Supplementary Methods

Proteomics

Trypsin digestion. Protein samples were digested with sequencing grade modified trypsin (Promega Corporation) according to manufacturer's instructions. Briefly, 90 μL of 200 mM triethyl ammonium bicarbonate (TEAB) and 10 μL of 2% sodium dodecyl sulfate were added to aliquots of 200 μg of protein, and the samples were adjusted to a final volume of 200 μL with ultrapure water. Five microliters of 200 mM Tris (2-carboxyethyl)phosphine was added to the samples and incubated for 1 h at 55°C. Then, 5 μL of 375 mM iodoacetamide was added and incubated for 30 min without light. After incubation, 1.2 mL of prechilled (−20°C) acetone was added, and the precipitation was allowed to proceed overnight. The pellets were centrifuged at 8000 *g* for 10 min at 4°C. The acetone-precipitated protein pellets were suspended with 100 μL of 200 mM TEAB, and 5 μg of trypsin was added to digest the sample overnight at 37°C.

Desalination of fractionated samples. A C18 solid-phase extraction (SPE) column (Thermo Scientific Hyper-Sep SPE Columns; Thermo-Fisher Scientific) was used to desalt the samples the next day after trypsin digestion. The samples were each adjusted to a final volume of 1 mL containing 0.25% (v/v in water) trifluoroacetic acid (TFA). The C18 SPE column was conditioned before use by filling the SPE column with 1 mL acetonitrile and allowing the solvent to pass through the media slowly (~3 min). The column was then rinsed three times with 1 mL 0.25% (v/v in water) TFA solution. Each sample was loaded on to the top of an SPE cartridge and allowed to bind slowly. The flow through was collected and passed through the column a second time. Columns were washed four times with 1 mL of 0.25% TFA aliquots before the peptides were eluted with 2600 mL of 80% acetonitrile/0.1% formic acid (aqueous). The eluted samples were completely lyophilized in a CentriVap centrifugal vacuum concentrator (Labconco).

LC-MS/MS analysis on LTQ-Orbitrap. Peptides were analyzed on an LTQ-Orbitrap XL (Thermo-Fisher Scientific) instrument interfaced with an Ultimate 3000 Dionex LC system (Dionex) using high-mass resolution for peptide identification and high energy collision dissociation (HCD) for reporter ion quantification. The RP-LC system consisted of a peptide Cap-Trap cartridge (0.5×2.0 mm) (Michrom BioResources) and a prepacked BioBasic C18 PicoFrit analytical column (75 μm i.d., 15 cm length) (New Objective) fitted with a FortisTip emitter tip. Samples were loaded onto the trap cartridge and washed with mobile phase A (98% H_2O, 2% acetonitrile, and 0.1% formic acid) for concentration and desalting. Subsequently, peptides were eluted over 180 min from the analytical column via the trap cartridge using a linear gradient of 6%–100% mobile phase B (20% H_2O, 80% acetonitrile and 0.1% formic acid) at a flow-rate of 0.3 μL/min using the following gradient: 6% B for 5 min, 6%–60% B for 125 min, 60%–100% B for 5 min, hold at 100% B for 5 min, 100%–6% B in 2 min, hold at 6% B for 38 min. The LTQ-Orbitrap tandem mass spectrometer was operated in a data-dependent mode. Briefly, each full MS scan (60,000 resolving power) was followed by six MS/MS scans where the three most abundant molecular ions were dynamically selected and fragmented by collision-induced dissociation (CID) using a normalized collision energy of 35%. The same three molecular ions were also scanned three times by HCD-MS2 with collision energy of 45%. MS scans were acquired in profile mode and MS/MS scans in centroid mode. LTQ-Orbitrap settings were as follows: spray voltage 2.0 kV, 1 microscan for MS1 scans at 60,000 resolution (fwhm at m/z 400), microscans for MS2 at 7500 resolution (fwhm at m/z 400), full MS mass range, m/z 400–1400; MS/MS mass range, and m/z 100–2000. The "FT master scan preview mode," "charge state screening," "monoisotopic precursor selection," and "charge state rejection" were enabled so that only the 2+, 3+, and 4+ ions were selected and fragmented by CID and HCD.

Database search. The protein search algorithm used was Mascot (Matrix Science). Mascot format files were generated by the Proteome Discoverer 1.2 software (Thermo Fisher Scientific) using the following criteria: database, IPI.Human.fasta.v3.77; enzyme, trypsin; maximum missed cleavages, 2; static modifications, carbamidomethylation (+57 Da), N-terminal TMT6plex (+229 Da), lysyl TMT6plex (+229 Da). Dynamic modifications, N-terminal Cln-pyro-Glu (+17 Da); methionine oxidation (+16 Da); STY phosphorylation (+80 Da); MS peptide tolerance was set at 15 ppm; MS/MS tolerance at 0.05 Da. Peptides reported by the search engine were accepted only if they met the false discovery rate of $p < 0.05$ (target decoy database).

Western blot

Native and decellularized lung protein samples (25 μg protein, 1× NuPage sample buffer, 1× NuPage reducing agent) were incubated at 70°C for 10 min. Denatured samples were loaded in triplicate to NuPage 4%–12% Bis-tris gels (Invitrogen) and subjected to polyacrylamide gel electrophoresis at 200 V for 1 h. The proteins were transferred onto nitrocellulose membranes using a wet transfer apparatus with modified Towbin's transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3) at 25 V overnight at 4°C. Membranes were washed 2×5 min with Tris-buffered saline containing 0.1% Tween-20 (TBS-T) and blocked for 1 h in TBS-T containing 5% reagent-grade blocking dry milk (BioRad) and either 1% goat serum or 1% bovine serum albumin (BSA) for blots ultimately exposed to goat anti-mouse and bovine anti-rabbit secondary antibodies, respectively. After blocking, membranes were rinsed briefly with TBS-T followed by overnight incubation at 4°C with TBS-T containing 5% dry milk, 1% goat serum or 1% BSA, and primary antibodies raised against human fibronectin or actin at dilutions of 1:5000 or 1:750, respectively. Next day, membranes were washed 3× for 10 min in TBS-T, and the secondary antibody-containing solutions (TBS-T, 5% dry milk, 1% goat serum or 1% BSA, 1:1000 goat anti-mouse IgG- horseradish peroxidase (HRP)

or 1:1000 bovine anti-rabbit-HRP, respectively) were applied to the membranes and blots were incubated at room temperature for 1 h with gentle shaking. A final wash of 3× for 10 min with TBS-T and 1× for 10 min TBS preceded development with enhanced chemiluminescence reagent (ECL; Invitrogen) and detection using the GE ImageQuant LAS 4000 system. To demonstrate equal protein load, nitrocellulose membranes were stained with Ponceau S (Sigma-Aldrich), imaged with ImageQuant, and total protein content quantified by whole-lane densitometry using ImageJ.

Rhesus macaque stem cell isolation and culture

Rhesus macaque bone marrow-derived mesenchymal stem cells (BMSC) were isolated and cultured as previously described.[12] Briefly, bone marrow was harvested antemortem by aspiration from the greater trochanter of the femur or at necropsy by flushing the shaft of the femur of rhesus macaques. Mononuclear cells were collected using Ficoll-Hypaque method (density = 1.073 g/mL; Pharmacia). The cells were resuspended in complete-minimum essential medium (α-MEM; Invitrogen) supplemented with 20% fetal bovine serum (FBS; Atlanta Biologicals), and seeded in 175 cm2 tissue culture plates (Nalgene Nunc). Tissue culture plates were washed the next day to remove the nonadherent cells. Cells were incubated for an additional 5–10 days, until they had reached 70% confluence, with media changes occurring every 2–3 days. Cell stocks were cryopreserved and stored in liquid nitrogen vapor.

Rhesus macaque adipose-derived mesenchymal stem cells (ASC) were isolated and cultured as previously described.[13,14] Briefly, adipose tissue was obtained from normal healthy rhesus macaques under local anesthesia. The raw adipose tissue was processed according to established methodologies to obtain a stromal vascular fraction.[14] To isolate MSC, samples were digested at 37°C for 30 min with 0.075% collagenase (Sigma). The cells were resuspended in red cell lysis buffer. The MSC were cultured in complete medium (CCM) containing α-MEM (Invitrogen) supplemented with 20% FBS (Atlanta Biological), 1% L-glutamine (Invitrogen), and 1% penicillin/streptomycin (Invitrogen) in a 37°C incubator with a 5% CO_2 atmosphere. The cells were expanded to 70% confluence, cryopreserved, and stored in liquid nitrogen vapor.

Ki67 and TUNEL staining (immunohistochemistry)

Ki67 staining was accomplished using a mouse monoclonal primary antibody ($IgG_{1\kappa}$ isotype) against Ki67 followed by AlexaFluor 568 goat anti-mouse IgG secondary antibody (Invitrogen). Negative controls for Ki67 immunohistochemistry used only TBS without primary antibody for initial incubations followed by staining with the same secondary antibodies as above. Cells proliferating in standard 2D cell culture were stained as positive controls. After extensive washing with TBS, all sections were stained with 0.3 mM DAPI for 5 min followed by washing with DI H_2O, air-drying, and coverslip mounting with Prolong Gold Antifade Reagent (Invitrogen). TUNEL staining was performed using the In Situ Cell Death Detection Kit—Fluorescein from Roche as per manufacturer instructions. Briefly, paraffin-embedded samples of stem cell-seeded macaque lung scaffold were deparaffinized and rehydrated through ethanol to water. The sections were treated with proteinase K (Roche) followed by staining by the TUNEL label and enzyme solution. Negative controls consisted of samples treated with only label solution without enzyme. Positive controls were treated with 30 μg/mL DNase I from bovine pancreas (Sigma-Aldrich) before TUNEL staining. After TUNEL staining, all sections were stained with 0.3 mM DAPI for 5 min followed by washing with DI H_2O, air-drying, and coverslip mounting with Prolong Gold Antifade Reagent (Molecular Probes). Images from both Ki67 and TUNEL staining were captured by standard fluorescence microscopy using the Leica DMRXA2 deconvolution microscope operated with SlideBook software. Twelve random images at 50× magnification were acquired per section for each Ki67 and TUNEL stains. The total number of cells was counted by nuclear particle analysis using NIH ImageJ, and the number of Ki67- and TUNEL-positive cells was counted by eye. The percent of cells showing evidence of proliferation (Ki67+) or apoptosis (TUNEL+) was calculated by dividing the number of positively staining cells per field by the total number of cells per field.

Cell–extracellular matrix binding array

The propensity of rhesus macaque ASC and BMSC to bind to various individual extracellular matrix (ECM) proteins was assessed using the fluorometric ECM Cell Adhesion Array Kit (ECM545) from Millipore as per manufacturer's instructions. Briefly, rhesus BMSC and ASC were cultured in CCM for ~1 week before assay. On the day of the experiment, the cells were trypsinized from the culture plates using 0.25% Trypsin–EDTA (Invitrogen), washed consecutively in CCM and PBS, pelleted at 1500 rpm for 7 min, and held on ice until use. To perform the assay, the cells were resuspended in Hanks buffered salts solution (HBSS) to 2×10^6 cells/mL. Next, 100 μL of the cell suspension was added to the wells of the array plate that are coated with different individual ECM proteins. The array plate was then incubated at 37°C, 5% CO_2 for 2 h to allow upregulation of integrin expression and cell–ECM binding. Then, the media and nonadherent cells were discarded, and the remaining adherent cells were washed 3 times with assay buffer. The cells were lysed and stained with assay lysis buffer and CyQuant GR dye, respectively. The stained lysate was transferred to a 96-well plate suitable for fluorescence measurement, and the plate was read at 485 nm excitation and 530 nm emission spectra. Since fluorescence readings correlate directly with cell number, data representing the number of adherent cells in each ECM condition were reported by relative fluorescence units (RFU).