Supplementary Information

Matrix mechanics regulates epithelial defence against cancer by tuning dynamic localization of filamin

Pothapragada *et al.*

This document contains:

Supplementary Table 1. Composition of compliant polyacrylamide gels with different stiffness.

Stiffness (Young's Modulus)	**1.2 kPa**	**4 kPa**	**11 kPa**	**23 kPa**	**35 kPa**	**90 kPa**
milli-Q H_2O (µl)	820	807	697	695	650	542
40% acrylamide (µl)	137	125	250	187	200	300
2% bis-acrylamide (µl)	25	50	35	100	132	140
Fluorescent beads (µl)	12	12	12	12	12	12
10% APS (µl)	5.75	5.75	5.75	5.75	5.75	5.75
TEMED (µl)	0.25	0.25	0.25	0.25	0.25	0.25

Supplementary Table 2. Antibody information

Antibodies/Fluorophore	Catalog No.	Manufacturer	Dilution/Working concentration
DAPI (4′,6-diamidino-2-phenylindole)	D1306	Invitrogen	1 μg ml^{-1}
Alexa-Fluor-647 Phalloidin	8940	CST	1:40
Alexa-Fluor-555 Phalloidin	8953S	CST	1:40
Mouse anti-FilaminA (monoclonal)	F6682, clone PM6/317	Sigma	1:100
Rabbit anti-HA-tag (monoclonal)	3724S, clone C29F4	CST	1:800
anti-FLAG-M2 (monoclonal)	F1804	Sigma	1:500
Rabbit anti-Cofilin (monoclonal)	5175S, clone D3F9	CST	1:200
Rabbit anti-pMLC2(Ser19)	3671	CST	1:50
Mouse polyclonal anti-DNMBP (anti-Tuba)	ab88534	Abcam	1:200
Alexa Fluor Plus 594 donkey anti-mouse IgG secondary antibody	A32744	Invitrogen	Same as per primary antibody dilution
AlexaFluor 488, Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody	A-11001	Invitrogen	Same as per primary antibody dilution
Alexa Fluor 488, Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody	A-11008	Invitrogen	Same as per primary antibody dilution
Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor®555 Conjugate)	4413S	CST	Same as per primary antibody dilution
Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor-647 Conjugate)	4414S	CST	Same as per primary antibody dilution

Supplementary Table 3. Plasmid information

Plasmid	Source	Identifier	Comment
pcDNA4/TO/GFP-RasV12	Yasuyuki Fujita	DOI: 10.1038/ncb1853	Tetracycline-inducible $HRas^{V12}$ expression
Cortactin-pmCherryC1	Addgene	27676	Cytoskeletal protein
FusionRed-Fascin-C-10	Addgene	56117	Cytoskeletal protein
mApple-Alpha-Actinin-19	Addgene	54865	Cytoskeletal protein
mCherry-ARP3-C-12	Addgene	54981	Cytoskeletal protein
mCherry-VASP-5	Addgene	55151	Cytoskeletal protein
mEmerald-N-Wasp-C-18	Addgene	54199	Cytoskeletal protein
YFP-mDia2	Addgene	25420	Cytoskeletal protein
pECE-M2-BAIAP2 wt	Addgene	31656	Cytoskeletal protein
mApple-FilaminA-N-9	Addgene	54901	Filamin overexpression
mApple-N1	Addgene	54567	Destination vector for mutant sequence
mApple-C1	Addgene	54631	Destination vector for mutant sequence
FAM101B-HA	This work	N/A	Human FAM101B and mScarlet cloned into pcDNA3.1+/C-HA
mApple-dnFLNA	This work	DOI: 10.1073/pnas.1104211108	Human FLNA Repeat [19-22]-HA cloned into mApple-C1
mApple-dnFAM101B	This work	DOI: 10.1073/pnas.1104211108	Human FAM101B ΔBD2 cloned into mApple-N1
pcdna nesprin TS	Addgene	68127	Nesprin tensor sensor
pcdna nesprin HL	Addgene	68128	Nesprin headless control
mApple-dnNesprin1	This work	DOI: 10.1242/jcs.02471	aa. 8369-8749 of human Nesprin-1 cloned into mApple-C1
mApple-dnLaminB1	This work	PMID: 11683386	XLaminB1Δ2+ (34-420 aa from X06344) cloned into mApple-C1
Raichu-Cdc42	Kyoto Univ.	Raichu-1054X	Cdc42 FRET-biosensor
LifeAct-TagGFP2	iBidi GmbH	60101	F-actin binding peptide

Supplementary Video Legends

Supplementary Video 1. Extrusion of HRas^{V12}-expressing cells by normal cells post-induction with doxycycline. HRas^{V12}-expression was induced by doxycycline and tracked up to 23 hours by taking images at 1 hr interval. *Top panels*: DIC images; *bottom panels*: fluorescence images.

Supplementary Video 2. Dynamics of photo-converted mEos2-filamin molecules on soft ECM. mEos2-filamin cell interfacing with an HRas^{V12} cell was stimulated and tracked for 180 seconds. This video corresponds to the snapshots showed in Fig. 2f, *top panels*, on soft ECM.

Supplementary Video 3. Dynamics of photo-converted mEos2-filamin molecules on stiff ECM. mEos2-filamin cell interfacing with an HRas^{V12} cell was stimulated and tracked for 180 seconds. This video corresponds to the snapshots showed in Fig. 2f, *bottom panels*, on stiff ECM.

Supplementary Figure Legends

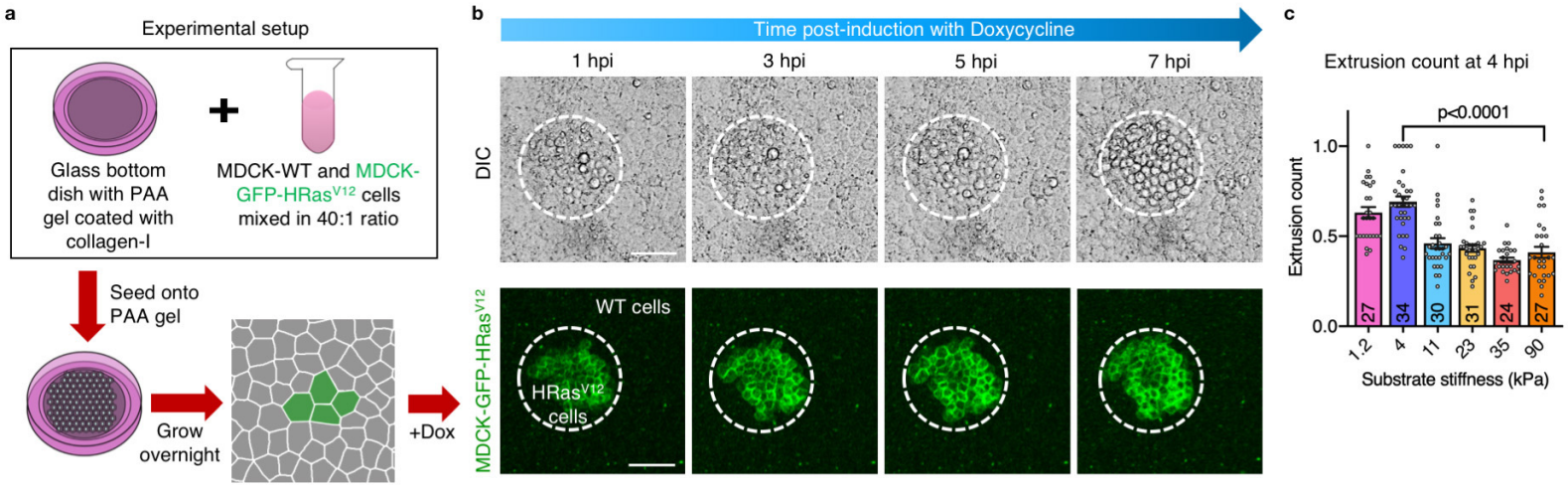
Supplementary Figure 1. Extrusion of MDCK-GFP-HRas^{V12}-transformed cells co-cultured with MDCK-WT cells. a. Illustration detailing the used experimental epithelial monolayer model of EDAC. **b.** Representative DIC images of MDCK-WT:MDCK-GFP-HRas^{V12} mosaic monolayer (*top row*), fluorescence images of GFP-HRas^{V12} expressing cells (*bottom row*). From left to right: snapshots of GFP-HRas^{V12} expressing cells at different time points post-induction with doxycycline. hpi: hour post-induction. One representative extruding colony of transformed cells encircled (dotted-white circle). Scale bars, 60μm. **c.** Scatter bar plots depicting the fraction of GFP-HRas^{V12}-expressing colonies extruded over substrates of varying stiffness at 4 hpi. The number of colonies counted is indicated inside each bar. Data are mean±s.e.m. collected over 3 independent biological replicates. Statistical significance was assessed using Mann-Whitney t-test (two-tailed).

Supplementary Figure 2. ECM-stiffness dependent intracellular localization of actin-binding proteins. a-g. Fluorescence images of MDCK-WT cells transiently transfected with Cortactin_pmCherry (**a**), FusionRed_Fascin (**b**), mApple-alpha-Actinin (**c**), mCherry-ARP3 (**d**), mCherry-VASP (**e**), mEmerald-N-WASP (**f**) and YFP-mDia2 (**g**). **h** Immunofluorescence images of MDCK-WT cells transiently transfected with BAIAP2-M2 and stained with anti-M2. **i,j** Immunofluorescence images of MDCK-WT monolayers stained with anti-cofilin (**i**) or anti-pMLC (**j**). **a-j** Top panels: Soft ECM; Bottom panels: Stiff ECM. Nucleus stained with DAPI. Scale bars, 10μm.

Supplementary Figure 3. ECM stiffness-dependent differential localization and dynamics of filamin. a. Immunofluorescence images of MDCK-WT cells immunostained for filamin cultured on soft (4 kPa, *top* panels) and stiff (90 kPa, *bottom* panels) ECM. Perinuclear and interfacial regions traced manually for quantifying mean fluorescence intensities. Representative grayscale images with traced-out ROIs displayed for cells cultured on soft (*top*) and stiff (*bottom*). Schematic showing the manual ROIs (red) used within each cell for perinuclear and interfacial regions. **b.** Immunofluorescence images of MDCK-WT cells culture on soft (4 kPa) and stiff (90 kPa) ECM stained with anti-FilaminA and AlexaFluor555-Phalloidin. Yellow arrowhead points to perinuclear co-localization of filamin and F-actin on stiff ECM. **c.** Photobleaching in mApple-FLNA cells cultured on soft (top panel) or stiff ECM (middle and bottom panels). White dotted-box indicates the photobleached regions: cell-cell interface (*top*, soft ECM), cell-cell interface (*middle*, stiff ECM) and perinuclear region (*bottom*, stiff ECM). Scale bars: 10 μm. Kymographs

for the dotted regions shown followed by the schematic depicting the differential and dynamic recovery of filamin. **d.** FRAP curve that depicts the recovery of mApple-FLNA post photobleaching at the ROIs used in (**c**).

Supplementary Figure 4. FAM101B and perinuclear cytoskeleton regulate perinuclear localization of filamin. a. Immunofluorescence images of MDCK-WT monolayer transfected with FAM101B-HA and stained with anti-HA and anti-Filamin, on soft (*top panels*) and stiff (*bottom panels*) substrates. Magnified view of boxed region shown in third panels. FAM101B-HA co-localizes with filamin to perinuclear region on stiff substrate. **b.** Nesprin tension sensor (Nesprin-TS) expressed in MDCK-WT cells cultured on soft (*top panels*) and stiff (*bottom panels*) substrates. The schematic underneath shows the construct, where the tension sensor module comprising of mTFP1 and mVenus FRET pair has been inserted between the actin binding domain and SUN binding domain of Nesprin. Increased force increases the distance between the FRET-pair causing a decrease in the FRET index. (*Right*) Box-and-whisker plot showing significant reduction of FRET index in cells cultured on stiff substrate, indicative of high LINC complex tension. **c.** Schematic representations of dominant-negative mutant constructs used. **d-g.** Immunofluorescence images of mApple-dnFLNA-MDCK cells (**d**), MDCK-mApple-dnFAM101B cells (**e**), mApple-dnNesprin1-MDCK cells (**f**), mApple-dnLaminB1-MDCK (**g**) stained with anti-filamin and corresponding grayscale images with traced-out ROIs displayed for cells cultured on stiff ECM. ROIs used for quantification of the fraction of filamin localization at perinuclear versus interfacial regions. Scale bars, 10μm.



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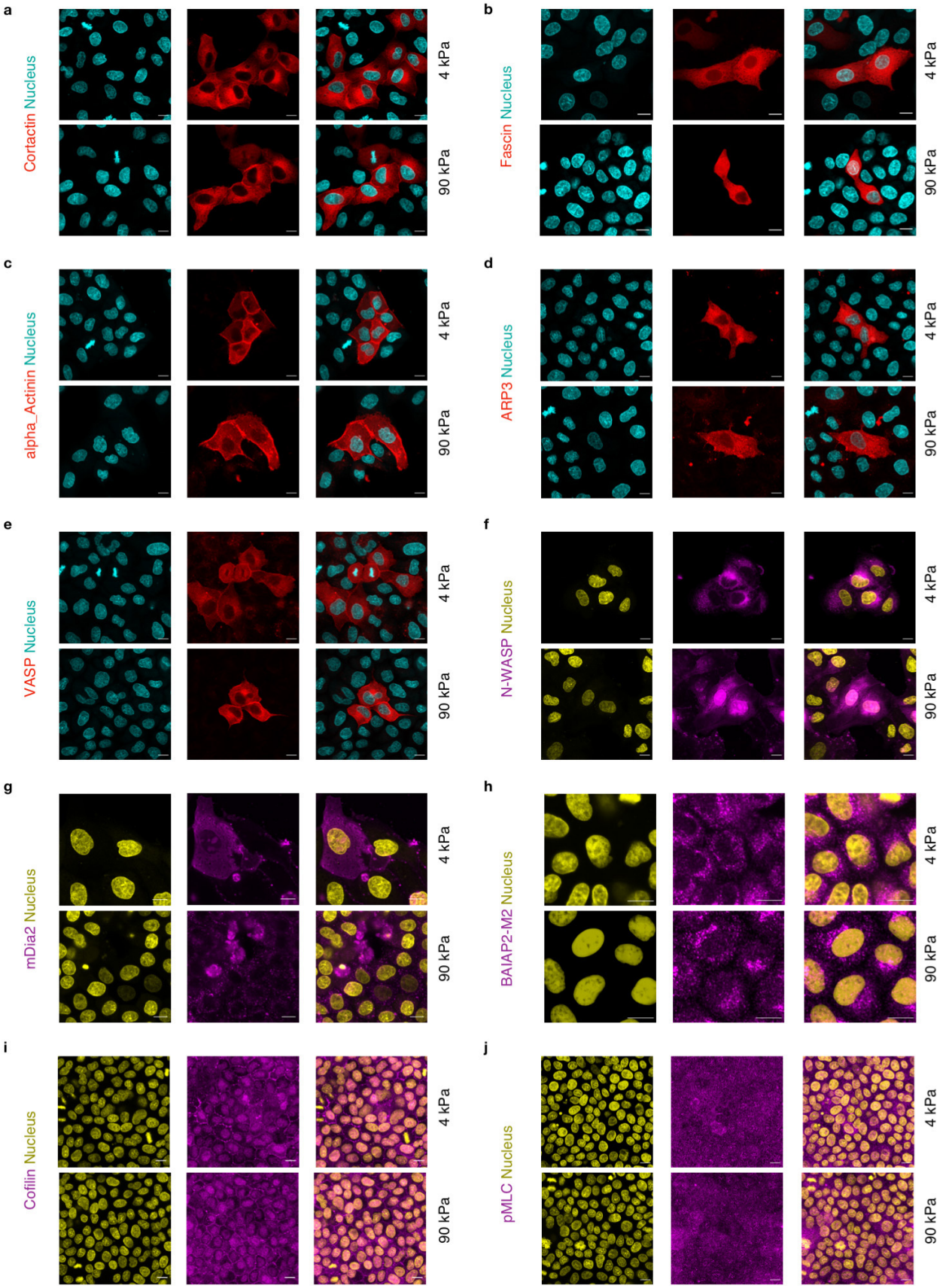

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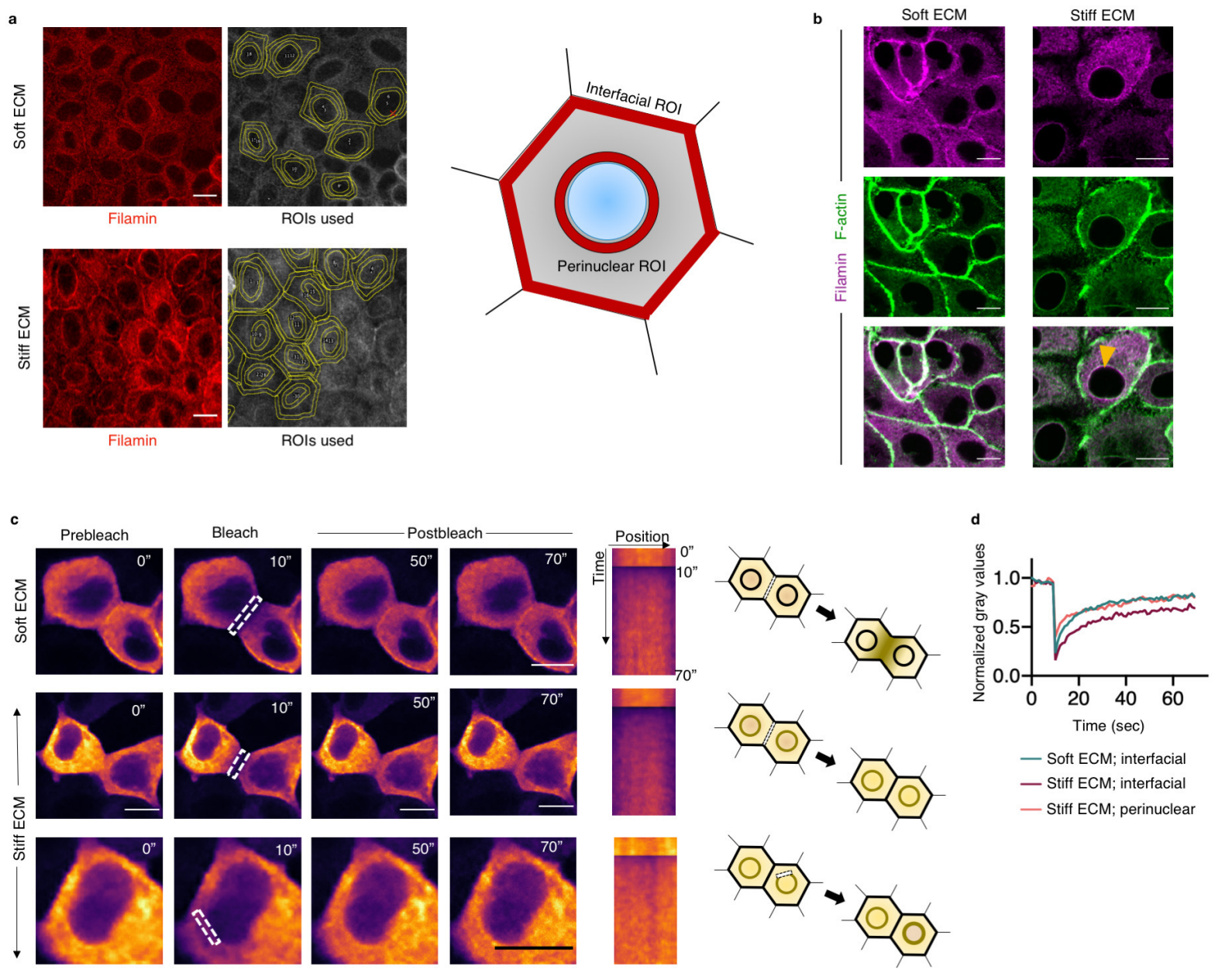

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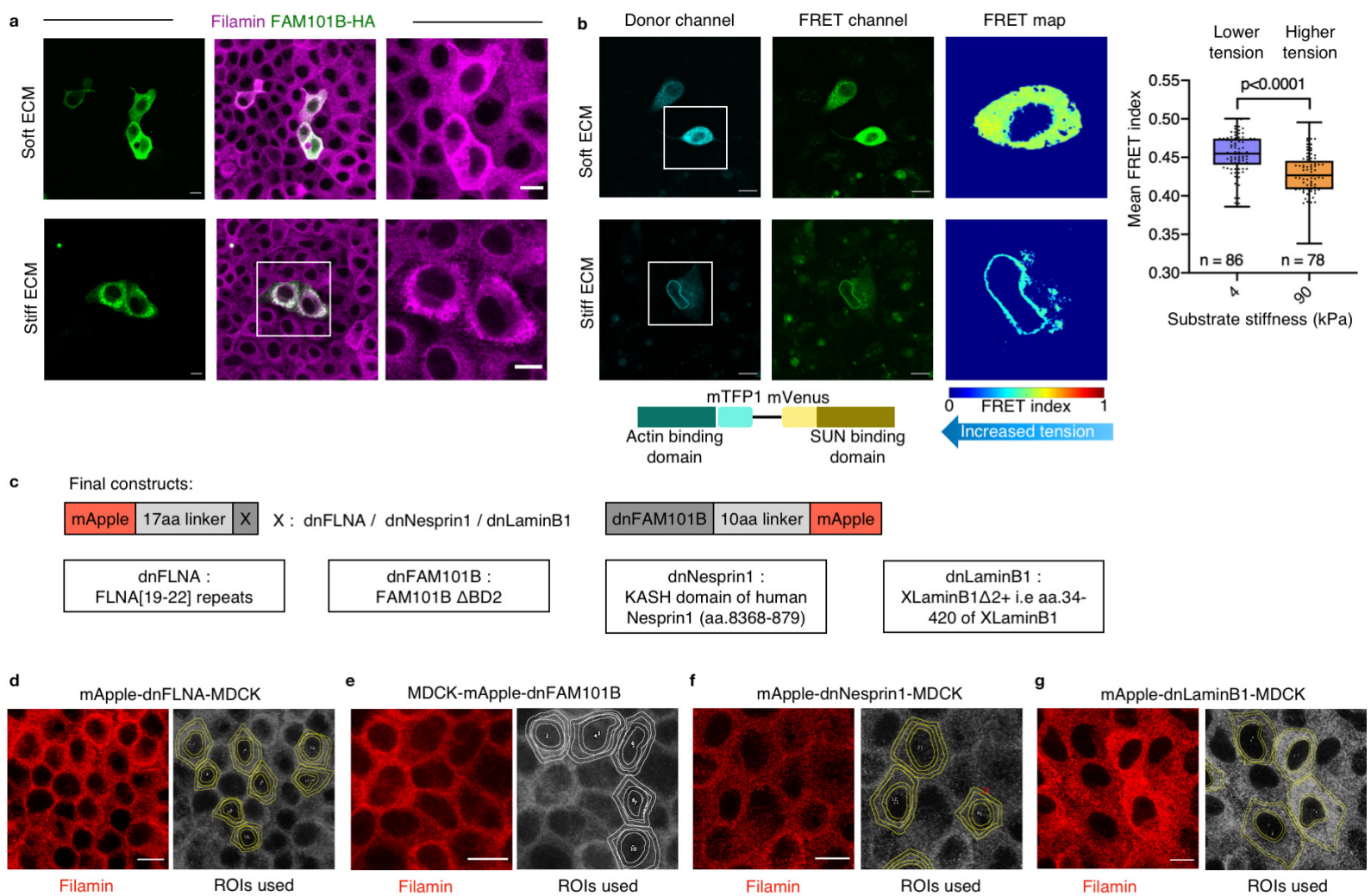

Supplementary Figure 4. Pothapragada *et al.*