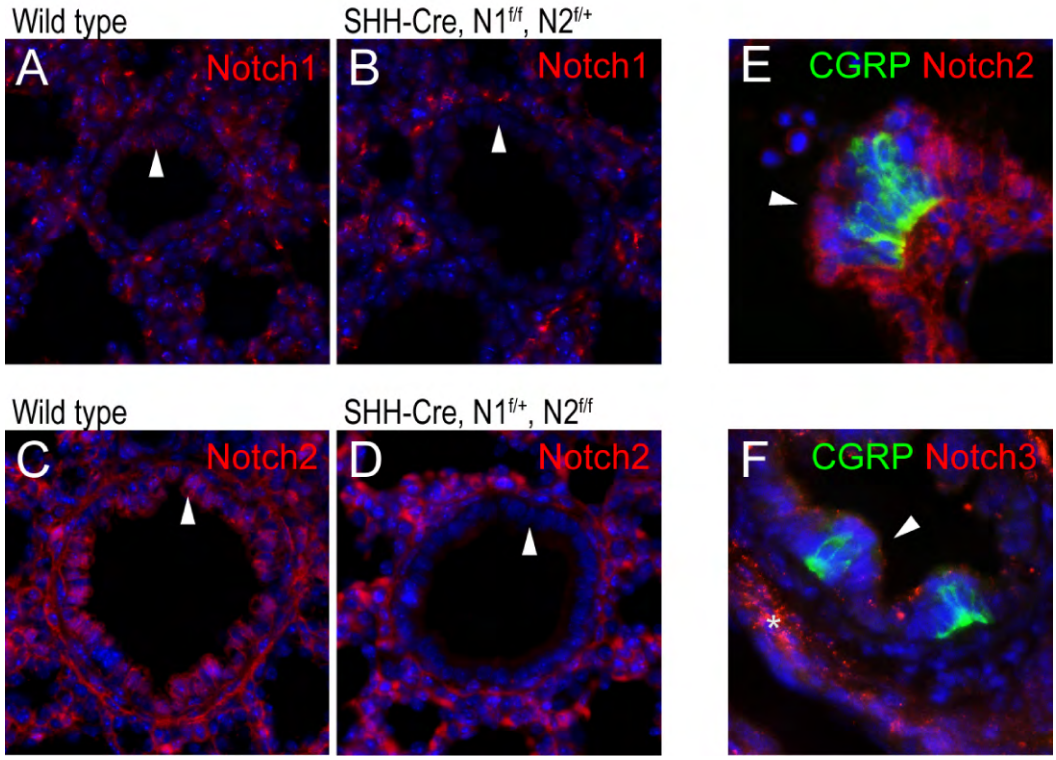

Fig. S1. Notch immunostaining. (**A-F**) Immunofluorescence staining for Notch1 (A,B), Notch2 (C-E) and Notch3 (F) of E18.5 lungs from wild type or mutants. Notch1 protein deletion from developing lung epithelium was confirmed in *SHH-Cre;N1$^{flox/flox}$;N2$^{flox/+}$* (B, arrowhead), as well as Notch2 protein deletion in *SHH-Cre;N1$^{flox/+}$;N2$^{flox/flox}$* (D, arrowhead). Expressions of Notch2 (E, red, arrowhead) and Notch3 (F, red, arrowhead) around pNEB (green) were identified in wild type. Asterisks in F indicate the Notch3 expression in vascular smooth muscle cells.

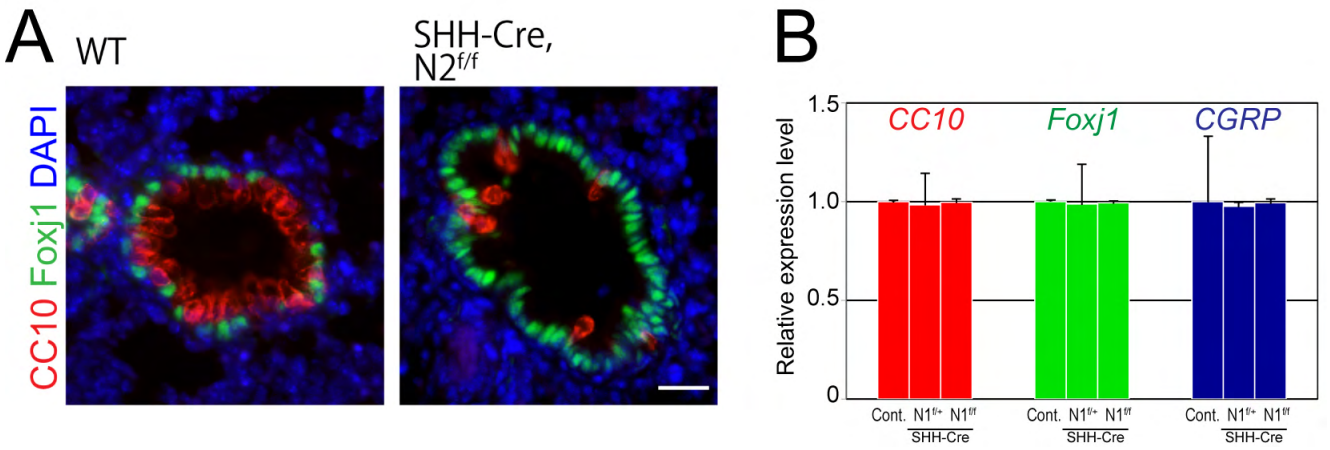

Fig. S2. Notch2 deletion expands ciliated cell population. (**A**) Double immunofluorescence staining for Foxj1 (green) and CC10 (red) in the distal conducting airways of E18.5 *SHH-Cre;N2$^{flox/flox}$* lung. A predominant distribution of Clara cells was observed in the control (left). However, ciliated cells were remarkably increased and Clara cells became a minority in the mutant (right). (**B**) qRT-PCR analysis for *SHH-Cre;N1$^{flox/flox}$* lung revealed that Notch1 ablation in developing epithelium does not alter the expressions of *CC10* (red), *Foxj1* (green) and *Cgrp* (blue) genes. The contribution of Notch1 receptor is minimal in pNEB size regulation in the presence of the other receptors.

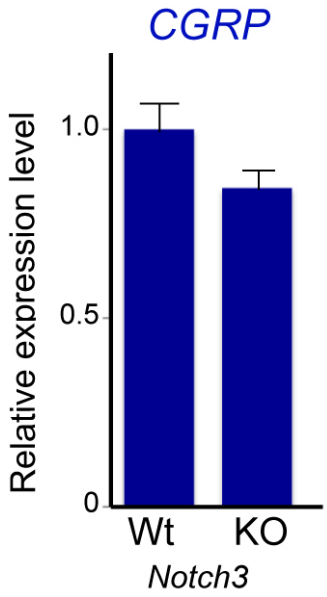

Fig. S3. Cgrp levels in the notch3 knockout. Total RNA was extracted from three whole lungs of Notch3 knockout or wild-type mice at E18.5. The relative gene expression levels of *Cgrp* in the lungs were measured by quantitative RT-PCR. CGRP was not increased in the Notch3 single knockout.

Fig. S4. *Dll1* and CGRP levels. Dll1 expression was visualized by X-gal staining in Dll1-*lacZ* lungs at 2 months (blue). Double staining with anti-CGRP (brown) revealed the expression of Dll1 in NE cells (arrowhead).

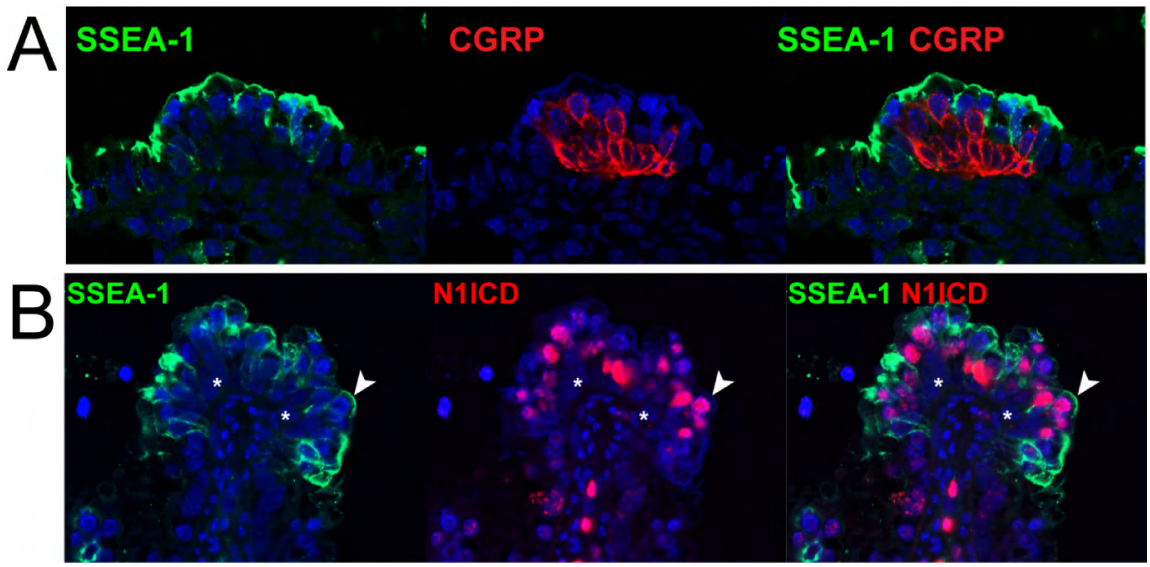

Fig. S5. Confocal imaging. (**A**,**B**) SSEA-1+ (green) and NE cells (A, red) or N1ICD (B, red) at E18.5. Confocal images of SSEA-1+ cells cannot be merged with images of NE cells, indicating a clear distinction between these cell types (A). These SSEA-1+, peri-pNEB cells display N1ICD in their nuclei (B, arrowhead).

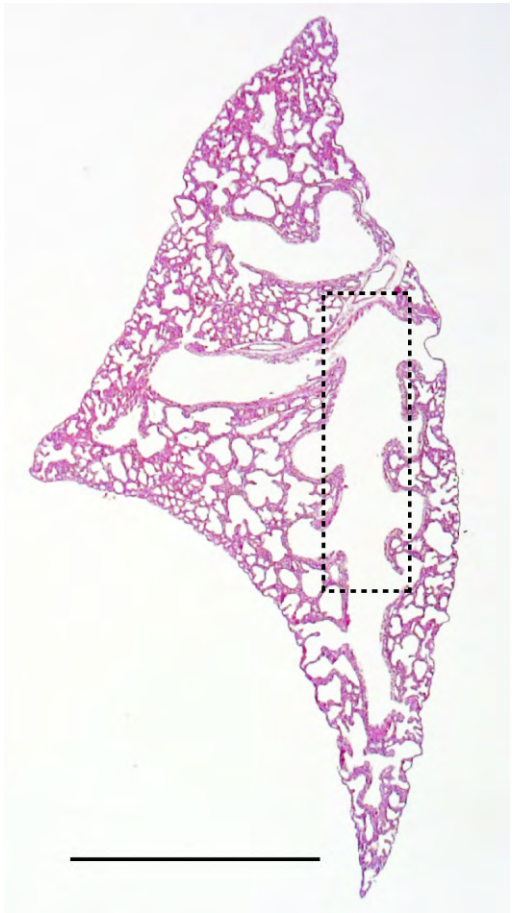

Fig. S6. Hematoxylin and Eosin staining of a lung section at E18.5 of *SPC-rtTA;TetO-Cre;Rosa-GFP-N1ICD.* Although epithelial tissue structure is altered by forced activation of Notch signaling (Guseh et al., 2009), it is still distinguishable, as indicated by a dotty rectangle. Scale bar: 0.5 mm.

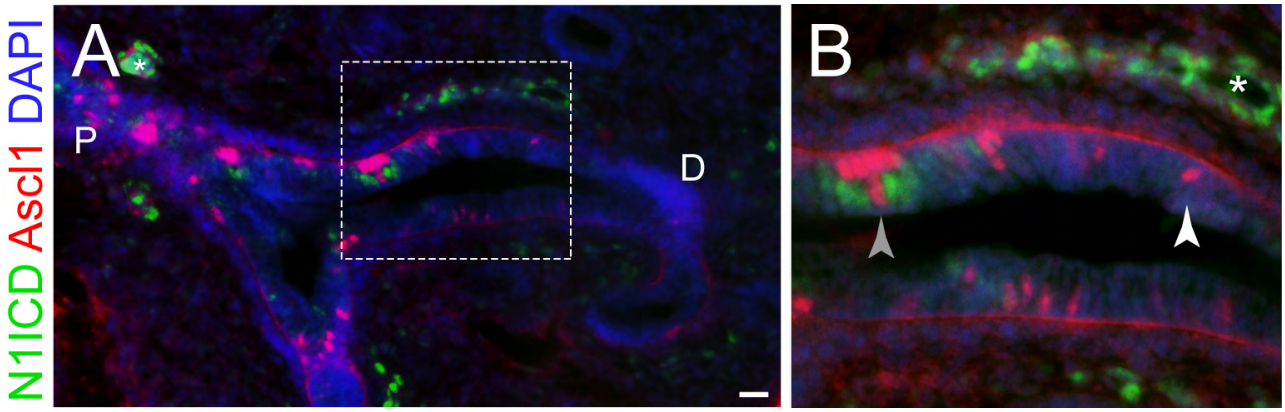

Fig. S7. Notch-active cells surround NE cell cluster but not a single NE cell at the early lung developmental stage. (**A**,**B**) Double staining for N1ICD (green) and Ascl1 (red) at E14.5. The dotted squares in A indicate the area magnified in B. N1ICD-positive cells are observed around pNEBs at E14.5 (B, gray arrowhead) but not in a newly formed single NE cell at the distal lung bud (B, white arrowhead). Notch signaling is stimulated in the neighboring clustered NE cells but not in nascent NE cells at the early developmental stage of the lung. Asterisks indicate N1ICD-positive vascular cells.

Table S1. The individual conditions for immunohistochemistry

Antibody, dilution	Company, catalog code	Fixative	Tissue preparation	Antigen retrieval	Secondary antibody	Amplification
CC10 (T-18), 1:150	Santa Cruz, sc-9769	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS-H[2]	Goat-Alexa594[4]	-
CGRP, 1:4000	Sigma, C8198	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS[3]	Rabbit-Alexa594[5]	-
CGRP, 1:8000	Sigma, C8198	4% PFA[1]	OCT-frozen	-	Rabbit-Alexa488[6]	-
CGRP (N-15), 1:300	Santa Cruz, sc-8856	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS[3]	Goat-Alexa594[4]	-
Foxj1, 1:100	Gift from Dr Steven L. Brody	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS[3]	Mouse-Alexa488[7]	-
Hes1, 1:1000	Gift from Dr N. Brown	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS-H[2]	Rabbit-Alexa594[5]	-
Mash1, 1:1000	BD Phar minutesgen, 556604	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS-H[2]	Rabbit-Biotin[8]	ABC[10] and TSA-Cy3[11]
N1ICD, 1:1000	Cell Signaling Technology, #4147	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS[3]	Rabbit-Biotin[8]	ABC[10] and TSA-Cy3[11]
Notch1, 1:300	Cell Signaling Technology, #3608	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS-H[2] and room temperature 60 minutes	Rabbit-Alexa594[5]	-
Notch2, 1:300	Cell Signaling Technology, #5732	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS-H[2] and room temperature 60 minutes	Rabbit-Alexa594[5]	-
SSEA-1, 1:300	Millipore, #MAB4301	4% PFA[1]	Paraffin	105°C for 15 minutes in AUS-H[2] and room temperature 60 minutes	Mouse-Alexa488[7]	-

[1]4% paraformaldehyde (Sigma)
[2]Antigen Unmasking Solution, High pH (Vector Laboratory)
[3]Antigen Unmasking Solution, Citric Acid Based (Vector Laboratory)
[4]Alexa Fluor 594 donkey anti-Goat IgG (Invitrogen)