# Supplementary Information

## Oxidative stress damages rRNA inside the ribosome and differentially affects the catalytic center

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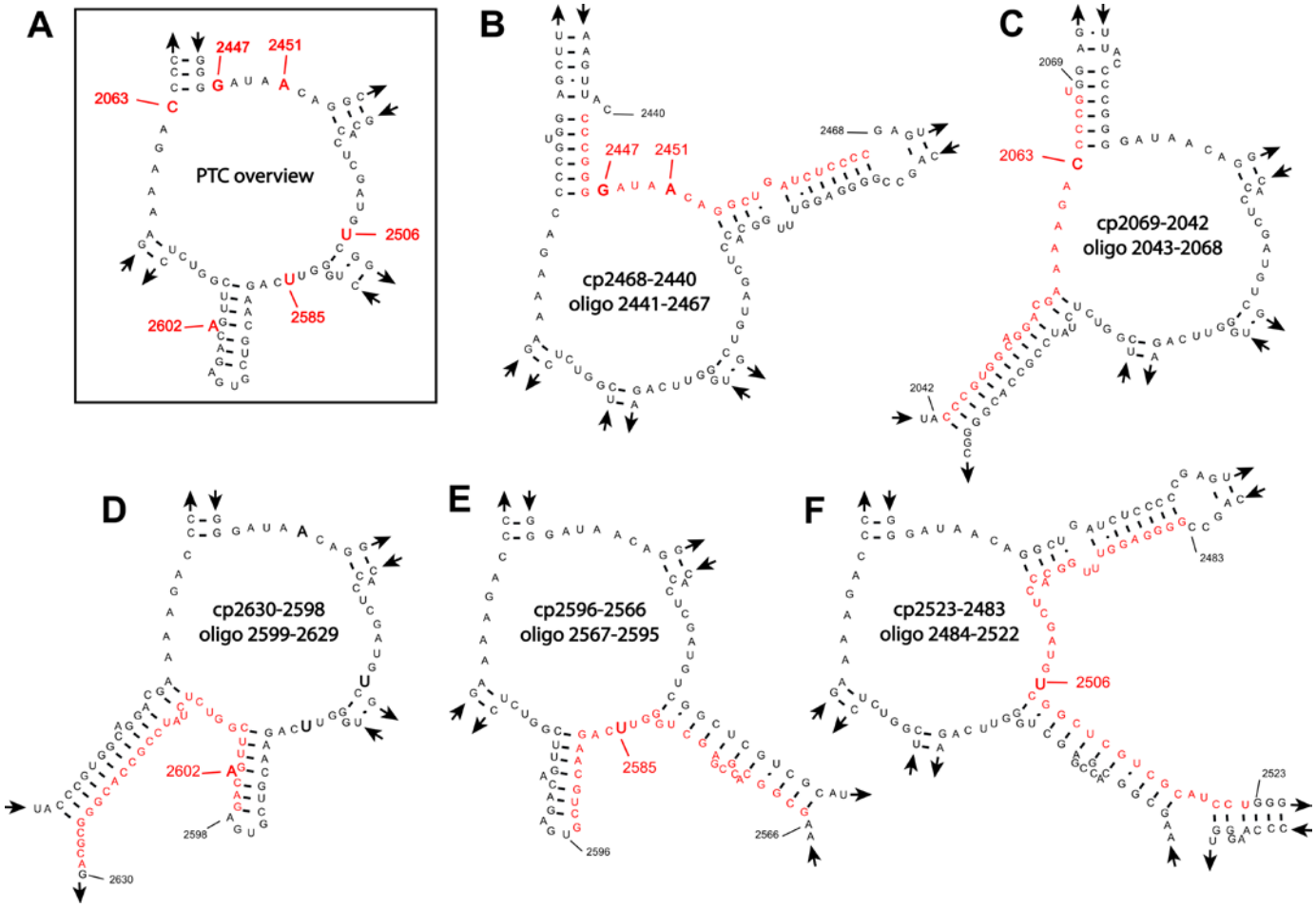
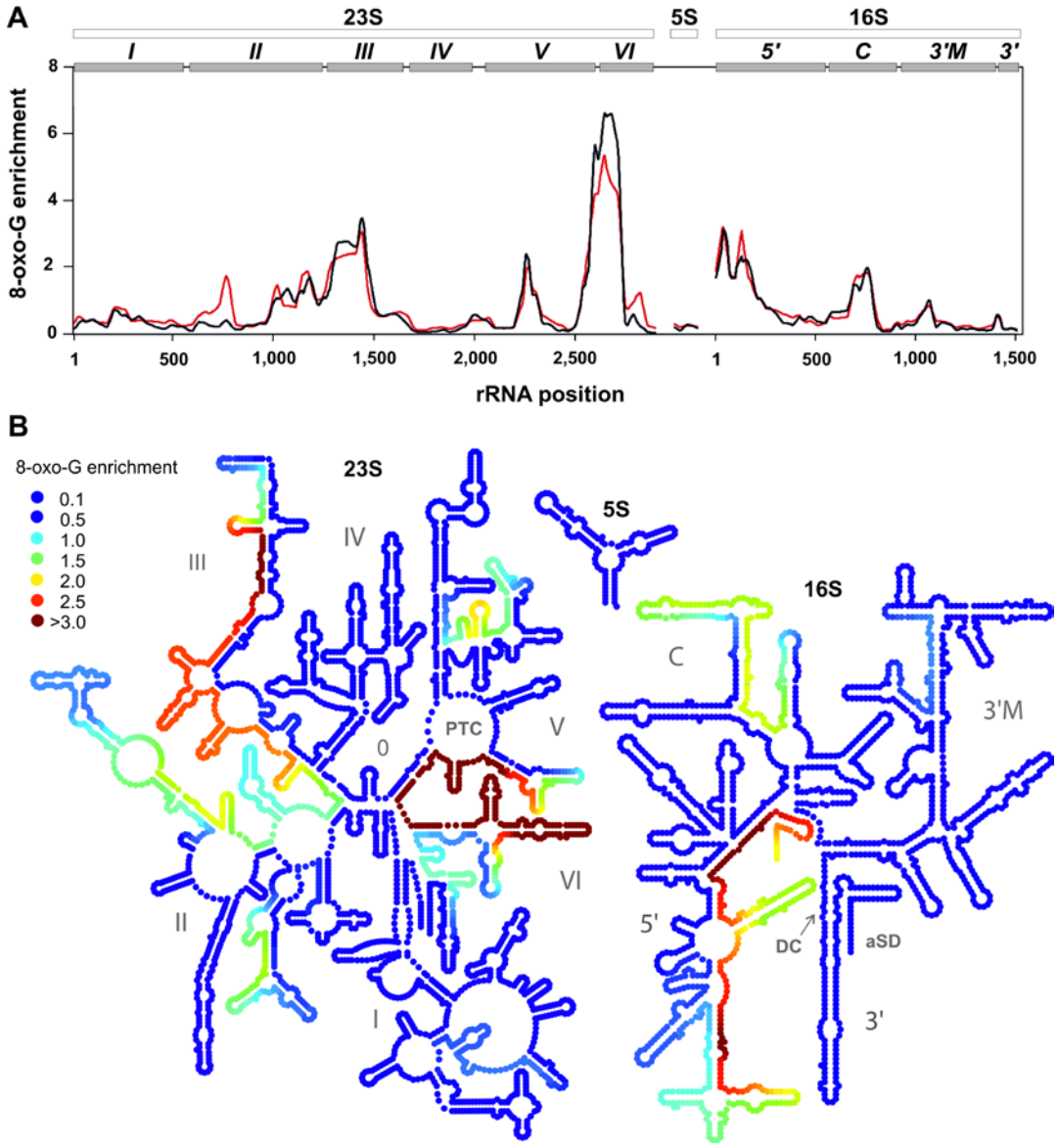
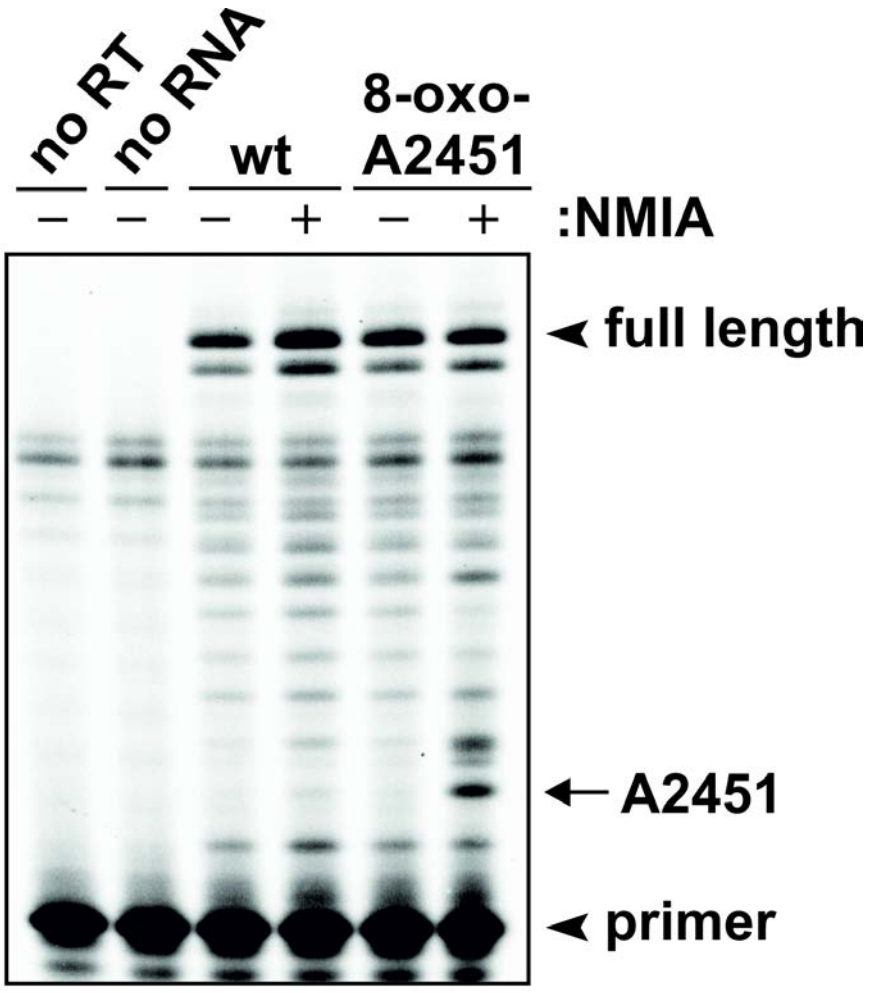



**Supplementary Figure S1. Structures of cp-23S rRNA constructs used in this study.** (**A**) The central loop of domain V of native 23S, which harbors the PTC. The most central PTC residues and the second tier nucleoside G2447 are marked in red. (**B-F**) In order to form cp-23S rRNA for atomic mutagenesis, 23S rRNA is circularly permuted so that the natural ends are covalently connected and new ends (5', 3') form a gap in the PTC region. This missing 23S segment is complemented by addition of a short synthetic RNA fragment (red), which contains one oxidized RNA nucleobase in the desired position. In order to address different PTC positions, different sets of circular permutation constructs and complementing RNA fragments were used (also see Supplementary Table S1). The constructs used in this study are (**B**) cp2468-2440 for manipulating either A2451 or G2447, (**C**) cp2069-2042 for manipulating C2063, (**D**) cp2630-2598 for manipulating A2602, (**E**) cp2596-2566 for manipulating U2585, and (**F**) cp2523-2483 for manipulating U2506. The new 5' and 3' ends of the cp-23S define the name of every cp-construct and are indicated (black). The corresponding synthetic RNA fragments (red) contain the position of interest (bold).




**Supplementary Figure S2.** (**A**) *In vivo* oxidation of 23S, 5S and 16S rRNA, measured by enrichment in anti-8-oxo-G IP-Seq. Total RNA of *E. coli* before (black) and after oxidative stress (red) was extracted. Both fractions were fragmented and subsequently enriched by immunoprecipitation with an antibody against 8-oxo-G. After sequencing, mapping of reads to the *E. coli* rRNA operon A and normalization, the sequence read enrichment was calculated by dividing IP-enriched reads by total input fragment reads. The 23S, 5S and 16S rRNA sequences are depicted as white bars, their domains (23S domains I-VI, and 16S domains 5', C, 3'M and 3' respectively) are indicated as grey bars. **(B)** *In vivo* oxidation of 23S, 5S and 16S rRNA under oxidative stress conditions (data from (A), red line) mapped onto the secondary structure of *E. coli* 23S, 5S and 16S rRNA. The heat map depicts regions enriched with the anti-8-oxo-G antibody on a rainbow scale from low (blue) to high oxidation (red). The domains (as in A), the central loop of 23S domain V forming the PTC and the 16S 3'-end containing the anti-Shine-Dalgarno (aSD) region and the decoding center (DC) are indicated.




**Supplementary Figure S3. SHAPE probing of the 8-oxo-A2451 oribonucleoside in solution.** The synthetic 26-mer oligoribonucleoside carrying the wt sequence or 8-oxo-A at a position corresponding to A2451 in the 23S rRNA was treated with NMIA. The reactivities of the ribose 2'-OH groups were assessed by primer extension analysis. The location of the radiolabeled primer, the full length reverse transcription product and the position corresponding to A2451 of 23S rRNA in the denaturing polyacrylamide gel are indicated by arrow heads and an arrow, respectively. Note that the NMIA reaction product halts reverse transcription one position 3' of the reacted site. Primer extension reactions in the absence of reverse transcriptase (no RT) or without RNA template (no RNA) served as negative controls.

## Supplementary Table S1:

## Synthetic RNA oligonucleotides

| Name | Sequence (5' → 3') | Position and type of modification | Mass m/z calculated measured |
|---|---|---|---|
| 2451_A<br>2447_G | CCCGGG**G**AUA**A**CAGGCUGAUCUCCCC | wild type<br>(2451 and 2447) | |
| 2451_8-oxo-A | CCCGGGGAUA-**8-oxo-A**-CAGGCUGAUCUCCCC | 8-oxo-Adenin<br>position 2451 | 8,293.04<br>8,293.00 |
| 2447_8-oxo-G | CCCGGG-**8-oxo-G**-AUAACAGGCUGAUCUCCCC | 8-oxo-Guanin<br>position 2447 | 8289.15<br>8288.20 |
| 2063_C | CCCGUGGCAGGACGAAAAGA**C**CCCGU | wild type | |
| 2063_5-HO-C | CCCGUGGCAGGACGAAAAGA-**5-HO-C**-CCCGU | 5-HO-Cytosin<br>position 2063 | 8,379.09<br>8,378.45 |
| 2585_U | GCGGCACGCGAGCUGGGU**U**CAGAACGUCG | wild type | |
| 2585_5-HO-U | GCGGCACGCGAGCUGGGU-**5-HO-U**-CAGAACGUCG | 5-HO-Uracil<br>position 2585 | 9,408.65<br>9,407.82 |
| 2506_U | GGGGAGGUUUGGCACCUCGAUG**U**CGGCUCGUCGCAUCCU | wild type | |
| 2506_5'end | GGGGAGGUUUGGCACCU | wild type | |
| 2506_3'end | pCGAUG-**5-OH-U**-CGGCUCGUCGCAUCCU | 5-HO-Uracil<br>position 2506 | 7,042.21<br>7,042.50 |
| 2506_5-HO-U | GGGGAGGUUUGGCACCUCGAUG-**5-OHU**-CGGCUCGUCGCAUCCU | 5-HO-Uracil<br>position 2506 | |
| 2602_A | GAC**A**GUUCGGUCUCUAUCCGCCACGGGCGCA | wild type | |
| 2602_8-oxo-A | GAC**-8-oxo-A-**GUUCGGUCUCUAUCCGCCACGGGCGCA | 8-oxo-Adenin<br>position 2602 | 9,901.05<br>9,902.00 |

## Supplementary Table S2:

## RNAs and DNA primers used for assays

| Name | Sequence (5' → 3') | Source |
|---|---|---|
| AUG-Stop | UUCAUGUAA | Dharmacon Research, Inc |
| 8c.s. mRNA[1] | GGCAAAGGAGGUAUUAUUAAUGUUCAAACGAUCAAUCUACGUAUAAU AAAAGAAAAGAAAAGAAAAGAAAAGAAAAGGACAUCACACAUUAACG | T7 *in vitro* transcribed |
| 8 c. s. rev | CGTTAATCTGTGATG | Promega |
| Taq2451_rev | GGGGAGATCAGC | Promega |
| DNA splint[2] | AAACCGTGGAGCTACAGCCG | Promega |

[1] short ORF underlined [2] for splint ligation of RNAs 2506_5'end and 2506_3'end

## Supplementary Table S3:

## PCR primers used to generate various cp-23S rRNA constructs

| Name | Sequence (5' → 3') | Construct (position of modification) |
|---|---|---|
| extT7-2069 | GGATCCTAATACGACTCACTATAGGAGCTTTACTGCAGCCTG | **cp2069-2042** (2062) |
| Taq2024 | TAGGCCGCATCTTCACGG | |
| extT7-2596 | GGATCCTAATACGACTCACTATAGGTGAGACAGTTCGGTCTCTATCCG | **cp2596-2566** (2585) |
| Taq2542 | TTTAATGGGCGAACAGCCCAACCC | |
| extT7-2523 | GGATCCTAATACGACTCACTATAGGGGCTGAAGAAGGTCCC | **cp2523-2483** (2506) |
| Taq2465 | GCCGCTGTGGACGCTCGG | |
| extT7-2630 | GGATCCTAATACGACTCACTATAGGAGGCTTGAGGGGGGCT | **cp2630-2598** (2602) |
| Taq2581 | TCACGACGTTCTGAACCC | |

T7 RNA polymerase promotor sequence is underlined. Cp-23S rRNA constructs are named the following way: first number marks the 5'-end of the cp-23S rRNA and the second number indicates the 3'-end (Supplementary Figure 1). The construct for modification of A2451 and G2447 (cp2468-2440) with T7 promoter was directly cloned into pUC19. After opening with Eco105L, this plasmid can be directly used for *in vitro* transcription.