

Supplementary Tables and Figures

Supplementary Table 1: Tandem Mass-Spectrometry Analysis

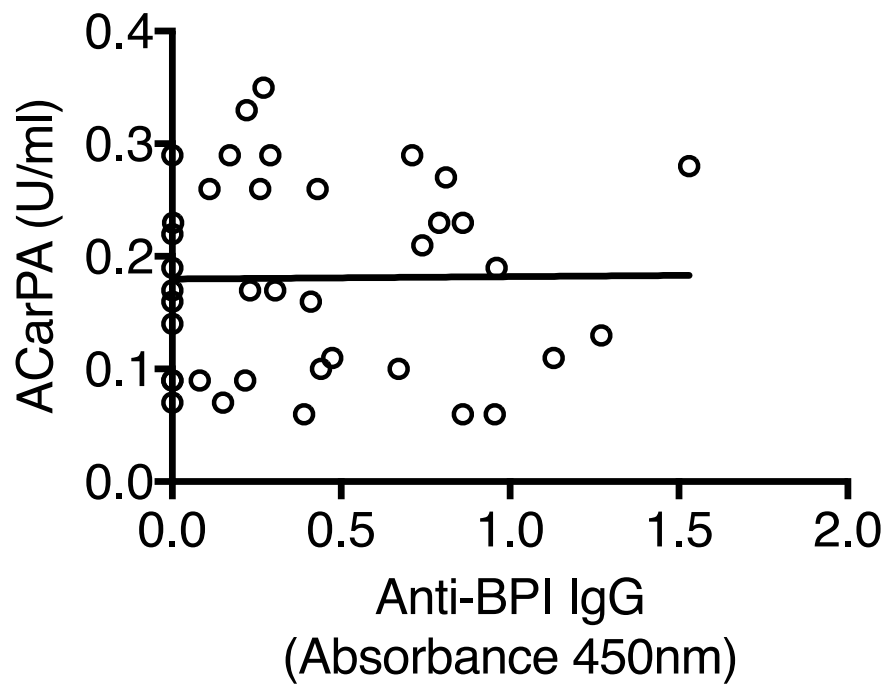
Protein ID	BPI_HUMAN									
Protein Name	Bactericidal permeability-increasing protein OS=Homo sapiens GN=BPI PE=1 SV=4									
Percent Coverage	66.1									
46 peptides identified with score greater than identity score										
Score	Expectation	Peptide Sequence	Start	End	M/Z	Ion Mass	Delta	ppm	Charge	
113.18	5.90E-11	R.EFQLPSSQISMVNPVGLK.F + Oxidation (M)	91	108	995.5	1989.0249	0.0008	0.4	2	
106.85	3.00E-10	K.GLDYASQQGTAALQK.E	44	58	775.9	1549.7761	0.0025	1.6	2	
97.4	2.50E-09	K.VTNSVSSELQPYFQTLPMVTK.I + Oxidation (M)	209	229	1193	2384.1944	0.0011	0.5	2	
93.17	6.50E-09	R.MVYLGLSDYFFNTAGLVYQEAGVLK.M + Oxidation (M)	284	308	938.8	2813.3986	0	0	3	
91.39	2.90E-09	K.GHYSFYSDIR.E	80	90	688.3	1374.6047	0.002	1.5	2	
82.91	1.50E-08	K.GHYSFYSDIR.E + Oxidation (M)	80	90	696.3	1390.5973	-0.0003	-0.2	2	
79.06	1.00E-07	R.LVGELKLDL.R	406	414	521.8	1041.6202	0.0019	1.8	2	
75.91	8.50E-08	K.HSNIGFPVVELLDIMNYIVPILVLR.V	421	447	772.7	3086.6995	0.0004	0.1	4	
71.76	7.30E-07	K.FSISNANIK.I	109	117	497.3	992.5283	-0.0007	-0.7	2	
69.51	0.000014	R.EFQLPSSQISMVNPVGLK.F + Oxidation (M)	91	108	664	1989.026	0.0019	1	3	
68.85	0.000015	K.FFGTFLPEVAK.K	328	338	628.3	1254.6652	0.0004	0.3	2	
68.24	0.000018	R.IKIPDYSDSFK.I	63	73	656.8	1311.6714	0.0004	0.3	2	
58.05	0.000022	R.MVYLGLSDYFFNTAGLVYQEAGVLK.M	284	308	933.5	2797.4047	0.001	0.4	3	
55.91	0.000034	K.LGSNPTSGK.P	150	158	430.7	859.4411	0.0011	1.3	2	
54.85	0.000011	K.HSNIGFPVVELLDIMNYIVPILVLR.V	421	447	1030	3086.6998	0.0007	0.2	3	
53.52	0.000047	R.EFQLPSSQISMVNPVGLK.F	91	108	987.5	1973.046	0.0168	8.5	2	
53.49	0.00003	K.IPDYSDSFK.I	65	73	536.3	1070.492	-0.0001	-0.1	2	
49.77	0.000056	K.GEFYSENHHNPPFPAPPVMEFFAAHDR.M + Oxidation (M)	257	283	622.1	3105.3845	0.0017	0.5	5	
49.7	0.00013	R.IKIPDYSDSFK.I	63	73	438.2	1311.674	0.0029	2.2	3	
48.91	0.0002	K.GLDYASQQGTAALQK.E	44	58	517.6	1549.7739	0.0003	0.2	3	
48.25	0.0001	K.KFPNMK.I + Oxidation (M)	339	344	390.7	779.4018	0.0018	2.3	2	
45.26	0.0002	K.IPDYSDSFK.I	65	73	1071	1070.4908	-0.0012	-1.1	1	
43.99	0.00025	K.GEFYSENHHNPPFPAPPVMEFFAAHDR.M	257	283	618.9	3089.3895	0.0016	0.5	5	
43.46	0.000095	R.LLLELH.H	415	420	364.8	727.4853	0.001	1.4	2	
42.67	0.00042	K.GFPLTPAR.V	455	463	478.3	954.5291	0.0004	0.4	2	
41.95	0.00036	K.VGWLIQLFHK.K	182	191	620.9	1239.715	0.0022	1.8	2	
41.67	0.00043	K.GEFYSENHHNPPFPAPPVMEFFAAHDR.M	257	283	1031	3089.3875	-0.0004	-0.1	3	
40.75	0.00045	R.DDMIPK.E + Oxidation (M)	313	318	367.7	733.333	0.0014	1.9	2	
39.5	0.00035	K.GHYSFYSDIR.E + Oxidation (M)	80	90	464.5	1390.5948	-0.0028	-2	3	
39.02	0.00062	R.VQLYNVVLQPHQNFLFGADVYK.-	464	487	935.5	2803.5122	0.0061	2.2	3	
37.95	0.0016	R.DDMIPK.E	313	318	718.3	717.3375	0.0008	1.1	1	
37.75	0.0011	R.DDMIPK.E + Oxidation (M)	313	318	734.3	733.3307	-0.0009	-1.2	1	
35.26	0.0032	K.KFPNMK.I	339	344	382.7	763.4061	0.0011	1.4	2	
34.55	0.002	K.VGWLIQLFHK.K	182	191	414.2	1239.7138	0.001	0.8	3	
34.35	0.0058	K.MTLRDDMIPK.E	309	318	407.2	1218.6114	0.0013	1.1	3	
33.34	0.0017	K.GHYSFYSDIR.E	80	90	459.2	1374.6029	0.0002	0.1	3	
33.2	0.0057	K.KIESALR.N	192	198	408.8	815.4862	-0.0003	-0.4	2	
31.94	0.0034	K.GEFYSENHHNPPFPAPPVMEFFAAHDR.M + Oxidation (M)	257	283	777.4	3105.3817	-0.0011	-0.4	4	
31.69	0.0014	R.LLLELH.H	415	420	728.5	727.4845	0.0002	0.3	1	
30.76	0.0071	R.LVGELKLDL.R	406	414	348.2	1041.6218	0.0036	3.5	3	
30.15	0.0098	R.DDMIPK.E	313	318	359.7	717.3381	0.0014	2	2	
28.49	0.016	K.MTLRDDMIPK.E + 2 Oxidation (M)	309	318	417.9	1250.5995	-0.0003	-0.2	3	
27.88	0.021	K.FSISNANIK.I	109	117	993.5	992.5273	-0.0018	-1.8	1	
25.9	0.033	K.MTLRDDMIPK.E + Oxidation (M)	309	318	618.3	1234.6048	-0.0002	-0.2	2	
25.75	0.029	K.MSGNFDLSIEGMSISADLK.L + Oxidation (M)	131	149	1016	2029.94	0.0064	3.2	2	
21.03	0.028	K.MNSQVCEK.V + Oxidation (M); Propionamide (C)	201	208	513.2	1024.4326	0.0008	0.8	2	
10 peptides identified with score between homology score and identity score										
Score	Expectation	Peptide Sequence	Start	End	M/Z	Ion Mass	Delta	ppm	Charge	
21.68	0.081	K.MTLRDDMIPK.E + 2 Oxidation (M)	309	318	626.3	1250.6019	0.002	1.6	2	
21.6	0.077	K.FFGTFLPEVAK.K	328	338	419.2	1254.6643	-0.0006	-0.5	3	
21.04	0.1	K.MTLRDDMIPK.E + Oxidation (M)	309	318	618.3	1234.6047	-0.0003	-0.2	2	
20.94	0.12	K.MTLRDDMIPK.E	309	318	610.3	1218.6125	0.0024	2	2	
20.83	0.11	K.MTLRDDMIPK.E + Oxidation (M)	309	318	412.5	1234.6061	0.0011	0.9	3	
18.53	0.11	K.GFPLTPAR.V	455	463	955.5	954.5293	0.0006	0.6	1	
17.37	0.11	R.VQLYNVVLQPHQNFLFGADVYK.-	464	487	701.9	2803.5033	-0.0028	-1	4	
16.68	0.071	K.LQKGFPLTPAR.V	452	463	442.3	1323.7664	0.0001	0.1	3	
15.72	0.24	K.MSGNFDLSIEGMSISADLK.L + 2 Oxidation (M)	131	149	1024	2045.9356	0.0071	3.5	2	
0.86	2.5	K.HSNIGFPVVELLDIMNYIVPILVLR.V + Oxidation (M)	421	447	1035	3102.7006	0.0066	2.1	3	

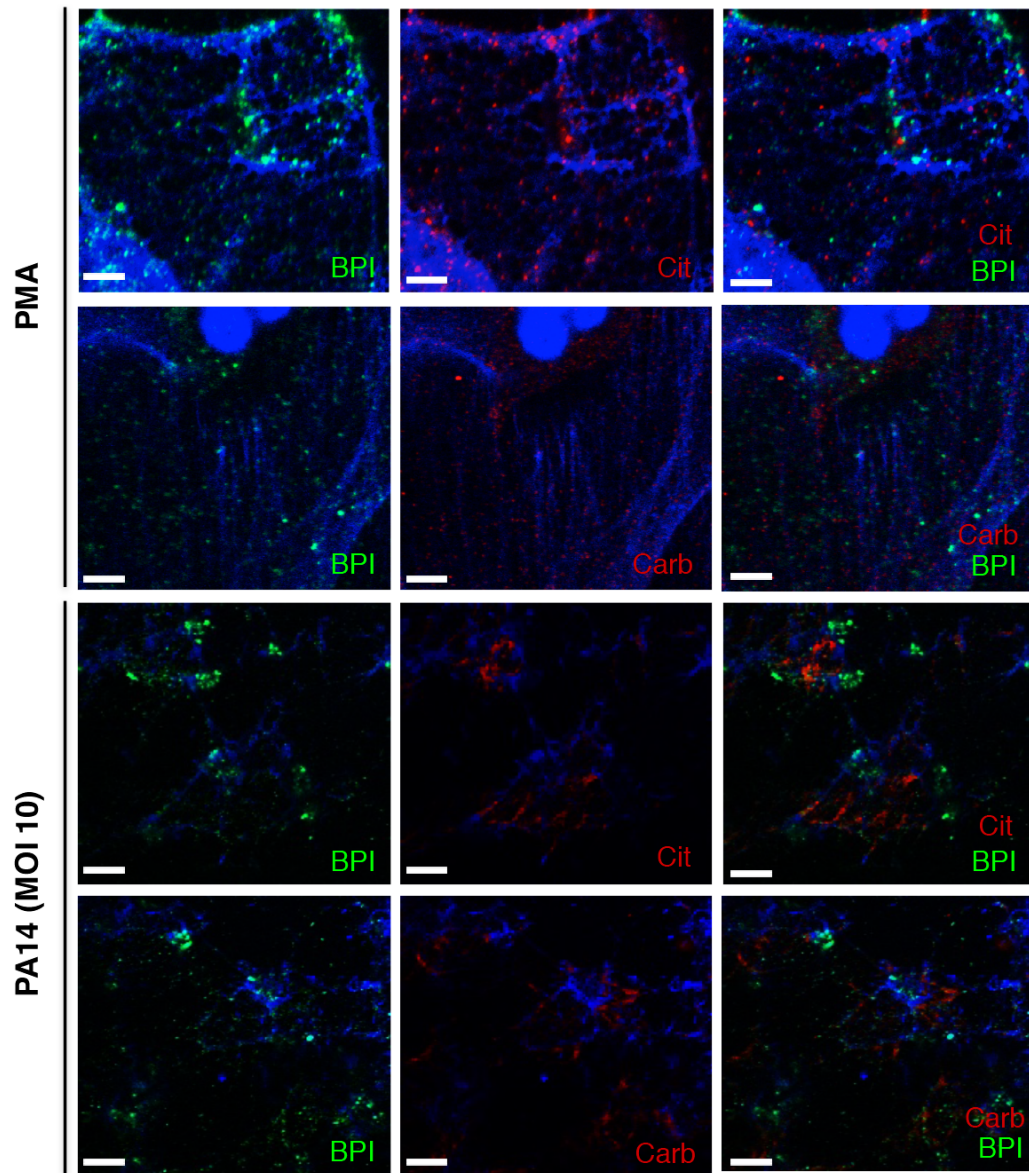
Supplementary Table 2: Purified BPI Isoaspartylation Assay Data

human BPI purified in Buffer A (10mM Tris, pH6.8, 100mM NaCl) BPI purity>95%	positive control	4124 cpm
	negative control	462 cpm
	BPI (52 pmole)	491 cpm
human BPI purified in Buffer B (10mM citrate phosphate , pH5.6, 700mM NaCl) BPI purity >90%	positive control	3722 cpm
	negative control	674 cpm
	BPI (60 pmole)	718 cpm

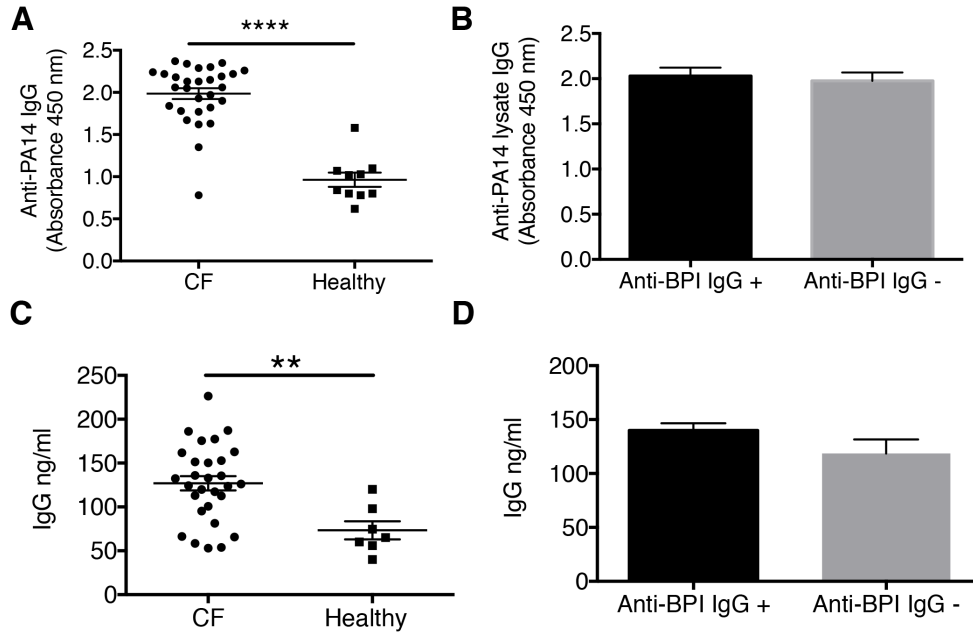
Supplementary Table 3

	CF sera (n=38)				RA sera (n=50)			
	ACPA U/ml	IgM RF U/ml	BPI-ANCA OD450	ACarPA U/ml	ACPA U/ml	IgM RF U/ml	BPI-ANCA OD450	ACarPA U/ml
Mean	6.47	10.87	0.41	17.2	448.2	121.9	0.1	50.34
Range	0 - 37.96	0 - 178	0 - 1.53	3.6- 46.6	3 - 1037	3-236	0-0.3	0 - 294
% positive	0	7.8	42.1	40	94	92	0	28

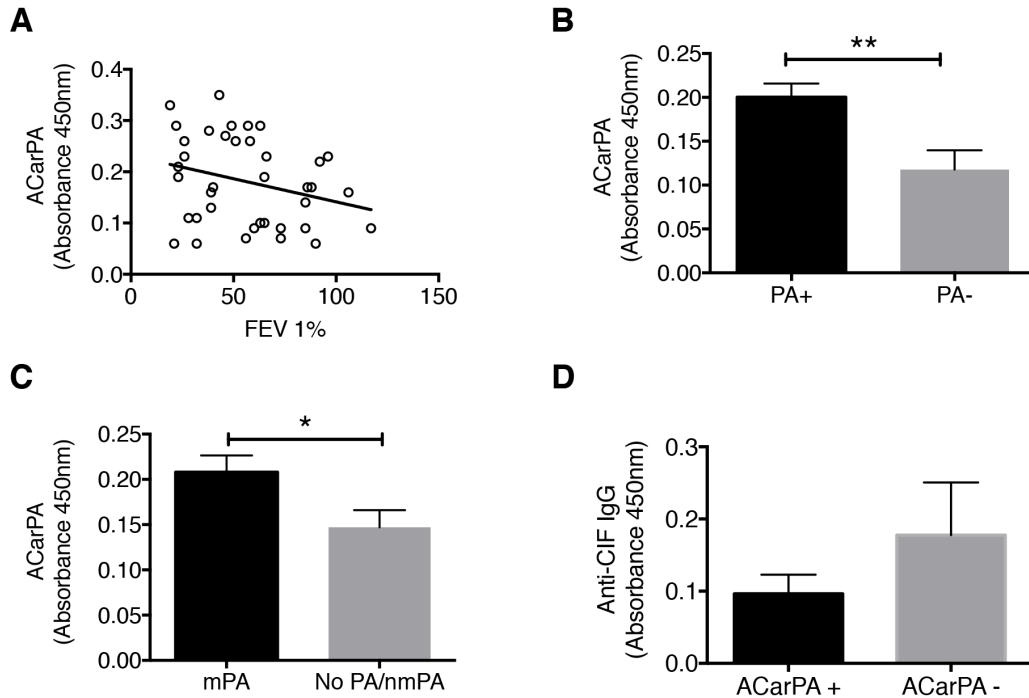




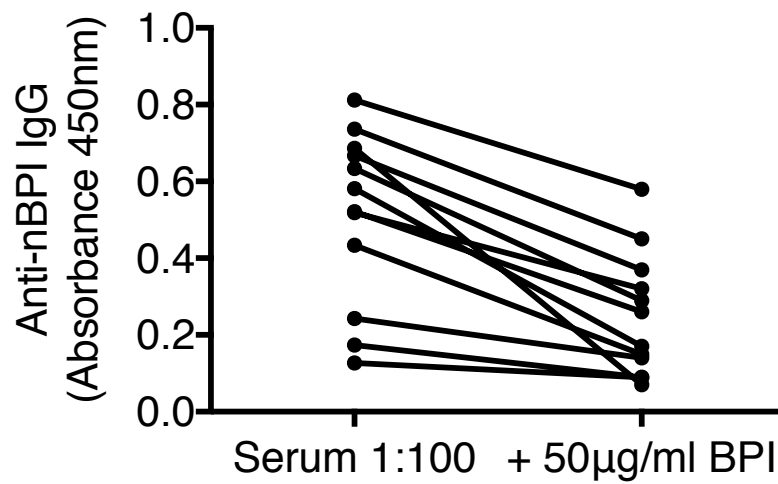
Supplementary Figure 2: BPI is not post-translationally modified. NETs were generated following 2hr treatment with 600nM PMA (A-F) or PsA PA14 (MOI 10) (G-L). (A, D, G, J) BPI-AI488 (green), (B, H) citrullinated proteins-Cy3 (aCit, red) and (E, K) carbamylated proteins-Cy3 (aCarb, red) were detected on the extruded DNA strands (DAPI, blue) via immunocytochemistry; merged images demonstrate there is no co-localization between (C, I) BPI and citrullinated proteins or between (F, L) BPI and carbamylated proteins; LSM 510 META, Zeiss confocal microscope, 60X, Scale bars represent 10 μ m.



Supplementary Figure 3: CF patients generate anti-PA14 IgG response and are hypergammaglobulinemic. (A) Anti-PA14 lysate IgG titers are higher in CF sera compared to healthy sera, $*p < 0.05$, (CF, $n=38$; Healthy sera, $n=10$). **(B)** IgG levels specific for *P. aeruginosa* PA14 lysate do not associate with anti-BPI auto reactivity in CF patients ($n = 38$, $p = 0.684$) **(C)** Total IgG titers are higher in CF sera ($n=38$) when compared to healthy sera ($n=7$), $p < 0.01$. **(D)** Total IgG titers are equivalent in CF patients independent of anti-BPI IgG status ($n = 38$, $p = 0.157$).



Supplementary Figure 4: ACarPA levels correlate with clinical parameters in CF, independent of anti-CIF immune response to *P. aeruginosa*. (A) ACarPA IgG titers negatively correlate with FEV1% (Pearson correlation analysis, $r = -0.278$, $p = 0.046$). (B) ACarPA IgG serum titers are higher in CF patients with positive *Pseudomonas aeruginosa* sputum culture (PA+) ($n = 38$, $**p < 0.01$) (C) and in those colonized with the mucoid *Pseudomonas aeruginosa* bacterial strains ($n = 38$, $*p < 0.05$). (D) Anti-CIF IgG titers do not differ between CF patients positive for anti-carbamylated protein autoantibodies (ACarPA+) vs. negative for ACarPA (ACarPA-) ($n = 38$, $p = 0.393$). ACarPA measured by the in-house ELISA assay.



Supplementary Figure 5: Confirmation of anti-BPI IgG presence in CF sera by fluid-phase ELISA. Incubation of 50µg/ml BPI with CF sera decreases the reactivity of anti-nBPI IgG to the plate bound protein by $\geq 30\%$ relative to the serum control with no nBPI inhibition in the fluid phase (n = 11).