Enhancing S-adenosyl-methionine catabolism extends \textit{Drosophila} lifespan

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Methionine restriction extends the lifespan of various model organisms. Limiting S-adenosyl-methionine (SAM) synthesis, the first metabolic reaction of dietary methionine, extends longevity in \textit{Caenorhabditis elegans} but accelerates pathology in mammals. Here, we show that, as an alternative to inhibiting SAM synthesis, enhancement of SAM catabolism by glycine \textit{N}-methyltransferase (Gnmt) extends the lifespan in \textit{Drosophila}. Gnmt strongly buffers systemic SAM levels by producing sarcosine in either high-methionine or low-sams conditions. During ageing, systemic SAM levels in flies are increased. Gnmt is transcriptionally induced in a dFoxO-dependent manner; however, this is insufficient to suppress SAM elevation completely in old flies. Overexpression of \textit{gnmt} suppresses this age-dependent SAM increase and extends longevity. Pro-longevity regimens, such as dietary restriction or reduced insulin signalling, attenuate the age-dependent SAM increase, and rely at least partially on Gnmt function to exert their lifespan-extending effect in \textit{Drosophila}. Our study suggests that regulation of SAM levels by Gnmt is a key component of lifespan extension.
The most successful regimen to delay ageing and extend longevity is dietary restriction (DR), which was shown to increase lifespan from yeast to mammals\(^1\). However, a total reduction in calories may not be required, as reductions in specific macromolecules, such as amino acids or proteins, alone can also have an effect on organismal lifespan. In particular, methionine restriction (MR) has been shown to be sufficient for lifespan extension in yeast, worms, flies and rodents\(^2\)-\(^5\). The evolutionarily conserved enzyme methionine adenosyltransferase (MAT), also known as S-adenosyl-methionine synthase (Sams), first converts methionine into S-adenosyl-methionine, or SAM, a versatile methyl donor required for almost all methyltransferase (MTase) activity. Since various MTases catalyse the transfer of methyl groups to nucleic acids, proteins and metabolites, changes in SAM levels can result in altered epigenetic regulation, lipid metabolism or protein function\(^6\). Moreover, downstream metabolites of SAM include polyamines and cysteine, both of which are related to longevity by inducing autophagy\(^7\) and conferring oxidative stress resistance, respectively\(^8\). Therefore, SAM and/or its downstream metabolites, rather than methionine, seems to be the main effector of the MR-dependent increase in lifespan. Indeed, knockdown of sams\(^1\) in Caenorhabditis elegans, which results in decreased SAM, extends longevity, possibly through a reduction in protein translation\(^9\),\(^10\). However, loss of MAT1A function in mice induced liver damage, which led to steatosis and eventually developed into hepatocellular carcinoma\(^6\). Human patients with chronic liver disease are reported to have decreased capacity to synthesize SAM\(^11\), suggesting that a reduction in SAM synthesis is harmful in humans.

The goal of our study was to determine whether alteration of SAM metabolism resulted in positive or negative effects on the Drosophila lifespan. We found that glycine N-methyltransferase (Gnmt) is a predominant regulator of SAM levels in Drosophila. Overexpressing Gnmt could rescue the age-dependent SAM increases and extend Drosophila lifespan. Our data demonstrate that enhanced SAM catabolism by Gnmt is a key factor for lifespan control.

**Results**

Knockdown of SAM synthase shortened Drosophila lifespan. Drosophila melanogaster has a single SAM synthase (sams) (Fig. 1a). We first knocked down sams to test whether it increases the lifespan in Drosophila, as reported in C. elegans. UAS-sams-RNA interference (RNAi) crossed with an ubiquitous driver, da-Gal4, showed developmental lethality, indicating that sams is an essential gene for proper development. Next, we checked the lifespan of male flies with adult-specific knockdown of sams by Tub\(_{GS}\)-Gal4, which is the RU486-inducible binary expression system in the whole body. We were able to bypass the developmental lethality of sams-RNAi, and thus, we analysed the adult lifespan in the presence or absence of RU486. In contrast to

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**Figure 1** | Gnmt is a predominant regulator of systemic SAM levels. (a) Schematic view of methionine metabolism in Drosophila melanogaster. Gly, glycine; Sar, sarcosine; Met, methionine; SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; Hcy, homocysteine; Sams, S-adenosyl-methionine synthase; Gnmt, glycine N-methyltransferase. (b) Lifespan analysis of ubiquitous sams-RNAi male flies under 10% SY diet. In total, 200 μM RU486 (RU) is used to knock down sams after adult eclosion in Tub\(_{GS}\) > sams-RNAi. Statistics, log-rank test, \(P < 0.0001\) (\(N = 70\) for -- RU, \(N = 79\) for + RU). (c) Western blot analysis of Sams and Gnmt in day-5 male flies with either sams-RNAi or gnmt-RNAi driven by the fat-body drivers: \(r^4\)-Gal4 and FB-Gal4, or no driver. + Indicates UAS-only or Gal4-only controls. (d-I) UPLC-MS/MS analysis of SAM, methionine (Met) and sarcosine (Sar) levels in day-5 male flies with either sams-RNAi or gnmt-RNAi driven by the fat-body drivers (FB-Gal4, \(r^4\)-Gal4) or no driver. + Indicates UAS-only or Gal4-only controls. Error bars represent mean ± s.d. (\(N = 4\)). Statistics, one-way analysis of variance with Bonferroni’s multiple comparison test. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\) from the biological replicates. NS, not significant.
sams-RNAi in *C. elegans*, we observed a significantly shortened lifespan in *Drosophila* (Fig. 1b).

It was possible that sams-RNAi had tissue-specific negative effects. Because methionine metabolism is active in the liver of mammals, we knocked down sams specifically in the fat body, a counterpart of the liver and white adipose tissue in *Drosophila*. We compared the lifespan of FB > sams-RNAi flies to UAS-only control flies, as FB-Gal4, but not UAS-sams-RNAi, was backcrossed in this experiment. Both the female and male lifespans were significantly decreased by knocking down sams in fat body (Supplementary Fig. 1a,b). Although this manipulation is not adult specific, as FB-Gal4 is also expressed during the developmental stage, inhibiting SAM synthesis in *Drosophila* did not demonstrate positive effects on lifespan, raising a question about what causes the different outcomes between flies and worms.

**Gnmt is a dominant regulator of systemic SAM levels.** We suspected that there were differences in the metabolic regulation of SAM. To find out how SAM metabolism was affected by sams-RNAi, we analysed the metabolites of methionine metabolism by ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) (Fig. 1c–l). Surprisingly, sams-RNAi in the fat body using two different fat-body Gal4 drivers (FB-Gal4 and r4-Gal4) did not result in a systemic SAM reduction (Fig. 1d,g), despite the marked decrease in Sams proteins (Fig. 1c). Methionine was drastically elevated, indicating that the synthesis of SAM from methionine was indeed inhibited (Fig. 1e,h). Fruit flies, mice and humans, but not worms, have Gnmt (http://www.genome.jp/kegg/) that catalyses the conversion of glycine to sarcosine (N-methyl-glycine) by methyl group transfer using SAM (Fig. 1a). Gnmt works as a regulator of SAM levels in metabolic organs such as liver in mammals or the fat body in *Drosophila*. Knockdown of *gnmt* in fat body led to reduced Gnmt proteins (Fig. 1c), elevated SAM levels (Fig. 1d,g) and decreased Sar levels (Fig. 1f,i), although there is an unexpected slight increase in all three metabolites in negative-control flies (Fig. 1j–l). When methionine was added to food, the increase in SAM levels was limited, whereas the amount of sarcosine was increased in a dose-dependent manner (Supplementary Fig. 1c–e). Conversely, sarcosine levels significantly decreased in sams knockdown male flies (Fig. 1f,i),

![Image](image1)

**Figure 2 | The effect of overexpression of Sams and Gnmt on metabolite levels.** (a–i) UPLC–MS/MS analysis of SAM, Met and Sar levels in day-5 male flies with sams, *sam5275H*, gnmt and gnmt*S145A* overexpression by ubiquitous (da-Gal4) or fat-body (r4-Gal4) drivers or no driver. + Indicates UAS-only or Gal4-only controls. Statistics, two-tailed unpaired t-test. Error bars represent mean ± s.d. (N = 5 for da-Gal4, N = 3 for r4-Gal4, N = 4 for no-driver control). *P<0.05, **P<0.01, ***P<0.001 from the biological replicates. NS, not significant.
in contrast to negative-control flies (where they were increased for unknown reasons) (Fig. 1l), indicating that Gnmtn maintains SAM levels by reducing SAM consumption. Interestingly, sam5-RNAi in the fat body led to a reduction in Gnmtn protein (Fig. 1c), which corresponded to a reduced amount of sarcosine, suggesting the existence of transcriptional or post-transcriptional regulation of Gnmtn expression for maintaining SAM levels. Considering that sam5-RNAi resulted in a decrease in the amount of SAM in C. elegans, the buffering of SAM levels by Gnmtn may explain the different sam5-RNAi phenotype. However, there are other possibilities, including differences in the knockdown efficiency of sam5 or the number of sam5 genes (four in C. elegans, but only one in D. melanogaster).

We further analysed how these metabolites are affected by overexpressing either sam5 or gnmtn ubiquitously or in a fat-body-specific manner (Fig. 2a–i; supplementary Fig. 2a). sam5 overexpression decreased Met and increased SAM. Introducing the R275H mutation, which was observed in several human hypermethioninemia patients who had a dominant MAT1A<sup>R264H</sup> mutation, led to increased Met without affecting SAM levels. This result resembled the sam5-RNAi phenotype. MAT1A in mammals forms dimers (called MAT III) or tetramers (called MAT I). R264 is essential for dimerization, and the R264/R264H dimer is enzymatically inactive. Thus, we considered that Sams-R275H may behave as a dominant-negative form in Drosophila as well. On the other hand, gnmtn overexpression decreased SAM levels, although subtly, as well as methionine levels. The reason methionine was also reduced in gnmtn-overexpressing male flies was unknown; however, we thought that the reduction in SAM accelerated SAM synthesis from methionine as a feedback mechanism for maintaining SAM levels. We also established a mutant version of a gnmtn overexpression line by introducing a serine 145 to alanine substitution. Since S145A is located at the SAM-binding site of Gnmtn, we speculated that this mutation might result in reduced enzymatic Gnmtn function. Indeed, the effect of gnmtn<sup>S145A</sup> overexpression on
SAM and methionine was attenuated, although it was not complete (Fig. 2a–i; supplementary Fig. 2b–d).

**Gnmt overexpression increased longevity.** We then assessed whether SAM reduction in gnmt-overexpressing flies had a positive impact on lifespan. When gnmt was overexpressed using TubGS-Gal4, we observed a slight but statistically significant increase in male lifespan (Fig. 3a,b). Several enzymes, including Gnmt, have functions other than enzymatic activity, making us question whether Gnmt-induced longevity is dependent on its enzymatic activity. Overexpression of gnmtS145A by TubGS-Gal4 did not show any significant effect on lifespan, confirming that enhancing enzymatic activity of Gnmt is necessary for lifespan extension (Fig. 3a,b). Unexpectedly, we did not observe the lifespan increase in female flies when we compared lifespans of gnmt with those of gnmt+ RU (Supplementary Fig. 3a,b). However, there was a significant increase in gnmt when compared with gnmtS145A, even in the absence of RU. It is possible that leaky expression of the TubGS driver was enough to extend lifespan in females, or that the position (appP40) effect of UAS-gnmt could have different effects on males and females.

Therefore, we also checked the effect of Gnmt overexpression on lifespan using a different gnmt overexpression line, which was inserted at the attP2 site (gnmt-2). In line with this, we observed a significant increase in male and female lifespans (Fig. 3c,d), suggesting that Gnmt overexpression can be beneficial for both sexes.

**Gnmt is essential for lifespan-extending regimens.** Genetic and pharmacological interventions targeting key metabolic pathways such as insulin/IGF-1 signalling (IIS) or the target of rapamycin (TOR) pathway also increase organismal lifespan. Despite accumulating genetic studies using model organisms, our knowledge of the mechanisms underlying lifespan extension by DR or IIS/TOR inhibition is not complete. For example, inhibiting the IIS pathway exerts its effect on longevity through the transcription factor FoxO. However, the precise molecular targets and mechanism of FoxO-dependent lifespan increase have not been fully revealed. Interestingly, studies in C. elegans indicated sams-1 expression is negatively regulated by daf-16, an orthologue of FoxO, implying that activation of FoxO under reduced IIS/TOR signalling or DR can extend longevity through
repression of sams-1 messenger RNA9,17–19. In Drosophila, dFoxO transcriptionally induces gnmt12, but it did not repress sams at least at the whole-body level (Supplementary Fig. 3c), implying that Gnmt may contribute to IIS/FoxO-dependent lifespan extension. We observed in our experimental conditions that adult-specific overexpression of InRDN in male flies significantly increased lifespan (Fig. 3e,f). As expected, we showed that knockdown of gnmt partially attenuated the lifespan extension of tubGS>inRDN (Fig. 3e,f), suggesting that Gnmt mediates lifespan extension under reduced IIS activity. This was not due to the dilution of Gal4 by introducing two UAS lines simultaneously, as we observed the same degree of InRDN induction by RU treatment in both lines (Fig. 3g). Interestingly, gnmt-RNAi alone did not affect lifespan, indicating that increases in SAM do not have a negative impact on the lifespan (Fig. 3e,f). This was also supported by the data that sams overexpression in male flies did not affect the lifespan (Fig. 3h).

We also tested whether DR-induced longevity requires Gnmt. We used 20% SY diet as nutrient-rich food and 5% SY diet as DR diet. When compared with nutrient-rich condition, DR significantly extended the lifespans of both male and female flies in our lab conditions (Fig. 3i–l). Then, we subjected the gnmtMi loss-of-function mutant, which is the protein null mutant that we characterized previously and in which neither Gnmt protein nor sarcosine could be detected12, to DR lifespan analysis.

Although gnmtMi has a different genetic background since it showed lethality when backcrossed to w1118, we found that DR-induced longevity was not observed in gnmtMi mutant (Fig. 3i–l), suggesting the possibility that Gnmt-dependent SAM catabolism mediates DR longevity. In addition, gnmt expression is positively regulated by the oxidative and xenobiotic stress-responsive factor CncC/Nfr2 (Supplementary Fig. 3d–h)20, which is also suggested as a mediator of DR longevity21, indicating that Gnmt is a common downstream target for longevity pathways.

**Figure 5 | Age-dependent SAM increase is attenuated by lifespan-extending regimens.** (a,b) UPLC-MS/MS analysis of SAM and Met levels in yw and gnmtMi young (1 weeks old) or old (5 weeks old) male flies maintained under dietary restriction or a nutrient-rich condition. Error bars represent mean ± s.d. (N = 3–4). Statistics: one-way analysis of variance (ANOVA) with Bonferroni’s multiple comparison test. (c–e) UPLC-MS/MS analysis of SAM levels in lacZ-, InRDN- or TORTED-overexpressing male flies at young (1 week old) or old (5 weeks old) stages. Error bars represent mean ± s.d. (N = 3–4). Statistics: one-way ANOVA with Bonferroni’s multiple comparison test. (f) Schematic view of the relationship between SAM metabolism and longevity control.

**SAM levels increase during ageing despite Gnmt induction.** Although Gnmt overexpression extended longevity, and Gnmt is required for DR-induced longevity, it is still unknown whether Gnmt activity and SAM metabolism changes during physiological ageing. We quantified Met and SAM in two different wild-type strains analysing both young and aged male flies. Met levels decreased during ageing, whereas SAM levels increased (Fig. 4a–d). We also found that the amount of sarcosine decreased during ageing (Fig. 4e). suggesting Gnmt activity to buffer SAM declines in an age-dependent manner. However, the expression of Gnmt was induced in aged male flies, as determined by quantitative real-time PCR (qRT–PCR) and western blotting using whole-body homogenates (Fig. 4f,g; Supplementary Fig. 4a).
Sarcosine levels are negatively regulated by sarcosine dehydrogenase (sardh)\textsuperscript{15,16}, and Sardh was also induced transcriptionally during ageing (Fig. 4f.h). Interestingly, when sardh expression was knocked down, sarcosine levels were high in young male flies and further induced by ageing (Fig. 4i), suggesting that total Gnmt activity is indeed increased during ageing. Gnmt was increased in the fat body, because fat-body-specific knockdown of gnmt abrogated elevation of Gnmt in whole-body homogenates (Supplementary Fig. 4b). This increase was dependent on dFoxO (Fig. 4j; Supplementary Fig. 4c). Induction of Gnmt expression during ageing might be an adaptive response against an increase in SAM levels. Indeed, when dFoxO was knocked down in the fat body, SAM levels in aged male flies were further elevated (Fig. 4k). Therefore, we concluded that the dFoxO–Gnmt pathway is activated, but not sufficiently for complete suppression of the SAM increase in aged flies. Importantly, overexpression of gnmt, but not gnmt\textsuperscript{S145A}, inhibited the age-dependent SAM increase (Fig. 4k; Supplementary Fig. 4d,e), indicating that Gnmt-induced lifespan extension is caused by suppression of the age-dependent increase in SAM.

**SAM levels are maintained under pro-longevity regimens.**

Since Gnmt is required for DR-dependent lifespan extension, we analysed how DR altered systemic SAM levels. We analysed young (1 weeks (w)) and aged (5 w) male flies from two wild-type strains as well as a gnmt mutant strain maintained on a DR- (5% SY) or nutrient-rich (20% SY) diet. Since the 20% SY diet contains much Met, young flies maintained on 20% SY diet showed a slight increase in Met levels; however, changes in SAM levels were not statistically significant (Fig. 5a,b; Supplementary Fig. 5a,b), suggesting that Gnmt buffered SAM increase. Indeed, the lack of regulation by Gnmt resulted in increased SAM in male flies on the 20% SY diet compared with that of flies on the 5% SY diet (Fig. 5a). As previously mentioned, Met levels tended to decrease with ageing in all three genotypes, but this phenotype was not affected by the diet (Fig. 5b; Supplementary Fig. 5b). In contrast, an age-dependent increase in SAM levels was suppressed by DR (Fig. 5a; Supplementary Fig. 5a). The gnmt mutants showed high SAM levels in young flies, but this was not further elevated by ageing (Fig. 5a), suggesting that a threshold for SAM increases exists in aged flies.

In addition to DR, we checked whether reduced IIS activity affected systemic SAM levels by analysing tub\textsuperscript{G535D} InR\textsuperscript{DN}. Compared with control flies (tub\textsuperscript{G535D} lacz), in which SAM was increased during ageing regardless of RU486 treatment (Fig. 5c), SAM levels were rather significantly decreased in tub\textsuperscript{G535D} InR\textsuperscript{DN} old male flies upon RU486 treatment than that in the control flies (old flies without RU486 or young flies with RU486), although the reason behind the RU486-induced SAM elevation in tub\textsuperscript{G535D} InR\textsuperscript{DN} young flies is unknown (Fig. 5d). Interestingly, an age-dependent SAM increase was also rescued by overexpressing the dominant-negative form of the TOR (tub\textsuperscript{G535D} \textsuperscript{TOR\textsuperscript{T546 A}}) (Fig. 5e). These data suggested that the suppression of SAM increases in aged flies was a common mechanism underlying lifespan extension by DR or reduced IIS/TOR pathway.

**Discussion**

Our study indicates that the enhancement of SAM catabolism by Gnmt is an essential component for lifespan extension (Fig. 5f). Although Gnmt is transcriptionally induced during ageing at a site downstream of dFoxO activity in the fat body, this seemed to be insufficient to maintain SAM levels in aged flies. The reason behind the increase in SAM during ageing has yet to be elucidated; however, strengthening Gnmt activity attenuates the elevation of SAM and, importantly, extends longevity. Moreover, our data implied that DR and reduced IIS signalling (probably TOR and CncC as well) commonly target SAM metabolism to extend lifespan by inducing Gnmt. In humans, whether SAM levels increase in an age-dependent manner remains unknown, since only a few studies have tested this. However, one report suggested that serum SAM levels were higher in older individuals than in middle-aged individuals, at least in some populations\textsuperscript{22}.

In our experimental conditions, sams-RNAi resulted in shorter lifespans. If present in excess, Met is a toxic compound in Drosophila\textsuperscript{8}. It is therefore possible that hypermethioninemia in sams knockdown flies, the MAT1A knockout mice and patients with MAT1A deficiency causes adverse health effects\textsuperscript{23,24}. However, whether sams-RNAi in C. elegans results in the accumulation of methionine is unknown. Unexpectedly, loss of Gnmt function and subsequent SAM elevation did not have a negative effect on lifespan. The fact that the correlation of SAM levels and lifespan is not bidirectional implies a threshold in SAM levels that modulate organismal lifespan. One explanation is the biochemical character (for example, Km) of methyltransferases or other enzymes related to SAM-dependent metabolic pathways such as polyamine biosynthesis, methionine salvage pathway or trans-sulfuration pathway (TSP), as excess SAM does not always lead to elevated methylation or increased downstream metabolites.

The fact that Gnmt overexpression increases Drosophila lifespan suggests that decreases in SAM (and Met) and/or increase in SAM catabolites have a positive effect on longevity. For example, the acceleration of SAM catabolism by Gnmt may enhance the TSP, which will increase anti-oxidative capacity by upregulating cysteine, taurine and glutathione synthesis. In addition, TSP is critical for producing hydrogen sulfide, H\textsubscript{2}S, which is suggested to be the mediator of DR-induced benefits in both hepatic damage from ischaemia/reperfusion in mice and longevity in worms\textsuperscript{26}. A study in Drosophila also suggests that TSP mediates DR-induced longevity\textsuperscript{25}. Therefore, TSP or H\textsubscript{2}S might represent an underlying mechanism for Gnmt-dependent lifespan extension. In the sams-overexpressing flies in our study, SAM and probably downstream metabolites are increased. However, we did not observe any effect on lifespan in these flies, suggesting that not only enhancing SAM catabolism but also reducing SAM under the threshold is required for lifespan extension. In contrast, Gnmt overexpression reduces SAM and simultaneously enhances the generation of SAH and downstream metabolites. Whether reduction of SAM without enhancing SAM catabolism is for lifespan extension is not known, although it is suggested by the fact that sams-RNAi in worms can extend lifespan. Since lifespan represents a total sum of both positive and negative effect of different pathways, it is difficult to pinpoint the SAM-related pathway(s) essential for lifespan control, until we elucidate how each component affects lifespan.

It is also possible that SAM amount in host cells is recognized as a hallmark of nutrition availability. Thus, SAM reduction triggers the ‘fasting’ response. For example, in yeast, nutrient poor diet induced autophagy, which was inhibited by methionine at least partially through the regulation of SAM-dependent PP2A methylation by ppm1 methyltransferase\textsuperscript{26}. Autophagy, induced by MR, was reported to be a direct cause of lifespan extension\textsuperscript{9}, suggesting that SAM reduction-induced autophagy extends longevity, although no orthologue of ppm1 is found in Drosophila. SAM-dependent transmethylation, including ribosomal RNA methylation, that affects lifespan through modulating translation\textsuperscript{27} is another possible connection between SAM and longevity. The exact molecular mechanisms behind the SAM effect on lifespan need to be investigated.
Ames dwarf mice are long-lived mutants that have defects in the production of growth hormone (GH) with consequent reductions in IGF-1 levels. Interestingly, Ames dwarf mice also show elevated GNMT expression and activity in addition to reduced SAM levels in their liver. Administration of GH to Ames dwarf reduced GNMT activity while GH receptor knockout mice showed increased GNMT expression, indicating that GH signaling negatively regulates GNMT. Although the contribution of GNMT in longevity was not studied, MR did not further extend lifespan in Ames dwarf mice, suggesting that altered methionine metabolism is responsible for longevity in these animals. GNMT is one of seven genes commonly upregulated under DR (or resveratrol treatment) conditions in flies and mice, further demonstrating that the positive effects of enhanced gnmt activity on longevity in mammals is conserved.

Methods

Fly stocks. Flies were reared on a standard diet containing 4% cornmeal, 4% baker’s yeast (Oriental Yeast), 10% glucose and 0.8% agar with propionic acid and nipagin at constant 25°C, 60% humidity under 12–12 light–dark conditions. For most biochemical analysis other than lifespan study, all flies were collected within 2 days after adult eclosion and maintained for 5 days with free access to food and mating for adult maturation, unless otherwise stated.

w118, yw and Canton S were utilized as wild-type strain. UAS-gnmt-RNAi were generated in our previous study. Tub-Gal4 was kindly provided by S. Pletcher. UAS-CasC was kindly provided by D. Bohmann. r4-Gal4, UAS-IndeK (K1409A), UAS-dfox-O-RNAi (Hsm0422) and UAS-dfox were obtained from the Bloomington Drosophila stock center. UAS-gnmt-RNAi (v25988), UAS-sams-RNAi (v103143) and UAS-keap1-RNAi (v107052) were obtained from the Vienna Drosophila resource center. da-Gal4, FB-Gal4, Mef2-Gal4, pxn-Gal4, Christmas, UAS-gnmt-RNAi and UAS-lacz-RNAi were all backcrossed at least six generations into w118, yw118. GNMT was maintained on its original genetic background, because it shows lethality when backcrossed onto Canton S. For full blot representations, full blot images are shown in Supplementary Fig. 6. For qRT–PCR, total RNA was purified from five adult males using the Qiazol and RNAeasy micro kit (Qiagen). cDNA was made from 100 or 200 ng DNase-treated total RNA by the Takara PrimeScript RT Reagent Kit with gDNA Eraser. Quantitative PCR was performed using Takara Premix Ex Taq (The Tli RNase H Plus) and the LightCycler 480 system (Roche). For the analysis, 20% SY diet was predominantly used, but RNA pol II was also checked to rule out the possibility that changes in target gene expression resulted from altered expression of internal controls. Primer sequences are available in Supplementary Table 1.

Measurement of metabolites by LC-MS/MS. Methionine, SAM and sarcosine levels were measured by UPLC equipped with tandem MS, TQD (UPLC–MS/MS, Waters) briefly. Five adult males were homogenized in 50% methanol, deproteinized by acetonitrile and evaporated completely. Pellets were solubilized in 10 mM HCl followed by filtration using 0.22-μm polyvinylidyne difluoride filters (Millipore). Samples were mixed in equal volume of 50 mM Tris-HCl pH 8.8 with 100 μM dithiothreitol for Met and SAM measurements. For the sarcosine measurement, samples were derivatized after filtration by the AccQ-Tag Ultra Derivatization Kit (Waters). Samples were subjected to UPLC system with Acquity UPLC BEH C18 column. Separated solutions were ionized by electrospray ionization in positive-ion mode (ESI +) and detected by TQD with the following m/z transitions: Methionine, 149.98 > 132.90, SAM, 399.20 > 250.00 and derivatized sarcosine, 260.00 > 171.50.

Because absolute metabolite amounts can easily vary between experiments due to technical (for example, machine conditions) as well as biological (for example, season, food, genotype and so on) conditions, we always prepared a control sample in the same analytical round to compare the quantities as ratios relative to the control. To analyse the metabolites precisely, the quality of parents, the developmental environment and sampling time (to avoid circadian changes) were carefully controlled, and each graph was constructed from the same round of analysis.

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**Author contributions**

F.O. and M.M. designed the study, performed experiments and wrote the manuscript.

**Additional information**

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