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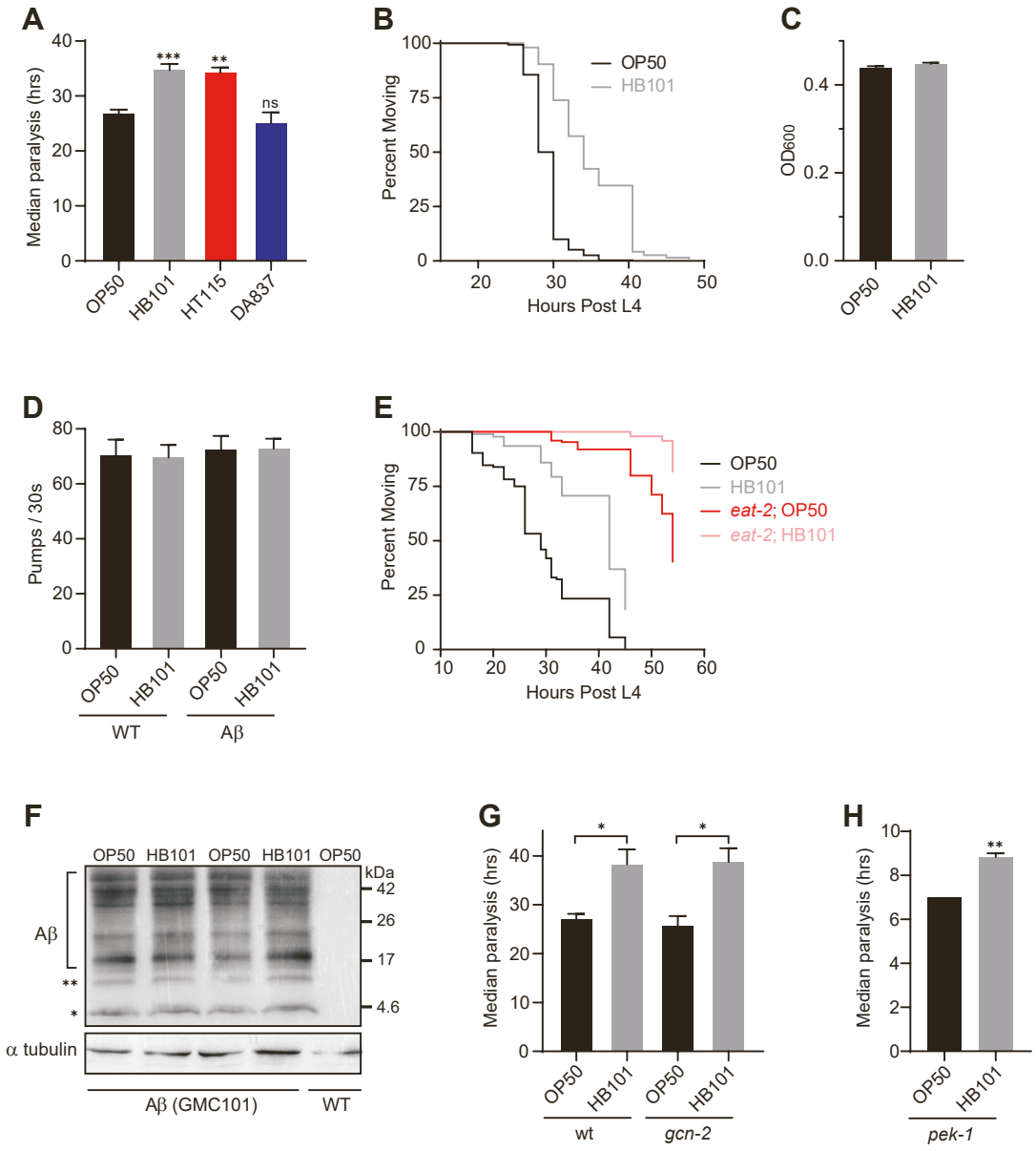
Supplemental information

Vitamin B_{12} impacts amyloid beta-induced proteotoxicity by regulating the methionine/S-adenosylmethionine cycle

Andy B. Lam, Kirsten Kervin, and Jessica E. Tanis

Supplemental Figure 1



Supplemental Figure 1: The impact of diet on Aβ-induced paralysis is not due to dietary restriction or differences in Aβ accumulation, related to Figure 1.

(A) Median time to paralysis for Aβ animals fed OP50 (black), HB101 (grey), HT115 (red), and DA837 (blue) *E. coli* (n≥3; one-way ANOVA with Dunnett's post-test).

(B) Dietary protective effects were not limited to the GMC101 Aβ-expressing strain. CL4176 nematodes, which also express Aβ in the body wall muscles, paralyzed slower when fed HB101 (grey) compared to OP50 (black; n≥260).

(C) OD_{600} measurements showed no significant difference in bacterial growth between OP50 (black) and HB101 (gray) indicating that the Aβ nematodes were exposed to the same amount of bacteria when grown on these diets (n=9; ns two-tailed t-test with Welch's correction).

(D) Pharyngeal pumping rates were not different between WT and Aβ nematodes raised on OP50 (black) and HB101(gray) bacteria (n≥25; ns, one-way ANOVA with Dunnett's post-test).

(E) Loss of *eat-2(ad465)* delayed Aβ-induced paralysis in animals grown on OP50 (red) and HB101 (pink), but HB101 still caused a delay (n≥49).

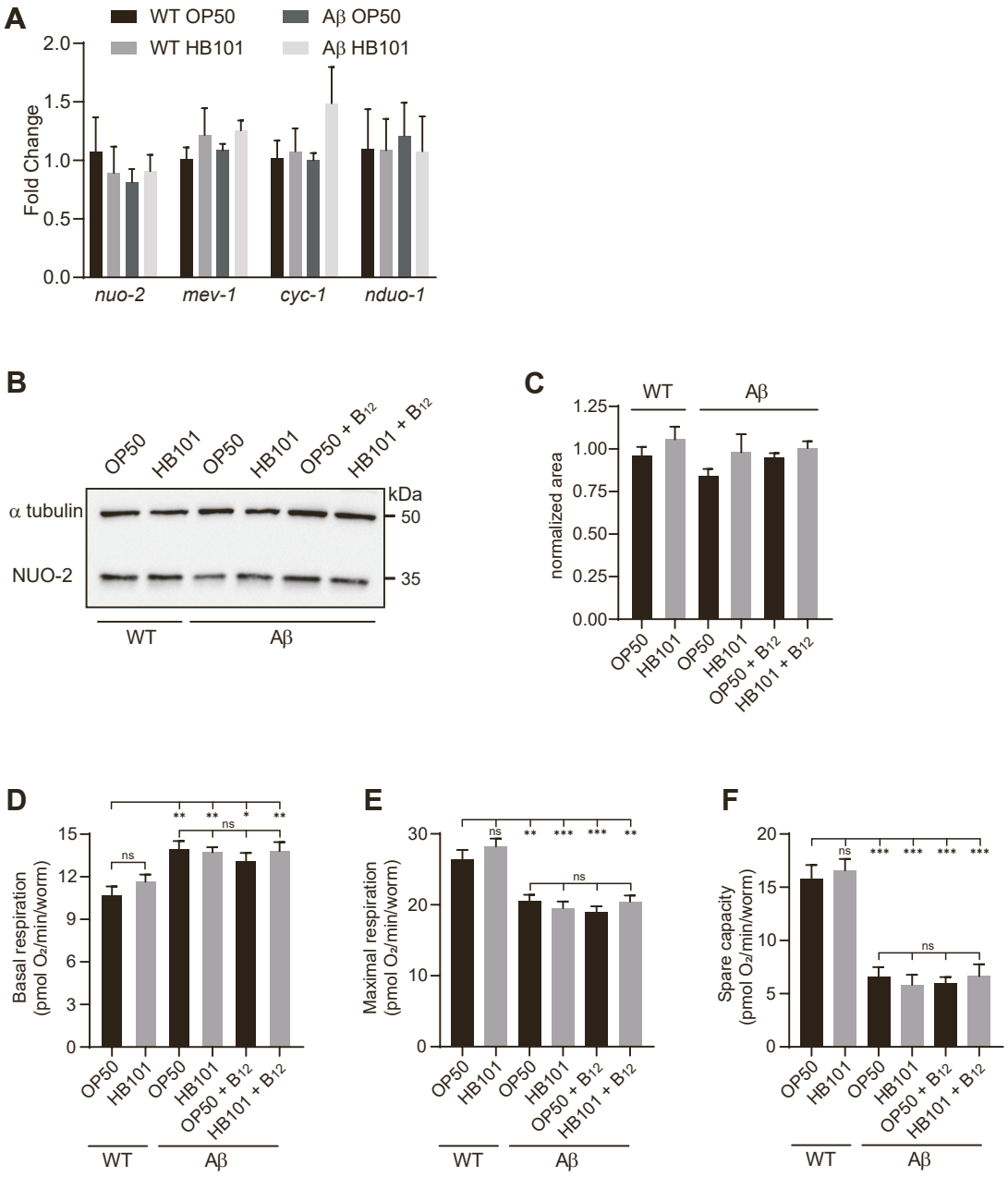
(F) As in Figure 1D, two additional Western replicates presented here showed no effect of diet on Aβ accumulation; five biological replicates performed in total. Aβ signal is detected in GMC101 Aβ animals, but not in the wild type (WT). The Aβ monomer (•), assembles into dimers (••) and higher order oligomers (bracket).

(G) Two mutants were used to determine if repression of protein synthesis is required for the HB101 protective effect. The GCN-2 kinase phosphorylates elongation initiation factor 2 alpha (eIF2α) during times of nutrient deprivation and mitochondrial stress (Baker *et al.* 2012; Rousakis *et al.* 2013). Loss of *gcn-2(ok871)* in Aβ animals raised on either diet did not affect median time to paralysis (n=4; one-way ANOVA with Dunnett's post-test).

(H) In response to misfolded protein accumulation, the endoplasmic reticulum UPR sensor PEK-1 phosphorylates eIF2α (Shen et al., 2005). Loss of *pek-1(ok275)* accelerated Aβ-induced paralysis, but HB101 still caused a delay compared to OP50 (n=3; two-tailed t-test with Welch's correction).

Error bars show SEM; **p<0.01, ***p<0.001.

Supplemental Figure 2



Supplemental Figure 2: Diet has no impact on oxidative phosphorylation gene transcript levels, mitochondrial protein content, or respiration, related to Figures 1 and 2.

(A) qRT-PCR analysis of transcripts from representative genes required for oxidative phosphorylation (n≥3). No significant differences were observed between wild type and Aβ animals grown on the two different diets. Error bars represent SEM.

(B) Western analysis of NUO-2, a component of mitochondrial complex I; the α-tubulin control was detected on the same blot as NUO-2.

(C) Quantification of NUO-2 signal, normalized to the α-tubulin control from the same blot. There was no significant difference in NUO-2 protein between animals grown on OP50 and HB101, with or without vitamin B_{12} supplementation. Analysis was performed using Image J on five biological replicates, all run on different gels.

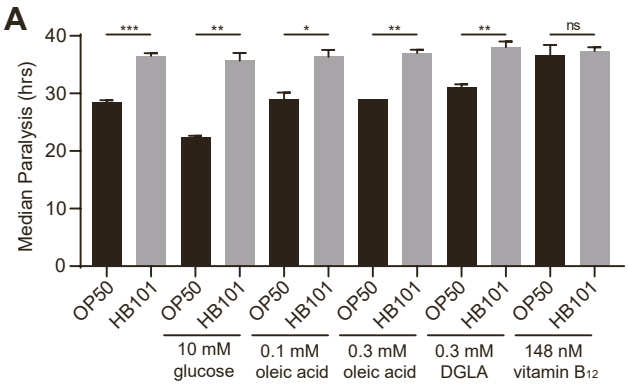
(D) Different parameters of mitochondrial respiration analyzed in wild type and Aβ animals with a Seahorse XFe96 Analyzer. For each condition, n≥18 wells with 10-18 animals per well. Diet had no impact on basal respiration, however Aβ expression did cause an increase in oxygen consumption under basal conditions. The ATP synthase inhibitor oligomycin works poorly in *C. elegans*, thus it is not possible to determine if the increased oxygen consumption in Aβ animals results from a change in ATP production linked respiration or an increase in proton leak.

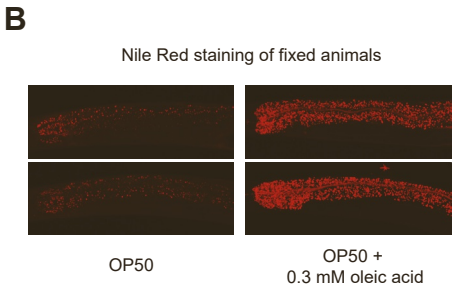
(E) Addition of FCCP dissipates the proton gradient and causes a collapse of the mitochondrial membrane potential, causing complex IV oxygen consumption to reach a maximal rate as cells try to re-establish the proton gradient. While diet had no effect on maximal respiration, Aβ expression resulted in significantly lower oxygen consumption following FCCP exposure.

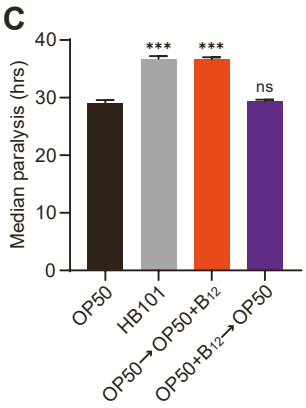
(F) Spare respiratory capacity, determined as the difference between the basal and maximal respiration states, was significantly reduced in Aβ animals. *E. coli* diet and vitamin B_{12} had no impact on spare capacity.

Error bars show SEM; **p<0.01, ***p<0.001, one-way ANOVA with Dunnett's post-test.

Supplemental Figure 3







Supplemental Figure 3: Dietary vitamin B_{12} in adulthood delays Aβ-induced paralysis, related to Figure 2.

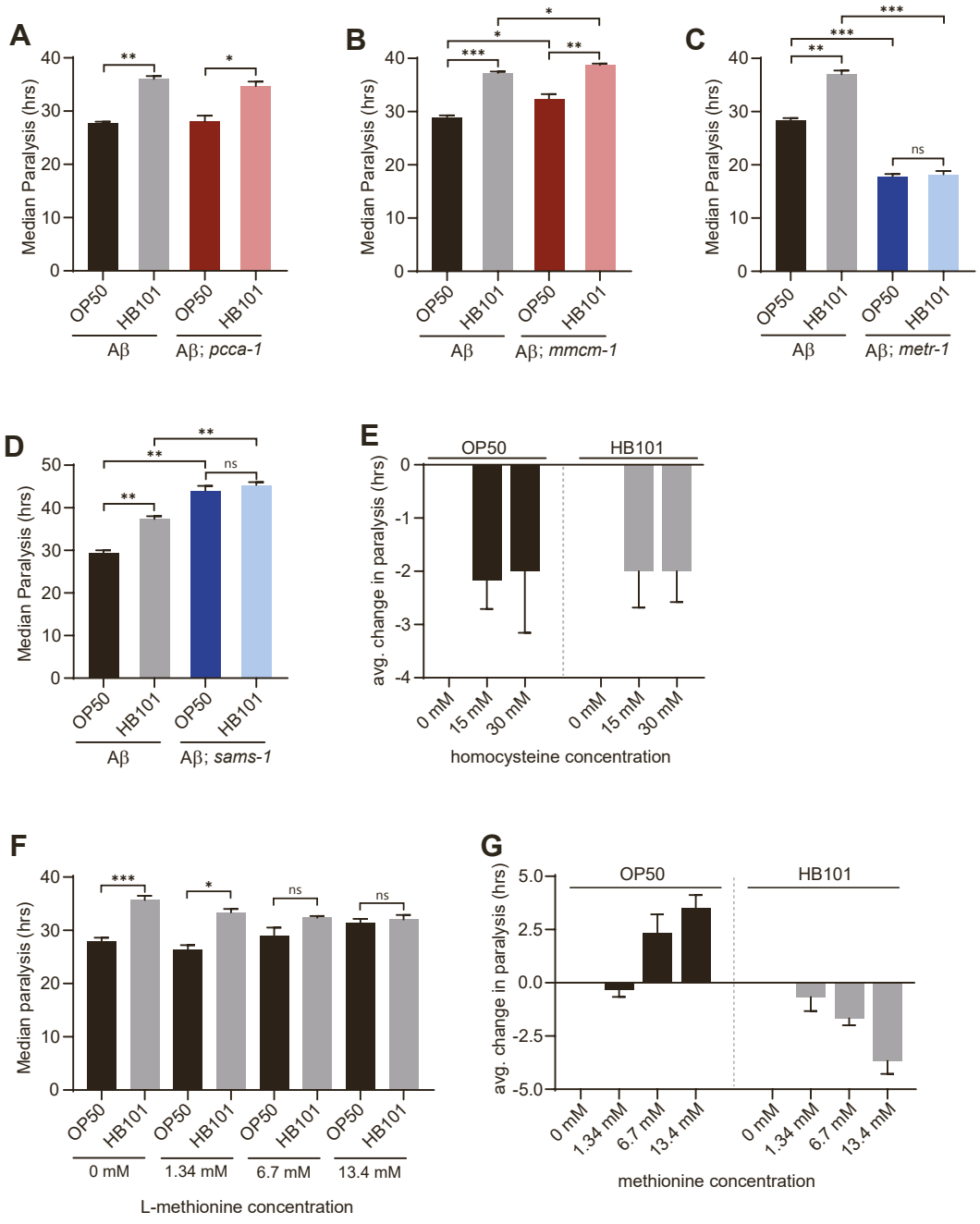
(A) Median time to paralysis for Aβ nematodes fed either OP50 (black) or HB101 (grey) on NGM plates with glucose, oleic acid, dihomo-gamma-linoleate (DGLA), vitamin B_{12}, or no supplementation ($n \geq 3$; one-way ANOVA without correction for multiple comparisons).

(B) Nile Red staining of paraformaldehyde-fixed animals. Supplementation with 0.3 mM oleic acid increased the intensity of Nile-Red stained droplets, which has been shown to correlate with an increase in triacylglycerol stores.

(C) Median time to paralysis for Aβ nematodes fed OP50 without supplementation (black) or with B_{12} introduced (orange) or removed (purple) at the end of the L4 stage; compare to those given the OP50 diet (n=3; one-way ANOVA with Dunnett's post-test)

Error bars show SEM; *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 4



Supplemental Figure 4: B_{12} deficiency causes defects in the methionine/SAM cycle, related to Figures 3 and 4.

(A) Loss of *pcca-1* had no impact on the median time to paralysis for Aβ animals grown on OP50 or HB101 (n=3).

(B) Median time to paralysis for Aβ animals grown on OP50 or HB101 was delayed by loss of *mmcm-1*, however, the diet-induced shift was not affected (n=5).

(C) Median time to paralysis was not significantly different between Aβ-expressing *metr-1* mutants raised on OP50 or HB101 (n=5).

D) Loss of *sams-1* eliminated the impact of diet on median time to paralysis in Aβ animals (n=3).

E) Average change in median time to paralysis (hrs) for Aβ nematodes on plates supplemented with 15 mM or 30 mM homocysteine compared to the respective diet controls (n=3).

F) Dose-dependent effects of 1.34 mM, 6.7 mM and 13.4 mM L-methionine supplementation on Aβ animals (n≥3).

G) Average change in median time to paralysis for Aβ animals grown on plates supplemented with 1.34 mM, 6.7 mM, or 13.4 mM L-methionine (n≥3).

Error bars show SEM; *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA with Dunnett's post-test.