

SUPPLEMENTAL MATERIAL

Figure S1. Ectopic dynein activity in *she1Δ* cells can cause unequal distribution of spindle poles at the end of mitosis. **(A)** Images from a time-lapse video featuring a *she1Δ GFP-TUB1* cell undergoing anaphase. One spindle pole leaves the bud and enters the mother cell. At the end of the Video, both spindle poles reside in the same cell. Scale bar, 5 μm. **(B)** Analysis of Chr III distribution in wild-type and *she1Δ* cells arrested in G1 with alpha factor. Cells that have faithfully segregated their chromosomes and nuclei possess only one Chr III in G1, which appears as a single fluorescent dot per cell. *she1Δ* cells frequently display two Chr III's, indicating missegregation of chromosomes likely caused by spindle curling seen in **(A)**. Scale bar, 4 μm.

Figure S2. Loss of She1 has a modest impact on dynein localization. **(A)** Two-color images showing Dyn1-3GFP (green) and mCherry-Tub1 (red) localization in wild-type and *she1Δ* cells. Scale bar, 5 μm. **(B)** Percentage of cells with Dyn1-3GFP present at an astral MT plus end. Loss of She1 slightly enhances Dyn1-3GFP localization at the plus end, but the change is not statistically significant for pre-anaphase and anaphase cells.

Figure S3. Expression levels of tagged dynactin subunits. **(A)** Western blots of Nip100-3GFP, Arp1-3GFP, Jnm1-3GFP and Pgk1p as a control from asynchronous wild-type or *she1Δ* whole cell extracts. **(B)** Whole cell extract from a JNM1-3GFP/JNM1 diploid immunoblotted with anti-Jnm1p antibody. The 3GFP tag has little effect on Jnm1 stability or expression.

Video S1. Spindle positioning in a wild-type cell expressing GFP-Tub1.

Video S2. An oscillating spindle in a *she1Δ* cell expressing GFP-Tub1.

Video S3. Spindle elongation in a wild-type cell expressing GFP-Tub1.

Video S4. Spindle elongation in a *she1Δ* cell expressing GFP-Tub1.

Video S5. Spindle elongation in a *she1Δ* cell expressing GFP-Tub1. Note that the two spindle poles reside in the mother (on the right) at the end of the Video.

Table S1. aMT dynamics and lengths in wild-type and *she1Δ* cells.

All values are mean \pm standard deviation with sample number (n)			
	Wild-type	<i>she1Δ</i>	P value
aMT growth rate ($\mu\text{m}/\text{min}$) (G1 & pre-anaphase)	1.37 \pm 0.5 (15)	1.46 \pm 0.7 (13)	0.69
aMT shrinkage rate ($\mu\text{m}/\text{min}$) (G1 & pre-anaphase)	2.54 \pm 0.9 (21)	2.34 \pm 1.0 (20)	0.49
Metaphase aMT length (μm) (<i>cdc20</i> arrest)	2.10 \pm 0.9 (18)	2.00 \pm 1.1 (23)	0.76
Anaphase aMT length (μm) (<i>cdc15</i> arrest)	3.56 \pm 1.4 (44)	4.18 \pm 1.6 (29)	0.11

Growth and shrinkage rates were determined by measuring the length of an aMT every 10 s in living cells expressing GFP-Tub1. aMT lengths were determined using indirect immunofluorescent labeling of endogenous alpha tubulin in fixed cells. Haploid cells contained either a *cdc20-3* or *cdc15-2* allele and were arrested in metaphase or anaphase, respectively, by incubating at 37° C for 2 hours.

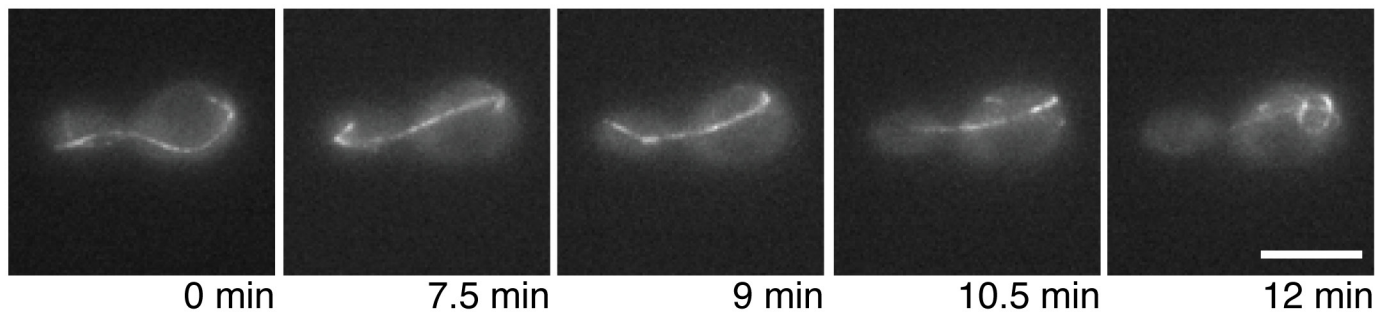
Table S2. Yeast Strains and Plasmids.

Yeast strains			
Strain name		genotype	source
JBY 1	<i>MATα</i>	<i>she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 65	<i>MATa</i>	<i>SPC42-mCherry::KanMX SHE1-3GFP::HIS3 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 39	<i>MATa</i>	<i>ura3-52::GFP-TUB1::URA3 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 70	<i>MATa</i>	<i>ura3-52::GFP-TUB1::URA3 she1Δ::LEU2 dyn1Δ::KanMX leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 75	<i>MATa</i>	<i>ura3-52::GFP-TUB1::URA3 she1Δ::LEU2 kar9Δ::KanMX leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 89	<i>MATa</i>	<i>DYN1-3GFP::HIS3 pHIS::mCherry-TUB1::URA3 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 87	<i>MATα</i>	<i>DYN1-3GFP::HIS3 pHIS::mCherry-TUB1::URA3 she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 98	<i>MATa</i>	<i>NIP100-3GFP::HIS3 she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 100	<i>MATα</i>	<i>NIP100-3GFP::HIS3 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 102	<i>MATa</i>	<i>ARP1-3GFP::HIS3 she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 104	<i>MATa</i>	<i>ARP1-3GFP::HIS3 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 106	<i>MATa</i>	<i>JNM1-3GFP::HIS3 she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 108	<i>MATa</i>	<i>JNM1-3GFP::HIS3 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 117	<i>MATa/α</i>	<i>ura3-52::pHIS::mCherry-TUB1::URA3/ura3-52; NIP100-3GFP::HIS3/NIP100-3GFP::HIS3; she1Δ::LEU2/she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 118	<i>MATa/α</i>	<i>ura3-52::pHIS::mCherry-TUB1::URA3/ura3-52; NIP100-3GFP::HIS3/NIP100-3GFP::HIS3 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 119	<i>MATa/α</i>	<i>ura3-52::pHIS::mCherry-TUB1::URA3/ura3-52; ARP1-3GFP::HIS3/ARP1-3GFP::HIS3; she1Δ::LEU2/she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 120	<i>MATa/α</i>	<i>ura3-52::pHIS::mCherry-TUB1::URA3/ura3-52; ARP1-3GFP::HIS3/ARP1-3GFP::HIS3 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 121	<i>MATa/α</i>	<i>ura3-52::pHIS::mCherry-TUB1::URA3/ura3-52; JNM1-3GFP::HIS3/JNM1-3GFP::HIS3; she1Δ::LEU2/she1Δ::LEU2 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 122	<i>MATa/α</i>	<i>pHIS::mCherry-TUB1::URA3/ura3-52; JNM1-3GFP::HIS3/JNM1-3GFP::HIS3</i>	this study
JBY 57	<i>MATα</i>	<i>she1Δ::LEU2 ura3-52::GFP-TUB1::URA3 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 10	<i>MATα</i>	<i>she1Δ::LEU2 HIS3::pCu-LacI-GFP leu2-3,112::lacO::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 16	<i>MATa</i>	<i>HIS3::pCu-LacI-GFP leu2-3,112::lacO::LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 136	<i>MATα</i>	<i>dyn1Δ::KanMX his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Res. Gen Collection
JBY 137	<i>MATα</i>	<i>kar9Δ::KanMX his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Res. Gen Collection

JBY 131	<i>MATa</i>	<i>JNM1-tdtomato::KanMX NIP100-3GFP::HIS3 LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 178	<i>MATa</i>	<i>JNM1-3HA::HIS3 NIP100-3GFP::HIS3 LEU2 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 180	<i>MATa/α</i>	<i>dyn1Δ::KanMX/dyn1(ko)::KanMX pHIS::mCherry-TUB1::URA3/ura3-52; NIP100-3GFP::HIS3/NIP100-3GFP::HIS3; she1Δ::LEU2/she1(KO)::LEU2</i>	this study
JBY 181	<i>MATa/α</i>	<i>dyn1Δ::KanMX/dyn1Δ::KanMX pHIS::mCherry-TUB1::URA3/ura3-52; NIP100-3GFP::HIS3/NIP100-3GFP::HIS3</i>	this study
JBY 184	<i>MATa</i>	<i>SHE1-GFP::HIS3 pHIS::mCherry-TUB1::URA3 leu2-3,112 lys2-801 his3Δ200 ura3-52</i>	this study
JBY 213	<i>MATa/α</i>	<i>LDB18-3GFP::HIS3/LDB18-3GFP::HIS3; pHIS::mCherry-TUB1::URA3/ura3-52 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 214	<i>MATa/α</i>	<i>LDB18-3GFP::HIS3/LDB18-3GFP::HIS3; pHIS::mCherry-TUB1::URA3/ura3-52; she1(KO)::LEU2/she1(KO)::LEU2 leu2-3,112 lys2-801 his3Δ200</i>	this study
JBY 175	<i>MATa/α</i>	<i>pHIS::mCherry-TUB1::URA3/+; JNM1-3GFP::HIS3/+ LEU2 leu2-3,112 lys2-801 his3Δ200</i>	this study
Plasmids			
Plasmid name			source
pAK011		pRS306-mCherry-TUB1	Khmelinskii et al., 2007
pYS47		3GFP::HIS3	Wong et al., 2007
		PFA6a::tdTomato::KanMX	Shaner et al., 2004

Figure-S1 (Barnes)

A *she1* Δ TUB1-GFP



B

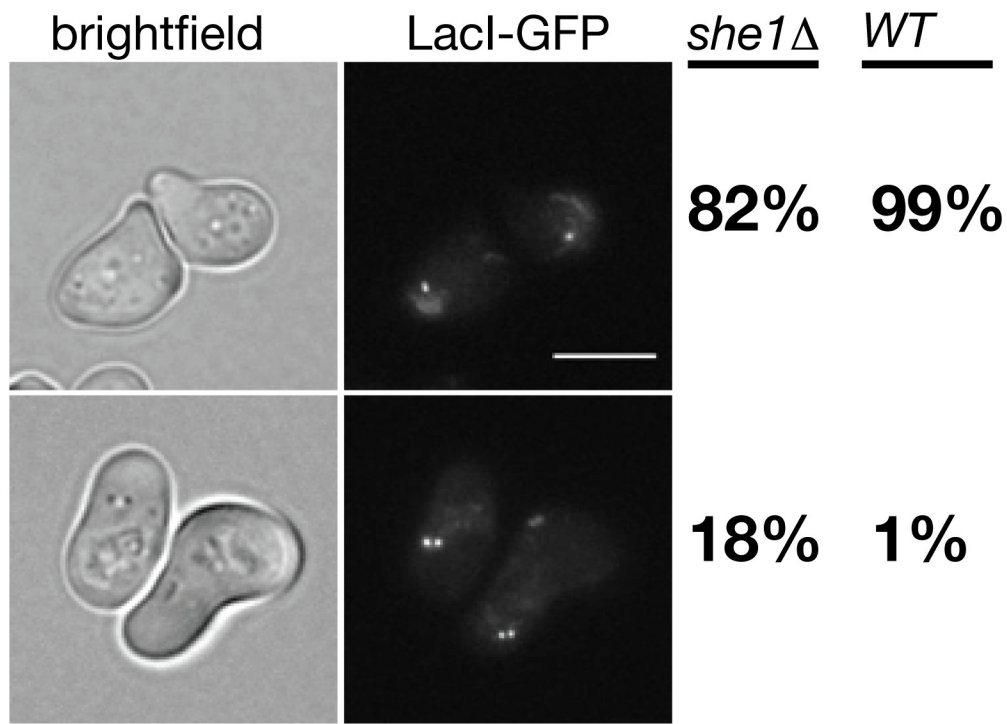


Figure-S2(Barnes)

A

Dyn1-3GFP
mCherry-Tub1

WT

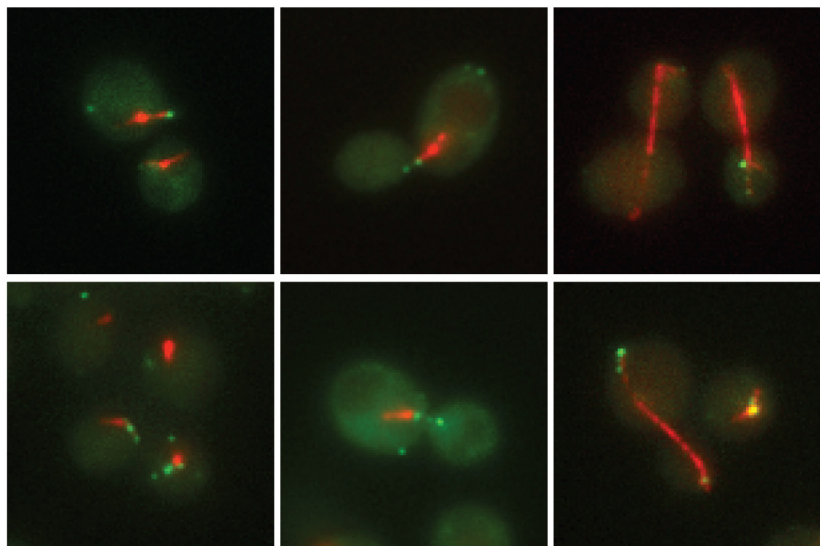
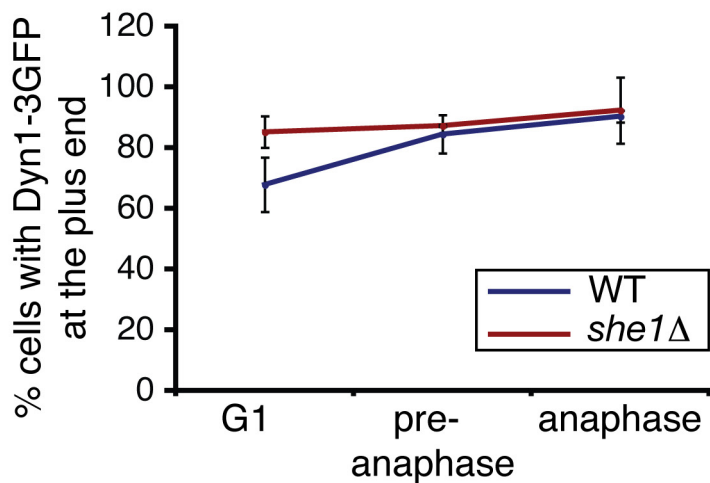
she1 Δ **B**

Figure-S3(Barnes)

