

Figure S1. Phosphorylation of S533 and S563 by Dbf2-Mob1 is essential in the absence of Cyk3.

A) Genetic interactions of the indicated genotypes. Cells contain an *URA3* based plasmid carrying wild type *CYK3* and were grown in glucose (2%) containing medium at 23°C. Serial dilutions were spotted onto glucose (2%) containing plates without or with 5-FOA, which selects against the wild type *CYK3* containing *URA3* plasmid. Plates were incubated at 23°C.

B) Kinase assay show the *in vitro* phosphorylation of Hof1-(aa521-586), Hof1-(aa521-586)-S533A and Hof1-(aa521-586)-S563A by Dbf2-Mob1.

C) Quantification of (B). See material and methods for details.

Figure S2. Chs2 and Inn1 are stabilized at the cell division site in *hof1-4A* upon depletion of *CYK3*.

A-B) Localization of Inn1-GFP and Chs2-GFP after overexpression (galactose, Gal) or repression (glucose, Glu) of pGal1-*CYK3* in *HOF1*, *hof1-4A*, *hof1-4E* mutants. Immunoblots show Inn1-GFP, Chs2-GFP and 3HA-Cyk3 levels after overexpression and repression of pGal1-3HA-*CYK3*. Scale bars 5µm.

Table S1. Yeast strains used in this study

ESM356	MATa <i>ura3-52 leu2Δ1 his3Δ200 trp1Δ63</i>	(Pereira et al., 2001)*
MFY289	ESM356 <i>hof1-4A-GFP-hphNT1</i>	this study
MFY572	YPH499 <i>hof1-4A-GFP-hphNT1 Δcyk3::HIS3MX6 pRS316-CYK3</i>	this study
MFY577	ESM356 <i>hof1-4E-GFP-hphNT1</i>	this study
MFY622	ESM356 <i>hof1-4A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY623	ESM356 <i>hof1-4E-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY1061	ESM356 <i>dbf2-2 dbf20Δ::HIS3MX6 HOF1-GFP-hphNT1 SHS1-Cherry-kanMX6</i>	(Meitinger et al., 2011)
MFY1062	ESM356 <i>dbf2-2 dbf20Δ::HIS3MX6 hof1-4E-GFP-hphNT1 SHS1-Cherry-kanMX6</i>	this study
MFY1113	ESM356 <i>dbf2-2 dbf20Δ::HIS3MX6 HOF1-GFP-hphNT1 CDC10-TAP-kanMX6</i>	this study
MFY1126	ESM356 <i>HOF1-GFP-hphNT1</i>	this study
MFY1136	ESM356 <i>hof1-W637A-GFP-hphNT1</i>	this study
MFY1138	ESM356 <i>dbf2-2 dbf20Δ::HIS3MX6 ura3-52::Yiplac211-Gal1-SIC1 (T5V, T33V, S76A)-HA hof1-4E-GFP-hphNT1</i>	(Meitinger et al., 2011)
MFY1143	ESM356 <i>dbf2-2 dbf20Δ::HIS3MX6 HOF1-3HA-TRP1 CDC10-TAP-kanMX6</i>	this study
MFY1144	ESM356-1, <i>dbf2-2 dbf20Δ::HIS3MX6 hof1-4E-3HA-TRP1 CDC10-TAP-kanMX6</i>	this study
MFY1160	ESM356 <i>hof1-4E-GFP-hphNT1 SHS1-Cherry-kanMX6</i>	(Meitinger et al., 2011)
MFY1164	ESM356 <i>HOF1-GFP-hphNT1 SHS1-Cherry-kanMX6</i>	(Meitinger et al., 2011)
MFY1182	ESM356 <i>Δhof11::HIS3MX6 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY1185	ESM356 <i>hof1-4A-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	(Meitinger et al., 2011)
MFY1186	ESM356 <i>hof1-4E-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	(Meitinger et al., 2011)
MFY1187	ESM356 <i>hof1-W637A-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	(Meitinger et al., 2011)
MFY1189	ESM356 <i>HOF1-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	(Meitinger et al., 2011)
MFY1237	ESM356 <i>hof1-Δ2-299-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	(Meitinger et al., 2011)
MFY1238	ESM356 <i>hof1-Δ2-399-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY1271	ESM356 <i>hof1-4A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3 SHS1-Cherry-kanMX6</i>	this study
MFY1374	YPH499 <i>hof1-4A-GFP-hphNT1 Δcyk3::HIS3MX6 pRS316-CYK3 Δcts1::TRP1</i>	this study
MFY1375	YPH499 <i>hof1-4A-GFP-hphNT1 Δcyk3::HIS3MX6 pRS316-CYK3 Δegt2::TRP1</i>	this study
MFY1376	YPH499 <i>hof1-4A-GFP-hphNT1 Δcyk3::HIS3MX6 pRS316-CYK3 Δdse2::TRP1</i>	this study
MFY1377	YPH499 <i>hof1-4A-GFP-hphNT1 Δcyk3::HIS3MX6 pRS316-CYK3 Δscw11::TRP1</i>	this study
MFY1466	ESM356 <i>hof1-S313E-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY1467	ESM356 <i>hof1-S421E-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY1469	ESM356 <i>hof1-S533E-S563E-GFP-hphNT1 SHS1-Cherry-kanMX6 ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY1420	ESM356 <i>dbf2-2 dbf20Δ::HIS3MX6 ura3-52::Yiplac211-Gal1-SIC1 (T5V, T33V, S76A)-HA hof1-313E-GFP-hphNT1</i>	this study
MFY1433	ESM356 <i>HOF1-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY1472	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δcla4::HIS3MX6</i>	this study
MFY1474	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δgin4::HIS3MX6</i>	this study
MFY1475	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δkcc4::HIS3MX6</i>	this study
MFY1476	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δrts1::HIS3MX6</i>	this study
MFY1477	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δhsl1::HIS3MX6</i>	this study
MFY1505	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δshs1::HIS3MX6</i>	this study
MFY1868	ESM356 <i>hof1-W637A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY1870	ESM356 <i>hof1-4A-W637A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY2003	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δgin4::HIS3MX6 pRS315-HOF1 SHS1-GFP-hphNT</i>	this study
MFY2004	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δgin4::HIS3MX6 pRS315-hof1-4A SHS1-GFP-hphNT</i>	this study
MFY2005	YPH499 <i>Δhof1::TRP1 pRS316-HOF1 Δgin4::HIS3MX6 pRS315-hof1-4E SHS1-GFP-hphNT</i>	this study
MFY2007	ESM356 <i>hof1-S313E-GFP-hphNT1 SHS1-Cherry-kanMX6</i>	this study
MFY2008	YPH499 <i>pRS316-HOF1 Δgin4::HIS3MX6 hof1-4A-GFP-hphNT</i>	this study
MFY2009	YPH499 <i>pRS316-HOF1 Δgin4::HIS3MX6 hof1-4E-GFP-hphNT</i>	this study
MFY2010	YPH499 <i>pRS316-HOF1 Δgin4::HIS3MX6 hof1-S313E-GFP-hphNT</i>	this study

MFY2011	YPH499 pRS316- <i>HOF1</i> Δ <i>shs1::HIS3MX6 hof1-4A-GFP-hphNT</i>	this study
MFY2012	YPH499 pRS316- <i>HOF1</i> Δ <i>shs1::HIS3MX6 hof1-4E-GFP-hphNT</i>	this study
MFY2013	YPH499 pRS316- <i>HOF1</i> Δ <i>shs1::HIS3MX6 hof1-S313E-GFP-hphNT</i>	this study
MFY2084	ESM356 <i>hof1-S533A-S563A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY2085	ESM356 <i>hof1-S313A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY2086	ESM356 <i>hof1-S517A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY2087	ESM356 <i>hof1-S517A-W637A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY2088	ESM356 <i>hof1-S533A-S563A-W637A-GFP-hphNT1 natNT1-Gal1-3HA-CYK3</i>	this study
MFY2089	ESM356 <i>hof1-Δ2-279-GFP-hphNT1 SHS1-Cherry-kanMX6</i> <i>ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY2090	ESM356 <i>hof1-Δ293-333-GFP-hphNT1 SHS1-Cherry-kanMX6</i> <i>ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY2098	ESM356 <i>hof1-Δ2-279-S313E-GFP-hphNT1 SHS1-Cherry-kanMX6</i> <i>ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
MFY2099	ESM356 <i>hof1-S313E-W637A-GFP-hphNT1 SHS1-Cherry-kanMX6</i> <i>ura3-52::URA3-Gal1-CLB2-ΔDB</i>	this study
SGY37	<i>MATa leu2 ADE2 ura3-52::URA3-lexA-op-LacZ his3 trp1</i>	(Geissler et al., 1996)
YPH499	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1</i>	(Sikorski and Hieter, 1989)
YPH500	<i>MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1</i>	(Sikorski and Hieter, 1989)

Table S2. Plasmids used in this study

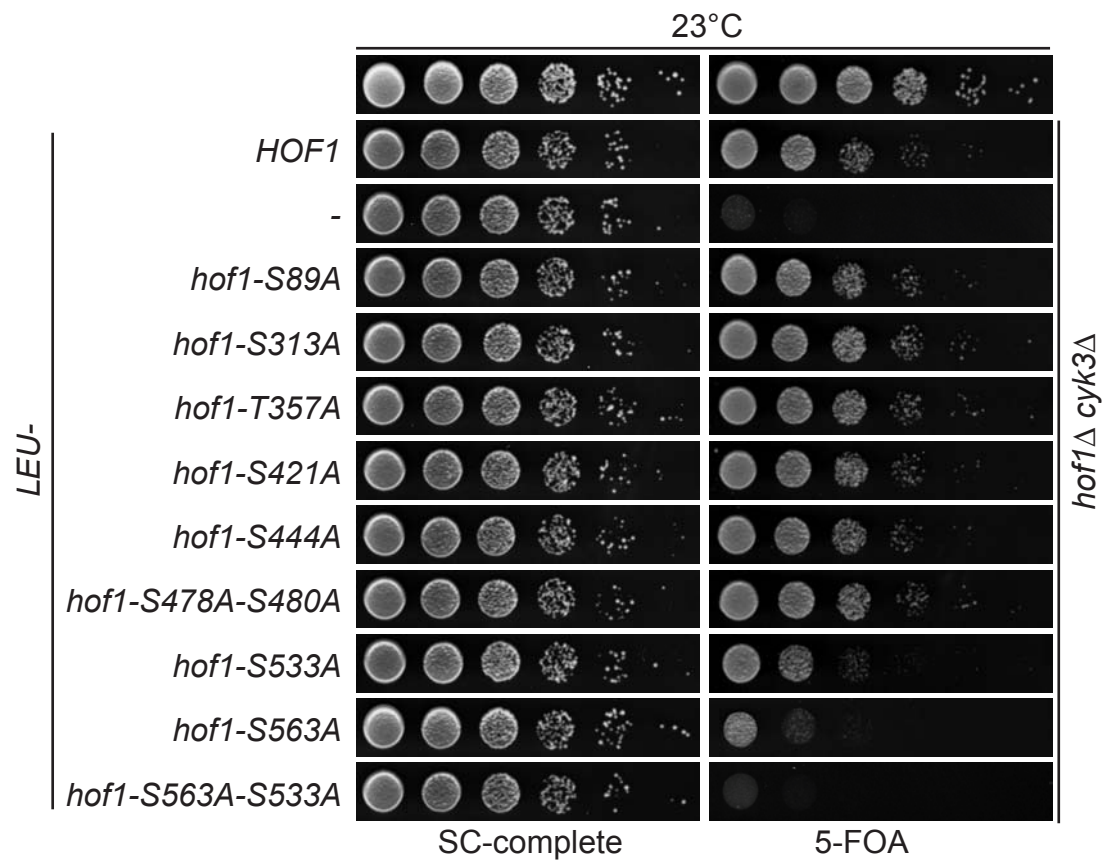
pBPE38-1	pRS315- <i>HOF1</i>	(Meitinger et al., 2010)
pDK028	pRS425- <i>CHS2</i>	(Palani et al., 2012)
pHA130	pET28c- <i>HOF1-200-355</i>	this study
pHA131	pET28c- <i>HOF1-200-355-S313E</i>	this study
pILM26	pRS425- <i>INN1</i>	(Meitinger et al., 2010)
pILM53	pRS425- <i>CYK3</i>	(Palani et al., 2012)
pMF056	pRS316- <i>HOF1</i>	(Meitinger et al., 2010)
pMF115-2	pRS316- <i>hof1-4A-GFP-hphNT1</i>	this study
pMF143	pRS315- <i>hof1-4A</i>	this study
pMF193	pRS315- <i>CYK3</i>	(Meitinger et al., 2011)
pMF194	pRS316- <i>CYK3</i>	this study
pMF205	pRS315- <i>hof1-4E</i>	(Meitinger et al., 2011)
pMF217	pRS316- <i>hof1-4E-GFP-hphNT1</i>	this study
pMF346	pRS315- <i>hof1-S89A</i>	this study
pMF347	pRS315- <i>hof1-S313A</i>	this study
pMF348	pRS315- <i>hof1-S421A</i>	this study
pMF349	pRS315- <i>hof1-S533A</i>	(Meitinger et al., 2011)
pMF350-2	pRS315- <i>hof1-S563A</i>	(Meitinger et al., 2011)
pMF355	pRS315- <i>hof1-S533A-S563A</i>	(Meitinger et al., 2011)
pMF370	pRS315- <i>hof1-S357A</i>	this study
pMF371	pRS315- <i>hof1-S444A</i>	this study
pMF372	pRS315- <i>hof1-S478A-S480A</i>	this study
pMF419	pRS316- <i>hof1-W637A-GFP-hphNT1</i>	this study
pMF496	pRS316- <i>hof1-S313E-GFP-hphNT1</i>	this study
pMF690	pRS316- <i>hof1-4A-W637A-GFP-hphNT1</i>	this study
pMF812	pRS315-pGal1- <i>GFP-HOF1-293-355</i>	this study
pMM5	pRS423-pGal1-LexA-MYC	(Geissler et al., 1996)
pMM6	pRS425-pGal1-Gal4-HA	(Geissler et al., 1996)
pRS306	integration, <i>URA3</i>	(Sikorski and Hieter, 1989)
pRS315	<i>CEN, LEU2</i>	(Sikorski and Hieter, 1989)
pRS316	<i>CEN, LEU2</i>	(Sikorski and Hieter, 1989)
pRS425	2 μ , <i>LEU2</i>	(Christianson et al., 1992)
pSP279	pGEX-5X1- <i>HOF1-521-586</i>	this study
pSP280	pGEX-5X1- <i>HOF1-521-586-S533A</i>	this study
pSP281	pGEX-5X1- <i>HOF1-521-586-S563A</i>	this study

* Table references:

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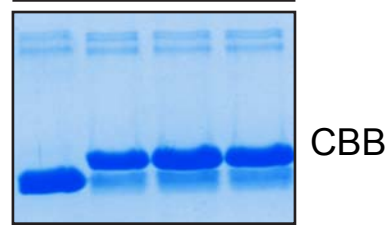
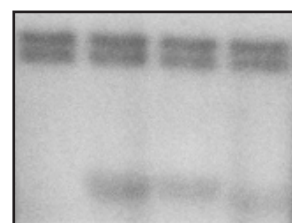
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A

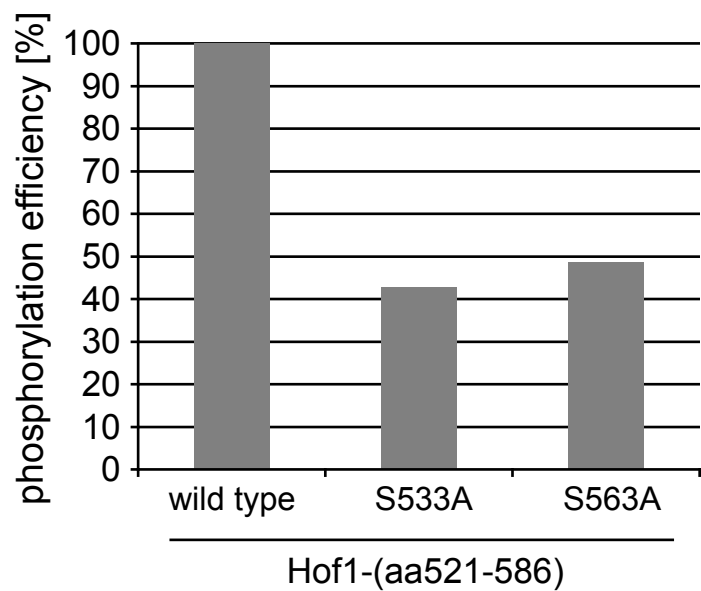


B

	Dbf2-Mob1	+	+	+	+
GST-Hof1-(aa521-586)	WT	-	+	-	-
	S533A	-	-	+	-
	S563A	-	-	-	+
	GST	+	-	-	-



C



Meitinger_Figure_S2

