

ONLINE SUPPLEMENT

MATERIAL AND METHODS:

Primer sequences used in this study:

COL1A1 sense, 5'-CACGACAAAGCAGAAACAT-3', antisense 5'-TTCAGCAACACAGTTACAC-3'; *COL1A2* sense, 5'-GGATGAGGAGACTGGCAAC-3', antisense 5'-GTGATGGCTTATTTGTTTTG-3'; *FN1* sense 5'-GAAAGACCAGCAGAGGCATA-3', antisense 5'-ACTCATCTCCAACGGCATAA-3'; *TNC* sense 5'-ACCCACTACACAGCCAAGA-3', antisense 5'-TCAGGAACACAATCCATCCA; *SPP1* sense 5'-CTACCTTCGCCTATGTTGTCA-3', antisense 5'-GTGATTAGTGATGTTGAGCA-3'; *SPARC* sense 5'-TGAGAGGGATGAGGACAACAA-3', antisense 5'-ATGAGGGGAGCACGCAGT-3'; *DCN*: sense 5'-TTGGAACGACTTTATCTGTCC-3', antisense 5'-TAAGGGAAGGAGGAAGACCT-3'; *BGN*: sense 5'-CCTGGGTCTGAAGTCTGT-3', antisense 5'-GTGGTTCTTGGAGATGTAGA-3'; *ITGB1*: sense 5'-CCTTTTTGACCTTTTCTTCCTG-3', antisense 5'-GACATTTACTTTGGATTCT-3'; *PAX7*: sense 5'-CAGTCAACAAGACATTCCTA-3', antisense 5'-TGAAGATGAGAAACAGGTA-3'; *MYOD1*: sense 5'-AGACCCTCGCAGACCTAA-3', antisense 5'-CTGGCAAAGCAACTCTTAT-3'; *Myostatin*: sense 5'-ACCATGCCTACAGAGTCTGA-3', antisense 5'-GATTCAGGTTGTTTGAGCCAA-3'. Real-time PCR was performed with SYBRGreen I (Molecular Probes) and Platinum Taq DNA polymerase (Invitrogen) in a StepOnePlus RT-PCR system (Applied Biosystems). Analysis was performed using the $2^{-\Delta\Delta Ct}$ method [1]. All values were normalized against expression of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*: sense 5'-GAAGGTGAAGGTCGGAGT-3', antisense 5'-CATGGGTGGAATCATATTGGAA-3') [2].

Protein analyses using ELISA.

Proteins were analysed using ELISA, which sensitivity and detection range are described for the following kits: collagen I (ab285250, Abcam, 0.188 ng/ml, 0.31 – 20 ng/ml); collagen IV (orb562147, 0.188 ng/ml, 0.31 – 20 ng/ml) and integrin beta 1 (orb563558, 9.375 pg/ml, 15.6 – 1000 pg/ml) from Biorbyt; osteopontin (DOST00, 0.024 ng/ml, 0.3 – 20 ng/ml), fibronectin (DFBN10, 0.579 ng/ml, 3.1 – 200 ng/ml), SPARC (DSP00, 0.269 ng/ml, 1.6 – 50 ng/ml) from R&D Systems; tenascin C (EH446RB, 6.1 pg/ml, 6.1 – 1500 pg/ml), decorin (EHDCN, 1.50 pg/ml, 0.96 – 700 pg/ml), and biglycan (EH45RB, 0.51 ng/ml, 0.51 – 125 ng/ml) from Thermo Fisher Scientific. Acquired standard curves for individual ELISA kits are shown in Supplementary Figure 3.

Supplementary Table 1. Demographic characteristics of healthy individuals and COPD patients

Characteristics	COPD	Healthy
Individuals (n)	29	14
Age (years)	65.68 ± 1.42 [*]	21.80 ± 0.60
Weight (kg)	72.89 ± 2.62	74.20 ± 2.10
BMI (kg • m⁻²)	25.94 ± 0.80	23.45 ± 0.58
FFM index (kg • m⁻²)	17.76 ± 0.40	23.70 ± 0.67
FEV₁ (L)	1.13 ± 0.08	-
FEV₁ (% predicted)	43.32 ± 3.29	-
FVC (L)	2.73 ± 0.14	-
FVC (% predicted)	78.80 ± 3.91	-

Data are presented as mean ± SEM. BMI: body mass index; FFM: fat-free mass; FEV₁: forced expiratory volume in the first second; FVC: forced vital capacity; Asterisks indicate $p < 0.05$ significance level between COPD and healthy groups.

Supplementary Table 2. Muscle fibre morphological characteristics of healthy individuals and COPD patients.

Muscle fibre characteristics	COPD	Healthy
Individuals (n)	29	14
Fibre type distribution (%)		
Type I	33.0 ± 2.9	39.8 ± 4.6
Type II	65.9 ± 2.4	59.2 ± 5.9
Type IIa	52.0 ± 1.9*	38.9 ± 2.8
Type IIx	15.5 ± 0.7	20.2 ± 3.1
Cross sectional area (CSA, μm²)		
Mean CSA	4100 ± 106.9*	4582 ± 155.6
Type I	4763 ± 171.9*	3974 ± 146.8
Type IIa	4154 ± 138.7*	5656 ± 205.8
Type IIx	3339 ± 165.2*	4176 ± 180.2
Capillary/fibre ratio	1.43 ± 0.07*	2.02 ± 0.56

Data are presented as mean ± SEM. Asterisks indicate $p < 0.05$ significance

level between COPD and healthy control group.

Supplementary Table 3. Protein expression of ECM molecules in vastus lateralis muscle of COPD patients and healthy controls.

Protein (pg/ml)	COPD	Healthy	p-value
COL1	380.5* (256.0-457.4)	6441 (1423-11580)	<0.0001
COL4	372.3 (281.1-530.8)	416.9 (309.0-624.7)	0.3621
Fibronectin	9418.0* (6271.0-13150.0)	3918.0 (2867.0-5972.0)	0.0002
ITGβ1	55.9 (40.8-96.9)	61.6 (49.9-73.5)	0.4852
Osteopontin	70.34* (30.60-146.9)	26.78 (20.65-66.8)	0.0194
Tenascin C	10.4* (5.1-14.2)	16.8 (6.1-22.0)	0.0403
SPARC	1612.0* (1186.0-1880.0)	1224.0 (940.5-1342.0)	0.0095
Decorin	42.8* (38.68-48.1)	71.1 (57.4-85.9)	<0.0001
Biglycan	230.8 (137.2-376.6)	169.9 (111.2-250.3)	0.1430

Protein expression was measured with ELISA. Data are shown as median (25 – 75 % percentile). Asterisks indicate $p < 0.05$ significance level between COPD and healthy control group.