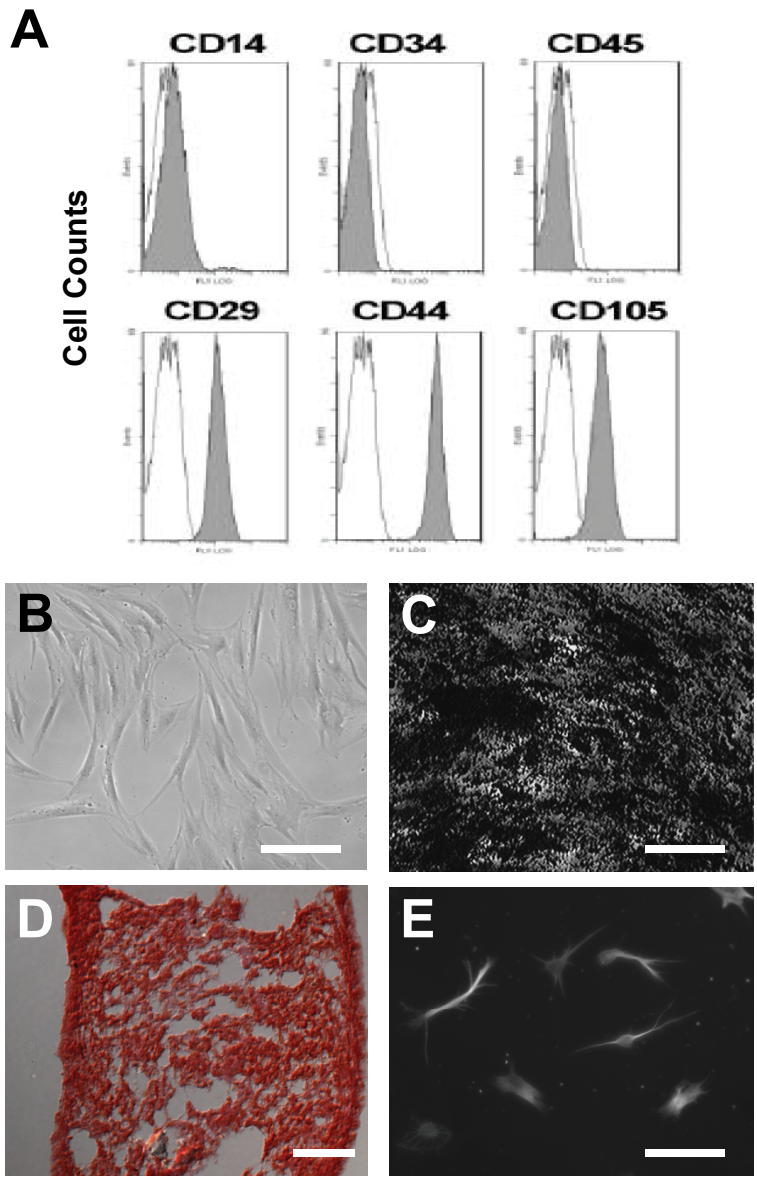


**Figure S1**. Characterization of MSCs. (A) Expression of cell surface markers in MSCs. The filled histogram curves show the staining with respective primary and secondary antibodies. The open histogram curves show the staining with the control antibodies and secondary antibodies. (B) Phase contrast image of undifferentiated MSCs. (C) MSCs were cultured in osteogenic medium for 1 month, and calcified matrix was stained by using silver nitrate. (D) MSCs were cultured as cell pellets in chondrogenic medium for 3 weeks, and glycosaminoglycans (GAGs) were stained with Safranin O. (E) MSCs were cultured in Schwann cell differentiation medium for 2 weeks, and stained for Schwann cell marker S100β. Scale bars are 100 μm.

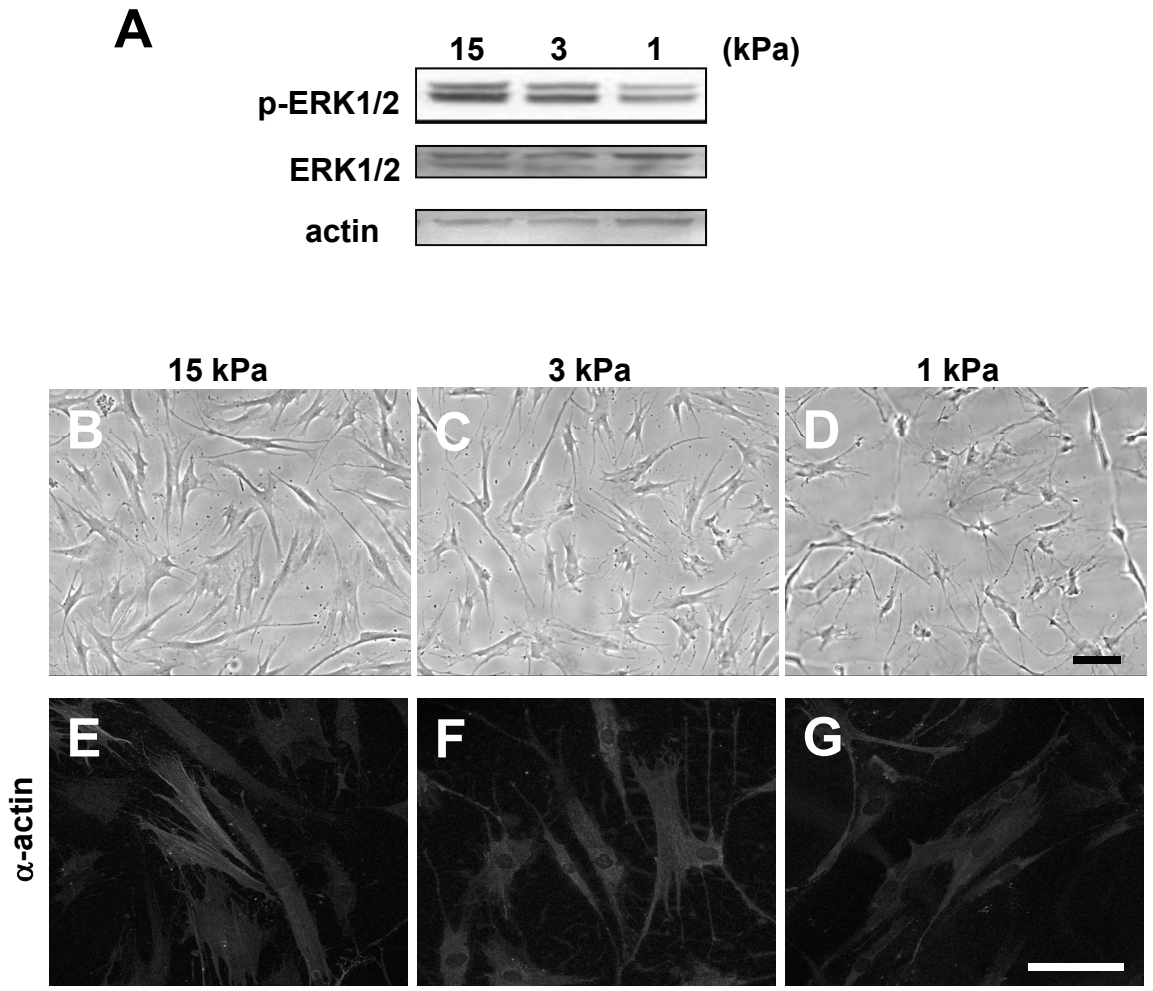


**Figure S2.** Effects of substrate stiffness on ERK phosphorylation, MSC morphology, and α-actin assembly. MSCs were grown on PA gel for 1 day in DMEM with 1% FBS. (A) Cells were lysed for immunoblotting analysis of ERK1/2 phosphorylation. The immunoblots of ERK1/2 and actin were used for normalization. (B-D) Phase contrast microscopy showing the morphology of MSCs on PA gels. (E-G) MSCs were stained for SM α-actin. Scale bars are 100 μm.

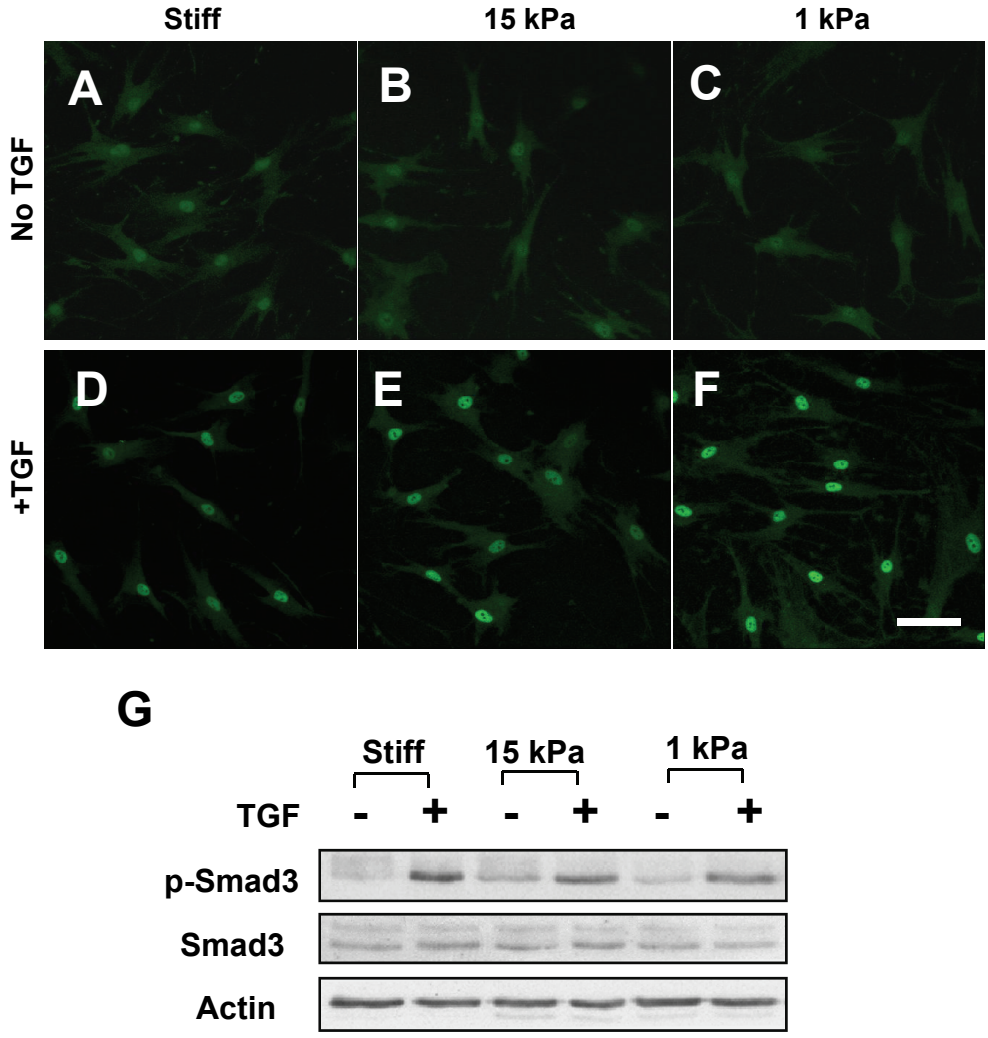


**Figure S3**. Effects of matrix stiffness on TGF-β signaling. MSCs were grown on collagen-coated culture dishes (stiff substrates) or PA gels coated with collagen for 1 day in DMEM with 1% FBS, and treated with TGF-β for 1 hour. (A-F) Cells were fixed and stained for Smad2. Scale bar=100 μm. (G) Cells were lysed for immunoblotting analysis of the phosphorylation of Smad3.

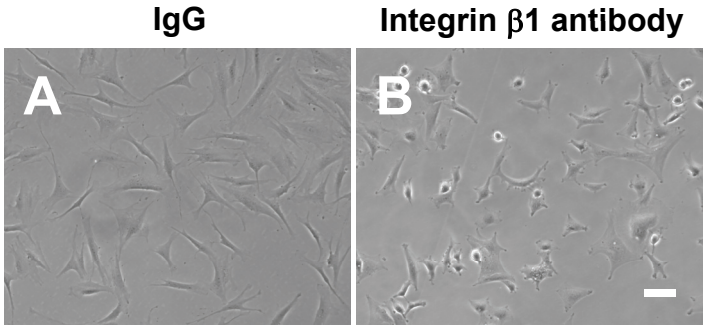


**Figure S4**. Effect of blocking integrin on cell spreading. MSCs were treated with a blocking antibody against integrin β1 (mAb13) or a control IgG for 30 minutes, and seeded on collagen-coated culture dishes for 6 hours. Images were collected by phase contrast microscopy. Scale bar=100 μm.

## Table S1. Commercial Sources of Reagents

| | |
|---|---|
| **Cell Culture Media and Supplement** | |
| Human MSCs | Lonza Corp. |
| MSCGM | Lonza Corp. |
| N2 medium | Invitrogen, Inc. |
| DMEM | Invitrogen, Inc. |
| αMEM | Invitrogen, Inc. |
| fetal bovine serum | Invitrogen, Inc. |
| penicillin/streptomycin | Invitrogen, Inc. |
| TGF-β1 | Peprotech, Inc. |
| Insulin | Invitrogen, Inc. |
| Dexamethasone | Sigma-Aldrich, Inc. |
| isobutylxanthine | Sigma-Aldrich, Inc. |
| ascorbic acid | Sigma-Aldrich, Inc. |
| β-glycerol phosphate | Sigma-Aldrich, Inc. |
| CNTF | Invitrogen, Inc. |
| bFGF | Invitrogen, Inc. |
| dibutyryl-cAMP | Invitrogen, Inc. |
| neuregulin | Invitrogen, Inc. |
| **Chemicals** | |
| rat tail collagen-I | BD BioSciences, Inc. |
| 3-aminopropyltrimethoxysilane | Sigma-Aldrich, Inc. |
| glutaraldehyde | Electron Microscopy Sciences |
| acrylamide | Polysciences, Inc. |
| bis-acrylamide | Polysciences, Inc. |
| ammonium persulfate | Bio-Rad Laboratories |
| N,N,N',N'-Tetramethylethylenediamine | Sigma-Aldrich, Inc. |
| Sulfo-SANPAH | Pierce, Inc. |
| Rho affinity precipitation assay kit | Millipore, Inc. |
| BrdU | Amersham Biosciences |
| Lipofectamine™2000 | Invitrogen, Inc. |
| G418 | Invitrogen, Inc. |
| Alcian blue | Sigma-Aldrich, Inc. |
| Oil red | Sigma-Aldrich, Inc. |
| Silver nitrate | Sigma-Aldrich, Inc. |
| Safranin O | Sigma-Aldrich, Inc. |
| phalloidin-FITC | Invitrogen, Inc. |

**Antibodies for Flow Cytometry Analysis**

| | |
|---|---|
| CD29 | BD Biosciences Pharmingen, Inc. |
| CD44 | Biosource, Inc. |
| CD105 | Chemicon, Inc. |
| CD34 | BD Biosciences Pharmingen, Inc. |
| CD14 | Santa Cruz Biotechnologies, Inc. |
| CD45 | BD Biosciences Pharmingen, Inc. |

**Antibodies for Immunostaining**

| | |
|---|---|
| SM $\alpha$-actin | Sigma-Aldrich, Inc. |
| calponin-1 | Epitomics, Inc. |
| Smad2 | Cell Signaling, Inc. |
| vinculin | Sigma-Aldrich, Inc. |
| S100$\beta$ | Sigma-Aldrich, Inc. |
| Secondary antibodies | Invitrogen, Inc. |

**Antibodies for Immnoblotting**

| | |
|---|---|
| calponin-1 | Sigma-Aldrich, Inc. |
| β-actin | Santa Cruz Biotechnologies, Inc. |
| p-Smad3(Ser423/425) | Cell Signaling, Inc. |
| Smad3 | Cell Signaling, Inc. |
| p-ERK1/2(Thr202/Tyr204) | Cell Signaling Inc. |
| ERK1/2 | Santa Cruz Biotechnologies, Inc. |
| RhoA | Santa Cruz Biotechnologies, Inc. |

**Antibodies for Blocking Cell Adhesion**

| | |
|---|---|
| integrin $\beta$1, mAb13 | BD BioSciences, Inc. |
| normal mouse IgG | BD BioSciences, Inc. |

**Instrument**

| | |
|---|---|
| Comparator | B.C. Ames Company, Waltham, MA |

## Table S2. Primers used in qPCR

| Gene Name | Forward Primer (5' to 3') | Reverse Primer (5’ to 3’) |
|---|---|---|
| 18S rRNA | CGCAGCTAGGAATAATGGAATAGG | CATGGCCTCAGTTCCGAAA |
| SM α-actin | CAGCTCCAGCTATGTGTGAAGAA | GCAAAGCCGGCCTTACAGA |
| Calponin-1 | GCATGTCCTCTGCTCACTTCAA | GGGCCAGCTTGTTCTTAACCT |
| Collagen-II | GGAAGAGTGGAGACTACTGGATTGAC | TCCATGTTGCAGAAAACCTTCA |
| LPL | TCTCTTGGGATACAGCCTTGGA | GCCAGTAATTCTGTTGACTTTCTTATTG |