Histone Acetylation Regulator Gcn5 Mediates Drug Resistance and Virulence of *Candida glabrata*

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SUPPLEMENTARY MATERIALS

FKS mutant construction methods
FKS mutant strains were constructed as described in Healey et al., 2020 (1). Briefly, Fks1-625delF, Fks1-S629P, Fks2-659delF, and Fks2-S663P were generated in strain ATCC 2001 through transformation of a purified PCR product. Desired mutations were PCR-amplified along with regions flanking the FKS1 or FKS2 hotspot 1 region (approximately 400 bp) from mutant isolates (see Table S1 for primers). Transformants were selected on low levels of caspofungin- (0.3 µg/mL) or micafungin- (0.03 µg/mL) containing YPD agar medium. All FKS1 and FKS2 hotspots were sequenced in each transformant to confirm the expected mutation was present and all other amino acids remained unaltered.

Figure S1. Growth curves of Candida glabrata wild type (WT), gcn5∆ and gcn5∆::GCN5 strains in YPD media.

Figure S2. (A) Immunoblots showed visibly decreased abundance of both H3K9Ac and H3K14Ac in gcn5∆, compared to WT and complemented strains. (B) Acetylation level comparison in WT, gcn5∆, and gcn5∆::GCN5. Signals of H3K9Ac and H3K14Ac were normalized to that of H3 in corresponding strain. Acetylated H3K9 and H3K14 in gcn5∆ decreased to 60% and 46% of that in WT, respectively. Representative blots from two independent experiments and mean ratios ± SD are shown.
**Figure S3.** qRT-PCR verification of representative DEGs in gcn5Δ cells. Expression was normalized to reference gene PGK1 and shown as a fold change compared to WT. Plots represent the mean ± SD from ≥ 3 independent experiments (*P<0.05, **P<0.01, ***P<0.001).

**Figure S4.** Venn diagram of upregulated gene sets in WT and gcn5Δ associated with fluconazole treatment.
**Figure S5.** qRT-PCR verification of representative DEGs in WT and gcnpΔ cells in response to flucanazole (FLC) pressure. Expression was normalized to reference gene PGK1 and shown as a fold change compared to WT no drug control (NDC). Plots represent the mean ± SD from ≥ 3 independent experiments (*P <0.05, **P<0.01, ***P<0.001).

**Figure S6.** Venn diagram of micafungin triggered up- and down-regulated gene sets in WT and gcnpΔ cells.

Upregulation

Downregulation

| Gene | WT NDC | WT FLC | gcnpΔ NDC | gcnpΔ FLC |
|------|--------|--------|-----------|-----------|
| CAGL0L06776g | ![](CAGL0L06776g.png) | ![](CAGL0L06776g.png) | ![](CAGL0L06776g.png) | ![](CAGL0L06776g.png) |
| CAGL0L03828g | ![](CAGL0L03828g.png) | ![](CAGL0L03828g.png) | ![](CAGL0L03828g.png) | ![](CAGL0L03828g.png) |
| CAGL0H09614g | ![](CAGL0H09614g.png) | ![](CAGL0H09614g.png) | ![](CAGL0H09614g.png) | ![](CAGL0H09614g.png) |
| AWP7 | ![](AWP7.png) | ![](AWP7.png) | ![](AWP7.png) | ![](AWP7.png) |
| ERG11 | ![](ERG11.png) | ![](ERG11.png) | ![](ERG11.png) | ![](ERG11.png) |
| PDR1 | ![](PDR1.png) | ![](PDR1.png) | ![](PDR1.png) | ![](PDR1.png) |
| CDR1 | ![](CDR1.png) | ![](CDR1.png) | ![](CDR1.png) | ![](CDR1.png) |
| CDR2 | ![](CDR2.png) | ![](CDR2.png) | ![](CDR2.png) | ![](CDR2.png) |
| STR3 | ![](STR3.png) | ![](STR3.png) | ![](STR3.png) | ![](STR3.png) |
| CDR3 | ![](CDR3.png) | ![](CDR3.png) | ![](CDR3.png) | ![](CDR3.png) |

| Gene | WT NDC | WT FLC | gcnpΔ NDC | gcnpΔ FLC |
|------|--------|--------|-----------|-----------|
| 65   | 89     | 487    |           |           |
| 33   | 70     | 440    |           |           |
**Figure S7.** qRT-PCR verification of representative DEGs in WT and *gcn5Δ* cells triggered by micafungin (MCF) treatment. Expression was normalized to reference gene *PGK1* and shown as a fold change compared to WT no drug control (NDC). Plots represent the mean ± SD from ≥ 3 independent experiments (*P* < 0.05, **P**<0.01, ***P***<0.001, ns denotes for no statistical significance).

**Table S1. Sequences of primers and oligos used in this study**

| Oligo | Sequence (5’-3’) | Application |
|-------|------------------|-------------|
| GCN5-F364 | CGATTACAAATGTTTACCCG | GCN5 upstream PCR |
| pRS-GCN5_R-67 | CTCAGTAAATCCTGCCTGACGCAGCAA | GCN5 upstream PCR |
| CYC1_GCN5_F1757 | AGTATGTCACGCTTACACCCATGAT | GCN5 downstream PCR |
| GCN5-R2071 | GGTGAAACACGGGCAACAAA | GCN5 downstream PCR |
| pRS-F | CGCAGAACGGCAGATGTA | Amplification of *NAT* cassette from pCN-PDC1 |
| CYC1-R | GCCTGAATGTAAGCGTGAC | Amplification of *NAT* cassette from pCN-PDC1 |
| TEFp263F | TCTGATGACCTCCACCTTTAT | GCN5 knockout validation sequencing |
| NAT297R | GTACGAGACGACCACGAAAC | GCN5 knockout validation sequencing |
| Gcn5-crRNA | GAGAGGUGAACAAUCCACCG | CRISPR crRNA, N20 sequence underlined |
| DNA-crRNA | CGUGACACCCUUCUCGAGUUAGA | CRISPR crRNA, N20 sequence underlined |
| CgFKS1c1757F | ACGTTACCAATGTTTACCCG | FKS1 mutant construction, hotspot 1 PCR |
| CgFKS1c2225R | GGTGAAACACGGGCAACAAA | FKS1 mutant construction, hotspot 1 PCR/sequence |
| CgFKS1c1674F | CGCAGAACGGCAGATGTA | screen/sequence |
| CgFKS1c3918F | GCCTGAATGTAAGCGTGAC | FKS1 mutant construction, hotspot 2 PCR |
| CgFKS1c4225R | TCTGATGACCTCCACCTTTAT | FKS1 mutant construction, hotspot 2/sequence |
| CgFKS2c1790F | GTACGAGACGACCACGAAAC | FKS2 mutant construction, hotspot 1 PCR |
| CgFKS2c2165R | GAGAGGUGAACAAUCCACCG | FKS2 mutant construction, hotspot 1 PCR/sequence |
| CgFKS2c1419F | CGUGACACCCUUCUCGAGUUAGA | screen/sequence |
| CgFKS2c3930F | ACGTTACCAATGTTTACCCG | FKS2 mutant construction, hotspot 2 PCR |
| CgFKS2c4312R | GGTGAAACACGGGCAACAAA | FKS2 mutant construction, hotspot 2 PCR/sequence |
| PDR1u175F | CGUGACACCCUUCUCGAGUUAGA | PDR1 amplification |
| PDR1d110-R | ACGTTACCAATGTTTACCCG | PDR1 amplification and sequencing |
| Primer ID  | Sequence                  | Description       |
|-----------|---------------------------|-------------------|
| PDR1-c828R | AAGTGACTTAGTGTTGGCAC      | PDR1 sequencing   |
| PDR1-c1703R| GCAACAGCTACATCTAACGAAACC | PDR1 sequencing   |
| PDR1-c2600R| CTCTCATTTTGGTGGTGGCAC    | PDR1 sequencing   |
| PGK1-F     | CAACAGGTTGAAAGAGACGGA    |                    |
| PGK1-R     | CGTCACACCGTTGCAAGGAAG    |                    |
| GCN5-F     | GGTGCTAAACCGCGAGAAAAG    |                    |
| GCN5-R     | CTCTATTGTCGCTGCTGATCTT   |                    |
| EPA13-F    | CAACATTGCTCTTTGGCTTTC    |                    |
| EPA13-R    | CTTTCATTGACTACGTTGCA     |                    |
| EPA6-F     | GACTGACGCGAGCCCTACTGAA   |                    |
| EPA6-R     | GCTTGATTGAGGATAAATTCG    |                    |
| BMT5-F     | TCCATTGACTACGTTGCA       |                    |
| BMT5-R     | TTCTTTGCAAAACGCTTGTC     |                    |
| BMT2-F     | ACCGACACCAAAATCAATC      |                    |
| BMT2-R     | AAGATGCACCTTCCGTCATC     |                    |
| HBN1-F     | TGATGACACACGCGAGAAAAG    |                    |
| HBN1-R     | AACAGGCTTGAGATCTGGAT     |                    |
| CAGL09018g-F| ACTGGGAAACTGGCTTGGTG     |                    |
| CAGL09018g-R| TTCTTACTGACACGCTACC     |                    |
| CAGL00157g-F| ACTGTGACGCGCAGCTCAC     |                    |
| CAGL00157g-R| AGCTTGTTTGGCTTTTCTG     |                    |
| FKS1-F     | CTCCTACTGTGCTAAGAATT    | qRT-PCR verification|
| FKS1-R     | AGGATCTTGATATCACATACC   |                    |
| FKS2-F     | ATATGCTGTTTGTTCTACATTCC |                    |
| FKS2-R     | ACGGACCTTGTGGATGCA      |                    |
| CDR1-F     | TACGCATAACACTACAGGAAGCT |                    |
| CDR1-R     | AGAAGGAACATTTAGAAGTGAATC |                    |
| CDR2-F     | GTCTTTTATGAAAGGCTACAGATTT |                    |
| CDR2-R     | TTTTGCCCGGAGATAATCACG   |                    |
| CRZ1-F     | AGGCTGCTAAAGGAGGCTAT    |                    |
| CRZ1-R     | AGGCTGCTAAAGGAGGCTAT    |                    |
| STR3-F     | GTCTAGTGGATGAGAGAAGAAG |                    |
| STR3-R     | TCTTTGATATTTGCTAAGGACCC |                    |
| CAGL00977s-F| CTGCTGATGCAAAATCTCAA    |                    |
| CAGL00977s-R| GCCATAGGCTTCTCCTCCTCC   |                    |
| CAGL03828s-F| CGGTCAGCTTCTGGACTATTG   |                    |
| CAGL03828s-R| CCAACTTACACCACCCTTCG    |                    |
| CAGL0H06414-F| CCATCTTCTCTAAAGGCTTTC  |                    |
| CAGL0H06414-R| TCACCGATTTTGGAGATAGC    |                    |
| AWP7-F     | TGCTCAGAAACCGACAAATCACA |                    |
| AWP7-R     | CTGTGATGCGCTCAGTCA      |                    |
| ERG11-Fn   | ACGGTACCAAGCAGCAGAG     |                    |
| ERG11-Rn   | GAACACTGGGGTGGTGAAGT    |                    |
| PDR1-F     | AAGAGGATGACACGCGAGAAAG  |                    |
| PDR1-R     | ATGGATGTCATTGGATATT     |                    |
| CAGL0M03377s-F| TGGATCCCTCTTGATTATCCA  |                    |
| CAGL0M03377s-R| CGTTGTAAGTCAGCGGTAAA   |                    |
| CAGL0K10626-F| CAACAAAGTGTCGGGATTATTAC|                    |
| CAGL0K10626-R| ACAGGTTAGACCTCTCTGAG    |                    |
| CAGL000484-F| CTGTAAGTCAGGCGGCTAAG    |                    |
| CAGL000484-R| CTCCTGAATGAGAATGGAATGTA |                    |
| SUT2-F     | CTAAACACGTGGCAGCATTG    |                    |
| SUT2-R     | GCAGACAAACAAAGGGGAGAAGA |                    |

### Reference

1. Healey KR, Paderu P, Hou X, Jimenez Ortigosa C, Bagley N, Patel B, Zhao Y, Perlin DS. 2020. Differential Regulation of Echinocandin Targets Fks1 and Fks2 in Candida glabrata by the Post-Transcriptional Regulator Ssd1. J Fungi (Basel) 6.