SUPPLEMENTARY INFORMATION

Supplementary Materials and Methods.

Bleomycin and Napthalene Lung Injury. 6 week old ***Fβ4SC*** mice or littermate controls were instilled intratracheally with saline or 2.3 units/kg of bleomycin (Sigma). Lungs were harvested 5-21 days after injury and either snap frozen in OCT for imaging or used for isolation of AECs. Lung permeability was determined by extravasation of Evans blue dye (1). Total protein (mg/ml) from bronchoalveolar lavage fluid was measured using the BCA assay (Pierce). Each experiment had 4-6 mice per group. In some experiments mice were injected ip with naphthalene (250 mg/kg) dissolved in corn oil and then sacrificed 8 or 14 days later(2).

***Mouse Type II and $\alpha 6\beta 4$ AEC Isolation*.** Isolation of primary AECs was performed initially as previously described(3). Saline perfused mouse lungs were intratracheally infused with Dispase (50units/ml)(Roche) followed by 1% agarose to minimize airway cell contamination. Crude cell suspensions were prepared by mechanical disaggregation of the lung, teasing major airways and vessels from the parenchyma, and then filtered progressively through 70μm, 40μm (BD), and 20μm filters (Spectrum Labs). The recovered cells were subjected to negative selection for hematopoietic cells using biotinylated CD45 and CD16/32 antibodies (BD), and streptavidin-coated biomagnetic beads (Dynal). To obtain high purity of epithelial cells, the suspended cells were labelled with a rat monoclonal anti-mouse E-cadherin, and positively selected using goat anti-rat IGG MACS microbeads and MACS LS cell separation columns (Miltenyi). The E-cadherin positive population was then labelled with a rat monoclonal β4 antibody (clone 346-11a BD) directly conjugated with Alexa 647, and sorted for β4+ and β4- populations using a FACS ARIA Sorter (BD) or MoFlo (DAKO). Dead cells were eliminated with Sytox Blue Dead Cell Stain (Invitrogen).

AEC cultures on Matrigel. Cells were seated on top of chamber slides or tissue culture plates coated with 70% Matrigel, supplemented with 1.5% collagen type 1 and 28.5% Small Airway Basal Medium (Clonetics), or 100 μg/mL fibronectin in PBS. Cells were maintained in Small Airway Growth Medium (Clonetics) without hydrocortisone containing 5% charcoal/dextran-

treated FBS and 10 ng/ml KGF in a 37°C, 5% CO_2.

SPCcreT2rtTA mice. BAC RP23-216B15 (Children's Hospital Oakland Research Institute), which contains the SPC genomic locus(4), was used as a template for PCR amplification of short regions of genomic sequences and recombineering. A "minitargeting" cassette in pBSKII was created by linking an amplicon of SPC homology (371bp) immediately 5' to the SPC stop codon with Cre-ERT2 (gift of P. Chambon) and rtTA-M2 (gift of S.Woods) separated by E2A and T2A sequences respectively using an internal AatII site in the 2A sequences (5-7). A new stop codon was inserted in the rtTA-M2 sequence prior to subcloning in pBSKII. A FRT-flanked neomycin resistance gene (fNEO-R; from recombineering plasmid PL451) and a 3' amplicon of SPC homology (333bp) immediately downstream of the stop codon completed the minitargeting vector. The completed targeting vector was removed from pBSKII and transformed into SW105 E coli strain containing the BAC followed by transient cre induction with L-arabinose to initiate recombination (see http://recombineering.ncifcrf.gov for details). DNA from bacterial clones containing the corrected targeted insert in the SPC gene were retrieved into a shuttle vector by "gap repair" using short genomic sequences spanning ~ 10kb of genomic DNA comprised of a 8kb 5' homology arm, the targeted region, and 1.5 kb 3' homology arm as indicated in Fig 6A. Linearized DNA containing the targeting vector was then electroporated into 129/C57bl6 hybrid ES cells (iGeniousTL, Inc., Stony Brook, NY). ES cell DNA was screened on both ends by Southern blotting (probe sites indicated in Fig 6A) and correctly targeted ES cells (Fig 6B) expanded and normal karyotype cells used to inject C57bl6 mice and achieve germline transmission. Mice were genotyped with the following primers: rtTA forward: 5'TCGCCTTCTATCGCCTTCTTG3' and wt reverse: 5' CCTTTTGCTCTGTTCCCCATTA3' (3' arm); wt forward: 5' TGGTTCCGAGTCCGATTCTTC3' (exon 5) with same wt reverse primer. Functionality of the mutant SPC allele was validated by immunoblotting of homozygous mice for pro-SPC protein and by crossing the mutant mice with the *loxp*-tm-TR/GFP reporter strain (Fig 6).

SUPPLEMENTARY FIGURES AND FIGURE LEGENDS.

Figure S1

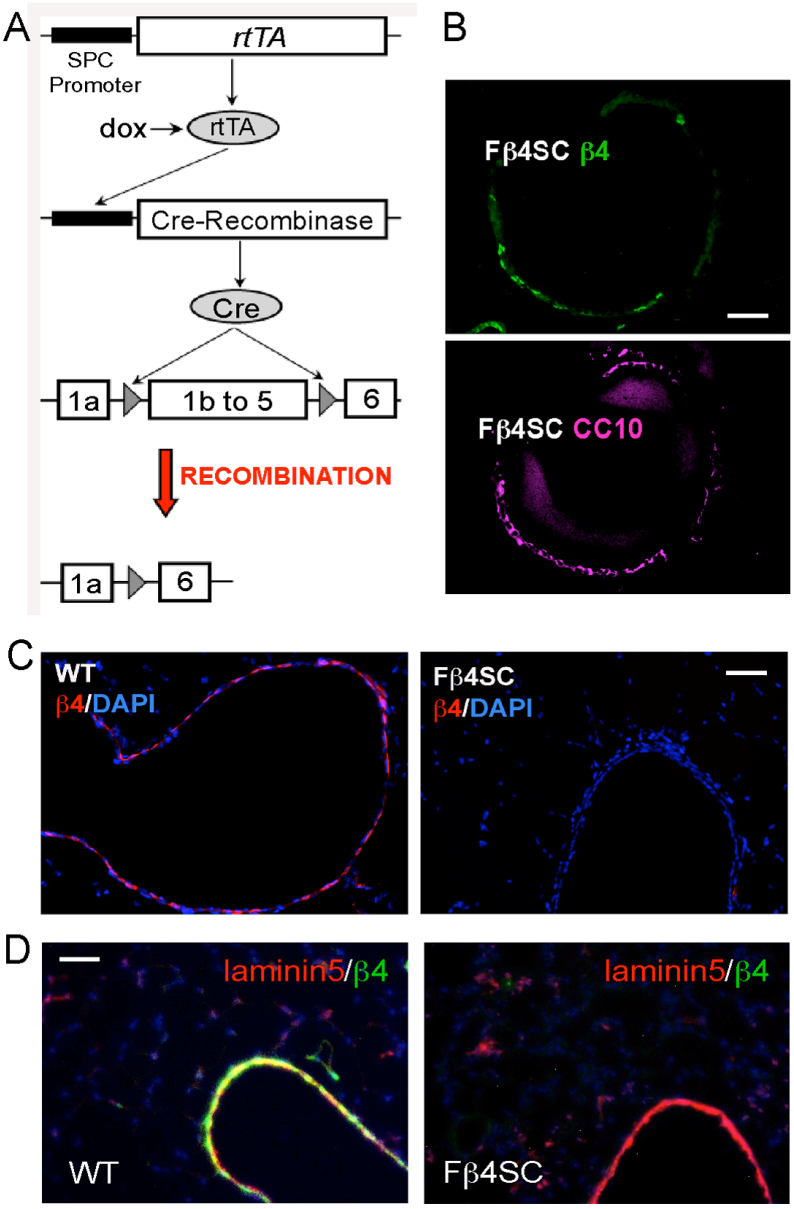

Figure S1. Generation of lung epithelial cell specific β4 null mice. (**A**) Schematic of strategy togenerate lung epithelial specific deletion of integrin β4, termed Fβ4SC mice. Triple transgenic mice and littermate controls were exposed to doxycycline (dox) throughout gestation and dox removed at the time of weaning. (**B**) β4 and CC10 staining of WT and Fβ4SC lung sections. (**C**) β4 immunostaining of WT and Fβ4SC lung sections illustrating near complete loss of β4 in mutant mice. (**D**) Co-localization of β4 with laminin-5. Scale bars = 50 μm.

Figure S2

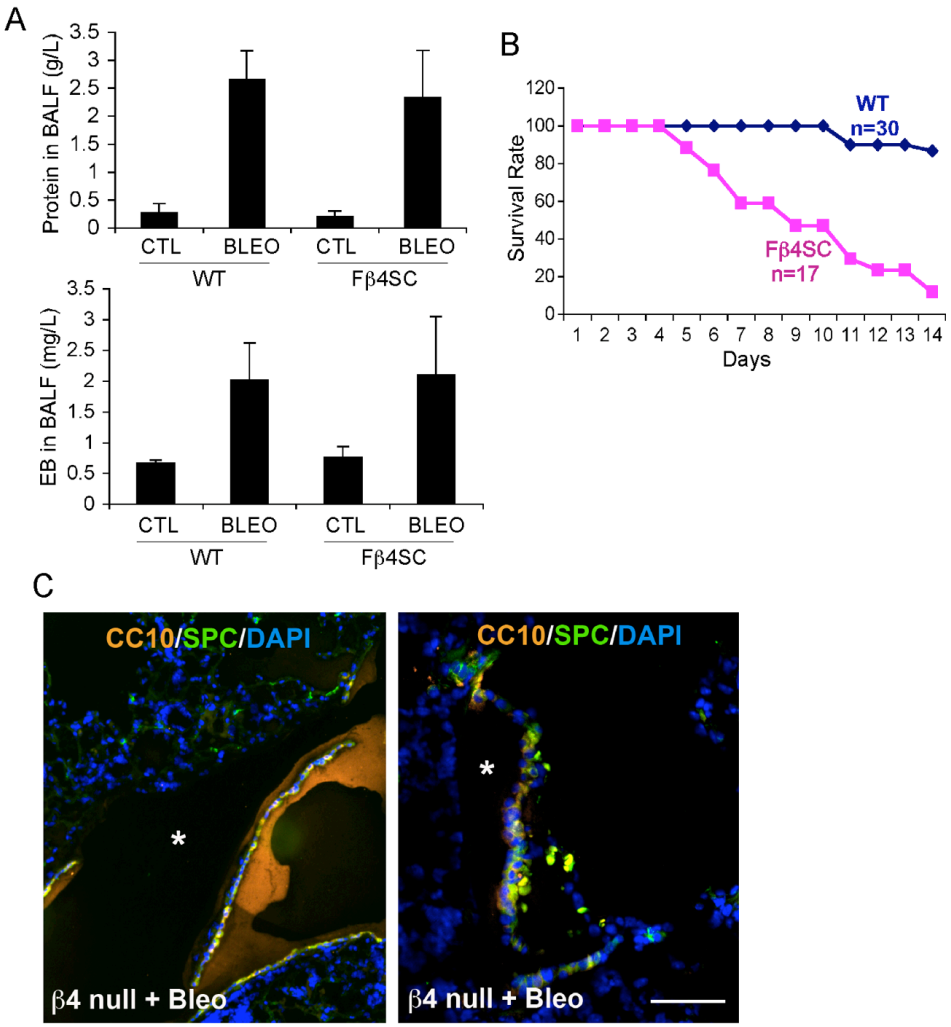

Figure S2. Fβ4SC mice succumb to injury after bleomycin. (**A**) Total protein and Evans blue dye (EB) in BAL fluid 5 days after saline (CTL) or bleomycin (BLEO) injection are not different between WT and Fβ4SC mice. (**B**) Fβ4SC mice die at a faster rate than WT mice after bleomycin injection. (**C**) Immunostaining of CC10 (orange), SPC (green), and nuclei (blue) in unlavaged lungs of Fβ4SC mice at the time of death 11 days after bleomycin injection. Asterisks indicate regions of sloughed epithelial cell monolayers. Scale bar = 50 μm.

Figure S3

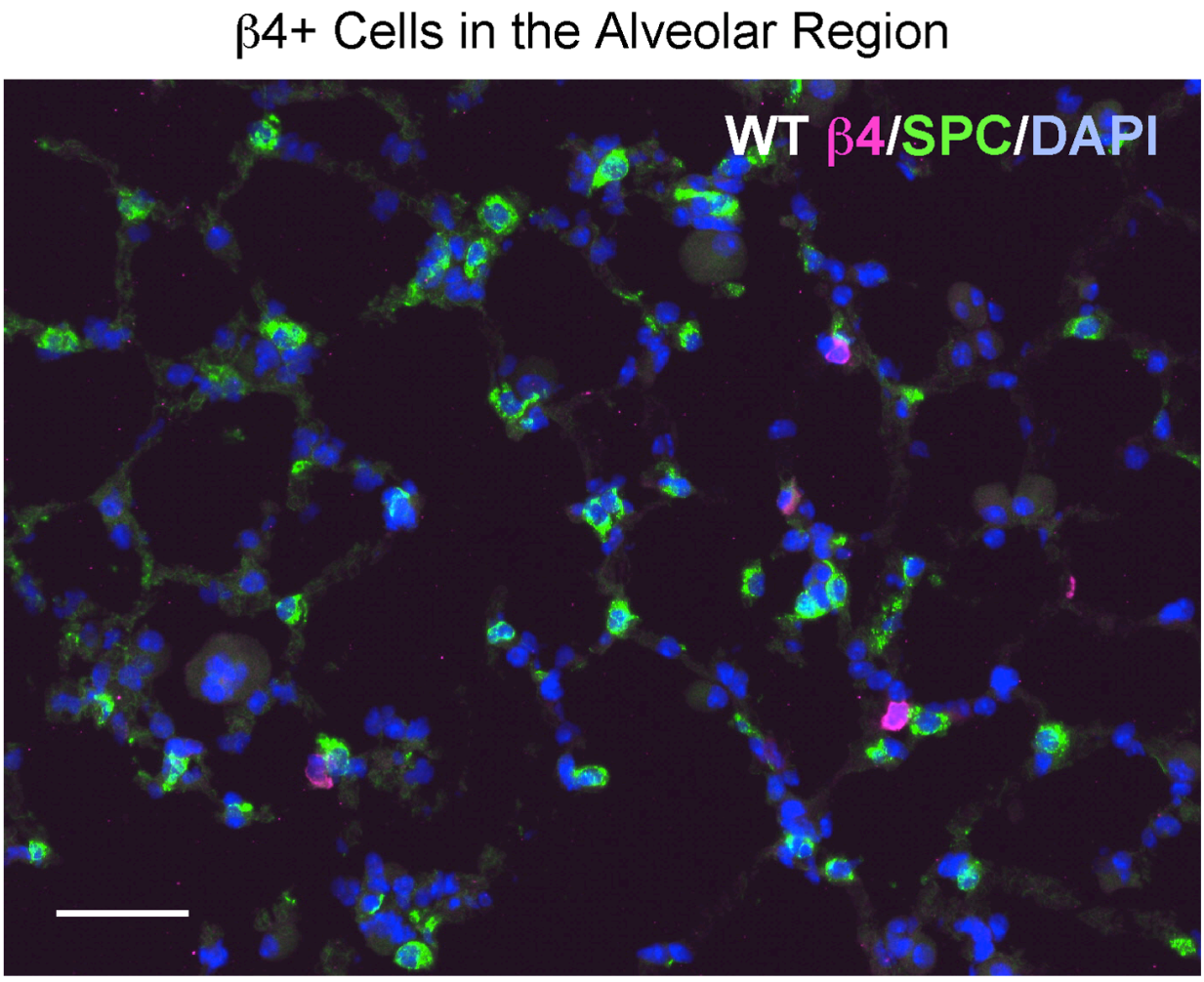

Figure S3. Enlargement of Fig 1C bottom right panel depicting β4+ cells in the alveolar region. Scale bar = 50 μm.

Figure S4

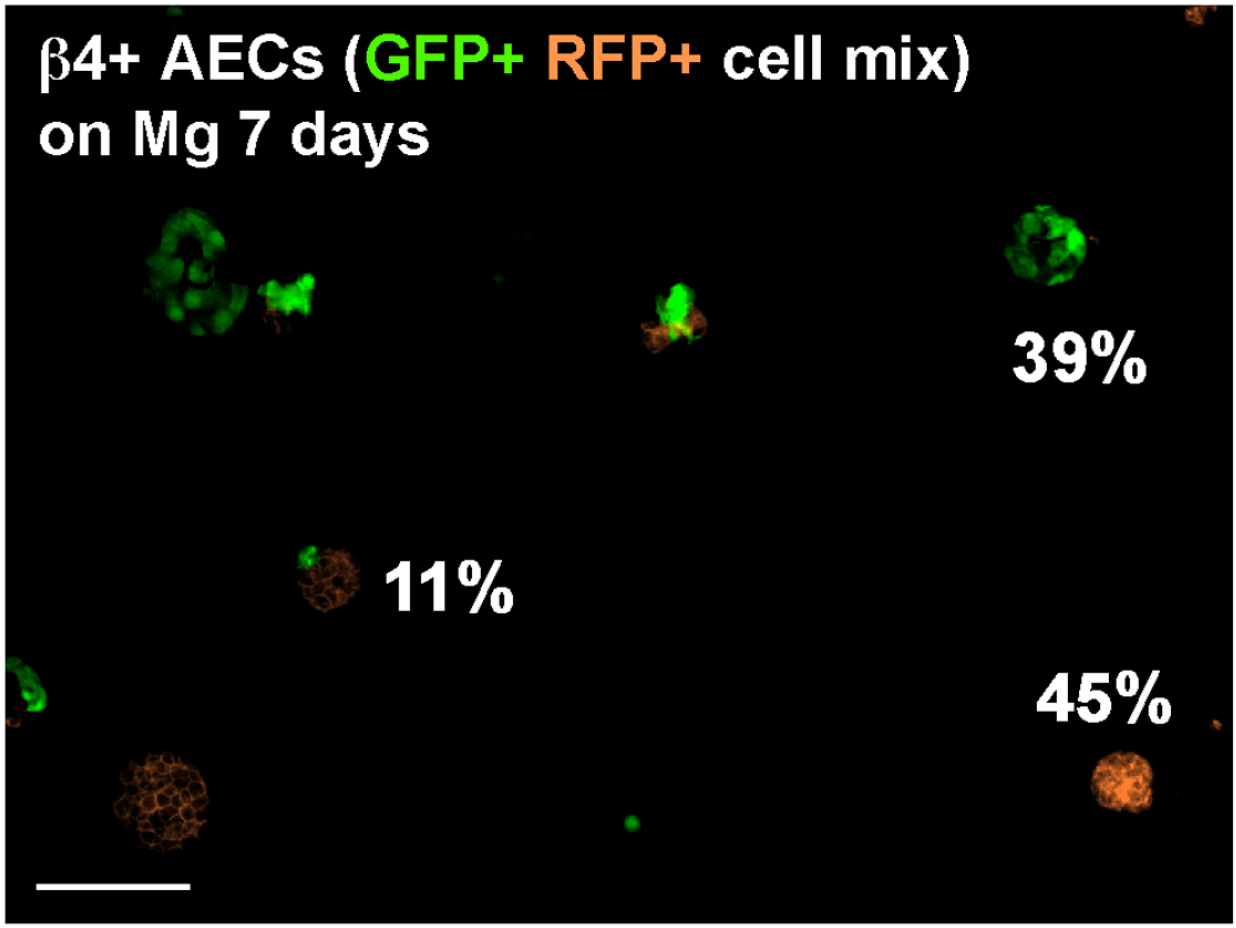

Figure S4. Clonal growth of β4+ AECs. Purified β4+ cells expressing either GFP or RFP were mixed and placed in culture on Matrigel for 7 days. Colonies (n = 65) were entirely exclusive GFP+ or RFP+ indicating clonal expansion and not simple aggregation of cells. About 10% of the colonies touched as shown in the figure but none of the spherical colonies had mixing of red and green cells. Scale bar = 100 μm.

Figure S5

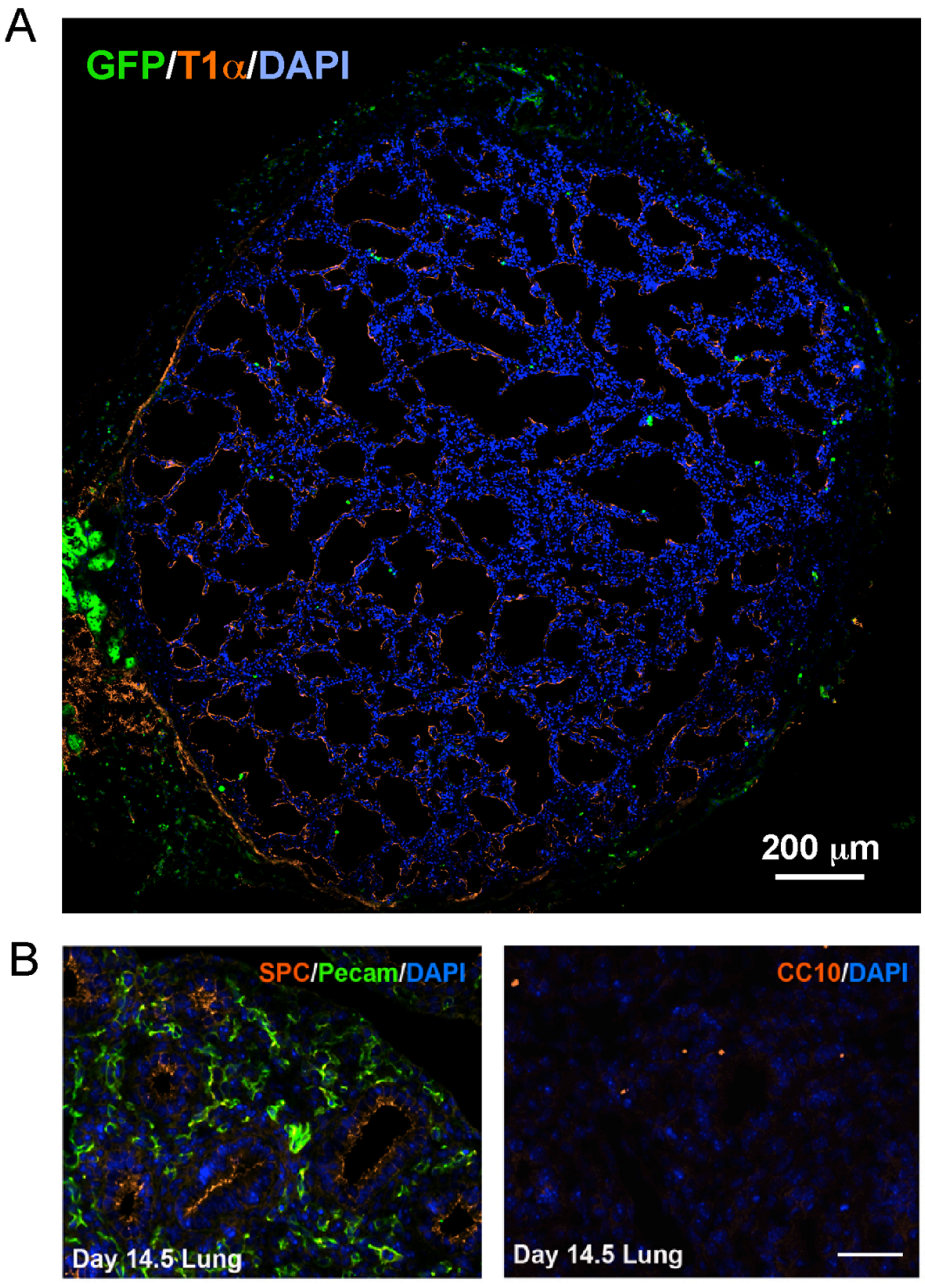

Figure S5. (**A**) Low power view of 5 day organoid developed from admixture of embryonic lung cells and β4 – AECs from GFP+ mice as described in the text. Multiple 20X images were captured and tiled into a single mosaic image. The section is stained with DAPI (blue) and antibodies to T1α (red). Single GFP+ cells are present in the organoid but no GFP+ clusters or structures develop in the β4 – population. (**B**) Distribution of Pecam-1 and SPC (left) and absence of CC10 staining in E14.5 lungs. Scale bar = 50 μm.

Figure S6

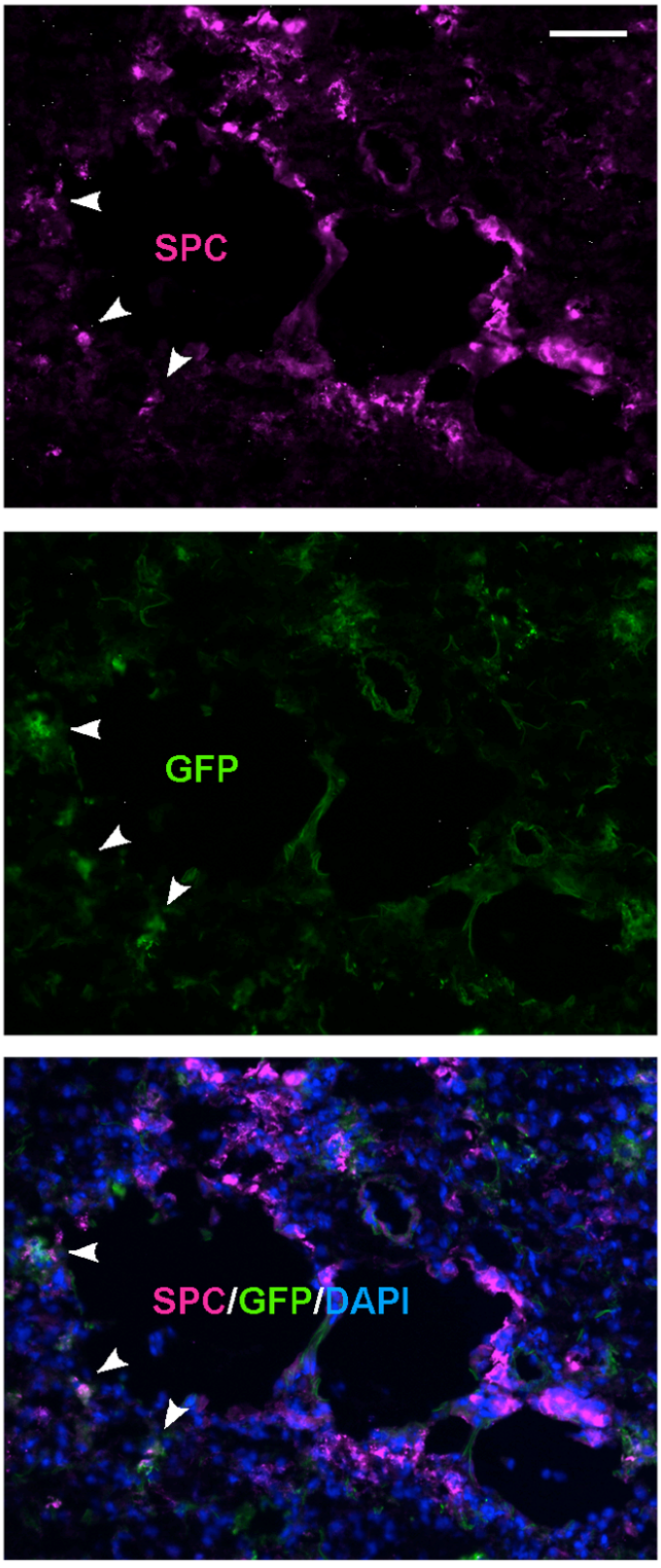

Figure S6. Few GFP+ cells among SPC+ type II cells lining alveoli of fibrotic area of bleomycin injured lungs. Single channel and merged images of pro-SPC staining (red) and GFP fluorescence (green) of injured lung area of SPCcreT2/loxp-tm-TR/GFP mice 30 days after tamoxifen and 14 days after intratrachael bleomycin injection. Almost all of the SPC+ cells lining alveoli of this injured area have no GFP fluorescence indicating the cells likely did not develop from mature type II cells existing at the time of injury. A few GFP+SPC+ cells are present in the far left of the photograph (see arrows). Scale bar = 50 μm.

Figure S7

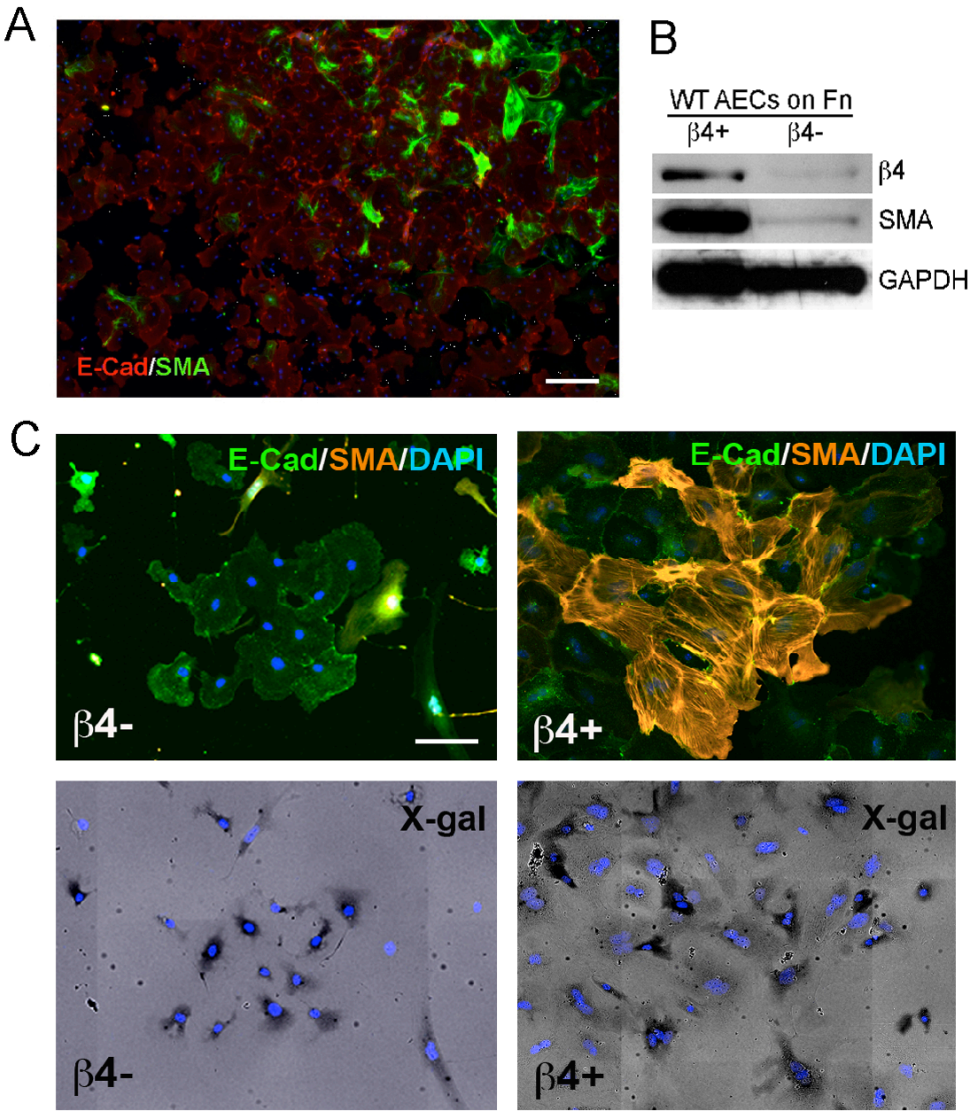

Figure S7. β4+ AECs preferentially undergo EMT when cultured on Fn. (**A**) Immunostaining for E-cadherin (red) or α-SMA (green) in purified AECs plated for 6 days on Fn. AECs were purified by standard methods followed by flow cytometry for E-cadherin expression. EMT develops only in a subfraction of AECs. Multiple 20X images were captured and tiled into a single mosaic image. Scale bar = 200 μm. (**B**) Immunoblot confirming α-SMA preferentially develops in β4+ AECs. (**C**) *Upper panels*. EMT (α-SMA+, E-cadherin-) develops mainly in the β4+ fraction of AECs. *Lower panels*. X-gal staining of the same cells shown in the upper panels to confirm epithelial origin (see Ref 26). Scale bar = 100 μm.

Supplemental References

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