Supplementary information

1. Analytical analysis of the impact of GAGs on the cellular force transmission

To derive an analytical expression for the range of force transmission, we considered a spherical cell (of radius $R_0$) exerting isotropic contractile stress on the surrounding swollen matrix (Fig. 5). Choosing the swollen state as the reference, the strain is tensile (compressive) in the radial (transverse) direction. In the vicinity of the cell, the radial strain induced by cell contraction is large enough to start to align fibers in the matrix. Due to spherical symmetry, the region where the fibers are aligned is also spherical with radius $R_a$. Inside this region, we can write the matrix stress $\sigma_{ij}$ as

$$\sigma = \sigma^b + \sigma^f + \sigma^{GAG} + \Pi I.$$  \hspace{1cm} (S1)

Here, $\sigma^b$, $\sigma^f$, $\sigma^{GAG}$, and $\Pi I$ denote the stress tensors that arise from the randomly-aligned collagen, aligned collagen, GAGs, and the swelling pressure induced by GAGs. Inside the aligned region, for linear bulk and fibrous response ($m = 0$ in Eq. 15),

$$\sigma^b = s\kappa_{col} t r(\epsilon) I + 2s\mu_{col} d e v(\epsilon)$$  \hspace{1cm} (S2)

$$\sigma^f = \frac{2}{3} \sum_{a=1}^{3} E_f (\epsilon_a - \epsilon_c) H(\epsilon_a - \epsilon_c) n_a \otimes n_a \approx \frac{2}{3} \sum_{a=1}^{3} E_f \epsilon_a H(\epsilon_a) n_a \otimes n_a$$  \hspace{1cm} (S3)

$$\sigma^{GAG} = \frac{c_{GAG}}{c_{GAG}^{ref}} \kappa_{GAG} t r(\epsilon) I + 2 \frac{c_{GAG}}{c_{GAG}^{ref}} \mu_{GAG} d e v(\epsilon)$$

Here, $H(\cdot)$ denotes the Heaviside step function, and $\epsilon_c = \lambda_c - 1 < 1$ denotes the critical strain for the collagen matrix to align. Note that in the swollen configuration the bulk modulus $\kappa$ and shear modulus $\mu$ are scaled by the scaling factor $s(J)$ as shown in Eq. S2.

Using a spherical coordinate system $(r, \theta, \varphi)$, we write the strains as

$$\epsilon_r = \frac{d u}{d r}, \quad \epsilon_\theta = \epsilon_\varphi = \frac{u}{r}.$$  \hspace{1cm} (S4)

Eq. S1 can then be rewritten as

$$\sigma_r = \left(\kappa - \frac{2}{3} \mu\right) \left(\frac{d u}{d r} + \frac{2 u}{r}\right) + 2 \mu \frac{d u}{d r} + \frac{2}{3} E_f \frac{d u}{d r} + \Pi,$$  \hspace{1cm} (S5)

$$\sigma_\theta = \left(\kappa - \frac{2}{3} \mu\right) \left(\frac{d u}{d r} + \frac{2 u}{r}\right) + 2 \mu \frac{u}{r} + \Pi.$$  \hspace{1cm} (S6)

Here in these equations, $\kappa = s\kappa_{col} + \frac{c_{GAG}}{c_{GAG}^{ref}} \kappa_{GAG} + d \Pi / d J$ and $\mu = s\mu_{GAG} + \frac{c_{GAG}}{c_{GAG}^{ref}} \mu_{GAG}$ denote the effective isotropic bulk and shear moduli of the network. The condition for mechanical equilibrium $\frac{d \sigma_r}{d r} + \frac{2}{r} (\sigma_r - \sigma_\theta) = 0$ can then be written as,

$$\left(1 + \frac{2E_f/3}{\kappa + 4\mu/3}\right) \left(\frac{d^2 u}{d r^2} + \frac{2}{r} \frac{d u}{d r}\right) - \frac{2 u}{r^2} = 0$$  \hspace{1cm} (S7)

with boundary conditions

$$u(R_0) = u_0, \quad \frac{d u}{d r}(R_a) = \epsilon_c.$$  \hspace{1cm} (S8)

Here $u_0$ denotes the displacement at $r = r_0$ induced by cell contraction. When $\epsilon_c << 1$, the solution can be approximated by
\[ u(r) \approx u_0 \left( \frac{r_0}{r} \right)^\gamma \text{ and } R_a \approx \sqrt{\frac{\gamma u_0 R_a^\gamma}{\epsilon_c}} \]  

(S9)

where \( \gamma = \frac{1}{2} \left( 1 + \frac{9 + \xi}{1 + \xi} \right) \in (1,2] \) and \( \xi = \frac{2E_f/3}{\kappa + 4\mu/3} \). When the matrix has no fibrous component with \( E_f = 0, \xi = 0, \gamma = 2 \), the displacement decays fast as \( \frac{1}{r^2} \). On the other hand, when the matrix is highly fibrous with \( E_f \to \infty, \xi \to \infty, \gamma \to 1 \), the displacement decays much more slowly as \( \frac{1}{r} \), showing a long range of force transmission.

For the region \( r > R_a \), the collagen fibers are not aligned and therefore the matrix stress \( \sigma \) has contributions only from GAGs and the unaligned fibers, i.e.

\[ \sigma = \sigma^b + \sigma^{\text{GAG}} + \Pi, \]  

(S10)

The expression of displacement in this region \( u(r) \) can be derived following a similar approach as demonstrated in Eq. S1-S9, with the boundary condition \( \frac{du}{dr}(R_a) = \epsilon_c \) and \( u(\infty) = 0 \). The solution follows the same form as shown in Eq. S9 with \( \xi = 0 \) and \( n = 2 \). Therefore, the overall displacement field can be written as

\[ u(r) = \begin{cases} 
    u_0 \left( \frac{r_0}{r} \right)^\gamma, & r_0 < r < R_a \\
    u_0 \left( \frac{r_0}{R_a} \right)^\gamma \left( \frac{R_a}{r} \right)^2, & r \geq R_a
  \end{cases} \]  

(S11)

Combining Eq. S2, S3, and S11, we can derive the stress field. Here we write out the expression for \( \sigma_r \),

\[ \sigma(r) = \begin{cases} 
    -\left[ (\kappa + \frac{4}{3}\mu) sy - 2(\kappa - \frac{2}{3}\mu) + \frac{2}{3} \gamma E_f \right] \frac{u_0}{r_0} \left( \frac{r_0}{r} \right)^{\gamma+1}, & r_0 < r < R_a \\
    -4\mu \frac{u_0}{r_0} \left( \frac{r_0}{R_a} \right)^{\gamma+1} \left( \frac{R_a}{r} \right)^3, & r \geq R_a
  \end{cases} \]  

(S12)

From Eq. S12, we can see that the force transmission is efficient in the aligned region \( r_0 < r < R_a \), with stress decaying as \( \frac{1}{r^{\gamma+1}} \). Outside the aligned region, stress decays much faster with \( \frac{1}{r^3} \).

Rescaling \( u_0 \) and \( R_a \) by the radius of the cell \( r_0 \), we can write the normalized radius of the alignment region as \( \bar{R}_a = \frac{R_a}{r_0} = \sqrt[\gamma+1]{\frac{\bar{u}_0}{\epsilon_c} \gamma} \), where \( \bar{u}_0 = -\frac{u_0}{r_0} \). From this expression we can see \( \bar{R}_a \) depends on \( \gamma \), which characterizes the rate of decay of displacement in the matrix. Small (large) \( \gamma \) corresponds with slow (fast) decay of displacement, and thus long (short) range of force transmission. Since \( \epsilon_c \ll 1, \frac{\bar{u}_0}{\epsilon_c} > 1 \), and

\[ \frac{d(\log \bar{R}_a)}{d\gamma} = \frac{1}{(\gamma+1)^2} \left( \gamma + 1 - \gamma \log \gamma - \gamma \log \left( \frac{\bar{u}_0}{\epsilon_c} \right) \right) < 0 \text{ when } \frac{\bar{u}_0}{\epsilon_c} > e^2, \]  

(S13)

the radius of the aligned region also increases as \( \gamma \) decreases.
2. Relation between range of force transmission and collagen network properties

In the main text we presented the impact of GAG concentration on force transmission. Here, we show a comprehensive parametric study on the properties of the collagen network, as well as the magnitude of the cell contraction. As shown in Fig. S1, the range of force transmission increases with the magnitude of cell contraction $\epsilon_0$, and the matrix fibrous modulus $\chi = \frac{E_f}{\kappa_{col} + 4\mu_{col}/3}$.
Figure S1 (a) Impact of cell contraction $\varepsilon_0$ and matrix fibrosity $\chi$ on force transmission. The colored area denotes the region of fiber alignment ($\tilde{\lambda}_1 > \lambda_c$). (b) Force-transmission distance $(D_c/h)$ as a function of cell contraction $\varepsilon_0$, with $\chi = 1$ and $\chi = 10$.
distance normalized by the long-axis radius of the cell as a function of cell contraction $\varepsilon_0$.

(c) (d) Same as (a) and (d) but with $\lambda_c = 1.02$.

3. Simplification of the constitutive law when acidic groups fully dissociate

When the pKa is much smaller than the pH in the surrounding environment of the ECM, the derivations given in Eq. 2-8 can be greatly simplified by assuming that the fixed acidic groups fully dissociate. That is, $K_a \to \infty$ (or $pK_a \to 0$), and Eq. 2 becomes

$$c_{\text{coo}}^- = \frac{c_{\text{AG}}D}{J} \quad (S14)$$

In this case, we do not need to explicitly specify the concentration of proton ($c_{H^+}$ is used to determine the dissociation of acidic groups by Eq. 2). Therefore, we can group all the cations together, using $c_+$, $c_-$, and $c_{\text{coo}}^-$ denote the concentration of cations, anions, and the fixed charges, respectively. Similarly, we use $\bar{c}_+$ and $\bar{c}_-$ to denote the concentration of cations and anions in the surrounding environment. The electroneutrality equations can be written as

$$c_+ = c_- + c_{\text{coo}}^- \quad \text{and} \quad \bar{c}_+ = \bar{c}_- \quad (S15)$$

The Donnan osmotic pressure given in Eq. 3 can be calculated using

$$\Pi = kT(c_+ + c_- - \bar{c}_+ - \bar{c}_-). \quad (S16)$$

And Eq. 4 reduces to

$$c_+/\bar{c}_+ = \bar{c}_-/c_- \quad (S17)$$

Solving Eq. S14-S16 gives a simple relation between the swelling pressure and the acidic group on GAGs,

$$\Pi = kT(\sqrt{(c_{\text{AG}}D/J)^2 + 4\bar{c}_+^2} - 2\bar{c}_+) \quad (S18)$$

4. Local accumulation of GAGs creates barriers for cell mechanosensing

Distribution of GAGs is often inhomogeneous in vivo. For example, in the tumor microenvironment, stromal cells have been found to release abnormally high amounts of HA, which can potentially affect cellular force transmission around the cancer cells. To investigate the impact of such local accumulation of GAGs, we simulated the increase of the concentration of GAGs in a circular region of radius $R_a$ located at a distance $L_a$ from a cell (or spheroid), defined by $x^2 + y^2 + (z - L_a)^2 \leq R_a$ (Fig. S2b). We show that the accumulation of GAGs can disrupt the force transmission locally.

When there is no overproduction of GAGs, cell contraction aligned matrix fibers in a gourd-shaped region (Fig. S2b), consistent with our results in the main text. As GAGs start to accumulate in the small circular region (increasing $c_{\text{AG}}$), we found that within the region of GAG accumulation, the strains significantly increase because of the swelling (Fig. S2b-S2d). Normally, the cell-induced strain is tensile in the radial direction, and compressive in the transverse direction. With the presence of GAGs, the strain state is overridden by swelling. This suggests that cells located in the accumulated region cannot
receive the mechanical signals from the other cells in the surrounding, which can potentially alter their mechanosensitive behaviors.

Figure S2 (a) Schematic showing the impact of local accumulation of GAG on force transmission. As the over-synthesized GAG leaves the cell surface and moves into a region close to the cell, it increases the local osmotic pressure and causes the collagen network to swell. The swollen region compresses the surrounding collagen network and disrupts the normal force transmission. (b) The deviatoric part of the first principal stretch in the ECM under 20% contraction of the cell. Only the region with strain larger than 0.04 is shown, indicating matrix that becomes aligned under the cell contraction. As the local accumulation of HA increases, it creates a barrier for transmission of the cell contractile force. (c) The Z-direction strain and (d) X-direction strain along the cell long axis passing through the area with increased level of GAGs.
5. Swelling-induced stiffening of fibrous networks cannot be reproduced by increasing fiber bending modulus

Our model confirms that fibers will not readily buckle in the expanded network as they would not in a network with unrealistically thick fibers of very high bending stiffness. However, we point out that the stresses developed during swelling or network expansion are fundamentally different from change of network stiffness by change of the fiber bending stiffness or a suppression of fiber buckling. In the expanded networks, residual stresses arise from the straightening and subsequent stiffening of fibers. The network remarkably stiffens in volumetric expansion, signified by a surprisingly large exponent of 11 (Fig. S6c), i.e., $K \sim (V/V_0)^{11}$, in distinct contrast to increasing network stiffness by changes of fiber bending stiffness, associated with much smaller exponents. Network stiffening by fiber bending stiffness is significantly smaller than what is caused by fiber stretching. A modest 10% volumetric swelling strain is comparable with the case when the fiber bending stiffness is increased by a very large factor of 100.

In the network model, fiber bending stiffness, $E_f I_f \propto d_f^4$, and axial stiffness $E_f A_f \propto d_f^2$, where $d_f$ is fiber diameter. $I_f$ and $A_f$ are fiber cross-sectional second moment of inertia and surface area, and the ratio of the bending to axial stiffness, $\propto d_f^2$, is an important parameter, controlling the behavior of the network model. We computed changes of shear strain-stiffening at 25% volumetric strain and 100-fold bending stiffness (Fig. S6d). We note that the two trends differ both in their functional form and magnitude. No realistic increase of bending stiffness can produce the observed increase in volumetric stiffness in the limits of small and large strains. Thus, the magnitude and trends of the observed effects cannot be attained by changes of fiber bending stiffness alone.

6. Table of parameters

$\mu_{GAG}$, $\kappa_{GAG}$, $\mu_{col}$, $\kappa_{col}$, and $E_f$ are determined by fitting the model to match the free swelling ratio and the shear moduli measured by rheology.

| Parameter | Physical meaning | Value | Source |
|-----------|------------------|-------|--------|
| $c_{GAG}$ | Concentration of GAGs | 0~2.5 mg/mL | 6, 7 |
| $c_{GAG}^{ref}$ | Reference concentration of GAGs for measuring stiffness | 1 mg/mL | Chosen |
| $\mu_{GAG}$ | Shear modulus of GAGs at $c_{GAG}^{ref}$ | 60 Pa | Fit to experiment |
| $\kappa_{GAG}$ | Bulk modulus of GAGs at $c_{GAG}^{ref}$ | 50 Pa | Fit to experiment |
| $c_{H^+}$ | Concentration of hydrogen ions in the external solution | 0.001 mmol/L | Physiological condition |
| $c_{+}$ | Concentration of cations in the external solution | 150 mmol/L | Physiological condition |
| Symbol | Description | Value | Notes |
|--------|-------------|-------|-------|
| \( \bar{c}_- \) | Concentration of anions in the external solution | 150 mmol/L | Physiological condition |
| \( K_a \) | Acidic strength | \( 10^{-3.3} \) | 22 |
| \( D \) | Number of acidic groups per mol of GAGs | 1 mol | Estimate |
| \( E_f \) | Fiber modulus of collagen network (2.5 mg/mL) | 100 Pa | Fit to experiment |
| \( \mu_{col} \) | Shear modulus of collagen network (2.5 mg/mL) | 80 Pa | Fit to experiment |
| \( \kappa_{col} \) | Bulk modulus of collagen network (2.5 mg/mL) | 100 Pa | Fit to experiment |
| \( m \) | Collagen stiffening exponent | 20 | 2, 3, 18 |
| \( n \) | Collagen stiffening exponent | 10 | 2, 3, 18 |
| \( \lambda_c \) | Critical stretch for collagen alignment | 1.02 | 2, 3, 18 |
| \( \alpha \) | Stiffening exponent of collagen upon swelling | 4.5 | Fit to experiment |
| \( \Pi_{col} \) | Collagen swelling pressure | 2 Pa | Fit to experiment |

**Figure S3** Stiffness of crosslinked HA gel as a function of swelling ratio.
**Figure S4** Amount of HA that remained in the collagen-HA co-gel after swelling.

**Figure S5** Alignment of the collagen under uniaxial tension (Left). The stress from the aligned fibers and randomly-aligned fibers (Right). The highly non-linear function $f()$ captures the stiffening behavior of the collagen network.
Figure S6. Comparison of the swelling (triaxial or volume expansion) and shear modes of deformation of discrete collagen networks. Network snapshots (a) before deformation and (b) at 25% swelling or volumetric strain. (c) Stiffening of the network with a surprisingly large exponent in volumetric deformation. (d) Comparison of strain-stiffening after swelling or increase of bending stiffness.
Figure S7 (a) Theoretical prediction of intensity of aligned fibers between two cells in collagen-GAG co-gels with increasing GAG concentration in 3D. (b) Theoretical prediction and experimental measurement of intensity of aligned collagen fibers between cell spheroids in 3D. (c) The average intensity of aligned fibers in the box specified in (b), n=33 for collagen gels and n=17 for collagen-HA gels, p < 0.001. The concentrations of collagen and HA were 1.5 mg/ml and 1.0 mg/ml, respectively.
Figure S8 The SHG intensity of fibroblast-free collagen and collagen-HA gels.