Supplemental Materials

Molecular Biology of the Cell

Rabie *et al*.

Supplementary Material

Table S1. Primers for qRT-PCR.

gene	**sequence**
18S	Forward: TCAGATACCGTCGTAGTTC Reverse: CCTTTAAGTTTCAGCTTTGC
Snai1	Forward: CCTGGTTCCTGCTTGGCTCTC Reverse: GCTCTGGGCGGGTACAAAG
Snai1 (virus)	Forward: CAGATGAGGACAGTGGGAAAG Reverse: GTAGAGGAGAGGGACGAAGGA
Sept6	Forward: GTCTCTGGACCTAGTGAC Reverse: AGAAATGGCATCTGACTTGGC
Anln (mouse)	Forward: AGAACTGGCATGTCTTCGTG Reverse: CATCAAACTGTCGCAAGCAC
Kif23 (mouse)	Forward: CATGCCATCACAGTATCTGTTG Reverse: ACTCTGCACCATCTGGTTGG
Anln (human)	Forward: AGTGCTGATGATGCGTCTTTG Reverse: TCCTATTAGTCCTCTGAAAACGGG
Kif23 (human)	Forward: ACCCCAAGGCTGAAGATTATG Reverse: CGAGGCTGGTTTCTGTATCTC
ILK	Forward: CTGAGAATCATTCTGGAGAGCTTTG Reverse: TGTACTCCAGTCTCGAACCTTCAG
ITGB1	Forward: GGAGATGGGAAACTTGGTGG Reverse: CCCATTCACCCCATTCTTGC

Figure S1

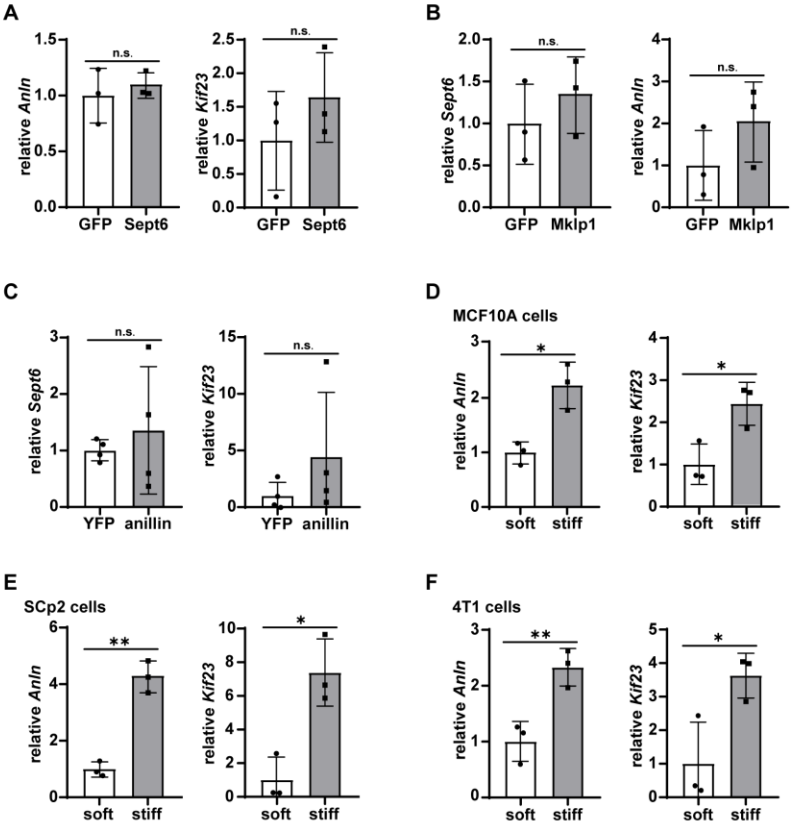
A
relative Anln
n.s.
GFP Sept6
relative Kif23
n.s.
GFP Sept6
B
relative Sept6
n.s.
GFP Mklp1
relative Anln
n.s.
GFP Mklp1
C
relative Sept6
n.s.
YFP anillin
relative Kif23
n.s.
YFP anillin
D
MCF10A cells
relative Anln
*
soft stiff
relative Kif23
*
soft stiff
E
SCp2 cells
relative Anln
**
soft stiff
relative Kif23
*
soft stiff
F
4T1 cells
relative Anln
**
soft stiff
relative Kif23
*
soft stiff

Figure S1. **Substratum stiffness increases the expression of anillin and Mklp1 in several mammary epithelial cell lines**. qRT-PCR analysis for (**A**) *Anln* or *Kif23* in NMuMG cells ectopically expressing septin-6. qRT-PCR analysis for (**B**) *Sept*6 or *Anln* in NMuMG cells ectopically expressing Mklp1. qRT-PCR analysis for (**C**) S*ept6* or *Kif23* in NMuMG cells ectopically expressing anillin. qRT-PCR analysis for *Anln* and *Kif23* in (**D**) MCF10A, (**E**) SCp2, and (**F**) 4T1 mammary epithelial and carcinoma cells, respectively, cultured on soft or stiff substrata. Shown are mean ± s.d. of n = 3-4 independent experiments. **P* < 0.05, ***P* < 0.01 using two-sided Welch's t-test.

Figure S2

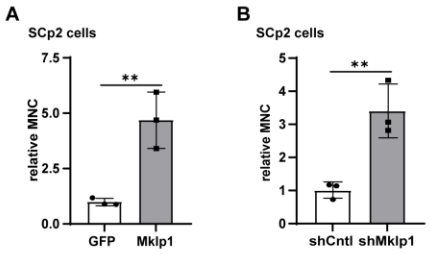

Figure S2. **Dysregulation of Mklp1 leads to an increase in MNC**. Quantification of MNC in SCp2 mouse mammary epithelial cells ectopically expressing (**A**) Mklp1 or (**B**) shMklp1. Shown are mean ± s.d. of n = 3 independent experiments. $^{**}P < 0.01$ using two-sided Student's t-test.

Figure S3

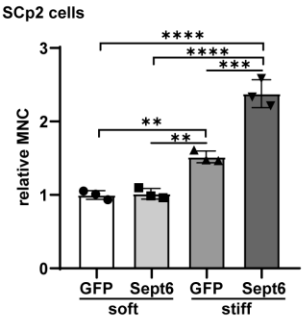

Figure S3. **Quantification of MNC in SCp2 mouse mammary epithelial cells ectopically expressing septin-6 cultured on soft or stiff substrata**. Shown are mean ± s.d. of n = 3 independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ using two-way ANOVA with Tukey's post-hoc test.

Figure S4

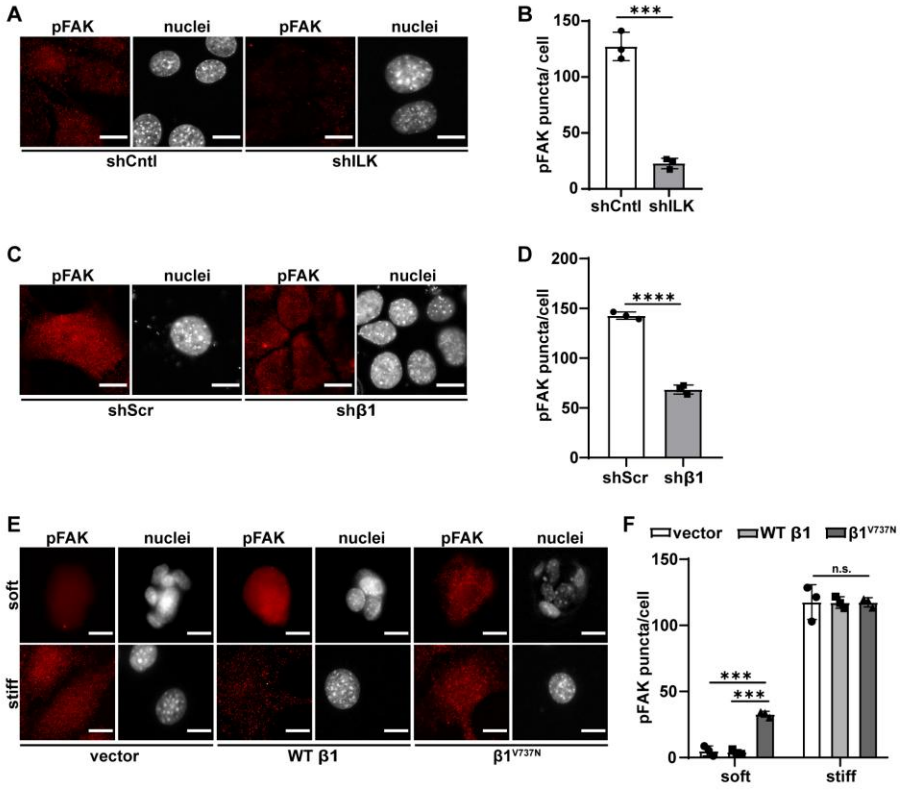
A
pFAK
nuclei
pFAK
nuclei
shCntl
shILK
B
***
pFAK puncta/ cell
150
100
50
0
shCntl
shILK
C
pFAK
nuclei
pFAK
nuclei
shScr
shβ1
D
****
pFAK puncta/cell
200
150
100
50
0
shScr
shβ1
E
pFAK
nuclei
pFAK
nuclei
pFAK
nuclei
soft
stiff
vector
WT β1
β1V737N
F
vector
WT β1
β1V737N
pFAK puncta/cell
150
100
50
0
n.s.
***
***
soft
stiff

Figure S4. Depletion of ILK and β1-integrin reduces, while expression of autoclustering mutant of β1-integrin promotes, focal adhesion formation. (**A**) Immunofluorescence analysis for pFAK to label focal adhesions in shCntl and shILK NMuMG mouse mammary epithelial cells (red, pFAK; white, nuclei; scale bars, 10 μm). (**B**) Quantification of pFAK puncta in shCntl or shILK cells. (**C**) Immunofluorescence analysis for pFAK to label focal adhesions in shScr- or shβ1-expressing cells (red, pFAK; white, nuclei; scale bars, 10 μm). (**D**) Quantification of pFAK puncta in shScr- or shβ1-expressing cells. (**E**) Immunofluorescence analysis for pFAK to label focal adhesions in cells expressing vector control, wild-type β1-integrin (WT β1), or $\beta1^{V737N}$ cultured on soft or stiff substrata (red, pFAK; white, nuclei; scale bars, 10 μm). (**F**) Quantification of focal adhesions in the cells in (E). Shown are mean ± s.d. of n = 3 independent experiments. ***$P < 0.001$, ****$P < 0.0001$ using two-sided Student's t-test (D) or two-way Anova with Tukey's post-hoc test (F).

Figure S5

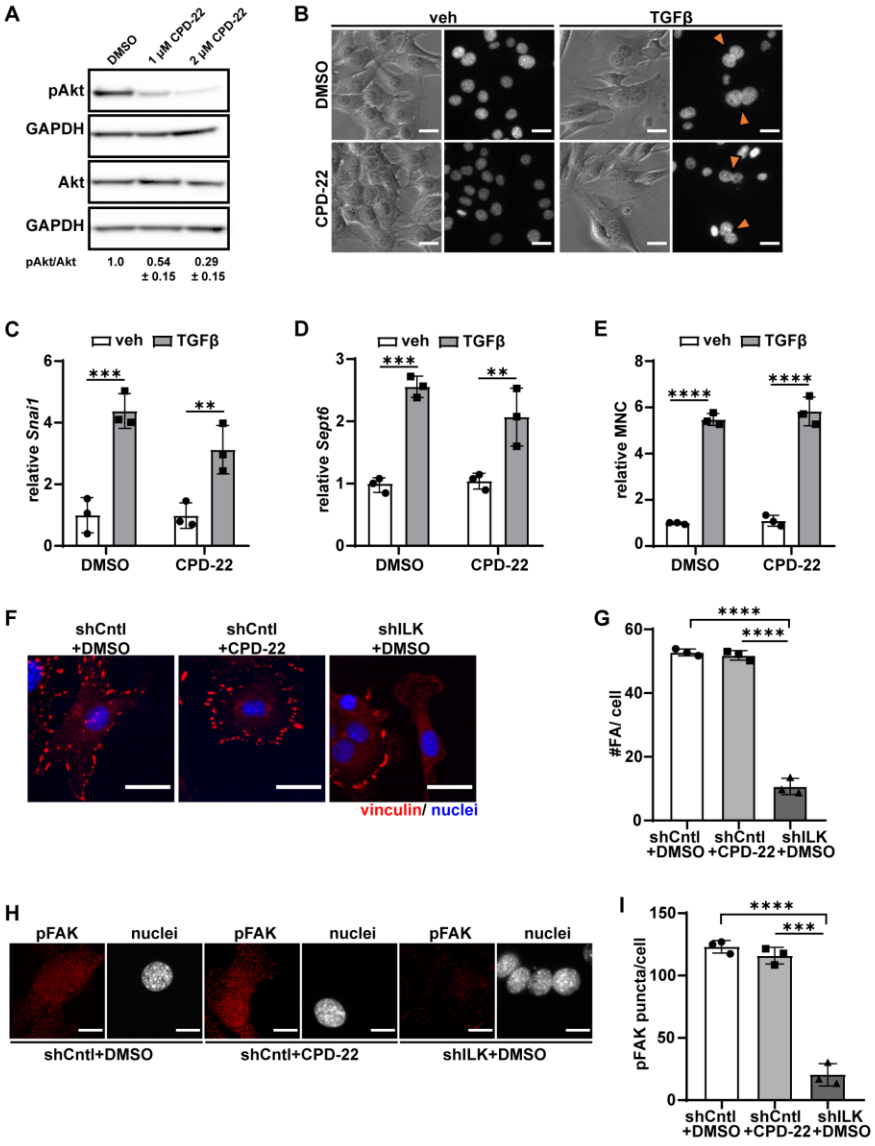
A
DMSO
1 μM CPD-22
2 μM CPD-22
pAkt
GAPDH
Akt
GAPDH
pAkt/Akt
1.0
0.54 ± 0.15
0.29 ± 0.15
B
veh
TGFβ
DMSO
CPD-22
C
veh
TGFβ
relative Snai1
DMSO
CPD-22
D
veh
TGFβ
relative Sept6
DMSO
CPD-22
E
veh
TGFβ
relative MNC
DMSO
CPD-22
F
shCntl +DMSO
shCntl +CPD-22
shILK +DMSO
vinculin/ nuclei
G
#FA/ cell
shCntl +DMSO
shCntl +CPD-22
shILK +DMSO
H
pFAK
nuclei
shCntl+DMSO
shCntl+CPD-22
shILK+DMSO
I
pFAK puncta/cell
shCntl +DMSO
shCntl +CPD-22
shILK +DMSO

Figure S5. **Inhibiting ILK kinase activity does not affect focal adhesion formation, EMT, or MNC**. (**A**) Immunoblotting analysis for pAkt and total Akt in cells treated with or without the ILK inhibitor, CPD-22. (**B**) Phase-contrast and fluorescence images of NMuMG mouse mammary epithelial cells treated with or without TGFβ in the presence or absence of CPD-22 (1 μM). (**C**) Quantification of MNC in cells treated with or without TGFβ in the presence or absence of CPD-22 (scale bars, 25 μm). qRT-PCR analysis for (**D**) *Snai1* or (**E**) *Sept6* in cells treated with or without TGFβ in the presence or absence of CPD-22. (**F**) Immunofluorescence analysis for vinculin to label focal adhesions in cells treated with or without CPD-22 (red, vinculin; white, nuclei; scale bars, 10 μm.) (**G**) Quantification of focal adhesions in cells treated with or without CPD-22. (**H**) Immunofluorescence analysis for pFAK to label focal adhesions in cells treated with or without CPD-22. (**I**) Quantification of pFAK puncta in cells treated with or without CPD-22. Shown are mean ± s.d. of n = 3 independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ using two-way ANOVA with Sidak's multiple comparisons test (C, D, E) or Tukey's post hoc test (G, I).

Figure S6

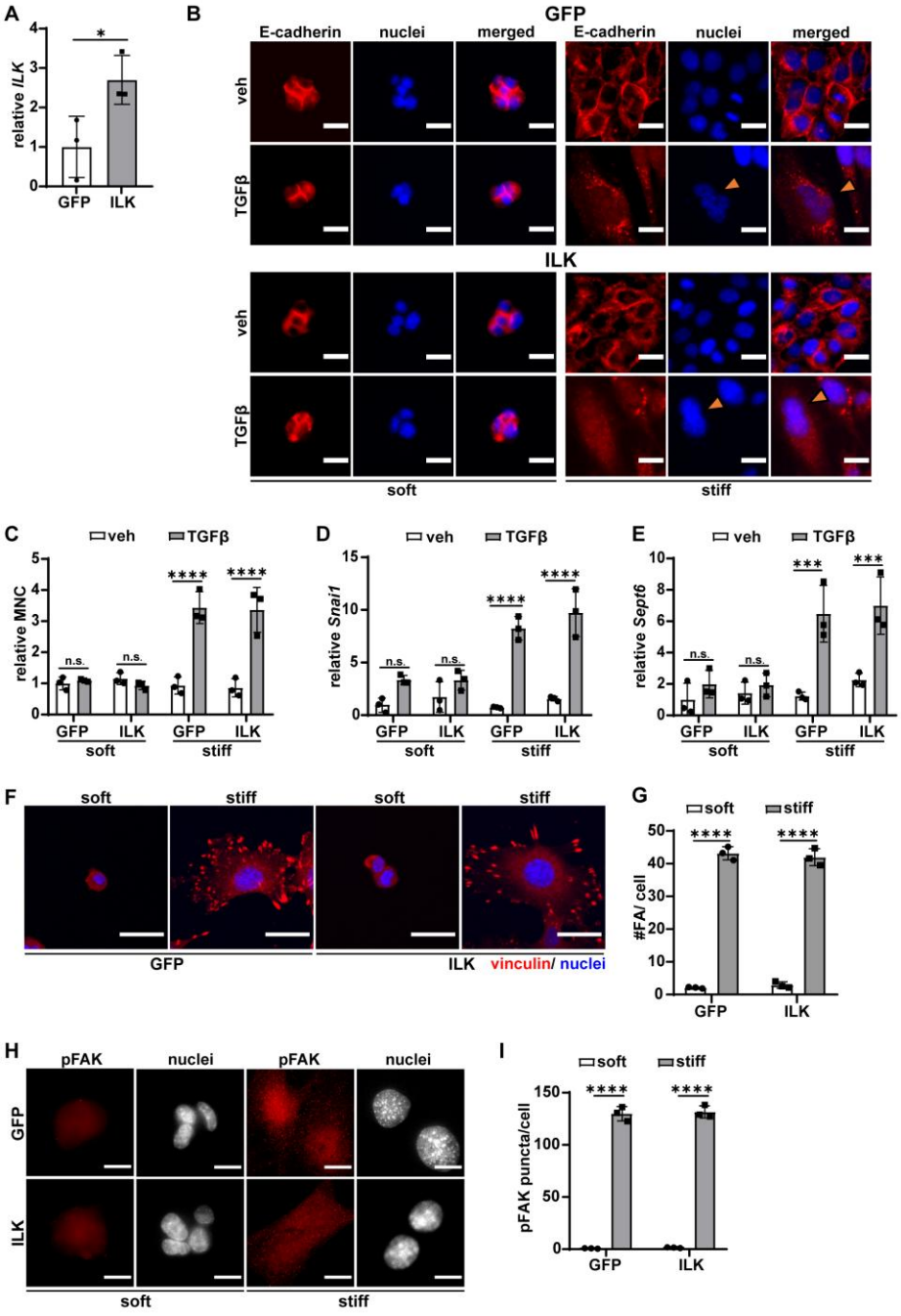
A
relative ILK
GFP
ILK
B
GFP
ILK
E-cadherin
nuclei
merged
veh
TGFβ
soft
stiff
C
veh
TGFβ
relative MNC
n.s.
****
GFP
ILK
soft
stiff
D
relative Snai1
E
relative Sept6
***
F
soft
stiff
GFP
ILK
vinculin/ nuclei
G
#FA/ cell
H
pFAK
nuclei
I
pFAK puncta/cell

Figure S6. **Ectopic expression of ILK does not permit EMT signaling to induce MNC in cells on soft substrata**. (**A**) qRT-PCR analysis for *ILK* in cells ectopically expressing GFP or ILK. (**B**) Immunofluorescence analysis for E-cadherin in NMuMG mouse mammary epithelial cells that ectopically express ILK cultured on soft or stiff substrata and treated with or without TGFβ (red, E-cadherin; blue, nuclei; scale bar, 10 μm). (**C**) Quantification of MNC in cells ectopically expressing ILK cultured on soft or stiff substrata and treated with or without TGFβ. qRT-PCR analysis for (**D**) *Snai1* or (**E**) *Sept6* in cells ectopically expressing ILK cultured on soft or stiff substrata and treated with or without TGFβ. (**F**) Immunofluorescence analysis for vinculin to label focal adhesions in cells ectopically expressing ILK cultured on soft or stiff substrata (red, vinculin; white, nuclei, scale bar, 10 μm). (**G**) Quantification of focal adhesions in cells ectopically expressing ILK cultured on soft or stiff substrata. (**H**) Immunofluorescence analysis for pFAK to label focal adhesions in cells ectopically expressing ILK cultured on soft or stiff substrata (red, pFAK; white, nuclei; scale bars, 10 μm). (**I**) Quantification of pFAK puncta in cells ectopically expressing ILK on soft or stiff substrata. Shown are mean ± s.d. of n = 3 independent experiments. **P* < 0.05, ****P* < 0.001, *****P* < 0.01 using Welch's t-test (A) or two-way ANOVA with Sidak's multiple comparisons test (C, D, E, G) or Tukey's post-hoc test (I).

Figure S7

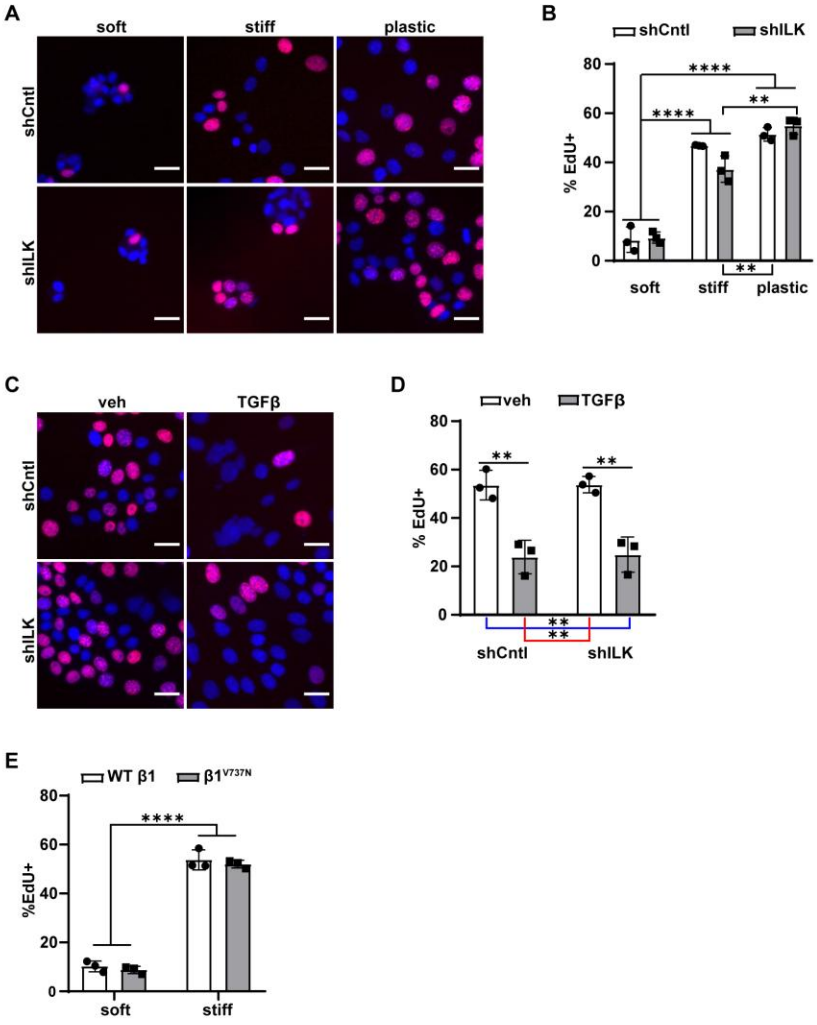
A
soft
stiff
plastic
shCntl
shILK
B
shCntl
shILK
% EdU+
80
60
40
20
0
****
****
**
**
soft
stiff
plastic
C
veh
TGFβ
shCntl
shILK
D
veh
TGFβ
% EdU+
80
60
40
20
0
**
**
**
**
shCntl
shILK
E
WT β1
β1V737N
%EdU+
80
60
40
20
0
****
soft
stiff

Figure S7. **Manipulating ILK expression or β1-integrin clustering does not affect proliferation**. (**A**) EdU analysis in shCntl- and shILK-expressing NMuMG mouse mammary epithelial cells cultured on soft or stiff substrata or plastic (red, EdU+ cells; blue, nuclei). (**B**) Quantification of EdU incorporation in shCntl and shILK cells. (**C**) EdU analysis in shCntl and shILK cells treated with or without TGFβ (red, EdU+ cells; blue, nuclei). (**D**) Quantification of EdU incorporation in (C). (**E**) Quantification of EdU incorporation in cells expressing WT β1 or $\beta1^{V737N}$ cultured on soft or stiff substrata. Shown are mean ± s.d. of n = 3 independent experiments. $^{**}P < 0.01$, $^{****}P < 0.0001$ using two-way ANOVA with Tukey's post-hoc test (B, D) or Sidak's multiple comparison test (E). Scale bars, 25 μm.

Figure S8

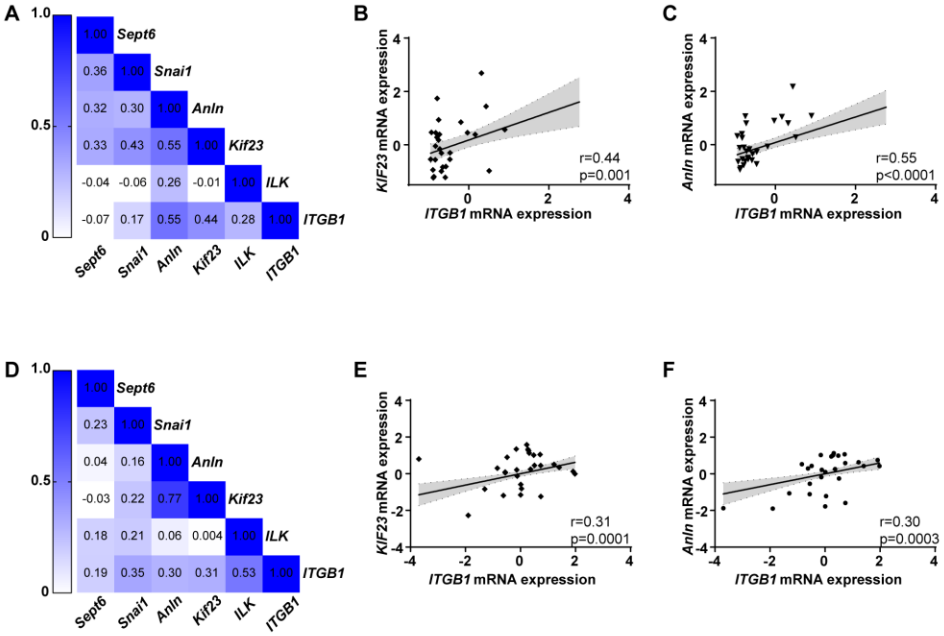
A
1.0
0.5
0
1.00 Sept6
0.36 1.00 Snai1
0.32 0.30 1.00 Anln
0.33 0.43 0.55 1.00 Kif23
-0.04 -0.06 0.26 -0.01 1.00 ILK
-0.07 0.17 0.55 0.44 0.28 1.00 ITGB1
Sept6 Snai1 Anln Kif23 ILK ITGB1
B
KIF23 mRNA expression
ITGB1 mRNA expression
r=0.44
p=0.001
C
Anln mRNA expression
ITGB1 mRNA expression
r=0.55
p<0.0001
D
1.0
0.5
0
1.00 Sept6
0.23 1.00 Snai1
0.04 0.16 1.00 Anln
-0.03 0.22 0.77 1.00 Kif23
0.18 0.21 0.06 0.004 1.00 ILK
0.19 0.35 0.30 0.31 0.53 1.00 ITGB1
Sept6 Snai1 Anln Kif23 ILK ITGB1
E
KIF23 mRNA expression
ITGB1 mRNA expression
r=0.31
p=0.0001
F
Anln mRNA expression
ITGB1 mRNA expression
r=0.30
p=0.0003

Figure S8. *ITGB1* expression is correlated with the expression of midbody proteins in both breast cancer cell lines and in breast tumor samples. (**A**) Correlation matrix of the transcript levels of 6 genes (*Sept6*, *Snai1*, *Anln*, *Kif23*, *ILK*, and *ITGB1*) in breast cancer cell lines. Scatter plots of the expression of *ITGB1* and (**B**) *Kif23* or (**C**) *Anln* from (A). (**D**) Correlation matrix of the transcript levels of 6 genes (*Sept6*, *Snai1*, *Anln*, *Kif23*, *ILK*, and *ITGB1*) in breast tumor samples. Scatter plots of the expression of *ITGB1* and (**E**) *Kif23* or (**F**) *Anln* from (D). The Pearson correlation coefficient was used to determine statistical significance.