Integrin β6 Mediates Phospholipid and Collectin Homeostasis by Activation of Latent TGF-β1

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Online Data Supplement

Immunohistochemistry staining for surfactant protein A (SPA) and D (SPD) – Formalin-fixed, paraffin embedded lung tissue was cut in 5 μm sections and mounted on polylysine-coated glass slides. The lung sections were deparafinized in xylene washes and rehydraded by incubating in graded alcohol solutions from 100% down to 70% and then placed in PBS. Antigen retrieval was performed using boiling sodium citrate (Antigen Unmasking Solution, Vector Laboratories, Inc., Burlingame, CA, USA). Sections were then blocked with 3% hydrogen peroxide in PBS for 5 minutes, washed in PBS, then incubated in 5% goat serum in TBS/T solution (20 mM Tris, 100 mM NaCL, 0.05% Tween 20) for 1-2 hours. Primary polyclonal antibody (diluted 1:1000 in TBS/T) for rabbit anti-human SPA or rabbit anti-mouse SPD (Chemicon International, Temecula, CA, USA) was incubated on lung sections for 1 hour at room temperature. Sections were washed three times with TBS/T and then incubated for one hour at room temperature with 1:200 diluted biotinylated goat anti-rabbit IgG (BA-100, Vector Laboratories, Inc., Burlingame, CA, USA). On each slide, there were two sections of lung tissue from each mouse. The second section was used as a control for nonspecific binding by adding only secondary antibody and no primary antibody. After incubation with the secondary antibody, the sections were washed three times with TBS/T and then incubated with the streptavidin complex (ABC reagent, Vector Laboratories, Inc., Burlingame, CA, USA) for 30 minutes at room temperature, followed by three final wash steps. Staining was performed using Dab reagent (Vector Laboratories, Inc., Burlingame, CA, USA) and counterstained with methyl green (Vector Laboratories, Inc., Burlingame, CA, USA) for 10 minutes. Slides were mounted and visualized under oil immersion. Lung sections from *Itgb6*$^{-/-}$ mice and wild-type littermates that were incubated only with secondary antibody revealed no nonspecific staining (data not shown).

Supplemental Data References:

E1. Morris, D. G., X. Huang, N. Kaminski, Y. Wang, S. D. Shapiro, G. Dolganov, A. Glick, and D. Sheppard. 2003. Loss of integrin avb6-mediated TGF-b activation causes Mmp-12-dependent emphysema. *Nature* 422(6928):169-73.

SUPPLEMENTAL FIGURE LEGENDS

Figure E1. Representative cytospins of BAL fluid from 2-month-old (**a**) wild-type and (**b-c**) *Itgb6*$^{-/-}$ mice carrying a lung-specific tetracycline-inducible transgene for constitutively active TGFβ1. Dox = doxycycline treatment which was fed to the mice starting at 3 weeks of age and continued until the mice were sacrificed at 2 months of age.

Figure E2. 2-month-old *Smad3*-deficient mice demonstrate normal levels of surfactant constituents. Total phospholipid and surfactant proteins A (SPA) and D (SPD) in BAL fluid from wild-type (*Smad3*$^{+/+}$) and *Smad3*-deficient (*Smad3*$^{-/-}$) littermate mice are expressed as mean +/- SEM (N = 5-9 per group).

Figure E3. Surfactant constituents are increased in BAL fluid from 2-month-old *Itgb6*$^{-/-}$ mice on a 129 genetic background. Total phospholipid and surfactant proteins A (SPA) and D (SPD) in

BAL fluid from wild-type (WT) and integrin β6-deficient (*Itgb6*$^{-/-}$) littermate mice. Data are expressed as mean +/- SEM (N = 5 for WT and 3 for *Itgb6*$^{-/-}$; * P ¡Ü 0.002).

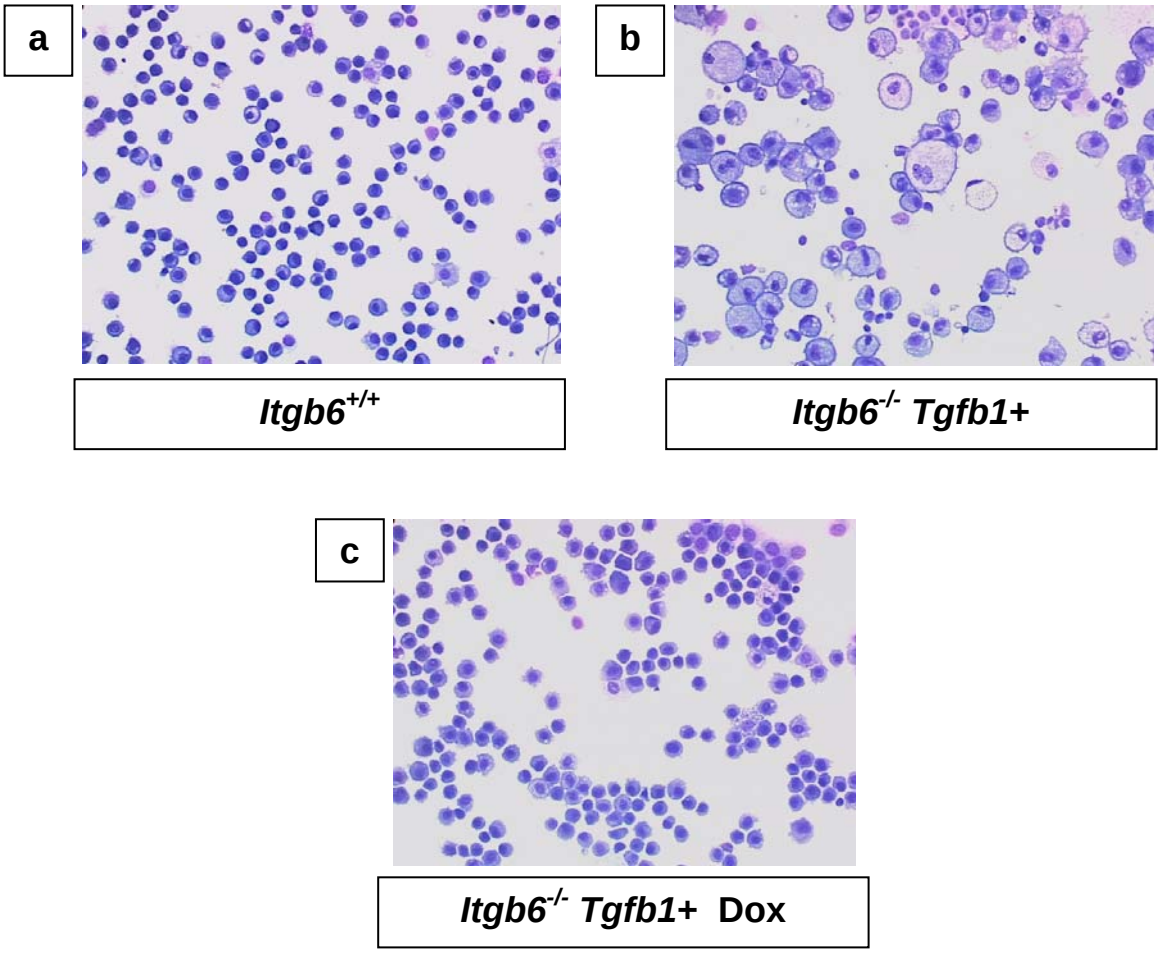
Figure E4. Alveolar epithelial type II cells in 6-month-old wild-type mice. Representative high power microscopic images show varying sizes of darkly staining lamellar bodies. Arrows indicate type II cells. Magnification 63X.

Figure E5. Alveolar epithelial type II cells in 6-month-old *Itgb6*$^{-/-}$ mice. Representative high power microscopic images show varying sizes of darkly staining lamellar bodies. Arrows indicate type II cells. Magnification 63X.32

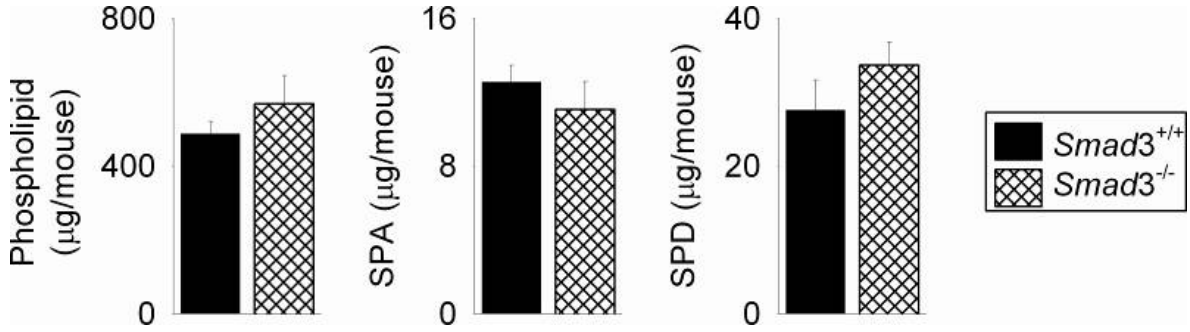
Figure E6. Surfactant protein A identified by immunohistochemical staining in *Itgb6*$^{-/-}$ and wildtype littermates. Brown staining indicates surfactant protein A immunoreactivity. Magnification 63X.

Figure E7. Surfactant protein D identified by immunohistochemical staining in *Itgb6*$^{-/-}$ and wildtype littermates. Brown staining indicates surfactant protein D immunoreactivity. Magnification 63X.

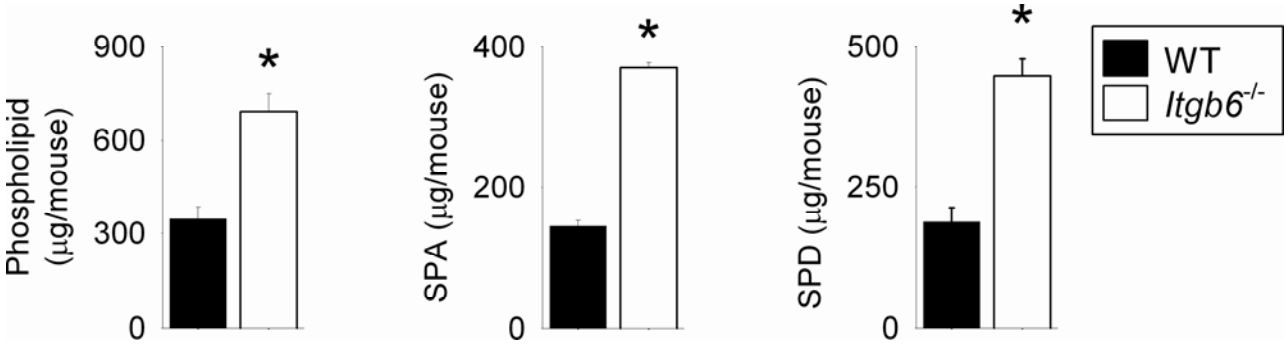
Figure E8. BAL cells from 2-month-old *Smad3*-deficient mice have increased matrix metalloproteinase-12 mRNA transcript levels. Data are normalized to Gapdh levels and expressed as mean +/- SEM (N = 9-12 per group; * P ¡Ü 0.005). Conversion of cycle threshold to fold change reveals ~8-fold increase in transcript levels in *Smad3*-deficient mice compared to littermate controls.



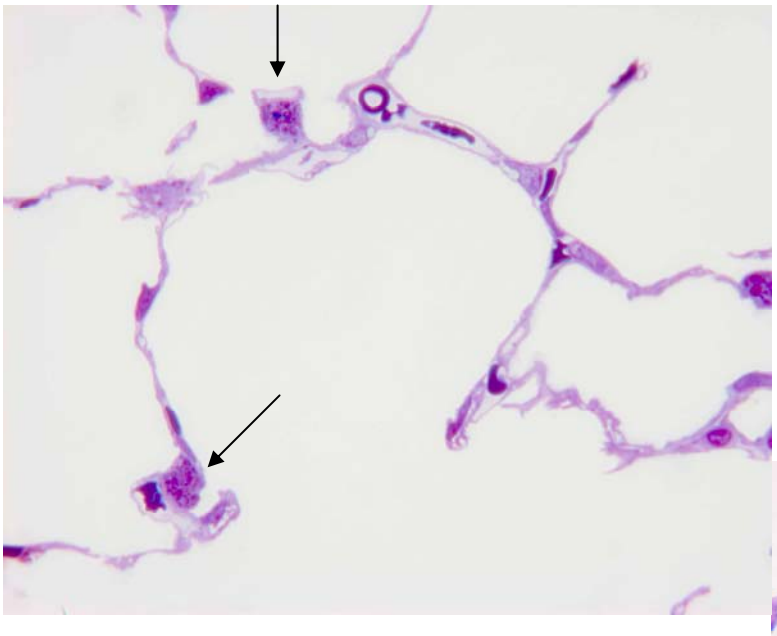
SUPPLEMENTAL FIGURE E1

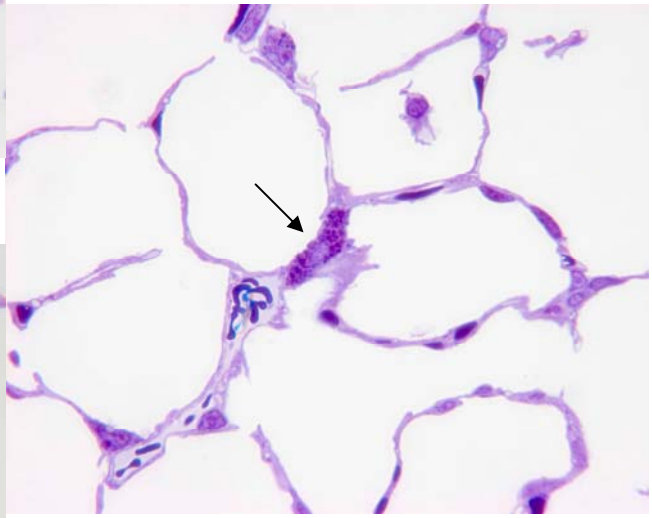


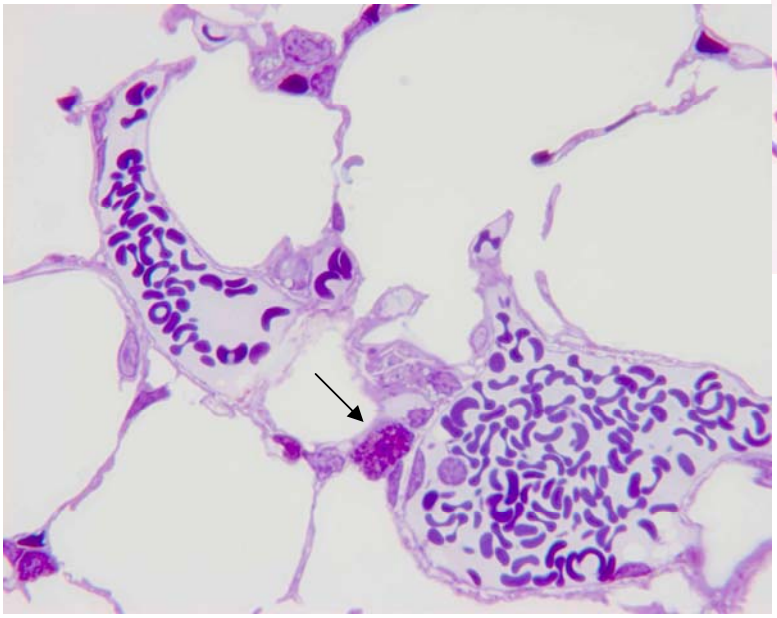
SUPPLEMENTAL FIGURE E2



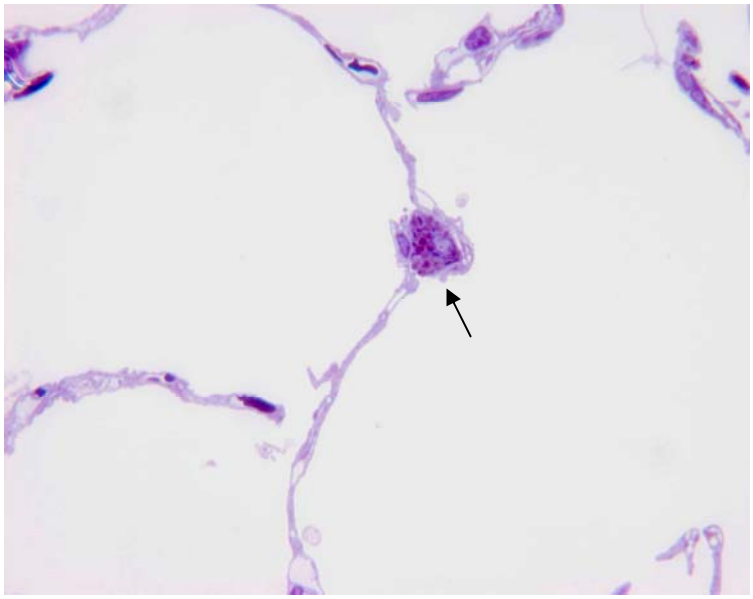
SUPPLEMENTAL FIGURE E3

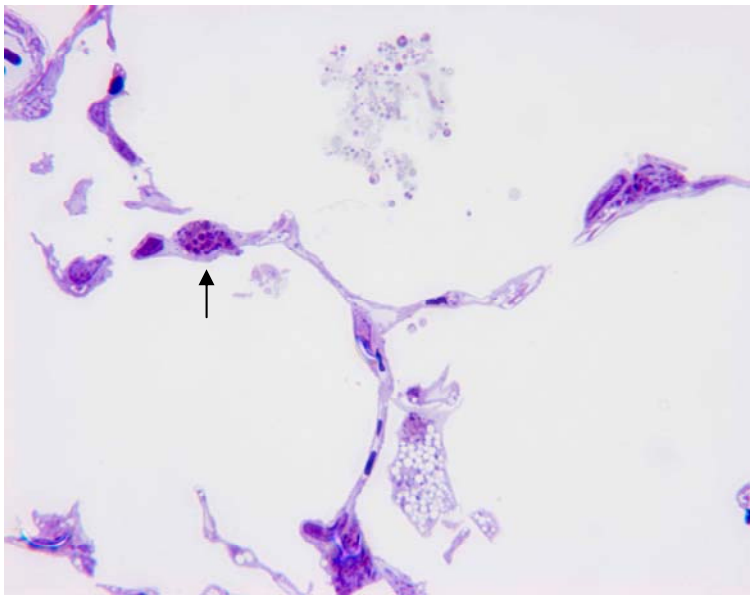


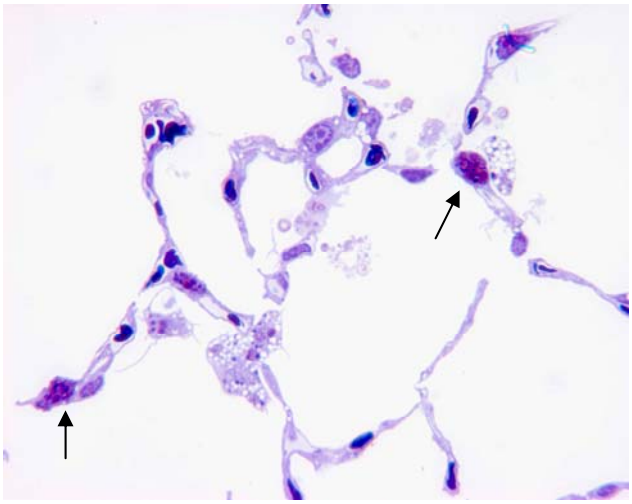




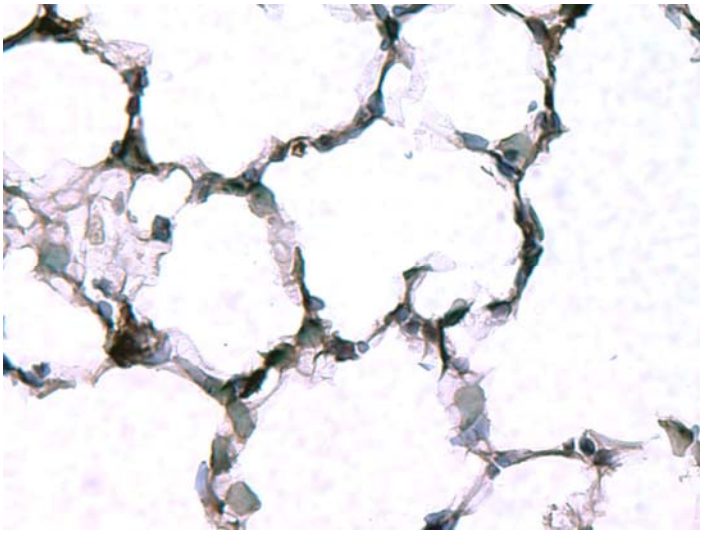
SUPPLEMENTAL FIGURE E4



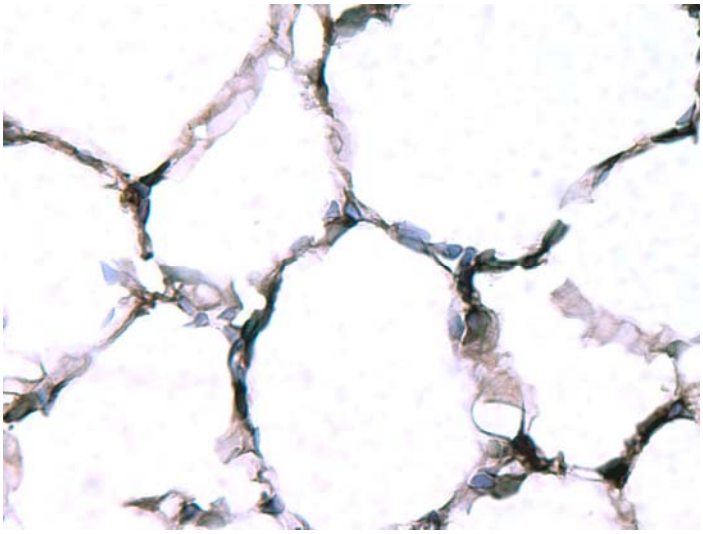




SUPPLEMENTAL FIGURE E5

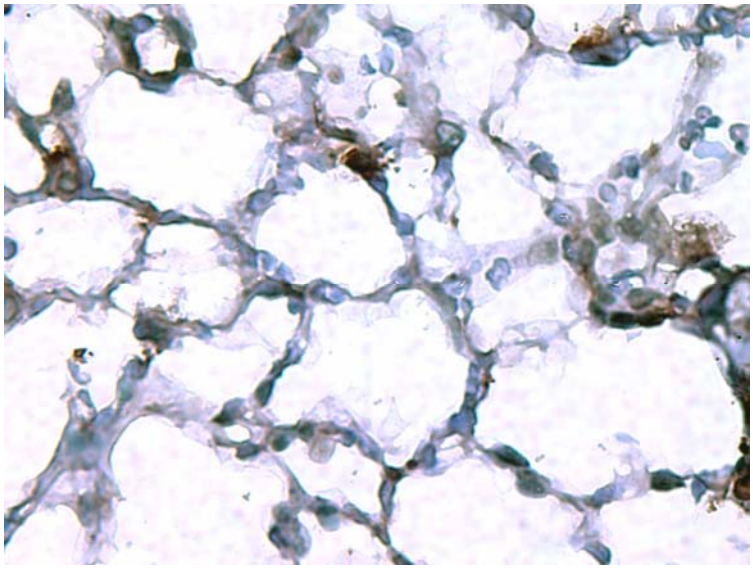


Itgb6$^{+/+}$



Itgb6$^{-/-}$

SUPPLEMENTAL FIGURE E6



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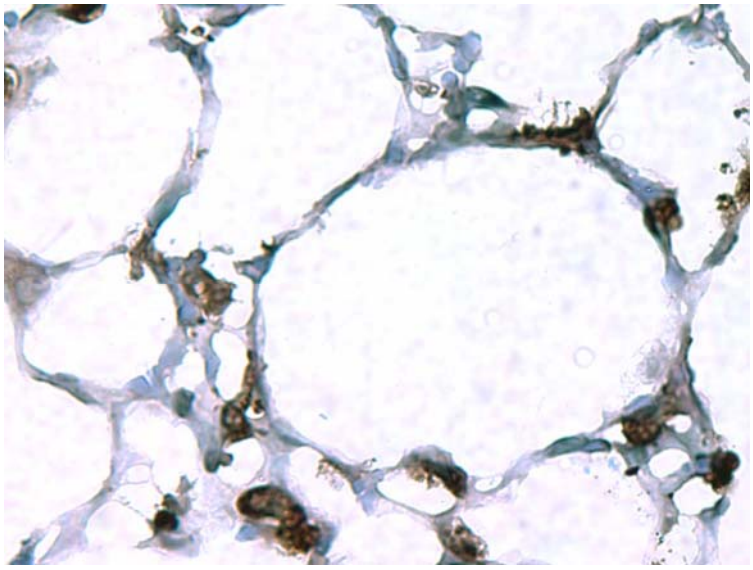
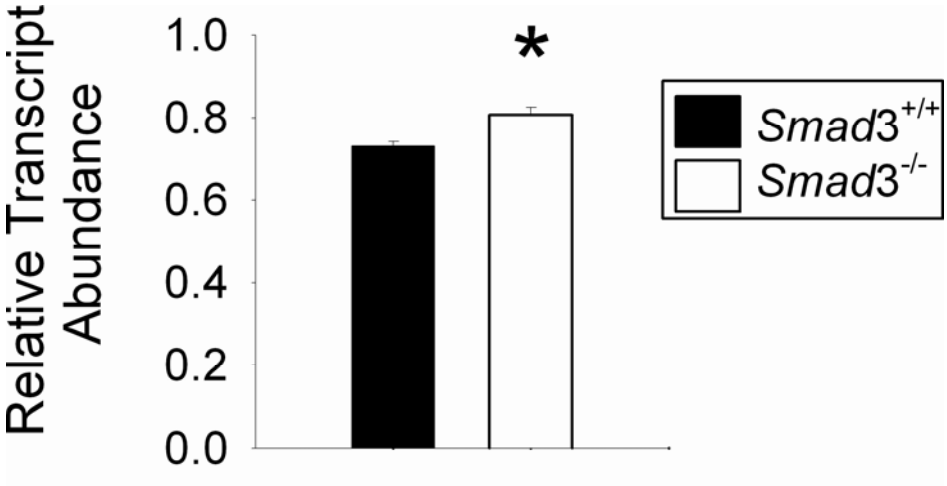


Itgb6$^{-/-}$

SUPPLEMENTAL FIGURE E7



SUPPLEMENTAL FIGURE E8

Supplemental Table E1: Primers and Probes Used for qRT-PCR*

Gene	Primer Title	Primer Sequence (5' to 3')
Surfactant A	m-Sftpa1-160-TMF	TGCAATGGGACAGAAGTTTGTG
	m-Sftpa1-234-TMR	TCTGCCGGGCAAACCA
	m-Sftpa1-197-TMP	TCCCTGGCACTCCCGGAAACC
	m-Sftpa1-100-RTF	GCCATGTCACTAGGCTCTTTGG
	m-Sftpa1-435-RTR	GAGTGCAGTCTGAAGCTCCTCAT
Surfactant C	m-Sftpc-RTF	GGGCCTCCACATGAGTCAAA
	m-Sftpc-RTR	GTTCCTGGAGCTGGCTTATAGG
	m-Sftpc-TMF	GGAGCACCGGAAACTCAGAA
	m-Sftpc-TMP	CGCCTAGCCCCGAGTGAGCG
	m-Sftpc-TMR	GGAAAAGGTAGCGATGGTGTCT
Surfactant D	m-Sftpd-138-TMF	CACCTGCACCCTAGTCATGTGT
	m-Sftpd-227-TMR	TTCTCACCCCGTGGACCTT
	m-Sftpd-178-TMP	CTGCCTGGTCGTGATGGACGGG
	m-Sftpd-59-RTF	CCATGCTTGTCTTGCTTGTACAG
	m-Sftpd-518-RTR	CCTTTTGCCCCTGTAGATCCT
GAPDH	m-GAPDH-RTF	CATGGCCTTCCGTGTTCCTA
	m-GAPDH-RTR	GGTCCTCAGTGTAGCCCAAGAT
	m-GAPDH-TM769F	TGTGTCCGTCGTGGATCTGA
	m-GAPDH-TM793P	CCGCCTGGAGAAACCTGCCAAGTATG
	m-GAPDH-TM845R	CCTGCTTCACCACCTTCTTGAT

TMF = Taqman forward amplification primer
TMR= Taqman reverse amplification primer
TMP = Taqman probe
RTF = preamplification forward primer
RTR = preamplication reverse primer
*Primers and probes used for Mmp12 qRT-PCR in Supplemental Figure E7 have been previously published (1)