Human DIMT1 generates $N_{\text{2,6,6}}$A-dimethylation-containing small RNAs

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Supporting Information

Included Supporting Information: Supplementary Figures S1-S7.
Figure S1. Purification of multiple RNA species. Purification of (A) tRNAs and (B) 18S and 28S rRNA. (C) RNA bioanalyzer results of the purification process of polyadenylated RNAs. This gel-like image is the simulated image of the transfer of the raw migration time and intensity data to mimic electrophoretic assays. qRT-PCR of 18S rRNA from total RNA, after two rounds of poly-dT extracted, and after one more round of ribominus purification. (D) LC-MS/MS channels, peak areas, and standard curves of adenosine, guanosine, and m$_2$$^6$A.
Figure S2. ESI-CID data for m$_2$$^6$A standard (paired with chromatogram peaks), measured using (A) synthetic standards (upper panels) and 18S rRNA isolated from HEK 293T cells (lower panels).
Figure S3. NLPY DIMT1 is catalytically inactive. (A) Left: Western blots showing the expression of DIMT1 (both Flag-tagged and endogenous) in $DIMT1^{+/+}$ + E85A, $DIMT1^{+/+}$ + wild type (wt), $DIMT1^{+/+}$ + NLPY (wt), and $DIMT1^{+/+}$ + empty vector cells with anti-DIMT1 antibody. Right: Quantification of the expression of DIMT1 in these cells. (B) Western blots showing the expression of FLAG-tagged DIMT1 in $DIMT1^{+/+}$ + E85A, $DIMT1^{+/+}$ + wild type (wt), $DIMT1^{+/+}$ + NLPY, and $DIMT1^{+/+}$ + empty vector with anti-Flag antibody. (C) LC-MS/MS channels and peak areas of adenosine and m$_{6,6}$$^6$A in small RNA (< 40 nt) extracted from $DIMT1^{+/+}$ + wild type DIMT1 (left) and $DIMT1^{+/+}$ + E85A DIMT1.
Figure S4. (A) Western blots showing the expression of endogenous DIMT1 in *DIMT1*+/+ and *DIMT1*+/- HEK 293T cells. (B) Gel purification of small RNAs from *DIMT1*+/+ and *DIMT1*+/- HEK 293T cells. (C) LC-MS/MS channels and peak areas of adenosine and m$_2$A in small RNA (< 40 nt) extracted from *DIMT1*+/+(left) and *DIMT1*+/- (right) HEK 293T cells. (D) quantification of m$_2$A in small RNA (< 40 nt) from *DIMT1*+/+(ctrl) and *DIMT1*+/- HEK 293T cells.
Figure S5. NLPY DIMT1 is catalytically inactive. (A) SDS-PAGE results of purified wild type and DIMT1 NLPY variant. (B) Thermostability of WT-DIMT1 and NLPY DIMT1 variant. RFU means relative fluorescence units. LC-MS/MS channels and peak areas of guanosine and m$_2^{6,6}$A in the in vitro reactions with (C) wild type or (D) NLPY DIMT1 variant. LC-MS/MS channels and peak areas of adenosine and m$_2^{6,6}$A in (E) DIMT1$^{wt}$ + wild type (wt) or (F) DIMT1$^{d/d}$ + NLPY cells.
Figure S6. Expression of the catalytically inactive NLPY DIMT1 decreases $m_{2,6}^A$ in 18S rRNA and impair protein synthesis but does not lead to obvious changes in the levels of 18S rRNA or 40S monosome. (A) LC-MS/MS quantification of $m_{2,6}^A$ in 18S rRNA extracted from $DIMT1^{+/−}$ + empty vector, $DIMT1^{+/−}$ + wild type (wt), and $DIMT1^{+/−}$ + NLPY cells. $p$ values were determined using a two-tailed Student’s t-test for unpaired samples. Error bars represent mean ± SD. ***$p < 0.001$, and n.s. = $p > 0.05$. (B) qRT-qPCR quantification of 18S rRNA as normalized the level of GADPH in $DIMT1^{+/−}$ + wild-type DIMT1 and $DIMT1^{+/−}$ + NLPY DIMT1 cells. $p$ values were determined using a two-tailed Student’s t-test for unpaired samples. Error bars represent mean ± SD; n.s. = $p > 0.05$. (C) Polysome profiles of $DIMT1^{+/−}$ + wild type and $DIMT1^{+/−}$ + NLPY variant cells. (D) Imaging and quantification of fluorescent-labeled nascent protein in $DIMT1^{+/−}$ + wild type and $DIMT1^{+/−}$ + NLPY cells. Nuclei were stained with NuclearMask.
Figure S7. Downregulation of DMT1 in MOLM-13C leads to a decreased level of m$_2$$^{6,6}$A in small RNAs. LC-MS/MS channels and peak areas of adenosine and m$_2$$^{6,6}$A MOLM-13 cells transfected with sgCtrl, sgDMT1#17, or sgDMT1#84.