

# Supplemental Materials

*Molecular Biology of the Cell*

Ren et al.

### Supplementary figure legends.

Figure S1. Localization kinetics of Spa2. A) Images from a movie of a *spa2-GFP cdc15-mCherry sid4-RFP* cell captured at 2.5-minute intervals. Scale bar, 5  $\mu\text{m}$ . B) Images at 5-minute intervals from independent representative movies (right and left panels) of *spa2-GFP cdc15-mCherry sid4-RFP* that were begun at different cell cycle stages. Scale bars, 5  $\mu\text{m}$ .

Figure S2. Localization kinetics of Rgf3 to the CR. A) Live cell images of the indicated strain. Scale bar, 5  $\mu\text{m}$ . B) Montages of images a movie of the indicated strain at 4-min intervals. Scale bars, 5  $\mu\text{m}$ .

Figure S3. Sequence comparison of Rgf3 homologs. Rgf3 sequences from the 4 indicated *Schizosaccharomyces* species were aligned with amino acid numbers corresponding to the last residue of each line provided to the right. Residues identical between all 4 proteins are in red, conserved residues are in blue, and the three Cdc15 and Imp2 SH3-binding sites are highlighted in cyan.

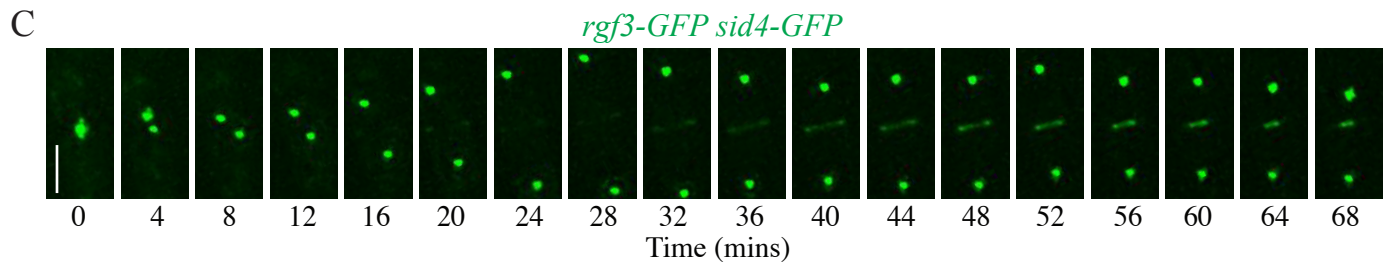
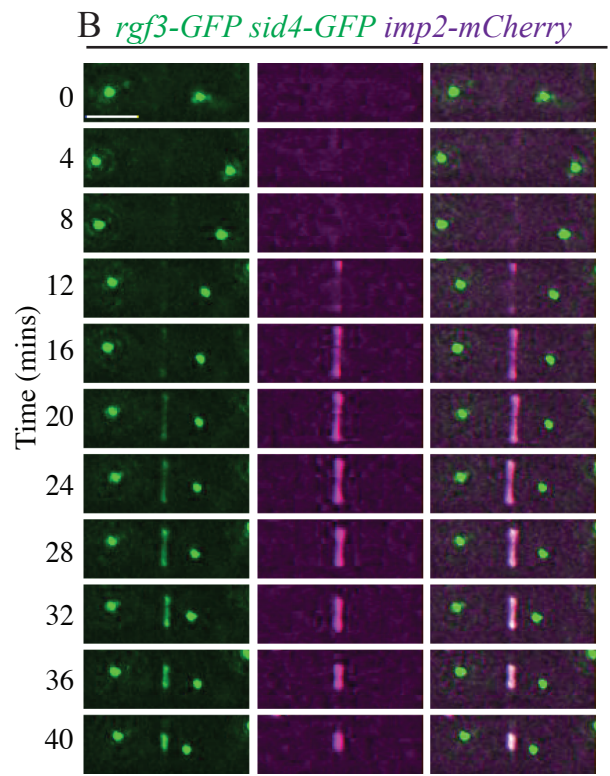
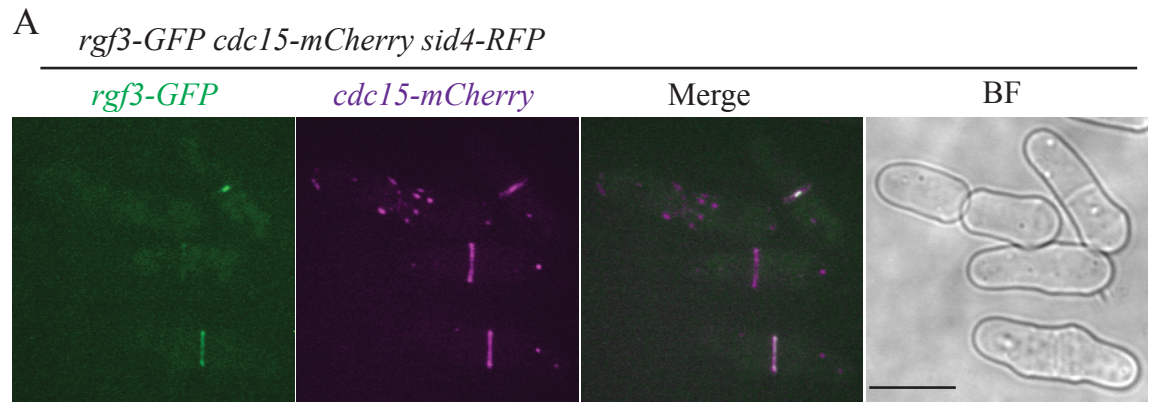
Figure S4. Characterization of Rgf3 and Spa2 association with Cdc15. A) All three predicted Cdc15<sub>SH3</sub> binding sites in Rgf3 at amino acids 154-160, 169-175 and 264-270 were mutated at their first proline position to alanines to make the 3PA mutant in a fragment of *rgf3* encoding residues 1-290 and tested for localization as GFP fusion proteins. Scale bar, 3  $\mu\text{m}$ . B) *spa2* $\Delta$  cells were grown at 32°C in YE medium, fixed and stained with methyl blue, and imaged. Scale bar, 3  $\mu\text{m}$ . C) The growth patterns of wildtype (wt) and *spa2* $\Delta$  cells at 32°C was assessed by staining the cells with calcofluor and determining the percentage of cells that were growing at one end (monopolar), both ends (bipolar), or undergoing septation. D) The indicated strain was grown at 25°C and stained live. Scale bar, 3  $\mu\text{m}$ .

Figure S5. Sequence comparison of Spa2 homologs. Spa2 sequences from the 4 indicated *Schizosaccharomyces* species were aligned with amino acid numbers corresponding to the last residue of each line provided to the right. Residues identical in all 4 proteins are in red, conserved residues are in blue, and the Cdc15 and Imp2 SH3-binding site is highlighted in cyan.

Figure S6. Pos1 and Spa2 localization dependencies. A) *pos1-GFP spa2* $\Delta$  cells were stained with 50  $\mu\text{M}$  Mitotracker and imaged live. B) The indicated strain was grown at 25°C and imaged live. Scale bar, 3  $\mu\text{m}$ .

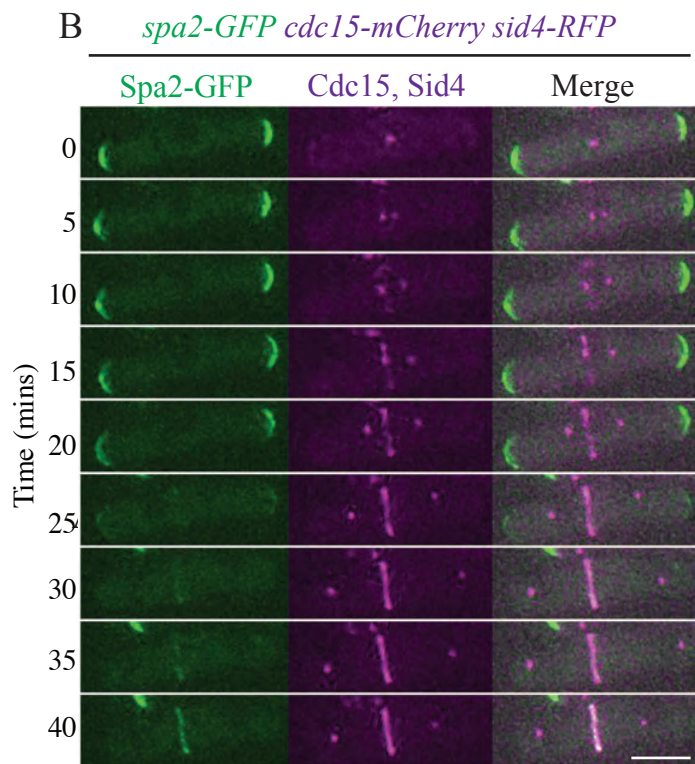
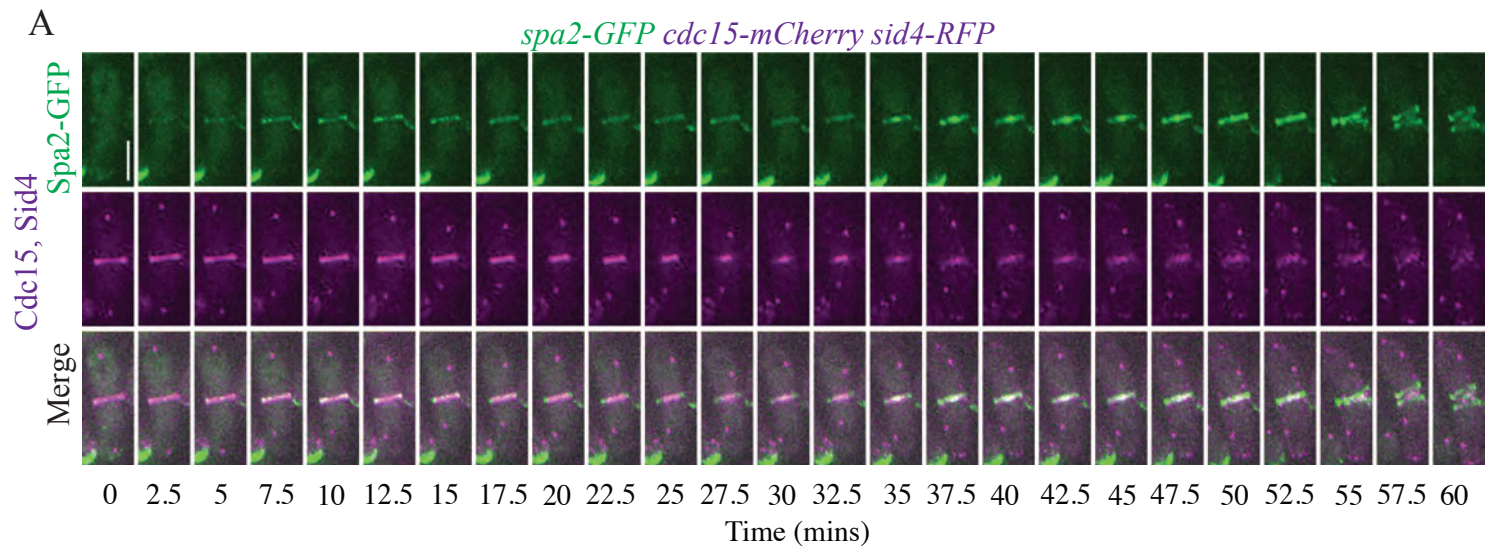
Figure S7. Genetic interactions among SH3 network components. A) Tetrad analysis of the indicated crosses with the relevant genotypes of mating partners indicated. Schematics of 3 tetrads are provided with the relevant genotypes of progeny indicated. Underneath, images of representative germinated spores or colonies of the indicated genotypes are shown. B) Top panel: The indicated strains were grown at 25°C and shifted to 36°C for 6 hours and the percent of cells that remained attached following septation were counted.  $n \geq 300$  cells for each strain. Bottom panel: Representative

fixed and DAPI-stained images of the indicated strains are also shown from the 6 hour shift.



**Figure S1**

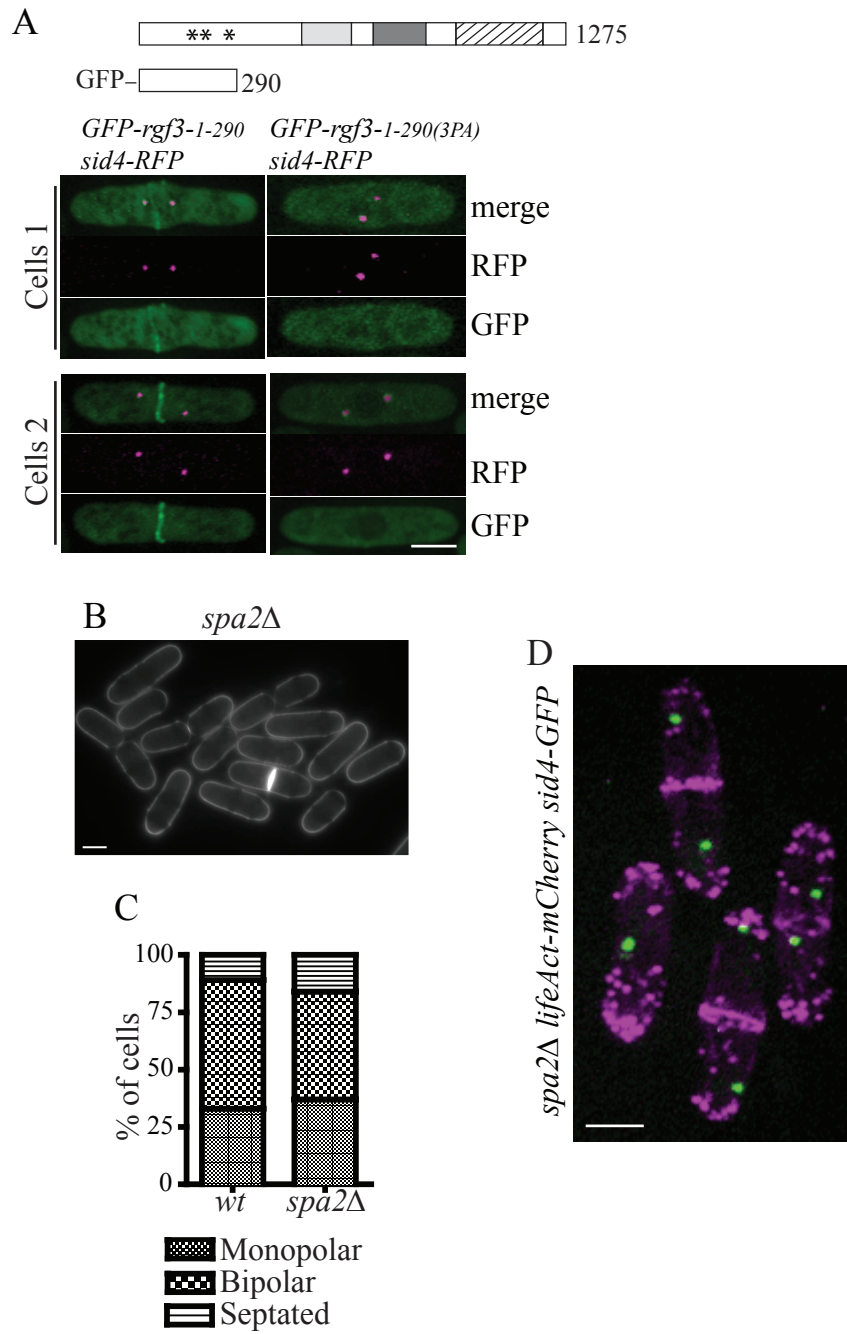




**Figure S2**

<i>S. pombe</i>	MKLSNELFHR	SSKDHGGKSR	ICLDSSSEDY	PPHSSSP---	----PSFQKR	LSFSDFSSTR	LFSPPFLSKR	SNNSPH-RFS	72
<i>S. cryophilus</i>	MKLSNDIFRK	NSRDERRRKR	TSIDYPDDL	ASPVSTP---	-NISVPSPKR	LSISDFSSFR	LFSSPFNSPK	RYDSTHSRQL	76
<i>S. octosporus</i>	MKLSNDIFRK	PSRHEHRNKR	TSIDYPDDL	ASPVSTPSS	PNTSPVSPKR	LSISDFSSFR	LFSSPFNSPK	RHESHTSRRL	80
<i>S. pombe</i>	YSPPOHPASI	NSRRVASVTV	QSSPSRTTYR	QLNPENQNSA	AYTTYSSFPN	ALFDDFSPNN	PLDTPDFLTS	PGNKQNTVDS	152
<i>S. cryophilus</i>	PMTPOSSRSL	PSRRTASVTV	RSSPALPTTS	S--VYSYDAD	PNGTVSSFPN	ALHSNFSFSD	PLDTPDPYLM	PGGQYT--DV	152
<i>S. octosporus</i>	PTTPPERPRL	PSRRTTSVTV	RSSPALPTMS	S--NYLYDAD	PNGTVSSFPN	ALHTNFSPSD	PLDIDPYLMS	PG---T--DA	153
<i>S. pombe</i>	FRPLPETPVS	PGGSLVHPLP	RPPLPSSVSS	HSSPYSTTSS	TSLYSLYNDI	SLSCSPEPYL	PLSPTRSPAR	TPSPIRLYSS	232
<i>S. cryophilus</i>	HRPLPETP-S	PTSPTS HPLP	RPPLLNTHSN	NSSPYSSSSS	NSFYTLYNEL	PLSFSPEPYL	PLSPTRSPSR	STSPFR TAMN	231
<i>S. octosporus</i>	HRPLPETP-S	PTSFTV HPLP	RPPLLSAHSN	NSSPQSTSSS	NSFYSLYNEQ	PLSFSPEPYL	PLSPSRSPSR	STSPFR TAIN	232
<i>S. pombe</i>	DALRPQSP--	-----LSPS	VEYLTP-PNP	YSLKS---DI	SSTRQLPKIP	VQDYASGKIS	SPLITRTHRR	AQSETLFS	300
<i>S. cryophilus</i>	EADNYSLSHI	DSFGCWSDFP	FTDLTSSPSP	ANFPSPSQEL	HISRELKVP	VQDYTSGEIS	SPLVSRSSRA	TQANALFS	311
<i>S. octosporus</i>	DANVNSVSQR	DSYGYWSDPS	FTDLTSSPSP	VSFSSPNKEL	HIPRELKVP	ARQYASGDIS	S LMSRSSRP	TQANALFS	312
<i>S. japonicus</i>								MKC	3
<i>S. pombe</i>	REPWLVGKLY	KWCKEEVFTA	LGGLVHEGVS	RREVAQVMAT	LFTIHIASME	FLIAEIIAKN	ILGDWYINYL	VEVINLEKQ	380
<i>S. cryophilus</i>	REPWSLKRLF	SWCKEEVFPA	LSATTTD GAP	RQEVAQIMAI	LFTMSVASMD	FLIAEVLAKN	IISDWIQNGY	MKVTNLEKLY	391
<i>S. octosporus</i>	REPWSLKRLF	MWCCKEEVFPA	LSATTTD GAP	RQDLAQIMAM	LFTIIVSPMD	FLIAEVLAKN	IISDWIQNGF	MKVINLEKLY	392
<i>S. japonicus</i>	RDPWSLNSLY	KWCRDVLAPL	MVKQEEGGYY	HYEVAQAMAT	LFTLRIPKLD	FLLAEMLAKN	ILIDWIREGV	VNVLNFEKLL	83
<i>S. pombe</i>	IAFTSNPEPS	GSGLVPLFTN	GGCYSYICRS	RSCPSKYQCY	SCRCARNSSL	EFTSLPGQ-S	SDTWSIFWNI	SSLNSLPS	459
<i>S. cryophilus</i>	ITFVDDQFPS	SSGVLPIILN	GGCYSYTCRS	KASSSNYTCY	SCRCVRIPAH	RLTSTQGDFC	DDSWNMFNWL	SPQDSLPS	471
<i>S. octosporus</i>	ITFVDDQFPS	NGGVLPIILN	GGCYSYTCRS	KSTSSKYTCY	TCRCVRKSTN	GLTRSQGDTS	DDSWNMFNWL	SPQDSLPS	472
<i>S. japonicus</i>	IEFNHKEDLE	HAGVLPALTT	GGCYSYTCCS	QDRSDKCACY	SSRCLHSPLO	KCFENAD--L	SNSWNNYWNL	PLSDELPL	161
<i>S. pombe</i>	SKREIARQNN	IHELICKESD	YVADLNTLAE	LFRDGI VQOQ	DAIVPSNRVA	DFIQSVFGNV	ESIRQLHSRL	FLPQLIMRER	539
<i>S. cryophilus</i>	TKKEVARQNN	IHELICKESD	YVADLHTLAE	LFRDGI VQEE	NSIVPSNRVA	DFIQSVFGNV	ESIRQLHSQF	ILPQLIIRER	550
<i>S. octosporus</i>	TKKEVARQNN	IHELICKESD	YVADLHTLAE	LFRDGI VQEE	NSIVPSNRVA	DFIQSVFGNV	ESIRQLHSQF	ILPQLIIRER	551
<i>S. japonicus</i>	TKKEITRQNN	IHELICKESE	YVADLDSLAD	LFRDGLLKSE	TDIIPLGRLA	DFIQSVFGNV	ETIRTIHSHR	FLPQLLVREK	241
<i>S. pombe</i>	LQGPVVSIIIG	DILLEWIHAA	KSSYINYAKQ	FPLADETYKL	ECQRNTYFAR	WLAACRSDPR	CRRLDFQHFL	QRPTQRLQRY	619
<i>S. cryophilus</i>	LQGPVVSIIIG	DILLEWIQMA	KVSYINYAKQ	FPLADETYKL	ECQRNTYFAR	WLAACRSDPR	CRRLDFQHFL	QRPTQRLQRY	630
<i>S. octosporus</i>	LQGPVVSIIIG	DILLEWVHLA	KTSYINYAKQ	FPLADETYKL	ECQRNTYFAR	WLAACRSDPR	CRRLDFQHFL	QRPTQRLQRY	631
<i>S. japonicus</i>	LQGPVVSIIIG	DVILEWIRVA	RDGYINYARQ	FPLADETYKF	ECQRNTCFAK	WLAGCRMDPR	CRRLDFQHFL	QRPTQRLQRY	321
<i>S. pombe</i>	TLELDTILKH	TEQSSWDFQL	ITQAVKELRA	TCEECDAVIA	TVLEANRIRD	LSYQLLFKNH	ESVNLELRDP	EREFFFEQIV	699
<i>S. cryophilus</i>	TLEWDTILKH	TDPTSWDYQL	ISQAEKELRA	TCEECDAVIA	TVLEANRIRD	LSYQLLFKNH	ESINLELRDP	EREFFFEQIV	710
<i>S. octosporus</i>	TLEWDTILKH	TDPTSWDYQL	ISQAEKELRA	TCEECDAVIA	TVLESNRIRD	LSYQLLFKNH	EGINLELRDP	EREFFFEQIV	711
<i>S. japonicus</i>	TLELNTILKH	TDPESLDYKF	LTQALKDIRA	TCEECDAVIA	TVLETNRIRD	LSYQLLFKNH	EHVNLDLRSP	LREFFFEQEV	401
<i>S. pombe</i>	QRRSDSRLDW	LDIHLFLLDN	YLIMAKARKD	KRTNASRYVV	SKRPIPLDLL	VLSPKMDDFQ	LKSNTNKFLG	SLAGNLPQE-	778
<i>S. cryophilus</i>	FRKSESRLDW	IEIHIFLLDN	YFIMAKPRRD	KRTNAMKFLV	SKRPIPMDDL	VLNPLTEEWN	SRNSNKFLG	SLTGNFPYDG	790
<i>S. octosporus</i>	FRKSESRLDW	IEIHIFLLDN	YFIMAKARRD	KRTNSMKYLV	SKRPIPMDDL	VLNPLIEEWN	SRSS--KFLG	TLASNFPYEG	789
<i>S. japonicus</i>	LKRSNSRLEW	LNHLFLLDN	YLVMTKVKRE	RKSSATKYLV	SKRPIPLDLL	VVERIDDLSL	HRSSPSRMFG	NFSLTTSEGT	481
<i>S. pombe</i>	SLTTKSKRKS	KV--NLELMP	DATAEKNNEN	SMNSAVFEKS	QLYPFTIRHL	GAYTASYTLY	VESLQRLKLV	VEKINVAKRR	856
<i>S. cryophilus</i>	STNVRSKRKS	RL--NLDILY	DYNSDKMNEG	SLNTLALAKQ	HFYPTVVRHL	GGLSSTYTYL	TESLQRLKLV	MDKINFARKR	868
<i>S. octosporus</i>	SLNARSKRKS	RL--NLDIRN	DYNSDRMNE	SWNNTLPEKP	HFYPTVVRHL	GGLSSTYTYL	TESSQRLKLV	MDKINFARKR	867
<i>S. japonicus</i>	FRTRRSSMRL	NKFQSQSTLS	EKHKRSNSDN	TLSPGRSEKQ	QLFAFAVRHL	GRTPMTYTFY	TETAQARKIW	IEKLNFAQKR	561
<i>S. pombe</i>	HSQKINIKNP	FALKVVDVA	FQYPPSDLVN	GNEPLNSFNE	ITLVEGSSID	RALNEVAWKH	PIVSEELPE	PIAYGDISCI	936
<i>S. cryophilus</i>	HAQKMHKSP	FALQIVSDVS	FQYPPADLSH	TFESSRSPE	ISLVEGSAVD	RALTMAAWRY	PIHNEDDLPE	AINYGDITCI	948
<i>S. octosporus</i>	HAQKMRKNP	FTLQIVSDVS	FQYPPADISG	TLDSSRSPE	ISLVEGSAVD	RALTMAAWRY	PIHNEDDLPE	AINYGDITCI	947
<i>S. japonicus</i>	HAQKQHDKVP	FAAKVLDVVL	FKDTSDBGYF	NSSSQDSSAK	IDLVEGSAVD	RALQEASWKY	PIPTAGDLPN	AIASGPVFSI	641
<i>S. pombe</i>	AQFNDYEGHV	SVLIATSTGI	FLGAFGDSSD	IRDWKKISSQ	RRVTQLGVVE	EPDILLELRD	KTLYAHKLSR	IIEMLIESK	1016
<i>S. cryophilus</i>	AQFQGGADGKL	SICIATTTGV	FLGFFGGNSN	IREWRKISSH	RRVMQIGLLE	EPDCLLELRD	RSMYAHKLSR	VIEGTLEPK	1028
<i>S. octosporus</i>	SQFQGGTDGKL	SICISTTTGV	FLGFFGGNSN	IREWRKISSH	RRVTQIGFLE	EPDCLLELRD	RSMYAYRLSR	VIESGTLEPK	1027
<i>S. japonicus</i>	AQFCLEGNTV	SFFAATCNGV	FYGEHGKDKD	IRSWRKVFDH	RRVTQLGVLE	EPDCLLELRD	KTLYAHKLSQ	IIELTNPEPF	721
<i>S. pombe</i>	I----AVVIG	TPHAVSFFKI	GKLSEGASVK	RERTLVFYKE	GLGNTTIIIC	CEPVIGLGHN	YQKTYAFKRR	DVTSFR TLDD	1092
<i>S. cryophilus</i>	V----AIPVG	TSHNISFFKI	SKLTEGASIK	RERTLIFFKQ	GSGNASSIHC	CEPVVGLGHH	NQKAYNFKQK	NVSSFRILEE	1104
<i>S. octosporus</i>	V----AIPVG	NSHNISFFKI	SKLTEGASLK	RERTLIFFKQ	GSGNSSSIHC	CEPVVGLGHH	NQKAYNFKLK	NVSSFRILEE	1103
<i>S. japonicus</i>	VERGTAQVLG	TPHAVSQFKI	AKVEEGSLLK	RSRTYILYKE	MTGTFTFIRC	CEPVVGFHGH	YQKAYGFROK	DVISFRVVDQ	801
<i>S. pombe</i>	FHVTANCHSI	DCFKYSIALC	HNKGIDVLR	DPKLAVGFPS	PSVLNDTLFR	NRINNSKPLG	VFRVHDP SLF	ACCYQFGAVF	1172
<i>S. cryophilus</i>	FRVLADCYSM	NCFKYSIAIS	RQKGDIVLR	DPKLAVGFPS	PSVLTDPFYR	SRINNSKPLG	VFRVHDP SLF	ACCYHNGALF	1184
<i>S. octosporus</i>	FRVLADCYSI	NCFKYSIAIS	RQKGDIVLR	DPKLAVGFPS	PSVLADPLYR	SRLNSSKPLG	VFRVHDP SLF	ACCYQTGALF	1183
<i>S. japonicus</i>	FQVTNDTSFA	NVFKYSFAVT	NSKGIEVIRL	DPKLARNNIPS	QVSLYD SPAR	NFAMSSKPLS	LLRTSKRGIF	ACCYETGAVF	881
<i>S. pombe</i>	VNNEGSMVKN	ECWFDWIGKP	NSVTSCHGYL	IAFNDEFVEI	WNTRTRKLNQ	IIQGNDIKYY	PSNSDWLANG	KYIMFGMVHP	1252
<i>S. cryophilus</i>	VNNEGSLIKK	DAWLQWIGRP	NSVACYEGYL	LAFSDDFIEI	WNSRSMKQVQ	VIQGNNIRLH	ASNSDLLSDG	KHVMFSMTHP	1264
<i>S. octosporus</i>	VNNEGSLIKK	DAWLQWIGRP	NSIACHEGYL	MAFSDDFIEI	WNSRSMKQVQ	IIQGNNIRLH	SSNSDLLSDG	KHVMFSMTHP	1263
<i>S. japonicus</i>	VTSEGLTTRT	TEFINWLGTP	EHVCMVGDYL	LSPDKNFVEI	WSTETLQLEQ	IIQGNISIQYH	PSGSEWHPSG	IMPVFSMTHP	961
<i>S. pombe</i>	QYHDRHLILA	LNKAKTNSFI	IED	1275					
<i>S. cryophilus</i>	QFHDRHLILA	LDLVKEENLM	DV	1286					
<i>S. octosporus</i>	QFHDRHLILA	LDLVKEENLM	D	1284					
<i>S. japonicus</i>	VYPDRRLIMA	LELTKLGNEL	P	982					

Figure S3



**Figure S4**

*S. pombe* MQSIIIRQYR FLARYLEPEF VKDPALRNAN ARPREDLQRL SHIQFSELLT DVADELQRI NNDPKVRFPL PVDSYHPKRN 80  
*S. octosporus* MQNIIIRQYR FLAKYLEPEF FKEPALRNAN ARPREDLQRL SHVQFSELLT DVADELQRI NNDPKVPFPL PVESYHPKRN 80  
*S. cryophilus* MQNIIIRQYR FLAKYLEPEF FKEPTLRNAN ARPREDLQRL SHVQFSELLT DVADELQRI NNDPKVPFPL PVESYHPKRN 80  
*S. japonicas* MRSIIIRQFR FLSRYLEPEF ARDPNL--VN SRSREKLQRL SHSQFNEELLT DVSEDLHRII NNDPNIPLPL SVDTFHPKRN 78

*S. pombe* HARQKLSSLA PTRLRDLCMD VFFEVSRYG GALKEVSSNT P-PNMTRAAS QPPQVQRLP ASAPKHDLYS SANASSASLH 159  
*S. octosporus* HARQKLASLA PSRLRDLCMD VYFEVQARYG NALKEVDTP TSLAQPSRTNS HPQVASQRNP SATHGVKAEG S---SSSSLH 157  
*S. cryophilus* HARQKLASLA PSRLRDLCMD VYFEVQARYN NALKEVDTP TMAQPNRTIS HPQLSSQRST QAAHGSKIEG P---SSSSLH 157  
*S. japonicas* HARQKMSTLA PLRLRDLCID VFELQTRYP AVVAE SPSNV SSSSLPNSSG APRTPTNATA SNASTASLGK A---SSPQYM 155

*S. pombe* PTEQRTTSSP TIPSYANRTH TDSPTSLSHR LPMVP----D TNALNTVQAN NSSTSSLSQG AQTGIDSSN YLSRIEMLES 235  
*S. octosporus* PSERRTTSSP NVV-NSKIAS REAPSSSSHA LPMVP----D AAPKSP---P HRVAE----- ----DSTTK YLAKIEMLEN 219  
*S. cryophilus* PLDRRTTSSP NVV-YPKKAS RETPSSSSHA LPMVP----D ATPKSPHRAP VPQTE----- ----DPSK YLAKIEMLEN 222  
*S. japonicas* PATTAINNQ PPRLPTPSSS SPSVSNMHR LPKA PHGRED VSISSYMTS PTASPSQQA IPVRSESPK FLSRIELLES 235

*S. pombe* SLAKSNSLLD SSKSEMEALK AKSISDATKH KNEIFQLEEK LHEASHEAEI SIKKLNDAN RIKEL----- ----ENNP 304  
*S. octosporus* NLAKSNSLLD VSKEIEELK AKLNSEAVHH KSRMFNMEEK LHEASLQITT LNEKLKESQL T----- ----RSTS 284  
*S. cryophilus* SLAKSNSLLD ISKSELEALK TKLNSEAVHH KGQLFNMEEK LHEMSLHVTT LNEKLKEAQN Q----- ----RSSS 287  
*S. japonicas* NLAkansLLD ANKAEMEAMT RQHTADQAKH KNELFQLEEK LHEAVHEKTT LSSQLEAAQK KIEELTKEVE KVNAABEERQ 315

*S. pombe* TLSFNPELEK NLKLMQELIV AESSKQHVIV ELESCLDLSK AETERWKKVA INAK-----S AKIDEDIRLF TMTTVPMSKI 379  
*S. octosporus* PAGVDGEFEK SLKMMQGLIL SETTKVHHIN ELEKSFEAQK DETERWKRVA INAK-----A SKIDDDVRLF TMNTIPMKTIV 359  
*S. cryophilus* PGLDSEVER SLKMMQGLIL SETAKVHHIN ELEKSFEAQK GETERWKRIA INAK-----A SKIDDDVRLF TMNTIPMKTIV 362  
*S. japonicas* TKRGNMELEE HLSQMRMLIQ AESKHLNELD EMQSQLERVK RESFQWKEKY YQVKLQNES A SKVDATACLF QNPPVSPETL 395

*S. pombe* RELISPNGLF DEQLYIRYFV EMNVFVASLR RDSPSQWMT AKDIALTLEA IVSSLREKSL LEELPDLGKK VDDLCSFTNS 459  
*S. octosporus* RELVSPNGIV DEQLYVRYFV EMNVFVSSLR KDSPSQWMT AKDIALTLEA IVSGLREAVL TKELLGLGSS IDDLCTFTNN 439  
*S. cryophilus* RELISPKGIV DEQLYVRYFV EMNVFVSSLR KDNP SQWMT AKDLALTLEA IVSSLREAAAL SDELPLRSS IDDLCTFTNN 442  
*S. japonicas* DKCTSDDGVF NRKDFVRYLF EMNEFIAATR SENSQLLTK MKSLAIVLDT LATQAREKSF ADS-SELMKH MDEVYIHADT 474

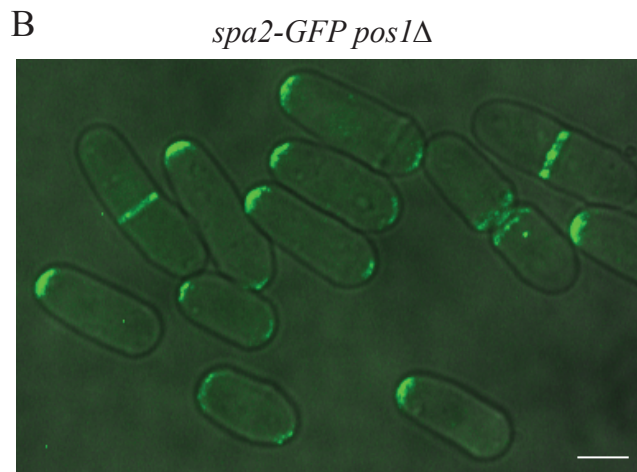
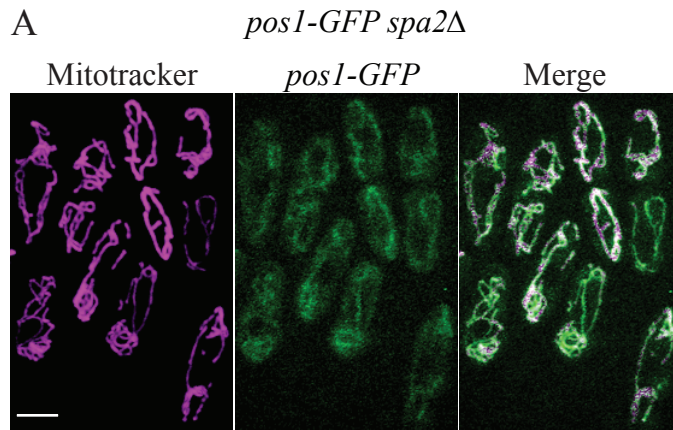
*S. pombe* LMEQVRLAVI NGALLPIYHV DASACISIS LMDIAKTYGL TNTGKSSLSV GRNSLHPVDD LKALSVAKTL LSEKSKQFSE 539  
*S. octosporus* LMQQVRVASS NGAF-PISHI DASACISIT LSEIVKKHGL LDSGKSSASY ARTSLHPVDD LKALSAAKTI LGDKTRRYNE 518  
*S. cryophilus* LMQQVRVTSS NGAL-PIGHI DASACISVA LLEIVKKYGL LDSGKSIASY ARTSLHPVDD LKALSAAKVI LADKTRRYNE 521  
*S. japonicas* LMLQVRNFAI NKGLTPLLHI DATAYTLSSV LINIVKQHQL HPDEMTRSSR G-DGTRAVNQ VETLSHVKEI VSKETDNLVA 553

*S. pombe* TNQKLLDAIS SDSSSSTIQ RVHEDINIVR DTLFDVSSPL ELMRDRAAYA VIHNALQEMR RYCMQLIEQE GHGENFGFTD 619  
*S. octosporus* VNQHLLNAIS DGRATYEVQ LIHVLTSIIR DTLFDVSAPL ELMRDRAAFG IIVHVSLEL R KCCMLLS DYE NQEQ----PS 594  
*S. cryophilus* VNQQVLNAIS DGRATYEVQ LIHISTSIIR DTLFDVSAPL EMMRDRAAFG IIVHVSLEL R KSCMLLC EYE RHEQ----PS 597  
*S. japonicas* GVQALVDSVH EHRSDADVYE GVRNIAAIVR DVLPDITPLV NQVSFAVDAS TVHDTLKALR DNRLRLDAQ DFDK----- 627

*S. pombe* NNSVPLTDL AFSAACFKD ILRAIEDAEF SVQRTQFSTR 659  
*S. octosporus* DPTIPPLTDL AFSTAKCFKN ILSIEDAEF TVQKTQFSNK 634  
*S. cryophilus* DSTIPPLTDL AFTTVKCFKN ILSIEDAEF TVQKTQFSSK 637  
*S. japonicas* EQSNTQLPDI AIATVAAYKQ LLRLIDDAEF QAQFGKPS 666

Figure S5

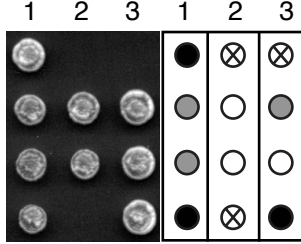




**Figure S6**

A

*imp2ΔSH3 x cdc15ΔSH3(1-869)*

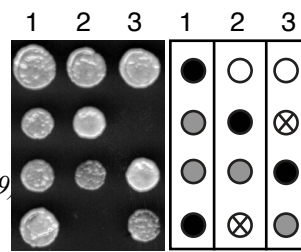


- wildtype
- *cdc15ΔSH3(1-869)*
- *imp2ΔSH3*
- ⊗ *imp2ΔSH3 cdc15ΔSH3(1-869)*

*imp2ΔSH3 cdc15ΔSH3(1-869)*

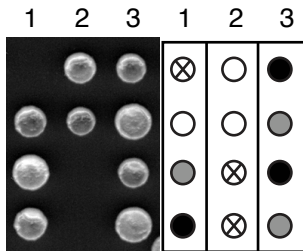


*imp2ΔSH3 x pxl1Δ*



- wildtype
- *pxl1Δ*
- *imp2ΔSH3*
- ⊗ *imp2ΔSH3 pxl1Δ*

*pxl1Δ x cdc15ΔSH3(1-869)*

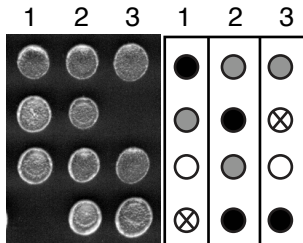


- wildtype
- *pxl1Δ*
- *cdc15ΔSH3(1-869)*
- ⊗ *pxl1Δ cdc15ΔSH3(1-869)*

*pxl1Δ cdc15ΔSH3(1-869)*



*cyk3Δ x cdc15ΔSH3(1-869)*

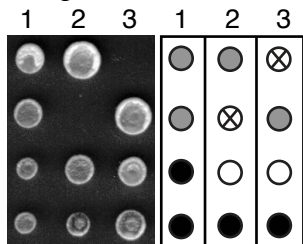


- wildtype
- *cdc15ΔSH3(1-869)*
- *cyk3Δ*
- ⊗ *cyk3Δ cdc15ΔSH3(1-869)*

*cyk3Δ cdc15ΔSH3(1-869)*



*pxl1Δ x art1Δ*

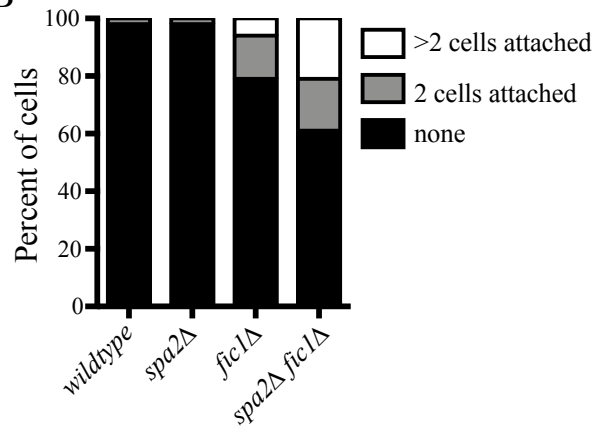


- wildtype
- *art1Δ*
- *pxl1*
- ⊗ *pxl1Δ art1Δ*

*pxl1Δ art1Δ*



B



*spa2Δ fic1Δ, 36°C*

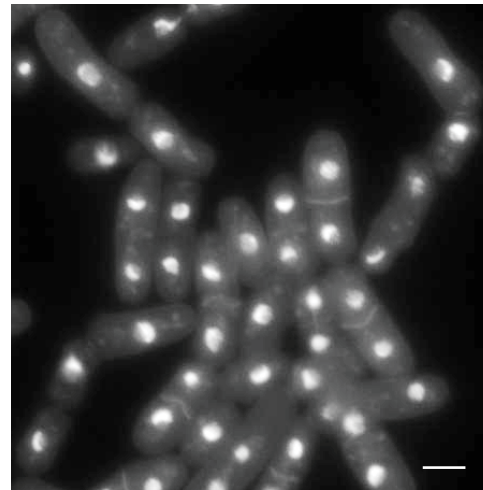


Figure S7

**TABLE S4** *S. pombe* strains used in this study

<b>Figure 2</b>		
KGY4401	<i>rgf3-MYC<sub>13</sub>:kan<sup>R</sup> ura4-D18 ade6-M210 leu1-32 h<sup>-</sup></i>	Lab stock
KGY6894	<i>spa2-FLAG<sub>3</sub>:kan<sup>R</sup> ura4-D18 ade6-M210 leu1-32 h<sup>-</sup></i>	This study
KGY7654	<i>rgf3-TAP:kan<sup>R</sup> nda3-KM311 ade6-M21X leu1-32 ura4-D18 h<sup>+</sup></i>	Lab stock
KGY6919	<i>spa2-TAP:kan<sup>R</sup> nda3-KM311 ade6-M21X leu1-32 ura4-D18 h<sup>?</sup></i>	Lab stock
<b>Figure 3</b>		
KGY11545	<i>spa2-GFP:kan<sup>R</sup> cdc15-mCherry:kan<sup>R</sup> sid4-RFP:kan<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
KGY11704	<i>spa2-GFP:kan<sup>R</sup> cdc15-mCherry:kan<sup>R</sup> nda3-KM311 ade6-M21X ura4-D18 leu1-32 h<sup>?</sup></i>	This study
KGY15778	<i>cdc15-mCherry:kan<sup>R</sup> spa2-GFP:kan<sup>R</sup> ade6-M21X ura4-D18 leu1-32 h<sup>?</sup></i>	This study
KGY14225	<i>spa2-mCherry<sub>3</sub>:kan<sup>R</sup> sid4-RFP:kan<sup>R</sup> Imp2-GFP:kan<sup>R</sup> ade6-M21X ura4-D18 leu1-32 h<sup>?</sup></i>	This study
KGY6670	<i>sid4-GFP:kan<sup>R</sup> fic1-GFP:kan<sup>R</sup> ade6-M21X leu1-32 ura4-D18 h-</i>	Lab stock
KGY7970	<i>cyk3-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> leu1-32 ade6-M21X ura4-D18 h-</i>	Lab stock
KGY16760	<i>pos1-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> leu1-32 ade6-M21X ura4-D18 h<sup>?</sup></i>	This study
KGY16759	<i>pxl1-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> leu1-32 ade6-M21X ura4-D18 h<sup>?</sup></i>	This study
<b>Figure 4</b>		
KGY9004	<i>rgf3-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> cdc15Δ(1-752)-FLAG<sub>3</sub>:kan<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
KGY9007	<i>rgf3-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> imp2ΔSH3-FLAG<sub>3</sub>:hyg<sup>R</sup> ade6-M216 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
KGY9012	<i>spa2-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> cdc15Δ(1-752)-FLAG<sub>3</sub>:kan<sup>R</sup> ade6-M216 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
KGY9014	<i>spa2-GFP:kan<sup>R</sup> sid4-GFP:kan<sup>R</sup> imp2ΔSH3-FLAG<sub>3</sub>:hyg<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
<b>Figure 5</b>		
KGY1296	<i>PJ69-4A MATa trp1-190 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL-lacZ</i>	Lab stock
KGY11936	<i>rgf3-GFP:kan<sup>R</sup> sid4-RFP:kan<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
KGY16185	<i>rgf3-3PA(P160A P172A P267A)-mGFP:kan<sup>R</sup> sid4-RFP:kan<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
KGY16265	<i>rgf3-GFP:kan<sup>R</sup> sid4-RFP:kan<sup>R</sup> nda3-KM311 ade6-M210 ura4-D18 leu1-32 h<sup>?</sup></i>	This study
KGY16266	<i>rgf3-3PA(P160A P172A P267A)-mGFP:kan<sup>R</sup> sid4-RFP:kan<sup>R</sup> nda3-KM311 ade6-M210 ura4-D18 leu1-32 h<sup>?</sup></i>	This study
KGY15643	<i>rgf3-3PA(P160A P172A P267A)-mGFP:kan<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
KGY246	<i>ade6-M210 ura4-D18 leu1-32 h<sup>-</sup></i>	Lab stock
<b>Figure 6</b>		
KGY15974	<i>spa2-GFP:kan<sup>R</sup> ade6-M216 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
KGY15754	<i>spa2-P191A-GFP:kan<sup>R</sup> ade6-M210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study

