SETDB1 regulates microtubule dynamics

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Abstract

Objectives: SETDB1 is a methyltransferase responsible for the methylation of histone H3-lysine-9, which is mainly related to heterochromatin formation. SETDB1 is overexpressed in various cancer types and is associated with an aggressive phenotype. In agreement with its activity, it mainly exhibits a nuclear localization; however, in several cell types a cytoplasmic localization was reported. Here we looked for cytoplasmic functions of SETDB1.

Methods: SETDB1 association with microtubules was detected by immunofluorescence and co-sedimentation. Microtubule dynamics were analysed during recovery from nocodazole treatment and by tracking microtubule plus-ends in live cells. Live cell imaging was used to study mitotic kinetics and protein–protein interaction was identified by co-immunoprecipitation.

Results: SETDB1 co-sedimented with microtubules and partially colocalized with microtubules. SETDB1 partial silencing led to faster polymerization and reduced rate of catastrophe events of microtubules in parallel to reduced proliferation rate and slower mitotic kinetics. Interestingly, over-expression of either wild-type or catalytic dead SETDB1 altered microtubule polymerization rate to the same extent, suggesting that SETDB1 may affect microtubule dynamics by a methylation-independent mechanism. Moreover, SETDB1 co-immunoprecipitated with HDAC6 and tubulin acetylation levels were increased upon silencing of SETDB1.

Conclusions: Taken together, our study suggests a model in which SETDB1 affects microtubule dynamics by interacting with both microtubules and HDAC6 to enhance tubulin deacetylation. Overall, our results suggest a novel cytoplasmic role for SETDB1 in the regulation of microtubule dynamics.

1 | INTRODUCTION

SETDB1 (also known as ESET and KMT1E) is a lysine methyltransferase (KMT) that belongs to the suppressor of variegation 3–9 (SUV39) family of KMTs that mainly methylate lysine 9 in histone H3 (H3K9). The SUV39 family members are characterized by cysteine-rich pre- and post-SET domains flanking a central SET (Su(var)3–9, Enhancer-of-zeste and Trithorax) domain that is responsible for the catalytic activity. SETDB1 also contains a methyl-CpG-binding domain (MBD) and a triple Tudor domain responsible for binding of H3K9me/K14ac to promote histone deacetylation by histone deacetylases (HDACs). SETDB1 can mono-, di- and tri-methylate H3K9. H3K9

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me2/3 are usually associated with gene repression and heterochromatin formation, and indeed SETDB1 is involved in silencing the X chromosome, repetitive elements and specific genes. SETDB1 is important for various developmental processes including embryogenesis, neurogenesis, immune cell development and germ line development. SETDB1 was also shown to methylate non-histone proteins such as inhibitor of growth protein 2 (ING2), p53 and upstream binding factor (UBF).

SETDB1 is considered a nuclear protein; and indeed, its substrates either histone H3 or non-histone proteins, are mainly nuclear proteins. However, SETDB1 contains both nuclear localization signal (NLS) motifs and nuclear export signal (NES) motifs. In addition, a cytoplasmic pool of SETDB1 has been found in several types of cells including HeLa cells, HEK293 cells, mouse embryonic fibroblasts, differentiated myoblasts and human melanoma biopsies. Cytoplasmic localization of SETDB1 is thought to facilitate methylation of newly synthesized histones before their incorporation into nucleosomes or to restrict the enzyme of methylating nucleosomal H3K9.

In recent years, SETDB1 has been considered an oncogene. Its genomic location is commonly amplified in melanoma and its expression levels are increased in various types of cancer to support cancer cell proliferation, migration and invasion. These types of cancer include melanoma, colorectal cancer, liver cancer and lung cancer. More recently, SETDB1 was also linked to adaptive neurogenesis, colorectal cancer, repetitive elements or to restrict the enzyme of methylating nucleosides. SETDB1 is considered a nuclear protein; and indeed, its substrates either histone H3 or non-histone proteins, are mainly nuclear proteins. However, SETDB1 contains both nuclear localization signal (NLS) motifs and nuclear export signal (NES) motifs. In addition, a cytoplasmic pool of SETDB1 has been found in several types of cells including HeLa cells, HEK293 cells, mouse embryonic fibroblasts, differentiated myoblasts and human melanoma biopsies. Cytoplasmic localization of SETDB1 is thought to facilitate methylation of newly synthesized histones before their incorporation into nucleosomes or to restrict the enzyme of methylating nucleosomal H3K9.

Currently, SETDB1 is thought to promote cancer mainly by its nuclear activity of methylating H3K9 or transcription factors such as p53. However, since a substantial amount of SETDB1 can be translated into cytoplasm, we looked for cytoplasmic activity of SETDB1 that may also promote cancer formation and progression. Here we show that SETDB1 co-sedimented with microtubules (MTs) and that cytoplasmic SETDB1 partially co-localized with the MT network. Reduced SETDB1 levels increased MT stability as measured by EB1-tracking and MT recovery from nocodazole treatment and interfered with mitotic progression. Over-expression of either wild type (WT) or catalytic dead (CD) SETDB1 increased MT polymerization rate to the same extent, suggesting that SETDB1 can affect MT dynamics by an additional mechanism to substrate methylation. Finding interaction between SETDB1 and the tubulin deacetylase histone deacetylase 6 (HDAC6), along with increased tubulin acetylation levels after reduction in SETDB1 levels, suggest that SETDB1 may affect MT dynamics by supporting HDAC6 activity.

2 MATERIALS AND METHODS

2.1 Plasmids and molecular cloning

Plasmids expressing GFP-fused WT and H1224K SETDB1 fused to GFP were generated by PCR using pREV-SETDB1 as a template (a kind gift from Slimane Ait-Si-Ali, Centre National de la Recherche Scientifique [CNRS], Université de Paris, Université Paris Diderot, Paris, France). WT SETDB1 was amplified by KOD Hot Start DNA Polymerase (71086, Merck) and the oligonucleotides 5′-cagagaTTGCTTCTCCTG GGTGCTAT-3′ and 5′-gtgtcagCTAAA-GGAGACGTCTCGCATCATT-3′. The PCR product was ligated into SacI-SaiI sites in pEGFP-C3. Site-directed mutagenesis to generate SETDB1 H1224K was performed by PCR using the above two oligonucleotides and the oligonucleotides: 5′-GGGCCGCTACCTCAAC aagAGTTGCAGCCCCAACC-3′ and 5′-GGTTGGGCGCTG AACATT GTTAGAGTCGACC-3′. Cloning procedures were confirmed by DNA sequencing. pcDNA-HDAC6-FLAG was a gift from Tso-Pang Yao (Addgene plasmid # 30482). EB1-GFP was a kind gift from Orly Reiner, Weizmann Institute of Science, Rehovot, Israel. pEGFP-Moesin was a kind gift from Peter Vilmos, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary.

2.2 Cell culture, transfections and proliferation assay

B16-F1 cells (ATCC, CRL-6323), WM266.4 cells (a kind gift from Gal Markel, Sheba Medical Center, Israel), HeLa cells (ATCC, CCL-2) and HEK293 cells (ATCC, CRL-1573) were cultured in DMEM (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 10% fetal calf serum, 0.5% penicillin - streptomycin solution mix, and 1% L-glutamine, at 37°C in a 7% CO2 environment. Transfections of DNA plasmids were carried out with the NanoJuice Transfection Kit (71900-3, Haemek, Israel), according to the manufacturer’s instructions. Cells were incubated for 24 h before further analysis. For gene silencing, cells were transfected twice with siRNA (IDT, Coralville, IA, USA), with a time interval of 48 h using INTERFERin transfection reagent (Polyplus-transfection, Illkirch-Graffenstaden, France) according to the manufacturer’s instructions. Cells were incubated for 24 h after the second transfection before further analysis. IRNAs used were mouse SETDB1 (mm. Rl.Setdb1.13.1), human SETDB1 (hs.Rl.SETDB1.13.1) and negative control (51-01-14-04). IRNA transfection efficiency was >90% as verified by transfection of Cy3 Transfection Control DsiRNA (51-01-03-06). Proliferation rate was measured with Cell Proliferation Kit (XTT based) (20-300-1000, Biological Industries, Kibbutz Beit-Haemek, Israel), according to the manufacturer’s protocol.

2.3 Protein lysate preparation and Western blot analysis

For nuclear–cytoplasmic fractionation, cells were washed in PBS, re-suspended in STM buffer: 50 mM Tris pH 7.4, 250 mM sucrose, 5 mM MgSO4, 10 mM NaF, 2 mM DTT, 0.025% Triton X-100 and 1× protease inhibitor cocktail (539134, Millipore, Burlington, MA, USA) and lysed by a Dounce homogenizer. Following cell membrane
breakage (as monitored under the microscope), the nuclei fraction was precipitated at 700 g for 10 min at 4 °C. The cytoplasmic supernatant was collected to a new tube and the nuclear pellet was re-suspended in TP buffer: 10 mM Tris pH 7.4, 10 mM phosphate buffer pH 7.4, 5 mM MgSO4, 10 mM NaF and 1× protease inhibitor cocktail supplemented with 5% glycerol. Protein concentrations were measured using the Bradford assay. Samples were stored at −80 °C. For whole cell extracts, cells were washed in PBS, re-suspended in 2× SDS sample buffer (100 mM Tris pH 6.8, 10% glycerol, 2% SDS, 100 mM bromophenol blue, 0.1 M DTT and 1× protease inhibitor cocktail) and sonicated. Samples were then heated at 95 °C for 10 min and kept at −20 °C until use.

Protein extracts were separated in SDS-PAGE and analysed by Western blot analysis using the following antibodies: rabbit anti-SETDB1 (1:500, Santa Cruz Biotechnology sc-66884), rabbit anti-SUV39H2 (1:1000, Abcam ab190270), rabbit anti-histone H3 (1:10,000, Millipore 05-928), mouse anti-SETDB1 (1:500, Santa Cruz Biotechnology sc-66884), mouse anti-acetylated tubulin (1:1000, Santa Cruz Biotechnology sc-23950), mouse anti-FLAG (1:1000, Sigma-Aldrich F7425). Where indicated, quantitative data analysis was performed with the ImageJ/Fiji software (National Institute of Health, Bethesda, USA) using three repetitions and statistical significance was determined by the Student’s t-test.

### 2.4 | MT co-sedimentation assay

MT co-sedimentation assay was performed as reported previously. Briefly, cells were lysed in PIPES buffer: 80 mM PIPES pH 6.8, 1 mM MgCl2, 1 mM EGTA, 100 mM NaCl, 1% Triton X-100, and 1× protease inhibitor cocktail (539134, Millipore, Burlington, MA, USA) for 30 min on ice. Cell lysates were cleared by two repetitive centrifugations at 20,000g for 20 min at 4 °C. The supernatants supplemented with 1 mM GTP and 40 μM Paclitaxel were incubated at 4 °C or 37 °C for 30 min for tubulin polymerization before centrifugation at 20,000g for 30 min at 4 °C or 37 °C, respectively. The resulting pellets and supernatants were subject to Western blot analysis.

### 2.5 | Immunostaining

Cells plated on fibronectin-coated coverslips (03-090-1-05, Biological Industries, Beit-Haemek, Israel) were fixed in methanol supplemented with 1 mM EGTA at −20 °C for 6 min. Antibodies included rabbit anti-SETDB1 (1:50, Santa Cruz Biotechnology sc-66884), rabbit anti-SETDB1 (1:500, Cell Signaling Technology 93212), rabbit anti-SUV39H2 (1:120, Abcam ab190270), mouse anti-α-tubulin (1:200, Thermo Fisher Scientific 62204), goat anti-GFP (1:2000, Abcam ab5450) and rabbit anti-γ-tubulin (1:400, Abcam ab1132). All images were collected using an Olympus 1X81 fluorescent microscope with a coolSNAP HQ2 CCD camera (Photometrics, Tuscon, AZ, USA).

### 2.6 | MT recovery assay

Cells plated on fibronectin-coated coverslips were treated with 7 μg/ml of nocodazole for 3 h. Following three washings with cold DMEM to remove the nocodazole, the cells were incubated at 37 °C in pre-warmed complete growth medium for the indicated periods of time, fixed and immunostained as described above. Quantitative data analysis was performed with ImageJ/Fiji software (NHI) by manual delineation of the area covered by MTOC-linked MTs. At least 30 cells were measured for each condition in each repetition and the average size of treated cells was normalized to the one of control cells. Average scores of three repetitions were calculated and statistical significance was determined by the Student’s t-test.

### 2.7 | Time-lapse imaging

For live-cell imaging, cells were plated in 35-mm glass-bottom dishes. Time-lapse images were collected with a coolSNAP HQ2 CCD camera (Photometrics, Tuscon, AZ, USA) mounted on an Olympus 1X81 fluorescent microscope at 37 °C and 7% CO2. Frames were captured every 3 s for 1.5 min (movies to track growing MT ends) or every 3 min for 10 h (movies to monitor mitotic progression). Acquired images were analysed by ImageJ/Fiji software. To analyse growing MT ends, EB1-GFP comets were tracked manually using the MTrackJ ImageJ plugin. Comets were analysed in each frame considering the distal site of the comet as the comet point. Data analysis was done according to duration and length tracked. To analyse mitosis progression, mitotic events were followed in terms of time and success rate.

### 2.8 | Co-immunoprecipitation

HEK293 cells were harvested 48 h following co-transfection and lysed in extraction buffer (50 mM Tris pH 8, 150 mM NaCl, 20 mM EDTA, 50 mM NaF, 1% Triton X-100) supplemented with 1× protease inhibitor cocktail. Cells were lysed on ice for 30 min and centrifuged at 20,000g for 30 min at 4 °C. A 5% input control sample was taken from each cleared lysate and boiled in SDS sample buffer for 10 min at 98 °C. For immunoprecipitation, clarified lysates were supplemented with GFP Trap Magnetic Agarose (gtma-20, Chromotek, Planegg-Martinsried, Germany) and incubated with rotation for 1 h at 4 °C. The beads were washed once in extraction buffer and three times in PBS and boiled in SDS sample buffer for 10 min at 98 °C, before being loaded on an 8% acrylamide gel for subsequent Western blot analysis.

### 3 | RESULTS

#### 3.1 | SETDB1 associates with the MT network

To identify a cytoplasmic role for SETDB1, we first verified its cytoplasmic localization in both mouse and human melanoma cells:
B16-F1 and WM266.4 cells, respectively. Biochemical fractionation followed by Western blot analysis identified a substantial amount of SETDB1 in the cytoplasmic fraction of these cells in contrast to another H3K9 methyltransferase, suppressor of variegation 3-9.
homologue 2 (SUV39H2), which was found only in the nuclear fraction (Figure 1A). Immunostaining for SETDB1 verified this observation and revealed a partial co-localization of SETDB1 with MTs in B16-F1 cells (Figure 1B,C). In WM266.4 cells, the cytoplasmic fraction of SETDB1 was smaller than in B16-F1 cells, though some co-localization of cytoplasmic SETDB1 with MTs could still be detected (Figure 1D,E). To validate this observation, we used an additional antibody against SETDB1 that revealed a similar pattern of partial co-localization (Figure 1D,E). Co-localization of SETDB1 with MTs was also found in HeLa cells (Figure 1F). Moreover, this pattern was kept during mitosis in B16-F1 cells and in HeLa cells (Figure 1G). Analysis of the localization of over-expressed GFP-fused SETDB1 revealed a similar pattern of partial co-localization with MTs (Figure 1H,I).

**FIGURE 2** Co-sedimentation of SETDB1 with MTs. MT co-sedimentation assay of HeLa cells and WM266.4 cells. The pellet (P) of 37°C incubation indicates the MT-bound fraction, and supernatant (S) indicates the unbound fraction. Incubation at 4°C serves as a MT-free control.

**FIGURE 3** Reduced SETDB1 levels enhance MT polymerization rate. (A,B) MTs were disrupted in B16-F1 cells transfected with either Ctl siRNA (A) or siRNA against SETDB1 (B) by nocodazole treatment for 3 h. Following nocodazole removal, cells were further incubated for the indicated time periods to allow MT polymerization. After fixation, cells were immunostained with antibodies against α-tubulin, γ-tubulin and DNA was stained with Hoechst 33342. The white arrows indicate the localization of the MTOCs in the recovery micrographs. The MTOCs in the orange rectangles are magnified at the left top corner of the micrographs of the 3 min of recovery from nocodazole treatment. The scale bar: 20 μm. (C) Quantification of MT recovery rate after nocodazole washout. The area covered by MTOC-linked MTs was delineated manually and measured by ImageJ. The MT covered area in cells transfected with Ctl siRNA was set as 1. The bar graph shows the average relative area covered by MTs of three repetitions ±SE. At least 30 cells were measured for each condition in each repetition. Statistical significance was calculated with Student’s t-test, **p < 0.01.
To verify this, we assessed MTs binding by co-sedimentation. As shown in Figure 2, incubation of cell lysates at 37°C in conditions that promote MTs polymerization led to co-sedimentation of SETDB1 in lysates prepared from HeLa and WM266.4 cells. Taken together, these results may suggest a role for SETDB1 in MT functions.

3.2 | SETDB1 affects MT growth rate

To evaluate whether SETDB1 can affect MT functions, we first tested the impact of SETDB1 levels on the rate of MT growth during recovery from nocodazole treatment. B16-F1 cells were transfected with control short interfering RNA (siRNA) or SETDB1 siRNA, which reduced SETDB1 levels by about 50%, probably due to its long half-life (Figure S1). Then cells were treated with nocodazole for 3 h to depolymerize MTs. Following nocodazole washout, the cells were further incubated for an additional 3 or 7 min, fixed and immunostained for α-tubulin. Measurements of the area covered by MTs originated from the MTOC (centrosomal MTs) revealed an increase of 82% and 60% in SETDB1 siRNA-transfected cells, in comparison to control siRNA (Ctl siRNA)-transfected cells at the 3 and 7 min time points, respectively (Figure 3). This finding suggests that SETDB1 may attenuate MT growth rate.

To verify the ability of SETDB1 to attenuate MT growth rate, we repeated the experiment in cells over-expressing SETDB1. Interestingly, over-expressed (OE) SETDB1 hardly accumulates in the nucleus and is localized mainly to the cytoplasm. This observation was reported before and repeated also in our hands (Figures 1H, 3B). For that experiment we used GFP-Moesin as a negative control and found that the amount of MTOC-linked MTs 3 min after nocodazole washout in GFP-SETDB1 OE cells were reduced by half, compared to GFP-Moesin OE cells (Figure 4).

FIGURE 4 SETDB1 over-expression enhances MT polymerization rate. (A,B) MTs were disrupted in B16-F1 cells transfected with either pEGFP-Moesin (A) or pEGFP-SETDB1 (B) by nocodazole treatment for 3 h. Following nocodazole removal, cells were further incubated for 3 min to allow MT polymerization. After fixation, cells were immunostained with antibodies against GFP, α-tubulin and γ-tubulin and DNA was stained with Hoechst 33342. The white arrows indicate the localization of the MTOCs in the recovery micrographs. The MTOCs in the orange rectangles are magnified at the right bottom corner of the micrographs of recovery from nocodazole treatment. The merged images show the merged signals of α-tubulin (yellow), γ-tubulin (magenta) and Hoechst (cyan). Scale bar: 20 μm. (C) Quantification of MT recovery rate after nocodazole washout. The area covered by MTOC-linked MTs was delineated manually and measured by ImageJ. The MT covered area in cells transfected with pEGFP-Moesin was set as 1. The bar graph shows the average relative area covered by MTs of three repetitions ±SE. At least 30 cells were measured for each condition in each repetition. Statistical significance was calculated with Student’s t-test, **p < 0.01.
opposite direction to the effect of SETDB1 siRNA (Figure 3), strengthen-
ing the idea that SETDB1 attenuated MT growth rate.

To validate the effects of SETDB1 on MT growth we monitored the MT plus end dynamics by tracking GFP-fused end-binding protein
1 (EB1) in SETDB1 siRNA-transfected cells (Figures S1 and 5, Movies S1 and S2). Notably, MT growth duration, growth length and growth rate were increased in SETDB1 siRNA-transfected cells by 25%, 38% and 11%, respectively, in comparison to control cells (Figure 5A–C). On the other hand, MT catastrophe rate in SETDB1 siRNA-treated cells was reduced by 15% in comparison to control cells (Figure 5D). These results suggest that SETDB1 could be a negative regulator of MT growth.

3.3 SETDB1 is important for proper cell division

The finding that SETDB1 regulates MT dynamics led us to test if SETDB1 is important for cell division, a process that is heavily dependent on MT organization. Indeed, reduced SETDB1 levels attenuated the proliferation of HeLa cells by 20% (Figure 6A), while increasing unsuccessful mitotic events from 4.1% to 22.1% (Figure 6B). Measuring the lengths of the different cell division stages in cells that were able to finish mitosis successfully revealed a significant increase in the duration of both early and late mitosis in SETDB1 siRNA transfected cells in comparison to Ctrl siRNA-transfected cells. In SETDB1 siRNA-transfected cells, the durations from nuclear envelope breakdown (NEB) to anaphase and from anaphase to the appearance of a cleavage furrow were increased by 17% and 40%, respectively, in comparison to control cells (Figure 6C–H).

3.4 Catalytic-dead SETDB1 can affect MT growth rate

SETDB1 is a well-established methyltransferase that was found to methylate both histones and non-histone proteins. Moreover, recently α-tubulin was found to be methylated at lysine 40 by SET domain containing 2 (SETD2). To evaluate if the effect of SETDB1 on MT growth rate is dependent on its methyltransferase activity, we repeated the MT recovery assay while over-expressing either WT
As shown in Figure 8, SETDB1 interacts with HDAC6 (Figure 8A-C), thus supporting the hypothesis that SETDB1 may serve as a co-factor of HDAC6 in the cytoplasm to support tubulin deacetylation to regulate MT dynamics.

3.5 | SETDB1 affects tubulin acetylation

The molecular mechanism by which SETDB1 affects MT dynamics seemed to be at least partially independent of its methyltransferase activity. To identify a molecular mechanism by which SETDB1 could affect MT dynamics, we took into account two features: the first is that nuclear SETDB1 is known to participate in a complex with HDAC1 and HDAC2 to repress transcription. This suggests that the oncogenic function of SETDB1 is partially contributed by methyltransferase independent activities of the protein. These results suggest that the effects of SETDB1 on MTs did not require its methyltransferase activity. This observation is in agreement with studies on SETDB1 oncogenic activity in the zebrafish model: over-expression of SETDB1 was found to accelerate melanoma onset in a zebrafish model for melanoma formation and progression. Notably, over-expression of the enzymatically inactive H1224K SETDB1 (CD SETDB1) still significantly accelerated melanoma onset, although to a lower extent than WT SETDB1. More recently, the Caenorhabditis elegans homologue of SETDB1, MET-2, was shown to be able to affect transcription even when its methyltransferase activity was ablated.

This suggests that the oncogenic function of SETDB1 is partially contributed by methyltransferase independent activities of the protein. One of these activities may be the modulation of MT dynamics. Based on the findings that SETDB1 interacted with HDAC6 and that reduced levels of SETDB1 led to increased tubulin acetylation, we hypothesize that SETDB1 can modulate MT dynamics by affecting the activity of the tubulin deacetylase HDAC6. Tubulin acetylation is associated with more stable MTs, as shown by others and also in our hands. Thus, effect of OE SETDB1 on MT dynamics suggests that the impact of SETDB1 on MT growth rate is due to the activity of its cytoplasmic pool rather than the nuclear SETDB1.

SETDB1 or H1224K point mutated SETDB1 (SETDB1-CD) (Figure 6). The H1224K mutation was shown before to completely impair the methyltransferase activity of SETDB1. As shown in Figure 7, overexpression of the CD SETDB1 attenuated MT recovery from nocodazole treatment to the same extent as over-expression of WT SETDB1. Thus, SETDB1 could affect MT dynamics by a mechanism that is independent of its enzymatic activity.
increased MT catastrophe rate and slower recovery from nocodazole treatment. Notably, altered levels of both tubulin acetylation and HDAC6 were detected in several types of cancer. In some cases, such as in breast cancer and glioblastoma, HDAC6 was shown to support tumour progression.51

Our results, taken together with previous reports, suggest that SETDB1 may affect various aspects of tumour progression such as cell division and cell migration, at multiple levels by different mechanisms. At the chromatin level, it may affect transcriptional control of key factors to support cell migration and proliferation28,30,52,53 and it may promote global chromatin condensation to support cell migration52,54; while at the MT level, it may modulate MT dynamics possibly by affecting the activity and/or the binding of microtubule-associated proteins (MAPs) to MTs such as HDAC6.

Cross-talk between interphase chromatin and MTs was found before: MT-driven mechanical forces were shown to alter chromatin organization,55,56 the motor protein kinesin KIF4 was found to be involved in heterochromatin formation, transcription and DNA repair.57 Dynein light chain 1 (DLC1) was found to be involved in transcriptional control.56 Tau nuclear subcellular localization is thought to associate with DNA damage protection.58 LIS1, a regulator of cytoplasmic dynein, was shown to interact with histone H1 and MeCP1 and to affect the chromatin binding of the latter.59 Thus, SETDB1 seems to join this growing group of factors with activities in both the chromatin and the MT worlds.

AUTHOR CONTRIBUTIONS
Conceptualization, G.G., R.H.V. and T.L.; methodology, R.H.V., J.S., T.L and G.G.; investigation, R.H.V., J.S. and N.P.; formal analysis, R.H.V.; writing—original draft, G.G.; writing—review and editing, R.H.V., J.S., N.P., T.L. and G.G.; supervision; T.L. and G.G.; funding acquisition, G.G.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
No codes or sequencing datasets were generated in this study. All materials and protocols will be available upon request.

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SUPPORTING INFORMATION
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