Supplemental information

Mammalian NSUN2 introduces 5-methylcytidines to mitochondrial tRNAs

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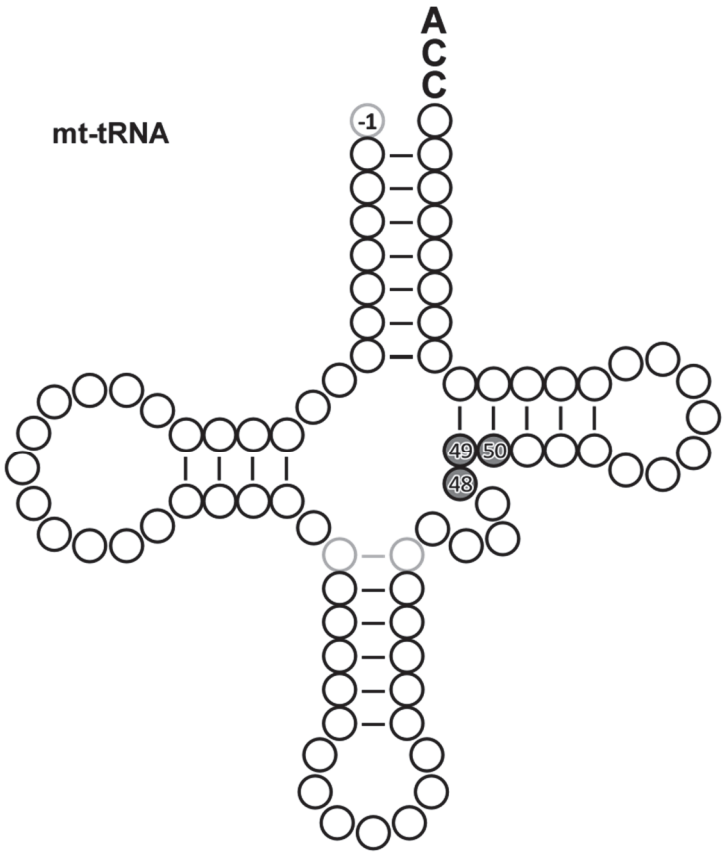
A



B

	Human	Mouse	Bovine
m^5C48	Phe, His, Leu(UUR), Ser(AGY), Tyr	His, Met, Leu(UUR), Asn, Tyr	Leu(UUR), Asn, Trp
m^5C49	Glu, Ser(AGY)	Glu, Leu(CUN), Ser(AGY)	Glu, Ser(AGY)
m^5C50	Ser(AGY)	-	-

Figure S1. m^5C distribution in mt-tRNAs of human, mouse, and cow.

(A) Secondary structure of mt-tRNAs. Positions of m^5C modifications are indicated by shaded circles.

(B) tRNA species bearing m^5C at the corresponding position in three mammals.

Bovine mt-tRNA data are from a previous study by our group (5).

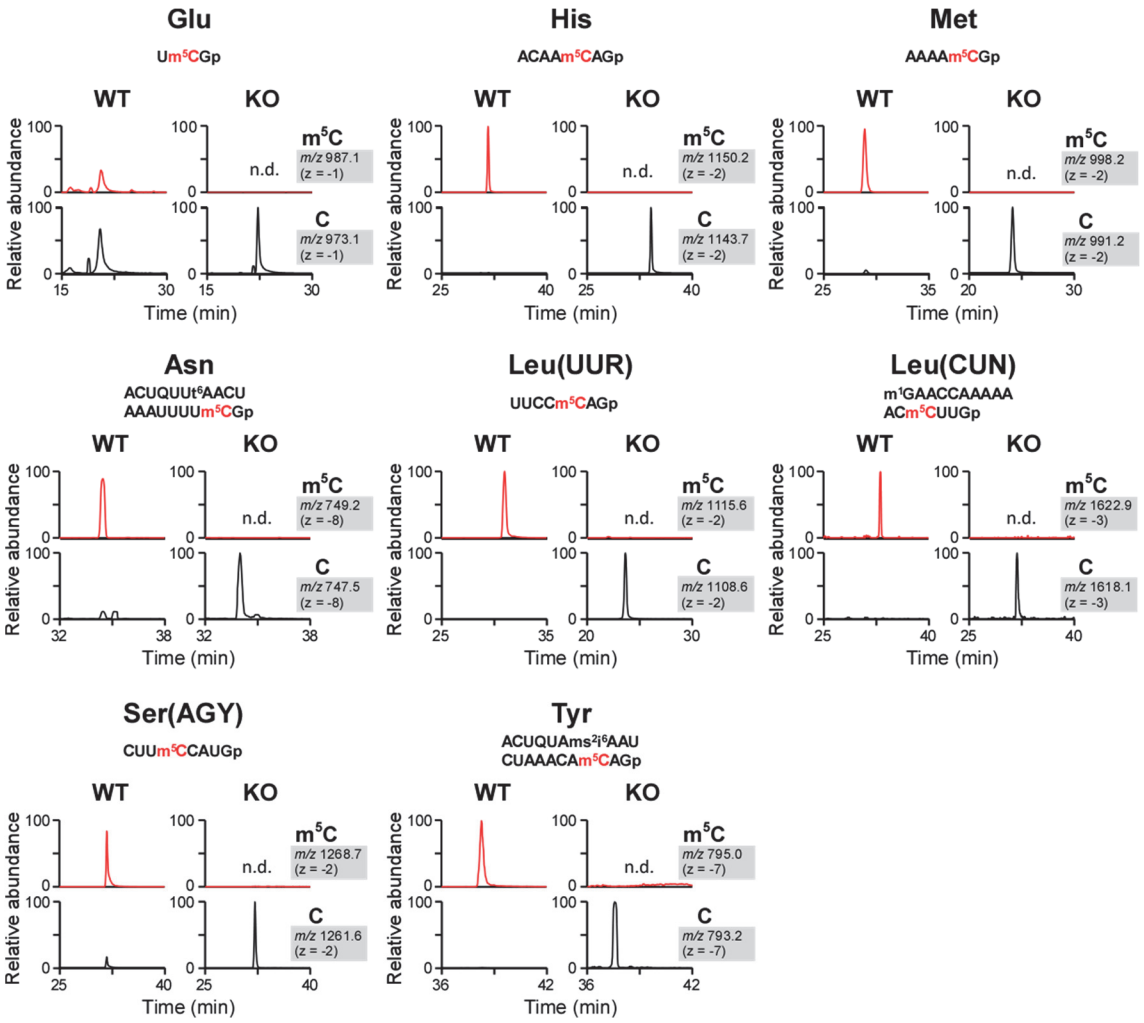


Figure S2. m^{5}C status in eight mt-tRNAs isolated from WT and *Nsun2* KO mouse livers

XICs of RNase T$_1$-digested fragments containing m^{5}C (top) and C (bottom) in eight mt-tRNAs isolated from WT and *Nsun2*$^{-/-}$ (KO) mouse liver.

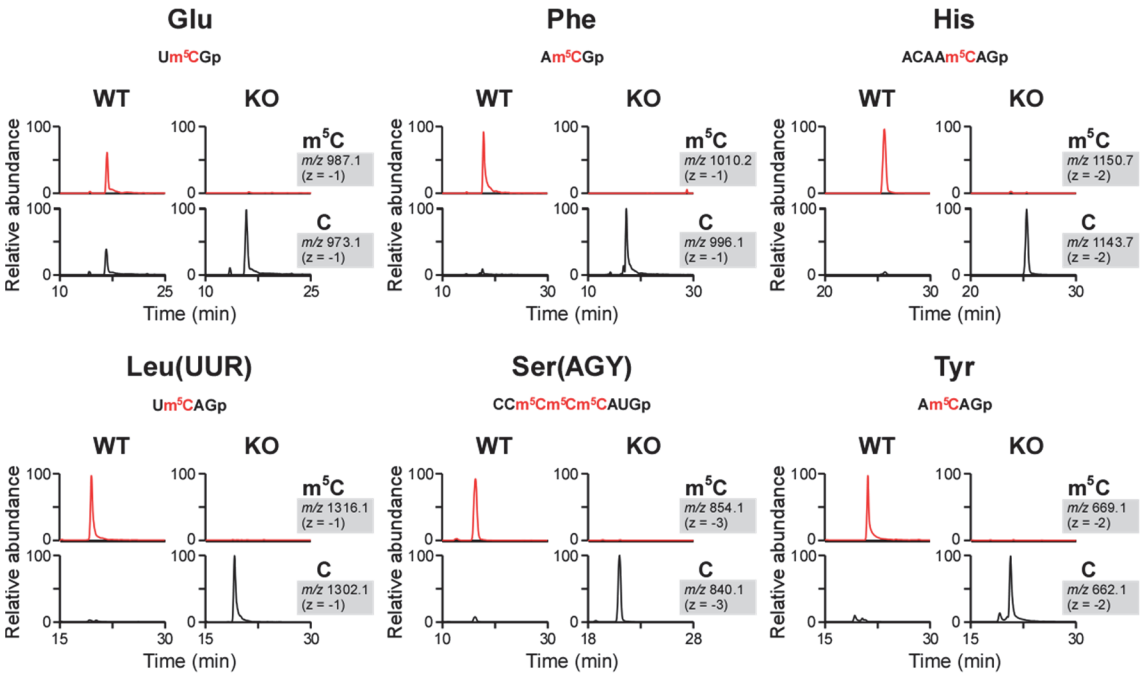


Figure S3. m^{5}C status in six mt-tRNAs isolated from WT and *NSUN2* KO human culture cells.

XICs of RNase T$_1$-digested fragments containing m^{5}C (top) and C (bottom) in six mt-tRNAs isolated from WT and *NSUN2* KO HEK293T cells. *m/z* value with the charge state for each fragment is shown to the right of each panel.

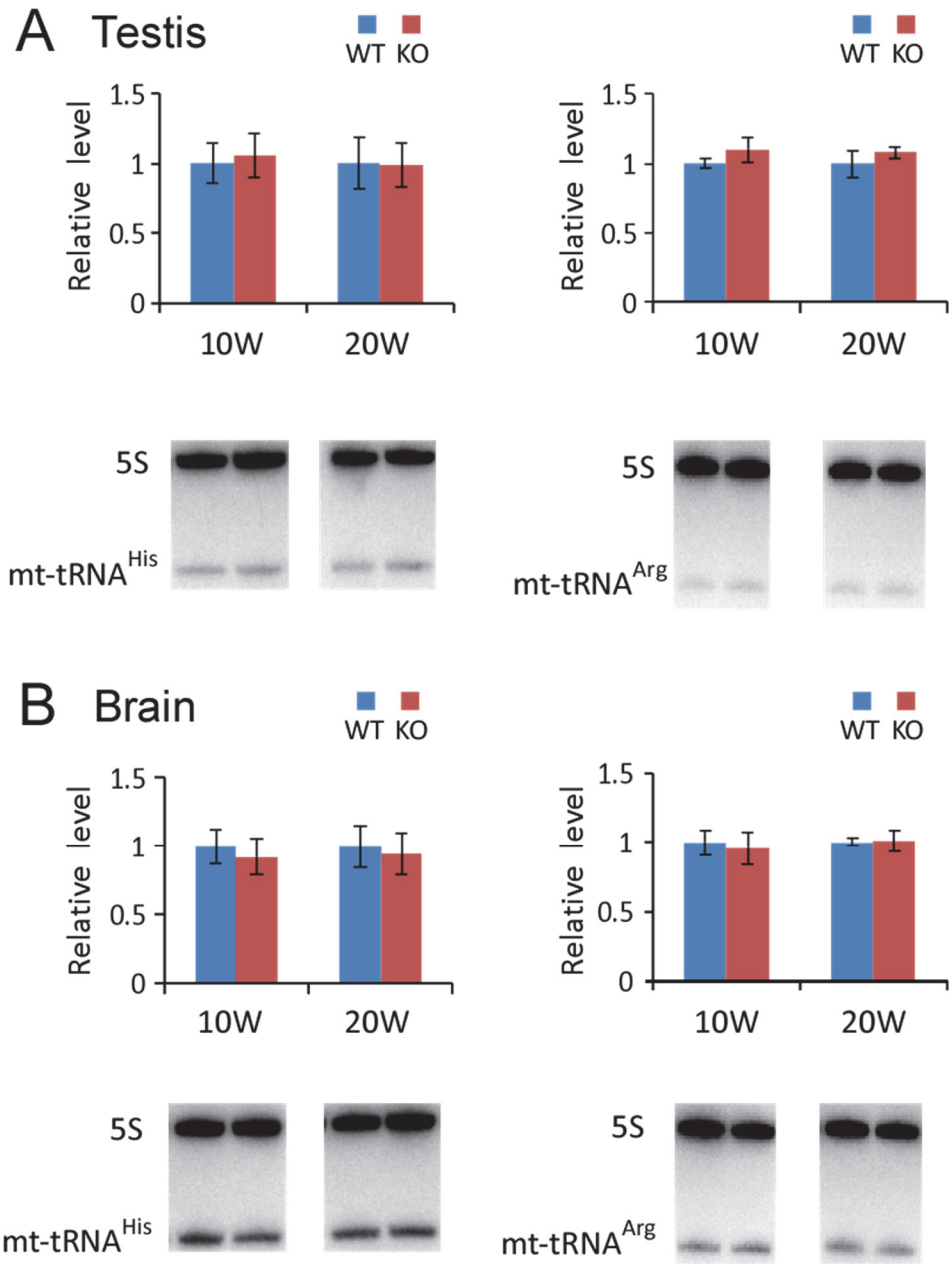


Figure S4. Northern blotting of mouse mt-tRNAs

Northern blots of mt-tRNAs for His (left panels) and Arg (right panels) in total RNA isolated from testis (A) and brain (B) of 10- and 20-week-old male mice (WT, blue; *Nsun2*$^{-/-}$ (KO), red). Bar graphs represent relative steady-state levels of each tRNA, normalized against 5S rRNA (used as a loading control). Means ± S.D. were calculated from three biological replicates.

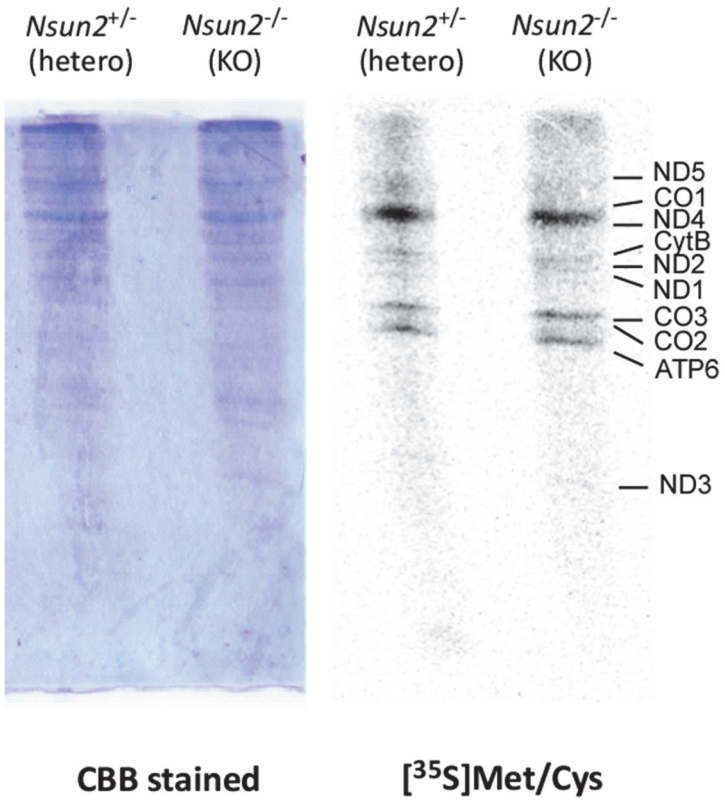


Figure S5. Pulse labeling of mitochondrial protein synthesis in MEF cells.
Nsun2$^{+/-}$ (hetero) and *Nsun2*$^{-/-}$ (KO) MEFs were labeled with ([^{35}S] Met/Cys) and chased for 1 h under emetine treatment (right). Total proteins were visualized by CBB staining (left).

Table S1 List of primers and probes used in this study

Purpose	Name	5' to 3' sequence
Inducible mutagenesis with CRISPR/Cas9		
sgRNA for human NSUN2 gene	sgRNA_NSUN2_fw	CTTAAAGTGGCCGGGAGCG
	sgRNA_NSUN2_rv	ATGGCGCCGAGGGTGGTGGAAA
Surveyor assay	genome_NSUN2_fw2	ACGGTTCTCTGGCACTGTAACAC
	genome_NSUN2_rv2	GTGTAGGGCTAGAGTTCTGGC
Isolation of mitochondrial tRNA		
For human	RCC_h-mtSer(AGY)	TGGTGAGAAAGCCATGTTGTTAGACATGGG
	RCC_h-mtLeu(UUR)	TATGCGATTACCGGGCTCTGCCATCTTAAC
	RCC_h-mtTyr	CAGTCCAATGCTTCACTCAGCCATTTTACC
	RCC_h-mGlu	TGGTATTCTCGCACGGACTACAACCACGAC
	RCC_h-mtHis	GGTAAATAAGGGGTCGTAAGCCTCTGTTGT
	RCC_h-mtPhe	TGGTGTTTATGGGGTGATGTGAGCCCGTCT
For mouse	RCC_m-mtLeu(UUR)	GAGTCTGGCGCCTTAGACCACTCGGCCATCCTGAC
	RCC_m-mtAsn	TTTAGTTAACAGCTAAATACCCTATTACTGGCTTCAATCT
	RCC_m-mGlu	ACAGCATTCAACTGCGACCAATGACATGAAAAATCATCGT
	RCC_m-mtHis	AAGGAGGTTTATTTCCTGTTGTCAGATTCACAGTCTAATG
	RCC_m-mtLeu(CUN)	TTGCACCAAGGTTTTTGGTTCCTAAGACCAATGGATTACT
	RCC_m-mtMet	TTCGGGGTATGGGCCCGATAGCTTAATTAGCTGAC
	RCC_m-mSer(AGY)	GCCATGTTTAAACATGGAAGCATGAATTAGCAGTTCTTGC
	RCC_m-mtTyr	AACCTCTGTGTTTAGATTTACAGTCTAATGCTTACTCAGC
	RCC_m-cytoGly	GAAGGGAGCTATGCTATGCTCACCACTATACCACCAACGC
	RCC_m-cytoLeu(CAA)	GAGTCTGGCGCCTTAGACCACTCGGCCATCCTGAC
PCR primer		
Template of T7 transcription	T7 forward primer	GCTAATACGACTCACTATA
	mt tRNASer(AGY) WT reverse	TGGTAAGAAAGCCATGTTTAAACATGGAAGCATGAATTAGCAGTTCTTGCAATCTTTCTTTATAG
RT primer for cloning human NSUN2 gene	RTcDNANSUN2Rv	AGCTTGGCCAAAGAAACAAA
Cloning human NSUN2 gene from cDNA by nested PCR	NSUN2_1st_Fw	CCCTTAGAGCTGTTCGCTGT
	NSUN2_1st_Rv	CCAGAAGAAGCCAGTTTTGC
	NSUN2_2nd_Fw	CACCATGGGGCGGCGGTCGC
	NSUN2_2nd_Rv	CCACCGGGGTGGATGGACC
Northern blotting		
	NT_MmmtHis_3'	TGGGGTGAATAAGGAGGTTTATTTC
	NT_MmmtArg	TGGTTGGTAATTATGAACAGCATCATAATC
	NT_MmmtLeu(UUR)	GATAGCTTAATTAGCTGACCTTACT
	NT_MmmtSerAGY_5'	CATGAATTAGCAGTTCTTGCAATCTTTCTT
	NT_Mmcyto_Gly_GCC	TCTACCACTGAACCACCCAT
	NT_Mmcyto_Arg_ACG	TGGCGAGCCAGCCAGGAGTCGAACCTGGAA
	NT_Mm_5S_rRNA	GGGTGGTATGGCCGTAGAC