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          10          20          30          40          50          60
Af51B      ....|....| ....|....| ....|....| ....|....| ....|....|
-----MG  LIAFILDGIC KHCSTQSTWV LVGIGLLSIL AVSVIINVLQ QLLFK--NPH
Trub51     ....|....| ....|....| ....|....| ....|....| ....|....|
-----MG  LLADIVSRFC ENCSTLSTAA LVASAISAFI VLSIVINVLQ QLLFK--DPT
Mgram51    ....|....| ....|....| ....|....| ....|....| ....|....|
-----MG  LLQEVLQAQFD AQFGQTSWV LVGLGFLAFS TLAILLNVLS QLLFRG-KLS
Af51A      ....|....| ....|....| ....|....| ....|....| ....|....|
-----    -----    ---MVPMLW LTAYMAVAVL T-AILLNVVY QLFFRLWNRT
Cneo51     MSAIIPQVQQ LLGQVAQFFP PWFAALPTSL KVAIAVVGIP ALIIGLNVFQ QLCLPR-RKD
Mglob51    ....|....| ....|....| ....|....| ....|....| ....|....|
-----    MLQEIG-AWP VWQQALT--- ---FLVGGL ALIVGINVLV QVLVPR-NKS
Calb51     ....|....| ....|....| ....|....| ....|....| ....|....|
-----MA  IVETVIDGIN YFLSLSVT-- ---QQISILL GVPFVYNLWV QYLYSL-RKD
ClustalX
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          70          80          90          100         110         120
Af51B      ....|....| ....|....| ....|....| ....|....| ....|....|
EPVVFWHWP FIGSTISYGI DPKYKFFDCK AKYGDIFTFI LLGKKTTVYL GTKGNDFILN
Trub51     ....|....| ....|....| ....|....| ....|....| ....|....|
KPPVVFWHWP IIGSTISYGI DPKYKFFDDCK EKYGDITFTFI LLGKKTTVFL GTKGNDFILN
Mgram51    ....|....| ....|....| ....|....| ....|....| ....|....|
DPLVFWHWP  FIGSTITYGI DPKYKFFSCCR EKYGDVFTFI LLGKKTTVCL GTKGNDFILN
Af51A      ....|....| ....|....| ....|....| ....|....| ....|....|
EPPMVFWHVP YLGSTISYGI DPKYKFFACR EKYGDDITFTFI LLGQKTTVYL GVQGNEFILN
Cneo51     ....|....| ....|....| ....|....| ....|....| ....|....|
LPPVVFHYIP WFGSAAYYGE DPKYKFLFECR DKYGDDLTFTFI LMGRRITVAL GPKGNNLSLG
Mglob51    ....|....| ....|....| ....|....| ....|....| ....|....|
LPPMVFWHVP VVGSAITYGM DPKYRFFFNCR EKYGDVFTFK LFGRNVTVAL GPKGSNLVFN
Calb51     ....|....| ....|....| ....|....| ....|....| ....|....|
RAPLVFYWIP WFGSAASYGQ QPYEFFESCR QKYGDVFSFM LLGKIMTVYL GPKGHEFVFN
ClustalX  .*::::* .**:* * :***.*: * :****:* * *: * * * * : * :: :.

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          130         140         150         160         170         180
Af51B      ....|....| SRS-1 | ....|....| ....|....| ....|....|
GKLRDVCAE E VYSPLTTPVF GRHVVYDCPN AKLMEQKKFV KYGLTSDALR SYVPLITDEV
Trub51     ....|....| E VYSPLTTPVF GRHVVYDCPN SKLMEQKKFV KFGLTSEALR SYVTLITKEV
Mgram51    ....|....| E IYSPLTTPVF GKDVVYDCPN SKLMEQKKFV KYGLTTSALQ SYVTLIAAET
Af51A      ....|....| E VYSPLTTPVF GSDVVYDCPN SKLMEQKKFI KYGLTQSALE SHVPLIEKEV
Cneo51     ....|....| E AYTHLTTPVF GKGVVYDCPN EMLMQKKFI KSLTSENFR SYPPMITSEC
Mglob51    ....|....| E AYTSLTTPVF GKGVVYDVPN AVLMEQKRFV KSLSMENFR MYVTQIESEV
Calb51     ....|....| E AYKHLTTPVF GKGVIYDCPN SRLMEQKKFA KFALTTDSEK RYVPKIREEI
ClustalX  .:: :* ** : * . ***** * * :** ** ** :***:* * .*: . :. : . * *

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          190         200         210         220         230         240
Af51B      ....|....| ....|....| ....|....| ....|....| ....|....| SRS-2
ESFVKNS--- PAFQG--HKG VFDVCKTIAE ITIYTASRSL QGKEVRSKFD STFAELYHNL
Trub51     ....|....| ....|....| ....|....| ....|....| ....|....|
EQFFESS--- PIFKG--DSG VFNVSVMMAE ITIYTASRSL QGKEVRGKFD SSFAELYSDL
Mgram51    ....|....| ....|....| ....|....| ....|....| ....|....|
RQFFDRNNPH KKFAS--TSG TIDLPPALAE LTIYTASRSL QGKEVREGFD SSFADLYHYL
Af51A      ....|....| ....|....| ....|....| ....|....| ....|....|
LDYLRDS--- PNFQG--SSG RVDISAAMAE ITIFTAARAL QGQEVRSKLT AEFADLYHDL
Cneo51     ....|....| ....|....| ....|....| ....|....| ....|....|
EDFFTKEVGI SPQKP---SA TLDLLKAMSE LIILTASRTL QGKEVRESLN GQFAKYYEDL
Mglob51    ....|....| ....|....| ....|....| ....|....| ....|....|
KDFINNDAAF LPLQKGATSV TVDIFNVFSE ITILTASRTL QGKEVRESLD KTFAKLYHDL
Calb51     ....|....| ....|....| ....|....| ....|....| ....|....|
LNYFVTDSEF KLKEK--THG VANVMKTQPE ITIFTASRSL FGDEMRRIFD RSFAQLYSDL
ClustalX  ... . : : . * : * **:* * * .*: * : ** * *

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          250         260         270         280         290         300
Af51B      DMGFAPINFM LPWAPLPHNR KRDAAQRKLT ETYMEIIKAR RQAGSKKDSE -DMVWNLMSC
Trub51     DMGFAAINFM FPWFFPHNR KRDRAQRKMA QVYTDIIRQR RAAGGEKDSE -DMVWNLMSS
Mgram51    DMGFTPINFM LPWAPLPQNR RRDYAQKKMS ETYMSIIQKR RESKTGEHEE -DMIHNLMQC
Af51A      DKGFTPINFM LPWAPLPHNK KRDAAHARMR SIYVDIITQR RLDGEKDSQK SDMIWNLMNC
Cneo51     DGGFTPLNFM FPNLPLPSYK RRDEAQKAMS DFYLKIMENR RK-GESD-HE HDMIENLQSC
Mglob51    DSGFTPINFV IPNLPLPNNF RRDRAQRLMS DFYLGIIKKR RE-GNTEGTE HDMISALMEQ
Calb51     DKGFTPINFV FPNLPLPHYW RRDAAQKKIS ATYMKEIKSR RERGDIDPNR DLIDSLLIHS
ClustalX  * **:::* : * ** : ** * : : * : * * . . : *

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          310          320          330          340          350          360
      ....|....| .. SRS-4 ....|....| ....|....| ....|....|
Af51B  VYKNGTPVPD EEIAHMMIAL LMAGQHSSSS TASWIVLRLA TRPDIMEELY QEQIRVLG-S
Trub51 VYKNGTPIPD IEVAHMMIAL LMAGQHSSSS TGSWIVLRLA SRPDILEELY EEQKRVLG-E
Mgram51 KYKDGNAIPD KEIAHMMIAL LMAGQHSSSA TESWITLRLA SRPDIQDELL QEQKDMLG-V
Af51A  TYKNGQQVPD KEIAHMMITL LMAGQHSSSS ISAWIMLRLA SQPKVLEELY QEQLANLGA
Cneo51  KYRNGVPLSD RDIAHIMIAL LMAGQHTSSA TSSWTLLHLA DRPDVVEALY QEQKQKLG--
Mglob51 SYKNGRNIND REIAHMMIAL LMAGQHTSSA TGSWAMLRLA SRPEIEELY EEQKRVS--
Calb51  TYKDGVKMTD QEIANLLIGI LMGGQHTSAS TSAWFLHLG EKPHLQDVIY QEVVELLK--
ClustalX  *:* : * :*:*:*: : **.**:*: : * *:* . : : : : *

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          370          380          390          400          410          420
      ....|....| ....|....| ....|....| .. SRS-5 ....|....| ....|....|
Af51B  DLP----PLT YDNLQ-KLDL HAKVIKETLR LHAPIHSIIR AVKNPMAVDG -----
Trub51 DLP----PLT YEALQ-KLDL HNNVIKETLR LHAPIHSILR AVKSPMPVEG -----
Mgram51 NADGSIKELT YANLS-KLTL LNQVVKETLR IHAPIHSILR KVKSPMPIEG -----
Af51A  GPDGSLPPLQ YKDLQ-KLPF HQHVIRETLR IHSSIHSIMR KVKSPMPVPG -----
Cneo51  NPDGTFRDYR YEDLK-ELPI MDSIIRETLR MHAPIHSIYR KVLSDIPVPP SLSAP-----
Mglob51 DGTGGFAPLD YDIQKSSVPV LDAVIRETLR LHPPHSIMR KVKSDMVVPP TLAAPISSKG
Calb51  EKGDDLNDLT YEDLQ-KLPS VNNTIKETLR MHMPLHSIFR KVTNPLRIPE -----
ClustalX  * . : : :*:*: :* . :*: * * . : :

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          430          440          450          460          470          480
      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Af51B  ---TSYVIPT SHNVLSSPGV TARSEEHFPN PLEWNPWRWD EN----- -IAASAEDD-
Trub51  ---TNYVVPT SHNLLAAPGV PSRDPQYFPD PLVWNPWRWE NN----- -VGVTVVEAS
Mgram51 ---TAYVIPT THTLLAAPGT TSRMDEHFPD CLHWEPHRWD ESPSEKYKHL SPTTALGSIA
Af51A  ---TPYMIPP GRVLLASPGV TALSDEHFPN AGCWDPHRWE NQ----- -ATKEQEN
Cneo51  SENGQYIIPK GHYIMAAPGV SQMDPRIWQD AKVWNPWRWH DEKG-----F AAAAMVQYTK
Mglob51 SRDETYVIPK GHYVIAAPGV SQVDPRIWED ASRFDPHRWL GDK----- -ANVMNQTD
Calb51  ---TNYIVPK GHYVLVSPGY AHTSERYFDN PEDFDPTRWD TAAA----- -KANVSFNS
ClustalX  *:* : : :*: . . : : :* **

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          490          500          510          520          530          540
      ....|....| ....|....| Heme-Cys ...| ....|....| ....|....|
Af51B  -EKVDYGYGL VSKGTNSPYL PFGAGRHRCI GEQFAYLQLG TITAVLVRLF RFRNLPG-VD
Trub51  EEKTDYGYGL VSKGANSPYL PFGSGRHRCI GEQFAYVQLG TVTATLARLM RWKQVEGTKD
Mgram51 EEKEDYGYGL VSKGAASPYL PFGAGRHRCI GEQFAYVQLQ TITATMVRDF KFYNVDS-SD
Af51A  DKVVDYGYGA VSKGTSSPYL PFGAGRHRCI GEKFAYVNLG VILATIVRHL RLFNVDS-KK
Cneo51  AEQVDYGFSG VSKGTESPYQ PFGAGRHRCV GEQFAYTQLS TIFTYVVRNF TLKLAVP---
Mglob51 DAQEDFGWGM VSTGANSPYL PFGAGRHRCI GEQFAYLQLG TIISTFVRAF DWRLLET----
Calb51  SDEVYDYGFK VSKGVSSPYL PFGGRHRCI GEQFAYVQLG TILTFVYVNL RWTIDGY---
ClustalX  *:*: *:*.* ** **.**:*: :* :* : : :

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          550          560          570
      ....| SRS-6 ....| ....|....| ...
Af51B  GIPDTDYSSL FSKPLGRSFV EFEKRESATK A--
Trub51  VVPPTYDYSSL FSKPFGNPMV SWEKRKQASQ K--
Mgram51 NVVGTDYSSL FSRPLSPAVV KWERRREEKEE KN-
Af51A  GVPETYDYSSL FSGPMKPSII GWEKRKNTS K--
Cneo51  KFPETNYRTM IVQPNPL-V TFTLRNAEVK QEV
Mglob51 KLPAPDYTSM VVLPTQPANL VFTPRKNKA- ---
Calb51  KVPDPDYSSM VVLPTPEAEI IWEKRETCMF ---
ClustalX  . :*: : : . * : : *

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**Supplementary figure S1.** Sequence alignment of selected fungal CYP51 enzymes. This alignment shows the six substrate recognition sites (SRS) according to Strushkevich et al (1) and the twenty-three conserved CYP51 amino acid residues (2) in orange as well as the conserved heme-binding cysteine residue in red. The fungal CYP51 sequences aligned were *Aspergillus fumigatus* CYP51 isoenzyme A (Af51A – UniProt accession number Q4WNT5), *Aspergillus fumigatus* CYP51 isoenzyme B (Af51B – Q96W81), *Candida albicans* CYP51 (Calb51 – P10613), *Cryptococcus neoformans* CYP51 (Cneo51 – Q5KQ65), *Malassezia globosa* CYP51 (Mglob51 – A8Q317), *Mycosphaerella graminicola* CYP51 (Mgram51 – Q5XWE5) and *Trichophyton rubrum* CYP51 (Trub51 – F2SHH3). ClustalX consensus sequence indicates absolutely conserved residues (\*), conserved strong (STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW) groups (:), and conserved weaker (CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, HFY) groups (.) (<http://www.clustal.org/>).

## REFERENCES

1. Strushkevich N, Usanov SA, Park HW. 2010. Structural basis of human CYP51 inhibition by antifungal azoles. *J Mol Biol* 397:1067-1078.
2. Lepesheva GI, Waterman MR. 2011. Structural basis for conservation in the CYP51 family. *Biochim Biophys Acta* 1814:88-93.