SUPPORTING INFORMATION

Platinum-RNA modifications following drug treatment in S. cerevisiae identified by click chemistry and enzymatic mapping

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Table S1. Estimation of Pt accumulation in different RNAs based on click fluorescent labeling following treatment with 250 μM picazoplatin (5 μg reaction). The in-gel detection limit of RNA-Pt-clicked Alexa Fluor 488 alkyne (in 10% 29:1 mono/bis polyacrylamide) is approximately 1 pmol and we estimate that we can visualize concentrations as low as 1 Pt per ~10,000 nt in these RNA species. Mol (nt) RNA determined assuming 80% rRNA and 15% tRNA in a total RNA preparation from growing yeast (51) using an average nucleotide molecular weight of 340 g/nt.

RNA (μg)	Mol (nt) RNA	Total nt	mol RNAs	mol AF488*	Pt/RNA
rRNA 4.0 μg	1.2e-8	~6000/ribosome	2.0e-12	0.94e-12	~0.5
tRNA 0.75 μg	2.2e-9	~100/tRNA	2.2e-11	0.80e-12	~0.4

*Mol of Alexa Fluor 488 in bands corresponding to rRNA (sum of all bands) or tRNA (single band), based on comparison with calibration gel. Each Alexa Fluor 488 corresponds to one Pt atom, assuming 100% click efficiency.

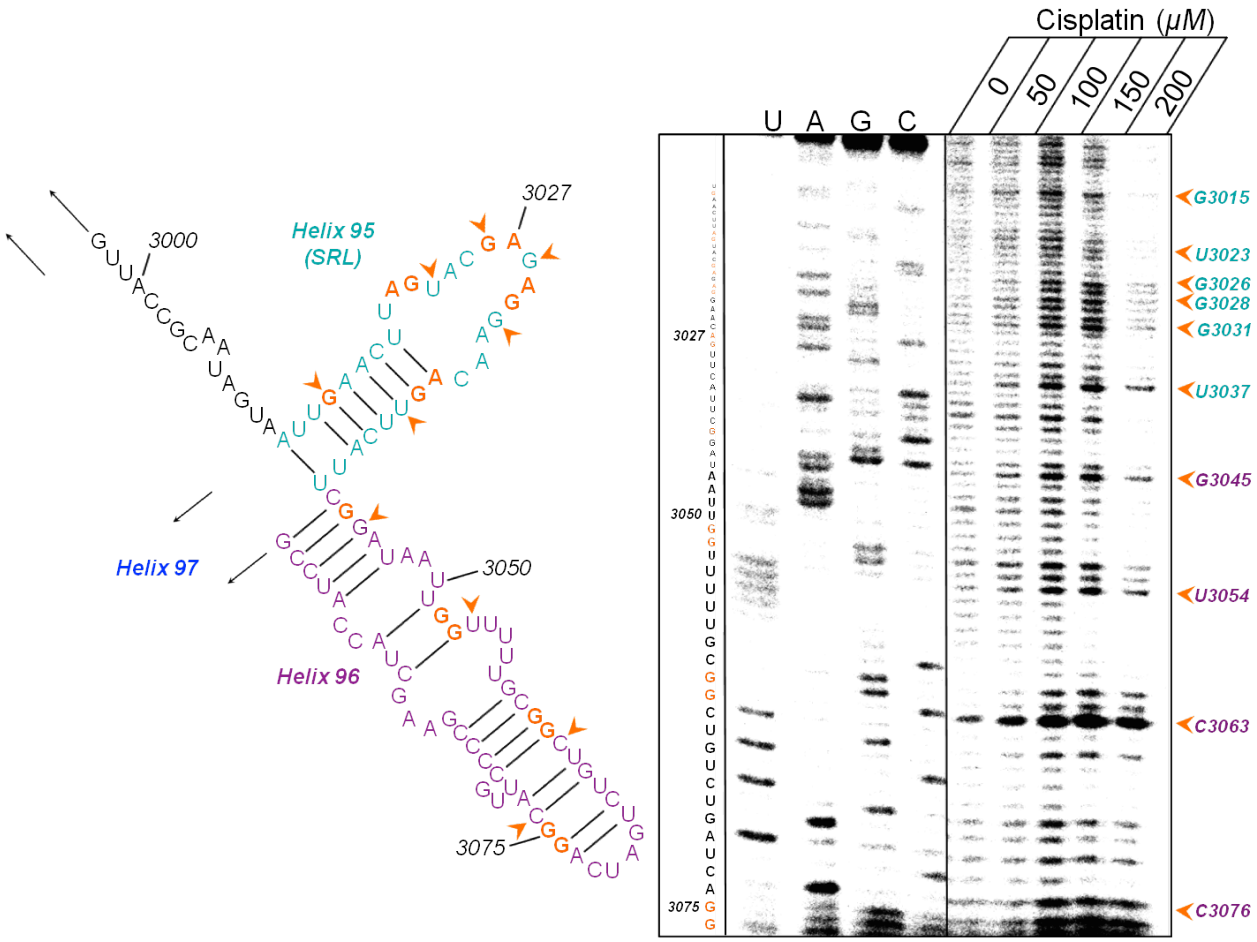

Figure S1. Full sequence analyzed by primer extension analysis of the *S. cerevisiae* sarcin ricin loop and adjacent helices from ribosomal RNA treated with 0-200 μM cisplatin. Dideoxy sequencing ladder labeled by A, U, C, and G. Cisplatin-induced stop sites are denoted by orange arrowheads and summarized in the secondary structure with orange lettering. Nucleotides which were not analyzed in this particular experiment are in black lettering in the secondary structure.

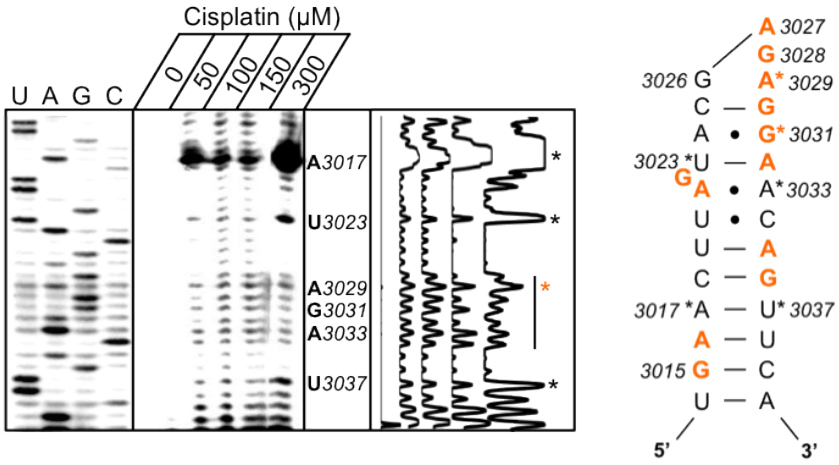

Figure S2. Primer extension analysis of the sarcin-ricin loop (SRL) region in ribosomal RNA extracted from *S. cerevisiae* treated with 0-300 μM cisplatin. This experiment used SRL primer B, a primer designed to probe further upstream than the one in Figure 3. Dideoxy sequencing ladder labeled by A, U, C, and G. Cisplatin-induced stop sites are denoted by asterisks (*) and represent nucleotides 3' to a stable platinum adduct. Orange asterisk adjacent to the line plot analysis denotes the primary stop site in the terminal SRL region (A3029). Predicted platinum crosslinks between adjacent purines based on experimental results are colored orange and in bold on the *S. cerevisiae* secondary structure map.

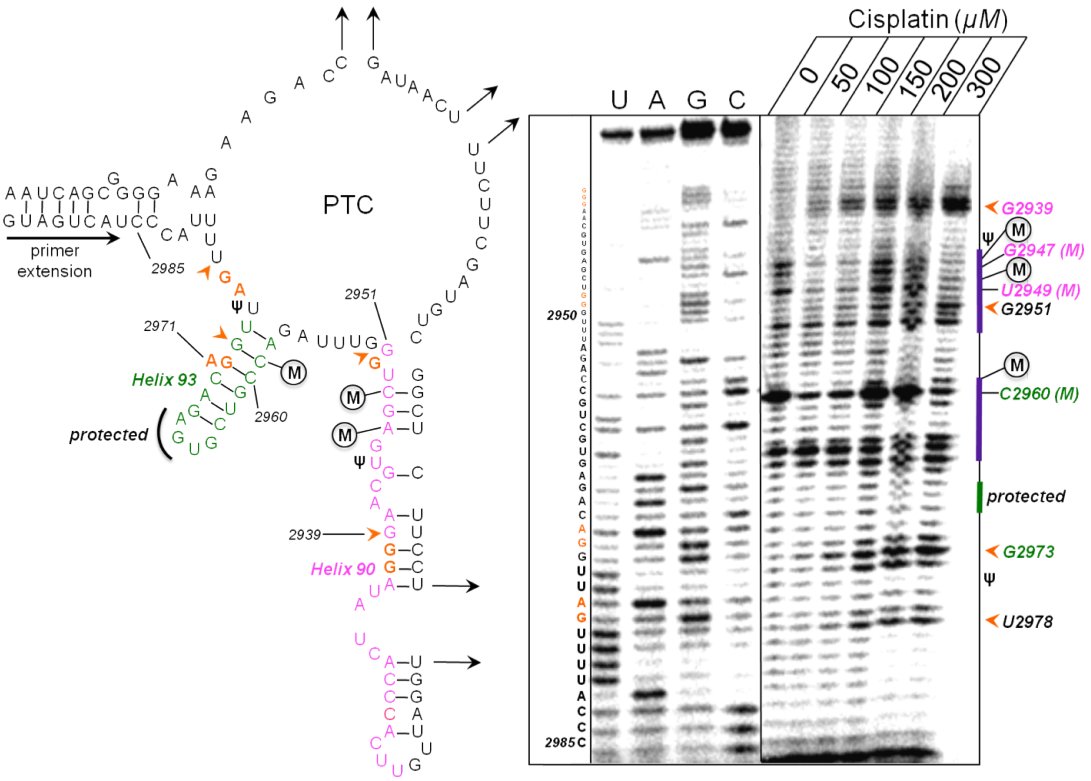

Figure S3. Primer extension analysis of the *S. cerevisiae* peptidyl transferase center from ribosomal RNA treated with 0-300 μM cisplatin. Cisplatin-induced stop sites are denoted by orange arrowheads and summarized in the secondary strucutre with orange lettering. Endogenous ribosomal RNA modifications are specifically denoted. 2'-OCH_3 modifications are marked with circled M's or by (M) at the nucleotide position corresponding to the stop site 3' to the modification. Pseudouridine substitutions are labeled with "ψ". Regions of stable structure that nonspecifically inhibit primer extension in the absence of platinum are denoted on the sequencing gel by vertical purple lines. Nucleotides protected from Pt binding are marked on the sequencing gel by a vertical green line. Nucleotides which were not analyzed in this particular experiment in black lettering on the secondary structure.