Supplementary Material

Supplementary Methods

GeLC-MS/MS

Plasma samples from the 10 COPD and 10 control subjects in the discovery group were pooled for further analysis by mixing 50 µL of each individual's sample. The final volume of the pooled sample was 500 µL for each group. Each of the pooled COPD and control samples was diluted at a 1:2 ratio with Laemmli sample buffer (BioRad, Hercules, CA) containing 5% β-mercaptoethanol, heated for 10 minutes at 90°C and loaded onto a 10–14% polyacrylamide gel. Electrophoresis was performed using a mini Protean II system (BioRad) at 200 V for 45 minutes. Separation was confirmed by staining with SimplyBlue

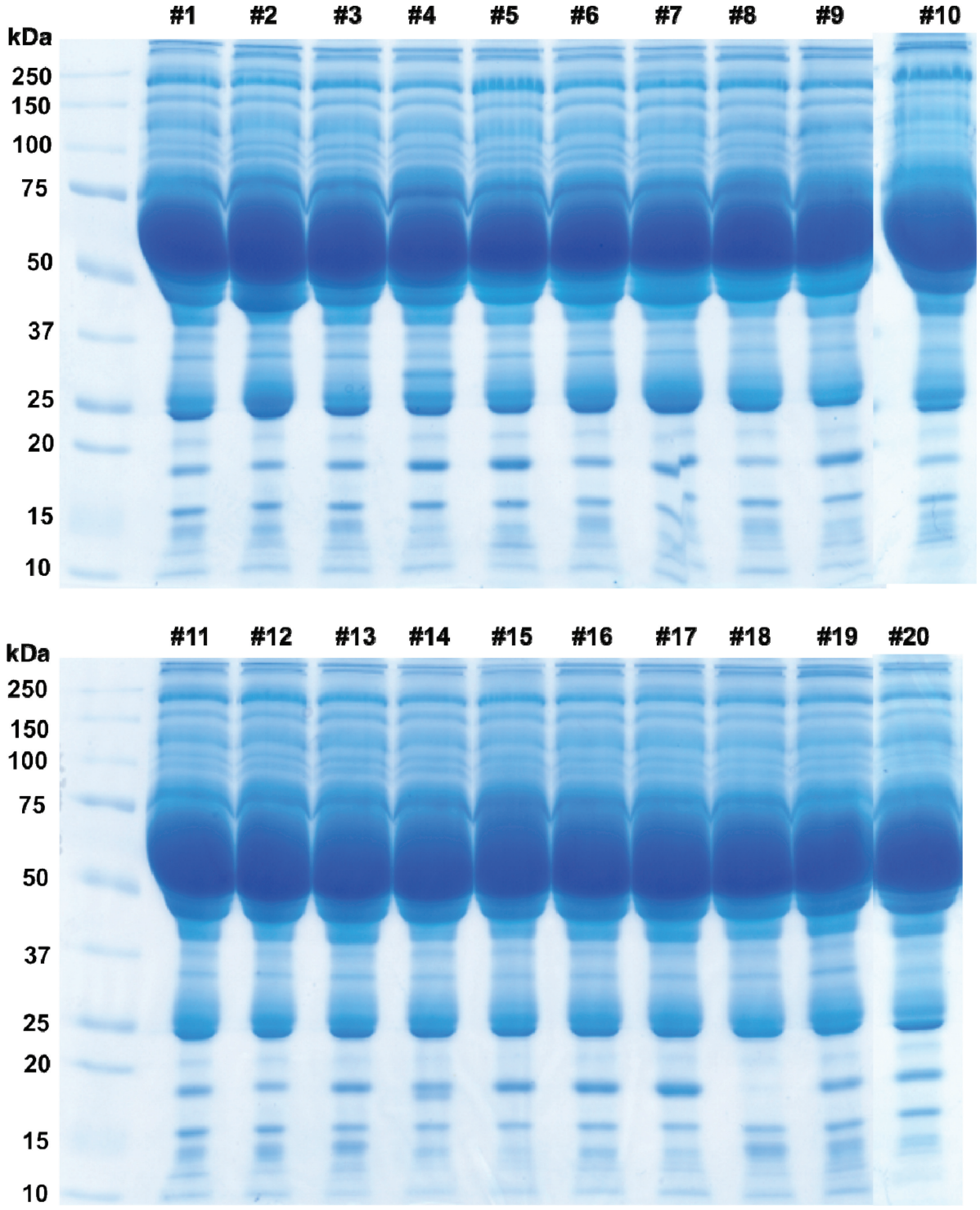

Figure S1. Individual 1D SDS PAGE gel of 10 COPD (rows #1 to #10) and 10 control subjects (rows #11 to #20) who comprise the discovery group. Gels show plasma samples in each subject prior to pooling.

Supplementary Table S1.

	Control (n = 10)	COPD (n = 10)	*p*-value
Age (yrs)	64 ± 2	63 ± 4	0.42
Smoking History (pack-yrs)	61 ± 11	57 ± 19	0.61
Smoking Cessation (yrs)	13 ± 4	7 ± 4	<0.01
FEV_1 (% pred)	94 ± 10	23 ± 9	<0.01
FVC (% pred)	95 ± 10	57 ± 13	<0.01
FEV_1/FVC (%)	75 ± 3	30 ± 8	<0.01
Emphysema (%)	3 ± 1	31 ± 4	<0.01
Height (cm)	173 ± 12	170 ± 10	0.55
Weight (kg)	101 ± 32	93 ± 30	0.56
BMI (kg/m^2)	34 ± 10	31 ± 7	0.53

SafeStain (Invitrogen). Each sample lane was sliced into 20 sections, and each section further cut into ~1 mm^3 slices in preparation for tryptic digestion.

The desalted tryptic peptides were dried in a vacuum centrifuge and resolubilized in 30 µL of 0.1% (vol/vol) trifluoroacetic acid. The tryptic peptide sample was loaded onto a 2 µg capacity peptide trap (CapTrap™; Michrom Bioresources, Auburn, CA), separated by a C18 capillary column (15 cm 75 µm, Agilent) at 300 nL/min (delivered by an Agilent 1100 LC pump). A mobile-phase gradient was run using mobile phase A (1% acetonitrile/0.1% formic acid) and B (80% acetonitrile/0.1% formic acid) from 0 to 10 minutes with 0–15% B followed by 10–60 minutes with 15-60 % B and 60–65 minutes with 60–100% B.

Nanoelectrospray ionization (ESI) tandem MS was performed using a Brukers HCT Ultra ion trap mass spectrometer. ESI was delivered using a distal-coating spray Silica tip (ID 20 µM, tip inner ID 10 µM, New Objective) at a spray voltage of -1300 V. Using automatic switching between MS and MS/MS modes, MS/MS fragmentation was performed on the two most abundant ions on each spectrum using collision–induced dissociation with active exclusion (excluded after two spectra, and released after 2 min). The complete system was fully controlled by HyStar 3.1 software.

Mass spectra (MS) processing was performed using Brukers Biotools (Version 2.3.0.0) with search and quantitation toolbox options. The generated de-isotoped peak list was submitted to an in-house Mascot server 2.2 and searched against the Swiss-Prot database (version 56.6 of 16-Dec-2008, 405506 sequences). Mascot search parameters were set as follows: Homo sapiens (20413 sequences); enzyme, trypsin with maximum 1 missed cleavage; fixed modification, cysteine carbamidomethylation; variable modification, methionine oxidation; 0.50 Da mass tolerance for precursor peptide ions; and 0.6 Da for MS/MS fragment ions. All peptide matches were filtered using an ion score cutoff of 10.

Supplementary Table S2. Proteins differentially expressed in COPD

Protein AC	Gene Name	Protein ID	Protein Name	Molecular Weight	MOWSE Score Ratio	Peptides Ratio COPD/CON	Sequence Coverage Ratio	emPAI Ratio
P26927	MST1	HGFL	Hepatocyte growth factor-like	80268	1.81	1.90	1.93	2.16
P43121	MCAM	MUC18	Cell surface glycoprotein	71563	2.7	3	2.4	3.5
P11021	HSPA5	GRP78	78 kDa glucose-regulated protein	72288	2.76	2	2.3	2.25
P12259	F5	FA5	Coagulation factor V	251514	3.6	4	5	4
Q86VB7	CD163	C163A	Scavenger receptor cysteine-rich type 1 protein M130	125355	0.37	0.25	0.25	0.25
P13591	NCAM1	NCAM1	Neural cell adhesion molecule	94515	0.45	0.67	0.56	0.429
Q92954	PRG4	PRG4	Proteoglycan 4	150984	0.50	0.50	0.51	0.53
Q15113	PCLCE	PCOC1	Procollagen C-endopeptidase enhancer 1	47942	0.56	0.5	0.59	0.583
P13796	LCP1	PLSL	Plastin-2 OS=Homo sapiens	70245	0.57	0.57	0.89	0.74
P00488	F13A1	F13A	Coagulation factor XIII A chain	83215	0.60	0.33	0.36	0.429
Q9UGM5	FETUB	FETUB	Fetuin-B	42028	0.65	0.31	0.54	0.589

Protein AC	Gene Name	Protein ID	Protein Name	Molecular Weight	MOWSE Score	Peptides	Sequence Coverage	emPAI
Q96JM7	LMBL3	LMBL3	Lethal(3)malignant brain tumor-like 3 protein	88280	82	2	3	0.04
P49913	CAMP	CAMP	Cathelicidin antimicrobial peptide	19289	112	2	10	0.36
Q12860	CNTN1	CNTN1	Contactin-1	113249	112	3	4.3	0.03
P19320	VCAM1	VCAM1	Vascular cell adhesion protein 1	81224	120	3	4.5	0.08
Q9NPH3	IL1RAP	IL1AP	Interleukin-1 receptor accessory protein	65377	145	5	7	0.10
AF144011	DCD	DCD	Dermcidin	11277	70	1	10	0.08
P22891	PROZ	PROZ	Vitamin K-dependent protein Z	44715	197	6	16.5	0.46

The following two criteria were used to evaluate protein identification: one peptide with ion score ≥ 35, two or more peptides with at least one ion score ≥ 20 ($p < 0.05$ threshold) and the cumulative Mascot scores ≥ 35. For all proteins with cumulative MOWSE scores ≥ 20 and ≤ 35, the theoretical and experimental gel molecular weights had to be consistent. When these criteria were used to search against a reversed decoy Swiss-Prot database, no false positive match was obtained (false discovery < 0.5%). For added stringency, proteins with scores above 40 were used for comparisons between samples. Protein quantitation using label-free methods was performed by averaging the results of: 1) the relative intensities of extracted ion chromatograms determined by Mascot Distiller; 2) the number of peptide fragments of a given protein isolated; 3) sequence coverage; and 4) the exponentially modified Protein Abundance Index (emPAI).

Western Blotting/ELISA of Individual Samples

Western blotting was used to assess expression of 3 proteins increased in the COPD pooled sample i.e., glucose regulated protein 78 (GRP 78), interleukin 1 receptor accessory protein (IL1AP), and macrophage stimulating protein 9 (MSTP9). Proteins (30 µg) were separated by 10–14% gradient SDS-PAGE and transferred to a nitrocellulose membrane in a semi-dry blotting chamber (Biorad, Hercules, CA). Blots were blocked with 5% milk in Tris-buffer saline solution (pH 7.6) containing 0.05% Tween-20 (TBS/T), and probed with the following rabbit anti-human antibodies (Santa Cruz Biotechnology, Santa Cruz, CA): GRP78, IL1AP and MSTP9. Primary antibodies were used at a concentration of 0.4 µg/mL. Goat anti-human IgG HRP (Santa Cruz Biotechnology) was used as a loading control. Blots were incubated with primary antibody overnight at 4°C with gentle shaking and then incubated with a mouse, anti-rabbit HRP-conjugated secondary Ab (1:10000) (Biomeda Corp, Foster City, CA) for 1 hr at room temperature. Blots were exposed by a chemiluminescent method (Enhanced ECL Detection System, Amersham Biosciences) and scanned by FLA 5100 (FujiFilm, Edison, NJ). The density of protein bands was determined using NIH free-ware (ImageJ software).

Soluble CD163 (sCD163) was measured by ELISA using a commercially available 96-well plate immunoassay kit according to the manufacturers' directions (Macro163, Trillium Diagnostics, Groningen, Netherlands). A 1:500 dilution of plasma was assayed in each well in a final volume of 100 µL in each well. Assays were run in duplicate. Optical density changes were read in a microtiter plate reader (BioRad, model 550).