Supplementary material
A novel non-invasive method allowing for discovery of pathologically relevant proteins from small airways.
Clinical Proteomics, Östling et. al. 2022

PExA sampling method
The PExA method and PExA 1.0 instrument herein used have previously been described in detail [1]. In short, the PExA 1.0 instrument report amount of PE in real-time and counts exhaled particles in seven diameter intervals between 0.41 and 4.55 µm, (Grimm model 1.108; Grimm Aerosol Technik GmbH & Co., Ainring, Germany) and sample them by impaction (Dekati Ltd., Tampere, Finland) on a removable substrate made of hydrophilic PTFE-membrane (Millipore FHLC02500, Merck, Darmstadt, Germany). The exhalation flow and volume is monitored using an ultrasonic flow meter (OEM flow sensor; Spiroson-AS, Medical Technologies, Zürich, Switzerland).

Subjects were asked to perform a standardized breathing manoeuvre, initiated by a full exhalation to residual volume and followed by breath-holding for five seconds, a maximal inhalation to total lung capacity and finalized by a normal exhalation to functional residual capacity.

Subjects were breathing HEPA-filtered air via a mouthpiece and a two-way, non-rebreathing valve into the instrument. To avoid contamination of particles from ambient air, the air present in the airways before sampling was diluted out by a minimum of three breaths of HEPA filtered air before sampling was initiated. All subjects wore a nose clip throughout the sampling session.

PEx sample preparation
Protein were extracted from the PE substrate by adding sample buffer (proprietary SomaLogic SB17 buffer with addition of Tween-20 to 0.1% final concentration) to the centrifugal filter insert (Millipore Ultrafree-MC LH Centrifugal Filter) containing the PE substrate membrane. The volume of sample buffer was adjusted to reach a final concentration of PE at either 1 or 2 µg/ml, as stated elsewhere. After addition of sample buffer, the sample tubes were briefly vortexed and placed in a thermal rotary shaker for 1 h at 30 °C and 400 rpm. After incubation on the thermal shaker, protein extracts were retrieved in the lower part of the centrifugal filter unit by centrifugation at 10,000 ×g for 10 min. Protein extracts were stored at -80 °C before sent to SomaLogic (Boulder, Co, USA) for analysis. Blank samples were made by extraction of the material on PE substrates subjected to the same sampling and sample preparation procedure as all other samples, omitting the breathing manoeuvre.

SOMAscan analysis
The SOMAscan assay (SomaLogic Inc, Boulder, USA) uses slow off-rate modified DNA aptamers (SOMAmers [2] as high affinity protein capture reagents to simultaneously quantify more than 1100 human proteins in only 150 µl of serum, plasma or equally small amounts of a variety of other biological matrices. SOMAscan allows measurement of proteins across a very broad dynamic range, from low abundant cytokines to albumin, with excellent reproducibility [3-6]. To account for systematic differences due to possible variability in final PE concentration as well as potential pipetting errors etc, the set of detected proteins were subjected to group median based normalization and log2 transformation before statistical analysis was performed.
References

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Table S1. Proteins considered being detected in PEx samples using the SOMAscan 1.3K platform

| Target Full Name                                                                 | Entrez Gene Symbol | Target, alternative name                        | SomalId   | UniProt   |
|----------------------------------------------------------------------------------|--------------------|-------------------------------------------------|-----------|-----------|
| 14-3-3 protein epsilon                                                           | YWHAE              | 14-3-3E                                         | SL004984  | P62258    |
| 6-phosphogluconate dehydrogenase, decarboxylating                               | PGD                | 6-Phosphogluconate dehydrogenase                | SL000247  | P52209    |
| Adapter molecule crk                                                              | CRK                | CRK                                             | SL013240  | P46108    |
| Adhesion G protein-coupled receptor E2                                            | ADGRE2             | EMR2                                            | SL008822  | Q9UHX3    |
| Adiponectin                                                                      | ADIPOQ             | Adiponectin                                     | SL004258  | Q15848    |
| Advanced glycosylation end product-specific receptor, soluble                     | AGER               | sRAGE                                           | SL003680  | Q15109    |
| Afamin                                                                           | AFM                | Afamin                                          | SL004742  | P43652    |
| Agouti-related protein                                                            | Agrp               | ART                                             | SL006924  | O00253    |
| Alcohol dehydrogenase [NADP(+)]                                                  | AKR1A1             | AK1A1                                           | SL008039  | P14550    |
| Alpha-1-antichymotrypsin                                                         | SERPINA3           | a1-Antichymotrypsin                            | SL000248  | P01011    |
| Alpha-1-antichymotrypsin complex                                                  | SERPINA3           | alpha-1-antichymotrypsin complex                | SL018548  | P01011    |
| Alpha-1-antitrypsin                                                              | SERPINA1           | a1-Antitrypsin                                 | SL000249  | P01009    |
| Alpha-2-antiplasmin                                                              | SERPINF2           | a2-Antiplasmin                                 | SL000250  | P08697    |
| Alpha-2-HS-glycoprotein                                                          | AHSG               | a2-HS-Glycoprotein                             | SL000251  | P02765    |
| Angiostatin                                                                      | PLG                | Angiostatin                                     | SL000268  | P00747    |
| Angiotensinogen                                                                  | AGT                | Angiotensinogen                                 | SL000271  | P01019    |
| Ankyrin-2                                                                        | ANK2               | ANK2                                            | SL014896  | Q01484    |
| Antithrombin-III                                                                | SERPINC1           | Antithrombin III                               | SL000272  | P01008    |
| Apolipoprotein E (isofrom E2)                                                     | APOE               | Apo E2                                          | SL000277  | P02649    |
| Apolipoprotein E (isofrom E3)                                                     | APOE               | Apo E3                                         | SL004668  | P02649    |
| Apolipoprotein E (isofrom E4)                                                     | APOE               | Apo E4                                         | SL004669  | P02649    |
| Apolipoprotein L1                                                                | APOL1              | Apo L1                                         | SL005699  | Q14791    |
| Beta-2-microglobulin                                                              | B2M                | b2-Microglobulin                                | SL000283  | P61769    |
| Beta-Ala-His dipeptidase                                                           | CNDP1              | CNDP1                                          | SL006694  | Q96KN2    |
| C3a anaphylatoxin                                                                | C3                 | C3a                                            | SL000313  | P01024    |
| C3a anaphylatoxin des Arginine                                                    | C3                 | C3adesArg                                      | SL003220  | P01024    |
| cAMP-dependent protein kinase catalytic subunit alpha                            | PRADA              | PRADA                                          | SL010513  | P17612    |
| Carbohydrate sulfotransferase 15                                                  | CHST15             | ST456                                          | SL007502  | Q7LFK5    |
| Carboxypeptidase B2                                                               | CPB2               | TAFI                                           | SL004015  | Q96IY4    |
| Cathepsin G                                                                       | CTSG               | Cathepsin G                                    | SL000345  | P08311    |
| Cathepsin H                                                                       | CTSH               | Cathepsin H                                    | SL000346  | P09668    |
| Cathepsin Z                                                                       | CTZ                | CATZ                                           | SL008830  | Q9UBR2    |
| Cation-independent mannose-6-phosphate receptor                                   | IGF2R              | IGF-II receptor                                | SL003679  | P11717    |
| C-C motif chemokine 18                                                            | CCL18              | PARC                                           | SL003323  | P55774    |
| C-C motif chemokine 23                                                            | CCL23              | MPIF-1                                         | SL003302  | P55773    |
| CD166 antigen | ALCAM | ALCAM | SL003166 | Q13740 |
|---------------|-------|-------|----------|--------|
| CD226 antigen | CD226 | CD226 | SL011100 | Q15762 |
| Cell adhesion molecule-related/down-regulated by oncogenes | CDON | CDON | SL014092 | Q4KMG0 |
| Chitinase-3-like protein 1 | CHI3L1 | YKL-40 | SL003340 | P36222 |
| CMRF35-like molecule 6 | CD300C | CLM6 | SL014270 | Q08708 |
| Coagulation factor IX | F9 | Coagulation Factor IX | SL000357 | P00740 |
| Coagulation factor IXab | F9 | Coagulation Factor IXab | SL004400 | P00740 |
| Coagulation Factor X | F10 | Coagulation Factor X | SL000360 | P00742 |
| Coagulation factor Xa | F10 | Coagulation Factor Xa | SL003324 | P00742 |
| Collectin-12 | COLEC12 | COLEC12 | SL007471 | Q5KU26 |
| Complement C1q subcomponent | C1QA C1QB C1QC | C1q | SL000309 | P02745, P02746, P02747 |
| Complement C1s subcomponent | C1S | C1s | SL000311 | P00871 |
| Complement C2 | C2 | C2 | SL002525 | P06681 |
| Complement C3 | C3 | C3 | SL000312 | P01024 |
| Complement C3b, inactivated | C3 | iC3b | SL000456 | P01024 |
| Complement C3d fragment | C3 | C3d | SL003362 | P01024 |
| Complement C4 | C4A C4B | C4 | SL000316 | P0C0L4, P0C0L5 |
| Complement C4b | C4A C4B | C4b | SL000318 | P0C0L4, P0C0L5 |
| Complement C5 | C5 | C5 | SL000319 | P01031 |
| Complement C5b-C6 complex | C5 C6 | C5b, 6 Complex | SL000321 | P01031, P13671 |
| Complement component C1q receptor | CD93 | C1QR1 | SL007696 | Q8NPY3 |
| Complement component C6 | C6 | C6 | SL000322 | P13671 |
| Complement component C7 | C7 | C7 | SL000323 | P10643 |
| Complement component C9 | C9 | C9 | SL000325 | P02748 |
| Complement decay-accelerating factor | CD55 | DAF | SL004556 | P08174 |
| Complement factor B | CFB | Factor B | SL000414 | P00751 |
| Complement factor D | CFD | Factor D | SL003327 | P00746 |
| Complement factor H | CFH | Factor H | SL000415 | P08603 |
| Complement factor I | CFI | Factor I | SL003328 | P05156 |
| Connective tissue-activating peptide III | PPBP | CTAP-III | SL004708 | P02775 |
| Contactin-1 | CNTN1 | contactin-1 | SL004855 | Q12860 |
| Contactin-4 | CNTN4 | Contactin-4 | SL010454 | Q8WIW2 |
| C-type mannose receptor 2 | MRC2 | MRC2 | SL08416 | Q9UBG0 |
| C-X-C motif chemokine 16 | CXCL16 | CXCL16, soluble | SL004016 | Q9HA27 |
| Cytoskeleton-associated protein 2 | CKAP2 | CKAP2 | SL006675 | Q8WWK9 |
| Desmocollin-2 | DSC2 | DSC2 | SL008631 | Q02487 |
| Desmoglein-2 | DSG2 | Desmoglein-2 | SL004857 | Q14126 |
| Dickkopf-related protein 3 | DKK3 | DKK3 | SL009412 | Q9UBP4 |
| Ectonucleoside triphosphate diphosphohydrolase 5 | ENTPD5 | ENTP5 | SL014028 | Q75356 |
| EGF-containing fibulin-like extracellular matrix protein 1 | EFEMP1 | FBLN3 | SL006527 | Q12805 |
| Endostatin | COL18A1 | Endostatin | SL000403 | P39060 |
| Gene Name                                      | Accession | Description                     |
|-----------------------------------------------|-----------|---------------------------------|
| Endothelial cell-selective adhesion molecule ESAM | ESAM      | SL005160 Q96AP7                 |
| Endothelin-converting enzyme 1 ECE1           | Endothelin-converting enzyme 1 SLO04060 P42892 |
| Ephrin-B                                      | EFNB1     | SLO08614 P98172                 |
| Epidermal growth factor receptor EGFR         | ERBB1     | SLO02644 P00533                 |
| Eukaryotic translation initiation factor 4 gamma 2 EIF4G2 | IF4G2 | SLO11211 P78344                 |
| Extracellular matrix protein 1 ECM1           | ECM1      | SLO06550 Q16610                 |
| Extracellular superoxide dismutase [Cu-Zn] SOD3 | SOD3      | SLO03672 P08294                 |
| Fatty acid-binding protein, heart FABP3       | FABP      | SLO01774 P05413                 |
| Ferritin                                      | FTH1 FTL  | SLO00420 P02794, P02792         |
| Fetuin-B                                      | FETUB     | SLO06777 Q9UGM5                 |
| Fibroblast growth factor receptor 1 FGFR1     | bFGF-R    | SLO03060 P11362                 |
| Fibroblast growth factor receptor 3 FGFR3     | FGFR-3    | SLO04063 P22607                 |
| Fibronectin                                   | FN1       | SLO00426 P02751                 |
| Fibronectin Fragment 3 FN1                    | FN1.3     | SLO10349 P02751                 |
| Fibronectin Fragment 4 FN1                    | FN1.4     | SLO10348 P02751                 |
| Follistatin-related protein 1 FSTL1           | FSTL1     | SLO09349 Q12841                 |
| Follistatin-related protein 3 FSTL3           | FSTL3     | SLO09324 Q05633                 |
| Fructose-bisphosphate aldolase A ALDOA        | aldolase A | SLO04910 P04075                 |
| Gelsolin                                      | GSN       | SLO05572 P06396                 |
| Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic GPD1 | GPDA | SLO07151 P21895                 |
| Granulins                                      | GRN       | SLO07173 P29299                 |
| Heat shock protein HSP 90-alpha/b HSP90AA1    | HSP90b    | SLO17612 P07900, P08238         |
| Heat shock protein HSP 90-beta HSP90AB1       | HSP90b    | SLO00454 P08238                 |
| Hemopexin                                     | HPX       | SLO00440 P02790                 |
| Heparin cofactor 2                            | SERPIND1  | Heparin cofactor II SLO04466 P05546 |
| Hepatocyte growth factor activator HGFAC      | HGF       | SLO06512 Q04756                 |
| Hepatocyte growth factor receptor MET         | Met       | SLO00134 P08581                 |
| Hepatocyte growth factor-like protein MST1    | MSP       | SLO05202 P26927                 |
| HERV-H LTR-associating protein 2 HHLA2        | HHLA2     | SLO20171 Q9UM44                 |
| Heterogeneous nuclear ribonucleoprotein A/B HNRNPAB | hnRNP A/B | SLO09791 Q99729                 |
| High affinity immunoglobulin gamma Fc receptor I FCR1A | FCR1 | SLO10461 P12314                 |
| Histidine triad nucleotide-binding protein 1 HINT1 | HINT1    | SLO09431 P49773                 |
| Histidine-rich glycoprotein HRG                | HRG       | SLO06448 P04196                 |
| Immunoglobulin G                              | IGHG1     | SLO00467 P01857                 |
| Immunoglobulin M                              | IGHM      | SLO00468 P01871                 |
| Inhibin beta A chain                          | INHBA     | SLO01938 P08476                 |
| Inhibin beta A chain:Inhibin beta B chain heterodimer INHBA INHB8 | Activin A | SLO04837 P08476, P09529         |
| Inorganic pyrophosphatase PPA1                | PPase     | SLO04914 Q15181                 |
| Insulin-like growth factor-binding protein 2 IGFBP2 | IGFBP-2 | SLO00466 P18065                 |
| Insulin-like growth factor-binding protein 3 IGFBP3 | IGFBP-3 | SLO00045 P17936                 |
| Insulin-like growth factor-binding protein 4 IGFBP4 | IGFBP-4 | SLO05171 P22692                 |
Insulin-like growth factor-binding protein 6
IGFBP6  IGFBP-6  SL005172  P24592
Inter-alpha-trypsin inhibitor heavy chain H4
ITIH4  ITI heavy chain H4  SL004739  Q14624
Intercellular adhesion molecule 1
ICAM1  siCAM-1  SL002922  P05362
Intercellular adhesion molecule 5
ICAM5  siCAM-5  SL005169  Q9UMF0
Interferon alpha/beta receptor 1
IFNAR1  IFN-a/b R1  SL004475  P17181
Interleukin-1 Receptor accessory protein
IL1RAP  IL-1 R AcP  SL004588  Q9NP8H
Interleukin-6 receptor subunit alpha
IL6R  IL-6 sRa  SL001943  P08887
Interleukin-6 receptor subunit beta
IL6ST  gp130, soluble  SL003872  P40189
Kallistatin
SERPINA4  Kallistatin  SL004876  P29622
Kininogen-1
KNG1  Kininogen, HMW  SL017189  P01042
Lactadherin
MFGE8  MFGM  SL006523  P05362
Leucine-rich repeat transmembrane protein FLRT3
FLRT3  FLRT  SL008372  Q9UMF0
Leukocyte immunoglobulin-like receptor subfamily B member 1
LILRB1  ILT-2  SL005190  Q8NH6L
Leukocyte immunoglobulin-like receptor subfamily B member 2
LILRB2  ILT-4  SL005191  Q8N423
Leukotriene A-4 hydrolase
LTA4H  LKHA4  SL007100  P09960
Lipopolysaccharide-binding protein
LBP  LBP  SL003309  P18428
Lactostathine-1-alpha
REG1A  PSP  SL005357  P05451
L-lactate dehydrogenase B chain
LDHB  LDH-H  SL000493  P07195
Low affinity immunoglobulin gamma Fc region receptor III-B
FCG3B  FCG3  SL008609  Q75015
Low-density lipoprotein receptor-related protein 1B
LRP1B  LRP1B  SL003872  Q9N2R2
L-Selectin
SELL  sL-Selectin  SL002823  P14451
Lumican
LUM  Lumican  SL006230  P51884
Lymphocyte antigen 86
LY86  LY86  SL007059  Q57111
Macrophage colony-stimulating factor 1 receptor
CSF1R  M-CSF R  SL004153  P07333
Macrophage mannose receptor 1
MRC1  Macrophage mannose receptor  SL004579  P22897
Macrophage-capping protein
CAPG  CAPG  SL008099  P40121
Metalloproteinase inhibitor 2
TIMP2  TIMP-2  SL005092  P16035
Myoglobin
MB  Myoglobin  SL000164  P02144
Neurexin-3-beta
NRXN3  NRX3B  SL008728  Q9HD8S
Neurogenic locus notch homolog protein 1
NOTCH1  Notch 1  SL005703  P46531
Neurogenic locus notch homolog protein 3
NOTCH3  Notch-3  SL005209  Q9UM47
Neuropilin-1
NRP1  NRP1  SL005493  P22392
Nucleoside diphosphate kinase B
NME2  NDP kinase B  SL004921  P01042
Phosphoglycerate kinase 1
PGK1  phosphoglycerate kinase 1  SL003653  P00558
Pigment epithelium-derived factor
SERPINF1  PEDF  SL003666  P36955
Plasma kallikrein
KLKB1  Prekallikrein  SL000545  P03952
Plasma protease C1 inhibitor
SERPING1  CI-Esterase Inhibitor  SL000308  P0155
Plasma serine protease inhibitor
SERPINA5  PCI  SL000550  P05154
Plexin-B2
PLXNB2  PLX82  SL009948  O15031
Polymeric immunoglobulin receptor
PIGR  PIGR  SL005797  P01833
| Protein Name                                      | Gene Symbol | NCBI Reference | UniProt Reference |
|--------------------------------------------------|-------------|----------------|-------------------|
| Pro-opiomelanocortin                             | POMC        | SL009210       | P01189            |
| Properdin                                        | CFP         | SL003192       | P27918            |
| Proprotein convertase subtilisin/kexin type 9    | PCSK9       | SL012707       | Q8NB7P3           |
| Protein kinase C alpha type                      | PRKCA       | SL000551       | P17252            |
| Protein Rev_HV2BE                                |             |                |                   |
| Prothrombin                                      | F2          | SL000558       | P00734            |
| Ras GTase-activating protein 1                   | RASA1       | SL013754       | P20936            |
| Ras-related C3 botulinum toxin substrate 1       | RAC1        | SL004009       | P63000            |
| Repulsive guidance molecule A                    | RTN4        | SL008309       | Q9NQG3            |
| Reticulon-4                                      | RTN4R       | SL005208       | Q9BZR6            |
| Retinoid acid receptor responder protein 2       | RARRES2     | SL010445       |                   |
| RGM domain family member B                       | RGM8        | SL010467       |                   |
| Scavenger receptor class F member 1              | SCARF1      | SL000551       | Q14162            |
| Secreted and transmembrane protein 1             | SECTM1      | SL000558       | Q8WVN6            |
| Serine/threonine-protein kinase WNK3              | WNK3        | SL010524       | Q9BYP7            |
| Serotransferrin                                  | TF          | SL000601       | P02787            |
| Serum albumin                                    | ALB         | SL000254       | P02768            |
| Serum amyloid P-component                        | SAP         | SL000573       | P02743            |
| Sex hormone-binding globulin                     | SHBG        | SL005102       | P04278            |
| Sialic acid-binding Ig-like lectin 14            | SIGLEC14    | SL014292       | Q08ET2            |
| Sialic acid-binding Ig-like lectin 7             | SIGLEC7     | SL005218       | Q9Y286            |
| Signal transducer and activator of transcription 3| STAT3       | SL007221       | P40763            |
| SPARC                                            | SPARC       | SL000532       | P09486            |
| Stanniocalcin-1                                  | STC1        | SL005789       | P52823            |
| Tartrate-resistant acid phosphatase type 5       | ACPS        | SL004118       | P13686            |
| Testican-2                                       | SPOCK2      | SL010471       | Q92563            |
| Thrombospondin-4                                 | THBS4       | SL007207       | P35443            |
| Thyroxine-binding globulin                       | SERPIN7     | SL000590       | P05543            |
| Tissue factor pathway inhibitor                   | TFPI        | SL001998       | P10646            |
| Transforming growth factor beta receptor type 3   | TGFBR3      | SL005059       | Q31673            |
| Transforming growth factor-beta-induced protein Ig-h3| TGFBI     | SL006544       | Q15582            |
| Transgelin-2                                     | TAGLN2      | SL004811       | P37802            |
| Tropomyosin alpha-4 chain                        | TPM4        | SL003646       | P67936            |
| Tumor necrosis factor receptor superfamily member 10B| TNFRSF10B  | SL004157       | Q14763            |
| Tumor necrosis factor receptor superfamily member 1A| TNFRSF1A   | SL001992       | P19438            |
| Tumor necrosis factor receptor superfamily member 1B| TNFRSF1B   | SL001800       | P20333            |
| Tumor necrosis factor receptor superfamily member 21| TNFRSF21   | SL004871       | Q75509            |
| Tumor necrosis factor receptor superfamily member 25| TNFRSF25   | SL004791       | Q93038            |
| Urokinase plasminogen activator surface receptor  | PLAUR       | SL002506       | Q03405            |
| Protein Name                                                                 | Abbreviation | Protein ID | Accession Number |
|------------------------------------------------------------------------------|--------------|------------|------------------|
| Vascular endothelial growth factor A, isoform 121                            | VEGFA        | VEGF121    | SL003310         |
| Vascular endothelial growth factor D                                         | FIGF         | VEGF-D     | SL003320         |
| Vitamin K-dependent protein C                                                | PROC         | Protein C  | SL000048         |
| Vitamin K-dependent protein S                                                | PROS1        | Protein S  | SL000049         |
| Vitronectin                                                                  | VTN          | Vitronectin | SL000616         |
| von Willebrand factor                                                        | VWF          | vWF        | SL000017         |
| Wnt inhibitory factor 1                                                      | WIF1         | WIF-1      | SL004652         |
| WNT1-inducible-signaling pathway protein 1                                   | WISP1        | WISP-1     | SL004689         |

Names of the 207 proteins that were detected with SOMAscan 1.3K platform, in at least 80% of 30 PEx samples used for assessment of pathological relevance of proteins detected in PEx samples, as described in Material and Methods.
Table S2. Result from differential abundance analysis

| Target Full Name                                      | Entrez Gene Symbol | A-hLCI vs. NA | A-hLCI vs. A-nLCI | A-hLCI vs. A-nLCI + NA |
|-------------------------------------------------------|--------------------|--------------|-------------------|------------------------|
|                                                       |                    | p-value      | q-value           | Fold change            | p-value      | q-value           | Fold change            | p-value      | q-value           | Fold change            |
| Vascular endothelial growth factor D                  | FIGF               | 0.0003       | 0.069            | 1.184                | n.s                    | n.s           | 1.099                |                  | 0.001       | 0.038            | 1.147                |
| Alpha-1-antitrypsin                                   | SERPINA1           | 0.0008       | 0.083            | 1.182                | 0.002                   | n.s           | 1.283                | 0.001       | 0.038            | 1.202                |
| Complement component C1q receptor                     | CD93               | 0.0017       | 0.120            | 1.320                | 0.017                   | n.s           | 1.291                | 0.001       | 0.038            | 1.286                |
| C-C motif chemokine 18                                | CCL18              | 0.0034       | 0.152            | 1.917                | n.s                    | n.s           | 1.457                | 0.021       | 0.167            | 1.718                |
| Fructose-bisphosphate aldolase A                      | ALDOA              | 0.0037       | 0.152            | 0.631                | 0.018                   | n.s           | 0.615                | 0.002       | 0.040            | 0.650                |
| Complement C4                                         | C4A, C4B          | 0.0052       | 0.170            | 0.633                | n.s.                   | n.s           | 0.704                | 0.002       | 0.043            | 0.660                |
| Coagulation Factor X                                  | F10                | 0.0068       | 0.170            | 1.305                | 0.038                   | n.s           | 1.265                | 0.007       | 0.092            | 1.277                |
| Immunoglobulin M                                      | IGHM               | 0.0070       | 0.170            | 1.216                | 0.007                   | n.s           | 1.303                | 0.001       | 0.038            | 1.247                |
| Interleukin-1 Receptor accessory protein              | IL1RAP             | 0.0074       | 0.170            | 1.260                | 0.020                   | n.s           | 1.292                | 0.001       | 0.038            | 1.275                |

Statistics for proteins found to be differentially abundant when comparing SOMAscan data for 207 proteins between the Asthma with high LCI (A-hLCI, n=10) and Non-asthma control group (NA, n=10), using a significance criteria including p-value below 0.05 and Benjamini–Hochberg corrected p-value (q) below 0.2, as described in Material and Methods. Statistics for comparison with asthma with normal LCI (A-nLCI) is also shown. Significance values above the threshold are indicated with n.s. Fold change was defined as 2 to the power of the difference difference being defined as the difference of the mean log2 of "group1" data and mean log2 of "group2" data. Fold change values >1 and < 1 indicate higher and lower levels in the first mentioned group, respectively. No significant difference were found for the A-nLCI vs. NA group comparison. Uniprot protein IDs and synonyms can be found in Table S1A.
Table S3. Result from manual reviewing the scientific literature on proteins found to be differentially abundant in A-hLCI as compared to NA group.

| Protein name | Abundance profile | Relevant literature findings, summary and reference |
|--------------|------------------|---------------------------------------------------|
| Alpha-1-antitrypsin | NA L A-nLCI L A-hLCI H | - Found to be the major inhibitor of neutrophil elastase in the lower respiratory system [1, 2].  
- Associated with an increased risk of pulmonary and extrapulmonary disease [3].  
- Protects alveoli against the destructive effects of neutrophil elastase, proteinase 3 and cathepsin G [4].  
- Increased levels in circulation is associated with pathology localised to the Small airway and alveolar space [5]. |
| Interleukin-1 receptor accessory protein | NA L A-nLCI L A-hLCI H | - Localized to the cytosol & vesicles. Involved in e.g. immune and inflammatory response, innate immune response, IL-1 and IL-33-mediated signalling (GO annotations) [6, 7].  
- Soluble isoform exist (sIL1RAP) [8].  
- Elevated levels is associated with reduced FEV1/FVC. Suggested to be a marker and potential therapeutic target in patients with neutrophilic asthma and airflow obstruction [9].  
- Elevated in patients with COPD [10].  
- Is required for IL-33 signalling which is important in promoting and maintaining the asthma phenotype [11]. |
| C-C motif chemokine 18 | NA L A-nLCI IM A-hLCI H | - mRNA particularly abundant in lung (Protein Atlas) [12, 13]  
- Levels in serum, BALF and Alveolar macrophage culture supernatant are markedly increased in various interstitial lung diseases [14].  
- Suggested to play a predominant role in allergic asthma [15, 16].  
- Suggested to serve as a circulating biomarker in non-small cell lung cancer diagnosis [17]. |
| Complement component C1q receptor | NA L A-nLCI IM A-hLCI H | - mRNA and protein particularly abundant in lung (Protein Atlas) [12, 13].  
- Involved in e.g. macrophage activation and neutrophil degranulation (GO annotations) [6, 7].  
- Have been observed to be expressed at high levels in lung and at elevated levels in circulation of asthmatics.  
- Have been suggested as a circulating biomarker with potential to aid in asthma diagnosis [18, 19]. |
| Gene Name                              | Accession Number | Description                                                                 |
|----------------------------------------|------------------|-----------------------------------------------------------------------------|
| IgM                                    | P01871           | Involved in innate immune response & complement activation, classical pathway (GO annotations) [6, 7]. Play role in host defence, immune regulation and immunological tolerance. Locally **synthesized in airways**. Elevated levels have been observed in RTLF from **asthmatic** subjects. Suggested to play role in pathogenesis of **asthma**. [20, 21]. |
| Vascular endothelial growth factor D   | O43915           | Localized to the extracellular space, platelet alpha granule lumen. Involved in platelet degranulation (GO annotations) [6, 7]. Contributes to the **small-airway** remodelling in a rat model of COPD [22]. Increased levels of vascular endothelial growth factor in induced sputum of asthmatics. Suggested to play important role in the pathogenesis of bronchial **asthma** [23]. |
| Coagulation Factor X                   | P00742           | Uncontrolled activation of the coagulation cascade contributes to the pathophysiology of several conditions, including acute and chronic lung diseases. Protease-activated receptors have been implicated as the molecular link between coagulation and allergic inflammation in **asthma** [24]. In contradiction with our finding showing lower levels of FX in PEx from asthmatic subjects as compared healthy controls, some studies report increased levels of FX in BALF. However, other studies report similar result with significantly decreased levels in airways in **severe asthma** and intermediate levels in **moderate asthma**, as compared to healthy controls [25]. |
| Fructose-bisphosphate aldolase A       | P04075           | Localized to the extracellular region, platelet alpha granule lumen, extracellular exosome. Involved in platelet degranulation, neutrophil degranulation (GO annotations) [6, 7]. Patients with asthma have high presence of nitrotyrosine in both the airways and the lung parenchyma [25]. Has been observed to undergo post-translational regulation by protein tyrosine nitration in mast cells. Suggested to be an important pathway that regulates mast cell phenotype and function [26-28]. Nitrotyrosine formation in airway epithelial and inflammatory cells is elevated in asthma and COPD [37]. The ability to detect post-translational modifications is an important advantage of aptamers as tools for identification and detection of biomarkers, as exemplified by Ray. P et al [29]. The lower levels of ALDOA in A-hLCI group may be explained by tyrosine nitration of ALDOA proteins in mast cells in the A-hLCI group, resulting in weaker binding of the SOMAscan aptamer to the nitrated isoform of ALDOA, giving rise to weaker signal in A-hLCI group. |
| Complement C4                          | P0COL4           | Localized to extracellular region, blood microparticle. Involved in innate immune response, inflammatory response, complement activation (GO annotations) [6, 7]. Pulmonary **alveolar type II epithelial cells** synthesize and secrete complement proteins C2, C3, C4, C5, and Factor B. Studies have demonstrated that complement may serve as a key link between innate and adaptive immunity in a **variety of pulmonary conditions** [30]. |
Footnote: Abundance profile indicate the level of the protein in PEx in one group in relation to the other groups; Non-Asthma (NA), Asthma with normal LCI (A-nLCI), Asthma with high LCI (A-hLCI), higher level (H), intermediate level (IM), lower level (L). Reference in brackets refer to references below and not to references in the main article.

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Table S4. Demographic and clinical characteristics of groups based on smoking history

| Parameter                          | Nevers smokers | Ex-smokers |
|-----------------------------------|----------------|------------|
| Number                            | 16             | 14         |
| Asthma y/n                        | 7/9            | 11/3       |
| Packyear                          |                | 3.36 (1.29) [0-12] |
| Gender (Male/Female)              | 8/8            | 8/6        |
| Age                               | 40.1 (3.38) [20-63] | 52.4 (3.5) [28-68] |
| Age at onset of asthma, yrs       | -              | 17.4 (5.16) [5-55] |
| BMI                               | 24.9 (0.73) [19.3-31.2] | 24.7 (0.7) [21.2-29.1] |
| Allergy y/n                       | 11/5           | 8/6        |
| FEV1 (% pred)                     | 91.6 (3.05) [77-117] | 82.6 (6.13) [39-123] |
| FEV1/FVC (%)                      | 74.3 (4.84) [7.8-93.8] | 69.2 (3.74) [35-88] |
| Reversibility (%)                 | 8.1 (2.08) [-5-24] | 9.7 (2.24) [2-28] |
| ACQ, mean (1-6) >1 (y/n)          | 3/4            | 5/5        |
| B-neutrophils (%)                 | 3.26 (0.27) [1.5-5.4] | 4.37 (0.4) [2.2-7.3] |
| B-eosinophils (%)                 | 0.22 (0.04) [0.06-0.6] | 0.29 (0.05) [0.1-0.6] |
| FENO, ppb                         | 39.64 (7.13) [6-113] | 30 (7.7) [10-103] |
| hsCRP (n=29)                      | 0.81 (0.26) [0.14-4.2] | 1.47 (0.48) [0.14-5.4] (n=13) |
| Average mass pg/particle          | 0.21 (0.01) [0.18-0.29] | 0.23 (0.01) [0.17-0.3] |

Data are presented as means with standard error given in parenthesis and range given in brackets. Incomplete data is indicated by n-number given in parenthesis. Dash (-) indicate "not applicable"