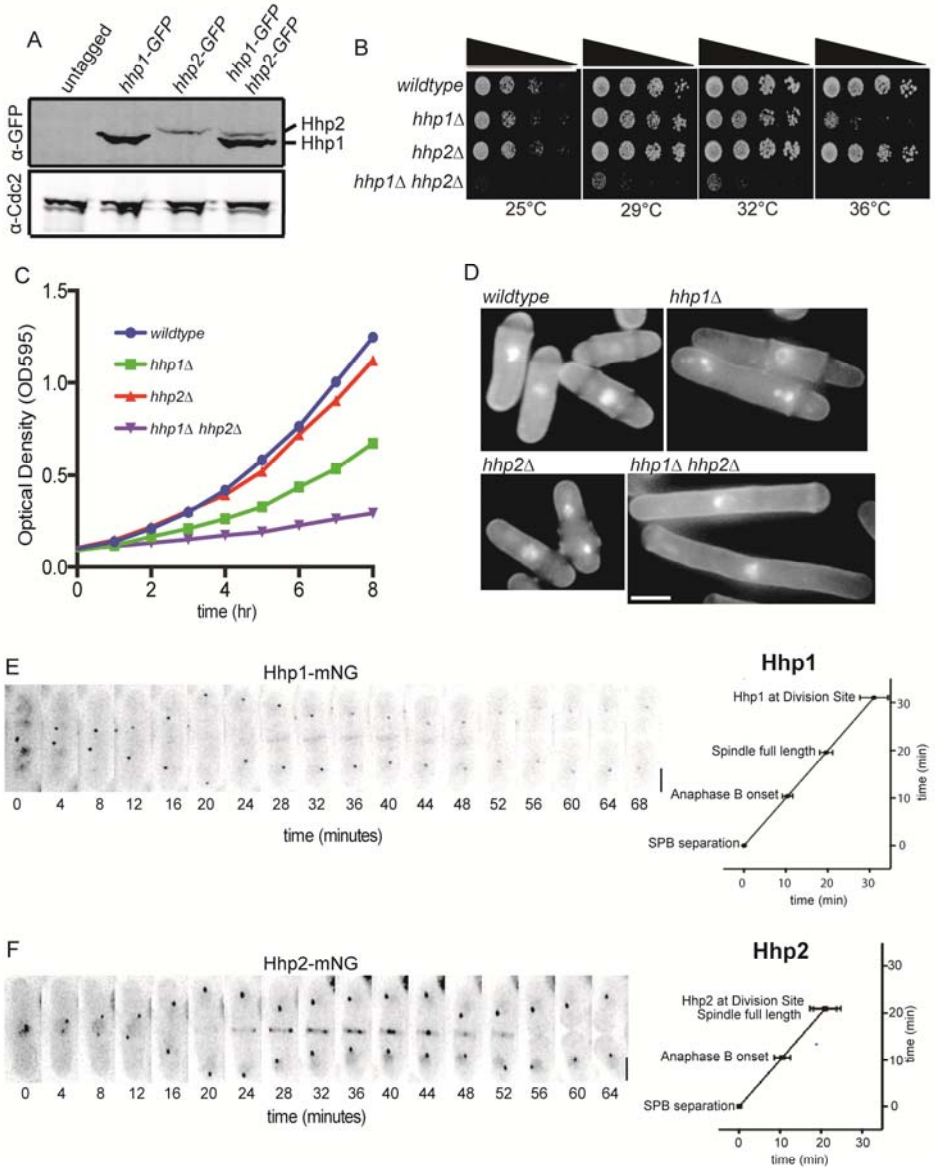
Supplemental Materials

Molecular Biology of the Cell

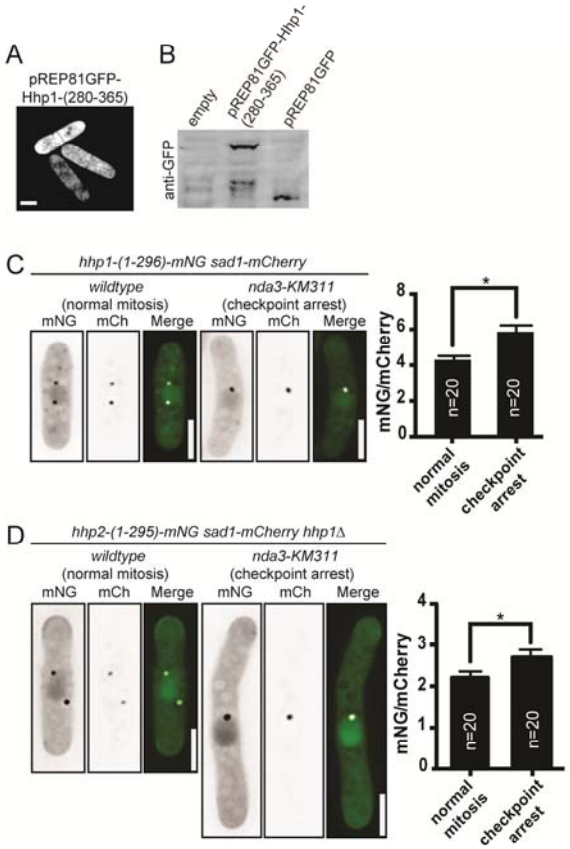
Elmore et al.



Supplemental Figure 1

Figure S1. Comparison of Hhp1 and Hhp2 functions and localizations. (A) Anti-GFP immunoblot of whole-cell extracts prepared from untagged and the indicated GFP-tagged strains. Anti-PSTAIREantibody served as loading control for lysates. (B) Serial dilutions (10-fold) of the indicated strains were spotted on YE plates and incubated at the indicated temperatures. (C) Liquid growth assay of wildtype,*hhp1*Δ*, hhp2*Δ and *hhp1*Δ *hhp2*Δ mutants. Cells were grown in liquid

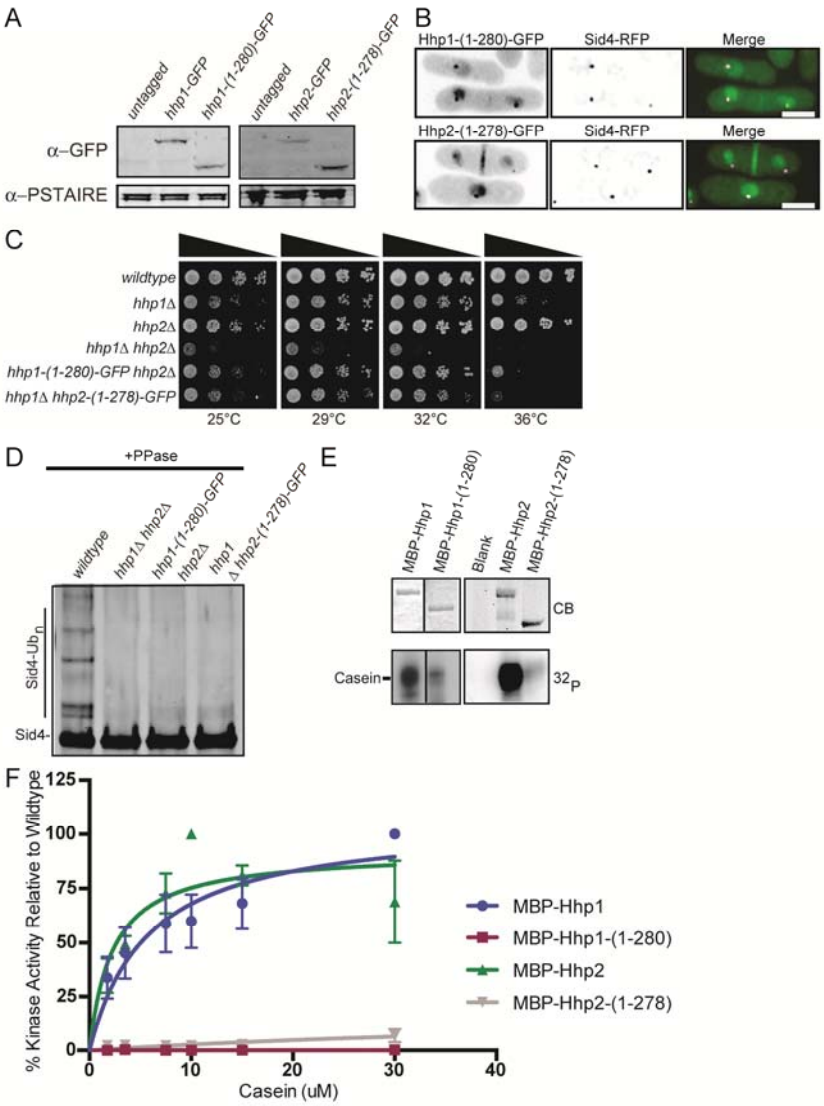
YE at 32°C to log phase and cut back to an OD_{595}=0.1. OD_{595} measurements were taken every hour for 8 h to determine growth rates of mutants. (D) *wildtype, hhp1*Δ*, hhp2*Δ*,* and *hhp1*Δ *hhp2*Δ mutant phenotypes were analyzed by DAPI (DNA) and methyl blue (cell wall) staining. (E and F) (Left panels) Representative montages of Hhp1-mNG (E) and Hhp2-mNG (F) movies. Images are shown in 4 min intervals with T=0 defined as the time of SPB separation. Right panels are timelines showing when Hhp1-mNG and Hhp2-mNG are detected at the division site. The mean time of detection of each protein ± SD is plotted from 20 cells each. The onset of anaphase B was determined to be 10.4 ± 1.2 min for *hhp1-mNG* and 10.5 ± 1.9 min for *hhp2-mNG* cells. Hhp1-mNG was detected at the division site at 31 ± 3.3 min and Hhp2-mNG at 21 ± 3.8 min. Scale bars, 5 μm.



Supplemental Figure 2

Figure S2. SPB targeting information of Hhp1/2 resides within the kinase domain(A) Live cell imaging of wildtype cells expressing *pREP81-GFP-hhp1-(280-365)* grown in the absence of thiamine for 20 hours at 32°C. (B) Anti-GFP immunoblot of whole-cell extracts prepared from wildtype cells carrying*pREP81*, *pREP81-GFP*, and *pREP81-GFP-hhp1-(280-365)*grown in the absence of thiamine for 20 h at 32°C. (C and D) Representative images of the indicated

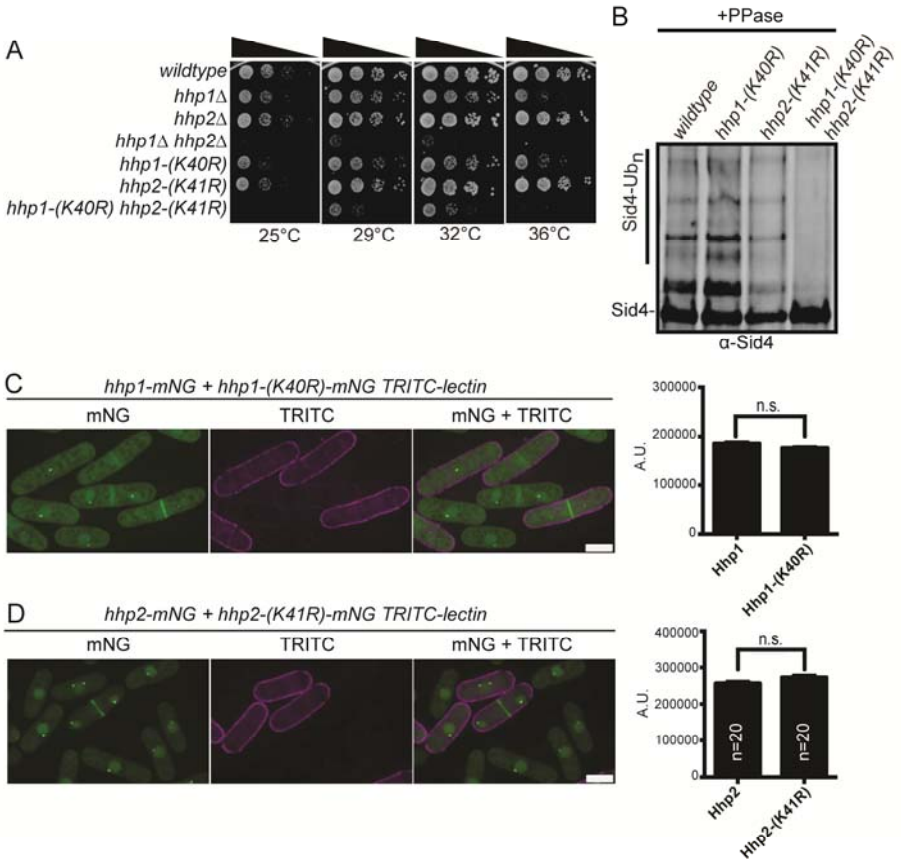
strains. Inverted grayscale images are shown for mNG and mCherry (mCh). Quantitation of Hhp1-(1-296)-mNG and Hhp2-(1-295)-mNG at SPBs is shown on the right represented as mNG/Sad1-mCherry ratios. *p<0.001 determined using Students *t*-test. Error bars represent SEM. Scale bars, 5 µm.



Supplemental Figure 3

Figure S3. The KDE of Hhp1/2 is required for enzymatic function *in vivo* and *in vitro*(A)Anti-GFP immunoblot of whole-cell extracts prepared from untagged

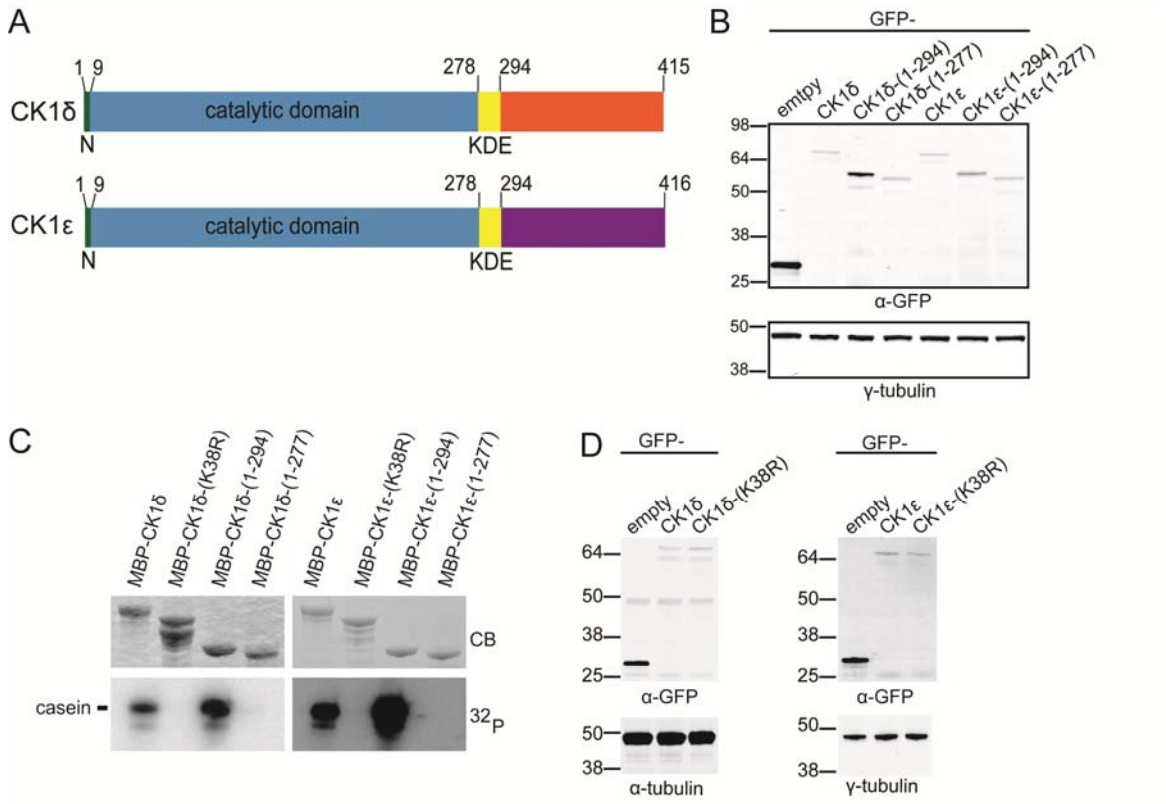
and the indicated GFP-tagged strains. Anti-PSTAIRE antibody served as loading control for lysates.(B) Live-cell imaging of endogenously tagged Hhp1-(1-280)-GFP and Hhp2-(1-278)-GFP with Sid4-RFP. Bars, 5 μm.(C) Serial 10-fold dilutions of the indicated strains were spotted on YE plates and incubated at the indicated temperatures.(D)Sid4 was immunoprecipitated from denatured cell lysates of the indicated strains, treated with phosphatase, and visualized by immunoblotting. (E) *In vitro* phosphorylation of casein by recombinant MBP-Hhp1-(1-280) and MBP-Hhp2-(1-278). Kinases were detected by CB staining of SDS-PAGE gels, and phosphorylated casein was detected by autoradiography (^{32}P). (F) Recombinant full length Hhp1/2 and the indicated Hhp1/2 truncation mutants were dephosphorylated with λ-phosphatase and used in *in vitro* kinase assays with increasing levels of casein (0-60μM). ^{32}P incorporationof casein was quantified by Phophorimager.



Supplemental Figure 4

Figure S4.Hhp1/2 kinase-dead mutants localize to the SPB. (A) Growth assay of *hhp1-(K40R)*, *hhp2-(K41R)*, and *hhp1-(K40R) hhp2-(K41R)* mutants. Serial dilutions (10-fold) of the indicated single and double mutant strains were spotted on YE plates and incubated at the indicated temperatures. (B) Sid4 from the indicated strains was immunoprecipitated from denatured cell lysates, treated with phosphatase, and visualized by immunoblotting. (C and D)*hhp1-(K40R)-*

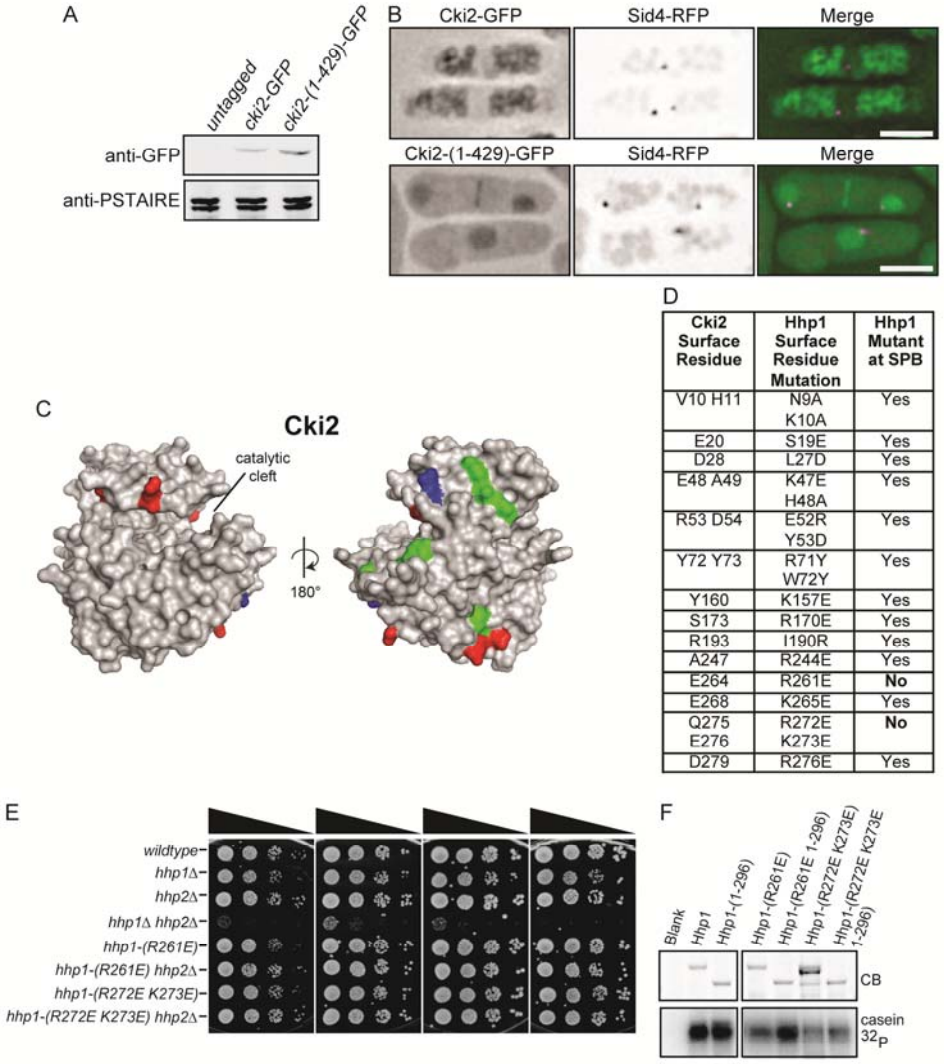
mNG(C) or *hhp2-(K41R)-mNG* (D) cells were labeled with tetramethylrhodamineisothiocyanate (TRITC)–lectin and mixed with *hhp1-mNG* (C) or *hhp2-mNG* (D) cells and imaged live. Representative images are shown in the left panels and quantitation of fluorescence intensities are shown on the right.A.U.=arbitrary units. Scale bar, 5 μm. p values determined using Student's *t*-test. ns=not significant. Error bars represent SEM.



Supplemental Figure 5

Figure S5. Expression of CK1δ/ε centrosomal localization mutants. (A) Schematic diagrams of CK1δ/ε with relative positions of N-terminal extension (N)

(green), catalytic domains (blue), kinase domain extensions (yellow) and unrelated C-termini in pink or purple indicated. (B) Anti-GFP immunoblot of whole-cell extracts prepared from the indicated transfected RPE1 cell lines. Anti-γ-tubulin antibody served as loading control for lysates. (C) *In vitro* phosphorylation of casein by recombinant MBP-CK1δ/ε and the indicated MBP-CK1δ/ε mutants. Phosphorylated proteins were detected by CB staining of SDS-PAGE gels, followed by autoradiography (^{32}P). (D) Anti-GFP immunoblot of whole-cell extracts prepared from the indicated transfected RPE1 cell lines. Anti-γ-tubulin and anti-α-tubulin antibodies served as loading control for lysates.

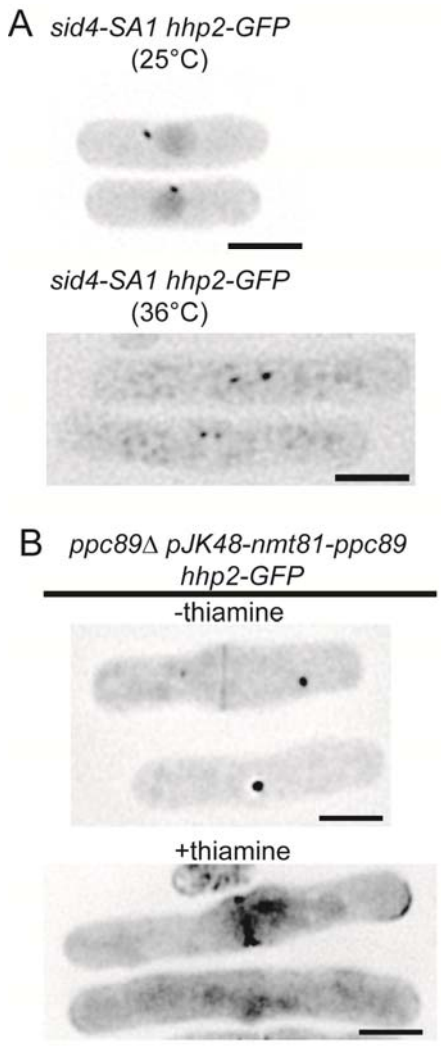


Cki2 Surface Residue	Hhp1 Surface Residue Mutation	Hhp1 Mutant at SPB
V10 H11	N9A K10A	Yes
E20	S19E	Yes
D28	L27D	Yes
E48 A49	K47E H48A	Yes
R53 D54	E52R Y53D	Yes
Y72 Y73	R71Y W72Y	Yes
Y160	K157E	Yes
S173	R170E	Yes
R193	I190R	Yes
A247	R244E	Yes
E264	R261E	**No**
E268	K265E	Yes
Q275 E276	R272E K273E	**No**
D279	R276E	Yes

Supplemental Figure 6

Figure S6.Cki2 does not localize to the SPB. (A) Anti-GFP immunoblot of whole-cell extracts prepared from untagged and the indicated GFP-tagged strains. Anti-PSTAIRE antibody served as loading control for lysates. (B) Live-cell imaging of endogenously tagged Cki2-GFP and Cki2-(1-429)-GFP with Sid4-RFP. Scale bars, 5um. (C) Homology model of Cki2 catalytic domain generated from Phyre[2] software and visualizedwith MacPymol.Surface residues with charge

differences between Cki2 and Hhp1 are colored. Green=hydrophobic residues, Red=acidic residues, Blue=basic residues. (D) Table of Cki2 surface residues with the corresponding mutations made in Hhp1and the effect of those mutations on SPB localization.(E)Growth assay of *hhp1-(R261E)*, *hhp1-(R261E) hhp2*Δ, *hhp1-(R272E K273E)*, and *hhp1-(R272E K273E) hhp2*Δ mutants. Serial 10-fold dilutions of the indicated single and double mutant strains were spotted on YE plates and incubated at the indicated temperatures. (F) *In vitro* kinase assays of recombinant MBP-Hhp1, MBP-Hhp1-(1-296), MBP-Hhp1-(R261E), MBP-Hhp1-(R261E 1-296), MBP-Hhp1-(R272E K273E) and MBP-Hhp1-(R272E K273E 1-296), detected by CB staining of SDS-PAGE gels, with casein as substrate. Phosphorylated casein was detected by autoradiography (^{32}P).



Supplemental Figure 7

Figure S7. Hhp2 requires Ppc89 to localize to the SPB.(A) Localization of Hhp2-GFP in a *sid4-SA1* temperature sensitive strain at the permissive (25°C) and restrictive (36°C) temperatures. (B)Localization of Hhp2-GFP in a *ppc89* shut-off strain (-thiamine, ppc89 expression on; +thiamine, ppc89 expression off). Scale bars, 5 μm.

Table S1. Strains used in this study.

Strain	Genotype	Source
Figure 1		
KGY17313	*hhp1-mNG:kan^R sid4-RFP:kan^R ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY16868	*hhp2-mNG:kan^R sid4-RFP:kan^R ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY17899	*hhp1-mNG:kan^R sad1-mCherry:kan^R ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY17900	*hhp1-mNG:kan^R sad1-mCherry:kan^R nda3-km311 ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY18725	*hhp2-mNG:kan^R sad1-mCherry:kan^R ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY18726	*hhp2-mNG:kan^R sad1-mCherry:kan^R nda3-km311 ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY19353	*hhp2-mNG:kan^R sad1-mCherry:kan^R hhp1::ura4$^+$ ade6-M210 leu1-32 h+*	This study
Figure 2		
KGY246	*ade6-M210 leu1-32 ura4-D18 h-*	Lab stock
KGY14697	*hhp1-GFP:kan^R ade6-M210 leu1-32 ura4-D18 h-*	Lab stock
KGY15972	*hhp1-(1-296)-GFP:kan^R ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY12838	*hhp2-GFP:kan^R ade6-M210 leu1-32 ura4-D18 h-*	Lab stock
KGY16977	*hhp2-(1-295)-GFP:kan^R ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY19140	*hhp1-(1-296)-GFP:kan^R sid4-RFP:kan^R ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY18815	*hhp2-(1-295)-GFP:kan^R sid4-RFP:kan^R leu1-32 ura4-D18 h-*	This study
KGY6415	*hhp1::ura4$^+$ leu1-32 h-*	Bimbo et al., 2005
KGY7081	*hhp2::ura4$^+$ leu1-32 h-*	Bimbo et al.,2005
KGY13958	*hhp1::ura4$^+$ hhp2::kan^R leu1-32 h+*	This study
KGY16775	*hhp1-(1-296)-GFP:kan^R hhp2::ura4$^+$ade6-M210 leu1-32 h-*	This study
KGY17078	*hhp1::ura4$^+$ hhp2-(1-295)-GFP:kan^R ade6-M210 leu1-32 h+*	This study
Figure3		
KGY16776	*hhp1-(K40R)-GFP:kan^{R}ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY17628	*hhp2-(K41R)-GFP:kan^{R}ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY19354	*hhp1-(K40R)-mNG:kan^R sid4-RFP:kanR ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY19444	*hhp1-(K40R)-mNG:kan^R hhp2::ura4$^+$ sid4-RFP:kanR ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY19445	*hhp2-(K41R)-mNG:kan^R hhp1::ura4$^+$ sid4-RFP:kanR ade6-M210 leu1-32 ura4-D18h?*	This study
Figure5		
KGY17627	*hhp1-(R261E)-mNG:kan^R sid4-RFP:kanR leu1-32 ura4-D18 h-*	This study
KGY17628	*hhp1-(R272E K273E)-mNG:kan^R sid4-RFP:kanR leu1-32 ura4-D18 h-*	This study
KGY14522	*hhp1-(R261E)-GFP:kan^R leu1-32 ura4-D18 h-*	This study
KGY14523	*hhp1-(R272E K273E)-GFP:kan^R leu1-32 ura4-D18h-*	This study
Figure 6		
KGY1296	*PJ69-4A MATa trp1-190 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2:GAL1-HIS3 GAL2-ADE2 met2::GAL-lacZ*	Lab stock
Figure 7		
KGY246	*ade6-M210 ura4-D18 leu1-32 h-*	Lab stock
KGY5663	*nda3-km311 ade6-M21x ura4-D18 leu1-32 h+*	Lab stock
KGY13805	*nda3-KM311 dma1::ura4+ ade6-M21x leu1-32 ura4-D18 h+*	Lab stock
KGY14395	*nda3-KM311 hhp2::kan^R ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY19352	*hhp1-(R272E K273E)::kan^R nda3-km311 ade6-M210 leu1-32 ura4-D18 h-*	This study

KGY18331	*hhp1-(R272E K273E)::kan^{R} hhp2::ura4^{+} nda3-KM311 ade6-M210 ura4-D18 leu1-32 h-*	This study
KGY3104-2	*hhp1-(R261E)::kan^{R} nda3-KM311 ade6-M210 ura4-D18 leu1-32 h-*	This study
KGY3105-2	*hhp1-(R261E)::kan^{R} hhp2::ura4+ nda3-KM311 ade6-M210 ura4-D18 leu1-32 h-*	This study
KGY14525	*hhp1-(R272E K273E)-GFP::kan^{R} hhp2::ura4+ ura4-D18 leu1-32 h+*	This study
KGY14526	*hhp1-(R261E)-GFP::kan^{R} hhp2::ura4+ ade6-M210 ura4-D18 leu1-32 h+*	This study
Figure S1		
KGY16343	*hhp1-GFP:kan^{R} hhp2-GFP:kan^{R} ade6-M210 leu1-32 ura4-D18h+*	This study
KGY16788	*hhp1-mNG:kan^{R} ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY16831	*hhp2-mNG:kan^{R} ade6-M210 leu1-32 ura4-D18 h-*	This study
Figure S2		
KGY17901	*hhp1-(1-296)-mNG:kan^{R}sad1-mCherry:kan^{R}ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY17902	*hhp1-(1-296)-mNG:kan^{R}sad1-mCherry:kan^{R}ade6-M210 leu1-32 ura4-D18 nda3-km311 h-*	This study
Figure S3		
KGY15971	*hhp1-(1-280)-GFP:kan^{R}ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY16143	*hhp2-(1-278)-GFP:kan^{R}ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY19157	*hhp1-(1-280)-GFP:kan^{R} sid4-RFP:kan^{R}ade6-M210 leu1-32 ura4-D18 h+*	This study
KGY17079	*hhp1-(1-280)-GFP:kan^{R}hhp2::ura4^{+}ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY16869	*hhp2-(1-278)-GFP:kan^{R}hhp1::ura4^{+}ade6-M210 leu1-32 ura4-D18 h-*	This study
Figure S4		
KGY19337	*hhp1-(K40R):kan^{R}leu1-32 ura4-D18 h-*	This study
KGY19349	*hhp2-(K41R):kan^{R}ade6-M210leu1-32 ura4-D18 h+*	This study
KGY19342	*hhp1-(K40R):kan^{R} hhp2-(K41R):kan^{R}ade6-M210 leu1-32 ura4-D18 h+*	This study
Figure S6		
KGY17283	*cki2-GFP:kanR ade6-M210leu1-32 ura4-D18 h-*	This study
KGY17461	*cki2-(1-429)-GFP:kanR ade6-M210leu1-32 ura4-D18 h-*	This study
KGY19323	*cki2-GFP:kanRsid4-RFP:kan^{R}ade6-M210leu1-32 ura4-D18h-*	This study
KGY19324	*cki2-(1-429)-GFP:kan^{R} sid4-RFP:kan^{R} ade6-M210leu1-32 ura4-D18h-*	This study
	hhp1-(R261E):kan^{R}ade6-M210leu1-32 ura4-D18 h-	
KGY19159	*hhp1-(R261E):kan^{R}hhp2::ura4^{+}leu1-32 ura4-D18 h?*	This study
KGY18807	*hhp1-(R272E K273E):kan^{R}ade6-M210leu1-32 ura4-D18 h-*	This study
KGY19344	*hhp1-(R272E K273E):kan^{R} hhp2::ura4^{+}ade6-M210leu1-32 ura4-D18*	This study
KGY19443	*h+*	This study
Figure S7	*hhp2-GFP:kan^{R}sid4-SA1 ade6-M210leu1-32 ura4-D18h+*	
KGY14155	*hhp2-GFP:kan^{R} leu1::pJK148-nmt81-ppc89 ppc89::ura4^{+}ade6-*	This study
KGY19398	*M210h+*	This study