SUPPLEMENTAL MATERIALS

Supplemental Table S1. *S. pombe* strains used in this study

Strain	Genotype	Source/reference
JW703	*h⁻ myp2-Δ2::kanMX6 ade6-M210 leu1-32 ura4-D18*	Lab stock
JW729	*h⁺ ade6-M210 leu1-32 ura4-D18*	Lab stock
JW739	*h⁻ ade6-M216 leu1-32 ura4-D18*	Lab stock
JW1825	*h⁺ blt1Δ::kanMX4 ade6 leu1-32 ura4-D18*	Kim *et al.*, 2010
JW1826	*h⁺ gef2Δ::kanMX4 ade6 leu1-32 ura4-D18*	Kim *et al.*, 2010
JW2255	*h⁺ mid1-366 ade6-M210 leu1-32 ura4-D18*	This study
JW2556	*h⁻ ade6-M210 leu1-32*	This study
JW2603	*h⁻ mid1(1-580)-6His-mECitrine-kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
JW2858	*gef2Δ::kanMX4 myp2-Δ2::kanMX6 ade6 leu1-32 ura4-D18*	This study
JW2923	*myp2-Δ2::kanMX6 rlc1-tdTomato-natMX6 leu1⁺::GFP-psy1 ade6 ura4*	This study
JW2948	*h⁺ gef2Δ::hphMX6 myp2-Δ2::kanMX6 rlc1-tdTomato-natMX6 leu1⁺::GFP-psy1 ade6 ura4*	This study
JW2972	*cdc11-136 gef2Δ::hphMX6 ade6 leu1-32 ura4-D18*	This study
JW2997	*h⁺ gef2-mECitrine-natMX6 ade6 leu1-32 ura4-D18*	This study
JW3009	*gef2Δ::hphMX6 sid2-250 ade6 leu1-32 ura4-D18*	This study
JW3066	*plo1.ts18::ura4⁺ kanˢ-mYFP-myo2 sad1-CFP-kanMX6 ade6 leu1-32 ura4*	This study
JW3067	*plo1.ts18::ura4⁺ gef2Δ::hphMX6 kanˢ-mYFP-myo2 sad1-CFP-kanMX6 ade6 leu1-32 ura4*	This study
JW3076	*plo1.ts18::ura4⁺ kanˢ-mYFP-4Gly-cdc15 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study
JW3077	*h⁻ gef2Δ::hphMX6 plo1.ts18::ura4⁺ kanˢ-mYFP-4Gly-cdc15 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study
JW3078	*h⁻ gef2Δ::hphMX6 plo1.ts18::ura4⁺ ade6 leu1-32 ura4-D18*	This study
JW3079	*h⁻ plo1.ts18::ura4⁺ ade6 leu1-32 ura4-D18*	This study
JW3184	*h⁻ gef2Δ::hphMX6 ade6 leu1-32*	This study
JW3185	*gef2Δ::hphMX6 kanˢ-mYFP-4Gly-cdc15 Patb2-CFP-atb2 ade6 leu1-32*	This study
JW3186	*kanˢ-mYFP-4Gly-cdc15 Patb2-CFP-atb2 ade6 leu1-32*	This study
JW3204	*h⁻ gef2-13Myc-hphMX6 ade6-M210 leu1-32 ura4-D18*	This study
JW3206	*h⁻ mid1-13Myc-hphMX6 ade6-M210 leu1-32 ura4-D18*	This study
JW3229	*gef2Δ::hphMX6 plo1.ts18::ura4⁺ 41nmt1-GFP-CHD (rng2)-leu1⁺ rlc1-tdTomato-natMX6 ade6 leu1-32 ura4-D18*	This study
JW3230	*plo1.ts18::ura4⁺ 41nmt1-GFP-CHD (rng2)-leu1⁺ rlc1-tdTomato-natMX6 ade6 leu1-32 ura4-D18*	This study
JW3237	*gef2Δ::hphMX6 plo1.ts18::ura4⁺ kanMX6-Purg1-mYFP-mid1 ade6 leu1-32 ura4-D18*	This study
JW3242	*gef2Δ::hphMX6 plo1.ts18::ura4⁺ ade6 leu1-32 ura4-D18* + pAP146 Pmid1-mid1-GFP integrated at leu1⁺	This study
JW3254	*h⁺ mid1-13Myc-kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
JW3255	*mid1-13Myc-kanMX6 gef2-mECitrine-natMX6 ade6 leu1-32 ura4-D18*	This study

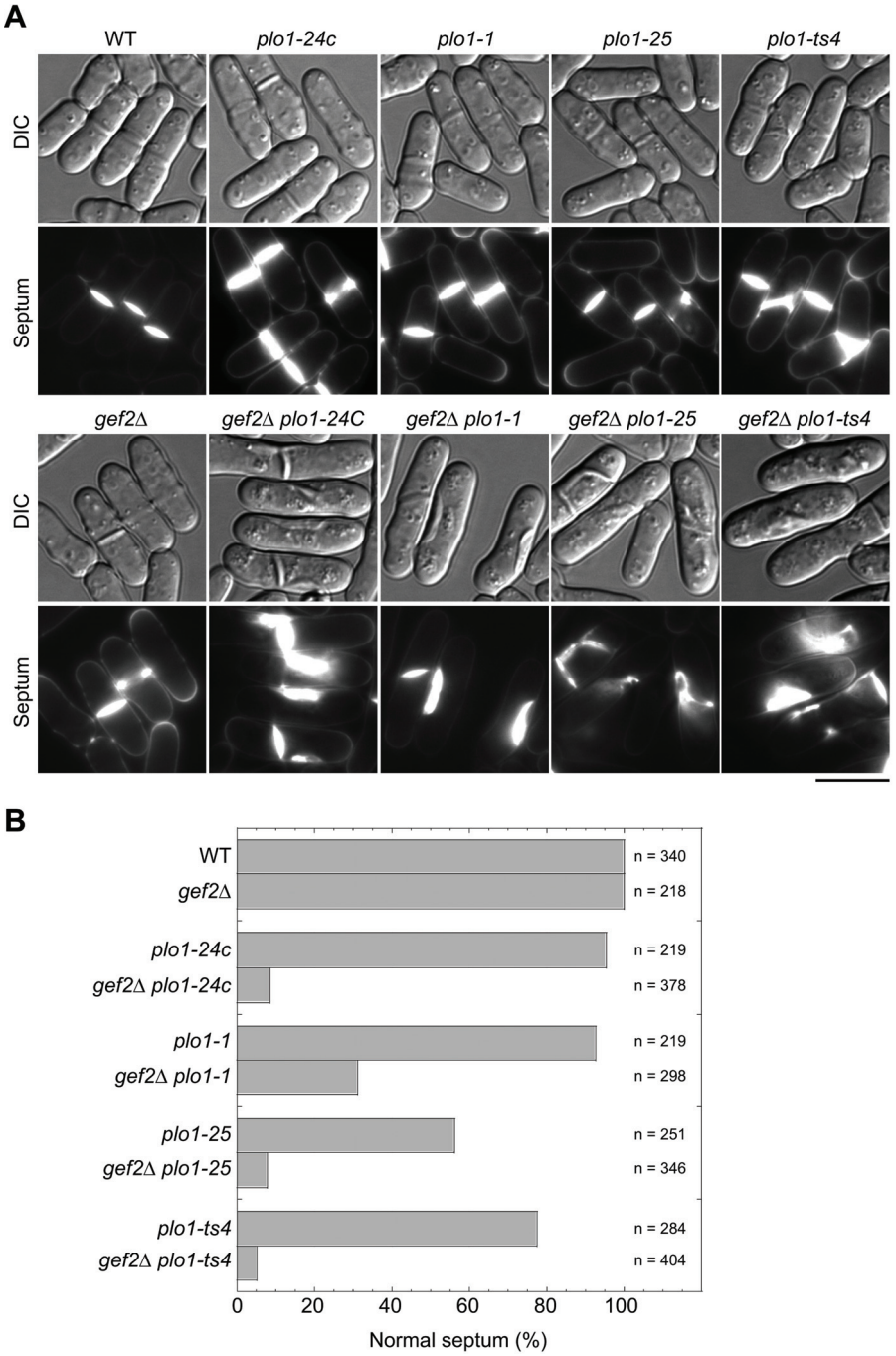
JW3276	*mid1-13Myc-kanMX6 ade6 leu1-32*	This study
JW3277	*gef2Δ::hphMX6 mid1-13Myc-kanMX6 ade6 leu1-32*	This study
JW3281	*gef2Δ::hphMX6 plo1.ts18::ura4*$^{+}$ *mid1-mECitrine-kanMX6 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study
JW3323	*mid1-mECitrine-kanMX6 ade6 leu1-32*	Laporte *et al.*, 2011
JW3324	*gef2Δ::hphMX6 mid1-mECitrine-kanMX6 ade6 leu1-32*	This study
JW3333	*cdr2Δ::kanMX6 gef2-mECitrine-natMX6 mid1-13Myc-hphMX6 ade6 leu1-32 ura4-D18*	This study
JW3364	*h*$^{-}$ *kanMX6-P81nmt1-mECitrine-mid1 ade6-210 leu1-32 ura4-D18*	This study
JW3383	*plo1.ts18::ura4*$^{+}$ *gef2Δ::hphMX6 mid1-mECitrine-kanMX6 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18* + pAP146 Pmid1-mid1-GFP integrated at leu1^{+}	This study
JW3426	*gef2Δ::hphMX6 plo1.ts18::ura4*$^{+}$ *mid1-cdr2-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3497	*h*$^{-}$ *gef2(Δ211-1101)-mECitrine-kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
JW3498	*h*$^{-}$ *gef2(Δ211-443)-mECitrine-natMX6 ade6-M216 leu1-32 ura4-D18*	This study
JW3546	*gef2Δ::hphMX6 kan*^{s}*-mYFP-myo2 sad1-CFP-kanMX6 ade6 leu1-32*	This study
JW3547	*kan*^{s}*-mYFP-myo2 sad1-CFP-kanMX6 ade6 leu1-32*	Lab stock
JW3550	*gef2Δ::hphMX6 plo1-24C ade6 leu1-32 ura4-D18*	This study
JW3551	*gef2Δ::hphMX6 plo1-1 ade6 leu1-32 ura4-D18*	This study
JW3552	*gef2Δ::hphMX6 plo1-25 ade6 leu1-32 ura4-D18*	This study
JW3553	*gef2Δ::hphMX6 plo1.ts4::ura4*$^{+}$ *ade6 leu1-32 ura4-D18*	This study
JW3554	*41nmt1-GFP-CHD (rng2)-leu1*$^{+}$ *rlc1-tdTomato-natMX6 ade6 leu1-32*	This study
JW3555	*gef2Δ::hphMX6 41nmt1-GFP-CHD (rng2)-leu1*$^{+}$ *rlc1-tdTomato-natMX6 ade6 leu1-32*	This study
JW3558	*gef2Δ::hphMX6 plo1.ts18::ura4*$^{+}$ *mid1-mECitrine-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3559	*plo1.ts18::ura4*$^{+}$ *mid1-mECitrine-kanMX6 ade6 leu1-32 ura4-D18 his2*	This study
JW3560	*gef2Δ::hphMX6 plo1.ts18::ura4*$^{+}$ *mid1-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3567	*mid1Δ::ura4*$^{+}$ *gef2Δ::kanMX4 ade6 leu1-32 ura4-D18* + pAP159 Pmid1 Helix* mid1-GFP integrated at leu1^{+}	This study
JW3568	*mid1Δ::ura4*$^{+}$ *gef2Δ::kanMX4 ade6 leu1-32 ura4-D18* + pAP167 Pmid1 NLS* mid1-GFP integrated at leu1^{+}	This study
JW3569	*mid1Δ::ura4*$^{+}$ *gef2Δ::kanMX4 ade6 leu1-32 ura4-D18* + pAP144 Pmid1 Cter-GFP integrated at leu1^{+}	This study
JW3570	*mid1Δ::ura4*$^{+}$ *gef2Δ::kanMX4 ade6 leu1-32 ura4-D18* + pSM26 Pmid1 GFP-Nter integrated at leu1^{+}	This study
JW3573	*rlc1-tdTomato-natMX6 leu1*$^{+}$*::GFP-psy1 ade6 ura4*	This study
JW3574	*gef2Δ::hphMX6 rlc1-tdTomato-natMX6 leu1*$^{+}$*::GFP-psy1 ade6*	This study

	ura4	
JW3575	*gef2Δ::hphMX6 cdr2Δ::kanMX6 mid1-mECitrine-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3576	*gef2Δ::hphMX6 plo1.ts18::ura4*$^+$ *cdr2Δ::kanMX6 mid1-mECitrine-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3577	*plo1.ts18::ura4*$^+$ *cdr2Δ::kanMX6 mid1-mECitrine-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3578	*cdr2Δ::kanMX6 mid1-mECitrine-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3623	*mid1Δ::ura4*$^+$ *gef2-13Myc-hphMX6 ade6 leu1-32 ura4-D18* + pSM26 Pmid1 GFP-Nter integrated at leu1$^+$	This study
JW3624	*mid1Δ::ura4*$^+$ *gef2-13Myc-hphMX6 ade6 leu1-32 ura4-D18* + pAP144 Pmid1 Cter-GFP integrated at leu1$^+$	This study
JW3628	*mid1Δ::ura4*$^+$ *rlc1-tdTomato-natMX6 ade6 leu1-32 ura4-D18* + pSM26 Pmid1 GFP-Nter integrated at leu1$^+$	This study
JW3629	*mid1Δ::ura4*$^+$ *gef2Δ::hphMX6 rlc1-tdTomato-natMX6 ade6 leu1-32 ura4-D18* + pSM26 Pmid1 GFP-Nter integrated at leu1$^+$	This study
JW3647	*cdr2Δ::kanMX6 mid1Δ::ura4*$^+$ *ade6 leu1-32 ura4-D18* + pSM26 Pmid1 GFP-Nter integrated at leu1$^+$	This study
JW3670	*gef2Δ::hphMX6 mid1(1-580)-6His-mECitrine-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW3705	*plo1.ts18::ura4*$^+$ *mid1Δ::kanMX4 ade6 leu1-32 ura4-D18* + pMA23 [pJK148-Pmid1-mid1(Δ300-350)-4GFP-stopnmt] integrated at leu1$^+$	This study
JW3721	*gef2Δ::kanMX4 mid1Δ::ura4*$^+$ *ade6 leu1-32 ura4-D18* + pAP163 Pmid1 Helix* NLS* mid1-GFP integrated at leu1$^+$	This study
JW3722	*plo1-24C ade6 leu1-32 ura4-D18*	This study
JW3723	*plo1-1 ade6 leu1-32 ura4-D18*	This study
JW3724	*plo1-25 ade6 leu1-32 ura4-D18*	This study
JW3825	*h- kanMX6-Pgef2-mECitrine-4Gly-gef2 ade6-M216 leu1-32 ura4-D18*	This study
JW3826	*h- kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ1-956) ade6-M216 leu1-32 ura4-D18*	This study
JW3827	*h- kanMX6-Pgef2-mECitrine-gef2(Δ1-443) ade6-M216 leu1-32 ura4-D18*	This study
JW3828	*h- kanMX6-Pgef2-mECitrine-gef2(Δ1-600) ade6-M216 leu1-32 ura4-D18*	This study
JW3829	*mid1-ΔF::ura4*$^+$ *gef2-mECitrine-natMX6 ade6 leu1-32 ura4-D18* + pMA23 [pJK148-Pmid1-mid1(Δ300-350)-13Myc-hphMX6] integrated at leu1 locus (but not leu1$^+$)	This study
JW3830	*mid1-ΔF::ura4*$^+$ *gef2-mECitrine-natMX6 ade6 leu1-32 ura4-D18* + pMA21 [pJK148-Pmid1-mid1(Δ400-450)-13Myc-hphMX6] integrated at leu1 locus (but not leu1$^+$)	This study
JW3843	*plo1.ts18::ura4*$^+$ *kanMX6-Pgef2-mECitrine-4Gly-gef2 ade6 leu1-32 ura4-D18*	This study
JW3846	*cdr2Δ::kanMX6 kanMX6-Pgef2- mECitrine-4Gly-gef2 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study

JW3848	*blt1Δ::kanMX4 kanMX6-Pgef2-mECitrine-4Gly-gef2 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study
JW3852	*kanMX6-Pgef2-mECitrine-4Gly-gef2 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study
JW3853	*kanMX6-Pgef2-mECitrine-4Gly-gef2 kan^{s}-Pmyo2-mCFP-myo2 Patb2-CFP-atb2 ade6 leu1-32 ura4-D18*	This study
JW3856	*plo1.ts18::ura4^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ1-956) ade6 leu1-32 ura4-D18*	This study
JW3857	*plo1.ts18::ura4^{+} kanMX6-Pgef2-mECitrine-gef2(Δ1-443) ade6 leu1-32 ura4-D18*	This study
JW3858	*plo1.ts18::ura4^{+} kanMX6-Pgef2-mECitrine -gef2(Δ1-600) ade6 leu1-32 ura4-D18*	This study
JW3949	*gef2Δ::hphMX6 mid1-366 ade6 leu1-32 ura4-D18*	This study
JW3950	*gef2Δ::hphMX6 kanMX6-P81nmt1-mECitrine-mid1 ade6 leu1-32 ura4-D18*	This study
JW3972	*h^{-} gef2Δ::hphMX6 ade6 leu1-32 ura4-D18*	This study
JW4226	*h^{+} kanMX6-Pgef2-tdTomato-4Gly-gef2 ade6 leu1-32 ura4-D18*	This study
JW4231	*rlc1-tdTomato-natMX6 mid1-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4232	*gef2Δ::hphMX6 rlc1-tdTomato-natMX6 mid1-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4233	*plo1.ts18::ura4^{+} rlc1-tdTomato-natMX6 mid1-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4234	*gef2Δ::hphMX6 plo1.ts18::ura4^{+} rlc1-tdTomato-natMX6 mid1-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4235	*kanMX6-Pgef2-tdTomato-4Gly-gef2 plo1-3GFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4236	*kanMX6-Pgef2-tdTomato-4Gly-gef2 mid1-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4237	*blt1Δ::kanMX4 kanMX6-Pgef2-mEGFP-4Gly-gef2 sad1-tdTomato-natMX6 ade6 leu1-32 ura4-D18*	This study
JW4238	*h^{-} gef2(Δ211-443)-Tgef2-kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
JW4241	*plo1.ts18::ura4^{+} gef2(Δ211-443)-Tgef2-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4307	*h^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ211-1101)-T$_{ADH1}$-natMX6 ade6 leu1-32 ura4-D18*	This study
JW4308	*h^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ444-1101)-T$_{ADH1}$-natMX6 ade6 leu1-32 ura4-D18*	This study
JW4309	*h^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ601-1101)-T$_{ADH1}$-natMX6 ade6 leu1-32 ura4-D18*	This study
JW4310	*h^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ957-1101)-T$_{ADH1}$-hphMX6 ade6 leu1-32 ura4-D18*	This study
JW4312	*plo1.ts18::ura4^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ211-1101)-T$_{ADH1}$-natMX6 ade6 leu1-32 ura4-D18*	This study
JW4313	*plo1.ts18::ura4^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ444-1101)-T$_{ADH1}$-natMX6 ade6 leu1-32 ura4-D18*	This study
JW4314	*plo1.ts18::ura4^{+} kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ601-*	This study

	1101)-T_{ADH1}-natMX6 ade6 leu1-32 ura4-D18	
JW4315	*plo1.ts18::ura4$^+$ kanMX6-Pgef2-mECitrine-4Gly-gef2(Δ957-1101)-T_{ADH1}-hphMX6 ade6 leu1-32 ura4-D18*	This study
JW4317	*plo1.ts18::ura4$^+$ mid1-13Myc-kanMX6 ade6 leu1-32 ura4-D18*	This study
JW4318	*gef2Δ::hphMX6 plo1.ts18::ura4$^+$ mid1-13Myc-kanMX6 ade6 leu1-32 ura4-D18*	This study
AP583	*h$^-$ mid1Δ::ura4$^+$ ade6-M216 leu1-32 ura4-D18* + pAP159 Pmid1 Helix* mid1-GFP integrated at leu1$^+$	Celton-Morizur *et al.*, 2004
AP621	*h$^-$ mid1Δ::ura4$^+$ ade6-M216 leu1-32 ura4-D18* + pAP144 Pmid1 Cter-GFP integrated at leu1$^+$	Celton-Morizur *et al.*, 2004
AP630	*h$^-$ mid1Δ::ura4$^+$ ade6-M216 leu1-32 ura4-D18* + pAP163 Pmid1 Helix* NLS* mid1-GFP integrated at leu1$^+$	Celton-Morizur *et al.*, 2004
AP734	*h$^-$ mid1Δ::ura4$^+$ ade6-M216 leu1-32 ura4-D18* + pAP167 Pmid1 NLS* mid1-GFP integrated at leu1$^+$	Celton-Morizur *et al.*, 2004
AP998	*h$^-$ mid1Δ::ura4$^+$ ade6-M216 leu1-32 ura4-D18* + pSM26 Pmid1 GFP-Nter integrated at leu1$^+$	Celton-Morizur *et al.*, 2004
AP1943	*h$^-$ mid1Δ::kanMX4 ade6-M216 leu1-32 ura4-D18* + pMA21 [pJK148-Pmid1-mid1(Δ400-450)-4GFP-stopnmt] integrated at leu1$^+$	Almonacid *et al.*, 2009
AP1977	*h$^-$ mid1Δ::kanMX4 ade6-M216 leu1-32 ura4-D18* + pMA23 [pJK148-Pmid1-mid1(Δ300-350)-4GFP-stopnmt] integrated at leu1$^+$	Almonacid *et al.*, 2009
FC164	*h$^-$ mid1-366*	Chang *et al.*, 1996
FY12587	*h$^-$ ade6-M210 ura4 leu1$^+$::GFP-psy1*	Nakamura *et al.*, 2001
IH1600	*h$^+$ plo1.ts18::ura4$^+$ ade6-M210 his2 leu1-32 ura4-D18*	MacIver *et al.*, 2003
IH1752	*h$^-$ plo1.ts4::ura4$^+$ ade6-M210 leu1-32 ura4-D18*	Tanaka *et al.*, 2001
JB201	*h$^+$ plo1-25 ura4-D18*	Bähler *et al.*, 1998a
JM346	*h$^+$ cdr2-mEGFP-kanMX6 ade6 leu1-32 ura4-D18*	Moseley *et al.*, 2009
JM578	*h$^+$ cdr2Δ::kanMX6 ade6 leu1-32 ura4-D18*	Moseley *et al.*, 2009
TP47	*h$^-$ cdc11-136 his7-366 leu1-32 ura4-D18*	Bezanilla *et al.*, 1997
YDM110	*h$^+$ plo1-1 leu1-32 ura4-D18*	Bähler *et al.*, 1998a
YDM114	*h$^+$ plo1-24C ade6-M210 leu1-32 ura4-D18*	Bähler *et al.*, 1998a
YDM429	*h$^+$ sid2-250 ade6-M210 leu1-32 ura4-D18*	Sparks *et al.*, 1999

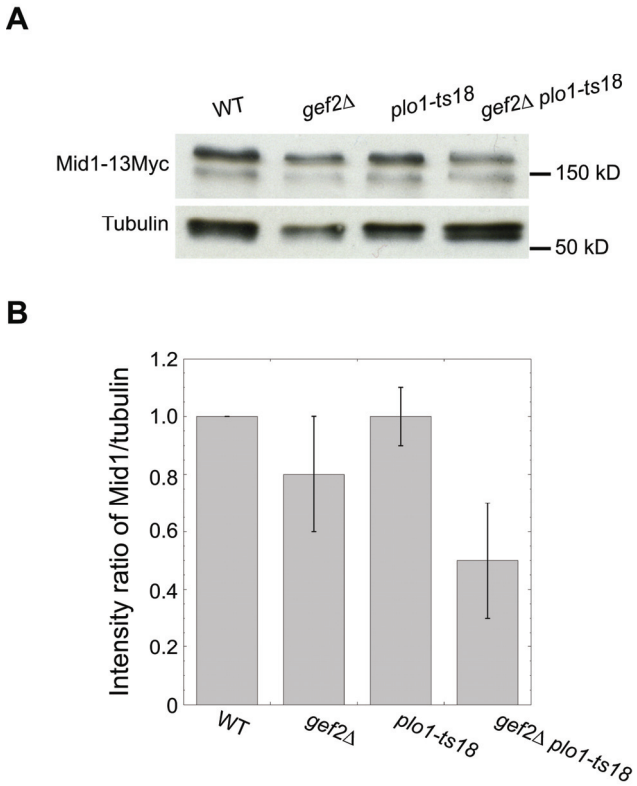
Supplemental Figure 1, Ye et al.



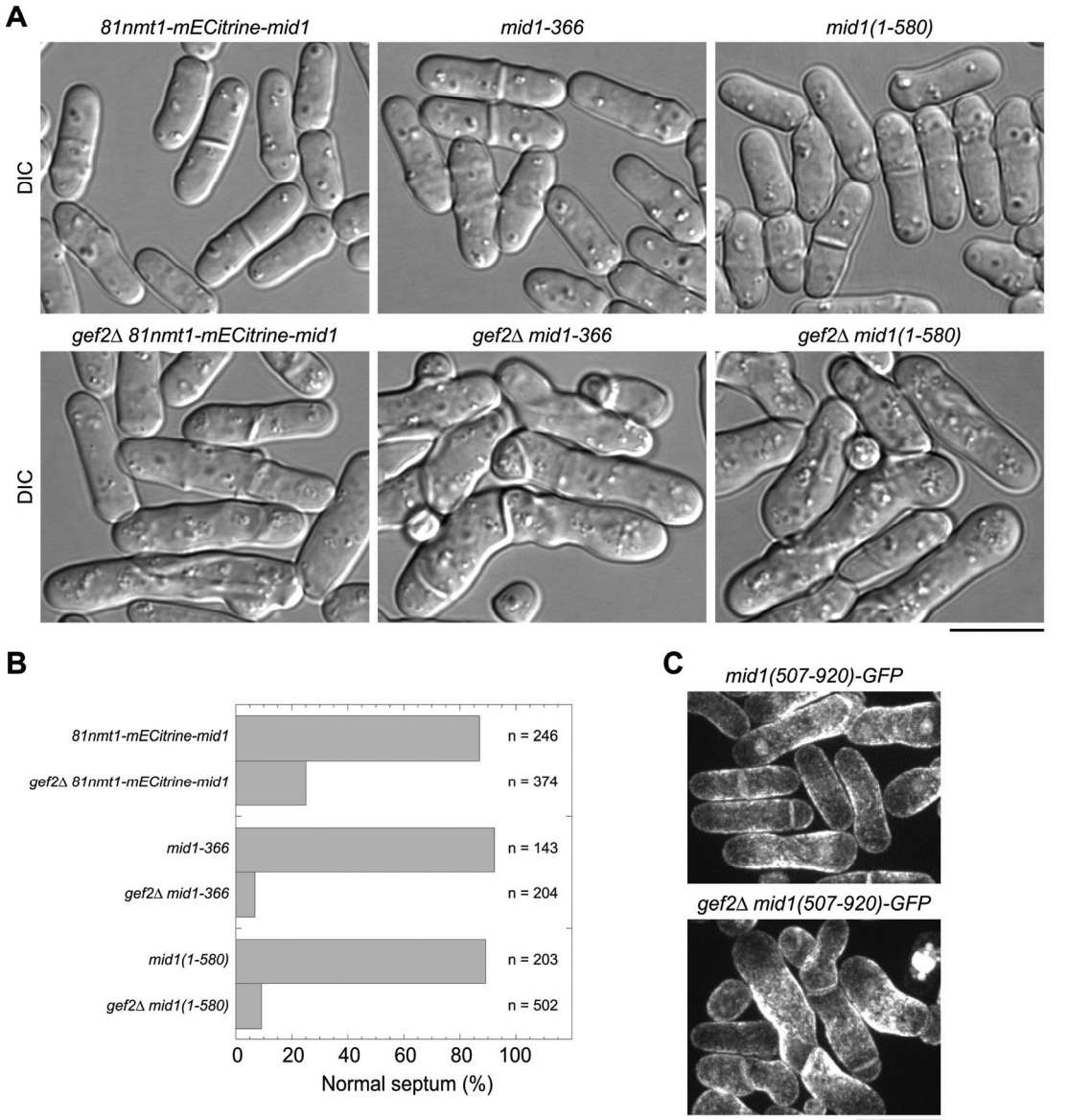
SUPPLEMENTAL FIGURE S1: Gef2 is required for division-site positioning when Polo kinase Plo1 function is compromised. (A and B) Phenotype and quantification of *gef2*Δ *plo1* mutants in septum positioning. 24 h after grown in YE5S medium at 25°C, cells (JW2556, JW3184, JW3722, JW3550, JW3723, JW3551, JW3724, JW3552, IH1752, and JW3553) were shifted to 32°C for 5 h before staining and imaging. (A) DIC and Calcoflour staining of septa. (B) Quantification of % normal septum observed in

Calcoflour-stained cells. In this and other figures, we defined a septum formed within the central 20% of the cell as a normal septum. Bars, 10 μm.

Supplemental Figure 2, Ye et al.



SUPPLEMENTAL FIGURE S2: Mid1 protein level revealed by western blotting. (A and B) Western blotting and quantification of Mid1-13Myc total protein level in cells. Cells (JW3276, JW3277, JW4317, and JW4318) were grown in YE5S at 25°C. (A) Western blotting of total extracted proteins using anti-Myc and anti-tubulin TAT1 antibodies (Woods *et al.*, 1989). (B) Quantification of Mid1 level. The intensity ratio (mean ± SD; n = 3) of Mid1/tubulin in each mutant relative to that in wt, which was set as 1.0, is shown.



SUPPLEMENTAL FIGURE S3: Gef2 is required for division-site positioning when Mid1 is depleted or Mid1 function is compromised. (A and B) Phenotype and quantification of *gef2*Δ *mid1* mutants in septum positioning. Cells (JW3364, JW3950, JW2255, JW3949, JW2603, and JW3670) were grown in YE5S at 25°C for 20 h before staining and imaging. (A) DIC image. (B) Quantification of % normal septum observed in Calcoflour-stained cells. (C) Gef2 is dispensable for the localization of Mid1(507-920) to the plasma membrane. AP621 and JW3569 cells were grown at 25°C. Bars, 10 μm.