Supplemental material

Preclinical validation and imaging of Wnt/beta-catenin-induced lung repair in human 3D lung tissue cultures

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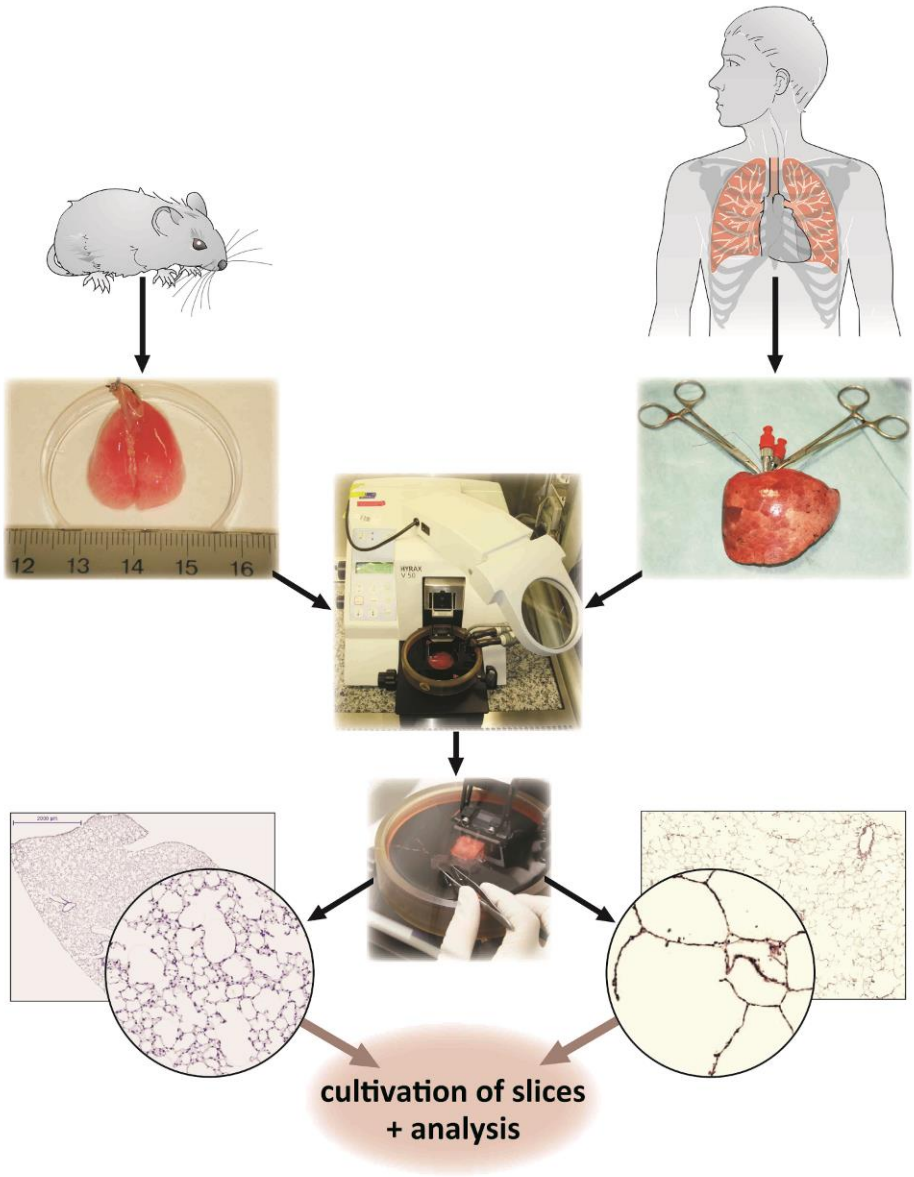
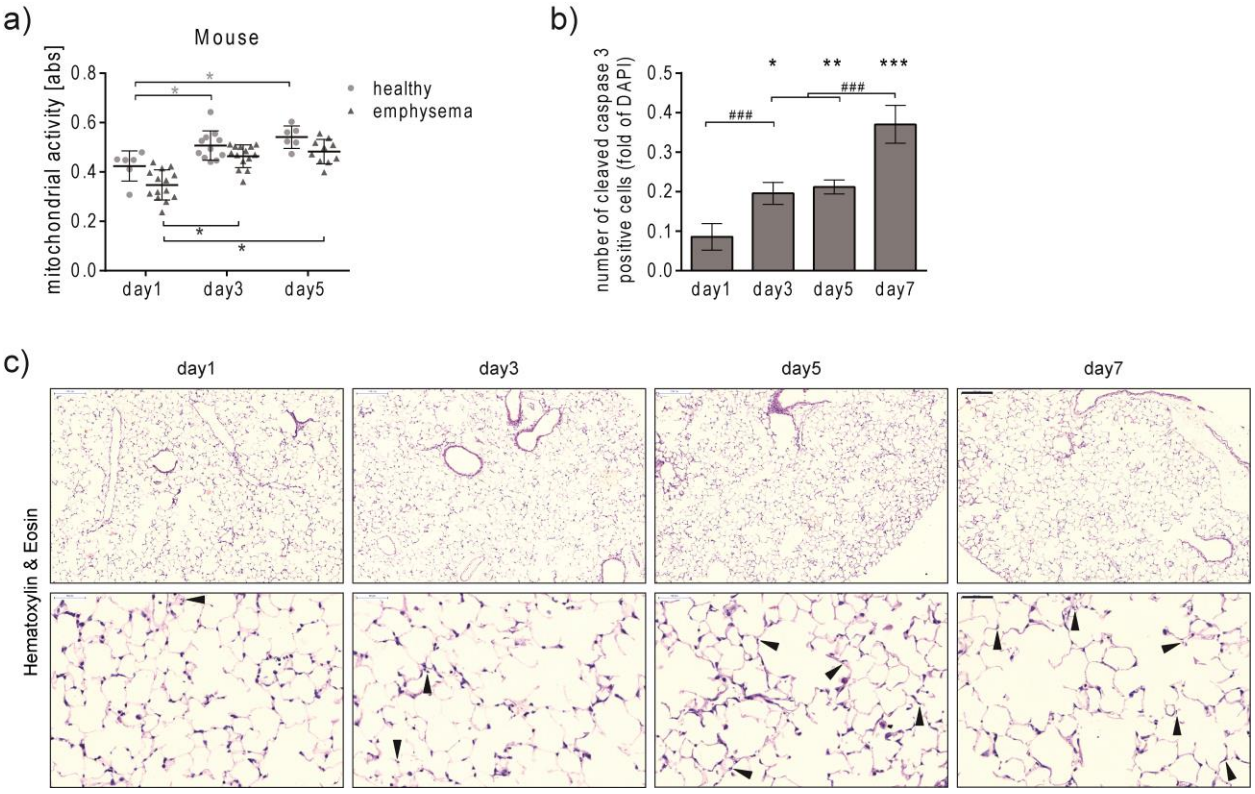
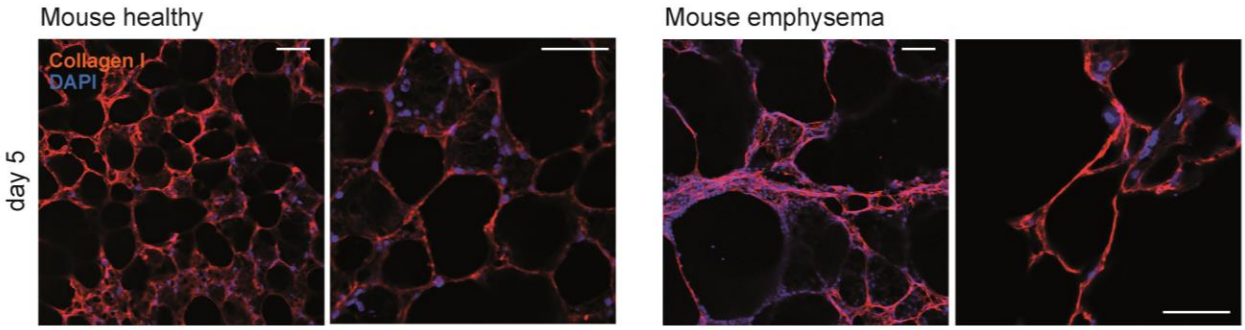


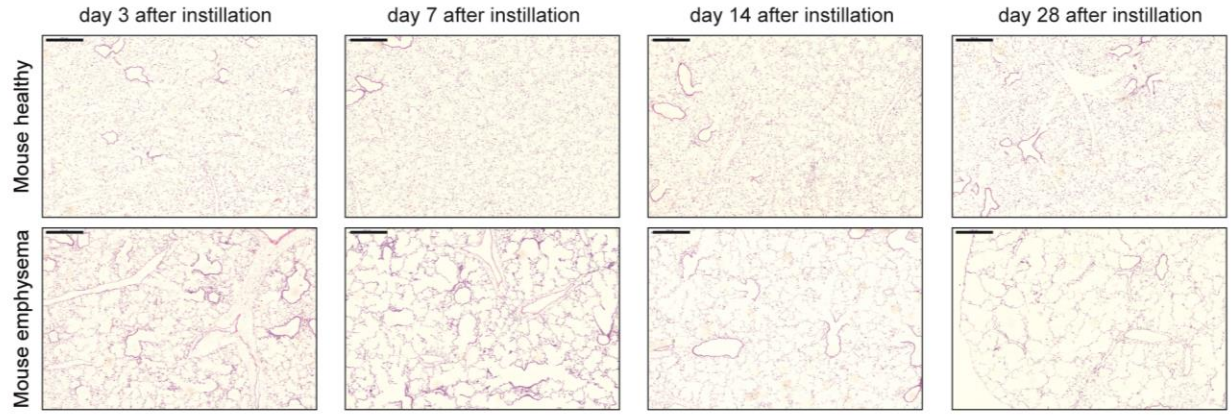
Supplemental Figure 1: Schematic overview of the generation of 3D *ex vivo* tissue cultures (3D-LTC) from murine and human samples. Lung tissue was filled with agarose and sliced with a vibratome to 300 (murine) or 500 µm sections (human). A detailed protocol is provided in the Material and Methods section.



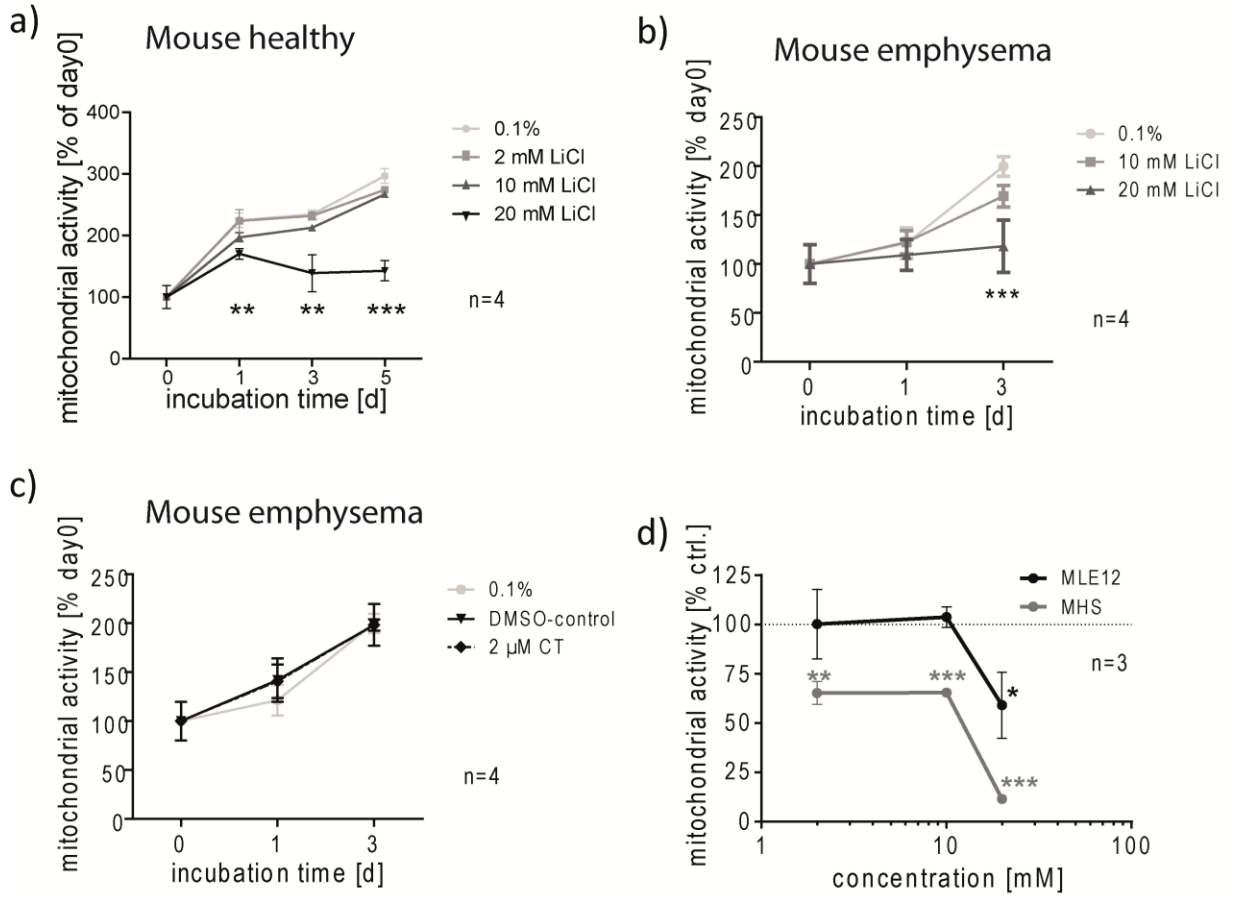
Supplemental Figure 2: Characterization of cell viability in 3D-LTC over culture time. a) WST-1 conversion of the cells in 3D-LTC from control and emphysematous C57Bl/6 mice cultivated for five days. n=6-14. *: $p<0.05$. b) Quantification of the cleaved caspase 3 positive stained cells in 3D-LTC from healthy C57Bl/6 mice at different time points of cultivation. *: significant to day1, #: significant to other time point, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. c) H&E staining of 3D-LTC from healthy C57Bl/6 mice during cultivation. Black arrows show fragmented nucleii. Scale bars 200 µm and 50 µm.



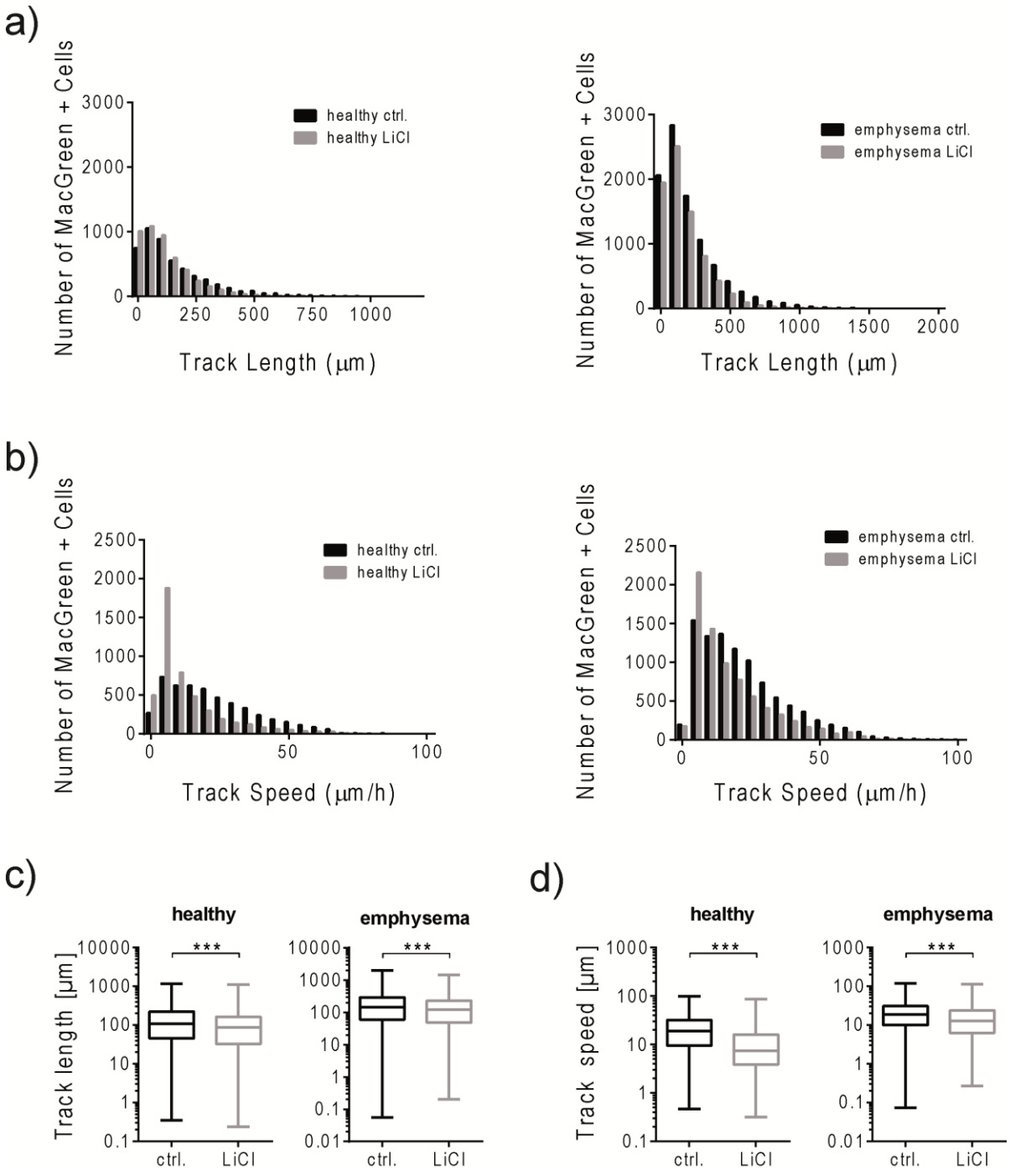
Supplemental Figure 3: Lung structure in murine 3D-LTC over culture time. Immunofluorescence staining of collagen I in 3D-LTC from healthy and emphysematous C57Bl/6 mice at day 5 of cultivation. Scale bars 50 µm.



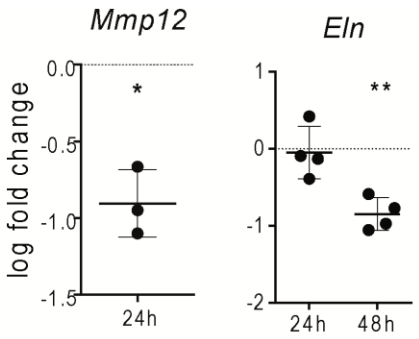
Supplemental Figure 4: Time course of elastase-induced emphysema. H&E staining of 3D-LTC at day 0 from healthy and emphysematous C57Bl/6 mice at different time points after elastase or PBS instillation. Scale bar 500 µm.



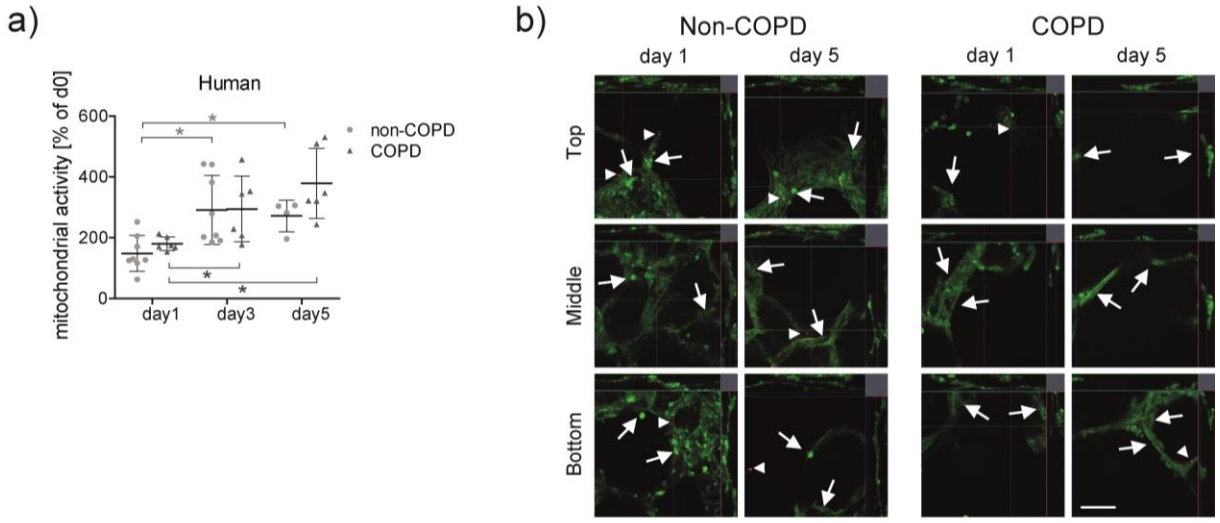
Supplemental Figure 5: Mitochondrial activity upon LiCl and CT treatment in 3D-LTC and 2D cell culture. WST-1 conversion of the cells in 3D-LTC from healthy (a) and emphysematous (b, c) C57Bl/6 mice treated with different concentrations of LiCl and CT for different periods. d) WST-1 conversion of the MLE12 epithelial cells and MHS macrophages treated with different concentrations of LiCl for 24 h compared to control (=100%). n=3-4. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.



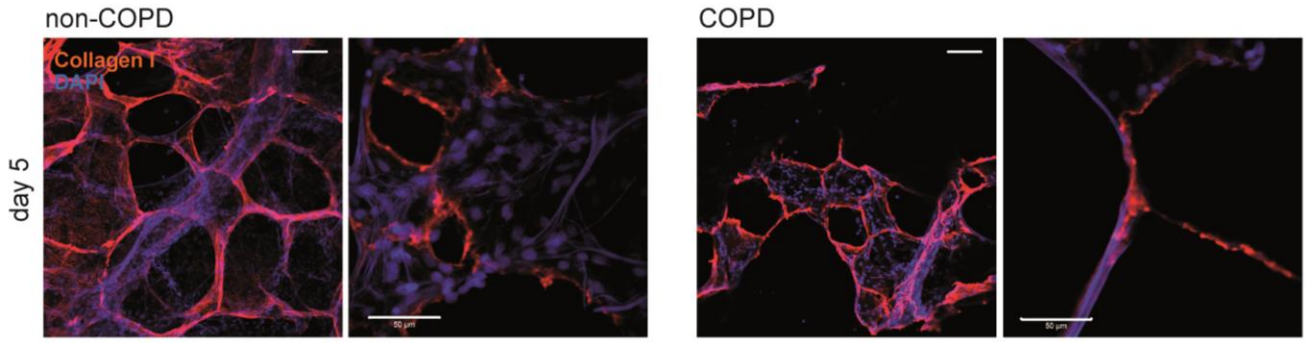
Supplemental Figure 6: Assessment of macrophage speed and length in healthy and emphysematous 3D-LTC by 4D confocal live tissue imaging. Quantification of track length (a) and track speed (b) of macrophages in 3D-LTC from healthy and emphysematous MacGreen animals with LiCl treatment for 48 h. Box plots of track length (c) and track speed (d). ***: $p<0.001$. Quantification derived from Video 3.



Supplemental Figure 7: *Mmp12* and *Eln* level upon Wnt/beta-catenin activation by 2 µM CT in murine 3D-LTC. Gene expression analysis by qPCR in 3D-LTC from emphysematous C57Bl/6 mice treated with CT for different periods. n=3-4. *: p<0.05, **: p<0.01.



Supplemental Figure 8: Characterization of cell viability in human 3D-LTC over culture time. a) WST-1 conversion of the cells in 3D-LTC from non-COPD and COPD patients cultivated for five days. n=4-8. *: p<0.05. b) Representative Live/Dead confocal images of the different planes of 3D-LTC from non-COPD and COPD patients at the indicated time points. Ortho views of the top, middle, and bottom plane. Healthy cells are indicated by green cytoplasm (arrows) and dead cells by red nucleus (arrowheads).



Supplemental Figure 9: Lung structure in patient-derived 3D-LTC over culture time. IF staining of collagen I of 3D-LTC from non-COPD and COPD patients at day 5 of cultivation. Scale bars 50 µm.

Supplemental Table 1. Clinical characteristics of patients included in this study

	COPD	Non-COPD
	(n = 11)	(n = 14)
Patient age at time of surgery (years)	66 ± 7.8	62.1 ± 8.6
Gender (M:F)	7:4 (63.6%/36.4%)	7:7 (50%/50%)
Smoking status		
Current smoker	8 (72.7%)	5 (35.7%)
Former smoker	2 (18.2%)	5 (35.7%)
Never	0 (0%)	2 (14.2%)
Unknown	1 (9.1%)	2 (14.2%)
Physiology		
%FEV_1	74.2 ± 13.6	107.1 ± 15.1
FEV_1/FVC %	64.7 ± 5.6	80.1 ± 5.6
%DLCO/VA	63.7 ± 25.3†	74.2 ± 17.6†
COPD stage (GOLD Guidelines)		
I	5 (45.5%)	
II	6 (54.5%)	
Cancer type		
Adenocarcinoma	5 (45.5%)	10 (71.4%)
Squamous cell carcinoma	2 (18.2%)	3 (21.4%)
Other adenocarcinomas*	4 (36.4%)	1 (7.1%)

Data are given as mean ± SD or as number of patients (%)
** metastasis from organ other than the lung*
† *three unknown values*

Supplemental Table 2. Human primers used for qPCR

Gene		Sequence 5' - 3'
ACTA2	fw	CGAGATCTCACTGACTACCTCATGA
	rv	AGAGCTACATAACACAGTTTCTCCTTGA
AXIN2	fw	AGAAATGCATCGCAGTGTGAAG
	rv	GGTGGGTTCTCGGGAAATG
COL1A1	fw	CAAGAGGAAGGCCAAGTCGAG
	rv	TTGTCGCAGACGCAGATCC
DKK2	fw	GATGTCACATATAAAAGGGCATGAAG
	rv	AATGACGAGCACAGCAAAACC
HOPX	fw	GCCCCACAGAGGACCAGGTG
	rv	GCTTGGTTAAGCGGAGGAGAG
HPRT	fw	AAGGACCCCACGAAGTGTTG
	rv	GGCTTTGTATTTTGCTTTTCCA
MMP12	fw	TGCTGATGACATACGTGGCA
	rv	AGGATTTGGCAAGCGTTGG
NKD1	fw	CACCCTGTATGACTTTGACAACAAC
	rv	CAGAGGAGTCCACCACCTCATAG
PDPN	fw	GAGAAAGATGGTTTGTCAACAGTG
	rv	GGCGTAACCCTTCAGCTCT
SFTPC	fw	GCCCAGTGCACCTGAAACGC
	rv	TCTCCAGAACCATCTCCGTGTGT

Supplemental Table 3. Murine primers used for qPCR

Gene		Sequence 5' - 3'
Axin2	fw	AGCAGAGGGACAGGAACCA
	rv	CTGAACCGATTCATGACCAC
Col1a1	fw	AGCTTTGTGGATACGCGGACT
	rv	TCGTACTGATCCCGATTGCA
Dkk2	fw	GAGATCGCAACCATGGTCACT
	rv	GGGTCTCCTTCATGTCCTTTTATATG
Eln	fw	GGCGTCTTGCTGATCCTCT
	rv	ATAATAGACTCCACCGGGAACT
Fgfr2	fw	ACACAGATAGCTCCGAAGACGTTGT
	rv	CCCAGCCGGACAGCGGAACT
Fn	fw	GGTGTAGCACAACTTCCAATTACG
	rv	GGAATTTCCGCCTCGAGTCT
Hopx	fw	TCTCCATCCTTAGTCAGACGC
	rv	GGGTGCTTGTTGACCTTGTT
Hprt	fw	CCTAAGATGAGCGCAAGTTGAA
	rv	CCACAGGACTAGAACACCTGCTAA
Mmp12	fw	TGTACCCCACCTACAGATACCTTA
	rv	CCATAGAGGGACTGAATGTTACGT
Nkd1	fw	TGTTCTCATCCACGCAATGG
	rv	GAGCCCCACTCAGGTTCCA
Pdpn	fw	ACAGGTGCTACTGGAGGGCTT
	rv	TCCTCTAAGGGAGGCTTCGTC
Sftpc	fw	AGCAAAGAGGTCCTGATGGA
	rv	GAGCAGAGCCCCTACAATCA
Wnt2	fw	AGCCCTGATGAACCTTCACAAC
	rv	TGACACTTGCATTCTTGTTTCAAG

Supplemental Table 4. Antibodies for Western Blot

Name	Source	Manufacturer	Order nr.	Dilution	Size [kDa]
β-actin	Mouse	Sigma-Aldrich; Taufkirchen Germany	A3854	1:50,000	42
β-catenin, active	Mouse	Millipore; Billerica, MA, USA	05-665	1:500	92
Elastin	Mouse	Millipore; Billerica, MA, USA	MAB2503	1:1,000	68
Hopx	Rabbit	Santa Cruz; Santa Cruz, CA, USA	sc-30216	1:1,000	13
Podoplanin	Goat	R&D; Minneapolis, MN, USA	AF3244	1:1,000	40
Podoplanin	Rat	Acris; Herford, Germany	AM01133PU-N	1:1,000	37

Supplemental Table 5. Antibodies for immunofluorescence

Name	Source	Manufacturer	Order nr.	Dilution
Aquaporin 5	Rabbit	Calbiochem, Millipore; Billerica, MA, USA	178615	1:100
CD45	Rat	BD; San Jose, CA, USA	553076	1:500
Cleaved caspase3	Rabbit	Cell Signalling Technology; Boston, MA, USA	9661	1:250
Collagen 1	Rabbit	Rockland; Gilbertsville, PA, USA	600-401-103	1:100
E-cadherin	Mouse	BD; San Jose, CA, USA	610181	1:100
Podoplanin	Goat	R&D; Minneapolis, MN, USA	AF3244	1:100
Podoplanin	Rat	Acris; Herford, Germany	AM01133PU-N	1:100
Sftpc, pro-	Rabbit	Abcam; Cambridge, UK	ab40879	1:500
Sftpc, pro-	Rabbit	Millipore; Billerica, MA, USA	AB3786	1:500

Supplemental Videos

Video 1: Ciliated cell of a murine 3D-LTC at day seven. Cilia are still beating, demonstrating cell viability and functionality.

Video 2: 4D confocal live tissue imaging of a 3D-LTC from an emphysematous TCF/LEF-GFP animal showing increases in GFP positive cells following 10 mM LiCl treatment.

Video 3: 4D confocal live tissue imaging of 3D-LTC from healthy MacGreen animals treated with ctrl. (left panel) or 10 mM LiCl (right panel) for 48 h showing subjective decreases in macrophage speed and distance travelled following LiCl treatment (quantitative characterization in Supplemental Figure 6).

Video 4: 4D confocal live tissue imaging of 3D-LTC from emphysematous MacGreen animals treated with ctrl. (left panel) or 10 mM LiCl (right panel) for 48 h showing subjective decreases in macrophage speed and distance travelled following LiCl treatment (quantitative characterization in Supplemental Figure 6).