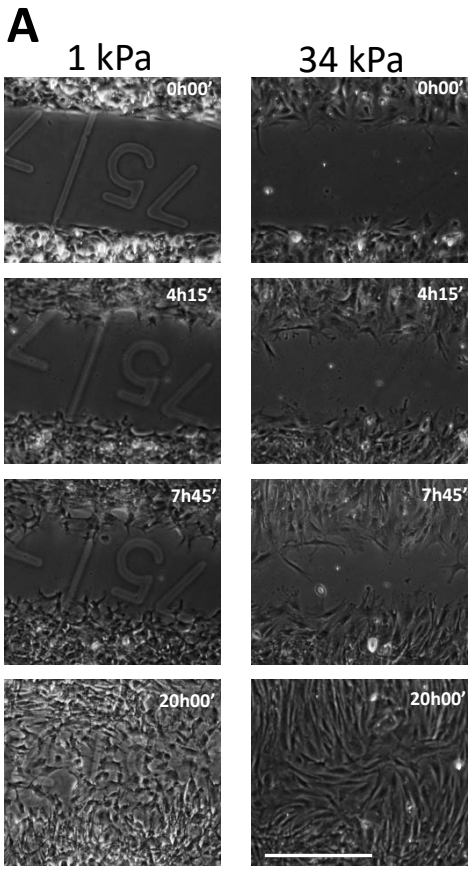
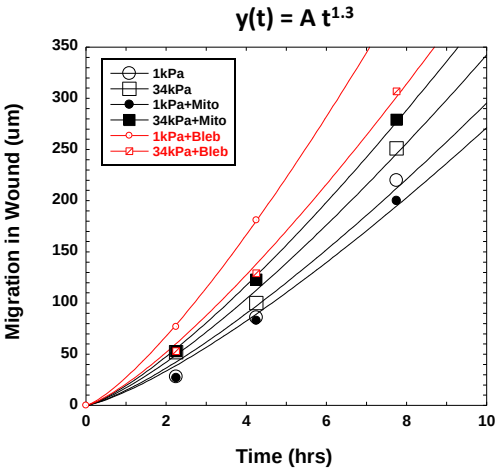
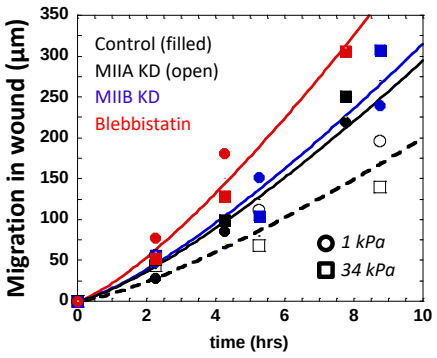
Fig. S1





	A (μm/hr$^{1.3}$)
1 kPa	14.8
34 kPa	17.2
1 kPa + Mitomycin-C	13.6
34 kPa + Mitomycin-C	19.3
1 kPa + Blebbistatin	21
34 kPa + Blebbistatin	27.5



	A (μm/hr$^{1.3}$)
Control	14.7
MIIA KD	10
MIIB KD	15.7
Blebbistatin	21.8

Fig. S1. MSCs close wounds atop soft and stiff matrix.

(A) Time-lapse imaging of MSCs closing a 500 μm gap on soft (1 kPa) or stiff (34 kPa gels). Gridded coverslips were used to find the same region for some experiments. Phase contrast imaging shows cells appearing flatter and more spread on the stiffer matrix. Scale bar 500 μm. (B) Curves were fit to the distance the cell sheet edges migrated into the gap versus time following a function that varies with time$^{1.3}$ which incorporates both components of acceleration and constant velocity during the wound closure. The coefficients in the table are from the fitted equations for soft and stiff conditions along with mitomycin C or blebbistatin treatment. (C) Mask wound assays were also performed with MIIA and MIIB isoforms knocked down separately. Black filled in data are for untreated, black open points are for MIIA KD, blue for MIIB KD, and red for blebbistatin. Squares indicate 34 kPa, circles 1 kPa. Table shows the results of the curve fits using both 1 kPa and 34 kPa data for each curve.

Fig. S2

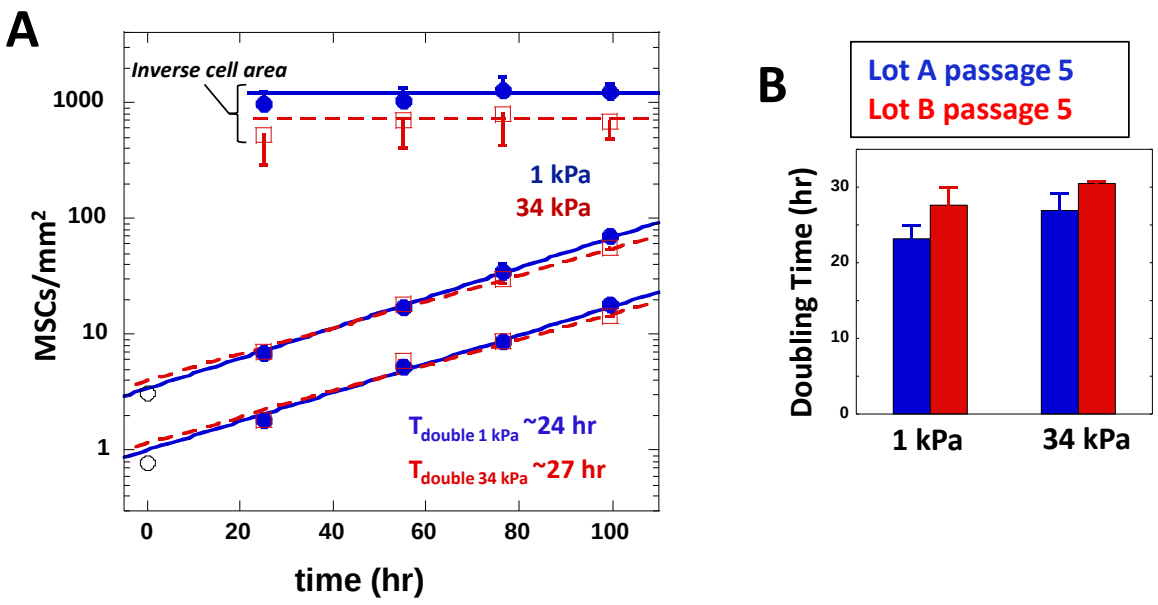


Fig. S2. MSCs proliferate at similar rates despite differences in matrix stiffness.

(A) MSCs were plated sparsely (open black circles) at a density of 80 or 300 cells/cm^2 (both independent experiments, indicated at t=0). Each day after plating at least 15 mm^2 of random area on the gel was imaged and the number of cells counted directly. Exponential growth curves were fit to all datasets. The reciprocal of the mean cell areas is also plotted for the MSCs, showing that MSCs on stiff matrix are always larger and that cell densities used in most of the experiments throughout this study are at least 10-100 fold below fully confluent. (B) Doubling times calculated for MSCs from 2 separate donor lots on soft or stiff gels, determined from the exponential fits in (A).

Fig. S3

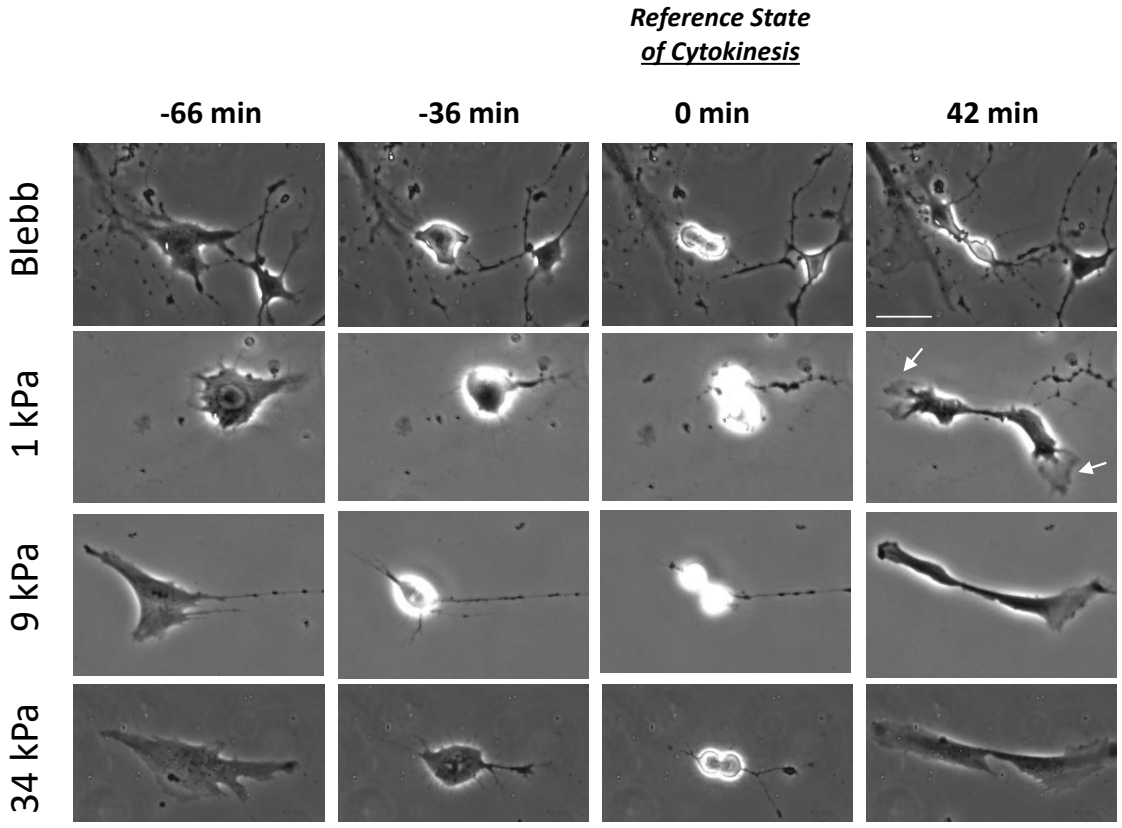


Fig. S3. MSCs round up, divide, and begin to migrate away from each other despite differences in matrix compliance.

Phase contrast time-lapse imaging shows similar rates of cytokinesis among gels of different stiffness. A reference point was taken as the cells first split into 2 as t = 0 min. Blebbistatin treatment on stiff matrix was also evaluated and slowed the division process. White arrows indicate the typical unstable lamellapodia observed on soft matrix. Scale bar 20 μm.

Fig. S4

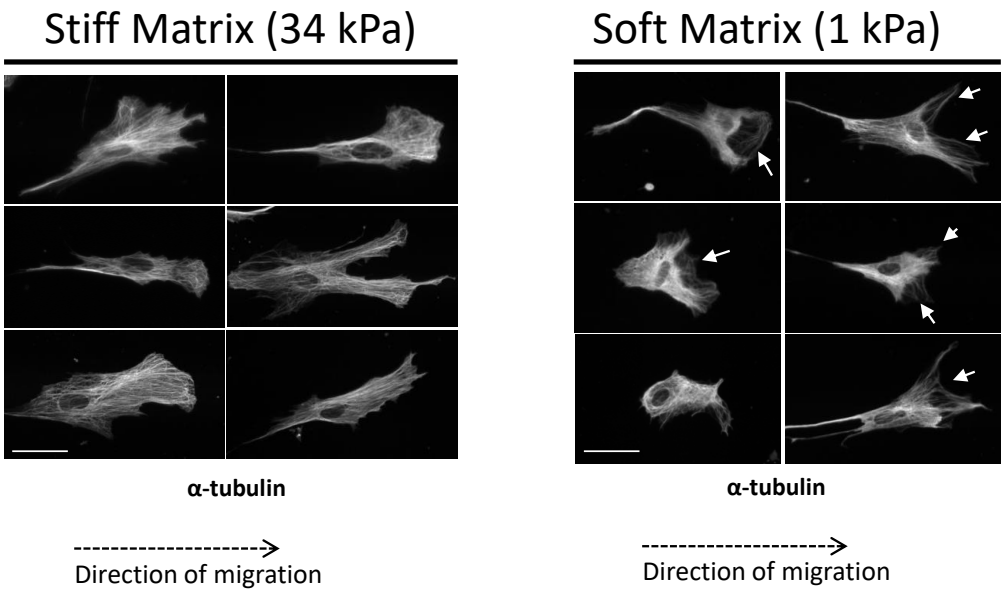


Fig. S4. Lamellipodia in MSCs on soft gels often are depleted of MTs.

MSCs on soft matrix often exhibit lamellipodia with low amounts of microtubules (white arrows). This microtubule depletion in outstretched lamellipodia was never observed in cells on stiff matrix. Scale bar 20 μm.