Supplementary Methods and Materials:

Yeast strains

The wild-type strains BY4742 (MAT**α** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and BY4743 (MAT**α**/**a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) were obtained from Open Biosystems (Huntsville, AL, USA). Unless otherwise stated all gene deletion strains were obtained from the Yeast Knock-Out MAT**a** Collection (Open Biosystems). The Aim17p-GFP expressing strain was obtained from the Yeast GFP Clone Collection (Invitrogen, Carlsbad, CA, USA). In experiments utilising genes deletion strains, a wild-type control strain bearing a neutral deletion of *HIS3* was used in order to ensure medium consistency. YCG326 (referred to herein as *pdr*Δ) (MAT**α** *pdr1*Δ::*NAT pdr3*Δ::*URA3 can1*Δ::*STE2pr-Sp_HIS5 lyp1*Δ *his3*Δ*1 leu2*Δ*0 ura3*Δ*0 met15*Δ*0*) and YCG387 (MAT**a** *pdr1*Δ:: *KanR pdr3*Δ::*URA3 can1*Δ::*STE2pr-Sp_HIS5 lyp1*Δ *his3*Δ*1 leu2*Δ*0 ura3Δ0 met15Δ0*) were a kind gift from N. Coorey (Victoria University of Wellington, Wellington, NZ). Unless stated otherwise all yeast strains were maintained on YPD agar plates containing appropriate antibiotics. G418 (Invitrogen) and cloNAT (Werner BioAgents, Jena, Germany) were used at 200 µg/ml and 100 µg/ml respectively.

Compounds and reagents

Unless otherwise noted, all reagents were purchased from Sigma-Aldrich Life Science and Biochemicals. Neothyonidioside was a kind gift from P. Northcote (School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington, New Zealand).

Liquid dose-response growth assays

Yeast cultures of BY4742 (wt) and *pdr*Δ were grown overnight in 2 mL SC media at 30 °C on a rotating drum. The cell density of each culture were determined by measuring optical density (OD) at 600 nm and diluted to 5 x 10^5 cells/mL in one of four different media solutions: 1) SC (normal pH ~4.0), 2) pH 8.0 SC, buffered with 25mM HEPES (SC-H), 3) YPD (normal pH ~7.0), and 4) pH 4.0 YPD, adjusted with 18.6 M HCl (YPD-HCl). Then, 100 µL aliquots of each media solution were dispensed into 96-well microtitre plates to give a final concentration of 5 x 10^4 cells/mL. An eight-point serial dilution of TA-289 was prepared from the 10 mM working stock, from which a 1 µL aliquot of each dilution point was added in triplicate to the wells containing 100 µL of media containing yeast, to achieve a final concentration range of 100 µM – 1 µM. DMSO (1% final concentration) served as a

solvent control. Plates were mixed by vortexing and incubated at 30 °C for 18 h. Cell growth was quantified by measuring OD at 600 nm using a Wallac EnVision 2102 Multilabel Plate Reader (Perkin Elmer™, Waltham, Mass.). The Δ*crd1* dose-response growth assays were performed as per normal dose-response assays described above, in SC media only, against the concentrations of TA-289 as indicated. The ability of various antioxidants to confer resistance to TA-289 was assessed according to the above procedure in SC medium with 50 mM citrate buffer (pH 4) containing 5 mM glutathione (GSH), 5 mM L-cysteine (Cys), 5 mM N-acteyl-L-cysteine (NAC), 10 mM sodium ascorbate (Asc) or 1 mM quercetin (Quer).

Colony forming unit assay

After treatment with 100 µM TA-289 for 24 h, *pdrΔ* cells were washed twice with sterile ddH_2O and 30 cells were plated onto YPD agar plates and incubated for 2 days at 30 °C. The number of viable colonies was compared to those remaining after treatment with the carrier solvent (1% DMSO) and negative and positive controls for cell death (cycloheximide and neothyonidioside respectively).

Cell cycle analysis by flow cytometry

Yeast cell DNA was stained according to standard procedures[51] samples were analysed with the FACScan flow cytometer (BD Biosciences), histogram plots were generated with CellQuest Pro software (BD Biosciences).

Cell morphology

Cells (*pdr*Δ) from the end-point liquid dose-response assays were assessed for morphological changes using an inverted phase contrast microscope at 60X magnification (Olympus inc., P.O. Box 610, Centre Valley, PA, USA) and then photographed (Nikon Corporation, Tokyo 100-8331, Japan). The *pdr*Δ cells treated at 100 µM and 30 µM of TA-289, as well as the DMSO control were photographed. Control cells were diluted 100 fold prior to photographing for ease of manual cell counting. Cell diameters of a random sample of 50 cells from each treatment were measured on ImageJ[72]. A two-tailed t-test assuming equal variances was performed in Excel, and fold decrease in cell size due to TA-289 treatment was calculated.

Cell death assays

Cultures of *pdr*Δ cells were grown overnight at 30 °C on a rotating drum in either synthetic complete media with 2% glucose (SC), or SC containing 2% glycerol and 2% ethanol (SC-Gly-EtOH). The cultures were diluted in either SC or SC-Gly-EtOH to give a final cell concentration of 1 x 10^6 cells/mL and a 100 µL aliquot of diluted cells in either SC or SC-Gly-EtOH were dispensed into 96-well microtitre plates, after which 1 µL aliquots of the previously described serial dilution of TA-289 were added, giving a final concentration range of 100 µM to 10 µM. The plates were then mixed by vortexing, and a 4 µL aliquot of the treated cells were removed and spotted onto a Singer© plate containing either SC agar or SC-Gly-EtOH agar, every 30 min for 4 h and then incubated at 30 °C. For the TA8 and *pdrΔ* cell death assays in H_2O_2, cells were treated with a serial dilution of 9 - 3 mM H_2O_2 (final concentrations), a 1.5 µL aliquot of cells was harvested and spotted onto the SC agar plate every 30 min for 3 h.

Resistant mutant generation

Liquid cultures of *pdr*Δ were grown overnight in 2 mL SC media at 30 °C on a rotating drum. Mutagenesis was adapted from the standard protocol according to Cold Spring Harbour Laboratory Press[73]. A selection of mutants, both spontaneous and EMS-generated, of various sizes, were picked and re-streaked onto SD –Ura +NAT agar plates containing 100 µM TA-289.

The strongest confirmed mutant was mated with the isogenic MAT**a** wild-type strain (YCG387), and the resulting diploids streaked onto fresh SD –Ura +NAT +G418 agar plates and grown for two days at 30 °C to attain single colonies. These heterozygous diploids were used to determine dominance and recessiveness of the mutation. Cell concentrations of overnight cultures of TA8, the *pdr*Δ parental, along with the corresponding heterozygous diploids were determined by measuring OD at 600 nm on a spectrophotometer (Genova Life Science), and subsequently diluted to 5 x 10^5 cells/mL. Each yeast culture was then serially diluted ten-fold, four times, after which a 10 µL aliquot from each dilution was spotted onto SC agar plates containing 60 µM TA-289, as well as the corresponding untreated control plates containing only SC agar. These were incubated for two days at 30 °C, and then photographed. The level of resistance was determined by comparing colony growth of heterozygous diploid mutants to original haploid resistant mutants on drug-containing plates.