

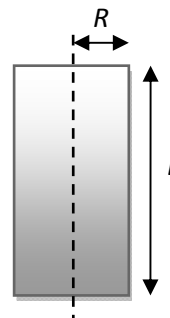
## SUPPLEMENTARY METHODS:

### I. EQUATIONS FOR THE SURFACE AREA (excluding the top base) AND VOLUME OF COMMON INVASIVE GEOMETRIES

(i) Surface area of a cylinder of radius  $R$  and length  $L$  (excluding the top base), and corresponding volume

$$S_{cylinder} = \pi R^2 + 2\pi RL \quad (\text{Eq. S1a})$$

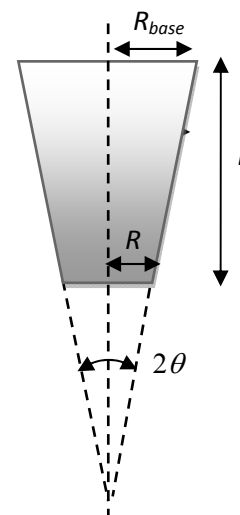
$$V_{cylinder} = \pi R^2 L \quad (\text{Eq. S1b})$$



(ii) Surface area of a truncated cone of smallest radius  $R$ , length  $L$ , and semiincluded angle  $\theta$  (excluding the top base), and corresponding volume:

$$S_{cone} = \pi R^2 + \pi(R + R_{base})\sqrt{L^2 + (R_{base} - R)^2} \quad (\text{Eq. S2a})$$

$$V_{cone} = \frac{\pi L}{3} (R^2 + R \cdot R_{base} + R_{base}^2) \quad (\text{Eq. S2b})$$



$R_{base}$  can be expressed in terms of the other parameters as:

$$R_{base} = R + L \tan \theta \quad (\text{Eq. S3})$$

Substituting Eq.S3 into Eq.S2a and Eq.S2b and rearranging:

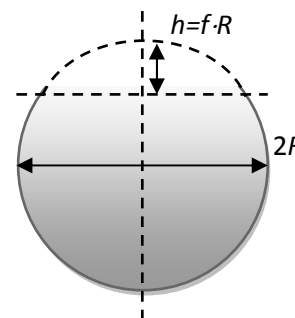
$$S_{cone} = \pi R^2 + \pi L(2R + L \tan \theta) \sqrt{1 + \tan^2 \theta} \quad (\text{Eq. S4a})$$

$$V_{cone} = \frac{\pi L}{3} [3R^2 + 3RL \tan \theta + (L \tan \theta)^2] \quad (\text{Eq. S4b})$$

(iii) Surface area of a truncated sphere of radius  $R$  (excluding the top base), and corresponding volume:

$$S_{sphere} = 4\pi R^2 - 2\pi R h \quad (\text{Eq. S5a})$$

$$V_{sphere} = \frac{4\pi R^3}{3} - \frac{\pi h^2}{3} (3R - h) \quad (\text{Eq. S5b})$$



where  $h$  is the height of the spherical cap, which can be expressed as a fraction  $f$  of  $R$  as:

$$h = fR \tag{Eq. S6}$$

Substituting Eq.S6 into Eq.S5a and Eq.S5b:

$$S_{sphere} = 4\pi R^2 \left(1 - \frac{f}{2}\right) \tag{Eq. S7a}$$

$$V_{sphere} = \frac{4\pi R^3}{3} \left(1 - \frac{3f^2}{4} + \frac{f^3}{4}\right) \tag{Eq. S7b}$$

II. VALUES FOR THE TRANSLATIONAL FRICTION COEFFICIENT

The general expression of the translational friction coefficient for tube-like bodies can be described as:

$$b_{tubelike} = \frac{2\pi\eta L}{\ln\left(\frac{L}{2R}\right) + \gamma}$$

Where  $\gamma$  depends on the ratio  $L/2R$ . Reported values for  $\gamma$  and the corresponding references are given in the following table

Geometry	L/2R	$\gamma$	Average $\gamma$
Cylinder	3	+0.1 <sup>a</sup>	-0.06
	5	-0.02 <sup>a</sup> (*)	
	7	-0.08 <sup>a</sup> (*)	
	9	-0.11 <sup>a</sup>	
	>50	-0.2 <sup>a</sup>	
	na	-0.03 <sup>b</sup> (*)	
	na	-0.12 <sup>b</sup> (*)	
Ellipsoid	na	+0.19 <sup>b,c</sup> (*)	-0.15
	na	-0.5 <sup>d</sup> (*)	

(\*) Values used to calculate the average  $\gamma$ , which correspond the conditions of our simulations

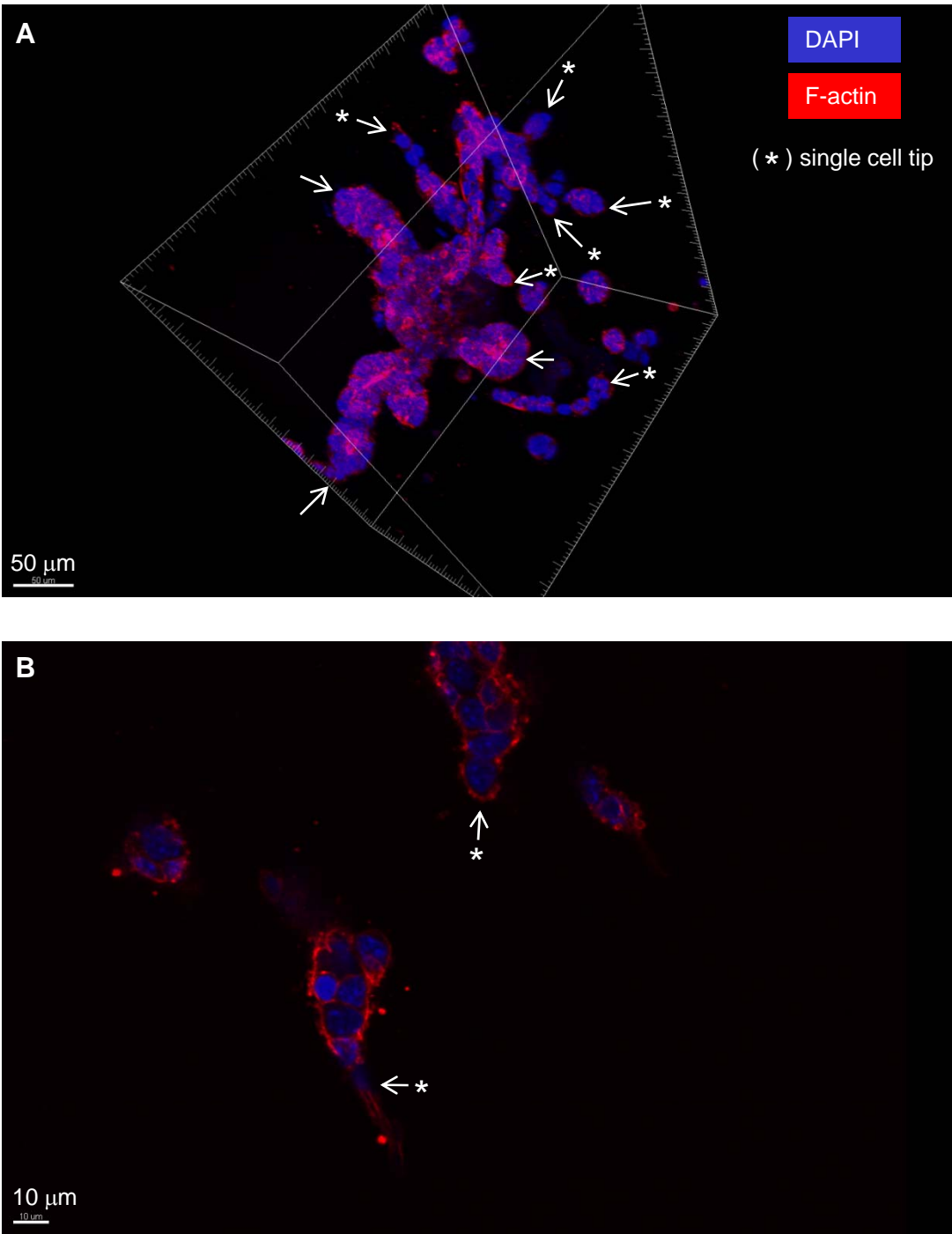
<sup>a</sup>M. M. Tirado and J. G. De la Torre, *J Chem Phys*, 1979, **71**, 2581:2587.

<sup>b</sup>H. Yamakawa and G. Tanaka, *J Chem Phys*, 1972, **57**, 1537:1542.

<sup>c</sup>C. M. Tchen, *J Appl Phys*, 1954, **25**, 463:473.

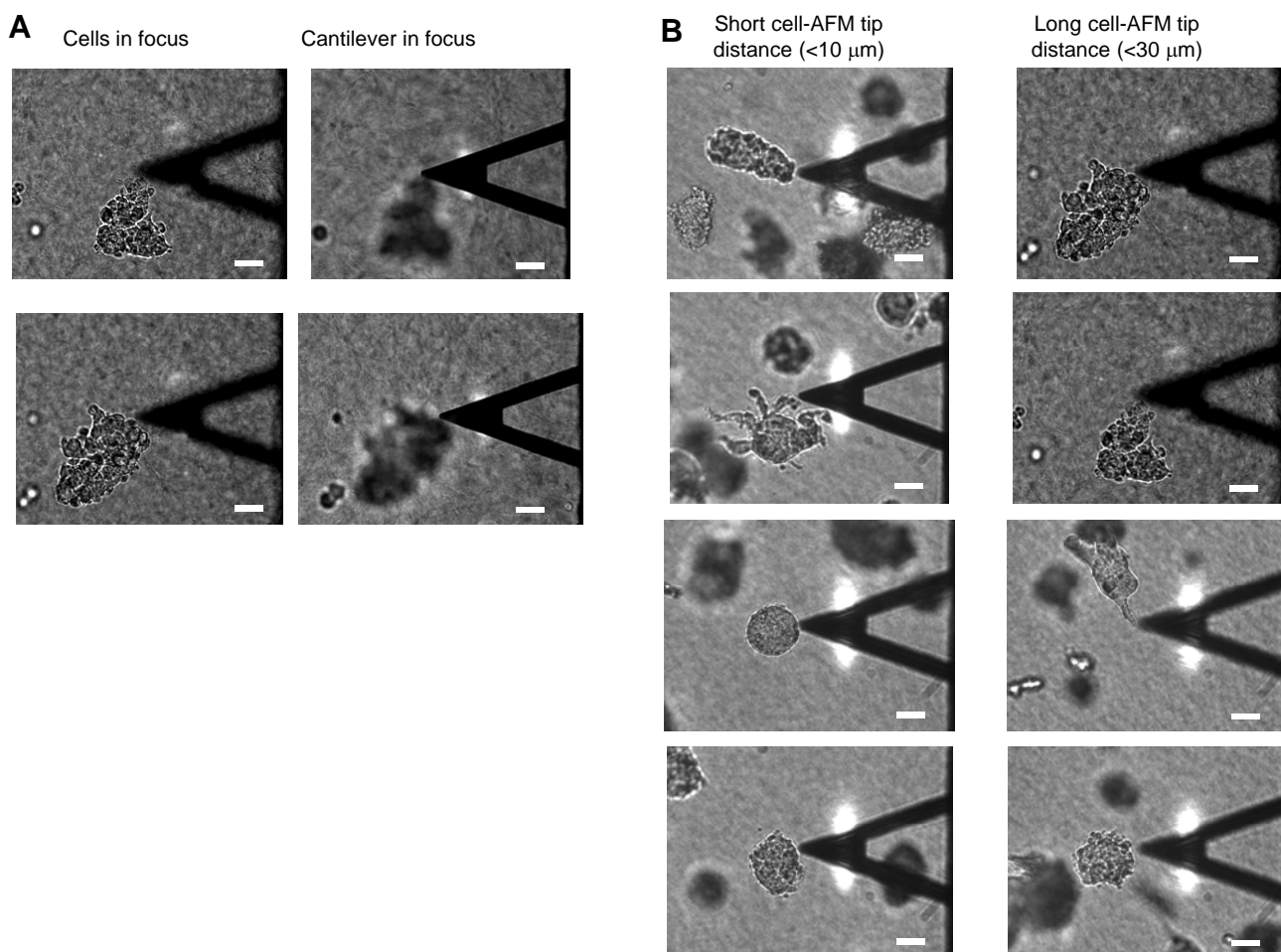
<sup>d</sup>J. Howard, *Mechanics of motor proteins and the cytoskeleton*, 2001 edn., Sinauer Associates, Sunderland, 1957.

**SUPPLEMENTARY FIGURE 1**



Visualization by confocal microscopy of the nuclear distribution of branching MECs grown in 3D collagen-I gels. Cell cultures were fixed and stained with DAPI and phalloidin to visualize the nucleus and F-actin, respectively, as described in Methods. (A) 3D reconstruction of a confocal stack carried out with Imaris (Bitplane). (B) Confocal image of a different cluster of branching cells. Arrows point to the tip of branching structures, whereas stars identify branches where a single nuclei is visible at the very tip.

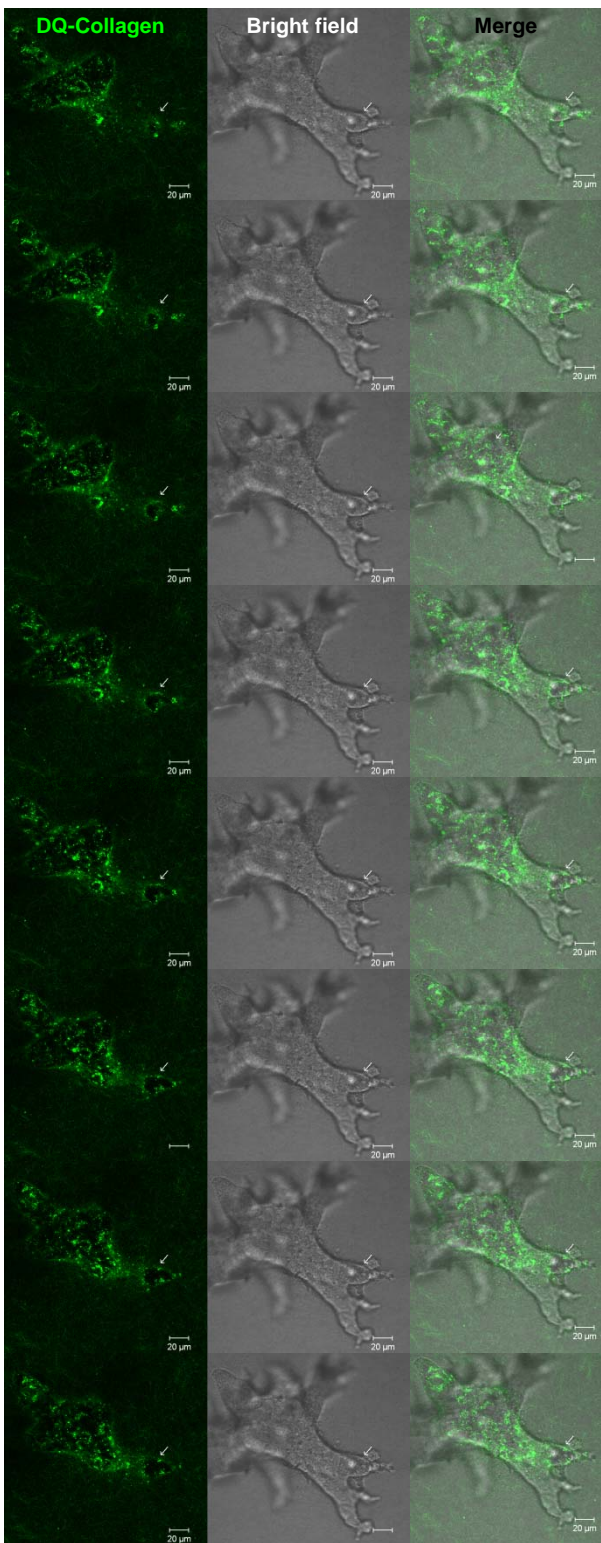
## SUPPLEMENTARY FIGURE 2



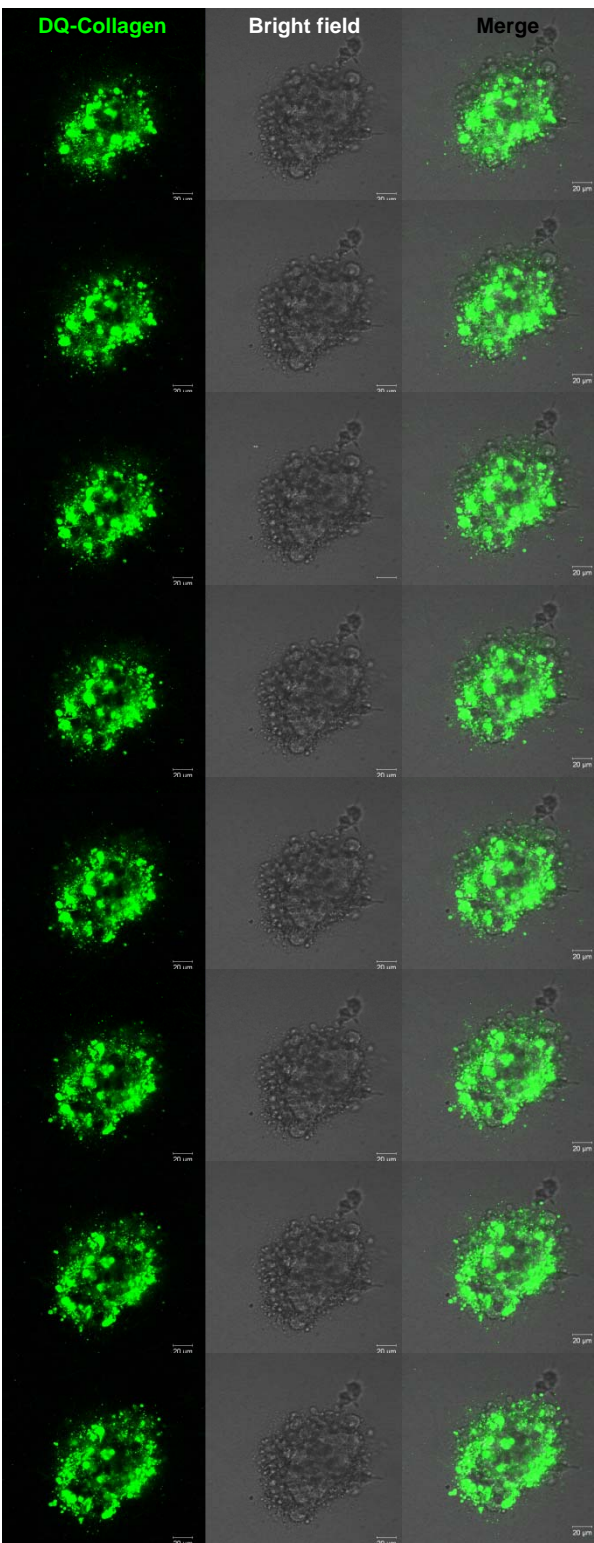
Visualization by bright field microscopy of the relative positions between cell clusters and the AFM cantilever during AFM measurements. (A) Each row corresponds to an illustrative example where either the cells (left images) or the cantilever (right images) were in focus. (B) Representative images of AFM measurements on branching cells illustrating the range of depths between the invasive front and the AFM tip used in our calculations. Left column shows images where the depth was < 10 μm, whereas right column corresponds to the maximum depth considered in this study (< 30 μm). Occasionally, focusing an invasive front rendered the tip slightly out of focus. In these conditions, we discarded cluster edges that required a vertical movement of the objective to bring the cantilever back into focus that were larger than half the cantilever height ( $0.5h_c$ ), which is estimated as ~25 μm. This distance was calculated considering the actual geometry of the cantilever as  $h_c = h_{tip} + L_c \cdot \tan\beta$ , where  $h_{tip}$  is the height of the tip,  $L_c$  is the length of the cantilever and  $\beta$  is the tilting angle of the cantilever with respect to the base of the cantilever holder, which were taken as 3 μm, 220 μm and 12 deg, respectively, according to the manufacturer's data sheet. By using these selection rules we guaranteed that the depth of the top of each cluster were < 25 μm. The suitability of this selection rule was supported by the examination of DQ-collagen by Confocal Reflection Microscopy, which reported areas void of DQ-signal with an average width of 25-40 μm at invasive fronts. Since this width is slightly larger than the 25 μm depth of invasive bodies probed with AFM, it is conceivable that we detected ECM softening at invasive fronts (see Fig. 4B) because this depth was very likely included within the degraded collagen region. All scale bars are 20 μm.



**SUPPLEMENTARY FIGURE 3**  
**- GM6001**

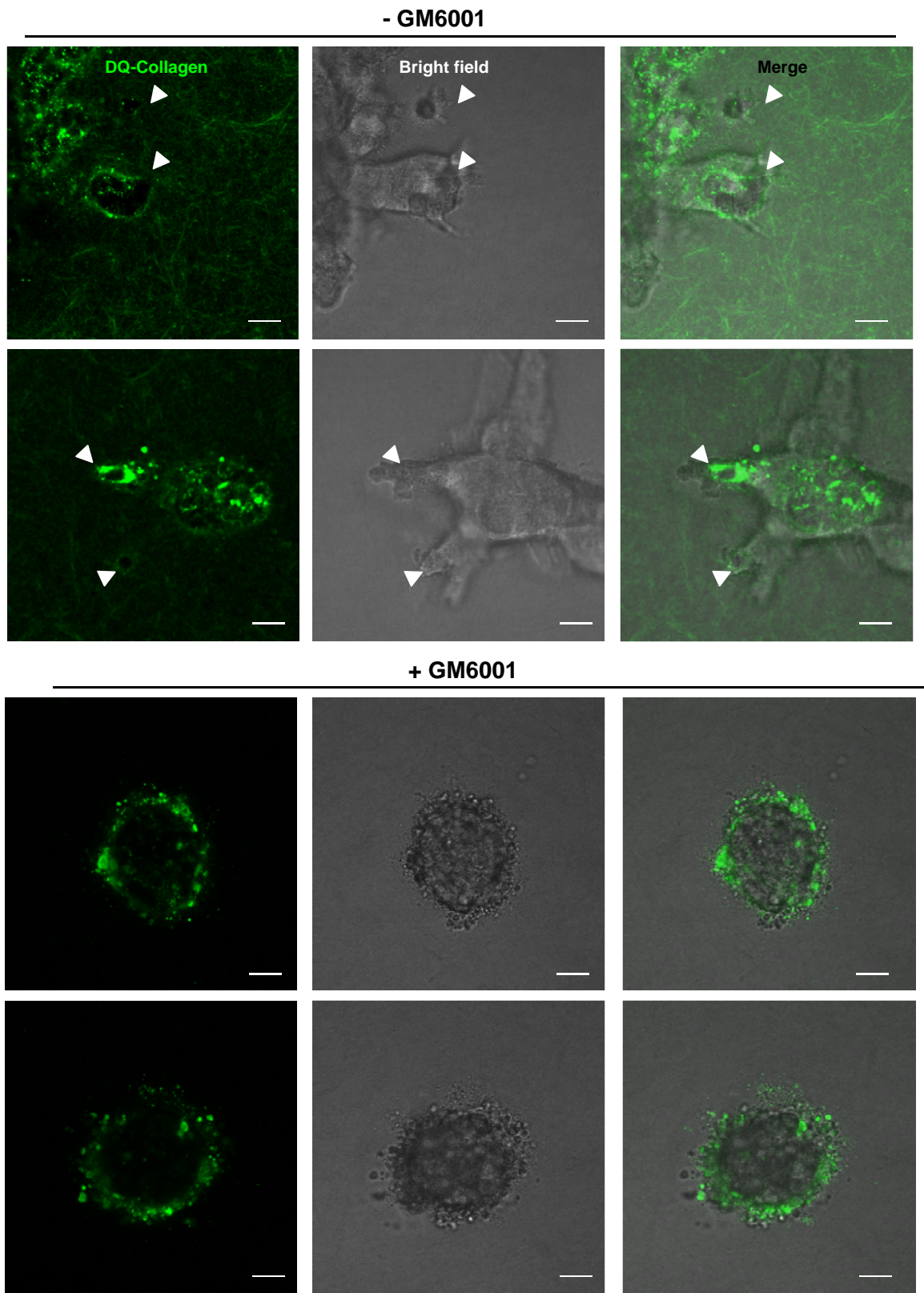


**+ GM6001**



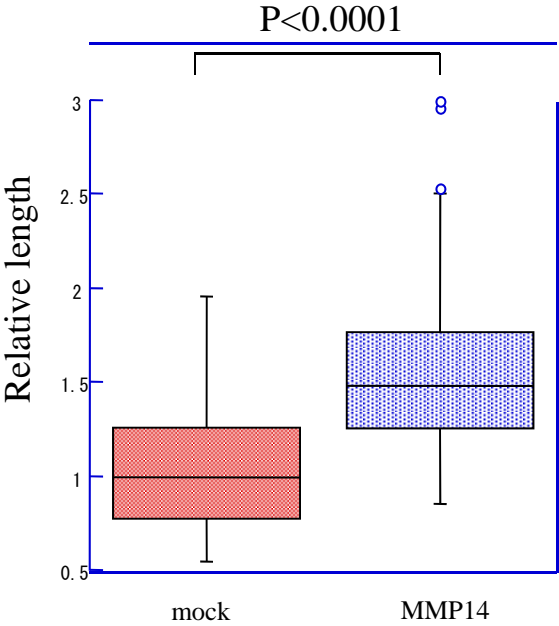
Full series of consecutive confocal stacks corresponding to the confocal images of the DQ signal shown in Fig. 4C, which were recorded on EpH4 cell clusters induced to branch in the absence or presence of the MMP inhibitor GM6001. Each row shows a confocal image (left), phase contrast image (center) and the merged image (right). All scale bars are 20 µm.

SUPPLEMENTARY FIGURE 4



Each row shows images corresponding to different Eph4 cell clusters induced to branch in the absence or presence of the MMP inhibitor GM6001, including a confocal image (left), phase contrast image (center) and the merged image (right). All scale bars are 20  $\mu\text{m}$ .

SUPPLEMENTARY FIGURE 5

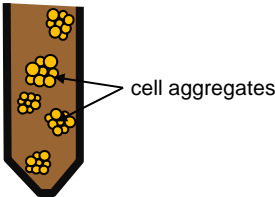


SUPPLEMENTARY FIGURE 6

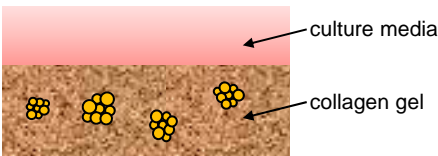
1. Form Mammary epithelial cell aggregates on poly-HEMA coated dishes



2. Resuspend cell aggregates in collagen I solution (3 mg/ml) on ice



3. Transfer solution of collagen + cells into well and incubate 30 min at 37C



4. Add growth factors (bFGF, 9 nM) for 4 days

