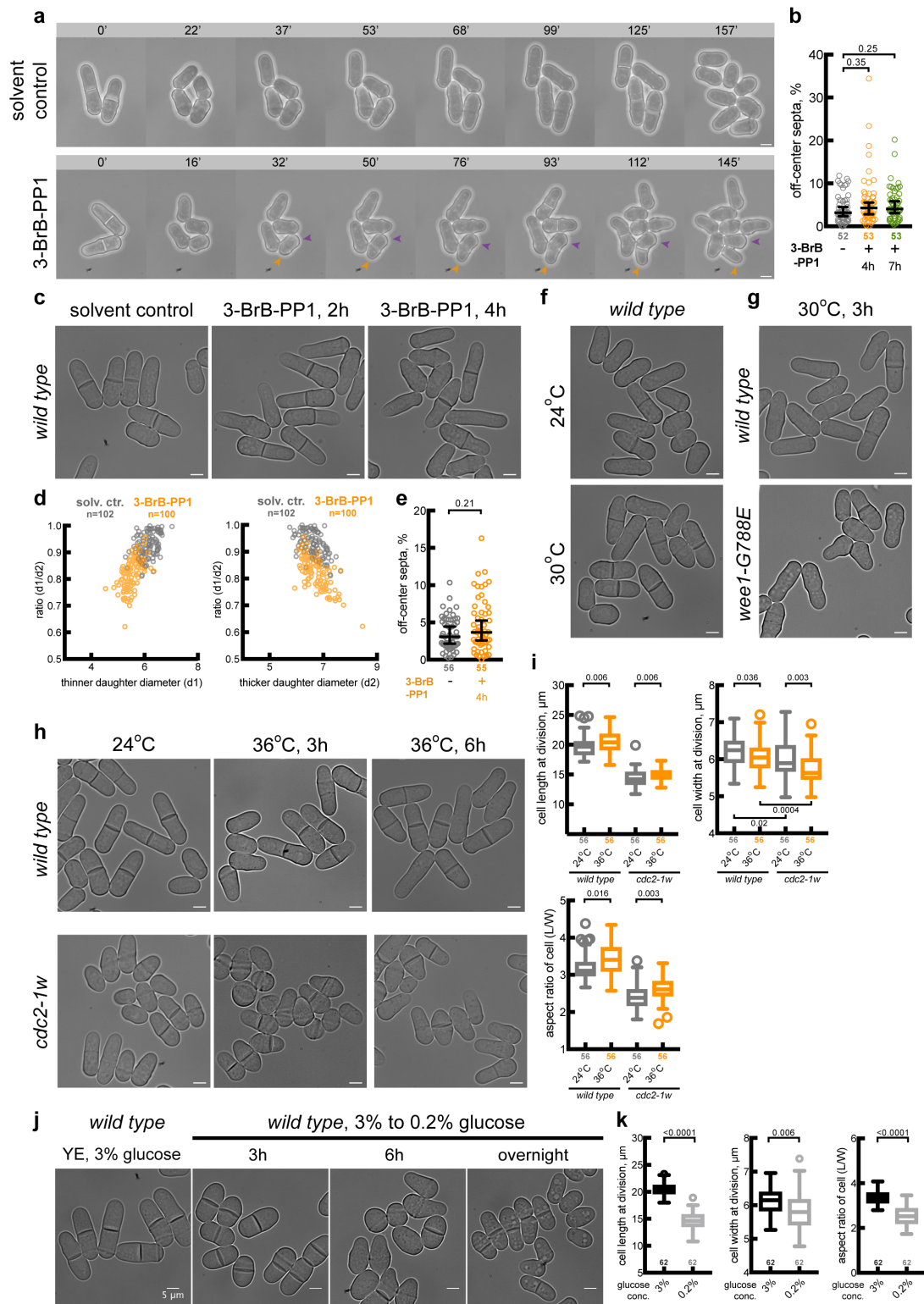


Supplementary information

Cellular geometry scaling ensures robust division site positioning
Ying Gu and Snezhana Oliferenko

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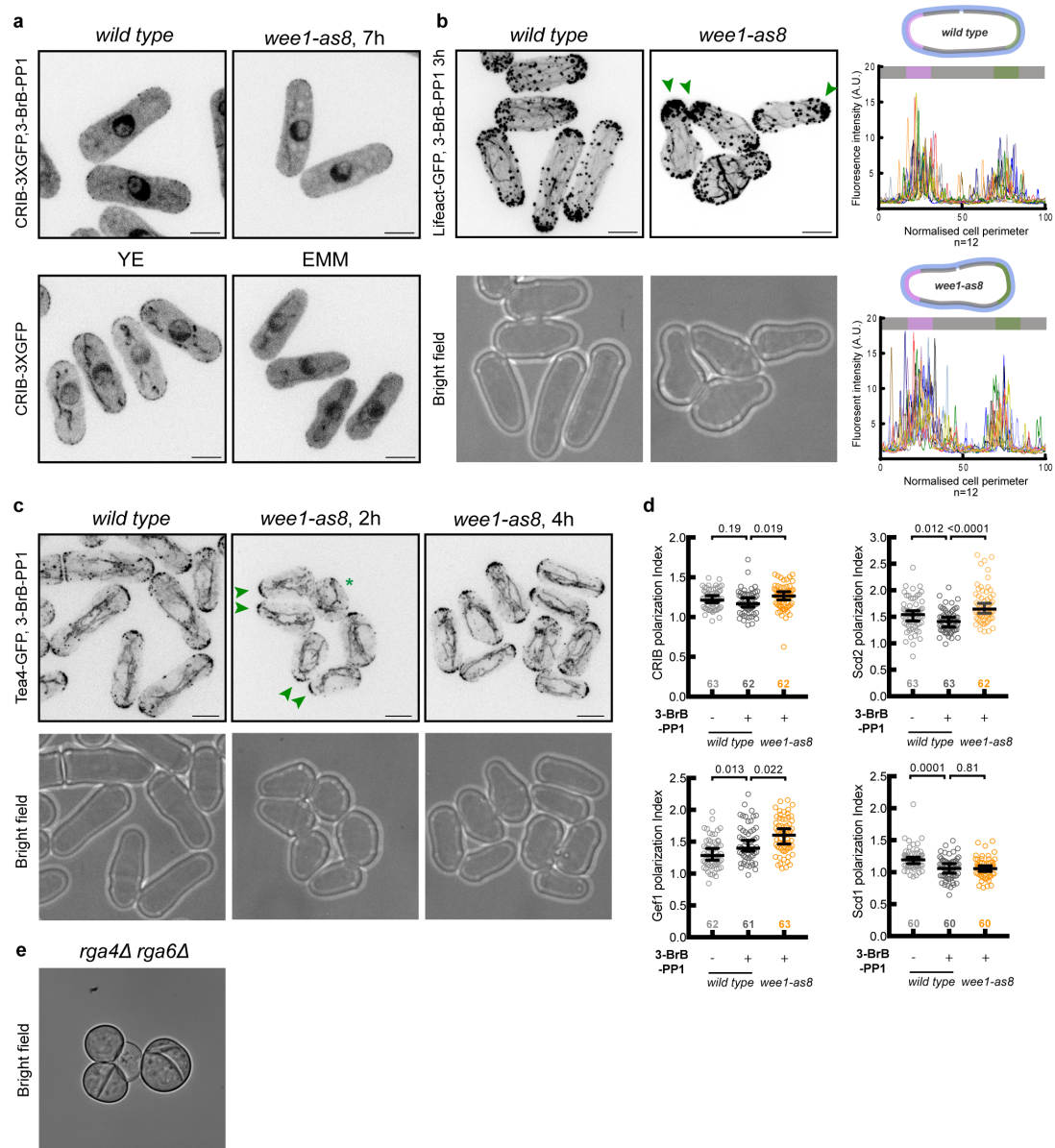
Supplementary figures 1 to 5
Supplementary tables 1 and 2



Supplementary figure 1. *S. japonicus* scales its geometry in a variety of experimental conditions. (a) Time-lapse montages of maximum intensity z-projected bright-field images of *S. japonicus* *wee1-as8* cells in methanol

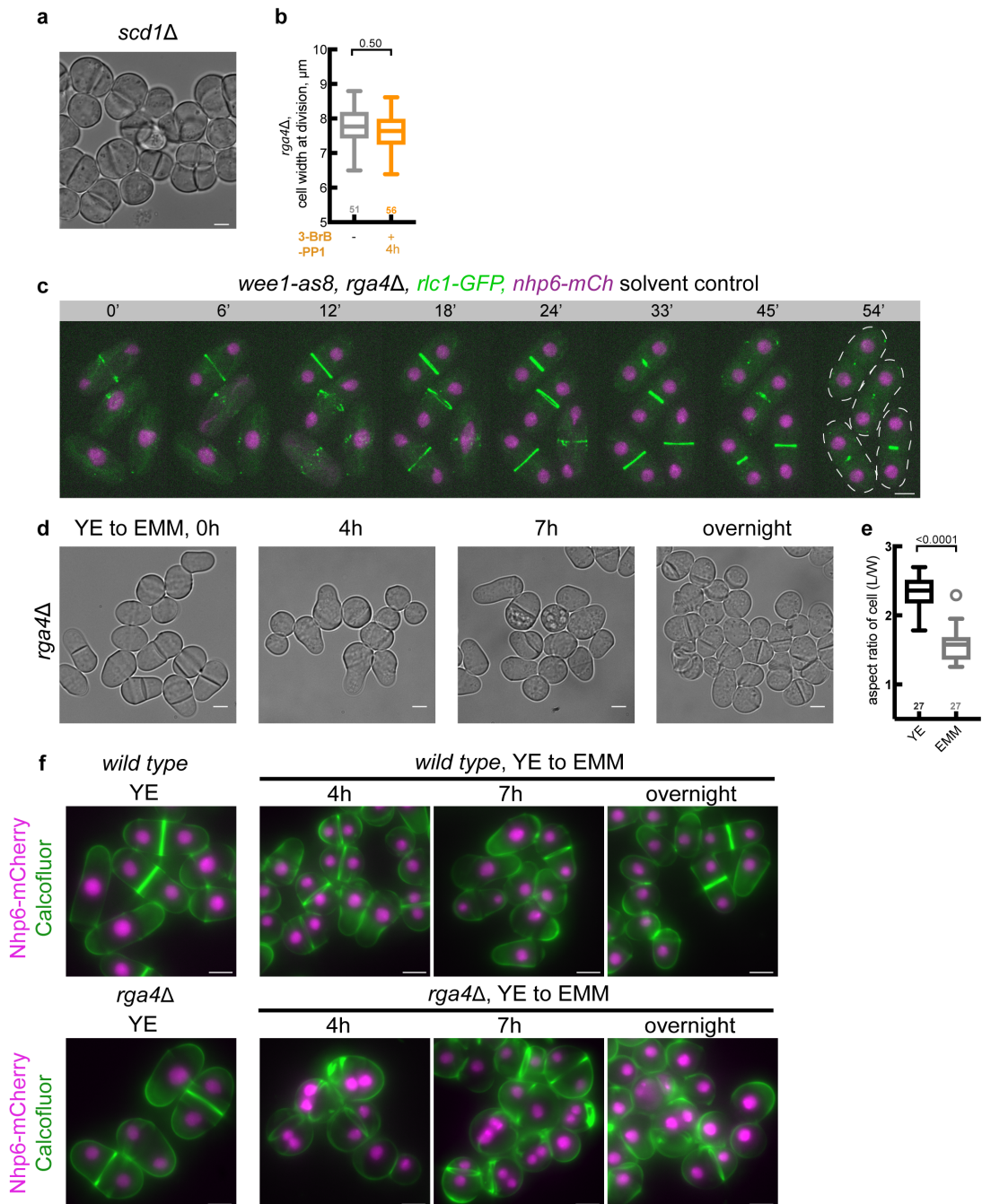
(solvent control) (*top*) or 20 μ M 3-BrB-PP1 (*bottom*). Methanol or 3-BrB-PP1 was administered one hour prior to imaging. Orange arrowheads point toward a hyperpolarizing cell end whereas magenta arrowheads indicate the other, thicker cell end. **(b)** A plot summarizing the accuracy of division plane positioning in methanol- or 3-BrB-PP1-treated *wee1-as8* cells shown in Fig. 1a. **(c)** *S. japonicus* wild type cells incubated with 20 μ M 3-BrB-PP1 for indicated time. **(d)** A 2-hour treatment with 3-BrB-PP1 causes mild hyperpolarization at one cell tip in wild type cells, indicative of off-target effects. Shown are the scatter plots, where either 'thinner' (*top*) or 'thicker' (*bottom*) daughter cell diameter measurements are on x-axis, and the ratios between diameters of the daughters are on y-axis. **(e)** A plot summarizing the accuracy of division plane positioning shown in **(c)**. **(f)** Representative images of wild type cells cultured at 24°C (*top*) and 30°C (*bottom*). **(g)** *S. japonicus* wild type and *wee1-G788E* mutant (*bottom*) cells shifted from 24°C to 30°C for 3 hours. **(h)** *S. japonicus* wild type (*top*) and *cdc2-1w* mutant (*bottom*) cells cultured at 24°C, and upon the shift to 36°C for 3 and 6 hours. **(i)** Measurements of cellular length, width and aspect ratio of the wild type as compared to *cdc2-1w* cells at the 6-hour time point, shown in **(h)**. **(j)** *S. japonicus* cells grown in YE with 3% glucose and shifted to YE-0.2% glucose for 3, 6 hours and overnight. **(k)** Quantifications of cell length, width and aspect ratio at division in YE with 3% glucose and 0.2% glucose overnight, for cells shown in **(j)**. **(c, f, g, h, j)** Shown are single z-plane bright-field micrographs of live cells; scale bars represent 5 μ m. **(b and e)** Shown as 1D-scatter plots. Deviation from the geometric center of the cell is indicated on y-axis. Black bars represent sample median with error bars indicating 95%

confidence intervals. **(i, k)** Quantifications presented as box plots with whiskers calculated by the Tukey method. *n* indicated in figures; *p* values derived from Kolmogorov-Smirnov test.



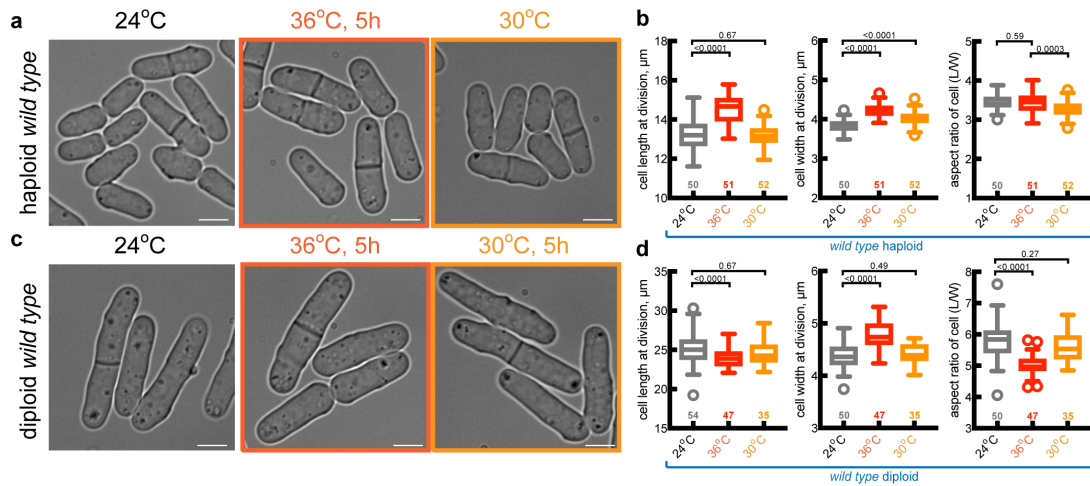
Supplementary figure 2. Localization of cellular polarity factors in control *S. japonicus* cells and during the process of scaling. (a) Following scaling, cells do not show relative enrichment of Cdc42 activity at cell tips. Spinning disk confocal micrographs of *S. japonicus* CRIB-3xGFP-expressing wild type and *wee1-as8* cells grown in indicated experimental conditions. (b) *S. japonicus* wild type and *wee1-as8* cells expressing LifeAct-GFP and the corresponding bright-field images. Green arrowheads indicate hyperpolarized cell tips. Cortical LifeAct-GFP fluorescence intensities (y-axis) normalized to cell

perimeter (x-axis) (*right*) indicate F-actin enrichment at hyperpolarizing tips in Wee1-inhibited cells. (c) 3-BrB-PP1-treated *S. japonicus* wild type and *wee1-as8* cells expressing Tea4-GFP and the corresponding bright-field images. Tea4 is preferentially enriched at the hyperpolarized cell tip in Wee1-inhibited cells (indicated by green arrowheads). Note that Tea4 does not exhibit specific cortical localization in a depolarized Wee1-inhibited cell at a 2-hour time-point (indicated by a green star). (d) Graphs showing relative fluorescence intensities of indicated marker proteins at thinner cell tips, for cells shown in Fig. 2c. Shown as 1D-scatter plots. Black bars represent sample median with error bars indicating 95% confidence intervals. n indicated in figures; *p* values derived from Kolmogorov-Smirnov test. (e) Single z-plane bright-field micrographs of *S. japonicus rga4Δrga6Δ* cells. Simultaneous removal of both Cdc42 GAPs Rga4 and Rga6 in *S. japonicus* gives rise to virtually spherical cells, indicating cooperation between these two GAPs in maintaining polarized growth. (a-c) shown are maximum intensity z-projections of spinning-disk confocal micrographs of cells. (a-c, e) Scale bars represent 5 μm .

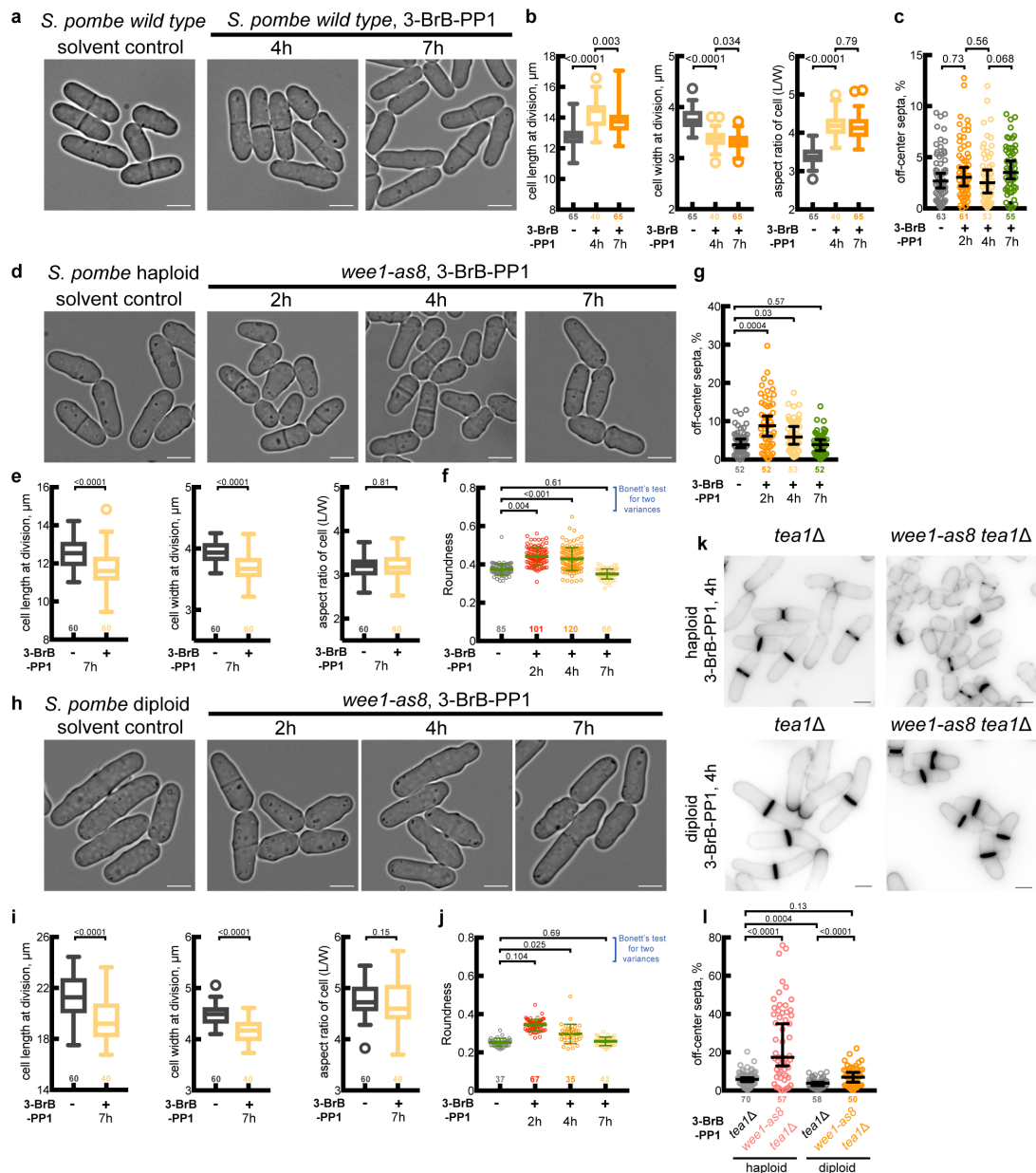


Supplementary figure 3. Rga4 is required for geometrical scaling in *S. japonicus*. (a) Representative image of *S. japonicus scd1Δ* cells. *S. japonicus* cells grown on YES agar lacking Cdc42 GEF Scd1 are defective in establishing polarized growth and cannot proliferate in the liquid YES medium. (b) Graph representing cell width at division for *rga4Δ S. japonicus* cells treated with solvent or 3-BrB-PP1 for 4 hours. (c) Time-lapse montage of

maximum intensity z-projected spinning-disk confocal micrographs of methanol-treated (as solvent control) *wee1-as8 rga4Δ S. japonicus* cells expressing Rlc1-GFP and Nhp6-mCherry. Myosin complexes assemble into medially positioned rings in these cells. Cell boundaries are outlined by white dashed lines. **(d)** *S. japonicus rga4Δ* cells after the shift from YE to EMM medium for 0, 4 and 7 hours followed by overnight incubation in EMM. **(e)** Graph showing cellular aspect ratio of cells with division septa shown in **(d)** before and after overnight incubation in EMM. **(f)** Pseudocolored maximum intensity z-projected epifluorescence images of wild type (*top*) and *rga4Δ S. japonicus* cells (*bottom*) expressing nuclear Nhp6-mCherry (magenta) stained with Calcofluor White (green) to visualize division septa under indicated conditions. Note that *rga4Δ* cells fail to rescale cell geometry after nutritional shift and display off-center division septa giving rise to bi-nucleated daughter cells after incubation in EMM. **(a and d)** Shown are single z-plane bright-field micrographs. **(a, c, d and f)** Scale bars represent 5 μm . **(b, e)** Box plots with whiskers; *n* indicated in figures; *p* values derived from Kolmogorov-Smirnov test.



Supplementary figure 4. Analyses of wild type *S. pombe* cells grown at different physiological temperatures. **(a)** *S. pombe* wild type haploid cells grown at 24°C overnight (grey), shifted to 36°C for 5 hours (red) or grown at 30°C overnight (orange). **(b)** Quantifications of cell length, width and aspect ratio at division of wild type *S. pombe* haploid cells at indicated temperatures shown in **(a)**. **(c)** *S. pombe* wild type diploid cells grown at 24°C overnight (grey), shifted to 36°C for 5 hours (red) or grown at 30°C for 5 hours (orange). **(d)** Quantifications of cell length, width and aspect ratio at division of wild type *S. pombe* diploid cells at indicated temperatures shown in **(c)**. **(a and c)** Scale bars represent 5 μm. **(b and d)** Presented are box plots with whiskers calculated by the Tukey method. n indicated in figures; *p* values derived from Kolmogorov-Smirnov test.



Supplementary figure 5. Analyses of wild type and *wee1-as8* *S. pombe* cells grown in the presence of the ATP analog 3-BrB-PP1. (a) *S. pombe* wild type cells incubated with 20 μM 3-BrB-PP1 for indicated time. (b) Quantifications of cell length, width and aspect ratio at division of cell population represented in (a). (c) A plot summarizing the accuracy of division plane positioning shown in (a). (d) *S. pombe* *wee1-as8* haploid cells incubated with methanol (solvent control) or 20 μM ATP analog 3-BrB-PP1 for 2, 4 and 7 hours. (e)

Quantifications of cell length, width and aspect ratio at division after Wee1 inhibition in *S. pombe wee1-as8* haploids shown in (d). (f) Cell shape at division was assessed using roundness parameter as a proxy, where spherical cells are assigned a roundness value of 1. Shown is a plot of the distribution of cell roundness values within an *S. pombe wee1-as8* haploid population treated with 20 μ M 3-BrB-PP1 for indicated time points shown in (d). (g) A plot summarizing the accuracy of division plane positioning in methanol- or 20 μ M 3-BrB-PP1-treated *S. pombe wee1-as8* haploid cells at different time points shown in (d). Deviation from the geometric center of the cell is indicated on y-axis. (h) *S. pombe wee1-as8* diploid cells incubated with methanol or 20 μ M 3-BrB-PP1 for 2, 4 and 7 hours. (i) Quantifications of cell length, width and aspect ratio at division after Wee1 inhibition in *S. pombe wee1-as8* diploid cells shown in (h). (j) A plot showing the distribution of cell roundness values within an *S. pombe wee1-as8* diploid population treated with 20 μ M 3-BrB-PP1 for indicated time points shown in (h). (k) Maximum intensity z-projections of epifluorescence images of Calcofluor White-stained live *S. pombe tea1 Δ* and *wee1-as8 tea1 Δ* haploid (top) and diploid (bottom) cells incubated with 20 μ M 3-BrB-PP1 for 4 hours. (l) A plot summarizing the accuracy of division plane positioning in 3-BrB-PP1-treated cells shown in (k). (a, d, h) Shown are single z-plane bright-field micrographs. Scale bars represent 5 μ m. (b, e, i) Presented are box plots with whiskers; n indicated in figures; *p* values derived using Kolmogorov-Smirnov test. (f, j) *p* values derived using Bonett's test for two variances. Green bars represent sample mean. Error bars represent standard deviation. (c, g, l) Shown are 1D scatter plots;_black bars represent sample median with error bars indicating 95%

confidence intervals. n indicated in figures; p values derived using Kolmogorov-Smirnov test.

<i>Schizosaccharomyces japonicus</i>	
Collection No.	Genotype
SOJ5	NIG2028 matsj-P2028 h- (prototroph)
SOJ2876	<i>wee1-as8::kanR</i> h- (prototroph)
SOJ3414	<i>wee1-G788E::ura4+ ura4sj-D3</i> h-
SOJ3312	<i>cdc2-1w::ura4+ ura4sj-D3</i>
SOJ1345	CRIB-3xGFP:: <i>ura4+ ura4sj-D3</i> h+
SOJ2443	<i>wee1-as8::ura4+ CRIB-3xGFP::ura4+ ura4sj-D3</i> h-
SOJ638	Lifeact-GFP:: <i>ura4+ nhp6-mCherry::ura4 ura4sj-D3</i>
SOJ2459	<i>wee1-as8::ura4+ Lifeact-GFP::ura4+ nhp6-mCherry::ura4 ura4sj-D3</i> h-
SOJ2465	<i>tea4-GFP::ura4+ nhp6-mCherry::ura4+ ura4sj-D3</i>
SOJ2464	<i>wee1-as8::ura4+ tea4-GFP::ura4+ ura4sj-D3</i>
SOJ2504	<i>gef1-mNeonGreen::kanR nhp6-mCherry::ura4+ ura4sj-D3</i>
SOJ2503	<i>wee1-as8::ura4+ gef1-mNeonGreen::kanR ura4sj-D3</i>
SOJ2507	<i>scd1-mNeonGreen::kanR nhp6-mCherry::ura4+ ura4sj-D3</i>
SOJ2506	<i>wee1-as8::ura4+ scd1-mNeonGreen::kanR ura4sj-D3</i>
SOJ2509	<i>rga4-mNeonGreen::kanR nhp6-mCherry::ura4+ ura4sj-D3</i> h+
SOJ2510	<i>wee1-as8::ura4+ rga4-mNeonGreen::kanR ura4sj-D3</i> h+
SOJ2602	<i>rga6-GFP::ura4+ ura4sj-D3</i> h+
SOJ2622	<i>wee1-as8::ura4+ rga6-GFP::ura4+ rlc1-mCherry::ura4+::kanR ura4sj-D3</i> h+
SOJ2825	<i>scd2-mNeonGreen::ura4+ ura4sj-D3</i> h-
SOJ2834	<i>wee1-as8::kanR scd2-mNeonGreen::ura4+ ura4sj-D3</i> h-
SOJ3615	<i>gef1Δ::ura4+ ura4sj-D3</i>
SOJ3316	<i>wee1-as8::kanR gef1Δ::ura4+ ura4sj-D3</i>
SOJ3269	<i>rga4Δ::kanR</i> (prototroph)
SOJ2477	<i>wee1-as8::ura4+ rga4Δ::kanR ura4sj-D3</i>
SOJ3608	<i>rga6Δ::natR</i> (prototroph)
SOJ3317	<i>wee1-as8::ura4+ rga6Δ::natR ura4sj-D3</i>
SOJ3628	<i>rga4Δ::kanR gef1Δ::ura4+ ura4sj-D3</i>
SOJ3336	<i>wee1-as8::ura4+ rga4Δ::kanR gef1Δ::ura4+ ura4sj-D3</i>
SOJ1360	<i>rga4Δ::ura4+ pom1-GFP::ura4+::kanR ura4sj-D3</i>
SOJ2532	<i>wee1-as8::ura4+ rga4Δ::ura4+ pom1-GFP::ura4+::kanR ura4sj-D3</i>
SOJ3324	<i>wee1-as8::ura4+ rga4Δ::kanR rlc1-GFP::kanR nhp6-mCherry::ura4+ ura4sj-D3</i>
SOJ3329	<i>wee1-as8::kanR scd1Δ::ura4+ ura4sj-D3</i>
SOJ3338	<i>rga4Δ::kanR rga6Δ::natR</i> (prototroph)
SOJ3610	<i>nhp6-mCherry::ura4+ ura4sj-D3</i>
SOJ3607	<i>rga4Δ::kanR nhp6-mCherry::ura4+ ura4sj-D3</i>
SOJ3420	<i>wee1-G788E::kanR rga4Δ::ura4+ ura4sj-D3</i>
SOJ3686	<i>wee1-G788E::kanR rga4Δ::kanR nhp6-mCherry::ura4+ ura4sj-D3</i>
<i>Schizosaccharomyces pombe</i>	
Collection No.	Genotype
SO7812	wild type strain 972 h-
From Kaz Shiozaki	CRIB-3xGFP:: <i>ura4+ ura4-294 leu1-32</i> h-
From Fulvia Verde	<i>rga4-GFP::kanR ade6-704 leu1-32 ura4D-18</i> h+
From Mohan Balasubramanian	<i>wee1-50 leu1-32</i> h-
SO8327	<i>wee1-50 ade6-210/216 ura4-D18? leu1-32?</i> (diploid)
SO7404	<i>tea1Δ::ura4+ ade6-21x leu? ura?</i> h+

SO7405	<i>tea1Δ::ura4+ ade6-21x leu? ura? h-</i>
From Iain Hagan	<i>wee1-as8 (V644G, M700F)::kanR h-</i>
SO8235	<i>wee1-as8::kanR ade6-210/216 leu1-32? (diploid)</i>
SO8286	<i>wee1-as8::kanR tea1Δ::ura4+ ade6-210 ura4-D18 leu1-32 h-</i>
SO8298	<i>wee1-as8::kanR tea1 Δ::ura4+ ade6-210/216 ura4-D18? leu1-32? (diploid)</i>
SO8243	<i>ade6-210/216 ura4-D18 leu1-32 (diploid)</i>
SO8308	<i>wee1-50 tea1 Δ::ura4+ ade6-216 ura4-D18 leu1-32 h-</i>
SO8309	<i>wee1-50 tea1 Δ::ura4+ ade6-210/216 ura4-D18 leu1-32? (diploid)</i>

Supplementary table 1.

List of *Schizosaccharomyces japonicus* and *Schizosaccharomyces pombe* strains

ATACTCGAGGGCATGGTTTGGCTGAG	XhoI_promoter_pak1_sj (Schizosaccharomyces japonicus) fwd
TATCCCGGGCTTGAAGACTAAAAGCCTG	Smal_promoter_pak1_sj rev
ATACCCGGGATGACTAGTGCAAGTATTAC	Smal_gic2_sc (Saccharomyces cerevisiae) fwd
TACCCGGGcCTTATTTTCGTGCGATCTTG	Smal_gic2_sc rev
TATCCCGGGCAGCTTGTTAATTAAC	Smal_gef1_sj C-terminus tagging fwd
ATAGGATCCGGCCTCCTCAGCAGAT	BamHI_gef1_sj C-terminus tagging rev
TATGGGCCCGAGGCCTAACTTTATGATG	ApaI_gef1_sj 3'UTR fwd
ATACCCGGGACGACACGGTTTCA	Smal_gef1_sj 3'UTR rev
TATCCCGGGAATTATTCAAAGAACCT	Smal_rga4_sj C-terminus tagging fwd
ATAGGATCCGAAAAGCTCCTCAACGTG	BamHI_rga4_sj C-terminus tagging rev
TATGGGCCCAATCATAAGAAACATATG	ApaI_rga4_sj 3'UTR fwd
ATACCCGGGAGGAGTGACAGAGT	Smal_rga4_sj 3'UTR rev
TATCCCGGGAATGAGCAAGACTG	Smal_scd1_sj C-terminus tagging fwd
ATAGGATCCGGGGCTAAGCTTGTGAA	BamHI_scd1_sj C-terminus tagging rev
TATGGGCCATAATGCTTGTCTAC	ApaI_scd1_sj 3'UTR fwd
ATACCCGGGAGTTGAATCAGAATC	Smal_scd1_sj 3'UTR rev
TATCTCGAGAAAGTTCGGCTTG	XhoI_rga6_sj C-terminus tagging fwd
ATACCCGGGAGTTTTTTCTTCTTAAAAAGTC	Smal_rga6_sj C-terminus tagging rev
TATGGGCCCTACCGACTTTTTGAC	ApaI_rga6_sj 3'UTR fwd
ATACTCGAGGTTCAAGGTAATTCC	XhoI_rga6_sj 3'UTR rev
TATATCGATTGCGGTATATCCCTT	ClaI_scd2_sj C-terminus tagging fwd
ATAGGATCCTTCCGAAATGAAGCCGTC	BamHI_scd2_sj C-terminus tagging fwd
TATGGGCCGGTTTGCTTGTTCATG	ApaI_scd2_sj 3'UTR fwd
ATAATCGATAGGGAATAACGTTGTCG	ClaI_scd2_sj 3'UTR rev
ATACTCGAGTTATCACGTACGTTTTTC	XhoI_tea4_sj C-terminus tagging fwd
AAAAAGATCTTAAAGATTTACAGCAGTT	BglII_tea4_sj C-terminus tagging fwd
TATGGTACCTAAAAACAATCGGTTCT	KpnI_tea4_sj 3'UTR fwd
ATACTCGAGATGTGCATTTGTCCAAA	XhoI_tea4_sj 3'UTR rev
TATGGGCCGTGAAAAACTAAAAG	ApaI_wee1-G788E_sj mutagenic fragment 1 fwd
CTTCGTAATTTGTTGCTCGGCCCTGCGTC	wee1-G788E_sj mutagenic fragment 1 rev
aaGCAACAATTTACGAAGGA	wee1-G788E_sj mutagenic fragment 2 fwd
AAACCCGGGACATTAGACATATCAAGG	Smal_wee1_sj_3'UTR rev
TATGGGCCACTCCCATTATGCCTCT	ApaI_wee1-as8_sj fragment 1 fwd
ACCTTGCATGTACAAGTAG	wee1-as8_sj fragment 1 rev
TGGCTACTTGTACATGCAAggtGAGCTTTGCGAAAAT GGAAG	wee1_as8_sj mutagenic fragment fwd
GGTGCCCTCAAACGTAATgaaAATGTTTGCGGGCTT CAAATC	wee1_as8_sj mutagenic fragment fwd
TTCATTACGTTTGAGGGCA	wee1-as8_sj fragment 2 fwd
ATACCCGGGTCAGAAGTAGGAAGCCAG	Smal_wee1-as8_sj fragment 2 rev
TATGGTACCTGGCTTCTACTTCTGA	KpnI_wee1_as8_sj 3'UTR fwd
ATAGGGCCCGTTTCTCGTAACAGCTAT	ApaI_wee1_as8_sj '3'UTR rev
TATCCCGGTATTGAGAGTCGGT	Smal_cdc2_sj 5'UTR to amplify ORF fwd
ATACCGCGCAAGTGTGACAGGAGTG	SacII_cdc2_sj 3'UTR to amplify ORF rev
TATGGGCCACCCCTTTTCTTCTGTTCC	ApaI_cdc2_sj 3'UTR fwd

ATACCCGGGAACATTTTCTGCACCACCA	Smal_cdc2_sj 3'UTR rev
GCGAGTTTCAGATTGtCTTCTTTGTGCATCAGCAA	cdc2_1w_sj mutagenic fragment 1 rev
AAGaCAATCTGAAACTCGCCGA	cdc2_1w_sj mutagenic fragment 2 fwd
TATCCCGGGATGCAGCTGCTTC	Smal_scd1_sj 5'UTR to knockout ORF fwd
ATAGGATCCGTCATCAACGTTGCGAGA	BamHI_scd1_sj 5'UTR to knockout ORF rev
TATGGGCCATAATGCTTGTCTAC	ApaI_scd1_sj 3'UTR to knockout ORF fwd
ATACCCGGGAGTTGAATCAGAATC	Smal_scd1_sj 3'UTR to knockout ORF rev
TATCCCGGGTCTTTACAATTCTCTG	Smal_gef1_sj 5'UTR to knockout ORF fwd
ATAGGATCCGTGTCGTAACGTTTGAA	BamHI_gef1_sj 5'UTR to knockout ORF rev
TATGGGCCGAGGCCTAACTTTATGATG	ApaI_gef1_sj 3'UTR to knockout ORF fwd
ATACCCGGGACGACACGGTTTCA	Smal_gef1_sj 3'UTR to knockout ORF rev
ATACCCGGGCCGTCAGATCATTG	Smal_rga4_sj 5'UTR to knockout ORF fwd
ATAGGGCCCGCTTATGAAATGAACGGTG	ApaI_rga4_sj 5'UTR to knockout ORF rev
TATTCTAGATTGACCACGTTGAGGAGC	XbaI_rga4_sj 3'UTR to knockout ORF fwd
ATACCCGGGAGGAGTGACAGAGTTC	Smal_rga4_sj 3'UTR to knockout ORF rev
TGTTCTAAATCCGTCATATTGGCTATAAAAACTACGT TACGTATACGAAATCTTAATGGACGATACTTCTCTTG CCTCGTgcccacttctaaataagc	rga4_sj kanMX6 cassette knockout fwd
TCGAACGTCATCATACCTATCTAGAGTCATAGTAAG GAAACATAACATCATATGTTTCTTATGATTGGGGTTA AGATTAaattcgagctcgittaaac	rga4_sj kanMX6 cassette knockout rev
CGGTACAGGTTGTGACGTCTTCCAGCACTCACAGGT TTACTTATTTTTCCATCTTATCCTGTCATTTTTTGT CTTCTTGgttagctgcctcgtc	rga6_sj natMX6 cassette knockout fwd
AATTATACACTTTCCTTTGCAACACAATATACAGAAA AAGGATTGTCAAAAAGTCGGTAGGGAGGTAATTTAC CACATCATtgatggcggcgttagtat	rga6_sj natMX6 cassette knockout rev
GTTCTCAGCAACTCCTT	rga4_sj 5'UTR genotyping fwd
CACAAGTTATGCATCTGA	rga4_sj ORF genotyping rev
CTACTCATCCGAGATC	rga4_sj ORF genotyping fwd
TGGAGCACCTCTAGATAT	rga4_sj 3'UTR genotyping rev
GCTTTTATAACAACACTGGAAT	rga6_sj 5'UTR genotyping fwd
TCGTTACCTGAGGACGAA	rga6_sj ORF genotyping rev
TCGCCCTTATCGCCTTC	rga6_sj ORF genotyping fwd
AGACGGATGCTTGAATCA	rga6_sj 3'UTR genotyping rev
TTCACTGCGTCAGCTTGT	gef1_sj 5'UTR genotyping fwd
TCATCGTGAACGCTCGT	gef1_sj ORF genotyping rev
CAGATGCAGATGTAACGTG	gef1_sj ORF genotyping fwd
GAGTCTCACCTAAACGAAG	gef1_sj 3'UTR genotyping rev
TACTAATCAGCAGCGCTG	scd1_sj 5'UTR genotyping fwd
CACGCGGATGTTTTACAAC	scd1_sj ORF genotyping rev
GCCTTTGAGTATTGCGTGT	scd1_sj ORF genotyping fwd
CTCAGATGAAGTTGTTTCTC	scd1_sj 3'UTR genotyping rev

Supplementary table 2.

List of primers