitochondrial respiration. Notably, these phenotypes are independent of mtDNA length, as maintenance of mutant mtDNAs with either a large deletion or a single base-pair substitution require LONP1 function.

Discussion
The underlying mechanisms that govern heteroplasmy dynamics are largely unknown; however, it has been proposed that the mutant
Fig. 6 | LONP1 inhibition improves heteroplasmy and OXPHOS function in cybrid cells. a, Schematic comparing human WT, KSS deletion (ΔmtDNA) and CoxI G6930A mtDNAs. b, LONP1 immunoblots from KSS heteroplasmic cells treated with hLONP1 or control (NC) siRNA. Tubulin served as the loading control. Representative immunoblots from three biological repeats. c, WT mtDNA quantification in KSS cells treated with control or hLONP1 siRNA. n = 4, biologically independent samples. **P = 0.0021. d, Quantification of KSS ΔmtDNA in cells treated with control or hLONP1 siRNA. n = 4, biologically independent samples. *P = 0.0185. e, Chemical structure of CDDO. f, Quantification of G6930A mtDNA percentage following treatment with dimethylsulfoxide (DMSO; as control), 0.1 μM CDDO or 0.25 μM CDDO at the indicated time points up to 130 days. n = 3, biologically independent samples. *P < 0.05. g, ΔmtDNA quantification in KSS heteroplasmic cells treated with DMSO, 0.1 μM CDDO or 0.25 μM for 4 or 13 weeks. h, Cell viability of 143b(WT) and CoxI G6930A cells exposed to the indicated concentration of CDDO for 72 h. n = 3 in 143b(WT) cell or CoxI G6930A cell, biologically independent samples. Every dot represents the averaged value from three technical replicates in c and d; two-tailed Student’s t-test was used in c, d and f; data shown in c, d and h represent the mean ± s.e.m.
Collectively, these results suggest that mitochondrial accumulation of ATFS-1 promotes the recruitment of the mitochondrial replisome to mtDNA during OXPHOS dysfunction. ATFS-1 is also required for the increased quantity of mtDNAs in heteroplasmic worms. The *uadfs* strain harbours approximately 60% ΔmtDNAs and 40% wild-type mtDNAs. However, ATFS-1 and POLG interact with ninefold more ΔmtDNAs than wild-type mtDNAs, which suggests that the increase in total mtDNA is due to increased ΔmtDNA replication. As both mtDNAs harbour the ATFS-1 binding site, how is the specificity attained? We found that heteroplasmic worms harbour distinct populations of functional and dysfunctional mitochondria. Moreover, ATFS-1 primarily
accumulates within the dysfunctional population. LONP-1 inhibition results in ATFS-1 accumulation in all mitochondria, which suggests that the replicative advantage is due to LONP-1-dependent degradation of ATFS-1 in functional mitochondria, which probably harbour wild-type mtDNAs. Somatic cells in *C. elegans* harbour between one and three mtDNAs. Thus, we speculate that the dysfunctional mitochondria in which ATFS-1 accumulates harbour all, or nearly all, AmtDNAs that impair OXPHOS and LONP-1, resulting in ATFS-1 accumulation.

Because LONP-1 is an ATP-dependent protease that degrades proteins damaged by reactive oxygen species, mitochondrial dysfunction may impede degradation of ATFS-1 by LONP-1, which results in the recruitment of POLG to mtDNA. We propose that this mechanism evolved to coordinate mtDNA replication with expansion of the mitochondrial network during normal cell growth or recovery from mitochondrial dysfunction. However, if compartmental dysfunction is caused by an enrichment of AmtDNAs, they are inadvertently, but preferentially, replicated. Our data suggest that inhibiting LONP1 throughout the mitochondrial network negates this preferential replication, which leads to a reduction in the heteroplasmic ratio and recovery of mitochondrial function.

There are currently no regulatory-agency approved treatments for diseases caused by mutant mtDNAs. However, the mTOR inhibitor rapamycin improves heteroplasy in cybrid cells by increasing autophagy. Of note, inhibition of several TORC1 components also inhibits atfs-1-dependent mitochondrial biogenesis in *C. elegans*, which suggests that TORC1 may function upstream of ATFS-1 in maintaining heteroplasy. Here we report that inhibition of LONP1 through siRNA-mediated knockdown or the small-molecule inhibitor CDDO reduces AtmtDNA abundance in cybrid cells that harbour patient-derived mutant mtDNAs. Moreover, this decrease in AmtDNAs was accompanied by improved mitochondrial respiration, which suggests that LONP1 inhibition may represent a therapeutic strategy for diseases caused by mutant mtDNAs.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-021-00840-5.

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Methods

Worm strains. The reporter strain hsp-6::gfp for visualizing UPR activation has been previously described\(^1\). N2 (wild-type) and AtmDna (or wild5) strains were obtained from the Caenorhabditis Genetics Center. The atf6(+188) strain was a gift from M. Pilon\(^1\). The atf6(+188), or atf6(+188) strain was generated via CRISPR-Cas9 in wild-type worms as previously described\(^1\). The CRNAs (Integrated DNA Technologies) were co-injected with purified Cas9 protein, tracrRNA (Integrated DNA Technologies) and the dpy-10 co-injection marker as previously described\(^1\). atf6(+188) was introduced into both wild-type worms and the hsp-6::gfp reporter strain using the CRISPR-Cas9 technique\(^1\) (crRNA and the replacement sequence are listed in Supplementary Table 1). lomp-1(+Lm) was introduced into both wild-type worms and the hsp-6::gfp reporter strain via CRISPR-Cas9. atf6(+188) was introduced into atf6(+188) worms to generate the atfs-1(+Lm)+Lm strain. Each strain was outcrossed at least five times. Unless otherwise noted, all worms were collected between the L3 and early L4 stages. All stages were maintained at 20 °C.

C. elegans mtDNA and human hybrid cell K5 mtDNA quantification. L4 wild-type or wild5 worms were placed on agar plates seeded with control (RNAi) or RNAi specific to the described OXPHOS genes, and the F1 generation was collected at the L4 stage. Wild-type mtDNA or AtmDna quantification was performed using qPCR-based methods as previously described\(^1\). Approximately 40–60 worms were collected in 35 μl of lysate buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 0.5% NP-40, 0.5% Tween 20, 0.01% gelatin) with freshly added 200 μg ml⁻¹ proteinase K and frozen at −80 °C for 20 min before lysis at 4 °C for 30 min. Absolute quantification was used for determining the fold changes in mtDNA between samples. A volume of 1 μl of lysate was used in each triplicate qPCR assay. qPCR was performed using iQ SYBR Green Supermix and a Bio-Rad qPCR CFX96 (Bio-Rad). Primers that specifically amplify wild-type or AtmDna are listed in Supplementary Table 1, as are primers that amplify both wild-type and AtmDna (total mtDNA). Primers that amplify a NCR near the nuclear-encoded g-f-g are used as an internal control for normalization (Supplementary Table 1).

For human patient fibroblast cell lines, wild-type and AKSS primers were used to detect wild-type mtDNA or AKSS mtDNA. Primers that amplify a sequence between the B2M (human β2 myoglobin) gene were used as an internal control for normalization. Absolute quantification was also performed to determine the percentage or ratio of KSS AtmDna relative to total mtDNA (KSS AtmDna and wild-type mtDNA) as previously described\(^1\). Primers that specifically amplify wild-type or AtmDna are listed in Supplementary Table 1. Standard curves for each qPCR primer set were generated using purified plasmids that individually contain approximately 1 kb of the mtDNA fragments specific for each primer set.

ChIP assays. ChIP assays for ATFS-1 and LONP-1FLAG were performed as previously described\(^1\). Synchronized worms were cultured in liquid and collected at early L4 stage by sucrose flotation. The worms were lysed via a Teflon homogenizer in cold PBS with protease inhibitors (Roche). Crosslinking of DNA and protein was performed by treating the worms with 1.85% formaldehyde with proteinase K at 55 °C for 2 h. Last, the immunoprecipitated and input DNA were purified with ChIP DNA Clean & Concentrator (Zymo Research, D5205) and used as templates for qPCR or next-generation sequencing.

ChIP–mtDNA and mtDNA quantification. mtDNA immunoprecipitation assays were performed similar to the previously described ATFS-1 ChIP assay\(^1\). Synchronized worms were cultured in liquid and collected at the L4 stage by sucrose flotation. The worms were lysed via a Teflon homogenizer in cold PBS with protease inhibitors (Roche). Crosslinking of DNA and protein was performed by treating the worms with 1.85% formaldehyde with protease inhibitors for 15 min. Glycine was added to a final concentration of 125 mM and incubated for 5 min at room temperature to quench the formaldehyde. The DNA fragments were sequenced using MiSeq at the Broad Institute. MiSeq data analysis for editing at target sites or off-target sites was performed using a suite of Unix-based-software tools. First, the quality of the paired-end sequencing reads (R1 and R2 fastq files) was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw paired-end reads were combined using paired end read merger (PEAR)\(^1\) to generate single merged high-quality full-length reads. The reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads. Reads were then filtered by quality (using Filter FASTQC\(^1\)) to remove those with a mean PHRED quality score under 30 and a high-quality full-length reads.

QuantStudio 3D digital PCR. A detailed method has been previously described\(^1\). All primers and probes were ordered from Integrated DNA Technologies. The 3D digital PCR was used according to the manufacturer’s protocol. QuantStudio 3D digital PCR master mix v2 and individual QuantStudio 3D digital PCR 20K Chip kit v2 were purchased from Thermo Scientific (Integrated Biosystems). Prepared sample mix was loaded into the ChIP system using QuantStudio 3D digital PCR chip loader (Thermo Scientific). Chip PCR amplification was performed in a ProFlex PCR system (96°C for 10 min; 39 cycles of 60°C for 20 sec and 98°C for 20 sec; and 60°C for 2 min). After amplification, each chip was loaded into a QuantStudio 3D Digital Reader. Data were analysed using QuantStudio 3D Analysis Suite (Thermo Scientific). All biological repeats were performed at least in triplicate.

AtmDNA primers: E: TGTTGTCTTTTTTATATGTTT; R: TTTATTTATTTTGTTAAACAGAGGT. AtmDNA probes: 5′-6FAM/ZEN′3′ IBFQ/-56FAM/AGATGCTA/ZEN/ACATTTTATTTTTTTGCTTTA/3IABkFQ/.
RNA isolation and qRT–PCR. Total RNA was isolated from worm pellets using TRIzol reagent (Invitrogen). cDNA was then synthesized from total RNA using a iScript cDNA synthesis kit (Bio-Rad). qPCR was performed to determine the expression levels of the indicated genes using SYBR GREEN supermix (Bio-Rad). Primer sequences are listed in Supplementary Table 1. The relative expression of target genes was normalized to the control. Fold changes in gene expression were calculated using the comparative CtΔΔct method as previously described17.

Chemicals and antibodies. CDDO was purchased from Cayman Chemicals (81035). ATSFS-1 polyclonal antibodies were generated and validated as previously described17. Polyclonal antibodies were generated against amino acids 1054–1072 of C. elegans POLG and subsequently affinity purified by Thermo Fisher Scientific. Polyclonal antibodies were generated against amino acids 191–204 of C. elegans HMG-3 (TFAM) and subsequently affinity purified by Thermo Fisher Scientific. Polyclonal antibodies were generated against amino acids 953–971 of C. elegans LONP-1 and subsequently affinity purified by Thermo Fisher Scientific. Monoclonal anti-FLAG M2 antibody (Sigma, F1804), α-tubulin (Sigma) and NDUF53 (NUO-2 in C. elegans, complex I, Abcam) were also used. A complete list is provided in Supplementary Table 2.

Cell culture. The K56 cell line was a gift from C. Moraes36,37. The CoxI C6930A cell line was a gift from G. Manfredi38. Cells were cultured in DMEM (4 mM L-glutamine, 4.5 g per litre glucose; Gibco, Thermo Fisher Scientific) plus 10% FBS with 1% penicillin-streptomycin. Total cellular mtDNA was prepared as previously described39. Cells were incubated continuously in the described concentration of CDDO for the indicated number of days. The cells were subcultured before confluence every 48 h.

Cell viability. At the indicated time points, cells were stained with trypan blue40 and quantified with an automated cell counter TC-20 (Bio-Rad). The results are an average of three independent assays.

siRNA. Cells were grown in 6-well plates and siRNAs were transfected with Dharmacon (L-003979-00-0005). Lipofectamine RNAiMAX (Thermo Fisher Scientific, 13778150) following the manufacturer’s instructions. Human LONP1 siRNA was purchased from Dharmacon (L-003979-00-0005).

Respiration assays. For mitochondrial respiration assays, the OCR was measured using a Seahorse Extracellular Flux Analyzer XFc496 (Seahorse Biosciences) as described17. A total of 14,000 Cosx C6930A cells were seeded per well with fresh medium. The OCR was measured using a Cell MitoStress Kit (as described by the manufacturer). A volume of 180 μl of XF-Media was added to each well and then the plates were subjected to analysis following sequential introduction of 1.5 μM oligomycin, 1.0 μM FCCP and 0.5 μM rotenone/antimycin as indicated. Data are normalized to total protein as determined by the BCA protein assay.

Western blots and mitochondrial fractionation. Mitochondrial fractionation and western blots were performed as previously described41. Synchronized L4 worms were transferred to plates seeded with control(RNAi) bacteria for 3 h to remove TMRE-containing bacteria from the digestive tract. Images were acquired using identical exposure times with a Zeiss LSM800 microscope with Airyscan. TMRE staining analysis was performed as previously described42,43. In brief, the average pixel intensity values were calculated by sampling images of different worms. The average pixel intensity for each animal was calculated using ImageJ (http://rsb.info.nih.gov/ij/). The fluorescence pixel intensity was quantified by using the threshold-adjusted images from worms at each condition in biological triplicates. Mean values were compared using Student’s t-test or one-way analysis of variance (ANOVA) followed by the post-hoc Tukey’s test where appropriate.

ATFS-1-GFP transgenic worms were stained with TMRE as described above. Colocalization of TMRE stain and ATFS-1-GFP was determined by object-based colocalization analysis as previously described44. The amount of colocalization was calculated for each sample. Statistical analysis was performed using the Prism software package (GraphPad Software).

Statistics and reproducibility. All data are reported as the mean ± s.e.m. Significance was accepted at P < 0.05. Asterisks denote corresponding statistical significance as follows: *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. No statistical method was used to predetermine the sample size. Results were analysed using Student’s t-test with a two-tailed distribution or one-way ANOVA (for multiple comparison) where appropriate using GraphPad Prism software with corrected Pvalues of <0.05 considered significant. For multiple comparisons, Pvalues were adjusted using the Tukey’s post hoc test. The statistical analyses for OCR and ChIP-mtDNA assays were all performed using two-sided Student’s t-test. The statistical testing and definition of n numbers in this study are indicated in the figure legends. For Figs. 1d,g,h, 2c–e, 3b,e,f,i, 4a,e,g–i, 5a–d,f,g, 6c,d,g,h and 7a–d, and Extended Data Figs. 1a, 1c–f, 3g, 4e–g, 5c,e, 6b–h and 7b–d, n means biologically independent samples. For C. elegans western blotting and gene expression, each sample within each biological replicate corresponds to a sample pooled from 3,000 to 5,000 animals. For the immunoblotting data, each blot (Figs. 2a,d, 3a,c and 6b) was independently repeated at least three times. For cell culture western blotting, each sample within each biological replicate corresponds to one well from a tissue culture plate. For Fig. 7b–d and Extended Data Fig. 7d, n means biological replicates, and each sample within each biological replicate corresponds to a sample pooled from 14,000 cells in Fig. 7b–d, 18,000 cells in Extended Data Fig. 7d (4 weeks) and 10,000 cells (13 weeks). The deep sequencing data used to quantify heteroplasmy of the CoxI C6930A mtDNA following exposure to CDDO was double-blinded. The researchers involved in the experiments were not completely blinded during sample obtainment or data analyses.

Data availability

The ChIP-seq data have been deposited to the Gene Expression Omnibus database under the BioProject accession code PRJNA590136. The next-generation sequencing data for mtDNA have been deposited in the NCBI Sequence Read Archive database under the BioProject accession code PRJNA780293. All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Author contributions
Q.Y. and C.M.H. planned the experiments. Q.Y., Y.D., T.S., N.U.N., J.L., R.D.Z. and P.C. generated the worm strains. R.L. and L.J.Z. analysed ATFS-1::GFP and TMRE quantification. Q.Y. performed the C. elegans and cybrid mtDNA analysis including ChIP and respiratory function. P.L., K.L. and S.A.W. performed and analysed mtDNA sequencing. Q.Y., N.S.A. and C.M.H. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | OXPHOS dysfunction increases mtDNAs. **a**, Quantification of total mtDNA in wildtype and nduf-7(et19) worms. n = 3, biologically independent samples (Each sample contains 40-60 animals; every dot stands for averaged value from 3 technical replicates; data shown represent mean ± S.E.M.). **P = 0.0015, Two-tailed Student’s t test.** b, POLG immunoblot of wildtype worms following fractionation into total lysate (T), post-mitochondrial supernatant (S), and mitochondrial pellet (M). Tubulin (Tub) and the OXPHOS protein (NDUFS3) serve as loading controls. Representative immunoblots from four biological repeats. c, POLG immunoblot of lysates from wildtype worms raised on control(RNAi) or polg(RNAi). Tubulin (Tub) serves as a loading control. Representative immunoblots from seven biological repeats.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | atfs-1-dependent transcription is impaired in atfs-1\(^{nuc(-)}\) worms. a, Schematic highlighting the R (Arginine) to A (Alanine) substitution to impair the nuclear localization sequence (NLS) within ATFS-1 yielding ATFS-1\(^{nuc(-)}\) confirmed by Sanger sequencing. b, UPR\(^\text{TM}\) signaling schematic highlighting the ATFS-1\(^{nuc(-)}\) with an impaired NLS. c, Expression level of hsp-6 mRNA in wildtype and atfs-1\(^{nuc(-)}\) worms raised on control(RNAi) or spg-7(RNAi) examined by qRT-PCR. \(n = 3\), biologically independent samples. **\(P = 0.0028\), One-way ANOVA. d–e, Photomicrographs of wildtype, atfs-1(\textit{et}18) and atfs-1(\textit{et}18)\(^{nuc(-)}\);\textit{hsp-6::gfp} worms (Scale bar 0.1 mm) (d); Quantification of fluorescence pixel intensity in wildtype (\(n = 117\); Max: 12.51; Min: 1.87; Median: 5.12), atfs-1(\textit{et}18) (\(n = 74\); Max: 75.251; Min: 15.790; Median: 32.021) and atfs-1(\textit{et}18)\(^{nuc(-)}\) strains (\(n = 12\); Max: 15.100; Min: 1.55; Median: 5.43). Box & whiskers plots Min to Max. ****\(P < 0.0001\), One-way ANOVA. n means the number of sampling areas. Average pixel intensity signals were calculated from sampling areas at each condition in biological triplicates (e). f, hsp-6 mRNA expression in wildtype, atfs-1(\textit{et}18) or atfs-1(\textit{et}18)\(^{nuc(-)}\) worms examined by qRT-PCR. \(n = 4\) (wildtype), \(n = 5\) (atfs-1(\textit{et}18)), \(n = 3\) (atfs-1(\textit{et}18)\(^{nuc(-)}\)), biologically independent samples. *\(P = 0.0114\) (wildtype vs. atfs-1(\textit{et}18)), *\(P = 0.0407\) (atfs-1(\textit{et}18) vs. atfs-1(\textit{et}18)\(^{nuc(-)}\)), one-way ANOVA. g, hsp-6 mRNA expression in wildtype and atfs-1\(^{nuc(-)}\) worms raised on control(RNAi) or cco-1(RNAi) examined by qRT-PCR. \(n = 3\), biologically independent samples. ****\(P = 0.0004\), one-way ANOVA. h, polg mRNA expression in atfs-1\(^{nuc(-)}\) worms raised on control(RNAi) or cco-1(RNAi) examined by qRT-PCR. \(n = 3\), biologically independent samples. Two-tailed Student’s t test. i, POLG immunoblots of lysates from wildtype, atfs-1\(^{nuc(-)}\) and atfs-1(\textit{null}) worms raised on control or cco-1(RNAi). Representative immunoblots from four biological repeats. j, Immunoblots of lysates from wildtype and atfs-1\(^{nuc(-)}\) worms raised on control or lonp-1(RNAi). ATFS-1 or ATFS-1\(^{nuc(-)}\) are indicated with an arrowhead. Representative immunoblots from four biological repeats. In c, f–h, each dot represents the average from 3 technical replicates; data shown represent mean ± S.E.M.
Extended Data Fig. 3 | LONP-1 inhibition promotes mtDNA content via ATFS-1. a, FLAG immunoblots of lysates from wildtype and LONP-1\textsuperscript{FLAG} wildtype worms. Tubulin (Tub) serves as a loading control. Representative immunoblots from four biological repeats. b, Images of wildtype or LONP-1\textsuperscript{FLAG} worms 48 hours after synchronization indicating worms expressing LONP-1\textsuperscript{FLAG} at the endogenous locus develop normally (Scale bar 1 mm). Representative images from four biological repeats. c, Fluorescent photomicrographs of wildtype hsp-6::gfp or lonp-1\textsuperscript{FLAG};hsp-6::gfp worms 48 hours after synchronization indicating worms expressing LONP-1\textsuperscript{FLAG} do not cause UPR\textsuperscript{me} activation (Scale bar 0.05 mm). Representative images from four biological repeats. d, Schematic of the putative ATFS-1 and LONP-1 binding sites within the mtDNA non-coding region (NCR) highlighting the proximity of both sites (~200 base pairs). e, POLG Immunoblots of lysates from wildtype worms raised on control or lonp-1(RNAi). Representative images from four biological repeats. f, Total mtDNA quantification in wildtype homoplasmic atfs-1\textsuperscript{nuc(−)} worms raised on control(RNAi) or lonp-1(RNAi), \( n = 5 \), biologically independent samples. ***\( P = 0.0004 \), Two-tailed Student’s t test. g, Total mtDNA quantification in wildtype homoplasmic atfs-1\textsuperscript{nuc(−)};nuc(−) worms raised on control(RNAi) or lonp-1(RNAi), \( n = 3 \), biologically independent samples. Two-tailed Student’s t test. In f and g, each biologically independent sample contained 40-60 animals; every dot stands for averaged value from 3 technical replicates; data shown represent mean ± S.E.M. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.0001 \).
Extended Data Fig. 4 | Mitochondrial ATFS-1 is required to maintain ∆mtDNA in heteroplasmic worms. a, Crossing strategy of atfs-1(null);pdr-1(tm598);uaDf5 strain. b, TMRE quantification of heteroplasmic (∆mtDNA) worms raised on control (RNAi) (n = 475; Max: 1.052; Min: 0.21; Median: 0.618), or wildtype worms raised on control (n = 232; Max: 1.318; Min: 0.725; Median: 0.995) or spg-7 (RNAi) (n = 114; Max: 0.798; Min: 0.134; Median: 0.402). Box & whiskers plots Min to Max. n means the number of sampling areas. Average pixel intensity signals were calculated from sampling areas at each condition in biological triplicates. c,d, Photomicrographs of uaDf5 and atfs-1nuc−;uaDf5;hsp-6pr::gfp worms (Scale bar 0.1 mm) (c); Quantification of fluorescence pixel intensity in uaDf5 (n = 199; Max: 30.89; Min: 3.430; Median: 11.590) and atfs-1nuc−;uaDf5;hsp-6pr::gfp (n = 234; Max: 15.640; Min: 2.540; Median: 6.915). Box & whiskers plots Min to Max. n means the number of sampling areas. Average pixel intensity signals were calculated from sampling areas at each condition in biological triplicates (d). e, ∆mtDNA quantification as determined by qPCR in heteroplasmic uaDf5 worms, atfs-1(null);uaDf5 worms and atfs-1nuc−;uaDf5 worms. n = 3, biologically independent samples. f, ∆mtDNA quantification as determined by qPCR in heteroplasmic atfs-1nuc−;uaDf5 worms and atfs-1nuc−;uaDf5. n = 3 (atfs-1nuc−;uaDf5) and n = 4 (atfs-1nuc−;uaDf5), biologically independent samples. ***P = 0.0007. g, Quantification of total mtDNA following POLG ChIP-mtDNA in homoplasmic wildtype or uaDf5 worms. n = 4 (wildtype) and n = 3 (uaDf5), biologically independent samples. *P = 0.0229. In e and f, each biologically independent sample contained 40-60 animals; in g, each biologically independent sample contained about 150,000 animals; each dot stands for averaged value from 3 technical replicates in f,g; Two-tailed Student’s t test was used in d, f and g. One-way ANOVA was used in b; data shown represent mean ± S.E.M. *p < 0.05, **p < 0.01, ****p < 0.0001.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | ATFS-1 and POLG primarily interact with ΔmtDNAs in heteroplasmic worms. a, Overview of the qPCR strategy to quantify the ΔmtDNA percentage in heteroplasmic worms or heteroplasmic cells. Plasmids containing a sequence specific to the ΔmtDNA or wildtype mtDNA were created. Standard curves were generated using the indicated concentration of each plasmid harboring sequences specific to either wildtype or ΔmtDNAs. Both PCR reactions were carried out simultaneously in the same qPCR machine. b–c, Scatter plots (b) and results (c) of 3D digital PCR quantification of wildtype mtDNA and ΔmtDNA following ATFS-1 ChIP-mtDNA in heteroplasmic uaDF5 worms. n = 4, biologically independent samples. d–e, Scatter plots (d) and results (e) of 3D digital PCR quantification of wildtype mtDNA and ΔmtDNA following POLG ChIP-mtDNA in heteroplasmic uaDF5 worms. n = 4, biologically independent samples. f, HMG-5/TFAM immunoblot of wildtype worms following fractionation into total lysate (T), post-mitochondrial supernatant (S), and mitochondrial pellet (M). Tubulin (Tub) and the OXPHOS component (NDUFS3) serve as loading controls. Representative immunoblots from two biological repeats. g, HMG-5/TFAM immunoblots of lysates from wildtype worms raised on control or hmg-5/tfam(RNAi). Tubulin (Tub) serves as a loading control. Representative immunoblots from three biological repeats. Each biologically independent sample contained 150,000 animals in c,e; data shown represent mean ± S.E.M.
Extended Data Fig. 6 | Inhibition of LONP-1 improves the deleterious heteroplasmy ratio. **a**, LONP-1 immunoblots of lysates from wildtype worms raised on control(rNAi) or lonp-1(rNAi). Tubulin (Tub) serves as a loading control. Representative immunoblots from four biological repeats. 

**b**, ChIP-mtDNA using ATFS-1 or LONP-1 antibodies in wildtype worms followed by quantification of total mtDNA. n = 3, biologically independent samples. 

**c**, ChIP-mtDNA using LONP-1 antibodies in wildtype or heteroplasmic worms followed by quantification of total mtDNA. n = 3, biologically independent samples. 

**d**, ∆mtDNA quantification in atfs-1(nuc−);uaDf5 worms raised on control(rNAi) or lonp-1(rNAi). n = 3, biologically independent samples. 

**e**, The brood size of heteroplasmic worms raised on control or lonp-1(rNAi). n = 9 worms. 

**f**, ∆mtDNA and wildtype mtDNA quantification following HMG-5/TFAM ChIP-mtDNA in uaDf5 heteroplasmic worms raised on lonp-1(rNAi) indicating that the binding of HMG-5/TFAM to wildtype mtDNAs or ∆mtDNAs is similar the input ratio. n = 4, biologically independent samples. 

**g**, wildtype mtDNA quantification in uaDf5 heteroplasmic worms raised on control(rNAi) or cco-1(rNAi). n = 3, biologically independent samples. 

**h**, wildtype mtDNA quantification in uaDf5 or clk-1(qm30);uaDf5 heteroplasmic worms. n = 3, biologically independent samples. Two-tailed Student’s t test was used; data shown represent mean ± S.E.M.
Extended Data Fig. 7 | Pharmacological inhibition of LONP1 improves heteroplasmy and OXPHOS function in heteroplasmic cybrid cells. a, Mutant (G6930A) mtDNA ratio confirmation by sanger sequencing in CoxI G6930A cells treated by CDDO. b, Oxygen consumption rates (OCR) of 143B (wildtype) cells treated with DMSO (ctrl), 0.1 μM or 0.25 μM CDDO for 3 days. n = 22 (ctrl) and n = 24 (0.1 μM and 0.25 μM CDDO), biologically independent samples. c, Cell viability of 143b (WT) and KSS ΔmtDNA cells exposed to various concentrations of CDDO for 72 hours. n = 3, biologically independent samples. d, Basal respiration of KSS heteroplasmic cells treated with DMSO (ctrl), 0.1 μM or 0.25 μM CDDO for 4 or 13 weeks. n = 14 (ctrl) and n = 16 (0.1 μM and 0.25 μM CDDO), biologically independent samples. ****P < 0.0001, Two-tailed Student’s t test. Data shown represent mean ± S.E.M.
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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

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☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Images were collected with Zeiss Zen 2.3 blue edition. Western blot images were acquired with Bio-Rad molecular imager ChemiDoc XRS+.

Data analysis
Images were analyzed with the Zen2.3 blue edition from Zeiss. Western blots and images were analyzed with ImageJ 1.52a. PRISM 9 was used. Deep sequencing data analysis is described in method section.

BWA (version 0.7.5) and SAMtools (version0.1.19).

Fastqc {0.11.5} (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

ChIP-seq data was mapped to the C. elegans genome (ce10 from UC Santa Cruz) by Burrows-Wheeler Aligner (BWA MEM, BWA version 0.7.15).

Picard tools v1.96 (https://broadinstitute.github.io/picard/).

The bigwig files were generated with the signal as fold enrichment by macs2 following the procedure at https://github.com/taoliu/MACS/wiki/Build-Signal-Track.

MEME (http://meme.sdsu.edu).

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy.

All discussed data are present in the paper or the Extended Data Materials. The ChIP-sequencing data has been deposited to the Gene Expression Omnibus database under the BioProject accession code PRJNA590136 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA590136). The next-generation mtDNA sequencing data has been deposited in the NCBI Sequence Read Archive database under the BioProject accession code PRJNA780293 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA780293).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical method was used in predetermining sample sizes. Sample size was used based on previous literature and recognized in the field. For each experiment, the n values were described in the figure legends and Statistics and Reproducibility. For C. elegans western blotting and mRNA quantification, each sample within each biological replicate corresponds to a sample pooled from 3000-5000 animals. For the immunoblotting data, each blot (Figs. 2a, 2d, 3a, 4c, 6b and Extended Data Figs. 1b-c, 2i-j, 3e, 5g) was repeated at least three times and the blot (Extended Data Figs. 2f) was repeated twice with similar results. In Figs. 1c, 1e, 1f, 2c, 3g, 4b, 4d; 5e, and Extended Data Figs. 2d, 3b, 3c, 4c, the experiments were independently repeated at least three times. For cell culture western blotting, each sample within each biological replicate corresponds to one well from a tissue culture plate. For Figs. 7b-d and Extended Data Figs. 7d, “n” means biological replicates and each sample within each biological replicate corresponds to a sample pooled from 14000 cells in Fig. 7b-d, 18000 cells in Extended Data Fig. 7d (4 weeks) and 10000 cells (13 weeks). |
| Data exclusions | No data was excluded from the analysis. |
| Replication | All experimental replications were indicated in the figure legends and Statistics and Reproducibility section. |
| Randomization | Randomization was not used and relevant in this study. For all experiments, worms or cells were clearly grouped for comparisons according to genotypes and treatments. In the experiments involving a time course treatment, the samples were collected at the indicated time points. |
| Blinding | The C. elegans experiments were not performed blindly. The deep sequencing to quantify heteroplasmy of the Cox1 G6930A mtDNA following exposure to CDDO was double-blinded. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Human research participants
- Clinical data
- Dual use research of concern

Methods

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging
Antibodies

**Antibodies used**
- Anti-HMG-5 (TFAM), rabbit, dilution 1:1000
- Anti-POLG, rabbit, rabbit, dilution 1:1000
- Anti-LONP-1, rabbit, dilution 1:1000
- Anti-ATFS-1, rabbit, dilution 1:1000

ANTI-FLAG® M2, mouse, monoclonal, dilution 1:2000, [https://www.sigmaaldrich.com/us/en/substance/monoclonalantiflagm2antibodyproducedinmouse1234598765](https://www.sigmaaldrich.com/us/en/substance/monoclonalantiflagm2antibodyproducedinmouse1234598765)

Anti-α-Tubulin, mouse, monoclonal, dilution 1:500, [https://www.sigmaaldrich.com/us/en/product/sigma/t9026](https://www.sigmaaldrich.com/us/en/product/sigma/t9026)

Normal Rabbit IgG, Rabbit, dilution 1:1000, [https://www.cellsignal.com/products/primary-antibodies/normal-rabbit-igg/2729](https://www.cellsignal.com/products/primary-antibodies/normal-rabbit-igg/2729)

Mouse mAb IgG1, Rabbit, dilution 1:2000, [https://www.cellsignal.com/products/primary-antibodies/mouse-g3a1-mab-igg1-isotype-control/s415](https://www.cellsignal.com/products/primary-antibodies/mouse-g3a1-mab-igg1-isotype-control/s415)

LONP1, rabbit, dilution 1:1000, [https://www.cellsignal.com/products/primary-antibodies/prss15-lonp1-d8w1-rabbit-mab/280207?site-search-type=Products&N=4294956287&n=-lonp1++rabbit+mab&fromPage=pip](https://www.cellsignal.com/products/primary-antibodies/prss15-lonp1-d8w1-rabbit-mab/280207?site-search-type=Products&N=4294956287&n=-lonp1++rabbit+mab&fromPage=pip)

NDUFS3, mouse monoclonal [17D95], dilution 1:1000, [https://www.abcam.com/ndufs3-antibody-17d95-ab14711.html](https://www.abcam.com/ndufs3-antibody-17d95-ab14711.html)

**Validation**

Validation statement from the company: Recognizes the ~60 kDa tubulin protein found in all eukaryotic cells. Validation statement from the company for Monoclonal ANTI-FLAG® M2: Validation for the ATFS-1 antibody was described previously (PMID: 22700657) as was the conducted RNAi assay. Validation for the POLG, LONP1 and HMG-5 antibodies were conducted via RNAi-specific knockdown as well as mitochondrial fractionation to confirm localization in C. elegans. Validation for the LONP1 antibody was conducted via RNAi-specific knockdown in human cell lines.

Eukaryotic cell lines

**Policy information about cell lines**

**Cell line source(s)**
The KSS and control/parental 143B cell lines were gifts from Carlos Moraes (PMID: 254133332, PMID: 2541710). The Coxi G6950A cell line was a gift from Giovanni Manfred (PMID: 10441567)

**Authentication**
Each cell line used was authenticated via PCR assay to detect the mutant or deletion mtDNA.

**Mycoplasma contamination**
All cell lines were confirmed negative for mycoplasma contamination.

**Commonly misidentified lines (See ICLAC register)**
The study did not use any commonly misidentified cell lines.

Animals and other organisms

**Policy information about studies involving animals, ARRIVE guidelines** recommended for reporting animal research

**Laboratory animals**
Caenorhabditis elegans used in this study:
- N2 Bristol strain.
- uadfs5
- hsp-6pr::gfp
- hsp-6pr::gfp::uadfs5
- LONP-1::FLAG
- LONP-1::FLAG;hsp-6pr::gfp
- isp-1(qm150)
- clk-1(qm30)
- nduf-2.1(fc21)
- atfs-1(null)
- atfs-1::nuc-1
- nduf-2.1::gfp
- pdr-1(tm598);uaadfs5
- atfs-1(null);uadfs5
- nduf-7(et19)
- atfs-1::nuc-1;hsp-6pr::gfp
- atfs-1::nuc-1
- atfs-1::nuc-1;uaadfs5
- atfs-1::nuc-1
- atfs-1::nuc-1;uaadfs5
null