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Supplemental Information

Rif1 Regulates the Fate of DNA Entanglements during Mitosis

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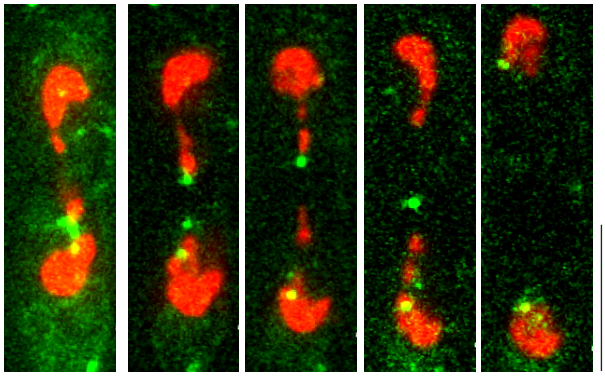
Figure S1

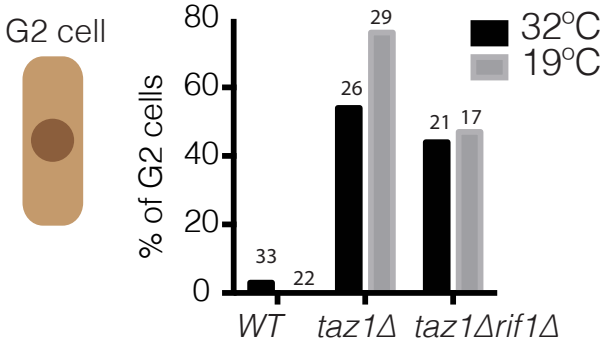
A

taz1Δ - 19°C - **Tpz1 Histone H3**



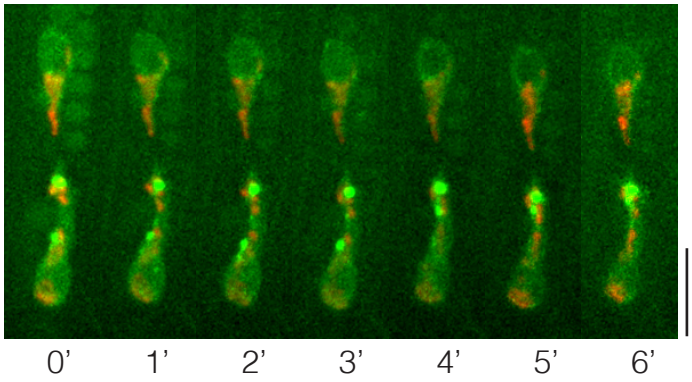
B

Polα persistence in G2 cells



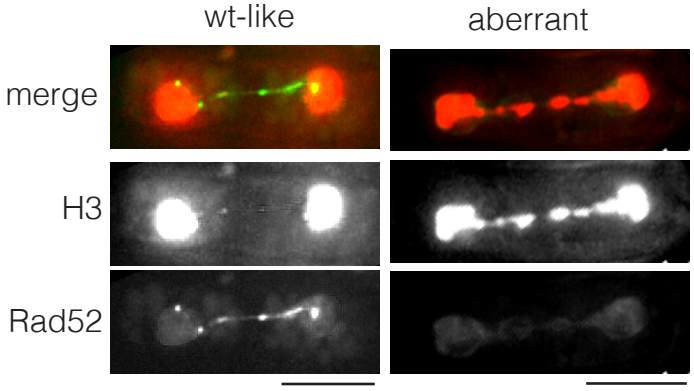
taz1Δ 19°C

Polα **Histone H3**



C

taz1Δ - 19°C **Rad52 Histone H3**



Rad52 presence in the midzone in *taz1Δ* cells

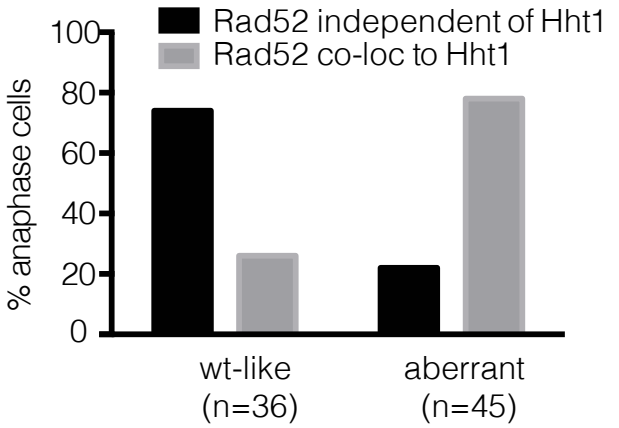


Figure S1, related to Figure 3: DNA replication and repair proteins appear in the anaphase mid-region in a *taz1Δ* setting.

A) Stills from a representative film of *taz1Δ* cells harboring endogenously tagged Tpz1-GFP and histone H3 as in Figure 1. Images were captured every 2 minutes through anaphase.

B) Cells harboring endogenously tagged Polα-GFP and histone H3 were grown to log phase at 32°C, then shifted to 19°C for 24 hours. Left panel: percentage of G2 cells showing Polα -GFP foci. Right panel: frames from film of a *taz1Δ* cell completing anaphase. Numbers below panels indicate time since filming began.

C) *taz1Δ* cells tagged at endogenous loci for Rad52 and histone H3 were followed through anaphase and categorized as showing either wt-like or aberrant chromosome segregation. Left: representative images. Right: Quantitation of presence of Rad52 between segregating chromosomes.

Figure S2

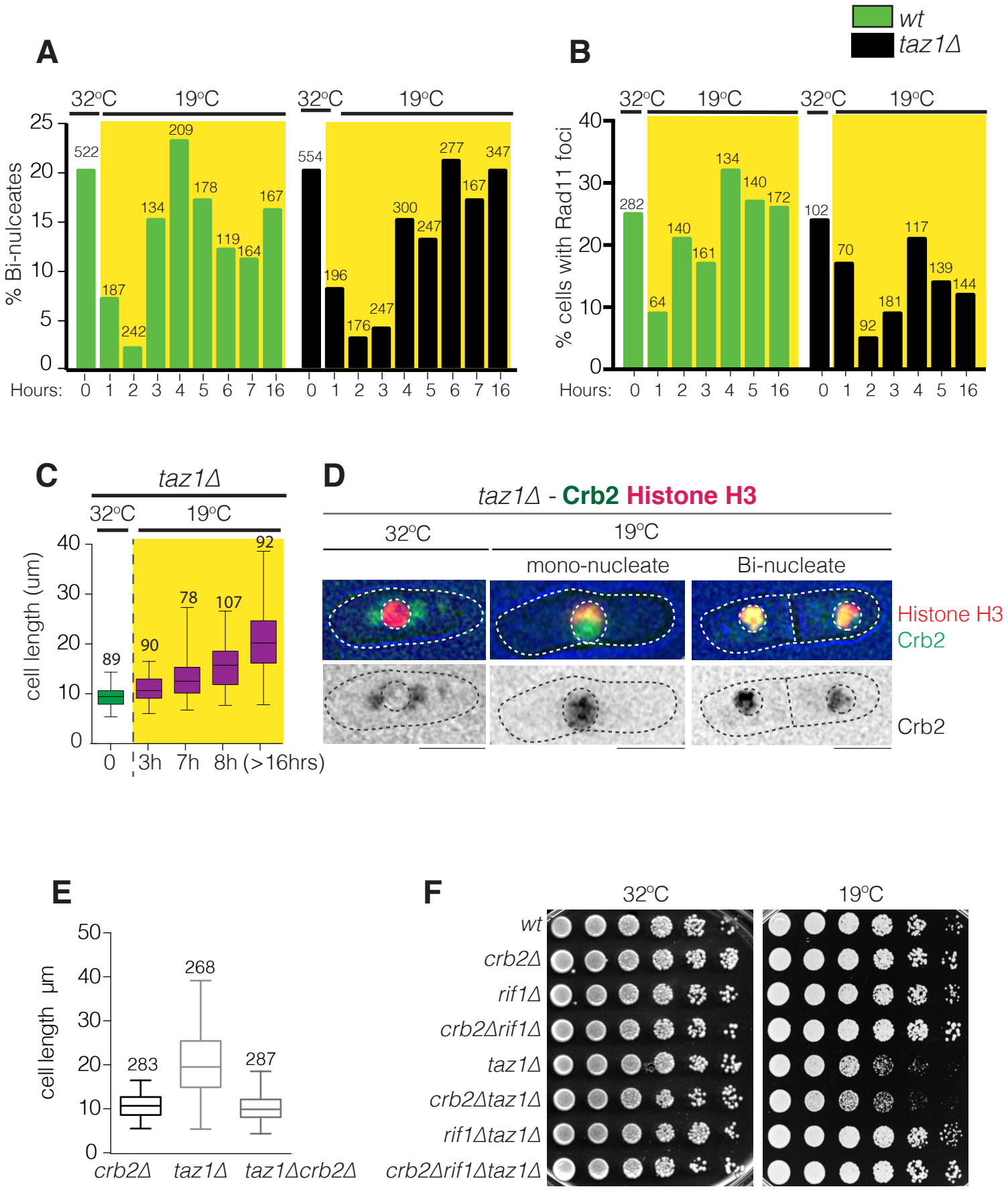

A
B
wt
taz1Δ
32°C
19°C
% Bi-nucleates
% cells with Rad11 foci
Hours:
C
taz1Δ
cell length (um)
0
3h
7h
8h (>16hrs)
D
taz1Δ - Crb2 Histone H3
mono-nucleate
Bi-nucleate
Histone H3
Crb2
E
cell length µm
crb2Δ
taz1Δ
taz1Δcrb2Δ
F
wt
crb2Δ
rif1Δ
crb2Δrif1Δ
taz1Δ
crb2Δtaz1Δ
rif1Δtaz1Δ
crb2Δrif1Δtaz1Δ

Figure S2, related to Figure 4: *taz1Δ* cells trigger the Crb2^{53BP1}-dependent checkpoint only in the second cell cycle following shift to cold temperature.

A) Bi-nucleate status (indicative of G1/S phase) is monitored in *wt* and *taz1Δ* cultures. Numbers below the x-axis indicate hours after shift from 32°C to 19°C. See text for details.
B) The presence of Rad11^{RPA}-GFP foci (indicative of S phase) is monitored as in (A).

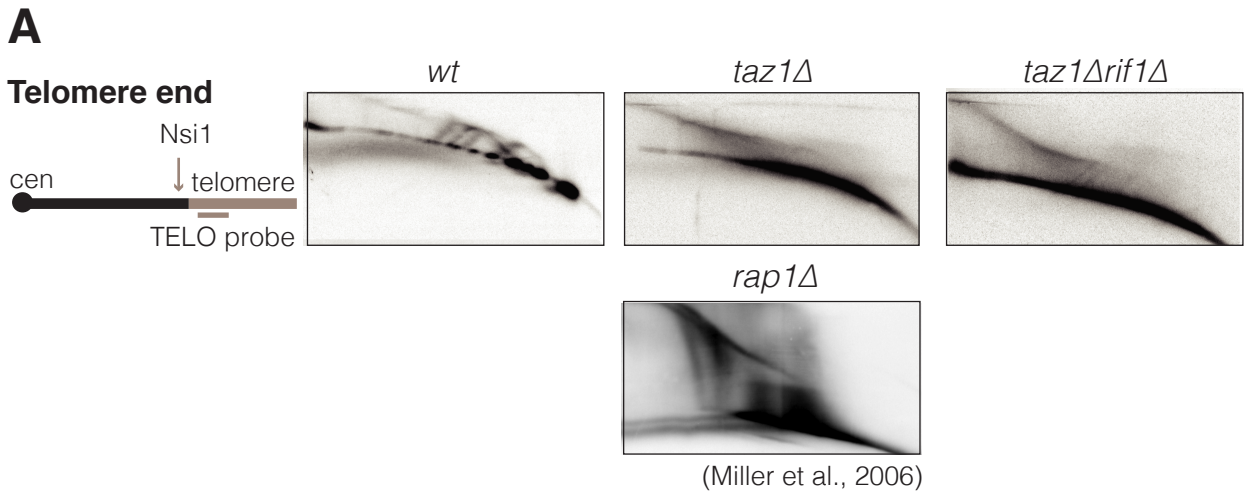
C) *taz1Δ* cell length over time in the cold. Cells were grown to log phase at 32°C, then shifted to 19°C. X-axis labels indicate hours after the temperature shift.

D) Crb2^{53BP1} localization is viewed in log phase at 32°C and >8 hours after shift to 19°C.

E) Cells grown to log-phase at 32°C were shifted to 19°C for 24 hours; then, cell lengths were measured.

F) Five-fold serial dilutions of log-phase cultures grown at 32°C were spotted onto rich medium and incubated at 32°C for 2 days or 19°C for 5 days.

Figure S3


A
Telomere end
Nsi1
cen
telomere
TELO probe
wt
taz1Δ
taz1Δrif1Δ
rap1Δ
(Miller et al., 2006)

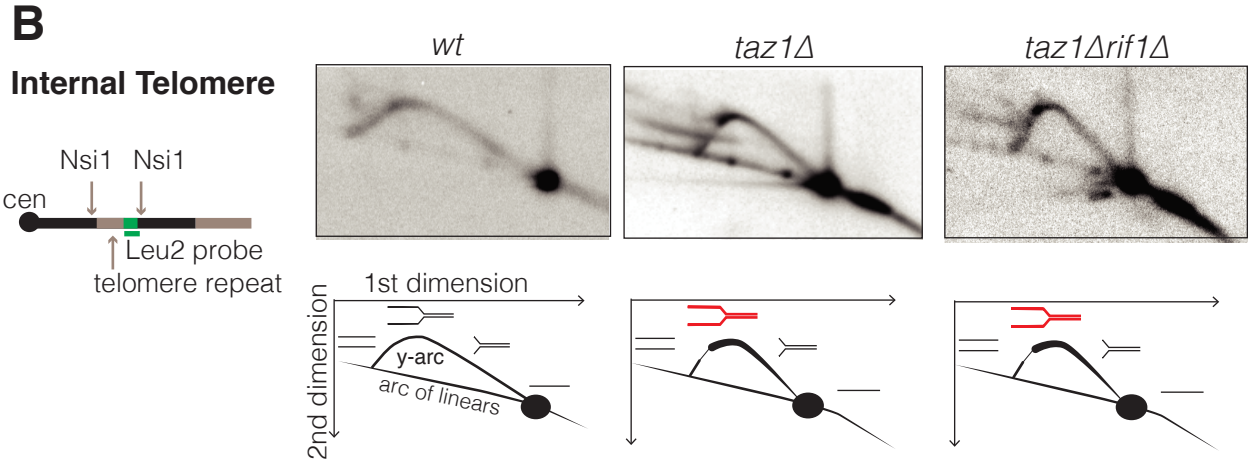

B
Internal Telomere
Nsi1
Nsi1
cen
Leu2 probe
telomere repeat
wt
taz1Δ
taz1Δrif1Δ
1st dimension
2nd dimension
y-arc
arc of linears

Figure S3, related to Figure 4: Rif1 neither causes nor suppresses *taz1Δ* telomere replication defects.
(A and B) Left: Diagrams indicating positions of NsiI restriction sites and probes. The internal telomere was constructed by inserting a cassette containing a synthetic telomere repeat tract and the *ScLEU2* gene at the *ura4+* locus on Chr III (Miller et al., 2006). Right: Southern blots of two-dimensional gels of NsiI fragments, hybridized with the telomere probe (A) or the Leu2 probe (B). Below each blot is an interpretive diagram.

A) While Y-arcs indicative of complete replication are seen for telomeric DNA isolated from *wt* cells, the corresponding arcs for *taz1Δ* telomeres fail to return to the arc of linears, indicating stalled, incomplete replication. *rap1Δ* telomeres are shown for comparison; while *rap1Δ* telomeres are long and heterogeneous, the corresponding Y-arcs both ascend and descend back to the arc of linears (*rap1Δ* image is re-printed from (Miller et al., 2006)).

B) The internal telomere was specifically assessed by hybridizing with a probe for *ScLEU2*. Prominent fork stalling is seen at this region in *taz1Δ* and *taz1Δrif1Δ* cells alike.

Figure S4

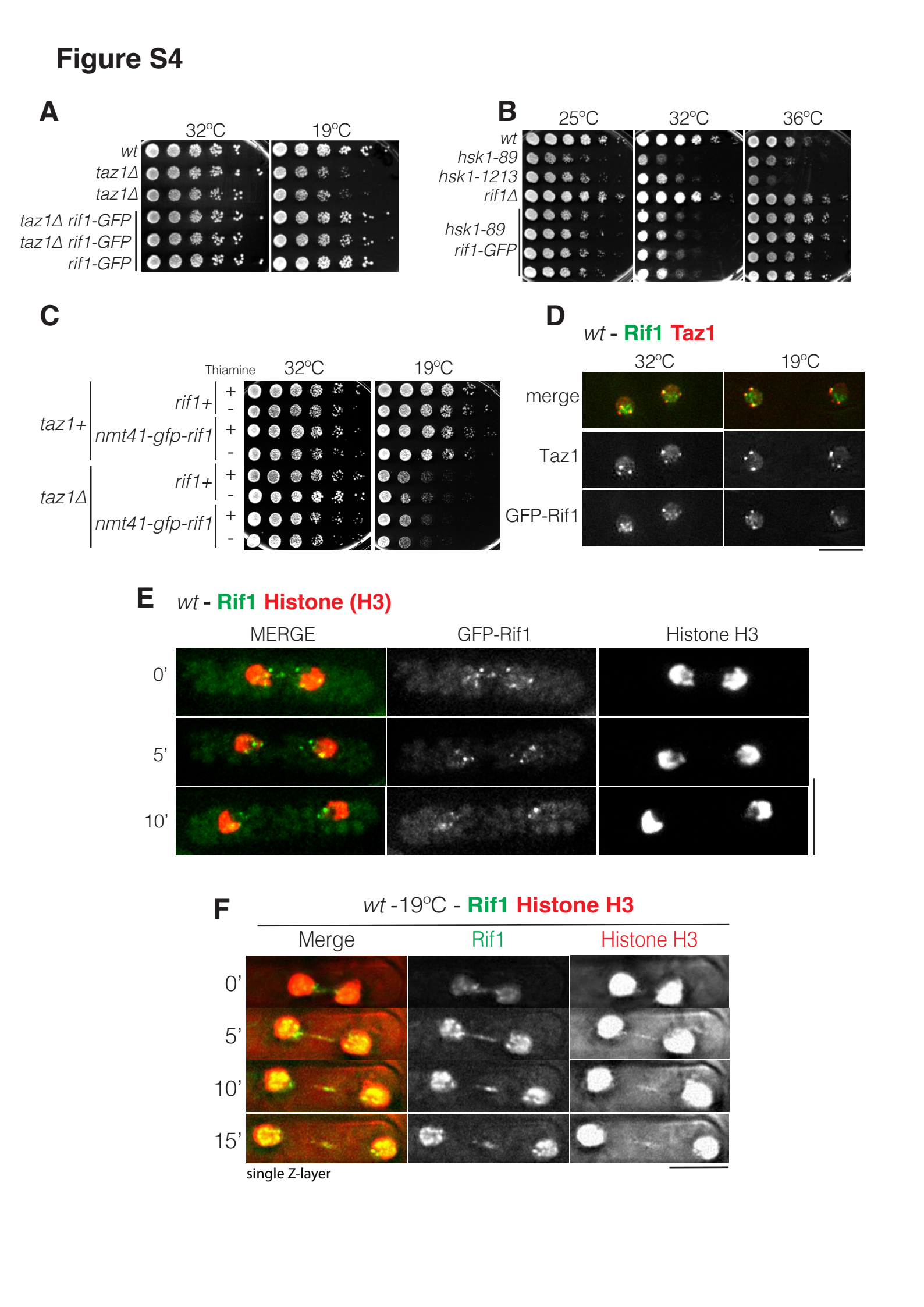

A
32°C
19°C
wt
taz1Δ
taz1Δ
taz1Δ rif1-GFP
taz1Δ rif1-GFP
rif1-GFP
B
25°C
32°C
36°C
wt
hsk1-89
hsk1-1213
rif1Δ
hsk1-89
rif1-GFP
C
Thiamine
32°C
19°C
taz1+
rif1+
nmt41-gfp-rif1
taz1Δ
rif1+
nmt41-gfp-rif1
D
wt - Rif1 Taz1
32°C
19°C
merge
Taz1
GFP-Rif1
E
wt - Rif1 Histone (H3)
MERGE
GFP-Rif1
Histone H3
0'
5'
10'
F
wt -19°C - Rif1 Histone H3
Merge
Rif1
Histone H3
0'
5'
10'
15'
single Z-layer

Figure S4, related to Figure 5: Behavior of N- and C-terminally GFP tagged Rif1.

A) Five-fold serial dilutions of log-phase cultures were spotted onto rich medium and incubated at 32°C for 2 days or 19°C for 5 days.

B) Serial dilutions were plated as in (A) and grown at 25°C for 5 days, or 32°C or 36°C for 2 days

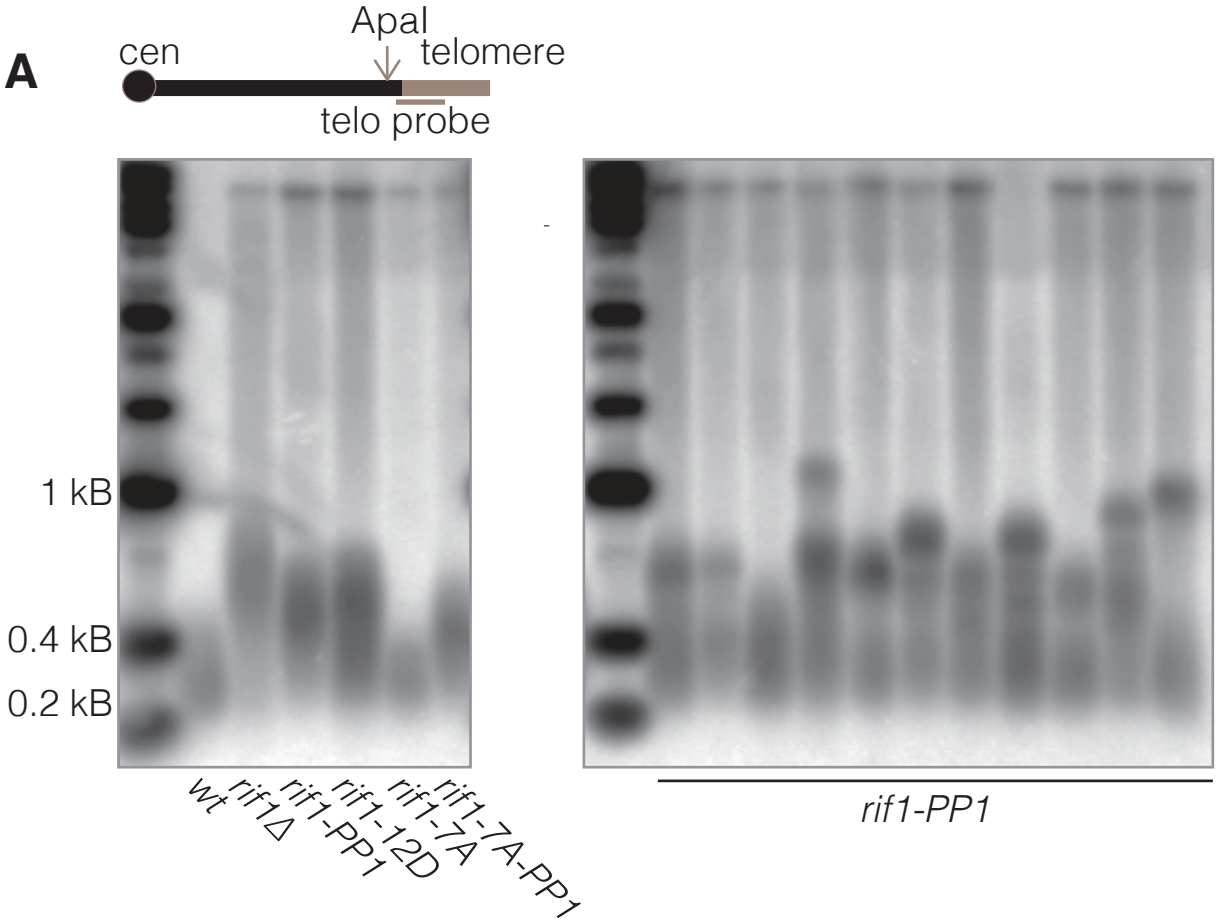
C) Cells were maintained in log phase in minimal liquid media with or without 60 μM thiamine (the absence of which confers induction of expression from the *nmt41* promoter) for 3 days at 32°C. Five-fold serial dilutions were then spotted onto rich media and incubated at 32°C for 2 days or 19°C for 5 days.

D) Representative images of G2-phase cells harboring endogenously tagged Taz1-mCherry and GFP-Rif1. Cells were grown to log phase at 32°C, then shifted to 19°C for 24 hours.

E) Frames from representative film of a cell as it progresses from G2 to anaphase at 32°C. No GFP-Rif1 is detected in the mid-region between segregating chromosomes. Compare with Figure 5A.

F) Frames from a representative film of a cell grown to log phase at 32°C, then shifted to 19°C for 24 hours. In contrast to all other microscopy images in which multiple stacks in Z-axis were collapsed into one, these images represent a single plane in the Z-axis.

Figure S5


A
cen
ApaI
telomere
telo probe
1 kB
0.4 kB
0.2 kB
wt
rif1Δ
rif1-PP1
rif1-12D
rif1-7A
rif1-7A-PP1
rif1-PP1

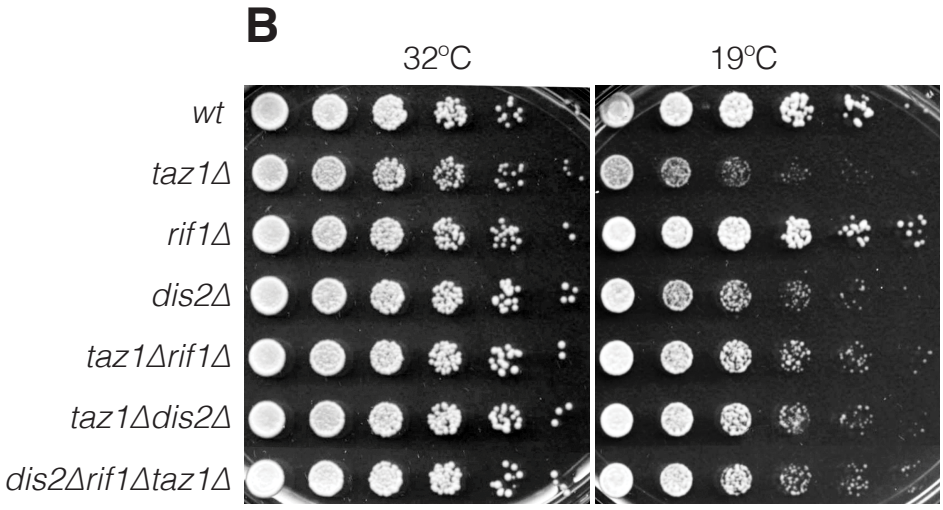

B
32°C
19°C
wt
taz1Δ
rif1Δ
dis2Δ
taz1Δrif1Δ
taz1Δdis2Δ
dis2Δrif1Δtaz1Δ

Figure S5, related to Figure 6: The loss of Rif1's PP1-binding ability, or the loss of PP1 phosphatase, rescues *taz1Δ* cold sensitivity.

A) Telomere lengths were analyzed by Southern blot analysis of Apa1 digested genomic DNA, hybridized with a telomere probe. The multiple *rif1-pp1* single clones in the right panel were constructed by crossing a *rif1Δ* strain against a *rif1-pp1* strain (Dave et al., 2014).

B) Five-fold serial dilutions of log-phase cultures were spotted onto rich medium and incubated at 32°C for 2 days or 19°C for 5 days. Dis2 is one of the two fission yeast PP1 phosphatases; see text for details.

Supplemental Movie Legends:

Movie S1: Film (stills in Figure 1A), showing a *taz1Δ* cell, harboring fluorescent histone H3 (see Figure 1A) and grown at 19˚C, with a "*wt*-like" chromosome segregation pattern.

Movie S2: Film of a *taz1Δ* cell tagged as in Figure 1A exhibiting the "pointing finger" segregation phenotype (see Figure 1A and text).

Movie S3: Film of a *taz1Δ* cell tagged as in Figure 1A demonstrating the "irreversibly entangled" segregation pattern (see Figure 1A and text).

Movie S4: Film (stills in Figure 3E) of *taz1Δ* cell carrying mRFP-tagged histone H3 and GFP-tagged RPA^{Rad11}. This cell, grown at 19˚C, shows a strand of RPA between segregating chromosomes retracting into one of the daughter nuclei.

Table S1. Strains constructed and analysed in this study

JCF N°	Mating type	*Genotype*	Origin
11	h^+	*taz1::ura4$^+$ ade6-M216 leu1-32 ura4-D18*	Lab Stock
108	h^-	*ade6-M210 his3-D1 leu1-32 ura4-D18*	Lab Stock
109	h^+	*ade6-M216 his3-D1 leu1-32 ura4-D18*	Lab Stock
282	h^-	*rif1::kanMX6 taz1::ura4$^+$ ade6-(M210 or M216) leu1-32 ura4-D18*	Lab Stock
300	h^-	*rif1::kanMX6 ade6-M216*	Lab stock
481	h^-	*rif1-GFP:kanMX6*	Lab Stock
1076	h^+/h^-	*trt1$^+$/trt1::hygMX6 ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18*	Lab Stock
1582	h^+/h^-	*taz1::kanMX6/taz1::kanMX6 trt1$^+$/trt1::his3 ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18*	Lab Stock
1675	h^+	*ura4::synthetic-telo (500bp) his3- leu1-32 ade6-(M210 or M216)*	Lab stock
1678	h^+	*ura4::synthetic-telo (500bp) taz1::kanMX6 his3- leu1-32 ade6-(M210 or M216)*	Lab stock
1689	*h?*	*ura4::synthetic-telo (500bp) rif1::kanMX6 taz1::hygMX6 his3- leu1-32 ade6-(M210 or M216)*	Lab stock
2906	h^-	*lys1:kanMX6:nmt1:GFP-atb2 leu1-32 ura4-D18*	Nurse Lab (PN3774)
4496	h^-	*hht1-mRFP:kanMX6 ade6-M210 leu1-32 ura4-D18 his3-D1*	Lab stock
6382	h^-	*hsk1-1312 ade6-M216 leu1-32 ura4-D18*	Forsburg lab
9202	h^-	*crb2::ura4$^+$ ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
9203	h^+	*rif1::hygMX6 his3-D1 leu1-32 ura4-D18*	This study
9204	h^+	*crb2::ura4$^+$ rif1::hygMX6 ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
9205	h^-	*taz1::natMX6 his3-D1 leu1-32 ura4-D18*	This study
9206	h^+	*taz1::natMX6 crb2::ura4$^+$ ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
9207	*h?*	*taz1::natMX6 rif1::hygMX6 ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
9208	h^+	*taz1::natMX6 rif1::hygMX6 crb2::ura4$^+$ his3-D1 leu1-32 ura4-D18*	This study
9236	h^+/h^-	*taz1::kanMX6/taz1::kanMX6 rif1+/rif1::hygMX6 trt1$^+$/trt1::his3$^+$ ade6-M201/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18*	This study
9273	h^-	*rif1-GFP:kanMX6 taz1::hygMX6*	This study
9292	$h^?$	*hht1-mRFP:kanMX6 reb1-GFP:natMX6 taz1::hygMX6 leu1-32 ura4-D18 his3-D1*	This study
9298	h^-	*hht1-mRFP:kanMX6 taz1::hygMX6 ade6-M210 leu1-32 ura4-D18 his3-D1*	This study
9301	$h^?$	*hht1-mRFP:kanMX6 taz1-GFP:kanMX6 ade6-(M210 or M216) leu1-32 ura4-D18 his3-D1*	This study
9306	h^-	*hht1-mRFP:kanMX6 taz1::hyg rif1::natMX6 ade6-M210 leu1-32 ura4-D18 his3-D1*	This study
9317	h^-	*kanMX6:nmt41:GFP-rif1 ade6-M210 his3-D1 leu1-32 ura4-D18*	This study
9319	$h^?$	*kanMX6:nmt41:GFP-rif1 taz1::natMX6 ade6-M210 his3-D1 leu1-32 ura4-D18*	This study
9328	*h?*	*rif1-GFP:kanMX6 hsk1-89:ura4$^+$*	This study
9339	$h^?$	*kanMX6:nmt41:GFP-rif1 taz1-mCherry:natMX6 ade6-(M210 or M216) his3-D1 leu1-32 ura4-D18*	This study
9363	$h^?$	*ade6-216 leu1-32 lys1-131 ura4-D18 sod2[::kanMX6-ura4+-lacOp] his7+::lacI-GFP hht1-mRFP:kanMX6 taz1::natMX6*	This study
9375	h^+	*tpz1-GFP:kanMX6 hht1-mRFP:kanMX6 taz1::natMX6 his3-D1 leu1-32 ura4-D18*	This study
9378	$h^?$	*hht1-mRFP:kanMX6 rad11-GFP:kanMX6 taz1::hygMX6 ade6-M210 his3-D1 leu1-32 ura4-D18*	This study

Table S1. Strains constructed and analysed in this study

10368	h^{90}	*z:NatR-P-adh131-tetR-tomato cnt2::TetO::ura4+*	Lab stock
11330	h^-	*hht1-mRFP:kanMX6 rad22-GFP:kanMX6 taz1::ura4+ ade6-M210 his3-D1 leu1-32 ura4-D18*	This study
11337	h^+	*crb2-D2::ura4+ leu1-32::2xYFP-crb2:leu1+ ura4-D18 taz1::kanMX6 hht1-mRFP:kanMX6*	This study
11357	$h^?$	*hht1-mRFP:kanMX6 kanMX6:nmt41-GFP-rif1 ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
11382	h^-	*swi7-GFP:kanMX6 hht1-mRFP:kanMX6 his3-D1*	This study
11384	h^+	*swi7-GFP:kanMX6 hht1-mRFP:kanMX6 taz1::hygMX6 his3-D1 leu1-32*	This study
11394	h^-	*swi7-GFP:kanMX6 hht1-mRFP:kanMX6 taz1::hygMX6 rif1::natMX6 his3-D1*	This study
11429	$h^?$	*hht1-mRFP:kanMX6 kanMX6:nmt41-GFP-rif1 taz1::natMX6 ade6-(M210 or M216)*	This study
11475	$h^?$	*hht1-mRfP:kanMX6 rad11-GFP:kanMX6 taz1::hyg rif1::natMX6 ade6-(M210 his3-D1 leu1-32 ura4-D18*	This study
12320	h^+	*rif1-PP1 ade6-216 his3-D1 ura4-D18 leu1-32*	Bianchi lab
12322	h^-	*rif1-12D ade6-216 his3-D1 ura4-D18 leu1-32*	Bianchi lab
12323	h^+	*rif1-7a ade6-216 his3-D1 ura4-D18 leu1-32*	Bianchi lab
12324	h^-	*rif1-7a-PP1 ade6-216 his3-D1 ura4-D18 leu1-32*	Bianchi lab
12333	h^-	*taz1::kanMX6 rif1-PP1 his3-D1*	This study
12361	$h^?$	*hht1-mRFP:kanMX6 rad11-GFP:kanMX6 ade6-M210 ura4-D18*	This study
12372	$h^?$	*rif1-PP1 z:NatR-Padh131-TetR-tomato cnt2::TetO*2::ura4 ura4-D18*	This study
12373	h^{90}	*rif1::hygMX6 z:NatR-Padh131-TetR-tomato cnt2::TetO*2::ura4 ura4-D18*	This study
12374	$h^?$	*rif1::hygMX6 z:NatR-Padh131-TetR-tomato cnt2::TetO*2::ura4 ura4-D18*	This study
12392	$h^?$	*z:NatR-Padh131-TetR-tomato*	This study
13431	h^+	*rif1::bsd hsk1-89:ura4+ ura4-D18 leu1-32*	Bianchi lab
13433	h^-	*hsk1-89:ura4+ ura4-D18 leu1-32*	Bianchi lab
13524	*h?*	*hht1-mRFP:kanMX6 reb1-GFP:natMX6 leu1-32 ura4-D18 his3-D1*	This study
13546	h^-	*pcdt1-rif1 ura4-D18 leu1-32 ade6-M210 his3-D1*	This study
13547	h^-	*pcdt1-rif1 ura4-D18 leu1-32 ade6-M210 his3-D1*	This study
13579	*h?*	*taz1::ura4+ pcdt1-rif1 ade6-(M210 or M216) leu1-32 ura4-D18*	This study
13616	h^+	*taz1::ura4+ pcdt1-rif1 ade6-M216 leu1-32 ura4-D18*	This study
13632	h^-	*hsk1-89:ura4+ pcdt1-rif1 ura4-D18 leu1-32*	This study
13670	$h^?$	*pcdt1-rif1 z:NatR-Padh131-TetR-tomato cnt2::TetO*2::ura4 ura4-D18*	This study
13829	h^-	*rif1::hygMX6 ade6-M210 his3-D1 ura4-D18 leu1-32*	This study
13831	h^+	*dis2::ura4+ ura4-D18 leu1-32 ade6-M216 his3-D1*	This study
13835	$h^?$	*taz1::natMX6 rif1::hygMX6 ade6-M216 his3-D1 ura4-D18 leu1-32*	This study
13837	h^-	*dis2::ura4+ taz1::natMX6 ura4-D18 leu1-32*	This study
13840	h^-	*dis2::ura4+ rif1::hygMX6 ade6-M216 his3-D1 ura4-D18 leu1-32*	This study
13843	h^-	*dis2::ura4+ rif1::hygMX6 taz1::natMX6 ade6-M216 his3-D1 ura4-D18 leu1-32*	This study
13846	$h^?$	*taz1::natMX6 ade6-M216 his3-D1 ura4-D18 leu1-32*	This study

Supplemental Experimental Procedures

Strains and media

Media and growth conditions were as previously described (Moreno et al., 1991). Strains are listed in Table S1. Single gene deletions and tag insertions were generated as described (Bahler et al., 1998) and used to create further strains through crossing and sporulation. The *taz1Δ rif1-pp1* clones were generated by crossing a *taz1Δrif1Δ* strain to a *rif1-pp1* strain; single *taz1Δ rif1-PP1* clones were selected.

Molecular Biology Techniques

Southern blot and two-dimensional gel electrophoresis were performed as previously described (Miller et al., 2006) using described probes (Rog et al., 2009).

qRT PCR:

RNA was harvested from cultures using MasterPure Yeast RNA Purification kit as described in the manufacturer's protocol (Epicentre). cDNA was generated using 2μg RNA, oligo dT primers and Superscript III Reverse Transcriptase kit (ThermoFisher). Transcripts were quantified using EXPRESS SYBR GreenER (ThermoFisher) and the StepOnePlus Real-Time PCR System (ThermoFisher). Primer specificity was confirmed by both melting-curve analysis and agarose gel electrophoresis of PCR products. Transcript levels for *rif1+* were analyzed by the standard curve method and normalized to *act1+* transcript levels.

Live Cell Microscopy

Cells were adhered to 35-mm glass culture dishes (MatTek Corporation) using 0.2 mg/ml of soybean lectin (Sigma-Aldrich) and immersed in YES, or alternatively places on solid media pads (2% agarose melted in YES) on glass slides, with a coverslip sealed on top.

Time-lapse imaging was performed at 27°C in an Environmental Chamber with a DeltaVision Spectris (Applied Precision) comprising a widefield inverted epifluorescence microscope (IX70; Olympus), a 100x NA 1.4 oil immersion objective (UPlanSapo; Olympus), and a charge coupled device CoolSnap HQ camera (Photometrics). Images were acquired over 20 focal planes at a 0.2µm step size with frames taken at different time points. Images were deconvolved (conserved ratio method) and combined into a 2D image using the maximum intensity projection setting for analysis using SoftWorx (Applied Precision). Structural Illumination microscopy was carried out using a DeltaVision OMX (Applied Precision) comprising an OMX optical microscope (version 4), an Olympus PLAPON60xO, NA 1.42, oil immersion objective, and a sCMOS camera.

Supplemental References

Bahler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast *14*, 943-951.

Moreno, S., Klar, A., and Nurse, P. (1991). Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods Enzymol *194*, 795-823.