Supplementary Materials

Smoking, COPD and 3-Nitrotyrosine Levels of Plasma Proteins

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Supplementary Material Contents

Supplementary Material Table 1. Selected candidate plasma biomarkers and related references for smoking studies.

Capture antibodies	Abbrev.	Catalog # and Supplier	References
angiotensinogen	AGT	MAB3156 [1]	(Mollsten et al. 2008;Seno et al. 1997)
amphiregulin	AmR	MAB262 [1]	(Du et al. 2005;Lemjabbar et al. 2003)
CD14	CD14	MAB3833 [1]	(Choudhry et al. 2005;Droemann et al. 2005;Zhou et al. 2009)
ceruloplasmin	CP	sc-69767 [2]	(Bridges et al. 1986;Galdston et al. 1984;Pignatelli et al. 2001)
C-reaction protein	CRP	MAB17071[1]	(Fujimori et al. 1993;Levitzky et al. 2008)
epidermal growth factor (EGF)	EGF	DY236 kit [1]	(Leikauf et al. 2002;Richter et al. 2002;Willey et al. 1987)
EGF receptor (extracellular domain)	EGFR	AF-231 [1]	(Leikauf et al. 2002;Richter et al. 2002;Schneider et al. 1999)
E-selectin	Esel	AF-724 [1]	(Chen et al. 2004;Churg et al. 2003;Gonzalez et al. 1996)
basic fibroblast growth factor	FGFb	MAB233 [1]	(Morimoto et al. 1999;Noordhoek et al. 2003)
fibrinogen	Fibr	ID6-250310 [3]	(Kourisky and Happert 1964;Pignatelli et al. 2001;Weitz et al. 1992)
heparin-binding epidermal growth factor	HBEGF	AF-292 [1]	(Basbaum et al. 2002;Newland and Richter 2008;Richter et al. 2002)
hepatocyte growth factor	HGF	MAB694 [1]	(Gunella et al. 2006;Stabile et al. 2002)
intracellular adhesion molecular 1	ICAM	MAB720 [1]	(Floreani et al. 2003;Gonzalez et al. 1996;Li et al. 2008)
insulin-like growth factor 1	IGF-1	MAB291 [1]	(Cook et al. 1993;Kim et al. 2007;Liu et al. 2003)
leptin	Leptin	MAB398 [1]	(Chen et al. 2006;Chen et al. 2008;Hansel et al. 2009;Vernooy et al. 2009)
matrix metalloprotease 1	MMP1	AF901 [1]	(Cawston et al. 2001;DeMeo et al. 2007;Klinchid et al. 2009)
matrix metalloprotease 2	MMP2	AF902 [1]	(Kader et al. 2007;Zhang et al. 2009)
matrix metalloprotease 9	MMP9	AF911 [1]	(Nakamaru et al. 2009;Sampsonas et al. 2007;Zayas et al. 2004)
platelet-derived growth factor AA	PDGF	MAB221 [1]	(Churg et al. 2006;Francus et al. 1992;Rom et al. 1991)
RANTES	RANTES	MAB678 [1]	(Bracke et al. 2007;Brozyna et al. 2009;Ma et al. 2005;Oltmanns et al. 2005)
lung surfactant protein A	SP-A	LS-C17957 [4]	(Behera et al. 2005;Honda et al. 1996;Kida et al. 1997;Robin et al. 2002)
Transforming growth factor alpha	TGFa	AF-239 [1]	(Huang et al. 2008;Klein-Szanto et al. 1992;Vlahos et al. 2006)
tumor necrosis factor alpha	TNFa	MAB610 [1]	(Churg et al. 2002;Shao et al. 2004;Wright et al. 2007)
vascular endothelial growth factor	VEGF	AF-293 [1]	(Marwick et al. 2006;Plataki et al. 2006;Suzuki et al. 2008)

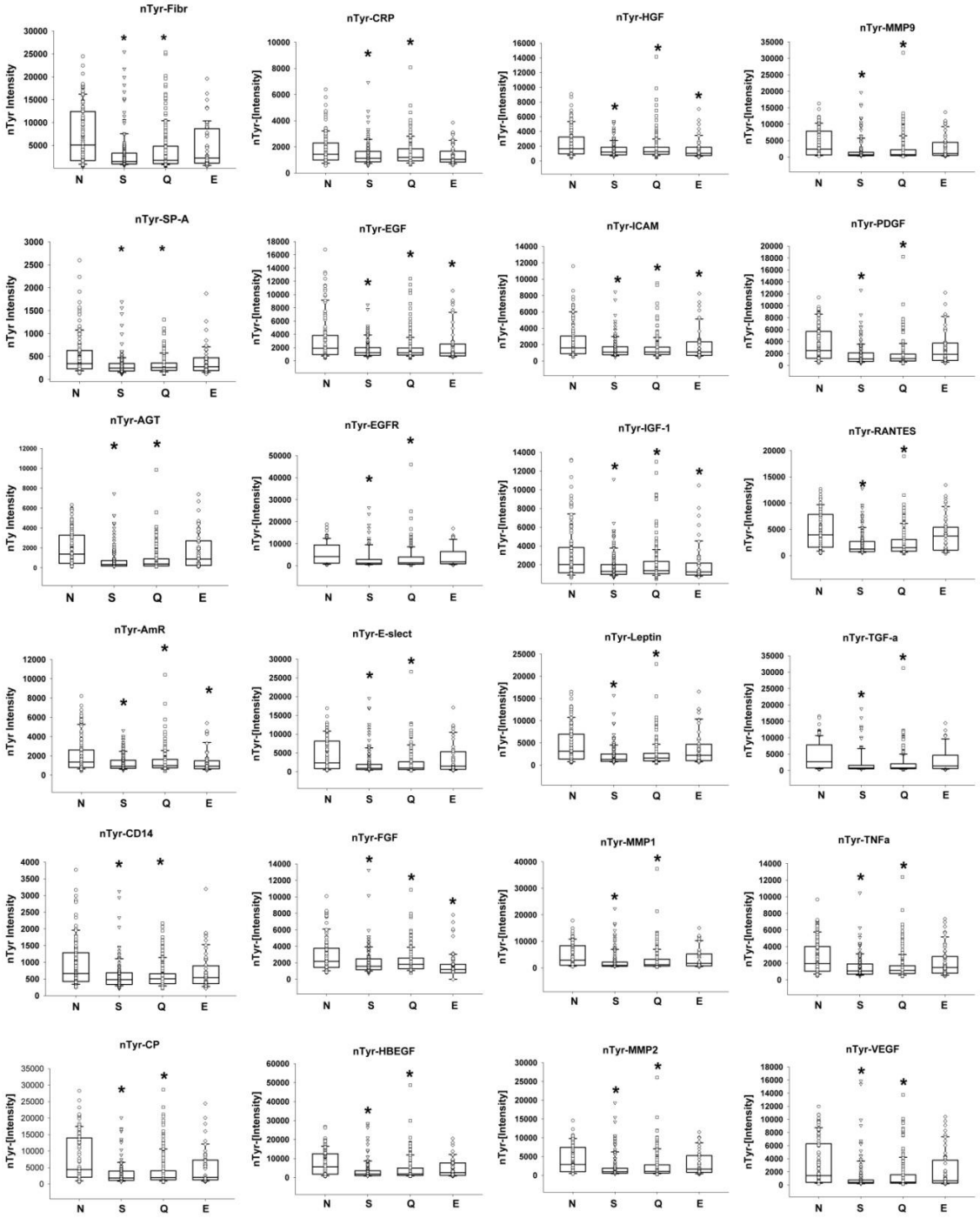
Commercial supplier:
[1]R&D Systems; Minneapolis, MN, USA.
[2]Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA.
[3]ABBiotek, San Diego, CA, USA
[4]Lifespan Biosciences, Seattle, WA, USA.

Supplementary Material Table 2. 3-nitrotyrosine antibodies that were tested in the ELISA microarray platform.

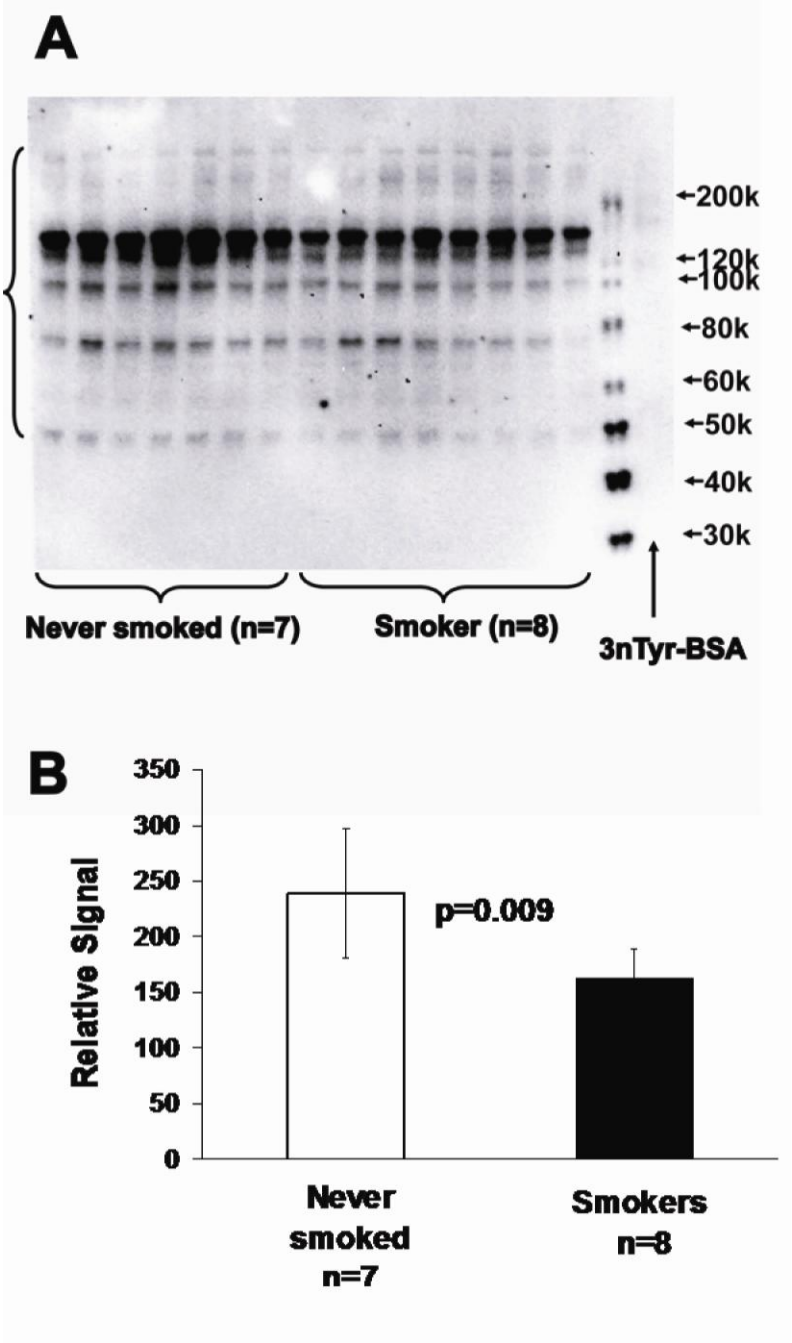
Detection antibodies for 3-nitrotyrosine	Catalog number	Antibodies Resources
1	HM5002	Biotinylated monoclonal antibody to 3-nitrotyrosine (HM11) Hycult Biotechnology b.v., from Netherlands
2	A-21285	Rabbit anti-3-nitrotyrosine polyclonal Antibody from Molecular Probes
3	K97520G	Goat anti-3-nitrotyrosine polyclonal antibody from Meridian Life Science, Inc, Saco, ME
4	Ab27648	Goat anti 3-nitrotyrosine polyclonal from Abcam, Cambridge, MA

Supplementary Material Table 3. Relationship between nitrotyrosine levels and characteristics of smokers. ELISA microarray analysis on the linear relationship between 3-nitrotyrosine levels for 24 plasma proteins against five clinical parameters that are associated with smoking, including years since quitting smoking, age of first cigarette, total number of years smoking, diastolic and systolic blood pressure (BP). The regression model used in the statistical analyses tests the hypothesis that all coefficients are equal to 0 (i.e., $H_O : \beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta_5 = 0$). This analysis indicates that there is very poor association between the measured log-transformed nitrotyrosine signal intensity and the independent variables included in the model.

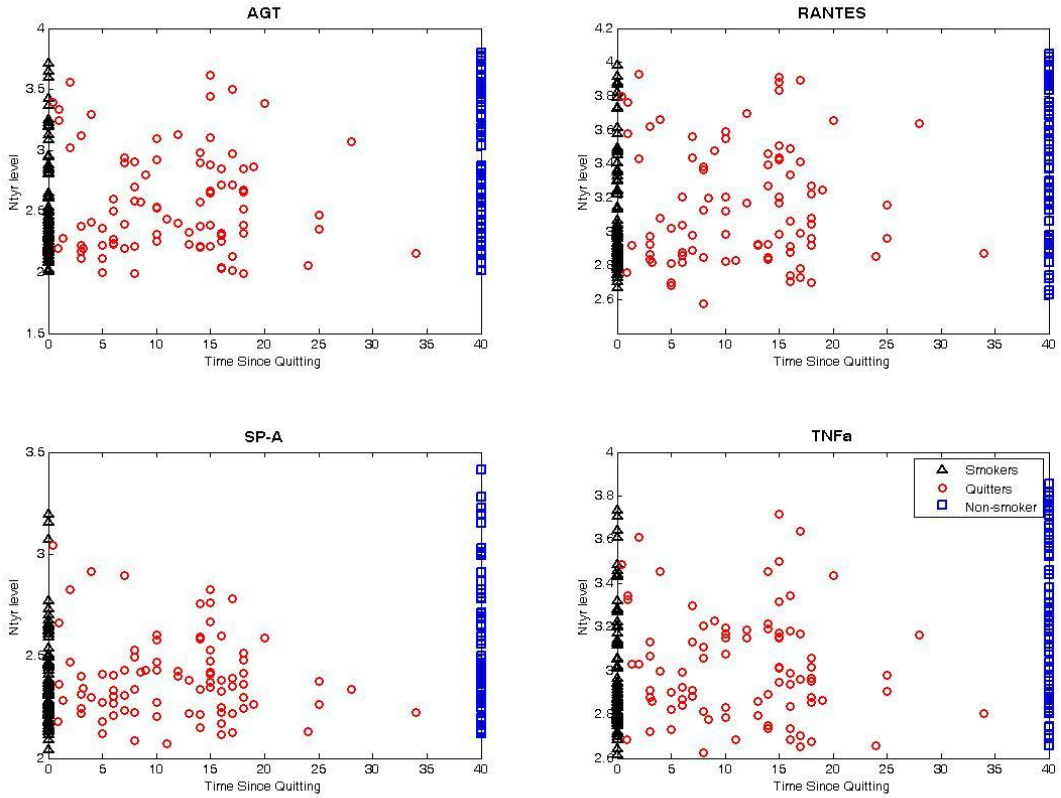
	Years Quit	Age first Cigarette	Years Smoking	BP Diastolic	BP Systolic
Protein	β_1	β_2	β_3	β_4	β_5
AGT	-0.0016	0.0120	-0.0071	-0.0010	-0.0012
AmR	0.0039	0.0078	0.0007	0.0045	-0.0017
CD14	-0.0017	0.0072	-0.0032	0.0021	-0.0007
CP	0.0015	0.0139	-0.0034	-0.0001	-0.0003
CRP	0.0008	0.0045	-0.0007	0.0022	0.0001
EGF	0.0037	0.0072	-0.0013	0.0052	-0.0011
EGFR	0.0014	0.0148	-0.0062	-0.0026	0.0010
E-Selct	0.0005	0.0146	-0.0072	-0.0031	-0.0007
FGF	0.0004	0.0039	-0.0022	0.0013	0.0006
Fibr	-0.0011	0.0087	-0.0060	-0.0014	-0.0005
HBEGF	0.0016	0.0076	-0.0046	0.0007	-0.0010
HGF	0.0030	0.0071	0.0009	0.0048	-0.0019
ICAM	0.0034	0.0053	-0.0008	0.0033	0.0001
IGF 1	0.0026	0.0049	-0.0009	0.0045	-0.0022
Leptin	0.0027	0.0083	-0.0028	0.0016	-0.0016
MMP1	-0.0010	0.0124	-0.0057	-0.0001	-0.0013
MMP2	-0.0005	0.0129	-0.0072	-0.0018	0.0003
MMP9	-0.0009	0.0168	-0.0080	-0.0025	-0.0002
PDGF	0.0001	0.0095	-0.0043	0.0007	-0.0005
RANTES	0.0009	0.0117	-0.0058	-0.0013	0.0003
SP-A	-0.0010	0.0055	-0.0039	0.0004	-0.0001
TGFa	0.0004	0.0160	-0.0069	-0.0023	0.0012
TNFa	0.0017	0.0092	-0.0025	0.0025	-0.0006
VEGF	-0.0024	0.0172	-0.0101	-0.0042	0.0016



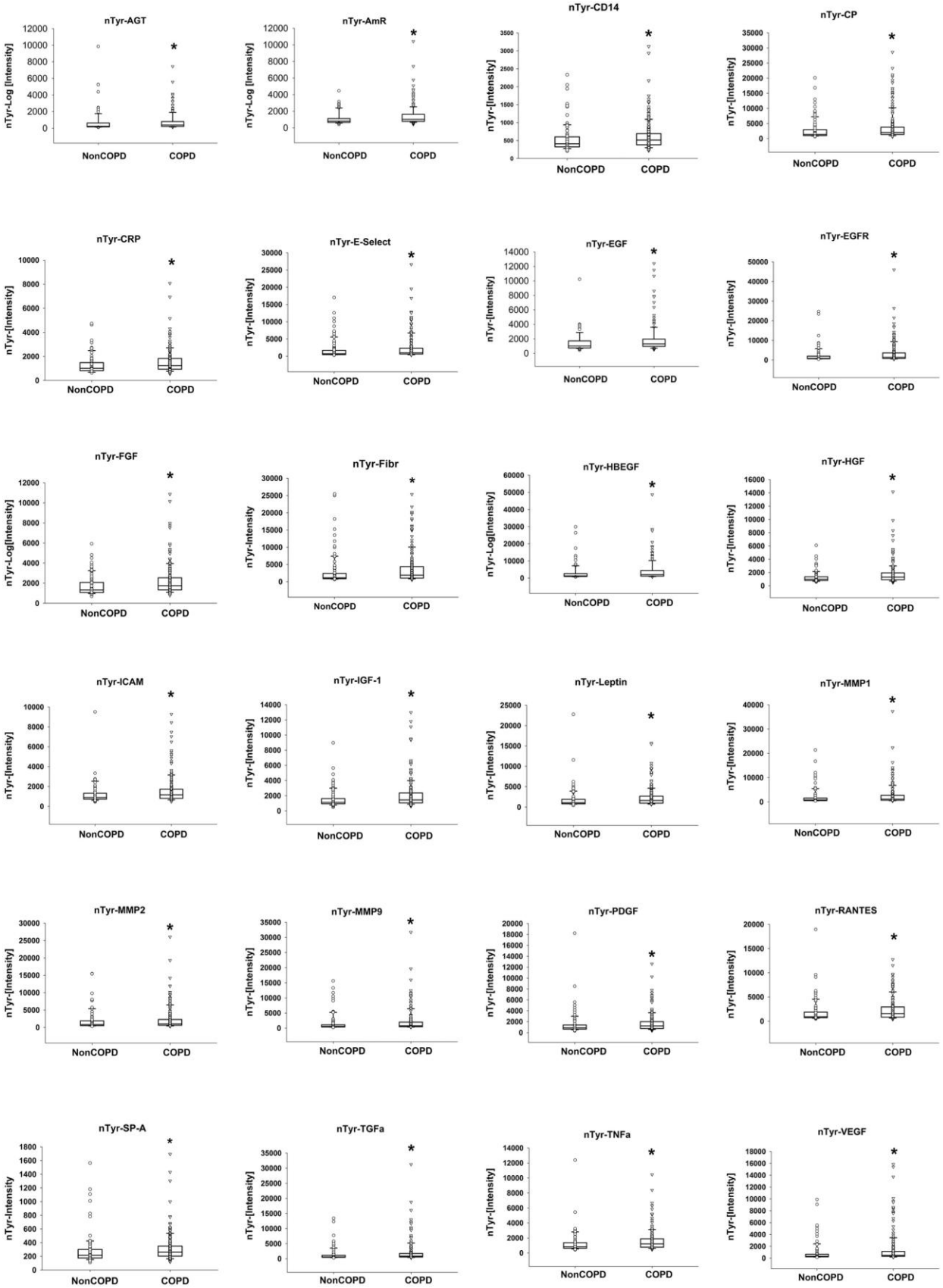
Supplementary Material Figure 1. Smoking status and 3-nitrotyrosine levels, as measured by ELISA microarray, for 24 circulating proteins. The horizontal line within the box represent the median values, the box edges represent the 25th and 75th quartiles, and whiskers represent for the 5th and 95th percentiles of each group. Individual intensity values are shown as individual symbols. Key: current smokers (S), former smokers (Q), never smoked (N), or high risk for environmental smoke exposure (E)* Indicated group value is significantly different ($p < 0.01$) from N group, based on a Kruskal Wallis analsis and a nonparametric Tukey's post-hoc test. (E, diamond). Stars (*) represent $p<0.01$ when S or Q are compared to N based on a Kruskal Wallis analysis and a nonparametric Tukey's post-hoc test.



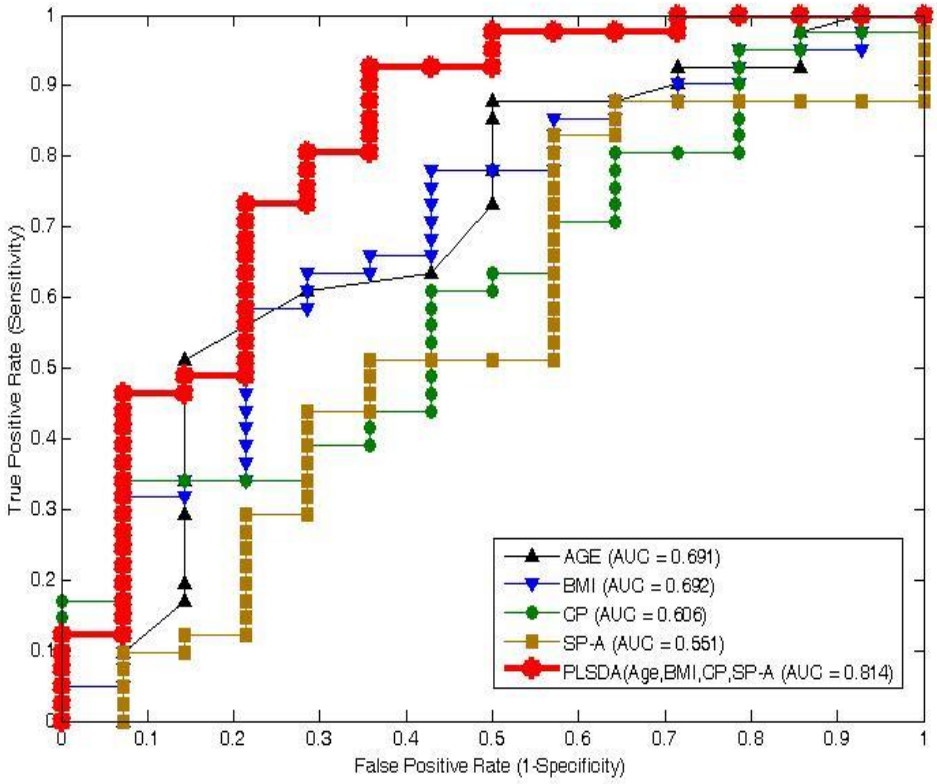
Supplementary Material Figure 2. Immunoblot analysis of the 3-nitrotyrosine levels of plasma proteins. Plasma samples from individuals who had never smoked and or were current smokers were randomly selected and compared. **A**. Image of the immunoblot from ~30 to 250 kDa. **B**. Results from densitometric analysis spanning the bracketed area of the immunoblot that is shown in panel A. Columns and crossbars represent the median density and standard error, respectively, of all the individual lanes per group. The p value (0.009) shows the statistical difference between the two groups, and was determined using a t-test.



Supplementary Material Figure 3. Nitrotyrosine levels are not associated with time-since-quitting in past smokers. ELISA microarray analysis of current nitrotyrosine levels for select, representative, plasma proteins in past smokers (red diamonds), graphed against the years since quitting. Data from current smokers (black triangles, on left side of graphs) and never smokers (blue squares, on right side of graphs) are also shown. The nitrotyrosine signal intensity (Y-axis) is graphed as the log-transformed data.



Supplementary Material Figure 4. 3-nitrotyrosine levels in 24 plasma proteins from smokers with chronic obstructive pulmonary disease (COPD) and smokers without COPD (nonCOPD). The horizontal bars within the boxes represent the median values, the boxes extend to the 25th and 75th percentiles, and the whiskers extend to the 5th and 95th percentiles, and all individual values are shown as stated symbols. * The COPD group is significantly different ($p<0.01$) from NonCOPD group based on a Wilcoxon test.



Supplementary Material Figure 5. A receiver operator characteristic (ROC) curve demonstrating the classification potential of integrating nitrotyrosine protein assay and clinical data. Key: AUC. Area under the ROC curve; BMI, body mass index; CP, ceruloplasmin; SP-A, surfactant protein A; PLSDA, partial least squares discriminant analysis.

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