**Supplemental data:** One table and six Supplemental Figures are included.

**Table S1. Sequences of oligonucleotides used in this study**

| Probe | Sequence |
|---|---|
| y540 (25S rRNA) | 5'-TCCTACCTGATTTGAGGTCAAAC |
| y521 (25S rRNA) | 5'-ATGGATTTATCCTGCCACCA |
| y503 (25S rRNA) | 5'-ACCCACGTCCAACTGCTGT |
| y530 (25S rRNA) | 5'-ACATCACTTTCTGACCATCGC |
| y537 (25S rRNA) | 5'-AGCAGATCGTAACAACAAGGC |
| y531 (18S rRNA) | 5'-ACTGGCAGGATCAACCAGAT |
| y500 (18S rRNA) | 5'-AGAATTTCACCTCTGACAATTG |
| y545 (18S rRNA) | 5'-TCTCAATCTGTCAATCCTTATTGTG |
| y532 (18S rRNA) | 5'-GTCCAAATTCTCCGCTCTGA |
| y534 (5.8S rRNA) | 5'-TTACGTATCGCATTTCGCTG |
| y506 (5S rRNA) | 5'-TAACTACAGTTGATCGGACGG |

**Supplemental Figure legends:**

**Figure S1. 25S and 18S RNAs' degradation requires both, $Fe^{2+}$ and ascorbic acid in the *in vitro* reaction.**

Northern blot analysis of *in vitro* cleavage of rRNAs purified from wild-type cells using probes for 25S (y540) or 18S (y532) rRNAs. Samples were treated with or without 0.5mM ascorbic acid and/or 1 μM $Fe(NH_4)_2(SO_4)_2$ or remained untreated. The sequences of all probes used in this study are listed in Table S1.

**Figure S2. Northern blot analysis of the rRNA degradation patterns using additional probes against 25S and 18S rRNAs.**

Ribosomes purified from wild-type cells were subjected to the *in vitro* $Fe^{2+}$/ascorbic acid assay. Reaction products ("*WT in vitro*") were resolved on 1.2% agarose-formaldehyde gels. Adjacent to these, we loaded RNA extracted from *grx5Δ* cells treated for 2 h with 50 μM menadione.

**A.** To analyze degradation products of 25S rRNA, we used probes y521, y503, y530, y505, y537.

**B.** For 18S rRNA, we used probes y500, y545. Where indicated, reactions were pretreated with 0.5 mM iron chelator DFO.

**C.** Schematic representation of the annealing sites of the probes used. See Supplemental Table 1 for probe sequences.

**Figure S3. ICP-MS measurements of metals in purified ribosomes.**

Ribosome-enriched fractions were extracted from wild-type (WT) and *grx5*Δ cells (see Figure 3A legend for more details) and analyzed by ICP-MS to detect levels of Fe, Mn, and Mg. The samples' ribosomal content was assessed by spectroscopic measurement of RNA concentration following the extraction, and RNA amounts were used to normalize the ICP-MS data. While Fe was consistently detected in these samples, precise quantification was not achieved, and these values are considered as estimates. (*P*-values: *, <0.05, two-tailed two-sample unequal variance *t*-test).

**Figure S4. Exogenously supplied manganese protects 18S rRNA from oxidant-induced degradation in cells.**

**A.** Mid-log *grx5*Δ cultures grown in YPDA were shifted to medium supplemented with the indicated concentrations of $MnCl_2$ and grown for 4 h at 30°C. Cultures were adjusted to the same $OD_{600}$~ 0.6 and incubated for 2 h in the presence (+) or absence (-) of 50 μM menadione. RNA was isolated and analyzed by northern hybridization with the 18S rRNA probe y531. Three biological replicates were analyzed, representative blots are shown.

**B.** Quantifications of the northern hybridization data from (A), done as detailed in Figure 6B. The $K_{RS}$ values were calculated for 18S rRNA from each culture treated or untreated with menadione and/or $MnCl_2$. The data show the mean value of three experimental replicates; error bars represent S.D. For samples treated with menadione (the set of bars on the right), the differences between $K_{RS}$ values in $MnCl_2$-free medium and in 0.5 mM, 1 mM, and 2 mM $MnCl_2$-containing medium were significant (*P*-values: **, <0.01; *, <0.05; NS, not significant). Two-tailed two-sample unequal variance *t*-test was used for statistical analysis.

**C** and **D.** A similar experiment was performed with wild-type (*WT*) cells and the data were quantified as in panels A and B, except that concentrations of menadione used were 0, 100, 200 and 500 μM. Three biological replicates were analyzed, representative blots are shown. As in (A), the data show the mean value of three independent experiments; error bars represent S.D. Restoration of full-length 18S rRNA in samples treated with 200 and 500 μM menadione in the presence of 1 mM and 2 mM $MnCl_2$ was significant (P-values: *, <0.05; NS, not significant).

**Figure S5. snoRNA U3 is stable during drug-induced oxidative stress.**

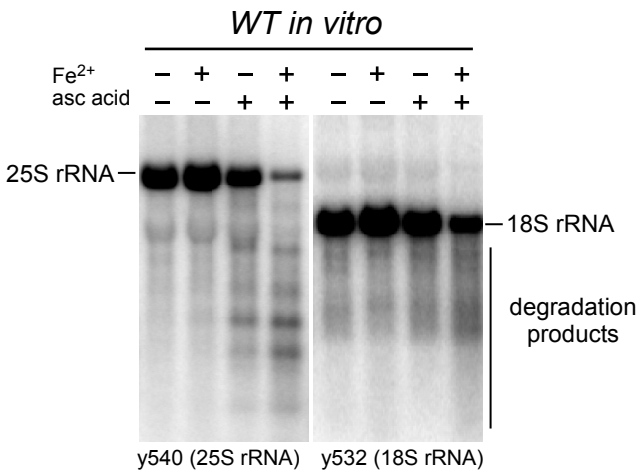
**A.** Mid-log WT cultures grown in YPD were shifted to medium supplemented with the indicated concentrations of $MnCl_2$ and grown for 4 h at 30°C. Cultures were adjusted to the $OD_{600}$~ 0.6 and incubated for 2 h in the indicated concentrations of menadione. RNA was isolated, resolved on 10% polyacrylamide, 8M urea-containing gels (as described in Figure 1C-D) and analyzed by northern hybridization with probe for snoRNA U3. Three replicates were analyzed, representative blots are shown.

**B.** Quantifications of the northern hybridization data from (A), as detailed in Figure 6B. The $K_{RS}$ values were calculated for full length RNA from each culture treated or untreated with menadione and/or $MnCl_2$. The data show the mean value of three replicates; error bars represent S.D. Two-tailed two-sample unequal variance t-test was used for statistical analysis. Difference between values was not statistically significant.

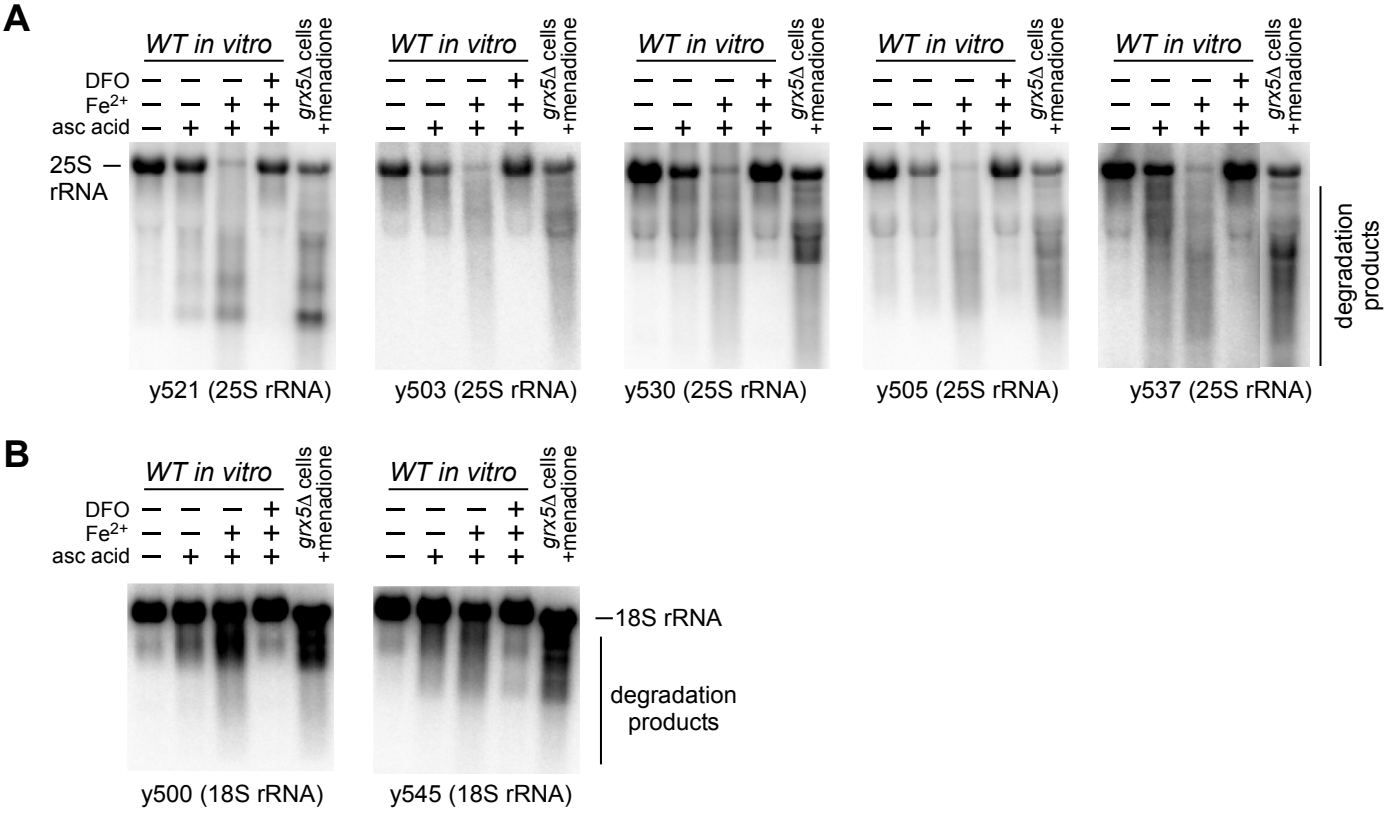
**Figure S6. Manganese and iron chelator PHL improve cell viability to a similar extent.**

Overnight wild-type (*WT*) and *grx5*Δ cultures were diluted with YPDA to $OD_{600}$~ 0.3, grown for 4 h, and treated with 1 mM or 2 mM $MnCl_2$ for 2 h, or with 80 μM PHL for 30 min, or remained untreated. Where indicated, 50 μM menadione was added for 2 h, and cells were incubated at 30°C with shaking. Cell pellets were washed in YPDA twice, adjusted to the same cell count ($2\times10^6$ cells/ml), serially diluted (1:5), and plated on YPDA agar plates. The plates were incubated at 30°C for 2 days (*WT*) or 3 days (*grx5*Δ). Viability assays were repeated three times, representative images are shown.

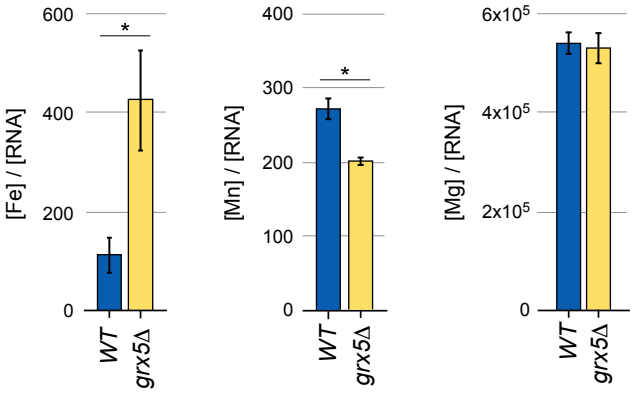
## Supplemental Figure 1 (S1)



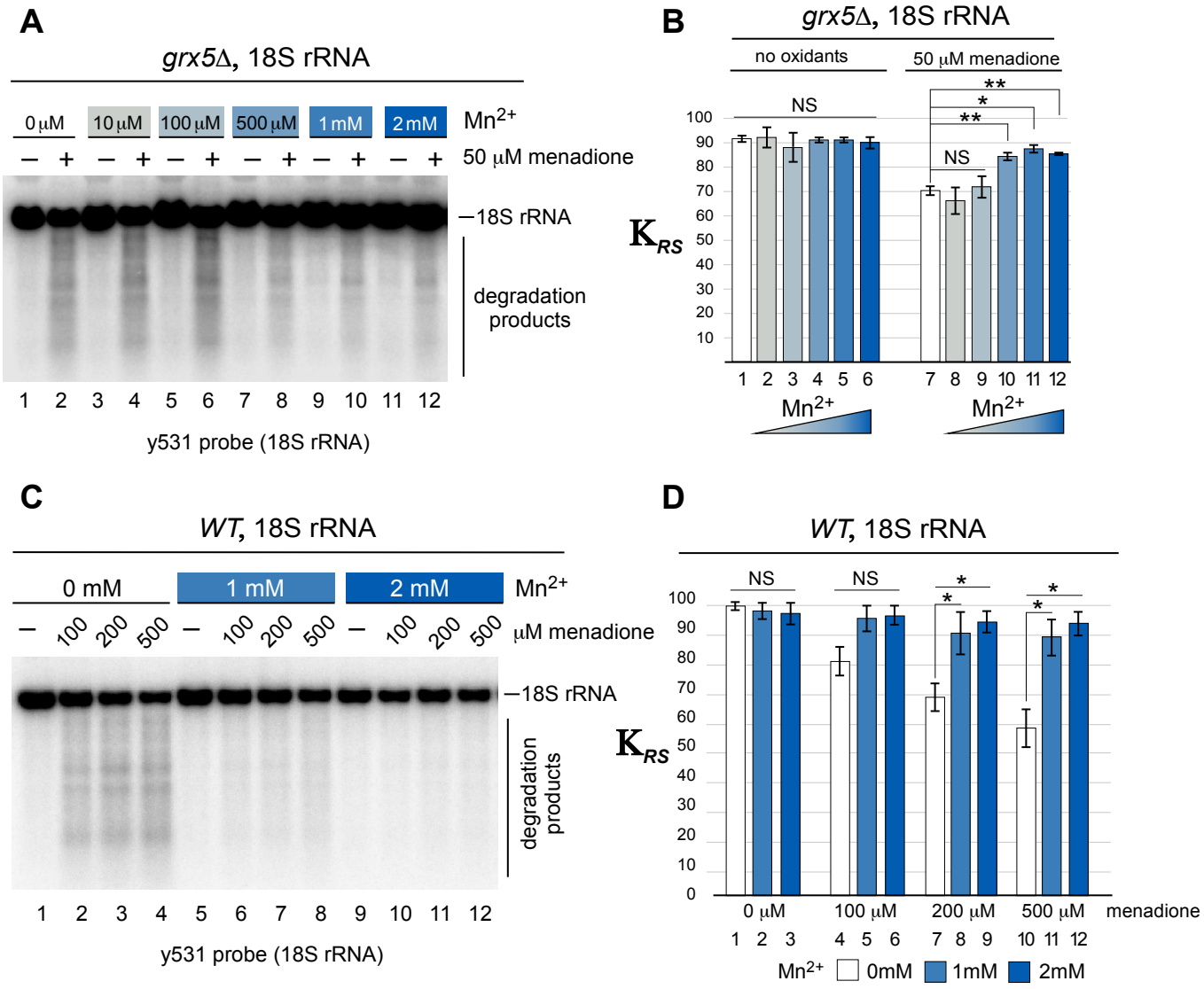
## Supplemental Figure 2 (S2)



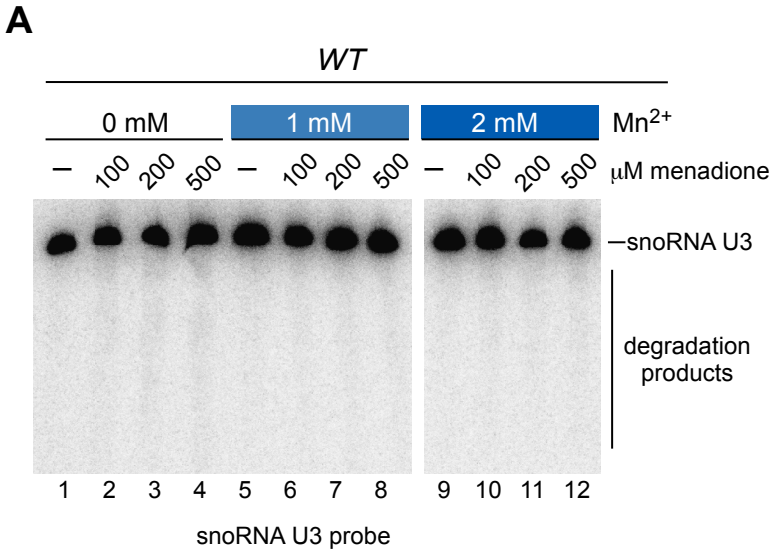
## Supplemental Figure 3 (S3)

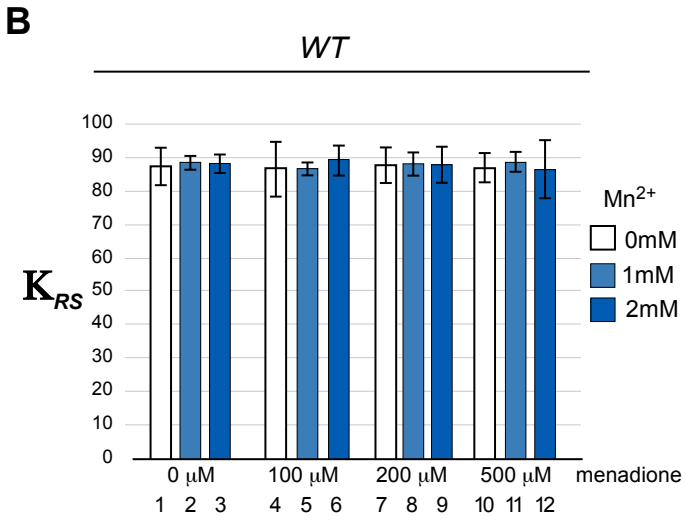


## Supplemental Figure 4 (S4)



## Supplemental Figure 5 (S5)



## Supplemental Figure 6 (S6)