

Figure S1. Alignments of δ_{802} and δ_2 human ENaC variants.

1) Nucleotide alignment of human δ_{802} and δ_2 ENaC variants.

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DQ898176.1_704_-----
NM_001130413.3_802_ ATGAGGGCAGTGCTGTCACAGAAGACAACACCGCTCCCTCGTTACCTGTGGCCCGGCCAC 60

DQ898176.1_704_-----
NM_001130413.3_802_ CTCAGCGGCCCAAGGAGGCTCACCTGGTCATGGTGCAGTGACCACAGGACCCCCACATGC 120

DQ898176.1_704_-----
NM_001130413.3_802_ CGGGAGCTGGGTTCGCCCCACCCACCCCTGCACCGGGCCAGCGAGGGGATGGCCCA 180

DQ898176.1_704_-----
NM_001130413.3_802_ AGAGGGGGAGGACCATGTGGATTCACCAGTGCTGGACATGTGCTCTGTGGCTACCCCTC 240

DQ898176.1_704_-----ATGGCT 6
NM_001130413.3_802_ TGCCTACTCTCTGGCCCGATACAGGGGTGTGGGACAGGCCTGGGTGACTCCAGCATGGCT 300
                        *****

DQ898176.1_704_ TTCCTCTCCAGGACGTCACCGGTGGCAGCTGCTTCCTTCCAGAGCCGGCAGGAGGCCAGA 66
NM_001130413.3_802_ TTCCTCTCCAGGACGTCACCGGTGGCAGCTGCTTCCTTCCAGAGCCGGCAGGAGGCCAGA 360
                        *****

DQ898176.1_704_ GGCTCCATCCTGCTTCAGAGCTGCCAGCTGCCCCCGCAATGGCTGAGCACCGAAGCATGG 126
NM_001130413.3_802_ GGCTCCATCCTGCTTCAGAGCTGCCAGCTGCCCCCGCAATGGCTGAGCACCGAAGCATGG 420
                        *****

DQ898176.1_704_ ACGGGGAATGGAAGCAGCCACACGGGGGGGCTCTCACCTCCAGATCGCCTGGGCCTGTG 186
NM_001130413.3_802_ ACGGGAGAATGGAAGCAGCCACACGGGGGGGCTCTCACCTCCAGATCGCCTGGGCCTGTG 480

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DQ898176.1_704_ GCTCCCCAGAGGCCCTGCCACCTGAAGGGATGGCAGCACAGACCCGCTCAGCACAACGCT 246
NM_001130413.3_802_ GCTCCCCAGAGGCCCTGCCACCTGAAGGGATGGCAGCACAGACCCACTCAGCACAACGCT 540

*****.*****

DQ898176.1_704_ GCCTGCAAACAGGGCCAGGCTGCAGCCCAGACGCCCCCAGGCCGGGGCCACCATCAGCA 306
NM_001130413.3_802_ GCCTGCAAACAGGGCCAGGCTGCAGCCCAGACGCCCCCAGGCCGGGGCCACCATCAGCA 600

DQ898176.1_704_ CCACCACCACCACCAAGGAGGGGCACCGGGAGGGGCTGGTGGAGCTGCCCGCCTCGTTC 366
NM_001130413.3_802_ CCACCACCACCACCAAGGAGGGGCACCGGGAGGGGCTGGTGGAGCTGCCCGCCTCGTTC 660

*****.*****

DQ898176.1_704_ CGGGAGCTGCTCACCTTCTTCTGCACCAATGCCACCATCCACGGCGCCATCCGCCTGGTC 426
NM_001130413.3_802_ CGGGAGCTGCTCACCTTCTTCTGCACCAATGCCACCATCCACGGCGCCATCCGCCTGGTC 720

DQ898176.1_704_ TGCTCCCGCGGGAACCGCCTCAAGACGACGTCCTGGGGGCTGCTGTCCCTGGGAGCCCTG 486
NM_001130413.3_802_ TGCTCCCGCGGGAACCGCCTCAAGACGACGTCCTGGGGGCTGCTGTCCCTGGGAGCCCTG 780

DQ898176.1_704_ GTCGCGCTCTGCTGGCAGCTGGGGCTCCTCTTTGAGCGTCACTGGCACCGCCGGTCCTC 546
NM_001130413.3_802_ GTCGCGCTCTGCTGGCAGCTGGGGCTCCTCTTTGAGCGTCACTGGCACCGCCGGTCCTC 840

DQ898176.1_704_ ATGGCCGTCTCTGTGCACTCGGAGCGCAAGCTGCTCCCGCTGGTCACCCTGTGTGACGGG 606
NM_001130413.3_802_ ATGGCCGTCTCTGTGCACTCGGAGCGCAAGCTGCTCCCGCTGGTCACCCTGTGTGACGGG 900

DQ898176.1_704_ AACCCACGTCGGCCGAGTCCGGTCCTCCGCCATCTGGAGCTGCTGGACGAGTTTGCCAGG 666
NM_001130413.3_802_ AACCCACGTCGGCCGAGTCCGGTCCTCCGCCATCTGGAGCTGCTGGACGAGTTTGCCAGG 960

DQ898176.1_704_ GAGAACATTGACTCCCTGTACAACGTCAACCTCAGCAAAGGCAGAGCCGCCCTCTCCGCC 726
NM_001130413.3_802_ GAGAACATTGACTCCCTGTACAACGTCAACCTCAGCAAAGGCAGAGCCGCCCTCTCCGCC
1020

DQ898176.1_704_ ACTGTCCCCCGCCACGAGCCCCCTTCCACCTGGACCGGGAGATCCGTCTGCAGAGGCTG 786
NM_001130413.3_802_ ACTGTCCCCCGCCACGAGCCCCCTTCCACCTGGACCGGGAGATCCGTCTGCAGAGGCTG
1080

DQ898176.1_704_ AGCCACTCGGGCAGCCGGGTCAGAGTGGGGTTCAGACTGTGCAACAGCACGGGCGGCGAC 846
NM_001130413.3_802_ AGCCACTCGGGCAGCCGGGTCAGAGTGGGGTTCAGACTGTGCAACAGCACGGGCGGCGAC
1140

DQ898176.1_704_ TGCTTTTACCGAGGCTACACGTCAGGCGTGGCGGCTGTCCAGGACTGGTACCACTTCCAC 906
NM_001130413.3_802_ TGCTTTTACCGAGGCTACACGTCAGGCGTGGCGGCTGTCCAGGACTGGTACCACTTCCAC
1200

DQ898176.1_704_ TATGTGGATATCCTGGCCCTGCTGCCCCGCGGCATGGGAGGACAGCCACGGGAGCCAGGAC 966
NM_001130413.3_802_ TATGTGGATATCCTGGCCCTGCTGCCCCGCGGCATGGGAGGACAGCCACGGGAGCCAGGAC
1260

DQ898176.1_704_ GGCCACTTCG CCTCTCCTGCAGTTACGATGGCCTGGACTGCCAGGCCCGACAGTTCCGG
1026
NM_001130413.3_802_ GGCCACTTCGT CCTCTCCTGCAGTTACGATGGCCTGGACTGCCAGGCCCGACAGTTCCGG
1320

DQ898176.1_704_ ACCTTCCACCACCCACCTACGGCAGCTGCTACACGGTCGATGGCGTCTGGACAGCTCAG
1086

NM_001130413.3_802_
1380

ACCTTCCACCACCCACCTACGGCAGCTGCTACACGGTCGATGGCGTCTGGACAGCTCAG

DQ898176.1_704_
1146

CGCCCCGGCATCACCCACGGAGTCGGCCTGGTCCTCAGGGTTGAGCAGCAGCCTCACCTC

NM_001130413.3_802_
1440

CGCCCCGGCATCACCCACGGAGTCGGCCTGGTCCTCAGGGTTGAGCAGCAGCCTCACCTC

DQ898176.1_704_
1206

CCTCTGCTGTCCACGCTGGCCGGCATCAGGGTCATGGTTCACGGCCGTAACCACACGCC

NM_001130413.3_802_
1500

CCTCTGCTGTCCACGCTGGCCGGCATCAGGGTCATGGTTCACGGCCGTAACCACACGCC

DQ898176.1_704_
1266

TTCCTGGGGCACCACAGCTTCAGCGTCCGGCCAGGGACGGAGGCCACCATCAGCATCCGA

NM_001130413.3_802_
1560

TTCCTGGGGCACCACAGCTTCAGCGTCCGGCCAGGGACGGAGGCCACCATCAGCATCCGA

DQ898176.1_704_
1326

GAGGACGAGGTGCACCGGCTCGGGAGCCCCTACGGCCACTGCACCGCCGGCGGGGAAGGC

NM_001130413.3_802_
1620

GAGGACGAGGTGCACCGGCTCGGGAGCCCCTACGGCCACTGCACCGCCGGCGGGGAAGGC

DQ898176.1_704_
1386

GTGGAGGTGGAGCTGCTACACAACACCTCCTACACCAGGCAGGCCTGCCTGGTGTCTCTGC

NM_001130413.3_802_
1680

GTGGAGGTGGAGCTGCTACACAACACCTCCTACACCAGGCAGGCCTGCCTGGTGTCTCTGC

DQ898176.1_704_
1446
NM_001130413.3_802_
1740

TTCCAGCAGCTGATGGTGGAGACCTGCTCCTGTGGCTACTACCTCCACCCTCTGCCGGCG
TTCCAGCAGCTGATGGTGGAGACCTGCTCCTGTGGCTACTACCTCCACCCTCTGCCGGCG

DQ898176.1_704_
1506
NM_001130413.3_802_
1800

GGGGCTGAGTACTGCAGCTCTGCCCGGCACCCTGCCTGGGGACACTGCTTCTACCGCCTC
GGGGCTGAGTACTGCAGCTCTGCCCGGCACCCTGCCTGGGGACACTGCTTCTACCGCCTC

DQ898176.1_704_
1566
NM_001130413.3_802_
1860

TACCAGGACCTGGAGACCCACCGGCTCCCCTGTACCTCCCGCTGCCCCAGGCCCTGCAGG
TACCAGGACCTGGAGACCCACCGGCTCCCCTGTACCTCCCGCTGCCCCAGGCCCTGCAGG

DQ898176.1_704_
1626
NM_001130413.3_802_
1920

GAGTCTGCATTCAAGCTCTCCACTGGGACCTCCAGGTGGCCTTCCGCCAAGTCAGCTGGA
GAGTCTGCATTCAAGCTCTCCACTGGGACCTCCAGGTGGCCTTCCGCCAAGTCAGCTGGA

DQ898176.1_704_
1686
NM_001130413.3_802_
1980

TGGA CTCTGGCCACGCTAGGTGAACAGGGGCTGCCGCATCAGAGCCACAGACAGAGGAGC
TGGA CTCTGGCCACGCTAGGTGAACAGGGGCTGCCGCATCAGAGCCACAGACAGAGGAGC

DQ898176.1_704_
1746
NM_001130413.3_802_
2040

AGCCTGGCCAAAATCAACATCGTCTACCAGGAGCTCAACTACCGCTCAGTGGAGGAGGCG
AGCCTGGCCAAAATCAACATCGTCTACCAGGAGCTCAACTACCGCTCAGTGGAGGAGGCG

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DQ898176.1_704_      CCGGTGTACTCGGTGCCGCAGCTGCTCTCGGCCATGGGCAGCCTCTGCAGCCTGTGGTTT
1806
NM_001130413.3_802_  CCGGTGTACTCGGTGCCGCAGCTGCTCTCGGCCATGGGCAGCCTCTGCAGCCTGTGGTTT
2100
*****

DQ898176.1_704_      GGGCCTCCGTCCTCTCCCTCCTGGAGCTCCTGGAGCTGCTGCTCGATGCTTCTGCCCTC
1866
NM_001130413.3_802_  GGGCCTCCGTCCTCTCCCTCCTGGAGCTCCTGGAGCTGCTGCTCGATGCTTCTGCCCTC
2160
*****

DQ898176.1_704_      ACCCTGGTGCTAGGCGGCCGCGGCTCCGCAGGGCGTGGTTCTCCTGGCCCAGAGCCAGC
1926
NM_001130413.3_802_  ACCCTGGTGCTAGGCGGCCGCGGCTCCGCAGGGCGTGGTTCTCCTGGCCCAGAGCCAGC
2220
*****

DQ898176.1_704_      CCTGCCTCAGGGGCGTCCAGCATCAAGCCAGAGGCCAGTCAGATGCCCCCGCCTGCAGGC
1986
NM_001130413.3_802_  CCTGCCTCAGGGGCGTCCAGCATCAAGCCAGAGGCCAGTCAGATGCCCCCGCCTGCAGGC
2280
*****

DQ898176.1_704_      GGCACGTCAGATGACCCGGAGCCCAGCGGGCCTCATCTCCACGGGTGATGCTTCCAGGG
2046
NM_001130413.3_802_  GGCACGTCAGATGACCCGGAGCCCAGCGGGCCTCATCTCCACGGGTGATGCTTCCAGGG
2340
*****

DQ898176.1_704_      GTTCTGGCGGGAGTCTCAGCCGAAGAGAGCTGGGCTGGGCCCCAGCCCCTTGAGACTCTG
2106
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NM_001130413.3_802_
2400

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DQ898176.1_704_
NM_001130413.3_802_

GACACCTGA 2115

GACACCTGA 2409

2) Amino acid sequence alignment of human δ_{802} and δ_2 human ENaC variants.

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NP_001123885.2_802_      MRAVLSQKTTPLPRYLWPGHLSGPRRLTWSWCS DHRTPTCRELGSPHPTPCTGPARGWPR 60
ABI64069.1      704
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NP_001123885.2_802_      RGGGPCGFTSAGHVLCGYPLCLLSGPIQGC GTGLGDSSMAFLSRTSPVAAASFQSRQEAR 120
ABI64069.1      -----MAFLSRTSPVAAASFQSRQEAR 22
                                           *****

NP_001123885.2_802_      GSILLQSCQLPPQWLSTEAWTGEWKQPHGGALTSRSPGPVAPQRPCHLKGWQHRPTQHNA 180
ABI64069.1      GSILLQSCQLPPQWLSTEAWTGEWKQPHGGALTSRSPGPVAPQRPCHLKGWQHRPAQHNA 82
                                           *****:*****

NP_001123885.2_802_      ACKQGQAAAQTPPRPGPPSAPPPPPKEGHQEGLVELPASFRELLTFFCTNATIHGAI RLV 240
ABI64069.1      ACKQGQAAAQTPPRPGPPSAPPPPPKEGHREGLVELPASFRELLTFFCTNATIHGAI RLV 142
                                           *****:*****

                                           M1
NP_001123885.2_802_      CSRGNRLKTTSWGLLSL GALVALCWQLGLL FERHWHRPVLMAVSVHSE RKL LPLVTLCDG 300
ABI64069.1      CSRGNRLKTTSWGLLSL GALVALCWQLGLL FERHWHRPVLMAVSVHSE RKL LPLVTLCDG 202
                                           *****

NP_001123885.2_802_      NPRRPSVLRHLELLDEFARENIDSLYNVNLSKGRAALSATVPRHEPPFHL DREIRLQRL 360
ABI64069.1      NPRRPSVLRHLELLDEFARENIDSLYNVNLSKGRAALSATVPRHEPPFHL DREIRLQRL 262
                                           *****

NP_001123885.2_802_      SHSGSRVRVGFRLCNSTGGDCFYRGYTS GVAAVQDWYHFHYVDILALLPAAWEDSHGSQD 420
ABI64069.1      SHSGSRVRVGFRLCNSTGGDCFYRGYTS GVAAVQDWYHFHYVDILALLPAAWEDSHGSQD 322
                                           *****

NP_001123885.2_802_      GHFVLSCSYDGLDCQARQFRTFHHPTYGSCYTVDG VWT AQRP GITHGVGLVLRVEQQPHL 480
ABI64069.1      GHFVLSCSYDGLDCQARQFRTFHHPTYGSCYTVDG VWT AQRP GITHGVGLVLRVEQQPHL 382
                                           ***.*****

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NP_001123885.2_802_ 540
ABI64069.1 PLLSTLAGIRVMVHGRNHTPFLGHHSFSVRPGTEATISIREDEVHRLGSPYGHCTAGGEG 442

NP_001123885.2_802_ 600
ABI64069.1 VEVELLHNTSYTRQACLVSCFQQLMVETCSCGYLHPLPAGAEYCSSARHPAWGHCFYRL 502

NP_001123885.2_802_ 660
ABI64069.1 YQDLETHRLPCTSRCPRESAFKLSTGTSRWPSAKSAGWTLATLGEQGLPHQSHRQRS 562

NP_001123885.2_802_ 720
ABI64069.1 SLAKINIVYQELNYRSVEEAPVYSVPQLLSAMGSLCSLWFGASVLSLLELLELLLDASAL 622

M2

NP_001123885.2_802_ 780
ABI64069.1 TLVLGGRRLRRRAWFSWPRASPASGASSIKPEASQMPPPAGGTSDDPEPSGPHLPRVMLPG 682

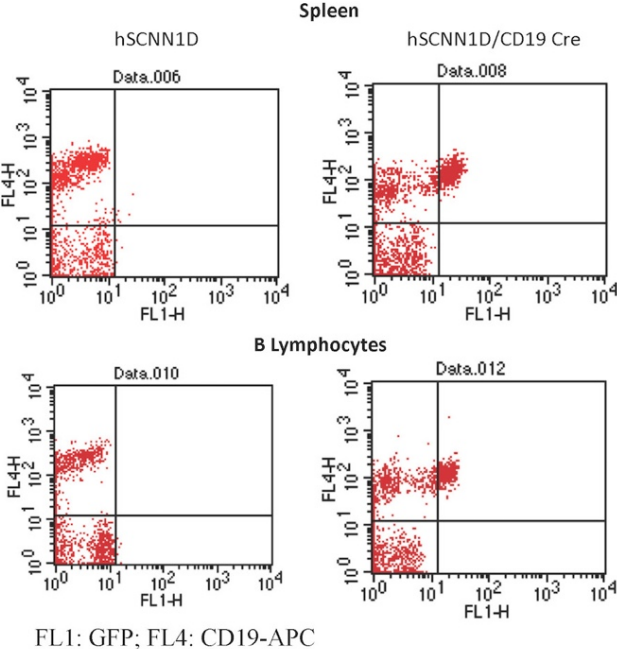
NP_001123885.2_802_ 802
ABI64069.1 VLAGVSAEESWAGPQPLETLDT 704

Figure S2. Amino acid sequence of human α -13 and δ -15 peptides.

- 1) Human α -13 peptide, C-terminal amidation, N-terminal acetylation.
"LRGTLPHPLQRLR".

- 2) Human δ -15 peptide, C-terminal amidation, N-terminal acetylation.
"LSATVPRHEPPFHLD".

Figure S3. Confirmation of conditional expression by cross breeding CD19^{Cre} and hscnn1d^{T9} lines. To confirm the conditional expression of human δ_{802} construct, the Biocytogen (<http://www.biocytogen.com>) bred δ_{802}^{Tg} with CD19^{Cre} and detected GFP⁺ cells in spleen and blood by FACS.



Spleen	Abs	Spleen
hSCNN1D	CD19+/GFP+	0.46%
hSCNN1D/CD19-Cre	CD19+/GFP+	31.60%

Blood	Abs	Spleen
hSCNN1D	CD19+/GFP+	0.02%
hSCNN1D/CD19-Cre	CD19+/GFP+	24.54%

FL1: GFP; FL4: CD19-APC

Figure S4. Mass spectrometry analysis of two δ 802 ENaC bands. AT2 cell lysates were immunoprecipitated with anti-HA antibody and immunoblotted with anti-his antibody. Then the blot was stained with Coomassie blue. Two bands near 100 kDa (selected by dashed line) were dissected for mass spectrometry. Corresponding MS1 spectra were shown on the right side. Both bands contained a human δ ENaC-specific peptide sequence (HLELLDEFAR).

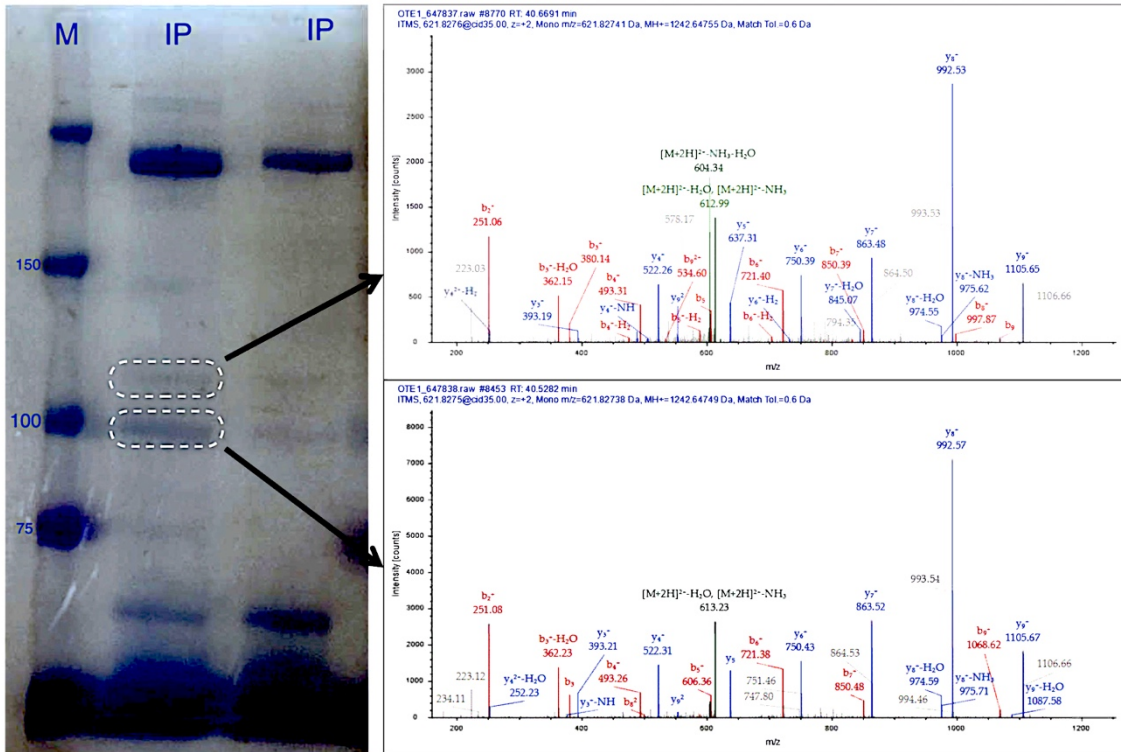


Figure S5. Visualization of tight junction of mouse AT2 monolayers. Image of mouse AT2 monolayers 5 days post seeding with anti-claudin 8 antibody. Scale bar, 100 μm .

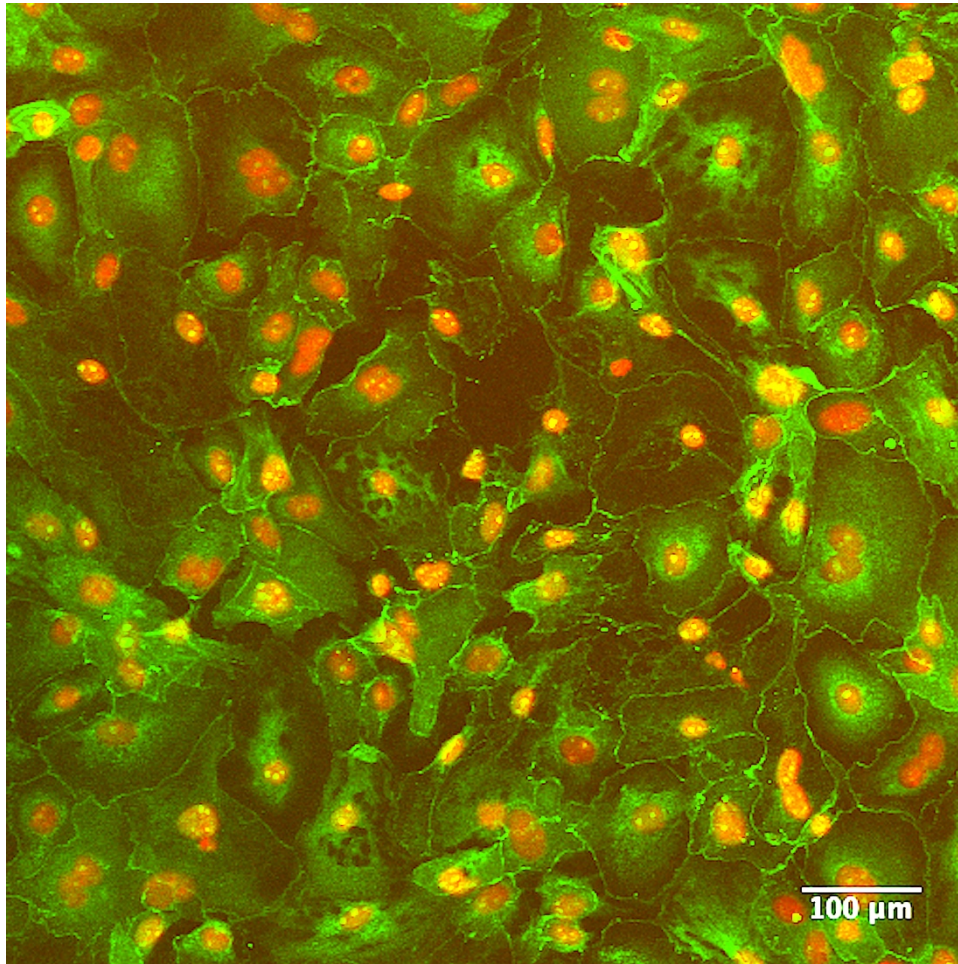


Figure S6. Images of an alveolus-like organoid formed by 3D cultured primary mouse AT2 cells. The organoid grown in 3D Matrigel for 12 days was imaged following stained with pdpn and sftpc antibodies. Dash lines, sideview level. Scale bar, 50 μ m.

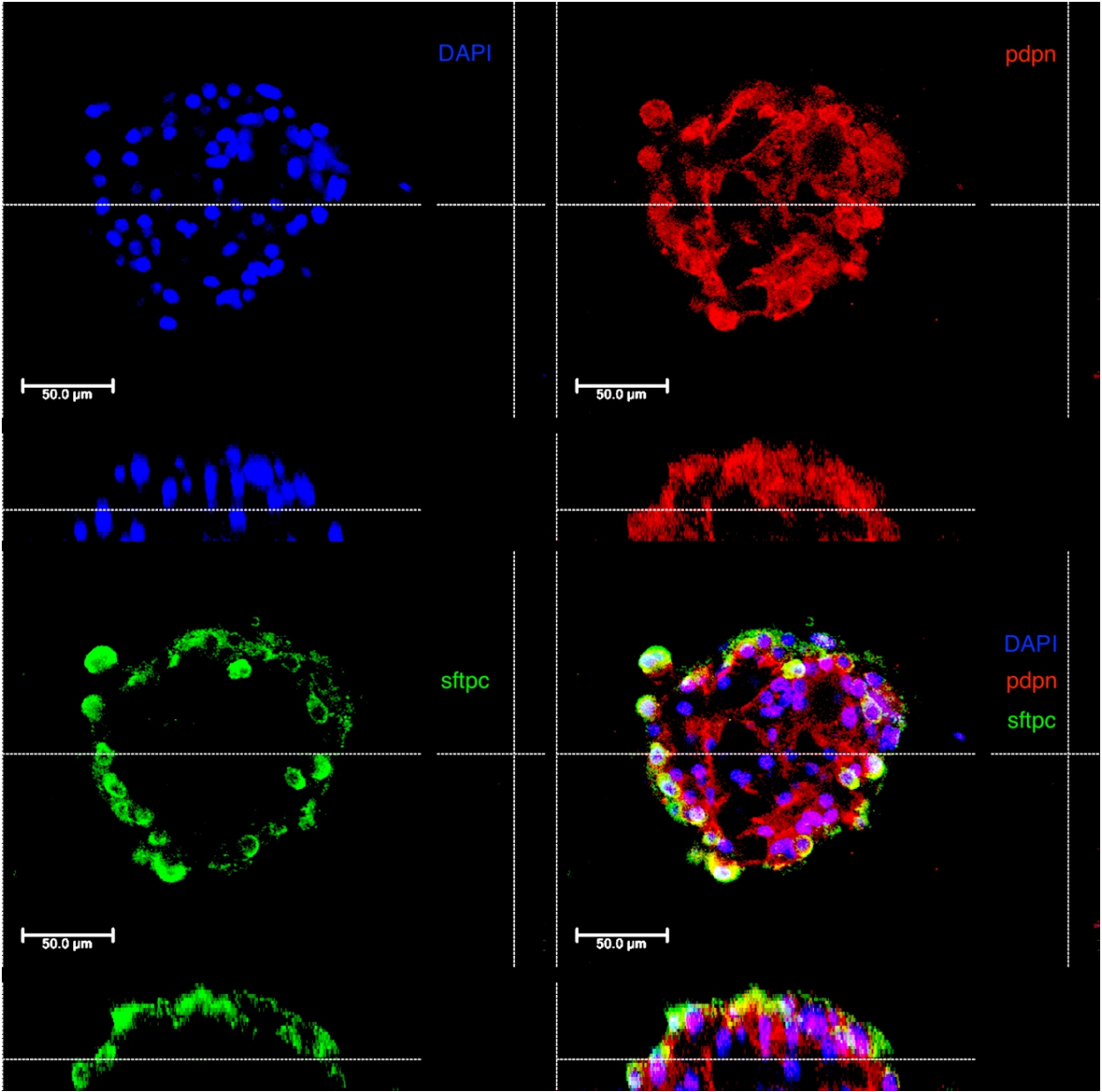


Figure S7. Image of isolated mouse AT2 cells. Top, freshly isolated mouse AT2 cells were stained with DAPI and anti-human pro-SPC antibody (sftpc) following cytopspining. IgG without primary antibody was used for negative control. Scale bar, 50 μm . Bottom, PAP stain of freshly isolated mouse AT2 cells. Scale bar, 20 μm .

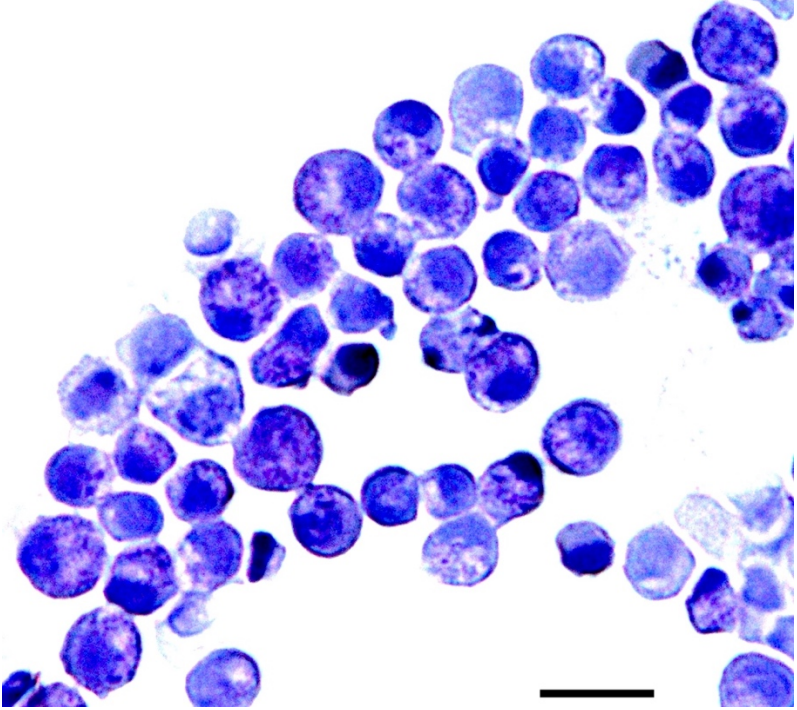
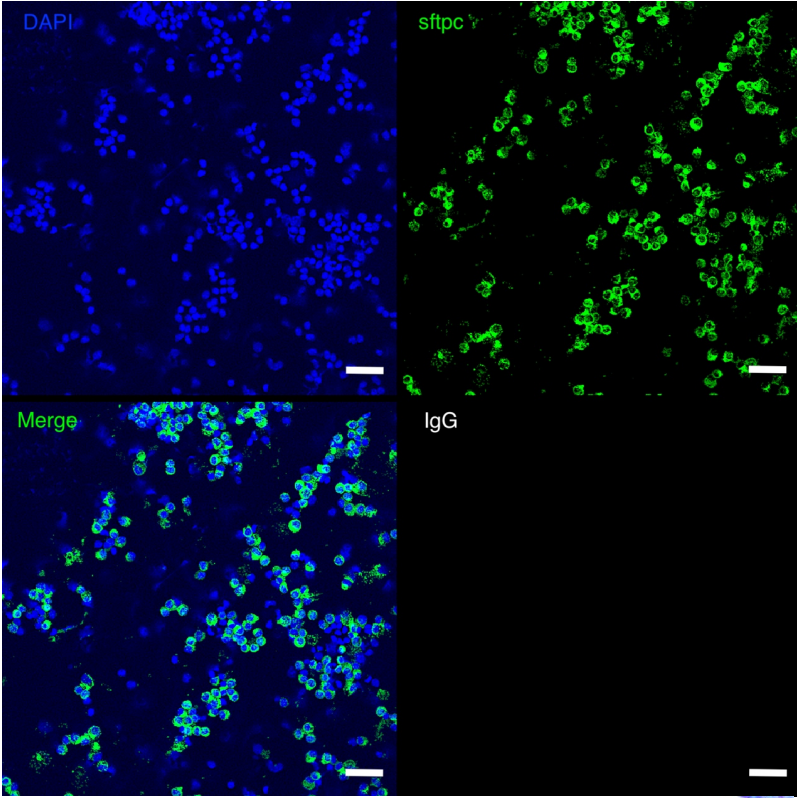


Figure S8. AT2 cells purity analyzed by FACS. Freshly isolated mouse AT2 cells were stained with EpCAM antibody (CD326). The purity is 96.58% (95 - 98%). n=8.

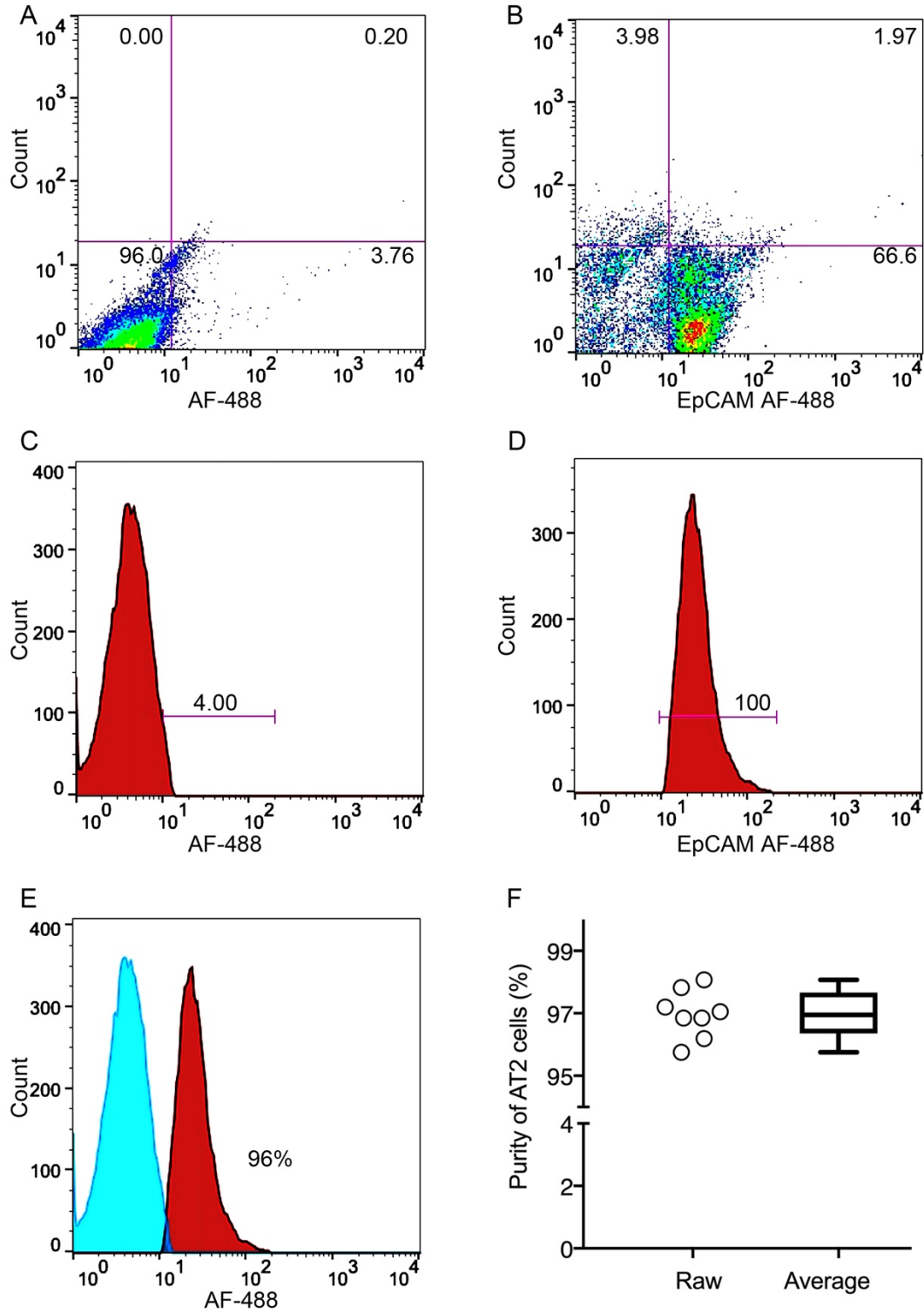


Figure S9. Histology of mouse lungs. Images of H & E stain of lung sections for wild type (wt) and human δ_{802}^{T9} mice (Scnn1d Tg/sox2).

WT

Scnn1d Tg/sox2

