SARS-CoV-2 and its ORF3a, E and M viroporins activate inflammasome in human macrophages and induce of IL-1α in pulmonary epithelial and endothelial cells

Magdalena Ambrożek-Latecka¹, Piotr Kozlowski², Grażyna Hoser¹, Magdalena Bandyszewska¹, Karolina Hanusek³, Dominika Nowis⁴, Jakub Gołąb⁴, Małgorzata Grzanka³, Agnieszka Piekiełko-Witkowska³, Luise Schulz⁵, Franziska Hornung⁵, Stefanie Deinhardt-Emmer⁵, Ewa Kozlowska⁶, Tomasz Skirecki^{1*}

Supplementary results

Supplementary Figure 1. NLRP3 inflammasome is engaged in IL- β and gasdermin D maturation upon SARS-CoV-2 viroporin expression. Densitometric analysis of Western blots from U937 WT and U937 NLRP3 KO cells transfected with lentiviral particles carrying ORF3A, E and M proteins. A. IL-1 β . B. Gasdermin D (GSDMD). Graphs represent means \pm SEM, n=3. Multiple comparisons were performed with two-way ANOVA test. ****p<0.0001.





Supplementary Figure 2. Validation of the novel inflammasome reporter cell lines. Immortalized cell lines expressing the ASC::GFP protein were stimulated with LPS and nigericin. A. human bronchial epithelial cells -3 KT cell line. B. human umbilical vein endothelial cells/TERT cell line.



Supplementary Figure 3. Analysis of the activation of apoptotic caspases in human pulmonary epithelial cells and human pulmonary microvascular endothelium by the viroporins A. Activation of caspase-3/7 in HPAEpiCs by the viroporins; representative pictures (left) and fluorometric readouts (right) are shown. F. Activation of caspase-3/7 in HPMECs by the viroporins; representative pictures (left) and fluorometric readouts (right) are shown. F. Activation of caspase-3/7 in shown. Graphs represent means \pm SEM, n=3-4. Two groups comparisons were analyzed with t-test. Multiple comparisons were performed with two-way ANOVA test. *p<0.05.

