Supplementary materials

Table S1: Strains used in this study.

Strain	Function	*Species*	Gene	Background	Selective marker	Source
H99O	Wild-type	*C. neoformans*		Clinical		(Janbon *et al.*, 2014)
CJS24	*hfi1*Δ	*C. neoformans*	*HFI1*	H99O	*NEO*	This study
KY06	*HFI1* complementation	*C. neoformans*	*HFI1*	CJS24	*NAT*	This study
Mach1	Cloning	*E. coli*		Mach1	*bla*	Thermo Scientific

Table S2: Primers used in this study.

Fragment / Gene	ID	Sequence
HFI1 – 5' flanking region	UQ5699	5'- CTATAGGGCGAATTGGGTACCCTTTGCTAACTGCCGTGCTGA -3'
	UQ5700	5'- TCCAGCTCACATCCTCGCAGCTAGAAGTGTGGAAAAAGGCCG -3'
HFI1 – 3' flanking region	UQ5702	5'- CCGTGTTAATACAGATAAACCGGAGGTGTTTGGTTGGTGTAG -3'
	UQ5704	5'- CCGCTCTAGAACTAGTGGATCACCTCTGGAGATGGAGATAGG -3'
HFI1 H99O gene overhang with pSDMA25	UQ5999	5'- GGGTACCGGGCCCCCCCTCGACTTTGCTAACTGCCGTGCTGA -3'
	UQ6000	5'- GTGGCGGCCGCTCTAGAACTAGACCTCTGGAGATGGAGATAGG -3'
HFI1 – NEO deletion plasmid sequencing	UQ6001	5'- GAGCATCGATGTCCTTCAAAC-3'
	UQ6002	5'- CTTCCAACCTACATCAAATAG-3'
	UQ6003	5'- CAGCAGCAGCGGAAAAGGATAT -3'
	UQ6004	5'- TCCACAGGCTAATGACAGCAC -3'
	UQ6005	5'- GTACGGTTATAATCTCACAGC -3'
HFI1 in pSDMA25 sequencing	UQ6001	5'- GAGCATCGATGTCCTTCAAAC -3'
	UQ6003	5'- CAGCAGCAGCGGAAAAGGATAT -3'
	UQ5746	5'- AAATATTAACGCTTACAATTT -3'
	UQ5747	5'- CGACTGGAAAGCGGGCAGTGA -3'
	UQ6002	5'- CTTCCAACCTACATCAAATAG -3'
	UQ6004	5' TCCACAGGCTAATGACAGCAC -3'
	UQ6005	5'- GTACGGTTATAATCTCACAGC -3'
HFI1 RT-PCR	UQ6258	5'- GTTAAAAGGCGATCATTTACACC -3'
	UQ6259	5'- CGTTGTTGGAGGGAGGA -3'
ADA2 RT-PCR	UQ6260	5'- CAGAGTATCGGGCTATACAAGC -3'
	UQ6261	5'- CTTCGGCAGTTTGCAACTTT -3'
NGG1 RT-PCR	UQ6300	5'- AGTGGATGTGGTTGATTTGG -3'
	UQ6301	5'- TTATTCGGGTCAAACTCTTCG -3'
CHD1 RT-PCR	UQ6262	5'- CTTGCCGATGAAATGGGTCTC -3'
	UQ6263	5'- GAGGGACAACAACAAGGAAAGG -3'
GCN5 RT-PCR	UQ6264	5'- CCGAACAAATCAAGGGTTACG -3'
	UQ6265	5'- TGTCCGCATAAGTCAGAAAGA -3'
SGF11 RT-PCR	UQ6202	5'- ATCAACTGGTGTGGGTAGTG -3'
	UQ6203	5'- ATATCGGTTGGAGGCAATAGG -3'
SGF29 RT-PCR	UQ6266	5'- CAAGTGGCGATGATTGGATTC -3'
	UQ6267	5'- GGGTAGTGTTGTATGTGTTGC -3'
SGF73 RT-PCR	UQ6268	5'- CCGGAAATGAAGAGGGAAGG -3'
	UQ6269	5'- CATGATTACACACCCGTCGAT -3'
SPT3 RT-PCR	UQ6270	5'- GGCACAGGCCCAAGTAAA -3'
	UQ6271	5'- CTCCAAACACAAACATCATCTGC -3'
SPT7 RT-PCR	UQ6302	5'- GTAGATCATCTGGATGGGTTGG -3'
	UQ6303	5'- GGCATGTAGAATGATCTCCTCG -3'
SPT8 RT-PCR	UQ6272	5'- GATGGAACCGTCAGAGAATGG -3'
	UQ6273	5'- GGAAGAGAGTTGGGCTTTGT -3'
SPT20 RT-PCR	UQ6274	5'- GATGGTCCAATGAAGCCATTCC -3'
	UQ6275	5'- GATGGGTGGTTGGATGTCATAAAG -3'
SUS1 RT-PCR	UQ6276	5'- GTTGATGTTACGGTCGATGAG -3'
	UQ6277	5'- AGTAGCTTTTGTATTCGCTCC -3'

TAF5 RT-PCR	UQ6278	5′- CGATGGTGGATGTCAGTGAA -3′
	UQ6279	5′- AGATTATCCAGTTCAGCCTTCC -3′
TAF6 RT-PCR	UQ6280	5′- CGAGTATTGGGCATTTGGTAGA -3′
	UQ6281	5′- GGAGGAGTTGATGCATGTATGG -3′
TAF9 RT-PCR	UQ6222	5′- GAAGCTCCTCCAAGAGATTACC -3′
	UQ6223	5′- GTAGACGGACGAGGTCAAATG -3′
TAF10 RT-PCR	UQ6282	5′- GGTACAATCGTCAATGTGTCC -3′
	UQ6283	5′- ATTAGGTGTGTAGGGTTCATCT -3′
TAF12 RT-PCR	UQ6304	5′- GGCTTTCCGCCAAAGTACC -3′
	UQ6305	5′- GGTTCTGATGTTGGGCGTTATT -3′
TRA1 RT-PCR	UQ6284	5′- TTCTTTCTGTCGGGATTCTCG -3′
	UQ6285	5′- CGCCTTGACGTGTGTAAGATA -3′
UBP8 RT-PCR	UQ6286	5′- GACAAGTTGTCGTGGTCTGC -3′
	UQ6287	5′- TGCTTTGAGAAGCGGATTATGG -3′
ACT1 RT-PCR	UQ482	5′- CCTACAACTCTATCATGAAGTGTGATCTC -3′
	UQ728	5′- TCTGCATACGGTCGGCAATAC -3′
TUB2 RT-PCR	UQ484	5′- AGTCGCTTTTCAAGCGTATCG -3′
	UQ729	5′- GGATTCGGCTTCAGAGAATTCA -3′
GPD1 RT-PCR	UQ486	5′- GTCTCTACTGATTTCGTTGGCACTAC -3′
	UQ730	5′- GTAACCGTACTCATTGTCATACCAGCTA -3'
HHT1 RT-PCR	UQ488	5′- GAAATCCGACGATACCAGAAGTCTAC -3′F
	UQ731	5′- GGAATCGAAGGTCGGTCTTG -3′
*sgf29*Δ testing	UQ3574	5′- CTGTAGAGCTCCTCGAAATAC -3′
	UQ3575	5′- CCATACCTAGGCCATCCATAC -3′

Table S3: Plasmids used in this study.

Strain	Function	Plasmid backbone	Marker	Selective marker	Source
pJAF1	*NEO* marker	pBluescript SK(-)	*NEO*	*bla*	Fraser *et al.* (2003)
pSDMA25	Safe Haven 1	pBluescript SK(-)	*NAT*	*bla*	Arras *et al.* (2015)
pCJS48	*hfi1*Δ	pBluescript SK(-)	*NEO*	*bla*	This study
pKY04	*HFI1* complementation	pSDMA25	*NAT*	*bla*	This study

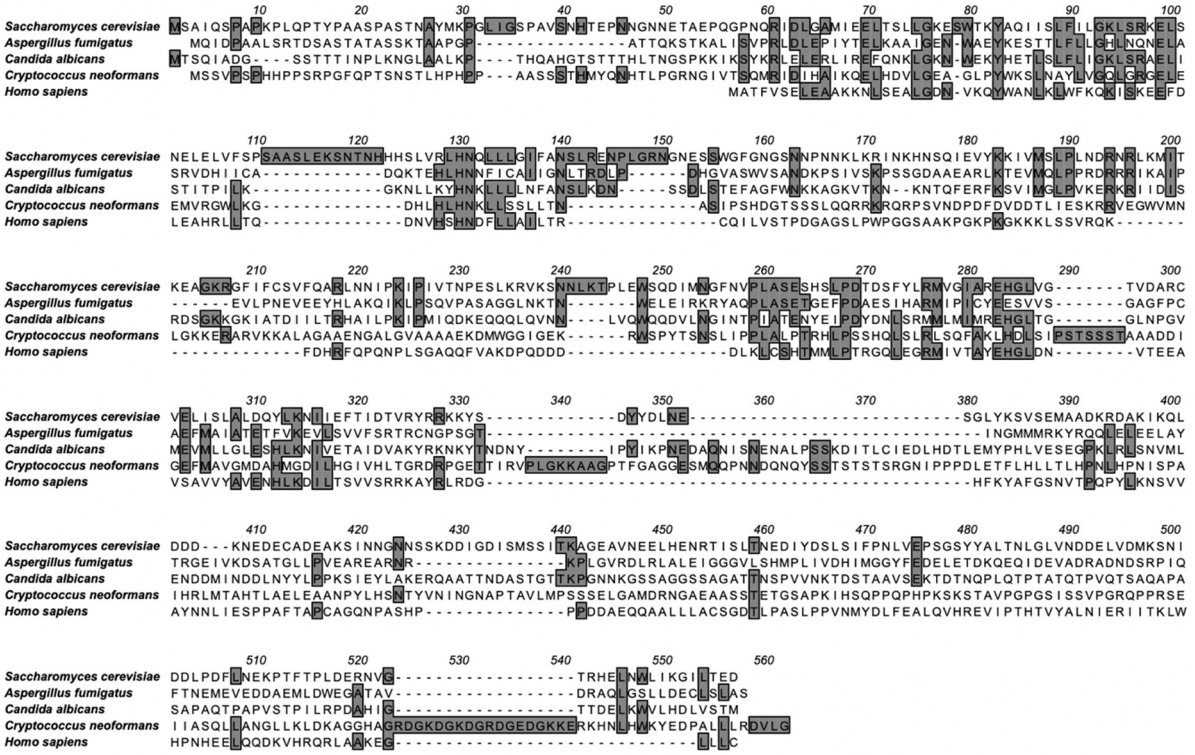

Figure S1: Protein alignments of Hfi1 from *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans* and *Homo sapiens*. Numbering for is based on the *C. neoformans* sequence.

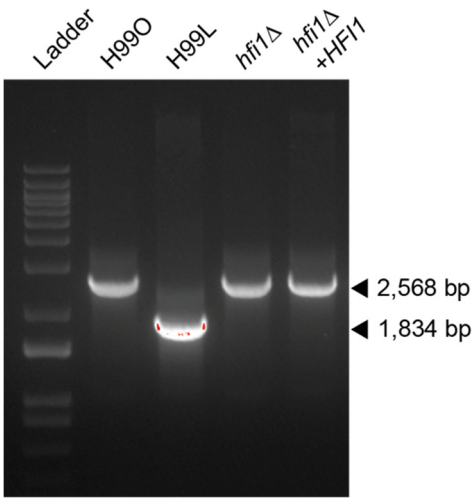

Figure S2: H99L *sgf29*Δ allele background verification. PCR using primers UQ3574 and UQ3575 amplifies a 2568 bp *SGF29* fragment from wild-type (H99O, lane 2) and a 1834 bp band from strains bearing the H99L *sgf29*Δ allele (H99L, lane 3) easily visible on a 1% agarose TAE gel. Lane 1, Invitrogen 1 Kb Plus DNA Ladder. Lane 3 and Lane 4, a 2568 bp fragment from the mutant and complemented strains.

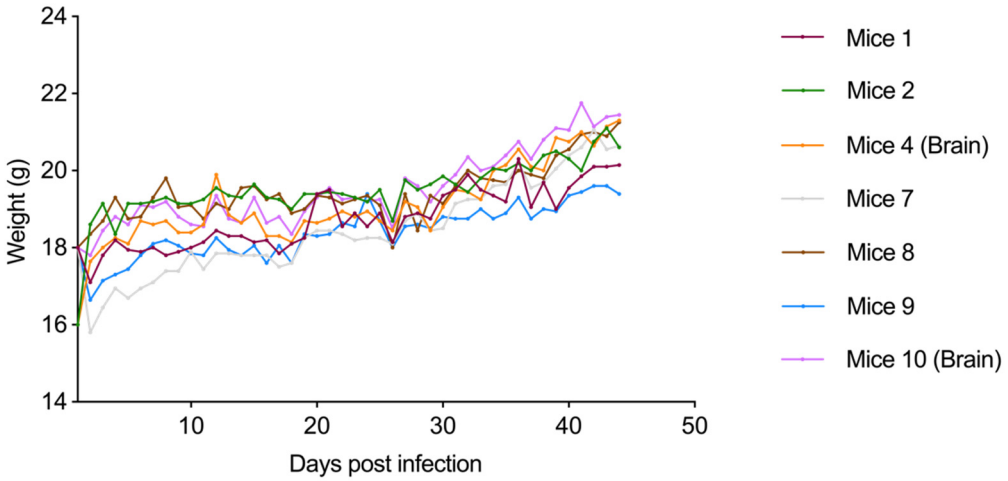

Figure S3: Changing of weight for seven mice during the experiment. Mice 1, 2, 7, 8, 9 had colonies identified in the lung. Mice 4 and 10 had colonies identified in both lung and brain.