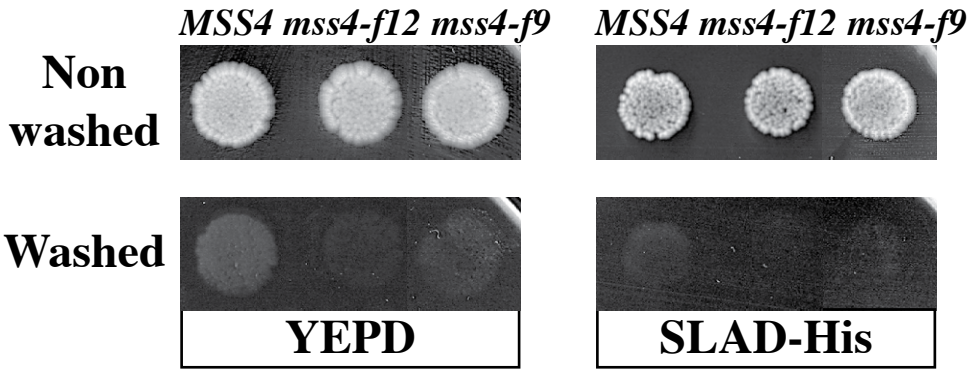

Fig. S1. The *mss4-f9* and *mss4-f12* mutants are defective for haploid invasive growth in glucose and low ammonia media. Indicated strains were spotted on YEPD and SLAD-His media and grown for 5 days. Cells on the surface of the agar were washed off revealing cells that had invaded the agar surface. Spots are from same agar plate. Similar results were observed in 2 independent experiments and representative images are shown.

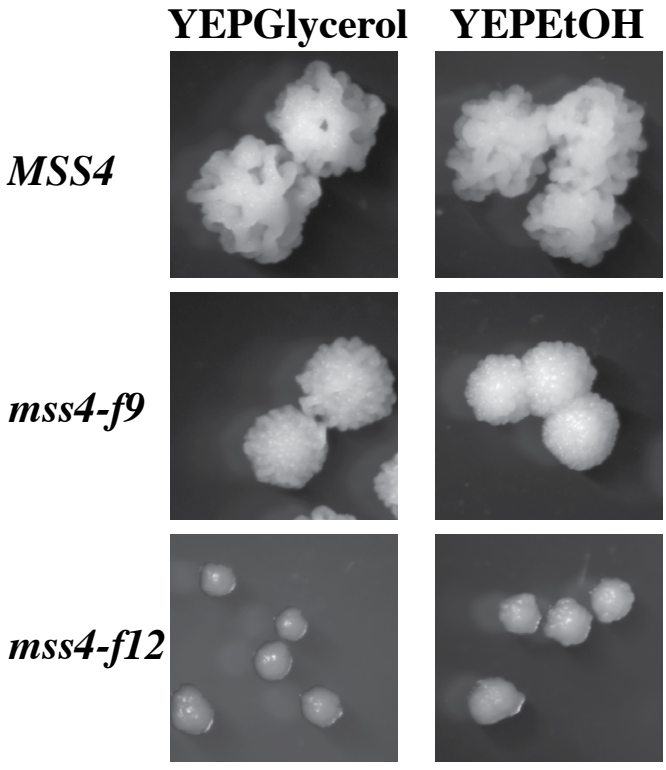

Fig. S2. *mss4* mutants are defective in colony crenelation on glycerol and EtOH containing media. Enlarged images of colonies of indicated strains grown on YEPGlycerol and YEPEtOH media for 5 days are shown. Similar results were observed in 3 independent experiments and representative images are shown.

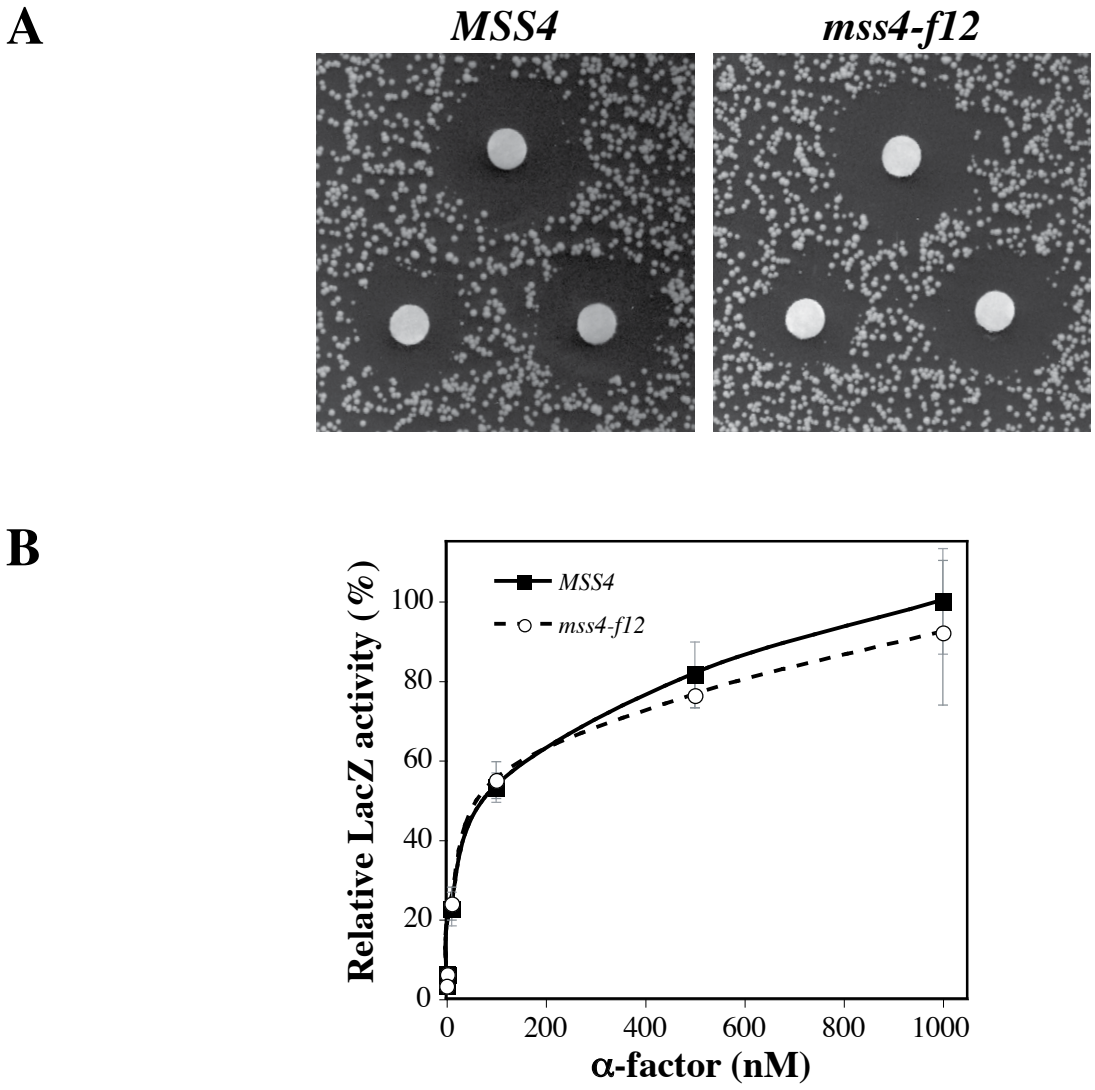

Fig. S3. *mss4-f12* cells respond to mating pheromone. *mss4-f12* cells arrest growth in the presence of mating pheromone similar to wild-type cells. α-factor (1, 0.5, and 0.2 μg) was spotted on filters placed on a lawn of the indicated *MAT**a*** strain. Plates were incubated for 2 days. Measurements of the halo diameter indicated <5% difference between *mss4-f12* and *MSS4* halos. Similar results were observed in 2 independent experiments with 2 independent *mss4-f12* and *MSS4* strains. B) *mss4-f12* cells induce the mating-specific *FUS1* gene similar to wild-type cells. Cells containing a *FUS1-lacZ* plasmid (pSG231) were incubated with the indicated α-factor concentration for 1 h and LacZ activity was determined. Values were normalized to the wild-type treated with 1000 nM α-factor (0.48 β-galactosidase units [μg protein]$^{-1}$ min^{-1}) and are the averages of two independent experiments each with two clones, each in duplicate and triplicate determinations, s.d. indicated.

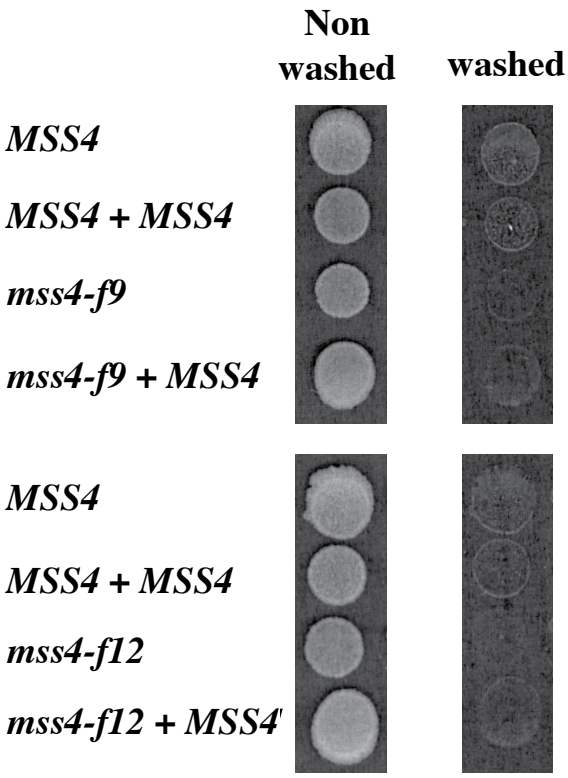

Fig. S4. *Mss4* mutant invasive growth defect is recessive. Complementation of the invasive growth defect was examined using an *mss4*Δ strain carrying either *MSS4*, *mss4-f9* or *mss4-f12* (RAY1986, RAY1991 and RAY2005, respectively) with or without the p415MSS4pMSS4. Indicated strains were grown on YEPD media for 5 days and invasive growth was assessed as in Fig. S1. Similar results were observed in 2 independent experiments and representative images are shown.

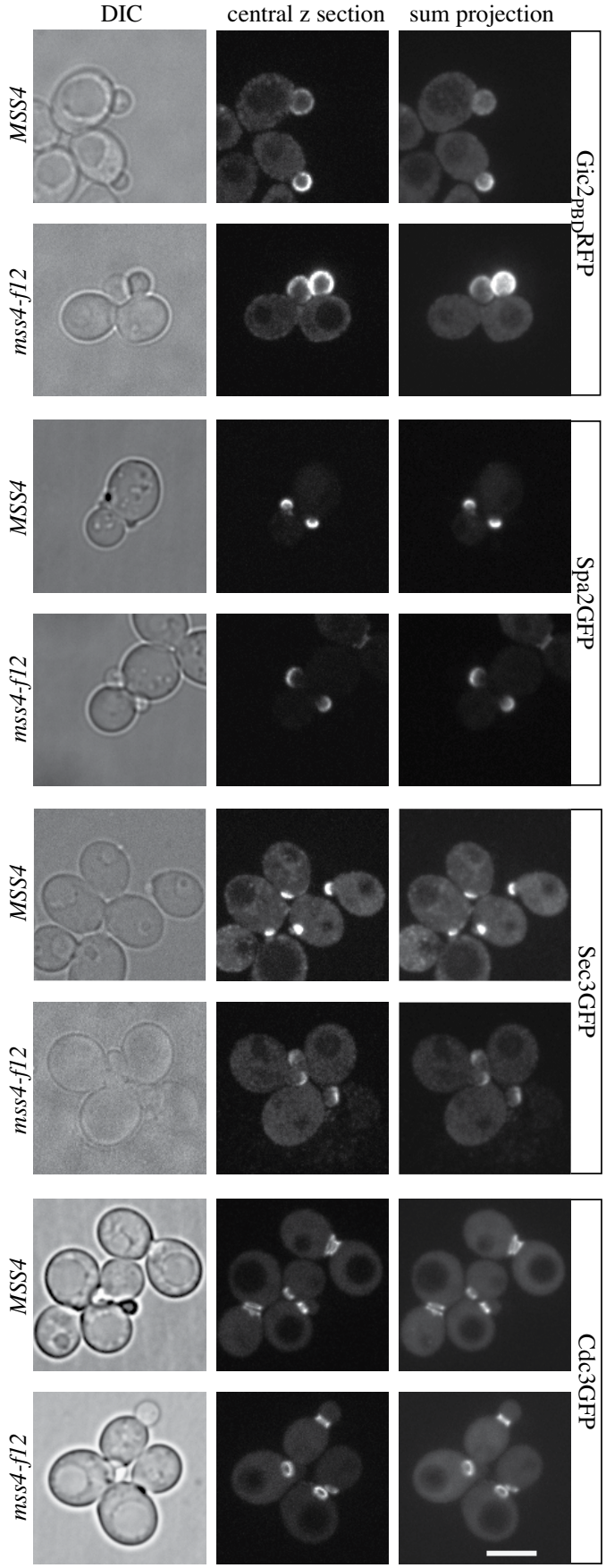

Fig. S5. Active Cdc42p, polarisome, exocyst and septin subunits localization is unaffected in *mss4-f12* cells. Indicated strains expressing the indicated RFP/GFP fusion protein were grown in selective media with 2% glucose. Spinning disk confocal fluorescence and DIC images were taken and central z-sections and sum projections (8-12 z-sections) of representative cells are shown. Similar results were observed in 3 independent experiments.

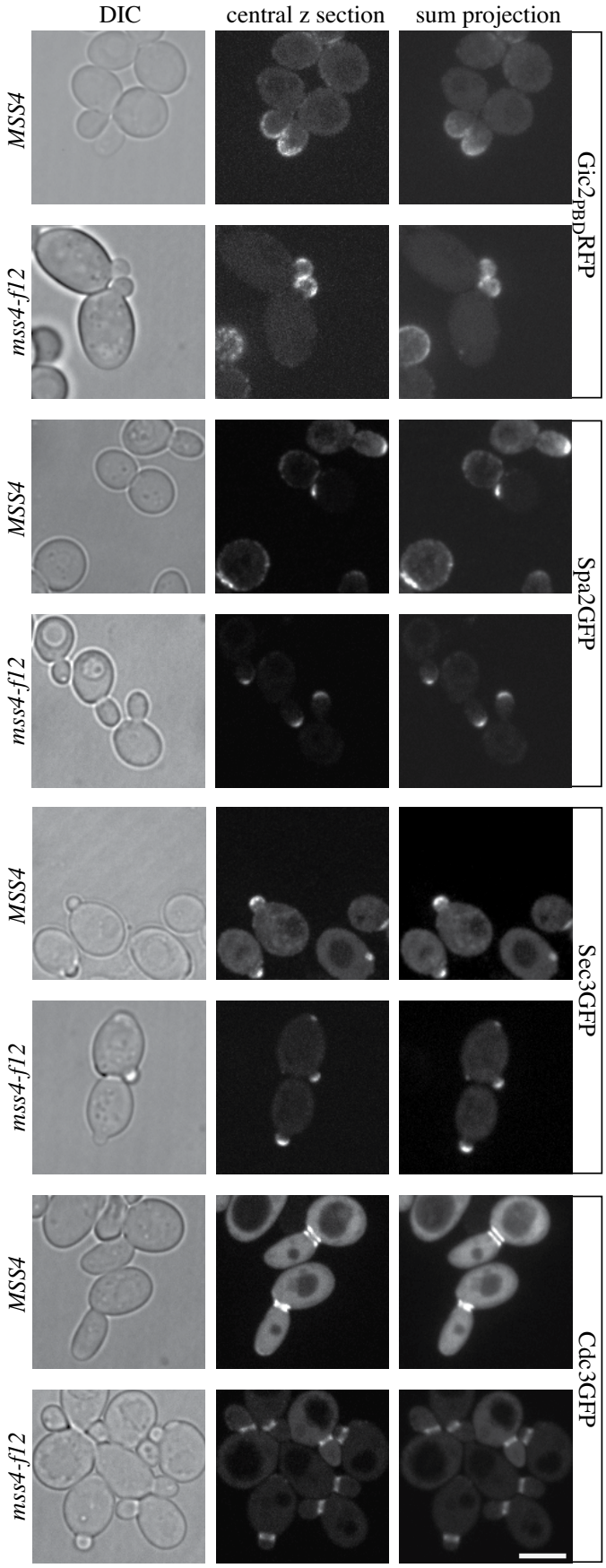

Fig. S6. Active Cdc42p, polarisome, exocyst and septin subunits localization is unaffected in *mss4-f12* cells in limiting glucose. Indicated strains expressing the indicated RFP/GFP fusion protein grown in selective media were back diluted into media containing 0.2% glucose and grown for an additional 6-7 hr. Spinning disk confocal fluorescence and DIC images were taken and central z-sections and sum projections (8-12 z-sections) of representative cells are shown. Similar results were observed in 2 independent experiments.

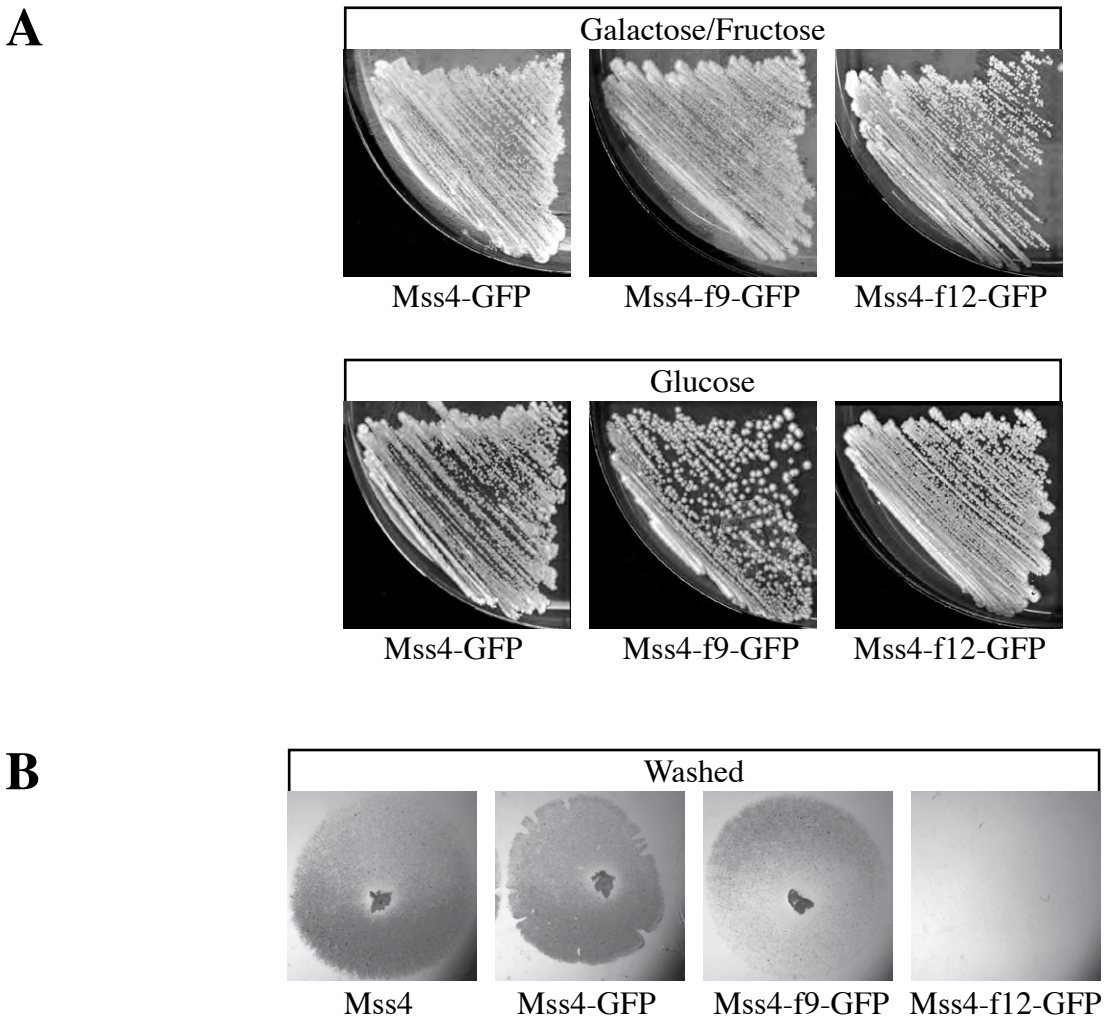

Fig. S7. Mss4, Mss4-f9 and Mss4-f12 GFP fusions are functional. A) RAY1885 carrying Mss4-GFP, Mss4-f9-GFP or Mss4-f12-GFP were grown on galactose or glucose containing media for 2 days. B) An *mss4*Δ strain carrying Mss4, Mss4-GFP, Mss4-f9-GFP or Mss4-f12-GFP was grown on YEP0.2%D media for 5 days and invasive growth was assessed as in Fig. S1.

Table S1. Yeast strains used in this study

Strain	Genotype	
10560-6B	*MAT ura3-52 trp1::hisG leu2::hisG his3::hisG*	(Roberts et al., 1997)
JY426	*MAT**a**, leu2-3,-112 ura3-52 his4-34 fus1- 1 fus2- 3*	Cold Spring Harbor
SEY6211	*MAT**a**, leu2-3,-112 ura3-52 his3- 200 trp1- 901 ade2 suc2- 9*	S. Emr
RAY876	SEY6211 *URA3*	This study
RAY1563	JY426 with pRS406GFPBud1	This study
RAY1885	10560-6B *mss4- 1::HIS5Sp* with p416GALpMSS4	This study
RAY1940	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pMSS4GFP	This study
RAY1941	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pmss4f9GFP	This study
RAY1942	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pmss4f12GFP	This study
RAY1945	*MAT /**a** ura3-52/ura3-52 trp1::hisG/trp1::hisG leu2::hisG/leu2::hisG his3::hisG/his3::hisG mss4- 1::HIS5Sp/mss4- 1::HIS5Sp* p414MSS4pMSS4	This study
RAY1949	*MAT /**a** ura3-52/ura3-52 trp1::hisG/trp1::hisG leu2::hisG/leu2::hisG his3::hisG/his3::hisG mss4- 1::HIS5Sp/mss4- 1::HIS5Sp* p414MSS4pmss4f12	This study
RAY1986	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pMSS4	This study
RAY1990	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pMSS4	This study
RAY1991	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pmss4f12	This study
RAY1992	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pmss4f12	This study
RAY1993	10560-6B *mss4- 1::HIS5Sp URA3::GALpGFP-PH^{Plc}-PH^{Plc}-GFP* with p414MSS4pmss4f12	This study
RAY1999	10560-6B *mss4- 1::HIS5Sp URA3::GALpGFP-PH^{Plc}-PH^{Plc}-GFP* with p414MSS4pmss4f12	This study
RAY2001	10560-6B *mss4- 1::HIS5Sp URA3::GALpGFP-PH^{Plc}-PH^{Plc}-GFP* with p414MSS4pMSS4	This study
RAY2003	10560-6B *mss4- 1::HIS5Sp URA3::GALpGFP-PH^{Plc}-PH^{Plc}-GFP* with p414MSS4pMSS4	This study
RAY2005	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pmss4f9	This study
RAY2006	10560-6B *mss4- 1::HIS5Sp* with p414MSS4pmss4f9	This study
RAY2012	*MAT**a** ura3-52 trp1::hisG leu2::hisG his3::hisG mss4- 1::HIS5Sp*	This study

	p414MSS4pMSS4	
RAY2013	*MAT**a** ura3-52 trp1::hisG leu2::hisG his3::hisG mss4- 1::HIS5Sp* p414MSS4pMSS4	This study
RAY2014	*MAT**a** ura3-52 trp1::hisG leu2::hisG his3::hisG mss4- 1::HIS5Sp* p414MSS4pmss4f12	This study
RAY2015	*MAT**a** ura3-52 trp1::hisG leu2::hisG his3::hisG mss4- 1::HIS5Sp* p414MSS4pmss4f12	This study

Table S2. Primers used in this study

#	Primer name	Primer sequence	Mutation	Restriction site
1	Mss4pBamHI	CGCGGATCCATGTCAGTCTTGCGATCAC		
2	Mss4mNotI	ATAGTTAGCGGCCGCACTCAGTCTTTATAATTTTTC		
3	Mss4pSalI	CGCCTTGTCGACCATCGTGAGTTAAGG		
4	Mss4pAatIInostop	CCTAACCAGAAAAATTATAAAGACGTCGTGCGGCCGCCACCGCGGTGG		
5	Mss4mAatIInostop	CCACCGCGGTGGCGGCCGCACGACGTCTTTATAATTTTTCTGGTTAGG		
6	AatIIpGAyeGFP	AAAGACGTCGGAGCAGGTGCTGGTGCTGG		
7	ADHtNotI	TCCCCGCGGTGGCGGCCGCGTTATCCCTAGCGGATCTG		
8	Mss4C12pT1717C	GATTTAAAAGGTCCCACATGGGGCCGTTTTACCAATCTAGATAAAG	S573P	XbaI
9	Mss4C12mT1717C	CTTTATCTAGATTGGTAAAACGGCCCCATGTGGGACCTTTTAAATC	S573P	XbaI
10	Mss4KOp	GTTTACACCCCCCGAGACAGTTGCCCTATATCGCTTTTCCCTATCAATAGTTTCTAACTCCGGTGGCGGCCGCTCTAG		
11	Mss4KOm	ATTAAATCAAAGTAGATTAGACTGAGTACATAGACGATAGGTTATTTACCTGTGCCCTACCCTCGAGGTCGACGGTATC		
12	Mss4pNheI-3	TCCCTATCAATAGTTTCGCTAGCATGTCAGTCTTGCGATCACAAC		NheI
13	Mss4mNheI-3	GTTGTGATCGCAAGACTGACATGCTAGCGAAACTATTGATAGGGA		NheI
14	Mss4pBglII607	TTTACTAAACAAGCGCGTTTCGAGGAGATCTTCCAGAATATCGGC		BglII
15	Mss4mBglII607	GCCGATATTCTGGAAGATCTCCTCGAAACGCGCTTGTTTAGTAAA		BglII
16	Mss4pAatII1401	GATTATTTGGTTTCGTTGACGTCCAAATACATTTTGAGTGAGTTGA		AatII
17	Mss4mAatII1401	TCAACTCACTCAAAATGTATTTGGACGTCAACGAAACCAAATAATC		AatII

18	Mss4pXhoI1804	GAAAGATTTAAATTGGCTCGAGGAAGGTCAGAAAATTAAATTCGG		XhoI
19	Mss4mXhoI1804	CCGAATTTAATTTTCTGACCTTCCTCGAGCCAATTTAAATCTTTC		XhoI
20	Mss4C9pA1768T	GGCGAAAGATTGATCATATAGGCCTGTGATGAGAGATCTAAATTGGC	R590*	BglII
21	Mss4C9mA1768T	GCCAATTTAGATCTCTCATCACAGGCCTATATGATCAATCTTTCGCC	R590*	BglII
22	Mss4pAatII1767	GGATAAAGAAAGGTTGGCGAAAGACGTCTCATATAGGCCTGTGATGAAAGATTTAAATTGGC	R590D S591V	AatII
23	Mss4mAatII1767	GCCAATTTAAATCTTTCATCACAGGCCTATATGAGACGTCTTTCGCCAACCTTTCTTTATCC	R590D S591V	AatII
24	Mss4pAatIIstop	CCTAACCAGAAAAATTATAAAGACGTCTGAGTGCGGCCGCCACCGCGGTGG	*780V 781*	AatII
25	Mss4mAatIIstop	CCACCGCGGTGGCGGCCGCACTCAGACGTCTTTATAATTTTTCTGGTTAGG	*780V 781*	AatII
26	Mss4C22pT1697A	CCACCACACTTAGACATTCACAATACTTATGATTTAAAAGGATCCATATGGGG	I566N	BamHI
27	Mss4C22mT1697A	CCCCATATGGATCCTTTTAAATCATAAGTATTGTGAATGTCTAAGTGTGGTGG	I566N	BamHI
28	Mss4C22pC1721T	CTTATTTCCACCACATCTAGACATTCACAATACTTATGATTTAAAAGGTTCC	T574I	XbaI
29	Mss4C22mC1721T	GGAACCTTTTAAATCATAAGTATTGTGAATGTCTAGATGTGGTGGAAATAAG	T574I	XbaI
30	MSS4clone9p	GCGAAAGATAGATCATATAGGCCTGTGATGAGAGATTTAAATTGGCTTGAAGAAGGTCAG	K597R	
31	MSS4clone9m	CTGACCTTCTTCAAGCCAATTTAAATCTCTCATCACAGGCCTATATGATCTATCTTTCGC	K597R	

Table S3. Plasmids used in this study

Plasmid	Vector	Insert	Source
pGEX-6P	pGEX-6P		Amersham
p406	pRS406		(Sikorski and Hieter, 1989)
p415	pRS415		(Sikorski and Hieter, 1989)
p416	pRS416		(Sikorski and Hieter, 1989)
pCM188	pCM188		(Gari et al., 1997)
pBSLoxPHisSpLoxp	pBS	LoxPHis5SpLoxP	(Nern and Arkowitz, 1998)
p406GALp	pRS406	GAL1-10 promoter	This study
p416GALp	pRS416	GAL1-10 promoter	This study
p416GalCdc24HAGFP	pRS416Gal	Cdc24HAGFP	(Nern and Arkowitz, 2000a)
p416GALpMSS4	pRS416GALp	MSS4	This study
pCM188TetpMSS4	pCM188	Tetp-MSS4	This study
p414MSS4pMSS4	pRS414	MSS4pMSS4	This study
p415MSS4pMSS4	pRS415	MSS4pMSS4	This study
p414MSS4pMSS4-AII	pRS414	MSS4pMSS4-AII	This study
p414MSS4pMSS4GFP	pRS414	MSS4pMSS4GFP	This study
p414MSS4pmss4f9GFP	pRS414	MSS4pmss4f9 GFP	This study
p414MSS4pmss4f12GFP	pRS414	MSS4pmss4f12GFP	This study
pExpARG-pADH1GFP-PH^{Plc}-PH^{Plc}-GFP	pExpARG-pADH1	GFP-PH^{Plc}-PH^{Plc}-GFP	(Vernay et al., 2012)
p406GALpGFP-PH^{Plc}-PH^{Plc}-GFP	pRS406	GFP-PH^{Plc}-PH^{Plc}-GFP	This study
p414MSS4pMSS4*	pRS414	MSS4pMSS4*	This study
p414MSS4pmss4f9	pRS414	MSS4p-mss4-f9	This study
p414MSS4pmss4f12	pRS414	MSS4pmss4-f12	This study

p414MSS4pMSS4-590AII	pRS414	MSS4pMSS4-590AII	This study
p414MSS4pMSS4-590AII-AII	pRS414	MSS4pMSS4-590AII-AII	This study
p414MSS4pmss4f9	pRS414	MSS4pmss4f9	This study
pGEX-6PMSS4	pGEX-6P	MSS4	This study
Flo11 lacZ (B3782)	YEp355	3 kbp-*FLO11::LacZ* in YEp355	(Rupp et al., 1999)
pSG231	*URA3* CEN	FUS1-LacZ	(Trueheart et al., 1987)
YIp211-GIC2-PBD-RFP	YIp211	GIC2-PBD-1.5tdTomato	(Tong et al., 2007)
p406GFPBud1	pRS406	GFPBUD1	(Nern and Arkowitz, 2000b)
p406Spa2GFP	pRS406	Spa2	(Arkowitz and Lowe, 1997)
p316Sec3GFP	pRS316	Sec3GFP	(Finger et al., 1998)
p316Cdc3GFP	pRS316	Cdc3GFP	(Caviston et al., 2003)
pSL1509	*URA3* CEN	*ste11-4*	(Stevenson et al., 1992)