Supporting Information

Cowen *et al.* 10.1073/pnas.0813394106

SI Text

Strain Construction. *C. albicans* transformations were performed according to standard protocols. Details on strain construction are provided below.

CaLC367 (*HSP90*/*hsp90*Δ). The plasmid pLC53 was digested with *Bss*HII to liberate the cassette to target the tetracycline-repressible transactivator *tet-R* to the *HIS1* locus and was transformed into CaLC239 [SN95, (1)]. Proper integration was verified by PCR with primers oLC239 (CaHIS1–587-F, GTAATTTGGTAGTGAAAACCC) and oLC218 (CartTA + 300-R, CCGTGTACCTAAATGTAC). The *SAP2* promoter was induced to drive expression of FLP recombinase (2, 3) to excise the NAT marker cassette and the clone was verified for presence of the transactivator with the same primers as above. This strain was transformed with the *C. dubliniensis HIS1* marker and ≈350 bases of homology upstream and downstream of *HSP90*, amplified from plasmid pLC64 with primers oLC268 (CaHsp90-FP-1, cggagatgtattgactgtgg) and oLC271 (CaHsp90-FP-6, CTCTATGTTATGTTACTGGG). Proper deletion of *HSP90* was confirmed by PCR with primers oLC255 (CdHIS1 + 1032-F, GTCCATTAACTTCAAACC) and oLC277 (CaHsp90 + 2627-R, CCACCCTGAACCTAATTTGG) as well as oLC276 (CaHsp90–427-F, GAGATGGCAAATCGTTAGG) and oLC254 (CdHIS1–710-R, CGTCTCTTGATGTATATGG). Genotype: *arg4*Δ/*arg4*Δ *his1*Δ/*his1*Δ *URA3*/*ura3*::*imm*^{434} *IRO1*/*iro1*::*imm*^{434} *HIS1*/*his1*::*tetR-FRT HSP90*/*hsp90*::*CdHIS1*.

CaLC436 (*tetO-HSP90*/*hsp90*Δ). The plasmid pLC330 was digested with *Bss*HII to liberate the cassette to replace the native *HSP90* promoter with the *tetO* promoter and was transformed into CaLC367. Proper integration was verified by PCR using primers oLC308 (CaHSP90–698-F, GATTATTTGCTCACGGAACC) and oLC275 (pJK863up-R, AAAGTCAAAGTTCCAAGGGG) as well as oLC309 (CaHsp90 + 415-R, CTTGGACGTGATCAGCAACC) and oLC274 (pJK863down-F, CTGTCAAGGAGGGTATTCTGG). The *SAP2* promoter was induced to drive expression of FLP recombinase to excise the NAT marker cassette. Genotype: *arg4*Δ/*arg4*Δ *his1*Δ/*his1*Δ *URA3*/*ura3*::*imm*^{434} *IRO1*/*iro1*::*imm*^{434} *HIS1*/*his1*::*tetR-FRT FRT-tetO-HSP90*/*hsp90*::*CdHIS1*.

CaLC946 (*tetO-HSP90*/*hsp90*Δ + *HSP90*). To complement the deleted *HSP90* allele in CaLC436, pLC455 was sequentially digested with SacII and ApaI to liberate a cassette to replace the deleted allele with a wild-type allele. NAT-resistant transformants were PCR tested for proper integration of the cassette at the *HSP90* locus with primers oLC274 (pJK863down-F, CTGTCAAGGAGGGTATTCTGG) and oLC335 (CaHsp90 + 3116-R, gttggttgttgattactcc). To determine whether the cassette integrated at the deleted allele or the *tetO-HSP90* allele, presence of *CdHIS1* at the *HSP90* locus was tested using primers oLC255 (CdHIS1 + 1032-F, GTCCATTAACTTCAAACC) and oLC277 (CaHsp90 + 2627-R, CCACCCTGAACCTAATTTGG). Lack of a band suggested that the deleted allele was replaced and the *tetO-HSP90* allele remained intact. To confirm this, presence of *tetO* within the *HSP90* promoter was tested by PCR using primers oLC267 (CaHsp90upR-BamHI, CGGGATCCCATAATGAACTATTGATTTG) and oLC609 (CaHsp90 + 308R-SacI, CGAGCTCCCAGATTTAGCAATAGTACC). If *tetO* was still present (i.e., the deleted allele was replaced), then the expected band would be ≈1.2 kb; if *tetO* was absent then the expected band would be 785bp. This complemented strain has the deleted allele replaced with a wild-type *HSP90* allele and the *tetO-HSP90* allele remains intact. The NAT marker was excised. Genotype: *arg4*Δ/*arg4*Δ *his1*Δ/*his1*Δ *URA3*/*ura3*::*imm434 IRO1*/*iro1*::*imm434 HIS1*/*his1*::*TAR-FRT FRT-tetO-HSP90*/*HSP90-FRT*.

Plasmid Construction. Recombinant DNA procedures were performed according to standard protocols. Details on plasmids construction are described below. Plasmids were sequenced to verify the absence of any nonsense mutations.

pLC53. [*tet-R* integration at *HIS1* vector, NAT, ampR (4)]. The *C. albicans* tetracycline-repressible transactivator (*tet-R*) was PCR amplified using primers oLC221 (CaTA-ApaI-F, TTGCGGGCCCTTGAGATGGAGCCGTCAAATATCC) and oLC222 (CaTA-ApaI-R, ACCGGGGCCCGACATTTTATGATGGAATGAATGG) and cloned into plasmid pLC49 [pJK863, FLP-CaNAT, ampR (3)] at ApaI. Clones were PCR tested for the orientation of the insert using primers oLC221 (CaTA-ApaI-F TTGCGGGCCCTTGAGATGGAGCCGTCAAATATCC) and oLC203 (CaSAP-65R, CCAAGAGGAATCAACTATCC). Approximately 300 base pairs upstream of *HIS1* were amplified from SC5314 genomic DNA using primers oLC231 (CaHIS1UPKpnI-F, CGGGGTACCCAATTTGGGGACAGAAGAGG) and oLC232 (CaHIS1UPKpnI-R, CGGGGTACCCATTATCGGTAGTTGGTGG) and was cloned into pLC49 at KpnI. The proper orientation of the homology was PCR verified with primers oLC231 (CaHIS1UPKpnI-F, CGGGGTACCCAATTTGGGGACAGAAGAGG) and oLC203 (CaSAP-65R, CCAAGAGGAATCAACTATCC). Approximately 300 base pairs downstream of *HIS1* were amplified with primers oLC233 (CaHIS1DOWNSacII-F, TCCCCGCGGTAAAAGAAGTGATAGTTTCTC) and oLC234 (CaHIS1DOWNSacII-R, TCCCCGCGGGAATTGGATAGATTATTTGGG) from SC5314 genomic DNA and was cloned into pLC49 containing the upstream homology at SacII. The proper orientation of the homology was PCR verified with primers oLC202 (CaNAT1 + 544F, GCTTTGTATATGTCTATGCC) and oLC234 (CaHIS1DOWNSacII-R, TCCCCGCGGGAATTGGATAGATTATTTGGG). The cassette to target *tet-R* to the *HIS1* locus can be excised with *Bss*HII, which leaves an additional ≈40 bases of plasmid sequence.

pLC64 (*CaHSP90 knock-out vector, CdHIS1*, ampR). Approximately 350 base pairs of homology upstream of *HSP90* were amplified from SC5314 genomic DNA with primers oLC268 (CaHsp90-FP-1, CGGAGATGTATTGACTGTGG) and oLC269 (CaHsp90-FP-3, TGCTGTGATTTATACCAGGGAGAGACCTCGTGGACATCCATAATGAACTATTGATTTG). Approximately 350 base pairs of homology downstream of *HSP90* were amplified from SC5314 genomic DNA with primers oLC272 (CaHsp90-FP-4, TATCTCAGCACACGGGACAGCTACGAGACCGACACCGTAAACACCAGAAGGGCTAC) and oLC271 (CaHsp90-FP-6, CTCTATGTTATGTTACTGGG). The *Candida dubliniensis HIS1* selectable marker was amplified from pSN52 (1) with primers oLC270 (CaHsp90-FP-2, CCCTGGTATAAATCACAGCACCAGTGTGCTGGAATTCG) and oLC273 (CaHsp90-FP-5, CTGTCCCGTGTGCTGAGATAGGATATCTGCAGAATTCGC). The 3 components were assembled by fusion PCR using primers oLC268 (CaHsp90-FP-1, CGGAGATGTATTGACTGTGG) and oLC271 (CaHsp90-FP-6, CTCTATGTTATGTTACTGGG).

This PCR product was TA cloned into pCRII-TOPO vector (Invitrogen Co.).

pLC330 (*CaHSP90* promoter replacement vector, *tetO-CaHSP90*, NAT, ampR). The tetracycline-responsive promoter was amplified from pNIM1 (5) with oLC300 (Tetp-F-NotI, ATAAGAATGCGGCCGCGTTTGGTTCAGCACCTTGTCG) and oLC301 (Tetp-R-SacII, GGCACCGCGGCGACTATTTATATTTGTATGTGTGTAGG) and cloned into pLC49 at NotI and SacII. Approximately 350 base pairs of homology upstream of the promoter were amplified from SC5314 genomic DNA with primers oLC294 (CaHsp90–597-F-KpnI, CGGGGTACCGATTTCAGGTTGAAGAATTTGC) and oLC295 (CaHsp90–250-R-ApaI, TTGCGGGCCCCTGTTATAGGTAGTAATATGG) and was cloned into pLC49 containing the *tetO* promoter at KpnI and ApaI. Approximately 350 base pairs of homology downstream of the promoter were amplified from SC5314 genomic DNA with primers oLC296 (CaHsp90–1-F-SacII, TCCCCGCGGTATGGCTGACGCAAAAGTTG) and oLC297 (CaHsp90 + 348-R-SacI, CCCGAGCTCTCAGCACCAGCACTTAAAGC) and cloned into pLC49 containing the *tetO* promoter and the upstream homology at SacII and SacI. The cassette to replace the native *HSP90* promoter with the *tetO* promoter can be excised with *Bss*HII.

***pLC455 (CaHSP90* complementation vector, NAT, ampR).** The *HSP90* ORF, in addition to ≈330 base pairs of promoter region and some terminator region was amplified from SC5314 genomic DNA by PCR with oLC867 (CaHsp90–331F-ApaI, TTGCGGGCCCGTATTGAGACAGAAGAAGTG) and oLC332 (CaHsp90 + 2491-R-ApaI, TTGCGGGCCCAACAAGATCAATACCTTACTACC) and cloned into pLC49 at ApaI. Homology downstream of *HSP90* was amplified from SC5314 genomic DNA with primers oLC333 (CaHsp90 + 2492-F-NotI, ATAAGAATGCGGCCGCGATCTATCTTGAAACTCAGCG) and oLC334 (CaHsp90 + 2884-R-SacII, GGCACCGCGGctctgatcgtatacctatcc) and cloned into pLC49 containing *HSP90* at NotI and SacII. Presence of the inserts was tested by PCR with oLC274 (pJK863down-F, CTGTCAAGGAGGGTATTCTGG) and oLC334 (CaHsp90 + 2884-R-SacII, GGCACCGCGGCTCTGATCGTATACCTATCC) as well as oLC275 (pJK863up-R, AAAGTCAAAGTTCCAAGGGG) and oLC200 (CaHsp90 + 1831F, GAAATTTCTCCATCTTCC). The complementation cassette can be liberated by digestion with ApaI and SacII.

Formulation of Drug Stocks for *Galleria mellonella* Killing Assays. Fluconazole (Sequoia Research Products Ltd.) was dissolved in water and filter sterilized; caspofungin (courtesy of R. Bagatell, University of Arizona) was provided as a formulation for i.v. delivery; geldanamycin was formulated in DMSO (tissue culture grade, Sigma Aldrich Co.); 17-DMAG was formulated in water and filter sterilized; 17-AAG was formulated in a complex vehicle: 5% N, N-dimethylacetamide (DMA, Sigma Aldrich Co.): 7.5% Cremophor-EL (Sigma Aldrich Co.): 87.5% saline solution (0.9% saline); specifically, 17-AAG was dissolved in DMA and vortexed vigorously; cremophor was added and vortexed until the 2 layers were completely mixed; the saline solution was added in 3 equal parts with intermittent vortexing until a clear emulsion was achieved; the final solution was filter sterilized.

***Aspergillus fumigatus* Species Identification.** *A. fumigatus* was identified by macroscopic morphology on Sabouraud medium and by microscopic appearance of conidiophores stained with lactophenol cotton blue, showing uniseriate phialides emerging from the upper portion of the vesicle parallel to the conidiophore axis (6).

Histology of *A. fumigatus*-Infected *G. mellonella* Larvae. Larvae were embedded in paraffin, cut at 5 μm, and stained with Grocott's methenamine-silver nitrate fungus stain (7, 8).

Dose-Finding Survival Experiment to Standardize *C. albicans* Inocula for Murine Model of Disseminated Disease. In an attempt to standardize day-4 fungal burden across *Candida* strains, increased inocula of the *tetO-HSP90*/*hsp90*Δ strain were evaluated in a survival experiment. An inoculum size of 2×10^7 CFU (200 μl of 1×10^8 CFU/ml suspension) produced significant morbidity at 48 h after inoculation, necessitating sacrifice of all mice (n = 5). An inoculum size of 2×10^6 CFU (200 μl of 1×10^7 CFU/ml) produced an average log CFU/g kidney of 4.36 +/– 1.33 without mortality and was chosen for the dose of this strain for subsequent experiments.

Quantitative RT-PCR. Cells were grown overnight in YPD at 30 °C, diluted to an OD_{600} of 0.2 in 50-ml YPD and grown for an additional 3 h. Then 15 ml of the culture was pelleted and resuspended in either: 50-ml YPD at 30 °C, 50-ml YPD at 37 °C, or 50-ml YPD with 4-μg/ml FL. Cultures were sampled by removing 7 ml, placing it on ice for 5 min, pelleting it at 1,308 × g for 5 min at 4 °C, and freezing the pellet at −80 °C. RNA was isolated using the QIAGEN RNeasy kit and RNase-free DNase (QIAGEN), and cDNA synthesis was performed using the AffinityScript cDNA synthesis kit (Stratagene). PCR was performed using SYBR Green JumpStart Taq ReadyMix (Sigma Aldrich Co.) with the following cycling conditions: 94 °C for 2 min, 94 °C for 15 s, 60 °C for 1 min, 72 °C for 1 min, for 40 cycles. All reactions were performed in triplicate, using oLC752 (GPD1 + 570-F, AGTATGTGGAGCTTTACTGGGA) and oLC753 (GPD1 + 766-R, CAGAAACACCAGCAACATCTTC) for *GPD1* and oLC756 (HSP90 + 1051-F, GCTGAAGAGTTGATTCCAGAAT) and oLC757 (HSP90 + 1236-R, GGAGAAAGCAGTGTAGAATTGG) for *HSP90*. Data were analyzed using iQ5 Optical System Software Version 2.0 (Bio-Rad Laboratories, Inc).

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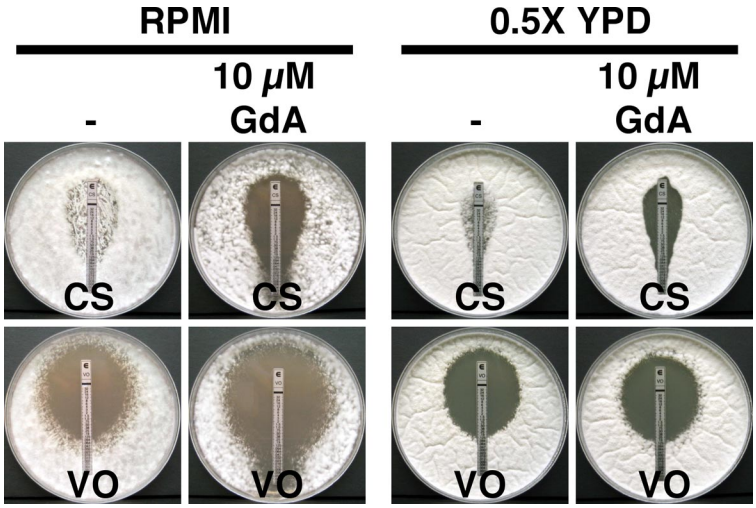

Fig. S1. Pharmacological inhibition of Hsp90 enhances the efficacy of antifungal drugs against *Aspergillus fumigatus*. Resistance of an *A. fumigatus* clinical isolate to the echinocandin caspofungin (CS) or the azole voriconazole (VO) on defined RPMI medium or rich 0.5X YPD medium, as indicated. Antifungal test strips (Etest, AB Biodisk) produce a gradient of drug concentration with the highest at the top. Plates contained vehicle control (DMSO) or geldanamycin (GdA), as indicated.

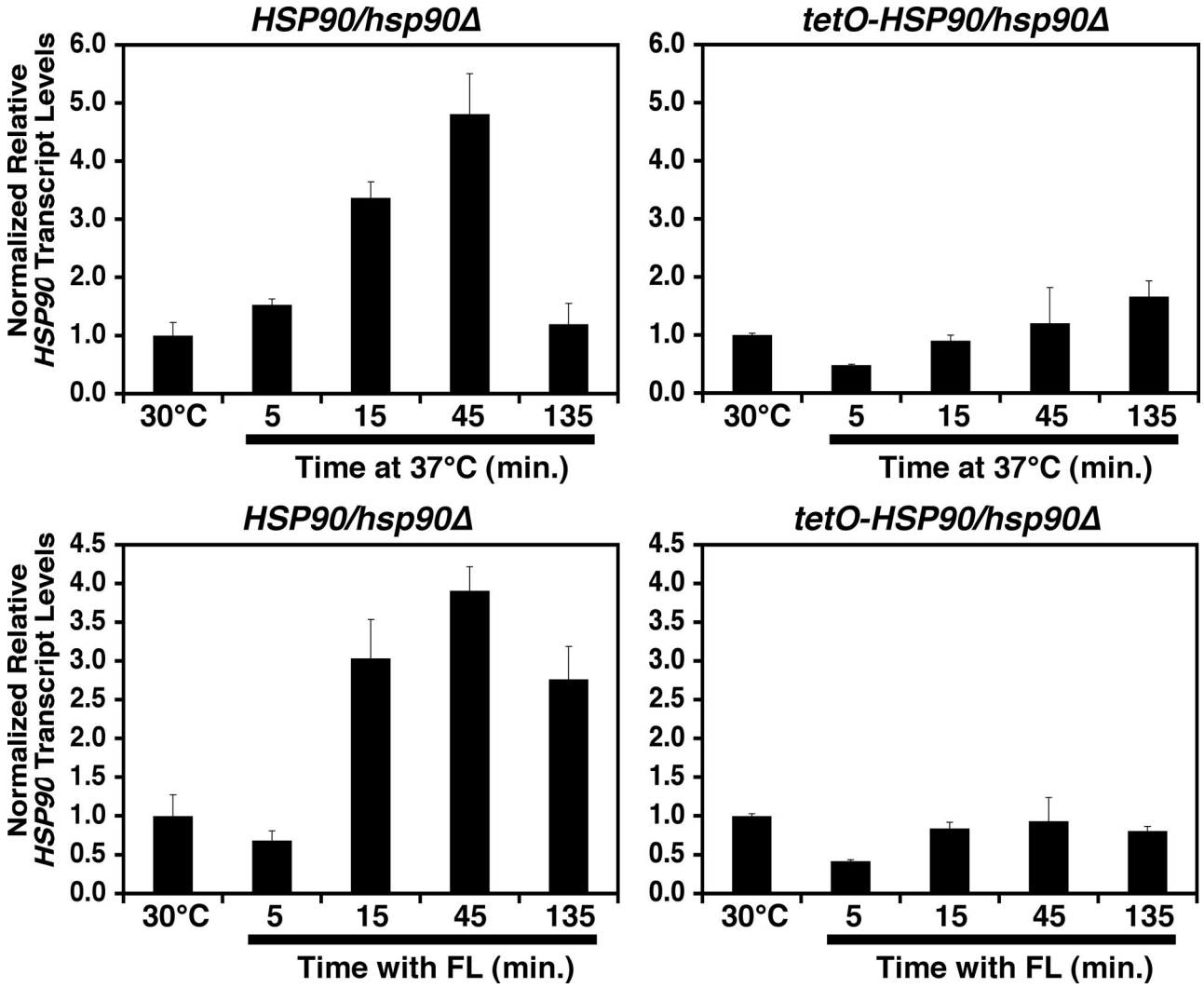

Fig. S2. Replacing the native stress-inducible *HSP90* promoter with the tetracycline-repressible promoter (*tetO*) blocks induction of *HSP90* upon exposure to increased temperature (37 °C) or FL (4 μg/ml). *HSP90* transcript levels were measured by quantitative RT-PCR and normalized relative to the *GPD1* control. Levels are expressed relative to the control sample at 30 °C with no treatment. Data are means for triplicate samples and standard deviations.