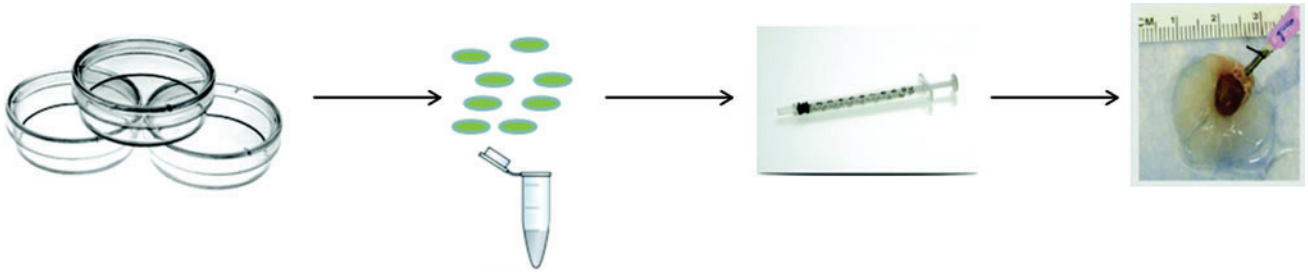
Supplementary Data

Immunofluorescence

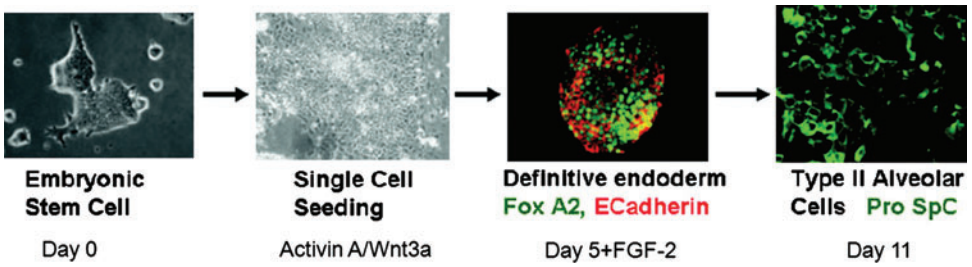
De-cellularized lung scaffolds were fixed in 4% paraformaldehyde followed by processing and paraffin embedding. Sections were deparafinized, permeabilized, and serum-blocked in a phosphate-buffered saline containing 0.1% Triton-X (Sigma) and 1% bovine serum albumin (Fisher Scientific) and 1% Tween-20 (Biorad). The primary antibody was applied overnight at 4°C. Primary polyclonal antibodies were collagen I 1:100 (Santa Cruz), collagen IV 1:200 (Abcam), elastin 1:100 (Santa Cruz), laminin 1:1000 (Abcam), smooth muscle alpha 1:200 (Abcam), pro-surfactant protein-C (Pro-SPC) 1:500 (Chemicon), PDGFRα 1:200 (Abcam), thyroid transcription factor 1 (TTF1) 1:200 (Millipore), FOXJ1 1:200 (Santa Cruz), mouse CD31 1:100 (BD), mouse MHC-I 1:100 (BD), and Isolectin B4 Dylite 546 1:200 (Jackson Immunoresearch). As a control, the primary antibodies were omitted to assess for nonspecific binding. The secondary antibody (Donkey Anti-Rabbit Alexa 594 or Donkey Anti-Goat Alexa 488; Invitrogen) was applied at room temperature. Samples were counterstained with Topro-3 (Invitrogen) or propidium iodide (Abcam) and mounted with Shandon Immuno-Mount (Thermo-Scientific). Samples were imaged using a Zeiss Axiovert 200M Confocal Microscope with an LSM 510 Meta Laser Module and Carl Zeiss LSM Image Browser Software (Carl Zeiss MicroImaging, LLC). The same imaging software was used to process the images in addition to Adobe Photoshop CS5 (Adobe Systems).

Supplementary Reference

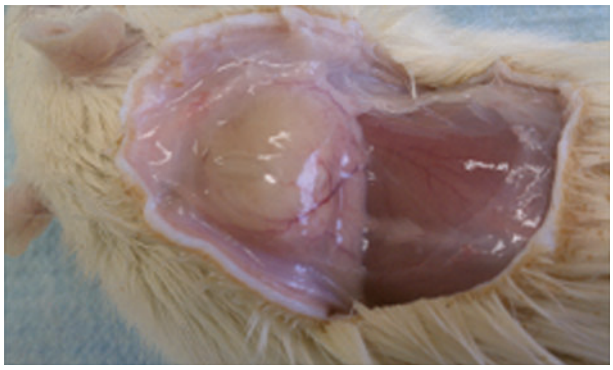
1. Roszell, B., Mondrinos, M.J., Seaton, A., Simons, D.M., Koutzaki, S.H., Fong, G.H., *et al.* Efficient derivation of alveolar type II cells from embryonic stem cells for *in vivo* application. Tissue Eng Part A **15,** 3351, 2009.



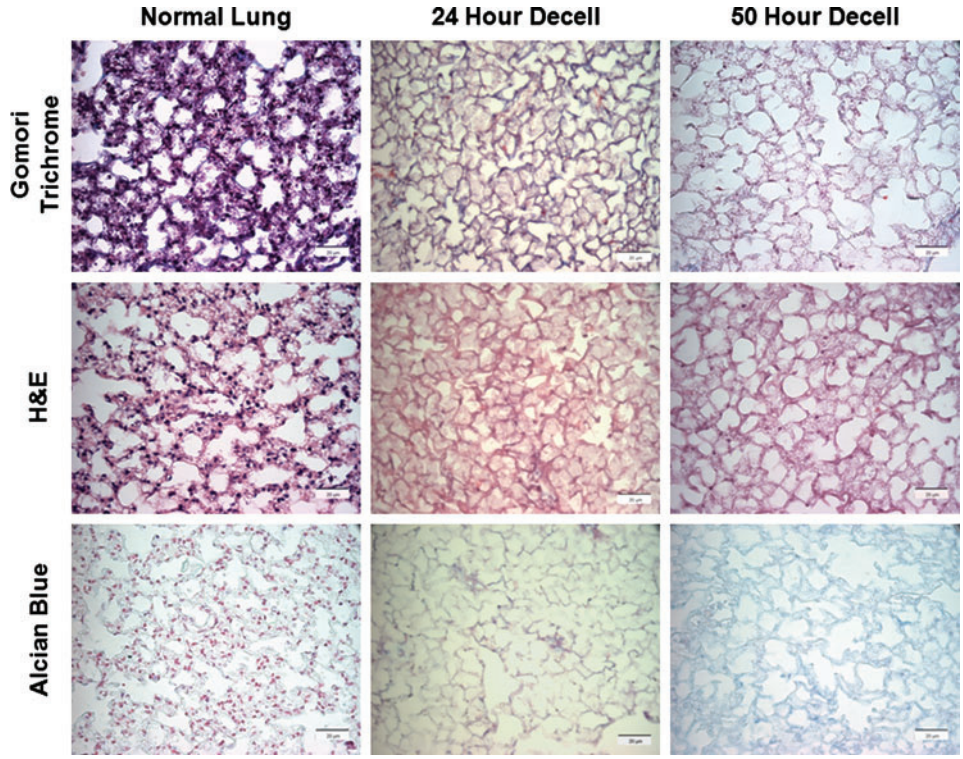
SUPPLEMENTARY FIG. S1. Subcutaneous-implanted construct. Note blood vessel in-growth around the construct.



SUPPLEMENTARY FIG. S2. Schematic of seeding procedure. pro-SPC and pro-surfactant protein C.



SUPPLEMENTARY FIG. S3. Schematic of differentiation protocol.[1]



SUPPLEMENTARY FIG. S4. The 24-h de-cellularization protocol effectively removes cells and maintains architecture, but increases alveolar spacing (higher magnification). Histology of 50-h de-cellularization and 24-h de-cellularization protocols compared to normal lung with gomori trichrome, H&E, and alcian blue staining. Representative images for all conditions are shown (40×magnification). H&E, hematoxylin and eosin.