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## Vitamin B<sub>12</sub> impacts amyloid beta-induced proteotoxicity by regulating the methionine/S-adenosylmethionine cycle

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### SUMMARY

Alzheimer's disease (AD) is a devastating neurodegenerative disorder with no effective treatment. Diet, as a modifiable risk factor for AD, could potentially be targeted to slow disease onset and progression. However, complexity of the human diet and indirect effects of the microbiome make it challenging to identify protective nutrients. Multiple factors contribute to AD pathogenesis, including amyloid beta (A $\beta$ ) deposition, energy crisis, and oxidative stress. Here, we use *Caenorhabditis elegans* to define the impact of diet on A $\beta$  proteotoxicity. We discover that dietary vitamin B<sub>12</sub> alleviates mitochondrial fragmentation, bioenergetic defects, and oxidative stress, delaying A $\beta$ -induced paralysis without affecting A $\beta$  accumulation. Vitamin B<sub>12</sub> has this protective effect by acting as a cofactor for methionine synthase, impacting the methionine/S-adenosylmethionine (SAMe) cycle. Vitamin B<sub>12</sub> supplementation of B<sub>12</sub>-deficient adult A $\beta$  animals is beneficial, demonstrating potential for vitamin B<sub>12</sub> as a therapy to target pathogenic features of AD triggered by proteotoxic stress.

### Graphical Abstract

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#### AUTHOR CONTRIBUTIONS

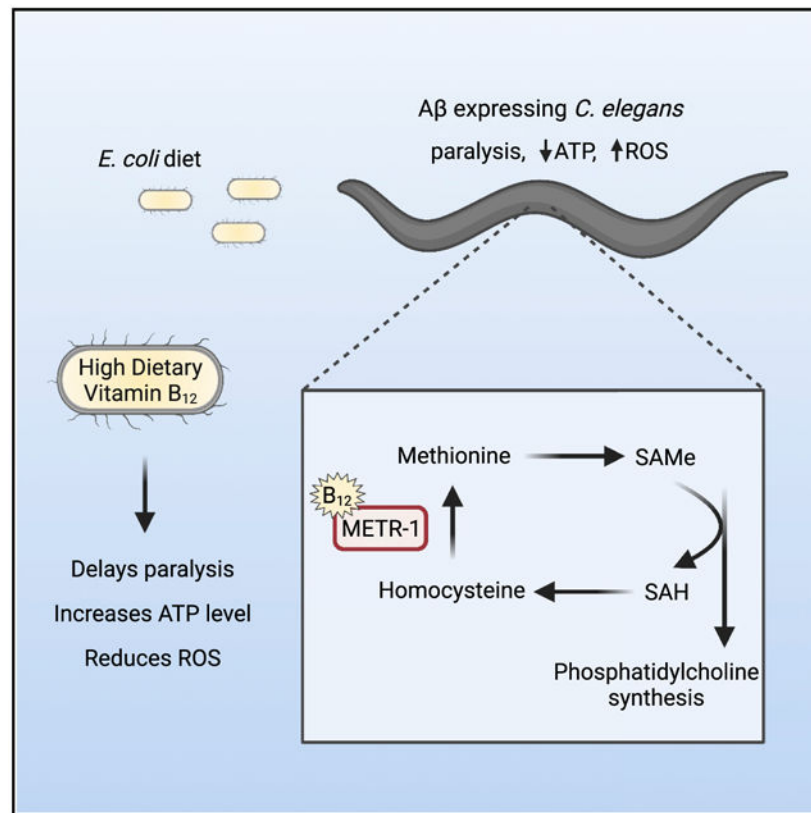
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#### SUPPLEMENTAL INFORMATION

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#### DECLARATION OF INTERESTS

The authors declare no competing interests.



### In brief

Lam et al. discover that feeding a vitamin-B<sub>12</sub>-deficient diet to amyloid beta ( $A\beta$ )-expressing *C. elegans* accelerates paralysis, reduces ATP levels, and increases oxidative stress. Their results indicate that vitamin B<sub>12</sub> affects the methionine/SAMe cycle to protect against  $A\beta$ -induced proteotoxicity.

## INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is a multifactorial neurodegenerative disorder characterized by accumulation of amyloid beta ( $A\beta$ ) plaques, hyperphosphorylated tau, oxidative stress, mitochondrial defects, and impaired glucose metabolism (Butterfield and Halliwell, 2019; Chakravorty et al., 2019; Lin and Beal, 2006; Long and Holtzman, 2019). Some AD risk factors, including genetic predisposition and aging, are non-modifiable, while other risk factors, such as diet, can be altered to impact disease onset and progression (Thelen and Brown-Borg, 2020). Complex diets consist of macronutrients, including carbohydrates, fats, and proteins, as well as vitamin and mineral micronutrients. It is challenging to determine which individual nutrients are neuroprotective in humans as well as other mammals due to organismal complexity, genetic diversity, and consumption of complex diets. Indirect dietary effects of gut microbiota, which provide micronutrients to the host, further complicate studies.

*Caenorhabditis elegans* eat a simple diet of *E. coli* in the laboratory, and consumption of different bacterial strains affects nematode gene expression, metabolic profile, development, fertility, and fat storage (Brooks et al., 2009; Cabreiro et al., 2013; Cogliati et al., 2020; Han et al., 2017; MacNeil et al., 2013; Revtovich et al., 2019; Virk et al., 2012, 2016; Watson et al., 2014). With its genetic tools and short lifespan, *C. elegans* is an ideal system for the study of age-related diseases and has been used extensively to identify factors that influence A $\beta$  proteotoxicity (Fang et al., 2019; Han et al., 2017; Hassan et al., 2015; Lublin and Link, 2013; Sorrentino et al., 2017; Teo et al., 2019). Transgenic expression of toxic human A $\beta$  peptides in *C. elegans* body wall muscles generates robust time-dependent paralysis as well as AD-like pathological features, including defects in mitochondrial morphology, reduced ATP levels, and oxidative stress (Drake et al., 2003; Fonte et al., 2011; Link, 1995; McColl et al., 2012; Sorrentino et al., 2017). Our goal was to use *C. elegans* to define the impact of diet on A $\beta$ -induced proteotoxicity.

We discovered that A $\beta$ -expressing *C. elegans* fed HB101 *E. coli* exhibited delayed paralysis, higher ATP levels, decreased mitochondrial fragmentation, and reduced reactive oxygen species (ROS) compared to those raised on OP50 *E. coli*. Mild vitamin B<sub>12</sub> deficiency was observed in animals grown on OP50, but not HB101. B<sub>12</sub> supplementation delayed A $\beta$ -induced paralysis and protected against the increase in ROS and energy crisis observed in animals fed OP50 but did not have additive beneficial impact on those that consumed HB101. The protective effects of vitamin B<sub>12</sub> required methionine synthase, indicating function as an enzyme cofactor rather than an antioxidant, and the methionine/S-adenosylmethionine (SAME) cycle. Vitamin B<sub>12</sub> supplementation in adulthood was beneficial for B<sub>12</sub>-deficient *C. elegans*, suggesting that administration even late in life has potential as a therapeutic intervention.

## RESULTS

### Diet alters A $\beta$ -induced paralysis in *C. elegans*

Genetic evidence suggests that A $\beta$ -induced proteotoxicity is a causal factor in AD development (Chartier-Harlin et al., 1991; Goate et al., 1991; Long and Holtzman, 2019). Little correlation between the density of extracellular A $\beta$  plaques and AD severity (Chen et al., 2017; De Strooper and Karran, 2016) suggests that the toxic effects likely result from small soluble A $\beta$  oligomers, which can enter lipid bilayers (Cline et al., 2018; Pagani and Eckert, 2011). Transgenic expression of human A $\beta$ <sup>1-42</sup> in *C. elegans* muscles causes AD-like cellular abnormalities, including reduced ATP levels, defects in mitochondrial morphology, and oxidative stress, as well as robust time-dependent paralysis (Drake et al., 2003; Fonte et al., 2011; McColl et al., 2012). Altered time to paralysis has been used extensively to identify genes and pharmacological agents that influence A $\beta$ -induced proteotoxicity (Cacho-Valadez et al., 2012; Han et al., 2017; Hassan et al., 2015; Lublin and Link, 2013; Sorrentino et al., 2017). While investigating the impact of genetic factors on A $\beta$ -induced paralysis, we noticed that animals fed OP50 B-type *E. coli* consistently paralyzed faster than those given HT115(DE3), an RNase-III-deficient K12-derived *E. coli* used for RNA interference experiments. To determine if diet was impacting A $\beta$  proteotoxicity, we raised A $\beta$  animals on different *E. coli* diets and discovered that those

fed HT115 or HB101, a B × K12 hybrid, exhibited a significant delay in paralysis compared to those that consumed OP50 (Figures 1A, S1A, and S1B).

Since caloric restriction reduces A $\beta$  toxicity (Steinkraus et al., 2008), we first sought to determine if the altered time to paralysis was due to differences in bacterial growth or *C. elegans* feeding. We found no difference in bacterial concentration on OP50 and HB101 plates (Figure S1C) or pharyngeal pumping rates (Figure S1D) in *C. elegans* grown on the different diets. A $\beta$  animals fed DA837, another *E. coli* B strain that is hard for worms to ingest (Shtonda and Avery, 2006), exhibited paralysis indistinguishable from those given OP50 (Figures 1B and S1A). Finally, the HB101 diet was still protective in an *eat-2* mutant, which exhibits delayed A $\beta$ -induced paralysis due to dietary restriction (Figure S1E; Steinkraus et al. 2008). Together, these results suggest that the diet-induced shift in paralysis was not due to changes in ingestion or dietary restriction.

Reducing A $\beta$  levels delays paralysis (Sorrentino et al., 2017), so we next sought to establish if diet impacted A $\beta$  content. We observed no difference in A $\beta$  accumulation between animals raised on OP50 and HB101 using immunoblotting (Figures 1C and S1F). Through a genetic approach, we found that *pek-1(ok275)* and *gcn-2(ok871)*, which alter protein synthesis (Baker et al., 2012; Rousakis et al., 2013; Shen et al., 2005), did not impact the HB101 protective effect (Figures S1G and S1H). Our results suggest that the HB101 diet delays A $\beta$ -induced paralysis without affecting A $\beta$  accumulation.

### Diet impacts ATP levels, mitochondrial morphology, and ROS in A $\beta$ animals

Bioenergetic deficits, which cause synaptic dysfunction in AD individuals, are exacerbated by A $\beta$  accumulation (Chakravorty et al., 2019). *C. elegans* expressing A $\beta$  exhibit a decrease in ATP levels and defects in electron transport chain complex activity, indicating that energy crisis is a fundamental consequence of A $\beta$  buildup (Fang et al., 2019; Fong et al., 2016; Sorrentino et al., 2017; Teo et al., 2019). Therefore, we tested the impact of diet on ATP levels and found that A $\beta$  animals fed HB101 had significantly higher ATP compared to those on OP50 (Figure 1D). Diet had no effect on ATP levels in wild-type *C. elegans* (Figure 1D), indicating that the dietary protection was only required for animals under proteotoxic stress.

To elucidate the mechanism underlying the diet-induced change in ATP level, we assessed mitochondrial gene transcripts, protein levels, morphology, and respiration activity. Transcript levels of genes required for oxidative phosphorylation (Figure S2A) and mitochondrial protein content (Figures S2B and S2C) were unchanged between A $\beta$  animals fed different diets. A $\beta$  expression disrupts mitochondrial network integrity, which is important for bioenergetic efficiency (Figure 1E; Fonte et al., 2011; Sorrentino et al., 2017). *C. elegans* muscle mitochondria are arranged in a periodic pattern that can be visualized with red fluorescent protein (RFP)-tagged TOMM-20 (Wei and Ruvkun, 2020). We quantified mitochondria length and found that the HB101 diet reduced fragmentation in both wild-type and A $\beta$  animals (Figure 1F). To assess if these changes in mitochondrial morphology impacted cellular respiration, we used a Seahorse XFe96 Analyzer. A $\beta$  expression caused a significant change in basal respiration rate, maximal respiration rate, and spare respiratory capacity; however, diet did not alter mitochondrial respiration in

either wild-type or A $\beta$  nematodes (Figures S2D–S2F). While it remains possible that the severe mitochondrial fragmentation in A $\beta$  animals alters respiration, the dietary impact on morphology did not change mitochondrial function. Instead, diet likely affects ATP levels by altering glycolytic output or energy expenditure.

Ab accumulation promotes production of ROS including super-oxide (O $_2^-$ ) and hydrogen peroxide (H $_2$ O $_2$ ). Oxidative stress, stemming from an imbalance between ROS formation and antioxidant defenses, plays a causal role in AD pathogenesis (Butterfield and Halliwell, 2019; Drake et al., 2003; Wang et al., 2014). Therefore, we investigated the impact of A $\beta$  and diet on O $_2^-$  and H $_2$ O $_2$  levels using ROS indicators. Expression of A $\beta$  resulted in a substantial increase in O $_2^-$ , but A $\beta$  animals fed HB101 had significantly reduced O $_2^-$  levels compared to those raised on OP50 (Figure 1G). Both wild-type and A $\beta$  *C. elegans* raised on OP50 exhibited significantly higher H $_2$ O $_2$  than those on HB101 (Figures 1H and 1I). In conclusion, diet impacts ATP level, mitochondrial fragmentation, and ROS accumulation in A $\beta$ -expressing *C. elegans*.

### Vitamin B $_{12}$ protects against A $\beta$ -induced paralysis and energy deficits

OP50 and HB101 differ in carbohydrate content and fatty acid composition (Brooks et al., 2009; Neve et al., 2020; Revtovich et al., 2019). Thus, we considered whether differences in macronutrients could alter the paralysis of A $\beta$  worms. Supplementation of plates with glucose (Figures 2A and S3A) or oleic acid (Figures 2B, S3A, and S3B) did not eliminate the dietary shift in A $\beta$ -induced paralysis, suggesting that these macronutrients are not responsible for the impact of diet. However, A $\beta$  animals raised on OP50 supplemented with glucose paralyzed significantly faster than those fed OP50 alone, consistent with reports of excess glucose shortening lifespan (Lee et al., 2009; Schulz et al., 2007). Supplementation of HB101 plates with glucose had no effect (Figures 2A and S3A), suggesting that the nutrient in HB101 that protects against A $\beta$  proteotoxicity may also defend against the detrimental consequences of high glucose.

*E. coli* strains also differ in micronutrient content and *C. elegans*, like humans, must obtain several essential vitamins from their diet. Mild vitamin B $_{12}$  deficiency is observed in animals fed OP50, which has reduced expression of the *tonB* transporter that mediates B $_{12}$  uptake (Revtovich et al., 2019; Watson et al., 2014). *C. elegans* vitamin B $_{12}$  status can be assessed using a *Pacdh-1::GFP* reporter, which is expressed in response to propionic acid accumulation resulting from B $_{12}$  deficiency (MacNeil et al., 2013; Revtovich et al., 2019; Watson et al., 2014, 2016; Wei and Ruvkun, 2020). GFP was highly expressed in animals grown on OP50 and DA837, the diets that led to faster paralysis of A $\beta$  worms, and B $_{12}$  supplementation suppressed GFP expression (Figure 2C). In contrast, GFP expression was repressed in *C. elegans* raised on the HB101 and HT115 protective diets, suggesting high B $_{12}$  levels (Figure 2C). Supplementation of OP50 plates with 148 nM vitamin B $_{12}$  eliminated the dietary shift in A $\beta$ -induced paralysis (Figure 2D). Further increasing B $_{12}$  concentration in OP50 plates by 5-fold as well as addition of vitamin B $_{12}$  to HB101 plates had no added protective effect (Figures 2E and S3A). Misfolded aggregation prone proteins underlie many neurodegenerative diseases. We found that B $_{12}$  supplementation also had a protective effect in *C. elegans* models of amyotrophic lateral sclerosis (ALS) and

Huntington's disease (Figure 2F), suggesting that B<sub>12</sub> may protect against shared cellular defects resulting from proteotoxic stress.

We next sought to determine the impact of vitamin B<sub>12</sub> on ATP availability, mitochondrial morphology, and ROS levels. A $\beta$  animals fed B<sub>12</sub> supplemented OP50 had significantly higher ATP levels compared to non-supplemented counterparts, while B<sub>12</sub> had no effect on animals raised on HB101 (Figure 2G). Vitamin B<sub>12</sub> supplementation did not alter mitochondrial gene transcription, protein levels, or respiration (Figures S2B–S2F) but did reduce mitochondrial fragmentation in A $\beta$  animals fed OP50 without impacting mitochondria length in those raised on HB101 (Figure 2H). Severe B<sub>12</sub> deficiency in *C. elegans* increases H<sub>2</sub>O<sub>2</sub> content and decreases antioxidant defense (Bito et al., 2017). We found that addition of vitamin B<sub>12</sub> to OP50 plates significantly reduced H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> in A $\beta$  animals to levels observed in those raised on HB101 (Figures 2I and 2J). Together, these results show that vitamin B<sub>12</sub> supplementation has a protective effect on A $\beta$  animals fed the OP50 diet.

### Vitamin B<sub>12</sub> given during adulthood has protective effects

Subclinical B<sub>12</sub> deficiency is common primarily in older individuals (Green et al., 2017). Thus, we sought to determine if manipulation of B<sub>12</sub> availability during adulthood affected A $\beta$  proteotoxicity in *C. elegans*. Animals grown on OP50 plates supplemented with vitamin B<sub>12</sub> and then transferred to B<sub>12</sub>-free OP50 plates at the end of the fourth larval stage (L4) exhibited A $\beta$ -induced paralysis indistinguishable from animals raised on OP50 their entire life (Figures 2K and S3C). This demonstrates that a decrease in vitamin B<sub>12</sub> during adulthood exacerbates A $\beta$  proteotoxicity. Furthermore, A $\beta$  animals first grown on OP50 and then transferred to OP50 plates supplemented with B<sub>12</sub> at the end of L4 exhibited the same delay in paralysis as those fed a vitamin-B<sub>12</sub>-rich diet their entire lifespan (Figures 2L and S3C). These results suggest potential benefit for dietary vitamin B<sub>12</sub> supplementation later in life.

### Protective effects of dietary B<sub>12</sub> require methionine synthase

Vitamin B<sub>12</sub> is an essential cofactor for methylmalonyl-coenzyme A (methylmalonyl-CoA) mutase (*C. elegans* MMCM-1), which converts methylmalonyl-CoA to succinyl-CoA in the propionyl-CoA breakdown pathway, and methionine synthase (*C. elegans* METR-1), which converts homocysteine (Hcy) to methionine in the methionine/SAMe cycle (Figure 3A). To define which pathway is required for the B<sub>12</sub> protective effects, we determined the impact of mutations in *mmcm-1(ok1637)*; propionyl-CoA carboxylase *pcca-1(ok2282)*, which acts upstream of *mmcm-1*; and *metr-1(ok521)* on A $\beta$ -induced paralysis. Loss of *pcca-1* and *mmcm-1* had no effect on the diet-induced shift in paralysis observed for A $\beta$  animals grown on the different *E. coli* (Figures 3B, 3C, S4A, and S4B). However, the dietary shift was eliminated in *metr-1* mutants, which exhibited an even more severe phenotype than OP50-raised A $\beta$  animals, which have only mild vitamin B<sub>12</sub> deficiency (Figures 3D and S4C). Consistent with the behavioral data, loss of *metr-1* in A $\beta$  animals reduced ATP levels and abolished the HB101 protective effect (Figure 3E). ROS content was also indistinguishable between *metr-1* A $\beta$  animals raised on OP50 and HB101, though loss of *metr-1* caused an overall reduction in ROS levels (Figures 3F and 3G).

The methionine/SAMe cycle, transsulfuration pathway, folate cycle, and polyamine biosynthesis are all disrupted in the *metr-1* mutant. We found that loss of the SAMe synthetase SAMS-1, which catalyzes conversion of methionine to SAMe, eliminated the dietary shift and delayed A $\beta$ -induced paralysis (Figures 3H and S4D). The opposite effect of the *metr-1* and *sams-1* mutations on time to paralysis is consistent with other previously observed phenotypes (Cabreiro et al., 2013). The elimination of dietary impact on A $\beta$ -induced paralysis in both mutants suggests that the methionine/SAMe pathway is required for vitamin B<sub>12</sub> protection.

### Methionine and choline supplementation is protective in B<sub>12</sub>-deficient A $\beta$ animals

B<sub>12</sub> deficiency reduces methionine synthase activity and can lead to Hcy accumulation (Figure 4A; Andra et al., 2021). We found that A $\beta$  animals grown on OP50 had significantly increased Hcy, which was reduced by B<sub>12</sub> supplementation (Figure 4B). Hyperhomocysteinemia is a modifiable risk factor for AD and results in oxidative stress (Bito et al., 2017; Smith and Refsum, 2016); thus, we sought to determine the effect of Hcy on A $\beta$ -induced paralysis. Hcy supplementation accelerated paralysis of A $\beta$  animals raised on OP50 and HB101 but did not change the dietary shift (Figures 4C and S4E). This suggests that the increased Hcy in animals fed OP50 did not underlie the effect of low dietary vitamin B<sub>12</sub>. We next considered whether methionine level could impact A $\beta$  toxicity. Methionine supplementation eliminated the dietary shift in paralysis (Figure 4D), having a beneficial effect on A $\beta$  animals fed OP50 and a detrimental impact on those grown on HB101 (Figures 4D, S4F, and S4G). To establish if methionine acts directly in *C. elegans* or indirectly by altering bacterial metabolism, we determined if methionine supplementation could rescue the *metr-1* mutant, which is completely dependent on exogenous methionine (Hannich et al., 2009). Addition of methionine significantly delayed A $\beta$ -induced paralysis and increased ATP in the *metr-1* mutant to the level observed in A $\beta$  animals raised on OP50 (Figures 4E–4G). Bacterial diet did not affect the paralysis of A $\beta$ -expressing *metr-1* mutants grown on plates supplemented with methionine (Figure 4F). Together, these results suggest that the impact of methionine supplementation is not due to a change in bacterial metabolism.

The loss of the dietary shift in A $\beta$ -induced paralysis in the *sams-1* mutant (Figure 3H) suggested that the methionine/SAMe cycle is required for the protective effects of B<sub>12</sub>. SAMe, the main methyl donor in cells, is utilized by many methyltransferases, which transfer methyl groups to nucleic acids, histones, and phospholipids. Since addition and removal of vitamin B<sub>12</sub> beginning at adulthood has beneficial and detrimental effects, respectively (Figures 2K and 2L), we reasoned that it was unlikely that SAMe impacted paralysis by having an effect on DNA and protein methylation that regulates transcription. Phosphatidylcholine (PtdCho), a major component of membranes, can be synthesized by SAMe-dependent methylation of phosphoethanolamine or directly from choline through the Kennedy pathway (Figure 4A; Brendza et al., 2007; Palavalli et al., 2006), and reduced PtdCho has been observed in individuals with AD (Whiley et al., 2014). Supplementation of OP50 plates with choline delayed A $\beta$ -induced paralysis, eliminating the dietary shift (Figure 4H). Choline provided no protection for A $\beta$  animals fed HB101, and further increasing choline concentration did not have additional benefit for those fed OP50 (Figures 4H and

4I). These data are consistent with a model in which vitamin-B<sub>12</sub>-dependent methionine/SAMe cycle activity increases PtdCho production to reduce A $\beta$  proteotoxic effects.

## DISCUSSION

Diet is a modifiable risk factor for AD; however, the impact of specific nutrients on disease onset and progression is difficult to define due to human genetic diversity and diet complexity. Here, we used the genetically tractable nematode *C. elegans*, which consumes a simple *E. coli* diet, and discovered that vitamin B<sub>12</sub> reduces A $\beta$  proteotoxicity. In A $\beta$ -expressing animals, vitamin B<sub>12</sub> increased ATP levels, reduced mitochondrial fragmentation, and decreased ROS but did not impact A $\beta$  levels. Currently, there is no effective disease-modifying treatment for AD, and therapeutics designed to reduce A $\beta$  levels have failed (Long and Holtzman, 2019). Our results suggest that vitamin B<sub>12</sub> supplementation could be a therapeutic approach to target energy crisis and oxidative stress, pathogenic features of AD triggered by A $\beta$  accumulation.

Subclinical B<sub>12</sub> deficiency is common, with a prevalence of 10%–15% among individuals older than 60 years and up to 35% among those older than 80 years (Green et al., 2017), and low B<sub>12</sub> status may be a modifiable risk factor for AD (Vogiatzoglou et al., 2008). Meta-analyses of clinical trials have suggested that administration of B vitamins does not prevent cognitive decline (Clarke et al., 2014; Ford and Almeida, 2019). However, most participants did not exhibit preexisting cognitive defects, trial durations were insufficient to observe cognitive decline, and prior B<sub>12</sub> status was not considered. Our results show that vitamin B<sub>12</sub> supplementation was beneficial for A $\beta$ -expressing *C. elegans* with mild B<sub>12</sub> deficiency but did not offer additional protection for non-deficient animals. Consistent with our work, studies focused on individuals with low dietary vitamin B showed that supplementation with B vitamins preserved cognition (Kang et al., 2008) and slowed brain atrophy (Smith et al., 2010). Thus, the therapeutic potential for vitamin B<sub>12</sub> is likely dependent on preexisting B<sub>12</sub> status. How genetic profile and other components of the complex human diet further impact probability of vitamin B<sub>12</sub> therapeutic success will need to be resolved.

Reduced ATP level hinders cellular ability to maintain ionic gradients, leading to increased intracellular Ca<sup>2+</sup>, synaptic dysfunction, and neuronal death, as observed in AD pathogenesis. Mild vitamin B<sub>12</sub> deficiency led to reduced ATP in A $\beta$ -expressing, but not wild-type, *C. elegans*, suggesting that low B<sub>12</sub> status is detrimental during proteotoxic stress. Despite decreasing mitochondrial fragmentation, we found vitamin B<sub>12</sub> did not prevent A $\beta$ -induced defects in cellular respiration. Rather, B<sub>12</sub> status appears to have an impact on the A $\beta$ -induced energy crisis that precedes mitochondrial dysfunction (Fong et al., 2016). Both A $\beta$  oligomers and oxidative stress can induce membrane damage, which leads to ion leakage and necessitates increased ATP expenditure by ATPases to maintain ionic gradients and membrane potential (Butterfield and Halliwell, 2019; Ronquist and Waldenström, 2003; Yang et al., 2017). Glycolysis is the dominant ATP production pathway used to fuel plasma membrane ATPases (Ronquist and Waldenström, 2003; Yellen, 2018), and studies suggest a link between impaired glucose metabolism and the severity of neurodegenerative diseases (An et al., 2018; Zhang et al., 2021). We found that dietary vitamin B<sub>12</sub> reduced energy crisis and was protective in *C. elegans* models of AD, Huntington's disease, and ALS.



Vitamin B<sub>12</sub> causes resistance to pathogen stress in *C. elegans*, and this requires the methylmalonyl-CoA mutase MMCM-1 (Revtovich et al., 2019). In contrast, our work showed that loss of *metr-1* or *sams-1* eliminated the effect of diet on A $\beta$ -induced paralysis, suggesting that B<sub>12</sub> defends against proteotoxic stress by acting as a cofactor for methionine synthase, impacting the methionine/SAMe cycle. While methionine restriction results in epigenetic changes that extend lifespan in many organisms, including *C. elegans* (Grandison et al., 2009; Orentreich et al., 1993; Parkhitko et al., 2019), our work suggests that a reduction in methionine synthase activity is detrimental for nematodes under proteotoxic stress. Reduced methionine lowers SAMe, which is required for methylation-dependent synthesis of PtdCho. Altered ratios of phospholipids can influence energy metabolism as well as ion leak across the plasma membrane (Ronquist and Waldenström, 2003; van der Veen et al., 2017), and reduced PtdCho has been observed in individuals with AD (Whiley et al., 2014). Here, we found that choline supplementation, which increases PtdCho synthesis via the Kennedy pathway (Walker et al., 2011), eliminated the effect of vitamin B<sub>12</sub> on paralysis of A $\beta$  animals. Together, our results support a model in which vitamin B<sub>12</sub> status impacts methionine/SAMe-cycle-dependent phospholipid homeostasis to protect against A $\beta$ -induced proteotoxicity.

## STAR★METHODS

### RESOURCE AVAILABILITY

**Lead contact**—Further information and reagent requests should be directed to and will be fulfilled by the lead contact, Jessica Tanis (jtanis@udel.edu).

**Materials availability**—*C. elegans* strains generated from our studies will be provided upon request. The University of Delaware Material Transfer Agreement Request Webform will be completed when research materials are transferred to an outside party.

**Data and code availability**—All original data have been deposited at Mendeley Data and are publicly available as of the date of publication; DOI is listed in the key resources table.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

**Nematode Culture**—All *C. elegans* strains were maintained on nematode growth medium (NGM) at 20°C as described (Brenner, 1974). The wild-type strain was Bristol N2; all other strains used in this study can be found in the key resources table. *eat-2(ad465)* was detected with SuperSelective genotyping (Touroutine and Tanis, 2020); standard duplex PCR genotyping was used for all deletion mutants.

**Bacterial Strains**—*Escherichia coli* strains used in this study include OP50, HB101, HT115, and DA837. Bacterial cultures for assay plates were grown in LB overnight shaking at 37°C to an OD<sub>600</sub> of 1.0 (Eppendorf BioPhotometer D30) and seeded onto NGM plates.

All plates were dried for three days at room temperature and stored at 4°C before use. Relative amount of bacteria on seeded plates was measured by washing bacteria off plates with M9 buffer following a 24 hour temperature shift to 25°C and OD<sub>600</sub> was measured using an Eppendorf BioPhotometer D30.

## METHOD DETAILS

**A $\beta$ -induced Paralysis Assay**—Animals were synchronized by a pulse egg lay for paralysis assays, however, we note that the dietary shift in paralysis was also observed with worms synchronized by bleaching or picking of late fourth larval stage (L4) animals to assay plates (not shown). Four adult animals were placed on 6 cm NGM plates seeded with different bacteria for 4 hours and then removed. The animals grew at 20°C and then were shifted to 25°C when the majority reached the late L4 stage, identified by a white crescent with black central dot at the vulva. Starting 12 to 20 hours post temperature upshift, depending on the genotype of the strain being assayed, the number of paralyzed and non-paralyzed animals were counted every two hours. Due to substantial acceleration of A $\beta$ -induced paralysis in *pek-1* mutants, animals were assessed for movement every hour following temperature upshift until all paralyzed. To determine the impact of B<sub>12</sub> post development, animals grown on OP50+B<sub>12</sub> plates were transferred to B<sub>12</sub>-free OP50 plates at the end of the L4 stage immediately prior to temperature upshift. For the inverse experiment, animals grown on B<sub>12</sub>-free plates were transferred to B<sub>12</sub> supplemented plates at the end of L4 stage. A minimum of three biological replicates were performed per *C. elegans* strain / bacterial condition. Data were used to generate Kaplan-Meier survival plots and determine median time to paralysis (GraphPad Prism 9).

**Nutrient and Metabolite Supplementation**—Nutrient supplemented plates for paralysis assays contained 10mM D(+)-glucose (Fisher Scientific), 0.3mM sodium homogamma linolenate (Nu-Chek Prep), 0.1mM or 0.3mM oleic acid (ThermoFisher Scientific), 148nM or 740 nM methylcobalamin (Millipore Sigma), 1.34 mM, 6.7 mM, 13.4mM, or 67 mM L-methionine (Fisher Scientific), 1 mM, 10 mM, or 20 mM choline chloride (Millipore Sigma), and 15mM or 30 mM DL-Homocysteine (Millipore Sigma). Supplements were added to autoclaved NGM media at 55°C. Specific concentrations used for each supplementation experiment are indicated in the figures and legends. Some of these concentrations for glucose (Alcántar-Fernández et al., 2018), fatty acids (Deline et al., 2013), methylcobalamin (Revtovich et al., 2019), L-methionine (Wei and Ruvkun, 2020), homocysteine (Wei and Ruvkun, 2020), and choline (Walker et al., 2011) were used in prior *C. elegans* work; additional concentrations were used to determine dose-dependent effects for certain treatments. Plates were stored at 4°C. Fatty acid supplemented plates were covered with foil to prevent light oxidation. Plates were seeded with the different *E. coli* cultures (OD<sub>600</sub> = 1.0) and dried for three days before use.

**Mitochondrial imaging and analysis**—Mitochondrial morphology was visualized by imaging *C. elegans* expressing RFP-tagged TOMM-20 (*P<sub>myo-3</sub>::tomm-20::mKate2::HA::tbb-2 3' UTR*) 24 hours after temperature upshift of late L4 animals to 25°C. Animals were immobilized with 10  $\mu$ M levamisole on 3% agar pads and Z stack images were obtained with a Zeiss LSM880 confocal microscope.

Images were analyzed with ImageJ by drawing a ROI around muscles and using the script below to determine mitochondrial length. Values below 1  $\mu\text{m}$  were excluded, then average branch length was calculated. Images from at least thirty animals were analyzed for each condition. The following script was used: run("Clear Results"); run("Median...", "radius=1.5"); run("Unsharp Mask...", "radius=2.5 mask=0.90"); setAutoThreshold("Li dark"); //run("Threshold..."); setOption("BlackBackground," true); run("Convert to Mask"); run("Skeletonize (2D/3D)"); run("Analyze Skeleton (2D/3D)," "prune=[shortest branch] prune\_0 calculate show display"); run("Summarize");

**Vitamin B<sub>12</sub> reporter imaging**—Images of *Pacdh-1::gfp* expression were taken of adult animals, 24 hr post L4, that had been immobilized on 3% agarose pads with 10  $\mu\text{M}$  levamisole. All images were collected under identical exposure conditions using a Zeiss AxioZoom V16 microscope with AxioCam 702 mono camera and ZEN 2.3 Digital Imaging System.

**Nile Red Staining**—Nile Red staining of fixed *C. elegans* was performed as previously described with modifications (Brooks et al., 2009). Synchronized populations of animals were raised on plates  $\pm$  0.3 mM oleic acid and then suspended in 1 mL of water 24 hr post L4. 50  $\mu\text{L}$  of fresh 10% paraformaldehyde solution (Electron Microscopy Sciences) was added, and the worms were placed on a nutator for 1 hr at room temperature. The PFA solution was removed, 1 mL of 1  $\mu\text{g}/\text{mL}$  Nile Red (ThermoFisher Scientific) in 1x M9 was added, and the worms were incubated for 30 minutes at room temperature. After removal of the Nile Red solution, the animals were washed 1x with M9 and mounted on 3% agarose pads. Z stack images were obtained with a Zeiss LSM880 confocal microscope using identical settings.

**ATP, ROS, and Homocysteine Quantification**—Approximately 1000 first larval stage (L1) animals synchronized by bleaching were grown on 10 cm plates at 20°C. Once the animals reached late L4, plates were shifted to 25°C for 24 hours (Figure 2) or 18 hours (Figures 3 and 4). Animals were washed 3x with 1x M9 and sonicated on ice with Tris-EDTA buffer (100mM Tris, 4mM EDTA pH 7.75) using a model 150V/T Ultrasonic Homogenizer for 5 minutes, then centrifuged at 14,000 RPM for 15 minutes at 4°C. The supernatant was collected and moved to a fresh tube. ATP quantitation was performed with the ATP Bioluminescence Assay Kit CLS II (Roche Diagnostics) using a Glomax Multi-Detection System reader as described (Chaya et al., 2021). ATP was normalized to protein content measured with the Pierce BCA protein assay kit (ThermoFisher Scientific). Triplicate technical replicates were performed for each sample; at least four biological samples were assayed for each dietary condition.

For ROS quantification, animals were prepared as for ATP quantification except animals were sonicated on ice in M9 buffer. To normalize samples, the Pierce BCA protein assay kit was used to determine supernatant volume required for 25 mg of protein. Hydrogen peroxide levels were measured using 2',7'-Dichlorofluorescein diacetate/H<sub>2</sub>DCFDA (Sigma-Aldrich) as described (Yoon et al., 2018). Briefly 50  $\mu\text{L}$  of 50  $\mu\text{M}$  H<sub>2</sub>DCFDA was added to normalized worm samples in black 96 well plates and incubated at room temperature for 6 hours before fluorescence was measured. The Amplex Red Hydrogen Peroxide/

Peroxidase Assay Kit (ThermoFisher Scientific) was also used to measure hydrogen peroxide levels. Superoxide levels were assessed with the MitoSox Red Mitochondrial Superoxide Indicator Kit (ThermoFisher Scientific) following manufacturers protocols. Fluorescence and absorbance values were measured using a Glomax Multi-Detection System. Triplicate technical replicates were performed for each sample; at least six biological samples were assayed for each dietary condition.

For homocysteine quantification, animals were prepared as for ROS quantitation. Homocysteine levels in supernatants containing 25  $\mu\text{g}$  of protein were determined using the Homocysteine Assay Fluorometric Kit (Abcam) following manufacturer instructions. Fluorescence was measured with use of a Glomax Multi-Detection System. Triplicate technical replicates were performed for each sample; four biological samples were assayed for each condition.

**Oxygen Consumption Rate Measurements**—Oxygen consumption was determined using a Seahorse XFe96 Analyzer (Agilent) as described (Ng and Gruber, 2019) with some modifications. Compounds to be injected were made and pipetted into the sensor cartridge from the Seahorse XFe96 FluxPak (Agilent) as follows. 40 mM Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; Millipore Sigma) stock was made from 10 mg FCCP in 984  $\mu\text{l}$  DMSO and 25  $\mu\text{l}$  aliquots were stored at  $-20^{\circ}\text{C}$ . 25  $\mu\text{l}$  of FCCP stock was diluted in 2475  $\mu\text{l}$  M9 buffer immediately before use and 22  $\mu\text{l}$  of this 400  $\mu\text{M}$  FCCP was loaded into drug port A of the sensor cartridge for a final well concentration of 40  $\mu\text{M}$  following injection. 800 mM sodium azide (Acros Organics) stock was made from 130 mg sodium azide in 2.5 mL  $\text{dH}_2\text{O}$ ; 24  $\mu\text{l}$  was loaded into drug port B of the sensor cartridge for a final well concentration of 80 mM following injection. Animals were grown as indicated for the ATP assays, then washed 3x with 1x M9 buffer 24 hours post  $25^{\circ}\text{C}$  temperature shift and diluted to  $\sim 1$  worm/ $\mu\text{l}$  in M9. 20  $\mu\text{l}$  of nematodes in M9 solution were pipetted into 180  $\mu\text{l}$  of M9 buffer. 10–18 animals in a total volume of 200  $\mu\text{l}$  was the target number of nematodes for each well of a Seahorse XFe96 Cell Culture Microplate; four blank wells without animals were left for background correction.

Basal respiration measurements were conducted for 6 loops, then FCCP was injected and maximal respiration was measured for 6 loops, followed by sodium azide injection and non-mitochondrial oxygen consumption was measured for 6 loops. Each loop consisted of mix for 3 minutes, wait for 2 minutes, measure for 3 minutes. Data analysis was performed on wells with than contained 10–18 animals similar to previously described (Koopman et al., 2016). Basal respiration was calculated by averaging oxygen consumption rate (OCR) from loops 3–6 then subtracting non-mitochondrial oxygen consumption as determined from loop 18, the last measurement following sodium azide addition. Maximal respiration was determined following FCCP injection by averaging OCR from loops 9–11, then subtracting non-mitochondrial OCR from loop 18. For each well, basal and maximal OCR were divided by the number of animals in that well and are presented as  $\text{pmol O}_2/\text{min}/\text{worm}$ . Spare capacity was determined by subtracting basal from maximal OCR. Three independent assays were conducted over different days; at least 18 wells were analyzed per strain / diet.

**Western Blotting**—Eggs from gravid adults were isolated by bleaching and allowed to hatch rocking in M9 buffer overnight. Approximately 1000 starved L1s were pipetted onto each plate the following day. Plates were moved to 25°C when a majority of animals were late L4 and 24 hours later, worms were washed 3x with M9 and the pellet was flash frozen. 100  $\mu$ L lysis buffer (100mM NaCl, 100mM Tris pH 7.5, 1% NP-40) supplemented with an EDTA-free Protease Inhibitor cocktail tablet (Roche) was added to the pellet, sonicated on ice (Model 150V/T Ultrasonic Homogenizer), and centrifuged 15 minutes at 4°C. The supernatant was transferred to a new tube and 20  $\mu$ L was set aside for protein quantification (Pierce BCA protein assay kit). 2x protein sample buffer (80 mM Tris-HCl, 2% SDS, 10% glycerol, 0.0006% Bromophenol blue with 10%  $\beta$ -mercaptoethanol and 8M Urea) was added to the supernatant. Samples were heated at 55°C for 5 minutes and 30  $\mu$ g of protein per sample was run on Tris-Tricine 16.5% precast polyacrylamide gels (BioRad) for A $\beta$  or 10% Tris-Glycine (TGX) precast protein gels (BioRad) for NUO-2. Samples were transferred onto 0.2  $\mu$ m (A $\beta$ ) or 0.45  $\mu$ m (NUO-2) nitrocellulose membranes (BioRad) for 40 minutes at 70V. Membranes were blocked in 5% non-fat milk in TBS with 0.1% Tween-20 one hour at room temperature. Primary antibody incubation was overnight at 4°C; A $\beta$  (6E10 Biolegend cat#803001, 1:1000), NUO-2 (Abcam cat#ab14711, 1:5000), and  $\alpha$ -tubulin (Sigma cat#T9026, 1:5000) antibodies were used. Incubation with a goat anti-Mouse IgG-HRP (ThermoFisher Scientific cat#31430, 1:5000) secondary antibody conjugated to horseradish peroxidase was 1 ½ hr. at room temperature. Chemiluminescence detection used SuperSignal West Pico PLUS (ThermoFisher Scientific) and a Chemidoc MP Imaging System (BioRad). NUO-2 and the  $\alpha$ -tubulin loading control were detected together on the same membrane, however, A $\beta$  membranes had to be stripped and re-probed with the loading control antibody to ensure equal protein loading across gels. All experiments were done with five biological replicates.

**qRT-PCR**—*C. elegans* were synchronized as described for western blotting. 24 hr after temperature upshift, animals were washed three times with M9 and one time with non-DEPC treated RNase free water (Fisher Scientific), then transferred to a microcentrifuge tube. 400  $\mu$ L of Trizol (ThermoFisher Scientific) was added and samples were flash frozen. RNA was isolated using the RNeasy Mini Kit (QIAGEN). Briefly, 200  $\mu$ L of Trizol was added to thawed samples along with 140  $\mu$ L of chloroform (ThermoFisher Scientific). Samples were centrifuged, the aqueous layer was removed, and equal volume of 70% ethanol was added. RNeasy spin columns (Quiagen) were used for on-column DNase treatment. Total RNA was transcribed into cDNA using the iScript cDNA Synthesis kit (BioRad). qRT-PCR was performed with PowerUp SYBR Green Master Mix (Applied Biosystems) using a Quantstudio 6 Flex Real-time PCR System (ThermoFisher Scientific) following the standard cycling mode with an anneal/extend temperature at 58°C followed by a default dissociation step. *act-2* was used as the housekeeping reference. The  $C_t$  method was used to determine relative expression. Triplicate technical replicates were performed for each sample; data presented are from at least three biological replicates per condition.

**Pharyngeal Pumping Rate Measurements**—Animals were grown at 20°C to early L4 stage. Using the Zeiss AxioZoom V16 microscope the number of pharyngeal pumps per 30 s was counted. At least 25 animals were measured per diet.

**Thrashing assay**—Motility was assessed by quantitating thrashing rate in liquid. The wild-type was compared to animals expressing either a mutated form of human SOD-1 or a polyglutamine expansion transgene 24 hours post L4 on day 1 of adulthood. Nematodes were individually placed into a 10  $\mu$ l drop of M9 buffer on a microscope slide and allowed to acclimate for 1 minute. The number of body bends completed in 1 minute was determined for 15 animals per strain / condition.

**Preparation of Figures**—Main text and supplemental figures were made with Adobe Illustrator; the graphical abstract was created with [BioRender.com](https://www.biorender.com).

## QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed with one-way ANOVA, performing multiple comparisons with the Dunnett test except where otherwise noted. Statistical analyses and graphing were performed with GraphPad Prism 9. Significant differences are indicated as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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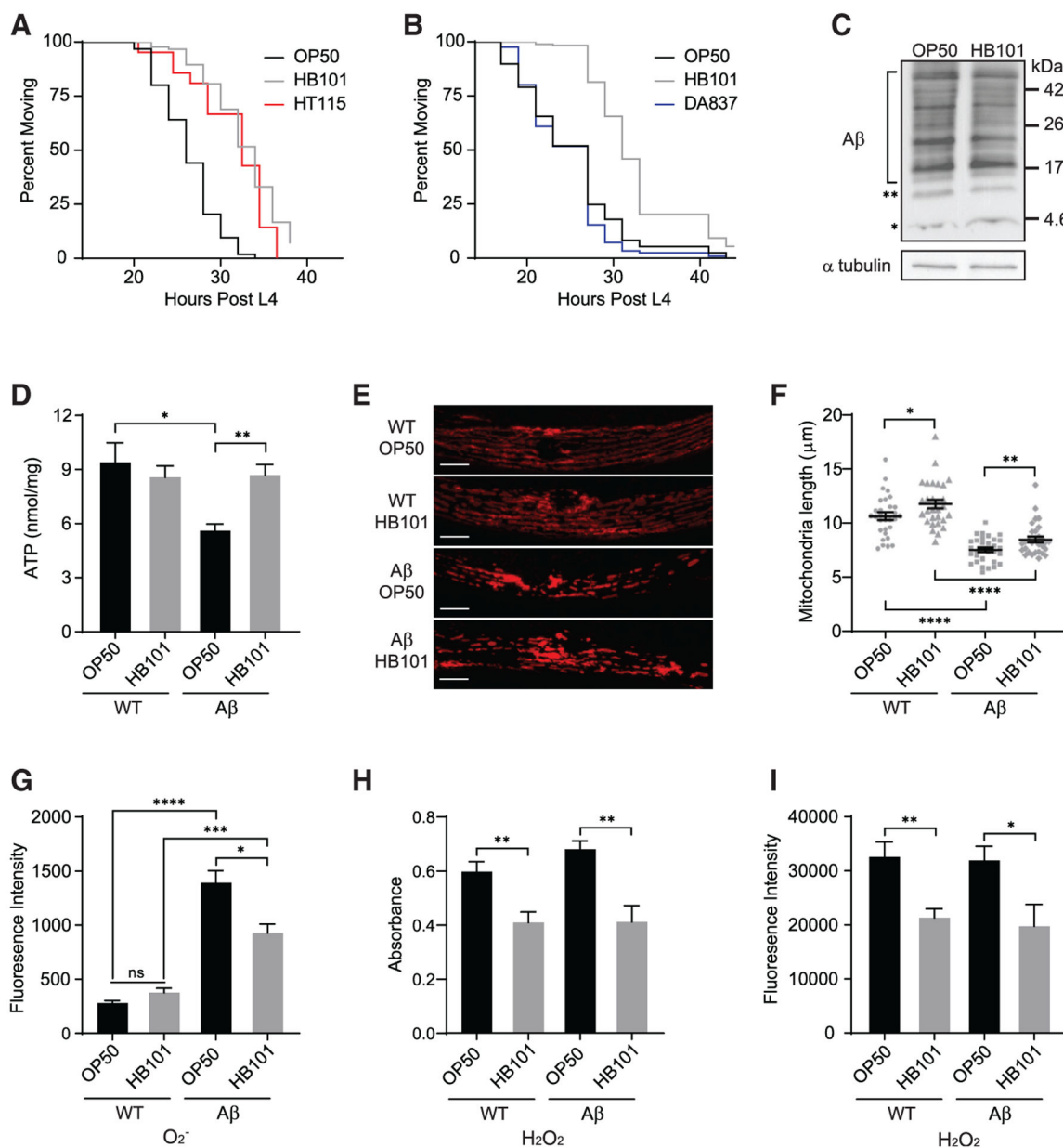


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**Highlights**

- Dietary vitamin B<sub>12</sub> reduces the proteotoxic effects of A $\beta$  in *C. elegans*
- Vitamin B<sub>12</sub> is protective even when given to deficient worms only during adulthood
- B<sub>12</sub> has this impact by acting in *C. elegans* as a cofactor for methionine synthase



**Figure 1. Diet impacts A $\beta$ -induced paralysis, energy availability, oxidative stress, and mitochondrial morphology without altering A $\beta$  accumulation**

(A) A $\beta$  animals fed HB101 (gray) and HT115 (red) exhibited delayed paralysis compared to those on OP50 (black) (n = 89).

(B) A $\beta$  animals fed DA837 (blue) and OP50 (black) became paralyzed at the same time (n = 183).

(C) Western analysis showed no effect of diet on A $\beta$  accumulation. The A $\beta$  monomer and dimer are indicated by asterisks (\* and \*\*, respectively); bracket shows A $\beta$  oligomers.

Additional replicates are in Figure S1F.

(D) ATP levels in A $\beta$  *C. elegans* fed OP50 were reduced compared to wild type (WT); the HB101 diet increased ATP in A $\beta$  animals (n = 10).

(E) Representative images of mitochondria, visualized with TOMM-20::RFP, in WT and A $\beta$  animals. Scale bars, 10  $\mu$ m.

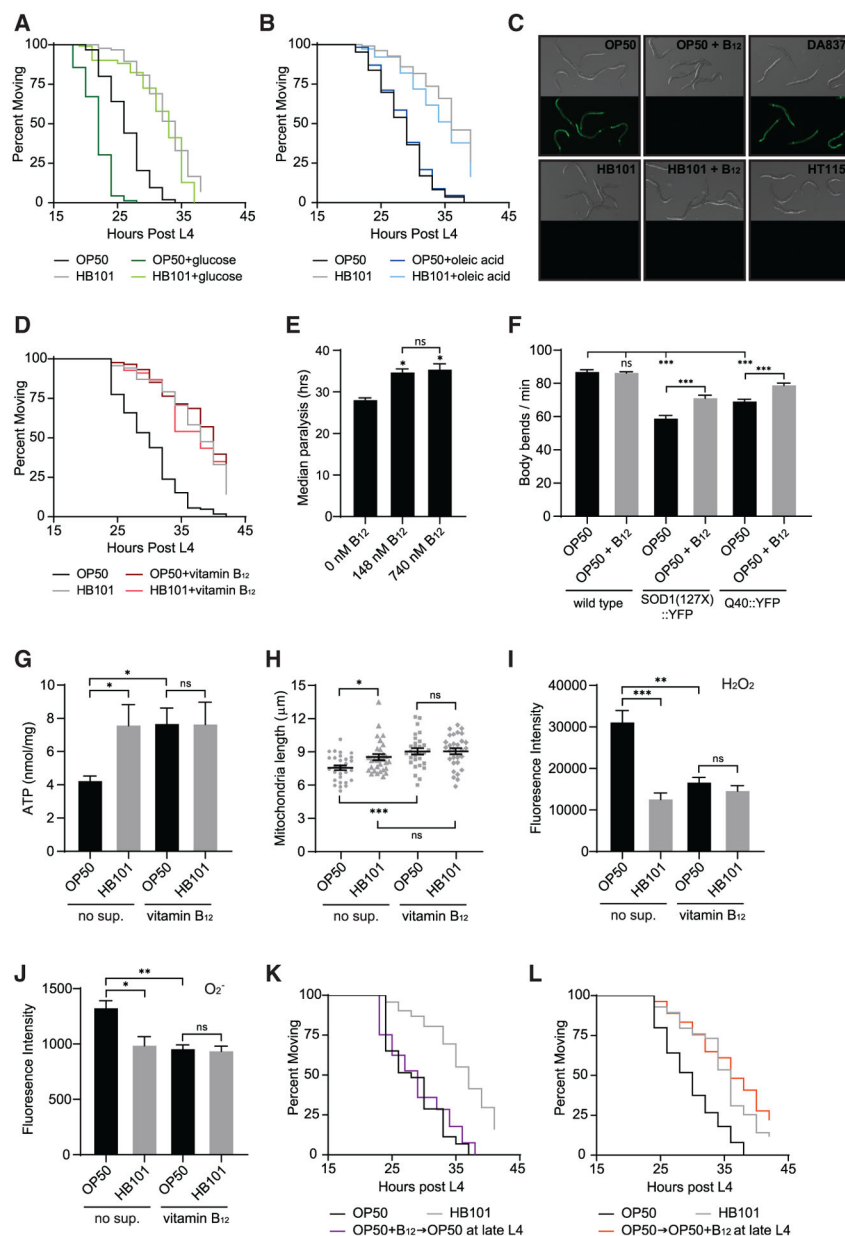
(F) Average mitochondrial length was affected by A $\beta$  accumulation and diet; individual measurements (gray symbols, n = 30) indicated.

(G) Superoxide (O $_2^-$ ), measured with MitoSox, was higher in A $\beta$  animals fed OP50 versus HB101 (n = 11).

(H) Hydrogen peroxide (H $_2$ O $_2$ ) level, quantified with Amplex red, was greater in both WT and A $\beta$  nematodes given OP50 (n = 7).

(I) H $_2$ O $_2$ , measured with H $_2$ DCFDA, was increased in WT and A $\beta$  animals fed OP50 (n = 8).

Error bars show SEM; \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001, one-way ANOVA with Dunnett's post-test. See also Figures S1 and S2.



**Figure 2. Vitamin B<sub>12</sub> is the protective factor in the HB101 diet**

(A) Supplementation with 10 mM glucose accelerated paralysis of A $\beta$  animals fed OP50 but did not eliminate the dietary shift (n = 102).

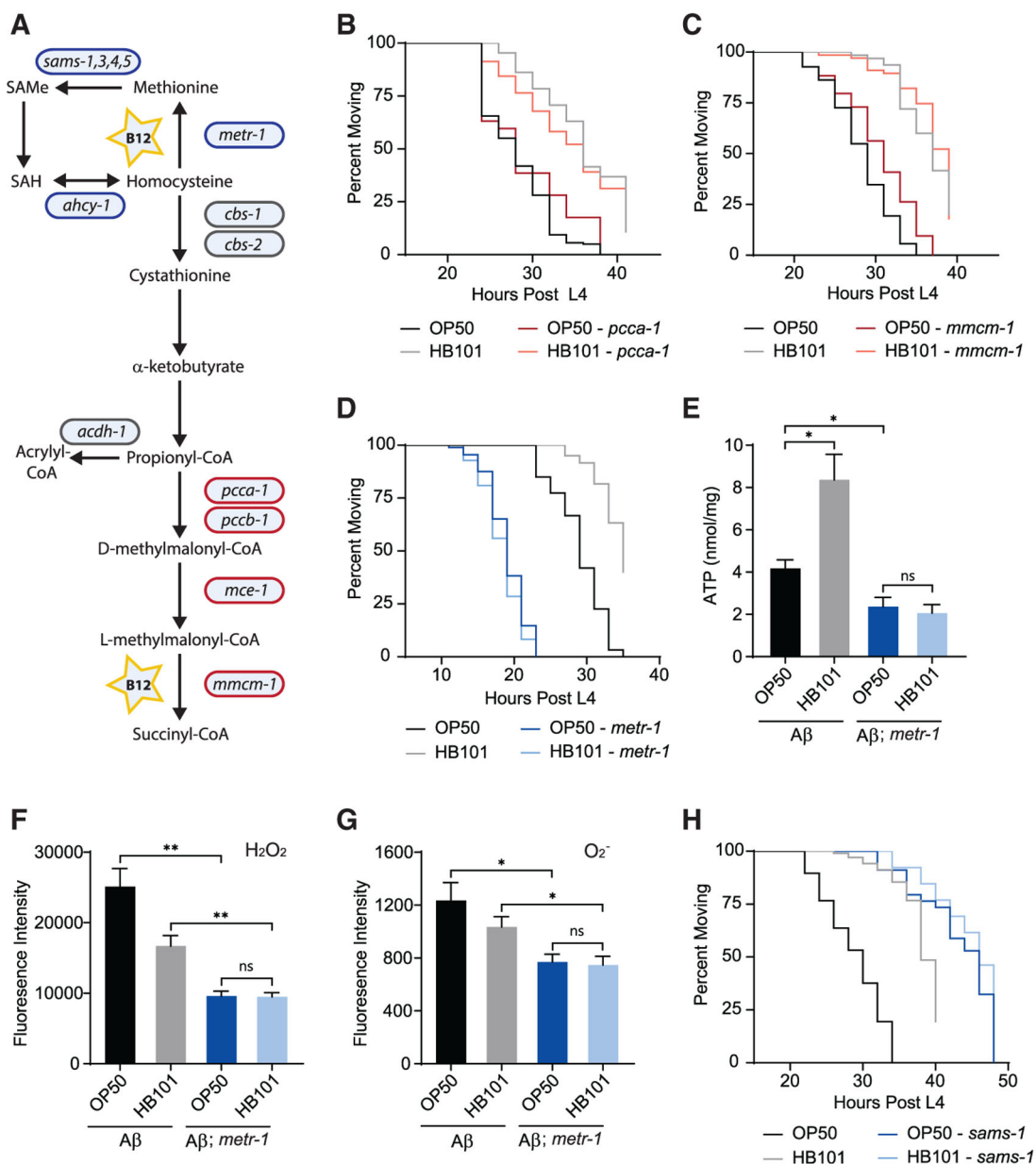
(B) Supplementation with 0.3 mM oleic acid did not affect A $\beta$ -induced paralysis (n = 174).

(C) *Pacdh-1::GFP* expression was induced in animals fed OP50 and DA837, but not OP50+B<sub>12</sub>, HB101, HB101+B<sub>12</sub>, and HT115.

(D) Supplementation with 148 nM vitamin B<sub>12</sub> eliminated the impact of bacterial diet on paralysis of A $\beta$  animals (n = 139).

(E) Supplementation with 740 nM B<sub>12</sub> had no added protective effect compared to 148 nM B<sub>12</sub> (n = 3); \*p < 0.05 compared to the no-supplementation control.

- (F) B<sub>12</sub> supplementation enhanced motility in *C. elegans* models of amyotrophic lateral sclerosis (ALS) and Huntington's disease but did not affect the WT (n = 15).
- (G) Vitamin B<sub>12</sub> increased ATP levels in A $\beta$  animals fed OP50 compared to those without B<sub>12</sub> supplementation (no sup.; n = 6).
- (H) Vitamin B<sub>12</sub> increased average mitochondrial length in A $\beta$  animals fed OP50 (n = 30).
- (I) Vitamin B<sub>12</sub> reduced H<sub>2</sub>O<sub>2</sub>, measured with H<sub>2</sub>DCFDA, in A $\beta$  animals fed OP50 (n = 9).
- (J) Vitamin B<sub>12</sub> decreased O<sub>2</sub><sup>-</sup>, measured with MitoSox, in A $\beta$  animals fed OP50 (n = 6).
- (K) Transfer of A $\beta$  animals from OP50 plus vitamin B<sub>12</sub> plates to B<sub>12</sub>-free OP50 plates at the end of L4 eliminated the B<sub>12</sub> protective effect (n = 132).
- (L) Transfer of A $\beta$  animals fed OP50 to OP50 plates supplemented with B<sub>12</sub> at the end of L4 delayed paralysis (n = 54).
- Error bars show SEM; \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001 one-way ANOVA with Dunnett's post-test. See also Figures S2 and S3.



**Figure 3. The methionine/SAMe cycle is required for the protective effects of vitamin B<sub>12</sub>**

(A) Diagram of vitamin-B<sub>12</sub>-dependent pathways in *C. elegans*. Genes encoding enzymes that function in the methionine/SAMe cycle (blue) and the canonical B<sub>12</sub> pathway (red) are indicated. SAMe, S-adenosylmethionine; SAH, s-adenosyl-L-homocysteine.

(B) *pcca-1(ok2282)* had no effect on the diet-induced shift in paralysis of A $\beta$  animals (n = 57).

(C) Loss of *mmcm-1(ok1637)* did not eliminate the dietary shift (n = 67).

(D) *metr-1(ok521)* accelerated A $\beta$ -induced paralysis and eliminated the impact of *E. coli* diet (n = 60).

(E) In *metr-1* mutant A $\beta$  animals, ATP levels were low and unaffected by diet (n = 7).

(F) H<sub>2</sub>O<sub>2</sub> levels were unaffected by diet in *metr-1* mutant A $\beta$  animals (n = 4).



(G)  $O_2^-$  levels were unaffected by diet in *metr-1* mutant  $A\beta$  animals (n = 6).

(H) *sams-1(ok2946)* delayed  $A\beta$ -induced paralysis and eliminated the dietary shift (n = 26).

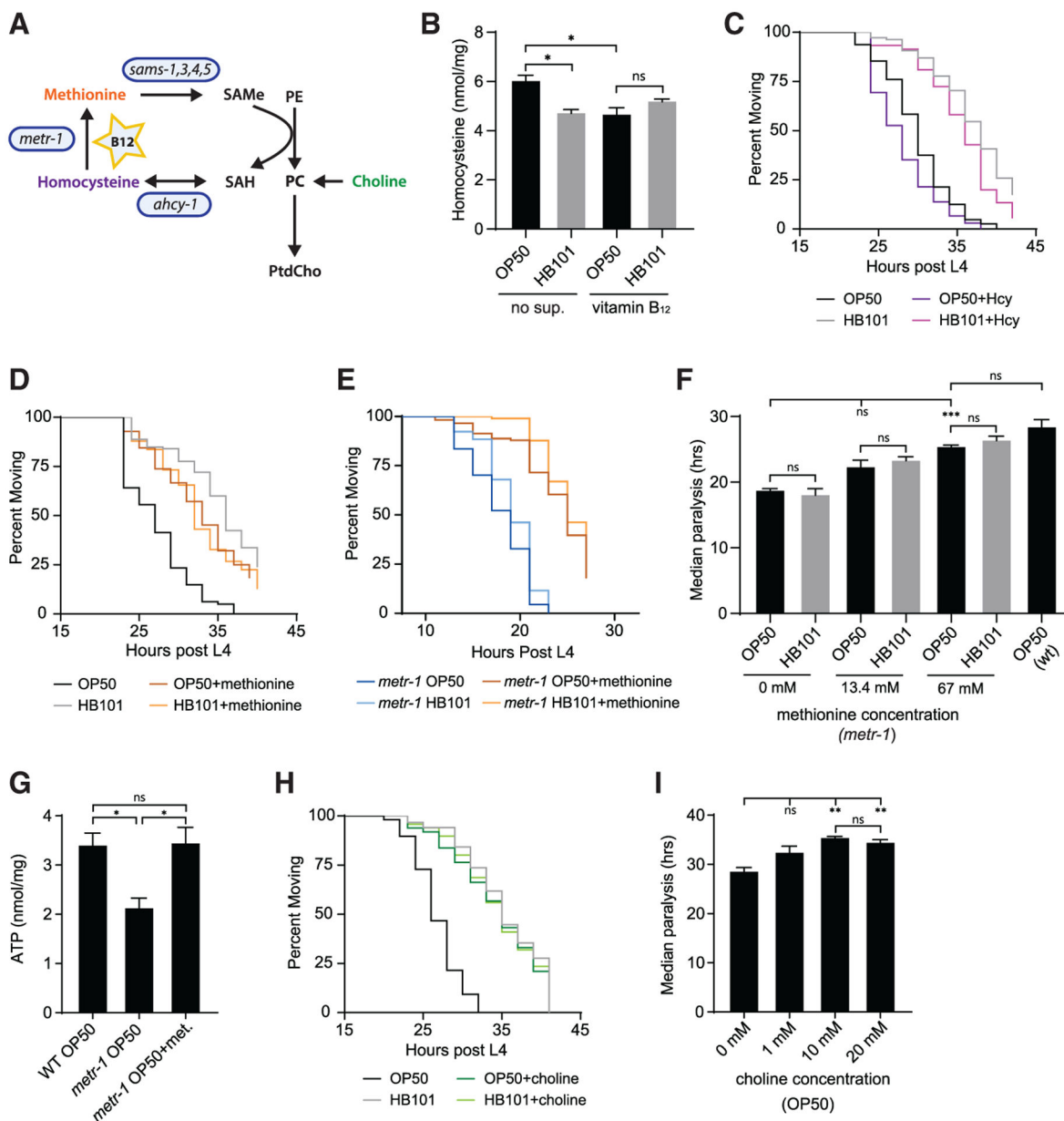
Error bars show SEM; \*p < 0.05 and \*\*\*p < 0.001, one-way ANOVA with Dunnett's post-test. See also Figure S4.

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**Figure 4. Methionine and choline supplementation reduce A $\beta$  proteotoxic effects in B<sub>12</sub>-deficient animals**

(A) Schematic diagram shows link between the vitamin-B<sub>12</sub>-dependent methionine/SAMe cycle (blue) and phospholipid biosynthesis. SAH, s-adenosyl-l-homocysteine; PE, phosphoethanolamine; PC, phosphocholine; PtdCho, phosphatidylcholine. PtdCho can be synthesized from choline or methylation of PE.

(B) A $\beta$  animals grown on OP50 had higher levels of Hcy compared to those raised on HB101- or B<sub>12</sub>-supplemented plates (n = 4).

(C) Supplementation with 15 mM Hcy accelerated paralysis of A $\beta$  animals grown on both OP50 and HB101 but did not eliminate the dietary shift (n = 105).

(D) Supplementation with 13.4 mM L-methionine eliminated the diet-induced shift in paralysis (n = 116).

(E) Methionine supplementation (67 mM) delayed A $\beta$ -induced paralysis in the *metr-1* mutant (n = 67).

(F) Supplementation of the *metr-1* mutant with 13.4 mM and 67 mM methionine delayed the median time to A $\beta$ -induced paralysis (n = 3).

(G) Supplementation with 67 mM methionine caused a significant increase in ATP in the *metr-1* mutant (n = 4).

(H) Supplementation with 10 mM choline eliminated the impact of bacterial diet on A $\beta$ -induced paralysis (n = 107).

(I) Choline concentration above 10 mM had no additional protective effect (n = 3).

Error bars show SEM; \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001, one-way ANOVA with Dunnett's post-test. See also Figure S4.

## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti- $\beta$ -Amyloid 6E10	BioLegend	Cat#803001; RRID: AB_2564653
anti- $\alpha$ -tubulin A11	Millipore Sigma	Cat#T9026; RRID: AB_477593
Anti-NDUFS3	Abcam	Cat#ab14711; RRID: AB_301429
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	ThermoFisher Scientific	Cat#31430; RRID: AB_228307
Bacterial and virus strains		
<i>Escherichia coli</i> OP50	<i>Caenorhabditis</i> Genetics Center (CGC)	N/A
<i>Escherichia coli</i> HB101	CGC	N/A
<i>Escherichia coli</i> HT115	CGC	N/A
<i>Escherichia coli</i> DA837	CGC	N/A
Chemicals, peptides, and recombinant proteins		
D(+)-glucose	Fisher Scientific	Cat#: AC410955000 CAS: 50-99-7
Sodium Homogamma Linolenate	Nu-Chek Prep	Cat#: S-1143, CAS: 65881-87-0
Oleic Acid	ThermoFisher Scientific	Cat#:31997, CAS: 112-80-1
Methylcobalamin	Millipore Sigma	Cat#: M9756, CAS: 13422-55-4
L-methionine	Fisher Scientific	Cat#: AC166160025 CAS: 63-68-3
DL-Homocysteine	Millipore Sigma	Cat#: H4628, CAS: 454-29-5
Choline Chloride	Millipore Sigma	Cat#: C7017-5G, CAS:67-48-1
Levamisole hydrochloride	Fisher Scientific	Cat#: AC187870100 CAS: 16595-80-5
EDTA-free Protease Inhibitor cocktail	Roche	Cat#: 4693159001
NP-40	ThermoFisher Scientific	Cat#: 85124, CAS: 9016-45-9
Sodium Dodecyl Sulfate	Fisher Bioreagents	Cat#: BP1311-200, CAS: 151-21-3,7732-18-5
Sodium Chloride (NaCl)	Fisher Bioreagents	Cat#: BP358-1 CAS:7647-14-5
1M Tris-HCl pH 8.3	Teknova	Cat#: T5083
Tris Base	Fisher Bioreagents	Cat#: BP152-500, CAS: 77-86-1
TRIS-buffered saline (TBS, 10x)	Alfa Aesar	Cat#: J62938-K2, CAS: 77-86-1
Tween-20	Fisher Bioreagents	Cat#: BP337-100 CAS: 9005-64-5
Non-Fat Powdered Milk	Boston BioProducts	Cat#: P-1400
$\beta$ -mercaptoethanol	Fisher Scientific	Cat#: AC125470100 CAS: 60-24-2
Bromophenol Blue	Fisher Scientific	Cat#: AC403140050 CAS: 115-39-9
Urea	ThermoFisher Scientific	Cat#: 29700, CAS: 57-13-6
Glycerol	Fisher Bioreagents	Cat#: BP229-1, CAS: 56-81-5
10x Tris/Glycine/SDS Buffer	BioRad	Cat#: 1210732
Glycine	Fisher Bioreagents	Cat#: BP381-500 CAS:56-40-6
Methanol	Fisher Chemical	Cat#: A454-4, CAS: 67-56-1
16.5% Mini-Protean Tris-Tricine Gel	BioRad	Cat#: 4563063
10% Mini-Protean TGX Precast Protein Gels	BioRad	Cat#: 4561033
Trizol	ThermoFisher Scientific	Cat#: 15596018

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chloroform	Crescent Chemical Co.	Cat#: 39553.01, CAS: 67-66-3
RNase-Free DNase Set	QIAGEN	Cat#: 79254
PowerUp SYBR Green Master Mix	Applied Biosystems	Cat#: A25741
0.5M EDTA pH 8.0	Invitrogen	Cat#: 15575-038
2'7'-Dichlorofluorescein diacetate	Millipore Sigma	Cat#: D6883-250, CAS: 4091-99-0
Nile Red	ThermoFisher Scientific	Cat#: N1142
Agarose	Fisher Bioreagents	Cat#:BP160-500, CAS: 9012-36-6
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP)	Millipore Sigma	Cat#: C2920-10, CAS: 370-86-5
DMSO (Dimethyl Sulfoxide) LC-MS Grade	ThermoFisher Scientific	Cat#: 85190, Cas:67-68-5
Sodium Azide	Acros Organics	Cat#: 19038-1000, CAS: 26629-22-8
Paraformaldehyde (16%) Solution EM Grade	Electron Microscopy Sciences	Cat#: 15710, CAS: 30525-89-4
Water, Molecular Grade, Sterile, DEPC Free	Fisher Scientific	Cat#: R91450001G, CAS: 7732-18-5
Critical commercial assays		
Pierce BCA Protein Assay Kit	ThermoFisher Scientific	Cat#: 23327
ATP Bioluminescence Assay Kit CLS II	Roche Diagnostics	Cat#: 11699695001
SuperSignal West Pico PLUS Chemiluminescent Substrate	Thermo Scientific	Cat#: 34580
iScript cDNA Synthesis Kit	BioRad	Cat#: 1708890
RNeasy Mini Kit	QIAGEN	Cat#: 74104
MitoSox Red Mitochondrial Superoxide Indicator	ThermoFisher Scientific	Cat#: M36008
Amplex red Hydrogen Peroxide/Peroxidase Assay Kit	ThermoFisher Scientific	Cat#: A22188
Homocysteine Fluorometric Kit	Abcam	Cat#: Ab228559
Seahorse XFe96 FluxPak	Agilent	Cat#: 102416-100
Seahorse XFe96 Cell Culture Microplate	Agilent	Cat#: 101085-004
Deposited data		
Mendeley Data	This study	<a href="https://doi.org/10.17632/djv7tg4rv.1">https://doi.org/10.17632/djv7tg4rv.1</a>
Experimental models: Organisms/strains		
<i>Caenorhabditis elegans</i> (WT)	CGC	N2
<i>dvIs100 [unc-54p::A-beta-1-42::unc-54 3' UTR + mtl-2p::GFP]V</i>	CGC	GMC101
<i>smg-1(cc546)I; dvIs27 [myo-3p::A-Beta (1-42)::let-851 3' UTR] + rol-6(su1006)]X</i>	CGC	CL4176
<i>eat-2(ad465)II; dvIs100[unc-54p::A-beta-1-42 + mtl-2p::GFP]V</i>	This study	UDE96
<i>gcn-2(ok871)II; dvIs100 [unc-54p::A-beta-1-42::unc-54 3' UTR + mtl-2p::GFP]V</i>	This study	UDE90
<i>dvIs100[unc-54p::A-beta-1-42::unc-54 3' UTR + mtl-2p::GFP]V; pek-1(ok275)X</i>	This study	UDE21
<i>foxSi16 [myo-3p::tomm-20::mKate2::HA::tbb-2 3' UTR]1</i>	CGC	SJZ47
<i>foxSi16 [myo-3p::tomm-20::mKate2::HA::tbb-2 3' UTR]1; dvIs100 [unc-54p::A-beta-1-42 + mtl-2p::GFP]V</i>	This study	UDE123

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>wwIs24 [acdh-1p::GFP + unc-119(+)]</i>	CGC	VL749
<i>dvIs100[unc-54p::A-beta-1-42::unc-54 3' UTR + mtl-2p::GFP]V; pcca-1(ok2282)X</i>	This study	UDE148
<i>metr-1(ok521)II; dvIs100 [unc-54p::A-beta-1-42::unc-54 3' UTR + mtl-2p::GFP]V</i>	This study	UDE146
<i>mmcm-1 (ok1637)III; dvIs100[unc-54p::A-beta-1-42 + mtl-2p::GFP]V</i>	This study	UDE194
<i>dvIs100[unc-54p::A-beta-1-42 + mtl-2p::GFP]V; sams-1 (ok2946)X</i>	This study	UDE205
<i>rmIs133[unc-54p::Q40::YFP]I</i>	CGC	AM141
<i>rmIs290 [unc-54p::Hsa-sod-1 (127X)::YFP]IV</i>	CGC	AM725
Oligonucleotides		
<i>eat-2(ad465)II</i>	<i>eat-2</i> forward: GCTCATTCGCACTGTTGAA TAACACTTATAGCGGTTTCT	Common Reverse: GTTACTTA AGGCGTACGAGCC
Wild type at <i>eat-2(ad465)II</i> locus	WT forward: GCTCATTCGCACTGTTGAATAACA CTTATAGC GGTTC	Common Reverse: GTTACTTA AGGCGTACGAGCC
<i>gcn-2(ok871)II</i>	<i>gcn-2</i> forward: GTGTTCACCGCTG AATCGG	Common Reverse: GACCACA TCCATCGCAACAC
Wild type at <i>gcn-2(ok871)II</i> locus	WT forward: GCAGTGTCTTCGCTTCTC	Common Reverse: GACCACA TCCATCGCAACAC
<i>pek-1(ok275)X</i>	<i>pek-1</i> forward: CTCTTGACAGCGTACTCG	Common Reverse: GCCAACA GTACATCGATG
Wild type at <i>pek-1(ok275)X</i> locus	WT forward: GACACCAATCTCAGCTGTAG	Common Reverse: GCCAACA GTACATCGATG
<i>pcca-1(ok2282)X</i>	<i>pcca-1</i> forward: CAGGTGAGGGTAATGGCATC	Common Reverse: GCTGGA GCCAAGTTCATC
Wild type at <i>pcca-1(ok2282)X</i> locus	WT forward: CCTGAAGACGAGTGTTTCATC	Common Reverse: GCTGGA GCCAAGTTCATC
<i>metr-1(ok521)II</i>	<i>metr-1</i> forward: GAGGAGTCGACGTG CTTCTC	Common Reverse: CGTCCG ACAAAGGAAGGC
Wild type at <i>metr-1(ok521)II</i> locus	WT forward: GTCGTTGGAGAGCTGTTC	COMMON REVERSE: CGTCCGA CAAAGGAAGGC
<i>mmcm-1(ok1637)III</i>	<i>mmcm-1</i> forward: GATGGCATTCTTGG GACCAG	Common Reverse: GAGAGACGC GGAGCAAATGC
Wild type at <i>mmcm-1(ok1637)III</i> locus	WT forward: GAGAGACGCGGAG CAAATGC	Common Reverse: GAGAGAC GCGGAGCAAATGC
<i>sams-1(ok2946)X</i>	<i>sams-1</i> forward: GGAGAAATTACCT CCAAGGC	Common Reverse: GCGATAGC AGATGTGGCTGG
Wild type at <i>sams-1(ok2946)X</i> locus	WT forward: GGTCTCTACCCAACACTCTC	Common Reverse: GCGATAGC AGATGTGGCTGG
<i>nuo-2</i> qRT-PCR primers	GTGCTGACTGTTTGTGAGC	GACCTCGTTGTATCCCC
<i>mev-1</i> qRT-PCR primers	GGACAGATCTACAAATCGGG	CTTGTTGCTCTTGTCTCGGC
<i>cyc-1</i> qRT-PCR primers	CGACATTGCTTCTGTACG	CAGTCATGATTGTGTCAACG
<i>nduo-1</i> qRT-PCR primers	GCCATCCGTGCTAGAAGAC	CAAATGGCGCCCGGTTAAG
<i>act-2</i> qRT-PCR primers	GCGCAAGTACTCCGTCTGGATCG	GGGTGTGAAAATCCGTAAGGCAGA
Software and algorithms		
ImageJ	National Institutes of Health	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
Graph Pad Prism 9	GraphPad Software Inc.	<a href="https://www.graphpad.com/">https://www.graphpad.com/</a>

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Quantstudio6 Real-time PCR	ThermoFisher Scientific	Cat: 4485694
ZEN 2.3 Digital Imaging System	Zeiss	<a href="https://www.zeiss.com/corporate/int/home.html?vaURL=www.zeiss.de/en">https://www.zeiss.com/corporate/int/home.html?vaURL=www.zeiss.de/en</a>

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