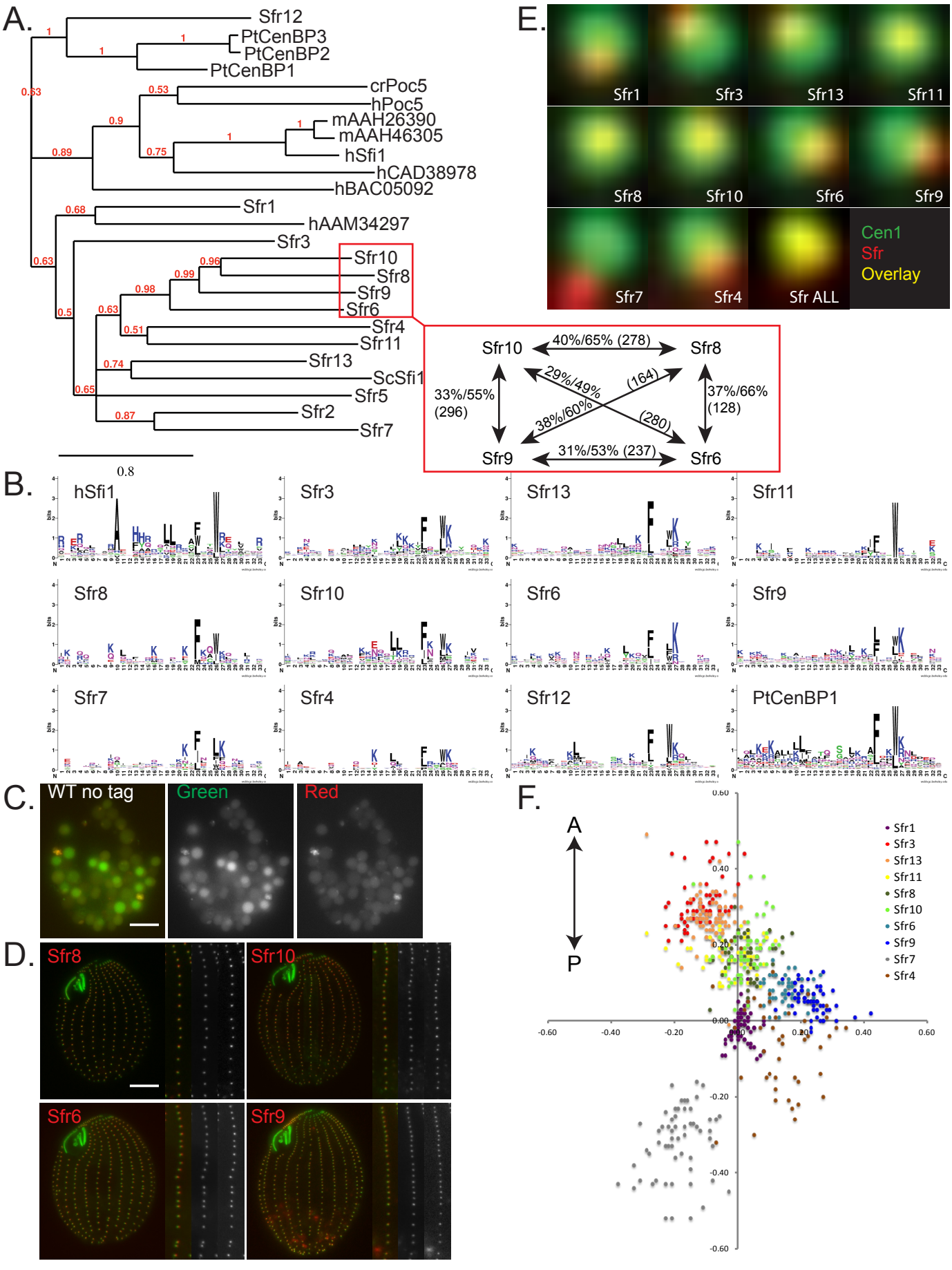
Fig. S1. Sfi1-repeat proteins in *Tetrahymena*. (**A**) A phylogenetic tree of the *Tetrahymena thermophila* Sfi1-repeat proteins, and other known Sfi1-repeat proteins. Sc = *Saccharomyces cerevisiae,* Pt = *Paramecium tetraurelia*, h = *Homo sapiens,* m = *Mus musculus*, cr = *Chlamydamonas reinhardtii*. Bootstrap values are in red, and the scale bar indicates the number of changes per amino acid site. The boxed region shows the amount of homology in the N-termini between Sfr6, Sfr8, Sfr9 and Sfr10. Percent identity and similarity are shown, and the number of amino acids over which homology was found is within the parenthesis. (**B**) LOGOS of the 33 amino acid sequences containing the centrin-binding sites within each protein as indicated. The *Homo sapiens*, and *Paramecium tetraurelia* LOGOS are included for comparison. (**C**). Background vacuolar fluorescence in an untagged strain in both the green and red channels. . Scale bar = 10 μm. (**D**) Sfr8, Sfr10, Sfr6, and Sfr9 tagged with mCherry are present at all cortical row basal bodies in cells starved for 24 hours. GFP-Poc1 marks all basal bodies. The insets are from left to right the merged images, GFP-Poc1 and Sfr-mCherry. . Scale bar = 10 μm. (**E**) Overlays of image averages from >45 basal bodies of GFP-Cen1 (green) with the Sfr proteins C-terminally tagged with mCherry, or N-terminally GFP tagged in the case of Sfr1 (red). The last panel is an overlay of all the Sfr image averages (red) with GFP-Cen1 (green). Panels are 0.96×0.96 μm. (**F**) The position of the tagged Sfr protein for each basal body analyzed was plotted relative to Poc1 (denoted by the axes origins). A = anterior, P = posterior. The scale is in μm.

Fig. S2. Characterization of Sfr10 localization. (**A**) Sfr10 accumulates at basal bodies as they mature. Cells containing the Sfr10-mCherry construct were fixed and stained with antibodies directed against Cen1 (green) and K-like (blue). White arrows indicate mature basal bodies that are positive for Cen1, Sfr10, and K-like signal. The red arrowheads show an immature basal body that is positive for Cen1, but has very little Sfr10 or K-like signal. The blue arrowheads show a basal body that is positive for both Cen1 and Sfr10, but has not yet acquired K-like, suggesting that Sfr10 accumulates as basal bodies mature. Scale bar = 10 μm. (**B**) N- and C-terminally tagged Sfr10 display similar localization patterns. The panel on the left is of a cell expressing N-terminally tagged mCherry-Sfr10 expressed from the Cadmium inducible *MTT1* promoter. The panel on the right is of a cell with C-terminally tagged Sfr10-mCherry. Both tags are integrated at the endogenous *SFR10* locus. Scale bar = 10 μm. (**C**) The position of C-terminally tagged full-length Sfr10 and GFP-Sfr10 C-terminal domain only are plotted relative to Poc1 (denoted by the axes origins) for each basal body analyzed. A = Anterior, P = Posterior. The scale is in μm.

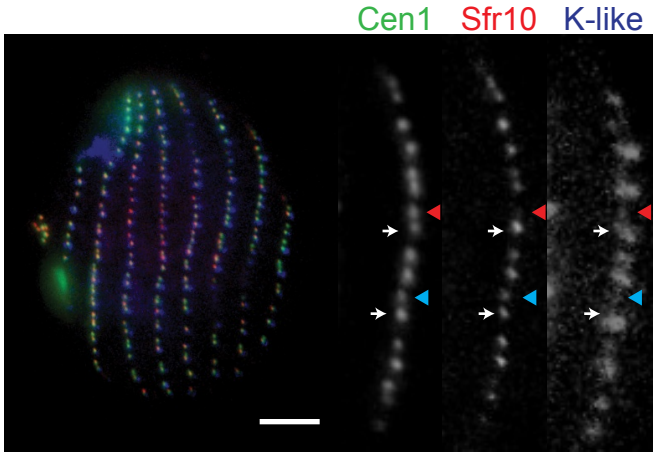
Fig S3. N and C-terminally tagged Sfr13 have similar localization patterns. (**A**) mCherry on either the N- or C-terminus of Sfr13. The N-terminal tag is under the control of the cadmium responsive *MTT1* promoter. Both tags are integrated into the endogenous *SFR13* locus. Scale bar = 10 μm. (**B**) Sfr13-mCherry colocalized with Cen1. Sfr13 is present throughout the developing oral apparatus but lost from most membranelle basal bodies in the mature oral apparatus. Scale bar = 10 μm. (**C**) The position of the C-terminal and N-terminal mCherry tags relative to Poc1 (represented by the origin) in each basal body analyzed. A = anterior, P = posterior. The scale is in μm. (**D**) GFP signal in the N-terminally tagged GFP-Sfr13 strain used in the immuno-electron microscopy experiment. Scale bar = 10 μm.

Fig. S4. Complete micronuclear knockouts of *SFR10* and *SFR13.* (**A**) PCR confirming the complete micronuclear knockouts of *SFR10* and *SFR13*. The left panel shows PCR fragments for the *sfr10*Δ. The schematic represents both the wild type and deletion alleles and the regions to which primers were designed. The vertical lines represent the boundaries of the transforming fragment used to generate the knockout strains. Strains from which template DNA was isolated are shown above the gels, and the primers used are shown beneath the gels. The panel on the right shows PCR confirming both the *sfr13*Δ strain and the rescue strain. The schematic shows three alleles: the wild type allele, the *sfr13*Δ allele, and an intermediate allele for the rescue construct, which has the 5′ end of *SFR13* cloned into the *sfr13*Δ strain along with a Blasticidin resistance gene (BSR) (see Materials and Methods). Regions of primer annealing are displayed above each allele schematic and the vertical lines represent the boundaries of the transforming fragment. Strains from which template DNA was isolated are shown above the gel, and primers used are shown below the gel. Because the rescue constructs were transformed into the macronucleus of the *sfr13*Δ strain, which carries roughly 45 copies of each chromosome, there is evidence for all three alleles in the rescue strain. (**B**) Swimming assays of wild type and *sfr13*Δ cells. n = 30 cells for each strain. (**C**) Wild type and *sfr13*Δ India ink positive vacuoles. The graphs depict the number of India ink positive vacuoles through the time course. n = 25 cells for each strain at each time-point. (**D**) Size measurements for India ink positive vacuoles. The graph depicts the diameter of the India ink positive vacuoles at the 30 minute time-point. To the right of the graph is an estimate of the volume of the vacuoles for each strain based on the measured diameter. n = 48 vacuoles for each strain.

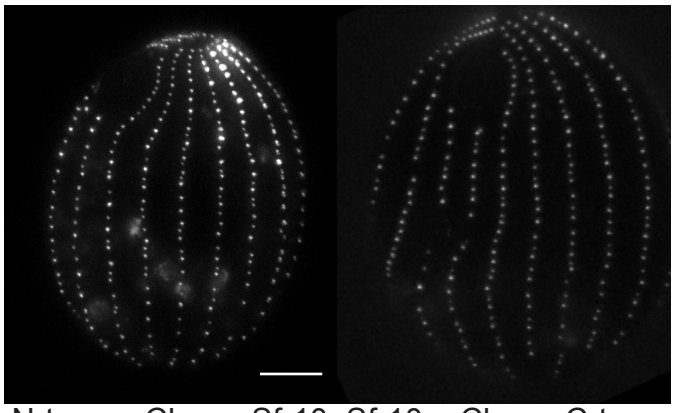
Table S1. Quantification of wild type and *sfr13Δ* cortical row basal bodies. Measurements to establish the number of cortical row basal bodies of wild type and *sfr13Δ* cells under growth and starvation conditions are presented. Cells were grown at 30°C and starved either at 30°C or 38°C. Cells were also grown at 38°C. The measurements include the basal body density, cell length and the number of cortical rows per cell. To calculate the basal body density along cortical rows, the number of basal bodies within 10 μm was counted in three different cortical rows for each cell. These were averaged and divided by 10. Each value is given ± the standard deviation. n = 30 cells for all 30°C measurements. n = 20 cells for 38°C growth. n = 25 cells for 38°C starvation.


A.
Sfr12
PtCenBP3
PtCenBP2
PtCenBP1
crPoc5
hPoc5
mAAH26390
mAAH46305
hSfi1
hCAD38978
hBAC05092
Sfr1
hAAM34297
Sfr3
Sfr10
Sfr8
Sfr9
Sfr6
Sfr4
Sfr11
Sfr13
ScSfi1
Sfr5
Sfr2
Sfr7
1
0.53
0.9
0.89
0.75
0.68
0.63
0.96
0.99
0.98
0.5
0.51
0.74
0.65
0.87
0.8
Sfr10
Sfr8
40%/65% (278)
29%/49%
(164)
33%/55%
(296)
37%/66%
(128)
38%/60%
(280)
Sfr9
Sfr6
31%/53% (237)
B.
hSfi1
Sfr3
Sfr13
Sfr11
Sfr8
Sfr10
Sfr6
Sfr9
Sfr7
Sfr4
Sfr12
PtCenBP1
C.
WT no tag
Green
Red
D.
Sfr8
Sfr10
Sfr6
Sfr9
E.
Sfr1
Sfr3
Sfr13
Sfr11
Sfr8
Sfr10
Sfr6
Sfr9
Sfr7
Sfr4
Sfr ALL
Cen1
Sfr
Overlay
F.
A
P
Sfr1
Sfr3
Sfr13
Sfr11
Sfr8
Sfr10
Sfr6
Sfr9
Sfr7
Sfr4
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60

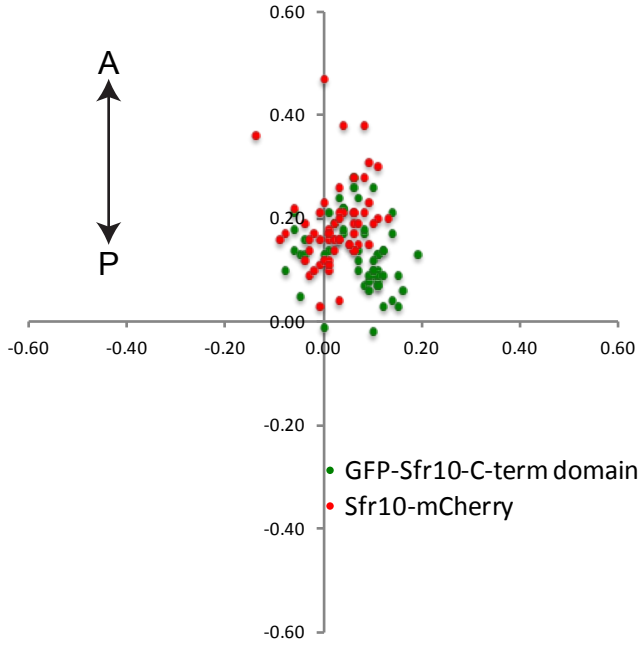
A.


Cen1
Sfr10
K-like

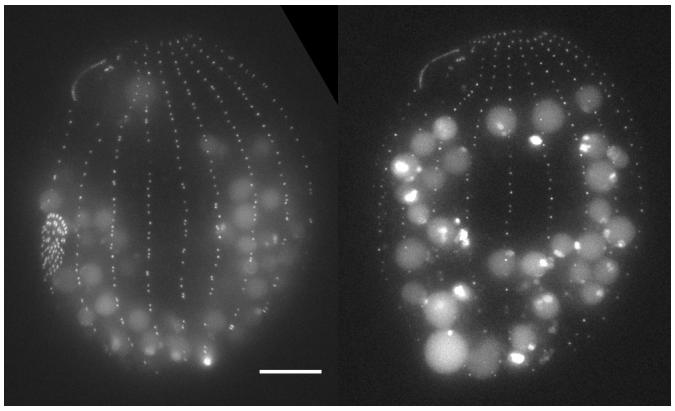
B.


N-term mCherry-Sfr10
Sfr10-mCherry C-term

C.

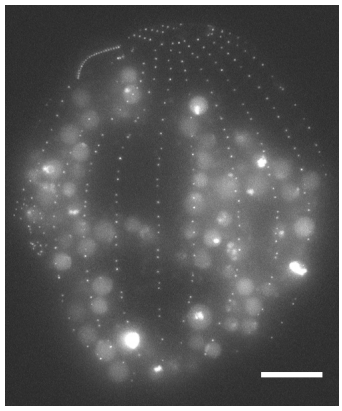

A
P
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.60
-0.40
-0.20
0.00
0.20
0.40
0.60
GFP-Sfr10-C-term domain
Sfr10-mCherry

A.



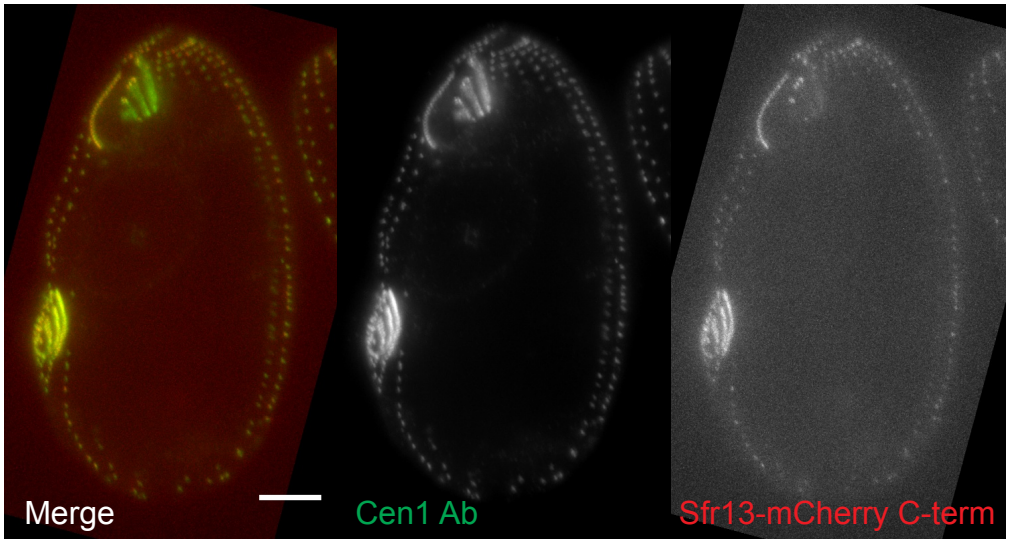
N-term mCherry-Sfr13 Sfr13-mCherry C-term

D.

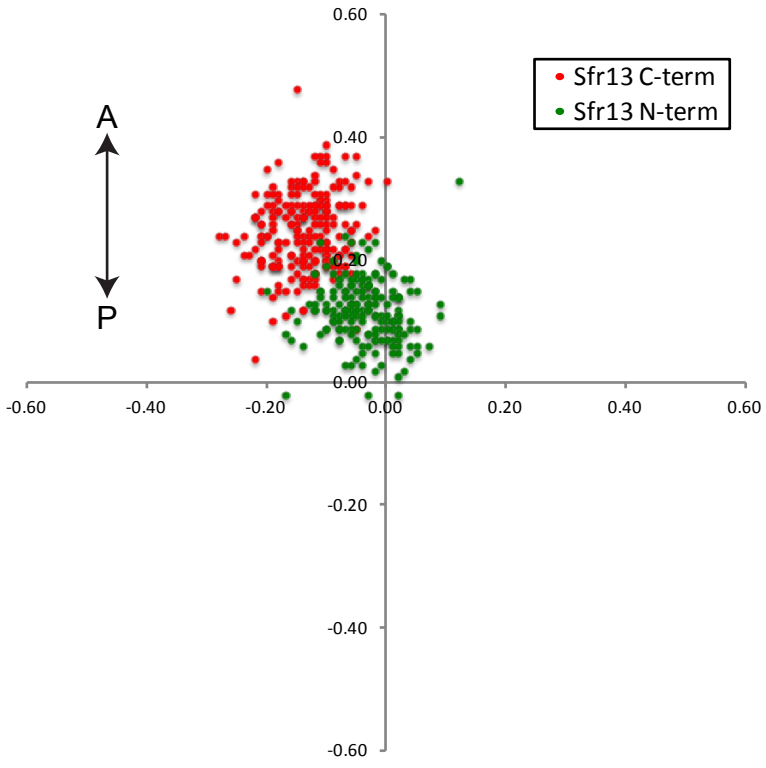


N-term GFP-Sfr13

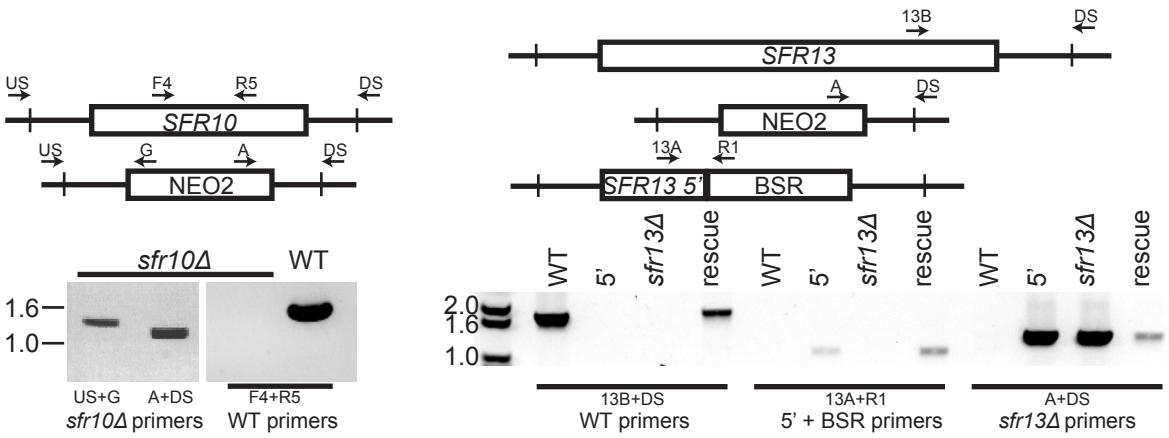
B.



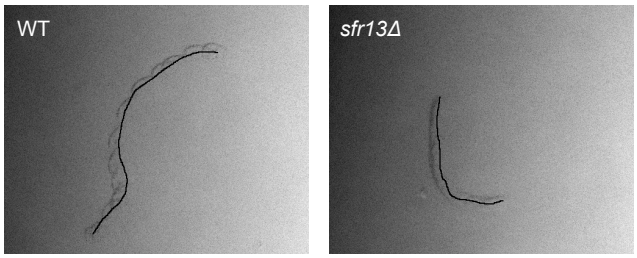
C.



A.



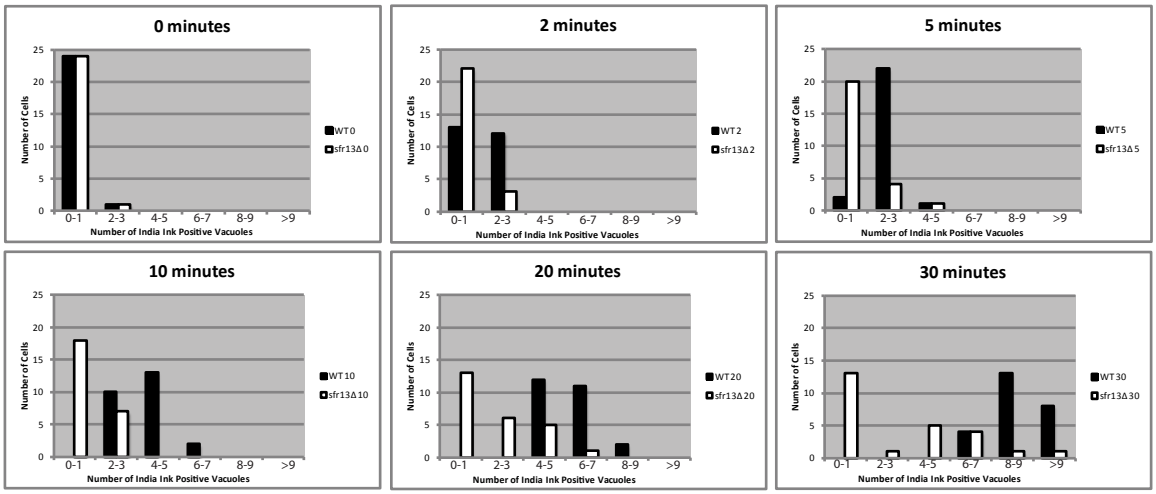
B.



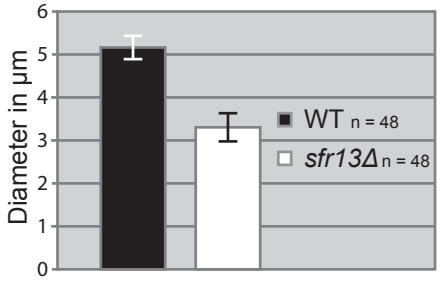
356.4 ± 124.4 µm/sec
n = 30

190.9 ± 106.36 µm/sec
n = 30

C.



D.



Vacuole Volume ($^4/_3\pi r3$)

WT	71.9µm3
sfr13Δ	18.8µm3

Table S1

		30ºC			38ºC			30ºC
		growth	starve 24 hr	starve 48 hr	growth	starve 24 hr	starve 48 hr	growth rescue
bb/µm	WT	0.83 ± 0.17	0.76 ± 0.13	0.71 ± 0.11	0.93 ± 0.14	0.81 ± 0.14	0.71 ± 0.10	0.87 ± 0.17
	sfr13Δ	0.78 ± 0.17	0.57 ± 0.12	0.40 ± 0.11	0.58 ± 0.21	0.52 ± 0.16	0.35 ± 0.14	
cell length (µm)	WT	37.17 ± 4.74	32.28 ± 2.96	32.69 ± 2.48	34.91 ± 3.99	29.92 ± 3.13	25.82 ± 2.16	40.85 ± 5.78
	sfr13Δ	31.97 ± 3.44	28.19 ± 4.15	25.33 ± 2.94	26.89 ± 5.13	19.04 ± 2.97	15.72 ± 2.48	
# cortical rows	WT	19.53 ± 1.09	19.50 ± 1.02	19.57 ± 0.76	19.95 ± .050	19.44 ± 2.97	19.44 ± 0.70	17.40 ± 0.71
	sfr13Δ	13.63 ± 0.98	14.27 ± 1.21	11.10 ± 2.13	15.20 ± 1.75	13.28 ± 1.54	12.72 ± 1.25	
bb/µm X cell length X # cortical rows	WT	626 ± 168	499 ± 103	473 ± 75	649 ± 137	466 ± 67	356 ± 50	623 ± 146
	sfr13Δ	340 ± 77	229 ± 54	114 ± 42	234 ± 81	133 ± 44	72 ± 26	