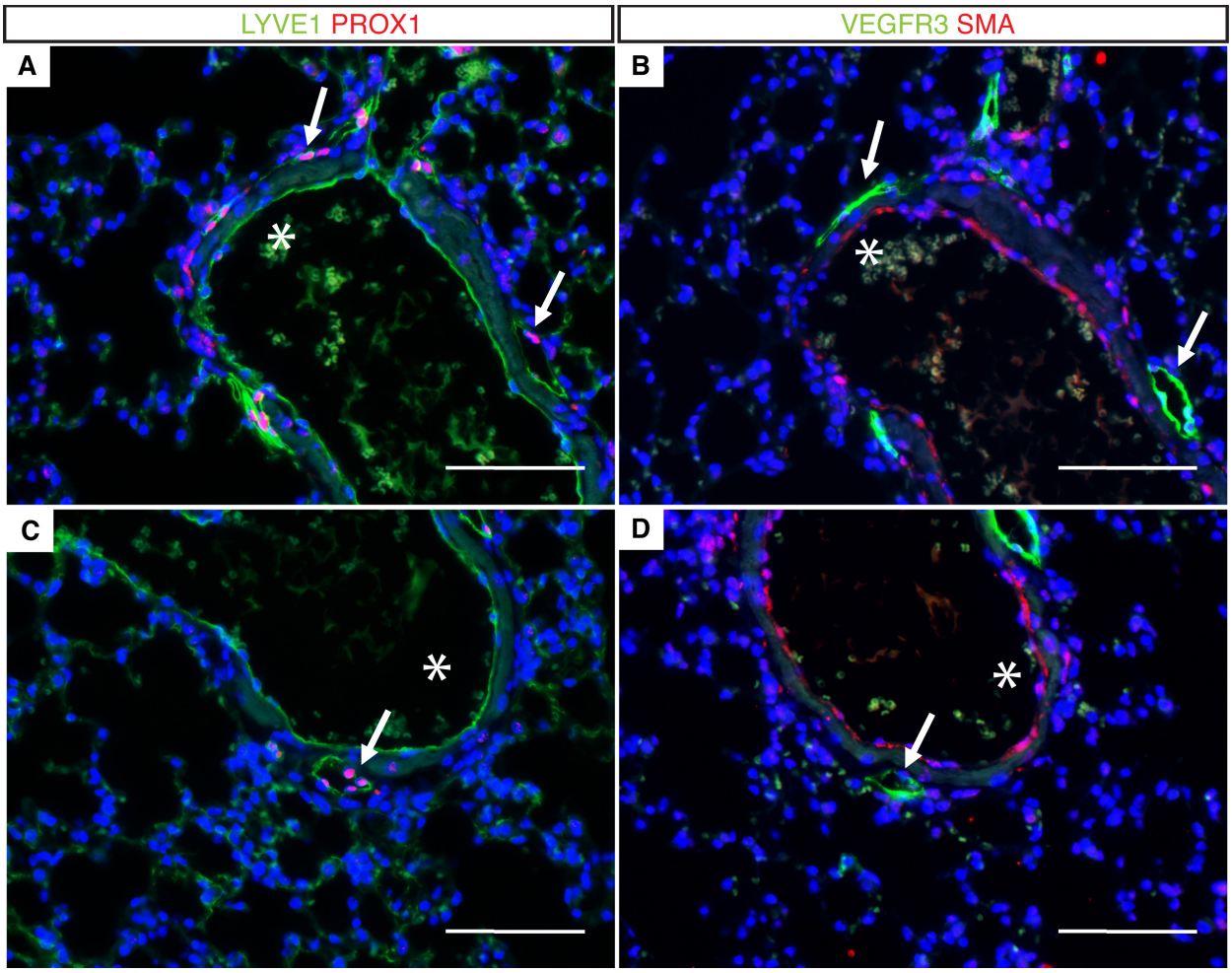
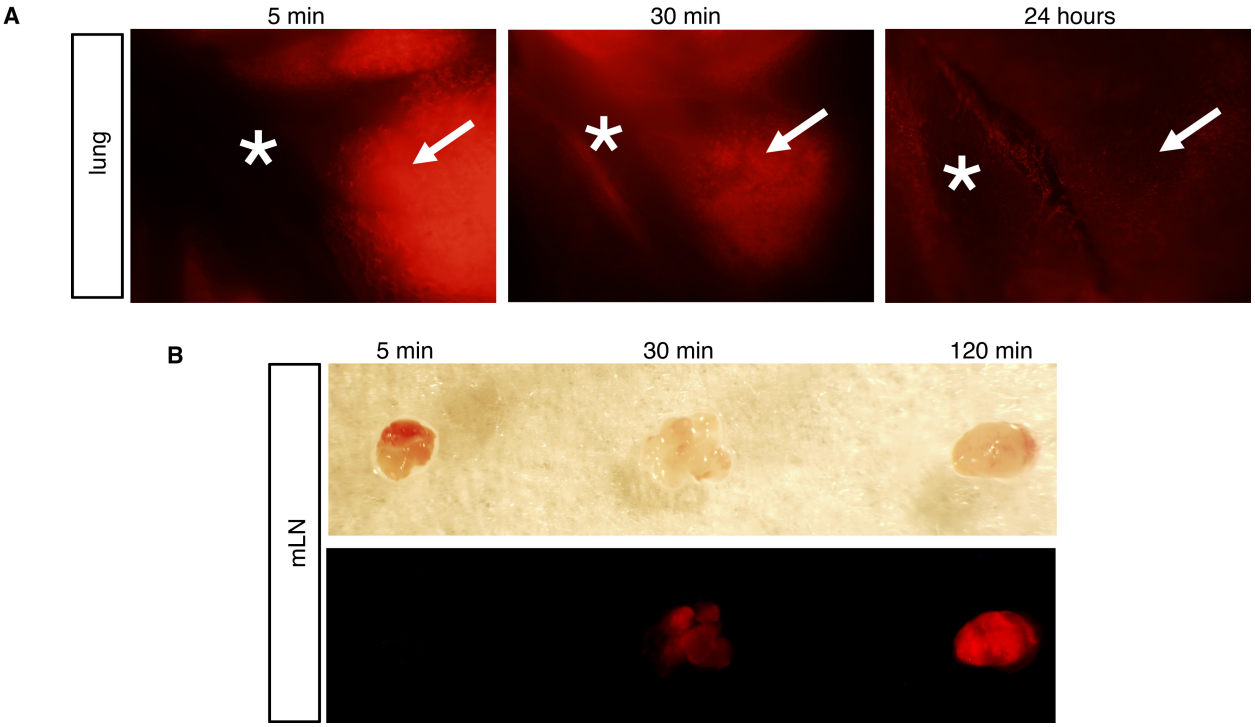
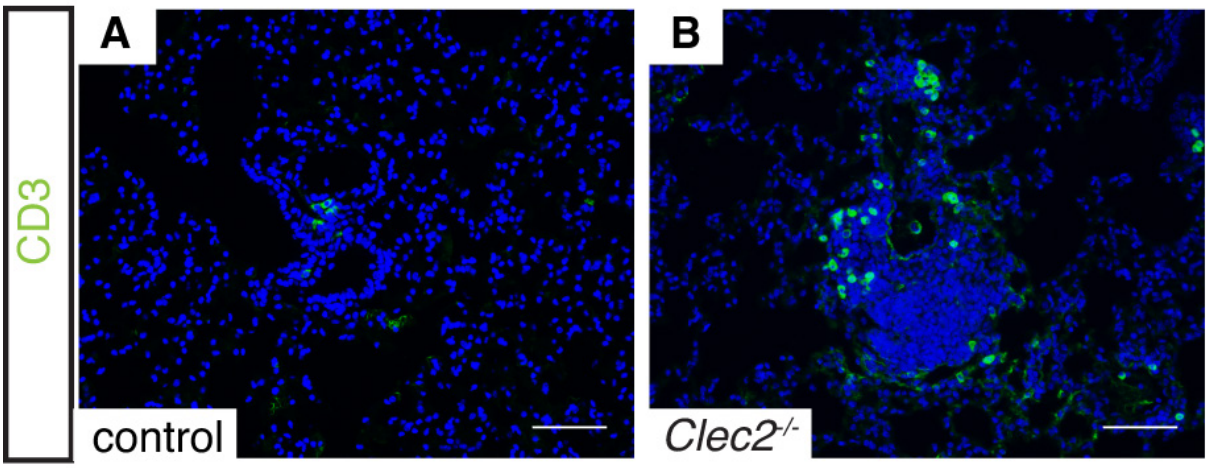


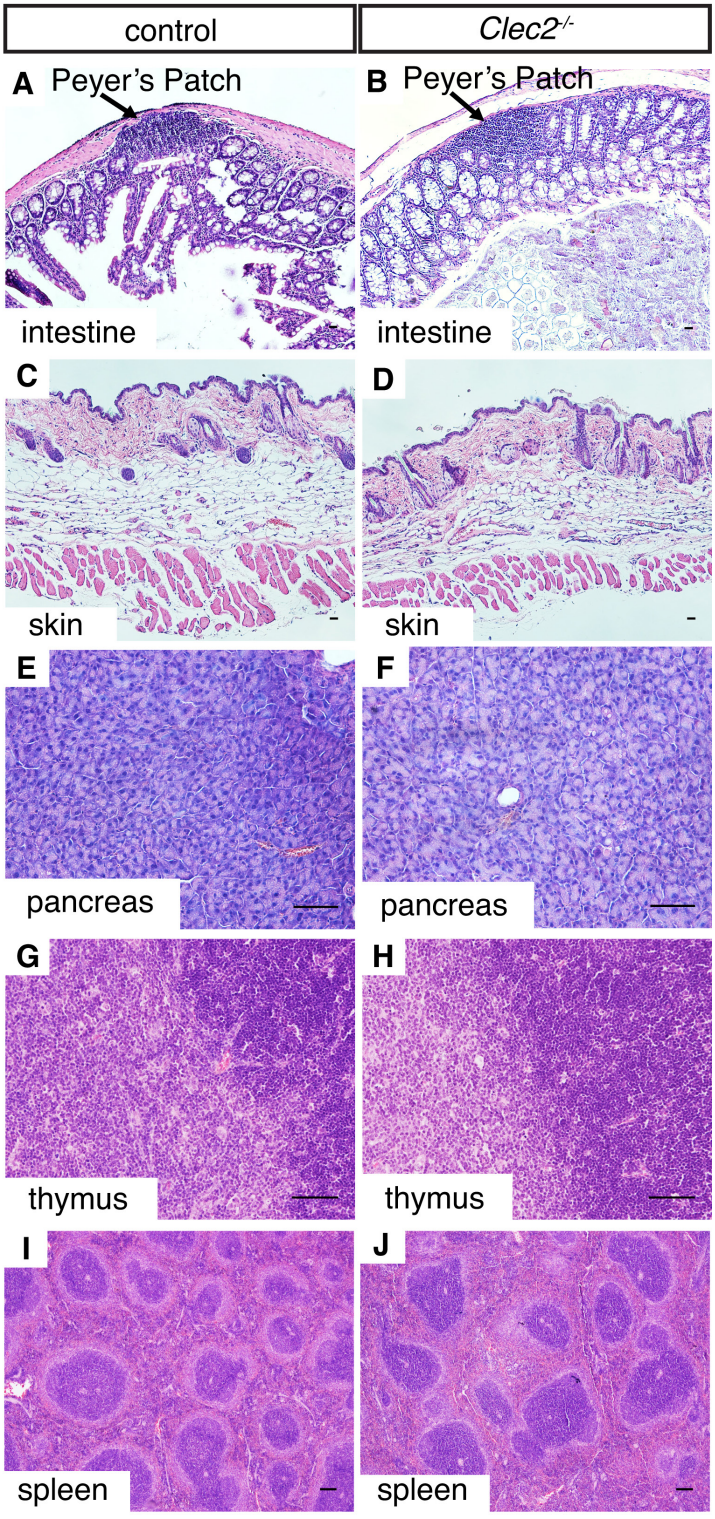
Supplemental Figure 1: Pulmonary lymphatics lack smooth muscle cell coverage. (A-D) Immunohistochemistry of normal mouse lung tissue. Lyve1/Prox1 double staining (A,C) distinguishes pulmonary lymphatics from blood vessels, which also express Lyve1. (B,D) Serial sections of the tissue shown in A and C using the lymphatic-specific marker VEGFR3. SMA staining is shown in red. Arrows indicate lymphatic vessels, with asterisks marking adjacent blood vessels. Scale bars = 25μm.



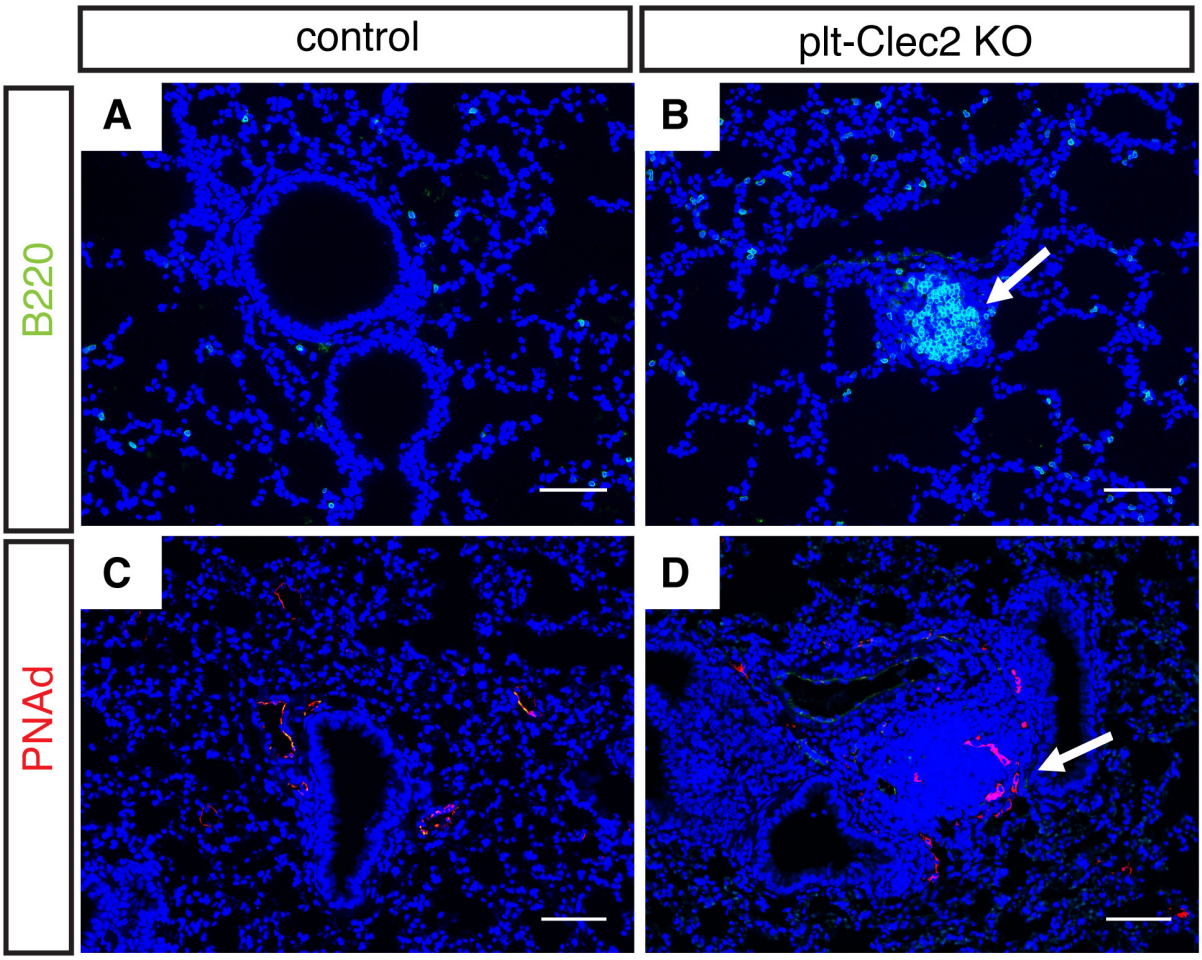
Supplemental Figure 2: Dextran drainage assay for pulmonary lymphatic flow. (A) Intra-tracheal delivery dextran-568 (red) to mice results in lung fluorescence (arrows) that declines over time with lymphatic drainage. Asterisks indicate right main bronchus. (B) Fluorescent microscopy of mediastinal lymph nodes (mLNs) after intra-tracheal delivery of dextran-568 demonstrates the time course of lymphatic drainage from the lungs of normal mice.



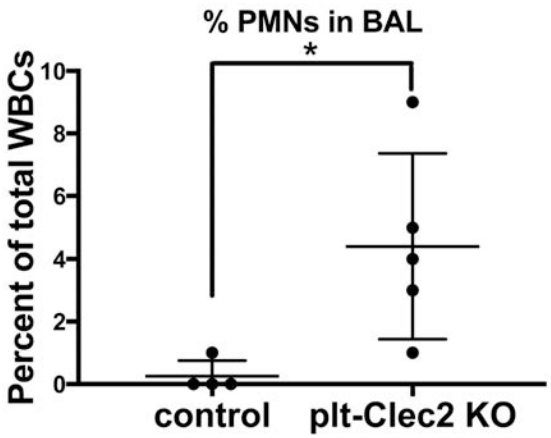
Supplemental Figure 3: Increased T cell infiltrate in the lungs of *Clec2*$^{-/-}$ mice. (A,B) Immunohistochemistry at areas of bronchovascular infiltrates in lung tissue from *Clec2*$^{-/-}$ and control mice was performed using the T cell marker CD3 (green). Scale bars = 25μm.



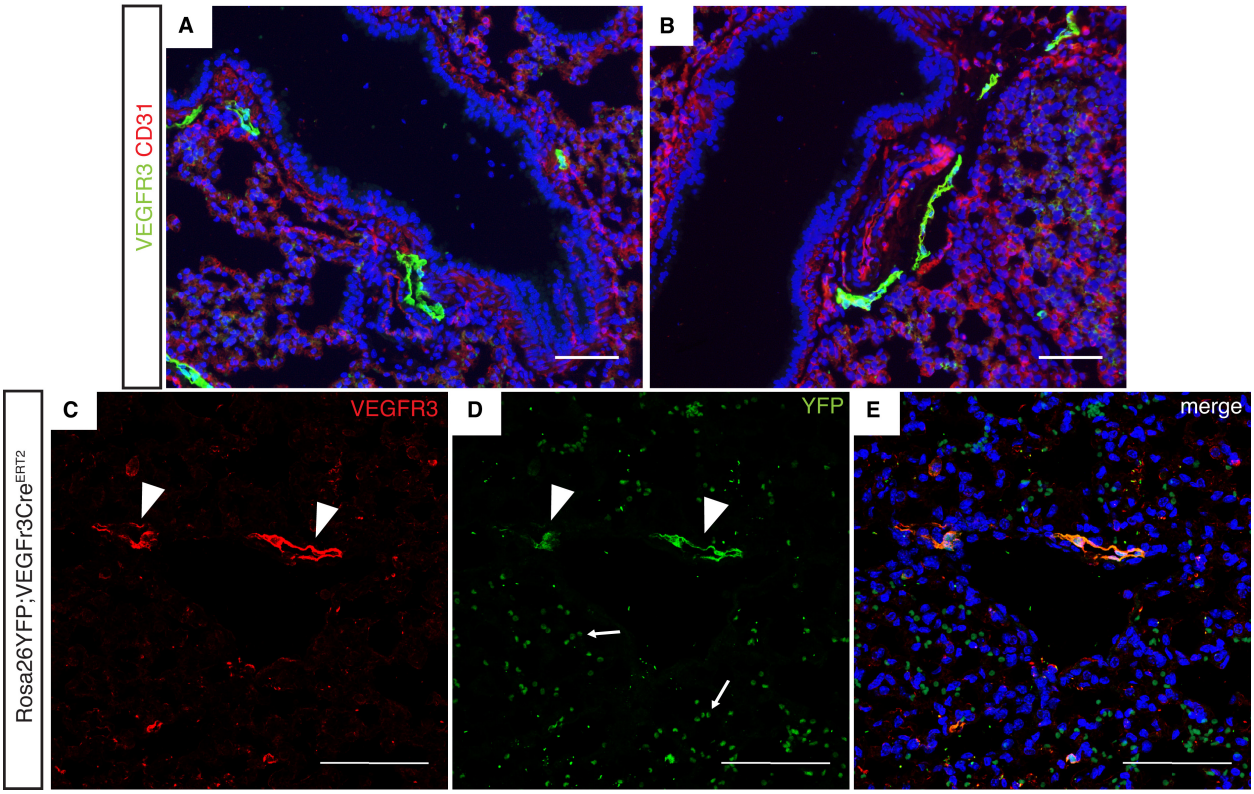
Supplemental Figure 4: Absence of TLOs in tissues outside of the lung in *Clec2*$^{-/-}$ mice. (A-J) H&E of tissue from 6-8 week old *Clec2*$^{-/-}$ and control mice is shown. (A,B) small intestine (C,D) skin (E,F) pancreas (G,H) thymus (I,J) spleen. Scale bars in A-H = 25μm. Scale bars in I,J = 100μm.



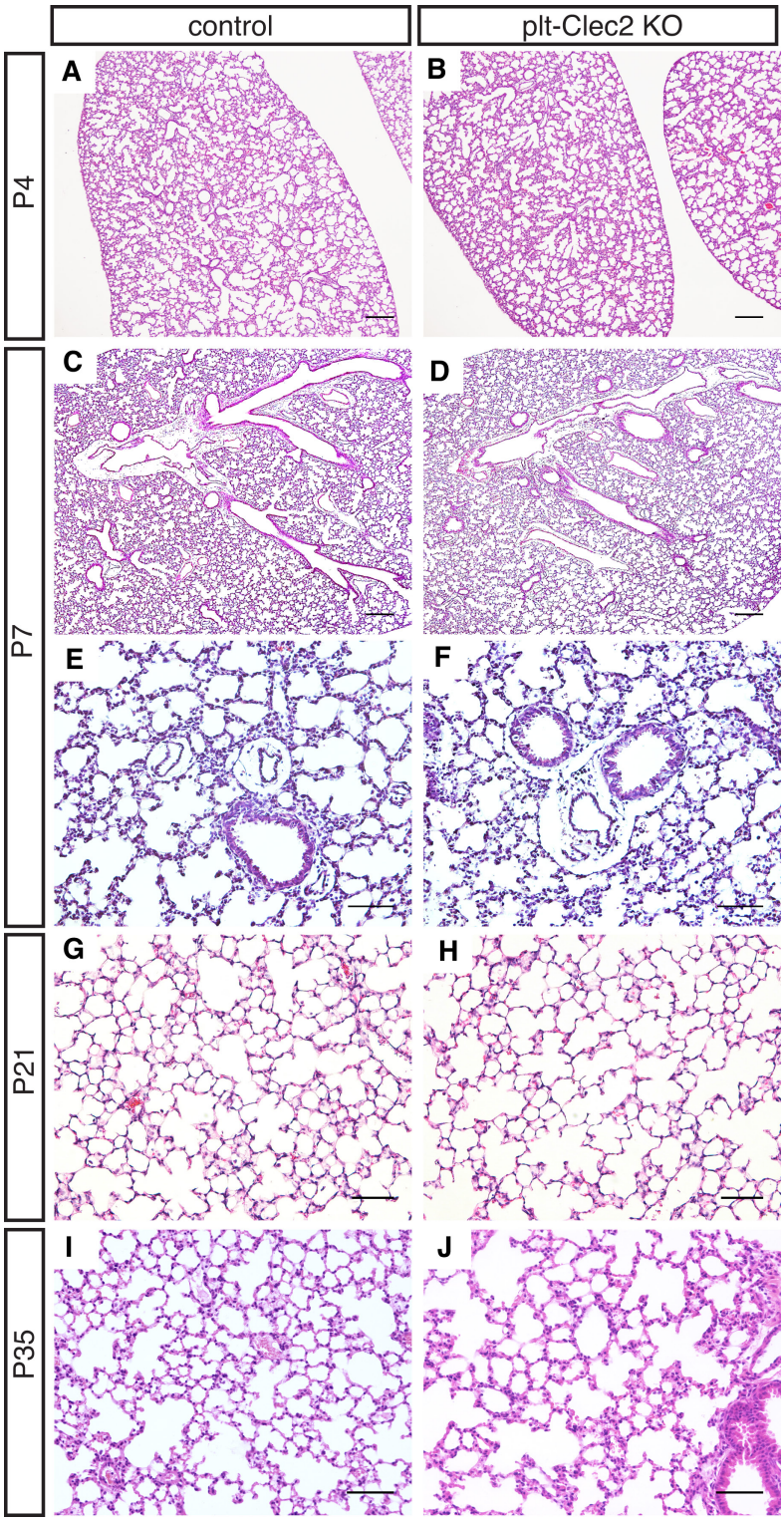
Supplemental Figure 5: Absence of B cells and HEVs in the lungs of control mice. (A-D) Immunohistochemistry for B cells (B220, green, arrows) and high endothelial venules (PNAd, red arrows) in plt-Clec2 KO and control mice. Scale bars = 25μm.



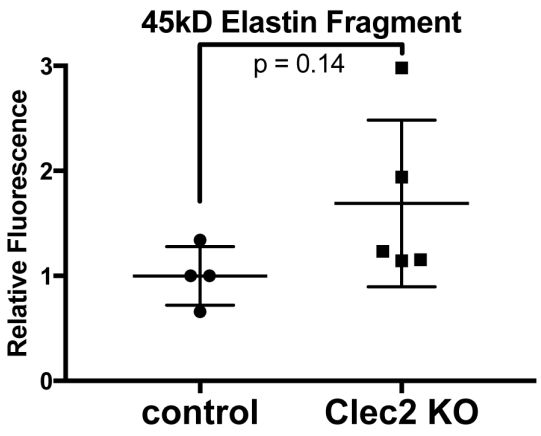
Supplemental Figure 6: Increased WBCs in the bronchoalveolar lavage (BAL) fluid of plt-Clec2 KO mice. WBC count (cells/ml) in BAL fluid was assessed in 12-16 week old control and plt-Clec2 KO mice. All values are means ± SEM. *P* value calculated by Student's *t* test. * $P < 0.05$.



Supplemental Figure 7: VEGFR3 expression is specific for lymphatic endothelial cells in the lung. (A,B) Immunohistochemistry of normal lung tissue for lymphatics (VEGFR3, green) and blood vessels/capillaries (CD31, red). (C-E) Immunohistochemistry of the lung for *Cre*-inducible YFP reporter expression (green) in mice expressing *VEGFR3Cre*^{ERT2} 4 weeks after tamoxifen administration. Arrowheads indicate VEGFR3^{+} lymphatics (red). Arrows indicate autofluorescent red blood cells. Scale bars = 25μm.



Supplemental Figure 8: Alveolar enlargement is not seen at early time points in plt-Clec2 KO mice. H&E of lung tissue from plt-Clec2 KO and control mice at P4 (A,B), P7 (C-F), P21 (G,H), and P35 (I,J). Scale bars in A-D = 100μm. Scale bars in E-F = 25μm. Data representative of at least 3 mice in each group.



Supplemental Figure 9: Detection of 45kD elastin fragment (EF) in the lungs of control and plt-Clec2 KO mice. Quantification 45kD EF in western blots of lung tissue from 6-8 month old plt-Clec2 KO and control mice, measured by fluorescence intensity (AU). Comparisons were made between matched littermates. All values are means ± SEM. *P* value calculated by Student's *t* test.

Mouse	# of VEGFR3+ Lymphatics Counted	# of VEGFR3+ Lymphatics with SMA staining
1	30	0
2	30	0
3	30	0
4	30	0
5	30	0
6	30	0

Supplemental Table 1: Quantification of SMA^{+} lymphatics in the lungs of control mice.

Mouse	# of VEGFR3+ Lymphatics Counted	# of VEGFR3+ lymphatics with RBCs in lumen
control 1	50	0
control 2	75	3
control 3	60	0
control 4	50	1
DT 1	14	1
DT 2	15	0
DT 3	30	0
DT 4	18	1

Supplemental Table 2: Quantification of lymphatics in transplanted lungs with any red blood cells (RBCs) within the lumen.