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Appendix Materials and Methods

Preparation of tRNAs and their corresponding mutants. The sequences of human cytosolic $tRNA^{Gly}$(GCC), $tRNA^{Gly}$(CCC), $tRNA^{Gly}$(UCC), $tRNA^{Pro}$(AGG), $tRNA^{Pro}$(CGG), $tRNA^{Pro}$(UGG), $tRNA^{His}$(GUG), $tRNA^{Lys}$(UUU), $tRNA^{Arg}$(ACG) and RNA^{Phe}(GAA) were obtained from the GtRNAdb, and the genes were inserted between the *Eco*RI and *Bam*HI in pTrc99B with a 5'-terminal T7 promoter. Site-directed mutagenesis of the tRNAs was generated by using a KOD-plus mutagenesis kit. All tRNAs were synthesized by *in vitro* T7 RNA polymerase transcription (Liu *et al*, 2017).

Construction of knockout cell lines. Sequence specific small guide RNAs (sgRNA) targeting *hTrmt13* were computationally designed for the selected genomic targets (http://crispr.mit.edu) and were cloned into vector pX330-mcherry (Addgene, 98750) which expresses red fluorescence protein. Two sgRNAs were used (sg-1: GGGTAGATGCGGTTACTATG, sg-2: AGATTTTGTGGTGAACACGC) in our experiment. For generating KO cell lines, 6 μg sgRNA plasmids were transfected in dishes (6 cm^2) of MDA-MB-231 cells using Lipofectamine 3000 (Invitrogen) as transfection reagent. After transfection for 24 h, MDA-MB-231 cells expressing red fluorescent protein were enriched by Fluorescence-Activated Cell Sorting Aria II (BD Bioscience) and plated into a well of a 10 cm^2 dish at a low density. After 5–8 d, single colonies were picked and plated into a well of a 96-well plate. The knockout efficiency of hTrmt13 was measured by Western blotting.

Cell culture. HEK293 (purchased from ATCC), MDA-MB-231 (This cell line was a gift from Dr. Mofang Liu's Lab), HeLa (purchased from ATCC), and HCC38 (purchased from ATCC) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) with 10% FBS (Gibco) and 1% penicillin–streptomycin (Gibco) at 37 °C with 5% CO_2.

Plasmid construction and cell transfection. Wild-type hTrmt13 was cloned from human cDNA into pcDNA3.1 vector (Invitrogen) with an additional N-terminal Flag tag or N-terminal HA tag and a stable expression vector pHAGE (Addgene) with HA or FLAG tag. The catalytically inactive hTrmt13 E463A mutant were generated by introducing mutations using a KOD-plus mutagenesis kit on the plasmid of pHAGE-hTrmt13. The promoter of *TGFB1*, *PKN2* or *MEF2A* was cloned into pGL3 (Promega). The shRNA targeting hTrmt13 was cloned into pLKO.1. The plasmids were transfected into HEK293 and HeLa cells using Lipofectamine 2000 (Invitrogen). The siRNA or synthetic oligonucleotides were transfected into MDA-MB-231, HeLa, and HCC38

cells using Lipofectamine™ RNAiMAX (Invitrogen). The pGL3 and pRL-TK were transfected into MDA-MB-231 cells using Lipofectamine 3000 (Invitrogen).

sh-1:

5'CCGGGGAGACATTATGTGGGCAACTCGAGTTGCCCACATAATGTCTCCTTTTTG3'

sh-2:

5'CCGGCCTTTAGCCAAACGCATAACTCGAGTTATGCGTTTGGCTAAAGGTTTTTG3'

si-1: 5'GGAGACAUUAUGUGGGCAATT

si-2: CCUUUAGCCAAACGCAUAATT

CLIP-seq. CLIP was performed in MDA-MB-231 cells as described (Konig *et al*, 2011) with some modifications. Cells were crosslinked at 254 nm, with 200 mJ/cm^2 using UV Stratalinker 1800 (StrataGene). After lysis, immunoprecipitation was carried out with hTrmt13 antibodies (10 µg) overnight at 4 °C. Precipitated RNA was de-modified by *Ec*AlkBs and then used for reverse transcription. RNA was extracted using Trizol and the RNeasy Mini Kit (Qiagen), and RNA was reverse transcribed with SuperScript™ IV (Thermo scientific). Each library was sequenced using TruSeq SBS kit v4-HS, in paired-end mode with a read length of 2x150bp.

CLIP-seq data analysis. CLIP-seq data have been analysis by adapted from PARalyzer (version 1.5) (Corcoran *et al*, 2011) and PIPE-CLIP (Chen *et al*, 2014). Briefly, raw reads were trimmed for adapters using Timmomatic (version 0.36) (Bolger *et al*, 2014) (parameters: "ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:25 HEADCROP:9") and then mapped to human genome hg19 (GRCh37) by bowtie2 (version 2.3.5.1, parameters "--local -k 1 --rdg 0,6 --rfg 13,1 -p 1 -U"). tRNA annotation were downloaded from GtRNAdb 2.0 (Chan & Lowe, 2016). Result mapped file were converted to bed format and counted by bedtools (version 2.24.0) at tRNA annotations (Quinlan & Hall, 2010). We confirmed the results from this pipeline were good by re-processing data from GSE109183 and compared with the supplementary data from their paper (Carter *et al*, 2019). Codes and tRNA annotation files could be found at https://doi.org/10.6084/m9.figshare.13369595. Two replicates of CLIP-seq were performed and analyzed, that each of our replicates have more than 40 million reads that were more than ENCODE recommendation for eCLIP (1 million) and 5 out of 6 of iCLIP data from GSE109183.

Western blotting. Cells were washed with cold PBS and lysed by cold RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium-deoxycholate) supplemented with protease inhibitor cocktails (Roche). Lysates were cleared by centrifugation at 12,000 rpm for 10 min at 4 °C. Proteins were loaded to SDS-PAGE and transferred to PVDF membranes (Bio-Rad).

Antibodies. The following antibodies were used: mouse anti-FLAG (Sigma), mouse anti-HA (CST), rabbit anti-ZEB1 (NOVUS), rabbit anti-E-cadherin (proteintech), anti-N-cadherin (ABclonal), anti-Snail1 (proteintech), anti-Snail2 (proteintech), mouse anti-GAPDH (CST), rabbit Vimentin anti-(ABclonal), rabbit anti-FOSL1 (ABclonal), Rabbit anti-USF1 (ABclonal), rabbit anti-E2F1 (proteintech). Rabbit anti-LaminA/C (CST), Rabbit anti-beta-actin (Abcam), mouse anti-Histone 3 (Abcam). Rabbit anti-hTrmt13 was customized from ABclonal (Wuhan, China).

Primer extension assay. Primer extension was conducted as described previously with little modifications (Qu *et al*, 2001). The 5' digitonin (DIG)-labeled primer (0.1 pmol) was incubated with 300 μg of purified HctRNA^{Gly}(GCC) or HctRNA^{Gly}(CCC) in a 10 μL solution containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA for 5 min at 85°C, and then cooled slowly to 42 °C. Subsequently, 1.5 μL ddH_2O, 4 μL 5× reverse transcription reaction buffer (Invitrogen), 0.5 μL dNTP mix (the final concentration of dNTPs was 40 μM in the High dNTP groups and 0.5 μM in the Low dNTP groups), 3 μL 25 mM $MgCl_2$, and 1 μL Moloney murine leukemia virus reverse transcriptase (Invitrogen) were added, and the mixture was incubated for 1 h at 50°C. Following incubation, 1 μL of RNase H (NEB) was added, and the mixture was incubated again for 10 min at 37°C. The reaction mixture was then subjected to 20% polyacrylamide gel electrophoresis (PAGE) containing 8 M urea and then transferred to nylon membrane (MILLIPORE). DIG antibody (Roche) was added and incubated for 30 min. The membrane was washed with wash buffer (100 mM Maleic acid, 150 mM NaCl, 0.3%(v/v) Tween 20, pH 7.5) and incubated in detection buffer (100 mM NaCl, 100 mM Tris-HCl 9.5). Lastly, the membrane was incubated with CDP-Star (Roche) and signal intensities were detected by Amersham Imager 680 (GE).

RT primer of HctRNA^{Gly}(GCC): 5'DIG-AATTCTACCACTGAACC-3'

RT primer of HctRNA^{Gly}(CCC): 5'DIG-CATGATACCACTACACC-3'

Polysome profiling. Polysome profiling was performed as described (Guzzi *et al*, 2018) with some modifications. Cells were incubated for 15 min at 37 °C in media supplemented with 10 μg/mL cycloheximide (CHX) (Sigma). Cells were immediately placed on ice, washed with cold PBS containing 10 μg/mL CHX. Cells were

resuspended in 425 μL hypotonic buffer (5 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$, 1.5 mM KCl, and 1x EDTA-free protease inhibitor cocktail (Roche) supplemented with 100 U of RNase inhibitor (Thermo scientific), 5 μL 10 mg/ml CHX, and 1 μL 1 M DTT, and vortexed for 5 s. Cells were then vortexed for another 5 s after the addition of 25 μL 10% Triton X-100. Lysates were centrifuged at 12,000 xg for 10 min at 4 °C and the cytosolic and endoplasmic reticulum-associated ribosomes were transferred to a new tube. The ribosomes were layered onto a linear sucrose gradient (10%–50% w/v) and centrifuged in a SW41Ti rotor (Beckman) for 2 h at 36,000 rpm at 4 °C. Polysome profiles were generated using a BioComp Gradient Station (BioComp).

Measurement of protein synthesis by puromycin incorporation. Cells were incubated in complete DMEM supplemented with 2.5 μg/mL puromycin for 45 min. After treatment, cells were washed twice in PBS and lysed by cold RIPA lysis buffer. 20 μg of cell lysate was loaded onto an SDS-PAGE gel and transferred to PVDF membranes. Immunoblots were detected by the Odyssey system (direct infrared fluorescence, LI-COR Biosciences). The following antibodies were used: mouse anti-Puromycin (Merck Millipore), rabbit anti-Actin (CST).

RNA-seq data analysis. Paired-end 150-cycle sequencing was performed on HiSeq X, per the manufacturer's directions (Illumina). After adapter trimming by cutadapt (Martin, 2011), RNA-Seq results were mapped onto the human genome hg19 (GRCh37) by STAR (version 2.5.2b, parameters "--outFilterMultimapNmax 20 --outFilterMismatchNmax 999 --alignIntronMin 20 --alignIntronMax 1000000 --alignMatesGapMax 1000000 --alignSJoverhangMin 8 --alignSJDBoverhangMin 1 --twopassMode Basic") (Dobin *et al*, 2013) and HTSeq (version 0.6.1 p1) (Anders *et al*, 2015) was used to count the gene-based reads using gene annotation from GENCODE (v24lift37) (Anders *et al*, 2015). For differential expression analysis, we performed trimmed mean normalization, then Voom (Law *et al*, 2014) was used to identify differentially expressed genes. Gene ontology analysis was performed using PANTHER and level 1 terms from Biological Process were used for presentation (Mi *et al*, 2019). For GSEA analysis (Subramanian *et al*, 2005), gene sets downloaded from the MSigDB database (v6.0) (Liberzon *et al*, 2015) were analyzed against the log2 fold change from differential analysis with GSEA prerank mode (version 3.0). Two replicates of RNA-seq were performed and analyzed. We followed ENCODE guideline for quality control that each our replicates have more than 40 million paired-end reads and the spearman correlation between replicates were larger than 0.9.

Nucleic acid immunoprecipitation. 6 x 10^7 MDA-MB-231 cells expressing FLAG-hTrmt13 were divided into cytoplasmic extracts and nuclear extracts, as stated above. For immunoprecipitation, 10 μL Anti-FLAG® M2 Magnetic Beads (Sigma) was added and rotated overnight at 4 °C. Beads were washed five times with ice-cold wash buffer A (1% NP-40, 0.5% sodium deoxycholate, 150 mM NaCl). The beads were then washed in elution buffer (1% SDS, 50 mM Tris-HCl, pH 8.0). The elution buffer was treated with proteinase K (Thermo Scientific) and the nucleic acids were extracted with Trizol reagent.

Sequential IF and RNA FISH. Cells were fixed by 4% paraformaldehyde for 10 min at RT and then permeabilized in PBS with 0.1% Triton X-100 for 5 min at RT. Primary and secondary antibody incubations were performed in PBS at RT for 2 h. Cells were washed in PBS for 10 min and repeat 3 times. Cells were fixed again by 4% paraformaldehyde for 10 min and heated at 80 °C for 5 min. Next, the RNA FISH were performed using Stellaris® RNA FISH Kit (BIOSEARCH TECHNOLOGIES).

Probe of HctRNA^{Gly}(GCC): 5'Cy5-TACCACTGAACCACCAATGC-3'

Probe of HctRNA^{Gly}(CCC)-1: 5'Cy5-TTGCATGATACCACTACACC-3'

Probe of HctRNA^{Gly}(CCC)-2: 5'Cy5-CGGGTCGCAAGAATGGGAAT-3'

ChIP-seq and ChIP-qPCR. ChIP-seq experiments were performed as described (Shan *et al*, 2016) with modifications. Approximately 4 x 10^7 MDA-MB-231 cells were used for each ChIP-seq assay. Cells were fixed by 1% formaldehyde (Sigma) for 9 min. The nuclear extracts were suspended in sonication buffer (10 mM EDTA, 0.1% SDS 20 mM Tris-HCl, pH 7.5) and the crosslinked DNA was sheared into 200 bp fragments with Covaris M220. The chromatin DNA was precipitated by either normal goat IgG (control) or polyclonal antibodies against hTrmt13 or FLAG. The DNA was purified with the CST ChIP-DNA purification kit. In-depth whole-genome sequencing was performed by GENWEIZ (Suzhou, China). The raw sequencing data were examined with the Illumina analysis pipeline. ChIP-qPCR was performed using Eastep® qPCR Master Mix.

ChIP-seq data analysis. 150 bp paired-end reads were obtained, trimmed for adapters by cutadapt (Guzzi *et al.*, 2018) and aligned to human genome hg19 (GRCh37) by BWA (version 0.7.12-r1039, default parameter) (Li & Durbin, 2009). Then duplicated reads were marked by biobambam2 (version 2.0.57) (Tischler & Leonard, 2014) and uniquely mapped reads were retained by samtools (parameter "-q 1 -F 1024" version 1.4) (Li *et al*, 2009). We followed ENCODE criterion for quality control and confirmed high quality (>30 million reads each replicates). Center 80bp of each fragment were

used for generating bigwig tracks and normalized to 15 million reads. Tracks were inspected on IGV (Robinson *et al*, 2011) for clear peak shape and confirmed for good reproducibility. Two replicates of ChIP-seq were analyzed. Peaks for each replicate were called by MACS2 (version 2.1.1.20160309, paired-end mode) twice independently using cutoff q-value 0.05 as high confidence peaks also cutoff q-value 0.5 as low confidence peaks. We then finalized reproducible peaks as called in high confidence peaks in one replicate also overlap the low confidence peak called from the other replicate. Reproducible peaks were then used for motif analysis by homer (v4.9.1) (Heinz *et al*, 2010)

ChIP-seq data for H3K4me3, H3K4me1, H3K27ac, FOXM1, MYC, E2F1, TP53, FOSL1, EZH2, were downloaded from GSE49651 (Rhie *et al*, 2014), GSE40762 (Sanders *et al*, 2013), GSE95303 (Gallenne *et al*, 2017). Methods and code could be found as described in (Yang *et al*, 2019). ATAC-seq data were downloaded from GSE72141 (Takaku *et al*, 2016) and analyzed following method in (Xu *et al*, 2020). DeepTools (v2.5.7) (Ramirez *et al*, 2016) was used to plot heatmap.

Luciferase reporter assay. Luciferase activity was performed in MDA-MB-231 cells using a dual luciferase kit (Promega) according to the manufacturer's protocol. Each experiment was performed in triplicate and repeated at least five times.

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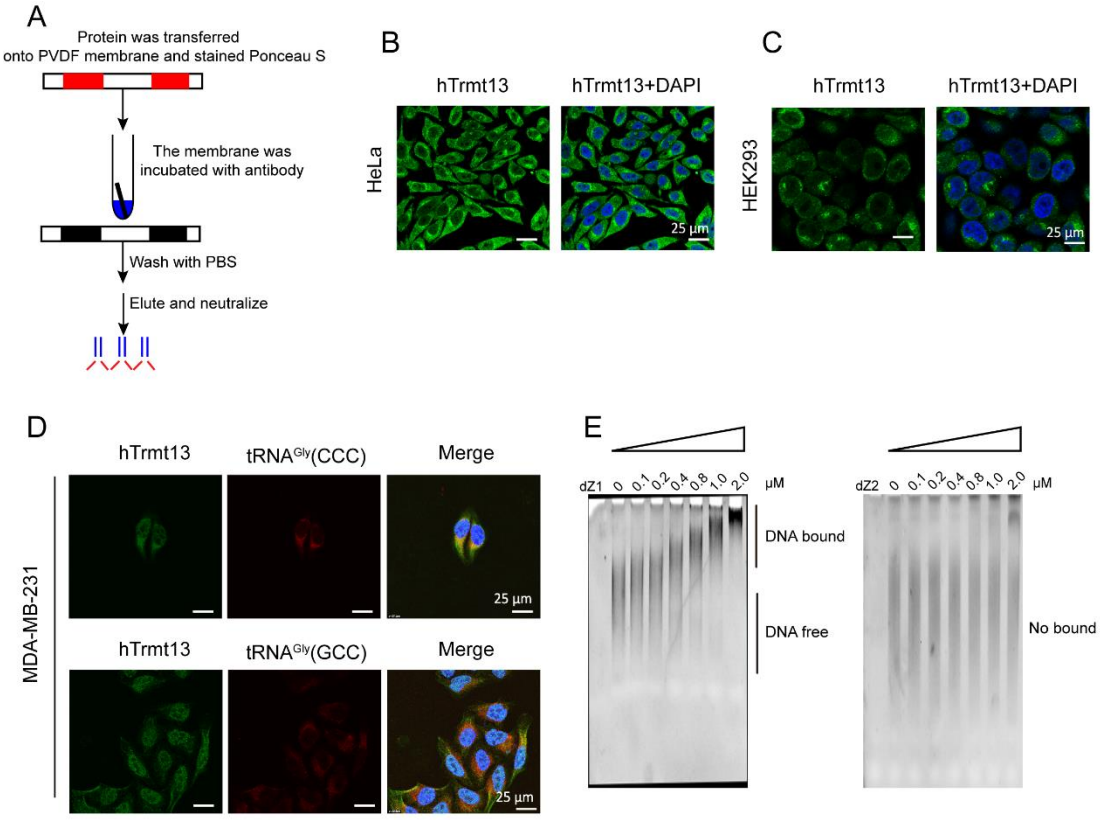


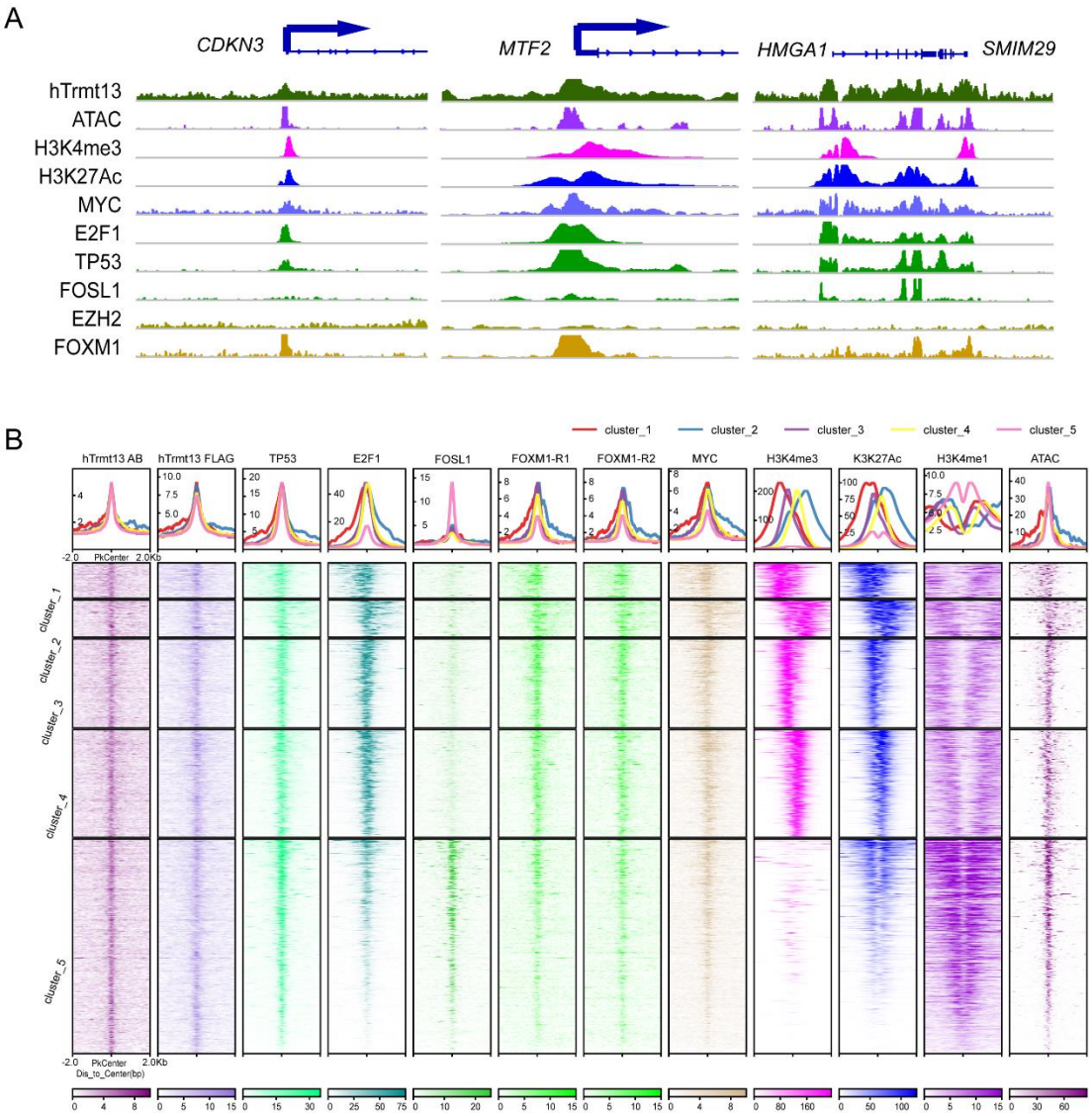
Appendix Fig. S1. hTrmt13 colocalizes with tRNA in cytoplasm and could bind with DNA through CHHC Zn-2 domain.

A. Workflow of antibody purification of anti-hTrmt13.

B, C. Confocal microscopy images of HeLa cells and HEK293 cells were immunolabeled with anti-hTrmt13.

D. Confocal microscopy images of MDA-MB-231 cells were labeled with HctRNA^{Gly}(CCC) and HctRNA^{Gly}(GCC) probes and simultaneously immunolabeled with hTrmt13 antibody.

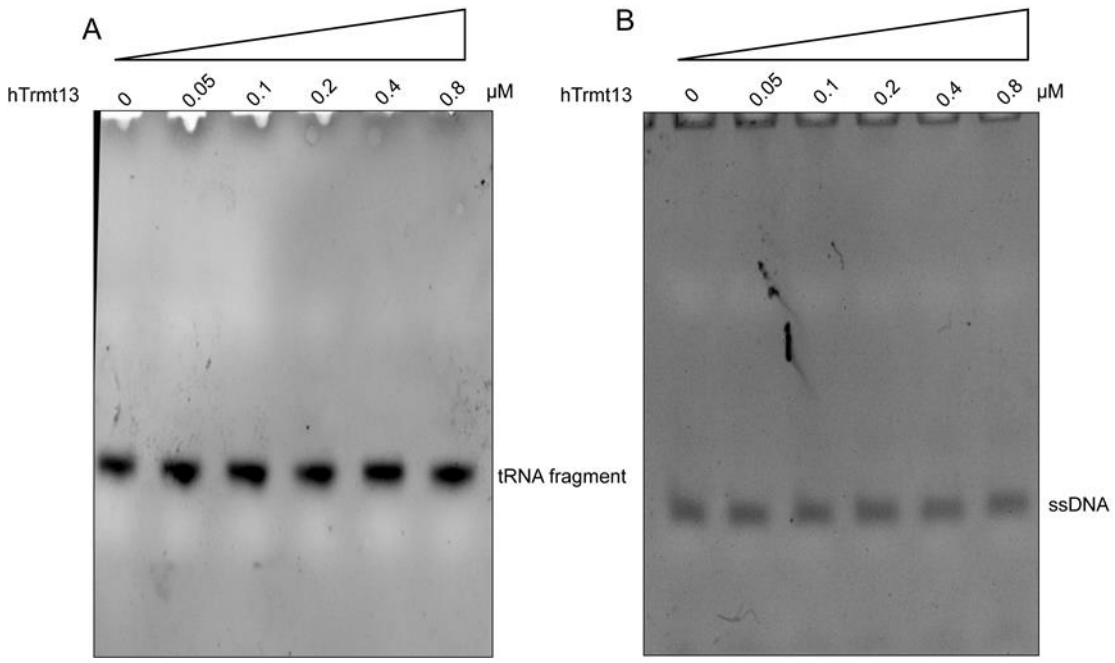
E. The binding affinities of hTrmt13-dZ1 (left) and hTrmt13-dZ2 (right) for DNA fragment analyzed by EMSA. The amount of DNA fragment is 300 ng.



Appendix Fig. S2. hTrmt13 binds DNA *in vivo*.

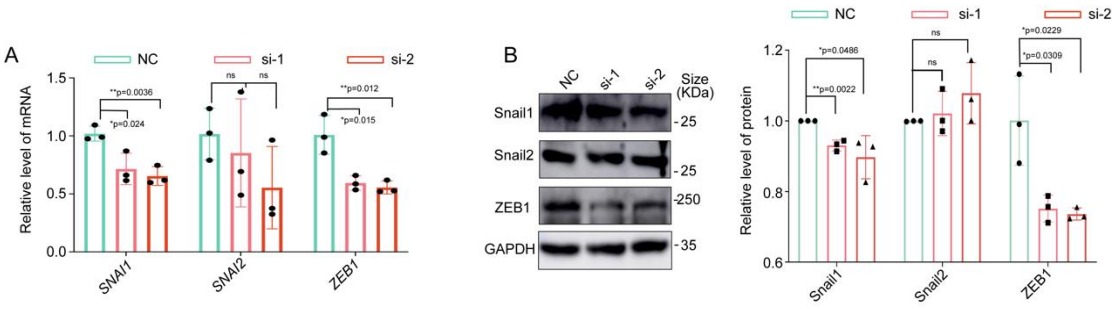
A. The binding profiles of hTrmt13, H3K4me3, H3K27Ac and transcription factors (MYC, E2F1, TP53, FOSL1, EZH2, FOXM1) from ChIP-seq and ATAC-seq on representative target genes *CDKN3*, *MTF2*, and *HMGA1*.

B. Heatmap and profiles of 5 clusters centered at reproducible hTrmt13 peaks (k-means clustering by deeptools2) for hTrmt13, H3K4me3, H3K27Ac, H3K4me1, and transcription factors (MYC, E2F1, TP53, FOSL1, EZH2, FOXM1) from ChIP-seq and ATAC-seq.



Appendix Fig. S3. hTrmt13 does not bind to tRNA fragment or single-stranded DNA

A, B. The binding shift assays of hTrmt13 for 5'-tRF from substrate HctRNA^{Gly}(GCC) (0.3 μM) **(A)** or single-stranded DNA (ssDNA) containing the hTrmt13 binding motif (15 ng/μL) **(B)**.



Appendix Fig. S4. hTrmt13 promotes transcription.

A, B. The expression level of *SNAI1*, *SNAI2*, and *ZEB1* were measured by RT-qPCR **(A)** and Western blot (**B**) respectively. Statistical analysis was performed using *t*-tests. Error bars represent mean ± SD for three independent experiments.