

FungAMR: a comprehensive database for investigating fungal mutations associated with antimicrobial resistance

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Antimicrobial resistance (AMR) is a global threat, especially in fungal pathogens. To optimize the use of available antifungals, we need rapid detection and monitoring tools that rely on high-quality AMR mutation data. Here we present FungAMR, a resource based on manual curation of 501 published studies on AMR mutations in clinically and agriculturally relevant fungal pathogens resulting in 35,792 entries covering 208 drugs, 246 genes and 95 fungal species. Each entry includes gene, mutation site and drug susceptibility data, with confidence scores indicating the strength of the supporting evidence. Data analysis revealed convergent mechanisms of resistance, indicating some potentially universal resistance mutations and mutations that lead to cross-resistance within and across antifungal classes. We also developed a computational tool, ChroQueTas, that leverages FungAMR to screen fungal genomes for AMR mutations. FungAMR is available as a web-searchable interface within the Comprehensive Antibiotic Resistance Database (CARD). These evolving resources promise to facilitate research on antifungal resistance.

Infections caused by fungal pathogens constitute an important health threat and socio-economic burden. Fungal pathogens have the potential to cause severe diseases, accounting for multiple million deaths annually^{1,2}. Yet, fungal infections are among the most overlooked infectious diseases. The burden of pathogenic fungi extends beyond human infections, encompassing challenges to both food security and biodiversity. Fungi are a leading cause of plant diseases, resulting in an estimated 20% worldwide crop yield loss³.

Another pressing threat to public health is antimicrobial resistance (AMR). The use of antifungals in agriculture and clinics, or in other sectors as preservatives, has led to the emergence of resistant strains and populations unresponsive to treatments⁴. AMR in human fungal pathogens poses a particular challenge because the medical community relies on only a handful major classes of antifungals to treat human systemic infections⁵. This is exacerbated by the overlap between the classes of antifungals used to treat infections in humans and the

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fungicides used on plants, which selects for resistance mutations in environmental fungi that can go on to infect humans, for instance, *Aspergillus fumigatus*⁶.

Despite ongoing efforts to extend our range of antifungal drugs and identify new targets, the rate at which new antimicrobials are discovered or engineered is far exceeded by the speed at which resistance emerges⁷. In addition, resistance will eventually probably emerge against these new drugs. While developing and discovering new antifungals is crucial, this alone will not address the problem of AMR. A better understanding of the different resistance mechanisms is essential to help guide the development of treatment strategies and hopefully, to better understand and control the evolution of resistance. Having a centralized antifungal mutation repository of high quality will also improve our capacity to detect resistance and pave the way for routine genotypic detection of antifungal resistance.

Antifungal resistance emerges through de novo mutations in the genome resulting in a wide array of possible resistance mechanisms⁸. The number of fungal AMR mutations documented in the literature remains relatively limited and dispersed, and in many cases, we lack an understanding of the precise impact or causal nature of resistance mutations. In-depth knowledge of resistance mutations across drugs and species would allow us to (1) better understand which mutations are directly linked with resistance; (2) determine the extent of cross-resistance among drugs; (3) assess how resistance mechanisms are conserved among fungi; and (4) enable us to streamline the development of antifungal agents that overcome resistance mutations and avoid cross-resistance characteristics by modifying currently available drugs. Finally, this knowledge could facilitate the development of genomic tools to track resistance in real time and help build predictive models to foresee resistance mutations before they spread in clinics and agriculture.

Databases on antibacterial resistance mutations, such as CARD⁹, have proved invaluable in addressing the serious threat posed by AMR. However, while some antifungal resistance databases exist, these are unfortunately outdated¹⁰ or contain a large fraction of inaccurate information when done using simple text mining¹¹. Since many studies are reporting potentially spurious associations between mutations and resistance, a careful annotation is needed to build a useful reference dataset (see below). Thus, there is a need to exhaustively and reliably gather all antifungal resistance mutations reported in the literature in one accessible repository.

To address these limitations, we manually curated 501 papers reporting fungal AMR mutations to create the FungAMR compendium (Supplementary Table 1, [FungAMR Mutation Data](#)). FungAMR contains 35,792 carefully curated entries across 208 drugs (including 118 antifungals) for 95 fungal species and includes amino acid substitutions as well as other genomic changes such as copy-number variations (CNVs). Every mutation is classified with the degree of evidence that supports its role in AMR. We have taken advantage of this resource to better understand resistance mechanisms among species and antifungals. We have combined variant effect predictions with resistance mutations to confirm that many genes confer resistance through loss-of-function mutations and show that such analyses could be used to help interpret the impact of mutations on

AMR. Furthermore, comparative analysis among species revealed a high level of convergence in resistance mechanisms at both the gene and the mutation levels. This analysis also confirmed that many resistance mutations provide cross-resistance to antifungals within the same class, but also between classes for mutations present in certain genes. Finally, we present Chromosome Query Targets (Chro-QueTas), a computational pipeline that utilizes the information contained in FungAMR to detect the presence of AMR mutations in fungal genomes. Overall, the analysis of the content of FungAMR revealed biases in the study of certain species, proteins and antifungals, and highlighted areas where more research is needed. These resources will help the scientific community to address the serious threat of antifungal resistance and have the potential to provide a launchpad for fundamental and applied research.

Results

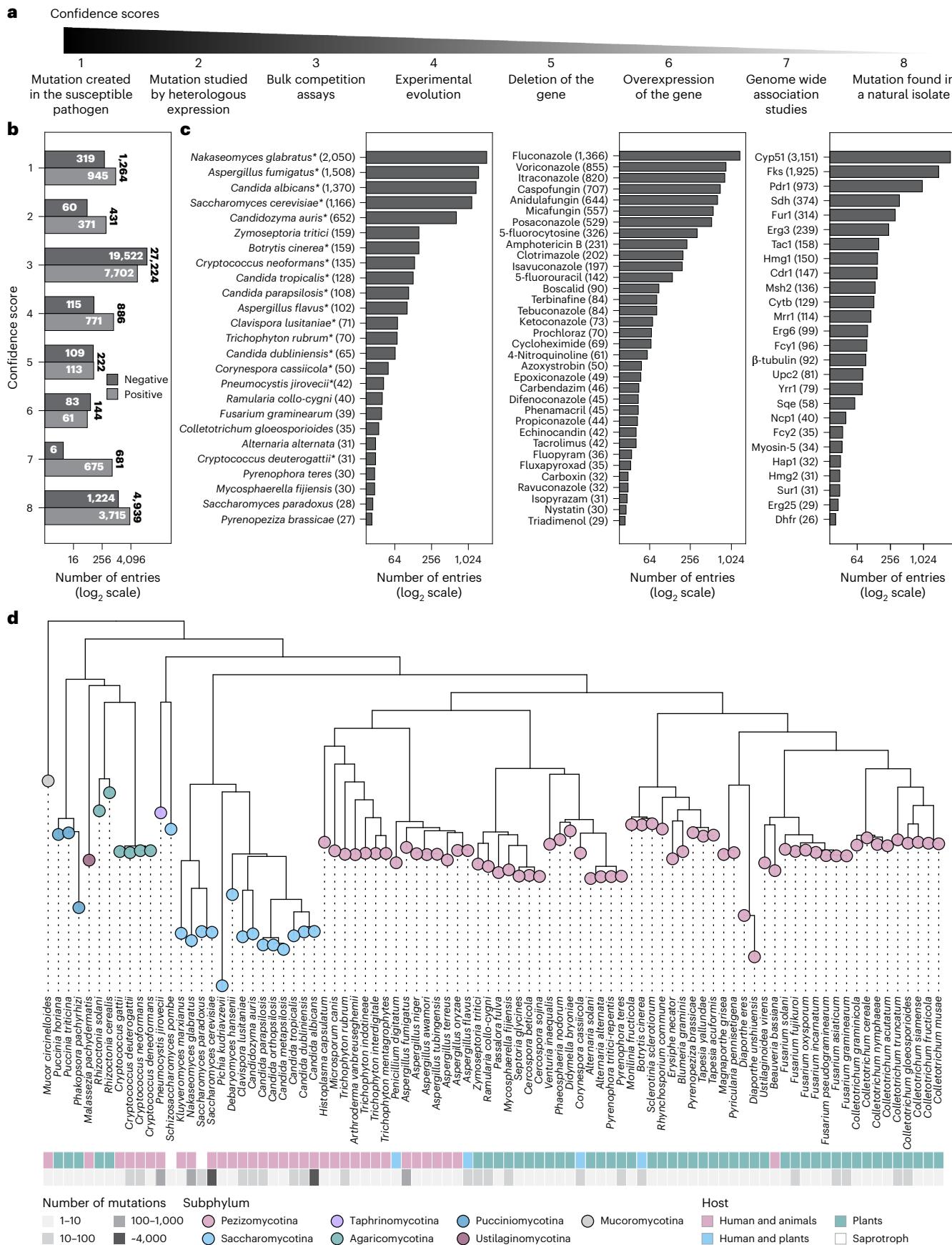
Content of FungAMR

A total of 501 papers were curated, leading to 35,792 mutation entries (Supplementary Table 1, [FungAMR Mutation Data](#)). All entries are associated with information on the publication from which it was curated, as well as the gene, the location of the mutation and the impact on drug susceptibility as experimentally assessed in the laboratory. Since the definition of a resistance mutation varies between studies, we defined a resistant strain as a strain presenting a minimum inhibitory concentration (MIC) to an antifungal above the established breakpoint when it was available or as a strain with significantly higher growth in the presence of an antifungal than the reference strain. Also, the level of evidence linking reported mutations to resistance varies between studies. Some reports demonstrate causality by introducing mutations in control backgrounds, while others simply identify mutations in resistant strains, often sequencing only known resistance genes. Therefore, we associated all entries with a confidence score assessing the degree of evidence that supports their role in drug resistance (Fig. 1a and Supplementary Table 2). A positive confidence score denotes mutations reported to confer resistance, while a negative confidence score relates to reported mutations that were not found to confer resistance. A low positive confidence score indicates that the evidence for the contribution of the mutation to resistance is strong, with 1 being the strongest and 8 being the weakest. Negative scores were excluded from our analyses due to potential confounding factors, such as assay sensitivity. For mutations with both positive and negative scores, we used the lowest positive score for our analyses. Ultimately, it is important to bear in mind that confidence scores do not translate into a probability of treatment failure, but only reflect the level of evidence we have that a mutation confers resistance under laboratory conditions.

We gathered more than 10,000 unique mutations for 208 drugs across 246 genes and 95 fungal species. Mutations assayed by deep mutational scanning (DMS, confidence score of 3) come from studies where large libraries of gene mutants were subjected to resistance testing. We have only four studies reporting mutations assayed by DMS, but they represent 76% of the entries (3: 21% and -3: 55%, Fig. 1b), showing how powerful systematic approaches can be at characterizing mutations. We set aside these entries for some analyses, since

Fig. 1 | Content of FungAMR per confidence score, species, antifungal and protein. **a**, Definition of the confidence scores assessing the degree of evidence that supports a mutation's role in drug resistance. A low positive confidence score means that the evidence for the effect of the mutation on resistance is strong, with 1 being the strongest and 8 being the weakest. A negative confidence score indicates a mutation that was found not to cause resistance using the same approach of the corresponding positive score. More details are available in Supplementary Table 2. **b**, Distribution of positive and negative confidence scores associated with mutations reported in the literature ($n = 35,792$ entries). Most entries come from high-throughput experiments and

have a confidence score of 3. Most remaining reports have limited experimental support. **c**, Number of observations per species, per drug and per protein. Species with * can cause human diseases (for example, asthma, dermatosis or invasive mycosis). Entries with a confidence score of 3 were excluded and cases with fewer than 25 reports are not shown to simplify the figure. **d**, Phylogenetic relationships of the species represented. On the right of species names are indicated which hosts these species can be isolated from and the number of unique mutations reported in the dataset in each case. The number of unique mutations can be lower than the number of mutation entries since many mutations have been reported multiple times.



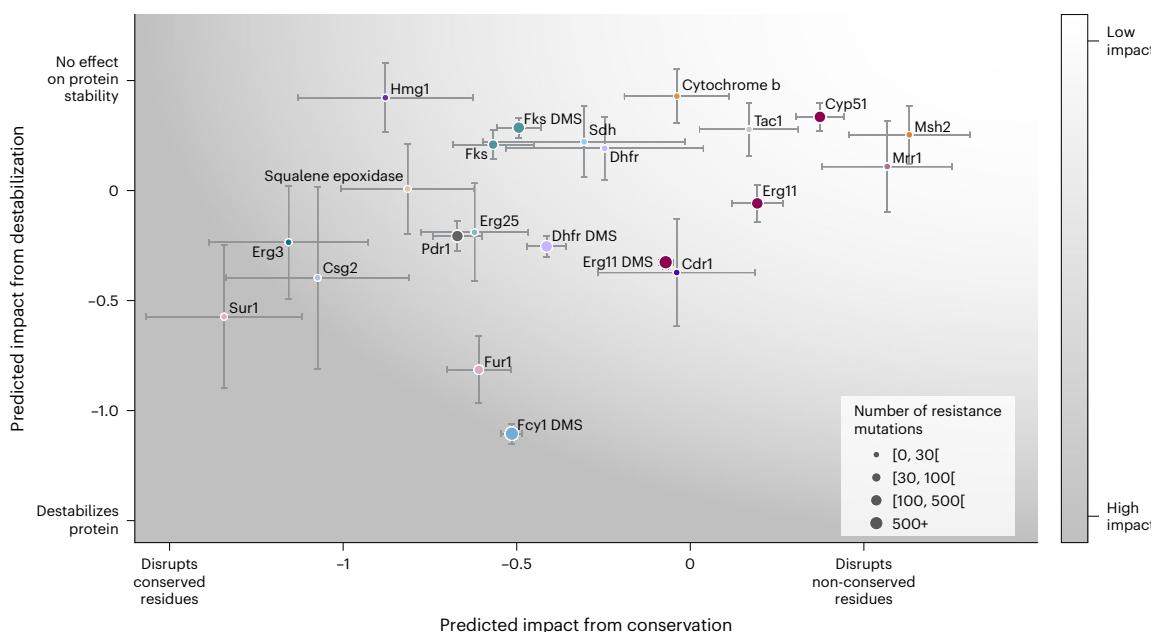


Fig. 2 | Prediction of mutations' effect on protein function and stability can aid differentiation between loss- and gain-of-function resistance mutations.

Relationship between the predicted impact of resistance mutations inferred from long-term evolutionary constraints (GEMME¹⁹) and protein stability (FoldX^{20,20}). Each dot represents the mean deviation of the predicted impact for resistance mutations in a given protein relative to all possible amino acid changes. The y axis presents the z-score of the normalized predicted change in the Gibbs free energy ($\Delta\Delta G$) upon mutation predicted by FoldX. Negative values predict destabilization of the protein. The x axis represents the z-score of the predicted impact of the mutation computed by GEMME, which uses patterns of protein conservation to

predict the tolerance of mutations. Negative values predict a higher impact on the protein function based on evolutionary conservation. For each gene, when mutations were available from DMS experiments (confidence score of 3), the DMS data are shown separately. The size of the dots shows the sample size (Erg11 DMS, 1,268; Fcy1 DMS, 940; Dhfr DMS, 354; Pdr1, 282; Fks DMS, 148; Erg11, 108; Cyp51, 105; Fur1, 84; Fks, 56; Tac1, 27; Erg25, 26; Erg3, 24; Mrr1, 21; Dhfr, 20; Sur1, 20; Cytochrome b, 19; Hmg1, 17; squalene epoxidase, 17; Cdr1, 13; Csg2, 12; Msh2, 12) and the colours show the different proteins. Error bars represent 1 standard error. The distribution of the data for each protein is presented in Extended Data Fig. 1.

they are not representative of the overall content. There are 498 studies reporting non-DMS data for 8,568 entries and over 3,000 unique mutations. The drugs listed include 42 antifungals used in the clinic (81% of entries), 76 fungicides used in agriculture (13% of entries) and 86 other molecules (6% of entries, for example, anticancer drugs and immunosuppressants). Most reported entries are of low confidence, with 58% having a confidence score of 8 (8: 43% and -8: 14%, Fig. 1b). Around 20% of the entries are mutations validated directly in the susceptible pathogen (1: 11% and -1: 4%, Fig. 1b) or by heterologous expression in the model yeast *Saccharomyces cerevisiae* (2: 4% and -2: 1%, Fig. 1b). This highlights the fact that although many AMR mutations have been reported in the literature, most have very little to no experimental support.

Nakaseomyces glabratus (formerly *Candida glabrata*), *Aspergillus fumigatus* and *Candida albicans* have the largest number of entries with more than 1,300 each. They are followed by *S. cerevisiae* (1,166 entries) and the emergent human fungal pathogen *Candidozyma auris* (formerly *Candida auris*, 652 entries) (Fig. 1c). Roughly 75% of the listed species have less than 25 entries, reflecting a strong bias towards the study of certain fungi. The drug targets of azoles and echinocandins, *CYP51* (also named *ERG11* in yeast) and *FKS*, are unsurprisingly the most studied genes, being listed in nearly 40% and 25% of the entries, respectively. This probably overestimates their contribution to resistance since many studies focus only on these genes and might falsely conclude they found a resistance mutation or might miss a resistance mutation in another gene. The other genes are much less studied, as ~90% of them are listed fewer than 25 times. Most of the biases in the entries stem from AMR in human fungal pathogens being better studied or more often reported than in plant pathogens. However, the diversity of species and antimicrobials reported for plant pathogens is larger than for human pathogens (Fig. 1c,d).

Identifying AMR mutations and their impact on protein function

Antifungal resistance frequently arises through loss-of-function mutations (LOF), for instance, by introducing premature stop codons, indels causing frameshifts or missense mutations at critical sites in proteins. LOF in *FCY1* and *FUR1* are well-established mechanisms of resistance to flucytosine (5-FC)^{12,13}, a prodrug that must be converted by the cytosine deaminase to gain potency. Experimental evolution of *S. cerevisiae* and *C. auris* in polyenes led to the identification of LOF causing resistance in *ERG3* and *ERG6* (refs. 14,15). LOF in other genes of the ergosterol pathway is also associated with resistance, including frameshifts in *ERG2* in *C. albicans* causing resistance to azoles¹⁶. LOF of *ERG4* has also been associated with azole and echinocandin resistance in *N. glabratus*¹⁷ but with a confidence score of 8, which means that this requires further examination. Interestingly, all of these cases occur in haploid strains, which is anticipated as most LOF mutations are expected to be recessive.

Amino acid substitutions in *Fcy1* or *Fur1*, typically at conserved sites, tend to destabilize the protein and sufficiently disrupt their function to confer resistance^{12,18}. It is therefore possible that conservation level and estimates of destabilization could help to better understand whether resistance emerges from LOF. To examine this, we integrated into the data two predictions of the impact of mutations: one based on conservation analysis¹⁹ and one based on the prediction of the destabilization by the mutation using protein structure²⁰ (Fig. 2). For genes that lead to resistance upon LOF, such as *FCY1* and *FUR1*, we indeed clearly see that resistance mutations are predicted to have a larger impact on protein function and stability in comparison with other mutations in the same genes. Other extreme cases are *Sur1* and *Csg2*, proteins involved in sphingolipid synthesis. Resistance-associated substitutions in these proteins are destabilizing and occur at conserved sites,

suggesting LOF. This observation is validated by recent experimental evolution results showing that stop codons in these two enzymes in *S. cerevisiae* cause resistance to clotrimazole²¹. For *ERG3* and *HMG1*, mutations are predicted to strongly impact function, but to have low or no impact on protein stability (*ERG6* did not have enough entries to perform this analysis). Nonetheless, this suggests that resistance mutations in *ERG3* and *HMG1* probably arose through LOF, which can be predicted through their potential effects on protein function.

Since other resistance genes for which we have enough data to perform such analysis do not show patterns consistent with LOF mutations, this analysis suggests that a large fraction of resistance genes does not evolve by simple inactivation.

Mapping AMR mutations on protein structures

Mutations leading to LOF through destabilization need to be located in regions of proteins important for stability. Alternatively, they could occur at the active sites of the proteins. To help rationalize how mutations may confer resistance, we coloured residues with listed resistance mutations on the protein structures to detect potential clustering. Fur1 and Fcy1 are tetrameric and dimeric proteins, respectively. In both cases, many of the mutations occur in structured and core residues, including at the interfaces, potentially disrupting complex assembly (Fig. 3a,b). For Erg3 (Fig. 3c), reported mutations are in transmembrane helices. For Hmg1, previous knowledge on the protein allows us to deduce that mutations are located in the conserved sterol sensing domain of the protein²². This could explain the results above (Fig. 2) where resistance mutations occur at conserved sites but are not predicted to destabilize the protein structure. Although these structures do not model the mutational effects, the mapping can help interpret the impact of mutations.

Mutations involved in preventing drug binding are expected to cluster in regions of proteins where the drug and the protein interact. For Fks, echinocandin resistance mutations are clustered in three different hotspots (Fig. 3e) as has been previously observed²³. The proximity of the hotspots on the enzyme structure suggests a putative binding site of echinocandin drugs. For Cyp51, azole resistance mutations are more dispersed, but some regions of the protein appear to be enriched with such mutations (Fig. 3f). This is consistent with findings from a DMS of *C. albicans* Erg11 (ref. 24).

One potential way to identify gain-of-function (GOF) mutations in transcription factors would be to examine whether mutations cluster in space in specific domains or regions associated with particular functions. For Upc2, resistance mutations cluster outside the DNA-binding region and probably increase activity by disrupting ergosterol binding in the sterol-binding pocket²⁵. Pdr1 resistance mutations are spread across the structure but exclude the DNA-binding site (Fig. 3h). There is evidence that GOF mutations in Pdr1 probably act by blocking regulatory inputs rather than enhancing DNA binding²⁶. Interestingly, Pdr1 mutations show intermediate predicted effects on conservation and stability (Fig. 2), suggesting that they may alter activity through minor destabilization. In *N. glabratus*, GOF mutations reduce Pdr1 stability compared with wild type²⁷. These observations from Upc2 and Pdr1 suggest that resistance mutations in transcription factors might often not directly alter DNA binding but rather other facets of their activity.

Fig. 3 | Mapping of resistance mutations on protein tertiary structures.

a–h, For each protein structure, residues are coloured by the best positive confidence score for all reported mutations at this residue. Multiple sequence alignments were used to map resistance mutations of orthologues. Confidence scores of 3 (DMS assays) were excluded. *C. albicans* Fur1 in complex with UTP (PDB ID 7RH8) (a), *S. cerevisiae* Fcy1 (PDB ID 1P6O) (b), *S. cerevisiae* Erg3 (AlphaFold 3 prediction with Uniprot P32353 protein sequence, pTM = 0.91) (c), *A. fumigatus* Hmg1 (AlphaFold 3 prediction with Uniprot Q4WHZ1 protein sequence and NADP, ipTM = 0.64 and pTM = 0.47) (d), *S. cerevisiae* Fks1 (PDB ID 7XE4) (e), *C. albicans* Erg11 bound to itraconazole (PDB ID 5VSZ) (f), *C. albicans* Upc2 (AlphaFold 3 prediction with Uniprot Q59QC7 protein sequence

Extreme convergence of AMR mutations among species

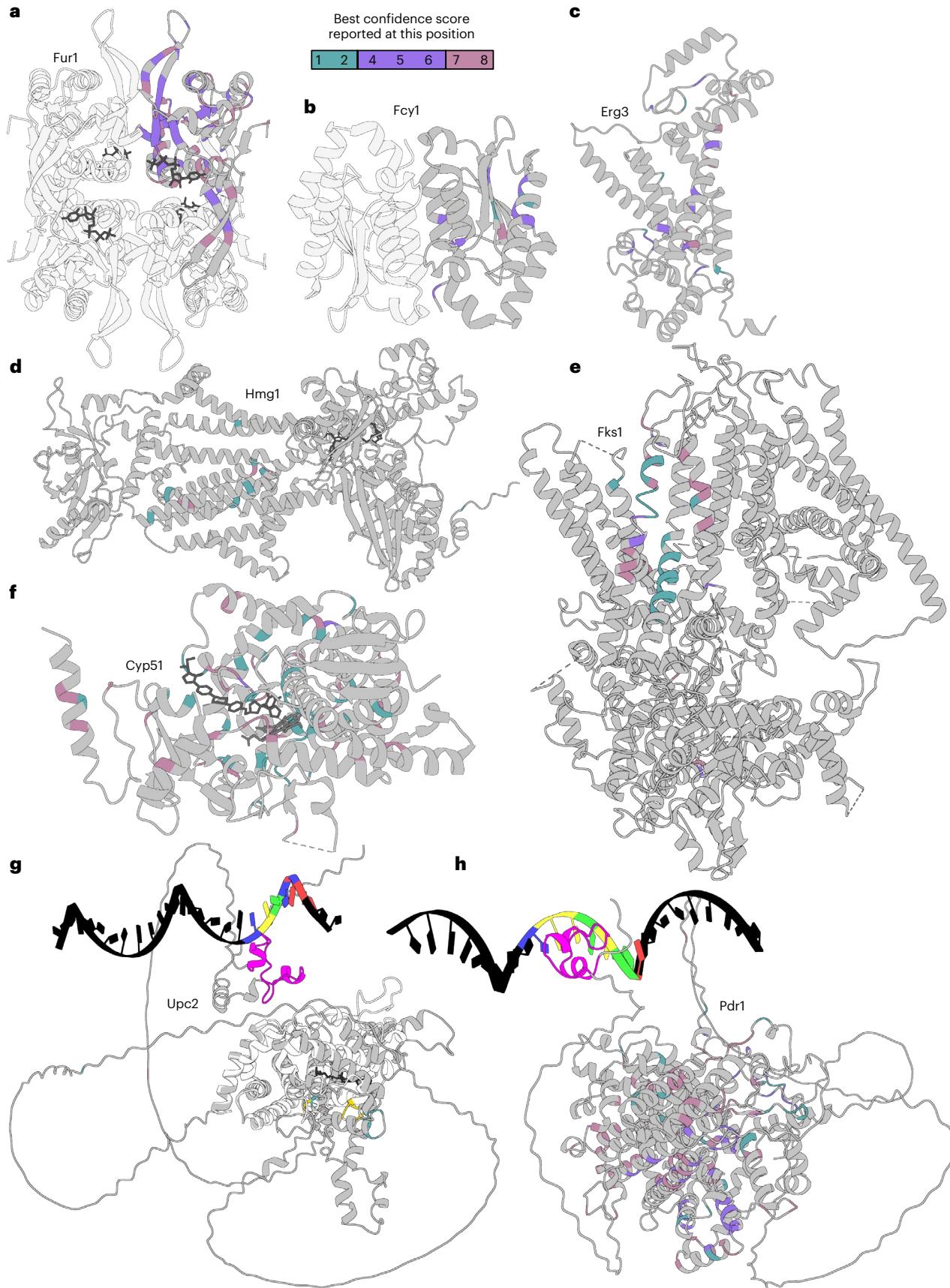
The use of the same antifungals to treat infections by different species allows us to examine evolutionary convergence in the mechanisms of resistance. Convergence is often used as evidence that a trait has been under selection, which in the case of AMR helps interpret and prioritize mutations for validation and for modifying drugs to overcome frequently encountered resistance mutations. Convergence in the molecular bases of resistance could occur in the orthologous genes at the same sites (mutational level); in orthologous genes but at different sites (gene level); or at the functional level, involving genes with similar functions but that are not homologous. We first examined the level of convergence at the gene and at the functional levels by comparing resistance genes between species (Fig. 4a,b and Extended Data Fig. 2). Outside of the specific drug targets such as *ERG11* and *FKS*, which are expected to be convergent, we found that, generally, fungi evolve resistance through mutations in homologous genes or in genes with similar functions. One example of this is efflux pumps of the ABC (for example, *CDR1* of *C. albicans* and *MDR2* of *A. fumigatus*) and MFS (for example, *MSF1* of *A. flavus*) transporter families²⁸, or transcription factors that regulate efflux pumps expression (for example, *TAC1* of *C. albicans*, *PDR1* of *N. glabratus* and *MRR1* of *C. albicans*). In several species, resistance can also result from variation in the number of gene copies²⁹. The absence of convergence, or species specificity in AMR mechanisms, is more difficult to assess due to incomplete data resulting from varying research efforts across species.

We found several cases where resistance mutations occur at the same sites in two or more species (Fig. 4c). The most impressive examples are amino acid (aa) substitutions in Cyp51. For instance, Y132H and F in Cyp51 (*C. albicans* aa numbering) have been found to confer azole resistance in more than 20 species, including the distantly related species *C. albicans*, *A. fumigatus* and *C. neoformans*, presumably by disrupting azole binding³⁰. Other striking examples are mutations at position S645 of Fks (*C. albicans* aa numbering) and G143 of cytochrome b (*A. fumigatus* aa numbering), which are associated with resistance in 10 species or more. In Fks, S645 is located in the echinocandin resistance hotspot 1 and is most likely involved in drug binding^{31,32}. Mutations at the residue S645 have been reported to decrease the maximum velocity of Fks and have been linked to increased echinocandin clinical failure³³. For cytochrome b, it is hypothesized that the G143A mutation alters the binding of Qo inhibitors through steric interactions³⁴. Mutations at residues where resistance mutations with a high level of convergence are observed can confidently be interpreted as resistance mutations across species. We provide the multiple sequence alignments (MSA) of the most frequently reported genes across multiple species as a resource (Supplementary Data 1).

Cross-resistance is common between and within classes of antifungals

One major question regarding AMR mutations is whether they confer cross-resistance to more than one drug. FungAMR allows quantifying the extent of cross-resistance. We found that if a mutation in a gene provides resistance to one antifungal, it will most likely confer cross-resistance to others within a class compared to those

and AATATCGTACCCGATTATGTCGTATAATT DNA sequence³¹, ipTM = 0.37 and pTM = 0.45, grey) superposed with *N. glabrata* Upc2 ligand-binding domain in complex with ergosterol (PDB ID 7VPR, white with orthologous resistance mutations in dark yellow) (g) and *S. cerevisiae* Pdr1 (AlphaFold 3 prediction with Uniprot P12383 protein sequence and GAGAAATGCTCCGGCGAACACTTCTAC DNA sequence³², ipTM = 0.4 and pTM = 0.69) (h). g,h, In bright pink are the Zn2-C6 fungal-type DNA-binding domains. On the DNA strands, colours represent the nucleotides (T, blue; C, yellow; G, green; A, red) of the core Upc2 DNA-binding site TCGTATA of *C. albicans* *ERG11* promoter³³ (g) and of the pleiotropic drug response element (PDRE) TCCGGCGGA present in *S. cerevisiae* *PDR5* promoter³² (h).



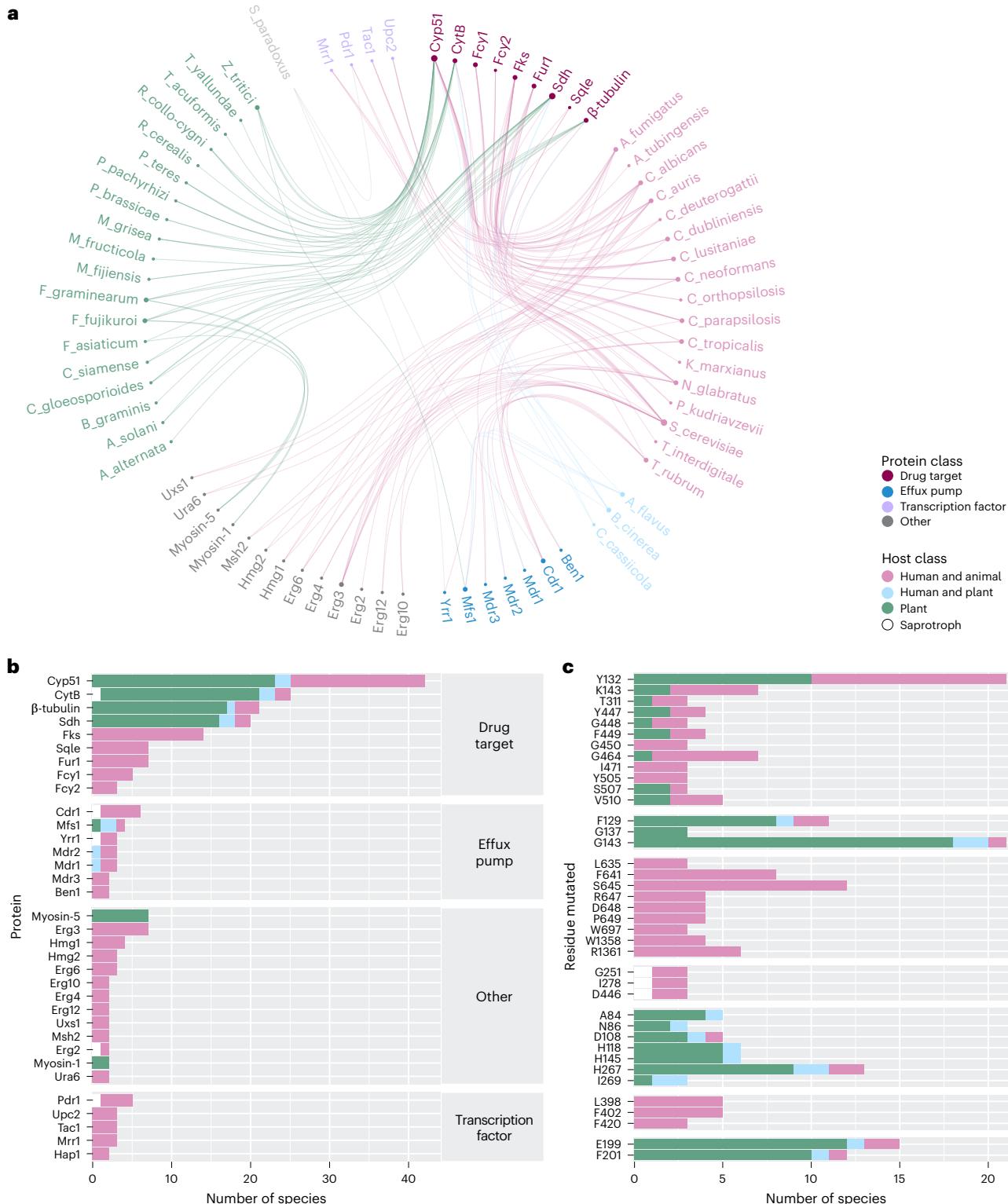


Fig. 4 | Massive convergence in the mechanisms of resistance among fungi at the gene and mutation levels. **a**, Edgebundle plot³⁴ of mutated proteins associated with resistance in more than one fungal pathogen. Only proteins and species with more than five entries were included in this analysis. The interactive html file is available in Supplementary Data 2. Gene classes were coloured on the basis of their function. **b**, Number of species with the same mutated protein associated with resistance. **c**, Orthologous mutations conferring resistance in several species show a high level of convergence at

the mutation level. Cyp51, Sqle (squalene epoxidase) and Fks use *C. albicans* numbering in the MSA. SdhB, SdhC, SdhD, β -tubulin and Cytb (cytochrome b) use *Z. tritici* numbering in the MSA. Pdr1 uses *S. cerevisiae* numbering. Only instances of mutations associated with resistance in more than two species are shown for clarity. Entries with mutations in multiple proteins were excluded as resistance causality cannot be assigned in these cases. The MSAs are available in Supplementary Data 1.

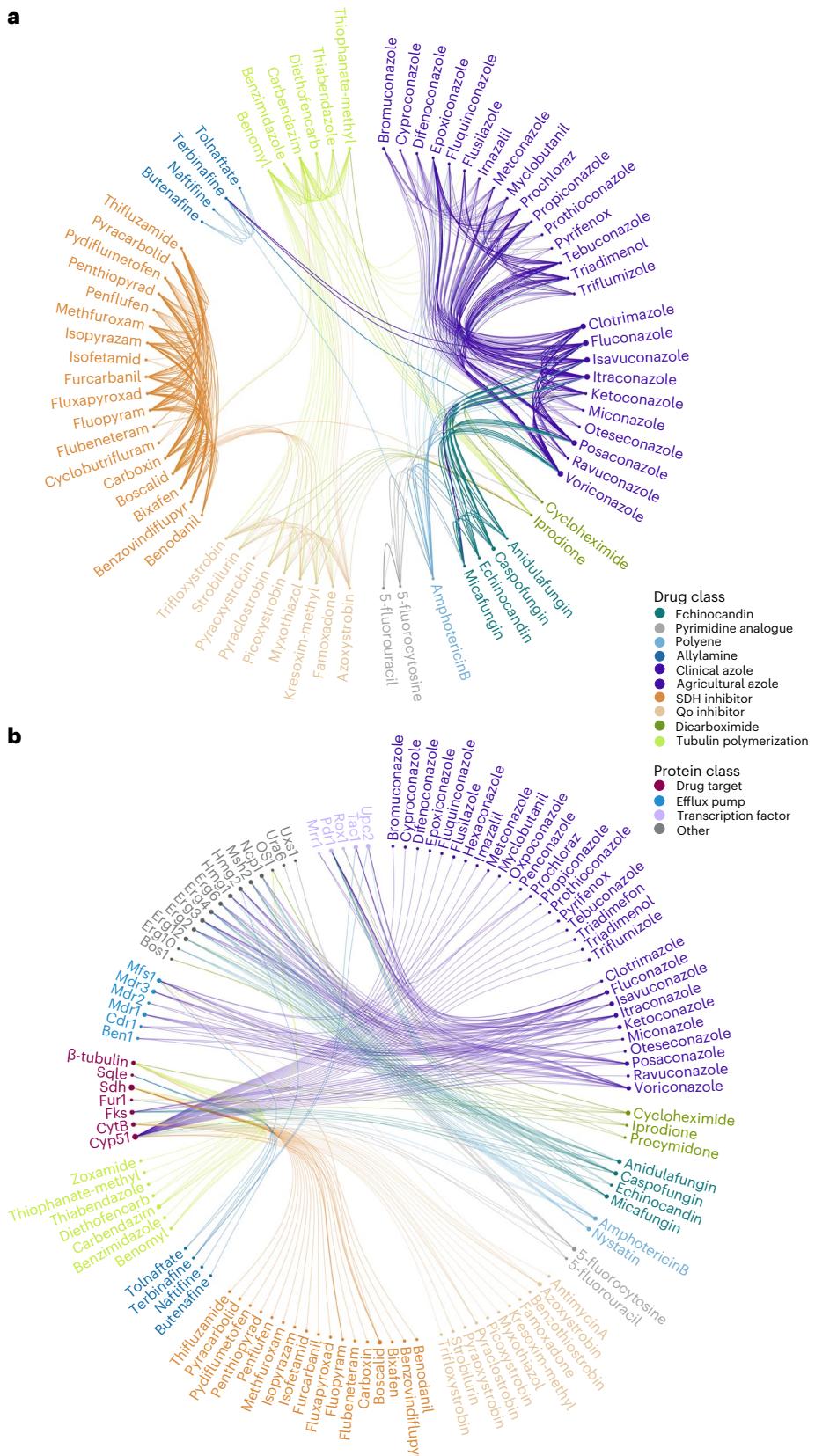


Fig. 5 | Rampant cross-resistance between and within classes of antifungals.

from different classes (Fig. 5a,b and Extended Data Fig. 2). This was expected given the similarities of molecules within a class (Extended Data Fig. 3) and their shared mode of action³⁵. For instance, we found extensive cross-resistance between clinical and agricultural azole antifungals. However, cross-resistance is not a systematic consequence of mutations, given the differences in the chemical structures of the drugs (Extended Data Fig. 3). Since not all mutations present in our dataset were systematically assayed for multiple drugs, it is difficult to identify clear cases of resistance mutations that are specific to only one drug.

Although within-class cross-resistance is more frequent, between-class cross-resistance also occurs³⁶. Mutations found in clinical isolates in proteins implicated in ergosterol biosynthesis such as Cyp51, Upc2, Hmg1, Erg3 and Erg6 can confer resistance to both azoles and polyenes, even if these antifungals target different elements of the ergosterol pathway (Fig. 5b). In addition, some mutations in Fks or Erg proteins seem to be involved in both azole and echinocandin resistance (Fig. 5b). This connection is more surprising since echinocandins do not target cell membranes, but target a β-glucan synthase, which produces a component of the fungal cell wall. Studies have shown that changes in the cell wall or membrane composition can provide an advantage for cells under drug-induced stress^{37,38}, possibly making resistance to the different clinical antifungal classes more interconnected than anticipated from the drugs' mode of action. On the side of agricultural antifungals, cross-resistance between the different classes is also present, but seems to be less frequent than among human pathogens. Such a result could also be due to lack of data, as resistance has been less frequently tested systematically among classes of fungicides. Although incomplete, these analyses are sufficient to further strengthen important concerns. First, cross-resistance between medical and agricultural azoles is frequent. Second, azoles, polyenes and echinocandins are connected through a core set of genes, meaning that cross-resistance to clinical drugs can evolve through single mutations. The full extent of antifungal cross-resistance remains to be assessed, but this analysis reveals that three out of the four classes of drugs used to treat fungal infections are not entirely orthogonal. This stresses the need for new molecular targets and drugs, and for increased stewardship between clinical and agricultural drugs³⁶.

ChroQueTas efficiently screens AMR in fungal genomes

To facilitate the analysis of AMR in fungal genomes from the FungAMR data, we developed ChroQueTas, a user-friendly bioinformatic tool that only necessitates a fungal genome, proteome or annotation file. As a practical example of how ChroQueTas can be used, we report here the results for Cyp51 (Erg11) of 46 *C. albicans* and 144 *Z. tritici* published isolates^{39–41} (Supplementary Table 9).

As a summary, we found that 83.3% of the *C. albicans* genomes in this dataset harboured at least one mutation in Erg11 known to cause AMR (Fig. 6a). The most commonly identified mutated positions were A114 ($n = 11$) and Y132 ($n = 11$). Nine of the *C. albicans* that harboured the mutation A114S also harboured Y257H, a combination that has been previously reported⁴² to cause AMR against different azoles. The other two genomes harboured the mutation A114V together with F145L, a combination known to confer azole resistance⁴³. For the *Z. tritici* genomes, 84.7% of the isolates showed at least one mutation in Cyp51 known to confer resistance (Fig. 6b). Notably, for both species, we identified AMR mutations that were not reported by the original studies (highlighted in green in Fig. 6). The results summarized here show the strength of ChroQueTas and FungAMR to efficiently screen AMR in fungal genomes and the potential to identify previously unknown mutations involved in AMR.

Discussion

Despite the threat that fungal pathogens and AMR represent, we were still missing a high-quality centralized antifungal mutations repository. Here we present FungAMR, a compendium of 35,792 carefully curated entries of clinical and agricultural importance. One major finding is that

many resistance mutations reported in the literature have very limited experimental support beyond identifying a mutation in a known resistance gene in a resistant isolate, which can be coincidental (confidence scores of 7 and 8, Fig. 1b). Such low-quality reports create an overconfidence in our knowledge of the identity of resistance mutations. Our observations call for more work focusing on the direct association of mutations to resistance. Reconstructing individual mutations in pathogens is more challenging than in model organisms, but recent progress with genome editing makes such experiments possible on an appreciable scale (confidence score of 1, Fig. 1b). We observed that heterologous expression of resistance genes (confidence score of 2, Fig. 1b) and high-throughput experiments such as deep mutational scanning (confidence score of 3, Fig. 1b) are powerful ways to characterize a large number of genotype–resistance phenotype associations^{18,44}.

FungAMR also highlights how reports are extremely skewed towards a few fungal species, genes and drugs (Fig. 1). The scientific community would benefit from diversifying studies, since focusing on only a few species and known resistance genes limits our understanding of the full range of resistance mechanisms. Although limited, the information we currently have is crucial in our fight against fungal pathogens and suggest that there is a high level of convergence in the mechanisms of resistance among fungi (Fig. 4) and that cross-resistance seems to be common within and between classes of antifungals (Fig. 5). FungAMR has the potential to help predict resistance mutations and develop new drugs, but more studies incorporating different species and different antifungals are needed to determine how universal our observations are. In the meantime, there is still an urgent need to identify new molecular targets and drugs for treatment, and the dual use of some antifungal classes in the clinic and in agriculture should be considered a critical threat to our ability to treat human fungal infections. Fisher et al.⁴⁵ propose a roadmap with actionable guidance for researchers to better understand and manage fungal antimicrobial resistance across One Health.

We developed ChroQueTas, a user-friendly bioinformatic tool based on the FungAMR resource, to facilitate the screening of AMR mutations in fungal genomes. We show that ChroQueTas and FungAMR together allow efficient screening of AMR in published fungal genomes by identifying all resistance mutations reported by the authors in addition to resistance mutations that were not reported initially.

It is important to note that FungAMR is intended to be a general resource to better understand resistance mechanisms rather than a guide to predict treatment outcomes. FungAMR data are based entirely on diverse in vitro assays reported by hundreds of researchers in the literature, which may not directly reflect clinical or field observations. In addition, treatment success or failure is influenced by several factors beyond the scope of this work, such as host immune responses, drug pharmacodynamics, biofilm formation and pathogen strain variability^{46–48}. Therefore, although in vitro susceptibility testing is useful for identifying isolates less likely to respond to a drug, it may not correlate with the outcome of an antifungal treatment⁴⁹. Future studies will be needed to link in vitro susceptibility testing and the complex parameters in infected hosts to potential treatment outcomes. Nonetheless, comprehensive datasets such as FungAMR are an important step towards this objective.

Finally, FungAMR has been incorporated as a web-searchable interface within the Comprehensive Antibiotic Resistance Database (CARD) ([FungAMR Mutation Data](#)). The data will be updated as new reports of fungal AMR are released. We created an email address for FungAMR (fungamr.db@gmail.com) that will allow the community to send us the same curation sheet the curators team used (Supplementary Table 3).

Methods

Literature review, data curation and development of the FungAMR resource

All papers were read by a scientist in the field and the information was extracted in a systematic manner. In addition to the laboratories

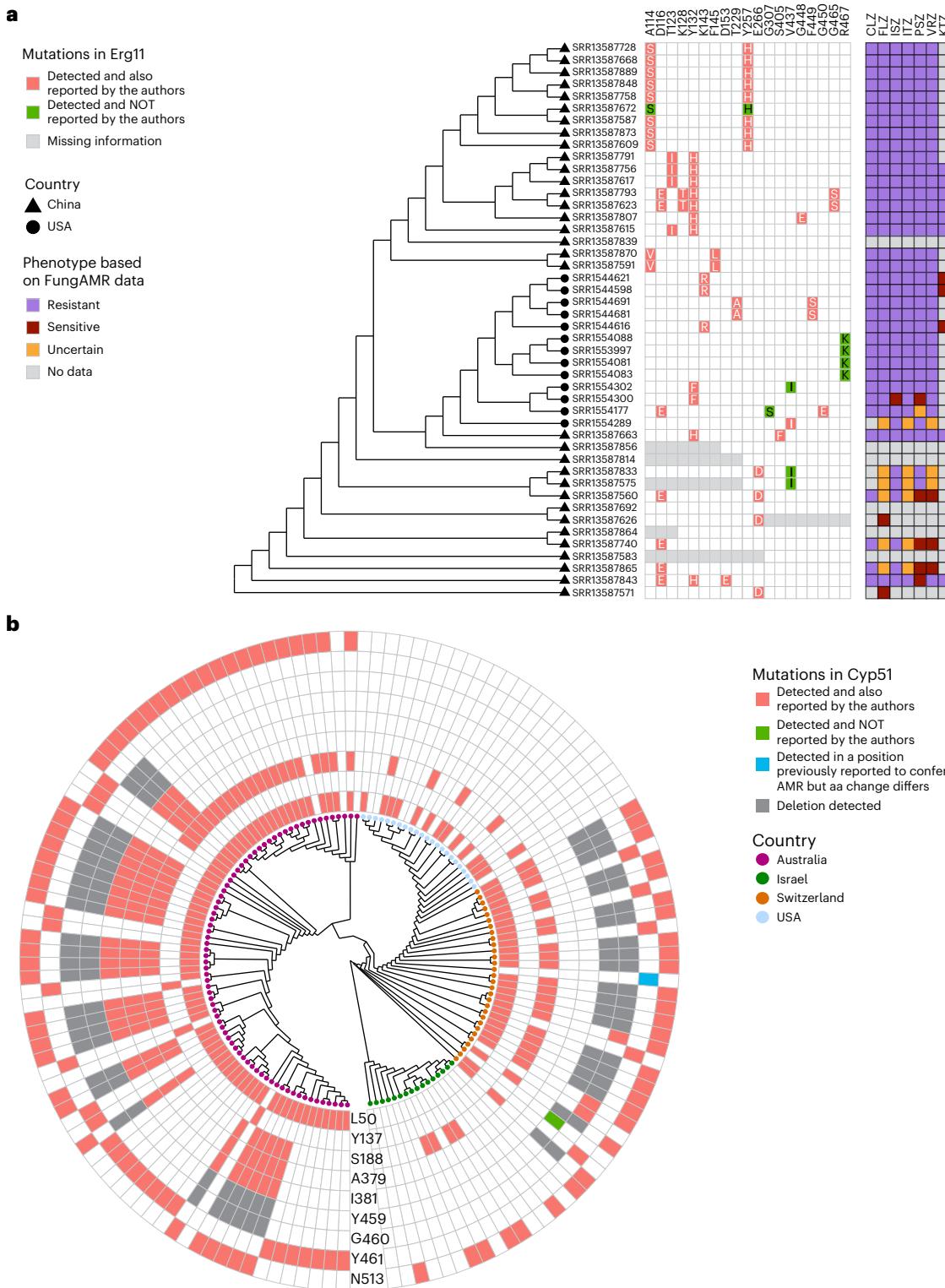


Fig. 6 | Practical example of AMR in silico mutation screening using ChroQueTas and FungAMR. a,b, Phylogenetic comparison of *C. albicans* (a) and *Z. tritici* (b) datasets based on the average nucleotide identity (ANI). The trees' tips are coloured or shaped according to the country in which strains were isolated. Each heatmap represents the position within the query protein sequences (Erg11 for *C. albicans* and Cyp51 for *Z. tritici*) and is coloured

when a mutation was detected with ChroQueTas. AA, amino acids. 'Missing information' in a stands for proteins that could only be resolved partially due to genome fragmentation, and 'Uncertain' refers to mutations that are associated with low positive and high negative evidence scores making the phenotype uncertain. CLZ, clotrimazole; FLZ, fluconazole; ISZ, isavuconazole; ITZ, itraconazole; PSZ, posaconazole; VRZ, voriconazole; and KTZ, ketoconazole.

involved, some of the curators were recruited among graduate students and postdoctoral fellows from laboratories working on fungal genetics and antifungal resistance, which are part of a pan-Canadian training

programme on the evolution of fungal pathogens, **EvoFunPath**. Two undergraduate students were involved in curating papers that more senior scientists also curated. Curation was conducted between April

2023 and March 2025 and included publications from 1988 to 2024. All curators collected papers reporting antimicrobial resistance using standard literature searching and mining references from other papers. Curators initially used general terms related to fungal antimicrobial resistance such as Resistance, Antifungal, Fungicide, Mutation, *Candida*, *Aspergillus*, *Cryptococcus*, *Zymoseptoria*, *Azoles*, *Echinocandins*, *Polyenes*, 5-fluorocytosine, Flucytosine, Fungi, SNP, Deletion, Over-expression, Aneuploidy and so on. Then, other relevant references were searched in the research papers or reviews and included in the curation. We examined human, animal and plant pathogens in addition to model species such as the laboratory model *Saccharomyces cerevisiae*, which is used as a model system to study drug resistance. We also re-curated all papers reported in the MARDy database¹⁰, as these did not come with any measure of confidence levels (see below).

Each paper was read by one of the curators, and mutations reported to be associated with resistance were extracted. Mutations also reported to probably not cause resistance were included in FungAMR but not used in the analysis since it is difficult to judge whether this is useful information, as assays rarely contain positive controls in parallel. Each entry (Supplementary Table 1) is associated with the first author, the journal, the publication year and the PubMedID (PMID) of the paper in which the mutation was reported, the fungal species and its host, the gene name and its protein's accession number, the location of the mutation, its impact on the gene (deletion, insertion, amino acid change, duplication) and the impact on susceptibility to the drugs assayed. We also recorded the origin of the strains (environmental, clinical, laboratory or evolved) as well as the quantitative measures of resistance or susceptibility when provided by the authors. These were often MICs, but many more measures were reported such as the half maximal effective concentration (EC_{50}), a more common quantitative measure used in the agricultural field. We tried to record these measures as faithfully as possible. Still, we found that the diversity of methods used made any systematic recording of the quantitative impact of the mutations difficult. We therefore relied on the author's assessment to record the mutation as being associated, or not, with resistance. When the authors did not assess whether a mutation was associated with resistance, we define strains as resistant when they have a significantly higher growth in the presence of an antifungal than the reference strain, or when they present with a minimal inhibitory concentration to an antifungal above the established breakpoint. Each reported entry concerned a particular strain and its associated phenotype. For instance, if a strain was reported and had multiple mutations in the gene or genes sequenced, those multiple substitutions were reported together in a single entry as it was impossible to discern which one could have been causal. When available, we also recorded the identifier of the genes reported so we could track the reference sequences for species of interest. These identifiers came from various databases such as the Candida Genome Database or NCBI. However, this was not systematically done by the authors, so some entries are left blank. Not all papers reported the gene identifier for the reference genes the mutations were reported to impact. We therefore performed a quality check by comparing the mutations reported (change from the wild-type genotype to the mutant one) with the standard reference genes from the species of interest (Supplementary Table 4). A commentary column is included where curators noted information relevant to the entry, such as the experimental method, the culture media or the antifungal susceptibility testing method (for example, EUCAST, CLSI). Finally, we extracted from all curated papers any information relevant to the clinical outcome, and this information is listed in the 'clinical outcome' column.

All curator annotation sheets (Supplementary Table 3) were combined into a single table after refining and editing the nomenclature of species, genes or proteins, mutations, drugs, journal names and strain origins to facilitate the comparison of mutations. MIC values were edited and converted to the same units whenever possible. Two

additional columns were added, one with an alternative species name and another one with a gene name that corresponds to orthologues or homologues of that gene in other species. In cases where the study was curated by multiple curators, entries of both curators were first compared for consistency and corrected if necessary. Redundant entries from the same study (mutations in the same gene, drug and strain) were combined or one of the duplicate entries was removed.

For each mutation or group of mutations, we assigned a score that assesses the degree of evidence that supports their role in drug resistance. The scoring scheme was inspired by the use of Multiplexed Assays of Variant Effects (MAVEs) to classify human pathogenic variants^{55,56} and based on examples of resistance reports that we had seen in the literature and were agreed upon and discussed among the curators. The list of confidence scores associated with their description is presented in Supplementary Table 2. A positive confidence score denotes mutations reported to confer resistance, while a negative confidence score relates to mutations reported in susceptible strains. A low positive confidence score indicates that the evidence for the contribution of the mutation to resistance is strong, with 1 being the strongest and 8 being the weakest. Similarly for negative scores, the evidence of susceptibility is stronger for -1 than for -8.

When a mutation is reported in several independent studies or strains within a study, the strongest resistance evidence (that is, the best positive confidence score reported) and the strongest sensitivity evidence (that is, the strongest negative confidence score reported) are provided in two separate columns.

Variant effect predictors

For all protein reference sequences retrieved, we used HHblits⁵⁷ from HH-suite3 (ref. 58) with each sequence as a query to create MSAs representative of the orthologue diversity. Initial HHblits iterative sequence search filter criteria were: a minimum coverage of 80% with query sequence, at least 20% and maximum 98% identity to the query, and an e-value cut-off for inclusion of 0.0001. Filter criteria were then gradually relaxed to get a minimum of 200 sequences in the final alignment. A total of 267 alignments were produced, with a median of 847 sequences per alignment. With these MSA files as input, we ran GEMME¹⁹ locally from docker desktop to compute the predicted effect of every single amino acid substitution across the proteins.

The protein structures from the proteins contained in FungAMR were obtained from the AlphaFold database (AlphaFold Protein Structure Database, Supplementary Table 5). We used these structures to estimate the effects of amino acid substitutions on protein stability using FoldX⁵⁰. The RepairPDB function was run 10 times with water prediction to repair incorrect torsion angles, VanderWaals clashes and total energy of the structure. We then used the MutateX Python package²⁰ to perform an in silico deep mutational scanning of the whole protein, running the BuildModel function from FoldX and computing the difference in Gibbs free energy between the mutant and the wild type.

Tertiary protein structure predictions

When experimentally determined protein structures were available, they were retrieved from the Protein Data Bank (RCSB PDB, PDB). No complete tertiary protein structures were available on the PDB for Erg3, Hmg1, Upc2 and Pdr1, therefore we used the Alphafold 3 (ref. 59) web server (AlphaFold Server) to predict the entire structures (seed; Erg3: 382113193, Hmg1: 1841159774, Upc2: 601343710 and Pdr1: 264428788). Protein sequences were retrieved from UniProt (Erg3: P32353, Hmg1: Q4WHZ1, Upc2: Q59QC7 and Pdr1: P12383). For Hmg1, the NAP (nicotinamide-adenine-dinucleotide-phosphate (NADP)) ligand was included in the prediction. For the transcription factors Upc2 and Pdr1, a DNA sequence containing one of the described DNA-binding sites of the enzymes was included in the prediction (Upc2: AATATCGTACCCGATTATGTCGTATATT from *C. albicans* ERG11 promoter^{51,53} and Pdr1: GAGAATGCTCCGCGGAACTCTTCTAC from *S. cerevisiae* PDR5 promoter⁵²).

Overall data description

We constructed tables of correspondence of amino acid sequences among the orthologues of the proteins reported in the data. Reference sequences for all proteins were retrieved from the Uniprot or NCBI database, and MSAs were performed on orthologous sequences using Muscle5 (ref. 60). All alignments for each family of orthologues are available in Fasta format as Supplementary Data 1. These MSA files were used to assign a group number to orthologous mutations in different species, which can be found in an additional column. Alignment positions were also used to produce figures and to map resistance mutations on protein structures. The classification of the host of the fungal species, drug classes and gene types used in the figures are available in Supplementary Tables 6–8.

To display phylogenetic relationships among the species present in FungAMR, we used a phylogenetic tree constructed in ref. 61 which includes 67 species from the database. To include approximate positions of the 18 remaining taxa at the species level, subphylogenies of species subsets were built using internal transcribed spacer (ITS) sequences from either ITS RefSeq (BioProject number PRJNA177353), UNITE database v.9, or if these were missing, from GenBank. Specific host forms (*forma specialis*) and hybrids were omitted from the species tree. Any ambiguous bases were resolved randomly, then sequences were aligned with mafft (v.7.525)⁶² and trimmed using trimAl (v.1.4)⁶³ with the ‘gappyout’ option. Maximum likelihood trees were built with iq-tree (v.2.2.2.7)⁶⁴. Branches with missing species were added to the main phylogenetic tree, retaining the proportion of the branch lengths and position of the nodes with respect to related species from the ITS tree. The tree was visualized using the ggtree package⁶⁵ in R.

Visualization of protein structures was done with ChimeraX⁶⁶. Figures and analyses were done using the following Python packages: Matplotlib⁶⁷, NumPy⁶⁸, SciPy⁶⁹, pandas⁷⁰ and seaborn⁷¹, and the R packages: dplyr⁷², tidyR⁷³, stringr⁷⁴, igraph^{75,76}, ape⁷⁷, castor⁷⁸, RRphylo⁷⁹ and ggplot2 (ref. 80).

Computational pipeline to detect mutations and indels causing resistance

In addition to the FungAMR repository, a bioinformatic tool named Chromosome Query Target (ChroQueTas) was developed to rapidly screen for AMR mutations in fungal genomes. ChroQueTas was devised to work as a command-line interface on UNIX environments (tested on Debian and Ubuntu-based operating systems) in a user-friendly manner so it can be run by following straightforward instructions and commands. ChroQueTas is open source and publicly available at <https://github.com/nmquijada/ChroQueTas>, together with instructions for its installation (available with Conda and Docker) and usage, and a wiki to get the most out of the software.

ChroQueTas requires as input either a fungal genome, proteome or annotation file (FASTA, FAA, GBF or GBK formats, and gz and bz2 compressions are allowed) and a ‘species’ flag (to be provided as ‘-s/--species’) corresponding to the species of the fungal genome as reported in the FungAMR repository (available species and associated proteins can be inspected by using the ‘--list_species’ flag). With that information, ChroQueTas will: (1) extract from the fungal genome the coding DNA sequence (CDS) and protein where a point mutation is known to cause AMR in that particular species by using miniprot (v.0.14-r265)⁸¹ and the information contained in FungAMR (the translation genetic code is automatically set to ‘standard’ for most fungi and to the ‘alternative yeast code’ for the CTG clade, but can be customized by enabling the ‘--trans_code’ flag); (2) evaluate sequence similarity against the reference by using Diamond v.2.0.4 (default option when using fungal proteomes as input) and BLASTP (v.2.14.1+)⁸², and discard low-confidence hits (to be specified by the user and the ‘--min_id,--min_cov’ flags); (3) deal with potential introns, exons and indels; (4) align polished sequences to their species-specific reference using MAFFT (v.7.525)⁶² and evaluate amino acid positions between the

query and the reference proteins accounting for FungAMR information; and (5) report amino acid changes and indels that could lead to AMR according to FungAMR, together with their confidence scores and the predicted resistance phenotype. The output from ChroQueTas consists of different text files with the information resulting from the AMR screening and further metadata contained in FungAMR, which are automatically downloaded and formatted by ChroQueTas during installation. ChroQueTas is intended to be a ‘living project’, being hosted in a public repository with continuous updates based on the state of the art and interaction with users towards potential further improvements and implementations.

To illustrate the power of the software, the Illumina raw sequencing data from 46 *C. albicans* and 144 *Z. tritici* isolates were downloaded using SRA Toolkit v.3.1.0 (<https://github.com/ncbi/sra-tools>) (Supplementary Table 9). These two species were selected to represent human and agricultural pathogens, respectively. The *C. albicans* dataset included isolates from two countries (China and the United States). The *Z. tritici* dataset included isolates recovered from wheat from four countries (Australia, Israel, Switzerland and the United States). The sequencing data quality was assessed using FastQC (v.0.11.9)⁸³. Residual adapters and barcodes were discarded, and quality filtering was performed using FastP (v.0.23.2)⁸⁴ by setting a minimum Phred score of 25 and a minimum length of at least 75% of the length of the raw FASTQ files. Genomes were assembled using SPAdes (v.3.15.5)⁸⁵ and contigs below 1,000 bp were discarded. The quality and completeness of each draft genome was assessed with QUAST (v.5.0.2)⁸⁶, BUSCO (v.5.4.3)⁸⁷ and by aligning the high-quality reads against the draft genomes using Bowtie2 (v.2.4.2)⁸⁸. The genomes that overcame quality control were submitted to ChroQueTas by choosing their species-associated scheme, which performed AMR screening over all the target proteins described in FungAMR for these two species, using the following command:

```
ChroQueTas --input <genome>.fasta --species <Candida_albicans/Zymoseptoria_tritici> --threads <num. CPUs> --output <genome>_ChroQueTas
```

The average nucleotide identity (ANI) of the fungal genomes was calculated using fastANI (v.1.32)⁸⁹ and the distance matrix was imported into the R environment⁹⁰ to build a phylogenetic tree using the ape, ggnnewscale, ggplot2 (ref. 80), ggtree⁶⁵, ggtreeExtra and reshape⁹¹ packages. For visualization purposes, only the results from mutations affecting Cyp51 (Erg11) are reported here.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The FungAMR resource is available in Supplementary Table 1 and in a web-searchable interface on the CARD database website ([The Comprehensive Antibiotic Resistance Database](#)). The most up-to-date version of FungAMR can always be found on GitHub at <https://github.com/Landrylab/FungAMR> (ref. 92). The multiple sequence alignment files for the genes present in FungAMR are available in Supplementary Data 1. The Protein Data Bank is available at <https://www.rcsb.org/>. UniProt is available at [UniProt](#). The AlphaFold database is available at [AlphaFold Protein Structure Database](#). The accession ID of the public genomes used for Fig. 6 and ChroQueTas are described in Supplementary Table 9. Source data are provided with this paper.

Code availability

All scripts for figures and for FungAMR content analysis are available on GitHub at <https://github.com/Landrylab/FungAMR> (ref. 92) (<https://doi.org/10.5281/zenodo.15742040>). The ChroQueTas code is available on GitHub, together with instructions for installation and usage, at <https://github.com/nmquijada/ChroQueTas> (ref. 93).

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Author contributions

C.R.L. and N.M.Q. designed the study. Data curation was performed by C.B., P.C.D., R.D., S.P., EM.M.A., F.D.R., D.F.J., A.J., M.G., M.B., J.S., L.M., N.C.G., A.C.T.M., D.M.-S., A.C.G., R.S.S., C.R.L. and D.G.D.L. Data analysis was done by A.P., C.B., A.F., I.G.-A., C.R.L., S.B., N.M.Q., D.M.-S. and J.R. Software development was done by N.M.Q., D.M.-S. and A.J.A. Figures were prepared by C.B., A.P., A.F., D.M.-S., A.J.A. and N.M.Q. The manuscript was drafted by C.B. and C.R.L. and editing was done by all authors. Funding was provided by C.R.L., A.C.G., R.S.S., A.S., P.J.D., A.B. and N.M.Q. Data contributions were made by A.B. and D.G.D.L.

Competing interests

The authors declare no competing interests.

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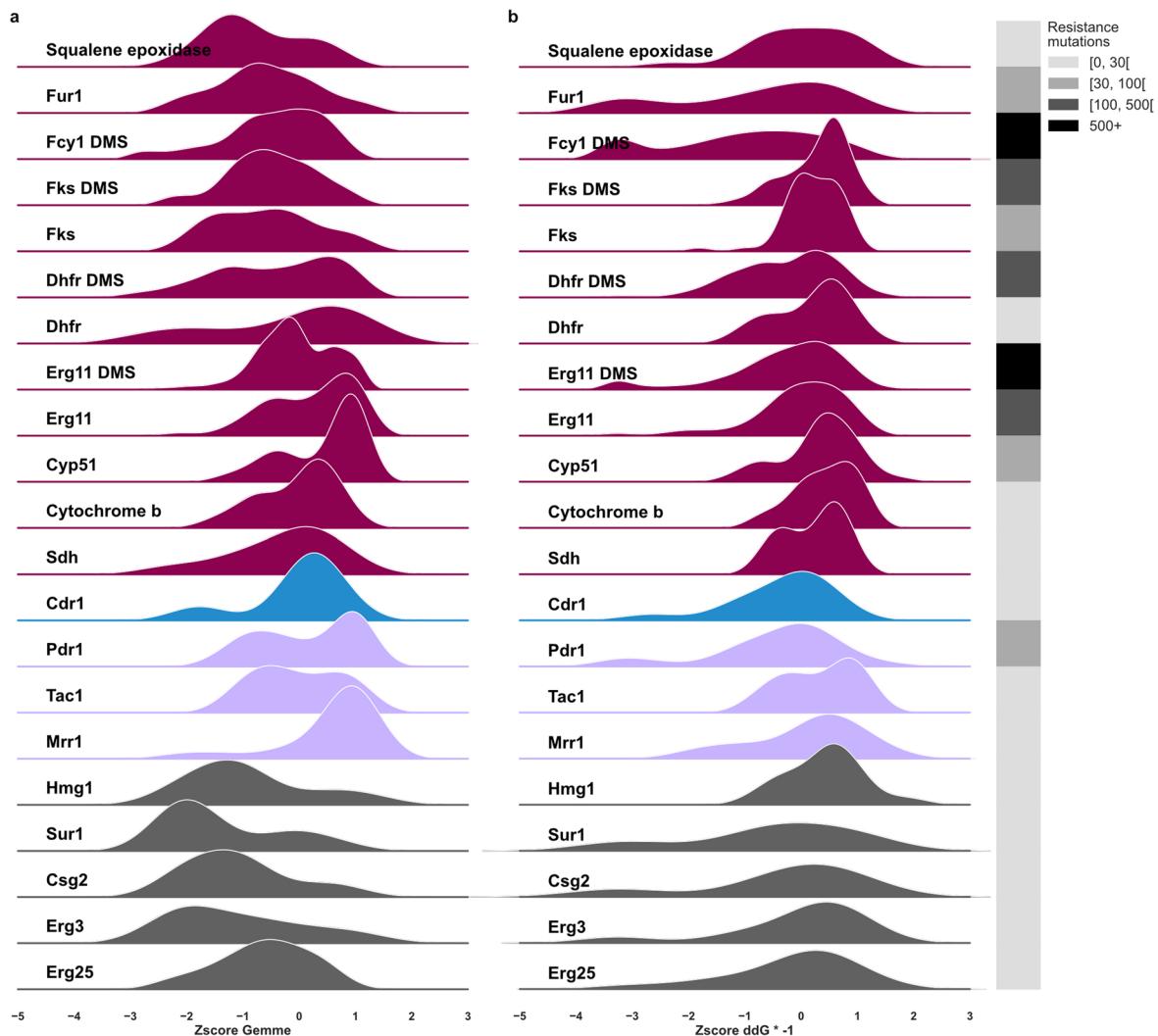
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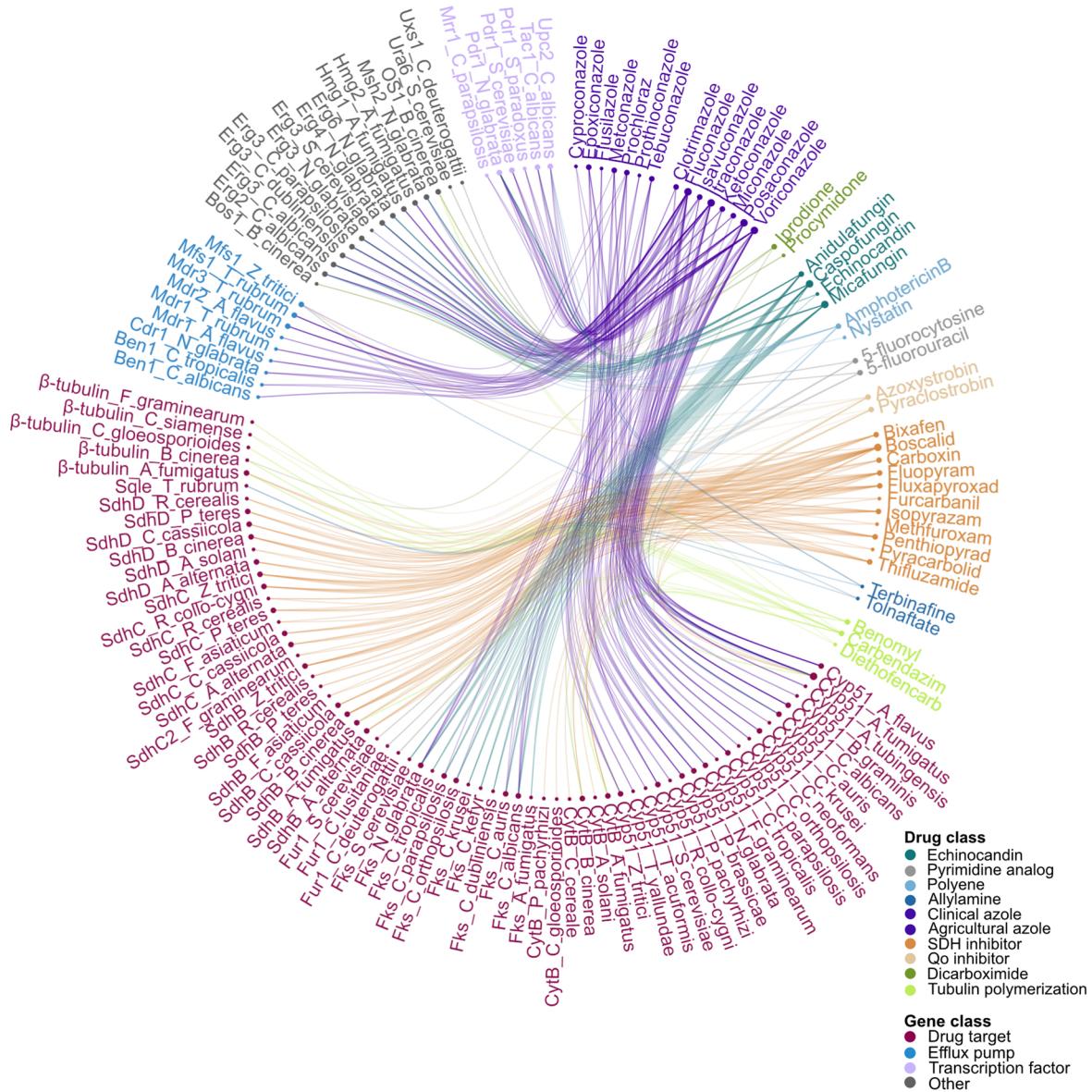
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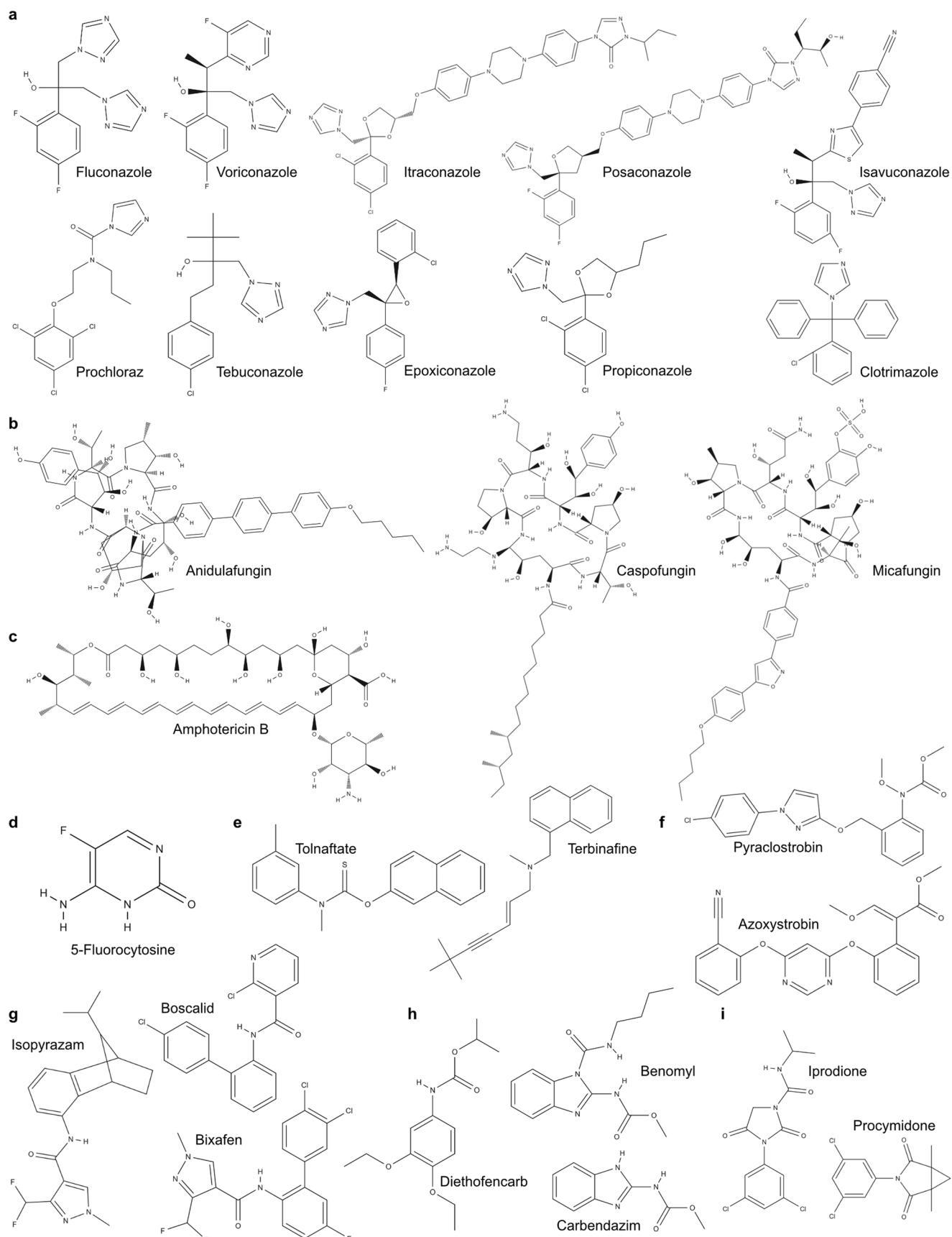
Extended Data Fig. 1 | Distribution of predicted effect of AMR mutations on protein function and stability. **a**, Distribution of the standardized GEMME score for all unique resistance mutations across proteins. Negative values predict a higher impact on the protein function based on evolutionary conservation. **b**, Distribution of the standardized negated predicted change in the Gibbs

free energy ($\Delta\Delta G$) upon mutation predicted by FoldX for all unique resistance mutations across proteins. Negative values predict destabilization of the protein. For each protein, when mutations were available from DMS experiments (confidence score of 3), the DMS data is shown separately. The grayscale shows the number of unique resistance mutations per protein.



Extended Data Fig. 2 | Convergence of resistance mechanisms and cross-resistance between antifungals. Edgebundle plot⁵⁴ linking species specific proteins to antifungal resistance. Orthologous proteins are associated with drug resistance across multiple fungal pathogens, demonstrating evolutionary

convergence. Furthermore, mutations in the same protein are linked to resistance against multiple drugs and drug families, indicating cross-resistance. Only proteins and drugs with more than five entries were included. The interactive html file is available in Supplementary Data 5.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Chemical structures of antifungals from different classes. **a**, Fluconazole, voriconazole, itraconazole, posaconazole and isavuconazole are triazoles used in clinics. Tebuconazole, epoxiconazole and propiconazole are triazoles used in agriculture. Clotrimazole and prochloraz are imidazole used in clinics and in agriculture respectively. **b**, Anidulafungin, caspofungin and micafungin are echinocandins used in clinics. **c**, Amphotericin B is a polyene used in clinics. **d**, 5-Fluorocytosine (5-FC) is a pyrimidine analog

used in clinics. **e**, Tolfanate and terbinafine are allylamines used to treat topical infections. **f**, Pyraclostrobin and azoxystrobin are quinone outside inhibitors (QoI) used in agriculture. **g**, Isopyrazam, boscalid and bixafen are succinate dehydrogenase inhibitors (SDHI) used in agriculture. **h**, Diethofencarb, carbendazim and benomyl are inhibitors of tubulin polymerization used in agriculture. **i**, Iprodione and procymidone are dicarboximides used in agriculture. Molecular structures were retrieved from PubChem¹²³.

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Data collection	No softwares were used for data collection.
Data analysis	All scripts for figures 1 to 5, Extended data Fig., and for FungAMR content analysis are available on GitHub at https://github.com/Landrylab/FungAMR . In figure 1, to display phylogenetic relationships, mafft v7.525, trimAl v1.4, iq-tree v2.2.2.7 and the following R packages (R v4.2.2) : ggtree v3.7.2, dplyr v1.1.4, tidyr v1.3.0, ape v5.6-2, castor v1.8.0 and RRphylo v2.8.0 were used. For figure 2, pandas 1.5.3, matplotlib 3.7.1, numpy 1.24.2, seaborn 0.13.0 and scipy 1.10.1 (Python v3.11.3) were used and in silico mutation scans were done using HHblits v3.3.0, MutateX v0.8 (foldX suite 5), pyfold paramx v0.1.4 and we ran GEMME locally from docker desktop v4.29.0 (elodelaine/gemme:gemme). Other figures and analysis were done using the following Python packages: matplotlib 3.7.2 numpy 1.23.5, pandas 1.5.2 and seaborn 0.12.2 (Python v3.9.7), and the following R packages: dplyr v1.1.4, tidyr v1.3.1, stringr v1.5.0, igraph v2.0.3, edgebundleR v0.1.4 and ggplot2 v3.5.1 (R v4.1.3). ChroQueTas and scripts for figure 6 are available on GitHub at https://github.com/nmquijada/ChroQueTas and its wiki. ChroQueTas uses miniprot v.0.14-r265, mafft v.7.525 and BLASTP v2.16.0. For the analysis displayed in figure 6, FastQC v0.11.9, FastP v0.23.2, SPAdes v3.15.5, QUAST v5.0.2, BUSCO v5.4.3, Bowtie2 v2.4.2 and fastANI v1.32 were used. The phylogenetic trees and heatmaps for visualization were made using ape v5.8.1, ggplot2 v3.4.3, ggrepel v0.5.2, ggtree v3.16.0, ggtreeExtra v1.18.0 and reshape v1.4.4 packages (R v4.3.1).

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The FungAMR resource is available in Supplementary table 1 and on the CARD database website. The multiple sequence alignment files for the genes present in FungAMR are available in Supplementary data 1. The dataset used for the Supplementary note 1 is available at 10.5281/zenodo.12583470. The Protein Data Bank is available at <https://www.rcsb.org/>. UniProt is available at UniProt. The AlphaFold database is available at AlphaFold Protein Structure Database. The accession number and metadata information for the public genomes used for Figure 6 are described in Supplementary Table 9.

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Sample size	No experimental work was done. Sample size determination was not applicable. AMR mutations were manually curated from papers by scientists.
Data exclusions	No data were excluded.
Replication	No experimental work was done. Replication was not applicable
Randomization	No experimental work was done. Randomization was not applicable
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