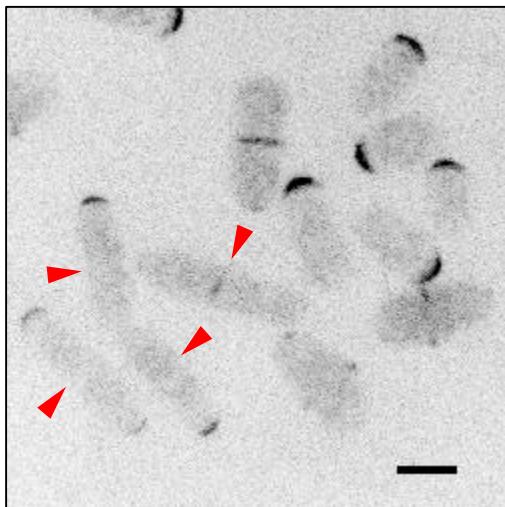
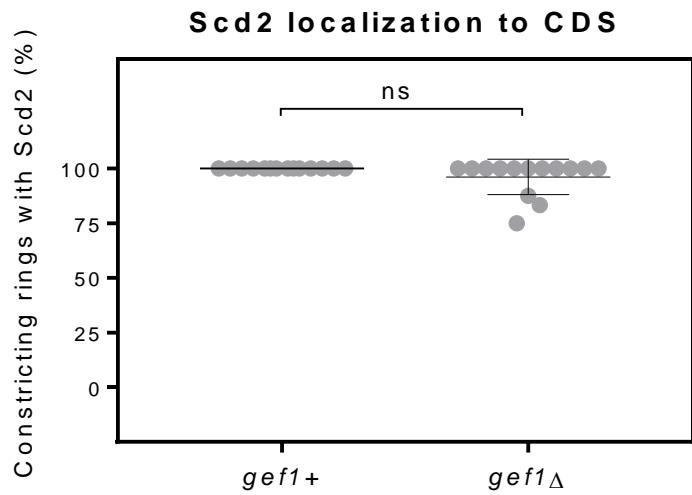


Scd1-3xGFP



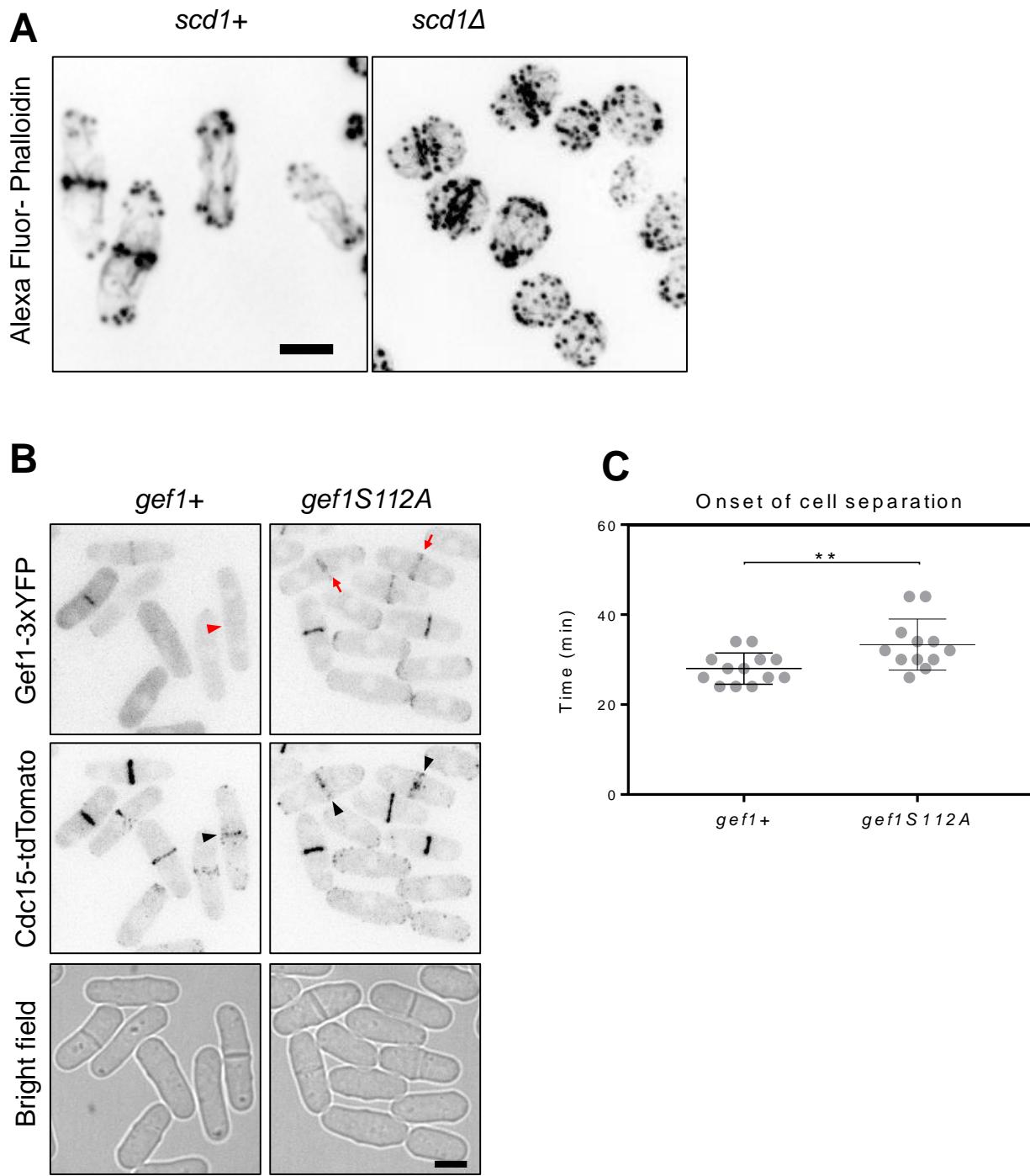
Supplemental Figure S1: The PAK kinase antagonizes Scd1 accumulation to limit Cdc42 activity. Scd1-3xGFP accumulation in *pak1+* and *nmt1:pak1* switch-off mutant cells. Cells were grown to an OD of 0.5 in minimal media + thiamine and mixed prior to imaging. Red arrow heads indicate *pak1+* cells. Images are inverted max projections. Scale bar=5 μ m.

Supplemental Figure 1



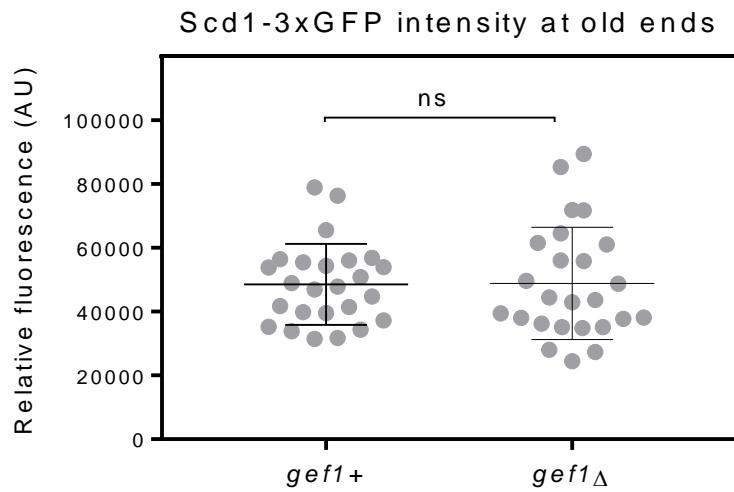
Supplemental Figure S2: Scd2 localization to the division site is delayed until the onset of ring constriction in *gef1* Δ . Quantification of Scd2-GFP localization to constricting rings in *gef1* $+$ and *gef1* Δ . (ns, not significant). Cell division site, CDS.

Supplemental Figure 2



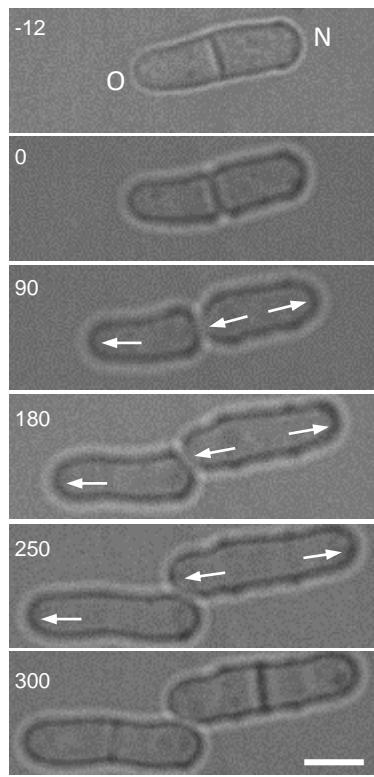
Supplemental Figure S3: (A) The actin cytoskeleton is disrupted in *scd1Δ* cells. Actin organization in fixed *scd1+* and *scd1Δ* cells stained with Alexa Fluor-phalloidin. **(B,C) Gef1 removal from the division site promotes cell separation.** (B) Gef1-3YFP and Gef1S112A-3YFP localization in cells expressing the ring marker Cdc15-tdTomato. Red arrow head indicates the absence of Gef1 from the division site after ring constriction. Red arrows indicate the presence of Gef1 at the division site after ring constriction. Black arrow heads mark the division site of cells that have completed ring constriction. **(C) Quantification of onset of cell separation after completion of ring constriction in *gef1+* and *gef1S112A* cells.** (**, p=0.009). Scale bar=5μm.

Supplemental Figure 3



Supplemental Figure S4: Scd1 localization to the old end is not impaired in *gef1Δ*.

Quantification of Scd1-3xGFP localization to old ends in *gef1+* and *gef1Δ* cells. n.s, not significant.



Supplemental Figure S5: 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

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 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	Δgef1::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 Δscd1::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	Δgef1::ura4+ pjk148-nmt41x-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD1002	Δgef1::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 Δscd1::ura4+ Gef1-NeonGreen:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18	This study
YMD1067	h90 Δscd2::ura4+ Gef1-NeonGreen:kanMX Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18 h210	This study
YMD1069	h90 Δscd2::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
YMD965	Δgef1::ura4+ gef1S112A-3xYFP:kanMX Cdc15-tdTomato:Nat ^r ade6 leu1-32 ura4-d18	This study
YMD208	Cdc15-tdTomato:NAT ^r Gef1-3xYFP:kanMX ade6 his7 ⁺	(Wei et al., 2016)

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