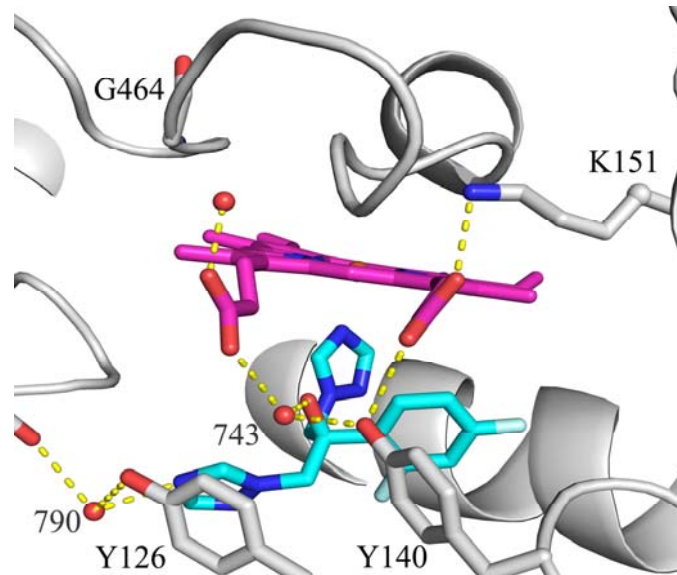


1	<i>S. cerevisiae</i>	MSATKSIVGEALEYVNI GLSHFLALPLAQRI--SLIIIIIPFIYNIWQLLYSLR-KDRPPLVFIWIPWVGS AVVYGMKPY	77
2	<i>C. albicans</i>	----MAIVETVID----GINYFLSLSVTQQI--SILLGVFPVYNLVWQYLYSLR-KDRAPLVFIWIPWFGSAASYGQQPY	69
3	<i>A. fumigatus</i> A	-----MVPML-----WLTAYMAV--AVLTAILLNVVYQLFFRLWNRTPEPPMVFWVYLGSTISYGIDPY	58
4	<i>A. fumigatus</i> B	MGLIAFILDGICKHCSTQST----WVLVIGIGLLSILAVSVI INVLQQLLFKN--PHEPPVVFHWFPFIGSTISYGIDPY	73
5		: : : ..: *:: * :: ..****:*:*:*:** ** .**	
6			
7	<i>S. cerevisiae</i>	EFFEECQKKYGDIFSFVLLGRVMTVYLGPKGHEFVFN AKLADVSAEAA YAHLTTPVFGKGVYDCPN SRLMEQKKFVKGA	157
8	<i>C. albicans</i>	EFFESCRQKYGDVFSFMLLGKIMTVYLGPKGHEFVFN AKLSDVSAEDAYKHLTTPVFGKGVYDCPN SRLMEQKKFAKFA	149
9	<i>A. fumigatus</i> A	KFFFACREKYGDIFTFILLGQKTTVYLG VQGNF ILNGKLDVNAEEVYSPLTTPVFGSDVVYDCPN SKLMEQKKFIKYG	138
10	<i>A. fumigatus</i> B	KFFFDCRAKYGDIFTFILLGKTTVYLGTKGNDF ILNGKLRDVCAEEVYSPLTTPVFGRHVVYDCPN AKLMEQKKFVKYG	153
11		::* * : ****:*:*:*:**: ***** :*:*:*:*:* ** * * * * * * * * * * * * * * * * *	
12			
13	<i>S. cerevisiae</i>	LTKEAFKSYVPLIAEEVYKYFRDSKNFRLNERTTGTIDVMVTQPEMTIFTASRSLLGKEMRAKLDTFAYLYSDLDKGFT	237
14	<i>C. albicans</i>	LTTDSFKRYVPKIREEILNYFVTDESFKLKEKTHGVANVMKTQPEITIFTASRSLFGDEMRRIFDRSFAQLYSDLDKGFT	229
15	<i>A. fumigatus</i> A	LTQSALESHVPLIEKEVLDYLRDSPNFQG---SSGRVDISAAMAEITIFTAARALQGQEVRSKLTAEFADLYHDLDKGFT	215
16	<i>A. fumigatus</i> B	LTSDALRSYVPLITDEVESFVKNSPAFQG---HKG VFDVCKTIAEITITYTASRSLQGKEVRSKFDSTFAELYHNLDMGFA	230
17		** .:.. : ** * .*: .. . * : * : : .*:**:*:*:* * *.*:* : ** * * : ** * * :	
18			
19	<i>S. cerevisiae</i>	PINFVFPNLPLEHYRKRDRHAQKAISGTYMSLIKERRKNNDIQD--RDLIDSL-MKNSTYKDGVKMTDQEIANLLIGVLMG	314
20	<i>C. albicans</i>	PINFVFPNLP LPHYWRRDAAQKKISATYMEIKSRRERGDIDPN-RDLIDSLLI-HSTYKDGVKMTDQEIANLLIGILMG	307
21	<i>A. fumigatus</i> A	PINFMLPWAPLPHNKRDAAHARMRSIYVDIITQRRLDGEKDSQKSDMIWNL-M-NCTYKNGQQVPDKEIAHMMITLLMA	293
22	<i>A. fumigatus</i> B	PINFMLPWAPLPHNRKRDAARQLTETEMEIKARRQAGSKKDS-EDMVWNL-M-SCVYKNGTPVPDEEIAHMMIALLMA	307
23		****:* * * * : ** * : : * : . * . ** .. . * : : * : ..**:* : .*:**:*:* * * * * *	
24			
25	<i>S. cerevisiae</i>	GQHTSAAISAWILLHLAERP DVQQELYEEQMRVLD----GGK KELTYDLLQEMPLLNTIKETLRMHHP LHSIFRKVMK	389
26	<i>C. albicans</i>	GQHTSASTSAWFLHLGKPHLQDVYQEVEVLLKEKG--GDLNDLTYEDLQKLPSVNNTIKETLRMHMPLHSIFRKVTN	385
27	<i>A. fumigatus</i> A	GQHSSSSISAWIMLRLASQPKVLEELYQEQLANLGPAGPDGSLPPLQYKDLDKLPFHQHVIRETLRIHSSIHSIMRKVKS	373
28	<i>A. fumigatus</i> B	GQHSSSSISAWIVLRLATRPDIMEELYQEQIRVLG----SDLPPLTYDNLQKLDLHAKVIKETLRLHAPIHSIIRAVKN	382
29		****:* : *:*:*:* * .*: : : *:* * : * .. * * .*::: : .*:**:*:* * .*:**:* * *	
30			
31	<i>S. cerevisiae</i>	DMHVPNTSYVIPAGYHVLVSPGYTHLRDEYFPAHQFNIHRWNNDSSASS---YSVGEEDVDYGF GAI SKGVSSPYLPFGG	465
32	<i>C. albicans</i>	PLRIPETNYIVPKGHYVLVSPGYAHTSERYFDNPEDFDPTRWDTAAKANSVSNSSDEVDYGF GKVSKGVSSPYLPFGG	465
33	<i>A. fumigatus</i> A	PLPVPGTPYMI PPGRVLLASPGVTALSDEHFPNAGCWDPHRWENQATKE---QENDKVV DYG YGAVSKGTSSPYLPFGA	449
34	<i>A. fumigatus</i> B	PMAVDGTSYVIPTSHNVLSSPGVTARSEEHPNPLEWNP HRWDENIAAS---AEDDEKVDYGYGLVSKGTNSPYLPFGA	458
35		: : * *:* * . : * ** * : : : * * . : : ** : : . . ****:* : ** .*****.	
36			
37	<i>S. cerevisiae</i>	GRHRCIGEHFAYCQLGVLMSIFIRTLKWHYPEGKT-VPPP DFTSMVTLPTGPAKIIWEKRNPEQKI	530
38	<i>C. albicans</i>	GRHRCIGEQFAYVQLGTILTFVYNLRWTI-DGYK-VPDPDYSSMVVLPTEPAEIIWEKRETCMF-	528
39	<i>A. fumigatus</i> A	GRHRCIGEFAYVNLGTVILATIVRHLRFRNVDGKKGVPETDYSSLSFGPMKPSIIGWEKRSKNTSK	515
40	<i>A. fumigatus</i> B	GRHRCIGEQFAYLQLGTITAVLVRFRNLPGV DGIPTDYSSLSFKPLGRSFVEFEKRESATKA	524
41		*****:* ** : * : : : : * : * .*:**:* * : : * * *	

42 **Figure S1** Sequence alignment of *S. cerevisiae* CYP51 (Uniprot: A6ZSR0), *C. albicans*
43 CYP51 (Uniprot: P10613) and *A. fumigatus* CYP51A (Uniprot: Q4WNT5) and B (Uniprot:
44 Q96W81). The alignment was carried out using the T-coffee server (Espresso) using pdb 4k0f as a
45 reference (1). The conserved tyrosine Y140 is highlighted in yellow while G310, T322 and G464 are
46 highlighted in red (*S. cerevisiae* numbering).
47



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49
50 **Figure S2** Sites of mutation in *C. albicans* and *A. fumigatus* CYP51 (residues G464 and K151
51 in ScErg11p). The main chain is indicated as a grey ribbon and fluconazole is indicated as
52 sticks (C atoms cyan, N atoms blue, O atoms red and F atoms pale blue). The heme cofactor is
53 also shown as sticks (magenta C atoms). Hydrogen bonds between the water molecule at G464
54 and the heme propionate as well as K151 and the second heme propionate are shown (yellow
55 dashed lines). Hydrogen bonding is also shown between HOH743, FLC, heme and Y140, as
56 well as between HOH790, FLC, and S382.

57
58

59 **Table S1.** Data collection and refinement statistics.

PDB code	4WMZ
Space group	P 1 2 ₁ 1
Resolution range (Å)	37.84 - 2.05 (2.11 - 2.05)
Unit cell axes (Å)	a = 77.24, b = 65.21 c = 81.02
Diffraction source	Australian Synchrotron MX1
Wavelength (Å)	0.954
Total reflections	293689
Unique reflections	47935
Average redundancy	6.1 (5.3)
Completeness (%)	95.6 (84.6)
<I/σ(I)>	12.4 (1.4)
Wilson B-factor	33.4
R _{merge} [†]	0.083 (0.953)
CC(1/2)	0.998 (0.634)
Refinement	
R _{cryst} [‡]	0.20 (0.30)
R _{free} [§]	0.23 (0.32)
Number of reflections	47899 (2351)
Number of atoms in model	
Protein	4304
Ligand	22
Water molecules	155
Deviation from ideal bond lengths* (Å)	0.008
Deviation from ideal bond angles* (°)	1.1
Ramachandran analysis (%)	
Preferred	96.5
Allowed	3.2
Residues in disallowed regions	0.2

60

61 [†] R_{merge} = $\sum_{hkl} \sum_i |I_i(hkl) - [I(hkl)]| / \sum_{hkl} \sum_i I_i(hkl)$.

62 ‡ $R_{\text{cryst}} = \frac{\sum_{\text{hkl}} |F_{\text{obs}} - F_{\text{calc}}|}{\sum_{\text{hkl}} |F_{\text{obs}}|}$ computed over a working set composed of 95% of data.

63 § $R_{\text{free}} = \frac{\sum_{\text{hkl}} |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum_{\text{hkl}} |F_{\text{obs}}|}$ computed over a test set composed of 5% of data.

64 *The parameters for ideal values for bond lengths and bond angles used were defined by
65 Engh and Huber (2). The validation statistics were obtained from Aimless (3) and Phenix (4).

66

67 **Table S2:**

68 CSD codes used for Fe-N and Fe-S distances respectively.

69 Fe-N

70 ABIVOX, ABIWAK, ABIWEO, AFADAN, BANTIV, COQHUM, COQJEY, DUPLOP,
71 EVINAZ, EVINED, EVINIH, EVINON, FENBAB, HEPREA, HERDOY, IXIDUO,
72 IXIFAU, JAQVOM, JAQWAZ, JEHVAU, JITSUB, KABWAN, KABWER, NEZXOG,
73 PAVDIA, PAVDOG, PAVDOG01, PAVDUM, POLNUA, QEHFIT, QIYROG, QUISAT,
74 QOFMUU, SAJFAL, SAJFAL01, TESLUY, TESMAF, TETWUK, , UCIVEH, UCIVIL,
75 WOQCOV, WOQCUB, WOQCUB01, WOQDIQ, WOQDOW, YIVSEB, DEDLAB,
76 DEDMEG,

77 Fe-S; KULWOD, PIMVFE01, SAXTEQ, YEHDAR

78

79

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