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Supplemental Information

KNL1/Spc105 Recruits PP1 to Silence the Spindle Assembly Checkpoint

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Supplemental Inventory

figure S1

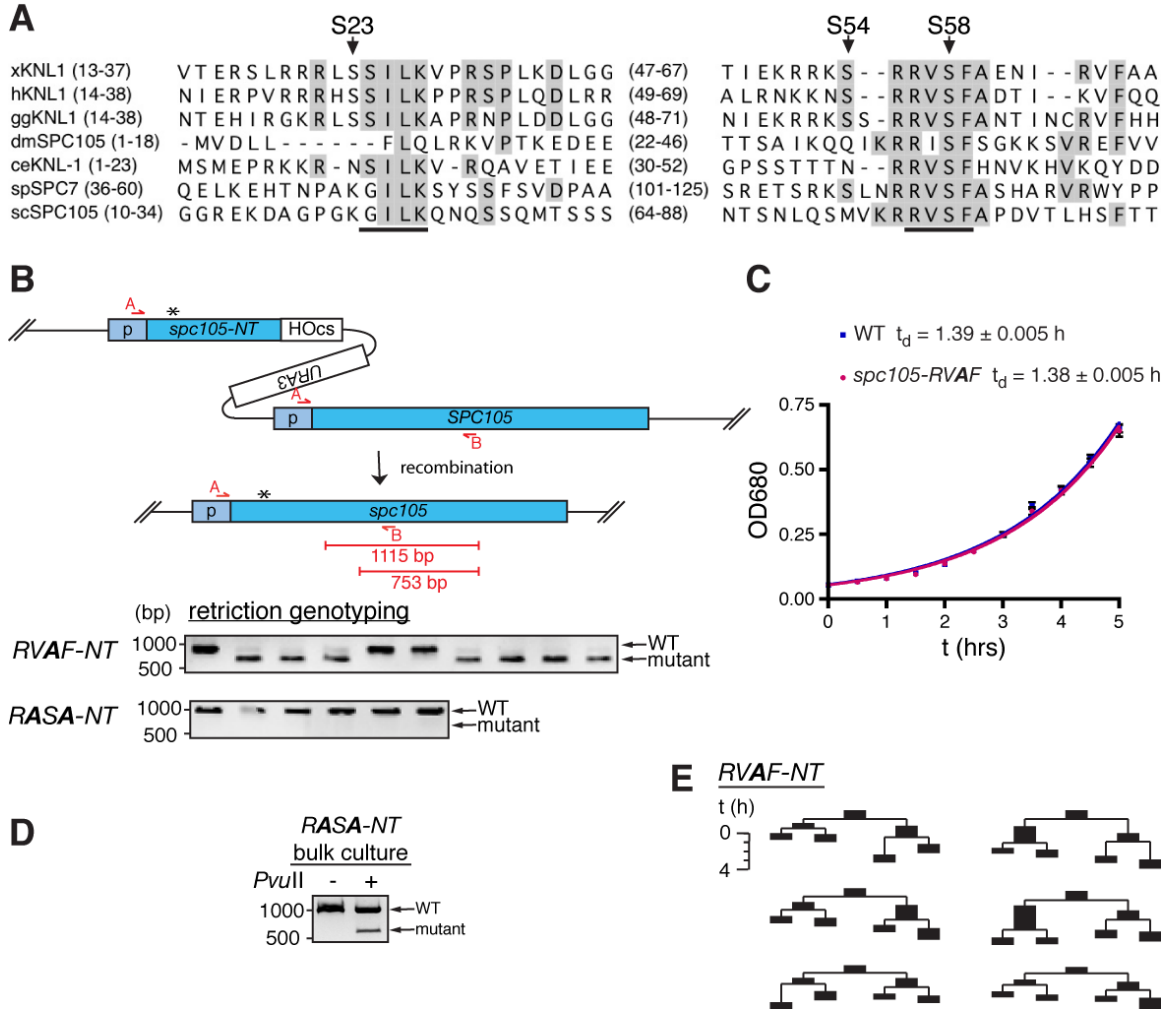
A
S23
S54
S58
xKNL1 (13-37) VTERSLRRRLSSILKVPRSPLKDLGG (47-67) TIEKRRKS--RRVSFAENI--RVFAA
hKNL1 (14-38) NIERPVRRRHSSILKPPRSPLQDLRR (49-69) ALRNKKNS--RRVSFADTI--KVFQQ
ggKNL1 (14-38) NTEHIRGKRLSSILKAPRNPLDDLGG (48-71) NIEKRRKSS-RRVSFANTINCRVFHH
dmSPC105 (1-18) -MVDLL------FLQLRKVPTKEDEE (22-46) TTSAIKQQIKRRISFSGKKSVREFVV
ceKNL-1 (1-23) MSMEPRKKR-NSILKV-RQAVETIEE (30-52) GPSSTTTN--RRVSFHNVKHVKQYDD
spSPC7 (36-60) QELKEHTNPAKGILKSYSSFSVDPAA (101-125) SRETSRKSLNRRVSFASHARVRWYPP
scSPC105 (10-34) GGREKDAGPGKGILKQNQSSQMTSSS (64-88) NTSNLQSMVKRRVSFAPDVTLHSFTT
B
p
spc105-NT
HOcs
URA3
SPC105
recombination
spc105
1115 bp
753 bp
(bp) retriction genotyping
RVAF-NT
RASA-NT
1000
500
WT
mutant
C
WT td = 1.39 ± 0.005 h
spc105-RVAF td = 1.38 ± 0.005 h
OD680
t (hrs)
D
RASA-NT
bulk culture
PvuII - +
E
RVAF-NT
t (h)
0
4

Figure S1. Genotyping of HGR Recombinants, Related to Figure 1

(A) Alignment of the SILK and RVxF motifs of KNL1 orthologs from *X. tropicalis* (xKNL1), *H. sapiens* (hKNL1), *G. gallus* (ggKNL1), *D. melanogaster* (dmSPC105), *C. elegans* (ceKNL-1), *S. pombe* (spSPC7), and *S. cerevisiae* (scSPC105). Arrows indicate S23, S54, and S58 of xKNL1.

(B) Representative genotyping of the single cell assay in 3B. The genomic *SPC105* locus was PCR amplified from recombinant colonies using the indicated primers A and B (red arrows), digested with mutation-specific restriction enzymes and resolved on an agarose gel.

(C) Growth curve of WT and *spc105-RVA**F*** at 30°C in YEPD medium. Average ± SEM of the doubling time of three independent experiments are also shown.

(D) Six hours after induction of HGR in the parent strain harboring the *R**A**S**A*-*NT* cassette, the genomic SPC105 locus was PCR amplified from bulk culture and digested with *Pvu*II to detect generation of *spc105-R**A**S**A***.

(E) Pedigree analysis of recombinants generated from the cells harboring the *RV**A**F-NT* cassette during live cell imaging (Movie S1). Each lineage starts from a single unbudded cell and the duration of budding to anaphase (black rectangle) and anaphase to budding (line) were measured for three generations. At each division, fates of the mother cell and the daughter cell are shown on the left and right, respectively.

figure S2

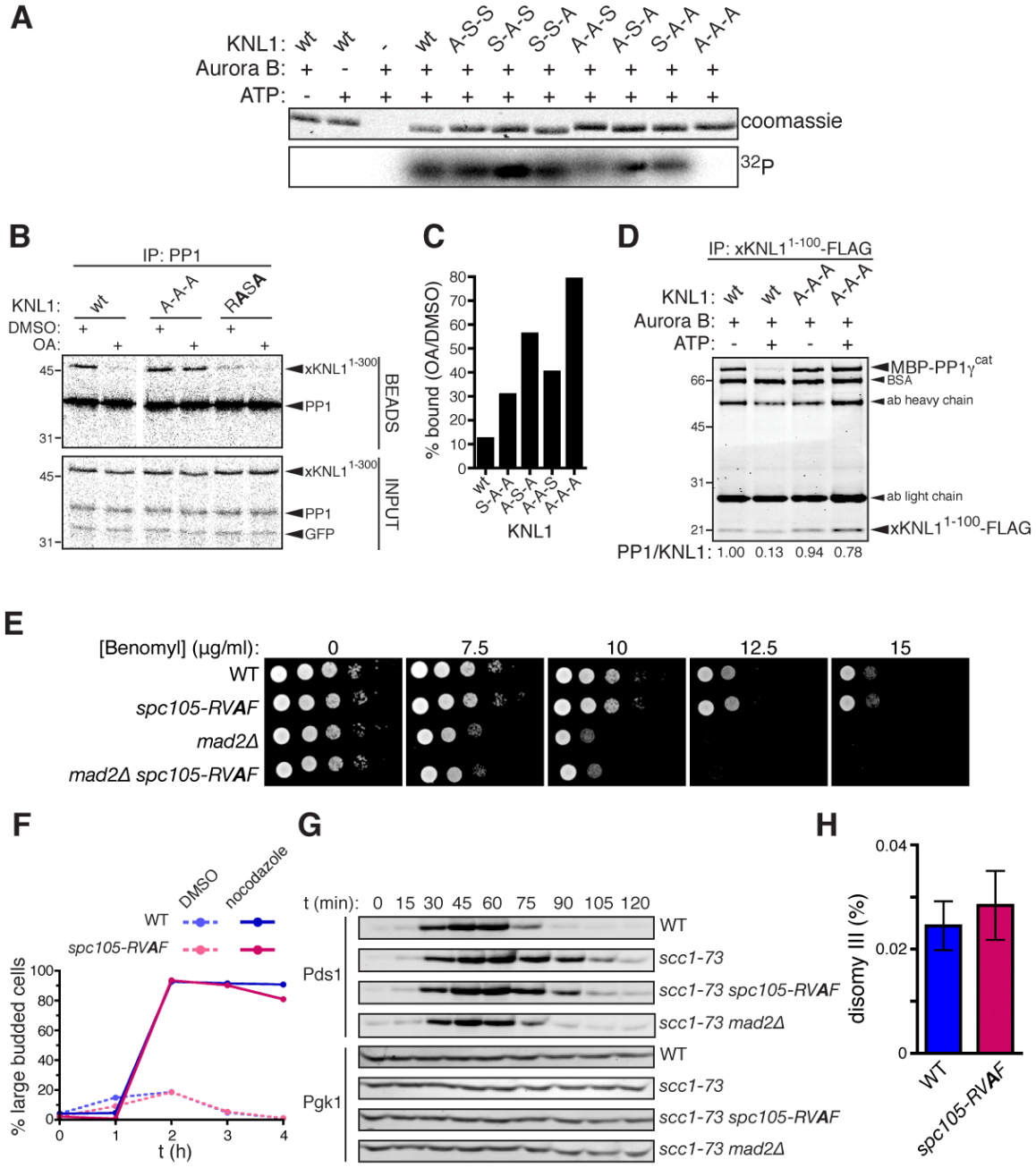
A
KNL1: wt wt - wt A-S-S S-A-S S-S-A A-A-S A-S-A S-A-A A-A-A
Aurora B: + - + + + + + + + + +
ATP: - + + + + + + + + + +
coomassie
32P
B
IP: PP1
KNL1: wt A-A-A RASA
DMSO: + + +
OA: + + +
xKNL1 1-300
PP1
BEADS
xKNL1 1-300
PP1
GFP
INPUT
C
% bound (OA/DMSO)
wt S-A-A A-S-A A-A-S A-A-A
KNL1
D
IP: xKNL1 1-100-FLAG
KNL1: wt wt A-A-A A-A-A
Aurora B: + + + +
ATP: - + - +
MBP-PP1γ cat
BSA
ab heavy chain
ab light chain
xKNL1 1-100-FLAG
PP1/KNL1: 1.00 0.13 0.94 0.78
E
[Benomyl] (µg/ml): 0 7.5 10 12.5 15
WT
spc105-RVAF
mad2Δ
mad2Δ spc105-RVAF
F
DMSO nocodazole
WT
spc105-RVAF
% large budded cells
t (h)
G
t (min): 0 15 30 45 60 75 90 105 120
Pds1
Pgk1
WT
scc1-73
scc1-73 spc105-RVAF
scc1-73 mad2Δ
H
disomy III (%)
WT
spc105-RVAF

Figure S2. Phosphorylation of the RVSF Motif of KNL1/Spc105 Can Abrogate PP1/Glc7 Binding but Is Dispensable for Mitosis, Related to Figure 2

(A) γ-^{32}P-ATP and recombinant Aurora B-INCENP$_{790\text{-}871}$ were incubated with N-terminal region of *Xenopus tropicalis* KNL1 (xKNL1$^{1\text{-}100}$-FLAG, a recombinant protein of the N-terminal 100 residues) containing various serine (S) to alanine (A) mutations as indicated at residues 23, 54, and 58 respectively. Coomassie staining of xKNL1$^{1\text{-}100}$-FLAG and autography are shown. Only the A-A-A mutant completely abolished ^{32}P incorporation, indicating that all the three serine residues can be phosphorylated by Aurora B.

(B) Metaphase *Xenopus laevis* egg extracts, containing ^{35}S-labeled PP1 (positive control), GFP (negative control), and xKNL1$^{1\text{-}300}$ (the N-terminal 300 residues of xKNL1), xKNL1$^{1\text{-}300,A\text{-}A\text{-}A}$, or xKNL1$^{1\text{-}300,R\mathbf{A}S\mathbf{A}}$, were treated with 0.4 μM okadaic acid (OA), which inhibits phosphatases antagonizing Aurora B and thus induces phosphorylation of KNL1 (data not shown), or with DMSO. ^{35}S-labeled proteins coimmunoprecipitated with anti-PP1 antibody (BEADS) were visualized by autoradiography. The R**A**S**A** mutation weakened the interaction with PP1, whereas the A-A-A mutation (S23A S54A S58A) desensitized the OA-induced PP1 dissociation.

(C) Phosphorylation at S23, S54 and S58 of xKNL1 additively weakens the binding of xKNL1 to PP1. Anti-PP1 coimmunoprecipitation was performed as in (B) with xKNL1$^{1\text{-}300}$ with the indicated serine (S) to alanine (A) mutations at residues 23, 54, and 58 respectively. Relative, normalized % ratios of KNL1 proteins copurified with PP1 from extracts treated with OA versus DMSO are shown.

(D) xKNL1$^{1\text{-}100}$-FLAG was treated with recombinant Aurora B-INCENP$_{790\text{-}871}$ in the presence or absence of ATP. xKNL1$^{1\text{-}100}$-FLAG was then isolated with anti-FLAG beads,

and incubated with 1 μM MBP-PP1γ^{cat} (the catalytically dead D95A mutant [26]). Fractions bound to the beads were visualized with Coomassie. Normalized PP1/KNL1 ratio is indicated below. Aurora B phosphorylation of xKNL1$^{1\text{-}100,wt}$-FLAG reduced binding of MBP-PP1γ^{cat} eight fold, but the binding with xKNL1$^{1\text{-}100,A\text{-}A\text{-}A}$-FLAG was resistant to Aurora B treatment.

(E) Ten-fold serial dilutions of WT, *spc105-RV**A**F*, *mad2Δ*, and *mad2Δ spc105-RV**A**F* were plated on YEPD with the indicated concentrations of benomyl.

(F) G1 synchronized WT and *spc105-RV**A**F* cells were released into nocodazole. The number of large budded cells was counted at the indicated timepoints, n > 500 cells each.

(G) WT, *scc1-73*, *scc1-73 spc105-RV**A**F*, and *scc1-73 mad2Δ* cells were G1 synchronized at the permissive temperature (23°C), released to the restrictive temperature (37°), and Pds1 and Pgk1 (loading control) levels were monitored by Western blotting at the indicated timepoints after release.

(H) Disomy III formation in WT and *spc105-RV**A**F* cells containing a chromosome III marked with a *leu2* locus disrupted by *URA3*. The mean frequency ± SEM of disomy formation (assessed by generation Leu$^+$, Ura$^+$ colonies) from five independent cultures are shown.

figure S3

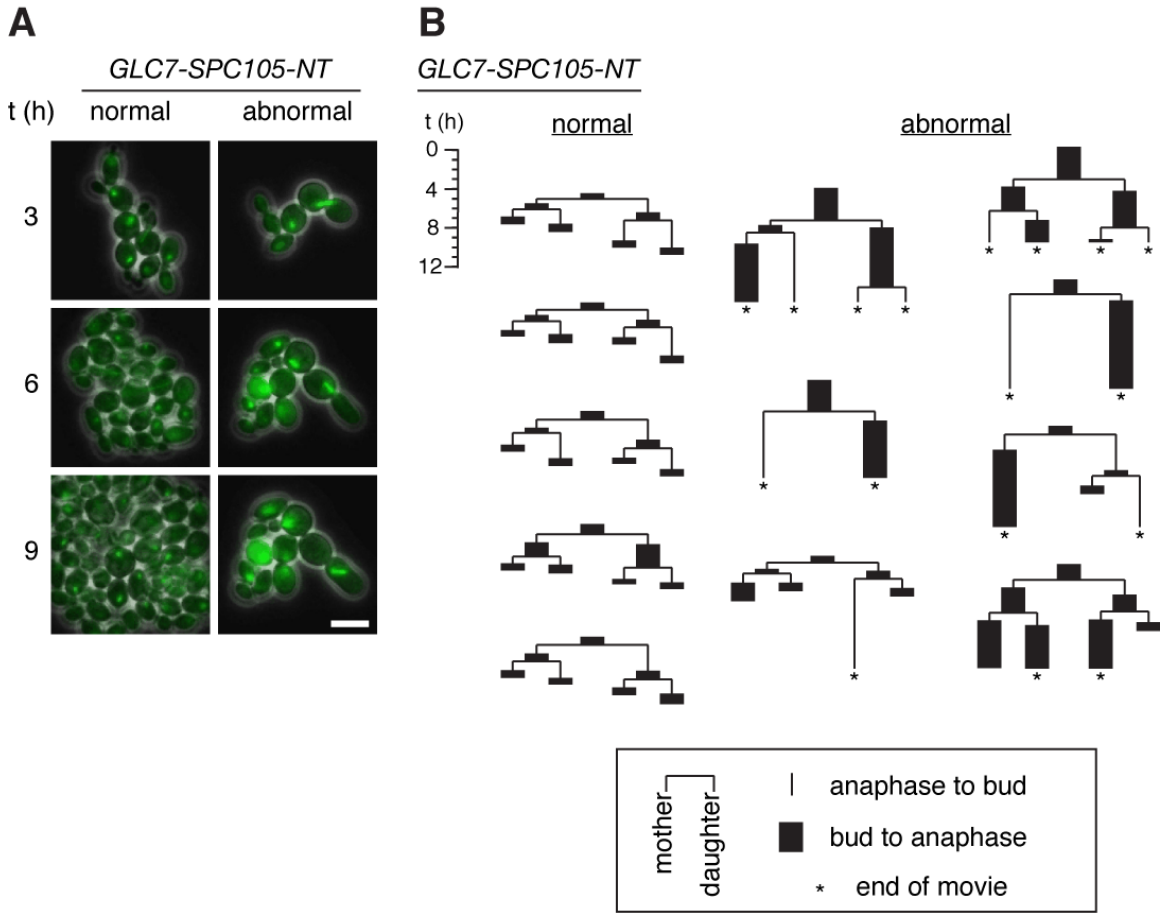

Figure S3. Live Microscopy of *GLC7-SPC105* Lethality, Related to Figure 3

(A) Time-lapse microscopy of GFP-Tub1 (green) was performed on *GLC7-SPC105-NT* cells beginning 6 hours after *GAL-HO* induction. Scale bar, 10 μm.

(B) Pedigree analysis of recombinants generated from cells harboring the *GLC7-SPC105-NT* cassette during live cell imaging (Movie S4, S5). Representative lineages showing normal cell divisions (left 6 examples) and abnormal cell divisions (right 5 examples) are shown.

figure S4

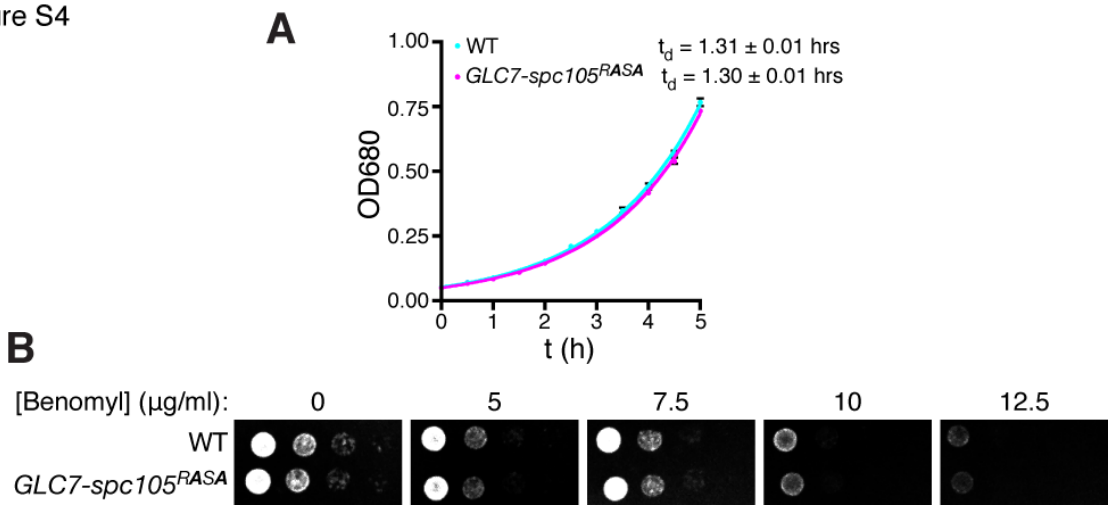

Figure S4. Characterization of *GLC7-SPC105*^{RASA}, Related to Figure 4

(A) Growth curve of WT and *GLC7-spc105*^{RASA} cells at 30°C in YEPD medium. Average ± SEM of the doubling time of three independent experiments are also shown.

(B) Ten-fold serial dilutions of WT and *GLC7-spc105*^{RASA} on YEPD with the indicated concentrations of benomyl.

Movie S1. Time-Lapse Microscopy of GFP-Tub1 (green) of *RVAF-NT* Cells after Recombination, Related to Figure 1

Movie duration: 12 hours.

Movie S2. Time-Lapse Microscopy of GFP-Tub1 (green) of *RASA-NT* Cells after Recombination, Related to Figure 1

A representative “normal” cell is shown. Movie duration: 12 hours.

Movie S3. Time-Lapse Microscopy of GFP-Tub1 (green) of *RASA-NT* Cells after Recombination, Related to Figure 1

A representative “abnormal” cell is shown, exhibiting long metaphase delays. Movie duration: 12 hours.

Movie S4. Time-Lapse Microscopy of GFP-Tub1 (green) of *GLC7-SPC105-NT* Cells after Recombination, Related to Figure 3

A representative “normal” cell is shown. Movie duration: 12 hours.

Movie S5: Time-Lapse Microscopy of GFP-Tub1 (green) of *GLC7-SPC105-NT* Cells after Recombination, Related to Figure 3

A representative “abnormal” cell is shown, exhibiting a pleiotropic cell cycle phenotype. Movie duration: 12 hours.

Name	sequence	purpose
MBP-PP1gamma_F	CGGATCCGCAGATGTTGACAAGCTCAAC	Cloning PP1γ into pMAL-c4g (5')
MBP-PP1gamma_R	CCGAAGCTTTTATTTCTTTGCTTGTTTTGTGATTATGCC	Cloning PP1γ into pMAL-c4g (3')
GST-Blink100_F	GCGGATCCGATGGCAATCCTTGGCTACAAG	Cloning xKNL1$^{1-100}$ intopGEX-6p2 (5')
GST-Blink100_R	GGAATTCTTACTTGTCATCGTCCTTGTAGTCGCTTCCAGTGATCTGGCAGCTAGATTC	Cloning xKNL1$^{1-100}$ intopGEX-6p2 (3')
PP1g_D95A_s	CCTAGGAGACTATGTAGCTCGAGGCAAGCAGTCT	PP1γ D95A mutation (sense)
PP1g_D95A_as	AGACTGCTTGCCTCGAGCTACATAGTCTCCTAGG	PP1γ D95A mutation (antisense)
Blinkin_RASA_sense	GCGGAGAAAAAGTCGTCGAGCTAGCGCTGCTGAGAATATAAGGGTTT	xKNL1 R**A**S**A** mutation (sense)
Blinkin_RASA_antisense	AAACCCTTATATTCTCAGCAGCGCTAGCTCGACGACTTTTTCTCCGC	xKNL1 R**A**S**A** mutation (antisense)
BlinkinS23A_sense	AGCCTCAGGAGGCGACTTGCCTCTATTTTAAAAGTTC	xKNL1 S23A mutation (sense)
BlinkinS23A_antisense	GAACTTTTAAAATAGAGGCAAGTCGCCTCCTGAGGCT	xKNL1 S23A mutation (antisense)
BlinkinS54A_sense	GATTCAACCATTGAAAAGCGGAGAAAAGCTCGTCGAGTTAGCT	xKNL1 S54A mutation (sense)
BlinkinS54A_antisense	AGCTAACTCGACGAGCTTTTCTCCGCTTTTCAATGGTTGAATC	xKNL1 S54A mutation (antisense)
BlinkinS58A_sense	GGAGAAAAAGTCGTCGAGTTGCCTTTGCTGAGAATATAAGGG	xKNL1 S58A mutation (sense)
BlinkinS58A_antisense	CCCTTATATTCTCAGCAAAGGCAACTCGACGACTTTTTCTCC	xKNL1 S58A mutation (antisense)
Blinkin_S54_to_DM_sense	GGAGAAAAGCTCGTCGAGTTGCCTTTGCTGAGAAATTAAGGG	xKNL1 S54A, S58A mutation (sense)
Blinkin_S54_to_DM_antisense	CCCTTATATTCTCAGCAAAGGCAACTCGACGAGCTTTTCTCC	xKNL1 S45A, S58A mutation (antisense)
Spc105_SG_F	TCCCCGCGGATTATCGAAGCGAAAGTATGGC	Cloning Spc105 synthetic genes into RS406 (5')
Spc105_SG_R	GGGGTACCGTCAACCACTCTACAAAACC	Cloning Spc105 synthetic genes into RS406 (3')
spc105_RVAF_wt_s	TACAGAGTATGGTAAAGAGAAGAGTTTCGTTCGCTCCCGA	Mutating spc105^{NT} construct to WT (sense)
spc105_RVAF_wt_as	TCGGGAGCGAACGAAACTCTTCTCTTTACCATACTCTGTA	Mutating spc105^{NT} construct to WT (antisense)
spc105_sg_seq	CGCGAAAGAGAAGGCGCC	Genotyping spc105 (F)
Spc105_R	CGCGAAAGAGAAGGCGCC	Genotyping spc105 (R)
spc105_SG_cut_asc1_SGTTTGAATGTGGATGAAAGAAGCCG	GATATCTAAGTGGGCTGACGAGCGCGCCGGTGATGGTGCTG	Adding Asc1 site and linker to spc105^{NT} constructs for GLC7 insertion (sense)
spc105_SG_cut_asc1_AS	CGGCTTCTTTCATCCACATTCAAACCAGCACCATCACCGGCGCGCCTGTCAGCCCACTTAGATATC	Adding Asc1 site and linker to spc105^{NT} constructs for GLC7 insertion (antisense)
glc7_fus_asc1_F	CGCGCGGGCGCGCCATGGACTCACAACCAGTTGAC	Cloning GLC7 into spc105^{NT} constructs (5')
glc7_fus_asc1_R	CGCGCGGGCGCGCCCTTTTTTCTTTCTACCCCC	Cloning GLC7 into spc105^{NT} constructs (3')
GLC7_sal1_QC_a	CTATTTTTGGGTGATTATGTTGACCGTGGTAAACAATCC	Eliminating SalI site in GLC7 (sense)
GLC7_sal1_QC_as	GGATTGTTTACCACGGTCAACATAATCACCCAAAAATAG	Eliminating SalI site in GLC7 (antisense)
glc7_D95A_s	CTATTTTTGGGTGATTATGTCGCCCGTGGTAAACAATCCTTAGAG	GLC7 D94A mutation (sense)
glc7_D95A_as	CTCTAAGGATTGTTTACCACGGGCGACATAATCACCCAAAAATAG	GLC7 D94A mutation (antisense)

Table S1. List of Primers Used

Strain	Genotype
JSR070	*MATa-inc lys2::GAL-HO-LYS2 SPC105::spc105$^{WT\text{-}NT}$-URA3*
JSR002	*MATa-inc lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 SPC105::spc105$^{RVAF\text{-}NT}$-URA3 ADE2*
JSR001	*MATa-inc lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 SPC105::spc105$^{RASA\text{-}NT}$-URA3 ADE2*
JSR057	*MATa-inc lys2::GAL-HO-LYS2 SPC105::spc105$^{RASA\text{-}NT}$-URA3 ipl1-1 TRP1*
JSR069	*MATa-inc lys2::GAL-HO-LYS2 spc105-R**A**S**A** ipl1-1 TRP1*
JSR069-1	*MATa-inc lys2::GAL-HO-LYS2 SPC105 ipl1-1 TRP1*
JSR006	*MATa-inc mad2Δ::KanMX lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 SPC105::spc105$^{RASA\text{-}NT}$-URA3 ADE2*
JSR083	*MATa-inc bar1Δ his3::TUB1-GFP-HIS3 PDS1-18MYC-LEU2 LYS2**
JSR084	*MATa-inc bar1Δ his3::TUB1-GFP-HIS3 PDS1-18MYC-LEU2 LYS2* trp1::tet^{off}-CDC20-127-TRP1*
JSR085	*MATa-inc bar1Δ his3::TUB1-GFP-HIS3 PDS1-18MYC-LEU2 LYS2* trp1::tet^{off}-CDC20-127-TRP1 spc105-R**A**S**A***
JSR004	*MATa-inc lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 SPC105-RV**A**F ADE2*
JSR004-1	*MATa-inc lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 SPC105 ADE2*
JSR049	*MATa-inc mad2Δ::KanMX lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 spc105-RV**A**F*
JSR078	*MATa-inc bar1Δ leu2::tetR-GFP-LEU2 ura3::TetOs-URA3 LYS2**
JSR079	*MATa-inc bar1Δ leu2::tetR-GFP-LEU2 ura3::TetOs-URA3 LYS2* SPC105-RV**A**F ADE2*
JSR080	*MATa-inc bar1Δ leu2::tetR-GFP-LEU2 ura3::TetOs-URA3 LYS2* scc1-73 TRP1 ADE2*
JSR081	*MATa-inc bar1Δ leu2::tetR-GFP-LEU2 ura3::TetOs-URA3 LYS2* SPC105-RV**A**F scc1-73 TRP1 ADE2*
YL044	*MATa-inc bar1Δ mad2::KanMX scc1-73 leu2::GAL-PDS1-mdb-LEU2*
JSR103	*MATa-inc mad2Δ::KanMX lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 spc105-R**A**S**A** ADE2*
JSR103-1	*MATa-inc mad2Δ::KanMX lys2::GAL-HO-LYS2 his3::TUB1-GFP-HIS3 SPC105 ADE2*
JSR093	*MATa-inc bar1Δ mad2::KanMX leu2::tetR-GFP-LEU2 ura3::TetOs-URA3*
JSR094	*MATa-inc bar1Δ mad2::KanMX leu2::tetR-GFP-LEU2 ura3::TetOs-URA3 spc105-R**A**S**A***
JSR096	*MATα lys2::GAL-HO-LYS2 mad2::kanMX leu2-Δ101::URA3::leu2-Δ102 ADE2*
JSR097	*MATα spc105-R**A**S**A** lys2::LYS2-GAL-HO mad2::kanMX leu2-Δ101::URA3::leu2-Δ102 ADE2*
JSR102	*MATa-inc lys2:: GAL-HO-LYS2 SPC105::spc105$^{GLC7\text{-}RASA\text{-}NT}$-URA3*
JSR113	*MATa-inc lys2:: GAL-HO-LYS2 SPC105::spc105$^{glc7cat\text{-}RASA\text{-}NT}$-URA3*
JSR112	*MATa-inc lys2:: GAL-HO-LYS2 SPC105::spc105$^{GLC7\text{-}SPC105\text{-}NT}$-URA3*
JSR114	*MATa-inc lys2:: GAL-HO-LYS2 SPC105::spc105$^{glc7cat7\text{-}SPC105\text{-}NT}$-URA3*
JSR128	*MATa-inc lys2:: GAL-HO-LYS2 SPC105::spc105$^{GLC7\text{-}WT\text{-}NT}$-URA3 mad2::KanMX his3::TUB1-GFP-HIS3 ADE2*
JSR105	*MATa-inc lys2::GAL-HO-LYS2 Glc7-spc105-R**A**S**A***
JSR105-1	*MATa-inc lys2::GAL-HO-LYS2 SPC105*
JSR106	*MATa-inc lys2 leu2-Δ101::URA3::leu2-Δ102*
JSR107	*MATa-inc lys2 leu2-Δ101::URA3::leu2-Δ102 GLC7-spc105^{RASA}*
JSR127	*MATa-inc lys2:: GAL-HO-LYS2 SPC105::spc105$^{GLC7\text{-}SPC105\text{-}NT}$-URA3 his3::TUB1-GFP-HIS3 ADE2*

Table S2. Yeast Strains Used in This Study

All strains are W303 background. Asterisks indicates either *LYS2* or *lys2::GAL-HO-LYS2*.

Supplemental Experimental Procedures

Plasmids and Constructs

cDNA endcoding *Xenopus tropicalis* KNL1 was purchased from Open Biosystems (IMAGE clone 7794105) and the full-length sequence was determined (Genbank Accession JF804775). MBP-PP1γ was made by cloning PP1γ from a *X. laevis* cDNA library into pMAL-c4g using the *Bam*HI and *Hin*DIII sites. KNL1$^{1-100}$-FLAG was made by cloning KNL1 from a cDNA library into pGEX-6p2 using the *Bam*HI and *Eco*RI sites and inserting a C-terminal FLAG tag by PCR. KNL1$^{1-300}$ was made by splicing the endogenous *Eco*R1 site in KNL1 at residue 300 and the *Eco*R1 site in the polylinker. All point mutants were made using Quikchange site-directed mutagenesis (Agilent). For primers used see table S1.

Recombinant Proteins

All proteins were expressed in BL-21 rosetta cells. For MBP-PP1γ: protein was purified on Amylose Resin according to manufacturers instructions (NEB), and dialyzed into PP1 storage buffer (50 mM TRIS pH 8.0, 200 mM NaCl, 0.1 mM $MnCl_2$, 0.1 mM EDTA, 5 mM DTT). For xKNL1$^{1-100}$-FLAG: protein was purified on Glutathione Sepharose 4B and cleaved with precision protease according to manufacturers instructions (GE Healthcare), and dialyzed into sperm dilution buffer (5 mM HEPES pH 8.0, 150 mM sucrose, 100 mM KCl, 1mM $MgCl_2$).

Generation of Peptide Antibodies

Methods previously described [32] were followed. Peptides corresponding to the C termini of xPP1 (QSRPVTPPRNKNKQSK) were synthesized and conjugated to KLH, and polyclonal antibodies were raised in rabbits (PTG labs). Antibodies were affinity

purified using SulfoLink Coupling Gel (Pierce) according to the manufacturer's directions.

In Vitro Kinase Assay and Immunoprecipitation

AuroraB-INCENP$_{790-871}$ (A gift from A. Kelly) (0.2 μM) and KNL1$^{1-100}$-FLAG (10 μM) were incubated for 30 minutes at 20°C in kinase buffer (20 mM HEPES, 150 mM NaCl, 10 mM MgCl2, 1 mM DTT, 1 mM MnCl2, 0.025% Tween-20) with either 1 mM ATP or 30 μM ATP plus 0.02 μM ^{32}P-ATP (approx. 6000 Ci/mmol).

In vitro immunoprecipitation was performed in binding buffer (BB = PBS, 0.01% NP-40, 0.1 mg/ml BSA, 0.25 mM TCEP, 10 % glycerol). 5 μl of an *in vitro* kinase reaction (see above) was added to 10 μl of anti-FLAG M2 agarose (Sigma) in 50 μl TBS and incubated for 30 minutes at room temperature. The agarose was washed 2 times in TBS, 2 times in BB, resuspended in BB with 1 μM MBP-PP1γ^{cat}, and incubated 2 hours at 4°C. Beads were washed 3 times in BB and eluted with SDS sample buffer.

Immunoprecipitation in *Xenopus laevis* Egg Extracts

Anti-PP1 antibodies (see above) were crosslinked to protein A Dynabeads (Invitrogen) using BS^3 according to the manufacturer's directions (Pierce). GFP, PP1, and KNL1$^{1-300}$ were translated and ^{35}S-labeled in rabbit reticulocyte lysate with SP6 RNA polymerase according to manufactures directions (Promega). Proteins were added to CSF extracts prepared from *Xenopus laevis* eggs [33] (1/10 of total extract volume, approx 1:1:6 PP1:GFP:KNL1$^{1-300}$ by volume) and incubated for 1 hour at 20°C, then added to an equal volume of antibody crosslinked beads and incubated for 30 minutes at 4° C. Beads were washed 6 times with PBS + 0.1 % triton and eluted with SDS sample buffer. ^{35}S-labeled labeled proteins were imaged and quantified using a phosphorimager.

Immunoblots

The antibodies anti- MYC 9E10 (Santa Cruz), anti- Pgk1 (Invitrogen), anti- Pds1 (a gift from A. Rudner), anti- Mad1 (a gift from K. Hardwick) were used. Primary antibodies were diluted in Blocking Buffer (Li-Cor) + 0.05% Tween-20. IRDye 800 goat anti-rabbit or donkey anti-goat or IRDye 680 goat anti-mouse secondary antibodies were used according to manufacturers instructions. Blots were detected using the Odyssey Infrared Imaging System (Li-Cor). For figure 2G, proteins were resolved on a low crosslinking SDS-polyacrylamide gel (8%; acrylamide to bisacrylamide ratio 33.5:0.3).

Yeast Strains

All strains are derivative of the W303 background, see table S2. *RVAF-NT* and *RASA-NT* constructs were synthesized with a *Sal*I site in the promoter for integrating (Epoch Biolabs) and cloned into pRS406 [6] using the *Sac*II and *Kpn*I sites. For *WT-NT*, the RVAF mutation was eliminated using site-directed mutagenesis. For Glc7 fusion constructs: An *Asc*I site and a linker (GDGAGL) were inserted into the *WT-NT* or *RASA-NT* plasmids via PCR, *GLC7* was PCR amplified from genomic DNA and inserted into the *Asc*I site, and the *Sal*I site in *GLC7* was eliminated and the catalytically dead mutation D94A was created using site directed mutagenesis.

HGR and Single-Cell Colony Assay

For live cell imaging and bulk culture genotyping, parent cells were grown to log phase before *GAL-HO* induction for 6 hours, the time needed to guarantee cells have performed recombination [6]. For the single cell colony assay, $spc105^{NT}$ strains were streaked on galactose plates and left for 6 hours at 30°C. Single budded cells were isolated and allowed to grow to colonies. In cases where macroscopic colonies formed, DNA was

isolated by standard protocol. The genotype was assessed by PCR amplification and restriction digest with an enzyme specific to each mutation.

Disomy III Assay

Cells containing *ura3-52* and *leu2-Δ101::URA3::leu2-Δ102* at the endogenous *LEU2* locus on chromosome 3 [21] were grown in unselective YEPD for 48 hours, diluted appropriately and plated on selective medium lacking leucine, or that lacking both leucine and uracil. Percent disomy III is defined as frequency of Leu^+, Ura^+ cell formation.

Time Courses

Cells were arrested in G1 in YEPD + 10 nM α-factor for 1.5 (30°C) or 2 (23°C) hours. For tet^{off}-CDC20-127 experiments, 10 μg/ml doxycycline (sigma-aldirch) was added for 2 hours. Cells were washed into YEPD with doxycycline and without α-factor. For all others, after arrest cells were washed into YEPD without α-factor at 30°C or the indicated temperatures. Samples for Western blotting were prepared as previously described [34] every 15 minutes for 2 hours after release, and 10 nM α-factor was added 45 minutes after release to prevent cells from entering the next cell cycle.

Nocodazole Block

For Figures 4 B, C, asynchronous cultures grown to log phase in YEPD were treated with 15 μg/ml nocodazole and 10 μg/ml benomyl or an equivalent amount of DMSO for 3 hours, and cell morphology was counted. Cells were then washed 2 times in YEPD, plated onto YEPD, and colony formation was counted after 48 hours. For Figure S2F, cells were arrested in G1 in YEPD + 10 nM α-factor for 1.5 hours, then washed into

YEPD without α-factor with 15 μg/ml nocodazole and 10 μg/ml benomyl or an equivalent amount of DMSO, and cell morphology was counted every hour.

Time-Lapse Microscopy

A previously described method [35] was used.

Supplemental References

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