Pathological activation of MAPK causes lymphangiectasia treatable with Ravoxertinib

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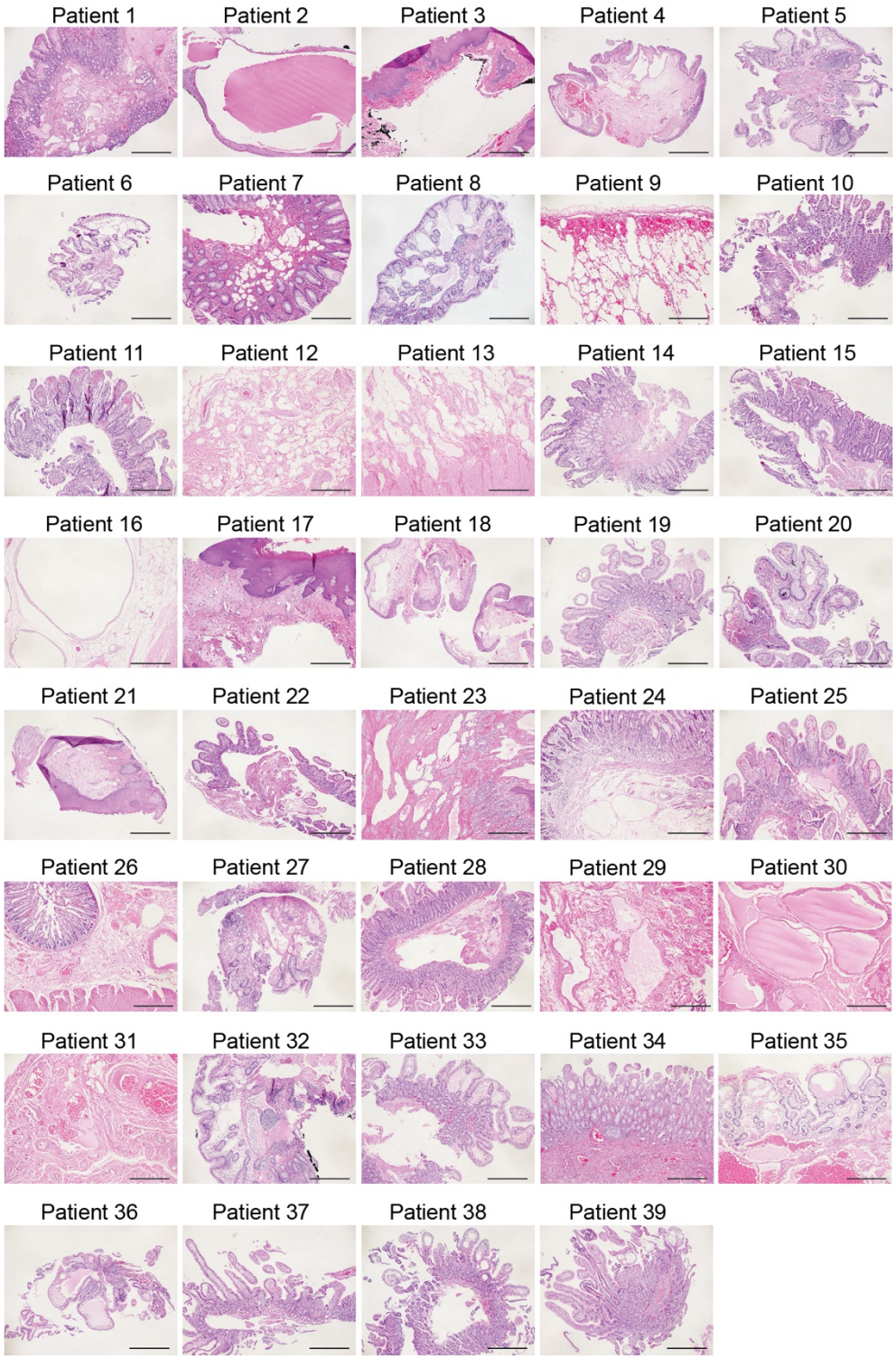
List of Supplementary Materials

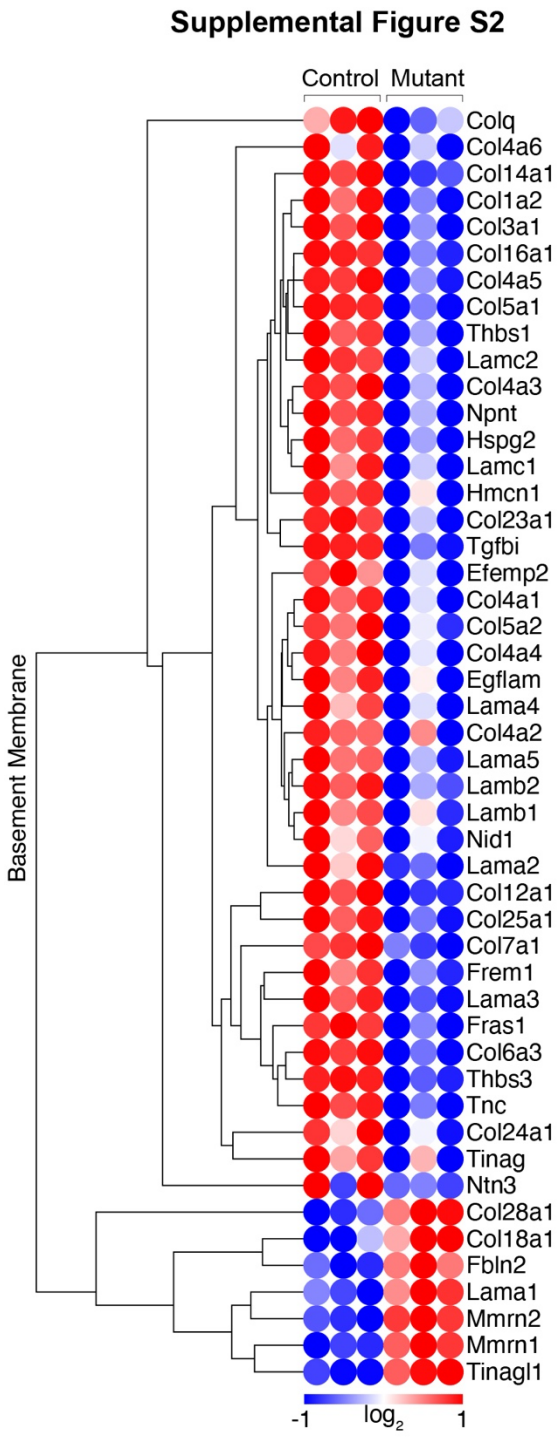
Supplemental figures 1 to 4

Supplemental table 1

Supplemental Figure S1

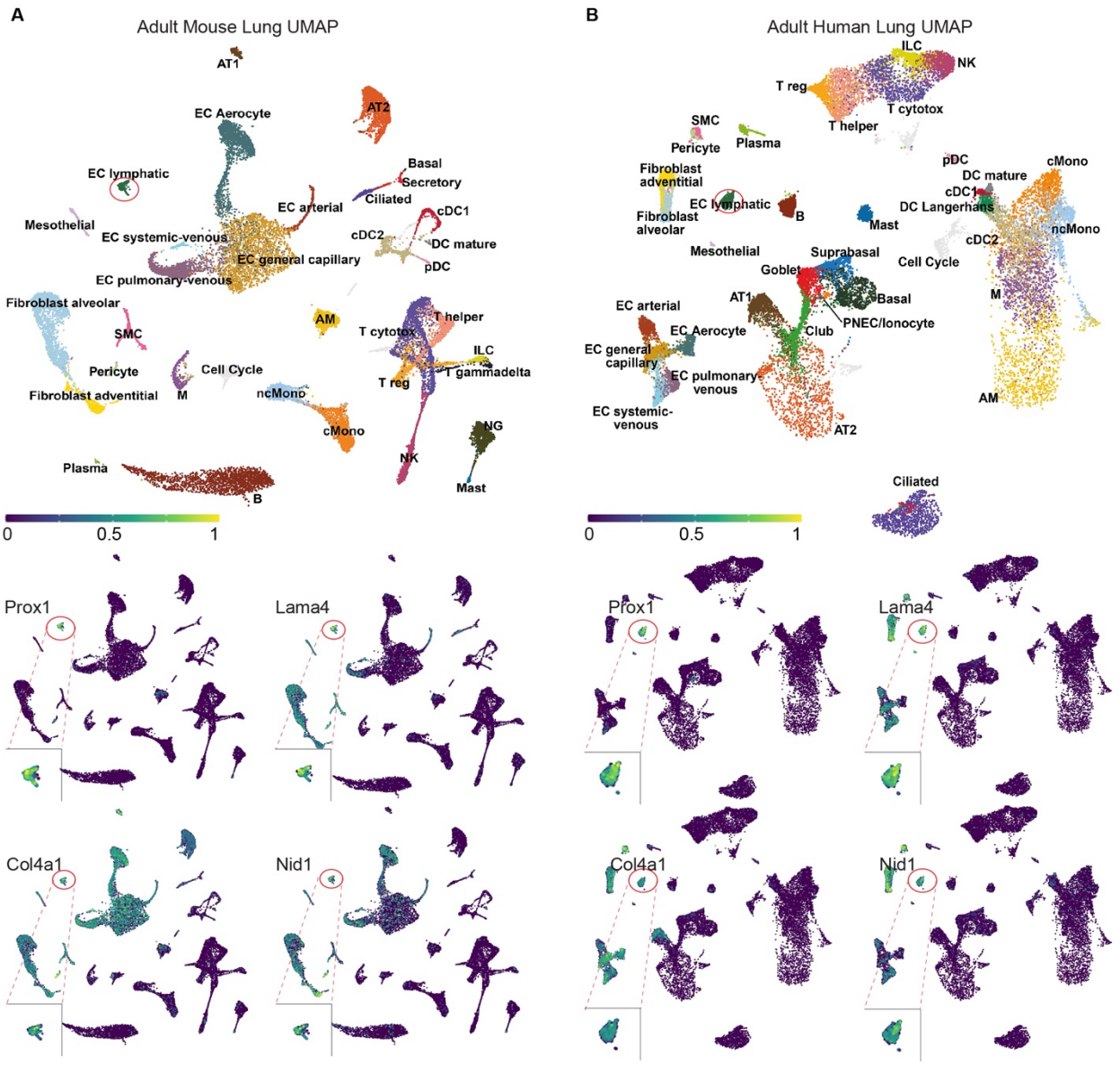


Supplemental Figure S1. Characterization of human lymphangiectasia tissue samples: Hematoxylin & Eosin-stained pathological human tissue section shows lymphangiectasia (n=39). Scale bar 500μm.



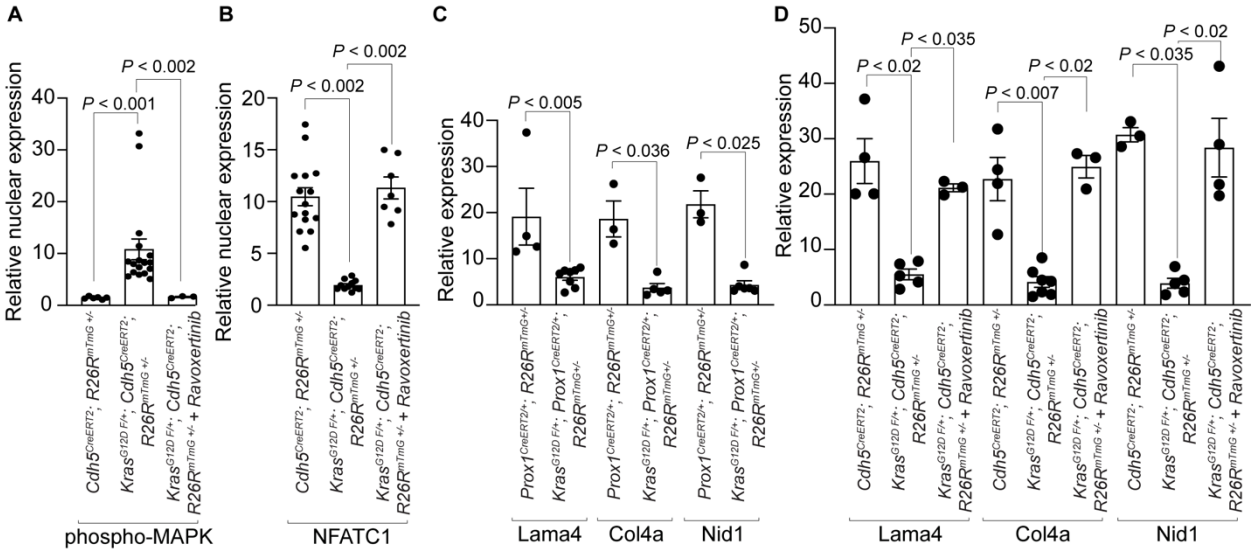
Supplemental Figure S2. Pathological Mapk activation downregulates basement gene transcription. Heatmap of top differentially regulated transcripts within the pathway categories of basement membrane in P12 $Kras^{G12D\ F/+}$; $Cdh5^{CreERT2}$ lungs treated with Tamoxifen compared with control lungs (n = 3).

Supplemental Figure S3



Supplemental Figure S3. Basement membrane gene expression in human and murine lungs at a single cell level: (A-B) Uniform manifold approximation and projection representation of the lung cell dataset (1) of 57,974 cells from 18 control mouse lungs **(A)** and 278,648 cells from 68 control human lungs **(B)**. Each dot represents a single cell. Basement membrane genes show robust expression in Prox1^{+} lymphatic endothelial cells (red oval). Green to yellow color dot represent higher expression of gene transcript. Purple color dot represent low to no expression of gene transcript. AM, alveolar macrophage; AT1/2, alveolar cell type 1/2; cDC1/2, classical dendritic cell type 1/2; cMono, classical monocyte; DC, dendritic cell; ILC, innate lymphoid cell; M, macrophage; ncMono, nonclassical monocyte; NK, natural killer; pDC, plasmacytoid dendritic cell; PNEC, pulmonary neuroendocrine cell; and SMC, smooth muscle cell.

Supplemental Figure 4



Supplemental Figure S4. (A-B) Quantification of nuclear phosphorylated-MAPK protein (A) or nuclear NFATC1 protein (B) expression relative to Hoechst nuclear staining in intercostal lymphatic vessels of *Kras*$^{G12D\ F/+}$*; Cdh5*^{CreERT2} mutant mice treated with or without Ravoxertinib. **(C-D)** Quantification of relative protein expression in pulmonary and intercostal lymphatic vessels of *Kras*$^{G12D\ F/+}$*; Prox1*^{CreERT2} (C) and *Kras*$^{G12D\ F/+}$*; Cdh5*^{CreERT2} treated with or without Ravoxertinib (D) mutant mice. Data represent the mean ± SEM. *P* values were determined by unpaired non-parametric Mann-Whitney test.

Supplemental Table S1. Antibodies used

Antibody	Company	Catalogue number	Species	Dilution
Lyve1	R&D Systems	AF2125	Goat	1:100
Prox1	Angiobio	11-002	Rabbit	1:100
GFP	Santa Cruz	sc-9996	Mouse	1:100
VE-Cadherin	R&D Systems	AF1002	Goat	1:100
Ki-67	abcam	ab15580	Rabbit	1:100
VegfR3	R&D Systems	AF743	Goat	1:100
Pdpn	DSHB	8.1.1	Hamster	1:100
SMA	abcam	ab5694	Rabbit	1:100
SMA	Sigma	A2547	Mouse	1:133
Collagen IV	Novus Bio	NB120-6586	Rabbit	1:200
Entactin/NID	abcam	ab254325	Rabbit	1:100
Laminin a4	R&D Systems	AF3837	Goat	1:100
Laminin a4	Novus Bio	NBP2-45582	Mouse	1:50
phospho-MAPK	Cell signaling	4370	Rabbit	1:50
Nfatc1	Cell signaling	8032	Rabbit	1:100
Nfatc1	Novus Bio	NB100-56732	Rabbit	1:50
IgG	Cell signaling	66362	Rabbit	1:10
Lyve1	R&D Systems	AF2089	Goat	1:100
Pdpn	Sigma	322M-1	Mouse	1:25
Phospho-Histone H3	Cell signaling	9706	Mouse	1:100
GFP	Santa Cruz	sc-8334	Rabbit	1:100
DyLight 488	Life Technologies	SA5-10086	Donkey	1:500
Alexa Fluor 488	Life Technologies	A-21202	Donkey	1:500
Alexa Fluor 488	Life Technologies	A-21206	Donkey	1:500
Anti-Hamster FITC	Life Technologies	31587	Rabbit	1:500
Alexa Fluor 546	Life Technologies	A-10036	Donkey	1:500
Alexa Fluor 568	Life Technologies	A-11057	Donkey	1:500
Alexa Fluor 568	Life Technologies	A-10042	Donkey	1:500
Alexa Fluor 568	Life Technologies	A-11079	Rabbit	1:500
Alexa Fluor 647	Life Technologies	A-31573	Donkey	1:500
Alexa Fluor 647	Life Technologies	A-21447	Donkey	1:500
Alexa Fluor 647	Life Technologies	A-32787	Donkey	1:500

Reference:

1. Schupp JC, Adams TS, Cosme Jr C, Raredon MSB, Yuan Y, Omote N, et al. Integrated Single Cell Atlas of Endothelial Cells of the Human Lung. *Circulation.* 2021.