ONLINE SUPPLEMENT

Metabolic re-patterning in chronic obstructive pulmonary disease airway smooth muscle cells

Charalambos Michaeloudes, Chih-Hsi Kuo, Gulam Haji, Donna K Finch, Andrew J Halayko,
Paul Kirkham, Kian Fan Chung and Ian M Adcock on behalf of COPDMAP

MATERIALS AND METHODS

Reagents

2-deoxy-D-glucose was purchased from Sigma Aldrich (Dorset, UK) and buthionine sulfoximine (BSO) from Cayman Chemicals (Ann Arbor, MI, USA).

ASMC isolation and culture

ASMCs were isolated from biopsies or resected airways of healthy non-smoker and healthy smoker subjects, and patients with COPD (Table 1).

The bronchoscopic procedures were performed under a research protocol approved by the local Ethics Committee and all participating subjects gave informed signed consent. The healthy subjects volunteered to take part in the study and were reimbursed for their participation. The amount of reimbursement for their travel and time was agreed with the Ethics Committee that approved the study. Biopsies were divided into small pieces of less than 1mm^2 and then transferred to empty 6-well tissue culture plates pre-coated with cell culture medium containing 10% fetal bovine serum (FBS). The tissue pieces were incubated in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 4mM L-glutamine, 100U/ml of penicillin, 100µg/ml streptomycin, 2.5µg/ml amphotericin B and 10% FBS, at 37^{0}C, 5% CO_2 and humidified atmosphere, to allow attachment and growth of ASMCs.

ASMCs were also isolated from macroscopically healthy sections of main bronchi resected from surgical patients as described previously [1-3]. Specimens were collected from subjects who provided informed consent in accordance with protocols approved by the Human Research Ethics Board of the University of Manitoba, Winnipeg, Canada. Briefly, primary human ASMCs were isolated from macroscopically healthy segments of second- to -fourth generation bronchi obtained after lung resection surgery from patients with a diagnosis of adenocarcinoma. Cells were plated on uncoated plastic dishes in DMEM supplemented with 50U/ml streptomycin, 50μg/ml penicillin, and 10% FBS.

ASMC phenotype was confirmed by observing the characteristic "hill and valley" morphology under a light microscope. Cells between passages 3 and 6 were used for experimentation. Prior to treatment, cells were serum-starved in medium containing phenol-free DMEM supplemented with 1mM sodium pyruvate, 4mM L-glutamine, 1:100 non-essential amino acids, 0.1% bovine serum albumin (BSA) and antibiotics as described above.

Untargeted metabolomics analysis

ASMCs were seeded in 75cm^2 flasks and at approximately 80% confluence were serum-starved overnight. After treatment cells were detached by trypsinisation and pelleted by centrifugation at 1500 rpm for 5 mins. Cells were re-suspended in FBS-containing medium and counted. 2x10^6 cells were transferred to cryovials, pelleted and snap-frozen on dry ice. Samples were stored at -80°C until processed. Sample preparation and analysis using ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) and gas chromatography-MS (GC-MS) was performed by Metabolon Inc. (Durham NC, USA) as described previously[4;5]. Using both UHPLC-MS and GC-MS enabled detection of a greater range of metabolites.

Determination of differentially-expressed metabolites

Data pre-processing and normalization was performed by Metabolon Inc. Data are presented as "scaled intensity" which represents the concentration of a specific metabolite normalised to the total protein concentration of the lysate, determined by Bradford assay, and then scaled so that the median for the specific metabolite across all sample groups is equal to one. Missing values were imputed with the minimum observed value. Differential expression analysis was performed by fitting a linear model of the scaled intensity with phenotype and batch/technical effect as covariates, using the Bioconductor R package limma. Differentially-expressed metabolites were filtered by nominal p-value <0.05 and 50% increase in the scaled intensity.

Supervised learning algorithm for phenotype classification

The nearest shrunken centroid method was used as a machine learning algorithm to further define the optimal number of metabolites that best differentiated the phenotypes, as previously described[6]. Using this method many differentially-expressed metabolites are eliminated from phenotype prediction leaving a sparse model, a set of surviving differentially-expressed metabolites, which is representative of each phenotype. Briefly, the mean (centroid) of each metabolite within each group as well as across all groups was calculated. Standardization of the centroids of each group was performed through dividing the difference of the group centroids and overall centroids by the within-group standard deviation of each metabolite. This standardized value was treated as an absolute value which was subsequently shrunken by an amount Δ (threshold value). If the value of a given standardized centroid was shrunken to zero by Δ for all groups, then this metabolite did not contribute to the nearest-centroid classification. Otherwise, a non-zero value of a standardized centroid after shrinkage was retained as a centroid classifier for the given group. The inherent property of this method is that metabolites will be eliminated from group prediction as Δ increases. Given that each amount of Δ shrunken will result in a set of surviving centroids for each group, the amount of shrinkage is chosen by iterative cross-validation on the performance of which each set of surviving centroids correctly predicts the group classification of each sample. This analysis was carried out using an algorithm available as free R software for prediction analysis of microarrays in The Comprehensive R

Archive Networks (CRAN-pamr package). The identified sparse model was further adjusted for sex and age using the R package Surrogate Variable Analysis in Bioconductor where principle component analysis (PCA) was later applied to inspect the phenotype boundaries along the first and second principal component coordinates.

Pathway analysis

The Pathway Activity Profiling (PAPi) algorithm was used to quantify the activity of metabolic pathways using the KEGG database. Briefly, all KEGG pathways to which metabolites from the database were mapped to were selected. Each pathway was then summarised as an activity score for each sample, based on the abundance of the metabolites that link to it, thus enabling conventional statistical tests between the groups. The algorithm is available as a Bioconductor R package and described in detail elsewhere[7].

Determination of mitochondrial ROS levels

Mitochondrial ROS levels were determined using the mitochondrial-targeted, redox-sensitive fluorescent probe MitoSOX Red (Invitrogen, Paisley, UK). Following treatment, ASMCs were washed with Hank's Balanced Salt Solution (HBSS) containing calcium and magnesium (Sigma-Aldrich, Dorset, UK) and incubated for 30 mins with a 5µM MitoSOX Red solution prepared in the same buffer. ASMCs were then washed using HBSS, detached using Accutase (Sigma-Aldrich) and pelleted by centrifugation at 1500 rpm for 5mins. Cells pellets were re-suspended in HBSS and the median fluorescence intensity (MFI) was determined by flow cytometry.

Measurement of mRNA expression

Total RNA was isolated using an RNeasy Mini Kit (Qiagen, West Sussex, UK) and reverse-transcribed into cDNA using random primers and AMV reverse transcriptase (Promega, Southampton, UK). mRNA was quantified by real-time PCR (Rotor Gene 6000; Qiagen) using SYBR Green PCR Master Mix Reagent and normalised to 18S rRNA expression. The primers used were:

α-SMA (ACTA2), forward 5’-TTCAATGTCCCAGCCATGTA-3’ and
reverse 5’-GAAGGAATAGCCACGCTCAG-3’ [8];
PDK1, forward 5’-GTCGCCACTCTCCATGAAG-3’ and
reverse 5’-TGGGGTCCTGAGAAGATTATC-R’;
LDHA, forward 5’-GGCCTGTGCCATCAGTATCT-3’ and
reverse 5’-GGAGATCCATCATCTCTCCC-3’ [9],
18S rRNA, forward 5’-CTTAGAGGGACAAGTGGCG-3’ and
reverse 5’-ACGCTGAGCCAGTCAGTGTA -3’.

QuantiTect primer assays (Qiagen) were used for PGC-1α (Hs_PPARGC1A_1_SG; QT00095578) and PGC-1β (Hs_PPARGC1B_1_SG; QT00081865).

Reference List

1. Gosens R, Stelmack GL, Dueck G, Mutawe MM, Hinton M, McNeill KD, Paulson A, Dakshinamurti S, Gerthoffer WT, Thliveris JA, Unruh H, Zaagsma J, Halayko AJ: **Caveolae facilitate muscarinic receptor-mediated intracellular Ca2+ mobilization and contraction in airway smooth muscle**. *Am J Physiol Lung Cell Mol.Physiol* 2007, **293:**L1406-L1418.

2. Tran T, Ens-Blackie K, Rector ES, Stelmack GL, McNeill KD, Tarone G, Gerthoffer WT, Unruh H, Halayko AJ: **Laminin-binding integrin alpha7 is required for contractile phenotype expression by human airway myocytes**. *Am J Respir.Cell Mol.Biol.* 2007, **37:**668-680.

3. Sharma P, Tran T, Stelmack GL, McNeill K, Gosens R, Mutawe MM, Unruh H, Gerthoffer WT, Halayko AJ: **Expression of the dystrophin-glycoprotein complex is a marker for human airway smooth muscle phenotype maturation**. *Am J Physiol Lung Cell Mol.Physiol* 2008, **294:**L57-L68.

4. Fessel JP, Hamid R, Wittmann BM, Robinson LJ, Blackwell T, Tada Y, Tanabe N, Tatsumi K, Hemnes AR, West JD: **Metabolomic analysis of bone morphogenetic protein receptor type 2 mutations in human pulmonary endothelium reveals widespread metabolic reprogramming**. *Pulm.Circ.* 2012, **2:**201-213.

5. Reitman ZJ, Jin G, Karoly ED, Spasojevic I, Yang J, Kinzler KW, He Y, Bigner DD, Vogelstein B, Yan H: **Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome**. *Proc.Natl.Acad.Sci.U.S.A* 2011, **108:**3270-3275.

6. Tibshirani R, Hastie T, Narasimhan B, Chu G: **Diagnosis of multiple cancer types by shrunken centroids of gene expression**. *Proc.Natl.Acad.Sci.U.S.A* 2002, **99:**6567-6572.

7. Aggio RB, Ruggiero K, Villas-Boas SG: **Pathway Activity Profiling (PAPi): from the metabolite profile to the metabolic pathway activity**. *Bioinformatics.* 2010, **26:**2969-2976.

8. Roach KM, Feghali-Bostwick C, Wulff H, Amrani Y, Bradding P: **Human lung myofibroblast TGFbeta1-dependent Smad2/3 signalling is Ca(2+)-dependent and regulated by KCa3.1 K(+) channels**. *Fibrogenesis.Tissue Repair* 2015, **8:**5.

9. Liang J, Cao R, Zhang Y, Xia Y, Zheng Y, Li X, Wang L, Yang W, Lu Z: **PKM2 dephosphorylation by Cdc25A promotes the Warburg effect and tumorigenesis**. *Nat.Commun.* 2016, **7:**12431.

FIGURE LEGENDS

Figure E1: Proliferation and α-SMA mRNA expression in ASMCs from healthy smokers and COPD patients. (A-C) ASMCs isolated from healthy smokers (n=7) and patients with COPD (n=6) were serum-starved overnight and incubated with TGF-β (1ng/ml) and FBS (2.5%) for 4 or 6 days. The number of viable cells was determined by counting after trypan blue staining. Data are expressed as fold-change compared to untreated cells (**A**). The effect of TGF-β/FBS on cell number was correlated to the % FEV_1 (**B**) and FEV_1/FVC of the subjects using the Spearman's correlation coefficient (**C**). **(D-E)** ASMCs isolated from healthy smokers (n=7) and patients with COPD (n=7) were serum-starved overnight and incubated with TGF-β (1ng/ml) and FBS (2.5%) for 24 hrs. Baseline α-SMA (ACTA2) mRNA expression **(D)** and the effect of TGF-β (1ng/ml) and FBS (2.5%) on ACTA2 mRNA expression **(E)** were compared between the two groups. Data are expressed as relative mRNA expression **(D)** and as fold-change compared to untreated cells (**E**). Bars represent mean ± SEM. In panel **(D)** the horizontal lines represent the median for each group. * $p<0.05$. The clinical characteristics of the subjects that provided ASMCs for these experiments are provided in Supplementary Table E1.

Supplementary Figure E1

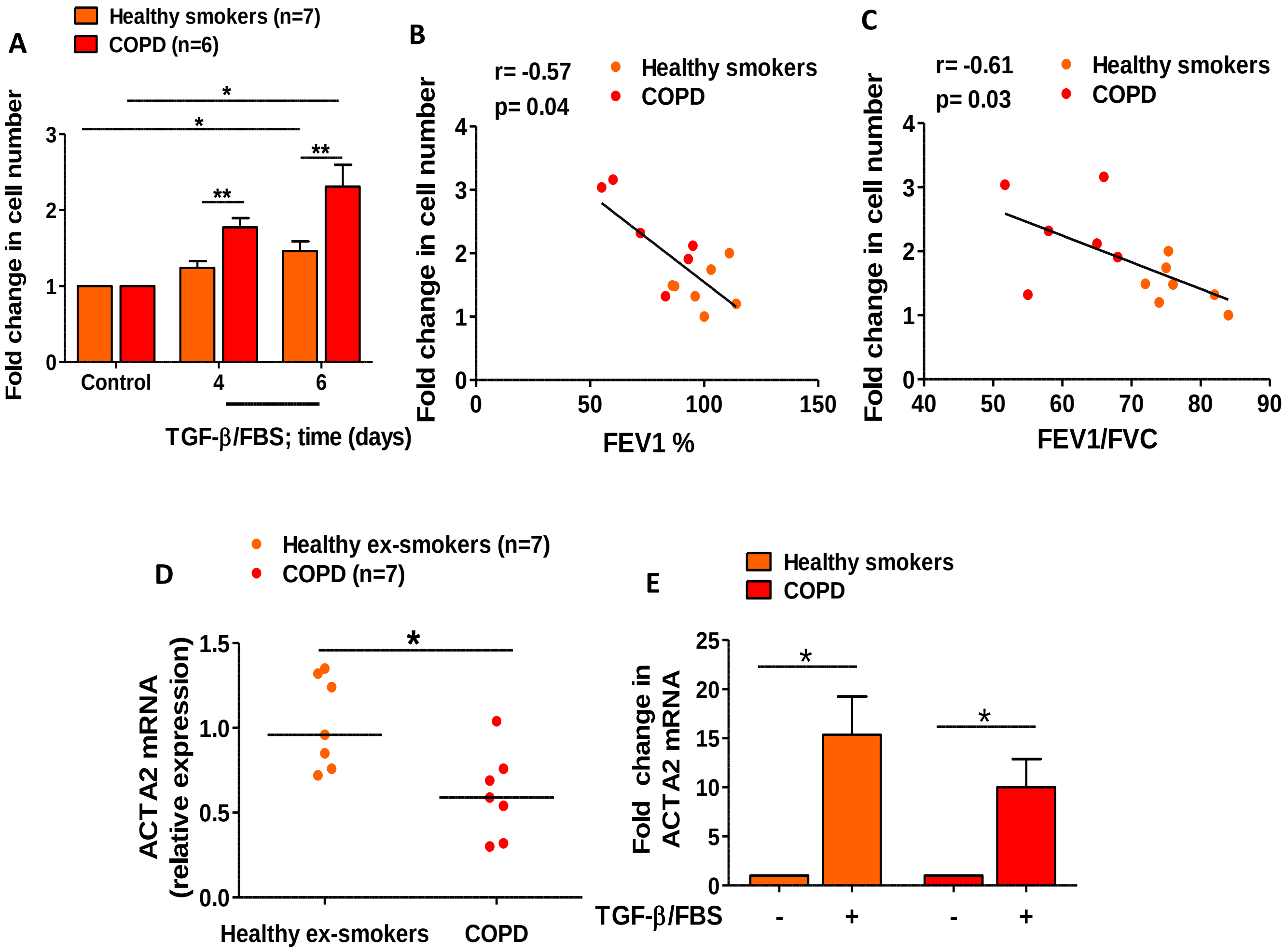


Figure E2: Number of differentially-expressed metabolites between the study groups. Venn diagrams showing the number of differentially-expressed metabolites between COPD ASMCs and healthy non-smoker and healthy smoker ASMCs, under unstimulated (**A**) and TGF-β/FBS-stimulated (**B**) conditions.

Supplementary Figure E2

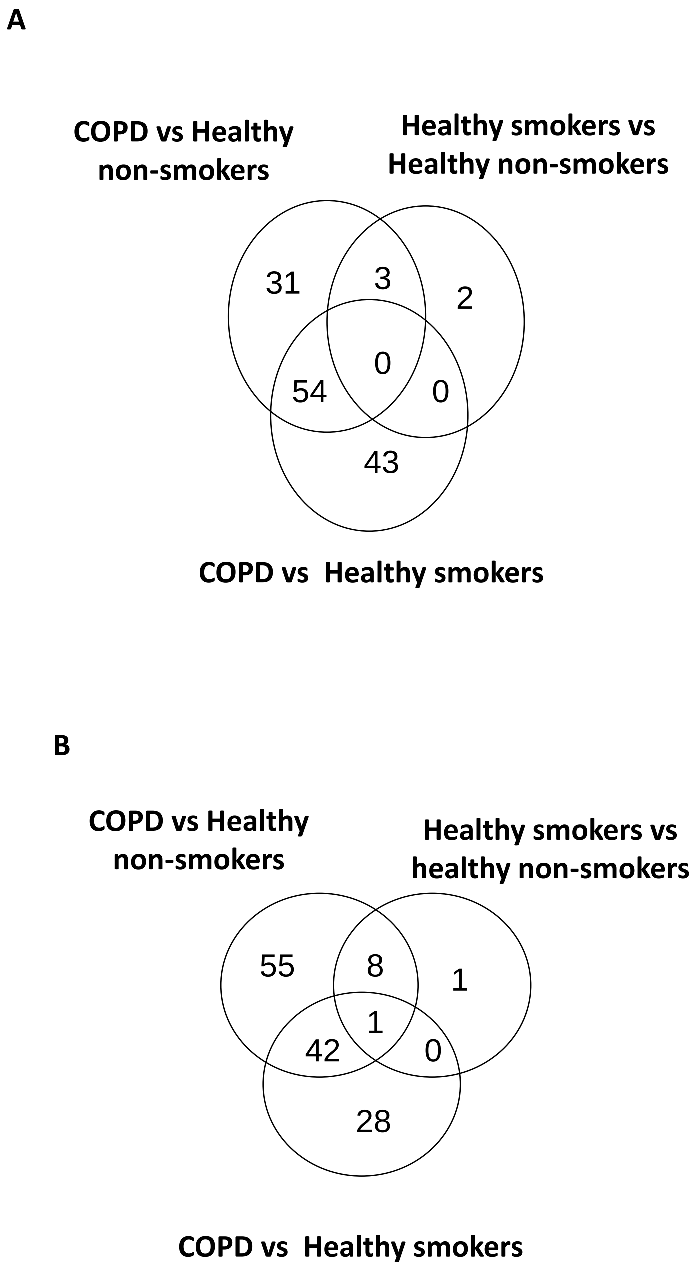


Figure E3: Expression of key mitochondrial and glycolytic genes and the effect of glycolysis inhibition on TGF-β/FBS-induced proliferation in healthy smoker and COPD ASMCs. (A-H) ASMCs isolated from healthy smokers (n=7) and patients with COPD (n=7) were serum-starved overnight and incubated with TGF-β (1ng/ml) and FBS (2.5%) for 24 hrs. Baseline mRNA expression **(A-D)** and the effect of TGF-β (1ng/ml) and FBS (2.5%) on the mRNA expression **(E-H)** of PGC-1α **(A and E)**, PGC-1β **(B and F)**, PDK-1 **(C and G)** and LDHA (**D and H**). Data are expressed as relative mRNA expression **(A-D)** and as fold-change compared to untreated cells (**E-H**). **(I)** Effect of glycolysis inhibition on healthy smoker and COPD ASMC proliferation. ASMCs from healthy smokers (n=9) and COPD patients (n=9) were pre-treated with the glycolysis inhibitor 2-deoxy-D-glucose (1-10μM) for 1hr and then stimulated with TGF-β (1ng/ml) and FBS (2.5%) for 72hrs. Changes in DNA synthesis were determined by measuring BrdU incorporation and expressed as percentage change compared to TGF-β/FBS- treated cells. Bars represent mean ± SEM. In panels **(A-D)** the horizontal lines represent the median for each group * $p<0.05$, ** $p<0.01$, *** $p<0.001$. The clinical characteristics of the subjects that provided ASMCs for these experiments are provided in Supplementary Table E1.

Supplementary Figure E3

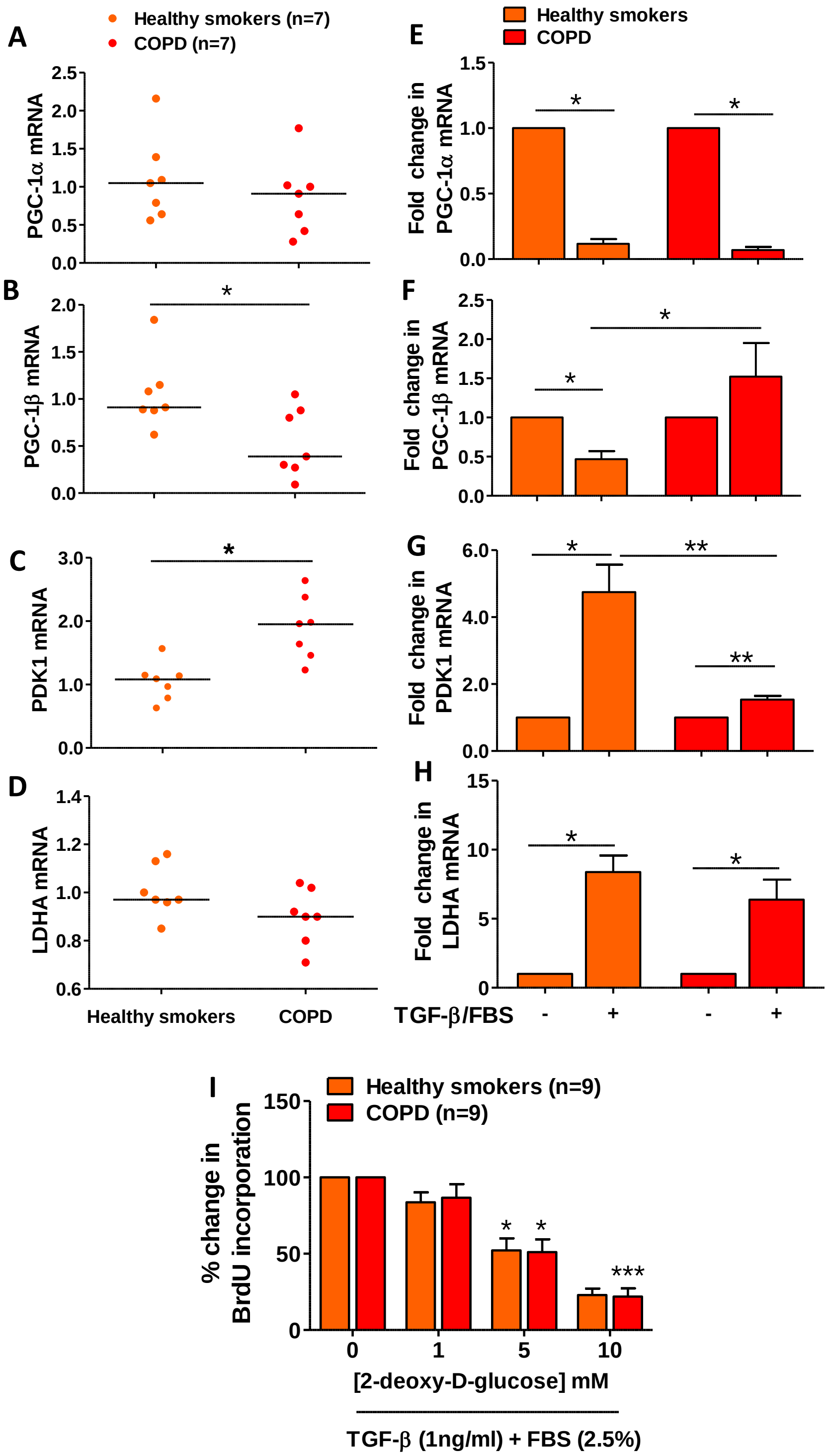


Figure E4: Correlation of nucleoside levels to lung function. ASMCs isolated from healthy non-smokers (n=6, ●), healthy smokers (n=6, ●) and patients with COPD (n=6, ●) were serum-starved overnight. Cell pellets were collected after incubation with TGF-β (1ng/ml) and FBS (2.5%) for 48hrs. The scaled intensities of uridine (**A**), cytidine (**B**) and thymidine (**C**) were determined in cell lysates by LC-MS and were correlated to the FEV_1/FVC of the subjects using the Spearman's correlation coefficient.

Supplementary Figure E4

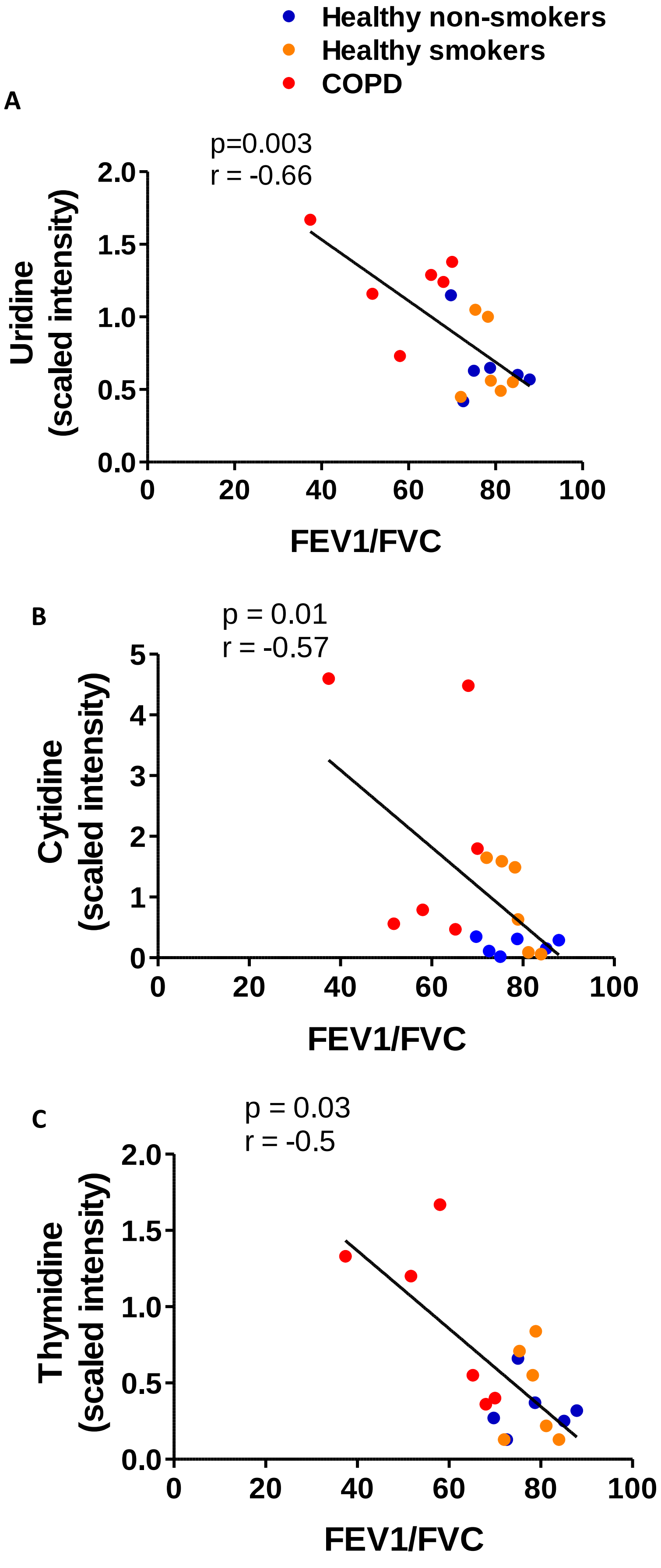


Figure E5: Correlation of glutamine levels to lung function and the effect of glutamine depletion on TGF-β/FBS-induced proliferation of COPD ASMCs. ASMCs isolated from healthy non-smokers (n=6, ●), healthy smokers (n=6, ●) and patients with COPD (n=6, ●) were serum-starved overnight. Cell pellets were collected immediately after starvation (t=0; **A**) or after incubation in the absence (**B**) or presence of TGF-β (1ng/ml) and FBS (2.5%; **C**) for 48hrs. The scaled intensities of glutamine were determined in cell lysates by LC-MS and were correlated to the FEV$_1$/FVC of the subjects using the Spearman's correlation coefficient. **(D)** Effect of glutamine on COPD ASMC proliferation. ASMCs from COPD patients (n=6) were stimulated with TGF-β (1ng/ml) and FBS (2.5%) for 72hrs in the presence or absence of glutamine (4mM) supplementation. Changes in DNA synthesis were determined by measuring BrdU incorporation. Data are expressed as percentage change compared to glutamine-supplemented, TGF-β/FBS-treated cells. Bars represent mean ± SEM. * $p<0.05$.

Supplementary Figure E5

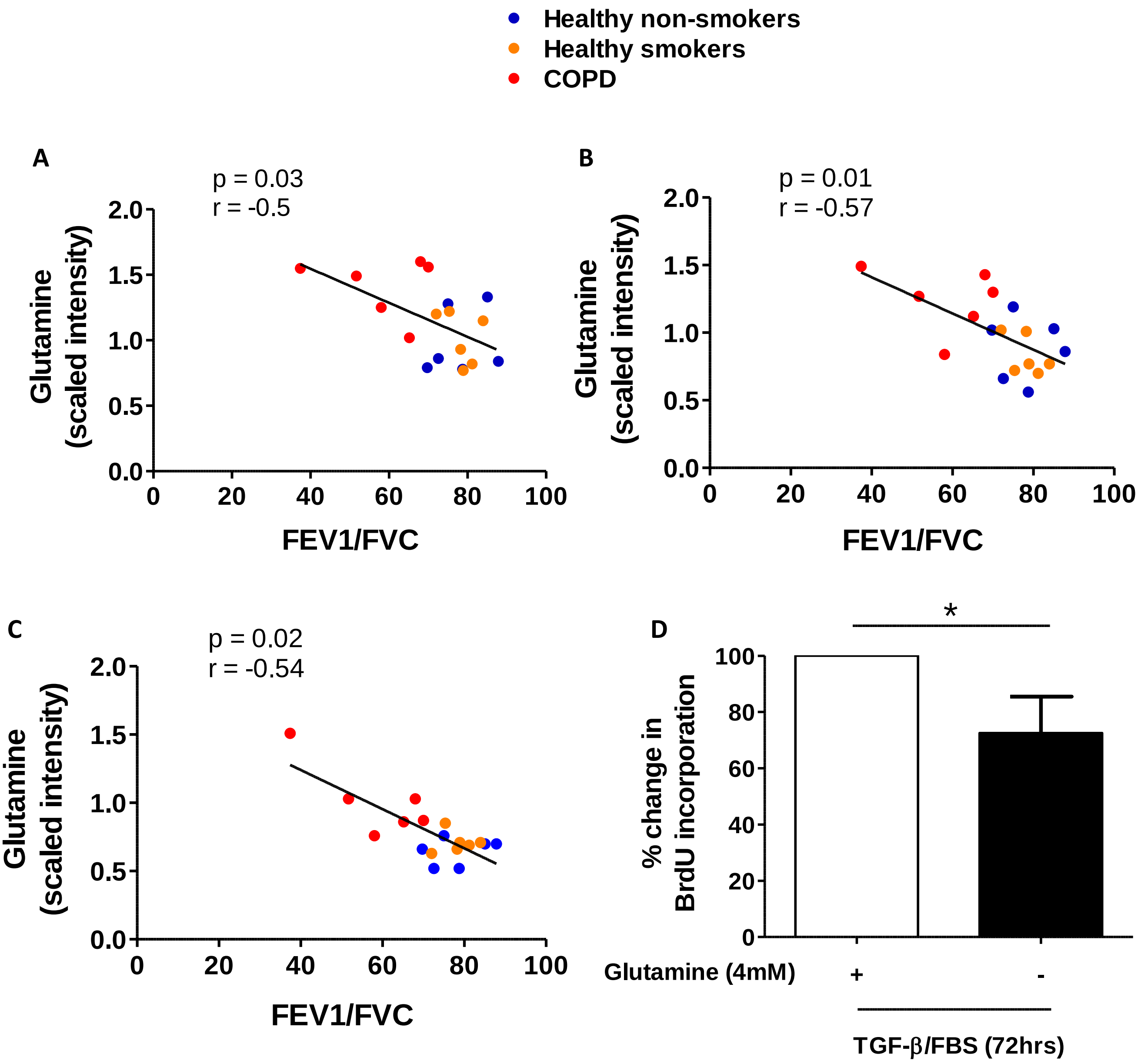


Figure E6: Correlation of fatty acid oxidation index to lung function and age. ASMCs isolated from healthy non-smokers (n=6, ●), healthy smokers (n=6, ●) and patients with COPD (n=6, ●) were serum-starved overnight. Cell pellets were collected after incubation for 48hrs in the absence of stimulation. The scaled intensities of acetylcarnitine (C2), propionylcarnitine (C3) and free carnitine (C0) were determined in cell lysates by LC-MS. The (C2+C3)/C0 ratios were correlated to the FEV$_1$/FVC (**A**) and age (**B**) of the subjects using the Spearman's correlation coefficient.

Supplementary Figure E6

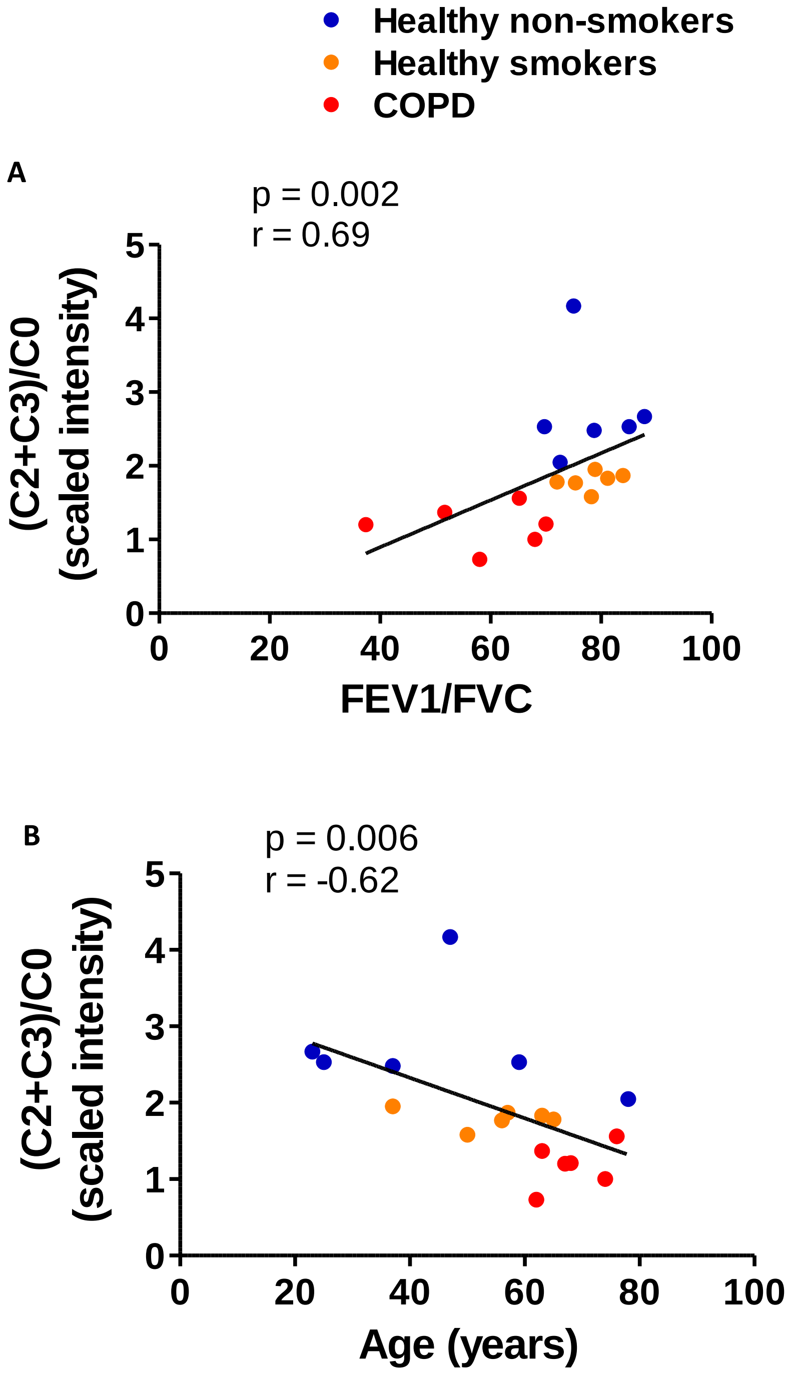
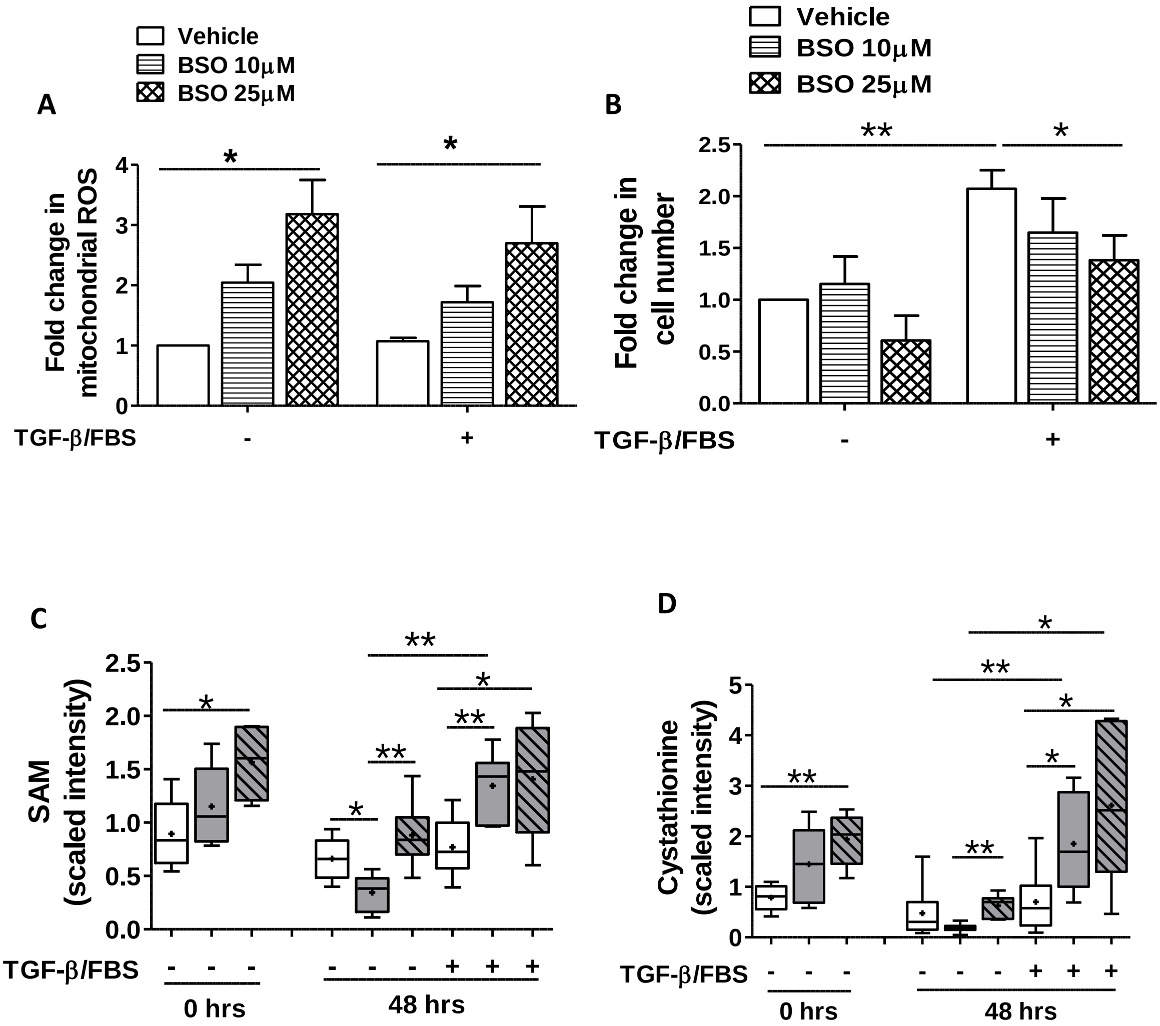


Figure E7: The effect of glutathione synthesis inhibition on the mitochondrial ROS levels and number of COPD ASMCs, and relative levels of S-adenosylmethionine and cystathionine. (A-B) Effect of glutathione synthesis inhibition on mitochondrial ROS levels and the number of COPD ASMCs. ASMCs from COPD patients (n=4-5) were pre-treated with the glutathione synthesis inhibitor buthionine sulfoximine (BSO; 10 and 25μM) for 1hr and then stimulated with TGF-β (1ng/ml) and FBS (2.5%). Mitochondrial ROS levels were determined after 48hrs by MitoSOX staining (**A**) and number of viable cells after 72hrs by counting after trypan blue staining (**B**). Bars represent mean ± SEM. **(C-D)** Relative levels of S-adenosylmethionine (SAM) and cystathionine. ASMCs isolated from healthy non-smokers (n=6), healthy smokers (n=6) and patients with COPD (n=6) were serum-starved overnight. Cell pellets were collected immediately after starvation (t=0) or after incubation in the absence or presence of TGF-β (1ng/ml) and FBS (2.5%; TGF-β/FBS) for 48hrs, and the scaled intensities of SAM (**C**) and cystathionine (**D**) were determined in cell lysates by LC-MS. Whiskers represent the spread of the data points. Horizontal lines indicate the median value and the + symbol indicates the mean of the values. * $p<0.05$, ** $p<0.01$.

Supplementary Figure E7



Supplementary Table E1. Clinical characteristics of subjects that provided ASMCs usedfor determining proliferation and mRNA expression.

Subjects that provided ASMCs used for measuring proliferation		
	Healthy smokers	**COPD**
Number	7	6
Age (years)	68 ± 3.45	70.33 ± 3.41
Gender (M/F)	4/3	6/0
Smoking (current/ex)	0/7	6/0
Smoking pack years	29.37 ± 5.11	61.40 ± 23.12
FEV$_1$ (L)	2.6 ± 0.17	2.43 ± 0.21
FEV$_1$ (% predicted)	99.57 ± 4.1	76.3 ± 6.86 *
FVC (L)	3.4 ± 0.26	4.02 ± 0.30
FEV$_1$/FVC (%)	76.91 ± 1.66	60.62 ± 2.71 **
Subjects that provided ASMCs used for measuring mRNA expression		
	Healthy smokers	**COPD**
Number	7	7
Age (years)	68 ± 3.45	70.50 ± 2.72
Gender (M/F)	4/3	5/2
Smoking (current/ex)	0/7	7/0
Smoking pack years	29.37 ± 5.11	76.40 ± 22.25 *
FEV$_1$ (L)	2.6 ± 0.17	2.40 ± 0.21
FEV$_1$ (% predicted)	99.57 ± 4.1	74 ± 6.76 *
FVC (L)	3.4 ± 0.26	3.56 ± 0.32
FEV$_1$/FVC (%)	76.91 ± 1.66	66.87 ± 1.00 **

Data are expressed as mean ± SEM. FEV1, forced expiratory volume in 1s; FVC, forced vital capacity. $^{*}p < 0.05$, ** p < 0.01 compared to healthy smokers.

Supplementary Table E2A: Differentially regulated metabolites between ASMCs from COPD patients and healthy non-smokers under unstimulated conditions

Metabolite	Sub-pathway	Super-pathway	KEGG	HMDB number	Fold change	p value
5-methyluridine (ribothymidine)	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism		HMDB00884	6.97	0.01197
1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide metabolism	C02494	HMDB03331	4.04	0.00114
ornithine	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C00077	HMDB03374	3.77	0.013445
galactonate	Fructose, Mannose,Galactose Metabolism	Carbohydrate Metabolism	C00880	HMDB00565	3.72	0.022714
homocitrulline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C02427	HMDB00679	3.54	0.007792
2-docosahexaenoyl glycerophosphoethanolamine*	Lysolipid	Lipid and fatty acid metabolism			3.32	0.026702
X - 22776 $^{\#}$	Unknown	Unknown			3.31	0.000409
N-palmitoyl-sphingosine*	Sphingolipid Metabolism	Lipid and fatty acid metabolism		HMDB04949	3.22	0.005381
gamma-glutamylleucine	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11171	3.21	0.005038
gamma-glutamylvaline	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11172	3.16	0.022138
2-hydroxystearate	Fatty acid, monohydroxy	Lipid and fatty acid metabolism	C03045		3.15	0.001016
X - 12097 $^{\#}$	Unknown	Unknown			3.14	0.00035
2-aminoadipate	Lysine metabolism	Amino acid and protein metabolism	C00956	HMDB00510	2.94	0.003054
citrulline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C00327	HMDB00904	2.86	0.017535
caprylate (8:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C06423	HMDB00482	2.79	0.003041
pipecolate	Lysine Metabolism	Amino acid and protein metabolism	C00408	HMDB00070	2.75	0.00166
X - 17010 $^{\#}$	Unknown	Unknown			2.73	0.00033
1-oleoylglycerophosphoserine	Lysolipid	Lipid and fatty acid metabolism			2.7	0.017037

2-hydroxypalmitate	Fatty acid, monohydroxy	Lipid and fatty acid metabolism		HMDB31057	2.68	0.005287
2-hydroxydecanoate	Fatty acid, monohydroxy	Lipid and fatty acid metabolism		HMDB06725	2.67	0.003514
6-oxopiperidine-2-carboxylic acid	Drug	Xenobiotics			2.58	0.000196
gamma-glutamylthreonine*	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB29159	2.41	0.006603
X - 21757 $^{\#}$	Unknown	Unknown			2.4	0.014598
deoxycarnitine	Carnitine metabolism	Lipid and fatty acid metabolism	C01181	HMDB01161	2.33	0.000217
gamma-glutamylisoleucine*	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11170	2.3	0.009687
4-guanidinobutanoate	Guanidino and Acetamido Metabolism	Amino acid and protein metabolism	C01035	HMDB03464	2.25	0.017796
2-hydroxyadipate	Fatty acid, dicarboxylate	Lipid and fatty acid metabolism	C02360	HMDB00321	2.22	0.003147
glutarate (pentanedioate)	Lysine metabolism	Amino acid and protein metabolism	C00489	HMDB00661	2.1	0.000395
myristoleate (14:1n5)	Long chain fatty acid	Lipid and fatty acid metabolism	C08322	HMDB02000	2.09	0.00804
caproate (6:0)	Medium chain fatty acid	Lipid and fatty acid metabolism	C01585	HMDB00535	2.08	0.000503
beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00099	HMDB00056	2.06	0.001725
1-stearoylglycerophosphoserine*	Lysolipid	Lipid and fatty acid metabolism			2	0.01922
pyroglutamine*	Glutamate metabolism	Amino acid and protein metabolism			1.97	0.002622
X - 16271 $^{\#}$	Unknown	Unknown			1.94	0.045686
2-methylcitrate	TCA cycle	Energy	C02225	HMDB00379	1.91	0.012576
benzoate	Benzoate Metabolism	Xenobiotics	C00180	HMDB01870	1.9	0.014801
tyrosylvaline	Dipeptide	Amino acid and protein metabolism			1.89	0.029233
N-acetylarginine	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C02562	HMDB04620	1.88	0.003359
X - 23086 $^{\#}$	Unknown	Unknown			1.88	0.001418
nervonoyl sphingomyelin*	Sphingolipid Metabolism	Lipid and fatty acid metabolism			1.86	0.000445
maltose	Glycogen Metabolism	Carbohydrate Metabolism	C00208	HMDB00163	1.84	0.045368
phenylacetylglycine	Phenylalanine and Tyrosine metabolism	Amino acid and protein metabolism	C05598	HMDB00821	1.84	0.009343
uridine-2',3'-cyclic	Pyrimidine metabolism, Uracyl	Nucleotide metabolism	C02355	HMDB11640	1.8	0.027908

monophosphate	containing					
glycine	Glycine, Serine and Threonine Metabolism	Amino acid and protein metabolism	C00037	HMDB00123	1.8	0.035971
3-indoxyl sulfate	Tryptophan Metabolism	Amino acid and protein metabolism		HMDB00682	1.79	0.001765
cysteine-glutathione disulfide	Glutathione Metabolism	Amino acid and protein metabolism		HMDB00656	1.78	0.037991
alpha-hydroxyisovalerate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB00407	1.77	0.048525
myo-inositol	Inositol Metabolism	Lipid and fatty acid metabolism	C00137	HMDB00211	1.73	0.047074
inositol 1-phosphate (I1P)	Inositol metabolism	Lipid and fatty acid metabolism	C04006	HMDB00213	1.7	0.008072
1-arachidonoyl-GPE* (20:4)*	Lysolipid	Lipid and fatty acid metabolism		HMDB11517	1.69	0.018094
heptanoate (7:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C17714	HMDB00666	1.69	0.000637
3-(4-hydroxyphenyl)lactate (HPLA)	Phenylalanine and Tyrosine Metabolism	Amino acid and protein metabolism	C03672	HMDB00755	1.68	0.001951
kynurenine	Tryptophan metabolism	Amino acid and protein metabolism	C00328	HMDB00684	1.63	0.016818
vaccenate (18:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08367	HMDB03231	1.63	0.040716
taurochenodeoxycholate	Primary Bile Acid Metabolism	Lipid and fatty acid metabolism	C05465	HMDB00951	1.62	0.048198
1-methylimidazoleacetate	Histidine Metabolism	Amino acid and protein metabolism	C05828	HMDB02820	1.59	0.00591
myristate (14:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C06424	HMDB00806	1.58	0.017948
phosphocholine	Lysolipid	Lipid and fatty acid metabolism	C00588	HMDB01565	1.58	0.017124
glycochenodeoxycholate	Primary Bile Acid Metabolism	Lipid and fatty acid metabolism	C05466	HMDB00637	1.58	0.042949
stearoyl-arachidonoyl-glycerophosphoinositol (1)*	Lysolipid	Lipid and fatty acid metabolism			1.57	0.007459
stearate (18:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C01530	HMDB00827	1.57	0.019851
oleate (18:1n9)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C00712	HMDB00207	1.56	0.016201
X - 16071 [#]	Unknown	Unknown			1.56	0.031293
arachidate (20:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C06425	HMDB02212	1.55	0.046628
palmitate (16:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C00249	HMDB00220	1.54	0.032418
sulfate*	Chemical	Xenobiotics	C00059	HMDB01448	1.54	0.020297
taurocholate	Primary Bile Acid Metabolism	Lipid and fatty acid metabolism	C05122	HMDB00036	1.54	0.038617

margarate (17:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism		HMDB02259	1.53	0.030698
glycocholate	Primary Bile Acid Metabolism	Lipid and fatty acid metabolism	C01921	HMDB00138	1.53	0.044535
3-methylglutaconate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB00522	1.53	0.007822
nonadecanoate (19:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C16535	HMDB00772	1.52	0.038521
pelargonate (9:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C01601	HMDB00847	1.52	0.014941
methylsuccinate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB01844	1.52	0.023483
stearoyl-arachidonoyl-glycerophosphoethanolamine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.51	0.012055
palmitoyl-palmitoyl-glycerophosphocholine (2)*	Lysolipid	Lipid and fatty acid metabolism			1.5	0.012538
palmitoyl-palmitoyl-glycerophosphocholine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.5	0.021283
3-hydroxyisobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism	C06001	HMDB00336	1.44	0.010204
N-acetylhistidine	Histidine metabolism	Amino acid and protein metabolism	C02997	HMDB32055	1.43	0.013644
nicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	C00153	HMDB01406	1.43	0.011378
amphotericin B	Drug	Xenobiotics	C06573	HMDB14819	1.42	0.048217
phosphate	Oxidative Phosphorylation	Energy	C00009	HMDB01429	1.39	0.009716
X - 12688 #	Unknown	Unknown			0.56	0.004675
X - 14255 #	Unknown	Unknown			0.42	0.040394
methyl glucopyranoside (alpha + beta)	Food Component/Plant	Xenobiotics			0.38	0.041489
methionylphenylalanine	Dipeptide	Amino acid and protein metabolism			0.3	0.033668
isoleucylvaline	Dipeptide	Amino acid and protein metabolism			0.26	0.027382
N-acetyl-aspartyl-glutamate (NAAG)	Glutamate Metabolism	Amino acid and protein metabolism	C12270	HMDB01067	0.17	0.028924
phosphoethanolamine (PE)	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00346	HMDB00224	0.12	0.004473

* Indicates compounds that have not been officially confirmed based on a standard, but we are confident in their identity

\# Unidentified metabolites

Supplementary Table E2B: Differentially regulated metabolites between ASMCs from COPD patients and healthy smokers under unstimulated conditions

Metabolite	Sub-pathway	Super-pathway	KEGG	HMDB number	Fold change	p value
1-stearoyl-GPC (18:0)	Lysolipid	Lipid and fatty acid metabolism		HMDB10384	6.43	0.032001
gluconate	Food Component/Plant	Xenobiotics	C00257	HMDB00625	4.7	0.043102
3'-dephosphocoenzyme A	Pantothenate and CoA Metabolism	Cofactors and Vitamins	C00882	HMDB01373	4.32	0.046942
sphingosine	Sphingolipid Metabolism	Lipid and fatty acid metabolism	C00319	HMDB00252	4.03	0.026914
5-methyluridine (ribothymidine)	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism		HMDB00884	3.74	0.027635
uracil	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00106	HMDB00300	3.55	0.02946
2-hydroxystearate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism	C03045		3.08	0.00111
X - 21861 [#]	Unknown	Unknown			2.99	0.011491
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism		HMDB31057	2.95	0.003617
X - 22776 [#]	Unknown	Unknown			2.92	0.000698
N-palmitoyl-sphingosine*	Sphingolipid Metabolism	Lipid and fatty acid metabolism		HMDB04949	2.88	0.007668
glucuronate	Aminosugar Metabolism	Carbohydrate	C00191	HMDB00127	2.8	0.041295
X - 17010 [#]	Unknown	Unknown			2.73	0.000332
gamma-aminobutyrate (GABA)	Glutamate Metabolism	Amino acid and protein metabolism	C00334	HMDB00112	2.73	0.048425
1-oleoylglycerophosphoserine	Lysolipid	Lipid and fatty acid metabolism			2.69	0.017222
1-stearoylglycerophosphoserine*	Lysolipid	Lipid and fatty acid metabolism			2.65	0.005129
2-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism			2.63	0.003735
S-adenosylmethionine (SAM)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid and protein metabolism	C00019	HMDB01185	2.56	0.002649
palmitoyl sphingomyelin	Sphingolipid Metabolism	Lipid and fatty acid metabolism			2.56	0.008971
sedoheptulose-7-phosphate	Pentose Phosphate Pathway	Carbohydrate	C05382	HMDB01068	2.51	0.017047
1-oleoylglycerophosphoglycerol*	Lysolipid	Lipid and fatty acid metabolism			2.44	0.016683
gamma-glutamylvaline	Gamma-glutamyl Amino Acid	Amino acid and protein		HMDB11172	2.38	0.047442

		metabolism				
caprylate (8:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C06423	HMDB00482	2.33	0.006819
X - 21755 #	Unknown	Unknown			2.26	0.009284
tyrosylvaline	Dipeptide	Amino acid and protein metabolism			2.2	0.013348
caproate (6:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C01585	HMDB00535	2.09	0.000478
phosphocholine	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00588	HMDB01565	2.09	0.001689
alpha-hydroxyisovalerate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB00407	2.09	0.021114
inositol 1-phosphate (I1P)	Inositol Metabolism	Lipid and fatty acid metabolism	C04006	HMDB00213	2.06	0.00168
myristoleate (14:1n5)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08322	HMDB02000	2.03	0.009503
cholestanol	Sterol	Lipid and fatty acid metabolism	C12978	HMDB00908	2.03	0.039842
gamma-glutamylisoleucine*	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11170	2.02	0.018381
X - 21756 #	Unknown	Unknown			2.02	0.0269
guanosine	Purine Metabolism, Guanine containing	Nucleotide metabolism	C00387	HMDB00133	1.91	0.04431
1-oleoyl-GPE (18:1)	Lysolipid	Lipid and fatty acid metabolism		HMDB11506	1.91	0.010694
imidazole propionate	Histidine Metabolism	Amino acid and protein metabolism		HMDB02271	1.88	0.019445
10-heptadecenoate (17:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism		HMDB60038	1.87	0.032919
1-palmitoyl-GPE (16:0)	Lysolipid	Lipid and fatty acid metabolism		HMDB11503	1.86	0.044247
pyridoxal	Vitamin B6 Metabolism	Cofactors and Vitamins	C00250	HMDB01545	1.85	0.035159
2-oleoyl-GPE* (18:1)*	Lysolipid	Lipid and fatty acid metabolism			1.83	0.02813
pentadecanoate (15:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C16537	HMDB00826	1.83	0.006154
hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide metabolism	C00262	HMDB00157	1.82	0.034854
benzoate	Benzoate Metabolism	Xenobiotics	C00180	HMDB01870	1.81	0.020187
oleate (18:1n9)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C00712	HMDB00207	1.8	0.004276
kynurenine	Tryptophan Metabolism	Amino acid and protein metabolism	C00328	HMDB00684	1.79	0.007417

phosphate	Oxidative Phosphorylation	Energy	C00009	HMDB01429	1.78	0.000299
palmitoyl-palmitoyl-glycerophosphocholine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.77	0.004041
2-arachidonoyl-GPE* (20:4)*	Lysolipid	Lipid and fatty acid metabolism			1.76	0.024152
4-acetamidobutanoate	Polyamine Metabolism	Amino acid and protein metabolism	C02946	HMDB03681	1.75	0.031077
1-arachidonoyl-GPE* (20:4)*	Lysolipid	Lipid and fatty acid metabolism		HMDB11517	1.74	0.014111
arachidate (20:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C06425	HMDB02212	1.74	0.020642
uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00299	HMDB00296	1.74	0.006457
uridine-2',3'-cyclic monophosphate	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C02355	HMDB11640	1.74	0.034844
prolylhydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism		HMDB06695	1.74	0.030657
glycocholate	Primary Bile Acid Metabolism	Lipid and fatty acid metabolism	C01921	HMDB00138	1.72	0.018595
N-acetylarginine	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C02562	HMDB04620	1.71	0.007602
gamma-glutamylthreonine*	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB29159	1.71	0.042595
stearoyl-arachidonoyl-glycerophosphoethanolamine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.71	0.002882
phenylacetylglycine	Phenylalanine and Tyrosine Metabolism	Amino acid and protein metabolism	C05598	HMDB00821	1.71	0.016456
pipecolate	Lysine Metabolism	Amino acid and protein metabolism	C00408	HMDB00070	1.7	0.026618
vaccenate (18:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08367	HMDB03231	1.7	0.030545
beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00099	HMDB00056	1.68	0.009542
palmitoyl-palmitoyl-glycerophosphocholine (2)*	Lysolipid	Lipid and fatty acid metabolism			1.67	0.00351
palmitoyl-linoleoyl-glycerophosphocholine (2)*	Lysolipid	Lipid and fatty acid metabolism			1.67	0.003005

X - 23086 #	Unknown	Unknown			1.66	0.004994
myristate (14:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C06424	HMDB00806	1.66	0.011071
X - 16071 #	Unknown	Unknown			1.66	0.018224
pelargonate (9:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C01601	HMDB00847	1.65	0.005994
glycochenodeoxycholate	Primary Bile Acid Metabolism	Lipid and fatty acid metabolism	C05466	HMDB00637	1.65	0.031429
inosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide metabolism	C00294	HMDB00195	1.64	0.010534
palmitate (16:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C00249	HMDB00220	1.64	0.019589
amphotericin B	Drug	Xenobiotics	C06573	HMDB14819	1.64	0.01243
stearoyl-arachidonoyl-glycerophosphoinositol (1)*	Lysolipid	Lipid and fatty acid metabolism			1.63	0.004849
xanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide metabolism	C00385	HMDB00292	1.63	0.035235
2-methylcitrate	TCA Cycle	Energy	C02225	HMDB00379	1.62	0.038251
2'-deoxyuridine	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00526	HMDB00012	1.62	0.048381
nicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	C00153	HMDB01406	1.61	0.002197
glutarate (pentanedioate)	Lysine Metabolism	Amino acid and protein metabolism	C00489	HMDB00661	1.6	0.005949
heptanoate (7:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C17714	HMDB00666	1.59	0.001447
FMN	Riboflavin Metabolism	Cofactors and Vitamins	C00061	HMDB01520	1.59	0.035055
deoxycholate	Secondary Bile Acid Metabolism	Lipid and fatty acid metabolism	C04483	HMDB00626	1.59	0.025202
N-acetylhistidine	Histidine Metabolism	Amino acid and protein metabolism	C02997	HMDB32055	1.58	0.003764
margarate (17:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism		HMDB02259	1.57	0.025139
deoxycarnitine	Carnitine Metabolism	Lipid and fatty acid metabolism	C01181	HMDB01161	1.54	0.010411
glycerol	Glycerolipid Metabolism	Lipid and fatty acid metabolism	C00116	HMDB00131	1.51	0.025842
stearate (18:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C01530	HMDB00827	1.51	0.028664
pyroglutamine*	Glutamate Metabolism	Amino acid and protein metabolism			1.49	0.030919
palmitoyl ethanolamide	Endocannabinoid	Lipid and fatty acid metabolism	C16512	HMDB02100	1.49	0.04449

2-piperidinone	Food Component/Plant	Xenobiotics			1.48	0.011474
choline	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00114	HMDB00097	1.47	0.034488
3-hydroxyisobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism	C06001	HMDB00336	1.46	0.008365
N-acetylthreonine	Glycine, Serine and Threonine Metabolism	Amino acid and protein metabolism	C01118	METPA0134	1.43	0.017742
xanthosine 5'-monophosphate (xmp)	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide metabolism	C00655	HMDB01554	0.27	0.001844
guanosine 5'-triphosphate	Purine Metabolism, Guanine containing	Nucleotide metabolism	C00044	HMDB01273	0.24	0.029345
uridine 5'-diphosphate (UDP)	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00015	HMDB00295	0.22	0.025457
uridine 5'-triphosphate (UTP)	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00075	HMDB00285	0.19	0.02205
phosphoethanolamine (PE)	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00346	HMDB00224	0.16	0.038751

* Indicates compounds that have not been officially confirmed based on a standard, but we are confident in their identity

[#] Unidentified metabolites

Supplementary Table E2C: Differentially regulated metabolites between ASMCs from healthy smokers and healthy non-smokers under unstimulated conditions

Metabolite	Sub-pathway	Super-pathway	KEGG	HMDB number	Fold change	p value
1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide metabolism	C02494	HMDB03331	5.15	5.73E-05
X - 12097 #	Unknown	Unknown			2.76	0.00188
gamma-glutamyllysine	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB03869	1.93	0.009454
X - 12688 #	Unknown	Unknown			0.43	0.000586
prolylglutamate	Dipeptide	Amino acid and protein metabolism			0.29	0.007795

Unidentified metabolites

Supplementary Table E3A: Differentially regulated metabolites between ASMCs from COPD patients and healthy non-smokers under TGF-β/FBS-stimulated conditions

Metabolite	Sub-pathway	Super-pathway	KEGG	HMDB number	Fold change	p value
prolylvaline	Dipeptide	Amino acid and protein metabolism			25.56	1.75E-08
cytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide metabolism	C00475	HMDB00089	10.26	0.009653
X - 21861 [#]	Unknown	Unknown			10.02	0.000672
X - 22216 [#]	Unknown	Unknown			8.73	0.040884
X - 14838 [#]	Unknown	Unknown			5.44	0.011481
X - 14380 [#]	Unknown	Unknown			4.38	0.021122
X - 17299 [#]	Unknown	Unknown			4.05	0.013788
kynurenine	Tryptophan Metabolism	Amino acid and protein metabolism	C00328	HMDB00684	3.8	0.006948
cystathionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid and protein metabolism	C02291	HMDB00099	3.72	0.006517
1-oleoylglycerophosphoserine	Lysolipid	Lipid and fatty acid metabolism			3.66	0.002953
1-arachidonoyl-GPI* (20:4)*	Lysolipid	Lipid and fatty acid metabolism		HMDB61690	3.37	0.029812
2-aminoadipate	Lysine Metabolism	Amino acid and protein metabolism	C00956	HMDB00510	3.32	0.000256
2-hydroxystearate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism	C03045		3.1	0.000359
1-oleoyl-GPI (18:1)*	Lysolipid	Lipid and fatty acid metabolism			3.1	0.016291
valylglutamate	Dipeptide	Amino acid and protein metabolism			3.1	0.00182
prolylhydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism		HMDB06695	3.09	0.0003
X - 21755 [#]	Unknown	Unknown			2.99	2.58E-06
valyltyrosine	Dipeptide	Amino acid and protein metabolism			2.94	0.00821
2-hydroxyadipate	Fatty Acid, Dicarboxylate	Lipid and fatty acid metabolism	C02360	HMDB00321	2.87	0.000592
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism		HMDB31057	2.79	0.00428
X - 23308 [#]	Unknown	Unknown			2.76	2.78E-05

thymidine	Pyrimidine Metabolism, Thymine containing	Nucleotide metabolism	C00214	HMDB00273	2.76	0.01732
AMP	Purine Metabolism, Adenine containing	Nucleotide metabolism	C00020	HMDB00045	2.75	0.029519
cytidine 2',3'-cyclic monophosphate	Pyrimidine Metabolism, Cytidine containing	Nucleotide metabolism	C02354	HMDB11691	2.71	0.002479
uridine-2',3'-cyclic monophosphate	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C02355	HMDB11640	2.68	0.00019
4-acetamidobutanoate	Polyamine Metabolism	Amino acid and protein metabolism	C02946	HMDB03681	2.63	0.002803
X - 23086 [#]	Unknown	Unknown			2.63	7.15E-05
saccharopine	Lysine Metabolism	Amino acid and protein metabolism	C00449	HMDB00279	2.49	0.033937
2-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism			2.44	0.003145
prolylglutamate	Dipeptide	Amino acid and protein metabolism			2.37	5.20E-05
X - 15666 [#]	Unknown	Unknown			2.31	0.000794
choline	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00114	HMDB00097	2.29	0.003296
6-oxopiperidine-2-carboxylic acid	Drug	Xenobiotics			2.28	0.000429
glycylisoleucine	Dipeptide	Amino acid and protein metabolism		HMDB28844	2.23	0.01799
docosatrienoate (22:3n3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid and fatty acid metabolism	C16534	HMDB02823	2.21	0.030842
phosphocholine	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00588	HMDB01565	2.2	3.31E-05
gamma-glutamylphenylalanine	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB00594	2.19	0.035737
histidylleucine	Dipeptide	Amino acid and protein metabolism			2.19	0.045504
X - 14318 [#]	Unknown	Unknown			2.14	0.022413
N-acetylhistidine	Histidine Metabolism	Amino acid and protein metabolism	C02997	HMDB32055	2.13	0.00102
caproate (6:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C01585	HMDB00535	2.08	0.000494
glutarate (pentanedioate)	Lysine Metabolism	Amino acid and protein metabolism	C00489	HMDB00661	2.08	0.001331
X - 12688 [#]	Unknown	Unknown			2.07	5.42E-05
N-acetyl-beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C01073		2.05	0.036724

1-methylnicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	C02918	HMDB00699	2.04	7.68E-06
ribose 5-phosphate	Pentose Phosphate Pathway	Carbohydrate metabolism	C00117	HMDB01548	2.04	0.015261
gamma-aminobutyrate (GABA)	Glutamate Metabolism	Amino acid and protein metabolism	C00334	HMDB00112	2.03	0.034775
imidazole propionate	Histidine Metabolism	Amino acid and protein metabolism		HMDB02271	2.02	0.000588
X - 17010 [#]	Unknown	Unknown			2.02	0.004625
X - 16271 [#]	Unknown	Unknown			1.98	0.049729
eicosenoate (20:1n9 or 1n11)	Long Chain Fatty Acid	Lipid and fatty acid metabolism		HMDB02231	1.96	0.018956
streptomycin	Drug	Xenobiotics			1.96	0.008618
isobutyrylcarnitine (C4)	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB00736	1.94	0.009949
myo-inositol	Inositol Metabolism	Lipid and fatty acid metabolism	C00137	HMDB00211	1.94	0.012512
glucuronate	Aminosugar Metabolism	Carbohydrate metabolism	C00191	HMDB00127	1.94	0.003859
valylvaline	Dipeptide	Amino acid and protein metabolism			1.94	0.047909
docosadienoate (22:2n6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid and fatty acid metabolism	C16533	HMDB61714	1.92	0.023589
galactonate	Fructose, Mannose and Galactose Metabolism	Carbohydrate metabolism	C00880	HMDB00565	1.87	0.030656
vaccenate (18:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08367	HMDB03231	1.87	0.005936
X - 21343 [#]	Unknown	Unknown			1.86	0.049896
phosphate	Oxidative Phosphorylation	Energy	C00009	HMDB01429	1.86	0.001667
uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide metabolism	C00299	HMDB00296	1.86	0.003362
scyllo-inositol	Inositol Metabolism	Lipid and fatty acid metabolism	C06153	HMDB06088	1.85	0.020454
1-methylguanosine	Purine Metabolism, Guanine containing	Nucleotide metabolism		HMDB01563	1.84	0.045582
palmitoyl-palmitoyl-glycerophosphocholine (2)*	Lysolipid	Lipid and fatty acid metabolism			1.84	0.001084
S-adenosylmethionine (SAM)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid and protein metabolism	C00019	HMDB01185	1.83	0.012262

phenylacetylglycine	Phenylalanine and Tyrosine Metabolism	Amino acid and protein metabolism	C05598	HMDB00821	1.83	0.010832
1-stearoylglycerophosphoserine*	Lysolipid	Lipid and fatty acid metabolism			1.83	0.03098
hexanoylcarnitine (C6)	Fatty Acid Metabolism(Acyl Carnitine)	Lipid and fatty acid metabolism		HMDB00705	1.82	0.037487
erucate (22:1n9)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08316	HMDB02068	1.82	0.039016
arachidate (20:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C06425	HMDB02212	1.82	0.026736
inositol 1-phosphate (I1P)	Inositol Metabolism	Lipid and fatty acid metabolism	C04006	HMDB00213	1.8	0.003649
1-docosahexaenoylglycerophosphoethanolamine*	Lysolipid	Lipid and fatty acid metabolism			1.8	0.017882
gamma-glutamylleucine	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11171	1.77	0.036858
pelargonate (9:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C01601	HMDB00847	1.76	0.001717
heptanoate (7:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C17714	HMDB00666	1.75	0.011659
pentadecanoate (15:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C16537	HMDB00826	1.75	0.007828
X - 22776 [#]	Unknown	Unknown			1.73	0.028896
5-oxoproline	Glutathione Metabolism	Amino acid and protein metabolism	C01879	HMDB00267	1.73	0.000473
10-heptadecenoate (17:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism		HMDB60038	1.72	0.047705
palmitoyl-palmitoyl-glycerophosphocholine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.7	0.002635
oleoyl-oleoyl-glycerophosphocholine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.7	0.000587
gamma-glutamylvaline	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11172	1.69	0.020424
nicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	C00153	HMDB01406	1.68	0.002557
hydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C01157	HMDB00725	1.67	0.00263
pyridoxate	Vitamin B6 Metabolism	Cofactors and Vitamins	C00847	HMDB00017	1.66	0.020969
myristate (14:0)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C06424	HMDB00806	1.66	0.020166
gamma-glutamylisoleucine*	Gamma-glutamyl Amino Acid	Amino acid and protein metabolism		HMDB11170	1.59	0.03728

cytidine 5'-monophosphate (5'-CMP)	Pyrimidine Metabolism, Cytidine containing	Nucleotide metabolism	C00055	HMDB00095	1.58	0.032795
deoxycarnitine	Carnitine Metabolism	Lipid and fatty acid metabolism	C01181	HMDB01161	1.56	0.012617
gulonic acid*	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins			1.53	0.012559
pyridoxal	Vitamin B6 Metabolism	Cofactors and Vitamins	C00250	HMDB01545	1.49	0.036953
stearoyl-arachidonoyl-glycerophosphoinositol (2)*	Lysolipid	Lipid and fatty acid metabolism			1.44	0.010192
X - 13529 #	Unknown	Unknown			1.35	0.038874
5-phosphoribosyl diphosphate (PRPP)	Pentose Phosphate Pathway	Carbohydrate metabolism	C00119	HMDB00280	0.58	0.039071
X - 21757 #	Unknown	Unknown			0.54	0.000329
xanthosine 5'-monophosphate (xmp)	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide metabolism	C00655	HMDB01554	0.49	0.017049
sorbitol	Fructose, Mannose and Galactose Metabolism	Carbohydrate metabolism	C00794	HMDB00247	0.42	0.028928
1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide metabolism	C02494	HMDB03331	0.41	0.000283
leucylisoleucine	Dipeptide	Amino acid and protein metabolism			0.4	0.043145
ergothioneine	Food Component/Plant	Xenobiotics	C05570	HMDB03045	0.35	0.00392
CDP-ethanolamine	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00570	HMDB01564	0.29	0.001941
maltopentaose	Glycogen Metabolism	Carbohydrate metabolism	C06218	HMDB12254	0.21	0.047814
X - 13871 #	Unknown	Unknown			0.19	0.046547
N6-succinyladenosine	Purine Metabolism, Adenine containing	Nucleotide metabolism		HMDB00912	0.18	0.000588
phosphoethanolamine (PE)	Phospholipid Metabolism	Lipid and fatty acid metabolism	C00346	HMDB00224	0.03	0.014636

* Indicates compounds that have not been officially confirmed based on a standard, but we are confident in their identity

[#] Unidentified metabolites

Supplementary Table E3B: Differentially regulated metabolites between ASMCs from COPD patients and healthy smokers under TGF-β/FBS-stimulated conditions

Metabolite	Sub-pathway	Super-pathway	KEGG	HMDB number	Fold change	p value
5'- GMP	Purine Metabolism, Guanine containing	Nucleotide Metabolism	C00144	HMDB01397	3.68	0.011791
Gluconate	Food Component/Plant	Xenobiotics	C00257	HMDB00625	2.89	0.047083
AMP	Purine Metabolism, Adenine containing	Nucleotide Metabolism	C00020	HMDB00045	2.73	0.030071
1-oleoylglycerophosphoserine	Lysolipid	Lipid and fatty acid metabolism			2.54	0.010056
2-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism			2.48	0.002871
X - 17717 [#]	Unknown	Unknown			2.44	0.04793
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism		HMDB31057	2.43	0.007753
Kynurenine	Tryptophan Metabolism	Amino acid and protein metabolism	C00328	HMDB00684	2.42	0.025845
X - 17677 [#]	Unknown	Unknown			2.4	0.047381
uridine-2',3'-cyclic monophosphate	Pyrimidine Metabolism, Uracil containing	Nucleotide Metabolism	C02355	HMDB11640	2.4	0.000377
Glycylisoleucine	Dipeptide	Amino acid and protein metabolism		HMDB28844	2.26	0.017202
UMP	Pyrimidine Metabolism, Uracil containing	Nucleotide Metabolism	C00105	HMDB00288	2.19	0.043287
2-hydroxystearate	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism	C03045		2.18	0.002481
X - 23827 [#]	Unknown	Unknown			2.11	0.046137
ribose 5-phosphate	Pentose Phosphate Pathway	Carbohydrate metabolism	C00117	HMDB01548	2.03	0.015762
docosatrienoate (22:3n3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid and fatty acid metabolism	C16534	HMDB02823	2	0.046154
Prolylvaline	Dipeptide	Amino acid and protein metabolism			1.99	8.33E-05
N1-methylguanosine	Purine Metabolism, Guanine containing	Nucleotide Metabolism		HMDB01563	1.94	0.03556
cytidine 2',3'-cyclic monophosphate	Pyrimidine Metabolism, Cytidine containing	Nucleotide Metabolism	C02354	HMDB11691	1.94	0.014613
2-aminoadipate	Lysine Metabolism	Amino acid and protein metabolism	C00956	HMDB00510	1.91	0.006114
palmitoleate (16:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08362	HMDB03229	1.89	0.040829

3-methylhistidine	Histidine Metabolism	Amino acid and protein metabolism	C01152	HMDB00479	1.89	0.046537
Adenosine	Purine Metabolism, Adenine containing	Nucleotide Metabolism	C00212	HMDB00050	1.88	0.027972
4-methyl-2-oxopentanoate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism	C00233	HMDB00695	1.87	0.019706
Glucuronate	Aminosugar Metabolism	Carbohydrate metabolism	C00191	HMDB00127	1.87	0.00512
Phenylacetylglycine	Phenylalanine and Tyrosine Metabolism	Amino acid and protein metabolism	C05598	HMDB00821	1.86	0.009515
1-stearoylglycerophosphoserine*	Lysolipid	Lipid and fatty acid metabolism			1.85	0.029205
X - 14318 [#]	Unknown	Unknown			1.83	0.047725
Uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide Metabolism	C00299	HMDB00296	1.82	0.004009
palmitoyl-palmitoyl-glycerophosphocholine (1)*	Lysolipid	Lipid and fatty acid metabolism			1.81	0.001394
N-acetylarginine	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C02562	HMDB04620	1.81	0.011887
myo-inositol	Inositol Metabolism	Lipid and fatty acid metabolism	C00137	HMDB00211	1.8	0.020213
oleoyl-linoleoyl-glycerophosphoinositol (1)*	Lysolipid	Lipid and fatty acid metabolism			1.78	0.013668
vaccenate (18:1n7)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08367	HMDB03231	1.77	0.00925
4-hydroxybutyrate (GHB)	Fatty Acid, Monohydroxy	Lipid and fatty acid metabolism	C00989	HMDB00710	1.77	0.004181
2-hydroxyadipate	Fatty Acid, Dicarboxylate	Lipid and fatty acid metabolism	C02360	HMDB00321	1.76	0.01234
X - 22776 [#]	Unknown	Unknown			1.74	0.028185
oleate (18:1n9)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C00712	HMDB00207	1.74	0.020615
Galactonate	Fructose, Mannose and Galactose Metabolism	Carbohydrate metabolism	C00880	HMDB00565	1.73	0.047164
X - 17010 [#]	Unknown	Unknown			1.73	0.014483
caproate (6:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C01585	HMDB00535	1.73	0.002881
Glycerol	Glycerolipid Metabolism	Lipid and fatty acid metabolism	C00116	HMDB00131	1.72	0.004759
3-methyl-2-oxovalerate	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism	C00671	HMDB03736	1.69	0.045223
inositol 1-phosphate (I1P)	Inositol Metabolism	Lipid and fatty acid metabolism	C04006	HMDB00213	1.69	0.006313

eicosenoate (20:1n9 or 1n11)	Long Chain Fatty Acid	Lipid and fatty acid metabolism		HMDB02231	1.69	0.04608
scyllo-inositol	Inositol Metabolism	Lipid and fatty acid metabolism	C06153	HMDB06088	1.66	0.042129
myristoleate (14:1n5)	Long Chain Fatty Acid	Lipid and fatty acid metabolism	C08322	HMDB02000	1.66	0.033387
N6-carbamoylthreonyladenosine	Purine Metabolism, Adenine containing	Nucleotide Metabolism		HMDB41623	1.65	0.018591
N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB11757	1.64	0.017533
isobutyrylcarnitine (C4)	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism		HMDB00736	1.62	0.035606
cytidine 5'-monophosphate (5'-CMP)	Pyrimidine Metabolism, Cytidine containing	Nucleotide Metabolism	C00055	HMDB00095	1.62	0.026801
methionine sulfone	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid and protein metabolism		HMDB59805	1.59	0.01332
Hydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism	C01157	HMDB00725	1.58	0.005189
6-oxopiperidine-2-carboxylic acid	Drug	Xenobiotics			1.58	0.011554
glutarate (pentanedioate)	Lysine Metabolism	Amino acid and protein metabolism	C00489	HMDB00661	1.58	0.014867
palmitoyl-palmitoyl-glycerophosphocholine (2)*	Lysolipid	Lipid and fatty acid metabolism			1.56	0.006706
Deoxycarnitine	Carnitine Metabolism	Lipid and fatty acid metabolism	C01181	HMDB01161	1.56	0.012191
X - 19561 [#]	Unknown	Unknown			1.53	0.015144
heptanoate (7:0)	Medium Chain Fatty Acid	Lipid and fatty acid metabolism	C17714	HMDB00666	1.53	0.035617
5-oxoproline	Glutathione Metabolism	Amino acid and protein metabolism	C01879	HMDB00267	1.49	0.003611
Prolylhydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino acid and protein metabolism		HMDB06695	1.48	0.04446
3-(4-hydroxyphenyl)lactate (HPLA)	Phenylalanine and Tyrosine Metabolism	Amino acid and protein metabolism	C03672	HMDB00755	1.47	0.029697
X - 15666 [#]	Unknown	Unknown			1.47	0.034405
imidazole propionate	Histidine Metabolism	Amino acid and protein metabolism		HMDB02271	1.41	0.026828
X - 22102 [#]	Unknown	Unknown			1.4	0.021831
X - 13529 [#]	Unknown	Unknown			1.35	0.039876
Succinate	TCA Cycle	Energy	C00042	HMDB00254	0.67	0.033072

creatine phosphate	Creatine Metabolism	Amino acid and protein metabolism	C02305	HMDB01511	0.42	0.03378
X - 16932 [#]	Unknown	Unknown			0.36	0.044638
6-keto prostaglandin F1alpha	Eicosanoid	Lipid and fatty acid metabolism	C05961	HMDB02886	0.3	0.048733
prostaglandin F2alpha	Eicosanoid	Lipid and fatty acid metabolism	C00639	HMDB01139	0.21	0.045729

* Indicates compounds that have not been officially confirmed based on a standard, but we are confident in their identity

[#] Unidentified metabolites

Supplementary Table E3C: Differentially regulated metabolites between ASMCs from healthy smokers and healthy non-smokers under TGF-β/FBS-stimulated conditions

Metabolite	Sub-pathway	Super-pathway	KEGG	HMDB number	Fold change	p value
prolylvaline	Dipeptide	Amino acid and protein metabolism			12.85	0.000172
X - 23308 $^{\#}$	Unknown	Unknown			2.66	5.34E-05
X - 21755 $^{\#}$	Unknown	Unknown			2.55	5.01E-05
prolylglutamate	Dipeptide	Amino acid and protein metabolism			2.02	0.000961
X - 12688 $^{\#}$	Unknown	Unknown			1.89	0.000365
1-methylnicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	C02918	HMDB00699	1.71	0.000479
beta-hydroxyisovaleroylcarnitine	Leucine, Isoleucine and Valine Metabolism	Amino acid and protein metabolism			0.44	0.008008
X - 21757 $^{\#}$	Unknown	Unknown			0.43	3.96E-05
1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide Metabolism	C02494	HMDB03331	0.22	1.41E-05
N6-succinyladenosine	Purine Metabolism, Adenine containing	Nucleotide Metabolism		HMDB00912	0.14	0.000378

$^{\#}$ Unidentified metabolites

Supplementary Table E4: Differentially regulated pathways between unstimulated ASMCs from COPD patients and healthy non-smokers

Pathway	Raw p value	Adj p val
Lysine degradation	5.23E-06	0.00046007
ABC transporters	0.0001308	0.00383775
Arginine biosynthesis	0.0002396	0.00501302
Propanoate metabolism	0.0002848	0.00501302
Fatty acid biosynthesis	0.0004187	0.00518382
Pyrimidine metabolism	0.0004459	0.00518382
Glutathione metabolism	0.0007073	0.00691533
Ubiquinone and other terpenoid-quinone biosynthesis	0.0010352	0.00697883
Tyrosine metabolism	0.0010415	0.00697883
Protein digestion and absorption	0.0010918	0.00697883
Lysine biosynthesis	0.0011533	0.00697883
Arginine and proline metabolism	0.0024609	0.01353468
Fatty acid degradation	0.0029899	0.01547725
Biosynthesis of amino acids	0.0048843	0.0231622
Histidine metabolism	0.0052108	0.0231622
Purine metabolism	0.0052743	0.0231622
beta-Alanine metabolism	0.0061421	0.0231622
Pantothenate and CoA biosynthesis	0.0061589	0.0231622
Inositol phosphate metabolism	0.0071217	0.0231622
Phosphatidylinositol signaling system	0.0072895	0.0231622
Nicotinate and nicotinamide metabolism	0.007515	0.0231622
Vitamin digestion and absorption	0.007633	0.0231622
Valine, leucine and isoleucine degradation	0.0101671	0.02795946
Oxidative phosphorylation	0.0109523	0.02920601
Parkinson's disease	0.0113772	0.02944683
Mineral absorption	0.0124012	0.03118012
D-Arginine and D-ornithine metabolism	0.0133456	0.03262256
Galactose metabolism	0.0145748	0.03380623
Primary bile acid biosynthesis	0.0172494	0.0371046
Sphingolipid metabolism	0.0172858	0.0371046
Sphingolipid signaling pathway	0.0172874	0.0371046
Glycerophospholipid metabolism	0.0191965	0.03928584
Phenylalanine metabolism	0.0216698	0.04333955
Cysteine and methionine metabolism	0.0243639	0.04726706
Sulfur metabolism	0.0252449	0.04726706
2-Oxocarboxylic acid metabolism	0.0267629	0.04906537

ad p val, adjusted p value

Supplementary Table E5: Differentially regulated pathways between TGF-β/FBS-treated ASMCs from COPD patients and healthy non-smokers

Pathway name	Raw p value	Adj p val
Bile secretion	7.81E-06	0.00062455
Arginine and proline metabolism	3.09E-05	0.00097489
Metabolic pathways	3.66E-05	0.00097489
Nicotinate and nicotinamide metabolism	5.74E-05	0.00114804
Choline metabolism in cancer	7.57E-05	0.00121045
Mineral absorption	0.0002696	0.00228165
Lysine degradation	0.0002852	0.00228165
Oxidative phosphorylation	0.0004849	0.00352678
Pyrimidine metabolism	0.0008483	0.00565556
Glutathione metabolism	0.0012638	0.00722186
Lysine biosynthesis	0.0013881	0.00731208
Glycine, serine and threonine metabolism	0.0014624	0.00731208
Histidine metabolism	0.0020719	0.00939947
Biosynthesis of amino acids	0.0021149	0.00939947
beta-Alanine metabolism	0.0025672	0.01026894
Ascorbate and aldarate metabolism	0.0039441	0.01502533
Inositol phosphate metabolism	0.0048976	0.01712414
Cholinergic synapse	0.0049232	0.01712414
Fatty acid degradation	0.0062185	0.02072831
Biosynthesis of secondary metabolites	0.0065611	0.02099544
Vitamin digestion and absorption	0.0072224	0.02222268
Phosphatidylinositol signaling system	0.00921	0.02688649
Amino sugar and nucleotide sugar metabolism	0.0117956	0.03072879
Pentose and glucuronate interconversions	0.0119074	0.03072879
Vitamin B6 metabolism	0.0128797	0.03219929
Cysteine and methionine metabolism	0.0133658	0.03240189
Parkinson's disease	0.0164849	0.03878805
Fatty acid biosynthesis	0.0252056	0.05487038
Biosynthesis of unsaturated fatty acids	0.0253776	0.05487038

Adj p val, adjusted p value

Supplementary Table E6: Differentially regulated pathways between unstimulated ASMCs from COPD patients and healthy smokers

Pathway name	Raw p value	Adj p value
Fatty acid biosynthesis	0.0003182	0.00954266
Glycerophospholipid metabolism	0.0005229	0.00954266
Mineral absorption	0.0005881	0.00954266
Parkinson's disease	0.0007491	0.00965852
Cysteine and methionine metabolism	0.0008049	0.00965852
Arginine and proline metabolism	0.0012855	0.01153504
Oxidative phosphorylation	0.0013217	0.01153504
Sphingolipid metabolism	0.0020112	0.01266583
Sphingolipid signaling pathway	0.0020162	0.01266583
Phosphatidylinositol signaling system	0.0020697	0.01266583
Propanoate metabolism	0.0035304	0.01694613
Sulfur metabolism	0.0041658	0.01904358
Nicotinate and nicotinamide metabolism	0.0047659	0.02054418
Vitamin digestion and absorption	0.0051152	0.02054418
Lysine degradation	0.005136	0.02054418
Valine, leucine and isoleucine degradation	0.0077669	0.0286778
Choline metabolism in cancer	0.009637	0.03304098
ABC transporters	0.0135363	0.04071158
Metabolic pathways	0.0144729	0.04210301
Pantothenate and CoA biosynthesis	0.0152956	0.04217665
Fatty acid degradation	0.0158018	0.04217665
Biosynthesis of unsaturated fatty acids	0.0169974	0.04294092
Retrograde endocannabinoid signaling	0.0183808	0.04384023
Pyrimidine metabolism	0.0185627	0.04384023
beta-Alanine metabolism	0.018903	0.04384023
Inositol phosphate metabolism	0.0193468	0.04384023
Secondary bile acid biosynthesis	0.0211561	0.04530641
Tryptophan metabolism	0.0212374	0.04530641
Bile secretion	0.0234229	0.0488825
African trypanosomiasis	0.0243746	0.0496986
Protein digestion and absorption	0.0248493	0.0496986

Adj p val, adjusted p value

Supplementary Table E7: Differentially regulated pathways between TGF-β/FBS-stimulated ASMCs from COPD patients and healthy smokers

Pathway name	Raw p value	Adj p value
Pyrimidine metabolism	0.0001584	0.01393801
Ascorbate and aldarate metabolism	0.0027011	0.04700726
Pentose and glucuronate interconversions	0.0031293	0.04700726
Glutathione metabolism	0.0052065	0.04700726
Purine metabolism	0.0058171	0.04700726
Regulation of lipolysis in adipocytes	0.0059663	0.04700726
Valine, leucine and isoleucine degradation	0.0064451	0.04700726
Valine, leucine and isoleucine biosynthesis	0.006754	0.04700726
Inositol phosphate metabolism	0.0069662	0.04700726
Phosphatidylinositol signaling system	0.0084747	0.04700726
ABC transporters	0.0088066	0.04700726
Glycerolipid metabolism	0.0088263	0.04700726
Retrograde endocannabinoid signaling	0.0096782	0.04700726
Metabolic pathways	0.0111355	0.04700726
Amino sugar and nucleotide sugar metabolism	0.011763	0.04700726
Starch and sucrose metabolism	0.0118735	0.04700726
Biosynthesis of amino acids	0.012979	0.04700726
Glyoxylate and dicarboxylate metabolism	0.0163473	0.04700726
Propanoate metabolism	0.0165134	0.04700726
Central carbon metabolism in cancer	0.0166335	0.04700726
Pyruvate metabolism	0.0168307	0.04700726
Sulfur metabolism	0.0169254	0.04700726
Alanine, aspartate and glutamate metabolism	0.0169807	0.04700726
Lysine degradation	0.0170325	0.04700726
Glucagon signaling pathway	0.0171112	0.04700726
Citrate cycle (TCA cycle)	0.017771	0.04700726
Fatty acid biosynthesis	0.0178669	0.04700726
Oxidative phosphorylation	0.0186961	0.04700726
Biosynthesis of unsaturated fatty acids	0.0196661	0.04733969
Galactose metabolism	0.0202581	0.04733969

Adj p val, adjusted p value

Supplementary Table E8: Differences in medium and long chain fatty acid levels between ASMCs from healthy non-smokers, healthy smokers and patients with COPD (fold-change)

Sub Pathway	Biochemical Name	KEGG number	HMDB number	Healthy smokers vs. Healthy non-smokers (0h)	COPD vs. Healthy non-smokers (0h)	COPD vs. Healthy smokers (0h)	Healthy smokers vs. Healthy non-smokers untreated (48h)	COPD vs. Healthy non-smokers untreated (48h)	COPD vs. Healthy smokers untreated (48h)	Healthy smokers vs. Healthy non-smokers TGF-β/FBS (48h)	COPD vs. Healthy non-smokers TGF-β/FBS (48h)	COPD vs. Healthy smokers TGF-β/FBS (48h)
Medium Chain Fatty Acid	caproate (6:0)	C01585	HMDB00535	0.83	1.44	**1.74**	0.99	**2.08**	**2.09**	1.2	**2.08**	**1.73**
	heptanoate (7:0)	C17714	HMDB00666	0.72	2.01	**2.8**	1.06	**1.69**	**1.59**	1.14	**1.75**	1.53
	caprylate (8:0)	C06423	HMDB00482	1.14	1.86	1.63	1.2	**2.79**	**2.33**	1.01	1.78	1.76
	pelargonate (9:0)	C01601	HMDB00847	1.11	**1.51**	1.36	0.92	1.52	**1.65**	1.21	**1.76**	**1.46**
	5-dodecenoate (12:1n7)		HMDB00529	0.87	0.62	0.71	0.94	1.36	1.44	1.33	1.43	1.08
Long Chain Fatty Acid	myristate (14:0)	C06424	HMDB00806	1.05	1.4	1.33	0.95	**1.58**	**1.66**	1.2	**1.66**	1.38
	myristoleate (14:1n5)	C08322	HMDB02000	0.78	1.25	1.6	1.03	**2.09**	**2.03**	0.88	1.45	**1.66**
	pentadecanoate (15:0)	C16537	HMDB00826	1.14	**1.52**	1.34	0.78	1.42	**1.83**	1.35	**1.75**	1.3
	palmitate (16:0)	C00249	HMDB00220	1.1	1.26	1.14	0.94	1.54	1.64	1.26	**1.63**	1.29
	palmitoleate (16:1n7)	C08362	HMDB03229	0.93	1.13	1.22	0.67	1.35	**2**	0.92	1.73	**1.89**
	margarate (17:0)		HMDB02259	1.08	1.38	1.27	0.98	1.53	1.57	1.36	1.53	1.12
	10-heptadecenoate (17:1n7)		HMDB60038	1.17	1.11	0.95	0.77	1.44	**1.87**	1.21	1.72	1.42
	stearate (18:0)	C01530	HMDB00827	1.15	1.37	1.19	1.04	1.57	1.51	1.42	**1.69**	1.19
	oleate (18:1n9)	C00712	HMDB00207	0.99	1.24	1.25	0.87	**1.56**	**1.8**	0.84	1.46	**1.74**
	cis-vaccenate (18:1n7)	C08367	HMDB03231	0.88	1.12	1.27	0.96	**1.63**	**1.7**	1.06	**1.87**	**1.77**
	nonadecanoate (19:0)	C16535	HMDB00772	1.12	1.41	1.26	1.05	1.52	1.45	1.42	**1.75**	1.23
	10-nonadecenoate (19:1n9)		HMDB13622	1.08	1.2	1.1	0.78	1.38	1.77	1.11	1.72	1.54
	arachidate (20:0)	C06425	HMDB02212	1.48	**1.81**	1.22	0.89	1.55	**1.74**	1.63	**1.82**	1.11
	eicosenoate (20:1n9 or 11)		HMDB02231	1.11	1.2	1.08	0.89	1.52	1.71	1.16	**1.96**	1.69
	erucate (22:1n9)	C08316	HMDB02068	1.5	1.52	1.02	0.78	1.25	1.6	1.12	1.82	1.62

Red boxes indicate statistically-significant up-regulation ($p<0.05$).
Inter-group comparisons performed by Welch's two sample t-test.
KEGG; Kyoto Encyclopedia of Genes and Genomes

Supplementary Table E9: Differences in amino acid levels between ASMCs from healthy non-smokers, healthy smokers and patients with COPD (fold-change)

Super Pathway	Biochemical Name	KEGG number	HMDB number	Healthy smokers vs. Healthy non-smokers (0h)	COPD vs. Healthy non-smokers (0h)	COPD vs. Healthy smokers (0h)	Healthy smokers vs. Healthy non-smokers untreated (48h)	COPD vs. Healthy non-smokers untreated (48h)	COPD vs. Healthy smokers untreated (48h)	Healthy smokers vs. Healthy non-smokers TGF-β/FBS (48h)	COPD vs. Healthy non-smokers TGF-β/FBS (48h)	COPD vs. Healthy smokers TGF-β/FBS (48h)
Amino Acid	glycine	C00037	HMDB00123	1.12	**1.79**	1.6	**1.56**	**1.8**	1.15	0.93	1.23	1.33
	serine	C00065	HMDB00187	0.75	0.97	1.3	0.63	0.92	**1.46**	0.91	1.26	**1.38**
	threonine	C00188	HMDB00167	0.85	1.25	**1.46**	0.76	1.19	**1.57**	1.01	**1.53**	**1.51**
	alanine	C00041	HMDB00161	0.61	0.82	1.34	0.46	0.68	**1.47**	0.75	0.99	**1.33**
	aspartate	C00049	HMDB00191	0.88	1.15	1.3	0.97	**1.34**	**1.39**	1.14	**1.52**	**1.34**
	asparagine	C00152	HMDB00168	0.68	0.92	1.36	0.54	0.78	**1.46**	0.8	1.08	**1.34**
	glutamine	C00064	HMDB00641	1.03	**1.44**	**1.39**	0.94	**1.4**	**1.49**	1.1	**1.57**	**1.43**
	histidine	C00135	HMDB00177	0.81	1.11	1.38	0.71	0.99	**1.39**	0.94	1.41	**1.5**
	lysine	C00047	HMDB00182	0.8	1.05	1.31	0.61	0.91	**1.5**	1.23	**1.62**	**1.32**
	phenylalanine	C00079	HMDB00159	0.82	1.07	1.3	0.78	1.03	**1.32**	0.95	1.36	**1.43**
	tyrosine	C00082	HMDB00158	0.83	1.11	1.33	0.79	1.06	**1.34**	0.97	1.4	**1.44**
	tryptophan	C00078	HMDB00929	0.84	1.13	**1.35**	0.81	1.08	**1.33**	0.99	**1.41**	**1.43**
	leucine	C00123	HMDB00687	0.77	1.01	1.32	0.72	0.97	**1.34**	0.95	1.34	**1.41**
	isoleucine	C00407	HMDB00172	0.89	1.15	1.28	0.86	1.09	1.26	1.04	**1.44**	1.38
	valine	C00183	HMDB00883	0.8	1.03	1.28	0.78	1.01	1.3	0.98	1.38	**1.41**
	methionine	C00073	HMDB00696	0.7	0.91	1.3	0.63	0.89	**1.42**	0.95	1.4	**1.48**
	cysteine	C00097	HMDB00574	0.98	**1.59**	1.62	1.33	1.34	1.01	0.9	1.25	1.39
	arginine	C00062	HMDB00517	0.74	1.01	1.36	0.6	0.85	**1.43**	0.91	1.37	**1.5**
	proline	C00148	HMDB00162	0.78	1.06	1.36	2.45	**3.77**	1.54	0.93	1.3	1.39

Red boxes indicate statistically-significant up-regulation (p<0.05).
Comparisons performed by Welch's two sample t-test.
KEGG; Kyoto Encyclopedia of Genes and Genomes
HMDB; The Human Metabolome Database