

Supplemental Information

Figure S1 (Huang et al)

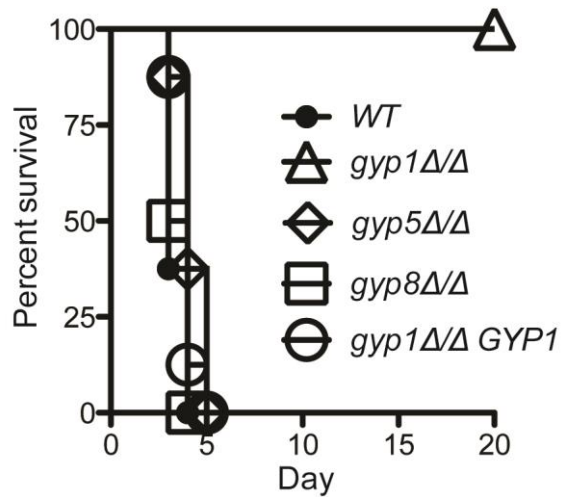


FIG S1. Requirement of Ypt1 GAPs for the virulence of *C. albicans*. Survival curve of mice injected with 1×10^6 cells with indicated genotype (n = 8 per strain). Gyp1, but not Gyp5 or Gyp8, is required for the virulence. Strains used were HZX201, HZX250, and HZX251.

Figure S2 (Huang et al)

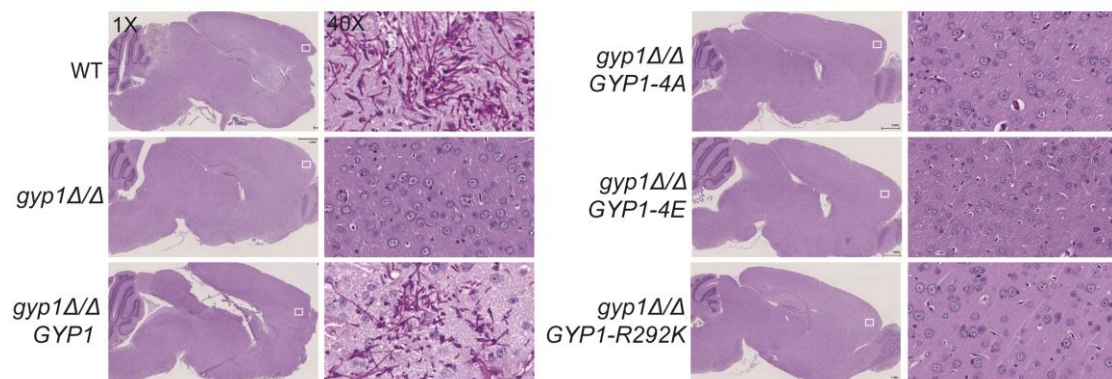


FIG S2. Histology analysis of the brains of infected mice. Necropsy and staining were performed as described in Figure 7C. *C. albicans* hyphae were only observed in the brain of the mice infected with WT (SC5314) or GYP1-reintegrant (HZX202) cells. Arrows indicate the region seen in the 40X images.

Table S1: Plasmids used in this study.

Name	Description
CIP10U	Yeast integration vector (1)
CIP10A	<i>CaURA3</i> in CIP10U was replaced with <i>CaARG4</i>
CIP10SAT	<i>CaURA3</i> in CIP10U was replaced with <i>CaSAT1</i> (2)
ZXP201	<i>GYP1ΔURA3-FLP/pBKS</i> ; <i>GYP1</i> promoter region (-571 to -1 bp) and terminator region (1695 to 2117 bp) were cloned to flank the <i>CaURA3</i> flipper in pBKS vector. The knock-out cassette was released by <i>KpnI</i> and <i>SacII</i> for transformation
ZXP202	<i>GYP1ΔHIS1/pBKS</i> ; <i>GYP1</i> promoter region (-571 to -1 bp) and terminator region (1695 to 2117 bp) were cloned to flank the <i>CaHIS1</i> in pBKS vector. The knock-out cassette was released by <i>KpnI</i> and <i>SacII</i> for transformation
ZXP203	<i>GYP5ΔURA3-FLP/pBKS</i> ; <i>GYP5</i> promoter region (-605 to -1 bp) and terminator region (1616 to 2149 bp) were cloned to flank the <i>CaURA3</i> flipper in pBKS vector. The knock-out cassette was released by <i>KpnI</i> and <i>SacII</i> for transformation
ZXP204	<i>GYP8ΔURA3-FLP/pBKS</i> ; <i>GYP8</i> promoter region (-588 to -1 bp) and terminator region (2216 to 2715 bp) were cloned to flank the <i>CaURA3</i> flipper in pBKS vector. The knock-out cassette was released by <i>KpnI</i> and <i>SacII</i> for transformation
ZXP205	Pro- <i>GYP1</i> -WT-GFP-UTR/CIP10A; DNA fragment containing <i>GYP1</i> promoter and coding region (-653 to 1692 bp) was cloned in frame with a C-terminal GFP epitope (followed by <i>UTR</i>) into CIP10A. The plasmid was linearized by <i>PmeI</i> (generated by mutagenesis at promoter region -390 bp) for integration
ZXP206	Pro- <i>GYP1</i> -R292K-GFP-UTR/CIP10A; ZXP205 was used as the template for mutagenesis to introduce the mutation R292K
ZXP207	Pro- <i>GYP1</i> -4A-GFP-UTR/CIP10A; ZXP205 was used as the template for mutagenesis to introduce the mutations: S32A, S96A, S105A and S167A
ZXP208	Pro- <i>GYP1</i> -4E-GFP-UTR/CIP10A; ZXP205 was used as the template for mutagenesis to introduce the mutations: S32E, S96E, S105E and S167E
ZXP209	Pro- <i>GYP1</i> -WT-HA-UTR/CIP10A; GFP epitope in ZXP205 was replaced with HA epitope.
ZXP210	Pro- <i>GYP1</i> -R292K-HA-UTR/CIP10A; GFP epitope in ZXP206 was replaced with HA epitope.
ZXP211	Pro- <i>GYP1</i> -4A-HA-UTR/CIP10A; GFP epitope in ZXP207 was replaced with HA epitope.
ZXP212	Pro- <i>GYP1</i> -4E-HA-UTR/CIP10A; GFP epitope in ZXP208 was replaced with HA epitope.
ZXP213	<i>GYP1c</i> -GFP-UTR/CIP10U; DNA fragment containing <i>GYP1</i> coding region (628 to 1692) was cloned in frame with a C-terminal GFP epitope (followed by <i>UTR</i>) into CIP10U. The plasmid was linearized by <i>XcmI</i> for integration
ZXP214	<i>MYO2c</i> -GFP-UTR/CIP10U; DNA fragment containing <i>MYO2</i> coding region (4075 to 4683) was cloned in frame with a C-terminal GFP epitope (followed by <i>UTR</i>) into CIP10U. The plasmid was linearized by <i>BamHI</i> for integration
ZXP214A	<i>MYO2c</i> -HA-UTR/CIP10U; DNA fragment containing <i>MYO2</i> coding region (4075 to 4683) was cloned in frame with a C-terminal HA epitope (followed by <i>UTR</i>) into CIP10U. The plasmid was linearized by <i>BamHI</i> for integration
ZXP216	<i>SEC7c</i> -GFP-UTR/CIP10U; DNA fragment containing <i>SEC7</i> coding region (4584 to 5517) was cloned in frame with a C-terminal GFP epitope (followed by <i>UTR</i>) into CIP10SAT. The plasmid was linearized by <i>AatII</i> (generated by mutagenesis) for integration
ZXP216A	<i>SEC7c</i> -HA-UTR/CIP10U; DNA fragment containing <i>SEC7</i> coding region (4584 to 5517) was cloned in frame with a C-terminal HA epitope (followed by <i>UTR</i>) into CIP10U. The plasmid was linearized by <i>AatII</i> (generated by mutagenesis) for integration
CIP-V1-GFP	GFP-tagged <i>CaVRG4</i> (3)
CIP-ER-GFP	HDEL-tagged <i>KAR2</i> -GFP fusion (3)

Table S2: Strains used in this study

Strain	Parent	Relevant genotype	Source
SC5314		Wild type	Clinical isolate
BWP17	SC5314	SC5314; <i>ura3/ura3 his1/his1 arg4/arg4</i>	(4)
HZX201	BWP17	BWP17; <i>gyp1Δ::FRT/gyp1Δ-HIS1</i>	This study
HZX202	HZX201	HZX201; <i>GYP1-GFP-ARG4</i>	This study
HZX203	HZX201	HZX201; <i>GYP1-R292K-GFP-ARG4</i>	This study
HZX204	HZX201	HZX201; <i>GYP1-4A-GFP-ARG4</i>	This study
HZX205	HZX201	HZX201; <i>GYP1-4E-GFP-ARG4</i>	This study
HZX206	HZX201	HZX201; <i>GYP1-HA-ARG4</i>	This study
HZX207	HZX201	HZX201; <i>GYP1-R292K-HA-ARG4</i>	This study
HZX208	HZX201	HZX201; <i>GYP1-4A-HA-ARG4</i>	This study
HZX209	HZX201	HZX201; <i>GYP1-4E-HA-ARG4</i>	This study
HZX210	BWP17	BWP17; <i>VRG4-GFP-URA3</i>	This study
HZX211	HZX201	HZX201; <i>VRG4-GFP-URA3</i>	This study
HZX215	HZX206	HZX206; <i>SEC7-GFP-URA3</i>	This study
HZX216	HZX201	HZX201; <i>SEC7-GFP-URA3</i>	This study
HZX217	HZX207	HZX207; <i>SEC7-GFP-URA3</i>	This study
HZX218	HZX208	HZX208; <i>SEC7-GFP-URA3</i>	This study
HZX219	HZX209	HZX209; <i>SEC7-GFP-URA3</i>	This study
WYL2	BWP17	BWP17; <i>bni1Δ-ARG4/bni1Δ-HIS1</i>	(5)
HZX225	WYL2	WYL2; <i>GYP1-GFP-URA3</i>	This study
IIHH6-4a	CAI4	CAI4; <i>tpk1Δ::hisG/tpk1Δ::hisG</i>	(6)
AS1	CAI4	CAI4; <i>tpk2Δ::hisG/tpk2Δ::hisG</i>	(7)
HZX226	IIHH6-4a	IIHH6-4a; <i>GYP1-GFP-URA3</i>	This study
HZX227	AS1	AS1; <i>GYP1-GFP-URA3</i>	This study
HZX228	HZX202	HZX202; <i>MYO2-HA-URA3</i>	This study
HZX229	HZX203	HZX203; <i>MYO2-HA-URA3</i>	This study
HZX230	HZX204	HZX204; <i>MYO2-HA-URA3</i>	This study
HZX231	HZX205	HZX205; <i>MYO2-HA-URA3</i>	This study

HZX232	BWP17	BWP17; MYO2-HA-URA3	This study
HZX233	HZX201	HZX201; <i>GYP1-4A-R292K-GFP-ARG4</i>	This study
BC1	CAI4	CAI4; <i>cyr1Δ::hisG/cyr1Δ::hisG</i>	(8)
HZX234	BC1	BC1; GYP1-GFP-URA3	This study
HZX250	BWP17	BWP17; <i>gyp5Δ::FRT/gyp5Δ::FRT</i>	This study
HZX251	BWP17	BWP17; <i>gyp8Δ::FRT/gyp8Δ::FRT</i>	This study

SUPPORTING REFERENCE:

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6. **Bockmuhl DP, Krishnamurthy S, Gerads M, Sonneborn A, Ernst JF.** 2001. Distinct and redundant roles of the two protein kinase A isoforms Tpk1p and Tpk2p in morphogenesis and growth of *Candida albicans*. *Mol Microbiol* **42**:1243-1257.
7. **Sonneborn A, Bockmuhl DP, Gerads M, Kurpanek K, Sanglard D, Ernst JF.** 2000. Protein kinase A encoded by TPK2 regulates dimorphism of *Candida albicans*. *Mol Microbiol* **35**:386-396.
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