Supplemental Materials

Molecular Biology of the Cell

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Supplementary Figure Legends

Supplementary Figure 1

A, B. Representative immunofluorescence images (A) and quantification (B) of vinculin staining show increased presence of vinculin at cell-matrix adhesions (CM) and adherens junction (AJ) at 30 min of stretch (mean ±SD; n=3 independent experiments; *p=0.043 (CM), *p=0.046**p=0.0023, ANOVA with Dunnett's; scale bars 30 μm). **C, D.** Representative segmented images and quantification of cell aspect ratio and cell spread area show no substantial differences in response to stretch (mean ±SD; n=3 independent experiments; scale bars 30 μm). **E, F**. Quantification of adherens junction (AJ) remodeling from VE-Cadherin staining (E) and pMLC2 intensity (F) in response to specific stretch frequencies (mean ±SEM; n=5 independent experiments; (mean ±SEM; n=5 independent experiments; ***p=0.002 and **p=0.0050 (VE-Cadherin 600mHz and 1200mHz) **p=0.0037 and *p=0.0266 (pMLC2 600mHz 30min and 1h) *p=0.0424 and *p=0.0233 (pMLC2 1200mHz 30min and 1h); ANOVA, Dunnett).

Supplementary Figure 2

A. Principle component (PC) analyses of VE-Cadherin BioID2 pulldowns in response to 0, 30 and 360 min of stretch show subtle changes in VE-Cadherin interactome upon 30 min and 3h of stretch. **B.** Examples of significantly altered VE-Cadherin interacting proteins upon stretch. **C.** Pie chart annotating Gene Ontology (GO) terms of significantly different proteins in proximity of VE-Cadherin at 30min (left) and 3h (right) of stretch.

Supplementary Figure 3

A. Representative western blot analysis of total Rho assay (activity measured calorimetrically with G-LISA). **B.** Representative western blot analysis of active Rac pulldown assay. **C.** Quantification of cell spread area in response to treatment of HUVEC monolayers with 10μm Y37632 or 5μm IPA3 for 15 minutes without stretch or subjected to 20% biaxial stretch for 30 or 3hrs (3 independent experiments; *p=0.0315, ***p=0.0005 ANOVA) **D.** Representative live imaging snapshots of calcium influx (gCamp3) in response to ionophore application. Scale bars 30 μm. **E.** Representative immunofluorescence images of VE-Cadherin and pMLC2 staining in DMSO-, 10μm Y37632- or 5μm IPA3-treated HUVEC monolayers (left) and quantification of pMLC2 intensity (right) **p=0.0024 ANOVA Kruskal-Wallis**.**

Supplementary Figure 4

A. RT-qPCR (left) and Western blot (right) analysis of Piezo1 mRNA and protein levels in control and Piezo1 RNAi cells (mean ±SD; n=6 independent experiments; ***p<0.001, Student's t-test for RT-qPCR; ***p=0.0004 for protein analysis). **B.** RT-qPCR analysis of Piezo1 mRNA in control and stretched cells (mean ±SD; n=3 independent experiments). **C, D.** Representative Western blots (C) and quantification (D) of Piezo1 protein levels show that inhibition of the proteasome by MG132 prevents Piezo1 degradation while inhibition of the lysosome with bafilomycin A1 (BF) has no effect (mean ±SD; n=3 independent experiments; ***p=0.002 (BF 3h); *p=0.0253 (BF 30m) ANOVA; Holm-Sidak)

Supplementary Figure 5

A. Schematic illustration of the atomic force microscopy experiments to quantify monolayer cortical stiffness. **B.** Frequency distribution of monolayer elastic moduli show that stiffening in response to stretch is most effectively prevented by cytochalasin D (cytoD) treatment. Blebbistatin has a similar but less pronounced effect (n=100-120 force curves pooled across 3 independent experiments; *p<0.002 (CytoD), Kolmogorov-Smirnov test). **C.** Representative immunofluorescence images of tubulin and vimentin show no substantial effects of stretch on these cytoskeletal elements. Scale bars 30 μm. **D**. RT-qPCR analysis of Filamin A and B mRNA in control and Filamin A and B RNAi cells (mean ±SD; n=3 independent experiments; **p<0.0075 (Filamin A),**p<0.009 (Filamin B), Student's t-test). **E.** Frequency distribution of monolayer elastic moduli in Filamin-depleted HUVEC monolayers.

Supplementary Table 1

Differential protein abundance analysis of VE-Cadherin BioID2 pulldown experiments

Supplementary Table 2

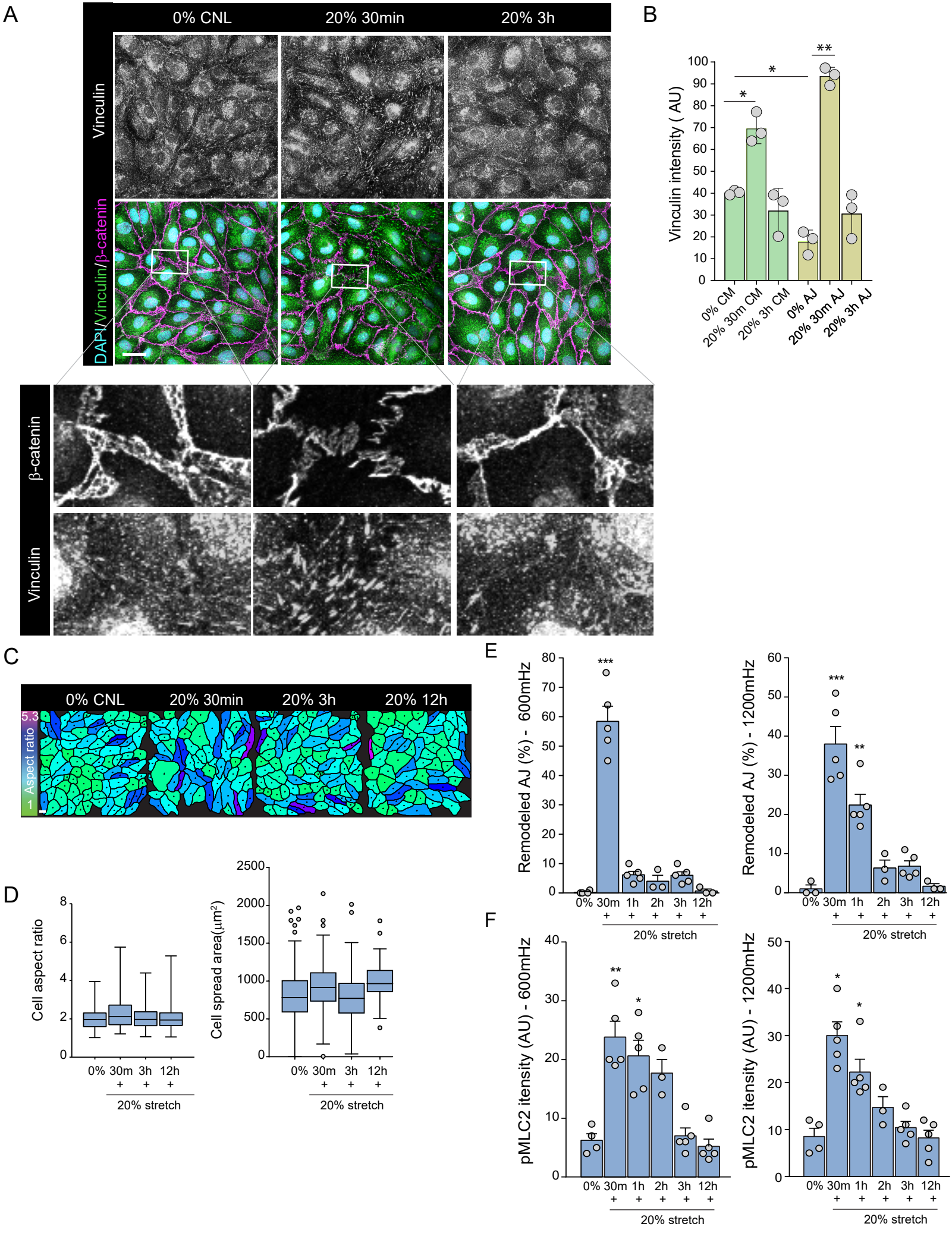
Differential protein abundance analysis in cells stretched for 60 min

Supplementary Table 3

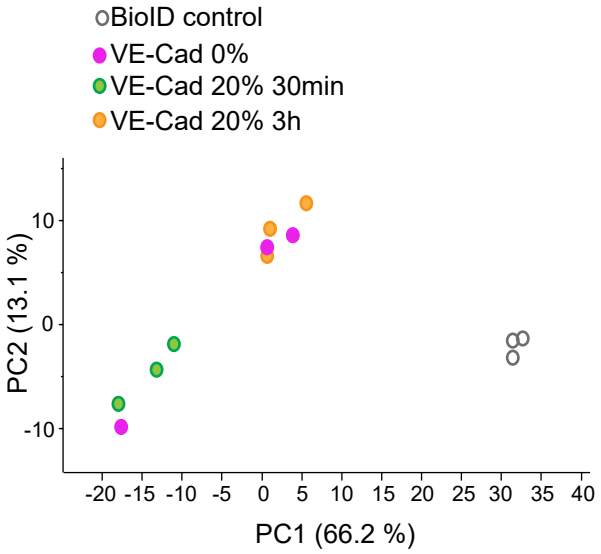
Differential phosphoprotein abundance analysis in cells stretched for 30 min

Supplementary Table 4

Differential phosphoprotein abundance analysis in cells stretched for 60 min


A
0% CNL
20% 30min
20% 3h
Vinculin
DAPI/Vinculin/β-catenin
β-catenin
Vinculin
B
Vinculin intensity (AU)
0% CM
20% 30m CM
20% 3h CM
0% AJ
20% 30m AJ
20% 3h AJ
C
0% CNL
20% 30min
20% 3h
20% 12h
Aspect ratio
D
Cell aspect ratio
Cell spread area(μm²)
0% 30m 3h 12h
20% stretch
E
Remodeled AJ (%) - 600mHz
Remodeled AJ (%) - 1200mHz
0% 30m 1h 2h 3h 12h
20% stretch
F
pMLC2 itensity (AU) - 600mHz
pMLC2 itensity (AU) - 1200mHz
0% 30m 1h 2h 3h 12h
20% stretch

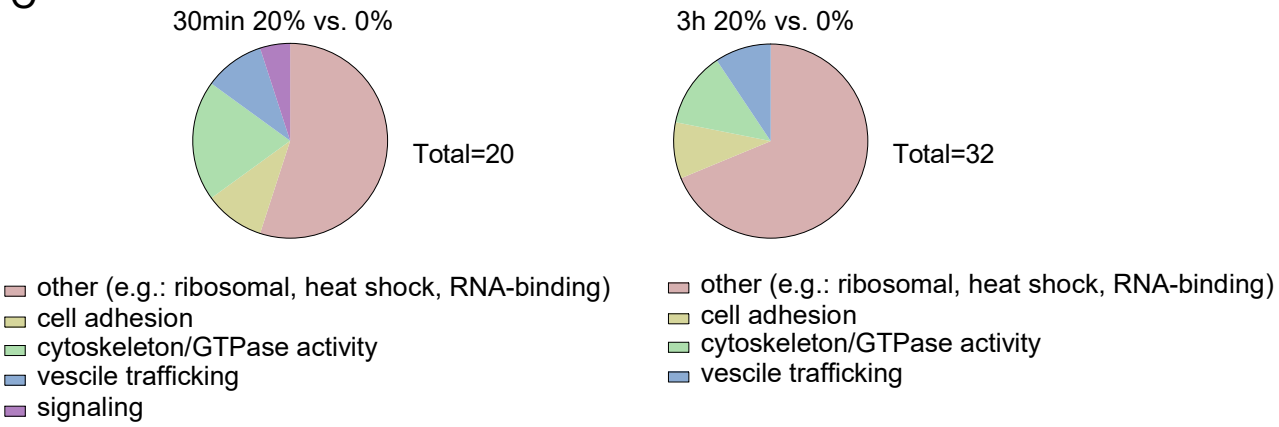
A

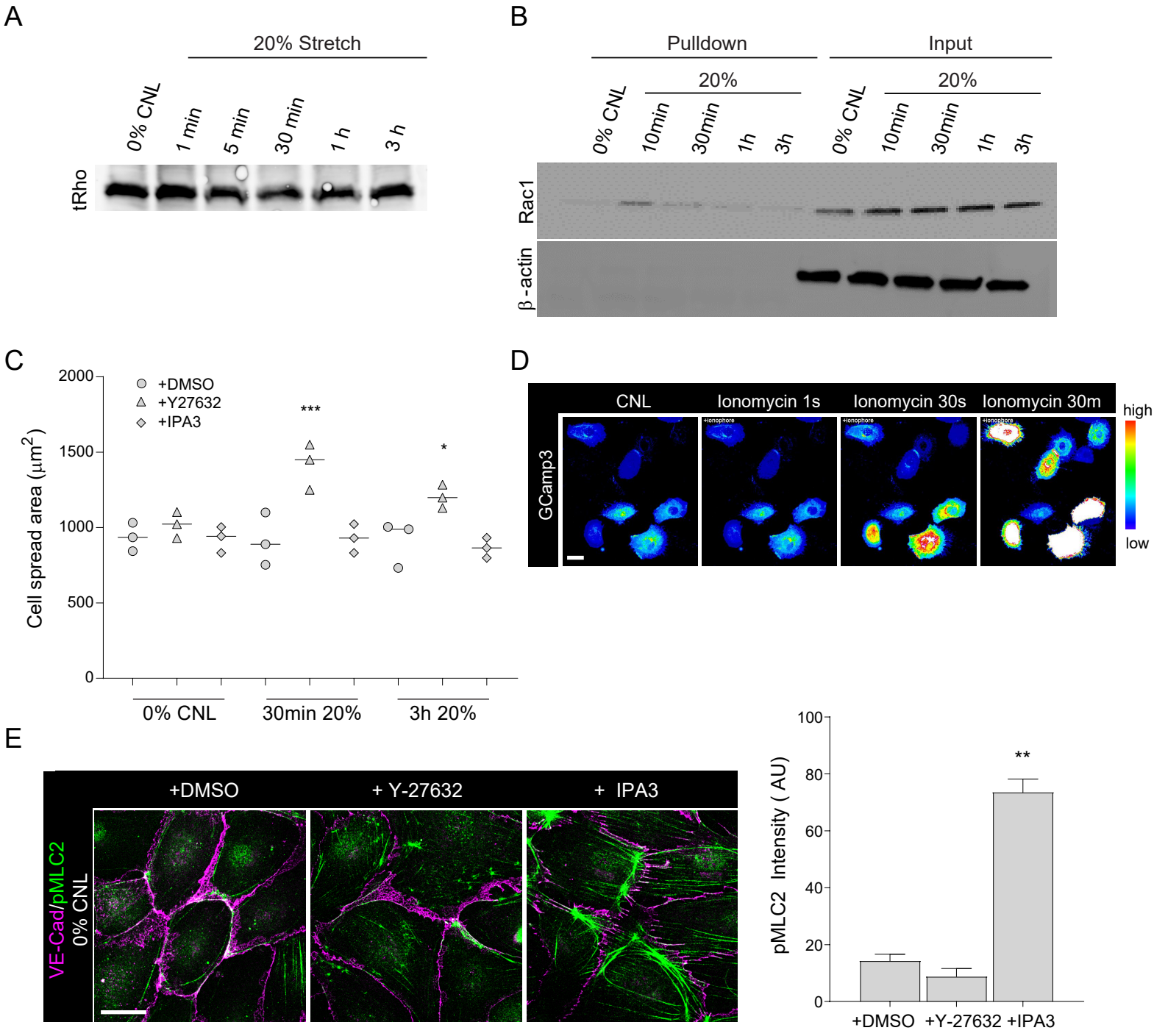


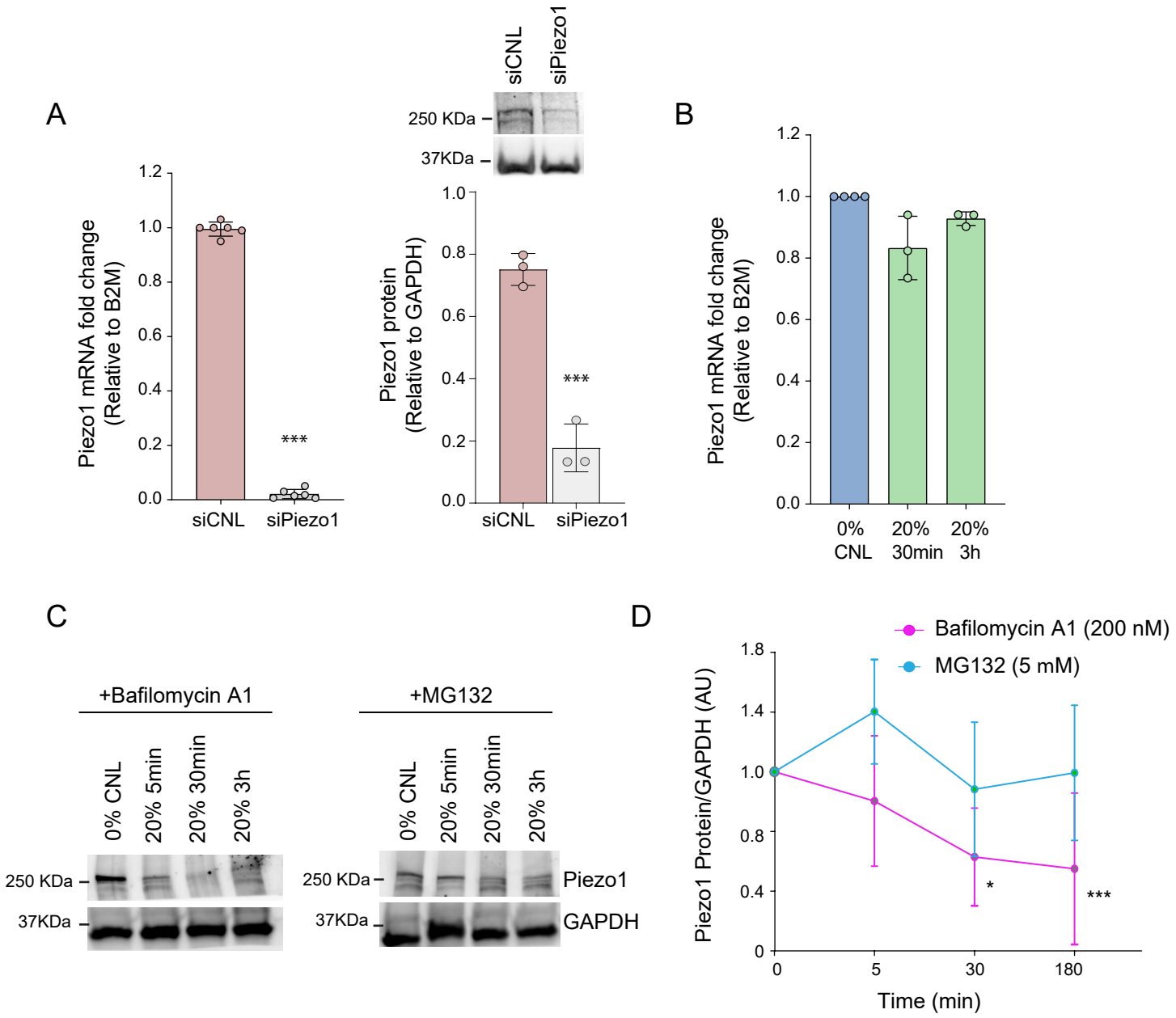
B

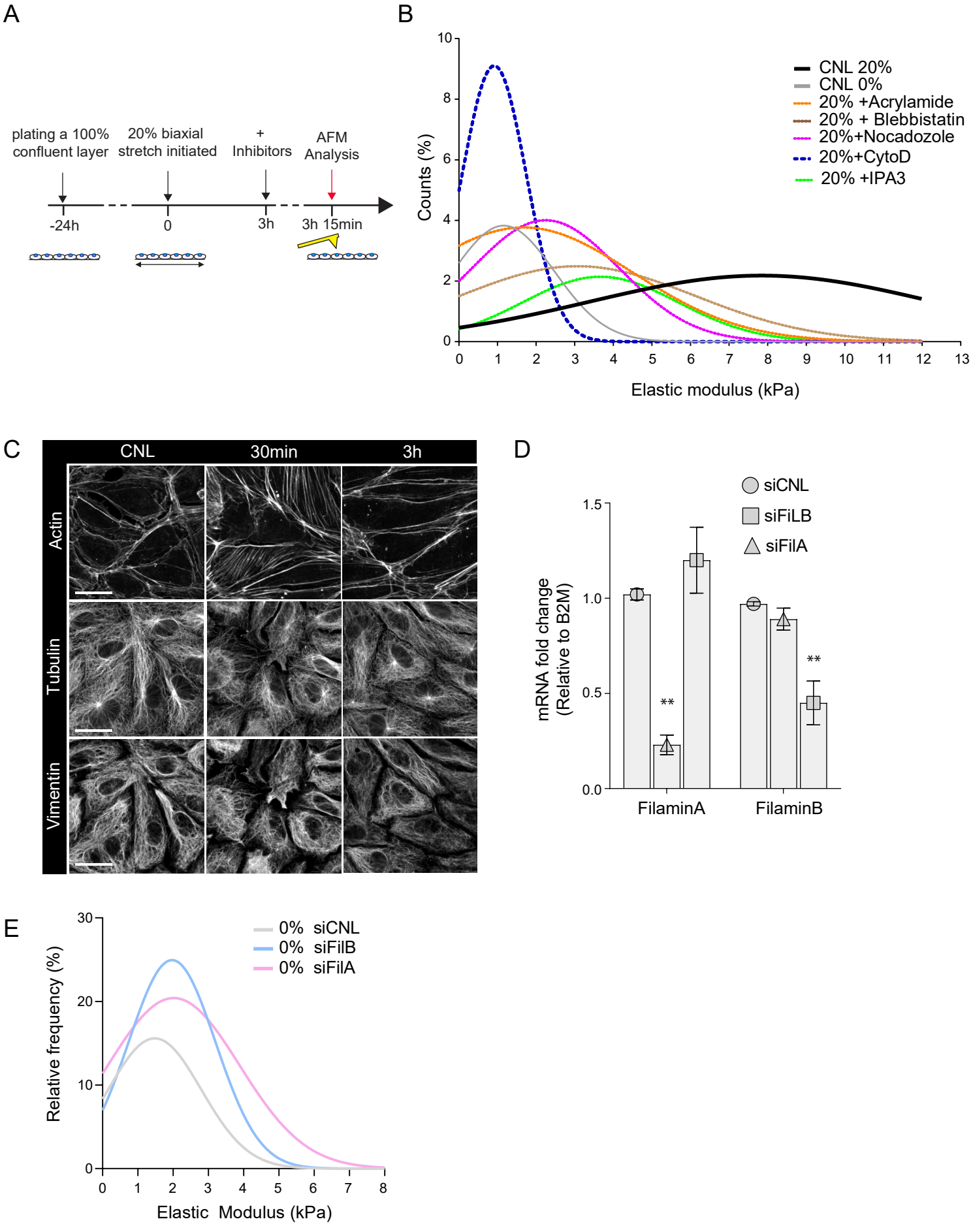
30min	3h
EEA1	SNTB2
DOCK9	ILK
CGN	FSCN
ILK	VCL
	THBS1
	UTRN

C




A
20% Stretch
0% CNL
1 min
5 min
30 min
1 h
3 h
tRho
B
Pulldown
Input
20%
20%
0% CNL
10min
30min
1h
3h
0% CNL
10min
30min
1h
3h
Rac1
β-actin
C
+DMSO
+Y27632
+IPA3
Cell spread area (μm2)
2000
1500
1000
500
0
***
*
0% CNL
30min 20%
3h 20%
D
CNL
Ionomycin 1s
Ionomycin 30s
Ionomycin 30m
GCamp3
high
low
E
+DMSO
+ Y-27632
+ IPA3
VE-Cad/pMLC2
0% CNL
pMLC2 Intensity (AU)
100
80
60
40
20
0
**
+DMSO
+Y-27632
+IPA3


A
Piezo1 mRNA fold change (Relative to B2M)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
siCNL
siPiezo1
siCNL
siPiezo1
250 KDa
37KDa
Piezo1 protein (Relative to GAPDH)
1.0
0.8
0.6
0.4
0.2
0.0
***
siCNL
siPiezo1
B
Piezo1 mRNA fold change (Relative to B2M)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0% CNL
20% 30min
20% 3h
C
+Bafilomycin A1
+MG132
0% CNL
20% 5min
20% 30min
20% 3h
250 KDa
37KDa
250 KDa
37KDa
Piezo1
GAPDH
D
Bafilomycin A1 (200 nM)
MG132 (5 mM)
Piezo1 Protein/GAPDH (AU)
1.8
1.4
1.0
0.8
0.4
0
*
***
0
5
30
180
Time (min)


A
plating a 100% confluent layer
20% biaxial stretch initiated
+ Inhibitors
AFM Analysis
-24h
0
3h
3h 15min
B
Counts (%)
Elastic modulus (kPa)
CNL 20%
CNL 0%
20% +Acrylamide
20% + Blebbistatin
20%+Nocadozole
20%+CytoD
20% +IPA3
C
CNL
30min
3h
Actin
Tubulin
Vimentin
D
siCNL
siFiLB
siFilA
mRNA fold change (Relative to B2M)
FilaminA
FilaminB
**
**
E
0% siCNL
0% siFilB
0% siFilA
Relative frequency (%)
Elastic Modulus (kPa)