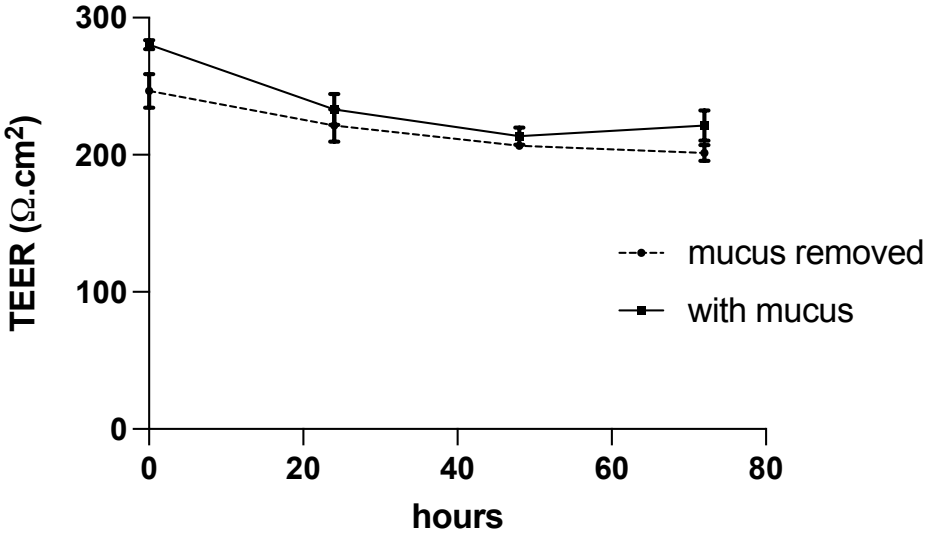
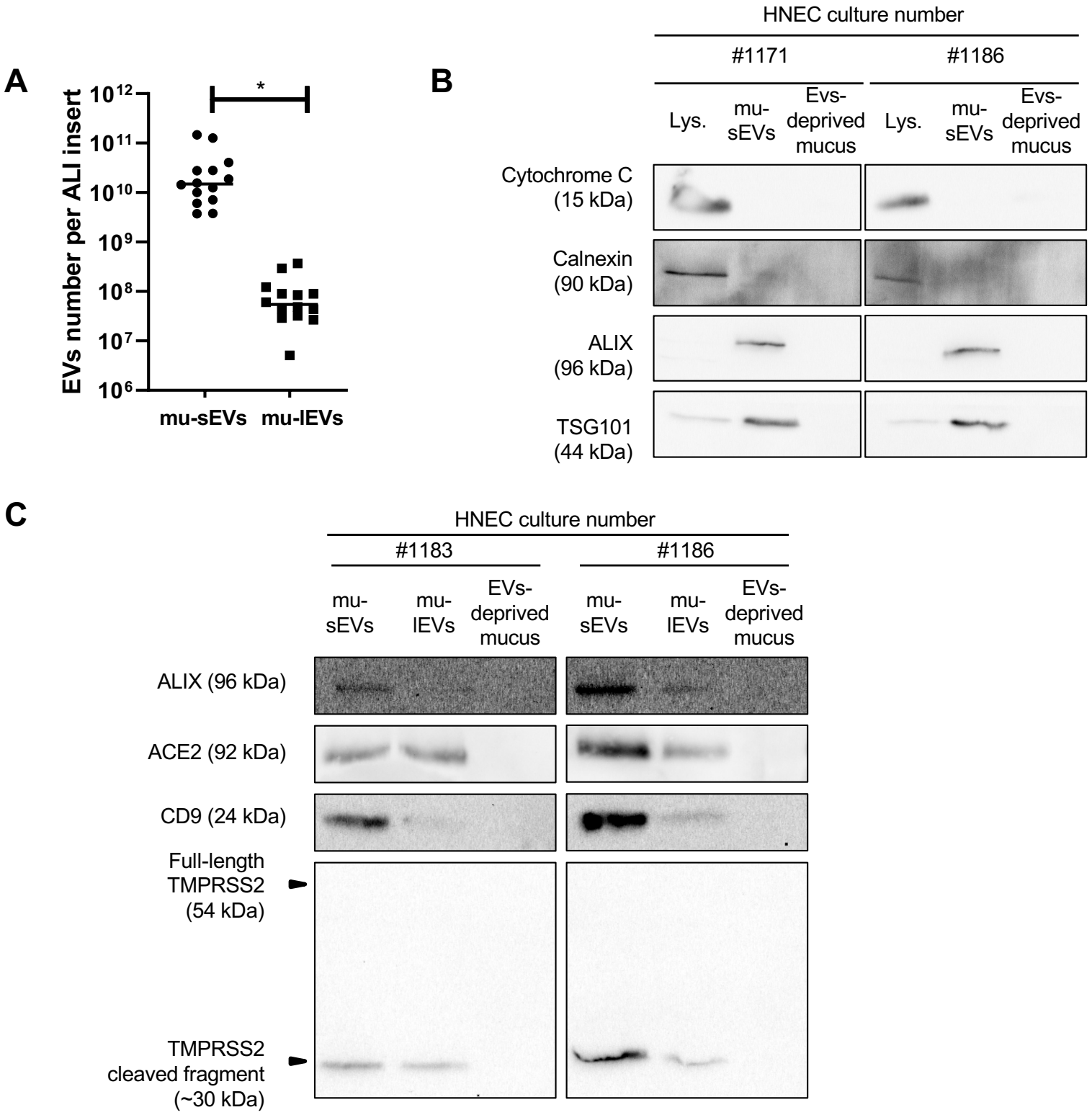
Supplementary Figure 1



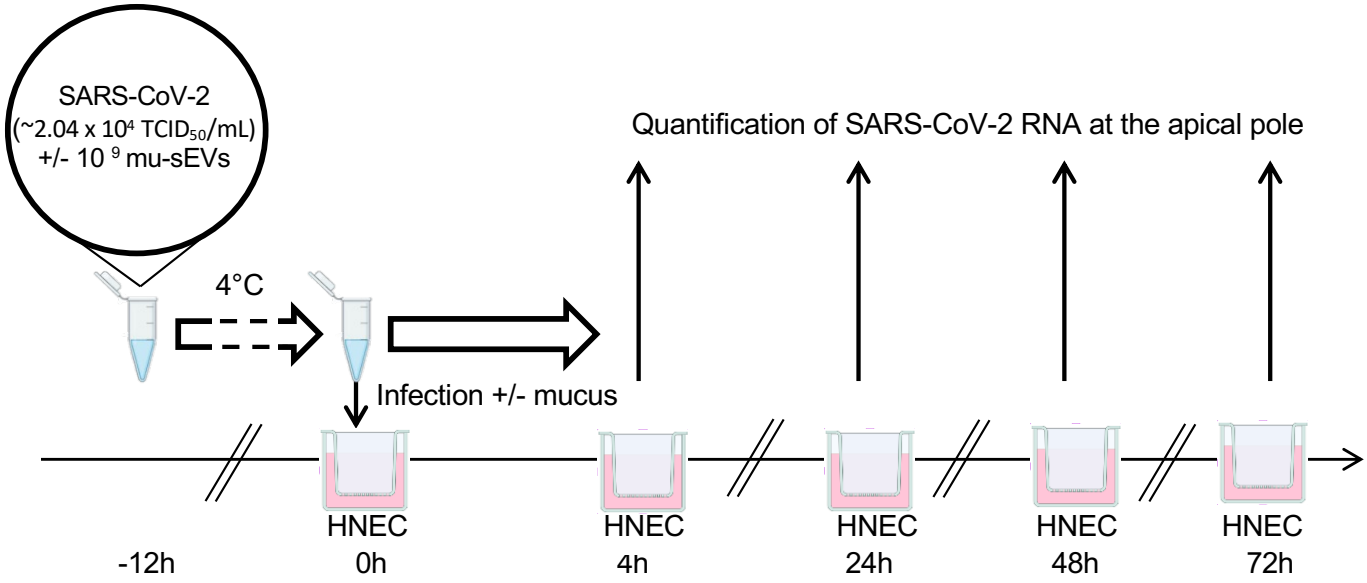
Supplementary Figure 1 legend. Effect of mucus removal on HNECs vitality. HNECs vitality as reflected by the trans-epithelial electrical resistance (TEER) measured in untreated or mucus-removed samples from two distinct patients, at different time-points.

Supplementary Figure 2



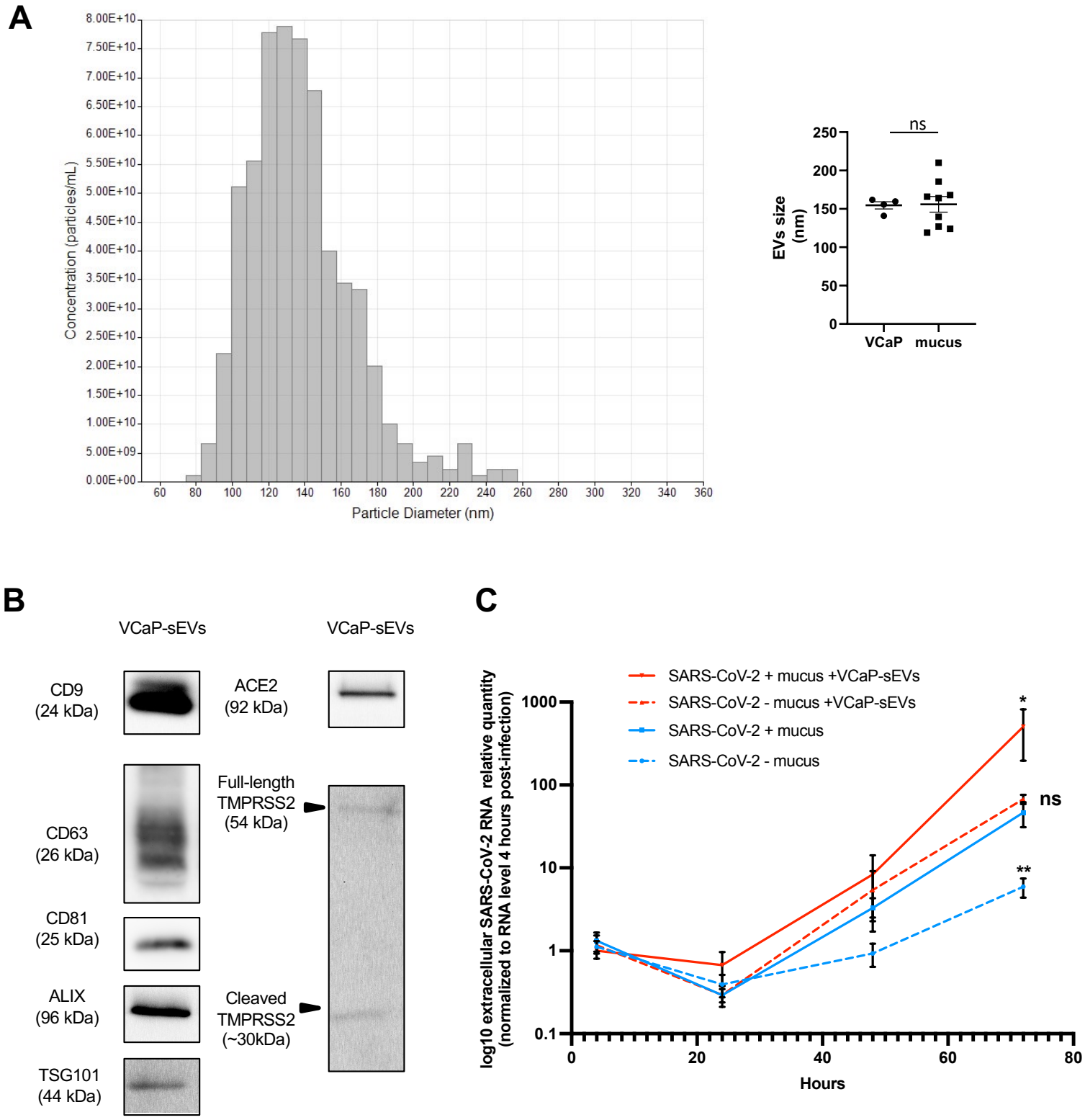
Supplementary Figure 2 legend. Characterization of EVs isolated from HNECs from distinct patients. (A) EVs produced by HNECs from 14 different patients analyzed by tunable resistive pulse sensing (TRPS) showing mean particles concentrations of 3.3±1.2 x 10^{10} mu-sEVs (small extracellular vesicles) and 9.6±2.8 x 10^{7} mu-lEVs (large extracellular vesicles) per ALI insert. **(B)** Western blot analysis of cytochrome C, Calnexin, ALIX and TSG101 using 10 µg of protein of HNECs lysates, mu-sEVs and EVs-deprived mucus. **(C)** Western blot analysis of CD9, ALIX, ACE2 and full-length and cleaved TMPRSS2 using 5 µg of protein of mu-sEVs, mu-lEVs and EVs-deprived mucus. *p <0.05.

Supplementary Figure 3



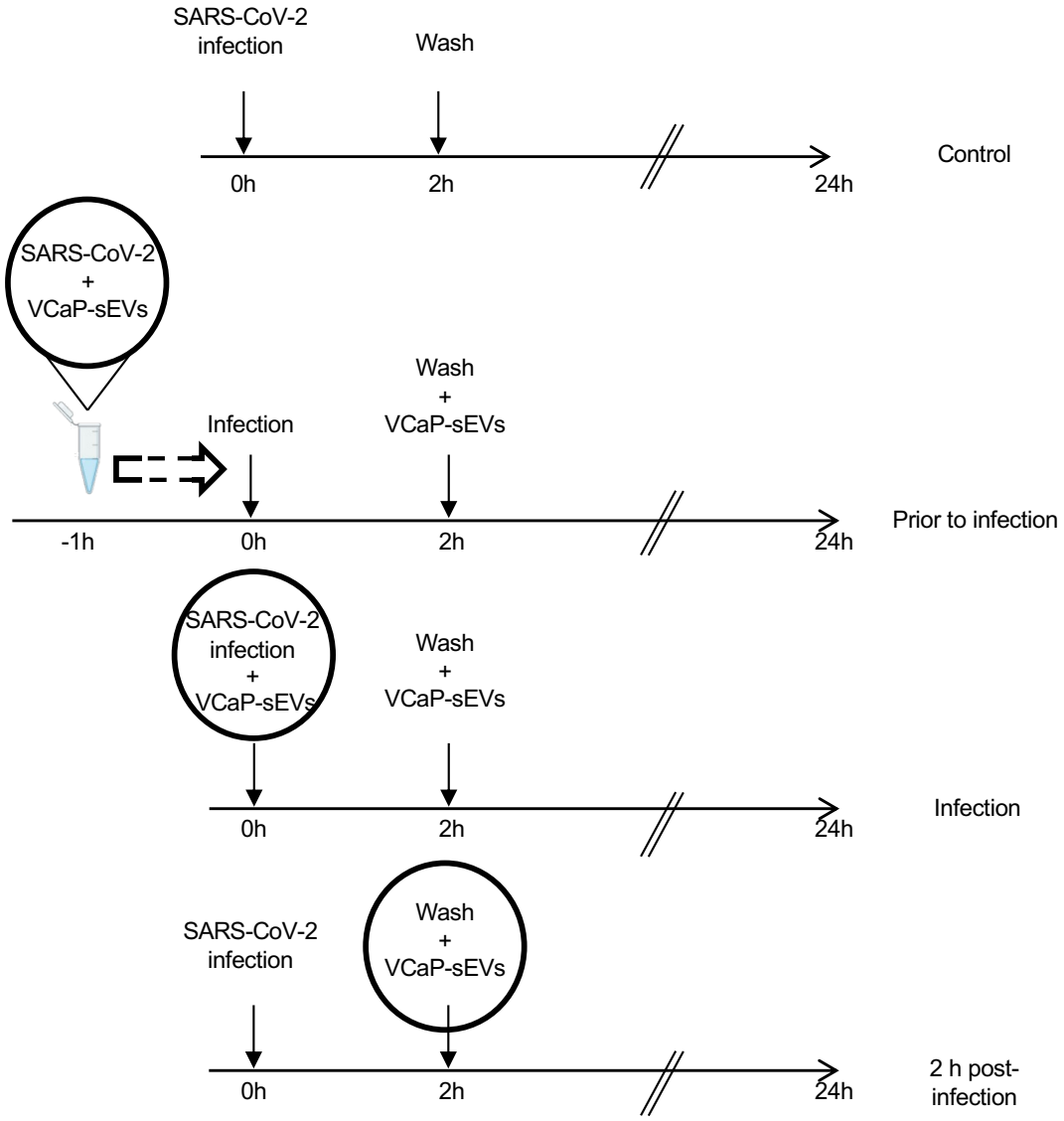
Supplementary Figure 3 legend. Effect of mu-sEVs and mucus-containing sEVs on HNEC infection by SARS-CoV-2: Experimental design. SARS-CoV-2 particles (10 µL of viral inoculum, ~2.04 x 10^5 $TCID_{50}$/mL) were incubated overnight at 4°C with 10^9 mu-sEVs from two different patients or with PBS in 100 µL final volume. Infection (20 µL of the mixture) was performed at the apical pole of HNECs isolated from a third, different, patient, in the presence or after removal of recipient cell mucus. Viral RNA was recovered at the apical pole at different time points post-infection and quantified.

Supplementary Figure 4



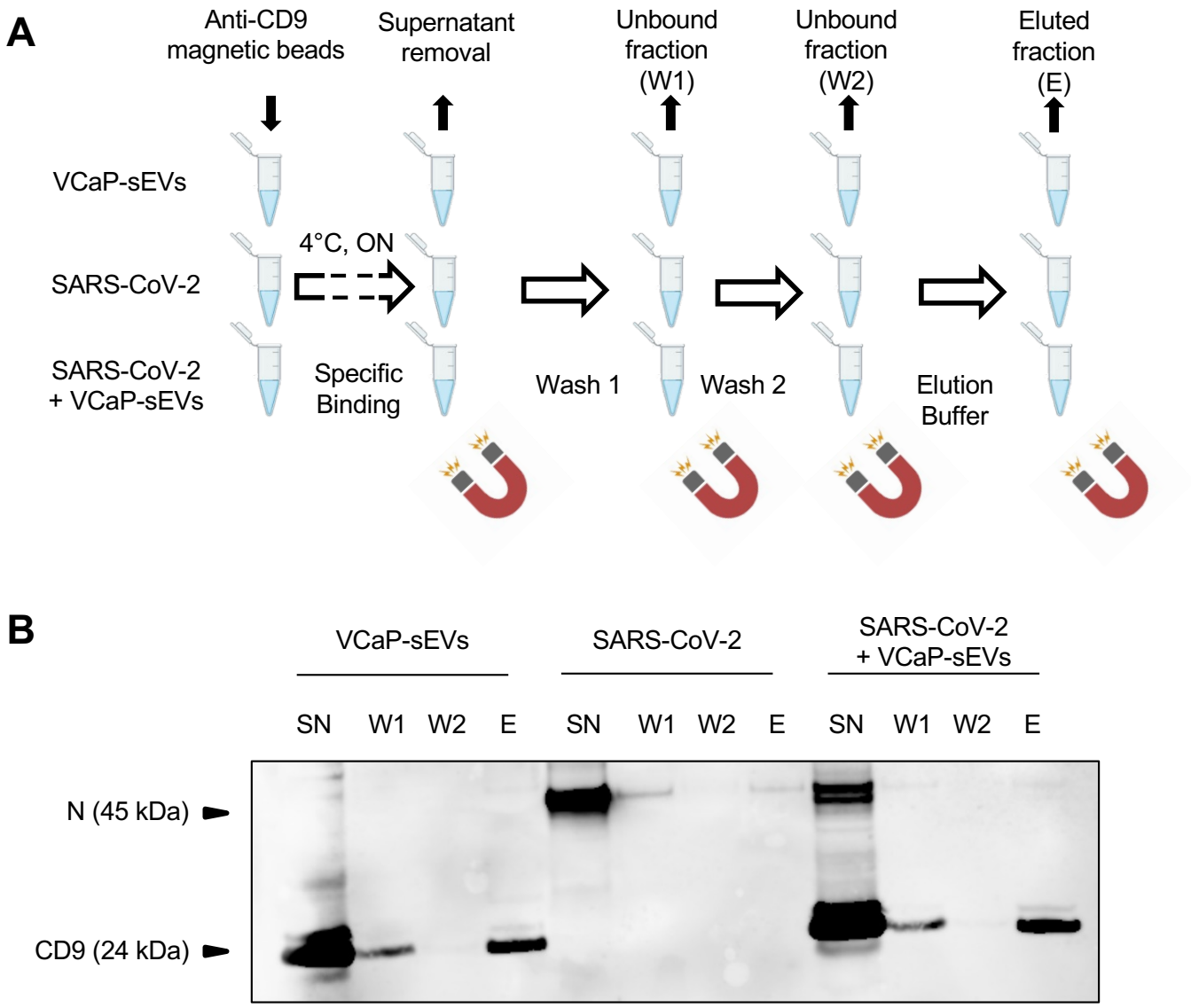
Supplementary Figure 4 legend. Characterization of VCaP-sEVs and effect on HNEC infection by SARS-CoV-2. (A) sEVs isolated from 4 independent VCaP cell cultures analyzed by tunable resistive pulse sensing (TRPS) showing a mean peak VCaP-sEV size of approximately ~150 nm in diameter. Left panel: histogram generated from one representative VCaP culture. Right panel: comparison of VCaP-sEV *vs* mu-sEV (mucus) sizes. **(B)** Western blot analysis of VCaP-sEV markers (CD9, CD63, CD81, ALIX, TSG101) and SARS-CoV-2 entry factors (ACE2 and TMPRSS2). **(C)** Dynamics of SARS-CoV-2 RNA production at the apical pole of HNECs isolated from two patients assessed by RT-qPCR. SARS-CoV-2 viral particles were preincubated with 10^9 VCaPs or PBS, overnight at 4°C, and HNECs infection in absence or presence of recipient cell mucus was performed as described in figure 2A. Results were normalized to the time-point 4 hours for each condition and expressed as log10 mean ± SEM of two independent experiments. *p <0.05, **p <0.01 (Mann-whitney U-test).

Supplementary Figure 5



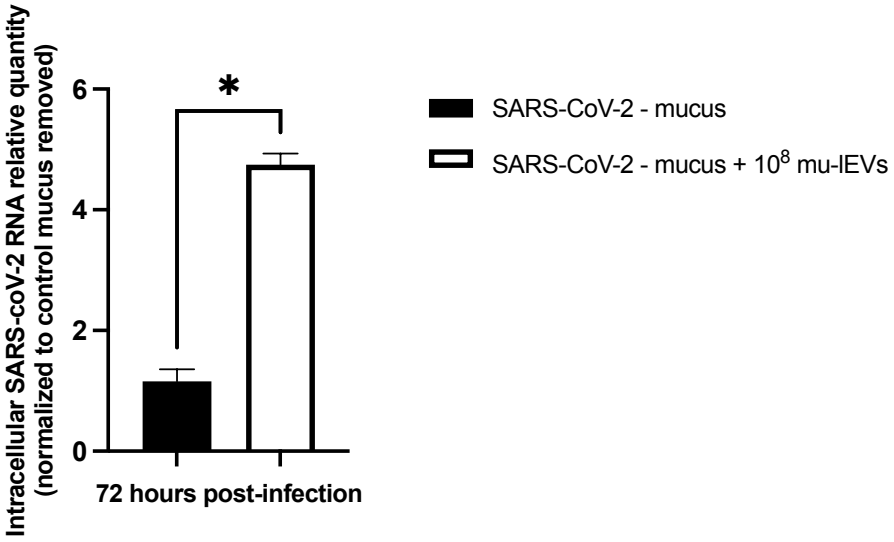
Supplementary Figure 5 legend. Design of time-of-addition experiments. Calu-3 cells were infected with SARS-CoV-2 virus not incubated with VcaP-sEVs (control) or incubated with VCaP-sEVs for 1 hour prior to infection, added at the time of infection or added 2 hours post-infection. Cells were washed 2 hours post-infection, and culture medium containing VCaP-sEVs was added. Infections were performed at a final MOI of 1 and 10^9 VCaP-sEVs were used. Intracellular RNA was extracted 24 hours post-infection and quantified by means of RT-qPCR.

Supplementary Figure 6



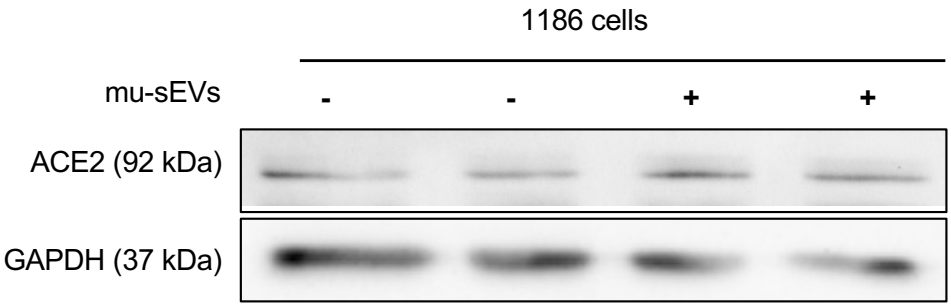
Supplementary Figure 6 legend. SARS-CoV-2/VCaP-sEV fusion assay. (A) Experimental Design. Exosome-human CD9 isolation beads (100 µL) were incubated with 10^{10} VCaP-sEVs, SARS-CoV-2 viral particles (10 µL viral inoculum, ~2.04 x 10^4 $TCID_{50}$) or a mixture of both in PBS -/- (final volume 100 mL) at 4°C overnight (ON) on a rotating wheel. All samples were placed on a magnetic separator and supernatants (SN) were collected. Two washings (300 µL PBS) were performed sequentially and collected using a magnetic separator (W1, W2). Finally, beads were resuspended in 50 µL EZ buffer (E) before denaturation at 95°C and analysis by western blot. **(B)** Western blot analysis of CD9 and N proteins in all fractions collected in (A).

Supplementary Figure 7



Supplementary Figure 7 legend. Effect of mu-lEVs on HNECs infection by SARS-CoV-2. Preincubations were performed under gentle agitation, overnight at 4°C, using SARS-CoV-2 viral particles (10 µL of viral inoculum, ~2.04 x 105 TCID50/mL) and PBS or 10^8 mu-lEVs in 100 µL final volume. Infection was performed at the apical pole of HNECs, after removal of cell produced-mucus. Intracellular SARS-CoV-2 RNA was extracted 72 hours post-infection and was quantified by RT-qPCR. Results were normalized to 18S rRNA, then to control without mucus and viral particles preincubation with PBS (black bar). They are expressed as mean±SEM of two independent experiments. * p <0.05.

Supplementary Figure 8



Supplementary Figure 8 legend. Effect of mu-sEVs incubation on ACE2 expression in HNECs cells. Western blot analysis of ACE2 and GAPDH proteins in HNECs lysates, after incubation of HNECs with 10^9 mu-sEVS or PBS for 4 hours.