genes

MDPI

Article

Stress-Dependent Dynamics of the *S. cerevisiae* tRNA and rRNA Modification Profile

Yasemin Yoluç [1], Erik van de Logt [1,2] and Stefanie Kellner [1,*]

[1] Department of Pharmaceutical Chemistry, Goethe University Frankfurt, Max-von-Laue str 9, 60438 Frankfurt; yoluc@pharmchem.uni-frankfurt.de
[2] Department of Chemistry, Ludwig-Maximilians University Munich, Butenandtstr. 5-13, 81377 Munich, Germany; vandelogt@genzentrum.lmu.de
* Correspondence: Kellner@pharmchem.uni-frankfurt.de; Tel.: +49-069-798-29919

Content:

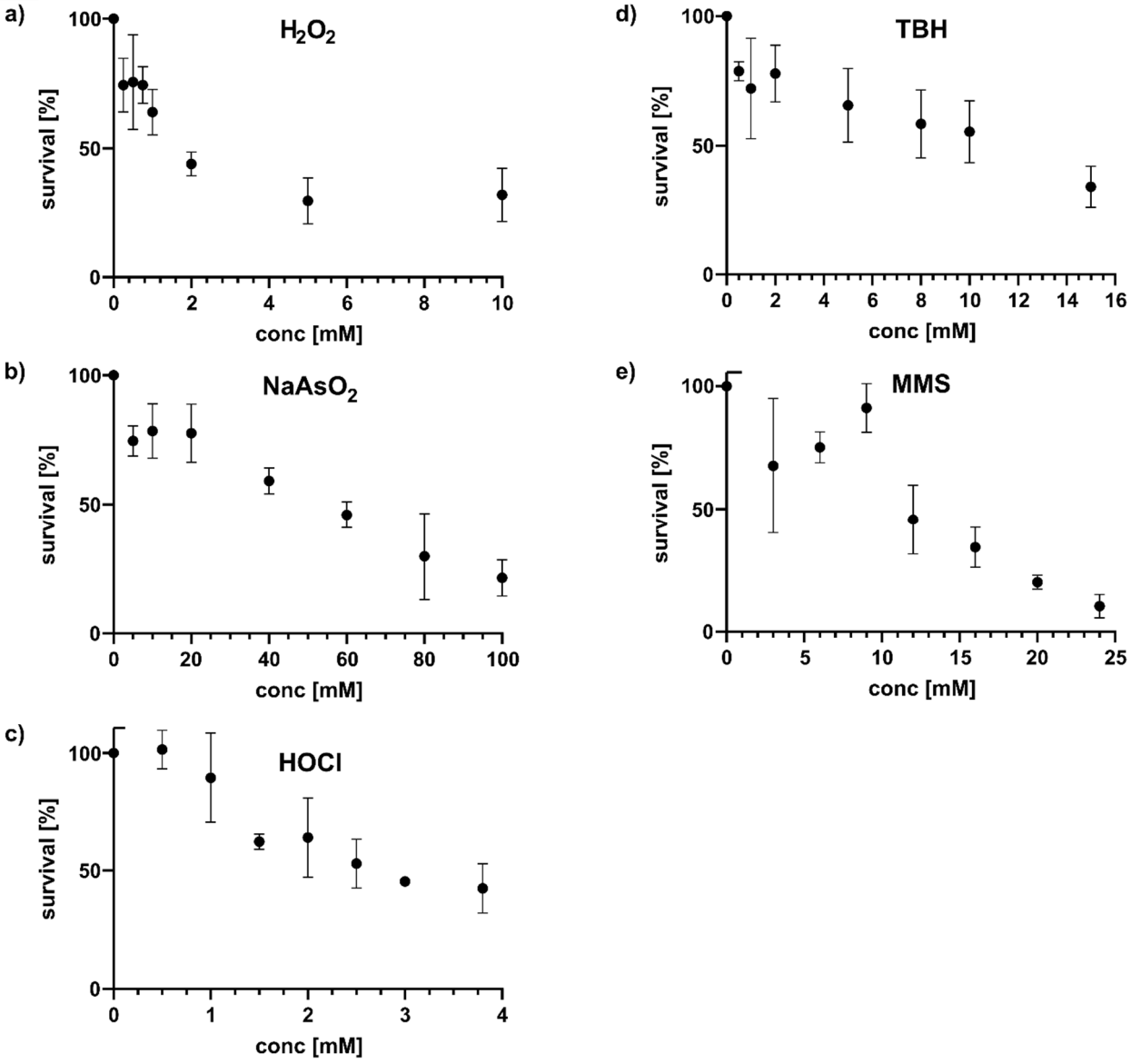

Figure S1: *LD_{50} for* S. cerevisiae *BY4741 after 60 min of stress exposure to chemicals with different concentrations a) H2O2: hydrogen peroxide; 0, 0.25, 0.5, 0.75, 1, 2, 5, 10 mM b) NaAsO2: sodium arsenite; 0, 5, 10, 20, 40, 60, 80, 100 mM c) HOCl: hypochloric acid; 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.8 mM d) TBH: tert-butyl hydroperoxide; 0, 0.5, 1, 2, 5, 8, 10, 15 mM e) MMS: methyl-methanesulfonate; 0, 3, 6, 9, 12, 16, 20, 24 mM; determined LD_{50} concentrations: H_2O_2 (2 mM), $NaAsO_2$(40 mM), HOCl (3 mM), MMS (12 mM), TBH (10 mM); data from n=3 biological replicates with standard deviation*

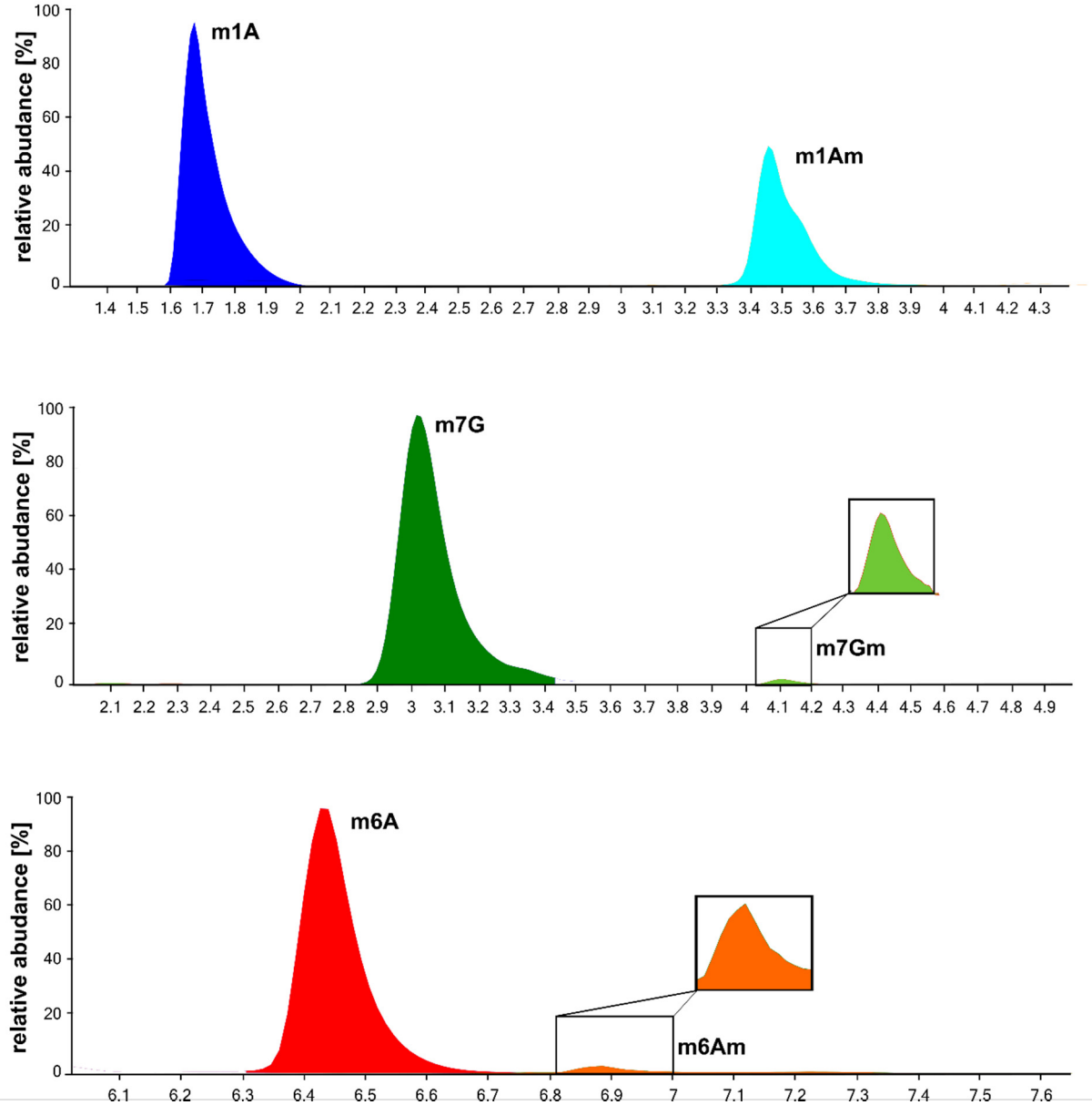

Figure S2: *Overlay of chromatograms from found damage products in total RNA after MMS treatment for 60 min. Peaks were scaled to largest in each chromatogram, m^3C (Rt: 1.65 min), m^1A (Rt: 1.67 min), m^7G (Rt: 3.02 min), m^1Am (Rt: 3.46 min), m^7Gm (Rt: 4.11 min), m^6A (Rt: 6.43), m^6Am (Rt: 6.90 min)*

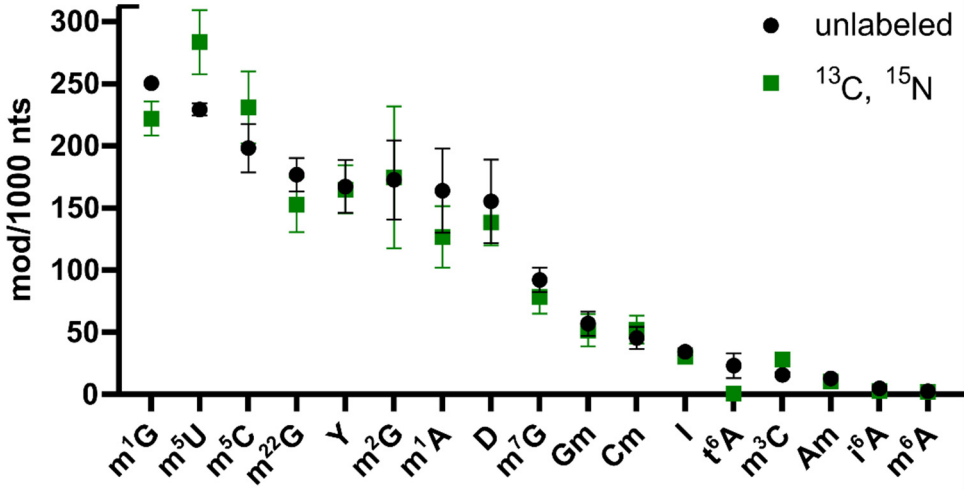

Figure S3: *comparative NAIL-MS of total RNA for labeling validation of $^{13}C_6$-glucose and $^{15}N_2$-uracil labeling (green) in comparison to unlabeled RNA (black), data from n=2 biological replicates with standard deviation*

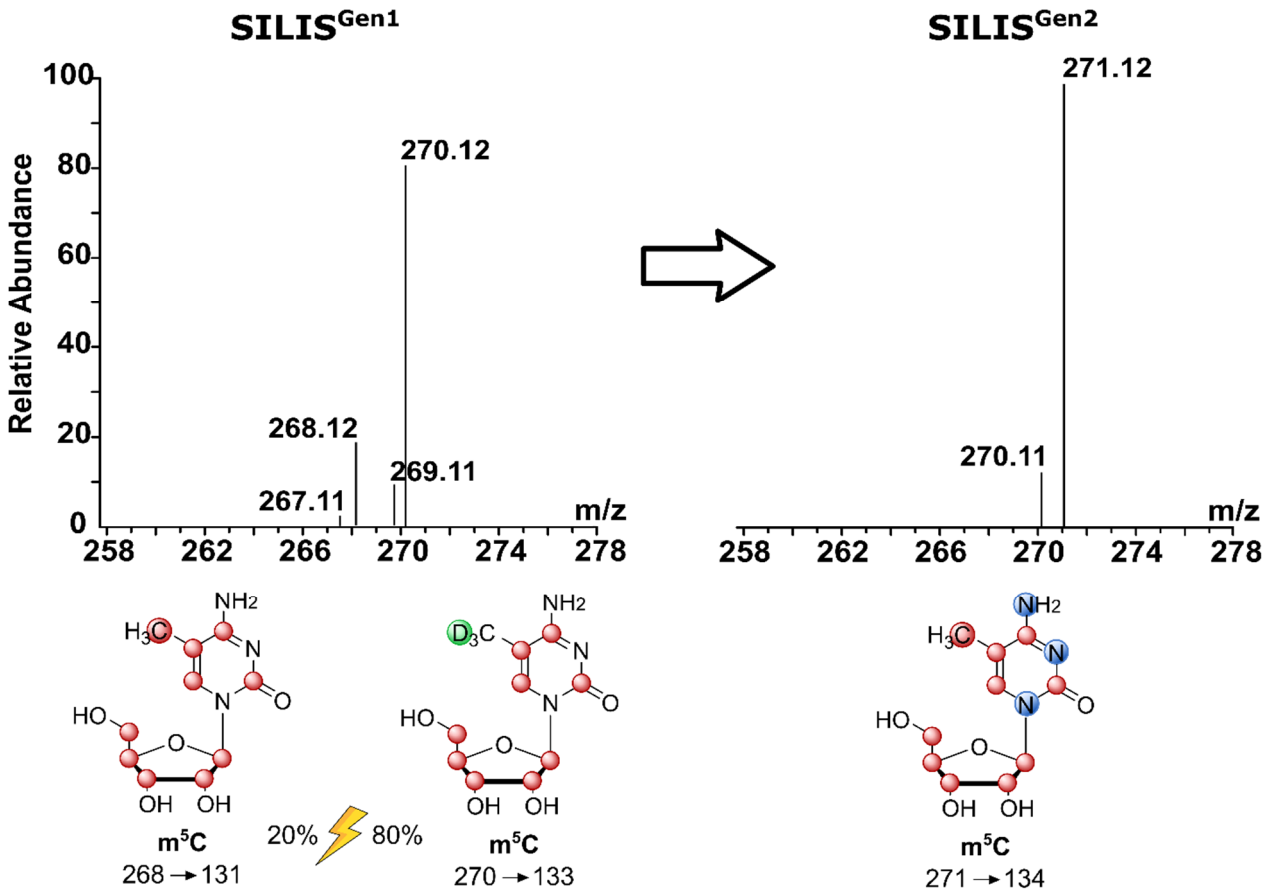

Figure S4: *Improvement of defined labeling of methylated nucleosides in SILIS^{Gen2}. Left: SILIS^{Gen1} was labeled using ^{13}C Silantes rich growth medium supplemented with ^{13}C-glucose and L-methionine-[$^{2}H_3$]-methyl which led to incomplete labeling of methylated nucleosides. Right: SILIS^{Gen2} was labeled using ^{13}C, ^{15}N Silantes rich growth medium supplemented with ^{13}C-glucose where all respective atoms are ^{13}C- and ^{15}N-labeled.*

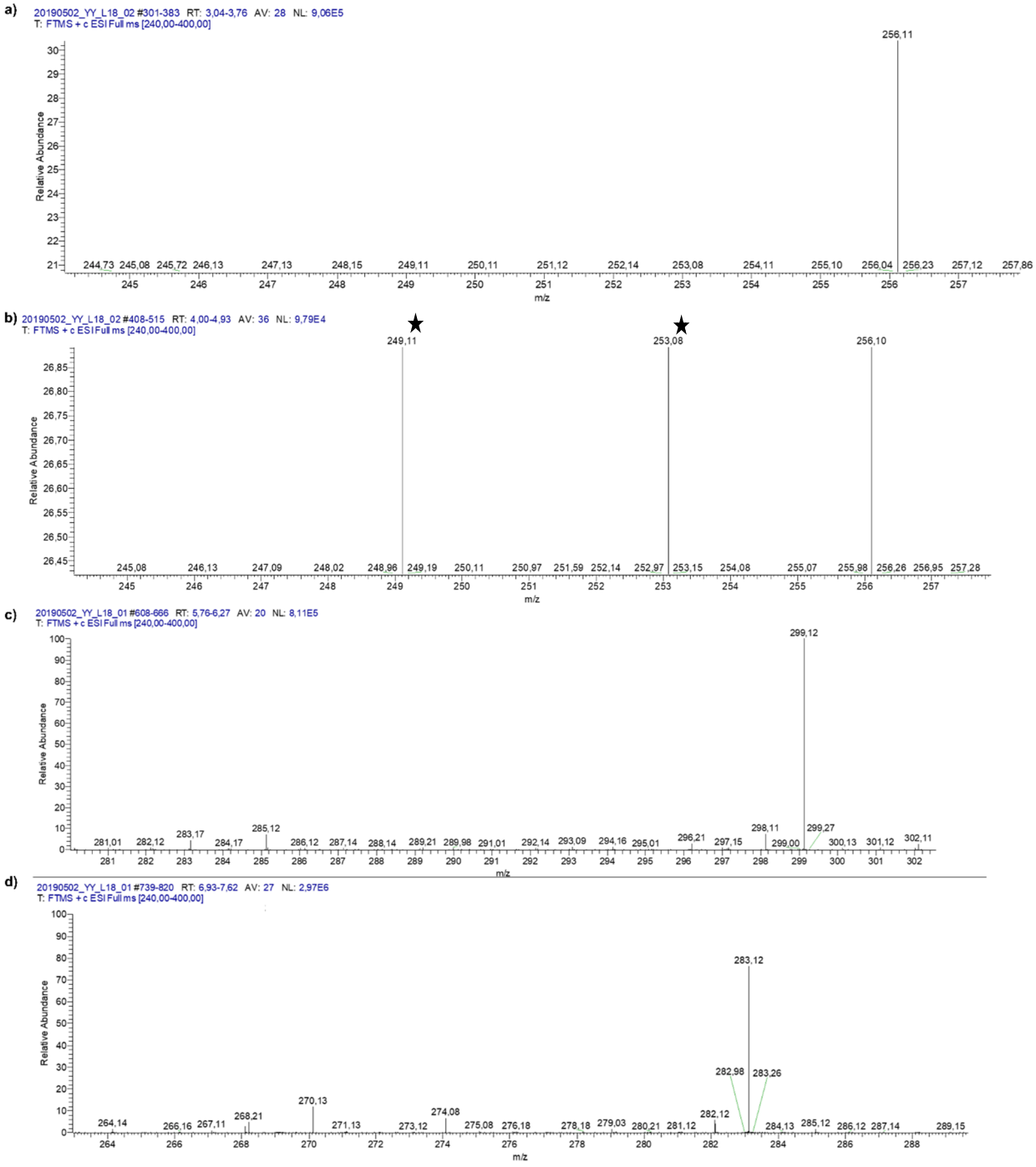

Figure S5: *High-resolution mass spectra of SILIS^{Gen2} shows representative nucleosides from the produced labeled RNA digests. a) cytosine m/z 256.11 b) uridine m/z 256.10; * indicates background contaminations c) guanosine m/z 299.12 d) adenosine m/z 283.12*

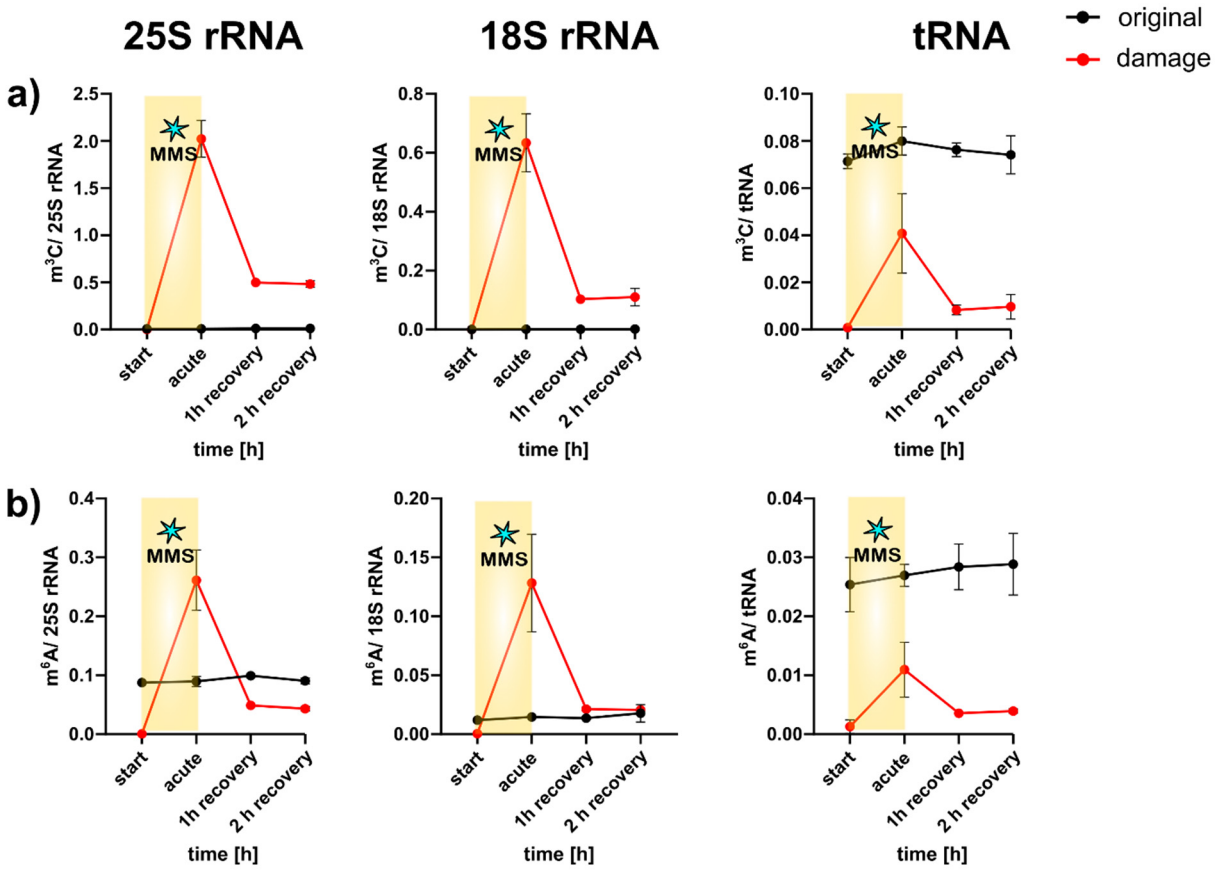

Figure S6: *Methylome discrimination based pulse chase experiment (a) number of m^3C per respective RNA (b) number of m^6A per respective RNA, red line shows damage induced methylation level, black line shows enzymatic methylation, data for tRNA from n=3 biological replicates with standard deviations, data for 25S and 18S rRNA from n=2 biological replicates with standard deviation*

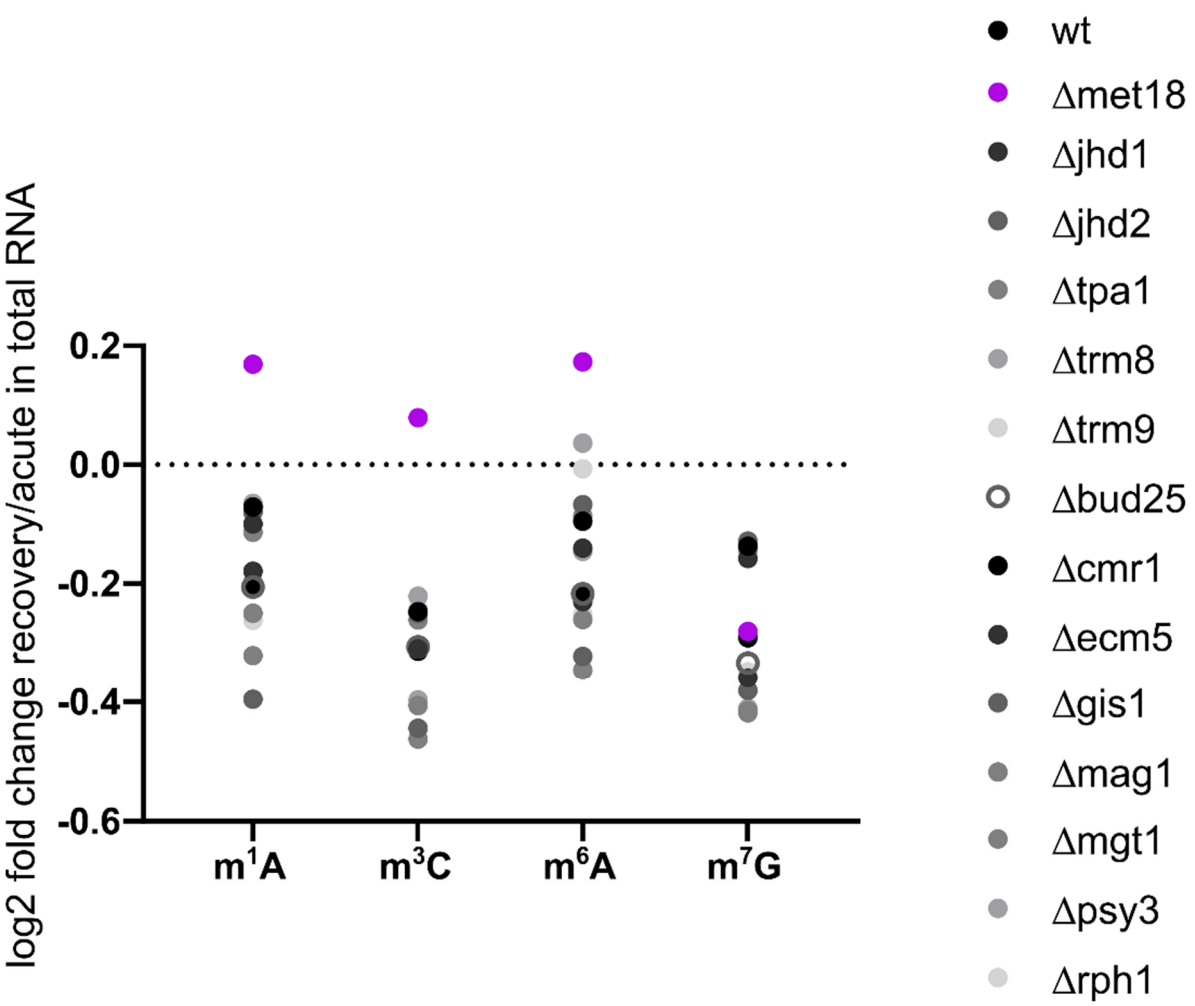

Figure S7: *log2 fold change from nucleoside abundance in acute stress phase in contrast to 1h recovery, Y < 0 indicates decrease of nucleoside level in recovery and Y > 0 indicates increase of nucleoside level in recovery, data from one biological replicate*

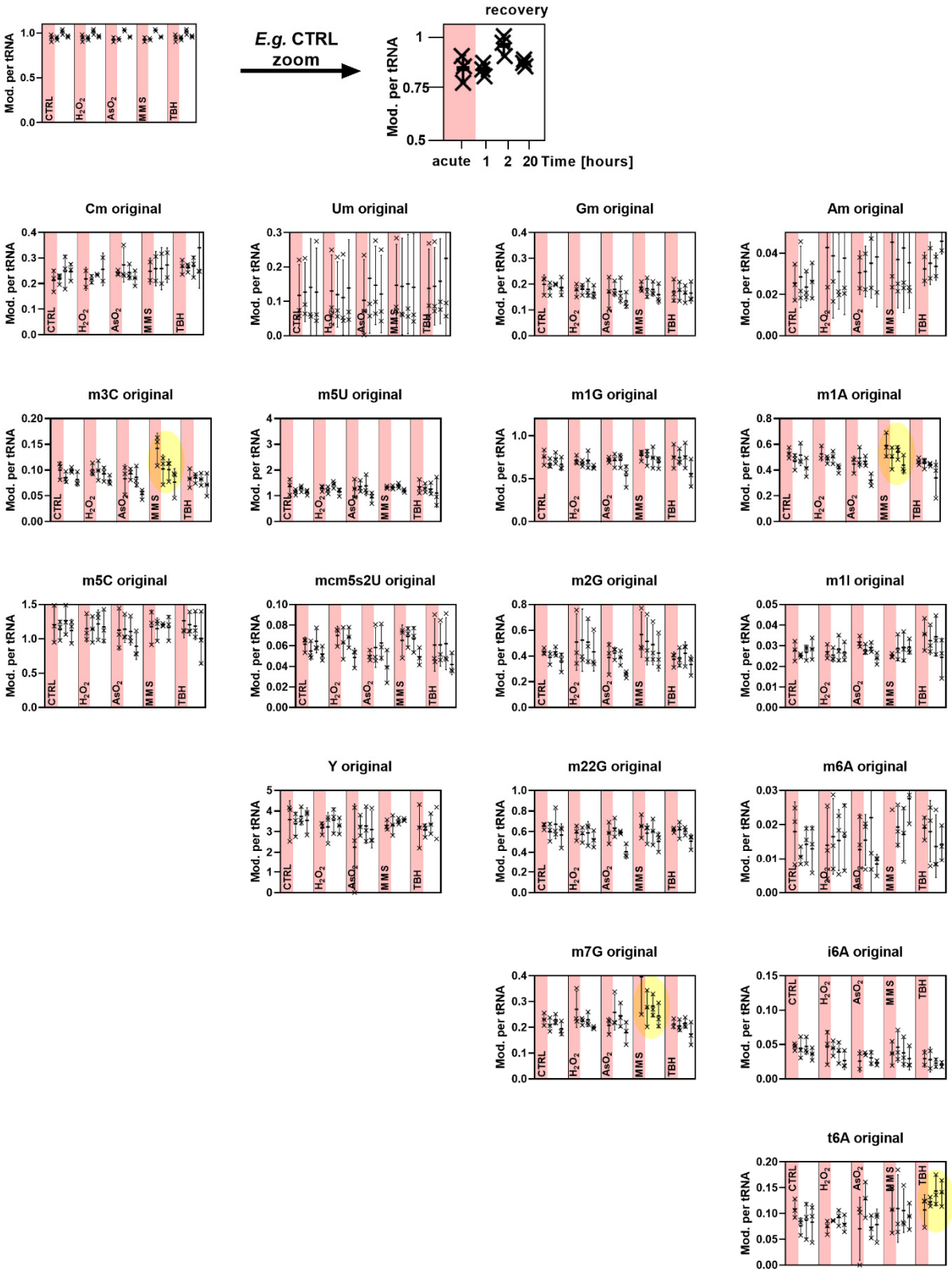

Figure S8: *Absolute abundance of modified nucleosides per original tRNA from NAIL-MS pulse chase experiment. Stress exposure phase (acute) is highlighted in red and recovery timepoints (1, 2 and 20 hrs) in white. Top plot gives an explanation for a fictive dataset. Yellow circles highlight interesting abundance changes in original tRNAs.*

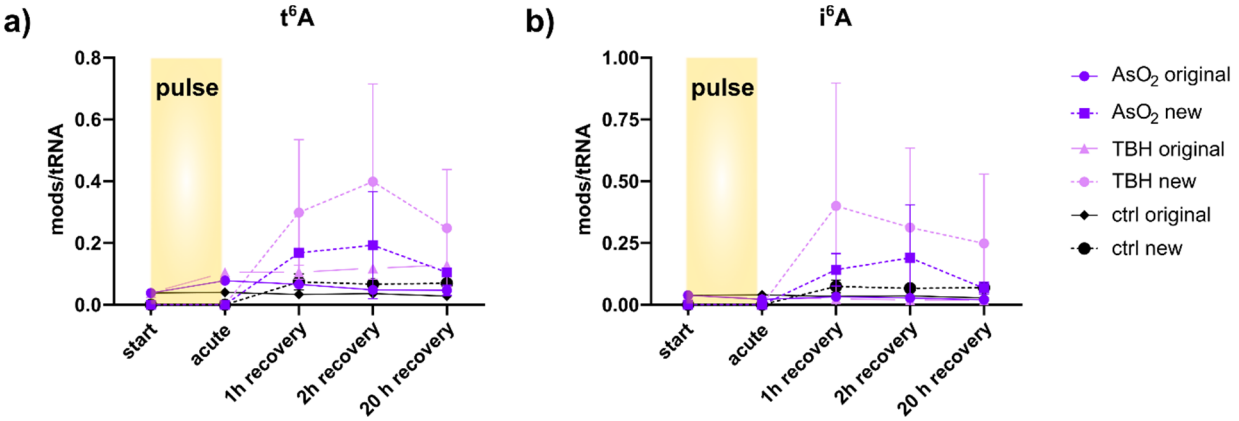

Figure S9: *NAIL-MS pulse chase experiment (a) comparison of original (solid line) and new (dashed lines) transcripts of ctrl (black), AsO_2 (violet) and TBH (pink) exposed yeast cultures. data from n=2 biological replicates with error bars reflecting standard deviation.*

Table S1: Overview of used synthetic standards, their vendors and alternative vendors. Sigma: Sigma, Aldrich, Munich, Germany; Carbosynth, Newbury, UK; TRC: Toronto Research Chemicals, Toronto, CA; Berry & Associates, Dexter, MI, USA.

Nucleoside	Abbreviation	Obtained from:	Also available from:
cytidine	C	Sigma	
2′ -O-methylcytidine	Cm	Carbosynth	
3-methylcytidine	m3C	Carbosynth	Carbo and TRC
5-methylcytidine	m5C	Carbosynth	
2-thiocytidine	s2C	Berry & Associates	
N4-acetylcytidine	ac4C	Carbosynth	
pseudouridine	Y	Carbosynth	
uridine	U	Sigma	
dihydrouridine	D	Apollo scientific, UK	
2′ -O-methyluridine	Um	Carbosynth	
3-methyluridine	m3U	Dedon Lab	
5-methyluridine	m5U	Carbosynth	
4-thiouridine	s4U	ordered from TRC	
5-methoxycarbonylmethyl-2-thiouridine	mcm5s2U	Helm Lab	
guanosine	G	Sigma	
1-methylguanosine	m1G		
2′ -O-methylguanosine	Gm	Carbosynth	
N2-methylguanosine	m2G	Dedon Lab	
7-methylguanosine	m7G	Carbosynth	
N2,N2-dimethylguanosine	m2,2G	Carbosynth	
adenosine	A	Sigma	
inosine	I	Carbosynth	
1-methyladenosine	m1A		Carbosynth
2-methyladenosine	m2A	Dedon Lab	
2′ -O-methyladenosine	Am	Carbosynth	Carbo and TRC
N6-methyladenosine	m6A	Carbosynth	Carbo, TRC and B&A
N6,N6-dimethyladenosine	m6,6A	Alfa Chemistry	TRC
N6-isopentenyladenosine	i6A	Dedon Lab	TRC
N6-threonylcarbamoyladenosine	t6A	TRC	TRC

Table S2: Comparison of S. cerevisiae 18S and 25S rRNA modification levels determined in this work and literature [Yang, J. et al. Mapping of complete set of ribose and base modifications of yeast rRNA by RPHPLC and mung bean nuclease assay. PloS one 11, e0168873 (2016).; Lovejoy, A. F., Riordan, D. P. & Brown, P. O. Transcriptome-wide mapping of pseudouridines: pseudouridine synthases modify specific mRNAs in S. cerevisiae. PloS one 9, e110799 (2014).] , data from n=10 biological replicates with standard deviation, n.d. = not detected.

	literature		experimental	
Modification	**18S**	**25S**	**18S**	**25S**
Cm	3	7	3.06 ± 0.34	6.78 ± 0.90
Um	2	8	1.90 ± 0.30	6.44 ± 1.05
Gm	5	10	4.34 ± 0.55	8.43 ± 1.25
Am	8	12	6.03 ± 1.12	8.84 ± 3.24
Ψ	13	30	12.24 ± 1.64	27.27 ± 3.32
ac^4C	2	absent	1.23 ± 0.32	0.15 ± 0.14
m^7G	1	absent	$0.77 \pm 9.85 \cdot 10^{-2}$	$4.7 \cdot 10^{-2} \pm 1.42 \cdot 10^{-2}$
$m^6{}_2A$	2	absent	$0.75 \pm 1.52 \cdot 10^{-2}$	$2.38 \cdot 10^{-2} \pm 4.84 \cdot 10^{-3}$
$m^1acp^3\Psi$	1	absent	n.d.	n.d.
m^5C	absent	2	$0.13 \pm 5.27 \cdot 10^{-2}$	1.84 ± 0.37
m^3U	absent	2	$0.11 \pm 3.50 \cdot 10^{-2}$	1.50 ± 0.32
m^1A	absent	2	$0.15 \pm 7.76 \cdot 10^{-2}$	1.90 ± 0.20
m^3C	absent	absent	$4.12 \cdot 10^{-4} \pm 4.05 \cdot 10^{-4}$	$6.10 \cdot 10^{-3} \pm 3.53 \cdot 10^{-3}$
m^6A	absent	absent	$2.04 \cdot 10^{-2} \pm 7.28 \cdot 10^{-3}$	$0.14 \pm 3.12 \cdot 10^{-2}$
m^6Am	absent	absent	$1.40 \cdot 10^{-4} \pm 3.10 \cdot 10^{-4}$	$1.50 \cdot 10^{-4} \pm 4.50 \cdot 10^{-2}$

Table S3: absolute number of enzymatically placed methylation (original, blue) and damage methylation (damage, red) per respective RNA after 60 min of MMS treatment (n=2)

		m^1A		m^3C		m^6A		m^7G	
tRNA	**original**	0.6568	0.5542	0.0865	0.0786	0.0262	0.0291	0.3682	0.3163
	damage	0.3027	0.1842	0.0560	0.0438	0.0146	0.0126	0.5864	0.3841
25S rRNA	**original**	1.9522	1.9539	0.0029	0.0039	0.0973	0.1338	0.0477	0.0438
	damage	1.7528	1.9876	0.5315	0.6391	0.0992	0.1471	7.7250	8.1068
18S rRNA	**original**	0.1755	0.1775	0.0005	0.0004	0.0163	0.0162	0.8018	0.7478
	damage	0.8561	0.9351	0.3085	0.3475	0.0592	0.0618	3.2262	3.2513

Table S4: *absolute number of modification per G in total RNA after 60 min of MMS exposure (acute, yellow) and after 1h of recovery (green) in different strains of* S. cerevisiae *BY4741 (n=1)*

	m1A		**m3C**		**m6A**		**m7G**	
	acute	**1h recovery**	**acute**	**1h recovery**	**acute**	**1h recovery**	**acute**	**1h recovery**
wt	0.01003	0.00802	0.00262	0.00149	0.00108	0.00065	0.01373	0.00845
Δjhd1	0.00885	0.00703	0.00264	0.00130	0.00058	0.00042	0.01184	0.00824
Δjhd2	0.00883	0.00735	0.00244	0.00137	0.00029	0.00050	0.01157	0.00860
Δtpa1	0.00905	0.00696	0.00238	0.00131	0.00959	0.00052	0.01133	0.00818
Δtrm8	0.00782	0.00673	0.00214	0.00128	0.00828	0.00048	0.00810	0.00580
Δtrm9	0.01031	0.00864	0.00266	0.00153	0.00789	0.00054	0.00053	0.15220
Δbud25	0.01047	0.00648	0.00245	0.00121	0.00104	0.00063	0.01235	0.00881
Δcmr1	0.01150	0.00731	0.00268	0.00130	0.00101	0.00061	0.01515	0.00775
Δecm5	0.01123	0.00728	0.00273	0.00136	0.00112	0.00066	0.01588	0.00695
Δgis1	0.01502	0.00636	0.00331	0.00119	0.00136	0.00065	0.01745	0.00726
Δmag1	0.00591	0.00330	0.00191	0.00075	0.00060	0.00033	0.01543	0.00594
Δmet18	0.00493	0.00740	0.00165	0.00140	0.00041	0.00062	0.01613	0.00846
Δmgt1	0.00622	0.00306	0.00206	0.00071	0.00058	0.00026	0.01679	0.00641
Δpsy3	0.00460	0.00285	0.00152	0.00061	0.00040	0.00029	0.01372	0.00532
Δrph1	0.00559	0.00315	0.00185	0.00073	0.00049	0.00027	0.01372	0.00532