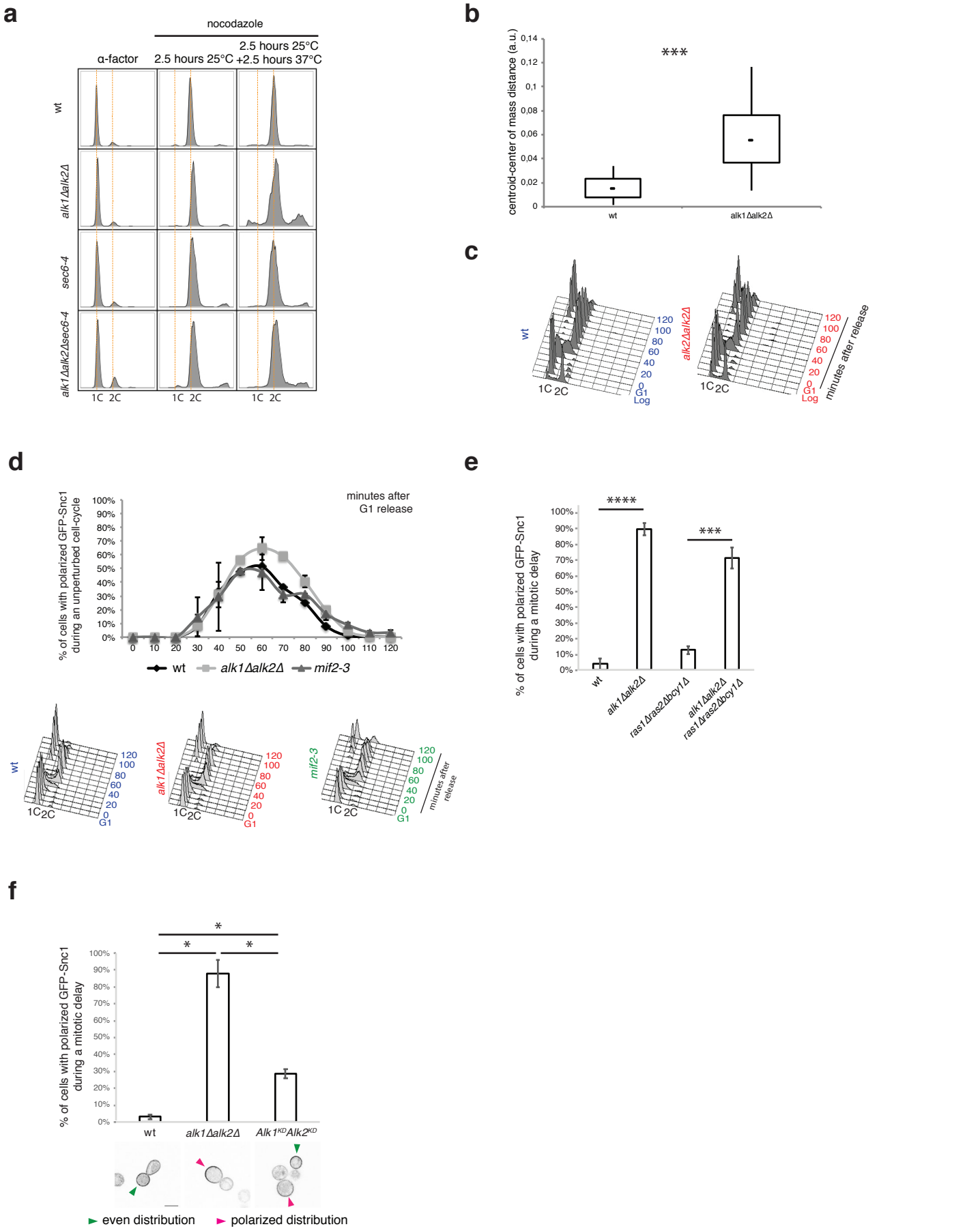
a
nocodazole
2.5 hours 25°C
α-factor
2.5 hours 25°C +2.5 hours 37°C
wt
alk1Δalk2Δ
sec6-4
alk1Δalk2Δsec6-4
1C 2C
b
centroid-center of mass distance (a.u.)
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
***
wt
alk1Δalk2Δ
c
wt
alk2Δalk2Δ
120
100
80
60
40
20
0
G1
Log
1C 2C
minutes after release
d
% of cells with polarized GFP-Snc1 during an unperturbed cell-cycle
minutes after G1 release
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 10 20 30 40 50 60 70 80 90 100 110 120
wt
alk1Δalk2Δ
mif2-3
wt
alk1Δalk2Δ
mif2-3
120
100
80
60
40
20
0
G1
1C 2C
minutes after release
e
% of cells with polarized GFP-Snc1 during a mitotic delay
****
***
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
wt
alk1Δalk2Δ
ras1Δras2Δbcy1Δ
alk1Δalk2Δ ras1Δras2Δbcy1Δ
f
% of cells with polarized GFP-Snc1 during a mitotic delay
*
*
*
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
wt
alk1Δalk2Δ
Alk1KDAlk2KD
even distribution
polarized distribution

**Fig S5. Related to Figure 5.**

(a) Cell cycle analysis by FACS of experiment in Fig. 5a. The plot in (b) represents the centroid-center of fluorescence mass distance of Snc1-GFP, normalized on the daughter cell area and average daughter cell strain circularity, calculated on 20 cells per strain; boxes include 50% of data points, lines represents the average distance and whiskers report the minimum and maximum values. (c) Cell cycle analysis by FACS of experiment in Fig. 5b and relative quantification (blue: wt; red: *alk1Δalk2Δ*). (d) cells of given strains were synchronized in G1 at 25°C and then released at 33°C, taking samples every 10 minutes to monitor Snc1 distribution by fluorescence microscopy and cell cycle progression by FACS (for quantification: blue: wt; red: *alk1Δalk2Δ*, green: *mif2-3*; The data derive from two independent experiments, counting 100 cells per repeat). (e) Cells of the indicated strains, expressing GFP-Snc1, were treated with nocodazole for 3 hours. Samples were then analyzed to monitor distribution of the SNARE. The panel reports the percentage of cells with polarized Snc1, calculated in 3 independent experiments, counting 100 cells per repeat. (f) Cells of given strains were synchronized in G1 and then released for 2.5 hours in nocodazole-containing medium. At the end of the treatment cells were fixed and analyzed by fluorescence microscopy. The graph shows the percentage of cells exhibiting polarized Snc1-GFP, calculated in 2 independent experiments, counting 100 cells per repeat. Green and magenta arrows point to cells with even or polarized Snc1-GFP signal, respectively. t-test was applied as a statistical measurement in (b,d,e,f); n.s.: not significant; * : p-value <0.05; ** : p-value <0.01; *** : p-value <0.005; **** : p-value <0.001.