

Supplementary Material

Supplementary Table S1. Fungi phosphatases studied in this work. *A. fumigatus*, *C. albicans* phosphatases were identified by the ontology classification tool and then confirmed by Blastp into the FungiDB and CGD. *C. neoformans* were obtained from Blastp searching. STP is serine/threonine phosphatase; PTP is phospho-tyrosine phosphatase; LP is lipid phosphatase.

| <i>A. fumigatus</i> | Phosphatase Family | Gene Name | Viable in AF | <i>C. albicans</i> | Gene Name | Viable in CA* | <i>C. neoformans</i> |
|---------------------|--------------------|-----------------------|--------------|--------------------|-----------------|---------------|----------------------|
| AFUA_1g13040 | PTP | dspA | Y | None | | | CNAG_00263 |
| AFUA_1g05640 | PTP/LP | ymlA | N | C1_08860C_A | uncharacterised | Y | CNAG_00390 |
| AFUA_1g03540 | PTP | dspB | Y | C1_04640W_A | uncharacterised | Y | CNAG_00498 |
| AFUA_3g12250 | PTP | cdcA | Y | C6_00670W_A | CDC14 | | CNAG_00498 |
| AFUA_2g03760 | PTP | ssuA | N | CR_03210C_A | SSU72 | N | CNAG_01054 |
| AFUA_4g07080 | PTP | dspC | N | CR_03570C_A | YVH1 | N | CNAG_01203 |
| AFUA_2g02760 | PTP | dspD | Y | C1_10000C_A | CPP1 | Y | CNAG_01357 |
| AFUA_6g08200 | PTP | nimT | N | C3_00800W_A | MIH1 | Y | CNAG_01572 |
| AFUA_4g07000 | PTP | yphA | Y | CR_06830C_A | SIW14 | Y | CNAG_03296 |
| AFUA_6g06650 | PTP/LP | ptyA | Y | C2_09250W_A | uncharacterised | Y | CNAG_03296 |
| AFUA_2g01880 | PTP | ltpA | Y | C1_08260C_A | LTP1 | Y | CNAG_03871 |
| AFUA_2g11990 | PTP/LP | tepA | Y | C7_02650W_A | TEP1 | Y | CNAG_04833 |
| AFUA_3g10970 | PTP | ptpB | Y | CR_08070W_A | PTP1 | Y | CNAG_05155 |
| AFUA_4g04710 | PTP | ptpA | Y | CR_10340W_A | PTP3 | Y | CNAG_05155 |
| AFUA_5g11690 | PTP | pps1 | Y | C7_00840C_A | PPS1 | Y | CNAG_05639 |
| AFUA_5g12010 | STP | pphA | Y | CR_03800C_A | PPH3 | Y | CNAG_00408 |
| AFUA_1g06860 | STP | ptcF | Y | CR_08160W_A | PTC5 | Y | CNAG_00427 |
| AFUA_4g00720 | STP | ptcH | Y | CR_07770C_A | PTC6 | Y | CNAG_00500 |
| AFUA_5g13340 | STP | ptcG | Y | C1_09260C_A | PTC1 | Y | CNAG_00500 |
| AFUA_1g09460 | STP | nemA | Y | C4_01300W_A | NEM1 | Y | CNAG_01177 |
| AFUA_6g11470 | STP | sitA | Y | C1_04380W_A | SIT4 | Y | CNAG_01436 |
| AFUA_5g06700 | STP | pptA | Y | C3_01710C_A | PPT1 | Y | CNAG_01496 |
| AFUA_6g10830 | STP | pphB | N | C3_01600W_A | PPH21 | | CNAG_02177 |
| AFUA_5g11370 | STP | ppgA | Y | C3_01600W_A | PPH21 | | CNAG_02236 |
| AFUA_5g08620 | STP | ppefA | Y | C7_03500W_A | uncharacterised | Y | CNAG_02470 |
| AFUA_1g09280 | STP | ptcB | Y | CR_01520W_A | PTC2 | y | CNAG_03052 |
| AFUA_2g03890 | STP | ptcE | Y | CR_01520W_A | PTC2 | Y | CNAG_03052 |
| AFUA_8g04580 | STP | ppmA | Y | C4_00340W_A | PTC7 | Y | CNAG_03541 |
| AFUA_2g03950 | STP | phzA | Y | CR_06420W_A | PPZ1 | Y | CNAG_03673 |
| AFUA_1g04950 | STP | glcA | N | CR_07650W_A | GLC7 | N | CNAG_03706 |
| AFUA_3g11410 | STP | fcpA | N | C3_07460W_A | FCP1 | Y | CNAG_04120 |
| AFUA_1g04790 | STP | psrA | Y | C3_00570C_A | PSR1 | Y | CNAG_04224 |
| AFUA_5g09360 | STP | calA/ cnaA | Y | C1_00730C_A | CMP1 | Y | CNAG_04796 |
| AFUA_1g15800 | STP | ptcA | Y | CR_07770C_A | PTC6 | Y | CNAG_06418 |
| AFUA_5g13740 | STP | ptcD | Y | None | | | None |

* Based on large scale null data provided at CGD

Supplementary Table S2. Oligonucleotides used in this study.

| Oligo Name | Oligo Sequence |
|-------------------|--|
| (AFUA_1g05640)P1 | GGGTAACCACACTACTGCCG |
| (AFUA_1g05640)P2 | TAGTTCTGTTACCGAGCCGGCAAACCTTAGCGATCCGTGT |
| (AFUA_1g05640)P3 | GCTCTGAACGATATGCTCCAACCTGCGACGATTACAACAAAGG |
| (AFUA_1g05640)P4 | CTTGAGCAGATTCCCCAAAG |
| (AFUA_1g05640)P5 | TAAATCCCTACCGAACGACG |
| (AFUA_1g05640)P6 | CCGCTCTCCATGATGAATCT |
| (AFUA_2g11990)P1 | GGGTTCAACACTACGTGCAA |
| (AFUA_2g11990)P2 | TAGTTCTGTTACCGAGCCGGGATGGGAGAGGAAGAAAGGG |
| (AFUA_2g11990)P3 | GCTCTGAACGATATGCTCCAACACCAAGCTTGTTCGTCTGTT |
| (AFUA_2g11990)P4 | GAGTATCTCCGGTCCATGGA |
| (AFUA_2g11990)P5 | CATCTTCCATGCGGAACTTT |
| (AFUA_2g11990)P6 | GCTTTCTGTCGTGGATGGAT |
| (AFUA_5g08620)P1 | GTACCGATGACCCTCTGTCTG |
| (AFUA_5g08620)P2 | TAGTTCTGTTACCGAGCCGGGCAAGGTCGGAGATTTCGTA |
| (AFUA_5g08620)P3 | GCTCTGAACGATATGCTCCAACGGTGTGGGGCGTCTACAAT |
| (AFUA_5g08620)P4 | TGCTCTCACTTCCATCACG |
| (AFUA_5g08620)P5 | GCTGTTTTCCGATACACGGT |
| (AFUA_5g08620)P6 | TATTTCTGCGTCAGGCACAC |
| (AFUA_5g11370)P1 | ATGTGACCGAAAGAGCGACT |
| (AFUA_5g11370)P2 | TAGTTCTGTTACCGAGCCGGAATGGACAACGTGCCAAACT |
| (AFUA_5g11370)P3 | GCTCTGAACGATATGCTCCAACAGTACTTCCCGATGCAGAGC |
| (AFUA_5g11370)P4 | GATCTCGTTGCCTCGAAGAC |
| (AFUA_5g11370)P5 | GGCGAAGACTAAAACGCTTG |
| (AFUA_5g11370)P6 | ACCAGAATGACGAATCCCTG |
| (AFUA_2g03760)P1 | CGGTGGGCAGAAATCTTCT |
| (AFUA_2g03760)P2 | TAGTTCTGTTACCGAGCCGGGGAAGTGACAGAGTGGGAGC |
| (AFUA_2g03760)P3 | GCTCTGAACGATATGCTCCAACAACCTCCAACCTAAGCAAAAGGGT |
| (AFUA_2g03760)P4 | TTGGCCTGAACATCACCTCG |
| (AFUA_2g03760)P5 | TCATGAGTTAGGCGTGACGG |
| (AFUA_2g03760)P6 | ACTGGAACCCTTCGCATGAC |
| (AFUA_6g08200)P1 | ATGGACTATGGAGTCGTCCG |
| (AFUA_6g08200)P2 | TAGTTCTGTTACCGAGCCGGGGATTTCCTCGGTAAAGGA |
| (AFUA_6g08200)P3 | GCTCTGAACGATATGCTCCAACAGCCGGTTGACTTTCCTTTT |
| (AFUA_6g08200)P4 | CGATGCCTCAGTTTTTCTTC |
| (AFUA_6g08200)P5 | GCGCCCTACCTACGTAACAA |
| (AFUA_6g08200)P6 | CCCAATTTTCTCGCCAAATA |
| AFUA_1G05640_PPF | GAGCAGCGAAAAGTGGTTTC |
| AFUA_1G05640_PPR | GGGGACTGTCCGAGTCATAA |
| AFUA_2G11990_PPF | GAACTTCCTCGACACCAAGC |
| AFUA_2G11990_PPR | CCATCTGGACCATCGAGTCT |
| AFUA_5G08620_PPF | ATTCAGTCCGAGATTCGAT |
| AFUA_5G08620_PPR | CACTGTCCGCATCAATCAGT |
| AFUA_5G11370_PPF | TGAAGGAGAGCAATGTGGTG |
| AFUA_5G11370_PPR | CGTAGTCGCCTACACAACGA |

Supplementary Table S3. Top scoring compounds obtained in the targeted docking with the Chemdiverset library, for each AfPPase and cluster. The table shows the compound structure, compound library ID, molecular weight (Mw), polar surface area (PSA), Gibbs free energy (ΔG) and ligand efficiency indices calculated by VSpice (BEI, SEI). Compounds show diversity except for those binding at the active site pocket (C1) of GlcA and PphB due to the high conservation between their active sites.

| AfPPases | Cluster | Compound Structure | ID | Mw | PSA | ΔG | BEI | SEI |
|-------------|---------|--------------------|---------|-------|------|------------|------|------|
| NimT | C1 | | 5236413 | 470.5 | 68.3 | -9.5 | 14.8 | 10.2 |
| | C2 | | 5641450 | 404.5 | 25.2 | -10.4 | 19.0 | 30.5 |
| PphB | C1 | | 5213442 | 456.4 | 58.2 | -10.2 | 16.5 | 12.9 |
| | C2 | | 6633679 | 493.4 | 80.2 | -8.2 | 12.2 | 7.5 |
| | C3 | | 5569062 | 489.5 | 95.6 | -9.5 | 14.2 | 7.3 |
| | C4 | | 7966036 | 393.4 | 85.1 | -9.4 | 17.5 | 8.1 |
| GlcA | C1 | | 5213442 | 456.4 | 58.2 | -9.9 | 15.8 | 12.4 |
| | C2 | | 7673412 | 446.5 | 96.5 | -9.5 | 15.6 | 7.2 |
| | C3 | | 7702995 | 371.4 | 74.2 | -8.6 | 16.9 | 8.5 |
| | C4 | | 7743093 | 485.0 | 85.1 | -10.3 | 15.5 | 8.8 |
| | C5 | | 5784178 | 389.5 | 99.2 | -8.9 | 16.7 | 6.6 |
| DspC | C2 | | 7784277 | 431.3 | 80.9 | -7.8 | 13.3 | 7.1 |
| | C3 | | 6629705 | 476.4 | 66.5 | -7.3 | 11.2 | 8.0 |

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Supplementary Table S4. Human PP1 biological partners present in the crystal structures and their homologues in *S. cerevisiae*, *C. albicans* and *A. fumigatus*. Percentage of identity is respect of the human proteins. Validated *S. cerevisiae* PP1 (Glc7) interactions are marked with (*).

| PDB | Gene in Human | <i>S. cerevisiae</i> | Gene ID | ID/Cover (%) | <i>C. albicans</i> | Gene ID | ID/ Cover (%) | <i>A. fumigatus</i> | Gene ID | ID/Cover (%) |
|------|------------------------|----------------------|---------|--------------|-------------------------------|------------------|---------------|-------------------------|--------------|--------------|
| 1S70 | PPP1R12A (MBS, MYPT1) | Hos4 ^V | YIL112W | 37/17 % | Uncharacterised protein | CAALFM_C304920CA | 30/22 % | Pfs | AFUA_1G01020 | 23/36 % |
| 2O8A | PPP1R2 | Glc8 ^{*V} | YMR311C | 20/100% | GLC8 | CAALFM_C402860WA | 23/100 % | Uncharacterised protein | AFUA_5G03700 | 32/100 % |
| 3V4Y | PPP1R8 | PML1 ^V | YLR016C | 14/100 % | Ubiquitin-conjugating protein | CAALFM_C600260WA | 50/10 % | SNIP1 | AFUA_3G11540 | 31/100 % |
| 4MOY | PPP1R10 (Cat53, Pnuts) | SKI2 ^V | YLR398C | 33/7 % | SKI2 | CAALFM_CR08570WA | 6/100 % | Pab1 ^U | AFUA_1G04190 | 41/10 % |
| 4V0U | ACTA1 | ACT1 ^U | YFL039C | 88/99 % | ACT1 | CAALFM_C113700WA | 83/99 % | ACT1 | AFUA_6G04740 | 87/90 % |
| 6DNO | PPP1R3A (PP1G) | Gac1 ^{*V} | YOR178C | 28/12 % | Gac1 | CAALFM_C700660WA | 37/8 % | Gac1 | AFUA_2G13850 | 29/19% |
| 6GHM | TP53BP2 (ASPP2, BBP) | Nas6 ^V | YGR232W | 36/11% | Uncharacterised protein | CAALFM_C108700WA | 36/10 % | Ankyrin repeat protein | AFUA_8G02140 | 31/14% |

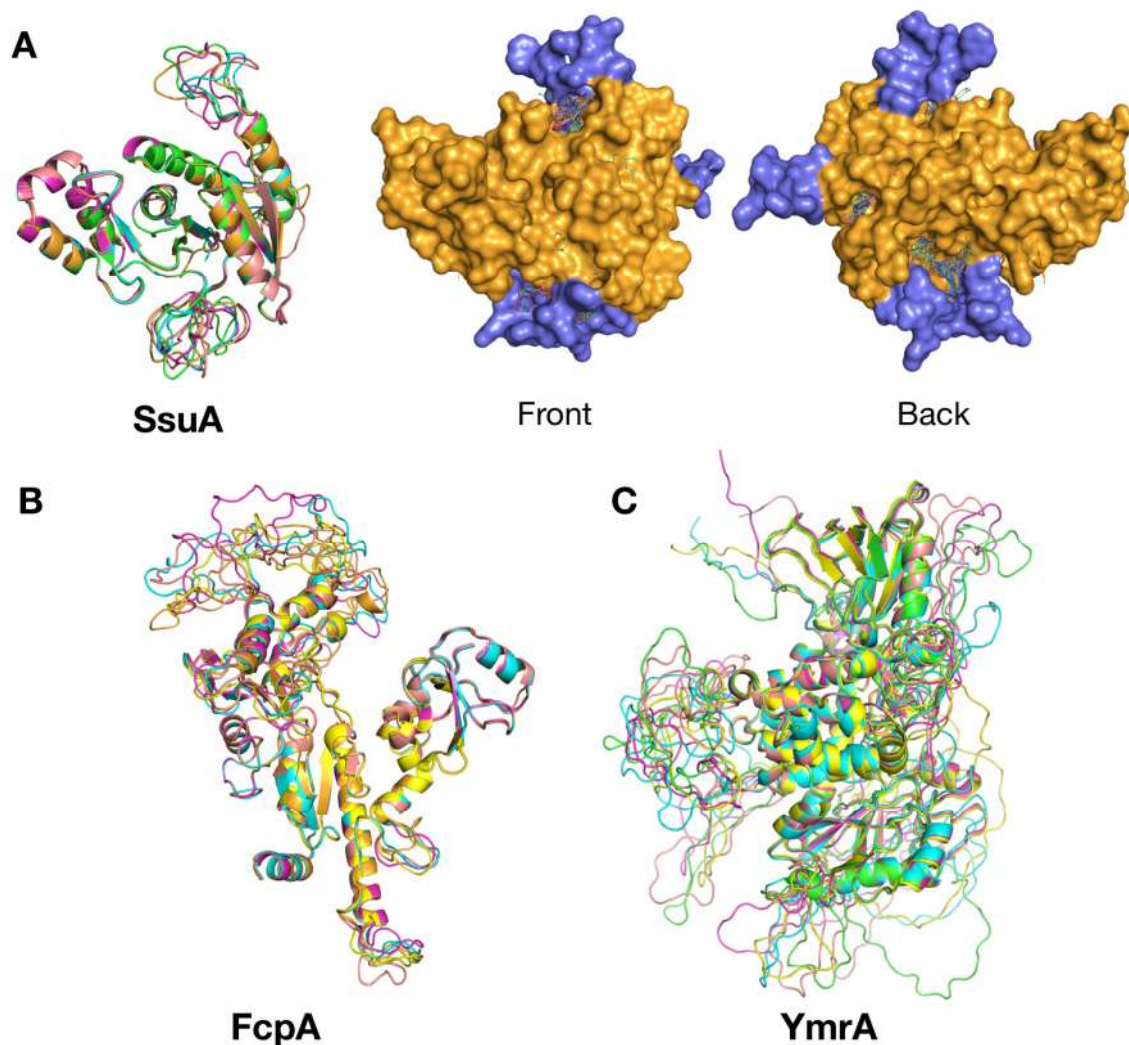
*: Glc7 partners (SGC database); V: KO viable; U: KO unviable

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Supplementary Table S5. Human PP2A biological partners present in the crystal structures and their homologues in *S. cerevisiae*, *C. albicans* and *A. fumigatus*. Percentage of identity is respect of the human proteins. Validated *S. cerevisiae* PP2A (Pph21/22) interactions are marked with (*).

| PDB | Gene in human | <i>S. cerevisiae</i> | Gene ID | ID/Cover (%) | <i>C. albicans</i> | Gene ID | ID/ Cover (%) | <i>A. fumigatus</i> | Gene ID | ID/Cover (%) |
|------|--------------------|-----------------------------|---------|--------------|--------------------|------------------|---------------|----------------------|--------------|--------------|
| 2IE3 | PPP2R1A | TPD3* ^v | YAL016W | 44/98 % | TPD3 | CAALFM_C306910CA | 42/97 % | PPP2R1A ^u | AFUA_1G05610 | 53/98 % |
| 2NPP | PPP2R5C | RTS1* ^v | YOR014W | 56/78 % | RTS1 | CAALFM_C206420CA | 57/81 % | SSC1 ^v | AFUA_5G02560 | 67/77 % |
| 3DW8 | PPP2R2A | CDC55* ^v | YGL190C | 56/88 % | CDC55 | CAALFM_C201600CA | 58/97 % | PPP2RB ^v | AFUA_8G05560 | 57/96 % |
| 4IYP | IGBP1 (IBP1) | Tap42* ^u | YMR028W | 26/86 % | - | CAALFM_C401670CA | 23/96 % | TapA ^u | AFUA_5G11780 | 26/90 % |
| 5W0W | Tip41-like (TIPRL) | Tip41* ^v | YPR040W | 35/89 % | Tip41 | CAALFM_C504520WA | 39/93 % | TipA ^v | AFUA_2G07540 | 41/89 % |
| 3C5W | PPME1 (PME1) | PPE1/ YmS2* ^v | YHR075C | 29/93 % | YmS2 | CAALFM_C201520WA | 32/92 % | PPE1 ^v | AFUA_1G03080 | 37/80 % |
| 4I5L | PPP2R2D (CDC6) | CDC6 ^u | YJL194W | 27/70 % | CDC6 | CAALFM_C112220WA | 37/41 % | CDC6 | AFUA_7G04310 | 30/65 % |
| 4LAC | PTPA | RRD1* ^v | YIL153W | 43/75 % | RRD1 | CAALFM_C307080WA | 39/91 % | RRD2 ^v | AFUA_1G13420 | 32/93 % |

*: Pph21/22 partners (SGC database); V: KO viable; U: KO unviable



Supplementary Figure S2. Molecular models for AfPPases. **(A)**. Cartoon representation of the molecular models generated by Modeller for SsuA superimposed. Insertions regions, respect to the template structure, appear as disordered loops at the top and bottom of the phosphatase core. To the right is the surface representation of SsuA (orange), insertion regions are coloured lilac and blind docking ligand clusters obtained with VSpine are shown in lines binding near the insertion regions. **(B)**. Molecular models for FcpA are shown in cartoon representation, unstructured insertions are visible at the top and bottom of the core of the structure. **(C)**. Molecular models for YmrA shown as cartoons and unstructured regions shown in different regions of the protein.