

SUPPLEMENTARY FIGURES

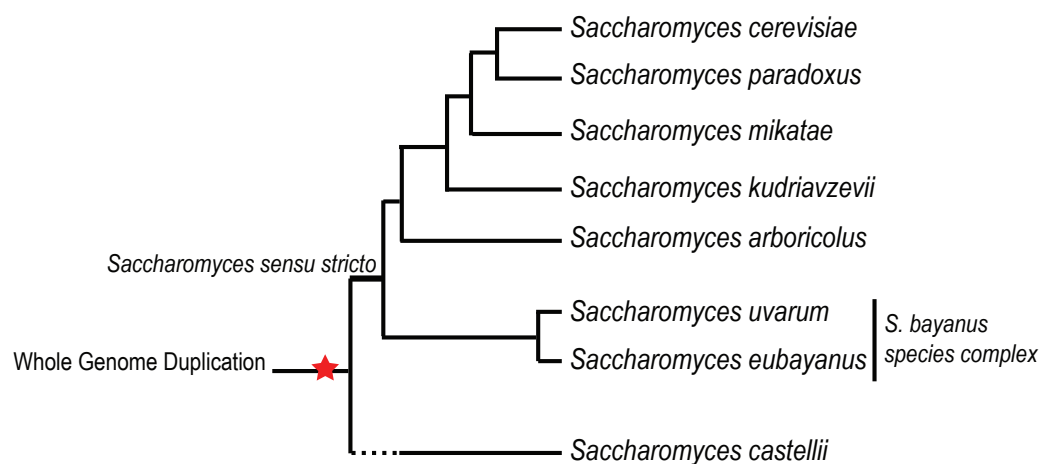


Figure S1 Closely related *Saccharomyces* species. *Saccharomyces* species inhabit a broad range of environments and exhibit different physiologies despite the short estimated-divergence time between lineages (5-20 mya).

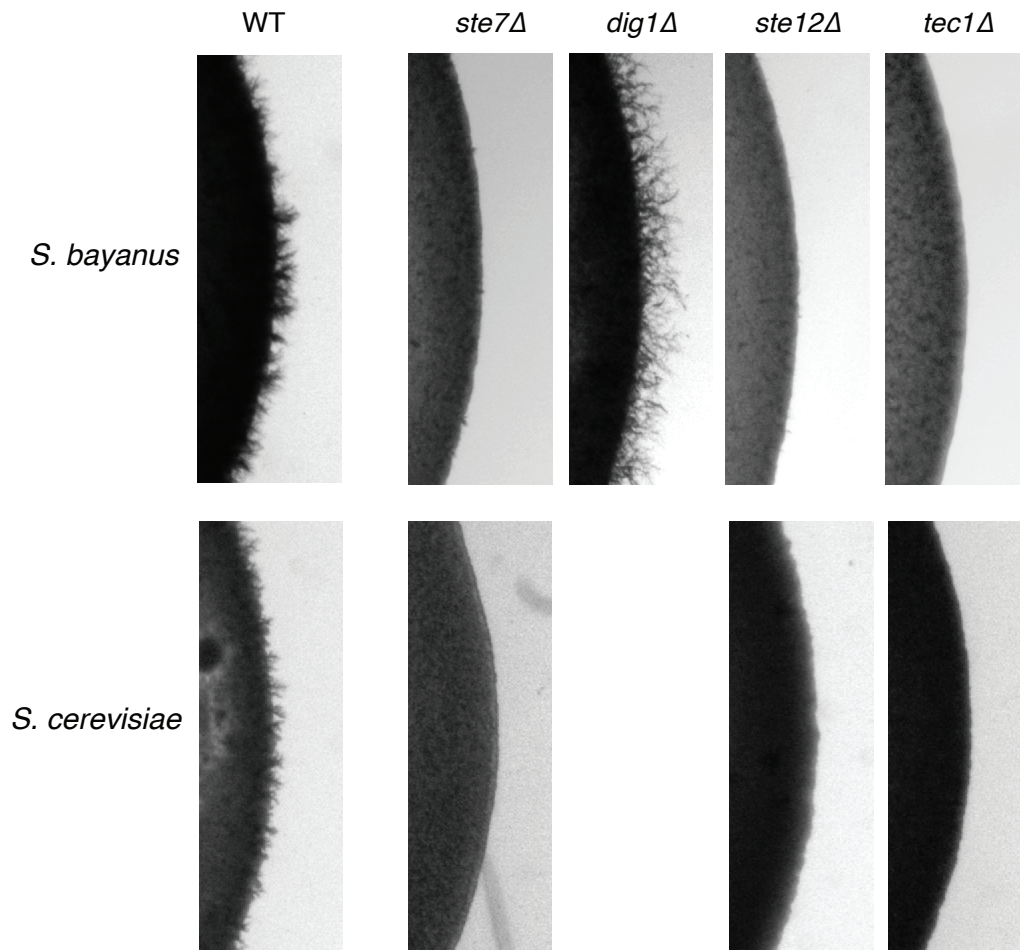


Figure S2 Disruption of MAPK signaling eliminates pseudohyphal growth in *S. cerevisiae* and *S. bayanus*, indicating that the cascade regulates the response positively in the two species. Mutants are in the Σ 1278b and NCYC365 backgrounds for *S. cerevisiae* and *S. bayanus*, respectively.

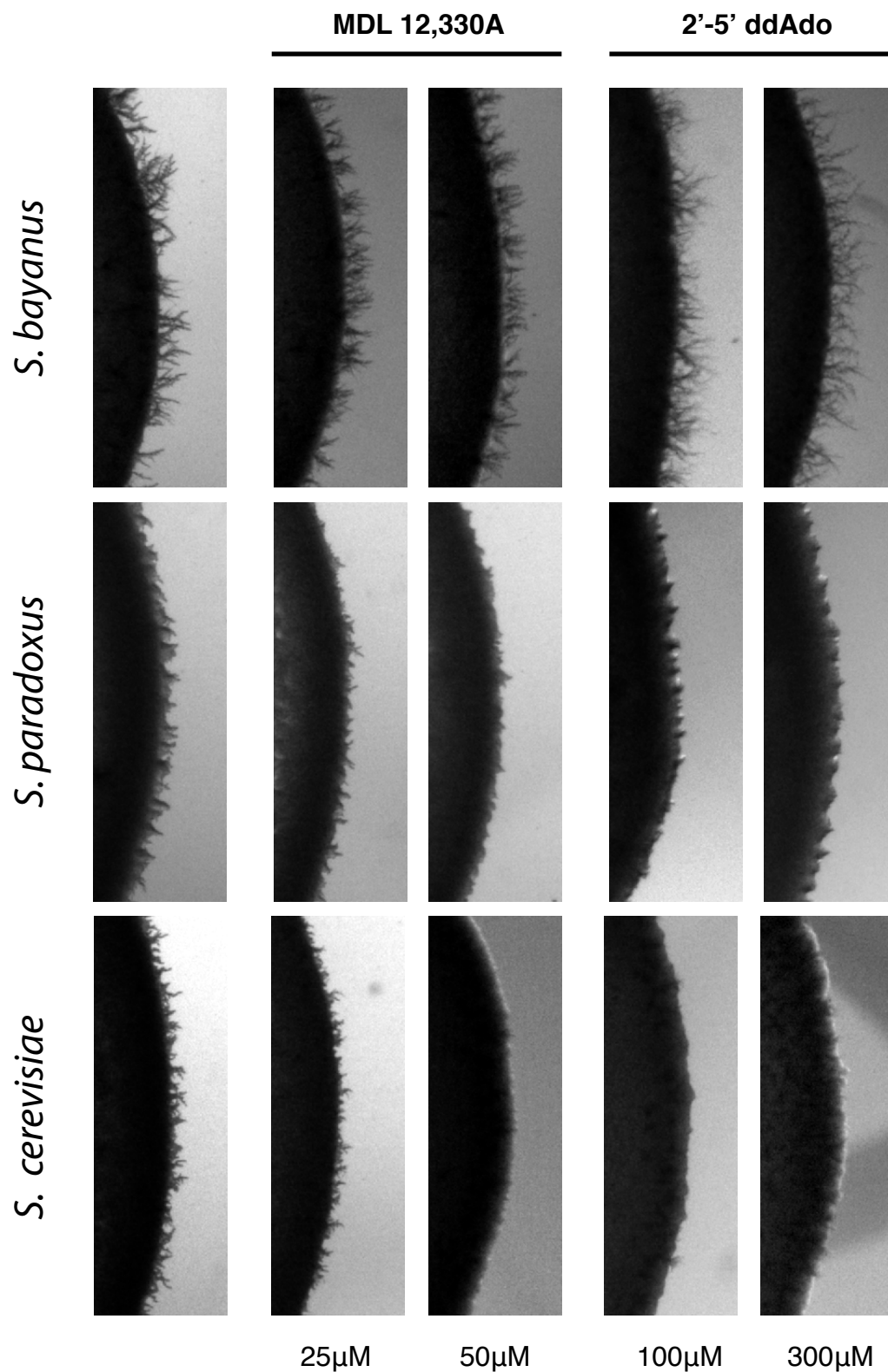


Figure S3 Pharmacological inhibition of cAMP synthesis and has divergent effect on pseudohyphal growth in *S. cerevisiae* and *S. bayanus*. MDL-12,330A and 2'-5' Dideoxyadenosine inhibit adenylate cyclase activity in different ways. MDL-12,330A prevents the membrane localization of adenylate cyclase; 2'-5' Dideoxyadenosine blocks the catalytic domain of adenylate cyclase.

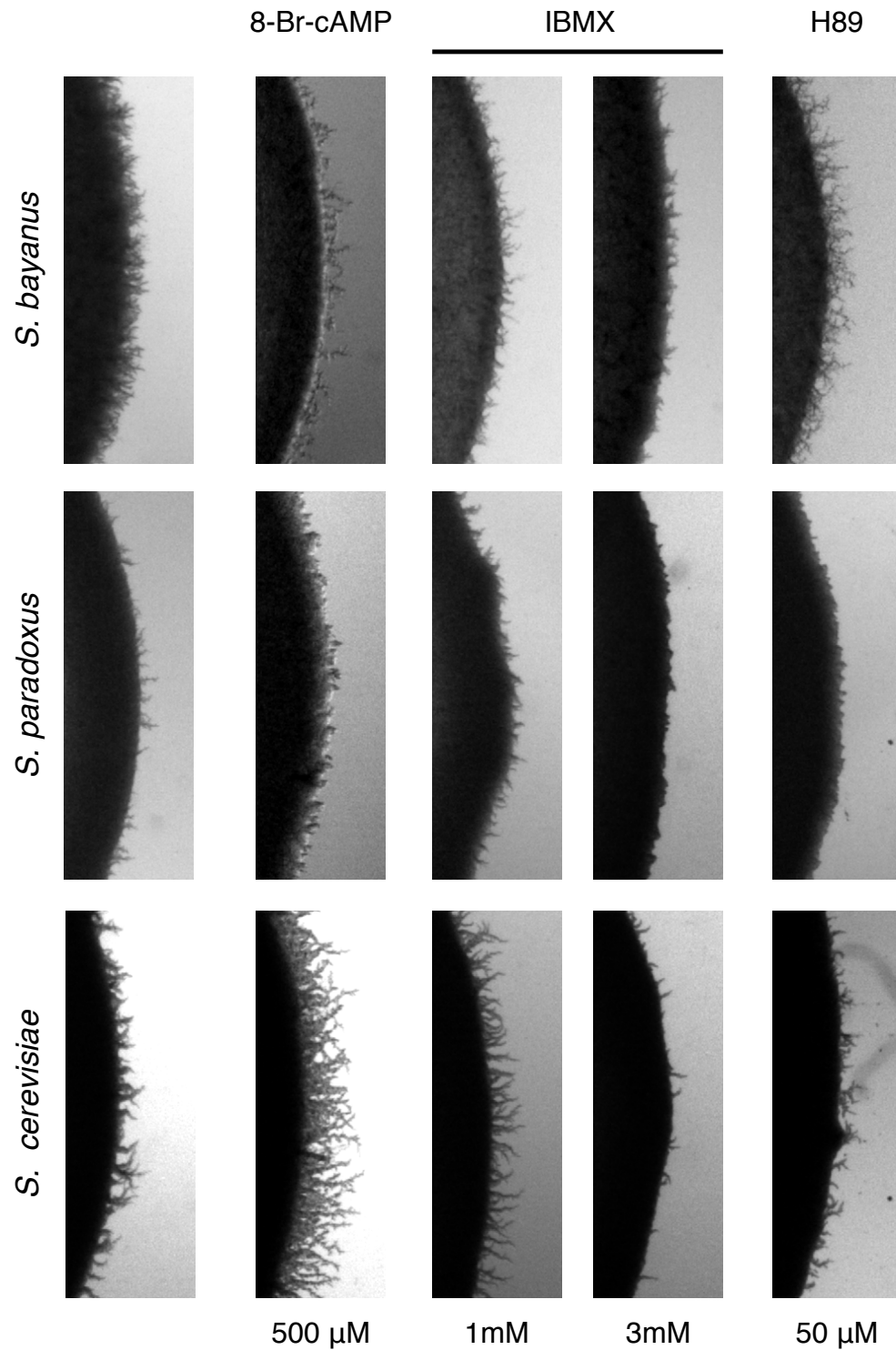


Figure S4 Pharmacological modulations of cAMP levels. Intracellular cAMP levels were modified using indicated drugs in three *Saccharomyces* species. Effects of 8-Br-cAMP were similar to exogenous cAMP treatment. Low concentrations of IBMX reflect the effects of increased intracellular cAMP levels but a higher concentration blocks the response likely due to collapse of the cAMP signaling. PKA inhibitor H89 had a strong effect on *S. paradoxus*.

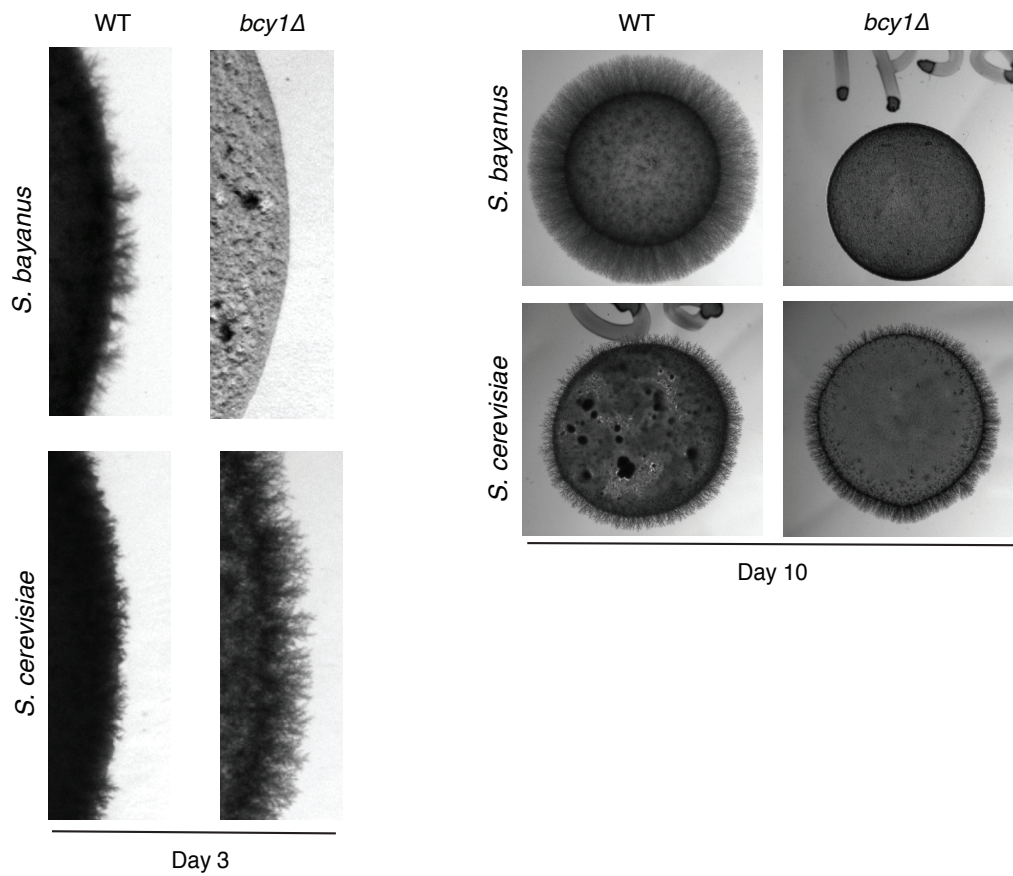


Figure S5 Hyperactivation of PKA and divergent pseudohyphal responses, *bcy1Δ* at day 3 and 10. The *bcy1Δ* mutation causes a complete loss of pseudohyphal differentiation in *S. bayanus*, consistent with effects of increased cAMP levels. The *bcy1Δ* mutant exhibits hyperfilamentation in *S. cerevisiae*.

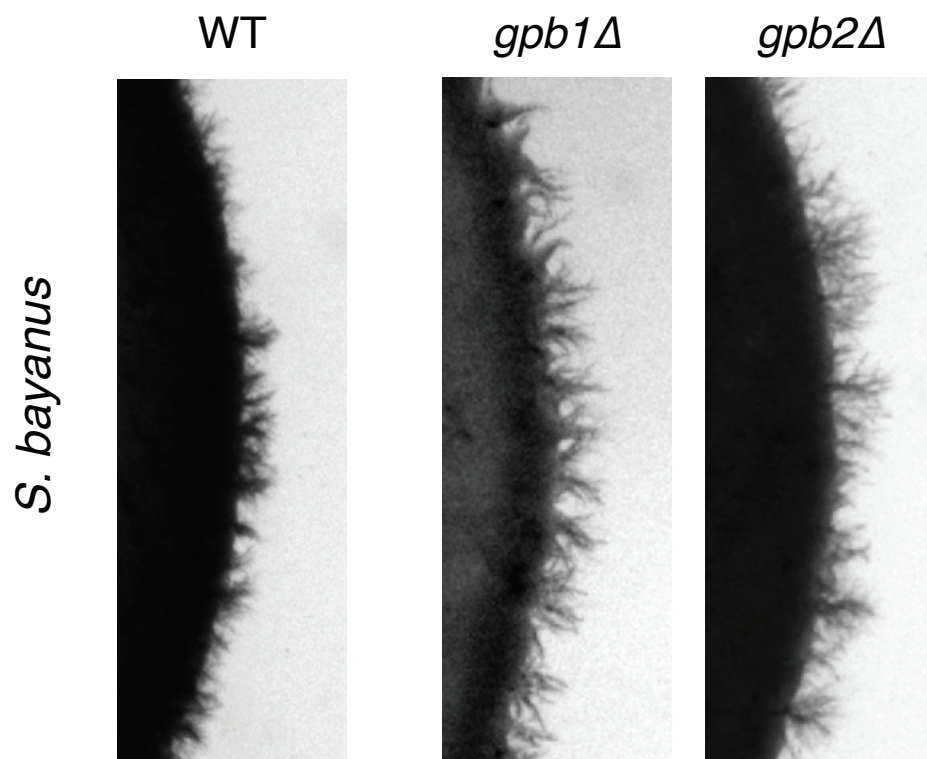


Figure S6 Deletions of GPB1 and GPB2 lead to a slight increase in filamentous growth in *S. bayanus*. The change is comparable to reported observations for the same deletion mutants in *S. cerevisiae* (see Fig. 3 in [Harashima and Heitman \(2002\)](#)).

SUPPLEMENTARY TABLES

S. cerevisiae strains surveyed for Psh response in this study

Strain Number	Strain Name	Origin	Psh	1 mM cAMP	3 mM cAMP	10 mM cAMP
PMY 011	YPS602	Oak	0	1	1	1
PMY 012	YPS606	Oak	0	1	1	1
PMY 014	YPS623	Oak	0	1	1	1
PMY 015	YPS630	Oak	0	1	1	1
PMY 017	YPS670	Oak	0	1	1	1
PMY 018	YPS681	Oak	0	1	1	1
PMY 070	EM93	Fig	1	1	1	1
PMY 072	PMY072	Vineyard	1	1	1	1
PMY 074	PMY074	Vineyard	1	1	1	1
PMY 083	PMY083	Vineyard	1	1	1	1
PMY 084	PMY084	Vineyard	1	1	1	1
PMY 086	PMY086	Vineyard	0	1	1	1
PMY 087	PMY087	Vineyard	0	1	1	1
PMY 088	PMY088	Vineyard	0	1	1	1
PMY 093	PMY093	Vineyard	0	1	1	1
PMY 094	PMY094	Vineyard	0	1	1	1
PMY 095	PMY095	Vineyard	0	1	1	1
PMY 110	PMY110	Vineyard	1	1	1	1
PMY 111	PMY111	Vineyard	1	1	1	1
PMY 112	PMY112	Vineyard	1	1	1	1
PMY 113	YJM336	Clinical	1	1	1	1
PMY 116	YJM431	Laboratory	0	0	1	1
PMY 119	YJM454	Clinical	1	1	1	1
PMY 120	F4852	Clinical	1	1	1	1
PMY 121	Y55	Laboratory	1	1	1	1
PMY 123	SK1	Laboratory	1	1	1	1
PMY 127	YJM128	Clinical	1	1	1	1
PMY 128	YJM128	Clinical	1	1	1	1
PMY 129	CBS1227	Clinical	1	1	1	1
PMY 131	YJM222	Clinical	1	1	1	1
PMY 133	YJM224	Distillery	1	1	1	1
PMY 137	VMC132B	Clinical	1	1	1	1
PMY 140	YJM277	Clinical	1	1	1	1
PMY 141	90-59	Clinical	1	1	1	1
PMY 144	YJM311	Clinical	1	1	1	1
PMY 147	YJM334	Vineyard	1	1	1	1

■ **Table S1** *S. cerevisiae* strains assayed for pseudohyphal growth (Psh) ability (0 = no pseudohyphal growth, 1 = pseudohyphal) on SLAD plates, and in the presence of exogenous cAMP added to the growth medium.

S. paradoxus strains surveyed for Psh in this study

Strain Number	Strain Name	Origin	Psh	1 mM cAMP	3 mM cAMP	10 mM cAMP
PMY 361	YPS138	Oak	0	0	1	0
PMY 362	N-43	Oak	0	1	1	1
PMY 363	CBS432	Oak	1	1	1	1
PMY 364	Y7	Oak	0	1	1	1
PMY 365	Q89.8	Oak	0	0	1	1
PMY 366	Q74.4	Oak	0	1	1	1
PMY 367	Z1	Oak	0	1	1	1
PMY 368	DBVPG6304	Drosophila	1	1	1	1
PMY 369	N-44	Oak	0	0	1	1
PMY 370	N-17	Oak	1	1	1	1
PMY 371	Y6.5	Oak	0	1	1	1
PMY 372	Q95.3	Oak	0	0	0	1
PMY 373	Q96.8	Oak	0	1	1	1
PMY 374	Y8.1	Oak	0	1	1	1
PMY 375	A4	Oak	1	1	1	1
PMY 376	N-45	Oak	0	1	1	1
PMY 377	CBS5829	Soil	1	1	1	1
PMY 378	Q32.3	Oak	1	1	1	1
PMY 379	S36.7	Oak	0	1	1	1
PMY 380	LD7	Oak	1	1	1	1
PMY 381	A12	Oak	0	0	0	1
PMY 382	IFO1804	Oak	0	0	1	1
PMY 383	DBVPG4650	Guano	1	1	1	1
PMY 384	Q59.1	Oak	0	0	1	1
PMY 385	Z1.1	Oak	0	1	1	1
PMY 386	Q31.4	Oak	1	1	1	1
PMY 387	KPN3828	Oak	1	1	1	1
PMY 388	UFRJ50791	Drosophila	0	0	0	0
PMY 389	T21.4	Oak	0	1	1	1
PMY 390	Q62.5	Oak	0	1	1	1
PMY 391	Y9.6	Oak	0	0	1	1
PMY 392	Y8.5	Oak	0	0	0	1
PMY 393	KPN3829	Oak	1	1	1	1
PMY 394	UFRJ50816	Drosophila	0	0	1	1
PMY 395	UWOPS91-917.1	Flux, <i>Myoporum</i>	0	1	1	1

■ **Table S2** *S. paradoxus* strains assayed for pseudohyphal growth (Psh) ability (0 = no pseudohyphal growth, 1 = pseudohyphal) on SLAD plates, and in the presence of exogenous cAMP added to the growth medium.

S. bayanus strains surveyed for Psh response in this study

Strain Number	Strain Name	Origin	Psh	1mM cAMP	3mM cAMP	10mM cAMP
PMY 640	NCYC365	Apple juice	1	1	0	0
PMY 641	NCYC2578	Turbid Beer	1	1	0	0
PMY 642	CBS7001	Mesophylax adopersus (insect)	1	1	0	0
PMY 643	YJM520	Fermenting juice of Taffert apples	1	1	0	0
PMY 644	YJM519	Pear Juice	0	0	0	0
PMY 645	NCYC509	Juice of Ribes nigrum (Blackcurrant)	1	1	0	0
PMY 660	GL 222	Unknown	1	1	0	0
PMY 661	GL 274	Unknown	0	0	0	0
PMY 668	VKM Y-361	Czech Wine	0	0	0	0
PMY 669	NRRL Y-969	Unknown	1	1	0	0
PMY 670	VKM Y-1146	Grape berries	1	1	0	0
PMY 734	NBRC539	Unknown	1	1	0	0
PMY 735	NCYC114	Unknown	1	1	0	0
PMY 736	NBRC10558	Must of soft fruit	0	0	0	0
PMY 911	ZP555	Oak	1	0	0	0
PMY 912	ZP556	Oak	0	0	0	0
PMY 937	NCYC686	Spoiled Coca-Cola	1	1	0	0
PMY 938	NCYC762	Palm Wine	0	0	0	0
PMY 939	NCYC1322	Irish Brewery	0	0	0	0
PMY 940	NCYC1323	Australian Brewery	1	0	0	0
PMY 941	NCYC1324	Scottish Brewery	0	0	0	0
PMY 942	NCYC1341	British Brewery	0	0	0	0
PMY 943	NCYC3066	Fermenting Saurkraut	0	0	0	0
PMY 944	NCYC3359	Cider apples	1	1	0	0
PMY 947	NCYC1326	British Brewery	0	0	0	0
PMY 948	NCYC1342	British Brewery	1	1	1	1
PMY 949	NCYC965	British Brewery	1	1	1	1
PMY 952	NCYC966	British Brewery	1	1	1	1
PMY 953	NCYC967	British Brewery	1	1	1	1
PMY 955	NCYC969	British Brewery	0	0	0	0
PMY 956	NCYC984	European Brewery	0	0	0	0
PMY 957	NCYC985	European Brewery	1	1	1	1
PMY 958	NCYC986	European Brewery	1	1	1	1
PMY 959	NCYC987	European Brewery	1	1	1	1
PMY 969	NCYC988	European Brewery	0	0	0	0
PMY 970	NCYC989	European Brewery	1	1	1	1

■ **Table S3** *S. bayanus* strains assayed for pseudohyphal growth (Psh) ability (0 = no pseudohyphal growth, 1 = pseudohyphal) on SLAD plates, and in the presence of exogenous cAMP added to the growth medium.

S. bayanus cAMP treatment

	Pseudohyphal	Non-pseudohyphal
Decrease	12	N/A
Increase	0	0
No-observable Change	10	14

S. paradoxus cAMP treatment

	Pseudohyphal	Non-pseudohyphal
Decrease	0	N/A
Increase	11	24
No-observable Change	0	0

S. cerevisiae cAMP treatment

	Pseudohyphal	Non-pseudohyphal
Decrease	0	N/A
Increase	23	13
No-observable Change	0	0

■ **Table S4** Summary of the effects of exogenous cAMP treatment on pseudohyphal growth for strains of *S. cerevisiae*, *S. paradoxus*, and *S. bayanus*.

Category	Gene	<i>S. cerevisiae</i>	<i>S. bayanus</i>
1	<i>gpa2Δ</i>	-	-
1	<i>pde2Δ</i>	∅	∅
1	<i>phd1Δ</i>	-	-
1	<i>sfl1Δ</i>	+	+
1	<i>tpk1Δ</i>	+	+
1	<i>tpk2Δ</i>	-	-
1	<i>tpk3Δ</i>	+	+
1	<i>gpb1Δ</i>	+	∅/+
1	<i>gpb2Δ</i>	+	∅/+
1	<i>ras1Δ</i>	∅	∅/-
1	<i>ras2Δ</i>	-	∅/-
2	<i>bcy1Δ</i>	+	-
2	<i>flo8Δ</i>	-	+
2	<i>flo11Δ</i>	-	∅
2	<i>ira2Δ</i>	+	-
2	<i>pde1Δ</i>	+	-

■ **Table S5** The effects on pseudohyphal growth of null mutations of genes involved in cAMP-PKA signaling in *S. cerevisiae* and *S. bayanus*. The pseudohyphal response in mutant strains was evaluated relative to the wild type phenotype in the same genetic background after 72 hours of growth on SLAD medium. Phenotypes were classified as increasing (+), decreasing (-), no change (∅), no change or slight increase (∅/+), and no-change or slight decrease (∅/-).

	% ID / Similarity S. paradoxus	% ID / Similarity S. uvarum	% ID / Similarity S. bayanus	Comparison Matrix
Bcy1	98.3 / 98.3	93.8 / 96.6	93.8 / 96.6	BLOSUM 62
Cyr1	95.1 / 97.1	89.4 / 93.9	89.4 / 93.9	BLOSUM 62
Flo8	87.0 / 90.6	NA	66.7 / 78.3	BLOSUM 62
Gbp2	72.4 / 76.8	75.8 / 85.7	75.8 / 85.7	BLOSUM 62
Gpa2	92.7 / 95.4	79.6 / 87.2	79.6 / 87.2	BLOSUM 62
Gpb1	91.1 / 95.0	79.4 / 88.9	79.4 / 88.9	BLOSUM 62
Ira2	95.5 / 98.0	NA	87.3 / 94.4	BLOSUM 62
Pde1	95.9 / 98.4	84.9 / 92.2	84.9 / 92.2	BLOSUM 62
Pde2	95.1 / 99.0	84.3 / 91.7	84.3 / 91.7	BLOSUM 62
Phd1	91.3 / 94.8	71.2 / 81.7	69.5 / 80.1	BLOSUM 62
Ras2	93.2 / 96.0	83.0 / 88.2	83.0 / 88.2	BLOSUM 62
Sfl1	92.2 / 94.7	65.4 / 71.4	75.9 / 83.9	BLOSUM 62
Tpk1	97.0 / 97.5	91.7 / 95.0	91.7 / 95.0	BLOSUM 62
Tpk2	97.9 / 99.2	82.8 / 84.2	93.5 / 95.1	BLOSUM 62
Tpk3	96.7 / 98.0	93.0 / 95.5	91.7 / 94.2	BLOSUM 62

■ **Table S6** Protein sequence alignment for key components of the cAMP-PKA signaling network indicates a high degree of conservation across the *Saccharomyces sensu stricto* clade.

Species	Strain	Genotype	Reference
<i>S. bayanus</i>	PMY 0640	NCYC365	Ludo & McCusker, 1999
	PMY 1392	<i>gpa2Δ:: kanMX</i>	This study
	PMY 1394	<i>ste7Δ:: kanMX</i>	This study
	PMY 1396	<i>flo8Δ:: kanMX</i>	This study
	PMY 1442	<i>dig1Δ:: kanMX</i>	This study
	PMY 1445	<i>ras2Δ:: kanMX</i>	This study
	PMY 1446	<i>tec1Δ:: kanMX</i>	This study
	PMY 1448	<i>tpk1Δ:: kanMX</i>	This study
	PMY 1450	<i>ste12Δ:: kanMX</i>	This study
	PMY 1455	<i>pde1Δ:: kanMX</i>	This study
	PMY 1459	<i>tpk3Δ:: kanMX</i>	This study
	PMY 1460	<i>sch9Δ:: kanMX</i>	This study
	PMY 1461	<i>pde2Δ:: kanMX</i>	This study
	PMY 1462	<i>bcy1Δ:: kanMX</i>	This study
	PMY 1479	<i>tpk2Δ:: kanMX</i>	This study
	PMY 1480	<i>rim15Δ:: kanMX</i>	This study
	PMY 1481	<i>ras1Δ:: kanMX</i>	This study
	PMY 1482	<i>gpb1Δ:: kanMX</i>	This study
	PMY 1483	<i>gpb2Δ:: kanMX</i>	This study
	PMY 1484	<i>ira2Δ:: kanMX</i>	This study
	PMY 1596	<i>msn2Δ:: kanMX</i>	This study
	PMY 1600	<i>flo11Δ:: kanMX</i>	This study
	PMY 1603	<i>phd1Δ:: kanMX</i>	This study
	PMY 1606	<i>sfl1Δ:: kanMX</i>	This study
<i>S. cerevisiae</i>	PMY 0485	<i>gpa2Δ:: kanMX</i>	Lorenz & Heitman, 1997
	PMY 0487	<i>ste12Δ:: kanMX</i>	Lorenz & Heitman, 1997
	PMY 0491	<i>flo8Δ:: kanMX</i>	Liu, <i>et.al.</i> , 1996
	PMY 0498	<i>ira2Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0499	<i>ras2Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0501	<i>tec1Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0506	<i>tpk1Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0507	<i>tpk2Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0508	<i>tpk3Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0746	<i>pde1Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0748	<i>flo11Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0750	<i>pde2Δ:: kanMX</i>	Drees, <i>et.al.</i> , 2005
	PMY 0827	<i>ste7Δ:: kanMX</i>	Lorenz & Heitman, 1997
	PMY 0843	<i>phd1Δ:: kanMX</i>	Lorenz & Heitman, 1998
	PMY 0861	<i>bcy1Δ:: kanMX</i>	Pan & Heitman, 1999
	PMY 0982	<i>rim15Δ:: kanMX</i>	Pan & Heitman, 1999
	PMY 1039	<i>msn2Δ:: kanMX</i>	Pan & Heitman, 1999
	PMY 1048	<i>sch9Δ:: kanMX</i>	Lorenz, <i>et.al.</i> , 1999
	PMY 1064	<i>sfl1Δ:: kanMX</i>	Pan & Heitman, 2002

■ **Table S7** List of mutant strains used in this study. All strains were MAT a/ α diploids.

Primer	Sequence (5'-3')
KanB	CTG CAG CGA GGA GCC GTA AT
KanC	TGATTT TGA TGA CGA GCG TAA T
SB_BCY1_AC	CCCGCTCTCGCTCAGAGGA
SB_BCY1_DC	CCCCTGCGAAATCGTGTCCATCG
SB_DIG1_AC	CCTGTGCGTGAGTTTGTGTGTTTG
SB_DIG1_DC	GGCAGGAAAATGTTTCAGAC
SB_FLO8_AC	GGCAATTCTTTGATAGCAAC
SB_FLO8_DC	CCCGTTCCTATTGTGGAAGTCG
SB_FLO11_AC	GGCATTGCTTACAATAGGTTCCCTG
SB_FLO11_DC	GGTATTGTTGAACACGGAATGG
SB_GPA2_AC	CGTCCGTGTACAGCTTCAAG
SB_GPA2_DC	GGGTATAGGACATTCACTG
SB_GPB1_AC	CCGTTTCGTTAGAATGTTT
SB_GPB1_DC	GGTCTGCGTTCAGGACTTAAC
SB_GPB2_AC	CCGTTTCGTTAGAATGTTT
SB_GPB2_DC	CCTGCGCGCAATCTGTAATC
SB_IRA2_AC	CGCAGCGTCGGTACACCCTG
SB_IRA2_DC	CTTCAACAGAAGAATGCTCCC
SB_MSN2_AC	ACACGAACACAGGTACCAC
SB_MSN2_DC	GTATCTTACTAGTTACAGGC
SB_PDE1_AC	GGTAGGTTGATTACTCATGG
SB_PDE1_DC	GGCTCAGCCAGATGCTTTGTCC
SB_PDE2_AC	GACCAAGAAATGGTATTTGC
SB_PDE2_DC	CCTCTGCATAATTCGACCTC
SB_PHD1_DC	CGGACGCTATTCTTGGCAGC
SB_PHD1_DC	CGCTTAGGTGTGGGAGATCCG
SB_RAS1_AC	GCATATGGTACCCGTAATGG
SB_RAS1_DC	GATTCGTGACAATTTCCC
SB_RAS2_AC	GCGTGTCACCAAGATTGG
SB_RAS2_DC	GGCATGCGACACCAGGAC
SB_RIM15_AC	GCCTTGGGAGAATACGTGAAAC
SB_RIM15_DC	CCTCGGTCTTAAACATGCATATG
SB_SCH9_AC	GCCTATCTTAAATCTCCTCC
SB_SCH9_DC	CGCATCGGAAAGACCAGCC
SB_SFL1_AC	CCATCGTGCATCTCCGGC
SB_SFL1_DC	CCCATGGACATCCAGTC
SB_STE7_AC	GGGAATATTCAATGCAGT
SB_STE7_DC	GGGAAACCATTCAAGTATGG
SB_STE12_AC	GGTATATCAGAAACAAGTCG
SB_STE12_DC	TGTAGTTGGTATCACCGC
SB_TEC1_AC	CGGCTCGAAGAATTTCTC
SB_TEC1_DC	GGCTAAATTCATGCAAATCC
SB_TPK1_AC	CCCAAGGCTGACCTCACC
SB_TPK1_DC	GCGTGAAAGCTTCTCATCTCC
SB_TPK2_AC	CTACATTCGGCCTATTAAAC
SB_TPK2_DC	GTGACTCGGCACTTCTGTCTG
SB_TPK3_AC	CGCTGGCTCATGTATCCAT
SB_TPK3_DC	GCCAGGTTGCTTTTAGC

■ **Table S8** List of primers that were used for confirmation-PCR to validate deletion of the targeted locus for each null mutant.