Supporting Information

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SI Methods

Cell Culture and Cell Engraftment. Normal human bronchial epithelial cells (EPs) cryopreserved with retinoic acid, human aortic endothelial cells (ECs), human umbilical vein endothelial cells (HUVECs), normal human lung fibroblast (NHLF), and all growth medium were obtained from Lonza. Bronchial epithelial growth medium (BEGM) was supplemented with 0.4% bovine pituitary extract, 0.1% hydrocortisone, 0.1% human EGF (hEGF), 0.1% epinephrine, 0.1% transferring, 0.1% insulin, 0.1% retinoic acid, 0.1% triiodothyronine, 0.1% gentamicin, amphotericin-B (GA-1000), and 20 units/ml penicillin-streptomycin. EGM-2 was supplemented with 10% FBS, 0.4% hEGF, 0.04% hydrocortisone, 0.1% R3-insulin-like growth factor-1, 0.1% vascular EGF, 0.1% human FGF-B (hFGF-B), 0.1% ascorbic acid, 0.1% Heparin 0.1% GA-1000, and 20 units/ml penicillin-streptomycin. Also, fibroblast growth medium (FGM) was supplemented with 5% FBS, 0.1% hFGFr-B, 0.1% insulin, 0.1% GA-1000, and 20 units/ml penicillin-streptomycin.

Cells were grown on uncoated polystyrene tissue culture polystyrene (TCPS) and denatured collagen-coated TCPS (DCCT; Becton-Dickinson; 10 mg/ml gelatin; Sigma). Two human donors for both EPs and ECs were used throughout these studies, but were not pooled for specific experiments. Levels of PGE_2 (Cayman Chemical), TGF-β2 (R&D Systems), GM-CSF, and TGF-β1 (Invitrogen) were used as internal controls between the donors for each cell type. Measurements were made by using commercially available ELISA kits and were found to be not significantly different between the two donors for both EPs and ECs (data not shown). Cells were used between passages 2 and 7 and cultured at 5% CO_2 at 37°C.

Depending on the specific experiment, denatured collagen matrix sheets were cut into blocks measuring $1.5 \times 1.0 \times 0.3$ to $3.5 \times 1.0 \times 0.3$ cm^3. Matrices were then hydrated in BEGM and/or EGM-2 for at least 30 min depending on the cell type(s) to be seeded. Immediately before seeding, matrices were placed horizontal on TCPS with no liquid. ECs (0.9×10^5) and/or EPs (2×10^5) were then concentrated to 100 μl, added to the surface of the matrices, and incubated for 3 h (5% CO_2 at 37°C). Matrices were then transferred to free-standing 15-ml polypropylene tubes containing 10 ml of EGM-2 and/or BEGM. Under standard culture conditions, cells were allowed to invade the blocks, lining the interstices of the matrices for up to 2 weeks. Cell viability was determined by trypan blue exclusion.

Animals and Surgical Procedure. This study conformed to the guidelines specified in the National Institutes of Health's *Guide for Care and Use of Laboratory Animals* and was approved by the Harvard Medical Area Standing Committee on Animals. At day 0, female New Zealand White rabbits (3.5–4.7 kg and 6 months to 1 year old) were sedated with 1 mg/kg acepromazine i.m., 0.01 mg/kg glycopyrrolate i.m., and 0.2 mg/kg butorphanol i.m. Induction was performed by using 0.25 mg/kg medetomidine s.c., followed by a general anesthetic of isoflorane (1–2%) and oxygen on a pediatric nonrebreathing anesthetic system. The rabbits were started on isoflurane and oxygen as its anesthetic during the surgical procedure. Before intubation, a 2% cetacaine spray was used to prevent larygnospasm. The animals were placed in the supine position on a heated operating table, and heart rate and PO_2 were monitored throughout the procedure. After airway epithelial injury and matrix implantation, the midline incisions were sutured closed, and the animals were observed in separate cages during recovery. The rabbits were observed daily for respiratory distress, stridor, general health, and activity. At any sign of airway obstruction, the rabbit was killed. At day 9 after injury, the presence or lack of respiratory stridor was recorded. The rabbits were then killed, and samples were removed for histological and morphological examination.

Tissue Processing and Analysis. Multiple segments proximal to the injury, at the injury, and distal to the injury were paraffin-embedded. Photomicrographs were taken with a DC-70 digital camera (Olympus). The lumen (L), epithelium (E), mesenchyme (M), mesenchyme injury (I), cartilage (C), and vascular (V) areas were measured in Adobe Photoshop CS3 software by an observer blinded to the treatment groups. For each trachea, all area measurements in the injured tracheal segments were represented as percent control by using the measurements of the uninjured tracheal segments proximal and distal to the injury as internal controls. The extent of mesenchyme injury was based on the combined areas of inflammation, fibrosis, and necrosis with the area being normalized for the size of the entire mesenchyme area.

Biosecretory Characterization. ECs and EPs were grown to confluence in all conditions. The same fresh medium (BEGM:EGM-2, 1:1) was then added with or without TNF-α (10 ng/ml; Sigma) for all conditions. Cells were trypsinized from TCPS and DCCT and counted with a cell counter (Coulter). Cells in matrices were detached by collagenase type I (1 mg/ml; Worthington) digestion and counted using a hemocytometer. Release of TGF-β2 (R&D Systems), PGE_2 (Cayman Chemical), sICAM-1, sVCAM-1, IL-8, GM-CSF, MCP-1, and TGF-β1 (Invitrogen) were measured by using commercially available ELISA kits according to the manufacturers' instructions. Fresh medium was incubated without cells for 24 h, and a baseline level for each soluble factor was measured at the same time as medium with cells. The baseline levels were then subtracted from the experimental raw data to obtain the cellular expression of each soluble factor. The results for each soluble factor were then normalized by cell number and expressed in either picograms or nanograms per 10^5 cells.

***In Vitro* Tube Formation Assay.** Reduced growth factor basement membrane extract purified from an Engelbreth–Holm–Swarm tumor was pipetted into wells of a 48-well plate (100 μl/well) and incubated at 37°C for 1 h to allow the extract to solidify. Eight hours after seeding, images of tube formation were taken at ×40 on an inverted microscope (Nikon Diaphot) and camera (Nikon D50). The branch points of the formed tubes were counted, and the length of the tube network was measured for each image. The total number of branch points and total length of the network from the four images per well were calculated. Medium from each coculture was collected and analyzed. FGF-2 (Invitrogen) levels were measured by using a commercially available ELISA kit according to the manufacturer's instructions.

Scanning EM. ECs, EPs, and EPs/ECs were grown in matrices for 12 days, and then scanning EMs were obtained for each cell type. In preparation for image capture, the ME cells were washed once in warm PBS and fixed overnight at 4°C with 3% glutaraldehyde. The matrices were then washed twice with PBS for 10 min. A 2-mm biopsy punch was used to cut cores from the middle of the matrices. The cores were washed four times with ultrapure water for 5 min. Cores were then stained for 30 min with a 0.5% uranyl

acetate solution, followed by four washes with ultrapure water for 5 min. Individual cores were placed in Quantomix TM QX-102 capsules. Sealed capsules were placed on the specimen stage to be viewed in the wet state with an S4300 SE-N (Hitachi) variable pressure scanning electron microscope equipped with a Schottky emitter. Imaging was performed by using a backscatter detector with emission energy of 15 kV and emission current of 90–100 mA at a working distance of 9.8–9.9 mm.

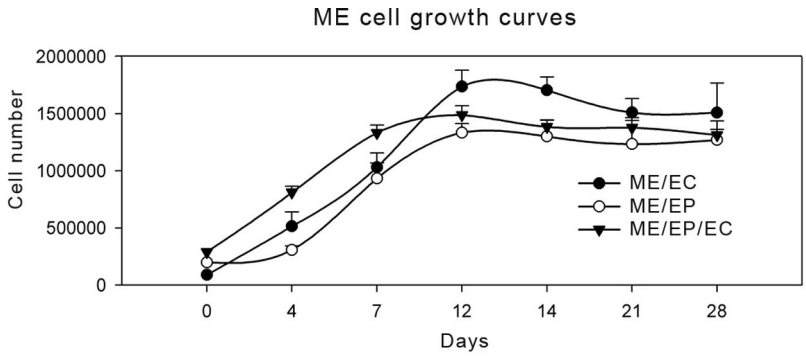

Fig. S1. EPs (2×10^5), ECs (9×10^4), and EPs/ECs (2×10^5 and 9×10^4) exhibit identical growth kinetics when embedded in denatured collagen matrices ($n = 3$). Cells reached peaked density (mean ± SE) at 12–14 days. To obtain similar growth kinetics over 28 days, cells were seeded in matrices ($2.5 \times 1.0 \times 0.3$ cm^3) with initial seedings of 2×10^5 EPs or 0.9×10^5 ECs. Cells reached a peaked density between 12–14 days. Thereafter, cell numbers remained fairly constant between days 15 and 28. Cell viability, by trypan blue exclusion, averaged 91.9 ± 0.47% for ME-EP, 92.6 ± 0.33% for ME-EC, and 91.2 ± 0.19% for ME-EP/ECs over a 28-day culture period.

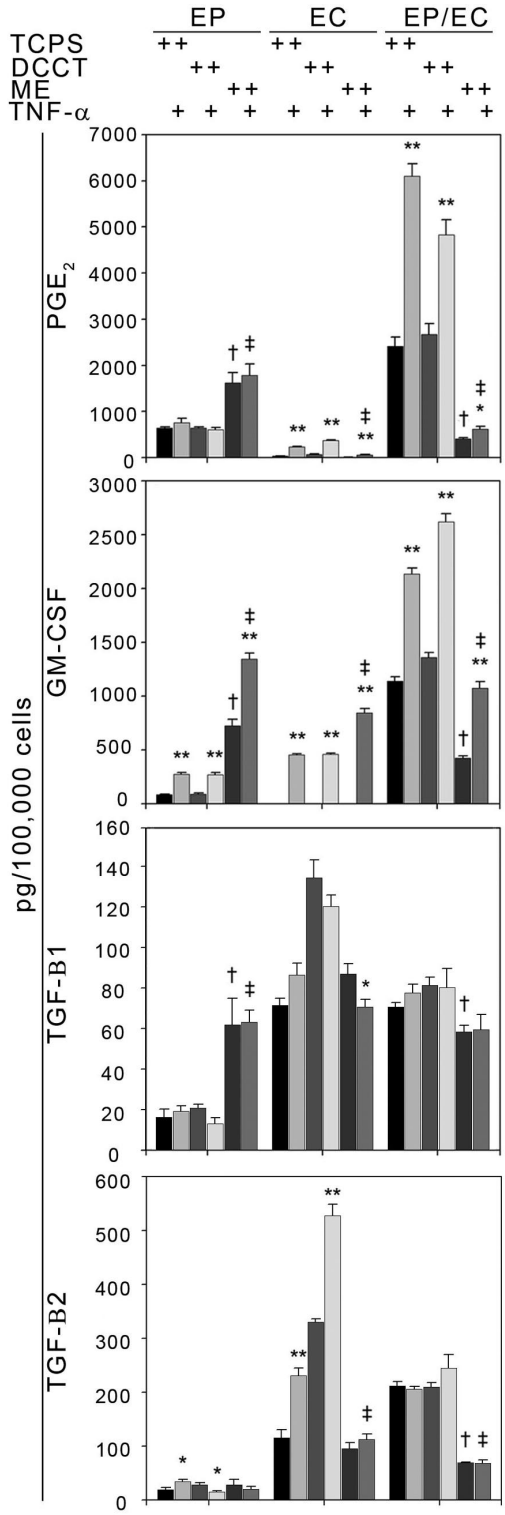

Fig. S2. Expansion of Fig. 2 *A* with protein secretion levels of PGE_2, GM-CSF, TGF-β1, and TGF-β2 from EPs, ECs, and EPs/ECs in TCPS, DCCT, and ME with or without TNF-α stimulation (10 ng/ml) for 24 h. Secretion levels were substratum and cell-specific (mean ± SE, $n = 5–6$). *, $P < 0.05$; **, $P < 0.001$ vs. unstimulated; †, $P < 0.01$ vs. TCPS and DCCT unstimulated for the same cell type; ‡, $P < 0.001$ vs. TCPS:TNF-α and DCCT:TNF-α for the same cell type.

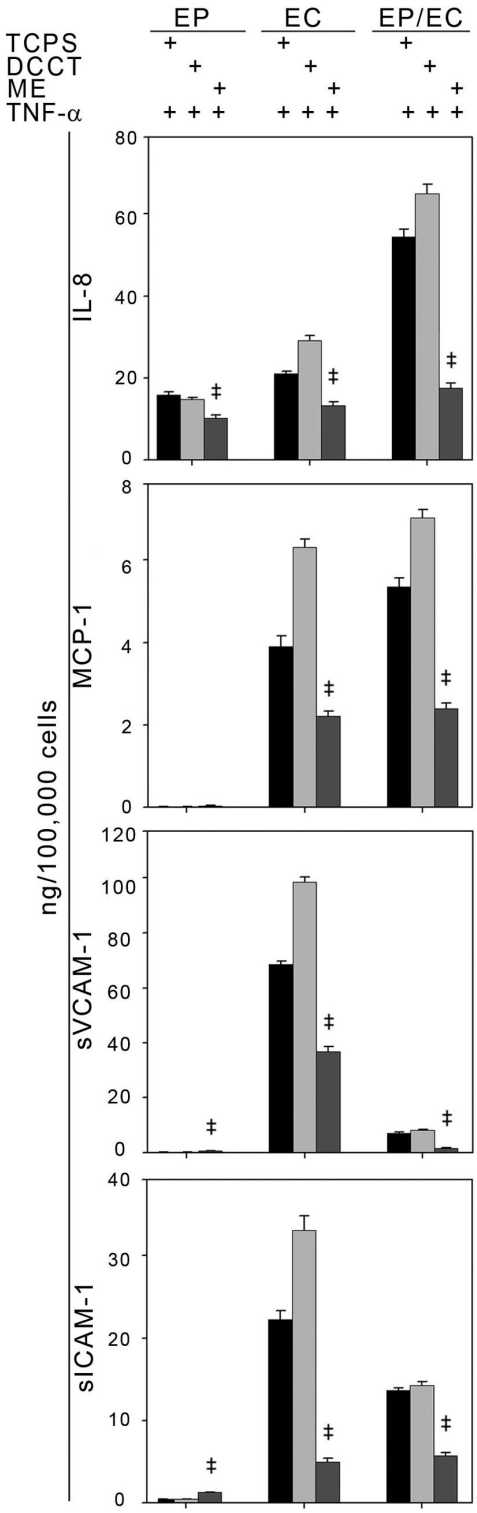

Fig. S3. Expansion of Fig. 2*B* with protein secretion levels of proinflammatory chemokines IL-8 and MCP-1 as well as adhesion molecules sICAM-1 and sVCAM-1 from EPs, ECs, and EPs/ECs in TCPS, DCCT, and ME stimulated with TNF-α (10 ng/ml) for 24 h. Matrix embedding limited up-regulation of all molecules. Data expressed as mean $\pm$ SEM from six independent experiments. ‡, $P < 0.0001$ vs. TCPS:TNF-α and DCCT:TNF-α for the same cell type.

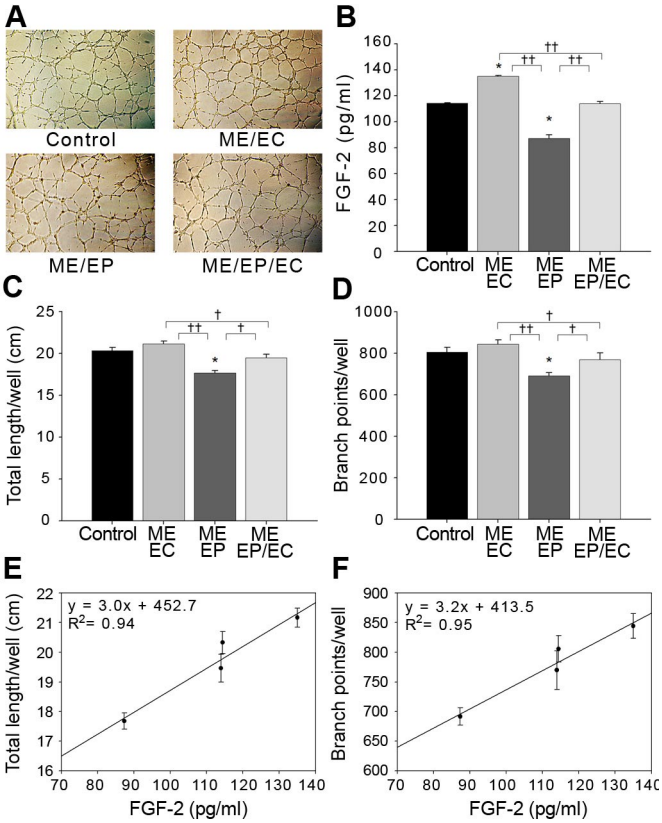

Fig. S4. Inhibition of tube formation by ME-EP conditioned medium (CM). HUVECs (2×10^4 cells per well) were seeded into 48-well plates that had been precoated with reduced growth factor basement membrane extract. The cells were treated with either BEGM:EGM-2 (1:1) medium unconditioned (incubated at 37°C without cells for 48 h) or conditioned for 48 h from matrices engrafted with ECs, EPs, or EPs/ECs. Eight hours after seeding, images of tube formation were taken with a ×40 objective lens on an inverted microscope. (*A*) Photomicrographs depict tube formation of HUVECs under defined CM. (*B*) (*C* and *D*) Expression levels of FGF-2 correlated with total length of tube network (*C*) and total number of branch points (*D*) from four microscopic fields per well ($n = 4$–5). *, $P < 0.01$ vs. control; †, $P < 0.05$; ††, $P < 0.001$ vs. compared condition. (*E* and *F*) Graphs depicting strong correlation between FGF-2 levels and total length of tube network (*E*) and total number of branch points per well (*F*).