SUPPORTING INFORMATION

Characterization of RNA-Pt Adducts Formed from Cisplatin Treatment of *Saccharomyces cerevisiae*

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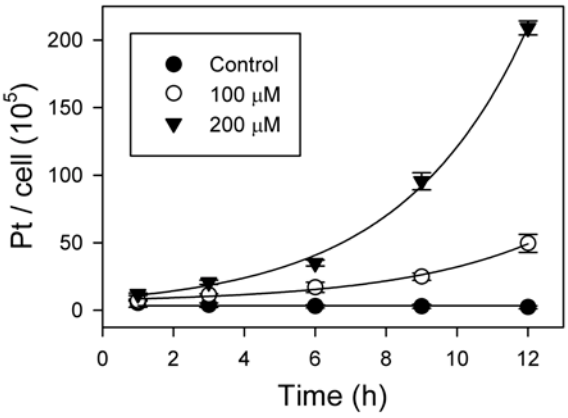

Figure S1. Accumulation of Pt atoms per yeast cell in yeast treated with cisplatin for 1, 3, 6, 9, and 12 h. Results averaged from four independent experiments, presented as the means ± SD.

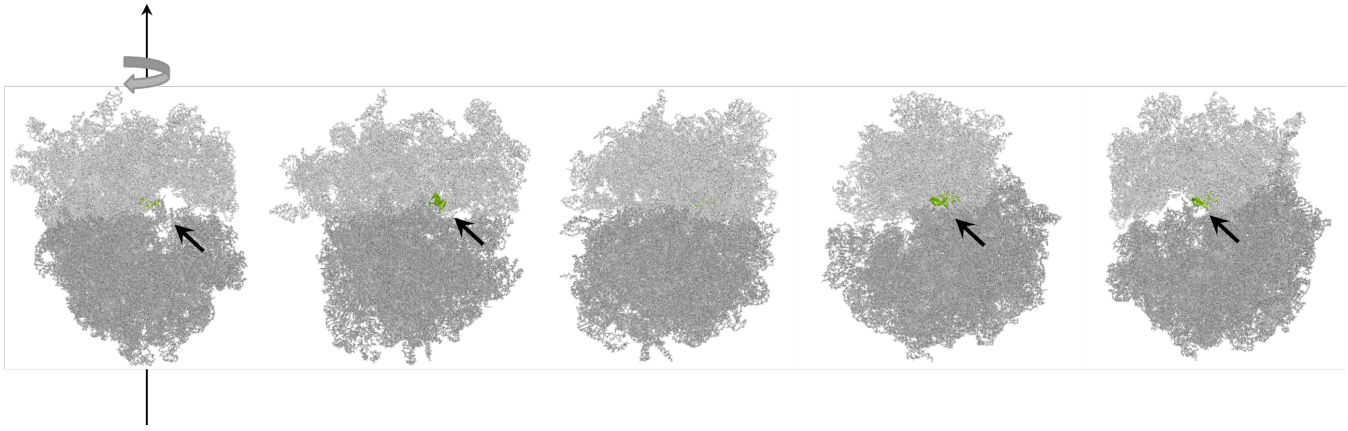

Figure S2. Helix 18 of the yeast ribosome (green) is located in close proximity to the peptidyltransferase center within the small ribosomal subunit (light gray). Image created with PyMol from PDB files 3O30 and 3O5H (Ben-Shem, A., Jenner, L., Yusupova, G., Yusupov, M. (2010) Crystal structure of the eukaryotic ribosome. *Science 330*, 1203-1209).

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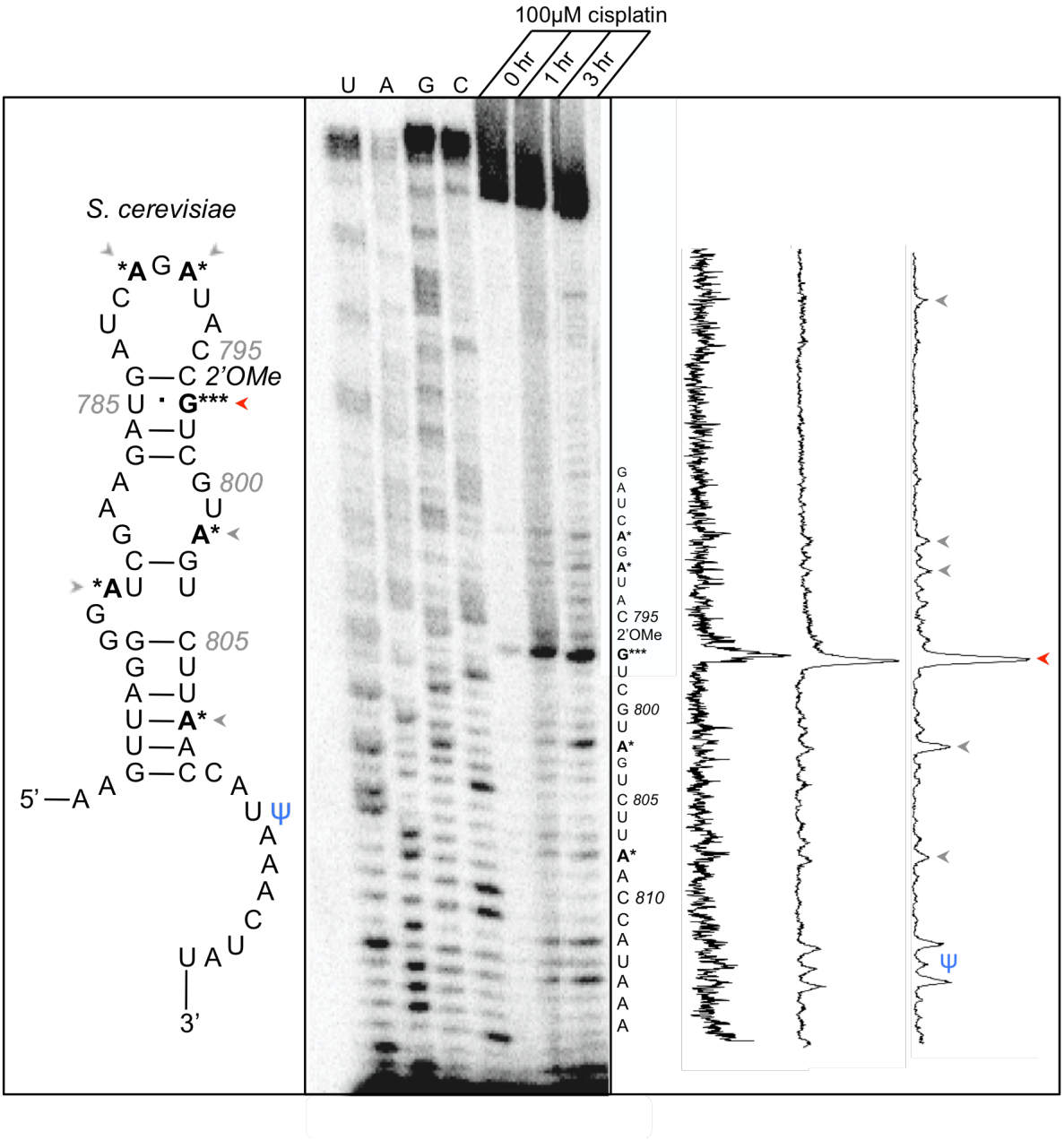

Figure S3. Primer extension analysis of RNA isolated from 100 μM cisplatin-treated BY4741 at 0, 1, and 3 h shows a time-dependent accumulation of Pt^{2+} at the indicated purine residues (red arrowheads) beginning as early as 1 h following treatment. Specific platination sites are conserved after 6 h continuous exposure to drug (Figure 5).

Full TUNEL protocol. Yeast cells (3.0×10^7) were pelleted, washed 3x with PBS, fixed with 3.7% (v/v) formaldehyde for 2 h at 24 °C, and then digested with 2.5 U zymolyase 100T (US biological) for 30 min at 30 °C in 1 mL sorbitol buffer (1.2 M sorbitiol, 0.5 mM $MgCl_2$, 35 mM phosphate buffer pH 6.8). A portion of the sample was applied to a polylysine-coated slide and let dry for 1 h at 37 °C. The slides were incubated with freshly prepared permiabilization solution (0.1% Triton X-100, 0.1 sodium citrate) for 1 min at 4 °C, then rinsed 3x with PBS. Positive control samples were incubated with 0.2, 1, and 5 U DNase I (Fermentas) for 1 h at 37 °C in the rxn buffer provided by the manufacturer. Samples were incubated with 10 μM TUNEL reaction mixture (in situ cell death detection kit, fluorescein, Roche) for 30 min at 37 °C in the dark, rinsed 3x with PBS.

Table S1. Pt atoms accumulated in yeast RNA or DNA

	[Cisplatin]	
	100 μM	**200 μM**
mRNA (Pt/nt, 6 h)	$(1.05 \pm 0.58) \times 10^{-5}$	$(3.53 \pm 0.40) \times 10^{-5}$
rRNA (Pt/nt, 6 h)[a]	$(5.77 \pm 0.96) \times 10^{-5}$	$(12.5 \pm 0.91) \times 10^{-5}$
Total RNA (Pt/nt, 6 h)[a]	$(6.97 \pm 0.69) \times 10^{-5}$	$(18.2 \pm 1.74) \times 10^{-5}$
Total RNA (Pt/nt, 12 h)	$(15.7 \pm 1.59) \times 10^{-5}$	$(56.6 \pm 6.46) \times 10^{-5}$
DNA (Pt/nt, 12 h)	$(60.2 \pm 8.22) \times 10^{-5}$	$(199 \pm 30.2) \times 10^{-5}$
Pt atoms on DNA from one yeast cell (10^4)[b]	2	6
Pt atoms on RNA from one yeast cell (10^4)[b]	7–34	24–120

[a] There is a small but statistically significant increase in Pt/nt values in total RNA vs. rRNA at 200 μM cisplatin treatment, but not at 100 μM cisplatin treatment. A major difference between these two cell populations is the number of viable, dividing cells (60% at 100 uM and only 30% at 200 uM cisplatin treatment, manuscript Table 1). It is possible that the cells in 200 uM cisplatin have begun processing the most heavily platinated rRNA, depleting it from the 'full-length' population.

[b] At 12 h, calculation based on the mass of DNA and RNA in one haploid *S. cerevisiae* cell (*60*)