Supplemental data:

Supplementary methods

Lung histological and morphometric analyses.

For histologic examinations, the lungs of 3 days, 10 days, 2 months and 6 months old *nrf2* +/+ and -/- mice (n = 3) were inflated with 0.5% agarose under 25 cm water pressure, fixed in 10% buffered formalin for 24 h. Lung processing and morphometry was performed as described in the Methods section of the text and outlined in detail elsewhere (1). The lung tissues were embedded in paraffin and multiple tissue sections (5 μm) were stained with Verhoeff and Hart's stain and Reticulin II staining Kit (at the Johns Hopkins Hospital Division of Surgical Pathology, Johns Hopkins Hospital, Baltimore, USA) to characterize elastic fibers and collagen (preferentially type III), respectively.

Total lung capacity.

To measure the total lung capacity, each animal (2 months old) was weighed and anesthetized with intraperitoneal injection of sodium pentobarbital (75 mg/kg body weight). A tracheostomy was performed and the lungs were ventilated with 100% oxygen. After 10 minutes, the tracheal cannula was occluded for an additional 5 minutes to de-gas the lungs. Immediately after de-gassing, quasistatic lung pressure volumes curves were performed in situ with the rate of inflation and deflation standardized by using an infusion/withdrawal pump (model 22, Harvard Apparatus, Dover, MA). Airway pressure (using differential pressure transducer, model 8510B-2, Endevco Corporation,

San Juan Capistrano, CA, USA) and lung volume (using linear displacement transducer, model 0244, Trans – Tek Incorporated, Ellington, CT, USA) were measured during inflation and deflation and the initial inflation rate was low (0.009 ml/s) to ensure that initial lung recruitment from the degassed state did not result in excessive pressures. Once a pressure of 30 cmH_2O was reached, the flow rate was increased to 0.035 ml/s for the remaining inflation and deflation maneuvers. Total Lung Capacity (TLC) was determined as the volume at $30cmH_2O$ (2, 3) .

Proliferating cell nuclear antigen immunohistochemistry and quantification.

The PCNA immunostaining of the lung sections from the air-exposed (6 months) *nrf2* +/+ and -/- mice were performed by following the procedure described earlier (4). The number of PCNA positive cells in the lung sections (n = 5 per group and 10 fields/lung section) were counted manually and normalized by alveolar length.

Transmission electron microscopy.

Transmission electron microscope was used to characterize the lungs of air exposed *nrf2* +/+ and -/- mice (n = 3) at the ultrastructural level. The lungs from the 3 days, 10 days, 2 months and 6 months old mice were cut into small pieces and fixed in 3% gluteraldehyde and then post-fixed with 1% osmium tetroxide. The sections were stained en bloc in 2% uranyl acetate, dehydrated in an ascending series of ethanol, and embedded in 100% Spurrs resin. Ultrathin sections (70-90 nm) were counterstained with lead citrate and examined using a Philips CM 120 transmission electron microscope (FEI Corporation Hillsboro, OR, USA).

Neutrophil and Lymphocyte immunohistochemistry and quantification.

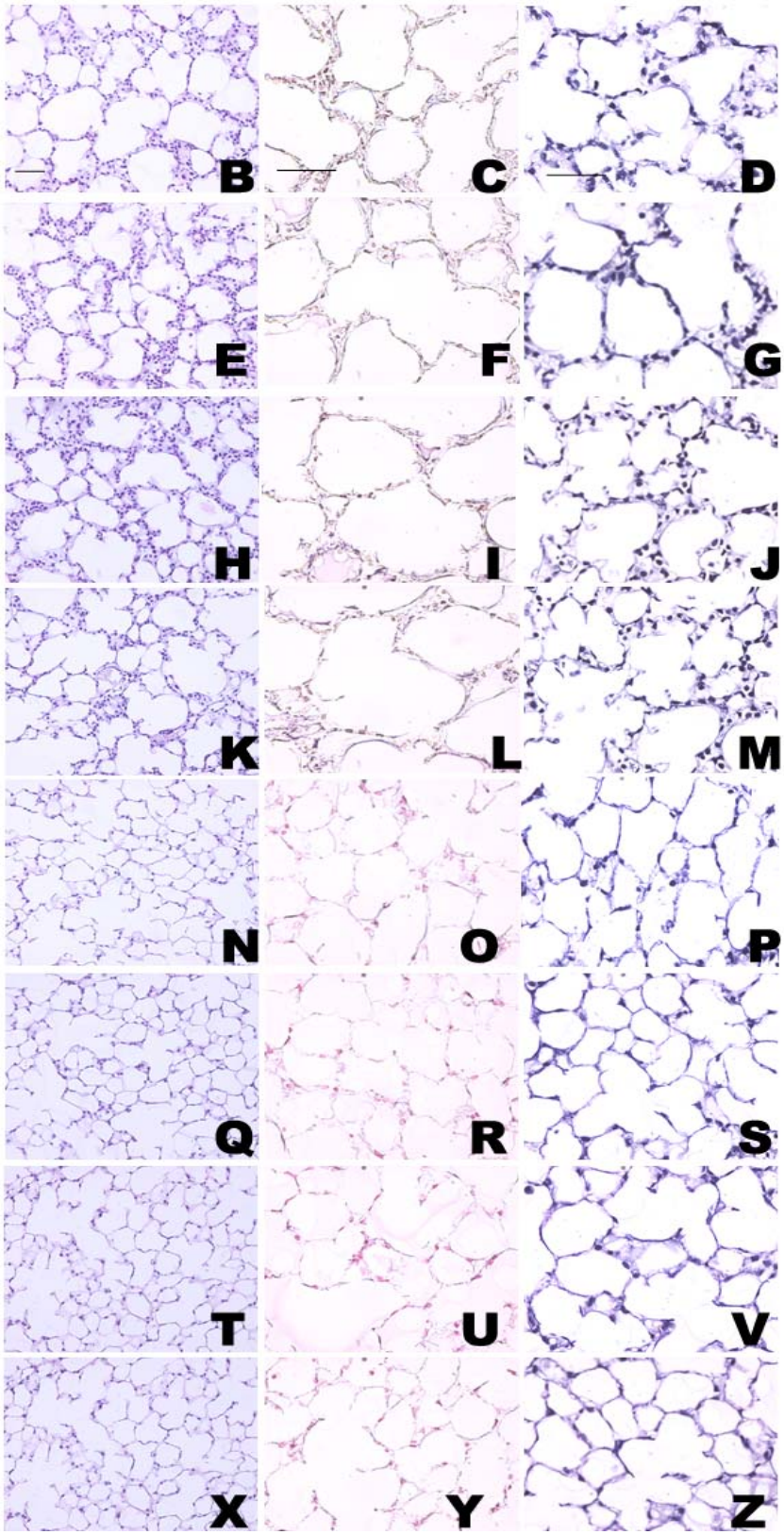
The neutrophils were identified using rat anti-mouse neutrophil antibody, biotinalated anti-rat secondary antibody and HRP-streptavidin complex. Lymphocytes were identified using rat anti-mouse CD45R primary antibody, biotinalated anti-rat secondary antibody and HRP-streptavidin complex. The number of neutrophils and lymphocytes in the lung sections (n = 3 per group and 10 fields/lung section) were counted manually and normalized by alveolar length.

Supplementary references

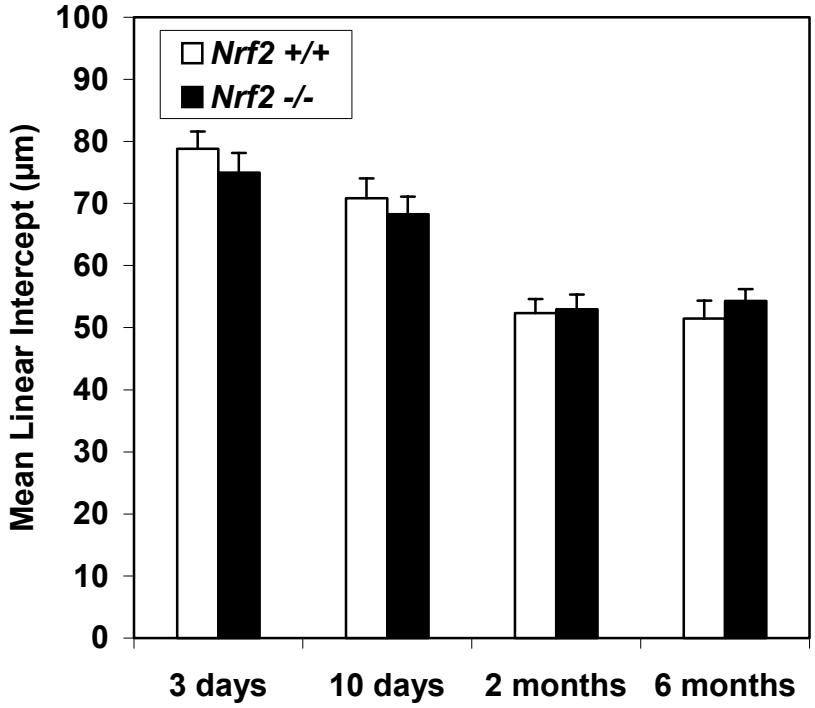
1. Tuder, R.M., Zhen, L., Cho, C.Y., Taraseviciene-Stewart, L., Kasahara, Y., Salvemini, D., Voelkel, N.F., and Flores, S.C. 2003. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 29:88-97.
2. Mitzner, W., Johnson, J.W., Beck, J., London, W., and Sly, D. 1982. Influence of betamethasone on the development of mechanical properties in the fetal rhesus monkey lung. *Am Rev Respir Dis* 125:233-238.
3. Soutiere, S.E., and Mitzner, W. 2004. On defining total lung capacity in the mouse. *J Appl Physiol* 96:1658-1664.
4. Kasahara, Y., Tuder, R.M., Taraseviciene-Stewart, L., Le Cras, T.D., Abman, S., Hirth, P.K., Waltenberger, J., and Voelkel, N.F. 2000. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 106:1311-1319.

Supplementary Figure 1. Lung histology (hematoxylin-eosin, right panel), lung reticulin (reticulin stain, middle panel), and lung elastin (Verhoeff stain, left panel) of postnatal day 3 (B-G), day 10 (H-M), 2 months old (N-S) and 6 months old (T-Z) lungs of *nrf2* +/+ (B-D, H-J, N-P and T-V) or *nrf 2* -/- (E-G, K-M, Q-S and X-Z) mice **(A)**. Note that similar pattern of maturation between the wild-type and knockout lungs, with thinning of reticulin and elastin frameworks in adult mouse lungs (O, P, R, S, U, V, Y and Z). These changes are paralleled by a concordant remodeling of the thin-threaded reticulin (which stains mostly collagen types III and II) and of elastin in both mice (right panel, Bar = 25 μm; middle and left panels, Bar = 25 μm). Images of the H&E stained lung sections were acquired with a 20X lens and reticulin and Verhoeff van Gieson stained lung sections were acquired with a 40X lens. Lung morphometric measurements revealed no statistically significant differences in mean linear intercept **(B)** and alveolar diameter **(C)** in the lungs of age-matched *nrf2* +/+ and -/- mice (ANOVA on ranks, Dunn's posthoc multiple comparison test). **(D)** Immunohistochemical staining with anti-PCNA antibodies revealed that there were no significant differences in cell proliferation in *nrf2* +/+ lungs (16.1 ± 1.7 PCNA positive cells/mm alveolar length) when compared with *nrf2* -/- lungs (16.1 ± 1.1 PCNA positive cells/mm alveolar length) under room air condition for 6 months.Anti-PCNA reactive cells are indicated by arrows. Magnification, 40X.

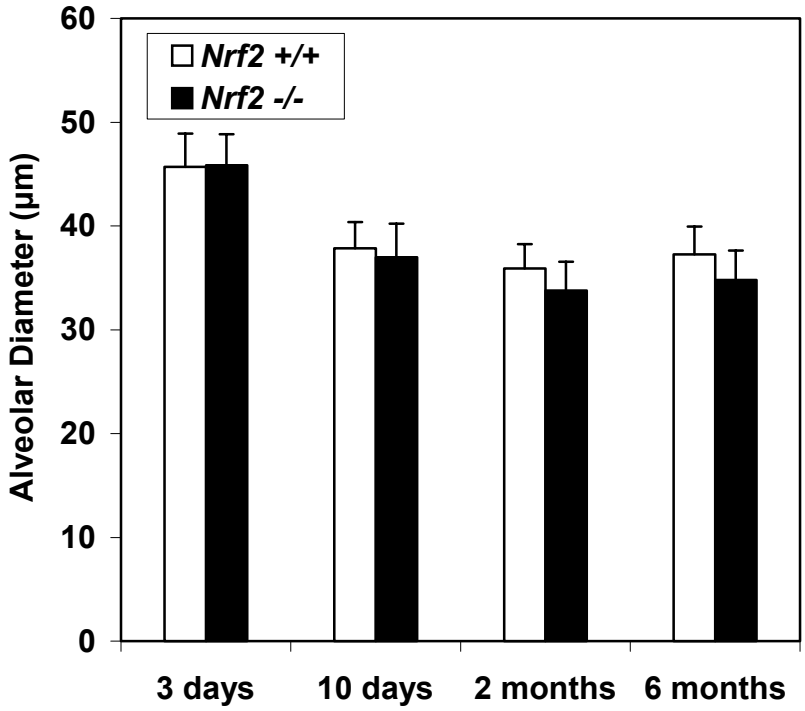
Figure 1 (A)



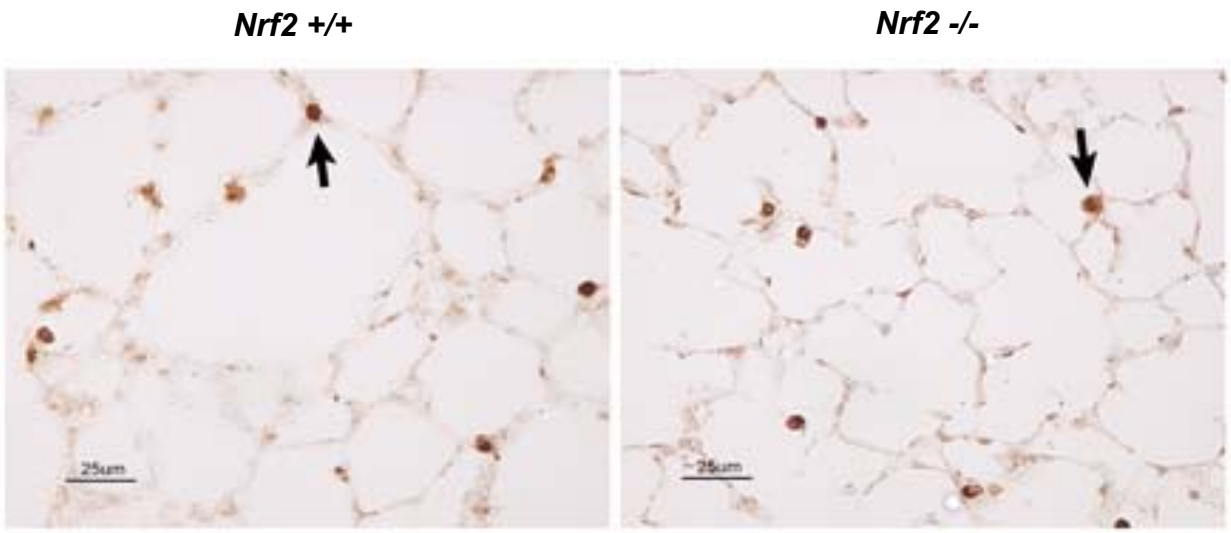
(B)


Nrf2 +/+
Nrf2 -/-
Mean Linear Intercept (µm)
100
90
80
70
60
50
40
30
20
10
0
3 days
10 days
2 months
6 months

(C)

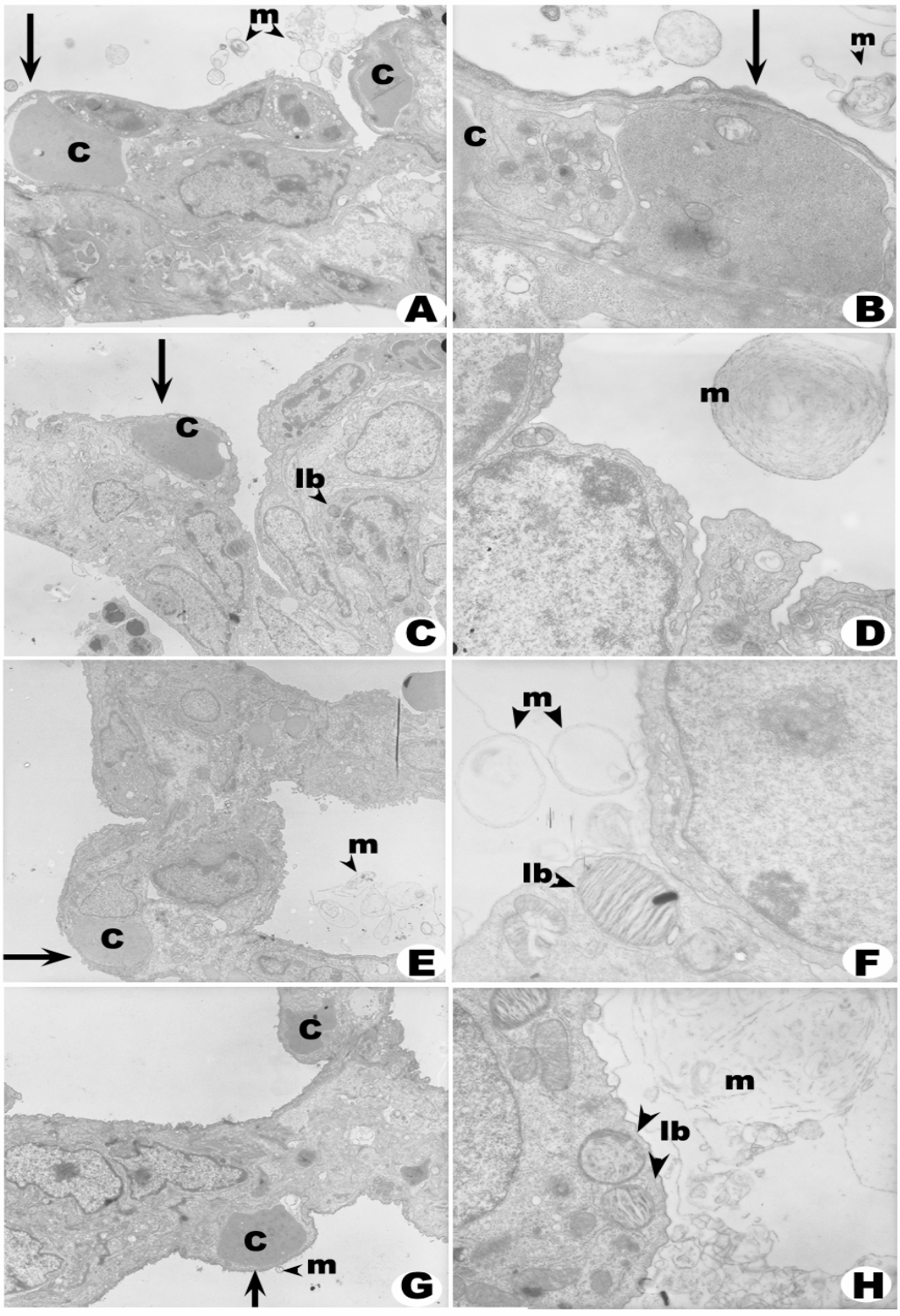

Nrf2 +/+
Nrf2 -/-
Alveolar Diameter (µm)
60
50
40
30
20
10
0
3 days
10 days
2 months
6 months

(D)

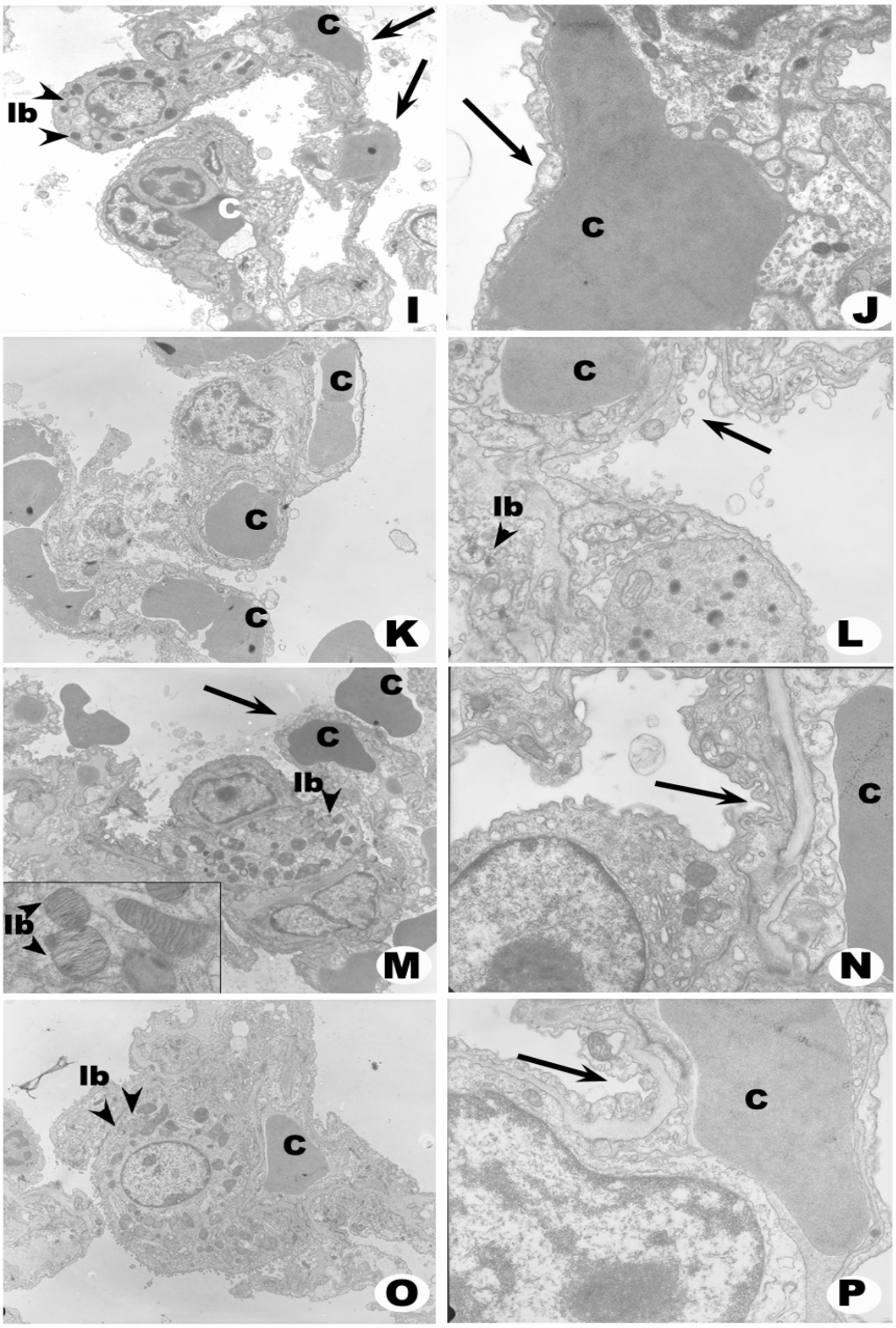


Supplementary Figure 2 (A) and (B). Ultrastructural lung morphology of postnatal day 3 (A-D), day 10 (E-H), 2 months (I-L), and 6 months (M-P) of *nrf2* +/+ (A-B, E-F, I-J and M-N) and *nrf2* -/- (C-D, G-H, K-L and O-P) mice. Note the overall similar septal cellular organization, with thin alveolar-capillary membranes (arrows, c = capillary), made of type I cell cytoplasm, basement membrane and capillary endothelial cell cytoplasm. Intracellular myelin bodies (lb) and extracellular myelin (m, arrowheads) were present in both wild-type and knockout lungs from all ages. Insert in M shows a close up of lamellar bodies. In both the wild-type and knockout mice, there was an overall pattern of focally immature septa in days 3 and 10 lungs, with prominent cuboidal cells, which became less evident as the lungs matured. There were no differences in collagen content or septal thickness between wild-type and knockout lungs in all ages examined (A, C, E, G, I, K, M and O: 3800X; B, D, F, H, J, L, N and P: 15000X).

(A)

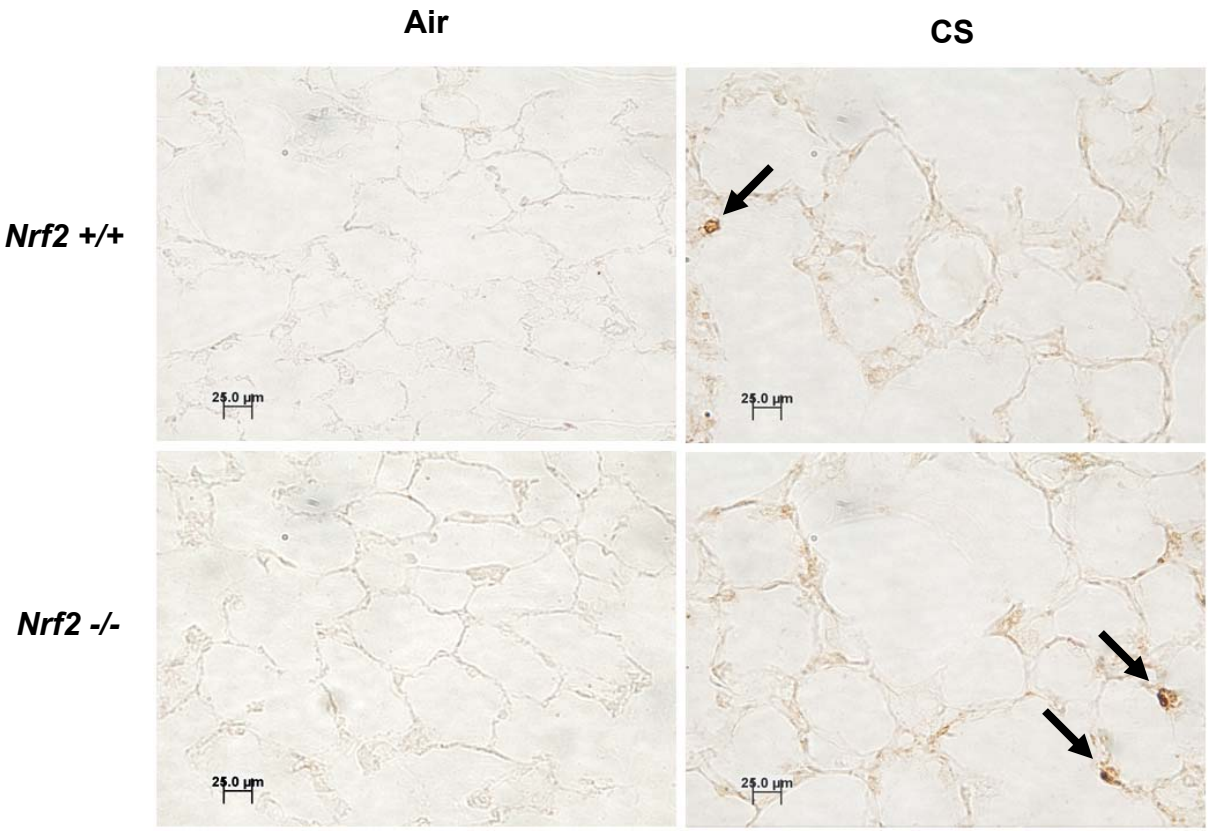


(B)

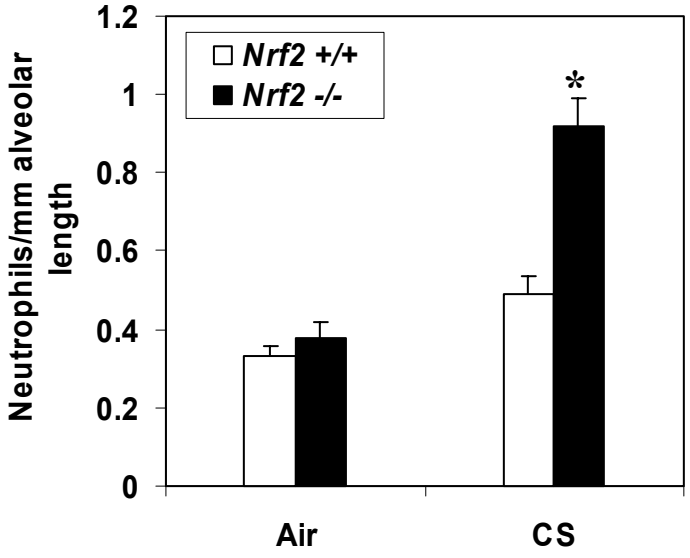


Supplementary Figure 3. Immunohistochemical localization of neutrophils **(A)** and lymphocytes **(C)** in the lung tissues of air or CS exposed *nrf2* +/+ and -/- mice. Immunohistochemical analysis showed scanty amount of neutrophils and lymphocytes in the lungs of CS exposed *nrf2* +/+ and -/- mice. Note the presence of neutrophils (arrows) and lymphocytes (arrows) in the alveolar septa of both CS exposed *nrf2* +/+ and -/- mice. Images were acquired with a 40X lens. Bar graphs representing a significantly (*) increased number of neutrophils **(B)** and lymphocytes **(D)** in the lungs of CS exposed *nrf2* -/- mice than its wild-type counterpart. Values represent mean ± SEM.

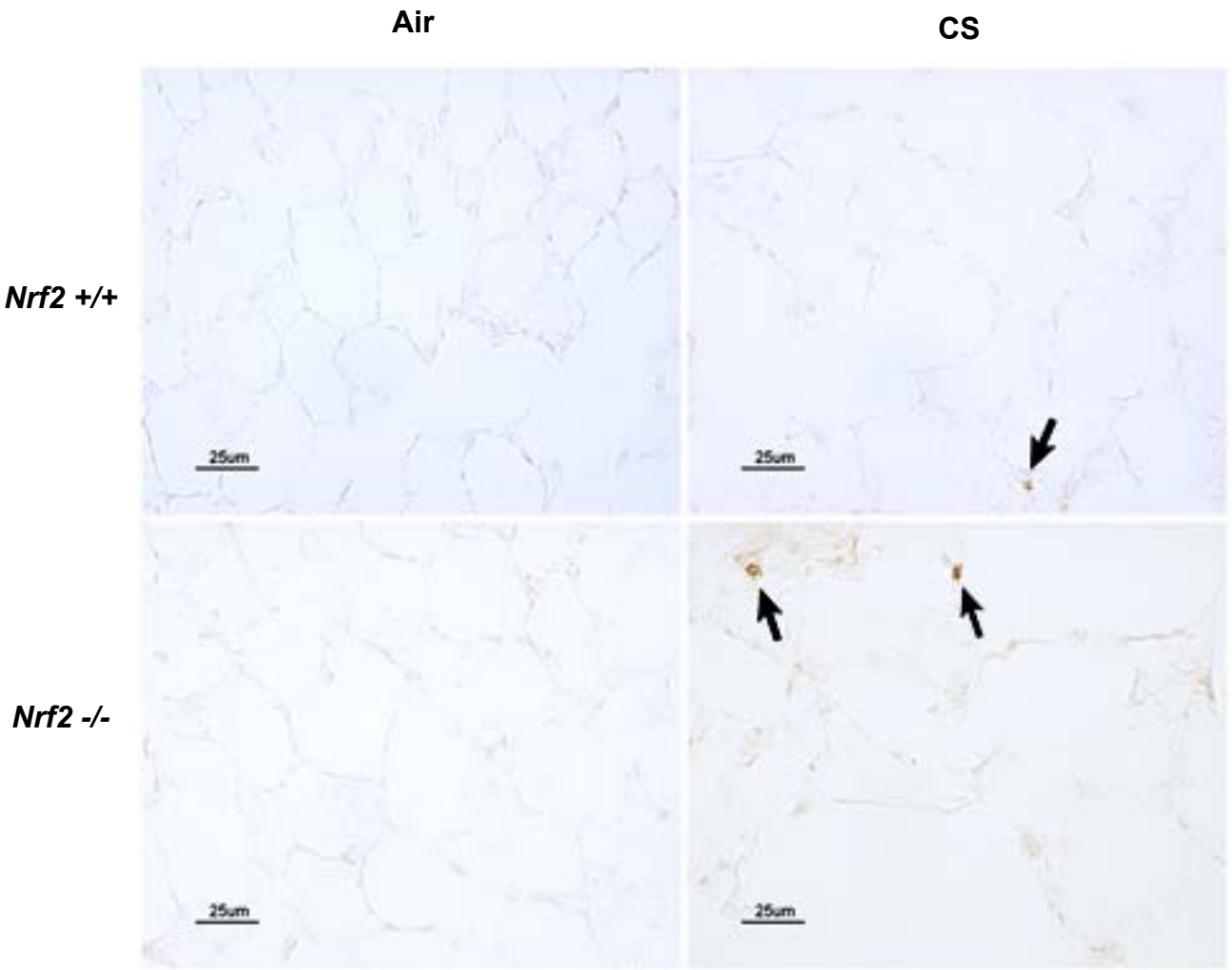
(A)



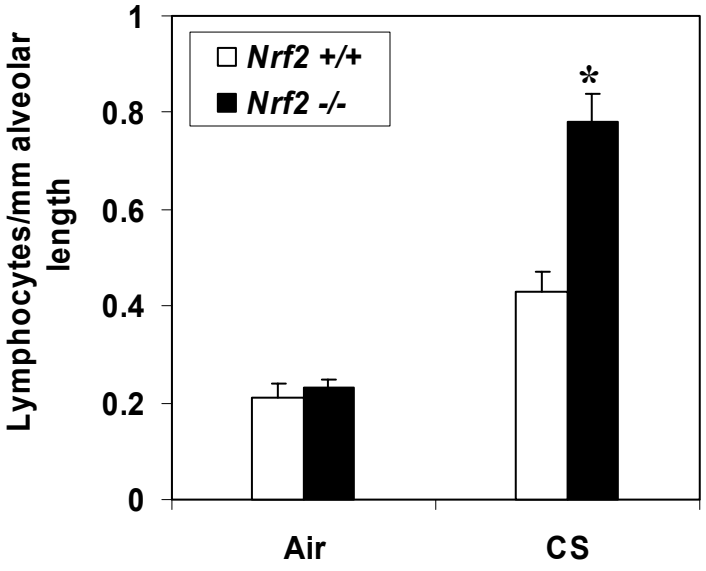
(B)


Neutrophils/mm alveolar length
1.2
1
0.8
0.6
0.4
0.2
0
Nrf2 +/+
Nrf2 -/-
*
Air
CS

(C)



(D)


Lymphocytes/mm alveolar length
1
0.8
0.6
0.4
0.2
0
Nrf2 +/+
Nrf2 -/-
*
Air
CS