Supporting Information

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SI Materials and Methods

Yeast Knockout Library Screen. The homozygous diploid knockout collection from Thermo Scientific was used for sensitivity screen as described (1) with some modifications. Briefly, the strains from the collection were replicated by using the Singer HTS Rotor onto 96-deep-well plates containing liquid 1% yeast extract (wt/vol), 2% (wt/vol) peptone and 2% (wt/vol) dextrose (YPD). Cells were grown for 20 h in a GeneMachines HiGRo shaker at 250 rpm and 30 °C and then printed with the rotor onto Nunc OmniTrays containing solid YPD media with or without 1 μM trichothecin (Tcin), which did not inhibit the growth of BY4743. Sensitivity was scored by identifying those strains that failed to grow after 2 d at 30 °C. The screen was repeated four times, and the identified strains were grown in liquid YPD media on 0, 0.5, 1, and 2 μM Tcin. Liquid growth assays were repeated twice before selecting and characterizing the deletion mutants as Tcin-sensitive.

Liquid Growth Assay. Yeast cells were grown in YPD. Following treatment, cells were grown for the specified time in a shaker at 30 °C, and growth at OD_{600} was recorded at every 60 min for a period of 18–24 h by using a Synergy 4 multiplate reader (BioTek).

Reactive Oxygen Species Measurement. Following treatment with trichothecenes or antioxidants, equal number of cells was collected, washed, and resuspended in 1× PBS with 2% glucose. Cells were stained with 2′,7′-dichlorfluorescein-diacetate (DCFH-DA) (Sigma-Aldrich) for 30 min at 30 °C. Following staining, cells were washed with water and then analyzed by using the Accuri C6 Flow Cytometer (Accuri Cytometers). For each sample, 15–20,000 events were recorded. Cells were gated by using the forward (FSC) and side (SSC) scatter before assessing for fluorescence. Cells with fluorescence signal above the background were detected in the FL1 channel [excitation (Ex) at 488 nm and emission (Em) at 530 nm] as staining positive for DCF and identified as reactive oxygen species (ROS) positive. Channel gating and histogram plots were made by using the CFlow Plus Analysis software (Accuri Cytometers).

Hydrogen peroxide levels were measured by using the Amplex Red/peroxidase assay as described (2). Briefly equal number of trichothecene treated or untreated cells were pelleted and washed with distilled water. Cells were then resuspended in an incubation mixture containing 145 mM NaCl, 5 mM KCl, 10 mM MOPS (pH 7.4), 1 mM potassium phosphate, 0.05% glucose, 2 μM Amplex Red (Life Technologies), and 5 μg/mL horseradish peroxidase. Following incubation for 30 min, fluorescence was measured at 530 nm (Ex) and 590 nm (Em) by using a Synergy 4 reader (BioTek).

1. McLaughlin JE, et al. (2009) A genome-wide screen in *Saccharomyces cerevisiae* reveals a critical role for the mitochondria in the toxicity of a trichothecene mycotoxin. *Proc Natl Acad Sci USA* 106(51):21883–21888.

2. Ojovan SM, et al. (2011) Accumulation of dodecyltriphenylphosphonium in mitochondria induces their swelling and ROS-dependent growth inhibition in yeast. *J Bioenerg Biomembr* 43(2):175–180.

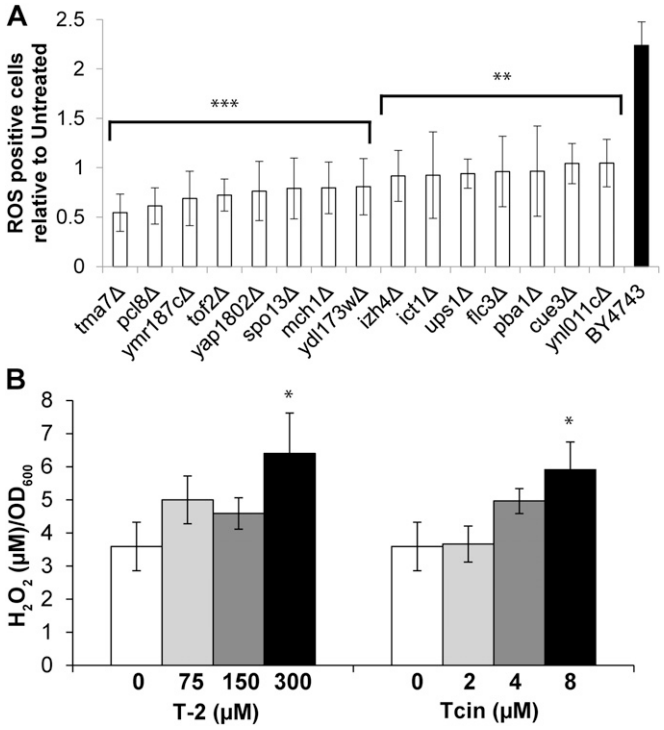

Fig. S1. Effect of trichothecenes on ROS generation in Tcin-resistant yeast mutants. (*A*) Yeast knockout mutants conferring the highest resistance to Tcin (12) were treated with 4 μM Tcin for 1 h. Equal OD_{600} cells were stained with DCFH-DA, and fluorescence was measured by using an Accuri flow cytometer. Differences relative to BY4743 were assessed by using analysis of variance (ANOVA) followed by post hoc LSD tests (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (*B*) The parental BY4743 cells were treated with increasing concentrations of T-2 (0-300 μM) or Tcin (0-8 μM) for 3 h. Intracellular H_2O_2 was measured fluorometrically by staining treated and untreated treated cells with the Amplex Red/peroxidase mix for 30 min. Readings were normalized to OD_{600} for each treatment. Error bars represent SE calculated from three independent experiments. Differences relative to untreated cells were assessed by using ANOVA followed by post hoc LSD tests (*$P < 0.05$).

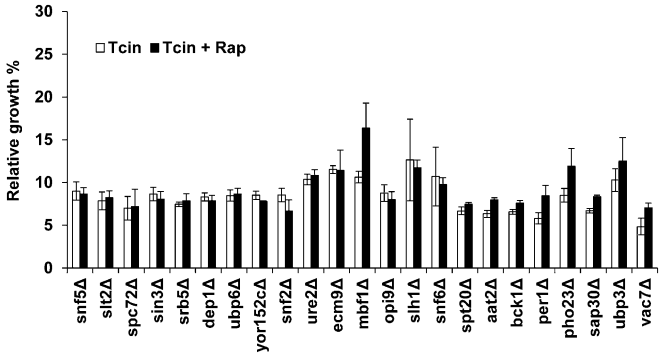

Fig. S2. Effect of rapamycin on the growth of Tcin-sensitive mutants. Tcin-sensitive mutants were treated with 1 μM Tcin alone or cotreated with 5 nM rapamycin (Rap) overnight. The ratio of OD_{600} of treated cells relative to the untreated cells was plotted. Error bars represent SE calculated from three independent experiments.

Other Supporting Information Files

Table S1 (DOC)