Expanded View Figures

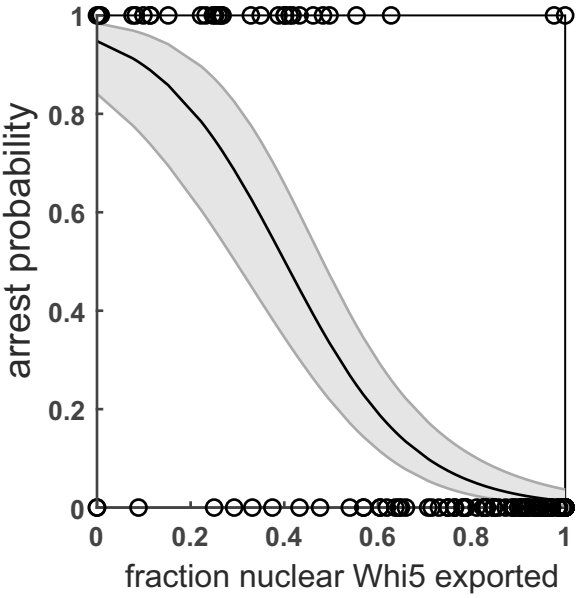

Figure EV1. Commitment to mating pheromone occurs at Whi5 exit.

Cells were grown for 120 min on glucose minimal medium and then exposed to 1 μM α-factor. *x*-axis depicts the fraction of Whi5 that had been exported (relative to the peak value in the last G1) at the time point of the switch. Open circles represent 244 individual cells that either directly arrest in G1 ($y = 1$) or complete one more division before inducing a mating arrest ($y = 0$). A logistic regression was performed on these data to estimate the probability of arrest based on nuclear Whi5. Black line represents the result of the regression; the shaded areas represent the 95% confidence intervals.

Figure EV2. Extended information for Fig 6.

A We introduced wild-type or the 7A phosphorylation site mutant (S78, S113, T114, S149, S276, T281, S288 mutated to alanine) Whi5 into a whi5-deletion strain and determined its response to starvation. Bars indicate the fraction of cells that re-import Whi5 when starved within 15 min after Start. Numbers over the bars indicate total number of cells that were analyzed.

B The histograms depicting Whi5 nuclear-to-cytoplasm ratios of Fig 6C are plotted as cumulated frequencies for direct comparison.

C Replicate of the Phos-tag gel in Fig 6D (without time point 4). An independent experiment was performed as described in Fig 6A.

D Representative Western blot showing the amount of Whi5 protein pulled down in ChIP experiments (Fig 6E).

E Quantification of Western blots from three independent ChIP experiments as described in Fig 6E.

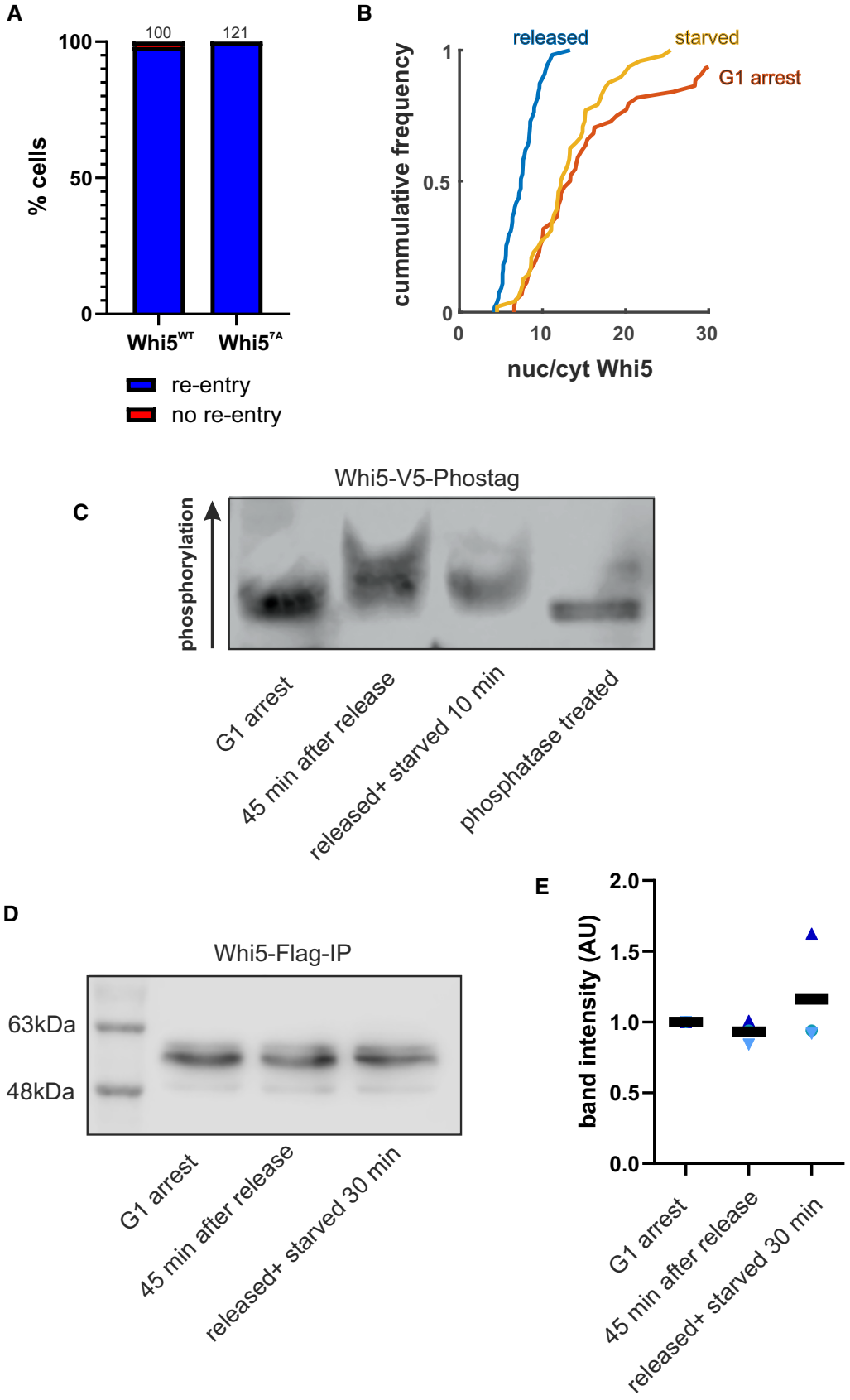
A
100
121
100
50
0
% cells
Whi5WT
Whi5 7A
re-entry
no re-entry
B
released
starved
G1 arrest
cummulative frequency
1
0.5
0
0
10
20
30
nuc/cyt Whi5
C
Whi5-V5-Phostag
phosphorylation
G1 arrest
45 min after release
released+ starved 10 min
phosphatase treated
D
Whi5-Flag-IP
63kDa
48kDa
G1 arrest
45 min after release
released+ starved 30 min
E
band intensity (AU)
2.0
1.5
1.0
0.5
0.0
G1 arrest
45 min after release
released+ starved 30 min

Figure EV2.

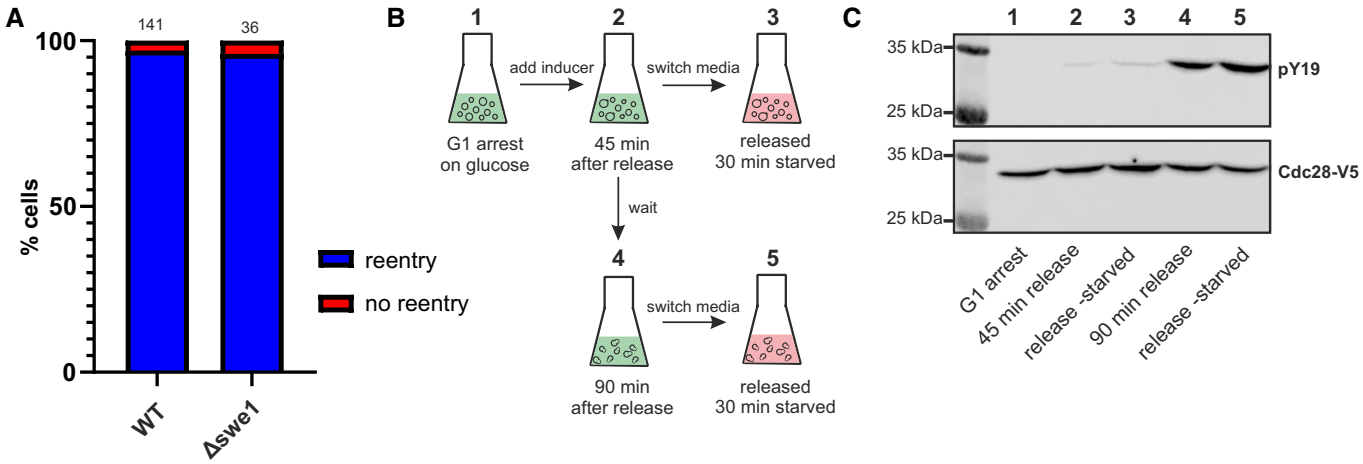

Figure EV3. The Swe1 kinase is not essential for Whi5 re-entries.

A We constructed a *swe1* deletion mutant and monitored its Whi5 response to starvation (wild-type control is the same data as in Fig 5A). Depicted is the fraction of cells that re-imports Whi5 when starved within 15 min after Start. Numbers over the bars indicate total number of cells that were analyzed.

B Experimental setup for (C): Cells in liquid culture were arrested in G1 (time point 1) as described in Fig 6A. Cells were released by inducing Cln1 expression. After 45 min (2), half of the culture was transferred to starvation medium (3), and the other half continued to progress until 90 min (4). These cells were then also transferred to starvation medium (5).

C Cells from the experiment described in (B) were sampled at the indicated times and protein lysates were used for Western blotting. Upper blot shows the signal of a commercial antibody against phosphorylated tyrosine 19 on CDK. After the detection, the blot was stripped and re-analyzed (lower blot) with an antibody detecting the V5-tagged total CDK1 (Cdc28).