

Coordinated control of both microtubule ends regulates mitotic spindle length

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The mitotic spindle is a biomechanical structure whose length must be precisely controlled to ensure faithful chromosome segregation. The treadmilling of microtubules towards spindle poles, termed poleward flux, is involved in spindle length control. However, poleward flux velocity has been shown to be both inversely and directly proportional to spindle length, a contradiction that remains unexplained by current mechanisms. Here we introduce a model which demonstrates that length-dependent regulation at both microtubule ends allows plus- and minus-end dynamics to synchronize with one another, enabling pathways of poleward flux-based spindle length control which rectify previous results. Moreover, our model predicts that spindle length and poleward flux velocity can vary independently via simultaneous perturbations at both microtubule ends, which we validate with protein depletion experiments in human cells. Finally, we provide experimental evidence for length-dependent accumulation of proteins at microtubule minus-ends. Our results thus resolve a longstanding paradox and provide mechanistic insight into the reciprocal control of spindle length and poleward flux.

A fundamental prerequisite for the successful propagation of life is the ability for cells to divide and pass on their genetic material to the next generation. To facilitate this process and minimize any potential errors, cells employ the mitotic spindle, a micromachine composed of microtubules and associated proteins¹. The spindle generates forces on chromosomes that are regulated over both space and time to ensure a high level of fidelity throughout mitosis^{2,3}. Due to its biological importance, the mechanisms controlling spindle length and function have been extensively studied both *in vivo* and *in vitro*^{4–18}. Given the complexity of the mitotic spindle and experimental resolution limits, theoretical modeling has also been a powerful tool for investigating how the spindle sets its length^{4–6,19–23} and shape^{24,25}. However, the mitotic spindle utilizes and precisely coordinates a myriad of feedback loops and regulatory processes, which are difficult to elucidate the individual contributions of, and so our understanding

of the physical mechanisms responsible for spindle length regulation remains incomplete.

Despite exhibiting many conserved features, spindles across species and cell types vary in the exact strategies employed to self-regulate length and chromosome positioning throughout mitosis²⁶. In metazoan spindles, a combination of motor sliding and continual depolymerization of microtubule minus-ends at spindle poles leads to a net velocity towards the poles referred to as poleward flux^{27–31}, and microtubules maintain a constant length via compensatory polymerization at plus-ends. Poleward flux velocity is precisely modulated throughout mitosis^{32–34} and is important for facilitating chromosome movement^{35–37} and positioning³⁸ as well as preventing erroneous kinetochore attachments³⁹. Spindle length has also been shown to depend on poleward flux velocity^{40–42}, but its exact role has been obfuscated by seemingly contradictory experiments in which reducing poleward flux velocity leads to spindle length decreasing^{41,43–46},

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increasing^{35,42,47}, or remaining approximately unchanged^{36,48}. Additionally, poleward flux velocity can span over an order of magnitude between species, e.g., 0.5 $\mu\text{m}/\text{min}$ in LLC-PK1 cells³⁰, 1.3 $\mu\text{m}/\text{min}$ in human RPE-1 cells⁴⁴, and 5.2 $\mu\text{m}/\text{min}$ in *Drosophila* embryos³², despite comparable spindle lengths of 18.8, 13.9, and 10–14 μm , respectively. This suggests that cells can tolerate vastly different poleward flux velocities and maintain their spindle length relatively independently of the quantity.

Theoretical models have been used to study how poleward flux is involved in chromosome movement, positioning, and error-correction^{44,49–53}. The physical mechanisms proposed to drive poleward flux are depolymerization at microtubule minus-ends together with motor activity which either pulls at the spindle poles^{49,50} or slides apart anti-parallel overlaps in the midzone^{44,53}. In these models, spindle poles have fixed positions, and forces at the poles are either not considered⁵¹ or are balanced by polar ejection forces^{49,50,52} or motor sliding forces^{44,53}. One recent model studied the direct relationship between spindle length and poleward flux velocity, expanding upon these previous works by incorporating spindle pole movement in addition to length-dependent depolymerization at microtubule minus-ends²³. However, experimental data in which spindle length and poleward flux velocity exhibit an inverse relationship or are independent of each other are still unexplained. Therefore, how spindle length is regulated in systems with poleward flux remains an open question.

In this paper, we use a combination of theory and experiment to explore how the mitotic spindle regulates its length despite changes in poleward flux velocity. We introduce an analytic model that incorporates length-dependent mechanisms at both the plus- and minus-end of microtubules in addition to core ingredients, such as motor sliding and interkinetochore forces. We find that this combination of length-dependent mechanisms allows each microtubule end to sense and respond to changes at the opposite end, resulting in mutual synchronization. Consequently, perturbations of plus- and minus-end dynamics influence poleward flux velocity in opposite ways for a given change in spindle length, allowing our model to explain both direct and inverse relationships between poleward flux velocity and spindle length. Our model also predicts that by perturbing both ends of the microtubule simultaneously, these changes can cancel each other out, allowing poleward flux velocity and spindle length to vary independently of one another. We confirm these predictions with *in vivo* experiments, which show that co-depletion of the plus-end polymerization inhibitor KIF18A and the minus-end severing protein KATNB1 rescues poleward flux velocity for larger spindles. Finally, we provide experimental evidence for length-dependent accumulation of KATNB1 at minus-ends by showing enhanced intensity near spindle poles in RPE-1 *KIF18A*^{-/-} cells. Our results demonstrate that length-dependent mechanisms at both microtubule ends allow plus- and minus-end dynamics to synchronize with one another by communicating through microtubule length, facilitating robust spindle length control over a wide range of poleward flux velocities.

Results

A model incorporating length-dependent mechanisms at both microtubule ends predicts mutual synchronization of plus- and minus-end dynamics

To investigate mitotic spindle length regulation, we introduce a model which includes length-dependent mechanisms at both the plus- and minus-end of microtubules (Fig. 1 and “Methods”). The central idea of this model is to study the potential interactions between these two length-dependent mechanisms and explore how they influence the relationship between poleward flux velocity and spindle length. The model includes a pair of microtubule fibers originating from opposite spindle poles that are bound to sister kinetochores, referred to as *k-fibers*, and a pair of microtubule fibers that interdigitate with other microtubules to form anti-parallel overlaps, referred to as *bridging*

*fibers*⁵⁴. This simplified geometry is supported by experiments which show that at least one microtubule within *k-fibers* is directly connected to both the kinetochore and the spindle pole⁵⁵. We use the antenna model to describe the length-dependent regulation of microtubule plus-ends⁵⁶ (Fig. 1, inset 3) and extend it to microtubule minus-ends as well (Fig. 1, inset 1). Length-dependent plus-end regulation by proteins, such as KIF18A, is already well-established in the mitotic spindle^{56–59}, and we justify the extension to minus-ends with previous experiments which have shown that the abundance of minus-end proteins KATNB1 and KIF2A at spindle poles correlates with the length of microtubules emanating from them⁶⁰. Forces in the system are generated laterally along microtubules by plus-end-directed crosslinking motors that accumulate in anti-parallel overlaps and also arise from interactions with the kinetochore and spindle pole at microtubule plus- and minus-ends, respectively (Fig. 1, insets 1–3). In line with previous work^{23,44,49–53}, these forces modulate both plus-end polymerization and minus-end depolymerization. Overlap length is kept fixed in our model because the mechanisms which regulate it are outside the scope of this paper and not well understood. This model provides a framework for studying the role of length-dependent regulation of microtubule plus- and minus-end dynamics in poleward flux velocity and spindle length control.

We first compare the model with and without length-dependent mechanisms in order to elucidate the contribution of each component. In the version of our model with no length-dependent regulatory mechanisms, there are three key parameters that set spindle length: (i) baseline polymerization velocity at the plus-end, v_0^+ , (ii) baseline depolymerization velocity at the minus-end, v_0^- , and (iii) the per-microtubule motor sliding velocity, $v_0/2$. We begin with the simplest parameter choice: all three velocities are set equal, i.e., $v_0^+ = v_0^- = v_0/2$. Under these conditions, the microtubule minus- and plus-ends remain at fixed positions, resulting in a constant spindle length (Fig. 2a). Next, we solve the case with faster baseline polymerization at plus-ends, i.e., $v_0^+ / gtv_0^- = v_0/2$, which results in the spindle growing indefinitely. Finally, we solve the model with slower baseline polymerization at plus-ends, i.e., $v_0^+ < v_0^- = v_0/2$. In this case, the spindle poles continually approach each other until the spindle collapses. We chose $v_0^+ = v_0^- = v_0/2$ in this example for the sake of simplicity, as it automatically fulfills the steady state requirement of plus-end polymerization, minus-end depolymerization, and poleward flux velocity being equal for all microtubules. We note that there exist in principle, other combinations of these three parameters which also fulfill the steady state requirement, formally defining a manifold (Supplementary Information). However, the probability of occupying this manifold is infinitesimal and, as in our illustrative example, individually perturbing any parameter and deviating from this manifold would result in spindle collapse or indefinite growth. Taken together, these results show that a model with no length-dependent mechanisms leads to a failed spindle which does not have a well-defined steady state length and cannot withstand any perturbations (Fig. 2b). Therefore, our model suggests that some length-dependent mechanism must be present.

Plus-end length-dependent mechanisms are well-established in the mitotic spindle^{56–59}, so we next explore a version of our model where only a plus-end length-dependent mechanism is included (Fig. 2c). We encode this mechanism into our model as a linear decrease in plus-end polymerization velocity as microtubule length increases, becoming negative above a critical length to represent plus-end depolymerization outweighing polymerization for very long microtubules (“Methods”). With this mechanism added, we see that the spindle now reaches a well-defined steady state for each value of polymerization velocity used, which was not possible in the version of the model with no length-dependent mechanisms (Fig. 2c, top). This occurs because the polymerization velocities are modulated by the length-dependent mechanism until they all converge to the same depolymerization velocity (Fig. 2c, bottom). To obtain a meaningful

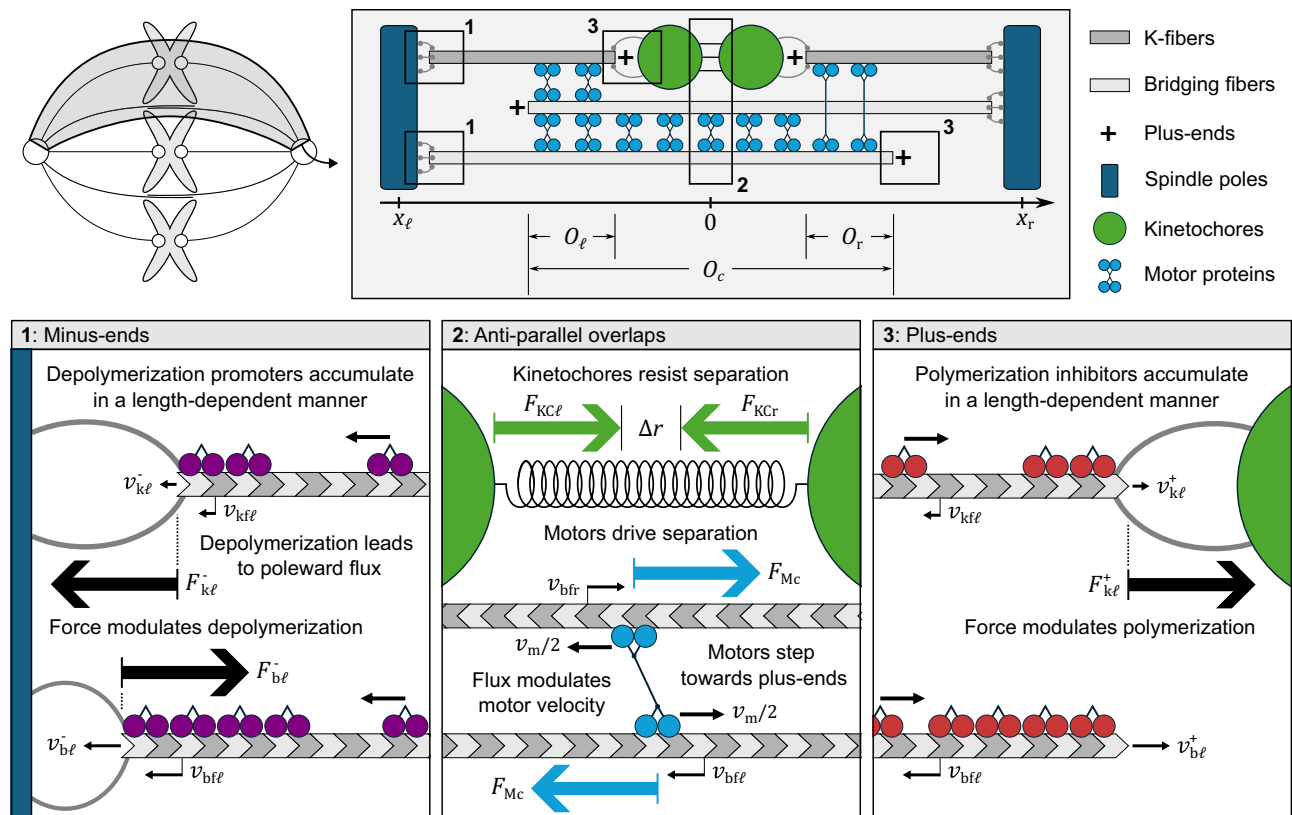


Fig. 1 | Model schematic. Our one-dimensional model represents a single chromosome pair out of the full mitotic spindle. Left and right spindle poles (dark blue) each have two microtubules (grey) attached to them. One microtubule is permanently affixed to the nearest sister kinetochore (green) to model k-fibers, while the other extends past the spindle midzone to model bridging fibers. This configuration leads to three distinct anti-parallel overlap regions: one central overlap, O_c , and two smaller overlaps to the left and right, O_ℓ and O_r , respectively. Motors (light blue) bind to these overlap regions and produce forces that work to extend the spindle by sliding microtubule plus-ends towards one another. The linkage between sister kinetochores provides a resistive force that opposes the sliding by motors. (Inset 1) Scheme demonstrating minus-end behavior in our model. There is a constant baseline depolymerization that is modulated by both force (large black

arrows) and length-dependent accumulation of depolymerization promoters (purple). Depolymerization of microtubule minus-ends leads to poleward flux. (Inset 2) Scheme demonstrating force generation. As motors step towards the plus-ends of microtubules in anti-parallel overlaps, they produce forces (large blue arrows) that move minus-ends apart. The interkinetochore force (large green arrows) resists motor force. Motor stepping velocity depends on the poleward flux velocities of the two microtubules it is bound to. (Inset 3) Scheme demonstrating plus-end behavior in our model. There is a constant baseline polymerization that is modulated by both force (large black arrow) and length-dependent accumulation of polymerization inhibitors (red). No force is exerted on bridging fiber plus-ends. Polymerization at plus-ends balances depolymerization at minus-ends to facilitate poleward flux at steady state.

change in poleward flux velocity, minus-end perturbations which directly change the depolymerization velocity must be used (Fig. 2d). These results demonstrate that plus-end perturbations have no effect on poleward flux velocity when only plus-end length-dependence is included in the model.

The version of the model with only plus-end length-dependence has major weaknesses when compared to previously published experiments. We see that it is sufficient to explain experiments in which minus-end perturbations, e.g., WDR62 or KATNB1 depletions, resulted in spindle length increasing but poleward flux velocity decreasing⁴². However, it is unable to explain experiments in which plus-end perturbations, e.g., KIF18A or KIF4A depletions, resulted in both spindle length and poleward flux velocity increasing⁴⁴. If we vary central overlap length in this version of the model to match what was observed in these experiments, changes in poleward flux velocity are still minimal (Supplementary Fig. 1a). Importantly, the changes are in the opposite direction to what is observed experimentally, with larger spindle and overlap lengths in this version of the model resulting in decreased flux velocity rather than increased⁴⁴. These results demonstrate that force-dependent effects cannot explain how microtubule minus-ends react to perturbations at the plus-end, even when central overlap length is varied to match the experiment. Therefore, plus-end

length-dependent regulatory mechanisms on their own are not sufficient to fully recreate observed experimental results.

Finally, we consider the full model with both plus- and minus-end length-dependent mechanisms included. We encode the minus-end length-dependent mechanism in our model as a linear increase in minus-end depolymerization velocity as microtubule length increases, representing continually increasing depolymerization for longer microtubules (“Methods”). With this mechanism added in conjunction with the plus-end length-dependent mechanism described earlier, we see that the spindle once again reaches a well-defined steady state for the three different plus-end perturbations (Fig. 2e, top). However, the polymerization velocity in each case now converges to a different depolymerization velocity (Fig. 2e, bottom). This leads to a direct relationship between spindle length and poleward flux velocity for plus-end perturbations (Fig. 2f). Additionally, varying central overlap length to match the experiment now results in larger overlaps having increased poleward flux velocity, consistent with previous work (Supplementary Fig. 1b)⁴⁴. Furthermore, the inverse relationship between spindle length and poleward flux velocity for minus-end perturbations is retained. This demonstrates that a minus-end length-dependent mechanism allows the minus-end to respond to perturbations at the plus-end, in line with previous theoretical work²³. More

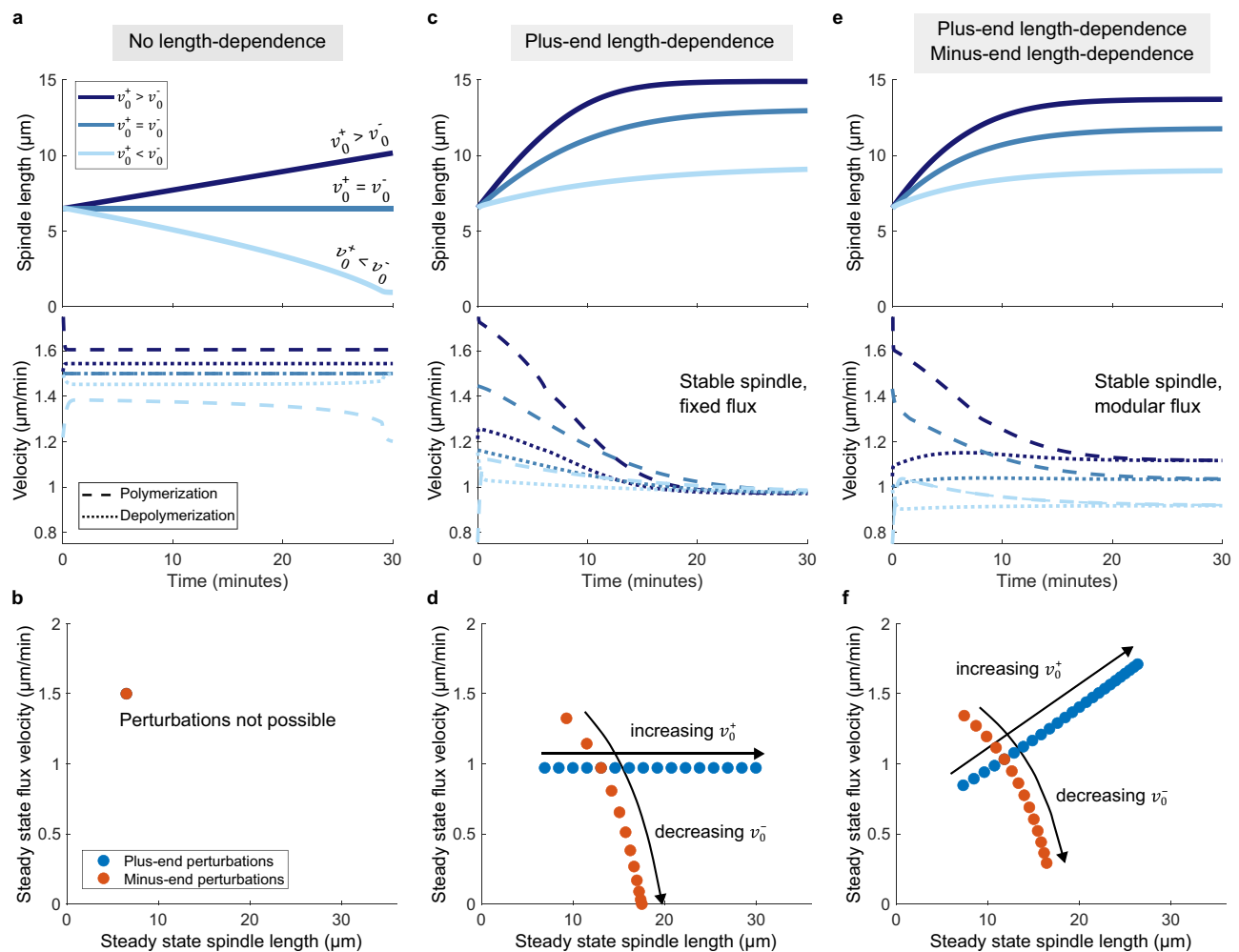


Fig. 2 | Length-dependent mechanisms at both microtubule ends facilitate the synchronization of plus- and minus-end dynamics. **a** Plots of spindle length (top) and velocities of polymerization and depolymerization (bottom) over time for a version of the model that has no length-dependent mechanisms. Polymerization, depolymerization, and single motor head stepping velocity are all set to $1.5 \mu\text{m}/\text{min}$ to produce a constant geometry (blue). Increasing polymerization by 25% results in unbounded growth (dark blue, top) because polymerization is always larger than depolymerization (dark blue, bottom). Decreasing polymerization by 20% results in spindle collapse (light blue, top) because depolymerization is always larger than polymerization (light blue, bottom). **b** Plot of steady state spindle length and k-fiber poleward flux velocity for different polymerization and depolymerization values, which only has one point since perturbations are not possible when no length-dependent mechanisms are present. **c, d** Same plots as **(a, b)**, but for a version of the model with a plus-end length-dependent mechanism present. In **c**, perturbations correspond to polymerization being increased by 100% (dark blue)

and decreased by 50% (light blue) relative to baseline (blue). All perturbations result in a stable spindle length (top), because polymerization velocities converge to the same depolymerization velocity (bottom) in all cases. In **d**, plus-end scaling factor A_k^+ and minus-end depolymerization velocity v_0^- are varied from 10 to 500% of their nominal values, showing that plus-end perturbations are unable to influence flux velocity when only a plus-end length-dependent mechanism is present. **e, f** Same plots as **(a, b)**, but for a version of the model with both plus- and minus-end length-dependent mechanisms present. In **e**, perturbations are the same as **(c)**. Spindle length is still stable for all perturbations (top), but polymerization velocities now converge to a different depolymerization velocity (bottom) in each case. In **d**, plus-end scaling factor A_k^+ and minus-end scaling factor A_k^- are varied from 10 to 500% of their nominal values, showing that a length-dependent mechanism at the minus-end allows plus-end perturbations to influence poleward flux velocity. Parameters used for plots **(c–f)** appear in Table S1.

generally, these results show that length-dependent mechanisms at both microtubule ends facilitate synchronization of plus- and minus-end dynamics by allowing them to communicate with each other through the overall length of the microtubule. Based on these results, we propose that a combination of plus- and minus-end length-dependent mechanisms is necessary to explain past experiments.

Synchronization of plus- and minus-end dynamics facilitates robust spindle length control over a wide range of poleward flux velocities

We have shown that a combination of length-dependent mechanisms at both the plus- and minus-end of microtubules allows poleward flux velocity to react to perturbations at both ends (Fig. 2f). Notably, perturbations at either the plus- or minus-end result in poleward flux

velocity either increasing or decreasing for larger spindle lengths, respectively. Here, we explore the implications of these opposite trends through simultaneous perturbations of both microtubule ends. We do this by modulating parameters in our model meant to represent the number of proteins that accumulate in a length-dependent manner and regulate plus- or minus-end dynamics (Fig. 3 and “Methods”). By linking our model with protein number in this way, we can better interpret experimental results and make predictions that can be directly tested.

Consistent with our previous results, independently modulating the number of plus- or minus-end regulatory proteins leads to spindle length and poleward flux velocity having correlated or anti-correlated changes, respectively (Fig. 3a, b). Notably, the change in either quantity has a comparable magnitude for the same percent change in the

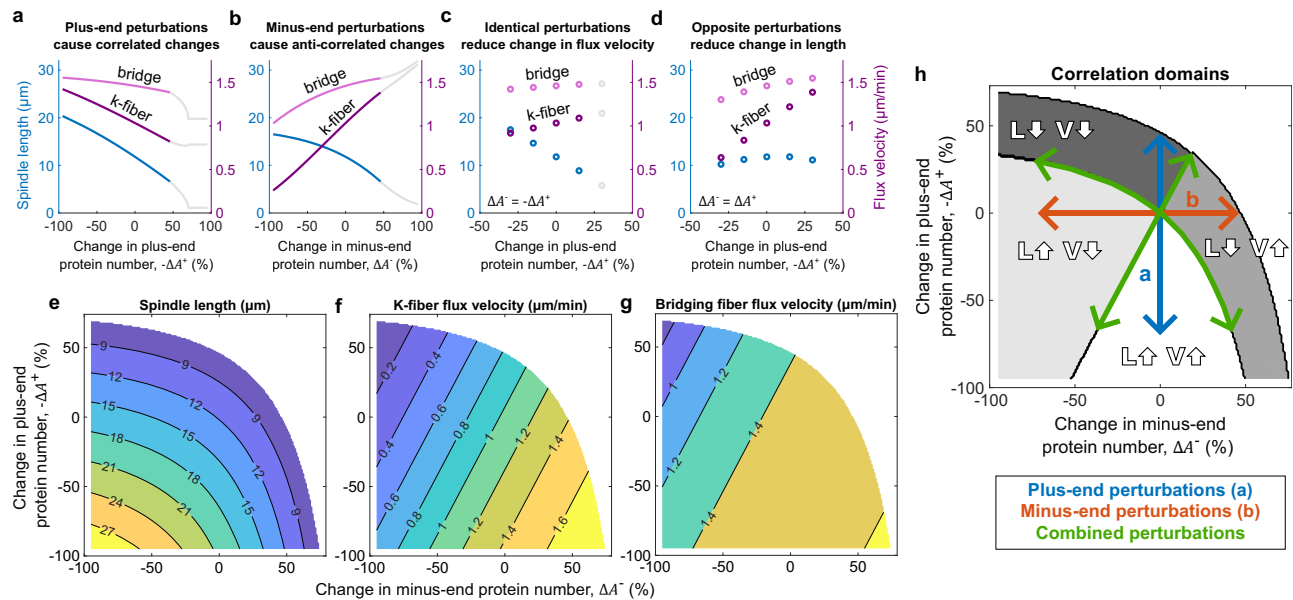


Fig. 3 | Synchronization of plus- and minus-ends through length-dependent mechanisms leads to rich dynamics in spindle length vs flux velocity. Plots of spindle length (blue) and poleward flux velocity (purple for k-fiber, magenta for bridging fiber) in the model for **a** varying plus-end protein number, **b** varying minus-end protein number, **c** varying both plus- and minus-end protein number by identical amounts, and **d** varying both plus- and minus-end protein number by equal but opposite amounts. Data which correspond to a spindle length less than 6.5 microns are shown in grey. In these plots, we assume that decreasing the parameter A_k^+ corresponds to increasing the number of plus-end-associated proteins which inhibit polymerization, e.g., KIF18A, whereas increasing A^- corresponds to increasing the number of minus-end-associated proteins which promote depolymerization, e.g., KIF2A, since both perturbations lead to smaller spindles in our

model. The positive direction of the x-axis for plus- and minus-end perturbations, therefore, corresponds to $-\Delta A^+$ and ΔA^- , respectively. In **a**, **b**, the scaling constants A_k^+ and A^- are varied between 5 and 195% of their nominal values (Table S1). In **c**, **d**, the scaling constants are varied between 70 and 130% of their nominal value in discrete 15% steps. Contour plots of **e** spindle length, **f** k-fiber flux velocity, and **g** bridging fiber flux velocity showing lines of constant value for each quantity as plus- and minus-end protein numbers are varied. Only data which corresponds to a spindle length greater than 6.5 microns are shown. **h** Plot of correlation domains set by (e–g). Cross sections which correspond to (a, b) appear as blue and orange lines, respectively, while lines of constant value from combined perturbations appear as green.

number of plus- or minus-end-associated proteins. As a result, the change in poleward flux velocity is substantially reduced if the number of plus- and minus-end-associated proteins are both varied by the same amount (Fig. 3c). Conversely, the change in spindle length is substantially reduced if the number of plus- and minus-end-associated proteins are instead varied in equal but opposite ways (Fig. 3d). The difference between plus- and minus-end perturbations thus allows for changes in either spindle length or poleward flux velocity to be partially cancelled out by combining these perturbations in different ways.

Seeing this difference in behavior motivated us to explore the full landscape of how spindle length and poleward flux velocity vary with simultaneous plus- and minus-end perturbations (Fig. 3e–g). We see that spindle length can be held fixed by compensating for an increase in the number of proteins at one microtubule end with a decrease at the other end (Fig. 3e and Supplementary Movies 1, 2). On the other hand, poleward flux velocity can be held fixed by increasing or decreasing the number of proteins at both ends simultaneously (Fig. 3f, g, and Supplementary Movies 3, 4). Therefore, our model suggests that the synchronization of plus- and minus-end dynamics through length-dependent mechanisms allows spindle length and poleward flux velocity to be uncoupled from one another through simultaneous perturbations, facilitating robust spindle length control over a wide range of poleward flux velocities.

The opposing response to changes in protein number prompted us to define four different domains relative to control values obtained with nominal parameters: spindle length and poleward flux velocity both increase, spindle length and poleward flux velocity both decrease, spindle length increases while flux velocity decreases, and

spindle length decreases while flux velocity increases (Fig. 3h). Our model predicts that overexpression or depletion of plus- or minus-end proteins each have distinct outcomes which correspond to one of these four correlation domains. We substantiate this prediction by testing perturbations of other parameters related to microtubule dynamics in our model as a control. Indeed, we observe the same qualitative trends if we independently vary either the strength of the length-dependent mechanisms (Supplementary Fig. 2a–h) or the constant baseline polymerization and depolymerization velocities (Supplementary Fig. 2i–p). Additionally, the same trends appear if central overlap length is allowed to vary freely (Supplementary Fig. 3), i.e., the plus-end polymerization velocity of bridging fibers is not constrained (Eq. 12 in “Methods”). Notably, pathways for constant spindle length and constant poleward flux velocity, in addition to the four distinct correlation domains, appear in all cases, indicating that this behavior occurs for generalized perturbations of microtubule dynamics and is independent of central overlap length being held fixed. Therefore, the conclusions presented here do not depend on our exact choice of perturbations or the constraint of having a fixed overlap length.

Length- and force-dependent mechanisms work together to make bridging fiber flux velocity more resilient to change

In our results so far, bridging fiber flux velocity has been larger in value and more resistant to change than k-fiber flux velocity (Fig. 3 and Supplementary Figs. 2, 3). Additionally, changes in bridging fiber flux velocity appear to be asymptotic, becoming substantially reduced as higher values are approached (Fig. 3g). This is consistent with previous experiments, which showed that bridging fiber flux velocity did not

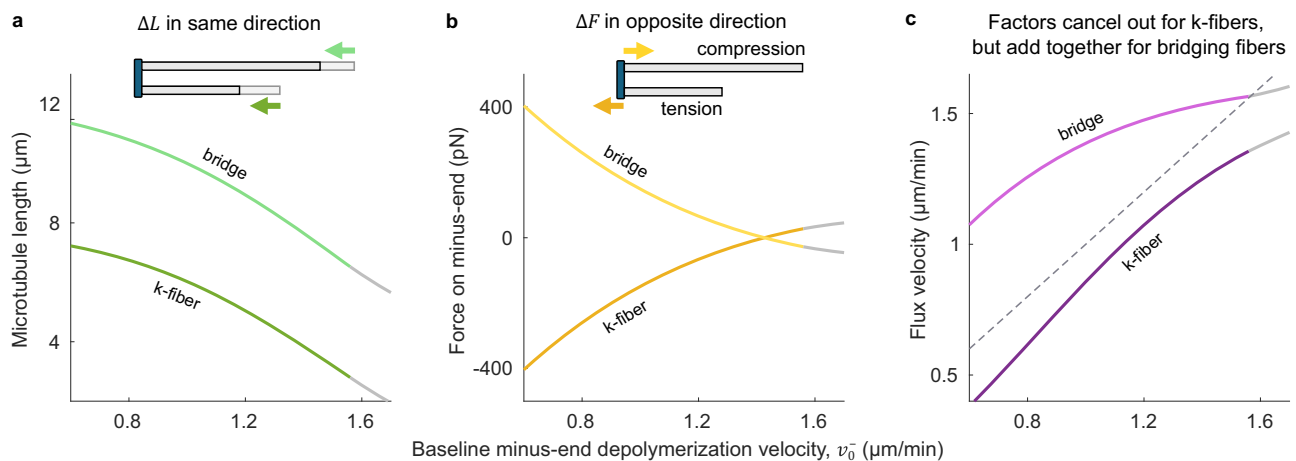


Fig. 4 | Length- and force-dependent mechanisms work together to keep the bridging fiber flux velocity constant as k-fiber flux velocity changes. **a** Plot of microtubule lengths for the left bridging (light green) and left k-fiber (dark green) as baseline depolymerization velocity v_0 is varied. The schematic shows that the changes in length for both the bridging and k-fiber are in identical directions. **b** Plot of force experienced by minus-ends of the left bridging (light yellow) and left k-fiber (dark yellow) as baseline depolymerization velocity v_0 is varied. The

schematic shows that changes in force for the left bridging and k-fiber are equal in magnitude but opposite in direction. **c** Plot of steady state poleward flux velocity for the left bridging (magenta) and left k-fiber (purple) as baseline depolymerization velocity v_0 is varied. Dashed grey line corresponds to baseline depolymerization velocity, i.e., has a slope of 1, which both flux velocities would be equal to if neither length- nor force-dependent mechanisms were present.

substantially increase even as k-fiber flux velocity did⁴⁴. These results suggest that there is some process which makes bridging fiber flux velocity resilient to perturbations and establishes an upper limit.

To investigate these trends in poleward flux velocity, we vary baseline depolymerization velocity in our model and observe how microtubule length and force at the minus-ends respond, since both quantities modulate poleward flux velocity (Eqs. 10 and 11 in “Methods”). We see that both microtubule lengths decrease as baseline depolymerization velocity increases, and therefore the minus-end length-dependent factor decreases for both k-fibers and bridging fibers (Fig. 4a). Force generation from motors also decreases as depolymerization velocity increases (Eqs. 5 and 8 in “Methods”) and so the force-dependent factor decreases as well. However, the forces experienced by bridging and k-fiber minus-ends are in opposite directions due to the intrinsic geometry of our model, so this decrease in force-dependent factors leads to k-fiber flux velocity increasing but bridging fiber flux velocity decreasing (Fig. 4b). Thus, the force- and length-dependent mechanisms partially cancel each other out for k-fibers, leading to a near-linear increase in flux velocity as baseline depolymerization velocity increases (Fig. 4c). Conversely, these two mechanisms add together and substantially decrease bridging fiber flux velocity as baseline depolymerization velocity increases, leading to much smaller changes. Force- and length-dependent factors therefore work together to keep bridging fiber flux velocity constant through perturbations of baseline depolymerization velocity in our model.

These results also show that as poleward flux velocity exceeds half the sliding velocity of motors, which is the stepping velocity of a single motor head, the forces produced by motors invert and begin to pull spindle poles together rather than push them apart. We observe this in Fig. 4b when bridging and k-fiber forces cross over zero, indicating that the direction of motor forces in the spindle has reversed. Shortly after this crossover, the spindle collapses. Our model thus predicts that the upper limit of flux velocity ultimately comes from the stepping velocity of motors. A corollary of this result is that the length-dependent mechanism at the minus-end modulates forces within the spindle by increasing poleward flux velocity for longer microtubules with larger overlaps. Without this modulation, increasing motor forces would decrease k-fiber flux velocity, resulting in a feedback loop that leads to

significantly increased forces (Supplementary Fig. 4). Therefore, a length-dependent mechanism at microtubule minus-ends helps maintain constant forces within the spindle over a wider range of conditions.

In vivo experiments confirm model predictions

Our model makes three key predictions: (1) perturbations at microtubule plus-ends lead to correlated changes in spindle length and poleward flux velocity while perturbations at minus-ends lead to anti-correlated changes, (2) simultaneous perturbations at both microtubule ends can allow one quantity to remain constant while the other varies, and (3) an increase in spindle length leads to a greater amount of minus-end depolymerization promoters near the spindle poles due to length-dependent accumulation. To test these predictions, we analyzed spindles in live human cells following perturbations of proteins with well-known biological functions: the plus-end polymerization inhibitor KIF18A⁵⁸, the minus-end severing protein KATNB1⁶¹, and the minus-end depolymerizing protein KIF2A⁶².

We first performed siRNA-mediated single depletion experiments in vivo using hTERT RPE-1 cells and measured the resulting spindle length and poleward flux velocity in live cells (Fig. 5a, b, and Supplementary Figs. 5a, 6a, 7a–h, Supplementary Movies 5, 6). To exclude confounding effects, we first confirmed that the live-cell tubulin probe SPY555-Tubulin, a taxol derivative, did not affect spindle length at the dilution level used in our experiments (Supplementary Fig. 5b, c, and Supplementary Movie 7). We found that depletion of KIF18A, a plus-end-associated protein, led to both spindle length and poleward flux velocity increasing. Conversely, depletion of KATNB1 or KIF2A, two minus-end-associated proteins, led to spindle length increasing but poleward flux velocity decreasing. These measured changes in spindle length and poleward flux velocity for KIF18A^{44,58} and KATNB1^{42,48} depletions, as well as the increase in spindle length for KIF2A⁶³ depletion, are in line with previous observations. Consistent with the first prediction of our model, these results demonstrate that depletion of plus- or minus-end-associated proteins in vivo leads to changes in spindle length and poleward flux velocity, which are correlated or anti-correlated, respectively.

To test the second prediction of our model, we next co-depleted microtubule plus- and minus-end regulating proteins in hTERT RPE-1

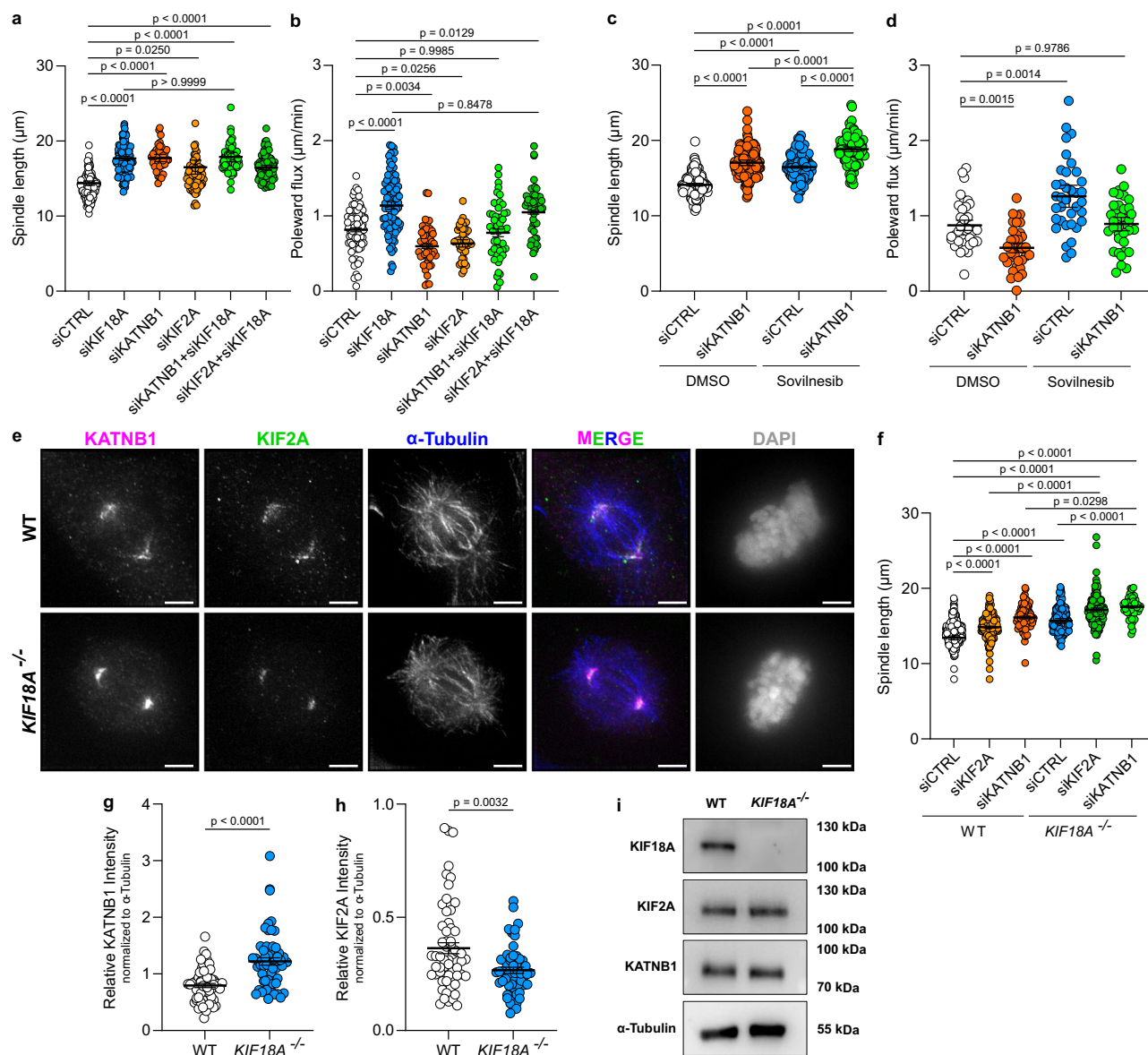


Fig. 5 | In vivo experiments confirm model predictions. **a** Quantification of spindle length in hTERT RPE-1 cells treated with indicated siRNAs: siCTRL ($N = 8$ independent experiments, $n = 198$ cells), siKIF18A ($N = 8$, $n = 133$), siKATNB1 ($N = 4$, $n = 42$), siKIF2A ($N = 4$, $n = 64$), siKATNB1+siKIF18A ($N = 3$, $n = 72$), and siKIF2A+siKIF18A ($N = 4$, $n = 90$). **b** Quantification of poleward flux velocity in hTERT RPE-1 PA GFP α -Tubulin cells treated with indicated siRNAs: siCTRL ($N = 8$, $n = 83$), siKIF18A ($N = 8$, $n = 96$), siKATNB1 ($N = 4$, $n = 50$), siKIF2A ($N = 4$, $n = 41$), siKATNB1+siKIF18A ($N = 4$, $n = 48$), and siKIF2A+siKIF18A ($N = 4$, $n = 51$). **c** Quantification of spindle length in hTERT RPE-1 cells treated with KATNB1 siRNA and/or Sovilnesib: siCTRL+DMSO ($N = 3$, $n = 197$), siKATNB1+DMSO ($N = 3$, $n = 174$), siCTRL+Sovilnesib ($N = 3$, $n = 129$), and siKATNB1+Sovilnesib ($N = 3$, $n = 114$). **d** Quantification of poleward flux velocity in hTERT RPE-1 PA GFP α -Tubulin cells treated with KATNB1 siRNA and/or Sovilnesib ($N = 3$, $n = 36$ for all). **e** Immunofluorescence images of WT and *KIF18A*^{-/-} hTERT RPE-1 cells stained for

KATNB1, KIF2A, α -Tubulin, and DAPI. Scale bars are 5 μ m. **f** Quantification of spindle length in WT and hTERT RPE-1 *KIF18A*^{-/-} cells treated with indicated siRNAs: siCTRL in WT ($N = 5$, $n = 364$), siKIF2A in WT ($N = 3$, $n = 225$), siKATNB1 in WT ($N = 3$, $n = 81$), siCTRL in *KIF18A*^{-/-} ($N = 5$, $n = 215$), siKIF2A in *KIF18A*^{-/-} ($N = 3$, $n = 191$), and siKATNB1 in *KIF18A*^{-/-} ($N = 3$, $n = 43$). Quantification of relative **g** KATNB1 and **h** KIF2A intensity at the poles normalized to α -Tubulin. $n = 58$ –62 cells. **i** Western blot of WT vs hTERT RPE-1 *KIF18A*^{-/-} whole cell extracts probed with KIF18A, KIF2A, KATNB1, and α -Tubulin (loading control) antibodies; this experiment was repeated 3 times with similar results. Statistical tests used to obtain p -values: linear mixed-effects model analysis (REML) with Tukey's multiple comparisons in (**a**–**c**, **f**); a RM two-way ANOVA with Dunnett's multiple comparisons in (**d**); Mann-Whitney in (**g**, **h**). In all cases, statistical tests were two-sided. Reported p -values in (**a**–**d**, **f**) were adjusted to account for multiple comparisons. Error bars indicate SEM. All source data are provided as a Source Data file.

cells and measured the resulting spindle length and poleward flux velocity in live cells (Fig. 5a, b, and Supplementary Figs. 5a, 6a, 7a–h, Supplementary Movies 5, 6). When KIF18A and KATNB1 were co-depleted, spindle length increased relative to control, but remarkably, no significant change in poleward flux velocity occurred. On the other hand, when KIF18A and KIF2A were co-depleted, both spindle length and poleward flux velocity increased relative to control. Notably, the same trends were observed when Sovilnesib, a pharmacological

inhibitor of KIF18A, was used instead of KIF18A siRNA, eliminating siRNA efficiency in co-depletions as a factor (Fig. 5c, d, and Supplementary Figs. 5d, 6b, 7i, j, Supplementary Movies 8, 9). These results therefore show that depletion or inhibition of plus- and minus-end-associated proteins can be combined to uncouple spindle length and poleward flux velocity from one another in vivo, confirming our second model prediction.

The third prediction of our model is that an increase in spindle length will lead to enhanced accumulation of proteins which promote microtubule minus-end depolymerization near or at spindle poles. To test whether KATNB1 or KIF2A might fulfill this prediction, we utilized an RPE-1 *KIF18A*^{-/-} cell line that has increased spindle length relative to its parental wild-type RPE-1 cell line (Fig. 5e, f, Supplementary Fig. 5e, and Supplementary Movie 10)⁶⁴. Consistent with our model prediction, we found by immunofluorescence that KATNB1 abundance near the spindle poles increased in RPE-1 *KIF18A*^{-/-} cells by 53% compared to the parental cell line (Fig. 5e, g). In contrast, KIF2A abundance at the spindle poles decreased in RPE-1 *KIF18A*^{-/-} cells by 27% compared to the parental cell line (Fig. 5e, h). Importantly, the increased abundance of KATNB1 at spindle poles was not caused by a change in protein expression (Fig. 5i) but rather increased recruitment at spindle poles. These results suggest that KATNB1, but not KIF2A, could facilitate the length-dependent regulation of microtubule minus-end depolymerization at poles that we propose in our model.

Based on these findings, we further predicted that the increase in microtubule poleward flux velocity seen in cells with depleted or inhibited KIF18A ultimately depends on length-dependent regulation at minus-ends, i.e., on the levels of KATNB1 at the poles. Moreover, we predicted that depleting KATNB1 in a KIF18A-depleted or -inhibited background would further increase spindle length. Indeed, when we depleted KATNB1 in a KIF18A-depleted or -inhibited background, we found a decrease in poleward flux velocity, whereas KIF2A depletion barely affected flux velocity in a KIF18A-depletion background (Fig. 5b). This confirmed that the increase in microtubule poleward flux velocity seen in KIF18A-depleted or inhibited cells depends on KATNB1. Furthermore, we found that KATNB1 depletion also increased spindle length in KIF18A-inhibited cells but not in KIF18A-depleted cells (Fig. 5a, c). We suspected that this difference was due to a less efficient KIF18A depletion in double depletion cells (Supplementary Fig. 7d, g, h). We thus repeated spindle length measurements in hTERT RPE-1 *KIF18A*^{-/-} cells and found that KATNB1 depletion indeed increased spindle length, as expected (Fig. 5f). We conclude that, as predicted by our model, an increase in spindle length due to perturbations at microtubule plus-ends leads to an associated increase in minus-end depolymerization via a length-dependent mechanism.

Quantitative mapping of experiments onto the model rectifies previous work

We have demonstrated that proteins influence spindle length and poleward flux velocity in distinct ways based on whether they predominantly affect plus- or minus-end dynamics. A consequence of this is that each pair of spindle length and poleward flux velocity values corresponds to a unique coordinate of plus- and minus-end protein numbers in our model (Fig. 3e, f). Based on this, we develop a method for quantitatively comparing our model against experiments which perturb proteins known to regulate microtubule dynamics. We use experimental measurements of spindle length and poleward flux velocity as input values, and our model assigns to them a unique coordinate which predicts the extent to which plus- and minus-end dynamics are affected without any prior knowledge of biological function. This allows us to not only test our model against proteins with known functions but also extrapolate and infer the function of less well-understood proteins.

We begin by mapping protein depletions from this work onto unique coordinates in the correlation domains predicted by our model (Fig. 6 and Supplementary Fig. 8). Based on their locations in the plot, our theory suggests that KIF18A predominantly affects plus-end dynamics, whereas both KATNB1 and KIF2A predominantly affect minus-end dynamics. This is consistent with their known biological function, corroborating the basic assumptions of our approach. The two co-depletions are mapped onto locations which suggest that KIF18A+KATNB1 co-depletion affects plus- and minus-end dynamics to

a similar extent, whereas KIF18A+KIF2A co-depletion predominantly affects plus-end dynamics. In other words, our model suggests that KATNB1 influences minus-end dynamics to a greater extent than KIF2A in KIF18A-depleted cells.

Next, we map depletions of relevant proteins from previous works onto the correlation domains predicted by our model (Fig. 6 and Supplementary Fig. 8). We include experiments which measured spindle length and poleward flux velocity for different perturbations that affected spindle dynamics^{36,42–48}. Depletions, which involved NDC80, are excluded since they eliminate k-fibers from the spindle⁴⁶, and our model is unable to represent such geometry. In these experiments, the proteins known to be associated with plus-end regulation are KIF18A⁵⁸, KIF4A⁶⁵, and CLASPs⁶⁴. Since the plus-end length-dependent mechanism in our model is based on proteins which inhibit polymerization, depletions involving KIF18A and KIF4A are mapped onto locations which directly correspond to a reduction in plus-end polymerization inhibitors in our model. On the other hand, depletions involving CLASPs, which promote plus-end polymerization rather than inhibit it, are mapped onto locations which correspond to an increase in plus-end polymerization inhibitors in our model. Proteins known to be associated with minus-end dynamics in these experiments are KATNB1⁶¹, KIF2A⁴⁰, NuMA⁶⁶, WDR62⁶⁷, ASPM⁴⁸, and NuSAP⁴⁷. All these proteins effectively increase minus-end depolymerization, with the exception of NuSAP, which inhibits depolymerization by KIF2A in addition to other functions. Consistent with our expectations, the first five depletions are mapped onto locations which directly correspond to a decrease in minus-end depolymerization promoters in our model, whereas NuSAP depletion is instead mapped onto a location which corresponds to an increase in minus-end depolymerization promoters in our model. Co-depletion experiments are mapped onto locations that are between the locations of their constituent single depletions, and so our model suggests that they partially cancel each other out. Depletion of proteins which affect both microtubule ends, e.g., MCAK⁶⁸, or do not directly influence microtubule dynamics, e.g., Haus8⁶⁹ or PRC1⁷⁰, are difficult to interpret using our model but are still included for the sake of completeness. This mapping shows that our model can rectify the seemingly contradictory results of how spindle length and poleward flux velocity change relative to one another in these experiments.

We see that depletions of proteins with similar biological function naturally cluster together when quantitatively mapped onto the domains of our model. Furthermore, almost all depletions are mapped onto domains which are consistent with known protein function, with the KIF4A and KIF2A depletions from Steblyanko et al. being two major exceptions. We note that other depletions of these proteins, KIF4A from Risteski et al. and KIF2A from this work, which agree with previously reported spindle length measurements⁶³, are mapped onto locations which are consistent with known protein function. We attribute these differences between individual depletions of the same protein to the different microtubule regulation dynamics between cell lines, as this work and Risteski et al. used RPE-1 cell lines, whereas Steblyanko et al. used U2OS cell lines. The two depletions from Jiang et al., which are borderline but still not located in the expected domains, also used U2OS cell lines. The only datum point which lies outside the explored bounds of our model domains is the MCAK +KIF2A co-depletion from Ganem et al., which has a measured poleward flux velocity that is slightly smaller than the lower bound in our model. We attribute this to a breakdown of the assumptions in our model for exceedingly low minus-end protein concentrations, i.e., nearly 100% reduction. Finally, we note that the mapped coordinates of some single depletion experiments correspond to changes in both plus- and minus-end protein concentration in our model. We propose that this ultimately arises from simplifications used in our model, which describe complex physical processes as linear relationships. Altogether, these results show that our model can explain the vast

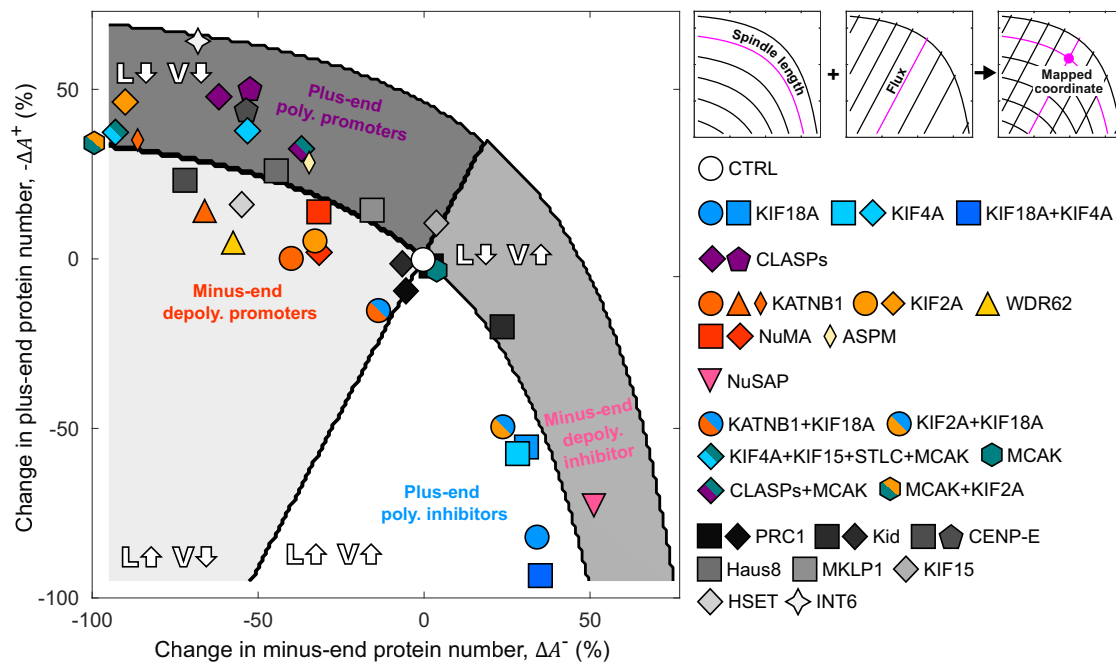


Fig. 6 | Quantitative mapping of experimental measurements unifies previous work. Data from this work (circles) and previously published experiments (other symbols) mapped onto the correlation domains predicted by our model (Fig. 3g) using overall flux velocity values from theory (Supplementary Fig. 8). Depletions of proteins known to inhibit or promote plus-end polymerization are colored in shades of blue or purple, respectively, whereas proteins known to inhibit or promote minus-end depolymerization are colored in shades of pink or red/orange, respectively. The protein MCAK, which affects both microtubule ends, is colored green. Co-depletions are represented by the colors of their constituent single depletions. Other proteins which do not directly affect microtubule dynamics are depicted in shades of grey. Circles correspond to original data from

this work (Fig. 5a, b), and other symbols represent data from previously published work: squares are Risteski et al.⁴⁴; square diamonds are Steblyanko et al.⁴⁶; thin diamonds are Jiang et al.⁴⁸; triangles are Guerreiro et al.⁴²; pentagons are Maffini et al.⁴⁵; hexagons are Ganem et al.³⁶; upside-down triangles are Sun et al.⁴⁷; and four-pointed stars are Renda et al.⁴³. All experimental measurements are normalized relative to theory such that the reported control value corresponds to simulation output using nominal parameter values (Table S1). Proteins with known biological function naturally cluster together in this quantitative mapping, and the vast majority of perturbations appear in the expected correlation domains, showing that our model rectifies these results across past works.

majority of depletion experiments across previous works, thereby unifying them.

Discussion

We have shown that length-dependent mechanisms can synchronize the dynamics of microtubule plus- and minus-ends, resulting in them working together rather than length being regulated entirely by the plus-end (Figs. 1 and 2)^{56,58,59}. This synchronization can explain changes in poleward flux velocity resulting from depletion of plus-end-associated proteins and, in general, how the spindle regulates changes in flux and maintains a stable geometry over a wide range of flux velocities (Fig. 3 and Supplementary Movies 1–4). Our model predicted that plus-end perturbations lead to correlated changes in spindle length and poleward flux velocity, whereas minus-end perturbations lead to anti-correlated changes, and we validated these predictions with in vivo depletion experiments of the plus-end-associated protein KIF18A and minus-end-associated proteins KIF2A and KATNB1 (Fig. 5). We also demonstrated that simultaneous perturbations of plus- and minus-ends can cancel out changes in either spindle length or flux velocity in our model, which we corroborated with an in vivo co-depletion experiment that rescued poleward flux velocity for larger spindle length in human cells. Finally, we showed that KATNB1 levels near spindle poles are strongly enhanced in KIF18A *KIF18A*^{-/-} cells that have longer spindles than the corresponding parental cell, providing experimental evidence for length-dependent accumulation of a minus-end regulating protein, as predicted by our model.

Our results provide an explanation for past experiments in which spindle length and poleward flux velocity were observed to change in the same direction^{41,43–46}, in the opposite direction^{35,42}, or vary independently of one another^{36,48} (Fig. 6). We demonstrated that synchronization of both microtubule ends through length-dependent mechanisms allows these seemingly contradictory results to be rectified through perturbations that predominantly affect microtubule plus-ends, minus-ends, or a combination of both, respectively. This expands upon previous work, which proposed a direct relationship between spindle length and poleward flux velocity^{23,44,46}. Additionally, our results shed light on how poleward flux velocity can vary by an order of magnitude between species despite having similar spindle lengths^{30,32,44}. More generally, we propose that this synchronization mechanism may allow cells to better maintain their spindle length across varying conditions (Fig. 7). Loss of proteins at either end would lead to a larger spindle, causing more proteins to accumulate at the opposite end and exert increased length regulation, reducing overall spindle size at the cost of poleward flux velocity. Additionally, this synchronization would allow cells to directly upregulate minus-end-associated proteins and compensate for a loss of plus-end-associated proteins or vice versa, maintaining a constant spindle length in exchange for poleward flux velocity (Supplementary Movies 1 and 2). Thus, our work not only resolves a paradox of how spindle length and poleward flux velocity relate to each other by unifying different observed trends, but it also reveals a fundamental mechanism of spindle length control.

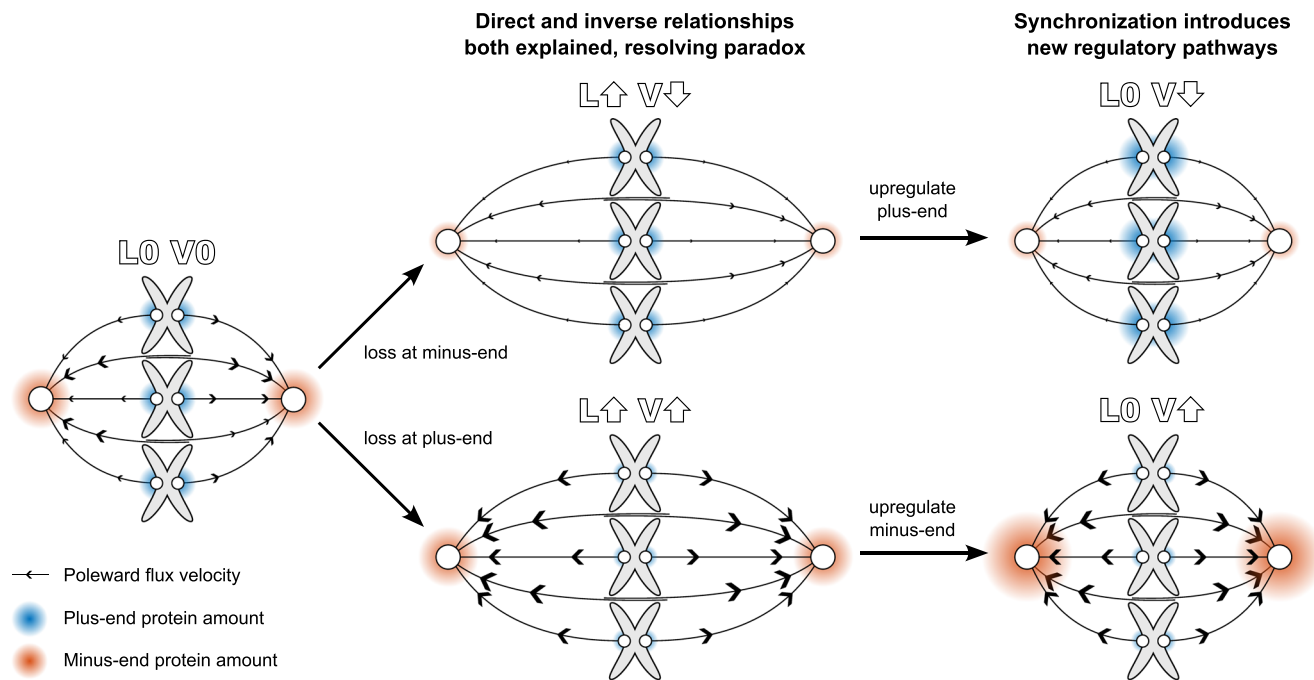


Fig. 7 | Schematic demonstrating robust spindle length control via the synchronization of microtubule plus- and minus-end dynamics. Length-dependent mechanisms at both microtubule ends allow each end to sense and respond to changes at the opposite end. Loss of either minus- (top) or plus-end-associated (bottom) proteins leads to larger spindles and decreased or increased poleward

flux velocity, respectively. The synchronization of plus- and minus-end dynamics facilitated by length-dependent mechanisms allows cells to upregulate proteins at the opposite end to exert increased length regulation, partially or fully rescuing spindle length at the cost of poleward flux velocity decreasing (top) or increasing (bottom).

The synchronization mechanism uncovered in this paper answers a longstanding question of how microtubule ends coordinate with one another despite being separated by microns. In anaphase, for example, the coordinated action of both plus- and minus-end-associated proteins^{71–73} is thought to control chromosome segregation, a process which is followed by a reduction in poleward flux velocity and spindle elongation across species^{34,74}. Our model shows that synchronization of plus- and minus-end dynamics through length-dependent mechanisms at both microtubule ends could facilitate this type of coordinated behavior. Furthermore, it is known that the reduction of poleward flux velocity and subsequent spindle elongation is driven by downregulation of kinesin-13 proteins^{75,76}, which is consistent with the predictions of our model. Additionally, our model suggests that lowering poleward flux velocity allows motor proteins in anti-parallel overlaps to exert significantly more force, further contributing to rapid spindle elongation and segregation of chromosomes^{77,78}. Our proposed synchronization mechanism, therefore, provides additional insight into how transitions in spindle length are controlled and how plus- and minus-end-associated proteins work together in a precise way. Furthermore, the conclusions presented here can be extended to other systems which exhibit a flux of microtubules, e.g., developing neurons where continual retrograde flow of microtubules towards the soma is involved in axon formation^{79,80}.

Our theoretical model also offers additional perspective on the ongoing debate of the primary drivers of microtubule poleward flux. Previous experiments have shown that poleward flux persisted when crosslinking motors were depleted, suggesting that depolymerization at microtubule minus-ends is the main driver⁸¹. Conversely, other experiments have shown that laser-severed k-fibers exhibited poleward movement despite no depolymerization occurring at the newly exposed minus-ends, implicating motor forces as a driving mechanism^{37,82}. Our model suggests that both factors contribute to poleward flux, explaining why it persisted when either minus-end depolymerization or motor forces were eliminated in previous

experiments. Furthermore, our model predicts that these two factors directly influence one another: forces from motors affect minus-end depolymerization, and minus-end depolymerization modulates motor forces (Fig. 4). We have shown that the different interactions between these two components can explain why the poleward flux velocity of bridging fibers and k-fibers have distinct values and responses to perturbations^{44,46}, and we further posit that it introduces a way for the spindle to actively regulate motor force generation through minus-end dynamics. This hypothesis is supported by previous experiments which showed that reducing minus-end depolymerization led to spindle elongation due to motor sliding^{40,83}. We therefore propose that motor force generation and minus-end depolymerization both work together to drive poleward flux, and the balance between them plays a direct role in spindle length control.

This work lays the foundation for a fundamental mechanism of spindle length regulation and opens new directions for future studies. Follow-up experiments that combine depletion of one protein with overexpression of another could yield additional insight into the cellular function of the regulatory pathways proposed in this paper. Additionally, further exploration of our proposed synchronization mechanism, e.g., the timescale required for one end to react to perturbations at the opposite end, could also shed light on the precise molecular mechanisms involved. Expanding our model, such as incorporating more realistic microtubule distributions, would allow us to explore the effects of our proposed synchronization mechanism in different stages of mitosis, e.g., prometaphase when k-fibers are forming or anaphase when sister kinetochores are no longer linked. With this more general geometry, we could also study the role of microtubule branching in the reciprocal control of spindle length and poleward flux velocity. Furthermore, adding dynamic kinetochore-microtubule attachments would let us investigate chromosome oscillations and how they respond to the perturbations described in this paper. Finally, incorporating additional spatial dimensions and steric interactions between chromosomes would let us study aberrant

architectures, such as multipolar spindles, and the mechanisms of length control proposed here may help characterize how these structures evolve over time and congregate their excess centrosomes^{84–86}.

Methods

Model equations of motion

To describe the geometry of the system, we follow the position of the left and right spindle poles, denoted as x_ℓ and x_r , respectively, over time t . We choose an initial condition such that $x = 0$ is the center of the spindle and define our coordinate system such that the left (right) region corresponds to negative (positive) positions and direction. Each term is denoted with an ℓ or r subscript to indicate which region it is in. The length of the spindle, L , is defined as the separation between spindle poles, $L = x_r - x_\ell$, which is strictly positive due to our choice of coordinate system. The coordinates x_ℓ and x_r also correspond to the position of minus-ends for microtubules originating from the left and right spindle poles, respectively, since we assume that they are permanently bound together. Microtubule fibers are thus fully defined by their lengths, L_j for index $j = b\ell, k\ell, br, kr$ corresponding to left bridging, left k-fiber, right bridging, and right k-fiber, respectively, which we use to calculate plus-end positions in conjunction with spindle pole position, e.g., $x_{b\ell}^+ = x_\ell + L_{b\ell}$ is the position of the left bridging fiber plus-end. With this information, we can calculate the length of all anti-parallel overlaps in the system and determine the sliding forces generated by motors, e.g., $O_c = L_{b\ell} + L_{br} - L$ is the length of the central anti-parallel overlap formed by the left and right bridging fibers. Similarly, we can use the position of k-fiber plus-ends to calculate interkinetochore stretch. The geometry of the system is thus fully defined by the two spindle pole positions and four microtubule lengths, which we can use to calculate forces arising from sliding motors and interkinetochore stretch.

We assume that the forces on each spindle pole are balanced, i.e., the drag force is equal to the net force exerted by microtubules:

$$\gamma \frac{dx_{\ell,r}}{dt} = F_{b\ell,r} + F_{k\ell,r}. \quad (1)$$

where γ is the spindle pole drag coefficient, and the forces exerted on spindle poles by the bridging and k-fibers are denoted as $F_{b\ell,r}$ and $F_{k\ell,r}$, respectively. For bridging fibers, these forces come exclusively from motors in the two anti-parallel overlaps formed with opposing microtubules. For k-fibers, the reactionary force from interkinetochore stretch must also be considered. For the sake of both simplicity and clarity, we only discuss equations for the left spindle pole here. The full set of equations and parameter values used appears in Supplementary Information. The force on the left spindle pole from the left bridging fiber is:

$$F_{b\ell} = F_{Mc} + F_{Mr}. \quad (2)$$

Here, F_{Mc} is the force generated by motors in the central overlap, and F_{Mr} is the force generated by motors in the right overlap. Similarly, the force exerted on the left spindle pole by the left k-fiber is:

$$F_{k\ell} = F_{M\ell} + F_{KC}, \quad (3)$$

where $F_{M\ell}$ is the force generated by motors in the left overlap and F_{KC} is the tensile force between sister kinetochores. The velocity of each spindle pole can therefore be calculated directly from the constituent forces of the system.

We find the tensile force between sister kinetochores by considering the stretch of the linkage connecting them. Experiments have shown that this linkage can be characterized by an effective stiffness that increases with interkinetochore stretch⁸⁷, which we incorporate

into our model as a nonlinear elastic spring:

$$F_{KC} = (\kappa + \sigma \Delta r) \Delta r, \quad (4)$$

where κ is the stiffness of the spring, σ controls the nonlinear response to stretching, and $\Delta r = r - r_0$ is the stretch from rest length, r_0 , of the spring connecting sister kinetochores. This functional form ensures that interkinetochore distance, and therefore the left and right overlap length, remain comparable to experiment in our model. The positions of sister kinetochores are set by the position of k-fiber plus-ends, allowing us to calculate the interkinetochore distance from system geometry, $r = L - L_{k\ell} - L_{kr} - 2D_{KC}$, where $L_{k\ell}$ and L_{kr} are the lengths of the left and right k-fibers, respectively, and D_{KC} is the diameter of each kinetochore. Here, we have assumed that the drag force of kinetochores is negligible. Together, Eqs. 3 and 4 establish a link between the active forces generated by motor proteins and the passive response of interkinetochore stretch.

To describe the force generated by motors in anti-parallel overlaps, we use a mean-field approach. First, we assume that all motors exert an equal force f_m such that $F_M = N_m f_m$, where N_m is the total number of motors in the overlap. Second, we assume that the number of motors is equal to a linear density c_m multiplied by the length of the overlap O , or $N_m = c_m O$. Finally, we use a linear force velocity relationship, $v_m = v_0(1 - f_m/f_0)$, to describe the force generated by each motor as a function of its stepping velocity v_m . Here, v_0 is the stepping velocity of motors in the absence of force, and f_0 is the motor stall force. Putting this all together, the motor forces on the left spindle pole are:

$$F_{Mi} = c_m O_i f_0 (-1 - v_{mi}/v_0), \quad (5)$$

where O_i denotes the length of the overlap region and v_{mi} denotes the stepping velocity of motors for index $i = \ell, c, r$ corresponding to left, central, and right overlap regions, respectively. The central overlap is calculated as $O_c = L_{b\ell} + L_{br} - L$ and the off-center overlaps are calculated as $O_{\ell,r} = L_{k\ell,r} + L_{br,\ell} - L$, where $L_{b\ell}$, L_{br} , $L_{k\ell}$, and L_{kr} are the lengths of the left bridging, right bridging, left k-fiber, and right k-fiber microtubules, respectively. We enforce that all overlaps are bounded between 0 and L , i.e., $O_i = \max(0, \min(L, O_i))$. In our model, the linear density c_m remains constant through time. In the absence of any relative movement between microtubules, motors have a stepping velocity of zero and exert a maximum force. The stepping velocity of motors is related to the poleward flux velocity of the two microtubules they are bound to, as discussed in the next section⁸³. Therefore, Eqs. 1–5 establish a link between system geometry, microtubule poleward flux, and the active force generation of motors.

Model description of microtubule plus- and minus-end dynamics

To fully describe spindle length evolution over time, we also include microtubule growth dynamics. We do not model the full stochastic process involving catastrophe, pause, and rescue events, but instead streamline these into an average polymerization velocity at the plus-end of microtubules. We also assume that the minus-ends of microtubules are continually depolymerized by proteins at the spindle pole, yielding

$$\frac{dL_j}{dt} = \pm (v_j^+ + v_j^-), \quad (6)$$

where L_j is the length of microtubules for index $j = b\ell, k\ell, br, kr$ that corresponds to left bridging, left k-fiber, right bridging, and right k-fiber, respectively. Here, $\pm$ indicates a positive (negative) sign for left (right) microtubules, v_j^+ is the polymerization velocity of the microtubule plus-end, and v_j^- is the depolymerization velocity of the

microtubule minus-end. Minus-ends remain fixed to spindle poles in our model, so the length change calculated from Eq. 6 is applied entirely at the plus-end, and the sign of each individual velocity is given by the direction it causes the plus-end to move. Thus, for microtubules originating from the left (right) spindle pole, the depolymerization velocity is negative (positive), and the polymerization velocity is positive (negative). For all microtubules, regardless of which spindle pole they originate from, a positive value of dL_j/dt indicates an increase in length, whereas a negative value indicates a decrease in length.

In our model, microtubule plus-ends move toward the spindle pole they originate from if $v^+ < v^-$, away if $v^+ > v^-$, and stay at a constant distance if $v^+ = v^-$. Any given segment along the microtubule will move towards the spindle pole with velocity v^- regardless of what occurs at the plus-end, resulting in microtubule poleward flux (Fig. 1, inset 1). To obtain the poleward flux velocity as measured from the lab reference frame, we also include the corresponding spindle pole velocity:

$$v_{f/r} = v_{f/r}^- + \frac{dx_{f/r}}{dt} \quad (7)$$

Note that, at steady state, poleward flux velocity and depolymerization velocity are identical. It can be shown that the stepping velocity of motors in an anti-parallel overlap is equal to the difference in poleward flux velocities of the microtubules which compose that overlap⁵³. For example, the motor velocity in the central overlap from the perspective of the left spindle pole is given by:

$$v_{mc} = v_{bfl} - v_{bfr} \quad (8)$$

where v_{bfl} and v_{bfr} are the poleward flux velocities of the left and right bridging fibers, respectively, that make up the central overlap. The steady state sliding velocity of motors in the central overlap from the perspective of the left spindle pole is therefore $v_{mc} = v_{bfl}^- - v_{bfr}^-$. In the case of a symmetric spindle, $v_{bfl}^- = -v_{bfr}^-$, leading to a net motor stepping velocity of $2v_{bfl}^-$ with each motor head having a stepping velocity of v_{bfl}^- at steady state. Note that, due to our choice of coordinate system, the flux velocity of microtubules originating from the left spindle pole is always negative, whereas the flux velocity for microtubules originating from the right spindle pole is always positive. If we were considering the motor velocity from the perspective of the right spindle pole, the two velocities in Eq. 8 would be swapped, flipping its sign. We see that in the special case where flux velocity is equal to the stepping velocity of an individual motor head, or $v_{bfl}^- = -v_0/2$ and $v_{bfr}^- = v_0/2$, the overall motor stepping velocity is $-v_0$ and it produces zero force as per Eq. 5. Likewise, if both poleward flux velocities are zero, the overall motor stepping velocity is zero and it produces a maximum force of $-f_0$, which will drive the left spindle pole further to the left and therefore elongate the spindle. Intuitively, this can be understood as poleward flux velocity partially canceling out the stepping of motors, reducing the force they can produce.

To investigate the physical mechanisms involved in microtubule length regulation, we also allow the baseline polymerization and depolymerization velocities to be modulated by both force- and length-dependent effects. We encode these into separate factors that scale the baseline velocities as follows:

$$v_j^\pm = v_{0j}^\pm (F_j^\pm) \varphi^\pm(L_j) \quad (9)$$

where $v_{0j}^\pm$ is the constant baseline polymerization and depolymerization velocity of the plus- and minus-ends, respectively, and $\theta^\pm(F_j^\pm)$ and $\varphi^\pm(L_j)$ are functions that return a unitless scaling factor based on

the force $F_j^\pm$ at the plus- or minus-end and length L_j of the microtubule, respectively.

It has been shown in vitro that forces affect the growing rate of microtubule plus-ends^{88,89}. The depolymerization of minus-ends at spindle poles is a less well-understood topic, but it is thought that kinesin-13 proteins, which localize at spindle poles, may ultimately be responsible for the effect^{36,81}. In this case, we assume that forces on microtubules displace the minus-ends such that they are exposed to a greater or lesser amount of kinesin-13 proteins at the spindle pole, leading to a similar force dependence as plus-ends. An alternative explanation for poleward flux is that forces from sliding motors drive depolymerization at the spindle pole^{44,53,75,90}. In this case, we assume a direct force dependence arises. We use a linear force-velocity relationship to encode these effects:

$$\theta_j^\pm(F_j^\pm) = \mp 1 - F_j^\pm / F_0^\pm \quad (10)$$

where $j = b\ell, k\ell, br, kr$ once again corresponds to left bridging, left k-fiber, right bridging, and right k-fiber, respectively, ∓ 1 indicates a negative (positive) sign for microtubules originating from the left (right) spindle pole, and $F_0^\pm$ is a scaling factor that sets force sensitivity for the plus- and minus-ends, respectively. The force term $F_j^\pm$ corresponds to the force experienced by the plus- or minus-end, and therefore the same external force will have a different sign for either end. For example, pulling on the plus-end of the left k-fiber would result in a positive force on the plus-end and a negative force on the minus-end. A microtubule under tension would therefore experience increased polymerization at the plus-end and decreased depolymerization at the minus-end. This behavior is consistent with past experiments, which showed that pulling forces from kinetochores increased the polymerization velocity of k-fibers microtubule plus-ends⁸⁹.

It is known that proteins can bind along the length of microtubules and then accumulate at the plus-end, leading to a length-dependent mechanism wherein protein number increases with microtubule length⁵⁶. Once microtubules reach a critical length, these proteins accumulate to a threshold level that either stalls further microtubule growth or induces catastrophe. In this manner, they establish a mechanism that sets microtubule length. Previous studies have shown that the plus-end-associated KIF18A, a protein from the kinesin-8 family, plays a dominant role in length-dependent regulation, with depletion of these proteins resulting in longer microtubules in vivo⁵⁷ and longer spindles during mitosis in both fission yeast^{91–93} and higher eukaryotes^{58,59}. At the time of formulating this model, the accumulation of regulating proteins at minus-ends was not well characterized, but we hypothesized that one or more of the many proteins which localize at spindle poles could exhibit this behavior. For example, it has been shown that the abundance of both KATNB1 and KIF2A at a given centrosome correlates with the length of microtubules originating from it⁶⁰. We further propose that any protein with a long residence time can accumulate on microtubule minus-ends in a length-dependent manner due to poleward flux, consistent with previous theoretical work²³. We note that this behavior does not strictly depend on the presence of a centrosome, as these proteins have been shown to localize to microtubule minus-ends independently of centrosomes^{42,81}. Similar to how we handled force dependence, we also encode these effects into a linear function:

$$\varphi_j^\pm(L_j) = A_j^\pm \mp L_j / L_{j0}^\pm \quad (11)$$

where $\mp$ indicates a negative (positive) sign for plus-ends (minus-ends), $L_{j0}^\pm$ is a scaling factor that sets length sensitivity for the plus- and minus-ends, respectively, and $A_j^\pm$ is a unitless scaling constant. For plus-ends, this function yields a positive value until $L_j = L_{j0}^+ A_j^+$, at which point it

becomes negative, representing depolymerization outweighing polymerization at the plus-end for very long microtubules. For minus-ends, this function is always positive, representing a continually increasing depolymerization velocity for longer microtubules. We assume that bridging and k-fibers can have different plus-end parameters due to interactions with kinetochores, but that they have identical minus-end parameters since they interact with spindle poles in the same way in our model.

Overlap regulation within the mitotic spindle is currently not well understood, and therefore, we choose to fix the central overlap length in our model so that effects from changing force do not influence our results. This is achieved by setting the change in length of bridging fibers equal to the velocity of the spindle pole they are attached to, or

$$\frac{dL_{b,r}}{dt} = \mp \frac{dx_{r}}{dt}, \quad (12)$$

which keeps the plus-ends of bridging fibers fixed in place and therefore maintains central overlap length. This allows us to study length regulation of the spindle independently of how anti-parallel overlaps are established or maintained. However, the main conclusions of this paper are unchanged if the overlap length is not fixed and instead allowed to vary freely (Supplementary Fig. 3).

Cell culture and drug treatment

hTERT RPE-1 cells (ATCC: CRL-4000), hTERT RPE-1 PA GFP α -Tubulin cells⁹⁴, hTERT RPE-1 *KIF18A*^{-/-} cells⁶⁴ along with its parental line (kind gift from J. Stumpff, University of Vermont, United States of America), and hTERT RPE-1 mEGFP α -Tubulin⁹⁵ cells (kind gift from D.W. Gerlich, Institute of Molecular Biotechnology, Vienna BioCenter, Austria) were cultured in Dulbecco's Modified Eagle Medium (DMEM; ThermoFisher, 41965-039) supplemented with 10% fetal calf serum (FCS; Labforce, S181T), 100 U/mL Penicillin and 100 mg/mL Streptomycin (ThermoFisher, 15140-122) at 37 °C with 5% CO₂ in a humidified incubator. The culture media of hTERT RPE-1 PA GFP α -Tubulin and hTERT RPE-1 mEGFP α -Tubulin cells were supplemented with 500 μ g/mL G418 (InvivoGen, ant-gn-1) and 0.5 μ g/mL Puromycin (Sigma-Aldrich, P8833), respectively. For live cell imaging, DMEM was replaced by Leibovitz's L-15 medium without phenol red (ThermoFisher, 21083-027) supplemented with 10% FCS. Where indicated, cells were incubated with 250 nM Sovilnesib (MedChemExpress, HY-132840) for 24 h prior to imaging or fixation.

siRNA transfections

Cells were transfected for 48 h with 20 nM of indicated siRNAs using Opti-MEM (ThermoFisher, 31985-062) and Lipofectamine RNAiMAX (ThermoFisher, 13778030) according to the manufacturer's instructions. The following sense strands of validated siRNA duplexes were used: KIF2A⁹⁶ (Ambion, 5'-GUUGUUUACUUUCCACGAATT-3'), KIF18A⁹⁸ (Dharmacon, 5'-CCAACAACAGUGCCAUAATT-3'), KATNB1⁴² (Qiagen, 5'-GCCUGGAUUUCCACCCGUATT-3'), and as a negative control (All-Stars Negative Control siRNA, Qiagen, 1027281, Proprietary). The depletion efficiencies of all siRNA treatments were validated using immunofluorescence and immunoblotting (Supplementary Fig. 7).

Microtubule poleward flux velocity measurements

hTERT RPE-1 PA GFP α -Tubulin cells were cultured in 4-well glass-bottom μ -slides (Ibidi, Vitaris) and treated with siRNAs as described above. Prior to imaging, cells were incubated with 50 nM SiR-DNA (Spirochrome, SC007) and 10 μ M MG132 for 2 h and 30 min, respectively. The images were acquired using the 60 $\times$ (N.A. 1.4) CFI Plan Apochromat oil objective on a Nikon A1r point scanning confocal microscope equipped with a 37 °C heating chamber and running NIS elements software. A rectangular ROI (250px-300px long and 5px wide) of the spindle parallel to the metaphase plate was

photoactivated using a laser of wavelength 405 nm at 50–100% intensity, depending on the GFP expression levels. Single focal planes were imaged every 20 s for 4 min. The subsequent movement of the photoactivated fluorescence towards the spindle pole was tracked over 2 min, and the rate of displacement was quantified using the metaphase plate as a reference point to determine the velocity of microtubule poleward flux.

Spindle length measurements in live cells

For live cell imaging, cells were cultured in glass-bottom μ -slides of 2, 4, or 8 wells (Ibidi, Vitaris) and treated with siRNAs as described above. The cells were incubated with 50 nM SiR-DNA (Spirochrome, SC007) and 0.5 $\times$ SPY555-Tubulin probe (Spirochrome, SC203) for 2 h prior to imaging. The images were recorded using either a 40 $\times$ (N.A. 0.75) or a 60 $\times$ (N.A. 1.4) objective of a Nikon Eclipse Ti-E wide-field microscope equipped with a GFP/mCherry/Cy5 filter set (Chroma Technology Corp.), an Orca Flash 4.0 complementary metal-oxide-semiconductor camera (Hamamatsu), and a 37 °C heating chamber using NIS software. Spindle length was quantified on Imaris 7.4.2 from the time-lapse series as the distance between the two spindle poles, visualized by fluorescently labeled tubulin, in the frame immediately preceding anaphase onset.

Immunofluorescence

To stain for KIF2A, KATNB1, and α -Tubulin, hTERT RPE-1 cells were fixed at -20 °C for 10 min with ice-cold methanol stored at -20 °C. To stain for KIF18A and CREST, hTERT RPE-1 cells were fixed at room temperature for 15 min with an aqueous solution containing 4% Formaldehyde (Applichem, A3592), 20 mM PIPES (Applichem, A1079.0500), pH 6.8, 10 mM EGTA (Applichem, A0878.100), 0.2% Triton X-100 (Applichem, A4975.0100), and 1 mM MgCl₂. Following fixation, cells were rinsed with PBS and blocked at room temperature for 1 h in blocking buffer (3% BSA (Labforce, S181T) and 1% NaN₃ (Applichem, A1430) in PBS). Cells were then incubated for 1 h with primary antibodies diluted in blocking buffer, rinsed with PBS, and incubated for 1 h with secondary antibodies diluted in blocking buffer. The coverslips were mounted on microscope slides (Epremedia, AA00000112E01MNZ10) using Vectashield mounting medium containing DAPI (Vector Laboratories, H1200).

The following primary antibodies were used: recombinant human anti- α -Tubulin (1:500)⁹⁷, rabbit anti-KIF18A (1:1000; Bethyl, A301-080A), mouse anti-KIF2A (1:250; Santa Cruz, sc-271471) in Fig. 5e, rabbit anti-KIF2A in Supplementary Fig. 7b (1:1000; Invitrogen, PA3-16833), rabbit anti-KATNB1 (1:250; Proteintech, 14969-1-AP), mouse anti-Centrin1 (1:1000; Sigma-Aldrich, 04-1624), and human anti-CREST (1:500; Antibodies Incorporated). Appropriate cross-adsorbed Alexa-Fluor conjugated secondary antibodies (1:1000; Invitrogen) were used. Images were acquired using a 60 $\times$ (N.A. 1.4) oil objective on an Olympus DeltaVision wide-field microscope (GE Healthcare) equipped with a DAPI/FITC/TRITC/Cy5 filter set (Chroma Technology Corp.) and Coolsnap HQ2 CCD camera (Roper Scientific) running Softworx (GE Healthcare). Z-stacks of 12.80 μ m thickness were imaged with z-slices separated by 0.2 μ m. 3D image stacks were deconvolved using Softworx (GE Healthcare) in conservative mode.

Immunoblotting

Whole-cell extracts were prepared by cell lysis in an aqueous solution containing 20 mM Tris (pH 7.5), 150 mM NaCl, 20 mM glycerol- β -phosphate, 5 mM MgCl₂, 10% glycerol, 20% NP-40 supplemented with 1 $\times$ protease inhibitor cocktail (Roche, 11873580001), 1 $\times$ phosphatase inhibitor cocktail (Roche, 04906845001), and 1 mM DTT. Samples were denatured at 96 °C for 5 min prior to SDS-PAGE, and subsequent wet transfer onto nitrocellulose membranes (Macherey-Nagel, 741280). Membranes were blocked with 5% non-fat dry milk in TBS 0.1% Tween 20 (TBST) for 1 h. After blocking, membranes were

incubated with primary antibodies in blocking buffer overnight at 4 °C. Membranes were washed thrice with TBST before a 1-h incubation with the appropriate horse radish peroxidase-conjugated secondary antibody diluted in blocking buffer. All blots were imaged through chemiluminescence detection using Amersham™ ECL Prime Western Blotting Detection Kit (Cytiva, RPN2232) using a Fusion FX7 Spectra Multispectral Imaging system (Witec AG, Switzerland). PageRuler Plus Prestained Protein Ladder (ThermoScientific, 26619) was used as the molecular weight marker. The following primary antibodies were used: rabbit anti-KIF18A (1:5000; Bethyl, A301-080A), rabbit anti-KIF2A (1:10,000; Invitrogen, PA3-16833), rabbit anti-KATNB1 (1:1000; Proteintech, 14969-1-AP), and mouse anti- α -Tubulin (1:10,000; Sigma-Aldrich, T9026).

Image processing and statistical analysis

For the morphological illustration of spindle length and microtubule poleward flux, the minimum and maximum display intensity thresholds of individual images were adjusted linearly and globally in Fiji to optimize structural visibility. These adjustments were made strictly for illustrative purposes, and no intensity-based comparisons or conclusions were derived from these specific experimental data. Conversely, for immunofluorescence, display intensity thresholds were kept constant and identical across all conditions compared within each figure to ensure valid visual comparison. All data were plotted as mean $\pm$ SEM. All statistical tests were performed and plotted in GraphPad Prism 11.0.0.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw data related to Fig. 5b, d, e, g–i, Supplementary Figs. 6a, b, and 7a–j in addition to representative images and movies, have been deposited on the Yareta data archive of the University of Geneva (<https://doi.org/10.26037/yareta:h6wkqxccljc6bikijfm7x4fqie>). The raw live-cell imaging data used to generate Figs. 5a, c, f, and S5a–e are greater than 5 terabytes in size and therefore are only available for non-commercial research purposes upon request to the corresponding authors. Source data are provided with this paper.

Code availability

All code is publicly available on GitHub (https://github.com/shfiorenza/spindle_synchro), and the exact release version used in this paper has been archived on Zenodo (<https://doi.org/10.5281/zenodo.22128179>)⁹⁸.

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Author contributions

N.P. and P.M. designed and supervised this study. S.A.F. and N.P. developed the theory. S.A.F. developed the code and performed theoretical research. S.C. and E.D. performed experimental research and analyzed experimental data. S.A.F. and S.C. assembled figures. N.P., P.M., and I.M.T. contributed with ideas and discussions. The manuscript was written by S.A.F. with feedback from N.P., P.M., and I.M.T.

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Competing interests

The authors declare no competing interests.

Additional information

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