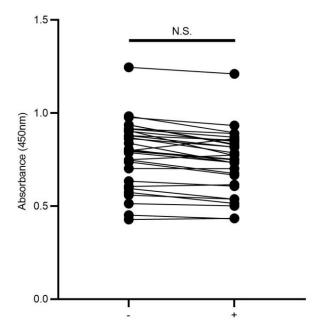
SUPPLEMENTAL MATERIAL

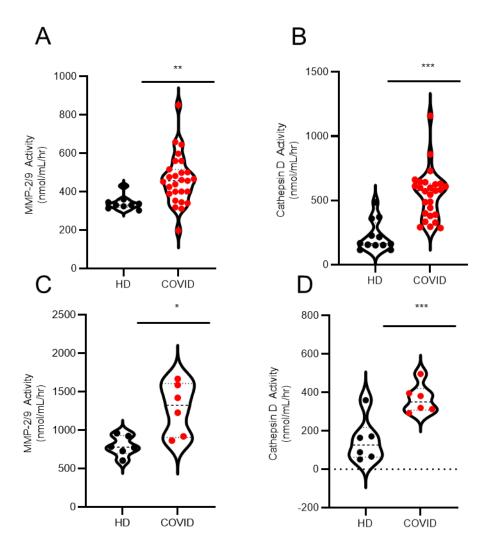
Supplemental Figures

Figure S1

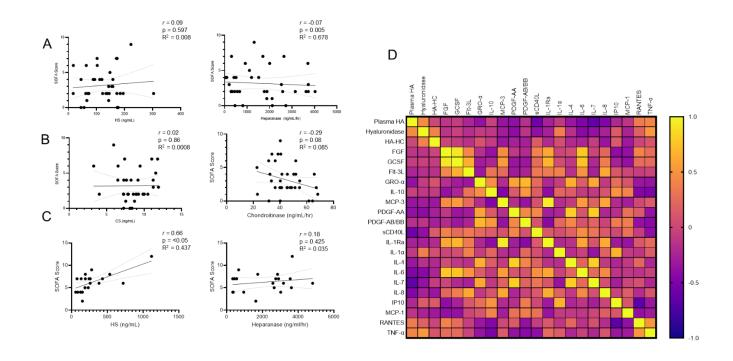


Supplemental Figure 1. JM403-based ELISA Detection of Plasma HS Does Not Detect Exogenous Low Molecular Weight Heparin Anticoagulant Enoxaparin. Plasma samples from healthy donors (n=10) or COVID-19 patients (n=32) were measured without (closed circles) or with (open circles) 200ng exogenous enoxaparin. Data are reported as mean \pm SEM. Differences between the groups were calculated using Mann-Whitney U tests.

Figure S2

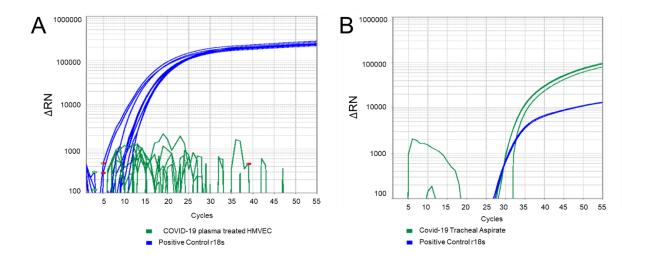


Supplemental Figure 2. Sheddase activity is increased in COVID-19 patient plasma and released from plasma treated endothelial cells compared to healthy donor plasma. Protease activity was measured for (A) MMP2/9 and (B) cathepsin D in healthy donors (n = 10) and COVID-19 patient plasma (n = 27). LMVECs were exposed to plasma from healthy donors or COVID-19 patients (n = 6 each). Differences between the groups were calculated using Mann-Whitney U test, **p<0.01, ***p<0.001. Sheddase activity released into the media was measured for (C) MMP2/9 and (D) cathepsin D. Data are reported as mean \pm SEM. Differences between the groups were calculated using unpaired Student's t-test, 2 tailed, *p<0.05, ***p<0.001.



Supplemental Figure 3. Circulating levels of HA and hyaluronidase activity correlate with clinical and inflammatory signatures of COVID-19. Linear regression analysis of SOFA scores with (A) HS or heparanase and (B) CS or chondroitinase in COVID-19 patients (n=46). (C) Corellation analysis of SOFA scores with HS or heparanase in sepsis patients (n=23). Spearman correlation matrix (D) of plasma HA, hyaluronidase activity, and cytokine levels in healthy donors (n=18). Yellow indicates a positive correlation and purple indicates a negative correlation. Pearson correlation coefficient was used to determine the *r* value of the correlation between the 2 groups.

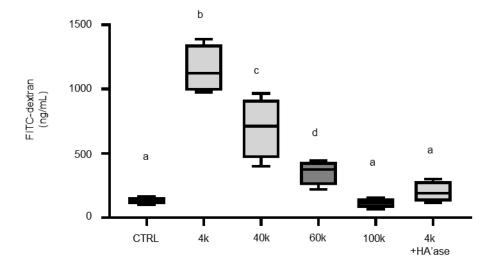
Figure S4.



Supplemental Figure 4. RT-PCR Analysis of LMVECs Treated With COVID-19 Plasma.

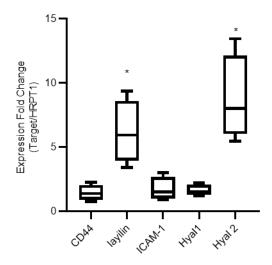
Cells were cultured in the presence or absence of COVID-19 patient plasma for 24 hours and analyzed for the presence of the SARS-CoV-2 N1 gene. (A) Representative tracing from ECs treated with COVID-19 patient plasma samples (n = 12) for SARS-CoV-2 mRNA (green) or r18s mRNA (blue) as a positive control. (B) mRNA isolated from COVID-19 patient tracheal aspirates (n = 3) served as a positive control (green) for the detection of viral mRNA.

Figure S5.



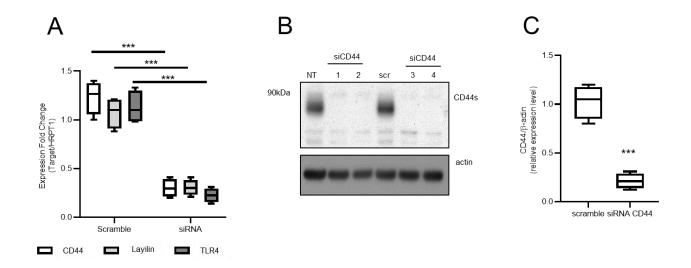
Supplemental Figure 5. HA fragments <100 kDa induce a size-dependent disruption of the endothelial barrier permeability in LMVECs. LMVEC were seeded on permeable supports (3 μ m pore size) placed into a 24-well plate and grown to confluence. Cells were treated with or without biosynthetic HA polymers of specific sizes (1000 ng) for 16 hours at 37°C to induce endothelial barrier disruption. The upper chamber was replaced with FITC-conjugated dextran (1 mg/mL, 40kDa) in PBS and a sample of medium from the lower chamber was measured after 1 hour. In some experiments, HA treatments were pre-digested with *Streptomyces* hyaluronidase (HA'ase) as a specificity control. Data is reported as mean \pm SEM; n = 5 independent experiments, statistics were calculated using a 1-way ANOVA followed by Tukey's multiple comparison tests. Different alphabetical superscripts are significantly different from each other, p < 0.05.

Figure S6.



Supplemental Figure 6. **HA 4kDa treatment increases mRNA expression of layilin and Hyal- 2.** LMVEC were seeded in a 6-well plate and grown to confluence. Cells were treated with or without biosynthetic HA 4kDa (1500 ng/mL) for 4 hours at 37°C. RNA was isolated from LMVEC and cDNA was made from RNA. qRT-PCR was used to measure relative mRNA expression levels of HA receptors (CD44, layilin), adhesive receptor ICAM-1, and hyaluronidases (Hyal-1, Hyal-2), normalized to housekeeping gene HPRT1. Biosynthetic HA 4kDa was pre-digested with *Streptomyces* hyaluronidase (HA'ase) as a specificity control. *p<0.05, Student's t-test, 2-tailed, n = 5 independent experiments. Data are reported as mean \pm SEM.

Figure S7.



Supplemental Figure 7. **Targeted siRNA knockdown of HA-receptors.** LMVEC were seeded in a 6-well plate and grown to confluence. Cells were treated with either vehicle (NT), scrambled control siRNA (scr, 10nM), or siRNA to indicated HA receptors (10nM). (A) mRNA expression normalized to HPRT1. (B) Representative western blot analysis of CD44 protein levels from cells treated for 48hrs with 10nM CD44 siRNA. NT (no treatment), scr (scrambled control siRNA), numeric lanes indicate independent biological replicates. (C) Densitometry quantification of CD44 knock down. ***p<0.001, unpaired Student's t-test, 2-tailed, n = 5 independent experiments. Data are reported as mean ± SEM.

Supplemental Tables

A Supplemental Table 1

	Plasma HA	Hyaluronidase	HA-HC	FGF	GCSF	Flt-3L	GRO	IL-10	MCP-3	PDGF-AA	PDGF-AB/BB	sCD40L	IL-1RA	IL-1a	IL-4	IL-6	IL-7	IL-8	IP10	MCP-1	RANTES	TNFa
Plasma HA	1	0.418681979	0.640112775	0.145478721	0.283974168	-0.332446419	0.073197362	0.513474029	0.348639002	0.144464857	0.384950636	0.259672613	0.55420658	0.339773839	0.174604844	0.717066753	0.273568971	0.661657305	0.684985894	0.778090584	-0.324258103	0.62502706
Hyaluronidase	0.418681979	1	0.693359234	0.250575193	0.269596972	-0.44257053	0.50284883	0.337271388	0.066992575	0.612471773	0.603198823	0.49630061	0.188938308	0.437286546	0.511313508	0.345191527	0.411797065	0.280687591	0.503250762	0.322685192	-0.111876508	0.370579623
HA-HC	0.640112775	0.693359234	1	0.587536832	0.561555581	-0.303425481	0.185790439	0.612802653	0.330531833	0.30382428	0.598136877	0.41626888	0.531584727	0.441968278	0.280889506	0.633535318	0.338105064	0.469653362	0.667509549	0.508303604	-0.351898256	0.440642826
FGF	0.145478721	0.250575193	0.587536832	1	0.681796741	0.091850704	0.067698063	0.139376693	0.388888018	0.115546439	0.198697473	0.384843485	0.349947157	0.388895124	0.20926382	0.257850046	0.183234861	0.209464846	0.15138799	0.078192667	0.086469281	0.38078776
GCSF	0.283974168	0.269596972	0.561555581	0.681796741	1	0.11812028	0.057568014	0.206183504	0.32213346	0.073302548	0.209078506	0.351565189	0.248445878	0.499744862	0.245320281	0.179479583	0.341577577	0.322836946	0.286623235	0.205467496	0.032248324	0.432593315
Flt-3L	-0.332446419	-0.44257053	-0.303425481	0.091850704	0.11812028		-0.220707645	-0.230655893	0.005376261	-0.18666754	-0.145309285	-0.04366724	-0.151264301	0.021683426	-0.242190704	-0.217106	-0.099387674	0.000988902	-0.274498716	-0.155074931	0.120908257	-0.165718968
GRO	0.073197362	0.50284883	0.185790439	0.067698063	0.057568014	-0.220707645	1	-0.032493906	-0.06858982	0.591472016	0.565922189	0.542865122	-0.232329516	0.205562087	0.741005085	0.008068879	0.436177407	0.171140169		0.027822074	-0.161734346	0.214117078
IL-10	0.513474029	0.337271388	0.612802653	0.139376693	0.206183504	-0.230655893	-0.032493906	1	0.373366404	0.177436393	0.243986347	0.216006837	0.570384482	0.289278515	0.091361299	0.646032022	0.331109117	0.338894333		0.447887115	-0.370035568	0.343133589
MCP-3	0.348639002	0.066992575	0.330531833	0.388888018	0.32213346		-0.06858982	0.373366404	1	0.122397998	0.296344712	0.397599197	0.675483501	0.472233054	0.062937969	0.576270924	0.483597598	0.72455472	0.323784948	0.248149173	-0.121352024	0.384197082
PDGF-AA	0.144464857	0.612471773	0.30382428	0.115546439	0.073302548		0.591472016	0.177436393	0.122397998		0.680315106	0.667101164	0.021184165	0.232974931	0.69921875	0.154067452		0.117206608		0.053097309	0.131009343	0.155417622
PDGF-AB/BB	0.384950636	0.603198823	0.598136877	0.198697473	0.209078506	-0.145309285	0.565922189	0.243986347	0.296344712	0.680315106	0.050000450	0.850396456	0.178449825	0.293505962	0.414211045	0.294636592		0.43325408	0.274829474	0.132119722	0.062488201	0.353488218
sCD40L IL-1RA	0.259672613	0.49630061 0.188938308	0.41626888	0.384843485	0.351565189		0.542805122	0.216006837	0.397599197	0.007101104	0.850390450	0.005064565	0.285061565	0.475487476	0.496690705	0.338212664		0.496503224	0.28191998	0.193390304	0.018279168 -0.26445315	0.475948282
IL-1NA	0.339773839	0.437286546	0.441968278	0.349947 157	0.248445878		-0.232329516 0.205562087	0.289278515	0.472233054	0.021184165	0.178449825	0.285061565 0.475487476	0.348822582	0.348822582	0.008946478	0.700414000	0.376885125	0.509024390	0.495205504 0.711436713	0.532451866	-0.20445515	0.533704627 0.36834519
IL-14	0.339773839	0.437280040	0.280889506	0.20926382	0.245320281	-0.242190704	0.741005085	0.289278515		0.69921875	0.414211045	0.475487476	0.008946478	0.471775371	0.471775371	0.09522802		0.200319523		0.419740002	0.049371193	0.242438542
IL-6	0.174004644	0.345191527	0.633535318	0.257850046	0.179479583		0.008068879	0.646032022		0.154067452	0.294636592	0.338212664	0.760414066	0.399288915	0.09522802	0.09322802	0.334979214	0.581453768	0.590575533	0.709753753	-0.371167931	
IL-7	0.273568971	0.411797065	0.338105064	0.183234861	0.341577577	-0.099387674	0.436177407	0.331109117	0.483597598	0.134007432	0.562404563	0.658145691	0.376885125	0.599208915	0.653507963	0.334979214	0.334979214	0.507922005	0.44072491	0.325819811	0.02694895	0.419539712
IL-8	0.661657305	0.280687591	0.469653362	0.209464846	0.322836946		0.171140169		_	0.117206608	0.43325408	0.496503224	0.569624396	0.518942486	0.200319523	0.581453768	0.597922005	0.557522005	0.520034755	0.570755038	-0.121060836	0.675110068
IP10	0.684985894	0.503250762	0.667509549	0.15138799	0.286623235		0.182290439		0.323784948	0.185163418	0.274829474	0.28191998	0.495205504	0.711436713	0.394302355	0.590575533	0.44072491	0.520034755	1	0.701874015	-0.305792832	0.383724012
MCP-1	0.778090584	0.322685192	0.508303604	0.078192667	0.205467496		0.027822074	0.447887115	0.248149173			0.193390304	0.532451866	0.419746602	0.103942168	0.709753753	0.325819811	0.570755038	0.701874015	1	-0.409632268	
RANTES	-0.324258103	-0.111876508	-0.351898256	0.086469281	0.032248324	0.120908257	-0.161734346	-0.370035568	-0.121352024	0.131009343		0.018279168	-0.26445315	-0.081343514	0.049371193		0.02694895	-0.121060836	-0.305792832	-0.409632268		-0.135483893
TNFa	0.62502706	0.370579623	0.440642826	0.38078776	0.432593315	-0.165718968	0.214117078	0.343133589	0.384197082	0.155417622		0.475948282	0.533704627	0.36834519	0.242438542	0.608728567	0.419539712	0.675110068	0.383724012	0.602255344	-0.135483893	1

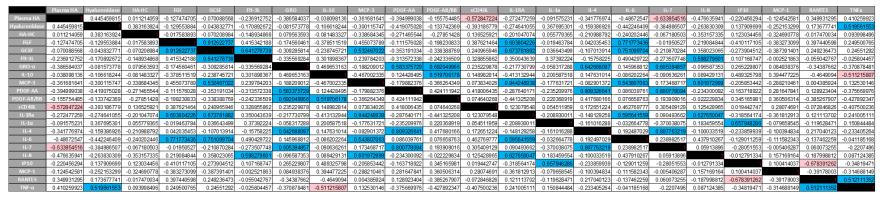
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Supplemental Table 1. Spearman correlations for HA, hyaluronidase, HA-HC, and inflammatory cytokines in COVID-19 patients.

Spearman's ranked correlations are indicated for COVID-19 patients (n=46), R>0.5 in blue, R<-0.5 in red. Significant correlations (P<0.05) are indicated in yellow highlight. A) R values. B) P values.

A Supplemental Table 2



В

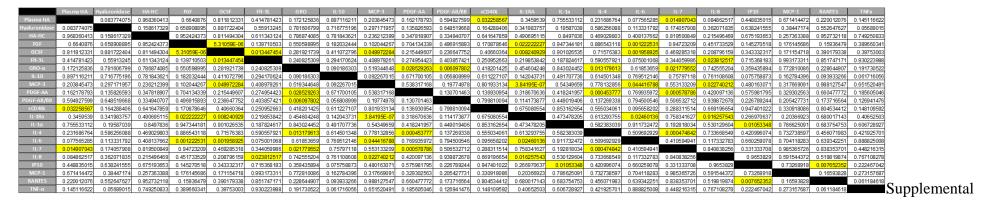


Table 2. **Spearman correlations for HA, hyaluronidase, HA-HC, and inflammatory cytokines in healthy donors.** Spearman's ranked correlations are indicated for healthy donors (n=18), R>0.5 in blue, R<-0.5 in red. Significant correlations (P<0.05) are indicated in yellow highlight. A) R values. B) P values.