

Table S1. Strains list.

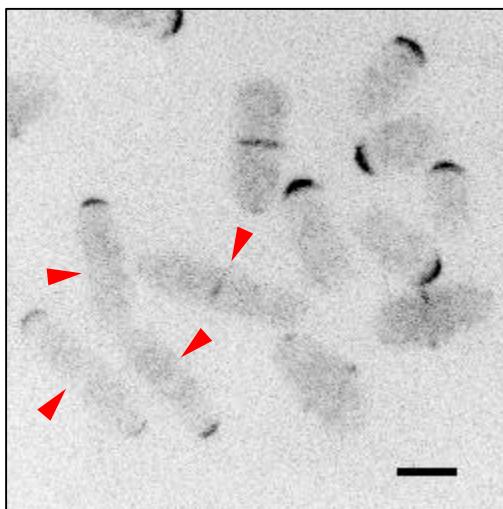
Strain	Genotype	Source
PN567	h+ ade6-704 leu1-32 ura4-d18	Paul Nurse
JX125	h90 Δscd1::ura4+ade6 leu1-32 ura4-d18 h210	(Hirota et al., 2003)
FV1218	Gef1-3xYFP:kanMX ade6-704 leu1-32 ura4-d18	(Das et al., 2009)
YSM947	Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	(Bendezu and Martin, 2013)
MBY3451	P3 nmt1:3HA-Shk1:KANr leu1-32 ura4-d18	(Loo and Balasubramanian, 2008)
YMD317	CRIB-3xGFP:ura4+ Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 his7+	(Wei et al., 2016)
YMD432	Δgef1::ura4+ pjk148-nmt41x:cdc42G12V-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD488	Δgef1::ura4+ CRIB-3xGFP:ura4+ Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18 his7+	(Wei et al., 2016)
YMD530	h90 Δscd1::ura4+ CRIB-3xGFP:ura4+ Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 his7+	(Wei et al., 2016)
YMD602	pjk148-nmt41x:cdc42G12V-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD635	Δfor3::kanMX Gef1-3xYFP:kanMX ade6-704 leu1-32 ura4-d18	This study
YMD761	Δgef1::ura4+ Scd1-3xGFP:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD773	Scd1-3xGFP:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD795	nmt1:3HA-Shk1 scd2-GFP:kanMX	This study
YMD840	Δgef1::ura4+ Scd2-GFP:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18	This study
YMD842	Scd2-GFP:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX kanMX ade6 leu1-32 ura4-d18	This study
YMD910	Gef1-NeonGreen:kanMX leu1-32 ura4-d18	This study
YMD926	Gef1-NeonGreen:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18	This study

YMD936	Δ gef1::ura4+ pjk148-nmt41x:cdc42G12V-leu1+ Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD994	pjk148-nmt41x:cdc42G12V-leu1+ Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD996	h90 Δ scd1::ura4+ Scd2-GFP:kanMX Rlc1-tdTomato:NATr ade6 leu1-32 ura4-d18	This study
YMD998	pjk148-nmt41x-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD1000	Δ gef1::ura4+ pjk148-nmt41x-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD1002	Δ gef1::ura4+ pjk148-nmt41x-leu1+ Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD1004	pjk148-nmt41x-leu1+ Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD1030	h90 Δ scd1::ura4+ Gef1-NeonGreen:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18	This study
YMD1067	h90 Δ scd2::ura4+ Gef1-NeonGreen:kanMX Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18 h210	This study
YMD1069	h90 Δ scd2::ura4+ Scd1-3xGFP:kanMX Rlc1-tdTomato:NATr ade6-m216 leu1-32 ura4-d18	This study
YMD1088	gef1s112a:kanMX Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study

REFERENCES:

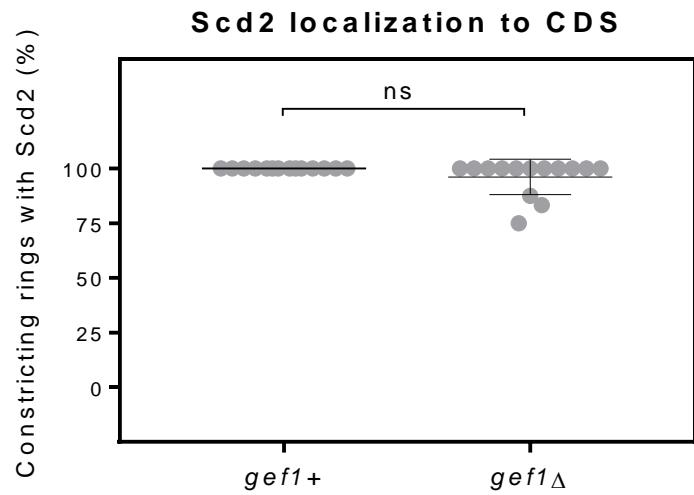
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Scd1-3xGFP



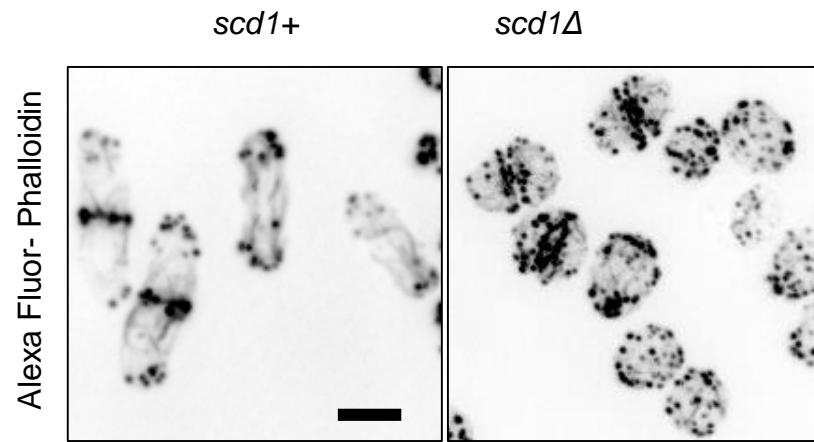
Supplemental Figure 1: The PAK kinase antagonizes Scd1 accumulation to limit Cdc42 activity. Scd1-3xGFP accumulation in *pak1+* and *nmt1:pak1* switch-off mutant cells. Cells were grown to an OD of 0.5 in minimal media + thiamine and mixed prior to imaging. Red arrow heads indicate *pak1+* cells. Images are inverted max projections. Scale bar=5 μ m.

Supplemental Figure 1



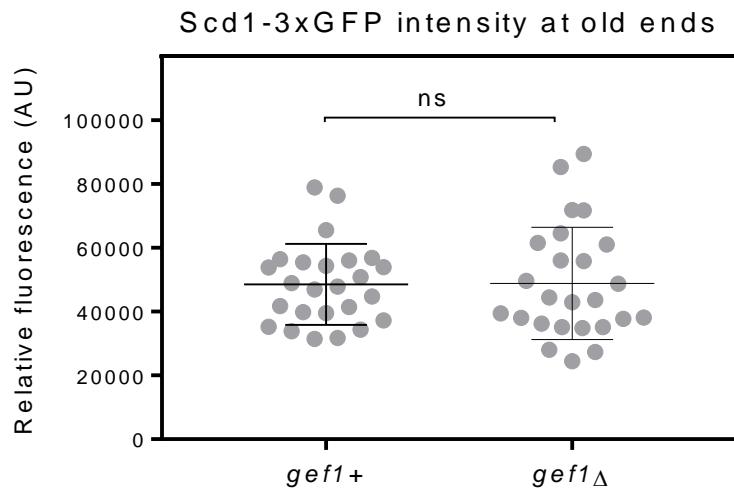
Supplemental Figure 2: Scd2 localization to the division site is delayed until the onset of ring constriction in *gef1 Δ* . Quantification of Scd2-GFP localization to constricting rings in *gef1+* and *gef1 Δ* . Cell division, CDS.

Supplemental Figure 2



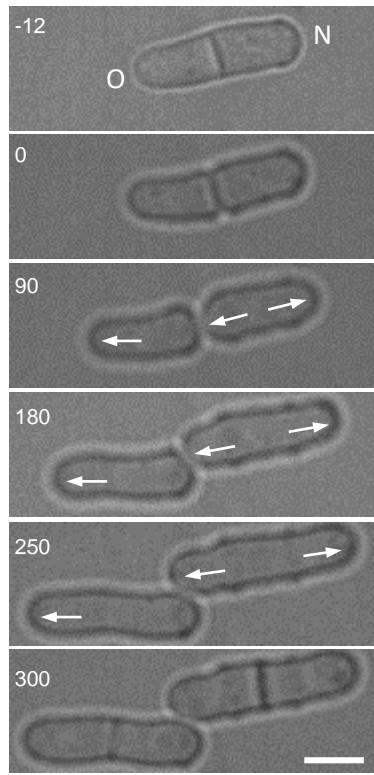
Supplemental Figure 3: The actin cytoskeleton is disrupted in *scd1Δ* cells. Actin organization in fixed *scd1+* and *scd1Δ* cells stained with Alexa Fluor-phalloidin. Scale bar=5 μ m.

Supplemental Figure 3



Supplemental Figure 4: Scd1 localization to the old end is not impaired in *gef1* Δ .
Quantification of Scd1-3xGFP localization to old ends in *gef1* $+$ and *gef1* Δ cells.

Supplemental Figure 4



Supplemental Figure 5: Growth pattern in the progeny of a monopolar *gef1Δ* cell. Image at -12 mins shows a monopolar *gef1Δ* cell that in the previous generation grew only from the old end (O) while the new end (N) did not have any prior history of growth. Time stamps are minutes elapsed since completion of division. Arrows indicate direction of growth at cell ends. Scale bar=5 μ m.

Supplemental Figure 5