

Functional analysis of Hif1 histone chaperone in *Saccharomyces cerevisiae*

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Supplementary Data

Supplementary table S1: Yeast strains used in this work

Supplementary table S2: Primers used in this work

Supplementary table S3: Accession number of the sequences used in this study

Supplementary figure legends

Supplementary figure 1: A- Structural comparison of fungal and human sNASP homologs. Acidic region which interrupts TPR2 is indicated as a groove. **B-E:** TPR sequence alignment of predicted TPR1 to TPR4 (B-E, respectively), across various fungal lineages. TPR2 and TPR4 insertions are indicated as gaps and are highlighted with red arrows. **F:** Sequence alignment of acidic region which interrupts TPR2 from representative fungal lineages. **G:** Sequence alignment of TPR4 interruption region from representative fungal lineages. Note: Residues are colored according to Clustal X coding system (1).

Supplementary figure 2: Sequence alignment of C-terminal amino acids of the protein encompassing the putative nuclear localization signal from representative fungal lineages. Residues are colored according to ClustalX coding system (1).

Supplementary figure 3: Schematic representation of Hif1 deletion mutants. **A:** Hif1 internal deletions lacking different TPR regions or acidic region and C-terminal external deletions are depicted. Acidic region which interrupts TPR2 is indicated as a groove. Note: The positions of deletions on the Hif1 are provided in the table as figure **A.1**.

Supplementary figure 4: A: Indirect immunofluorescence analysis of 12MYC tagged full length Hif1 and truncated mutants carrying C-terminal external deletions. **B:** IF analysis of 12MYC tagged full length Hif1 and truncated mutants carrying internal deletions. Top panels were stained with DAPI to capture the nuclei orientation. Middle panels were probed with anti-MYC antibody to examine the localization of either full length Hif1 (F.L) or truncated

mutants. Cells transfected with an empty vector were used as a control. The bottom panel represents merge of DAPI and anti-MYC staining. Red arrows represent the position of nuclei.

Supplementary figure 5: Analysis of *hif1Δ hat2Δ* double knockout cells for growth defects on YPD media. (A) Strains were grown to an OD at 600nm of $\cong 0.5$ before being plated at five-fold serial dilutions on YPD. (B) Strains were cloned into pRB4151-2MYC lacking the Leucine amino acid (-Leu) for selectivity. Hif1 F.L. was transformed into *hif1Δ/hat2Δ* and *hif1Δ* to rescue the phenotype. (C, D): Various Hif1 truncations were expressed back into *hif1Δ hat2Δ* double knockout cells to examine their ability to rescue the phenotype. Note: C1 and D1 represent 3 days of growth. (E) Western blot analysis of whole cell extracts to examine the expression of Hif1 truncation mutants in *hif1Δ hat2Δ* double knockout cells.

Supplementary figure 6: Sensitivity of *hif1Δ*, *hat1Δ*, *hat2Δ* and *lsm1Δ* cells to genotoxic agent hydroxyurea. **A-D:** Ten-fold serial dilutions of strains WT, *hif1Δ*, *hat1Δ*, *hat2Δ*, *lsm1Δ* and *spt2Δ* were spotted on YPD or YPD medium containing the indicated dose of genotoxic agent hydroxyurea (HU).

Supplementary figure 7: **A:** Network representation of protein-protein and genetic interaction data of Hif1. Nodes and edges represent genes/proteins and interactions between them, respectively. Edges connecting genetic interactions are shown in green whereas physical interactions are depicted in pink. The width of edges represents the confidence for a given functional link. The width of the node is proportional to the number of functional links in the network. The Hif1 node is represented in black, **B:** Network representation of protein-protein and genetic interaction data of Hif1, Hat1, Hat2 and Asf1 (depicted in blue). Nodes and edges represent genes/proteins and interactions between them, respectively. **C:** Venn diagram of shared nodes among Hif1, Hat1, Hat2 and Asf1.

Supplementary citation

1. Larkin,M.A., Blackshields,G., Brown,N.P., Chenna,R., McGettigan,P.A., McWilliam,H., Valentin,F., Wallace,I.M., Wilm,A., Lopez,R., *et al.* (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947–8.

Table S1

#	Code	Strain Name	Marker	Strain Version	Source
1	JF087	pRB415ADH1-12MYC+ Δ(+TPR3Domain)	Amp	Bacterial strain	This study
2	JF086	pRB415ADH1-12MYC+ Δ(+TPR4Domain)	Amp	Bacterial strain	This study
3	JF076	pRB415ADH1-12MYC+ Δ(+TPR4)	Amp	Bacterial strain	This study
4	JF080	pRB415ADH1-12MYC+ Δ(+TPR4 +35)	Amp	Bacterial strain	This study
5	JF077	pRB415ADH1-12MYC+ ΔB.P.(Basic Patch)	Amp	Bacterial strain	This study
6	JF078	pRB415ADH1-12MYC+ Full Length F.L.	Amp	Bacterial strain	This study
7	JF079	pRB415ADH1-12MYC+ ΔTPR1	Amp	Bacterial strain	This study
8	JF082	pRB415ADH1-12MYC+ ΔTPR2- 1 st	Amp	Bacterial strain	This study
9	JF084	pRB415ADH1-12MYC+ ΔACD (Acidic region)	Amp	Bacterial strain	This study
10	JF088	pRB415ADH1-12MYC+ ΔTPR2 Entire	Amp	Bacterial strain	This study
11	JF083	pRB415ADH1-12MYC+ ΔTPR3	Amp	Bacterial strain	This study
12	JF085	pRB415ADH1-12MYC+ ΔTPR4	Amp	Bacterial strain	This study
13	JF200	W303+ plasmid	-LEU	Yeast strain	This study
14	JF201	Hat1-TAP <i>Δhif1+</i> plasmid	-LEU	Yeast strain	This study
15	JF202	12MYC-Hif1(F.L.)	-LEU	Yeast strain	This study
16	JF243	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR3Domain)	-LEU	Yeast strain	This study
17	JF242	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4Domain)	-LEU	Yeast strain	This study
18	JF203	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4)	-LEU	Yeast strain	This study
19	JF204	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4 +35)	-LEU	Yeast strain	This study
20	JF206	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔB.P.	-LEU	Yeast strain	This study
21	JF205	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 +Full Length F.L.	-LEU	Yeast strain	This study
22	JF207	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR1	-LEU	Yeast strain	This study
23	JF208	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR2- 1 st	-LEU	Yeast strain	This study
24	JF209	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔACD	-LEU	Yeast strain	This study
25	JF241	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR2 Entire	-LEU	Yeast strain	This study
26	JF210	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR3	-LEU	Yeast strain	This study
27	JF211	Hat1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR4	-LEU	Yeast strain	This study
28	JF167	pYES+ WT	-URA	Yeast strain	This study
29	JF169	pYES+ <i>hif1Δ::KAN</i>	-URA	Yeast strain	This study
30	JF175	pYES+ <i>halΔ::KAN</i>	-URA	Yeast strain	This study

31	JF177	pYES+ <i>hat2Δ::KAN</i>	-URA	Yeast strain	This study
32	JF257	pYES+ <i>spt2Δ::KAN</i>	-URA	Yeast strain	This study
33	JF171	pYES+ <i>lsm1Δ</i>	-URA	Yeast strain	This study
34	JF168	pYES+ H3+ WT	-URA	Yeast strain	This study
35	JF170	pYES+ H3+ <i>hif1Δ::KAN</i>	-URA	Yeast strain	This study
36	JF176	pYES+ H3+ <i>ha1Δ::KAN</i>	-URA	Yeast strain	This study
37	JF178	pYES+ H3+ <i>hat2Δ::KAN</i>	-URA	Yeast strain	This study
38	JF258	pYES+ H3+ <i>spt2Δ::KAN</i>	-URA	Yeast strain	This study
39	JF172	pYES+ H3+ <i>lsm1Δ::KAN</i>	-URA	Yeast strain	This study
40	JF248	pYES+ H3+ 12MYC-Hif1 Δ(+TPR3Domain)	-LEU -URA	Yeast strain	This study
41	JF247	pYES+ H3+ 12MYC-Hif1 Δ(+TPR4Domain)	-LEU -URA	Yeast strain	This study
42	JF246	pYES+ H3+ 12MYC-Hif1 Δ(+TPR4)	-LEU -URA	Yeast strain	This study
43	JF245	pYES+ H3+ 12MYC-Hif1 Δ(+TPR4 +35)	-LEU -URA	Yeast strain	This study
44	JF244	pYES+ H3+ 12MYC-Hif1 ΔB.P.	-LEU -URA	Yeast strain	This study
45	JF250	pYES+ H3+ 12MYC-Hif1 +Full Length (F.L.)	-LEU -URA	Yeast strain	This study
46	JF251	pYES+ H3+ 12MYC-Hif1 ΔTPR1	-LEU -URA	Yeast strain	This study
47	JF252	pYES+ H3+ 12MYC-Hif1 ΔTPR2- 1 st	-LEU -URA	Yeast strain	This study
48	JF253	pYES+ H3+ 12MYC-Hif1 ΔACD	-LEU -URA	Yeast strain	This study
49	JF254	pYES+ H3+ 12MYC-Hif1 ΔTPR2 Entire	-LEU -URA	Yeast strain	This study
50	JF255	pYES+ H3+ 12MYC-Hif1 ΔTPR3	-LEU -URA	Yeast strain	This study
51	JF256	pYES+ H3+ 12MYC-Hif1 ΔTPR4	-LEU -URA	Yeast strain	This study
52	JF249	pYES+ H3+ 12MYC-Δ <i>hif1</i>	-LEU -URA	Yeast strain	This study
53	JF110	Spt2-TAP+ Hif1-13MYC	-	Yeast strain	This study
54	JF102	Spt2-TAP+ Hat1-13MYC	-	Yeast strain	This study
55	JF111	Spt2-TAP+ Hat2-13MYC	-	Yeast strain	This study
56	JF300	<i>hif1Δ::NAT</i> + plasmid	-LEU	Yeast strain	This study
57	JF301	<i>hif1Δ::NAT</i> + 12MYC-Hif1(F.L.)	-LEU	Yeast strain	This study
58	JF306	<i>hif1Δ::NAT</i> + 12MYC-Hif1 Δ(+TPR3Domain)	-LEU	Yeast strain	This study
59	JF305	<i>hif1Δ::NAT</i> + 12MYC-Hif1 Δ(+TPR4Domain)	-LEU	Yeast strain	This study
60	JF304	<i>hif1Δ::NAT</i> + 12MYC-Hif1 Δ(+TPR4)	-LEU	Yeast strain	This study
61	JF303	<i>hif1Δ::NAT</i> + 12MYC-Hif1 Δ(+TPR4 +35)	-LEU	Yeast strain	This study

62	JF302	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔB.P.</i>	-LEU	Yeast strain	This study
63	JF307	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔTPR1</i>	-LEU	Yeast strain	This study
64	JF308	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔTPR2- 1st</i>	-LEU	Yeast strain	This study
65	JF309	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔACD</i>	-LEU	Yeast strain	This study
66	JF310	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔTPR2 Entire</i>	-LEU	Yeast strain	This study
67	JF311	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔTPR3</i>	-LEU	Yeast strain	This study
68	JF312	<i>hif1Δ::NAT+ 12MYC-Hif1 ΔTPR4</i>	-LEU	Yeast strain	This study
69	JF313	Hat2-TAP <i>Δhif1+</i> plasmid	-LEU	Yeast strain	This study
70	JF314	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1(F.L.)	-LEU	Yeast strain	This study
71	JF319	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR3Domain)	-LEU	Yeast strain	This study
72	JF318	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4Domain)	-LEU	Yeast strain	This study
73	JF317	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4)	-LEU	Yeast strain	This study
74	JF316	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4 +35)	-LEU	Yeast strain	This study
75	JF315	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔB.P.	-LEU	Yeast strain	This study
76	JF320	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR1	-LEU	Yeast strain	This study
77	JF321	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR2- 1 st	-LEU	Yeast strain	This study
78	JF322	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔACD	-LEU	Yeast strain	This study
79	JF323	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR2 Entire	-LEU	Yeast strain	This study
80	JF324	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR3	-LEU	Yeast strain	This study
81	JF325	Hat2-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR4	-LEU	Yeast strain	This study
82	JF326	Asf1-TAP <i>Δhif1+</i> plasmid	-LEU	Yeast strain	This study
83	JF327	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1(F.L.)	-LEU	Yeast strain	This study
84	JF332	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR3Domain)	-LEU	Yeast strain	This study
85	JF331	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4Domain)	-LEU	Yeast strain	This study
86	JF330	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4)	-LEU	Yeast strain	This study
87	JF329	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 Δ(+TPR4 +35)	-LEU	Yeast strain	This study
88	JF328	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔB.P.	-LEU	Yeast strain	This study
89	JF333	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR1	-LEU	Yeast strain	This study
90	JF334	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR2- 1 st	-LEU	Yeast strain	This study
91	JF335	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔACD	-LEU	Yeast strain	This study
92	JF336	Asf1-TAP <i>Δhif1+</i> 12MYC-Hif1 ΔTPR2 Entire	-LEU	Yeast strain	This study

93	JF337	Asf1-TAP <i>Δhif1</i> + 12MYC-Hif1 ΔTPR3	-LEU	Yeast strain	This study
94	JF338	Asf1-TAP <i>Δhif1</i> + 12MYC-Hif1 ΔTPR4	-LEU	Yeast strain	This study
95	JF339	<i>hif1Δ hat1Δ</i>	-	Yeast strain	This study
96	JF340	<i>hif1Δ hat2Δ</i>	-	Yeast strain	This study
97	JF341	<i>hif1Δ</i> + plasmid	-LEU	Yeast strain	This study
98	JF342	<i>hat1Δ</i> + plasmid	-LEU	Yeast strain	This study
99	JF343	<i>Hat2Δ</i> + plasmid	-LEU	Yeast strain	This study
100	JF344	<i>hif1Δ hat2Δ</i> + plasmid	-LEU	Yeast strain	This study
101	JF345	<i>hif1Δ hat2Δ</i> + Hif1(F.L.)	-LEU	Yeast strain	This study
102	JF346	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔB.P.	-LEU	Yeast strain	This study
103	JF347	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔTPR1	-LEU	Yeast strain	This study
104	JF348	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔTPR2- 1 st	-LEU	Yeast strain	This study
105	JF349	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔACD	-LEU	Yeast strain	This study
106	JF350	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔTPR2 Entire	-LEU	Yeast strain	This study
107	JF351	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔTPR3	-LEU	Yeast strain	This study
108	JF352	<i>hif1Δ hat2Δ</i> + 12MYC-Hif1 ΔTPR4	-LEU	Yeast strain	This study

Table S2

A: DNA sequencing primers

Sequencing primers	Sequence
HJ559	5'-cggtgtaaaacgacggccag-3'
AJJ296	5'-tggactgaagtttagccaattc-3'
AJL320	5'-cagatcatttcaaagtaat-3'

B: PCR primers

PCR primers	Sequence
HIF1F(BAMH1)	5'-CCCGGATCCatgaaactaaggcagaagac-3'
HIF1F(dTPR1)	5'-ggttgtgcctgatcctgaa-3'
HIF1R(dTPR1)	5'-aggatcaggcaacaaccatcaattgaactttatg-3'
HIF1F(dACD)	5'-cttcgcaagtctggttccac-3'
HIF1R(dACD)	5'-gtaaatgtggaaaccagactgcaagccccaaacagattaccaga-3'
HIF1F(dTPR2a)	5'-gacgcttctggcaggt-3'
HIF1R(dTPR2a)	5'-acctgccagaagagcgtcatcaggcaacaaccatc-3'
HIF1F(dTPR2entire)	5'-ttggacacctggtcggcag-3'
HIF1R(dTPR2entire)	5'-gtccgccagtaggtccaaatcaggcaacaaccatc-3'
HIF1F(dTPR3)	5'-aaacccgcagaacaaga-3'
HIF1R(dTPR3)	5'-tacttgttctgcgggtttagtgcgcaatctgctgtt-3'
HIF1F(dTPR4)	5'-atgactacaagacccaag-3'
HIF1R(dTPR4)	5'-cttgggttctgttgtcatcacccctcagttacttg-3'
HIF1R(+TPR3Domain)	5'-CCCCTGCAG tc agatgcgcaatctgttctc-3'
HIF1R(+TPR4Domain)	5'-CCCCTGCAG tc acacccctcagttactgttc-3'
HIF1R(+TPR4-PSTI)	5'-CCCCTGCAG tc agtgtttcaagcagacgcctt-3'
HIF1R(+TRP4+35-PSTI)	5'-CCCCTGCAG tc accccttggagccgtgt-3'
HIF1R(-BASIC-PSTI)	5'-CCCCTGCAG tc agaccaggatggagagatcatt-3'
HIF1R(PSTI)	5'-CCCCTGCAG tc aatgccttcaggcttctt-3'
yHIF1F	5'-aaggacagcgagttacagcaggcaa-3'
yHIF1R	5'-gctagtgttcttgctcattatgaa-3'

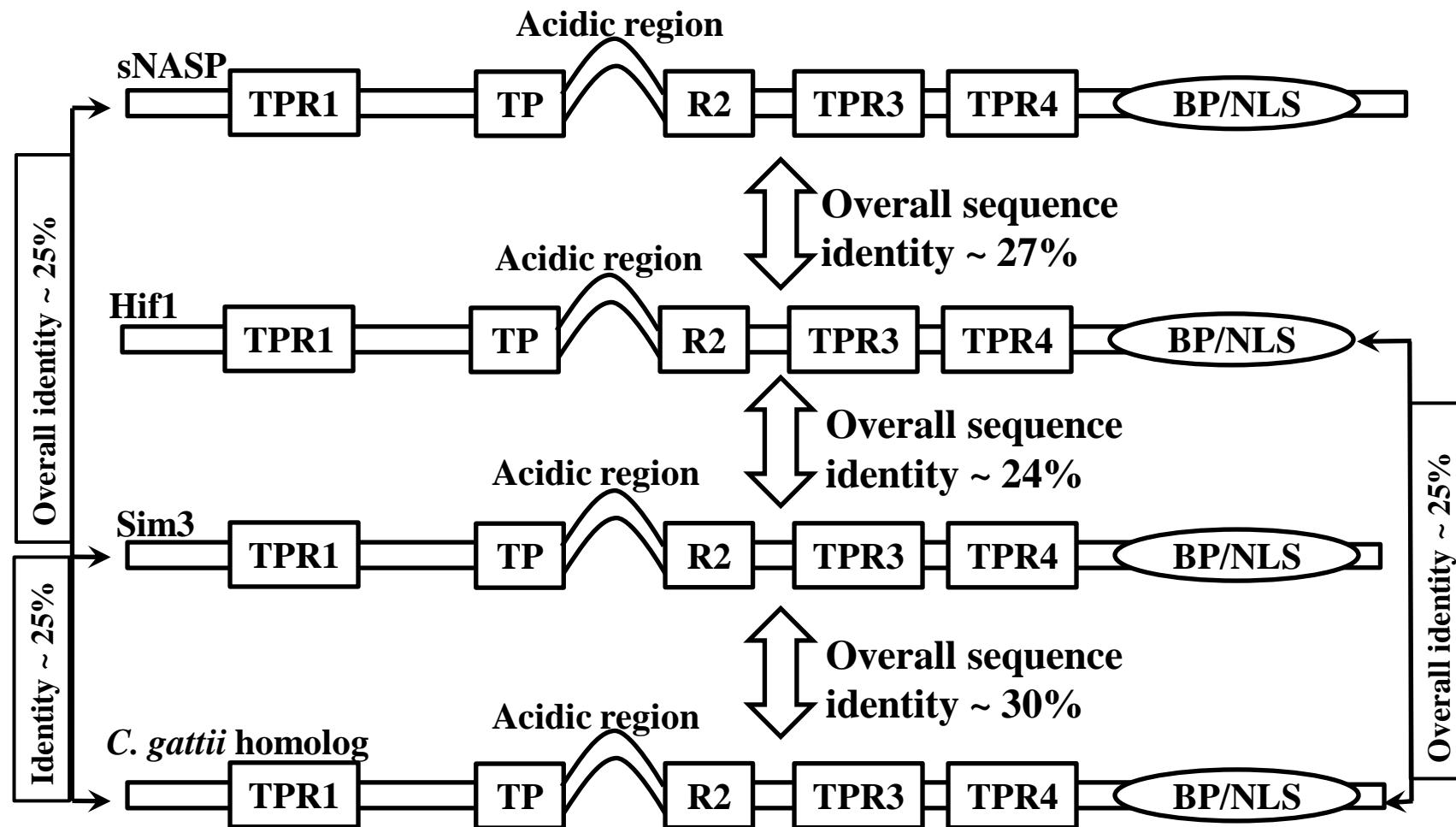
C: PCR primers- deletion

PCR primers	Sequence
HIF1F	5'-cggcagtggaaatcttaccacttctcag-3'
HIF1R	5'-gtagtaagtatgtcattcaggatg-3'
HIF1conF	5'-acttgcagaaggactcgtagtccc-3'
NATF	5'-acatggaggcccagaataccct-3'
NATR	5'-cagtagatgcgaccaggattca-3'

Supplementary table S3

Accession (UniProt KB)	Fungal lineage
M7PAE8	<i>Pneumocystis murina</i>
B6K743	<i>Schizosaccharomyces japonicus</i>
S9VTK9	<i>Schizosaccharomyces cryophilus</i>
Q9USQ4	<i>Schizosaccharomyces pombe</i>
S9Q1K3	<i>Schizosaccharomyces octosporus</i>
Q12373	<i>Saccharomyces cerevisiae</i>
K0KPL0	<i>Wickerhamomyces ciferrii</i>
Q6CS41	<i>Kluyveromyces lactis</i>
W0T796	<i>Kluyveromyces marxianus</i>
W0W828	<i>Zygosaccharomyces bailii</i>
C5DQJ4	<i>Zygosaccharomyces rouxii</i>
G8BZL2	<i>Tetrapisispora phaffii</i>
Q754F8	<i>Ashbya gossypii</i>
A5DWT8	<i>Lodderomyces elongisporus</i>
Q6BWS5	<i>Debaryomyces hansenii</i>
Q6C817	<i>Yarrowia lipolytica</i>
G8B7Q7	<i>Candida parapsilosis</i>
Q59RB9	<i>Candida albicans</i>
J3K3P4	<i>Coccidioides immitis</i>
B6Q370	<i>Penicillium marneffei</i>
A0A017SC82	<i>Aspergillus ruber</i>
W7ECX2	<i>Bipolaris victoriae</i>
W6ZKF9	<i>Bipolaris oryzae</i>
A0A014QWQ9	<i>Metarhizium robertsii</i>
E9F7L3	<i>Metarhizium anisopliae</i>
W9CAV8	<i>Sclerotinia borealis</i>
G2XVN4	<i>Botryotinia fuckeliana</i>
U4LBR5	<i>Pyronema omphalodes</i>
D5GBR5	<i>Tuber melanosporum</i>
S8A4G4	<i>Dactyellina haptotyla</i>
F4NW57	<i>Batrachochytrium dendrobatidis</i>
I1BKA7	<i>Rhizopus delemar</i>
S2JYJ6	<i>Mucor circinelloides f. circinelloides</i>
A0A015LBY5	<i>Rhizophagus irregularis</i>
J9VLV7	<i>Cryptococcus neoformans var. grubii</i>
J8TYB0	<i>Trichosporon asahii var. asahii</i>
D5KY45	<i>Tremella fuciformis</i>
E6QYX1	<i>Cryptococcus gattii</i>
M7XBY6	<i>Rhodosporidium toruloides</i>
K5Y1H8	<i>Agaricus bisporus var. burnettii</i>
B0CU79	<i>Laccaria bicolor</i>
D6RNQ5	<i>Coprinopsis cinerea</i>
I2G0Q0	<i>Ustilago hordei</i>
Q4PBI3	<i>Ustilago maydis</i>
U5H4M9	<i>Microbotryum violaceum</i>

Supplementary Figure 1-A

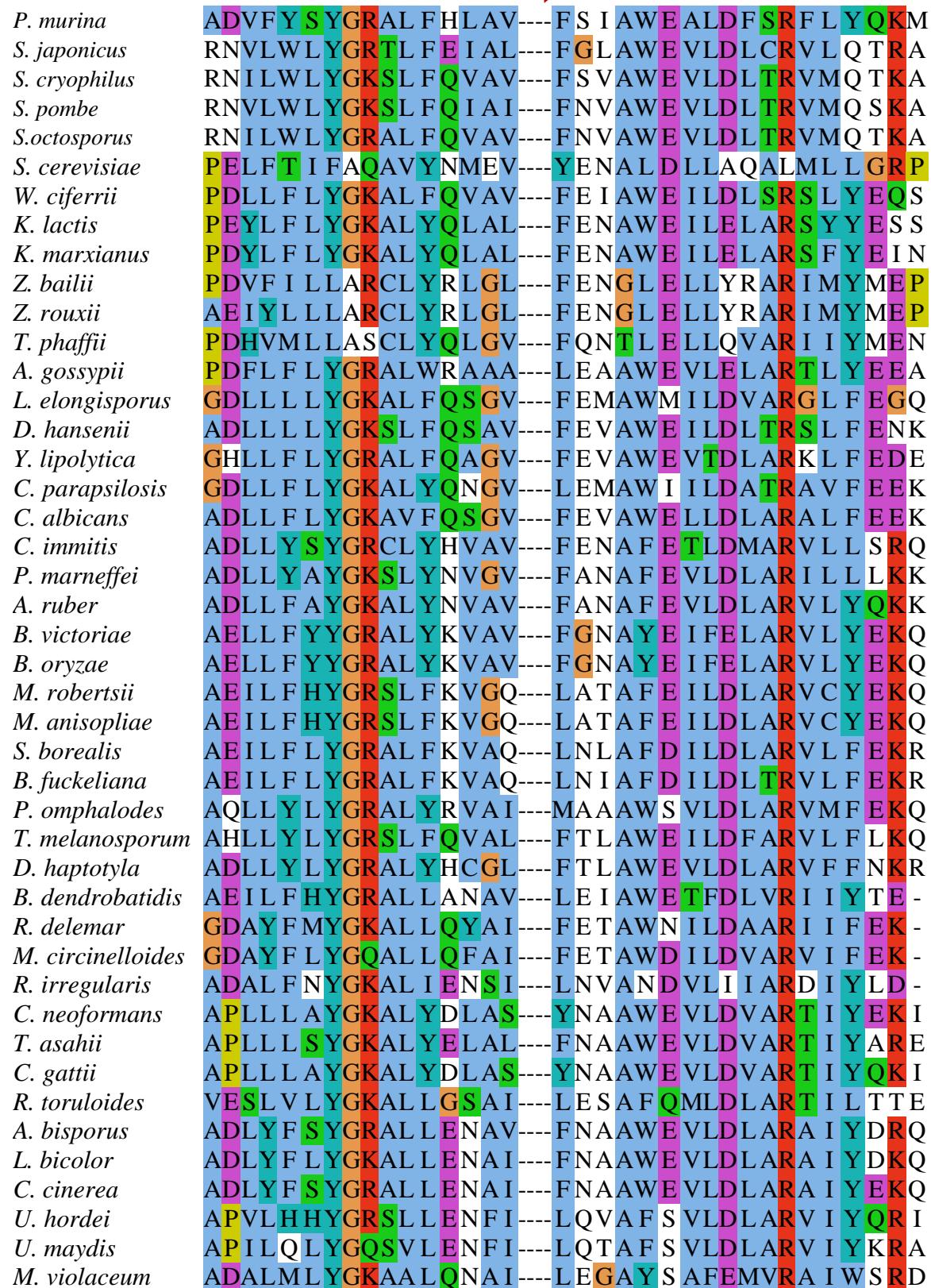


Supplementary Figure 1-B

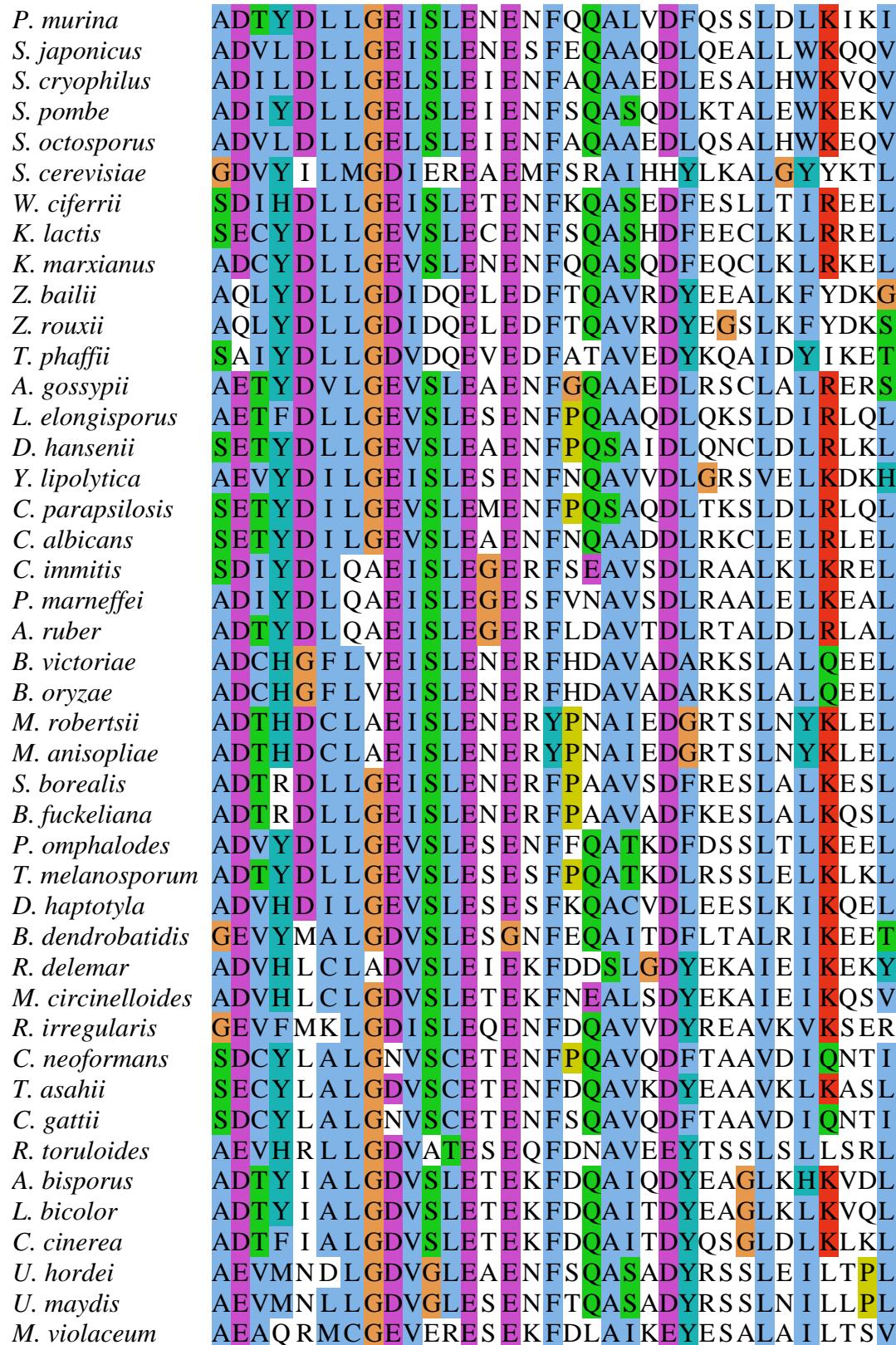
Pneumocystis murina
Schizosaccharomyces japonicus
Schizosaccharomyces cryophilus
Schizosaccharomyces pombe
Schizosaccharomyces octosporus
Saccharomyces cerevisiae
Wickerhamomyces ciferrii
Kluyveromyces lactis
Kluyveromyces marxianus
Zygosaccharomyces bailii
Zygosaccharomyces rouxii
Tetrapisispora phaffii
Ashbya gossypii
Lodderomyces elongisporus
Debaryomyces hansenii
Yarrowia lipolytica
Candida parapsilosis
Candida albicans
Coccidioides immitis
Penicillium marneffei
Aspergillus ruber
Bipolaris victoriae
Bipolaris oryzae
Metarhizium robertsii
Metarhizium anisopliae
Sclerotinia borealis
Botryotinia fuckeliana
Pyronema omphalodes
Tuber melanosporum
Dactylellina haptotyla
Batrachochytrium dendrobatidis
Rhizopus delemar
Mucor circinelloides
Rhizophagus irregularis
Cryptococcus neoformans
Trichosporon asahii
Cryptococcus gattii
Rhodosporidium toruloides
Agaricus bisporus
Laccaria bicolor
Coprinopsis cinerea
Ustilago hordei
Ustilago maydis
Microbotryum violaceum

AYSVVNEADR LYKKDYEKAVEKYSLALER I VRE
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IEQLVTQGNMAYAQKNYEAVDKYQGQALMQSES
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VQTLLIEGA KYSASDDAEKA AKCYARILDLES--
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VAKLISEGS RAYS SKDFDLASEEKYGEACEEYSKS
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IPTL LAAGAQAYAMSNFSLAAEKLISIASQLQTEM
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AQELFEQGKLA FNNGEYESVTKLGEACQLL - QL
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VAKLVS EGGKKAIALRQWEEGVGKYADALDLQREL
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VELAVEQAKRAFALKKYEQAVEHYATALEIATKK
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Supplementary Figure 1-C



Supplementary Figure 1-D



Supplementary Figure 1-E

<i>P. murina</i>	SEAHYKLA ^L A ^L A ^L EFLQ---REKAIEH ^I HWA ^I K ^S LEKR
<i>S. japonicus</i>	SEAHYKLA ^L A ^L A ^L FTA---KKEALKHV ^E AAADI ^I IQHV
<i>S. cryophilus</i>	SEAHYKLA ^L A ^L A ^L FAD---KDRAREHV ^E MAAE ^I LRGI
<i>S. pombe</i>	SEAHYKLA ^L A ^L A ^L FTN---KSRACEHV ^E KAAE ^I LKNV
<i>S. octosporus</i>	TEAHYKLA ^L A ^L A ^L FAN---KDRAREHV ^E TAANI ^I LRDI
<i>S. cerevisiae</i>	I ^Q AEFLVCDALRWVD---QVKDKLKR ^F KHAKALLEK
<i>W. ciferrii</i>	SEAHYKLSLALEFNF---KIKAINHLTKAI ^E S ^I KLK
<i>K. lactis</i>	I ^E SY ^Y KI ^S LALEFDP---FKKCTSNLQKC ^I ELLKQR
<i>K. marxianus</i>	I ^E SHYKISLAYE ^F DP---FEKCKANLVKC ^I ELLKKR
<i>Z. bailii</i>	I ^K TYLKLA ^D ALRWSN---KEKRLEHLQLNLQEL ^I RTR
<i>Z. rouxii</i>	VDVYLKLTDALRWSD---KEQRQRHLQELEKL ^I RSR
<i>T. phaffii</i>	L ^T SLKLIEALRWLT---KDKHKE ^I LNSTQ ^I LLKKR
<i>A. gossypii</i>	VEAHYKLA ^L ALEYVP---AAECCAQLQRCVDMLGAR
<i>L. elongisporus</i>	SESHYKLA ^L A ^L FCV---RKQAAQH ^I QMA ^I K ^S LEER
<i>D. hansenii</i>	SESHYKLSLALEFCV---RSKACEQMRLAI ^E SVRDR
<i>Y. lipolytica</i>	SEAHYKYSLALEFCP---KQKAVDQM ^I LAIDSVVKKR
<i>C. parapsilosis</i>	SESHYKLA ^L A ^L FCV---RKKAEEH ^I QSA ^I K ^S TEAR
<i>C. albicans</i>	SESHYKLA ^L A ^L FQS---RKNAAEQMKLAI ^E SVERR
<i>C. immitis</i>	AECHYKLSLALEFSS---RAEAAKHMEAA ^I QCCKLR
<i>P. marneffei</i>	SECHYKLSLALEFAS---REQSAKHM ^I QSA ^I E ^S CKLR
<i>A. ruber</i>	AECHYKLSLALEFGA---RKEAAVQMEKA ^I E ^S CQAR
<i>B. victoriae</i>	TEAHYSLSLALEFAS---RKEAAEQTDLA ^I QSLEAR
<i>B. oryzae</i>	TEAHYSLSLALEFAS---RKEAAEQTDLA ^I QSLEAR
<i>M. robertsii</i>	AAEHYKLSLALEFAS---RDEAIKEMS ^L A ^I K ^S FKLK
<i>M. anisopliae</i>	AAEHYKLSLALEFAS---RDEAIKEMS ^L A ^I K ^S FKLK
<i>S. borealis</i>	AAEHYKLSLALEFAS---RAEAVKEL ^E LA ^I K ^S TKLK
<i>B. fuckeliana</i>	AAEHYKLSLALEFAS---RAEAVKEL ^E LA ^I K ^S TKLK
<i>P. omphalodes</i>	SEAHFKMSLALEYSS---RNAAAEEH ^I DKA ^I DSCKAR
<i>T. melanosporum</i>	SEAHFKLSLALEFAA---REEAAVQME ^L A ^I ASCRAR
<i>D. haptotyla</i>	TEAHYKLSLALEFAS---REQAVKHMR ^S A ^I ASCKKR
<i>B. dendrobatidis</i>	AAEHYKLA ^L ALEY ^S ---IEDAI ^V QVTQT ^T TVLEKH
<i>R. delemar</i>	AAEAYRHALALEFSS---YDDALPAL ^Q KAI ^S VLKKR
<i>M. circinelloides</i>	AAEHYKYALALEFST---ADQALPEL ^Q KAVNVLKKR
<i>R. irregularis</i>	TEAHWR ^L ALALSAST---IDQAI ^E HVERAMEVLNKC
<i>C. neoformans</i>	ASAHYQLATALEFTP---RTSALT ^H VESALSSLVRR
<i>T. asahii</i>	ASAEYQLGTALEFTP---RPAALVHV ^Q AALDGFKAR
<i>C. gattii</i>	ASAHYQLATALEFTP---RTSALT ^H VESALSSLVRR
<i>R. toruloides</i>	SELHMLTALALEFVP---TSRAVSHA ^E KAKSVLVSK
<i>A. bisporus</i>	AAEHYKLSMVLDLTS---LSDAI ^I SHAENALE ^S VEAR
<i>L. bicolor</i>	AAEHYKLSMVLDLTS---LADAIVHV ^E KALE ^S VETR
<i>C. cinerea</i>	AAEHYKLSMVLDLTS---LADAIGHV ^E KALE ^S VEHR
<i>U. hordei</i>	ADAHLRLGLALEFHP---RKGAKSHVQSASDVL ^G KR
<i>U. maydis</i>	ADAHLRLGLALEFHP---RKGAKPHVQAASDVLAAR
<i>M. violaceum</i>	SELHMLIALAYDMIP---VPQAVHAE ^Q SKAVLLTK

Supplementary Figure 1-F

<i>S. cryophilus</i>	1	TGQKMENRYHV PETSEQADTSARSQ - - - - -	DRKEEGGS P	34
<i>S. pombe</i>	1	SGQK I ENKYTVNEENSS IAHP EK - - - - -	ESEEKETNE	32
<i>S. octosporus</i>	1	TGQK I ENRYAAPTN I ENVGGSTS SQGKK - - - - -	EEGQE GQE Q	37
<i>S. cerevisiae</i>	1	TGDVDSGDS EDS GEGSEEEEENVEKEERLALHELAF SPAN - - - - -	EHDDE I EDV	51
<i>W. ciferrii</i>	1	SEDVPLAEEDEE - - DEEEDKE - - - - -	QNNEEEE EENNDDDQ	36
<i>K. lactis</i>	1	GEEEEEE EEEEEE - - EEEEEEEEEE EEEEEE - - - - -	EE E EQKQ DQE QEE EDQ	45
<i>K. marxianus</i>	1	GEEEEEE EAEEE - - EEEEEEEEEE EEEEEE - - - - -	EEAEEGK EVEQKAQGE	45
<i>Z. bailii</i>	1	DHEEEEV D ENNAQ - - TFEDTTNASNFEDTTNASNYEDARGESMLQGLQKQAQEM	52	
<i>Z. rouxii</i>	1	DHEEEED I D E STLQ - - NFEDATNASAYVDARG - - ES I LQGG - - NGQDESKQQ	45	
<i>T. phaffii</i>	1	DQEEED E DPTDGE - - DGDEQAA I DEEVYDGE - - SEL EQDN - - SDNE NHDP E	45	
<i>A. gossypii</i>				
<i>L. elongisporus</i>	1	YDAEP VPEGEDGKT - - DDEDKV - - DTQEG - - NDG NEEE - - EEEEQEE Q	40	
<i>D. hansenii</i>	1	CDDAPLAEE D DDE - - GGAAAEEEEEESGN - - - - -	EADKQDAGG	38
<i>Y. lipolytica</i>	1	EEAPEEV EEEVEV - - EEEVS ESKGKEKA - - - - -	EEGDAGEAE	35
<i>C. parapsilosis</i>	1	YDAEPI E GEEEEEK - - EDKEKPTEGTNKNQE I - - RE S ENGN - - DQQEDEEE E	45	
<i>C. albicans</i>	1	YADGDGE EEDGEE - - - - -	NEEPNEDEN	22
<i>A. ruber</i>	1	NGAAKKEAGANEK - - GQSEKQNT PFFQFTGD - - EN F D ESE - - EEEEDEGA Q	45	
<i>B. victoriae</i>	1	AKSPEPKT E SVENK - - PYFQLQGD - - ENWT DSE - - DEDEETQDG	37	
<i>B. oryzae</i>	1	AKSQPKT E SVENK - - PYFQLQGD - - ENWT - - DSEDEDEEA	34	
<i>M. robertsii</i>	1	GQAGDAHEKKEDKTKEGGPDEKKPLFQFTGD - - EN F D D S S - - DEEQQEEAP	47	
<i>M. anisopliae</i>	1	GQAGDAHEKKEDKTKEGGPDEKKPLFQFTGD - - EN F D D S S - - DEEQQEEAP	47	
<i>S. borealis</i>	1	ATIANG AAKKEE - - SSSTD LKKPLFTFTGD - - EN F EDSD - - EEEEAE GEG	45	
<i>B. fuckeliana</i>	1	E EKADKVABEEVAI I ANGGAAKKEES SNTNSNKPLFT TGDE - - NFEDSDEEE E	51	
<i>P. omphalodes</i>	1	HGDGE YDES DEED - - - - -	EDEEGGE GE	22
<i>T. melanosporum</i>	1	GGE GND E KLKA P - - APVVGAKCGFFSFTGD - - ENWDDSE - - EEEGDADAG	44	
<i>D. haptotyla</i>	1	KGKGKAKRKA EEL - - TEGGNLKKGVFSFQGD - - ENY DVED - - EEE DADD AD	45	
<i>B. dendrobatidis</i>	1	NGDEENS EDD D ECAEEQVERADV - - - - -	DENGDDGV	32
<i>R. delemar</i>	1	ESIPEFRDENDVGC - - - - -	EEEGEEEE E	22
<i>M. circinelloides</i>	1	SGVPEFKESE EDD - - - - -	EEDEENDE E	22
<i>R. irregularis</i>	1	E EGP S F E DEMV E ELTEESLEKE IN - - - - -	NNDCESHA E	33
<i>C. neoformans</i>	1	SGDAPS DDEGE ADDP EPEPESEPQASSSSAP - - - - -	AAA PAGTDA	40
<i>T. asahii</i>	1	EGDGDDG D EEE ECG EGE EEE - - - - -	GEGEK GQ Q	28
<i>C. gattii</i>	1	SGDAPS DDEGEADEPE PEPEVE P QASSSS - - - - -	APAPAPDAA	36
<i>R. toruloides</i>	1	GGDVEDAS G D EGD - - - - -	GADS D E DGE	22
<i>A. bisporus</i>	1	SGDAE EED P AVDLFASAENAV - - - - -	VEEKAAAAA	30
<i>L. bicolor</i>	1	SGDAE D E AVDL FA - QATH - - - - -	LEEE EG DDE	26
<i>C. cinerea</i>	1	SGDGDDG D D P VVD - LSA - - - - -	QPEDDDGSD	25
<i>U. hordei</i>	1	SGDAE D D D D D E Q E - - - - -	EGAPGAAGG	22
<i>U. maydis</i>	1	TGDAE D D D G D D E E - - - - -	SAPDAGAA E	22
<i>M. violaceum</i>	1	GGDAE D E D D D E E - - - - -	EGAEGDDDE	21
<i>S. cryophilus</i>	35	EEQE QE - - - - -	QDEDD	45
<i>S. pombe</i>	33	ASPASE - - - - -	EDEDD	43
<i>S. octosporus</i>	38	DEGED - - - - -	EDEDD	47
<i>S. cerevisiae</i>	52	SQLRKS G F H I Y - - - - -	FENDL	67
<i>W. ciferrii</i>	37	EQE Q E PQ - - - - -	EEQSD	48
<i>K. lactis</i>	46	VEGQEE E - - - - -	VDKDD	56
<i>K. marxianus</i>	46	EEGED E E EQHDHANCQ GHCHGEEKDD - - - - -	71	
<i>Z. bailii</i>	53	EAEEDE G N Q TDMS I GES FEG FLEGNL - - - - -	78	
<i>Z. rouxii</i>	46	DEDE E E QE ADMS I GDT FED F LQGNL - - - - -	71	
<i>T. phaffii</i>	46	DD I D E E GL I S Y VD Y - - - - -	LEGDH	64
<i>A. gossypii</i>	1	- - - - -	EEQSD	5
<i>L. elongisporus</i>	41	EEQEE E - - - - -	AEE SD	51
<i>D. hansenii</i>	39	EEE EDE - - - - -	EEQSD	49
<i>Y. lipolytica</i>	36	EEGEDE E - - - - -	AP QTD	47
<i>C. parapsilosis</i>	46	EEEEE - - - - -	EEPGD	55
<i>C. albicans</i>	23	NDED - - - - -	ANKSD	31
<i>A. ruber</i>	46	ADAGD - - - - -	EDEDD	55
<i>B. victoriae</i>	38	EQE - - - - -	EEDDD	45
<i>B. oryzae</i>	35	QEDEQE - - - - -	EDDDD	45
<i>M. robertsii</i>	48	E - - - - -	EEDDD	53
<i>M. anisopliae</i>	48	E - - - - -	EEDDD	53
<i>S. borealis</i>	46	EGDEDE - - - - -	EEDDP	56
<i>B. fuckeliana</i>	52	EEAEEED - - - - -	EEDDP	63
<i>P. omphalodes</i>	23	- - - - -	DDDDD	27
<i>T. melanosporum</i>	45	GDGDVEK - - - - -	EGDDDP	57
<i>D. haptotyla</i>	46	DDGE Q G - - - - -	EDDDD	56
<i>B. dendrobatidis</i>	33	ALGD I DE - - - - -	EDP ED	44
<i>R. delemar</i>	23	EGNEE E G - - - - -	ETEDD	34
<i>M. circinelloides</i>	23	EEGAGN - - - - -	TADED	33
<i>R. irregularis</i>	34	TTNE SQT - - - - -	DQDSD	45

<i>C. neoformans</i>	41	AEGDE V G - - - - -	E L EDD- - - - -	52
<i>T. asahii</i>	29	EEGE E - - - - -	E P EDD- - - - -	38
<i>C. gattii</i>	37	EGDE M E- - - - -	E L EDD- - - - -	47
<i>R. toruloides</i>	23	GEGEDA P S G - - - - -	DREDD- - - - -	36
<i>A. bisporus</i>	31	EEEED- - - - -	E P EDD- - - - -	40
<i>L. bicolor</i>	27	EV G ED G - - - - -	E P EDD- - - - -	37
<i>C. cinerea</i>	26	EEGDDD G - - - - -	E P EDD- - - - -	37
<i>U. hordei</i>	23	E - - - - -	DDEDD- - - - -	28
<i>U. maydis</i>	23	A- - - - -	DDEDD- - - - -	28
<i>M. violaceum</i>	22	DGGDEAMMV- - - - -	DR D E- - - - -	35

Supplementary Figure 1-G

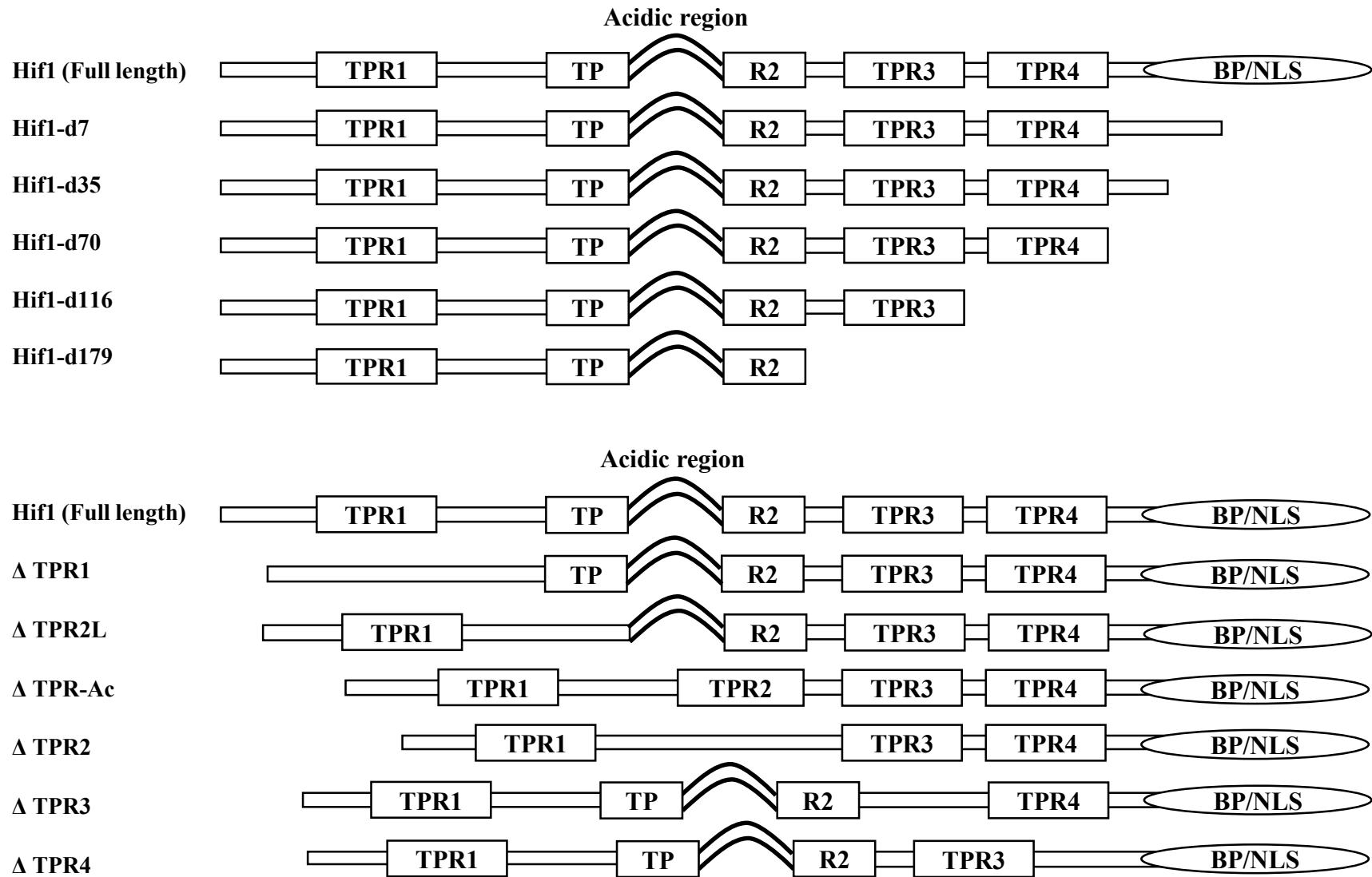
<i>Coccidioides immitis</i>	- - - - - T Q T S G E D S H L T - - - - - S
<i>Penicillium marneffei</i>	- - - - - S Q P D E E N G E G K P - - - - - T
<i>Aspergillus ruber</i>	- - - - - S K T E E S G E Q D S H G Q - - - S
<i>Bipolaris victoriae</i>	SKVREDQ T G Q S T D A P P E A E Q Q K E D E D
<i>Bipolaris oryzae</i>	SKVREDQ T G Q S T D A P A E T E Q Q K E D E D
<i>Metarhizium robertsii</i>	- - - - - T M S D D E G K N T K R - - - - - E
<i>Metarhizium anisopliae</i>	- - - - - T M S D D E G K N T K R - - - - - E
<i>Sclerotinia borealis</i>	- - - - - T Q T E S E D K K P N A G A - - - D
<i>Botryotinia fuckeliana</i>	- - - - - I T Q T E T D D K K P N A G A D
<i>Pyronema omphalodes</i>	- - - - - G E G I S - - - - - - - - - K
<i>Tuber melanosporum</i>	- - - - - G E G V T - - - - - - - - - N
<i>Dactylellina haptotyla</i>	- - - - - Q G G G E G D A D N E V S P

Supplementary Figure 2

<i>Pneumocystis murina</i>	NDVNALV	-KKKKKS I
<i>Schizosaccharomyces japonicus</i>	NDLGS LV	-KRKR PKT
<i>Schizosaccharomyces cryophilus</i>	NDLGGLV	-KRKR PKQ
<i>Schizosaccharomyces pombe</i>	NDLGGLV	-KRKRT KQ
<i>Saccharomyces cerevisiae</i>	NDLSQLV	-KKKP RRH
<i>Wickerhamomyces ciferrii</i>	NDLTS IV	-KKRK SKP
<i>Kluyveromyces lactis</i>	NDLTSMV	-KKRK S ND
<i>Kluyveromyces marxianus</i>	NDLTSKI	-KKRKAN
<i>Zygosaccharomyces bailii</i>	NDLSKVI	-KKKKNKL
<i>Zygosaccharomyces rouxii</i>	NDLSKMV	-KKKKT KS
<i>Tetrapisispora phaffii</i>	NDLSSMV	FKKKKGKK
<i>Ashbya gossypii</i>	NDLTSRV	-RRRG GAP
<i>Lodderomyces elongisporus</i>	NNLNLSV	-RKKR PSK
<i>Debaryomyces hansenii</i>	NDLSSVV	-KKKP SKP
<i>Yarrowia lipolytica</i>	NDLSGLA	-VRKKAPK
<i>Candida parapsilosis</i>	NDLSGLV	-KKRK QSK
<i>Candida albicans</i>	NNLQTMV	-KKKQNKE
<i>Coccidioides immitis</i>	NDLNAFV	-RKRKRNP
<i>Aspergillus ruber</i>	TDLSAFV	-KRKPTNG
<i>Bipolaris victoriae</i>	NDVSGMV	-KKKA KPA
<i>Bipolaris oryzae</i>	NDVSGMV	-KKKA KPA
<i>Metarhizium robertsii</i>	TDLTGLV	-RKKKA KE
<i>Metarhizium anisopliae</i>	TDLTGLV	-RKKKA KE
<i>Mucor circinelloides</i>	NDLSTLV	-KRKP AND
<i>Cryptococcus neoformans</i>	NDLTGMV	-KKKKPKA
<i>Trichosporon asahii</i>	NDLTSMV	-KKKKKPS
<i>Tremella fuciformis</i>	NDLSGLV	-KKKPA KK
<i>Cryptococcus gattii</i>	NDLTSMV	-KKKKPKA
<i>Agaricus bisporus</i>	NDLTGIV	-KKKKKVA
<i>Coprinopsis cinerea</i>	NDLTSIV	-KKKKKNP
<i>Ustilago hordei</i>	NNLSSMV	-KRKKKAE
<i>Ustilago maydis</i>	NNLSSMV	-KRKKKPE

Supplementary Figure 3

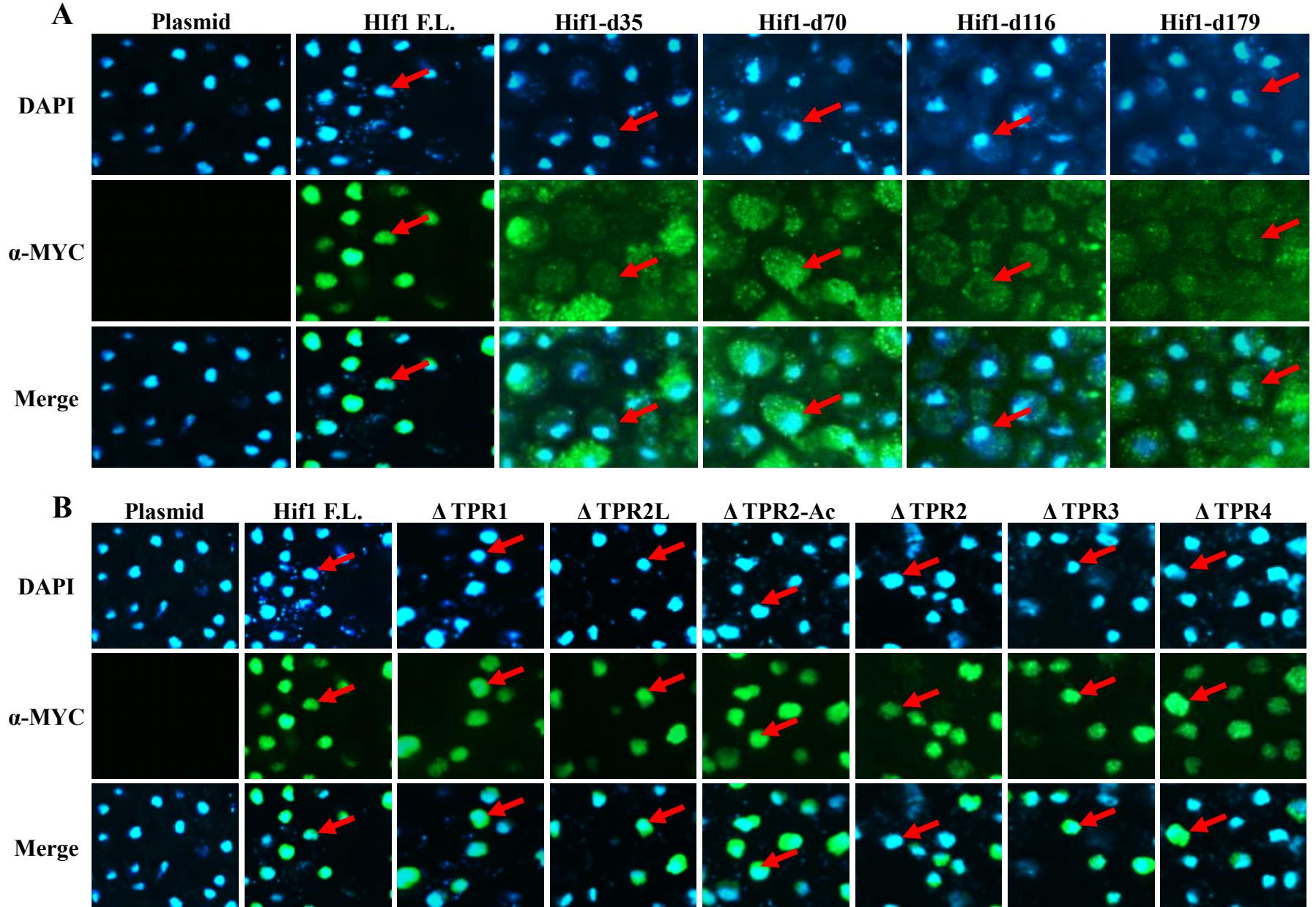
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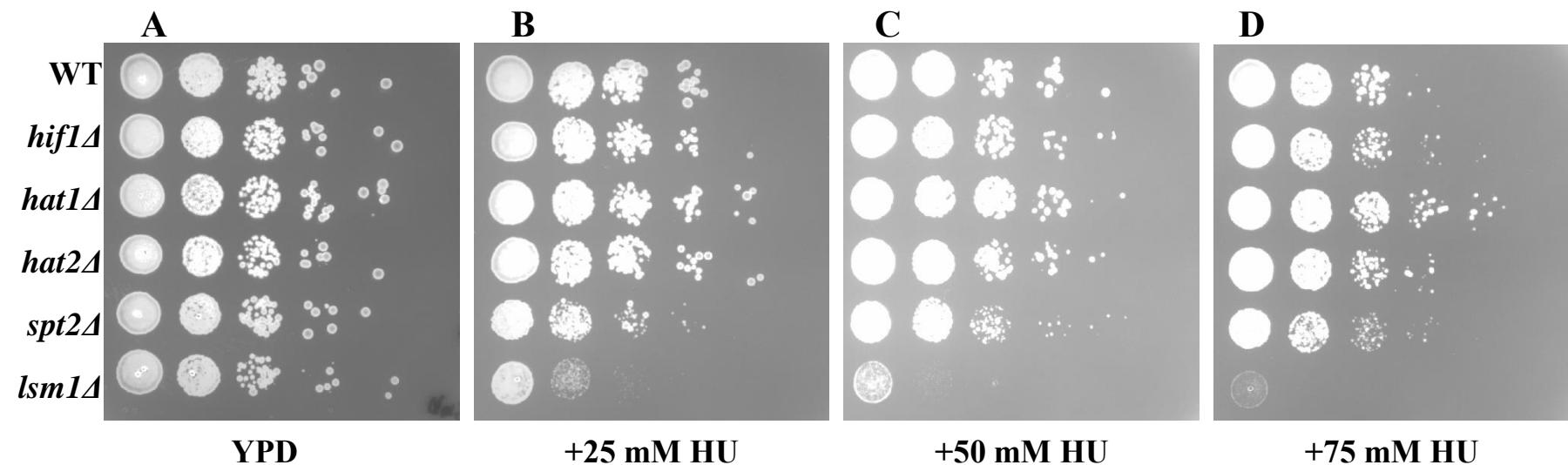
A.1

Hif1 mutants name	Deleted segment	Protein length (amino acids)
Hif1 (Full length)	-	385
Hif1-d7	Hif1: 378- 385	378
Hif1-d35	Hif1: 350- 385	350
Hif1-d70	Hif1: 315- 385	315
Hif1-d116	Hif1: 269- 385	269
Hif1-d179	Hif1: 206- 385	206
Δ TPR1	Hif1: 22- 55	352
Δ TPR-2L	Hif1: 61- 84	362
Δ TPR-Ac	Hif1: 85- 188	282
Δ TPR2	Hif1: 61- 206	240
Δ TPR3	Hif1: 236-259	352
Δ TPR4	Hif1: 280-315	350

Supplementary Figure 4

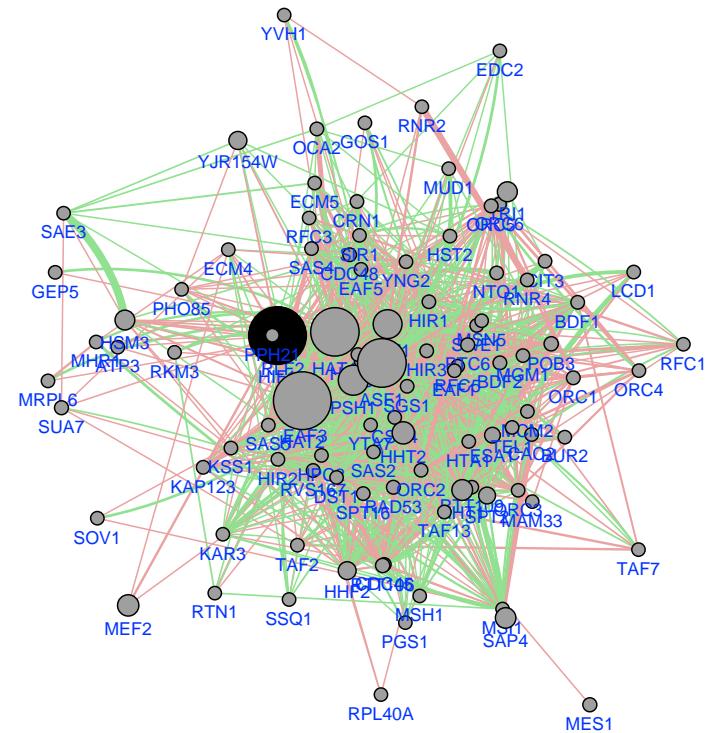


Supplementary Figure 5



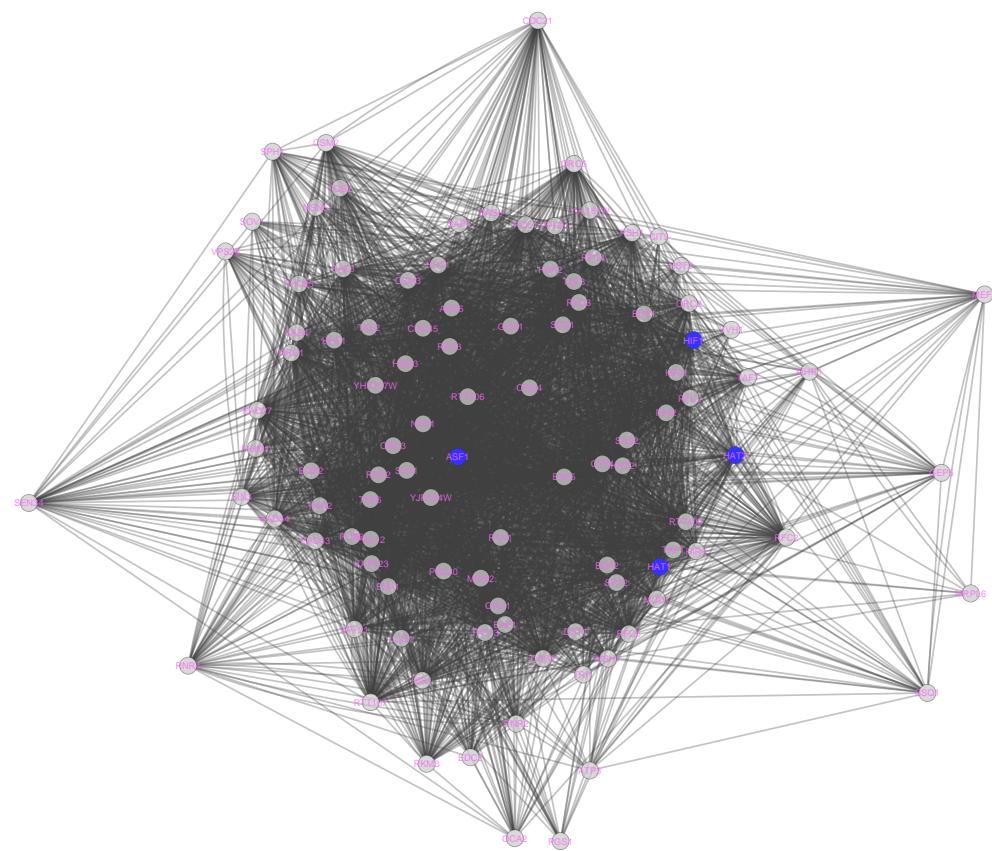
Supplementary Figure 6

A



Supplementary Figure 6

B



Supplementary Figure 6

C

