

Transcriptional response of *Candida auris* to the Mrr1 inducers methylglyoxal and benomyl

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22 **Abstract**

23 *Candida auris* is an urgent threat to human health due to its rapid spread in healthcare
 24 settings and its repeated development of multidrug resistance. Diseases that put individuals at
 25 a higher risk for *C. auris* infection, such as diabetes, kidney failure, or immunocompromising
 26 conditions, are associated with elevated levels of methylglyoxal (MG), a reactive dicarbonyl
 27 compound derived from several metabolic processes. In other *Candida* species, expression of
 28 MG reductase enzymes that catabolize and detoxify MG are controlled by Mrr1, a multidrug
 29 resistance-associated transcription factor, and MG induces Mrr1 activity. Here, we used
 30 transcriptomics and genetic assays to determine that *C. auris MRR1a* contributes to MG
 31 resistance, and that the main Mrr1a targets are an MG reductase and *MDR1*, which encodes an
 32 drug efflux protein. The *C. auris* Mrr1a regulon is smaller than Mrr1 regulons described in other
 33 species. In addition to MG, benomyl (BEN), a known Mrr1 stimulus, induces *C. auris* Mrr1
 34 activity, and characterization of the *MRR1a*-dependent and independent transcriptional
 35 responses revealed substantial overlap in genes that were differentially expressed in response
 36 to each compound. Additionally, we found that an *MRR1* allele specific to one *C. auris*
 37 phylogenetic clade, clade III, encodes a hyperactive Mrr1 variant, and this activity correlated with
 38 higher MG resistance. *C. auris MRR1a* alleles were functional in *Candida lusitanae* and were
 39 inducible by BEN, but not by MG, suggesting that the two Mrr1 inducers act via different
 40 mechanisms. Together, the data presented in this work contribute to the understanding Mrr1
 41 activity and MG resistance in *C. auris*.

42

43 Importance

44 *Candida auris* is a fungal pathogen that has spread since its identification in 2009 and is
 45 of concern due to its high incidence of resistance against multiple classes of antifungal drugs. In
 46 other *Candida* species, the transcription factor Mrr1 plays a major role in resistance against
 47 azole antifungals and other toxins. More recently, Mrr1 has been recognized to contribute to
 48 resistance to methylglyoxal (MG), a toxic metabolic byproduct. Here, we show that *C. auris*
 49 *MRR1a*, the closest ortholog to *MRR1* in other species, contributes to resistance to MG, and
 50 that Mrr1a strongly co-regulates expression of *MGD1*, encoding a methylglyoxal reductase
 51 enzyme and *MDR1*, encoding an efflux protein involved in resistance to azole drugs,
 52 antimicrobial peptides and bacterial products. We found that one major clade of *C. auris* has a
 53 constitutively active Mrr1 despite high azole resistance due to other mutations, and that this high
 54 Mrr1a activity correlates with higher MG resistance. Finally, we gain insights into the activities of
 55 MG and another Mrr1 inducer, benomyl, to better understand *C. auris* regulation of phenotypes
 56 relevant *in vivo*.

57

58 Keywords

59 *Candida auris*, *Candida*, methylglyoxal, benomyl, Mrr1, RNA-seq, transcriptomics

60

61 Introduction

62 Although *Candida albicans* has historically been the most prominent *Candida* species
 63 associated with both superficial and invasive fungal infections, worldwide incidence of non-
 64 *albicans Candida* (NAC) species is increasing (1-10). Of particular concern is *Candida auris*,
 65 which the CDC classifies as an urgent threat due to its relatively high frequency of resistance to

multiple different classes of drugs including amphotericin B, echinocandins and azoles (reviewed in (11)). Since its recognition as a novel *Candida* species in 2009, *C. auris*, has been reported in at least 40 countries (12-14). Whole-genome sequencing (WGS) analyses of *C. auris* isolates collected from across the globe indicate the concurrent emergence of four genetically distinct clades (15) with a potential fifth clade defined more recently (16). *C. auris* is thought to primarily colonize the skin (17-19) in addition to a diverse array of body sites, and most clinical isolates to date have been isolated from blood (20). Once *C. auris* has disseminated to the bloodstream, it can cause potentially fatal candidemia which has an estimated global mortality rate ranging from about 30 – 60% (15, 21, 22).

The resistance to azoles in *C. auris* is multifactorial; it has been shown that certain mutations in *ERG11* (15, 23-31) and overproduction of Cdr1p (32-36) contribute to resistance to fluconazole (FLZ). In multiple *Candida* species, the transcriptional regulator Mrr1 also plays a role in FLZ resistance (37-45), and Mayr and colleagues (46) found three *C. auris* homologs of the transcriptional regulator Mrr1, and showed that one of them, *MRR1a*, modestly affected fluconazole resistance. Previously, we demonstrated that in *Candida (Clavispora) lusitanae*, which is more closely related to *C. auris* relative to other well-studied *Candida* species (12, 47), Mrr1 regulates the expression of *MDR1*, and overexpression of *MDR1* confers resistance to FLZ (40, 48-55), the host antimicrobial peptide histatin-5 (40, 56), bacterially produced phenazines (40), and other toxic compounds (57) in multiple *Candida* species. *C. lusitanae* Mrr1 also regulates dozens of other genes with two of the most strongly regulated genes encoding methylglyoxal (MG) reductase enzymes, *MGD1* and *MGD2* (37, 40, 58). Mrr1 contributes to *C. lusitanae* resistance to MG (58), which is a spontaneously formed dicarbonyl electrophile generated as a byproduct of several metabolic processes by all living cells (reviewed in (59)).

Via its carbonyl groups, MG reacts non-enzymatically with biomolecules, which can lead to cellular stress and toxicity (reviewed in (59)). Some of the risk factors (60-69) for candidiasis caused by *C. auris* or other *Candida* spp., such as diabetes (70-72), kidney disease (73-76), or septic shock (77), are associated with elevated MG in human serum. MG resistance across clinical isolates of the same *Candida* species, including *C. auris*, can vary (58).

Through specific regulators, MG and other reactive electrophiles induce stress responses in bacteria (78-80), plants (reviewed in (81)), mammals (reviewed in (82)), and the yeasts *Saccharomyces cerevisiae* (83-87) and *Schizosaccharomyces pombe* (88, 89) at subinhibitory concentrations. We found in *C. lusitanae*, MG induces expression of *MGD1* and *MGD2* as well as *MDR1*, through a mechanism that involved Mrr1 (58), and that MG increased fluconazole (FLZ) resistance. *C. auris* displays nosocomial transmission (61-63, 65-69), in part due to its resistance to high temperatures (90) and common surface antiseptics (91), and persistence on abiotic surfaces including latex and nitrile gloves (92), plastics (90), and axillary temperature probes (93). The factors that control *C. auris* stress resistance are not yet known.

In the present study, we show that *C. auris MRR1a* regulates resistance to MG and that MG is an inducer of Mrr1-regulated gene expression. Mrr1a regulates the gene orthologous to the methylglyoxal reductase genes *C. lusitanae MGD1* in addition to *MDR1*, which regulates FLZ efflux, but the Mrr1a regulon is smaller than that described for other species. Furthermore, we characterize Mrr1a in both Clade I and Clade III isolates and show that the Mrr1 variant in Clade III is constitutively active. Transcriptomics analysis shows that MG elicits a large transcriptional response that is similar in both Clade I and Clade III, and that there are commonalities in the responses elicited by MG and the Mrr1 inducer benomyl. These data support the model that Mrr1 is a regulator of MG resistance in coordination with efflux proteins

such as Mdr1 and provides the basis for future studies on the roles of Mrr1 and MG in survival of *C. auris* in hospital settings.

Results

Mrr1a regulates expression of orthologs to *MDR1* and *MGD1* in *C. auris* strain B11221 and is involved in MG resistance.

To determine whether the *C. auris* *MRR1* orthologs *MRR1a*, *MRR1b*, and *MRR1c* contributed to resistance to MG, we performed growth kinetic assays in YPD +/- 5 mM, 10 mM, or 15 mM MG. At MG concentrations of 10 mM (**Fig. 1A**) and 15 mM (**Fig. S1**), the *mrr1a*Δ mutant displayed a substantial growth defect relative to WT, while the *mrr1b*Δ and *mrr1c*Δ mutants exhibited growth comparable to WT. None of the mutants (*mrr1a*Δ, *mrr1b*Δ, and *mrr1c*Δ) differed from the parental isolate B11221 (WT) in YPD alone or in the presence of 5 mM MG (**Fig. S1**). Like *C. lusitanae*, the *C. auris* genome encodes multiple putative MG reductases; the closest orthologs to *MGD1* and *MGD2* were *CJI97_000658* and *CJI97_004624*, respectively, in the B11221 genome assembly (58) and we will henceforth refer to these genes as *MGD1* and *MGD2*. For reference, *MGD1* and *MGD2* correspond to B9J08_000656 and B9J08_004828 respectively. By quantitative real-time PCR (qRT-PCR), basal expression of *MGD1* was significantly decreased 24-fold in the *mrr1a*Δ mutant relative to B11221 WT (**Fig. 1B**), and expression of *MGD2* trended lower in the *mrr1a*Δ mutant (~1.2-fold) but this difference did not reach statistical significance (**Fig. 1C**). *MGD1* was also more highly expressed than *MGD2* in the WT B11221 as in *C. lusitanae* (58). Consistent with the transcriptional patterns, *C. auris* Mgd1 shares slightly more identity with *C. lusitanae* Mgd1 than does *C. auris* Mgd2 (63% identity versus 61% identity).

In the *C. auris* B11221 background, expression of *MDR1*, another target of Mrr1 in other species including *C. lusitanae*, also depended on Mrr1a, as the *mrr1aΔ* mutant exhibited a significant 21-fold decrease in *MDR1* expression compared to the WT parent (**Fig. 1D**). These results indicate that in *C. auris* *MDR1* and *MGD1* are co-regulated, as has been reported in *C. albicans* (44, 45, 94-96), *C. parapsilosis* (97), and *C. lusitanae* (37, 39, 40, 58, 98) and that higher expression of *MGD1* and/or *MDR1* contributes to growth in high concentrations of MG (**Fig. 1A**).

In *C. lusitanae* and other *Candida* species, Mrr1 regulates dozens of genes in addition to *MDR1* and *MGD1* (37, 40). To further elucidate the Mrr1a regulon in *C. auris* isolate B11221, we performed an RNA-seq analysis of in B11221 WT and its *mrr1aΔ* derivative in cells from exponential phase cultures grown at 37°C in YPD. In the control condition (YPD + dH₂O), only four genes, including *MDR1* and *MGD1*, were differentially expressed between the two strains with the cutoff of a log₂ fold change (log₂FC) ≥ 1.00 or ≤ -1.00 and a p-value less than 0.05 (**Fig 1E** and **Supplementary File S1** for all data). *MGD1* and *MDR1* showed a 22- and 24-fold decrease, respectively, in *mrr1aΔ* compared to WT, consistent with our qRT-PCR data. *CJI97_005632*, which was 2.25-fold lower in *mrr1aΔ*, is orthologous to the *C. albicans* genes *RIM11* and *C2_04280W_A*, both of which are predicted to encode proteins with serine/threonine kinase activity, though it is worth noting that levels of the transcript were much lower than levels of *MDR1* and *MGD1*. *CJI97_000852*, which was 2.77-fold higher in *mrr1aΔ* than in WT, has 16 orthologs of diverse predicted or known functions in *C. albicans*, including *USO5*, *USO6*, and *RBF1* (**Fig. 1E** and **Supplementary File S1**). Notably, *MGD2* was not differentially expressed between B11221 WT and the *mrr1aΔ* mutant in our RNA-seq data (**Supplementary File S1**), consistent with our qRT-PCR results described above.

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159 **Mrr1a regulates only *MDR1* and *MGD1* in response to MG and benomyl**

160 We have previously shown in *C. lusitaniae* that MG induces expression of the Mrr1-
 161 regulated genes *MGD1* and *MGD2* in an Mrr1-dependent manner, and *MDR1* in a partially Mrr1-
 162 dependent manner (58). To determine if MG would induce expression of *MGD1*, *MGD2*, and/or
 163 *MDR1* in *C. auris*, we purified RNA for qRT-PCR from exponential-phase cultures of B11221 WT
 164 and *mrr1aΔ* treated with 5 mM MG or an equal volume of dH₂O for 15 minutes. We found that
 165 MG treatment significantly enhanced expression of *MGD1* in WT by 2.4-fold but not in *mrr1aΔ*
 166 (**Fig. 2A**). *MGD1* was also induced by a 30-min treatment with 25 μg/mL benomyl (BEN), a
 167 known inducer of Mrr1-regulated genes in other *Candida* species (37, 41, 43, 95, 99-104), by
 168 7.5-fold in the WT (**Fig. 2A**). The different treatment times for MG and BEN were used to be
 169 consistent with previous studies using either compound in the related species *C. lusitaniae* (37,
 170 58). Expression of *MDR1* was also more highly induced by treatment with either MG or BEN in
 171 WT compared to the *mrr1aΔ* mutant by 6- and 14.5- fold respectively (**Fig. 2B**). Although *MDR1*
 172 expression was significantly induced by MG and BEN in the *mrr1aΔ*, transcript levels of *MDR1*
 173 were approximately 20-fold higher in the WT than in the *mrr1aΔ* under these conditions (**Fig.**
 174 **2B**), suggesting that Mrr1a is required for maximum expression of *MDR1* in response to stimuli.

175 To describe the complete Mrr1-dependent MG- and BEN- response regulon under our
 176 test conditions in *C. auris*, we also performed RNA-seq on exponential-phase cultures of B11221
 177 WT and *mrr1aΔ* treated with MG or BEN as described above. In B11221 WT, MG led to the
 178 upregulation of 319 genes and downregulation of 133 genes compared to the control condition
 179 (**Fig. 2C** and **Supplementary File S2**). In the *mrr1aΔ* mutant, MG led to the upregulation of 349
 180 genes and downregulation of 143 genes compared to the control condition (**Fig. S2A** and

Supplementary File S2). Consistent with our qRT-PCR data in **Fig. 2A**, MG induced expression of *MGD1* in the WT but not in the *mrr1a*Δ mutant (**Table S1** and **Supplementary File S2**). Although expression of *MDR1* was significantly induced by MG in both the WT and the *mrr1a*Δ mutant (**Table S1** and **Supplementary File S2**), levels of *MDR1* were substantially lower in the *mrr1a*Δ mutant even in the presence of MG (**Fig. 2D** and **Supplementary File S2**), also in agreement with our qRT-PCR data. *MGD1* and *MDR1* strongly stood out as the only two genes in the MG response that were strongly dependent on Mrr1a (**Fig. 2D**).

Treatment with BEN led to upregulation of 160 genes and downregulation of 163 genes in the WT (**Fig. 2E** and **Supplementary File S3**). In the *mrr1a*Δ mutant, 181 genes were upregulated, and 229 genes were downregulated in response to BEN (**Fig. S2B** and **Supplementary File S3**). Like MG, induction of *MGD1* by BEN was completely dependent on Mrr1a (**Table S1** and **Supplementary File S3**) and *MGD2* expression was not induced by BEN (**Supplementary File S3**). Expression of *MDR1* was also induced by BEN in both the WT and the *mrr1a*Δ mutant, but as with MG, *MDR1* levels in the *mrr1a*Δ mutant did not reach that of the WT even with BEN treatment (**Fig. 2F** and **Supplementary File S3**). Again, *MGD1* and *MDR1*, appear to be the only genes in *C. auris* whose induction of expression by either MG or BEN is dependent on Mrr1a. The Mrr1a-independent responses to MG and BEN are discussed further below.

B11221 has higher basal expression of *MDR1* and of putative MG reductase genes compared to the Clade I isolate AR0390.

Many Clade III isolates, including B11221, contain an N647T single nucleotide polymorphism (SNP) in *MRR1a* (25, 105). In (105), this SNP was proposed to be a gain-of-

function mutation due to the resistance of Clade III isolates against azoffluxin, a novel antifungal compound that inhibits expression and activity of *C. auris* efflux pumps. As a first step to determine if there were activity differences between the Mrr1a variant that was found Clade III strains was different from that encoded by the alleles found in Clade I, II, and IV strains, we compared MG sensitivity of B11221 to that of Clade I isolate AR0390. Interestingly, AR0390 grew substantially better than B11221 in the YPD control but showed a greater reduction in growth in YPD with 5 mM MG than did B11221 (**Fig. S3**). At concentrations of 10 mM (**Fig. 3A**) and 15 mM MG (**Fig. S3**), AR0390 exhibited a profound growth defect compared to B11221. To determine if differences in MG sensitivity were due to differences in *MGD1* expression, we measured basal expression of *MGD1* and its co-regulated gene *MDR1* in B11221 and AR0390 using qRT-PCR. Both genes were significantly more highly expressed in B11221 by 42- and 4.2-fold, respectively (**Fig. 3B-C**).

To gain a deeper understanding of the broader transcriptional differences between B11221 and AR0390, we compared the basal global gene expression in YPD of the two strains using RNA-seq. First, we matched the 5227 syntenic orthologs between the genomes of B11221 and the Clade I reference strain B8441 to compare expression of each gene under the control condition. Of these, 755 genes were differentially expressed between B11221 and AR0390 in the control condition ($|\log_2FC| \geq 1.00$, FDR-corrected $p < 0.05$) (**Fig. 3D, Supplementary File S4**). The top twenty differentially expressed genes whose orthologs have known or predicted functions in *C. albicans* are reported in **Table S3**. Strikingly, the two genes which exhibited the largest difference in expression between B11221 and AR0390 were *MGD2* ($\log_2FC = 11.29$) and *MGD1* ($\log_2FC = 8.53$) (**Fig. 3D, Table S3, and Supplementary File S4**). A third gene with homology to MG reductases, *CJI97_001800/B9J08_002257*, was also more highly expressed

in B11221, although the log₂FC in expression of this gene in B11221 vs AR0390 was only 1.41 (Supplementary File S4). Low expression of *MGD1*, *MGD2*, and/or *B9J08_002257* may contribute to the severe growth defect of AR0390 in the presence of MG. Consistent with our qRT-PCR data, *MDR1* was also significantly more highly expressed in B11221 relative to AR0390 (log₂FC = 4.42) (Fig. 3D and Table S3). Although *MGD2* and *B9J08_002257* do not appear to be regulated by Mrr1a in our studies, it is nonetheless interesting to note the elevated expression of three putative MG reductases in the *MDR1*-overexpressing *C. auris* isolate B11221, as the co-expression of *MDR1* with at least one MG reductase has been reported in numerous studies in other *Candida* species (37, 40, 44, 45, 58, 94-97).

Clade III Mrr1a^{N647T} exhibits a gain-of-function phenotype compared to Clade I Mrr1a when expressed in *C. lusitaniae*.

To compare the activities of the proteins encoded by the *MRR1a* alleles of B11221 and AR0390 more directly, we heterologously expressed each allele, henceforth referred to as *CauMRR1a*^{N647T} and *CauMRR1a* respectively, independently in a *C. lusitaniae* *mrr1*Δ mutant previously generated and characterized by our lab (37, 40, 58). All three *C. lusitaniae* clones expressing *CauMRR1a*^{N647T} which we tested exhibited a four-fold increase in fluconazole (FLZ) MIC relative to the U04 *mrr1*Δ parent (16 μg/mL versus 4 μg/mL, Table S4), confirming that *C. auris* Clade III *MRR1a* can complement *MRR1*-dependent FLZ resistance in *C. lusitaniae* and adding support to the hypothesis that the N647T substitution in Clade III *MRR1a* confers increased activity. However, the FLZ MIC of the three tested *C. lusitaniae* clones expressing *CauMRR1a* did not differ from that of U04 *mrr1*Δ (4 μg/mL; Table S4), so FLZ MIC alone could not indicate whether this allele is functional in *C. lusitaniae*. One clone expressing each *C. auris*

250 *MRR1a* allele was chosen at random for the remaining experiments described in this paper:
 251 clone #1 for *CauMRR1a*^{N647T} and clone #5 for *CauMRR1a*. Using qRT-PCR, we then examined
 252 basal expression levels of *C. lusitaniae* *MGD1* (*CLUG_01281*) and *MDR1*
 253 (*CLUG_01938/CLUG_01939*) in the heterologous complements and the U04 *mrr1Δ* parent.
 254 Complementation with *CauMRR1a*^{N647T} conferred a significant increase in basal expression of
 255 both *MGD1* (**Fig. 3E**) and *MDR1* (**Fig. 3F**) compared to the *mrr1Δ* parent, while complementation
 256 with *CauMRR1a* led to a small, but significant, decrease in expression of both genes relative to
 257 *mrr1Δ* (**Fig. 3E-F**). These results are consistent with our previous observations that *C. lusitaniae*
 258 strains expressing certain Mrr1 variants with low basal activity demonstrate lower expression of
 259 some Mrr1-regulated genes, including *MDR1* and *MGD1*, compared to an isogenic *mrr1Δ* strain
 260 suggesting that Mrr1 has both repressing and activating roles (37, 58). Finally, we assessed the
 261 relative MG resistance of the isogenic *C. lusitaniae* strains expressing *CauMRR1a*^{N647T} or
 262 *CauMRR1a* and the U04 *mrr1Δ* parent. The *CauMRR1a*^{N647T} complement grew markedly better
 263 in 15 mM MG compared to U04 *mrr1Δ* whereas the *CauMRR1a* complement grew substantially
 264 worse than U04 *mrr1Δ* (**Fig. 3G**), consistent with the pattern of *MGD1* expression we observed
 265 in these strains via qRT-PCR. None of the *C. lusitaniae* strains demonstrated growth differences
 266 in the YPD control, or in the presence of MG at concentrations of 5 mM or 10 mM (**Fig. S4**).

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268 **MG induces expression of *MGD1* and *MDR1* in *C. auris* B11221 and AR0390, but not**
 269 **in *C. lusitaniae* strains expressing *C. auris* *MRR1a* alleles.**

270 Next, we compared induction of *MGD1* and *MDR1* by MG in the *C. auris* strains B11221
 271 and AR0390 via qRT-PCR. MG significantly induced expression of *MGD1* by 2.4-fold in *C. auris*
 272 strain B11221 and by 4.0-fold in *C. auris* strain AR0390 (**Fig. 4A**) and expression of *MDR1* by

6.0-fold in B11221 and 9.3-fold in AR0390 (**Fig. 4B**). AR0390 displayed lower expression of both genes in MG, but a higher fold change compared to B11221, further supporting the hypothesis that the N647T allele is gain-of function.

Finally, we compared induction of *MGD1* and *MDR1* by MG in the isogenic *C. lusitaniae* strains expressing either *CauMRR1a*^{N647T} or *CauMRR1a* and the *mrr1Δ* parent. Additionally, we tested induction by BEN in these strains as a control. While the *mrr1Δ* parent exhibited a significant 1.8- fold induction of *MDR1*, neither *C. lusitaniae* strain expressing a *C. auris* Mrr1a allele demonstrated a significant change in *MGD1* or *MDR1* expression in response to MG (**Fig. 4C-D**), indicating that *C. auris* Mrr1a may repress *MRR1*-independent MG induction of *MDR1* in *C. lusitaniae* and that induction of *MGD1* by MG in *C. lusitaniae* requires a functional *MRR1* allele from its own species. Treatment with BEN led to significant increase in expression of *MGD1* (**Fig. 4E**) and *MDR1* (**Fig. 4F**) in all three *C. lusitaniae* strains. In response to BEN, *MGD1* was induced by 1.9-fold in *mrr1Δ*, 2.9-fold in the *CauMRR1a*^{N647T} complement, and 6.1-fold in the *CauMRR1a* complement (**Fig. 4E**). Likewise, expression of *MDR1* was induced by 2.3-fold in *mrr1Δ*, 3.5-fold in the *CauMRR1a*^{N647T} complement, and 5.0-fold in the *CauMRR1a* complement in response to BEN (**Fig. 4F**). The striking difference in the ability of the *C. lusitaniae* strains expressing *C. auris* *MRR1a* alleles to respond to BEN versus MG suggests that there are differences in the mechanisms by which BEN and MG induce Mrr1-dependent transcriptional activation and that MG induction of *C. auris* Mrr1a is not supported by *C. lusitaniae* factors. These potential differences are a topic of future study and may shed light on mechanisms of Mrr1 activation in *Candida* species.

MG and BEN induced Mrr1a-independent transcriptional responses in *C. auris*

We have previously observed heterogeneity in MG resistance as well as MG-induced FLZ resistance among several *C. auris* isolates from different clades (58), and thus we were interested in whether the overall transcriptional response to MG was more similar or different in B11221 and AR0390. AR0390 had greater number of genes differentially expressed by MG compared to B11221; 438 genes were significantly upregulated, and 242 genes were significantly downregulated by MG (see **Fig. S5** for the volcano plot of all genes). More genes had a larger fold change in response to MG in AR0390 compared to B11221, including *MGD1* and *MDR1* (**Fig. 5A**), consistent with the qRT-PCR results in **Fig. 4A-B**. However, there was a large overlap of 254 genes which were induced by MG in both strains (**Fig. 5B**), suggesting a common response across these two genetically distinct clades. These commonly induced genes include many with putative roles in amino acid biosynthesis; transmembrane transport; or acquisition and usage of sulfur (**Fig. 5C** and **Table S5**). The complete comparison is available in **Supplementary File S6**).

Only 68 genes with syntenic orthologs across both strains were commonly repressed by MG (**Fig 5B**). These genes include some with putative roles in metal transport or carbohydrate uptake and metabolism (**Fig. 5C** and **Table S6**). We did not observe obvious patterns in genes that were only induced or repressed in one strain, and some genes that are listed as only induced or repressed in one strain were close to the cutoff in the other strain. The groups of genes that were differentially expressed in response to MG in both B11221 and AR0390 were also evident in the response of B11221 to BEN as well as the response of the *mrr1a*Δ mutant in response to MG and BEN. In B11221, a total of 46 genes exhibited significant induction by both MG and BEN, including *MGD1* and *MDR1*. Many of the 44 other genes have predicted roles in assimilation and biosynthesis of sulfur-containing compounds or xenobiotic

transport (**Fig. 5C** and **Table S1**). MG also induced expression of many genes with predicted roles in the biosynthesis of amino acids. The two genes most highly upregulated upon MG treatment, in terms of fold change, in this strain were orthologous to the arginine biosynthesis genes *ARG3* ($\log_2FC = 4.77$) and *ARG1* ($\log_2FC = 4.72$) (**Fig. 2C** and **Table S1**). Conversely, BEN had a limited effect on expression of amino acid biosynthesis genes (**Table S1**). There were also common themes among the genes that were significantly repressed by both MG and BEN in B11221. Genes that were repressed by both MG and BEN included four orthologs of the *HGT* glucose transporter family, five genes with a predicted role in uptake of iron and/or copper, and *ERG6*, which encodes an enzyme in the ergosterol biosynthesis pathway (**Fig. 5C** and **Table S2**). The genes that were repressed by only one stimulus, MG or BEN, also included those involved in ergosterol biosynthesis and the uptake of iron, copper, or glucose (**Fig. 5C**, **Table S2**). In general, the transcriptional response of the *mrr1a* Δ mutant to MG and BEN was similar to that of B11221 WT (**Fig. S2** and **Tables S1-S2**). The complete datasets for MG and BEN responses are available in **Supplementary Files S2** and **S3**, respectively.

Discussion

In this work, we have demonstrated that in *C. auris*, the zinc-cluster transcription factor Mrr1a, which is orthologous to Mrr1 in other *Candida* species, strongly regulates expression of a putative MG reductase *MGD1* in addition to *MDR1*, and that Mrr1a plays a role in MG resistance, highlighting a function of Mrr1 that is distinct from antifungal resistance. We also compared basal global gene expression in B11221 and AR0390 and found that *MDR1*, *MGD1*, and *MGD2* were among the genes significantly more highly expressed in B11221, consistent with the higher MG resistance of this isolate relative to AR0390. These differences were

explained by our finding that *MRR1a* from B11221 encoded a higher activity variant than that from AR0390 as evidenced by a higher FLZ MIC, higher expression of *MDR1* and *MGD1*, and higher MG resistance in the strain expressing *CauMRR1a*^{N647T} compared to the isogenic strain expressing *CauMRR1a*. The allele from B11221, which contains an N647T amino acid substitution (25, 105) which is in the central region of the regulator where other gain of function substitutions have been found. Both alleles result in induction of *MDR1* and *MGD1* in response to BEN but not to MG in *C. lusitaniae*, suggesting that these two compounds activate Mrr1-dependent transcription through different mechanisms.

Under the conditions tested, Mrr1a regulation in the *C. auris* B11221 background was mainly of *MGD1* and *MDR1*. Homologs of *MDR1* and at least one gene encoding a known or predicted MG reductase are co-regulated by Mrr1 in *C. albicans* (44, 45, 94-96), *C. parapsilosis* (97), and *C. lusitaniae* (37, 40, 58), suggesting that the co-regulation of these two genes has been conserved throughout multiple *Candida* species. Gaining a deeper understanding of the evolutionary and biochemical relationship between methylglyoxal reductases and efflux pumps, particularly Mdr1, may shed light on how *Candida* species sense and respond to environmental or physiological stresses, evade host defense mechanisms, and develop antifungal resistance. In all other *Candida* species with published Mrr1 regulons, however, Mrr1 appears to regulate expression of many more genes than the four we have described here in the *C. auris* strain B11221 (37, 40, 44, 45, 97). The surprisingly small number of *C. auris* genes whose expression was significantly altered by genetic deletion of *MRR1a* may be due to possible redundancy between *MRR1a* and the other two *MRR1* orthologs in *C. auris*, *MRR1b* and *MRR1c*, although further studies would be necessary to test this hypothesis. It is striking, however, that *MRR1a* alone seems to be necessary for expression and induction of *MGD1*, which is further supported

365 by our observation that only the *mrr1a*Δ mutant had a growth defect in MG compared to parental
366 B11221 (**Fig. 1A**).

367 Our demonstration of increased basal activity of the *CauMRR1a*^{N647T} allele compared to
368 the allele from AR0390 supports the hypothesis put forth by Iyer et al. that the N647T substitution
369 found in many Clade III isolates is a gain-of-function mutation (105). Furthermore, this may
370 explain why deletion of *MRR1a* leads to a mild decrease in azole resistance in B11221, but not
371 in the Clade IV isolate B11243 (46). In *C. albicans*, knocking out gain-of-function *MRR1* causes
372 a significant decrease in FLZ resistance, but knocking out *MRR1* with wild-type transcriptional
373 activity does not alter FLZ resistance (41, 44, 45, 106). Similarly, knocking out gain-of-function
374 *MRR1* in *C. lusitaniae* also decreases FLZ resistance, although knocking out *MRR1* alleles that
375 do not encode a constitutively active protein generally leads to increased FLZ resistance (37).

376 Although Mrr1a does not appear to play a major role in *C. auris* azole resistance (46), our
377 findings suggest that it contributes to resistance against MG, which may be encountered in the
378 host environment. We have previously shown that Mrr1 also contributes to MG resistance in *C.*
379 *lusitaniae* in a manner that is partially dependent on *MGD1* and *MGD2* (58). Indeed, gain-of-
380 function mutations in *MRR1* may arise in various *Candida* species due to selective pressures
381 other than azoles. In *C. lusitaniae*, we have reported the emergence of gain-of-function
382 mutations in *MRR1* among isolates from a patient with no prior history of clinical antifungal use
383 (40). In *C. auris*, most sequenced clade III isolates exhibit both the *MRR1a*^{N647T} allele and the
384 *ERG11*^{F126L} allele (25), the latter of which has been shown to be a major contributor to azole
385 resistance (31). Although it is not known whether the *MRR1a* or *ERG11* mutation occurred first
386 in the clade III lineage, it seems plausible that if the *ERG11* mutation did occur first, evolution of
387 the *MRR1a*^{N647T} allele in *C. auris* is likely to be the result of selection for *MGD1* expression and/or

an unknown role for Mdr1 that is unrelated to azole resistance. Therefore, we hypothesize that Mrr1 may act, either directly or indirectly, as a response regulator for carbonyl stress in *Candida* species, and future studies will investigate a possible role for Mrr1 in resistance against other physiologically relevant reactive carbonyl compounds.

Curiously, although both variants of *C. auris* Mrr1a were inducible by BEN when expressed in *C. lusitanae*, they were not inducible by MG under the conditions tested (**Fig. 4E-F**). One possible hypothesis for this observation is that Mrr1 must interact with at least one particular binding partner to induce transcription in response to MG, and that *C. auris* Mrr1a does not bind efficiently to this *C. lusitanae* Mrr1-binding protein or complex. Differential requirements for Mrr1-dependent transcriptional activation by chemical stressors have reported in *C. albicans*. For example, the transcription factor Mcm1 is required for Mrr1-dependent induction of *MDR1* in response to BEN but not to H₂O₂ (101), and the redox-sensing transcription factor Cap1 is required for *MDR1* induction by H₂O₂ and may play a role in *MDR1* induction by BEN (44). Furthermore, gain-of-function Mrr1 in *C. albicans* requires the Swi/Snf chromatin remodeling complex to maintain promoter occupancy, and the kinase Ssn3, which is a subunit of the Mediator complex, may act in opposition to Mrr1 or its coactivators (38). Thus, although *C. auris* Mrr1a can complement Mrr1-dependent basal and BEN-induced expression of *MDR1* and *MGD1* in *C. lusitanae*, it may be incompatible with certain elements of the *C. lusitanae* MG-responsive transcriptional machinery. Further studies on the differences between *C. lusitanae* and *C. auris* Mrr1, particularly in the presence of MG, may elucidate more detailed mechanisms of Mrr1 activation.

In general, we observed substantial upregulation of genes with predicted roles in transmembrane transport, sulfur metabolism, and amino acid biosynthesis in response to MG in

all three strains tested. Many genes downregulated in response to MG in all three strains have predicted roles in metal acquisition, particularly iron, and carbohydrate metabolism. In both B11221 WT and *mrr1a*Δ, BEN treatment led to differential expression of similar groups of genes as MG in addition to induction of genes with predicted roles in oxidative stress response. Our studies of the transcriptional response of *C. auris* to MG and BEN contribute to the understanding of how *Candida* species may adapt to oxidative and/or carbonyl stress, two types of stress that a pathogen is likely to encounter in the host environment. In humans, elevated serum MG has been reported in diabetes as well as in renal failure, which are both risk factors for *Candida* infection (107, 108). There is also evidence that neutrophils (109) and macrophages (110, 111) generate MG during the inflammatory response, consistent with elevated levels of MG in sepsis patients (77). In our transcriptomics analysis of three *C. auris* strains exposed to 5 mM MG for 15 min, upregulation of numerous genes involved in amino acid uptake, metabolism, and biosynthesis was one of the most striking responses to MG (**Tables S1** for comparison of MG and BEN and **S5** for the comparison of genes induced by MG in B11221 and/or AR0390). In particular, induction of *ARG* genes is interesting considering the report that *C. albicans* upregulates expression of arginine biosynthesis genes when phagocytosed by macrophages or in response to sublethal concentrations of hydrogen peroxide, tert-butyl hydroperoxide, or menadione *in vitro* (112). This induction of *ARG* genes in *C. albicans* by macrophages is dependent on the *gp91^{phox}* subunit of the macrophage oxidase, and thus is likely a direct response to oxidative stress rather than arginine depletion (112). In our dataset, *ARG3* and *ARG1* exhibited the highest log₂FC in response to MG in the B11221 background, independently of *MRR1a* (**Table S1**). We also observed, in all three *C. auris* strains, induction of several *MET* genes, which are involved in methionine synthesis and are an important branch of sulfur

assimilation in yeast. Other genes involved in sulfur acquisition and assimilation that were induced by MG include the sulfate importer *SUL2*, a gene orthologous to both *CYS3* and *STR3* of *S. cerevisiae*, and numerous genes associated with iron-sulfur cluster formation (**Table S1**). A gene orthologous to *MUP1* of *S. cerevisiae* and *C. albicans* was induced by MG in B11221 WT and AR0390 but fell short of the $\log_2FC \geq 1.00$ cutoff in *mrr1a* Δ (**Table S1** and **Supplementary File S2**). Induction of genes involved in sulfur metabolism, including the *MET* pathway, *SUL2*, *CYS3*, *STR3*, and *MUP1*, has previously been observed in *Saccharomyces cerevisiae* exposed to 1g/L acetaldehyde (113), another reactive aldehyde metabolite that is structurally similar to MG. Thus, sulfur acquisition and metabolism may be an important part of the carbonyl stress response in yeast.

In the B11221 background, we observed modest overlap in the genes and groups of genes that were up- or down- regulated in response to either MG or BEN. *MDR1* and *MGD1* were among the genes induced by both compounds, and induction of *MGD1* by either MG or BEN was completely dependent on *MRR1a*. Although BEN, which originated as an agricultural fungicide, is widely recognized as an inducer of expression of Mrr1-regulated genes in *Candida* species (37, 41, 43, 95, 99-104), the mechanism by which this induction occurs is not yet known. BEN is thought to cause oxidative stress in yeast (114, 115), which is consistent with our observation of an upregulation of genes with a predicted role in oxidative stress response in BEN-treated *C. auris* cultures (**Table S1**). Additionally, in mammalian cells, BEN exposure has been shown to inhibit aldehyde dehydrogenase enzymes (116-119), which may lead to an accumulation of reactive aldehydes, although this possible mechanism has not yet been investigated in fungi.

We also note similarities between the results of our study of MG- and BEN- treated *C. auris* and the recently published transcriptional analysis of the Clade I *C. auris* strain NCPF 8973 exposed to 75 μ M farnesol (120). In response to farnesol, the authors reported upregulation of many genes with predicted roles in transmembrane transport, such as *MDR1* and *CDR1*, and downregulation of numerous genes predicted to be involved in metal acquisition and homeostasis, including multiple ferric reductases and iron permeases (120). As farnesol may cause oxidative stress in *Candida* species (120-123) and in *S. cerevisiae* (124, 125), the overlap in transcriptional changes in response to MG, BEN, and farnesol likely provides valuable insight into how *C. auris* and other *Candida* species sense and adapt to physiologically relevant stressors. In fact, MG itself may serve as a stress signal in various organisms. In plants, for example, intracellular MG increases in response to drought (126, 127), salinity (126, 128-131), cold stress (126), heavy metals (128), or phosphorous deficiency (131), and overexpression of certain genes involved in MG detoxification has been shown to enhance salt tolerance in tobacco (126) and in *Brassica juncea* (132). Investigating whether MG detoxification is linked to abiotic stressors such as salt, temperature, or desiccation in *Candida* species would be an interesting avenue of future research, particularly in *C. auris* due to its persistence on hospital surfaces and high salt tolerance.

Methods

Strains, media, and growth conditions

The sources of all strains used in this study are listed in **Table S7**. All strains were stored long term in a final concentration of 25% glycerol at -80°C and freshly streaked onto yeast extract peptone dextrose (YPD) agar (10 g/L yeast extract, 20 g/L peptone, 2% glucose, 1.5% agar)

once every seven days and maintained at room temperature. Unless otherwise noted, all overnight cultures were grown in 5 mL YPD liquid medium (10 g/L yeast extract, 20 g/L peptone, 2% glucose) on a rotary wheel at 30°C. Media was supplemented with 25 µg/mL BEN (stock 10 mg/ml in DMSO) or 5 mM, 10 mM, or 15 mM MG (Sigma-Aldrich, 5.55 M) as noted. *E. coli* strains were grown in LB with 15 µg/mL gentamycin (gent).

Plasmids for complementation of *C. auris* *MRR1a*

Plasmids for complementing *C. auris* *MRR1a* into *C. lusitaniae* were created as follows: the open reading frame of *MRR1a* was amplified from the genomic DNA of *C. auris* isolates B11221 (for *CauMRR1a*^{N647T}) and AR0390 (for *CauMRR1a*) using a forward primer with homology to the 5' flank of *C. lusitaniae* *MRR1* and a reverse primer with homology to the 3' flank of *C. lusitaniae* *MRR1* for recombination into the *C. lusitaniae* *MRR1* complementation plasmid pMQ30^{MRR1-L1191H+Q1197*} (58). Plasmid pMQ30^{MRR1-L1191H+Q1197*} was digested with *Ascl* (New England BioLabs) and *AgeI*-HF (New England BioLabs). The PCR products and digested plasmid were cleaned using the Zymo DNA Clean & Concentrator kit (Zymo Research) and assembled using the *S. cerevisiae* recombination technique described in (133). Recombined plasmids were isolated from *S. cerevisiae* using a yeast plasmid miniprep kit (Zymo Research) before transformation into NEB®5-alpha competent *E. coli* (New England BioLabs). *E. coli* containing pMQ30-derived plasmids were selected for on LB containing 15 µg/mL gentamycin. Plasmids from *E. coli* were isolated using a Zyppy Plasmid Miniprep kit (Zymo Research) and subsequently verified by Sanger sequencing with the Dartmouth College Genomics and Molecular Biology Shared Resources Core. *MRR1a* complementation plasmids containing the correct sequences were linearized with *NotI*-HF (New England BioLabs), cleaned up with the

Zymo DNA Clean & Concentrator kit (Zymo Research) and eluted in molecular biology grade water (Corning) before transformation of 1.5 µg into *C. lusitaniae* strain U04 *mrr1*Δ as described below. All plasmids used and created in this study are listed in **Table S7**. All primers used in this study are listed in **Table S8**.

Transformation of *C. lusitaniae* with *C. auris* *MRR1a* complementation constructs

Mutants in *C. lusitaniae* were generated using an expression-free CRISPR-Cas9 method as previously described (37, 58, 134). In brief, cells suspended in 1M sorbitol were electroporated immediately following the addition of 1.5 µg of *C. auris* *MRR1a* complementation plasmid that had been previously linearized with NotI-HF (New England BioLabs) and Cas9 ribonucleoprotein containing crRNA targeting the *NAT1* gene. Transformants were selected on YPD agar containing 600 µg/ml hygromycin B (HygB). Successful transformants were identified via PCR of the *C. lusitaniae* *MRR1* locus as previously described (37, 58). CRISPR RNAs (crRNAs; IDT) and primers used to validate transformants are listed in **Table S8**.

Minimum Inhibitory Concentration (MIC) Assay

MIC assays for FLZ were performed in RPMI-1640 medium (Sigma, containing L-glutamine, 165 mM MOPS, 2% glucose at pH 7) as described in (40) and (58) using the broth microdilution method. The final concentration of FLZ in each well ranged from 64 µg/mL to 0.125 µg/mL. Plates were incubated at 35°C and scored for growth at 24 and 48 hours; the results are reported in **Table S4**. The MIC was defined as the drug concentration that abolished visible growth compared to a drug-free control.

Growth Kinetics

Growth kinetic assays were performed as previously described in (58). In brief, exponential-phase cultures of *C. auris* or *C. lusitaniae* were washed and diluted in dH₂O to an OD₆₀₀ of 1; 60 µL of each diluted cell suspension was added to 5 mL fresh YPD. To each well of a clear 96-well flat-bottom plate (Falcon) was added 100 µL of YPD or YPD with MG at twice the desired final concentration and 100 µL of cell inoculum in YPD. Plates were arranged in technical triplicate for each strain and condition and incubated in a Synergy Neo2 Microplate Reader (BioTek, USA) according to the following protocol: heat to 37°C, start kinetic, read OD₆₀₀ every 60 minutes for 36 hours, end kinetic. Results were calculated in Microsoft Excel and plotted in GraphPad Prism 9.0.0 (GraphPad Software).

Quantitative Real-Time PCR

Overnight cultures of *C. auris* or *C. lusitaniae* were diluted 1:50 into 5 mL fresh YPD, and grown to for four hours at 37°C. To each culture was added MG to a final concentration of 5 mM (4.5 µL stock), BEN to a final concentration of 25 µg/mL (12.5 µL stock), or 4.5 µL molecular biology grade dH₂O. Cultures were returned to the roller drum at 37°C for 15 min (MG or dH₂O) or 30 min (BEN), then centrifuged at 5000 rpm for 5 min. The differences in time of exposure in the experimental scheme was used to maintain consistency with published experiments in other species, and not because of known differences in kinetics of activity for the two inducers. RNA isolation, gDNA removal, cDNA synthesis, and quantitative real-time PCR were performed as previously described (40). Transcripts were normalized to *C. auris* or *C. lusitaniae* *ACT1* expression as appropriate. Results were calculated in Microsoft Excel and plotted in GraphPad Prism 9.0.0 (GraphPad Software). Primers are listed in **Table S8**.

548

549 **RNA sequencing**

550 Overnight cultures of *C. auris* were diluted to an OD₆₀₀ of 0.1 in 5 mL fresh, pre-warmed
551 YPD, and incubated on a roller drum at 37°C for 5-6 doublings (approx. 6 hours). Cultures were
552 diluted once more to an OD₆₀₀ of 1 in 5 mL fresh, pre-warmed YPD and returned to the roller
553 drum at 37°C for another 5-6 doublings. To each culture was added MG to a final concentration
554 of 5 mM (4.5 µL), BEN to a final concentration of 25 µg/mL (12.5 µL), or 4.5 µL molecular biology
555 grade dH₂O. Cultures were returned to the roller drum at 37°C for 15 min (MG or dH₂O) or 30
556 min (BEN), then centrifuged at 5000 rpm for 5 min. Supernatants were discarded and RNA
557 isolation was performed on cell pellets as described above for qRT-PCR. gDNA was removed
558 from RNA samples as described above. DNA-free RNA samples were sent to the Microbial
559 Genome Sequencing Center (<https://www.migscenter.com/>) for RNA sequencing.

560

561 **Analysis of RNAseq**

562 RNAseq data were analyzed by the Microbial Genome Sequencing Center
563 (<https://www.migscenter.com/>) as follows: Quality control and adapter trimming was performed
564 with bcl2fastq ([https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-](https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html)
565 [conversion-software.html](https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html)). Read mapping was performed with HISAT2 (135). Read
566 quantification was performed using Subread's featureCounts (136) functionality. Read counts
567 were loaded into R (<https://www.R-project.org/>) and normalized using edgeR's (137) Trimmed
568 Mean of M values (TMM) algorithm. Subsequent values were then converted to counts per
569 million (cpm). Differential expression analysis was performed using edgeR's Quasi Linear F-
570 Test. In the supplementary files, sheets named "All Quantified Genes" contain the results of the

exact test for all genes in addition to the normalized counts per million. Differentially expressed genes were determined using the cutoff of $|\log FC| > 1$ and $p < .05$.

Identification of orthologs

Orthologs of *C. auris* genes in *C. albicans*, *C. lusitaniae*, and *S. cerevisiae*, as well as orthologs between B11221 and the Clade I reference strain B8441, were identified using FungiDB (<https://fungidb.org>) (138, 139).

Generation of Venn diagrams

Venn diagrams of differentially expressed genes across different strains and conditions were computed using the Venn diagram tool from UGent Bioinformatics & Evolutionary Genomics, which is accessible at <https://bioinformatics.psb.ugent.be/webtools/Venn/>.

Statistical Analysis and Figure preparation

All graphs were prepared with GraphPad Prism 9.0.0 (GraphPad Software). Ratio paired t-tests and one-way ANOVA tests were performed in Prism; details on each test are described in the corresponding figure legends. All p-values were two-tailed and $p < 0.05$ were considered significant for all analyses performed and are indicated with asterisks in the text: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Data availability

The data supporting the findings in this study are available within the paper and its supplemental material and are also available from the corresponding author upon request. The

raw sequence reads from the RNA-Seq analysis have been deposited into NCBI sequence read archive under BioProject PRJNA801628.

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Author contributions. ARB and DAH conceived and designed the experiments and wrote the paper. ARB performed the experiments. ARB and DAH analyzed the data.

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Figure Legends

Figure 1. Mrr1a regulates expression of *MGD1* and *MDR1* in *C. auris* isolate B11221. A) Growth curves of B11221 WT (blue) and its *mrr1a*Δ (red), *mrr1b*Δ (green), and *mrr1c*Δ (purple) derivatives in YPD + 10 mM MG. Data shown represent the mean ± SD for three independent experiments. **B-C)** qRT-PCR assessment of *MGD1* (**B**) and *MDR1* (**C**) expression in B11221 WT (blue) and *mrr1a*Δ (red) cultures grown to exponential phase in YPD at 37°C. Data shown represent the mean ± SD for three independent experiments. Ratio paired t-test was used for statistical evaluation; * p < 0.05. **D)** Volcano plot of all quantified genes in B11221 WT vs *mrr1a*Δ in the control condition. Each point represents a single gene; blue points indicate genes significantly more highly expressed in WT; red points indicate genes significantly more highly expressed in *mrr1a*Δ. Numbers adjacent to each colored point indicate the log₂FC in *mrr1a*Δ versus WT.

Figure 2. MG and BEN both lead to a vast transcriptional response in *C. auris* B11221, which includes upregulation of *MDR1* and *MGD1*. A-B) qRT-PCR analysis for expression of *MGD1* (**A**) and *MDR1* (**B**) in exponential-phase cultures of B11221 WT (blue) or *mrr1a*Δ (red) treated with MG or BEN as indicated. Data shown represent the mean ± SD for three independent experiments. Ratio paired t-test was used for statistical evaluation; ns p > 0.05, * p < 0.05, ** p < 0.01. **C-D)** Volcano plots of all quantified genes in B11221 WT treated with either MG (**C**) or BEN (**D**). Each point represents a single gene; magenta points indicate genes that were significantly upregulated compared to the control condition, teal points indicate genes that were significantly downregulated compared to the control condition. *MDR1* and *MGD1* are

shown along with the two most up- and down- regulated genes in each condition. **E-F)** Scatter plots of the average CPMs of all quantified genes in *mrr1Δ* vs. B11221 WT treated with MG (**E**) or BEN (**F**). Each point represents a single gene. Points below the dotted line indicate genes that were more highly expressed in the WT, and points above the dotted line indicated genes that were more highly expressed in the *mrr1Δ* mutant. *MDR1* and *MGD1* are shown with red dots for reference.

Figure 3. *MDR1* and *MGD1* are among the genes significantly more highly expressed in isolate B11221 compared to isolate AR0390. **A)** Growth curves of B11221 (blue) and AR#0390 (orange) in YPD + 10 mM MG. Data shown represent the mean ± SD for three independent experiments. **B-C)** qRT-PCR assessment of *MGD1* (**B**) and *MDR1* (**C**) expression in B11221 (blue) and AR0390 (orange) grown to exponential phase in YPD at 37°C. Data shown represent the mean ± SD for three independent experiments. Ratio paired t-test was used for statistical evaluation; * $p < 0.05$, **** $p < 0.0001$. **D)** Volcano plot of all quantified genes, matched by syntenic ortholog, in B11221 and AR0390 in the control condition (YPD). Each point represents a single gene; blue points indicate genes significantly more highly expressed in B11221; orange points indicate genes significantly more highly expressed in AR0390. **E-F)** qRT-PCR expression analysis for *MGD1* (**E**) and *MDR1* (**F**) in *C. lusitaniae* U04 *mrr1Δ* (grey) and its derivatives expressing *CauMRR1a*^{N647T} (dark blue) or *CauMRR1a* (brown). Data shown represent the mean ± SD for three independent experiments. One-way ANOVA was used for statistical evaluation; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **G)** Growth curves of *C. lusitaniae* U04 *mrr1Δ* (grey) and its derivatives expressing *CauMRR1a*^{N647T} (dark blue) or *CauMRR1a* (brown) in YPD + 15 mM MG. One representative experiment of three independent

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Figure 4. MG induces expression of *MGD1* and *MDR1* in *C. auris* isolates B11221 and AR0390, but *C. auris MRR1a* is not inducible by MG when heterologously expressed in *C. lusitaniae*. A-B) qRT-PCR analysis for expression of *MGD1* (A) and *MDR1* (B) in exponential-phase cultures of B11221 (blue) or AR0390 (orange) treated with MG as indicated. Data shown represent the mean $\pm$ SD for three independent experiments. Ratio paired t-test was used for statistical evaluation; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$. (C-F) qRT-PCR analysis for expression of *MGD1* (C,E) and *MDR1* (D,F) in exponential-phase cultures of *C. lusitaniae* U04 *mrr1* Δ (grey) and its derivatives expressing *CauMRR1a*^{N647T} (dark blue) or *CauMRR1a* (brown) treated with 5 mM MG for 15 min (C,D) or 25 μ g/mL BEN for 30 min (E,F). Data shown represent the mean $\pm$ SD for three independent experiments. Ratio paired t-test was used for statistical evaluation; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

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Figure S2. The transcriptional response of *mrr1a*Δ to either MG or BEN is overall similar to that of the B11221 WT parent strain. Volcano plots of all quantified genes in the *mrr1a*Δ mutant treated with either MG (**A**) or BEN (**B**). Each point represents a single gene; magenta points indicate genes that were significantly upregulated compared to the control condition, teal points indicate genes that were significantly downregulated compared to the control condition. *MDR1* is shown along with the two most up- and down- regulated genes in each condition.

Figure S3. *C. auris* strain AR0390 has a growth advantage over B11221 in YPD but loses that advantage in the presence of increasing concentrations of MG. Growth curves of B11221 (blue) and AR0390 (orange) in YPD (left), or YPD supplemented with 5 mM (middle), or 15 mM (right) MG. Data shown represent the mean ± SD for three independent experiments.

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Figure S5. Treatment with 5 mM MG leads to the differential expression of more genes in AR0390 than in B11221. Volcano plot of all quantified genes in AR0390 treated with MG. Each point represents a single gene; magenta points indicate genes that were significantly upregulated compared to the control condition, teal points indicate genes that were significantly downregulated compared to the control condition. *MDR1* and *MGD1* are shown for reference.

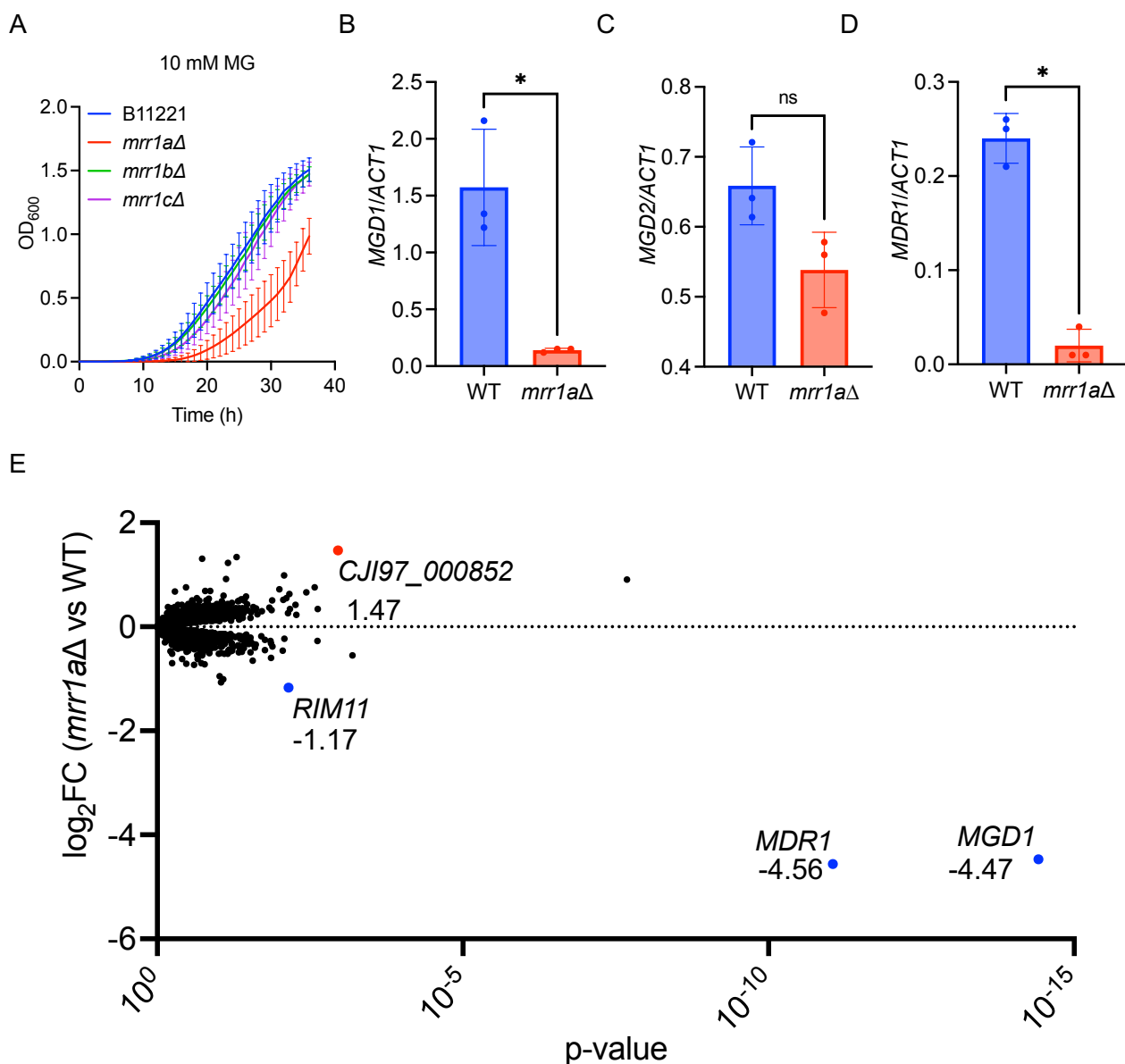


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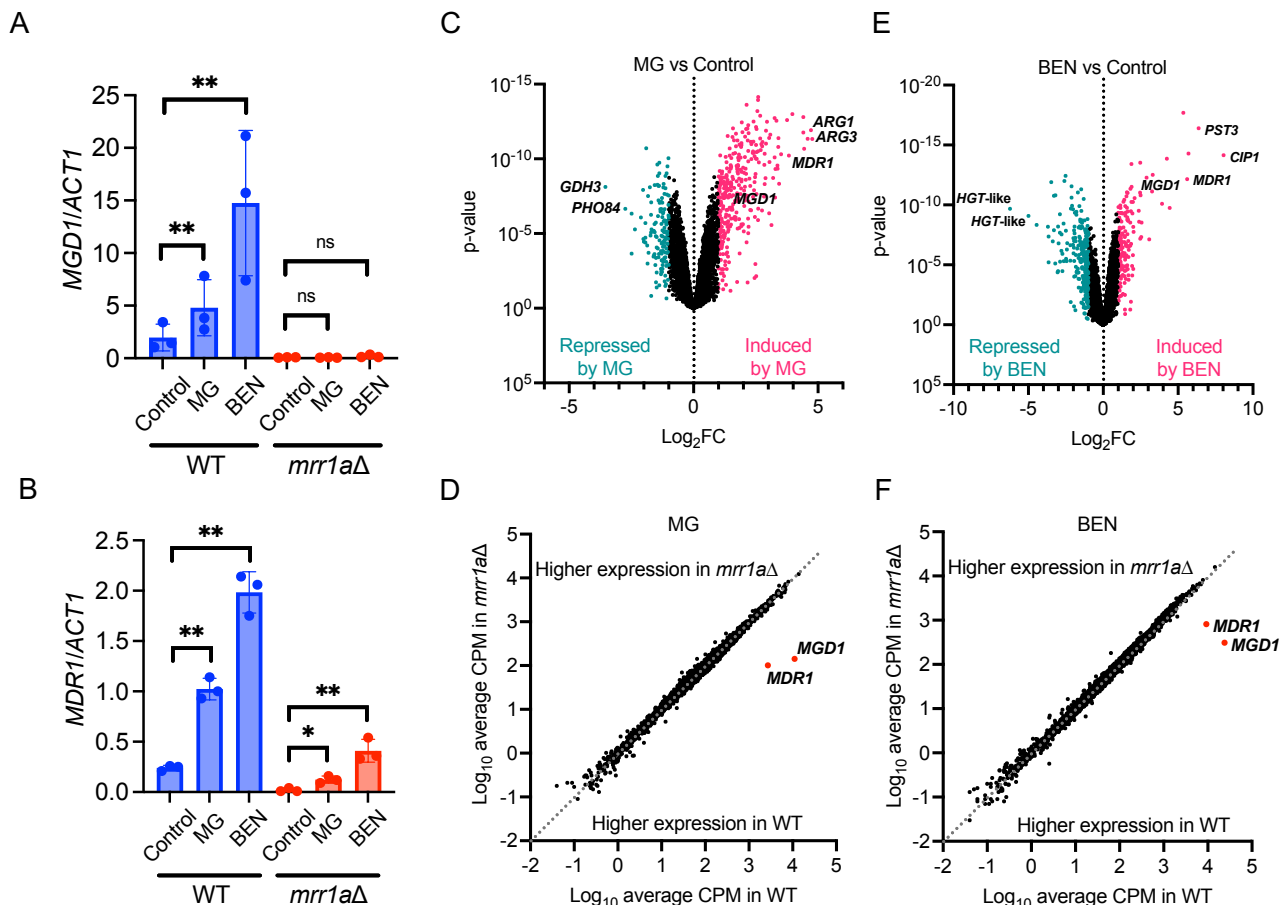


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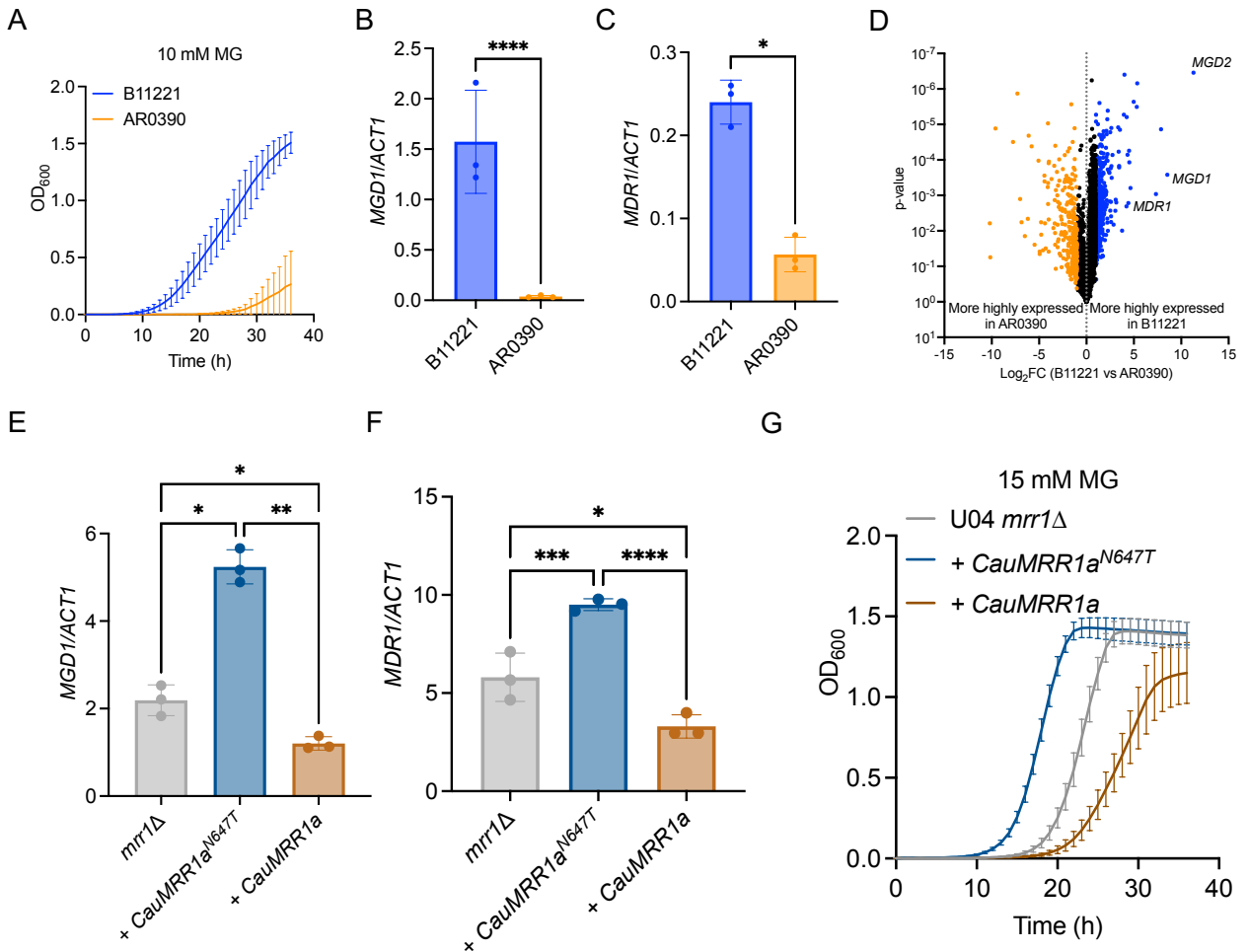


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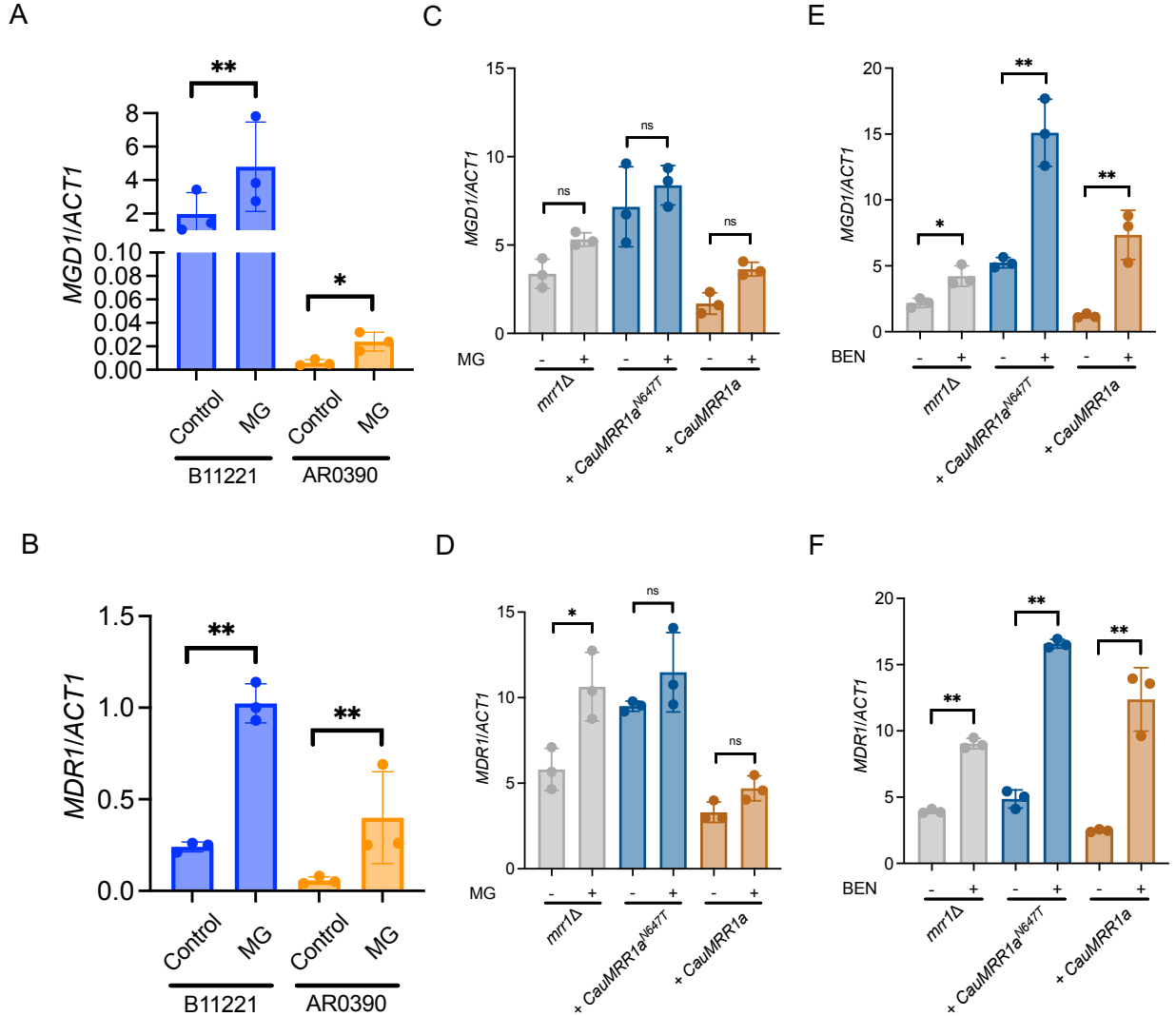


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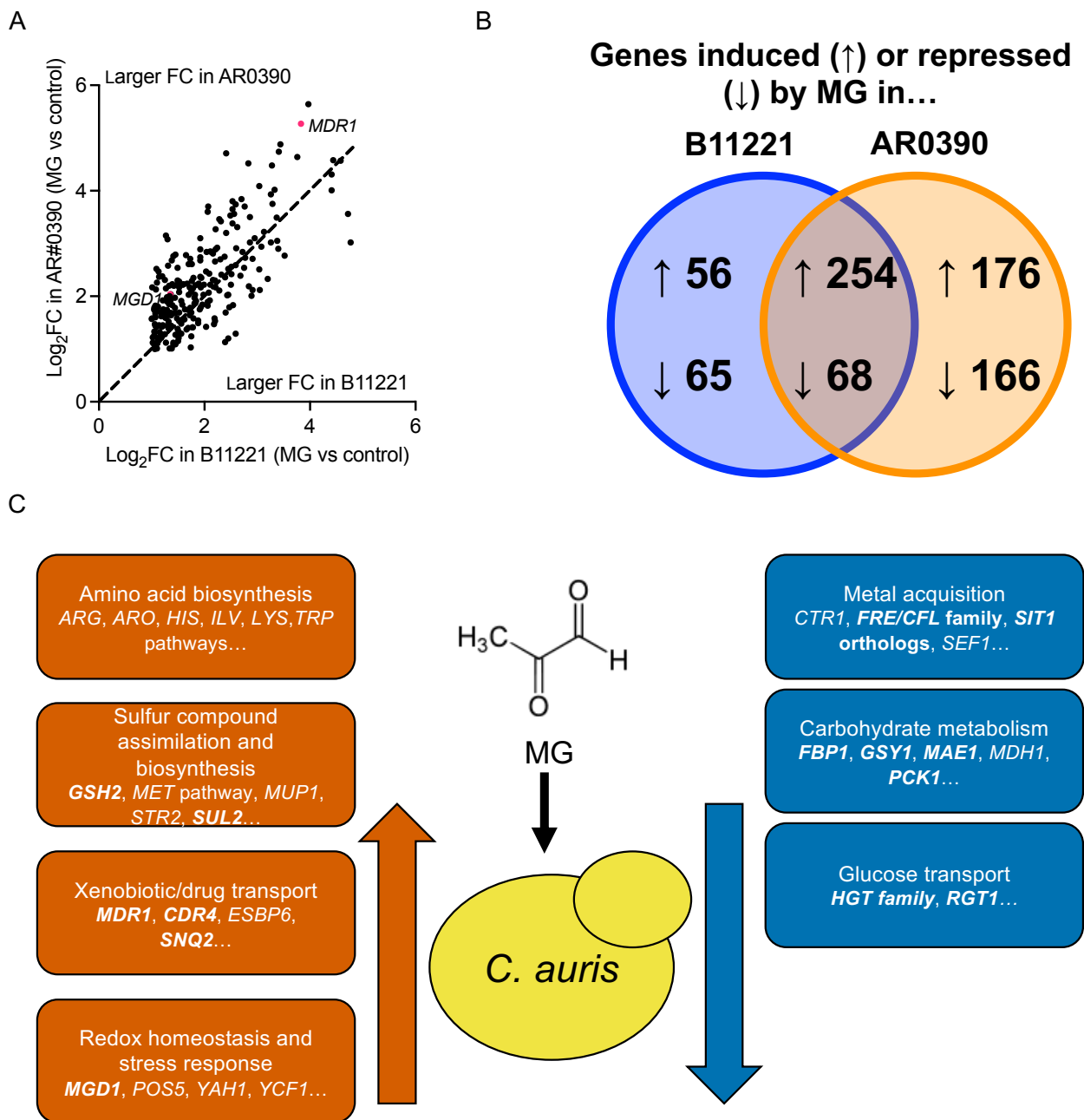


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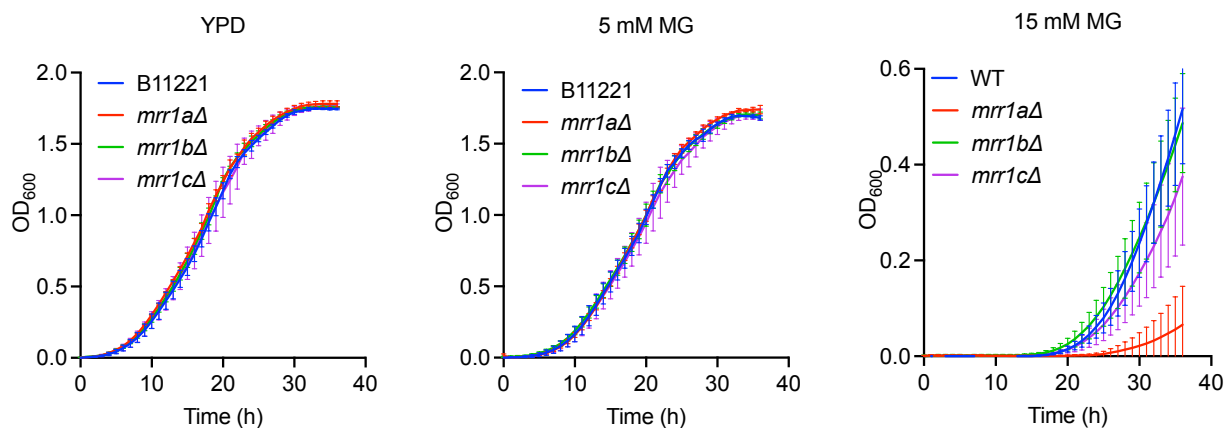


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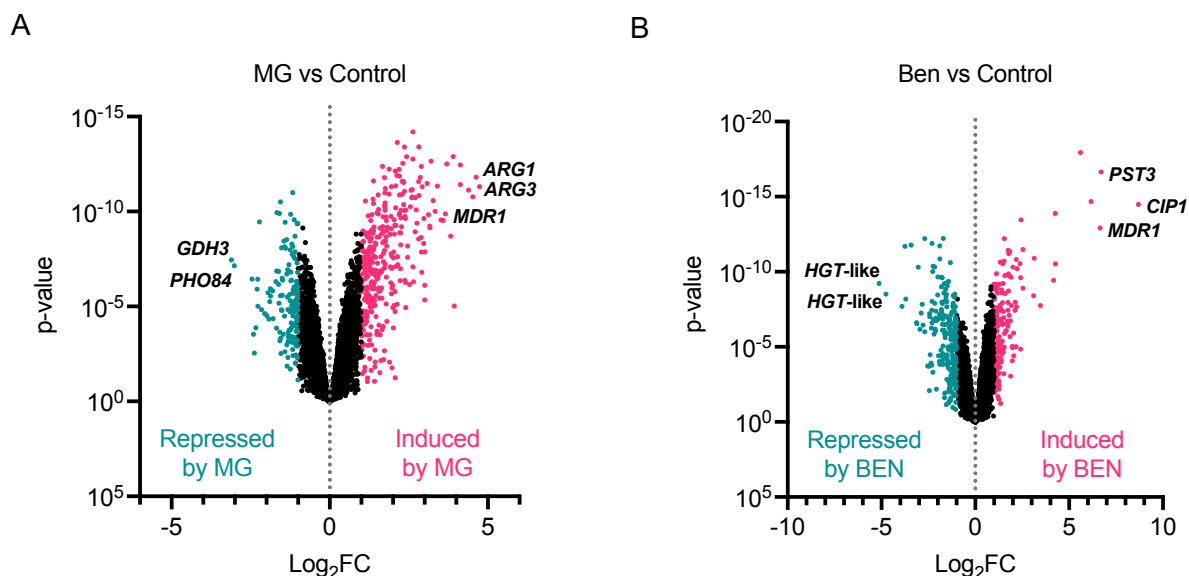


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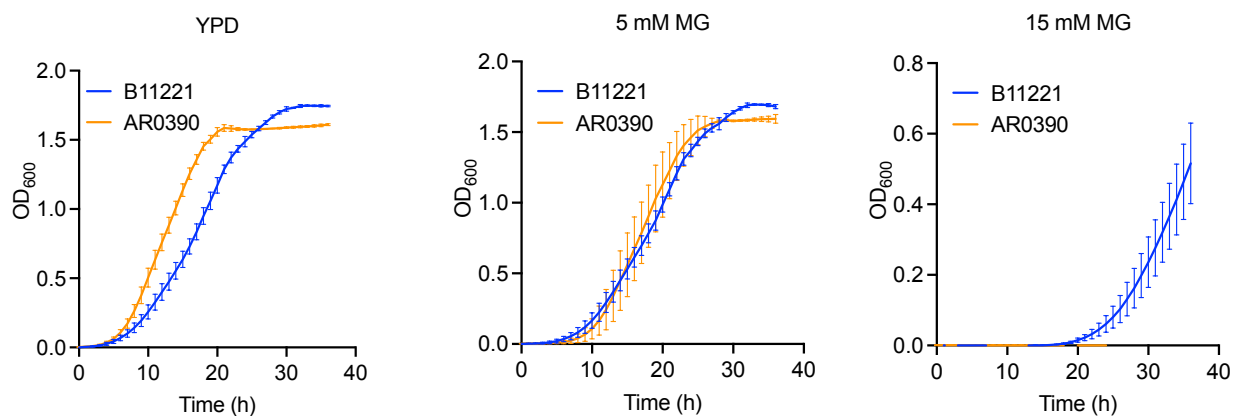


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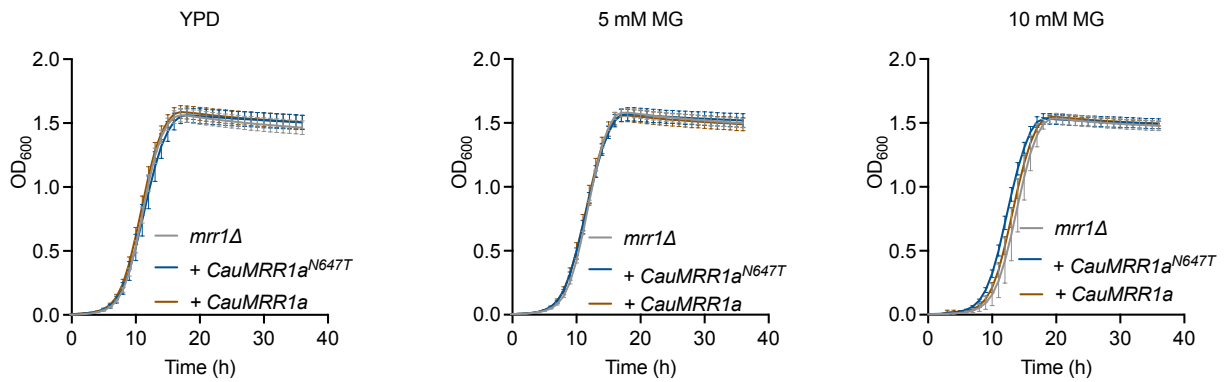


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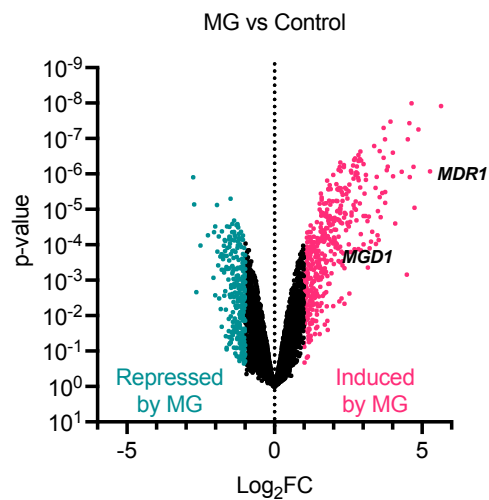


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Table S1. Select genes significantly upregulated by MG and/or BEN in the B11221 background.

Sulfur compound assimilation and biosynthesis						
Locus Tag	Gene Name	Predicted function	WT MG Log₂FC	WT BEN Log₂FC	<i>mrr1a</i>Δ MG Log₂FC	<i>mrr1a</i>Δ BEN Log₂FC
CJI97_001242	<i>AGP3</i>	Serine transporter	1.06	0.80	1.36	0.42
CJI97_002494	<i>DUG1</i>	Glutathione hydrolase	1.42	-0.02	1.38	-0.46
CJI97_001665	<i>CYS3</i>	Peroxisomal cystathionine beta-lyase	1.37	0.42	1.39	0.30
CJI97_001939	<i>CFD1</i>	Role in Fe-S cluster assembly	-0.02	1.17	0.26	1.31
CJI97_001514	<i>CIA1</i>	Role in protein maturation by Fe-S cluster transfer	0.40	1.12	0.34	1.20
CJI97_004156	<i>DRE2</i>	Cytosolic Fe-S protein assembly protein	1.09	1.08	1.15	0.98
CJI97_001503	<i>ECM4</i>	Cytoplasmic glutathione S-transferase	0.48	1.55	0.33	1.73
CJI97_001382	<i>ECM17</i>	Sulfite reductase beta subunit	1.14	0.76	1.45	0.33
CJI97_001705	<i>GCS1</i>	Gamma-glutamylcysteine synthetase	0.78	2.00	1.06	1.75
CJI97_003892	<i>GLR1</i>	Glutathione reductase	-0.04	1.17	0.06	1.11
CJI97_005081	<i>GSH2</i>	Glutathione synthase	1.07	1.41	0.98	1.13
CJI97_003274	<i>GTO1</i>	Cytoplasmic glutathione S-transferase	1.39	3.93	1.93	4.27
CJI97_001739	<i>JLP1</i>	Sulfonate dioxygenase	0.38	1.44	0.42	1.32
CJI97_002076	<i>MET1</i>	Uroporphyrin-3 C-methyltransferase	1.18	1.55	1.99	1.33
CJI97_002761	<i>MET2</i>	Homoserine acetyltransferase	2.20	0.49	2.31	0.30
CJI97_004689	<i>MET8</i>	Dehydrogenase, ferrochelatase	1.83	0.30	1.65	0.65
CJI97_003625	<i>MET10</i>	Sulfite reductase	1.01	0.94	1.31	0.86
CJI97_003066	<i>MET14</i>	Adenylylsulfate kinase	1.08	0.27	1.50	0.61
CJI97_005391	<i>MET16</i>	3'-phosphoadenylylsulfate reductase	1.63	1.44	1.95	1.29
CJI97_003613	<i>MUP1</i>	High affinity methionine permease	1.05	0.36	0.94	-0.06
CJI97_004493	<i>MUP3</i>	L-methionine transmembrane transporter	0.31	1.65	0.20	1.59
CJI97_004842	N/A	Role in Fe-S cluster assembly	-0.08	1.00	0.08	0.60
CJI97_005600	N/A	Cystathionine gamma-synthase	1.56	0.34	1.45	0.52
CJI97_001635	N/A	Glutathione S-conjugate transporter	0.05	1.10	0.12	0.90
CJI97_000433	<i>SPE2</i>	S-adenosylmethionine decarboxylase	1.11	0.19	1.14	0.05
CJI97_000171	<i>SRX1</i>	Sulfiredoxin	2.21	3.07	2.03	3.47
CJI97_003300	<i>STR2</i>	Cystathionine gamma-synthase	1.15	0.21	1.11	0.15
CJI97_001014	<i>SUL2</i>	Sulfate transporter	1.98	1.42	3.01	1.17
CJI97_003257	<i>TES1</i>	Acyl-CoA thioesterase	1.27	0.59	1.06	0.38
CJI97_001516	<i>TRR1</i>	Thioredoxin reductase	0.40	1.60	0.48	1.82
CJI97_000545	<i>TRX1</i>	Thioredoxin	-0.33	1.20	-0.82	2.05
Xenobiotic/Drug Transport						
Locus Tag	Gene Name	Predicted function	WT MG Log₂FC	WT BEN Log₂FC	<i>mrr1a</i>Δ MG Log₂FC	<i>mrr1a</i>Δ BEN Log₂FC
CJI97_002597	<i>AMF1</i>	MFS family transporter	0.35	1.28	0.71	1.29

CJI97_000167	<i>CDR1</i>	ABC family multidrug transporter	0.30	1.88	0.40	1.76
CJI97_000479	<i>CDR4</i>	ABC family multidrug transporter	1.34	1.13	1.27	1.14
CJI97_004181	<i>ERC1</i>	Xenobiotic transmembrane transporter	1.47	1.46	1.96	1.44
CJI97_004982	<i>ESBP6</i>	MFS membrane transporter	3.97	0.66	3.90	0.20
CJI97_002850	<i>FLU1</i>	Multidrug efflux pump of the plasma membrane	0.18	1.25	0.35	1.17
CJI97_002639	<i>MCH2</i>	MFS membrane transporter	1.36	0.04	1.24	-0.01
CJI97_000609	<i>MCH4</i>	MFS membrane transporter	2.13	0.47	2.14	0.33
CJI97_004042	<i>MDR1</i>	Plasma membrane MDR/MFS multidrug efflux pump	3.83	5.60	3.65	6.65
CJI97_000797	N/A	MFS membrane transporter	3.29	1.68	3.17	0.98
CJI97_005702	N/A	ABC family multidrug transporter	1.22	1.94	1.10	2.42
CJI97_005706	N/A	ABC family multidrug transporter	0.86	1.93	0.78	1.99
CJI97_005256	<i>QDR3</i>	MFS membrane transporter	2.83	-1.94	2.63	-2.88
CJI97_005513	<i>ROA1</i>	PDR-subfamily ABC transporter	2.86	0.05	3.58	0.01
CJI97_001481	<i>SNQ2</i>	ABC family multidrug transporter	1.50	2.56	1.34	2.19
CJI97_001817	<i>VBA1</i>	MFS transporter	0.68	1.18	0.64	0.95

Amino acid biosynthesis, excluding sulfur-containing amino acids

Locus Tag	Gene Name	Predicted function	WT MG Log ₂ FC	WT BEN Log ₂ FC	<i>mrr1Δ</i> MG Log ₂ FC	<i>mrr1Δ</i> BEN Log ₂ FC
CJI97_000687	<i>ARG1</i>	Argininosuccinate synthase	4.72	0.81	4.63	0.55
CJI97_004654	<i>ARG3</i>	Ornithine carbamoyltransferase	4.77	1.52	4.74	0.91
CJI97_002308	<i>ARG4</i>	Argininosuccinate lyase	1.75	0.39	1.67	0.21
CJI97_001846	<i>ARG5,6</i>	Arginine biosynthetic enzyme	1.50	0.60	1.46	0.19
CJI97_005293	<i>ARG8</i>	Acetylornithine aminotransferase	1.58	0.23	1.67	0.17
CJI97_002234	<i>ARO1</i>	Pentafunctional arom enzyme	1.49	-0.06	1.32	-0.20
CJI97_000465	<i>ARO2</i>	Chorismate synthase	2.40	0.53	2.37	0.12
CJI97_001597	<i>ARO3</i>	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase	1.74	-0.12	1.64	-0.02
CJI97_003954	<i>ARO4</i>	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase	2.50	0.36	2.38	0.18
CJI97_003913	<i>ARO7</i>	Chorismate mutase	1.51	0.08	1.30	0.37
CJI97_001973	<i>ASN1</i>	Asparagine synthetase	2.32	-0.20	2.06	-0.39
CJI97_001997	<i>BAT21</i>	Branched chain amino acid aminotransferase	2.51	0.24	2.61	0.10
CJI97_002013	<i>CPA2</i>	Carbamoyl-phosphate synthase subunit	1.78	0.15	1.83	-0.37
CJI97_005329	<i>HIS1</i>	ATP phosphoribosyl transferase	3.40	0.97	3.27	0.57
CJI97_003017	<i>HIS3</i>	Imidazoleglycerol-phosphate dehydratase	3.20	1.31	2.97	1.46
CJI97_003537	<i>HIS4</i>	Phosphoribosyl-AMP cyclohydrolase, phosphoribosyl-ATP diphosphatase, and histidinol dehydrogenase	2.86	0.94	2.73	0.33
CJI97_003604	<i>HIS5</i>	Histidinol-phosphate aminotransferase	2.72	0.73	2.60	0.49
CJI97_003946	<i>HIS7</i>	Imidazole glycerol phosphate synthase	2.39	0.63	2.34	0.55
CJI97_003721	<i>HOM2</i>	Aspartate-semialdehyde dehydrogenase	1.52	0.23	1.40	0.20
CJI97_003292	<i>HOM3</i>	L-aspartate 4-P-transferase	3.04	0.61	3.08	0.35

CJI97_003178	<i>ILV1</i>	Threonine dehydratase	2.06	0.53	1.93	0.28
CJI97_002682	<i>ILV2</i>	Acetolactate synthase	2.59	0.23	2.40	-0.17
CJI97_000020	<i>ILV3</i>	Dihydroxyacid dehydratase	2.77	0.75	2.60	0.37
CJI97_003514	<i>ILV5</i>	Ketol-acid reductoisomerase	1.99	-0.29	2.04	-0.50
CJI97_004523	<i>ILV6</i>	Regulatory subunit of acetolactate synthase	1.50	0.30	1.25	0.31
CJI97_004671	<i>LEU1</i>	3-isopropylmalate dehydratase	1.45	-0.06	1.30	-0.31
CJI97_001329	<i>LEU4</i>	2-isopropylmalate synthase	4.41	0.44	4.13	-0.12
CJI97_003280	<i>LYS1</i>	Saccharopine dehydrogenase	2.89	0.33	2.81	0.11
CJI97_003346	<i>LYS2</i>	Alpha-aminoacidopate reductase, large subunit	2.47	0.38	2.24	-0.31
CJI97_002417	<i>LYS4</i>	Homoaconitase	3.08	1.06	2.92	0.50
CJI97_002151	<i>LYS5</i>	Phosphopantetheinyl transferase	2.70	0.24	2.55	0.29
CJI97_001920	<i>LYS9</i>	Saccharopine dehydrogenase	1.13	0.07	0.96	-0.06
CJI97_003796	<i>LYS22</i>	Homocitrate synthase	2.91	0.07	2.82	-0.33
CJI97_003176	<i>SER1</i>	3-phosphoserine aminotransferase	2.11	0.16	2.05	0.15
CJI97_003156	<i>SER2</i>	Phosphoserine phosphatase	1.29	0.14	1.34	0.34
CJI97_000320	<i>SER33</i>	Enzyme of amino acid biosynthesis	1.05	-0.29	1.03	-0.51
CJI97_003863	<i>SHM1</i>	Mitochondrial serine hydroxymethyltransferase	1.30	-0.11	1.22	-0.01
CJI97_001157	<i>THR1</i>	Homoserine kinase	1.82	0.41	1.75	0.25
CJI97_000379	<i>TRP2</i>	Anthranilate synthase	1.05	0.42	0.91	0.08
CJI97_003979	<i>TRP3</i>	Indole-3-glycerol-phosphate synthase, anthranilate synthase	1.33	-0.02	1.27	0.12
CJI97_003855	<i>TRP4</i>	Enzyme of amino acid biosynthesis	1.25	0.06	1.17	-0.05
CJI97_003424	<i>TRP5</i>	Tryptophan synthase	2.45	0.28	2.25	0.17

Redox homeostasis and stress response

Locus Tag	Gene Name	Predicted function	WT MG Log ₂ FC	WT BEN Log ₂ FC	<i>mrr1Δ</i> MG Log ₂ FC	<i>mrr1Δ</i> BEN Log ₂ FC
CJI97_001841	<i>CAT1</i>	Catalase	0.54	2.49	0.67	2.38
CJI97_003095	<i>CIP1</i>	Oxidoreductase	0.51	8.04	1.33	8.70
CJI97_002824	<i>FDH3</i>	Oxidoreductase and zinc ion binding activity	-0.61	1.14	1.22	1.22
CJI97_000658	<i>MGD1</i>	NAD(H)-linked methylglyoxal oxidoreductase	1.35	2.46	-0.46	0.66
CJI97_002022	N/A	Quinone oxidoreductase	0.36	1.98	0.53	2.18
CJI97_002187	N/A	Oxidoreductase	-0.04	1.07	-0.07	0.97
CJI97_004869	N/A	Oxidoreductase	0.33	1.58	0.16	1.57
CJI97_004704	<i>POS5</i>	Mitochondrial NADH kinase	1.41	-0.46	1.54	-0.59
CJI97_004613	<i>PST3</i>	Flavodoxin-like protein	0.41	6.37	0.38	6.71
CJI97_000530	<i>SOD2</i>	Mitochondrial superoxide dismutase	-0.26	1.02	-0.39	1.41
CJI97_001282	<i>YAH1</i>	Oxidoreductase	1.01	0.99	0.87	1.07
CJI97_002560	<i>YCF1</i>	Glutathione S-conjugate transporter	4.58	0.85	0.12	0.90
CJI97_004612	<i>YCP4</i>	Flavodoxin-like protein	0.63	5.35	0.60	5.61

Significantly induced genes were determined using a cutoff of Log₂FC ≥ 1.00 and p-value < 0.05.

Table S2. Select genes significantly downregulated by MG and/or BEN in the B11221 background
Ergosterol biosynthesis

Locus Tag	Gene Name	Predicted function	WT MG Log ₂ FC	WT BEN Log ₂ FC	<i>mrr1a</i> Δ MG Log ₂ FC	<i>mrr1a</i> Δ BEN Log ₂ FC
CJI97_000262	<i>ERG1</i>	Squalene epoxidase	-0.85	-1.43	-0.68	-1.38
CJI97_003811	<i>ERG3</i>	C-5 sterol desaturase	-0.96	-1.11	-0.70	-0.73
CJI97_005423	<i>ERG6</i>	Delta(24)-sterol C-methyltransferase	-1.01	-1.53	-0.78	-1.09
CJI97_005634	<i>ERG10</i>	Acetyl-CoA acetyltransferase	-0.93	-1.04	-1.07	-0.77
CJI97_005638	<i>ERG10</i>	Acetyl-CoA acetyltransferase	-1.48	-0.63	-0.71	-1.63
CJI97_001156	<i>ERG11</i>	Lanosterol 14- α -demethylase	-0.24	-1.40	-0.13	-1.09

Metal acquisition, including regulation

Locus Tag	Gene Name	Predicted function	WT MG Log ₂ FC	WT BEN Log ₂ FC	<i>mrr1a</i> Δ MG Log ₂ FC	<i>mrr1a</i> Δ BEN Log ₂ FC
CJI97_002517	<i>CTR1</i>	Copper transporter	-1.13	-0.26	-1.24	0.01
CJI97_000015	<i>FRE7</i>	Ferric reductase	-1.23	-0.11	-1.43	-0.40
CJI97_003972	<i>FRE8</i>	Iron/copper reductase	-1.03	-1.18	-0.90	-1.30
CJI97_004532	<i>FRP1</i>	Ferric reductase	-1.41	-1.39	-0.85	-1.66
CJI97_001154	N/A	Ferric or cupric reductase	-0.57	-1.79	-0.97	-2.16
CJI97_004566	N/A	Ferric or cupric reductase	-1.43	-1.46	-1.51	-1.81
CJI97_005148	N/A	Ferric or cupric reductase	-1.56	-0.18	-1.81	-0.50
CJI97_002299	N/A	High affinity iron transporter for intravacuolar stores of iron	-0.19	-1.79	-0.16	-1.54
CJI97_001085	N/A	Transporter of ferrochrome siderophores	-0.06	-1.10	-0.57	-1.83
CJI97_001117	N/A	Transporter of ferrochrome siderophores	-1.90	-2.52	-1.67	-2.30
CJI97_001762	N/A	Transporter of ferrochrome siderophores	-1.94	-2.41	-1.87	-2.31
CJI97_004100	N/A	Transporter of ferrochrome siderophores	-0.26	-1.47	-0.21	-1.70
CJI97_004165	N/A	Transporter of ferrochrome siderophores	0.58	-1.14	0.83	-0.62
CJI97_001499	<i>SEF1</i>	Zn2-Cys6 transcription factor, regulates iron uptake	-1.13	0.31	-1.10	0.02
CJI97_000010	<i>ZRT2</i>	Zinc transporter	-0.38	-2.50	-0.80	-2.32

Carbohydrate metabolism and biosynthesis

Locus Tag	Gene Name	Predicted function	WT MG Log ₂ FC	WT BEN Log ₂ FC	<i>mrr1a</i> Δ MG Log ₂ FC	<i>mrr1a</i> Δ BEN Log ₂ FC
CJI97_003911	<i>DAC1</i>	N-acetylglucosamine-6-phosphate deacetylase	-0.35	-2.02	0.20	-2.05
CJI97_005247	<i>FBP1</i>	Fructose-1,6-bisphosphatase	-1.33	-1.11	-1.06	-0.96
CJI97_003057	<i>GLC3</i>	1,4-glucan branching enzyme	-1.05	-1.86	-0.95	-1.62
CJI97_001045	<i>GSY1</i>	Glycogen synthase	-1.17	-1.40	-1.10	-1.25
CJI97_003909	<i>HXK1</i>	N-acetylglucosamine kinase	-0.53	-1.15	-0.36	-1.37
CJI97_005579	<i>MAE1</i>	Mitochondrial malic enzyme	-1.44	-1.41	-1.36	-1.49
CJI97_000695	<i>MDH1</i>	Mitochondrial malate dehydrogenase	-1.23	-0.73	-1.09	-0.51
CJI97_003910	<i>NAG1</i>	Glucosamine-6-phosphate deaminase	-0.28	-1.76	0.03	-1.42

CJI97_001805	N/A	Role in beta-1,6 glucan biosynthesis	-1.16	-0.30	-0.95	-0.84
CJI97_000696	<i>NTH1</i>	Neutral trehalase	-1.07	-1.46	-1.20	-1.43
CJI97_002722	<i>PCK1</i>	Phosphoenolpyruvate carboxykinase	-2.03	-1.92	-1.68	-1.77
CJI97_002654	<i>PMM1</i>	Phosphomannomutase	-1.05	-1.33	-1.12	-0.73

Glucose transport, including regulation

Locus Tag	Gene Name	Predicted function	WT MG Log ₂ FC	WT BEN Log ₂ FC	<i>mrr1a</i> Δ MG Log ₂ FC	<i>mrr1a</i> Δ BEN Log ₂ FC
CJI97_000584	<i>HGT16</i>	MFS glucose transporter	1.38	-1.96	1.28	-2.14
CJI97_002713	<i>HGT17</i>	MFS glucose transporter	0.76	-3.05	-0.60	-2.46
CJI97_005108/ CJI97_005109	<i>HGT19</i>	MFS glucose/myo-inositol transporter	-1.71	-2.78	-1.95	-2.14
CJI97_001793	N/A	MFS glucose transporter	-2.00	-6.23	-2.27	-4.76
CJI97_001794	N/A	MFS glucose transporter	0.85	-2.17	-0.21	-1.41
CJI97_002023	N/A	MFS glucose transporter	-1.57	-5.00	-1.58	-5.13
CJI97_002024	N/A	MFS glucose transporter	-2.02	-2.80	-1.69	-2.70
CJI97_002448	<i>RGT1</i>	Transcriptional repressor of glucose transport	-1.26	-1.50	-1.29	-1.56
CJI97_005617	<i>SHA3</i>	Ser/thr kinase involved in glucose transport	-1.14	-0.65	-0.87	-1.09

Significantly repressed genes were determined using a cutoff of Log₂FC ≤ -1.00 and p-value < 0.05.

Table S3. Top 20 genes with predicted functions differentially expressed between B11221 and AR0390 in the control condition

Genes more highly expressed in B11221						
B11221 Locus Tag	AR0390 Locus Tag	Gene Name	Predicted Function	Log ₂ FC (B11221 vs AR0390)	B11221 average (CPM)	AR0390 average (CPM)
CJI97_004624	B9J08_004828	<i>MGD2</i>	NAD(H)-linked methylglyoxal oxidoreductase	11.29	1657	0.66
CJI97_000658	B9J08_000656	<i>MGD1</i>	NAD(H)-linked methylglyoxal oxidoreductase	8.53	4335	11.7
CJI97_004768	B9J08_004684	N/A	Role in histone deacetylation	7.87	51.9	0.22
CJI97_000946	B9J08_000928	<i>AQY1</i>	Aquaporin water channel, osmotic shock resistance	7.33	581	3.60
CJI97_004767	B9J08_004685	N/A	Curved DNA-binding protein	5.36	279	6.78
CJI97_003833	B9J08_003761	N/A	DNA topoisomerase	5.31	96.8	2.43
CJI97_002880	B9J08_002824	N/A	DNA replication licensing factor required for pre-replication complex assembly	4.49	316	14.1
CJI97_004042	B9J08_003981	<i>MDR1</i>	Plasma membrane MDR/MFS multidrug efflux pump	4.42	190	8.88
CJI97_002740	B9J08_002688	<i>FDH1</i>	Formate dehydrogenase	4.33	131	6.50
CJI97_004770	B9J08_004682	<i>ECM42</i>	Ornithine acetyltransferase	4.24	99.9	5.28
Genes more highly expressed in AR0390						
Locus Tag	AR0390 Locus Tag	Gene Name	Predicted Function	Log ₂ FC (B11221 vs AR0390)	B11221 average (CPM)	AR0390 average (CPM)
CJI97_001302	B9J08_001303	<i>BDF1</i>	Essential chromatin-binding bromodomain protein	-10.21	0.48	563
CJI97_004515	B9J08_004451	N/A	ALS family protein	-9.61	1.98	1546
CJI97_004556	B9J08_005565	<i>PRD1</i>	Proteinase	-7.29	0.14	22.6
CJI97_001865	B9J08_002322	<i>BMH1</i>	Role in morphology	-7.01	12.7	1639
CJI97_002974	B9J08_002900	<i>RMD9</i>	Mitochondrial protein with a predicted role in respiratory growth	-6.86	6.41	745
CJI97_003073	B9J08_003002	N/A	Iron permease	-6.51	36.8	3350
CJI97_002817	B9J08_002762	<i>INO1</i>	Inositol-1-phosphate synthase	-5.48	28.7	1281
CJI97_004514	B9J08_004450	<i>THI13</i>	Thiamin pyrimidine synthase	-4.95	2.34	72.4
CJI97_004654	B9J08_004798	<i>ARG3</i>	Ornithine carbamoyltransferase	-4.95	0.70	21.5
CJI97_000838	B9J08_000820	<i>SAM4</i>	S-adenosylmethionine-homocysteine methyltransferase	-4.43	1.93	41.5

Differentially expressed genes were determined using a cutoff of Log₂FC ≥ 1.00 or ≤ -1.00 and p-value < 0.05.

Table S4. Minimum inhibitory concentration (MIC) of fluconazole (FLZ) for *C. lusitaniae* U04 *mrr1*Δ and isogenic strains complemented with either *CauMRR1a*^{N647T} or *CauMRR1a* from *C. auris*

<i>C. lusitaniae</i> strain	FLZ MIC (μg/mL)	
	24h	48h
U04 <i>mrr1</i> Δ	4	4
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> ^{N647T} clone # 1	16	16
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> ^{N647T} clone # 2	16	16
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> ^{N647T} clone # 8	16	16
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> clone # 4	4	4
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> clone # 5	4	4
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> clone # 7	4	4

Table S5. Comparison of select genes induced by MG in B11221 and/or AR0390

B11221 Locus Tag	AR0390 Locus Tag	Gene Name	Predicted Function	B11221 Log ₂ FC	AR0390 Log ₂ FC
CJI97_005163	B9J08_005078	<i>AAH1</i>	Adenine deaminase, purine salvage and nitrogen catabolism	0.29	1.10
CJI97_002719	B9J08_002666	<i>AGP2</i>	Amino acid permease	0.45	1.16
CJI97_001242	B9J08_001362	<i>AGP3</i>	Serine transporter; sulfur assimilation	1.06	0.00
CJI97_004654	B9J08_004798	<i>ARG3</i>	Ornithine carbamoyltransferase	4.77	3.02
CJI97_003828	B9J08_003754	<i>ARG11</i>	Ornithine transporter of the mitochondrial inner membrane	0.94	1.70
CJI97_003954	B9J08_003882	<i>ARO4</i>	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase; aromatic amino acid biosynthesis	2.50	2.22
CJI97_001997	B9J08_002453	<i>BAT21</i>	Branched chain amino acid aminotransferase	2.51	2.56
CJI97_005185	B9J08_005101	<i>BNA1</i>	3-hydroxyanthranilic acid dioxygenase; NAD biosynthesis	1.54	-0.33
CJI97_000479	B9J08_000479	<i>CDR4</i>	ABC transporter superfamily	1.34	1.01
CJI97_003095	B9J08_003024	<i>CIP1</i>	Oxidoreductase	0.51	1.30
CJI97_004156	B9J08_004088	<i>DRE2</i>	Cytosolic Fe-S protein assembly protein	1.09	2.52
CJI97_004181	B9J08_004118	<i>ERC1</i>	Xenobiotic transmembrane transporter	1.47	0.00
CJI97_002824	B9J08_002769	<i>FDH3</i>	Oxidoreductase and zinc ion binding activity	1.25	0.90
CJI97_005329	B9J08_005247	<i>HIS1</i>	ATP phosphoribosyl transferase; histidine biosynthesis	3.40	2.90
CJI97_001933	B9J08_002388	<i>HST6</i>	ABC transporter related to mammalian P-glycoproteins	0.66	1.18
CJI97_003449	B9J08_003374	<i>ICL1</i>	Isocitrate lyase; glyoxylate cycle enzyme	0.79	1.33
CJI97_000020	B9J08_000013	<i>ILV3</i>	Dihydroxyacid dehydratase	2.77	2.73
CJI97_004268	B9J08_004204	<i>JEN1</i>	Lactate transporter	1.84	-0.24
CJI97_001329	B9J08_001277	<i>LEU4</i>	2-isopropylmalate synthase	4.41	4.31
CJI97_001920	B9J08_002375	<i>LYS9</i>	Saccharopine dehydrogenase; lysine biosynthesis	1.13	0.52
CJI97_004689	B9J08_004763	<i>MET8</i>	Bifunctional dehydrogenase and ferrochelatase; siroheme biosynthesis	1.83	0.94
CJI97_003625	B9J08_003552	<i>MET10</i>	Sulfite reductase; sulfur amino acid metabolism	1.01	0.90
CJI97_000409	B9J08_000409	<i>MET13</i>	Methionine biosynthesis protein	0.04	1.65
CJI97_003066	B9J08_002995	<i>MET14</i>	Adenylylsulfate kinase; sulfur metabolism	1.08	0.93
CJI97_005391	B9J08_005307	<i>MET16</i>	3'-phosphoadenylylsulfate reductase; sulfur amino acid metabolism	1.63	1.75
CJI97_004042	B9J08_003981	<i>MDR1</i>	Plasma membrane MDR/MFS multidrug efflux pump	3.83	5.27
CJI97_000658	B9J08_000656	<i>MGD1</i>	NAD(H)-linked methylglyoxal oxidoreductase	1.35	2.04
CJI97_004624	B9J08_004828	<i>MGD2</i>	NAD(H)-linked methylglyoxal oxidoreductase	-0.06	1.60
CJI97_002730	B9J08_002677	<i>MIS12</i>	Mitochondrial C1-tetrahydrofolate synthase precursor	1.05	0.87
CJI97_004904	B9J08_004548	<i>NAR1</i>	Cytosolic iron-sulfur protein assembly machinery protein	0.84	2.13
CJI97_003488	B9J08_003413	<i>OPT7</i>	Oligopeptide transporter, may transport GSH or related compounds	1.09	0.90
CJI97_001481	B9J08_001125	<i>SNQ2</i>	Putative ABC transporter superfamily	1.50	2.25
CJI97_003300	B9J08_003225	<i>STR2</i>	Cystathionine gamma-synthase; sulfur compound metabolism	1.15	1.32

CJI97_001014	B9J08_000995	<i>SUL2</i>	Sulfate transporter	1.98	2.03
CJI97_004677	B9J08_004775	<i>TPO3</i>	Polyamine transporter, MFS-MDR family	0.91	1.24
CJI97_002495	B9J08_001834	<i>TPO4</i>	Spermidine transporter	0.48	1.49
CJI97_003424	B9J08_003349	<i>TRP5</i>	Tryptophan synthase	2.45	2.52
CJI97_002560	B9J08_001899	<i>YCF1</i>	Glutathione S-conjugate transporter	4.58	4.57
CJI97_005451	B9J08_005368	<i>YDJ1</i>	Type I HSP40 co-chaperone	0.25	1.20

Significantly induced genes were determined using a cutoff of $\text{Log}_2\text{FC} \geq 1.00$ and p-value < 0.05.

Table S6. Comparison of select genes repressed by MG in B11221 and/or AR0390

B11221 Locus Tag	AR0390 Locus Tag	Gene Name	Predicted Function	B11221 Log₂FC	AR0390 Log₂FC
CJI97_001435	B9J08_001171	<i>ADH5</i>	Alcohol dehydrogenase	-1.54	-1.34
CJI97_002591	B9J08_001930	<i>AOX1</i>	Alternative oxidase, cyanide-resistant respiration	-1.31	-0.01
CJI97_004799	B9J08_004653	<i>ARP2</i>	Component of the Arp2/3 complex	-0.79	-1.11
CJI97_001469	B9J08_001137	<i>ARP3</i>	Protein with Myo5p-dependent localization to cortical actin patches at hyphal tip	-0.55	-1.05
CJI97_000089	B9J08_000084	<i>ATP14</i>	Mitochondrial F1F0 ATP synthase subunit	-0.45	-1.02
CJI97_002664	B9J08_002610	<i>ATP17</i>	Mitochondrial ATPase complex subunit	-0.33	-1.25
CJI97_003777	B9J08_003702	<i>CDG1</i>	Cysteine dioxygenases, role in conversion of cysteine to sulfite	-2.12	-2.65
CJI97_004336	B9J08_004273	<i>COX6</i>	Cytochrome c oxidase	-0.64	-1.03
CJI97_002184	B9J08_001993	<i>COX11</i>	Cytochrome oxidase assembly protein	-0.43	-1.43
CJI97_004817	B9J08_004635	<i>COX19</i>	Cytochrome c oxidase assembly protein	-0.39	-1.16
CJI97_002517	B9J08_001856	<i>CTR1</i>	Copper transporter	-1.13	-2.07
CJI97_005423	B9J08_005340	<i>ERG6</i>	Delta(24)-sterol C-methyltransferase, converts zymosterol to fecosterol, ergosterol biosynthesis	-1.01	-0.46
CJI97_005321	B9J08_005239	<i>FBA1</i>	Fructose-bisphosphate aldolase	-0.72	-1.13
CJI97_005247	B9J08_005163	<i>FBP1</i>	Fructose-1,6-bisphosphatase, key gluconeogenesis enzyme	-1.33	-1.48
CJI97_000015	B9J08_000008	<i>FRE7</i>	Ferric reductase	-1.23	-0.86
CJI97_003972	B9J08_004052	<i>FRE8</i>	Iron/copper reductase	-1.03	-0.23
CJI97_004532	B9J08_004468	<i>FRP1</i>	Ferric reductase	-1.41	-0.53
CJI97_002942	B9J08_002886	<i>GIT3</i>	Glycerophosphocholine permease	-1.18	-0.13
CJI97_003057	B9J08_002986	<i>GLC3</i>	1,4-glucan branching enzyme	-1.05	-0.93
CJI97_004438	B9J08_004375	<i>GPM1</i>	Phosphoglycerate mutase	-0.53	-1.40
CJI97_001045	B9J08_001025	<i>GSY1</i>	Glycogen synthase	-1.17	-1.36
CJI97_005108/ CJI97_005109	B9J08_005025	<i>HGT19</i>	MFS glucose/myo-inositol transporter	-1.71	-0.98
CJI97_002699	B9J08_002646	<i>HXT5</i>	Sugar transporter	0.13	-1.13
CJI97_002817	B9J08_002762	<i>INO1</i>	Inositol-1-phosphate synthase	-0.36	-1.41
CJI97_001658	B9J08_001652	<i>JAC1</i>	ATPase activator activity	-0.25	-1.04
CJI97_005579	B9J08_005497	<i>MAE1</i>	Mitochondrial malic enzyme	-1.44	-1.10
CJI97_000695	B9J08_000694	<i>MDH1</i>	Mitochondrial malate dehydrogenase	-1.23	-1.26
CJI97_002683	B9J08_002630	<i>MEP1</i>	Ammonium permease	-1.81	-1.38
CJI97_003663	B9J08_003590	<i>MIG2</i>	Transcription factor involved in glucose repression	-1.17	-1.44
CJI97_002101	B9J08_002556	<i>MIX14</i>	Role in aerobic respiration and mitochondrial intermembrane space localization	-0.20	-1.38
CJI97_002993	B9J08_002919	<i>MLS1</i>	Malate synthase, glyoxylate cycle enzyme	-0.84	-1.11
CJI97_001141	B9J08_001463	<i>MYO1</i>	Component of actomyosin ring at neck of newly emerged bud	-0.98	-1.22
CJI97_001117	B9J08_001487	N/A	Transporter of ferrochrome siderophores	-1.90	-1.06
CJI97_001762	B9J08_001547	N/A	Transporter of ferrochrome siderophores	-1.94	-1.07
CJI97_000596	B9J08_000675	N/A	Adhesin-like protein	-1.12	0.15

CJI97_002126	B9J08_002582	N/A	Adhesin-like protein	-1.48	1.00
CJI97_003987	B9J08_004037	N/A	Adhesin-like protein	-1.19	-0.35
CJI97_004240	B9J08_004176	N/A	Secreted lipase	-1.69	-0.78
CJI97_001776	B9J08_001533	N/A	NAD-aldehyde dehydrogenase	-1.57	-1.55
CJI97_003161	B9J08_003088	N/A	NAD-aldehyde dehydrogenase	-1.76	-1.89
CJI97_001793	B9J08_002250	N/A	MFS glucose transporter	-2.00	-1.41
CJI97_002024	B9J08_002481	N/A	MFS glucose transporter	-2.02	-1.52
CJI97_004566	B9J08_004886	N/A	Protein similar to ferric reductases and cupric reductases	-1.43	-1.43
CJI97_005148	B9J08_005064	N/A	Protein similar to ferric reductases and cupric reductases	-1.56	-1.84
CJI97_000696	B9J08_000695	<i>NTH1</i>	Neutral trehalase	-1.07	-0.98
CJI97_002722	B9J08_002669	<i>PCK1</i>	Phosphoenolpyruvate carboxykinase	-2.03	-2.01
CJI97_002521	B9J08_001860	<i>PGK1</i>	Phosphoglycerate kinase	-0.77	-1.19
CJI97_001140	B9J08_001464	<i>PHO84</i>	High-affinity phosphate transporter	-2.74	-2.72
CJI97_001580	B9J08_002202	<i>PHO89</i>	Phosphate permease	-1.48	-0.73
CJI97_001697	B9J08_001613	<i>PHO100</i>	Putative inducible acid phosphatase	-1.38	-1.60
CJI97_004666	B9J08_004786	<i>PIR1</i>	1,3-beta-glucan-linked cell wall protein	-0.44	-1.12
CJI97_002654	B9J08_002600	<i>PMM1</i>	Phosphomannomutase, enzyme of O- and N-linked mannosylation	-1.05	-0.94
CJI97_002321	B9J08_002130	<i>PUT1</i>	Putative proline oxidase	-2.49	-1.79
CJI97_004379	B9J08_004317	<i>PUT2</i>	Putative delta-1-pyrroline-5-carboxylate dehydrogenase	-1.01	-1.45
CJI97_002448	B9J08_001787	<i>RGT1</i>	Transcriptional repressor involved in the regulation of glucose transporter genes	-1.26	-1.62
CJI97_002974	B9J08_002900	<i>RMD9</i>	Mitochondrial protein with a predicted role in respiratory growth	-0.25	-1.09
CJI97_004415	B9J08_004352	<i>SAH1</i>	S-adenosyl-L-homocysteine hydrolase	-1.46	-1.22
CJI97_004940	B9J08_004512	<i>SCO1</i>	Copper transporter	-0.79	-1.06
CJI97_001499	B9J08_001107	<i>SEF1</i>	Zn2-Cys6 transcription factor, regulates iron uptake	-1.13	-0.81
CJI97_005617	B9J08_005567	<i>SHA3</i>	Ser/thr kinase involved in glucose transport	-1.14	0.10
CJI97_002536	B9J08_001875	<i>TPI1</i>	Triose-phosphate isomerase	-0.36	-1.42
CJI97_003198	B9J08_003126	<i>QCR8</i>	Ubiquinol cytochrome c reductase	-0.22	-1.12
CJI97_002481	B9J08_001820	<i>QCR10</i>	Ubiquinol-cytochrome-c reductase	-0.31	-1.01
CJI97_003997	B9J08_004027	<i>WOR1</i>	Transcription factor of white-opaque phenotypic switching in <i>C. albicans</i>	-1.22	-0.33

Significantly repressed genes were determined using a cutoff of $\text{Log}_2\text{FC} \leq -1.00$ and $p\text{-value} < 0.05$.

Table S7. Strains used in this study

Strain	Lab #	Species	Parent	Relevant Characteristics or Genotype	Source
Fungal Strains					
AR0390	DH2777	<i>C. auris</i>		Clinical isolate, clade I	(1)
B11221	DH3880	<i>C. auris</i>		Clinical isolate, clade III	(2)
<i>mrr1a</i> Δ	DH3881	<i>C. auris</i>	B11221	<i>mrr1a</i> Δ::caSAT1	(2)
<i>mrr1b</i> Δ	DH3882	<i>C. auris</i>	B11221	<i>mrr1b</i> Δ::caSAT1	(2)
<i>mrr1c</i> Δ	DH3883	<i>C. auris</i>	B11221	<i>mrr1c</i> Δ::caSAT1	(2)
U04 <i>mrr1</i> Δ	DH3306	<i>C. lusitaniae</i>	U04	<i>mrr1</i> Δ::NAT1	(3)
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> ^{N647T} clone #1	DH3914	<i>C. lusitaniae</i>	U04 <i>mrr1</i> Δ	<i>CauMRR1a</i> ^{N647T} -HygB	This study
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> ^{N647T} clone #2	DH3915	<i>C. lusitaniae</i>	U04 <i>mrr1</i> Δ	<i>CauMRR1a</i> ^{N647T} -HygB	This study
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> ^{N647T} clone #8	DH3916	<i>C. lusitaniae</i>	U04 <i>mrr1</i> Δ	<i>CauMRR1a</i> ^{N647T} -HygB	This study
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> clone #4	DH3917	<i>C. lusitaniae</i>	U04 <i>mrr1</i> Δ	<i>CauMRR1a</i> -HygB	This study
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> clone #5	DH3918	<i>C. lusitaniae</i>	U04 <i>mrr1</i> Δ	<i>CauMRR1a</i> -HygB	This study
U04 <i>mrr1</i> Δ + <i>CauMRR1a</i> clone #7	DH3919	<i>C. lusitaniae</i>	U04 <i>mrr1</i> Δ	<i>CauMRR1a</i> -HygB	This study
Plasmids in <i>E. coli</i> (DH5α)					
pMQ30 ^{MRR1-L1191H+Q1197*}	DH3829	<i>E. coli</i>		<i>MRR1</i> ^{L1191H+Q1197*} -HygB complementation, Gent ^R	(5)
pMQ30 ^{CauMRR1a^{N647T}}	DH3912	<i>E. coli</i>		<i>CauMRR1a</i> ^{N647T} -HygB complementation, Gent ^R	This study
pMQ30 ^{CauMRR1a}	DH3913	<i>E. coli</i>		<i>CauMRR1a</i> -HygB complementation, Gent ^R	This study

Table S8. Oligonucleotides and primers used in this study

Name	Description	Sequence	Reference
ED222	<i>C. auris</i> ACT1 qRT Fwd	5' – GAA GGA GAT CAC TGC TTT AGC C – 3'	This study
ED223	<i>C. auris</i> ACT1 qRT Rev	5' – GAG CCA CCA ATC CAC ACA G – 3'	This study
ED224	<i>C. auris</i> MDR1 qRT Fwd	5' – GAA GTA TGA TGG CGG GTG – 3'	This study
ED225	<i>C. auris</i> MDR1 qRT Rev	5' – CCC AAG AGA GAC GAG CCC – 3'	This study
AB126	<i>C. auris</i> MGD1 qRT Fwd	5' – TTC CCC TGA AAT GGA TTT GA – 3'	This study
AB127	<i>C. auris</i> MGD1 qRT Rev	5' – GTC TTG GAG CCA TAG TAA CC – 3'	This study
AB130	Amplify <i>C. auris</i> MRR1a for heterologous complementation, Fwd	5' – CTT CAA CTC CGC AAC ACC TGG AAA CTT CAT TAC TAA AGA TGA TGG TAT CTT CGA AAG ATC – 3'	This study
AB131	Amplify <i>C. auris</i> MRR1a for heterologous complementation, Fwd	5' – CTT TAC CAG TAA AGT ATC CTT GCC AAA TTT CGT TCC ATA ATT ACA CAT CAA GCA TCT CTT C – 3'	This study
ED125	Forward upstream of MRR1 to validate complement	5' – GAA AAA GAA GCC AGC AGA CC – 3'	(5)
ED126	Reverse downstream of MRR1 to validate complement	5' – GGG TAA AGC CAT TGC AGA C – 3'	(5)
ACT1-F	<i>C. lusitaniae</i> ACT1 qRT Fwd	5' – GTA TCG CTG AGC GTA TGC AA – 3'	(6)
ACT1-R	<i>C. lusitaniae</i> ACT1 qRT Rev	5' – GAT GGA TGG TCC AGA CTC GT – 3'	(6)
ED058	<i>C. lusitaniae</i> MDR1 qRT Fwd	5' – TCC ATC CAT GGG TCC ATT ATT C – 3'	(3)
ED059	<i>C. lusitaniae</i> MDR1 qRT Rev	5' – CTC AAC ACA AGG AAA GCA CAT C – 3'	(3)
AB039	<i>C. lusitaniae</i> MGD1 qRT Fwd	5' – CGC AGA AAT CCC TAA AGT AAA T – 3'	(5)
AB040	<i>C. lusitaniae</i> MGD1 qRT Rev	5' – TAC CCT TTG CTT CGT TCT T – 3'	(5)
NAT1 crRNA	crRNA for NAT1; used to complement <i>C. auris</i> MRR1a alleles into <i>C. lusitaniae</i>	5' – GGG AAA ACC TTA GTC AAT GG – 3'	(5)

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