Genetic Deletion of *Muc5b* Reduces Interstitial Lung Disease in Neonatal *Nedd4-2* Deficient Mice

N. Smyczek[1,2,3], J.C. Meyer auf der Heyde[1,2,3], D.H.W. Leitz[1,2,3], L. Schaupp[1,2,3], A. Loewe[1,2,3], D. Kunkel[4], Y. Rodriguez Sillke[4], M. Daniltchenko[1,2,3], A. González[1,2,3], C.M. Evans[5], M.A. Mall[1,2,3], J. Duerr[1,2,3]

[1]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany; [2]German Center for Lung Research (DZL), associated partner site, Berlin, Germany; [3]German Center for Child and Adolescent Health (DZKJ) – Partner Site, Berlin, Germany; [4]Berlin Institute of Health at Charite´- Universitätsmedizin Berlin, Flow & Mass Cytometry Core Facility, Berlin, Germany; [5]Department of Medicine, University of Colorado, Aurora, CO, USA

Introduction

MUC5B has been implicated as a major risk factor for the development of pulmonary fibrosis [1]. However, insights into its role in the *in vivo* pathogenesis of interstitial lung disease (ILD) remain incomplete. We previously developed a mouse model (*Nedd4-2*$^{-/-}$) exhibiting severe spontaneous ILD driven by congenital *Nedd4-2* deletion [2]. To functionally assess the contribution of MUC5B to ILD pathogenesis, we generated mice with a lung-specific *Muc5b* knockout on the *Nedd4-2*$^{-/-}$ background (*Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$).

Methods

We conducted a comparative analysis of neonatal *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ mice against *Nedd4-2*$^{-/-}$, *Muc5b*$^{-/-}$, and control littermates during the first month of life. Assessments included survival, oxygen saturation, lung mechanics, bronchoalveolar lavage (BAL) fluid analysis, and lung histology. Muc5b protein levels were quantified by Western blot, and cellular phenotypes were spatially analyzed using Imaging Mass Cytometry (IMC).

Results

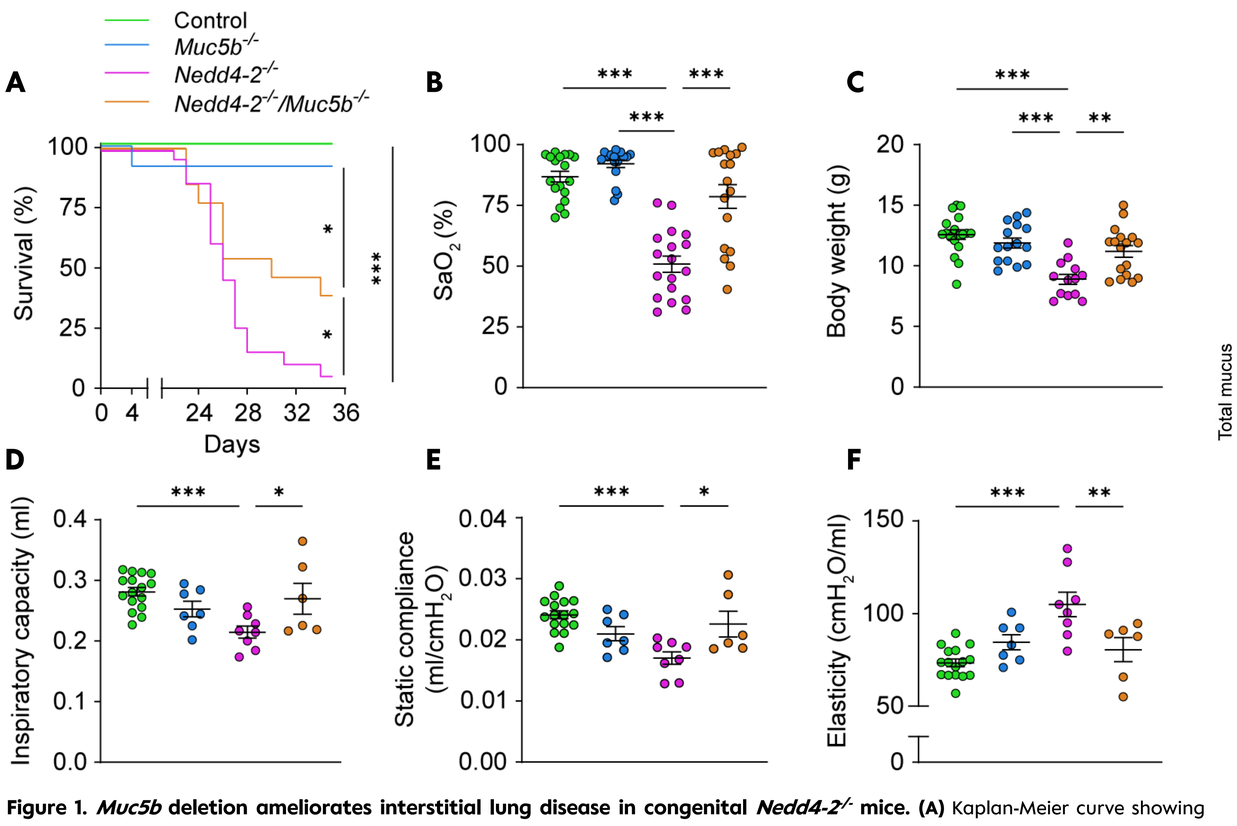

Figure 1. *Muc5b* deletion ameliorates interstitial lung disease in congenital *Nedd4-2*$^{-/-}$ mice. (A) Kaplan-Meier curve showing survival of *Nedd4-2*$^{-/-}$, *Muc5b*$^{-/-}$, *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ mice and littermate controls within first five weeks of life. Survival was analyzed via pairwise Log-Rank tests, with *p* values adjusted by Benjamini-Hochberg method. **(B)** Oxygen saturation was obtained at day of endpoint study. Multiple comparison Kruskal-Wallis testing. **(C)** Body weight was measured on day 25 of life for all groups. Pulmonary function testing on day 25 of life shown as **(D)** inspiratory capacity, **(E)** static lung compliance and **(F)** elasticity in all four groups (n = 6-8 animals per group). Ordinary one-way ANOVA testing, n = 5–18 animals per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are shown as mean ± S.E.M.

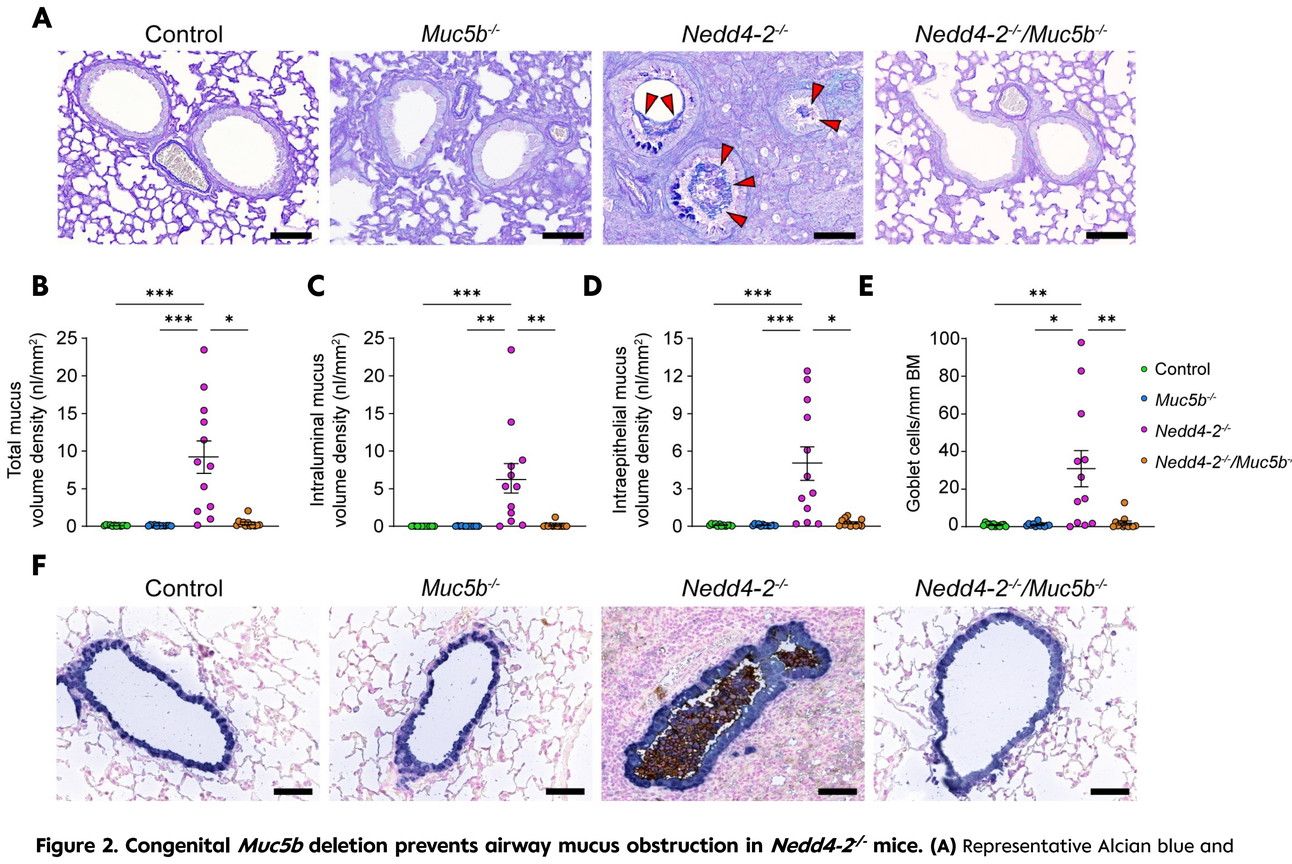

Figure 2. Congenital *Muc5b* deletion prevents airway mucus obstruction in *Nedd4-2*$^{-/-}$ mice. (A) Representative Alcian blue and periodic acid-Schiff (AB-PAS) stained lung sections, red errors indicating mucus filled airspaces in the distal lung, scale bars 50 µm. Mucus volume density quantification shown as **(B)** total, **(C)** intraluminal and **(D)** intraepithelial AB-PAS positive material and **(E)** goblet cells per millimeter basement membrane in *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ compared to *Nedd4-2*$^{-/-}$, *Muc5b*$^{-/-}$ mice and littermate controls. **(E)** Immunohistochemistry of distal airways stained with CCSP (blue) and Muc5b (brown). Scale bars, 50 µm. Multiple comparison Kruskal-Wallis testing, n = 12 animals per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are shown as mean ± S.E.M.

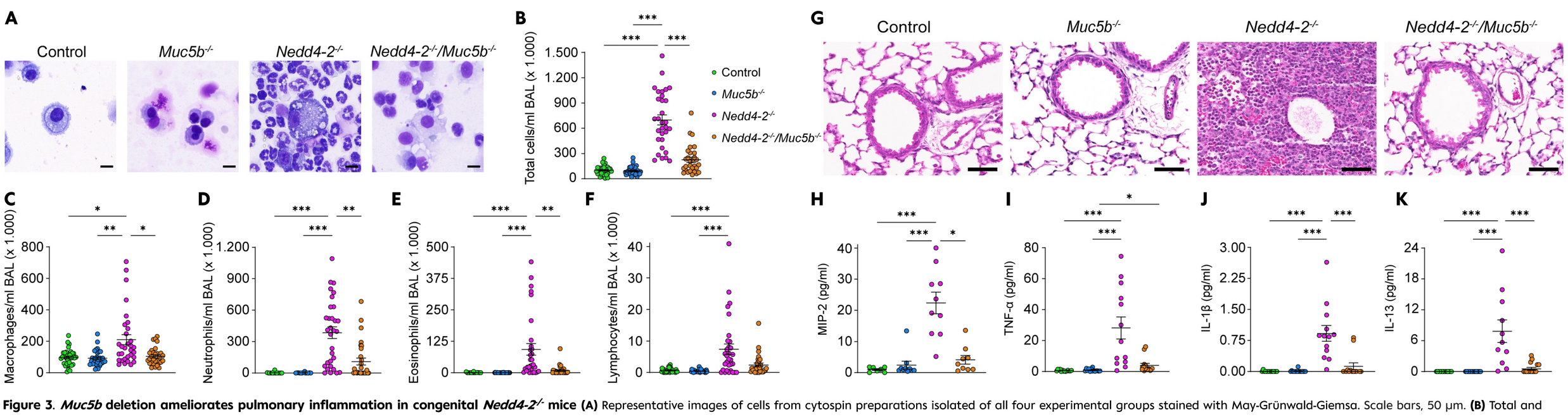

Figure 3. *Muc5b* deletion ameliorates pulmonary inflammation in congenital *Nedd4-2*$^{-/-}$ mice (A) Representative images of cells from cytospin preparations isolated of all four experimental groups stained with May-Grünwald-Giemsa. Scale bars, 50 µm. **(B)** Total and differential cell count in BAL of investigated mice depicted by **(C)** Macrophages, **(D)** Neutrophils, **(E)** Eosinophils and **(F)** Lymphocytes. **(G)** Representative hematoxylin and eosin (H&E) stained lung sections of distal airways. Scale bars, 50 µm. Inflammation markers in BAL supernatant of mice up to 5-weeks-old, depicted as of **(H)** MIP-2, **(I)** TNF-α, **(J)** IL-1β and **(K)** IL-13. Multiple comparison Kruskal-Wallis testing, n = 6-30 animals per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are shown as mean ± S.E.M.

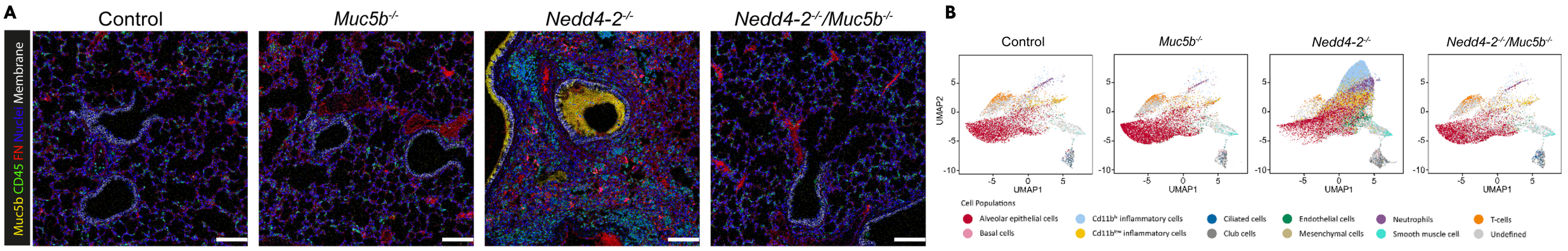

Figure 4. Imaging mass cytometry reveals effect of *Muc5b* deletion on cellular composition in the lungs of *Nedd4-2*$^{-/-}$ mice. (A) Representative lung sections ablated by Imaging Mass Cytotometry, stained for Muc5b (yellow), CD45 (green), Fibronectin (red), EpCAM (white) and DNA1 (blue). Scale bar; 100 µm. **(B)** UMAP projection of individual cells derived from imaging mass cytometry, color-coded by identified cell populations. n = 4 mice per group.

Conclusion

Taken together, deletion of *Muc5b* ameliorates the severity of spontaneous ILD in the neonatal *Nedd4-2*$^{-/-}$ model by preventing mucus obstruction and subsequent pulmonary inflammation. These data highlight a central pathogenic role for Muc5b in this ILD model and support its potential as a therapeutic target.

References: [1] Hunninghake GM, et al. (2013) MUC5B promoter polymorphism and interstitial lung abnormalities. N Engl J Med. 368(23):2192-200.
[2] Leitz DHW, et al. (2021) Congenital Deletion of *Nedd4-2* in Lung Epithelial Cells Causes Progressive Alveolitis and Pulmonary Fibrosis in Neonatal Mice. Int J Mol Sci. 22(11):6146

Contact: Nathalie Smyczek | nathalie.smyczek@charite.de