Effects of zinc transporters on *Cryptococcus gattii* virulence

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SUPPLEMENTARY MATERIAL

Supplementary Figure 1

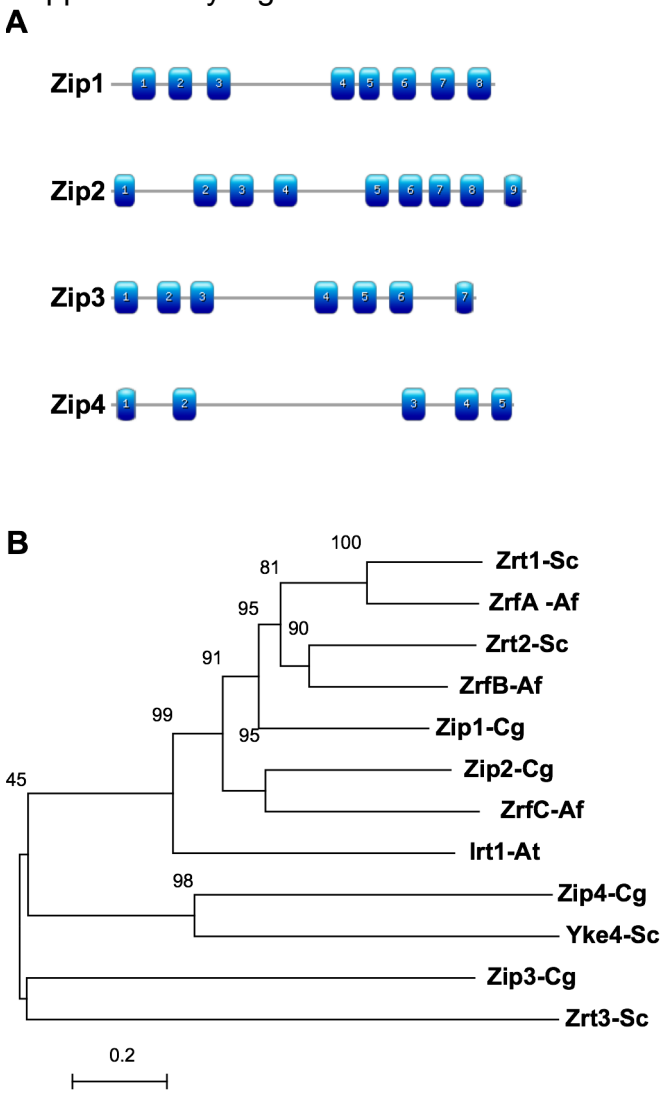

Figure S1. Some characteristics of *C. gattii* zinc transporters of the ZIP family. A Predicted transmembrane helices of the four Zip proteins encoded by the *C. gattii* genome were evaluated with the TMHMM tool and are illustrated as numbered blue boxes. **B** Phylogenetic analysis applying the neighbor-joining method and including Zip zinc transporters sequences from related fungi (Sc – *S. cerevisiae;* Af – *A. fumigatus*; Cg – *C. gattii*) and Irt1 from *A. thaliana*.

Supplementary Figure 2.

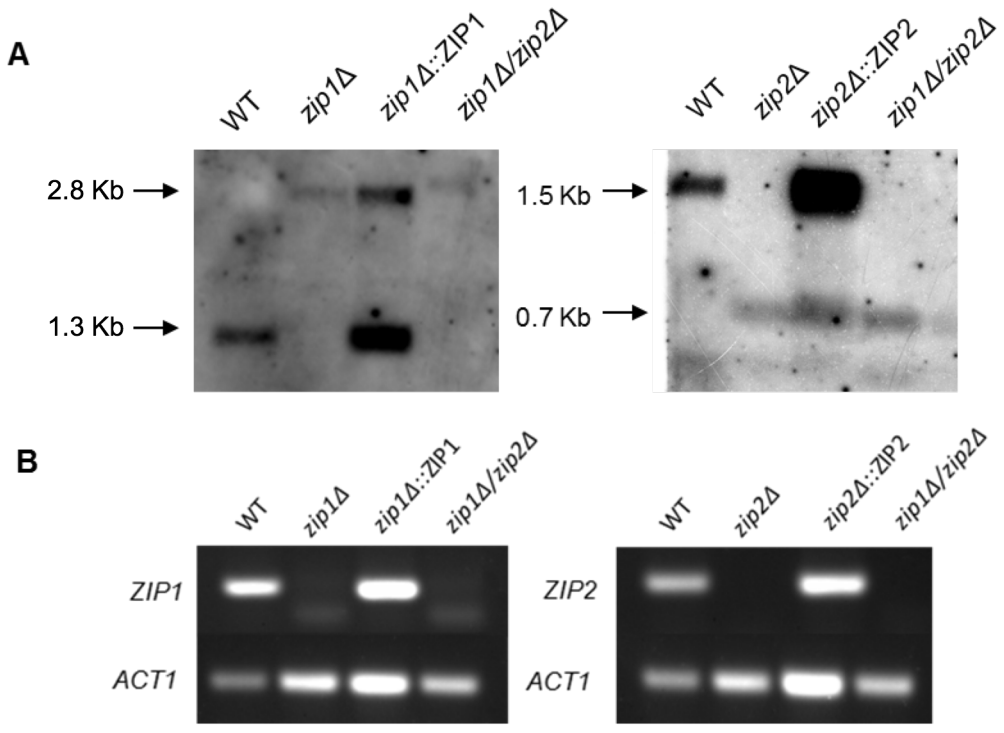

Figure S2. Construction of knockout and complemented strains. A Confirmatory Southern blot. Genomic DNA (10 µg) from the WT, *zip1*Δ mutant, *zip1*Δ::ZIP1 complemented and *zip1*Δ *zip2*Δ double mutant strains were digested with EcoRV. The 5′ gene flanking region was used as a probe for Southern hybridization. DNA (10 µg) from the WT, *zip2*Δ mutant, *zip2*Δ::ZIP2 complemented and *zip1*Δ *zip2*Δ double mutant strains were digested with the PstI restriction enzyme. In this experiment, the 3′ gene flanking region was used as a probe for Southern hybridization. Numbers at the left indicate the hybridization signal sizes based on the position of the molecular size marker. **B** Semi-quantitative RT-PCR using cDNA from the WT, *zip1*Δ mutant, *zip1*Δ::ZIP1 complemented and *zip1*Δ *zip2*Δ double mutant strains (left panel). RNA samples from the WT, *zip2*Δ mutant, *zip2*Δ::ZIP2 complemented and *zip1*Δ *zip2*Δ double mutant strains (right panel) were used

as templates for a reaction employing reverse transcriptase. The upper panel shows the *ZIP* amplicons, and the lower panel shows the *ACT1* amplicons used as loading controls.

Supplementary Figure 3

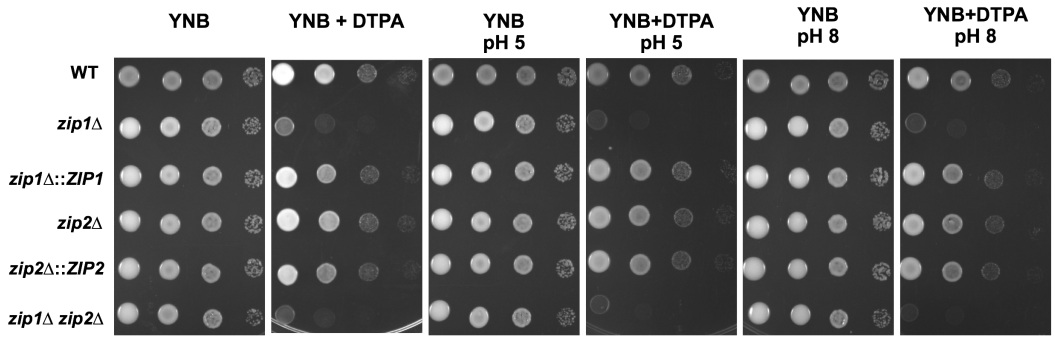

Figure S3. *C. gattii ZIP2* is not required for cryptococcal growth at different pHs Growth of the WT, *zip1*Δ, *zip1*Δ::ZIP1, *zip*2Δ, *zip*2Δ::ZIP2 and *zip1*Δ *zip2*Δ strains in YNB, YNB supplemented with 100 µM DTPA with or without 400 µM $ZnCl_2$ at alkaline pH (8) or a control pH (5) obtained using 20 mM phosphate buffer.

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50 **Supplementary Table 1: List of primers used in this work**

Primer name	Sequence (5'-3')	Purpose
ZIP1-5F	AAAATAGGGATAACAGGGTAATGGAAAATGTGGATTGGATGG	5'-flanking amplicon of *ZIP1* knockout construct
ZIP1-5R	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGAAATGACTGACGGGTCGTT	5'-flanking amplicon of *ZIP1* knockout construct
ZIP1-3F	GGGGACCACTTTGTACAAGAAAGCTGGGTACATTGTAGAAAATCCGTGAGAAG	3'-flanking amplicon of *ZIP1* knockout construct
ZIP1-3R	AAAAATTACCCTGTTATCCCTACGACAGGCTCTCAACGAGAC	3'-flanking amplicon of *ZIP1* knockout construct
ZIP2-5F	AAAATAGGGATAACAGGGTAATAGCAAGATAGGGGCTTACCC	5'-flanking amplicon of *ZIP2* knockout construct
ZIP2-5R	GGGGACAAGTTTGTACAAAAAAGCAGGCTATCATGCATCAGCGTGAGGA	5'-flanking amplicon of *ZIP2* knockout construct
ZIP2-3F	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAAAAGGGGGAAGAGTTTGG	3'-flanking amplicon of *ZIP2* knockout construct

ZIP2-3R	AAAAATTACCCTGTTATCCCTATGAGTGGGAGCATGGAACT	3'-flanking amplicon of *ZIP2* knockout construct
ZIP1compF	AGGGCGAATTCTGCAGATGCAAGAGACTAAGTCAGAGACTAAGTCAGAATCCA	*ZIP1* amplicon for complementation
ZIP1compR	CGGCCGCCAGTGTGATGGATCTCTTCGACAGGCTCTCAAC	*ZIP1* amplicon for complementation
ZIP2compF	AGGGCGAATTCTGCAGATTTACCCGCAGAGGCTCTTT	*ZIP2* amplicon for complementation
ZIP2compR	CGGCCGCCAGTGTGATGGATCTGGAGGACGACGATGAAGAA	*ZIP2* amplicon for complementation
CGACTF	CGGTATCGTCACAAACTGG	Amplification of *ACT1* for RT-PCR/qRT-PCR
CGACTR	GGAGCCTCGGTAAGAAGAAC	Amplification of *ACT1* for RT-PCR/qRT-PCR
CNBG5361F	GTGTAGCCCTTCTTTTCTCC	Amplification of *ZIP3* for qRT-PCR
CNBG5361R	CAATCCTGTCCGTTGGTAC	Amplification of *ZIP3* for qRT-PCR
CNBG6066F	GCTGAAGTCGCCGCTTATC	Amplification of *ZIP1* for qRT-

		PCR
CNBG6066R	GGGAGGGATGGATGTGATG	Amplification of *ZIP1* for qRT-PCR
CNBG2209F	GTCATATTGGGCAAACTGG	Amplification of *ZIP2* for qRT-PCR
CNBG2209R	AGGGGCAACAGACTCATAG	Amplification of *ZIP2* for qRT-PCR

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