1 # Supplemental Material

2

3 ## Materials and Methods

4 ### MADL and Z-score calculation

5 The MADL score for strain *Y* was defined as follows: let *ID* be the intensity of strain *Y* in a
6 sample that is treated with Compound D and *IC* the average intensity of the control samples.
7 Furthermore, let *mD* be the median and *madD* be the MAD of the logarithmic ratio ln*ID/IC*
8 overall strains for the sample treated with Compound *D*. The MADL score *sY* for strain *Y* is now
9 given by $sY = (\ln ID/IC - mD) / madD$. The aim of the analysis is to detect outliers; these should
10 influence the normalization as little as possible. In addition to the MADL score for each gene, a
11 Z-score was calculated from the TAG4 microarray intensities by taking the logarithm of the ratio
12 of mean experiment versus mean control tag-averaged intensities allowing for 15% outliers.

13

14 ### Targeted metabolomics profiling

15 *S. cerevisiae* cell pellets were resuspended in 100 µl PBS and adding the cell suspension to 1 ml
16 methanol/$CHCl_3$ (2:1 v/v) supplemented with 0.01% (w/v) butylated hydroxytoluene. Acid
17 washed glass beads (diameter 212-300 µm) were added and the yeast cells were homogenized by
18 agitation for 10 min. Cell debris was then removed by centrifugation at 16,000 g for 10 min. The
19 supernatant was transferred into a new tube and 400 µl of 50 mM citric acid in water and 600 µl
20 $CHCl_3$ were added. After agitation for 10 min, samples were centrifuged at 16,000 g for 5 min.
21 Lipids in the lower organic phase of each extraction were dried under a nitrogen stream and then

22 re-dissolved in ethanol. Relative concentrations of ergosterol and lanosterol in *S. cerevisisae*
23 cells were determined by LC-MS analysis. For each compound treatment three biological
24 replicates were analyzed with four technical replicates on an Agilent 1100 Series LC system
25 (Agilent Technologies, Santa Clara, CA, USA) coupled to a 4000 QTRAP (AB Sciex, Foster
26 City, CA, USA), which was equipped with a APCI-Turbo Spray ion source (AB Sciex).
27 Chromatography was carried out on an Atlantis dC18, 3 µm, 1 x 50 mm column (Waters,
28 Milford, MA, USA) with a flow rate of 200 µl/min and a gradient from 30% A (H_2O with 0.1%
29 (v/v) formic acid) and 70% B (acetonitrile with 0.1% (v/v) formic acid) to 2% A and 98% B in
30 2.5 min. Solvent composition remained for 5 min at 2% A and 98% B before the column was
31 reconditioned to 30% A and 70% B with a flow rate of 300 µl/min. The total runtime for one
32 analysis was 14 min. To reduce ion source contaminations, a switch valve was used to direct the
33 flow to waste for the first 4 min and the last 5 min of the LC method. When the flow of the LC
34 system was directed to waste, a second LC system was directed to the ion source with a flow rate
35 of 200 µl/min and solvent composition 30% A and 70% B. Ion source parameters and
36 compound-dependent instrument parameters were optimized by infusing pure standard solutions
37 of ergosterol and lanosterol. Ergosterol was detected in positive ion mode by selected reaction
38 monitoring (SRM) using the mass transitions 379.3 $[M-H_2O+H]^+$ → 69.2 and 379.3 → 83.1 and
39 lanosterol was detected using the mass transitions 409.4 $[M-H_2O+H]^+$ → 109.1 and 409.4 →
40 191.2. All mass transitions were monitored for 150 msec per SRM scan with a unit/unit
41 resolution for Q1/Q3. The IntelliQuan algorithm in Analyst 1.5 (AB Sciex) was used for peak-
42 finding and peaks were smoothed with a width of three points. For the relative comparison of
43 ergosterol and lanosterol concentrations in yeast cells, the peak areas were determined for the

44 ergosterol mass transition 379.3 → 69.2 and for the lanosterol mass transition 409.4 → 191.2.
45 The normalized lanosterol peak area of a sample was calculated by multiplying the lanosterol
46 peak area with the ratio of the median ergosterol peak area of all biological replicates for all
47 tested compound concentrations divided by the ergosterol peak area of the sample.
48

49 **Human CYP inhibition**

50 Dilution ranges of test compounds were obtained by dispensing 2.5 to 100 nl of a 10 mM stock
51 solutions of the compound being tested (0.5, 1, 2, 3, 5, 10, 15 and 20 µM final concentrations)
52 into the wells of a 384-well microplate by acoustic dispensing with an ECHO 520 apparatus
53 (Labcyte Inc., CA, USA). For the definition of enzyme activity range (0-100%), 16 wells were
54 filled with 100 nl DMSO only (100%) and 16 wells with 100 nl of 5 mM ketoconazole (0%
55 activity, 5 µM final concentration). A 10 nl sample of 10 mM midazolam (10 mM in DMSO, 2
56 µM final concentration) and 5 nl D4-1'-hydroxy-midazolam (0.28 mM in DMSO, 0.028 µM
57 final concentration) were added as substrate and internal standard into every well of the 384
58 microplate. All wells were adjusted to final volume of 100 nl with DMSO using the ECHO 520
59 (0.2% final DMSO content). The incubation was started by the addition of 50 µl of a mix of 0.05
60 mg/ml human liver microsomes and 1 mM NADPH dissolved in 50 mM sodium phosphate
61 buffer (pH 7.5) using a Multidrop Combi Reagent Dispenser (Thermo Fisher Electron
62 Corporation, Vantaa, Finland). The plate was immediately placed in an incubator (ELMI Skyline
63 DTS-4, LTF Labortechnik, Wasserburg, Germany) and incubated for 10 min before being
64 stopped by the addition of 50 µl ice-cold acetonitrile containing 1 µM alprenolol, which was
65 included as an additional internal standard for LC-MS analysis. Analysis of samples was

66 performed on a high-performance liquid chromatography-tandem mass spectrometry system
67 consisting of a TSQ Quantum Discovery MAX mass spectrometer controlled by QuickQuan 2.0
68 and equipped with an electrospray ion source (Ion Max electrospray interface) from Thermo
69 Fisher Scientific (Reinach, Switzerland), a CTC-HTS Pal autosampler (CTC Analytics, Zwingen,
70 Switzerland) with a sample cooling unit (10°C), and a Rheos pump (model 2000; Thermo Fisher
71 Scientific). Samples were separated on a Polar-RP column (2.1 x 50 mm, 3.5 µm; Phenomenex,
72 Torrance, CA) protected by a guard column (2 x 4 mm) containing the same material (provided
73 by Brechbühler AG, Schlieren, Switzerland) using an isocratic mobile phase of water-acetonitrile
74 (65:35) containing 0.1% formic acid at a flow rate of 400 µl/min for 2 min. The injection volume
75 was 20 µl, and the first 0.5 min of eluent was diverted to waste to protect the ion source from
76 salts and polar impurities. 1-Hydroxymidazolam and 1-hydroxymidazolam-D4 were detected in
77 positive ion mode by selective reaction monitoring using the mass transition of 342 to 324 and
78 346 to 328, respectively, at collision energy of 20 eV.

79

80

Supplementary Figure Legends

SUPPLEMENTARY FIGURE 1 | Growth inhibitory action of Compound-1 is static but not cidal. **A)** Minimal growth- inhibitory concentration of Compound-1 using the *S. cerevisiae* BYΔ8 strain (BY4743 strain deleted for 8 genes involved in drug resistance) was determined to be around 250 nM in a pre-experiment. Plating 1000 *S. cerevisiae* BYΔ8 cells on a 5 cm dish containing DMSO, 1 μM or 10 μM of Compound-1, did not result in observable colonies on the compound plates, after 48 hours. **B)** The surfaces of the 1 and 10 μM Compound-1 plates were then rinsed with buffer and spread on non-selective rich medium where within 36 hours >500 clonies were formed.

SUPPLEMENTARY FIGURE 2 | Sequence alignment of Human CYP51 with *S. cerevisiae* and *C. albicans* Erg11p using CLUSTAL W2. Sites proposed to interact with ketconazole in Human CYP51 are indicated with red letters, sites identified to confer azole-resistance in *C. albicans* (ATCC 64124) are indicated with green letters, and sites identified to confer azole-resistance in *C. albicans* (NF2013) are indicated with blue letters. Boxed sites are placed within a 10Å radius from the heme based on the CYP51 crystal structure. Boxed residues show 67% identity, 88% similarity between fungi and human."*" marks identical residues, ":" marks conserved substitutions, "." marks semi-conserved substitutions.

SUPPLEMENTARY FIGURE 3 | *S. cerevisiae* Erg11p model based on the human CYP51 (PDB code 3JUV). **A)** Superimposed structure of *S. cerevisiae* Erg11p (yellow) and human CYP51 (cyan). **B)** Superimposed structure of *S. cerevisiae* Erg11p (yellow) and *A. fumigatus* CYP51 model (cyan). **C)** Supeimposition of the *S. cerevisiae* and human structure at the active site with the central heme co-factor. Sites within a 10 Å radius of the heme are labeled with an asterisk and are boxed in the alignment of Supplementary Figure 1.