Tissue fluidity mediated by adherens junction dynamics promotes planar cell polarity-driven ommatidial rotation

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The phenomenon of tissue fluidity—cells’ ability to rearrange relative to each other in confluent tissues—has been linked to several morphogenetic processes and diseases, yet few molecular regulators of tissue fluidity are known. Ommatidial rotation (OR), directed by planar cell polarity signaling, occurs during Drosophila eye morphogenesis and shares many features with polarized cellular migration in vertebrates. We utilize in vivo live imaging analysis tools to quantify dynamic cellular morphologies during OR, revealing that OR is driven autonomously by ommatidial cell clusters rotating in successive pulses within a permissive substrate. Through analysis of a rotation-specific nemo mutant, we demonstrate that precise regulation of junctional E-cadherin levels is critical for modulating the mechanical properties of the tissue to allow rotation to progress. Our study defines Nemo as a molecular tool to induce a transition from solid-like tissues to more viscoelastic tissues broadening our molecular understanding of tissue fluidity.
During morphogenesis, complex tissue patterns and structures are formed from simple sheets of cells. Cell shape change and positional rearrangements facilitate these morphogenetic processes and yet overall tissue integrity has to be maintained throughout\textsuperscript{1–3}. Cells must adhere to their neighbors to maintain the integrity and also to transmit mechanical forces throughout the developing tissue\textsuperscript{1,2,4,5}. Adherens junctions (AJs) are the critical site of cell adhesion and their linkage to the cytoskeleton allows cells to both respond to and generate tension at the tissue level. E-cadherin (E-cad) is a core component of AJs: through its extracellular domain it binds E-cad on neighboring cells and its intracellular domain provides an anchor point for the actin cytoskeleton through binding partners such as the catenins and vinculin\textsuperscript{6,7}. E-cad molecules form clusters of varying sizes that are dependent upon both \textit{trans} and \textit{cis} interactions with other E-cad molecules\textsuperscript{8–9}. AJs are dynamic structures and their regulated turnover mediates tissue remodeling. Depending on the size of E-cad clusters and their recycling rate to/from the cell surface, AJs can be stable complexes that withstand tension, or dynamic complexes that allow a remodeling of cell contacts to relax tension at the tissue level\textsuperscript{5,10}. Tissue fluidity in epithelia has been defined as the ability of cells in a confluent tissue to exchange neighbors and yet maintain intercellular junctions to ensure epithelial integrity and associated barrier functions\textsuperscript{3,11,12}. Throughout morphogenetic processes, tissues need sufficient fluidity to allow cell intercalation and tissue shaping: tissues that are solid-like resist the applied forces whereas more fluid-like tissues can respond to stresses by junctional remodeling and allowing processes such as tissue flow or shape change. Tissues are examples of active materials, where local interactions between the basic elements, in this case cells, consume energy and govern the large-scale behavior. Cell adhesion through AJs is a major mediator of such local interactions. Convergent extension is one example of how tissue fluidity drives axis elongation in both vertebrates and invertebrates\textsuperscript{2,4,5,10,13}. Cells undergo coordinated shape adjustment and movement with respect to their neighbors to change the dimensions of the tissue and elongate the anterior—posterior body axis. Although progress has been made at examining the biophysical dynamics of unidirectional movements of epithelia, such as convergent extension in the \textit{Drosophila} embryo or tissue flow in the \textit{Drosophila} thorax and wing\textsuperscript{5,10,11,13–15}, the molecular signals regulating the degree of fluidity at the tissue level remain largely unknown, particularly during tightly-localized and temporally-discrete morphogenetic events.

The \textit{Drosophila} eye is uniquely suited as a model to address these questions. The eye is comprised of \textasciitilde 700 identical individual units, termed ommatidia, which sequentially undergo morphogenetic processes, including ommatidial rotation\textsuperscript{16,17}. Each ommatidium contains a cluster of eight photoreceptors (R1–R8 cells) and additional accessory cells, which are progressively recruited to each cluster following the anterior progression of the morphogenetic furrow (MF) through the eye imaginal disc (Supplementary Fig. 1a–c)\textsuperscript{16}. As the furrow progresses across the tissue, it induces uncommitted cells to differentiate and recruit their neighbors in a stereotypical sequence to initially form 5-cell pre-clusters, which are separated from surrounding pre-clusters by 2–4 layers of neighboring interommatidial cells (ICs) (Fig. 1a and Supplementary Fig. 1a–c). Planar cell polarity (PCP)-signaling induces the differential specification of R3 and R4, which breaks the symmetry of the pre-cluster and is followed by the rotation process with the direction of rotation being governed by the R3/R4 positioning (in the R3 to R4 direction), clockwise dorsally or counterclockwise ventrally\textsuperscript{16}. This creates a line of mirror-symmetry along the D/V midline, the equator (Fig. 1a and Supplementary Fig. 1a–c). The rotation process is needed for proper axonal bundling of R-cells to allow their correct connectivity in the neurons in the optic lobes\textsuperscript{18,19}, and ceases after each ommatidium rotates 90°.

All cells in the developing eye are connected via E-cad-containing AJs, although the ommatidal (pre)cluster cells upregulate E-cad relative to the ICs to maintain cluster cohesion\textsuperscript{20}. In addition, other adhesion systems play a role in the eye with N-cadherin and the atypical cadherin Flamingo also being upregulated in the ommatidial cluster\textsuperscript{21,22}, whereas the cell-adhesion molecules Echinoid and Friend of Echinoid have more dynamic expression patterns throughout the eye during the rotation process\textsuperscript{23}. In addition to cell–cell adhesion, cell-extracellular matrix (ECM) interactions are also required for eye morphogenesis\textsuperscript{24}. Photoreceptor cells express the \textit{Drosophila} homolog of β1 Integrin, Myospheroid, which localizes to the outside of the R-cells and forms a cup-like shape around each cluster, where it interacts with the ECM\textsuperscript{24}. Mutations in any of these components lead to rotation defects, with clusters either rotating asynchronously, or too little or too much (reviewed in\textsuperscript{17,20–24}).

The genetic basis of cell specification and the signaling pathways governing ommatidial rotation have been well studied (reviewed in refs. \textsuperscript{17,25}, and also e.g., refs \textsuperscript{26–30}), however, the cell biological and biophysical aspects of rotation remain poorly understood. Indeed, due to the lack of specific genetic tools to manipulate signaling in the ICs, the vast majority of past studies have focused on rotation cues emanating from the ommatidial cluster itself, rather than its surrounding environment. From a biophysical standpoint, it remains unclear how the rotating groups of cells coordinate their collective movement within a field of, what has been considered to be, immobile epithelial ICs\textsuperscript{17,31}. Also, whether the forces driving rotation comes from the pre-cluster itself (autonomous) or from the surrounding ICs (non-autonomous) remains unresolved. Moreover, biophysical mechanisms controlling complex cellular behavior such as chiral rotation of groups of cells in a field of non-rotating confluent cells, and the effect of tissue fluidity on the dynamics of such movements have not been addressed. We show here that rotation requires a specific level of tissue fluidity to enable the precise progression and cessation of rotation. Tissue fluidity is controlled by Nemo (Nmo, a conserved MAPK family member)\textsuperscript{31–34}, which we show regulates junctional E-cad dynamics, and thus overall tissue fluidity and mechanical properties.

**Results**

An \textit{in vivo} live imaging approach to analyze ommatidal rotation. The primary method of analyzing and quantifying rotation has been to determine ommatidial orientation within the successive rows of pre-clusters posterior to the furrow in fixed tissue (Supplementary Fig. 1a–d). This identified the genetic basis of rotation and the importance of PCP factors in establishing rotation direction\textsuperscript{35,36}. However, a mechanistic understanding of ommatidial rotation, and PCP-directed movement more broadly, requires spatiotemporal measurements in real-time of cellular processes and associated behaviors, including force generation and transduction mechanisms\textsuperscript{13}. We established a live imaging protocol of pupal eye tissue in vivo during rotation, focusing on E-cad to monitor cellular junctions as a readout of cell behavior, and visualized the process with an E-cadherin:GFP knock-in \textit{Drosophila} line (see “Methods”). We observed active furrow progression generating new rows of ommatidial pre-clusters and their subsequent maturation and rotation (Supplementary Fig. 1e–i; Supplementary Movie 1). To investigate the mechanistic basis of the rotation process, we developed image processing software to quantify the temporal variation of various force-
generating mechanisms, including cell division, delamination (apoptosis), neighbor exchange, and cell movement in wild-type (wt) control animals, as well as quantifying rotation in genetic backgrounds in which force-generating mechanisms were perturbed.

**Rotation is a continuous, pulsatile process.** Rotation could be driven by forces intrinsic to the cluster and/or input derived from the surrounding ICs. We examined the spatial and temporal variation of cellular dynamics in wt eyes to determine whether any occur concomitantly with rotation and might therefore drive the process. Ommatidial rotation was measured over time, $R(t)$, as the change in the angle between the line connecting the R2/R5 centroids and the furrow (Fig. 1a–e). Upon the formation of ommatidial 5-cell pre-clusters (row 1), the R2/R5 pair was parallel to the furrow ($R(0) \sim 0^\circ$), and with time this angle increased (Fig. 1a–e). To obtain measurements of rotation for an extended time period, we tracked ommatidia from rows 1–4, allowing us to quantify rotation—and other cellular processes—for a period of up to 500 min (~8 h) (Supplementary Fig. 1d–i). Ommatidial clusters rotated with an average angular velocity of $\Omega = 11^\circ/h$, fitting a sigmoid curve (Fig. 1e). This continuous progression is in contrast to the proposed two phases of 45° rotations suggested in a previous study performed in fixed tissue. Moreover, the...
dynamics of rotation appear pulsatile in each individual cluster within the continuum of the process (Fig. 1g), immediately demonstrating insight from live imaging. Concomitant with rotation, the apical surface area of ommatidial clusters constricted (see pseudo-colored apical surface in Fig. 1b). The dynamics of constriction followed a similar profile to the rotation rate, with an average constriction of ~60% of the original size with a rate $C = 2.5 \mu m^2/h$ (Fig. 1c–f). A cross-correlation analysis of rotation and apical constriction indicates that these two dynamic, pulsatile processes are strongly correlated without a time lag ($r = 0.98$, $p = 1.58e-30$, Fig. 1g, h), suggesting that the force-generating mechanism driving both processes is shared, or that one directs the other. In an effort to examine the involvement of other force generation mechanisms within the cluster, we turned our attention to the migration of photoreceptor nuclei to the apical surface. This movement is initiated once ommatidia have been recruited to the ommatidial pre-cluster\textsuperscript{37}. Nuclear migration can be a force generator for cell motility in mammalian cells. We used the klarsicht mutant (klar\textsuperscript{A1–18}), which affects the apical positioning of pre-cluster cell nuclei, and exhibits randomized nuclear positioning along the apico-basal axis\textsuperscript{39,40}. Rotation proceeded in klar mutants as in wt (Supplementary Fig. 2), demonstrating that nuclear migration is not a major force generation mechanism during the rotation process.

Interommatidial cells appear to serve a permissive role during rotation progression. The rotation process requires both a force-generating feature, which appears to be autonomous to the cluster based on the above correlation with cluster constriction, and yet could also be directed by external forces from the immediate environment (e.g., via shear forces or cell adhesion), which would be a non-autonomous feature\textsuperscript{20–24,31,34}. Other non-autonomous aspects regulating rotation could include the cellular dynamics of the eye disc as a whole. Previous studies in fixed tissues have shown that ICs near the furrow divide (second mitotic wave) and delaminate\textsuperscript{41,42}. Further from the furrow, cell division and delamination cease\textsuperscript{41,42} (the photoreceptors themselves are post-mitotic and do not delaminate). The coordinated waves of division and delamination appear to coincide with the onset and progression of rotation. Cell division, in particular oriented cell division, and delamination can be force-generating mechanisms that drive tissue morphogenesis\textsuperscript{43,44}, and the behavior of ICs could thus directly impact the rotation of ommatidial clusters.

We, therefore, sought to analyze the temporal change in division and delamination of ICs directly adjacent to ommatidial clusters during rotation (Fig. 2a, b and Supplementary Movie 2). Our analyses revealed that cell division peaked upon the formation of pre-clusters close to the furrow, and the fraction of dividing cells decreased gradually and ceased after $t \sim 250$ min (Fig. 2b). Upon a decline in cell division, cell delamination peaked at $t \sim 350$ min, and subsequently ceased at $t \sim 450$ min (Fig. 2b). However, as the speed of rotation was consistent throughout both peaks of cell division and delamination, and rotation continued even after the decline of both, neither process appeared to directly influence rotation rate. Consistently with these data, adult eyes displayed a wild-type phenotype when cell division orientation was perturbed in the mud loss of function background (Supplementary Fig. 3a, d). It was also shown previously that the second mitotic wave can be uncoupled from patterning in the eye\textsuperscript{45}. Similarly, inhibition of cell delamination did not affect the morphology of the adult eye (Supplementary Fig. 3a–c), suggesting that these processes are not essential for the force generation that drives rotation and its progression.

Next, we asked whether ICs could be driving rotation by shear forces arising from the directed or patterned neighbor exchange and IC movement in the immediate environment. The movement of ICs has not been studied, and generally, ICs have been presumed to be immobile. To this end, we analyzed the neighbor exchange and trajectories of ICs that are in contact with the cluster (see “Methods”) to determine whether there was a relationship between rotation rate and the rate of junction remodeling, or a coherent flow of ICs relative to the direction of rotation. We measured the temporal variation in neighbor exchange, $N(t)$, of ICs adjacent to ommatidial clusters (Fig. 2c, d, and Supplementary Movie 2); cell neighbor exchange continuously declined during rotation from 7 events/ommatidium at the formation of the pre-clusters to 4 events/ommatidium by the end of our imaging period (Fig. 2d), while the rotation of the cluster proceeded with the same angular velocity (Fig. 1c–e; the initially higher levels of neighbor exchange seem to be related to the second mitotic wave\textsuperscript{13,44} and not to rotation progression). We can conclude that neighbor exchange is occurring throughout the rotation and that it declines as rotation nears completion.

During this period, we also measured the motion of ICs by tracking their centroids (Fig. 2e) and plotting the average displacement field of all analyzed ommatidia during a 3 h time window (Fig. 2f). If ICs were pulling the pre-cluster during rotation, we would expect to observe a coherent circular motion of ICs around the pre-cluster. Instead, these data revealed that ICs did not display such movement relative to ommatidial clusters, arguing against a role for ICs in driving the rotation process and instructing the direction of rotation (Fig. 2f). As such, taken together the data derived from our movies suggest that the clusters drive rotation autonomously (also Discussion) and that the surrounding ICs may serve a permissive role during the rotation process.

Nmo modulates tissue dynamics. To gain further insight into the mechanics of rotation, we wanted to examine these same parameters under conditions when rotation itself is specifically affected. With the wt data set serving as a baseline, we performed a comparative analysis of the rotation modulator nmo, a conserved member of the MAP kinase superfamily\textsuperscript{32,33}. Although many genes are known to affect rotation, several, such as Egfr, also affect other aspects of photoreceptor fate specification\textsuperscript{17,26,28}. We, therefore, wanted to analyze the function of a factor that largely only affects the rotation step, and use these phenotypes to understand which of the force generation mechanisms we identified in the wt eyes, are contributing to the nmo mutant phenotype. The nmo loss-of-function (LOF or nmo\textsuperscript{mut}) mutants show under-rotation of clusters, with rotation angle correlating with the amount of Nmo function\textsuperscript{33}. The Nmo gain-of-function (GOF) phenotype, in contrast, shows a large variability in rotation angles at the population level, with both under- and over-rotation of ommatidia detectable (Supplementary Fig. 4a–d, nmo mutant and eye-specific Nmo overexpression are shown; also “Methods” and ref. 33). Comparing Nmo LOF and GOF with wt should therefore allow us to identify cellular processes underlying the difference in rotation phenotypes.

We first measured $R_t$ in each genotype and confirmed the rotation defects observed in fixed tissue (Fig. 3a, b). Given the correlation between constriction and rotation in wt (Fig. 1d, g, h), we examined constriction in the nmo backgrounds to determine whether changes in constriction rate could explain the rotation defects (Fig. 3a, c and Supplementary Movies 3 and 4). Comparing constriction to wt, we observed that ultimately the clusters constricted equally successfully in all three genotypes, reaching a comparable surface area (~8 $\mu m^2$) (Fig. 3a, c). However, the dynamics of constrictions were rather different.
Fig. 2 Interommatidial cells display dynamic behaviors during ommatidial rotation. a Snapshots of the same cluster shown in Fig. 1 highlighting interommatidial cells (ICs) that divide (green) and delaminate (yellow; these cells can also divide, but at least one of the daughters delaminates subsequently). The ICs analyzed were first level neighbors of the cluster at the R7 recruitment stage. b Average number of dividing (green) and delaminating (orange) ICs that were the first-level neighbors of the photoreceptor cluster, as a function of time. Data were binned into 90 min time periods and were averaged over all ommatidia. The mean and standard error of the mean is shown. Gray dashed lines indicate the recruitment of the R1/R6 pair and R7, respectively (also in d). c Selected ICs, highlighted with random colors, illustrating junction remodeling and neighbor exchange. d Average number of ICs that were the first-level neighbors of the photoreceptor cluster and underwent neighbor exchange in between frames plotted as a function of time. Line shows mean, and shaded region indicates standard error of the mean. e Snapshots with trajectories of IC centroids, with dots representing the current centroid position in the respective image. f Average cell speed and displacement of ICs during ommatidial rotation. The trajectories of ICs from all ommatidia were pooled, and the average displacement (white arrows) and cell speed (heat map) were measured. A 5-cell pre-cluster schematic with gray cells is indicated in the middle of the plot with a curved red arrow representing the direction of rotation. Measurements in b, d, and f are averaged over 11 clusters from two pupae in >300 min movies. All scale bars: 3 µm.

between the three genotypes (Fig. 3d). In the LOF nmo<sup>mut</sup> tissue, constriction was a steadier process than in wt, exhibiting lower amplitudes of relaxation following consecutive constriction pulses (Fig. 3d–f). In contrast, in the Nmo GOF, pre-clusters failed to stabilize successive constriction pulses and exhibited higher amplitudes of relaxation between constriction pulses (Fig. 3d–f). Taken together these data suggested that the ability of the contractile apparatus to constrict the apical surface was largely unaffected in both LOF and GOF scenarios, but that the effective stabilization of contractions at the junctional level was altered. Actomyosin contractions are required for apical constriction, with a molecular ratchet required to stabilize size decrease<sup>46–48</sup>. Consistent with a failure of stabilization and not actomyosin contraction, MyosinII (Zipper::GFP) intensity and localization was altered. This would have a subsequent effect on the ability of the cluster to rotate. We, therefore, conclude that Nmo does not affect the contractile apparatus during rotation and our data suggest that the effect of Nmo on rotation might be to regulate junctional stability and/or dynamic remodeling following actomyosin contraction.

**Nmo levels affect tissue jamming and fluidity.** In addition to apical constriction, neighbor exchange was the other process that proceeded concomitantly with rotation. To analyze the impact of Nmo on this parameter, we monitored IC cell displacement and neighbor exchange in the nmo genotypes. We observed stark differences in neighbor exchange and cell displacement between wt, nmo<sup>mut</sup> LOF, and Nmo GOF scenarios. The neighbor...
exchange was markedly reduced in the nmo LOF mutant (Fig. 3g, h and Supplementary Movie 3) with cells maintaining the same neighbors over an extended time period, suggesting that there is more cohesion between cells, with cells appearing more adhesive to each other. In contrast, neighbor exchange was maintained at a high level in Nmo GOF tissue relative to wt, with cells more prone to exchanging neighbors frequently and consistently (Fig. 3g, h and Supplementary Movie 4). This cellular behavior was confirmed by centroid trajectory plots, in which nmo\textsuperscript{mut} LOF exhibited a mutual cell caging\textsuperscript{12,51} (or collective trapping) phenotype with markedly reduced cell displacement of ICs as compared to wt, whereas Nmo GOF revealed enhanced IC movement (Fig. 3i–k; Supplementary Fig. 5h). Further analyses showed that the dynamics of ommatidial rotation and constriction were consistent within each genotype across individual pupae (Supplementary Fig. 6). Taken together, these data indicate that Nmo...
GOF appeared to boost neighbor exchange (as well as the onset of cell delamination, Supplementary Fig. 5d, i), while also failing to stabilize apical constriction as discussed above. Conversely, in the LOF nmo^mut, neighbor exchange and cell delamination were both reduced (Fig. 3g, h and Supplementary Fig. 5a, c, i, g), while the stabilization of successive constriction pulses was increased (Fig. 3d, i).

Neighbor exchange and cell displacement are both key determinants of tissue fluidity, and these results suggest a gradient of fluidity from nmo^mut (lowest) to wt to Nmo GOF (highest). However, they are also impacted by other events such as cell division and delamination as seen above. To try to address tissue fluidity specifically, we turned to the geometry of the ICs. Whilst analyzing neighbor exchange, we noticed that the individual ICs had a different appearance in the nmo^mut and Nmo GOF genotypes, as compared to wt; they looked more compact in the mutant and more elongated in the GOF (compare IC outlines in Fig. 4a). To quantify these phenotypes, we measured the perimeter and area of each cell (Fig. 4b, c) and both metrics indicated that nmo^mut cells were indeed smaller and the Nmo GOF were larger than wt ICs. Recent studies defined that the associated shape index \( s^2 = p_0 / \sqrt{A} \) (where \( p_0 \) is the perimeter, \( A \) is the area) is also a measure of tissue jamming.\(^{52} \)

To the jamming transition point (Fig. 4d and color scheme in Fig. 4a). Conversely, Nmo GOF cells with their elongated appearance have a higher shape index, suggesting the tissue is more fluid (Fig. 4a-d). The standard deviation of the shape index is also a measure of tissue jamming.\(^{52} \) The more variable the shape index within a field of cells, the more fluid the tissue. Accordingly, by this measure, the nmo^mut cells are less variable, and Nmo GOF cells are more variable than the wt control cells (Fig. 4e). Subsequent work has suggested that tissue packing also impacts the jamming transition point.\(^{55} \) The pupal wing is the Drosophila model tissue that has been most studied in terms of cell packing and dynamics.\(^{11,37-59} \)

We thus analyzed whether nmo also affected cell packing and behavior in the wing. We generated nmo LOF clones or Nmo GOF patches of tissue next to wt cells and analyzed cell perimeter and area, and calculated the associated shape index for each group. Consistently with data from the eye, nmo^mut LOF cells were smaller and more tightly packed than wt, with a low shape index (Fig. 4f-i), and Nmo GOF cells were larger with a higher shape index than wt (Fig. 4f-i). Adherens junction remodeling has been shown to directly affect cell packing and movement in the pupal wing. Hexagonal packing is dependent upon the recycling of E-cad, with defects in endocytosis changing the polygon distribution as pentagonal cell junctions are less easily remodeled to form hexagonal shape. Accordingly, we find an altered polygon distribution in nmo^mut LOF wings (Supplementary Fig. 7e). Interestingly, this process is also dependent on PCP input\(^{37} \) and p120 catenin is also known to regulate E-cad internalization and thereby affect cell shape and tissue level stress response during wing morphogenesis.\(^{11} \)

Strikingly, p120 seems to have the opposite phenotype to nmo—p120 mutant cells are more elongated due to higher internalization rates of E-cad and thus remodeling in response to the tissue level tension.\(^{11} \)

Thus, changes in Nmo levels affect not only cell movement but also cell packing and dynamics in multiple tissues undergoing morphogenesis and might thus act generally in tissue packing and associated behavior (also "Discussion"). Nmo GOF tissue appeared to be more fluid-like compared to wt, based on both cellular geometry and behavior (Fig. 4). Conversely, nmo LOF cells behaved in a caged manner, barely moving beyond their size and adopting the morphology of locomotion-inhibited confluent cells in a solid-like tissue (Fig. 4f-1). In each case, the degree of tissue fluidity affects the ability of the cluster to rotate the full 90°, either restricting (nmo LOF) or removing the regulatory input that can determine the appropriate stopping point (Nmo GOF). Consistent with the notion that nmo^mut LOF cells are producing more tension, reflected in smaller apical areas and reduction in the most energy preferred cell shape, the hexagon,\(^{57} \), mutant wing cells are both smaller and display less prominent hexagonal appearance (Supplementary Fig. 7e, i; this notion is supported by previous theoretical and experimental studies arguing that higher cellular tension is linked to a smaller apical cell area and reduced junctional remodeling between cells, affecting the distribution of hexagons and pentagons.\(^{57,58} \)

Taken together these observations led us to examine steady-state surface E-cad levels and E-cad turnover rates in each of our genotypes.

**Nmo regulates E-cad levels at the AJs.** We used the pupal wing to analyze the level of E-cad at the junction, because of their homotypic and larger cell size, and the ability to tightly regulate the spatial pattern of Nmo overexpression, both features make it more amenable for such studies as compared to the eye. In order to compare E-cad levels accurately between the genotypes, we calculated the fold change in steady-state E-cad levels relative to neighboring wt tissue, for either the nmo^mut LOF or Nmo GOF (overexpression) genotypes. nmo^mut LOF clones were induced as above, and for the GOF experiment we used patched-GAL4 (driving Nmo expression in a stripe along the A/P boundary in the center of the wing). Compared to control, we saw an increase (~50%) in E-cad intensity at junctions in nmo^mut LOF cells (Fig. 5a, c and Supplementary Fig. 7g, h). Conversely, there was a decrease (~25%) in E-cad intensity in the Nmo GOF cells (Fig. 5b, c and Supplementary Fig. 7h). These data are consistent with reduced E-cad endocytosis leading to higher levels at the junctions in nmo^mut LOF cells, and increased endocytosis leading to lower levels of junctional E-cad in the GOF cells.

E-cad trafficking is known to play a pivotal role in junctional remodeling, cell migration, and morphogenesis.\(^{60} \) We sought to directly test whether Nmo regulated E-cad dynamics at AJs using Fluorescence Recovery After Photobleaching (FRAP) experiments.\(^{61} \) E-cad fluorescence recovery can be achieved either through lateral movement of E-cad molecules already present at the plasma membrane, or the arrival of newly trafficked E-cad molecules (either recycled from AJs or newly synthesized protein).\(^{62} \) To specifically address the trafficking of E-cad we bleached a 10 µm-long junction between four cells (two adjacent cell boundaries) and recorded at 20 s intervals to monitor E-cad fluorescence recovery at the junction of ICs at the center of the bleached region (Fig. 5d–f; Supplementary Fig. 7a, d and Supplementary Movies 5–7). The bleaching of entire junctions between cells ensures that only newly-trafficed E-cad contributes to recovery and not molecules diffusing within the junctional plasma membrane. LOF nmo^mut exhibited a slower initial recovery rate compared to wt controls, with a longer half recovery time (Fig. 5g–i). Conversely, Nmo GOF tissue demonstrated a faster initial recovery rate with a shorter half-recovery time (Fig. 5g–i). Of note, the recovery time here is of a similar scale to the pulses of constriction and rotation described earlier (Fig. 1g, h).
We next investigated the functional effect of altered E-cad recycling on rotation. Using the sev > Nmo genotype as background, we performed genetic interactions to identify factors that enhanced or suppressed the Nmo GOF variable rotation phenotype. To reduce E-cad recycling, we used a LOF allele of dynamin (known as shibire/shi in Drosophila). Removing one copy of \( shi \) (\( shi^{-/} \)) was sufficient to partially suppress the NmoGOF phenotype (Fig. 5j, k), suggesting that the phenotypic effect is in part dependent upon dynamin-mediated endocytosis. We also co-overexpressed an E-cad molecule that cannot bind to p120 catenin\(^63\), and compared it to co-overexpression of wt E-cad. As p120 binding to E-cad stabilizes E-cad complexes at junctions\(^64\), deletion of the p120 binding site should promote E-cad turnover. Indeed, co-expression of the E-cad molecule that cannot bind to p120 enhanced the sev > Nmo phenotype as compared to wt E-cad expression (Supplementary Fig. 7c), consistent with the notion that the variability in rotation angles observed with sev > Nmo GOF is dependent on increased E-Cad turnover. We saw that over-rotated clusters tended to center around 270°, supporting previous observations and the hypothesis that there is a stop signal in the antero-posterior axis\(^31\). Interestingly, Nmo GOF seemed to increase the variation around
Fig. 4 Nmo regulates cell shape in eye and wing tissues. a Snapshots of representative ommatidia (at stage of R7 joining clusters) in wt control, nmo\textsuperscript{mut}, and nmo\textsuperscript{GOF}, where interommatidial cells are colored based on their shape index (D\textsubscript{3} = cell perimeter/\sqrt{cell area}). The color map is shown. Scale bar: 3 μm. b Violin plots of the perimeter length of interommatidial cells for each respective genotype, black line shows the mean value and all subsequent panels (***p = 2.06 × 10\textsuperscript{-82} and 3.13 × 10\textsuperscript{-27}, respectively). c Violin plot of the area of interommatidial cells for the respective genotype (***p = 1.16 × 10\textsuperscript{-83} and 1.20 × 10\textsuperscript{-21}, respectively). d Violin plot of the shape index of interommatidial cells for the respective genotype (**p = 2.44 × 10\textsuperscript{-25} and 4.06 × 10\textsuperscript{-19}, respectively). e Standard deviation of shape index as a function of average shape index for all the first and second level neighbors of an ommatidium. Each ommatidium is represented as a single dot, and color-coded by respective genotype as indicated. The shaded ellipse covers the smallest area that is enclosed by the individual points (note the linear relation between these two quantities). f Mosaic wing tissue (fixed) at 22 h after pupariation formation (APF) with cells colored based on their shape index; a nmo\textsuperscript{mut} clone (nmo\textsuperscript{null} allele) and nmo\textsuperscript{GOF} cells are juxtaposed to wt control cells, as indicated on left. Blue line marks the boundary between experimental (nmo\textsuperscript{mut} or nmo\textsuperscript{GOF}) cells and control cells. The color map is shown. Scale bar: 5 μm. g Violin plot of the cell perimeter in control and nmo\textsuperscript{mut}, and in control and nmo\textsuperscript{GOF}. 22 h APF pupal wing tissues (**p = 7.64 × 10\textsuperscript{-62} and 4.49 × 10\textsuperscript{-181}, respectively). h Violin plot of the area in control and nmo\textsuperscript{mut}, and in control and nmo\textsuperscript{GOF} wing tissues (**p = 1.12 × 10\textsuperscript{-55} and 4.05 × 10\textsuperscript{-105}, respectively). i Violin plot of the shape index in control and nmo\textsuperscript{mut}, and in control and nmo\textsuperscript{GOF} wing tissues, respectively (**p = 2.37 × 10\textsuperscript{-13} and 1.35 × 10\textsuperscript{-80}, respectively). Note that the change in cell perimeter, area, and shape index between different genotypes are consistent in the eye and wing tissues. To calculate the p-values in (b-d) and (g-i), two-tailed Student’s t-test was used. In a-e, the n values were as follows: 290 cells/11 ommatidia (control); 430 cells/17 ommatidia (nmo\textsuperscript{mut}), and 450 cells/18 ommatidia (nmo\textsuperscript{GOF}), each from two pupae. In f-i, the n values were as follows: >700 cells from three mosaic wings (control and nmo\textsuperscript{mut}); and four mosaic wings (nmo\textsuperscript{GOF}).

Discussion

This work defines a role for Nmo kinase in modulating tissue fluidity, which is essential for proper tissue morphogenesis. Together with previous work\textsuperscript{33}, these data revealed that Nmo regulates the dynamics of E-cad turnover at AJs directly, thus impacting junctional remodeling and tissue fluidity (see model in Fig. 6i). Our results are consistent with a model in which rotation is powered autonomously from within the ommatidial cluster, and that the surrounding ICs serve as a permissive environment, whose fluidity regulates the degree of rotation. Actomyosin contractility is required within the cluster itself for apical constriction and rotation\textsuperscript{50}, and myosin heavy chain progressively localizes to the interface of cluster-ICs as more cells are added\textsuperscript{49}. Intercellular adhesion is also important with adhesion molecules, including E-cad, being upregulated as cells join the cluster\textsuperscript{20}. Consistent with the role of cell adhesion in rotation, it has been shown that clones of cells expressing the altered level of adhesion molecules can negatively impact the rotation of neighboring wt clusters\textsuperscript{20,22}. Beyond the mechanical anchoring aspect of IC-cluster adhesion though, our data suggest that the fluidity of the local IC neighbors may also play a role. As the cluster rotates, its asymmetrical shape necessitates displacing ICs. The more motile the ICs, the less effort is required for the cluster to rotate. It is worth noting that basolateral Integrin-ECM mediated adhesion also affects ommatidial rotation and is linked to PCP signaling\textsuperscript{24}, and interestingly, it acts in the opposite manner to Nmo (Supplementary Fig. 7). Basolateral input also regulates the cell rearrangements studied extensively during Drosophila germ band extension\textsuperscript{66}. Together these data suggest that an interplay of adhesion systems provides the right level of tissue fluidity. ICs not only insulate each ommatidial (pre)cluster from forces coming from other surrounding clusters, possibly helping to relax tension, but also serve as a permissive substrate with an ideal fluidity in wt to allow rotation to proceed properly. As such changes in the fluidity level of the entire eye disc tissue are critical and when this is outside the correct range it is deleterious to the process. Regulation of the IC environmental fluidity is a parsimonious way to provide robustness to the development of the eye as a whole: over 700 identical units undergo the same 90° rotation step and regulation of the IC environment is one way to ensure uniformity in morphology and functionality.

In contrast to previous studies investigating the relationship between E-cad and the actomyosin cytoskeleton showing that mechanical forces affect junctional dynamics\textsuperscript{57–69}, our data indicate that the control of junctional E-cad levels and dynamics can directly modulate tissue tension, viscoelasticity, and stress, thus affecting tissue fluidity and cell motility. Although we cannot exclude regulation of the actomyosin cytoskeleton by Nmo through its phosphorylation of E-cad/\beta-catenin complexes, the largely normal apical appearance of R-cells within the cluster, which is in contrast to direct effects on the actomyosin cytoskeletal regulation causing severe disruption to the R-cell clusters\textsuperscript{50}, suggests that it is indirect at best; addressing this question here is
difficult due to the inherent spatial and temporal resolution requirements imposed by the tissue (small cell size, and long-timescale), which prohibit effective monitoring of the rapid cytoskeletal dynamics. Moreover, manipulating actin or myosin function directly alters the gross morphology of the clusters, precluding accurate rotation analysis of mutant phenotypes. Our data support a model of linear coupling between E-cad and actomyosin that could explain the change in the mechanical properties resulting from the modulation of E-cad levels at the junction: the more stable E-cad at the junction the more the tension generated by the cytoskeleton can be transmitted across the tissue, which results in a more solid-like, jammed tissue behavior.

Junctional dynamics have been well studied in the convergent extension model of germ band elongation in *Drosophila* embryos, as the A-P axis lengthens. Here, actomyosin force-generating...
Fig. 5 Nmo controls E-cad recycling and affects junctional E-cad steady state levels. a, b Mosaic 22 h after puparium formation pupal wings (fixed tissue) with either nmo

 networks undergo periodic contractility and direct intercalary movements. Although similar to the pulsatile contraction and rotation we see in ommatidia, the timescales are different: of the order of seconds in the contractility of single cells, whereas each pulse is several minutes in the context of whole ommatidia. However, the phenotype observed in the Nmo GOF, of apical constriction followed by periods of relaxation, strikingly resembles that of Rab35 LOF during germ band extension; apical area oscillations do occur and contractile steps are functioning, but there is a reversal of movement and junctions ultimately fail to shorten Rab35-dependent ratchet-like behavior also occurs during mesoderm invagination, where Rab35 is planar polarized and dynamic, and is enriched at the interfaces of intercalating cells. Rab35 functions as an endocytic hub, and has been suggested to direct E-cad complexes for endocytosis and allow junctional remodeling. Regulation of tissue fluidity has also been shown to be important during germ band extension and mesoderm invagination (see below). Our combined studies (here and ref. 33) on the role of Nmo kinase in phosphorylating the E-cad/β-catenin complex also closely mirror the work of Tamada and colleagues in showing that Abl kinase phosphorlates β-catenin during axis elongation in the *Drosophila* embryo. More recent work has shown that convergent extension movements in *Xenopus* require local heterogeneity in junctional mechanics at the level of individual junctions. Local stiffness at vertices dictates shortening and fluid-like directed motion on a sub-cellular scale. Cadherin-cis-interactions determine local junctional stiffness and impact on vertex activity. Cadherin3 clusters undergo dynamic fluctuations in size and these temporally correlate with, and precede, pulsatile junctional shortening. It will be interesting therefore to determine how Nmo impacts E-cad clustering at the molecular level.

AJ stability and dynamics are required for junctional remodeling and cell shape changes, cell processes that are central to tissue fluidity during morphogenesis. Our observations support the hypothesis that a more fluid-like tissue, i.e., permissive, would allow a higher degree of rotation due to a decrease in resistance between the cluster and ICs, whereas a more rigid tissue would decelerate, if not block, the rotation process as the forces between the cluster and the ICs cells increase. Further, our data suggest that changes in Nmo levels directly affect AJs downstream of PCP (this work, and ref. 33), thereby controlling tissue fluidity and acting as a catalyst for a transition from a solid-like, jammed tissue (in the absence of Nmo) to a fluid-like environment (increased Nmo levels). The contrast between the solid-like and fluid tissues explains the differences in neighbor exchange and cell caging dynamics, as well as onset of cell delamination (Supplementary Fig. 5): increased junctional remodeling facilitates each of these processes.

Tissue jamming has been defined as a divergence of the viscosity of a tissue with step changes in tissue fluidity, with behavior changing among amorphous solid-like and fluid-like states. The phenomenon of tissue jamming has been well documented in tissue culture models of various epithelial cell types, particularly from human bronchial epithelial cells (HBECs). In culture, HBECs behave as a fluid at first and become more like an amorphous glassy solid over time due to cell adhesion and internal friction. Cells become caged whereby they can hardly move beyond their size and display decreased velocity, due in part to increased E-cad at AJs. Tissue jamming can be described as a phase transition and it also occurs in reverse, which has been described as “reawakening.” Overexpression of the key endocytic regulator RAB5A is sufficient to induce an unjamming transition, with increase in locomotion of cells and shape index. Similarly to our results with Nmo, RAB5A overexpression does not affect actomyosin, rather it regulates the dynamic-dependent trafficking of E-cad. RAB5A has a similar effect during wound closure and zebrafish gastrulation (see also below). Using HBECs, others have shown that the unjamming transition is molecularly distinct from epithelial-mesenchymal transition, which underlies other instances of increased cell motility. HBECs underwent a change in appearance from a cobblestone-like pattern of confluent cells to more elongated and variable cell shape, similar to the change in cell shape we observed between the *nmo* LOF and Nmo GOF. The dynamics and structural signatures of the unjamming transition further resemble the Nmo GOF with increased motility, cell elongation, intact junctional integrity and epithelial A/B polarity markers, and increased stochastic but cooperative motion. The link between jamming and geometry transcends systems—from HBECs to gastrulation in the *Drosophila* embryo, and from healthy homeostasis to airway disease. Moreover, shape index and cell packing can be used as a predictor of when tissue will undergo fluidization, as shown in convergent extension and
mesoderm invagination in Drosophila. During anterior-posterior axis elongation in zebrafish, posterior tissues undergo a jamming transition: they are fluid-like at the extending end and become more solid in the presomitic mesoderm to support the extending tissue. Actively-generated stress fluctuates, and this variability helps to fluidize the tissue at the posterior. Temporal and spatial regulation of jamming transitions are therefore critical to development and homeostasis; it has been proposed that organisms have evolved to occupy space around such transitions, which allow functional changes in tissue fluidity with relatively little effective energy expended. Here we identify the developing Drosophila eye tissue as occupying a similar space, with changes in Nmo levels regulating the balance around the transition point. At a phenomenological level, modeling based on shape index and cell packing is able to make accurate predictions about the level of tissue fluidity. However, discovering the biological underpinning of this relationship, will require experimental manipulations of fluidity and thus Nmo provides a genetic tool to study the mechanics of tissues during morphogenesis in vivo.

Our data combined with previous observations suggest a multi-tiered requirement of Nmo in the rotation process. The heterogeneous localization of core PCP factors within the cluster,
with Prickle and VanGogh localizing to the R4 side of the R3/R4 junction with their enrichment in R4 and their junctional recruitment of Nmo (ref. 3)). Confluent Z series of 10–12 planes spanning 7–8 µm at the level of adherens junctions were acquired, focused at z+3 along the MZ at intervals of 0.8 µm. Deconvolution for 4–6 h was combined for analysis. In all movies, laser power and offset conditions were optimized to get the best signal while minimizing photobleaching.

Image analysis and quantifications. For the analysis of live microscopy movies shown in Figs. 1–4 and Supplementary Figs. 5, 6, images were first processed using a custom-written macro in Fiji to correct nonlinear Y:Z drift. On average, individual ommatidial pre-clusters, including their surrounding neighbor cells, were used to select the first five best focus planes for a maximum-intensity projection and, if needed, further drift corrections were applied. These crops were chosen such that ommatidial clusters analyzed are far from each other and have no overlap. Therefore, measured events for each ommatidium are considered independent, both biologically and statistically. Drift-corrected movies were then segmented using the Fiji plugin, Tissue Analyzer

Methods

Fly strains, genetics, and histology. E-cad::GFPK1 was a knock in line generated by homologous recombination. Both, the hypomorphic allele (nmo(0)) and the null allele (nmoDB) were characterized in ref. 3). All live imaging/movies representing these mutant genotypes(s) were generated with nmoDB/+/trans-allelic viable combination.

To generate the clonal tissue for nmoDB mutation the following strains were used:

Hs-FPL112, Ecad::GFPK1, His2Av mRFP,FRT79D/TM6 and Ecad::GFPK1, nmoDB;FRT79D/TM5a;TM6b. F1 generation was heat shocked at 35 °C for 60 min on days 2 and 3 after egg laying.

Image analysis and quantification. For the analysis of live microscopy movies shown in Figs. 1–4 and Supplementary Figs. 5, 6, images were first processed using a custom-written macro in Fiji to correct nonlinear Y:Z drift. On average, individual ommatidial pre-clusters, including their surrounding neighbor cells, were used to select the first five best focus planes for a maximum-intensity projection and, if needed, further drift corrections were applied. These crops were chosen such that ommatidial clusters analyzed are far from each other and have no overlap. Therefore, measured events for each ommatidium are considered independent, both biologically and statistically. Drift-corrected movies were then segmented using the Fiji plugin, Tissue Analyzer

Live imaging. Pupae were prepared as described in Supplementary Fig. 1 and imaged at room temperature (–22 °C) using a confocal scanning laser microscope, Zeiss LSM 780, 63X/1.4 oil-immersion objective with zoom x3. Confocal Z series of 10–12 planes spanning 7–8 µm at the level of adherens junctions were acquired, focused at z+3 along the MZ at intervals of 0.8 µm. Deconvolution for 4–6 h was combined for analysis. In all movies, laser power and offset conditions were optimized to get the best signal while minimizing photobleaching.

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of bleaching. The normalized intensity time traces were fitted, using MATLAB, to a single exponential as follows:

$$I(t) = A(1 - e^{-\lambda t})$$  \hspace{1cm} (2)

where A is the mobile fraction, t is the time after bleaching and k is a fit parameter. The recovery rate is calculated as $A \times k$, and the half recovery time, $T_{1/2}$, was calculated as follows:

$$T_{1/2} = -\ln(0.5)/k$$  \hspace{1cm} (3)

**Immunofluorescence microscopy on fixed tissues.** The primary antibodies used in this study from the Developmental Studies Hybridoma Bank: mouse anti-Elav antibody (Elav 9F8A9, 1:30), rat anti-acman (DCAD2, 1:20), mouse anti-Fmi antibody (APA1, 1:23), rabbit anti-Patj (1:1000) was a gift from Hugo Bellen. Secondary antibodies are from Jackson Immuno Research Laboratories and used at 1:1000. Nuclear stain via Hoechst 33342 (10 mg/ml, 1:1000). Confocal images of fixed tissue were acquired on a Zeiss LSM 880 at the Microscopy CoRE of the Ichan School of Medicine at Mount Sinai.

**Laser ablation.** Pups for laser ablation were prepared as for live imaging. For ablation of the adherens junctions, a custom-build system with a femtosecond near-infrared Ti:sapphire pulsed laser (Mai-Tai, Spectra-Physics, Mountain View, CA) combined with a focussed tissue were acquired on a Zeiss LSM 880 at the Microscopy CoRE of the Ichan School of Medicine at Mount Sinai.

**Statistical methods.** Statistical analyses to determine p-values were the two-tailed Student t-test for data in panels Figs. 3c, f, j, 3b–d, g–i, 3c, h, i and Supplementary Fig. 7E. To calculate the p-value for data displayed in panels Fig. 5k, and Supplementary Fig. 7B, c, we used the Two-sample Kolmogorov–Smirnov test.

Please note that the statistical analyses in most cases compare the distribution of values, not the averages, and as such the n values are either individual ommatidia, cells, or cellular junctions, consistent with many previous studies. For example, in the context of a distribution analysis, the n number is the number of individuals, either ommatidia or single cells in our case, depending on the measured parameter. Each ommatidium or cluster is independent from its neighbors in its phenotypic decision making in the PCP and ommatidial rotation context, as such the n value is the number of individual junctions analyzed in that context (and not the number of individual cells). It is not the average across animals that matters but the distribution of phenotypic values of individual ommatidia between genotypes. Similarly, when analyzing cellular behavior and cell shape, individual cells are the n values, as was described and used many times in the elegant and groundbreaking work of the Eaton and Julicher labs.

**Data availability**

The data generated in this study have been deposited in the Figshare data repository under the following URL: https://figshare.com/articles/dataset/Data_Source/16866450. This URL is freely accessible with no restrictions. Source data are provided with this paper. The source data of experiments presented in this paper are organized in figure panel-specific Excel sheets and available at the above URL. Source data are provided with this paper.

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**References**

1. Tada, M. & Heisenberg, C. P. Convergent extension: using collective cell migration and cell intercalation to shape embryos. Development 139, 3897–3904 (2012).

2. Heisenberg, C. P. & Bellaiche, Y. Forces in tissue morphogenesis and patterning. Cell 153, 948–962 (2013).

3. Tetley, R. J. & Mao, Y. The same but different: cell intercalation as a driver of tissue deformation and fluidity. Philos. Trans. R. Soc. Lond. B Biol. Sci. https://doi.org/10.1098/rstb.2017.0328 (2018).

4. Cavey, M. & Lecuit, T. Molecular bases of cell–cell junctions stability and dynamics. Cold Spring Harb. Perspect. Biol. 1, a002998 (2009).

5. Ishihara, T. & Takeichi, M. Remodeling of the adherens junctions during morphogenesis. Curr. Top. Dev. Biol. 89, 33–54 (2009).

6. Buckley, C. D. et al. Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. Science 346, 1254211 (2014).

7. Duc, Q. et al. Vinculin potentiates E-cadherin mechano sensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. J. Cell Biol. 199, 1107–1115 (2011).

8. Halbheie, J. M. & Nelson, W. J. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. Genes Dev. 20, 3199–3214 (2006).

9. Yap, A. S., Gomez, G. A. & Parton, R. G. Adherens junctions revisualized: organizing cadherins as nanoassemblies. Dev. Cell 35, 12–20 (2015).

10. Wirtz-Peitz, F. & Zallen, J. A. Junctional trafficking and epithelial morphogenesis. Curr. Opin. Genet. Dev. 19, 350–356 (2009).

11. Iyer, K. V., Piscitello-Gomez, R., Paijmans, J., Julicher, F. & Eaton, S. Epithelial viscoelasticity is regulated by mechanosensitive E-cadherin turnover. Curr. Biol. 29, 578–591 e575 (2019).

12. Mongera, A. et al. A fluid-to-solid jamming transition underlies vertebrate kidney elongation. Nature 543, 540–543 (2017).

13. Dahmann, C. & Classen, A. K. Charting the unknown currents of cellular flows and forces. Development https://doi.org/10.1242/dev.186403 (2020).

14. Herrera-Perez, R. M. & Kasza, K. E. Biophysical control of the cell rearrangements and cell shape changes that build epithelial tissues. Curr. Opin. Genet. Dev. 51, 88–95 (2018).

15. Bozwell, F. et al. Mechanism of morphogenesis by Fat/Dachsous/Four-jointed planar cell polarity pathway. Science 336, 724–727 (2012).

16. Mlodzik, M. Planar polarity in the Drosophila eye: a multifaceted view of signaling specificity and cross-talk. EMBO J. 18, 6873–6879 (1999).

17. Jenny, A. Planar cell polarity signaling in the Drosophila eye. Curr. Top. Dev. Biol. 93, 189–227 (2010).

18. Claudini, T. R. & Zipursky, S. L. Making connections in the fly visual system. Nature 389, 86–872 (2001).

19. Lee, R. C. et al. The protocadherin Flamingo is required for axon target selection in the Drosophila visual system. Nat. Neurosci. 6, 557–563 (2003).

20. Mirkovic, I. & Mlodzik, M. Cooperative activities of drosophila DE-cadherin and DN-cadherin regulate the cell motility process of ommatidial rotation. Development 133, 3283–3293 (2006).

21. Das, G., Reynolds-Kenncally, J. & Mlodzik, M. The atypical cadherin flamingo links frizzled and notch signaling in planar polarity establishment in the Drosophila eye. Dev. Cell 2, 656–666 (2002).

22. Ho, Y. H. et al. Echinoid regulates Flamingo endocytosis to control ommatidial rotation in the Drosophila eye. Development 137, 745–754 (2010).

23. Fetting, J. L., Spencer, S. A. & Wolff, T. The cell adhesion molecules Echinoid and Friend of Echinoid coordinate cell adhesion and cell signaling to regulate the fidelim of ommatidial rotation in the Drosophila eye. Development 136, 3323–3333 (2009).

24. Thuysen, M. et al. Integrins are required for synchronous ommatidial rotation in the Drosophila eye linking planar cell polarity signaling to the extracellular matrix. Open Biol. 9, 190148 (2019).

25. Seijert, J. R. & Mlodzik, M. Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat. Rev. 8, 126–138 (2007).

26. Gaengel, K. & Mlodzik, M. Efg signaling regulates ommatidial rotation and cell motility in the Drosophila eye via MAPK/Pnt signaling and the Ras effector Canoe/Af6. Development 130, 5413–5423 (2003).

27. Brown, K. E. & Freeman, M. Efg signalling defines a protective function for ommatidial orientation in the Drosophila eye. Development 130, 5401–5412 (2003).

28. Weber, U., Pataki, C., Mihaly, J. & Mlodzik, M. Combinatorial signaling by the Frizzled/PCP and Efg pathways during planar cell polarity establishment in the Drosophila eye. Dev. Biol. 316, 110–123 (2008).

29. Wolff, T. & Rubin, G. M. strabismus, a novel gene that regulates tissue polarity and cell fate decisions in Drosophila. Development 125, 1149–1159 (1998).

30. Koca, Y., Housden, B. E., Gault, W. J., Bray, S. J. & Mlodzik, M. Notch signaling coordinates ommatidial rotation in the Drosophila eye via transcriptional regulation of the EGF-Receptor ligand Argos. Sci. Rep. 9, 18628 (2019).

31. Fehler, R. W. & Wolff, T. Nemo is required in a subset of photoreceptors regulating the speed of ommatidial rotation. Dev. Biol. 313, 533–544 (2008).

32. Choi, K.-W. & Benzer, S. Rotation of photoreceptor clusters in the developing Drosophila eye requires the nemo gene. Cell 78, 125–136 (1994).
33. Mirkovic, I. et al. Nemo kinase phosphorylates beta-catenin to promote ommatidial rotation and connect core PCP factors to E-cadherin-beta-catenin. Nat. Struct. Mol. Biol. 18, 132–142 (2011).
34. Munoz-Soriano, V., Ruiz, C., Perez-Alonso, M., Mlodzik, M. & Paricio, N. Nemo regulates cell dynamics and represses the expression of mple, a midline/pleiotrophin cytokine, during ommatidial rotation. Dev. Biol. 377, 113–125 (2013).
35. Czaplinski, J., Beretta, C. A., Berns, N. & Riechmann, V. Three mechanisms control E-cadherin localization to the zonula adherens. Nat. Commun. 7, 10834 (2016).
36. Pacquelet, A. & Rorth, P. Regulatory mechanisms required for DE-cadherin function in cell migration and other types of adhesion. J. Cell Biol. 170, 903–812 (2005).
37. Larson, D. E., Liberman, Z. & Cagan, R. L. Cellular behavior in the developing Drosophila pupal retina. Mech. Dev. 125, 223–232 (2008).
38. Petridou, N. I. & Heisenberg, C. P. Tissue rheology in embryonic organization. EMBO J. 38, e102497 (2019).
39. Sun, Z. et al. Basolateral positioning and apical contraction cooperatively drive Drosophila germ-bund extension. Nat. Cell Biol. 19, 375–383 (2017).
40. Levery, R., Pelissier-Monier, A. & Leclut, T. Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. Nat. Cell Biol. 13, 529–540 (2011).
41. Engl, W., Arasi, B., Yap, L. L., Thiery, J. P. & Vianvo, V. Actin dynamics modulate mechanosensitive immobilization of E-cadherin at adherens junctions. Nat. Cell Biol. 16, 587–594 (2014).
42. Chu, Y. S. et al. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. J. Cell Biol. 167, 1183–1194 (2004).
43. Yamada, M., Farrell, D. L. & Zallen, J. A. Abh regulates planar polarized functional dynamics through beta-catenin tyrosine phosphorylation. Dev. Cell 22, 309–319 (2012).
44. Hueber, R. N., Malini-Kakkada, A. N., Sarkaya, S., Weng, S., Thirumalai, D. & Wallingford, J. B. Mechanical heterogeneity along single cell-cell junctions is driven by lateral clustering of cadherins during vertebrate axis elongation. Elife 10, e65390 (2021).
45. Lecut, T. & Yap, A. S. E-cadherin junctions as active mechanical integrators in tissue dynamics. Nat. Cell Biol. 17, 533–539 (2015).
46. Oswald, L., Grosser, S., Smith, D. M. & Kas, J. A. Jamming transitions in cancer. J. Phys. D. Appl. Phys. 50, 483001 (2017).
47. Malinverno, C. et al. Endocytic reactivation of motility in jammed epithelia. Nat. Mater. 16, 587–596 (2017).
48. Nishimura, T., Honda, H. & Takeichi, M. Planar cell polarity links axes of spatial dynamics in neural-tube closure. Cell 149, 1084–1097 (2012).
49. Thorpe, C. J. & Moon, R. T. nemo-like kinase is an essential co-activator of Wnt signaling during early zebrafish development. Development 131, 2099–2009 (2004).
50. Ulrich, F. et al. Wnt11 functions in gastrulation by controlling cell cohesion during Rab5c and E-cadherin. Dev. Cell 9, 555–564 (2005).
51. Levery, R. & Leclut, T. Oscillation and polarity of E-cadherin asymmetries control actomyosin flow patterns during morphogenesis. Dev. Cell 26, 162–175 (2013).
52. Warrington, S. J., Strutt, H. & Strutt, D. The frizzled-dependent planar polarity pathway locally promotes E-cadherin turnover via recruitment of RhoGEF2. Development 140, 1045–1054 (2013).
53. Lv, M. et al. Lentivirus-mediated knockdown of NLK inhibits small-cell lung cancer growth and metastasis. Drug Des. Dev. Ther. 10, 3737–3746 (2016).
54. Sun, Z. et al. NLK is a key regulator of proliferation and migration in gallbladder carcinoma cells. Mol. Cell Biochem. 369, 27–32 (2013).
55. Tetley, R. J. et al. Tissue fluidity promotes epithelial wound healing. Nat. Phys. 15, 1195–1203 (2019).
56. Huang, J., Zhou, W., Dong, W., Watson, A. M. & Hong, Y. From the cover: directed, efficient, and versatile modifications of the Drosophila genome by genomic engineering. Proc. Natl Acad. Sci. USA 106, 8284–8289 (2009).
57. Lye, C. M., Naylor, H. W. & Sanson, B. Subcellular localisations of the CPTI midkine/pleiotrophin cytokine, during ommatidial rotation. Development 208, 270–279 (2009).
58. Mitchel, J. A. et al. In primary airway epithelial cells, the unjamming transition up. We thank the Bloomington Stock Center, Michael Welte, and Esther Verheyen for part supported by the Tisch Cancer Institute P30 CA196521 grant from the NCI.
Author contributions
N.F., R.F. and M.M. conceived and designed the study, also based on discussions with GMC. N.F., R.F., G.M.C. and U.W. performed experiments and/or analyzed the experimental data. N.F., R.F., G.M.C. and M.M. analyzed and interpreted the data and wrote the manuscript. M.J.S. and M.M. provided funding and oversight for the project.

Competing interests
The authors declare no competing interests.

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