

## **Sputum proteomics and airway cell transcripts of current and ex-smokers with severe asthma in U-BIOPRED:**

### **an exploratory analysis**

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#Consortium project team members are listed under Acknowledgements

### **Supplementary Tables S1- S10**

Supplementary Table S1. Gene Sets for GSVA.

Gene signatures	Cell types	Gene symbols
Xenobiotic metabolism by CYP450 <sup>#</sup>	Human cells	ADH1A ADH1B ADH1C ADH4 ADH5 ADH6 ADH7 AKR1C1 AKR1C2 AKR1C3 AKR1C4 ALDH1A3 ALDH3A1 ALDH3B1 ALDH3B2 CYP1A1 CYP1A2 CYP1B1 CYP2B6 CYP2C18 CYP2C19 CYP2C8 CYP2C9 CYP2E1 CYP2F1 CYP2S1 CYP3A4 CYP3A43 CYP3A5 CYP3A7 DHDH EPHX1 GSTA1 GSTA2 GSTA3 GSTA4 GSTA5 GSTK1 GSTM1 GSTM2 GSTM3 GSTM4 GSTM5 GSTO1 GSTO2 GSTP1 GSTT1 GSTT2 GSTZ1 MGST1 MGST2 MGST3 UGT1A1 UGT1A10 UGT1A3 UGT1A4 UGT1A5 UGT1A6 UGT1A7 UGT1A8 UGT1A9 UGT2A1 UGT2A3 UGT2B10 UGT2B11 UGT2B15 UGT2B17 UGT2B28 UGT2B4 UGT2B7
Glutathione metabolism <sup>#</sup>	Human cells	ANPEP G6PD GCLC GCLM GGCT GGT1 GGT5 GGT6 GGT7 GPX1 GPX2 GPX3 GPX4 GPX5 GPX6 GPX7 GSR GSS GSTA1 GSTA2 GSTA3 GSTA4 GSTA5 GSTK1 GSTM1 GSTM2 GSTM3 GSTM4 GSTM5 GSTO1 GSTO2 GSTP1 GSTT1 GSTT2 GSTZ1 IDH1 IDH2 LAP3 MGST1 MGST2 MGST3 ODC1 OPLAH PGD RRM1 RRM2 RRM2B SMS SRM TXNDC12
Response to oxidative stress <sup>*</sup>	Human cells	ABCC2 ABL1 ADA ADAM9 ADIPOQ ADNP2 ADPRHL2 AGER AIF1 AIFM1 AKR1B1 AKR1C3 AKT1 ALAD ALDH3A2 ALDH3B1 ALS2 ANGPTL7 ANKRD2 ANXA1 APEX1 APOA4 APOD APOE APP APTX AQP1 AREG ARG1 ARL6IP5 ARNT ARNTL ATF4 ATOX1 ATP13A2 ATP2A2 ATP7A ATRN BAD BAK1 BCL2 BMP4 BNIP3 C20orf111 CA3 CASP3 CAT CBX8 CCL19 CCL5 CCNA2 CCR7 CCS CD36 CD38 CDK1 CDK2 CHD6 CHRNA4 CHUK CLN8 COL1A1 CPEB2 CRYAB CRYGD CYBA CYBB CYCS CYGB CYP1B1 CYP2E1 DDIT3 DGKK DHCR24 DHRS2 DIABLO DNM2 DPEP1 DUOX1 DUOX2 DUSP1 ECT2 EDN1 EEF2 EGLN1 ENDOG EP300 EPAS1 EPX ERCC1 ERCC2 ERCC3 ERCC6 ERCC8 ERO1L ETFDH ETS1 ETV5 EZH2 F3 FABP1 FAS FER FGF8 FKBP1B FN1 FOS FOSL1 FOXO1 FOXO3 FXN G6PD GAB1 GCLC GCLM GJA3 GJB2 GLRX2 GNAO1 GPX1 GPX2 GPX3 GPX4 GPX5 GPX6 GPX7 GPX8 GSK3B GSR GSS GSTP1 GUCY1B3 HAO1 HBA1 HBA2 HBB HDAC2 HDAC6 HIF1A HMOX1 HNRNPD HP HSPA1A HSPA1B HTRA2 HYAL1 HYAL2 IDH1 IL18BP IL18RAP IL1B IL1R1 IL6 IMPACT IPCEF1 JAK2 JUN KAT2B KCNA5 KCNC2 KDM6B KLF2 KLF4 KLF6 KPNA4 KRT1 LDHA LIAS LONP1 LPO LRRK2 MAP3K5 MAPK7 MAPT MB MBL2 MDM2 MELK MGMT MGST1 MICB MMP14 MMP3 MPO MPV17 MSRA MSRB2 MSRB3 MTA5 MT3 MTF1 MTR MUTYH NAPRT1 NDUFA12 NDUFA6 NDUFB4 NDUFS2 NDUFS8 NEFH NEIL1 NET1 NFE2L1 NFE2L2 NFKB1 NGFR NOS1 NOS3 NOX4 NOX5 NQO1 NR4A2 NR4A3 NUDT1 NUDT2 OGG1 OLR1 OXR1 OXSR1 P4HB PARK2 PARK7 PARP1 PAX2 PCGF2 PDCD10 PDGFD PDGFRA PDGFRB PDK1 PDK2 PDLIM1 PENK PINK1 PKD2 PLA2G4A PLA2R1 PLEKHA1 PLK3 PML PNKP PNPT1 PON2 PPARGC1A PPARGC1B PPIF PPP1R15B PPP2CB PPP5C PRDX1 PRDX2 PRDX3 PRDX5 PRDX6 PRKAA1 PRKCD PRKD1 PRKRA PRNP PRODH PSEN1 PSIP1 PSMB5 PTGS1 PTGS2 PTK2B PTPRK PTPRN PXDN PXDNL PXN PYCR1 PYCR2 RAD52 RBM11 RELA RGS14 RHOB ROMO1 RPS3 RRM2B S100A7 SCARA3 SCGB1A1 SDC1 SELK SELS SEPNI SEPP1 SEPP1 SETX SGK2 SHC1 SIN3A SIRT1 SIRT2 SLC11A2 SLC23A2 SLC25A24 SLC7A11 SLC8A1 SNCA SOD1 SOD2 SOD3 SPHK1 SRC SRXN1 STAR STAT6 STAU1 STC2 STK24 STK25 STX2 STX4 TACR1 TAT TMEM161A TNFAIP3 TOR1A TP53 TP53INP1 TPM1 TPO TRAF2 TRPA1 TRPC6 TRPM2 TXN TXN2 TXNDC2 TXNDC3 TXNDC8 TXNIP TXNLI TXNRD1 TXNRD2 UCN UCP2 UCP3 VKORC1L1 VNN1 VRK2 WNT16 WRN XBP1 XPA ZC3H12A ZNF277 ZNF580 ZNF622
Endoplasmic reticulum stress [1]	Mouse embryo fibroblasts	WFS1 ERO1A CLCN3 ACAT2 CHKA DDIT3 ETS2 RBM38 TCEA1 IFI16 PPP1R15A POLR1A PPARA CA6 MTM1 CDCA7 PTX3 STBD1 ADH7 RAD1 LONP1 AMPD3 IFRD2 PARD6A KITLG PTRH2
Unfolded protein response <sup>§</sup>	Human cells	ACADVL ADD1 ARFGAP1 ASNS ATF3 ATF4 ATF4P3 ATF6 ATP6V0D1 C19orf10 CALR CCL2 CTDSP2 CUL7 CXXC1 DCP2 DCTN1 DDIT3 DDX11 DDX12P DIS3 DNAJB11 DNAJB9 DNAJC3 EDEM1 EIF2AK3 EIF2S1 ERN1 EXOSC1 EXOSC2 EXOSC3 EXOSC4 EXOSC5 EXOSC6 EXOSC7 EXOSC8 EXOSC9 EXTL3 FKBP14 GOSR2 GSK3A HDGF HERPUD1 HSP90B1 HSPA5 HYOU1 IGFBP1 IL8 KDELR3 KHSRP KLHDC3 LMNA LOC730136 MBTPS1 MBTPS2 NFYA NFYB PARN PDIA5 PDIA6 PPP2R5B PREB SEC31A SERP1 SHC1 SRPR SRPRB SSR1 SULT1A3 SULT1A4 SYVN1 TATDN2 TLN1 TPP1 TSPYL2 WFS1 WIPI1 XBP1 YIF1A ZBTB17
Lysosome <sup>#</sup>	Human cells	ABCA2 ABCB9 ACP2 ACP5 AGA AP1B1 AP1G1 AP1M1 AP1M2 AP1S1 AP1S2 AP1S3 AP3B1 AP3B2 AP3D1 AP3M1 AP3M2 AP3S1 AP3S2 AP4B1

		AP4E1 AP4M1 AP4S1 ARSA ARSB ARSG ASAH1 ATP6AP1 ATP6V0A1 ATP6V0A2 ATP6V0A4 ATP6V0B ATP6V0C ATP6V0D1 ATP6V0D2 ATP6V1H CD164 CD63 CD68 CLN3 CLN5 CLTA CLTB CLTC CLTCL1 CTNS CTSA CTSB CTSC CTSD CTSE CTSF CTSG CTSH CTSK CTSL1 CTSL2 CTSO CTSS CTSW CTSZ DNASE2 DNASE2B ENTPD4 FUCA1 GAA GALC GALNS GBA GGA1 GGA2 GGA3 GLA GLB1 GM2A GNPTAB GNPTG GNS GUSB HEXA HEXB HGSNAT HYAL1 IDS IDUA IGF2R LAMP1 LAMP2 LAMP3 LAPT4A LAPT4B LAPT5 LGMN LIPA M6PR MAN2B1 MANBA MCOLN1 MFSD8 NAGA NAGLU NAGPA NAPSA NEU1 NPC1 NPC2 PLA2G15 PPT1 PPT2 PSAP PSAPL1 SCARB2 SGSH SLC11A1 SLC11A2 SLC17A5 SMPD1 SORT1 SUMF1 TCIRG1 TPP1
Glycolysis and gluconeogenesis <sup>#</sup>	Human cells	ACSS1 ACSS2 ADH1A ADH1B ADH1C ADH4 ADH5 ADH6 ADH7 AKR1A1 ALDH1A3 ALDH1B1 ALDH2 ALDH3A1 ALDH3A2 ALDH3B1 ALDH3B2 ALDH7A1 ALDH9A1 ALDOA ALDOB ALDOC BPGM DLAT DLD ENO1 ENO2 ENO3 FBP1 FBP2 G6PC G6PC2 GALM GAPDH GCK GPI HK1 HK2 HK3 LDHA LDHAL6A LDHAL6B LDHB LDHC PCK1 PCK2 PDHA1 PDHA2 PDHB PFKL PFKM PFKP PGAM1 PGAM2 PGAM4 PGK1 PGK2 PGM1 PGM2 PKLR PKM2 TPI1
Current-smoking [2]	Human bronchial epithelial cells	GMDS ZNF323 GALNT12 AP2B1 HN1 GMDS ABCC1 RAB11A MSMB MAFG ABHD2 ANXA3 VND2 FTH1 UGT1A3 TSPAN-1 CTGF PGD HTATIP2 CYP4F11 GCLM ADH7 GCLC UPK1B PLEKHB2 TCN1 TRIM16 UGT1A9 UGT1A1 UGT1A6 HDO1 TXNRD1 PRDX1 ME1 PIR TALDO1 GPX2 HDO1 HDO1 AKR1C3 AKR1C1 AKR1C-pseudo AKR1C2 ALDH3A1 CLDN10 TXN TKT CYP1B1 AKR1C1 CBR1 AKR1B1 KLF4 NET-6 NUDT4 GALNT3 GALNT7 CEACAM6 AP1G1 CA12 FLJ20151 BCL2L13 SRPUL FLJ13052 GALNT6 OASIS MUC5B MUC5B S100P NUDT4 ME1 SDR1 PLA2G10 DPYSL3
Smoking index-related [2]	Human bronchial epithelial cells	CST6 EIF2C3 BRD2 HBP17 PCDHGC3 MGC13053 C22orf3 SFN TCF20 C9orf7 EPS8L1 BDKRB2 FAXDC1 ARIH2 EPS8L1 FLJ10404 PRG1 RPN2 GMPPA TCIRG1 BAIAP2 S100A8 DKFZp564B0769 TRAP95 SRRM2 MUC5AC MYL6 PURA BAIAP3 ARFGAP1 FLJ10849 PCDHGC3 LMNA MGC14376 LYPLA2 KIAA0992 C11orf13 CPT1B EPHA2 MUC5AC QSCN6 RPA1 PPAP2B KIAA0971 ZNF258

Curated gene sets from <sup>#</sup>KEGG, <sup>\*</sup>GO or <sup>§</sup>Reactome in MSigDB.

Supplementary Table S2. GO enrichment analysis for biological process using differentially expressed proteins in ESA or NSA.

<b>GO biological process</b>	<b>Fold enrichment</b>	<b>P value</b>
<b><i>ESA-NH</i></b>		
Regulation of phagocytosis	21.11	3.71E-02
Response to hydrogen peroxide	15.45	2.33E-02
Response to monosaccharide	13.38	8.55E-03
Regulation of chemotaxis	12.8	1.93E-03
Protein autophosphorylation	10.8	3.47E-02
Myeloid cell differentiation	10.75	3.59E-02
Leukocyte migration	8.87	2.90E-02
Positive regulation of cell migration	8.15	8.49E-04
Response to bacterium	7.26	1.98E-04
Leukocyte activation	7.02	1.31E-02
Inflammatory response	6.94	4.18E-03
Response to hormone	6.83	1.73E-07
Activation of immune response	6.71	1.95E-02
Response to wounding	6.27	3.50E-03
Positive regulation of kinase activity	6.07	4.71E-02
Immune effector process	6.07	4.71E-02
Positive regulation of cell proliferation	5.93	2.03E-05
Tube development	5.78	2.41E-02
Regulation of cytokine production	5.72	2.66E-02
Regulation of proteolysis	5.55	4.29E-03
Regulation of protein kinase activity	5.38	6.02E-03
Innate immune response	5.38	4.83E-02
Response to cytokine	5.26	7.77E-03
Positive regulation of transport	4.78	3.30E-03
Positive regulation of protein phosphorylation	4.72	1.01E-02
Negative regulation of catalytic activity	4.66	2.94E-02
Positive regulation of intracellular signal transduction	4.63	1.28E-02
Positive regulation of cell differentiation	4.52	4.08E-02
Negative regulation of protein metabolic process	4.38	1.01E-02
Negative regulation of multicellular organismal process	4.17	4.25E-02
Positive regulation of multicellular organismal process	3.75	6.50E-03
Cellular response to organic substance	3.74	5.00E-05
Cell adhesion	3.74	3.22E-02
Cell surface receptor signaling pathway	3.62	1.85E-05
Regulation of multicellular organismal development	3.5	4.11E-03
Negative regulation of cellular process	2.73	1.51E-06
Regulation of biological quality	2.56	1.92E-03
<b><i>NSA-NH</i></b>		
Acute-phase response	36.41	4.09E-02
Platelet degranulation	20.61	3.91E-02
Response to carbohydrate	13.94	3.83E-02
Response to wounding	7.51	5.59E-03
Cell adhesion	4.7	6.65E-03
Immune system process	4.46	8.67E-06
Positive regulation of response to stimulus	3.52	3.99E-02
Regulation of biological quality	2.82	5.93E-03

GO: Gene Ontology.

Supplementary Table S3. Patient characteristics in transcriptomic analyses (bronchial brushings)

	Severe asthma			Healthy	P value
	Current-smoker (CSA) (n=6)	Ex-smoker (ESA) (n=12)	Non-smoker (NSA) (n=40)	Non-smoker (NH) (n=37)	
Female	4 (66.7)	3 (25.0)	22 (55.0)	13 (35.1)	1.05E-01*
Age (y)	49.7±7.6	53.0±8.8	48.6±13.9	36.2±13.9 <sup>¶</sup>	<b>2.95E-04</b> <sup>§</sup>
Onset age of asthma (y)	27.3±20.0	29.8±17.1	22.1±19.9	N.R.	5.38E-01 <sup>§</sup>
Age at starting smoking (y)	16.5±3.7	16.2±3.7	N.R.	N.R.	9.61E-01 <sup>†</sup>
Years of smoking cessation (y)	N.R.	17.7±9.5	N.R.	N.R.	
Smoking pack-year	22.4±8.6	29.9±23.1	0.0±0.0	0.0±0.0	8.51E-01 <sup>†</sup>
BMI (kg/m <sup>2</sup> )	22.4±2.4 <sup>¶</sup>	32.5±4.4	31.5±6.3	24.5±3.0 <sup>¶</sup>	<b>9.99E-09</b>
Atopic (%)	2 (66.7) [3]	10 (83.3)	28 (71.8) [1]	12 (40.0) [7] <sup>¶</sup>	<b>1.44E-02</b> *
Blood eosinophil (*10 <sup>3</sup> /μL)	178±68	308±321	298±246	(158±153)	5.18E-01 <sup>§#</sup>
Blood neutrophil (*10 <sup>3</sup> /μL)	8.14±4.02 <sup>¶</sup>	6.19±2.46	4.45±1.61	(3.34±2.13)	<b>8.24E-03</b> <sup>§#</sup>
Sputum eosinophil (%)	0.87±1.03 [3]	17.0±20.2 [8]	12.6±17.2 [24]	(0.33±0.50) [16]	4.44E-01 <sup>§#</sup>
Sputum neutrophil (%)	61.8±26.0 [3]	39.6±18.1 [8]	51.4±17.9 [24]	(35.0±26.7) [16]	3.39E-01 <sup>§#</sup>
IgE (IU/mL)	184±198 [1]	807±1851	300±456	(60.4±99.7) [2]	7.49E-01 <sup>§#</sup>
FeNO (ppb)	14.1±13.5 <sup>¶</sup>	35.1±27.4 [1]	41.0±30.1 [4]	(21.9±15.5) [2]	<b>1.12E-02</b> <sup>§#</sup>
Periostin (ng/mL)	44.1±6.7 [3]	47.9±14.8 [1]	51.4±13.9 [5]	(51.9±10.0) [5]	5.18E-01 <sup>§#</sup>
%FEV1 post-bronchodilator (%) <sup>##</sup>	76.8±7.3	70.7±19.1	84.6±21.4	(100.8±12.7)	9.33E-02 <sup>§#</sup>
FEV1/FVC post-bronchodilator (%) <sup>##</sup>	61.4±6.5	62.2±12.7	69.8±12.1	(79.4±6.1)	5.94E-02 <sup>§#</sup>
Airway reversibility (%)	16.8±7.1	11.4±11.4	12.5±16.2	N.R.	8.07E-02 <sup>§</sup>
Airflow limitation [92]	5 (83.3)	8 (66.7)	15 (37.5)	N.R.	5.30E-02 <sup>§</sup>
Average ACQ-7	3.12±0.79 [1]	2.27±1.29 [4]	2.19±1.16 [9]	N.R.	2.06E-01 <sup>§</sup>
Average AQLQ	4.25±1.19	5.01±1.47 [5]	4.98±0.98 [6]	N.R.	2.97E-01 <sup>§</sup>
Exacerbation in previous year (n/y)	2.6±3.3	2.1±1.9	2.4±1.9	N.R.	3.80E-01 <sup>§</sup>
ER visit due to breathing problems	2 (33.3)	7 (58.3)	25 (62.5)	N.R.	3.80E-01*
<b>Comorbidities</b>					
Allergic rhinitis (%)	1 (16.7)	5 (41.7)	21 (60.0) [5]	N.R.	1.14E-01*
Nasal polyp (%)	1 (16.7)	6 (54.5) [1]	12 (34.3) [5]	N.R.	3.05E-01*
Sinusitis (%)	3 (50.0)	2 (16.7)	11 (30.6) [4]	N.R.	3.44E-01*
Chronic bronchitis (%)	0 (0.0) [1]	4 (33.3)	6 (15.0)	N.R.	2.54E-01*
Psychiatric disease (%)	3 (50.0)	3 (25.0)	6 (16.2) [3]	N.R.	1.52E-01*
GERD (%)	4 (80.0) [1]	7 (58.3)	21 (56.8) [3]	N.R.	7.51E-01*
<b>Medications</b>					
Inhaled corticosteroids (%)	6 (100.0)	12 (100.0)	40 (100.0) [5]	N.R.	1.00E+00*
Systemic corticosteroids (%)	1 (16.7)	8 (72.7) [1]	13 (35.1) [3]	N.R.	<b>4.41E-02</b> *
Oral corticosteroid dose (mg/day)	1.67±4.08	14.0±17.6 [2]	3.81±7.00 [4]	N.R.	<b>4.17E-02</b> <sup>§</sup>
Anti-IgE therapy (%)	0 (0.0)	1 (12.5) [4]	2 (5.7) [5]	N.R.	6.45E-01*
Long-acting beta agonist (%)	6 (100.0)	12 (100.0)	40 (100.0)	N.R.	1.00E+00*
Leukotriene modifiers (%)	2 (33.3)	6 (60.0) [2]	22 (59.5) [3]	N.R.	5.67E-01*
Tiotropium (%)	2 (33.3)	3 (42.9) [5]	4 (11.4) [5]	N.R.	5.72E-02*
Macrolide (%)	1 (16.7)	1 (8.3)	5 (12.5)	N.R.	1.00E+00*

Data are presented as mean±SD or n (%). The numbers of missing values are shown in square brackets. <sup>##</sup>Spirometry data without bronchodilator were used for healthy subjects. Statistical analysis was performed by Fisher's exact test\*, Kruskal-Wallis test<sup>§</sup> or Mann-Whitney U test<sup>†</sup>. <sup>#</sup>Healthy subjects were excluded from statistical analyses of several items. Variables with P values in bold were significantly different (<0.05). <sup>¶</sup>p<5.00E-02 vs CSA, <sup>‡</sup>p<5.00E-02 vs ESA, <sup>§</sup>p<5.00E-02 vs NSA. BMI: body mass index, IgE: immunoglobulin E, FeNO: fractional exhaled nitric oxide, FEV: forced expiratory volume, FVC: forced vital capacity, ERS: European Respiratory Society, ACQ: Asthma Control Questionnaire, AQLQ: Asthma Quality of Life Questionnaire, ER: emergency room, GERD: gastroesophageal reflux disease, N.R.: not relevant.

Supplementary Table S4. Patient characteristics in transcriptomic analyses (bronchial biopsies)

	Severe asthma			Healthy	P value
	Current-smoker (CSA) (n=7)	Ex-smoker (ESA) (n=6)	Non-smoker (NSA) (n=34)	Non-smoker (NH) (n=22)	
Female	5 (71.4)	0 (0.0) <sup>‡¶</sup>	21 (61.8)	9 (40.9)	<b>1.55E-02*</b>
Age (y)	50.0±7.0	58.5±7.4	49.8±13.1	39.6±15.9	<b>2.11E-02</b>
Onset age of asthma (y)	29.9±19.5	28.5±21.6 [1]	21.8±20.6	N.R.	6.56E-01 <sup>§</sup>
Age at starting smoking (y)	17.0±3.7	18.0±4.1	N.R.	N.R.	7.72E-01 <sup>†</sup>
Years of smoking cessation (y)	N.R.	21.3±11.4	N.R.	N.R.	
Smoking pack-year	22.6±7.9	29.8±18.6	0.0±0.0	0.0±0.0	4.74E-01 <sup>†</sup>
BMI (kg/m <sup>2</sup> )	23.6±3.7	33.2±4.8	30.9±5.8	24.5±2.9	<b>3.14E-06</b> <sup>§</sup>
Atopic (%)	2 (50.0) [3]	5 (83.3)	24 (72.7) [1]	9 (42.9) [1]	9.45E-02*
Blood eosinophil (*10 <sup>3</sup> /μL)	210±104	457±404	278±229	(157±184)	5.06E-01 <sup>§#</sup>
Blood neutrophil (*10 <sup>3</sup> /μL)	7.92±3.71 <sup>¶</sup>	5.37±2.45	4.65±1.56	(3.70±2.57)	<b>4.80E-02</b> <sup>§#</sup>
Sputum eosinophil (%)	1.99±2.40 [3]	N.D. [6]	11.7±16.3 [16]	(0.43±0.53) [9]	2.49E-01 <sup>†#</sup>
Sputum neutrophil (%)	46.7±36.9 [3]	N.D. [6]	51.3±16.1 [16]	(32.8±28.0) [9]	9.32E-01 <sup>†#</sup>
IgE (IU/mL)	153±192 [1]	1418±2567	288±385	(61.8±88.8)	4.99E-01 <sup>§#</sup>
FeNO (ppb)	16.5±13.9 <sup>¶</sup>	32.5±29.8	38.8±29.5 [5]	(27.2±18.5) [3]	<b>3.22E-02</b> <sup>§#</sup>
Periostin (ng/mL)	42.1±6.7 [3]	51.4±16.6 [1]	49.9±12.9 [4]	(55.0±11.6) [5]	4.05E-01 <sup>§#</sup>
%FEV1 post-bronchodilator (%) <sup>##</sup>	76.7±6.6	73.9±22.6	82.4±21.1	(98.6±13.8)	4.77E-01 <sup>§#</sup>
FEV1/FVC post-bronchodilator (%) <sup>##</sup>	61.4±6.0	61.8±15.5	67.4±12.7	(80.0±6.2)	3.02E-01 <sup>§#</sup>
Airway reversibility (%)	21.9±15.0	7.0±14.8	14.2±16.7	N.R.	8.32E-02 <sup>§</sup>
Airflow limitation [92]	6 (85.7)	4 (66.7)	15 (44.1)	N.R.	1.03E-01*
Average ACQ-7	3.12±0.79 [2]	2.37±1.54 [1]	2.17±1.21 [5]	N.R.	2.28E-01 <sup>§</sup>
Average AQLQ	4.25±1.19 [1]	4.48±1.36 [1]	5.02±1.06 [3]	N.R.	2.32E-01 <sup>§</sup>
Exacerbation in previous year (n/y)	4.1±3.1	2.0±1.7	2.3±2.2 [1]	N.R.	2.26E-01 <sup>§</sup>
ER visit due to breathing problems	3 (42.9)	3 (50.0)	20 (58.8)	N.R.	8.04E-01*
<b>Comorbidities</b>					
Allergic rhinitis (%)	2 (28.6)	2 (33.3)	19 (61.3) [3]	N.R.	2.29E-01*
Nasal polyp (%)	1 (14.3)	4 (66.7)	10 (31.3) [2]	N.R.	1.51E-01*
Sinusitis (%)	4 (57.1)	0 (0.0)	9 (28.1) [2]	N.R.	6.12E-02*
Chronic bronchitis (%)	0 (0.0) [1]	2 (33.3)	5 (14.7)	N.R.	4.11E-01*
Psychiatric disease (%)	3 (42.9)	1 (16.7)	6 (18.2) [1]	N.R.	3.92E-01*
GERD (%)	4 (66.7) [1]	4 (66.7)	16 (48.5) [1]	N.R.	7.05E-01*
<b>Medications</b>					
Inhaled corticosteroids (%)	7 (100.0)	6 (100.0)	34 (100.0)	N.R.	1.00E+00*
Systemic corticosteroids (%)	2 (28.6)	3 (50.0)	14 (43.8) [2]	N.R.	7.99E-01*
Oral corticosteroid dose (mg/day)	2.86±4.88	3.00±4.47 [1]	4.98±7.82 [3]	N.R.	8.47E-01 <sup>§</sup>
Anti-IgE therapy (%)	0 (0.0) [1]	0 (0.0) [1]	2 (6.5) [3]	N.R.	1.00E+00*
Long-acting beta agonist (%)	7 (100.0)	6 (100.0)	34 (100.0)	N.R.	1.00E+00*
Leukotriene modifiers (%)	3 (42.9)	4 (66.7)	18 (56.3) [2]	N.R.	8.02E-01*
Tiotropium (%)	2 (33.3) [1]	2 (40.0) [1]	7 (22.6) [3]	N.R.	6.10E-01*
Macrolide (%)	1 (14.3)	0 (0.0)	5 (14.7)	N.R.	1.00E+00*

Data are presented as mean±SD or n (%). The numbers of missing values are shown in square brackets. <sup>##</sup>Spirometry data without bronchodilator were used for healthy subjects. Statistical analysis was performed by Fisher's exact test\*, Kruskal-Wallis test<sup>§</sup> or Mann-Whitney U test<sup>†</sup>. <sup>#</sup>Healthy subjects were excluded from statistical analyses of several items. Variables with P values in bold were significantly different (<0.05). <sup>‡</sup>p<5.00E-02 vs CSA, <sup>¶</sup>p<5.00E-02 vs ESA, <sup>¶¶</sup>p<5.00E-02 vs NSA. BMI: body mass index, IgE: immunoglobulin E, FeNO: fractional exhaled nitric oxide, FEV: forced expiratory volume, FVC: forced vital capacity, ERS: European Respiratory Society, ACQ: Asthma Control Questionnaire, AQLQ: Asthma Quality of Life Questionnaire, ER: emergency room, GERD: gastroesophageal reflux disease, N.R.: not relevant.

Supplementary Table S5. Patient characteristics in transcriptomic analyses (sputa)

	Severe asthma			Healthy	P value
	Current-smoker (CSA) (n=8)	Ex-smoker (ESA) (n=15)	Non-smoker (NSA) (n=47)	Non-smoker (NH) (n=15)	
Female	4 (50.0)	8 (53.3)	28 (59.6)	3 (20.0) <sup>¶</sup>	6.15E-02*
Age (y)	48.6±11.9	54.8±10.5	52.3±12.7	37.5±13.6	<b>2.56E-03</b> <sup>§</sup>
Onset age of asthma (y)	25.6±20.7	40.7±18.7 <sup>¶</sup>	21.9±18.6	N.R.	<b>8.97E-03</b> <sup>§</sup>
Age at starting smoking (y)	18.8±4.4	15.9±3.1	N.R.	N.R.	2.29E-01
Years of smoking cessation (y)	N.R.	13.6±8.8	N.R.	N.R.	
Smoking pack-year	19.6±9.6	23.1±18.8	0.0±0.0	0.0±0.0	9.49E-01
BMI (kg/m <sup>2</sup> )	26.7±4.0	30.8±4.9	27.6±5.5	25.8±2.9	<b>4.82E-02</b> <sup>§</sup>
Atopic (%)	5 (83.3) [2]	8 (72.7) [4]	36 (85.7) [5]	4 (44.4) [6]	<b>4.96E-02</b> *
Blood eosinophil (*μL)	294±157	303±226	393±339 [2]	(113±74)	6.17E-01 <sup>§#</sup>
Blood neutrophil (*10 <sup>3</sup> /μL)	6.31±2.66	5.92±2.38	5.10±2.31 [2]	(3.66±1.40)	2.28E-01 <sup>§#</sup>
Sputum eosinophil (%)	9.4±17.5	14.6±15.0	14.4±22.3	(0.13±0.26)	2.70E-01 <sup>§#</sup>
Sputum neutrophil (%)	53.8±19.3	57.1±20.4	60.3±28.5	(45.0±25.6)	6.19E-01 <sup>§#</sup>
IgE (IU/mL)	238±202 [2]	324±547	266±471 [3]	(119±178)	5.47E-01 <sup>§#</sup>
FeNO (ppb)	18.3±18.9 <sup>¶</sup>	42.9±31.2 [1]	40.8±35.1 [2]	(20.1±10.2) [2]	<b>1.41E-02</b> <sup>§#</sup>
Periostin (ng/mL)	45.4±10.7 [1]	54.6±20.4 [4]	53.2±19.5 [8]	(47.4±5.6) [2]	6.85E-01 <sup>§#</sup>
%FEV1 post-bronchodilator (%) <sup>###</sup>	81.6±12.6	75.0±22.8	66.7±22.2	(106.4±10.4)	1.08E-01 <sup>§#</sup>
FEV1/FVC post-bronchodilator (%) <sup>###</sup>	63.2±7.9	59.4±11.5	58.1±14.3	(78.5±6.0)	6.57E-01 <sup>§#</sup>
Airway reversibility (%)	16.6±9.8	17.0±14.2 [1]	17.2±18.5	N.R.	8.46E-01 <sup>§</sup>
Airflow limitation [92]	5 (62.5)	10 (66.7)	36 (76.6)	N.R.	5.46E-01*
Average ACQ-7	2.53±1.42 [1]	2.36±0.92 [2]	2.68±1.40 [3]	N.R.	7.24E-01 <sup>§</sup>
Average AQLQ	4.51±1.65 [1]	5.05±1.07 [3]	4.56±1.23 [2]	N.R.	4.31E-01 <sup>§</sup>
Exacerbation in previous year (n/y)	2.9±3.4	2.1±2.1	2.2±1.9	N.R.	8.99E-01 <sup>§</sup>
ER visit due to breathing problems	3 (37.5)	9 (60.0)	30 (63.8)	N.R.	3.95E-01*
<b>Comorbidities</b>					
Allergic rhinitis (%)	2 (28.6) [1]	5 (35.7) [1]	19 (48.7) [8]	N.R.	5.36E-01*
Nasal polyp (%)	2 (28.6) [1]	6 (40.0)	16 (35.6) [2]	N.R.	9.29E-01*
Sinusitis (%)	2 (33.3) [2]	5 (33.3)	11 (25.0) [3]	N.R.	6.81E-01*
Chronic bronchitis (%)	0 (0.0) [2]	2 (13.3)	8 (18.2) [3]	N.R.	1.94E-01*
Psychiatric disease (%)	2 (28.6) [1]	2 (13.3)	6 (13.0) [1]	N.R.	4.90E-01*
GERD (%)	4 (80.0) [2]	9 (60.0)	17 (37.8) [2]	N.R.	2.03E-01*
<b>Medications</b>					
Inhaled corticosteroids (%)	8 (100.0)	15 (100.0)	47 (100.0)	N.R.	1.00E+00*
Systemic corticosteroids (%)	3 (42.9) [1]	10 (71.4) [1]	20 (43.5) [1]	N.R.	2.17E-01*
Oral corticosteroid dose (mg/day)	3.57±5.37 [1]	8.75±7.83 [1]	4.06±6.25 [2]	N.R.	6.29E-02 <sup>§</sup>
Anti-IgE therapy (%)	0 (0.0) [1]	1 (7.7) [2]	1 (2.2) [2]	N.R.	5.24E-01*
Long-acting beta agonist (%)	8 (100.0)	14 (93.3)	47 (100.0)	N.R.	3.29E-01*
Leukotriene modifiers (%)	4 (50.0)	7 (50.0) [1]	23 (48.9)	N.R.	1.00E+00*
Tiotropium (%)	3 (42.9) [1]	2 (15.4) [2]	14 (31.1) [2]	N.R.	3.89E-01*
Macrolide (%)	2 (25.0)	2 (13.3)	6 (12.8)	N.R.	5.90E-01*

Data are presented as mean±SD or n (%). The numbers of missing values are shown in square brackets. <sup>###</sup>Spirometry data without bronchodilator were used for healthy subjects. Statistical analysis was performed by Fisher's exact test\*, Kruskal-Wallis test<sup>§</sup> or Mann-Whitney U test<sup>†</sup>. <sup>#</sup>Healthy subjects were excluded from statistical analyses of several items. Variables with P values in bold were significantly different (<0.05). <sup>¶</sup>p<5.00E-02 vs CSA, <sup>‡</sup>p<5.00E-02 vs ESA, <sup>§</sup>p<5.00E-02 vs NSA. BMI: body mass index, IgE: immunoglobulin E, FeNO: fractional exhaled nitric oxide, FEV: forced expiratory volume, FVC: forced vital capacity, ERS: European Respiratory Society, ACQ: Asthma Control Questionnaire, AQLQ: Asthma Quality of Life Questionnaire, ER: emergency room, GERD: gastroesophageal reflux disease, N.R.: not relevant.

Supplementary Table S6. GO enrichment analysis for biological process using differentially expressed genes from bronchial brushings in comparison between CSA and NSA.

<b>GO biological process</b>	<b>Fold enrichment</b>	<b>P value</b>
Pentose biosynthetic process	> 100	8.37E-03
Cellular response to jasmonic acid stimulus	> 100	8.37E-03
Doxorubicin metabolic process	> 100	4.19E-06
Daunorubicin metabolic process	> 100	4.19E-06
Primary alcohol catabolic process	92.66	4.44E-02
Cellular aldehyde metabolic process	32.03	1.89E-06
Retinoid metabolic process	19.43	9.02E-05
Cellular hormone metabolic process	14.55	5.32E-03
Response to endoplasmic reticulum stress	8.07	1.67E-02
Oxidation-reduction process	4.74	1.99E-05

GO: Gene Ontology.



Supplementary Table 7. Functional clustering of annotated probes by DAVID using differentially expressed gene probes from bronchial brushings in comparison between CSA and NSA.

<b>DAVID Function cluster</b>	<b>Enrichment score</b>
Endoplasmic reticulum	5.71
Cytoplasmic membrane-bounded vesicle	4.37
Oxidation reduction	4.18
Signal peptide	3.51
Redox	2.56
Xenobiotic metabolic process	2.55
Terpenoid metabolic process	2.34
Hormone metabolic process	2.16
NADP and nicotinamide metabolic process	1.96
Glycosylation	1.84
Lysosome	1.37

DAVID: Database for Annotation, Visualization and Integrated Discovery. NADP: nicotinamide adenine dinucleotide phosphate. Only significant clusters (enrichment score > 1.3) are shown.

Supplementary Table S8. Critical genes whose absolute fold change was more than or equal to 2.0 in limma in comparison between ESA and NSA.

Probe ID	Gene symbol	Gene name	Function
<b><i>Sputum</i></b>			
215101_s_at	CXCL5	C-X-C motif chemokine ligand 5	The encoded protein is proposed to bind the G-protein coupled receptor chemokine (C-X-C motif) receptor 2 to recruit neutrophils, to promote angiogenesis and to remodel connective tissues.
204580_at	MMP12	matrix metalloproteinase 12	The encoded protease degrades soluble and insoluble elastin. The mutations in this gene are associated with lung function and COPD.
212298_at	NRP1	neuropilin 1	Neuropilins affect cell survival, migration, and attraction. Some of the ligands and co-receptors bound by neuropilins are VEGF and semaphorin family members. This encoded protein plays versatile roles in angiogenesis, axon guidance, cell survival, migration, and invasion.
214146_s_at	PPBP	pro-platelet basic protein	This platelet-derived growth factor is a potent chemoattractant and activator of neutrophils.
1554091_a_at	TIRAP	Toll-interleukin 1 receptor domain containing adaptor protein	The protein encoded by this gene is a Toll-interleukin 1 receptor adaptor protein involved in the TLR4 signaling pathway of the immune system. It activates NF- $\kappa$ B, MAPK1, MAPK3 and JNK, which then results in cytokine secretion and the inflammatory response.
<b><i>Bronchial Brushings</i></b>			
205624_at	CPA3	carboxypeptidase A3	The encoded preproprotein is proteolytically processed to generate a mature protease that is released by mast cells and may be involved in the degradation of endogenous proteins and the inactivation of venom-associated peptides.
206224_at	CST1	cystatin SN	This protein is a member of type 2 cystatin proteins that are a class of cysteine proteinase inhibitors found in a variety of human fluids and secretions, where they appear to provide protective functions. This gene is located in the cystatin locus and encodes a cysteine proteinase inhibitor found in saliva, tears, urine, and seminal fluid.
224795_x_at 221651_x_at	IGKC	immunoglobulin kappa constant	This is a human gene that encodes the constant domain of kappa-type light chains for antibodies. It is associated with humoral immunity to a variety of self and non-self antigens, and it is also a risk factor for several immune-mediated diseases, including some cancers.
204673_at	MUC2	mucin-2	This protein coats the epithelia of the intestines, airways, and other mucus membrane-containing organs. It is thought to provide a protective, lubricating barrier against particles and infectious agents at mucosal surfaces.
216474_x_at	TPSAB1	tryptase alpha/beta 1	This gene encodes tryptase alpha-1 and tryptase beta-1, that have been implicated as mediators in the pathogenesis of asthma and other allergic and inflammatory disorders. Beta tryptases appear to be the main isoenzymes expressed in mast cells; whereas in basophils, alpha tryptases predominate.
<b><i>Biopsies</i></b>			
229152_at	FDCSP	follicular dendritic cell secreted protein	This protein secreted from follicular dendritic cells specifically binds to activated B cells, and functions as a regulator of antibody responses.
215214_at	IGLC1	immunoglobulin lambda constant 1	This encoded protein is the constant domain of lambda-type light chains for antibodies. It is associated with humoral immunity to a variety of self and non-self antigens.
234764_x_at	IGLV@	immunoglobulin lambda variable cluster	This gene encodes the variable region protein of lambda-type light chains for antibodies. It is associated with humoral immunity to a variety of self and non-self antigens.
210809_s_at 1555778_a_at	POSTN	periostin	The encoded protein is a secreted extracellular matrix protein which functions in tissue development and regeneration through IL-4/13 signalling.
214146_s_at	PPBP	pro-platelet basic protein	This platelet-derived growth factor is a potent chemoattractant and activator of neutrophils.

COPD: chronic obstructive pulmonary disease, VEGF: vascular endothelial growth factor, TLR: Toll-like receptor, NF- $\kappa$ B: nuclear factor kappa-light-chain-enhancer of activated B cells, MAPK: mitogen-activated protein kinase, JNK: Jun N-terminal kinase.

Supplementary Table S9. GO enrichment analysis for biological process using current-smoking signature gene set [2].

<b>GO biological process</b>	<b>Fold enrichment</b>	<b>P value</b>
Pentose biosynthetic process	> 100	7.34E-04
Cellular response to thyroxine stimulus	> 100	1.34E-02
Cellular response to jasmonic acid stimulus	> 100	7.18E-04
Extracellular matrix constituent secretion	> 100	1.91E-02
NADP biosynthetic process	> 100	2.58E-02
Doxorubicin metabolic process	> 100	1.08E-04
Daunorubicin metabolic process	> 100	1.02E-04
Xenobiotic glucuronidation	> 100	1.10E-04
Flavonoid glucuronidation	> 100	1.05E-04
Response to prostaglandin D	99.02	3.32E-02
Pentose-phosphate shunt, non-oxidative branch	82.52	4.05E-02
Vitamin K metabolic process	82.52	4.00E-02
Cobalamin transport	70.73	4.88E-02
Cellular response to follicle-stimulating hormone stimulus	61.89	5.50E-03
Retinal metabolic process	57.13	6.42E-03
Progesterone metabolic process	53.05	7.24E-03
Regulation of cell migration involved in sprouting angiogenesis	32.29	1.87E-02
Prostaglandin metabolic process	25.61	2.99E-02
Arachidonic acid metabolic process	22.50	1.13E-03
O-glycan processing	20.98	1.51E-03
Cell redox homeostasis	13.75	2.67E-02
Positive regulation of JAK-STAT cascade	13.03	3.10E-02
Cellular response to oxidative stress	10.95	5.84E-05
Response to corticosteroid	9.46	8.40E-03
Oxidation-reduction process	4.70	5.69E-05
Response to growth factor	4.48	2.23E-02
Response to wounding	4.00	4.06E-02
Chemical homeostasis	3.07	4.75E-02
Regulation of apoptotic process	2.81	1.36E-02

GO: Gene Ontology. JAK: Janus kinase. STAT: Signal Transducers and Activator of Transcription.

Supplementary Table S10. Functional clustering of annotated probes by DAVID using current-smoking signature gene set [2].

<b>DAVID Function cluster</b>	<b>Enrichment score</b>
Oxidation reduction	4.86
Metallothionein	3.93
Metabolism of xenobiotics by cytochrome P450	3.89
CTCK domain	2.74
Glycosyltransferase	2.54
Response to reactive oxygen species	1.92
Calcium ion binding	1.69
Pentose-phosphate shunt	1.57
Extracellular space	1.44

DAVID: Database for Annotation, Visualization and Integrated Discovery. CTCK: C-terminal cystine knot-like. Only significant clusters (enrichment score > 1.3) are shown.

## References

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