SI Materials and Methods

Fly Stocks and Genetics. Embryos were generated at 23°C and analyzed at room temperature. Wild-type control embryos were *yw* with one maternal copy of a sqh-gap43:mCherry transgene to label cell membranes (1). *snail twist* embryos were zygotic *snail*^{IIG05} *twist*^{DfS60} mutants and expressed Spider:GFP to visualize cell outlines (2, 3). The *bcd nos tsl (bnt)* maternal mutant embryos were the progeny of *bcd*^{E1} *nos*^{L7} *tsl*¹⁴⁶ homozygous females and expressed Resille:GFP to visualize cell outlines.

Time-Lapse Imaging. Embryos aged 2-4 hours were dechorionated for 2 min in 50% (vol/vol) bleach, washed in distilled water, and mounted in halocarbon oil 27 and 700, 1:1 (Sigma) between a coverslip and an oxygen-permeable membrane (YSI). Embryos were oriented with the cephalic furrow and ventral furrow just visible at the edges of the field of view (*SI Appendix*, Fig. S1). A 212 μ m x 159 μ m ventrolateral region of the embryo was imaged on a Zeiss LSM880 laser scanning confocal microscope with a 40X/1.2 NA water-immersion objective. Z-stacks were acquired at 1- μ m steps and 15-s time intervals. Maximum intensity z-projections of 3 μ m in the apical junctional plane were analyzed.

Tissue Elongation Measurement. Tissue elongation was measured by particle image velocimetry (PIV) using PIVlab in MATLAB (4). Each image was divided into 2-pass Fast-Fourier-Transform windows (120×120 pixels) with 50% overlaps. A displacement vector field for each window and each time point was determined by cross-correlating each window in the current time point and the image in the next time point. Tissue length change was measured by quantifying the cumulative sum of the anterior-directed displacement at the anterior end of the germband and the posterior-directed displacement at the posterior end of the germband. The onset of tissue elongation (t = 0) was the time point when the derivative of the tissue elongation curve intersects zero.

Automated Image Segmentation and Cell Rearrangement Analysis. Time-lapse movies were projected and despeckled using ImageJ. Processed movies were segmented and computationally analyzed using the MATLAB based software SEGGA, and errors were corrected manually with the interactive user interface (3). Cells were tracked and analyzed between t = -10 min and t = 30 min for each movie. To be included in cell rearrangement analysis, cells must be in the region of interest for at least 5 minutes after t = 0. The cell rearrangement rate shown is a uniformly weighted average over 1.5 minutes.

Cell Shape Index and Cell Shape Alignment Analysis. Based on the cell segmentation data, we computed the average cell shape index \bar{p} by quantifying for each segmented cell both the perimeter *P* and area *A* of the polygon defined by the cell vertices (i.e. the points where at least 3 cells meet). The average cell shape index \bar{p} at a time point is the average of P/\sqrt{A} over all segmented cells.

Cell shape alignment Q was quantified using the triangle method following Ref. (5). A triangular tiling was created based on the barycenters of the cellular polygons, where each vertex of the cellular network gives rise to a triangle whose corners are defined by the barycenters of the three abutting cells. In the case of a manyfold vertex, i.e. a vertex abutting M > 3 cells, M

triangles are created, where each triangle has one corner defined as the average position of the barycenters of all M abutting cells and the other two corners are the barycenters of two adjacent cells. For each triangle, we computed a symmetric, traceless tensor q quantifying triangle elongation. To compute the tensor q for a given triangle m, we first define a shape tensor s that corresponds to the affine deformation transforming an equilateral reference triangle into the observed triangle m. With the corners of the triangle being at positions r^A , r^B , r^C in counter-clockwise order, the shape tensor s can be computed as follows:

$$\mathbf{s} = \begin{pmatrix} r_x^B - r_x^A & r_x^C - r_x^A \\ r_y^B - r_y^A & r_y^C - r_y^A \end{pmatrix} \cdot \begin{pmatrix} 1 & 1/2 \\ 0 & \sqrt{3}/2 \end{pmatrix}^{-1} .$$
(S1)

From the triangle shape tensor, the triangle elongation tensor q is extracted, which characterizes the anisotropic component of the deformation characterized by s. It is computed by first splitting s into trace part t, symmetric, traceless part \tilde{s} , and antisymmetric part s^a :

$$\boldsymbol{s} = \boldsymbol{t} + \tilde{\boldsymbol{s}} + \boldsymbol{s}^a. \tag{S2}$$

Then first a triangle rotation angle θ is extracted such that:

$$\binom{\cos\theta}{\sin\theta} = a \binom{t_{xx}}{s_{yx}^a} \tag{S3}$$

with some prefactor *a*. In practice, θ can be extracted using the "arctan2" function that exists in many programming languages as $\theta = \arctan(s_{yx}^a, t_{xx})$. Finally, the triangle elongation tensor q is computed as:

$$\boldsymbol{q} = \frac{1}{|\tilde{\boldsymbol{s}}|} \operatorname{arcsinh}\left(\frac{|\tilde{\boldsymbol{s}}|}{(\det \boldsymbol{s})^{1/2}}\right) \, \tilde{\boldsymbol{s}} \cdot \boldsymbol{R}(-\theta),\tag{S4}$$

where $|\tilde{s}| = [s_{xx}^2 + s_{xy}^2]^{1/2}$ is the magnitude of the symmetric, traceless tensor \tilde{s} , det s is the determinant of the shape tensor s, and $R(-\theta)$ is a clockwise rotation by angle θ :

$$\boldsymbol{R}(-\theta) = \begin{pmatrix} \cos\theta & \sin\theta\\ -\sin\theta & \cos\theta \end{pmatrix}.$$
 (S5)

The cell shape alignment tensor

$$\boldsymbol{Q} = \begin{pmatrix} Q_{xx} & Q_{xy} \\ Q_{xy} & -Q_{xx} \end{pmatrix}$$
(S6)

is then the average of the symmetric, traceless elongation tensors q of all triangles:

$$\boldsymbol{Q} = \langle \boldsymbol{q} \rangle. \tag{S7}$$

The average is an area-weighted average $\langle \boldsymbol{q} \rangle := (\sum_m a_m \boldsymbol{q}_m)/(\sum_m a_m)$, where the sums are over all triangles *m* with area a_m and elongation tensor \boldsymbol{q}_m . The cell shape alignment parameter *Q* in the main text is the magnitude of this tensor defined by $Q = \left[Q_{xx}^2 + Q_{xy}^2\right]^{1/2}$.

Our cell shape alignment parameter Q combines information about both cell shape anisotropy and cell shape alignment. It can be split accordingly into a product:

$$Q = |\langle \boldsymbol{q} \rangle| = Q_s Q_a. \tag{S8}$$

The first factor $Q_s = \langle |\mathbf{q}| \rangle$ is the average magnitude of triangle anisotropy, which is a proxy for cell shape anisotropy, and the second factor $Q_a = \left| \langle \frac{q}{|\mathbf{q}|} \frac{|\mathbf{q}|}{\langle |\mathbf{q}| \rangle} \rangle \right|$ is the norm of the average triangle elongation axis $\mathbf{q}/|\mathbf{q}|$ weighted by the norm of \mathbf{q} . This second factor thus corresponds to an alignment separate from cell shape, which similarly to a nematic order parameter varies between zero (random shape orientation) and one (perfectly aligned shapes).

Vertex Model. Our vertex model describes an epithelial tissue as a planar tiling of N cellular polygons, where the degrees of freedom are the vertex positions $r_{k\alpha}$ (6). We use Latin indices starting with k to refer to vertices and Greek indices starting with α to refer to spatial dimensions. Forces are defined such that cell perimeters and areas act as effective springs with a preferred perimeter p_0 and a preferred area of one. This is implemented via the following effective energy functional, which in dimensionless form is (7):

$$E = \sum_{i=1}^{N} [(p_i - p_0)^2 + k_A (a_i - 1)^2]$$
(S9)

Here, the sum is over all cells *i*, with perimeter p_i and area a_i . The parameter k_A is a dimensionless number comparing area and perimeter rigidity. We use periodic boundary conditions with box size $L_x \times L_y$ such that the average cell number density is one: $L_x L_y = N$. The boundary conditions can accommodate a skew (as in Lees-Edwards boundary conditions) with a corresponding simple shear γ . Hence, the system energy is a function of all vertex positions and the periodic box parameters: $E = E(\{r_{k\alpha}\}, L_x, L_y, \gamma)$. We focus on stable, force-balanced states of the system, which corresponds to local minima of E. To numerically find such states, we use the BFGS2 multidimensional minimization routine of the Gnu scientific library (GPL) with a cutoff on the average residual force of 10^{-6} . We allow for manyfold vertices – i.e. vertices are allowed to be in contact with more than three cells at once. During the minimization, the vertices belonging to an edge are fused to a single vertex whenever the edge length is below a cutoff of 10^{-3} , and a vertex with at least four edges attached to it splits into several vertices can change the transition point in vertex models (8), we checked that the energy-minimized states we obtained rarely contained any manyfold vertices. In all simulations, we have N = 512 cells and $k_A = 1$.

For a given local energy minimum, we compute the simple shear modulus G as described in (9):

$$G = \frac{1}{N} \left(\frac{\partial^2 E}{\partial \gamma^2} - \sum_m \frac{1}{\omega_m^2} \left[\sum_{k,\alpha} \frac{\partial^2 E}{\partial \gamma \partial r_{k\alpha}} u_{k\alpha}^m \right]^2 \right)$$
(S10)

In the second term, the outer sum is over all positive eigenvalues ω_m^2 and the corresponding eigenvectors $u_{k\alpha}^m$ of the Hessian matrix $(\partial^2 E/\partial r_{k\alpha} \partial r_{l\beta})$. In practice, we include all eigenvalues smaller than 10^{-14} in the sum. The inner sum in the second term is over all vertices and both spatial dimensions.

Anisotropic vertex model. In all our simulations, we initialize the system with the Voronoi tessellation of a uniformly random point pattern on a squared domain $(L_x = L_y = L_0)$. For the first set of simulations of anisotropic tissue (Fig. 3D), we apply an external pure shear strain ε by setting $L_x = e^{\varepsilon}L_0$ and $L_y = e^{-\varepsilon}L_0$. We start with $\varepsilon = 0$ and increase in steps of 0.02 up to a value of $\varepsilon = 2$, minimizing the energy after each step. For these minimizations, we vary all vertex positions, keep the box dimensions fixed, but also allow the simple shear variable γ to vary (shear-stabilized

minimization). We follow this protocol for different values of p_0 , which we varied between 3.5 and 4.5 in steps of 0.01. For each value of p_0 we carry out 100 separate simulation runs.

For a second set of simulations of an anisotropic tissue (Fig. 3*E*, *inset*), we model the anisotropic myosin distribution in the germband by introducing an additional anisotropic line tension with amplitude λ_0 into the effective energy functional:

$$E = \sum_{i=1}^{N} [(p_i - p_0)^2 + k_A (a_i - 1)^2] + \sum_{\langle k, l \rangle} \lambda_{\langle k, l \rangle} \ell_{\langle k, l \rangle}$$
(S11)

While the first sum is the same as in Eq. (S9), we have added a second sum, which is over all edges in the system, connecting two vertices k, l. Here, $\ell_{\langle k, l \rangle}$ denotes the length the edge $\langle k, l \rangle$, and $\lambda_{\langle k, l \rangle}$ is a line tension associated with this edge. Before each minimization, we define each of these line tensions based on the respective edge angle $\theta_{\langle k, l \rangle}$ as follows:

$$\lambda_{\langle k,l \rangle} = \lambda_0 \cos(2[\theta_{\langle k,l \rangle} - \phi]) \tag{S12}$$

Thus, the line tension will be increased by λ_0 for edges parallel to lines with angle ϕ and decreased by λ_0 for edges perpendicular to that.

During each minimization run, we vary all vertex positions and the pure shear strain $\varepsilon = 1/2 \log(L_x/L_y)$, but keep the system area $L_x L_y$ and the simple shear strain γ fixed. While we set $\lambda_{\langle k,l \rangle}$ before a minimization run and keep it constant during the minimization, the angle $\theta_{\langle k,l \rangle}$ usually changes during the minimization as the vertex positions are varied. As a consequence, the state obtained after the minimization will not correspond to an energy minimum anymore once we update the line tensions $\lambda_{\langle k,l \rangle}$ with the new angles $\theta_{\langle k,l \rangle}$. Thus, to identify a force-balanced state where the line tensions are consistent with the directions of the cell edges, we iterate over several minimizations, where after each minimization we update $\lambda_{\langle k,l \rangle}$ based on the latest angles $\theta_{\langle k,l \rangle}$. We stop these iterations once the states do not significantly change anymore, or more precisely, when the average residual stress per degree of freedom *before* a minimization, but with the *new* $\lambda_{\langle k,l \rangle}$ is smaller than 2×10^{-6} . We intentionally *do not* include the explicit dependency of $\lambda_{\langle k,l \rangle}$ on $\theta_{\langle k,l \rangle}$ and thus the vertex positions in our energy minimizations, because this would create additional torques in our model, while here we merely want to study the effect of an anisotropic distribution of line tensions as provided for instance by an anisotropic myosin distribution.

We set the direction of line tension anisotropy parallel to the y axis, i.e. $\phi = \pi/2$. For Fig. 3*E*, we varied the magnitude of line tension anisotropy λ_0 between zero and one in steps of 0.1, and for Fig. 3*E* inset, we varied it in steps of 0.01. Again, the preferred perimeter p_0 is varied between 3.5 and 4.5 in steps of 0.01, where for each value of p_0 we run 100 separate simulations.

We found many states where the system flowed during a minimization until ε was so large that the system was only one cell thick in the y direction. In particular, this was the case in what was otherwise expected to be the floppy regime. This will probably not only occur in the floppy regime, but also in the solid regime whenever the anisotropic stress created by the line tension anisotropy is large enough to overcome the yield stress, which perhaps explains why there is a gap between mechanically stable solid states and the black line in Fig. 3*E* inset. Because we did not obtain any force-balanced state of bulk vertex model tissue in this regime, we have no way to determine from our simulations neither the shear modulus, nor the morphological quantities \bar{p} and Q, and thus this regime does not appear in Fig. 3E inset. To access this regime, one needs to include dynamics into the model, e.g. including a viscosity or a substrate friction.

In order to prevent the system from flowing indefinitely, we also ran a third set of simulations (Fig. 3*E*), where we combined anisotropic line tensions with a fixed system size. In other words, we ran simulations like the second set where we now fixed also the pure shear strain ε , which we successively increased and each time looked for a force-balanced state as described for the second simulation set. While our findings are consistent with our results from the other two simulation sets, we again do not obtain any fluid states for any nonzero value of the line tension anisotropy λ_0 , because the iterative procedure involving updating line tensions and minimizing the energy described above does not converge in this regime. To some extent this can even be understood analytically. We start from a fix point $(r^*, \theta^*) = (\{\vec{r}_k^*\}, \{\theta_{\langle k, l \rangle}\})$ of the iterative procedure described above, where $\partial E/\partial r(r^*, \theta^*) = 0$ and $\theta^* = \theta(r^*)$. A small deviation δr_1 from this state in the vertex positions leads to a change in the bond angles of $\delta \theta_1 = d\theta(r^*)/dr \cdot \delta r_1$. This leads in turn to an energy minimized state at vertex positions $r_2 = r^* + \delta r_2$ with

$$0 = \frac{\partial E(r^* + \delta r_2, \theta^* + \delta \theta_1)}{\partial r} = \frac{\partial^2 E(r^*, \theta^*)}{\partial r^2} \cdot \delta r_2 + \frac{\partial^2 E(r^*, \theta^*)}{\partial r \partial \theta} \cdot \frac{\partial \theta(r^*)}{\partial r} \cdot \delta r_1.$$
(S13)

Here, $\partial^2 E(\mathbf{r}^*, \boldsymbol{\theta}^*)/\partial \mathbf{r}^2$ is the Hessian of the system and $\partial^2 E(\mathbf{r}^*, \boldsymbol{\theta}^*)/\partial \mathbf{r}\partial \boldsymbol{\theta}$ scales linearly with the line tension anisotropy λ_0 . According to Eq. (S13), if eigenvalues of the Hessian are sufficiently small that have a nonzero overlap with the second term in Eq. (S13), then any initially small deviation $\delta \mathbf{r}_1$ from the fix point $(\mathbf{r}^*, \boldsymbol{\theta}^*)$ will grow during the iterative procedure. In particular, this would explain why we did not observe any stable fluid states for $\lambda_0 > 0$, where the Hessian is expected to contain non-trivial zero modes. We note that this is likely more than just a technical phenomenon related to our quasi-static simulations. This result could instead indicate that generally fluid tissues with anisotropic line tension may not be able to easily attain a stationary state even when boundary conditions prevent overall anisotropic tissue flow.

To obtain Fig. 3*D*,*E* & inset we binned all of our energy-minimized configurations with respect to \bar{p} and *Q* (which were computed as described above), and then computed the fraction of floppy configurations within each bin. A configuration was defined floppy when its shear modulus *G* was below a cutoff value of 10^{-5} .

Packing dependence of transition point. To study the packing-dependence of the transition point, we annealed the isotropic vertex model tissue at different temperatures prior to quenching the system to zero temperature, as this is a standard method for altering packing disorder in other materials such as structural glasses. To simulate the vertex model at a given temperature, we followed an Euler integration scheme updating all vertex positions $r_{k\alpha}$ as follows in each time step Δt :

$$r_{k\alpha} \rightarrow r_{k\alpha} + \mu F_{k\alpha} \Delta t + \eta_{k\alpha}$$
 (S14)

Here, we have non-dimensionalized time such that the dimensionless motility μ is one, $F_{k\alpha} = -\partial E/\partial r_{k\alpha}$ is the force on vertex k with the energy given by Eq. (S9), and $\eta_{k\alpha}$ is a normal distributed random force with zero average and variance $\langle \eta_{k\alpha} \eta_{l\beta} \rangle = 2\mu T \Delta t \delta_{kl} \delta_{\alpha\beta}$. To simulate these dynamics, we use the publicly available cellGPU code (10), with a time step of $\Delta t = 0.01$. For these simulations, vertices are always 3-fold coordinated and an edge undergoes a full T1 transition whenever its length is below a cutoff of 0.04.

We run 100 simulations for each set of parameters (T, p_0) , where T varies logarithmically between 5×10^{-6} and 1.5×10^{-1} , and p_0 varies between 3.7 and 3.9 in steps of 0.01. All simulations are thermalized at their target temperature for a time of 10^4 before recording the data. We then perform simulations for 10^6 at the target temperature before the temperature is quenched to T = 0. We calculate the decay of the self-overlap function for the slowest of the most solid states (low p_0 and T sets) and confirm that the vertices are displaced less than a characteristic distance of 1/e at time 10^6 , suggesting the states are relaxed. To quench the temperature to zero, we run Eq. (S14) for an additional time of 10^6 . Afterwards, we use the BFGS2 algorithm of the GSL to further minimize until the average residual force per degree of freedom is below 10^{-6} . The data are shown in *SI Appendix*, Fig. S3, demonstrating that the transition point p_0^* does depend systematically on the annealing temperature and therefore on the packing disorder.

The transition point we find occasionally decreases below the value of 3.81 (*SI Appendix* Fig. S3), which is the minimal transition point we would expect for disordered packings (11). To test whether this could be due to partial crystallization, we also quantified a hexatic order parameter:

$$\Phi_6 = \frac{1}{N_e} \sum_{\langle k,l \rangle} e^{6i\theta_{\langle k,l \rangle}} \tag{S15}$$

Here, the sum is over all N_e edges in the system, where $\theta_{\langle k,l \rangle}$ is the angle of the edge between vertices k and l. We find that the decreased transition point is indeed correlated with hexatic order $|\Phi_6|^2$ (SI Appendix, Fig. S3).

Theoretical Expectation for the Shift of the Transition Point. In a recent publication (7), some of us showed that the transition point \bar{p}_{crit} in the vertex model is expected to shift away from the isotropic transition point p_o^* as the material is anisotropically deformed with strain $\varepsilon = 1/2 \log(L_x/L_y)$ as:

$$\bar{p}_{\rm crit} = p_o^* + 4b\varepsilon^2 \tag{S16}$$

Here, *b* is a constant prefactor whose precise value depends on the packing disorder, but whose typical value was previously found to be 0.6 ± 0.2 (average \pm standard deviation). We use here the pure shear strain variable $\varepsilon = 1/2 \log(L_x/L_y)$, which is related to the strain variable γ used in Ref. (7) as $\varepsilon = \gamma/2$, and so we get an additional factor of 4 in front of *b* in Eq. (S16). However, Eq. (S16) has so far only been discussed without cell rearrangements, which do occur in our simulations.

To apply these ideas here, we start from an anisotropic configuration that results from some externally applied area-preserving anisotropic strain ε_{xx} that for simplicity we define here to extend the tissue along the x axis. It is possible that cell rearrangements occur during this deformation process, which are not taken into account in Eq. (S16). Thus, we need to disentangle overall strain from the cell rearrangements that it may cause. In Ref. (5), some of us have shown before that in the limit of homogeneous deformation without global rotations, the overall strain of a dense 2D cellular network can be decomposed into:

$$\varepsilon_{xx} = Q_{xx}^{\text{final}} - Q_{xx}^{\text{initial}} + \sum_i \Delta X_{xx}^i$$
(S17)

Here, Q_{xx}^{initial} and Q_{xx}^{final} are measures for nematic cell shape alignment before and after the deformation process, defined as described in section "Cell Shape Index and Cell Shape Alignment Analysis" (Eq. (S1) and following), projected on the x axis. The sum is over all T1 transitions *i*

that occur during the deformation process, where each T1 transition contributes an amount ΔX_{xx}^i to the overall strain. Ref. (5) more generally derives a relation about symmetric, traceless tensors, which we projected onto the x axis for simplicity here.

To apply Eq. (S16) to any anisotropic configuration with nematic cell shape alignment Q_{xx} , we ask for the amount of strain needed to deform this anisotropic configuration into an isotropic one *without* any cell rearrangements. Defining an isotropic configuration as one where the nematic cell shape alignment is zero $Q_{xx}^{\text{final}} = 0$, we obtain from Eq. (S17) that the strain needed to deform our starting configuration into an isotropic one without T1 transitions is $\varepsilon_{xx} = -Q_{xx}$. Conversely a strain of $\overline{\varepsilon}_{xx} = Q_{xx}$ is needed to get from that isotropic state back to the initial anisotropic state. With p_o^* being the transition point of the isotropic state, we thus obtain from Eq. (S16) that the transition point of our anisotropic tissue state is:

$$\bar{p}_{\rm crit} = p_o^* + 4b\bar{\varepsilon}_{xx}^2 = p_o^* + 4bQ_{xx}^2 \tag{S18}$$

Here we have assumed that all deformations are along the x axis, but the same line of argument applies to any arbitrary axis, such that we finally obtain

$$\bar{p}_{\rm crit} = p_o^* + 4bQ^2 \tag{S19}$$

Here, Q being the magnitude of the nematic cell shape alignment defined in section "Cell Shape Index and Cell Shape Alignment Analysis" above.

An alternative way to obtain Eq. (S19) is to Taylor expand \bar{p}_{crit} in terms of the cell shape alignment tensor Q, where the lowest-order term besides the constant allowed by symmetry is a term ~ Q^2 . However, with the approach above, we can also connect the value of the prefactor *b* to previous results.

Finally, the predictions in Ref. (7) strictly speaking refer to the non-dimensionalized average perimeter, i.e. the average of p_i over all cells, whereas here by \bar{p} we refer to the average shape index, i.e. the average of $p_i/\sqrt{a_i}$ over all cells. We verified that this difference does not play a role in our vertex model simulations.

Fit to simulation data. To fit Eq. (S19) to the simulation data where we apply the external anisotropic deformation (Fig. 3D), we compute the average transition point for each Q by interpreting the \bar{p} -dependent fraction of floppy configurations for fixed Q as a cumulative probability distribution function and extracting the average from it. For varying Q, the resulting plot together with a fit to Eq. (S19) is shown in *SI Appendix*, Fig. S4A.

We excluded a few data points from the fit, which were affected by the excess of rigid states observed around $\bar{p} \approx 4.15$ and $Q \approx 0.3$. This excess of rigid states very likely comes from the occurrence of higher coordinates vertices (*SI Appendix* Fig. S4*B*), which are known to increase the vertex model transition point (8).

Fits to experimental data and quality of fit. To compare our theoretical predictions to experimental data, we define a quality of fit measure n_{tot} , which we define as the number of experimental data points that are wrongly categorized as either solid or fluid by our theory. Experimentally, a data point is declared fluid if the instantaneous cell rearrangement rate averaged over a 1.5 minute time interval surpasses a cutoff value, which we set to 0.02 rearrangements per cell and minute for the plots in the main text Figs. 2*C*, 4*C*-*F*, 5*G*-*I*. A few of our theoretical

predictions include fit parameters. To determine their value from experimental data, we minimize the quality of fit measure n_{tot} varying those fit parameters.

In SI Appendix Fig. S8, we compare several theoretical predictions by plotting the quality of fit over the rearrangement rate cutoff. To obtain a reliable measurement for the cell rearrangement rate, we fixed the lower limit for the rearrangement rate cutoff requiring that the standard error $2\sigma_r$ of the cell rearrangement rate that we measure is at most as big as its average r. Assuming a Poissonian distribution of the number n_{T1} of cell rearrangements during the measurement interval of $\Delta t = 1.5$ min, we thus find:

$$1 \ge \frac{2\sigma_r}{r} = \frac{2\sigma_{n_{T_1}}}{n_{T_1}} = \frac{2}{\sqrt{n_{T_1}}} = \frac{2}{\sqrt{r\Delta t N_c}}.$$
(S20)

Hence, to get a reliable measurement for the cell rearrangement rate, we choose a minimal cutoff value of $r_{\min} = \frac{4}{\Delta t N_c} = 0.014 \text{ min}^{-1}$ per cell, where N_c is the total number of cells, which is on average $N_c = 190$.

Statistical Analysis. Unless otherwise noted, error bars are the standard deviation.

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SI Figures



Figure S1. Imaging and analysis of the germband epithelial tissue. (*Left*) Confocal images from time lapse movies of epithelial cell patterns in the ventrolateral region of the germband tissue during *Drosophila* body axis elongation. Cell outlines were visualized using the fluorescently-tagged cell membrane marker, gap43:mCherry. Anterior left, ventral down. Images, 212 μ m x 159 μ m. (*Right*) Zoomed-in regions from images at left with overlaid polygon representations used to quantify cell shapes (green). Images, 40 μ m x 40 μ m.

wild type







Figure S2. Behavior of the germband in individual wild-type and mutant embryos over time. The cell rearrangement rate per cell per minute, average cell shape index \bar{p} , cell shape alignment index Q, average vertex coordination number z, average cell neighbor number, and fraction of cells that are pentagonal f_5 .



Figure S3. The vertex model transition point depends on the packing disorder. Results of vertex model simulations, where the effect of packing disorder is studied by annealing the model tissue with thermal fluctuations prior to quenching to a force-balanced state to create packings with different degrees of disorder. Taken together, these simulation results show that the critical shape index in the vertex model depends on the cellular packing disorder, where fluctuations can help to decrease packing disorder and p_o^* . (A) The transition point p_o^* decreases with the annealing temperature T before increasing again for very high T, confirming a dependence of the transition point on packing disorder. The values for p_0^* decreased from 3.86 for low temperatures to 3.72 for higher temperatures, and increased again for even higher temperatures. (B) While the lower bound is below the previously reported value of 3.81, this may be related to partial crystallization of the tissue in this regime. The hexatic bond-orientational order parameter, shown here depending on the preferred shape index p_0 and annealing temperature T, indicates at least partial crystallization for intermediate temperatures, which correlates with lower transition points p_{o}^{*} . (C) We bin the simulations from panels A, B (100 simulations for each different combination p_0, T) with respect to average cell shape index \bar{p} and fraction of pentagonal cells f_5 . Within each bin we then compute the fraction of fluid states using a cutoff of 10^{-7} on the shear modulus. The black dashed line is a linear fit obtained by minimizing the number of simulations on the wrong side of the transition line: $p_0^* = 3.725 + 0.59 f_5$. White regions do not contain any simulation. Same plot as Fig. 2D in the main text. (D) Same plot as in panel C with a corrected fraction of pentagonal cells f'_5 . As the spatial resolution in our experiments is limited, we detect cell-cell interfaces with a length smaller than 0.11d as manyfold vertices, where d is the square root of the average cell area. We applied the same cutoff to interpret the energy-minimized configurations in panel C, which generally leads to a somewhat higher fraction of pentagonal cells $f'_5 > f_5$. However, the linear fit of the solidfluid transition (black dashed line) is only slightly altered to: $p_0^* = 3.726 + 0.57 f_5'$.



Figure S4. Fits of Eq. (1) in main text to simulations. (*A*) Fit of Eq. (1) (black line) to the vertex model simulation results for the case of external deformation (dots, cf. Fig. 3*D*). The average transition points \bar{p}_{crit} were extracted from the simulation data (Fig. 3*D*) by interpreting the fraction of fluid networks for fixed *Q* as a cumulative probability density and extracting the average from it (9). From the fit we find $p_o^* = 3.94$ and b = 0.43. The red data points were excluded from the fit. These points are related to an excess number of rigid states in the region (cf. panel B and Fig. 3*D*). (*B*) The excess number of rigid states around $Q \approx 0.3$ and $\bar{p} \approx 4.15$ (cf. panel A and Fig. 3*D*) is associated with an increase in the number of manyfold coordinated vertices in model tissues. Plotted here is the total number of manyfold vertices in configurations of 512 cells.



Figure S5. Effects of cell area variation in vertex model simulations. Effects of cell area variation on vertex model simulation results. A Gaussian distribution for the preferred cell areas was used with relative standard deviations of 0% (*A*), 10% (*B*), and 20% (*C*). Solid black lines represent fits to the quadratic relation Eq. (1) in the main text. Variation in cell area has only a very small effect on our theoretical findings and the parameters p_0^* and *b*. We find the following fit parameters: (A) $p_0^* = 3.96$, b = 0.57; (B) $p_0^* = 3.97$, b = 0.58; (C) $p_0^* = 3.97$, b = 0.57. These parameters differ somewhat from what we find in Figs. 3*D*, S4*A*, because there we allowed for manyfold vertices as intermediate states during the energy minimization, whereas in these simulations, we did not allow manyfold vertices in order to reduce simulation run time.



Figure S6. Vertex model tissue with anisotropic internal stresses. Force-balanced vertex model state with anisotropic cell-cell interfacial tensions to model the effects of planar polarized myosin II localization patterns. Cell edges color-coded by the tension anisotropy. Parameters: $p_0 = 3.5$, $\lambda_0 = 0.5$.



Figure S7. Cell shape alignment. Throughout the main text, we discuss the parameter Q (black curve), which describes both cellular shape anisotropy and alignment of cell shapes in the tissue. Indeed, Q can be decomposed as $Q = Q_s Q_a$ (cf. Eq. (S8)), where Q_s represents cellular shape anisotropy (red curve) and Q_a represents cell shape alignment (blue curve). The "pure" nematic alignment parameter Q_a ranges from zero (random cellular orientation) to one (all cells aligned along the same axis, although with potentially different magnitudes). Here we plot Q, Q_s , and Q_a for the wild-type germband. Q_a is almost one at around t = 0, which indicates almost perfect alignment of the cells at that time point.



Figure S8. Quality of fits. To compare theoretical predictions to experimental data, we define a quality of fit measure n_{tot} , which is the number of experimental data points that are wrongly categorized as either solid or fluid by the prediction. Thus, a better fit is associated with a smaller value of n_{tot} . (A) Comparison of several theoretical predictions by plotting the quality of fit, n_{tot} , over a range of cell rearrangement rate cutoffs. The predictions include: a constant value of p_0^* = 3.813 (black dashed line) (11), a constant value of $p_0^* = 3.813$ and Q-dependent correction according to Eq. (1) in the main text (black solid line), fraction-of-pentagon-dependent $p_0^*(f_5)$ extracted from vertex model simulations in Fig. 2D (green dashed line), same with Q-dependent correction (green solid line), vertex-coordination-dependent $p_0^*(z)$ according to Ref. (8) (red dashed line), and same with Q-dependent correction (red solid line; cf. Eq. (2) and Fig. 4D). Note that all of these six predictions are parameter free ("fp" in the legend indicates the respective number of fit parameters used). In addition, we plot the quality of one-parameter fits, where we either fit a constant p_0^* (blue dashed line; cf. Fig. 4C) or additionally include the Q-dependent correction (blue solid line; cf. Fig. 4C). Finally, we plot the quality of a two-parameter fit, where for each cell rearrangement cutoff we extract the fraction-of-pentagon dependence $p_0^*(f_5)$ from experimental data using a fit like Fig. 4E and include the Q-dependent correction of Eq. (1) (cyan solid line; cf. SI Appendix Fig. S9). We find that including the Q-dependent correction always improves the prediction, where each time we have fixed b = 0.43. Moreover, the best parameterfree prediction for small rearrangement rate cutoffs is given by the red solid line corresponding to Eq. (2) in the main text. (B) Quality of fit, n_{tot} , for different fixed values of the parameter b for one-parameter fits of Eq. (1) where we vary p_0^* (cf. blue dashed and solid lines in panel A and Fig. 4C). Throughout the manuscript, we used the value of b = 0.43 determined from vertex model simulations results. The quality of fit is only slightly improved by changing the value of b.



Figure S9. Accounting for packing disorder using the fraction of pentagonal cells. In the main text, we account for the effects of packing disorder on p_o^* by taking into account the vertex coordination number in the tissue (cf. Fig. 4F) using the previous prediction in Ref. (8), as shown in Eq. (2) in the main text. Alternately, one could use the fraction of pentagonal cells, which in our experiments correlates well with p_o^* (cf. Fig. 4E). Here we show the results if we instead correct the cell shape index by the fraction of pentagonal cells at the transition point, according to the linear fit in Fig. 4E. (A) Same experimental data as in Fig. 4D, but with the cell shape corrected by fraction of pentagonal cells observed in the tissue at the transition point. (B) Same experimental data as in Fig. 5H, but with the cell shape corrected by fraction of pentagonal cells observed in the tissue at the transition point.



Figure S10. bnt mutants. (A) bcd nos tsl (bnt) mutant embryos, which lack anterior-posterior patterning genes required for axis elongation, show severely disrupted myosin planar polarity compared to wild-type embryos. Scale bar, 10 μ m. (B) Same data as in Fig. 51, but with a different color scale to better distinguish rearrangement rate values within bnt embryos. Relationship between the corrected average cell shape index \bar{p}_{corr} and Q for 5 bnt embryos with each point representing a time point in a single embryo. Instantaneous cell rearrangement rate is represented by the color of each point. Solid line represents the prediction of Eq. (2) in the main text.