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Reduced Cytosolic Protein Synthesis Suppresses Mitochondrial Degeneration

Xiaowen Wang[1,2], **Xiaoming Zuo**[2], **Blanka Kucejova**[2], and **Xin Jie Chen**[1,2,3]

[1]Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, New York 13210, USA

[2]Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390-9148, USA

Abstract

Mitochondrial function degenerates during aging and in aging-related neuromuscular degenerative diseases, which leads to the physiological decline of the cell[1]. Factors that can delay the degenerative process are actively sought after. Here, we show that reduced cytosolic protein synthesis is a robust cellular strategy that suppresses aging-related mitochondrial degeneration. We modelled the adult- or later-onset degenerative disease, autosomal dominant Progressive External Ophthalmoplegia (adPEO), by introducing the A128P mutation into the yeast adenine nucleotide translocase, Aac2p. The *aac2*^{A128P} allele dominantly induces aging-dependent mitochondrial degeneration and phenotypically tractable degenerative cell death independent of its ADP/ATP exchange activity. Mitochondrial degeneration is suppressed by lifespan-extending nutritional interventions and by 8 longevity mutations, which are all known to reduce cytosolic protein synthesis. These longevity interventions also independently suppress aging-related mitochondrial degeneration in the pro-aging prohibitin mutants. The *aac2*^{A128P} mutant has reduced mitochondrial membrane potential ($\Delta\psi_m$) and is synthetically lethal to low $\Delta\psi_m$ conditions, including the loss of prohibitin. Mitochondrial degeneration is accelerated by defects in protein turnover on the inner membrane and is suppressed by cycloheximide, a specific inhibitor of cytosolic ribosomes. Reduced cytosolic protein synthesis suppresses membrane depolarization and defects in mitochondrial gene expression in *aac2*^{A128P} cells. Our finding thus provides a link between protein homeostasis (proteostasis), cellular bioenergetics and mitochondrial maintenance during aging.

Autosomal dominant Progressive External Ophthalmoplegia (adPEO) is a neuromuscular degenerative disease clinically manifested by ptosis, progressive muscle weakness, sensory ataxia, peripheral neuropathy and parkinsonism[2]. Phenotypically, it is characterised by multiple deletions in mitochondrial DNA (mtDNA), while the activities of respiratory chain enzymes remain either normal or mildly affected[3]. One of the inherited forms of adPEO is caused by specific missense mutations in *ANT1*, encoding the isoform 1 of the adenine nucleotide translocase (Ant)[4]. Ant promotes ADP/ATP exchange across the mitochondrial inner membrane. Ant1 knock-out mice accumulate multiple mtDNA deletions in skeletal and cardiac muscles[5]. Low Ant activity depletes the matrix ADP level. This sequentially causes ATP synthase stagnation, membrane hyperpolarization, increased free radical production and mtDNA damages. Interestingly, mutations in the yeast Aac2p, that mimic the pathogenic

3Correspondence should be addressed to X.J.C. (chenx@upstate.edu).

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adPEO mutations in human Ant1, have only limited effects on its basic nucleotide transport kinetics while dominant-negatively reduce cytochrome content[6]. The mutant proteins also have a noticeable preference towards the transport of ATP versus ADP. This raises the possibility that, like in the Ant1 knock-out mice, the adPEO-type mutations may also cause adenine nucleotide imbalance in mitochondria thereby leading to mtDNA deletions and respiratory deficiency.

Yeast cannot tolerate over-expression of the *aac2*^{A128P} allele, equivalent to the human pathogenic *ant1*^{A114P}, even on glucose medium where respiration is dispensable[7]. The Ala → Pro mutation might introduce a proline kink and conformational changes at the end of the α-helix 3 in the cytosolic gating region[8] (Fig. 1a). When incubated at cold-temperature (25°C), expression of the chromosomally integrated *aac2*^{A128P} from its native promoter completely inhibit cell growth (Fig. 1b). The cold-sensitivity is also observed in the presence of the wild-type *AAC2*, supporting the gain-of-function nature of A128P. Aac2^{A128P} is extractable by the detergent NP-40 but not by NaCl or Na_2CO_3 (Supplementary Information, Fig. S1), indicating that it is integrally inserted into the inner membrane. Cell growth inhibition by Aac2^{A128P} is independent of its ADP/ATP exchange activity, given that the *aac2*$^{A128P, R252I}$, *aac2*$^{A128P, R253I}$ and *aac2*$^{A128P, R254I}$ double mutants remain cold-sensitive. Arg252, Arg253 and Arg254, equivalent to Arg234, Arg235 and Arg236 in bovine Ant1 (see Fig. 1a), are located in an evolutionarily conserved arginine triplet that acts as a "two-way switch" for nucleotide binding from both sides of the membrane[8]. Mutations in any of the arginine residues abolish nucleotide transport activity[9]. As expected, mitochondria expressing *aac2*$^{A128P, R252I}$, *aac2*$^{A128P, R253I}$ and *aac2*$^{A128P, R254I}$ remain inactive in catalyzing ADP/ATP exchange or in supporting respiratory growth (Supplementary Information, Fig. S2).

Incubation of the *aac2*^{A128P} mutant at 25°C drastically reduces the mitochondrial membrane potential ($\Delta\psi_m$) (Supplementary Information, Fig. S3). Mitochondrial depolarization is accompanied with the accumulation of the unprocessed precursor of Hsp60, a nuclear-encoded subunit of the chaperonine complex in the mitochondrial matrix, suggesting that general protein import or proteolytic processing of protein precursors is compromised (Fig. 1c). This is preceded by the loss of the mtDNA-encoded subunit 2 of the cytochrome *c* oxidase, Cox2p, which may not be synthesised or rapidly degraded because of defective membrane targeting and/or assembly. In these cells, mtDNA profile remains little changed (Fig. 1d), indicating that defects in mitochondrial biogenesis are caused by factors other than mtDNA instability. Massive production of mtDNA mutants can be observed in cells moderately over-expressing *aac2*^{A128P} (Supplementary Note and Fig. S4). Taken together, our data support that *aac2*^{A128P} primarily induces defect(s) in general mitochondrial biogenesis by dissipating $\Delta\psi_m$. This subsequently compromises mitochondrial gene expression and ultimately, mtDNA stability at a late stage of the degenerative process.

Flow cytometry analysis revealed that a small fraction (2.3%) of diploid cells heterozygous for *AAC2/aac2*^{A128P} have drastically depolarized mitochondria at 30°C (Fig. 2a). The depolarized cells increase to 23.5% when two copies of *aac2*^{A128P} were integrated into the genome. In these cultures, mitochondrial depolarization follows a biphasic pattern. In addition to the drastically depolarized cells, the mean fluorescence intensity of the major subpopulation is also reduced by 2.2 fold. The frequency of *AAC2/aac2*^{A128P} heterozygous cells with severely depolarized mitochondria coincided with the observation that 4.5% of these cells formed barely visible micro-colonies when individually spotted onto YPD medium by micromanipulation (Fig. 2b). These micro-colonies contain maximally 2,000-4,000 cells, in which each cell is incapacitated in producing perpetually proliferating lineages. Thus, after an irreversible mitochondrial damage in each founder cell of a micro-colony, cells progressively degenerate in the following 12-13 cell divisions. We called the progressive loss of the cell's proliferating capability as degenerative cell death, which is a composite phenotype likely instigated by a

combination of low $\Delta\psi_m$-induced mtDNA mutations and the ρ°-lethal nature of *aac2*^{A128P} cells (see below).

By taking advantage of the phenotypically tractable formation of the degenerative micro-colonies, we asked whether *aac2*^{A128P}-induced mitochondrial degeneration is aging-dependent. Using pedigree analysis, we determined the commitment to degenerative cell death of all the daughter cells isolated from diploid mothers heterozygous for *AAC2/aac2*^{A128P}. In most pedigrees, all the daughters formed normal colonies (upper panel; Fig. 2c). However, 5% of the founding mothers produced daughters that formed degenerative micro-colonies (lower panel; Fig. 2c). In these cells, the appearance of the first degenerative daughter was often followed by all her younger siblings, suggesting that after an irreversible commitment in the dividing mother, all the following daughter cells likely inherited the permanently damaged mtDNA, which ultimately limits their proliferating potential.

In haploid cells co-expressing *AAC2* and *aac2*^{A128P}, about 50% of pedigrees were degenerative. Analysis of these pedigrees, which have a median replicative lifespan of 20 generations, revealed that 58.6% of the founding mothers produce their first degenerative daughters after 9-11 cell divisions (Fig. 2d). Mid-age onset is therefore an inherent property of *aac2*^{A128P}-induced mitochondrial degeneration.

We then directly isolated virgin cells and those that have undergone 5, 10 and 15 cell divisions. These cells of different replicative ages, which co-express *AAC2* and *aac2*^{A128P}, were then individually tested for the degenerative micro-colony formation (Fig. 2e). As age progresses, the micro-colony frequency steadily increases. Degenerative cell death considerably accelerates after the cells are >10 generation old. Thus, aging synergizes with *aac2*^{A128P} in accelerating mitochondrial degeneration.

We hypothesized that the putative aging-related factor(s) accelerating mitochondrial degeneration may be linked to those that also limit replicative lifespan. If so, mitochondrial degeneration might be delayed by lifespan-extending conditions. To test this, we determined whether a selected panel of currently known lifespan-extending mutations[10-14] can suppress *aac2*^{A128P}. In a steady-state cell population, the defect of *aac2*^{A128P} cells in forming viable colonies after exposure to 25°C was not suppressed by *SIR2* over-expression or *fob1Δ* (Fig. 3a) which extend replicative lifespan by inhibiting the formation of nucleolar extrachromosomal ribosomal DNA circles (ERCs)[14, 15]. However, the cold-induced degenerative cell death was significantly suppressed by *gpr1Δ* and *tor1Δ*, and almost completely suppressed by *sch9Δ, rei1Δ* and *rpl6BΔ. GPR1* encodes a G protein-coupled receptor upstream of the protein kinase A (PKA) pathway. Together with *tor1Δ* and *sch9Δ*, mutations in these three nutrient-sensing kinases promote longevity[10, 11]. *REI1* is involved in ribosomal biogenesis[16]. *RPL6B* encodes a component of the 60S ribosomal subunit. These longevity mutations have no significant effect on the expression levels of Aac2p and Aac2^{A128P} (Supplementary Information, Fig. S5).

The suppressor activity can also be directly observed based on growth phenotype at 30°C. For instance, by dissecting diploid strains heterozygous for *aac2*^{A128P} and *rpl6BΔ, aac2*^{A128P}-expressing segregants formed small, white and sectoring colonies indicative of cell growth inhibition, whereas double mutants bearing both *aac2*^{A128P} and *rpl6BΔ* produced regular red colonies like the wild type spores (Fig. 3b).

Caloric and amino acid restrictions also extend replicative lifespan[13, 17]. We found that the defect of nascent *aac2*^{A128P}-expressing meiotic segregants to form viable colonies at 25°C is weakly suppressed by reducing glucose concentration from 2% to 0.5%, and markedly suppressed by growing the cells on minimal synthetic medium lacking most amino acids (Fig. 3c).

Additional lifespan-extending mutations, including *tma19Δ*[18], *rpd3Δ*[19] and *rpl31AΔ*[11] also suppress the cold-induced degenerative cell death in *aac2*^{A128P}-expressing strains (Fig. 3a). Tma19p (or Mmi1p) affects polysome formation [20]. Rpd3p is a histone deacetylase known to affect rRNA processing and ribosomal biogenesis[21]. Rpl31Ap is a component of the 60S ribosomal subunit.

We next tested whether the longevity interventions can suppress the aging-dependent onset of degenerative cell death. Clearly, the aging-dependent formation of degenerative micro-colonies in *aac2*^{A128P} cells was dramatically suppressed by *rpl6BΔ, rei1Δ, sch9Δ*, and caloric restriction (Fig. 3d).

Replicative lifespan analysis also recapitulated an opposing effect between longevity interventions and *aac2*^{A128P}. The median lifespan of the cell is shortened by an average of 28% ($P < 0.005$, unpaired student's *t* test) in *aac2*^{A128P}-expressing cells compared with the wild type control (Fig. 3e). The median lifespan of *aac2*^{A128P} cells is extended for 76.5%, 45.0% and 55.5% by *rpl6BΔ, rei1Δ* and *sch9Δ* respectively (Wilcoxon Rank-Sum test, $P<0.0001$). Mitochondrial degeneration is therefore epistatic to *rpl6BΔ, rei1Δ* and *sch9Δ* in replicative lifespan control.

Aged yeast cells have low $\Delta\psi_m$[22]. We hypothesized that Aac2^{A128P} may synergise with the aging-dependent decline of $\Delta\psi_m$ and accelerate mitochondrial degeneration. To support this, we found that *aac2*^{A128P} cells are hypersensitive to low $\Delta\psi_m$ conditions. For instance, after meiosis, although *aac2*^{A128P} segregants can tolerate the loss of mitochondrial ATP synthesis by disrupting *ATP1*, encoding the α-subunit of the ATP synthase, they are synthetically lethal with the disruption of *CYT1* and *COX4*, encoding components of the complex III and IV in the electron transport chain (Fig. 4a). Expression of *aac2*^{A128P} is also synthetically lethal with *phb1Δ* and *phb2Δ*, which are known to cause low $\Delta\psi_m$[23]. The Phb1 and Phb2 complex has a chaperone-like activity that provides a scaffold in the inner membrane for the assembly of the $\Delta\psi_m$-generating respiratory complexes[24]. The synthetic lethality between *aac2*^{A128P} and *phb1Δ* is poorly suppressed by *gpr1Δ*, moderately suppressed by *tor1Δ*, and strongly suppressed by *sch9Δ, rpl6BΔ* and *rei1Δ* (Fig. 4b). It is also suppressed by lifespan-extending nutritional regimens including caloric and amino acid restrictions (Fig. 4c). The *gpr1Δ, tor1Δ, sch9Δ, rpl6BΔ* and *rei1Δ* alleles suppress mitochondrial depolarization in *aac2*^{A128P} cells grown at both 30°C and 25°C (Supplementary Information, Fig. S3).

Loss of prohibitin accelerates aging in yeast and plants[23, 25]. We found that longevity interventions can also suppress mitochondrial degeneration in prohibitin mutants independent of *aac2*^{A128P}. Prohibitin mutants cannot tolerate a further decrease of $\Delta\psi_m$ caused by mtDNA loss[26]. The ρ°-lethal phenotype can be suppressed by *gpr1Δ, tor1Δ, sch9Δ, rpl6BΔ* and *rei1Δ* (Fig. 4d). Most importantly, aging-dependent formation of degenerative micro-colonies is also a prominent phenotypic manifestation of *phb1Δ*, which is suppressed by *sch9Δ, rpl6BΔ, rei1Δ* and caloric restriction (Fig. 4e). Thus, degenerative cell death appears to be a common phenotypic manifestation of low $\Delta\psi_m$ cells. Suppression of aging-dependent mitochondrial degeneration is an intrinsic property of the longevity interventions.

A common property shared by the suppressor mutations is their connection with cytosolic protein synthesis. We speculated that a reduction of cytosolic protein synthesis may lower the overall loading of proteins onto the mitochondrial inner membrane and promote $\Delta\psi_m$ maintenance in aged *aac2*^{A128P} and *phb1Δ* cells. To support this, we first found that the longevity mutations having the strongest phenotypes for suppressing mitochondrial degeneration, namely *sch9Δ, rei1Δ, rpl6BΔ* and *rpl31AΔ* (see Fig. 3a), also have the most pronounced effects on cytosolic protein synthesis (Fig. 5a). Conditions such as caloric and amino acid restrictions that have different capacity to suppress cold-induced degenerative cell

death (Fig. 3c) also differ in cytosolic protein synthesis (Supplementary information, Fig. S5). Secondly, *aac2*^{A128P} is synthetically lethal with the disruption of *YME1* (Fig. 5b) encoding the i-AAA protease for protein turnover[27]. *yme1Δ* does not significantly affect Aac2p level (Supplementary Information, Fig. S5) but is known to cause protein over-accumulation on the inner membrane[28]. The *yme1Δ*–induced lethality in *aac2*^{A128P} cells is suppressed by *sch9Δ*, *rpl6BΔ* and *rei1Δ*. Finally, we demonstrated that partial inhibition of cytosolic protein synthesis by cycloheximide (Fig. 5a) phenocopies the longevity interventions in suppressing the cold-induced growth inhibition (Fig. 5c) and the synthetic lethality between *yme1Δ* and *aac2*^{A128P} (Fig. 5b). Cycloheximide, as well as *rpl6BΔ*, *sch9Δ* and *rei1Δ*, can all suppress the cold-induced loss of Cox2p in *aac2*^{A128P} cells (Fig. 5d). The relative suppressor activity of *rpl6BΔ*, *sch9Δ* and *rei1Δ* correlates with the extent by which they affect cytosolic protein synthesis (Fig. 5a).

In summary, in the yeast adPEO model and the pro-aging *phb1Δ* mutant, we found that reduced cytosolic protein synthesis suppresses mitochondrial degeneration. Like the prohibitin mutants, *aac2*^{A128P}-expressing cells have low $\Delta\psi_m$ and shortened replicative lifespan. Ant has an intrinsic membrane uncoupling activity independent of its ADP/ATP exchange function[29]. A128P may increase such a proton-conducting activity and actively depolarise the membrane, which leads to defects in general mitochondrial biogenesis and mtDNA instability. We captured an aging-dependent trait that accelerates mitochondrial degeneration in replicatively aged *aac2*^{A128P} and *phb1Δ* cells. In these mutants, the virgin cells have a low frequency of degenerative cell death, but cell death progressively increases during replicative aging. This aging-dependent deleterious trait likely reflects a progressive decline of $\Delta\psi_m$. It is suppressed by reduced cytosolic protein synthesis that may alleviate mitochondria from unassembled protein stress and reduce non-specific proton leakage across the inner membrane during aging (Supplementary Discussion).

The finding that reduced cytosolic protein synthesis robustly promotes mitochondrial maintenance during aging is reminiscent of the observations that longevity interventions not only delay aging-related pathologies, but also improve mitochondrial bioenergetic efficiency and increase stress resistance[30-32]. Our finding is also consistent with the observation that reduced TOR signalling maintains the robustness of the mitochondrial gene expression system and stimulates mitochondrial respiration[33]. Recent studies from several model organisms have shown that reduced cytosolic protein translation directly extends lifespan[34]. In yeast, reduction of the cytosolic ribosomal subunits and altered translation also promote longevity[11, 12, 35]. One of the underlying mechanisms is that specific translational alterations selectively change the expression of proteins such as the transcriptional factor Gcn4, which may benefit cell survival[12]. Our finding therefore opens the possibility that aged cells also benefit from reduced cytosolic protein synthesis by improving energy homeostasis.

Methods

Strains and media

Yeast strains used in this study are listed in Supplementary Information Table S1. Details for strain construction, plasmids and growth media are provided in Supplementary Methods.

Replicative lifespan and pedigree analysis

Yeast strains were grown in YPD at 30°C for overnight and seeded on YPD plates using an inoculation loop. Individual cells were arrayed on YPD plates by using a Singer yeast tetrad dissector and allowed to divide for 1-2 divisions. Virgin cells were retained for lifespan analysis. Subsequent daughter cells were removed and counted. All micromanipulations were carried out in a room conditioned at 26°C. For lifespan analysis, about 50 cells were examined

for each strain. A wild type strain is always examined in parallel to mutant strains, in order to minimize fluctuations of environmental factors that may influence data output. For pedigree analysis, only two virgin cells were analyzed on each plate. All the daughter cells generated were orderly arrayed. Cells were grown at 30°C during the day and stored at 4°C at night.

Degenerative micro-colony assay

For assaying micro-colony formation of cells in a steady state cell population, a fresh colony from YPD plates is inoculated in 5 ml YPD medium. After a growth for 15 hours at 25°C, cells were patched on YPD plates. 90 individual cells were arrayed onto fresh spots for colony formation. Cells were incubated at 30°C for five days. All the arrayed spots were inspected under microscopy for degenerative micro-colonies. Degenerative micro-colonies were defined for those that contain 4 – 4,000 cells after the 5-day incubation. The ratios of degenerative micro-colonies over total number of dividing cells were calculated. For examining aging-dependent micro-colony formation, precultures were grown in YPD at 30°C. Cells were arrayed on YPD. Virgin cells were prepared and allowed to form colonies (0 generation). Virgin cells were also allowed to divide and the generated daughter cells were removed. The removal of daughter cells was stopped after 5, 10 and 15 cell divisions, resulting cells of 5-, 10- and 15-generation old respectively. The mother cells of defined ages were allowed to form colonies at 30°C. The ratios of degenerative micro-colonies over total number of seeded cells were calculated.

Protein synthesis assay

Overnight YPD-grown cultures were diluted into synthetic complete medium lacking methionine to reach an OD_{600} of ~0.5. Cells were grown for 3 hours at 30°C or 25°C. The OD_{600} of the cultures were measured to determine cell numbers. 1 ml of the cultures were labelled with 6 μCi/ml [^{35}S]-methionine/cysteine (EXPRE^{35}S^{35}S Protein Labeling Mix; >1000 Ci/mmol; NEN Research Products) for 5-30 min at 30°C or 25°C. Labelling was stopped by the addition of 1/10 volume of 100% TCA to each culture and chilling on ice. After heating at 90°C for 20 min, TCA precipitates were collected on GFC filters (Whatman), washed sequentially with 10 ml of 10% TCA and 95% ethanol, and counted in 5 ml of UniverSol (ICN). For each sample, the counts per minute/OD_{600} was normalized to wild type which is set as 100%. Three independent experiments were carried out with the error bars representing the standard deviation. The *P*-values shown were determined using unpaired Student's *t* test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005;39:359–407. [PubMed: 16285865]
2. Spinazzola A, Zeviani M. Disorders of nuclear-mitochondrial intergenomic signaling. Gene 2005;354:162–168. [PubMed: 15921863]
3. Suomalainen A, Kaukonen J. Diseases caused by nuclear genes affecting mtDNA stability. Am J Med Genet 2001;106:53–61. [PubMed: 11579425]

4. Kaukonen J, et al. Role of adenine nucleotide translocator 1 in mtDNA maintenance. Science 2000;289:782–785. [PubMed: 10926541]
5. Esposito LA, Melov S, Panov A, Cottrell BA, Wallace DC. Mitochondrial disease in mouse results in increased oxidative stress. Proc Natl Acad Sci USA 1999;96:4820–4825. [PubMed: 10220377]
6. Fontanesi F, et al. Mutations in *AAC2*, equivalent to human adPEO-associated *ANT1* mutations, lead to defective oxidative phosphorylation in *Saccharomyces cerevisiae* and affect mitochondrial DNA stability. Hum Mol Genet 2004;13:923–934. [PubMed: 15016764]
7. Chen XJ. Induction of an unregulated channel by mutations in adenine nucleotide translocase suggests an explanation for human ophthalmoplegia. Hum Mol Genet 2002;11:1835–1843. [PubMed: 12140186]
8. Pebay-Peyroula E, et al. Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. Nature 2003;426:39–44. [PubMed: 14603310]
9. Heidkamper D, Muller V, Nelson DR, Klingenberg M. Probing the role of positive residues in the ADP/ATP carrier from yeast. The effect of six arginine mutations on transport and the four ATP versus ADP exchange modes. Biochemistry 1996;35:16144–16152. [PubMed: 8973186]
10. Fabrizio P, Pletcher SD, Minois N, Vaupel JW, Longo VD. Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in *Saccharomyces cerevisiae*. FEBS Lett 2004;557:136–142. [PubMed: 14741356]
11. Kaeberlein M, et al. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 2005;310:1193–1196. [PubMed: 16293764]
12. Steffen KK, et al. Yeast life span extension by depletion of 60S ribosomal subunit is mediated by Gcn4. Cell 2008;133:292–302. [PubMed: 18423200]
13. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and *SIR2* for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science 2000;289:2126–2128. [PubMed: 11000115]
14. Sinclair DA, Guarente L. Extrachromosomal rDNA circles--a cause of aging in yeast. Cell 1997;91:1033–1042. [PubMed: 9428525]
15. Defossez PA, et al. Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol Cell 1999;3:447–455. [PubMed: 10230397]
16. Lebreton A, et al. A functional network involved in the recycling of nucleocytoplasmic pre-60S factors. J Cell Biol 2006;173:349–360. [PubMed: 16651379]
17. Jiang JC, Jaruga E, Repnevskaya MV, Jazwinski SM. An intervention resembling caloric restriction prolongs life span and retards aging in yeast. FASEB J 2000;14:2135–2137. [PubMed: 11024000]
18. Rinnerthaler M, et al. *MMI1* (*YKL056c*, *TMA19*), the yeast orthologue of the translationally controlled tumor protein (TCTP) has apoptotic functions and interacts with both microtubules and mitochondria. Biochim Biophys Acta 2006;1757:631–638. [PubMed: 16806052]
19. Kim S, Benguria A, Lai CY, Jazwinski SM. Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol Biol Cell 1999;10:3125–3136. [PubMed: 10512855]
20. Fleischer TC, Weaver CM, McAfee KJ, Jennings JL, Link AJ. Systematic identification and functional screens of uncharacterized proteins associated with eukaryotic ribosomal complexes. Genes Dev 2006;20:1294–1307. [PubMed: 16702403]
21. Meskauskas A, et al. Delayed rRNA processing results in significant ribosome biogenesis and functional defects. Mol Cell Biol 2003;23:1602–1613. [PubMed: 12588980]
22. Lai CY, Jaruga E, Borghouts C, Jazwinski SM. A mutation in the *ATP2* gene abrogates the age asymmetry between mother and daughter cells of the yeast *Saccharomyces cerevisiae*. Genetics 2002;162:73–87. [PubMed: 12242224]
23. Coates PJ, Jamieson DJ, Smart K, Prescott AR, Hall PA. The prohibitin family of mitochondrial proteins regulate replicative lifespan. Curr Biol 1997;7:607–610. [PubMed: 9259555]
24. Arnold I, Langer T. Membrane protein degradation by AAA proteases in mitochondria. Biochim Biophys Acta 2002;1592:89–96. [PubMed: 12191771]
25. Chen JC, Jiang CZ, Reid MS. Silencing a prohibitin alters plant development and senescence. Plant J 2005;44:16–24. [PubMed: 16167892]

26. Dunn CD, Lee MS, Spencer FA, Jensen RE. A genomewide screen for petite-negative yeast strains yields a new subunit of the i-AAA protease complex. Mol Biol Cell 2006;17:213–226. [PubMed: 16267274]
27. Thorsness PE, White KH, Fox TD. Inactivation of *YME1*, a member of the *ftsH-SEC18-PAS1-CDC48* family of putative ATPase-encoding genes, causes increased escape of DNA from mitochondria in *Saccharomyces cerevisiae*. Mol Cell Biol 1993;13:5418–5426. [PubMed: 8355690]
28. Pearce DA, Sherman F. Degradation of cytochrome oxidase subunits in mutants of yeast lacking cytochrome *c* and suppression of the degradation by mutation of *yme1*. J Biol Chem 1995;270:20879–20882. [PubMed: 7673107]
29. Brand MD, et al. The basal proton conductance of mitochondria depends on adenine nucleotide translocase content. Biochem J 2005;392:353–362. [PubMed: 16076285]
30. Miller RA, et al. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell 2005;4:119–125. [PubMed: 15924568]
31. Lin SJ, et al. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature 2002;418:344–348. [PubMed: 12124627]
32. Tong JJ, Schriner SE, McCleary D, Day BJ, Wallace DC. Life extension through neurofibromin mitochondrial regulation and antioxidant therapy for neurofibromatosis-1 in *Drosophila melanogaster*. Nat Genet 2007;39:476–485. [PubMed: 17369827]
33. Bonawitz ND, Chatenay-Lapointe M, Pan Y, Shadel GS. Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. Cell Metab 2007;5:265–277. [PubMed: 17403371]
34. Tavernarakis N. Ageing and the regulation of protein synthesis: a balancing act? Trends Cell Biol 2008;18:228–235. [PubMed: 18346894]
35. Chiocchetti A, et al. Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast replicative life span. Exp Gerontol 2007;42:275–286. [PubMed: 17174052]

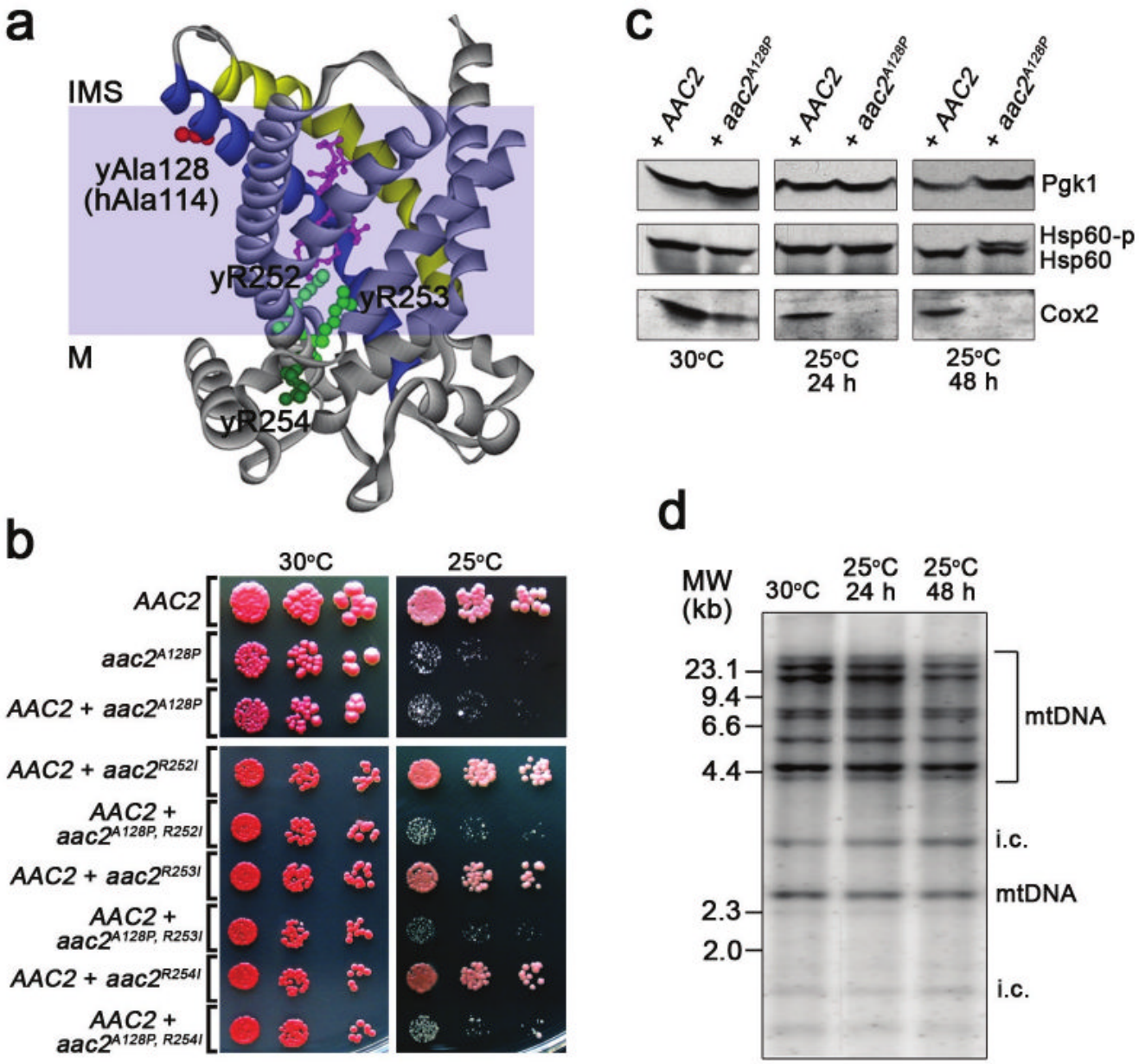

Figure 1. The *aac2*^{A128P} alleles induces defect in mitochondrial biogenesis independent of ADP/ATP exchange activity or mtDNA instability

a, Projected localization of Ala128 (red, yAla128, equivalent to Ala114 in human Ant1) and Arg252, 253 and 254 (green) in yeast Aac2p on the crystal structure of bovine Ant1 in the cytosolic conformation bound by carboxyatractyloside (magenta)[8]. M, matrix; IMS, inter-membrane space. **b**, *aac2*^{A128P} dominant-negatively inhibits cell growth on YPD medium at 25°C in a manner independent of nucleotide exchange activity. **c**, Cold-induced defects in Hsp60 processing and Cox2 biogenesis in *aac2*^{A128P}-expressing cells. Pgk1, 3-phosphoglycerate kinase; Hsp60, large subunit of mitochondrial chaperonine; Hsp60-p, precursor of Hsp60; Cox2, cytochrome *c* oxidase subunit 2. **d**, mtDNA profile in cells co-expressing *aac2*^{A128P} and the wild type *AAC2* incubated at 30°C and 25°C. i.c., internal control for sample loading.

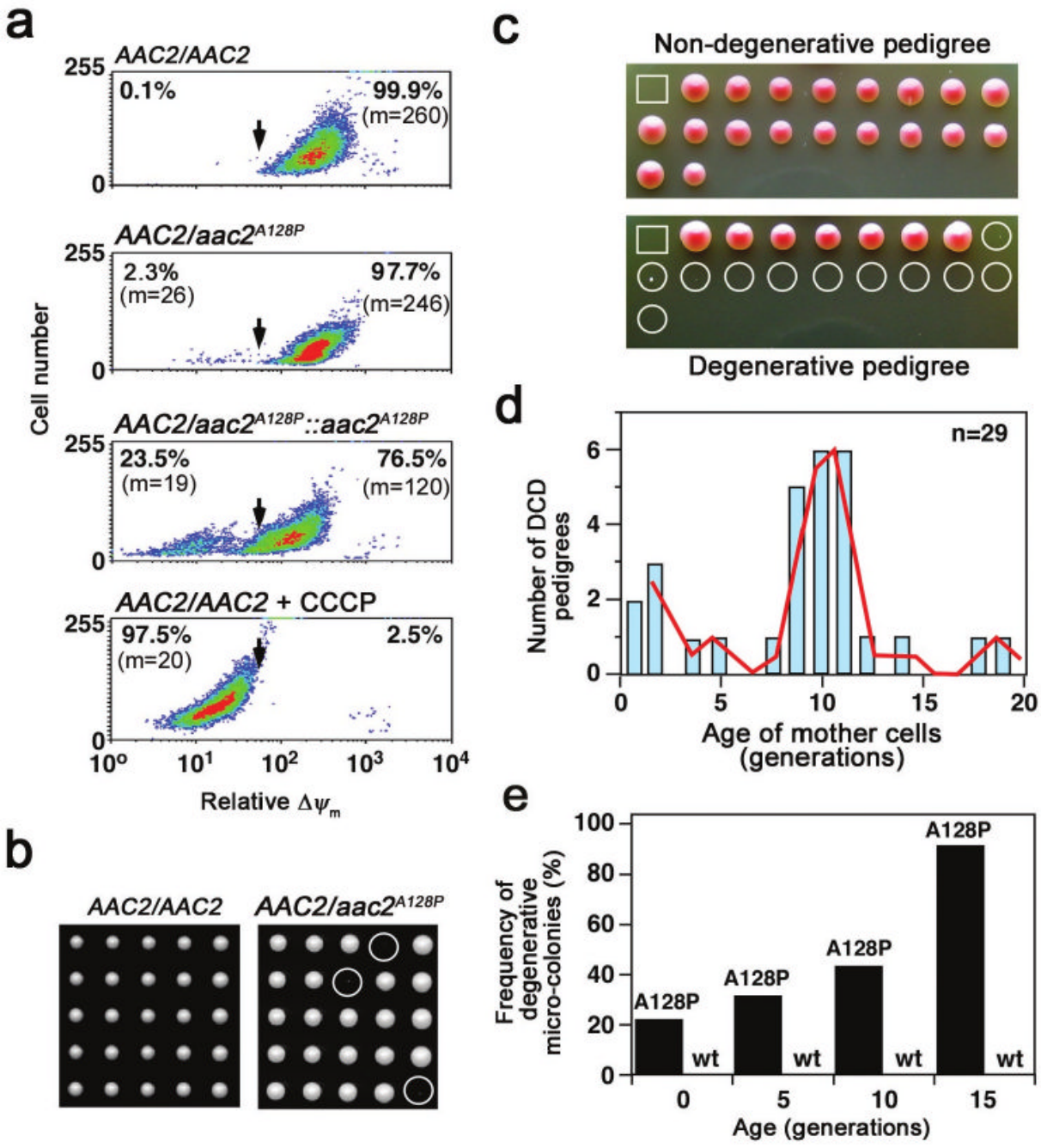

Figure 2. Aging accelerates mitochondrial degeneration and degenerative cell death

a, Flow cytometry analysis showing mitochondrial depolarization induced by increasing copy number of *aac2*^{A128P} in diploid cells. The mean fluorescent intensities of the subpopulations are indicated in parentheses. **b**, *aac2*^{A128P} induces the formation of degenerative micro-colonies (circles) on YPD medium in *AAC2/aac2*^{A128P} heterozygous diploid cells. Totally 270 individual cells were analysed and representative micro-colonies from the *AAC2/aac2*^{A128P} cells are circled. No micro-colony is detectable in the wild type control. **c**, Representative pedigree demonstrating irreversible commitment of mother cells co-expressing *AAC2* and *aac2*^{A128P} (boxed) in the production of daughter cells that form degenerative micro-colonies (circled) on YPD medium. Top, a full non-degenerative pedigree with 19 daughters (n=38);

Bottom, a full degenerative pedigree with 18 daughters (n-38). The daughters are orderly placed from left to right and top to bottom. **d**, The fraction of mother cells committed to produce their first degenerative daughters at a given age. A total of 59 pedigrees were analysed, among which 29 are degenerative. **e**, Frequency of degenerative micro-colony formation on YPD, in function of the replicative age of wild type (WT) and mutant cells co-expressing *AAC2* and *aac2*^{A128P} (A128P).

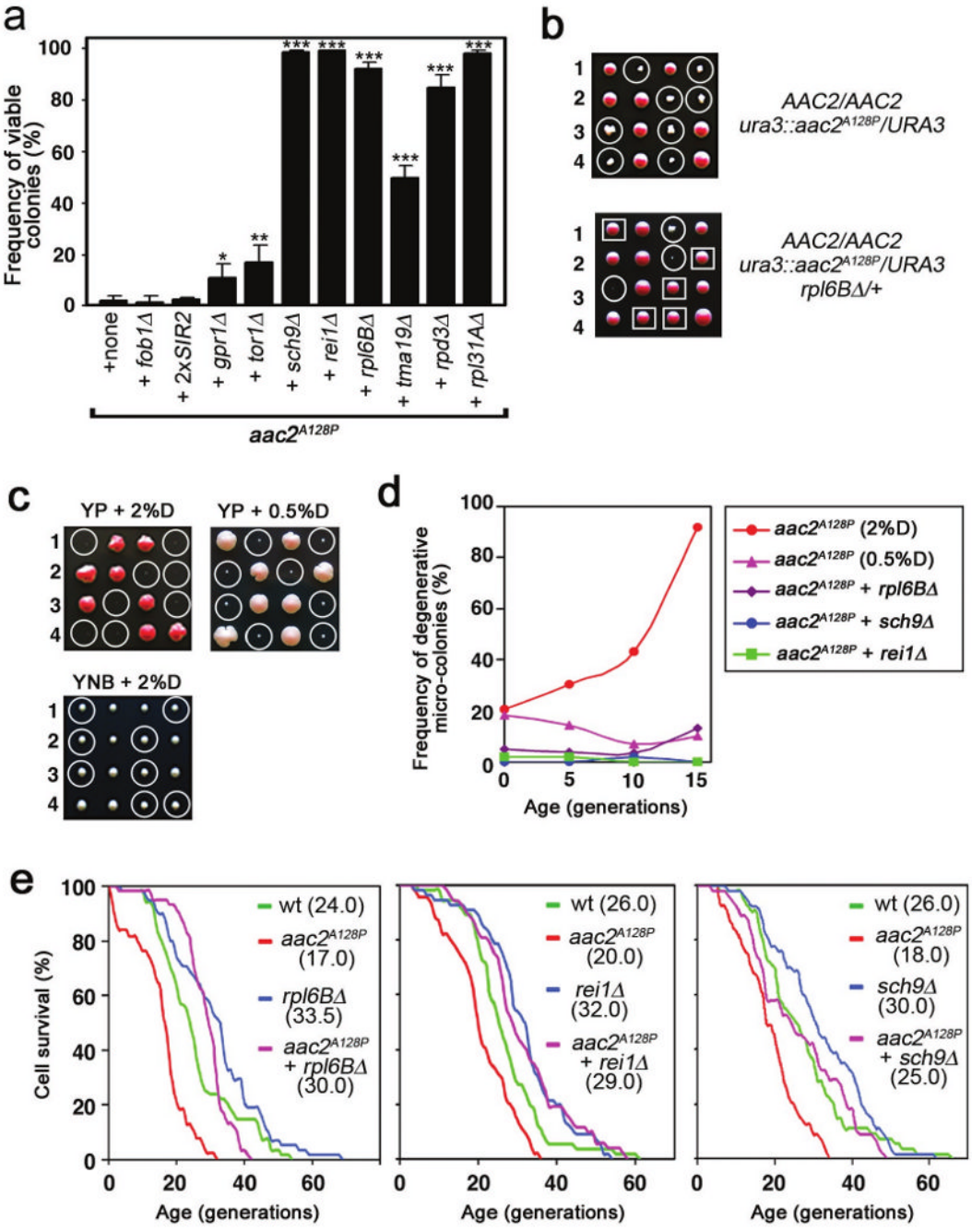

Figure 3. Longevity interventions suppress mitochondrial degeneration

a, The cold-induced defect of *aac2*^{A128P}-expressing cells to form viable colonies is suppressed by *gpr1Δ*, *tor1Δ*, *sch9Δ*, *rei1Δ*, *rpl6BΔ*, *tma19Δ*, *rpd3Δ* and *rpl31AΔ*, but not by *fob1Δ* or the introduction of an extra copy of *SIR2* (2XSIR2). Three independent experiments were carried out and error bars represent standard deviations. Unpaired Student's *t* test: *, $P<0,05$; **, $P<0.005$; ***, $P<0.0001$. **b**, Growth defect of nascent meiotic segregants co-expressing *AAC2* and *aac2*^{A128P} is suppressed by *rpl6BΔ* at 30°C. Four representative tetrads are shown. The *aac2*^{A128P}-expressing segregants are circled and those that co-segregated with *rpl6BΔ* are boxed. The plates were incubated for four days before being photographed. **c**, The formation of degenerative micro-colonies at 25°C by nascent meiotic segregants co-expressing *AAC2*

and *aac2*^{A128P} (circled) is suppressed by caloric restriction (0.5% D) and by growth on minimal medium supplemented with adenine, leucine and uracil. Four representative tetrads are shown for each growth condition. **d**, Longevity interventions suppress the aging-dependent formation of degenerative micro-colonies. **e**, The short replicative lifespan of haploid cells co-expressing *AAC2* and *aac2*^{A128P} is suppressed by *rpl6BΔ*, *rei1Δ* and *sch9Δ* respectively. Wild type (wt), *rpl6BΔ*, *rei1Δ* and *sch9Δ* single mutants are analysed in parallel as controls. Median lifespans are indicated in parentheses.

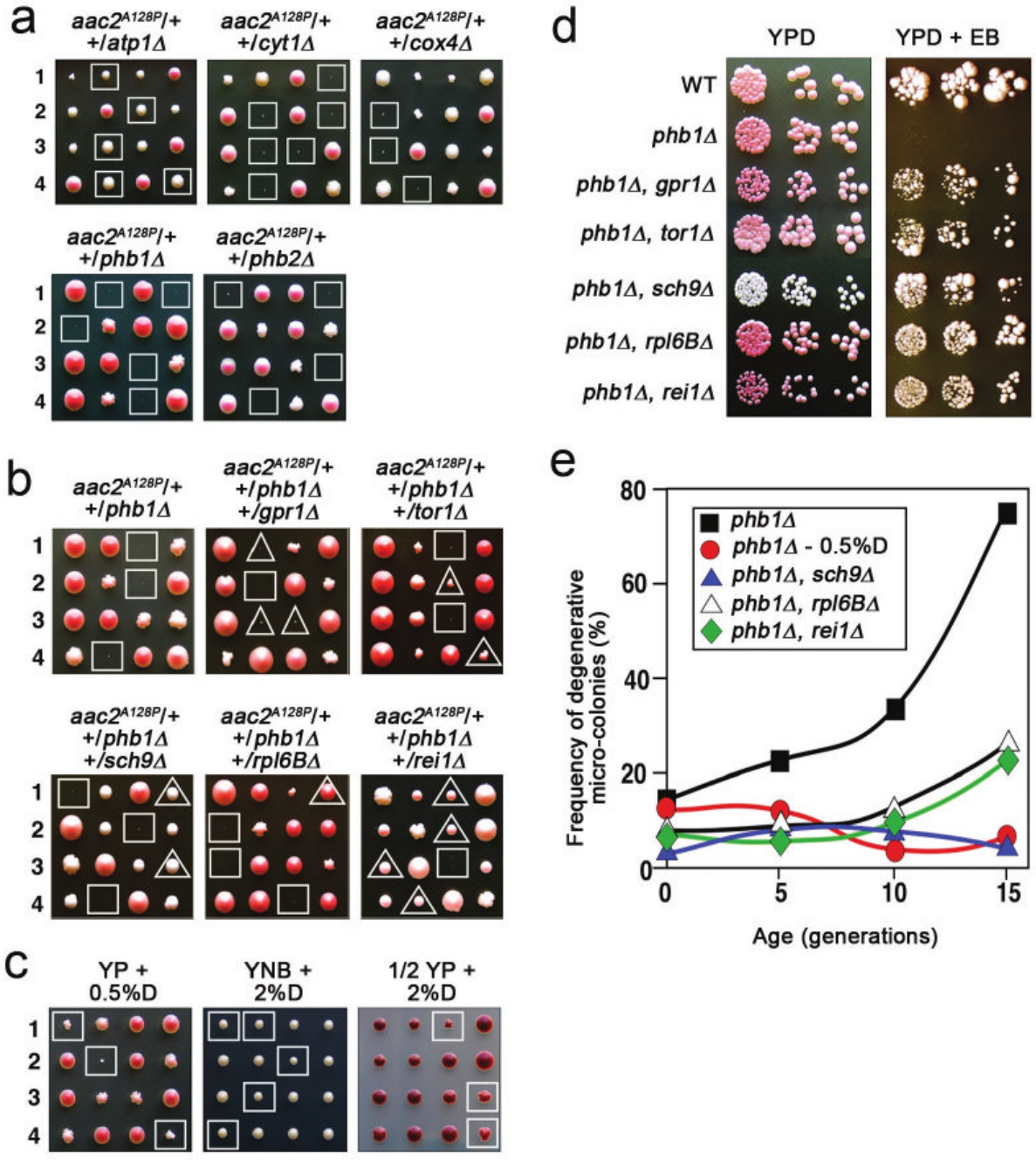

Figure 4. Longevity interventions suppress aging-dependent mitochondrial degeneration in prohibitin mutants

a, Growth phenotype of four representative tetrads. Meiotic spores co-segregating *aac2*^{A128P} with *cyt1Δ*, *cox4Δ*, *phb1Δ* and *phb2Δ*, but not with *atp1Δ*, form degenerative micro-colonies on YPD at 30°C (boxed). **b**, Growth phenotype of representative tetrads showing that synthetic lethality between *aac2*^{A128P} and *phb1Δ* among the meiotic segregants is suppressed by lifespan-extending mutations. Box, *aac2*^{A128P} *phb1Δ* double mutant. Triangle, triple mutants with *gpr1Δ*, *tor1Δ*, *sch9Δ*, *rpl6BΔ* or *rei1Δ*. **c**, Suppression of synthetic lethality between *aac2*^{A128P} and *phb1Δ* among the meiotic segregants by lifespan-extending nutritional conditions. Boxed are *aac2*^{A128P} *phb1Δ* double mutants. **d**, The sensitivity of *phb1Δ* cells to

ethidium bromide (EB)-induced mtDNA elimination is suppressed by longevity mutations. **e**, Aging-dependent formation of degenerative micro-colonies in *phb1Δ* cells is suppressed by longevity mutations.

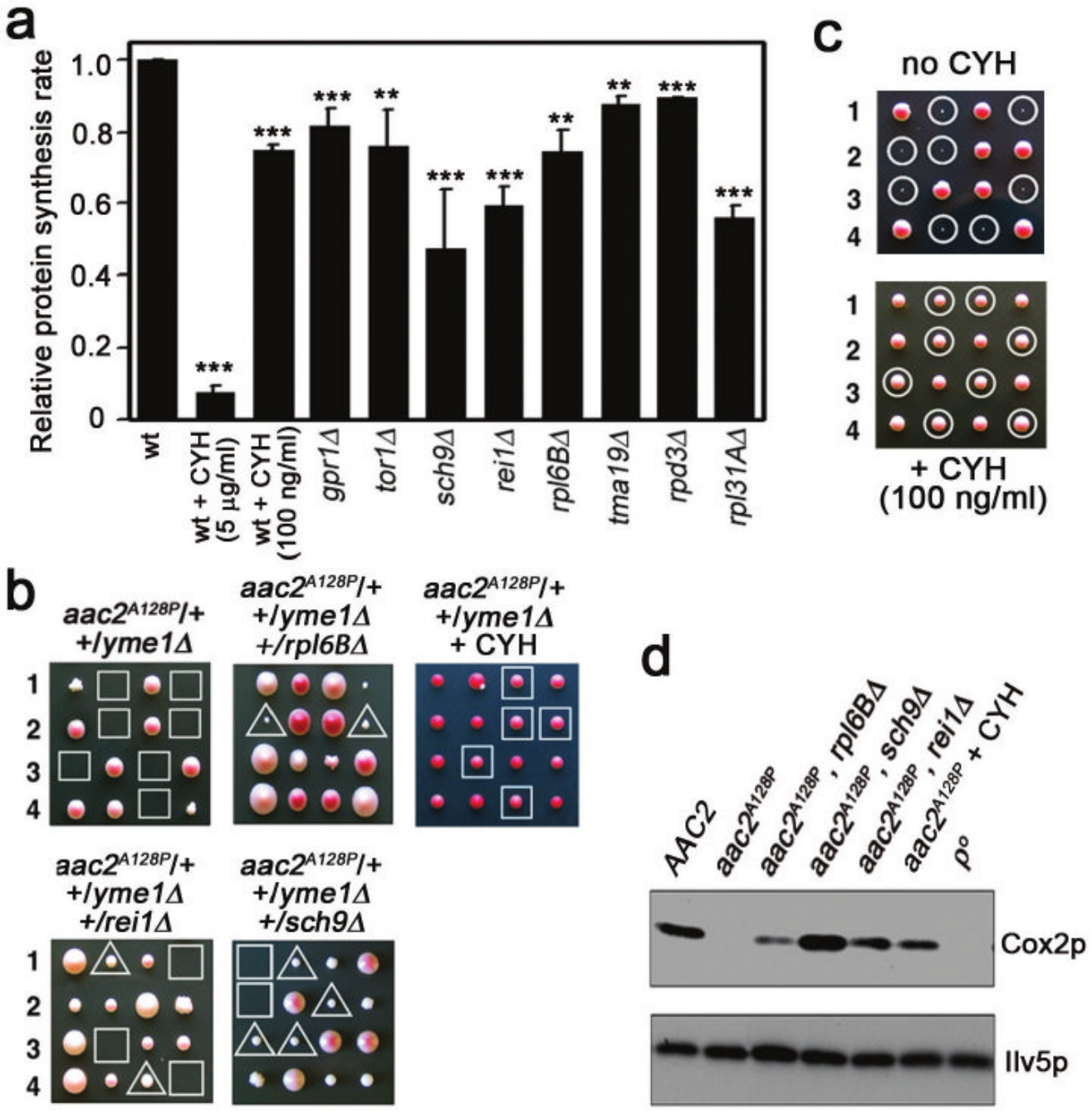

Figure 5. Modulation of *aac2*^{A128P}-induced cell death by mitochondrial protein loading

a, (*A*) *In vivo* protein synthesis assay showing the incorporation of ^{35}S-methionine in longevity mutants (15 min, 30°C). The standard deviations between three independent samples are shown. Unpaired Student's *t* test: **, *P*<0,05; ***, *P*<0.005. CYH, cycloheximide. **b**, Growth phenotype of representative tetrads showing the synthetic lethality between *aac2*^{A128P} and *yme1Δ* among the meiotic segregants and the suppression of the synthetic lethality by lifespan-extending mutations and by cycloheximide (CYH, 100 ng/ml). Box, *aac2*^{A128P} *yme1Δ* double mutant. Triangle, triple mutants with *rpl6BΔ*, *rei1Δ* or *sch9Δ*. **c**, The formation of degenerative micro-colonies at 25°C by nascent meiotic segregants co-expressing *AAC2* and *aac2*^{A128P} (circled) is suppressed by cycloheximide on YPD medium. **d**, Western-blot analysis of crude

mitochondrial preparations showing that the loss of Cox2p in *aac2*^{A128P}-expressing cells is suppressed by *rpl6BΔ*, *sch9Δ*, *rei1Δ*, and cycloheximide (25 ng/ml). The level of the matrix Ilv5p is an internal control for sample loading.