Received: 13 February 2023 | Accepted: 18 April 2023

DOI: 10.1002/jcp.31033

RESEARCH ARTICLE

Journal of Cellular Physiology WILEY

A gene-nutrient interaction between vitamin B6 and serine hydroxymethyltransferase (SHMT) affects genome integrity in *Drosophila*

Eleonora Pilesi[1] | Chiara Angioli[1] | Claudio Graziani[2] | Alessia Parroni[2,3] | Roberto Contestabile[2] | Angela Tramonti[2,3] | Fiammetta Vernì[1]

[1]Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome, Italy

[2]Department of Biochemical Sciences "A. Rossi Fanelli", Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome, Italy

[3]Institute of Molecular Biology and Pathology, National Research Council (IBPM-CNR), Rome, Italy

Correspondence
Fiammetta Vernì, Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome 00185, Italy.
Email: fiammetta.verni@uniroma1.it

Funding information
Grants from Sapienza University of Rome, Grant/Award Numbers: RP120172838CFF6C, RM122181618E2878, RM12117A610B653E, RM120172A76E4B78

Abstract

Pyridoxal 5′-phosphate (PLP), the catalytically active form of vitamin B6, participates as a cofactor to one carbon (1C) pathway that produces precursors for DNA metabolism. The concerted action of PLP-dependent serine hydroxymethyltransferase (SHMT) and thymidylate synthase (TS) leads to the biosynthesis of thymidylate (dTMP), which plays an essential function in DNA synthesis and repair. PLP deficiency causes chromosome aberrations (CABs) in *Drosophila* and human cells, rising the hypothesis that an altered 1C metabolism may be involved. To test this hypothesis, we used *Drosophila* as a model system and found, firstly, that in PLP deficient larvae SHMT activity is reduced by 40%. Second, we found that RNAi-induced SHMT depletion causes chromosome damage rescued by PLP supplementation and strongly exacerbated by PLP depletion. RNAi-induced TS depletion causes severe chromosome damage, but this is only slightly enhanced by PLP depletion. dTMP supplementation rescues CABs in both PLP-deficient and PLP-proficient $SHMT^{RNAi}$. Altogether these data suggest that a reduction of SHMT activity caused by PLP deficiency contributes to chromosome damage by reducing dTMP biosynthesis. In addition, our work brings to light a gene-nutrient interaction between SHMT decreased activity and PLP deficiency impacting on genome stability that may be translated to humans.

KEYWORDS
chromosome aberrations, gene-diet interactions, one carbon metabolism, SHMT, TS, vitamin B6

1 | INTRODUCTION

The catalytically active form of vitamin B6, the pyridoxal 5′-phosphate (PLP) serves as cofactor for about 160 enzymes (Percudani & Peracchi, 2003) involved in protein, lipid, sugar and one-carbon (1C) metabolism, the latter providing precursors for nucleic acid synthesis and methylation processes (Ducker & Rabinowitz, 2017). Vitamin B6 is also an antioxidant molecule able to counteract reactive oxygen species (ROS) and advanced glycation end-products (AGEs) (Contestabile et al., 2020). Animals do not

synthesize PLP, making it an essential nutrient in their diet; thus PLP is recycled from food in a salvage pathway which requires the activity of pyridoxal kinase and pyridoxine 5′-phosphate oxidase (di Salvo et al., 2011).

PLP deficiency has been associated with several pathologies including cancer mostly affecting gastrointestinal tract, although underlying mechanisms need to be still clarified (Contestabile et al., 2020; Mocellin et al., 2017). Growing evidence indicates that vitamin B6 plays a crucial role in genome integrity maintenance as its deficiency causes chromosome aberrations (CABs) in *Drosophila*, yeast, and HeLa cells (Kanellis et al., 2007; Marzio et al., 2014; Mascolo et al., 2020) as well as micronuclei in human lymphocytes (Wu et al., 2016). Thus, it is conceivable that one of the mechanisms through which PLP deficiency favors cancer development may involve the DNA damage. Studies performed in *Drosophila* suggested that chromosome damage induced by PLP deficiency depends in part upon PLP antioxidant properties (Marzio et al., 2014). It has been demonstrated, indeed, that PLP-deficient individuals develop a hyperglycemic condition leading to the accumulation of AGEs (Marzio et al., 2014; Mascolo et al., 2020), which in turn produce reactive oxygen species (ROS) responsible for DNA breaks, subsequently transformed into CABs by the DNA repair systems (Natarajan, 2002). It is, however, conceivable that the chromosome damage can also depend on the role of PLP in 1C pathway since this produces essential metabolites for DNA biosynthesis and repair.

The 1C pathway relies on the activity of three interconnected pathways: the folate cycle, the methionine cycle, and the transsulfuration pathway (Lyon et al., 2020) (Figure 1a). In folate cycle vitamin B6 serves as a cofactor for serine hydroxymethyltransferase (SHMT, EC 2.1.2.1) which converts serine into glycine, and transfers released 1C units to tetrahydrofolate (THF) giving rise to N5, N10- methylene THF. Then, this compound is both used for thymidylate (dTMP) synthesis, catalyzed by thymidylate synthase (TS), and reduced to methyl-THF, which enters the methionine cycle (Fox & Stover, 2008).

Mammalian genomes contain two *SHMT* genes *SHMT1* and *SHMT2*, encoding a cytoplasmic and a mitochondrial isoform, respectively (Anderson & Stover, 2009). SHMT2 seems to be preferentially involved in the synthesis of mitochondrial dTMP, however, its main role is probably to produce 1C units from serine, which are exported as formate into the cytosol to sustain 1C metabolism (Stover & Field, 2011). Giving its implication in nucleotide synthesis and cellular methylation, the 1C pathway takes part to metabolic reprograming which sustains cancer growth. Consistently, increased expression of *SHMT2* is associated with several tumors (Zeng et al., 2021). On the other hand, polymorphic variants of *SHMT1* gene have been studied to investigate their role in cancer susceptibility based on the hypothesis that a dysfunctional folate pathway may cause imbalances in pyrimidine synthesis, leading to an increased level of DNA double strand breaks, resulting in turn in point mutations, chromosomal translocations, and other preneoplastic alterations. Consistently, specific variants of *SHMT1* have been found associated with breast (Wu et al., 2016) lung (Wang et al., 2007) and rectal cancer (Komlósi et al., 2010) as well as with adult acute lymphocytic leukemia (Skibola et al., 2002) and malignant lymphoma (Hishida et al., 2003).

A crucial role in the folate pathways is played by thymidylate synthase (TS, EC 2.1.1.45), which catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). Once synthesized, dTMP is metabolized intracellularly to the dTTP form used for DNA biosynthesis and DNA repair. Similar to *SHMT*, *TS* upregulation has been observed in various tumors (Fu et al., 2019; Nomura et al., 2002; Song et al., 2021) and associated with more invasive and metastatic abilities of cancer cells (Kimura et al., 2011; Lu et al., 2013; Nomura et al., 2002). *TS* variants mostly deriving from tandem repeats variations in the promoter regions have been, instead, correlated with susceptibility to different tumors including acute lymphocytic leukemia, colon cancer, breast cancer, and gastric cancer (Curtin et al., 2007; Graziano et al., 2004; Lightfoot et al., 2005; Skibola et al., 2002).

The *Drosophila* genome harbors only one *SHMT* gene encoding alternative transcripts, which give rise to a cytoplasmic isoform and a mitochondrial isoform, proteolytically derived from a longer precursor with a putative N-terminal sequence that mediates the mitochondrial import (Winkler et al., 2017). *TS* function is also conserved in *Drosophila* (Carpenter, 1974) and sensitive to common TS inhibitors (Silber et al., 1985).

Using *Drosophila* as a model system, we investigated whether PLP may protect genome from damage working as cofactor of SHMT and also whether PLP deficiency combined to reduced activity of SHMT or TS enzymes may amplify genome instability. Understanding how micronutrients exert their protective action towards genome, as well as revealing gene-nutrients interactions which compromise genome stability is crucial to identify individuals at-risk to be treated with diet-based interventions.

2 | MATERIALS AND METHODS

2.1 | *Drosophila* stocks and crosses

The $SHMT^{v19206}$, $SHMT^{v19208}$, and TS^{v29354} lines were obtained from Vienna *Drosophila* Resource Center (VDRC). $SHMT^{v19206}$ and TS^{v29354} lines carry the hairpin RNA constructs on the second chromosome. The $SHMT^{v19208}$ line carries the hairpin RNA construct on the third chromosome. To silence *SHMT* or *TS* genes we crossed males from $SHMT^{v19206}$ and TS^{v29354} lines to females carrying the ubiquitous *act-Gal4/CyTb* driver and selected non-*Tubby* larval progeny. The *Oregon-R* strain was used as wild-type control. All stocks were maintained at 25°C and crosses were made at 29°C. The used balancers and genetic markers are described in detail in FlyBase (http://flybase.bio.indiana.edu/).

2.2 | Fly food recipes

Flies were raised on standard food containing (in 100 mL): agar (0.68 g), yeast (6.52 g), flour (3 g), propionic acid (600 µL) and sucrose

(5.13 g). The 4-deoxypyridoxine (4DP) drug (S-D0501; SIGMA) was added to the standard medium at the 2 mM final concentration. To test the effects of vitamin B6 supplementation, 1 mM pyridoxal 5′-phosphate (PLP) (82870; SIGMA) was dissolved into the standard food.

2.3 | Chromosome cytology

Colchicine-treated larval brain metaphases for CAB scoring were obtained as described in (Merigliano et al., 2017). Fixed preparations were mounted in Vectashield H-1200 with 4,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories) to stain the DNA. Cytological preparations were examined with a Carl Zeiss Axioplan fluorescence microscope, equipped with an HBO100W mercury lamp and a cooled charged-coupled device (CCD camera; Photometrics CoolSnap HQ). At least 500 cells for condition were scored in 5–10 brains (the exact number of brains and cells scored in each condition is reported in Table S1, Supporting Information).

2.4 | Deoxythymidine monophosphate (dTMP) treatment

Brains dissected from third instar larvae were incubated in 2 mL of saline supplemented with 10% fetal bovine serum (FBS, Gibco BRL) for 4 h with addition of 50 uM deoxythymidine monophosphate (dTMP) (Merck T7004-100MG). One hour before fixation colchicine (final concentration, 10^{-5} M) was added to the saline/FBS to collect metaphases. Brains were then fixed with the standard procedure (Section 2.3).

2.5 | SHMT activity measurement

Measurement of SHMT activity was performed using a radioisotope assay based on the ability of SHMT to catalyze the exchange of the pro-2S proton of glycine with solvent (P J Stover et al., 1997). Protein extracts obtained from about 20 larvae in 20 mM K-phosphate, pH 7.2, containing 150 mM NaCl, 0.1% NP-40 and 5 mM 2-mercapto ethanol, were incubated with tritiated [2^3H] glycine (23 nmol/L) at 30°C for 4 h and treated as previously described (Tramonti et al., 2021). The experiment was repeated four times, duplicates were used each time and the radioactivity was normalized to total protein content, determined with Bradford's assay.

2.6 | Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). The statistical significance in the analysis of chromosome damage was done with the χ^2 test in which we compared the total number of cells with and without chromosome damage for each condition. All the experiment were repeated at least three times. Enzymatic tests were performed independently four times and each experiment was performed in triplicate. Statistical analysis for enzymatic tests was done with the Student t test. $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | Reduced activity of SHMT results in chromosome aberrations (CABs)

We previously demonstrated that PLP deficiency causes CABs in *Drosophila* and human cells, largely dependent on the antioxidant role of PLP (Marzio et al., 2014; Mascolo et al., 2020). However, PLP also serves as a coenzyme for SHMT in 1C metabolism, a pathway that plays a critical role in DNA biosynthesis, repair, and methylation (Figure 1a). Thus, here we wondered whether chromosome damage induced by PLP deficiency might also be due to reduced SHMT activity, caused by the absence of cofactor, which would ultimately decrease the biosynthesis of dTMP (Figure 1a).

To address this issue, we used *Drosophila* as a model system and firstly measured the catalytic activity of SHMT enzyme in extracts from larvae reared on a medium containing 2 mM 4-deoxypyridoxine (4DP), a strong PLP inhibitor (Merigliano et al., 2018). This analysis demonstrated that PLP depletion caused a significant decrease of SHMT activity (40%) (Figure 1b). Then, we investigated whether reduced SHMT activity can cause chromosome damage, like PLP deficiency (Marzio et al., 2014). To this purpose, we exploited the RNA interference (RNAi) strategy to silence the *SHMT* gene using the *actin-Gal4* ubiquitous driver and examined mitotic chromosomes in larval neuroblasts. RNAi reduced SHMT enzymatic activity by 60% (Figure 1b). Preparation of chromosomes from brain cells of *actin-Gal4* larvae and larvae of the $SHMT^{RNAi}$ line (not crossed to driver) yielded frequencies of cells with CABs not higher than wild type (wt) (0.25% and 0.33% vs. 0.45%) (Supporting Information: Table S1). In contrast, the SHMT depletion caused a substantial increase in the number of cells with broken chromosomes compared to control (Figure 2). To better evaluate the extent of damage we classified the cells with CABs into three groups: SA cells showing single aberrations such as chromatid deletions, isochromatid deletions and exchanges, MA cells exhibiting multiple aberrations (two to five) and MF cells displaying metaphases with chromosome fragmentations (more than five breaks). The last class mainly includes cells in which chromosomes are highly condensed and completely pulverized. About 7% of $SHMT^{RNAi}$ neuroblasts displayed CABs with prevalence of SA and MA cells (Figure 2b). Rearing wild-type larvae on a medium containing 4DP yielded about 15% of neuroblasts with CABs, half of which were MA and MF cells (Figure 2). The increased severity of the damage in these larvae was probably due to the dual role of PLP as a cofactor of SHMT and as antioxidant (Martinez et al., 2000; Matxain et al., 2009).

As shown in Figure 2b, PIP supplementation rescued CABs in $SHMT^{RNAi}$ neuroblasts decreasing the frequencies of SA, MA, and MF cells, thus indicating a direct relation between PLP availability, SHMT

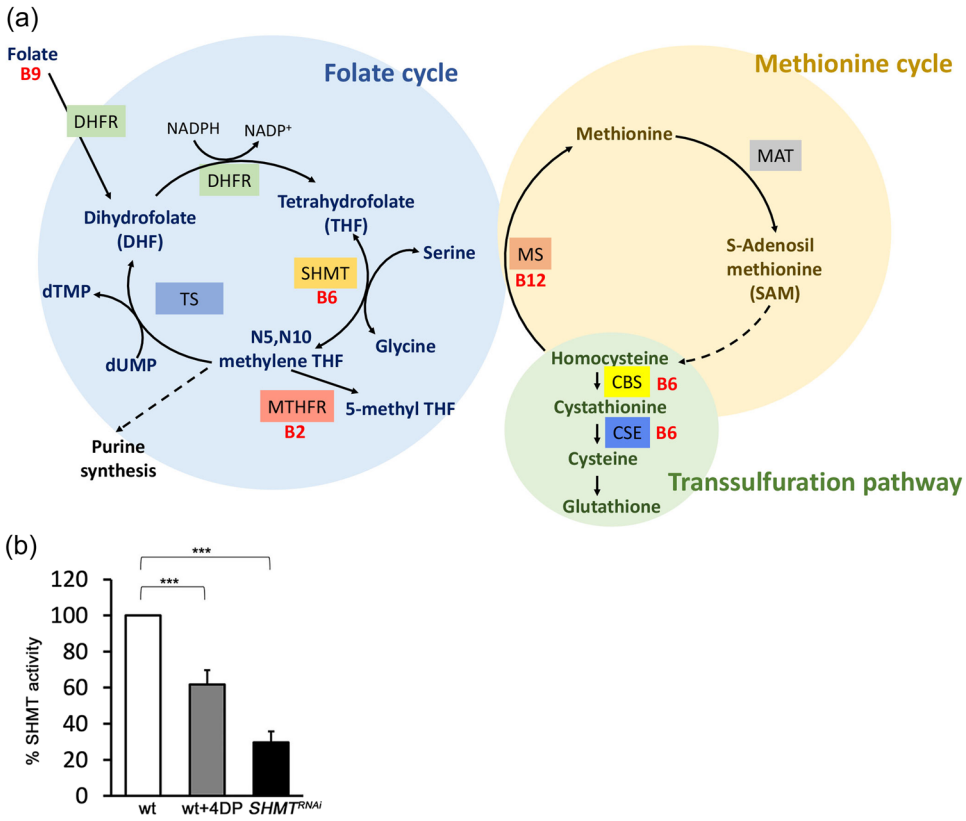

FIGURE 1 1C pathway and *Drosophila* SHMT activity (a) Simplified scheme of 1C metabolism. The dashed arrows indicate the final product (b) % of SHMT activity. Each column represents the mean value ± the standard error.*** significant in Student *t* test with $p < 0.001$. CBS, cystathionine beta-synthase; CSE, cystathionine γ-lyase; DHFR, dihydrofolate reductase; MAT, methionine adenosyltransferases; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SHMT, serine hydroxymethyltransferase; TS, thymidylate synthase.

activity and DNA damage. We confirmed these results also in another $SHMT^{RNAi}$ line ($SHMT^{v19208}$) (Supporting Information: Figure S1 and Table S1).

To further prove that SHMT activity can be modulated by PLP availability, we asked whether PLP depletion could amplify DNA damage in $SHMT^{RNAi}$ larvae.

As shown in Figure 2, rearing $SHMT^{RNAi}$ larvae in a medium containing 4DP strongly increased the frequency of cells with chromosome damage. In particular, we found a significant increase of MF cells (8.7%), clearly indicating a strong synergistic effect between PLP depletion and SHMT reduced activity on chromosome integrity. This frequency was significantly higher ($p < 0.001$) than the sum of MF cell frequency observed in PLP-proficient $SHMT^{RNAi}$ brains (0.31) and that found in brains from 4DP-fed wild-type larvae (3.49%). Administration of PLP to $SHMT^{RNAi}$ larvae reared on 4DP medium significantly reduced the frequency of MF cells (2.13%).

Taken together, these data indicate that reduced SHMT activity causes chromosome damage similarly to PLP depletion; moreover, they reveal a gene-nutrient interaction between reduced PLP availability and reduced SHMT activity that can heavily threaten genome integrity.

3.2 | The silencing of *thymidylate synthase (TS)* gene causes chromosome damage

SHMT produces N5,N10-methylene tetrahydofolate, which is used by both TS to produce dTMP from dUMP and by methylene tetrahydrofolate reductase (MTHFR) in the methionine cycle to provide methyl groups (Figure 1a). Thus, we supposed that the majority of CABs found in $SHMT^{RNAi}$ brains are due to reduced dTMP synthesis, although it is also conceivable that an altered chromatin methylation pattern can compromise DNA repair (Fernandez et al., 2021).

To verify this hypothesis, we firstly tested whether also *TS* gene silencing could cause CABs.

The frequency of neuroblasts with CABs in larvae from the TS^{RNAi} line (not crossed to driver) did not differ from wt (Supporting Information: Table S1). In contrast, the analysis of TS^{RNAi} brains in which the gene was silenced revealed a high frequency (28% vs. 0.55% in wild type brains) of cells with extensive chromosome damage, the majority belonging to MA (12%) and MF (11.9%) classes (Figure 3). Often, irregularly condensed chromosomes and precocious sister chromatid separation were observed in metaphases with broken chromosomes (Figure 3a).

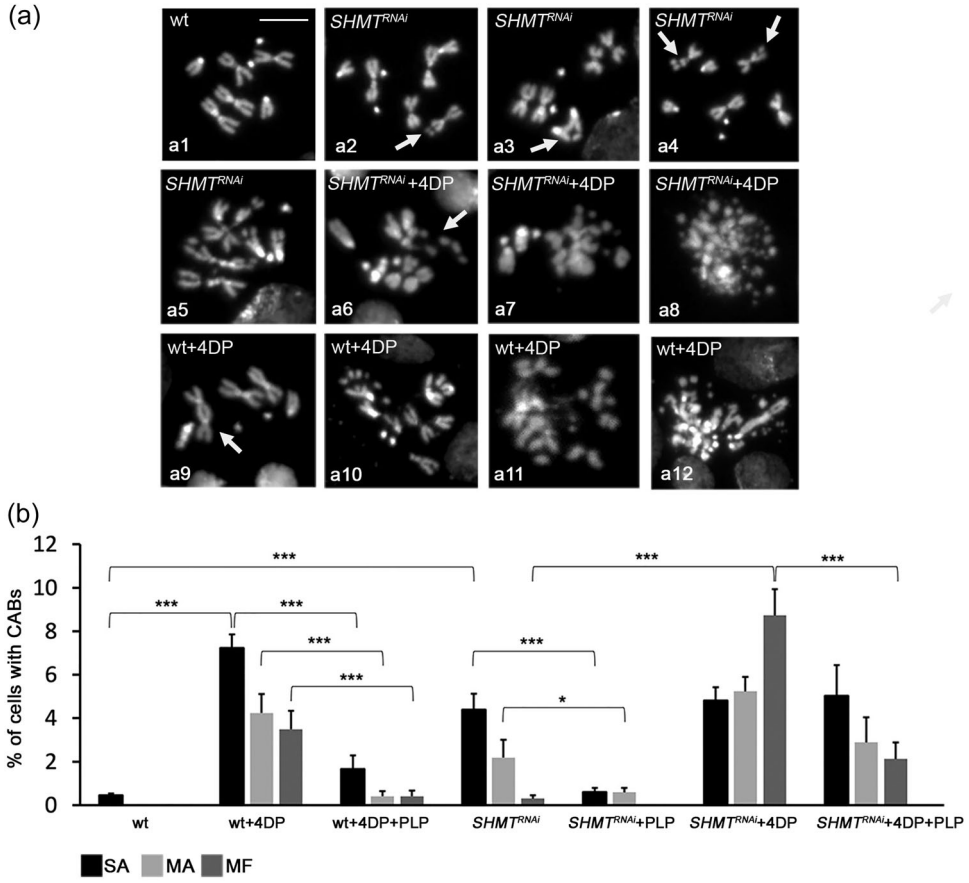

FIGURE 2 Silencing of *SHMT* causes CABs (a) Examples of CABs in $SHMT^{RNAi}$, 4DP-treated $SHMT^{RNAi}$, and 4DP-treated wild type larval brains (a1) wild-type (wt) female metaphase; (a2) isochromatid deletion of a major autosome, arrow; (a3) exchange between two X chromosomes, arrow; (a4) metaphase with both a chromatid and an isochromatid break, arrows; (a5) breaks affecting more chromosomes; (a6) fragmentation of two autosomes; (a7) metaphase with fragmented and irregularly condensed chromosomes; (a8) metaphase with pulverized chromosomes; (a9) dicentric chromosome, arrow; (a10–12) extensive chromosome fragmentation. Scale bar, 5 μm. (b) Quantification of CABs. Each column expresses the average ± standard error. *,*** significant in χ^2 test with $p < 0.05$, and $p < 0.001$ respectively. MA, multiple aberrations; MF, multifragmented chromosomes; SA, single aberrations.

PLP treatment to TS^{RNAi} larvae maintained unchanged the percentage of cells with CABs, whereas 4DP feeding significatively increased only the percentage of SA cells. These data Indicated that TS depletion causes severe chromosome damage not sensitive to PLP reduction.

3.3 | dTMP supplementation rescued CABs in SHMT depleted neuroblasts

Since TS impaired activity strongly impinges on genome integrity, it is conceivable that chromosome damage observed in SHMT depleted cells might derive from reduced dTMP synthesis. To verify it, we tested whether DNA damage in $SHMT^{RNAi}$ brains could be rescued by dTMP administration. As shown in Figure 4, treatment of isolated brains from $SHMT^{RNAi}$ larvae with 50 μM dTMP reported SA cell frequency to control values and reduced to zero MA and MF cell frequencies. dTMP treatment of brains from $SHMT^{RNAi}$ larvae reared in 4DP significantly rescued chromosome damage. MA and MF cell frequencies were also reduced in brains from TS^{RNAi} larvae, confirming the specificity of the treatment. Moreover, dTMP treatment of brains from 4DP-fed wild type larvae decreased both MA and MF cells (Figure 4). Chromosome damage in these brains remained, however, high compared to that of wild-type untreated brains according to the notion that PLP also acts as antioxidant.

Taken together these results support our working hypothesis that reduced vitamin B6 levels may induce the formation of chromosome damage not only by reducing the oxidative stress, but also by impairing the folate pathway.

4 | DISCUSSION

Compelling evidence indicates that micronutrient deficiency can compromise genome stability, because most of vitamins and minerals are antioxidants or cofactors for enzymes involved in DNA metabolism (Ames, 2001; Fenech & Ferguson, 2001). Vitamin B6 deficiency threatens genome stability by causing CABs in *Drosophila*, yeast, and

human cells (Kanellis et al., 2007; Marzio et al., 2014). In the past we provided evidence that in *Drosophila* CABs are in part attributable to the antioxidant properties of PLP (Marzio et al., 2014). However, we also found an increased dUTP/dTTP ratio in PLP-deficient larvae (Marzio et al., 2014) that led us to suppose that CABs may be also attributable to the role of PLP as cofactor of SHMT enzyme, which ultimately promotes the synthesis of dTMP in folate pathway.

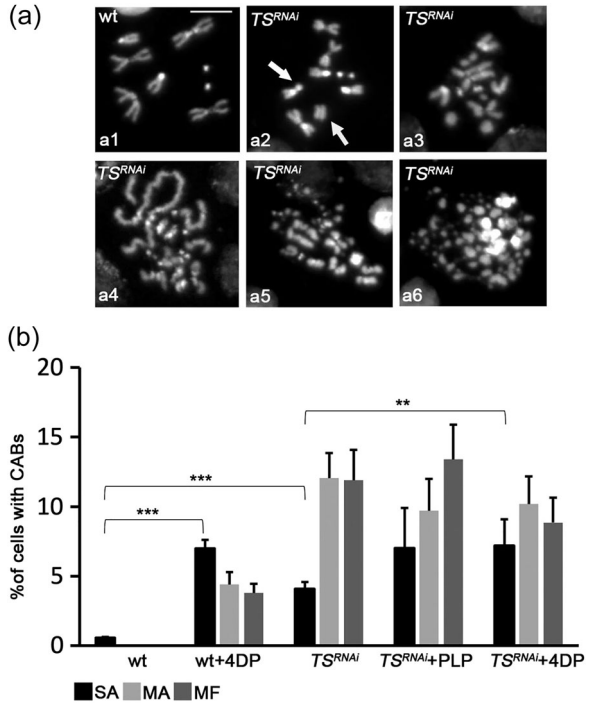

FIGURE 3 *TS* silencing causes CABs (a) Examples of CABs in TS^{RNAi} larval brains. (a1) wild-type (wt) female metaphase; (a2) isochromatid deletion of a major autosome, arrows; (a3) metaphase showing multiple rearrangements (a4) metaphase showing rearranged irregularly condensed chromosomes also displaying precocious sister chromatid separation (PSCS) (a5,6) metaphases showing overcondensed chromosomes displaying extensive chromosome fragmentation. Scale bar, 5 µm. (b) Percentage of cells showing chromosome damage. Each column represents the average value ± the standard error. **,*** significant in χ^2 test with $p < 0.01$ and $p < 0.001$ respectively. MA, cells with multiple aberrations; MF, cells with multifragmented chromosomes; SA, cells with single aberrations.

Although in literature it has been often taken for granted that vitamin B6 depletion impairs genome integrity due to its involvement in folate pathway (Ames, 2001; Ames & Wakimoto, 2002), direct proofs are lacking. In this report, we collected evidence validating this hypothesis in *Drosophila*, thus reinforcing the correlation between metabolism and genome integrity. First of all, we demonstrated that SHMT levels are reduced by 40% in PLP depleted larvae, consistently with the results obtained in other systems (Perry et al., 2007) and indicating that reduced cofactor availability can decrease SHMT function also in *Drosophila*. Then, we have shown that both SHMT and TS depletion can cause chromosome damage, like PLP deficiency, and that this can be rescued by dTMP administration. To further reinforce the validity of our hypothesis we have shown that dTMP treatment partially rescued CABs also in wild type PLP-deficient individuals, thus indicating that PLP deficiency impacts on genome integrity not only by compromising cellular defenses, as previously shown, but also by compromising thymidylate synthesis in the folate pathway. These data are further corroborated by the finding that PLP depletion strongly exacerbates chromosome damage in $SHMT^{RNAi}$ individuals increasing the frequency of cells with completely fragmented chromosomes. In contrast PLP treatment rescued CABs in both PLP proficient and deficient $SHMT^{RNAi}$ brains. This finding provides a clear example of gene-diet interaction which impacts on genome integrity that add to the increasing number of gene-nutrient interactions able to modulate risk of disease such as cancer, cardiovascular disease, and neurodegenerative diseases (Zinck & MacFarlane, 2014). Translated to humans, our data may suggest that people carrying SHMT variants with reduced enzymatic activity are more sensitive to an insufficient amount of vitamin B6 with respect to healthy people and consequently they face an increased risk of chromosomal damage, which is a strong predictor for cancer. Although PLP deficiency is rare in developed countries (Contestabile et al., 2020), is noteworthy that several conditions such as diabetes, pregnancy, celiac diseases, can reduce PLP levels; thus, in these

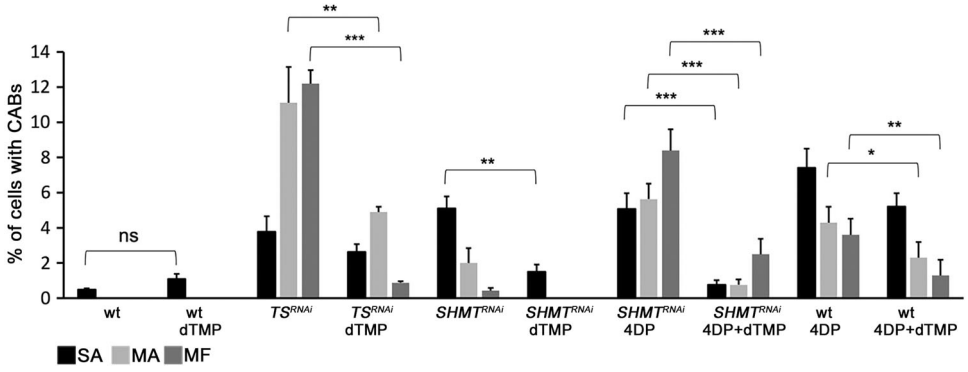

FIGURE 4 dTMP administration rescues CABs in $SHMT^{RNAi}$ cells Each column represents the average value ± the standard error. MA, cells with multiple aberrations; MF, cells with multifragmented chromosomes; ns, not statistically significant; SA, cells with single aberrations. *,**,*** significant in χ^2 test with $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively.

specific contexts, carriers of *SHMT* genetic polymorphisms may particularly benefit from vitamin B6 administration to safeguard their genome integrity.

Differently from *SHMT* silencing, TS depletion was not influenced by PLP supplementation and only slightly by 4DP treatment, according with the fact that it is not a PLP-dependent enzyme. However, by considering that PLP is also an antioxidant, we would have expected to find higher frequencies of cells with severe DNA damage in 4DP-treated TS^{RNAi} brains. Thus, we can speculate that some cells depleted for both TS and PLP would accumulate a chromosome damage incompatible with life.

Impaired dTMP synthesis damages DNA either through dUTP misincorporation or by promoting replicative stress. A futile cycle of reiterative dUTP misincorporation and uracil glycosylase-mediated excision causes DNA strand breaks. Alternatively, nucleotide imbalance caused by dTMP deficiency triggers replication stress and accumulation of stalled forks, thus providing a further source of CABs (Magdalou et al., 2014). Fly genome does not encode the uracil glycosylase. However, since depletion of dUTPase, which converts dUTP into dUMP preventing its incorporation into DNA, causes DNA double strand breaks (Muha et al., 2012), it is conceivable that another enzyme limits misincorporation, thus making plausible this mechanism even in *Drosophila*. Regarding the second mechanism we can speculate that such a mechanism may operate in *Drosophila* based on the consideration that most of MF neuroblasts from TS^{RNAi}, 4DP-fed $SHMT^{RNAi}$, and 4DP-fed wild-type larvae displayed pulverized dot-like chromosomes, which are highly reminiscent of those specifically produced by hydroxyurea (HU) treatment on *Drosophila* neuroblasts carrying *timeless (tim-2)* mutation, which affects fork stabilization (Benna et al., 2010).

Although our work did not deal directly with cancer, it provides a strong correlation between SHMT activity, vitamin B6 levels and genome integrity that could be investigated in future studies exploiting the *Drosophila* model to overcome the difficulties encountered in other systems. To date only one study tried to investigate in vitro whether an interaction between SHMT polymorphic variants and reduced vitamin B6 levels may impact on breast cancer through chromosome damage, but the small size of the examined sample prevented to reach definitive results (Wu et al., 2016).

5 | CONCLUSIONS

We provided evidence that vitamin B6 deficiency impacts on genome integrity not only as antioxidant molecule—as previously proposed—but also as cofactor of SHMT in 1C metabolism. In addition, we found that reduced levels of vitamin B6 strongly synergize with reduced SHMT activity, revealing how this nutrient-gene interaction seriously compromises the genome integrity. Implementing this field of research which correlates diet with genome stability is an important goal to increase the possibility, in some cases, to reduce cancer risk with a more refined individual approach involving targeted nutritional interventions.

AUTHOR CONTRIBUTIONS

Eleonora Pilesi, Chiara Angioli, Claudio Graziani, Alessia Parroni performed the experiments. Angela Tramonti performed the experiments, analyzed, and interpreted the data. Roberto Contestabile analyzed and interpreted the data. Fiammetta Vernì designed the project, analyzed the data, and wrote the paper.

ACKNOWLEDGMENTS

This research was supported by grants from Sapienza University of Rome: RP120172838CFF6C, RM122181618E2878, RM12117A6 10B653E, and RM120172A76E4B78.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Eleonora Pilesi http://orcid.org/0000-0001-6703-8001
Chiara Angioli http://orcid.org/0000-0002-7958-2327
Claudio Graziani http://orcid.org/0000-0001-9347-9732
Alessia Parroni http://orcid.org/0000-0001-5853-6690
Roberto Contestabile http://orcid.org/0000-0002-5235-9993
Angela Tramonti http://orcid.org/0000-0002-5625-1170
Fiammetta Vernì http://orcid.org/0000-0001-8866-3324

REFERENCES

Ames, B. N. (2001). DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *475*(1–2), 7–20. https://doi.org/10.1016/s0027-5107(01)00070-7

Ames, B. N., & Wakimoto, P. (2002). Are vitamin and mineral deficiencies a major cancer risk. *Nature Reviews Cancer*, *2*(9), 694–704. https://doi.org/10.1038/nrc886

Anderson, D. D., & Stover, P. J. (2009). SHMT1 and SHMT2 are functionally redundant in nuclear de novo thymidylate biosynthesis. *PLoS One*, *4*(6), e5839. https://doi.org/10.1371/journal.pone.0005839

Benna, C., Bonaccorsi, S., Wülbeck, C., Helfrich-Förster, C., Gatti, M., Kyriacou, C. P., Costa, R., & Sandrelli, F. (2010). Drosophila timeless2 is required for chromosome stability and circadian photoreception. *Current Biology*, *20*(4), 346–352. https://doi.org/10.1016/j.cub.2009.12.048

Carpenter, N. J. (1974). Properties and inhibition of thymidylate synthetase in *Drosophila melanogaster*. *Journal of Insect Physiology*, *20*(7), 1389–1401. https://doi.org/10.1016/0022-1910(74)90240-6

Contestabile, R., di Salvo, M. L., Bunik, V., Tramonti, A., & Vernì, F. (2020). The multifaceted role of vitamin B(6) in cancer: *Drosophila* as a model system to investigate DNA damage. *Open Biology*, *10*(3), 200034. https://doi.org/10.1098/rsob.200034

Curtin, K., Ulrich, C. M., Samowitz, W. S., Bigler, J., Caan, B., Potter, J. D., & Slattery, M. L. (2007). Thymidylate synthase polymorphisms and colon cancer: Associations with tumor stage, tumor characteristics and survival. *International Journal of Cancer*, *120*(10), 2226–2232. https://doi.org/10.1002/ijc.22603

di Salvo, M. L., Contestabile, R., & Safo, M. K. (2011). Vitamin B(6) salvage enzymes: Mechanism, structure and regulation. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1814*(11), 1597–1608. https://doi.org/10.1016/j.bbapap.2010.12.006

Ducker, G. S., & Rabinowitz, J. D. (2017). One-carbon metabolism in health and disease. *Cell Metabolism*, *25*(1), 27–42. https://doi.org/10.1016/j.cmet.2016.08.009

Fenech, M., & Ferguson, L. R. (2001). Vitamins/minerals and genomic stability in humans. *Mutation Research*, *475*, 1–6. https://doi.org/10.1016/s0027-5107(01)00069-0

Fernandez, A., O'Leary, C., O'Byrne, K. J., Burgess, J., Richard, D. J., & Suraweera, A. (2021). Epigenetic mechanisms in DNA double strand break repair: A clinical review. *Frontiers in Molecular Biosciences*, *8*, 685440. https://doi.org/10.3389/fmolb.2021.685440

Fox, J. T., & Stover, P. J. (2008). Folate-mediated one-carbon metabolism. *Vitamins and Hormones*, *79*, 1–44. https://doi.org/10.1016/S0083-6729(08)00401-9

Fu, Z., Jiao, Y., Li, Y., Ji, B., Jia, B., & Liu, B. (2019). TYMS presents a novel biomarker for diagnosis and prognosis in patients with pancreatic cancer. *Medicine*, *98*(51), e18487. https://doi.org/10.1097/MD.0000000000018487

Graziano, F., Kawakami, K., Watanabe, G., Ruzzo, A., Humar, B., Santini, D., Catalano, V., Ficarelli, R., Merriman, T., Panunzi, S., Testa, E., Cascinu, S., Bearzi, I., Tonini, G., & Magnani, M. (2004). Association of thymidylate synthase polymorphisms with gastric cancer susceptibility. *International Journal of Cancer*, *112*(6), 1010–1014. https://doi.org/10.1002/ijc.20489

Hishida, A., Matsuo, K., Hamajima, N., Ito, H., Ogura, M., Kagami, Y., Taji, H., Morishima, Y., Emi, N., & Tajima, K. (2003). Associations between polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and susceptibility to malignant lymphoma. *Haematologica*, *88*(2), 159–166.

Kanellis, P., Gagliardi, M., Banath, J. P., Szilard, R. K., Nakada, S., Galicia, S., Sweeney, F. D., Cabelof, D. C., Olive, P. L., & Durocher, D. (2007). A screen for suppressors of gross chromosomal rearrangements identifies a conserved role for PLP in preventing DNA lesions. *PLoS Genetics*, *3*(8), e134. https://doi.org/10.1371/journal.pgen.0030134

Kimura, M., Kuwabara, Y., Mitsui, A., Ishiguro, H., Sugito, N., Tanaka, T., Shiozaki, M., Naganawa, Y., & Takeyama, H. (2011). Thymidylate synthetase and dihydropyrimidine dehydrogenase mRNA levels in esophageal cancer. *Oncology Letters*, *2*(2), 297–301. https://doi.org/10.3892/ol.2010.227

Komlósi, V., Hitre, E., Pap, É., Adleff, V., Réti, A., Székely, É., Bíró, A., Rudnai, P., Schoket, B., Müller, J., Tóth, B., Ottó, S., Kásler, M., Kralovánszky, J., & Budai, B. (2010). SHMT1 1420 and MTHFR 677 variants are associated with rectal but not colon cancer. *BMC Cancer*, *10*, 525. https://doi.org/10.1186/1471-2407-10-525

Lightfoot, T. J., Skibola, C. F., Willett, E. V., Skibola, D. R., Allan, J. M., Coppede, F., Adamson, P. J., Morgan, G. J., Roman, E., & Smith, M. T. (2005). Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *14*(12), 2999–3003. https://doi.org/10.1158/1055-9965.EPI-05-0515

Lu, Y., Zhuo, C., Cui, B., Liu, Z., Zhou, P., Lu, Y., & Wang, B. (2013). TYMS serves as a prognostic indicator to predict the lymph node metastasis in Chinese patients with colorectal cancer. *Clinical Biochemistry*, *46*(15), 1478–1483. https://doi.org/10.1016/j.clinbiochem.2013.06.017

Lyon, P., Strippoli, V., Fang, B., & Cimmino, L. (2020). B vitamins and one-carbon metabolism: Implications in human health and disease. *Nutrients*, *12*(9), 2867. https://doi.org/10.3390/nu12092867

Magdalou, I., Lopez, B. S., Pasero, P., & Lambert, S. A. (2014). The causes of replication stress and their consequences on genome stability and cell fate. *Seminars in Cell & Developmental Biology*, *30*, 154–164. https://doi.org/10.1016/j.semcdb.2014.04.035

Martinez, M., Cuskelly, G. J., Williamson, J., Toth, J. P., & Gregory 3rd, J. F. (2000). Vitamin B-6 deficiency in rats reduces hepatic serine hydroxymethyltransferase and cystathionine β-synthase activities and rates of in vivo protein turnover, homocysteine remethylation and transsulfuration. *The Journal of Nutrition*, *130*(5), 1115–1123. https://doi.org/10.1093/jn/130.5.1115

Marzio, A., Merigliano, C., Gatti, M., & Vernì, F. (2014). Sugar and chromosome stability: Clastogenic effects of sugars in vitamin B6-deficient cells. *PLoS Genetics*, *10*(3), e1004199. https://doi.org/10.1371/journal.pgen.1004199

Mascolo, E., Amoroso, N., Saggio, I., Merigliano, C., & Vernì, F. (2020). Pyridoxine/pyridoxamine 5′-phosphate oxidase (Sgll/PNPO) is important for DNA integrity and glucose homeostasis maintenance in *Drosophila*. *Journal of Cellular Physiology*, *235*(1), 504–512. https://doi.org/10.1002/jcp.28990

Matxain, J. M., Padro, D., Ristilä, M., Strid, A., & Eriksson, L. A. (2009). Evidence of high *OH radical quenching efficiency by vitamin B6. *The Journal of Physical Chemistry. B*, *113*, 9629–9632. https://doi.org/10.1021/jp903023c

Merigliano, C., Marzio, A., Renda, F., Somma, M. P., Gatti, M., & Vernì, F. (2017). A role for the twins protein phosphatase (PP2A-B55) in the maintenance of drosophila genome integrity. *Genetics*, *205*(3), 1151–1167. https://doi.org/10.1534/genetics.116.192781

Merigliano, C., Mascolo, E., La Torre, M., Saggio, I., & Vernì, F. (2018). Protective role of vitamin B6 (PLP) against DNA damage in *Drosophila* models of type 2 diabetes. *Scientific Reports*, *8*(1), 11432. https://doi.org/10.1038/s41598-018-29801-z

Mocellin, S., Briarava, M., & Pilati, P. (2017). Vitamin B6 and cancer risk: A field synopsis and meta-analysis. *Journal of the National Cancer Institute*, *109*(3), djw230. https://doi.org/10.1093/jnci/djw230

Muha, V., Horváth, A., Békési, A., Pukáncsik, M., Hodoscsek, B., Merényi, G., Róna, G., Batki, J., Kiss, I., Jankovics, F., Vilmos, P., Erdélyi, M., & Vértessy, B. G. (2012). Uracil-containing DNA in *Drosophila*: Stability, stage-specific accumulation, and developmental involvement. *PLoS Genetics*, *8*(6), e1002738. https://doi.org/10.1371/journal.pgen.1002738

Natarajan, A. T. (2002). Chromosome aberrations: Past, present and future. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *504*(1–2), 3–16. https://doi.org/10.1016/s0027-5107(02)00075-1

Nomura, T., Nakagawa, M., Fujita, Y., Hanada, T., Mimata, H., & Nomura, Y. (2002). Clinical significance of thymidylate synthase expression in bladder cancer. *International Journal of Urology*, *9*(7), 368–376. https://doi.org/10.1046/j.1442-2042.2002.00479.x

Percudani, R., & Peracchi, A. (2003). A genomic overview of pyridoxal-phosphate-dependent enzymes. *EMBO Reports*, *4*(9), 850–854. https://doi.org/10.1038/sj.embor.embor914

Perry, C., Yu, S., Chen, J., Matharu, K. S., & Stover, P. J. (2007). Effect of vitamin B6 availability on serine hydroxymethyltransferase in MCF-7 cells. *Archives of Biochemistry and Biophysics*, *462*(1), 21–27. https://doi.org/10.1016/j.abb.2007.04.005

Silber, J., Bazin, C., & Le Menn, A. (1985). Inhibitors of thymidylate synthesis increase whereas thymidine decreases meiotic recombination in *Drosophila melanogaster*. *Molecular & General Genetics: MGG*, *199*(1), 53–54. https://doi.org/10.1007/BF00327508

Skibola, C. F., Smith, M. T., Hubbard, A., Shane, B., Roberts, A. C., Law, G. R., Rollinson, S., Roman, E., Cartwright, R. A., & Morgan, G. J. (2002). Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. *Blood*, *99*(10), 3786–3791. https://doi.org/10.1182/blood.v99.10.3786

Song, S., Tian, B., Zhang, M., Gao, X., Jie, L., Liu, P., & Li, J. (2021). Diagnostic and prognostic value of thymidylate synthase expression in breast cancer. *Clinical and Experimental Pharmacology & Physiology*, *48*(2), 279–287. https://doi.org/10.1111/1440-1681.13415

Stover, P. J., Chen, L. H., Suh, J. R., Stover, D. M., Keyomarsi, K., & Shane, B. (1997). Molecular cloning, characterization, and regulation of the human mitochondrial serine hydroxymethyltransferase gene. *Journal of Biological Chemistry*, *272*(3), 1842–1848. https://doi.org/10.1074/jbc.272.3.1842

Stover, P. J., & Field, M. S. (2011). Trafficking of intracellular folates. *Advances in Nutrition*, *2*(4), 325–331. https://doi.org/10.3945/an.111.000596

Tramonti, A., Cuyàs, E., Encinar, J. A., Pietzke, M., Paone, A., Verdura, S., Arbusà, A., Martin-Castillo, B., Giardina, G., Joven, J., Vazquez, A., Contestabile, R., Cutruzzolà, F., & Menendez, J. A. (2021). Metformin is a pyridoxal-5′-phosphate (PLP)-competitive inhibitor of SHMT2. *Cancers*, *13*(16). https://doi.org/10.3390/cancers13164009

Wang, L., Lu, J., An, J., Shi, Q., Spitz, M. R., & Wei, Q. (2007). Polymorphisms of cytosolic serine hydroxymethyltransferase and risk of lung cancer: A case-control analysis. *Lung Cancer*, *57*(2), 143–151. https://doi.org/10.1016/j.lungcan.2007.03.002

Winkler, F., Kriebel, M., Clever, M., Gröning, S., & Großhans, J. (2017). Essential function of the serine hydroxymethyl transferase (SHMT) gene during rapid syncytial cell cycles in *Drosophila*. *G3: Genes|Genomes|Genetics*, *7*(7), 2305–2314. https://doi.org/10.1534/g3.117.043133

Wu, X., Xu, W., Zhou, T., Cao, N., Ni, J., Zou, T., Liang, Z., Wang, X., & Fenech, M. (2016). The role of genetic polymorphisms as related to one-carbon metabolism, vitamin B6, and gene-nutrient interactions in maintaining genomic stability and cell viability in Chinese breast cancer patients. *International Journal of Molecular Sciences*, *17*(7), 1003. https://doi.org/10.3390/ijms17071003

Zeng, Y., Zhang, J., Xu, M., Chen, F., Zi, R., Yue, J., Zhang, Y., Chen, N., & Chin, Y. E. (2021). Roles of mitochondrial serine hydroxymethyltransferase 2 (SHMT2) in human carcinogenesis. *Journal of Cancer*, *12*(19), 5888–5894. https://doi.org/10.7150/jca.60170

Zinck, J. W. R., & MacFarlane, A. J. (2014). Approaches for the identification of genetic modifiers of nutrient dependent phenotypes: examples from folate. *Frontiers in Nutrition*, *1*, 8. https://doi.org/10.3389/fnut.2014.00008

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pilesi, E., Angioli, C., Graziani, C., Parroni, A., Contestabile, R., Tramonti, A., & Vernì, F. (2023). A gene-nutrient interaction between vitamin B6 and serine hydroxymethyltransferase (SHMT) affects genome integrity in *Drosophila*. *Journal of Cellular Physiology*, 1–9. https://doi.org/10.1002/jcp.31033