

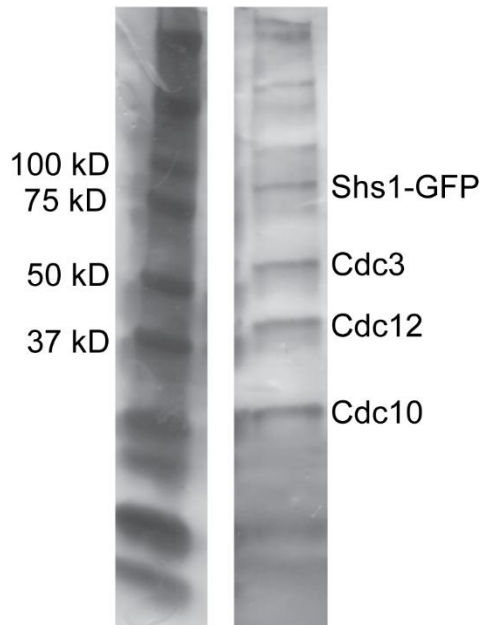
Supplemental Materials

Molecular Biology of the Cell

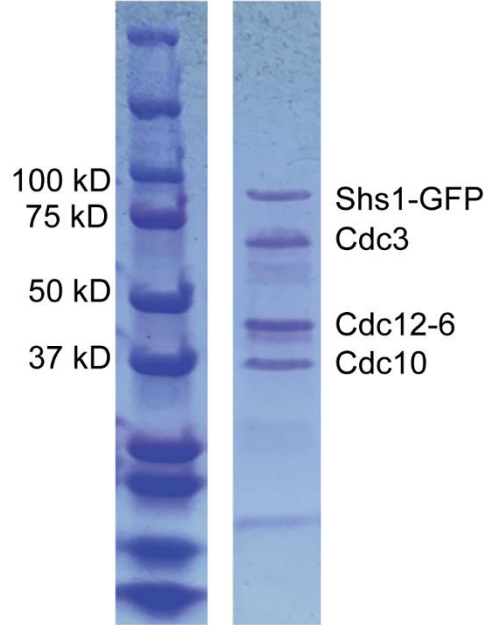
Woods *et al.*

A

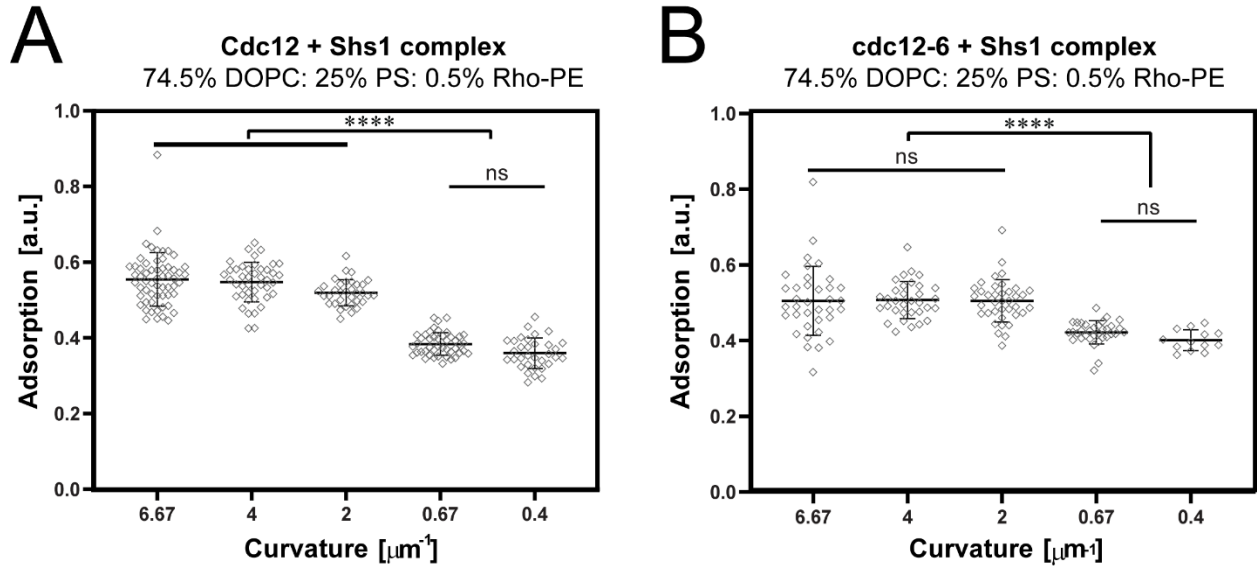
Cdc12 + Shs1-capped complex

**B**

Cdc12-6 Shs1-capped complex

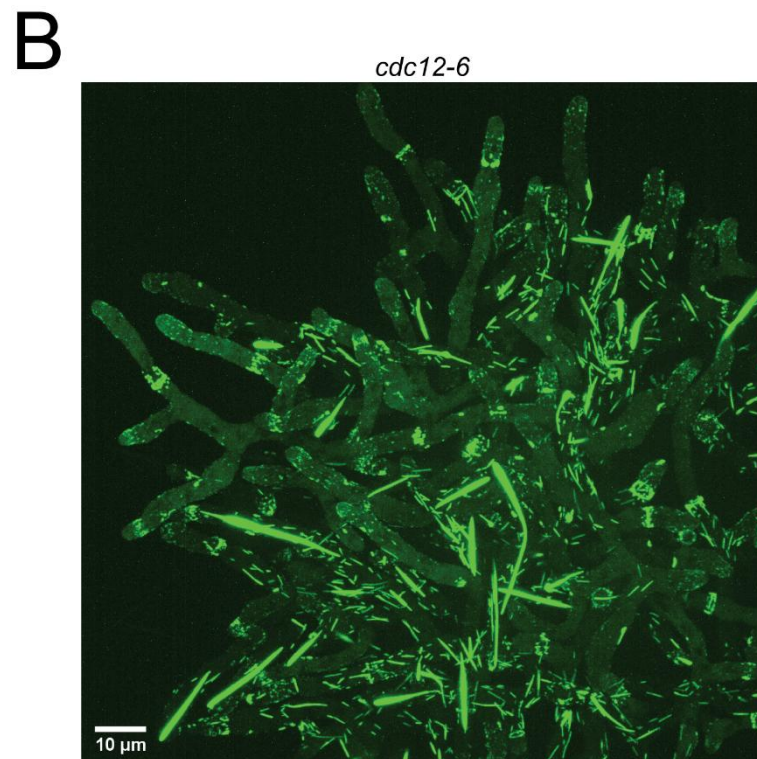
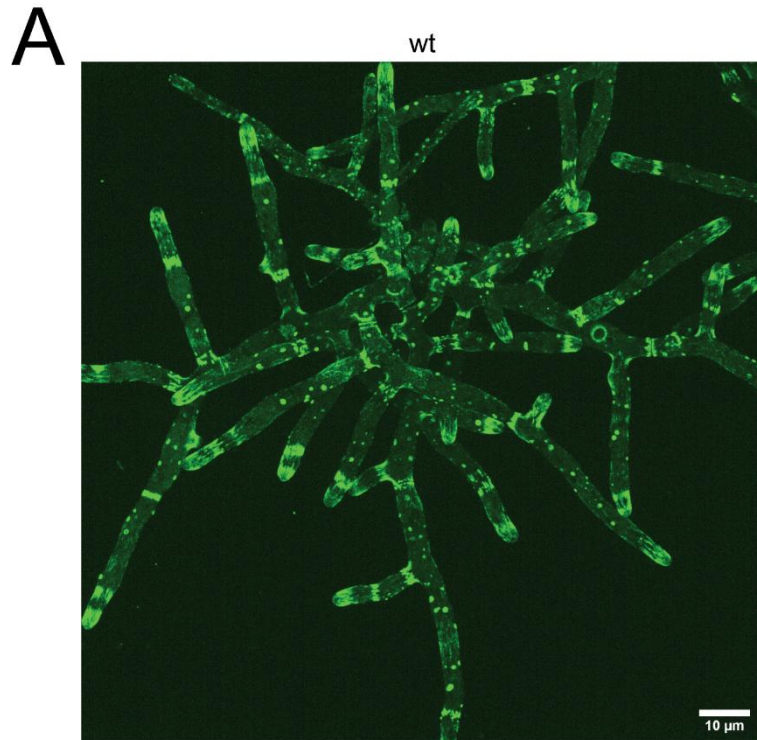
**Supplementary Figure 1. Purification of septin complexes**

- A. Silver stain of an SDS-PAGE Shs1-capped wild-type septin complex.
- B. Coomassie blue stain of an SDS-PAGE Shs1-capped cdc12-6 septin complex.



Supplementary Figure 2. Septin and AH domain curvature preference on different membrane compositions

- A. Cdc12 + Shs1 septin complex adsorption onto different membrane curvatures (composition is 74.5% DOPC, 25% PS, 0.5% Rhodamine-PE). Bar represents mean, error bar represents stand deviation of the mean (with >30 beads of each curvature measured across three replicates). Adsorption on membrane curvatures of 6.7, 4, or 2 μm^{-1} was greater than adsorption on curvatures of 0.67 or 0.4 μm^{-1} ****($p < 0.0001$). **ns**, adsorption was not significantly different.
- B. Shs1 septin complex with mutant cdc12-6 adsorption onto different membrane curvatures (composition is 74.5% DOPC, 25% PS, 0.5% Rhodamine-PE). Bar represents mean, error bar represents stand deviation of the mean (with >30 beads of each curvature measured across three replicates). Adsorption on membrane curvatures of 6.7, 4, or 2 μm^{-1} was greater than adsorption on curvatures of 0.67 or 0.4 μm^{-1} ****($p < 0.0001$). **ns**, adsorption was not significantly different.



Supplementary Figure 3. Comparing septin structures in wild-type and *cdc12-6* mutant *Ashbya gossypii* cells

A-B. Maximum projection images of wild-type cells (Cdc11a-GFP) and *cdc12-6* (*cdc12-6*-GFP) mutants grown at permissive temperature for 16-20 hours. Scale bars, 10μm.

Table S1. Yeast strains used in this study.

STRAIN	RELEVANT GENOTYPE	SOURCE
AGY411	MATa/ α <i>shs1::HIS3/SHS1 cdc12-6/CDC12 CDC3-mCherry:LEU2/CDC3 BEM1-GFP:LEU2/BEM1</i>	Daniel Lew
AGY436	MATa/ α <i>shs1^{Δ488-551}-GFP:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY441	MATa/ α <i>SHS1-GFP:HIS3/SHS1</i>	This study
AGY444	MATa/ α <i>shs1^{Δ341-551}-GFP:HIS3/SHS1</i>	This study
AGY450	MATa/ α <i>shs1^{Δ488-551}-GFP:HIS3/SHS1</i>	This study
AGY453	MATa/ α <i>shs1^{Δ506-551}-GFP:HIS3/SHS1</i>	This study
AGY460	MATa/ α <i>SHS1-GFP:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY462	MATa/ α <i>shs1^{Δ341-551}-GFP:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY463	MATa/ α <i>shs1^{Δ488-551}-GFP:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY464	MATa/ α <i>shs1^{Δ506-551}-GFP:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY478	MATa/ α <i>cdc12-6-3xHA:TRP1/CDC12</i>	This study
AGY479	MATa/ α <i>cdc12-6-(SpoVM-AH)-3xHA:TRP1/CDC12</i>	This study
AGY481	MATa/ α <i>cdc12-6-(SHS1⁴⁸⁸⁻⁵⁰⁵)-3xHA:TRP1/CDC12</i>	This study
AGY482	MATa/ α <i>cdc12-6-(RVS161-AH)-3xHA:TRP1/CDC12</i>	This study
AGY484	MATa/ α <i>cdc12-6-(RVS167-AH)-3xHA:TRP1/CDC12</i>	This study
AGY493	MATa/ α <i>shs1^{Δ523-551}:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY494	MATa <i>shs1^{Δ523-551}:HIS3 cdc12-6</i>	This study
AGY496	MATa/ α <i>shs1^{Δ341-551}:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY498	MATa/ α <i>shs1^{Δ488-551}:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY500	MATa/ α <i>shs1^{Δ506-551}:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY502	MATa/ α <i>shs1^{Δ508-551}:HIS3/SHS1 cdc12-6/CDC12</i>	This study
AGY508	MATa <i>shs1^{Δ488-551}:HIS3 cdc12-6</i>	This study
AGY512	MATa <i>shs1^{Δ506-551}:HIS3 cdc12-6</i>	This study
AGY516	MATa <i>shs1^{Δ508-551}:HIS3 cdc12-6</i>	This study
AGY524	MATa <i>cdc12-6 CDC3-mCherry:LEU2 bar1::URA3</i>	This study
AGY533	MATa/ α <i>cdc12^{ΔAH}-3xHA:TRP1/CDC12</i>	This study
AGY534	MATa/ α <i>cdc12^{Δ400-407}-3xHA:TRP1/CDC12</i>	This study
AGY537	MATa/ α <i>cdc12^{ΔAH}-(SpoVM-AH)-3xHA:TRP1/CDC12</i>	This study
AGY538	MATa/ α <i>cdc12^{ΔAH}-(SHS1⁴⁸⁸⁻⁵⁰⁵)-3xHA:TRP1/CDC12</i>	This study
AGY540	MATa/ α <i>cdc12^{ΔAH}-(RVS167-AH)-3xHA:TRP1/CDC12</i>	This study
AGY541	MATa/ α <i>cdc12^{ΔAH}-(S.p.RITC-AH)-3xHA:TRP1/CDC12</i>	This study
AGY542	MATa/ α <i>cdc12^{ΔAH}-(SHS1⁴⁸⁸⁻⁵⁰⁷)-3xHA:TRP1/CDC12</i>	This study
AGY548	MATa/ α <i>shs1^{Δ488-507}-GFP:HIS3/SHS1 cdc12-6/CDC12</i>	This study

Yeast strains were in the YEF473 genetic background (*his3-Δ200, leu2-Δ1, lys2-801, trp1-Δ63, ura3-5*; Bi and Pringle, 1996).

Table S2. Plasmids used in this study.

NAME	DESCRIPTION	SELECTION MARKER
AGB 548	pMVB133 ScCDC3/ ScSHS1-GFP	CAM
AGB 710	pMVB128 6-HIS-TEV-ScCDC12/ScCDC10	AMP
AGB 1055	pRSET-A-His-F2-2xCDC12-AHD-GFP	AMP
AGB 1084	pUC57 2xScShs1AH-GFP	AMP
AGB 1204	pET-duet-6HIS-TEV-ScCDC12-6/ScCDC10	AMP
AGB1205	pET-duet-6HIS-TEV-ScCDC12-6-SpoVM/ScCDC10	AMP
AGB 1388	pMVB133 ScCDC3/ ScSHS1 ^{ΔAH} -GFP	CAM