SUPPLEMENTARY INFORMATION

Endothelial cell-activating antibodies in COVID-19
Shi et al.
MATERIALS AND METHODS

Serum and plasma samples from patients with COVID-19 and sepsis. Blood was collected into either serum separator tubes containing clot activator and serum separator gel or EDTA tubes by a trained hospital phlebotomist. After completion of testing ordered by the clinician, the samples were released to the research laboratory. Serum and plasma samples were immediately divided into small aliquots and stored at -80°C until the time of testing. All 118 patients in the primary cohort and 126 patients in the expansion cohort had a confirmed COVID-19 diagnosis based on U.S. Food and Drug Administration (FDA)-approved RNA testing. All COVID-19 aspects of the study complied with all relevant ethical regulations and was approved by the University of Michigan Institutional Review Board (HUM00179409). For sepsis, consent was obtained from either the patient or their legally authorized representative (HUM00131596). Blood was collected through an existing catheter and handed to the study coordinator. The sample was walked back to the laboratory and processed by standard methods. Plasma was immediately divided into small aliquots and stored at -80°C until the time of testing. Patient sex and race were captured as reported in the electronic medical record. Healthy controls were recruited by posted flier (HUM00044257) and information on race was not available.

Cell culture. Human umbilical vein endothelial cells (HUVEC) were purchased from ATCC. For most experiments, HUVEC were cultured in Endothelial Cell Growth Basal Medium-2 (CC-3156, Lonza) supplemented with EGM-2 MV SingleQuots Kit (CC-4147, Lonza) without hydrocortisone. 10,000 cells/well were seeded into 0.2% gelatin-coated 96-well tissue culture plates the day before experiments. The following day, HUVEC were cultured with 2.5% human serum or IgG at a concentration of 100 μg/ml at 37°C in a humidified atmosphere of 5% CO₂. For the 2.5% human plasma experiments, HUVEC were cultured in Endothelial Cell Basal Medium (CC-3121, Lonza) supplemented with EGM-MV SingleQuots Kit (CC-4143, Lonza). All experiments were performed using HUVEC of passage 6 or fewer.

Neutrophil adhesion assay. 1×10⁴ HUVEC per well were seeded into a 96-well plate and cultured until confluent. In the BSL-3 facility, the HUVEC monolayer was cultured with 2.5% COVID serum or control serum for 6 hours. Fresh neutrophils isolated from healthy controls were labeled with calcein-AM (C1430, Thermo) for 30 minutes at 37°C. After washing the HUVEC monolayer, 3×10⁵ labeled neutrophils were then added and incubated for 20 minutes. After washing with pre-warmed HBSS, adherent neutrophil fluorescence was measured with a microplate fluorescence reader (BioTek) at 485 and 535 nm. After reading, the cells were fixed
with 4% PFA for 30 minutes, and images were captured with a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek) through the GFP channel.

**IgG depletion from serum.** IgG was purified from COVID-19 and control serum with protein G Agarose beads (Pierce). Briefly, 800 μl of protein G agarose beads were washed at least 10 times by PBS. Then, 200 μl of serum was added and incubated with the beads at 4°C overnight. The supernatant was collected after spinning the tube at 2500xg for 5 minutes. The supernatant was the IgG depleted serum. The same volume of serum was incubated without beads under the same conditions as a mock control. Depletion was confirmed by Coomassie-stained gels.

**Purification of human IgG fractions from serum.** Serum was diluted in IgG binding buffer and passed through a Protein G Agarose column (Pierce) at least 5 times. IgG was then eluted with 0.1 M glycine and neutralized with 1 M Tris. This was followed by overnight dialysis against PBS at 4°C. IgG purity was verified with Coomassie staining, and concentrations were determined by BCA protein assay (Pierce) according to manufacturer’s instructions. In some experiments, IgG was used to supplement control serum at a final concentration of 100 µg/ml. All IgG samples were determined to have endotoxin level below 0.1 EU/ml by the Pierce LAL Chromogenic Endotoxin Quantitation Kit (A39552) according to manufacturer’s instructions. This kit offers high sensitivity with linear detection range of 0.01-1.0 EU/mL.

**In-cell ELISA.** In the Biosafety Level 3 facility, endothelial cell activation was assessed by an in-cell ELISA, which measured surface expression of E-selectin, ICAM-1, VCAM-1 on endothelial cells. Briefly, confluent monolayers of HUVEC in 96-well microplates were incubated with 2.5% serum or 100 µg/ml purified IgG for 6 hours and then fixed using the same volume of 8% paraformaldehyde for 30 minutes. Cells were blocked with 2x blocking solution (Abcam) at room temperature for 2 hours. After washing with PBS, cells were incubated with 5 µg/ml primary mouse anti-human antibodies against E-selectin (catalog BBA26, R&D), VCAM-1 (catalog BBA5, R&D), or ICAM-1 (ab2213, Abcam) at 4°C overnight. Next, 100 μl of diluted horseradish peroxidase conjugated rabbit anti-mouse IgG (1:2000, Jackson ImmunoResearch) in 1x blocking solution was added and incubated at room temperature for 1 hour. After washing thoroughly, 100 μl of TMB substrate was added blue color development was measured at OD 650 nm with a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek). The signals were corrected by subtracting the mean signal of wells incubated in the absence of the primary antibody from
all other readings. Fold change was calculated by normalizing experimental wells to the mean signal of wells incubated with culture medium alone.

**Quantification of soluble E-selectin and soluble ICAM-1 in serum.** Soluble E-selectin and ICAM-1 were quantified using E-selectin ELISA (DY724, R&D Systems) and ICAM-1 ELISA (DY720, R&D Systems) kits according to the manufacturer’s instructions.

**Quantification of antiphospholipid antibodies (aPL).** aPL were quantified in serum or plasma using Quanta Lite® ACA IgG, ACA IgM, aβ₂GPI IgG, aβ₂GPI IgM, aPS/PT IgG, and aPS/PT IgM kits (Inova Diagnostics Inc.) according to the manufacturer’s instructions, as we did previously (1). All assays are approved for clinical use and received 510(k) clearance from the FDA. Quanta Lite® aPL ELISAs (Inova Diagnostics) are well recognized by the international APS research community and are utilized by the largest international APS clinical research network registry, APS ACTION, in its core laboratories as the “gold standard” for aPL testing (2, 3). Here, aCL assays were reported in GPL and MPL units; aβ₂GPI assays in standard β₂GPI IgG units (SGU) and standard β₂GPI IgM units (SMU); and aPS/PT assays in IgG and IgM units. All were per the manufacturer’s specifications.

**Quantification of myeloperoxidase-DNA complexes.** Myeloperoxidase-DNA complexes were quantified similarly to what has been previously described (4). This protocol used several reagents from the Cell Death Detection ELISA kit (Roche). First, a high-binding EIA/RIA 96-well plate (Costar) was coated overnight at 4ºC with anti-human myeloperoxidase antibody (Bio-Rad 0400-0002), diluted to a concentration of 1 µg/ml in coating buffer (Cell Death kit). The plate was washed two times with wash buffer (0.05% Tween 20 in PBS), and then blocked with 4% bovine serum albumin in PBS (supplemented with 0.05% Tween 20) for 2 hours at room temperature. The plate was again washed five times, before incubating for 90 minutes at room temperature with 10% serum or plasma in the aforementioned blocking buffer (without Tween 20). The plate was washed five times, and then incubated for 90 minutes at room temperature with 10x anti-DNA antibody (HRP-conjugated; from the Cell Death kit) diluted 1:100 in blocking buffer. After five more washes, the plate was developed with 3,3',5,5'-Tetramethylbenzidine (TMB) substrate (Invitrogen) followed by a 2N sulfuric acid stop solution. Absorbance was measured at a wavelength of 450 nm using a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek). Data were normalized to in vitro-prepared NET standards included on every plate, which were quantified based on their DNA content.
Quantification of cell-free DNA. Cell-free DNA was quantified in serum using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, P11496) according to the manufacturer’s instructions.

Quantification of Cit-H3. Cit-H3 was quantified in serum using the Citrullinated Histone H3 (clone 11D3) ELISA Kit (Cayman, 501620) according to the manufacturer’s instructions.

Quantification of S100A8/A9 (calprotectin). Calprotectin levels were measured with the Human S100A8/S100A9 Heterodimer DuoSet ELISA (DY8226-05, R&D Systems) according to the manufacturer’s instructions, as we have done previously (5).

Statistical analysis. Normally-distributed data were analyzed by 2-sided t test and skewed data were analyzed by Mann-Whitney test when two groups were analyzed. For more than two groups, one-way ANOVA or Kruskal-Wallis test with correction for multiple comparisons was utilized. Correlations were tested by Spearman’s correlation coefficient. These analyses were with GraphPad Prism software version 8, and statistical significance was defined as p<0.05. Univariate and multivariate logistic regression analysis was used to identify potential relationships between anticardioli pin IgG/M, anti-β2GPI IgG/M, anti-PS/PT IgG/M and HUVEC surface activation markers. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. All variables were included in the regression model. Variables with p <0.1 by univariate analysis were entered in multivariate analysis. A 2-sided p-value <0.05 was considered statistically significant. This analysis was performed using SPSS version 23.0 (IBM, Armonk, NY, USA).
REFERENCES

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## Supplemental Table 1: Patient characteristics (serum)

|                         | COVID-19 (primary cohort) | COVID-19 (expansion cohort for surface ICAM-1 measurement) | Control |
|-------------------------|--------------------------|-----------------------------------------------------------|---------|
| **Demographics**        |                          |                                                           |         |
| Number                  | 118                      | 126                                                       | 40      |
| Age (years)\(^a\)      | 61.9 ± 17.2 (25-96)      | 58.3 ± 16.7 (16-90)                                       | 38.3 ± 12.1 (21-64) |
| Hospital day\(^b\)     | 4. (1-25)                | 4 ± 5.8 (1-40)                                            |         |
| Female                  | 55 (47%)                 | 50 (40%)                                                  | 29 (73%)|
| Black/African American  | 50 (42%)                 | 52 (41%)                                                  |         |
| White                   | 47 (40%)                 | 55 (44%)                                                  |         |
| **Comorbidities**       |                          |                                                           |         |
| Diabetes                | 45 (38%)                 | 51 (40%)                                                  |         |
| Ischemic heart disease  | 36 (31%)                 | 67 (53%)                                                  |         |
| Renal disease           | 33 (28%)                 | 55 (44%)                                                  |         |
| Lung disease            | 29 (25%)                 | 67 (53%)                                                  |         |
| Autoimmune              | 3 (3%)                   | 3 (2%)                                                    |         |
| Cancer                  | 15 (13%)                 | 17 (13%)                                                  |         |
| History of stroke       | 11 (9%)                  | 5 (4%)                                                    |         |
| Obesity                 | 50 (42%)                 | 67 (53%)                                                  |         |
| Hypertension            | 73 (62%)                 | 63 (50%)                                                  |         |
| Immune deficiency       | 7 (6%)                   | 35 (28%)                                                  |         |
| History of smoking      | 23 (19%)                 | 29 (23%)                                                  |         |
| **Respiratory status**  |                          |                                                           |         |
| Mechanical ventilation  | 41 (35%)                 | 59 (47%)                                                  |         |
| High-flow oxygen        | 8 (7%)                   | 12 (10%)                                                  |         |
| Nasal-cannula oxygen    | 38 (32%)                 | 29 (23%)                                                  |         |
| Room air                | 31 (26%)                 | 26 (21%)                                                  |         |
| **Key medications**     |                          |                                                           |         |
| Dexamethasone           | 0 (0%)                   | 3 (2%)                                                    |         |
| Remdesivir              | 1 (1%)                   | 2 (2%)                                                    |         |

\(^a\) Mean ± standard deviation (range)  
\(^b\) Median = 3 (original) and 3 (expansion)
Supplemental Table 2: Patient characteristics (plasma)

|                        | Sepsis       | COVID-19     | Control     |
|------------------------|--------------|--------------|-------------|
| **Demographics**       |              |              |             |
| Number                 | 100          | 72           | 38          |
| Age (years)<sup>a</sup> | 62.6 ± 6.0   | 61.43 ± 18   | 43.2 ± 9.3  |
| (50-73)                |              | (25-96)      | (25-62)     |
| Hospital day<sup>b</sup> | 6.7 ± 6.9    | 5.1 ± 6.1    |             |
| (1-40)                 |              | (0-24)       |             |
| Female                 | 47 (47%)     | 33 (46%)     | 22 (58%)    |
| Black/African American | 4 (4%)       | 32 (44%)     |             |
| White                  | 92 (92%)     | 26 (36%)     |             |
| **Comorbidities**      |              |              |             |
| Diabetes               | 34 (34%)     | 29 (40%)     |             |
| Ischemic heart disease | 26 (26%)     | 25 (35%)     |             |
| Renal disease          | 51 (51%)     | 19 (26%)     |             |
| Lung disease           | 42 (42%)     | 16 (22%)     |             |
| Autoimmune             | 9 (9%)       | 2 (3%)       |             |
| Cancer                 | 37 (37%)     | 8 (11%)      |             |
| Obesity                | 50 (50%)     | 32 (44%)     |             |
| Hypertension           | 48 (48%)     | 43 (60%)     |             |
| History of smoking     | 49 (49%)     | 15 (21%)     |             |
| **Respiratory status** |              |              |             |
| Mechanical ventilation | 65 (65%)     | 33 (46%)     |             |
| High-flow oxygen       | 11 (11%)     | 5 (7%)       |             |
| Nasal-cannula oxygen   | 24 (24%)     | 21 (29%)     |             |
| Room air               | 0 (0%)       | 13 (18%)     |             |

<sup>a</sup> Mean ± standard deviation (range)

<sup>b</sup> Median = 4 (sepsis) and 2 (COVID-19)
### Supplemental Table 3: Prevalence of antiphospholipid antibodies in serum from patients with COVID-19 (n=118)

| aPL          | Number of positive (manufacturer’s cutoff) | %  | Number of positive (titer ≥40 units) | %  |
|--------------|---------------------------------------------|----|--------------------------------------|----|
| aCL IgG      | 4                                           | 3% | 0                                    | 0% |
| aCL IgM      | 29                                          | 25%| 9                                    | 8% |
| aβ2GPI IgG   | 2                                           | 2% | 2                                    | 2% |
| aβ2GPI IgM   | 5                                           | 4% | 3                                    | 3% |
| aPS/PT IgG   | 28                                          | 24%| 15                                   | 13%|
| aPS/PT IgM   | 18                                          | 15%| 12                                   | 10%|
| Any positive | 53                                          | 45%| 30                                   | 25%|

The manufacturer’s cutoff: aCL IgG >20 GPL, aCL IgM >20 MPL, aβ2GPI IgG >20 SGU, aβ2GPI IgM >20 SMU, aPS/PT IgG >30 IgG units, and aPS/PT IgM >30 IgM units; aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; aβ2GPI=anti-beta-2 glycoprotein I antibodies; aPS/PT=anti-phosphatidylserine/prothrombin antibodies.
**Supplemental Table 4:** Correlation amongst antiphospholipid antibodies in serum from patients with COVID-19 (n=118)

|          | aCL IgG | aCL IgM | aβ₂GPI IgG | aβ₂GPI IgM | aPS/PT IgG | aPS/PT IgM |
|----------|---------|---------|------------|------------|------------|------------|
| aCL IgG  | 0.44****| 0.10 ns | -0.04 ns   | 0.51****   | 0.15 ns    | 0.15 ns    |
| aCL IgM  | 0.44****| 0.11 ns | 0.14 ns    | 0.35****   | 0.53****   | 0.53****   |
| aβ₂GPI IgG | 0.10 ns | 0.11 ns | 0.25**     | 0.03 ns    | 0.23*      | 0.23*      |
| aβ₂GPI IgM| -0.04 ns| 0.14 ns | 0.25**     | -0.06 ns   | 0.31***    | 0.31***    |
| aPS/PT IgG| 0.51****| 0.35****| 0.03 ns    | -0.06 ns   | 0.19*      | 0.19*      |
| aPS/PT IgM| 0.15 ns | 0.53****| 0.23*      | 0.31***    | 0.19*      | 0.19*      |

aCL=anticardiolipin antibodies; aβ₂GPI=anti-beta-2 glycoprotein I antibodies; aPS/PT=anti-phosphatidylserine/prothrombin antibodies; ns=not significant; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 by Spearman’s method.
### Supplemental Table 5: Correlation of antiphospholipid antibodies with clinical and laboratory data (n=118)

|                | aCL IgG | aCL IgM | aβ₂GPI IgG | aβ₂GPI IgM | aPS/PT IgG | aPS/PT IgM |
|----------------|---------|---------|------------|------------|------------|------------|
| **r**          | *       | *       | r          | *          | r          | r          |
| FiO₂           | 0.20    | 0.05    | ns         | 0.12       | ns         | 0.05       |
| D-dimer        | 0.15    | ns      | 0.21       | *          | -0.02      | ns         |
| ANC            | 0.23    | *       | 0.21       | *          | 0.01       | ns         |
| Calprotectin   | 0.29    | **      | 0.24       | **         | 0.17       | ns         |
| MPO-DNA        | 0.23    | *       | 0.30       | ***        | 0.23       | *          |

aCL=anticardiolipin antibodies; aβ₂GPI=anti-beta-2 glycoprotein I antibodies; aPS/PT=anti-phosphatidylserine/prothrombin antibodies; ANC=absolute neutrophil count; MPO-DNA=myeloperoxidase-DNA complexes (NET remnants); ns=not significant; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 by Spearman's method.
**Supplemental Table 6:** Prevalence of antiphospholipid antibodies in serum from healthy controls (n=38)

| aPL                  | Number of positive (manufacturer’s cutoff) | %   | Number of positive (titer ≥40 units) | %   |
|----------------------|-------------------------------------------|-----|--------------------------------------|-----|
| aCL IgG              | 0                                         | 0%  | 0                                    | 0%  |
| aCL IgM              | 1                                         | 3%  | 0                                    | 0%  |
| aβ2GPI IgG           | 0                                         | 0%  | 0                                    | 0%  |
| aβ2GPI IgM           | 1                                         | 3%  | 0                                    | 0%  |
| aPS/PT IgG           | 0                                         | 0%  | 0                                    | 0%  |
| aPS/PT IgM           | 0                                         | 0%  | 0                                    | 0%  |
| Any positive         | 2                                         | 5%  | 0                                    | 0%  |

The manufacturer’s cutoff: aCL IgG >20 GPL, aCL IgM >20 MPL, aβ2GPI IgG >20 SGU, aβ2GPI IgM >20 SMU, aPS/PT IgG >30 IgG units, and aPS/PT IgM >30 IgM units; aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; aβ2GPI=anti-beta-2 glycoprotein I antibodies; aPS/PT=anti-phosphatidylserine/prothrombin antibodies.
Supplemental Table 7: Prevalence of antiphospholipid antibodies in plasma from healthy controls (n=36)

| aPL          | Number of positive (manufacturer’s cutoff) | %  | Number of positive (titer ≥40 units) | %  |
|--------------|-------------------------------------------|----|--------------------------------------|----|
| aCL IgG      | 0                                         | 0% | 0                                    | 0% |
| aCL IgM      | 1                                         | 3% | 0                                    | 0% |
| aβ₂GPI IgG   | 0                                         | 0% | 0                                    | 0% |
| aβ₂GPI IgM   | 0                                         | 0% | 0                                    | 0% |
| aPS/PT IgG   | 0                                         | 0% | 0                                    | 0% |
| aPS/PT IgM   | 0                                         | 0% | 0                                    | 0% |
| Any positive | 1                                         | 3% | 0                                    | 0% |

The manufacturer’s cutoff: aCL IgG >20 GPL, aCL IgM >20 MPL, aβ₂GPI IgG >20 SGU, aβ₂GPI IgM >20 SMU, aPS/PT IgG >30 IgG units, and aPS/PT IgM >30 IgM units; aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; aβ₂GPI=anti-beta-2 glycoprotein I antibodies; aPS/PT=anti-phosphatidylserine/prothrombin antibodies.
**Supplemental Table 8: Logistic regression analysis of adhesion molecule upregulation.**
Positive/negative threshold defined as 2 standard deviations above the control mean.

| E-selectin | univariate | multivariate |
|------------|------------|--------------|
|            | HUVEC+ (n=52) | HUVEC- (n=66) | p | OR (95% CI) | p | OR (95% CI) |
| aCL IgG (GPL) | 9.3 | 5.7 | **0.001** | 1.21 (1.08-1.35) | **0.001** | 1.21 (1.08-1.35) |
| aCL IgM MPL | 21.7 | 18.2 | 0.11 | 1.03 (0.99-1.07) |  |  |
| aβ2GPI IgG (SGU) | 1.7 | 1.4 | 0.85 | 1.01 (0.96-1.05) |  |  |
| aβ2GPI IgM (SMU) | 2.6 | 5.3 | 0.43 | 0.99 (0.96-1.02) |  |  |
| aPS/PT IgG (units) | 24.7 | 25.8 | 0.71 | 1.00 (0.97-1.02) |  |  |
| aPS/PT IgM (units) | 26.9 | 17.9 | 0.078 | 1.01 (1.00-1.03) |  |  |

| VCAM-1 | univariate | multivariate |
|--------|------------|--------------|
|         | HUVEC+ (n=47) | HUVEC- (n=71) | p | OR (95% CI) | p | OR (95% CI) |
| aCL IgG (GPL) | 9.6 | 5.8 | **0.001** | 1.22 (1.10-1.36) | **0.003** | 1.18 (1.06-1.31) |
| aCL IgM MPL | 23.8 | 17.1 | **0.008** | 1.08 (1.02-1.14) |  |  |
| aβ2GPI IgG (SGU) | 2.2 | 1.1 | 0.45 | 1.01 (0.97-1.07) |  |  |
| aβ2GPI IgM (SMU) | 3.1 | 4.7 | 0.64 | 1.00 (0.97-1.02) |  |  |
| aPS/PT IgG (units) | 29.3 | 22.6 | **0.037** | 1.03 (1.00-1.06) |  |  |
| aPS/PT IgM (units) | 23.1 | 21.1 | 0.71 | 1.00 (0.99-1.11) |  |  |

| ICAM-1 | univariate | multivariate |
|--------|------------|--------------|
|         | HUVEC+ (n=28) | HUVEC- (n=90) | p | OR (95% CI) | p | OR (95% CI) |
| aCL IgG (GPL) | 8.3 | 7.0 | 0.25 | 1.05 (0.97-1.13) |  |  |
| aCL IgM MPL | 22.6 | 18.9 | 0.15 | 1.03 (0.99-1.06) |  |  |
| aβ2GPI IgG (SGU) | 1.0 | 1.7 | 0.67 | 0.98 (0.91-1.06) |  |  |
| aβ2GPI IgM (SMU) | 1.6 | 4.8 | 0.48 | 0.98 (0.92-1.04) |  |  |
| aPS/PT IgG (units) | 32.3 | 23.1 | **0.018** | 1.03 (1.01-1.06) | **0.018** | 1.03 (1.01-1.06) |
| aPS/PT IgM (units) | 25.5 | 20.8 | 0.44 | 1.01 (0.99-1.02) |  |  |
### Supplemental Table 9: Prevalence of antiphospholipid antibodies in plasma from patients with sepsis (n=100)

| aPL       | Number of positive (manufacturer’s cutoff) | %   | Number of positive (titer ≥40 units) | %   |
|-----------|-------------------------------------------|-----|--------------------------------------|-----|
| aCL IgG   | 0                                         | 0%  | 0                                    | 0%  |
| aCL IgM   | 10                                        | 10% | 1                                    | 1%  |
| aβ2GPI IgG| 2                                         | 2%  | 2                                    | 2%  |
| aβ2GPI IgM| 4                                         | 4%  | 0                                    | 0%  |
| aPS/PT IgG| 2                                         | 2%  | 1                                    | 1%  |
| aPS/PT IgM| 24                                        | 24% | 21                                   | 21% |
| Any positive | 29                                         | 29% | 23                                   | 23% |

The manufacturer’s cutoff: aCL IgG >20 GPL, aCL IgM >20 MPL, aβ2GPI IgG >20 SGU, aβ2GPI IgM >20 SMU, aPS/PT IgG >30 IgG units, and aPS/PT IgM >30 IgM units; aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; aβ2GPI=anti-beta-2 glycoprotein I antibodies; aPS/PT=anti-phosphatidylserine/prothrombin antibodies.
Supplementary Table 10: Correlation of HUVEC surface ICAM-1 with antiphospholipid antibodies in patients with sepsis

| Antiphospholipid antibodies | ICAM-1 | p  |
|-----------------------------|--------|----|
| IgG anticardiolipin          | 0.283  | **|
| IgM anticardiolipin          | 0.191  | ns |
| IgG anti-β2GPI               | 0.056  | ns |
| IgM anti-β2GPI               | 0.161  | ns |
| IgG anti-PS/PT               | -0.021 | ns |
| IgM anti-PS/PT               | 0.293  | **|

**p<0.01; ns=not significant.
Supplemental Figure 1: Activation of human umbilical vein endothelial cells (HUVEC) by control or COVID-19 serum (presented as optical density rather than fold change).

HUVEC were cultured for 6 hours with serum from either healthy controls (collected pre-pandemic) (n=38) or patients hospitalized with COVID-19 (n=118). Cells were then fixed, and surface expression of E-selectin (A), VCAM-1 (B), or ICAM-1 (C) was quantified. Median values are indicated by horizontal lines. Data were presented as raw absorbance (optical density 650 nm). Groups were analyzed by Mann-Whitney test; ****p<0.0001.
Supplemental Figure 2: TNF-α upregulated surface adhesion molecules. A-C, HUVEC were cultured for 6 hours with TNF-α as indicated. Cells were then fixed, and surface expression of E-selectin (A), VCAM-1 (B), and ICAM-1 (C) was quantified. Mean and standard deviation are indicated for 3 independent experiments. Comparisons were by paired t test; *p<0.05 and **p<0.01.
Supplemental Figure 3: Similar ICAM-1 upregulation by original (n=118) and expansion (n=126) COVID-19 cohorts. HUVEC were cultured for 6 hours with serum from either healthy controls (collected pre-pandemic) (n=40) or patients hospitalized with COVID-19 from two similar cohorts as defined in Supplementary Table 1 (n=118 from original cohort in Figure 1D; and n=126 from expansion cohort). Cells were then fixed, and surface expression of ICAM-1 was quantified. Median values are indicated by horizontal lines. Groups were analyzed by Kruskal Wallis test with correction for multiple comparisons by Dunn’s method; ****p<0.0001 and ns=not significant.
Supplemental Figure 4: Association between soluble E-selectin in serum and clinical parameters. A, Serum from healthy controls (n=38) and COVID-19 patients (n=102) were assessed for soluble E-selectin. COVID-19 samples were compared to controls by Mann-Whitney test; ****p<0.0001. B, Soluble E-selectin was compared to HUVEC E-selectin expression as presented in Figure 1B. Correlation was determined by Spearman’s method. C-F, Soluble E-selectin in COVID-19 serum was compared to laboratory and clinical data when
available on the same day as the serum collection. Spearman’s correlations are presented for C-reactive protein (n=83) (C), D-dimer (n=72) (D), calprotectin (n=102) (E), oxygenation efficiency (n=99) (pulse oximetry/fraction of inspired oxygen, F).
Supplemental Figure 5: Association between soluble ICAM-1 and clinical parameters. A-C, Soluble ICAM-1 in COVID-19 serum was compared to laboratory and clinical data when available on the same day as the serum collection. Spearman’s correlations are presented for C-reactive protein (n=176) (A), D-dimer (n=174) (B), and oxygenation efficiency (n=228) (pulse oximetry/fraction of inspired oxygen, C).
**Supplemental Figure 6: Serum calprotectin levels remain stable after IgG depletion.**

Serum calprotectin levels from 3 COVID-19 patients were tested before and after IgG depletion. There were no statistically significant comparisons by paired t-test.
Supplemental Figure 7: IgG from serum with negative anticardiolipin (aCL) and anti-phosphatidlyserine/prothrombin (aPS/PT) antibodies does not trigger HUVEC activation.

Serum was pooled from 3 patients with negative testing for both aCL IgG and aPS/PT IgG. IgG (100 μg/ml) was purified from the pooled samples, and then spiked into control serum that had been depleted of IgG. Activation of HUVEC was determined after culture for 6 hours as defined by surface expression of ICAM-1. Groups were compared by one-way ANOVA with correction for multiple comparisons by Dunnett’s test; no comparisons were statistically significant.