1 **SUPPORTING INFORMATION**

2 **Table S1. *C. neoformans* strains used in this study.**

Strain	Additional information	Reference
RC2-wt (smooth phenotype)	Serotype D, Switch variant *MATα*	(1)
H99	Serotype A, *MATα*	(2)
RC2-*all2Δ*	*all2Δ*::*NEO*	This study
RC2-*all2Δ*+P$_{ACT1}$-*ALL2*	*ALL2* promoter replaced with *ACT1* promoter to generate P$_{ACT1}$-*ALL2*-*NAT* and inserted to RC2-*all2Δ* *all2Δ*::*NEO*	This study
H99-*all2Δ*	*all2Δ*::*NEO*	This study
H99-*all2Δ*+P$_{ACT1}$-*ALL2*	*ALL2* promoter replaced with *ACT1* promoter to generate P$_{ACT1}$-*ALL2*-*NAT* and inserted to H99-*all2Δ*	This study
RC2-*all1Δall2Δ*	*all2Δ*::*NEOall1Δ*::*NAT*	This study
RC2-*ALL1*::HA	*ALL1* tagged with HA at C-terminal	This study

3

4 **Table S2**
5 **Primers used in this study.** Primers were designed using the Primer3 software
6 package (http://frodo.wi.mit.edu/primer3/) for generating gene-deletion cassettes, gene-
7 reconstitution cassettes, or real-time PCR products.

8

Primer	Sequence (5'-3')	Function/ restriction site
All2-UpF	CCATAGATTGG– AGAAGAAGGGAGACGCAGCT	*ALL2* gene knock out cassette/*Van*91I
All2-UpR	CCATCATTTGG- GTTTGGTTCTGAAGTTGATATGTGGT	
All2-DwnF	CCATTTCTTGG- CTGCCTAAGAAGTAGTTTGCCATAT	*ALL2* gene knock out cassette/*Van*91I
All2-DwnR	CCATAAATTGG- ATGGCTCACCAGGAAGAACGA	
All2-GF	CATATGTCTACTGTTACTCAAGGTGTT AAGGA	*ALL2* gene reconstitution/*Nde*I
All2-GR	CTCGAGCGGGGATGTGATCTGTTTTC	*ALL2* gene reconstitution/*Xho*I
ACT1-PF	TCTAGAAGGCTGCGGGAGGTGAGCT	*ACT1* promoter for gene reconstitution/*Xba*I
ACT1-PR	CATATGAGACATGTTGGGCGAGTTTTA C	*ACT1* promoter for gene reconstitution/*Nde*I
ACT1-RT-f ACT1-RT-r	(3)	Real time PCR actin
All1-RT-f All1-RT-r	(3)	Real time PCR *ALL1*
All2-RTF All2-RTR	TGAAAGAAGGCCTCAAGAGCG AGTGGAAGCGGCATTGGTT	Real time PCR *ALL2*
ALL1prom- XbaI-F	TCTAGAAGGTTTGGGGTTGATTTCGAA GCT	*ALL1*::HA
ALL1-HA NCOI-rev	CCATGGTTAAGCGTAGTCTGGGACGT CGTATGGGTAAAGAGCCTGGGTCTTG CTG	
ALL1-3UTR- For-NCOI	CCATGGAAGAATGTGTAGTAGTTTATG GTATCTGAAGGTTAAAG	*ALL1*::HA
ALL1-3UTR- ECORV	GATATCGTGCACTGAACCACAAGCTC	
All2For-NHEI	GCTAGCAGGCTGCGGGAGGTGAGCT G	*ALL2*-mCherry
All2Rev-PSTI	CTGCAGGGCAGTAGGAACTTCCTTAG TGGA	
P_{Act}-XbaFor	TCTAGAAGGCTGCGGGAGGTGAGCTG	*ALL2*-mCherry
mcherry rev- ECORV	GATATCTTACTTGTAGAGCTCGTCCAT ACCAC	*ALL2*-mCherry
All2 3'UTR-for- ECORV	GATATCGAAGTAGTTTGCCATATATTT TTTGTCTTGGGG	*ALL2*-mCherry
All2 3'UTR- Rev XHOI	CTCGAGTTCATCGTGCGTGTCTTCACT TACT	

9

10

11   **Table S3. Expression levels of *ALL2* as determined by real time PCR in mutants.**

Sample	$-\Delta C_T$	$2^{-\Delta CT}$	$2^{-\Delta\Delta CT}$
ALL2 expression relative to reference gene – *ACT1*			
RC2 wt	3.6	0.07745	
	15.1	0.00003	0.00037
all2Δ			
	14.8	0.00003	0.00043
all1Δall2Δ			
ALL2 expression in reconstituted strain relative to reference gene – *ACT1*			
RC2-wt	3.4	0.094732	
all2Δ+P$_{ACT1}$-*ALL2*	3.9	0.066986	1.01942
ALL1 expression relative to reference gene – *ACT1*			
RC2-wt	2.6	0.16348	
	15.5	0.00002	0.00013
all1Δall2Δ			

12

13 **Table S4. Characteristics of *ALL2* null mutant in H99 background.**

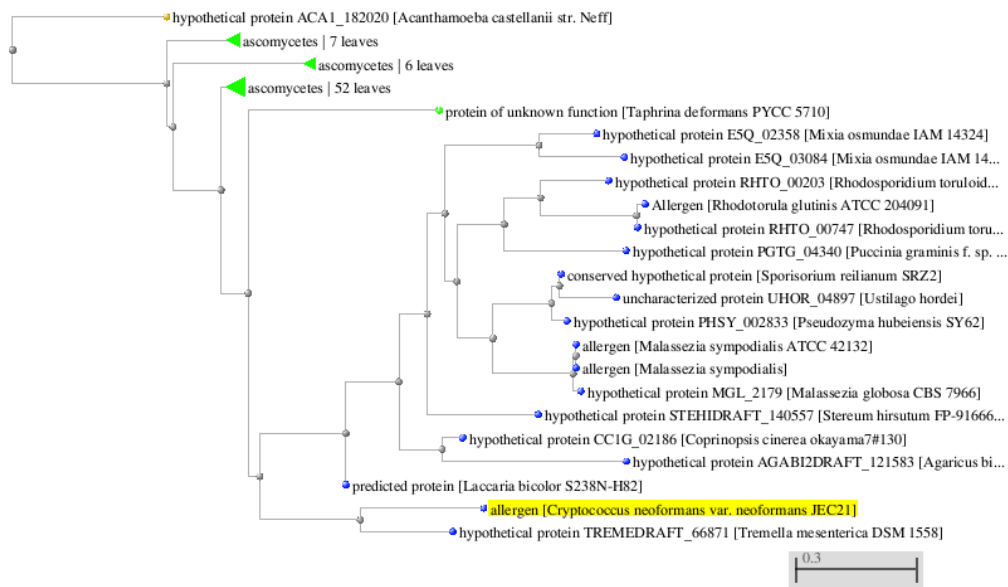| Characteristic	H99	H99-*all2Δ*	H99-*all2Δ*+P$_{ACT1}$-*ALL2*
Exo-polysaccharide viscosity (mL/g)	3238 $\pm$ 432.6	1958 $\pm$ 413.3	3512 $\pm$ 426.2
H$_2$O$_2$ sensitivity zone size (mm)	63	69	62
Phagocytosis index (%)	131.1 $\pm$ 55.8	101.8 $\pm$ 28.3	113.2 $\pm$ 20.3

14

15 To generate H99-*all2Δ*, the coding region of *ALL2* (945bp) in H99 was replaced with a
16 neomycin resistance marker by homologous recombination as described in Materials
17 and Methods. The exo-polysaccharide viscosity, H$_2$O$_2$ sensitivity and phagocytosis
18 index for H99, H99-*all2Δ*, and H99-*all2Δ*+P$_{ACT1}$-*ALL2* were measured using similar
19 methods described for RC2.

20 **Figure S1. All1p and All2p are homologous to fungi that affect plants.**
21 Both All1p and All2p exhibit up to 33-38% homology with hypothetical proteins in other
22 non-encapsulated fungi that are pathogenic to plants, but no homology to any fungi that
23 that are pathogenic to humans.
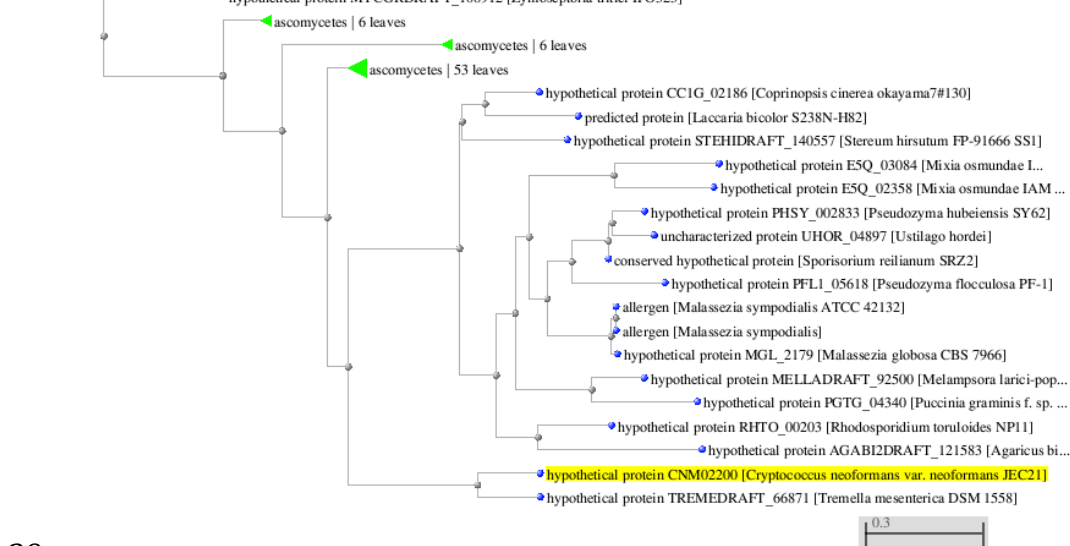24
25 All1p alignment tree

26

hypothetical protein ACA1_182020 [Acanthamoeba castellanii str. Neff]
ascomycetes | 7 leaves
ascomycetes | 6 leaves
ascomycetes | 52 leaves
protein of unknown function [Taphrina deformans PYCC 5710]
hypothetical protein E5Q_02358 [Mixia osmundae IAM 14324]
hypothetical protein E5Q_03084 [Mixia osmundae IAM 14...
hypothetical protein RHTO_00203 [Rhodosporidium toruloid...
Allergen [Rhodotorula glutinis ATCC 204091]
hypothetical protein RHTO_00747 [Rhodosporidium toru...
hypothetical protein PGTG_04340 [Puccinia graminis f. sp. ...
conserved hypothetical protein [Sporisorium reilianum SRZ2]
uncharacterized protein UHOR_04897 [Ustilago hordei]
hypothetical protein PHSY_002833 [Pseudozyma hubeiensis SY62]
allergen [Malassezia sympodialis ATCC 42132]
allergen [Malassezia sympodialis]
hypothetical protein MGL_2179 [Malassezia globosa CBS 7966]
hypothetical protein STEHIDRAFT_140557 [Stereum hirsutum FP-91666...
hypothetical protein CC1G_02186 [Coprinopsis cinerea okayama7#130]
hypothetical protein AGABI2DRAFT_121583 [Agaricus bi...
predicted protein [Laccaria bicolor S238N-H82]
allergen [Cryptococcus neoformans var. neoformans JEC21]
hypothetical protein TREMEDRAFT_66871 [Tremella mesenterica DSM 1558]

0.3

27

28 All2p alignment tree

hypothetical protein MYCGRDRAFT_108912 [Zymoseptoria tritici IPO323]
ascomycetes | 6 leaves
ascomycetes | 6 leaves
ascomycetes | 53 leaves
hypothetical protein CC1G_02186 [Coprinopsis cinerea okayama7#130]
predicted protein [Laccaria bicolor S238N-H82]
hypothetical protein STEHIDRAFT_140557 [Stereum hirsutum FP-91666 SS1]
hypothetical protein E5Q_03084 [Mixia osmundae I...
hypothetical protein E5Q_02358 [Mixia osmundae IAM ...
hypothetical protein PHSY_002833 [Pseudozyma hubeiensis SY62]
uncharacterized protein UHOR_04897 [Ustilago hordei]
conserved hypothetical protein [Sporisorium reilianum SRZ2]
hypothetical protein PFL1_05618 [Pseudozyma flocculosa PF-1]
allergen [Malassezia sympodialis ATCC 42132]
allergen [Malassezia sympodialis]
hypothetical protein MGL_2179 [Malassezia globosa CBS 7966]
hypothetical protein MELLADRAFT_92500 [Melampsora larici-pop...
hypothetical protein PGTG_04340 [Puccinia graminis f. sp. ...
hypothetical protein RHTO_00203 [Rhodosporidium toruloides NP11]
hypothetical protein AGABI2DRAFT_121583 [Agaricus bi...
hypothetical protein CNM02200 [Cryptococcus neoformans var. neoformans JEC21]
hypothetical protein TREMEDRAFT_66871 [Tremella mesenterica DSM 1558]

0.3

29

30

Figure S2. Validation of the *ALL2* gene disruption and complementation done by PCR.
(**A**) PCR Confirmation of the disruption of *ALL2* gene with the *NEO* disruption cassette. Lane 1: 1kb DNA marker, Lane 2: PCR amplification using primers All2homo-F and All2homo-R using RC2 genomic DNA yielded 3000 bp PCR product, Lane 3: PCR amplification using primers All2homo-F and All2homo-R using *all1Δall2Δ* genomic DNA yielded 4000 bp PCR product, Lane 4: PCR amplification using primers All1homo-F and All1homo-R using RC2 genomic DNA yielded 3000 bp PCR product, Lane 5: PCR amplification using primers All1homo-F and All1homo-R using *all1Δall2Δ* genomic DNA yielded 4000bp PCR product, Lane 6: 1kb DNA marker, Lane 7: PCR amplification using primers All2homo-F and All2homo-R using WT genomic DNA yielded 3000bp PCR product, Lane 8: PCR amplification using primers All2homo-F and All2homo-R using *all2Δ* genomic DNA yielded 4000bp PCR product. (**B**) PCR confirmation of insertion of a copy of *ALL2* under Actin promoter to generate *all2Δ*+P$_{ACT1}$-ALL2.
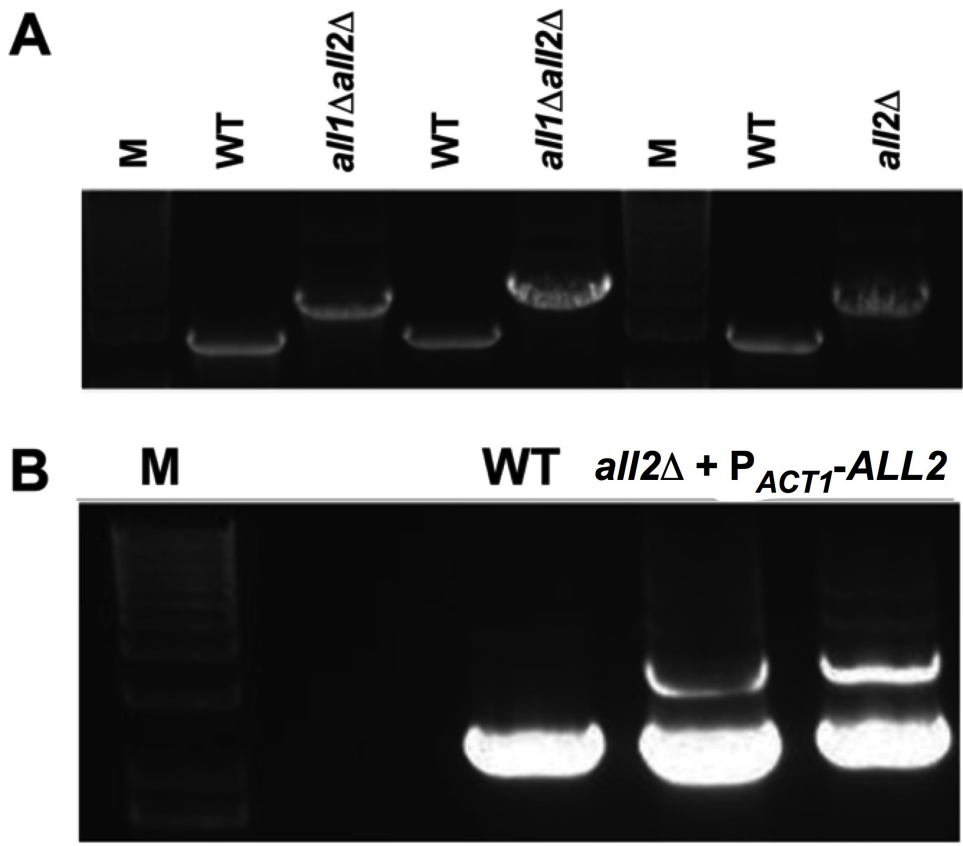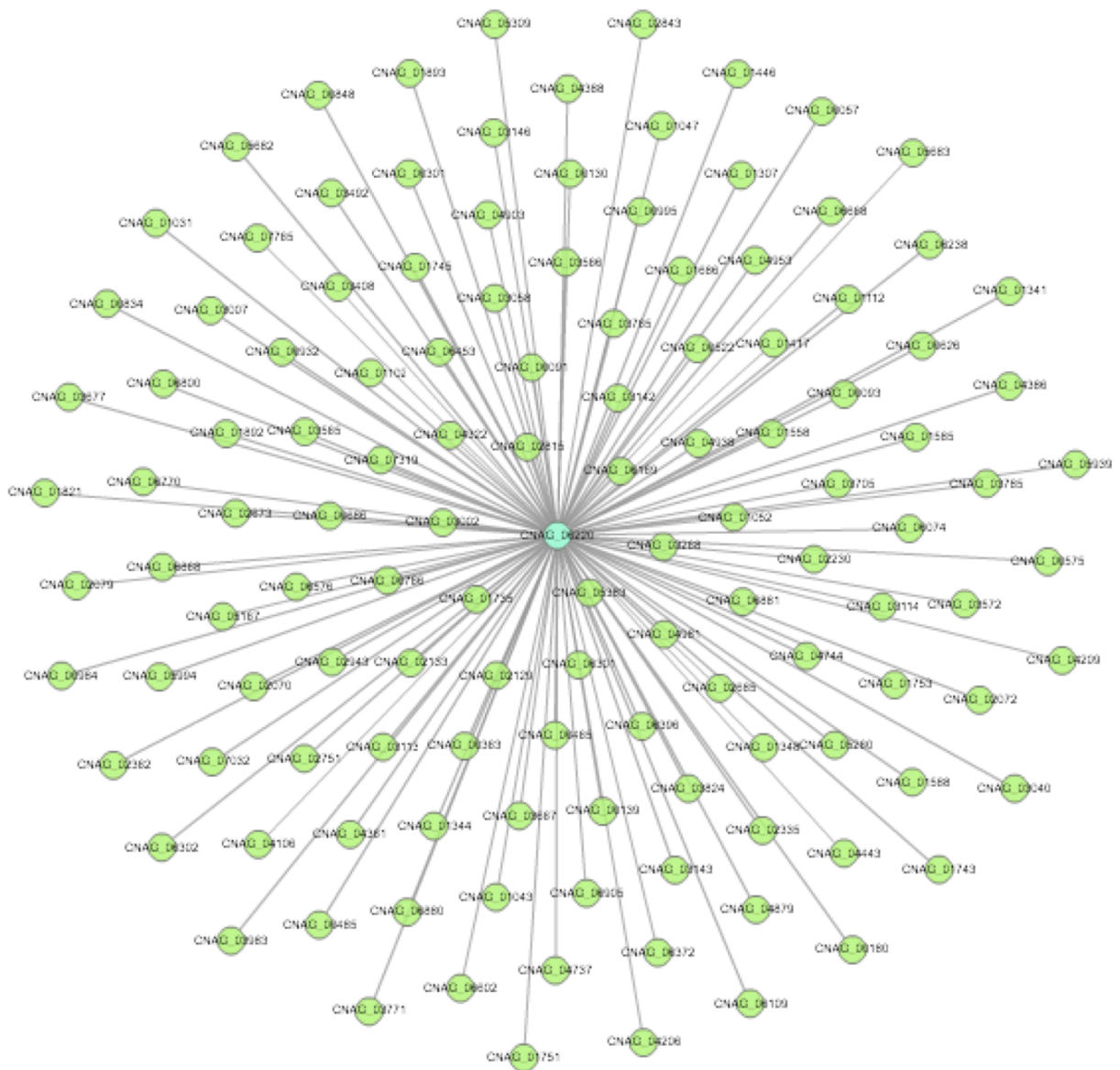
46 **Figure S3. All1-mCherry Cells recovered from murine tissue displayed All2p**
47 **localization (red) in vacuoles.**
48
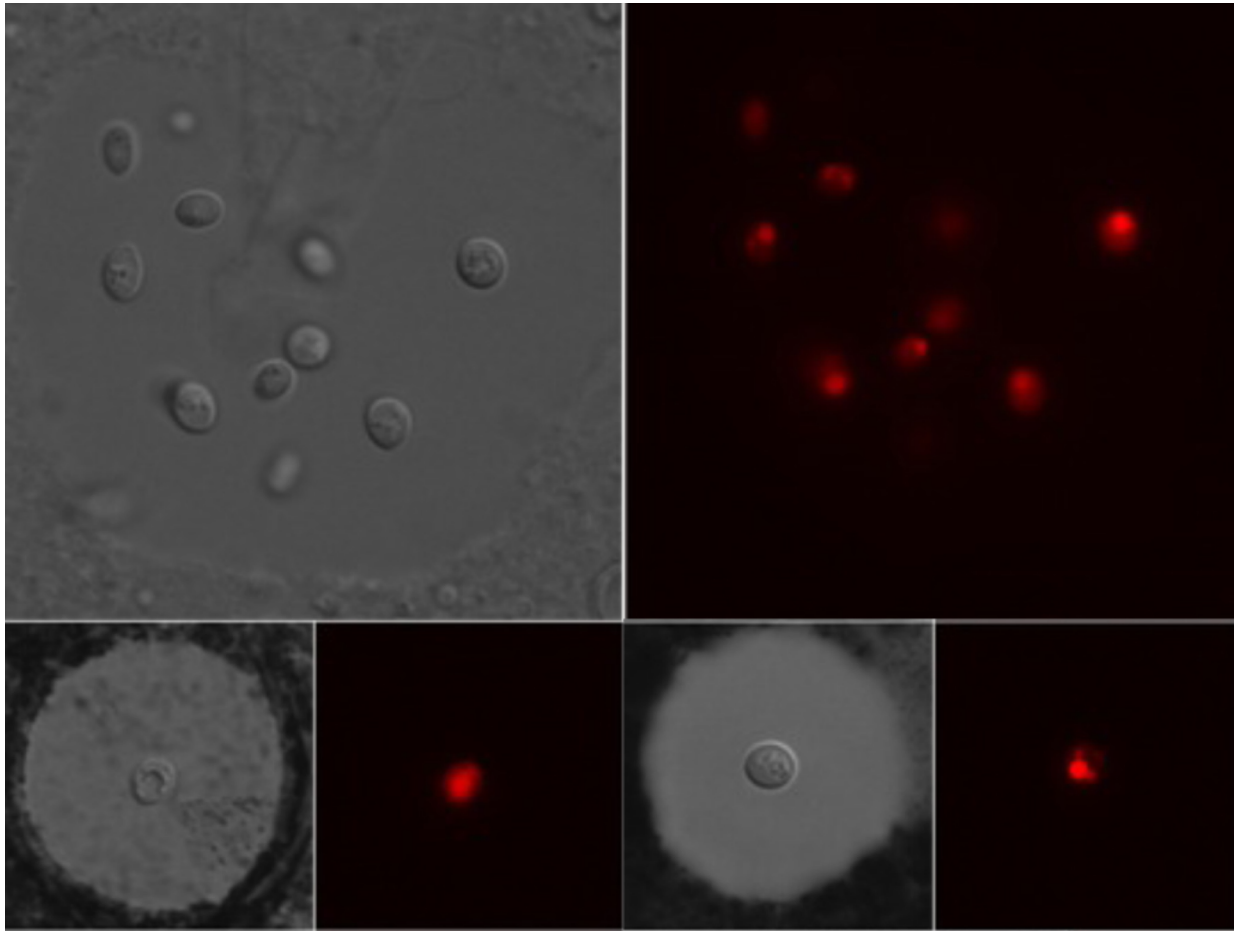
Figure S4. Functional network map of genes regulated by *ALL2*.

References

1. **Fries BC, Taborda CP, Serfass E, Casadevall A.** 2001. Phenotypic switching of Cryptococcus neoformans occurs in vivo and influences the outcome of infection. J Clin Invest **108:**1639-1648.
2. **Perfect JR, Lang SD, Durack DT.** 1980. Chronic cryptococcal meningitis: a new experimental model in rabbits. Am J Pathol **101:**177-194.
3. **Jain N, Li L, Hsueh YP, Guerrero A, Heitman J, Goldman DL, Fries BC.** 2009. Loss of allergen 1 confers a hypervirulent phenotype that resembles mucoid switch variants of Cryptococcus neoformans. Infect Immun **77:**128-140.