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1 **Ends and middle: global force balance and septum location in fission yeast**

2

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19

1 **Abstract**

2 *The fission yeast cell is shaped as a very regular cylinder ending by hemi-spheres at both cell*
3 *ends. Its conserved phenotypes are often used as read-outs for classifying interacting genes and*
4 *protein networks. Using Pascal and Young-Laplace laws, we proposed a framework where*
5 *scaling arguments predicted shapes. Here we probed quantitatively one of these relations which*
6 *predicts that the division site would be located closer to the cell end with the larger radius of*
7 *curvature. By combining genetics and quantitative imaging, we tested experimentally whether*
8 *altered shapes of cell end correlate with a displaced division site, leading to asymmetric cell*
9 *division. Our results show that the division site position depends on the radii of curvatures of*
10 *both ends. This new geometrical mechanism for the proper division plane positioning could be*
11 *essential to achieve even partitioning of cellular material at each cell division.*

12

13 **1. Introduction**

14

15 Biological screens are often based on the classification of shared phenotypes. This approach is
16 used successfully for a variety of model systems, ranging from yeast [1] to *C. elegans* [2],
17 Zebrafish [3] and *Drosophila* [4]. In fission yeast, for example, this approach has allowed to
18 reveal essential genes involved in a large class of phenomena such as cell shape [5,6], polarity
19 [7], cell fusion [8], cell cycle [9], nuclear volume [10], and septum position [11]. Interacting
20 proteins can be labeled fluorescently and their localisations in mutants mapped over time to
21 correlate placements and distribution of proteins with potential activating and inhibiting effects
22 [12–15].

23 However, these genetic changes in strains are often associated also with changes in cell shapes.
24 With this respect, differences in pressure between the inside and the outside of the cell together
25 with a local surface tension can be utilised to derive simple laws for read-outs, such as
26 localisation of polarity cues for lower tension, displacements of division planes, buckled
27 mutants [16]. This approach has proven its potential impact for new rules of self-organisations
28 and localisations [17].

29 We proposed in reference [16] scaling relations for shapes using Pascal principle and Young-
30 Laplace law. Here we test one of these relations quantitatively using fission yeast, the placement
31 of the septum. Its biological function is essential, since this physical separation between sister
32 cells secures the reliable partition of biological materials at the end of each cell cycle.

33 The cell is shaped as a very regular cylinder ending by hemi-spheres at both cell ends. Fission
34 yeast cells elongate during interphase keeping this regular shape, set by a balance between cell
35 wall stiffness and turgor pressure [16]. The cell is being remodelled locally at the cell ends to
36 promote cell extension. The nucleus is permanently maintained in the middle of the cell by
37 different forces, including microtubule pushing forces [18,19]. The central position of the
38 nucleus is used as a spatial cue to assemble a contractile actomyosin ring when cells entered
39 mitosis. This ring is used to drive the synthesis of a specific cell wall structure called the

1 division septum, which physically separates the daughter cells into two cells and leaving them
2 approximately half the mother cell after cytokinesis.

3 We proposed scaling arguments where the radii of curvatures of cell ends R_i influence
4 constraints on the cell wall [16]. They ultimately produce an effect on the division site position.
5 This relation predicts that a higher radius of curvature at one cell end should displace the
6 division site by a length L_{shift} due to unbalanced stress applied on the cell wall. The division site
7 would be located closer to the cell end with the larger radius (lower curvature, defined as $1/R$).
8 Qualitatively, the phenomenon can be captured in the following way: the difference in pressure
9 is normal to the cell surface. As a consequence, and by symmetry, the ends will be the important
10 contributors for a potential competition between forces of opposite directions. At some specific
11 localizations at the wall, they will compensate. The potential shift in the division site encodes
12 this possibility: if the ends have the same radius of curvature, the center will be the geometric
13 center; if not, it will be shifted towards the end with the larger radius of curvature.

14 We decided to test experimentally whether altered shapes of cell end actually correlate with a
15 displaced division site, leading to an asymmetric cell division. We combined genetics together
16 with live cell imaging. We used two strains modified from the wild type strain, *i.e.* a constitutive
17 deletion *tea4Δ* mutant and a conditional *kin1-as1* mutant, which affect cell ends. Our results
18 show that the division site position depends on the radii of curvatures of both ends.

19

20 **2. Experimental procedures**

21 **2.1. Yeast Strains and General Techniques**

22 *S. pombe* strains used in this study are XLG52 (*h- cdc15::GFP-ura4⁺ ura4-D18 leu1-32*, a kind
23 gift of V. Simanis, Switzerland), XLG540 (*h- tea4::ura4⁺ ura4-D18 cdc15::GFP-ura4⁺ leu1-*
24 *32*), XLG741 (*h- cdc15::GFP-ura4⁺ kin1-as1 leu1-32 ura4-D18*). Growth media and basic
25 techniques for *S. pombe* have been described [20].

26 **2.2. Microscopy**

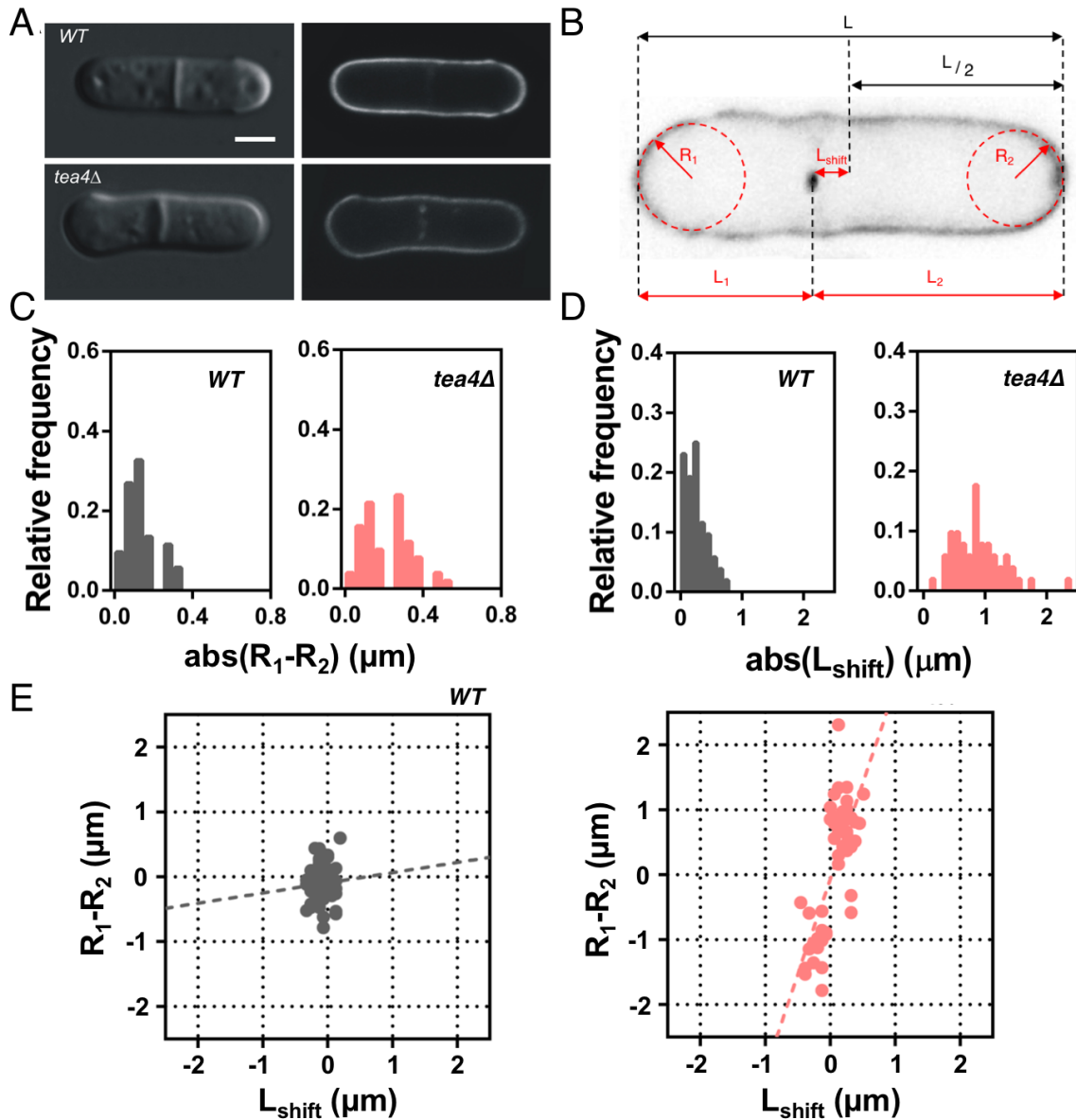
27 A spinning disk Nikon TE2000 microscope, equipped with a 100x 1.45 NA PlanApo oil-
28 immersion objective lens and a HQ2 Roper camera, was used for data acquisition. Cells
29 expressed the acto-myosin ring component Cdc15-GFP and were stained 10 minutes with
30 isolectin-488 (Molecular probes) that stains the global cell wall (but not the septum).
31 Metamorph software was used for capturing images. The “three point circular” ImageJ Plugin
32 allows to draw a ring with three points at a cell end and it gives the radius of curvature. We
33 used this Plugin to measure radii of curvature to obtain the best measurements. Cell lengths (L ,
34 L_1 and L_2) were measured with the Plot profile Plugin. For Transmission Electron Microscopy,
35 cells were stained with potassium permanganate and images were captured by a Jeol Jem-1010
36 (Peabody, MA).

37 **2.3. Statistical analyses and graphical representation**

1 Statistical analysis was done using GraphPad Prism. Results are presented as mean \pm s.e.m of
2 $N = 3$ experiments, $n_{WT} = 52$ cells, $n_{tea4\Delta} = 51$ cells, $n_{kin1-as1+DMSO} = 35$ cells and $n_{kin1-as1+NMPP1}$
3 $= 48$ cells. First, normality of the datasets was tested by the d'Agostino-Pearson normality test.
4 Statistical differences were analyzed by t-test (Gaussian distribution) and Mann-Whitney test
5 (non Gaussian distribution). The Pearson's r correlation coefficient (Gaussian distribution) and
6 the Spearman correlation coefficient (non Gaussian distribution) were used in order to test the
7 relation between (R_1-R_2) and L_{shift} for all the conditions. (R_1-R_2) as function of L_{shift} were fitted
8 using a linear regression. To obtain the mean surface tension γ for $tea4\Delta$ and $kin1-as1$ cells, γ
9 was taken constant *on average* at cell ends, and calculated by rewriting Equation 10 from
10 reference [16] to give $l_{shift} = (\langle \gamma_{end} \rangle / (\Delta P \cdot R_{side})) \cdot (R_1 - R_2)$ (Eq. 1), where L_{shift} , R , R_1 and R_2 are
11 obtained from the experiments and assuming $\Delta P = 0.85$ MPa for every cell. The distribution of
12 $\langle \gamma_{end} \rangle$ was plotted and fitted with a Gaussian distribution $y = A \cdot \exp(-(x-x_c)^2 / (2 \cdot w^2))$, where x_c
13 is the mean value for γ_{end} and w corresponds to the standard deviation.

14 **3. Results**

15 According to our scaling law, the cell end curvature would impact on cell division site position
16 at the time of septum ingression due to cell wall constraints. Therefore, we monitored the cell
17 division site localization using the expression of the cytokinetic ring component Cdc15-GFP
18 (Figure 1A). Cellular outlines were stained with the cell wall isolectin-488 label. Equation 10
19 of reference [16] showed that this shift with actual center does not depend on the longitudinal
20 cell length L (Figure 1B) and can be rewritten as $l_{shift} = (\langle \gamma_{end} \rangle / (\Delta P \cdot R_{side})) \cdot (R_1 - R_2)$, (Eq. 1),
21 where R is the mean radius of the long axis, ΔP the constant difference in pressure between the
22 inside and the outside of the cell, if we consider the mean cell wall surface tension $\langle \gamma_{end} \rangle$.



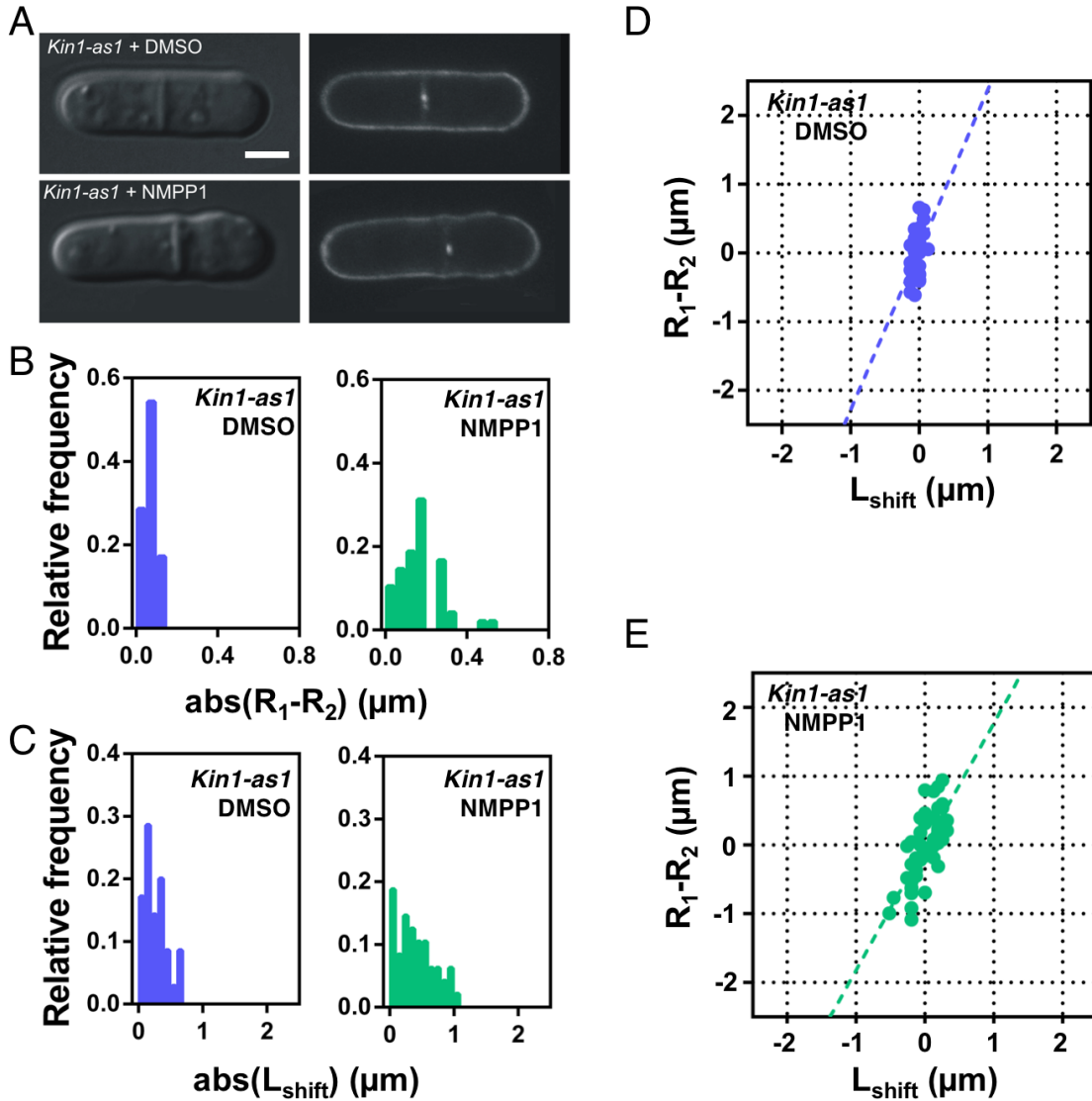
1
2 **Figure 1.** (A) DIC (left) and fluorescent (right) microscopy images (*cdc15-GFP*, *isolectin-488*)
3 of WT and *tea4Δ*, Scale bar 2 μm . (B) Schematics of the parameters measured in each cell: R_i
4 corresponds to the radii of curvature at the cell “end i ”, and L_i corresponds to the distance
5 between “end i ” and septum; L_{shift} is defined as the distance between the septum and the middle
6 plane ($L_{\text{shift}}=(L_2-L_1)/2$), L the total length. (C) Distributions of the absolute values of R_1-R_2 for
7 the WT and *tea4Δ* cells. R_1-R_2 distributions are statistically different (t-test, $p = 0.0003$). (D)
8 Distributions of the absolute values of L_{shift} values for the WT and *tea4Δ* cells. Distributions are
9 statistically different (Mann-Whitney, $p < 0.0001$). (E) Correlation between L_{shift} and R_1-R_2 for
10 the WT and *tea4Δ* cells. The fits of Equation 1 are shown in the graphs. $n_{\text{WT}}=52$ and $n_{\text{tea4}\Delta}=51$.
11 Spearman correlation coefficients are 0.03 and 0.61 respectively.

12

13 First, we compared wild type (WT) and the asymmetrically dividing mutant *tea4Δ* (Figure 1A).
14 The Tea4 protein is involved in bipolar activation of cell growth in the cell ends. *tea4Δ* cells

1 showed altered cell morphology including one more enlarged cell end than the other and an
2 asymmetrically positioned division site [21,22], that was determined with Cdc15-GFP. We
3 calculated L_{shift} value as $L_{shift} = (L_2 - L_1) / 2$ (Eq. 2), where L_1 stands for the distance between the
4 ring and one cell end (cell end1) and L_2 stands for the distance between the ring and the cell
5 end (cell end2). To calculate the $R_1 - R_2$ value, radii of curvatures were measured as follows: R_1
6 for cell end1 (or the end associated to L_1) and R_2 for the other cell end (or the end associated to
7 L_2). We observed an increased asymmetry in *tea4Δ* cells compared to WT, both for $R_1 - R_2$ value
8 (Figure 1C) and L_{shift} value (Figure 1D). On one hand, cell end radii are different in *tea4Δ* cells
9 compared to WT cells. Difference of radii of curvature at the cell ends ($R_1 - R_2$) augmented from
10 $0.13 \pm 0.01 \mu\text{m}$ for WT cells to $0.21 \pm 0.02 \mu\text{m}$ for *tea4Δ* (Figure 1C), showing an increase of the
11 asymmetry in the cap curvatures. Although a small asymmetry for WT cells was observed,
12 probably due to the intrinsic noise of the biological system, it significantly changed about two-
13 fold for *tea4Δ* mutant. On the other hand, the mean value of L_{shift} increased from $0.26 \pm 0.05 \mu\text{m}$
14 for WT cells to $0.62 \pm 0.06 \mu\text{m}$ for *tea4Δ* cells (Figure 1D). Again, a non-zero L_{shift} was observed
15 for WT cells attributed to the intrinsic variability of biological systems. However, the shift in
16 the division site significantly increased by two-fold in *tea4Δ* cells. Thus, there is clearly a larger
17 amplitude of L_{shift} in *tea4Δ* cells indicating that they divide more asymmetrically than WT cells.
18 Finally, the L_{shift} , plotted as a function of the $R_1 - R_2$ difference of radii of cell end curvatures
19 (Figure 1E), showed a positive correlation between L_{shift} and $R_1 - R_2$ for *tea4Δ* cells (correlation
20 coefficient 0.61), whereas no correlation was observed for WT cells (correlation coefficient
21 0.03). Therefore, the experimental results show that the division site is displaced towards the
22 end with the highest radius of curvature, which is consistent with our prediction.

23 *Tea4Δ* cells are constitutively misshapen and cell end curvatures differences may arise from
24 cell wall defects inherited through several generations independently of cell division site
25 selection. Thus, we used *kin1-as1*, a conditional allele of the cell wall regulating Kin1 kinase,
26 that promoted cell division site mispositioning within the duration of a cell division cycle. *Kin1-*
27 *as1* was inhibited using a small molecule called NMPP1 added into the culture medium [23].
28 *Kin1-as1* NMPP1 and *kin1-as1* DMSO cells are isogenic but cultured with or without the
29 inhibitor for 2 hours, respectively. *Kin1-as1* NMPP1 cells adopt an asymmetric cell division
30 pattern in less than a generation time (see Figure 2A).



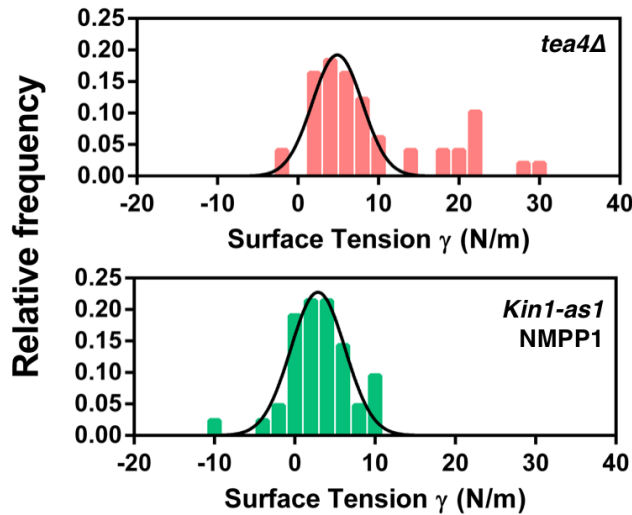
1

2 **Figure 2.** (A) DIC (left) and fluorescent (right) microscopy images (*cdc15-GFP*, *isolectin-488*)
 3 of *kin1-as1* DMSO and *kin1-as1* NMPP1 cells, Scale bar $2\mu\text{m}$. (B) Distributions of the absolute
 4 values of R_1-R_2 for *kin1-as1* DMSO and *kin1-as1* NMPP1 cells. R_1-R_2 distributions are
 5 statistically different (Mann-Whitney, $p < 0.0001$). (C) Distributions of the absolute values of
 6 L_{shift} for *kin1-as1* DMSO and *kin1-as1* NMPP1 cells. Distributions are statistically different (*t*-
 7 test, $p = 0.0122$). (D) Correlation between L_{shift} and R_1-R_2 for *kin1-as1* DMSO cells. The fit of
 8 Equation 1 is shown in the graph. (E) Correlation between L_{shift} and R_1-R_2 for *kin1-as1* NMPP1
 9 cells. The fit of Equation 1 is shown in the graph. $n_{\text{Kin1-as1 DMSO}}=35$ and $n_{\text{Kin1-as1 NMPP1}}=48$.
 10 Pearson's correlation coefficient is 0.44 for *kin1-as1* DMSO cells and Spearman correlation
 11 coefficient is 0.72 for *kin1-as1* NMPP1 cells.

12 We monitored division site position and cell end curvatures using the method described above.
 13 R_1-R_2 value is higher in *kin1-as1* NMPP1 cells ($0.17\pm 0.02\mu\text{m}$) compared to *kin1-as1* DMSO
 14 cells ($0.06\pm 0.01\mu\text{m}$), showing that cell end radii are less equivalent (Figure 2B). Furthermore,
 15 a larger L_{shift} is clearly observed in *kin1-as1* NMPP1 cells ($L_{\text{shift}} = 0.57\pm 0.04\mu\text{m}$) compared to
 16 *kin1-as1* DMSO cells ($L_{\text{shift}} = 0.25\pm 0.02\mu\text{m}$) (Figure 2C). This indicates that *kin1-as1* NMPP1

1 cells divide more asymmetrically than *kin1-as1* DMSO cells and WT cells. Again, L_{shift} , plotted
 2 as a function of the R_1-R_2 (Figure 2D and 2E), showed a positive correlation between L_{shift} and
 3 R_1-R_2 for *kin1-as1* NMPP1 cells compared to *kin1-as1* DMSO cells (correlation coefficient 0.72
 4 and 0.44 respectively). L_{shift} was again displaced towards the end with the highest radius of
 5 curvature, confirming the results with the *tea4Δ* mutant and strongly supporting the scaling
 6 laws of reference [16]. The mild correlation observed for *kin1-as1* DMSO cells (0.44, Figure
 7 2D) compared to the correlation seen for WT cells (0.03, Figure 1E) may reflect that the *kin1-*
 8 *as1* mutated allele is not fully functional in control condition as the wild type gene [24].

9 Applying Equation 1 and using the experimental values of L_{shift} , R_2 , R_1 and R (the radius of the
 10 cell at the middle plane) measured for *tea4Δ* cells and *kin1-as1* NMPP1 cells we could obtain
 11 the distribution of the mean wall surface tension $\langle\gamma_{end}\rangle$ for each cell [17] (Figure 3). We then
 12 fitted the distributions with a gaussian function, obtaining a mean surface tension $\langle\gamma_{end}\rangle =$
 13 4.9 ± 3.1 N/m for *tea4Δ* cells and $\langle\gamma_{end}\rangle = 2.9 \pm 3.4$ N/m for *kin1-as1* NMPP1 cells, in good
 14 agreement in order of magnitude with values obtained with independent methods [17].



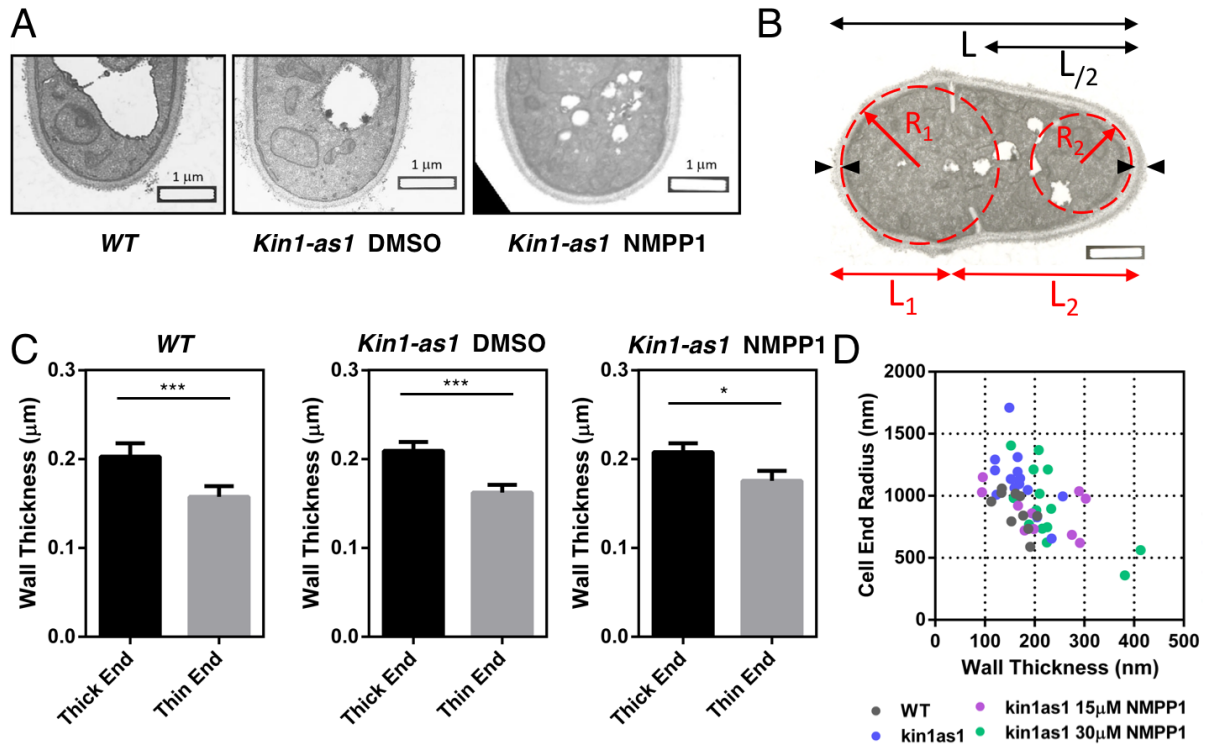
15

16 **Figure 3.** Distribution of mean surface tension $\langle\gamma\rangle$ for *tea4Δ* cells and *kin1-as1* NMPP1 cells.
 17 $\langle\gamma\rangle$ was calculated according to Equation 1 assuming $\Delta P = 0.85$ MPa [17]. The data were
 18 fitted assuming a Gaussian distribution, obtaining a value for the surface tension of $\langle\gamma_{end}\rangle =$
 19 4.9 ± 3.1 N/m for *tea4Δ* cells and $\langle\gamma_{end}\rangle = 2.9 \pm 3.4$ N/m for *kin1-as1* NMPP1 cells.

20 To further test the mechanism associated with shift in septum and curvatures at cell ends, we
 21 decided to measure wall thickness by performing measurements with Electron Microscopy
 22 ([25] and Figure 4A and 4B). The thickness was of the order of 200nm, as reported in other
 23 studies [27]. However, we systematically observed that one wall was thicker than the other
 24 (Figure 4C). This difference between the thick and the thin end was $(45 \pm 6$ nm) and $(47 \pm 9$
 25 nm) for WT cells and *Kin1-as1* DMSO cells respectively, but decreased by a 30% $(32 \pm 5$ nm)
 26 when *Kin1-as1* cells were incubated with NMPP1. Finally, we found an inverse correlation
 27 between the cell end radius of curvature and wall thickness (Figure 4D). This is consistent with
 28 the notion that wall assembly is deficient in these mutants, and wall behaves as an inert

1 ‘balloon’ with cylindrical shape similar to fission yeast: it undergoes thinning for larger radii
 2 of curvature.

3



4

5 **Figure 4.** (A) Typical E.M. images for wild type, *kin1-as1* and *kin1-as1*+NMPP1 cells. (B)
 6 Schematics of the parameters measured in each cell: R_i corresponds to the radii of curvature
 7 at the cell “end i ”, and L_i corresponds to the distance between “end i ” and septum; L the total
 8 length and the wall thickness w is defined by the black arrows. (C) Measurements of the wall
 9 thickness. $n_{WT}=8$, $n_{Kin1-as1\ DMSO}=13$ and $n_{Kin1-as1\ NMPP1}=6$. (D) Cell end radius as a function of
 10 wall thickness.

11 4. Discussion

12 Our study suggests an interplay between molecular actors of polarity, cell ends, and mechanics.
 13 The cell ends are defined by the concentration of polarity factors such as Tea1 and Bud6 that
 14 are absent from lateral sides. Tea1 acts to recruit other polarity factors, is itself delivered by
 15 microtubules and organizes the MT network, while Bud6 is known to bind to the F-actin
 16 nucleator For3 and regulates F-actin cable assembly and hence exocytic vesicle delivery
 17 [26,27]. In enlarged cell ends, these factors would tend to be less concentrated. The effect of
 18 increasing cell end radius on these aspects could be further studied through candidate mutant
 19 screens and imaging fluorescently tagged proteins. Cell end enlargement should also promote
 20 stretching of the cell wall, consistent with the thinning measured in mutants. This may activate
 21 mechanosensitive trans-membrane stress sensors such as Wsc1 and Mtl2 and/or the Cell Wall
 22 Integrity pathway [28,29]. Investigating their role by combining genetics, laser ablation, and
 23 soft-lithography techniques [30,31] would be valuable to link cell wall structure in contributing
 24 to the cell division control presented here.

1 Septum location has been a central topic of interest from the scientists studying fission yeast
2 with important reports identifying the genes and proteins implicated in the phenomenon and
3 their spatial distributions over time (for a review, [32]). In particular, distribution of Mid1 at
4 the wall in the vicinity of the nucleus is essential for the future localization of the septum [33].
5 In addition, the Pom1 kinase, which marks cell ends, generates a zone of inhibition for Mid1
6 and subsequent medial septum components [34–36]. Both examples suggest that cells regulate
7 the local composition at the cell wall.

8 It is interesting to conjecture connections between these proteins localizations at the cell wall
9 and our results. Mid1, the Pom1 kinase, together with their dense and complex protein partners,
10 may have an effect on the local wall thickness. In turns, this may change the surface tension at
11 the cell end and along the cell side, thereby ‘weakening’ the wall close to the nucleus. Future
12 study could connect the septum localization with local properties of the cell wall to test further
13 our scaling argument.

14 Connections between our mutants and *mid1/pom1* could also be involved in the phenomenon
15 [21,37]. This would reinforce connections between protein partners localizations at the cell wall
16 and the local surface tension. Future studies could measure locally the surface tension while
17 visualizing the local distribution of these proteins essential for the localization of septum.
18 Mechanical perturbations coupling local forces at the cell wall and local recruitments may also
19 show tighter connections between local mechanics and cell response [38,39].

20 A variety of mutants were reported with misplaced septa. They involved proteins having key
21 roles in microtubule-dependent nuclear centering, F-actin and septum structure, cell polarity
22 and cell cycle signaling pathways. Our results suggest that this way of correlating cell ends and
23 septum localization could be tested in the future, and if verified, this may provide quantitative
24 links between complexes at cell walls and local surface tensions. Since our results are consistent
25 with the scaling relation, new studies could be performed with these mechanical tests bridging
26 mesoscopic cellular structures and local mechanics. For example, cells could be placed in
27 microfabricated channels to evaluate the localizations of septa in confined conditions,
28 measurements with atomic force microscopes could map the distributions of surface tension
29 around the cell, or measurements of wall thickness distributions could be evaluated for each
30 strain. These experiments will allow to use further this relation between ends and septum
31 location with specific connections between local cellular composition and wall property.

32 Microtubules were also shown to control the localization of the nucleus [18]. Both mechanisms
33 could cooperate and compete. In this context, alternative mechanisms could be suggested.
34 Microtubules may change their distributions and dynamics [40] in asymmetric cells. In turn,
35 the localization of the nucleus could be shifted. Alternatively, microtubules dynamics *per se*
36 could be altered by the curvatures at cell ends; in this case, the cell wall would act as the
37 transmitter of asymmetry between ends directly. These alternative results could be tested with
38 relevant mutants with modified microtubule dynamics and their live observation during septum
39 formation.

40 The same approach could be used in other systems and for other biological functions, if we
41 consider the cell cortex in other cells as the equivalent of cell wall in fission yeast [16]. For

1 example, differentiation from stem cells has been associated to asymmetric cell division. In this
2 context, the radii of curvatures of cells could be monitored over time and the onset of
3 asymmetry may be associated as well with changes in radii of curvature at cell ends. To test
4 this hypothesis, these experiments could be conducted for example in *Drosophila* [41,42],
5 among other model systems. Similar approach may allow to give more quantitative substance
6 to cortical force generators during cell division, in the context of *C. elegans* or mammalian cells
7 [43]: this ‘gel’ localized at the cell poles spanning the cell membrane during cytokinesis may
8 affect as well the localization of the cytokinetic furrow along the mechanism presented in this
9 paper. In these different systems, future experiments could measure local curvatures at cell ends
10 and cytokinesis localizations along our line to correlate poles shapes with respect to cytokinetic
11 furrow plane. This could also be consistent with the notion that differences in tension between
12 poles and furrow could play essential roles in cytokinesis [44]. Altogether ends and middles
13 could be key read-outs in general for a variety of questions and model systems.

14

15 **5. Conclusions**

16 Our results show that fission yeast cell end shapes influence the division site position. In WT
17 cells, the small difference in both cell end radii promotes balanced global forces that place the
18 division site close to the geometric cell center. Accordingly, daughter cells divide at nearly
19 equal sizes and this might be crucial for cell population fitness regarding symmetric partitioning
20 of cellular components and damaged material inheritance [45]. We propose that two
21 mechanisms contribute to symmetry of division in fission yeast: an ‘external’ input from cell
22 wall driven forces and an ‘internal’ input driven by microtubule-dependent nuclear localization
23 [18,19]. In mutants where the cell wall synthesis machinery is depolarized from cell ends but
24 exhibit a normal microtubule network [22], the external cell wall contribution exceeds a
25 threshold and cells divide asymmetrically, suggesting that the internal input cannot compensate
26 the defect. The role of cell wall forces proposed here may be a generic mechanism in single
27 celled symmetrically dividing organisms to produce equally sized daughter cells at each cell
28 division.

29

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3

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