Mitotic spindle orientation and dynamics are fine-tuned by anisotropic tissue stretch via NuMA localisation

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Significance

Mechanical forces regulate cell division orientation, which is important for cell fate and tissue shaping. Cells generally orient divisions along their long axis of interphase shape and many studies have shown that mechanosensitive division orientation is an indirect response to force-induced cell shape changes. However, we show that, to achieve an accurate alignment of divisions with cell shape, force sensing via nuclear mitotic apparatus protein (NuMA) is necessary. Through its mechanosensitive cortical localisation, NuMA promotes increased spindle oscillations upon tissue stretch and subsequently enhances the accuracy of spindle orientation along the shape and stretch axis. This fundamental mechanism opens avenues for further exploration in the context of diseases such as cancer where spindle orientation tends to go awry.

Abstract

Cell division orientation, which is key to cell fate and shaping tissues, is influenced by mechanical forces. For example, in uniaxially stretched tissue most divisions orient along the stretch axis. This mechanosensitive division orientation can be achieved by distinct mechanisms involving a direct response to force or an indirect response to force via cell shape changes. Cells also generally align divisions along their long axis of interphase shape. Since stretching a tissue elongates cells, uncoupling whether divisions orient in response to force or to cell shape remains challenging. Here, we utilised the *Xenopus laevis* embryonic animal cap tissue and applied a reproducible external stretch to understand mechanosensitive division orientation. We focused on nuclear mitotic apparatus protein (NuMA), which is key to spindle orientation. We show that NuMA is dynamically localised to the cell cortex earlier during mitosis in uniaxially stretched tissues. This temporal cortical recruitment of NuMA coincides with the onset and subsequent stretch-induced amplification of spindle oscillations. Additionally, we show that knockdown of NuMA reduces spindle oscillations and disrupts division orientation according to stretch and cell shape. We also show that divisions dynamically align with cell shape under tissue stretch and this involves a direct response to force via NuMA localisation, rather than an indirect response to force via cell shape changes. Overall, using live tissue imaging, mathematical modelling, and tissue mechanics our results

indicate that NuMA fine tunes spindle dynamics and ensures the accurate alignment of divisions with cell shape and anisotropic tension.

Keywords: Mechanical force; cell division orientation; NuMA; mitotic spindle; oscillation

Introduction

Oriented cell division, dictated by the dynamic positioning of the mitotic spindle, is a key process that contributes to embryonic development. Oriented divisions are important not only for individual cell fates (1, 2) but also for tissue morphogenesis (3-5) and tissue homeostasis (6). Therefore, a failure to achieve the correct division alignment leads to abnormalities in morphogenesis and organogenesis (3, 5) and is linked to cancer development in adults (7, 8).

Division orientation is regulated by the external mechanical environment of the cell. For example, when tissues experience a stretch, most divisions orient along the axis of greatest tensile stress (6, 9-11). However, the molecular mechanism of how forces are sensed to orient divisions remains largely uncharacterised. Forces can be sensed either directly or indirectly, via cell shape changes, to orient divisions. A default mechanism of division orientation, seen in many cell types, is the alignment of the mitotic spindle along the long axis of cell shape, as dictated by Hertwig's rule (12). Since stretch elongates cell shape along the axis of stretch, many studies have reported a cell-shape sensing mechanism for mechanosensitive division orientation (10, 11, 13-19). However, a growing body of work has also shown that force can be sensed independently of cell shape to orient divisions (9, 20-23). A generalised mechanism therefore seems unlikely to capture the robustness of mechanosensitive division orientation, with one study indicating that both force and cell-shape sensing function in parallel within the same tissue to position the mitotic spindle (24).

For cell-shape sensing, it is thought that cells align divisions to their interphase shape rather than mitotic shape because most cells lose shape anisotropy when they round-up upon mitotic entry (25). In the *Drosophila* pupal notum, the memory of interphase shape is retained during mitosis by the core spindle orientation protein, Mushroom body defect (Mud). In this tissue, Mud localises to the tricellular junctions (TCJs), and this localisation pattern is maintained during mitotic rounding thereby providing a blueprint of interphase shape (26). However, Mud is not enriched at the TCJs in other *Drosophila* tissues (20, 23, 27), raising the question of how divisions are oriented in these tissues. Additionally, Mud has key differences with its vertebrate homologue, nuclear mitotic apparatus protein (NuMA). Whereas Mud is localised to the cortex throughout the cell cycle (28-30), NuMA has a more dynamic spatiotemporal localisation and is localised to the nucleus during interphase (31). Therefore, whether Mud-like mechanisms of shape-mediated spindle orientation translate to NuMA in vertebrates is an open question. We have previously shown that Leucine-Glycine-Asparagine-enriched protein (LGN), the binding partner of NuMA (32), is concentrated at the tricellular vertices (TCVs; the meeting point of 3 or more cells) throughout mitosis in the *Xenopus laevis* animal cap (10). This TCV localisation of LGN corresponds with the ability of divisions to align with cell shape in this tissue (10). However, whether NuMA localises similarly to LGN at the TCVs to orient divisions with cell shape remains unexplored.

Since the mitotic spindle is highly dynamic, a strong possibility is that, following the initial placement along the interphase shape axis, the spindle is reoriented via external cues, such as mechanical force. Indeed, recent studies have reported that the spindle may be dynamically repositioned during metaphase, independent of interphase shape (13, 23, 33). The likelihood of such mitosis-specific cues is further bolstered by the existence of highly dynamic stereotypical oscillations of the metaphase spindle that coincide with achieving the final division alignment (34-37). Additionally, mathematical modelling suggests that changes to the oscillations of the mitotic spindle may be used as a read out of the factors influencing spindle positioning (35, 36).

Considering a mechanism that ties spindle oscillations, division orientation and mechanical cues, NuMA emerges as a strong candidate. The spindle orientation machinery is highly conserved, consisting of a ternary complex containing NuMA, LGN, and $G\alpha_i$ (bound in the order NuMA-LGN-$G\alpha_i$) at the cortex (32, 38, 39). Simultaneously NuMA binds to, and cortically localises, dynein which exerts pulling forces on astral microtubules to orient the spindle (40). Previous studies on fixed keratinocytes have indicated that NuMA could be important for stretch-oriented divisions (41). Additionally, in the *Drosophila* pupal notum, the TCJ bipolarity, via localised Mud, aligns with division orientation as well as with the axis of a simulated stress on the tissue (26).

Here, we investigate the role of NuMA in mechanosensitive spindle orientation using a combination of tissue mechanics, mathematical modelling, and live tissue imaging. We use the *Xenopus laevis* embryonic animal cap, a multilayered vertebrate epithelial tissue that maintains its tissue architecture *ex vivo* and is amenable to mechanical manipulation and imaging (42). We discover that the cortical localisation of NuMA is sensitive to uniaxial stretch with earlier recruitment to the polar cortex during mitosis in stretched tissues. This temporal cortical recruitment is shown to coincide with the onset and subsequent amplification of spindle oscillations, both of which are shown to be reduced upon knockdown of NuMA. Furthermore, we show that NuMA is important for division alignment with anisotropic tension and cell shape. Finally, we demonstrate that NuMA specifically links anisotropic tension with the spindle to dynamically orient divisions and ensure an accurate alignment of divisions with cell shape and anisotropic stretch.

Results

Cortical localisation of NuMA is dynamic and cell cycle dependent

To understand whether NuMA plays a role in mechanosensitive division orientation, we focussed on cortical NuMA since it is the NuMA-dynein complex at the cortex which exerts the pulling forces on astral microtubules to orient the spindle (40). Previous studies have reported that the localisation of NuMA varies throughout the cell cycle (41, 43-45). Indeed, we were able to visualise this dynamic spatiotemporal localisation by live-imaging GFP-tagged NuMA in the *Xenopus* animal cap, and looking at cell divisions in the superficial layer of this tissue. During interphase, NuMA accumulates in the nucleus (**Fig. 1A**; **Movie S1**). However, when the nuclear envelope breaks down at the onset of mitosis, NuMA is released from the nucleus and localises to the spindle poles and weakly at the cortex (**Fig. 1A**; **Movie S1**). The cortical localisation of NuMA becomes more prominent as the cell progresses through mitosis, reaching a peak during anaphase at the cortex closest to the spindle poles. Following

anaphase, NuMA is recruited away from the cortex and relocalises to the nuclei of the daughter cells (**Fig. 1A**; **Movie S1**).

NuMA is not enriched at the tricellular vertices

To quantify the dynamics of GFP-NuMA localisation, we measured the fluorescence of GFP-NuMA at the cortex of each dividing cell at different stages of mitosis. Previously, it was shown that Mud (the *Drosophila* homologue of NuMA) localises to TCJs in the pupal notum tissue (26). Therefore, we investigated whether, in the *Xenopus* animal cap, NuMA localises similarly to the tricellular vertices (TCVs), where three or more cells meet (**Fig. 1B**). Moreover, we investigated whether this NuMA localisation was sensitive to mechanical force by measuring GFP fluorescence in both unstretched tissues and in tissues under a uniaxial stretch, applied using an approach described previously (10, 42). Interestingly, our data revealed that, in contrast to Mud, NuMA is not significantly enriched at the TCVs relative to the cell edges in either uniaxially stretched or unstretched tissues (**Fig. 1C**, **D**; **Fig. S1A**, **B**). In fact, we find that NuMA localisation is significantly reduced at the TCVs compared to the cell edges. Additionally, there was no difference in this localisation pattern between stretched and unstretched tissues (**Fig. 1C**, **D**; **Fig. S1A**, **B**).

Cortical localisation of NuMA is sensitive to tissue stretch

In addition to the TCVs, we measured GFP-NuMA fluorescence at two other regions of the cortex - the polar cortex (closest to the poles) and the equatorial cortex (closest to the metaphase plate) (**Fig. 1B**). We chose these regions to measure as, by eye, we could observe an enhanced cortical localisation of GFP-NuMA at the polar cortex during anaphase (**Fig. 1A**).

Our quantification indeed revealed that, in unstretched tissues, GFP-NuMA was significantly enriched at the polar cortical regions at early anaphase (**Fig. 1E**; **Fig. S1C**). Moreover, when a stretch was applied to the tissue, this preferential enrichment of GFP-NuMA at the polar cortex occurred earlier, at late metaphase, and was maintained throughout early anaphase (**Fig. 1F**; **Fig. S1D**). To prevent selection bias, we used two different ROIs (polygon and oval) to measure the fluorescence of cortical NuMA. Importantly, the cortical localisation trend of NuMA observed between unstretched and stretched tissues was similar irrespective of the type of ROI used for fluorescence measurements (**Fig. S1E**, **G**, **H**). Additionally, we did not observe any change to the total fluorescence of GFP-NuMA at the cortex between stretched and unstretched tissues (**Fig. S1E**, **F**). This suggests that rather than an increase in overall cortical NuMA, there is a redistribution of cortical NuMA between the equatorial and polar cortex in the presence of tissue stretch.

Together, these data demonstrate that rather than being enriched at the TCVs, NuMA is localised preferentially to the polar cortex. Uniaxial stretch, in turn, facilitates an earlier recruitment of NuMA at the polar cortex during mitosis.

Spindle oscillations are amplified by mitotic progression and by stretch

NuMA is known to function at the cell cortex as part of a complex with dynein which generates pulling forces on astral microtubules to position the spindle (43, 46). Mathematical modelling has shown that the combination of the attachment and detachment of these “cortical force generators” and the antagonistic centring forces of spindle microtubules can result in dynamic movements of the spindle pole (35, 36).

Coordinated dynamic movements of both spindle poles can result in rotational movements of the mitotic spindle (**Fig. 2A; Movie S2**). These movements can be split into two categories: overall spindle rotation from metaphase onset until the end of anaphase; and the finer, oscillatory, movements which overlay the larger-scale movement. Both rotational movements have been observed in the animal cap at this stage (36, 37). Crucially, mathematical modelling of spindle pole dynamics has shown that the oscillatory movement of the spindle is dependent on the number of cortical force generators (*N*) at the cortex, with small increases in *N* promoting a switch between non-oscillatory and oscillatory movement (**Fig. S2A**) (35, 36). Stochastic binding and unbinding of force generators promotes the onset of noise-induced oscillations outside of the stability boundary (purple shaded region to the left of the stability line, **Fig. S2A**), making oscillations viable at smaller values of *N* (36)**.** The same model can be used to predict the relationship between oscillation amplitude and *N* (**Fig. S2B**), which is also smoothed by the presence of noise. For a constant binding rate between cortical force generators and microtubules, a positive relationship between *N* and the amplitude of oscillation is predicted (**Fig. S2B**) (36). The period of oscillation around the stability boundary (**Fig. S2A**) is also shown to vary with *N* (**Fig. S2C**). With our observation that NuMA is recruited to the polar cortex earlier in stretched tissues (**Fig. 1E**, **F**), likely corresponding to an increase in *N* earlier in metaphase, we sought to determine if any related changes in spindle movement could be identified.

To track spindle movements, we used the position of the metaphase plate (**Fig. 2A**), which we found to be an accurate proxy for spindle movement (**Fig. S2D-I**). The angle of the metaphase plate was shown to lie perpendicular to the angle of the metaphase spindle (89.7 ± 0.3º, mean ± SEM; **Fig. S2H**) with highly correlated inter-frame angle displacements (**Fig. S2G**, **I**). We measured rotational movement of the mitotic spindle from metaphase onset until anaphase (**Fig. 2B**, **C**; **Fig. S2J**, **K**). As expected, we typically observed an overall spindle rotation (**Fig. 2C** blue line), superimposed with finer movements about this trend. To determine how these finer movements change over the course of mitosis, we compared movements between early metaphase (defined as the first half of metaphase) and late metaphase (the second half of metaphase) (**Fig. 2C**). We first analysed the spindle trend (**Fig. 2C** blue line) to determine whether the overall spindle rotation towards the ultimate division axis was impacted by the application of stretch. By calculating the root mean square (RMS) of the angular displacement between sequential timepoints, we see biphasic behaviour whereby the spindle moves most in early metaphase, followed by a reduction in overall rotation in late metaphase (**Fig. 2D**). Notably, this trend was unaffected by the application of stretch.

We next focussed on the smaller movements overlaying the larger rotation movement by removing the trend line (blue line, **Fig. 2C**) from the spindle angle measurements (**Fig. 2E**). Spindles were determined to be oscillatory or non-oscillatory using an autocorrelation function. The presence of significant spikes in correlated movements at increasing time-lags was used as an indicator for spindle oscillations. In unstretched tissues we observed an increase in the percentage of spindles undergoing oscillatory movements as mitosis progresses, with significantly more spindles undergoing oscillatory movements in late compared to early metaphase (**Fig. 2F**). Moreover, in stretched tissues we saw a significantly larger fraction of spindles undergoing oscillatory movements in both early and late metaphase compared to unstretched tissues. Notably, in stretched tissues, the percentage of oscillatory spindles in early metaphase was very similar to that seen in unstretched tissues in late metaphase (64 ± 4% and 54 ± 5%, respectively, mean ± SEM; **Fig. 2F**), indicating that stretch accelerates the increase in oscillatory spindles normally seen as mitosis progresses.

To further investigate the dynamics of spindle oscillations, we defined an oscillation range by taking the upper and lower bounds of the signal, omitting outliers (**Fig. 2E**). The range spanned by the remaining signal provides a measure related to the oscillation amplitude (**Fig. 2E**, oscillation range indicator). As mathematical modelling suggests that increasing *N* would result in an increase in the amplitude of a spindle pole oscillation (**Fig. S2B**), we utilised this measurement as an indicator of whether changes in the spindle movements could be a result of the temporal accumulation of NuMA. We observed that the oscillation range (amplitude) significantly increased between early and late metaphase in both stretched and unstretched tissues (**Fig. 2G**). Moreover, the oscillation range was observed to be significantly increased in stretched tissues compared to unstretched tissues in both early and late metaphase (**Fig. 2G**). In addition, just as we saw with the percentage of oscillatory spindles, the oscillation range seen in stretched tissues at early metaphase was very similar to the range seen in late metaphase in unstretched tissues (24.8 ± 0.8º and 23.7 ± 0.7º, respectively (mean ± SEM); **Fig. 2G**).

The mathematical model highlights that the period of oscillation around the neutral curve has a non-linear relationship with *N* (**Fig. S2C**). Experimentally, we observed that the application of stretch increased the period of oscillation from 66 ± 2 s to 72 ± 2 s (mean ± SEM) in late metaphase (**Fig. S2I**). In early metaphase we observed no significant difference in period between stretched and unstretched conditions. This general trend is congruent with increasing *N* leading to an increased period of oscillation (upper branch of **Fig. S2C**). We highlight that the measurement of the period of oscillation is sensitive to noise, and less accurate for shorter time series. As the time series length was limited by the length of metaphase, we use the oscillation range as a more comparable and measurable change to the spindle oscillations.

Overall, our analysis of spindle rotational movement indicates that stretching accelerates and accentuates the changes in spindle oscillations normally seen as mitosis progresses. This acceleration in the oscillatory dynamics of spindles in stretched tissues mirrors the earlier recruitment of NuMA to the polar cortex that we see following stretch.

Knockdown of NuMA affects embryonic development and spindle bipolarity

We next sought to functionally test the role of NuMA in mechanosensitive spindle orientation, by knocking down the expression of endogenous NuMA in early *Xenopus* embryos. Using translation blocking morpholinos (MOs), we achieved a 46% knockdown of NuMA expression at the early gastrula stage (**Fig. 3A**). We then characterised the effects of NuMA knockdown on embryo development. Despite the partial knockdown of NuMA expression via MOs, we observed markedly increased lethality at the end of gastrulation in embryos injected with NuMA MO compared to uninjected embryos and embryos injected with control MO (**Fig. S3A, B**). Therefore, NuMA is an essential gene during *Xenopus* development similar to that in mice (47) and in humans (based on data from genetic screens) (48-51). Importantly, embryos injected with NuMA MO were healthy at the early gastrula stage (9 h timepoint in **Fig. S3A**) at which the animal cap tissue was dissected for stretch experiments. Since NuMA is important for spindle structure (47), we tested the effect of NuMA knockdown on spindle morphology in fixed early gastrula embryos. In line with previous studies (52, 53), we observed an increased incidence of multipolar spindle formation upon knockdown of NuMA (**Fig. S3C, D**). Importantly, most spindles had a normal structure despite the knockdown (**Fig. S3D**). This allowed us to analyse normal spindles within the knockdown background in subsequent live imaging experiments.

NuMA is important for cell division orientation with mechanical stretch

Previously, NuMA was shown to be important for stretch-dependent spindle orientation in fixed cells (41). Therefore, we tested the extent to which knockdown of NuMA affects stretch-sensitive divisions in live cells within a tissue environment. We did not observe any significant effect of NuMA knockdown on cell division rate (**Fig. S3E**). Therefore, we focussed on how division orientation may be affected by knockdown of NuMA. To do this, we measured the angle between the spindle axis at late anaphase, when the final division orientation is set (54, 55), and the horizontal axis (the stretch axis in stretched tissues). Cells can divide at any angle between 0° (**Fig. 3B**) and 90° (**Fig. 3B'**) relative to the horizontal/stretch axis. In unstretched tissues, there was no significant difference between the distributions of control and NuMA knockdown tissues (**Fig. 3C, E**). In stretched control MO tissues, the majority of divisions oriented with the axis of stretch (within 30º) (**Fig. 3D**), corroborating previous studies showing that application of an external uniaxial stretch orients divisions (9-11). This was also the case in stretched NuMA knockdown tissues (**Fig. 3D**). Importantly, however, there was a significant difference between the distribution of division orientation and the stretch axis in the stretched control and NuMA knockdown tissues, with NuMA knockdown tissues showing a small but significant loss of alignment (**Fig. 3D, E**).

To further probe the nuances of each distribution, we partitioned the division angles into three 30° bins relative to the horizontal/stretch axis (**Fig. 3F**). In the unstretched tissues, divisions were uniformly distributed across each bin. In the stretched tissues, most divisions were concentrated within 30° of the stretch-axis. However, while 59% of the divisions oriented within 30° of the stretch-axis in the control tissues, this decreased to 52% in the NuMA knockdown tissues. Conversely, while 12% of divisions oriented at angles greater than 60º in control tissues, this increased to 19% in NuMA knockdown tissues (**Fig. 3F**).

Importantly, this division misalignment with the stretch axis in NuMA knockdown tissues was rescued by expressing GFP-NuMA over the endogenous knockdown background (**Fig. 3D, E, F**). The dose of exogenous GFP-NuMA was carefully titrated to match the level of NuMA expressed endogenously (**Fig. S3F**). Overall, these data show that NuMA is important for mechanosensitive cell division orientation with NuMA knockdown leading to the misalignment of divisions with the stretch axis.

NuMA is required for stretch-induced spindle oscillation amplification

Given the identification of an accentuation of spindle oscillations under stretch, coinciding temporally with increased GFP-NuMA localisation to the polar cortex, we sought to determine whether NuMA knockdown would impact spindle oscillations. Indeed, the overall percentage of spindles showing an oscillatory phenotype was significantly decreased upon NuMA knockdown in both early and late metaphase (**Fig. 3G**). The percentage of oscillatory spindles in stretched tissues is almost halved by the partial knockdown of NuMA (64 ± 4% control vs 32 ± 4% NuMA knockdown in early metaphase and 71 ± 5% control vs 44 ± 4% NuMA knockdown in late metaphase; mean ± SEM). Additionally, the percentage of spindles showing an oscillatory phenotype upon NuMA knockdown across both stretched and unstretched conditions remained similar to unstretched control levels (**Fig. 3G**).

Within the population of spindles which oscillate, the range (amplitude) of the oscillation was also seen to significantly decrease in NuMA knockdown compared to control, upon the application of a tissue stretch in both early and late metaphase (**Fig. 3H**), congruent with an

overall reduction of *N* (**Fig. S2B**). Just as with the percentage of spindles oscillating, knockdown of NuMA keeps oscillation amplitude in stretched tissue at a level similar to unstretched. Overall, these data show that a partial NuMA knockdown is sufficient to induce significant differences in the dynamic rotation of the mitotic spindle under stretch. In particular, with NuMA knockdown, we observe a dampening down of the normal increases in oscillation (percentage and amplitude) seen when a control tissue is stretched.

NuMA is required to fine-tune division alignment with cell shape under anisotropic stretch

Previous studies have shown that application of a uniaxial stretch changes the shape of cells, with more elongated cells in stretched tissues and an alignment of the long axis of cell shape with the stretch axis. Since cells are known to preferentially align their divisions with the interphase long axis of shape, the introduction of cell shape anisotropy upon stretch has been suggested to drive alignment of division with tensile force in many tissues (10, 11). We therefore hypothesised that the loss of division alignment with the stretch axis seen upon NuMA knockdown could result from reduced alignment with cell shape more generally. To test this, we explored the effect of NuMA knockdown on division alignment with cell shape.

As expected, we observed an increase in cell elongation upon application of a uniaxial stretch in both control and NuMA knockdown tissues (**Fig. 4A, B**). We determined the principal axis of shape (θ_V) at interphase just before nuclear envelope breakdown, based on the position of the TCVs (10). The orientation of cell division relative to cell shape was then determined by calculating the difference between the division angle (θ_D; measured at late anaphase) and θ_V. The closer the absolute value of θ_D-θ_V is to zero, the better the alignment between division orientation and cell shape (**Fig. 4A**).

Hertwig's rule dictates that most cells tend to orient their divisions with their long axis of shape (12). This was observed in both the unstretched and stretched tissues (**Fig. 4C**). In unstretched tissues, divisions were similarly aligned with cell shape in both control and NuMA knockdown tissues with no significant difference between the two distributions (**Fig. 4C**). Importantly, the application of stretch significantly improved division alignment with cell shape ($|\theta_D$-$\theta_V|$ moves closer to 0º) in the control tissues (**Fig. 4C**). However, this improvement in division alignment with cell shape was not observed in the NuMA knockdown tissues despite a stretch (**Fig. 4C**). Therefore, there was a significant difference in the distribution of division orientation with cell shape between stretched control and stretched NuMA knockdown tissues.

To further probe the extent to which divisions were aligned with shape, we tripartite binned each distribution relative to the axis of cell shape (**Fig. 4D**). This showed that the majority of divisions aligned within 30° of the cell shape-axis in all conditions. In the unstretched control and NuMA knockdown tissues this was very similar with 59% and 62% divisions orienting closest to cell shape, respectively. Stretch enhanced this alignment to 68% divisions in the control tissues. However, this was lower at 60% in the stretched NuMA knockdown tissues, similar to that in unstretched NuMA knockdown tissues (**Fig. 4D**). Crucially, the reduction in alignment of division orientation with cell shape observed in the stretched NuMA knockdown tissues could be rescued by introducing GFP-NuMA over the endogenous knockdown background (**Fig. 4C, D**).

Importantly, the misalignment of divisions with cell shape in the stretched NuMA knockdown tissues was a robust phenotype since this was observed regardless of whether cell shape was

measured based on TCVs, cell perimeter or cell area (**Fig. 4C; Fig. S4A, B**). We also investigated whether cell size, as measured by apical cell area, was a factor in the misorientation of divisions with cell shape in the stretched NuMA knockdown tissues. We found no significant effect of NuMA knockdown on cell area in either unstretched or stretched tissues (**Fig. S4C**). Additionally, there was no correlation between cell area and the alignment of division orientation with cell shape in any of the conditions (**Fig. S4D**).

Together, these data indicate that the alignment of the spindle with cell shape is significantly improved upon uniaxial stretch and that this process requires NuMA. Importantly, knockdown of NuMA does not reduce the alignment of division with cell shape in unstretched tissues. This suggests that NuMA is functioning to improve division alignment with cell shape but only in response to uniaxial stretch. However, a confounding factor here is that uniaxial stretch also changes the shape of cells, making them more anisotropic in shape (**Fig. 4A, B**). Since spindles are known to align more accurately with anisotropic cell shapes (10) this increased anisotropy could also explain the difference in NuMA knockdown phenotype between stretched and unstretched tissues. We therefore sought next to exclude, as far as possible, the confounding factor of shape anisotropy.

NuMA links anisotropic stretch to division orientation independently of cell shape

To exclude the confounding factor of shape anisotropy and address whether NuMA responds to force or cell shape, we computationally fractionated the whole population of cells into different shape subtypes based on cell elongation. In this way, we could compare cells of a similar shape across the different conditions. Elongation was mathematically determined based on the position of the TCVs (E_V) and ranged from 0 to 1 (the closer the value is to 1, the more elongated the cell) (**Fig. 4A**). We focussed on the most elongated cells from each condition, since it is with these most anisotropically shaped cells that the most accurate measurement of the long axis is achieved. To classify a cell as either more or less elongated, we employed an E_V cutoff value of 0.35 such that cells with $E_V > 0.35$ were considered more elongated (10) (**Fig. 4A**).

In these more elongated cells, division orientation with cell shape mirrored the effects seen in the overall cell population (**Fig. 4C, E**). In all cases, divisions were majorly oriented with cell shape with no significant difference between the unstretched control and NuMA knockdown tissues (**Fig. 4E, F**). Stretch improved division alignment with cell shape in the control tissues with 74% divisions orienting within 30° of the cell shape axis compared to 64% in the unstretched tissues (**Fig. 4F**). This improvement was negated in the stretched NuMA knockdown tissues but could be rescued by exogenous NuMA (**Fig. 4E, F**). Despite all the cells being of a similar shape anisotropy, the division misalignment with cell shape was only observed in the NuMA knockdown tissues specifically in the presence of a tissue stretch. This suggests that NuMA links spindle orientation to mechanical force directly rather than indirectly via cell shape.

Together, these data demonstrate that NuMA functions in mechanosensitive division orientation to finesse the ability of the spindle to find the long axis and therefore align with an axis of stretch. Moreover, this finessing by NuMA appears to be a direct response to mechanical force rather than an indirect response to a change in cell shape anisotropy.

Mechanosensitive spindle reorientation with cell shape occurs later in mitosis

We next sought to determine whether NuMA-mediated mechanosensitive finessing of spindle orientation with cell shape occurs at a particular time during mitosis. Additionally, given that cells generally align divisions with the long axis of interphase shape, it is important to determine whether spindle alignment with interphase shape is determined early in mitosis. The finessing of division alignment with cell shape that we observe in stretched control tissues was measured at the end of metaphase (**Fig. 4C, D**). This coincides with our observation that NuMA is significantly enriched at the polar cortex at late metaphase in stretched tissues (**Fig. 1; Fig. S1**). To check whether this is coincidental, we asked whether a similar stretch-induced fine-tuning of spindle alignment with cell shape could be observed early in mitosis, at prometaphase, when there is no significant enrichment of NuMA at the polar cortex (**Fig. 1; Fig. S1**).

To do this, we measured the axis of the spindle relative to the horizontal/stretch axis at prometaphase (θ_M) and used this to determine the absolute difference between spindle alignment and cell shape ($|\theta_M-\theta_V|$) at prometaphase (**Fig. 4G**). In contrast to the anaphase spindles that were predominantly oriented with cell shape (**Fig. 4C, D**), the spindles at prometaphase were less oriented with shape (**Fig. 4H, I**). This was the case for both control and NuMA knockdown tissues. For example, in the unstretched control tissues, 59% of spindles at anaphase were oriented within 30° of cell shape (**Fig. 4D**) whereas fewer spindles (44%) were oriented to this extent at prometaphase (**Fig. 4I**). This suggests that even though spindle alignment with cell shape is not entirely random at the onset of mitosis, there is further enhancement of division alignment with shape later in mitosis.

Interestingly, in contrast to the anaphase spindles (**Fig. 4C, D**), application of a tissue stretch did not improve spindle alignment with cell shape at prometaphase in the control tissues (**Fig. 4H, I**). The percentage of spindles aligning within 30° of the cell shape-axis remained similar at 44% and 47% for unstretched and stretched control tissues respectively (**Fig. 4I**). In addition, there was no misalignment upon NuMA knockdown (**Fig. 4H, I**).

Together, these data suggest that spindle orientation along the long axis of interphase shape is not strictly determined at the onset of mitosis but is rather modulated throughout mitosis. Furthermore, external mechanical forces finesse this spindle alignment with cell shape during metaphase which coincides with the time that we observe enrichment of NuMA at the polar cortex in stretched tissue.

Discussion

The fundamental mechanisms of cell division orientation are well characterised but considerably less is known about how mechanical forces are sensed by the mitotic spindle to orient divisions. In this work, we identify NuMA as a key link between external forces and spindle orientation. We show that NuMA has a mechanosensitive cortical localisation with earlier mitotic recruitment to the polar cortex in tissues under uniaxial stretch. This early recruitment is shown to have a direct impact on oscillatory spindle dynamics by increasing the likelihood of inducing a mitotic spindle oscillation and increasing the associated oscillation amplitude. Additionally, knockdown of NuMA disrupts division orientation with the global axis of stretch and cell shape. Furthermore, we show that NuMA is a dynamic cortical landmark

that links anisotropic tension with the spindle to precisely orient it with cell shape and external force.

A significant portion of our knowledge of spindle orientation mechanisms stems from studies in *Drosophila* (26, 28-30). In this context, TCJ-localised Mud is important in cell-shape mediated spindle alignment in the pupal notum tissue (26). We show that, unlike Mud in the pupal notum, NuMA is not enriched at the TCVs in the *Xenopus* animal cap and is rather distributed around the cell edges (**Fig. 1; Fig. S1**). More akin to Mud in the vertebrate tissue is LGN, which, according to our previous work, is enriched at the TCVs and is important for shape-dependent spindle orientation (10). Since NuMA is not enriched at the TCVs like LGN, this suggests that NuMA may be targeted to the cortex in an LGN-independent manner. Indeed, NuMA can be targeted to the cortex at anaphase independently of LGN (41). Additionally, NuMA contains two membrane binding domains which interact with phospholipids (45, 56). Whether the lack of NuMA enrichment at the TCVs is related to NuMA's direct interaction with membrane lipids is therefore interesting to consider. The differential localisation of NuMA and LGN may be indicative of distinct mechanisms involving shape-mediated alignment by LGN and force-mediated alignment by NuMA to efficiently orient the spindle with anisotropic tension. NuMA and LGN have been reported to act independently. For example, in the *Drosophila* pupal notum, spindle orientation depends on Mud and does not require Pins (LGN homologue) (26).

For a force-responsive mechanism of spindle orientation, it may be beneficial to enrich cortical NuMA in the vicinity of the spindle poles to generate short-range pulling forces which are translated to the spindle pole, to fine-tune spindle movements and efficiently position the spindle. In fact, we show that NuMA is more enriched at the polar cortex relative to the equatorial cortex, with uniaxial stretch facilitating this enrichment earlier in mitosis (**Fig. 1; Fig. S1**). Our findings are reinforced by studies where cells on adhesive micropatterns localise NuMA (in addition to LGN) at prometaphase to cortical regions that are in direct contact with the micropattern (57). Cortical distance-sensing mechanisms such as exclusion of NuMA from the equatorial cortex by a Ran-GTP gradient (58) and from the polar cortex by polo-like kinase 1 (PLK1) have been reported (59). As to how NuMA is reinforced specifically at the polar cortex, no mechanism has so far been identified. Interestingly, NuMA can be localised to the cortex dependent on its state of phosphorylation (44, 45, 60, 61). Additionally, interactions with actin-binding proteins may further facilitate NuMA/LGN localisation to the cortex (43, 57, 62, 63). Therefore, it is interesting to speculate whether phosphorylation or interaction with actin-binding proteins have a role in localising NuMA earlier to the polar cortex in stretched tissues and these will provide important avenues for future study.

The earlier recruitment of NuMA to the polar cortex under tissue stretch likely ensures that the spindle responds correctly to tension to orient divisions. Indeed, we observe a misalignment of divisions with the global axis of stretch (**Fig. 3**) and cell shape (**Fig. 4; Fig. S4**) in tissues where NuMA is knocked down. The misalignment of division orientation with the global stretch axis in the NuMA knockdown tissues agrees with a previous study on fixed keratinocytes (41) and coincides with a measurable change in the mobility of the spindle, as evidenced by the reduction of spindle oscillations (**Fig. 3)**. It is important to note that although this misalignment phenotype is evident, most of the divisions were still oriented very close to the stretch or cell shape axis. This could be a combination of two factors. The first is due to an experimental limitation of the morpholino-mediated knockdown which resulted in a partial suppression of NuMA expression rather than a complete knockdown. This partial knockdown of NuMA was

important to ensure that spindle structure remains normal in most cells (**Fig. S3**). However, the residual levels of NuMA present may still be sufficient to ensure relatively accurate division alignment with the stretch-axis. Second, other proteins are involved in the overall network underpinning mechanosensitive division orientation. To that end, cadherin and LGN are important candidates based on past studies on their mechanosensitive localisation and effect on division orientation (9, 27). Another key player in the process could be the actin cytoskeleton, since NuMA contains the 4.1R domain that interacts with actin-binding proteins (41). It has been shown that depolymerisation of F-actin in cells leads to the loss of cortical NuMA localisation (57). Additionally, a recent study in *Xenopus* suggested that a stable pool of F-actin coupled to the spindle pole may be involved in spindle movements and eventual positioning (64). Overall, there is a layering of multiple mechanisms, with a combination of both cell shape sensing and the force-mediated finessing from NuMA that we show here, to ensure robust and accurate division orientation.

To respond to anisotropic tension, it seems that NuMA directly links spindle orientation to force rather than indirectly via changes in cell shape. We uncovered this by looking at the most elongated cells where even in the unstretched tissues, divisions align mostly with cell shape (**Fig. 4**). In the case of elongated cells in other tissue models (6, 11, 14, 16, 18, 23), the extent of elongation generally overrides any direct force sensing mechanism. However, our study suggests that even in the most elongated cells, NuMA ensures accurate division orientation with shape under anisotropic tension. Our data shares interesting parallels with findings from the *Drosophila* pupal notum where cells in the midline, despite being the most elongated, are less efficient at orienting divisions with cell shape under low tissue tension (15). In these elongated cells, fewer spindles undergo Mud-dependent rotations toward the long axis compared to cells outside the midline under higher tissue tension. Additionally, upon increasing tension within the midline, the elongated cells become more efficient at orienting divisions with shape (15). We therefore suggest that NuMA functions as a spindle orientation landmark which dynamically responds to the external mechanical environment of the cell to fine-tune division orientation.

Importantly, the blueprint for spindle orientation with cell shape is not strictly determined at the onset of mitosis. Rather, the finessing of divisions with cell shape by tissue stretch occurs during metaphase and not at prometaphase (**Fig. 4**). Interestingly, the mechanosensitive finessing of divisions with cell shape that we observe coincides with the stretch-sensitive enrichment of NuMA at the polar cortex in metaphase (**Fig. 1; Fig S1**). Therefore, the polar cortical localisation of NuMA and cell division orientation under mechanical stretch are likely to be interlinked. Indeed, mathematical modelling of spindle pole dynamics suggests that a change in the concentration of NuMA (characterised by a change in the value of N, the number of cortical force generators) is sufficient to create observable oscillations of the mitotic spindle (**Fig. S2**) (35, 36), the amplitude of which will increase with NuMA concentration (**Fig. S2**) (36). Experimentally, both the occurrence of mitotic spindle oscillations and the associated amplitude of oscillation increases as metaphase proceeds in unstretched tissues (**Fig. 2**), reflecting the temporal accumulation of NuMA to the polar cortex. The application of stretch, which accelerates the accumulation of NuMA to the polar cortex (**Fig. 1**) also amplifies the occurrence and associated amplitude of mitotic spindle oscillations (**Fig. 2**). This is further supported by the reduction of oscillation amplitude in a declined fraction of oscillatory spindles upon a partial knockdown of NuMA (**Fig. 3**). Interestingly, we did not observe a complete eradication of spindle oscillations upon knockdown of NuMA (**Fig. 3**). We attribute this to the capability of the system to undergo noise-induced oscillations in the stable region at low values

of *N* (**Fig. S2**), in particular because we were only able to achieve a partial knockdown of NuMA in order to retain spindle structure. We conclude that spindle motion can be used to confirm that the altered localisation of NuMA under stretch is sufficient to create measurable changes to the dynamic movements of the mitotic spindle, which in turn impacts the final division orientation. In general, oscillations of the mitotic spindle may also aid the achievement of the correct final division orientation, as a more mobile mitotic spindle will be more responsive to positioning into the correct alignment by NuMA.

Taken together, our findings support a model whereby under anisotropic tissue tension, cells recruit NuMA to the polar cortex earlier during mitosis. This enhanced cortical localisation of NuMA modulates increased spindle oscillations. Ultimately, this mediates dynamic division alignment with the axis of stretch as well as with cell shape in a mechanism that relies on a direct response to anisotropic tension.

Materials and methods

DNA constructs and morpholino oligonucleotides

pCS2+/mCherry-Histone2B (65), pCS2+/GFP-α-tubulin (54), pCS2+/BFP-CAAX (37), and pCS2+/GFP (66) were gifts from Bill Bement (University of Wisconsin-Madison, US). pCS2+/GFP-CAAX (67) was a gift from Ann Miller (University of Michigan, US). pCS2+/GFP-NuMA was generated by excising the NuMA sequence from pEGFP/C1-NuMA (Addgene, 81029) and ligating into Bgl2 and EcoR1 sites in pCS2+/GFP. Morpholino (MO) sequences (Gene Tools, LLC) used were as follows: Standard Control (5'-CCTCTTACCTCAGTTACAATTTATA-3'), NuMA1.S (5'-GTCATTATGCTTCAGCACTTCTCCC-3'), and NuMA1.L (5'-GCCATTTTGTTTCACTACTTTTCCC-3').

Xenopus laevis ovulation induction and natural mating

Xenopus laevis were housed within tanks maintained by the in-house animal facility at the University of Manchester. The female and male frogs were used for embryo collection and natural mating respectively. Females were pre-primed 4-7 days in advance of egg collection with 50 U of pregnant mare's serum gonadotrophin (MSD Animal Health) injected into the dorsal lymph sac. Before the day of egg collection, females and males were primed with 200 U and 100 U of human chorionic gonadotrophin, Chorulon (MSD Animal Health) respectively, injected into the dorsal lymph sac. The primed frogs were maintained in individual tanks containing aquarium water, and 16 to 18 hours later, a female and male frog were transferred to the same tank for natural mating. Eggs were collected from the tank 2-5 hours later. All *Xenopus* work was performed using protocols approved by the UK Government Home Office and covered by Home Office Project Licences PFDA14F2D (Licence holder: Enrique Amaya) and PP1859264 (Licence holder: Karel Dorey) as well as Home Office Personal Licences held by N.T., D.H., G.G., and S.W.

Embryo processing and microinjection

Embryos collected from natural mating were processed as previously described (42, 55). mRNA was synthesised as described previously (68) and each cell was injected with a total volume of 4.2 nl or 2.1 nl for injections at 2-cell or 4-cell stages respectively. RNA was

microinjected into both cells at 2-cell stage at the following needle concentrations: 0.1 mg/ml mCherry-Histone2B, 0.1 mg/ml BFP-CAAX, 0.1 mg/ml GFP-CAAX, 0.5 mg/ml GFP-α-tubulin, 0.25 mg/ml GFP-human NuMA (overexpression experiments), 0.01 mg/ml GFP-NuMA (morphant rescue experiments). For NuMA knockdown/rescue and metaphase plate tracking experiments, MOs prepared as 1 mM stocks were microinjected at a needle concentration of 1 mM. NuMA1.S MO and NuMA 1.L MO were injected into all cells at 2-cell and 4-cell stages respectively. Control MO was injected similarly for respective control experiments. Following microinjection, embryos were incubated in 0.1% MMR at 16°C for approximately 20 hours.

Animal cap dissection and culture

Animal cap tissue was dissected from embryos at stage 10 of development (early gastrula stage) as previously described (69). Dissected animal caps were cultured *ex vivo* in Danilchik's for Amy explant culture media (53 mM NaCl, 5 mM Na_2CO_3, 4.5 mM Potassium gluconate, 32 mM Sodium gluconate, 1 mM $CaCl_2$, 1 mM $MgSO_4$, pH 8.3 with Bicine) with 0.1% BSA (Sigma, A7906) on a fibronectin-coated PDMS membrane chamber which was prepared as described previously (42). Explants were held in place by a coverslip fragment and allowed to adhere to the fibronectin at 18°C for 2 hours before imaging (42).

Animal cap stretch manipulation and imaging

The PDMS membrane chamber was placed on a stretch apparatus (custom-made by Deben UK Ltd.) fixed securely to the stage of the confocal microscope and then subjected to one of the following stretch manipulations: unstretched (0.5 mm displacement to remove sag on the membrane) or uniaxial stretch (8.6 mm displacement of one arm). Images were acquired with the Leica Application Suite X (LAS X) software using hybrid detectors (HyDs) in a 20-23°C environment. For full details on microscopy settings please see supplemental information.

Immunofluorescence

Immunofluorescence was performed as previously described (70). Stage 10 embryos injected with control MO and NuMA MO were fixed and incubated in primary and secondary antibodies in 1X Tris-buffered saline with Nonidet P-40 (TBSN) (155 mM NaCl, 10 mM Tris-HCl pH 7.4, 0.1% Nonidet P-40) containing 10 mg/ml BSA at the following dilutions: mouse anti-α-tubulin (Sigma, T9026) (primary; 1:200) and goat anti-mouse Alexa Fluor 488 (Thermofisher, A-11001) (secondary; 1:400). Embryos were also incubated with 10 µg/ml DAPI (Invitrogen, D2149). Samples were methanol dehydrated and then cleared and mounted in Murray's Clear (2:1 benzyl benzoate:benzyl alcohol). Embryos were imaged as described in the supplemental information.

Whole embryo imaging

To show that the metaphase plate can be used as a proxy for spindle movements, embryos at stage 10-11 were mounted as described previously (71) and imaged as described in the supplemental information.

Morphant embryo survival

Uninjected, control MO, and NuMA MO-injected embryos in 0.1% MMR were imaged during early development as described in the supplemental information.

Western blotting

Western blotting was performed as described previously (70). Stage 10 embryos injected with NuMA MO, control MO, and NuMA MO with GFP-NuMA were lysed and fractionated by SDS-PAGE on 4-15% Mini-PROTEAN TGX Stain-Free Protein Gels (Bio-rad, 4568083) at 120 V. Proteins were transferred to a 0.45 µm nitrocellulose membrane (GE Healthcare, 10600002) at 30 V for 16 hours at 4°C. The membrane was blocked by incubation with 5% non-fat milk in 1X Tris-buffered saline with tween (TBST) (10 mM Tris pH 8.0, 150 mM NaCl, 0.5% tween 20) and incubated with the following antibodies in blocking solution: rabbit anti-NuMA1 (1:500) (Prestige Antibodies, HPA029912), mouse anti-α-tubulin (1:1000), goat anti-rabbit IgG H&L IRDye 800CW (1:5000) (Abcam, ab216773), and donkey anti-mouse IgG H&L IRDye 680RD (1:5000) (Abcam, ab216778). Membranes were imaged on the Odyssey CLX LI-COR. Band intensity was measured using Image Studio (LI-COR Biosciences). The intensity of each band was normalised to background fluorescence. The band for NuMA was then normalised to α-tubulin. The fold change relative to the control MO was then calculated.

Image processing and quantification

Image processing and analysis were performed using ImageJ (Fiji) (72, 73). For full details of the image analysis performed please see the supplemental information.

Data presentation and statistical analysis

Rose histograms were generated using in-house Python scripts (10). All other graphs were plotted using GraphPad Prism 10 (GraphPad Software, San Diego, USA). Figures were prepared using Adobe Illustrator and (Adobe, San Jose, USA). Statistical analysis was performed using GraphPad Prism 10. Samples were tested for normality using the D'Agostino-Pearson normality test and the Shapiro-Wilk normality test. Normality tests were passed at $p>0.05$. Following the assessment of normality, statistical tests were chosen as appropriate. For all statistical analyses, p values less than 0.05 were considered statistically significant. All statistical analysis and p values are reported in the figure legends.

Acknowledgements

This work was supported by Wellcome Trust 4-year PhD Studentships for N.T. and D.H. (215210/Z/19/Z and 220054/Z/19/Z respectively), a Leverhulme Trust Research Project Grant (RPG-2021-394), a Wellcome Trust/Royal Society Sir Henry Dale Fellowship to S.W. (098390/Z/12/Z), and a Wellcome Trust Career Development Award to S.W. (225408/Z/22/Z). The Bioimaging Facility microscopes used in this study were purchased with grants from BBSRC, Wellcome, and the University of Manchester Strategic Fund. Thanks to Peter March, Roger Meadows, and Steve Marsden from the Biomaging team for their help with microscopy. Also, thanks to members of the Biological Services Facility for their help with the maintenance of *Xenopus laevis* colonies.

Author contributions

S.W., N.T., D.H., and O.E.J. designed research; N.T. and D.H. performed research; N.T. and D.H. contributed new reagents/analytic tools; N.T., D.H., D.I., G.G., and S.W. analysed data; N.T., D.H., O.E.J., and S.W. wrote the paper.

Competing interests

The authors declare no competing interest.

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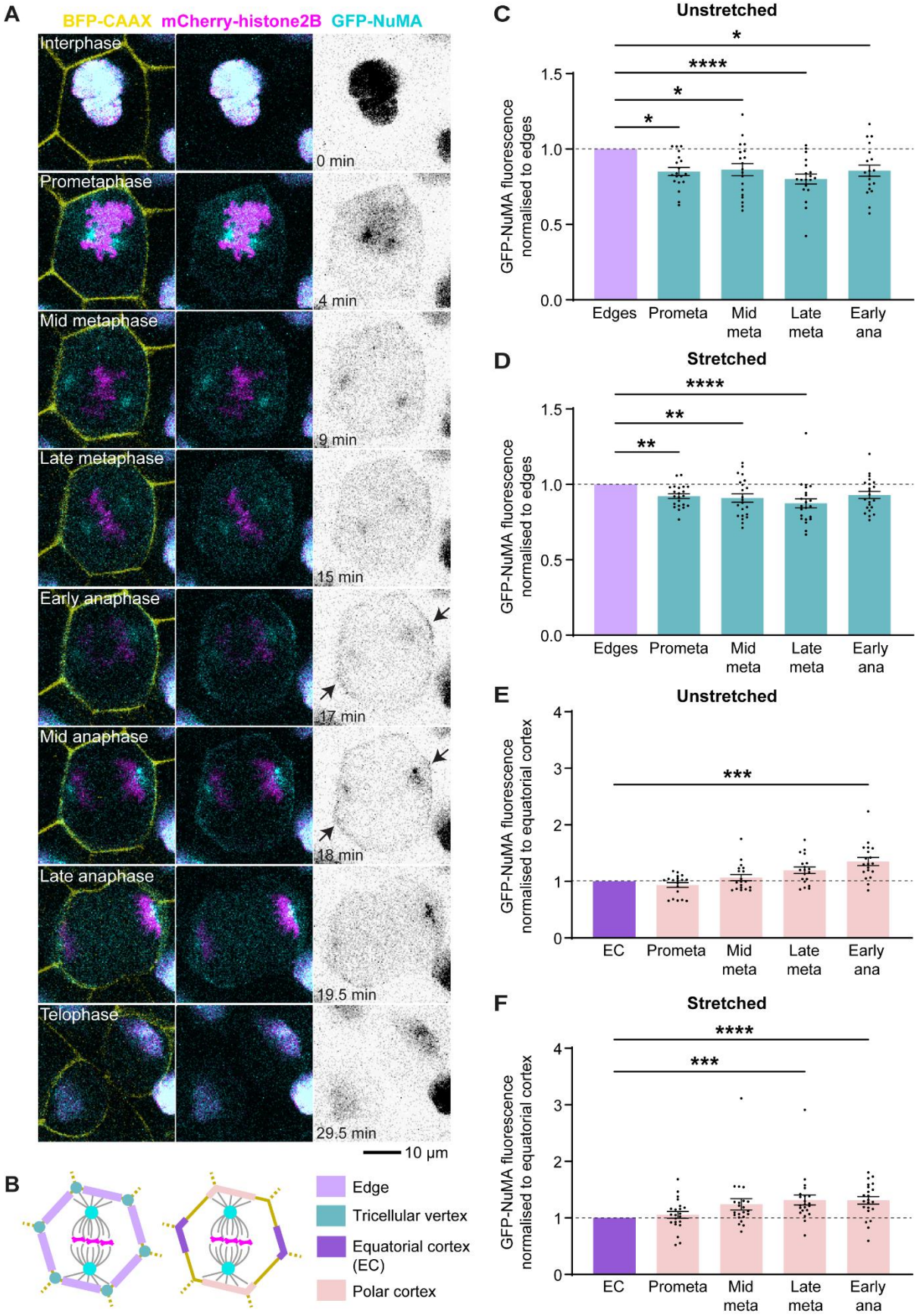
A
BFP-CAAX mCherry-histone2B GFP-NuMA
Interphase
0 min
Prometaphase
4 min
Mid metaphase
9 min
Late metaphase
15 min
Early anaphase
17 min
Mid anaphase
18 min
Late anaphase
19.5 min
Telophase
29.5 min
10 µm
B
Edge
Tricellular vertex
Equatorial cortex (EC)
Polar cortex
C
Unstretched
GFP-NuMA fluorescence normalised to edges
Edges
Prometa
Mid meta
Late meta
Early ana
D
Stretched
GFP-NuMA fluorescence normalised to edges
E
Unstretched
GFP-NuMA fluorescence normalised to equatorial cortex
EC
F
Stretched
GFP-NuMA fluorescence normalised to equatorial cortex

Fig. 1. NuMA localises to the cortex earlier in mitosis in tissues under uniaxial stretch.

(A) A representative cell at interphase and mitosis expressing GFP-NuMA (cyan/inverted grayscale), chromatin marker (mCherry-histone2B; magenta) and membrane marker (BFP-CAAX; yellow). Black arrows indicate pronounced cortical localisation of GFP-NuMA.

(B) Fluorescence measurements of GFP-NuMA were taken at the tricellular vertices, cell edges, polar, and equatorial cortex during mitosis. Polar and equatorial cortex were measured using a polygon ROI.

(C, D) Mean normalised GFP-NuMA fluorescence at tricellular vertices relative to cell edges in unstretched and stretched tissues respectively.

(E, F) Mean normalised GFP-NuMA fluorescence at polar cortex relative to equatorial cortex (polygon ROI) in unstretched and stretched tissues respectively. Data in C, D, E, and F analysed using Friedman test and Dunn's multiple comparisons test. For all data, n=19 cells, 5 embryos (unstretched); 22 cells, 6 embryos (stretched). Statistical significance: $p<0.0001$****, $p<0.001$***, $p<0.01$**, $p<0.05$*. All error bars represent mean ± SEM.

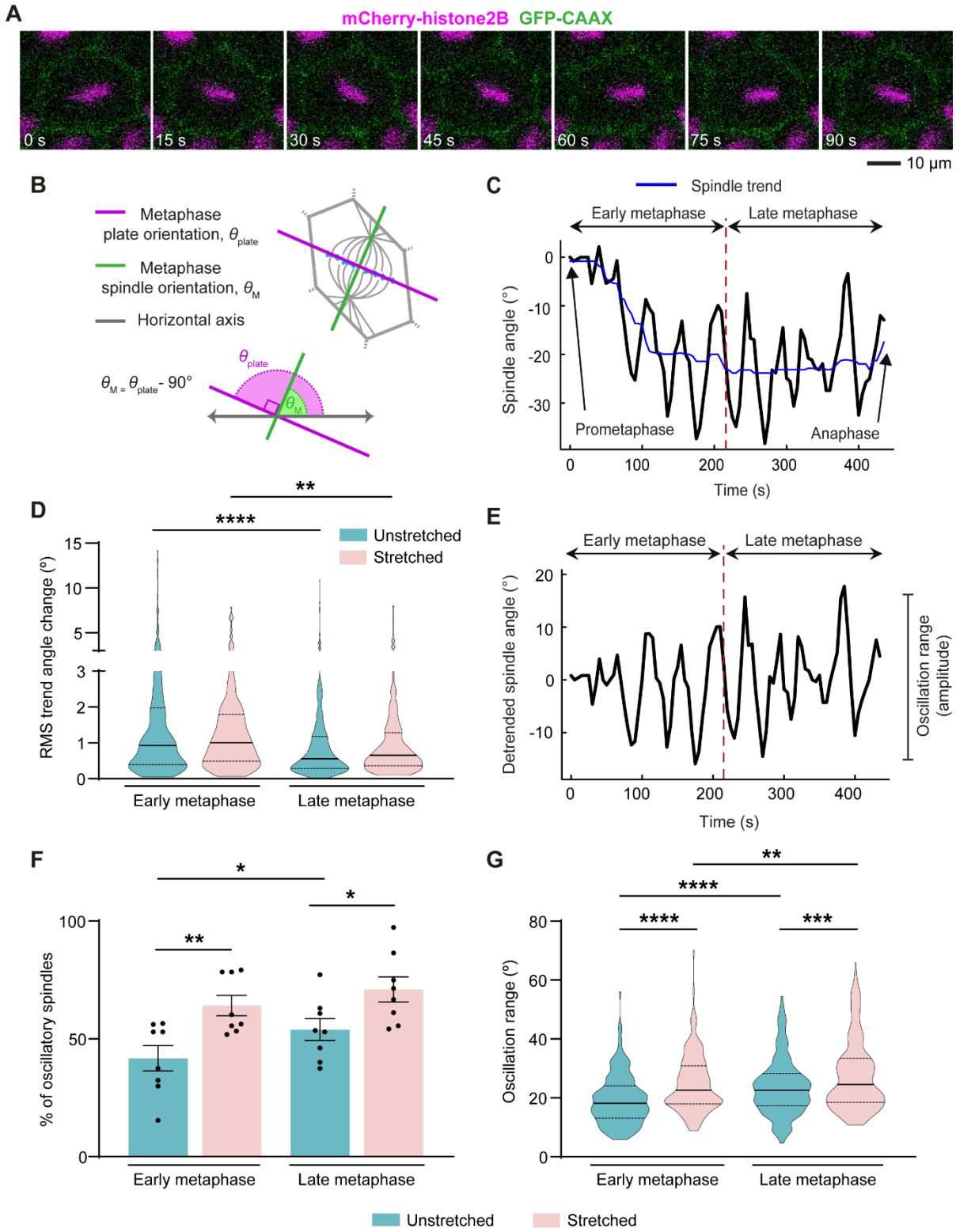
A
mCherry-histone2B GFP-CAAX
0 s
15 s
30 s
45 s
60 s
75 s
90 s
10 µm
B
Metaphase plate orientation, θ_{plate}
Metaphase spindle orientation, θ_M
Horizontal axis
$\theta_M = \theta_{plate} - 90°$
θ_{plate}
θ_M
C
Spindle trend
Early metaphase
Late metaphase
Spindle angle (°)
Prometaphase
Anaphase
Time (s)
D
Unstretched
Stretched
RMS trend angle change (°)
Early metaphase
Late metaphase
E
Early metaphase
Late metaphase
Detrended spindle angle (°)
Oscillation range (amplitude)
Time (s)
F
% of oscillatory spindles
Early metaphase
Late metaphase
G
Oscillation range (°)
Early metaphase
Late metaphase
Unstretched
Stretched

Fig. 2. The mitotic spindle oscillates in a stretch-dependent manner.

(A) A representative cell at metaphase expressing membrane marker (GFP-CAAX; green) and chromatin marker (mCherry-histone2B; magenta). One whole period of oscillation is shown between t=0 s and t=60 s.

(B) Schematic showing the measured orientation of the metaphase plate (θ_{plate}) and the corresponding metaphase spindle orientation (θ_M) lying perpendicular to one another.

(C) Track of a representative spindle undergoing rotational movements from prometaphase until the start of anaphase. Red dashed line corresponds to the mid metaphase timepoint. The overall spindle movement (blue line) is determined using a moving median with a window length Δt=125 s.

(D) Root mean square (RMS) of the change in the trend angle between successive timepoints in unstretched and stretched tissues in early and late metaphase. Data analysed using a mixed-effects model and Tukey's multiple comparisons test.

(E) The spindle track from C with the trend (blue) removed. The measured oscillation range is defined by the upper and lower bounds of the signal omitting outliers.

(F) Percentage of dividing cells with oscillatory spindles in stretched and unstretched tissues, in early and late metaphase. Data analysed using two-way ANOVA and Fisher's least significant difference test. Error bars represent mean ± SEM. For D and F, n=342 cells, 8 embryos (unstretched); 237 cells, 8 embryos (stretched).

(G) Range of oscillation for oscillatory spindles in stretched and unstretched tissues, in early and late metaphase. Data analysed using a mixed-effects model and Fisher's least significant difference test. n=152 cells, 8 embryos; 193 cells, 8 embryos (unstretched, early and late metaphase respectively); 156 cells, 8 embryos; 174 cells, 8 embryos (stretched, early and late metaphase respectively). For D and G, solid and dashed lines represent median and quartiles respectively. Statistical significance: $p<0.0001$****, $p<0.001$***, $p<0.01$**, $p<0.05$*.

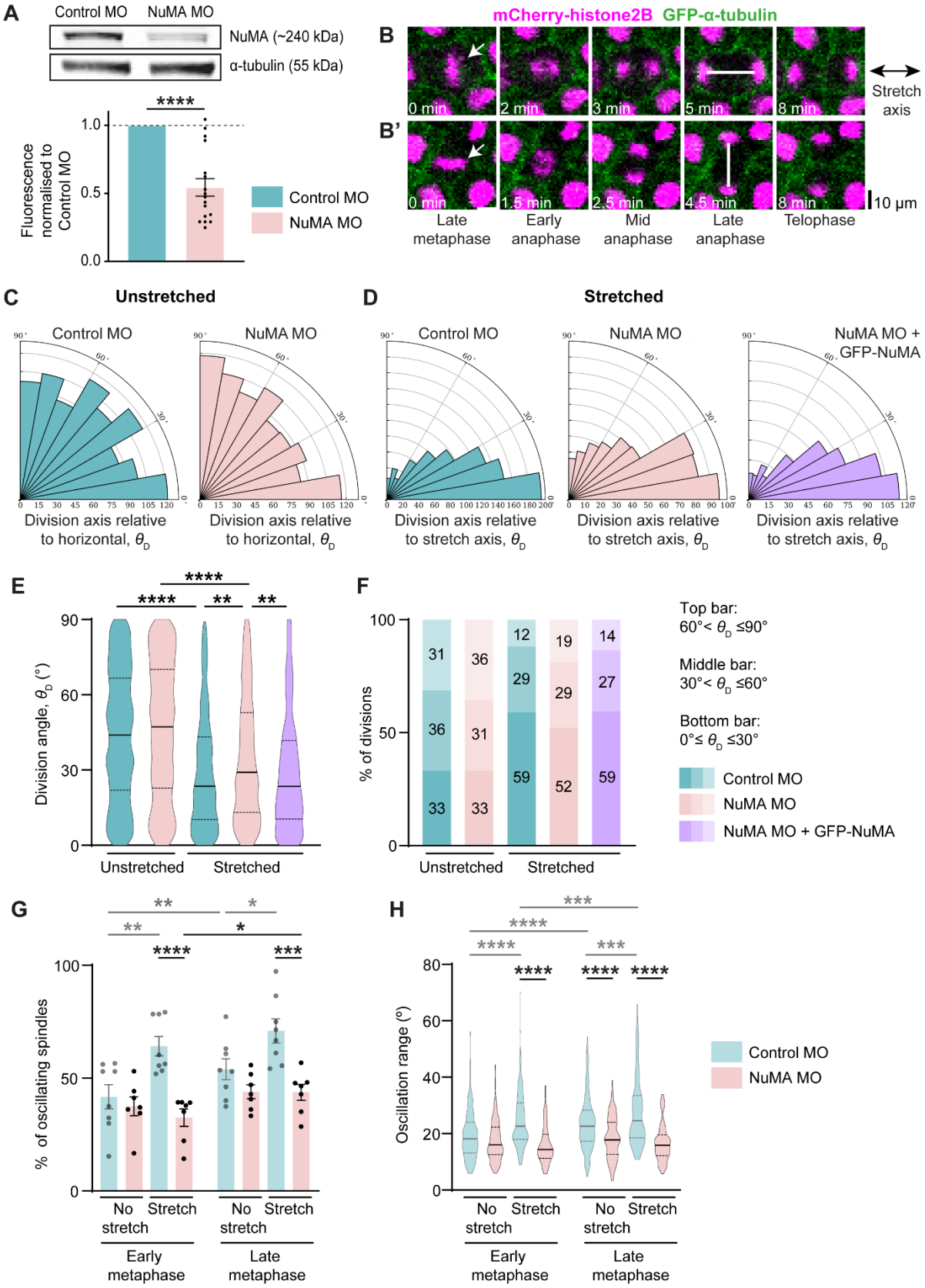
A
Control MO
NuMA MO
NuMA (~240 kDa)
α-tubulin (55 kDa)
Fluorescence normalised to Control MO
Control MO
NuMA MO
B
B'
mCherry-histone2B GFP-α-tubulin
0 min
2 min
3 min
5 min
8 min
1.5 min
2.5 min
4.5 min
Stretch axis
10 µm
Late metaphase
Early anaphase
Mid anaphase
Late anaphase
Telophase
C
Unstretched
D
Stretched
NuMA MO + GFP-NuMA
Division axis relative to horizontal, θD
Division axis relative to stretch axis, θD
E
Division angle, θD (°)
Unstretched
Stretched
F
% of divisions
Top bar: 60°< θD ≤90°
Middle bar: 30°< θD ≤60°
Bottom bar: 0°≤ θD ≤30°
G
% of oscillating spindles
No stretch
Stretch
Early metaphase
Late metaphase
H
Oscillation range (°)

Fig. 3. Knockdown of NuMA disrupts division orientation along the axis of stretch and spindle oscillation.

(A) Endogenous *Xenopus* NuMA expression following knockdown of NuMA. Data analysed using Kolmogorov-Smirnov test. n=17 independent repeats per condition from 170 embryos.

(B) A representative cell expressing chromatin marker (mCherry-histone2B; magenta), microtubule marker (GFP-α-tubulin; green) and dividing parallel to the axis of stretch (double sided black arrow) or **(B')** perpendicular to the stretch axis. White bars indicate the orientation of division. White arrows indicate the position of the metaphase plate at the end of metaphase.

(C, D) Division angles relative to the horizontal axis (the stretch axis in stretched tissues) in control MO, NuMA MO, and rescue tissues, with the number of divisions in 10° bins.

(E) Distribution of division angles in the unstretched and stretched control MO, NuMA MO, and rescue tissues. Data analysed using Kruskal-Wallis test and Dunn's multiple comparisons test. n=922 cells, 9 embryos; 887 cells, 13 embryos (unstretched, control MO and NuMA MO respectively); 810 cells, 9 embryos; 502 cells, 11 embryos (stretched, control MO and NuMA MO respectively); 495 cells, 11 embryos (rescue).

(F) Percentage of divisions from each distribution in E binned into three partitions relative to the horizontal axis (the stretch axis in stretched tissues).

(G) Percentage of dividing cells with oscillatory spindles in stretched and unstretched tissues injected with control MO and NuMA MO, in early and late metaphase. Data analysed using two-way ANOVA and Šidák's multiple comparisons test. Control MO data and comparisons as shown previously, shaded grey for clarity. n=342 cells, 8 embryos; 213 cells, 7 embryos (unstretched, control MO and NuMA MO respectively); 237 cells, 8 embryos; 201 cells, 7 embryos (stretched, control MO and NuMA MO respectively). For A and G, error bars represent mean ± SEM.

(H) Range of oscillation for oscillatory spindles in stretched and unstretched tissues injected with control MO and NuMA MO, in early and late metaphase. Data analysed using a mixed-effects model and Tukey's multiple comparisons test. Control MO data and comparisons as shown previously, shaded grey. n=152 cells, 8 embryos; 193 cells, 8 embryos (unstretched control MO, early and late metaphase respectively); 156 cells, 8 embryos; 174 cells, 8 embryos (stretched control MO, early and late metaphase respectively); 87 cells, 7 embryos; 96 cells, 7 embryos (unstretched NuMA MO, early and late metaphase respectively); 67 cells, 7 embryos; 89 cells, 7 embryos (stretched NuMA MO, early and late metaphase respectively). Statistical significance: $p<0.0001$****, $p<0.001$***, $p<0.01$**, $p<0.05$*. For E and H, solid and dashed lines represent median and quartiles respectively.

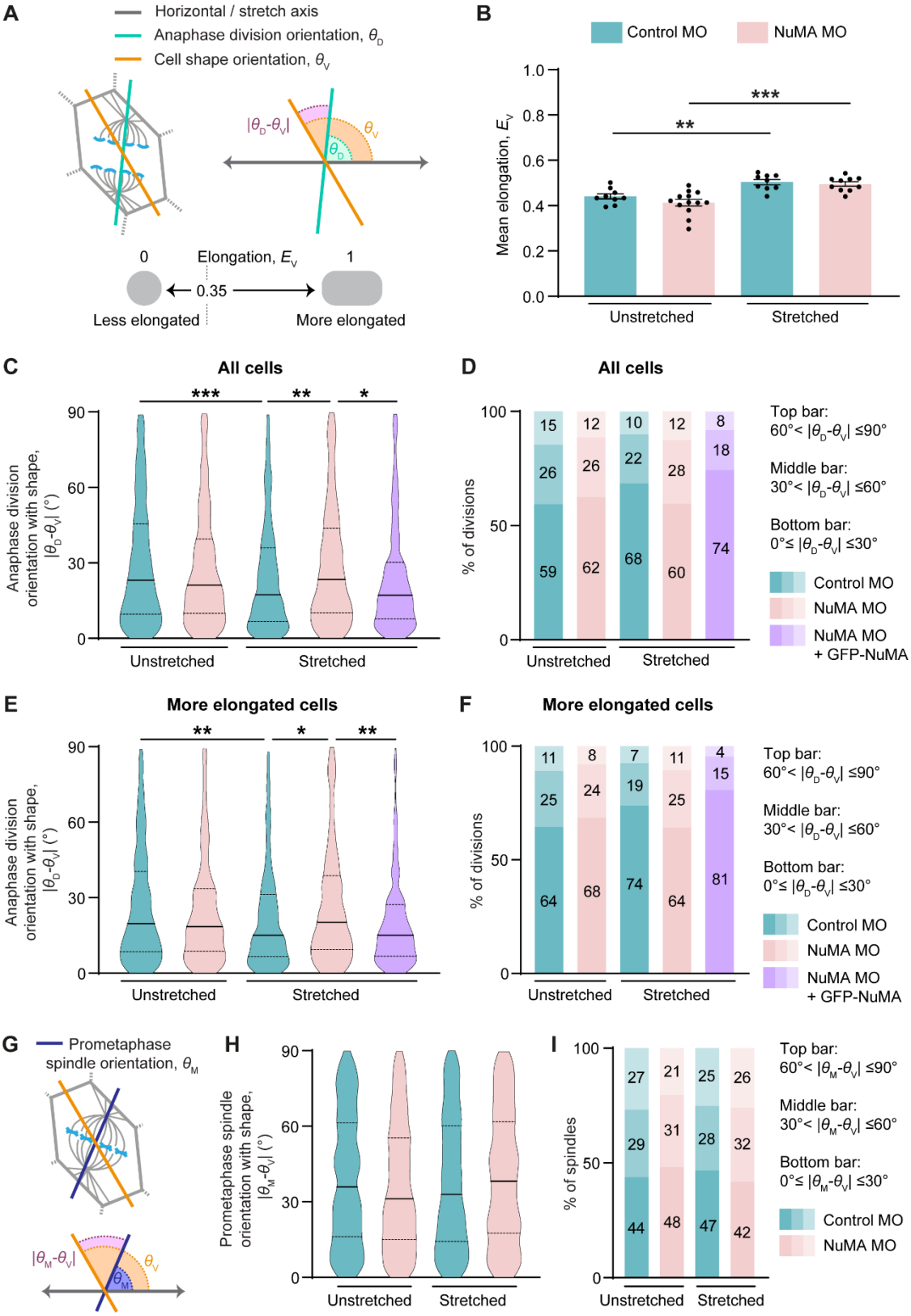
A
Horizontal / stretch axis
Anaphase division orientation, θ_D
Cell shape orientation, θ_V
$|\theta_D-\theta_V|$
θ_V
θ_D
0
Elongation, E_V
1
0.35
Less elongated
More elongated
B
Control MO
NuMA MO
Mean elongation, E_V
1.0
0.8
0.6
0.4
0.2
0.0
**
***
Unstretched
Stretched
C
All cells
***
**
*
Anaphase division orientation with shape, $|\theta_D-\theta_V|$ (°)
90
60
30
0
Unstretched
Stretched
D
All cells
% of divisions
100
50
0
15
26
59
12
26
62
10
22
68
12
28
60
8
18
74
Unstretched
Stretched
Top bar: 60°< $|\theta_D-\theta_V|$ ≤90°
Middle bar: 30°< $|\theta_D-\theta_V|$ ≤60°
Bottom bar: 0°≤ $|\theta_D-\theta_V|$ ≤30°
Control MO
NuMA MO
NuMA MO + GFP-NuMA
E
More elongated cells
**
*
**
Anaphase division orientation with shape, $|\theta_D-\theta_V|$ (°)
90
60
30
0
Unstretched
Stretched
F
More elongated cells
% of divisions
100
50
0
11
25
64
8
24
68
7
19
74
11
25
64
4
15
81
Unstretched
Stretched
Top bar: 60°< $|\theta_D-\theta_V|$ ≤90°
Middle bar: 30°< $|\theta_D-\theta_V|$ ≤60°
Bottom bar: 0°≤ $|\theta_D-\theta_V|$ ≤30°
Control MO
NuMA MO
NuMA MO + GFP-NuMA
G
Prometaphase spindle orientation, θ_M
$|\theta_M-\theta_V|$
θ_V
θ_M
H
Prometaphase spindle orientation with shape, $|\theta_M-\theta_V|$ (°)
90
60
30
0
Unstretched
Stretched
I
% of spindles
100
50
0
27
29
44
21
31
48
25
28
47
26
32
42
Unstretched
Stretched
Top bar: 60°< $|\theta_M-\theta_V|$ ≤90°
Middle bar: 30°< $|\theta_M-\theta_V|$ ≤60°
Bottom bar: 0°≤ $|\theta_M-\theta_V|$ ≤30°
Control MO
NuMA MO

Fig. 4. NuMA responds to anisotropic stretch to orient divisions with cell shape.

(A) Schematic anaphase cell showing the eventual axis of division (θ_D). The cell shape axis (θ_V) is mathematically determined based on the position of the TCVs. The absolute value of the difference between θ_D and θ_V ($|\theta_D-\theta_V|$) is used as a measure of division alignment with cell shape. Elongation is mathematically determined based on the position of the TCVs (E_V) and the value ranges from 0 to 1. Cells with $E_V > 0.35$ are classified as more elongated.

(B) Mean elongation in unstretched and stretched control MO and NuMA MO tissues. Data analysed using ordinary one-way ANOVA with Šidák's multiple comparisons test. n=9 embryos; 13 embryos (unstretched, control MO and NuMA MO respectively); 9 embryos; 11 embryos (stretched, control MO and NuMA MO respectively). Error bars represent mean ± SEM.

(C) Distribution of $|\theta_D-\theta_V|$ in all cells of the unstretched and stretched control MO, NuMA MO, and rescue tissues. n=677 cells, 9 embryos; 609 cells, 13 embryos (unstretched, control MO and NuMA MO respectively); 608 cells, 9 embryos; 357 cells, 10 embryos (stretched, control MO and NuMA MO respectively); 304 cells, 11 embryos (rescue).

(D) Percentage of divisions from each distribution in C binned into three partitions relative to cell shape.

(E) Distribution of $|\theta_D-\theta_V|$ in elongated cells of the unstretched and stretched control MO, NuMA MO, and rescue tissues. n=467 cells, 9 embryos; 411 cells, 13 embryos (unstretched, control MO and NuMA MO respectively); 489 cells, 9 embryos; 282 cells, 10 embryos (stretched, control MO and NuMA MO respectively); 238 cells, 11 embryos (rescue).

(F) Percentage of divisions from each distribution in E binned into three partitions relative to cell shape.

(G) Schematic prometaphase cell showing the spindle axis relative to the horizontal (θ_M). The absolute value of the difference between θ_M and θ_V ($|\theta_M-\theta_V|$) is used as a measure of spindle alignment with cell shape at prometaphase.

(H) Distribution of $|\theta_M-\theta_V|$ in all cells of the unstretched and stretched control MO and NuMA MO tissues. n=647 cells, 9 embryos; 557 cells, 13 embryos (unstretched, control MO and NuMA MO respectively); 594 cells, 9 embryos; 331 cells, 10 embryos (stretched, control MO and NuMA MO respectively). Data in C, E, and H analysed using Kruskal-Wallis test and Dunn's multiple comparisons test. For C, E, and H, solid and dashed lines represent median and quartiles respectively.

(I) Percentage of spindles from each distribution in H binned into three partitions relative to cell shape. Statistical significance: $p<0.001$***, $p<0.01$**, $p<0.05$*.