Supplemental Data

SARS-CoV-2 infection induces the activation of tissue factor-mediated coagulation via activation of acid sphingomyelinase

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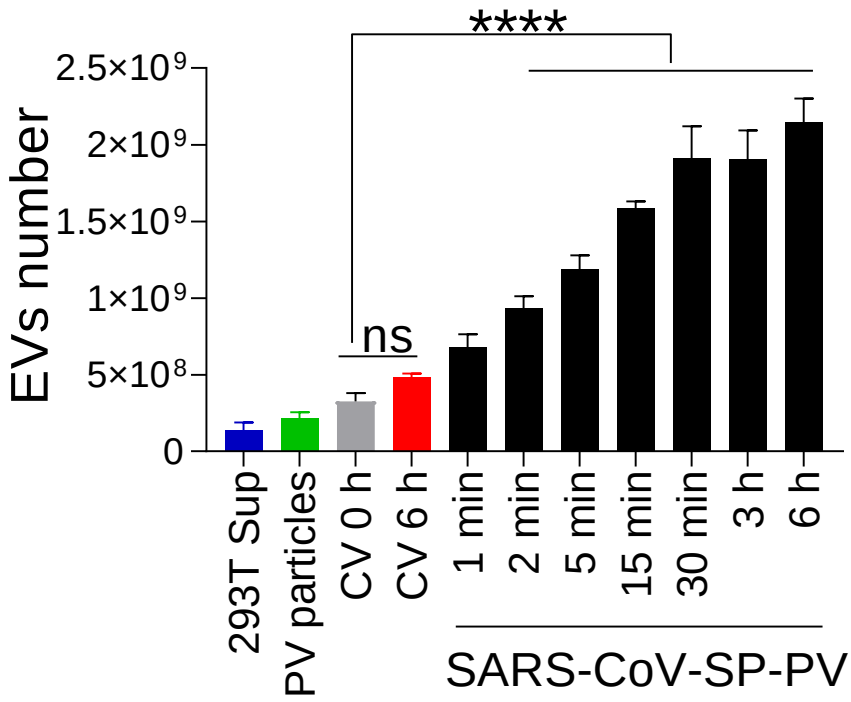
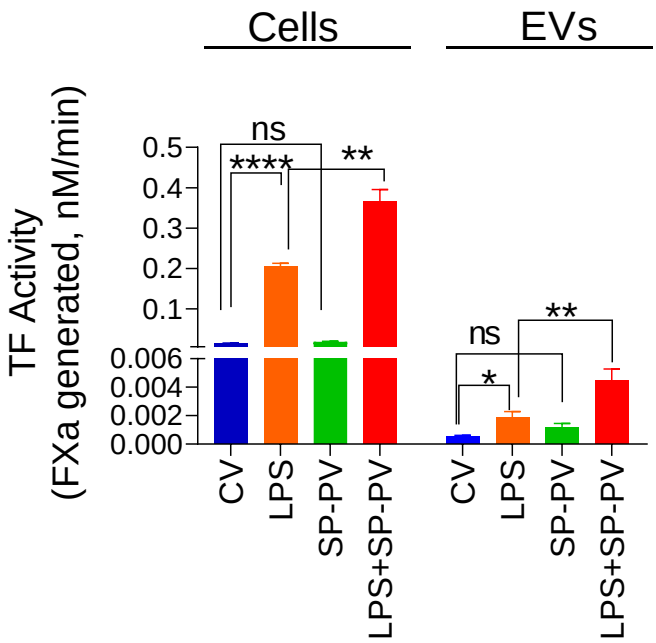
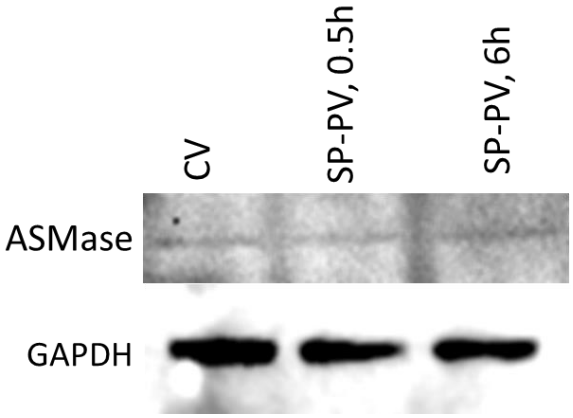
Supplemental Figure 1. SARS-CoV-2-spike protein pseudovirus (SARS-CoV-2-SP-PV) infection induces the release of extracellular vesicles (EVs) from human monocyte-derived macrophages (MDMs). MDMs were treated with a control vehicle (CV) or infected with SARS-CoV-2-SP-PV. At the indicated times, the cell supernatants were removed, and the EVs released into the conditioned medium were isolated by centrifugation at 21,000 x g for 1 h. EVs number was determined by nanoparticle tracking analysis using NanoSight NS 300 (Malvern Panalytical). Cell supernatants of 293T cells (293T Sup) that were not infected with the pseudovirus but processed in the same fashion as used for the generation of pseudovirus and the pseudovirus preparation used for experimental treatments (PV particles) were also subjected to nanoparticle tracking analysis. ****, p<0.0001; ns, no statistical significance.



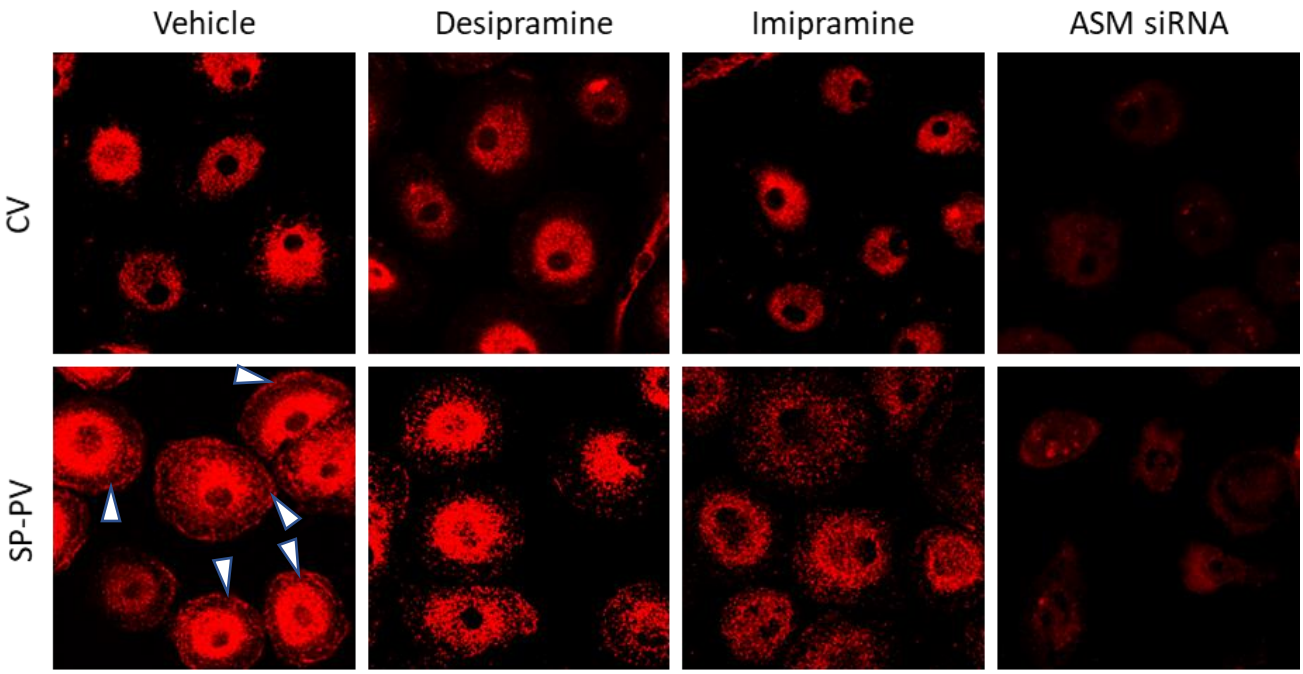
Supplemental Figure 2. SARS-CoV-2-SP-PV infection does not induce TF activity *per se* but enhances LPS-induced TF activity and the release of TF^{+} EVs from peripheral blood mononuclear cells. PBMCs were isolated from the blood of healthy human subjects using Ficoll-Hypaque density gradient. PBMCs were treated with a control vehicle (CV) or LPS (1 µg/ml) for 4 h to induce TF expression. Then, PBMCs were infected with SARS-CoV-2-SP-PV (SP-PV) for 6 h. EVs were isolated from cell supernatant medium. TF activity associated with intact cells and EVs was measured by adding FVIIa (10 nM) and the substrate factor X (175 nM), and measuring the rate of factor Xa generation. *p< .05; ** p<0.01; ****, p<0.0001; ns, no statistical significance.



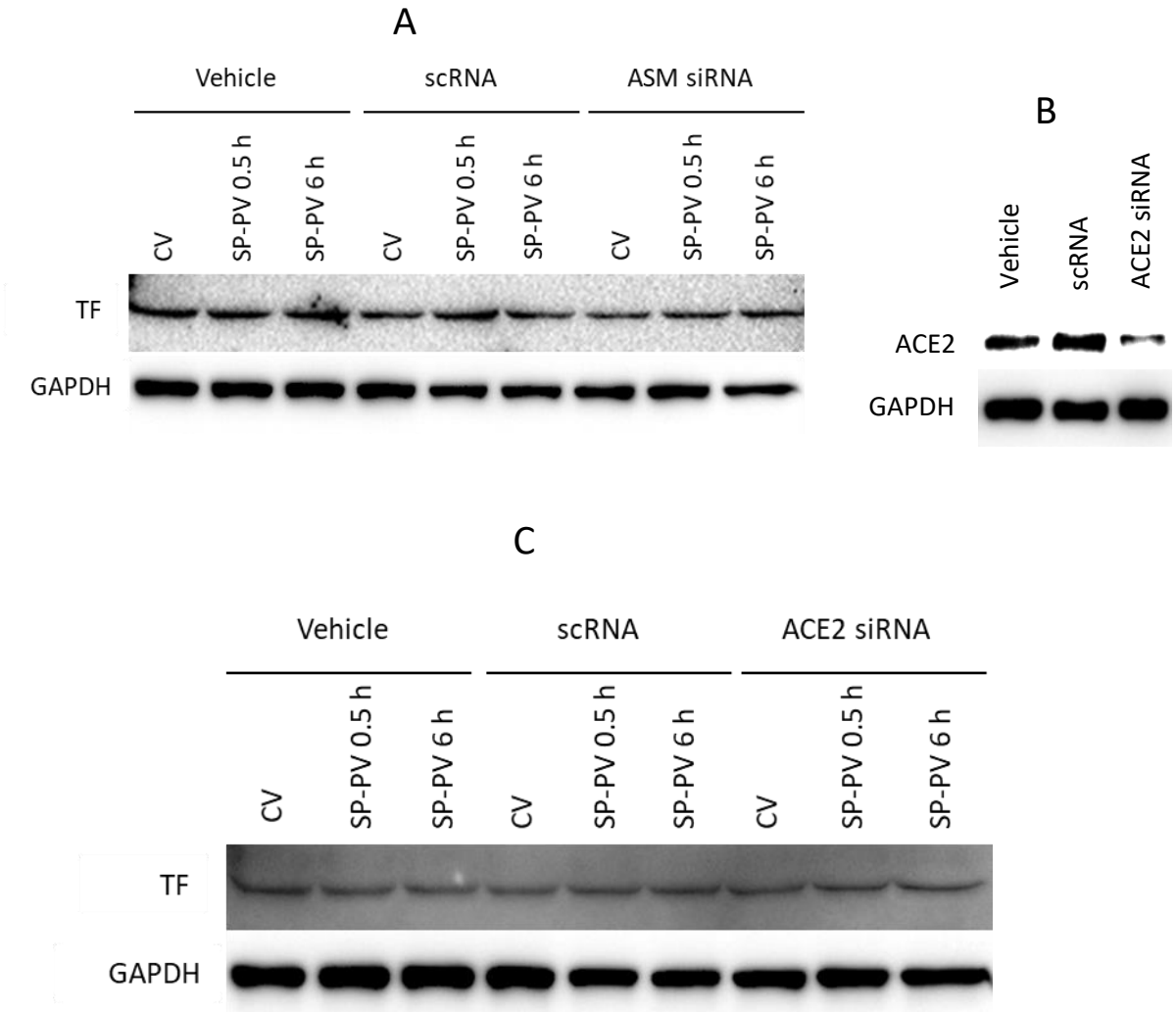
Supplemental Figure 3. SARS-CoV-2-SP-PV infection does not increase total ASMase protein levels. MDMs were treated with a control vehicle (CV) or infected with SARS-CoV2-SP-PV (SP-PV) for 0.5 or 6 h. Cell lysates were subjected to immunoblot analysis to probe for ASMase. GAPDH was used as a loading control.



Supplemental Figure 4. ASMase functional inhibitors or ASMase silencing blocks the SARS-CoV-2 spike protein pseudovirus-induced ASMase translocation in monocyte-derived macrophages (MDMs). MDMs were incubated with ASMase inhibitors - desipramine (1 μM) or imipramine (1 μM) - for 1 h or transfected with ASMase siRNA for 48 h, and then treated with a control vehicle (CV) or infected with the pseudovirus for 6 h. The cells were fixed, permeabilized, and immunostained with rabbit anti-human ASMase antibody (2 μg/ml) at 4°C overnight, followed by AF546-conjugated donkey anti-rabbit IgG (2 μg/ml) for 90 min. The immunofluorescence staining was analyzed by confocal microscopy. White arrowheads point out ASMase translocation to the plasma membrane.



Supplemental Figure 5. ASMase or ACE-2 silencing did not affect TF protein expression in monocyte-derived macrophages. (A) MDMs were transfected with transfection reagent (vehicle), scrambled oligonucleotide (scRNA), or siRNA specific for ASMase (100 nM). After 48 h, transfected MDMs were treated with a control vehicle (CV) or infected with SARS-CoV-2 spike protein pseudovirus (SP-PV) for 6 h. Cell lysates were subjected to immunoblot analysis to probe for TF or GAPDH (loading control). **(B)** MDMs were transfected with transfection reagent (vehicle), scrambled oligonucleotide (scRNA), or siRNA specific for ACE-2 (100 nM). After 48 h, cell lysates were harvested and probed for ACE-2 by immunoblot analysis. **(C)** ACE-2 silenced MDMs were treated with a control vehicle (CV) or infected with the pseudovirus for 0.5 or 6 h. Cell lysates were probed for TF protein by immunoblot analysis. **(D)** MDMs transfected with transfection reagent, scRNA, or ACE-2 siRNA were infected with the pseudovirus for 6 h and then immunostained for TF and ACE-2.



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