

## Supplementary data for Hyaluronic acid induces ROCK-dependent amoeboid migration in glioblastoma cells

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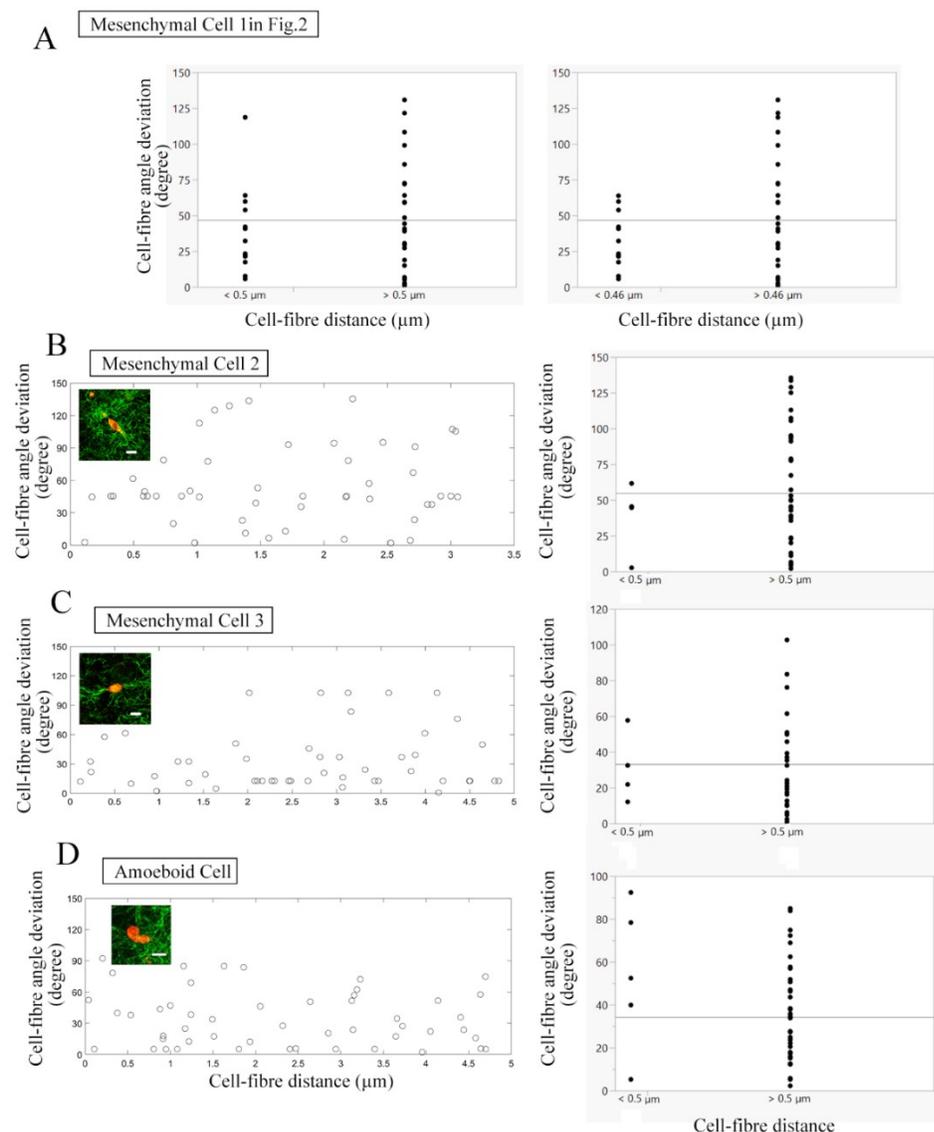
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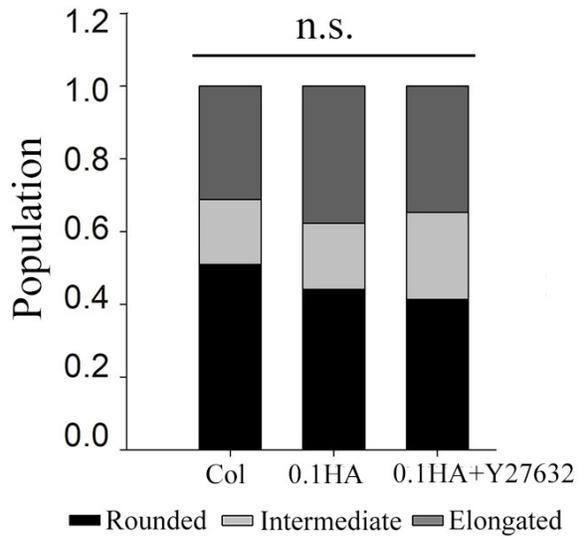
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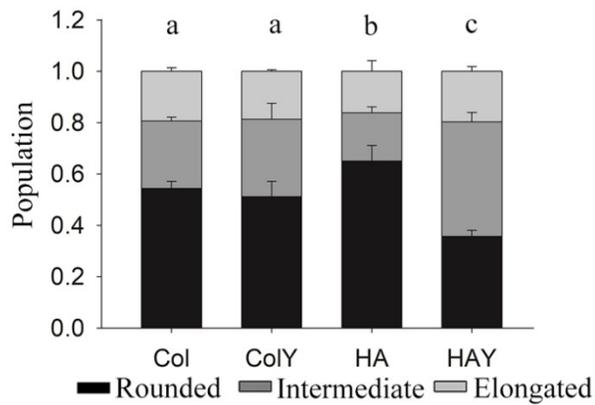
### Supplementary Figures



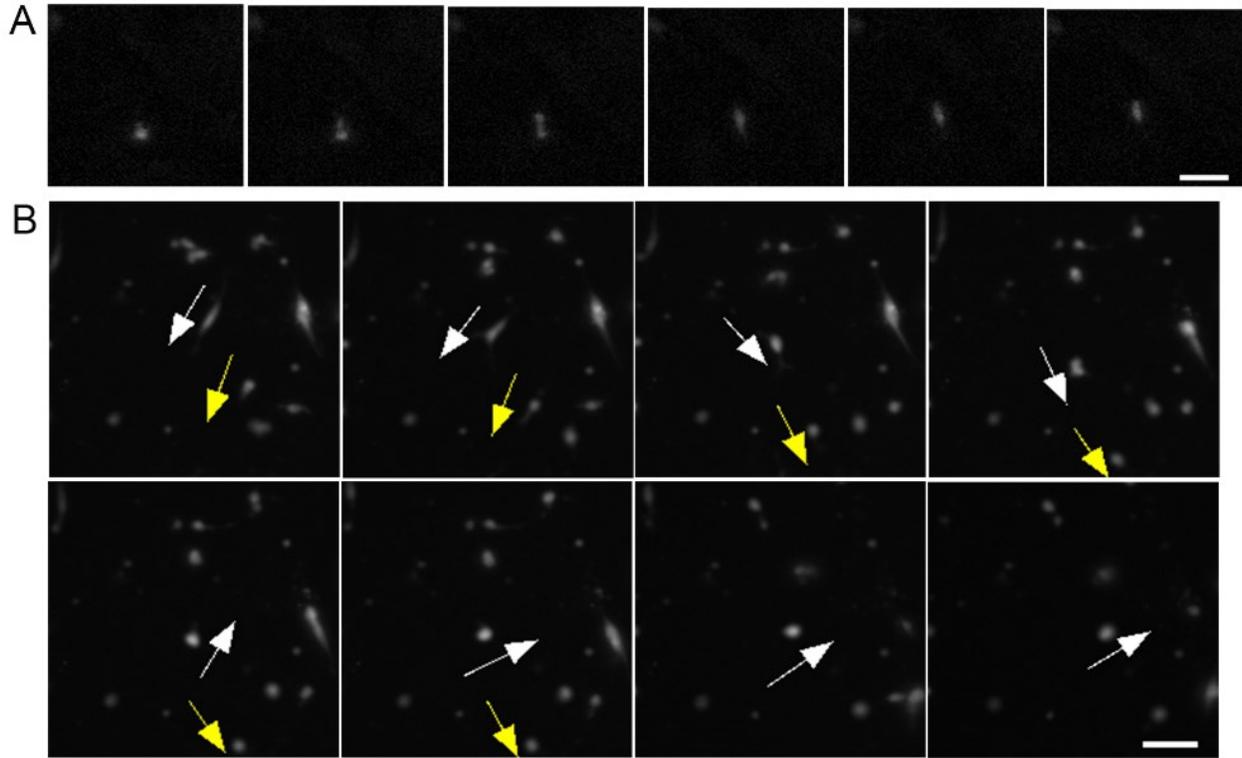
Supplementary Figure 1. Quantification of cell-fibre alignment for three mesenchymal-like cells (A-C) and one amoeboid-like cell (D) in collagen hydrogel. Fibers  $< 0.5 \mu\text{m}$  to the cell showed smaller variance in cell-fibre angle deviation than fibers  $> 0.5 \mu\text{m}$  from the cell for mesenchymal cells. For mesenchymal cell 1 the cut off was reduced to  $0.46 \mu\text{m}$  to exclude an outlier. Showing 50 nearest fibres surrounding each cell. Bar =  $10 \mu\text{m}$ .



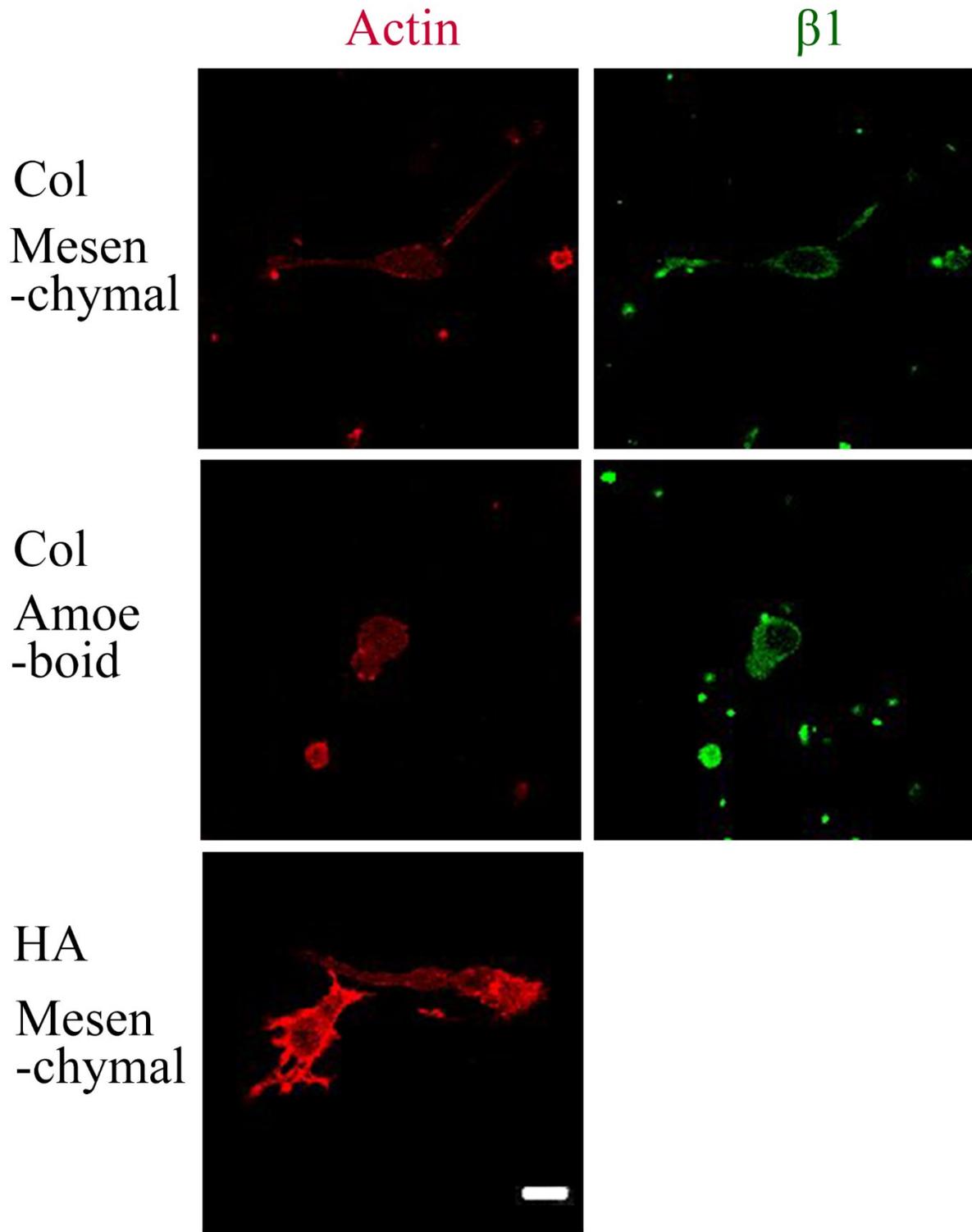
Supplementary Figure 2. No morphological change was detected between U87s cultured in pure collagen, 0.1HA, or 0.1HA with ROCK inhibition by Y27632. n.s. =non-significant.  $\alpha = 0.05$ .  $N > 150$  for all conditions.



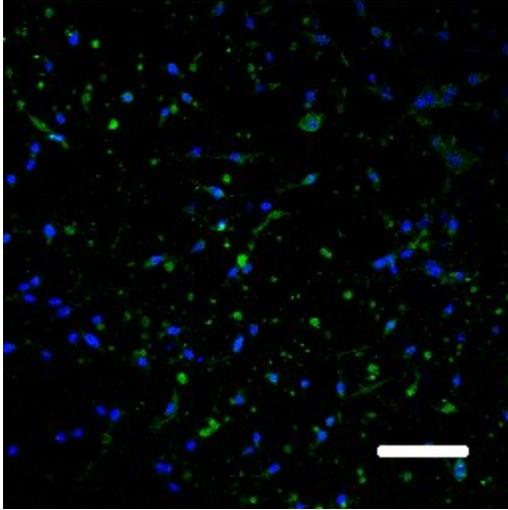
Supplementary Figure 3. Aspect ratio (AR) of U87 cultured in pure 1 mg/ml collagen (Col), 1 mg/ml collagen with 10 $\mu$ M Y27632 (ColY), 1 mg/ml collagen with 0.2 wt% HA (HA), or 1 mg/ml collagen with 0.2 wt% HA and 10 $\mu$ M Y27632 (HAY) hydrogels. Stacked bar plots showing populations of rounded (AR < 1.5), intermediate (1.5 < AR < 2), and elongated (AR > 2) cells.



Supplementary Figure 4. Cell migration during time lapse imaging at 10x. A). Example of migration behaviours that are hard to classify as mesenchymal or amoeboid because of poor resolution. B). Examples of mixed behaviours showing features of both mesenchymal and amoeboid migration. Arrows indicate the migration direction of the cell. Scale bar = 50  $\mu\text{m}$ .



Supplementary Figure 5. High resolution images of actin and  $\beta_1$  integrin staining of mesenchymal and amoeboid U87 cells cultured in Col and HA gels. Bar = 10  $\mu\text{m}$ .



Supplementary Figure 6.  $\beta_1$  integrin immunocytochemistry staining of U87s cultured in 2D on a thick 0.2HA gel. Blue: Nuclear counter staining with DAPI. Green:  $\beta_1$  integrin. Scale bar = 100  $\mu\text{m}$ .

### Supplementary Tables

Supplementary Table 1. Linear model fitting of aspect ratio (AR) data of U87 cells cultured in 1 mg/ml collagen or 1 mg/ml collagen with 0.2 wt% hyaluronic acid (HA) gels with or without Y27632 (Y).

	Estimate	Std. Error	t value	Pr (>  t )
Intercept	1.80	0.0218	82.6	<2e-16
HA	-0.163	0.0390	-4.18	3e-05
Y	0.0814	0.0319	2.56	0.0106
HA * Y	0.530	0.0509	10.4	<2e-16

Adjusted  $R^2 = 0.0369$ , p-value < 2.2e-16. Formula =  $AR \sim HA + Y + HA * Y$

Supplementary Table 2. Linear model fitting of migration speed data of U87 cells cultured in 1 mg/ml collagen or 1 mg/ml collagen with 0.2 wt% hyaluronic acid (HA) gels with or without Y27632 (Y).

	Estimate	Std. Error	t value	Pr (>  t )
Intercept	23.2	0.527	44.1	<2e-16
HA	-12.2	0.823	-14.8	<2e-16
Y	-2.41	0.741	-3.25	0.00118
HA * Y	-1.18	1.27	-0.928	0.354

Adjusted R<sup>2</sup> = 0.2506, p-value < 2.2e-16. Formula = migration speed ~ HA + Y + HA \* Y

Supplementary Table 3. Goodness of fitting compared by the sum of squared errors (SSE) and adjusted R<sup>2</sup> of cell migration data.

	1-term Gaussian		2-term Gaussian		1-term log normal	
	SSE	adjusted R <sup>2</sup>	SSE	adjusted R <sup>2</sup>	SSE	adjusted R <sup>2</sup>
Col	486.5	0.8669	359.7	0.8985	340.5	0.9069
ColY	907.4	0.7754	582	0.8513	466.4	0.8845
HA	268.5	0.8539	51.6	0.971	142.8	0.9223
HAY	695.2	0.8412	260.3	0.9386	341.8	0.9219

1-term Gaussian, 2-term Gaussian, and 1-term log normal fitting were done by Equation 1, Equation 2, and Equation 3, respectively.

1-term Gaussian equation:

$$f(x) = a1 \times \exp\left(-\frac{(x - b1)^2}{c1}\right)$$

Equation 1

2-term Gaussian equation:

$$f(x) = a1 \times \exp\left(-\left(\frac{x-b1}{c1}\right)^2\right) + a2 \times \exp\left(-\left(\frac{x-b2}{c2}\right)^2\right) \quad \text{Equation 2}$$

1-term log normal equation:

$$f(x) = a1 \times \frac{1}{x} \times \frac{1}{\sigma1\sqrt{2\pi}} \exp\left(-\frac{(\ln x - \mu1)^2}{2\sigma1^2}\right) \quad \text{Equation 3}$$

\*a1, b1, b2, c1, c2,  $\mu1$ , and  $\sigma1$  are parameters determined by Curve Fitting function in MATLAB.

### Supplementary Videos

Supplementary Video 1: Time lapse imaging of U87 mesenchymal migration behaviors in pure collagen.

Red: U87 cells. Green: collagen cells. Bar = 10  $\mu\text{m}$ . Time interval = 15 min. Duration = 150 min. Arrows indicate direction of cell migration.

Supplementary Video 2: Time lapse of collagen fiber remodeling during mesenchymal migration (green channel of Supplementary Video 1). Bar = 10  $\mu\text{m}$ . Time interval = 15 min. Duration = 2.5 hr. Arrows indicate direction of cell migration.

Supplementary Video 3: Time lapse imaging of U87 amoeboid migration behaviors in pure collagen. Red: U87 cells. Green: collagen cells. Bar = 10  $\mu\text{m}$ . Time interval = 15 min. Duration = 2.5 hr. Arrows indicate direction of cell migration.

Supplementary Video 4: Time lapse imaging of U87 mesenchymal migration behaviors in 0.1HA gels. Red: U87 cells. Green: collagen cells. Bar = 10  $\mu\text{m}$ . Time interval = 30 min. Duration = 3.5 hr. Arrows indicate direction of cell migration.

Supplementary Video 5: Time lapse imaging of U87 amoeboid 'squeezing' migration behaviors in 0.1HA gels. Red: U87 cells. Green: collagen cells. Bar = 10  $\mu\text{m}$ . Time interval = 30 min. Duration = 9 hr. Arrows indicate direction of cell migration.

Supplementary Video 6: Time lapse imaging of U87 amoeboid 'gliding' migration behaviors in 0.1HA gels. Red: U87 cells. Green: collagen cells. Bar = 10  $\mu\text{m}$ . Time interval = 30 min. Duration = 15 hr. Arrows indicate direction of cell migration.