Substratum stiffness signals through integrin-linked kinase and β1-integrin to regulate midbody proteins and abscission during EMT

Emann M. Rabie[a,b], Sherry X. Zhang[c], Connor E. Dunn[b], and Celeste M. Nelson[b,c,*]
[a]Rutgers Robert Wood Johnson Medical School, Piscataway, NJ 08854; [b]Departments of Molecular Biology and [c]Chemical & Biological Engineering, Princeton University, Princeton, NJ 08544

ABSTRACT **Abscission is the final stage of cytokinesis during which the parent cell physically separates to yield two identical daughters. Failure of abscission results in multinucleation (MNC), a sign of genomic instability and a precursor to aneuploidy, enabling characteristics of neoplastic progression. Induction of epithelial-mesenchymal transition (EMT) causes MNC in mammary epithelial cells cultured on stiff microenvironments that have mechanical properties similar to those found in breast tumors, but not on soft microenvironments reminiscent of the normal mammary gland. Here we report that on stiff microenvironments, EMT signaling through Snail up-regulates the midbody-associated proteins septin-6, Mklp1, and anillin, leading to abscission failure and MNC. To uncover the mechanism by which stiff microenvironments promote MNC in cells undergoing EMT, we investigated the role of cell–matrix adhesion through β1-integrin and integrin-linked kinase (ILK). We found that ILK expression, but not kinase activity, is required for EMT-associated MNC in cells on stiff microenvironments. Conversely, increasing focal adhesions by expressing an autoclustering mutant of β1-integrin promotes MNC in cells on soft microenvironments. Our data suggest that signaling through focal adhesions causes failure of cytokinesis in cells actively undergoing EMT. These results highlight the importance of tissue mechanics and adhesion in regulating the cellular response to EMT inducers.**

Monitoring Editor
Valerie Marie Weaver
University of California, San Francisco

Received: Feb 18, 2021
Revised: Apr 22, 2021
Accepted: May 20, 2021

INTRODUCTION

Multinucleation (MNC), or polyploidy, is commonly observed in several organs throughout the body, including the skin, gut, muscle, and blood (Fox and Duronio, 2013). Polyploid cells are also present in the lactating mammary gland, where the majority of secretory alveolar epithelial cells are found to be multinucleated after failing to complete cytokinesis (Rios *et al.*, 2016). These multinucleated cells are believed to have evolved to maximize milk production to improve survival of offspring (Rios *et al.*, 2016). At the same time, continued division of multinucleated cells can lead to an abnormal number of chromosomes, or aneuploidy, a sign of chromosomal instability (Weaver and Cleveland, 2006). Aneuploidy has been associated with multidrug resistance in tumors, which promotes more aggressive cancer phenotypes as well as a worse prognosis (Giam and Rancati, 2015). One of these aggressive phenotypes is epithelial-mesenchymal transition (EMT), a process observed both in development as well as in cancer. During EMT, epithelial cells attain mesenchymal characteristics in response to soluble factors including transforming growth factor beta (TGFβ) and matrix metalloproteinases (MMPs) (Heerboth *et al.*, 2015). Exposure to these stimuli leads to the up-regulation of Snail-family transcription factors, which was recently shown to promote MNC through failure of abscission, the final stage of cytokinesis (Comaills *et al.*, 2016; Simi *et al.*, 2018).

Cytokinesis is an ordered process that results in the production of two daughter cells (Mierzwa and Gerlich, 2014). Cytokinesis begins during anaphase as contraction of the actomyosin ring generates the cleavage furrow, allowing the chromosomes to move to opposite sides of the dividing cell (Normand and King, 2010). As the

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E21-02-0072) on May 26, 2021.

*Address correspondence to: Celeste M. Nelson (celesten@princeton.edu).

Abbreviations used: ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; FBS, fetal bovine serum; ILK, integrin-linked kinase; MMP, matrix metalloproteinase; MNC, multinucleation; PA, polyacrylamide; PBS, phosphate-buffered saline; PFA, paraformaldehyde; shRNA, short hairpin RNA; TGFβ, transforming growth factor-beta; WT, wild type.

daughter cells separate, the connection between them elongates and coalesces into an intercellular bridge with a central midbody, which is an accumulation of microtubules from the metaphase spindle (Mierzwa and Gerlich, 2014). The midbody serves as a scaffold for the recruitment of several proteins including septins, among many others, to allow abscission (Mierzwa and Gerlich, 2014). Septins are filament-forming GTPases that assemble into complexes with anillin, which serves as a scaffold for other abscission factors and also helps to mature and stabilize the intercellular bridge (Renshaw *et al.*, 2014). After the recruitment of abscission factors, including Mklp1, CEP55, and the ESCRT machinery, a secondary ingression forms on either side of the midbody, allowing the daughter cells to separate from each other (Agromayor and Martin-Serrano, 2013). Abscission can fail in response to the induction of EMT by TGFβ or MMPs, but also in response to other signals within the cellular microenvironment (Lafaurie-Janvore *et al.*, 2013; Sambandamoorthy *et al.*, 2015; Uroz *et al.*, 2019).

Both the biochemical and the mechanical properties of the extracellular matrix (ECM) can regulate progression through the cell cycle, including the completion of cytokinesis (Assoian and Marcantonio, 1996; Sechler and Schwarzbauer, 1998; Klein *et al.*, 2009; Lafaurie-Janvore *et al.*, 2013; Sambandamoorthy *et al.*, 2015; Simi *et al.*, 2018; Uroz *et al.*, 2019). The stiffness of the ECM, which cells sense and respond to via integrin-mediated interactions, regulates both gene expression as well as cellular behaviors (Northey *et al.*, 2017). For example, mammary epithelial cells cultured on soft microenvironments that match the compliance of the normal mammary gland fail to up-regulate Snail or undergo EMT when cultured in the presence of TGFβ or MMPs (Lee *et al.*, 2012; Leight *et al.*, 2012). In contrast, these same cells undergo robust EMT in response to both stimuli when cultured on stiff microenvironments that match the compliance of breast tumors (Lee *et al.*, 2012; Leight *et al.*, 2012). Activation of the EMT program also promotes MNC in mammary epithelial cells, but only when they are cultured on stiff substrata (Fenner *et al.*, 2014; Simi *et al.*, 2018). On these stiff microenvironments, treatment with TGFβ or MMP3 induces the expression of Snail, which leads to increased levels of the filament-forming GTPase, septin-6 (Simi *et al.*, 2018). Elevated expression of septin-6 leads to MNC by causing abscission failure (Simi *et al.*, 2018). Similarly, dysregulation of other abscission proteins has been shown to cause an increase in MNC in HeLa human cervical cancer cells by disrupting different stages of cytokinesis (Straight *et al.*, 2005; Zhao *et al.*, 2006; Gai *et al.*, 2011; Carlton *et al.*, 2012; Horgan *et al.*, 2012). Nonetheless, it remains unclear how the mechanical stiffness of the microenvironment signals to promote or protect against MNC in cells actively undergoing EMT.

Here we investigated the mechanism by which ECM stiffness couples with EMT signaling to regulate abscission in mammary epithelial cells. We cultured cells on synthetic polyacrylamide (PA) substrata with mechanical properties that mimic those of the normal murine mammary gland or the average breast tumor. We show here that, in cells cultured on stiff but not soft substrata, EMT-associated signaling through Snail up-regulates the expression of not only septin-6 but also the midbody proteins anillin and Mklp1. Furthermore, elevated expression of Mklp1 results in an increase in MNC of mammary epithelial cells on stiff microenvironments. In contrast, we found that culture on a soft microenvironment protects against MNC even in the presence of elevated levels of septin-6 or Mklp1. Consistently, expression of ILK and β1-integrin is required for EMT signaling to induce MNC in cells cultured on stiff substrata. We found that increasing cell–ECM adhesion in cells cultured on soft substrata by expressing an autoclustering mutant of β1-integrin permits EMT signaling through Snail to up-regulate the expression of midbody proteins and induce MNC. These results demonstrate the importance of tissue mechanics and adhesion in regulating the cellular responses to EMT stimuli.

RESULTS

Induction of EMT by exposure to TGFβ or ectopic expression of Snail leads to MNC downstream of septin-6

When exposed to TGFβ, NMuMG mouse mammary epithelial cells up-regulate the expression of the EMT-associated transcription factor, Snail (Figure 1A). Exposure to TGFβ also results in an increase in the number of multinucleated cells (Figure 1, B and C). Consistently, we found that ectopically expressing Snail in the absence of exogenous TGFβ (Figure 1D) induces MNC (Figure 1, E and F). We previously found that induction of EMT with MMP3 causes MNC in part by leading to elevated levels of the filament-forming GTPase, septin-6 (Simi *et al.*, 2018). Consistent with those results, here we found that ectopic expression of Snail causes an increase in the transcript levels of septin-6 in NMuMG cells (Figure 1G). Similarly, we found that ectopic expression of septin-6 itself (Figure 1H) increases MNC in NMuMG cells (Figure 1, I and J). Conversely, short hairpin RNA (shRNA)-mediated depletion of septin-6 (Figure 1K) reduces, but does not completely prevent, MNC in the presence of EMT inducers such as TGFβ (Figure 1, L and M). These data reveal that EMT-associated signaling through Snail induces MNC in NMuMG mouse mammary epithelial cells, at least in part by elevating the expression of the midbody protein septin-6.

Snail and substratum stiffness increase the expression of midbody proteins

We previously found that the mechanical stiffness of the microenvironment regulates the induction of MNC downstream of EMT signaling by affecting the completion of abscission (Lee *et al.*, 2012; Simi *et al.*, 2018). The abscission process is preceded by formation of the intercellular bridge and the midbody as well as recruitment of various proteins, including kinesin-like protein (*Kif23* or Mklp1), anillin, and septins (Figure 2A) (Mierzwa and Gerlich, 2014). Anillin is a scaffolding protein that localizes to the cleavage furrow during cytokinesis and forms complexes with septins to stabilize the actomyosin ring (Renshaw *et al.*, 2014). Both up-regulation (Gai *et al.*, 2011) and depletion (Straight *et al.*, 2005) of anillin are associated with defects in cytokinesis in HeLa cells. Mklp1 is required for formation and maturation of the midbody. Depletion of Mklp1 has been shown to lead to abscission failure in HeLa cells (Zhu *et al.*, 2005). Here we found that ectopic expression of Snail up-regulates the levels of *Sept6* in NMuMG cells cultured on stiff substrata, with mechanical properties similar to those of breast tumors, but not in cells on soft substrata, with mechanical properties similar to that of normal breast tissue (Figure 2B). These results are consistent with our previous findings in SCp2 mouse and MCF10A human mammary epithelial cells (Simi *et al.*, 2018).

Although elevated expression of septin-6 increases MNC, successful completion of abscission depends on several midbody proteins, any of which can cause abscission failure if dysregulated (Normand and King, 2010; Mierzwa and Gerlich, 2014). We therefore investigated whether EMT-associated signaling through Snail affects the expression of other components of the abscission machinery to cause an increase in MNC in mammary epithelial cells, given that depletion of septin-6 is not sufficient to completely prevent EMT-induced MNC. Similar to its effects on the levels of septin-6, we found that ectopic expression of Snail leads to an increase in the expression of anillin (*Anln*) and Mklp1 (*Kif23*) at both the transcript

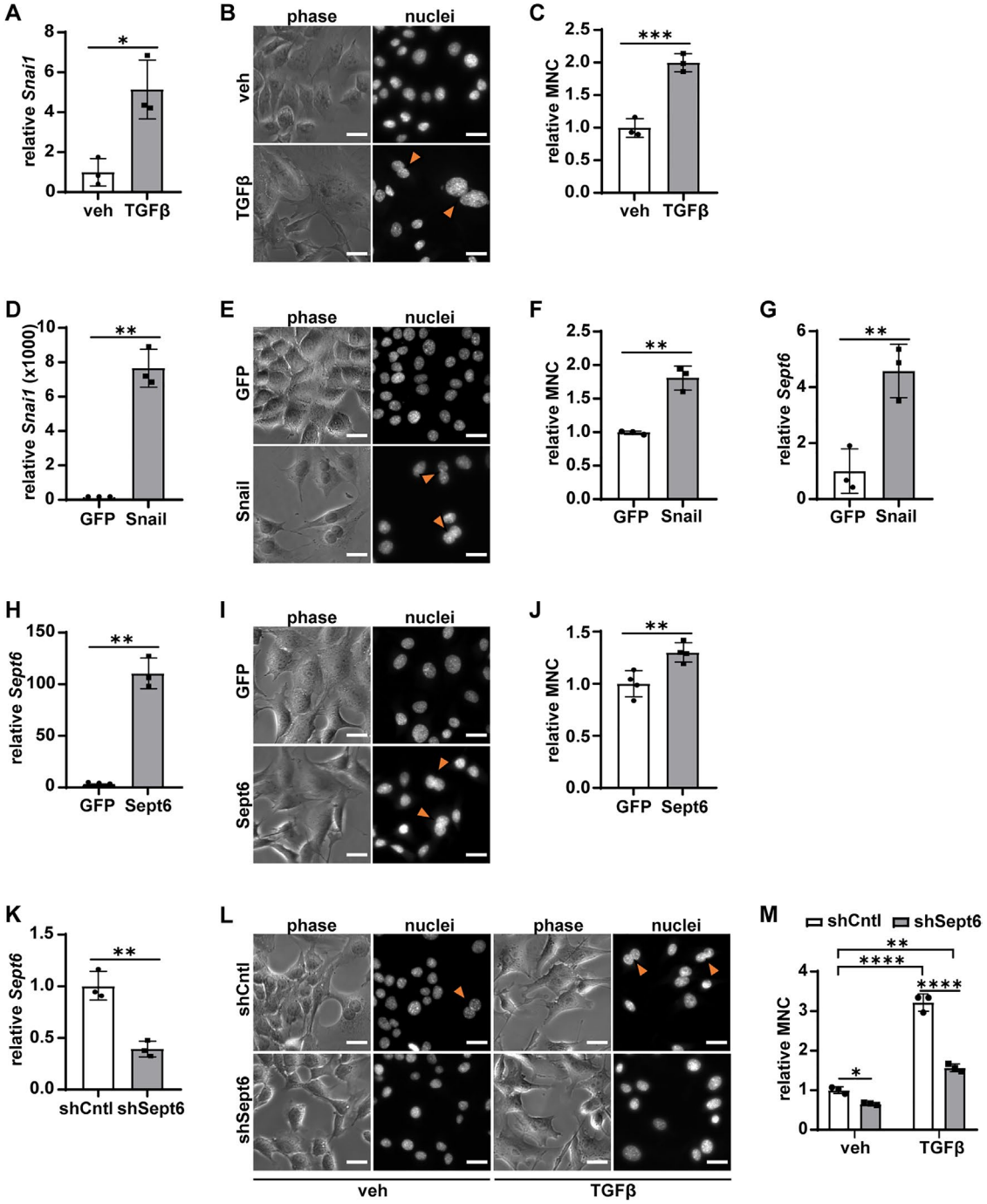

FIGURE 1: Inducing EMT by exposing cells to TGFβ or ectopically expressing Snail leads to MNC through septin-6. (A) qRT-PCR analysis for *Snai1* in NMuMG mouse mammary epithelial cells treated with or without TGFβ. (B) Phase-contrast and fluorescence images and (C) quantification of MNC in cells treated with or without TGFβ. (D) qRT-PCR analysis for *Snai1* in cells ectopically expressing GFP or Snail. (E) Phase-contrast and fluorescence images and (F) quantification of MNC in cells ectopically expressing GFP or Snail. (G) qRT-PCR analysis for *Sept6* in NMuMG cells ectopically expressing GFP or Snail. (H) qRT-PCR analysis for *Sept6* in cells ectopically expressing GFP or septin-6. (I) Phase-contrast and fluorescence images and (J) quantification of MNC in cells ectopically expressing GFP or septin-6. (K) qRT-PCR analysis for *Sept6* in cells ectopically expressing shCntl or shSeptin-6. (L) Phase-contrast and fluorescence images and (M) quantification of MNC in cells ectopically expressing shCntl or shSeptin-6 treated with or without TGFβ. Shown are mean ± SD of $n = 3$–4 independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ using two-sided Welch's *t* test (A, D, G, H, K), two-sided Student's *t* test (C, F, J), or two-way Anova with Tukey's post-hoc test (M). Scale bars, 25 μm.

(Figure 2C) and protein (Figure 2D) levels in NMuMG mouse mammary epithelial cells. To determine whether Snail affects the expression of *Anln* or *Kif23* by signaling through septin-6, we ectopically expressed septin-6 and used qRT-PCR analysis to evaluate the other midbody components. This analysis revealed no changes in the levels of either *Anln* or *Kif23* in response to septin-6 expression

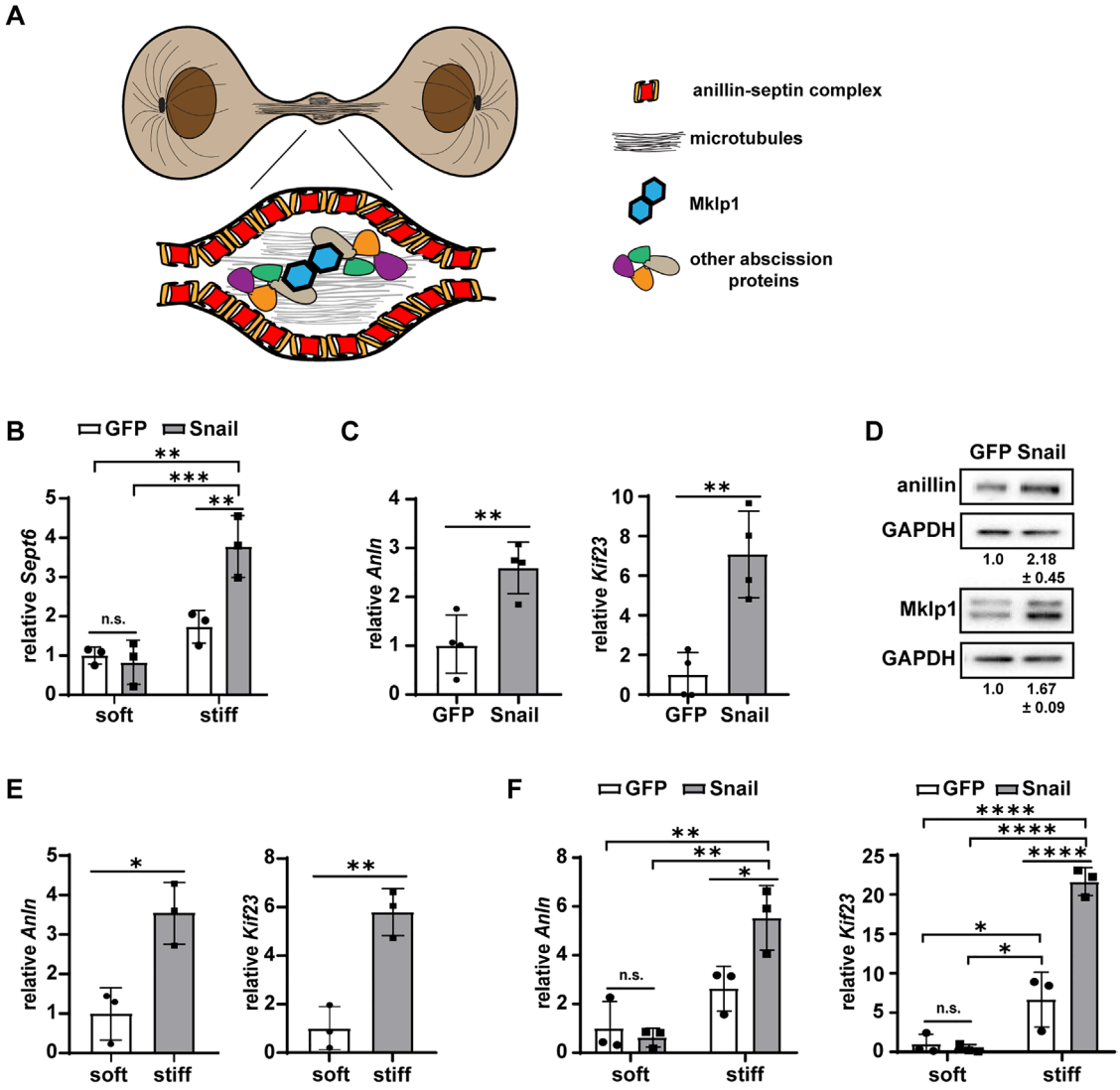

FIGURE 2: Snail and substratum stiffness increase the expression of midbody proteins. (A) Schematic of the abscission machinery. (B) qRT-PCR analysis for *Sept6* in NMuMG mouse mammary epithelial cells cultured on soft or stiff substrata and ectopically expressing GFP or Snail. (C) qRT-PCR analysis for *Anln* (anillin) and *Kif23* (Mklp1) in cells ectopically expressing GFP or Snail. (D) Immunoblotting analysis for anillin and Mklp1 in cells ectopically expressing GFP or Snail. (E) qRT-PCR analysis for *Anln* and *Kif23* in cells cultured on soft or stiff substrata. (F) qRT-PCR analysis for *Anln* and *Kif23* in cells cultured on soft or stiff substrata and ectopically expressing GFP or Snail. Shown are mean ± SD of $n = 3$-4 independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ using two-way ANOVA with Tukey's post-hoc test (B, F) or two-sided Welch's *t* test (C, E).

(Supplemental Figure S1A). Similarly, ectopic expression of Mklp1 or anillin has no effect on that of septin-6 (Supplemental Figure S1, B and C).

To determine whether the mechanical properties of the microenvironment affect the expression of these midbody proteins, we cultured mammary epithelial cells on soft or stiff substrata. We found that NMuMG mouse mammary epithelial cells as well as other human (MCF10A) and mouse (SCp2, 4T1) mammary epithelial cells express higher levels of both anillin and Mklp1 when cultured on stiff substrata than when cultured on soft substrata (Figure 2E; Supplemental Figure S1, D–F). We also found that the ability of Snail to up-regulate the expression of *Anln* and *Kif23* depends on substratum stiffness (Figure 2F). Specifically, cells cultured on soft microenvironments are impervious to ectopic expression of Snail and maintain their levels of anillin and Mklp1. These data reveal that stiff microenvironments permit EMT-associated signaling through Snail to alter the expression of several components of the abscission machinery.

Since Snail regulates the expression of Mklp1, we next sought to determine whether changes in the levels of Mklp1 cause an increase in MNC in mammary epithelial cells. We ectopically expressed a GFP-Mklp1 fusion protein, which revealed that increasing the levels of Mklp1 promotes MNC in NMuMG and SCp2 cells (Figure 3, A–C; Supplemental Figure S2A). To our knowledge, these data implicate elevated levels of Mklp1 in abscission failure for the first time. This finding suggests that tight regulation of Mklp1 expression is essential for the completion of cytokinesis and normal cell division. Similarly, we found that depleting Mklp1 using an shRNA-based approach also induces MNC in mammary epithelial cells (Figure 3, D–F; Supplemental Figure S2B), consistent with previous work in HeLa cells (Zhu *et al.*, 2005). We used a similar strategy to determine whether changes in the levels of anillin affect MNC in mammary

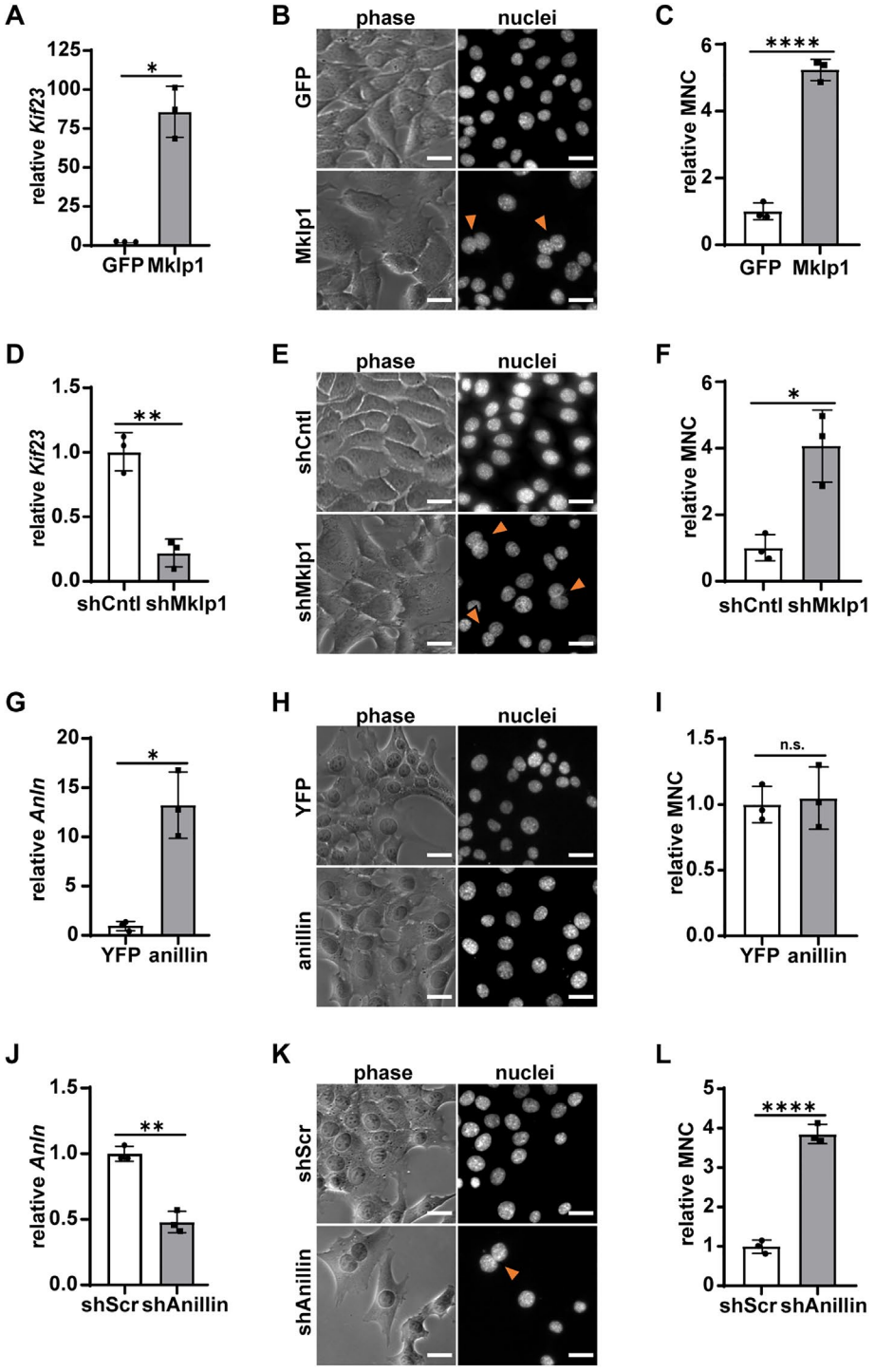

FIGURE 3: Disrupting the levels of midbody proteins causes an increase in MNC. (A) qRT-PCR analysis for *Kif23* in cells ectopically expressing GFP or Mklp1. (B) Phase-contrast and fluorescence images and (C) quantification of MNC in cells ectopically expressing GFP or Mklp1. (D) qRT-PCR analysis for *Kif23* in cells ectopically expressing shCntl or shMklp1. (E) Phase-contrast and fluorescence images and (F) quantification of MNC in cells ectopically expressing shCntl or shMklp1. (G) qRT-PCR analysis for *Anln* in cells ectopically expressing YFP or anillin. (H) Phase-contrast and fluorescence images and (I) quantification of MNC in cells ectopically expressing YFP or anillin. (J) qRT-PCR analysis for *Anln* in cells expressing shScr or shAnillin. (K) Phase-contrast and fluorescence images and (L) quantification of MNC in cells ectopically expressing shScr or shAnillin. Shown are mean ± SD of $n = 3$ independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ using two-sided Welch's *t* test (A, D, G, J), or two-sided Student's *t* test (C, F, I, L). Scale bars, 25 μm.

epithelial cells. In contrast to Mklp1, we found that ectopic expression of anillin does not induce MNC (Figure 3, G–I). However, depleting anillin leads to a significant increase in MNC in mammary epithelial cells (Figure 3, J–L), consistent with previous findings in HeLa cells (Straight *et al.*, 2005). These data confirm that altering the levels of components of the abscission machinery promotes abscission failure and MNC in mammary epithelial cells.

A soft microenvironment protects against MNC downstream of elevated levels of septin-6 or Mklp1

The stiffness of the breast tumor microenvironment is often elevated and has been shown to regulate both cytokinesis (Lafaurie-Janvore *et al.*, 2013; Sambandamoorthy *et al.*, 2015; Simi *et al.*, 2018) and induction of EMT (Lee *et al.*, 2012; Leight *et al.*, 2012; Wei *et al.*, 2015; Rice *et al.*, 2017). We previously found that mammary epithelial cells cultured on soft microenvironments are impervious to ectopic expression of Snail, undergo normal abscission, and remain mononucleated (Simi *et al.*, 2018). To determine whether substratum stiffness regulates MNC downstream of abnormal levels of abscission components, we ectopically expressed septin-6 or Mklp1 in NMuMG mouse mammary epithelial cells that were subsequently cultured on soft or stiff substrata (Figure 4A). As expected, we found that ectopic expression of septin-6 or Mklp1 induces MNC in cells cultured on stiff microenvironments (Figure 4, B and C; Supplemental Figure S3). In contrast, culture on soft microenvironments protects cells from MNC downstream of either septin-6 or Mklp1 (Figure 4, B and C). Consistently, ectopic expression of septin-6 promotes MNC in SCp2 mouse mammary epithelial cells that are cultured on stiff but not on soft microenvironments (Supplemental Figure S3). Soft microenvironments therefore protect against MNC, even when mammary epithelial cells express abnormally high levels of components of the abscission machinery.

The stiffness of the microenvironment regulates MNC in part through integrin-linked kinase (ILK)

Mechanical signals are transmitted from the ECM to focal adhesions through β1-integrin. The focal adhesion component ILK is upregulated in NMuMG cells cultured on stiff substrata (Han *et al.*, 2018). Furthermore, we recently found that ILK is required for induction of EMT in mammary epithelial cells cultured on stiff substrata (Kilinc *et al.*, 2021).

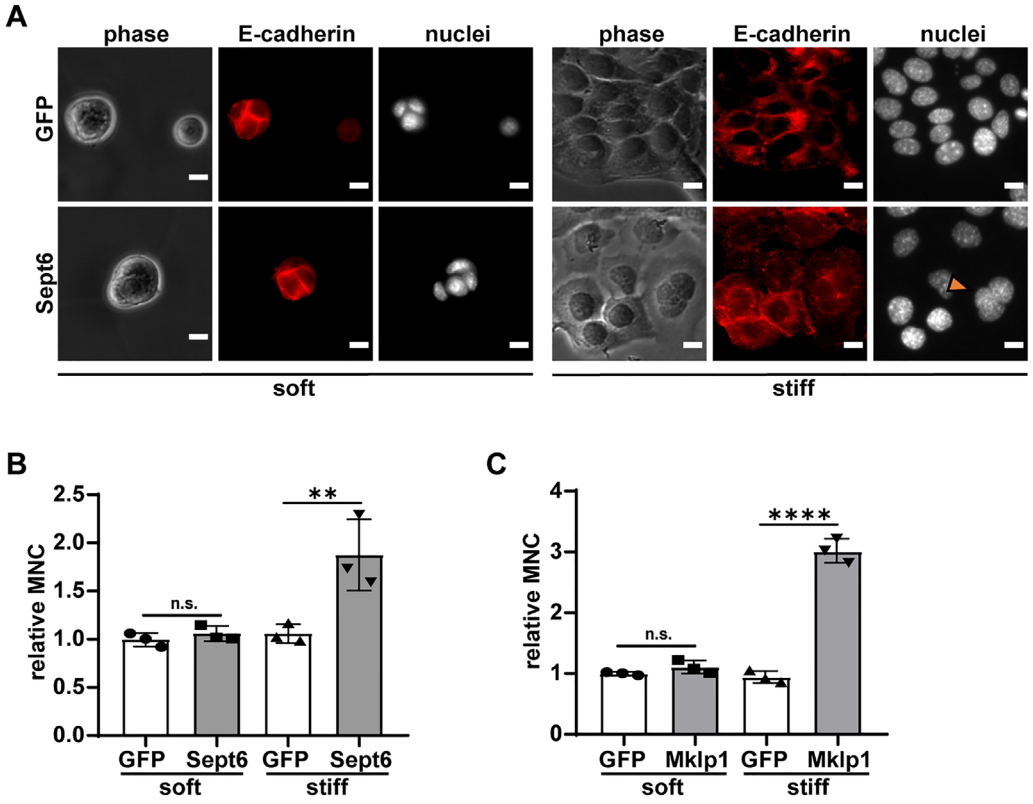

FIGURE 4: A soft microenvironment protects against MNC even in cells that ectopically express septin-6 or Mklp1. (A) Phase-contrast and fluorescence images of NMuMG mouse mammary epithelial cells ectopically expressing GFP or septin-6 and cultured on soft or stiff substrata. Red, E-cadherin; white, nuclei. Quantification of MNC in cells ectopically expressing (B) septin-6 or (C) Mklp1 cultured on soft or stiff substrata. Shown are mean ± SD of $n = 3$ independent experiments. $^{**}P < 0.01$, $^{****}P < 0.0001$ using two-way ANOVA with Tukey's post-hoc test. Scale bars, 10 μm.

Stable depletion of ILK in NMuMG cells using shRNA approaches (shILK) causes a reduction in cell–matrix adhesion, as inferred from quantifying the number of focal adhesions (Figure 5, A and B; Supplemental Figure S4, A and B) (Han *et al.*, 2018). To determine whether ILK plays a role in MNC downstream of EMT induction, we cultured control (shCntl) or shILK-expressing cells in the presence or absence of TGFβ (Figure 5C). Consistent with previous reports, we found that depleting ILK blocks the ability of TGFβ to induce morphological or transcriptional changes associated with EMT (Figure 5D). Additionally, we found that depleting ILK blocks the expression of *Septó* or induction of MNC downstream of TGFβ (Figure 5, E and F). Depleting ILK also decreases MNC in response to ectopic expression of septin-6 or Mklp1 (Figure 5, G and H). Furthermore, ectopic expression of septin-6 or Mklp1 induces MNC in shCntl cells cultured on stiff but not soft substrata (Figure 5, I and J), consistent with the results using parental cells (Figure 4). In contrast, shILK cells are insensitive to elevated levels of septin-6 or Mklp1 and remain mononucleated on both soft and stiff substrata. Therefore, stiff microenvironments signal through ILK to promote abscission failure in mammary epithelial cells that are treated with EMT inducers.

To parse whether it is the presence of ILK or its kinase activity that is required for TGFβ to induce MNC on stiff microenvironments, we compared the phenotype of cells in which ILK is blocked pharmacologically to that of shILK cells. We pretreated NMuMG cells with CPD-22, an ATP-competitive inhibitor of ILK that prevents downstream signaling and phosphorylation of Akt (Supplemental Figure S5A) (Lee *et al.*, 2011). We found that in cells pretreated with CPD-22, exposure to TGFβ still induces morphological changes associated with EMT (Supplemental Figure S5B), increases the expression of *Snai1* and *Septó* (Supplemental Figure S5, C and D), and promotes MNC (Supplemental Figure S5E). These data are consistent with previous observations, specifically that treatment with CPD-22 does not prevent the phosphorylation of Smad-2 downstream of TGFβ, which is required for the induction of EMT (Parker *et al.*, 2018). In contrast to depleting ILK levels using shRNA, blocking ILK activity pharmacologically has no effect on the formation of focal adhesions (Supplemental Figure S5, F–I). We therefore conclude that while the expression of ILK is required for focal adhesion formation and abscission failure downstream of EMT inducers, its kinase activity is not.

Based on the above findings, we hypothesized that the low levels of ILK in cells on soft substrata protect against abscission failure and MNC in response to EMT inducers. If correct, our hypothesis would suggest that increasing the expression of ILK should promote MNC in cells cultured on soft microenvironments. To test our hypothesis, we used an adenoviral approach to ectopically express ILK (adILK) in NMuMG cells (Supplemental Figure S6A). Surprisingly, we found that ectopic expression of ILK is not sufficient to permit TGFβ to induce MNC in cells cultured on soft substrata (Supplemental Figure S6, B and C). Consistently, ectopic expression of ILK also fails to permit treatment with TGFβ to induce expression of *Snai1* or *Septó* in cells cultured on soft substrata (Supplemental Figure S6, D and E). However, we noticed that adILK-transduced cells maintain a rounded morphology on soft substrata. We also observed that ectopic expression of ILK is not sufficient to promote focal adhesion formation in cells cultured on soft substrata (Supplemental Figure S6, F–I). Combined with our other observations, these data suggest that it is the presence of ILK

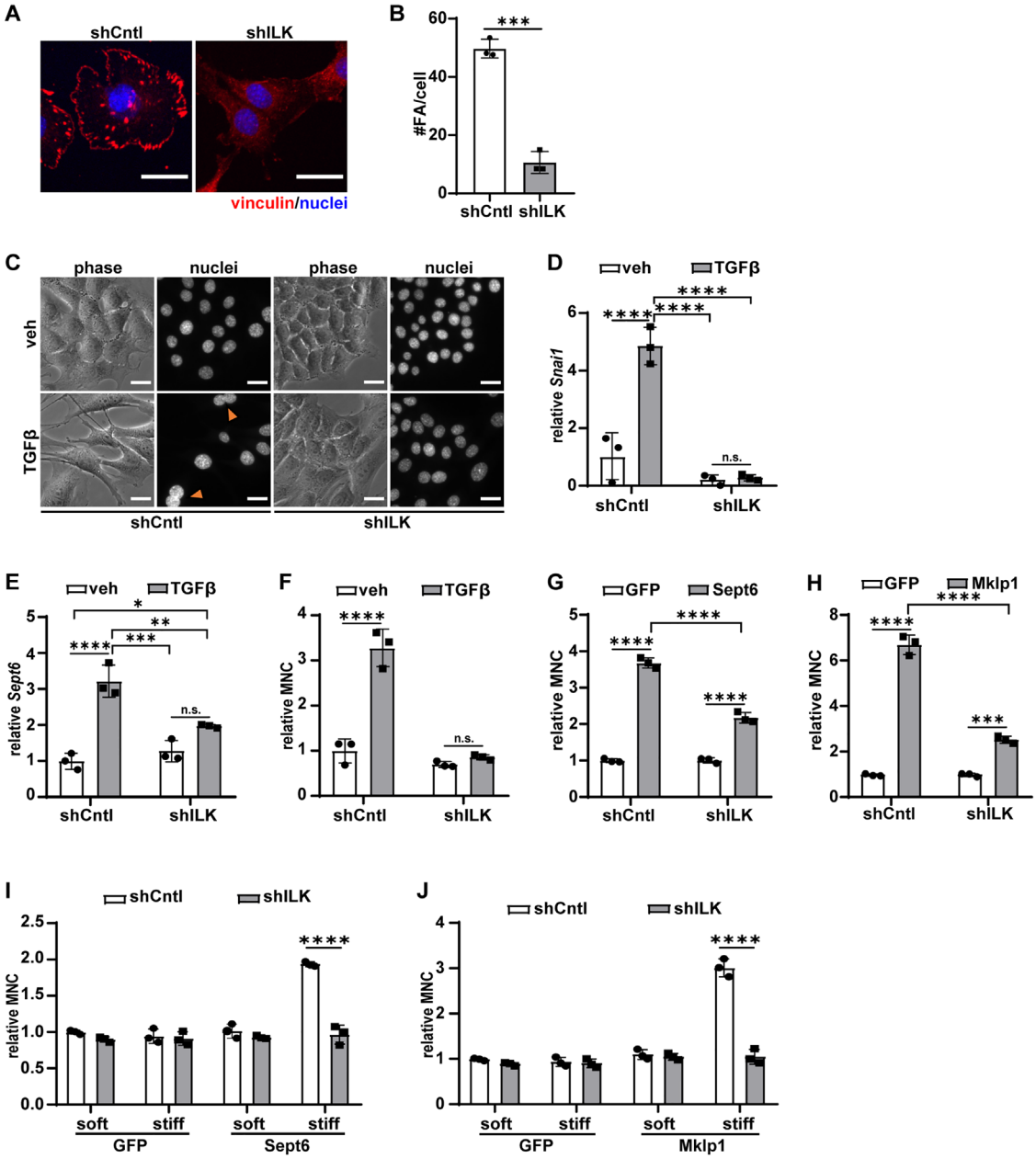

FIGURE 5: Expression of ILK is required for MNC in cells cultured on stiff microenvironments. (A) Immunofluorescence analysis for vinculin to label focal adhesions in shCntl and shILK-expressing NMuMG mouse mammary epithelial cells (red, vinculin; blue, nuclei; scale bars, 10 μm). (B) Quantification of focal adhesions in shCntl or shILK-expressing cells. (C) Phase-contrast and fluorescence images of shCntl and shILK-expressing cells treated with or without TGFβ (scale bars, 25 μm). qRT-PCR analysis for (D) *Snai1* and (E) *Sept6* in shCntl and shILK-expressing cells treated with or without TGFβ. (F) Quantification of MNC in shCntl or shILK-expressing cells treated with or without TGFβ. Quantification of MNC in shCntl or shILK-expressing cells cultured on tissue culture plastic that ectopically express (G) septin-6 or (H) Mklp1. Quantification of MNC in shCntl or shILK-expressing cells that ectopically express (I) septin-6 or (J) Mklp1 and are cultured on soft or stiff substrata. Shown are mean ± SD of $n = 3$ independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ using two-sided Student's *t* test (B), two-way ANOVA with Tukey's post-hoc test (D, E, F, G, H), or Sidak's multiple comparisons test (I, J).

in focal adhesions that promotes MNC in cells undergoing EMT on stiff microenvironments.

Clustering of β1-integrin increases MNC in cells cultured on soft substrata

We and others have previously reported that β1-integrin plays a key role in cellular responses to substratum stiffness, and that depleting β1-integrin itself prevents the induction of EMT on stiff microenvironments (Paszek *et al.*, 2005; Lee *et al.*, 2012). Here we found that depleting β1-integrin (Figure 6A) prevents statistically significant increases in the expression of *Snai1* (Figure 6B) and *Sept6* (Figure 6C) and reduces MNC (Figure 6, D and E) in response to treatment with TGFβ. Consistent with these observations, depleting β1-integrin reduces MNC when septin-6 or Mklp1 are expressed

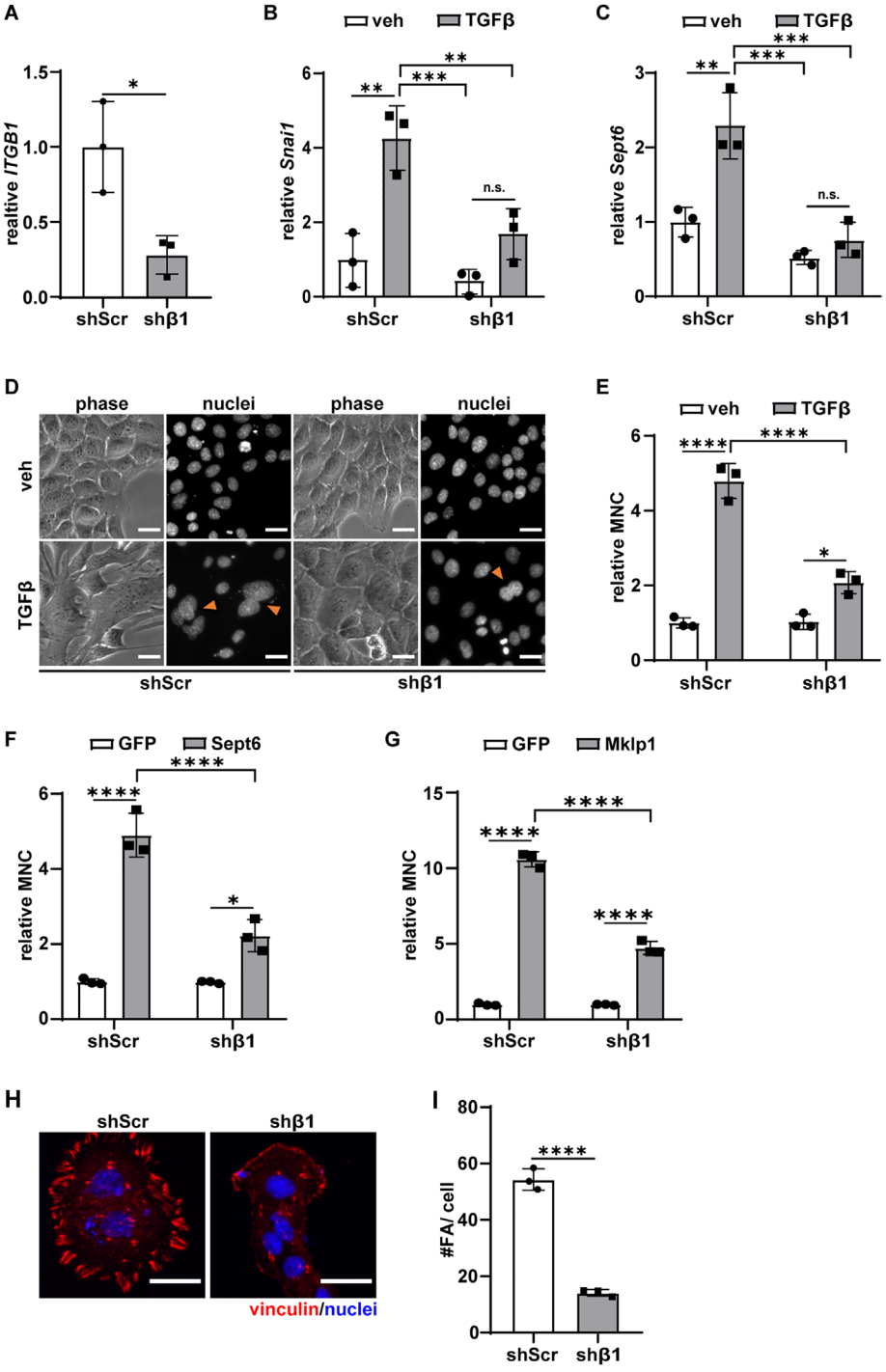

FIGURE 6: Depleting β1-integrin prevents EMT and reduces MNC in cells on plastic. (A) qRT-PCR analysis for *ITGB1* in cells expressing shScr or shβ1. qRT-PCR analysis for (B) *Snai1* or (C) *Sept6* in shScr- or shβ1-expressing cells treated with or without TGFβ. (D) Phase-contrast and fluorescence images of shScr- and shβ1-expressing cells treated with or without TGFβ (scale bars, 25 μm). Quantification of MNC in shScr- and shβ1-expressing cells (E) treated with or without TGFβ, or that ectopically express (F) septin-6 or (G) Mklp1. (H) Immunofluorescence analysis for vinculin to label focal adhesions in shScr- or shβ1-expressing cells (red, vinculin; blue, nuclei; scale bars, 10 μm). (I) Quantification of focal adhesions in shScr- or shβ1-expressing cells. Shown are mean ± SD of $n = 3$ independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ using two-sided Welch's *t* test (A), two-way ANOVA with Tukey's post-hoc test (B, C, E, F, G), or two-sided Student's *t* test (I).

ectopically (Figure 6, F and G). As expected, we also found that depleting β1-integrin causes a decrease in the number of focal adhesions in cells cultured on plastic (Figure 6, H and I; Supplemental Figure S4, C and D) (Kawahara *et al.*, 2018), consistent with our hypothesis that focal adhesion signaling is necessary for MNC of cells undergoing EMT.

We therefore took advantage of an autoclustering mutant of β1-integrin ($\beta1^{V737N}$) (Paszek *et al.*, 2005; Lee *et al.*, 2012), which increases the number of focal adhesions in NMuMG cells cultured on soft substrata, as compared with cells expressing wild-type (WT) β1-integrin or vector control (Figure 7, A and B; Supplemental Figure S4, E and F) (Paszek *et al.*, 2005; Yeh *et al.*, 2017). Consistent with our hypothesis, we found that treatment with TGFβ induces the expression of *Snai1* (Figure 7C) and *Sept6* (Figure 7D) in $\beta1^{V737N}$-expressing cells that are cultured on soft substrata. Furthermore, treatment with TGFβ promotes MNC in cells on soft substrata that express $\beta1^{V737N}$ but not in those that express WT β1-integrin (Figures 7, E and F). To determine whether expression of septin-6 is required for MNC downstream of $\beta1^{V737N}$, we depleted septin-6 in $\beta1^{V737N}$-expressing cells cultured on soft substrata in the presence of TGFβ. Consistent with our findings in cells cultured on plastic (Figure 1M), we found that depleting septin-6 in $\beta1^{V737N}$-expressing cells reduces but does not completely prevent MNC induced by TGFβ signaling (Figure 7G), indicating that septin-6 is not solely responsible for the phenotype we observe in cells undergoing EMT. Our data reveal that mechanical signaling through focal adhesions promotes MNC in cells undergoing EMT. These results suggest that soft microenvironments protect cells from abscission failure by decreasing ILK expression, β1-integrin clustering, and focal adhesion formation.

DISCUSSION

EMT is a dynamic process fundamental for development in which epithelial cells take on a mesenchymal phenotype and increase their motility and invasiveness (Heerboth *et al.*, 2015). This phenotypic transition is tightly regulated by both mechanical and chemical signals from the surrounding microenvironment (Scott *et al.*, 2019). EMT is often dysregulated in the context of breast cancer, where the ECM within the tumor microenvironment is drastically remodeled, leading to an increase in stiffness (Paszek *et al.*, 2005) that on its own can promote

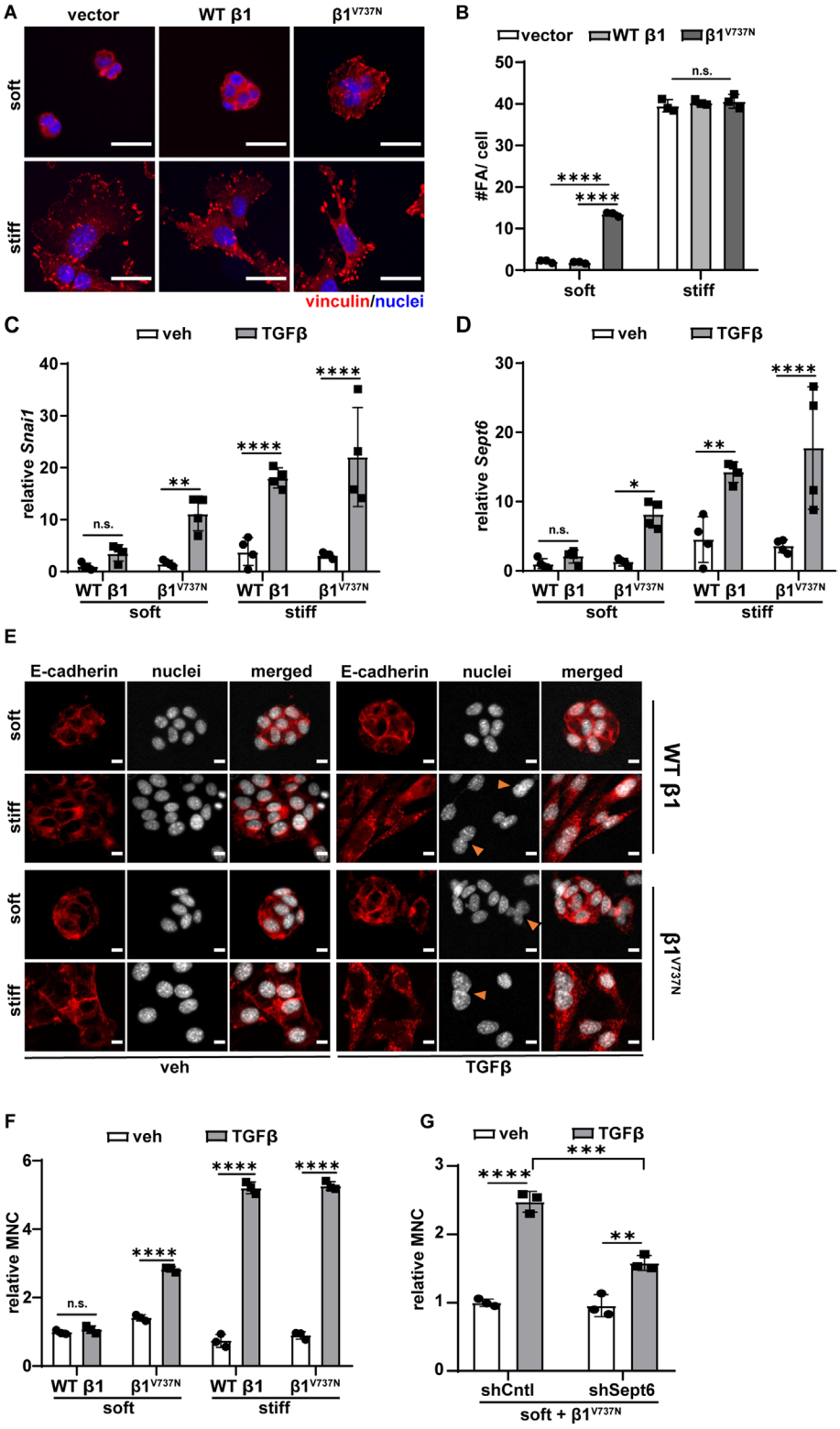

FIGURE 7: Expression of an autoclustering mutant of β1-integrin promotes focal adhesion formation, EMT, and MNC in cells cultured on soft substrata. (A) Immunofluorescence analysis for vinculin to label focal adhesions in cells expressing vector control, WT β1-integrin (WT β1), or $\beta1^{V737N}$ cultured on soft or stiff substrata (red, vinculin; blue, nuclei, scale bar, 10 μm). (B) Quantification of focal adhesions in the cells in A. qRT-PCR analysis for (C) *Snai1* or (D) *Sept6* in cells expressing WT β1 or $\beta1^{V737N}$ cultured on soft or stiff substrata and treated with or without TGFβ. (E) Immunofluorescence analysis for E-cadherin in cells expressing WT β1 or $\beta1^{V737N}$ cultured on soft or stiff substrata and treated with or without TGFβ (red, E-cadherin; white, nuclei; scale bar, 10 μm). (F) Quantification of MNC in cells expressing WT β1 or $\beta1^{V737N}$ cultured on soft or stiff substrata and treated with or without TGFβ. (G) Quantification of MNC in cells expressing $\beta1^{V737N}$ and shCntl or shSept6 cultured on soft or stiff substrata and treated with or without TGFβ. Shown are mean ± SD of $n = 3$–4 independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ using two-way ANOVA with Tukey's post-hoc test (B, G) or Sidak's multiple comparisons test (C, D, F).

EMT (Lee *et al.*, 2012; Leight *et al.*, 2012; Wei *et al.*, 2015; Rice *et al.*, 2017). We previously reported that stiff microenvironments disrupt normal cell division, leading to an increase in MNC downstream of EMT-associated up-regulation of septin-6 (Simi *et al.*, 2018). Here we found that signaling downstream of EMT inducers, specifically through Snail, also leads to an increase in the expression of other midbody proteins, including anillin and Mklp1, in a stiffness-dependent manner (Figure 2). Although Snail is commonly known as a transcriptional repressor, recent studies have shown that ~50% of target genes are transcriptionally activated upon Snail binding (Rembold *et al.*, 2014), indicating that Snail may potentially act as a transcriptional activator for septin-6, Mklp1, and anillin. In support of this hypothesis, we analyzed available Snail ChIP-seq datasets acquired from mouse breast cancer cells and found that Snail may potentially bind to the promoters of septin-6, Mklp1, and anillin (Ye *et al.*, 2015). Whether Snail directly binds to the promoters or collaborates with another transcription factor to drive the expression of these proteins will be the subject of a future study.

Increasing or decreasing the expression of midbody proteins has been shown to cause cytokinesis failure in HeLa cells, which results in an increase in MNC (Straight *et al.*, 2005; Zhu *et al.*, 2005; Zhao *et al.*, 2006; Gai *et al.*, 2011). In addition to disrupting cytokinesis, elevated levels of midbody proteins are associated with an increase in tumor growth and invasion as well as a decrease in overall survival of patients with breast cancer (Zou *et al.*, 2014; Kalimutho *et al.*, 2018; Li *et al.*, 2020; Wang *et al.*, 2020). Consistently, we found that ectopic expression of septin-6 or Mklp1 leads to MNC in mouse mammary epithelial cells (Figure 3). However, this potentially deleterious phenotype is blocked in cells cultured on soft microenvironments that mimic the stiffness of the normal mammary gland (Figure 4). These data reveal that soft matrices protect cells from abscission failure, MNC, and aneuploidy. It is interesting to note, however, that

while depletion of anillin and Mklp1 both result in a significant increase in MNC, depletion of septin-6 does not. One possible explanation is that different septin family members can substitute for each other to stabilize the septin complex and promote normal cytokinesis (Dolat *et al.*, 2014). Consistently, septin-6-knockout mice have no phenotype. In contrast, depletion of anillin results in improper localization of the contractile ring, causing regression of the cleavage furrow and cytokinesis failure (Straight *et al.*, 2005; Kim *et al.*, 2017). Similarly, depletion of Mklp1 prevents formation of the midbody, which is required for abscission (Zhu *et al.*, 2005). Therefore, although we observe that substratum stiffness affects the expression of several different components of the abscission machinery, each likely plays distinct roles.

Mechanical signals are transmitted from the matrix microenvironment through integrins and focal adhesions. ILK is an adaptor protein involved in transmitting cell–ECM signals and in regulating several cellular processes, including EMT (Gil *et al.*, 2011; Zheng *et al.*, 2019). Here we found an association between ILK and MNC in response to induction of EMT (Figure 5). Although ILK expression is required for induction of EMT and MNC in cells on stiff microenvironments, inhibiting its kinase activity does not affect either process. Similarly, ectopic expression of ILK in cells cultured on soft substrata is not sufficient to increase focal adhesions or induce MNC downstream of EMT-associated signaling. These data suggest that it is the scaffolding function of ILK, rather than its kinase activity, that permits EMT-associated signaling on stiff microenvironments.

In addition to serving as a scaffolding protein, ILK plays an important role in regulating proliferation (Han *et al.*, 2018), which is significantly reduced in cells on soft microenvironments (Schrader *et al.*, 2011; Razinia *et al.*, 2017; Kalli and Stylianopoulos, 2018). Since MNC results from failure of cytokinesis in this context, it remained possible that the lower levels of MNC that we observed on soft substrata resulted from lower rates of proliferation. If this were the case, then we would expect to observe an increase in proliferation in $\beta1^{V737N}$-expressing cells cultured on soft substrata (which show increased MNC in response to TGFβ) compared with vehicle-expressing controls (which do not). However, EdU analysis revealed that depleting ILK or ectopically expressing $\beta1^{V737N}$ has no effect on proliferation (Supplemental Figure S7). Consistent with these results, we previously found that increasing proliferation in cells cultured on soft substrata does not permit an induction of MNC downstream of EMT signaling (Simi *et al.*, 2018). From these data, we conclude that the lower rates of MNC that we observe in shILK-expressing cells and cells cultured on soft substrata result from weak cell–matrix interactions instead of from a reduction in proliferation. This conclusion is supported by the results of our experiments using the autoclustering $\beta1^{V737N}$ mutant, which permits treatment with TGFβ to induce an increase in the levels of *Snai1* and *Sept6* as well as MNC in cells cultured on soft substrata.

Although our results implicate ILK in regulating MNC downstream of EMT signaling, analysis of available breast cancer cell line and tumor sample datasets reveals a positive and statistically significant correlation between the expression of β1-integrin and midbody proteins (Supplemental Figure S8). In contrast, these datasets show no correlation between the expression of ILK and the midbody proteins analyzed. These correlative results are consistent with our findings that clustering of β1-integrin (and focal adhesion assembly) is essential for EMT signaling, abscission failure, and MNC and suggest that the expression of β1-integrin is more essential in regulating these processes in the context of cancer. These findings are corroborated by other studies implicating the importance of both integrin-ligand anchoring and stiffness in the responses of cells to their microenvironment (Trappmann *et al.*, 2012; Wen *et al.*, 2014).

Although previous studies have emphasized the role of integrin signaling and matrix compliance in the regulation of EMT, no study to date has explored the role of EMT signaling and integrin–ECM interactions in the formation of multinucleated cells. Depleting integrins or interfering with integrin signaling prevents the induction of EMT (Bhowmick *et al.*, 2001; Bianchi *et al.*, 2010; Lee *et al.*, 2012; Feldkoren *et al.*, 2017), which we found promotes MNC in cells on stiff microenvironments. The increase in multinucleated cells that we observe on stiff microenvironments is supported by previous work, which shows that increases in ECM stiffness, traction forces at the cytokinetic ring, or prolonged tension at the intercellular bridge all promote abscission failure leading to an increase in MNC (Lafaurie-Janvore *et al.*, 2013; Uroz *et al.*, 2019). Taken together, our results suggest the following: mammary epithelial cells form weak interactions with soft matrices, leading to a decrease in the response to EMT inducers and expression of midbody proteins, which permits the cells to complete cytokinesis and generate two daughter cells. In contrast, cells form stronger interactions with stiff matrices, leading to expression of Snail in the response to EMT inducers, which increases the expression of midbody proteins, causing downstream defects in abscission (Figure 8). This interplay between mechanical and chemical signaling from the microenvironment is especially significant in the context of cancer, including tumors of the breast, where increased integrin–ECM interactions have been implicated in cell survival and resistance to chemotherapy (Aoudjit and Vuori, 2012; Thibaudeau *et al.*, 2015; Cooper and Giancotti, 2019). Our data suggest that normalizing a stiff microenvironment, or blocking mechanical signaling through cell–matrix adhesions, as a strategy to prevent enhanced invasiveness, MNC, and genomic instability in cells undergoing EMT.

MATERIALS AND METHODS

Request a protocol through *Bio-protocol*.

Cell culture

NMuMG mouse mammary epithelial cells (ATCC) were cultured in high glucose DMEM (Hyclone) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin (Life Technologies), and 100 µg/ml streptomycin (Life Technologies). SCp2 mouse mammary epithelial cells were cultured in DMEM:F12 supplemented with 2% FBS, 10 µg/ml insulin (Sigma), and 50 µg/ml gentamicin (Life Technologies); 4T1 mouse mammary carcinoma cells (ATCC) were maintained in RPMI medium (Hyclone) supplemented with 10% FBS and 50 µg/ml gentamicin. MCF10A human mammary epithelial cells were cultured in DMEM:F12 supplemented with 5% horse serum (Fisher Scientific), 100 ng/ml cholera toxin (Sigma), 10 µg/ml insulin, 20 ng/ml epidermal growth factor (Sigma), 0.5 mg/ml hydrocortisone (Fisher Scientific), and 50 µg/ml gentamicin. Cells were treated for 24 h with vehicle (0.1% bovine serum albumin) or TGFβ1 (5 ng/ml; R&D Systems) starting 24 h after initial plating. For experiments using the ILK inhibitor CPD-22 (1 µM; Millipore), cells were pretreated with the inhibitor for 24 h before exposure to TGFβ1.

Synthetic substrata

PA gels were generated as previously described (Simi *et al.*, 2018). Briefly, PA gels were polymerized on 31-mm-diameter glass coverslips by mixing water and 12.5% (vol/vol) acrylamide with either 0.5% (vol/vol) or 17.5% (vol/vol) bisacrylamide. To initiate polymerization, 10% ammonium persulfate (Bio-Rad) at a 1:200 dilution and N,N,N′,N′-tetramethethylenediamine (Sigma-Aldrich) at a 1:2000

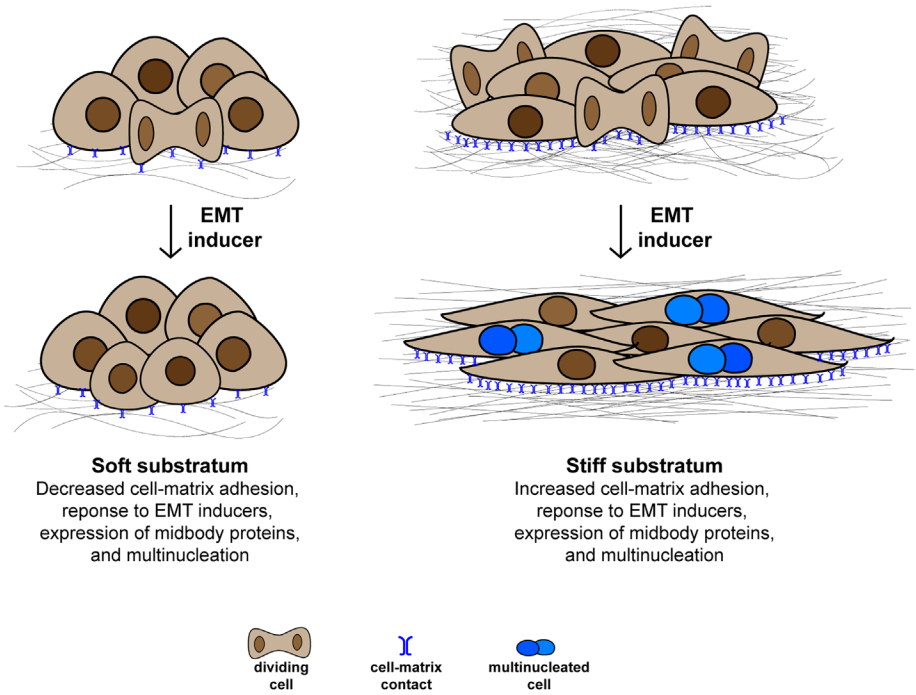

FIGURE 8: Substratum stiffness signals through focal adhesions to regulate MNC in cells undergoing EMT. Cells cultured on soft microenvironments have weak cell–matrix interactions leading to a decrease in the response to EMT inducers, levels of midbody proteins, and MNC. In contrast, cells cultured on stiff microenvironments have stronger cell–matrix interactions resulting in an increase in the response to EMT inducers, expression of midbody proteins, and MNC.

dilution were added; 36 µl of the mixture was sandwiched between the coverslips, and the mixture was allowed to polymerize for 45 min at room temperature, after which the top coverslip was carefully removed. The heterobifunctional crosslinker Sulfo-SANPAH was used to conjugate the surface of the PA gel with fibronectin (Fisher Scientific) as previously described (Simi *et al.*, 2018). Before cells were plated, the gels were rinsed at least twice with HEPES buffer or phosphate-buffered saline (PBS) and incubated with culture medium at 37°C for ~1 h.

Viral transductions and transfections

Recombinant adenoviruses encoding GFP-septin-6, GFP-septin-6-shRNA, GFP-Mklp1, GFP-Mklp1-shRNA, GFP-U6-shRNA, GFP-ILK, or GFP alone were purchased from Vector Biolabs. The recombinant adenovirus encoding GFP-Snail was described previously (Simi *et al.*, 2018). Adenovirus was added directly to culture medium at an MOI of 50.

ShCntl or shILK-expressing NMuMG cells were generated using a lentivirus expressing a scrambled shRNA sequence or a lentivirus expressing shRNA against ILK as previously described (Han *et al.*, 2018). Stable clones were selected using puromycin (5 µg/ml; Sigma).

NMuMG cells were transfected with the pTK88_GFP-Anillin plasmid (Addgene plasmid # 46354), the scramble-shRNA plasmid (shScr, Addgene plasmid #1864), the shAnillin plasmid (Sigma), or the shβ1 plasmid (Sigma) using FuGENE HD Transfection Reagent (Promega). The autoclustering β1-integrin mutant ($\beta1^{V737N}$) plasmid was generated as previously described (Lee *et al.*, 2012). FuGENE and the plasmid were mixed with Opti-MEM medium (3:1 FuGENE reagent:DNA ratio), incubated for 10 min at room temperature, and then added directly to culture medium. Cells were fixed with 4% paraformaldehyde (PFA) in PBS and analyzed 48 h later.

Quantitative reverse transcriptase PCR (qRT-PCR)

Gene expression was measured by qRT-PCR analysis 24 h after TGFβ treatment or 48 h after viral transduction. RNA was extracted using TRIzol reagent (Invitrogen), followed by reverse transcription to generate cDNA using a Verso cDNA synthesis kit (Thermo Scientific). Transcript levels were measured using an Applied Biosystems Step One Plus instrument and iTaq Universal SYBR Green SuperMix (Bio-Rad). Melt-curve analysis was used to confirm the amplification of only one PCR product. The expression level of each gene was normalized to that of 18S rRNA in the same sample. Primers were designed using PrimerQuest (IDT) and verified for specificity using BLAST (Supplemental Table S1).

Immunofluorescence analysis

Cells were fixed with 4% PFA in PBS for 15 min at room temperature and then blocked for 1 h at room temperature with 10% (vol/vol) goat serum (Sigma Aldrich) in 0.1% Triton X-100 in PBS (PBST). To label E-cadherin, samples were incubated overnight at 4°C with mouse anti-E-cadherin antibody (C36; BD Biosciences; 1:200). To label focal adhesions, samples were incubated overnight at 4°C with mouse anti-vinculin (Sigma; 1:200) or rabbit anti-pY397-FAK antibody (141-9; Invitrogen; 1:200). Samples were then washed four times with PBST for 15 min each time and incubated with Alexa 594 goat anti-mouse secondary antibody (Invitrogen, 1:200) overnight at 4°C. Nuclei were counterstained by incubating cells with a 1:5000 dilution of Hoechst 33342 (Invitrogen) for 30 min at room temperature and then washed three times with PBS for 15 min each wash. Samples were imaged using a Hamamatsu camera attached to a Nikon Ti-U inverted microscope at 20× magnification in air or at 40× magnification in oil (for focal adhesions).

To analyze MNC, the number of cells with at least two nuclei was divided by the total number of cells in each image to obtain the percentage of multinucleated cells. Focal adhesions were quantified as previously described (Lee *et al.*, 2012). Briefly, to count the number of focal adhesions per cell, original images were filtered to subtract background fluorescence and segmented with a threshold of 0.05 μm^2. The number of images required to quantify an experimental condition was determined using a running average, such that when the condition stabilized no further images were analyzed. All image analysis was performed in ImageJ.

Immunoblotting

Samples were lysed with RIPA buffer supplemented with phosphatase and protease inhibitors. Protein concentrations were measured using the DC protein assay (Bio-Rad). Samples were mixed with Laemmli buffer, boiled for 10 min, resolved by SDS–PAGE (4–12% Tris-glycine gels), and transferred to nitrocellulose membranes. Membranes were blocked in 5% nonfat milk for 30 min and then

incubated overnight at 4°C in blocking buffer containing antibodies against anillin (Santa Cruz, 1:500), Mklp1 (Abcam, 1:1000), Akt (Cell Signaling Technologies, 1:1000), pAkt (Ser473) (Cell Signaling Technologies, 1:2000), or GAPDH (Cell Signaling Technologies, 1:2000).

EdU incorporation assay

Proliferation was assessed by quantifying the percentage of EdU-positive cells using the Click-It EdU Alexa Fluor 594 kit (Thermo Fisher Scientific). Samples were exposed to EdU for 2 h and then immediately fixed with 4% PFA in PBS before applying the Click-iT cocktail reagent.

Breast cancer cell line and clinical tumor analysis

The Cancer Cell Line Encyclopedia dataset (Ghandi *et al.*, 2019) and The Metastatic Breast Cancer Project (using cBioPortal) (Wagle *et al.*, 2017) were used to extract the mRNA expression profiles of 53 breast cancer cell lines and 146 breast tumors, respectively. The transcript levels of six genes (*Sept6*, *Snai1*, *Anln*, *Kif23*, *ILK*, and *ITGB1*) were analyzed using a Pearson correlation matrix.

Statistical analysis

Statistical analysis was performed using Prism version 8 (GraphPad). Two-sided Welch's *t* test, two-sided Student's *t* test, Pearson's correlation, and one-way or two-way ANOVA with Tukey's post-hoc test or Sidak's multiple comparison test were used for all data analysis. All experiments were performed using at least three separate biological replicates. A p value < 0.05 was considered to be statistically significant.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (NIH HD099030, CA187692, CA214292) and a Faculty Scholars Award from the Howard Hughes Medical Institute. E.M.R. was supported in part by an NIH National Research Service Award F30 (GM134602).

REFERENCES

Agromayor M, Martin-Serrano J (2013). Knowing when to cut and run: mechanisms that control cytokinetic abscission. Trends Cell Biol 23, 433–441.

Aoudjit F, Vuori K (2012). Integrin signaling in cancer cell survival and chemoresistance. Chemother Res Pract 2012, 283181.

Assoian RK, Marcantonio EE (1996). The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis. J Clin Invest 98, 2436–2439.

Bhowmick NA, Zent R, Ghiassi M, McDonnell M, Moses HL (2001). Integrin beta 1 signaling is necessary for transforming growth factor-beta activation of p38MAPK and epithelial plasticity. J Biol Chem 276, 46707–46713.

Bianchi A, Gervasi ME, Bakin A (2010). Role of β5-integrin in epithelial-mesenchymal transition in response to TGF-β. Cell Cycle 9, 1647–1659.

Carlton JG, Caballe A, Agromayor M, Kloc M, Martin-Serrano J (2012). ESCRT-III governs the Aurora B-mediated abscission checkpoint through CHMP4C. Science 336, 220–225.

Comaills V, Kabeche L, Morris R, Buisson R, Yu M, Madden MW, LiCausi JA, Boukhali M, Tajima K, Pan S, *et al.* (2016). Genomic instability is induced by persistent proliferation of cells undergoing epithelial-to-mesenchymal transition. Cell Rep 17, 2632–2647.

Cooper J, Giancotti FG (2019). Integrin signaling in cancer: mechanotransduction, stemness, epithelial plasticity, and therapeutic resistance. Cancer Cell 35, 347–367.

Dolat L, Hu Q, Spiliotis ET (2014). Septin functions in organ system physiology and pathology. Biol Chem 395, 123–141.

Feldkoren B, Hutchinson R, Rapoport Y, Mahajan A, Margulis V (2017). Integrin signaling potentiates transforming growth factor-beta 1 (TGF-β1) dependent down-regulation of E-Cadherin expression—Important implications for epithelial to mesenchymal transition (EMT) in renal cell carcinoma. Exp Cell Res 355, 57–66.

Fenner J, Stacer AC, Winterroth F, Johnson TD, Luker KE, Luker GD (2014). Macroscopic stiffness of breast tumors predicts metastasis. Sci Rep 4, 5512.

Fox DT, Duronio RJ (2013). Endoreplication and polyploidy: insights into development and disease. Development 140, 3–12.

Gai M, Camera P, Dema A, Bianchi F, Berto G, Scarpa E, Germena G, Di Cunto F (2011). Citron kinase controls abscission through RhoA and anillin. Mol Biol Cell 22, 3768–3778.

Ghandi M, Huang FW, Jané-Valbuena J, Kryukov GV, Lo CC, McDonald ER, Barretina J, Gelfand ET, Bielski CM, Li H, *et al.* (2019). Next-generation characterization of the Cancer Cell Line Encyclopedia. Nature 569, 503–508.

Giam M, Rancati G (2015). Aneuploidy and chromosomal instability in cancer: a jackpot to chaos. Cell Div 10, 3.

Gil D, Ciołczyk-Wierzbicka D, Dulińska-Litewka J, Zwawa K, McCubrey JA, Laidler P (2011). The mechanism of contribution of integrin linked kinase (ILK) to epithelial-mesenchymal transition (EMT). Adv Enzyme Regul 51, 195–207.

Han S, Pang MF, Nelson CM (2018). Substratum stiffness tunes proliferation downstream of Wnt3a in part by regulating integrin-linked kinase and frizzled-1. J Cell Sci 131.

Heerboth S, Housman G, Leary M, Longacre M, Byler S, Lapinska K, Willbanks A, Sarkar S (2015). EMT and tumor metastasis. Clin Transl Med 4, 6.

Horgan CP, Hanscom SR, Kelly EE, McCaffrey MW (2012). Tumor susceptibility gene 101 (TSG101) is a novel binding-partner for the class II Rab11-FIPs. PLoS One 7, e32030.

Kalimutho M, Sinha D, Jeffery J, Nones K, Srihari S, Fernando WC, Duijf PH, Vennin C, Raninga P, Nanayakkara D, *et al.* (2018). CEP55 is a determinant of cell fate during perturbed mitosis in breast cancer. EMBO Mol Med 10, e8566.

Kalli M, Stylianopoulos T (2018). Defining the role of solid stress and matrix stiffness in cancer cell proliferation and metastasis. Front Oncol 8, 55.

Kawahara R, Niwa Y, Simizu S (2018). Integrin β1 is an essential factor in vasculogenic mimicry of human cancer cells. Cancer Sci 109, 2490–2496.

Kilinc AN, Han S, Barrett LA, Anandasivam M, Nelson CM (2021). Integrin-linked kinase tunes cell-cell and cell–matrix adhesions to regulate the switch between apoptosis and EMT downstream of TGFbeta1. Mol Biol Cell 32, 402–412.

Kim H, Johnson JM, Lera RF, Brahma S, Burkard ME (2017). Anillin phosphorylation controls timely membrane association and successful cytokinesis. PLoS Genet 13, e1006511.

Klein EA, Yin L, Kothapalli D, Castagnino P, Byfield FJ, Xu T, Levental I, Hawthorne E, Janmey PA, Assoian RK (2009). Cell-cycle control by physiological matrix elasticity and in vivo tissue stiffening. Curr Biol 19, 1511–1518.

Lafaurie-Janvore J, Maiuri P, Wang I, Pinot M, Manneville JB, Betz T, Balland M, Piel M (2013). ESCRT-III assembly and cytokinetic abscission are induced by tension release in the intercellular bridge. Science 339, 1625–1629.

Lee K, Chen QK, Lui C, Cichon MA, Radisky DC, Nelson CM (2012). Matrix compliance regulates Rac1b localization, NADPH oxidase assembly, and epithelial-mesenchymal transition. Mol Biol Cell 23, 4097–4108.

Lee SL, Hsu EC, Chou CC, Chuang HC, Bai LY, Kulp SK, Chen CS (2011). Identification and characterization of a novel integrin-linked kinase inhibitor. J Med Chem 54, 6364–6374.

Leight JL, Wozniak MA, Chen S, Lynch ML, Chen CS (2012). Matrix rigidity regulates a switch between TGF-β1-induced apoptosis and epithelial-mesenchymal transition. Mol Biol Cell 23, 781–791.

Li TF, Zeng HJ, Shan Z, Ye RY, Cheang TY, Zhang YJ, Lu SH, Zhang Q, Shao N, Lin Y (2020). Overexpression of kinesin superfamily members as prognostic biomarkers of breast cancer. Cancer Cell Int 20, 123.

Mierzwa B, Gerlich DW (2014). Cytokinetic abscission: molecular mechanisms and temporal control. Dev Cell 31, 525–538.

Normand G, King RW (2010). Understanding cytokinesis failure. Adv Exp Med Biol 676, 27–55.

Northey JJ, Przybyla L, Weaver VM (2017). Tissue force programs cell fate and tumor aggression. Cancer Discov 7, 1224–1237.

Parker SJ, Stotland A, MacFarlane E, Wilson N, Orosco A, Venkatraman V, Madrid K, Gottlieb R, Dietz HC, Van Eyk JE (2018). Proteomics reveals Rictor as a noncanonical TGF-β signaling target during aneurysm progression in Marfan mice. Am J Physiol Heart Circ Physiol 315, H1112–H1126.

Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, *et al*. (2005). Tensional homeostasis and the malignant phenotype. Cancer Cell 8, 241–254.

Razinia Z, Castagnino P, Xu T, Vázquez-Salgado A, Puré E, Assoian RK (2017). Stiffness-dependent motility and proliferation uncoupled by deletion of CD44. Sci Rep 7, 16499.

Rembold M, Ciglar L, Yáñez-Cuna JO, Zinzen RP, Girardot C, Jain A, Welte MA, Stark A, Leptin M, Furlong EE (2014). A conserved role for Snail as a potentiator of active transcription. Genes Dev 28, 167–181.

Renshaw MJ, Liu J, Lavoie BD, Wilde A (2014). Anillin-dependent organization of septin filaments promotes intercellular bridge elongation and Chmp4B targeting to the abscission site. Open Biol 4, 130190.

Rice AJ, Cortes E, Lachowski D, Cheung BCH, Karim SA, Morton JP, Del Río Hernández A (2017). Matrix stiffness induces epithelial-mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. Oncogenesis 6, e352.

Rios AC, Fu NY, Jamieson PR, Pal B, Whitehead L, Nicholas KR, Lindeman GJ, Visvader JE (2016). Essential role for a novel population of binucleated mammary epithelial cells in lactation. Nat Commun 7, 11400.

Sambandamoorthy S, Mathew-Steiner S, Varney S, Zuidema JM, Gilbert RJ, Van De Water L, LaFlamme SE (2015). Matrix compliance and the regulation of cytokinesis. Biol Open 4, 885–892.

Schrader J, Gordon-Walker TT, Aucott RL, van Deemter M, Quaas A, Walsh S, Benten D, Forbes SJ, Wells RG, Iredale JP (2011). Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells. Hepatology 53, 1192–1205.

Scott LE, Weinberg SH, Lemmon CA (2019). Mechanochemical signaling of the extracellular matrix in epithelial-mesenchymal transition. Front Cell Dev Biol 7, 135.

Sechler JL, Schwarzbauer JE (1998). Control of cell cycle progression by fibronectin matrix architecture. J Biol Chem 273, 25533–25536.

Simi AK, Anlą AA, Stallings-Mann M, Zhang S, Hsia T, Cichon M, Radisky DC, Nelson CM (2018). A soft microenvironment protects from failure of midbody abscission and multinucleation downstream of the EMT-promoting transcription factor snail. Cancer Res 78, 2277–2289.

Straight AF, Field CM, Mitchison TJ (2005). Anillin binds nonmuscle myosin II and regulates the contractile ring. Mol Biol Cell 16, 193–201.

Thibaudeau L, Taubenberger AV, Theodoropoulos C, Holzapfel BM, Ramuz O, Straub M, Hutmacher DW (2015). New mechanistic insights of integrin β1 in breast cancer bone colonization. Oncotarget 6, 332–344.

Trappmann B, Gautrot JE, Connelly JT, Strange DG, Li Y, Oyen ML, Cohen Stuart MA, Boehm H, Li B, Vogel V, *et al*. (2012). Extracellular-matrix tethering regulates stem-cell fate. Nat Mater 11, 642–649.

Uroz M, Garcia-Puig A, Tekeli I, Elosegui-Artola A, Abenza JF, Marín-Llauradó A, Pujals S, Conte V, Albertazzi L, Roca-Cusachs P, *et al*. (2019). Traction forces at the cytokinetic ring regulate cell division and polyploidy in the migrating zebrafish epicardium. Nat Mater 18, 1015–1023.

Wagle N, Painter C, Anastasio E, Dunphy M, McGillicuddy M, Kim D, Jain E, Buendia-Buendia J, Cohen O, Knelson E, *et al*. (2017). The Metastatic Breast Cancer (MBC) project: Accelerating translational research through direct patient engagement. J Clin Oncol 35, 1076–1076.

Wang D, Naydenov NG, Dozmorov MG, Koblinski JE, Ivanov AI (2020). Anillin regulates breast cancer cell migration, growth, and metastasis by non-canonical mechanisms involving control of cell stemness and differentiation. Breast Cancer Res 22, 3.

Weaver BA, Cleveland DW (2006). Does aneuploidy cause cancer? Curr Opin Cell Biol 18, 658–667.

Wei SC, Fattet L, Tsai JH, Guo Y, Pai VH, Majeski HE, Chen AC, Sah RL, Taylor SS, Engler AJ, *et al*. (2015). Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway. Nat Cell Biol 17, 678–688.

Wen JH, Vincent LG, Fuhrmann A, Choi YS, Hribar KC, Taylor-Weiner H, Chen S, Engler AJ (2014). Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat Mater 13, 979–987.

Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Ng Eaton E, Weinberg RA (2015). Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. Nature 525, 256–260.

Yeh YC, Ling JY, Chen WC, Lin HH, Tang MJ (2017). Mechanotransduction of matrix stiffness in regulation of focal adhesion size and number: reciprocal regulation of caveolin-1 and β1 integrin. Sci Rep 7, 15008.

Zhao WM, Seki A, Fang G (2006). Cep55, a microtubule-bundling protein, associates with centralspindlin to control the midbody integrity and cell abscission during cytokinesis. Mol Biol Cell 17, 3881–3896.

Zheng CC, Hu HF, Hong P, Zhang QH, Xu WW, He QY, Li B (2019). Significance of integrin-linked kinase (ILK) in tumorigenesis and its potential implication as a biomarker and therapeutic target for human cancer. Am J Cancer Res 9, 186–197.

Zhu C, Bossy-Wetzel E, Jiang W (2005). Recruitment of MKLP1 to the spindle midzone/midbody by INCENP is essential for midbody formation and completion of cytokinesis in human cells. Biochem J 389, 373–381.

Zou JX, Duan Z, Wang J, Sokolov A, Xu J, Chen CZ, Li JJ, Chen HW (2014). Kinesin family deregulation coordinated by bromodomain protein ANCCA and histone methyltransferase MLL for breast cancer cell growth, survival, and tamoxifen resistance. Mol Cancer Res 12, 539–549.