Table S1. Strains used in this study

C. neoformans strains	Genotype	Parent	Reference
H99	*MAT*α		(3)
KN99	*MAT***a**		(2)
YSB53	*MAT*α *ras1Δ::NAT STM#150*	H99	(1)
YSB42	*MAT*α *cac1Δ::NAT STM#159*	H99	(1)
YSB188	*MAT*α *pka1Δ::NAT STM#191*	H99	(1)
YSB13	*MAT*α *msl1Δ::NAT STM#123*	H99	This study
YSB62	*MAT***a** *msl1Δ::NEO*	KN99**a**	This study
YSB528	*MAT*α *msl1Δ::NAT STM#123 MSL1-NEO*	H99	This study
YSB1063	*MAT*α *cac2Δ::NEO*	H99	This study
YSB1064	*MAT*α *cac2Δ::NEO*	H99	This study
YSB1065	*MAT*α *cac2Δ::NEO*	H99	This study
YSB1066	*MAT*α *cac2Δ::NEO*	H99	This study
YSB1067	*MAT*α *cac2Δ::NEO*	H99	This study
YSB1693	*MATa cac2Δ::NEO*	KN99**a**	This study
YSB1694	*MATa cac2Δ::NEO*	KN99**a**	This study
YSB1695	*MATa cac2Δ::NEO*	KN99**a**	This study
YSB1696	*MATa cac2Δ::NEO*	KN99**a**	This study
YSB1697	*MAT*α *msl1Δ::NAT STM#123 cac2Δ::NEO*	YSB13	This study
YSB1698	*MAT*α *msl1Δ::NAT STM#123 cac2Δ::NEO*	YSB13	This study
YSB1699	*MAT*α *msl1Δ::NAT STM#123 cac2Δ::NEO*	YSB13	This study
YSB1700	*MAT*α *msl1Δ::NAT STM#123 cac2Δ::NEO*	YSB13	This study
YSB1775	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1776	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1777	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1778	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1779	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1780	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1781	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1782	*MAT*α *PH3::MSL1::NAT*	H99	This study
YSB1790	*MAT*α *msl1Δ::NATSTM#123 ras1Δ::NEO*	YSB13	This study
YSB1918	*MAT*α *msl1Δ::NATSTM#123 MSL1::GFP::NEO*	YSB13	This study
YSB2042	*MAT*α *pka1Δ::NATSTM#191* P_{H3}*::MSL1::NEO*	YSB188	This study
YSB2043	*MAT*α *pka1Δ::NATSTM#191* P_{H3}*::MSL1::NEO*	YSB188	This study
YSB2044	*MAT*α *pka1Δ::NATSTM#191* P_{H3}*::MSL1::NEO*	YSB188	This study
YSB2045	*MAT*α *pka1Δ::NATSTM#191* P_{H3}*::MSL1::NEO*	YSB188	This study
YSB2046	*MAT*α *pka1Δ::NATSTM#191* P_{H3}*::MSL1::NEO*	YSB188	This study
YSB2047	*MAT*α *cac1Δ::NATSTM#159* P_{H3}*::MSL1::NEO*	YSB42	This study
YSB2048	*MAT*α *cac1Δ::NATSTM#159* P_{H3}*::MSL1::NEO*	YSB42	This study
YSB1084	*MAT*α *cac1Δ::NAT STM#159 msl1Δ::NEO*	YSB42	This study
YSB1085	*MAT*α *pka1Δ::NAT STM#191 msl1Δ::NEO*	YSB188	This study
YSB1086	*MAT*α *pka1Δ::NAT STM#191 msl1Δ::NEO*	YSB188	This study

References

1. **Bahn YS, Hicks JK, Giles SS, Cox GM, Heitman J**. 2004. Adenylyl cyclase-associated protein Aca1 regulates virulence and differentiation of *Cryptococcus neoformans* via the cyclic AMP-protein kinase A cascade. Eukaryot. Cell **3**:1476-1491.
2. **Nielsen K, Cox GM, Wang P, Toffaletti DL, Perfect JR, Heitman J**. 2003. Sexual cycle of *Cryptococcus neoformans* var. *grubii* and virulence of congenic a and a isolates. Infect. Immun. **71**:4831-4841.
3. **Perfect JR, Ketabchi N, Cox GM, Ingram CW, Beiser CL**. 1993. Karyotyping of *Cryptococcus neoformans* as an epidemiological tool. J. Clin. Microbiol. **31**:3305-3309.

Table S2. Primers used in this study

Primer	Sequence (5′ to 3′)	Comments
JOHE8994	TGTGGATGCTGGCGGAGGATA	Universal diagnostic primer[a]
JOHE10425	TCGCATTTGAACCTCGCTC	*MSL1* 5′ flanking region primer 1
JOHE10426	CTGGCCGTCGTTTTACGGTTTAGTGGTGGCGTATTAC	*MSL1* 5′ flanking region primer 2[b]
JOHE10427	GTCATAGCTGTTTCCTGTGAGGACTCCCTATGAAGCC	*MSL1* 3′ flanking region primer 1[c]
JOHE10428	TTCTCCCACTACCCAAAACCCCTC	*MSL1* 3′ flanking region primer 2
JOHE10429	TACGGCATCTTCCACCAGTC	*MSL1* diagnostic screening primer 1
JOHE10430	CACTCATACCGTTCTCCTGAC	*MSL1* diagnostic screening primer 2
JOHE11454	CCATCCATCCATAAACACAC	*MSL1* Southern blot probe primer 1
JOHE11455	GATGGTAGTATCCTCACTGGC	*MSL1* Southern blot probe primer 2
JOHE11725	CTTGGTGGCAATGAGTTCTGGG	*MSL1* 5′-RACE primer 1
Bahn3100	GCTGTGTATGAGCCAATCTC	*MSL1* 5′-RACE primer 2
Bahn3643	CCTTCAACTTTCCTGGTCC	*MSL1* 3′-RACE primer 1
Bahn3677	TTGTATCTCTTTCGCCCC	*MSL1* 3′-RACE primer 2
Bahn1927	ATTGCCAAGTGTGTCCAGG	*CAC2* 5′ flanking region primer 1
Bahn1928	CTGGCCGTCGTTTTACGGAAATCGCACGAGTAGAC	*CAC2* 5′ flanking region primer 2[b]
Bahn1929	GTCATAGCTGTTTCCTGAAGAAGAAGAGGAGGGTCGC	*CAC2* 3′ flanking region primer 1[c]
Bahn1930	GGCAAACTCAAAGTCAAGC	*CAC2* 3′ flanking region primer 2
Bahn1931	TTGCCTTCACCTTCTGAGC	*CAC2* diagnostic screening primer
Bahn1932	TCTCCCCCGTGTTTTATG	*CAC2* probe primer
Bahn4017	GCATGCAGGATTCGAGTG	P_{H3}*::MSL1* primer 1
Bahn4018	GTGATAGATGTGTTGTGGTG	P_{H3}*::MSL1* primer 2
Bahn4019	TACGGCATCTTCCACCAGTC	P_{H3}*::MSL1* primer 3
Bahn4020	CACTCGAATCCTGCATGCTGTGTGTTTATGGATGGATG	P_{H3}*::MSL1* primer 4[a]
Bahn4021	CACCACAACACATCTATCACATGGCCCCCAACGAACCTAT	P_{H3}*::MSL1* primer 5[d]
Bahn4022	GATGGTAGTATCCTCACTGGC	P_{H3}*::MSL1* primer 6
Bahn4278	ACATACTCCATCCAGGTCGC	P_{H3}*::MSL1* diagnostic screening primer
Bahn4112	CTCGAGTACGGCATCTTCCACCAGTC	Msl1::GFP primer 1[e]
Bahn4113	CCCGGGAGAGCCACCGCCACCCTCCAAATCCTTTTCATCAA	Msl1::GFP primer 2[f]
Bahn4114	CCCGGGGTGAGCAAGGGCGAGGAGCT	Msl1::GFP primer 3[f]
Bahn4115	CTTGTACAGCTCGTCCATGC	Msl1::GFP primer 4
Bahn4116	GCATGGACGAGCTGTACAAGATGGATGGAGTTTAGGATGC	Msl1::GFP primer 5
Bahn4545	TGAGCGGCCGCTCATCGTTCTCCCACTACC	Msl1::GFP primer 6[g]
Bahn3066	CTCTATTCTTTCGCACGTT	*HSP78* qRT-PCR primer 1
Bahn3067	AAGGATGATGGAAGGCGGAAGC	*HSP78* qRT-PCR primer 2
Bahn3068	ATGGAAGAATGCGGGAGCC	*SNF3* qRT-PCR primer 1
Bahn3069	CATTCTGGTCAATCGCAAG	*SNF3* qRT-PCR primer 2
Bahn3070	ATCTGGAAAAGGTCGTCAA	*AGX1* qRT-PCR primer 1
Bahn3071	CAACGCATTTACAGTTTGGC	*AGX1* qRT-PCR primer 2
Bahn3072	TGGTTCTTATTCCCTGTGG	*SSL1* qRT-PCR primer 1
Bahn3073	CATCGGAAAACTCGCTATC	*SSL1* qRT-PCR primer 2
Bahn3074	GGATGGAACTTCGTAGCAAC	*YVC1* qRT-PCR primer 1
Bahn3075	GGTATTCGTCCGTCTCATTATC	*YVC1* qRT-PCR primer 2
Bahn3076	TGATTGTCGCTCCTGAGCT	*GDB1* qRT-PCR primer 1
Bahn3077	TCAAGGGTCTTCATACCAAG	*GDB1* qRT-PCR primer 2
Bahn3078	ATTCTGAAGTATTGAGGCTACG	*PMP3* qRT-PCR primer 1
Bahn3079	TAACATACAATCTCCCTGCC	*PMP3* qRT-PCR primer 2
Bahn3080	TTGCTGCGTTTGACCAACC	*CAN2* qRT-PCR primer 1
Bahn3081	GGCAAAGAATGCTTCAACC	*CAN2* qRT-PCR primer 2
Bahn3082	ACGACAACCAGGAGTCTCTT	*HSP12* qRT-PCR primer 1
Bahn3083	GTTTCTACACCATTTGTTTA	*HSP12* qRT-PCR primer 2
Bahn3086	AGCCCACTGCTTGCTGGATG	*VPS55* qRT-PCR primer 1
Bahn3087	AACTCTTCCTCGTTACCACC	*VPS55* qRT-PCR primer 2
Bahn3513	CCTTTCCTCTATGACACCG	*MSL1* Northern blot probe primer 1
Bahn3514	CTGTGAGGCGTCAGTCTTAC	*MSL1* Northern blot probe primer 2
Bahn3495	CTTCTCAACCTTGGCAAAG	*HSP78* Northern blot probe primer 1
Bahn3496	TTCGGTCTTACCTACACCAG	*HSP78* Northern blot probe primer 2
Bahn3511	CCACTACTACCACACCCAAC	*HSP12* Northern blot probe primer 1
Bahn3512	CAGTAAGAGACTCCTGGTTGTC	*HSP12* Northern blot probe primer 2

[a] Binding to *ACT1* promoter regions of the Nat^r or Neo^r marker

[b] The reverse-complementary sequence of M13 forward (M13F) primer is underlined.

[c] The reverse-complementary sequence of M13 reverse (M13R) primer is underlined.

[d] Binding to H3 promoter
[e] XhoI site is underlined.
[f] XmaI site is underlined.
[g] NotI site is underlined.

Table S4. List of Msl1-regulated genes having unknown functions

H99 ID (CNAG)	Fold change[a]	KOG Functional description
07367	-3.913	Amino acid transporters
05276	-1.463	Histone deacetylase complex, catalytic component RPD3
03454	-1.273	None
01494	-1.24	None
06759	-1.236	Zinc-binding oxidoreductase
04322	-1.012	None
06220	-0.968	1 FOG: Zn-finger
01348	-0.927	None
06453	-0.913	Peripheral-type benzodiazepine receptor and related proteins
04417	-0.896	None
00984	-0.866	Reductases with broad range of substrate specificities
06117	-0.847	None
01743	-0.831	None
07856	-0.661	None
03595	-0.634	Uncharacterized conserved protein
01735	-0.612	Cystathionine beta-lyases/cystathionine gamma-synthases
02722	-0.596	Aldo/keto reductase family proteins
00283	0.611	Phospholipase C
02484	0.612	Cyclophilin type peptidyl-prolyl cis-trans isomerase
05534	0.644	Voltage-gated shaker-like K+ channel, subunit beta/KCNAB
01993	0.669	None
00663	0.697	None
01540	0.79	Predicted oxidoreductase
04631	0.859	Ribulose kinase and related carbohydrate kinases
03599	0.88	None
06431	0.902	Short-chain acyl-CoA dehydrogenase
07837	0.923	None
05305	1.013	DNA damage inducible protein
05184	1.207	Glycosyl transferase, family 8 - glycogenin
05461	1.366	Glycolate oxidase
05258	1.519	None
05256	1.687	Catalase
00269	1.733	Sorbitol dehydrogenase
04025	1.907	Transaldolase
05831	1.96	Putative translation initiation inhibitor UK114/IBM1
04197	2.133	Dual-specificity tyrosine-phosphorylation regulated kinase

a, Fold change indicates Log_2 (*msl1Δ*/WT). Genes showing more than 1.5-fold expression changes are listed.

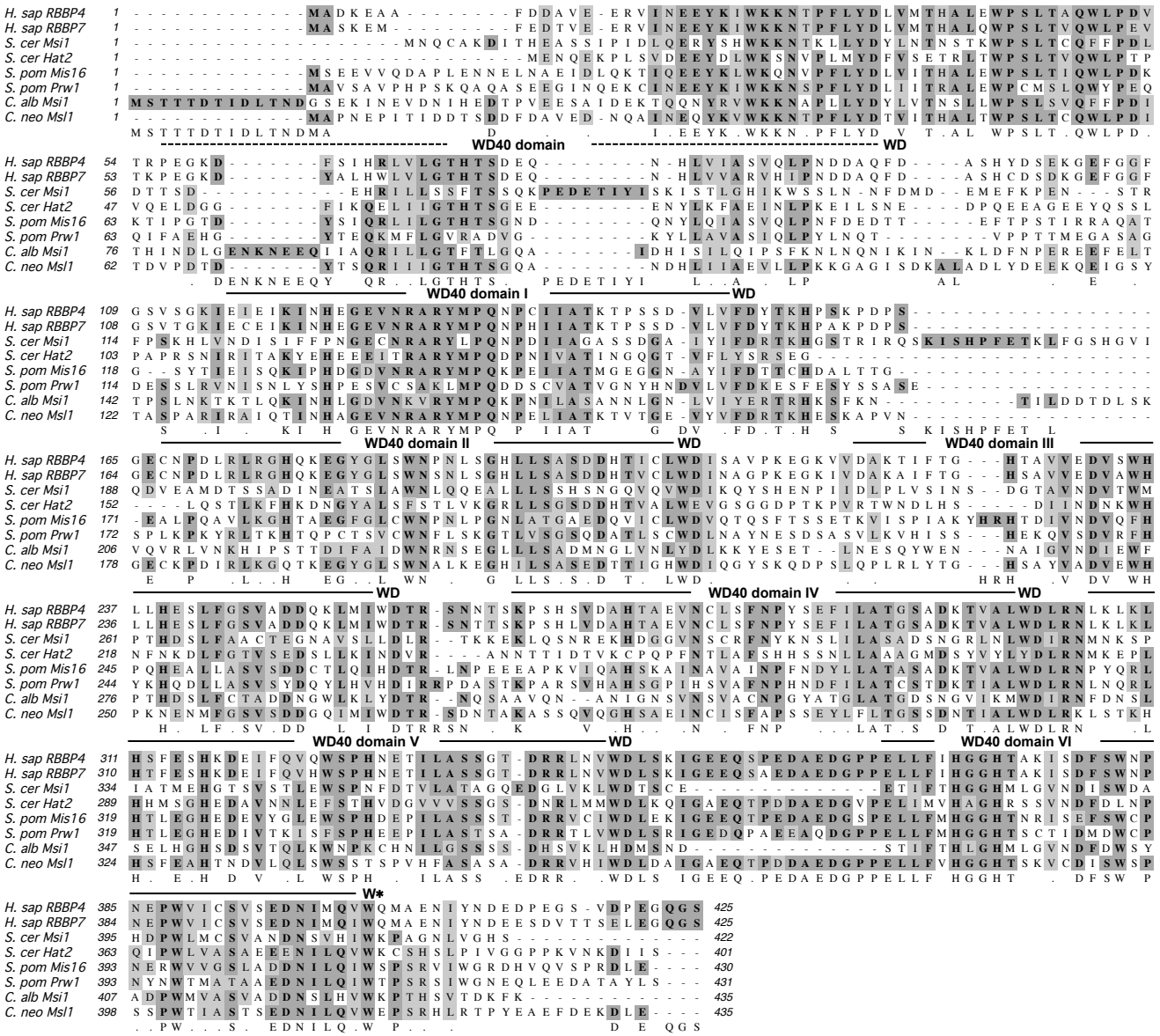

Fig S1. Alignment of MSIL proteins from several fungi and human. Multiple sequence alignment is depicted by Clustal W alignment from MacVector software (versions 7.2.3, Accelrys). The WD40 domains were marked based on the known WD40 domains in human homologs RBBP4 and RBBP7. *H. sap* RBBP4, NP_005601.1; *H. sap* RBBP7, NP_002884.1; *S. cer* Msi1, NP_009754; *S. cer* Hat2, NP_010858.3; *S. pom* Prw1, NP_594864.1; *S. pom* Mis16, NP_587881.1; *C. alb* Msi1, XP_715003; *C. neo* Msl1, CNAG_03297.2.

Figure S2 (Yang et al. 2012)

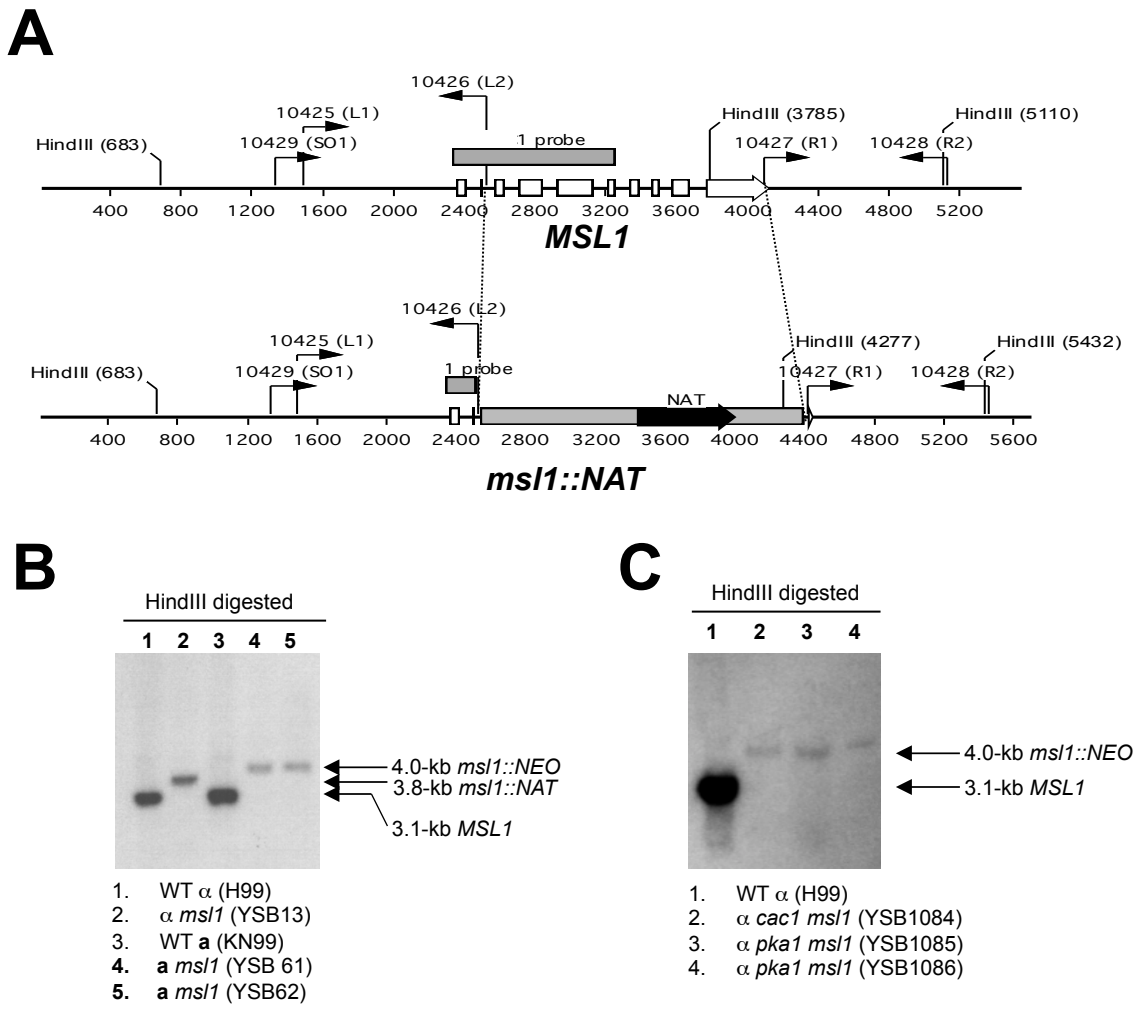

Fig S2. Disruption of the *MSL1* gene. (A) The *MSL1* disruption strategy. The genomic DNA structure of the *MSL1* gene is illustrated as white boxes for the first 9 exons and an arrow for exon 10 indicating the direction of transcription. Primers for overlap PCR and diagnostic PCR are indicated as bent arrows. (B) Southern blot analysis with HindIII-digested genomic DNA of wild-type α (H99, lane 1) and **a** (KN99**a**, lane 3) strains, α and **a** *msl1Δ* mutants (YSB13, lane 2; YSB61, lane 4; YSB61, lane 5) was performed with the *MSL1*-specific probe. (C) Southern blot analysis of *cac1Δ msl1Δ* and *pka1Δ msl1Δ* mutants was performed with HindIII-digested genomic DNA of wild type α strain (H99, lane 1), α *cac1Δ msl1Δ* mutant (YSB1084, lane 2), α *pka1Δ msl1Δ* mutants (YSB1085, lane 3; YSB1086, lane 4).

Figure S3 (Yang et al. 2012)

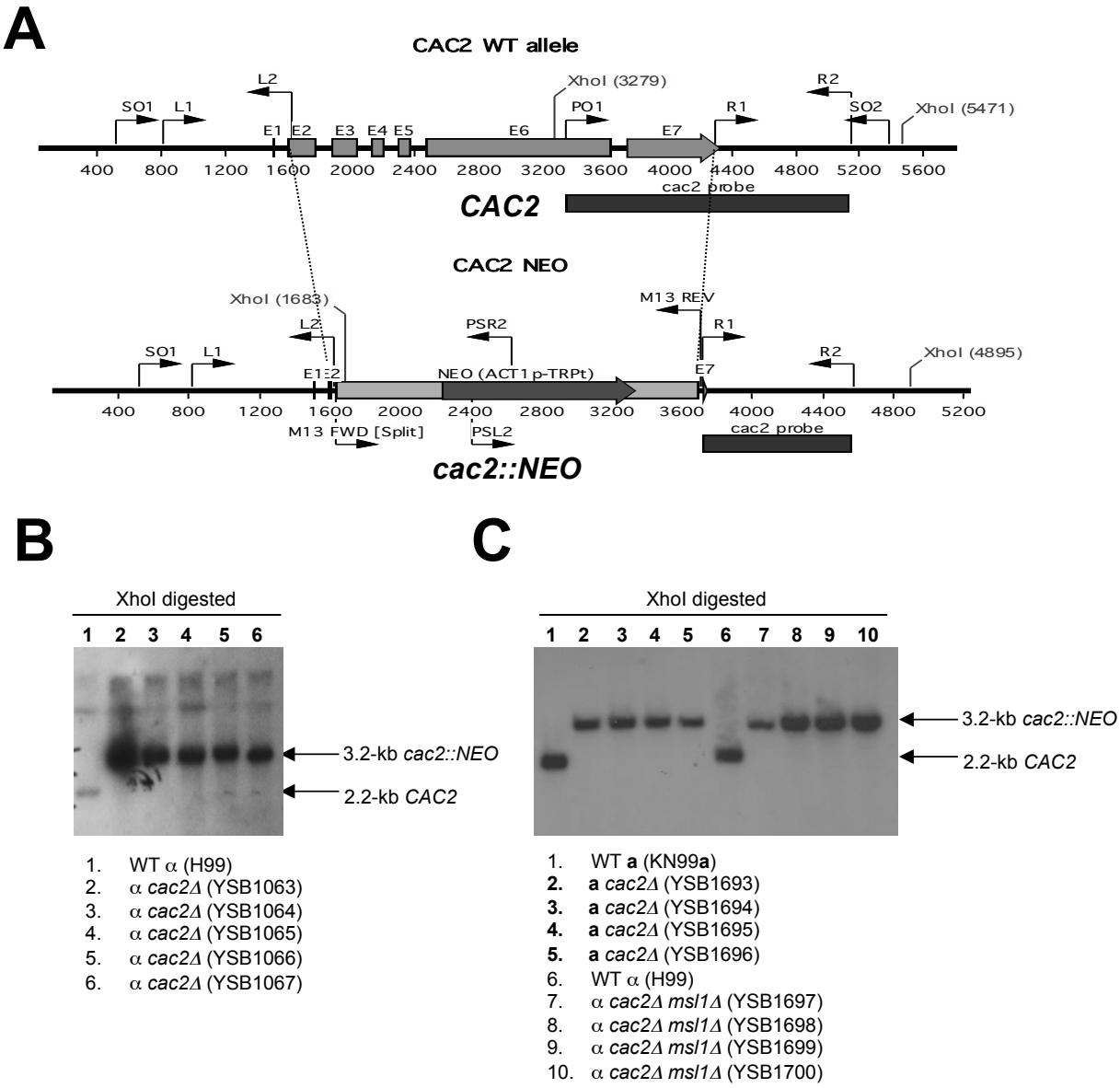

Fig S2. Disruption of the *CAC2* gene. (A) The *CAC2* disruption strategy. The genomic DNA structure of the *CAC2* gene is illustrated as shade boxes for the first 6 exons and an arrow for exon 7 indicating the direction of transcription. Primers for overlap PCR and diagnostic PCR are indicated as bent arrows. (B) Southern blot analysis with XhoI-digested genomic DNA of wild-type α strain (H99, lane 1), α *cac2Δ* mutants (YSB1063, lane 2; YSB1064, lane 3; YSB1065, lane 4; YSB1066, lane 5; YSB1067, lane 6) was performed with the *CAC2*-specific probe. (C) Southern blot analysis of **a** *cac2Δ* and α *cac2Δ msl1Δ* was performed with XhoI-digested genomic DNA of wild-type **a** and α (KN99**a,** lane 1; H99, lane 6), **a** *cac2Δ* mutants (YSB1693, lane 2; YSB1694, lane 3; YSB1695, lane 4; YSB1696, lane 5), α *cac2Δ msl1Δ* mutants (YSB1697, lane 7; YSB1698, lane 8; YSB1699, lane 9; YSB1700, lane 10).

Figure S4 (Yang et al. 2012)

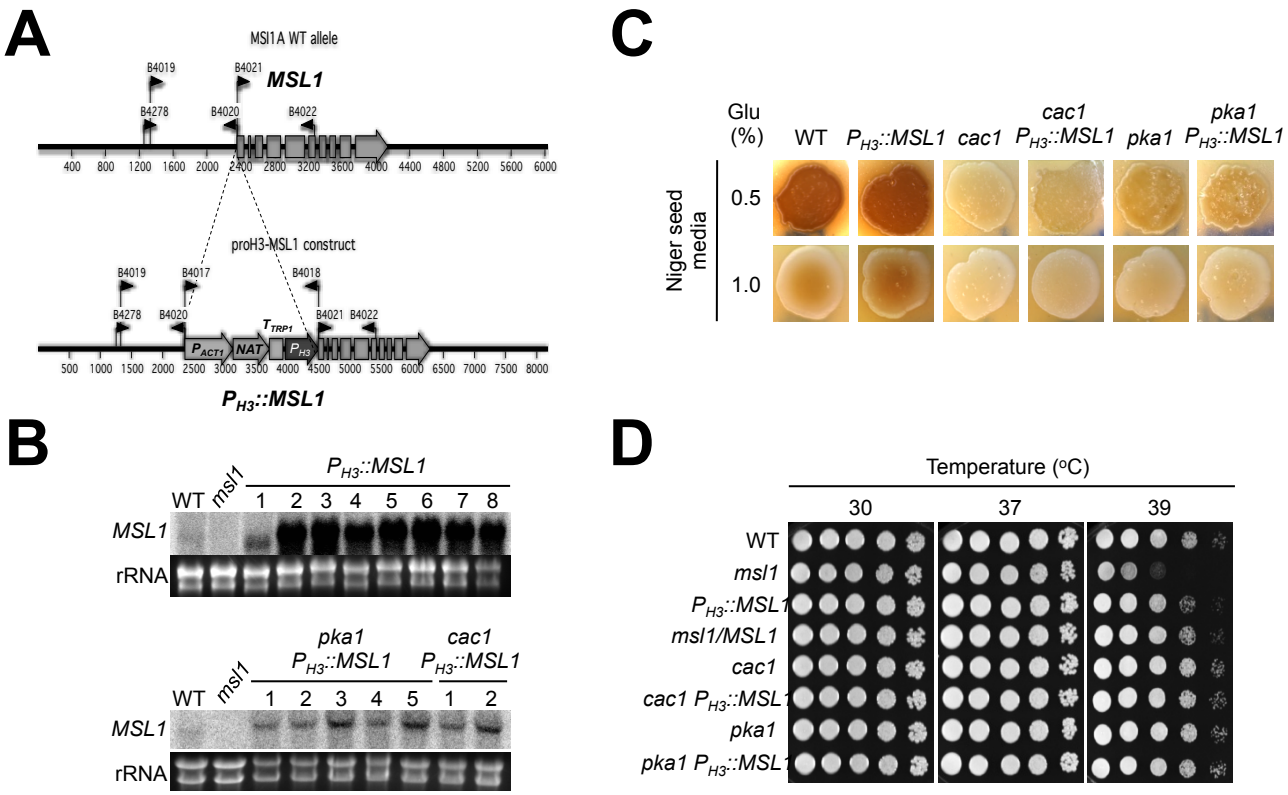

Fig S4. Construction and phenotypic analysis of *MSL1* overexpression strains.

(A) The construction strategy for the *MSL1* overexpression strains. The primers for overlap PCR and diagnostic PCR are indicated as bent arrow. The first arrow box on the P*H3::MSL1* construct indicates the *ACT1* promoter. The shade arrow box named *NAT* indicates the *NAT* (nourseothricin acetyltransferase) gene. The shade box next to the *NAT* gene indicates the *TRP1* terminator. The dark box named H3 indicates the histone H3 promoter. The P*H3::MSL1*, the *pka1Δ* P*H3::MSL1*, and the *cac1Δ* P*H3::MSL1* strains were confirmed by Southern blot analysis and the overexpression of *MSL1* was verified by Northern blot analysis with RNA extracted from wild-type (H99), *msl1Δ* (YSB13), P*H3::MSL1* (YSB1775, lane 1; YSB1776, lane 2; YSB1777, lane 3; YSB1778, lane 4; YSB1779, lane 5; YSB1780, lane 6; YSB1781, lane 7; YSB1782, lane 8), *pka1Δ* P*H3::MSL1* (YSB2042, lane 1; YSB2043, lane 2; YSB2044, lane 3; YSB2045, lane 4; YSB2046, lane 5), and *cac1Δ* P*H3::MSL1* (YSB2047, lane 1; YSB2048, lane 2). Note that *MSL1* overexpression levels were lower in the cAMP mutant backgrounds (both *cac1Δ* and *pka1Δ* mutants) than in WT, indicating that the *H3* promoter may be under control of the cAMP pathway. (B) The following strains were spotted and grown on Niger seed agar medium (0.5 % and 1.0 % glucose) at 30℃ for 3 days and photographed: WT H99 strain, P*H3::MSL1* (YSB1776), *cac1Δ*, *cac1Δ* P*H3::MSL1* (YSB2047), *pka1Δ*, *pka1Δ* P*H3::MSL1* (YSB2042). (C) Each *C. neoformans* strain [WT H99 strain, P*H3::MSL1* (YSB1776), *cac1Δ*, *cac1Δ* P*H3::MSL1* (YSB2047), *pka1Δ*, *pka1Δ* P*H3::MSL1* (YSB2042)] was cultured overnight at 30℃ in liquid YPD medium, 10-fold serially diluted (1-10^{-4} dilutions) and spotted (3 μl of dilution) on YPD agar medium and then further incubated at 37℃ and 39℃ for 2 days to monitor thermotolerance.