Supplemental methods

Histopathology and histochemistry

To assess for the presence of lung fibrosis, sections of lung were deparaffinized in xylene, and rehydrated through a graded alcohol series to water. Sections were then incubated in Bouin's picric-formalin and then stained using Masson's trichrome with aniline blue as the collagen stain and Weigert's iron hematoxylin as the nuclear counterstain. Sections were dehydrated through graded alcohols to xylene, and coverslips were mounted with permount. Stained slides were examined on a Leica DM LB2 microscope (Wetzlar, Germany), digital micrographs were captured at 40x magnification and imported into QCapture (Quantitative Imaging Corporation, Surrey, BC, Canada).

For immunohistochemical staining, sections were deparaffinized with xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed in citrate buffer, pH 6.0 (Electron Microscopy Sciences, Hatfield, PA) at 95ºC for 3 minutes in pressure cooker. Endogenous peroxidase activity was quenched with 0.3 % H_2O_2 in water for 5 minutes. Following blocking in 2.5 % normal horse serum for 1 hour, sections were incubated with the appropriate primary antibody for 1 hour at room temperature followed by a peroxidase conjugated secondary antibody. Following incubation with Impact 3,3'-diaminobenzidine (Vector Laboratories, Burlingame, CA), sections were washed, counterstained with hematoxylin (Sigma Aldrich, St Louis, MO), dehydrated, and mounted with Permount. Antibodies against CD68 (macrophage marker), CD3 (T-lymphocyte marker), and fibrin were purchased from Abcam (Cambridge, MA). An antibody for neutrophil elastase (neutrophil marker) was purchased from EMD Millipore (Billerica, MA). Secondary antibodies conjugated with HRP were obtained from Vector Laboratories (Burlingame, CA). The number of macrophages,

neutrophils, or CD3 positive cells was counted in five randomly selected high power fields (40x) in each lung (15 fields per group).

The β-galactosidase activity assay (Abcam, Cambridge, MA) was performed according to the manufacturer's instructions in frozen lung sections or primary pneumocyte cultures to assess for replicative senescence. To determine if senescent cells were AECII, lung tissue sections and primary cell cultures were incubated with an anti-prosurfactant-C antibody (Abcam, Cambridge, MA), a marker of AECII, and treated with a compatible secondary antibody conjugated to Alexa Flour 594 (Life technologies, Grand Island, NY). Slides were mounted with ProLong antifade reagent containing DAPI (Life technologies, Grand Island, NY).

Enrichment of primary pneumocytes

Briefly, lungs were collected from mice pre-treated heparin sodium (12.5 u/g body weight). Lungs were perfused with 10 ml of HBSS (containing 30 mM HEPES), filled with 1 mL enzyme cocktail (Elastase 3 u/ml, 0.01% DNase I and 0.2 % Collagenase in HBSS containing 30 mM HEPES, Sigma Aldrich), and incubated in 5 ml of enzyme cocktail at 37^{o}C for 30 minutes. The digested tissue was carefully teased from the airways and gently swirled for 5 to 10 min. The resulting suspension was successively filtered through 100 μm and 40 μm Falcon cell strainers, then centrifuged at 130 x *g* for 8 min at 4°C and resuspended in HBSS. The crude single cell suspension was applied to Ficoll density gradient isolation solution. Pneumocytes were collected from the layer of density 1.077 ~ 1.080, washed with HBSS, and then resuspended with DMEM media containing 10% fetal calf serum (FCS, Life Technologies) and 1 % antibiotic-antimycotic solution (Life Technologies).