
Supplementary information

Substrate selectivity of the human RNA m⁵C methyltransferase NSUN2

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Supplementary Information

Substrate selectivity of the human RNA m⁵C methyltransferase NSUN2

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Table of Contents:

Supplementary Information Figures

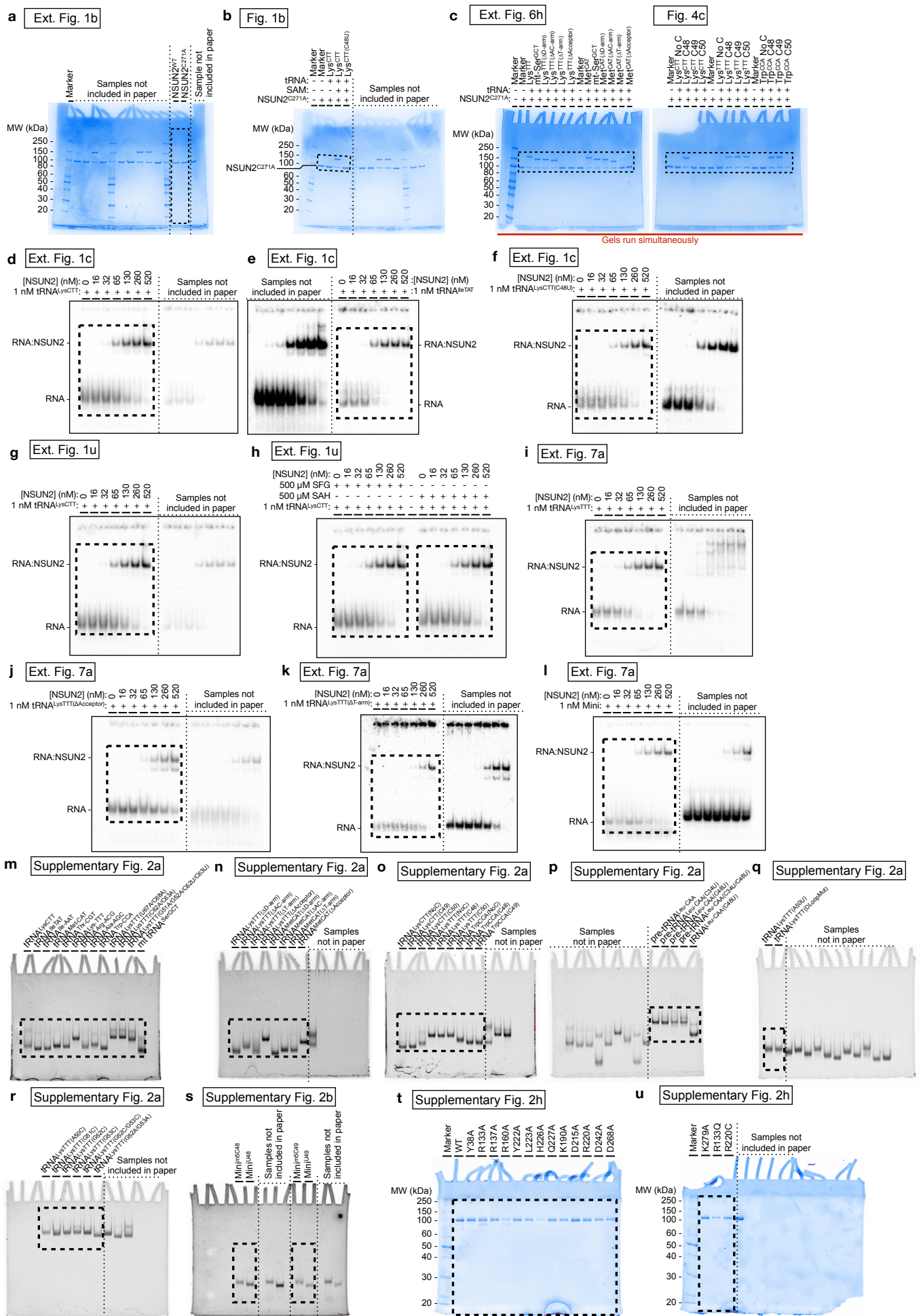
Supplementary Information Figure 1	3
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Supplementary Information Figure 2	4
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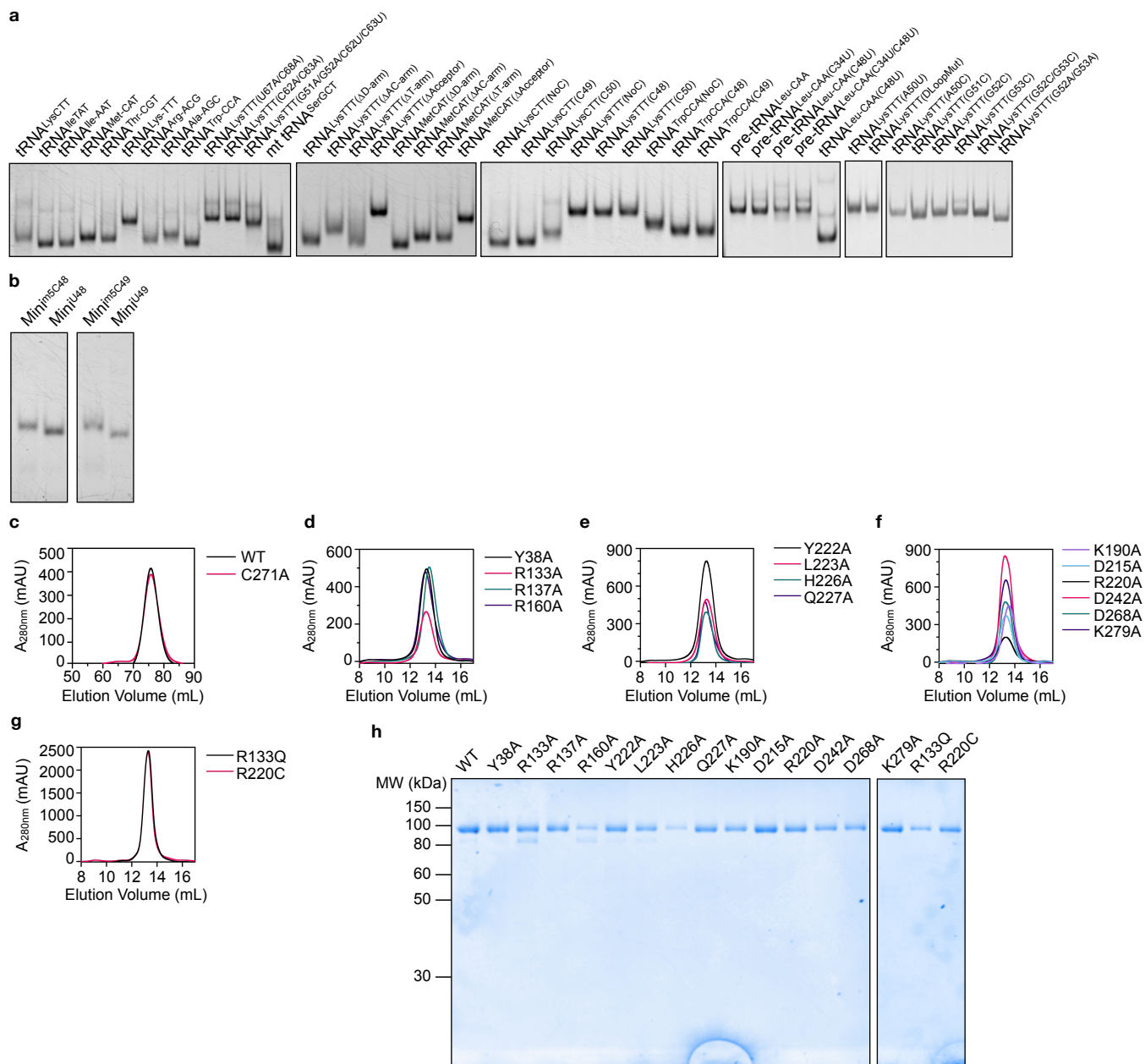
Supplementary Information Tables

Supplementary Information Table 1	5
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Supplementary Information Table 2	6
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Supplementary Information Fig. 1: Source gel images for figures a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u. Uncropped 4-20% gradient SDS-PAGE image for Extended Data Fig. 1b visualized with Coomassie Blue R-250 stain. **b-c,** Uncropped 4-20% gradient SDS-PAGE images for cross-linking assays in Fig. 1b (b) and Fig. 4c alongside Extended Data Fig. 6h (c) visualized with Coomassie Blue R-250 stain. The two gels in c were the same type and run simultaneously in the same cassette. **d-l,** Uncropped EMSA images visualized with a phosphor-imager used in Extended Data Fig. 1c (d-f), Extended Data Fig. 1u (g-h), and Extended Data Fig. 7a (i-l). **m-s,** Uncropped native 8% PAGE gels visualized with Ethidium Bromide used in Supplementary Fig. 2a (m-r) and Supplementary Fig. 2b (s). **t-u,** Uncropped 12% SDS-PAGE images visualized with Coomassie Blue R-250 stain used in Supplementary Fig. 2h. Dashed boxes indicate cropped areas



Supplementary Information Fig. 2: Quality control for tRNAs and protein purifications **a**, 8% acrylamide tris-borate native gels for tRNA substrates made via in vitro transcription. **b**, 10% acrylamide tris-borate native gels for Mini^{m5C48}, Mini^{U48}, Mini^{m5C49}, and Mini^{U49} substrates. **c-h**, Representative size exclusion chromatography profiles for WT NSUN2 and C271A catalytic mutant (**c**), alanine point mutants in the NTD from Fig. 2m (**d**), alanine point mutants in the MTD from Fig. 2m (**e**), alanine point mutants in the CTD from Fig. 3c (**f**), and identified cancer mutants from Fig. 2m and 3c (**g**). NSUN2 WT and C271A were run on a Superdex 200 pg HiLoad 16/600 column (Cytiva), and all other mutants were run on a Superdex 200 Increase 10/300 GL (Cytiva). **h**, 12% SDS-PAGE gel of WT NSUN2 and point mutants. SDS-PAGE gel of NSUN2^{C271A} is available in Extended Data Fig. 1b.

Name	Sequence (5'-3')
IleTAT_fwd	GCCTGCTAATACGACTCAC
IleTAT_rev	TGGTGTCTCCAGGTGAGGATCGAAGCTCACAACTCGGCATTATAAGTACCGCGCGCTAACCGATTGCGCCACTGGAGCTATAGTGAGTCGTATTAGCAGGC
LysCTT_fwd	GCTTGCATGCTAATACGACTCACTATAGCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTCTTAATC
LysCTT_rev	TGGCGCCCAACGTGGGGCTCGAACCCTGACCCTGGGATTAAAGTCCCAGTCTACCGGAC
LysCTT(NoC)_fwd	GCCTGCTAATACGACTCAC
LysCTT(NoC)_rev	TGGCGCCCAACGTGGGGCTCGAACCCTGACCCTGGGATTAAAGTCCCAGTCTACCGATTGAGCTAGCCGGGCTATAGTGAGTCGTATTAGCAGGC
LysCTT(C49)_fwd	GCCTGCTAATACGACTCAC
LysCTT(C49)_rev	TGGCGCCCAACCTGGGGCTCGAACCAGAACCTGGGATTAAAGTCCCAGTCTACCGATTGAGCTAGCCGGGCTATAGTGAGTCGTATTAGCAGGC
LysCTT(C50)_fwd	GCCTGCTAATACGACTCAC
LysCTT(C50)_rev	TGGCGCCCAACGTGGGGCTCGAACCCTGACCCTGGGATTAAAGTCCCAGTCTACCGATTGAGCTAGCCGGGCTATAGTGAGTCGTATTAGCAGGC
LysCTT(C50)_rev	TGGCGCCCAACGTGGGGCTCGAACCCTGACCCTGGGATTAAAGTCCCAGTCTACCGATTGAGCTAGCCGGGCTATAGTGAGTCGTATTAGCAGGC
LysTTT(NoC)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(C48)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(C48)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(C50)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(C50)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(ΔD-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(ΔD-arm)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(ΔD-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(ΔD-arm)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(ΔAC-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(ΔAC-arm)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(ΔT-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(ΔT-arm)_rev	TGGCGCTGAAGAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(ΔAcceptor)_fwd	ACTGCTAATACGACTCACTATAGTAGCTCAGTTGGTGAACATCAGACTTTTAATC
LysTTT(ΔAcceptor)_rev	ACAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACCACCTG
LysTTT(U67A/C68A)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(U67A/C68A)_rev	TGGCGCTTTACAGGGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(C62A/C63A)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(C62A/C63A)_rev	TGGCGCTGAACATTGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(G51A/G52A/C62U/C63U)_fwd	ACTGCTAATACGACTCACTATAGCCTGGATAGCTCAGTTGGTGAACATCAGACTTTTAATCTGACG
LysTTT(G51A/G52A/C62U/C63U)_rev	TGGCGCTGAACAAAGACTTGAACCTCCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(A50C)_rev	TGGCGCTGAACCGGACTTGAACCCGGCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(A50U)_rev	TGGCGCTGAACCTGGGACTTGAACCCAGCACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(G51C)_rev	TGGCGCTGAACCGGACTTGAACCTGACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(G52C)_rev	TGGCGCTGAACAGGACTTGAACCTGACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(G53C)_rev	TGGCGCTGAACAGGACTTGAACCTGACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(G52C/G53C)_rev	TGGCGCTGAACAGGACTTGAACCTGACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(G52A/G53A)_rev	TGGCGCTGAACAGGACTTGAACCTGACCCTCAGATTAAAGTCTGATGTTCTACC
LysTTT(DLoopMut)_fwd	CGCAAAATTAATACGACTCACTATAGCCTGGATAGCTCGATTAAATAGAATCAGACTTTTAATCTGACG
LysTTT(DLoopMut)_rev	TGGCGCTGAACAGGGACTTGAACCTGACCCTCAGATTAAAGTCTGATGTTCTATT
TrpCCA(NoC)_fwd	GCCTGCTAATACGACTCAC
TrpCCA(NoC)_rev	TGGTGACCCCGATGTGATTTGAACACACAACTTCTGATCTGGAGTCAGACGCGCTACCGTTGCGCCACGAGGCCCTATAGTGAGTCGTATTAGCAGGC
TrpCCA(C48)_fwd	GCCTGCTAATACGACTCAC
TrpCCA(C48)_rev	TGGTGACCCCGATGTGATTTGAACACACGACCTTCTGATCTGGAGTCAGACGCGCTACCGTTGCGCCACGAGGCCCTATAGTGAGTCGTATTAGCAGGC
TrpCCA(C49)_fwd	GCCTGCTAATACGACTCAC
TrpCCA(C49)_rev	TGGTGACCCCGATGTGATTTGAACACACAACTTCTGATCTGGAGTCAGACGCGCTACCGTTGCGCCACGAGGCCCTATAGTGAGTCGTATTAGCAGGC
TrpCCA_fwd	GCCTGCTAATACGACTCAC
TrpCCA_rev	TGGTGACCCCGATGTGATTTGAACACGAACTTCTGATCTGGAGTCAGACGCGCTACCGTTGCGCCACGAGGCCCTATAGTGAGTCGTATTAGCAGGC
MetCAT_fwd	ACTCGATAATACGACTCACTATAGCCTCGTTAGCGCAGTAGGTAGCGCTCAGTCTC
MetCAT_rev	TGGTGCCCCGTGTGAGGATCGAAGCTCAGCACTTTCAGATTATGAGACTGACGCGCTACC
MetCAT(ΔD-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTCGTTAGCGTCAGTCTCATAATCTGAAGG
MetCAT(ΔD-arm)_rev	TGGTGCCCCGTGTGAGGATCGAAGCTCAGCACTTTCAGATTATGAGACTGACGC
MetCAT(ΔAC-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTCGTTAGCGCAGTAGGTAGCGCTAAGGTC
MetCAT(ΔAC-arm)_rev	TGGTGCCCCGTGTGAGGATCGAAGCTCAGCACTTTCAGATTATGAGACTGACGC
MetCAT(ΔT-arm)_fwd	ACTGCTAATACGACTCACTATAGCCTCGTTAGCGCAGTAGGTAGCGCTCAGTCTC
MetCAT(ΔT-arm)_rev	TGGTGCCCCGTGCGACCTTCAGATTATGAGACTGACGCGCTACCTAC
MetCAT(ΔAcceptor)_fwd	ACTGCTAATACGACTCACTATAGTTAGCGCAGTAGGTAGCGCTCAGTCTCATAATC
MetCAT(ΔAcceptor)_rev	TGTGAGGATCGAAGCTCAGCACTTCAGATTATGAGACTGACGCGCTACC
IleAAT_fwd	ACTCGATAATACGACTCACTATAGGCTGGTTAGTTCAGTTGGTTAGAGCGTGGTGC
IleAAT_rev	TGGTGCGCGATATGGGGATCGAACCACGACCTTGGCGTTATTAGCACCACGCTCTAACCAAC
ThrCGT_fwd	CGATAATACGACTCACTATAGGCGCTGAGCTCAGCGGTTGGAGCGCTGGTCTCGTAAAC
ThrCGT_rev	TGGAGGCCCTGGTGAGATTGAACTCAGACCCCTTAGGTTTACGAGACGAGCGCTCCAAC
ArgACG_fwd	ACTGCTAATACGACTCACTATAGGCGCAGTGGCGCAATGGATAACGCGCTGACTACGG
ArgACG_rev	TGGCGAGCCAGCCAGGAGTCGAACCTAGAACTTCTGATCCGTAGTCAGACGCGTTATCC
AlaAGC_fwd	ACTGCTAATACGACTCACTATAGGGGATAGCTCAAAATGGTAGAGCGCTCGCTTAGCATGC
AlaAGC_rev	TGGTGAGGATGCGGCGCATCGATCCCGCTACCTCTCGCATGCTAAGCGAGCGCTCTACC
mtSerGCT_fwd	GCCTGCTAATACGACTCACTATAGAGAAAGCTCACAAGAACTGCTAACTCATG
mtSerGCT_rev	TGGTGAGAAAGCCATGTTGTTAGACATGGGGCATGAGTTAGCAGTTCTTGTGAGC
pre-tRNA ^{Leu} CAA_fwd	CGCAAAATTAATACGACTCACTATAGTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCAAGTAAGCACCTTGCCTGCG
pre-tRNA ^{Leu} CAA_rev	TGTCAGAAGTGGGATTTCGAACCCACGCTCCATCCGGAGACCCAGAAAGCCCGCAGGCAAGGTGCTTACCTT
pre-tRNA ^{Leu} CAA(C34U)_fwd	CGCAAAATTAATACGACTCACTATAGTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTAAGTAAGCACCTTGCCTGCG
pre-tRNA ^{Leu} CAA(C34U)_rev	TGTCAGAAGTGGGATTTCGAACCCACGCTCCATCCGGAGACCCAGAAAGCCCGCAGGCAAGGTGCTTACCTT
pre-tRNA ^{Leu} CAA(C48U)_fwd	CGCAAAATTAATACGACTCACTATAGTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCAAGTAAGCACCTTGCCTGCG
pre-tRNA ^{Leu} CAA(C48U)_rev	TGTCAGAAGTGGGATTTCGAACCCACGCTCCATCCGGAGACCCAGAAAGCCCGCAGGCAAGGTGCTTACCTT
pre-tRNA ^{Leu} CAA(C34U/C48U)_fwd	CGCAAAATTAATACGACTCACTATAGTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTAAGTAAGCACCTTGCCTGCG
pre-tRNA ^{Leu} CAA(C34U/C48U)_rev	TGTCAGAAGTGGGATTTCGAACCCACGCTCCATCCGGAGACCCAGAAAGCCCGCAGGCAAGGTGCTTACCTT
tRNA ^{Leu} CAA(C48U)_fwd	CGCAAAATTAATACGACTCACTATAGTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCAAGTCTCTG
tRNA ^{Leu} CAA(C48U)_rev	TGTCAGAAGTGGGATTTCGAACCCACGCTCCATCCGGAGACCCAGAACTTGAGTCTGGCGCTT
RP11(WT)_fwd	CGCAAAATTAATACGACTCACTATAGTCTCAGGAGACGTCTCCTGG
RP11(WT)_rev	TAGGACAGATGCCACGCCACTGTCCAGGAGACTGTCTCCTGAGAC
RP11(CtoU)_fwd	CGCAAAATTAATACGACTCACTATAGTCTTAGGAGACAGTCTCCTGG
RP11(CtoU)_rev	TAGGACAGATGCCACGCCACTGTCCAGGAGACTGTCTCCTAAGAC
RP11(CAGCU)_fwd	CGCAAAATTAATACGACTCACTATAGTCTCAGTGACAGTCAGCTGG
RP11(CAGCU)_rev	TAGGACAGATGCCACGCCACTGTCCAGTGTGCTCAGCTGAGAC

Supplementary Table 1: List of DNA primers used for RNA in vitro transcription
Sequences are listed 5' - 3'.

RNA	Name	Mutation	Sequence (5'-3')
IleTAT	IleTAT	WT	GCUCCAGUGGCGCAAUCGGUUGAGCGCGCGGUACUUAAUAGCCGAGGUUGUGAGUUCGAUCCUCACCUUGGAGCACCA
LysCTT	LysCTT	WT	GCCCGGCUAGCUCAGUCGGUAGAGCAUUGGACUCUUAAUCCAGGGUUCGUGGGUUCGAGCCCCACGUUGGGCGCCA
	LysCTT(NoC) or LysCTT(C48U)	G15A/C48U	GCCCCGGCUAGCUCAAUCGGUAGAGCAUUGGACUCUUAAUCCAGGGUUGUGGGUUCGAGCCCCACGUUGGGCGCCA
	LysCTT(C49)	G15A/C48U/U49C/C65G	GCCCGGCUAGCUCAAUCGGUAGAGCAUUGGACUCUUAAUCCAGGGUUCGUGGGUUCGAGCCCCAGGUUGGGCGCCA
	LysCTT(C50)	G15A/C48U/U50C/A64G	GCCCGGCUAGCUCAAUCGGUAGAGCAUUGGACUCUUAAUCCAGGGUUCGCGGGUUCGAGCCCCCGGUUGGGCGCCA
LysTTT	LysTTT(NoC)	C49G/G65C	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGGAGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(C48)	G48C/C49G/G65C	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT	WT	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(C50)	C49G/G65C/A50C/U64G	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(ΔD-arm)	Δ11-24	GCCUGGAUAGCAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(ΔAC-arm)	Δ28-42	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(ΔT-arm)	Δ50-64	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(ΔAcceptor)	Δ1-5,67-73	GAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(U67A/C68A)	U67A/C68A	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAAAGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(C62A/C63A)	C62A/C63A	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCAAUUGUUCAGGGCGCCA
	LysTTT(G51A/G52A)	G51A/G52A/C62U/C63U	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAAAGUUCAAGUCCCUUCUACGGCGCCA
	LysTTT(A50C)	A50C/U54G	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(A50U)	A50U/U64A	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(G51C)	G51C/C63G	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(G52C)	G52C/C62G	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(G53C)	G53C/C61G	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(G52C/G53C)	G52C/C62G/G53C/C61G	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(G52A/G53A)	G52A/G53A/C61U/C62U	GCCUGGAUAGCUCAGUUGGUAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
	LysTTT(DloopMut)	A14G/G15A/G18A/G19A	GCCUGGAUAGCUCGAUUAAGAACAUCAGACUUUUAAUCUGACGGUGCAGGGUUCAAGUCCCGUUCAGGGCGCCA
TrpCCA	TrpCCA(NoC)	C50U/G64A	GGCCUCGUGGGCGCAACGGUAGCGCGUCUGACUCCAGAUCAAGAGGUUGUGUUAACAAUCACUCCGGGUCACCA
	TrpCCA(C48)	A15G/U48C/C50U/G64A	GGCCUCGUGGGCGCAGCGGUAGCGCGUCUGACUCCAGAUCAAGAGGUUGUGUUAACAAUCACUCCGGGUCACCA
	TrpCCA(C49)	G49C/C50U/G64A/U65G	GGCCUCGUGGGCGCAACGGUAGCGCGUCUGACUCCAGAUCAAGAGGUUGUGUUAACAAUCACUCCGGGUCACCA
	TrpCCA	WT	GGCCUCGUGGGCGCAACGGUAGCGCGUCUGACUCCAGAUCAAGAGGUUGUGUUAACAAUCACUCCGGGUCACCA
MetCAT	MetCAT	WT	GCCUCGUUAGCGCAGUAGGUAGCGCGUCACUAUUAUCUGAAGGUUCGAGUUCGAUCCUCACACGGGGCACCA
	MetCAT(ΔD-arm)	Δ11-24	GCCUCGUUAGCGCAGUACUCUAUUAUCUGAAGGUUCGAGUUCGAUCCUCACACGGGGCACCA
	MetCAT(ΔAC-arm)	Δ28-42	GCCUCGUUAGCGCAGUAGGUAGCGCGUAAGGUUCGAGUUCGAUCCUCACACGGGGCACCA
	MetCAT(ΔT-arm)	Δ50-64	GCCUCGUUAGCGCAGUAGGUAGCGCGUCACUAUUAUCUGAAGGUUCGAGUUCGAUCCUCACACGGGGCACCA
	MetCAT(ΔAcceptor)	Δ1-5,67-73	GUUAGCGCAGUAGGUAGCGCGUCAGUCUAUUAUCUGAAGGUUCGAGUUCGAUCCUCACACGGGGCACCA
IleAAT	IleAAT	WT	GGCUGGUUAGGUUACGUUGGUUAGAGCGUGGUCUAAUAAACGCCAAGGUCGUGGGUUCGAUCCCAUAUCGGCCACCA
ThrCGT	ThrCGT	WT	GGCCCUUGAGCUCAGCGGUGGAGCGCGUGGUCUCGUAACCUUAGGGGUCGUGAGUUCAAAUCCUACACGGGGCUCCA
ArgACG	ArgACG	WT	GGCCAGUGGCGCAAUGGAUAACGCCGUCUGACUACGGAUACAAGAUUCUAGGUUCGACUCCUGGCGUGCGC
AlaAGC	AlaAGC	WT	GGGGGAUUAGCUCAAUUGGUAGAGCGCGUCGCUUAAGCAUGCGAGGUAAGCGGGAUAGUCCCGCAUCCUCCACCA
SerGCT	mtSerGCT	WT	GAGAAAGCUCACAAGAACUCUAACUCAUGGCCCAUGUCUAAACAACAUUGGCUUUUCACCA
LeuCAA	pre-LeuCAA	WT	GUCAGGAUGGCGAGUGGUUUAAGGCGCCAGACUUAAGGUAAGCACCUCUUGCCUGCGGGCUUUUCUGGUCUCCGGAUGGAGGC
	pre-LeuCAA	C34U	GUCAGGAUGGCGAGUGGUUUAAGGCGCCAGACUUAAGGUAAGCACCUCUUGCCUGCGGGCUUUUCUGGUCUCCGGAUGGAGGC
	pre-LeuCAA	C48U	GUCAGGAUGGCGAGUGGUUUAAGGCGCCAGACUUAAGGUAAGCACCUCUUGCCUGCGGGCUUUUCUGGUCUCCGGAUGGAGGU
	pre-LeuCAA	C34U/C48U	GUCAGGAUGGCGAGUGGUUUAAGGCGCCAGACUUAAGGUAAGCACCUCUUGCCUGCGGGCUUUUCUGGUCUCCGGAUGGAGGU
	Mature LeuCAA	C48U	GUCAGGAUGGCGAGUGGUUUAAGGCGCCAGACUUAAGGUAAGCACCUCUUGCCUGCGGGCUUUUCUGGUCUCCGGAUGGAGGU
	Mature LeuCAA	C48U	ACA
Mini	Mini or Minj ^{8nt}	N/A	GCCUGGAUAAUACGGUGCAGGGUUAAGUCCCUUGUUCAGGGCGCCA
	Minj ^{ΔssRNA} or Minj ^{GGG}	Δ42-45	GCCUGGAUAAUACGGUGCAGGGUUAAGUCCCUUGUUCAGGC
	Minj ^{7bp}	Δ1,41	CCUGGAUAAUACGGUGCAGGGUUAAGUCCCUUGUUCAGG
	Minj ^{Open-Bulge}	N/A	GGUGCAGGGUUAAGUCCCUUGUUCAGGGCAACCUUGGAUAAUAC
	Minj ^{Closed-stem}	N/A	UCAAGUCCCUUGUUCAGGGCAACCUUGGAUAAUACGGUGCAGGGU
	Minj ^{7nt}	Δ9-10	GCCUGGAUAAUACGGUGCAGGGUUAAGUCCCUUGUUCAGGGCGCA
	Minj ^{5nt}	Δ9-12	GCCUGGAUACGGUGCAGGGUUAAGUCCCUUGUUCAGGGCGCA
	Minj ^{3nt}	Δ9-14	GCCUGGAUGUGCAGGGUUAAGUCCCUUGUUCAGGGCGCA
	Minj ^{0nt}	Δ9-17	GCCUGGAUACAGGGUUAAGUCCCUUGUUCAGGGCGCA
	Minj ^{N-LoopMut}	A27G/G28A	GCCUGGAUAAUACGGUGCAGGGUUAAGUCCCUUGUUCAGGGCGCA
	Minj ^{U48}	N/A	GCCUGGAUAAUAGGGUUCAGGGUUAAGUCCCUUGUUCAGGC
	Minj ^{m5C48}	N/A	GCCUGGAUAAUAGGGU (m5C) CAGGGUUAAGUCCCUUGUUCAGGC
	Minj ^{U49}	N/A	GCCUGGAUAAUAGGGUUAAGGGUUAAGUCCCUUGUUCAGGC
	Minj ^{m5C49}	N/A	GCCUGGAUAAUAGGGU (m5C) AGGGUUAAGUCCCUUGUUCAGGC
Intron	WT	WT	CUCAGGUAAGCACCUCUUGCCUGCGGGCUUUC
	C34U	C3U	CUAAGGUAAGCACCUCUUGCCUGCGGGCUUUC
	CAACC	G6C/G7C/C14G/C15G	CUCACCUAAGCAGGUUGCCUGCGGGCUUUC
RP11	WT	WT	GUCUCAGGAGACAGUCUCCUGGACAGUGGCGUGGCAUCUGUCCUA
	CtoU	C5U	GUCUAGGAGACAGUCUCCUGGACAGUGGCGUGGCAUCUGUCCUA
	CAGCU	G8C/A9U/U17A/C18G	GUCUCAGCUGACAGUCAGCUGGACAGUGGCGUGGCAUCUGUCCUA

Supplementary Table 2: Complete list of RNA sequences used in this study