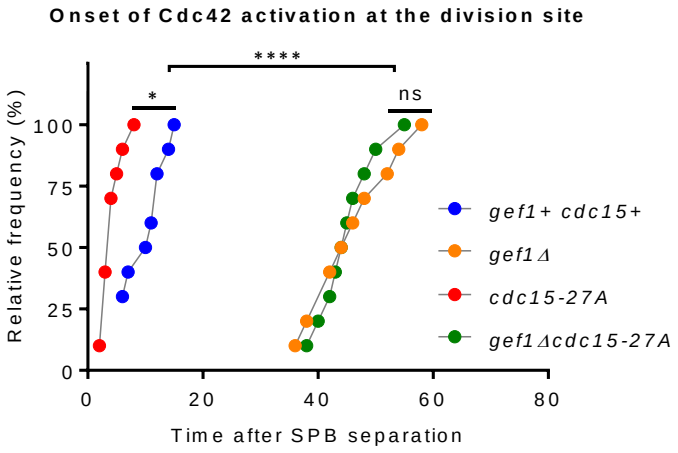
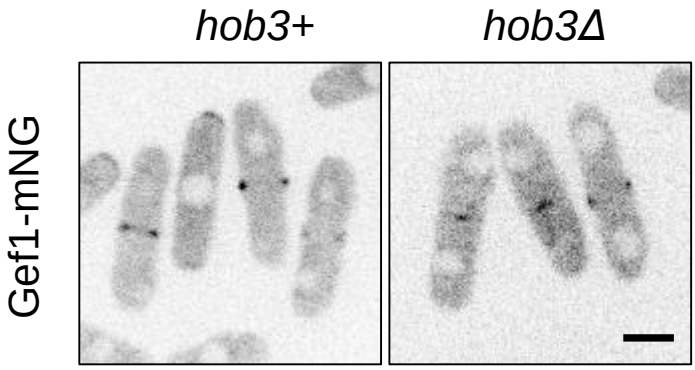


Supplemental Figure 1. Precocious Cdc42 activation at the division site in *cdc15-27A* cells is *gef1*-dependent. Quantification of Cdc42 activation at the division site from time lapse images the indicated genotypes expressing CRIB-3xGFP and Sad1-mCherry. ****, $p<0.001$, *, $p<0.05$, ns=not significant, one-way ANOVA with Tukey's multiple comparisons post hoc test.



Supplemental Figure 2. Gef1 localization is not impaired by loss of *hob3+*. Inverted medial plane images showing Gef1-mNG localization in the indicated genotypes. Scale bar represents 5µm.

Table S1. Strains list.		
Strain	Genotype	Source
PN567	*h+ ade6-704 leu1-32 ura4-D18*	Paul Nurse
YMD926	*Gef1-mNeonGreen:kanMX Rlc1-tdTomato NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-D18*	This study
FC2126	*pREP42:cdc12ΔC-GFP:ura4+ ade6-M216 leu1-32 ura4-D18 his7+*	(YONETANI AND CHANG 2010)
YMD1054	*pREP42:cdc12ΔC-GFP:ura4+ Gef1-tdTomato:kanMX ade6 leu1-32 ura4-D18*	This study
KGY1105	*sid2-250 ade6-M21X ura4-D18 leu1-32*	(BALASUBRAMANIAN *et al.* 1998)
KGY2090	*plo1-25*	(BAHLER *et al.* 1998)
YMD872	*Δmid1::ura4+ Gef1-3xYFP:kanMX ade6 leu1-32 ura4-D18*	This study
YMD844	*plo1-1 Gef1-3xYFP:kanMX ade6 leu1-32 ura4-D18*	This study
YMD847	*sid2-250 Gef1-3xYFP:kanMX ade6-M21X ura4-D18 leu1-32*	This study
YMD952	*plo1-25 Gef1-mNeonGreen:kanMX Rlc1-tdTomato NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-D18*	This study
YMD954	*sid2-250 Gef1-mNeonGreen:kanMX Rlc1-tdTomato NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-D18*	This study
YMD978	*mid1Δ Gef1-mNeonGreen:kanMX Rlc1-tdTomato NATr Sad1-mCherry:kanMX ade6-M21X ura4-D18 leu1-32*	This study
YMD317	*CRIB-3xGFP:ura4+ Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 his7+*	(WEI *et al.* 2016)
YMD488	*Δgef1::ura4+ CRIB-3xGFP:ura4+ Rlc1-tdTomato:NATr Sad1-mCherry:kanMX ade6 leu1-32 ura4-D18 his7+*	(WEI *et al.* 2016)
YMD133	*Cdc15-GFP:kanMX6 sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18*	(WEI *et al.* 2016)
YMD973	*cdc15ΔSH3-GFP:kanMX Gef1-tdTomato:kanMX Sad1-mCherry:kanMX ade6-M21X ura4-D18 leu1-32*	This study
YMD991	*Cdc15-GFP:kanMX Gef1-tdTomato:kanMX Sad1-mCherry:kanMX ade6-M21X ura4-D18 leu1-32*	This study
YMD929	*gef1S112A:ura4+ kanMX ade6-M210 ura4-D18 leu1-32*	This study
YMD710	*Δgef1::ura4+ ura4-D18 leu1-32*	This study

KGY7051	*cdc15ΔSH3 ade6-M210 leu1-34 ura4-D18*	(ROBERTS-GALBRAITH *et al.* 2009)
KGY10303	*cdc15-27A ade6-M21X leu1-32 ura4-D18*	(ROBERTS-GALBRAITH *et al.* 2010)
KGY9723	*cdc15-27D ade6-M21X leu1-32 ura4-D18*	(ROBERTS-GALBRAITH *et al.* 2010)
KGY10307	*cdc15-27A-GFP:kanMX ade6-M21X leu1-32 ura4-D18*	(ROBERTS-GALBRAITH *et al.* 2010)
YMD1155	*Cdc15-GFP:kanMX Gef1-tdTomato:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD1145	*cdc15-27A-GFP:kanMX Gef1-tdTomato:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD1243	*Cdc15-GFP:kanMX Scd1-tdTomato:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD1212	*cdc15-27A-GFP:kanMX Scd1-tdTomato:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD121	*CRIB-3xGFP:ura4+ Sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD1143	*cdc15-27A CRIB-3xGFP:ura4+ Sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD1242	*Δgef1::ura4+ cdc15-27A CRIB-3xGFP:ura4+ Sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18*	This study
YMD1097	*Δhob3::kanMX Gef1-mNeonGreen:kanMX ade6-M21X leu1-32 ura4-D18*	This study

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