Supplemental Materials

Molecular Biology of the Cell

Bharucha et al.

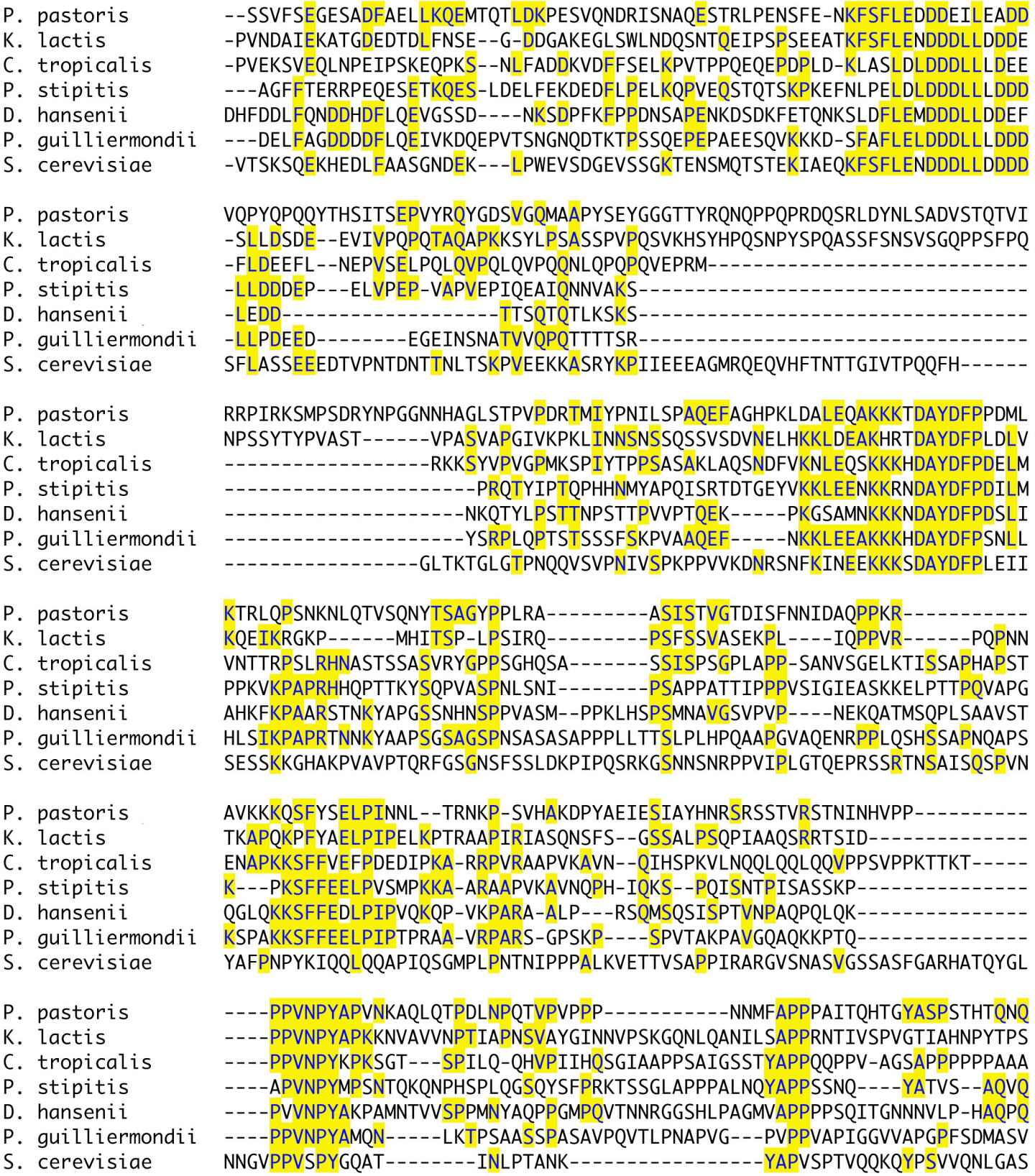
P. pastoris --SSVFSEGESADFAELLKQEMTQTLDKPESVQNDRISNAQESTRLPENSFE-NKFSFLEDDDEILEADD
K. lactis -PVNDAIEKATGDEDTDLFNSE--G-DDGAKEGLSWLNDQSNTQEIPSPSEEATKFSFLENDDDLLDDDE
C. tropicalis -PVEKSVEQLNPEIPSKEQPKS--NLFADDKVDFFSELKPVTPPQEQEPDPLD-KLASLDLDDDLLLDEE
P. stipitis ---AGFFTERRPEQESETKQES-LDELFEKDEDFLPELKQPVEQSTQTSKPKEFNLPELDLDDDLLLDDD
D. hansenii DHFDDLFQNDDHDFLQEVGSSD----NKSDPFKFPPDNSAPENKDSDKFETQNKSLDFLEMDDDLLDDEF
P. guilliermondii ---DELFAGDDDDFLQEIVKDQEPVTSNGNQDTKTPSSQEPEPAEESQVKKKD-SFAFLELDDDLLLDDD
S. cerevisiae -VTSKSQEKHEDLFAASGNDEK---LPWEVSDGEVSSGKTENSMQTSTEKIAEQKFSFLENDDDLLDDDD
P. pastoris VQPYQPQQYTHSITSEPVYRQYGDSVGQMAAPYSEYGGGTTYRQNQPPQPRDQSRLDYNLSADVSTQTVI
K. lactis -SLLDSDE--EVIVPQPQTAQAPKKSYLPSASSPVPQSVKHSYHPQSNPYSPQASSFSNSVSGQPPSFPQ
C. tropicalis -FLDEEFL--NEPVSELPQLQVPQLQVPQQNLQPQPQVEPRM----------
P. stipitis -LLDDDEP---ELVPEP-VAPVEPIQEAIQNNVAKS----------
D. hansenii -LEDD----------TTSQTQTLKSKS----------
P. guilliermondii -LLPDEED--------EGEINSNATVVQPQTTTTSR----------
S. cerevisiae SFLASSEEEDTVPNTDNTTNLTSKPVEEKKASRYKPIIEEEAGMRQEQVHFTNTTGIVTPQQFH------
P. pastoris RRPIRKSMPSDRYNPGGNNHAGLSTPVPDRTMIYPNILSPAQEFAGHPKLDALEQAKKKTDAYDFPPDML
K. lactis NPSSYTYPVAST------VPASVAPGIVKPKLINNSNSSQSSVSDVNELHKKLDEAKHRTDAYDFPLDLV
C. tropicalis ----------RKKSYVPVGPMKSPIYTPPSASAKLAQSNDFVKNLEQSKKKHDAYDFPDELM
P. stipitis ----------PRQTYIPTQPHHNMYAPQISRTDTGEYVKKLEENKKRNDAYDFPDILM
D. hansenii ----------NKQTYLPSTTNPSTTPVVPTQEK-----PKGSAMNKKKNDAYDFPDSLI
P. guilliermondii ----------YSRPLQPTSTSSSFSKPVAAQEF-----NKKLEEAKKKHDAYDFPSNLL
S. cerevisiae ----------GLTKTGLGTPNQQVSVPNIVSPKPPVVKDNRSNFKINEEKKKSDAYDFPLEII
P. pastoris KTRLQPSNKNLQTVSQNYTSAGYPPLRA---------ASISTVGTDISFNNIDAQPPKR----------
K. lactis KQEIKRGKP------MHITSP-LPSIRQ---------PSFSSVASEKPL----IQPPVR------PQPNN
C. tropicalis VNTTRPSLRHNASTSSASVRYGPPSGHQSA-------SSISPSGPLAPP-SANVSGELKTISSAPHAPST
P. stipitis PPKVKPAPRHHQPTTKYSQPVASPNLSNI--------PSAPPATTIPPPVSIGIEASKKELPTTPQVAPG
D. hansenii AHKFKPAARSTNKYAPGSSNHNSPPVASM--PPKLHSPSMNAVGSVPVP----NEKQATMSQPLSAAVST
P. guilliermondii HLSIKPAPRTNNKYAAPSGSAGSPNSASASAPPPLLTTSLPLHPQAAPGVAQENRPPLQSHSSAPNQAPS
S. cerevisiae SESSKKGHAKPVAVPTQRFGSGNSFSSLDKPIPQSRKGSNNSNRPPVIPLGTQEPRSSRTNSAISQSPVN
P. pastoris AVKKKQSFYSELPINNL--TRNKP-SVHAKDPYAEIESIAYHNRSRSSTVRSTNINHVPP----------
K. lactis TKAPQKPFYAELPIPELKPTRAAPIRIASQNSFS--GSSALPSQPIAAQSRRTSID----------
C. tropicalis ENAPKKSFFVEFPDEDIPKA-RRPVRAAPVKAVN--QIHSPKVLNQQLQQLQQVPPSVPPKTTKT-----
P. stipitis K---PKSFFEELPVSMPKKA-ARAAPVKAVNQPH-IQKS--PQISNTPISASSKP----------
D. hansenii QGLQKKSFFEDLPIPVQKQP-VKPARA-ALP---RSQMSQSISPTVNPAQPQLQK----------
P. guilliermondii KSPAKKSFFEELPIPTPRAA-VRPARS-GPSKP----SPVTAKPAVGQAQKKPTQ----------
S. cerevisiae YAFPNPYKIQQLQQAPIQSGMPLPNTNIPPPALKVETTVSAPPIRARGVSNASVGSSASFGARHATQYGL
P. pastoris ----PPVNPYAPVNKAQLQTPDLNPQTVPVPPP----------NNMFAPPPAITQHTGYASPSTHTQNQ
K. lactis ----PPVNPYAPKKNVAVVNPTIAPNSVAYGINNVPSKGQNLQANILSAPPRNTIVSPVGTIAHNPYTPS
C. tropicalis ----PPVNPYKPKSGT---SPILQ-QHVPIIHQSGIAAPPSAIGSSTYAPPQQPPV-AGSAPPPPPPAAA
P. stipitis ----APVNPYMPSNTQKQNPHSPLQGSQYSFPRKTSSGLAPPPALNQYAPPSSNQ----YATVS--AQVQ
D. hansenii ----PVVNPYAKPAMNTVVSPPMNYAQPPGMPQVTNNRGGSHLPAGMVAPPPSQITGNNNVLP-HAQPQ
P. guilliermondii ----PPVNPYAMQN-----LKTPSAASSPASAVPQVTLPNAPVG---PVPPVAPIGGVVAPGPFSDMASV
S. cerevisiae NNGVPPVSPYGQAT--------INLPTANK----------YAPVSPTVQQKQYPSVVQNLGAS

Figure S1

Figure S1. Alignment of UCR sequences from various yeasts. The UCR of *P. pastoris* Sec16 (residues 500 – 868) was aligned with corresponding Sec16 sequences from the following yeasts: *Kluyveromyces lactis* (residues 313 – 673), *Candida tropicalis* (residues 403 – 744), *Pichia stipitis* (residues 479 – 793), *Debaromyces hansenii* (residues 467 – 771), *Pichia guilliermondii* (residues 307 – 620), and *S. cerevisiae* (residues 457 – 824). An initial ClustalW alignment was edited extensively by hand to highlight similarities identified by BLAST searches. Residues highlighted in yellow match the consensus.

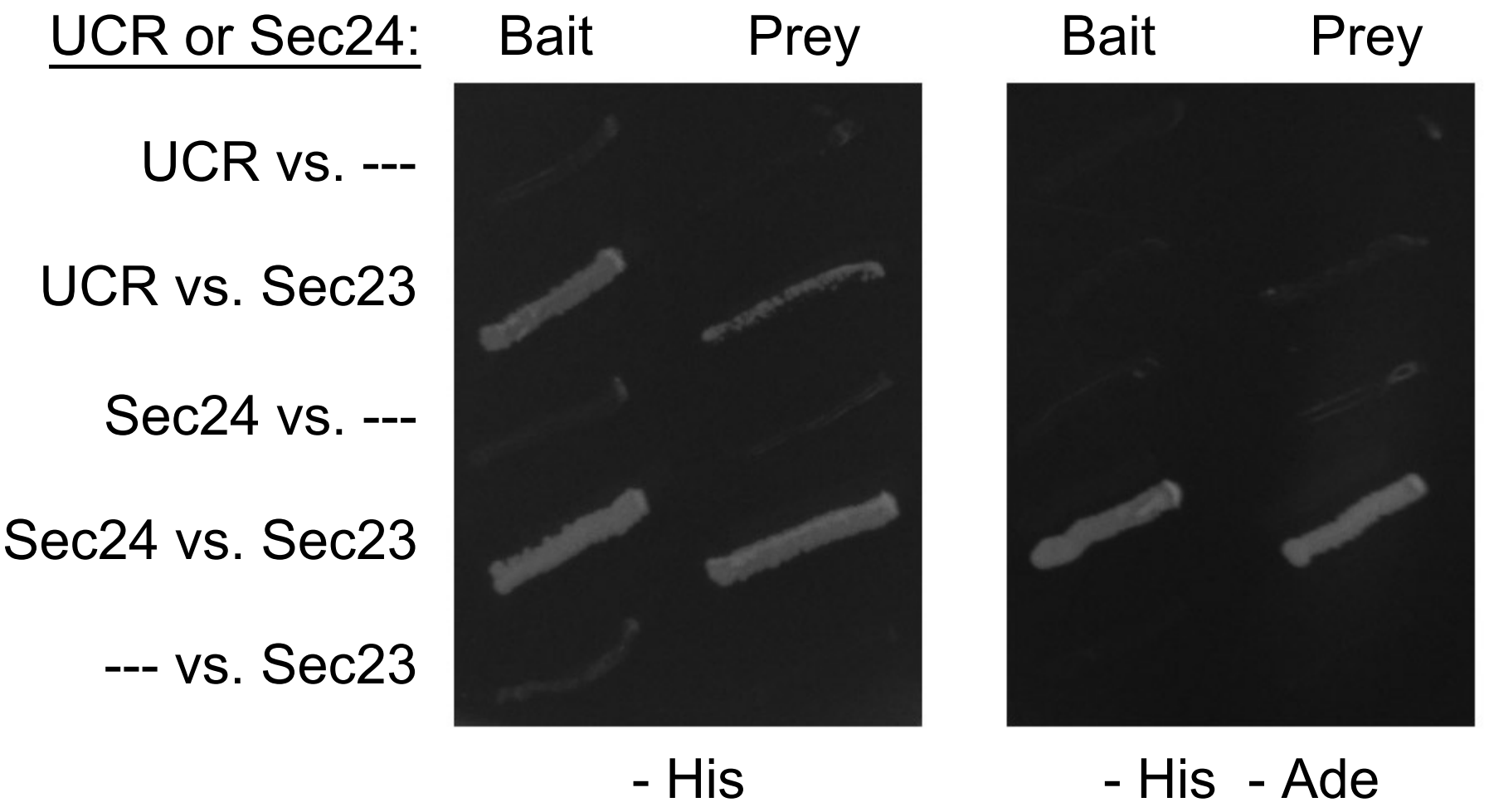
UCR or Sec24:
Bait
Prey
Bait
Prey
UCR vs. ---
UCR vs. Sec23
Sec24 vs. ---
Sec24 vs. Sec23
--- vs. Sec23
- His
- His - Ade

Figure S2

Figure S2. Two-hybrid interaction of the UCR with Sec23 using *S. cerevisiae* proteins. In the yeast streaks on the left side of each plate, a "prey" vector encoding *S. cerevisiae* Sec23 was tested against "bait" vectors encoding *S. cerevisiae* versions of either the UCR (residues 457-977) or Sec24. Empty vectors ("---") were used as controls. In the yeast streaks on the right side of each plate, the "bait" and "prey" vector backbones were swapped. A weak interaction is sufficient for growth on the plate lacking histidine ("– His"), whereas a strong interaction is needed for growth on the plate lacking both histidine and adenine ("– His – Ade").

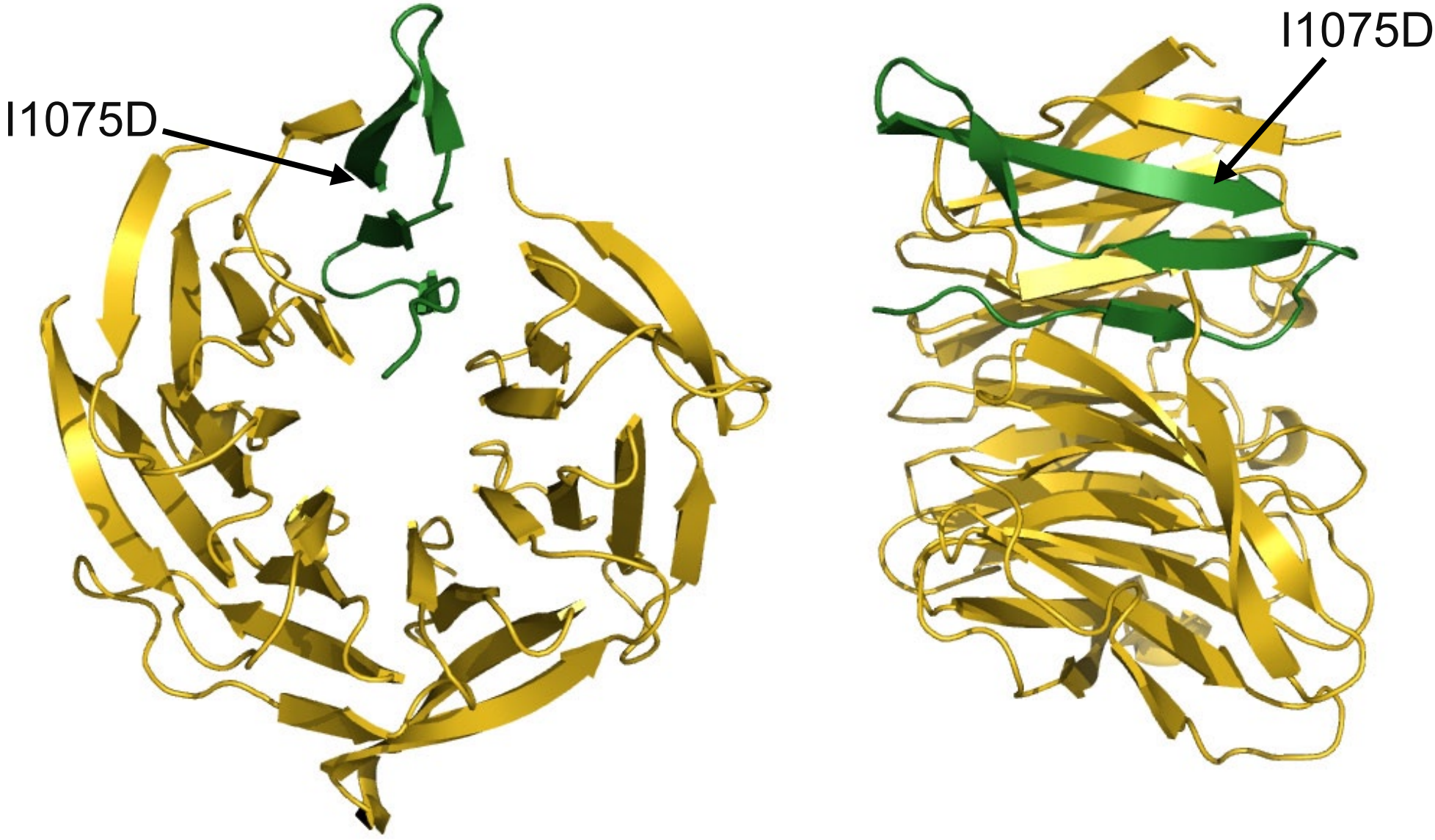

Figure S3A

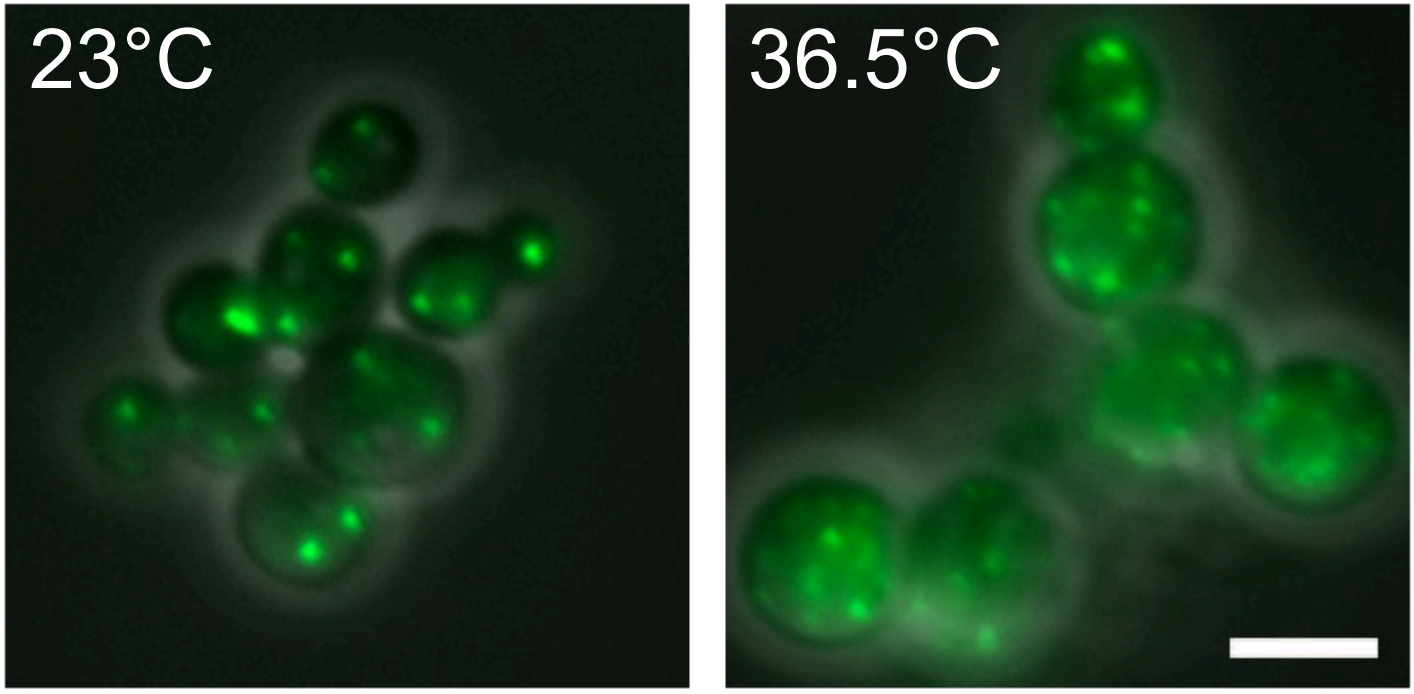

Figure S3B

Figure S3. tER dispersal caused by an I1075D mutation in the Sec13-binding blade of the CCD. (A) Crystal structure of a chimera between *P. pastoris* Sec13 and the β-stranded blade of the CCD from *P. pastoris* Sec16 (CCDβ). Shown on the left is a view from the top of the Sec13 β-propeller bound to CCDβ, with the N-terminal portion of CCDβ projecting into the foreground. A side view is shown on the right. Visible in the crystal structure are amino acids 1042-1076 of Sec16, colored in green, and 13-299 of Sec13, colored in yellow. The position of the I1075D mutation is indicated. (B) tER dispersal in a *sec16-I1075D* mutant at 36.5°C. This mutant allele was introduced by gene replacement into a strain expressing Sec13-GFP, and cells were imaged under the conditions described in Figure 4C. Scale bar, 5 μm.

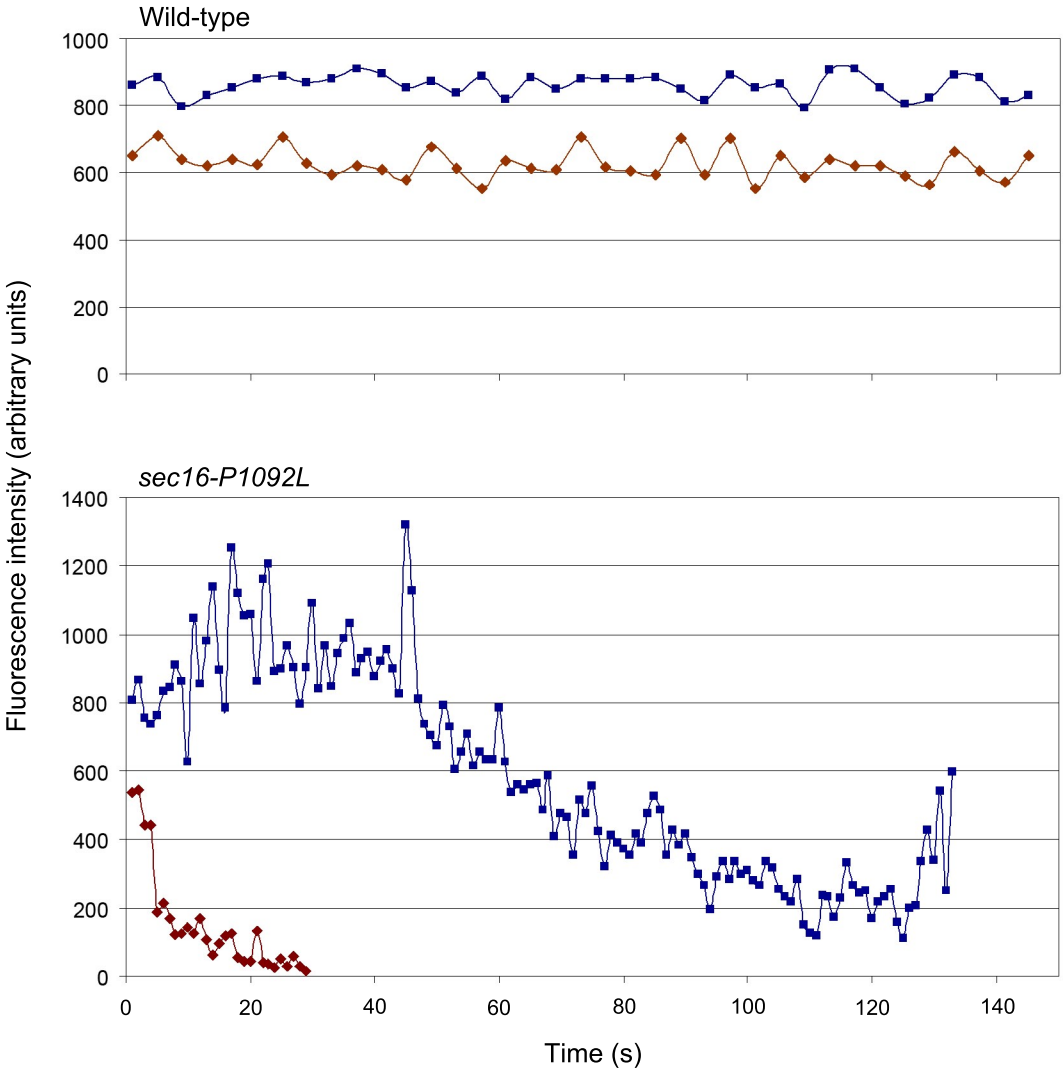
Wild-type
1000
800
600
400
200
0
sec16-P1092L
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
Time (s)
Fluorescence intensity (arbitrary units)

Figure S4A

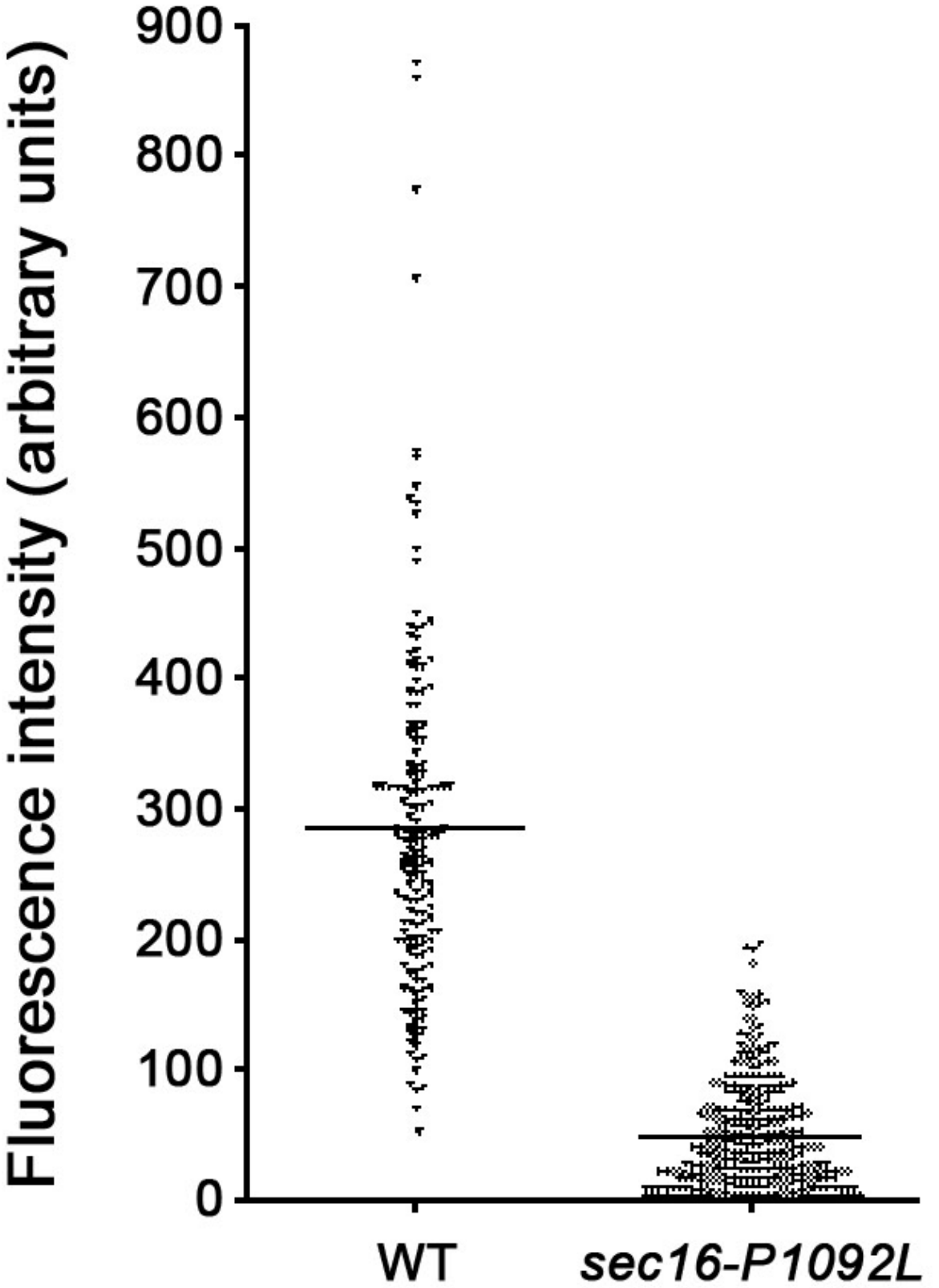
Fluorescence intensity (arbitrary units)
900
800
700
600
500
400
300
200
100
0
WT
sec16-P1092L

Figure S4B

Figure S4. Accelerated shrinkage and reduced fluorescence intensity of tER sites in the *sec16-P1092L* mutant. (A) Rapid shrinkage of tER sites after fusion events in *sec16-P1092L* cells. Two representative fused tER sites were analyzed by 4D microscopy at 36.5°C after fusion events in wild-type cells (top panel) or *sec16-P1092L* mutant cells (bottom panel). Fluorescence intensity is plotted as a function of time. In wild-type cells, a fused tER site typically requires tens of minutes to shrink to the steady-state size (Bevis *et al.*, 2002). (B) Quantitation of tER site fluorescence intensities in wild-type and *sec16-P1092L* cells at 36.5°C. 4D movie projections were examined, and fluorescence signals were measured for all resolvable tER sites. A total of 172 wild-type tER sites and 322 *sec16-P1092L* tER sites were analyzed. Each dot represents an individual tER site, and the horizontal line represents the average of the values obtained.

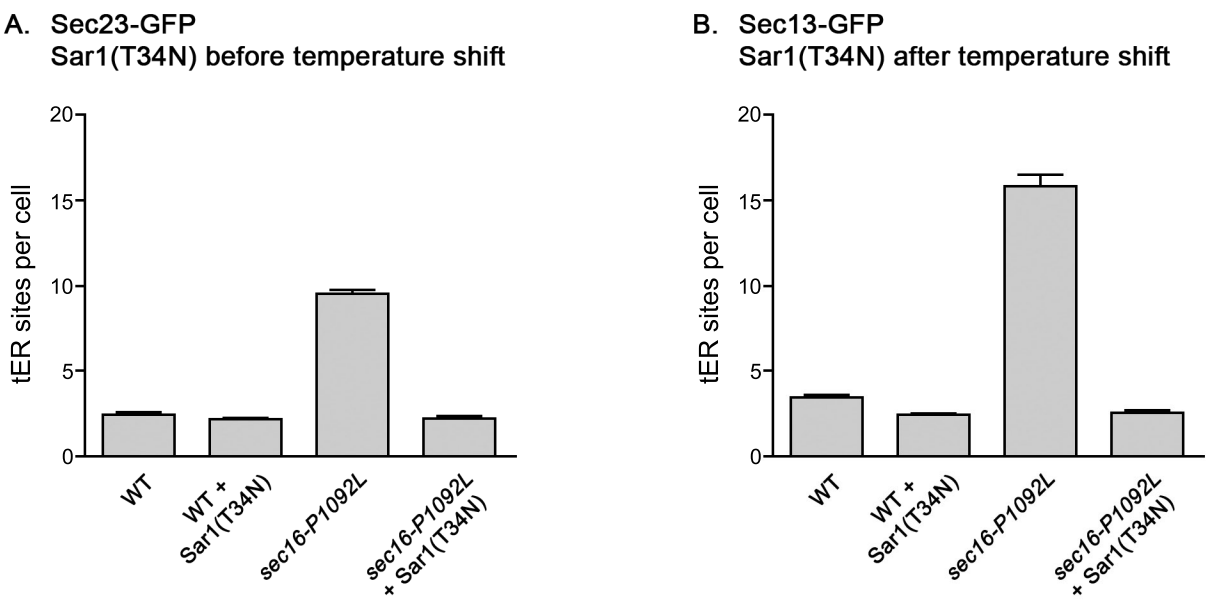
A. Sec23-GFP
Sar1(T34N) before temperature shift
tER sites per cell
20
15
10
5
0
WT
WT + Sar1(T34N)
sec16-P1092L
sec16-P1092L + Sar1(T34N)
B. Sec13-GFP
Sar1(T34N) after temperature shift
tER sites per cell
20
15
10
5
0
WT
WT + Sar1(T34N)
sec16-P1092L
sec16-P1092L + Sar1(T34N)

Figure S5

Figure S5. Prevention or reversal of tER dispersal by expression of Sar1(T34N). Where indicated, a shift to methanol-containing medium induced expression of Sar1(T34N). (A) The procedure was the same as in Figure 6D-E, except that tER sites were labeled with Sec23-GFP. (B) The procedure was the same as in Figure 6D-E, except that the cells were first grown for 1 h at 36.5°C to cause tER dispersal, and then shifted to methanol medium for 2.5 h at 36.5°C.

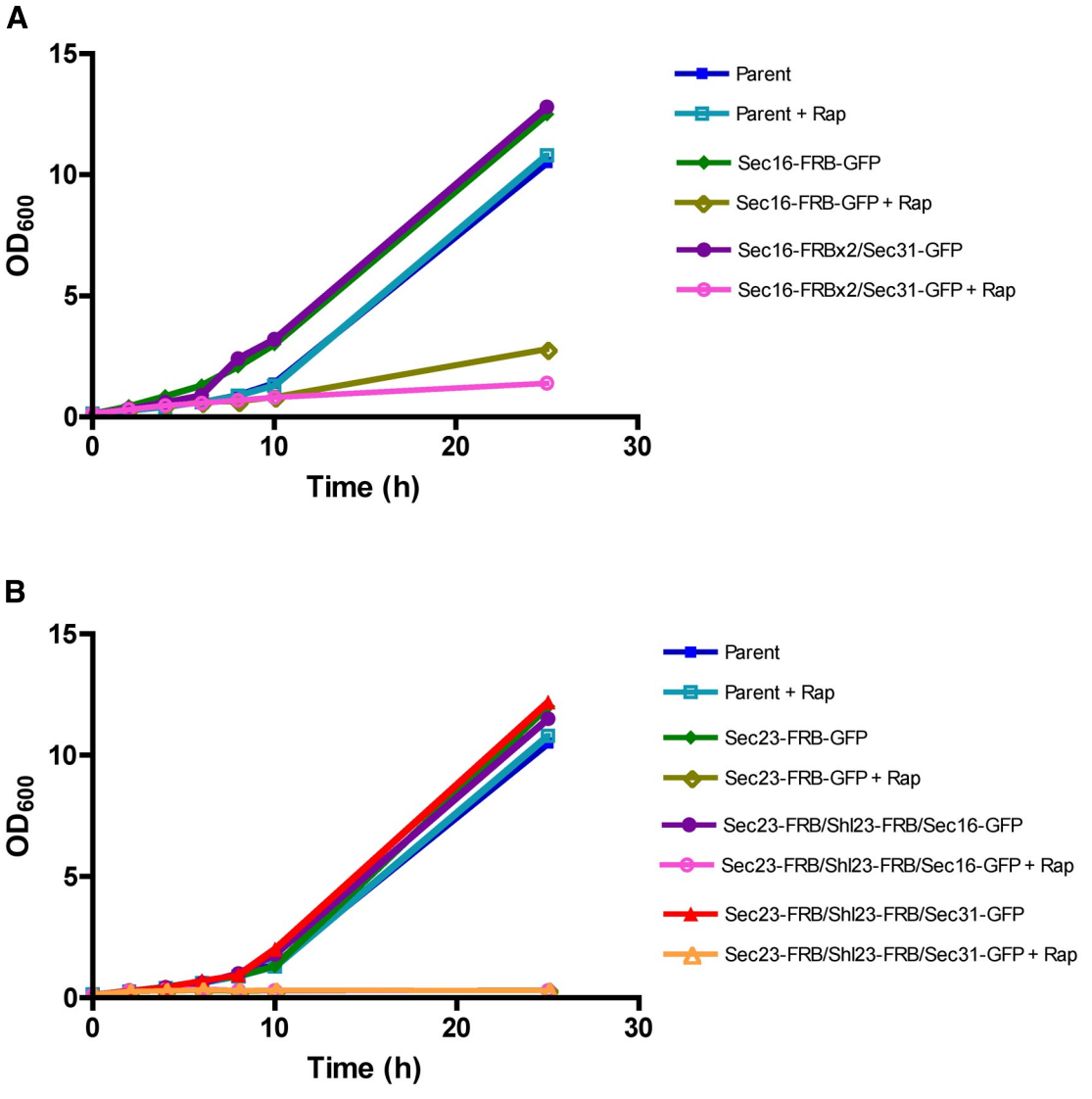
A
15
10
5
0
OD600
0
10
20
30
Time (h)
Parent
Parent + Rap
Sec16-FRB-GFP
Sec16-FRB-GFP + Rap
Sec16-FRBx2/Sec31-GFP
Sec16-FRBx2/Sec31-GFP + Rap
B
15
10
5
0
OD600
0
10
20
30
Time (h)
Parent
Parent + Rap
Sec23-FRB-GFP
Sec23-FRB-GFP + Rap
Sec23-FRB/Shl23-FRB/Sec16-GFP
Sec23-FRB/Shl23-FRB/Sec16-GFP + Rap
Sec23-FRB/Shl23-FRB/Sec31-GFP
Sec23-FRB/Shl23-FRB/Sec31-GFP + Rap

Figure S6

Figure S6. Growth inhibition by rapamycin in anchor-away strains. (A) The Sec16 anchor-away strains used in Figure 7, plus the parental rapamycin-resistant Rpl17-FKBPx4 strain ("Parent"), were grown in YPD at 30°C to an OD_{600} of 0.1. Then 1 μg/mL rapamycin ("Rap") was added to half of each culture, and the cultures were incubated for an additional 25 h. At the indicated time points, OD_{600} was measured. (B) Same as (A), except that the analysis was performed with the Sec23 anchor-away strains used in Figure 8.

Movie S1. tER dynamics in wild-type cells. tER sites in wild-type *P. pastoris* cells were labeled with Sec13-GFP, and cells were imaged by 4D confocal microscopy at intervals of 4 sec between each Z-stack. The fluorescence data for each time point were projected and merged with a DIC image of the cells. Time is indicated in min:sec format.

Movie S2. tER dynamics in *sec16-P1092L* mutant cells. Imaging and image processing were performed as for Movie S1, except that the cells carried the *sec16-P1092L* allele and the intervals between Z-stacks were 2 sec.

Table S1. X-ray data collection and refinement statistics for CCDβ-Sec13.

Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	70.51, 49.32, 90.90
α, β, γ (°)	90.0, 111.7, 90.0
Wavelength (Å)	1.1
Resolution (Å)	50-1.55 (1.61-1.55)
R_{sym} (%) [a]	8.5 (68.0)
$\langle I / \sigma_I \rangle$ [a]	12.9 (1.9)
Completeness (%) [a]	99.3 (93.2)
Redundancy [a]	6.9 (5.6)
Refinement	
Resolution (Å)	44-1.6
No. reflections	76777
R_{work} / R_{free}	0.180 / 0.212
No. atoms	
Protein (non-H)	5033
Riding hydrogens	4899
Waters	306
Ca^{2+}, Cl^-, EDO	25
B-factors (Å^2) [b]	
Main-chain	21.4
Side-chain	28.9
Non-protein	30.5
R.m.s. deviations [c]	
Bond lengths (Å)	0.013
Bond angles (°)	1.49
B-factors (m/c) (Å^2)	3.8
B-factors (s/c) (Å^2)	5.3
Ramachandran	
Within favored (%)	96.6
Within allowed (%)	99.7
Outliers (%)	0.3

a: Values in parentheses are for the highest resolution shell.

b: $R_{sym} = \Sigma_h \Sigma_i \mid I_{h,i} - I_h \mid / \Sigma_h \Sigma_i I_{h,i}$, where I_h is the mean intensity of the i observations of symmetry related reflections of h. $R = \Sigma \mid F_{obs} - F_{calc} \mid / \Sigma F_{obs}$, where $F_{obs} = F_P$, and F_{calc} is the calculated protein structure factor from the atomic model (R_{free} was calculated with 5% of the reflections).

c: The r.m.s.d. values in bond lengths and angles are the deviations from ideal values, and the r.m.s.d. in B factors is calculated between bonded atoms.