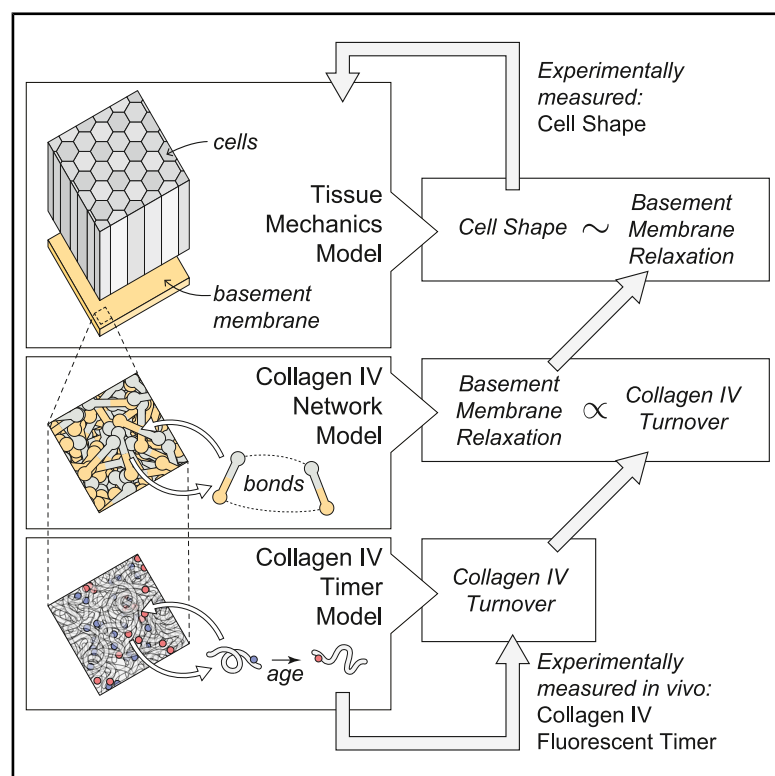


Basement membrane turnover controls cell shape

Graphical abstract



Authors

Ricardo Barrientos, Billie Meadowcroft, Besaiz J. Sánchez-Sánchez, ..., Shiladitya Banerjee, Andela Šarić, Yanlan Mao

Correspondence

ricardo.barrientos.14@ucl.ac.uk (R.B.), y.mao@ucl.ac.uk (Y.M.)

In brief

Basement membranes are scaffolds of proteins, like collagen, that give organs shape. During development, basement membranes must adapt to the growing organ. We infer how fast the basement membrane adapts by measuring collagen turnover. We find that perlecan, another basement membrane protein, regulates collagen turnover to control organ shape.

Highlights

- Increasing basement membrane (BM) stress relaxation time confines proliferating cells
- BM stress relaxation time is proportional to collagen IV lifetime in the BM
- A fluorescent timer is used to measure the *in vivo* lifetime of collagen IV in the BM
- Perlecan modulates BM collagen IV lifetime to impact BM relaxation time and cell shape



Article

Basement membrane turnover controls cell shape

Ricardo Barrientos,^{1,2,*} Billie Meadowcroft,^{1,2,3} Besaiz J. Sánchez-Sánchez,⁴ Brian M. Stramer,⁴ Ewa K. Paluch,⁵ Guillaume Charras,^{2,6,7} Shiladitya Banerjee,⁸ Andela Sarić,³ and Yanlan Mao^{1,2,9,*}

¹UCL Laboratory for Molecular Cell Biology, University College, London, Gower Street, London WC1E 6BT, UK

²Institute for the Physics of Living Systems, University College, London, Gower Street, London WC1E 6BT, UK

³Institute of Science and Technology, Austria, 3400 Klosterneuburg, Austria

⁴Randall Centre for Cell and Molecular Biophysics, King's College, London, London SE1 1UL, UK

⁵Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK

⁶London Centre for Nanotechnology, 17-19 Gordon street, London WC1H 0AH, UK

⁷Department of Cell and Developmental Biology, University College, London, Gower Street, London WC1E 6BT, UK

⁸School of Physics, Georgia Institute of Technology, Atlanta, GA 30306, USA

⁹Lead contact

*Correspondence: ricardo.barrientos.14@ucl.ac.uk (R.B.), y.mao@ucl.ac.uk (Y.M.)

<https://doi.org/10.1016/j.cell.2026.07.010>

SUMMARY

The regulation of 3D cell shape is a fundamental problem of life. In multicellular tissues, cell shape emerges through the balance of forces inside and outside the cell. In epithelia, the basement membrane (BM) is the first extracellular barrier that cells sense biochemically and mechanically. Despite this, little is known about how BM mechanical properties are regulated and how they impact cell shape. Through mathematical modeling, we show that the stress relaxation time of the BM can regulate cell shape. Using molecular dynamics simulations, we show that the stress relaxation time of a collagen IV network can be inferred from the lifetime of collagen IV molecules. To measure collagen IV lifetime *in vivo*, we develop a fluorescent timer reporter for collagen IV and show that perlecan modifies collagen IV lifetime. This cross-disciplinary approach establishes a multiscale framework to probe matrix turnover, and its regulation and function in cell shape control.

INTRODUCTION

During development, cells undergo complex three-dimensional (3D) shape changes to generate the precise form and function of organs. From the laws of physics, we know that cells must experience differential forces to drive morphological shape changes.^{1–6} These forces can be intrinsically generated by the cell or extrinsically applied to the cell by the external environment.⁷ The force balance within cells, between cells, and across tissue boundaries sets the shape of a cell in a multicellular tissue environment.⁸ This shape could be in a highly tensile stress state or in a relaxed, stress-free state. How cells relax stresses over timescales of minutes and hours has been well studied.^{9,10} However, tissues are composites of cells and extracellular structures that develop together over the course of days. How composite tissues generate and relax stresses over the longer timescales of days that are relevant to development is less clear and necessitates an understanding of the relaxation of the extracellular matrix (ECM) to which cells are bound.

In most organs, cells are tightly packed and adhere to one another or to their immediate surroundings, such as the ECM. The basement membrane (BM) is a type of ECM that forms thin, dense sheets.¹¹ In epithelia and endothelia, the BM is the first physical barrier that cells experience.¹² Therefore, the

molecular and physical properties of the BM are critical for cell and tissue morphogenesis.

The BM is formed of a network of secreted components. Collagen IV and laminin are core BM components known to form networks and are thought to provide the BM with structural integrity.^{13–15} There are many other BM components, such as perlecan,^{14,16} nidogen,^{17,18} and SPARC.^{14,19} It has become increasingly clear that the BM contributes mechanically to tissue morphogenesis.^{12,20} Studies of matrix mechanics have mostly focused on its elastic properties—its stiffness. However, few have considered the dynamic and viscoelastic properties of the BM, including how its components assemble, remodel, and dissipate stress over time, especially over developmentally relevant timescales.^{21–24} This is particularly important during tissue growth, where cells and the BM must expand over days, incorporate new material, and remodel in a coordinated manner. Therefore, understanding how coordinated cell-BM interactions dynamically generate, maintain, and dissipate mechanical stress is paramount to understanding cell shape control and tissue morphogenesis.

The mechanical properties of the BM likely emerge from the gel-like behavior of its network.¹¹ In gels, viscosity on short timescales emerges from friction and entanglement as polymers slide past one another.^{25,26} In the BM, viscosity on longer timescales may emerge from the remodeling (defined here as



breaking and making) of more persistent interactions between BM molecules, as suggested in other systems.^{27,28} However, how the BM molecularly remodels to give rise to viscoelastic properties that persist for days is unclear.

The wing disc of *Drosophila melanogaster*, a highly proliferative monolayer epithelium encased in a BM shell, is an ideal system to study cell-BM interactions during rapid growth.^{14,29–31} The area of this epithelium increases by around two orders of magnitude over 4 days,³² while cell number increases by around three orders of magnitude³³ over the same period. The BM is present and apposed to the basal surface of cells throughout this period of rapid growth; hence, the BM and epithelium must expand synchronously. This could be achieved by two methods: the BM could expand elastically, stretching by two orders of magnitude, or the BM network could remodel to conform to its increasing size while adding more material, thereby requiring molecular turnover to maintain its integrity as it expands. How the BM is regulated to expand with the proliferating cells will have a significant impact on cell shape and, in turn, the final tissue shape because of the geometric constraints the BM and proliferating cells impose on one another.

To uncover the mechanisms of BM regulation, we employ a cross-disciplinary multiscale approach, generating complementary physical models and novel molecular tools for measuring BM turnover *in vivo*. First, we construct a minimal mathematical model of the epithelium to generate quantitative predictions of the impact of BM mechanical properties on cell shape. One important prediction from this model is that the relaxation time of the BM plays a major role in regulating cell shape. To infer BM relaxation time, which cannot be directly measured *in vivo* over the days-long duration of wing disc growth, we develop molecular dynamics simulations that predict a proportional relationship between BM relaxation time and collagen IV lifetime in the BM. To verify the model predictions, we develop a novel molecular reporter for measuring the *in vivo* collagen IV lifetime by fusing a fluorescent timer protein to endogenous collagen IV in *Drosophila*. With this molecular reporter, we measure collagen IV lifetime in the BM *in vivo* and determine how the BM component perlecan can modify this lifetime. With this cross-disciplinary multiscale framework, we reveal new insights into the molecular regulation of BM turnover and the impact of BM relaxation on cell and tissue shape control.

RESULTS

Mathematical modeling predicts that BM relaxation time contributes to 3D cell shape

In epithelia, cells and the BM form two discrete adhered layers (Figure 1). As cells proliferate, they will become confined unless the BM also increases in area to accommodate them. Mechanically, cells can expand the BM by applying an expansive stress via adhesions such as integrins.^{34–36} How the BM responds to stress depends on its mechanical properties, namely stiffness (elasticity) and stress relaxation time (viscosity). Many studies have focused on the effect of BM stiffness on epithelial cell shape.^{14,29–31} We propose that the stress relaxation time of the BM has a critical impact on cell shape in growing epithelia. Conceptually, if the BM takes a long time to relax (relative to

cell proliferation time) but is very soft (relative to cells), then the BM will stretch with the cells, without confining them (scenario I, Figure 1A). However, if the BM is very stiff, then it will be hard for the cells to stretch the BM sufficiently as they proliferate, and consequently cells will become confined (scenario II, Figure 1A). On the other hand, if the BM takes a short time to relax stress compared with proliferation time, then regardless of how soft or stiff it is, it will expand with the proliferating cells, and cells will not be confined (scenario III, Figure 1B). Exactly where the *Drosophila* wing disc epithelium is along these extreme conceptual scenarios is yet to be determined.

To test the above intuition of the effect of matrix mechanics on cell shape in the context of growth, we developed three mathematical tissue mechanics models (supplemental methods, section S1). In the first, and simplest, model (supplemental methods, section S1.1), cells are approximated as hexagonal prisms of fixed volume^{30,37–39} packed onto a patch of BM (Figures 1C–1F). These cells are given apical, basal, and lateral surface tensions that act to minimize cell surface energy (Figure 1C). This minimum energy defines the preferred cell shape—a stress must be applied to force cells away from this preferred shape (Figure 1D, red arrows, red line). At the initial time point, the BM is treated as a sheet with an area equal to that of the packed cells (Figures 1E and 1F).^{14,37} We approximate BM mechanics as a viscoelastic fluid (Figure 1E and supplemental methods, section S1), which will deform reversibly on short timescales but irreversibly over longer timescales. Stresses of cells and the BM are coupled (Figure 1F), and we mimic cell proliferation by increasing the number of cells in the patch with time (Figures 1G–1J).

Using this model to gain insight into how BM mechanical parameters affect proliferating cell shape, we chose a constant, physiological cell-doubling rate,^{40,41} while varying BM relaxation time and relative cell-BM stiffness (Figure 1G). This confirms our intuition from Figures 1A and 1B that BM stiffness and relaxation time both impact cell confinement. The stiffer the BM, the more confined cells will become (moving right on the x axis in Figure 1G), but this confinement is reduced if the BM takes a shorter time to relax compared with proliferation time (moving down the y axis). Further, when the BM takes a sufficiently long time to relax compared with the proliferation time, BM deformation is mostly elastic and reversible (above the dotted line). In this reversible deformation domain, we identify scenarios I and II from Figure 1A. From the model, we also identify scenario III from Figure 1A, and a new fourth scenario, IV, where cells are confined with little, mostly irreversible, BM deformation. These phases are present even when cell volume is allowed to vary by up to 10% (Figure S1A). Three cases are considered where BM stiffness is constant, shown in Figures 1H–1J and labeled in Figure 1G. For a sufficiently short BM relaxation time, cells remain approximately in their preferred cell shape as cell number increases (Figure 1H; Video S1). For moderate (Video S2) or long (Video S3) BM relaxation time, cells become confined, appearing taller and thinner as cell number grows (Figures 1I and 1J).

To consider more realistic cell geometries and topologies, we extended the minimal model to account for heterogeneity in apical-basal cell shape and surface area (supplemental methods, section S1.2). When cell shape and surface area heterogeneity

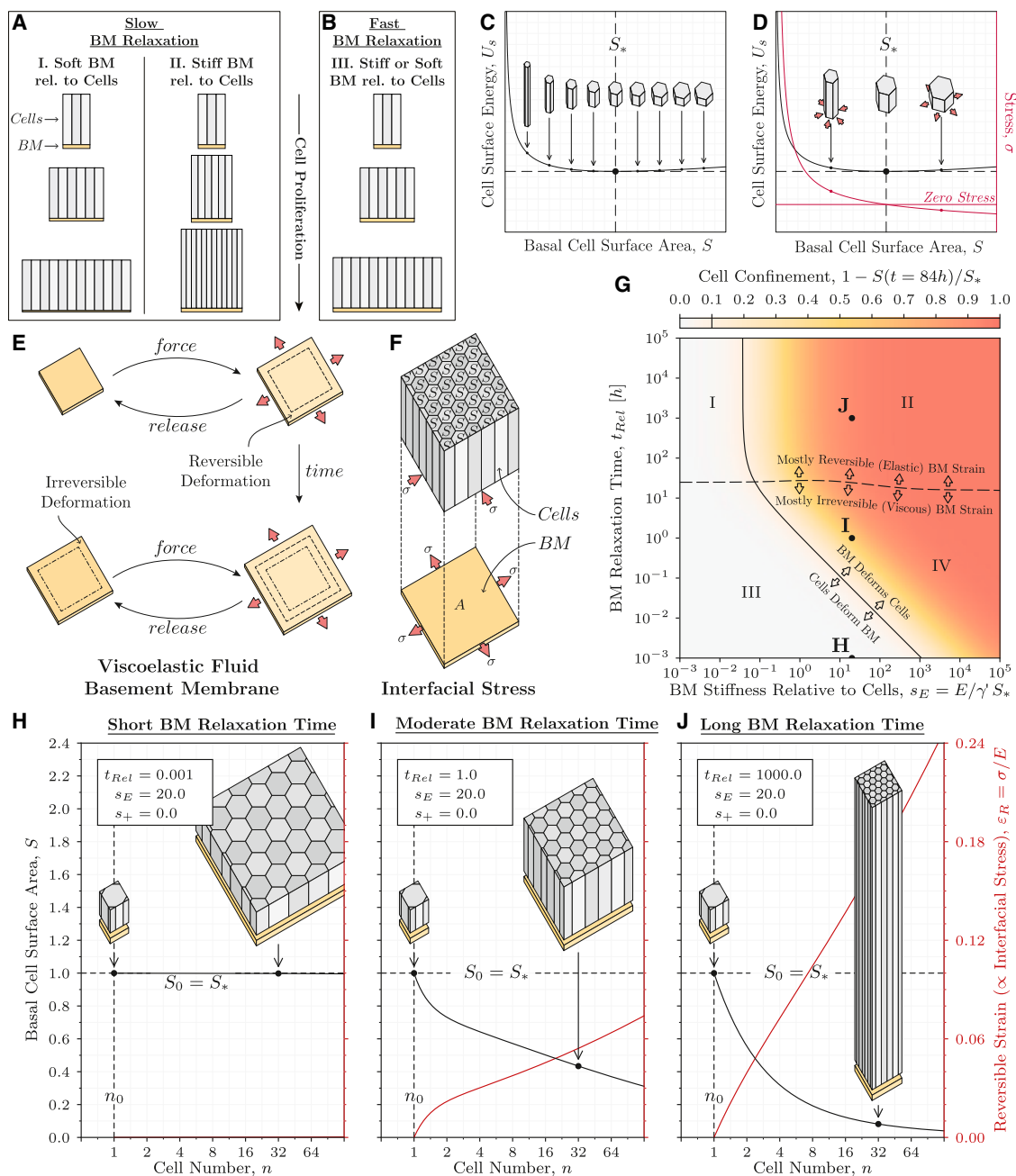


Figure 1. A tissue mechanics model shows that cell proliferation drives cell confinement when BM relaxation time is sufficiently long (A and B) Hypothesis.

(C) The shape of cells of fixed volume is defined by basal surface area. Cell surface energy has a minimum at which cells are in their optimum shape.

(D) Mechanical stress (red arrows and red line) is required to push cells away from their optimum shape.

(E and F) We developed a tissue mechanics model (see [supplemental methods](#)). (E) The BM is treated as a viscoelastic fluid: an irreversible strain develops if a stress is maintained over time. (F) Cells are tiled onto a BM patch and mechanically coupled via a shared interfacial stress.

(G) Solutions to the mechanical model for a range of parameters. Solid line indicates a soft transition between the BM and the BM deforming cells. Dashed line indicates a transition between mostly reversible and mostly irreversible strain. These transitions define four phases; the first three phases were hypothesized.

(H–J) Three example solutions varying BM relaxation time, also indicated in (G).

See also [Figure S1](#).

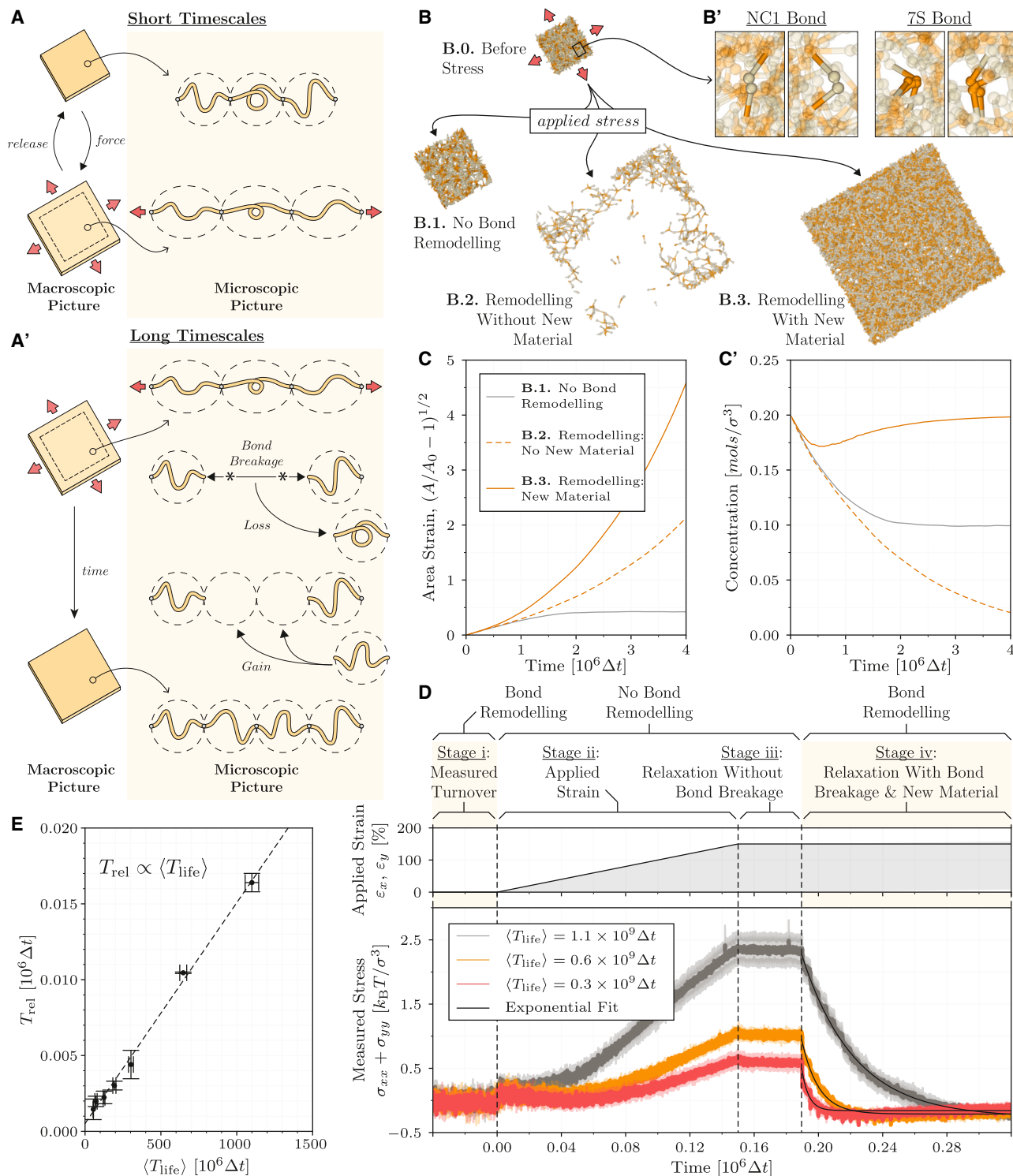


Figure 2. A collagen IV network model shows that BM relaxation time is proportional to the mean lifetime of collagen IV in the network
(A) Schematic of hypothesized microscopic and macroscopic pictures of collagen IV. On short timescales (top), BM elasticity emerges from entropic spring behavior of collagen IV molecules in a network. (A') On long timescales, bonds between collagen IV molecules break and new collagen IV molecules bind, allowing the BM to relax stress and behave as a viscoelastic fluid.

(legend continued on next page)

are low, all four phases are present (Figure S1B). However, as this heterogeneity increases, the reversible BM strain phases (I and II) shrink, suggesting that the cell layer becomes softer. We also extended the minimal model to include asymmetry between apical and basal cell surface areas (supplemental methods, section S1.3), leading to tissue curvature. Despite this asymmetry, the four phases are still present (Figure S1C) with a minor shift in their boundaries as relative apical-basal surface tension varies. Generally, we find that the apical surface area expands to allow for basal confinement (Figures S1D–S1G; Videos S4, S5, S6, and S7), and, interestingly, we note that no combination of parameters results in a stable trajectory with equal apical-basal surface areas, which only occurs transiently, for example, in Figure S1D.

In summary, our mathematical model predicts that the relaxation time of the BM relative to cell proliferation time has a strong influence on cell shape. We hence designed new experimental and computational tools to infer the *in vivo* BM relaxation time to determine whether BM viscoelasticity impacts cell shape changes during wing disc growth.

Inferring BM relaxation time from collagen IV lifetime

There are no established methods to measure the BM relaxation time *in vivo*. Instead, as a proxy, we measure the mean lifetime of collagen IV, a major BM component, which we define as the average time a collagen IV molecule spends in the BM network. Our motivation is as follows. We know that collagen IV is a network-forming component of the BM and contributes significantly to its stiffness and integrity.^{14,16} Many soft materials, including biopolymer networks, are viscoelastic fluids,⁴² behaving elastically at short timescales and exhibiting viscous dissipation at long timescales. Viscous dissipation can occur from network rearrangement on the timescale of minutes. However, on developmental growth timescales of days, we expect the collagen IV network to expand and remodel as interactions break and new interactions are made. We therefore propose the molecular picture outlined in Figures 2A and 2A' as the main mechanism responsible for BM stiffness and relaxation. We expect that when collagen IV bonds break, stress is relaxed (long timescales, Figure 2A'), and when new bonds are made, collagen IVs tend to be in a relaxed state. If the network is expanding, new collagen IV must be added to maintain the relaxed state. Hence, by breaking bonds, we expect the BM to relax stress over time, while new material is needed to allow expansion of the BM. Therefore, the faster the bonds break, the shorter the

amount of time a collagen IV molecule spends in the BM network, and the shorter the amount of time required to relax the stress. However, the exact relationship between these quantities is not immediately clear *a priori*.

To validate the above intuition and quantitatively explore how collagen IV lifetime is related to BM relaxation time, we extended a coarse-grained collagen IV network model, recently developed in Meadowcroft et al.⁴³ and implemented in a molecular dynamics simulation (Figures 2B–2E). Collagen IV molecules are modeled as two beads connected by a spring that interacts via NC1 bonds and 7S bonds to form networks (Figures S2A–S2C). The beads are confined to a flat box of a shape similar to the BM. Details of the coarse-grained model and how measurements are made are outlined in the supplemental methods, section S2. The x-y area of the box can be increased to simulate tissue growth, and the resulting network rheology can be measured (Videos S8 and S9). We simulate 3 types of stress relaxation scenarios after applying a fixed stress to the network (Figure 2B; Videos S12, S13, and S14) to see how this allows the network to expand. In scenario 1 (Figure 2B.1), we do not allow bonds to break or new bonds to form (no bond remodeling); the network expands to a limited extent (Figures 2C), and there is a decrease in overall concentration (Figure 2C'). In scenario 2 (Figure 2B.2), we allow bonds to break and reform, but we do not provide new material (collagen IV molecules) to the system; the network breaks and loses integrity as it expands, and the concentration of collagen IV drops dramatically. Only when we allow bonds to break and reform and provide new collagen IV (scenario 3, Figure 2B.3) can the network expand extensively while maintaining the integrity and concentration of collagen IV in the network, as observed during *in vivo* growth.

To better mimic gradual *in vivo* growth, we applied a gradual strain to the network (while adding new material) and measured the stress (Figure 2D). As increasing strain is applied, stress gradually increases (stage ii) and does not significantly relax if we do not allow bond breakage (stage iii). Once we allow bond breakage and the incorporation of new collagen IV molecules to form new bonds, stress relaxes (stage iv). The time it takes to relax this stress is directly proportional to the mean lifetime (T_{Life}) of collagen molecules in the network (Figure 2E). This holds true even if the tissue is slightly curved, as is the case in some regions of the wing disc (Figures S2E–S2G; Videos S10 and S11).

In summary, we find that network relaxation time (T_{Rel}) is directly proportional to collagen IV lifetime (T_{Life}), as shown in Figure 2E. Networks that can relax stress quickly due to

(B) Molecular dynamics simulation of a collagen IV network responding to applied stress using different stress relaxation mechanisms: B.1: no bond remodeling (breaking or remaking) is allowed; B.2: bond remodeling is allowed but no new collagen IV molecules are provided; B.3: bond remodeling is allowed, along with new incorporation of collagen IV molecules.

(C) Network area expansion of the 3 different stress relaxation mechanisms in (B) and (C') the corresponding collagen IV network concentration.

(D) Collagen IV network response to a gradually applied strain and resulting measured stress at different stages. Stage i: system initializes. Stage ii: gradually applied strain; relaxation without bond breakage is allowed during stage ii, hence very little stress can be further relaxed without bond breakage during stage iii. Stage iv: full stress relaxation with bond breaking and new collagen IV incorporation. Each line represents the mean measured stress over multiple runs with error bars indicating standard deviation. Repeated with different collagen IV mean lifetimes (T_{Life}) in the network, the shorter T_{Life} the faster the stage iv stress relaxation (T_{Rel} estimated from fitted black line).

(E) Stress relaxation time, T_{Rel} , the time taken for the stress in the network to reduce by $1/e$, is plotted against collagen IV mean lifetime in the network. Each point represents the mean collagen IV lifetime vs. network relaxation time over multiple runs of (D) with error bars indicating standard deviation, dashed line indicates a linear fit to the data.

See also Figure S2.

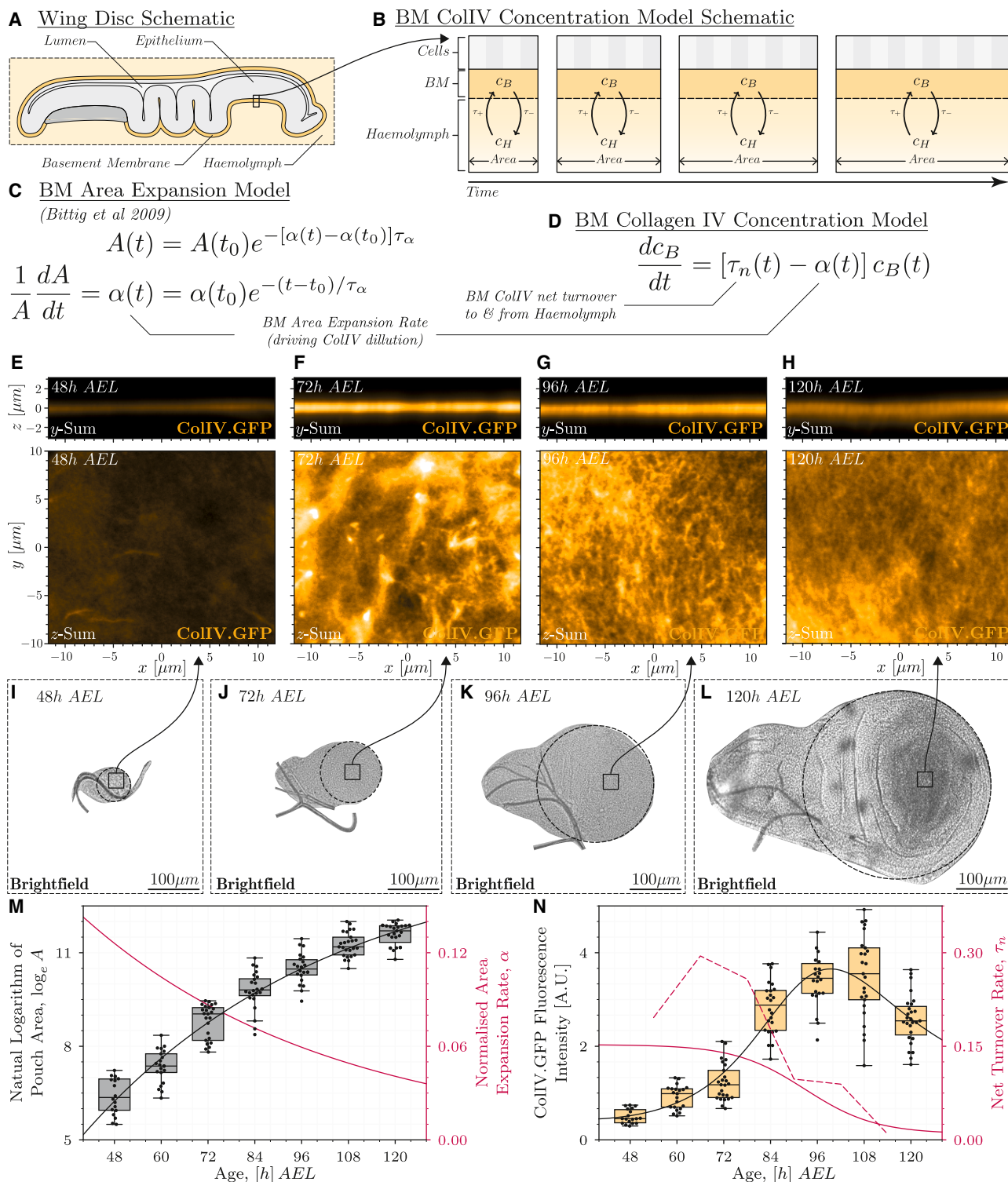


Figure 3. Measuring BM collagen IV concentration is insufficient to determine mean collagen IV lifetime in the BM

(A) Schematic of wing disc cross-section, showing immersion in hemolymph.

(B) Schematic of collagen IV transfer between wing disc BM and hemolymph, and BM expansion with time.

(C) Area expansion model used to estimate the wing disc BM expansion rate from the pouch area measured in (I)–(L), as shown in Bittig et al.³²

(D) BM collagen IV concentration model used to estimate BM collagen IV concentration dynamics given transfer to and from the hemolymph and dilution due to BM expansion.

(legend continued on next page)

increased bond breaking can also expand their area faster (Figure S2D), exactly as predicted by the tissue mechanics model (Figure 1H). These two models therefore link the molecular-scale turnover of the BM to stress relaxation of the BM and to cell shape in an epithelium.

Measuring collagen IV lifetime *in vivo*

To compare our models to *in vivo* data, we measured collagen IV lifetime in the wing disc BM. Collagen IV is not produced by wing disc cells but by a separate tissue, the fat body, from which it is secreted into the hemolymph and subsequently deposited onto the surface of the wing disc (Figures 3A and 3B).¹⁴ Consequently, dissected wing discs no longer have a source of collagen IV. We therefore sought an *in vivo* method of measuring collagen IV lifetime. We began by measuring how BM collagen IV levels change with wing disc growth. We measured colIV-GFP fluorescence intensity during wing disc development in 12-h intervals between 48 h AEL and 120 h AEL (Figures 3E–3H), while simultaneously measuring the size of the wing disc pouch (Figures 3I–3L, dashed circle). We observe that colIV-GFP increases in concentration from 48 h AEL up to 96 h AEL, suggesting the addition of collagen IV. However, from 96 h AEL to 120 h AEL, we observe a reduction in colIV-GFP, which could be due to changes in gain and loss of collagen IV, as well as dilution of collagen IV as the tissue grows.

To determine contributions from turnover and dilution, we adapted a model of collagen IV turnover developed in Matsubayashi et al.²² to account for collagen IV dilution due to BM expansion (Figure 3D and supplemental methods, section S3.1). To model BM expansion, we use the model of Bittig et al.,³² who showed that wing disc area growth rate follows an exponential decay during development (Figure 3C). We estimate BM expansion rate by fitting the area expansion model to the pouch area data (Figure 3M); using this estimated BM expansion rate, we then fit our BM collagen IV concentration model to the experimental fluorescence intensity data (Figure 3N). We found that a model that assumes a constant rate of loss or gain of collagen IV did not suitably fit the data. To gain insight into how the turnover rates would need to change to fit the data, we used a very approximate, linearized version of the BM collagen IV concentration model. From this model, we find net turnover, which we define as the rate of gain of collagen IV to the BM minus the rate of loss of collagen IV from the BM, switches from a high to a low value at around 80 h AEL (Figure 3N, dashed red line). So, instead, we described net turnover using a sigmoid function (solid red line), which gives a much better fit to the data (solid black line). To determine collagen IV lifetime, we need to know the collagen IV loss rate. However, we cannot differentiate between gain and loss from the colIV-

GFP fluorescence intensity alone. To address this problem, we turned to a fluorescent timer approach.

To measure BM collagen IV lifetime, we utilized a fluorescent timer. A fluorescent timer is a protein that changes fluorescent state over time or a pair of fluorescent proteins that mature with different kinetics.⁴⁴ We chose the fast monomeric fluorescent timer developed by Subach et al.⁴⁵ This fast fluorescent timer (FTF) starts in an immature non-fluorescent state, then transitions to a blue fluorescent state, then to an intermediate non-fluorescent state, and finally to a red fluorescent state (Figure S4A) with transition rates already determined (Figure S4B).⁴⁵ We chose this timer in particular due to its optimal transition rates, which provide a working timescale of days, commensurate with the long timescales of collagen IV dynamics. If we consider a batch of FTFs created at exactly the same time, then individual FTFs will stochastically transition through their states (Figure S4D), but at the population level, the fraction of FTF in each state reliably follows a trajectory through time (Figure S4C) determined by a model developed by Subach et al.,⁴⁵ reproduced in Figure S4A and supplemental methods, section S3.2. We can, therefore, use FTF to track the age of collagen IV molecules and determine their average lifetime within the wing disc BM.

We generated FTF-tagged collagen IV (colIV-FTF) by knocking FTF into the endogenous *viking* (*vkg*) gene—one of only two collagen IV subunits (see STAR Methods). As new collagen IV is continually produced and moves between different locations in the larva and wing disc (Figures 4A and 4B), the signal we measure on the wing disc will be the accumulation from old through newly deposited colIV-FTF. Therefore, to accurately interpret our experimental results from the timer, we developed a BM collagen IV timer model (see Figure 4C; supplemental methods, section S3.2).

We carefully measured the red and blue fluorescence intensity of colIV-FTF in the BM at the center of the wing disc pouch at 12-h intervals between 48 h and 120 h AEL, Figures 5 and 6 (for details see supplemental methods, section S4; Figure S5), as well as wing disc pouch size. ColIV-FTF red and blue measurements for control conditions are shown in Figures 5A and 6A, and example images are shown in Figures S6A–S6D. In the control, we find that the blue colIV-FTF state increases between 48 h and 84 h AEL, then decreases slightly and plateaus (Figures 5A' and 6A'), which is reminiscent of the colIV-GFP trajectory (Figure 3N). In contrast, the red colIV-FTF state steadily increases over the same period.

The difference in trajectories between blue and red states can be explained by the differing timescales they capture. If colIV-FTF enters the BM mostly in the blue state, then, because this state is short lived, only colIV-FTF that has entered recently will appear

(E–H) Sum projections of GFP-tagged collagen IV (ColIV-GFP, yellow) fluorescence in the BM at 48, 72, 96, and 120 h after egg laying (h AEL). Top, projections parallel to BM plane (y sum); bottom, projections perpendicular to BM plane (z sum).

(I–L) Bright-field images of whole wing discs at four ages. Squares with arrows indicate locations of images in (E)–(H). Dashed circles indicate wing disc pouch area measurement, A, used to estimate BM area and expansion rate in (C).

(M) Wing disc pouch area measurements with age; black line is fitted area expansion model, and red line is normalized area expansion rate from the fitted model in (C).

(N) colIV-GFP fluorescence intensity measurements with age; black solid line is the fitted BM collagen IV concentration model in (D) solid red line is net turnover from the fitted BM collagen IV concentration model, and dashed red line is net turnover from the fitted linearized BM collagen IV concentration model. Boxplots in (M) and (N) indicate first, second, and third quantiles; dots are individual wing disc measurements.

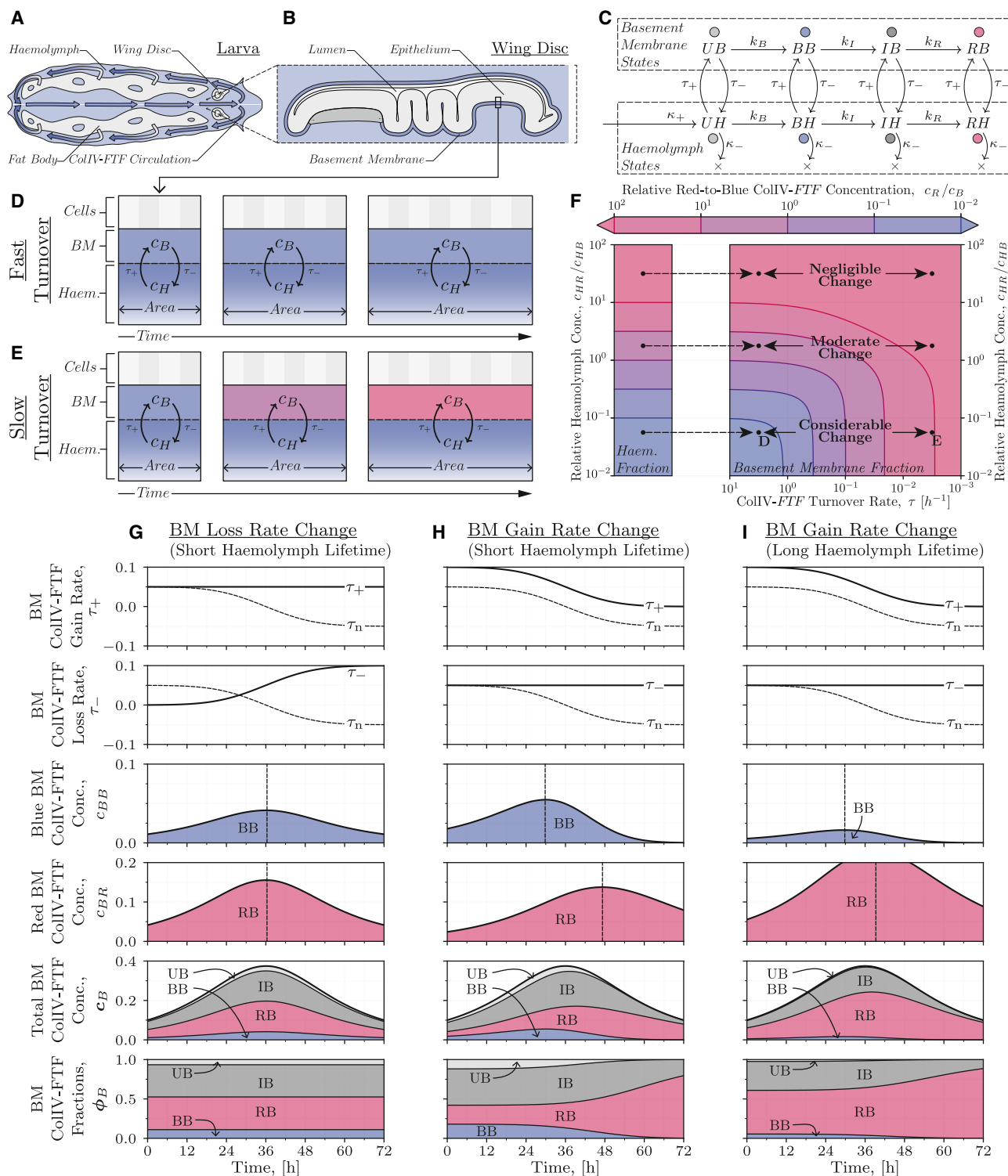


Figure 4. Collagen IV tagged with a fluorescent timer can be used to measure collagen mean lifetime in the BM

Using a fluorescent timer to measure collagen IV turnover (colIV-FTF).

(A and B) colIV-FTF is synthesized in the larval fat body and secreted into the circulating hemolymph.

(C) colIV-FTF dynamics are described by the BM colIV-FTF model. From the hemolymph, colIV-FTF is gained or lost from the wing disc BM with rates τ_+ and τ_- , respectively, which characterize turnover.

(D) For fast turnover colIV-FTF does not have sufficient time in the BM to progress beyond its original hemolymph colIV-FTF state, which, in this schematic, is blue.

(legend continued on next page)

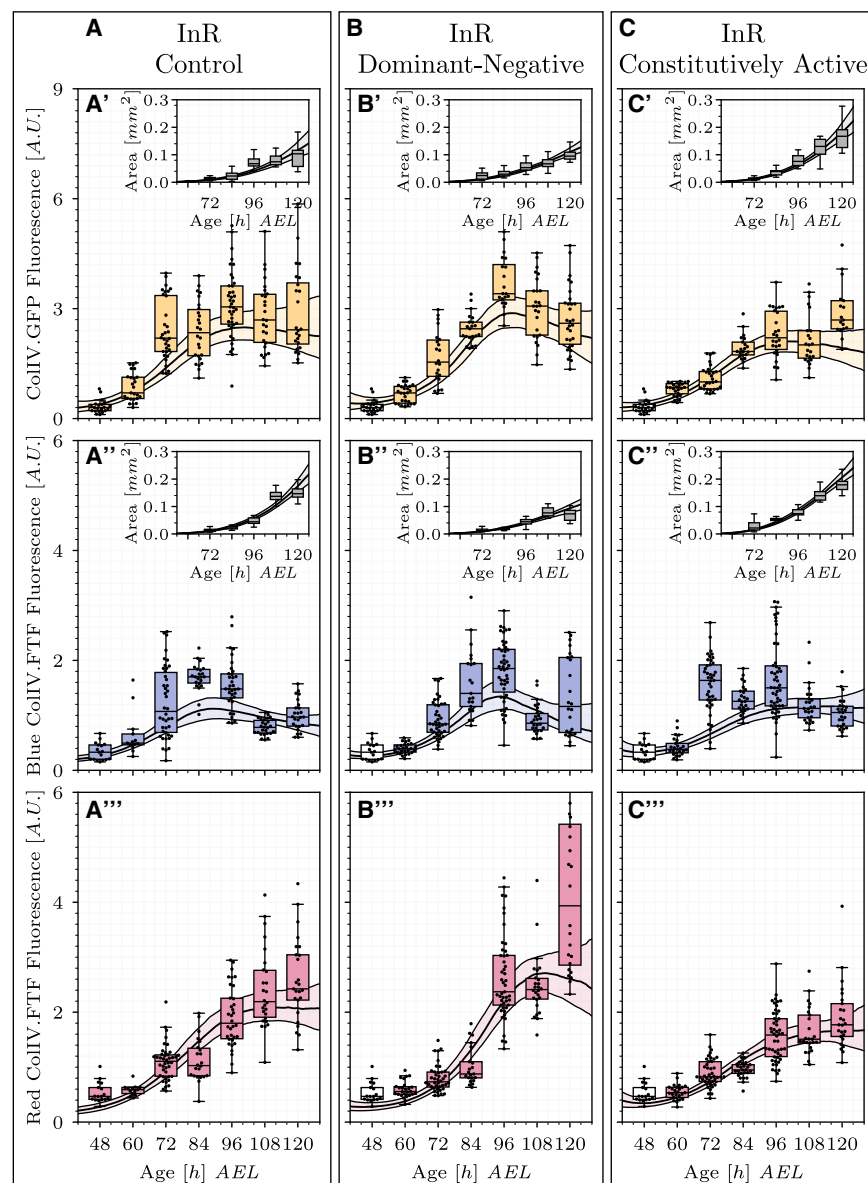


Figure 5. Increasing cell proliferation with a constitutively active insulin receptor reduces BM collagen IV mean lifetime

(A–C) colIV-GFP and colIV-FTF fluorescence intensity with age (after egg laying) data for three conditions: insulin receptor dominant negative (B), constitutively active (C), and their control (A). Dots represent wing discs; boxplots indicate first, second; and third quantiles. ColIV timer model estimates are given in terms of median (central solid black line) and first and third quantiles (solid black lines bounding a colored region). (A'), (B'), and (C') show BM colIV-GFP data overlaying the colIV timer model fit estimate for colIV-GFP. Inset plots show colIV-GFP wing disc pouch area overlaying colIV timer model estimate. (A''), (B''), and (C'') show blue BM colIV-FTF data overlaying colIV timer model fit estimate for blue colIV-FTF. Inset plots show colIV-FTF wing disc pouch area overlaying colIV timer model estimate. (A'''), (B'''), and (C''') show red BM colIV-FTF data overlaying colIV timer model fit estimate for red colIV-FTF. Boxplots at 48 h AEL for (B) and (C) are white to indicate that data at this time point are taken from the control (InR perturbations only switch on at around 60 h).

See also [Figures S5](#) and [S6](#).

the red state better characterizes how long colIV-FTF remains in the BM. Factors that decrease colIV-FTF concentration (dilution and loss) are more likely to affect the red state due to its prolonged exposure to these processes.

From our colIV-GFP data, we expect a drop in net turnover rate to around 80 h AEL ([Figure 3N](#)), which could be due to changes in either gain, loss, or both. Importantly, from the BM colIV-timer model, we find that the fraction of colIV-FTF in each fluorescent state depends only on the gain rate, not the loss rate ([supplemental methods](#), section S3.2); the fraction of blue

blue. For example, only a negligible amount of blue colIV-FTF gained at one time point will remain in the blue state 12 h later in the next time point, an effect that is exacerbated by loss and dilution ([Figure S4F](#)). The blue state therefore characterizes BM colIV-FTF gain, and the changing amount of blue state with age suggests that the rate of gain is changing over time. In contrast,

relative to red remains constant if we only change the loss rate ([Figure 4G](#), bottom row), whereas the fractions do change if we change only the gain rate ([Figure 4H](#), bottom row). Therefore, as we do observe a change in blue state relative to red with age in the *in vivo* wing discs ([Figures 5](#) and [6](#)), we conclude that the gain rate must be changing *in vivo*, so we proceed by modeling

(E) For slow turnover, colIV-FTF will have enough time in the BM to progress from the blue to the red state.

(F) Red-blue ratio steady state solutions for the BM colIV-FTF model in (C) are shown while varying the hemolymph colIV-FTF red-blue ratio along the x axis and varying the BM turnover rate along the y axis. Dashed lines with arrows indicate how variations in turnover rate lead to changes in the red-blue ratio. Greater variation in the red-blue ratio provides more accurate measurements of turnover rate.

(G–I) Three cases of the BM colIV-FTF model exhibiting differing gain- and loss-rate dynamics but the same net gain dynamics. Top two rows, solid line indicates gain rate and loss rate, respectively; dashed line indicates net gain rate. In middle two rows, vertical dashed lines indicate the location of the peak. (G) For sigmoidal loss rate and constant gain rate, colIV-FTF states form in-phase peaks, and distribution of timer state fractions remain constant. (H) For constant loss rate and sigmoidal gain rate, red peak is delayed relative to blue peak due to timer state redistribution. (I) Slowing the hemolymph colIV-FTF loss rate attenuates the blue state in favor of the red, while reducing delay between their peaks.

See also [Figure S4](#).

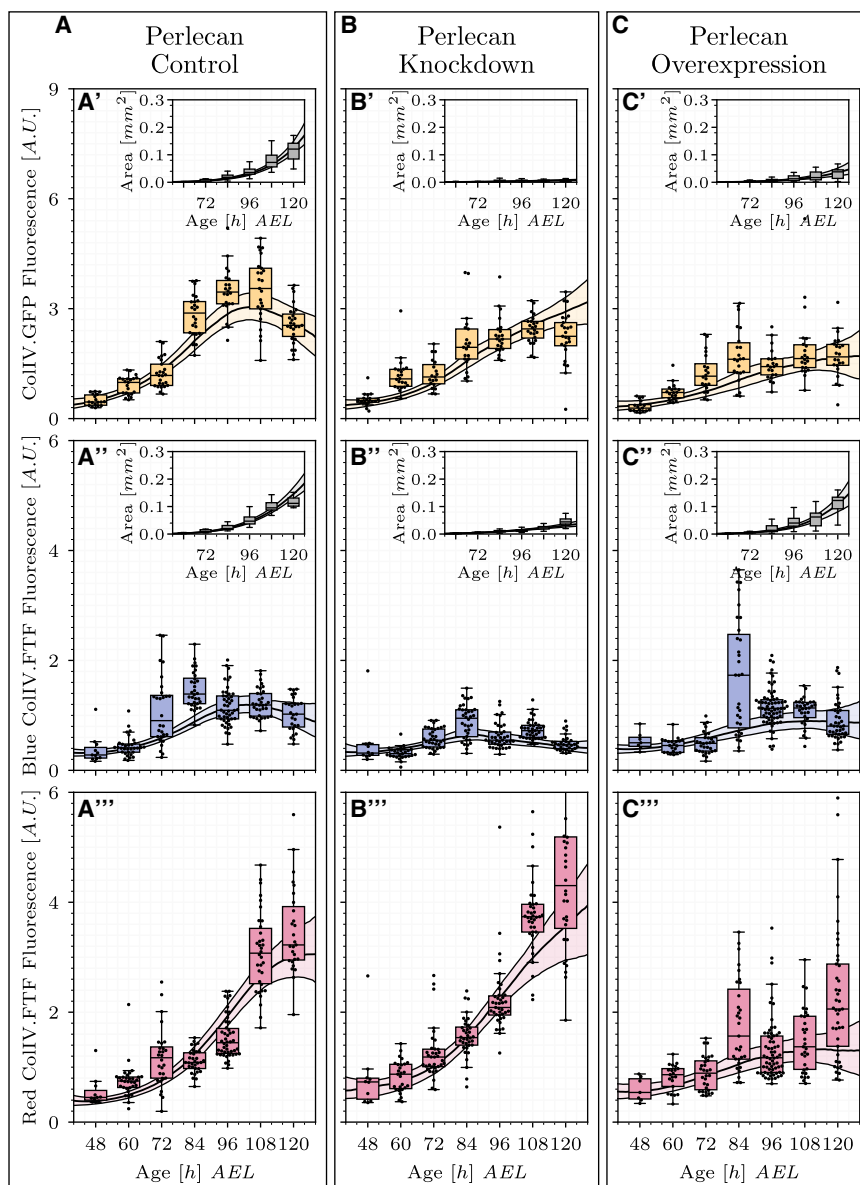


Figure 6. Gain or loss of perlecan leads to decreased or increased BM collagen IV mean lifetime, respectively

(A–C) colIV-GFP and colIV-FTF fluorescence intensity with age (after egg laying) data for three conditions: perlecan knockdown (B), perlecan overexpression (C), and their control (A). Dots represent wing discs, boxplots indicate first, second, and third quantiles. ColIV timer model estimates are given in terms of median (central solid black line) and first and third quantiles (solid black lines bounding a colored region). (A'), (B'), and (C') show BM colIV-GFP data overlaying colIV timer model fit estimate for colIV-GFP. Inset plots show colIV-GFP wing disc pouch area overlaying colIV timer model estimate. (A''), (B''), and (C'') show blue BM colIV-FTF data overlaying colIV timer model fit estimate for blue colIV-FTF. Inset plots show colIV-FTF wing disc pouch area overlaying colIV timer model estimate. (A'''), (B'''), and (C''') show red state BM colIV-FTF data overlaying colIV timer model fit estimate for red colIV-FTF.

See also [Figures S5](#) and [S6](#).

the gain rate with a sigmoid function. In summary, we believe the timer allows us to differentiate between gain and loss of BM collagen IV, and by fitting the collagen IV timer model to data, we can estimate BM collagen IV lifetime.

Next, we consider the impact of genetic perturbations on BM collagen IV lifetime. In principle, if the tissue grows faster, then collagen IV will be diluted more unless there is some compensation in collagen IV gain. To test this, we increase and decrease wing disc pouch growth by driving constitutively active and dominant-negative forms of insulin receptor (InR) ([Figures 5B](#) and [5C](#)). In principle, InR activity drives cellular growth, leading to an increase in tissue area, and, indeed, with constitutively active InR, we find area expansion is increased relative to control ([Figures 5A](#) and [5C](#), area inset plots). We also find colIV-GFP levels are generally reduced for constitutively active InR ([Figures 5A'](#) and [5C'](#)), suggesting that the increased growth drives greater collagen

IV dilution. This is consistent with the reduction in collagen IV concentration that we observe in the collagen IV network model when the network grows and re-models without introduction of new material ([Figure 2B](#), B.2). Blue colIV-FTF levels in the constitutively active InR are similar to control ([Figures 5A''](#) and [5C''](#)), implying that the amount of collagen IV entering the BM per unit area is maintained, while red colIV-FTF levels are reduced ([Figures 5A'''](#) and [5C'''](#)). Interestingly, the collagen IV timer model estimates elevated gain and loss rates for the constitutively active InR ([Figure S6N](#)), suggesting that InR activity is driving elevated turnover. We also observed elevated gain and loss rates in the collagen network

model when driving faster growth by increasing applied stress ([Figure S6O](#)), suggesting a possible BM-intrinsic physical origin for this growth-induced turnover. In summary, increased growth due to constitutively active InR leads to greater collagen IV dilution; however, the timer reveals an elevation in both collagen IV gain and reduction in collagen IV lifetime ([Figure S6M](#)), suggesting some interaction with turnover.

Perlecan changes collagen IV lifetime in the BM

We next considered BM components that may modify the mechanical properties of the BM. Perlecan is a highly conserved BM component that affects cell shape when modified¹⁴; hence, we wanted to explore whether it affects collagen IV lifetime. Perlecan requires collagen IV for BM localization in the wing disc, implying that perlecan binds collagen IV.^{14,16,21} Loss of perlecan causes wing disc cells to become constricted in the epithelial

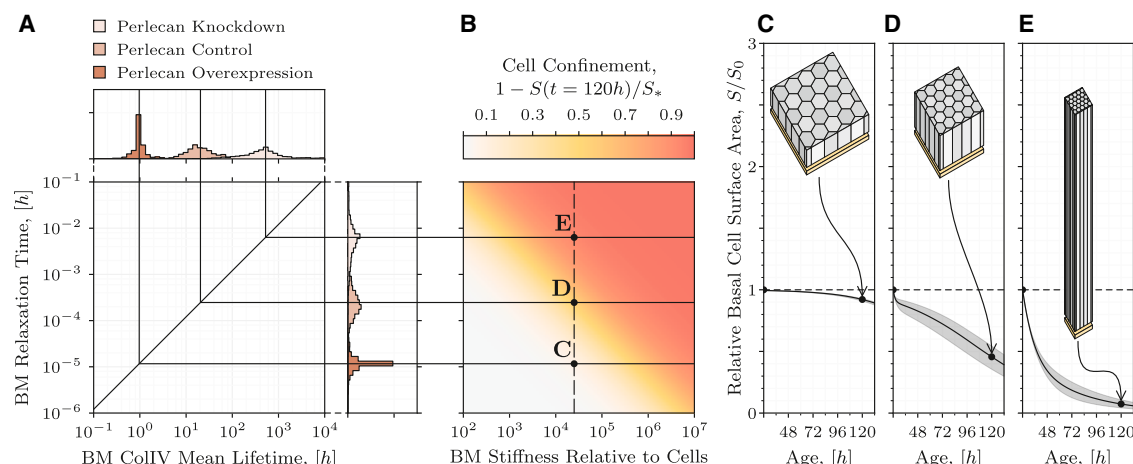


Figure 7. Connecting models using perlecan perturbations

(A) Using the linear relationship between collagen IV lifetime and BM relaxation time established by the collagen IV network model (Figure 2), distributions for BM collagen IV lifetimes estimated by fitting the collagen IV timer model to colIV-FTF data for perlecan perturbations (Figure 6) can be transformed into BM relaxation time distributions.

(B) Estimated relaxation time distributions for perlecan perturbations are transformed into cell shapes with the tissue mechanical model (Figure 1), given a BM stiffness relative to cells.

(C–E) Relative basal cell surface trajectories for the median BM relaxation times of the three perlecan conditions indicated in (B): perlecan overexpression (C), control (D), and knockdown (E), respectively. Gray shaded region indicates the distribution of trajectories, as quantified by the first and third quartile, due to the distribution of BM relaxation times.

plane and extended along the apicobasal axis, and the reverse occurs for perlecan overexpression.¹⁴ This suggests that perlecan is modulating the mechanical properties of the BM. We hypothesize from our tissue mechanics model that perlecan loss increases BM relaxation time, leading to the observed confinement of cells (as in Figure 1J), and vice versa when perlecan is overexpressed. From our collagen IV network model (MD simulations), we found that the lifetime of collagen IV in a network is proportional to the relaxation time of the network (Figure 2E); therefore, if perturbing perlecan affects BM relaxation rate, we should see a change in BM collagen IV lifetime as a readout. We therefore used the collagen IV timer model to probe the lifetime of collagen IV in perlecan knockdown and overexpression conditions.

Experimentally, we find perlecan knockdown, and to a much lesser extent perlecan overexpression, have a decreased area expansion rate compared with their control (Figures 6A–6C, inset area plots). With perlecan knockdown, we observe a decrease in blue colIV-FTF and an increase in red compared with the control (Figures 6A and 6B). With perlecan overexpression, we observe a decrease in red colIV-FTF levels and a slight decrease in blue (Figures 6A and 6C). For perlecan overexpression, we find a BM collagen IV mean lifetime of approximately 1 h, compared with approximately 20 h for its control and approximately 500 h mean lifetime for perlecan knockdown. Using the proportionality relationship derived from the collagen IV network model (MD simulations), these experimentally measured changes in collagen IV lifetimes give rise to changes in BM stress relaxation times: shorter for perlecan overexpression and longer for perlecan knockdown (Figure 7A). Our tissue mechanics model (Figure 7B) then predicts that the shorter BM relaxation time in perlecan overexpression will result in less cell confinement and

shorter, wider cells (Figure 7C), whereas the longer BM relaxation time in perlecan knockdown will result in more confined and taller, narrower cells (Figure 7E) compared with the control cell shapes (Figure 7D). This matches the previously reported relative changes in cell shape in perlecan overexpression and knockdown conditions.¹⁴ Together, these results suggest that perlecan promotes bond breakage in the collagen IV network, thereby promoting the BM to relax stress and expand during organ growth.

In summary, by coupling our collagen IV timer reporter measurements with different mathematical models, we can measure at the molecular scale the lifetime of collagen IV molecules in the BM and how this influences network-scale stress relaxation to impact tissue-scale cell shape.

DISCUSSION

By employing three complementary quantitative modeling approaches that bridge molecular to tissue scales, we show that the turnover of molecules in the BM regulates stress relaxation to control cell shape during organ growth.

We use mathematical modeling to show that, for proliferating epithelia, cell confinement can be generated when BM stress relaxation time is longer than cell proliferation time. We focused on developing methods to determine BM stress relaxation, a previously unexplored property. Using molecular dynamics simulations, we find that, in a BM-like network, the stress relaxation time of the network is proportional to collagen IV mean lifetime. We therefore experimentally measured collagen IV lifetime in the *Drosophila* wing disc—a highly proliferative epithelium—as a proxy for BM relaxation time. Nonlocal production of collagen IV in the wing disc necessitates an *in vivo* method of measuring

collagen IV lifetime. We endogenously tagged collagen IV with a fluorescent timer protein. We exploit slow-progressing fluorescent timers, often used to study fast turnover of intracellular proteins,^{46–48} to measure slow *in vivo* collagen IV turnover.^{45,49} The fluorescent timer essentially accumulates information about collagen IV *in vivo* as the organism develops over days. Through mathematical modeling of the timer behavior and quantitative analysis, we can estimate a lifetime for collagen IV in the wing disc BM and determine how this lifetime changes in genetic mutants.

Perlecan is a BM component of particular relevance due to its effect on wing discs: perlecan knockdown leads to apparent cell confinement and vice versa for overexpression.¹⁴ Using our timer-tagged collagen IV, we find that perlecan promotes collagen IV turnover, thereby promoting BM stress relaxation and therefore tissue expansion. Roles of collagen IV and perlecan are generally seen as opposing; collagen IV positively correlates with BM stiffness, whereas perlecan negatively correlates with BM stiffness.^{14,16} However, morphogenetic processes are dynamic, and tissue shape is impacted by the rate at which the BM adapts. We therefore propose new roles of collagen IV and perlecan: while collagen IV determines the stiffness of the BM, perlecan regulates collagen IV remodeling and, therefore, BM viscosity.

With respect to epithelial morphogenesis, we propose that mechanical interplay between the growing cellular layer and the resisting BM layer determines cell shape and, therefore, tissue shape. BM thickness is generally found to increase with age,^{50–53} particularly that of human collagen IV.^{52,54} However, factors that regulate BM turnover are largely unknown. It would be interesting to determine whether perlecan plays a role in aging-related effects on the BM. Being able to directly measure ECM remodeling rates and determine their regulating factors at different time points *in vivo* has been a long-standing challenge. To this end, our multi-scale framework opens new opportunities with respect to development, aging, and disease. Importantly, studying the molecular turnover of proteins will enable us to deduce the macroscopic emergent properties of the complex systems they create.

Limitations of the study

We do not consider how feedback affects parameters in our models, such as cell growth mechano-regulation, stress-induced dissociation, or stabilization of BM interactions.⁵⁵ We have also assumed that the BM is passive and that stresses are only generated actively by cells. However, it has been suggested that *de novo* polymerization of the BM could itself be a source of stress.²⁰ How the BM remodels in response to stress and whether it is even able to generate stress itself remain interesting open questions.

Another limitation of our study is that we use collagen IV as a proxy to estimate the stress relaxation dynamics of the whole BM. In reality, the BM consists of many other components, which can each contribute differently to the collective dynamics of the BM. How each of these proteins turns over and contributes to the BM's mechanical properties will require further systematic research.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yanlan Mao (y.mao@ucl.ac.uk).

Materials availability

The fly line generated in this study is available from the [lead contact](#) without restriction.

Data and code availability

- Collagen IV fluorescent timer image data and associated data analysis code have been deposited in the UCL Research Data Repository at <https://doi.org/10.5522/04/32647191> and are publicly available as of the date of publication.
- All original code associated with the tissue mechanics model has also been deposited in the UCL Research Data Repository at <https://doi.org/10.5522/04/32647191> and is publicly available as of the date of publication.
- All original code associated with the collagen IV network model has been deposited on Zenodo at <https://doi.org/10.5281/zenodo.20719515> and is publicly available as of the date of publication.
- Any additional information reported in this paper is available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

We thank all members of the Mao lab for insightful discussions throughout the project, and we thank Giulia Paci and Nargess Khailgharibi for critical reading of the manuscript. We thank Jonathan Chubb for molecular biology guidance and WellGenetics for generating the timer protein fly lines.

R.B. was funded by a UCL COMPLEX PhD studentship and a Leverhulme Trust project grant RPG-2020-068 awarded to Y.M. B.M. and A.S. were funded by the European Union Horizon 2020 research and innovation programme (ERC starting grant no. 802960). S.B. was funded by NIH R35 GM143042. G.C. was funded by EPSRC Physics of Life, grants EP/W023806 and EP/W023865. B.J.S.S. and B.M.S. are funded by the Wellcome Trust (grant no. 301803/Z/23/Z to B.M.S.). E.K.P. was funded by the Medical Research Council UK (MRC programme award MC_UU_12018/5). Y.M. was funded by MRC award MR/W027437/1, a Lister Institute Research Prize, and the EMBO Young Investigator Programme.

AUTHOR CONTRIBUTIONS

R.B. and Y.M. conceived the project. R.B. performed all experiments, data analysis, and mathematical modeling, except for the molecular dynamics simulations, which were performed by B.M. B.J.S.-S. and B.M.S. assisted with the design and generation of the timer protein fly lines. E.K.P. and G.C. assisted with data interpretation. R.B. and S.B. developed the tissue mechanics model. A.S. supervised B.M. R.B. and Y.M. interpreted the data and wrote the manuscript, with input from all authors. Y.M. coordinated and supervised the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS](#)
 - Fly Genetics
 - Fly Staging

- **METHOD DETAILS**
 - Tissue Mechanics Model
 - Collagen IV Network Molecular Dynamics Model
 - BM Collagen IV Concentration Model
 - BM Collagen IV Timer Model
 - Imaging
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
 - Image Analysis and Quantification

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cell.2026.07.010>.

Received: September 11, 2025

Revised: June 25, 2026

Accepted: July 8, 2026

Published: July 30, 2026

REFERENCES

1. Molnar, K., and Labouesse, M. (2021). The plastic cell: mechanical deformation of cells and tissues. *Open Biol.* 11, 210006. <https://doi.org/10.1098/rsob.210006>.
2. LeGoff, L., and Lecuit, T. (2015). Mechanical Forces and Growth in Animal Tissues. *Cold Spring Harb. Perspect. Biol.* 8, a019232. <https://doi.org/10.1101/cshperspect.a019232>.
3. Paci, G., and Mao, Y. (2021). Forced into shape: Mechanical forces in *Drosophila* development and homeostasis. *Semin. Cell Dev. Biol.* 120, 160–170. <https://doi.org/10.1016/j.semcdb.2021.05.026>.
4. Heisenberg, C.-P., and Bellaïche, Y. (2013). Forces in Tissue Morphogenesis and Patterning. *Cell* 153, 948–962. <https://doi.org/10.1016/j.cell.2013.05.008>.
5. Villeneuve, C., Hashmi, A., Ylivinkka, I., Lawson-Keister, E., Miroshnikova, Y.A., Pérez-González, C., Myllymäki, S.-M., Bertillot, F., Yadav, B., Zhang, T., et al. (2024). Mechanical forces across compartments coordinate cell shape and fate transitions to generate tissue architecture. *Nat. Cell Biol.* 26, 207–218. <https://doi.org/10.1038/s41556-023-01332-4>.
6. Savin, T., Kurpios, N.A., Shyer, A.E., Florescu, P., Liang, H., Mahadevan, L., and Tabin, C.J. (2011). On the growth and form of the gut. *Nature* 476, 57–62. <https://doi.org/10.1038/nature10277>.
7. Mao, Y., and Baum, B. (2015). Tug of war—The influence of opposing physical forces on epithelial cell morphology. *Dev. Biol.* 401, 92–102. <https://doi.org/10.1016/j.ydbio.2014.12.030>.
8. Mao, Y., and Wickström, S.A. (2024). Mechanical state transitions in the regulation of tissue form and function. *Nat. Rev. Mol. Cell Biol.* 25, 654–670. <https://doi.org/10.1038/s41580-024-00719-x>.
9. Khalilgharibi, N., Fouchard, J., Asadipour, N., Barrientos, R., Duda, M., Bonfanti, A., Yonis, A., Harris, A., Mosaffa, P., Fujita, Y., et al. (2019). Stress relaxation in epithelial monolayers is controlled by the actomyosin cortex. *Nat. Phys.* 15, 839–847. <https://doi.org/10.1038/s41567-019-0516-6>.
10. Wyatt, T., Baum, B., and Charras, G. (2016). A question of time: tissue adaptation to mechanical forces. *Curr. Opin. Cell Biol.* 38, 68–73. <https://doi.org/10.1016/j.ceb.2016.02.012>.
11. Yurchenco, P.D. (2011). Basement Membranes: Cell Scaffoldings and Signaling Platforms. *Cold Spring Harb. Perspect. Biol.* 3, a004911. <https://doi.org/10.1101/cshperspect.a004911>.
12. Khalilgharibi, N., and Mao, Y. (2021). To form and function: on the role of basement membrane mechanics in tissue development, homeostasis and disease. *Open Biol.* 11, 200360. <https://doi.org/10.1098/rsob.200360>.
13. Urbano, J.M., Torgler, C.N., Molnar, C., Tepass, U., López-Varea, A., Brown, N.H., de Celis, J.F., and Martín-Bermudo, M.D. (2009). *Drosophila* laminins act as key regulators of basement membrane assembly and morphogenesis. *Development* 136, 4165–4176. <https://doi.org/10.1242/dev.044263>.
14. Pastor-Pareja, J.C., and Xu, T. (2011). Shaping Cells and Organs in *Drosophila* by Opposing Roles of Fat Body-Secreted Collagen IV and Perlecan. *Dev. Cell* 21, 245–256. <https://doi.org/10.1016/j.devcel.2011.06.026>.
15. Yurchenco, P.D., and Ruben, G.C. (1987). Basement membrane structure in situ: evidence for lateral associations in the type IV collagen network. *J. Cell Biol.* 105, 2559–2568. <https://doi.org/10.1083/jcb.105.6.2559>.
16. Isabella, A.J., and Horne-Badovinac, S. (2015). Dynamic regulation of basement membrane protein levels promotes egg chamber elongation in *Drosophila*. *Dev. Biol.* 406, 212–221. <https://doi.org/10.1016/j.ydbio.2015.08.018>.
17. Dai, J., Estrada, B., Jacobs, S., Sánchez-Sánchez, B.J., Tang, J., Ma, M., Magadán-Corpas, P., Pastor-Pareja, J.C., and Martín-Bermudo, M.D. (2018). Dissection of Nidogen function in *Drosophila* reveals tissue-specific mechanisms of basement membrane assembly. *PLOS Genet.* 14, e1007483. <https://doi.org/10.1371/journal.pgen.1007483>.
18. Wolfstetter, G., Dahlitz, I., Pfeifer, K., Töpfer, U., Alt, J.A., Pfeifer, D.C., Lakes-Harlan, R., Baumgartner, S., Palmer, R.H., and Holz, A. (2019). Characterization of *Drosophila* Nidogen/entactin reveals roles in basement membrane stability, barrier function and nervous system patterning. *Development* 146, dev168948. <https://doi.org/10.1242/dev.168948>.
19. Shahab, J., Baratta, C., Scuric, B., Godt, D., Venken, K.J.T., and Ringette, M.J. (2015). Loss of SPARC dysregulates basal lamina assembly to disrupt larval fat body homeostasis in *Drosophila melanogaster*. *Dev. Dyn.* 244, 540–552. <https://doi.org/10.1002/dvdy.24243>.
20. Serna-Morales, E., Sánchez-Sánchez, B.J., Marcotti, S., Nichols, A., Bhargava, A., Dragu, A., Hirvonen, L.M., Díaz-de-la-Loza, M.-D.-C., Mink, M., Cox, S., et al. (2023). Extracellular matrix assembly stress initiates *Drosophila* central nervous system morphogenesis. *Dev. Cell* 58, 825–835.e6. <https://doi.org/10.1016/j.devcel.2023.03.019>.
21. Matsubayashi, Y., Louani, A., Dragu, A., Sánchez-Sánchez, B.J., Serna-Morales, E., Yolland, L., Gyoergy, A., Vizcay, G., Fleck, R.A., Heddleston, J.M., et al. (2017). A Moving Source of Matrix Components Is Essential for De Novo Basement Membrane Formation. *Curr. Biol.* 27, 3526–3534.e4. <https://doi.org/10.1016/j.cub.2017.10.001>.
22. Matsubayashi, Y., Sánchez-Sánchez, B.J., Marcotti, S., Serna-Morales, E., Dragu, A., Díaz-de-la-Loza, M.-D.-C., Vizcay-Barrena, G., Fleck, R.A., and Stramer, B.M. (2020). Rapid Homeostatic Turnover of Embryonic ECM during Tissue Morphogenesis. *Dev. Cell* 54, 33–42.e9. <https://doi.org/10.1016/j.devcel.2020.06.005>.
23. Fiore, V.F., Krajnc, M., Quiroz, F.G., Levorse, J., Pasolli, H.A., Shvartsman, S.Y., and Fuchs, E. (2020). Mechanics of a multilayer epithelium instruct tumour architecture and function. *Nature* 585, 433–439. <https://doi.org/10.1038/s41586-020-2695-9>.
24. Wuergeshen, D., Gindroz, E., Morita, R., Hashimoto, K., Abe, T., Kiyonari, H., and Fujiwara, H. (2024). An eGFP-Col4a2 mouse model reveals basement membrane dynamics underlying hair follicle morphogenesis. *J. Cell Biol.* 224, e202404003. <https://doi.org/10.1083/jcb.202404003>.
25. Gennes, P.-G. (1979). *Scaling Concepts in Polymer Physics, First Edition* (Cornell University Press).
26. Rubinstein, M., Colby, R.H., Rubinstein, M., and Colby, R.H. (2003). *Polymer Physics* (Oxford University Press). <https://doi.org/10.1093/oso/9780198520597.001.0001>.
27. Vernerey, F.J., Long, R., and Brighenti, R. (2017). A statistically-based continuum theory for polymers with transient networks. *J. Mech. Phys. Solids* 107, 1–20. <https://doi.org/10.1016/j.jmps.2017.05.016>.
28. Meng, F., Saed, M.O., and Terentjev, E.M. (2022). Rheology of vitrimers. *Nat. Commun.* 13, 5753. <https://doi.org/10.1038/s41467-022-33321-w>.
29. Harmansa, S., Erlich, A., Eloy, C., Zurlo, G., and Lecuit, T. (2023). Growth anisotropy of the extracellular matrix shapes a developing organ. *Nat. Commun.* 14, 1220. <https://doi.org/10.1038/s41467-023-36739-y>.

30. Sui, L., Alt, S., Weigert, M., Dye, N., Eaton, S., Jug, F., Myers, E.W., Jülicher, F., Salbreux, G., and Dahmann, C. (2018). Differential lateral and basal tension drive folding of *Drosophila* wing discs through two distinct mechanisms. *Nat. Commun.* 9, 4620. <https://doi.org/10.1038/s41467-018-06497-3>.
31. Kumar, N., Rangel Ambriz, J., Tsai, K., Mim, M.S., Flores-Flores, M., Chen, W., Zartman, J.J., and Alber, M. (2024). Balancing competing effects of tissue growth and cytoskeletal regulation during *Drosophila* wing disc development. *Nat. Commun.* 15, 2477. <https://doi.org/10.1038/s41467-024-46698-7>.
32. Bittig, T., Wartlick, O., González-Gaitán, M., and Jülicher, F. (2009). Quantification of growth asymmetries in developing epithelia. *Eur. Phys. J. E Soft Matter* 30, 93–99. <https://doi.org/10.1140/epje/i2009-10507-6>.
33. Bryant, P.J. (1970). Cell lineage relationships in the imaginal wing disc of *Drosophila melanogaster*. *Dev. Biol.* 22, 389–411. [https://doi.org/10.1016/0012-1606\(70\)90160-0](https://doi.org/10.1016/0012-1606(70)90160-0).
34. Domínguez-Giménez, P., Brown, N.H., and Martín-Bermudo, M.D. (2007). Integrin-ECM interactions regulate the changes in cell shape driving the morphogenesis of the *Drosophila* wing epithelium. *J. Cell Sci.* 120, 1061–1071. <https://doi.org/10.1242/jcs.03404>.
35. Delon, I., and Brown, N.H. (2009). The integrin adhesion complex changes its composition and function during morphogenesis of an epithelium. *J. Cell Sci.* 122, 4363–4374. <https://doi.org/10.1242/jcs.055996>.
36. Goodwin, K., Ellis, S.J., Lostchuck, E., Zulueta-Coarasa, T., Fernandez-Gonzalez, R., and Tanentzapf, G. (2016). Basal Cell-Extracellular Matrix Adhesion Regulates Force Transmission during Tissue Morphogenesis. *Dev. Cell* 39, 611–625. <https://doi.org/10.1016/j.devcel.2016.11.003>.
37. Tozluoğlu, M., Duda, M., Kirkland, N.J., Barrientos, R., Burden, J.J., Muñoz, J.J., and Mao, Y. (2019). Planar Differential Growth Rates Initiate Precise Fold Positions in Complex Epithelia. *Dev. Cell* 51, 299–312.e4. <https://doi.org/10.1016/j.devcel.2019.09.009>.
38. Gelbart, M.A., He, B., Martin, A.C., Thiberge, S.Y., Wieschaus, E.F., and Kaschube, M. (2012). Volume conservation principle involved in cell lengthening and nucleus movement during tissue morphogenesis. *Proc. Natl. Acad. Sci. USA* 109, 19298–19303. <https://doi.org/10.1073/pnas.1205258109>.
39. Venkova, L., Vishen, A.S., Lembo, S., Srivastava, N., Duchamp, B., Ruppel, A., Williard, A., Vassilopoulos, S., Deslys, A., Garcia Arcos, J.M., et al. (2022). A mechano-osmotic feedback couples cell volume to the rate of cell deformation. *eLife* 11, e72381. <https://doi.org/10.7554/eLife.72381>.
40. Neufeld, T.P., de la Cruz, A.F.A., Johnston, L.A., and Edgar, B.A. (1998). Coordination of Growth and Cell Division in the *Drosophila* Wing. *Cell* 93, 1183–1193. [https://doi.org/10.1016/S0092-8674\(00\)81462-2](https://doi.org/10.1016/S0092-8674(00)81462-2).
41. Mao, Y., Tournier, A.L., Hoppe, A., Kester, L., Thompson, B.J., and Tapon, N. (2013). Differential proliferation rates generate patterns of mechanical tension that orient tissue growth. *EMBO J.* 32, 2790–2803. <https://doi.org/10.1038/emboj.2013.197>.
42. Chen, D.T.N., Wen, Q., Janmey, P.A., Crocker, J.C., and Yodh, A.G. (2010). Rheology of Soft Materials. *Annu. Rev. Condens. Matter Phys.* 1, 301–322. <https://doi.org/10.1146/annurev-conmatphys-070909-104120>.
43. Meadowcroft, B., Sorichetti, V., Ratajczyk, E., Pérez-Verdugo, F., Khalilgharibi, N., Mao, Y., Palaia, I., and Šarić, A. (2025). Nonequilibrium Remodeling of Collagen IV Networks in Silico. *PRX Life* 3, 033019. <https://doi.org/10.1103/gdd5-mh7>.
44. Khmelinskii, A., Keller, P.J., Bartosik, A., Meurer, M., Barry, J.D., Mardin, B.R., Kaufmann, A., Trautmann, S., Wachsmuth, M., Pereira, G., et al. (2012). Tandem fluorescent protein timers for in vivo analysis of protein dynamics. *Nat. Biotechnol.* 30, 708–714. <https://doi.org/10.1038/nbt.2281>.
45. Subach, F.V., Subach, O.M., Gundorov, I.S., Morozova, K.S., Piatkevich, K.D., Cuervo, A.M., and Verkhusha, V.V. (2009). Monomeric fluorescent timers that change color from blue to red report on cellular trafficking. *Nat. Chem. Biol.* 5, 118–126. <https://doi.org/10.1038/nchembio.138>.
46. Barry, J.D., Donà, E., Gilmour, D., and Huber, W. (2016). TimerQuant: a modelling approach to tandem fluorescent timer design and data interpretation for measuring protein turnover in embryos. *Development* 143, 174–179. <https://doi.org/10.1242/dev.125971>.
47. He, L., Binari, R., Huang, J., Falo-Sanjuán, J., and Perrimon, N. (2019). In vivo study of gene expression with an enhanced dual-color fluorescent transcriptional timer. *eLife* 8, e46181. <https://doi.org/10.7554/eLife.46181>.
48. Durrieu, L., Kirmaier, D., Schneidt, T., Kats, I., Raghavan, S., Hufnagel, L., Saunders, T.E., and Knop, M. (2018). Bicoid gradient formation mechanism and dynamics revealed by protein lifetime analysis. *Mol. Syst. Biol.* 14, e8355. <https://doi.org/10.15252/msb.20188355>.
49. Subach, O.M., Tashkeev, A., Vlaskina, A.V., Petrenko, D.E., Gaivoronskii, F.A., Nikolaeva, A.Y., Ivashkina, O.I., Anokhin, K.V., Popov, V.O., Boyko, K.M., et al. (2022). The mRubyFT Protein, Genetically Encoded Blue-to-Red Fluorescent Timer. *Int. J. Mol. Sci.* 23, 3208. <https://doi.org/10.3390/ijms23063208>.
50. Xi, Y.P., Nette, E.G., King, D.W., and Rosen, M. (1982). Age-related changes in normal human basement membrane. *Mech. Ageing Dev.* 19, 315–324. [https://doi.org/10.1016/0047-6374\(82\)90015-X](https://doi.org/10.1016/0047-6374(82)90015-X).
51. Anderson, S., and Brenner, B.M. (1986). Effects of aging on the renal glomerulus. *Am. J. Med.* 80, 435–442. [https://doi.org/10.1016/0002-9343\(86\)90718-7](https://doi.org/10.1016/0002-9343(86)90718-7).
52. Candiello, J., Cole, G.J., and Halfter, W. (2010). Age-dependent changes in the structure, composition and biophysical properties of a human basement membrane. *Matrix Biol.* 29, 402–410. <https://doi.org/10.1016/j.matbio.2010.03.004>.
53. Vázquez, F., Palacios, S., Alemañ, N., and Guerrero, I. (1996). Changes of the basement membrane and type IV collagen in human skin during aging. *Maturitas* 25, 209–215. [https://doi.org/10.1016/S0378-5122\(96\)01066-3](https://doi.org/10.1016/S0378-5122(96)01066-3).
54. Uspenskaia, O., Liebetrau, M., Herms, J., Danek, A., and Hamann, G.F. (2004). Aging is associated with increased collagen type IV accumulation in the basal lamina of human cerebral microvessels. *BMC Neurosci.* 5, 37. <https://doi.org/10.1186/1471-2202-5-37>.
55. Kelley, L.C., Chi, Q., Cáceres, R., Hastie, E., Schindler, A.J., Jiang, Y., Matus, D.Q., Plastino, J., and Sherwood, D.R. (2019). Adaptive F-Actin Polymerization and Localized ATP Production Drive Basement Membrane Invasion in the Absence of MMPs. *Dev. Cell* 48, 313–328.e8. <https://doi.org/10.1016/j.devcel.2018.12.018>.
56. Dye, N.A., Popović, M., Spann, S., Etournay, R., Kainmüller, D., Ghosh, S., Myers, E.W., Jülicher, F., and Eaton, S. (2017). Cell dynamics underlying oriented growth of the *Drosophila* wing imaginal disc. *Development* 144, 4406–4421. <https://doi.org/10.1242/dev.155069>.
57. Thompson, A.P., Aktulga, H.M., Berger, R., Bolintineanu, D.S., Brown, W.M., Crozier, P.S., in 't Veld, P.J., Kohlmeyer, A., Moore, S.G., Nguyen, T.D., et al. (2022). LAMMPS – a flexible simulation tool for particle-based materials modeling at the atomic, meso, and continuum scales. In *Comput. Phys. Commun.*, 271, A. Kohlmeyer, S.G. Moore, and T.D. Nguyen, et al., eds., pp. 108171. <https://doi.org/10.1016/j.cpc.2021.108171>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
yw; Vkg.GFP.G00454;	BDSC 98343	FBti0153267; RRID:BDSC_98343
w; Vkg.FTF;	This paper	N/A
w; Act5C-Gal4	BDSC 3954	FBti0012292; RRID:BDSC_3954
w; Pdm2-Gal4	BDSC 49828	FBti0132980; RRID:BDSC_49828
w; UAS-TrolRNAi;	VDRC 24549	FBti0096489; RRID:Flybase_FBst0455475
yw, UAS-Trol;;	DGRC 201–233	FBti0207019; RRID:DGGR_201233
yw; UAS-InrDN;	BDSC 8252	FBti0040688; RRID:BDSC_8252
yw; UAS-InRCA;	BDSC 8263	FBti0040693; RRID:BDSC_8263
Chemicals, peptides, and recombinant proteins		
Grace's Media	Sigma-Aldrich	G9771-1L
Fetal Bovine Serum	Sigma-Aldrich	F4135-500ML
Penicillin-Streptomycin	ThermoFisher	15140-122
Cell Tak	Corning	354240
InSpec™ Blue (350/440)	ThermoFisher	I7221
InSpec™ Green (505/515)	ThermoFisher	I7219
InSpec™ Red (580/605)	ThermoFisher	I7224
Oligonucleotides		
Vkg Left Homology Arm Fwd Primer: ACTTCCGGCATGAAAACATC	This paper	N/A
Vkg Left Homology Arm Rev Primer: CGAATTGTGCATTGAGTTAACTG	This paper	N/A
Vkg Right Homology Arm Fwd Primer: nGCCATCATAAGTGTGCAGCT	This paper	N/A
Vkg Right Homology Arm Rev Primer: AATGGTGCAGAACGTCAAAA	This paper	N/A
Vkg gRNA Sense Oligo: CTTCGATGGCAAACACGTGAGCGA	This paper	N/A
Vkg gRNA Antisense Oligo: AAACTCGCTCACGTGTTTGCCATC	This paper	N/A
Recombinant DNA		
Plasmid: pFast-FT-N1	Addgene 31910	RRID: Addgene_31910
Plasmid: pSlow-FT-N1	Addgene 31912	RRID: Addgene_31912
Plasmid: pBS-KS-attB1-2-PT-SA-SD-0	DGRC 1297	RRID: DGRC_1297
Deposited Data		
Collagen IV fluorescent timer data and code, and tissue mechanics model code	UCL Research Data Repository	https://doi.org/10.5522/04/32647191
Collagen IV network model code	Zenodo	https://doi.org/10.5281/zenodo.20719515
Software and algorithms		
Fiji	https://imagej.net/software/fiji/	RRID:SCR_002285
Napari	https://github.com/napari/napari	RRID:SCR_022765
Scipy	https://scipy.org/	RRID:SCR_008058

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Fly Genetics

The following fly lines were used in this work (see [Key Resources Table](#)). Vkg.GFP (BDSC 98343), Pdm2-Gal4 (BDSC 49828), Act5C-Gal4 (BDSC 3954), UAS-InR.DN (BDSC 8252) and UAS-InR.CA (BDSC 8263) were sourced from Bloomington Drosophila Stock Center. UAS-Trol.RNAi (VDRC 24549) was sourced from Vienna Drosophila Resource Center. UAS-Trol (DGRC 201–233) was sourced from Drosophila Genomics Resource Center. Vkg.FTF was generated for this study (see below). Genotypes for each experiment are presented with reference to their figures in the table below. For all fluorescence intensity quantification experiments fluorescent transgenes were kept homozygous.

Genotypes by figure	
Genotype	Figures
w/w; Vkg.GFP/Vkg.GFP; Pdm2-Gal4/+	Figures 3E–3L and 5A
w/w; Vkg.GFP/Vkg.GFP; Act5C-Gal4/+	Figures 3M, 3N, and 6A
w/w; Vkg.FTF/Vkg.FTF; Pdm2-Gal4/+	Figure 5A
w/w; Vkg.GFP,UAS-InR.DN/Vkg.GFP; Pdm2-Gal4/+	Figure 5A
w/w; Vkg.FTF,UAS-InR.DN/Vkg.FTF; Pdm2-Gal4/+	Figure 5B
w/w; Vkg.GFP,UAS-InR.CA/Vkg.GFP; Pdm2-Gal4/+	Figure 5B
w/w; Vkg.FTF,UAS-InR.CA/Vkg.FTF; Pdm2-Gal4/+	Figure 5C
w/w; Vkg.FTF/Vkg.FTF; Act5C-Gal4/+	Figures 6A and S6A–S6D
w/w; Vkg.GFP,UAS-Trol.RNAi/Vkg.GFP; Act5C-Gal4/+	Figure 6B
w/w; Vkg.FTF,UAS-Trol.RNAi/Vkg.FTF; Act5C-Gal4/+	Figures 6B and S6E–S6H
yw,UAS-Trol/w; Vkg.GFP/Vkg.GFP; Act5C-Gal4/+	Figure 6C
yw,UAS-Trol/w; Vkg.FTF/Vkg.FTF; Act5C-Gal4/+	Figures 6C and S6I–S6L

ColIV-FTF knockin line (Vkg.FTF) was generated by first creating a landing site in the Vkg gene by inserting an RMCE-capable cassette by CRISPR-Cas9 mediated homology-dependent repair. The insertion cassette contains, in order, a forward attP site, an Mhc intron 18 splice acceptor, a ph0-4xGGS linker, the slow FT sequence, a 4xGGS linker, an Mhc intron 17 splice donor, a reverse attP site and a floxed white maker. FT sequences were obtained from plasmids pFast-FT-N1 (Addgene 31910) and pSlow-FT-N1 (Addgene 31912), gifts from Vladislav Verkhusha. The cassette was targeted 1589bp from the ATG of the Vkg gene, close to the GFP insertion point of the well-characterised Vkg.GFP (BDSC 98343) fly line. Homology arms were amplified using primers 5'-ACTTCCGGCATGAAAACATC, 5'-CGAATTGTGCATTGAGTTAACTG, 5'-GCCATCATAAGTGTGCAGCT and 5'-AATGGTGCA GAACGTCAAAA (see [Key Resources Table](#)). The gRNA fragment was generated by anealling oligos 5'-CTTCGATGGCAAAACAGT GAGCGA and 5'-AAACTCGCTCACGTGTTGCCATC (see [Key Resources Table](#)). After generating and validating successful insertions, one line was taken forward for RMCE. The Fast FT sequence was inserted into the protein trap cassette plasmid pBS-KS-attB1-2-PT-SA-SD-0 (DGRC 1297), and exchanged with slow FT by phC31-mediated RMCE to generate Vkg.FTF fly lines. Design and execution of CRISPR-Cas9 and subsequent RMCE was all performed by Well Genetics.

Fly Staging

For data shown in [Figures 3, 5, and 6](#), flies were staged and wing discs were dissected at different ages to determine how collagen IV evolves with time. Ages are given in hours after egg laying (h AEL). Flies were staged by allowing eggs to be laid within a 2h window at 25C. Staged flies were then raised at 25C for a time equal to 1h less than the target age. The dissection and imaging window was kept to a minimum, around 2h so that the staging error is approximately +/-2h of the target age. Wing discs were dissected and imaged in Grace's Media (Sigma-Aldrich, G9771-1L, prepared as described in Dye et al.⁵⁶), and supplemented with 5% fetal bovine serum (Sigma-Aldrich F4135-500ML) and 1% penicillin-streptomycin (ThermoFisher, 15140–122).

METHOD DETAILS

Tissue Mechanics Model

Development of the tissue mechanical model and its extensions, are presented in [Figures 1 and S1](#) is described in detail in [supplemental methods](#) Section S1. In brief, the symmetric homogeneous model ([supplemental methods](#) Section S1.1) is a mean field tissue model in which the mean cell shape is modelled as a prism, whose caps are defined by the mean apical-basal surface area (S) and mean cell shape parameter (α). We define a tissue energy for a tiling of n mean cells, whose apical-basal surface area is $A = nS$. This tissue energy includes terms for apical, basal and lateral cell surface tension, apical and basal perimeter tension, and volume conservation. Cell height is chosen such that tissue energy is optimised. From tissue energy we derive the cell stress required to drive the mean cell surface area

away from its optimum. We describe the basement membrane as a 2D viscoelastic fluid (Maxwell model) sheet with area, A , matching the cell layer. Cells and BM are mechanically coupled by setting cell stress equal and opposite to BM stress. This results in a single ordinary differential equation describing how mean apical-basal cell surface area (S) evolves with cell number (n) as cells proliferate with rate λ . Other key parameters that control this evolution are relative cell to BM stiffness, s_E , and BM relaxation time, t_{Rel} .

The symmetric heterogeneous model (supplemental methods Section S1.2) extends the symmetric homogeneous model by accounting for variation in apical-basal surface area ($CV[S]$) and cell shape ($CV[\alpha]$). Second order corrections are made to the mean tissue energy, which is then used as the tissue energy in the same way as the symmetric homogeneous model. In this model cell height is kept homogeneous, and instead the cell volume must vary to allow for variation in apical-basal cell surface area.

The asymmetric homogeneous model (supplemental methods Section 1.2) extends the symmetric homogeneous model by allowing for different apical and basal surface areas and surface tensions. This extension naturally leads to tissue curvature. In this model, tissue energy depends to two variables, apical cell surface area (S_A) and basal cell surface area (S_B). Only the basal cell surface area matches the basement membrane area, $A = nS_B$. The basal surface area is therefore confined by the basement membrane, while the apical cell surface area acts to minimise tissue energy. This leads to a pair of coupled ordinary differential equations describing the differing evolution of apical and basal cell surface areas. The new parameter, s_γ , is apical divided by basal surface tension.

All ordinary differential equations are solved using either a custom RK4 implementation or `solve_ivp` from the SciPy python library.

Collagen IV Network Molecular Dynamics Model

Collagen IV molecules are represented by two reactive ends (beads) which are connected by a rigid rod. The ends are denoted by 7S end and NC1 end reflecting the 7S domain and NC1 domain of a collagen IV molecule which have different bonding behaviours. The 7S end/domain reacts with 3 other 7S ends/domains to form a 7S tetramer bond and the NC1 end/domain reacts with one other NC1 end/domain to form an NC1 dimer bond. The model is implemented in molecular dynamics simulations using LAMMPS.⁵⁷ The velocity-verlet integrator is used with timestep $\Delta t = 0.001\tau_0$ and friction coefficient $\gamma = 0.1m/\tau_0$ where τ_0 is the simulation time unit and m is the mass of one bead. The dynamics are coupled to a Monte Carlo scheme for the addition and removal of monomers such that the simulations are in the grand canonical ensemble. Bonds can make or break with a given probability P_{make} and P_{break} , the ratio of which we keep constant and is chosen to be $P_{make}/P_{break} = 3$ such that the molecules spontaneously assemble into a percolating network. $N = 3125$ protomers are initialised in a simulation box of the shape of a slab with dimensions $L_z = 12a$, $L_x = L_y = 32a$. Periodic boundary conditions apply in the y and x dimensions, while Lennard-Jones walls apply in the z-boundaries. For further modelling details, see supplemental methods Section S2.

BM Collagen IV Concentration Model

Development of the BM collagen IV concentration model presented in Figure 3 is described in detail in supplemental methods Section S3.1. In brief, the evolution of collagen IV concentration in the BM (c_B) is modelled as an ordinary differential equation with three terms, Figure 3D. The first term in square brackets on the right describes the net rate of gain of collagen IV to the wing disc BM, (τ_n). It is the difference between BM collagen IV gain rate (τ_+) and loss rate (τ_-), although for the concentration model we cannot differentiate between these two. The second term describes the dilution of BM collagen IV concentration due to the increase in BM area, and depends on the current BM collagen IV concentration and the rate of change of BM area per unit area (or normalised expansion rate, α). The expansion rate can be determined from a wing disc area model developed by Bittig *et al.*,³² Figure 3C. We integrate this ordinary differential equation numerically using `solve_ivp` from the `integrate` library of SciPy.

To fit this model to our data we first determine the wing disc expansion rate. We fit the logarithm of the area model (Figure 3C) to the logarithm of our measured approximate wing disc pouch area data, Figure 3M, using `curve_fit` from the `optimize` library of SciPy. This fit estimates the parameters describing initial area (A_0), initial expansion rate (α_0) and expansion rate decay time (τ_a). We use the latter two parameters to estimate the BM expansion rate, which we use as fixed input for the BM collagen IV concentration model. To sufficiently describe the shape of the data we describe the net turnover by a sigmoid curve. Specifically a logistic function that transitions between initial ($\tau_{n,i}$) and final ($\tau_{n,f}$) net gain rates. We fit the model using `curve_fit` from the `optimize` library of SciPy. From this fit we get estimates for the parameters describing the net turnover sigmoid curve, as shown in Figure 3N (black solid line is the resulting fit, red solid line is the estimated net gain rate). To support the choice of net turnover model we linearised the BM collagen IV concentration model, and approximated changes between 12h data time points as linear. As a result we found an approximation of net turnover shown in Figure 3N (red dashed line).

BM Collagen IV Timer Model

Development of the BM collagen IV timer model presented in Figures 4, 5, and 6 is described in detail in supplemental methods Section S3.2. In brief, we extend the collagen IV concentration model (described in the previous section) to include transitions between fast fluorescent timer (FTF) states (Figure S4A). A schematic indicating both transition between FTF states and transition between haemolymph and BM is shown in Figure 4C. At each fluorescent state BM colIV-FTF can be gained from, or lost to, the haemolymph in the same way as the collagen IV concentration model. ColIV-FTF is introduced into the haemolymph (at rate κ_+) in the immature state UH, and lost from the haemolymph (κ_-) at all states. The resulting model is a system of ordinary differential equations.

For the haemolymph we assume the colIV-FTF states are at or near steady state. We assume the distribution of FTF states in the haemolymph is at steady state, and the exact distribution is defined by κ_- , Figure S4E. We cannot measure the concentration of

non-fluorescing FTF states, but we must estimate their initial states with the fitting procedure. These initial non-fluorescing concentrations, and the haemolymph loss rate are left as free parameters, which lowers the predictive power of the model. To combat this we include the colIV-GFP data in the fitting as an estimate of the total collagen IV concentration, which adds further constraint to the fit. For combined colIV-FTF and colIV-GFP fitting we begin by fitting the area expansion model to each dataset in order to estimate the BM area expansion rate. We describe the BM collagen IV gain rate (τ_+) with the same sigmoid function used for net turnover rate in the collagen IV concentration model. BM colIV-FTF loss rate (τ_-) is kept constant. We simultaneously fit the BM colIV-FTF and colIV-GFP models directly to their respective datasets, sharing gain and loss rate parameters, using `solve_ivp` and `curve_fit` from the SciPy library. To test the robustness of the model in estimating BM collagen IV lifetime (inverse of BM collagen IV loss rate), we perform global optimisation routines `basinhopping` and `dual_annealing` from the SciPy library. To get good estimates and confidence intervals for the fitted parameters we take our globally optimised fits and perform estimation of parameter distribution (EPD) REF. With EPD we obtain estimated distributions for our fitted parameters from which we can obtain statistics, such as those quoted in [Figure S6M](#). For more details on fitting procedure see [supplemental methods](#) Section S4.

Imaging

To accurately measure fluorescence in the wing disc basement membrane it was important to control for experimental factors affecting measured fluorescence intensity. It was therefore necessary to keep the basement membrane a consistent distance from the coverslip when imaging. To do this we stuck the peripodial epithelium side of wing discs on to a shard of coverslip using Cell Tak (Corning, 354240), then flipped over the shard in a 3.5mm glass-bottomed dish. This effectively sandwiched the wing disc between the dish bottom and the coverslip shard. The columnar epithelium basement membrane was hence maintained at a consistently close distance to the glass bottom of the dish through which we imaged using an inverted confocal microscope (Zeiss LSM 880 or LSM900). We also monitored for any changes in the effective laser power by sticking fixed-intensity fluorescent beads (ThermoFisher, green – I7219, blue – I7221 and red – I7224), of the relevant colour, to the bottom of the dish with Cell Tak. The beads were imaged once before imaging a dish of wing disc BMs and once after.

In each dish we mount and image roughly 8 to 12 wing discs. For each wing disc an over-sampled image stack is taken in roughly the center of the pouch. Fluorescent signal from the BM forms a sheet, for example see [Figures 3E–3H](#). We make sure that in *z*, the image stack begins well below, and ends well above, the fluorescent sheet. After taking fluorescent images of the BMs in the dish, we took zoomed-out brightfield images of the wing discs, for example see [Figures 3I–3L](#). These brightfield images were used to quantify wing disc pouch size. For younger wing discs the same 40x oil objective was used for imaging fluorescence and brightfield. However, for older wing discs it was necessary to use a 10x air objective to fit the whole wing disc within the field of view. For each dish we obtain for each wing disc one fluorescent image stack of the BM and one zoomed-out brightfield image, as well as two fluorescent image stacks of fixed-intensity beads, one at the beginning and one at the end of imaging.

For the quantification of GFP-tagged collagen IV (Vkg.GFP) fluorescence, [Figures 3, 5, and 6](#), a single channel with 488nm excitation was used on a Zeiss LSM 880 inverted microscope with airyscan. For the quantification of FTF-tagged collagen IV (Vkg.FTF) fluorescence, [Figures 5 and 6](#), two channels with 405nm and 561nm excitation respectively were used on a Zeiss LSM 900 inverted microscope with airyscan.

QUANTIFICATION AND STATISTICAL ANALYSIS

Image Analysis and Quantification

We quantify the total fluorescence per unit area of BM, which we call fluorescence intensity. If there were negligible background fluorescence and the BM were flat we could simply sum the image stack through *z* and take the *x-y* area of the image. However, for the fluorescent timer in particular we found considerable background signal, especially from within the tissue, see [Figures S6A–S6C](#) *y*-sum, and the BM in some images exhibited non-negligible curvature. We therefore implement an image analysis pipeline account for these effects, see [supplemental methods](#) Section S4 for details, but in brief we first split the image stack into blocks in *x-y*, then fit a fluorescence segmentation function to the resulting image block data, [Figures S5I and S5II](#). The fluorescence segmentation function assumes different fluorescence levels at three regions in *z*: outside the tissue, the BM, and inside the tissue. The fluorescence varies smoothly between these regions, and the *z*-height of the boundary between regions is allowed to vary to take into account BM curvature. For timer data where there are two fluorescence channels, when we fit the fluorescence segmentation function the boundary surfaces are shared. From the fluorescence segmentation function we extract an estimate of the background fluorescence inside and outside the tissue, [Figure S5III](#). We then subtract this background from both the block fluorescence data and the fluorescence segmentation function, [Figures S5IV and S5V](#), giving an estimate of the fluorescence from the BM alone. From the background-subtracted fluorescence function we obtain BM-bounding *z*-surfaces between which approximately 99% of the estimated fluorescence lies, [Figures S5VI and S5VII](#). We then take the sum of all the background-subtracted fluorescence data between these *z*-surfaces to estimate the total fluorescence from the BM in the data block, [Figures S5VIII and S5IX](#). We estimate the surface area of the BM from the central *z*-surface, taking into account BM curvature, [Figure S5X](#). The fluorescence intensity of the block is the estimated total BM fluorescence of the block divided by the estimated area of the BM within the block. The fluorescence intensity of the full image stack (or wing disc) is the mean of fluorescence intensities of the blocks.