A number of inflammatory lung diseases, including chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and pneumonia, are modulated by WNT/β-catenin signaling. However, the underlying molecular mechanisms remain unclear. Here, starting with a forward genetic screen in mouse, we identify the WNT coreceptor Related to receptor tyrosine kinase (RYK) acting in mesenchymal tissues as a cell survival and antiinflammatory modulator. Ryk mutant mice exhibit lung hypoplasia and inflammation as well as alveolar simplification due to defective secondary septation, and deletion of Ryk specifically in mesenchymal cells also leads to these phenotypes. By analyzing the transcriptome of wild-type and mutant lungs, we observed the up-regulation of proapoptotic and inflammatory genes whose expression can be repressed by WNT/RYK signaling in vitro. Moreover, mesenchymal Ryk deletion at postnatal and adult stages can also lead to lung inflammation, thus indicating a continued role for WNT/RYK signaling in homeostasis. Our results indicate that RYK signaling through β-catenin and Nuclear Factor kappa B (NF-κB) is part of a safeguard mechanism against mesenchymal cell death, excessive inflammatory cytokine production, and inflammatory cell recruitment and accumulation. Notably, RYK expression is down-regulated in the stromal cells of pneumonitis patient lungs. Altogether, our data reveal that RYK signaling plays critical roles as an antiinflammatory modulator during lung development and homeostasis and provide an animal model to further investigate the etiology of, and therapeutic approaches to, inflammatory lung diseases.

**Significance**

WNT/β-catenin signaling is critical for lung development, and homeostasis and it has also been implicated in inflammatory lung diseases. However, the underlying molecular mechanisms, especially those at play during inflammatory conditions, are unclear. Here, we show that loss of the WNT coreceptor Related to receptor tyrosine kinase (RYK) specifically in mesenchymal cells results in lung inflammation. Our data indicate that RYK signaling through β-catenin and Nuclear Factor kappa B (NF-κB) is part of a safeguard mechanism against mesenchymal cell death, excessive inflammatory cytokine production, and inflammatory cell recruitment and accumulation.
in vitro manipulations suggest that alterations of WNT/Ryk signaling through NF-kB contribute to the pathogenesis of inflammatory lung diseases. Two different mesenchymal Ryk deletion models display extensive inflammation at developmental and adult stages, thereby providing a useful platform to further investigate the etiology and treatment of inflammatory lung diseases.

Results

Ryk Mutant Mice Exhibit Lung Inflammation and Alveolar Simplification. In a previous study, we reported the identification and analysis of a SL mutant allele of Ryk, which leads to lung hypoplasia and inflammation (Fig. 1 A and B) (14). Epithelial-specific deletion of Ryk leads to goblet cell hyperplasia and mucus hypersecretion without lung inflammation (14). Here, we focus on the inflammation phenotype. To determine which immune cell types accumulate in RykSL/SL lungs, we performed histological analyses and immunostaining. At the histological level, RykSL/SL lungs display infiltration of macrophages and monocytes, neutrophils, and also lymphocytes (SI Appendix, Fig. S1A). The number of both alveolar and interstitial macrophages was markedly increased in RykSL/SL lungs at postnatal day 7 (P7) (Fig. 1C). In addition, immunostaining analyses revealed increased numbers in P7 RykSL/SL lungs of hematopoietic cells (CD45+) (Fig. 1D), including lymphocytes (CD3+ and CD19+) and macrophages (MAC2+) (SI Appendix, Fig. S1 B–D). We next investigated the stage at which an accumulation of inflammatory cells is first observed in RykSL/SL lungs. While RykSL/SL lungs were partially collapsed at P0, inflammatory cells were first recruited in mutant lungs starting around P1 (SI Appendix, Fig. S1 E and F), indicating that the loss of Ryk function causes the recruitment of inflammatory cells into the lung starting at postnatal stages. Moreover, many immune cells were proliferating in RykSL/SL lungs (SI Appendix, Fig. S2 A and B), indicating that the marked increase in the number of inflammatory cells is due to both recruitment from other tissues and local proliferation. We next performed α-smooth muscle actin (α-SMA) and elastin (ELN) immunostaining to visualize secondary septa in P7 lungs. RykSL/SL mice exhibited severe defects in secondary septa formation and a significant reduction in α-SMA and ELN expression at P7 (Fig. 1E and F), suggesting that Ryk participates in myofibroblast development or maintenance and, consequently, secondary septa formation during lung maturation. Loss of vascular integrity in inflammatory conditions can trigger immune cell infiltration into the lung parenchyma and alveolar spaces (18). To evaluate vessel integrity in RykSL/SL lungs, we injected Evans blue dye intracardially at P3. While Evans blue dye was retained in the blood vessels of wild-type (WT) lungs, it diffused into the RykSL/SL lung parenchyma (Fig. 1G). In addition, the postnatal expression of platelet endothelial cell adhesion molecule-1 (PECAM-1), an adhesion molecule that contributes to the maintenance of vascular integrity (19), appeared to be reduced in RykSL/SL lungs compared with Ryk+/+ siblings, and its localization at endothelial cell junction also appeared more punctate (Fig. 1H and SI Appendix, Fig. S2C). These data indicate
that Ryk deficiency leads to defects in lung vessel wall integrity as well as increased permeability at postnatal stages.

Since cell death can induce endothelial barrier dysfunction and inflammatory cell recruitment (3, 5), we examined cell death in RykSL/SL lungs using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The number of dead cells was significantly increased in RykSL/SL lungs at postnatal stages but not at embryonic day 18.5 (E18.5) (Fig. 1F and SI Appendix, Fig. S2D). Moreover, most dead cells were observed in the mesenchymal compartment surrounding blood vessels (Fig. 1F and SI Appendix, Fig. S2D), indicating that RYK is required for the survival of mesenchymal cells in the postnatal lung. Notably, RykSL/SL mice exhibited no obvious defects in other organs, including the heart, kidney, and liver (SI Appendix, Fig. S2E). Overall, these data indicate that in the postnatal lung, loss of Ryk function leads to cell death in the mesenchyme and triggers inflammatory cell recruitment and local proliferation.

Mesenchymal Ryk Deletion Leads to Lung Inflammation and Alveolar Simplification. To investigate the cell type–specific functions of Ryk during mouse lung development, we deleted the gene in mesenchymal cells using a Dermo1/Twist2-Cre line (hereafter Dermo1-Cre–Rykfl/ or Dermo1-RykWT and Dermo1-Cre–Rykfl/fl or Dermo1-RykcKO) and obtained specific loss of Ryk expression in lung mesenchymal cells (SI Appendix, Fig. S3A). Similar to Ryk KO and SL mice (14, 20), Dermo1-RykcKO mice exhibited growth retardation and hypoplastic lungs at P7 (Fig. 2 A–C) and subsequently died around P14. In addition, Dermo1-Rykfl/fl lungs displayed inflammatory cell accumulation and alveolar simplification at P7 (SI Appendix, Fig. S3 B–D). The inflammatory cells observed in Dermo1-Rykfl/fl lungs included macrophages, monocytes, neutrophils, and lymphocytes, as judged by cell morphology and marker analysis (Fig. 2D and SI Appendix, Fig. S3D). To quantitatively characterize the various inflammatory cell populations, we performed immunophenotyping using flow cytometry (SI Appendix, Fig. S3F). The number of immune cells (CD45+) was markedly increased in Dermo1-Rykfl/fl lungs compared with Dermo1-RykWT lungs (Fig. 2 D and E). Dermo1-Rykfl/fl lungs exhibited increased numbers of eosinophils (CD11c−SiglecF+), dendritic cells (CD11c+SiglecF−), and neutrophils (CD11b+Ly6G+) (Fig. 2E). In addition, the number of macrophages (CD11c+)...
RKYK Signaling Inhibits Apoptosis-Associated Gene Expression and Restricts the Immune Response. To identify mesenchymal genes regulated by Ryk function that are involved in postnatal lung development, we performed transcriptomic analysis of P0 Dermo1-RykKO\textsuperscript{WWT} and Dermo1-RykKO\textsuperscript{RKO} lungs. From gene ontology (GO) analysis, the up-regulated genes were mostly associated with immune response and apoptosis, and the down-regulated genes with WNT signaling (Fig. 3A and SI Appendix; Fig. S6A and Dataset S1). We focused on the up-regulated genes and first validated their expression by qPCR. The expression of immune response genes, including Bcl3, Cld2, Il11f9, Il11f2, and Serpin3f, was significantly up-regulated in Dermo1-RykKO\textsuperscript{RKO} lungs starting at P1 (Fig. 3B and SI Appendix; Fig. S6B). In addition, the expression of apoptosis genes, including Casp4, Fas, Ddit4, Pim1, and Gadd45g, was significantly up-regulated already at P0 in Dermo1-RykKO\textsuperscript{RKO} lungs compared with wild type (Fig. 3C and SI Appendix; Fig. S6C).

We next examined the expression of Ccl2/CCL2 and Bcl3/BCL3 by qPCR, in situ hybridization, and Western blotting or immunostaining. The expression of Ccl2 was strongly up-regulated and that of Bcl3 moderately up-regulated in P2 lungs at P3 and P7 but not at P0 (Fig. 2G and SI Appendix, Fig. S4A). In addition, we observed defects in vessel integrity in Dermo1-RykKO\textsuperscript{RKO} lungs at P3 and P7 but not at P0 (Fig. 2H and SI Appendix, Fig. S5A), indicating that mesenchymal cell death causes vessel integrity defects in Dermo1-RykKO\textsuperscript{RKO} lungs. We next examined cell death in lung sections. Arrows point to TUNEL-positive cells. (Fig. 2I and SI Appendix, Fig. S6C).

Overall, these data indicate that in the lung mesenchyme, RYK functions as a survival factor and/or immunomodulator starting at early postnatal stages.
Dermo1-Ryk\(^{KKO}\) lungs (SI Appendix, Fig. S6 D and E). CCL2 expression was also increased in mesenchymal cells and immune cells in P2 Dermo1-Ryk\(^{KKO}\) lungs (Fig. 3 D and E and SI Appendix, Fig. S7 A–C), indicating that RYK can repress CCL2 expression in the lung mesenchyme at postnatal stages. We also examined the expression of the apoptosis genes Casp4 and Fas in developing lungs. Casp4 and Fas expression was significantly increased in mesenchymal cells of P2 Dermo1-Ryk\(^{KKO}\) (SI Appendix, Fig. S6 D and F). Protein levels of Casp4 and cleaved CASP3, the latter a hallmark of active apoptosis (21), were significantly increased in Dermo1-Ryk\(^{KKO}\) lungs starting at P1, and even more strongly at P2 (Fig. 3 F and G). These data indicate that loss of Ryk function in lung mesenchymal cells can cause their death, and that subsequently the dying cells induce the expression of cytokines.

To test whether Ryk deficiency also leads to the up-regulation of downstream genes in vitro, we performed Ryk knockdown in NIH3T3 mouse embryonic fibroblasts (SI Appendix, Fig. S7 D). Since NF-kB signaling is a master regulator of the inflammatory response (22, 23), we first examined the activation status of NF-kB in Ryk knockdown cells. Ryk knockdown led to increased phosphorylation of NF-kB and IκB (a positive regulator of NF-kB signaling that is activated by phosphorylation), as well as IκB (a negative regulator of NF-kB signaling that is inactivated by phosphorylation) (Fig. 3 H and SI Appendix, Fig. S7 E and F). In addition, expression of CCL2, cleaved CASP3, and CASP4 was also increased when Ryk was knocked down in NIH 3T3 cells (Fig. 3 H). As Ryk is known to modulate both β-catenin–dependent and –independent signaling (12, 13), we examined whether RYK participates in WNT/β-catenin signaling by testing GSK-3β–induced phosphorylation of CTNNB1/β-catenin. Ryk knockdown led to increased phosphorylation of CTNNB1 (SI Appendix, Fig. S7 G) and could prevent the activation of WNT/β-catenin by WNT-3A, but it had no effect on the blockade of this pathway by WNT-5A (SI Appendix, Fig. S7 H). These data indicate that Ryk participates in β-catenin–dependent signaling in fibroblasts. WNT-3A treatment alone led to a reduction in NF-kB phosphorylation and lower levels of cleaved CASP3 in fibroblasts (Fig. 3 I). Conversely, Ryk knockdown in combination with WNT-3A treatment led to an increase in NF-kB phosphorylation and CCL2 expression as well as cleaved CASP3 levels, reverting the phenotype in combination with WNT-3A (hereafter Gli1-CreER\(^{T2}\)-Ryk floxed mice (24, 25), we tested Ryk expression in the lungs of five healthy controls and 10 pneumonitis patients. Similar to our previous findings (14), in the lungs of healthy controls, high levels of Ryk were detected in the airway epithelium and moderate levels in immune cells and stromal cells (Fig. 4 H and SI Appendix, Fig. S10 A and B). However, in the lungs of pneumonitis patients, Ryk expression was markedly reduced in stromal cells, even though it was still high in epithelial cells of the airway and alveoli (SI Appendix, Fig. S10 A and B). We also examined CCL2 expression in those lungs. While CCL2 expression was specifically detected in immune cells in the lungs of healthy controls, it was significantly increased in stromal cells of pneumonitis patients (Fig. 4 I and SI Appendix, Fig. S10 C). These results are consistent with our mouse data, further indicating that in the lung, Ryk restricts the immune response, including inflammatory cell recruitment.
Discussion

Here, we identify a role for RYK in the lung mesenchyme during lung development and maturation, as well as during homeostasis. In mesenchyme-specific Ryk-deficient lungs, inflammatory cells accumulate in airway lumens, alveolar spaces, and lung parenchyma, a phenotype accompanied by increased mesenchymal cell death and defective vessel integrity. We show that RYK inhibits proapoptotic and inflammatory genes in part through NF-κB-dependent and -independent signaling in a cell-autonomous manner. In contrast, our in vivo data suggest that Ryk deficiency in lung mesenchymal cells, but not lung endothelial cells, is sufficient to cause defects in endothelial cell junctions and vessel permeability/integrity. It has previously been reported that Ryk is expressed in the bone marrow and that its expression is regulated during hematopoietic development and maturation (28). In addition, Ryk deficiency in hematopoietic stem cells (HSCs) from the fetal liver can reduce HSC self-renewal and lead to proliferation-induced apoptosis (29). We found that mice with myeloid lineage-specific Ryk deletion (Lyz2-Ryk–/–) display no obvious inflammatory phenotypes in the lung, suggesting that RYK does not participate in myeloid cell proliferation and maturation in a cell-autonomous manner.

In the present study, the Ryk N-ethyl-N-nitrosourea (ENU) mutant mice (Ryk–/–) and the Dermo1-Ryk–/– mice, which express Cre recombinase in lung mesenchymal cells, all exhibit severe inflammation as well as developmental defects, including
lung hypoplasia and leaky blood vessels. In contrast, when we activated CreERT2 in GlI1-RykKO mice between P0 and P2 to conditionally delete Ryk in lung mesenchymal cells after birth, we observed lung inflammation and leaky blood vessels without obvious lung hypoplasia. The weaker phenotype in GlI1-RykKO mice compared with both the Ryk−/− and the Derma1-RykKO mice may be due to the fairly restricted vs. broader domain of Cre expression in GlI1-CreERT2 and Derma1-Cre mice, respectively. These observations, together with our results from the knockdown of Ryk in NIH 3T3 fibroblasts, indicate that the inflammation phenotype derives directly, or indirectly, from a cell-autonomous defect in lung mesenchymal cells. Nevertheless, we cannot exclude the possibility that the increased immune response is in part due to developmental defects, such as growth retardation.

The stimulation of WNT/β-catenin signaling leads to the up-regulation of antiapoptotic factors in hepatocytes (30). Activated WNT/β-catenin signaling inhibits apoptosis during development and tissue repair, as well as during tumorigenesis (31, 32). Moreover, WNT/β-catenin signaling has been shown to suppress the apoptosis of IFP myofibroblasts (7, 33). We found that Ryk deficiency leads to the increased expression of proapoptotic genes in mesenchymal cells, increased interstitial cell death, vessel permeability defects, and excessive immune cell recruitment and local proliferation. Inhibition of Caspase-3 activity can attenuate the inflammation phenotype in Ryk mutant mice, possibly in part by reducing NF-κB phosphorylation. Overall, these results suggest that the increased expression of inflammatory cytokines observed in Ryk mutants is due to both direct and indirect effects, including signals released by dying cells.

Recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus disease 19 (COVID-19) has been declared a pandemic. SARS-CoV-2 can, not only activate antiviral immune responses, but also cause uncontrolled inflammatory responses characterized by a cytokine storm (34, 35). Relevant to these processes, the mouse models in the present study recapitulate features of inflammatory lung diseases characterized by combined disruption of endothelial cell barrier function and excessive immune responses.

In conclusion, we propose that during lung development and homeostasis, WNT signaling, through RYK, β-catenin, and NF-κB, plays an important role in safeguarding the lung mesenchyme against cell death and an exacerbated immune response, including increased inflammatory cytokine production and inflammatory cell recruitment and accumulation. This signaling axis could represent an important therapeutic target against inflammatory lung diseases, such as pneumonia and COPD.

**Materials and Methods**

All animal care and experimental procedures in this study were approved by the local animal ethics committee at the Regierungspräsidium Darmstadt, Hessen, Germany. Human pneumoniainduced lung samples and healthy control lungs were provided from the Universities of Giessen and Marburg Lung Center Biobank, which is a member of the Deutsches Zentrum für Lungenforschung (DZL) Platform Biobanking. The study protocol was approved by the ethics committee of the Justus Liebig University School of Medicine (no. 58/2015), and informed consent was obtained in written form from each patient. All human studies were performed in adherence to the relevant ethical guidelines.

For the transcriptome analysis, total RNA was isolated from P0 lungs of three Derma1-RykWT and three Derma1-RykKO mice. For immune cell phenotyping, cells were obtained from P4 Derma1-RykWT and Derma1-RykKO lungs.

The materials and methods used in this study are described in detail in SI Appendix, Materials and Methods.

**Data Availability.** The RNA-Sequencing dataset produced in this study (36) is available in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141974).

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1. H. Alkhouri, J. W. Poppiung, N. P. Tanis, A. Ammit, M. Schuliga, Regulation of pulmonary inflammation by mesenchymal cells. Pulm. Pharmacol. Ther. 29, 156–165 (2016).
2. W. Urenam, et al., Mesenchymal stem cells: A novel therapy for the treatment of chronic obstructive pulmonary disease? Thorax 73, 565–574 (2018).
3. E. P. Schmidt, R. M. Tuder, Role of apoptosis in amplifying inflammatory responses in lung diseases. J. Cell Death 3, 41–53 (2010).
4. B. D. Luy, C. N. Sehnan, Resolution of acute inflammation in the lung. Annu. Rev. Physiol. 76, 467–492 (2014).
5. L. Zitvogel, D. Kopp, G. Kroemer, Decoding cell death signals in inflammation and immunity. Cell 140, 798–804 (2010).
6. D. Wallach, T. B. Kang, A. Kovalenko, Concepts of tissue injury and cell death. J. Cell Death 3, 41–53 (2010).
7. M. Sauler, J. S. Bazar, P. L. Lee. Cell death in the lung: The apoptosis-necrosis axis. Annu. Rev. Physiol. 81, 375–402 (2019).
8. M. Zhang, J. Shi, Y. Huang, L. Lai, Expression of canonical WNT/β-Catenin signaling components in the developing human lung. BMC Dev. Biol. 12, 21 (2012).
9. C. Ota, H. A. Baarsma, D. E. Wagner, A. Hilgendorff, M. K€onigshoff, Linking bronchopulmonary dysplasia to adult chronic lung diseases: Role of WNT signaling. Mol. Cell Pediatr. 81, 34 (2016).
10. M. Hussain, et al., Wnt1/catenin signaling links embryonic lung development and asthmatic airway remodeling. Biochem. Biophys. Acta Mol. Basis Dis. 1863, 3226–3242 (2017).
11. M. Königshoff, D. Eickelberg, WNT signaling in lung disease: A failure or a regeneration signal? Am. J. Respir. Cell Mol. Biol. 42, 21–31 (2010).
12. J. Green, R. Russe, R. van Amerongen, The role of Ryk and Receptor tyrosine kinases in Wnt signal transduction. Cold Spring Harb. Perspect. Biol. 6, 4 (2014).
13. J. P. Ray, M. M. Halford, S. A. Slacker, The biochemistry, signalling and disease relevance of RYK and other Wnt-binding receptors. Gene 459, 15–40 (2018).
14. H. T. Kim et al., WNT/RYK signaling restricts goblet cell differentiation during lung development and repair. Proc. Natl. Acad. Sci. U.S.A. 116, 25657–25706 (2019).
15. E. R. Hollis II et al., Ryk controls remapping of motor cortex during functional recovery after spinal cord injury. Nat. Neurosci. 19, 697–705 (2016).
16. S. Sarin et al., Role for Wnt signaling in retinal neuropl dop development: Analysis via RNA-Seq in vivo and in vivo somatic CRISPR mutagenesis. Neuron 98, 109–126 e8 (2018).
17. T. Skaia, E. Bachi, G. Schoedon, Wnt5A/Ryk signaling critically affects barrier function in human vascular endothelial cells. Cell Adhes. Migr. 11, 24–38 (2017).
18. C. E. Green, A. M. Turner, The role of the endothelium in asthma and chronic obstructive pulmonary disease (COPD). Respir. Rev. 20, 20 (2017).
19. P. Lentinkomakoglu, D. Liao, H. Mei, H. Hu, P. J. Newman, Endothelial functions of platelet/endothelial cell adhesion molecule 1 (CD103). Curr. Opin. Hematol. 23, 253–259 (2016).
20. M. M. Halford et al., Ryk-deficient mice exhibit craniofacial defects associated with perturbed Eph receptor crosstalk. Nat. Genet. 25, 414–418 (2000).
21. K. M. Dubois, B. T. Kile, Apoptotic caspases: Multiple or mistaken identities? Trends Cell Biol. 28, 475–493 (2018).
22. B. Ma, M. O. Hottiger, Crosstalk between Wnt/Catenin and NF-κB signaling pathway during inflammation. Front. Immunol. 7, 378 (2016).
23. Q. Zhang, M. J. Lenardo, D. Baltimore, 30 years of NF-κB: A blossoming of relevance to human pathobiology. *Cell* **168**, 37–57 (2017).
24. A. J. Hoogendijk, S. H. Diks, T. van der Poll, M. P. Peppelenbosch, C. W. Wieland, Kinase activity profiling of pneumococcal pneumonia. *PloS One* **6**, e18519 (2011).
25. W. Dai et al., Blockade of Wnt/β-Catenin pathway aggravated silica-induced lung inflammation through Tregs regulation on Th immune responses. *Mediators Inflamm.* **2016**, 6235614 (2016).
26. W. J. Chue, A. L. M. Bothwell, Canonical and non-canonical Wnt signaling in immune cells. *Trends Immunol.* **39**, 830–847 (2018).
27. M. M. Halford, M. L. Macheda, S. A. Stacker, “The RYK receptor family” in Receptor Tyrosine Kinases: Family and Subfamilies, D. L. Wheeler, Y. Yarden, Eds. (Humana, 2015), pp. 685–741.
28. D. K. Simoneaux et al., The receptor tyrosine kinase-related gene (Ryk) demonstrates lineage and stage-specific expression in hematopoietic cells. *J. Immunol.* **154**, 1157–1166 (1995).
29. F. Famili et al., The non-canonical Wnt receptor Ryk regulates hematopoietic stem cell repopulation in part by controlling proliferation and apoptosis. *Cell Death Dis.* **7**, e2479 (2016).
30. F. Götzschel et al., Inhibition of GSK3 differentially modulates NF-κappaB, CREB, AP-1 and beta-catenin signaling in hepatocytes, but fails to promote TNF-alpha-induced apoptosis. *Exp. Cell Res.* **314**, 1351–1366 (2008).
31. S. Chen et al., Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. *J. Cell Biol.* **152**, 87–96 (2001).
32. N. Pečina-Slaus, Wnt signal transduction pathway and apoptosis: A review. *Cancer Cell Int.* **10**, 22 (2010).
33. W. Chang et al., SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin. *J. Biol. Chem.* **285**, 8196–8204 (2010).
34. L. A. Teuwen, V. Geldhof, A. Pasut, P. Carmeliet, COVID-19: The vasculature unleashed. *Nat. Rev. Immunol.* **20**, 389–391 (2020).
35. C. Matacic, Blood vessel injury may spur disease’s fatal second phase. *Science* **368**, 1039–1040 (2020).
36. H. T. Kim, S. Guenther, D. Y. R. Stainier. Transcriptome of P0 Dermo1-RykWT and Dermo1-RykcKO lungs. Gene Expression Omnibus (GEO) database. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141974. Deposited 13 December 2019.