Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/33/eabb7238/DC1

Supplementary Materials for

Epithelial cell–specific loss of function of *Miz1* causes a spontaneous COPD-like phenotype and up-regulates *Ace2* expression in mice

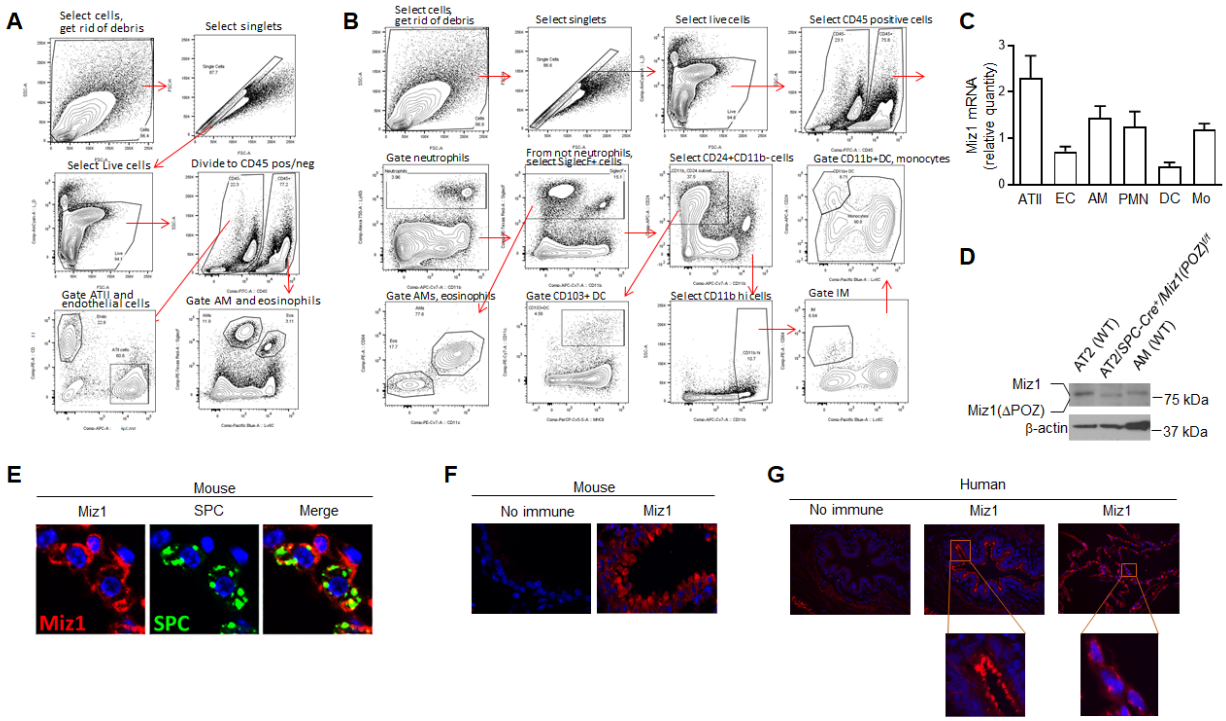
Hanh Chi Do-Umehara, Cong Chen, Qiao Zhang, Alexander V. Misharin, Hiam Abdala-Valencia, S. Marina Casalino-Matsuda, Paul A. Reyfman, Kishore R. Anekalla, Francisco J. Gonzalez-Gonzalez, Marc A. Sala, Chao Peng, Ping Wu, Catherine C. L. Wong, Ravi Kalhan, Ankit Bharat, Harris Perlman, Karen M. Ridge, Jacob I. Sznajder, Peter H. S. Sporn, Navdeep S. Chandel, Jindan Yu, Xiangdong Fu, Irina Petrache, Rubin Tuder, G. R. Scott Budinger*, Jing Liu*

*Corresponding author. Email: jinglius@uic.edu (J.L.); s-buding@northwestern.edu (G.R.S.B.)

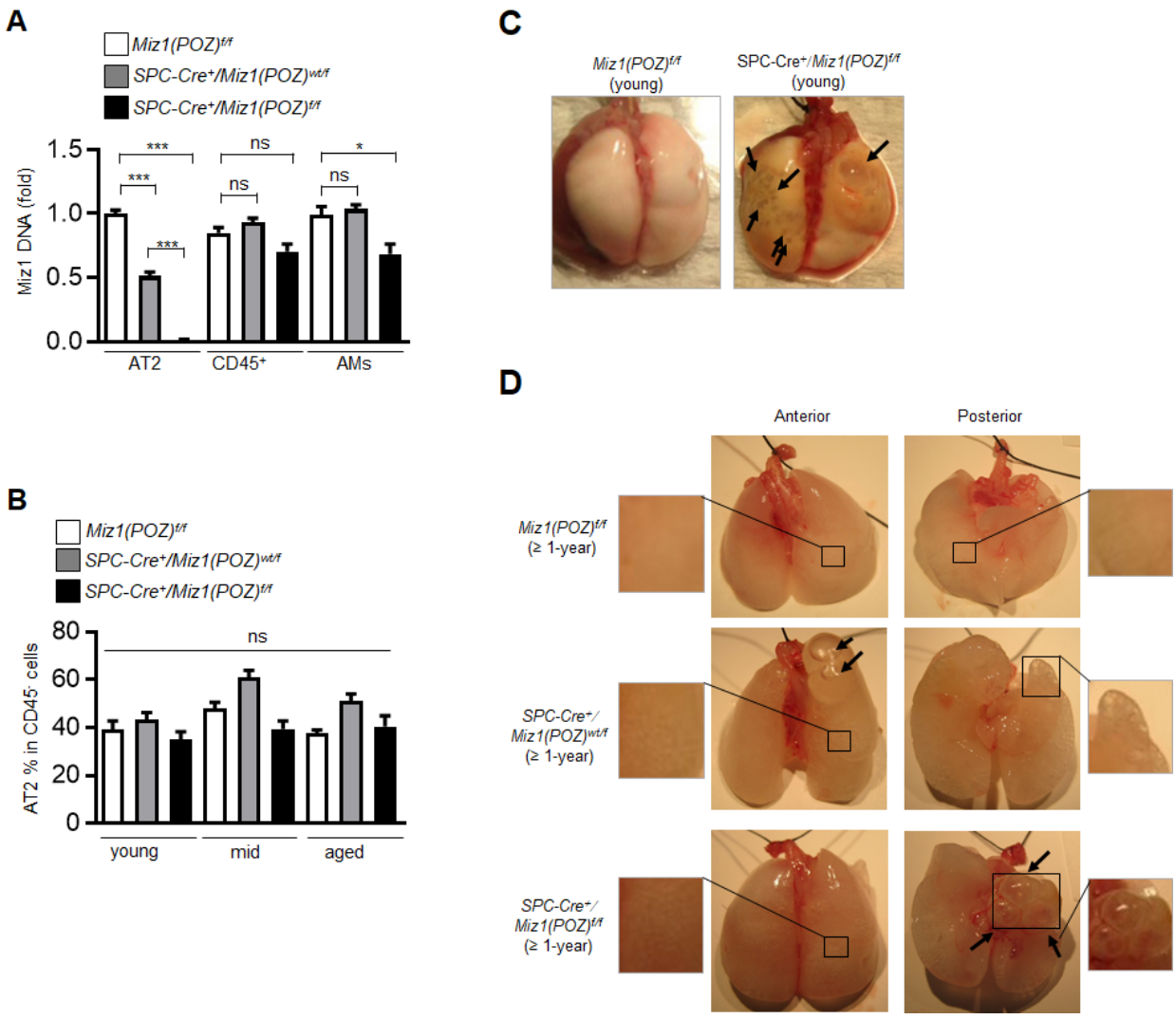
Published 14 August 2020, *Sci. Adv.* **6**, eabb7238 (2020)
DOI: 10.1126/sciadv.abb7238

This PDF file includes:

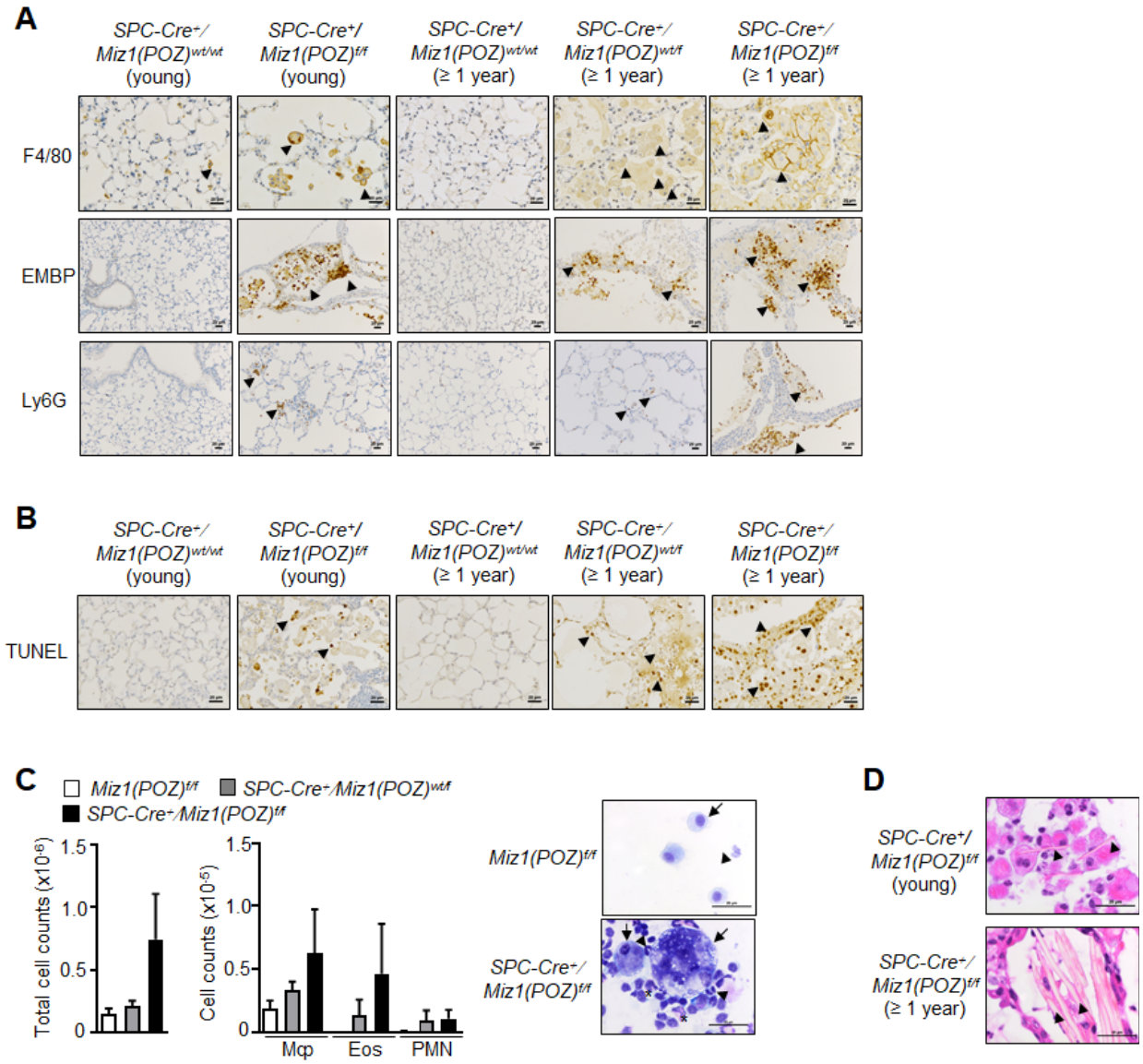
Supplementary Figures



Supplementary Fig. 1 Miz1 is ubiquitously expressed in most cell types in the lung. (**A,B**) Gating strategies for flow-sorted cell types from the mouse lung. (**C**) Miz1 mRNA levels in AT2, endothelial cells (EC), AMs (AM), neutrophils (PMN), DCs (DC), and monocytes (Mo) isolated from wild-type mouse lungs. Data were presented as mean with SEM from 2 wild-type mice with technical triplets. (**D**) Miz1 protein expression in AT2 cells or AMs (AM) isolated from control *Miz1(POZ)*$^{fl/fl}$ or homozygous *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{fl/fl}$ mice as indicated. AT2 cells from *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{fl/fl}$ mice express a truncated Miz1 protein ~ 76 kDa as compared to the full-length 87 kDa Miz1 protein. (**E**) IF staining of Miz1 and SPC in wild-type mouse lungs. (**F**) IF staining of Miz1 in the airways of wild-type mouse lungs. (**G**) IF staining of Miz1 in normal human lungs. Left, no immune control; Middle, airways; Right, alveoli. Data are representative of at least two independent experiments.

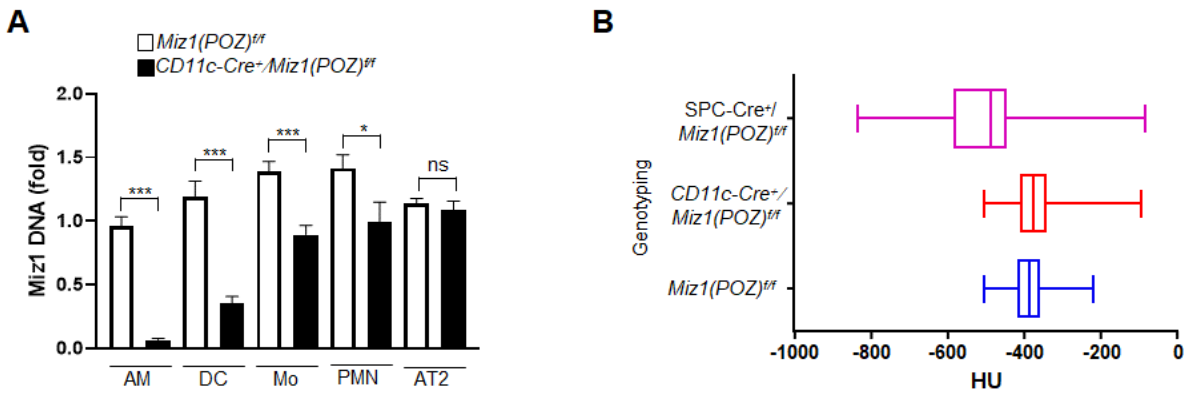


Supplementary Fig. 2 The Miz1 POZ domain is specifically deleted in lung epithelial cells of *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice and loss of function of Miz1 does not affect lung epithelial cell proliferation. (**A**) qPCR of the Miz1 POZ domain using genomic DNA in AT2, AMs, or other CD45^{+} cells (after gating out AMs) isolated from control *Miz1(POZ)$^{fl/fl}$*, or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$*, or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (n = 3-4). ns, non-significant; *, $p < 0.05$; ***, $p < 0.001$. (**B**) AT2 cell percentage in CD45-negative cells of young (~ 2-month-old), middle-aged (~ 6-month-old), or aged (≥ 1-year-old) control *Miz1(POZ)$^{fl/fl}$*, or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$*, or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice. n = 3-6. (**C,D**) Gross photograph of the lung from ≤ 4-month-old control *Miz1(POZ)$^{fl/fl}$* or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (**C**), or ≥ 1-year-old control *Miz1(POZ)$^{fl/fl}$*, or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$*, or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (**D**). Arrows indicate bullous emphysema-like changes. Data are representative of at least three independent experiments. Photo credits: Hanh Chi Do-Umehara (**C,D**).

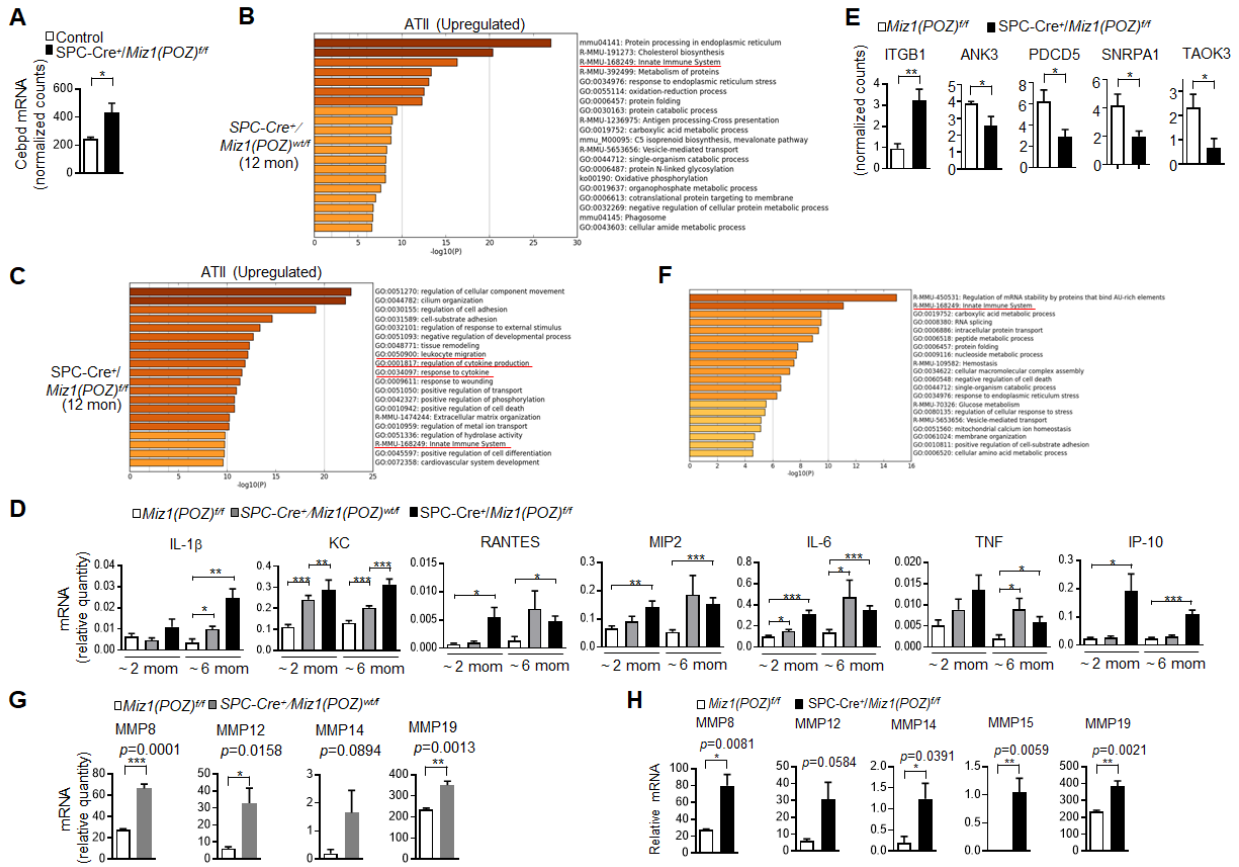


Supplementary Fig. 3 Mice with lung epithelial loss of function of Miz1 have inflammatory cell infiltrates and increased apoptosis in the lung. (**A**) Representative IHC staining of F4/80 (macrophages) (× 40 objective), EMBP (eosinophils) (× 20 objective), or Ly6G (PMNs) (× 20 objective) of lungs from young control *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{wt/wt}$, or homozygous *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{fl/fl}$ mice, or ≥ 1-year-old control *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{wt/wt}$, or heterozygous *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{wt/fl}$, or homozygous *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{fl/fl}$ mice. (**B**) Representative TUNEL (× 40 objective) staining of lungs from mice in (**A**). (**C**) BALF total cell counts (*left*), or BALF cell differentials (*middle*) from ≥ 1-year-old control *Miz1(POZ)*$^{fl/fl}$, or heterozygous *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{wt/fl}$, or homozygous *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{fl/fl}$ mice (n = 4-6). Note, macrophages (Mφ), eosinophils (Eos), and neutrophils (PMN). *Right*, representative picture of recovered BALF cells stained with Wright-Giemsa. Macrophages, neutrophils, and eosinophils were shown by arrows, arrow heads, and stars, respectively. Giant, multinucleated, and vacuolated macrophages were clearly evident. (**D**) Representative histological sections (× 100 objective) of lungs showing

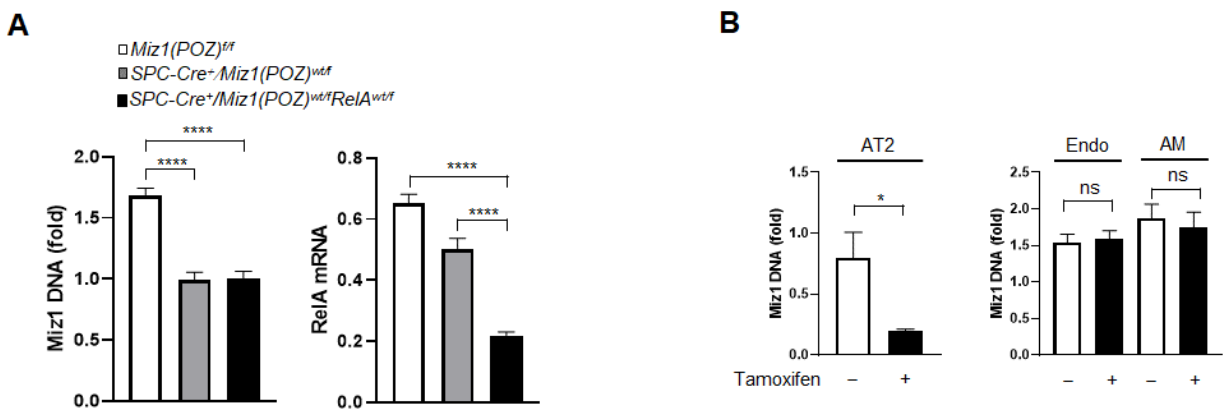
the presence of eosinophilic crystals (shown as arrow heads) in young or aged *SPC-Cre*$^{+}$*/Miz1(POZ)*$^{fl/fl}$ mice. Data are representative of at least three independent experiments.



Supplementary Fig. 4 Deletion of the Miz1 POZ domain in different cell types of the lung from *CD11c-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice and normal lung radiographic density in aged *CD11c-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice. (**A**) qPCR of the Miz1 POZ domain using genomic DNA of AMs (AM), DCs (DC), monocytes (Mo), neutrophils (PMN), or AT2 cells isolated from control *Miz1(POZ)$^{fl/fl}$* or *CD11c-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (n = 4-5). Data are representative of at least three independent experiments. (**B**) Representative Hounsfield units (HU) of the lung parenchyma from ≥ 1-year-old control *Miz1(POZ)$^{fl/fl}$* or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* or *CD11c-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mouse.



Supplementary Fig. 5 Deletion of the Miz1 POZ domain results in deregulated expression of inflammatory genes and candidate genes involved in COPD. (**A**) Cebpd mRNA levels analyzed by RNA-seq in AT2 cells from 6-month-old control *Miz1(POZ)$^{fl/fl}$* and homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (n = 4). (**B,C**) GO analysis of differentially expressed genes (analyzed by RNA-seq) in AT2 cells from 1-year-old control *Miz1(POZ)$^{fl/fl}$*, or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$* (**B**) or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (**C**). $n \geq 4$. (**D**) mRNA levels (analyzed by RT-qPCR) of inflammatory genes, including IL-1β, KC, RANTES, MIP2, IL-6, TNF, and IP-10, in primary AT2 cell isolated from control *Miz1(POZ)$^{fl/fl}$*, or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$*, or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice at ~ 2 or 6 months of age (n = 4-6). (**E,F**) Protein abundance of ITGB1, ANK3, PDCD5, SNRPA1, and TAOK3 (analyzed by proteomics) (**E**), or GO analysis of differentially expressed proteins (analyzed by proteomics) (**F**) in AT2 cells isolated from young (≤ 4-month-old) control *Miz1(POZ)$^{fl/fl}$* (n = 3) or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (n = 3). (**G,H**) mRNA levels (analyzed by RNA-seq) of various MMPs in AMs from 6-month-old control *Miz1(POZ)$^{fl/fl}$* or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$* (**G**), or homozygous *SPC-Cre^{+}/Miz1(POZ)$^{fl/fl}$* mice (**H**) (n = 3-4). Red underlines indicate pathways involved in innate immunity.



Supplementary Fig. 6 Characterization of the *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$/RelA$^{wt/fl}$* mice and tamoxifen-treated *SPC-CreER^{T2+}/Miz1(POZ)$^{f/f}$* mice. (**A**) qPCR of the Miz1 POZ domain using genomic DNA (**left**; n = 3-6) and RelA mRNA levels (**right**; n = 3-4) in AT2 cells of ≥ 1-year-old control *Miz1(POZ)$^{fl/fl}$* or heterozygous *SPC-Cre^{+}/Miz1(POZ)$^{wt/fl}$* or *SPC-Cre^{+}/Miz1(POZ)$^{wt/f}$RelA$^{wt/fl}$* mice. (**B**) qPCR of the Miz1 POZ domain using genomic DNA in AT2, AMs, or endothelial cells (Endo) of tamoxifen-treated control *SPC-CreER^{T2-}/Miz1(POZ)$^{f/f}$* or *SPC-CreER^{T2+}/Miz1(POZ)$^{f/f}$* mice. n = 2. Data are representative of at least three independent experiments.

Supplementary Table 1. Primer sequences used for qPCR.

Gene	primer name	Sequence
Miz1 POZ domain	Primer 3	CGTTGACTTCAAGGCTCACA
	Primer 4	GTCCACGTTCTCAGGGCTAA
IL-1β	mIL-1β-1-5'	GCCCATCCTCTGTGACTCAT
	mIL-1β-1-3'	AGGCCACAGGTATTTTGTCG
KC	KC-1-5'	ACTGCACCCAAACCGAAGTC
	KC-1-3'	TGGGGACACCTTTTAGCATCTT
RANTES	RANTES-1-5'	CCCTCACCATCATCCTCACT
	RANTES-1-3'	CCTTCGAGTGACAAACACGA
MIP2	MIP2-3-5'	CAAGGGCGGTCAAAAAGTT
	MIP2-3-3'	AGGCACATCAGGTACGATCC
IL-6	mIL-6-1-5'	AGTTGCCTTCTTGGGACTGA
	mIL-6-1-3'	TCCACGATTTCCCAGAGAAC
TNF	mTNFα-2-5'	GAACTGGCAGAAGAGGCACT
	mTNFα-2-3'	AGGGTCTGGGCCATAGAACT
IP10	IP10-1-5'	CCCACGTGTTGAGATCATTG
	IP10-1-3'	GAGGCTCTCTGCTGTCCATC