1 **SUPPLEMENTAL MATERIALS AND METHODS**

2

3 **Includes Supplemental Materials and Methods and Table S1. List of strains used**
4 **in this study, Table S2. List of plasmids used in this study, and Table S3. List of**
5 **plasmids used in this study.**

6

7 **Generation of *Cryptococcus neoformans* mutants**

8 YPD, supplemented with 1.5% agar and 100 µg ml-1 of nourseothricin (NAT), 200 µg
9 ml-1 of neomycin (G418) or 200 u ml-1 of hygromycin B (HYG) was used for colony
10 selection after biolistic transformation.

11

12 The *C. neoformans* Atm1-F strains (wimp and stud) (DTY947 and DTY967,
13 respectively) were generated as follows: the multiple cloning site of pSL1180
14 (Amersham) was amplified with the primer pair OLSG-49 and OLSG-50 (primers
15 sequences are described in Table S2), and HindIII/EcoRI cloned into pUC19 to yield
16 plasmid pDT1799. The *NAT* cassette from pAI3 (1) was amplified with the primer pair
17 OLSG-35 and OLSG-36 and NheI cloned into DTP1799 to generate the plasmid
18 pDT1800. The *4FLAG-HOG1* terminator sequence was amplified from plasmid p553
19 (gift from Dr. Sun-Bahn, Yonsei University, Seoul) with the primer pair OLSG-158 and
20 OLSG-107 and cloned into pDT1800 with the restriction sites SacII/StuI to generate the
21 plasmid pDT1802. The primer pair OLSG-196 and OLSG-197 was used to amplify

22 approximately 1kb upstream of the stop codon (not including the stop codon) of *ATM1*
23 (ATM1up) using genomic DNA as a template. This fragment was digested with AscI and
24 cloned into pDT1801 to generate the plasmid pDT1802. The primer pair OLSG-93 and
25 OLSG-94 was used to amplify approximately 1kb downstream of the stop codon (not
26 including the stop codon) of *ATM1* (ATM1d) using genomic DNA as a template. This
27 fragment was digested with XmaI and cloned into pDT1802 to generate the plasmid
28 pDT1803. A PCR product, including the sequence ATM1up-4FLAG-HOGt-NAT-ATM1d
29 was obtained with the primer pairs OLSG-276 and OLSG-54, using the plasmid
30 pDT1803 as a template, and transformed in *C. neoformans* strains H99 wimp (DTY758)
31 and H99 stud (DTY981) by biolistic transformation, to generate the strains DTY947 and
32 DTY967, as previously described.

33

34 The *C. neoformans* strains Gal7-Atm1-F wimp and stud (DTY949 and DTY969,
35 respectively) strains were generated as follows: the G418 cassette from pJAF1 (2) was
36 amplified with the primer pair OLSG-112 and OLSG-113 and NheI cloned into pDT1799
37 to generate the plasmid pDT1804. A fragment of approximately 1kb was amplified from
38 the genomic DNA using OLSG-294 and OLSG-295 as a primer pair. This fragment was
39 cut with the restriction enzymes SacII and StuI, and cloned into pDT1804 to generate
40 the plasmid pDT1805. A PCR product amplifying the *GAL7* promoter was obtained with
41 OLSG-297 and OLSG-298, using pCN69 as a template, a second PCR product, with
42 size approximately 1kb, and starting at the *ATM1* initiation codon, was obtained with the
43 primer pair OLSG-298 and OLSG-299 using genomic DNA as a template. These two
44 PCR products were cloned into pDT1805 using the infusion cloning kit (Clontech

45 Laboratories) to generate the plasmid pDT1806. The plasmid was linearized with SacII,
46 and transformed in both *C. neoformans* DTY947 to generate the strain DTY949, and in
47 *C. neoformans* DTY967 to generate the strain DTY969, by biolistic transformation.

48

49 The *C. neoformans* strain Gal7-Atm1-mCherry (DTY951) strain was generated
50 according to the following steps: the mCherry-HOGt sequence was amplified from p524
51 (gift from Dr. Sun-Bahn) with OLSG-194 and OLSG-195, and cloned after digestion with
52 the restriction enzymes SacII/NgoMIV into pDT1800. The primer pair OLSG-196 and
53 OLSG-197 was used to amplify approximately 1kb upstream of the stop codon (not
54 including the stop codon) of *ATM1* (ATM1up) using genomic DNA as a template. This
55 fragment was digested with AscI and cloned into pDT1807 to generate the plasmid
56 pDT1808. The primer pair OLSG-93 and OLSG-94 was used to amplify approximately
57 1kb downstream of the stop codon (not including the stop codon) of *ATM1* (ATM1d)
58 using genomic DNA as a template. This fragment was digested with XmaI and cloned
59 into pDT1808 to generate the plasmid pDT1809. A PCR product, including the
60 sequence ATM1up-mCherry-HOGt-NAT-ATM1d was obtained with the primer pairs
61 OLSG-276 and OLSG-277 and transformed in *C. neoformans* strain H99 (DTY758) by
62 biolistic transformation to generate the strain ATM1-mCherry. A maxiprep for pDT1806
63 was obtained, the plasmid was linearized with SacII, and transformed in Atm1-mCherry
64 by biolistic transformation, to generate the Gal7-Atm1-mCherry strain.

65

66 *C. neoformans* Atm1-F Rli1-HA (DTY955) and Gal7-Atm1-F Rli1-HA (DTY957) strains
67 were generated as follows: the primer pair OLSG-190 and OLSG-191 was used to
68 amplify the *HYG* cassette from the pHYG-KB1 vector (3), the PCR fragment obtained
69 was digested with KpnI and cloned into pDT1799 to generate the pDT1810 vector. The
70 Rli1-HA-Hog terminator sequence, including a SbfI site between the RLI1 sequence and
71 the HA, was synthesized by Integrated DNA Technologies (IDT), this sequence was
72 amplified with OLSG-358 and OLSG-107 and digested with the restriction enzymes
73 XhoI/StuI for cloning it into pDT1810 vector, to generate the pDT1811 vector. A PCR
74 fragment, approximately 1kb downstream of the stop codon (and not including the stop
75 codon) was amplified with OLSG-359 and OLSG-360 and cloned into pDT1811 after
76 digestion with XmaI/XbaI to generate the vector pDT1812. A maxiprep of pDT1812 was
77 obtained, and the harvested plasmid was digested with XhoI for linearization before
78 biolistic transformation in both DTY947 to obtain the strain Atm1-F Rli1-HA (DTY955)
79 and in DTY949 to obtain the strain Gal7-Atm1-F Rli1-HA (DTY957).

80

81 *C. neoformans* Atm1-F Leu1-HA (DTY959) and Gal7-Atm1-F Leu1-HA (DTY961) strains
82 were generated as follows: the primer pair OLSG-412 and OLSG-413 was used to
83 amplify approximately 1kb upstream the *LEU1* stop codon (without including it), the
84 PCR product obtained was digested with SpeI/SbfI and cloned into pDT1811 SpeI/SbfI
85 digested to yield the vector pDT1813. A PCR fragment, approximately 1kb downstream
86 of the stop codon (and not including the stop codon) was amplified with OLSG-414 and
87 OLSG-415 and cloned into DTP1813 after digestion with XbaI/BamHI to generate the
88 vector pDT1814. A PCR product was obtained by amplifying the sequence LEU1up-HA-

89 HOGt-HYG-LEU1d with OLSG-434 and OLSG-54 and transformed in both DTY947 to
90 obtain the strain Atm1-F Leu1-HA (DTY959) and in DTY949 to obtain the strain Gal7-
91 Atm1-F Leu1-HA (DTY961).

92

93 *C. neoformans* Atm1-F Lys4-HA (DTY963) and Gal7-Atm1-F Lys4-HA (DTY965) strains
94 were generated as follows: the primer pair OLSG-445 and OLSG-446 was used to
95 amplify approximately 1kb upstream the *LYS4* stop codon (without including it), the
96 PCR product obtained was cloned with the infusion kit (Clontech Laboratories) into
97 DTP1811 SpeI/SbfI digested to yield the vector pDT1815. A PCR fragment,
98 approximately 1kb downstream of the stop codon (and not including the stop codon)
99 was amplified with OLSG-447 and OLSG-448 and cloned into pDT1815 after digestion
100 with XbaI/BamHI to generate the vector pDT1816. A PCR product was obtained by
101 amplifying the sequence LYS4up-HA-HOGt-HYG-LYS4d with OLSG-465 and OLSG-54
102 and transformed in both DTY947 to obtain the strain Atm1-F Lys4-HA (DTY963) and in
103 DTY949 to obtain the strain Gal7-Atm1-F Lys4-HA (DTY965). Primers are described in
104 Table S3.

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108 **REFERENCES TO THE SUPPLEMENTAL MATERIALS AND METHODS**

109

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113 of the primary fungal pathogen Cryptococcus neoformans var. gattii: implications for an
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116 fungal pathogen Cryptococcus neoformans. Clin Diagn Lab Immunol 7:125-8.

117

118

Table S1. List of strains used in this study

NAME	YEAST	STRAIN	GENOTYPE	REFERENCE
DTY758	*C. neoformans*	H99	wild-type wimp	J. Heitman lab, Duke University, USA
DTY981	*C. neoformans*	H99	wild-type stud	J. Heitman lab, Duke University, USA
DTY761	*C. neoformans*	*ΔCuf1*	*cuf1::Neo* wimp	(1)
DTY762	*C. neoformans*	*ΔCuf1* + pCuf1-FLAG	*cuf1::Neo/CUF1-FLAG::HYG* wimp	(2)
DTY947	*C. neoformans*	Atm1-F	*ATM1-4FLAG::NAT* wimp	This study, derived from DTY758
DTY949	*C. neoformans*	Gal7-Atm1-F	*ATM1p::GAL7p::G418 ATM1-4FLAG::NAT* wimp isolate #1	This study, derived from DTY947
DTY949	*C. neoformans*	Gal7-Atm1-F	*ATM1p::GAL7p::G418 ATM1-4FLAG::NAT* wimp isolate #2	This study, derived from DTY947
DTY951	*C. neoformans*	Gal7-Atm1-mCherry	*ATM1p::GAL7p::G418 ATM1-mCherry::NAT* wimp	This study
DTY756	*C. neoformans*	*ΔMt1 ΔMt2*	*mt1::NAT mt2::NEO* wimp	(1)
DTY953	*C. neoformans*	*ΔMt1 ΔMt2* Gal7-Atm1	*mt1::NAT mt2::NEO ATM1p::GAL7p::G418* wimp	This study, derived from DTY756
DTY955	*C. neoformans*	Atm1-F Rli1-HA	*ATM1-4FLAG::NAT RLI1-HA::HYG* wimp	This study, derived from DTY947
DTY957	*C. neoformans*	Gal7-Atm1-F Rli1-HA	*ATM1p::GAL7p::G418 ATM1-4FLAG::NAT RLI1-HA::HYG* wimp	This study, derived from DTY949
DTY959	*C. neoformans*	Atm1-F Leu1-HA	*ATM1-4FLAG::NAT LEU1-HA::HYG* wimp	This study, derived from DTY947
DTY961	*C. neoformans*	Gal7-Atm1-F Leu1-HA	*ATM1p::GAL7p::G418 ATM1-4FLAG::NAT LEU1-HA::HYG* wimp	This study, derived from DTY949
DTY963	*C. neoformans*	Atm1-F Lys4-HA	*ATM1-4FLAG::NAT LYS4-HA::HYG* wimp	This study, derived from DTY947
DTY965	*C. neoformans*	Gal7-Atm1-F Lys4-HA	*ATM1p::GAL7p::G418 ATM1-4FLAG::NAT LYS4-HA::HYG* wimp	This study, derived from DTY949
DTY967	*C. neoformans*	Atm1-F	*ATM1-4FLAG::NAT* stud	This study, derived from DTY946
DTY969	*C. neoformans*	Gal7-Atm1-F	*ATM1p::GAL7p::G418 ATM1-4FLAG::NAT* stud	This study, derived from DTY946
	S. cerivisiae	GalL-Atm1	*MATa, ura3-1, ade2-1, trp1-1, his3-11,15 leu2-3,112 ATM1p::GalLp*	(3)
	S. cerivisiae	GalL-Atm1 Aft1-lux	*MATa, ura3-1, ade2-1, trp1-1, his3-11,15 leu2-3,112 ATM1p::GalLp AFT1::HIS3 AFT1lux*	Dr U. Muehlenhoff, Philipps-Universitat Marburg
	S. cerivisiae	Grx4-HA	*MATa, ura3-1, ade2-1, trp1-1, his3-11,15 leu2-3,112::GRX4-HA tetO2* promoter	(4)

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Table S2. List of plasmids used in this study

PLASMID	ORF	BACKBONE	REFERENCE
pRS416-NP-ScAtm1	ScATM1, MET25 promoter replaced by ATM1p	pRS416-MET25	(1)
p416-pGPD-CnAtm1	*C. neoformans* ATM1 cDNA under control of GPD promoter	p416-GPD	This work
p416-pCCC1-LUX	luc2 under control of CCC1 (-600 to -1)	p416-Luc2	(2)
p426-FDX2-HA	FDX2 (Homo sapiens); C-terminal HA	p426-TDH3	(3)
p426-NAR1-HA	NAR1, C-terminal HA	p426-TDH3	(4)
p424-NBP35-TAP	NBP35, C-terminal TAP	p424-TDH3	(5)
p426-RLI1-HA	RLI1; C-terminal HA; own promoter replacing TDH3 promoter	p426-TDH3	(6)
p414-SDH2-Myc	SDH2; C-terminal Myc	p414-MET25	D.R. Winge, University of Utah, USA
p416-SDH1	SDH1	p416-MET25	D.R.Winge, University of Utah, USA

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Table S3. List of primers used in this study

PRIMER NAME	SEQUENCE	COMMENTS
OLSG-35	ACGGCTAGCGTAAAACGACGGCCAGT	
OLSG-36	ACGGCTAGCAACAGCTATGACCATG	
OLSG-49	ACGAAGCTTAAGGTGCACGGCCC	
OLSG-50	CATGAATTCGAATGGCCATGGGAC	
OLSG-54	CAGCTGGCGAAAGGGGGATGT	
OLSG-93	ATGCCCGGGACTTAGAAGAAGAGGTTGG	
OLSG-94	ATGCCCGGGAATTATAGTTCACTTTCTC	
OLSG-107	ATGAGGCCTCAATCTATCCCTCTCTCC	
OLSG-112	ACGGCTAGCGACTCACTATAGGGCG	
OLSG-113	ACGGCTAGCCCACTAGTAACGGCCGC	
OLSG-158	ATGCCGCGGGGCGCGCCAGATTACAAGGATGACGAC	
OLSG-190	ATGGGTACCACTCGCTATTGTCCAGGCTG	
OLSG-191	ATGGGTACCGCAACCATGTGATGTGCAAA	
OLSG-194	TAGCCGCGGGGCGCGCCAGGTGGCGGTGGCTCTGTGA	
OLSG-195	ATGGCCGGCCAATCTATCCCTCTCTCCGA	
OLSG-196	ACGGGCGCGCCTTCCCTTCCCCTCAACTTCC	
OLSG-197	ACGGGCGCGCCCTGCTTTTTCTTCTCGTCCA	
OLSG-276	CAGTCTACCGCGAACTTCGTCAAA	
OLSG-277	TTGGGAAGGGCGATCGGTG	
OLSG-294	ATGCCGCGGACTCACTATGATCGAAAAAG	
OLSG-295	ATCAGGCCTAATGGTCGCTGACATTCTAC	
OLSG-296	CATTGCAATTGGATCCGGTACCGAGCTCGAGCTC	
OLSG-297	GGAGCCGAAGCCCATTCTCAGGAGAGAATTGAGTG	
OLSG-298	ATGGGCTTCGGCTCCTGC	
OLSG-299	AGGTAATTATAACCCGGGATGGTAATCGCAGTAACGG	
OLSG-358	ATTCTCGAGCATGACCAAGACTCTTGGCAAC	
OLSG-359	ATTTCTAGAAGCGATGAATCAAAAACCTGC	
OLSG-360	TTACCCGGGGATCAGATTCGTTTTCAGC	
OLSG-412	ACGACTAGTAACTTCGAAGGTCGACAAGGC	
OLSG-413	ATCCCTGCAGGCCAGTCCGTCTTCCTGACGCC	
OLSG-414	ACTTCTAGAGGGCAGATTGGAGATAATGGC	
OLSG-415	ACTGGATCCGCCGTAATGAGGCTTTCCGG	
OLSG-434	GTCGACAAGGCGCTGGTGGCC	
OLSG-445	CGGCCCACGTGGCCACTAGTACTTTGATTGACGCA	
OLSG-446	ACCGGCGGCCATTCCTGCAGGTAATGAAGCTCTAA	
OLSG-447	TCCTCTAGAGCATTTGTCATGATTAGAGC	
OLSG-448	TATGGATCCTTCATCACCAGGATCTCCAC	
OLSG-465	TGCAGGCTGCGGTCCTTGTATTG	
OLSG-542	TGAGTGAAAGTGGCTCATCG	*TUB2* ChIP PRIMER FORWARD
OLSG-543	AGCAAGCCAAAAACAACACC	*TUB2* ChIP PRIMER REVERSE
OLRF-304	CGGGATTAACGTGATTGCTT	*ATM1* ChIP PRIMER FORWARD
OLRF-310	TAGAACGAGGAACGCGCTAT	*ATM1* ChIP PRIMER REVERSE
OLRF-272	GCACCATACCTTCTACAATGAGC	*ACT1* qRT-PCR PRIMER FORWARD
OLRF-273	CAGGAAGCTCGTAAGACTTTTCA	*ACT1* qRT-PCR PRIMER REVERSE
OLRF-260	TCATCTGGAGCGTCAAACTG	*ATM1* qRT-PCR PRIMER FORWARD
OLRF-261	GCCAAATTTCCATGAGAGGA	*ATM1* qRT-PCR PRIMER REVERSE
OLRF-350	TACGAGCGAGGCTCAAGACA	*MT1* qRT-PCR PRIMER FORWARD
OLRF-351	GGCAGCGCCAGATCCGCTG	*MT1* qRT-PCR PRIMER REVERSE
OLRF-352	TTCAACCCCAACCCAGAAAAG	*MT2* qRT-PCR PRIMER FORWARD
ORF-353	CACCTTGGCAACCGCATGTG	*MT2* qRT-PCR PRIMER REVERSE
OLSG-361	CACATTCACCGCTGCCATGGCTG	*CIG1* qRT-PCR PRIMER FORWARD
OLSG-362	CTGTTGACCGGGTCGAAACCAC	*CIG1* qRT-PCR PRIMER REVERSE
OLSG-363	ATGCCAGACCAGCTCTATCCG	*SIT1* qRT-PCR PRIMER FORWARD
OLSG-364	GACAGAGCCATCCAGGCCATAGC	*SIT1* qRT-PCR PRIMER REVERSE