

**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

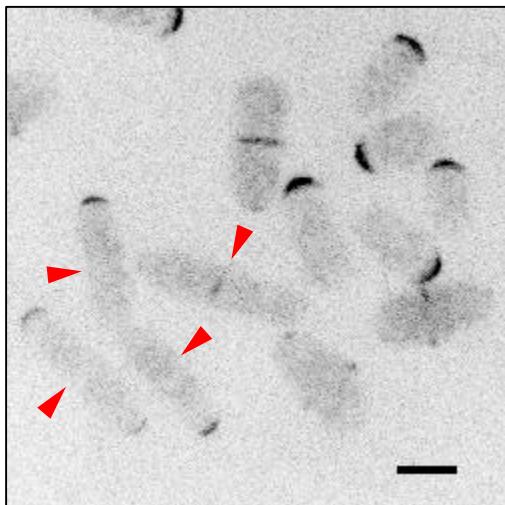
YMD936	$\Delta$ gef1::ura4+ pjk148-nmt41x:cdc42G12V-leu1+ Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD994	pjk148-nmt41x:cdc42G12V-leu1+ Scd1-3xGFP:kanMX ade6-m216 leu1-32 ura4-d18	This study
YMD996	h90 $\Delta$ scd1::ura4+ Scd2-GFP:kanMX Rlc1-tdTomato:NATr ade6 leu1-32 ura4-d18	This study
YMD998	pjk148-nmt41x-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD1000	$\Delta$ gef1::ura4+ pjk148-nmt41x-leu1+ CRIB-3xGFP:ura4+ ade6 leu1-32 ura4-d18	This study
YMD1002	$\Delta$ 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 $\Delta$ scd1::ura4+ Gef1-NeonGreen:kanMX Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18	This study
YMD1067	h90 $\Delta$ scd2::ura4+ Gef1-NeonGreen:kanMX Sad1-mCherry:kanMX ade6 leu1-32 ura4-d18 h210	This study
YMD1069	h90 $\Delta$ 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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- Das, M., D.J. Wiley, X. Chen, K. Shah, and F. Verde. 2009. The conserved NDR kinase Orb6 controls polarized cell growth by spatial regulation of the small GTPase Cdc42. *Curr Biol.* 19:1314-1319.
- Hirota, K., K. Tanaka, K. Ohta, and M. Yamamoto. 2003. Gef1p and Scd1p, the Two GDP-GTP exchange factors for Cdc42p, form a ring structure that shrinks during cytokinesis in *Schizosaccharomyces pombe*. *Mol Biol Cell.* 14:3617-3627.
- Loo, T.H., and M. Balasubramanian. 2008. *Schizosaccharomyces pombe* Pak-related protein, Pak1p/Orb2p, phosphorylates myosin regulatory light chain to inhibit cytokinesis. *J Cell Biol.* 183:785-793.

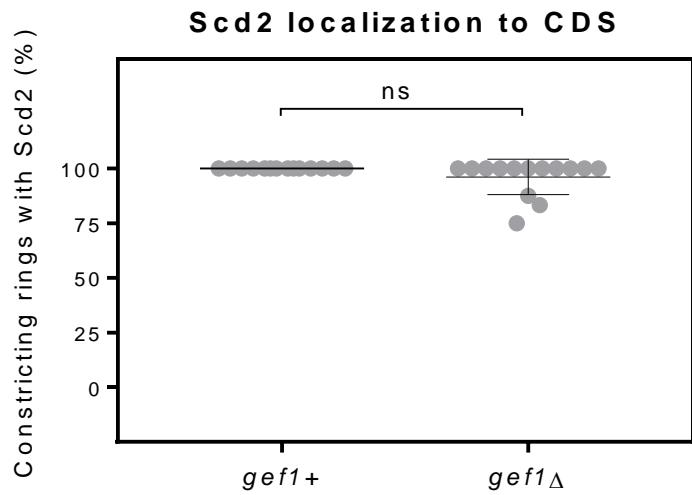
Wei, B., B.S. Hercyk, N. Mattson, A. Mohammadi, J. Rich, E. DeBruyne, M.M. Clark, and M. Das. 2016. Unique spatiotemporal activation pattern of Cdc42 by Gef1 and Scd1 promotes different events during cytokinesis. *Mol Biol Cell*. 27:1235-1245.

Scd1-3xGFP



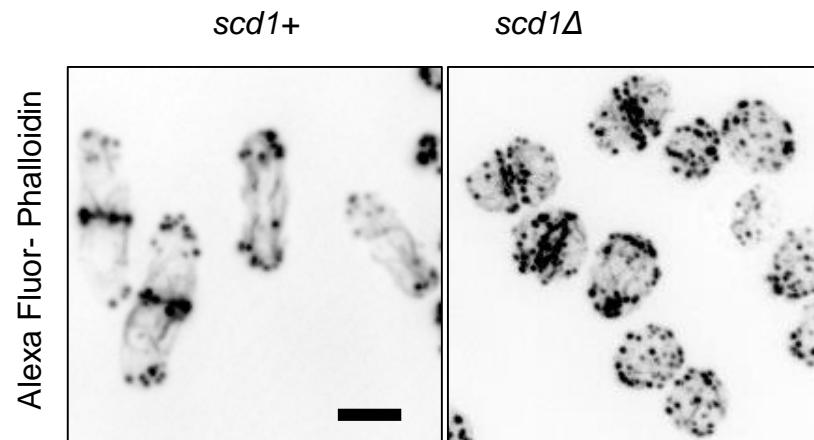
**Supplemental Figure 1: The PAK kinase antagonizes Scd1 accumulation to limit Cdc42 activity.** Scd1-3xGFP accumulation in *pak1*+ and *nmt1:pak1* switch-off mutant cells. Cells were grown to an OD of 0.5 in minimal media + thiamine and mixed prior to imaging. Red arrow heads indicate *pak1*+ cells.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

Supplemental Figure 1



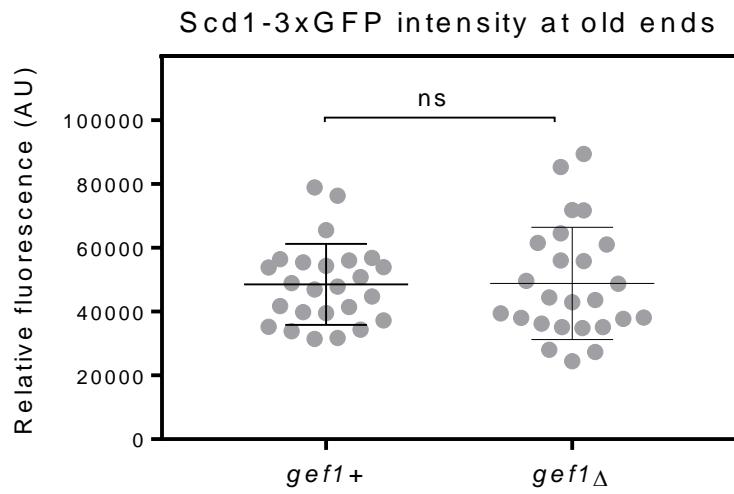
**Supplemental Figure 2: Scd2 localization to the division site is delayed until the onset of ring constriction in *gef1Δ*.** Quantification of Scd2-GFP localization to constricting rings in *gef1+* and *gef1Δ*. Cell division site abbreviated as CDS.

Supplemental Figure 2



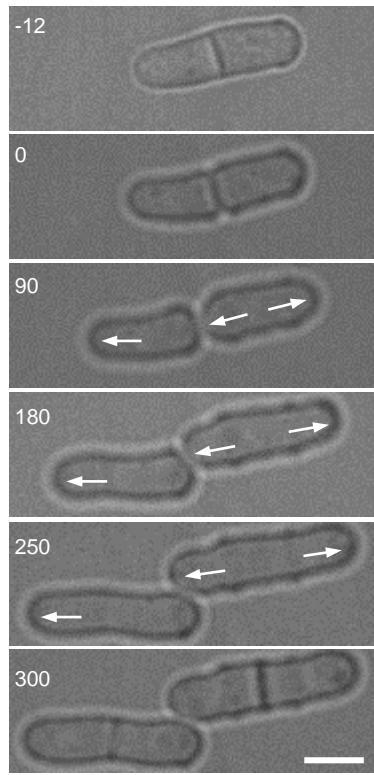
**Supplemental Figure 3: The actin cytoskeleton is disrupted in *scd1Δ* cells.** Actin organization in fixed *scd1+* and *scd1Δ* cells stained with Alexa Fluor-phalloidin. Scale bar=5μm.

Supplemental Figure 3



**Supplemental Figure 4: Scd1 localization to the old end is not impaired in *gef1Δ*.** Quantification of Scd1-3xGFP localization to old ends in *gef1+* and *gef1Δ* cells.

Supplemental Figure 4



**Supplemental Figure 5: Growth pattern in the progeny of a monopolar *gef1Δ* cell.** Time stamps are minutes elapsed since completion of division. Arrows indicate growth at cell poles. Scale bar=5 $\mu$ m.

Supplemental Figure 5