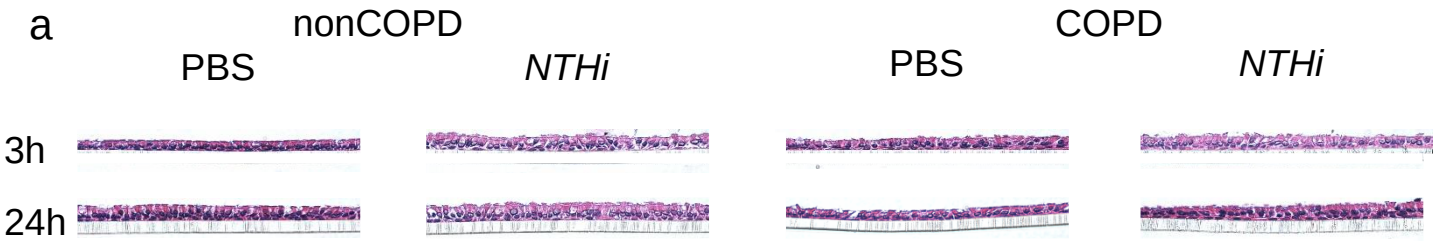
Supplementary Figure S1

There are no differences in characteristics between nonCOPD and COPD epithelial cells

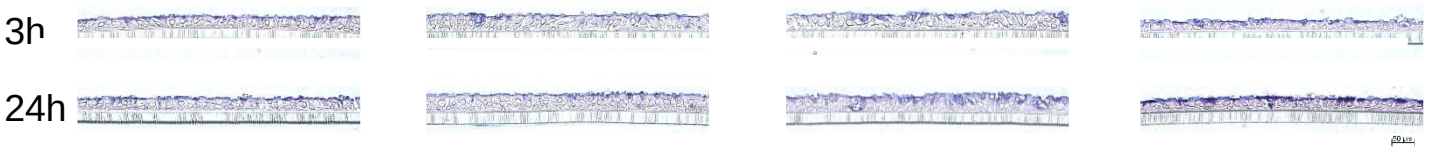
Representative images of (a) H&E staining, (b) Alcian Blue/PAS staining and (c) E-Cadherin IF staining of representative transwell cultures from nonCOPD and COPD SAEC exposed to PBS or *NTHi* for 3h or 24h. (d) Transepithelial electrical resistance (TEER) values across SAEC cells exposed to PBS or *NTHi* presented as a mean (n=9). Differences calculated with one-way ANOVA followed by Tukey's multiple comparison test. (e) mRNA expression of *TJP, SCGB1A1, VIM* in nonCOPD and COPD SAEC exposed to PBS of *NTHi* for 3h or 24h (each n=6). Relative gene expression, expressed as crossing point change (ΔCp = Cp for *GAPDH* - Cp for gene of interest) is presented as a mean. Differences calculated with one-way ANOVA followed by Tukey's multiple comparison test.

Figure S1

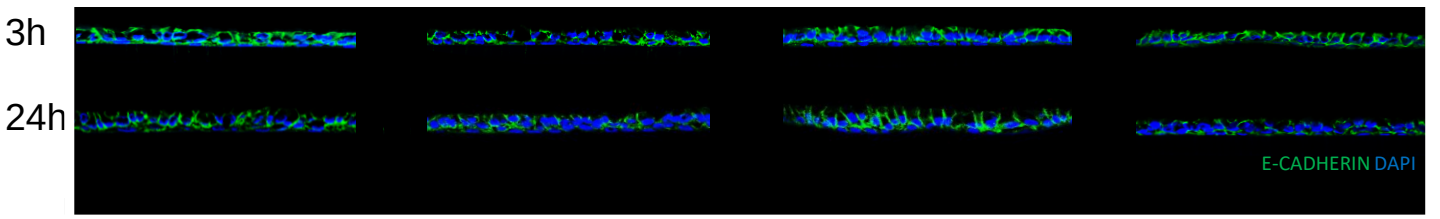
a



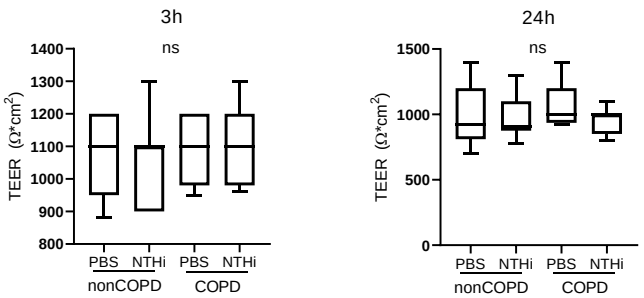
b



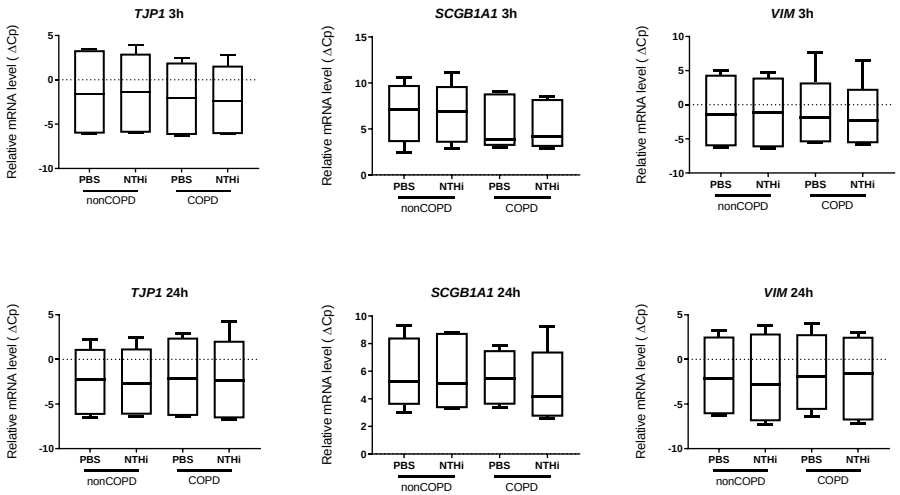
c



d



e



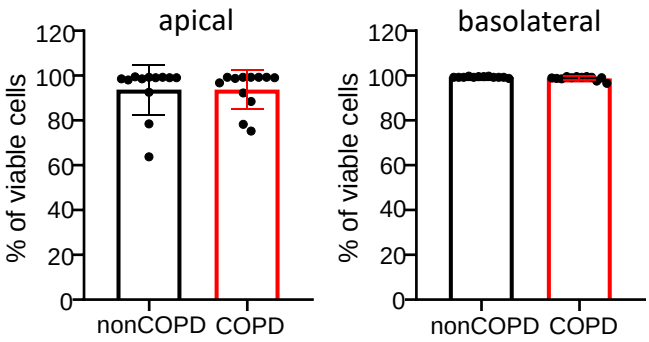
Supplementary Figure S2

There are no differences in viability and surface marker expression between macrophages exposed to nonCOPD and COPD epithelial cells supernatants

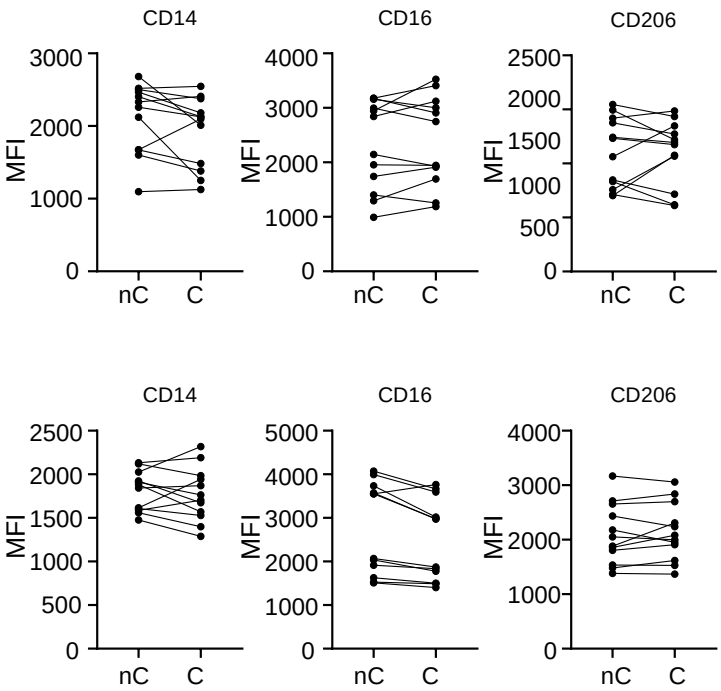
(a) Viability of MDMs exposed to apical (left) and basolateral (right) SN from nonCOPD and COPD SAEC (each n=12) measured by Flow Cytometry. (b) Expression of surface markers (CD14, CD16, CD206) depicted as Mean Fluorescence Intensity (MFI, geometric mean) on MDMs (2 donors) exposed for 24h to apical (top) and basolateral (bottom) SN from nonCOPD (nC; n=6) and COPD (C, n=6) SAEC (each MDM donor + SAEC donor represents a unique pair) measured by Flow Cytometry.

Figure S2

a


apical
basolateral
% of viable cells
120
100
80
60
40
20
0
nonCOPD
COPD

b

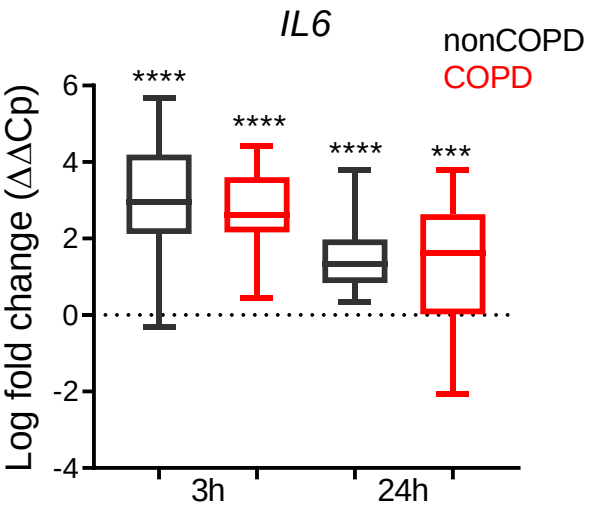

CD14
CD16
CD206
MFI
nC
C

Supplementary Figure S3

NTHi exposure increases expression of _IL6_ in SAECs

IL6 mRNA expression in nonCOPD- (black) and COPD-derived (red) SAEC exposed to *NTHi* for 3h or 24h relative to PBS-exposed SAEC. Gene expression comparative crossing point change (ΔΔCp) is presented as a mean (n=11-15). Impact of *NTHi* to corresponding PBS-exposed SAEC was calculated with paired t-test; ***P*<0.01, ****P<0.0001.

Figure S3



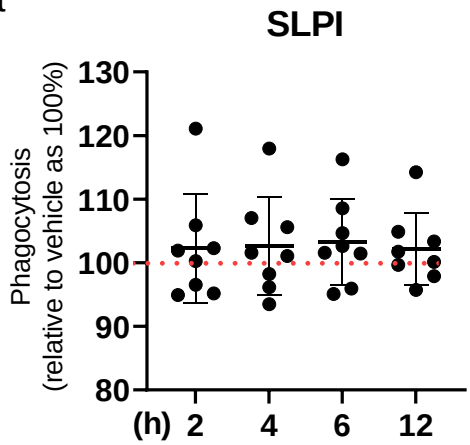
Supplementary Figure S4

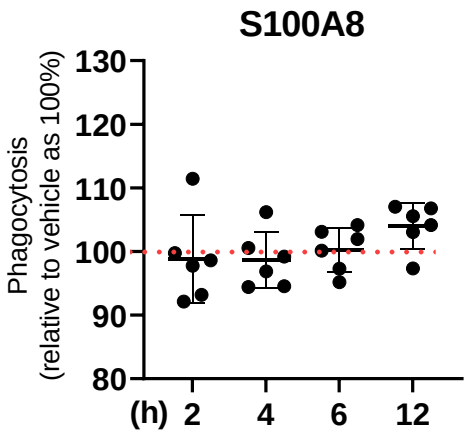
SLPI, S100A8 and S100A9 antimicrobial peptides do not influence phagocytosis in macrophages

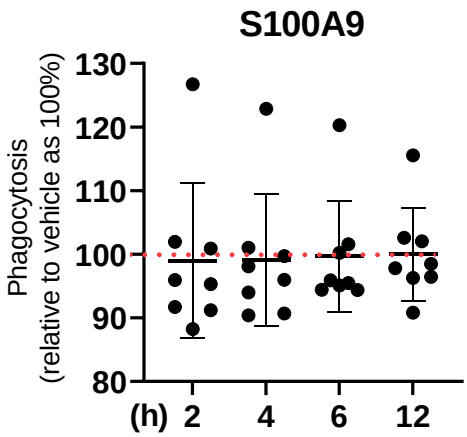
(a) Influence of recombinant AMPs: SLPI, S100A8 and S100A9 on phagocytic capacity of MDMs. Cells were pre-treated with indicated proteins or vehicle and progression of phagocytosis of the pHrodo® *E.coli* particles was monitored with IncuCyte S3 Live-Cell Analysis System. For each time point, data is presented as a % of phagocytosis to respective vehicle control (100%, dotted line) (SLPI n=8, S100A8 n=6, S100A9 n=8). (b) Viability of MDMs exposed to S100A8, S100A9, S100A8/A9, SLPI and BD2 (depicted as a % of vehicle control) measured by WST-1 assay, shown as a mean (n=8=13). Impact of the AMPs calculated with paired t-test, **P*<0.05.

Figure S4

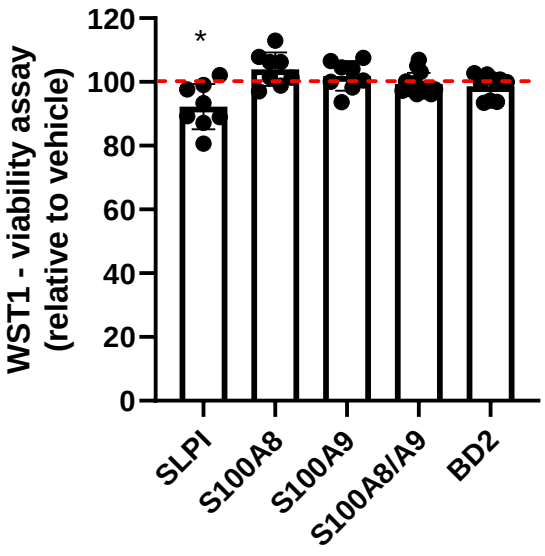
a


SLPI
Phagocytosis
(relative to vehicle as 100%)
130
120
110
100
90
80
(h) 2
4
6
12


S100A8
Phagocytosis
(relative to vehicle as 100%)
130
120
110
100
90
80
(h) 2
4
6
12


S100A9
Phagocytosis
(relative to vehicle as 100%)
130
120
110
100
90
80
(h) 2
4
6
12

b

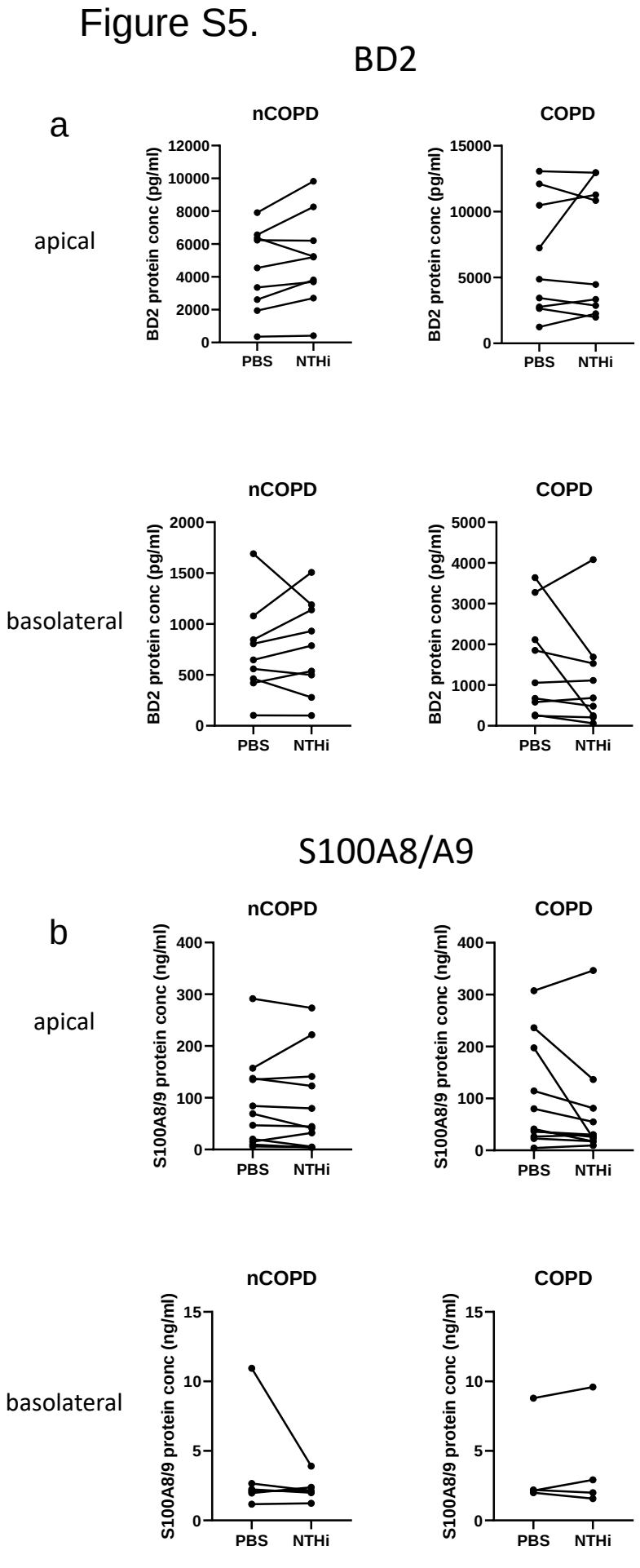

WST1 - viability assay
(relative to vehicle)
120
100
80
60
40
20
0
*
SLPI
S100A8
S100A9
S100A8/A9
BD2

Supplementary Figure S5

S100A8/A9 and BD2 proteins are differently regulated by *NTHi* in nonCOPD and COPD SAEC secretions

(a) BD2 and (b) S100A8/A9 protein expression in apical and basolateral SN from nonCOPD (left) and COPD (right) SAECs exposed to PBS or *NTHi* for 3h, measured by ELISA.

Figure S5.


BD2
a
apical
nCOPD
COPD
BD2 protein conc (pg/ml)
12000
10000
8000
6000
4000
2000
0
15000
5000
PBS
NTHi
basolateral
2000
1500
1000
500
5000
4000
3000
S100A8/A9
b
S100A8/9 protein conc (ng/ml)
400
300
200
100
15
10

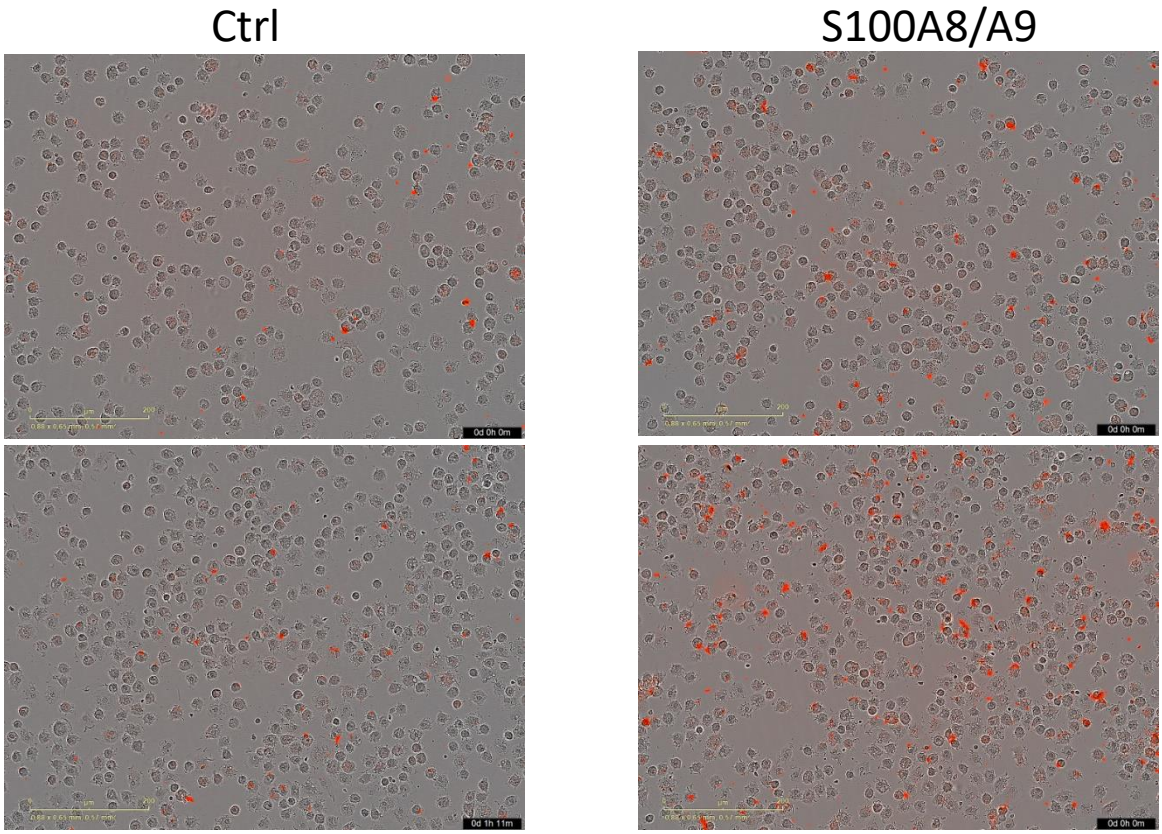
Supplementary Figure S6

S100A8/A9 recombinant protein binds to macrophages

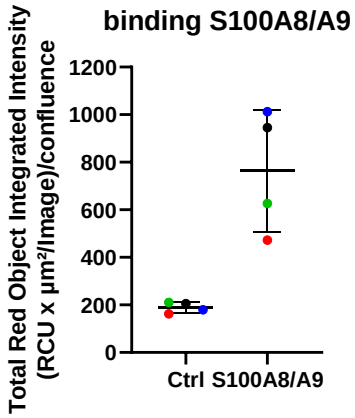
Binding of RPE-labelled control protein (soluble Thy1) and S100A8/A9 to MDMs: (a) pictures visualizing binding. (b) Quantification of the RPE signal relative to cell density in cells treated with control protein and S100A8/A9.

Figure S6

a


Ctrl
S100A8/A9

b


binding S100A8/A9
Total Red Object Integrated Intensity (RCU x µm²/Image)/confluence
1200
1000
800
600
400
200
0
Ctrl
S100A8/A9

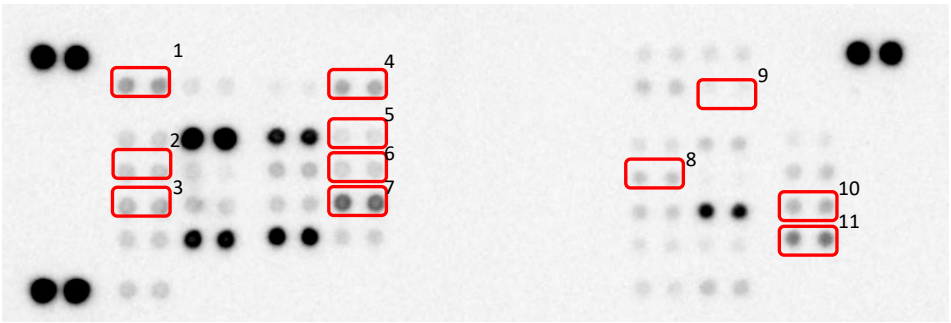
Supplementary Figure S7

S100A8/A9 induces phosphorylation of different molecules in macrophages

Representative blot showing MDM phosphorylation status of signalling molecules upon 30 mins exposure to PBS (upper blot) or S100A8/A9 (lower blot). Affected signalling molecules marked.

Figure S7

PBS



1. CREB
2. JNK1/2/3
3. P38a
4. ERK1/2
5. HSP27
6. MSK1/2
7. Src
8. P70 S6 Kinase
9. C-Jun
10. RSK1/2/3
11. STAT3

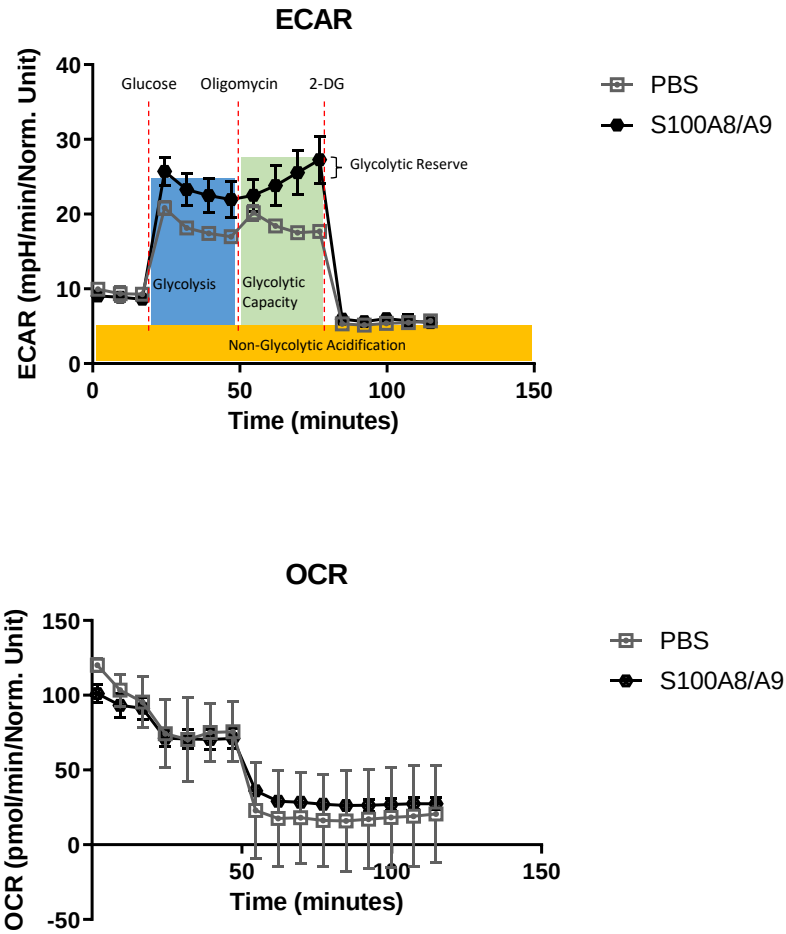
S100A8/A9

Supplementary Figure S8

S100A8/A9 impacts glycolysis in macrophages

Representative the Extracellular Acidification Rate (ECAR) profile plot (upper panel) and the Oxygen Consumption Rate (OCR; lower panel) upon PBS or S100A8/A9 treatment.

Figure S8


ECAR
ECAR (mpH/min/Norm. Unit)
Glucose
Oligomycin
2-DG
Glycolytic Reserve
Glycolysis
Glycolytic Capacity
Non-Glycolytic Acidification
Time (minutes)
PBS
S100A8/A9
OCR
OCR (pmol/min/Norm. Unit)
Time (minutes)
PBS
S100A8/A9

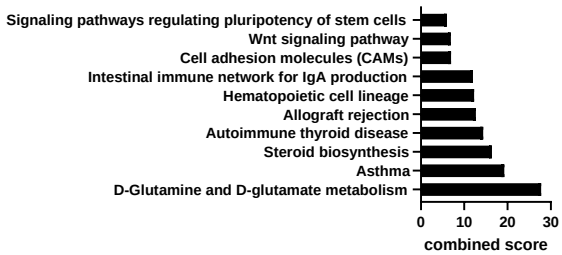
Supplementary Figure S9

WNT signalling pathway is one of the top deregulated pathways in COPD epithelium

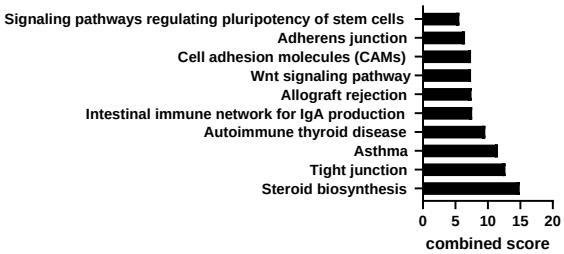
(a) *Kyoto Encyclopedia of Genes and Genomes (*KEGG) analysis of most deregulated pathways in COPD compared to nonCOPD small airway epithelial cells (GSE19407 dataset). (b) Promoter analysis of S100A8 and S100A9 for the TCF4 and LEF1 (β-catenin binding sites).

Figure S9

a



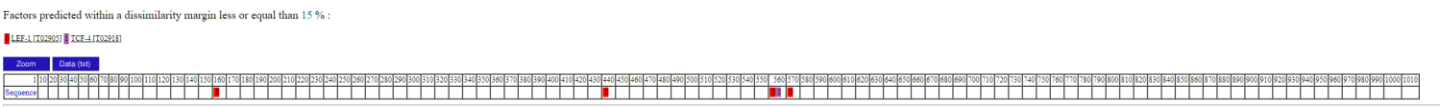
COPD vs non smokers



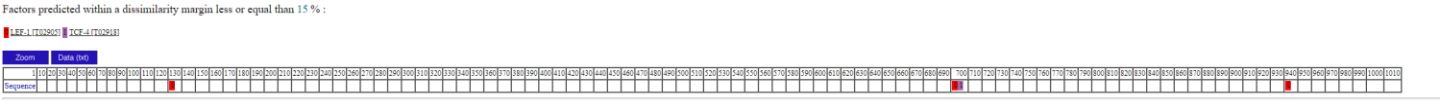
COPD vs smokers

b

S100A8
LEF1 – 4
TCF4 - 1



S100A9
LEF1 – 3
TCF4 - 1



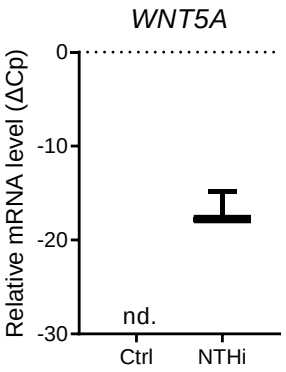
Supplementary Figure S10

NTHi modulates WNT pathway in macrophages and epithelial cells

(a) mRNA expression of WNT5A in MDMs treated with PBS or exposed to *NTHi* for 24h (n=2). (b) mRNA expression of AXIN2 in nonCOPD and COPD SAEC exposed to *NTHi* for 3h, relative to PBS-exposed SAEC. Gene expression comparative ΔΔCp is presented as a mean (n=7-10). Impact of *NTHi* calculated with paired t-test.

Figure S10

a



b