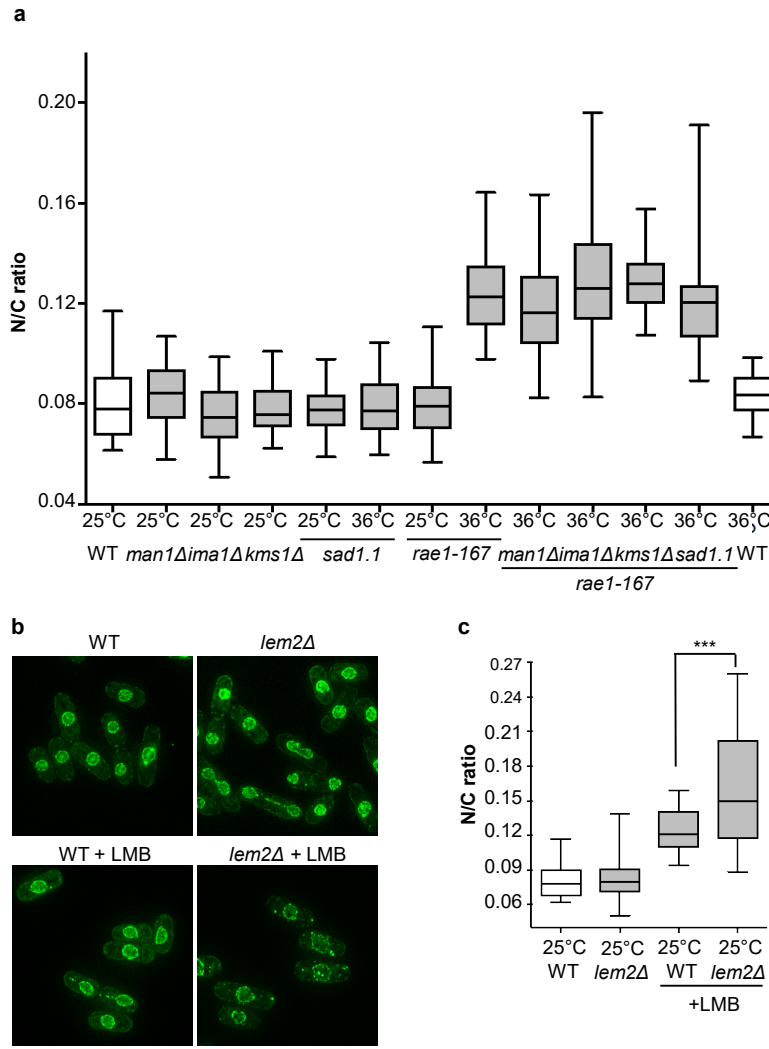


Nuclear membrane protein Lem2 regulates nuclear size through membrane flow

Kume *et. al.*



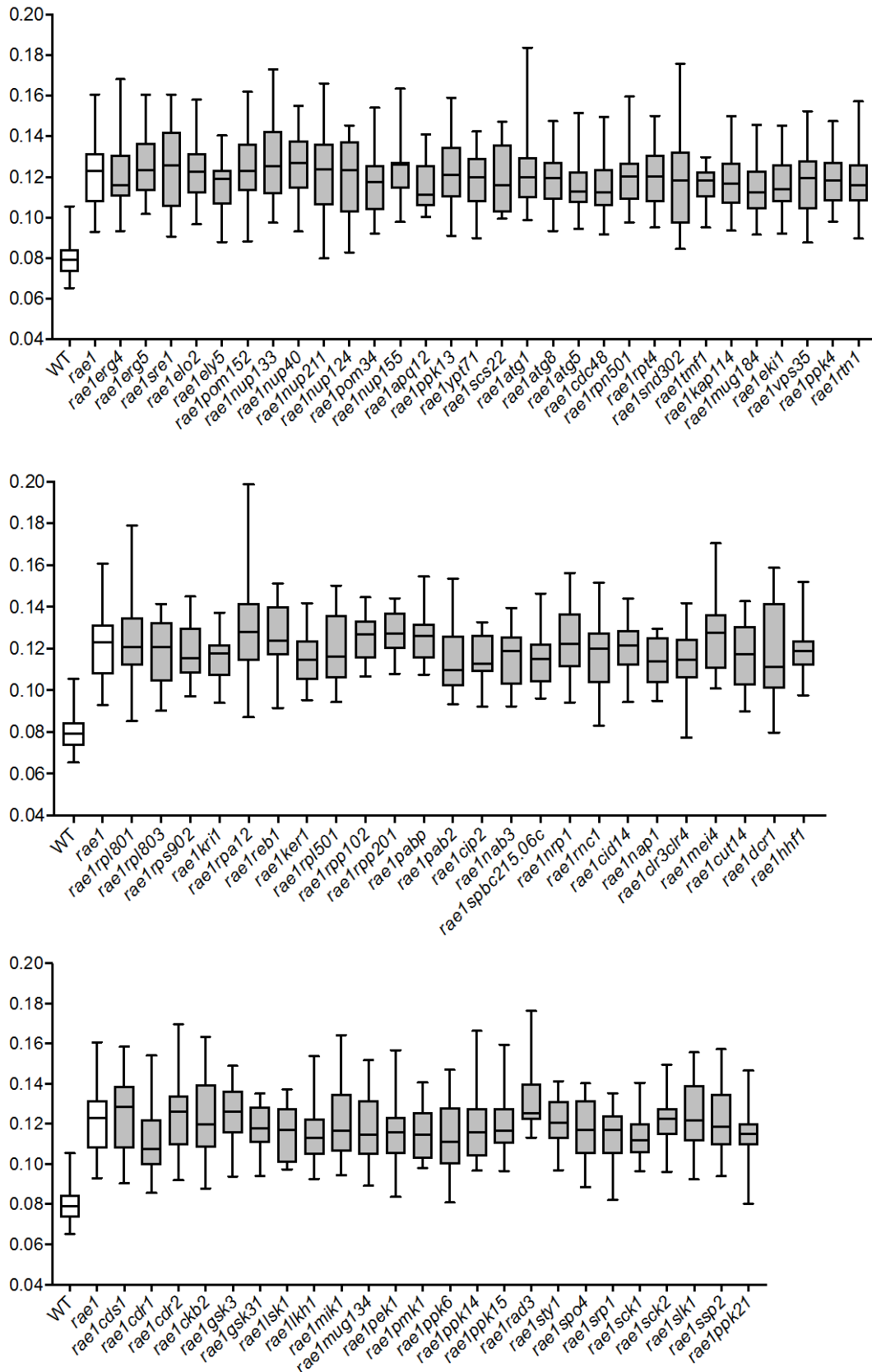
### Supplementary Figure 1: Lem2 deletion enhances nuclear size enlargement

(a) N/C ratio of wild type (WT), *man1Δ*, *ima1Δ*, *kms1Δ*, *sad1.1*, *rae1-167*, *rae1-167 man1Δ*, *rae1-167 ima1Δ*, *rae1-167 kms1Δ* and *rae1-167 sad1.1* cells (25°C) shifted to the indicated temperature for four hours ( $n > 30$  cells). Two-tailed Mann Whitney tests: *man1Δ* ( $n = 43$  cells), *ima1Δ* ( $n = 36$  cells), *kms1Δ* ( $n = 32$  cells) and *sad1.1* (36°C) ( $n = 31$  cells) against WT ( $n = 35$  cells),  $P = 0.1574$ ,  $0.1530$ ,  $0.7695$  and  $0.0761$  respectively; *rae1-167 man1Δ* ( $n = 34$  cells), *rae1-167 ima1Δ* ( $n = 42$  cells), *rae1-167 kms1Δ* ( $n = 31$  cells) and *rae1-167 sad1.1* ( $n = 35$  cells) against *rae1-167* ( $n = 43$  cells),  $P = 0.1282$ ,  $0.3742$ ,  $0.1609$  and  $0.2217$  respectively. The corresponding dot plot is available in Supplementary Fig. 10a.

(b) Images of the nuclear envelope (Cut11-GFP, green) of WT and *lem2Δ* cells (25°C) untreated or treated with 100 ng/ml leptomycin B (+ LMB) for three hours. Maximum intensity projections shown. Scale bar: 5  $\mu$ m.

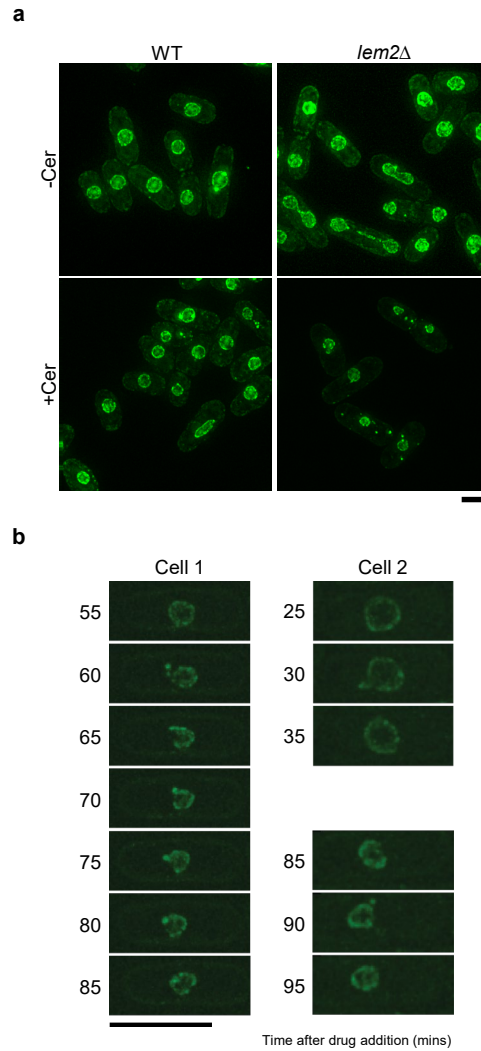
(c) N/C ratio of WT and *lem2Δ* cells (25°C) untreated or treated with 100 ng/ml leptomycin B (+LMB) for three hours ( $n > 30$  cells). Two-tailed Mann Whitney tests:  $**P = 0.0013$ , WT + LMB ( $n = 32$  cells) against *lem2Δ* + LMB ( $n = 32$  cells); *lem2Δ* ( $n = 52$  cells) against WT ( $n = 35$  cells),  $P = 0.8062$ . The corresponding dot plot is available in Supplementary Fig. 10b.

In all box-and-whiskers diagrams, boxes indicate median and upper and lower quartile and whiskers indicate range of data.

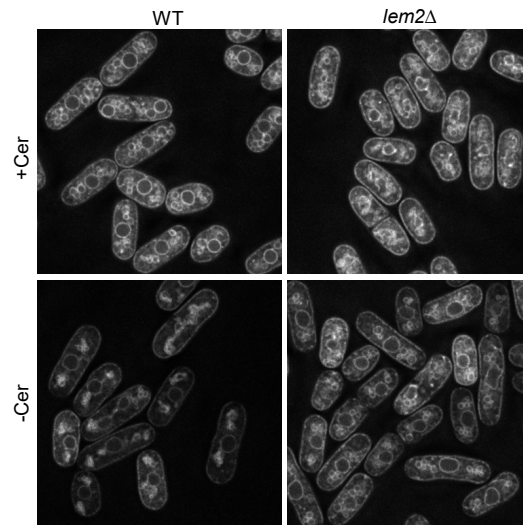


**Supplementary Figure 2: Double mutant analysis of *rae1-167* with proteins reported to localise to the nucleus, the endoplasmic reticulum and /or other organelles**

N/C ratio of WT, *rae1-167* and indicated double mutants. Cells grown at 25°C were shifted to 36°C for four hours ( $n \geq 20$  cells). In box-and-whiskers diagrams, boxes indicate median and upper and lower quartile and whiskers indicate range of data.



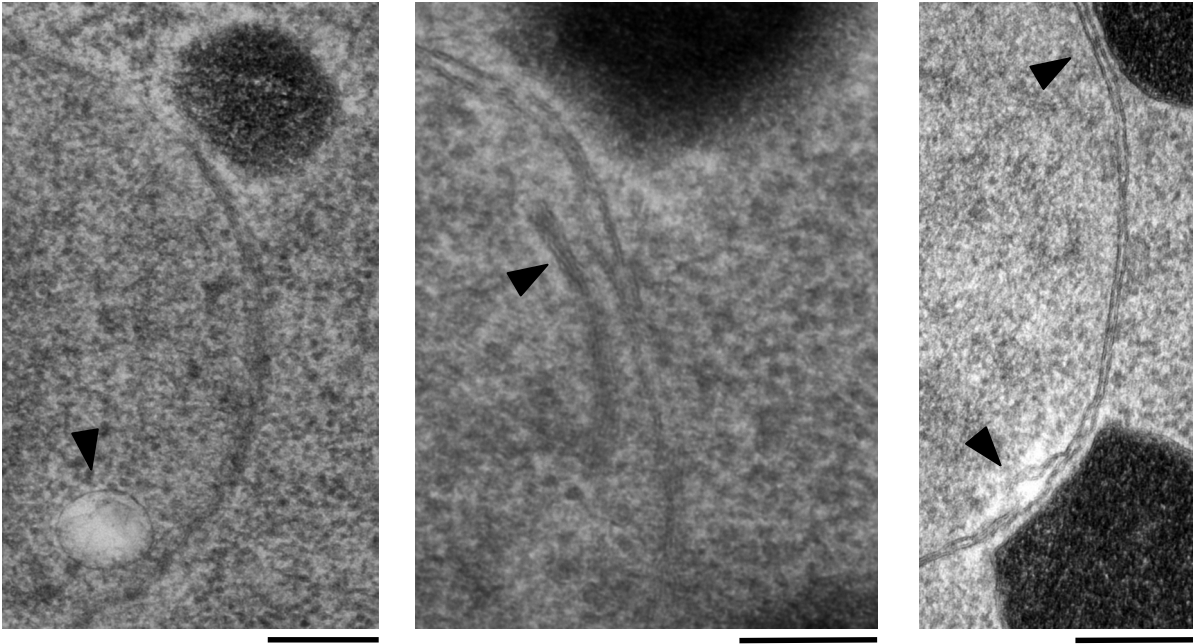
**Supplementary Figure 3: Cerulenin treatment causes shrinkage of interphase *lem2Δ* but not wild type nuclei**  
 (a) Images of the nuclear envelope (Cut11-GFP, green) of wild type (WT) and *lem2Δ* cells grown at 25°C untreated (-Cer) or treated (+Cer) with 10 µg/ml cerulenin for three hours. Maximum intensity projections shown. Scale bar: 5 µm.  
 (b) Stills from timelapse imaging of *lem2Δ* cells treated with 10 µg/ml cerulenin at 32°C that show blebbing. Time after drug addition indicated. Scale bar: 10 µm. Nuclear envelope (Cut11-GFP, green) maximum intensity projection.



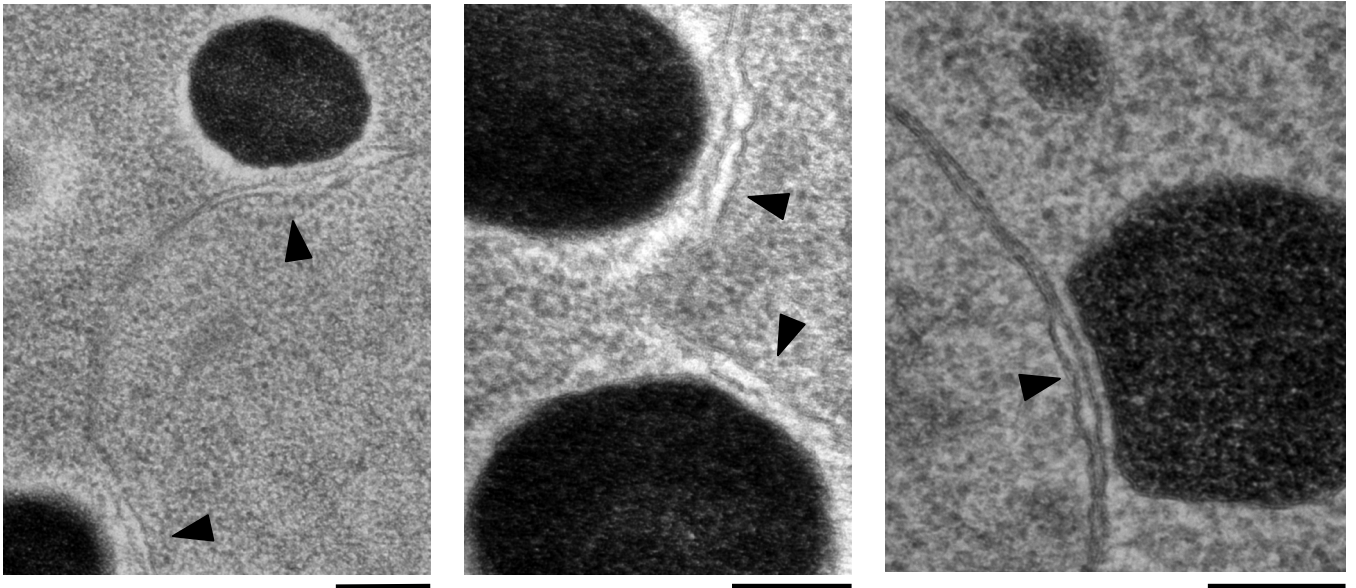
**Supplementary Figure 4: Membrane staining indicates aberrant nuclear shape and membrane foci on nuclear envelope of *lem2Δ* cells treated with cerulenin**

Images of wild type (WT) and *lem2Δ* cells, untreated (-Cer) and following 90 mins of treatment with 10  $\mu\text{g/ml}$  cerulenin (+Cer) at 32°C. Cellular membranes visualized by DiOC<sub>6</sub> staining. Single z-slice shown. Scale bar: 5  $\mu\text{m}$ .

**a**

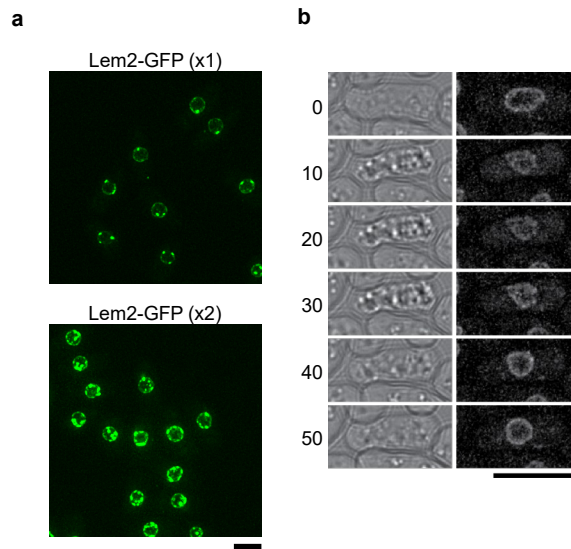


**b**



**Supplementary Figure 5: Membranous compartments in close proximity to the nuclear envelope and intranuclear membranous structures are visible in cerulenin treated *lem2Δ* cells.**

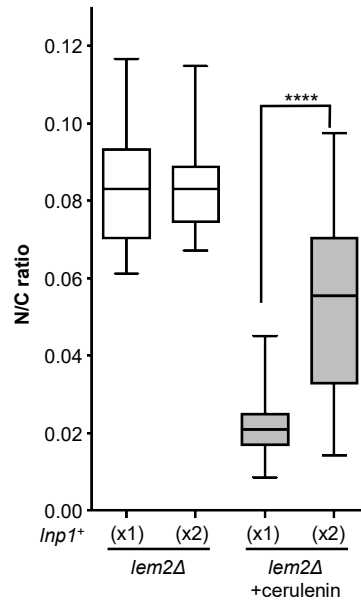
Transmission electron microscopy images of *lem2Δ* cells treated with 10  $\mu\text{g/ml}$  cerulenin for 90 minutes displaying intranuclear membranous structures (a) and membranous compartments in close proximity to the nuclear envelope (b) indicated by black arrowheads. Scale bars: 250 nm.



**Supplementary Figure 6: Lem2 localisation is not affected by increasing its copy number**

(a) Images of wild type cells grown at 25°C expressing 1 copy or 2 copies of Lem2-GFP (Lem2-GFP, green). Single z-slice shown. Scale bar: 5  $\mu$ m.

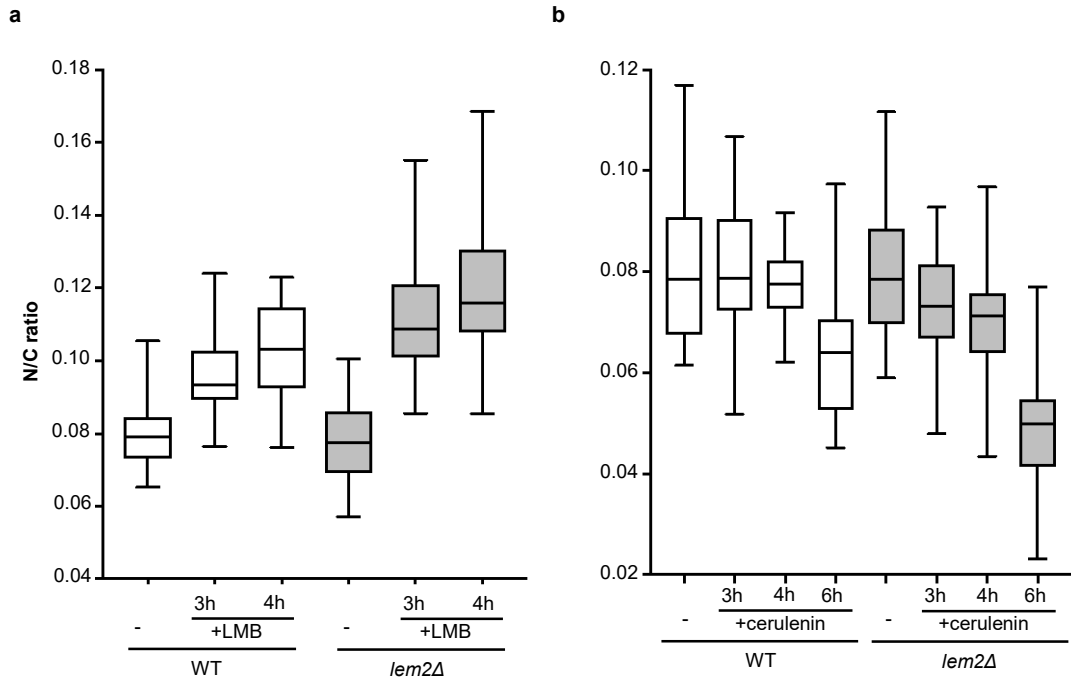
(b) Stills from timelapse microscopy of a *lem2* $\Delta$  cell grown at 25°C (EtOH treated as control) displaying nuclear shrinkage. Time in minutes indicated. Scale bar: 10  $\mu$ m. Cell (brightfield, grayscale, left hand column), nuclear envelope maximum intensity projection (Cut11-GFP, grayscale, right hand column).



**Supplementary Figure 7: Increased copy number of *lnp1* suppresses the reduction of N/C ratio caused by cerulenin treatment of *lem2Δ* cells**

N/C ratio of *lem2Δ* cells containing one (x1) or two (x2) copies of the gene encoding Lnp1 grown at 25°C untreated or treated with 10 µg/ml cerulenin (+Cer) for three hours. (n > 30 cells). Two-tailed Mann Whitney test: \*\*\*\* $P < 0.0001$ , *lem2Δ* + 1x*lnp1<sup>+</sup>* + cerulenin (n = 32 cells) against *lem2Δ* + 2x*lnp1<sup>+</sup>* + cerulenin (n = 33 cells). In box-and-whiskers diagrams, boxes indicate median and upper and lower quartile and whiskers indicate range of data. The corresponding dot plot is available in Supplementary Fig. 10c.

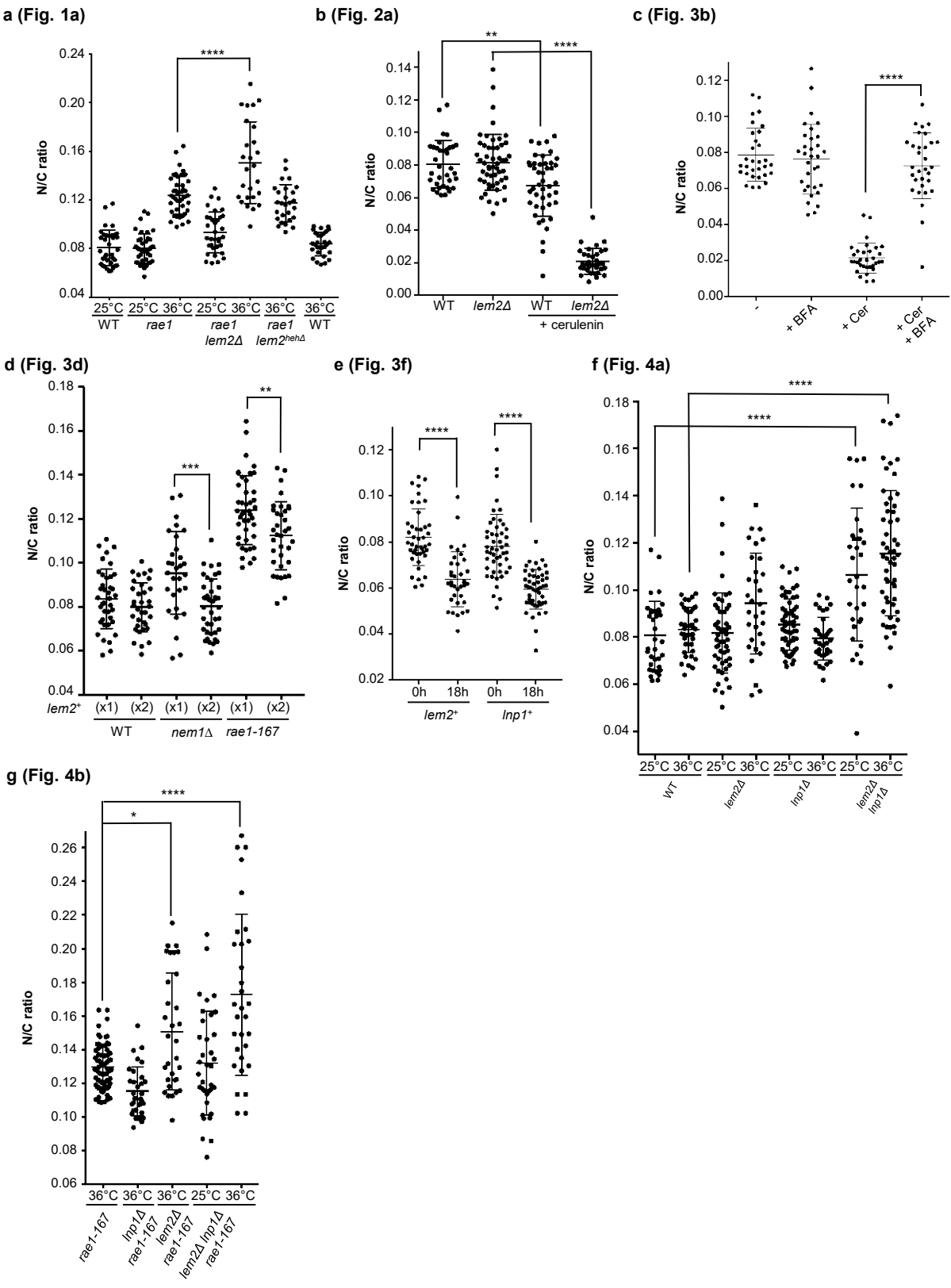




**Supplementary Figure 8: The nuclear size phenotypes of *lem2* deletion cells grown in EMM medium**

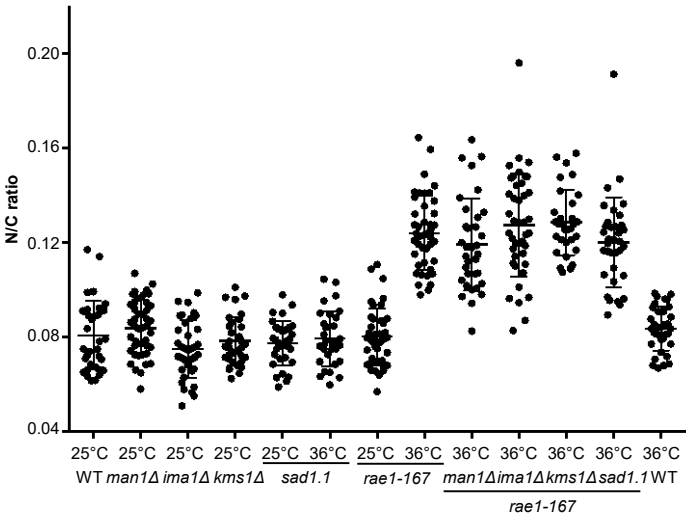
(a) N/C ratio of wild type (WT), and *lem2Δ* cells grown in EMM medium with or without 100 ng/ml leptomycin B (+LMB) for three hours or four hours (25°C) ( $n > 22$  cells). The corresponding dot plot is available in Supplementary Fig. 10d.

(b) N/C ratio of WT and *lem2Δ* cells grown in EMM medium with or without 10  $\mu$ g/ml cerulenin for three, four or six hours (25°C) ( $n > 30$  cells). The corresponding dot plot is available in Supplementary Fig. 10e. In all box-and-whiskers diagrams, boxes indicate median and upper and lower quartile and whiskers indicate range of data.

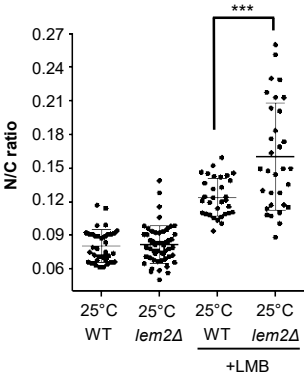


**Supplementary Figure 9: Dot plot of N/C ratios shown in the indicated Figures**  
 Error bars represent mean  $\pm$  s.d.

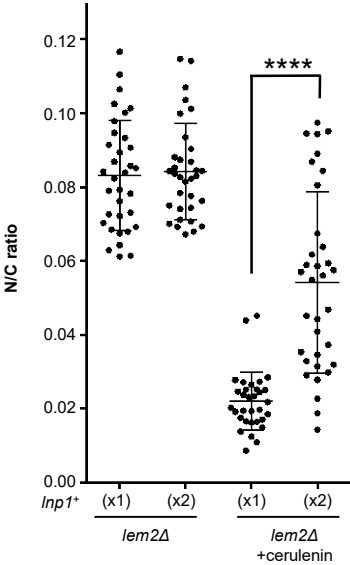
a (Supplementary Fig. 1a)



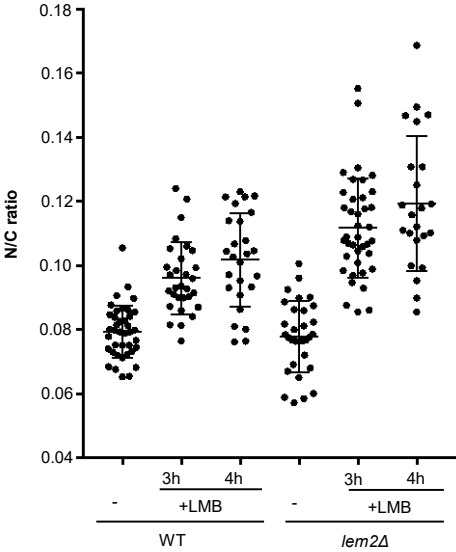
b (Supplementary Fig. 1c)



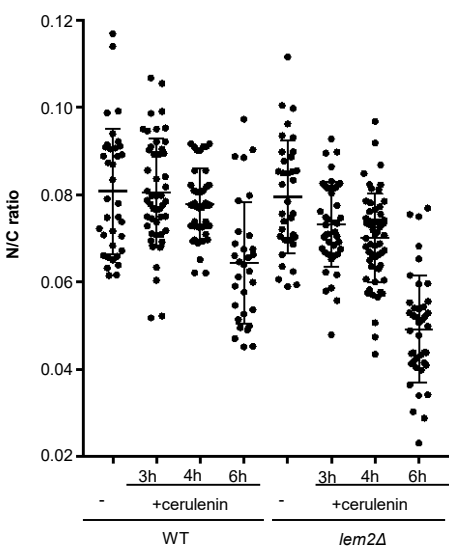
c (Supplementary Fig. 7)



d (Supplementary Fig. 8a)



e (Supplementary Fig. 8b)



Supplementary Figure 10: Dot plot of N/C ratios shown in the indicated Supplementary Figures  
Error bars represent mean  $\pm$  s.d.

**Supplementary Table 1: *S. pombe* strains used in this study**

| Strain      | Genotype                                                                                                                                                                       | Sources                         |                                                                                                           |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------|
| PN10345     | <i>h<sup>-</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18</i>                                                                                                           | <i>Neumann &amp; Nurse 2007</i> | Fig. 1a, 1b, 2a-c, 2g, 3c, 3d, 4a, 4c, Supplementary Fig. 1a-c, 2, 3a, 8, Supplementary Movie 1-4         |
| UKK412      | <i>h<sup>-</sup> rae1-167 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                                                     | <i>Kume et al. 2017</i>         | Fig. 1a, 1b, 3d, 4b, 4c, Supplementary Fig. 1a, 2                                                         |
| UKK737-2C   | <i>h<sup>-</sup> rae1-167 lem2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                              | This study                      | Fig. 1a, 1b, 4b, 4c                                                                                       |
| UKK925-5B   | <i>h<sup>2</sup> rae1-167 lem2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32:lem2<sup>hehΔ</sup> ura4-D18 ade6</i>                                          | This study                      | Fig. 1a                                                                                                   |
| UKK794-1A   | <i>h<sup>2</sup> rae1-167 lem2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> hht1<sup>+</sup>:mRFP:kan<sup>r</sup> ura4-D18 leu1-32 ade6</i>                        | This study                      | Fig. 1c                                                                                                   |
| UKK737-4A   | <i>h<sup>-</sup> lem2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                                       | This study                      | Fig. 2a-d, 2g, 2h, 3a-d, 4a, 4c, Supplementary Fig. 1b, 1c, 3a, 5b, 6b, 7, 8, and Supplementary Movie 1-4 |
| PN5603      | <i>h<sup>2</sup> sid4<sup>+</sup>:mRFP:kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                        | This study                      | Fig. 2e, Supplementary Fig. 3b, Supplementary Movie 5-8                                                   |
| PN5602      | <i>h<sup>2</sup> lem2Δ::kan<sup>r</sup> sid4<sup>+</sup>:mRFP:kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                 | This study                      | Fig. 2e, Supplementary Fig. 3b, Supplementary Movie 5-8                                                   |
| PN5605      | <i>h<sup>2</sup> sid4<sup>+</sup>:mRFP:kan<sup>r</sup> ura4-D18 leu1-32 ade6</i>                                                                                               | This study                      | Fig. 2f, Supplementary Fig. 4                                                                             |
| PN5604      | <i>h<sup>2</sup> lem2Δ::kan<sup>r</sup> sid4<sup>+</sup>:mRFP:kan<sup>r</sup> ura4-D18 leu1-32 ade6</i>                                                                        | This study                      | Fig. 2f, Supplementary Fig. 4                                                                             |
| UKK342-1A   | <i>h<sup>-</sup> nem1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                                       | <i>Kume et al. 2017</i>         | Fig. 3c, 3d                                                                                               |
| UKK842-1B   | <i>h<sup>2</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32:lem2<sup>+</sup>:leu1<sup>+</sup> ura4-D18</i>                                                                 | This study                      | Fig. 3c, 3d                                                                                               |
| UKK843-2A   | <i>h<sup>2</sup> nem1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32:lem2<sup>+</sup>:leu1<sup>+</sup> elo2<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18</i> | This study                      | Fig. 3c, 3d                                                                                               |
| UKK843-1A   | <i>h<sup>2</sup> rae1-167 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32:lem2<sup>+</sup>:leu1<sup>+</sup> ura4-D18</i>                                                        | This study                      | Fig. 3d                                                                                                   |
| UKK2214-3D  | <i>h<sup>2</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> pBip1:GFP:ADEL:ura4<sup>+</sup> ura4-D18 leu1-32</i>                                                                | This study                      | Fig. 3e                                                                                                   |
| UKK2210-10B | <i>h<sup>2</sup> nem1Δ::kan<sup>r</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> pBip1:GFP:ADEL:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                    | This study                      | Fig. 3e                                                                                                   |
| UKK2210-1C  | <i>h<sup>2</sup> nem1Δ::kan<sup>r</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> pBip1:GFP:ADEL:ura4<sup>+</sup> leu1-32:lem2<sup>+</sup>:leu1<sup>+</sup> ura4-D18 ade6</i>  | This study                      | Fig. 3e                                                                                                   |
| UKK2255-3B  | <i>h<sup>2</sup> nem1Δ::kan<sup>r</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> pBip1:GFP:ADEL:ura4<sup>+</sup> leu1-32:lnp1<sup>+</sup>:leu1<sup>+</sup> ura4-D18</i>       | This study                      | Fig. 3e                                                                                                   |
| UKK2212     | <i>h<sup>-</sup> kan<sup>r</sup>:nmt1:GFP:lem2<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>                                                      | This study                      | Fig. 3f                                                                                                   |
| UKK2215     | <i>h<sup>-</sup> kan<sup>r</sup>:nmt1:GFP:lnp1<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>                                                      | This study                      | Fig. 3f                                                                                                   |
| UKK1708-3A  | <i>h<sup>2</sup> lnp1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                                       | This study                      | Fig. 4a, 4c                                                                                               |
| UKK1867-1A  | <i>h<sup>2</sup> lem2Δ::kan<sup>r</sup> lnp1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 ade6</i>                                                        | This study                      | Fig. 4a, 4c                                                                                               |
| UKK1708-1B  | <i>h<sup>2</sup> rae1-167 lnp1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                                                              | This study                      | Fig. 4b, 4c                                                                                               |

|            |                                                                                                                                  |            |                      |
|------------|----------------------------------------------------------------------------------------------------------------------------------|------------|----------------------|
| UKK1879-1C | <i>h<sup>2</sup> rael-167 lem2Δ::kan<sup>r</sup> lnp1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 ade6</i> | This study | Fig. 4b, 4c          |
| UKK463-1B  | <i>h<sup>+</sup> man1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 ade6</i>                                 | This study | Supplementary Fig.1a |
| UKK546-2C  | <i>h<sup>2</sup> rael-167 man1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32 ura4-D18 ade6</i>                | This study | Supplementary Fig.1a |
| UKK986-1A  | <i>h<sup>2</sup> ima1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32 ura4-D18 ade6</i>                         | This study | Supplementary Fig.1a |
| UKK986-2C  | <i>h<sup>2</sup> rael-167 ima1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32 ura4-D18 ade6</i>                | This study | Supplementary Fig.1a |
| UKK985-2A  | <i>h<sup>2</sup> kms1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32 ura4-D18 ade6</i>                         | This study | Supplementary Fig.1a |
| UKK985-1B  | <i>h<sup>2</sup> rael-167 kms1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32 ura4-D18 ade6</i>                | This study | Supplementary Fig.1a |
| UKK1060-4C | <i>h<sup>+</sup> sad1.1 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32</i>                                              | This study | Supplementary Fig.1a |
| UKK1846-1B | <i>h<sup>+</sup> rael-167 sad1.1 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32</i>                                     | This study | Supplementary Fig.1a |
| UKK1806-3A | <i>h<sup>+</sup> rael-167 erg4Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>                 | This study | Supplementary Fig.2  |
| UKK542-1C  | <i>h<sup>2</sup> rael-167 erg5Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                | This study | Supplementary Fig.2  |
| UKK543-2B  | <i>h<sup>2</sup> rael-167 sre1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                | This study | Supplementary Fig.2  |
| UKK1827-1D | <i>h<sup>2</sup> rael-167 elo2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                | This study | Supplementary Fig.2  |
| UKK708-1D  | <i>h<sup>2</sup> rael-167 ely5Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                | This study | Supplementary Fig.2  |
| UKK818-1B  | <i>h<sup>2</sup> rael-167 pom152Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2  |
| UKK1046-6B | <i>h<sup>2</sup> rael-167 nup133Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2  |
| UKK1000-1D | <i>h<sup>2</sup> rael-167 nup40Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>               | This study | Supplementary Fig.2  |
| UKK1136-3D | <i>h<sup>2</sup> rael-167 nup211Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2  |
| UKK1313-5B | <i>h<sup>2</sup> rael-167 nup124Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2  |
| UKK2257-3A | <i>h<sup>+</sup> rael-167 pom34Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32</i>                    | This study | Supplementary Fig.2  |
| UKK2256-2B | <i>h<sup>2</sup> rael-167 nup155Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2  |
| UKK1400-1A | <i>h<sup>2</sup> rael-167 apq12Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>               | This study | Supplementary Fig.2  |
| UKK1614-1A | <i>h<sup>+</sup> rael-167 ppk13Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2 ade6</i>      | This study | Supplementary Fig.2  |
| UKK461-1A  | <i>h<sup>2</sup> rael-167 ypt71Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32</i>                    | This study | Supplementary Fig.2  |
| UKK1160-1A | <i>h<sup>2</sup> rael-167 scs22Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>               | This study | Supplementary Fig.2  |
| UKK1616-1C | <i>h<sup>+</sup> rael-167 atg1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>            | This study | Supplementary Fig.2  |
| UKK633-5B  | <i>h<sup>2</sup> rael-167 atg8Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                | This study | Supplementary Fig.2  |
| UKK457-3C  | <i>h<sup>2</sup> rael-167 atg5Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>                | This study | Supplementary Fig.2  |

|            |                                                                                                                                |            |                     |
|------------|--------------------------------------------------------------------------------------------------------------------------------|------------|---------------------|
| UKK1407-1A | <i>h<sup>2</sup> rae1-167 cdc48-353 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6<sup>?</sup></i>   | This study | Supplementary Fig.2 |
| UKK1379-3D | <i>h<sup>2</sup> rae1-167 rpn501Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK1401-2B | <i>h<sup>2</sup> rae1-167 rpt4Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK1408-2C | <i>h<sup>2</sup> rae1-167 snd302Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK1592-7C | <i>h<sup>2</sup> rae1-167 tmf1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK1540-2C | <i>h<sup>2</sup> rae1-167 kap114Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32</i>     | This study | Supplementary Fig.2 |
| UKK1393-1C | <i>h<sup>2</sup> rae1-167 mug184Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK1572-2A | <i>h<sup>2</sup> rae1-167 eki1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6</i>  | This study | Supplementary Fig.2 |
| UKK1468-1B | <i>h<sup>2</sup> rae1-167 vps35Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6</i> | This study | Supplementary Fig.2 |
| UKK1576-2A | <i>h<sup>+</sup> rae1-167 ppk4Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>          | This study | Supplementary Fig.2 |
| UKK2261-1A | <i>h<sup>+</sup> rae1-167 rtn1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK686-1B  | <i>h<sup>2</sup> rae1-167 rpl801Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 ade6</i>                    | This study | Supplementary Fig.2 |
| UKK1394-2A | <i>h<sup>2</sup> rae1-167 rpl803Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK1294-4B | <i>h<sup>2</sup> rae1-167 rps902Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK1395-2B | <i>h<sup>2</sup> rae1-167 kri1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK1597-1A | <i>h<sup>2</sup> rae1-167 rpa12Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6</i> | This study | Supplementary Fig.2 |
| UKK1594-3C | <i>h<sup>2</sup> rae1-167 reb1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6</i>  | This study | Supplementary Fig.2 |
| UKK1591-4C | <i>h<sup>2</sup> rae1-167 ker1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6</i>  | This study | Supplementary Fig.2 |
| UKK1382-2C | <i>h<sup>2</sup> rae1-167 rpl501Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK1390-2C | <i>h<sup>2</sup> rae1-167 rpp102Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4 leu1-32 ade6</i>                | This study | Supplementary Fig.2 |
| UKK1403-2C | <i>h<sup>2</sup> rae1-167 rpp201Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>            | This study | Supplementary Fig.2 |
| UKK707-1A  | <i>h<sup>2</sup> rae1-167 pabpΔ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK728-6D  | <i>h<sup>2</sup> rae1-167 pab2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK1381-4A | <i>h<sup>2</sup> rae1-167 cip2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK1375-1B | <i>h<sup>2</sup> rae1-167 nab3Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |
| UKK1414-3B | <i>h<sup>2</sup> rae1-167 SPBC215.06cΔ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>       | This study | Supplementary Fig.2 |
| UKK1396-3C | <i>h<sup>2</sup> rae1-167 nrp1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>              | This study | Supplementary Fig.2 |

|            |                                                                                                                            |            |                     |
|------------|----------------------------------------------------------------------------------------------------------------------------|------------|---------------------|
| UKK1626-3A | <i>h<sup>2</sup> rael-167 rnc1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18? leu1-32 ade6</i>         | This study | Supplementary Fig.2 |
| UKK677-1B  | <i>h<sup>2</sup> rael-167 cid14Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>         | This study | Supplementary Fig.2 |
| UKK1410-1B | <i>h<sup>2</sup> rael-167 nap1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>          | This study | Supplementary Fig.2 |
| UKK592-2D  | <i>h<sup>2</sup> rael-167 clr3Δ::kan<sup>r</sup> clr4Δ::LEU2 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32</i>   | This study | Supplementary Fig.2 |
| UKK729-1B  | <i>h<sup>2</sup> rael-167 mei4Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>          | This study | Supplementary Fig.2 |
| UKK884-2C  | <i>h<sup>2</sup> rael-167 cut14-208 cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32</i>                            | This study | Supplementary Fig.2 |
| UKK1634-5D | <i>h<sup>2</sup> rael-167 dcr1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18? leu1-32 ade6</i>         | This study | Supplementary Fig.2 |
| UKK1402-1D | <i>h<sup>2</sup> rael-167 hhf1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>          | This study | Supplementary Fig.2 |
| UKK535-1D  | <i>h<sup>2</sup> rael-167 cds1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>          | This study | Supplementary Fig.2 |
| UKK1661-2D | <i>h<sup>+</sup> rael-167 cdr1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>      | This study | Supplementary Fig.2 |
| UKK1666-1A | <i>h<sup>+</sup> rael-167 cdr2Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>           | This study | Supplementary Fig.2 |
| UKK1601-2D | <i>h<sup>2</sup> rael-167 ckb2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18? leu1-32 ade6</i>         | This study | Supplementary Fig.2 |
| UKK1615-1B | <i>h<sup>+</sup> rael-167 gsk3Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2 ade6</i> | This study | Supplementary Fig.2 |
| UKK1659-2B | <i>h<sup>+</sup> rael-167 gsk31Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>     | This study | Supplementary Fig.2 |
| UKK1585-7D | <i>h<sup>+</sup> rael-167 lsk1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 ade6</i>      | This study | Supplementary Fig.2 |
| UKK1587-1D | <i>h<sup>+</sup> rael-167 lkh1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>      | This study | Supplementary Fig.2 |
| UKK1589-2A | <i>h<sup>+</sup> rael-167 mik1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>           | This study | Supplementary Fig.2 |
| UKK1638-4B | <i>h<sup>2</sup> rael-167 mug134Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18? leu1-32 ade6</i>       | This study | Supplementary Fig.2 |
| UKK1663-5C | <i>h<sup>+</sup> rael-167 pek1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>           | This study | Supplementary Fig.2 |
| UKK1607-2D | <i>h<sup>+</sup> rael-167 pmk1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>           | This study | Supplementary Fig.2 |
| UKK1621-1B | <i>h<sup>+</sup> rael-167 ppk6Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>           | This study | Supplementary Fig.2 |
| UKK1683-2D | <i>h<sup>+</sup> rael-167 ppk14Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>     | This study | Supplementary Fig.2 |
| UKK1675-1A | <i>h<sup>+</sup> rael-167 ppk15Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>          | This study | Supplementary Fig.2 |
| UKK526-1D  | <i>h<sup>2</sup> rael-167 rad3Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18 leu1-32 ade6</i>          | This study | Supplementary Fig.2 |
| UKK1608-2D | <i>h<sup>+</sup> rael-167 stylΔ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>           | This study | Supplementary Fig.2 |
| UKK1632-1D | <i>h<sup>+</sup> rael-167 spo4Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 ade6</i>      | This study | Supplementary Fig.2 |
| UKK1645-3A | <i>h<sup>2</sup> rael-167 srp1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18? leu1-32 ade6</i>         | This study | Supplementary Fig.2 |

|            |                                                                                                                                                                                  |            |                       |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------------|
| UKK1482-5D | <i>h<sup>2</sup> rae1-167 sck1Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> ura4-D18<sup>?</sup> leu1-32 ade6</i>                                                    | This study | Supplementary Fig.2   |
| UKK1653-1C | <i>h<sup>+</sup> rae1-167 sck2Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>                                                            | This study | Supplementary Fig.2   |
| UKK1660-1C | <i>h<sup>+</sup> rae1-167 slk1Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2</i>                                                            | This study | Supplementary Fig.2   |
| UKK1574-1A | <i>h<sup>+</sup> rae1-167 ssp2Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32 his2 ade6</i>                                                       | This study | Supplementary Fig.2   |
| UKK1617-2A | <i>h<sup>-</sup> rae1-167 ppk21Δ::ura4<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 leu1-32</i>                                                                | This study | Supplementary Fig.2   |
| UKK1050-1A | <i>h<sup>-</sup> lem2<sup>+</sup>:GFP:hph<sup>r</sup> leu1-32</i>                                                                                                                | This study | Supplementary Fig. 6a |
| UKK2018-9C | <i>h<sup>2</sup> lem2<sup>+</sup>:GFP:hph<sup>r</sup> leu1-32 :lem2<sup>+</sup>:GFP:hph<sup>r</sup>:leu1<sup>+</sup> cut11<sup>+</sup>:mCherry:nat<sup>r</sup> ura4-D18 ade6</i> | This study | Supplementary Fig. 6a |
| UKK1883-4B | <i>h<sup>+</sup> lem2Δ::kan<sup>r</sup> cut11<sup>+</sup>:GFP:ura4<sup>+</sup> leu1-32:lnp1<sup>+</sup>:leu1<sup>+</sup> ura4-D18 ade6</i>                                       | This study | Supplementary Fig. 7  |

*sad1.1* mutant strain was provided by the National Bio-Resource Project (NBRP), Japan.