**SIN3 maintains adaptive capacity to different diets**

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**Abstract**

Diet has profound effects on metabolism and health, requiring animals to adapt their physiology to varying food sources. Here we show that in *Caehnorhabditis elegans* the conserved SIN3 transcriptional coregulator is required for animals to adapt to different bacterial diets. Shifting *sin-3* mutant animals from the commonly used *E. coli* OP50 strain to the *E. coli* HT115 strain used for RNAi knockdown experiments has a dramatic impact on survival, leading to lethality at the young adult stage. A major difference between OP50 and HT115 is vitamin B12 content, which is lower in OP50 than HT115. Feeding *sin-3* mutant animals on OP50 bacteria supplemented with vitamin B12 reproduced the lethality observed on HT115. Vitamin B12 functions in two metabolic pathways: the canonical propionate breakdown pathway and the methionine/S-adenosylmethionine (met/SAM) cycle. Loss of *metr-1*, encoding the methionine synthase enzyme that requires vitamin B12 as a cofactor in the met/SAM cycle repressed lethality on HT115. Conversely, complementation with metabolites of the met/SAM cycle enhanced lethality on OP50. These results show that SIN3 is required for animals to adapt to dietary changes through B12-dependent metabolic pathways.

## Introduction

Complex diseases arise through the interplay between genetic and environmental factors including diet (Curran et al. 2009). While interactions between the host, its diet and its microbiota are essential for the health of the organism, demonstrating reproducible gene–diet interactions can be challenging. In *C. elegans*, numerous diet-dependent effects on animal physiology and behaviour have been reported (Shtonda and Avery 2006; Soukas et al. 2009; Maier et al. 2010; Gracida and Eckmann 2013a; MacNeil et al. 2013; Macneil and Walhout 2013; Pang and Curran 2014; Frézal et al. 2023), but only a few diet- gene pairs have been identified where the consequence of mutating a specific gene is only observed on specific diets (Pang and Curran 2014; Verma et al. 2018; Amin et al. 2020; Nair et al. 2022). Here we report a diet-dependent phenotype associated with loss of the SIN3 transcriptional coregulator in *C. elegans.* In previous work we showed that SIN3 is required for fertility, and plays a role in mitochondrial dynamics and metabolic flux (Giovannetti et al. 2024). How diet, or the observed metabolic changes relate to *sin-3* mutant phenotypes and physiology was not investigated. Here we report that *sin-3* null mutant animals that are viable on OP50 bacteria routinely used to grow *C. elegans* (Brenner 1974), die as young adults when fed on the HT115 bacteria utilized to elicit an RNA interference (RNAi) response. Lethality on HT115 is due to an “exploded" phenotype resulting from extrusion of the intestine through the vulva. One well described difference between these two bacterial strains is vitamin B12 content, which is lower in OP50 than HT115 (Watson et al. 2013; Watson et al. 2014; Watson et al. 2016; Revtovich et al. 2019; Nair et al. 2022), and we show that complementing OP50 with vitamin B12 reproduces the "exploded" phenotype and lethality of *sin-3* mutants on HT115. As in humans, in *C. elegans* vitamin B12 is required in two metabolic pathways, the canonical mitochondrial propionate breakdown pathway, and one-carbon (1C) metabolism composed of the methionine/S- adenosylmethionine (met/SAM) and folate cycles. In the canonical propionate breakdown pathway, vitamin B12 acts as a co-factor for methyl malonyl-CoA mutase (MUT, MMCM-1 in *C. elegans*) that catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA. In the methionine cycle, it is a cofactor for methionine synthase (MS, METR-1 in *C. elegans*) that synthesizes methionine from homocysteine (Watson et al. 2013 ; Watson et al. 2014 ; Watson et al. 2016; Green et al. 2017). We show that RNAi knock-down of *metr-1*, but not *mmcm-1*, suppresses the lethality of *sin-3* mutant animals on

the B12-replete HT115 diet, indicating that the activity of the met/SAM cycle contributes to lethality. Supplementation with 1C cycle metabolites further showed that the met/SAM, but not the folate cycle, contributes to lethality in *sin-3* mutant animals on the vitamin B12-depleted OP50 strain. Although the vitamin B12- dependent activity of the canonical propionate breakdown pathway does not contribute to lethality in *sin-3* mutants, these animals are more sensitive to propionate on the OP50 diet, possibly due to reduced expression in these animals of genes in the alternative propionate shunt pathway active under low vitamin B12 conditions (Watson et al. 2016). Altogether these results suggest that metabolic rewiring in the absence of *sin-3* renders animals sensitive to vitamin B12, identifying a role for SIN-3 in adaptation to dietary changes.

## Results

### Dietary vitamin B12 affects *sin-3* viability

The first hint that food source can impact the physiology of *sin-3* mutant animals came from the observation that a high proportion of *sin-3(tm1276)* null mutant animals fed on HT115, but not OP50 bacteria, died as young adults with an exploded vulva resulting in extrusion of the intestine (Figure 1A, right panel). The exploded phenotype, which is one of multiple pathologies observed in aged wild-type animals, has also been referred to as “age-associated vulval integrity defects” phenotype (AVID) (Leiser et al. 2016). Here we refer to the phenotype we observe in young animals as "exploded", without any reference to the underlying cause, which was not investigated in this study. To quantify the exploded phenotype, synchronized animals at the L1 stage were grown on plates seeded with either OP50 or HT115 bacteria, and visually scored when they reached day 3 of adulthood. No diet-dependent difference was observed in wildtype N2 adult animals, which as expected never showed the exploded phenotype at this stage (Leiser et al. 2016) (Figure 1B). *sin-3* mutant animals grown on OP50 developed normally to adulthood, with fewer than 5% dying from a ruptured vulva. In contrast, when grown on HT115 bacteria, 28% of day-3 mutant adults died with this phenotype. The exploded phenotype correlates negatively with temperature on both OP50 and HT115: it was rarer in worms at 25°C and more frequent in worms at 15 °C (Figure S1A). We also observed more variability at 20°C. The reasons for these temperature effects are not known. The number of exploded *sin-3* mutant animals on HT115 peaked at day 5 of adulthood, at which time point we sometimes observed more than 75% lethality, while

wiltype animals were 100% viable at this stage (Figure S1B). We chose the day 3 time point for all subsequent experiments in order to be able to measure any potential enhancement of this phenotype.

OP50 and HT115 bacteria differ in both the amount and composition of nutrients and metabolites (Brooks et al. 2009; Reinke et al. 2010). Because of this, these two diets differentially affect nearly every aspect of worm biology and physiology, including development, metabolism, behavior, and aging (Jeong et al. 2009; Gracida and Eckmann 2013; Reinke et al. 2010; Yen and Curran 2016; Stuhr and Curran 2020; Frézal et al. 2023). One of the major differences identified between these two strains is the level of vitamin B12. *C. elegans* acquires vitamin B12 from its bacterial food source. However, the *E. coli* strain OP50 poorly expresses TonB (Bassford et al. 1976), a transporter required for vitamin B12 uptake from the environment, causing it to be relatively deficient in vitamin B12 compared to HT115 (Watson et al. 2013; Watson et al. 2014; Revtovich et al. 2019; Nair et al. 2022). Supplementation of OP50-seeded plates with 25 nM cobalamin (vitamin B12) significantly increased the proportion of *sin-3* exploded animals from less than 5% to 20% (Figure 1B). These results are consistent with vitamin B12 levels being responsible for the increased lethality observed on HT115.

**Activity of the vitamin B12-dependent enzyme *metr-1* contributes to the exploded phenotype of *sin-3* mutant animals**

Vitamin B12 is an important co-factor in two metabolic pathways: the mitochondrial propionate breakdown pathway that prevents the toxic accumulation of propionate, and 1C metabolism formed by the met/SAM and folate cycles (Figure 2A). In the canonical propionate breakdown pathway, vitamin B12 acts as a co-factor for MMC-1, leading to the production of succinyl-Co. In the met/SAM cycle it serves as a co-factor for METR-1 (Watson et al. 2014; Watson et al. 2016; Green et al. 2017). We found that RNAi inactivation of *metr-1* rescued *sin-3* mutant lethality on HT115, with the percent of exploded animals significantly decreasing to less than 5% (Figure 2B). *mmcm-1* knockdown by contrast had no significant impact on the *sin-3* exploded phenotype. RT-qPCR confirmed a similar efficacy of RNAi knock-down in both wildtype and mutant backgrounds for each independent replica (Figure S2 A and B). These results indicate that vitamin B12 acts through *metr-1* in the met/SAM cycle to give rise to the exploded phenotype in *sin-3* mutant animals.

**Loss of *sin-3* reshapes the mitochondrial network independently of *drp-1*, with no evidence of vitamin B12 involvement**

One of the many functions attributed to vitamin B12 in *C. elegans* physiology is the maintenance of mitochondrial homeostasis, most likely through multiple mechanisms (Revtovich et al. 2019; Wei and Ruvkun 2020). Notably, vitamin B12 deficiency was shown to suppress the increase in mitochondrial fusion that occurs when DRP-1-the protein responsible for mediating mitochondria fission (fragmentation) (Campbell and Zuryn 2024)- is inactivated (Wei and Ruvkun 2020). Suppression of increased fusion by vitamin B12 depletion in this context was attributed to a compensatory increase in mitochondrial fission and mitochondrial mass, leading to a reestablishment of normal mitochondria dynamics by unknown mechanisms independent of DRP-1. The effect of vitamin B12 on *sin-3* mutants, and the striking increase in mitochondrial fragmentation we previously reported in these animals (Giovannetti et al. 2024) prompted us to explore the relationship between vitamin B12, mitochondrial function, and *sin-3* mutant phenotypes. We first asked whether *drp-1* inactivation in *sin-3* mutants could rescue increased fragmentation, as reported in other *C. elegans* contexts and in animal models of mitochondrial disease (Wei and Ruvkun 2020; Campbell and Zuryn 2024; Chen et al. 2024). Because *sin-3* mutant animals carrying the *drp-1(tm1108)* loss of function mutation were sterile, we instead carried out *drp-1* knock down by RNAi. To keep food conditions as similar as possible in these experiments, we used the recently developed RNA interference competent strain iOP50 (Neve et al. 2020), which only differs from OP50 in that it contains the same two alleles that render HT115 RNAi competent. *drp-1*(RNAi) in wildtype animals resulted in reduced fertility, as previously reported (Machiela et al. 2021), while *sin-3;drp-1*(RNAi) animals were sterile, similar to double mutants (Figure S3A). We confirmed that RNAi treatment resulted in at least a 50% decrease in mRNA levels in both wildtype and mutant animals (Figure S3B). As previously reported, microscopy analysis of mitochondria morphology in muscle cells using the TOMM-20::RFP marker revealed that mitochondria of *drp-1*-depleted animals formed aggregated networks, including individual mitochondria connected by thin threads (Dong et al. 2018and Figure S3C), while *sin-3* mutants showed increased fragmentation or interconnections(Giovannetti et al. 2024). No obvious improvement in mitochondria morphology was observed in *sin-3;drp-1*(RNAi) animals, where mitochondria remained either fragmented, or in some

cases even more highly connected that in either single knock-down or mutant (Figure S3C). The highly abnormal morphology and variability of mitochondria in these animals did not allow us to reproducibly quantify mitochondrial defects. Nonetheless, these results strongly suggest that the changes in mitochondrial dynamics observed in the absence of *sin-3* are independent of DRP-1. Our results also reveal that *drp-1* inactivation enhances the sterility of *sin-3* mutant animals. Additional experiments in which we tried to compare mitochondria dynamics in *sin-3* mutant animals raised on OP50 versus HT115 were inconclusive. Whether and how the lethality of *sin-3* mutants on HT115 might be related to an effect of vitamin B12 on mitochondrial homeostasis in these mutants therefore remains to be investigated.

**1C metabolites affect lethality in *sin-3* mutants**

Having shown that vitamin B12 acts through *metr-1* in the met/SAM cycle to negatively impact survival of *sin-3* mutant animals, we carried out additional experiments to identify relevant metabolites in this pathway. In 1C metabolism, 5-methyltetrahydrofolate (5-methyl-THF) acts as a methyl donor for the synthesis of methionine from homocysteine through the activity of METR-1/MS. Methionine is then converted into SAM, the main methyl donor in cells (Ducker and Rabinowitz 2017; Ye et al. 2017) (Figure 2A) required for phosphatidylcholine (PC) synthesis (Ducker and Rabinowitz 2017), as well as DNA, RNA, histone and protein methylation (Chiang et al. 1996). We asked whether feeding *sin-3* mutant animals on OP50 bacteria supplemented with different 1C metabolites reproduced the exploded phenotype and lethality observed on HT115. Specifically, we tested folinic acid, a derivative of tetrahydrofolic acid that is converted into the different metabolites to enhance the folate cycle, as well as methionine and SAM to enhance the met/SAM cycle (Figure 2A). Both methionine and SAM enhanced the exploded phenotype of mutant animals on OP50 (Figure 2C). The methylation-dependent pathway represents only one of the 2 avenues to synthesize PC (DeLong et al. 1999), which can also be synthesized from choline (Figure 2A). Choline supplementation also increased lethality in *sin-3* mutants (Figure 2C), suggesting that increased PC synthesis by either pathway may be deleterious in the context of SIN-3 depletion. Altogether, these results suggest that in the absence of SIN-3, METR-1 activity contributes to the exploded phenotype through methionine, SAM and PC. By contrast, folinic acid

supplementation had no effect on the survival of *sin-3* mutants, suggesting that this branch of 1C metabolism is not implicated (Figure 2C).

**Increased propionate sensitivity in *sin-3* mutant animals**

RNAi knockdown of *mmcm-1*, which requires vitamin B12 as a cofactor in the canonical mitochondrial propionate breakdown pathway (Figure 2A), had no effect on HT115 induced lethality, suggesting that this pathway is not implicated. Nonetheless, sensitivity to propionate could be altered in *sin-3* mutant animals. *C. elegans* has an alternate propionate breakdown pathway, or shunt, that is used under low vitamin B12 dietary conditions, or when flux through the canonical pathway is genetically perturbed (Watson et al. 2013; Watson et al. 2014; Watson et al. 2016 and Figure 2A). Because the shunt pathway generates toxic intermediates such as acrylyl-CoA (Watson et al., 2016; Bulcha et al. 2019), it is kept off when there is sufficient flux through the canonical pathway. Survival assays revealed that *sin-3* mutants are significantly more sensitive to propionate than wild type, with an LD50 of 27.9 mM compared to 45.2 mM for wild type (Figure 3A and B). The acyl-CoA dehydrogenase *acdh-1* is differentially expressed depending on the vitamin B12/propionate axis: its transcript levels are very low when vitamin B12 is high (as on HT115) and the canonical breakdown pathway is active, and increase several hundred fold when propionate accumulates under low vitamin B12 levels (as on OP50) (Watson et al. 2013; Watson et al. 2014). *acdh-1* induction occurs normally on OP50 in *sin-3* mutants, indicating that the shunt pathway is properly activated (Figure S4). However, our previous transcriptomic analysis on *sin-3* mutants revealed downregulation of three of the four conserved genes downstream of *acdh-1* in the propionate shunt pathway: *hach-1*, a 3-hydroxyisobutyryl-CoA hydrolase, *hphd-1*, a hydroxyacid-oxoacid transhydrogenase, and *alh-8*, a decarboxylating dehydrogenase, (Watson et al. 2016) (FigureS5A). In addition, comparison with our previously published ChIP-seq data (Beurton et al. 2019) revealed the presence of SIN-3 at the promoter region of these genes (Figure S5B). Altogether, these results suggest that although the shunt pathway is properly activated in *sin-3* mutants, the downregulation of downstream components may make this pathway less efficient, leading to their increased sensitivity to propionate accumulation.

**Discussion**

In response to changing environments, metabolic networks can be rewired through the use of alternate enzymes to adjust pathway flux, thereby maintaining organismal homeostasis. Our results suggest that in *C. elegans*, the SIN-3 coregulator may play a role in maintaining adaptive capacity on different sources of food.

We found that *sin-3* mutant animals grown on vitamin B12-replete HT115 bacteria die with an exploded vulva, a phenotype that we only rarely observed on OP50. Knock-down of the vitamin B12-dependent METR-1 methionine-synthase in mutant animals suppressed the exploded phenotype and adult lethality on HT115. Conversely, methionine or SAM supplementation enhanced lethality on the vitamin B12-depleted OP50 diet. Together, these results suggest that met/SAM cycle activity contributes to lethality in the absence of SIN-3. Met/SAM cycle activity is tightly regulated by a gene regulatory network to support organismal homeostasis (Giese et al. 2020), and either impaired or excessive activity is deleterious, reflecting the role of SAM as the major methyl donor in cells (Finkelstein 1990). Our results indicate that *sin-3* mutants are particularly sensitive to vitamin B12-dependent 1C metabolism, specifically the channeling of methionine and SAM into the met/SAM cycle.

Because both SIN-3 and vitamin B12 impact mitochondria homeostasis (Janssen et al. 2019 ; Revtovich et al. 2019; Wei and Ruvkun 2020; Giovannetti et al, 2024), we investigated whether altered mitochondrial dynamics might play a role in the sensitivity of *sin-3* mutants to vitamin B12. In *drp-1*/DRP1 mutants that show defective mitochondrial fission, vitamin B12 deficiency was shown to be beneficial by partially re-establishing mitochondrial morphology and mass, a compensatory effect dependent on the activity of the met/SAM cycle (Wei and Ruvkun 2020). Our data suggests that the increased fission observed in *sin-3* mutants (Giovannetti et al, 2024) is most likely independent of *drp-1* activity, since *drp-1* inactivation did not reduce mitochondria fission in these mutants. In *C. elegans*, examples of *drp-1* independent fission have previously been reported, for example as a response to wounding of epithelial cells and in a model of Huntington disease (Machiela et al. 2021). In addition, *drp-1* inactivation in *sin-3* mutants resulted in sterility, suggesting that DRP-1 and SIN-3 act in parallel pathways to influence germ cell health. Additional studies are required to establish whether mitochondrial morphology and the observed vitamin B12-dependent metabolic changes in *sin-3* mutants are connected.

A specific vitamin B12 diet-dependent vulnerability was previously identified in a *C. elegans* model of a neomorphic mutations in isocitrate dehydrogenase (*idh-1neo*). In humans *idh1neo* causes increased levels of cellular D-2-hydroxyglutarate (D-2HG), a proposed oncometabolite (Losman and Kaelin 2013). Increased vitamin B12 levels enhanced embryonic lethality of *C. elegans idh-1neo* mutants through increased utilization of the met/SAM cycle, resulting in a decrease in the cellular 1C pool. In this context, supplementation with alternate sources of 1C donors suppressed lethality (Ponomarova et al., 2024). In *sin*-3 mutants by contrast, supplementation with the 1C donors methionine or SAM enhanced the exploded phenotype and lethality on OP50. Several explanations could account for this effect. In *sin-3* mutants, both chromatin landscape and transcription patterns are significantly altered, with evidence of changes in histone modification states and widespread misregulation of gene expression (Robert et al. 2023). Elevated SAM levels may intensify these disruptions, for example by inducing further changes in histone methylation, which in turn could profoundly influence transcriptional activity (Mentch et al. 2015). Alternatively, or in addition, increasing the cellular 1C pool may increase flux to the polyamine synthesis pathway that also uses SAM as a substrate. Like SAM synthesis, polyamine biosynthesis is tightly controlled, with changes in polyamine metabolism linked to human diseases including cancer and diabetes (Nakanishi and Cleveland 2021). Polyamine biosynthesis is significantly increased in *sin-3* mutant animals (Giovannetti et al, 2024), and a further increase may potentially have deleterious consequences on animal health.

Transcriptional misregulation of metabolic genes in *sin-3* mutants is likely to contribute to at least some of the phenotypes described in this study, including increased sensitivity to propionate. While induction of *acdh-1* revealed that the non-canonical vitamin B12 independent pathway is properly activated in *sin-3* mutants, *hach-1, hphd-1* and *alh-8*, three genes downstream of *acdh-1*, were found to be downregulated in our transcriptomic analysis of *sin-3* mutants (Giovanetti et al 2024), possibly reflecting a direct role of SIN3 at their promoters. This could affect the efficiency of the non-canonical pathway and lead to increased sensitivity to propionate accumulation in these animals. In conclusion, our results identify SIN-3 as a key regulator that rewires the *C. elegans* metabolic network in response to diet and vitamin B12 levels.

**Materiel and methods**

**Strains**

*C. elegans* strains were cultured on Nematode Growth Medium (NGM) agarose plates with *Escherichia coli* OP50 or HT115 and incubated at 20°C unless otherwise indicated. The following strains were used: wild type N2 Bristol, PFR590 *sin-3(tm1276)I*, EN7714 *krSi134[myo-3p::tomm-20Nter::wrmScarlet]*, PFR754 *krSi134[myo-3p::tomm-20Nter::wrmScarlet];sin-3(tm1276)I*, VL749 wwIs24*[acdh-1p::GFP + unc-119(+)]*, PFR761 *wwIs24[acdh-1p::GFP + unc-119(+)]; sin-3(tm1276)I*. PFR761 was obtained by crossing *sin-3(tm1276)* hermaphrodites with *acdh-1p*::GFP males.

**Effect of vitamin B12 on *sin-3* mutant animals on OP50**

Methylcobalamine (vitamin B12 analog, #M9756, Sigma-Aldrich) was added at 25 nM to liquid NGM before pouring plates, which were then seeded with either OP50 or HT115 bacterial culture. Wildtype and *sin-3(tm1276)* worms were synchronized by egg laying on OP50 plates with or without vitamin B12, or on unsupplemented HT115, and grown at 20°C. Animals with exploded vulvas were scored at day 3 of adulthood. For pairwise comparison, a two-proportions two-tailed z-test was carried out in R with the prop.test() function. The corresponding script is available upon request.

**Metabolite assays**

All metabolites were added in liquid NGM before pouring the plates at the following final concentrations: L-methionine (#A1340,0100-ITW, ITW reagents) 4 mM, S-(5′-Adenosyl)-L-methionine chloride dihydrochloride (SAM) (#A7007-25MG Sigma-Aldrich) 500 µM, choline chloride (#1102900500 Thermo Scientific) 40 mM, and folinic acid calcium salt hydrate (#F7878-100MG Sigma-Aldrich) 100 µM. For scoring the exploded vulva phenotype, wildtype and *sin-3(tm1276)* worms were synchronized by egg laying on OP50 plates with or without supplementation and grown at 20°C until day 3 of adulthood. For each metabolite, at least 3 independent experiments were performed for *sin-3* mutant animals and 2 for wild-type animals. For pairwise comparison, a two-proportions two-tailed z-test was carried out in R with the prop.test() function. The corresponding script is available upon request.

**RNAi experiments on HT115**

HT115 RNAi clones were grown in liquid LB with ampicillin (1 µg/ ml) and tetracycline (12.5 µg/ ml)

overnight. 220 µl of bacterial culture was then plated on NGM agar plates with IPTG (1 mM). RNAi was induced for 18 to 20 hours at room temperature. Three wildtype or *sin-3* mutant L4 stage animals were allowed to lay eggs for 24h on HT115 control (empty vector) or *metr-1*/*mmcm-1* RNAi before removing the mother. Three days later, three adults (F1) were transferred to a new RNAi plate and allowed to lay eggs for 6h at 20°C. Three days later 20 to 60 adults (F2) were transferred to a new RNAi plate and allowed to develop to day 5 of adulthood before scoring exploded vulvas. Three independent RNAi experiments were performed and for each of them, RNA was extracted from F1 worms and used in RT- qPCR experiments to check RNAi efficiency as described in (Giovanetti et al 2024).

**Propionate survival assay**

Wildtype or *sin-3* mutant animals were collected in M9 buffer from NGM plates and passed through a 40 µM cell strainer (Clearline #141308C). Gravid adults retained on the filter were recovered in 2-3 ml of M9 in a 50 ml conical tube and allowed to lay eggs overnight at 20°C with agitation (220 rpm) without food to obtain hatched animals arrest at the L1 larval stage. L1 were separated from adults by filtering through a 10 µM cell strainer (Pluriselect #43-50010-01), and approximatively 40-50 starved L1 were transferred to NGM plates containing 0, 20 mM, 40 mM and 60 mM propionic acid. Three days later, survivors were counted. Three independent experiments were performed and a dose response curve was generated for each experiment using the LD50 function from HelpersMG package (https://search.r- project.org/CRAN/refmans/HelpersMG/html/LD50.html). A global dose response curve combining all three experiments was generated and presented in Figure 3A. The barplot presented in Figure 3B was generated using the LD50 of each experiment and unpaired student's T tests were used to calculate p- values.

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**Competing Interests**

No competing interests declared

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**Data and resource availability:** All relevant data and details of resources can be found within the article and its supplementary information. All scripts are availaible upon request.

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**Figures legends**

**Figure 1. Vitamin B12 levels are responsible for the increased lethality of *sin-3* mutant animals on HT115. (**A) Representative images of worms at day 3 of adulthood for wildtype (left panel) and *sin-3* mutant animals (middle and right panels) fed on HT115 bacteria. The right panel shows a *sin-3* mutant adult with an exploded vulva phenotype resulting in extrusion of the intestine. (B) Lethality due to exploded vulva of *sin-3* mutant animals is significantly increased when worms are grown on HT115 bacteria, or on OP50 supplemented with vitamin B12 (25 nM). n = total number of animals scored. Circles represent independent plates with 15-64 animals (give number). A two-proportions two-tailed z- test was carried out to perform pairwise comparison. *** P<0.05.

**Figure 2. Methionine cycle perturbation is involved in survival of *sin-3* mutant animals.** (A) Cartoon showing *C. elegans* one-carbon and propionate breakdown metabolic pathways, and link of Met/SAM cycle to PC synthesis. The folate cycle was simplified to illustrate the role of vitamin B12 in tetrahydrofolate (THF) synthesis. Enzymes are written in red. Red stars correspond to metabolite tested in figure C. (B) Inactivation of *metr-1*, but not *mmcm-1* by RNAi rescued lethality of *sin-3* mutant animals on HT115. Three independent experiments were performed. A two-proportions two-tailed z-test was carried out to perform pairwise comparison. *** P<0.05. (C) Supplementation with methionine (4 mM), SAM (500 µM) or choline (40 mM), but not folinic acid (100 µM) increases lethality of *sin-3* mutant animals. n = total number of animals scored. A two-proportions two-tailed z-

test was carried out to perform pairwise comparison, *** P<0.05.

**Figure 3. *sin-3* mutant animals are more sensitive to propionate than wildtype** (A) Dose-response curves showing percent survival of *sin-3* and wildtype on increasing propionate concentrations. Three independent experiments were performed and the resulting curves were fitted using HelpersMG package in R. (B) Average LD50 and standard deviation of data shown in (A). Unpaired student's T tests were used to calculate p-values, ** P<0.01.

**Figure 1**

A

wt adult *sin-3* adult *sin-3* exploded

B

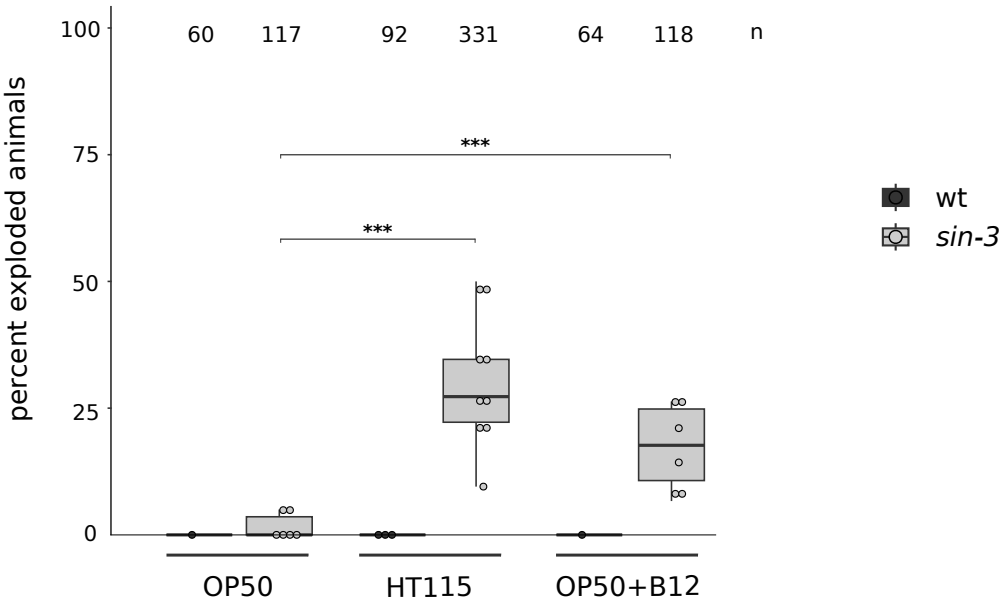
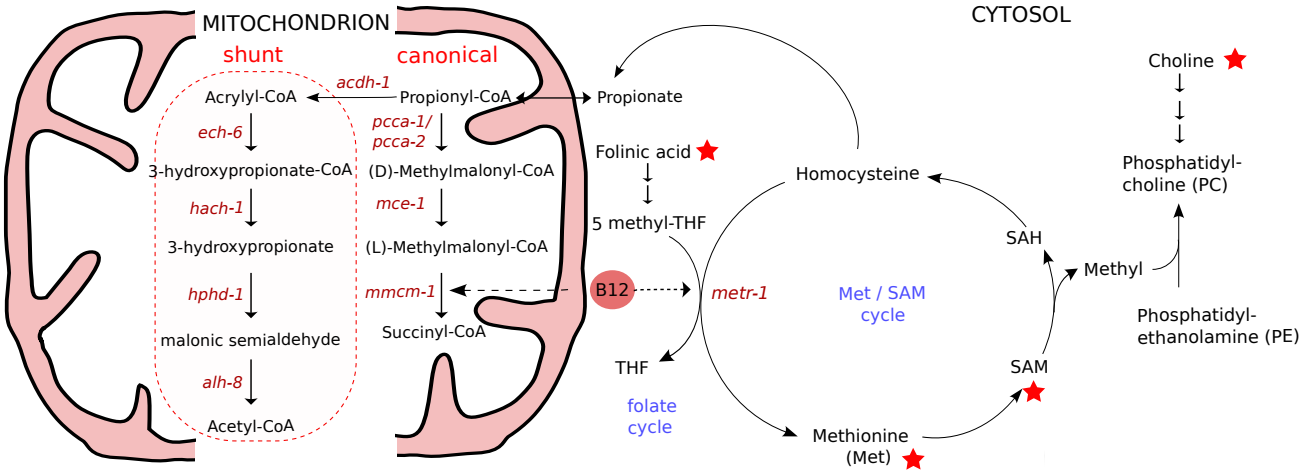
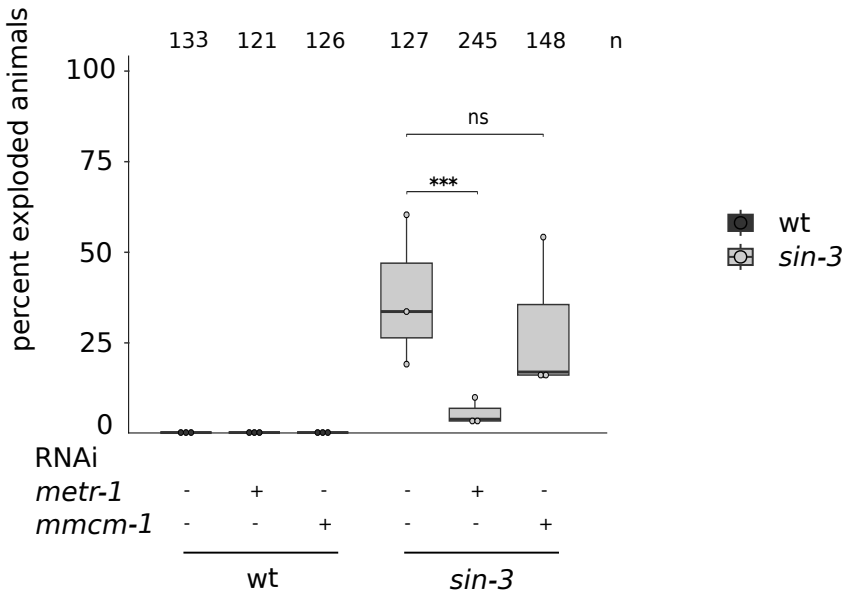


**Figure 2**

A


MITOCHONDRION
shunt
canonical
CYTOSOL
acdh-1
Acrylyl-CoA
Propionyl-CoA
Propionate
ech-6
pcca-1/
pcca-2
3-hydroxypropionate-CoA
(D)-Methylmalonyl-CoA
hach-1
mce-1
3-hydroxypropionate
(L)-Methylmalonyl-CoA
hphd-1
mmcm-1
malonic semialdehyde
Succinyl-CoA
alh-8
Acetyl-CoA
Folinic acid
5 methyl-THF
B12
metr-1
THF
folate
cycle
Homocysteine
Met / SAM
cycle
SAH
SAM
Methionine
(Met)
Choline
Phosphatidyl-
choline (PC)
Methyl
Phosphatidyl-
ethanolamine (PE)

B


percent exploded animals
133 121 126 127 245 148 n
100
75
50
25
0
ns
***
wt
sin-3
RNAi
metr-1 - + - - + -
mmcm-1 - - + - - +
wt
sin-3

C

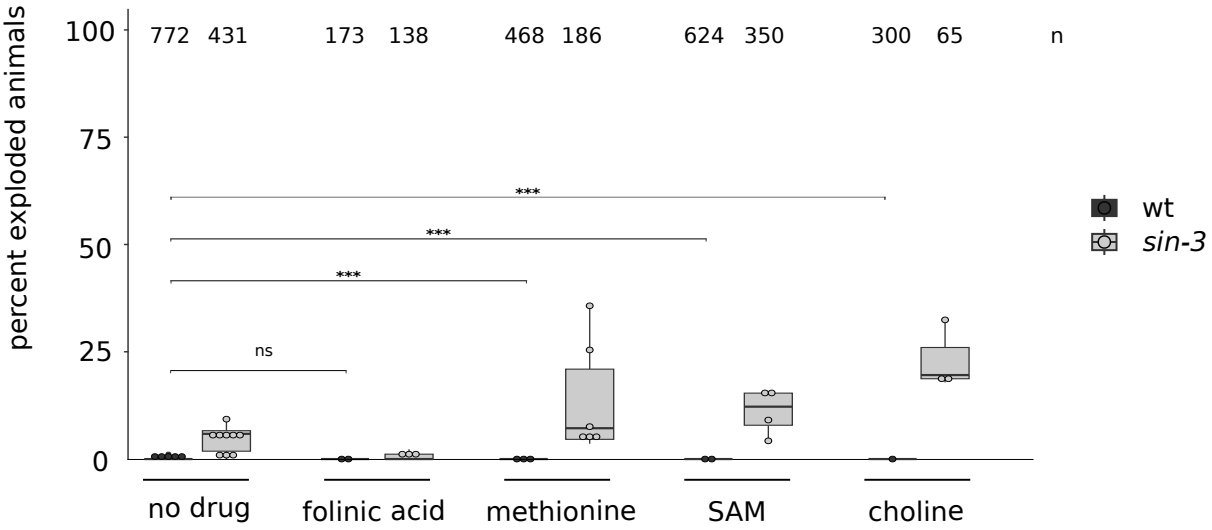
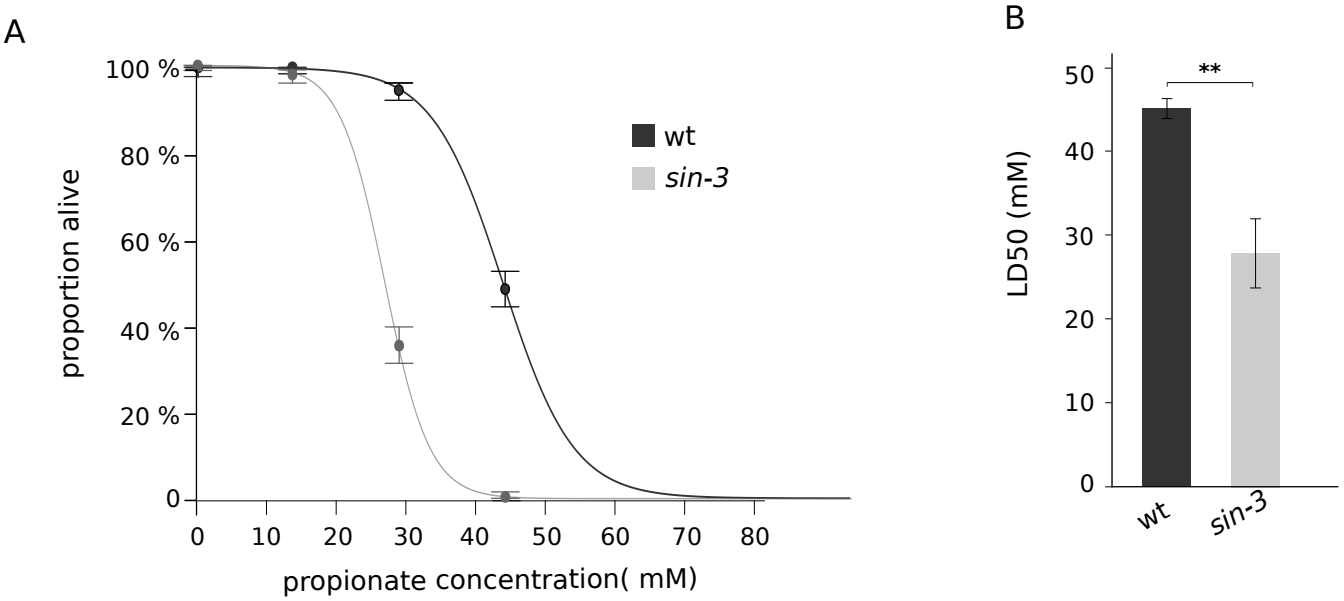

percent exploded animals
772 431 173 138 468 186 624 350 300 65 n
100
75
50
25
0
***
***
***
ns
wt
sin-3
no drug
folinic acid
methionine
SAM
choline

**Figure 3**


A
100 %
80 %
60 %
40 %
20 %
0
proportion alive
0
10
20
30
40
50
60
70
80
propionate concentration( mM)
wt
sin-3
B
50
40
30
20
10
0
LD50 (mM)
**
wt
sin-3