Supplementary Information

Enzymatic RNA Biotinylation for Affinity Purification and Identification of RNA-protein Interactions

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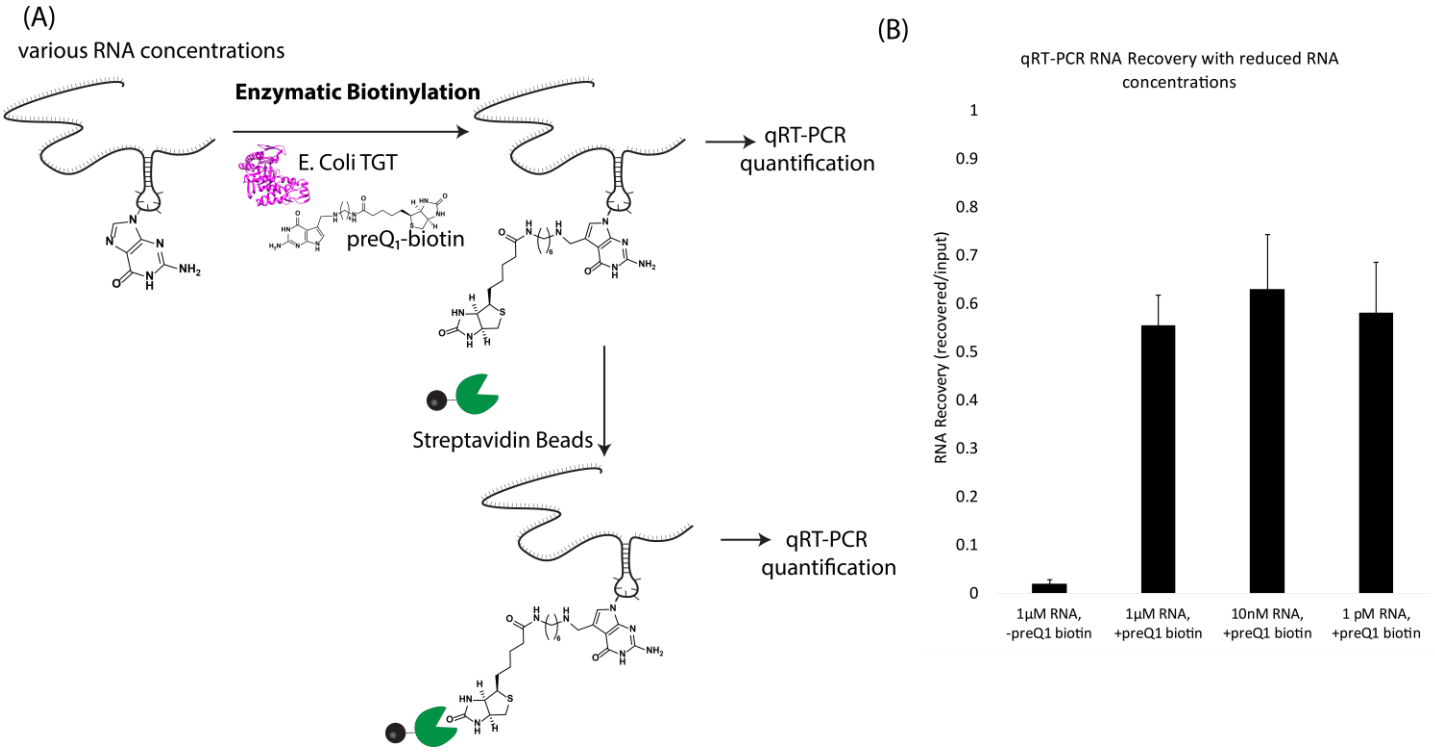
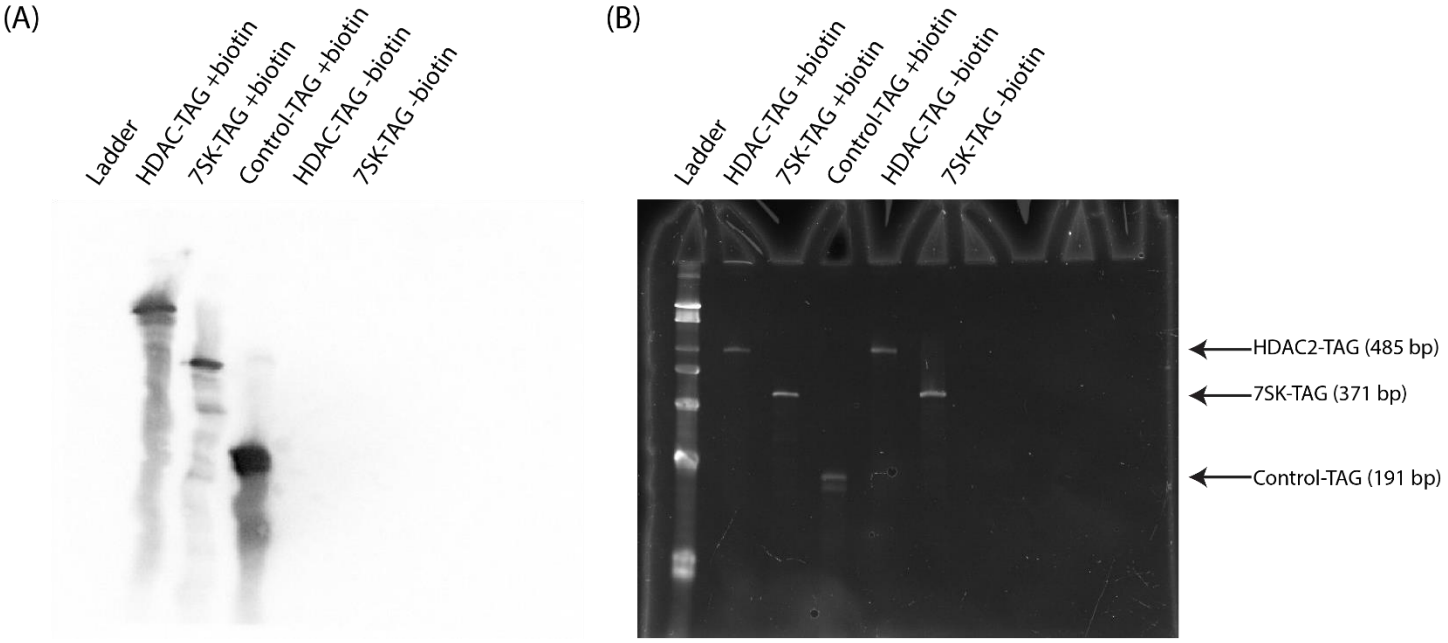
Supplementary Figures and Tables

Supplementary Figure S1: Quantification of RNA recovery with reduced RNA concentrations



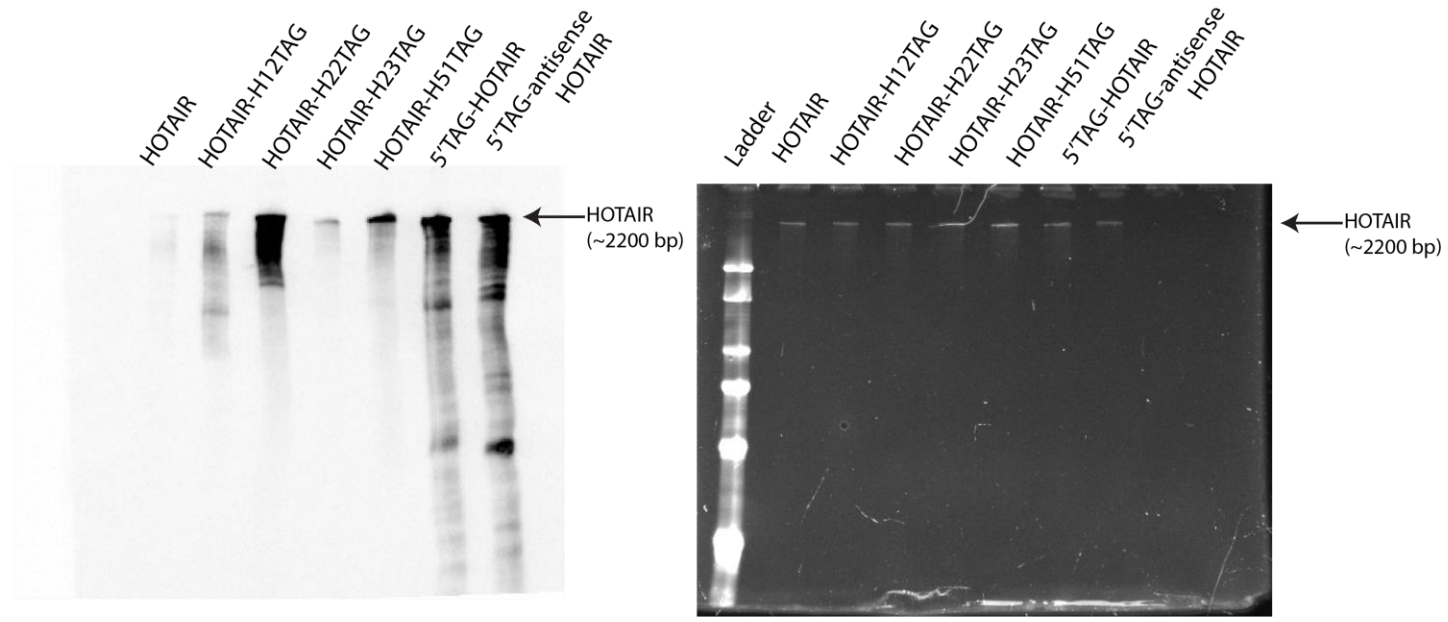
(A)HDAC2-TAG RNA at the indicated concentrations (1µM, 10 nM, 1 pM) was treated with preQ$_1$-biotin and TGT under standard labeling conditions. After precipitation of the RNA product, RNA was diluted to uniform concentrations for RT-qPCR analysis. Input RNA was quantified by qRT-PCR, alongside RNA that was affinity purified using Dynabeads M-280 Streptavidin. (B) RNA recovery values were determined by comparison of the input and recovered RNA C_T values, as described in Methods. Biotinylation of RNA was observed in RNA concentrations as low as 1 pM.

Supplementary Figure S2: Northern blot assay of HDAC2-TAG and 7SK-TAG RNA transcripts used in mass spectrometry experiments



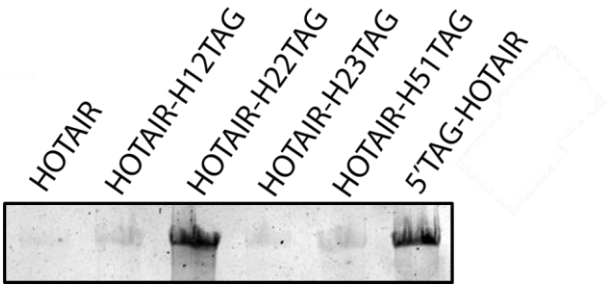
Northern blot results for transcripts used in HDAC2-TAG and 7SK-TAG proteomics experiments (A) Biotin detection (B) SYBR green staining.

Supplementary Figure S3: Northern blot assay of HOTAIR mutant labeling



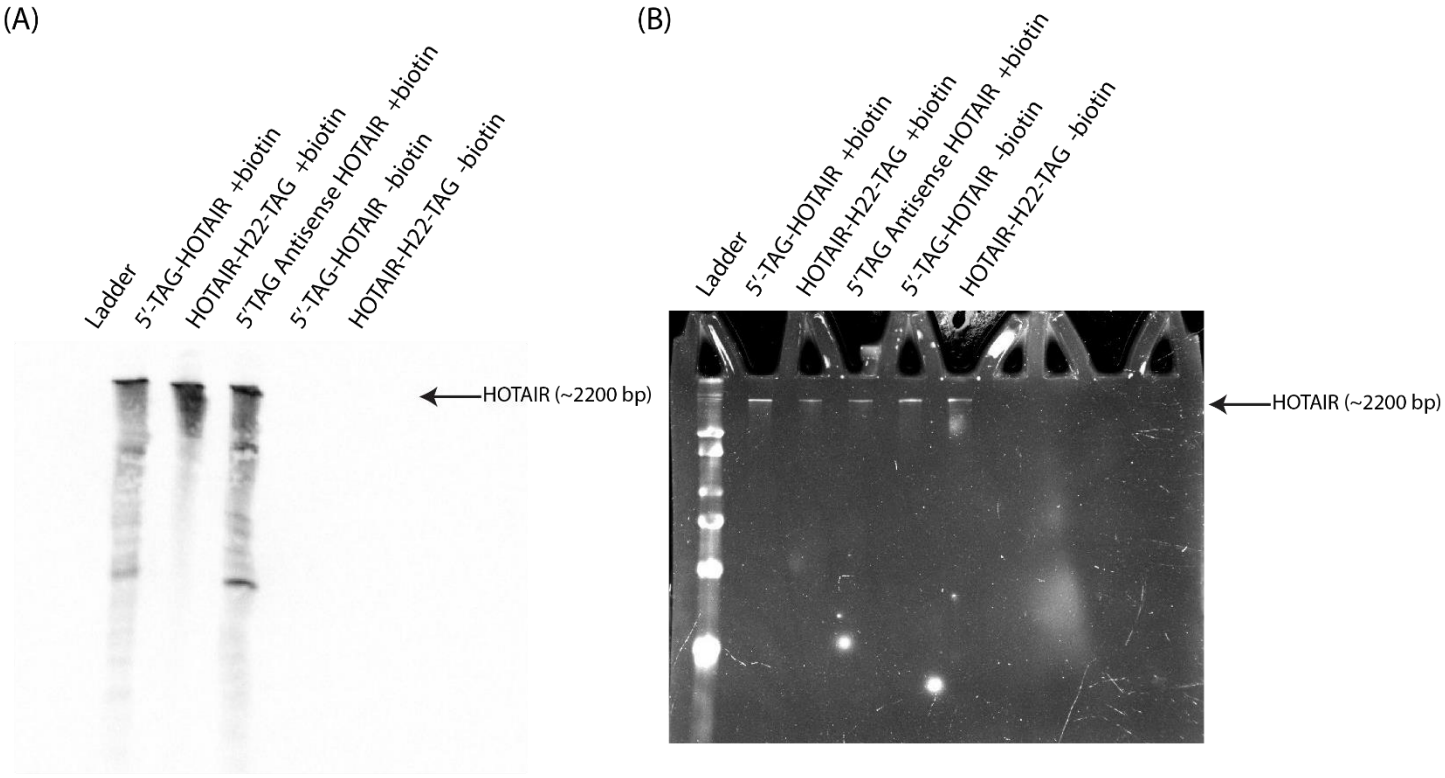
Northern blot results of HOTAIR labeling assay (A) Biotin detection (B) SYBR green staining.

Supplementary Figure S4: HOTAIR labeling with preQ1-BODIPY



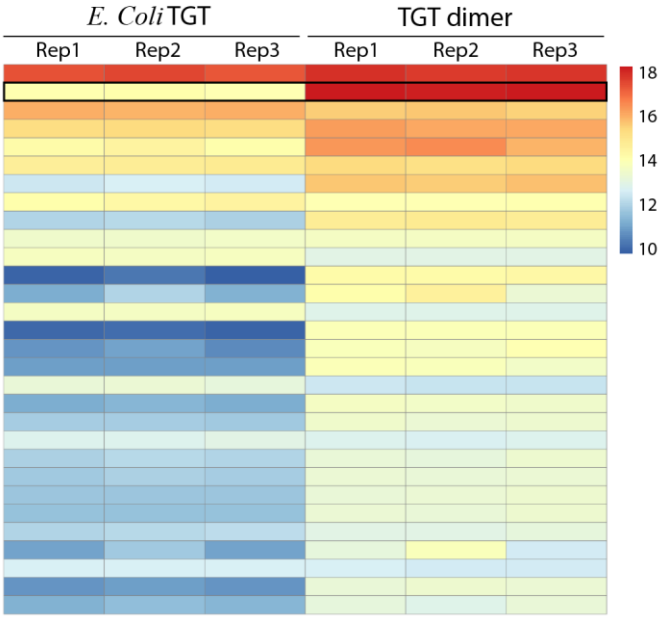
Polyacrylamide gel electrophoresis of HOTAIR constructs treated with TGT and preQ$_1$-BODIPY. 1µM RNA was treated with 1 µM *E. Coli* TGT and 10 µM preQ$_1$-BODIPY[1] for 2 h at 37°C. RNA was subsequently precipitated with lithium chloride and analyzed via 4% denaturing UREA-PAGE. BODIPY fluorescence was detected using a Bio-Rad ChemiDoc-MP imager.

Supplementary Figure S5: Northern blot assay of HOTAIR transcripts used in mass spectrometry experiments



Northern blot results for transcripts used in HOTAIR proteomics experiments (A) Biotin detection (B) SYBR green staining.

Supplementary Figure S6: Heatmap of the count matrix.



All genes are sorted based on their mean expression under TGT dimer treatment condition. The first thirty genes are visualized in this heatmap. "HDAC-TAG-Puro" gene is ranked second on the heatmap and denoted with a black rectangular box.

Supplementary Table 1: Putative TAG-like sequences in human transcripts

Gene	**Transcript accession number**	**TAG-like cDNA Sequence**
GAPDH	NM_001256799.3	'CATGG**GTGTGAA**CCATG'
LBR	NM_002296.4	'ACTCC**CTGTAAA**GGAGT'
MRPL51	NM_016497.4	'GGAAA**CTGTACT**TTTCC'
MALAT1	NR_002819.4	'AGAAA**ATGTTTT**TTTCT'

Supplementary Table 2: qPCR ΔCt data of initial selectivity screen

		ΔCt (purified-input)		
		E Coli TGT	TGT Dimer	No Enzyme
HDAC2-TAG	Rep1	0.34	0.85	13.03
	Rep2	0.21	1.5	15.29
	Rep3	0.75	1.31	16.98
GAPDH	Rep1	5.00	12.83	15.19
	Rep2	8.01	11.8	21.24
	Rep3	8.57	11.72	19.17
Actin	Rep1	10.13	16.45	17.03
	Rep2	14.24	13.83	18.18
	Rep3	12.5	17.54	17.16
LBR	Rep1	9.68	10.46	12.03
	Rep2	9.76	10.3	12.96
	Rep3	9.47	13.25	13.67
U1 snRNA	Rep1	16.8	20.65	13.89
	Rep2	19.09	21.17	20.15
	Rep3	17.61	15.14	12.72
18s rRNA	Rep1	11.37	18.2	11.6
	Rep2	13.56	17.82	18.81
	Rep3	13.85	14.95	10.61

Supplementary Table 3: Calculated qPCR Recovery of initial selectivity screen

	Calculated RNA Recovery (mean ± S.D., 3 replicates)		
	E Coli TGT	TGT Dimer	No Enzyme
HDAC2-TAG	75.0 ± 13.9%	43.7 ± 10.5%	0.0051 ± 0.0060%
GAPDH	1.25 ± 1.62%	0.024 ± 0.009%	0.0010 ± 0.0015%
Actin	0.037 ± 0.045%	0.0028 ± 0.0035%	0.0006 ± 0.0002%
LBR	0.13 ± 0.01%	0.054 ± 0.038%	0.015 ± 0.008%
U1 snRNA	0.0005 ± 0.0004%	0.0010 ± 0.0016%	0.0072 ± 0.0074%
18s rRNA	0.018 ± 0.018%	0.0013 ± 0.0016%	0.032 ± 0.032%

Supplementary Table 4: qPCR ΔCt data examining RNA-Seq Transcripts

		dCt (purified-input)		
		E Coli TGT	TGT Dimer	No Enzyme
HDAC2-TAG	Rep1	0.82	1.14	7.08
	Rep2	0.55	1.11	7.38
	Rep3	0.91	1.21	6.88
FTH1	Rep1	2.98	6.21	12.62
	Rep2	2.47	6.26	12.59
	Rep3	2.98	6.15	12.38
RPS6	Rep1	4.27	7.18	13.37
	Rep2	3.07	6.72	13.7
	Rep3	4.13	7.11	12.93
RPL41	Rep1	4.27	8.07	14.68
	Rep2	4.11	8.04	15.06
	Rep3	4.61	8.25	15.34
MRPL51	Rep1	2.65	4.75	10.34
	Rep2	1.68	4.22	11.1
	Rep3	2.66	4.74	11.33
MALAT1	Rep1	8.71	12.36	15.57
	Rep2	7.32	11.55	15.89
	Rep3	8.95	12.16	16.22

Supplementary Table 5: Calculated qPCR Recovery Data of RNA-Seq Transcripts

	Calculated RNA Recovery (mean ± S.D., 3 replicates)		
	E Coli TGT	TGT Dimer	No Enzyme
HDAC2-TAG	59.4 ± 7.9 %	45.0 ± 1.6%	0.73 ± 0.12%
FTH1	14.5 ± 3.1%	1.35 ± 0.05%	0.017 ± 0.002%
RPS6	7.60 ± 3.74%	0.79 ± 0.14%	0.010 ± 0.003%
RPL41	5.02 ± 0.86%	0.36 ± 0.03%	0.003 ± 0.001%
MRPL51	21.0 ± 8.9%	4.27 ± 0.95 %	0.054 ± 0.021%
MALAT1	0.36 ± 0.23%	0.025 ± 0.008%	0.0017 ± 0.0004%

Supplementary Table 6: qPCR Ct values from lysate purification

		Rep1	Rep2	Rep3
HDAC2-TAG	E Coli TGT	19.97304	19.4716	19.509
	TGT dimer	20.42991	19.9254	20.206
	No enzyme	27.73386	26.1111	27.77
GAPDH	E Coli TGT	21.16293	20.2132	20.522
	TGT dimer	24.16379	23.8605	23.997
	No enzyme	25.1854	24.3525	25.03
Actin	E Coli TGT	25.03013	24.2149	24.018
	TGT dimer	25.30845	25.0633	25.064
	No enzyme	25.39997	24.3181	25.256

Supplementary Table 7: Calculated Enrichment values from lysate purification

	E Coli TGT	TGT dimer
HDAC	130.2 ± 37.5	145.3 ± 22.0
GAPDH	12.9 ± 3.4	2.0 ± 0.3

Supplementary Notes

Gene Sequences

The gene sequences for each construct are listed below (from the T7 promoter to the restriction cut site, or transcription stop site), with the TAG recognition element underlined. Mutated HOTAIR constructs were prepared using Q5 site directed mutagenesis with the following primers:

Primer Name	Sequence
HOTAIR-H12TAG-Fwd	aaaCTGGTAGAAAAAGCAACCACGAAG
HOTAIR-H12TAG-Rev	acagCTGGCTTAGGCCCCAACG
HOTAIR-H22TAG-Fwd	aaaGCACCCGGCTCGGGTCAG
HOTAIR-H22TAG-Rev	acagGCACCCGCTCAGGTTTTTCCAG
HOTAIR-H23TAG-Fwd	aaaGCCCCGCCCCTCGCGGCC
HOTAIR-H23TAG-Rev	acagGCCCGGTGTGGGGCAGTGGC
HOTAIR-H51TAG-Fwd	aaaTCCCAATGCCTGAACTTC
HOTAIR-H51TAG-Rev	acagTCTCCATCTGCTGATTTTTTTTC

Control-TAG

GGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGTACCGAGCTCGGATCCTCTAACACAGACTCTCGGTACCATCATTTTCAT
ATCCCCGGGAGCAGACTGTAAATCTGCTCCCACCACCATCATTTAATGAATTCCATCAGGAATCCCTCACTTCTGCAGACTGGCCGTCG
TTTTACACTCGAGT

HDAC2-TAG

GGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGTACCGAGCTCGGATCCTGACAGTCTCACCAATTTCAGAAAATCATTAAA
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β-Actin-TAG

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7SK-TAG

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HOTAIR

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5’TAG-HOTAIR

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G

5'TAG Antisense HOTAIR

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Primers used for RT-qPCR

Primer	**Sequence**
HDAC2-TAG RT	CTCCCGGGGATATGAAAATG
GAPDH RT	GTGAAGACGCCAGTG
β-actin RT	GTGGATGCCACAGGAC
LBR RT	CTTCATAATAAAGTGAACTCCCAG
18S RT	GAGGGCCTCACTAAACC
U1 RT	CCCACTACCACAAATTATGC
HDAC2-TAG qPCR fwd	AATTTCTTTTCTCCACCATGCTTTATGTG
HDAC2-TAG qPCR rev	GAAAATGATGGTACCGAGAGTCTGTGTTAG
GAPDH qPCR fwd	AATCCCATCACCATCTTCCA
GAPDH qPCR rev	TGGACTCCACGACGTACTCA
β-actin qPCR fwd	AGAGCTACGACGTGCCTGAC
β-actin qPCR rev	CTCCATGCCCAGGAAGGAAGG
LBR qPCR fwd	TGCTGTGCGACTATTCTCC
LBR qPCR rev	CAGGCCATCGACCTCTTACC
18S qPCR fwd	GTAACCCGTTGAACCCC
18S qPCR rev	CCATCCAATCGGTAGTAGCG
U1 qPCR fwd	CCATGATCACGAAGGTGGTTT

U1 qPCR rev	ATGCAGTCGAGTTTCCCACAT
FTH1 qPCR fwd	CCAGAACTACCACCAGGACTC
FTH1 qPCR rev	GTCAAAGTAGTAAGACATGGACAGG
RPS6 qPCR fwd	AAGCACCCAAGATTCAGCGT
RPS6 qPCR rev	TAGCCTCCTTCATTCTCTTGGC
RPL41 qPCR fwd	AGCCAAGTGGAGGAAGAAGC
RPL41 qPCR rev	AGCGTCTGGCATTCCATGTT
MALAT1 qPCR fwd	TGGTGATGAAGGTAGCAGGC
MALAT1 qPCR rev	ATTGCCGACCTCACGGATTT
MRPL51 qPCR fwd	AAGCTTCTCTCTTGGTGTGC
MRPL51 qPCR rev	CCAGGATCCCGATGTTGTCA

preQ$_1$-biotin Characterization Data

^{1}H NMR (500 MHz, CD_3OD) δ 6.85 (s, 1H), 4.50 (dd, *J* = 7.8, 4.4 Hz, 1H), 4.31 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.24 – 3.15 (m, 4H), 3.06 (t, *J* = 9.5 Hz, 2H), 2.93 (dd, *J* = 12.6, 5.1 Hz, 1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.20 (t, *J* = 7.3 Hz, 3H), 1.79 – 1.32 (m, 18H). ^{13}C NMR (126 MHz, CD_3OD) δ 174.62, 166.64, 164.73, 161.25, 153.10, 152.57, 117.69, 108.43, 98.21, 61.98, 60.19, 55.66, 46.19, 43.37, 39.64, 38.57, 35.37, 28.76, 28.38, 28.12, 25.89, 25.62, 25.57.

HRMS [M+H]+ m/z calcd. for [C23H37N8O3S] + 505.2704, found 505.2707 (Δ = 0.6 ppm).

References

(1) Alexander, S. C.; Busby, K. N.; Cole, C. M.; Zhou, C. Y.; Devaraj, N. K. Site-Specific Covalent Labeling of RNA by Enzymatic Transglycosylation. *J. Am. Chem. Soc.* **2015**, *137* (40), 12756–12759. https://doi.org/10.1021/jacs.5b07286.