Electronic Supplementary Material (ESI) for Biomaterials Science.

Normal and HCC cell spreading dynamics in response to viscoelasticity

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Figure S1:

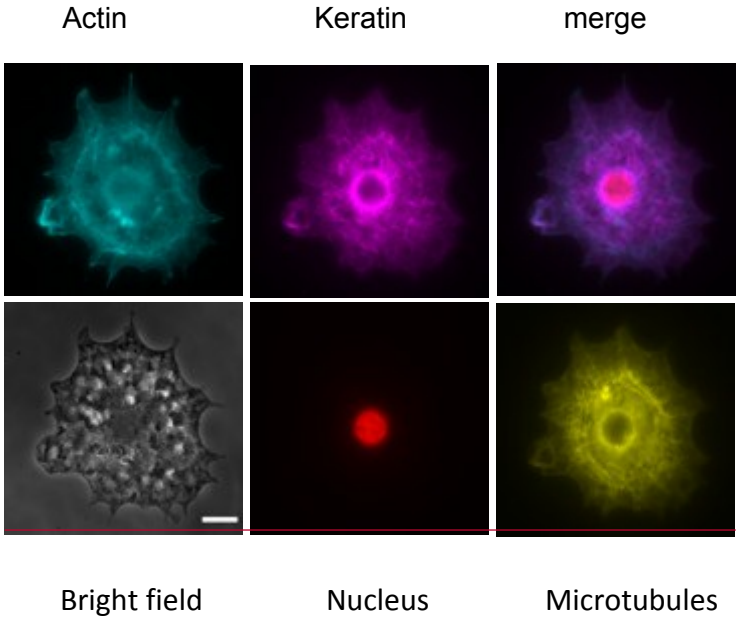


Figure S1: (A) Primary human hepatocyte on glass. Left to right: Actin (cyan), keratin (magenta), merge. lower panel: bright field, nucleus (red) and microtubules (yellow). Scale bar 10 μm.

Figure S2:

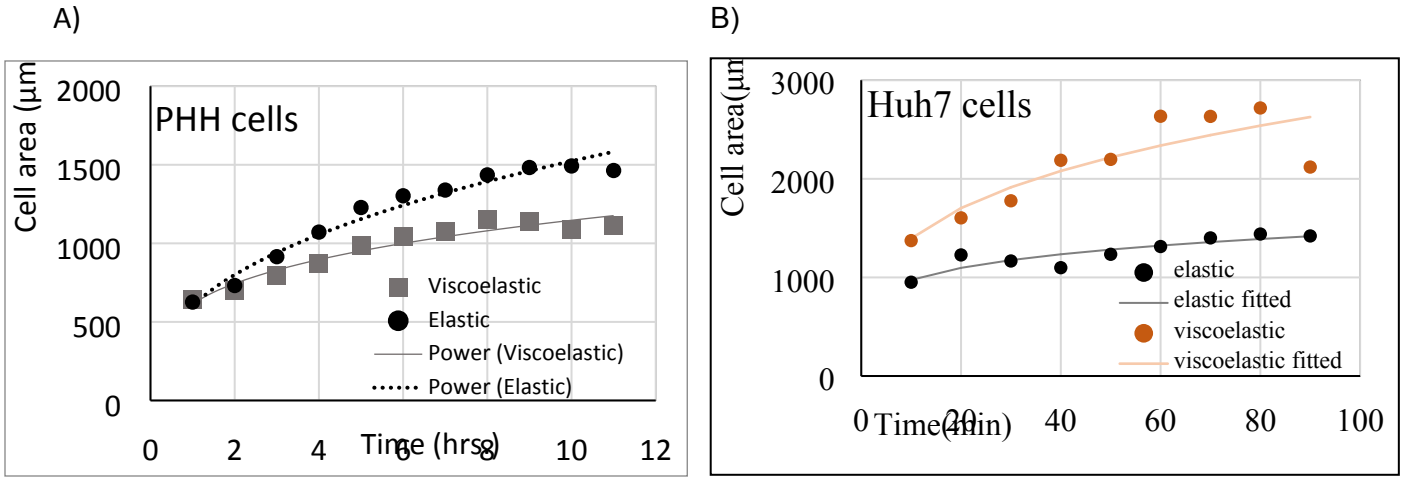
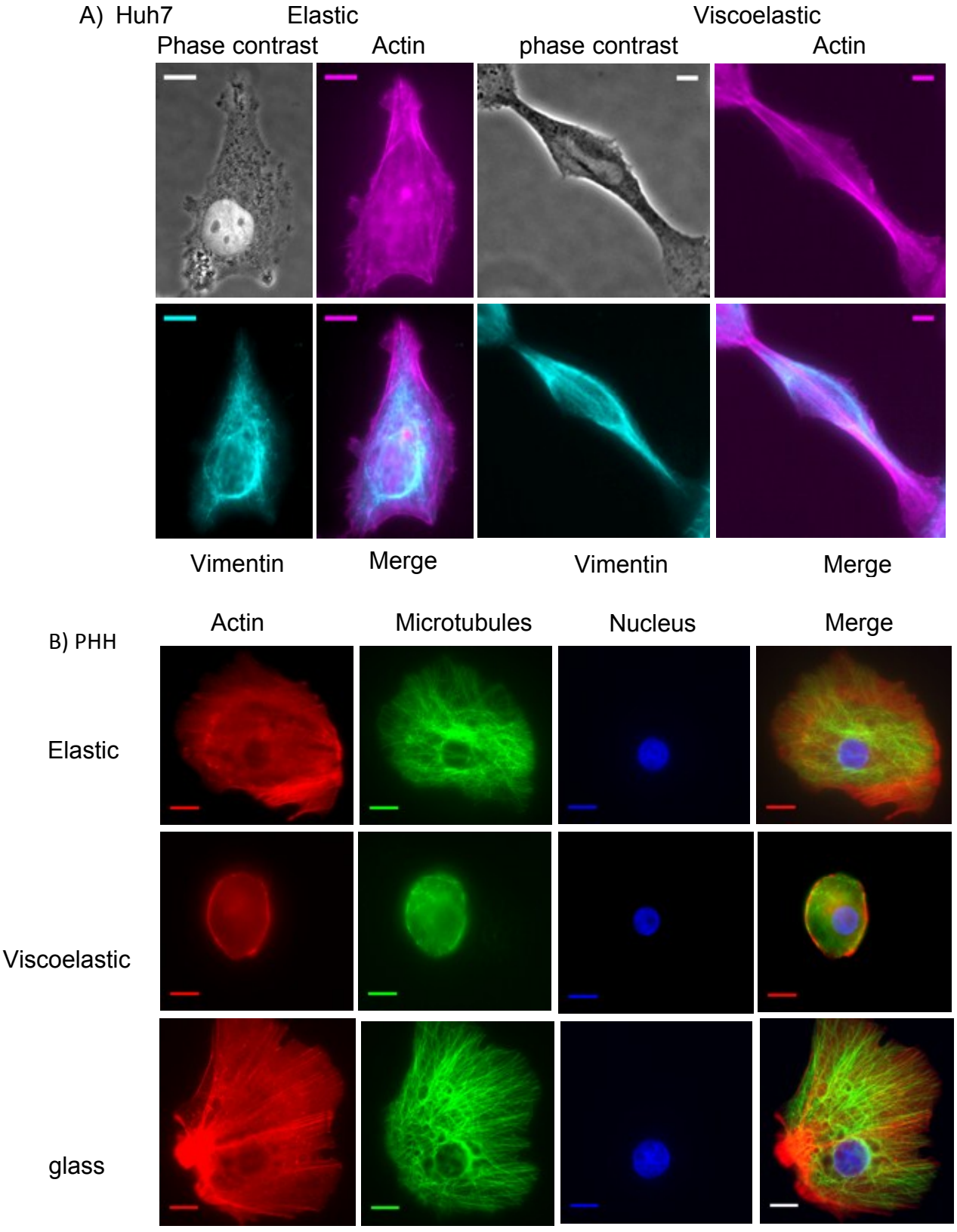


Figure S2: Typical primary human hepatocyte (left) and Huh7 cell (left) area growth curves on elastic (black) and viscoelastic (orange) substrates, fitted with power law model ($A \sim t^b$) for first 1.5hr.



FigureS3: Huh7and PHH cells are stained to visualize intracellular structure and imaged by epifluorescence microscopy.(a) Huh7 cells on elastic (left) and viscoelastic (right) substrates. Upper panel phase contrast image and actin (magenta), lower panel vimentin (cyan) and merge of actin and vimentin. (b) Primary human hepatocyte plated on elastic gel, viscoelastic gel, and glass and stained after 48 hrs. Left to right: Actin (red), Microtubules (green), nucleus (blue) and merged. Scale bar 10 μm.

Figure S4 :

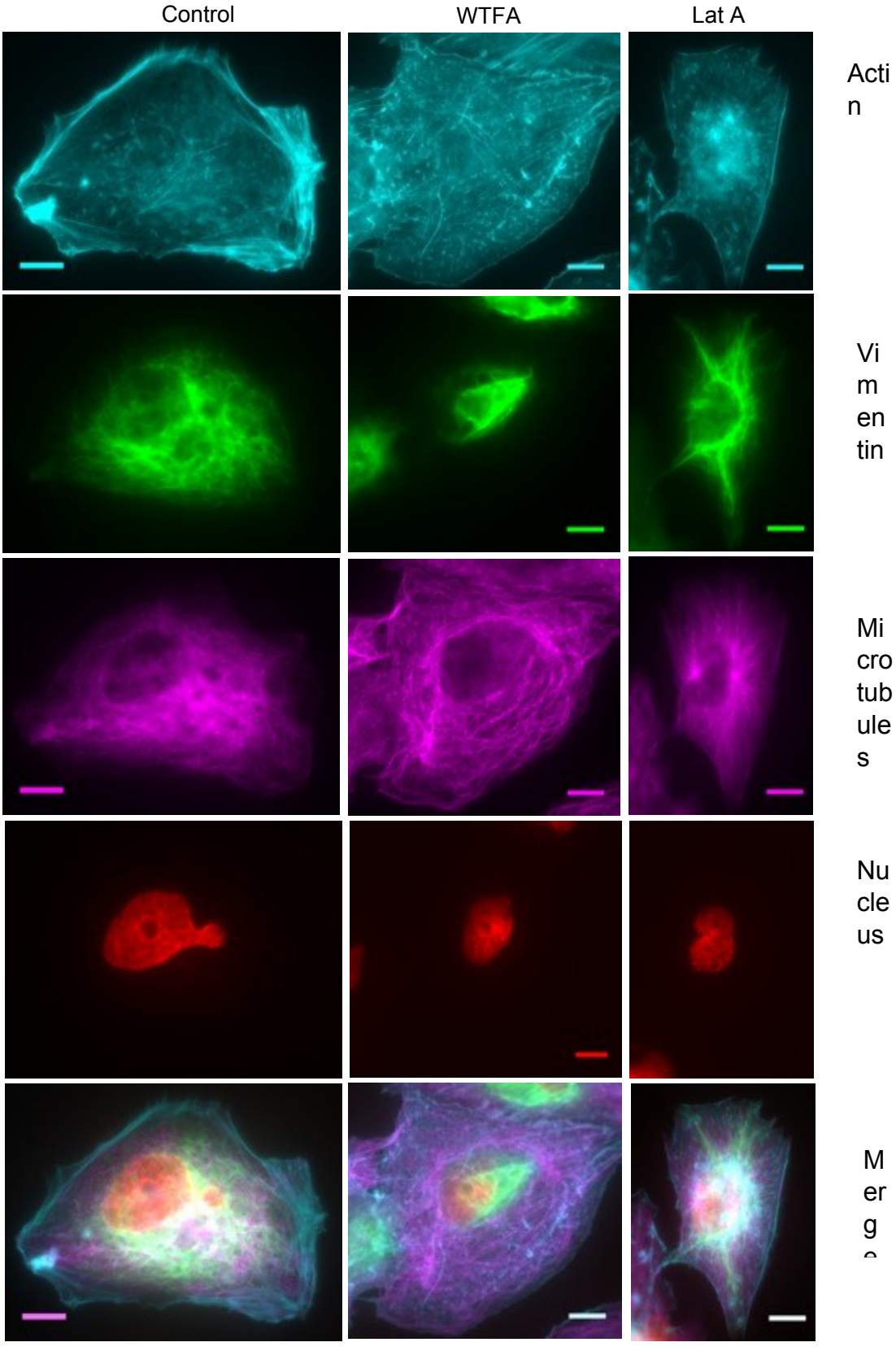


Figure S4: The effect of Withaferin A (WTFA) and Latrunculin A (Lat A) on Huh7 cell internal structure imaged by immunostaining when cells are plated on glass. Left to right: control, withaferin A and Latrunculin A. Top to bottom: Actin (cyan), vimentin (green), lower panel actin (magenta) and merge of actin and vimentin. Left to right: Actin(red), microtubules (magenta), nucleus (red) and merged. Scale bar 10 µm.

Table S1. Model parameters

Parameters	Meaning	Value	References
n_m	Myosin motor number	75	[36], [43]
F_m	Myosin force	2 pN	
n_c	Clutch number	75	
F_b	Characteristic breakage force	2 pN	
r_{on}^0	Binding rate	1 s^{-1}	
r_{off}^0	Zero force dissociation rate	0.1 s^{-1} for PHH cells 0.12 s^{-1} for Huh7 cells	Adjusted based on [36], [43]
k_c	Clutch stiffness	5 pN/nm	
v_u V_p	Unloaded retrograde flow velocity Polymerization speed	v_u,$V_p = 120\ nm/s$ for PHH cells v_u,$V_p = 200\ nm/s$ for Huh7 cells	Adjusted based on [36], [43]
k_E	Elastic PAA stiffness	0.5 pN/nm	Fitting by experiments
k_l	Left elastic stiffness	$0.1k_E$ for viscoelastic substrates $10^3 k_E$ for elastic substrates	Estimated
k_{a1}, k_{a2}	First, second additional stiffness	0.06 pN/nm, 0.04 pN/nm	Fitting by experiments
τ_{s1},τ_{s2}	Two relaxation timescales	0.9 s, 13 s	

Figure S5 :

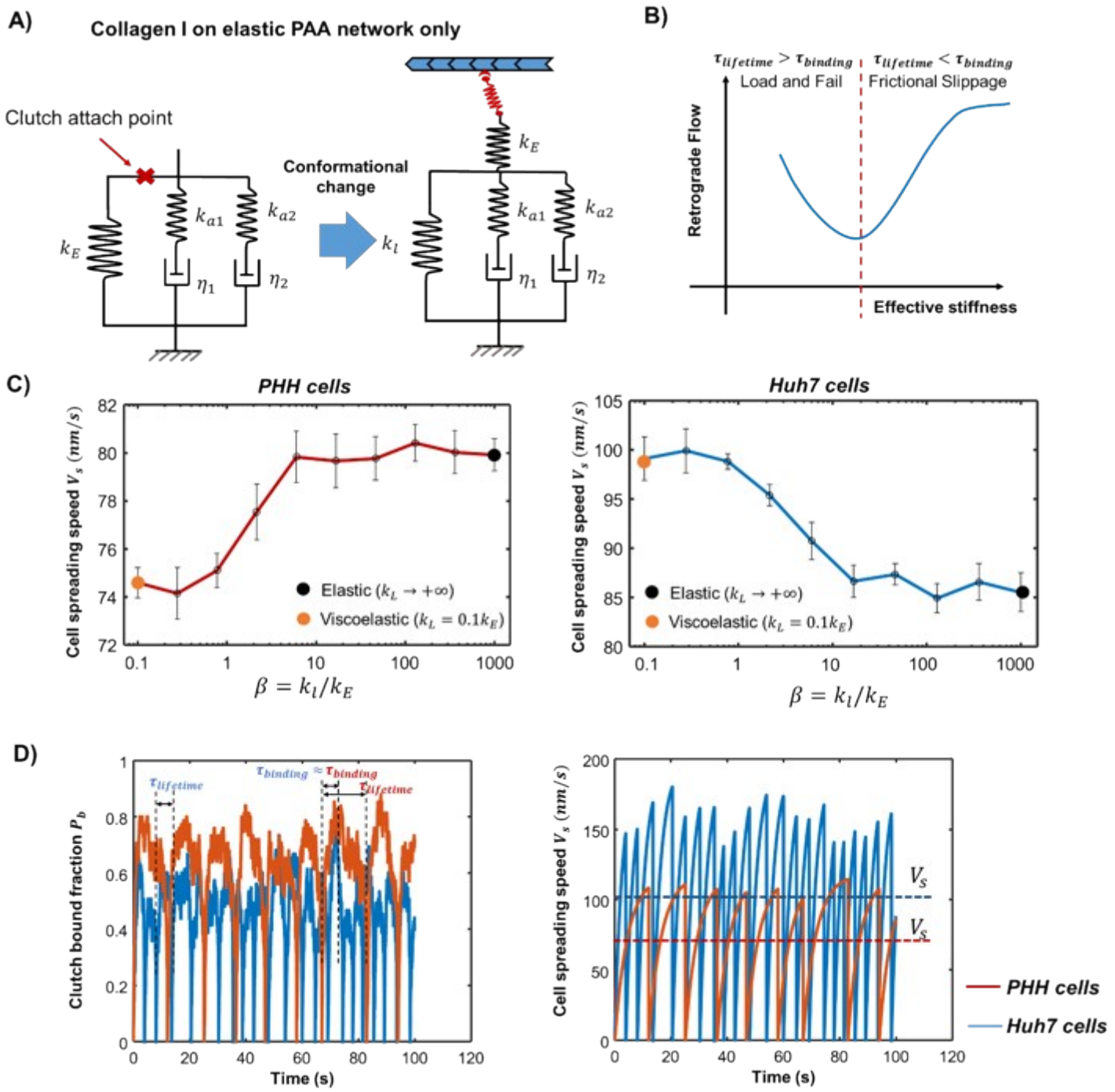


Figure S5: Theoretical model: (A) Schematic showing the linking mode. Collagen I is only coated on elastic PAA components, leading to a conformational change in PAA gels. (B) Load-and-fail clutch dynamics leads to slower time-averaged F-actin retrograde flow rates as the effective stiffness increases, whereas frictional slippage dynamics lead to increased retrograde flow rates with increasing effective stiffness. (c) Spreading speed V_s of PHH cells (left) and Huh7 cells (right) as a function of stiffness ratio $\beta = k_l/k_E$. When the stiffness ratio β becomes very large, the whole viscoelastic model (one spring in series with generalized Maxwell) converges to elastic (spring with stiffness k_E). Black and orange circled markers indicate the cell spreading speed on elastic and viscoelastic substrates, respectively. Error bars represent the standard deviation based on N=10 simulations. (D) Clutch bound fraction P_b (left) and cell spreading speed V_s (right) versus time for PHH cells (red) and Huh7 cells (blue). The dashed lines show the mean spreading velocity. Different timescales for PHH cells (red fonts) and Huh7 cells (blue fonts) are marked on the left panel.

Video files: **Supplementary Movies**

Movie1: Cell spreading on elastic substrate:
Huh7 cells were seeded on a polyacrylamide gel with G'= 5 kPa and G" = 0 Pa. The movie was taken at one frame per 5 minutes for 4 hrs or more.

Movie2: Cell spreading on viscoelastic substrate:
Huh7 cells were seeded on a polyacrylamide gel with G'= 5 kPa and G" = 600 Pa at 1Hz. The movie was taken at one frame per 5 minutes for 4 hrs or more.

Movie3: Cell spreading on elastic substrate when treated with withaferin A:
Huh7 cells were seeded on a polyacrylamide gel with G'= 5 kPa and G" = 0 Pa. Cells were treated with 8 μM withaferin A before the movie started. The movie was taken at one frame per 5 minutes for 4 hrs or more.

Movie4: Cell spreading on viscoelastic substrate when treated with withaferin A:
Huh7 cells were seeded on a polyacrylamide gel with G'= 5 kPa and G" = 600 Pa at 1Hz. Cells were treated with 8 μM withaferin A before the movie started. The movie was taken at one frame per 5 minutes for 4 hrs or more.

Movie5: Cell spreading on elastic substrate when treated with Latrunculin A:
Huh7 cells were seeded on polyacrylamide gel with G'= 5 kPa and G" = 0 Pa. Cells were treated with 2 μM Latrunculin A before the movie started. The movie was taken at one frame per 5 minutes for 4 hrs or more.

Movie6: Cell spreading on viscoelastic substrate when treated with Latrunculin A:
Huh7 cells were seeded on a polyacrylamide gel with G'= 5 kPa and G" = 600 Pa at 1Hz. Cells were treated with 2 μM Latrunculin A before the movie started. The movie was taken at one frame per 5 minutes for 4 hrs or more.