C.o. : C.g. : M.o. : N.c. : A.n. : S.p. : S.c. :	*  MNLSTKNTSSGS2	20 -MPPSPTRDPFD MPSP ARGRDPGTNAMP	* TPTHAFVQSRAQT MT IKESAPSLPPPAA LLSTAPGAPVPGP MSSQPPTQAQLNL 	40 TELLPPPSPR- TELLPPPSPG- TTLHPPPSPG- PQPAPPSPAAG AALAGSPSPR-	* 60	* THRALRKLQSAH THR-LRKLQSAH THRALRKLHSAH IGQRSLRRLQSAH TMRSLRKIQSHQ	80 NLGSKAALQ NLGSKAALQ INLGAKAYNLGGG SLGAKALPQ HLSSRPPLIS	* 1 MOPQLQPS LQPQHQPS SSLISQORQQQQQ PSLISQORLQLQQ QVSFDSASA	00 LISQQ : 68 LIAQQ : 45 QHQQQ : 69 QQQQH : 100 GGEEL : 59 : -
C.o. : C.g. : M.o. : N.c. : A.n. : S.p. : S.c. :	* RLTSRNI SPPRRI RLTQRNI SPTRRI RTTQRSLSPTRRI QEPQHQGQPQSQI SKPAQLESPVR	120 D DPS NVSNSYANPTAA PRRQPHQQPQHQ	* 140 SVSRINDNNTVRF HRLLPGLP	* XSST DPQQHSRKASS SPHTRHASFNY	160 AAPVTRPRGRSNS MVRHTRGRSNSDA GRSPQRGRTNGDA RSTPQRGRANSDA LRTHRRARSNSDA	* SDATNTHH APAPVAHH P-VLQQQTAAGA APPVPIPLGQQYS ASSRDF	180 ILNALAASKRSMMG IMTAMASSKRSMMG VAAVKGRRPGLPI TMAAANRRAMRG PKLTARRPGRKT	* 200 SINPRYTNMSTHO GINPKYATMSLOQ RRSAVADELSIER SSSIGLKRSLIES SGIGLKRSLIES MPSIDCKRLOK MTSIED 6	LIKDG : 134 LIKEG : 114 LIREG : 171 LIREG : 198 LIRDG : 118 LAPKS : 16 LISN- : 10 L
C.o. : C.g. : M.o. : N.c. : A.n. : S.p. : S.c. :	* 22 PSDGDAVEAIGM PPNGDTVEAIGM PPDGDVTSAIES PQGNVQEAIQEI PENQSKCISR - PPLLLHSSISQ p 6	20 * ARRKVISEGIKS ARRKVISEGIKS ARYRVIHDGIKS IRVINJEKTIDQEVKS IRVINJEGIPI R 66 6	240 SDCMST-IRIYV SDCMST-IRIYV NTECMSQ-IRIYV SDCMSS-IRIYV GDCMST-YRVYI DECCNSS-TRYVY SEDKQQQRTRCYV g s R Y6	* WMILLTFILP WLILLTFILP WLIMLCPVLE WLILLAFIIE WLILLJFIE WAVLLAFPRN WTVLSQTSMEA: W 661 p	260 TTDYIA THRC TDDYIG IHRC TDAYID THRC TTYIS THRC AD EYIRYROS STORYIA TKLC td Y6 16 G	Rab GA * 28 SPAYSKIRND SPAYSKIRND SPAYSKIRND SPAYSKIRND SPAYSKIRND 3P y KI ND	AP domain	300 RVSEASLIR LN RVSEASLIR LN RVSEASLIR LN RVSEASLIR LN RVTDASLR LN RVSESISR LN RVSEDALTRCIS RV32 SLIRLLN	AVAWR : 233 AVAWR : 213 AVAWR : 213 AVAWR : 270 AVAWR : 297 AVAWR : 217 AYVWK : 113 CFAMQ : 112 a AW
C.o. : C.g. : M.o. : N.c. : A.n. : S.p. : S.c. :	* 320 LHDAREEQRNRDI LHDSREEQRR LHDSREIRQ LHDARGDRTRDHS IHDAK-NRNNLX R TQQRQKTRFDR-	* RPSRPSTGRSSI ERPSTGRSSI SSTRQSLPQ SIVSLSRRSAPS FSSSRREMEL	340 SLDSSNSTATAYS SQDSSTSTATAHS LPESPARPRTATN EPGSRPGTSGH LINTPPSIAEEEP	* QTPSS-PSARN QNPASSPSARN DTPTSSPAVKN DSITGSPASKS SADDMTPDSMI	360 RARALTLTTEGSE RARALTLTTEGSE RARALTLTTEGSE SSNSSTSAVSPG	380 TS-HAAEPGTY TS-HAGEPGTY AG-AGESGTY ASGVASEPGTY ASGVASEPGTY TNETATY GALY YV	* QGMNVLA PFLY QGMNVLA PFLY QGMNVLA PFLY QGMNVLA PFLY QGMNVLA PFLY QGMNVLA PLLY QGMNVLA PLLY QGMNVL APLY	400 ARSESCAFIAFY ARSEACAFYAFH ARSEACAFYAFH ARSECAFALFH CKSENCAFALFH CKSENCAFQLFT ASE AS F	* TLLTR : 334 TLLTR : 311 QLLTQ : 365 QLLTQ : 398 TFITR : 315 RLLQN : 149 KLCYE : 161 1
C.o. : C.g. : M.o. : N.c. : A.n. : S.p. : S.c. :	420 CPGYIRGAMDG CPGYIRGAMDG LPGYIRGAMDG CPGYIRGAMDG CPGYIRGAMDG CPGYIRGAMDG MIPTYLFKNLNG e P Y6 61g	* VHKCLALVDRVL VHKCLALVDRVL VHKCLALVDRVL VHKCLALVDRVL VHKCLALVDRCL VHKCAKLLDISL VHKCAKLLDISL VH G L6D L	440 ATV SKISGYIHS ATV SKISGYIHS ATV EKISKYIMV SIV PKISLYIMS EVERTAAVIFS EVERTAAVIFS EVERTAAVIFS RII PKISKFISD 6d 4L 5L	* 460 KGLIAEIYAFP KGLIAEIYAFP KGLAEIYAFP KGLARIYAFP KGLARIYAFP KGLARIYAFP KGLARIYAFP KGLARIYAP NLLARIYGMP k L A GYA P	* SVLTLCACTPPL SVLTLCACTPPL SVLTLCACTPPL SVLTLCACTPPL SILTLSACTPL SILTLSSONKPL S6LTL ACT PL	480 EVIRLWDFIFAY EVICLWDFIFAY EVICLWDFIFAY EVIRLWDFIFAY EVIRLWDFIFAY OVIRLWDFMFAX OVIRLWDFMFAX 2v6 6WDF6FAY	* GPHLNILCIVAQ GPGLNILCIVAQ GPGLNILCIVAQ GPHLNILCIVAQ GIHLNILCIVAQ GFHMNILGVAA GFHMNILFVVAF G h6NILC66Aq	* MGURDQUMSSPS MGURDH MITRSQUMASPS JIMRTKIMESPS JIMRTKIMESPS JIMRTILKSQN FIFREQUIDHPS VKURSKVFKSDS 5 R	PNKLI : 437 PNKLI : 408 PNKLI : 468 PNKLI : 501 PNKII : 418 PMTLI : 252 PVNLI : 264 P 6L
C.o. : C.g. : M.o. : N.c. : A.n. : S.p. : S.c. :	520 RSFESIOADKVII RSFESIOADKVII RSFEAIOADLIKI RSFEAIOADLIKI RSFEPIDAKTII RIFFPINAKNIM ROFEPEDADEII R FP 1 A	* 54 ERTIWITERTPD ERTIWITERTPD TTIFTARNIPD RTTUVIRMIPD ALTVLIVRKIPE RLGVGFTAKIPA t6 6P	0 * DTVAATVAHAQ DTVAATVAHAH DVYAETVHAK PLYAETUHAK PLYAETUHAK ELYNTJARHAWDS QTVDLTVDHLTDP Y 6 Ha	560	: 472 : 443 : 503 : 536 : 453 : 299 : 306				

**Supplemental Figure 1.** Amino acid sequence alignment of Co Bub2 homologs.

Co Bub2 homologs (C.o, Colletotrichum orbiculare) were identified in Colletotrichum graminicola (C.g), Magnaporthe oryzae (M.o.), Neurospora crassa (N.c.), Aspergillus nidulans (A.n.), Schizosaccharomyces pombe (S.p.), and Saccharomyces cerevisiae (S.c.). The alignment was generated using Clustal W and shaded using Gene Doc. Numbers on the right indicate amino acid residue positions. Shading of residues represents percentage amino acid conservation: black, 100%; dark grey, 80%; light grey, 70%. Gaps introduced for alignments are indicated by a hyphen. The Rab GAP domain is indicated by a red line.

#### Supplemental Data. Fukada and Kubo (2015). Plant Cell. 10.1105/tpc.15.00179

C 0		110
C.a.		110
М.о.	: MMAWTQPAAMDTLRLKPRQPVVD-DIENWDDD-DELID-DDITFRSSGSNVTGQLTRRGSHSSFRSERESIQG-EEQCLQLPDVDDEKSTLDAIAAAAASAGIPLPSNITPSALMGGTIK :	115
N.c.	$: MMETIRLKTR - PSVEDDIEN MDD - pliegdd = IHSHSTAANAHP + HRD SHSSFR = DFESIHG - EEERQVHLPGDDEKSTLDALAAAERAGIPLEKNVSPSALMGGTIK : = PSVEDDIEN MDD + PSVEDIEN MDD + PSVEDDIEN MDD + \mathsf{PSVEDDIEN MDD + PSVEDDIEN MDD + PSVEDDIEN MDD + PSVEDDIEN MDD + \mathsf{PSVEDDIEN MDD + \mathsf{PSVEDIEN MDD + \mathsf{PSVEDIEN MDD + \mathsf{PSVEDIEN MDD + \mathsf{PSVEDDIEN MDD + \mathsf{PSVEDDIEN MDD + \mathsf{PSVEDDIEN MDD + \mathsf{PSVEDDIEN MDD + \mathsf{PSVEDIEN MD + \mathsf{PSVEDIEN MDD + \mathsf{PSVEDIEN MDD + \mathsf{PSVEDIEN MDD + \mathsf{PSVEDIEN MDD + \mathsf$	108
A.n.	:MAPSTVDVR9NSEGSIICADD-DELQCYEDIHIRAASSTTSVTNSSVRRSGHRDSISSRRSARSDLDSTTGGDEDWQIQLLDEDEFVNEEAITSVQNAGIPISVNIPRAAIVGGTIK :	117
S.p.	:	77
S.c.	:	64
	* 140 * 160 * 180 * 200 * 220 * 240 *	
C.o.	: RLGCTIKKINVDDADDELPDTIQGLRIKPQDCSKFPDMLRQVSGGSNHTSPTKALKTPPALSTLDWRESTDRRESTESRTSTLSTISALSGAINPDR :	210
C.g.	: RLGG_KIKKIMQDD:EDLVLPDSTQPLRIKPQDGSKFPDMLRQVSGGSTQTSPTKSMKTPPAVSLLDRRESTESTTSTLSGAINTER :	198
М.о.	: RLGG-KINKIIQEDDEDJFLPEPGRALRIKPQDGTRFPESLRQISS-STTTSPVKSTRPTLVTPEQKGRQSSAASSNINPDK :	197
N.C.	: RLGG KINKI GED VDD EFPSDAGOOIKKRDSSOFPEAIROVSGSFOHTOPPRTSGSFSGRLEPVRKISGSFGRPEAMRKASNTFPSRALPPOKLTVKPMDVVQVRVSRPSLTSGLDDER :	236
A.n. S.n	KLIGK KTIKKET ID INSUTEEPSYSVALE INKHETSIPEALIHUSISPIANN	188
S.c.	SNSTSAFWSNKÖA DDOUVEVDODE	94
	d dD 6	
	260 * 280 * 300 * 320 * 340 * 360 * 380	
C.o.	: FRONDDDD-FroeSerikVskrr-PLKFISLITPPTPSkkwRPLDKAFPEDFroeFLPSDEK/RPSAKKDIFKTP-LAQVDDDWGEGSIGTIFGGREARSKNSSSVSAMSPVSSSFTAESED:	335
C.g. Mo	: FRONDUDUDE COGSELENSER FREEDUIETETT - NUNALUDUETETELESUGETETETET VINLEDUWGGSTETERGENE ABSIKSSY AMSS SSISTESE VKNDNDNDE COGSELENSET VENERUIETETET VENERUIETETETETETETETETETETETETETETETET	319
N.C.	FROM DOND FIGURE AND	358
A.n.	: IPGDYSTSIDVD TEDIPTIKAAKQVPSQIPTISNLDFSKETASAENF N FELPANDS OTNPSASVLSP-APDDIDIDWSCS SV.FGG TR HRSNPSSSISVVSPSASSLTGESD :	311
S.p.	: NKYQDADVDE_NTIRA_ETASQRPPIPFSSDT_KKTYLSSNARY_SVSDSPYCESEGFSSFED_FEIDPDADLNSILHRKQ_RMDPKA_FS_VEQS_LR_PSSAHND :	224
S.c.	: FQEFQNKKDD_DDAIK_NFHLRNGCRTGPFKNDIFAEEFDR	200
	fd edpl6 d3r sss d	
<b>C A</b>		442
C.a.	TTPGLW PSGPWIFEERLSRR LSRSPVRSVEEP	430
M.o.	- AMOSLV PEGPVDFTERLOKR PSPSPEROPIOSPOLVOKPPPLKAALOTPTSRAEPEKE PLS LE GN EV DSSKL UHRNVKVKDTRTGS STPK AASTTF NKSSGAVSRLPRM :	441
N.c.	: E-FDGII PPGPLNWEERLHRR: OSRSSER-IREEPVIVERVOKKDREEQEDFLSCLDIGDGEVEDSKKLUPHRNIKVKDTRGDS: N.PKASVAPKF/NKP-VAASRLPRPMGSMGSL :	473
A.n.	: EGLOGUVIPEGPLNLGGRLTQLEKHNFPDTPYRVPEGRRQEQLRSDFFSCLDINSCDLSARRPSINPNLKYKTEPLGSPAR-RGATTITTINAAVSPKTRIPRLSG :	418
S.p.	: GFWDDFDDFNNETESIFR(KIRSPNTINQKHPYISSTISYQPNYHQDAK"YPLCKDFFPSDANENPHSDN NLKYSKTFSKRDTS	311
S.c.	: DREDOREFNYKIDNDTODTILAGFSSDDEG	277
	eD1 dgg6n p6	
	520 * 540 * 560 * 580 * 600 * 620 * 640	
C.o.	:SHERTMSAM PVSESGGP-FLSRTRRSOSRLGHOSOSSVTSIBSMPTPTLPSPAHPLLPAMPROCEPSSKTSATSIRNEPTTSSOLLRFK SL-AMWASHSPAKPISSR-FERPPSRTESGROO :	565
C.g.	: H-ERHERALSALEPVSESGGP-IFARSRRSQSRMGHASQSSVTSIHSMPTPTTPSPGS-LLPSTPRREDGPRTSTTSLRNEPTTTSSQLLRFKRSLFAMKASHSPAKPISSR-FDRPPSRTDPNRPA :	554
М.о.	$: \ GGHERTHTSSS_{LEPVS} ESGGP_{IPHP-RRS} SSRIGHSS_{SSSVT} SLPTPTTPSSTHSMLPATPR_{RRE} GHKTSNTSLHNEPTTTNAQLLRLKRSLFVM_{APISPARPPTRGGYDRPPSRSDTQSRP : SSGP_{SSS} SSGP_{SSSS} SSGP_{SSS} SSGP_{SSS} SSGP_{SSS} SSGP$	564
N.c.	: VSHERTQTQSS. PPYSETGEADTRSSSRRSLTRSGHHSQASISNVMSSGASQPAPSTTTRRNAQKTATSQKAEPTTTNAQLLRMKSM-GLRP-QSQTRSSTRSYERPPSRTEATRAQ :	594
A.n.	:HDRTRSTHIDT/SESGAPLSKFRTSPRPGHVSHSSVSSLPIAGSSQGSSIQFTPTRISUGSRVSKEALAGEHAPG-KQLLKTK:SM_SM_NAPHTTSSTSFACPPGQSGLNWLN :	532
S.p.		
		370
S.c.	:YDIVKHOLIPPGLHRRQRDWNTQQELDSFKEKSVRHCSNQNVQLMGPAKIKTIKQQIDHNT_MKKG	370 345
s.c.	YDIVKHD_LT#PGLHR	370 345
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C.o. C.g. M.o. N.c. A.n. S.p. S.c. C.o. C.g. M.o. N.c. A.n. S.p. S.c.	Junction       Individual Junci Indina Junci Indin Junci Individual Junci India Junci Individual Junci	370 345 682 668 679 715 640 388 366 794 779 800 842 763 485 459
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C.o. C.g. M.o. A.n. S.c. C.o. C.g. M.o. A.n. S.c. C.o. C.g. M.o. C.g. C.o. C.g. M.o. N.c. N.c.	<pre>Dirich Schuber Stratege Schuber Schub</pre>	370 345 682 668 679 715 560 388 366 794 779 800 842 2763 485 459 921 889 921 889 908
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S.c. C.o. C.g. M.c. A.n. S.p. S.c. C.o. C.g. M.c. A.n. S.p. S.c. C.o. C.g. M.c. A.n. S.p. S.c. S.c. S.c.	<pre></pre>	370 345 682 668 679 715 640 388 366 794 779 800 842 763 459 921 889 908 845 953 846 6577 537
S.c. C.o. C.g. M.o. N.c. S.p. S.c. C.o. C.g. M.o. N.c. S.c. C.o. C.g. M.o. N.c. S.c. S.c. S.c. S.c. S.c.	<pre></pre>	370 345 682 668 679 715 640 388 366 794 779 800 842 763 485 459 921 889 998 953 846 577 537
S.c. C.o. C.g. M.o. N.c. S.p. S.c. C.o. C.g. M.o. N.c. S.p. S.c. C.o. C.o. C.g. M.o. N.c. S.p. S.c. C.o. C.o. C.g. C.o. C.g. C.o. C.g. C.o. C.g. C.o. C.g. C.g	<pre></pre>	370 345 682 668 679 715 640 388 366 794 779 838 485 459 921 889 921 889 933 846 577 537
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S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. A.n. S.p. S.c. C.o. C.g. M.o. S.c. S.c. S.c. S.c. S.c. S.c. S.c. S		370 345 668 668 679 715 640 388 366 794 779 800 842 763 459 908 842 763 459 908 842 753 763 849 908 849 953 846 557 537

Supplemental Figure 2. Amino acid sequence alignment of Co Bfa1 homologs.

Co Bfa1 homologs were identified in *Colletotrichum graminicola* (*C.g*), *Magnaporthe oryzae* (*M.o.*), *Neurospora crassa* (*N.c.*), *Aspergillus nidulans* (*A.n.*), *Schizosaccharomyces pombe* (*S.p.*), and *Saccharomyces cerevisiae* (*S.c.*). The alignment was generated using Clustal W. Numbers on the right indicate amino acid residue positions. Shading of residues represents percentage amino acid conservation: black, 100%: dark grey, 80%; light grey, 70%. Gaps introduced for alignments are indicated by a hyphen. The two imperfect direct repeats are indicated by a blue line.



#### Supplemental Figure 3. Southern blot analysis of targeted gene deletion mutants.

Genomic DNA was extracted from each putative transformant and the wild-type strain and digested with restriction enzymes indicated in the schematic diagrams on the left. Blots were probed with a labeled restriction fragment after fractionation by gel electrophoresis. The size difference in each blot was consistent with successful gene disruption with the resistance cassette *HPH* between the left border (LB) and right border (RB) sequences. *HPH*, hygromycin.

(A) Targeted gene deletion of Co BUB2. HindIII- and EcoRI-digested genomic DNA from the wildtype 104-T strain and putative Co bub2∆ transformants were gel-fractionated and probed with a 1kb fragment from directly upstream of the start codon of Co BUB2. Through double-crossover, the 1.2-kb HindIII-EcoRI fragment of the wild-type strain was replaced with the 3.7-kb HindIII-EcoRI fragment.

**(B)** Targeted gene deletion of Co *BFA1*. EcoRI- and Xhol-digested genomic DNA from the wild-type 104-T strain and putative Co *bfa1* $\Delta$  transformants were gel-fractionated and probed with a 1-kb fragment from upstream of the start codon of Co *BFA1*. Through double-crossover, the 1.7-kb HindIII-EcoRI fragment of the wild-type strain was replaced with the 3.0-kb HindIII-EcoRI fragment. **(C)** Targeted gene deletion of Co *TEM1*. Xhol-digested genomic DNA from 104-T wild-type strain and putative Co *tem1* $\Delta$  transformants were gel-fractionated and probed with a 1-kb fragment from upstream of the start codon of Co *TEM1*. Through double-crossover, the 1.1-kb Xhol fragment from upstream of the start codon of Co *TEM1*. Through double-crossover, the 1.1-kb Xhol fragment of the wild-type strain was replaced with the 2.4-kb Xhol fragment.



**Supplemental Figure 4.** Co *BUB2* is not involved in G1/S progression during vegetative hyphal growth in *C. orbiculare*.

(A) Representative images of vegetative hyphae of the wild type and Co *bub2* $\Delta$  at 10 hours post incubation. DAPI-stained nuclei were visualized by fluorescence image with magenta or white. Hyphal cells from conidia were fixed and stained with DAPI. Bars = 10  $\mu$ m.

**(B)** Mean percentage ( $\pm$  SE, n = 3) of the number of nuclei in germlings of the wild type and Co *bub2* $\Delta$ . At least 200 germlings were examined at each time point.

C.o. C.g. M.o. N.c. A.n. S.p. S.c.	:::::::::::::::::::::::::::::::::::::::	* MEQDQISVQPHPDEN MEHDHGAAPPHPDDA 	20 LGGLEDAAHVA LGGFDASPQVI LGGIEGSPHVG MEDSPQIT VPEAEPQHQDQ	* QSNGYDYAEK( QNNGYDYSEK( SSAQIRGSD) PDQNGFPDRH YQENQPEHQH	40 QRHQSNPSLI QRHQPSQSLI RRHGQSQSLI YQQQFDQNLN HQTELDQPDQ	* QGLPQS QGFGSQ ISSPRSYSQHDN QMSQGLSHMSET	60 SPRAEEQAD PRMEDQQAD AHQSPHNEDME NFDSD FETPTIVPTAA	* RYNTPPLPM RYNTPPLPM RYNTPPIPP RYATPPAPQ SEEPTLPST	80 GAPPISRPASG INAPSISRPASG PGPSLSRPASG PSTSRPPSG SYHNGYSSDSR	LS : LS : LD : AH : :	83 83 63 86 -
							Rab fam	ily doma	in		
		* 100	*	120	*	140	*	160	*	1	
C.o.	:	GNGAQQGYA	DHASRSSAGAE	AAASNGR	NHVVIKVGM	GDAQIGKTSL	IVKYVEGSWDE	DYI <mark>QTLGVN</mark>	IFMEKTISIRNTI	эт :	163
C.g.	:	GAGAQQGYA	DHASRSSAGAE	AAASNGR	NHVVIKVGM	GDAQIGKTSL	IVKYVEGSWDE	DYIQTLGVN	FMEKTISIRNT	эт :	163
M.O.	:	GGIHNGNSHANNNSA GONPSO	SAENGSBUTTAG	GSSSEOANGE		GDAQIGKTSL	IVKYVEGSWDE NVKYVEGSWDE	DYIQTLGVN	IFMERTISIRNTI IFMERTISIRNTI		143
A.n.	:	YEYHTPTODPOPOPP	TSSRPSSGLSG	TOKOPAOTTK	NSVVIKVGM	GDAQIGKTSL	IVKYVEGSWDE	DYIQTLGVN	FMERTISIRNT	от :	175
S.p.	:			MADARK	NNVTIKVGMI	GDSSIGKTSL	1VTYVQGSFDE	<b>EST</b> QTLGVN	FMEKTISIRNT	эп :	59
S.c.	:		MATPST	GANNSIPAVR	NQVEVQVGLV	GDAQVGKTSLN	IVKYVQNIYDK	2YTQTLGVN	IFLKRKVSIRSTI	: 10	69
				41	N V 6kVG66	GDaq6GKTSLM	WkYV2gs5De	y QTLGVN	F6e4t6SIRnTe	ΞI	
		80 *	200	*	220	*	240	*	260		
C 0							-			_	
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C.g.	:	TFSIWDLGGQREFVN TFSIWDLGGQREFVN	MLPLVCNDAVA MLPLVCNDAVA	ILFMFDLTRK: ILFMFDLTRK:	STLNSIKEWY STLNSIKEWY	RQ <mark>GRGFNKTAI</mark> RQ <mark>GRGFNKTAI</mark>	IPILVGTKYDH IPILVGTKYDH	FVNFPREDQ FVNFPREDQ	EEISNQARRFAI EEISNQARRFAI	KA: KA:	252 252
C.g. M.o.	:	TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGOREFVN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA	ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY	RQGRGFNKTAI RQGRGFNKTAI RQGRGFNKTAI	IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH	FVNFPREDQ FVNFPREDQ FVNFPREDQ FVNLSREEQ	EEISNQARRFAR EEISNQARRFAR EEISNQARRFAR EEISNQARRFAR	KA : KA : KA :	252 252 261 232
C.g. M.o. N.c. A.n.	::	TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA	ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY	RQGRGFNKTAI RQGRGFNKTAI RQGRGFNKTAI RQGRGFNKTAI RQGRGFNKTAI	I PI LVGTKYDH I PI LVGTKYDH I PI LVGTKYDH I PVLVGTKYDH I PFLVGTKYDH	FVNFPREDQ FVNFPREDQ FVNFPREDQ FVNLSREEQ FVNFPREDQ	EEISNQARRFA EEISNQARRFA EEISNQARRFA EEISNQARRFA EEISIQAKRFA	KA : KA : KA : KA :	252 252 261 232 264
C.g. M.o. N.c. A.n. S.p.	::	TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPMVCNDAVA	ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLSRK	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY	(RQCRGFNKTA) (RQCRGFNKTA) (RQCRGFNKTA) (RQCRGFNKTA) (RQCRGFNKTA) (RQ <mark>A</mark> RGFNKTA)	IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH IPVLVGTKYDH IP <mark>F</mark> LVGTKYDH /PILIGTKYDH	FVNFPREDQ FVNFPREDQ FVNFPREDQ FVNLSREEQ FVNFPREDQ FMTFPREDQ	EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISIOAKRFA EEITKOARRYA	(A : (A : (A : (A : (A : (A :	252 252 261 232 264 148
C.g. M.o. N.c. A.n. S.p. S.c.	::	TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFIN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPUVCNDAVA MLPI <mark>ATVGSSV</mark>	ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLSRK IIFLFDLTR <mark>P</mark>	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY ETLSSIKEWY	'RQCRGFNKTA' 'RQCRGFNKTA' 'RQCRGFNKTA' 'RQCRGFNKTA' 'RQCRGFNKTA' 'RQ <mark>A</mark> RGFNKTA' 'RQ <mark>A</mark> RGFNKTA'	I P I LVGTKYDH I P I LVGTKYDH I P I LVGTKYDH I P I LVGTKYDH I P V LVGTKYDH I P I LIGTKYDH I P I LVGTKYD <mark>I</mark>	FVNFPREDQ FVNFPREDQ FVNFPREDQ FVNFPREDQ FVNFPREDQ FMTFPREDQ LIDLDPEYQ	EEISNOARRA EEISNOARRA EEISNOARRA EEISNOARRA EEISIOAKRA EEITKOARRA EEITKOARRA	(A) (A) (A) (A) (A) (A) (A) (A) (A) (A)	252 252 261 232 264 148 158
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C.g. M.o. N.c. A.n. S.p. S.c. C.o. C.g. M.o.		TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFIN tFSIWDLGGQREFEN * 280 MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPUCNDAVA MLPIATVGSSV MLP6vcndava * VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK	ILFMFDLTRK. ILFMFDLTRK. ILFMFDLTRK. ILFMFDLSRK. ILFMFDLSRK. IIFLFDLTRP IGFGFDL3Rk: 300 AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY IENVGEPLLI IENVGEPLLI	RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGAGFNKTA RQAYGLNDSA RQ rGFNK3A 320 YQSC YQSC YQSC	LPILVGTKYDH LPILVGTKYDH LPULVGTKYDH LPVLVGTKYDH LPELVGTKYDH LPILIGTKYDH LPILVGTKYDL 5PLL6GTKYDH: *	FVNFPREDO FVNFPREDO FVNFPREDO FVNFPREDO FVNFPREDO FMTFPREDO FMTFPREDO f6 rE Q 340	EEISNOARRFA BEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARFA EEISNOARRFA EEISNOA	(A : (A : (A : (A : (A : (A : (A : (A :	252 252 261 232 264 148 158
C.g. M.o. N.c. A.n. S.p. S.c. C.o. C.g. M.o. N.c.		TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFIN tFSIWDLGGQREFIN tFSIWDLGGQREFEN XAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPUCNDAVA MLPMVCNDAVA MLPMVCNDAVA MLPMVCNDAVA MLPGvcndava * VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK	ILFMFDLTRK. ILFMFDLTRK. ILFMFDLTRK. ILFMFDLTRK. ILFMFDLSRK. IIFLFDLTRPI IGFGFDLSRK. 300 AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY IENVGEPLLI IENVGEPLLI IENVGEPLLI IENVGEPLLI	RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQQRGFNKTA RQQAYGLNDSA RQ rGFNK3A 320 YQSC YQSC YQSC YQSC YQSC YQSC	IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH IPVLVGTKYDH IPELVGTKYDH IPILIGTKYDH IPILVGTKYDL 5PLL6GTKYDH *	FVNFPREDO FVNFPREDO FVNFPREDO FVNFPREDO FVNFPREDO FMTFPREDO FMTFPREDO f6 rE Q 340	EEISNOARRFA BEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA DARFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EEISNOARRFA EQISRTSMKYA EQISRTSMKYA EQISRTSMKYA EQISRTSMKYA	(A):         (A): <t< td=""><td>252 252 261 232 264 148 158 02 02 11 82 14</td></t<>	252 252 261 232 264 148 158 02 02 11 82 14
C.g. M.o. N.c. A.n. S.p. S.c. C.o. C.g. M.o. N.c. A.n. S.p.		TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFFN TFSIWDLGGQREF6N * 280 MRARLIFSSTSHSIN MRARLIFSSTSHSIN MRARLIFSSTSHSIN MRARLIFSSTSHSIN MRARLIFSSTSHSIN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPIATVGSSV MLP6vcndava * vQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK	ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLSRK IIFLFDLTRF IGFGFDL3RK 300 AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE	TINSIKEWY STINSIKEWY STINSIKEWY STINSIKEWY STINSIKEWY STINSIKEWY STINSIKEWY STINSIKEWY ENVGEPLLI IENVGEPLLI IENVGEPLLI ISNVGEPLLI ISNVGEPLLI IENIGEPLLI IENIGEPLLI	RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQARGFNKTA RQAYGLNDSA RQ rGFNK3A 320 YQSC YQSC YQSC YQSV YUSC YUSC YUSC YUSC YUSC YUSC YUSC YUSC YUSC	IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH IPILVGTKYDH IPILIGTKYDH IPILVGTKYDL 5PLLGTKYDH *	EVNFPREDO EVNFPREDO EVNFPREDO EVNFPREDO ENTFPREDO ENTFPREDO ENTFPREDO ETILDPEYO 66 rE Q 340	EEISNOARRFAI EEISNOARRFAI EEISNOARRFAI EEISNOARRFAI EEISTOAKRFAI EEISTOAKRFAI EEITROARRFAI EQISRTSMKYA (E2I3 qa 45A) *	(A : (A : (A : (A : (A : (A : (A : (A :	252 252 261 232 264 148 158 02 02 11 82 14 .98
C.o. N.c. S.p. S.c. C.o. C.g. N.c. A.n. S.c. N.c. S.p. S.c.		TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFVN TFSIWDLGGQREFFN TFSIWDLGGQREFFN * 280 MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN MRAALIFSSTSHSIN	MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPLVCNDAVA MLPGvcndava * vQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK VQKIFKIVLSK	ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK ILFMFDLTRK IFMFDLSRK IFFDLTRF IFFDLTRF AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE AFDLKCTIPE IFFLLTTIPE	STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLNSIKEWY STLSIKEWY S	RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQGRGFNKTA RQARGFNKTA RQAYGLNDSA RQ rGFNK3A 320 YQSC YQSC YQSC YQSV YQSV YKHLGGQQHRI	LPILVGTKYDH LPILVGTKYDH LPILVGTKYDH LPILVGTKYDH LPILVGTKYDH LPILVGTKYDH LPILVGTKYDH * *	VNFPREDO VNFPREDO VNFPREDO VNFREDO MTFPREDO MTFPREDO 11 DDPYO f6 rE Q 340	EEISNOARRFAI EEISNOARRFAI EEISNOARRFAI EEISNOARRFAI EEISTOAKRFAI EEISTOAKRFAI EEITTOARRYAI EQISRTSMKYA (E2I3 qa 45A) * *	KA       :         XA	252 252 261 232 264 148 158 02 02 011 882 14 .98 245

**Supplemental Figure 5.** Amino acid sequence alignment of Co Tem1 homologs.

Co Tem1 homologs were identified in *Colletotrichum graminicola* (*C.g*), *Magnaporthe oryzae* (*M.o.*), *Neurospora crassa* (*N.c.*), *Aspergillus nidulans* (*A.n.*), *Schizosaccharomyces pombe* (*S.p.*), and *Saccharomyces cerevisiae* (*S.c.*). The alignment was generated using Clustal W and shaded using Gene Doc. Numbers on the right indicate amino acid residue positions. Shading of residues represents percentage amino acid conservation: black, 100%; dark grey, 80%; light grey, 70%. Gaps introduced for alignments are indicated by a hyphen. The Rab family domain is indicated by a orange line.



**Supplemental Figure 6.** Co Bub2 interacts with Co Bfa1 in yeast two-hybrid assays.

(A) Schematic representation of *C. orbiculare* Co Bub2 and Co Bfa1. The 124 amino acid sequence of the Nterminal in Co Bub2 is specific to filamentous fungi and absent in yeast. Rab GAP of Co Bub2 is labeled in black; the imperfect direct repeats (IDR) of Co Bfa1 are labeled in gray.

(B) Interaction of Co Bub2 with Co Bfa1 assayed by a yeast two-hybrid experiment. Co Bub2 proteins were expressed in fusion with GAL4BD (BD) protein, and their interactions with Co Bfa1 proteins fused with GAL4AD (AD) were tested. For the positive control, the interaction of *S. cerevisiae* Bub2p with Bfa1p was analyzed. Yeast transformants were grown on SD medium lacking the indicated amino acids. Interaction was assessed from growth on SD –Trp –Leu + AbA (DDO/A), SD –Trp –Leu – Ade –His (QDO), and SD –Trp –Leu + AbA + X- $\alpha$ -Gal (DDO/A/X) medium. Empty-BD vector and empty-AD vector were used as the negative control. +, interaction; –, no interaction.



**Supplemental Figure 7.** Co  $tem1\Delta$  is impaired in septum formation but not in nuclear behavior during appressorium development.

(A) Mean percentage ( $\pm$  SE, n = 3) of cells with various patterns of nuclear distribution in the wild type and Co *tem1* $\Delta$ . Scoring: one nucleus retained in the conidium (black), two nuclei retained in the conidium (red), one nucleus in the conidium and the other in the appressorium through mitosis (blue), multiple nuclei in the conidium and appressorium through second or third mitosis (yellow), one nucleus in the appressorium and degradation of the remaining nucleus in the conidium through autophagy (green), two or three nuclei forming an aberrantly extra septum in the middle of appressorium-forming conidia (pink). At least 200 conidia were assessed at each time point.

(B) Representative images of septum formation during appressorium morphogenesis in Co  $tem1\Delta$  at 10 hpi. Germinating conidia were fixed and stained with DAPI and calcofluor white (40 ng/mL). Samples were observed by fluorescence microscope. White arrowhead shows an aberrant extra septum at the conidium. Bars = 10  $\mu$ m.



**Supplemental Figure 8.** Co Bub2/Co Bfa1 affects the localization of septin during appressorium development in *C. orbiculare*.

(A) Representative images of Sep6-GFP localization in fungal cells during appressorium development. Strains that carried Sep6-GFP were incubated on a glass slide and observed over time; septin was first observed in conidia at 0 h, germlings at 3 h, and initial appressoria at 6 h. Bars = 10  $\mu$ m.

**(B)** Mean percentage ( $\pm$  SE, n = 3) of cells with Sep6-GFP localization in the wild type and Co *bfa1* $\Delta$ . Sep6-GFP fluorescence in the each strain was localized in conidia (white bar), the tip of immature appressoria (grey bar), or the neck of appressoria in a ring (black bar). At least 200 conidia were assessed at each time point.



**Supplemental Figure 9.** A defense response in plants was induced by attempted penetration by appressoria of *C. orbiculare* mutants Co *bub2* $\Delta$  and Co *bfa1* $\Delta$ .

(A) Representative images of reactive oxygen species  $(H_2O_2)$  accumulation at sites of attempted penetration by appressoria of the wild type and Co *bub2* $\Delta$ . Epidermis of cucumber cotyledons removed 3 days after inoculation with Co *bub2* $\Delta$ , stained with DAB to detect reactive oxygen species, and then observed with bright-field microscopy. Bars = 10 um.

**(B)** Mean percentage (+ SE, n = 3) of reactive oxygen species accumulation at sites of attempted penetration by appressoria of the wild type, Co *bub2* $\Delta$ , Co *bfa1* $\Delta$ , and Co *ssd1* $\Delta$  at 3 dpi. Co *ssd1* $\Delta$  was used as the control. At least 200 appressoria were assessed for each fungal strain.

(C) Dark, yellowish sunken necrotic areas on cucumber cotyledons with resistance impaired by heat-shock treatment. Conidial suspensions of the wild type, Co *bub2* $\Delta$ , Co *bfa1* $\Delta$ , Co *ssd1* $\Delta$ , and Co *pks1* $\Delta$  were dropped onto a heat-shocked cucumber cotyledon (H.S. +) or without heat-shocked cotyledon (H.S. -) and incubated at 24°C for 7 days. Co *ssd1* $\Delta$  was used as the positive control, and Co *pks1* $\Delta$  was used as the negative control. Mock, distilled water control.

(D) Bar charts showing disease symptoms on heat-shock-treated cucumber cotyledons scored at 7 dpi. Symptom definitions: large = lesions larger than the inoculated spot, necrosis present; medium = lesions similar in size to the inoculated spot, necrosis present; small = lesions not larger than inoculated spot, no necrosis; tiny, lesions dot sized (< 1 mm); no = no disease symptoms.

# **Supplemental Table 1.** Growth rate and conidiation of mutants on PDA medium.

Strain <sup>a</sup>	Vegetative growth (mm/3 days) <sup>b</sup>	Conidia (×10 <sup>7</sup> /colony) <sup>c</sup>
WT	18.67±0.82	3.32±0.44
bub2∆	17.00±0.89	0.67±0.13**
bfa1∆	12.50±6.12	0.41±0.11**
bub2∆ bfa1∆ + Sc BUB2 Sc BFA1	16.67±0.52	1.00±0.35**
WT + Co Tem1 <sup>T128N</sup>	17.33±0.00	1.80±0.26
<i>bub2</i> ∆ + Co Tem1 <sup>T128N</sup>	17.00±0.82	1.24±0.29
<i>bfa1∆</i> + Co Tem1 <sup>T128N</sup>	17.67±0.52	1.32±0.3

<sup>a</sup> *C. orbiculare* wild type (WT), Co *bub2* $\Delta$  (*bub2* $\Delta$ ), Co *bfa1* $\Delta$  (*bfa1* $\Delta$ ), Co *bub2* $\Delta$  Co *bfa1* $\Delta$  expressing *S. cerevisiae BUB2* and *BFA1* (*bub2* $\Delta$  *bfa1* $\Delta$ +Sc *BUB2* Sc *BFA1*), and the Co Tem1<sup>T128N</sup> introduced isoform (WT+Co Tem1<sup>T128N</sup>, *bub2* $\Delta$ +Co Tem1<sup>T128N</sup>, and *bub2* $\Delta$ +Co Tem1<sup>T128N</sup>).

<sup>b</sup> Each strain was grown on PDA at 24°C. Growth rate was calculated based on the growth diameter between 3 days after incubation and 6 days after incubation. Five colonies in each strain were measured; means and standard deviations were calculated.

<sup>c</sup> Conidia produced by 7-day-old cultures were counted for five colonies of each strain; means and standard deviations were calculated.

\*\**P* < 0.05 by Tukey's test. (*n*=10)

#### Supplemental Table 2. Colletotrichum orbiculare strains used in this study.

Strain	Genotype (plasmid used for transformation)	Reference
104-T (MAFF240422)	Wild type	Ishida and Akai, 1969
104-T CoBub2∆-1	Wild type/ENH83696Δ (pBIcobub2SH)	This study
104-T CoBub2Δ-2	Wild type/ENH83696Δ (pBIcobub2SH)	This study
104-T CoBfa1∆-1	Wild type/ENH87866Δ (pBIcobfa1BH)	This study
104-T CoBfa1∆-2	Wild type/ENH87866Δ (pBIcobfa1BH)	This study
104-T CoBub2∆CoBfa1∆-1	Wild type/ENH83696Δ/ENH87866Δ (pBIcobub2SH, pBIcobfa1BH)	This study
104-T CoBub2∆CoBfa1∆-2	Wild type/ENH83696Δ/ENH87866Δ (pBIcobub2SH, pBIcobfa1BH)	This study
104-T CoTem1∆-1	Wild type/ENH82693∆ (pBIcotem1BH)	This study
104-T CoTem1∆-2	Wild type/ENH82693∆ (pBIcotem1BH)	This study
104-T CoBub2∆ CoBub2Com	Wild type/ENH83696Δ/ENH83696 (pBICoBub2S)	This study
104-T CoBfa1∆ CoBfa1Com	Wild type/EENH87866Δ/ENH87866 (pBICoBfa1B)	This study
104-TLacOLacIGFP	wild type/K02913/TEF_CP011342_GFP (pCT31S, pBITEFGFPLacIB)	This study
104-T∆CoBub2LacOLacIGFP	wild type/ENH83696Δ/K02913/TEF_CP011342_GFP (pCT31S, pBITEFGFPLacIB)	This study
104-T∆CoBfa1LacOLacIGFP	wild type/ENH87866∆/K02913/TEF_CP011342_GFP (pCT31S, pBITEFGFPLacIB)	This study
104-T CoHIH1GFP	Wild type/ ENH76847_GFP (pBICoHIH1-GFPB)	This study
104-T α-TUB1mRFP	Wild type/SCD1_Q9C413_RFP (pBI-SCD1pmRFP1-α-TUB1S)	Sakaguchi et al., 2008
104-T CoHIH1-GFP α-TUB1mRFP	Wild type/NH76847_GFP/SCD1_Q9C413_RFP (pBICoHIH1-GFPB, pBISCD1pmRFP1-α-TUB1S)	This study
104-T CoBub2 $\Delta$ CoHIH1-GFP $\alpha$ -TUB1mRFP	Wild type/ENH83696/NH76847_GFP/SCD1_Q9C413_RFP (pBICoHIH1-GFPB, pBI- SCD1pmRFP1-α-TUB1S)	This study
104-T CoBfa1Δ CoHIH1-GFP α-TUB1mRFP	Wild type/ENH87866/NH76847_GFP/SCD1_Q9C413_RFP (pBICoHIH1-GFPB, pBI-SCD1pmRFP1- $\alpha$ -TUB1S)	This study
104-TCoTEM1GFP	Wild type/ENH82693_GFP (pBICoTEM1-GFPB)	This study
104-T∆CoBub2CoTEM1GFP	Wild type/ENH83696Δ/ENH82693_GFP(pBICoTEM1-GFPB)	This study
104-T∆CoBfa1CoTEM1GFP	Wild type/ENH87866Δ/ENH82693_GFP(pBICoTEM1-GFPB)	This study
104-TCoTEM1GFPa-TUB1mRFP	Wild type/ENH82693_GFP/SCD1_Q9C413_RFP (pBI-SCD1pmRFP1-a-TUB1S)	This study
104-TΔCoBub2CoTEM1GFPα-TUB1mRFP	Wild type/ENH83696Δ/ENH82693_GFP/SCD1_Q9C413_RFP(pBI-SCD1pmRFP1-α TUB1S)	This study
104-T CoBub2∆ Bub2Com	Wild type/ENH87866Δ/ENH82693_GFP(pBICoTEM1-GFPB)	This study
104-T CoBfa1∆ Bfa1Com	Wild type/ENH87866Δ/NP_012587(pBI-Bfa1N)	This study
104-T CoTem1 <sup>T128N</sup>	Wild type/ENH82693_T128N (pBI-CoTem1_T128NSB)	This study
104-T CoBub2∆ CoTem1 <sup>T128N</sup>	Wild type/ENH83696∆/ENH82693_T128N (pBI-CoTem1_T128NSB)	This study
104-T CoBfa1∆ CoTem1 <sup>T128N</sup>	Wild type/ENH87866Δ/ENH82693_T128N (pBI-CoTem1_T128NSB)	This study
104-T Cdc12GFP	Wild type/MGG07466_GFP (Cdc12_GFPS)	This study
104-T CoBfa1∆ Cdc12GFP	Wild type/ENH87866Δ/MGG07466_GFP (Cdc12_GFPS)	This study
104-T LifeactRFP	Wild type/Lifeact_RFP (Lifeact_RFP)	This study
104-T CoBub2∆ LifeactRFP	Wild type/ENH83696Δ/Lifeact_RFP (Lifeact_RFP)	This study
104-T CoBfa1∆ LifeactRFP	Wild type/ENH87866Δ/Lifeact_RFP (Lifeact_RFP)	This study

#### Supplemental Table 3. Primers used in this study.

Name	Sequence (5' - 3')	Notes 1	Note 2	
g8005_F8	ATTCGCATTAATCAGTAAGTTCG			
g8005_R5	TATCAAGGACGGCCCGTCAGACGGCGATGC	0.000		
q8005 R6	GAGGAACAGCGCAACAGGGACCGCCCTAGC	Co BUB2		
q8005 R8	TCTGTCGTTCTGTTCGACAAGTG			
g3970 F1	TCTTTCGGGAAACGACC		_	
g3970_F2	GGCAAGTAAGCGGAGGT			
g3970_F3	TAATAGAAGCTCGTCCGTC	Co BEA1	For cDNA sequencing	
g3970_F4	CGGTGACCAGCATTCATA	COBIAT		
g3970_F5	GACAAAGCCTCACCGAAC			
g3970_R1	GTCGAAGCTGGGCGTATT		_	
g9031_F1		0.7514		
g9031_F2		Co TEM1		
<u>99031_R1</u>				
98005-FTA		Co <i>BUB2</i> 5' UTR		
g8005-R2D	ACGTCGACTACCGGCTCAGGACTGTAACATAT			
g8005-F2C	ACGTCGACTTGGGCGGATTGTAGTTTACGCAG	Co BUB2 3' UTR		
g8005-R1B	GCTTATCGTTATCCAGTTGGCGGATGTCGTTG		For pBICoBub2SH and	
g8005-hphR1C	CCGCCCAAGTCGACGTTAACTGGTTCCCGGTC		pBICoBub2S plasmids	
g8005-hphF1D	AGCCGGTAGTCGACGTTAACTGATATTGAAGG	Hygromycin resistant gene		
g8005-pBIF1A	CCATGCTAATACCGTCGACCTAAATCTCGGTG			
		pBI-G4MRSrev Binary vector		
g8005-pBIR1B	CTGGATAACGATAAGCTTGATATCGAATTCCT			
g3970_F1A	TCTAGAATTCGAGTCGGAAGCATCATCCGCCG			
g3970_R2D	ACGTCGACTGCCCAGTAGCCTCACTCACGAAC	COBFATS UTR		
q3970 F2C	ACGTCGACTTTTCCCGAAAGACTCCCGACGGC			
a3970 R1B	GCTTATCGTTAGACCTCGTTCCCGCCCACCTG	Co <i>BFA1</i> 3' UTR		
a3970 hphR1C	CGGGAAAAGTCGACGTTAACTGGTTCCCCGGTC		For pBICoBfa1BH and	
g3070_hphR10		Hygromycin resistant gene	percobia re plasifilius	
g3970_pBIF1A	CGACTCGAATTCTAGAAATACGACTCACTATA	nBL-G4MRBrey Binany vector		
g3970_pBIR1B	AGGTCTAACGATAAGCTTGATATCGAATTCCT	pbi-O+initblev binary vector		
a9031 F1A 2	TCTAGAATTTGGTACCTAGGTAAGGCACTATA			
a9031 R2D 2	ACGTCGACTAGTGACGAGGACAGCTGGCAGTC	Co <i>TEM1</i> 5' UTR		
g0031 E2C 2				
99031_120_2		Co <i>TEM1</i> 3' UTR		
g9031_R1B_2			For pBICoTem1BH	
g3970_hphR1C	CGGGAAAAGTCGACGTTAACTGGTTCCCCGGTC	Hygromycin resistant gene	plasmids	
g3970_hphF1D	ACTGGGCAGTCGACGTTAACTGATATTGAAGG	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		
g9031_pBIF1A_2	GGTACCAAATTCTAGAAATACGACTCACTATAGGGCGAAT			
a9031 pBIR1B 2	TCGCAGGACGATAAGCTTGATATCGAATTCCTGCAGCCCG	pBI-G4MRBrev Binary vector		
	GGGTGGTGGTGGTGGTataGTGAAACCAGTAACGT			
NIS R1	TTAGGCAACCTTTCTCTTCTTCTTTGGTGGAGTAC	Lacl-NLS	For nBITEEGEPI aclB	
alvGFPR1	ACCACCACCACCCTTGTACAGCTCGTC		plasmids	
pBI_NLS_R1	GAGAAAGGTTGCCTAAGGTACCGGGCCCCCCCCG	pBITEFGFPB vector		
glyGFPF1	GGAGGAGGAGGAGGAATGGTGAGCAAGGGC			
GFPR1	TTACTTGTACAGCTCGTCCATGCCGAGAGT	gly GFP		
g1862_F1A	TCTAGAATTTAGAACGACAGGCGGTAGGACCT		For pBICoHIH1-GFPR	
q1862 pBIR1B	ATGTTGAACGATAAGCTTGATATCGAATTCCT	Co HIH1 5' UTR	plasmids	
a1862 R1B	ACGTCGACTCGCTTGAAGTCGAGGAAAAGCCG			
g1862_REF1A	CGTTCTAAATTCTAGAAATACGACTCACTATA	Co HIH1 3' UTR		
alvGFPF1	GGAGGAGGAGGAGGAGGAATGGTGAGCAAGGGC			
GFPR1	TTACTTGTACAGCTCGTCCATGCCGAGAGT	glyGFP	For pBICoTEM1-GFPB	
CoTem1_pBIR5	TTCCTCCTCCTCCGCAAGACTGGTACAGCAGC		plasmids	
CoTem1_pBIF5	CGAGCTGTACAAGTAATGATCCCGGCTCCATGTGC	pBICOTEMTB plasmid		

## Supplemental Table 3. Primers used in this study.

Name	Sequence (5' - 3')	Notes 1	Note 2
bub2_F3	ACGCGCCAAGATGACCTCAATTGAAGATCTGATAT		
bub2_R3	GCCAACGCGGTTACGGTATATATATGTCTGGGTCC	SC BUBZ URF	For pDIDub2D ploomide
g8005_F11_Sc	TCTTCAATTGAGGTCATCTTGGCGCGTGTTGTACC	nDICoDUD2D plaamid	FOI PEIEUDZE plasifilus
g8005_R11_Sc	ATATATATACCGTAACCGCGTTGGCTCTGGAAAGA	рысововав ріазпій	
bfa1_F2	TACCAACGCCATGTCAATTAGGCCTCTCACGTTAA		
bfa1_R2	TGAAGTGAGGCTAATCTTTTGTCGAATTGATTACC	SC BFAT OKF	For pDIDfo1N plaamida
g3970_F7	CCTAATTGACATGGCGTTGGTAAGTTTTGTTTGGT	nDICoDEA1N plaamid	For perena in plasmius
g3970_R7	ACAAAAGATTAGCCTCACTCACGAACATCACATTT	рысовгати ріазпіц	
g9031_F1A_2	TCTAGAATTTGGTACCTAGGTAAGGCACTATA	Co Tom1T128N 5	
g9031_T128N_R1	ACTTGACCATCAGACTATTCTTGCCGATTTGCGCG	Co leilli 5	
g9031_R1B_2	GCTTATCGTCCTGCGATAGCAAGTCCTAGAAA	Co Tom1T128N 3	
g9031_T128N_F1	CGCGCAAATCGGCAAGAATAGTCTGATGGTCAAGT	Collemn 5	
g9031_pBIF1A_2	GGTACCAAATTCTAGAAATACGACTCACTATAGGGCGAAT		For pBICoTem1 <sup>T128N</sup> SB
g9031_pBIR1B_2	TCGCAGGACGATAAGCTTGATATCGAATTCCTGCAGCCCG	pBIG4MRBrev Binary vecto	<sup>r</sup> plasmids
Sur_R1B	GTCGACGTGAGAGCATGCAATTCCCGTGCA	<b>.</b>	
_ Sur_F1A	GTCGACGTGCCAACGCCACAGTGCCCCACAT	Sulfonylurea resistant gene	
g9031_pm_pBIF4A	TGCTCTCACGTCGACCTGCGACTGGCCAAGTCTTG	T129N	
g9031_pm_pBIR4B	CGTTGGCACGTCGACCGTTTTCAGTGCCACTAGTT	pBICoTem1 <sup>1126</sup> B plasmid	
Y_bub2_F1	CATGGAGGCCGAATTCATGCCGCCGAGTCCAACGA	Co BUB2 1-	
Y_bub2_R1	GCAGGTCGACGGATCCCTACTGGGCATGCGCGACA	Co BUB2 -472	
Y_bub2_R2	GCAGGTCGACGGATCCCTAGTTTGTGTATCGCGGG	Co <i>BUB2</i> -124	
Y_bub2_F2	CATGGAGGCCGAATTCATGTCTCTGCACCAGCTTA	Co BUB2 124-	
Y_bub2_F5	CATGGAGGCCGAATTCATGACCTCAATTGAAGATC	Sc BUB2 1-	
Y_bub2_R5	GCAGGTCGACGGATCCTTACGGTATATATATGTCT	Sc BUB2 -302	
Y_bfa1_F1	CATGGAGGCCGAATTCATGGAACCTTTGCGCCTAA	Co BFA1 1-	
Y_bfa1_R1	GCAGGTCGACGGATCCCTACTGGGCAGCCCTCTGG	Co <i>BFA1</i> -1020	
Y_bfa1_R2	GCAGGTCGACGGATCCCTACGGCACGCCGGGGCTG	Co BFA1 -783	
Y_bfa1_F2	CATGGAGGCCGAATTCCAATCCTCAATTCGGTCAA	Co BFA1 784-	
Y_bfa1_F3	CATGGAGGCCGAATTCATGTCAATTAGGCCTCTCA	Sc <i>BFA1</i> 1-	
Y_bfa1_R3	GCAGGTCGACGGATCCCTAATCTTTTGTCGAATTG	Sc <i>BFA1</i> -302	
Y_tem1_F1	CATGGAGGCCGAATTCATGGAGCAAGATCAGATCT	Co <i>TEM1</i> 1-	
Y_tem1_R1	GCAGGTCGACGGATCCTCAGCAAGACTGGTACAGC	Co TEM1 -302	
Y_tem1_R2	GCAGGTCGACGGATCCTCAACCGTTACTCGCGGCG	Co <i>TEM1</i> -109	
Y_tem1_F2	CATGGAGGCCGAATTCCGCAACCACGTCGTCATCA	Co <i>TEM1</i> 110-	
Y_tem1_F6	CATGGAGGCCGAATTCATGGCTACACCAAGCACGG	Sc <i>TEM1</i> 1-	For pGBKT7- and
Y_tem1_R6	GCAGGTCGACGGATCCTCATGTATTAACGCCCGGC	Sc TEM1 -302	pGADT7- plasmids
Y_BUB2_AD_F1	GGAGGCCAGTGAATTCCCGCCGAGTCCAACGAGAG	Co <i>BUB2</i> 1-	
Y_BUB2_AD_R1	TCATCTGCAGCTCGAGCTACTGGGCATGCGCGACA	Co BUB2 -472	
Y_BUB2_AD_F2	GGAGGCCAGTGAATTCATGACCTCAATTGAAGATC	Sc BUB2 1-	
Y_BUB2_AD_R2	TCATCTGCAGCTCGAGTTACGGTATATATATGTCT	Sc BUB2 -472	
Y_BFA1_AD_F1	GGAGGCCAGTGAATTCGAACCTTTGCGCCTAAAGC	Co <i>BFA1</i> 1-	
Y_BFA1_AD_R1	TCATCTGCAGCTCGAGCTACTGGGCAGCCCTCTGG	Co BFA1 -1020	
Y_BFA1_AD_R2	TCATCTGCAGCTCGAGCTACGGCACGCCGGGGCTG	Co BFA1 -783	
Y_BFA1_AD_F2	GGAGGCCAGTGAATTCCAATCCTCAATTCGGTCAA	Co BFA1 784-	
Y_BFA1_AD_F3	GGAGGCCAGTGAATTCATGTCAATTAGGCCTCTCA	Sc BFA1 1-	
Y_BFA1_AD_R3	TCATCTGCAGCTCGAGCTAATCTTTTGTCGAATTG	Sc BFA1 -1020	
Y_tem1_AD_F1	GGAGGCCAGTGAATTCGAGCAAGATCAGATCTCCG	Co <i>TEM1</i> 1-	
Y_tem1_AD_R1	TCATCTGCAGCTCGAGTCAGCAAGACTGGTACAGC	Co TEM1 -302	
pGB_F1	GGATCCGTCGACCTGCAGCGGCCGCATAACTAGCA	nGBKT7 vector	
pGB_R1	GAATTCGGCCTCCATGGCCATATGCAGGTCCTCCT		
pGD_R1	GAATTCACTGGCCTCCATGGCCATATGAGCGTAAT	nGADT7 vector	
pGD_F1	CTCGAGCTGCAGATGAATCGTAGATACTGAAAAAC		

## Supplemental Table 4. Saccharomyces cerevisiae strains used in this study.

Strain	Genotype	Reference
Y2H Gold	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200	Clontech
Y187	MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met-	Clontech
Y2H Gold: pGBKT7	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7	This study
Y2H Gold: pGBKT7-Co Bub2	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-CoBub2	This study
Y2H Gold: pGBKT7-Co Bub2 1-124	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-CoBub2 1-124	This study
Y2H Gold: pGBKT7-Co Bub2 125-473	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-CoBub2 125-473	This study
Y2H Gold: pGBKT7-Bub2	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-Bub2	This study
Y2H Gold: pGBKT7-Co Tem1 1-94	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-CoTem1 1-94	This study
Y2H Gold: pGBKT7-Co Tem1 95-302	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-CoTem1 95-302	This study
Y2H Gold: pGBKT7-Tem1	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-Tem1	This study
Y2H Gold: pGBKT7-Co Bfa1	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-CoBfa1	This study
Y2H Gold: pGBKT7-Bfa1	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200 pGBKT7-Bfa1	This study
Y187: pGADT7	MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met', pGADT7	This study
Y187: pGADT7-Co Bfa1	MAΤα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met, pGADT7- CoBfa1	This study
Y187: pGADT7-Co Bfa1 1-783	MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met, pGADT7- CoBfa1 1-783	This study
Y187: pGADT7-Co Bfa1 784-1020	MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met', pGADT7- CoBfa1 784-1020	This study
Y187: pGADT7-Bfa1	MAΤα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met, pGADT7- Bfa1	This study
Y187: pGADT7-Co Bub2	MAΤα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met, pGADT7- CoBub2	This study
Y187: pGADT7-Bub2	MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met, pGADT7- Bub2	This study
Y187: pGADT7-Co Tem1	MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met, pGADT7- CoTem1	This study