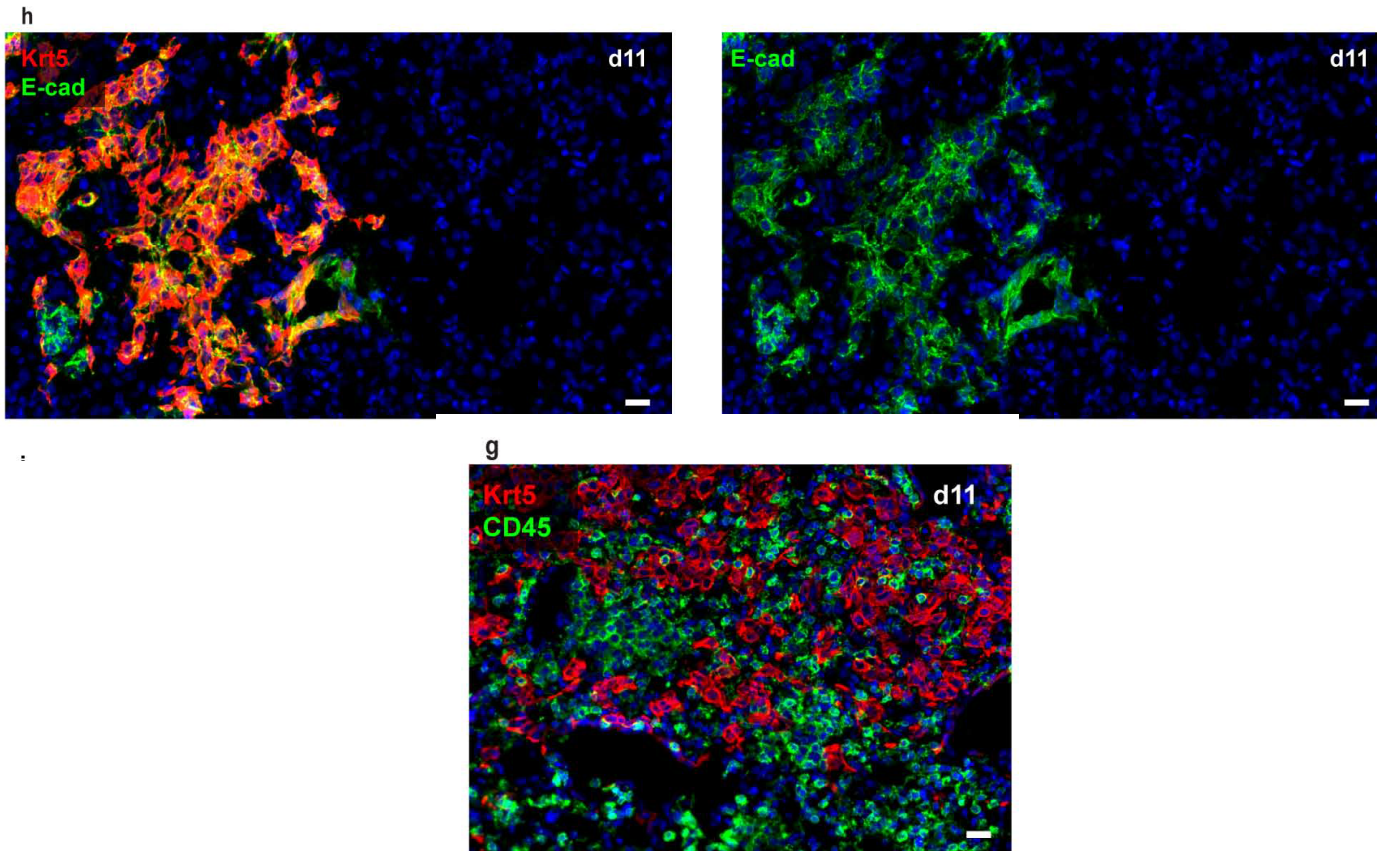


# Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury

Andrew E. Vaughan et al.,  
*Nature* 517, 621–625  
(29 January 2015)

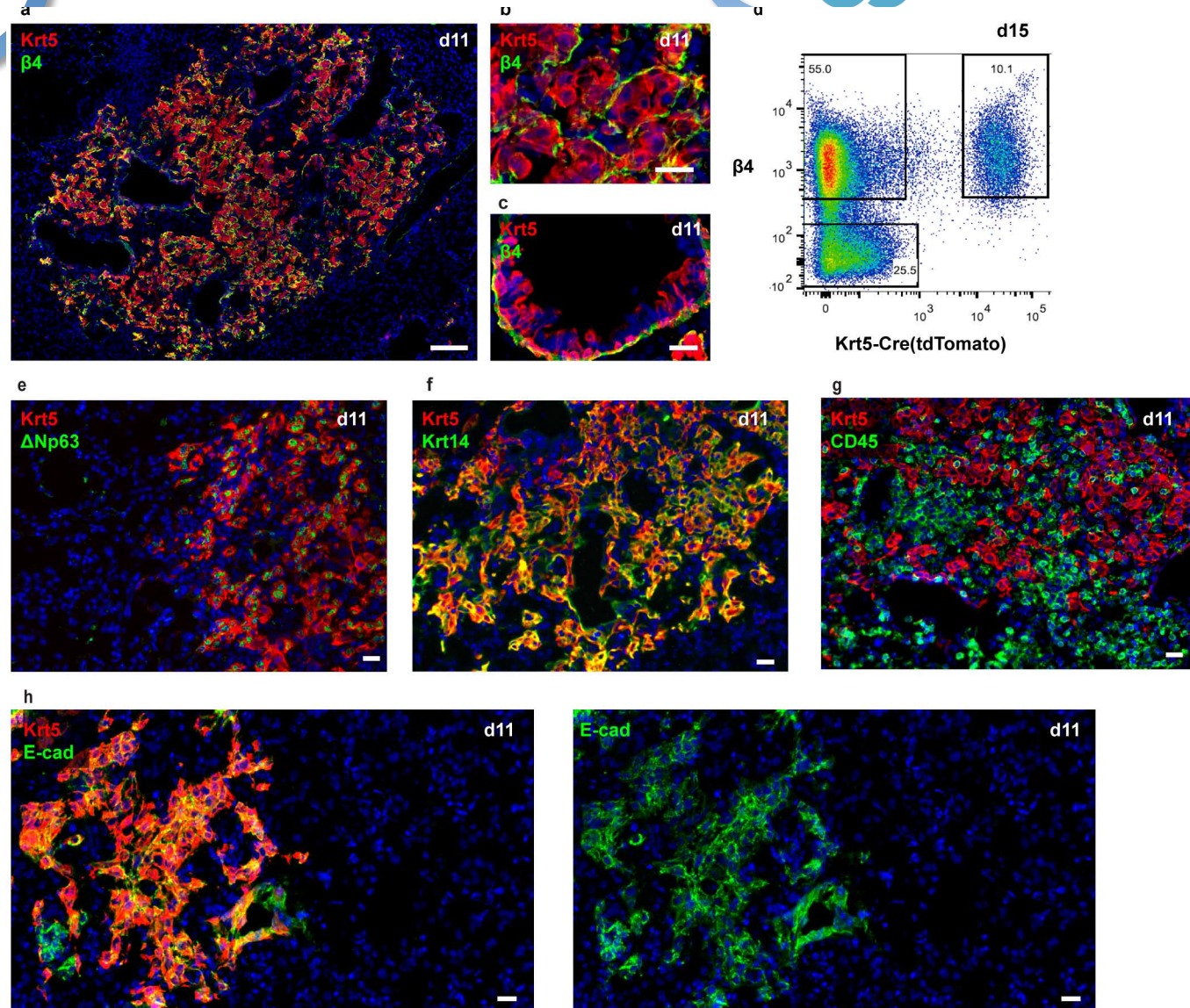
After influenza infection → ablation of epithelial cells



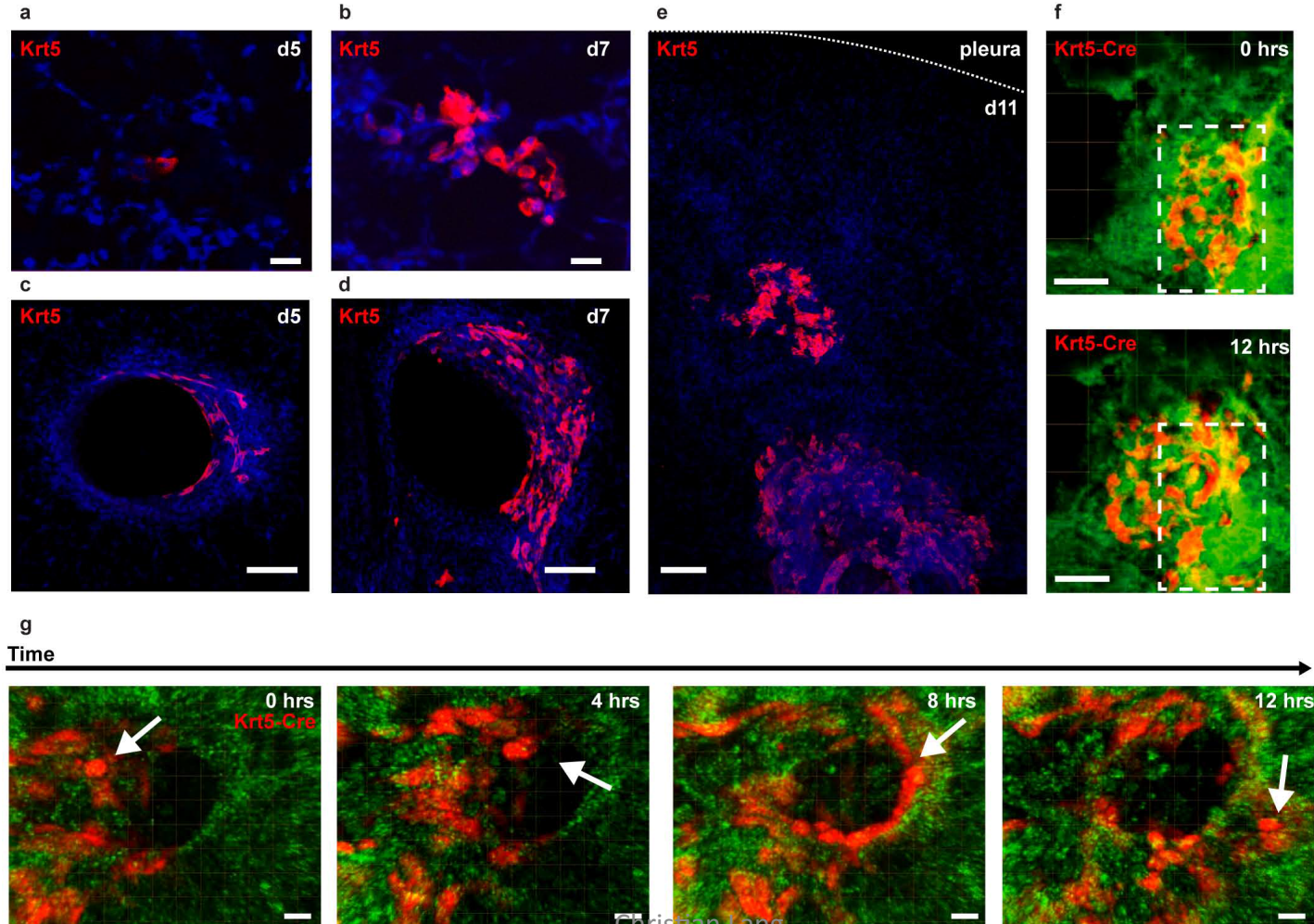


## After influenza infection...

- expansion of Krt5<sup>+</sup> cells
- Coexpression of integrin beta<sub>4</sub>



After bleomycin injury → also expansion of Krt5<sup>+</sup>-cells



Christian Lang

n1242366@students.meduniwien.ac.at

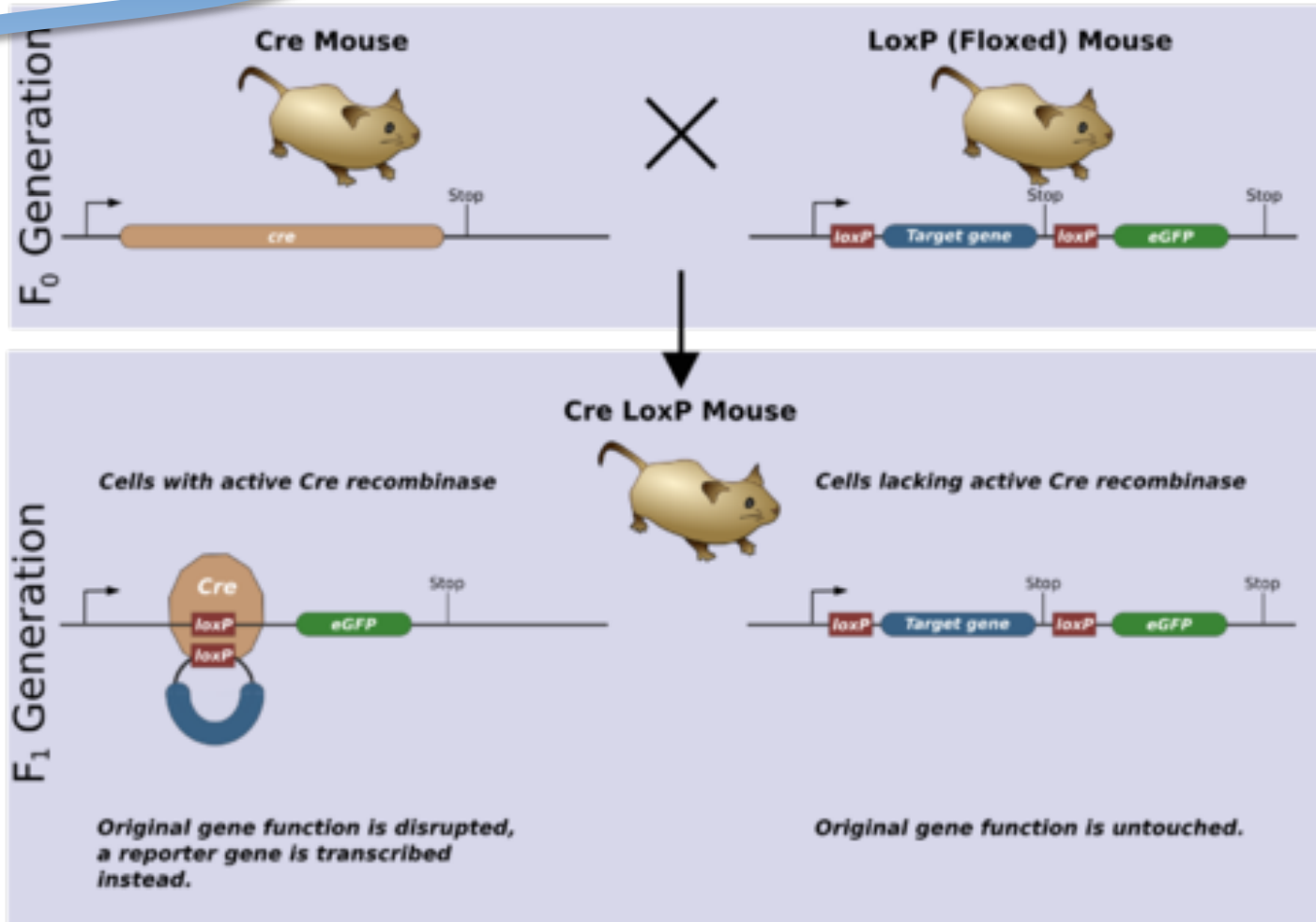
- After injury (influenza, bleomycin) → **expansion** of **Krt5<sup>+</sup> cells** in the lung parenchyma
- Cellular origin still unknown...



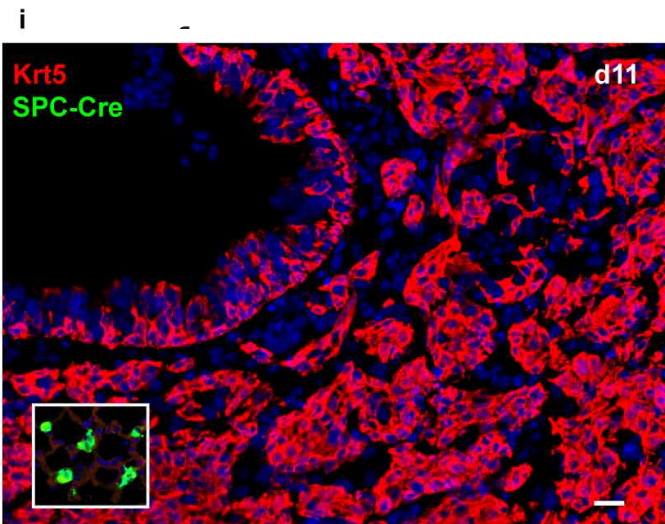
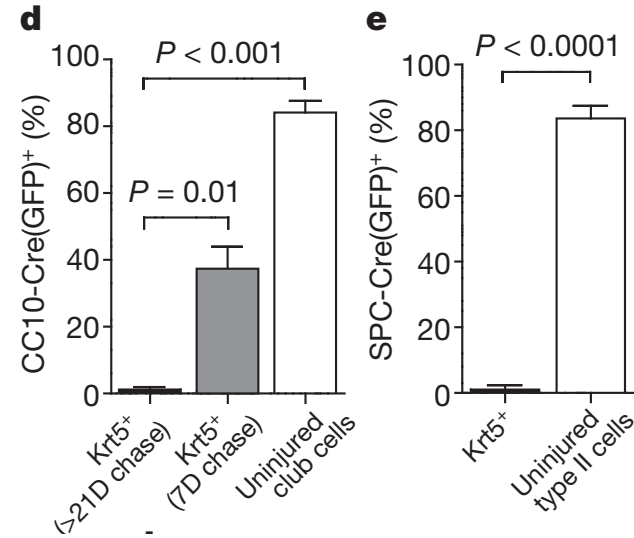
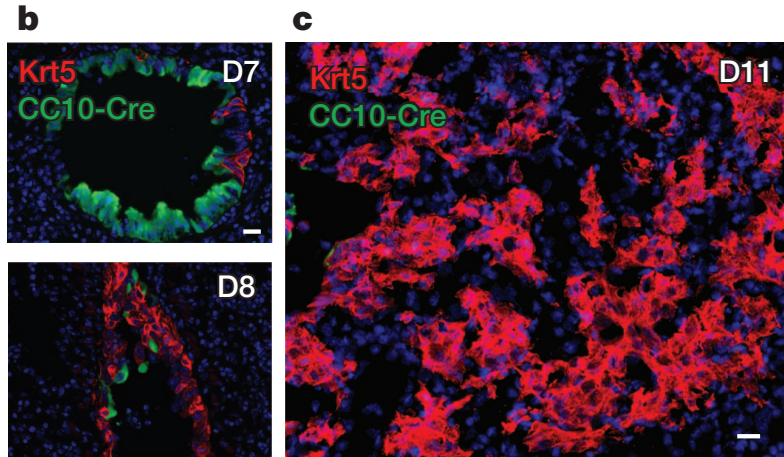
**Lineage-tracing**  
with lungspecific Cre-recombinase drivers (*CC10* and *SPC*)



## Lineage-tracing using the Cre-lox system



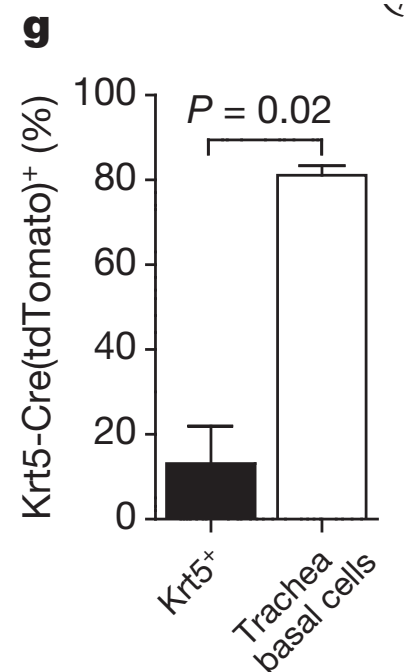
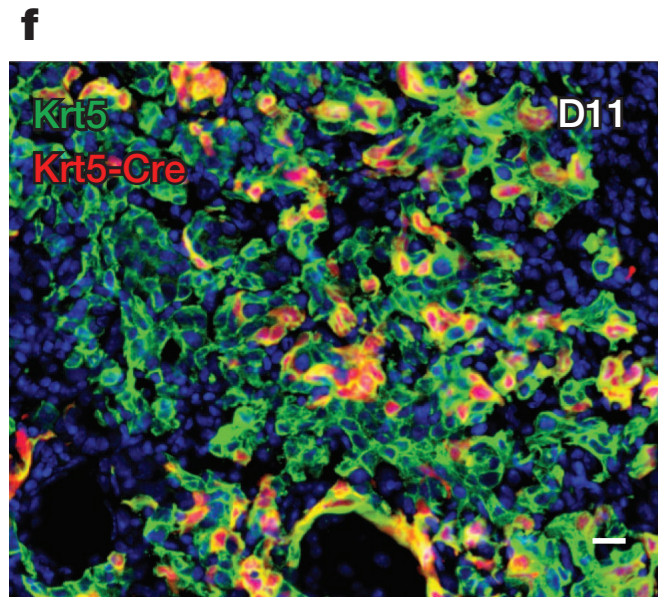
Source: [http://en.wikipedia.org/wiki/Cre-Lox\\_recombination#/media/File:CreLoxP\\_experiment.png](http://en.wikipedia.org/wiki/Cre-Lox_recombination#/media/File:CreLoxP_experiment.png)

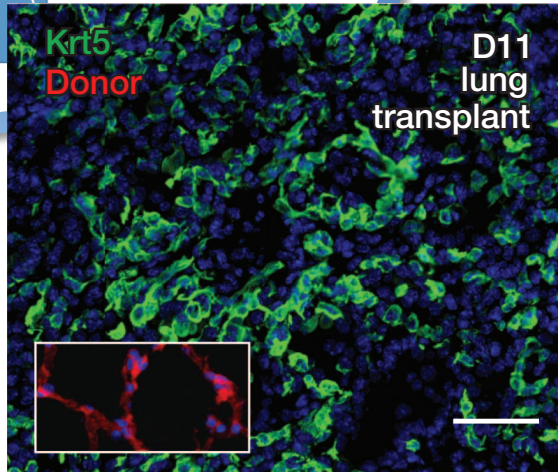


Krt5<sup>+</sup>-cells are **untraced**



Small fraction (13%) of expanded Krt5<sup>+</sup>-cells are **labelled** (*Krt5-CreERT2*)



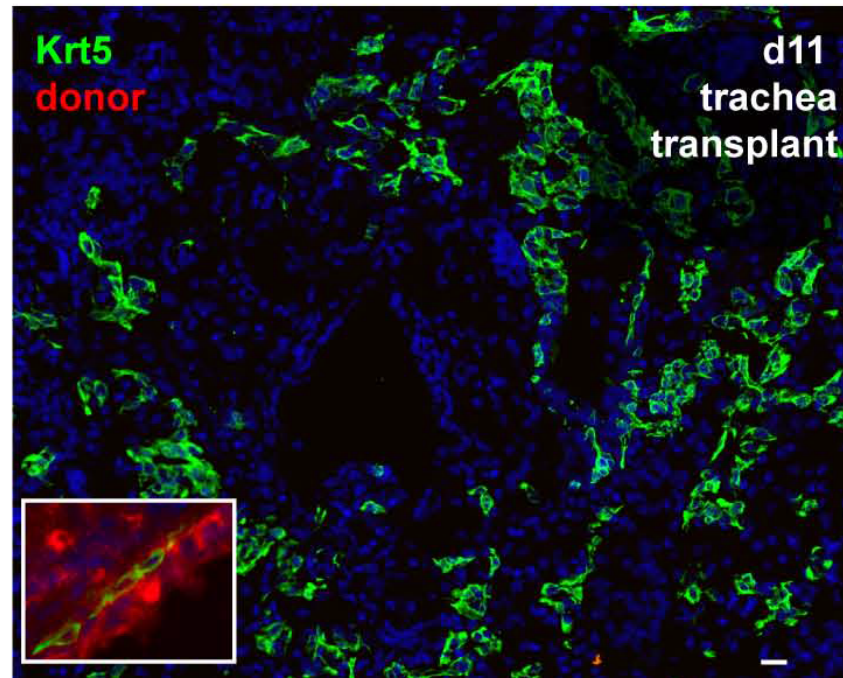


After transplantation  
→ expansion of Krt5<sup>+</sup>-cells  
→ But Krt5<sup>+</sup>-cells are not fluorescent

j



k

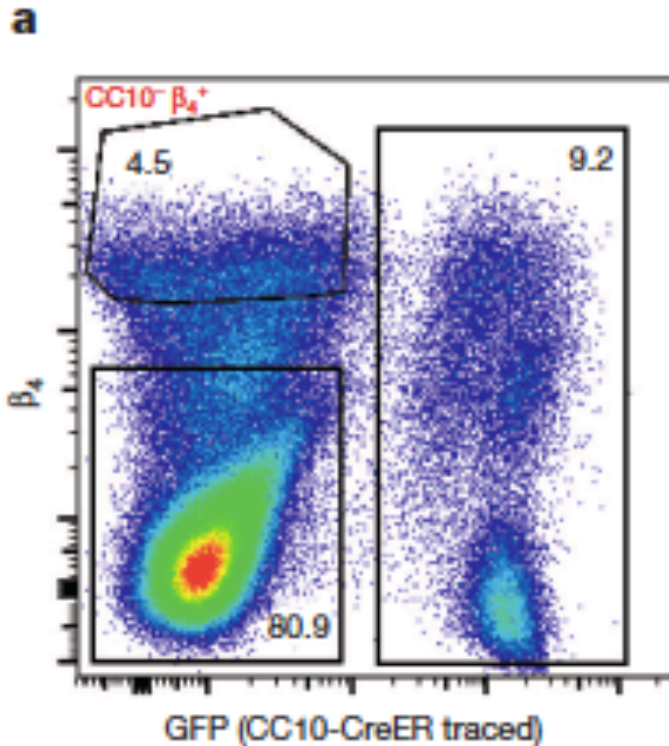


Major source of Krt5<sup>+</sup> (and Np63<sup>+</sup>) cells



**a lineage-negative epithelial progenitor  
(LNEP)**

Characterizing and segregating the LNEP  
-Beta<sub>4</sub>-expression in CC10-CreERT2 mice -



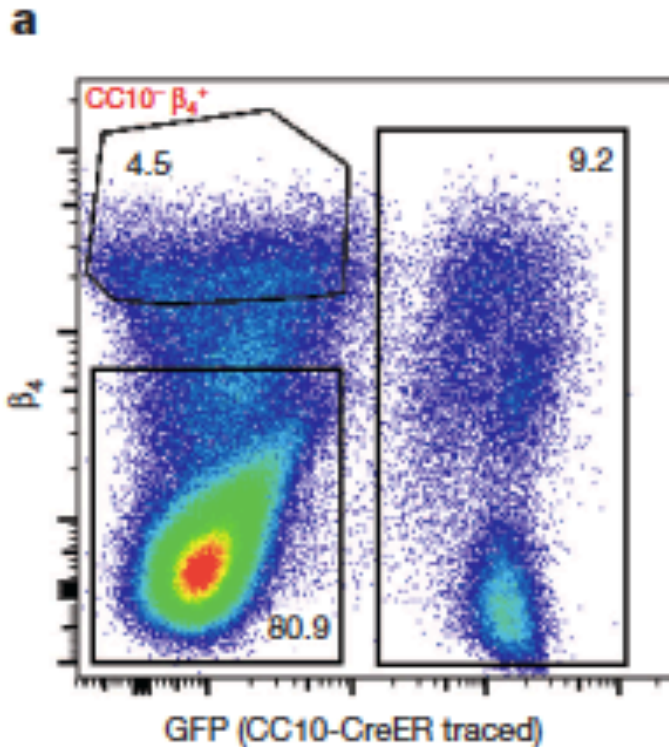
**fold change vs. type II cells**

	clara cells	CC10- $\beta_4^+$
SPC	0.25 ± 0.1	0.023 ± 0.01
ABCA3	0.46 ± 0.06	0.05 ± 0.01
CC10	89.2 ± 25.5	7.2 ± 2.1
Aqp5	0.49 ± 0.04	0.017 ± 0.01
CGRP	1.12 ± 0.12	0.19 ± 0.06
FoxJ1	85.8 ± 25.8	271 ± 73
$\Delta$ Np63	ND	detected
Hey1	66.2 ± 14.0	161 ± 82.0
Jag2	1.22 ± 0.41	4.46 ± 1.51
Sox2	481 ± 51	1444 ± 152

CC10- $\beta_4^+$  cells → LNEP-containing



## Np63 – expression in the LNEP-containing cells



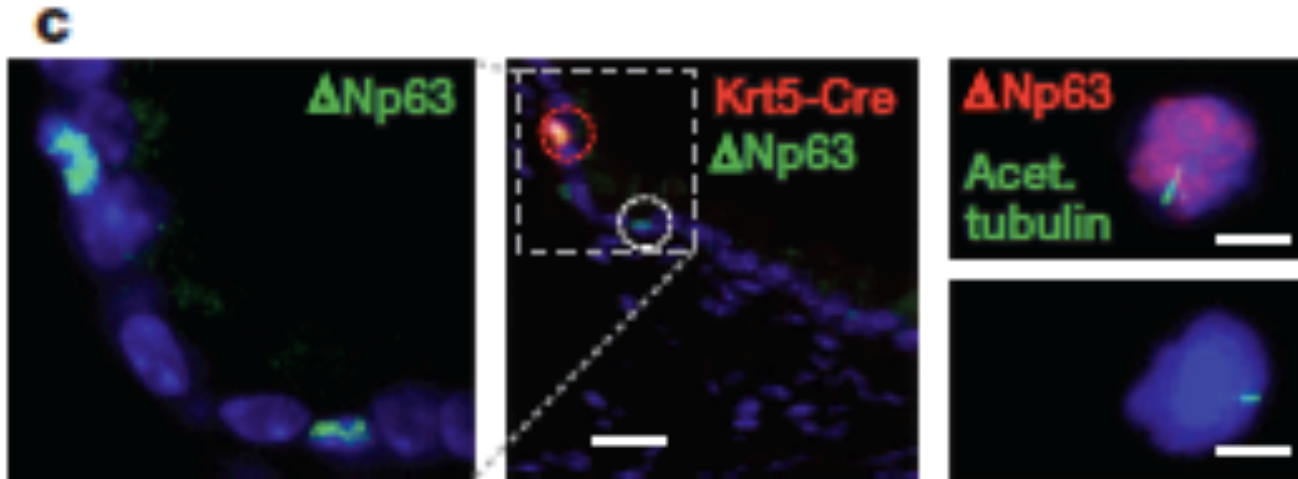
**c**

### fold change vs. type II cells

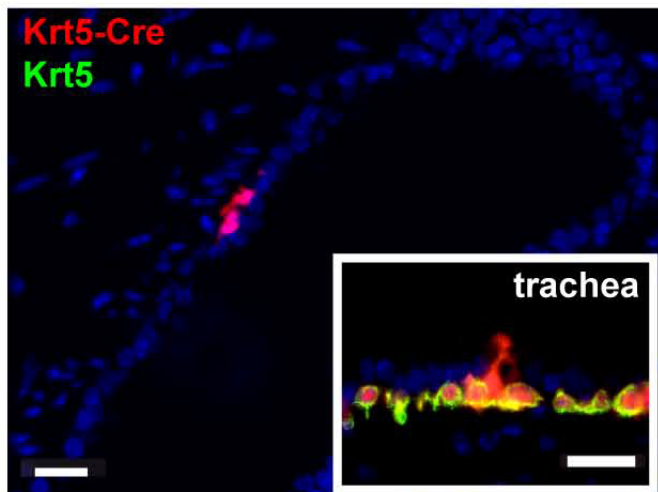
	clara cells	CC10- β <sub>4</sub> <sup>+</sup>
SPC	0.25 ± 0.1	0.023 ± 0.01
ABCA3	0.46 ± 0.06	0.05 ± 0.01
CC10	89.2 ± 25.5	7.2 ± 2.1
Aqp5	0.49 ± 0.04	0.017 ± 0.01
CGRP	1.12 ± 0.12	0.19 ± 0.06
FoxJ1	85.8 ± 25.8	271 ± 73
<b>ΔNp63</b>	ND	detected
Hey1	66.2 ± 14.0	161 ± 82.0
Jag2	1.22 ± 0.41	4.46 ± 1.51
Sox2	481 ± 51	1444 ± 152

CC10-β<sub>4</sub><sup>+</sup> cells → LNEP-containing





**a**



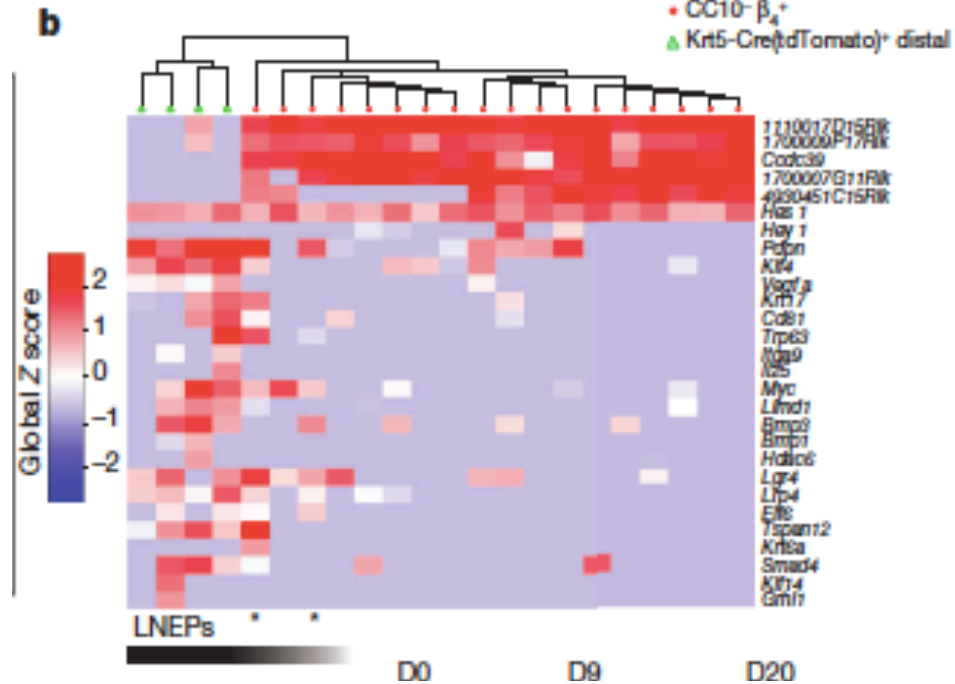
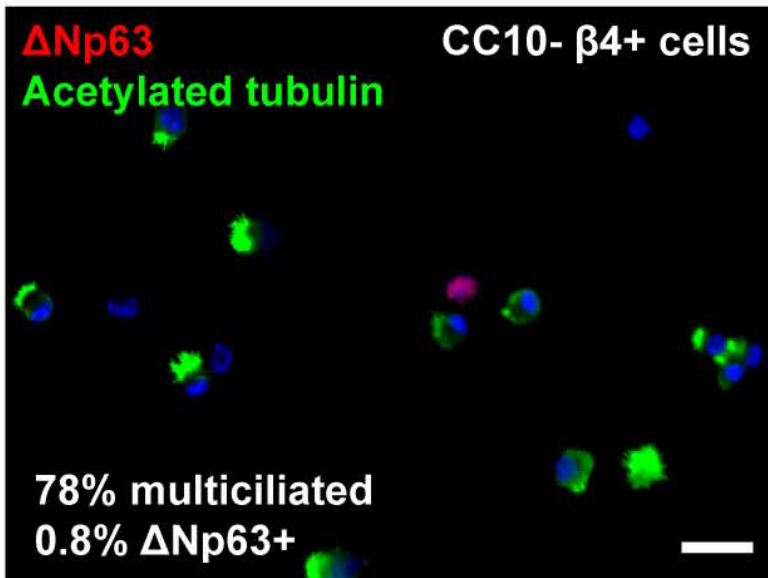
Np63<sup>+</sup>-cells → scattered sporadically in uninjured distal airways (figure c)

Np63<sup>+</sup>-cells → no expression of Krt5 protein (figure a)

*Maybe reason for weak labelling of Krt5<sup>+</sup>-cells...(Fig. 1f,g)*

Analyzing the  $CC10\text{-}\beta_4^+$  and the  $Krt5\text{-CreERT2}$ -population...

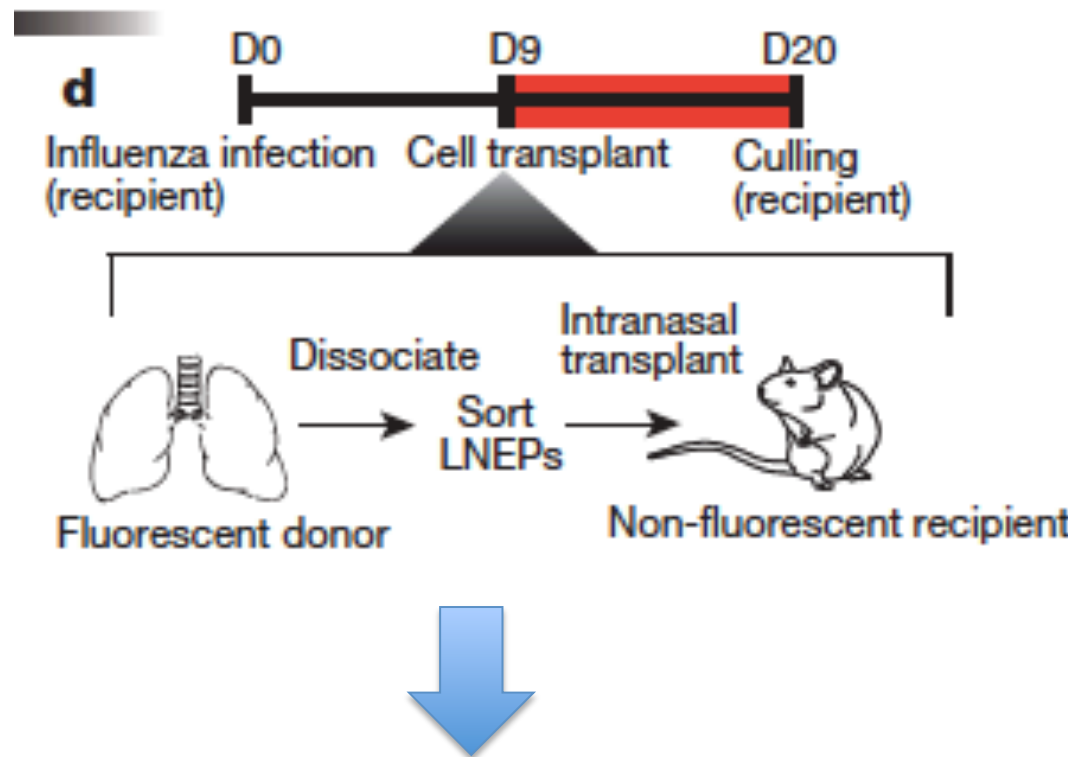
b



78% of  $CC10\text{-}\beta_4^+$  cells  $\rightarrow$  multiciliated

- Np63 transcript in
  - CC10-Beta<sub>4</sub><sup>+</sup>-cells
  - (rare) Krt-CreERT2-labelled cells *subset*
- Enrichment for **pluripotency**-associated transcription factors (Myc, Klf4) in the **LNBP** (Krt5-CreERT-2-labelled cells) group

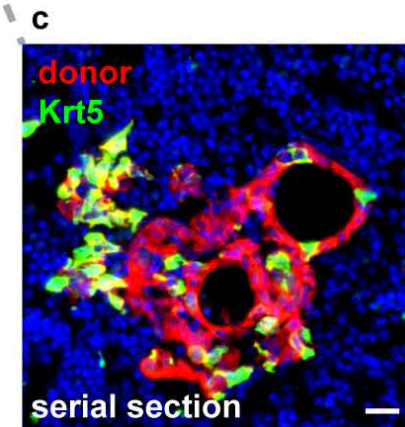
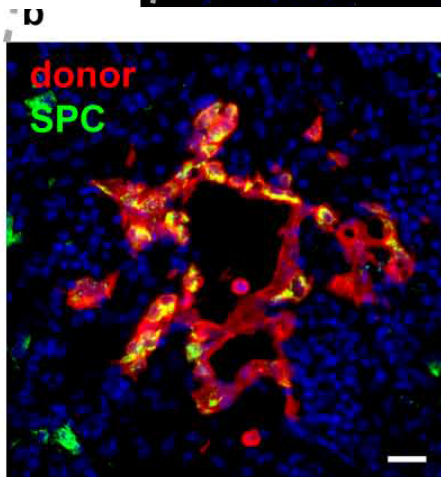
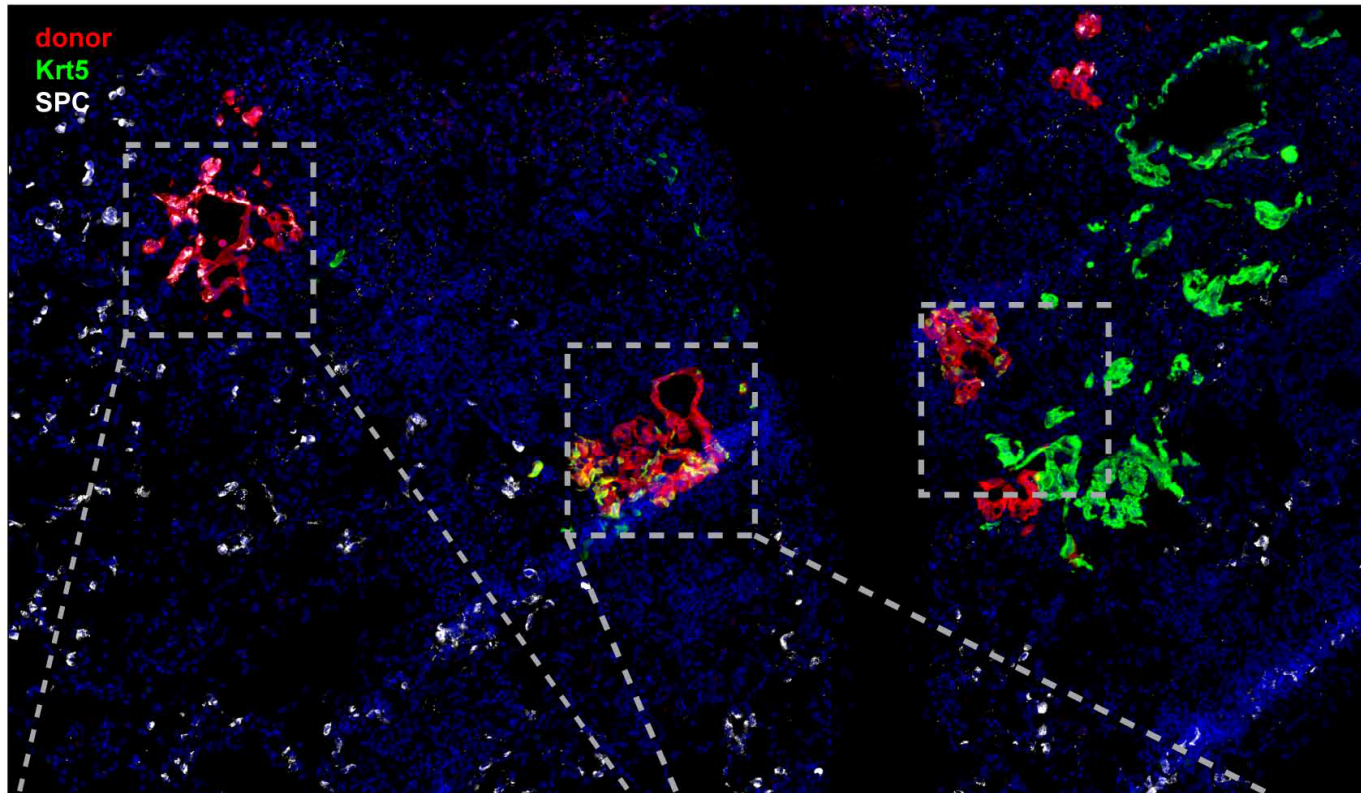
## Transplantation of **CC10-Beta<sub>4</sub><sup>+</sup>** cells (*LNEP containing*) into influenza-injured mice



Development of LDEPs → **dependent on local microenvironment**

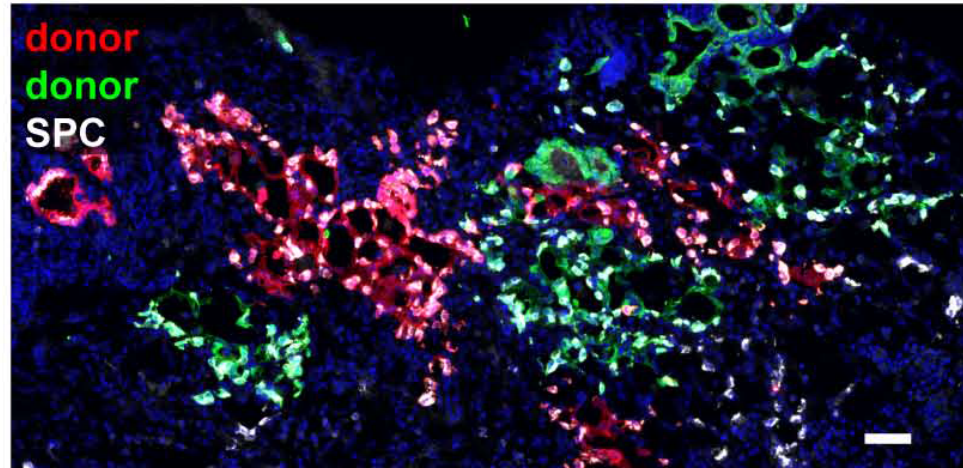
Christian Lang

n1242366@students.meduniwien.ac.at

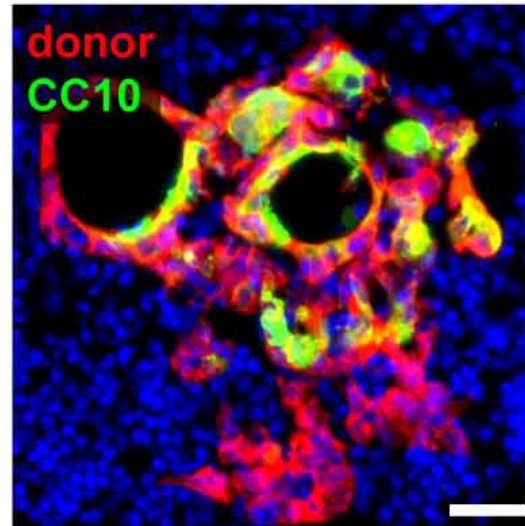




**h**



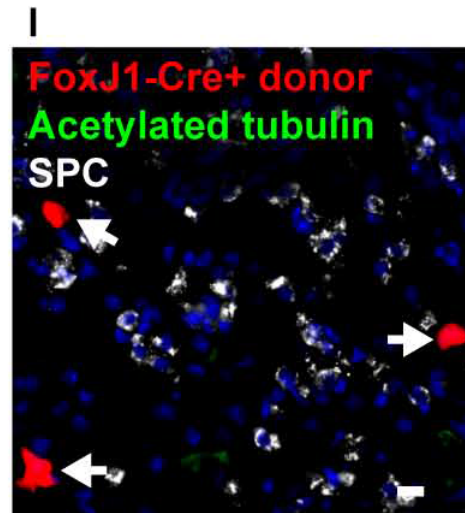
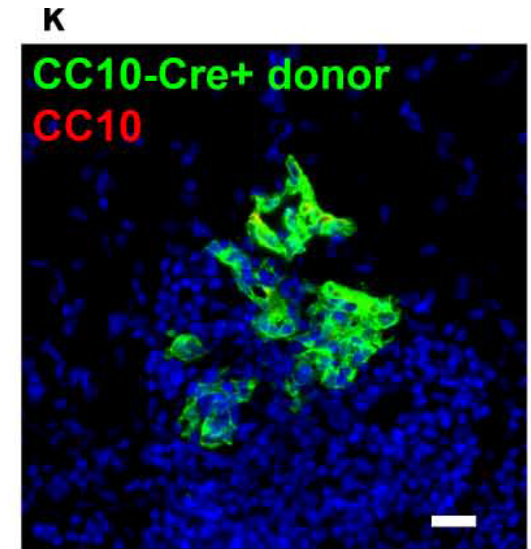
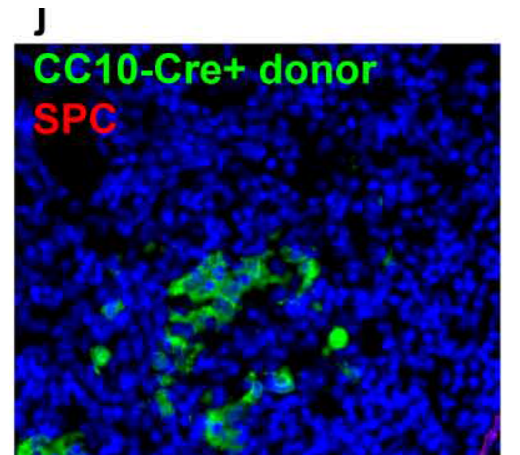
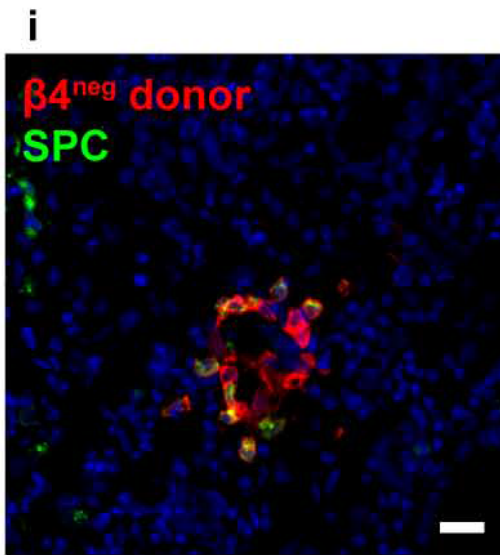
**g**



Beta<sub>4</sub><sup>-</sup> cells → small clusters and only expression of SPC (figure i)

CC10<sup>+</sup> cells → lost SPC and CC10-expression (figure j, k)

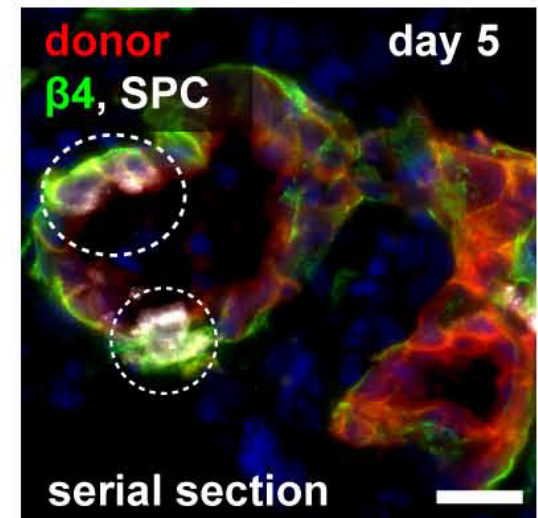
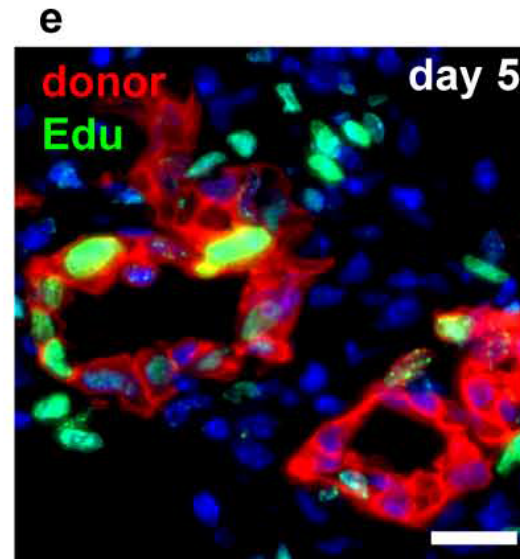
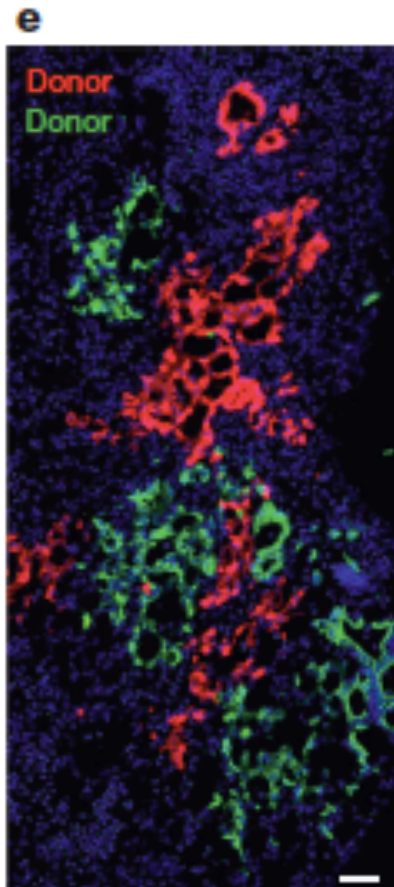
Transplantation of multi-ciliated cells → lack of progenitor → no development (figure l)



Transplantation of eGFP-labelled (greenN) and tdTomato-expressing LNEPs



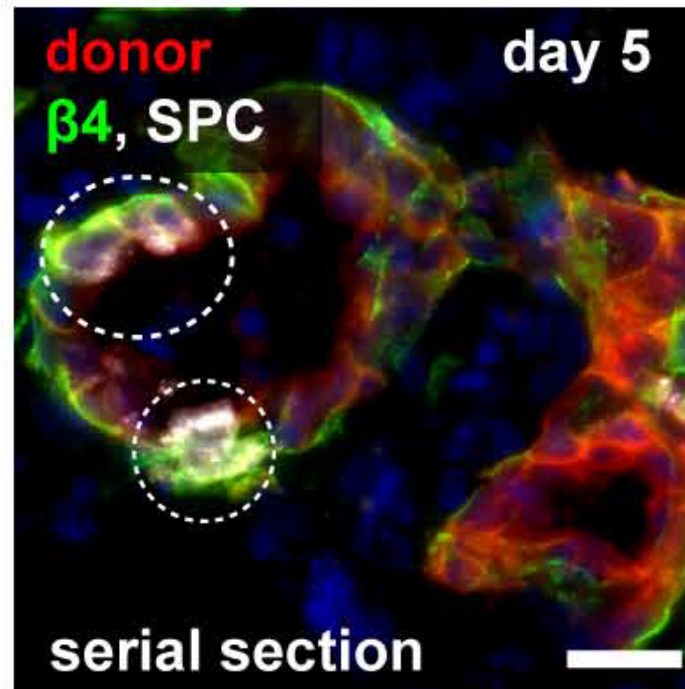
Non-overlapping (fig. E, left) & highly-proliferative engraftments (fig. E, right)



h

Normal mature type II cells → no Beta<sub>4</sub> expression

**LNEP-clones → Beta<sub>4</sub> expression (fig. E, right)**





## Summary:

**LNEPs**

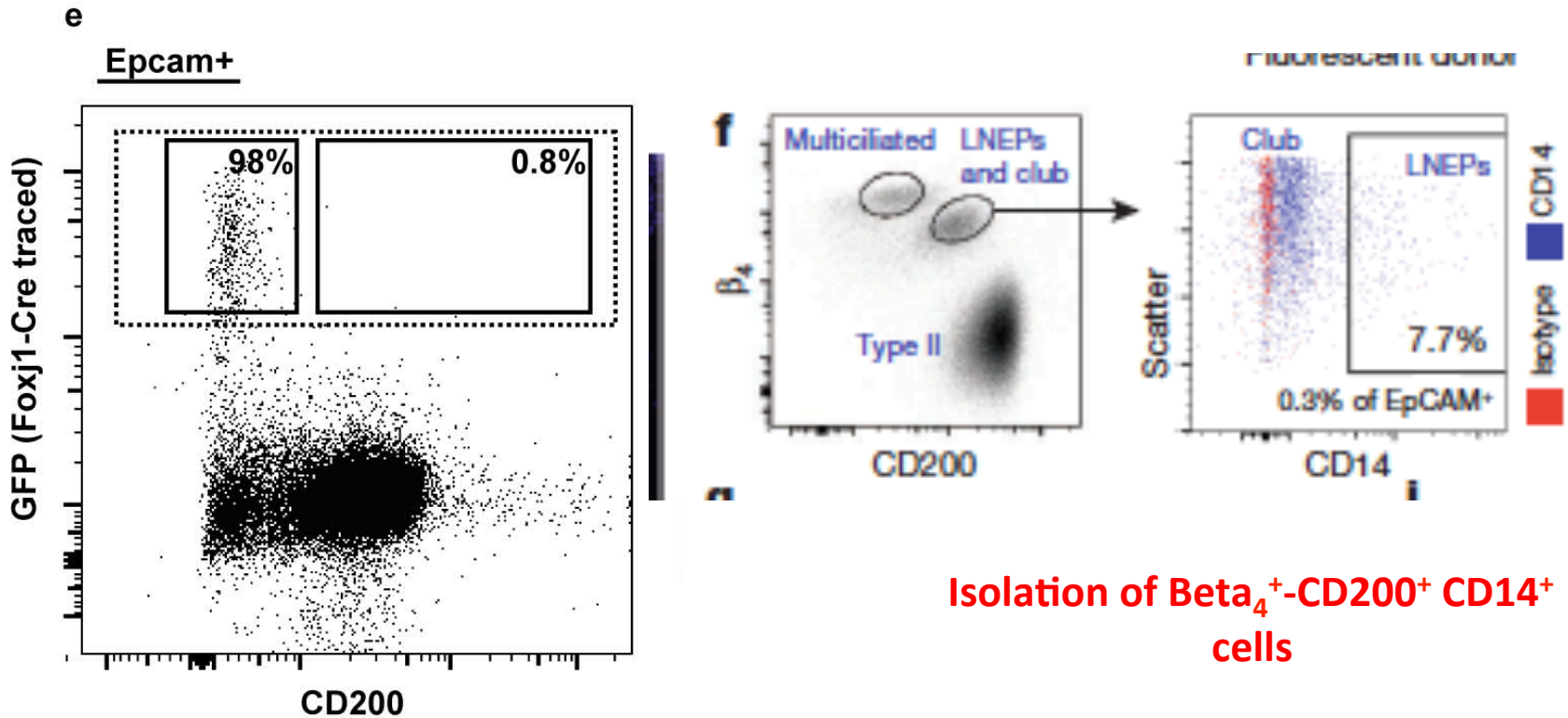


**proliferative & multipotent  
function after transplantation**

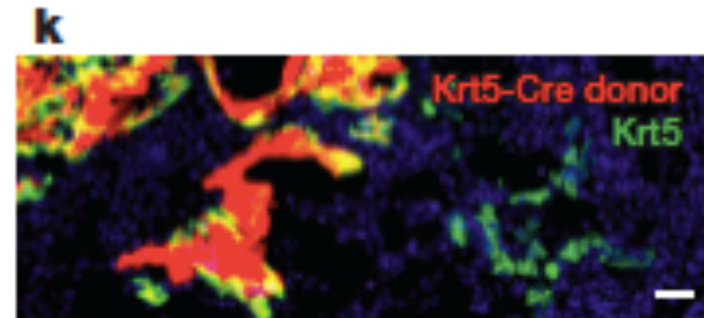
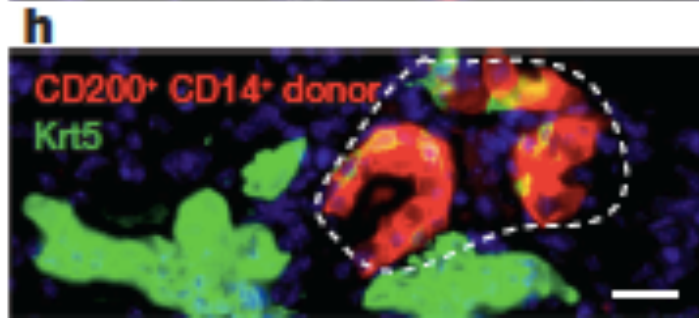
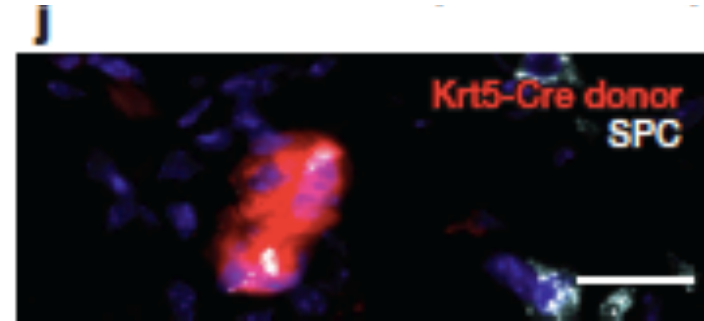
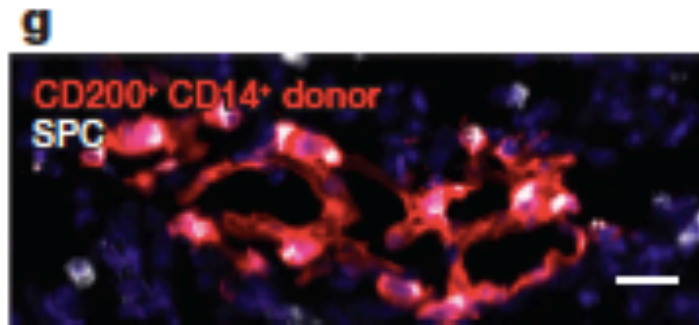


-RNA-analysis → **CD14-enrichment** in  $\text{Np63}^+$   $\text{CC10}^-$   $\text{Beta}_4^+$  cells (LNEP containing)

- **CD200** selects against multi-ciliated cells (fig. e)

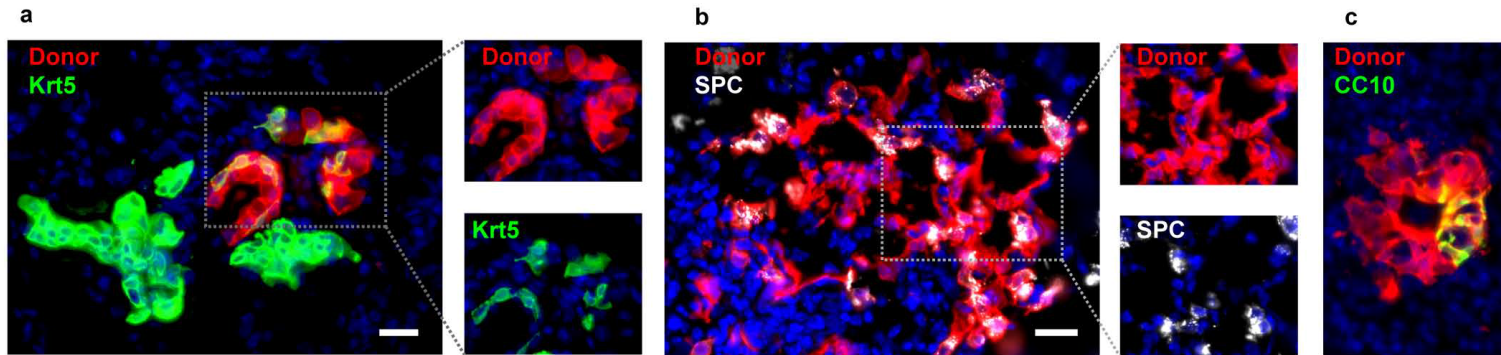


$\text{Beta}_4^+$ - $\text{CD200}^+$   $\text{CD14}^+$  cells  $\rightarrow$  represent active fraction of LNEPs

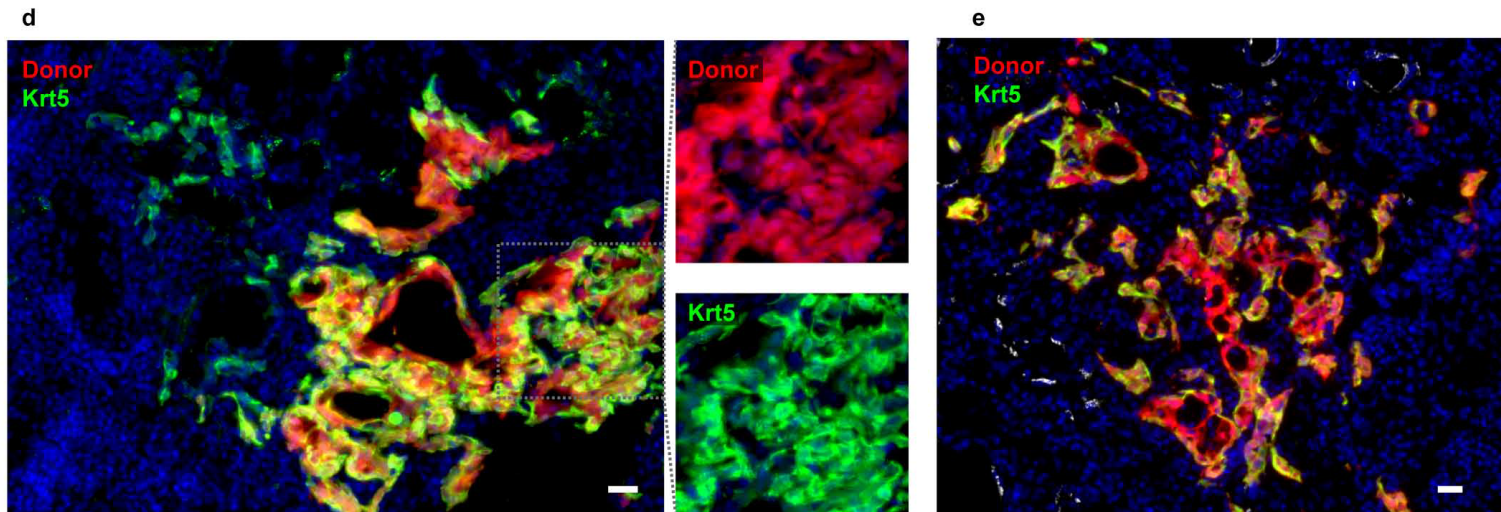


**Beta<sub>4</sub><sup>+</sup>-CD200<sup>+</sup> CD14<sup>+</sup> cells → represent active fraction of LNEPs**

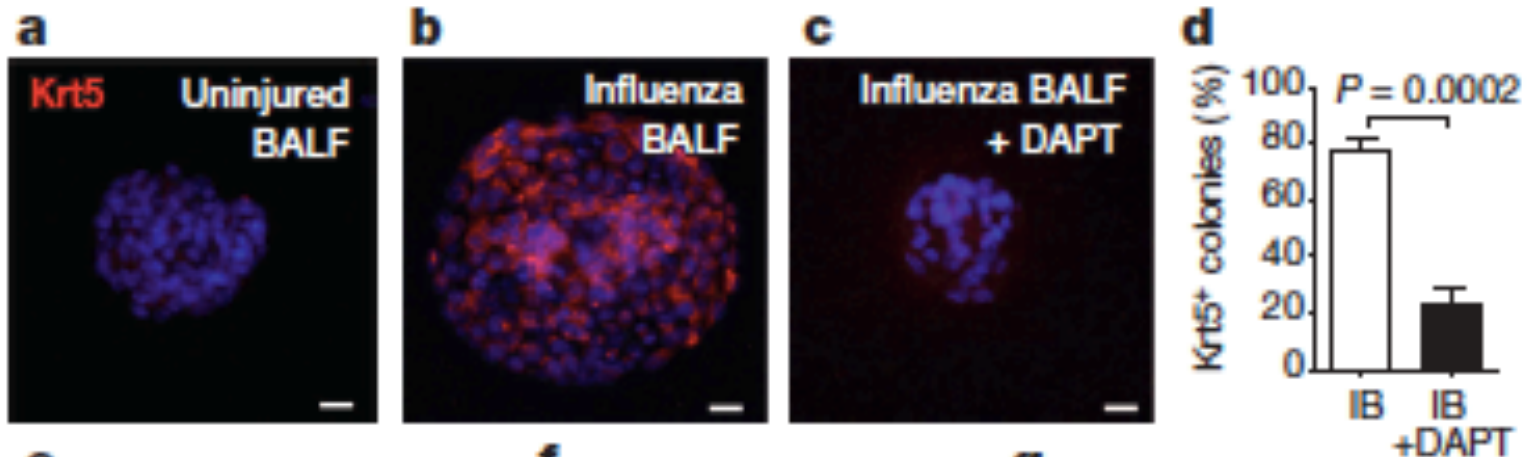
**β4+ CD200+ CD14+ transplant**



**Krt5-CreERT2 / tdTomato transplant**

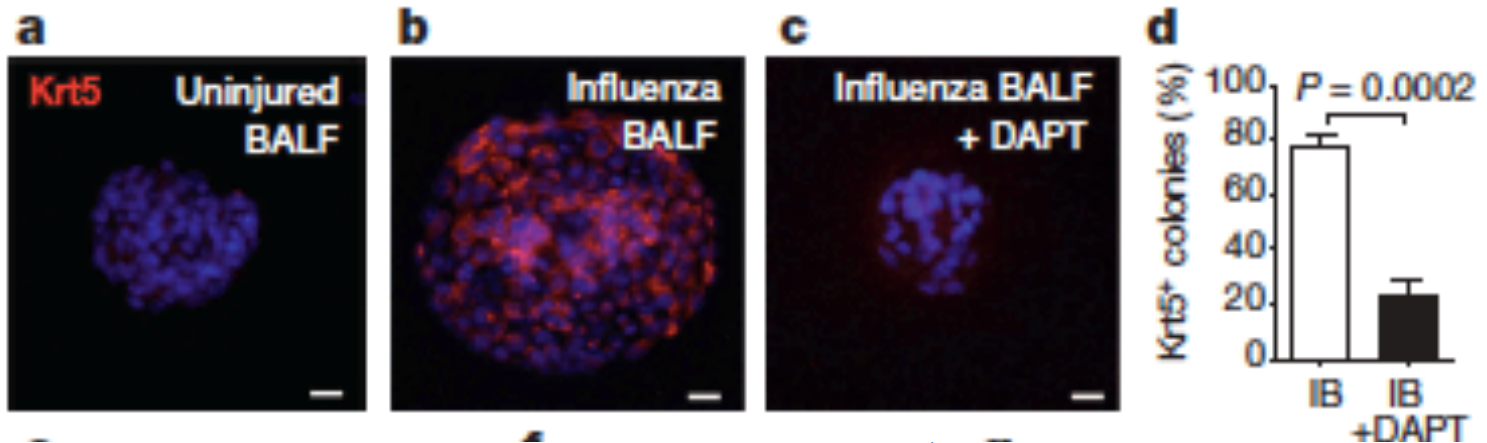


**LNEPs ex vivo → expression of Krt5**  
only after stimulation with BALF from influenza-injured mice





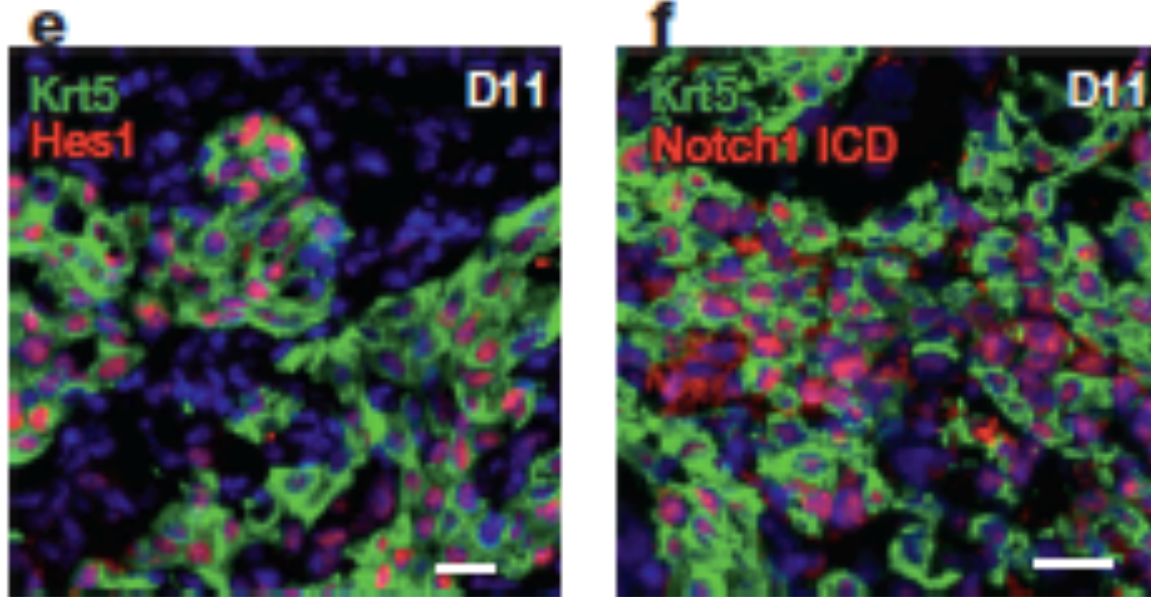
„Screen“ of pathway inhibitors → Notch signaling pathway involved



DAPT...  $\gamma$ -secretase inhibitor → inhibitor of Notch-signalling

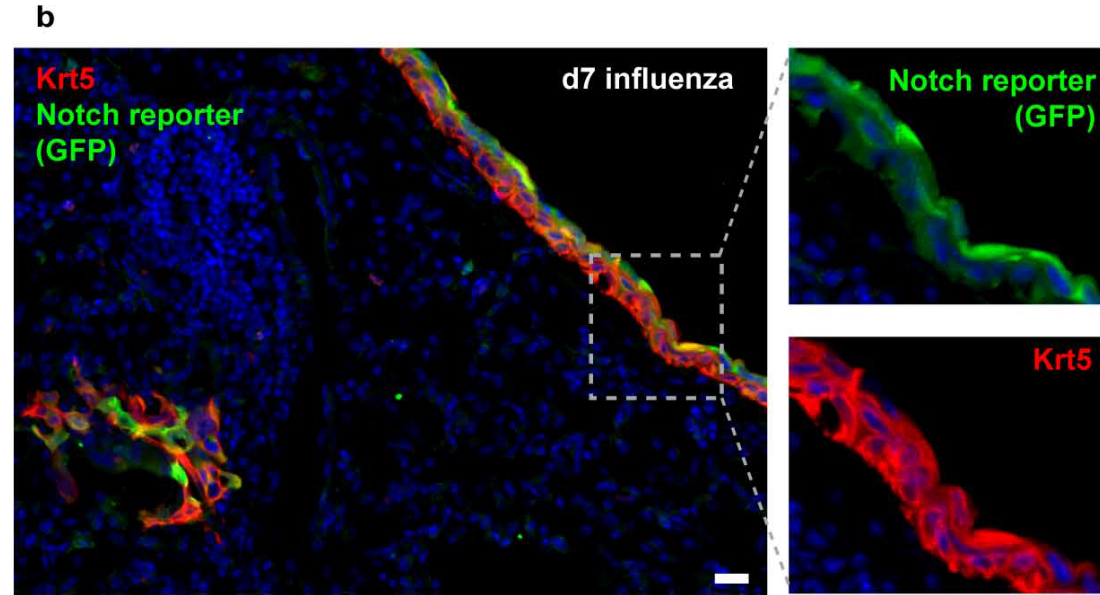
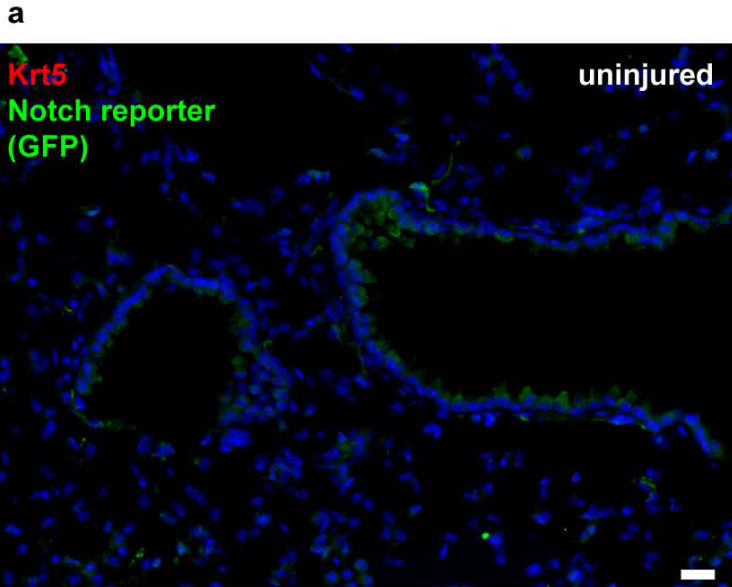


In vivo analysis → Notch-pathway involved!

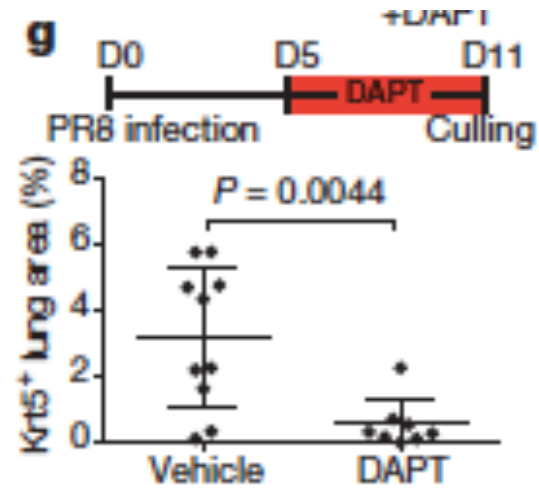


*ICD...Notch1 intracellular domain  
Hes1...Notch signalling target*

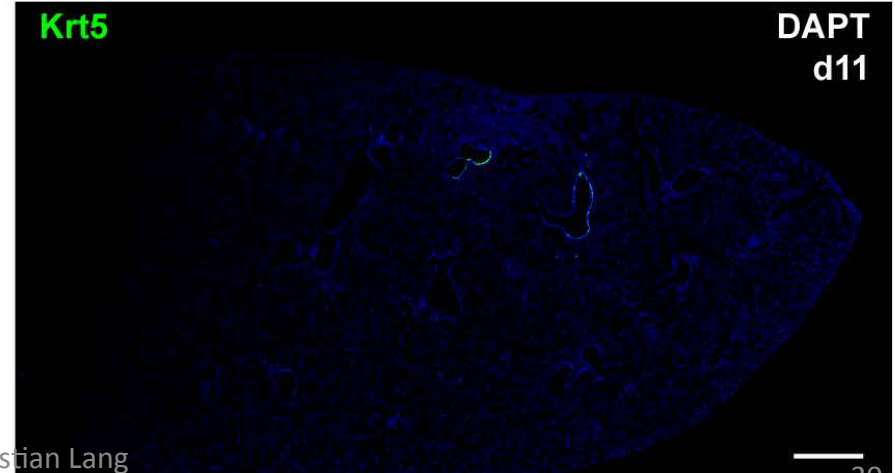
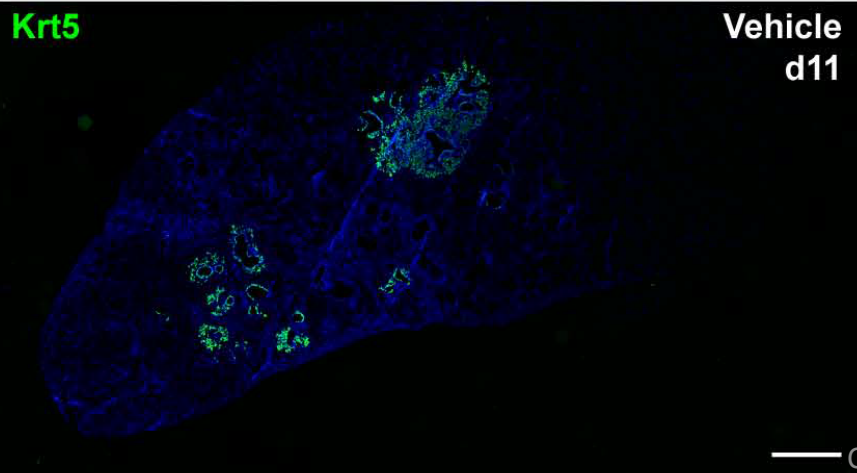
## Notch pathway is involved in LNEP-regulation



## Notch pathway is involved in LNEP-regulation



**h**

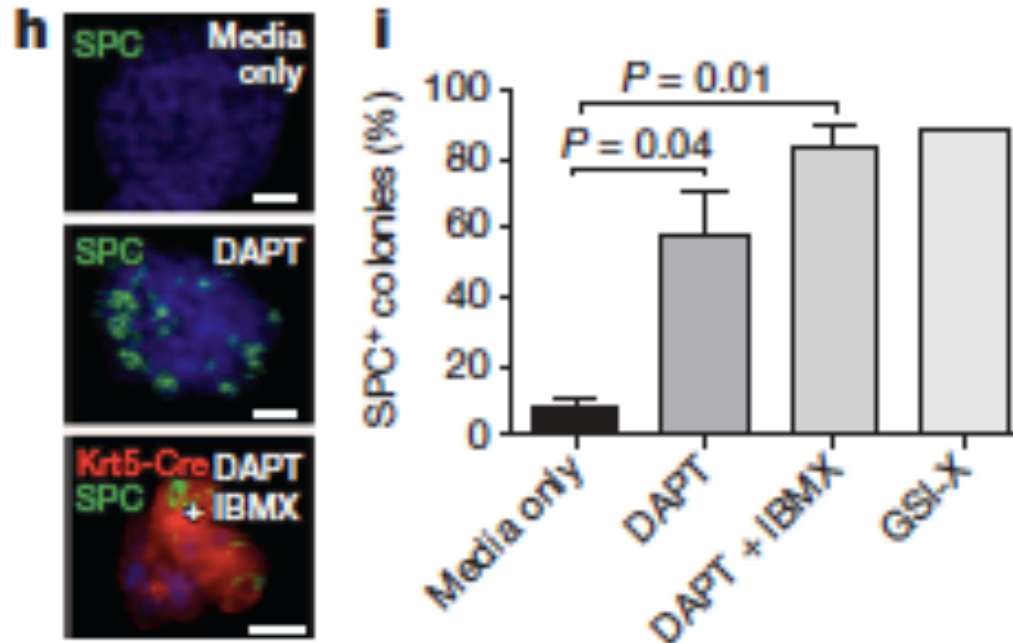


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During development:

Notch signalling —————| alveolar differentiation

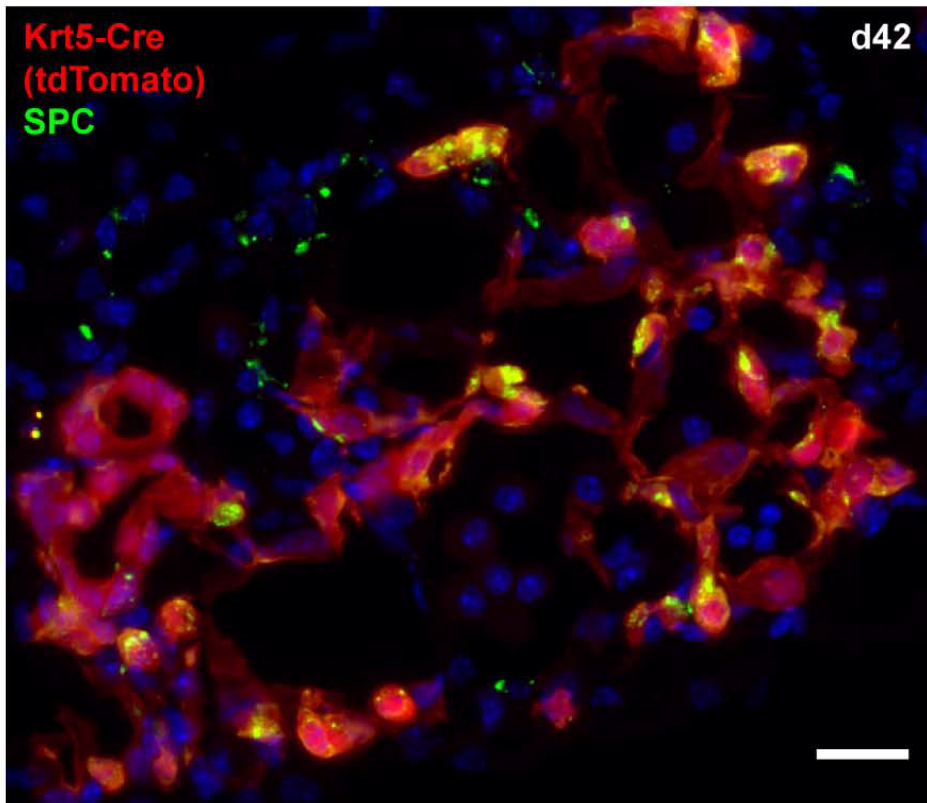
*when Notch inhibited → strong SPC expression*



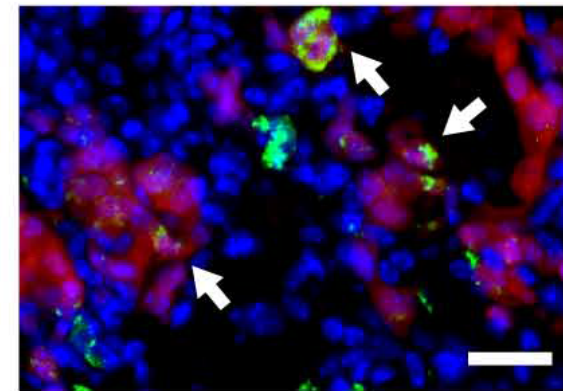
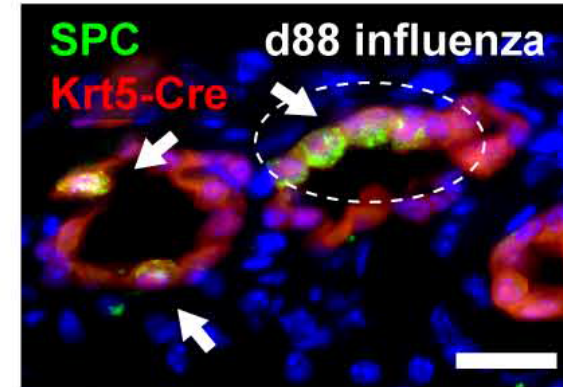
Persistent Notch-signalling → prevents alveolar differentiation  
Removal of Notch → maturation towards type II cells

After bleomycin injury

e



e After influenza injury

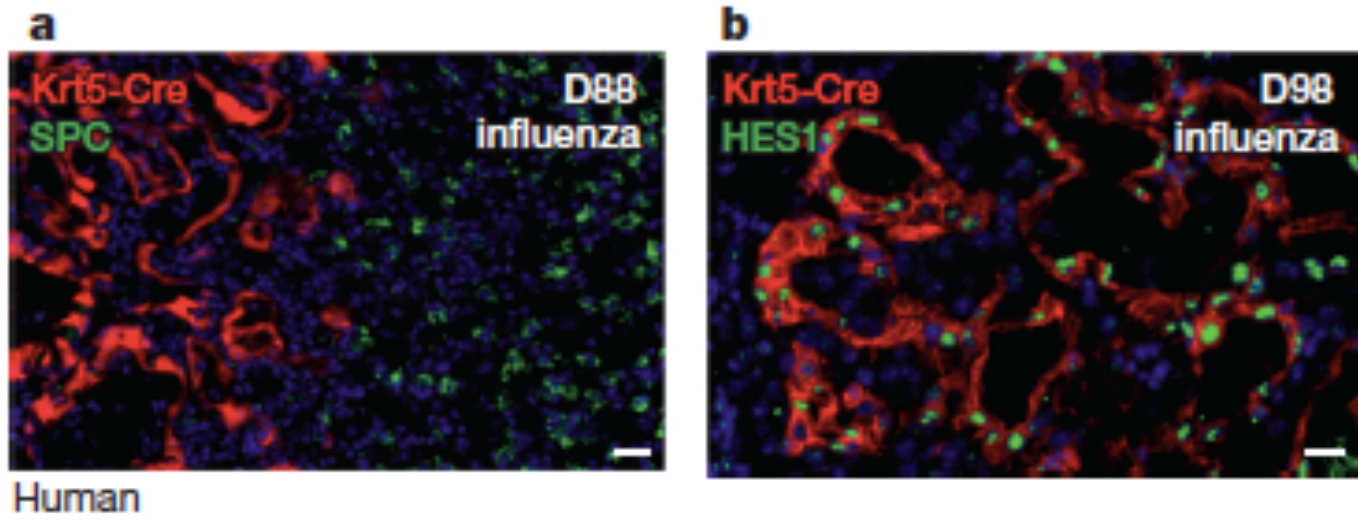


*Alveolar cysts*  
*(„micro-honeycombing“)*

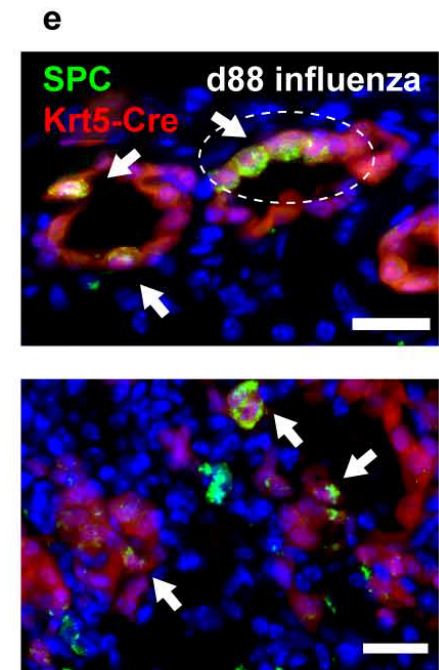
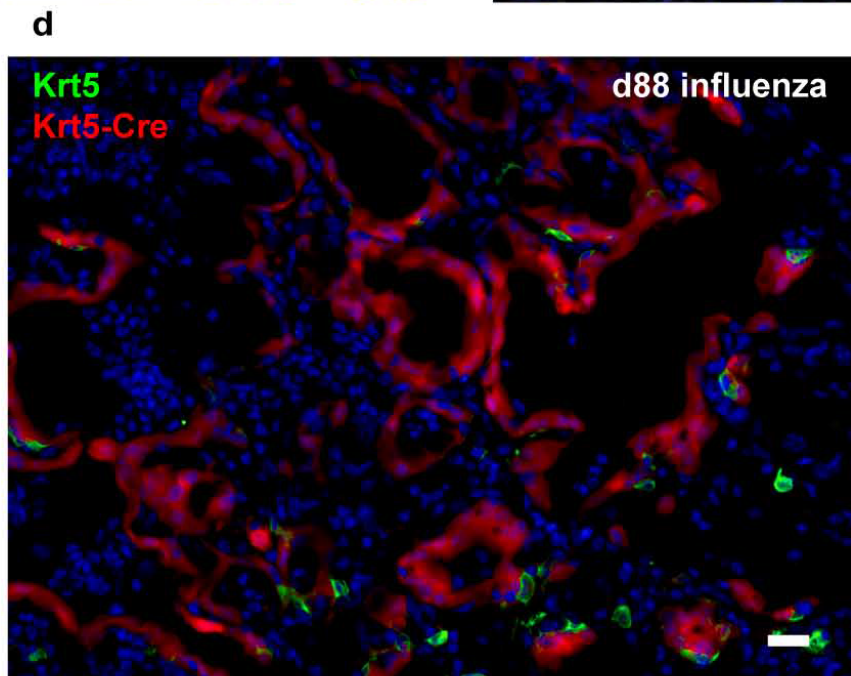
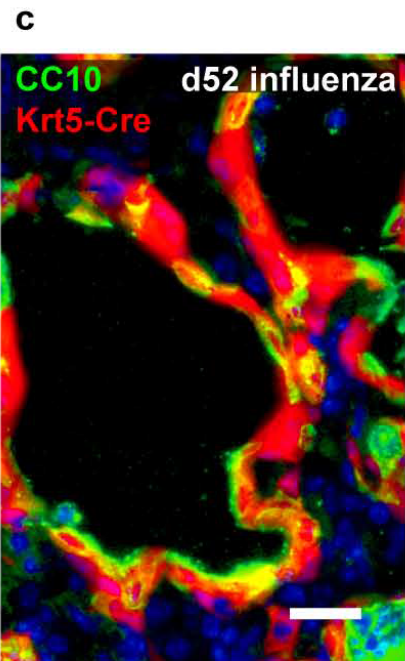


## Ongoing Notch activity in cystic epithelium

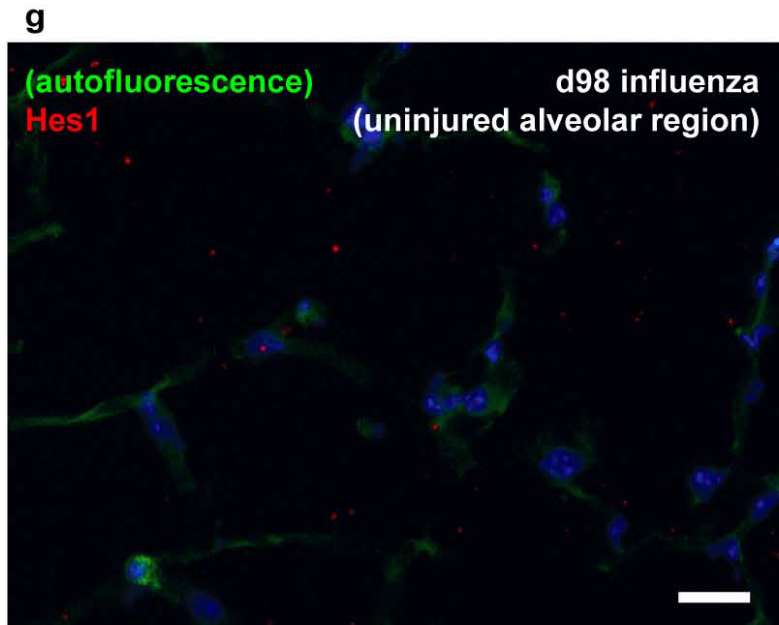
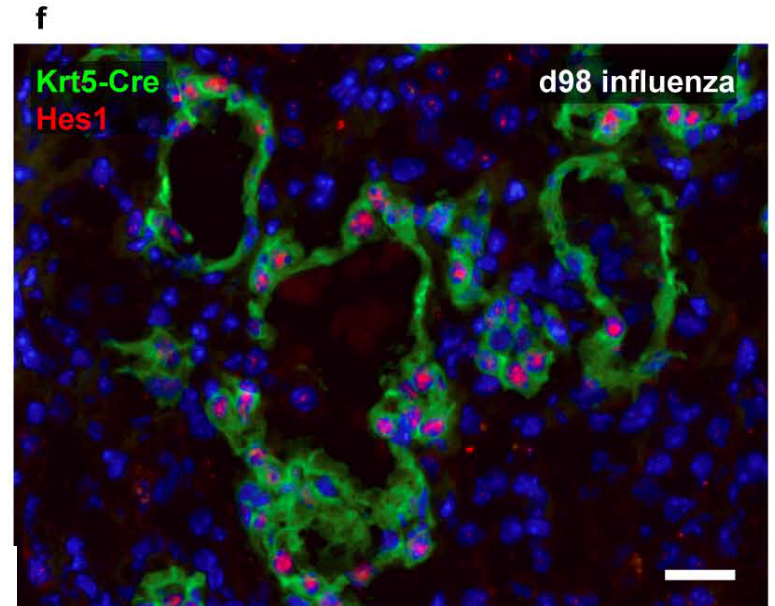
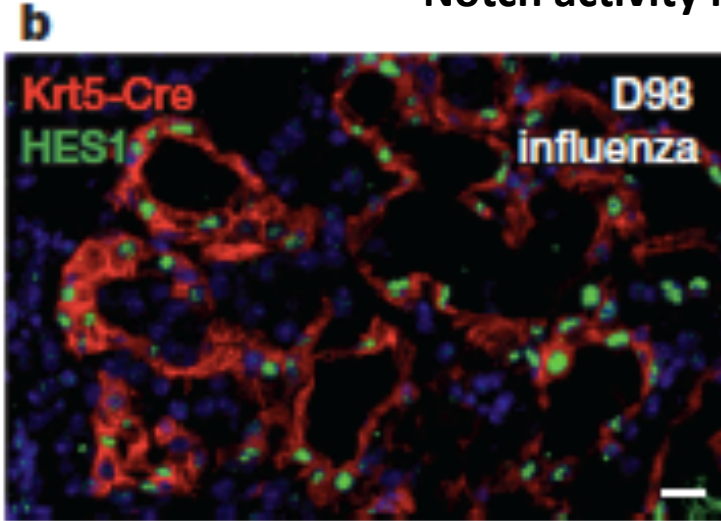
Mouse



## Ongoing Notch activity in cystic epithelium



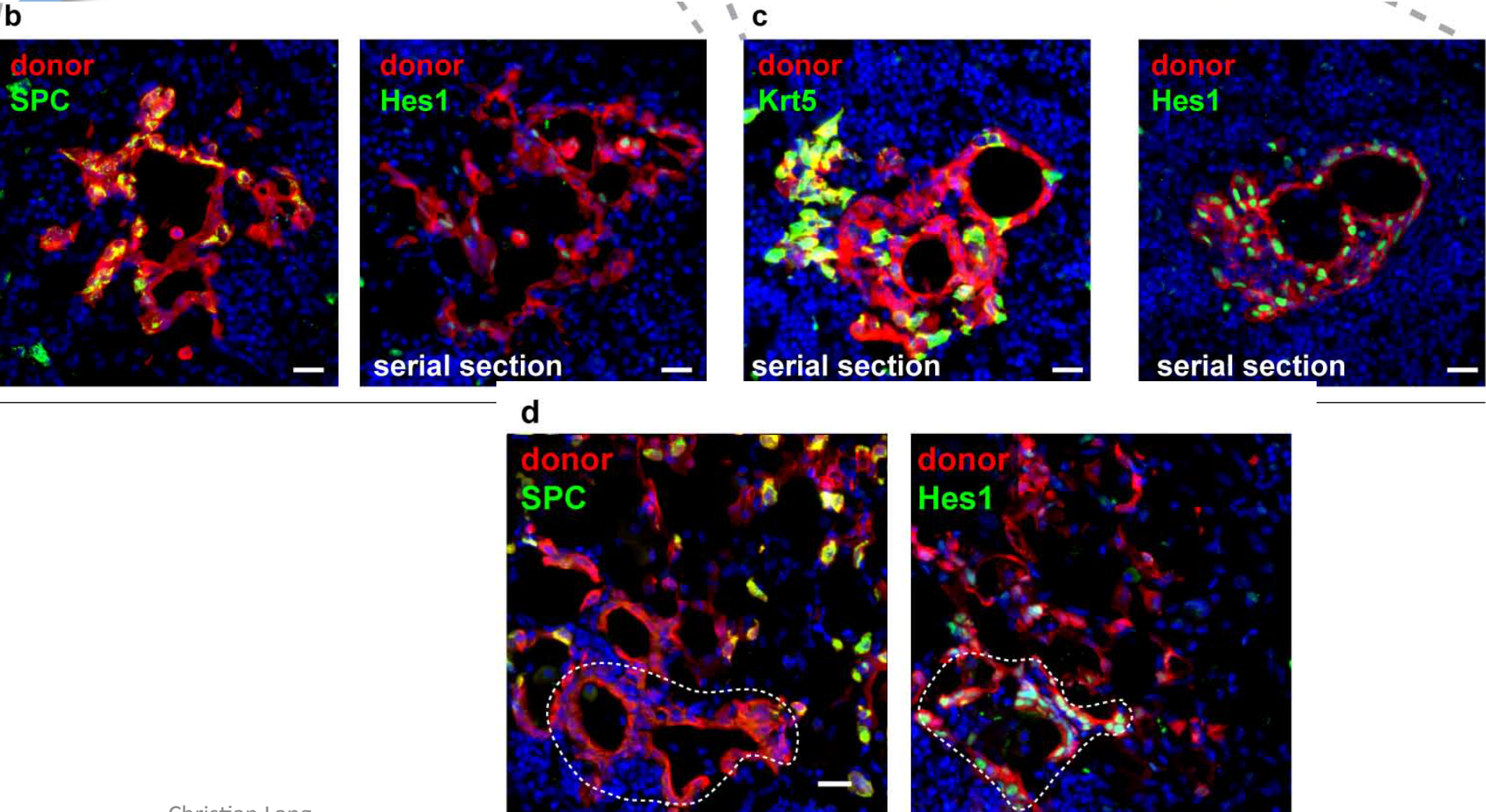
# Ongoing Notch activity in cystic epithelium



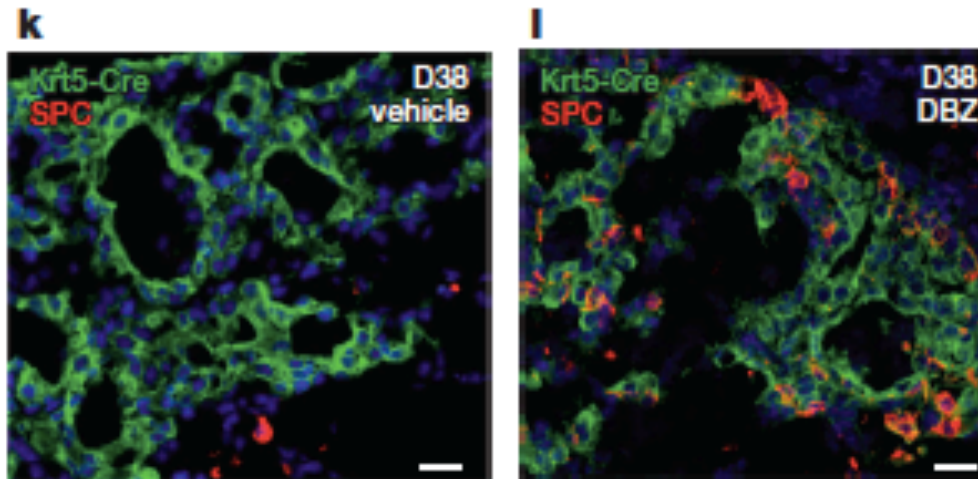
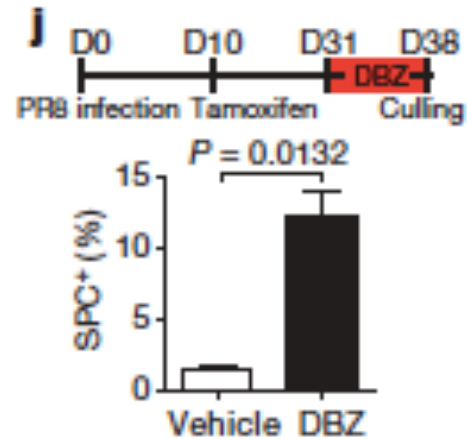
← No Notch activity in normal alveolar epithelium



## Ongoing Notch activity after transplantation of LNEP-derived Krt5<sup>+</sup> and CC10<sup>+</sup> cells



Notch antagonism *in vivo* → increase in the number of cyst-derived SPC<sup>+</sup> cells





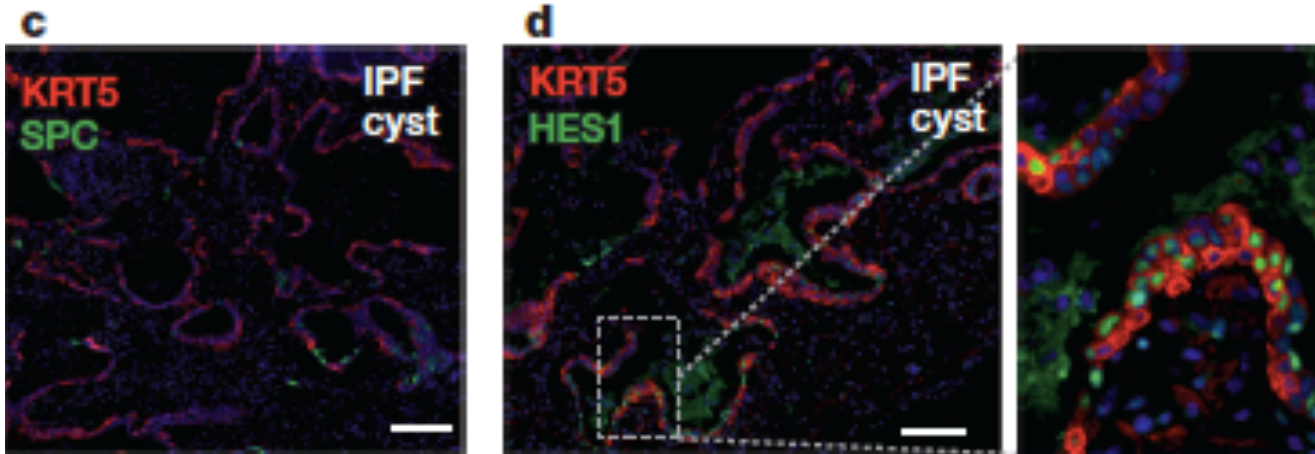
**Resemblance:**

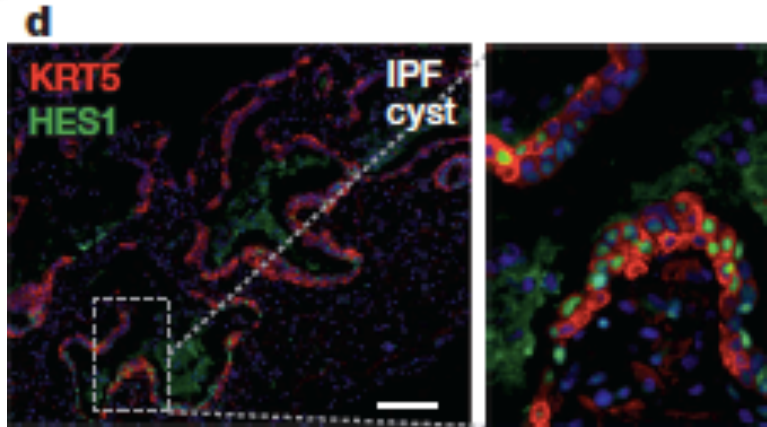
**Cysts in mice**



**Idiopathic pulmonary fibrosis (IPF) in human**

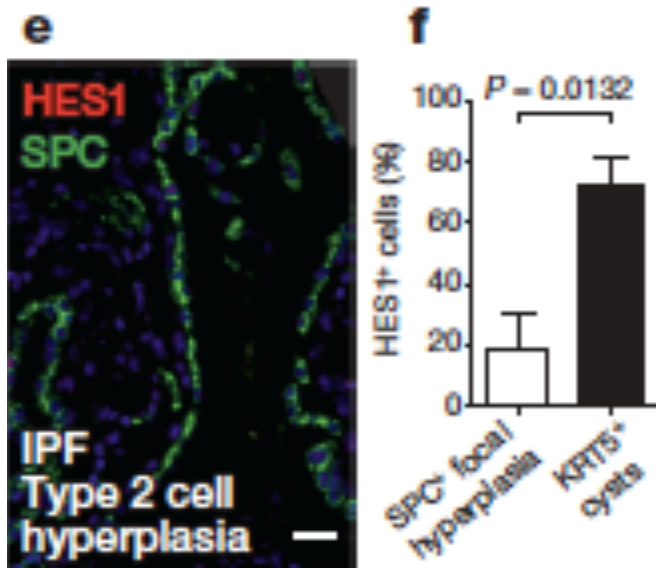
Human





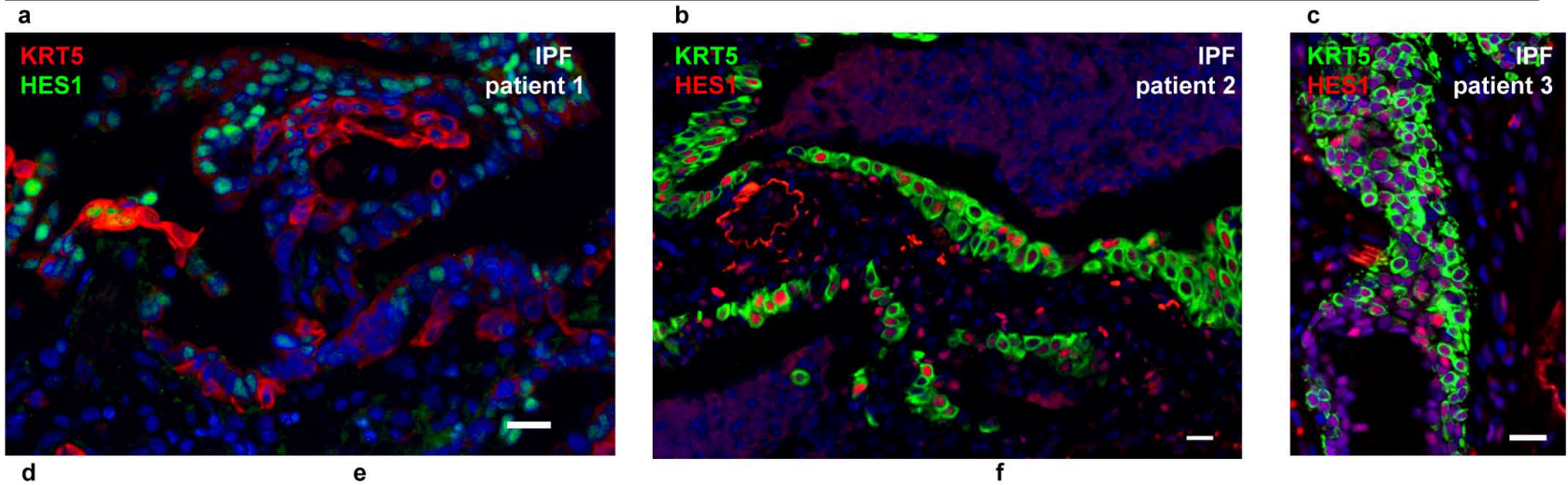
Ongoing **Notch activity** in **Krt5<sup>+</sup> cysts**  
(human with IPF)

No Notch activity in hyperplastic **SPC<sup>+</sup> cells**

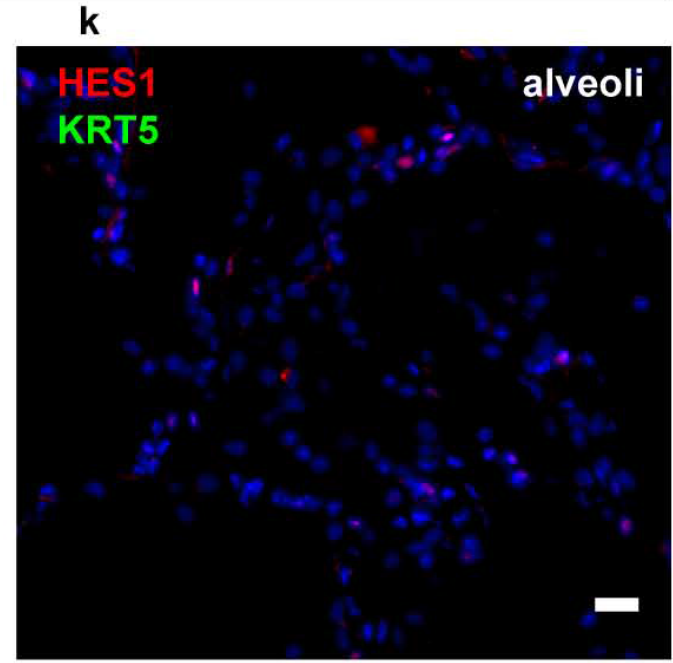


## Ongoing Notch activity in Krt5<sup>+</sup> cysts (human with IPF)

diseased human lung

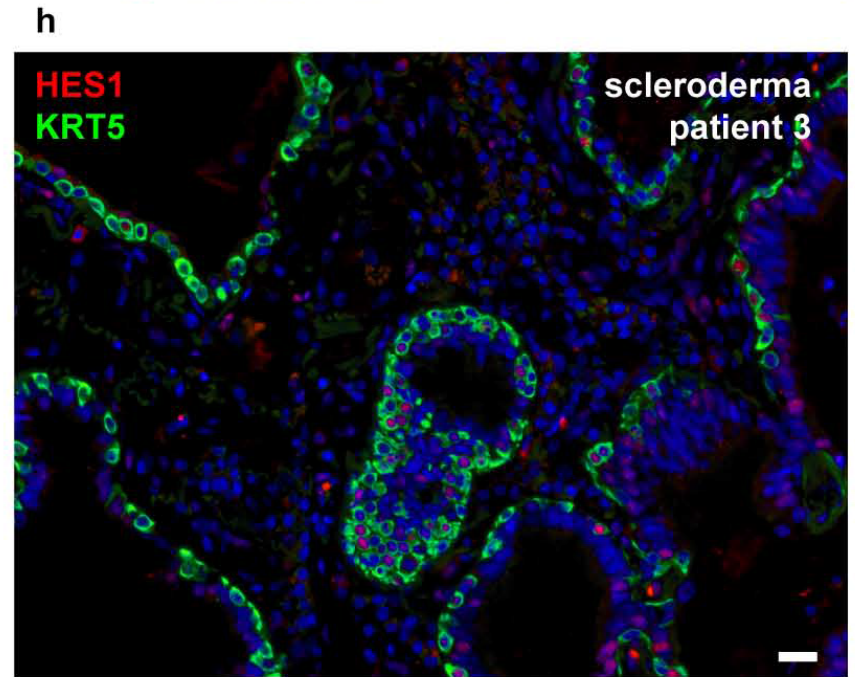
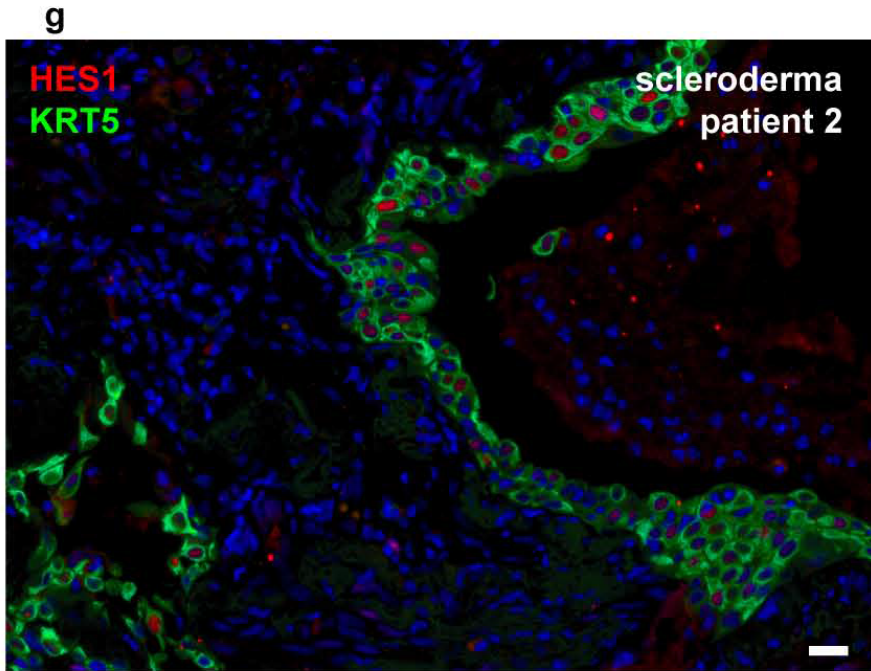
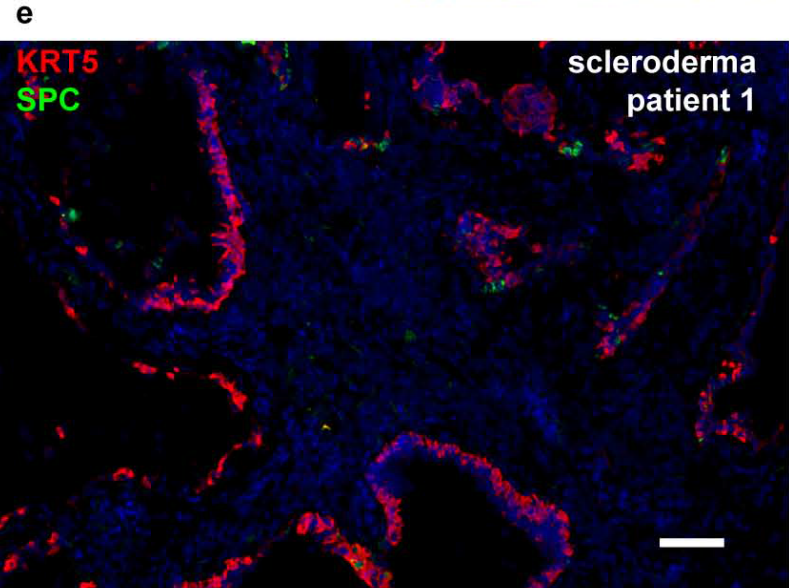


No Notch activity in normal human lung



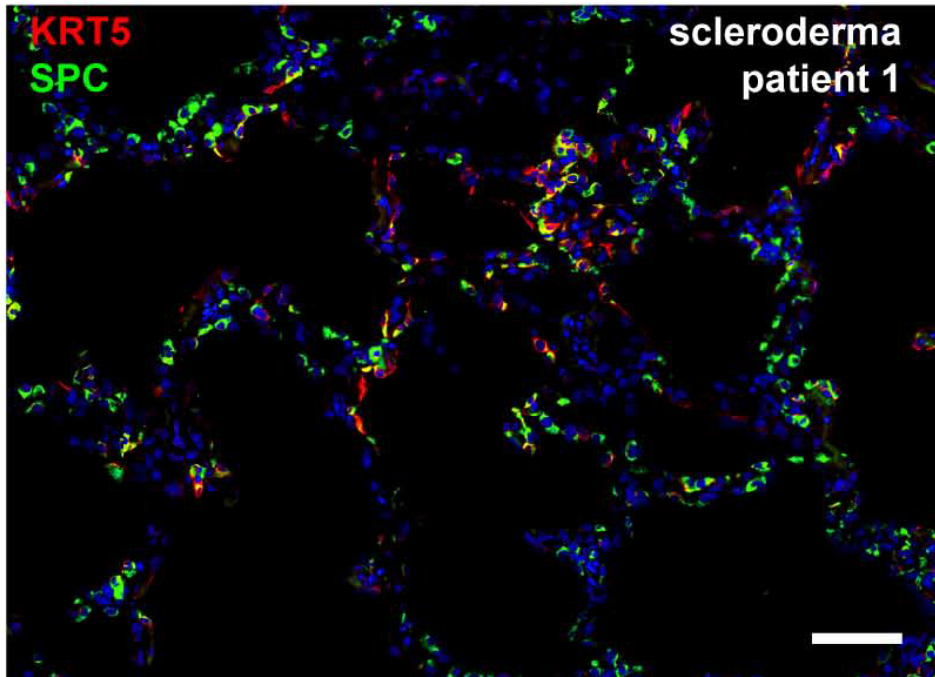


# Ongoing Notch activity in patients with scleroderma

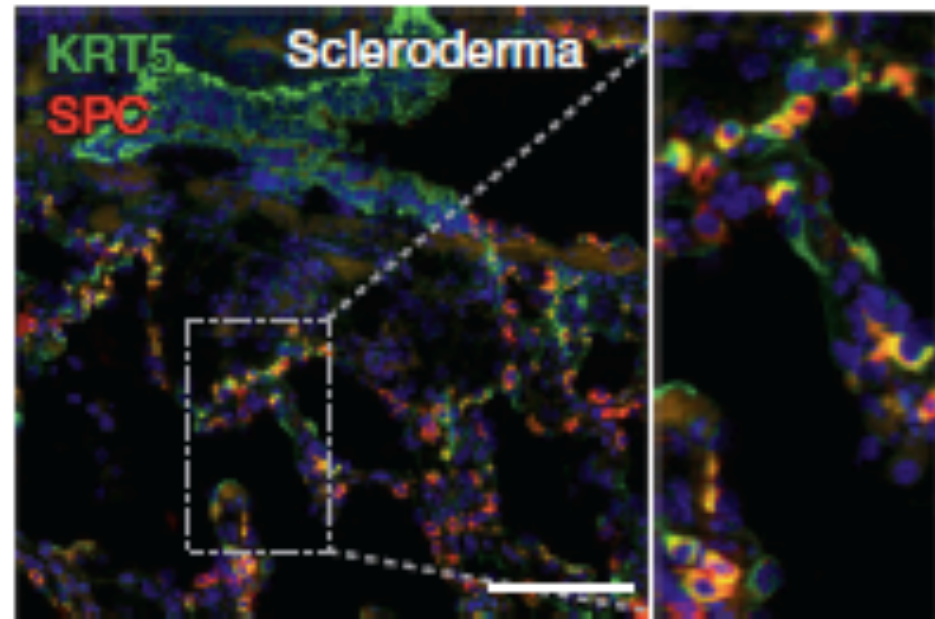


Co-expression of SPC<sup>+</sup> and Krt5<sup>+</sup> cell lines in patients with scleroderma (n=3)

†



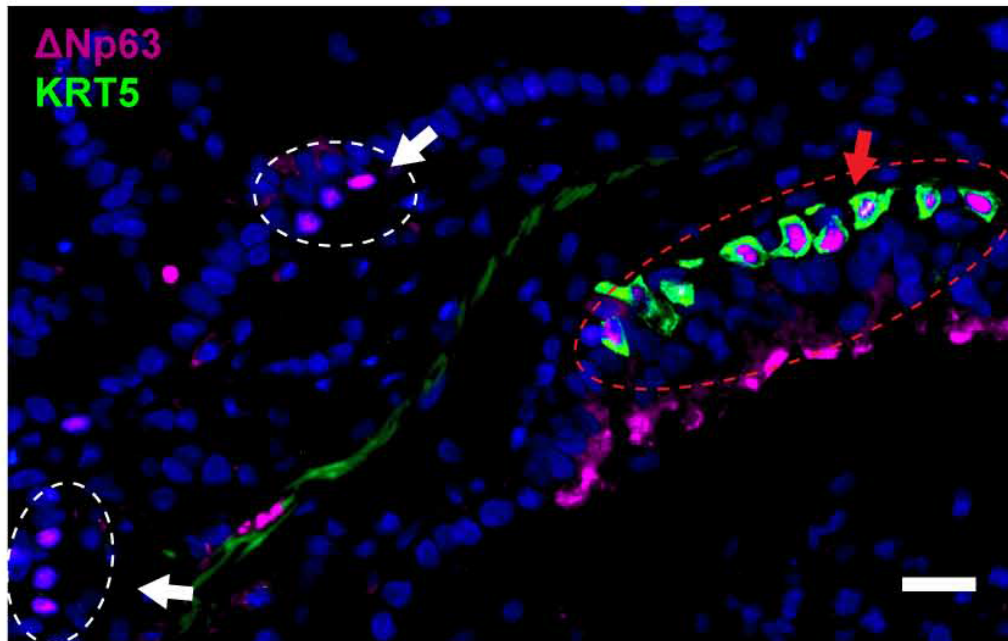
g

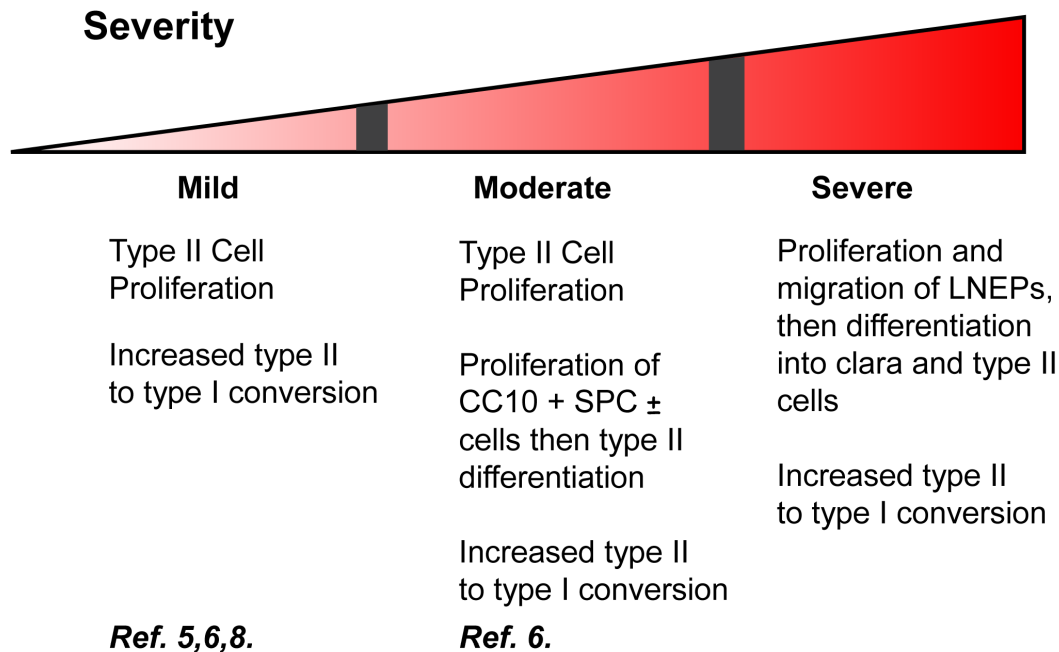


**Np63<sup>+</sup> Krt5<sup>-</sup> cells in normal terminal airways(human) → analogous to LNEPs in mice**

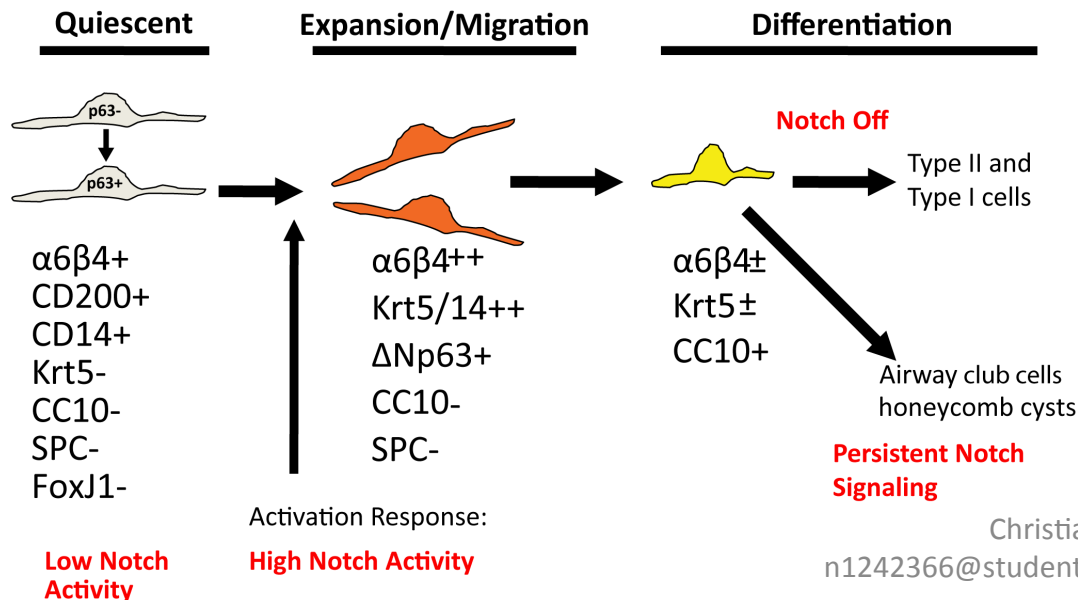
normal human lung

i





b






# Summary

- **LNEPs** cells present within normal distal lung
- LNEPs activate **remodelling program** after major injury (*Np63 and Krt5* )
- High **proliferative** capacity and **multipotency** of LNEPs
- LNEPs require **Notch signalling**
- Hyperactive Notch signalling → alveolar cysts, IPF and scleroderma

# Discussion

- LNEPs in human
- Notch signalling  chronic lung disease