Figure S1

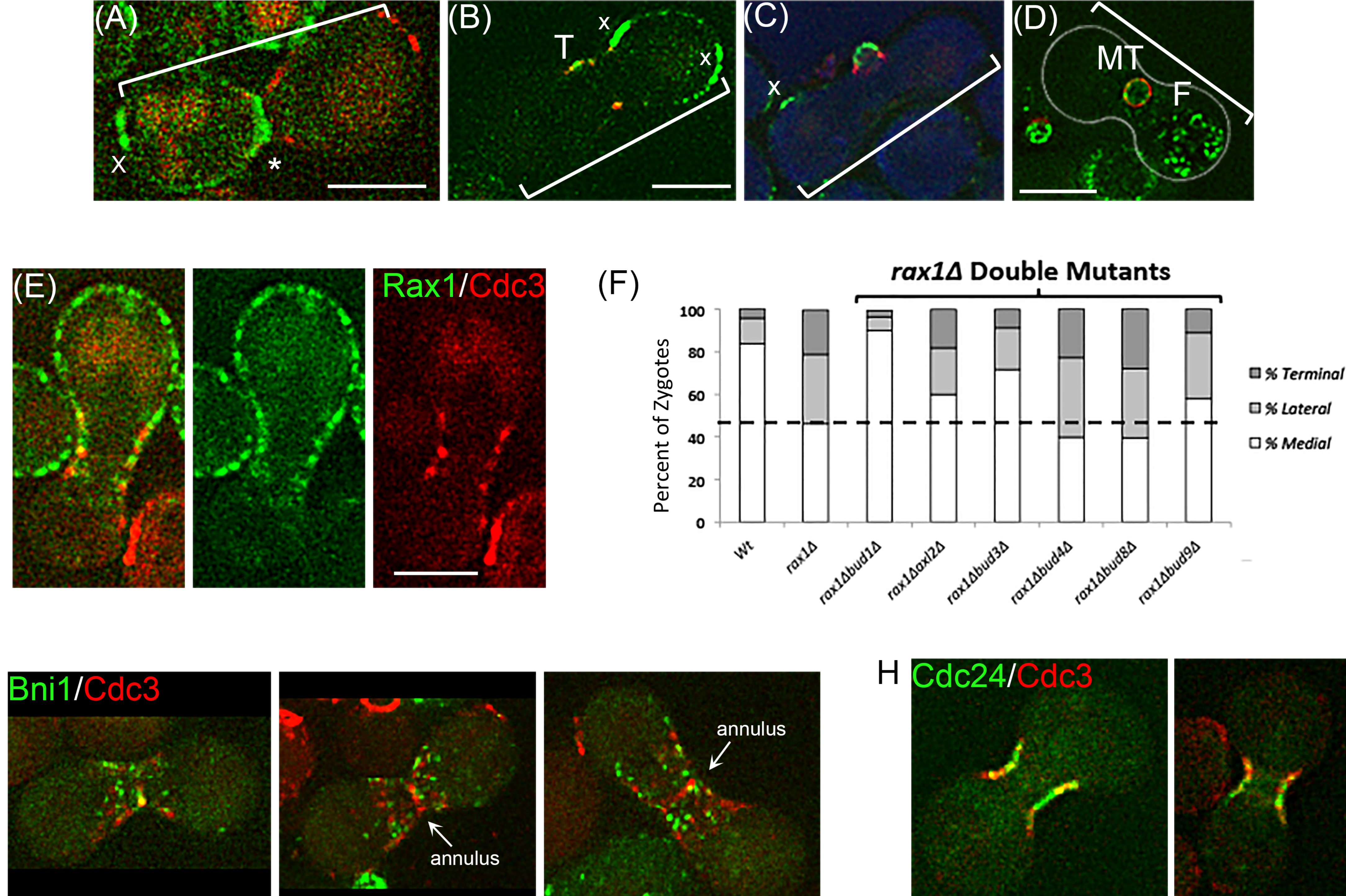


Figure S1. Rax1 and Other Polarity Specifying Proteins (related to Figure 1)

For A-E, a cell expressing functional GFP-Rax1 was crossed with a cell expressing the tagged septin, Cdc3-mCherry. The brackets designate the position of the zygotes.

A. Localization of GFP-Rax1 to the Zone of Contact (ZOC). Before cell fusion, GFP-Rax1 concentrates at the ZOC (*) and is also present as seemingly rough patches (x) which likely are sites of previous cytokinesis (Fujita et al., 2004).

B. **Localization just before triad formation.** Triad formation (T) is visible and two rough patches (x) persist.

C. **Localization to the triad**. With time, the triad (T) becomes more visible.

D. **Supramolecular structure at the cortex**. In face views, the rough patches often have a “floret”-like substructure (F). This same zygote is producing a medial bud (MT) which expresses GFP-Rax1 and is encircled by Cdc3-mCherry (constituting a triad). Strains: ATY4774 x ATY5562.

E. Limited diffusion of GFP-Rax1. The three images illustrate an intermediate time point equivalent to Figure 2E (“intermixed”). Note that GFP-Rax1 - that initially rims much of the upper parental cortex – is interspersed along with the Cdc3-mCherry throughout the ZMZ. GFP-Rax1 does not gain access to the cortex of the mating partner. In the pair of images on the right, the colors have been separated for clarity. Strains: ATY4774 x ATY5562.

F. Impact of deletion of landmarks on bud site specification in *rax1*Δ zygotes

A full panel of double mutants including the Rax1 deletion was generated, homotypic crosses were conducted, fixed and counted. Averages of the bud distributions (medial, lateral, terminal) from at least three independent experiments are plotted in each case. The dashed horizontal line indicates the control value for medial budding in *rax1*Δ zygotes as a point of reference. Quantitation is given in Table S2.

G/H. Distribution of cortical proteins in *rax1Δ* zygotes

Proteins that guide the orientation of actin concentrate at the ZOC and scatter through the ZMZ cortex upon cell fusion. Deletion of Rax1 results in an increase in non-medial budding. We therefore asked whether the characteristic scattering from the ZOC to the ZMZ is affected in *rax1Δ* zygotes. As shown in the representative images of *rax1Δ* zygotes expressing Bni1-GFP (G) or Cdc24-GFP (H), crosses with strains that express Cdc3-mCherry do not show obvious wide-spread delocalization of these proteins during the time interval after cell-cell fusion that precedes bud emergence. Nevertheless, well-formed triads do subsequently appear at non-medial sites, presumably following delayed relocation of their proteins. The images of Bni1 are grazing sections of the cortex. The images of Cdc24 are more nearly medial. The annulus that includes septins is indicated (A). Strains: ATY5105, ATY5563, ATY5562.

Figure S2

A

Landmarks

Axl1 Bud8 Bud9 Rax2

Fusion-related

Fus1 Prm1

Mating factor export/response

Ste6 Ste7 Ste20 Ste50

Tonicity sensor

Wsc2 Wsc3

Endo/Exocytosis

Sla2 Rvs161 Rvs167 Sec3

Pkc1-related

Ack1 Kel1 Kel2 Lrg1 Msb1 Mpk1 Pkc1

Glycerol Transport

Fps1

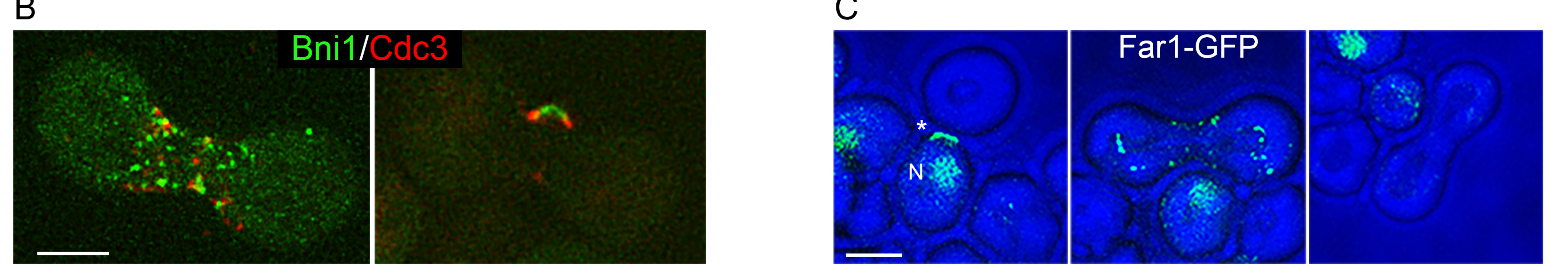


Figure S2. Organization of the Zone of Contact (Related to Figure 2)

A. Composition of the ZOC. In each case, crosses were examined after 4 hr to image prezygotes. The mating partner was either unlabeled (for Axl1, Bud8, Bud9 – brightfield images in blue), or expressed Cdc3-mCherry (for Rax2), or expressed cytoplasmic soluble dsRed (all other examples). In each case, the GFP-tagged protein accumulates at the ZOC (*). In some cases the signal forms a spot or a broad cap. The other examples form a line. Note that the tagged glycerol transporter, Fps1, like Pma1, is absent from the ZOC.

The following strains were crossed with ATY4373, ATY5545 (Cdc3-mCherry) or ATY4971 (Cytoplasmic dsRed): The comments in parenthesis describe the localization of each protein in cycling haploid cells. Axl1-GFP [ATY5252] (cytokinetic rings), Bud8-GFP [ATY5253] (occasional dots at the cortex), Bud9-GFP [ATY5255] (bud tip/cortex), Rax2-GFP [ATY4982] (cortex, bud neck), Fus1-GFP [ATY5160] (not visible), Prm1-GFP [ATY5144] (occasional spots at the cortex), Ste6-GFP [ATY5392] (spots in the vacuole), Ste7-GFP [ATY5381] (faint throughout), Ste20-GFP [ATY5379] (bud cortex), Ste50-GFP [ATY5380] (faint throughout), Wsc2-GFP [ATY5145] (cortical spots), Wsc3-GFP [ATY5146] (cortical spots), Rvs161-GFP [ATY5150] (bud cortex spots), Rvs167-GFP [ATY5151] (cortical spots), Sec3-GFP [ATY5873] (bud cortex, bud neck), Ack1-GFP [ATY5301] (bud cortex, bud neck), Kel1-GFP [ATY5340] (bud cortex, bud neck), Kel2-GFP [ATY5341] (bud cortex, bud neck), Lrg1-GFP [ATY5307] (bud cortex, cytokinetic rings), Msb1-GFP [ATY5338] (bud cortex, bud neck), Mpk1-GFP [ATY5345] (faint nuclear signal), Pkc1-GFP [ATY5149] (bud neck, sites of bud emergence), Fps1-GFP [ATY5299] (cortical spots).

In parallel experiments with the Invitrogen collection of GFP-tagged strains, we noted that Agp2, Bap2, Bgl2, Can1, Chs3, Dip5, Exg1, Ftr1, Fui1, Lyp1, Mup1 are uniformly present around the cortex in prezygotes. Abp1, Aga2, Axl2, Bud3, Bud4, Cch1, Cmp2,

Cna1, Cnb1, Fig1, Fus2, Rom2, Sho1, Vps1 and Wsc1 concentrate at the ZOC. The cortical proteins, Lsp1, Mep1, Mep3, Mss4, Nha1, Pdr12, Pil1, Sln1, Snq2, Trk1, Trk2, Vht1 and Yor1 avoid the ZOC.

B. Progressive clearing of ZOC proteins during bud emergence

Crosses were conducted between cells expressing Bni1-GFP and a cell expressing Cdc3-mCherry. After 4 hrs, the mating mixture was examined. Note in the images at the left the scattering and intermixing of foci of both green and red. With time (image at the right), little or no scattered signal remains and a characteristic triad appears. Strains: ATY5176/4312 x ATY5545.

C. Transience of Far1-GFP

A cross was conducted between a strain expressing Far1-GFP and an unlabeled strain. Note that the Far1-GFP signal at the ZOC (*, left) vanishes after cell fusion (middle and right images), prior to bud emergence. N: nucleus. Strains: ATY4979 x ATY4373.

Figure S3

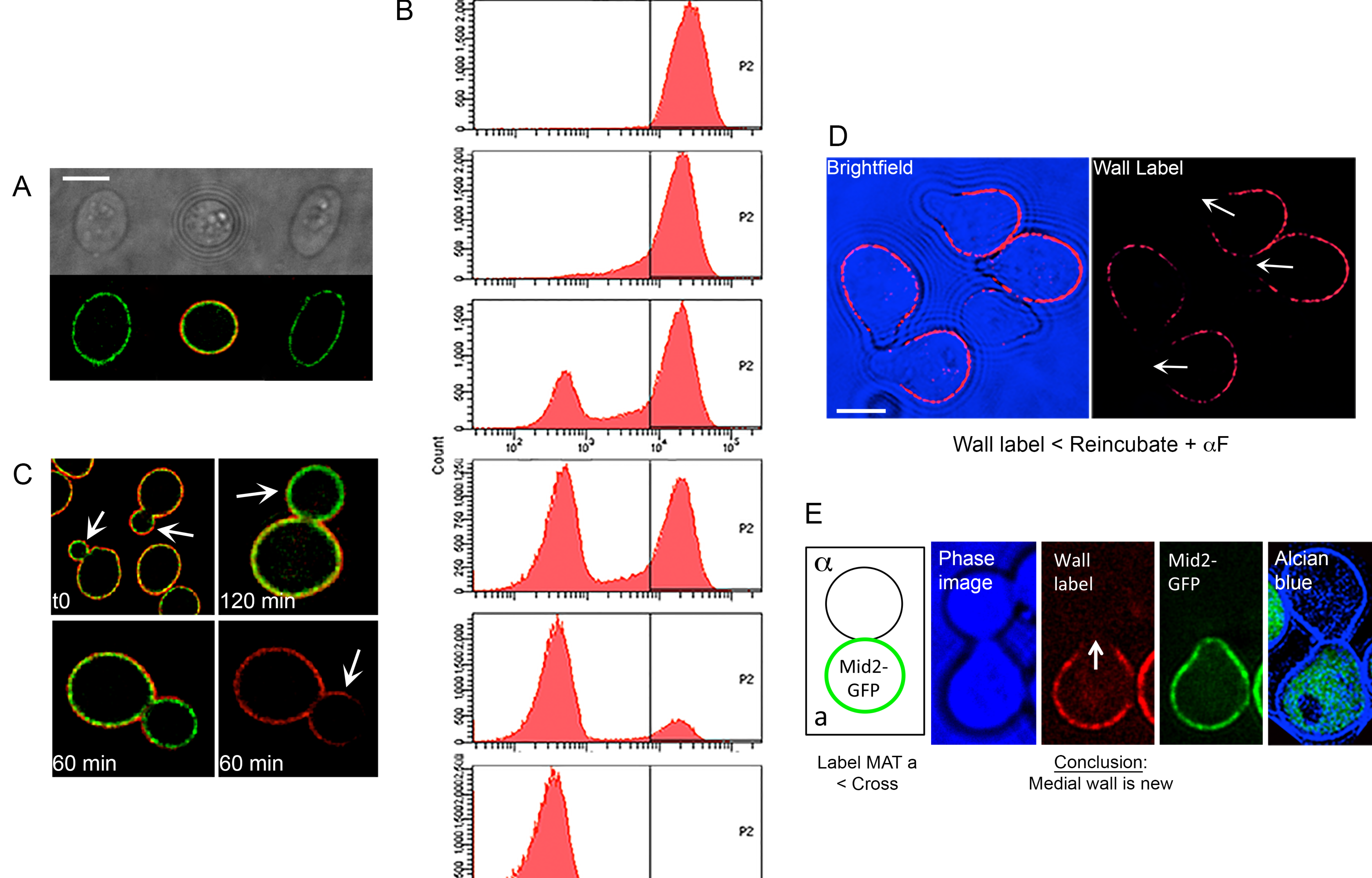


Figure S3. Site of Addition of New Cell Wall (Related to Figure 3)

A. The cell wall of cells expressing Mid2-GFP [ATY1469] was labeled with red hydrazide (see below) and then partially spheroplasted. Top row: brightfield images. Bottom row: fluorescent images. Spheroplasted cells (right, left) lack refractility and show no cell wall signal, although Mid2-GFP is still visible. The refractile cell in the middle that has not been spheroplasted shows both red and green signals.

B. As judged by flow cytometry, the labeled population loses a fraction of its fluorescence intensity initially (probably due to release of partially-labeled buds), but the intensity of labeled cells then remains stable for many hours. Top-to-bottom: cells reincubated for 0, 2, 4, 6, 12 or 20 hrs. Samples of the labeled cells were also cultured in parallel with unlabeled cells. Their rates of growth were comparable to each other.

C. The Mid2-GFP-expressing cells [ATY1469] at the upper left (t0) were labeled immediately before imaging and therefore both the mother and bud are red (arrows). The lower panels illustrate cells after reincubation for 60 min. Note that the extremity of the bud is not labeled, as expected if its wall was produced during the reincubation. The arrow marks the transition from the labeled to unlabeled regions. Upper right: after 120 min of reincubation, the mother cell is still uniformly labeled (arrow) but the bud is not labeled. It therefore came into existence after the labeling interval.

D. A MAT a cell [ATY3852] was labeled and then exposed to mating factor for 3 hr. Left: brightfield. Right: fluorescent image. Note that the ends of the mating projection are no longer labeled (arrows). Thus, its cell wall is newly-synthesized.

E. MAT a cells expressing Mid2-GFP [ATY1469] were labeled and then mixed with MAT a cells expressing Mid2-GFP [ATY3852]. In the illustrated prezygote, note that the ZOC, although green, is not red (arrow). Thus the cell wall overlying the ZOC is newly-

synthesized. The rightmost panel shows an equivalent preparation in which the wall is stained by including 0.5% Alcian blue in the agarose pad. The image proves that wall material is present at the ZOC. One of the partners expressed cytoplasmic GFP, thereby proving that cell-cell fusion had not occurred.

Labeling Method: Cultures of rapidly-growing cells were chilled on ice for 15 minutes, washed in PBS, and oxidized with 0.5 mM sodium m-periodate in PBS for 15 minutes on ice. After two washes, they were incubated on ice for 30 minutes with 0.5 mg/ml Texas Red- or fluorescein-hydrazide (Invitrogen) [or 13 mM CF488A Aminooxy (Biotium)] in PBS. Cells were then washed twice and returned to growth medium.

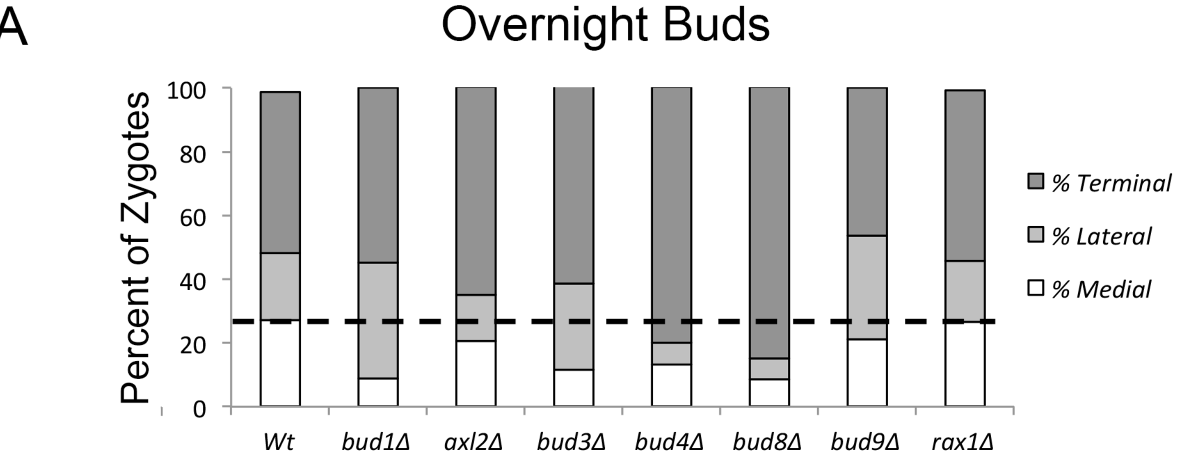
Comment: Earlier studies have not examined the timing of cell wall synthesis in prezygotes; however, similar approaches have been used to localize new cell wall in cycling cells and in cells exposed to isotropic mating factor. These studies conclude that new cell wall is added at the bud tip and at the tip of the mating projection (Chung et al., 1965; Schekman and Brawley, 1979; Tkacz and Lampen, 1972).

References:

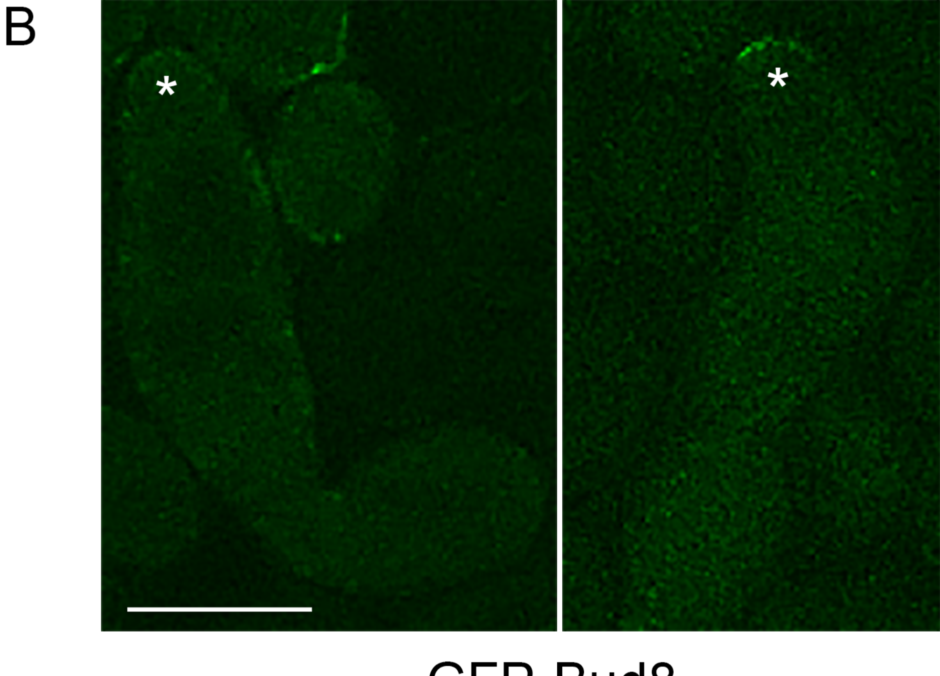
Chung, K.L., Hawirko, R.Z., and Isaac, P.K. (1965). Cell wall replication in Saccharomyces cerevisiae. Canadian journal of microbiology *11*, 953-957.
Fujita, A., Lord, M., Hiroko, T., Hiroko, F., Chen, T., Oka, C., Misumi, Y., and Chant, J. (2004). Rax1, a protein required for the establishment of the bipolar budding pattern in yeast. Gene *327*, 161-169.
Schekman, R., and Brawley, V. (1979). Localized deposition of chitin on the yeast cell surface in response to mating pheromone. Proc Natl Acad Sci U S A *76*, 645-649.
Tkacz, J.S., and Lampen, J.O. (1972). Wall replication in saccharomyces species: use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. J Gen Microbiol *72*, 243-247.

Figure S4

A



B



GFP-Bud8

Figure S4. Late Bud Emergence (Related to Figure 4)

A. Late bud site specification in deletion strains. In each case, corresponding deletion strains were crossed for 5 hr and then reincubated for 15 hr under conditions that do not allow further zygote formation. Note that budding has become extensively non-medial, regardless of whether Bud1 and landmarks are present. See Table S2 for quantitation. Strains: See legend of Figure 3G.

B. Distribution of Bud8 in late zygotes.

Two strains that express GFP-Bud8 were crossed for 5 hr and then reincubated for 15 hr. Although a cortical signal can be detected in haploid and diploid cells, the only signal in these late zygotes is very faint. In the example on the left, the signal is broadly distributed at the cortex, with some concentration at the end (*). In the example on the right, the restriction to the end of the zygote is more evident (*). Strains: ATY4308 x ATY4279.

Table S1. Distribution of Landmark Proteins

	Present in Haploid (H), Diploid (D) Cells ?	References	Distribution in Zygotes				
			Early*				Late**
			Cortical ring	ZOC	Bud tip/ cortex	2 rings at neck	2 rings at neck
Haploid Landmarks							
Axl1	H	(1)	-	+	-	-	-
Axl2	H, D	(2-4)	+	-	+	+	+
Bud3	H, D	(5)	+	-	-	+	+
Bud4	H, D	(6)	+	-	-	+	+
Diploid Landmarks							
Bud8	H, D	(7, 8)	-	+	+	-	-
Bud9	H, D	(7, 8)	-	+	+	-	-
Rax1	H, D	(8, 9)	Widespread along the cortex, ZOC				
Rax2	H, D	(8, 10)	Widespread along the cortex, ZOC				

* In each case, twenty zygotes from a 5 hr mating mixture were photographed at random. ** Equivalent observations on zygotes that were recultured for 15 hr. The distributions indicated are representative of the majority. The cortical ring is likely a remnant from haploid budding history. When two rings are at the neck, one becomes associated with the mother and one with the bud. Each of the following MAT a strains expressing a GFP-tagged landmark protein was studied after a cross with ATY4373: Axl1 (ATY5252), Axl2 (ATY5251), Bud3 (ATY5194), Bud4 (ATY5195), Bud8 (ATY5253), Bud9 (ATY5255), Rax1 (ATY5493), Rax2 (ATY4982).

Table S2.	Quantitation of Bud Site Distributions (after fixation)				
Cross	**Genotype**	**% Medial**	**% Lateral**	**% Terminal**	**Strains**
			Initial Buds		
1	Wt (S288C - BY4741)	83.7 +/- 4.3	12.1 +/- 2.1	4.2 +/- 2.2	ATY3852 x ATY4373
2	Wt (W303)	91.4 +/- 1.0	4.8 +/- 0.9	3.8 +/- 1.6	ATY2109 x ATY2110
3	*axl2Δ*	78.0 +/- 13.3	12.0 +/- 9.5	9.7 +/- 6.8	ATY5619 x ATY5620
4	*bud1Δ*	97.3 +/- 2.5	1.7 +/- 1.9	0.6 +/- 0.7	ATY4227 x ATY4256
5	*bud3Δ*	94.2 +/- 3.7	4.7 +/- 3.3	1.0 +/- 0.5	ATY4813 x ATY4851
6	*bud4Δ*	83.8 +/- 7.7	14.0 +/- 5.7	2.2 +/- 2.0	ATY4191 x ATY4275
7	*bud8Δ*	81.1 +/- 3.9	12.2 +/- 4.2	6.7 +/- 3.6	ATY4815 x ATY6283
8	*bud9Δ*	87.2 +/- 6.5	12.3 +/- 4.3	4.7 +/- 3.2	ATY4814 x ATY4832
9	*rax1Δ*	46.1 +/- 4.4	32.6 +/- 3.3	20.8 +/- 6.7	ATY4297 x ATY4304
10	*sst2Δ*	96.4 +/- 1.8	2.9 +/- 1.5	0.7 +/- 0.8	ATY4997 x ATY5125
11	*far1-H7*	85.6 +/- 6.5	9.5 +/- 4.4	4.9 +/- 2.6	ATY5269 x ATY5279
12	Wt (W303) x *kar1Δ15*	91.2 +/- 4.5	3.0 +/- 2.1	5.5 +/- 3.7	ATY4503 x ATY4263
13	*cdc12-6* (22-25°C)*	62.3 +/- 7.4	19.1 +/- 5.9	14.1 +/- 9.7	ATY2089 x ATY4028
			Initial buds in *rax1Δ* Double Mutants		
14	*rax1Δ axl2Δ*	59.9 +/- 9.2	21.8 +/- 4.9	18.2 +/- 5.0	ATY5931 x ATY5932
15	*rax1Δ bud1Δ*	89.9 +/- 2.2	6.5 +/- 0.5	3.0 +/- 1.6	ATY5037 x ATY5038
16	*rax1Δ bud3Δ*	71.5 +/- 6.5	19.7 +/- 4.3	8.7 +/- 4.0	ATY5879 x ATY5880
17	*rax1Δ bud4Δ*	39.9 +/- 7.7	37.2 +/- 1.9	22.9 +/- 6.6	ATY6019 x ATY6016
18	*rax1Δ bud8Δ*	39.6 +/- 7.0	32.6 +/- 6.6	27.9 +/- 5.9	ATY6017 x ATY6018
19	*rax1Δ bud9Δ*	57.8 +/- 4.4	31.1 +/- 5.0	11.1 +/- 2.7	ATY5928 x ATY5929
			Overnight Buds (+ 15 hr)		
20	Wt	27.2 +/- 8.6	20.9 +/- 13.1	50.7 +/- 13.1	ATY4303 x ATY4307
21	*fob1Δ*	14.5 +/- 8.2	16.0 +/- 4.3	69.5 +/- 8.7	ATY5887 x ATY5894

* Temperatures above 26°C could not be used for these crosses due to the dramatic increase in complex zygote forms (multiple buds, lack of cytokinesis, etc.).

Table S3. Contiguity of Medial Bud Scars; Relative Proportion of Lateral *vs* Terminal Scars
(5 + 15 hr protocols, stained with Calcofluor White)

Relevant Genotype	medial	twin	necklace	lanyard	Contiguity Index *	Terminal/Lateral *	Strain Numbers
	Average Percent of Zygotes						
wt x wt	39	33	13	0	0.53+/-0.08 (138)	1.16+/-0.16 (457)	ATY4307, ATY4303
bud1Δ x *bud1Δ*	95	0	0	0	0 (269)	0.77+/-0.07 (899)	ATY4602, ATY4907
axl2Δ x *axl2Δ*	93	0	0	0	0 (78)	2.6+/-0.86 (252)**	ATY5881, ATY5860
bud3Δ x *bud3Δ*	92	0	0	0	0 (61)	1.9+/-0.1 (195)	ATY5882, ATY5861
bud4Δ x *bud4Δ*	97	0	0	0	0 (58)	1.6+/-0.14 (302)	ATY5883, ATY5862
bud8Δ x *bud8Δ*	21	41	27	9	0.77+/-0.02 (63)	0.75+/-0.08 (397)	ATY5884, ATY6284
bud9Δ x *bud9Δ*	31	33	20	16	0.63+/-0.02 (150)	0.47+/-0.08 (882)	ATY5885, ATY5864
rax1Δ x *rax1Δ*	31	38	15	0	0.61+/-0.12 (107)	1.22+/-0.16 (528)	ATY5052, ATY5477
wt x GAL-Axl1, gal	13	5	29	55	0.91+/-0.05 (138)	NA	ATY3328, ATY6125
wt [pbud1^{dn}] x GAL-Axl1 [pbud1^{dn}], gal	98	0	0	0	0 (263)	NA	ATY5056, ATY6179

The contiguity index is the number of zygotes in which medial scars show contiguity divided by that number plus the number with a solitary medial bud. The terminal/lateral tabulation is the number of scars at the terminae divided by the number of lateral scars.
* The entries in parentheses indicate the total number of zygotes studied (for the contiguity index) or total number of scars counted (for the terminal/lateral tabulation).
** Many *axl2Δ* zygotes have bizarre shapes, making it difficult to classify their buds.

Table S4. Strain List

Strain	Relevant Genotype	Parent	Plasmid	Cell Source
Reference Strains				
ATY3852	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	S288C		Invitrogen, BY4741
ATY4373	MATα *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	S288C		ATY3852
ATY2111	MATa *leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 RAD5+*	W303		K. Runge
ATY2112	MATα *leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 RAD5+*	W303		K. Runge
Bud1 Deletion and Landmark Deletion Strains				
ATY5619	MATa *axl2::KanMX6*	S288C		Invitrogen
ATY5620	MATα *axl2::KanMX6*	S288C		ATY5619
ATY5881	MATa *axl2::KanMX6*	S288C	pRS316	ATY5620
ATY5860	MATα *axl2::KanMX6*	S288C	pRS315	ATY5619
ATY4227	MATa *bud1::KanMX6*	S288C		Invitrogen
ATY4256	MATα *bud1::KanMX6*	S288C		ATY4227
ATY6213	MATa *bud1::KanMX6*	S288C	pRS316	ATY4227
ATY4907	MATα *bud1::KanMX6*	S288C	pRS315	ATY4256
ATY4813	MATa *bud3::KanMX6*	S288C		Invitrogen
ATY4851	MATα *bud3::KanMX6*	S288C		ATY4813
ATY5882	MATa *bud3::KanMX6*	S288C	pRS316	ATY4813
ATY5861	MATα *bud3::KanMX6*	S288C	pRS315	ATY4851
ATY4191	MATa *bud4::KanMX6*	S288C		Invitrogen
ATY4275	MATα *bud4::KanMX6*	S288C		ATY4275
ATY5883	MATa *bud4::KanMX6*	S288C	pRS316	ATY4191
ATY5862	MATα *bud4::KanMX6*	S288C	pRS315	ATY4275
ATY4815	MATa *bud8::KanMX6*	S288C		Invitrogen
ATY6283	MATα *bud8::KanMX6*	S288C		ATY4827
ATY5884	MATa *bud8::KanMX6*	S288C	pRS316	ATY4815
ATY6284	MATα *bud8::KanMX6*	S288C	pRS315	ATY4827
ATY4814	MATa *bud9::KanMX6*	S288C		Invitrogen
ATY4832	MATα *bud9::KanMX6*	S288C		ATY4814
ATY5885	MATa *bud9::KanMX6*	S288C	pRS316	ATY4814
ATY5864	MATα *bud9::KanMX6*	S288C	pRS315	ATY4832
ATY5501	MATa *rax1::KanMX6*	S288C		Invitrogen
ATY5500	MATα *rax1::KanMX6*	S288C		ATY5501
ATY5052	MATa *rax1::KanMX6*	S288C	pRS316	ATY4814
ATY5477	MATα *rax1::KanMX6*	S288C	pRS315	ATY5501
Rax1 Double Deletion Strains				
ATY5544	MATa Cdc3-mCherry *rax1::KanMX6*	S288C	pAT1368	This study
ATY5562	MATα Cdc3-mCherry *rax1::KanMX6*	S288C	pAT1368	This study
ATY5931	MATa *axl2::KanMX6 rax1::HPHNT1*	S288C		This study
ATY5930	MATα *axl2::KanMX6 rax1::HPHNT1*	S288C		This study
ATY5037	MATa *bud1::KanMX6 rax1::KanMX6*	S288C		ATY4297, ATY4227
ATY5038	MATα *bud1::KanMX6 rax1::KanMX6*	S288C		ATY4297, ATY4227
ATY5880	MATa *bud3::KanMX6 rax1::HPHNT1*	S288C		This study
ATY5879	MATα *bud3::KanMX6 rax1::HPHNT1*	S288C		This study
ATY6019	MATa *bud4::KanMX6 rax1::HPHNT1*	S288C		This study
ATY6016	MATα *bud4::KanMX6 rax1::HPHNT1*	S288C		This study
ATY6017	MATa *bud8::KanMX6 rax1::HPHNT1*	S288C		This study
ATY6018	MATα *bud8::KanMX6 rax1::HPHNT1*	S288C		This study
ATY5929	MATa *bud9::KanMX6 rax1::HPHNT1*	S288C		This study
ATY5928	MATα *bud9::KanMX6 rax1::HPHNT1*	S288C		This study
Other Deletion Strains, Mutants, Strains with Fluorescent Markers, etc.				
ATY4307	MATa ATY3852	S288C	pRS316	ATY3852
ATY4303	MATα ATY4373	S288C	pRS315	ATY4373
ATY2089	MATa *cdc12-6*	S288C		E. Bi, #743
ATY4028	MATα *cdc12-6*	S288C		ATY2089

ATY5194	MATa Bud3-GFP	S288C		Invitrogen
ATY5195	MATa Bud4-GFP	S288C		Invitrogen
ATY4312	MATa Sec5-GFP	S288C		W. Guo, GY1143
ATY4774	MATα GFP-Rax1	S288C		pHP1109, H-O Park
ATY5124	MATa *far1-H7*	SEY6210		RAY1423, R. Arkovitz
ATY5279	MATα *far1-H7*	SEY6210		ATY5124
ATY5297	MATa Pma1-GFP	S288C		Invitrogen
ATY5637	MATa Cdc3-mCherry	S288C	pAT1368	ATY3852
ATY5545	MATα Cdc3-mCherry	S288C	pAT1368	ATY4373
ATY5887	MATa *fob1::KanMX6*	S288C	pRS316	Invitrogen
ATY5926	MATα *fob1::KanMX6*	S288C	pRS315	ATY5887
ATY5896	MATa Cdc3-mCherry Pma1-GFP	S288C	pAT1368	ATY5297
ATY4263	MATα *kar1Δ15*	W303		R. Rothstein W2108-14C
ATY5176	MATa Bni1-GFP	S288C		Invitrogen
ATY5563	MATα Bni1-3GFP *rax1Δ::KanMX6*	S288C		This study
ATY5001	MATa Cdc24-GFP	S288C		Invitrogen
ATY5105	Cdc24-GFP *rax1Δ::KanMX6*	S288C		This study
ATY1469	MATα Mid2-GFP	SEY6210		S. Emr
ATY4982	MATa Rax2-GFP	S288C		Invitrogen
ATY4308	MATa	S288C	pAT1301	This study
ATY4279	MATα	S288C	pAT1301	This study
ATY5253	MATa Bud8-GFP	S288C		Invitrogen
ATY5255	MATa Bud9-GFP	S288C		Invitrogen
ATY5493	MATa GFP-Rax1	S288C		This study
ATY4982	MATa Rax2-GFP	S288C		Invitrogen
ATY4971	MATα Cyt-dsRed	S288C	pAT1145	ATY4373
ATY5252	MATa Axl1-GFP	S288C		Invitrogen
ATY5251	MATa Axl2-GFP	S288C		Invitrogen
ATY5160	MATa Fus1-GFP	S288C		Invitrogen
ATY5144	MATa Prm1-GFP	S288C		Invitrogen
ATY5392	MATa Ste6-GFP	S288C		Invitrogen
ATY5381	MATa Ste7-GFP	S288C		Invitrogen
ATY5379	MATa Ste20-GFP	S288C		Invitrogen
ATY5380	MATa Ste50-GFP	S288C		Invitrogen
ATY5145	MATa Wsc2-GFP	S288C		Invitrogen
ATY5146	MATa Wsc3-GFP	S288C		Invitrogen
ATY5150	MATa Rvs161-GFP	S288C		Invitrogen
ATY5151	MATa Rvs167-GFP	S288C		Invitrogen
ATY5873	MATa Sec3-GFP	S288C		Invitrogen
ATY5301	MATa Ack1-GFP	S288C		Invitrogen
ATY5340	MATa Kel1-GFP	S288C		Invitrogen
ATY5341	MATa Kel2-GFP	S288C		Invitrogen
ATY5307	MATa Lrg1-GFP	S288C		Invitrogen
ATY5338	MATa Msb1-GFP	S288C		Invitrogen
ATY5345	MATa Mpk1-GFP	S288C		Invitrogen
ATY5149	MATa Pkc1-GFP	S288C		Invitrogen
ATY5299	MATa Fps1-GFP	S288C		Invitrogen
ATY4979	MATa Far1-GFP	S288C		Invitrogen
ATY1513	MATα Sik1-mRFP	S288C		W-K Huh
ATY4066	MATa Sik1-mRFP [pNup49-GFP]	S288C	pAT635	ATY1513
ATY3328	MATa YPH499	YPH		E. Cabib
ATY6125	MATα GAL-Axl1-GST Bud4-13Myc	YPH		M. Lord, ML627
ATY6179	MATα GAL-Axl1-GST Bud4-13Myc	YPH	p1352	ATY6125
ATY5056	MATa	YPH	p1352	ATY3328

Table S5. Plasmid List

Name	Expression Unit	Type	Source
pAT161	GAL-HO	URA3/CEN	I. Herskowitz
pAT635	pNup49-GFP	LEU2/CEN	V. Doye
pAT1045	pFA6a-hphNT1	N.A.	Euroscarf
pAT1125	GFP-Cdc3	URA3/CEN	E. Bi
pAT1301	GFP-Bud8	URA3/2-micron pRS426	H-U Moesch, pME1772
pAT1145	Cyt-dsRed	URA3/YiP	E. Grote, pEG223
pAT1303	GFP-Bud9	URA3/2-micron pRS426	H-U Moesch, pME1777
pAT1352	*bud1* (arg16)	YCp50	H-U Moesch/A. Bender
pAT1368	CDC3-mCherry	Yip128/LEU2	E1914, E. Bi
pAT1380	Bni1-3GFP	Yip/LEU2	PB1993, D. Pellman