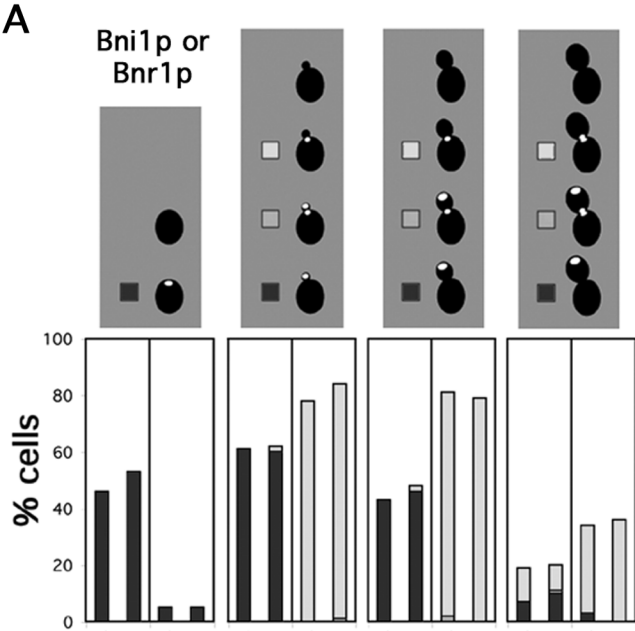
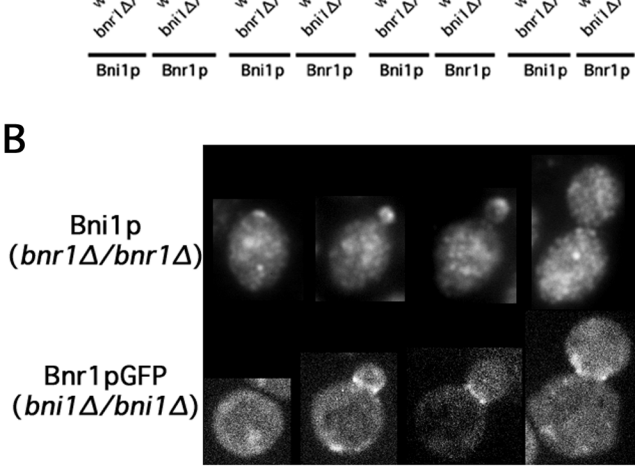
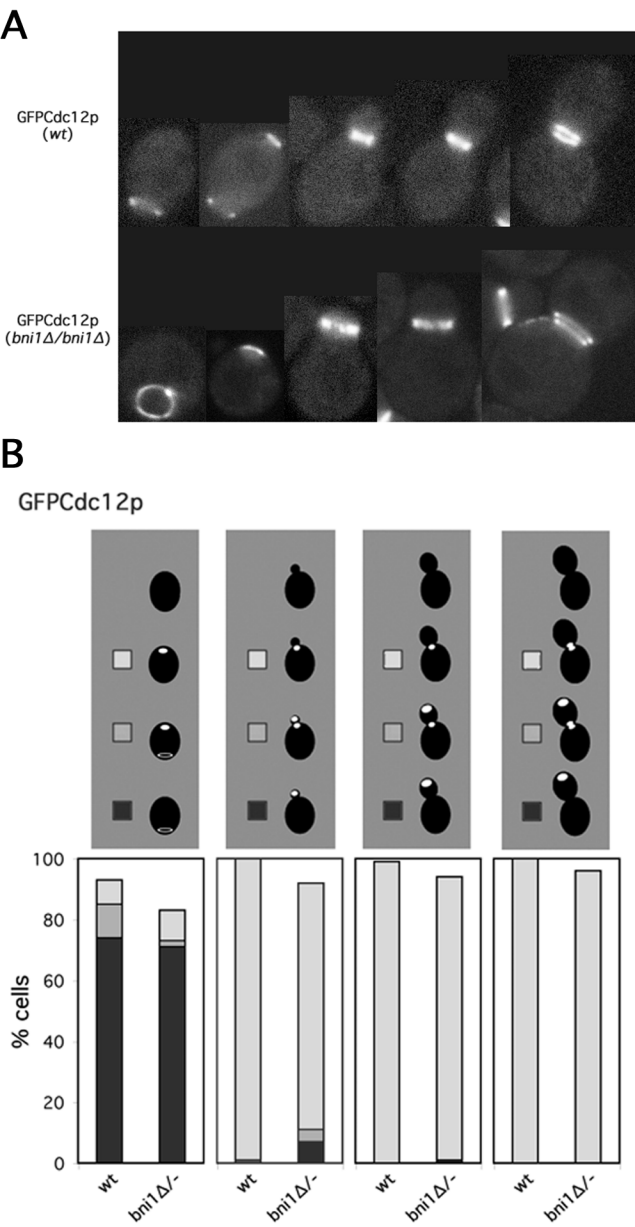
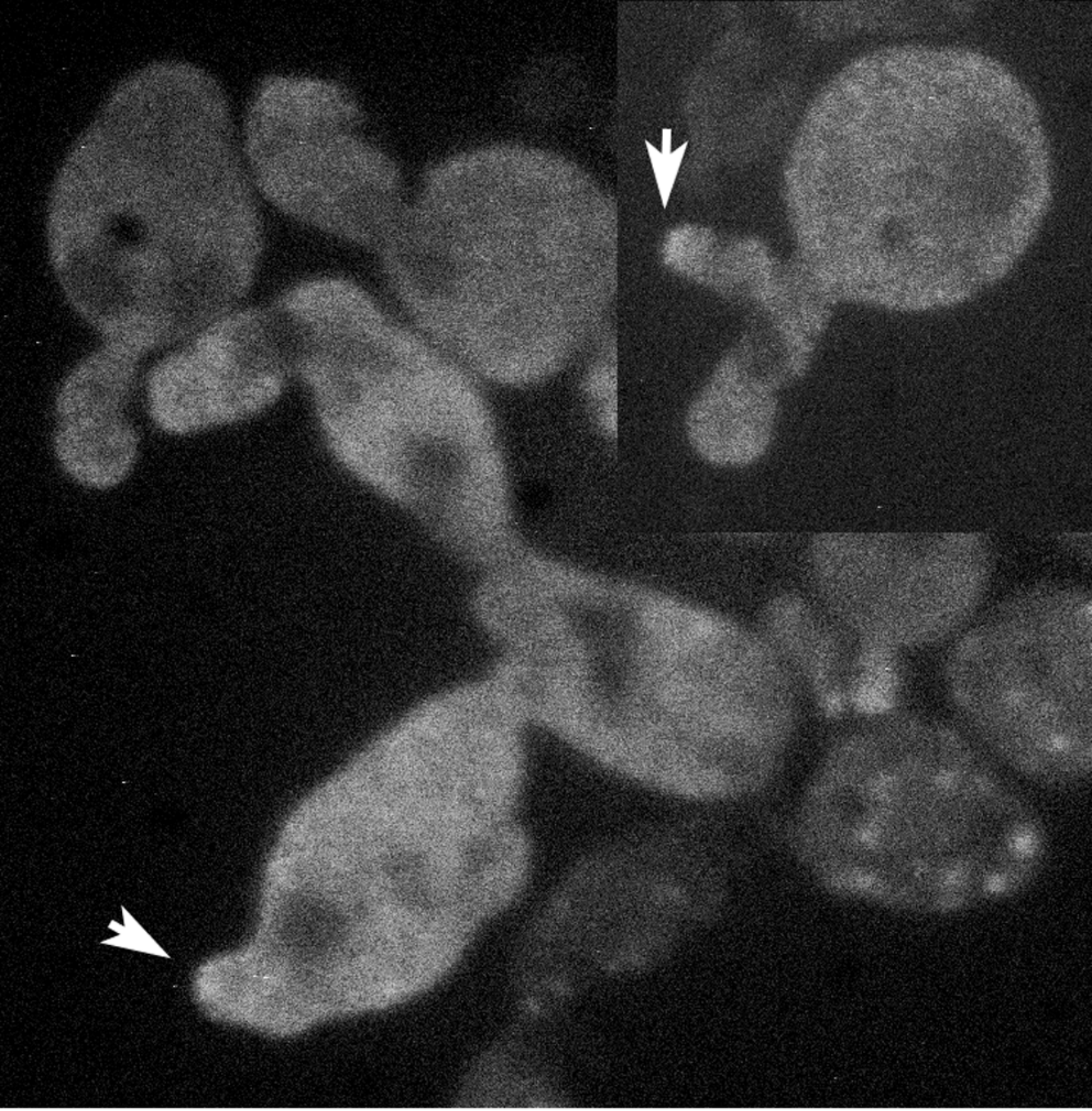

A
Bni1p or
Bnr1p
% cells
100
80
60
40
20
0
wt
bnr1Δ/-
wt
bni1Δ/-
Bni1p
Bnr1p


B
Bni1p
(bnr1Δ/bnr1Δ)
Bnr1pGFP
(bni1Δ/bni1Δ)


A
GFPCdc12p
(wt)
GFPCdc12p
(bni1Δ/bni1Δ)
B
GFPCdc12p
100
80
60
40
20
0
% cells
wt
bni1Δ/-
wt
bni1Δ/-
wt
bni1Δ/-
wt
bni1Δ/-



GFPCdc12p
(*cdc10-1/cdc10-1*)

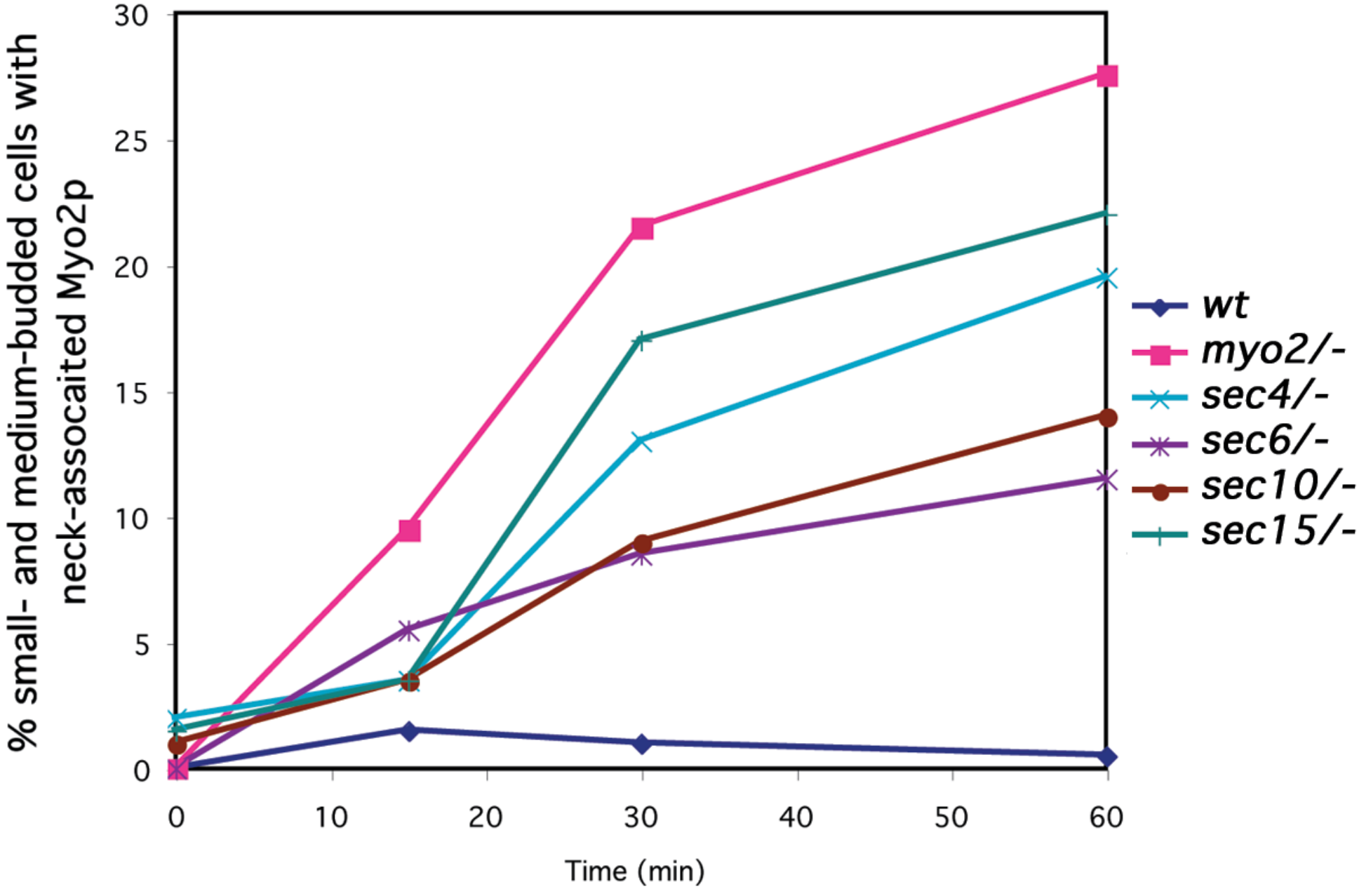

% small- and medium-budded cells with neck-assocaited Myo2p
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Time (min)
wt
myo2/-
sec4/-
sec6/-
sec10/-
sec15/-

Figure S1. Proper localizations of Bni1p and Bnr1p do not depend on the other formin isoform. (A) Wild-type (ABY1848) and *bnr1Δ/bnr1Δ* (ABY1801) cells stained to show Bni1p distribution and wild-type (YEF2255) and *bni1Δ/bni1Δ* (ABY2256) expressing Bnr1pGFP were scored for the distribution of the appropriate formin. One hundred cells of each strain and indicated budding index were categorized for the relevant formin associated with the bud tip (black), bud tip and neck (gray), bud neck (white), or delocalized (blank). (B) Examples of Bni1p staining in *bnr1Δ/bnr1Δ* (ABY1801) cells and Bnr1pGFP fluorescence in *bni1Δ/bni1Δ* (ABY2256) cells are shown.

Figure S2. Septin organization is only modestly perturbed by loss of Bni1p. (A) GFPCdc12p fluorescence is shown in selected wild-type (ABY1896) and *bni1Δ/bni1Δ* (ABY1898) cells. (B) One hundred cells of each indicated budding index of each strain in (A) were scored for their distribution of GFPCdc12p. For unbudded cells, cells were categorized as having fluorescence marking the cytokinetic remnant (visible as a large ring) (black), marking the nascent bud site (visible as a smaller ring or a single spot) and the cytokinetic remnant (gray), marking the nascent bud site only (white), or showing delocalized fluorescence (blank). For budded cells, cells were categorized as having fluorescence concentrated at the bud tip (black), the bud tip and neck (gray), the bud neck (white), or delocalized (blank).

Figure S3. GFPCdc12p fluorescence of *cdc10-1/cdc10-1* (ABY2257) cells shifted to 35˚C for 1 h is shown. White arrows indicate cell tips with a faint enrichment of GFPCdc12p.

Figure S4. Myo2p gradually redistributes to the bud neck when polarized secretion is disrupted. Wild-type (ABY501), *myo2-16/myo2-16* (ABY506), *sec4-8/sec4-8* (ABY994), *sec6-4/sec6-4* (ABY993), *sec10-2/sec10-2* (ABY995), and *sec15-*

1/sec15-1 (ABY996) cells were shifted to 35˚C for the indicated times before being stained for Myo2p distribution. One hundred each small- and medium-budded cells of each strain were scored for whether Myo2p was present at the bud neck.

Movie S1. GFPSec4p in wild-type (ABY1848) cells. Cells were imaged for 12 sec. Fluorescence can be seen concentrated at the bud tips, and as discrete particles in the mother that undergo directed movements toward the neck.

Movie S2. GFPSec4p in *bni1*Δ/*bni1*Δ (ABY1867) cells. Cells were imaged for 12 sec. Fluorescence can be seen concentrated at the bud neck and over the entire bud cortex, and as discrete particles in the mother that undergo directed movements toward the neck and, less-frequently, away from the neck.

Movie S3. GFPSec4p in *bnr1*Δ/*bnr1*Δ (ABY1801) cells. Cells were imaged for 12 sec. Fluorescence can be seen concentrated at the bud tips, and as abundant discrete particles in the mother that undergo directed movements toward the neck.