Supplementary Table S1. Padlock probes for m^5C detection in *E. coli*

Name	Sequence (5' → 3')
16S m^5C1407	Phos-ACAAACAATATATACAAAACTTTCGACACGACACGATTTGGAACTCTGCTCGACGGATTAAA TAATACAGTCTGCCCACAACCTTTAACCCACTCCCATAATAT
16S m^5C967	Phos-CATCAAATTAAACCACATTTTCGACACGACACGATTTGGAACTCTGCTCGACGGATTAAA TAATACAGTCTGCCCACAACCTTTCAAATAAAATTCTTCACATT
23S m^5C1962	Phos-ATCAAAACTTACCCAACATTTCGACACGACACGATTTGGAACTCTGCTCGACGGATTAAA TAATACAGTCTGCCCACAACCTTTATCATTACACCATTCATACA
m^5C tRNA TyrQUA II	Phos- TAACAACAAATTTACAATCTTTTCGACACGACACGATTTGGAACTCTGCTCGACGGATTAAA TAATACAGTCTGCCCACAACCTTT ATTCAAACCTTCAAAATCT

**Color code indicates primer binding regions within the padlock backbone sequence. Corresponding primers sequence are shown using the same color scheme.*

***Note: 5'-arm - F1c - F2c - SHSc - 3'-arm*

Supplementary Table S2. Primers for RCA and LAMP

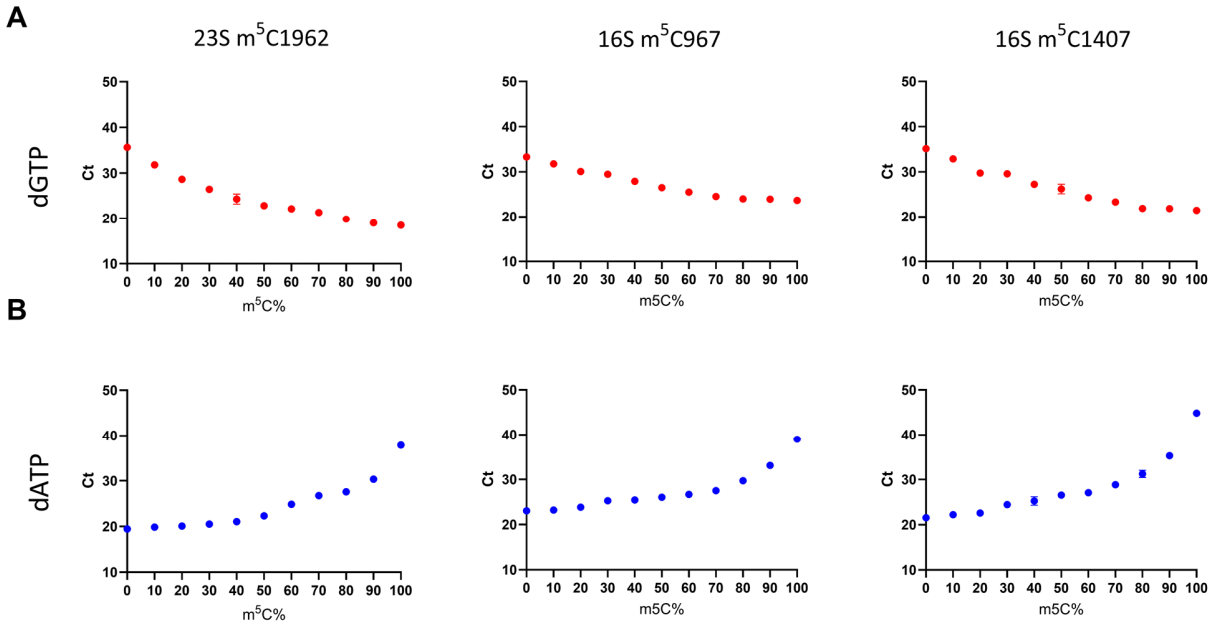
Name	Sequence (5' → 3')
SLP	ATCGTCGTGACTGTTTCCCTAACCCTAACCCTAACCCTTTCAGTCACGACGATTTTTAATACAGTCTGCCCACAACC
FIP	CGACACGACACGAAAAATCCGTCGAGCAGAGTTCC
BIP	ATCGTCGTGACTGTTTCCCTAACCCTAACCCTAACCC

Supplementary Table S3. RNA oligos

Name	Sequence (5' → 3')
23S m^5C1962	CCUUGUCGGGUAAGUUCCGACm^5CUGCACGAAUGGCGUAAUGAU
23S control	CCUUGUCGGGUAAGUUCCGACCUGCACGAAUGGCGUAAUGAU
16S m^5C967	GCAUGUGGUUUAAUUCGAUGm^5CAACGCGAAGAACCUUACC
16S control 967	GCAUGUGGUUUAAUUCGAUGCAACGCGAAGAACCUUACC
16S m^5C1407	GCCUUGUACACACCGCCCGUm^5CACACCAUGGGAGUGGGUUGC
16S control 1407	GCCUUGUACACACCGCCCGUCACACCAUGGGAGUGGGUUGC

Supplementary Table S4. Primers for mutant construction

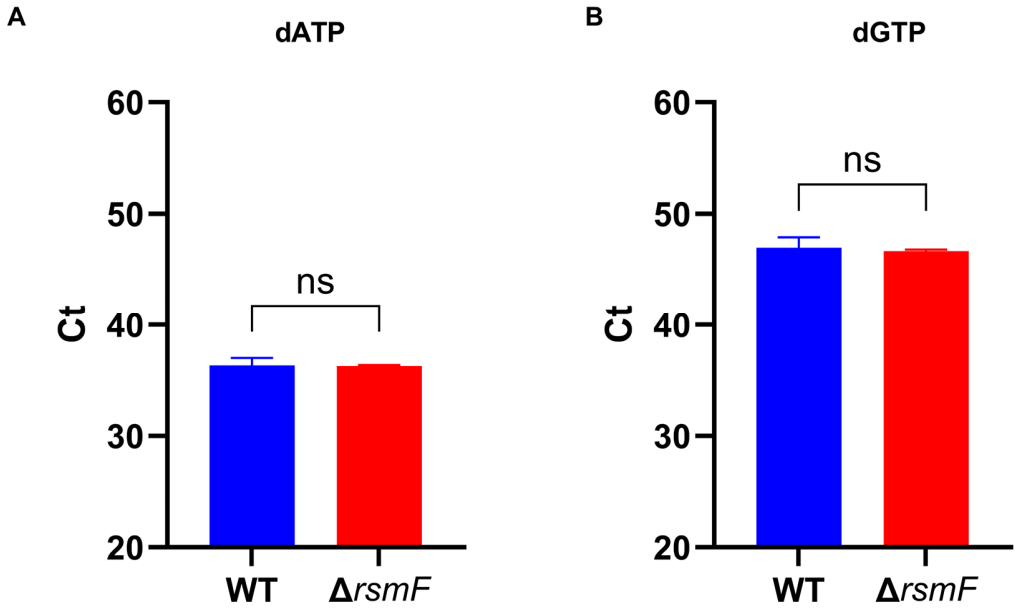
Name	Sequence (5′ → 3′)
DEL_rsmB_for_f1	GGCGCCAAGCTTCTCTGCAGGATCTGCCCGATTACTGCAGAAG
DEL_rsmB_rev_f1	CGGCATCAGCGGTACGGCAAGACCGGGCTTAGAAG
DEL_rsmB_for_f2	CCCGGTCTTGCCGTACCGCTGATGCCGAACTTTG
DEL_rsmB_rev_f2	GCTAGCGAATTCGTGGATCCAGATTGCTCTGGTGCGATCAGATG
DEL_rsmB_chk_for	GCGGGTGATGCAGAAACTGG
DEL_rsmB_chk_rev	GAAGTTCACCACCTGCAATG
DEL_rsmF_for_f1	GGCGCCAAGCTTCTCTGCAGGATAGGCCACCATTACTGATTCG
DEL_rsmF_rev_f1	TCACGCGGATAGTTATTGGGCCACGAGCATGTACC
DEL_rsmF_for_f2	CTCGTGGCCCAATAACTATCCGCGTGAACTGGTGC
DEL_rsmF_rev_f2	GCTAGCGAATTCGTGGATCCAGATTGTGGTGCCGTACCGTTAAG
DEL_rsmF_chk_for	CAATCCTCGCCGCGACTTTG
DEL_rsmF_chk_rev	CACAACCGCCATCCAGGTAG
DEL_rlmI_for_f1	GGCGCCAAGCTTCTCTGCAGGATTTCTCCGGCGATTCGTATGC
DEL_rlmI_rev_f1	CCCTTCCGGATAGAGAAATGGGCGTCATTGTCC
DEL_rlmI_for_f2	GACGCCCATTTCTCTATCCGGAAGGGCTATATC
DEL_rlmI_rev_f2	GCTAGCGAATTCGTGGATCCAGATCTGCCCGGCTATTGAGATCC



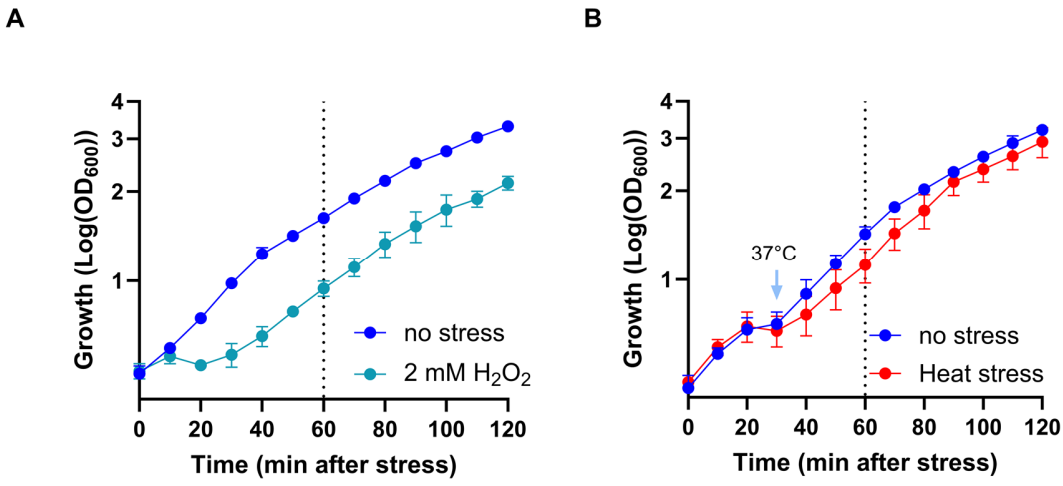
Supplementary Figure S1. Dynamic range determination of m⁵C-Rol-LAMP using synthetic RNAs with defined m⁵C content (0–100%). Synthetic RNA oligonucleotides containing defined fractions of methylated cytosine (0–100% m⁵C) at target sites were analyzed using m⁵C-Rol-LAMP with either dATP (detecting unmodified C→U after bisulfite conversion) or dGTP (detecting retained m⁵C). For each condition, triplicate reactions were performed. Ct values are plotted as a function of % m⁵C for: (A) 23S rRNA m⁵C1962, (B) 16S rRNA m⁵C967, and (C) 16S rRNA m⁵C1407. dATP reactions exhibited larger Ct shifts at high methylation level (>60%), while dGTP reactions were more sensitive at low methylation level (<40%). Data are mean ± SD (see Supplementary Table S1 for Ct values).

Supplementary Table S5. Ct values from dynamic range experiments using synthetic RNAs with defined m⁵C content (0–100%). Triplicate reactions were performed for the m⁵C methylation percentages shown. Values are reported as mean ± SD.

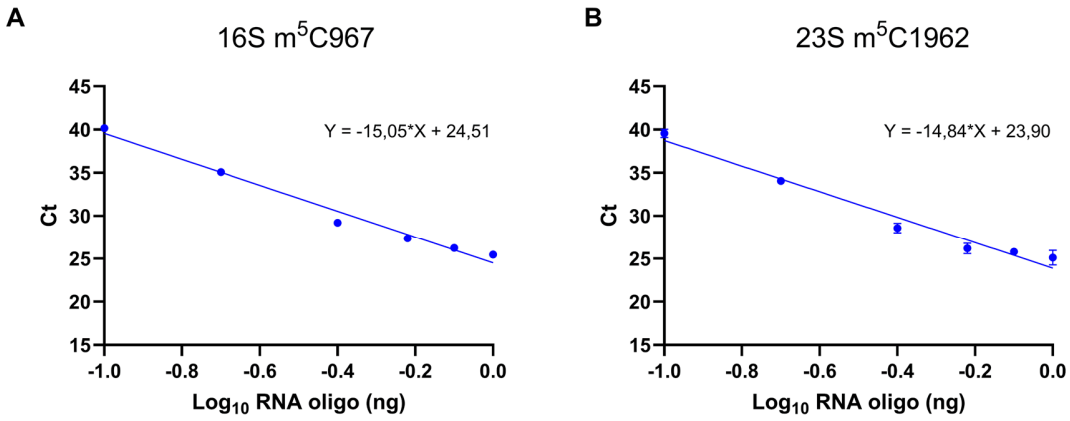
m⁵C%	23S m⁵C1962 dATP	23S m⁵C1962 dGTP	16S m⁵C967 dATP	16S m⁵C967 dGTP	16S m⁵C1407 dATP	16S m⁵C1407 dGTP
0	19.46 ± 0.58	35.65 ± 0.65	23.06 ± 0.41	33.32 ± 0.23	21.55 ± 0.53	35.16 ± 0.29
10	19.85 ± 0.43	31.83 ± 0.67	23.23 ± 0.31	31.77 ± 0.39	22.20 ± 0.78	32.89 ± 0.18
20	20.09 ± 0.38	28.63 ± 0.37	23.84 ± 0.14	30.10 ± 0.24	22.59 ± 0.16	29.75 ± 0.45
30	20.53 ± 0.30	26.44 ± 0.29	25.28 ± 0.05	29.48 ± 0.44	24.46 ± 0.20	29.59 ± 0.32
40	21.06 ± 0.07	24.32 ± 1.12	25.44 ± 0.16	27.90 ± 0.61	25.25 ± 0.94	27.91 ± 0.39
50	22.31 ± 0.16	22.85 ± 0.27	26.05 ± 0.15	26.50 ± 0.36	26.54 ± 0.34	26.88 ± 1.06
60	24.86 ± 0.20	22.14 ± 0.23	26.68 ± 0.15	25.51 ± 0.42	27.10 ± 0.28	24.29 ± 0.72
70	26.74 ± 0.44	21.35 ± 0.34	27.51 ± 0.23	24.54 ± 0.17	28.85 ± 0.57	23.32 ± 0.31
80	27.57 ± 0.13	19.86 ± 0.43	29.72 ± 0.40	24.01 ± 0.09	31.26 ± 0.81	21.87 ± 0.58
90	30.34 ± 0.18	18.98 ± 0.43	33.11 ± 0.62	23.94 ± 0.32	35.28 ± 0.21	21.84 ± 0.57
100	37.85 ± 0.39	18.50 ± 0.53	38.98 ± 0.34	23.69 ± 0.45	44.86 ± 0.59	21.46 ± 0.41



Supplementary Figure S2. Amplification of tRNA^{Tyr} by m^{5}C-Rol-LAMP. WT and Δ*rsmF* mutant cells were subjected to oxidative stress (20 mM H_2O_2 + 10 µM $FeCl_2$, 30 min) to induce ROS via the Fenton reaction. A) dATP reactions. B) dGTP reactions. Shown are the mean values of three biological replicates. Error bars represent standard deviations of the mean of three replicates. n.s. not significant by unpaired two-tailed t-test.



Supplementary Figure S3. Growth of *E. coli* under stress and recovery conditions. A) Cultures were treated with 2 mM H_2O_2 at time zero. B) Cultures were subjected to heat shock (45 °C, followed by return to 37 °C at 30 min). OD_{600} was monitored over time; Error bars represent standard deviations of the mean of three replicates. The dotted vertical line at 60 min marks the time point used as the recovery reference in subsequent assays.



Supplementary Figure S4. Calibration curves for quantification at 16S rRNA m^{5}C967 and 23S rRNA m^{5}C1962. A calibration curve was generated using m^{5}C-Rol-LAMP by amplifying increasing amounts of a synthetic 41-nucleotide RNA oligo representing the sequence around position C967 of *E. coli* 16S rRNA and C1962 of *E. coli* 23S rRNA with a methylated cytosine. Reactions were performed using dATP+dGTP to achieve maximum amplification of all correctly hybridized padlock probes. Ct values were plotted against the logarithmic values of the RNA inputs [RNA oligo (ng)]. Each data point represents the average of three independent replicates and error bars indicate the standard deviation of the mean.