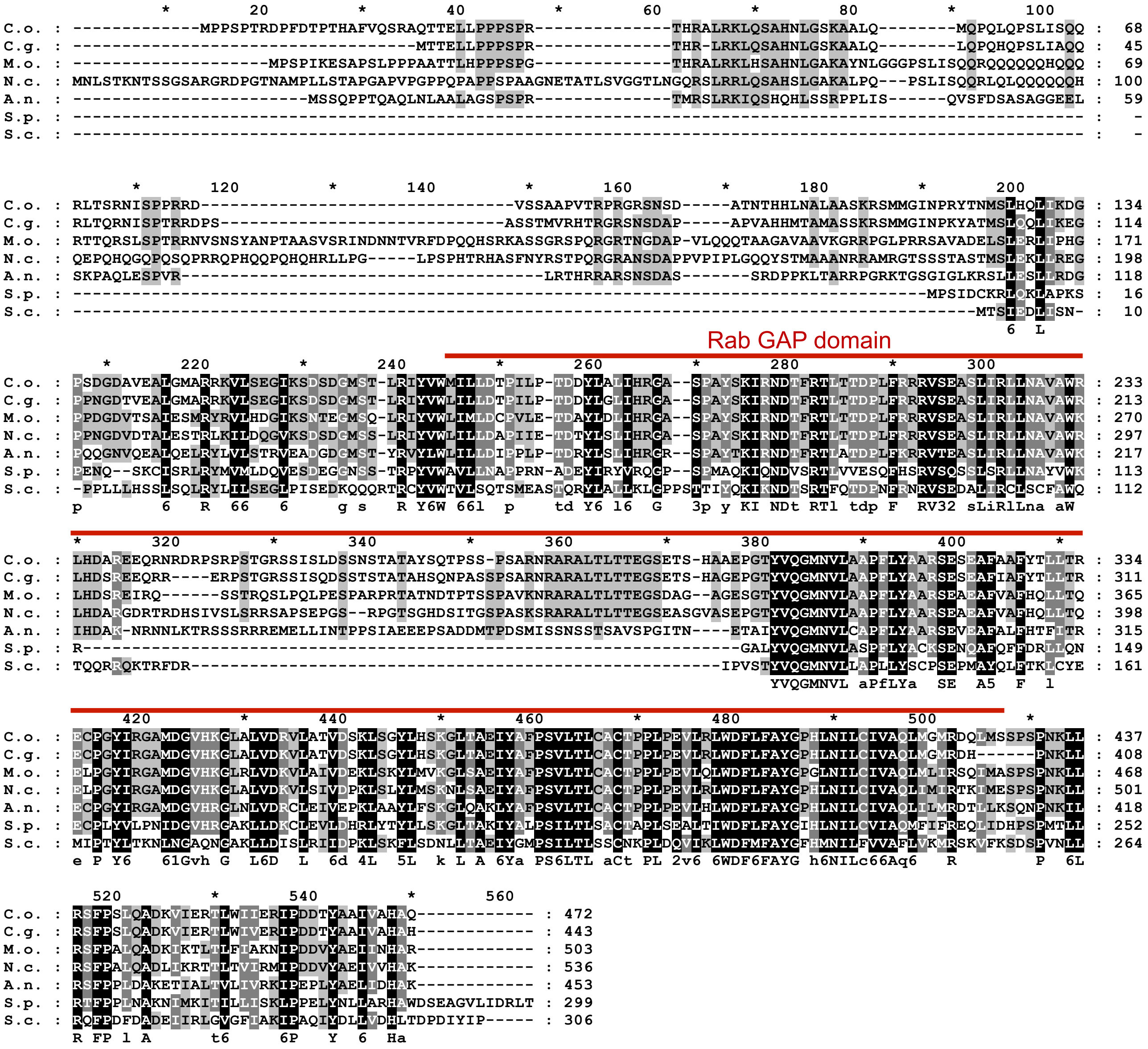
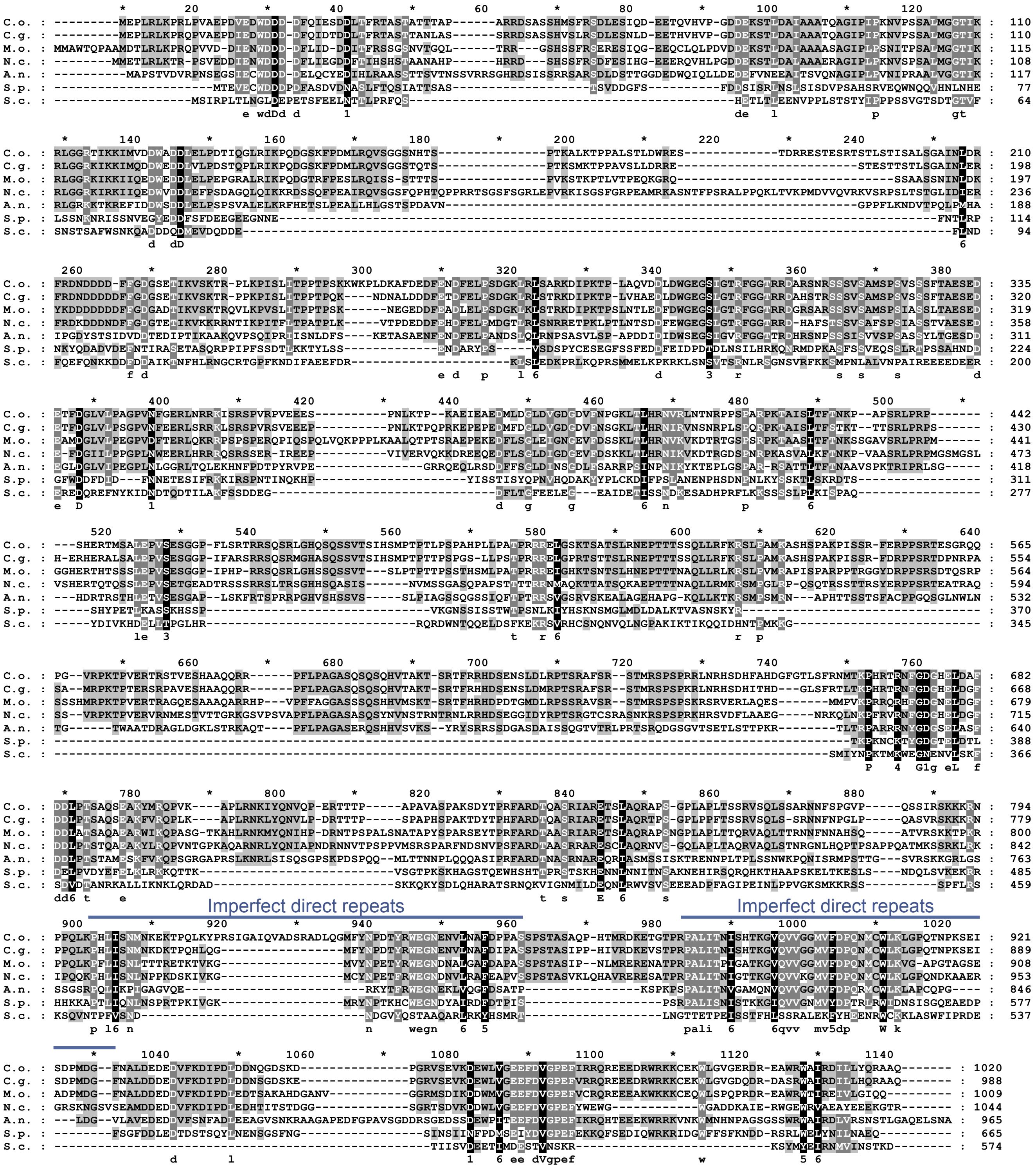


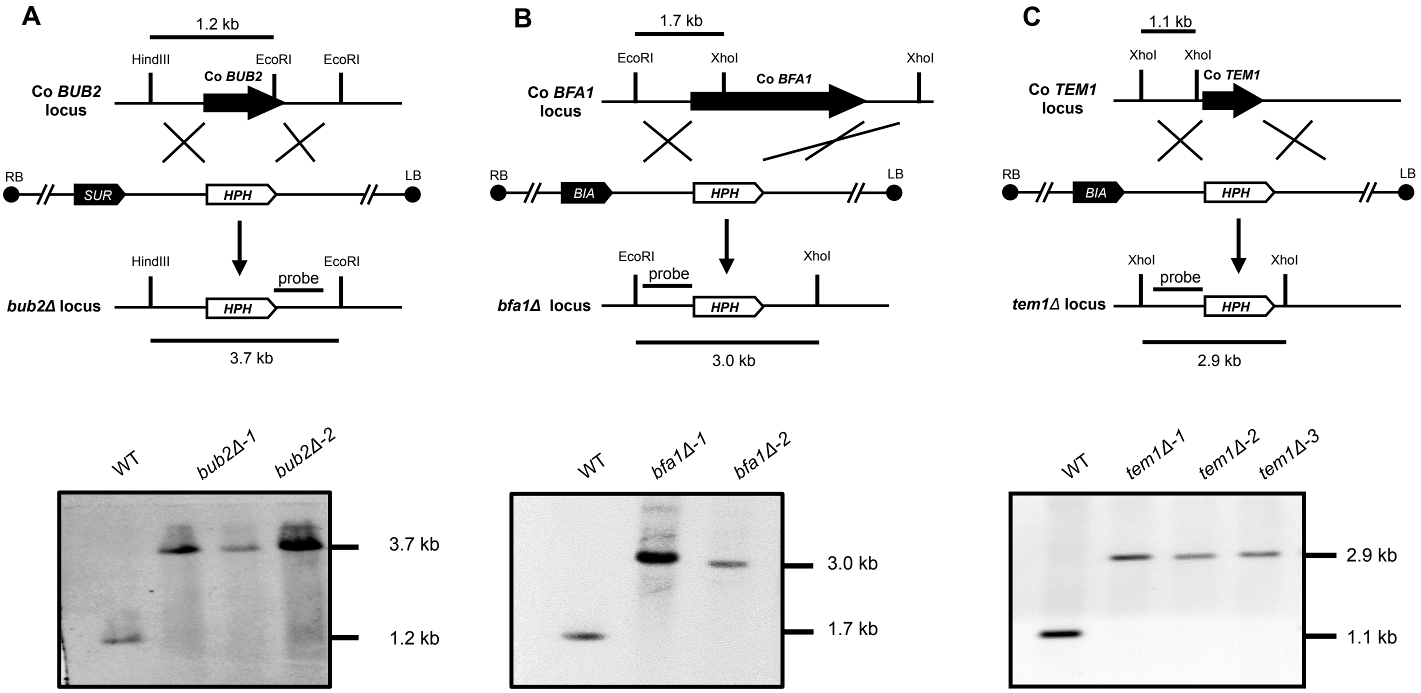
Supplemental Figure 1. Amino acid sequence alignment of Co Bub2 homologs.

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 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.



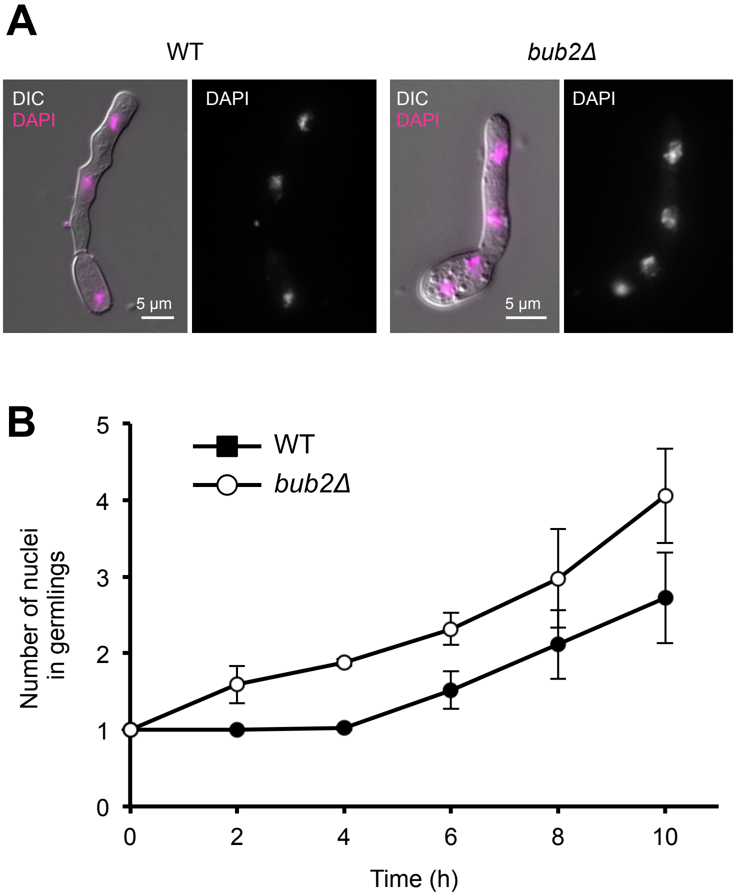
Supplemental Figure 3. Southern blot analysis of targeted gene deletion mutants.

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 C*o BUB2*. HindIII- and EcoRI-digested genomic DNA from the wild-type 104-T strain and putative Co *bub2Δ* transformants were gel-fractionated and probed with a 1-kb 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.

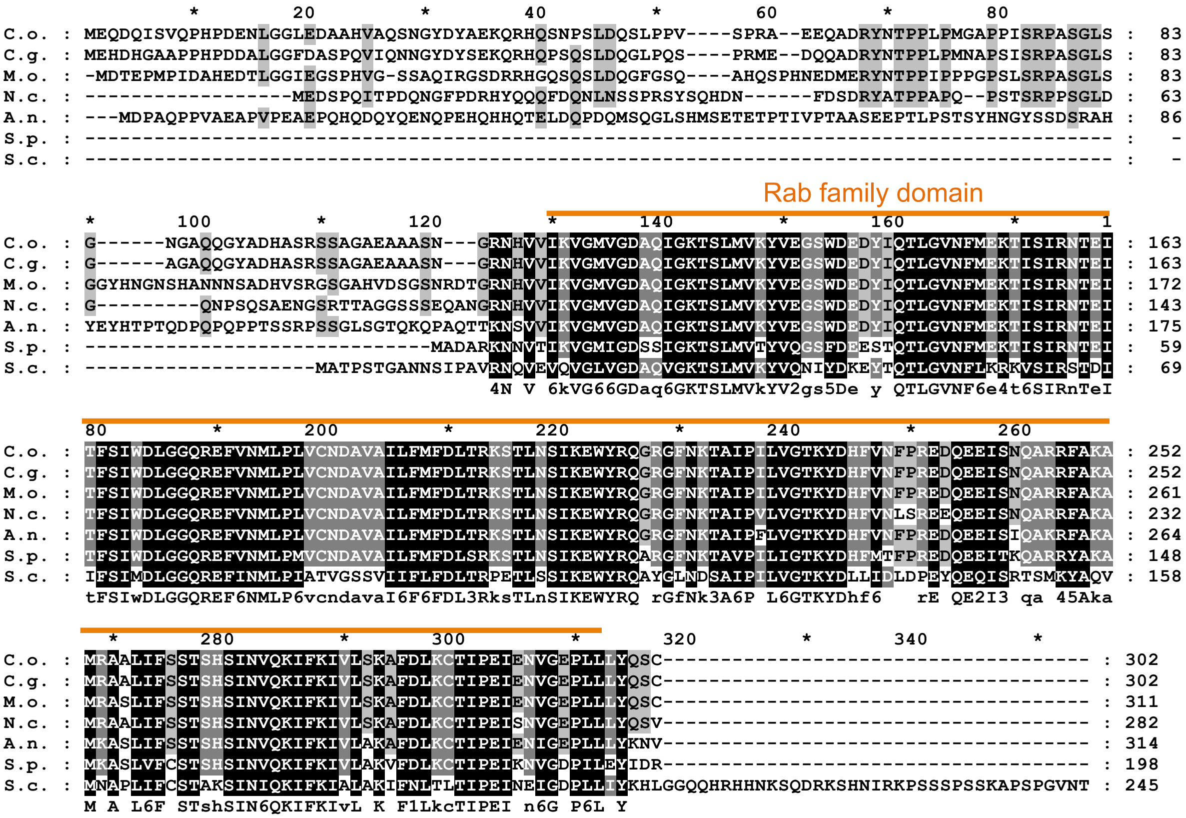
(B) Targeted gene deletion of Co *BFA1.* EcoRI- and XhoI-digested genomic DNA from the wild-type 104-T strain and putative Co *bfa1Δ* transformants were gel-fractionated and probed with a 1-kb fragment from upstream of the start codon of Co *BFA1*. Through double-crossover, the 1.7-kb HindIII-EcoRI fragment of the wild-type strain was replaced with the 3.0-kb HindIII-EcoRI fragment.

(C) Targeted gene deletion of Co *TEM1.* XhoI-digested genomic DNA from 104-T wild-type strain and putative Co *tem1Δ* 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 XhoI fragment of the wild-type strain was replaced with the 2.4-kb XhoI fragment.



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

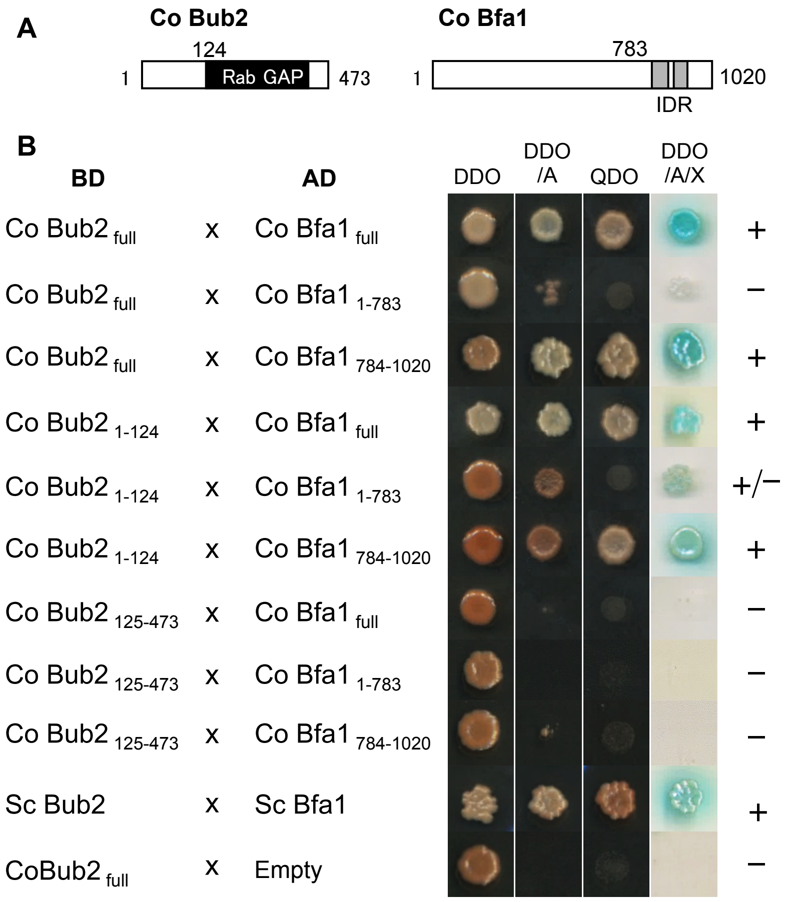
(A) Representative images of vegetative hyphae of the wild type and Co *bub2Δ* at 10 hours post incubation. DAPI-stained nuclei were visualized by fluorescence image with magenta or white. Hyphal cells from conidia were fixed and stained with DAPI. Bars = 10 μm.
(B) Mean percentage (± SE, *n* = 3) of the number of nuclei in germlings of the wild type and Co *bub2Δ.* At least 200 germlings were examined at each time point.



```
                *        20         *        40         *        60         *        80
C.o. : MEQDQISVQPHPDENLGGLEDAAHVAQSNGYDYAEKQRHQSNPSLDQSLPPV----SPRA--EEQADRYNTPPLPMGAPPISRPASGLS :  83
C.g. : MEHDHGAAPPHPDDALGGFDASPQVIQNNGYDYSEKQRHQPSQSLDQGLPQS----PRME--DQQADRYNTPPLPMNAPSISRPASGLS :  83
M.o. : -MDTEPMPIDAHEDTLGGIEGSPHVG-SSAQIRGSDRRHGQSQSLDQGFGSQ----AHQSPHNEDMERYNTPPIPPPGPSLSRPASGLS :  83
N.c. : ------------------MEDSPQITPDQNGFPDRHYQQQFDQNLNSSPRSYSQHDN------FDSDRYATPPAPQ--PSTSRPPSGLD :  63
A.n. : ---MDPAQPPVAEAPVPEAEPQHQDQYQENQPEHQHHQTELDQPDQMSQGLSHMSETETPTIVPTAASEEPTLPSTSYHNGYSSDSRAH :  86
S.p. : ----------------------------------------------------------------------------------------- :   -
S.c. : ----------------------------------------------------------------------------------------- :   -

                                                                 Rab family domain
       *       100         *       120         *       140         *       160         *       1
C.o. : G------NGAQQGYADHASRSSAGAEAAASN---GRNHVVIKVGMVGDAQIGKTSLMVKYVEGSWDEDYIQTLGVNFMEKTISIRNTEI : 163
C.g. : G------AGAQQGYADHASRSSAGAEAAASN---GRNHVVIKVGMVGDAQIGKTSLMVKYVEGSWDEDYIQTLGVNFMEKTISIRNTEI : 163
M.o. : GGYHNGNSHANNNSADHVSRGSGAHVDSGSNRDTGRNHVVIKVGMVGDAQIGKTSLMVKYVEGSWDEDYIQTLGVNFMEKTISIRNTEI : 172
N.c. : G---------QNPSQSAENGSRTTAGGSSSEQANGRNHVVIKVGMVGDAQIGKTSLMVKYVEGSWDEDYIQTLGVNFMEKTISIRNTEI : 143
A.n. : YEYHTPTQDPQPQPPTSSRPSSGLSGTQKQPAQTTKNSVVIKVGMVGDAQIGKTSLMVKYVEGSWDEDYIQTLGVNFMEKTISIRNTEI : 175
S.p. : ------------------------------MADARKNNVTIKVGMIGDSSIGKTSLMVTYVQGSFDEESTQTLGVNFMEKTISIRNTEI :  59
S.c. : --------------------MATPSTGANNSIPAVRNQVEVQVGLVGDAQVGKTSLMVKYVQNIYDKEYTQTLGVNFLKRKVSIRSTDI :  69
                                          4N V 6kVG66GDaq6GKTSLMVkYV2gs5De y QTLGVNF6e4t6SIRnTeI

       80         *       200         *       220         *       240         *       260
C.o. : TFSIWDLGGQREFVNMLPLVCNDAVAILFMFDLTRKSTLNSIKEWYRQGRGFNKTAIPILVGTKYDHFVNFPREDQEEISNQARRFAKA : 252
C.g. : TFSIWDLGGQREFVNMLPLVCNDAVAILFMFDLTRKSTLNSIKEWYRQGRGFNKTAIPILVGTKYDHFVNFPREDQEEISNQARRFAKA : 252
M.o. : TFSIWDLGGQREFVNMLPLVCNDAVAILFMFDLTRKSTLNSIKEWYRQGRGFNKTAIPILVGTKYDHFVNFPREDQEEISNQARRFAKA : 261
N.c. : TFSIWDLGGQREFVNMLPLVCNDAVAILFMFDLTRKSTLNSIKEWYRQGRGFNKTAIPVLVGTKYDHFVNLSREEQEEISNQARRFAKA : 232
A.n. : TFSIWDLGGQREFVNMLPLVCNDAVAILFMFDLTRKSTLNSIKEWYRQGRGFNKTAIPFLVGTKYDHFVNFPREDQEEISIQAKRFAKA : 264
S.p. : TFSIWDLGGQREFVNMLPMVCNDAVAILFMFDLSRKSTLNSIKEWYRQARGFNKTAVPILIGTKYDHFMTFPREDQEEITKQARRYAKA : 148
S.c. : IFSIMDLGGQREFINMLPIATVGSSVIIFLFDLTRPETLSSIKEWYRQAYGLNDSAIPILVGTKYDLLIDLDPEYQEQISRTSMKYAQV : 158
       tFSIwDLGGQREF6NMLP6vcndavaI6F6FDL3RksTLnSIKEWYRQ rGfNk3A6P L6GTKYDhf6   rE QE2I3 qa 45Aka

         *       280         *       300         *       320         *       340         *
C.o. : MRAALIFSSTSHSINVQKIFKIVLSKAFDLKCTIPEIENVGEPLLLYQSC------------------------------------- : 302
C.g. : MRAALIFSSTSHSINVQKIFKIVLSKAFDLKCTIPEIENVGEPLLLYQSC------------------------------------- : 302
M.o. : MRASLIFSSTSHSINVQKIFKIVLSKAFDLKCTIPEIENVGEPLLLYQSC------------------------------------- : 311
N.c. : MRAALIFSSTSHSINVQKIFKIVLSKAFDLKCTIPEISNVGEPLLLYQSV------------------------------------- : 282
A.n. : MKASLIFSSTSHSINVQKIFKIVLAKAFDLKCTIPEIENIGEPLLLYKNV------------------------------------- : 314
S.p. : MKASLVFCSTSHSINVQKIFKIVLAKVFDLKCTIPEIKNVGDPILEYIDR------------------------------------- : 198
S.c. : MNAPLIFCSTAKSINIQKIFKIALAKIFNLTLTIPEINEIGDPLLIYKHLGGQQHRHHNKSQDRKSHNIRKPSSSPSSKAPSPGVNT : 245
       M A L6F STshSIN6QKIFKIvL K F1LkcTIPEI n6G P6L Y
```

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

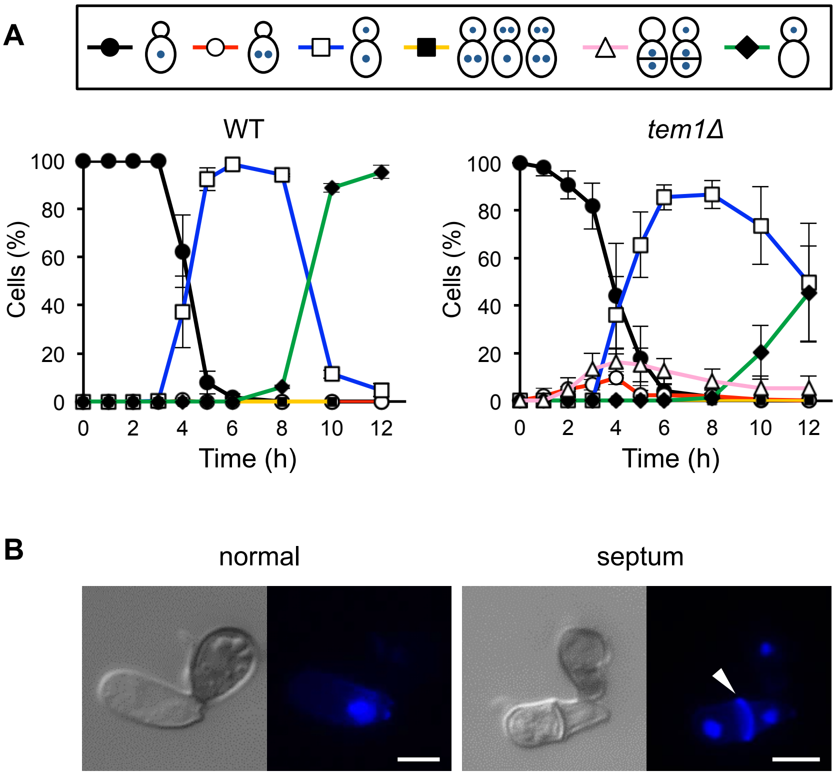
Co Tem1 homologs were identified in *Colletotrichum graminicola* (*C.g*), *Magnaporthe oryzae* (*M.o.*), *Neurospora crassa* (*N.c.*), *Aspergillus nidulans* (*A.n.*), *Schizosaccharomyces pombe* (*S.p.*), and *Saccharomyces cerevisiae* (*S.c.*). The alignment was generated using Clustal W and shaded 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 N-terminal 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.

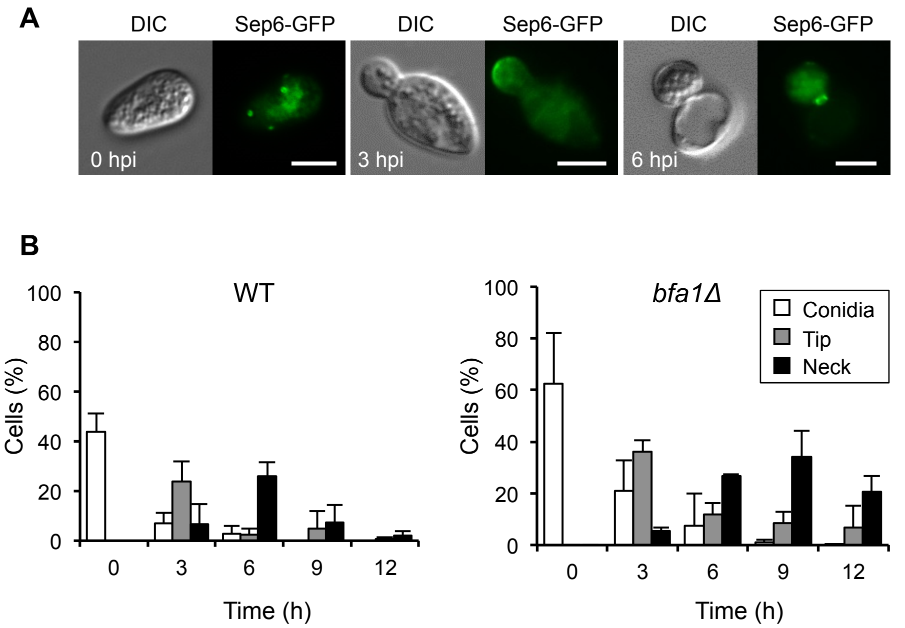
(B) Interaction of Co Bub2 with Co Bfa1 assayed by a yeast two-hybrid experiment. Co Bub2 proteins were expressed in fusion with GAL4BD (BD) protein, and their interactions with Co Bfa1 proteins fused with GAL4AD (AD) were tested. For the positive control, the interaction of *S. cerevisiae* Bub2p with 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-α-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 *tem1Δ* is impaired in septum formation but not in nuclear behavior during appressorium development.

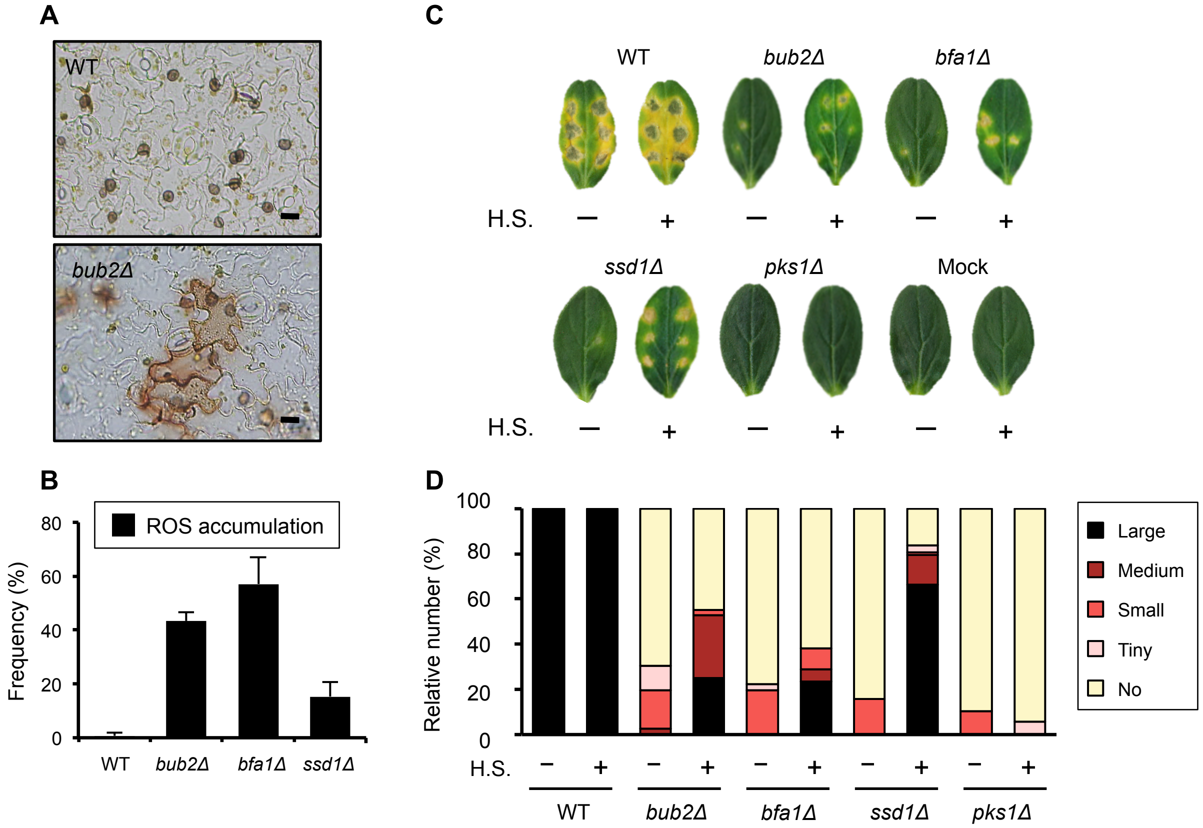
(A) Mean percentage (± SE, *n* = 3) of cells with various patterns of nuclear distribution in the wild type and Co *tem1Δ*. Scoring: one nucleus retained in the conidium (black), two nuclei retained in the conidium (red), one nucleus in the conidium and the other in the appressorium through mitosis (blue), 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 *tem1Δ* 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 μm.



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 μm.
(B) Mean percentage (± SE, *n* = 3) of cells with Sep6-GFP localization in the wild type and Co *bfa1Δ*. 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 *bub2Δ* and Co *bfa1Δ*.

(A) Representative images of reactive oxygen species (H_2O_2) accumulation at sites of attempted penetration by appressoria of the wild type and Co *bub2Δ*. Epidermis of cucumber cotyledons removed 3 days after inoculation with Co *bub2Δ*, 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 *bub2Δ*, Co *bfa1Δ*, and Co *ssd1Δ* at 3 dpi. Co *ssd1Δ* 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 *bub2Δ*, Co *bfa1Δ*, Co *ssd1Δ*, and Co *pks1Δ* 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 *ssd1Δ* was used as the positive control, and Co *pks1Δ* 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[a]	Vegetative growth (mm/3 days)[b]	Conidia ($\times 10^7$/colony)[c]
WT	18.67±0.82	3.32±0.44
bub2Δ	17.00±0.89	0.67±0.13**
bfa1Δ	12.50±6.12	0.41±0.11**
bub2Δ bfa1Δ + Sc *BUB2* Sc *BFA1*	16.67±0.52	1.00±0.35**
WT + Co Tem1^{T128N}	17.33±0.00	1.80±0.26
bub2Δ + Co Tem1^{T128N}	17.00±0.82	1.24±0.29
bfa1Δ + Co Tem1^{T128N}	17.67±0.52	1.32±0.3

[a] *C. orbiculare* wild type (WT), Co *bub2Δ* (*bub2Δ*), Co *bfa1Δ* (*bfa1Δ*), Co *bub2Δ* Co *bfa1Δ* expressing *S. cerevisiae BUB2* and *BFA1* (*bub2Δ bfa1Δ*+Sc *BUB2* Sc *BFA1*), and the Co Tem1^{T128N} introduced isoform (WT+Co Tem1^{T128N}, *bub2Δ*+Co Tem1^{T128N} ,and *bub2Δ*+Co Tem1^{T128N}).

[b] 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.

[c] 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 CoBub2Δ-1	Wild type/ENH83696Δ (pBIcobub2SH)	This study
104-T CoBub2Δ-2	Wild type/ENH83696Δ (pBIcobub2SH)	This study
104-T CoBfa1Δ-1	Wild type/ENH87866Δ (pBIcobfa1BH)	This study
104-T CoBfa1Δ-2	Wild type/ENH87866Δ (pBIcobfa1BH)	This study
104-T CoBub2ΔCoBfa1Δ-1	Wild type/ENH83696Δ/ENH87866Δ (pBIcobub2SH, pBIcobfa1BH)	This study
104-T CoBub2ΔCoBfa1Δ-2	Wild type/ENH83696Δ/ENH87866Δ (pBIcobub2SH, pBIcobfa1BH)	This study
104-T CoTem1Δ-1	Wild type/ENH82693Δ (pBIcotem1BH)	This study
104-T CoTem1Δ-2	Wild type/ENH82693Δ (pBIcotem1BH)	This study
104-T CoBub2Δ CoBub2Com	Wild type/ENH83696Δ/ENH83696 (pBICoBub2S)	This study
104-T CoBfa1Δ CoBfa1Com	Wild type/EENH87866Δ/ENH87866 (pBICoBfa1B)	This study
104-TLacOLacIGFP	wild type/K02913/TEF_CP011342_GFP (pCT31S, pBITEFGFPLacIB)	This study
104-TΔCoBub2LacOLacIGFP	wild type/ENH83696Δ/K02913/TEF_CP011342_GFP (pCT31S, pBITEFGFPLacIB)	This study
104-TΔCoBfa1LacOLacIGFP	wild type/ENH87866Δ/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 CoBub2Δ CoHIH1-GFP α-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-α-TUB1S)	This study
104-TCoTEM1GFP	Wild type/ENH82693_GFP (pBICoTEM1-GFPB)	This study
104-TΔCoBub2CoTEM1GFP	Wild type/ENH83696Δ/ENH82693_GFP(pBICoTEM1-GFPB)	This study
104-TΔCoBfa1CoTEM1GFP	Wild type/ENH87866Δ/ENH82693_GFP(pBICoTEM1-GFPB)	This study
104-TCoTEM1GFPα-TUB1mRFP	Wild type/ENH82693_GFP/SCD1_Q9C413_RFP (pBI-SCD1pmRFP1-α-TUB1S)	This study
104-TΔCoBub2CoTEM1GFPα-TUB1mRFP	Wild type/ENH83696Δ/ENH82693_GFP/SCD1_Q9C413_RFP(pBI-SCD1pmRFP1-α-TUB1S)	This study
104-T CoBub2Δ Bub2Com	Wild type/ENH87866Δ/ENH82693_GFP(pBICoTEM1-GFPB)	This study
104-T CoBfa1Δ Bfa1Com	Wild type/ENH87866Δ/NP_012587(pBI-Bfa1N)	This study
104-T CoTem1^{T128N}	Wild type/ENH82693_T128N (pBI-CoTem1_T128NSB)	This study
104-T CoBub2Δ CoTem1^{T128N}	Wild type/ENH83696Δ/ENH82693_T128N (pBI-CoTem1_T128NSB)	This study
104-T CoBfa1Δ CoTem1^{T128N}	Wild type/ENH87866Δ/ENH82693_T128N (pBI-CoTem1_T128NSB)	This study
104-T Cdc12GFP	Wild type/MGG07466_GFP (Cdc12_GFPS)	This study
104-T CoBfa1Δ Cdc12GFP	Wild type/ENH87866Δ/MGG07466_GFP (Cdc12_GFPS)	This study
104-T LifeactRFP	Wild type/Lifeact_RFP (Lifeact_RFP)	This study
104-T CoBub2Δ LifeactRFP	Wild type/ENH83696Δ/Lifeact_RFP (Lifeact_RFP)	This study
104-T CoBfa1Δ 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	Co *BUB2*	For cDNA sequencing
g8005_R5	TATCAAGGACGGCCCGTCAGACGGCGATGC		
g8005_R6	GAGGAACAGCGCAACAGGGACCGCCCTAGC		
g8005_R8	TCTGTCGTTCTGTTCGACAAGTG		
g3970_F1	TCTTTCGGGAAACGACC	Co *BFA1*	
g3970_F2	GGCAAGTAAGCGGAGGT		
g3970_F3	TAATAGAAGCTCGTCCGTC		
g3970_F4	CGGTGACCAGCATTCATA		
g3970_F5	GACAAAGCCTCACCGAAC		
g3970_R1	GTCGAAGCTGGGCGTATT		
g9031_F1	AGCCTCGAACTGAAACCTAC	Co *TEM1*	
g9031_F2	TGGACTATCTGGCAACG		
g9031_R1	GCCGAGTCGATCTGATAT		
g8005-F1A	GACGGTATTAGCATGGCCTCATTGTTTGGCAT	Co *BUB2* 5' UTR	For pBICoBub2SH and pBICoBub2S plasmids
g8005-R2D	ACGTCGACTACCGGCTCAGGACTGTAACATAT		
g8005-F2C	ACGTCGACTTGGGCGGATTGTAGTTTACGCAG	Co *BUB2* 3' UTR	
g8005-R1B	GCTTATCGTTATCCAGTTGGCGGATGTCGTTG		
g8005-hphR1C	CCGCCCAAGTCGACGTTAACTGGTTCCCGGTC	Hygromycin resistant gene	
g8005-hphF1D	AGCCGGTAGTCGACGTTAACTGATATTGAAGG		
g8005-pBIF1A	CCATGCTAATACCGTCGACCTAAATCTCGGTG	pBI-G4MRSrev Binary vector	
g8005-pBIR1B	CTGGATAACGATAAGCTTGATATCGAATTCCT		
g3970_F1A	TCTAGAATTCGAGTCGGAAGCATCATCCGCCG	Co *BFA1* 5' UTR	For pBICoBfa1BH and pBICoBfa1B plasmids
g3970_R2D	ACGTCGACTGCCCAGTAGCCTCACTCACGAAC		
g3970_F2C	ACGTCGACTTTTCCCGAAAGACTCCCGACGGC	Co *BFA1* 3' UTR	
g3970_R1B	GCTTATCGTTAGACCTCGTTCCCGCCCACCTG		
g3970_hphR1C	CGGGAAAAGTCGACGTTAACTGGTTCCCGGTC	Hygromycin resistant gene	
g3970_hphF1D	ACTGGGCAGTCGACGTTAACTGATATTGAAGG		
g3970_pBIF1A	CGACTCGAATTCTAGAAATACGACTCACTATA	pBI-G4MRBrev Binary vector	
g3970_pBIR1B	AGGTCTAACGATAAGCTTGATATCGAATTCCT		
g9031_F1A_2	TCTAGAATTTGGTACCTAGGTAAGGCACTATA	Co *TEM1* 5' UTR	For pBICoTem1BH plasmids
g9031_R2D_2	ACGTCGACTAGTGACGAGGACAGCTGGCAGTC		
g9031_F2C_2	ACGTCGACTTTTGTCTTTGCGACGCATGGCCC	Co *TEM1* 3' UTR	
g9031_R1B_2	GCTTATCGTCCTGCGATAGCAAGTCCTAGAAA		
g3970_hphR1C	CGGGAAAAGTCGACGTTAACTGGTTCCCGGTC	Hygromycin resistant gene	
g3970_hphF1D	ACTGGGCAGTCGACGTTAACTGATATTGAAGG		
g9031_pBIF1A_2	GGTACCAAATTCTAGAAATACGACTCACTATAGGGCGAAT	pBI-G4MRBrev Binary vector	
g9031_pBIR1B_2	TCGCAGGACGATAAGCTTGATATCGAATTCCTGCAGCCCG		
LacI_F1	GGGTGGTGGTGGTGGTatgGTGAAACCAGTAACGT	LacI-NLS	For pBITEFGFPLacIB plasmids
NLS_R1	TTAGGCAACCTTTCTCTTCTTCTTTGGTGGAGTAC		
glyGFPR1	ACCACCACCACCACCCTTGTACAGCTCGTC	pBITEFGFPB vector	
pBI_NLS_R1	GAGAAAGGTTGCCTAAGGTACCGGGCCCCCCCTCG		
glyGFPF1	GGAGGAGGAGGAGGAATGGTGAGCAAGGGC	gly GFP	For pBICoHIH1-GFPB plasmids
GFPR1	TTACTTGTACAGCTCGTCCATGCCGAGAGT		
g1862_F1A	TCTAGAATTTAGAACGACAGGCGGTAGGACCT	Co *HIH1* 5' UTR	
g1862_pBIR1B	ATGTTGAACGATAAGCTTGATATCGAATTCCT		
g1862_R1B	ACGTCGACTCGCTTGAAGTCGAGGAAAAGCCG	Co *HIH1* 3' UTR	
g1862_pBIF1A	CGTTCTAAATTCTAGAAATACGACTCACTATA		
glyGFPF1	GGAGGAGGAGGAGGAATGGTGAGCAAGGGC	glyGFP	For pBICoTEM1-GFPB plasmids
GFPR1	TTACTTGTACAGCTCGTCCATGCCGAGAGT		
CoTem1_pBIR5	TTCCTCCTCCTCCTCCGCAAGACTGGTACAGCAGC	pBICoTem1B plasmid	
CoTem1_pBIF5	CGAGCTGTACAAGTAATGATCCCGGCTCCATGTGC		

Supplemental Table 3. Primers used in this study.

Name	Sequence (5' - 3')	Notes 1	Note 2
bub2_F3	ACGCGCCAAGATGACCTCAATTGAAGATCTGATAT	Sc *BUB2 ORF*	For pBIBub2B plasmids
bub2_R3	GCCAACGCGGTTACGGTATATATATGTCTGGGTCC		
g8005_F11_Sc	TCTTCAATTGAGGTCATCTTGGCGCGTGTTGTACC	pBICoBUB2B plasmid	
g8005_R11_Sc	ATATATATACCGTAACCGCGTTGGCTCTGGAAAGA		
bfa1_F2	TACCAACGCCATGTCAATTAGGCCTCTCACGTTAA	Sc *BFA1 ORF*	For pBIBfa1N plasmids
bfa1_R2	TGAAGTGAGGCTAATCTTTTGTCGAATTGATTACC		
g3970_F7	CCTAATTGACATGGCGTTGGTAAGTTTTGTTTGGT	pBICoBFA1N plasmid	
g3970_R7	ACAAAAGATTAGCCTCACTCACGAACATCACATTT		
g9031_F1A_2	TCTAGAATTTGGTACCTAGGTAAGGCACTATA	Co Tem1^{T128N} 5'	For pBICoTem1^{T128N} SB plasmids
g9031_T128N_R1	ACTTGACCATCAGACTATTCTTGCCGATTTGCGCG		
g9031_R1B_2	GCTTATCGTCCTGCGATAGCAAGTCCTAGAAA	Co Tem1^{T128N} 3'	
g9031_T128N_F1	CGCGCAAATCGGCAAGAATAGTCTGATGGTCAAGT		
g9031_pBIF1A_2	GGTACCAAATTCTAGAAATACGACTCACTATAGGGCGAAT	pBIG4MRBrev Binary vector	
g9031_pBIR1B_2	TCGCAGGACGATAAGCTTGATATCGAATTCCTGCAGCCCG		
Sur_R1B	GTCGACGTGAGAGCATGCAATTCCCGTGCA	Sulfonylurea resistant gene	
Sur_F1A	GTCGACGTGCCAACGCCACAGTGCCCCACAT		
g9031_pm_pBIF4A	TGCTCTCACGTCGACCTGCGACTGGCCAAGTCTTG	pBICoTem1^{T128N}B plasmid	
g9031_pm_pBIR4B	CGTTGGCACGTCGACCGTTTTCAGTGCCACTAGTT		
Y_bub2_F1	CATGGAGGCCGAATTCATGCCGCCGAGTCCAACGA	Co *BUB2* 1-	For pGBKT7- and pGADT7- plasmids
Y_bub2_R1	GCAGGTCGACGGATCCCTACTGGGCATGCGCGACA	Co *BUB2* -472	
Y_bub2_R2	GCAGGTCGACGGATCCCTAGTTTGTGTATCGCGGG	Co *BUB2* -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 *BFA1* -1020	
Y_bfa1_R2	GCAGGTCGACGGATCCCTACGGCACGCCGGGGCTG	Co *BFA1* -783	
Y_bfa1_F2	CATGGAGGCCGAATTCCAATCCTCAATTCGGTCAA	Co *BFA1* 784-	
Y_bfa1_F3	CATGGAGGCCGAATTCATGTCAATTAGGCCTCTCA	Sc *BFA1* 1-	
Y_bfa1_R3	GCAGGTCGACGGATCCCTAATCTTTTGTCGAATTG	Sc *BFA1* -302	
Y_tem1_F1	CATGGAGGCCGAATTCATGGAGCAAGATCAGATCT	Co *TEM1* 1-	
Y_tem1_R1	GCAGGTCGACGGATCCTCAGCAAGACTGGTACAGC	Co *TEM1* -302	
Y_tem1_R2	GCAGGTCGACGGATCCTCAACCGTTACTCGCGGCG	Co *TEM1* -109	
Y_tem1_F2	CATGGAGGCCGAATTCCGCAACCACGTCGTCATCA	Co *TEM1* 110-	
Y_tem1_F6	CATGGAGGCCGAATTCATGGCTACACCAAGCACGG	Sc *TEM1* 1-	
Y_tem1_R6	GCAGGTCGACGGATCCTCATGTATTAACGCCCGGC	Sc *TEM1* -302	
Y_BUB2_AD_F1	GGAGGCCAGTGAATTCCCGCCGAGTCCAACGAGAG	Co *BUB2* 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 *BFA1* 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 *TEM1* 1-	
Y_tem1_AD_R1	TCATCTGCAGCTCGAGTCAGCAAGACTGGTACAGC	Co *TEM1* -302	
pGB_F1	GGATCCGTCGACCTGCAGCGGCCGCATAACTAGCA	pGBKT7 vector	
pGB_R1	GAATTCGGCCTCCATGGCCATATGCAGGTCCTCCT		
pGD_R1	GAATTCACTGGCCTCCATGGCCATATGAGCGTAAT	pGADT7 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	*MATα, 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	*MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, met*$^-$*, pGADT7-Bfa1*	This study
Y187: pGADT7-Co Bub2	*MATα, 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