Liver injury in COVID-19 and IL-6 trans-signaling-induced endotheliopathy

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I. Supplementary materials and methods

Histological Examination

Hematoxylin and Eosin (H&E) staining and immunohistochemical staining were performed on paraffin-embedded liver specimens. For immunohistochemical staining, the following antibodies were used: polyclonal rabbit anti-human vWF antibody (code A0082; dilution 1:4000; Dako), monoclonal mouse anti-human CD61 antibody (clone Y2/51; dilution 1:20; Dako). CD61 is a specific marker of platelets and megakaryocytes. Immunohistochemical examination was performed using an automated immunostainer (intelliPATH FLX; Biocare Medical), according to the manufacturer's instructions. Histological examination was performed blindly by an experienced pathologist confident with liver histopathology (R.K.). The average number of neutrophils in the sinusoid at 4 randomly selected sinusoidal regions was evaluated and scored as neutrophil infiltration in the sinusoids. The vWF positive area and CD61 positive area in each specimen were quantified in 4 randomly selected regions in an unbiased fashion using Fiji (ImageJ).

Immunofluorescence

vWF and platelets were detected by immunofluorescent labeling of paraffin-embedded human liver specimens. Fibrinogen was detected by immunofluorescent labeling of paraffin-embedded human liver specimens and mouse liver specimens. For immunofluorescence with vWF and CD41 (Integrin alpha IIb, a marker of platelets and megakaryocytes) using paraffin-embedded human liver specimens, we deparaffinized the tissues and performed heat-induced epitope retrieval with citrate Buffer (0.1 M citric acid and 0.1 M sodium citrate). For immunofluorescence for fibrinogen, we deparaffinized the tissues and performed proteinase K-induced epitope retrieval. The sections were blocked in 5% normal donkey serum in phosphate-buffered saline (PBS) for 1 h at room temperature and incubated with primary antibodies overnight at 4℃. The following antibodies were used: polyclonal rabbit anti-human vWF antibody (code A0082; dilution 1:200; Dako), monoclonal mouse anti-human CD41 (Integrin alpha IIb) antibody (sc-365938; dilution 1:100; Santa Cruz Biotechnology), and polyclonal rabbit anti-human fibrinogen antibody (code A0080; dilution 1:100; Dako). Primary antibodies were detected using Alexa Fluor 488-conjugated donkey anti-rabbit IgG, Alexa Fluor 647-conjugated donkey anti-mouse IgG, Alexa Fluor 488-conjugated donkey anti-rat IgG, or Alexa Fluor 647-conjugated donkey anti-rabbit IgG secondary antibody (dilution 1:300, Invitrogen), respectively. All samples were mounted with FluoroshieldTM containing DAPI (Sigma-Aldrich) and observed with a fluorescence microscope (Zeiss

Observer Z1, Zeiss). The antibody conditions are summarized in Table S1. The Fibrinogen positive area in each specimen was quantified in 6 randomly selected regions in an unbiased fashion using Fiji (ImageJ).

Human primary LSECs

Human liver sinusoidal microvascular endothelial cells were purchased from PELOBiotech (#PB-CH153-5511, Germany). The manufacturer reported more than 95% of the isolated cells to have cytoplasmic immunofluorescent staining for von Willebrand factor/Factor VIII, PECAM1, and Di-I-Ac-LDL uptake[1]. LSECs were cultured in a humidified atmosphere at 5% CO2 and 37 °C and were grown in microvascular endothelial cell growth medium kit enhanced (PELOBiotech, #PB-MH-100-4099), plated on 6-or 12-well plates or microscope cover glass (#12545100, Fisher Scientific), both pre-coated with speed coat solution (PELOBiotech, #PB-LU-000-0002-00), and used up to passage 6. In all experiments, LSECs were cultured in serum-free medium overnight and then stimulated by IL-6(20ng/ml), sIL-6R(20ng/ml), and IL-6/sIL-6R(20ng/ml) complex (referred to henceforth as “complex”).

Human umbilical vein endothelial cells (HUVECs) – *Supplementary results*

We obtained HUVECs from the Vascular Biology and Therapeutics Program (Yale School of Medicine, CT, USA). Briefly, all cells were grown in endothelial culture medium (ECM) (No. 1001, ScienCell; Carlsbad, CA, USA) containing 5%-fetal bovine serum (FBS) (No. 0025), 1% endothelial cell growth supplement (No. 1052) and 1% penicillin/streptomycin solution (No. 0503) in 5% CO2 at 37 °C. At passage 3–6, cells were used for experiments. Generally, 1×10^5 HUVECs per well were seeded in a six-well plate with fibronectin coating (No. 8248). HUVECs were cultured in serum-free medium overnight and then stimulated by IL-6 (20ng/ml), sIL-6R (20ng/ml), and IL-6/sIL-6R (20ng/ml) complex.

Primary mouse hepatocytes

Hepatocytes were isolated from WT mice by collagenase perfusion and maintained in a collagen sandwich culture system as described[2] with slight modifications. Briefly, cells were cultured on collagen-coated cell culture dishes in adherence culture media (William’s medium E; Gibco 12551-032 (45ml), HEPES-1M; Gibco 15630 (500µl), L-glutamine 200mM; Gibco 25030-081 (500µl), Gentamycin 10mg/ml; Gibco 15710-064 (50µl), 100xSP (500µl), Dexamethasone 25mM; Lonza CC-4021 (5µl), Insulin; Lonza CC-4021 (5.75µl), Heat Inactivated FBS 2.5ml). The initial

plating density was 4 × 10^5/ mL. Six hours later, cells were replaced with basal maintenance medium (William's medium E; 49.5ml, Gentamicin; 50µl, 100SP; 500µl, Insulin; 5.75µl) and incubated overnight. Cells were then subjected to experimental conditions as described.

Platelets

We obtained expired donor platelets from the Yale-New Haven Hospital Blood Bank (Yale-New Haven Hospital, CT, USA). Briefly, 1 × 10^5 LSECs were seeded on microscope cover glass (see above). 10 mL of the platelet suspension from the blood bank was centrifuged at 800 x g for 15 minutes at room temperature. The platelet pellet was subsequently resuspended in platelet wash buffer (103 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgCl2, 5 mmol/L glucose, 36 mmol/L citric acid, pH 6.5) containing 3.5 mg/ml BSA) and centrifuged again at 800 x g for 15 minutes at room temperature. The platelet pellet was resuspended in serum-free LSEC media and added to LSECs treated with either complex or control medium for 4 hours at a ratio of 100 platelets to 1 LSEC (final platelet concentration 1 x 10^7/mL). After co-culturing for 2 hours, the LSECs were gently washed three times with PBS. Cells were fixed with 4%-paraformaldehyde (PFA) solution for 15 min at room temperature and permeabilized for 5 min in 0.25% Triton-X/PBS. After blocking for 60 min in 5% donkey serum, cells were incubated with Alexa Fluor 488 Phalloidin (1:200, Molecular Probe) for 20 minutes at room temperature and then incubated with anti-Integrin alpha IIb (1:200, Santa-Cruz, Biotechnology, Dallas, TX), overnight at 4 °C. After washing cells three times with PBS, cells were incubated with secondary antibody Alexa 647 (1:300, Invitrogen, Carlsbad, CA) for 1 hour. Cells on coverslips were mounted with Antifade Reagent with DAPI (Sigma) and imaged utilizing a Zeiss Observer Z1 fluorescence microscope. For image analysis, fluorescence intensities were measured by ImageJ after defining the boundaries of the cells by setting a threshold.

Chemicals and reagents

Recombinant human IL-6(20ng/ml), soluble IL-6Rα(20ng/ml), and soluble gp130(100ng/ml) were supplied by R&D Systems (Minneapolis, MN, USA). Ruxolitinib (No. 23215) and Filgotinib (No. 17669) were purchased from Cayman Chemical (Ann Arbor, MI, USA). IL-6(20ng/ml) and soluble IL-6Rα(20ng/ml) complex formation was done by mixing in solution and letting stand for 30 minutes before using. Soluble gp130 was incubated with the IL-6/sIL-6Rα(20ng/ml) complex for 30 minutes at room temperature before using. Ruxolitinib or Filgotinib were added to cells 20 minutes before

experimental treatment.

Western blot analysis

Proteins were extracted using a lysis buffer containing 50mmol/L Tris-HCl, 0.1mmol/L EGTA, 0.1mmol/L EDTA, 0.1% SDS, 0.1% deoxycholic acid, 1% (vol/vol) Nonidet P-40, 5mmol/L sodium fluoride, 1mmol/L sodium pyrophosphate, 1mmol/L activated sodium vanadate, 0.32% protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany), and 0.027% Pefabloc (Roche Diagnostics). Protein concentrations were measured using a BCA assay method. Equal amounts of proteins were loaded and separated by SDS-PAGE. Proteins were transferred to a 0.2μm nitrocellulose membrane (Bio-Rad Laboratories) and were analyzed by immunoblotting with primary antibodies shown in Supplementary Table1. After washing with Tris buffered saline containing 0.1% Tween-20 (TBS/T), membranes were incubated with fluorophore-conjugated secondary antibodies (Li-Cor Biotechnology, Lincoln, NE) having 680nm or 800nm emission. Proteins were visualized and quantified using the Odyssey Infrared Imaging System (Li-Cor Biotechnology, Lincoln, NE). β-actin was used as a loading control.

Quantitative reverse-transcription Polymerase Chain Reaction

Total RNA was isolated from cells and liver tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA quantity and quality were assessed by micro-volume spectrophotometry on the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). Extracted total RNA was used as a template for reverse transcription into cDNA using Reverse Transcript Reagents kit (Roche Molecular Systems, Branchburg, NJ). Synthesized cDNA was mixed with iTaq Universal SYBR green supermix (Bio-Rad Laboratories, Hercules, CA) and amplified by a real time ABI 7500 PCR system (Applied Biosystems, Foster City, CA). The values of the human or mouse genes were normalized to the level of 18S mRNA. Forward and reverse primer sequences are shown in Table S2. The oligonucleotide primers were all custom designed and synthesized by the Keck Oligonucleotide facility at Yale School of Medicine.

Flow cytometry

LSECs were treated with IL-6/sIL-6Rα complex (20ng/ml) for 6 hours at 37°C. Trypsin (0.05%) with EDTA was used to detach LSECs and cells were then washed twice with PBS. LSECs were incubated with 3%-FBS in PBS for 30 min at 4°C to prevent any nonspecific binding. LSECs were not fixed and incubated with anti-WF

(1:50, A0082; Dako;) for 20 minutes at 4°C. And then incubated with Donkey anti-rabbit Alexa 647(1:50, A31573, Invitrogen) at 4°C for 20 minutes in the dark. Flow cytometric analysis was carried out using BD Accuri C6vFlow Cytometer (BD Biosciences). FACS data were analyzed with FlowJo sotware (FlowJo, Ashland, Oregon, USA)

Murine hepatitis virus (MHV)-A59 infected mice – *Supplementary results*

We obtained livers from MHV-A59-infected mice from our collaborator, Dr. Carlos Fernandez-Hernando (Yale University). C57BL/6J (Wide-type, WT) mice were purchased from Jackson Laboratories. The mice were housed in a biosafety level 2 (BSL2) facility through Yale Animal Resources Center. All the experiments were approved by the Institutional Animal Care Use Committee of Yale School of Medicine. Male WT mice (4 months old) were anesthetized by intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine, and intranasally inoculated with MHV-A59 (BEI Resources, Manassas, VA) at a dose of 1.25 X 10^6 PFU (sublethal dose). Body weight was monitored before and after infection. Arterial oxygen saturation and respiratory rate were measured via pulse oximetry using the MouseSTAT Jr. (Kent Scientific, Torrington, CT) after infection. Seven days after infection, the livers were harvested from MHV-A59 infected mice. Liver tissues were fixed with 4%-PFA, embedded in paraffin blocks and sectioned for further analysis. For frozen sections, liver tissues were fixed in 4%-PFA, dehydrated in 30% sucrose solution, and frozen as a block in OCT compound. Paraffin sections were used for fibrinogen staining. Paraffin sections were de-paraffinized with xylene and dehydrated with graded ethanol. Proteinase K was used for 5 minutes at 37 °C for antigen retrieval for fibrinogen staining.

Liver sections were incubated with blocking buffer (5% donkey serum and 0.3% Triton X-100 in PBS) for 1 hour. After blocking, the sections were incubated with anti-fibrinogen (1:100, A0080, Dako,) overnight at 4°C. After washing with PBS three times each for 5 minutes, samples were treated with Alexa Fluor 647 donkey anti-rabbit IgG (1:300, A31573, Invitrogen), for 30 minutes at room temperature. All samples were mounted with FluoroshieldTM containing DAPI (Sigma-Aldrich) and observed utilizing a fluorescence microscope (Zeiss Observer Z1, Oberkochen, Germany) or a confocal microscope (Leica SP5, Wetzlar, Germany).

Small interfering (siRNA) gene knockdown in human LSECs

To induce gene knockdown in human LSECs the siRNA approach was used. Sequence suggestions were made by Dharmacon and siR-STAT3 (#M-003544-02-005) and siR-STAT1 (#M-003543-01-005) were purchased from Dharmacon (Lafayette, CO,

USA). Sequences are shown in Table S3. Both siRNAs were used for the transfection of the cells, which was achieved by using ScreenFect (Wako Chemicals USA, Richmond, VA). Silencer Negative Control siRNA (Invitrogen, USA) was used as a control for nonspecific effects.

II. Supplementary figures

Fig. S1

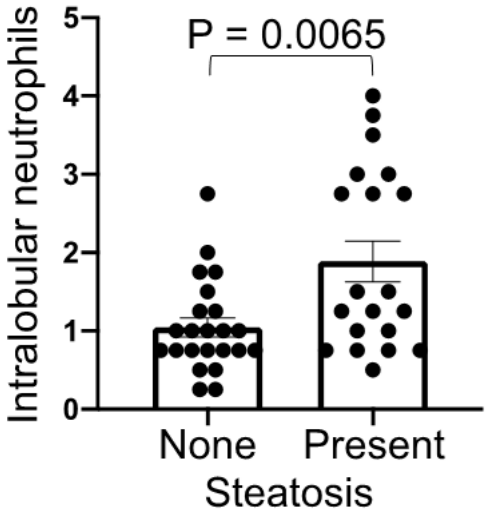

Fig. S1: Number of intralobular neutrophils in liver tissue from COVID-19 patients with steatosis (>5%) present (n = 20) and absent (n = 23). P = 0.0065. Welch's t-test was used.

Fig. S2

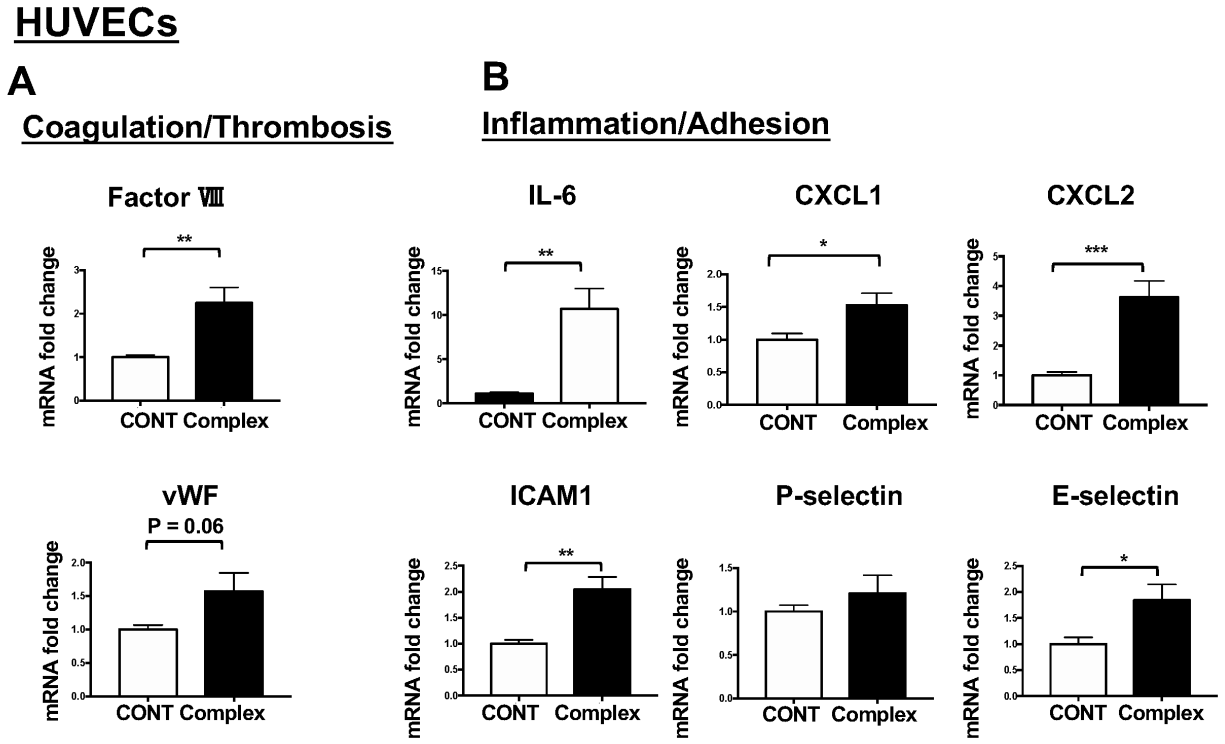

Fig. S2. IL-6 trans-signaling induces endotheliopathy in human umbilical vein endothelial cells (HUVECs).

HUVECs were incubated with control or IL-6/sIL-6R complex (20ng/ml) for 1 hour. Total RNA was collected and used for qPCR analysis. qPCR of markers for **(A)** procoagulant; Factor Ⅷ (2.25-fold, $P < 0.01$) and vWF (1.57-fold, $P = 0.06$) and **(B)** proinflammatory endotheliopathy; IL-6 (10.7-fold, $P < 0.01$), CXCL1/2 (1.53/3.62-folds, $P < 0.05$/ 0.001), ICAM1 (2.05-fold, $P < 0.01$), P-selectin (1.21-fold, $P = 0.37$), and E-selectin (1.84-fold, $P < 0.05$). Data is presented as mean ± SEM of at least 3 experiments. Unpaired t-test was used. *P<0.05, **P<0.01, ***P<0.001.

Fig. S3

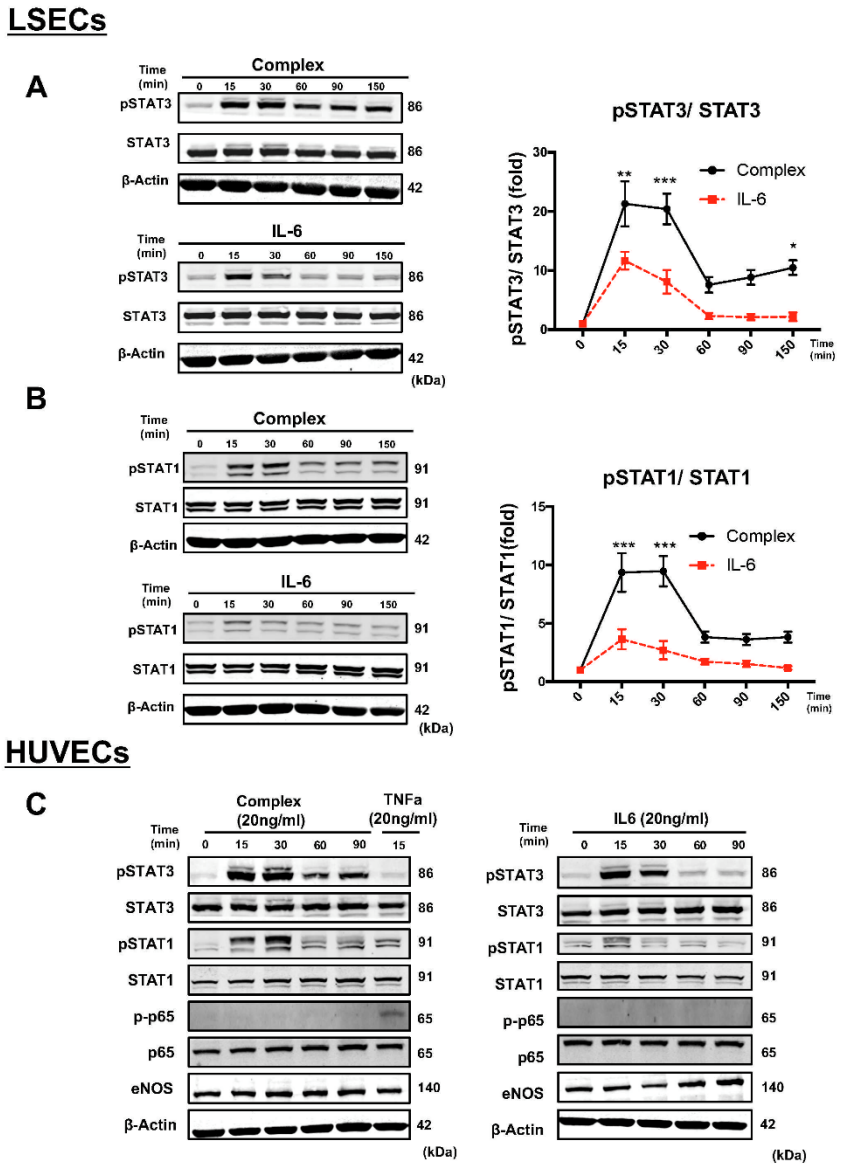

Fig. S3. Activation of signaling pathways by IL-6 in the presence or absence of soluble IL-6R in human liver sinusoidal endothelial cells (LSECs) and HUVECs.

Western blot analyses showing phosphorylation of **(A)** STAT3 and **(B)** STAT1 in primary human LSECs or **(C)** in HUVECs treated with IL-6 alone (20 ng/ml) or in combination with sIL-6R (20 ng/ml). One representative blot containing the phosphorylated protein, total protein, and β-actin (loading control) is shown for each pathway. The ratio of the phosphorylated proteins and the total proteins are calculated. The graphs show the fold change (control; 0 min is set to 1). Data is presented as mean ± SEM of at least 3 experiments. Two-way ANOVA with Sidak's multiple comparison test was used. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. S4

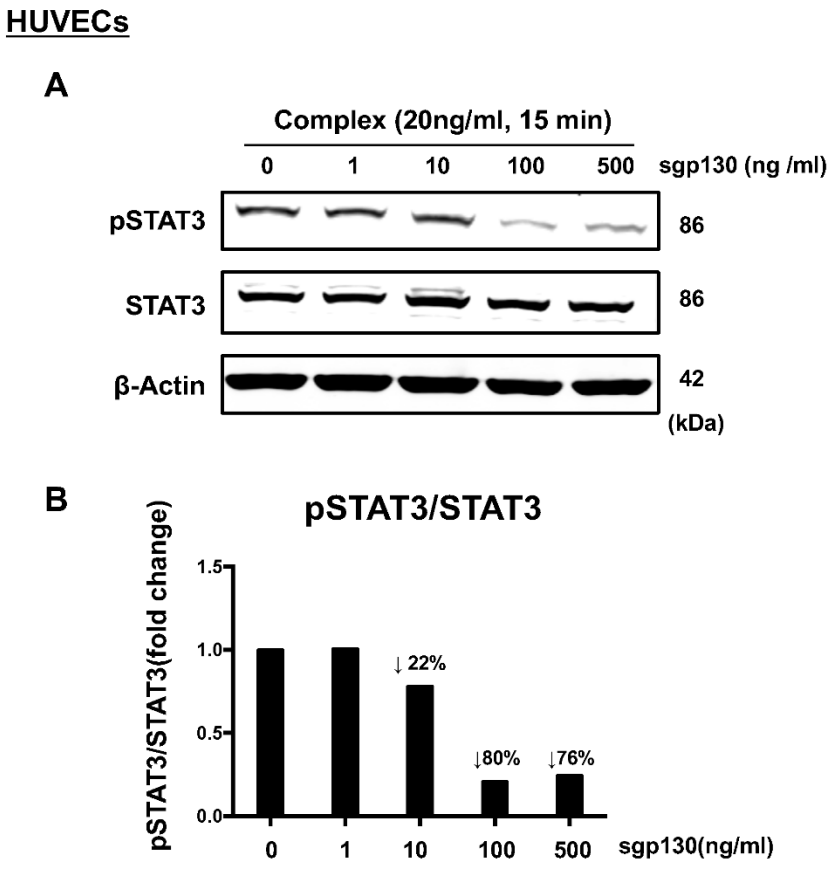

Fig. S4. Soluble gp130 (sgp130) blocks STAT3 phosphorylation induced by IL-6 trans-signaling in HUVECs.

A dose response (0, 1, 10, 100, 500 ng/ml) experiment to determine optimal concentrations of sgp130 to block activation of STAT3. HUVECs were treated with IL-6/sIL-6R complex (20 ng/ml) in the presence or absence of sgp130 Fc (0, 1, 10, 100, 500 ng/ml) for 15 minutes. **(A)** Western blot containing the phosphorylated protein, total protein, and β-actin (loading control) is shown. **(B)** The ratio of the phosphorylated proteins and the total proteins are calculated. The graphs show the fold change of the quantification of Western blot.

Fig. S5

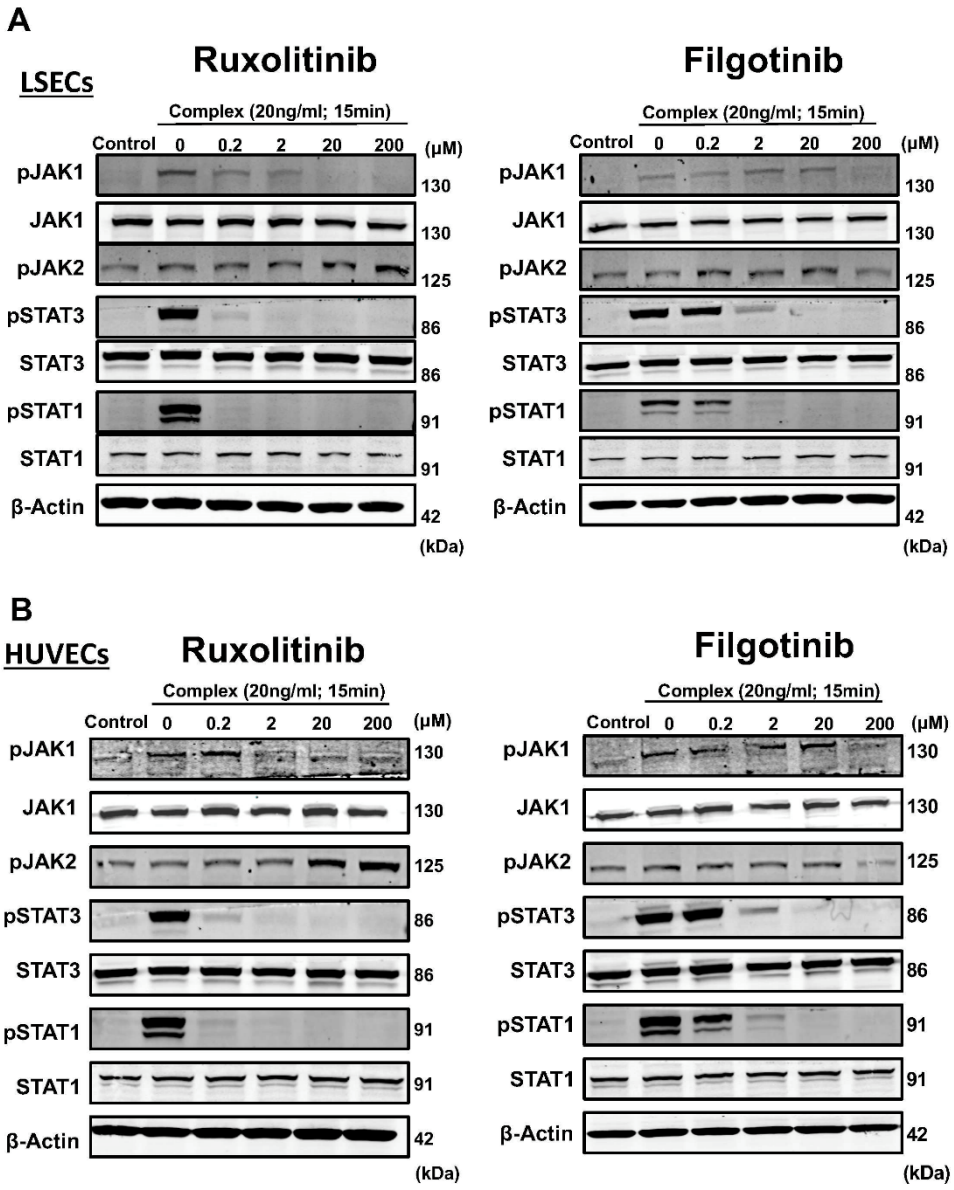

Fig. S5. JAK inhibitor, Ruxolitinib, efficiently blocks JAK1/STAT3 induction by IL-6 trans-signaling in LSECs and HUVECs.

A dose response (0, 0.2, 2, 20, 200μM) experiment to determine optimal concentrations of Ruxolitinib and Filgotinib to block IL-6-JAK/STAT activation in **(A)** Human primary LSECs and **(B)** HUVECs. They were treated with control and IL-6/sIL-6R complex (20 ng/ml) in the presence or absence of Ruxolitinib or Filgotinib for 15 minutes. Cells were incubated with Ruxolitinib or Filgotinib for 20 minutes before treatment with complex.

III. Supplementary tables

Table S1. Antibodies.

Antibody for Western Blot	Catalog Number	Source	Condition
Phospho-Jak1(Tyr1034/1035) (D7N4Z)	#74129	CST	1:1000
Jak1 (D1T6W) Mouse mAb	#50996	CST	1:1000
Phospho-Jak2 (Tyr1007/1008) (C80C3) Rabbit mAb	#3776	CST	1:1000
Jak2 (D2E12) XP® Rabbit mAb	#3230	CST	1:1000
pSTAT3 (Tyr705)	#9131	CST	1:1000
STAT3	#9139	CST	1:1000
pSTAT1 (Tyr701)	#9167	CST	1:1000
STAT1	#9176	CST	1:1000
β-actin	A2228	Sigma-Aldrich	1:6000
Factor VIII	NB100-91761	Novus	1:500
IRDye Donkey anti-Rabbit 680RD	925-68073	LI-COR	1:10000
IRDye Donkey anti-Mouset 800CW	926-32212	LI-COR	1:10000
Antibody for IF, IHC, and FACS	**Catalog Number**	**Source**	**Condition**
vWF	A0082	Dako	1:200 for IF, 1:50 for FACS, 1:4000 for IHC
Fibrinogen	A0080	Dako	1:100 for IF
CD61	M0753	Dako	1:20 for IHC
CD41 (Integrin alpha IIb)	sc365938	Santa Cruz	1:100 for IF
Donkey anti-rabbit Alexa 647	A31573	Invitrogen	1:300 for IF, 1:50 for FACS
Donkey anti-rat Alexa 488	A21208	Invitrogen	1:300 for IF
Alexa Fluor 488 phalloidin	A12379	moleculer probee	1:200 for IF
DAPI	F6057	SIGMA	

Table S2. Primers for qPCR.

Human Gene name	Primer Sequences (5'-3')
Factor VIII	F: GTGCCTTTTGCGATTCTGCT
	R: GCAGCTCACCGAGATCACTT
vWF	F: CCATCGAGGTGAAGCACAGT
	R: CCATGTTCCCACCCACGTAA
IL-6	F: ACCCCCAGGAGAAGATTCCA
	R: GATGCCGTCGAGGATGTACC
CXCL1	F: GCGCCCAAACCGAAGTCATA
	R: ATGGGGGATGCAGGATTGAG
CXCL2	F: GGCAGAAAGCTTGTCTCAACCC
	R: CTCCTTCAGGAACAGCCACCAA
ICAM1	F: AGGATGGCACTTTCCCACTG
	R: GGAGAGCACATTCACGGTCA
VCAM1	F: GTCTCATTGACTTGCAGCACC
	R: AGATGTGGTCCCCTCATTCGT
P-selectin	F: ATTGTACTCGATCGGGACGC
	R: AGAGAAATGGCAGGTGGAGC
E-selectn	F: AAGGCTTCATGTTGCAGGGA
	R: ATTCATGTAGCCTCGCTCGG
18S	F: GGCCCTGTAATTGGAATGAGTC
	R: CCAAGATCCAACTACGAGCTT
Mouse Gene name	**Primer Sequences (5'-3')**
IL-6	F: ACAAGTCGGAGGCTTAATTACACAT
	R: TTGCCATTGCACAACTCTTTTC
Fibrinogen α	F: GCCCAACGAGAGACTGTGAT
	R: CCATCCTCCCAAACTGGTCTC
18S	F: ACGGAAGGGCACCACCAGGA
	R: CACCACCACCCACGGAATCG

Table S3. siRNA Sequences Utilized.

Human STAT1(6722) siRNA	Target Sequence
D-003543-01	AGAAAGAGCUUGACAGUAA
D-003543-03	UAAAGGAACUGGAUAUAUC
D-003543-04	GAGCUUCACUCCCUUAGUU
D-003543-05	GAACCUGACUUCCAUGCGG
Human STAT3(6774) siRNA	**Target Sequence**
D-003544-02	GGAGAAGCAUCGUGAGUGA
D-003544-03	CCACUUUGGUGUUUCAUAA
D-003544-04	UCAGGUUGCUGGUCAAAUU
D-003544-19	CGUUAUAUAGGAACCGUAA

Table S4. A hypercoagulable thromboelastography profile in COVID-19 patients.

	ALT <3x	ALT>3x
Hypercoagulable	**30.00%**	**61.11%**
Not Hypercoagulable	**70.00%**	**38.89%**

Percentages of ICU patients from the cohort from Goshua, et .al.[3] who had a hypercoagulable thromboelastography (TEG) profile (defined as two or more parameters consistent with hypercoagulability) with and without ALT greater than or equal to three times the upper limit of normal (P = 0.07). Increased maximum amplitude (12/18 patients) and increased alpha angle (10/18 patients) were the most frequent hypercoagulable abnormalities in the elevated ALT group.

Table. S5. Demographics and selected covariates for the study population from the Yale DOM-CovX database.

	All Patients (n=3,780)	ALT <3x (n=2,634)	ATL >3x (n=1,006)	P value
Age (years)	65 (52/78)	68 (54/81)	60 (48/71)	P < 0.0001
Sex				
Female	50.50%	53.38%	39.86%	P < 0.0001
Male	49.50%	46.62%	60.14%	P < 0.0001
Race/Ethnicity				
Black	25.58%	26.23%	48.11%	P < 0.0001
White	48.52%	52.16%	38.97%	P < 0.0001
Latino	25.63%	22.06%	35.39%	P < 0.0001
Asian	1.85%	1.67%	2.58%	P = 0.07
Disease Severity				
ICU	24.68%	19.63%	41.15%	P < 0.0001
Vasopressors	13.70%	10.14%	24.45%	P < 0.0001
Treatment				
Tocilizumab	36.61%	28.40%	62.62%	P < 0.0001
Comorbidities				
Obesity	31.67%	32.92%	29.32%	P = 0.04
Congestive Heart Failure	21.77%	25.25%	12.62%	P < 0.0001
Lipid Disorder	53.15%	57.02%	44.73%	P < 0.0001
Hypertension, complicated	28.12%	32.49%	16.70%	P < 0.0001
Hypertension, uncomplicated	61.67%	66.34%	50.69%	P < 0.0001
Diabetes, complicated	27.51%	30.91%	19.66%	P < 0.0001
Diabetes, uncomplicated	34.31%	37.69%	26.48%	P < 0.0001
MI	11.67%	13.63%	6.86%	P < 0.0001
PVD	18.33%	21.37%	11.43%	P < 0.0001

Atrial Fibrillation/Flutter	16.01%	18.94%	8.35%	P < 0.0001
Liver Disease	11.30%	12.07%	9.84%	P = 0.06
Chronic Kidney Disease	21.88%	25.70%	12.62%	P < 0.0001
Metastatic Cancer	2.91%	3.26%	1.99%	P = 0.04
Solid Tumor	11.24%	12.60%	8.35%	P = 0.0003
Lymphoma	1.14%	1.14%	1.19%	P = 0.89
Alcohol Use Disorder	9.60%	10.59%	7.16%	p = 0.002
Mortality	14.87%	15.03%	14.71%	p = 0.81

Significant differences between patients with and without ALT greater than or equal to three times the upper limit of normal included younger age, male sex, intensive care unit (ICU) admission, vasopressor use, and tocilizumab treatment (more prevalent in the high ALT group), and congestive heart failure, lipid disorder, hypertension, diabetes, myocardial infarction (MI), peripheral vascular disease (PVD), atrial fibrillation/flutter, chronic kidney disease, alcohol use disorder, and solid tumor (more prevalent in the low ALT group). Patients with lower ALT had a higher prevalence of cardiovascular and metabolic comorbidities and alcohol use disorder, the converse of what would be expected if non-alcoholic fatty liver disease or alcohol-associated liver disease were responsible for the elevated ALT. No difference in the prevalence of pre-existing liver disease was present between groups. Age shown as median (IQR)

Table S6. Demographics, clinical, and treatment information for population of COVID-19 patients with post-mortem liver histology.

	Patients (n = 43)	ALT < 3x (n = 29)	ALT > 3x (n = 12)	P value
Age (median)	**75 (65/80)**	**79 (70/82)**	**71 (59/75)**	**P = 0.06**
Sex				
Female	**30.23% (13/43)**	**20.69%**	**41.67%**	**P = 0.25**
Male	**69.77% (30/43)**	**79.31%**	**58.33%**	
Treatment				
Tocilizumab	**2.33% (1/43)**	**3.45%**	**0.00%**	**P > 0.99**
Diabetes	**23.3% (10/43)**	**17.24%**	**41.67%**	**P = 0.12**
Obesity	**14.0% (6/43)**	**10.34%**	**25.00%**	**P = 0.33**
Hypertension	**53.5% (23/43)**	**51.72%**	**66.67%**	**P = 0.50**
Dyslipidemia	**20.9% (9/43)**	**17.24%**	**33.33%**	**P = 0.41**
ICU	**25.6% (11/43)**	**20.69%**	**33.33%**	**P = 0.44**
Neutrophils x $10^3/\mu L$	**11.7 (7.2/17.0)**	**12.3 (6.5/15.9)**	**11.0 (8.0/20.0)**	**P = 0.75**

Age and neutrophil count shown as median (IQR).

Neutrophil count is the value closest to patient death

Two patients did not have ALT values recorded

Table S7. Age and sex of patients with liver samples utilized for normal histology (Yale).

	Patients (n = 6)
Age (median)	**26.5 (18.25/32.5)**
Sex	
Female	**50% (3/6)**
Male	**50% (3/6)**

Age shown as median (IQR)

IV. Supplementary references

[1] Lorenz L, Axnick J, Buschmann T, Henning C, Urner S, Fang S, et al. Mechanosensing by beta1 integrin induces angiocrine signals for liver growth and survival. Nature 2018;562:128-132.

[2] Gao L, Utsumi T, Tashiro K, Liu B, Zhang D, Swenson ES, et al. Reticulon 4B (Nogo-B) facilitates hepatocyte proliferation and liver regeneration in mice. Hepatology 2013;57:1992-2003.

[3] Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol 2020;7:e575-e582.