

Supplementary tables

Supplementary Table 1. List of TaqMan assay IDs (Applied Biosystems) for quantitative RT-PCR

Gene name	TaqMan assay ID
ACTB	Hs01060665_g1
AGRN	Hs00394748_m1
BGN	Hs00959143_m1
CD44	Hs01075861_m1
COL1A2	Hs01028956_m1
COL5A1	Hs00609088_m1
COL5A2	Hs00893878_m1
ELN	Hs00355783_m1
NOS3	Hs01574659_m1
FBN1	Hs00171191_m1
HAPLN3	Hs00820260_m1
HYLA2	Hs00186841_m1
RPL19	Hs02338565_gH
TBP	Hs00427620_m1

Supplementary Table 2. List of primary antibodies for immuno-TEM

Antibody	Supplier & cat. no.	Host	Dilution	Immunogen
COL1A2	Abcam ab96723	Rabbit polyclonal	EM: 1:25	Recombinant protein fragment corresponding to a region within amino acids 1057 and 1339 of COL1A2 (NP_000080).
COL5A2	Invitrogen PA5-38880	Rabbit / IgG polyclonal	EM: 1:25	A synthetic peptide derived from the internal region of human Collagen V alpha2.

Supplementary Table 3. List of ECM genes included in gene expression analysis

Gene	Mean LSS	Mean Static	Difference	SE difference	P value	q value	>8% FDR
ACAN	3.71	2.74	0.96	1.11	0.43	0.67	No
ADAMTS1	10.03	8.55	1.47	0.28	0.01	0.05	Yes
ADAMTS15	3.61	1.83	1.78	0.43	0.01	0.08	Yes
ADAMTS18	7.75	5.44	2.31	0.28	0	0.02	Yes
ADAMTS3	1.96	2.02	-0.06	0.51	0.91	0.88	No
ADAMTS4	8.77	9.62	-0.85	0.21	0.02	0.09	No
AGRN	11.5	10.15	1.35	0.13	0	0.01	Yes
ARSA	9.7	9.42	0.29	0.11	0.06	0.21	No
ARSB	3.28	3.84	-0.56	0.7	0.47	0.67	No
ARSD	7.27	6.6	0.67	0.18	0.02	0.1	No
ARSE	6.22	5.29	0.93	0.26	0.02	0.11	No
ARSG	3.33	3.73	-0.4	0.42	0.39	0.66	No
ARSI	2.98	3.53	-0.55	0.67	0.45	0.67	No
ARSJ	3.15	2.94	0.2	0.77	0.81	0.84	No
ARSK	5.97	6.14	-0.17	0.49	0.74	0.82	No
ASPN	2.31	1.34	0.97	0.74	0.26	0.56	No

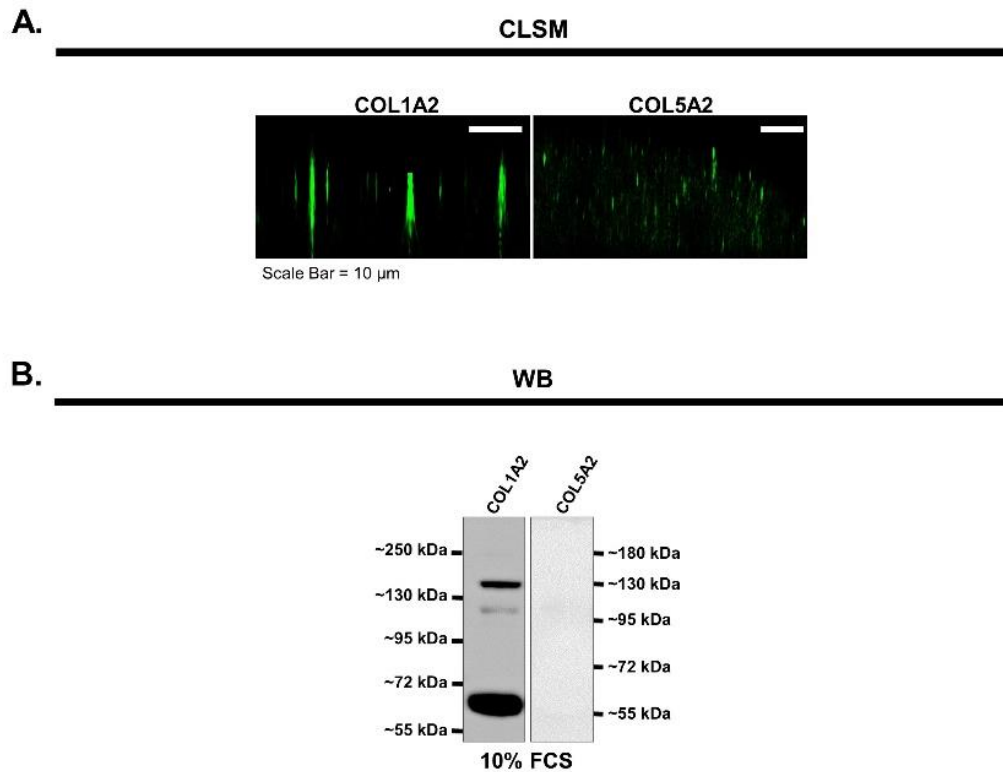
B3GALT1	2.18	1.78	0.4	1.16	0.75	0.82	No
B3GALT4	7.78	6.72	1.06	0.32	0.03	0.13	No
B3GALT6	9.54	10.08	-0.55	0.1	0	0.04	Yes
B3GAT1	4.54	4.24	0.3	0.32	0.4	0.66	No
B3GAT2	3.82	3.57	0.25	0.71	0.74	0.82	No
B3GLCT	6.85	6.28	0.57	0.28	0.11	0.34	No
B3GNT2	8.32	7.78	0.54	0.17	0.03	0.14	No
B3GNT3	3.42	1.75	1.67	0.81	0.11	0.33	No
B3GNT4	3.03	3.09	-0.06	0.57	0.92	0.88	No
B3GNT7	3.83	3.27	0.56	1.16	0.65	0.78	No
B4GALT4	2.12	1.83	0.3	0.53	0.61	0.75	No
B4GALT7	6.56	6.77	-0.21	0.23	0.4	0.66	No
BCAN	2.48	2.68	-0.2	0.61	0.76	0.82	No
BGN	15.6	14.55	1.05	0.08	0	0.01	Yes
CD44	4.41	5.75	-1.34	0.26	0.01	0.05	Yes
CHAD	2.89	2.84	0.06	0.7	0.94	0.89	No
CHADL	3.84	2.97	0.87	0.67	0.26	0.56	No
CHPF	4.47	4.91	-0.44	0.16	0.05	0.2	No
CHPF2	10.79	9.95	0.83	0.23	0.02	0.11	No
CHST1	3.05	3.61	-0.55	0.53	0.36	0.64	No
CHST10	8.48	8.38	0.11	0.15	0.51	0.71	No
CHST11	5.18	4.72	0.46	0.4	0.31	0.6	No
CHST12	9.78	9.29	0.49	0.18	0.05	0.2	No
CHST13	10.5	10.53	-0.03	0.27	0.91	0.88	No
CHST14	9.95	9.99	-0.04	0.11	0.71	0.82	No
CHST15	7.66	7.43	0.24	0.12	0.12	0.35	No
CHST2	6.79	6.28	0.51	0.51	0.37	0.66	No
CHST3	8.93	8.15	0.78	0.21	0.02	0.1	No
CHST4	3.09	2.52	0.57	0.28	0.11	0.33	No
CHST5	1.69	1.74	-0.05	0.41	0.91	0.88	No
CHST6	2.09	2.23	-0.14	0.87	0.88	0.87	No
CHST7	5.32	6.72	-1.4	0.2	0	0.03	Yes
CHST9	1.21	1.74	-0.53	0.33	0.18	0.5	No
CHSY1	9.76	9.65	0.11	0.05	0.1	0.33	No
CHSY3	4.57	4.85	-0.29	0.36	0.47	0.67	No
COL12A1	10.1	9.92	0.18	0.1	0.14	0.41	No
COL13A1	7.58	7.89	-0.31	0.32	0.38	0.66	No
COL14A1	6.3	6.4	-0.1	0.31	0.76	0.82	No
COL15A1	6.82	7.28	-0.46	0.34	0.25	0.56	No
COL18A1	12.34	12.22	0.11	0.13	0.43	0.67	No
COL1A2	6.92	2.09	4.83	0.3	0	0	Yes
COL5A1	11.06	10.32	0.74	0.17	0.01	0.07	Yes
COL5A2	10.47	9.55	0.92	0.14	0	0.03	Yes
COL6A1	10.32	10.6	-0.28	0.35	0.47	0.67	No
COL9A1	2.38	1.9	0.48	0.52	0.4	0.66	No
COL9A2	5.7	5.64	0.06	0.28	0.84	0.85	No
COL9A3	4.93	3.59	1.34	0.37	0.02	0.11	No
CSGALNACT1	4.05	4.67	-0.62	0.49	0.27	0.56	No
CSGALNACT2	4.64	5.15	-0.51	0.29	0.15	0.43	No
CSPG4	2.09	2.45	-0.36	0.49	0.5	0.7	No
DCN	3.29	3.41	-0.12	0.86	0.9	0.88	No
DSE	8.03	8.62	-0.58	0.22	0.06	0.21	No

DSEL	6.01	5.2	0.81	0.38	0.1	0.33	No
ECM2	2.17	1.92	0.26	0.79	0.76	0.82	No
ELN	10.22	5.43	4.79	0.27	0	0	Yes
EPYC	1.04	1.08	-0.04	0.07	0.57	0.75	No
ESM1	5.13	4.77	0.36	0.58	0.56	0.75	No
EXT1	9.84	9.93	-0.1	0.17	0.6	0.75	No
EXT2	9.04	8.5	0.54	0.13	0.01	0.08	Yes
EXTL1	5.04	5	0.04	0.29	0.88	0.88	No
EXTL2	7.25	7.08	0.17	0.15	0.3	0.59	No
EXTL3	9.56	9.61	-0.05	0.04	0.25	0.56	No
FAM20B	7.46	7.55	-0.09	0.42	0.84	0.85	No
FBLN2	7.93	3.13	4.81	0.4	0	0.01	Yes
FBN1	9.55	6.46	3.1	0.49	0	0.03	Yes
FMOD	3.81	1.44	2.37	0.53	0.01	0.07	Yes
FRAS1	5.53	5.39	0.14	0.44	0.77	0.82	No
FUT8	6.56	6.9	-0.33	0.26	0.27	0.56	No
GALNS	10.39	10.69	-0.3	0.36	0.46	0.67	No
GLB1	10.1	9.88	0.22	0.14	0.19	0.5	No
GLB1L2	3.22	4.4	-1.18	0.7	0.17	0.46	No
GLB1L3	2.18	2.9	-0.73	0.67	0.34	0.63	No
GLCE	9.23	8.67	0.56	0.2	0.05	0.2	No
GNS	10.66	10.84	-0.18	0.05	0.03	0.12	No
GPC1	10.69	10.8	-0.11	0.27	0.71	0.82	No
GPC2	3.98	5.67	-1.69	0.3	0	0.04	Yes
GPC3	4.3	4.61	-0.31	0.66	0.67	0.79	No
GPC4	4.1	4.29	-0.2	0.52	0.73	0.82	No
GPC5	6.04	5.04	1	0.37	0.05	0.2	No
GPC6	1.38	1.09	0.29	0.22	0.26	0.56	No
GUSB	9.37	9.65	-0.28	0.05	0.01	0.05	Yes
HAPLN1	2.03	2.2	-0.16	0.29	0.6	0.75	No
HAPLN2	5.99	6.18	-0.2	0.17	0.32	0.61	No
HAPLN2	9.88	10.14	-0.26	0.33	0.47	0.67	No
HAPLN3	11.34	9.39	1.95	0.08	0	0	Yes
HAPLN4	5.39	5.4	-0.01	0.34	0.97	0.91	No
HAS1	1.54	1.65	-0.11	0.09	0.31	0.6	No
HAS2	2.74	2.17	0.57	0.64	0.42	0.67	No
HAS3	3.3	2.87	0.42	0.47	0.42	0.67	No
HEXA	9.34	9.43	-0.09	0.1	0.41	0.66	No
HEXB	10.1	10.05	0.06	0.19	0.79	0.84	No
HEXDC	8.14	8.09	0.05	0.11	0.66	0.79	No
HGSNAT	6.01	6.04	-0.03	0.26	0.91	0.88	No
HMMR	7.71	8.74	-1.03	0.24	0.01	0.07	Yes
HPSE	4.13	3.82	0.31	0.59	0.63	0.76	No
HPSE2	4.23	4.15	0.08	0.85	0.93	0.89	No
HS2ST1	6.8	6.24	0.55	0.61	0.42	0.67	No
HS3ST1	6.05	5.4	0.65	0.15	0.01	0.07	Yes
HS3ST2	1.61	2.78	-1.17	0.2	0	0.04	Yes
HS3ST3B1	2.13	1.44	0.69	0.81	0.44	0.67	No
HS3ST4	2.47	3.29	-0.82	0.46	0.15	0.43	No
HS3ST5	4.34	4.65	-0.3	0.33	0.41	0.67	No
HS3ST6	5.03	4.75	0.28	0.42	0.54	0.73	No
HS6ST1	7.57	6.66	0.91	0.05	0	0	Yes

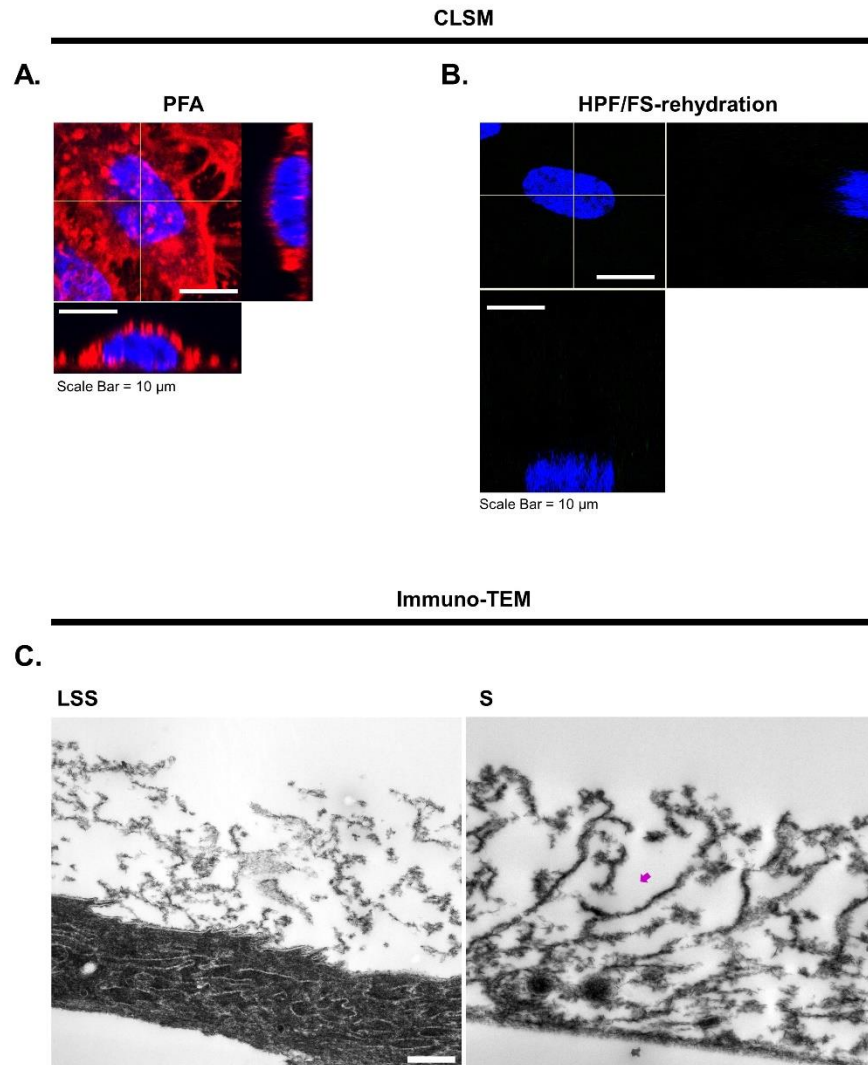
HS6ST2	4.81	5.49	-0.68	0.43	0.19	0.5	No
HSPG2	8.64	8.91	-0.27	0.36	0.5	0.7	No
HYAL1	8.08	8.41	-0.33	0.3	0.33	0.61	No
HYAL2	13.34	12.33	1.01	0.13	0	0.02	Yes
HYAL3	6.29	5.84	0.45	0.17	0.06	0.21	No
HYAL4	1.32	1.98	-0.66	0.51	0.27	0.56	No
IDS	6.59	7.02	-0.44	0.3	0.22	0.53	No
IDUA	7.2	6.88	0.32	0.15	0.1	0.32	No
IMMP1L	5	5.23	-0.23	0.15	0.21	0.52	No
IMPG1	1.78	2.08	-0.31	0.51	0.58	0.75	No
IMPG2	2.06	2.42	-0.37	0.45	0.47	0.67	No
ITGA1	3.85	3.14	0.71	0.45	0.19	0.5	No
ITGA2	9.07	9.49	-0.42	0.13	0.03	0.14	No
ITGA5	12.02	12.39	-0.37	0.1	0.02	0.1	No
ITGB1	13.1	13.24	-0.14	0.26	0.61	0.75	No
ITGB1BP1	11.11	10.99	0.12	0.19	0.57	0.75	No
KERA	2.88	2.79	0.09	0.99	0.93	0.89	No
LUM	3.44	3.67	-0.22	0.86	0.81	0.84	No
MATN2	8.51	2.57	5.94	0.18	0	0	Yes
MMP1	12.37	10.22	2.15	0.78	0.05	0.2	No
MMP10	5.56	5.29	0.27	0.49	0.61	0.75	No
MMP12	1.73	2.39	-0.66	0.43	0.2	0.51	No
MMP13	1.38	1.43	-0.05	0.18	0.8	0.84	No
MMP14	11.26	11.44	-0.18	0.17	0.35	0.64	No
MMP15	7.92	6.15	1.77	0.35	0.01	0.05	Yes
MMP16	6.84	7.23	-0.39	0.36	0.34	0.62	No
MMP17	4.72	4.26	0.46	0.33	0.23	0.54	No
MMP19	8.47	8.68	-0.22	0.43	0.64	0.78	No
MMP2	13.22	11.29	1.93	0.36	0.01	0.05	Yes
MMP20	1.33	1.64	-0.31	0.3	0.37	0.65	No
MMP23A	3.07	4.12	-1.06	0.5	0.1	0.33	No
MMP23B	2.99	2.13	0.86	0.49	0.15	0.43	No
MMP24	5.02	4.58	0.44	0.29	0.2	0.52	No
MMP24-AS1	10.51	10.03	0.49	0.07	0	0.03	Yes
MMP25	3.63	3.09	0.54	0.43	0.27	0.56	No
MMP26	1.64	1.83	-0.19	0.33	0.59	0.75	No
MMP27	2.32	1.84	0.48	1.12	0.69	0.8	No
MMP28	8.11	5.14	2.98	0.23	0	0.01	Yes
MMP3	1.71	1.89	-0.18	0.29	0.57	0.75	No
MMP7	2.13	2.81	-0.69	0.51	0.25	0.56	No
MMP8	1.24	1.86	-0.61	0.43	0.23	0.53	No
MMP9	2.46	2.54	-0.07	0.63	0.91	0.88	No
NAGLU	8.68	8.56	0.12	0.14	0.43	0.67	No
NCAN	7.05	7.33	-0.28	0.49	0.6	0.75	No
NDST1	10.93	11.2	-0.27	0.12	0.09	0.31	No
NDST2	7.63	7.13	0.5	0.07	0	0.02	Yes
NDST3	1.8	1.34	0.46	0.4	0.31	0.6	No
NDST4	2.24	2.37	-0.13	0.53	0.82	0.84	No
NOS3	12.54	11.75	0.79	0.16	0.01	0.05	Yes
NRP1	7.58	8.13	-0.55	0.04	0	0.01	Yes
NRP2	11.42	9.99	1.43	0.16	0	0.02	Yes
NYX	6.08	6.28	-0.19	0.43	0.68	0.79	No

OGN	1.15	1.18	-0.03	0.08	0.72	0.82	No
OMD	1.75	1.3	0.45	0.4	0.32	0.61	No
OPTC	4.35	3.04	1.32	1.26	0.35	0.64	No
P3H1	8.82	8.55	0.27	0.19	0.22	0.53	No
PODN	2.54	2.67	-0.13	0.54	0.82	0.85	No
PODNL1	3.05	2.69	0.36	0.61	0.59	0.75	No
PRELP	1.67	1.88	-0.22	0.37	0.59	0.75	No
PRG2	5.71	5.79	-0.08	0.31	0.8	0.84	No
PRG3	5.09	5.08	0.01	0.45	0.99	0.92	No
PRG4	5.47	5.73	-0.26	0.18	0.22	0.53	No
PTPRZ1	1.41	1.57	-0.17	0.26	0.56	0.75	No
SDC1	5.56	6.13	-0.56	0.28	0.11	0.34	No
SDC2	5.2	5.46	-0.26	0.46	0.6	0.75	No
SDC3	6.43	6.27	0.16	0.26	0.58	0.75	No
SDC4	8.33	8.77	-0.44	0.22	0.12	0.35	No
SERPINH1	11.57	10.52	1.06	0.06	0	0	Yes
SGSH	3.05	2.58	0.47	0.99	0.66	0.79	No
SPINT2	7.75	5.87	1.88	0.42	0.01	0.07	Yes
SPOCK1	12.17	12.52	-0.35	0.27	0.26	0.56	No
SPOCK2	2.39	2.81	-0.43	0.3	0.22	0.53	No
SRGN	13.04	12.84	0.19	0.16	0.29	0.59	No
ST3GAL1	8.63	8.06	0.57	0.16	0.02	0.11	No
ST3GAL2	5.05	4.66	0.4	0.27	0.21	0.52	No
ST3GAL3	6.02	6.33	-0.31	0.1	0.03	0.14	No
STAB2	5.17	5.43	-0.26	0.35	0.5	0.7	No
SULF1	10.46	5.79	4.66	0.45	0	0.01	Yes
SULF2	10.73	9.12	1.61	0.23	0	0.03	Yes
TGFBR3	8.36	7.62	0.74	0.23	0.03	0.14	No
THBD	10.54	7.97	2.57	0.22	0	0.01	Yes
TNXB	1.29	1.24	0.05	0.24	0.84	0.85	No
TSKU	8.96	8.45	0.51	0.14	0.02	0.11	No
UST	5.78	5.73	0.05	0.23	0.83	0.85	No
VCAM1	2.56	3.23	-0.67	0.86	0.48	0.69	No
VCAN	5.84	2.96	2.87	0.51	0	0.04	Yes
VEGFC	8.56	9.83	-1.27	0.18	0	0.03	Yes
WHAMMP1	9.29	8.96	0.33	0.28	0.3	0.59	No
WHAMMP3	8.89	8.5	0.39	0.25	0.19	0.5	No
XYLT1	4.85	4.33	0.51	0.24	0.09	0.32	No
XYLT2	8.68	8.55	0.13	0.11	0.32	0.61	No

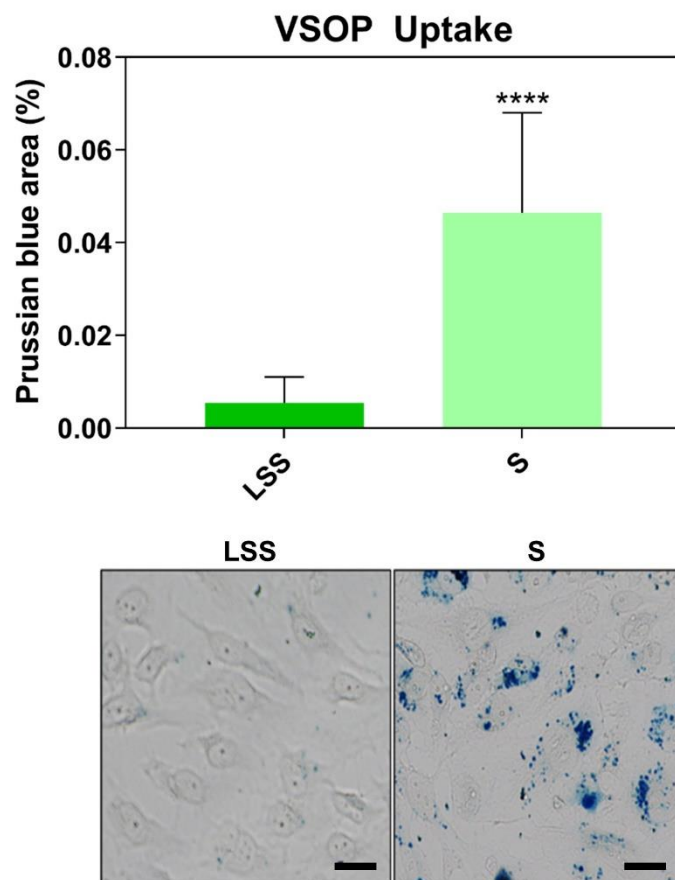
Supplementary figures



Supplementary figure 1. Traces of COL1A2 and COL5A2 signal were detectable in fetal calf serum. **A.** Orthogonal images depicting the signal distribution of COL1A2 and COL5A2 (green coloring) in cell culture media supplemented with 10% FCS on IbiTreat polymer coverslips processed by HPF/FS-RH and immunolabeled for CLSM analysis as described in the main text. **B.** Western blotting was used to analyze protein content of COL1A2 and COL5A2 in 10% FCS (method described in the main text).

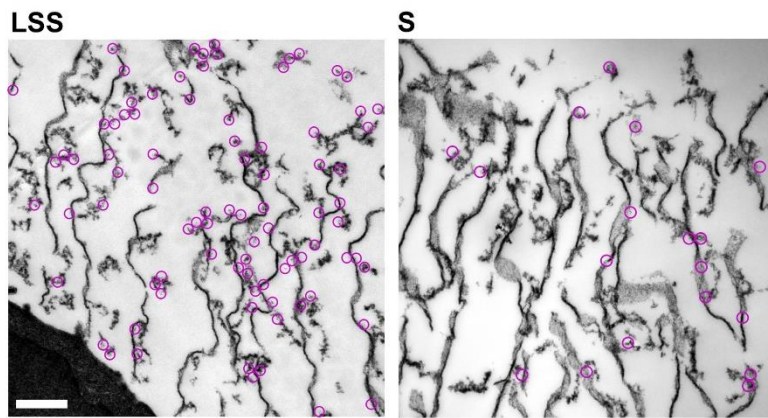


Supplementary figure 2. Unspecific binding of secondary antibodies in the ESL was not detected by confocal laser scanning microscopy or transmission electron microscopy. **A.** CLSM orthogonal image reconstruction shows formaldehyde fixed HUVECs labeled by Texas red-WGA (red) without primary antibodies incubation. Nuclei were labeled with DAPI (blue) **B.** HUVECs processed by HPF/FS. As a negative control, HUVECs were not incubated with primary antibodies. Blue coloring depicts the nuclei **C.** Immuno-EM of negative controls with omitted primary antibodies. Note the absence of immunoreactivity in the surface layer of LSS- and S-cultivated HUVECs, except single gold particle sporadically occurs (magenta thick arrow). Scale bar: c: 1000 nm.

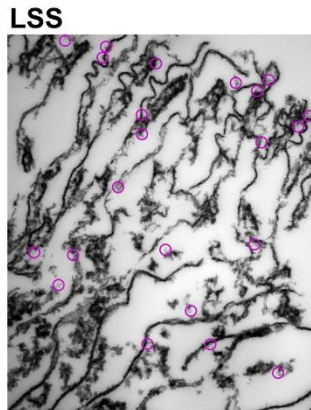


Supplementary figure 3. LSS cultivation decreased the internalization of perfused VSOPs in HUVECs. LSS- and S-cultivated HUVECs perfused with 0.75 mM VSOPs for 3 h, formaldehyde fixed and stained for Prussian blue. Graph depicts the quantification of the amount of Prussian blue stained area per ROI between LSS- and S-cultivated HUVECs. Images showing the amount of Prussian blue stained area in LSS- and S-cultivated HUVECs. Data are presented as mean \pm SD. ****P < 0.0001 vs. LSS by a ratio paired t-test. n = 9. Scale bar = 20 μ m.

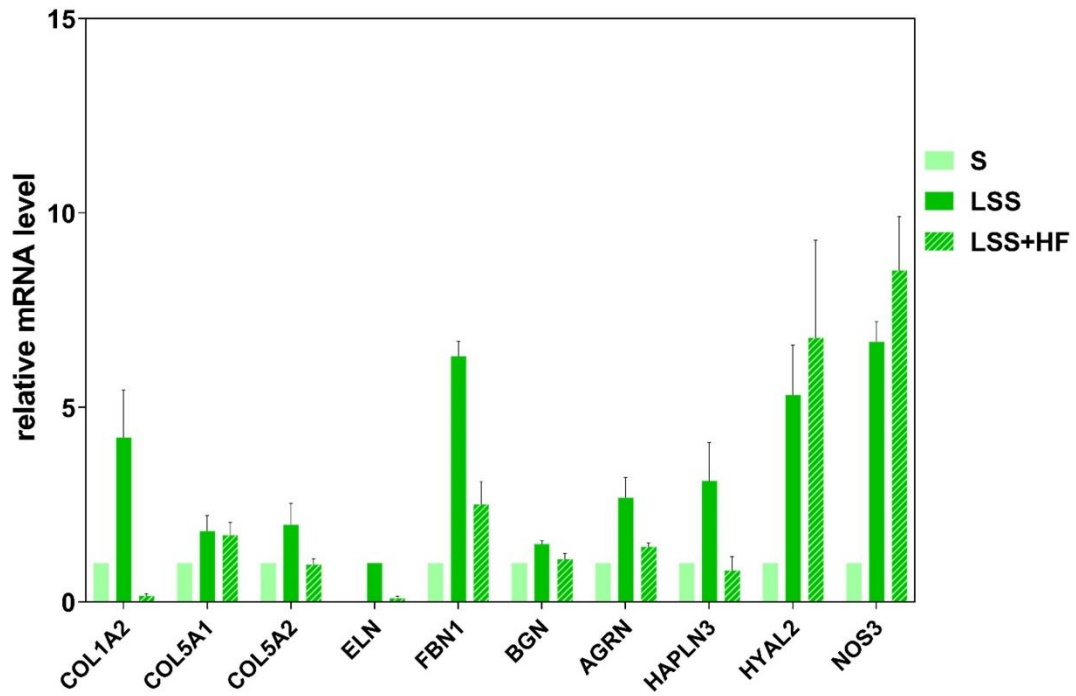
A. **COL1A2**



B. **COL5A2**



Supplementary figure 4. Pre-embedding immunogold labeling for COL1A2 and COL5A2 in the surface layer of LSS- and S-cultured HUVECs. Magenta circles highlight clusters of gold-coupled antibodies in the ESL against A. COL1A2 and B. COL5A2. Scale bar = 1 μ m



Supplementary figure 5. Halofuginone lactate reduced the expression of extracellular matrix proteins in LSS-cultivated HUVECs. Gene expression measured by RT-qPCR. Relative target gene expression was normalized to the housekeeping gene ACTB using the comparative $\Delta\Delta C_t$ analysis ($2^{-\Delta\Delta C_t}$). Data are presented as mean \pm SD. n=3.

Supplementary video legends

Supplementary video 1. CLSM 3D projection of LSS-cultivated HUVECs depicting the localization and labeling pattern of COL1A2 in the ESL. HUVECs were processed by HPF/FS in culture media supplemented with 10% FCS. Green coloring shows COL1A2 labeling and blue nuclei. Bounding box: 100 μm .

Supplementary video 2. CLSM 3D projection of S-cultivated HUVECs depicting the localization and labeling pattern of COL1A2 in the ESL. HUVECs were processed by HPF/FS in culture media supplemented with 10% FCS. Green coloring shows COL1A2 labeling and blue nuclei. Bounding box: 100 μm .

Supplementary video 3. CLSM 3D projection of LSS-cultivated HUVECs depicting the localization and labeling pattern of COL5A2 in the ESL. HUVECs were processed by HPF/FS in culture media supplemented with 1% BSA. Green coloring shows COL5A2 labeling and blue nuclei. Bounding box: 100 μm .

Supplementary video 4. CLSM 3D projection of S-cultivated HUVECs depicting the localization and labeling pattern of COL5A2 in the ESL. HUVECs were processed by HPF/FS in culture media supplemented with 1% BSA. Green coloring shows COL5A2 labeling and blue nuclei. Bounding box: 100 μm .