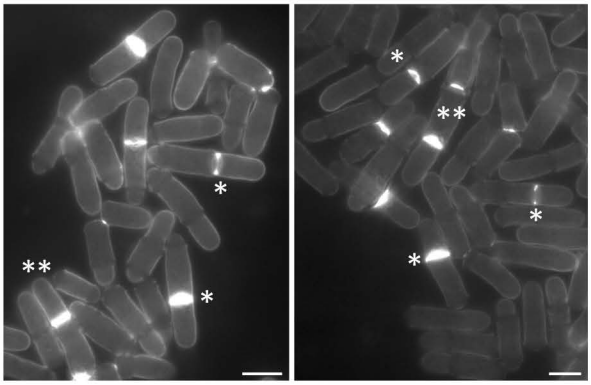
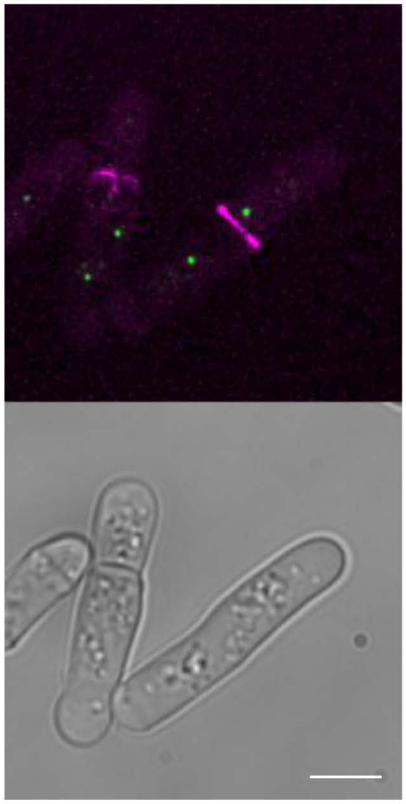
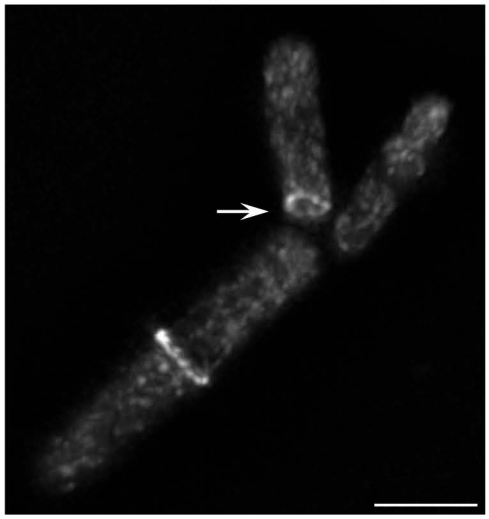
A



B



C

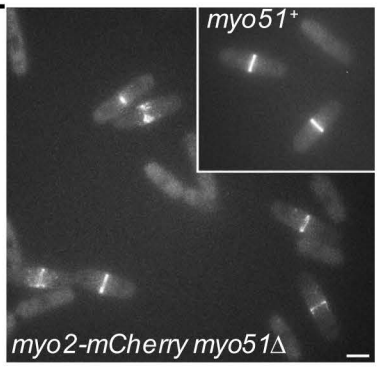


D

Genetic background	Duration of cytokinesis phases (min)				Timings of proteins to the CAR after start of phase II (min)		
	I	II	III	IV	Acp1-gfp	YFP-Myp2	Ste20-gfp
Wild type	14 ± 2.3	23 ± 1.5	26 ± 1.3	9 ± 1.5	7.50 ± 1.68	10.33 ± 1.34	4.36 ± 0.46
acp1Δ	11 ± 1.0	20 ± 1.2	35 ± 4.0	7 ± 0.6	-	8.63 ± 1.35	4.58
myp2Δ	15 ± 0.1	18 ± 4.5	51 ± 3.7* (35 ± 4.7)	8 ± 1.4	7.00 ± 1.15	-	- (4.80 ± 0.63)
ste20Δ	11 ± 1.5	30 ± 6.9	87 ± 13.1	13 ± 3.6	4.80 ± 0.1	21.00 ± 4.24	-
acp1-AA	9 ±1.2	20 ± 3.0	30 ± 2.7	7 ± 1.0	n.d.	n.d.	n.d.

* denotes mean length of phase III in *myp2Δ* cells in which Ste20 failed to localise to the cell equator. Bracketed numbers relate to length of phase III in *myp2Δ* cells in which Ste20 recruited to equator, and the associated timing TORC2 recruited there.

E



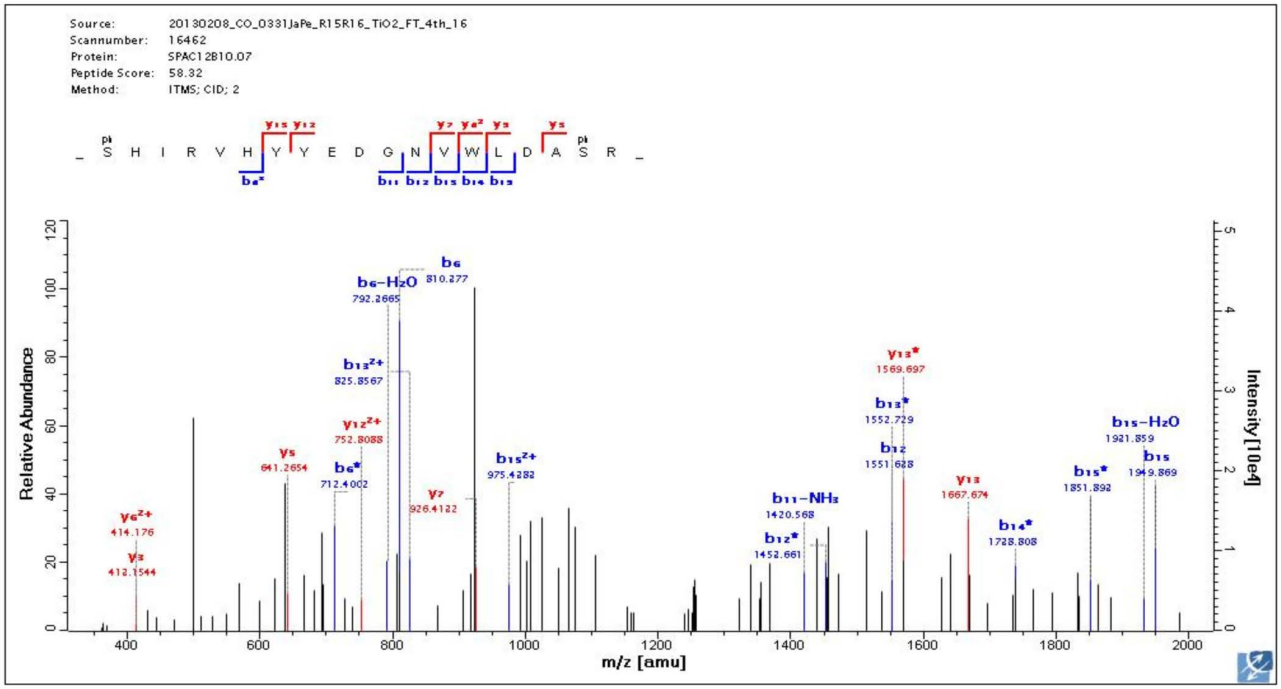
F

Phosphorylation sites identified (also published by Koch et al. 2011). All are unaffected by TOR signalling.				
Mid1	Blt1	Cdc12	Myp2	Myo51
S28	S493	S1496	S1407	No Sites identified
S328	S495	S1543	S1417	
S329	S496	S1600	S1421	
S331	S635		S1579	
S344	S640		S1582	
S347				

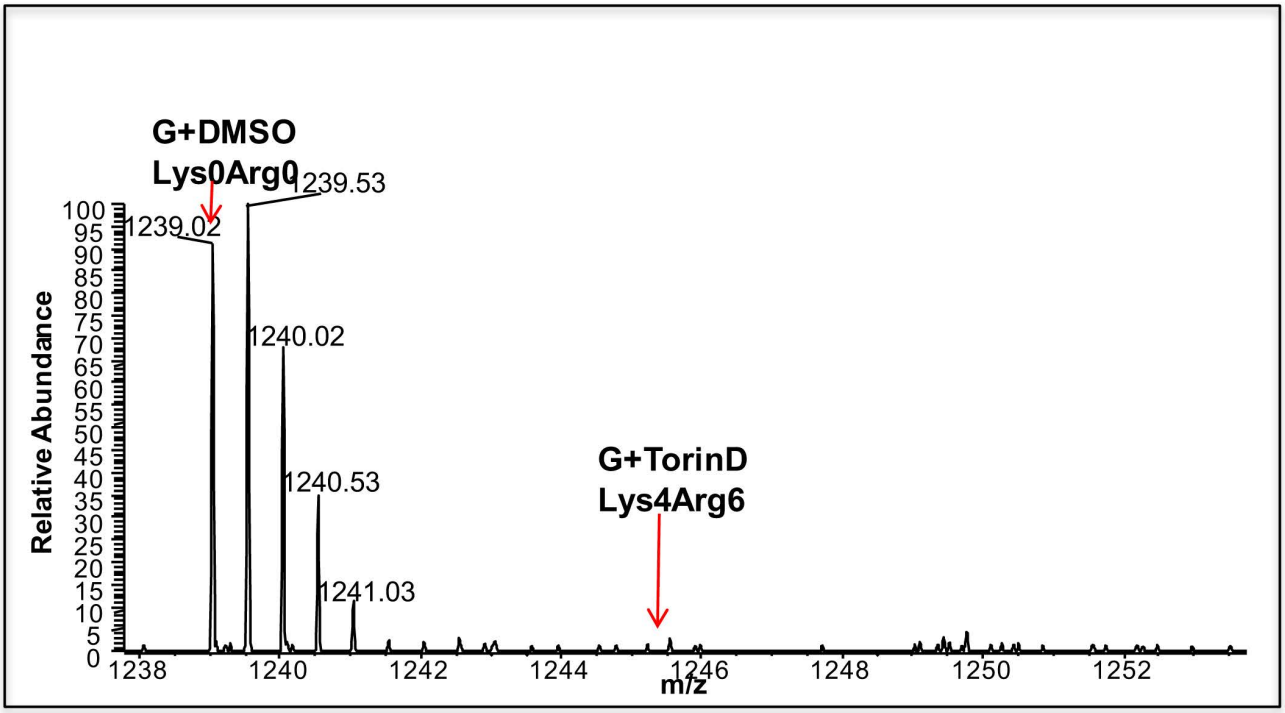
Supplemental figure 1:

(A) Micrograph of calcofluor stained *Rictor$^{ste20}\Delta$* cells. Cells display an assortment of cytokinesis defects including misplaced (*) or multiple (**) septa. (B) Micrographs of mCherry (magenta) and GFP (green) fluorescence (upper panel) and transmitted light (bottom panel) of *Rictor$^{ste20}\Delta$ myo2-mCherry cut12-gfp* cells, in which spindle poles have separated into the same compartment of the dividing cell. (C) Micrograph of *Rictor$^{ste20}\Delta$* cells subjected to anti-Tm^{Cdc8} immunofluorescence reveals an unconstructed actin ring on the end of a cell which has just completed cell division (arrow). (D) Summary of timings of phases of CAR lifecycle and Acp1, Myp2 and Rictor^{ste20} medial recruitment during mitosis. (E) Micrographs of *myo2*-mCherry fluorescence in *myo51Δ* main panel and *myo51^{+}* (inset) cells. (F) Summary of phosphosites in Tor1 associated proteins that are unaffected by TOR signalling. Scales – 5 μm.

A



B

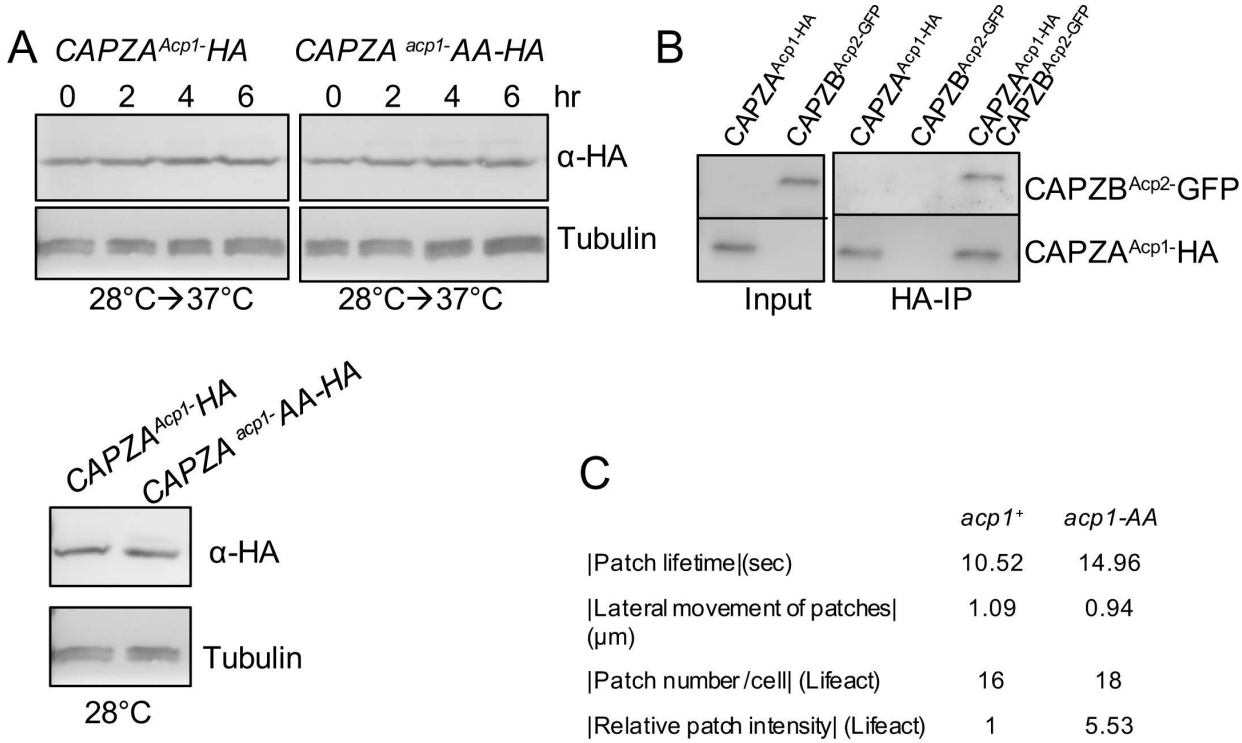


Position	Protein	Localization Prob	Number of Phospho STY.	Modified Sequence	m.z	Ratio H.L Normalized	Ratio H.L Normalized By Protein	Ratio H.L Normalized By Protein Significance	Protein Ratio.H.L
172 189	acp1	0.99922 0.999976	2S	S(ph)HIRVHYYEDGNVW LDAS(ph)R	1239.0254	0.000539	0.000435732	1.61E-46	1.237

Supplemental figure 2:

Serine residues within CAPZA^{Acp1} are subject to TOR dependent phosphorylation.

(A) TOR substrates using a SILAC (Stable isotope labelling by amino acids in cell culture) and Torin1 based approached identified a phosphorylated Acp1 peptide. The spectrum shows the fragmentation pattern of the Acp1 phosphopeptide S(ph)HIRVHYYEDGNVWLDAS(ph)R indicating S172 and S189 to be phosphorylated. The mass of the parent ion is 2476.03604, the measured mass error is -0.08 ppm. (B) The Acp1 phosphorylated peptide was compared in wild type cells grown in "light" medium with cells grown in medium containing "medium" labelled amino acids followed by inhibition of TOR signalling with Torin1 for 30 min. (B) The MS spectrum shows a (2 fold charged) peptide with an m/z value of 1239.02 that was extracted and further fragmented to get the MS/MS spectrum that led to the Acp1-peptide sequence: -S(pH)HIRVHYYEDGNVWLDAS(ph)R. The isotope distribution of the 2 fold charged peptide in the light labelled form can be seen (condition: G+DMSO) however no isotope pattern of the heavy labelled form at +6Th (condition: G+TorinD) can be observed, indicating that the Torin1 treatment led to down regulation of both phosphorylation sites. Table to shown the reduced Acp1 serine 172 and serine 189 phosphorylation following TOR inhibition.



C

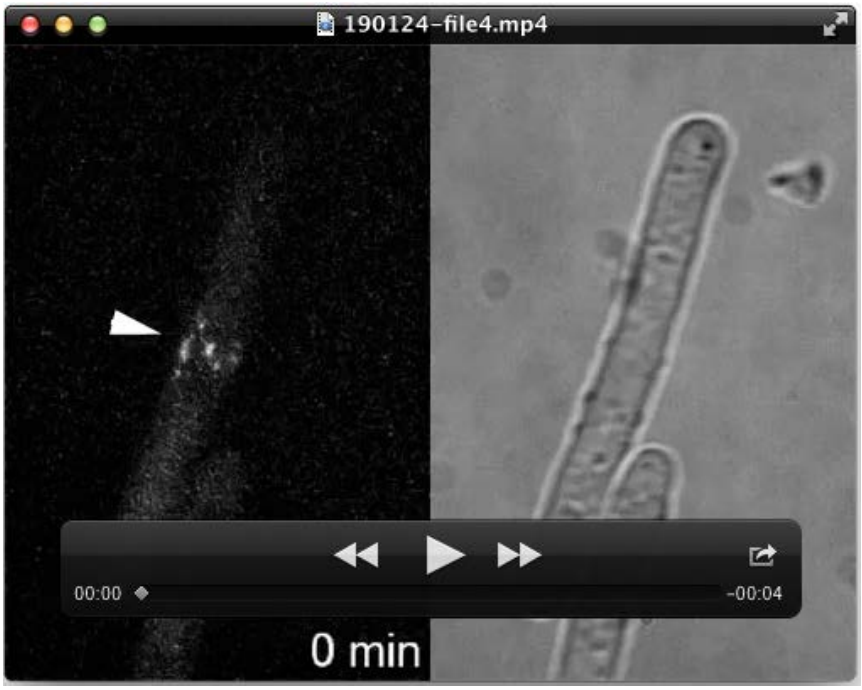
	acp1⁺	*acp1-AA*
|Patch lifetime|(sec)	10.52	14.96
|Lateral movement of patches| (μm)	1.09	0.94
|Patch number/cell| (Lifeact)	16	18
|Relative patch intensity| (Lifeact)	1	5.53
|Relative patch volume| (Lifeact)	1	4.01
% cellular signal within patches	1.65%	3.2%
|Relative Acp signal / patch|	1	1.29

Supplemental figure 3:

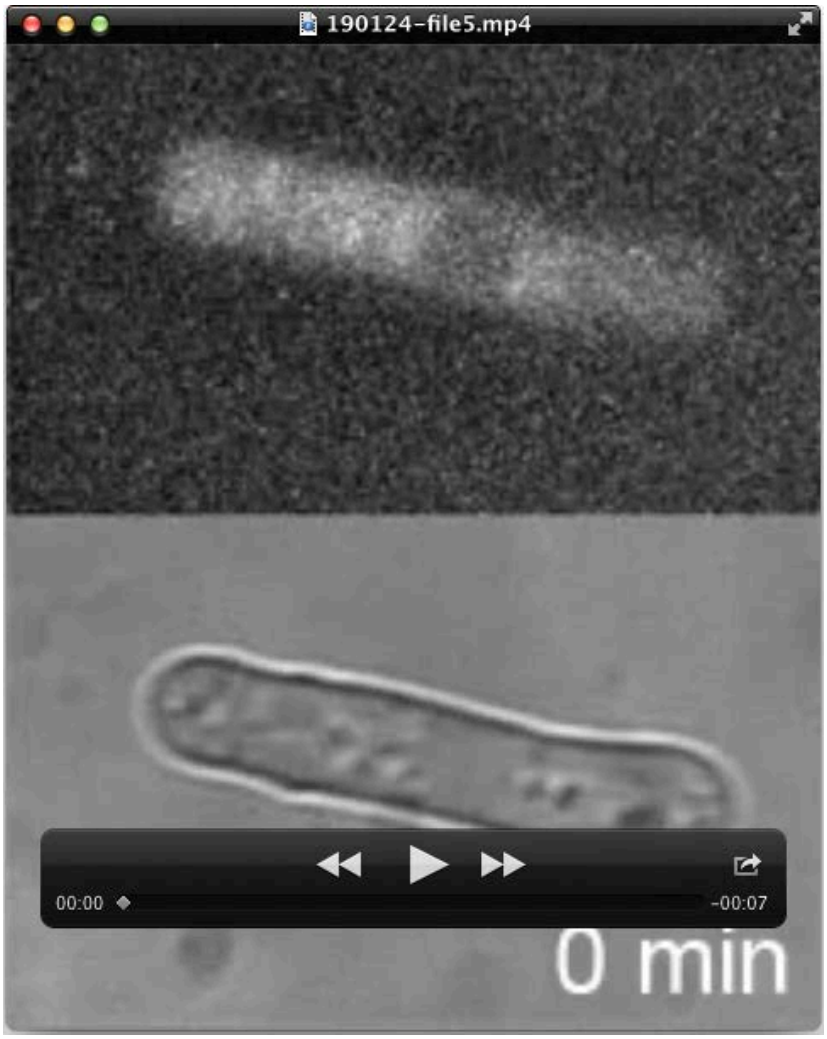
(A) CapZ^{Acp1} and CapZ^{Acp1}-AA are thermo stable. Cell extracts were generated from early-log phase *capZ^{acp1}-HA* (left) and *capZ^{acp1}-AA-HA* (right) cells that had been shifted from 28ºC and held at 37ºC for 6 hrs were subjected to anti-HA (upper panels) and Tat1 (lower panel) western blot analysis. (B) CapZA^{Acp1} associates with CapZB^{acp2} *in vivo.* Anti -GFP (upper panels) and -HA (lower panels) western blots of cell extract (left panels) and anti-HA immunoprecipiations (right panels) from *capZA^{acp1}-HA, capZB^{acp2}-GFP* and *capZ^{acp1}-HA capZB^{acp2}-GFP* cells.

(C) Summary of Lifeact and Acp1 dynamics from *capZ^{acp1}* and *capZ^{acp1}-AA* cells.

Movie 1: Time lapse of red fluorescence from 21 z-slice maximum projections of *myo2-mCherry sid4-tdTomato* prototroph cells cultured to early log phase at 25ºC in EMMG. The arrow highlights a cell undergoing a cycle of CAR formation and constriction. 3 min / frame.

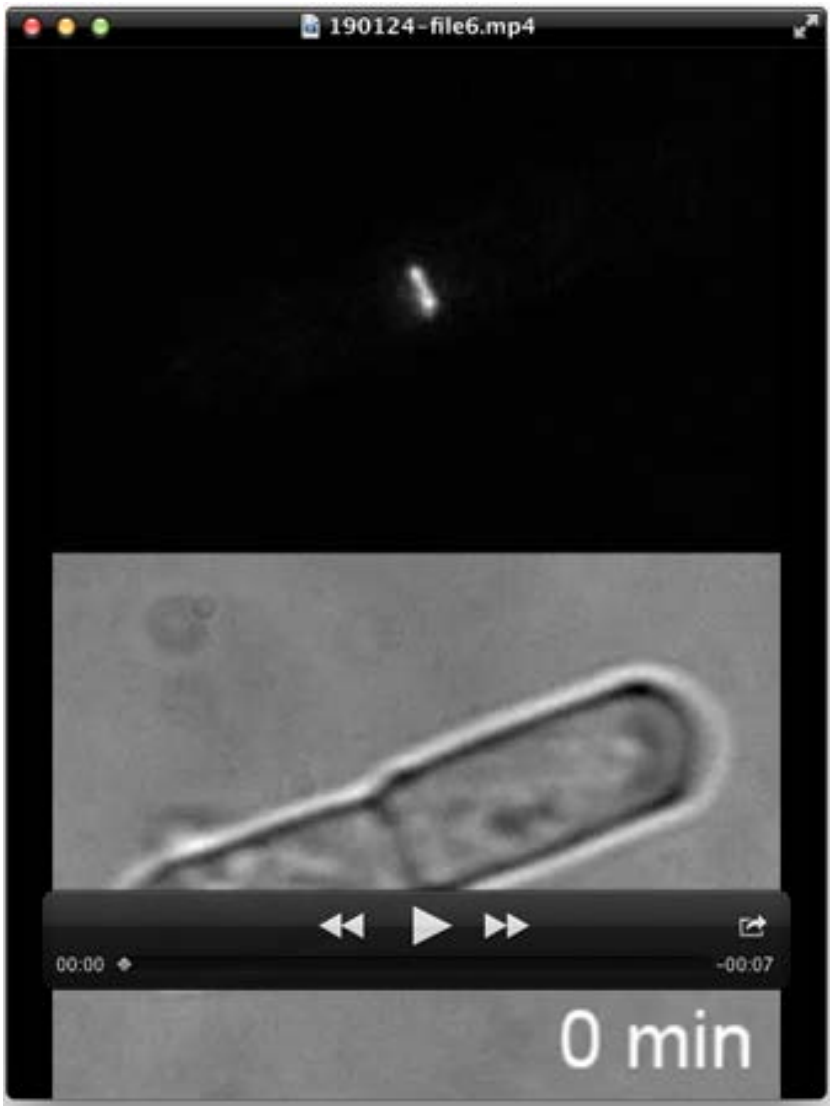


Movie 2: Time lapse of mCherry fluorescence from 21 z-slice maximum projections (left panel) and transmitted light images (right panel) of *Rictor*$^{ste20}\Delta$ *myo2-mCherry* prototroph cells cultured to early log phase at 25ºC in EMMG. At the start of the movie Myo2 foci can be seen to recruit to the cell equator (arrowhead). These take 60 mins to coalesce into 2 discrete ring structures (* @ 60 min), and a further 25 mins to merge into a single CAR structure (** @ 85 min). This CAR splits into two discrete rings (***) which synchronously constrict over the next 40 mins, resulting in the formation of an aberrant septum (arrow). In contrast to wild type cells, polar growth continues while the cell undergoes cytokinesis. Growth 5 min / frame.



Movie 3: Time lapse of mCherry fluorescence 21 z slice maximum projections (upper panel) and transmitted light images (lower panel) of *Rictor*$^{ste20}\Delta$ *myo2-mCherry* prototroph cells cultured to early log phase at 25ºC in EMMG. Myo2 foci can be seen to localise to foci at the cell equator and exhibit a dynamic behaviour over the next 3 hours, but fail to incorporate into a stable CAR structure. 3 min / frame.

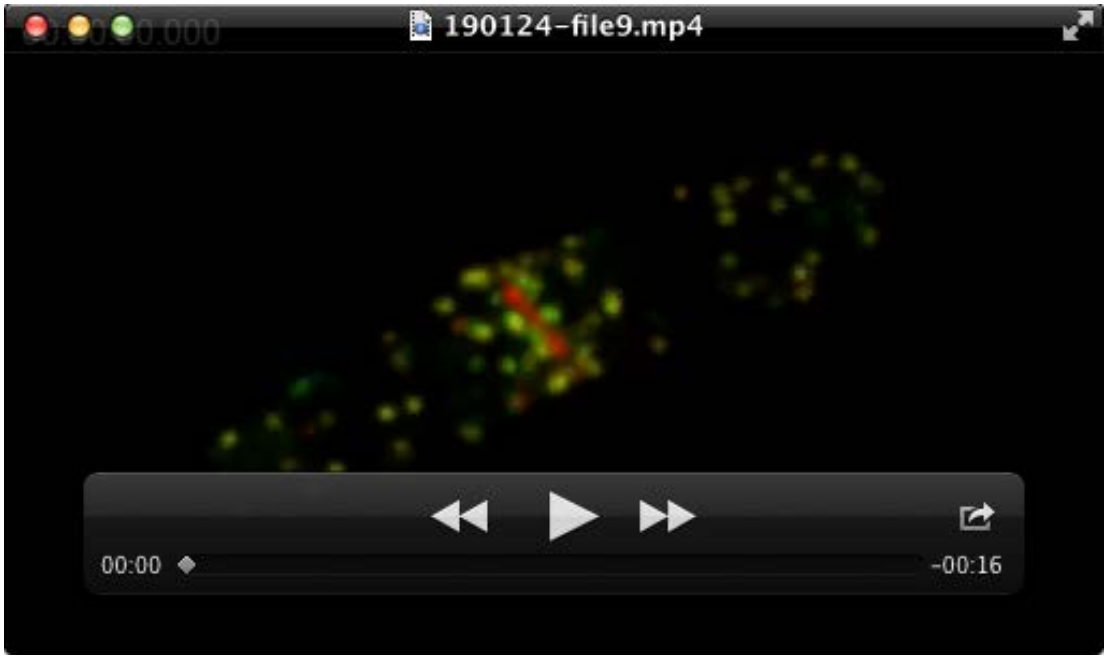
Journal of Cell Science • Supplementary information



Movie 4: Time lapse of mCherry fluorescence from 21-z slice maximum projections (upper panel) and transmitted light images (lower panel) of *Rictor*$^{ste20}\Delta$ *myo2-mCherry* prototroph cells cultured to early log phase at 25ºC in EMMG. At the start of the time-lapse a normal looking CAR constricts, but upon completion rather than disassembling, rings of Myo2 reform at the cell equator across the width of the cell, and remain associated with the cortex for more than 3 hours. 5 min / frame.

Movie 5: Time lapse of inverted 21 z slice maximum projections of tdTomato/ mCherry (left panel) and GFP signals (middle panel) and composite GFP (green) & red (magenta) fluorescence signals (right panel) of *Rictor*^{ste20}*-3GFP sid4-tdTomato myo2-mCherry* prototroph cells cultured to early log phase at 25ºC in EMMG. Rictor^{ste20} can be seen localised to the cell poles, and then redistributes to the cell equator during CAR formation. 3 min / frame.

Movie 6 Time lapse of mCherry (magenta) and YFP (yellow) fluorescence 21 z slice maximum projections of *myo2-mCherry YFP-myp2* prototroph cells. 3 min / frame.



Movie 7: Time lapse of mCherry (red) and GFP (green) fluorescence 21 z slice maximum projection composites of *myo2-mCherry* , *capZA*^{acp1}*-GFP* prototroph cells. 1.25 sec / frame.

Table S1. *Strains used in this study*

Strain #	Genotype	Source
JP3	*h*$^{-}$	Lab stock
JP350	*h*$^{+}$	Lab stock
JP1379	*ste20::kanMX6*	*ste20::kanMX6* from (Tatebe et al., 2010)
JP1377	*sin1::kanMX6*	*sin1::kanMX6* from (Tatebe et al., 2010)
JP598	*gad8::ura4*$^{+}$	*gad8::ura4*$^{+}$ from (Bimbo et al., 2005)
JP1942	*ste20::kanMX6 myo51-gfp:kanMX6*	This study
JP1994	*ste20::kanMX6 myo52-gfp:kanMX6*	This study
JP1999	*ste20::kanMX6 myo2-mCherry:hphMX6*	This study
JP2159	*ste20::kanMX6 myp2-YFP:kanMX6*	This study
JP2141	rpl42::cyhr(sP56Q)pku80::ura4 leu1.32	From (Fennessy et al., 2014)
JP2222	*acp1-NAT rpl42::cyhr(sP56Q) pku80::ura4 leu1.32*	This study
DM1903	*myo51-gfp:kanMX6*	This study
DM1923	*myo2-mCherry:hphMX6*	This study
DM1980	*myo52-gfp:kanMX6*	This study
DM2120	*myp2-YFP:kanMX6*	This study
DM1880	*myo51::ura4 ura4-d18*	This study
JP1919	*ste20-3gfp:kanMX6 h*$^{-}$	*ste20-3gfp:kanMX6* from (Tatebe et al., 2010)
DM1927	*ste20-3gfp:kanMX6 myo2-mCherry:hphMX6*	This study
DM2006	*ste20-3gfp:kanMX6 myo2-mCherry:hphMX6 myo51::ura4 ura4-d18*	This study
DM2102	*ste20-3gfp:kanMX6 myo2-mCherry:hphMX6 myp2::ura4 ura4-d18*	This study
DM2105	*ste20-3gfp:kanMX6 myo51-mCherry:hphMX6*	This study
DM1924	*myo51::ura4 ura4-d18 myo2-mCherry:hphMX6*	This study
DM2135	*myo51::ura4 kanMX6-Pmyp2-YFP-myp2 ura4-d18*	This study
DM824	*leu1::nmt41gfp-myo51-tail:ura4 ura4-d18*	This study
JP2191	*acp1::kanMX6*	*acp1::kanMX6* from (Kovar et al., 2005)
JP2169	*acp1::ura4*$^{+}$	*acp1::ura4*+ from (Kovar et al., 2005)
DM2122	*acp1::ura4 ste20-3gfp:kanMX6 myo2-mCherry:hphMX6 ura4-d18*	This study
DM2123	*acp1::ura4*$^{+}$ *myo2-mCherry:hphMX6 ura4-d18*	This study
DM2134	*acp1::ura4*$^{+}$ *kanMX6-Pmyp2-YFP-myp2 ura4-d18*	This study
DM2164	*acp1::ura4*$^{+}$ *myo51-gfp:kanMX6 ura4-d18*	This study
JP2487	*redlifeact:LEU2 leu1-32*	This study
JP2600	*ste20::kanMX6 redlifeact:LEU2 leu1-32*	This study

JP2343	*acp2-gfp:kanMX6*	*acp2-gfp:kanMX6* from (Kovar et al., 2005)
JP2199	*acp1-gfp:kanMX6*	This study
DM2216	*acp1-gfp:kanMX6 redlifeact:LEU2 leu1-32*	This study
DM2116	*acp1::kanMX6 redlifeact:LEU2 leu1-32*	This study
DM2154	*acp1-gfp:kanMX6 ste20::kanMX6*	This study
DM2146	*acp1-gfp:kanMX6 myo2-mCherry:hphMX6*	This study
DM2145	*acp1-gfp:kanMX6 myo51:ura4 ura4-d18*	This study
DM2156	*acp1-gfp:kanMX6 myp2::ura4 ura4-d18*	This study
JP2398	*acp1-AA-gfp:kanMX6*	This study
DM2264	*acp1-AA-gfp:kanMX6 myo2-mCherry:hphMX6*	This study
JP2438	*acp1-AA-gfp:kanMX6 ste20::kanMX6*	This study
JP2528	*acp1-AA-gfp:kanMX6 redlifeact:LEU2 leu1-32*	This study
DM2279	*acp1-AA-HA-kanMX6 kanMX6-Pmyp2-YFP-myp2 myo2-mCherry:hphMX6*	This study
JP2198	*acp1-HA:kanMX6*	This study
JP2441	*acp1-AA-HA:kanMX6*	This study
JP2388	*acp1-HA:kanMX6 acp2-gfp:kanMX6*	This study
JP2490	*acp1-AA-HA:kanMX6 acp2-gfp:kanMX6*	This study
DM2156	*acp1-gfp:kanMX6 myo2-mCherry:hphMX6 myp2::ura4 ura4-d18*	This study
DM2410	*myo2-mCherry:hphMX6 sid-tdTomato:hphMX6 ste20-gfp:kanMX6*	This study
DM2427	*myo2-mCherry:hphMX6 sid-tdTomato:hphMX6 ste20-gfp:kanMX6 myp2::ura4 ura4-d18*	This study
DM2414	*myo2-mCherry:hphMX6 cut12-gfp:ura4 ste20::kanMX6*	This study
DM2413	*acp1-AA-HA:kanMX6 cut12-gfp myo2-mCherry:hphMX6 ura4-d18*	This study
DM2408	*myo2-mCherry:hphMX6 kanMX6-Pmyp2-YFP-myp2*	This study
DM2415	*myo2-mCherry:hphMX6 kanMX6-Pmyp2-YFP-myp2 ste20::kanMX6*	This study
DM2270	*myo2-mCherry:hphMX6 kanMX6-Pmyp2-YFP-myp2 acp1::kanMX6*	This study
DM2415	*myo2-mCherry:hphMX6 acp1-gfp:kanMX6 ste20::kanMX6*	This study