SUPPLEMENTAL METHODS

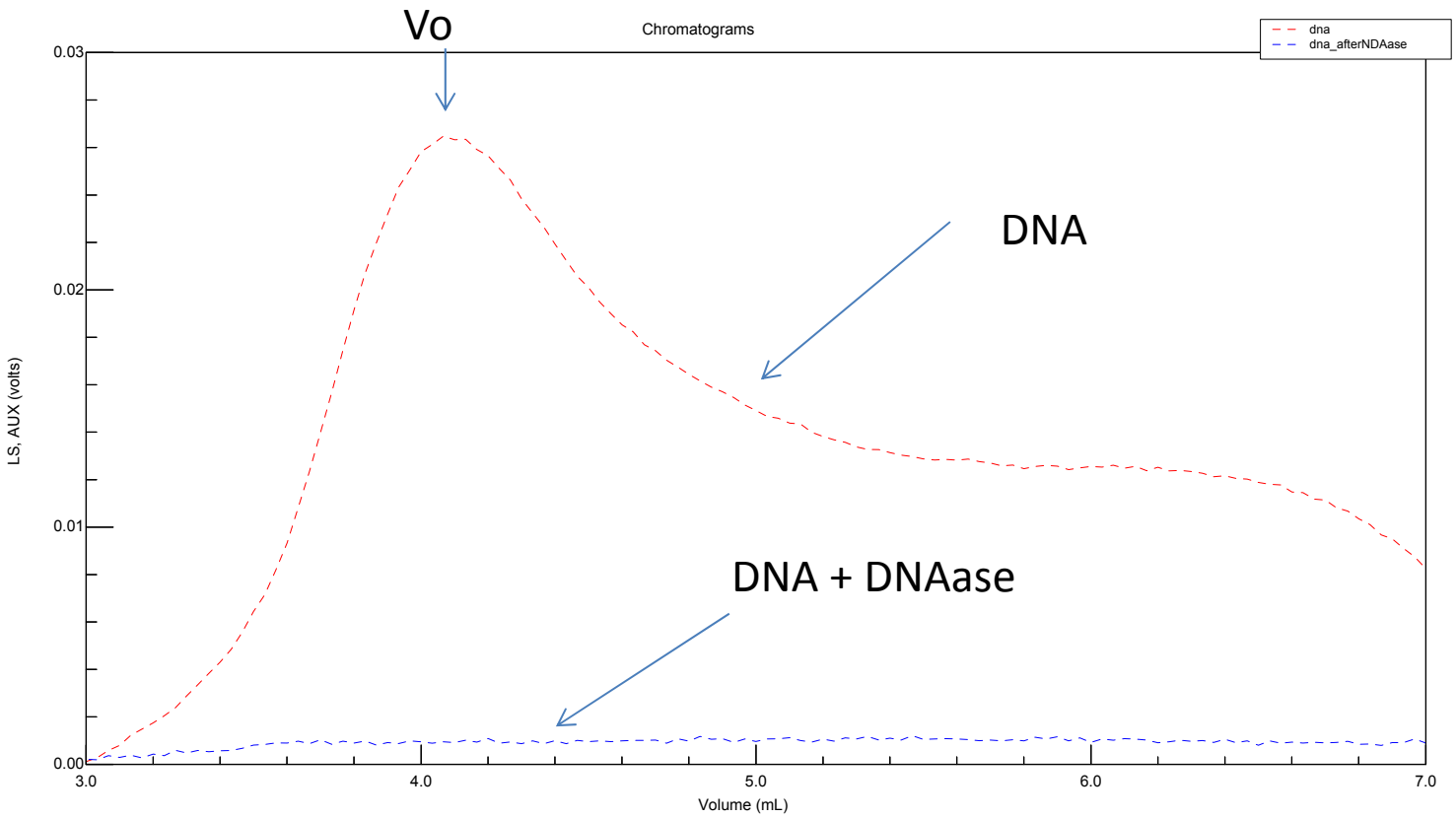
Collection of clinical samples

Normal and CF sputum collection: Sputum was collected from human subjects either by spontaneous expectoration or by sputum induction via three different protocols. For the first protocol, a large pool of sputum was collected from anonymized CF subjects with *Pseudomonas aeruginosa (PsA)* infection for studies of mucin proteolysis and immunogenicity (Figures 4, 5). For the second protocol, normal subjects were induced with hypertonic saline and spontaneous sputum was obtained from CF subjects for sputum mucin concentration analyses (Figures 1, 6). The spontaneously expectorated CF sputa were collected and stored in sterile cups on ice until diluted into 6M guanidine hydrochloride (GuHCl) at a 1:5 dilution. In this same protocol, sputum was collected from normal volunteers via induction as previously described (41). In brief, after obtaining informed consent, the subjects were administered nebulized albuterol followed by nebulized hypertonic saline at increasing doses of 3%, 4%, and 5% until able to produce a sample. All subjects performed throat clearance and nasal clearance prior to producing a sample. If an adequate sample was obtained at the lower doses of hypertonic saline, they did not progress to a higher concentration. The sputum samples were kept on ice until added to 6M GuHCl at a 1:5 dilution. For the third protocol, normal subjects were induced and CF subjects produced spontaneous sputum for osmotic pressure measurements (Figure 10). In this measurement, no GuHCl was added to the samples, and they were rapidly transported to the laboratory for analysis. The demographic data for each patient group are presented in Supplemental Table 1.

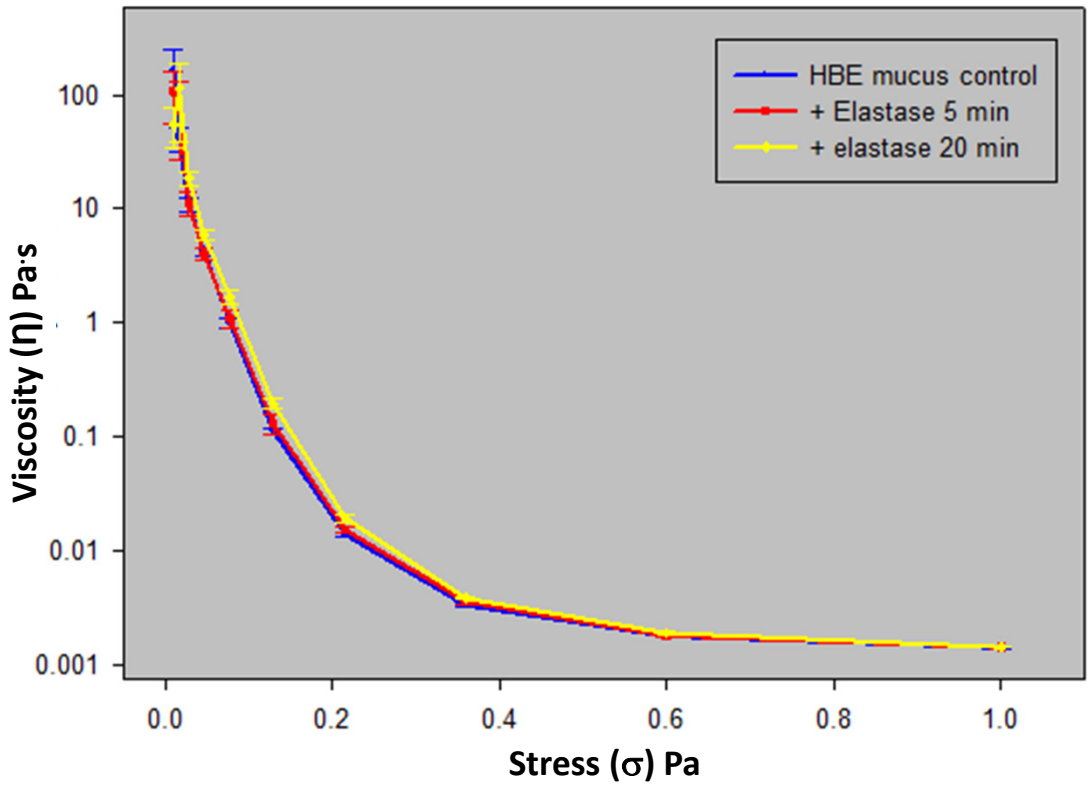
Normal and CF pediatric BAL samples (Figure 2): Informed consent was obtained from parents of children undergoing clinically indicated bronchoscopy to obtain excess bronchoalveolar lavage (BAL) fluid and to access clinical information linked to BAL samples. Bronchoscopy with BAL was performed for clinical indications by a pediatric pulmonologist according to standard clinical practices. The location for BAL was based on radiographic findings, clinical examination, or bronchoscopic findings and was determined by the bronchoscopist. BAL was performed according to the established standard at UNC-CH, with instillation of 10 ml of non-bacteriostatic normal saline into each lavage site for patients <10 kg, 1 ml/kg for patients 10 - 20 kg, and 20 ml for patients >20 kg. Quantitative bacterial cultures were performed in the UNC Hospitals Clinical Microbiology Laboratory by standard protocols. After obtaining an aliquot of the excess BAL fluid for total and differential cell counts, the remaining fluid was aliquoted into 2 ml vials and frozen at -80 C. BAL fluid cell counts were determined using a hemocytometer. After cytocentrifugation, a modified Wright–Giemsa stain was used and differential cell counts were assessed with 200 consecutive cells examined under light microscopy.

Viscosity measurement (supplementary figure 2): The complex viscosity of the neutrophil treated samples were determined by shear dependence of the viscosity on a Bohlin Gemini Rheometer (Malvern Instruments, Worcestershire, UK), with a 50 mm diameter parallel plate and with a gap thickness of 50 μm. Viscosity measurements were performed over a stress range of 0.01–1 Pa and at a frequency of 1 Hz. All analyses were performed at 23°C to minimize sample dehydration.

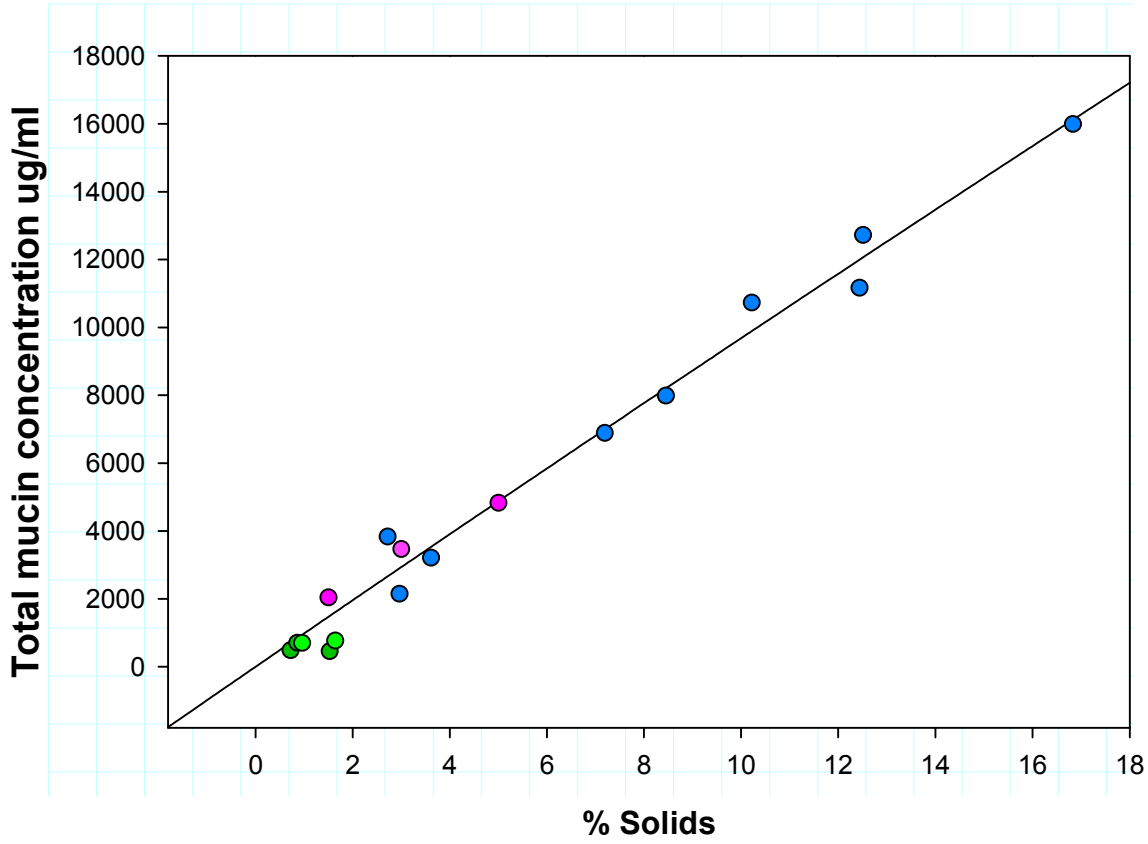
SUPPLEMENTARY FIGURES



Supplementary figure S1: Screen shot from the refractometer, showing the primary refractive index data from an analysis of the void fraction from the CL2B chromatography of DNA and DNA treated with DNAase. DNA completely diminished from the Vo after DNA treatment indicating our DNA eliminating protocol of the mucus samples are effective.



Supplementary figure S2: The affect of neutrophil elastase on HBE mucus viscosity. HBE mucus (% 2-3 solids) were incubated either with PBS or elastase for 5 and 20 minutes. The samples were then subjected to shear dependent viscosity measurement using a cone and plate rheometer. The output from a series of experiment is shown, where viscosity is plotted as a function of decreasing shear forces between 1- 0.001 Pa. Typically HBE mucus viscosity is dependent to shear stress and varies by 4-5 order of magnitude. No significant difference on shear dependent viscosity profile was observed on HBE mucus after 5 min and 20 elastase incubation, consistent with the result in the fig 3B in the main text.



Supplementary figure S3: Mucin concentration vs % solids of normal mucus and CF sputum: Percent solids and total mucin concentrations were measured as explained in the material methods. Typically, there is a linear relationship between % solids and total mucin concentration ($R^2 = 0.9855$) of airway mucus including normal in vivo mucus (green), CF sputum (blue) and different concentration of HBE mucus (magenta, 1.5%, 3% and 5%).

Demographics for Figures 1 and 6

NORMAL SAMPLES

	Subject #	age	gender	ht	wt	race	FVC	FVC %	FEV1	FEV1%	ratio
423V11001441	211	23	f	157.1	51	w	3.22	95	2.58	87	0.8
423V11001203	176	24	m	169	68.2	w	5.69	117	5.04	121	0.89
423V11002294	189	21	m	167.6	68.2	asian	3.45	80	2.88	78	0.83
423V11001369	187	50	f	160	73.5	w	3.74	122	3.11	122	0.83
423V11001924	150	28	f	160	68.2	w	3.58	104	2.67	90	0.74
423V11002035	214	22	m	170	60	aa	4.24	101	3.93	109	0.93
423V11001462	210	25	f	160	56.8	aa	3.74	113	2.86	108	0.83
423V11001700	213	27	m						3.96	103	

AVG	27.5	4m:4f	3.951429	104.5714	102.25	0.835714	
SD	9.396048		0.828622	14.36265	15.99777	0.061062	

CF SAMPLES

								PsA?	DNAse?
012612 K	39	f	w						
012612 J	44	m	w	3.07	76	2	0.65	y	n
011912 F	27	f	w	2.81	55	0.95	0.34	y	n
011912 D	21	f	w	1.66	38	1.25	0.76	y	y
011912 H	29	m	w	2.12	65	1.39	0.66	Y	y
011912 B	27	f	w	4.41	82	1.99	0.45	Y	n
011912 A	28	f	w	3.1	80	1.87	0.6	Y	n
011912 G	35	f	w	3.56	101	2.85	0.8	Y	y
011912 E	33	f	w	2.44	64	1.33	0.54	y	y
011912 I	26	f	w	2.71	74	1.68	0.62	y	y
				1.92	50	0.92	0.48	y	y
AVG	30.9	8f: 2m							
SD	6.854844								
				2.78	68.5	1.623	0.59	10y	4n: 6y
				0.816687	18.08775	0.584333	0.141107		

Demographics for Fig 10B and supplementary fig3

		AGE	Gender	FEV1	DNAse	PsA
NORMAL SAMPLES						
7/17/2012	IVS-215R	27	m	3.95	n/a	n/a
7/30/2012	IVS-235R	38	m	4.98	n/a	n/a
7/30/2012	IVS-216R	26	f	3.23	n/a	n/a
7/31/2012	IVS-237R	29	f	3.47	n/a	n/a
7/31/2012	IVS-239R	20	m	3.79	n/a	n/a
8/1/2012	IVS-233R	25	m	4.92	n/a	n/a
8/7/2012	IVS-228R	28	m	3.78	n/a	n/a
8/8/2012	IVS-187R	51	f	3.03	n/a	n/a
8/10/2012	IVS-240R	26	m	3.93	n/a	n/a
CF SAMPLES						
7/13/2012	Dsputum 001	24	F	2.61	no	no
9/5/2012	002-TACCO	31	M	2.23	no	yes
10/2/2012	CF sputum	50	F	1.08	no	yes
10/7/2012	KH mucus (2)	32	F	1.44	yes	yes
10/16/2012	CF sputum	50	F	1.15	no	yes
10/25/2012	CF suptum (AH-2)	27	M	0.95	yes	yes
1/25/2012	CF Sputum (006)	30	F	1.74	no	yes
11/1/2012	RRL	30	F	1.4	yes	yes
11/1/2012	CJC	30	F	1.51	yes	yes
11/1/2012	CLH	35	F	2.52	yes	yes
11/1/2012	ND	32	F	1.61	no	yes
11/1/2012	SLW	30	F	0.88	yes	yes
1/30/2013	013-TACCO	30	F	1.74	no	yes
1/30/2013	011-TACCO	30	F	1.65	no	yes
CF 121913 DK		40	F	0.98	yes	yes
CF 121913 JG		23	M	0.69	yes	yes
CF 120513JJ		28	F	0.68	yes	yes
CF 121913 CR		29	F	2.28	yes	yes

CF 121913 RCS			24	M	1.85	no	yes

AVG norm	30	6M/3F	3.897778	#REF!	
SD norm	9.192388		0.673364	#REF!	
AVG CF	32.92857	2M/12F	1.607857	#REF!	8N/6Y
SD CF	7.640623		0.540074	#REF!	