| Gene                | Forward primer                  | Reverse primer                 | Amplicon<br>length | Ta   |
|---------------------|---------------------------------|--------------------------------|--------------------|------|
| GAPDH               | 5'-CCCTTCATTGACCTCAACTACATGA-3' | 5'-TGGGATTTCCATTGATGACAAGC-3'  | 116bp              | 62°C |
| SCNNIA              | 5'-GCTGATAACCAGGACAAAACACAA-3'  | 5'-CGTCGCTGGGCAGGAA-3'         | 68bp               | 60°C |
| SCNN1B              | 5'-GAGCCCTGCAACTACCGGA-3'       | 5'-GCCGAAGGAAGTGCCTTCTC-3'     | 101bp              | 60°C |
| SCNNIG              | 5'-GCCCTGAAGTCCCTGTATGG-3'      | 5'-CGGTGGGAGAATCTAGGCTG-3'     | 101bp              | 60°C |
| CFTR<br>(Ex6-Ex7)   | 5'-TGGGAGTTGTTACAGGCGTCTGCC-3'  | 5'-AGGGAAATTGCCGAGTGACCGC-3'   | 421bp              | 60°C |
| CFTR<br>(Ex8-Ex10)  | 5'-ACAAAAGCAAGAATATAAGACATTG-3' | 5'-GAATGAAATTCTTCCACTGTGC-3'   | 346bp              | 60°C |
| CFTR<br>(Ex11-Ex13) | 5'-ACACTGAGTGGAGGTCAACG-3'      | 5'-CCATTTTAGAAGTGACCAAAATCC-3' | 184bp              | 60°C |
| CFTR<br>(Ex11-Ex15) | 5'-ACACTGAGTGGAGGTCAACG-3'      | 5'-AGCAAAGTGTCGGCTACTCC-3'     | 1142bp             | 60°C |

# Table S1 - Primers used for qualitative endpoint PCR.

# Table S2 - Primers used for quantitative real time PCR.

| Gene                | Forward primer                 | Reverse primer             | Amplicon<br>length | $T_{a}$ |
|---------------------|--------------------------------|----------------------------|--------------------|---------|
| Actin-β             | 5'-GCCGGGACCTGACTGACTA-3'      | 5'- TGGTGATGACCTGGCCGT -3' | 204bp              | 60°C    |
| SCNNIA              | 5'-GCTGATAACCAGGACAAAACACAA-3' | 5'-CGTCGCTGGGCAGGAA-3'     | 68bp               | 60°C    |
| SCNN1B              | 5'-GAGCCCTGCAACTACCGGA-3'      | 5'-GCCGAAGGAAGTGCCTTCTC-3' | 101bp              | 60°C    |
| SCNN1G              | 5'-GCCCTGAAGTCCCTGTATGG-3'     | 5'-CGGTGGGAGAATCTAGGCTG-3' | 101bp              | 60°C    |
| CFTR<br>(Ex10-Ex11) | 5'-AAGCGTCATCAAAGCATGCC-3'     | 5'TTGCTCGTTGACCTCCACTCA-3' | 110bp              | 60°C    |

### Table S3 - Primers used for HpaII / PCR.

# A) SCNN1A gene.

| Region  | Forward primer                | Reverse primer               | Amplicon<br>length | $T_{a}$ |
|---------|-------------------------------|------------------------------|--------------------|---------|
| а       | 5'-CAAGATTCAGCAGAGATGACACC-3' | 5'-TCCTGGTCCCTCCTCTTTCC-3'   | 875bp              | 66-59°C |
| b       | 5'-CTAGCTCCTGGAAGCACACTTG-3'  | 5'-TGTGTCCTGATTCTGTCTCTGC-3' | 711bp              | 66-59°C |
| c       | 5'-AGAGGAGAGGCCGTTGTTGTAGG-3' | 5'-GCTGAAGTACTCTCCGAAAAGC-3' | 636bp              | 66-59°C |
| control | 5'-ATCAACCTCAACTCGGACAAGC-3'  | 5'-GTGCTAGGATGGATTCACTGG-3'  | 265bp              | 66-59°C |

### B) SCNN1B gene.

| Region  | Forward primer               | Reverse primer               | Amplicon<br>length | Ta      |
|---------|------------------------------|------------------------------|--------------------|---------|
| а       | 5'-TGAGTCCAGGAGTTCCAGACC-3'  | 5'-CCACGAATATGTCCACAGACC-3'  | 924bp              | 66-59°C |
| b       | 5'-CAGCTCCCCAAAGGTAAACACC-3' | 5'-ATTCATGGGTCCGTATGTGAGC-3' | 655bp              | 66-59°C |
| с       | 5'-ATTTGAACCCAGGCAGTCC-3'    | 5'-ACACAGCTCAATGGGTAGGC-3'   | 487bp              | 66-59°C |
| d       | 5'-CCAGCCTACATGGTGAAACC-3'   | 5'-CCCATCGGTAGGCATTATCC-3'   | 355bp              | 66-59°C |
| control | 5'-AGTTCAGGCAATTCCCTTCC-3'   | 5'-GGCCATCTCCAGGTCTCC-3'     | 188bp              | 66-59°C |

### C) SCNN1G gene.

| Region  | Forward primer                 | Reverse primer               | Amplicon<br>length | $T_{a}$ |
|---------|--------------------------------|------------------------------|--------------------|---------|
| a       | 5'-AGACGCGTGGATCACCTG-3'       | 5'-AAGGGTCCAAGGCTCGTG-3'     | 871bp              | 66-59°C |
| b       | 5'-TGGAACCGAAAGGTGAGTTC-3'     | 5'-AGATTTGCCCCAAGTCTAGC-3'   | 670bp              | 66-59°C |
| control | 5'-GTGAAAATTAAATGAGGTGACAGC-3' | 5'-ACCTCCTCCCTCACTACAATCC-3' | 485bp              | 66-59°C |



#### Figure S1 – Quantitative CFTR gene expression analysis by real time PCR.

A) Results represent the expression of *CFTR* in H441, MCF10A, 16HBE, CFBE, HaCaT cell lines (respectively from 1 to 5). B) Results with (+) and without (-) dexamethasone treatment are shown for H441 and MCF10A cell lines. C) Results for nasal brushing (B), lymphocytes/monocytes (LM), granulocytes (PMN), lymphocytes (L) and monocytes (M) are shown (from 6 to 10). A relative quantification (RQ) is reported on y-axis, as fold changes in respect to *SCNN1B* expression in CFBE (Figure 1, panel D, column 4 of main text) used as reference condition (the numbers above the bars are the exact RQ values). For panels A and C, ANOVA p<0.01; for panel A the single \* indicates the only statistically significant difference following Bonferroni's multiple comparison test are as indicated (\*p<0.01). For panel B, Student's t-test of all dexamethasone treated cells (+) as compared to respective untreated cells (-) \*p<0.01.





Results represent the expression of *CFTR* in indicated cell lines (1-5) and *ex vivo* samples (6-10). The analysis was performed by a qualitative endpoint PCR protocol by studying the 4 CFTR exonic regions indicated (with the size of amplicons showed in base pairs (bp)). For some cell lines (1, 2) results with (+) and without (-) dexamethasone treatment are shown. Panels from A to D refer to *CFTR* expression analysis after 38 cycles of PCR amplification protocol. Panel E refers to *GAPDH* expression analysis after 28 cycles of PCR amplification protocol. In every panel: 1 = H441, 2 = MCF10A, 3 = 16HBE, 4 = CFBE, 5 = HaCaT, 6 = nasal brushing, 7 = lymphocytes + monocytes, 8 = granulocytes, 9 = lymphocytes, 10 = monocytes. The first and last lane of each panel contain the DNA ladder described in Materials and Methods.





Panels refer to the indicated number of cycles of PCR amplification protocol for *SCNN1A* (panels A, B and C), *SCNN1B* (panels D, E and F) and *SCNN1G* (panels G, H and I) genes. *GAPDH* gene was analyzed after 28 cycles of PCR amplification. The size of amplicons in base pairs (bp) is shown on the right of each panel. Cell lines (1-5, panels A, D and G), H441 and MCF10A treated (+) or untreated (-) with dexamethasone (panels B, E and H) and *ex vivo* samples (6-10, panels C, F and I) are indicated as follows. In every panel: 1 = H441, 2 = MCF10A, 3 = 16HBE, 4 = CFBE, 5 = HaCaT, 6 = nasal brushing, 7 = lymphocytes + monocytes, 8 = granulocytes, 9 = lymphocytes, 10 = monocytes. The first and last lane of each panel contain the DNA ladder described in Materials and Methods.



#### Figure S4 – Schematic representation of 5'-flanking regions of ENaC genes.

A) SCNN1A gene. Blue arrows indicate the 4 analyzed regions (a, b, c, Control). Black lines indicate CCGG sites. The entire region analyzed consists of 2926 base pairs of the 5'-flanking region of SCNN1A gene, covering a total of 7 CCGG sites. B) SCNN1B gene. Blue arrows indicate the 5 analyzed regions (a, b, c, d, Control). Black lines indicate CCGG sites. The entire region analyzed consists of 3842 base pairs of the 5'-flanking region of SCNN1B gene, covering a total of 14 CCGG sites. The position of the CpG island is shown. C) SCNN1G gene. Blue arrows indicate the 3 analyzed regions (a, b, Control). Black lines indicate CCGG sites. The entire region analyzed regions (a, b, Control). Black lines indicate CCGG sites. The position of the CpG island is shown. C) SCNN1G gene. Blue arrows indicate the 3 analyzed regions (a, b, Control). Black lines indicate CCGG sites. The entire region analyzed consists of 2537 base pairs of the 5'-flanking region of SCNN1G gene, covering a total of 21 CCGG sites. The position of the CpG island is shown.