SUPPLEMENTAL MATERIAL AND METHODS

Preparation of COL I gels.

Acid-soluble collagen stock (7.5 vol of 0.5% solution) was mixed gently on ice with 1 vol of 0.1N NaOH and 1 vol of 10X DMEM. Collagen solution was diluted in DMEM/F-12 medium to a final concentration of ~2 g/l.

Quantitative RT-PCR analysis.

The following primers were used to amplify β-casein cDNA and 18S rRNA sequences: forward primer of the β-casein gene 5'-GCT CAG GCT CAA ACC ATC TC-3' and reverse primer 5'-TGT GGA AGG AAG GGT GCT AC-3'; forward primer of the 18S rRNA gene 5'-TCGGAACTGAGGCCATGATT-3' and reverse primer 5'-CCTCCGACTTTCGTTCTTGATT-3'. Fragments of β-casein gene and 18S rRNA were amplified with following protocol: 95°C for 10 min (initial denaturation), and 45 amplification cycles (95°C for 5 s, 60°C for 10 s, 72°C for 5 s). Melting curve was analyzed to verify the presence of a single PCR product. GAPDH gene was amplified as described elsewhere (Xu et al, 2007)

Analysis of AFM measurements.

The zero-force offset was assessed from the non-contact region of the *F-z* curve. The tip-sample contact point z_c and E were estimated by least-squares fitting $z\text{-}d = z_c + (4(1-\nu^2)kd/3E\tan\theta)^{1/2}$ (where d is the cantilever deflection, ν is the Poisson's ratio, assumed to be 0.5, and θ is the semiincluded angle of the AFM tip) to the loading trace of the *F-z* curve. As described in methods, we apply two selection rules to E data. We discarded E values based on coefficient of variation CV > 15% and on Chauvenet's criterion.

SUPPLEMENTAL DISCUSSION

The problem of indentation measurements on the surface of a body formed by two layers with a different stiffness (Young modulus *E1* and *E2* for layer 1 and 2, respectively) have been studied both theoretically (Costa & Yin, 1999; Li & Chou, 1997; Sridhar & Sivashanker, 2003) and experimentally (Dimitriadis et al, 2002) in much detail. These previous studies concluded that mechanical measurements of the first layer (*E1*, the cell in our case) are not affected by the stiffness of the second layer (*E2*, ECM gel or glass in our study) provided that the relative indentation of the first layer (cell indentation divided by total cell height) is < 15%. Otherwise, *E1* will be underestimated if $E2 << E1$; conversely *E1* will be overestimated if $E2 >> E1$. The first scenario ($E2 << E1$) corresponds to cells on top of ECM gels, whereas the second ($E2 >> E1$) corresponds to cells on 2D-glass. Based on these previous studies, as we describe in the AFM elasticity measurements section, recordings from cells subjected to relative indentations > 15% of the total height were discarded to rule out any artifactual contribution from the underlying substratum. In support of our approach, we obtained several results that are not possible to observe assuming an artifactual contribution of the stiffness of the underlying substrata (either 2D or ECM gels): (1) *E* of SCp2 on 2D treated with latrunculin B was smaller than SCp2 on Matrigel (Figure 6A), even though the stiffness of 2D substratum was 8 orders of magnitude higher than Matrigel (Table 1); (2) SCp2 and EpH4 exhibited similar stiffness values on 100% LM1 and 40%LM1 mixed with COL I. In constrast, they exhibited different and opposing trends on Matrigel (Figure 2C, SCp2 stiffer than EpH4) and on 100% COL I (SCp2 softer than EpH4) (Figure 4D); (3) we found an inverse and nonlinear relationship between the stiffness of SCp2 and that of LM1 gels mixed with increasing concentration of COL I (Figure 4F): while gel stiffness increased dramatically with LM1 concentrations lower than 10%, the corresponding stiffness of SCp2 decreased. Since none of these findings are consistent with an artifactual contribution of the stiffness of the underlying substrata, we are confident that our selection rule was sufficient to discard this artifact.

SUPPLEMENTARY REFERENCES

Costa KD, Yin FC (1999) Analysis of indentation: implications for measuring mechanical properties with atomic force microscopy. *J Biomech Eng* **121**(5)**:** 462-471

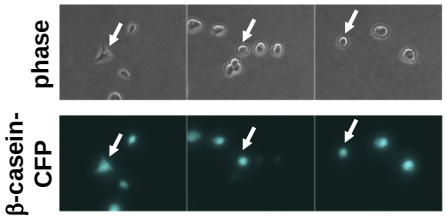
Dimitriadis EK, Horkay F, Maresca J, Kachar B, Chadwick RS (2002) Determination of elastic moduli of thin layers of soft material using the atomic force microscope. *Biophys J* **82**(5)**:** 2798-2810

Li J, Chou TW (1997) Elastic field of a thin-film/substrate system under an axisymmetric loading. *Int J Solids Structures* **34:** 4463-4478

Sridhar I, Sivashanker S (2003) On the adhesion mechanics of multi-layer elastic systems. *Surf Coat Tech* **167:** 181-187

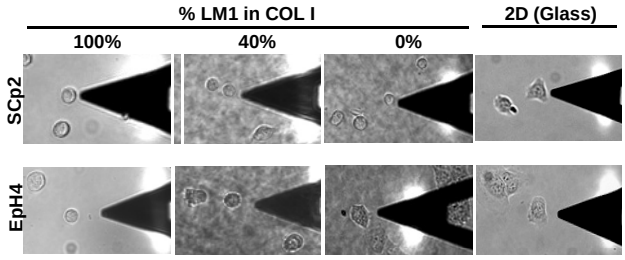
Xu R, Spencer VA, Bissell MJ (2007) Extracellular matrix-regulated gene expression requires cooperation of SWI/SNF and transcription factors. *J Biol Chem* **282**(20)**:** 14992-14999

SUPPLEMENTAL FIGURE 1



Visualization of the activity of the β-casein gene promoter in single MECs. Phase contrast (top) and corresponding CFP fluorescence intensity images (bottom panels) for three different fields of EpH4 cells stably transfected with 16 concatenated copies of the β-casein promoter fused to CFP cultured in 2D and overlaid for 24h with 2% Matrigel diluted in differentiation medium. Arrows mark single cells. Note that CFP expression was detected even in single EpH4 cells, thereby confirming that cell-cell contacts are not necessary for β-casein transcription.

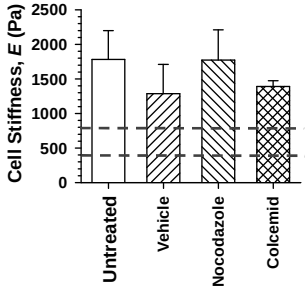
SUPPLEMENTAL FIGURE 2



Loss of LM1 signaling differentially affect the morphology of SCp2 and EpH4 cells. Representative images of the morphology of SCp2 and EpH4 cells in LM1:COL I gels and on glass substrata.

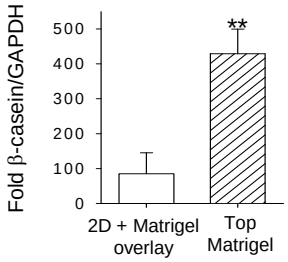
.

SUPPLEMENTAL FIGURE 3



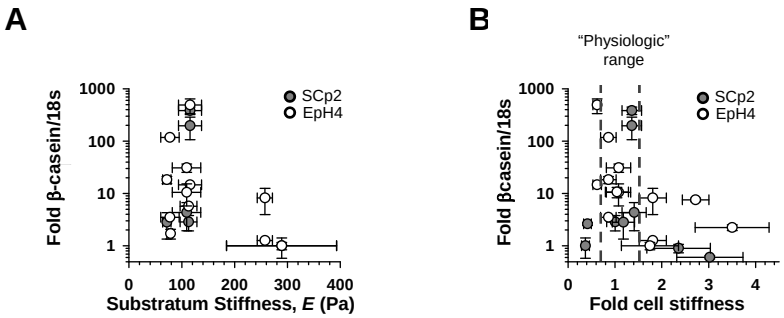
Inhibiting microtubule polymerization does not compromise the elasticity of MECs on Matrigel. Comparison of the elasticity of SCp2 cells cultured for 24h on glass after treatment for at least 30 min with either vehicle (DMSO) or two inhibitors of microtubule polymerization (nocodazole and colcemid) using concentrations described in the main text.

SUPPLEMENTAL FIGURE 4



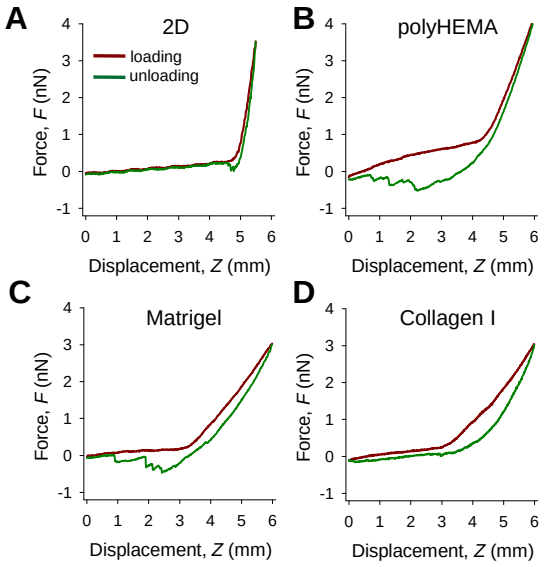
Increased induction of β-casein expression in cells on top of Matrigel in comparison to cells in 2D overlaid with Matrigel. EpH4 cells were cultured in differentiation media in either 2D, 2D overlaid with 2% Matrigel or on top of Matrigel for 48h. Plot shows b-casein mRNA levels with respect to GADPH mRNA assessed by quantitative RT-PCR and normalized by expression levels in 2D in the absence of Matrigel. Data shown are mean ± SD (two independent experiments). ** $p<0.05$ was determined by two-tailed Student t-test.

SUPPLEMENTAL FIGURE 5



Summary of β-casein, substratum stiffness and cell stiffness data from all the experiments carried out on SCp2 and EpH4 cells in this study. (A) β-casein as a function of the elastic modulus of the substratum. (B) β-casein as a function of the stiffness of the cells. All stiffness data were measured by AFM.

SUPPLEMENTAL FIGURE 6



Representative raw AFM force curve data on single cells on different substrata. Force versus displacement curves recorded on single SCp2 cells cultured on 2D (A), polyHEMA (B), top of Matrigel (C) and top of collagen I (2 g/l) (D). Red and green lines correspond to loading and unloading curves, respectively.