

SUPPLEMENTAL METHODS

To determine if longer incubations of *C. albicans* with 6-NDA would induce fatty acid β -oxidation genes, *C. albicans* SC5314 cells were exposed to either 0.25% DMSO or 6-NDA at the IC_{50} concentration for one doubling (OD_{600} of 0.4), two doubling (OD_{600} of 0.8), and four doubling (OD_{600} of 3.2) times. The media conditions, inoculum size, and culture size were identical to those used in the one doubling experiment described in the “Materials and Methods” section. Each treatment consisted of three biological replicates, resulting in 6 samples (three DMSO-treated and three 6-NDA treated) at each time point. Total RNA was isolated as described in the “Materials and Methods” section. The RNA samples were treated with DNase I “on column” as per manufacturer’s instructions using Qiagen RNeasy® (Qiagen, Inc., Valencia, CA) columns to remove residual DNA contamination. First strand cDNAs were synthesized from 2 μ g of total RNA in a 100 μ L reaction volume using the TaqMan Reverse Transcription Reagents Kit (Applied Biosystems, Foster City, CA) as per the manufacturer’s instructions. Quantitative real-time PCR reactions were performed in triplicate using the 7300 Real-Time PCR System (Applied Biosystems). Independent PCR reactions were performed using the same cDNA for both the gene of interest and 18S rRNA, using the SYBR® Green PCR Master Mix (Applied Biosystems). Gene-specific primers were designed for the gene of interest and 18S rRNA using Primer Express® software (Applied Biosystems). All related coding sequences within the *C. albicans* genome were identified via BLASTN queries of the complete coding sequences using the BLAST server at the *Candida* Genome Database. All sequences thus identified were then aligned using the ClustalW alignment function of MegAlign software (DNASTAR, Inc. Madison, WI). Gene-specific primer pairs were then manually selected such

that at least one primer per pair contained a minimum of two consecutive mismatches at the 3' end when compared against all related *C. albicans* sequences. For all of the genes analyzed, both primers contained numerous additional mismatches when compared against related sequences. The sequences of the primer pairs are listed in Supplemental Table S5. The genes analyzed consisted of five genes involved in fatty acid β -oxidation (*FAA2*, *POX1*, *FOX2*, *POT1*, and *TES1*), and one gene involved in sphingolipid biosynthesis (*LAG1*) as a positive control, which was induced by 5.3-fold in the one doubling microarray experiment (see Table 2 and Supplemental Table S4). The PCR conditions consisted of denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 1 min. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software provided with the 7300 Real-Time PCR System. A negative control reaction in the absence of template (no template control) was also routinely performed in triplicate for each primer pair. The change in fluorescence of SYBR® Green I dye in every cycle was monitored by the 7300 Real-Time PCR System software, and the threshold cycle (C_T) above background for each reaction was calculated. The C_T value of 18S rRNA was subtracted from that of the gene of interest to obtain a ΔC_T value. The C_T value of the calibrator (DMSO-treated sample) was subtracted from the ΔC_T value to obtain a $\Delta\Delta C_T$ value. The gene expression level relative to the calibrator was expressed as $2^{-\Delta\Delta C_T}$. Statistical significance was assessed by pairwise comparisons between treated and control sample $\Delta\Delta C_T$ values using an independent two-tailed t-test, assuming equal variance with $p < 0.005$ considered significant.