Supplemental Data and Demographic Tables for

SPLUNC1 Degradation by the Cystic Fibrosis Mucosal Environment Drives Airway Surface Liquid Dehydration

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Running Title: SPLUNC1 & CF Sputum

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Supplemental Methods

Proteins and peptides. SPLUNC1 cDNA was kindly provided by Colin Bingle (University of Sheffield, Sheffield, United Kingdom). Recombinant SPLUNC1 protein (rSPLUNC1) was produced using a mammalian expression system and lacked the cleavable N-terminal signal sequence (M1-M19) but was otherwise full length and contained the S18 ENaC inhibitory region (residues G22 through A39)(1). The SPX-101 peptide (aaLPIPLDQTaa) was prepared by the High-Throughput Peptide Synthesis and Array Facility at UNC-Chapel Hill.

Collection of normal and CF sputum samples. Induced and spontaneous sputum samples were obtained as previously described (2, 3). For induced sputum collection, subjects inhaled 3%, 4% and 5% hypertonic saline, each for a 7 min period. To reduce squamous cell contamination, all subjects performed a 3-step cleansing procedure, including (i) rinsing and gargling of the mouth with water, (ii) clearing of the throat, without coughing and (iii) blowing of his/her nose. Following cleansing, induced and spontaneous sputum samples were collected into specimen cups using a cough from the chest. All sputum samples were placed on ice. Samples were incubated in Dulbecco's Phosphate Buffered Saline (DPBS) solution with agitation for 15 min followed by centrifugation and collection of supernatent. Spontaneous sputum collection was similar, except the subjects did not inhale hypertonic saline prior to sputum production. Supernatants were stored at -80°C until required. Donor demographics are shown in supplementary table 1 and 2.

Determination of endogenous NE and SPLUNC1 protein levels in sputum samples. Neat sputum samples were denatured in the presence of 2.5% β-mercaptoethanol at 95°C for ~10

min and were subject to western blot. Samples were transferred to PDVF membranes and blocked using 5% skimmed milk in Tris buffered saline with Tween 20 (TBST-T). For detection of NE protein, membranes were probed using a mouse-monoclonal anti-hELA2 antibody, raised against residues M1 – N252 (1:3000, R&D systems), primary antibodies were detected using an anti-mouse horseradish peroxidase (HRP) conjugated secondary antibody (Thermo-Fisher Scientific). Membranes were stripped, re-blocked and re-probed for SPLUNC1 using a goat-polyclonal hPLUNC1 antibody raised against residues Q20 – V256 of hPLUNC1 (1:3000, R&D systems), A secondary anti-goat HRP (Thermo-Fisher Scientific) conjugated antibody was used for detection of hPLUNC1. Secondary antibodies were detected by enhanced chemiluminescence.

Degradation of rSPLUNC1. rSPLUNC1 was incubated in combination with normal and CF sputum (pooled from N = 6 donors), NE (Elastin Products Company, #SE563), PBS, and SMM (pooled from N ≥ 3 donors) for specified time periods, at 37ºC. Heat-denaturing of sample proteins, in the presence of 2.5% β-mercaptoethanol, at 95°C for ~10 min was performed to stop protease activity. Sputum samples were used neat, except for addition of rSPLUNC1, and SMM samples were diluted 1:2 with PBS. NE was used at indicated concentrations. For matched protein experiments, protein concentrations of pooled sputum (N = 6 normal or CF donors) were determined using the bicinchonicic acid (BCA) assay (Pierce). For inhibition experiments, sputum samples were pooled from N = 6 normal or CF donors and co-incubated with protease inhibitors for one hour prior to addition of rSPLUNC1. Inhibitors used were as follows: EDTA free, protease inhibitor cocktail (PIC, Roche); sivelestat sodium salt (Tocris); aprotinin from bovine lung (Sigma); EDTA (Sigma); E64 (Sigma); CA-074 (Sigma); pepstatin A; leupeptin**.** Resultant solutions were subject to western blot as described above.

Neutrophil elastase activity assay. NE activity in sputum samples from individual donors was determined using the elastase substrate Suc-Ala-Ala-Ala-MCA (#MAA-3133; Peptides International) at 100 μM. Cathepsin B, K and S/L activity was determined using the substrates Z-Arg-Arg-MCA, Z-Gly-Pro-Arg-MCA and Z-Val-Val-Arg-MCA respectively (Peptides International). Sputum was diluted 1:1 with assay buffer (final concentrations; 154 mM NaCl; 10 mM HEPES). 10 μl of sputum buffer mix was added per well in 384-well plates, and 10 μl of 100 μM MAA-3133 added to each well. Where specified 20 μM sivelestat was added to sputum: buffer mix, to give final concentration of 10 μM. Assays were performed at 37°C using a Tecan infinite M1000 plate-reader, with fluorescence readings taken every 15 minutes. Samples were excited at 380 ± 5 nm and emitted fluorescence collected at 460 ± 10 nm. Resultant AMC concentrations were determined from an AMC standard curve with linear regression analysis. MMP-2/9 activity was determined using a fluorogenic substrate that is cleaved by both MMP-2 and MMP-9 but not by other proteases (DNP-Pro-Leu-Gly-Met-Trp-Ser-Arg; SCP0191, Sigma Aldrich). The MMP-2/9 substrate was incubated with normal and CF sputum for 2.5 h and fluorescence measured kinetically using excitation 280 ± 5 nm and emission 360 ± 10 nm. Sputum protein concentrations were determined using the BCA (Pierce), and fluorescence values adjusted accordingly.

Human bronchial epithelial cell culture (HBECs). Cells were harvested via enzymatic digestion in the presence of antibiotics from human lungs deemed unsuitable for transplantation (non-CF donors) or post-transplantation (CF donors) as per the UNC protocol #03-1396 (4). Freshly isolated (passage zero) or passage one HBECs were seeded on 0.6 cm (24-well hanging inserts, 0.4 μm pore; Oxyphen, Germany) and were maintained at the air-liquid interface in a

modified bronchial epithelial growth medium (5) at 37°C/5% CO_2 in a humidified incubator. Cells were studied 21-28 days following initial seeding.

ASL height measurements and HBEC incubation with sputum. To determine if sivelestat could recover ability of rSPLUNC1 to regulate ASL volume, CF sputum, pooled from N = 6 donors) was incubated ± 30 μM rSPLUNC1 ± 10 μM sivelestat overnight at 37°C. For assessment of ability of rSPLUNC1 and SPX-101 to regulate ASL height 30 μM rSPLUNC1 or 30 μM SPX-101 were co-incubated with PBS or normal/CF sputum (pooled from N = 6 donors) overnight at 37°C. Prior to experimentation all HBECs were washed apically using PBS. 1mg/ml tetramethylrhodamine dextran (Invitrogen) was added to PBS/sputum and 14 μl of the resulting solution deposited onto the HBEC mucosal surface. ~30 μl perfluorocarbon (PFC; FC-77, 3M) was added mucosally. PFC does not displace ASL nor affect ASL height but prevents ASL evaporation (6). Images were acquired using XZ-scanning confocal microscopy (Leica SP5, glycerol 63 x immersion lens) as described previously (7) at a minimum of 10 predetermined points across the entire culture surface using an automated stage 2 hours post sputum addition to HBEC surface.

In some cases, sputum was passed through 10 kDa molecular weight cutoff spin columns (0.5 ml total volume; Millipore-Sigma). Here, sputum was spun in these columns at 13,000 x g for 45 min at 4ºC. After this time, the spin column was visually inspected to ensure that all supernatant had passed through. If solution remained in the upper chamber, an additional centrifugation step was performed. The filtrate was then diluted 1:1 with PBS, labeled with rhodamine-dextran and added mucosaly to normal HBECs in order to measure ASL height.

Harvesting of airway supernatant of mucopurulent material (SMM). SMM was harvested from the airways of excised human CF lungs as described (8). Harvested material was

centrifuged at 10^6 rpm (60 min, 4°C, Beckman TL-100 Tabletop Ultracentrifuge, with a TLA 100.2 rotor), and the supernatant collected. Supernatant was passed through a 0.2 μm filter and stored at -80°C until required. Donor demographics are shown in supplementary table 3. Endogenous expression of SPLUNC1 in SMM, and degradation of rSPLUNC1 by SMM were determined as described above.

Determination of ENaC and Na^+/K^+-ATPase expression in human bronchi. Human lungs unsuitable for transplantation (non-CF donors) or post-transplant (CF donors) were obtained under protocol #03-1396 approved by the UNC at Chapel Hill Biomedical Institutional Review Board. Informed consent was obtained from authorized representatives of all organ donors. Non-CF lungs were from donors with no history of chronic lung disease (supplementary table 4). Bronchi were dissected free from the underlying tissue at 4°C. CF bronchi were selected from relatively disease-free regions. Tissues were rinsed using a lactated Ringers solution and proteins extracted using lysis buffer containing NP40 (4). Donor demographics are shown in supplementary table 4.

Determination of NE mediated cleavage of rSPLUNC1 and SPX-101 by mass spectrometry. 10 μM rSPLUNC1 and 10 μM SPX-101 were incubated with normal or CF sputum (pooled from N = 6 donors) for either 6 or 24 hours. Samples were snap frozen at -80°C prior to processing. Proteomic sample preparation was performed utilizing filter-aided sample preparation (FASP) (9). First, each sample was spin-filtered at 14,000 x g through an Amicon Ultra 4 10 kDa spin filter (Millipore) to collect peptides produced by proteolytic activities under NE, C.F sputum or normal sputum treatment conditions. The remaining material in the filter of higher than 10 kDa molecular weight was reduced in 10 mM dithiothreitol (Sigma-Aldrich) and alkylated in 50 mM iodoacetamide (Sigma-Aldrich). Trypsin digestion (1 ng/μl), was performed

in 50 mM ammonium bicarbonate overnight at 37°C. The resulting peptide digests were filtered using the same Amicon Ultra 4 spin filters. Peptide mixes were vacuum-dried and dissolved in 25 µl of 2% acetonitrile and 0.1% trifluoroacetic acid. Five microliter of solubilized peptide material was injected for label-free quantitative proteomic analysis utilizing a Q Exactive (Thermo Scientific) mass spectrometer coupled to an UltiMate 3000 (Thermo Scientific) nanoHPLC system, and data acquisition was performed as described previously (10).

Proteomic data analysis. The acquired raw data was processed using the Proteome Discoverer 1.4 software (Thermo Scientific) and searched against the UniProt protein database (Homo sapiens, November 2016) and the custom sequence of the SPX-101 peptide using the MASCOT (Matrix Science) search engine with parameters set as follows: 5 ppm mass accuracy for parent ions and 0.02 Da accuracy for fragment ions, with 2 missed cleavages allowed. For the identification of peptides produced by NE activity, cleavage site detection was set to semi-elastase specificity and allowed for variable methionine oxidation and c-terminal amidation, of SPX-101.

For tryptic peptide detection, cleavage specificity was set to semi-tryptic, and Carbamidomethyl modification of cysteines was set to fixed, and methionine oxidation to variable. Scaffold 4.7.5 (Proteome Software Inc.) was used to validate MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (11). Protein quantification was performed by adding the total ion chromatogram intensities of identified peptide ions and fragments assigned to a protein and normalizing the total ion

chromatogram to the total intensity of all identified proteins in each sample. The data were normalized via natural log transformations.

Binding of SPLUNC1 and SPX-101 to HBECs. Prior to experimentation HBECs were washed apically using PBS. Cells were loaded with Calcein-AM for 30 minutes prior to apical addition of fluorescently labelled rSPLUNC1 or SPX-101. rSPLUNC1 fluorescently labelled with amine-reactive Dylight-633 (Thermo Fisher) and 5-TAMRA - SPX-101 were pre-incubated with PBS, normal and CF sputum (pooled from N = 6 donors) for 2 hours and further incubated for 2 hours on the apical surface of normal HBECs. Cells were then washed apically to remove excess, unbound, rSPLUNC1 and SPX-101 and were imaged using a Leica SP8 confocal microscope with a 63x glycerol immersion objective. rSPLUNC1-Dylight 633 nm fluorescence was acquired using a 633 nm laser, and SPX-101 5-TAMRA fluorescence acquired using a 561 nm laser, calcein-AM was imaged sequentially using an argon 488 nm laser.

Transepithelial Potential Difference Studies. A single-barreled transepithelial potential difference (V_t)-sensing microelectrode was positioned in the ASL by micromanipulator and used in conjunction with a macroelectrode in the serosal solution to measure V_t using a voltmeter (World Precision Instruments) as described (12). Perfluorocarbon was present throughout the readings to prevent evaporation of the ASL.

αENaC-GFP internalisation assay. We have previously shown that SPLUNC1 causes internalisation of αENaC-GFP and a resulting diminution of GFP fluorescence (13). Here, we used the decrease in αENaC-GFP as an indicator of SPLUNC1/SPX-101-induced ENaC internalisation. HEK293T cells were seeded onto 10 cm dishes and transiently transfected with 2 μg each of αENaC-GFP, βENaC-untagged and γENaC-untagged or GFP alone. 24 hours following transfection, cells were trypsinised and seeded on 96 well culture treated plate

(Costar). 30 μM of rSPLUNC and 30 μM SPX-101 were incubated overnight ± 0.1 μM NE and the reaction stopped by adding 10 μM sivelestat. Cells were then exposed to either PBS, 30 μM rSPLUNC ± 0.1 μM NE or 30 μM SPX-101 ± 0.1 μM NE in HEK293T cell media (DMEM-HG (4500 mg/l), 10% FBS, 5% Pen-strep) for 1 h at 37°C, and fluorescence measured using a Tecan infinite M1000 plate-reader. Fluorescence was adjusted against that of control, non-transfected, HEK293T cells. Cells were excited at wavelength 488 ± 5 nm and emission collected at 590 ± 5 nm, with a 5 nm bandwidth.

Statistics. When variances were homogeneously distributed, data were analyzed using ANOVA followed by the Tukey test or Student's t-test as appropriate. When data were not normally distributed, the non-parametric equivalents were used (e.g. Mann-Whitney U-test; Kruskal Wallis Test with Dunn's Multiple Comparisons Test). Data shown are mean ± SD, or mean ± SEM as indicated. SPLUNC1 degradation over time curves were fit with single exponentials and analyzed using the Extra Sum of Squares F Test. All data analysis was performed using GraphPad Prism 7.0. Details of experimental numbers are provided in the figure legends. Significance values are denoted as follows; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. All experiments were performed on a minimum of three separate occasions and are from ≥3 donors/group where applicable.

Supplemental Results

SPX-101, but not rSPLUNC1, internalises αENaC-GFP following exposure to NE. We have previously demonstrated that SPLUNC1 internalises αENaC-GFP, leading to a decrease in GFP fluorescence, likely as ENaC enters acidic organelles (13). Additionally, we have shown ability of SPX-101, to internalise ENaC subunits (14). Using a multiplate reader, we measured quenching of GFP fluorescence as an indicator of ENaC internalisation (figure S4). We found that SPLUNC1 exposure lead to a quenching of αENaC-GFP fluorescence in transiently transfected HEK293T cells. Using this approach, we next tested whether NE-exposed rSPLUNC1 was still capable of affecting ENaC. Whilst exposure of HEK293T cells to either 30 μM rSPLUNC1 or 30 μM SPX-101 reduced αENaC-GFP fluorescence, which is indicative of αENaC-GFP internalization, rSPLUNC1 pre-incubated with NE did not decrease fluorescence (figure S4). However, SPX-101 remained capable of affecting αENaC-GFP fluorescence even in the presence of NE. In contrast, HEK293T cells transfected with GFP alone, showed no significant change in fluorescence levels following exposure to SPLUNC1 or SPX-101 (figure S4).

Supplemental References

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Channel-targeted Therapeutic for Cystic Fibrosis That Restores Mucus Transport. *Am J Respir Crit Care Med.* 2017;196(6):734-44.

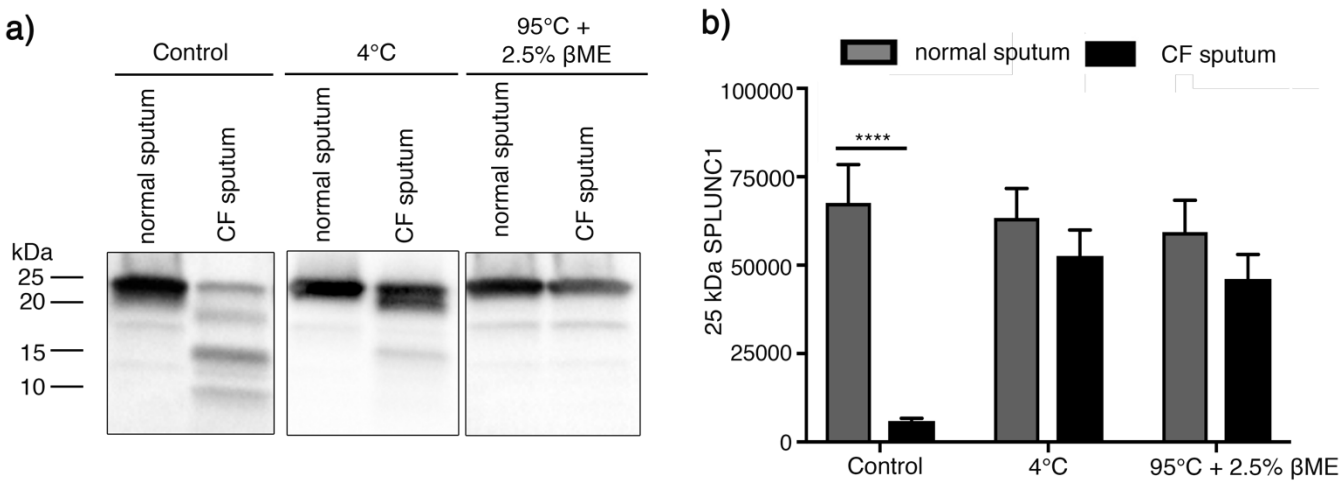

Figure S1 rSPLUNC1 degradation is prevented at 4°C and in the presence of heat denatured CF sputum. **a**) Representative Western blots showing rSPLUNC1 incubated at 37°C (control), 4°C and at 95°C with 2.5% β-mercaptoethanol in normal and CF sputum. **b)** Associated densitometry. Data show mean ± SEM from N = 6 individual experiments, **** $P < 0.001$ Two-way ANOVA analysis, with Sidak's multiple comparison test.

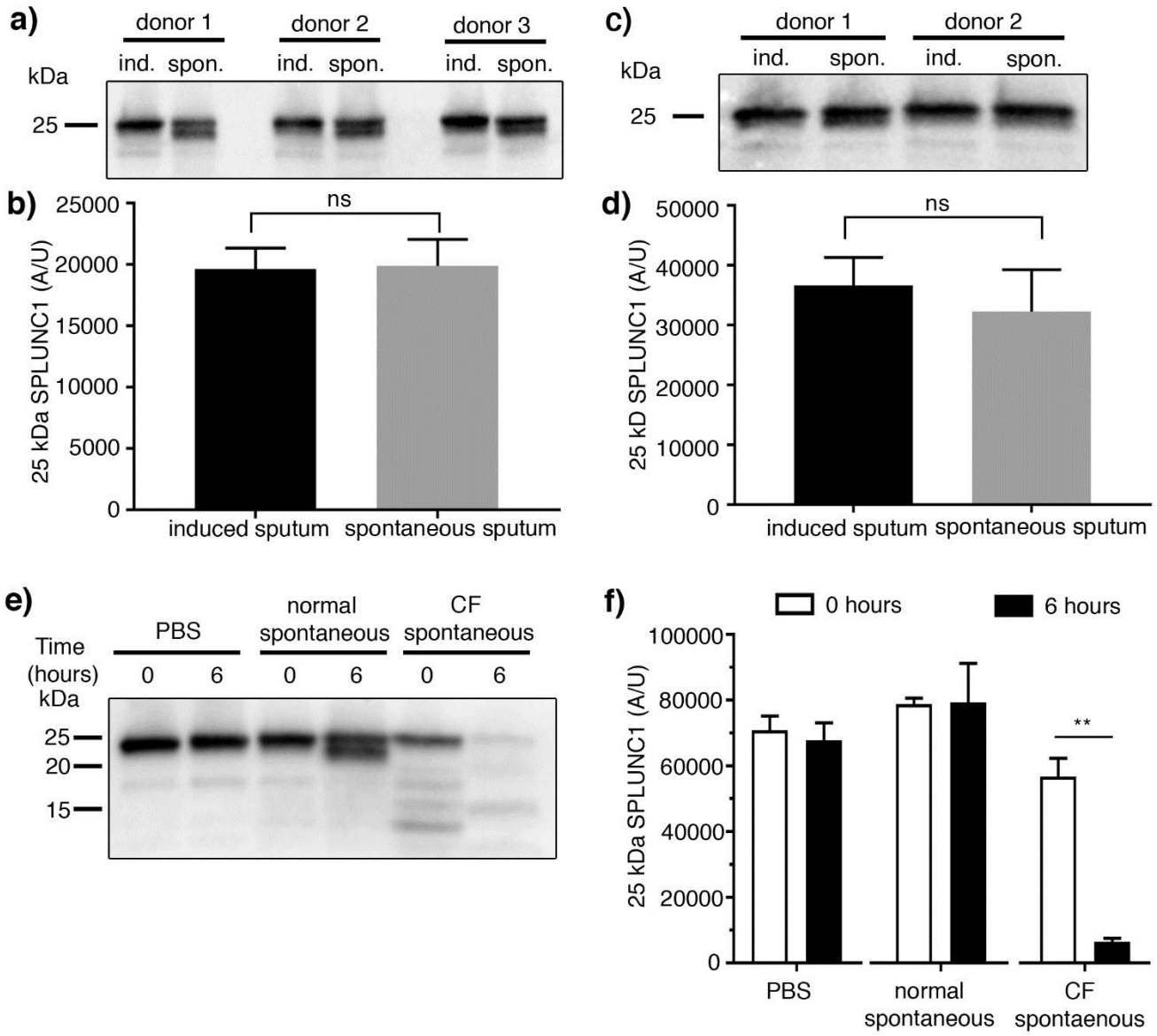

Figure S2. SPLUNC1 is not degraded by normal sputum. a) 10 μM rSPLUNC1 was incubated with 15 μl of paired normal sputum samples collected spontaneously and via saline induction from N = 3 individual donors and measured by Western blot and **b)** associated densitometry. Data show mean N = 3 ± SD, Mann-Whitney test analysis. **c)** 10 μM rSPLUNC1 was incubated with 15 μg of total protein from paired normal sputum collected spontaneously and via saline induction and **d)** associated densitometry. Data show mean N = 2 donors ± SD, Mann-Whitney test analysis. **e)** Representative western blot showing relative abundance of SPLUNC1 following incubation of 10μM rSPLUNC1 with both normal and CF sputum (pooled from ≥ N = 3 donors), collected spontaneously, for 6 h at 37°C and **f)** associated densitometry. Data show mean N = 3 – 6 individual experiments ± SEM. Mann-Whitney test, t = 0 vs. t = 6 for each respective group. ** $P < 0.001$, CF sputum t = 0 vs. t = 6 h.

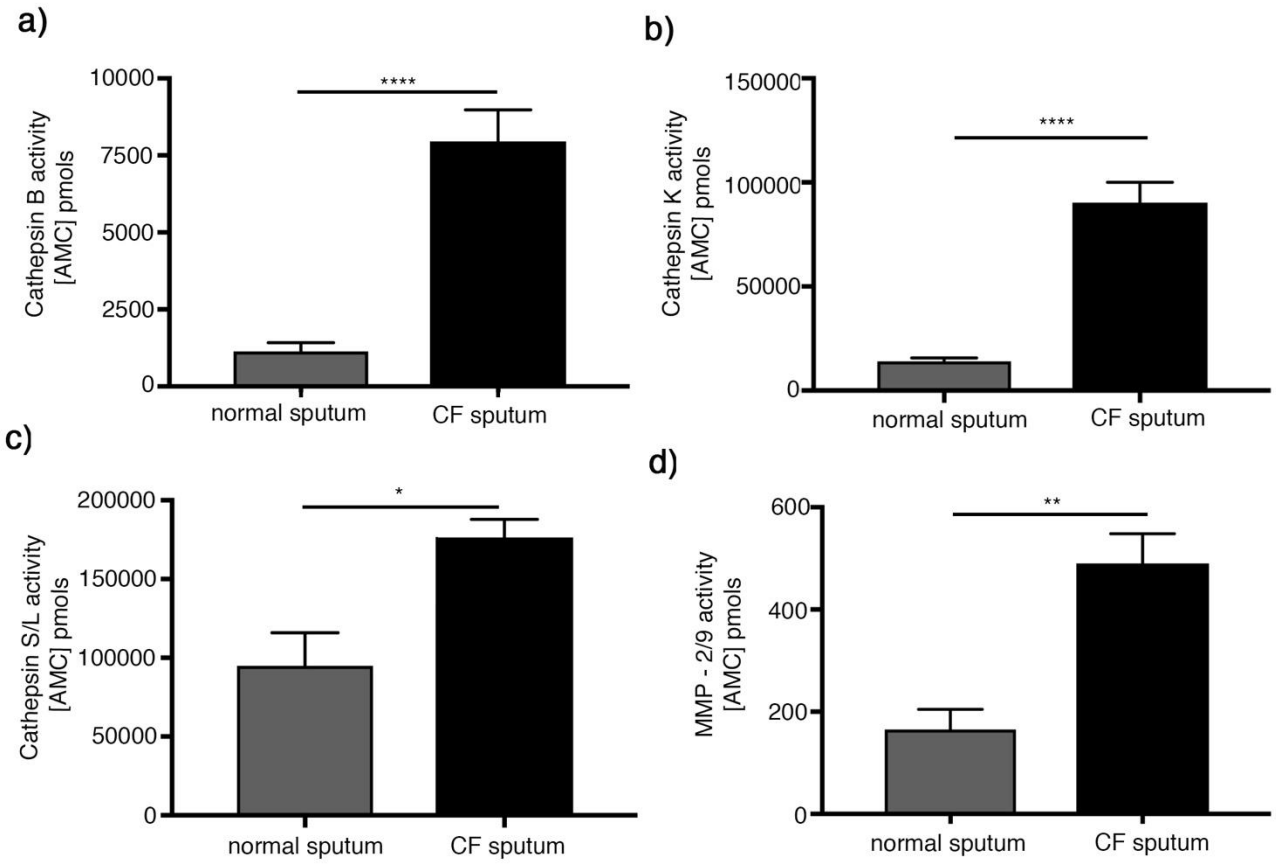

Figure S3. CF sputum cleaves cathepsins B, K, S/L and MMP-2/9 specific substrates. Fluorogenic substrates were incubated with normal and CF sputum over time at 37°C and the subsequent cleavage (i.e. AMC formation) was measured using a multiplate reader. (**a-c)** Show cleavage of 100 μM of fluorogenic substrates Z-Arg-Arg-vMCA (Cathepsin B), Z-Gly-Pro-Arg-MCA (Cathepsin K) and Z-Val-Val-Arg-MCA (Cathepsins S/L) following 6 h incubation with normal and CF sputum. Fluorescence excitation 380 ± 5 nm, emission 460 ± 10 nm. **d)** Shows the change in fluorescence after incubation of 100 μM of the fluorogenic substrate DNP-Pro-Leu-Gly-Met-Trp-Ser-Arg in normal and CF sputum for 2.5 h at 37°C. Fluorescence, excitation 280 ± 5 nm and emission 360 ± 10 nm. Data shown as mean ± SEM; n = 9 replicates from N = 3 independent experiments using sputum pooled from 6 donors. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.001$ normal vs. CF, Mann-Whitney U test. All data were normalized against total sputum protein concentration.

excited at 380 ± 5 nm and emitted fluorescence collected at 460 ± 10 nm

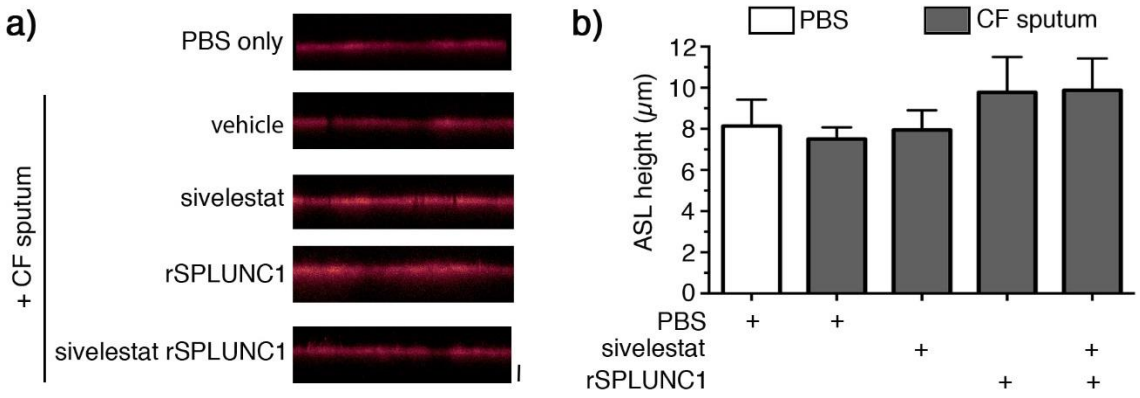

Figure S4. Sivelestat is unable to recover ability of rSPLUNC1 to increase ASL height. a) Representative ASL images, following mucosal addition of CF sputum ± 30 μM rSPLUNC1 and/or ± 10 μM sivelestat to normal HBECs. **b)** ASL height measurements from N = 15 - 16 HBECs per group. Data shown as mean ± SEM, no significant statistical difference.

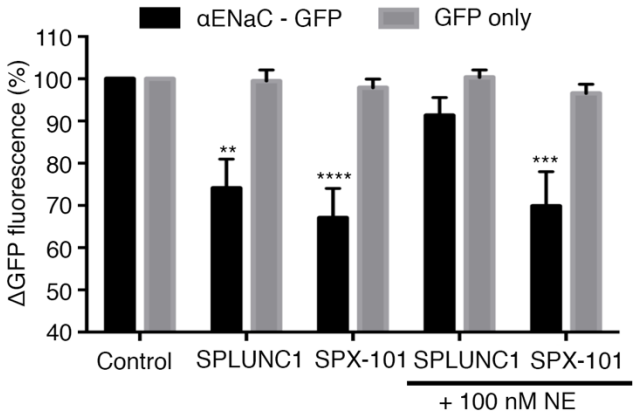

Figure S4. Neutrophil elastase-exposed SPLUNC1 is unable to alter αENaC-GFP fluorescence in HEK293T cells. HEK293T cells transiently co-transfected with αENaC-GFP, βENaC-untagged and γENaC-untagged in combination or with GFP alone were incubated with HEK293T cell media ± 30 μM rSPLUNC1 or 30 μM SPX-101, following pre-incubation in the absence and presence of 100 nM NE. GFP fluorescence was determined by plate reader analysis. Data shown mean n = 16-24 wells from N = 3 independent experiments ± SEM. ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$, Two-way ANOVA with Tukey's post-test analyses. No significant difference was apparent between GFP only fluorescence under any conditions.

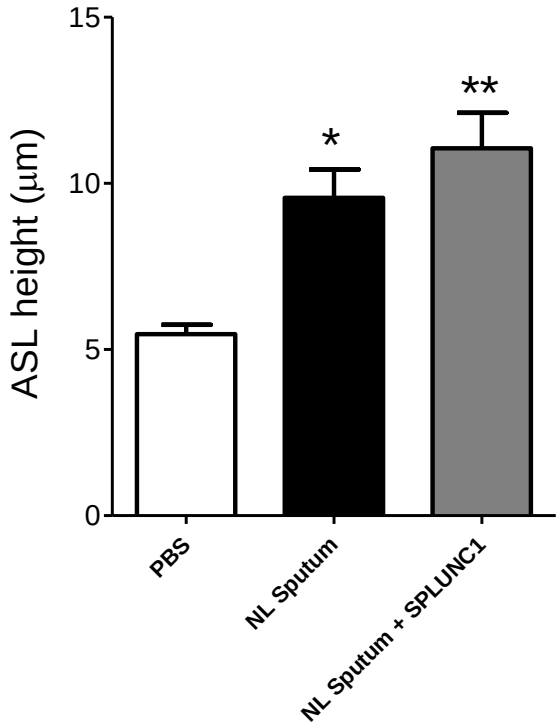

Figure S5. Endogenously-produced normal peptides are bioactive and increase ASL height. Freshly obtained normal sputum from three donors was pooled, centrifuged and filtered using a 10 kDa size exclusion spin column. The filtrate was diluted 1:1 with PBS, labeled with rhodamine-dextran and added mucosaly to normal HBECs in order to measure ASL height. Height was measured 6 h after sputum addition and rSPLUNC1 was added at 30 μM. Data shown as mean ± SEM. PBS, n = 10; sputum. n=6; sputum + SPLUNC1, n=6. Data from 3 separate experiments. * $P < 0.05$ and ** $P < 0.01$ vs PBS control, by Kruskal-Wallis test with Dunn's multiple comparison.

Supplemental Tables

Table S1: Donor demographics for CF sputum samples. Mean ± SD ages were 28.3 ± 6.1 and 31.8 ± 10.9 years for normal and CF donors respectively. P = 0.25, Student's t-test with Welch's correction.

	Donor	Age at sputum collection	Sex	Ethnicity	FEV1	FVC	Genotype
normal sputum samples	**1**	25	m	Caucasian	n/a	n/a	n/a
	2	32	f	Caucasian	n/a	n/a	n/a
	3	25	f	Caucasian	n/a	n/a	n/a
	4	33	f	Caucasian	n/a	n/a	n/a
	5	19	f	Caucasian	n/a	n/a	n/a
	6	33	f	mixed	n/a	n/a	n/a
	7	25	m	Caucasian	n/a	n/a	n/a
	8	21	m	Caucasian	n/a	n/a	n/a
	9	19	m	Asian	n/a	n/a	n/a
	10	30	f	Caucasian	n/a	n/a	n/a
	11	34	m	Caucasian	n/a	n/a	n/a
	12	31	f	Caucasian	n/a	n/a	n/a
	13	37	m	African American	n/a	n/a	n/a
	14	21	f	African American	n/a	n/a	n/a
	15	27	m	Hispanic/Latino	n/a	n/a	n/a
	16	40	f	African American	n/a	n/a	n/a
	17	30	f	Caucasian	n/a	n/a	n/a
	18	27	f	Caucasian	n/a	n/a	n/a
CF sputum samples	**1**	33	m	Caucasian	1.44	3.78	delF508/Q220X
	2	22	f	Caucasian	2.67	3.55	delF508/N1303K
	3	19	f	Caucasian	3.01	3.63	delF508/R347P
	4	28	m	Caucasian	2.66	6.09	delF508/delF508
	5	25	m	Caucasian	2.31	4.31	delF508/R553X
	6	31	m	Caucasian	2.66	4.36	delF508/delF508
	7	27	m	Caucasian	n/a	n/a	delF058/delF508
	8	36	f	Caucasian	1.21	2.28	delF058/delF508
	9	59	m	Caucasian	1.92	2.46	3849+10kbC>T/3659delC
	10	38	m	Caucasian	1.99	2.28	delF058/delF508
	11	20	f	Caucasian	2.89	4.08	delF058/delF508
	12	28	m	African American	1.59	3.38	deltaF508/2184insA
	13	30	f	Caucasian	2.71	3.67	delF058/delF508
	14	32	f	Caucasian	1.74	3.16	delF058/delF508
	15	35	f	Caucasian	1.21	2.28	delF058/delF508
	16	37	m	Caucasian	1.02	2.13	delF058/delF508
	17	18	f	Caucasian	2.61	3.2	deltaF508/R347P
	18	54	f	Caucasian	1.94	1.88	delF058/delF508

Table S2: Donor demographics for induced vs. normal sputum. Mean age was 39.0 ± 18.5 years.

Donor	Age at sputum collection	Sex	Ethnicity	FEV_1	FVC	Genotype
1	60	f	Caucasian	81%	84%	n/a
2	25	M	Caucasian	80%	87%	n/a
3	32	F	Caucasian	68.5%	178%	n/a

Table S3: Donor demographics for mucopurulent material. Mean age was 27.8 ± 15.6 years

Donor	Age at sputum collection	Sex	Ethnicity	FEV_1	FVC	Genotype
1	23	f	Caucasian	n/a	n/a	DF/E585X
2	39	f	Caucasian	n/a	n/a	delF058/delF508
3	34	m	unknown	n/a	n/a	delF508/1717-1G>A
4	14	m	unknown	n/a	n/a	delF058/delF508
5	49	f	unknown	n/a	n/a	delF058/delF508
6	8	f	unknown	n/a	n/a	delF508/G330X

Table S3: Donor demographics for normal and CF bronchi. Mean ± SD ages were 33.0 ± 10.4 and 31.4 ± 9.7 years for normal and CF donors respectively. P = 0.78, Student's t-test with Welch's correction.

Donor	Normal/CF	Diagnosis/Genotype	Age	Smoking History	Sex	Ethnicity
1	normal	Head Trauma 2nd Blunt Injury	26	non-smoker	m	Hispanic
2	normal	Head Trauma 2nd Blunt Injury	47	non-smoker	f	Caucasian
3	normal	Head Trauma 2nd Blunt Injury	25	non-smoker	f	Caucasian
4	normal	Head Trauma 2nd SIGSW	31	non-smoker	m	Caucasian
5	normal	Head Trauma 2nd Blunt Injury	19	non-smoker	m	Hispanic
6	normal	Head Trauma 2nd Blunt Injury	40	non-smoker	f	Caucasian
7	normal	Cerebrovascular/Stroke 2nd ICH	43	non-smoker	m	Caucasian
1	CF	delF058/G85E	22	n/a	m	Caucasian
2	CF	delF058/G542X	25	n/a	f	unknown
3	CF	delF058/delF508	22	n/a	f	unknown
4	CF	delF058/delF508	38	n/a	f	unknown
5	CF	delF058/delF508	48	n/a	m	unknown
6	CF	delF058/unknown	36	n/a	m	Caucasian
7	CF	delF058/delF508	29		m	Caucasian