Dynamic Interaction Between SARS-CoV-2 and Influenza A Virus Infection in Human Respiratory Tissues and Cells

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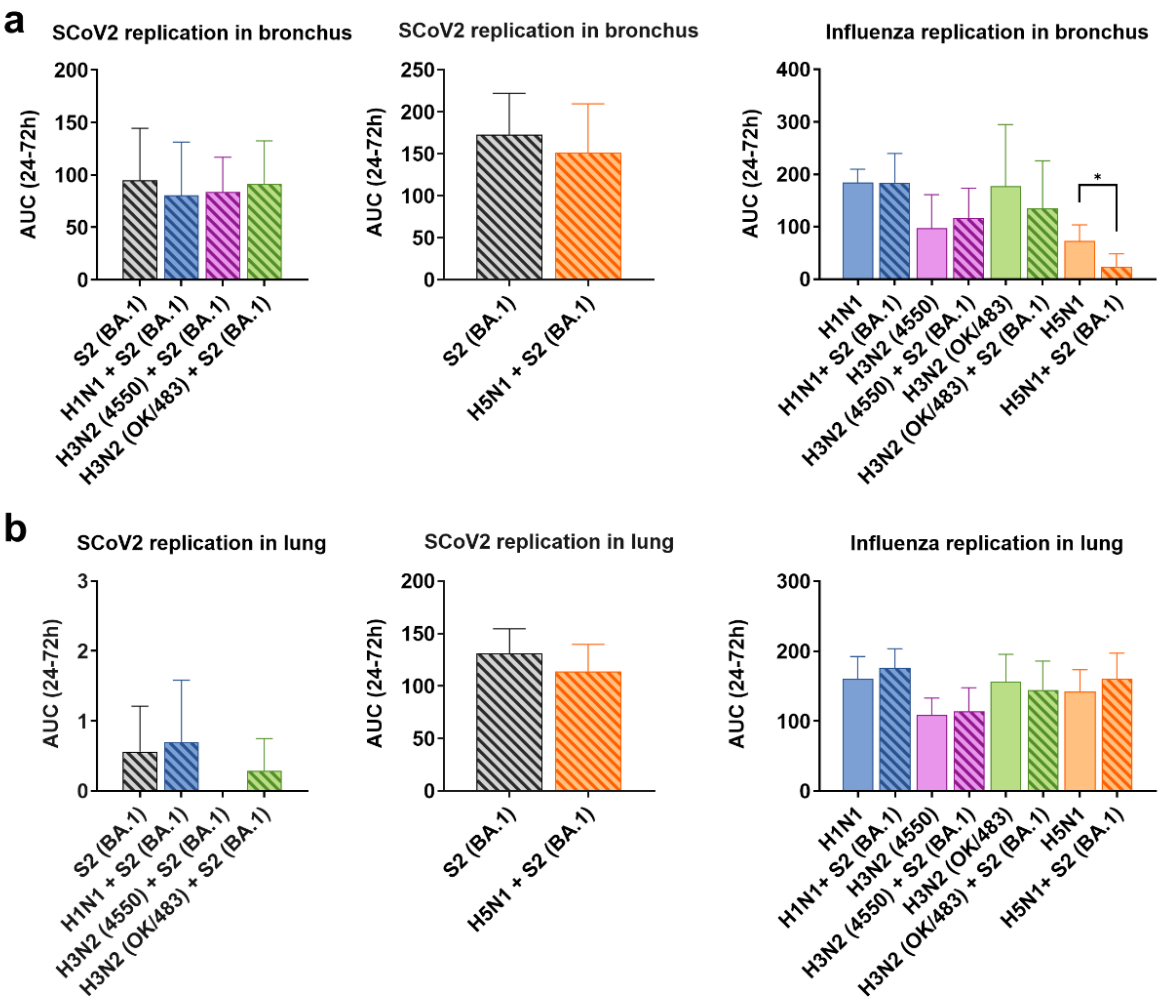
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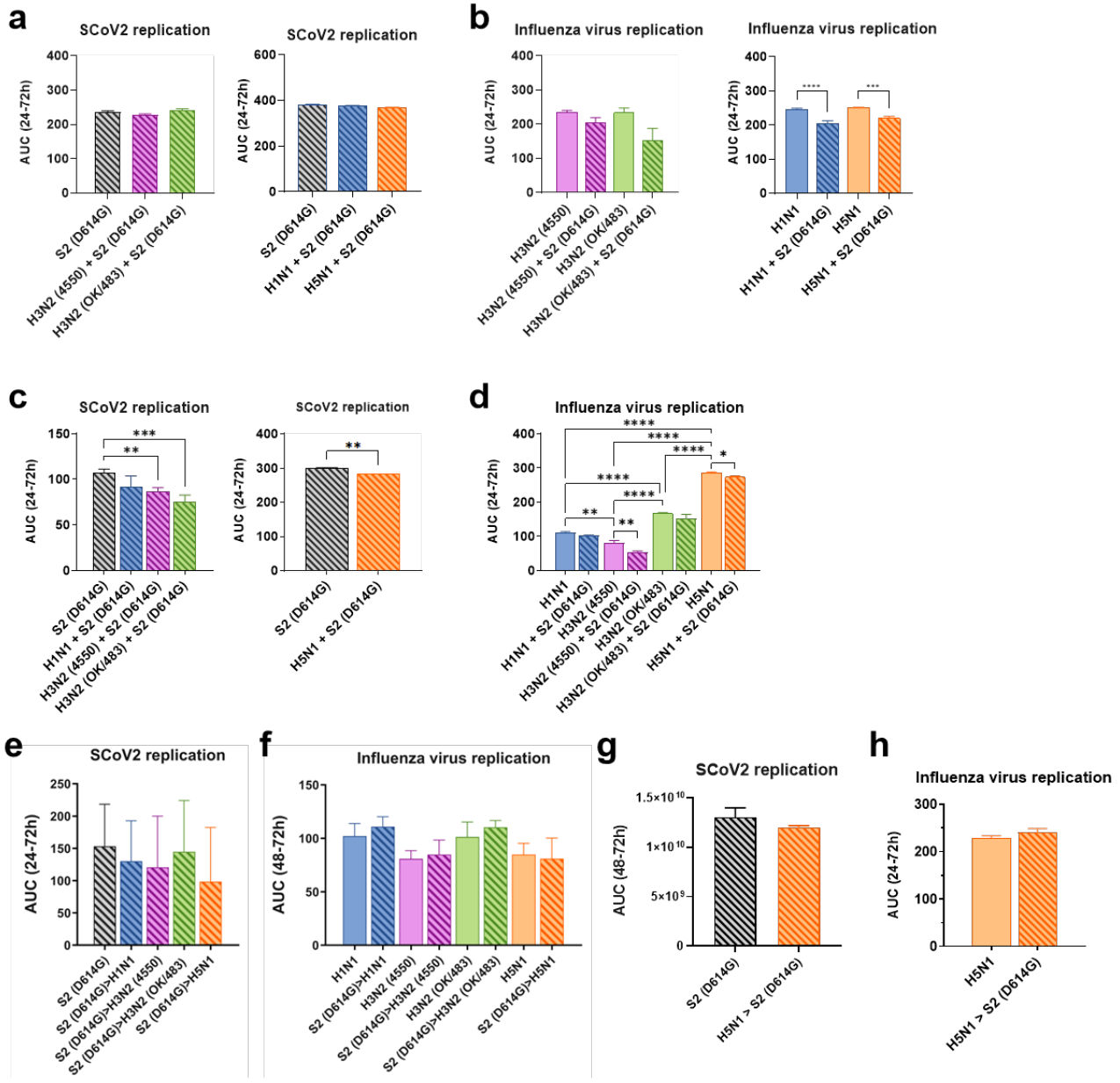
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Supplementary Table S1. List of primers used for quantitative PCR

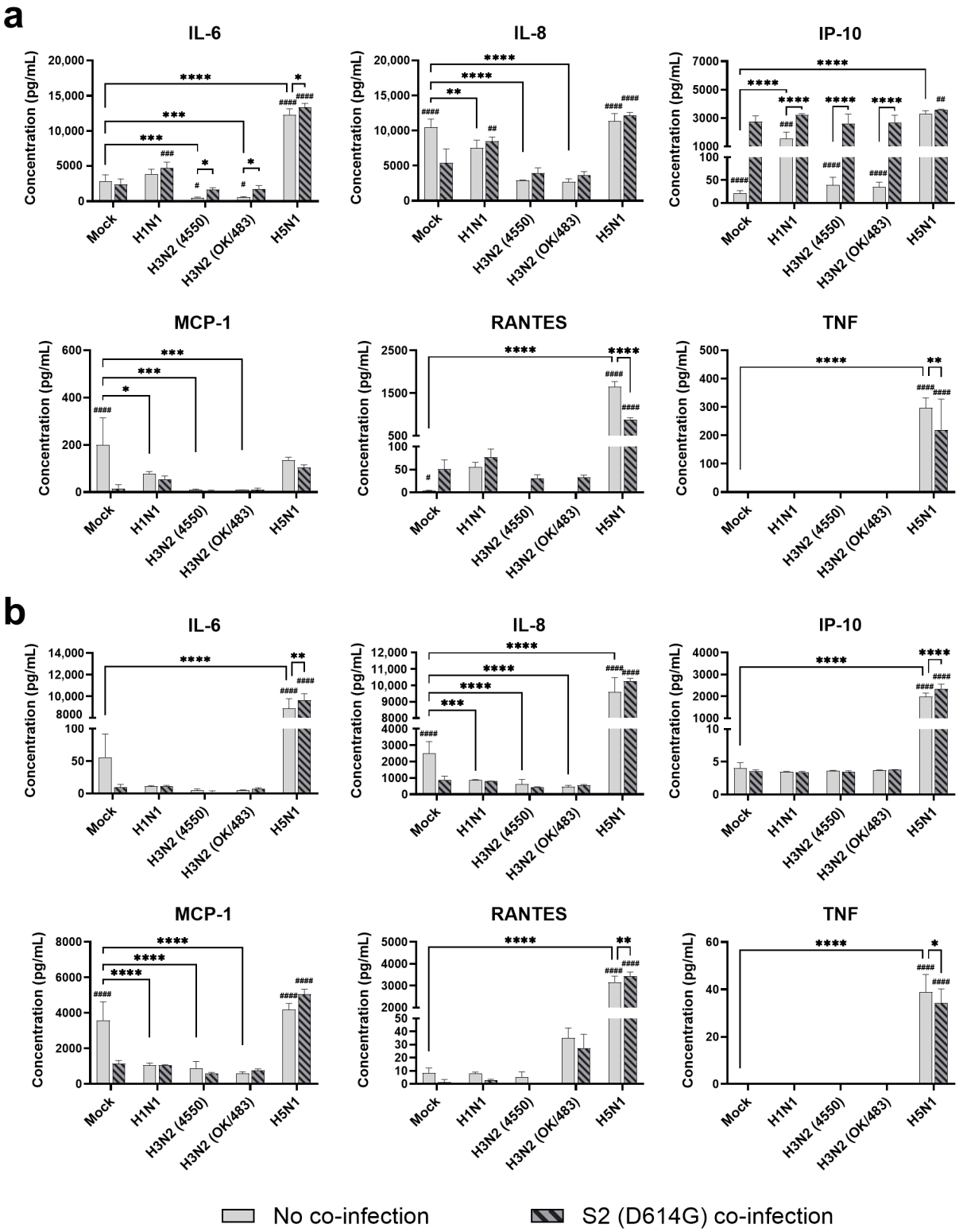
Gene	Primer sequence (5'-3')
β-actin	F: TGGATCAGCAAGCAGGAGTATG
	R: GCATTTGCGGTGGACGAT
SARS-CoV-2 ORF1b	F: TGGGGYTTTACRGGTAACCT
	R: AACRCGCTTAACAAAGCACTC
Influenza M-gene	F: CTTCTAACCGAGGTCGAAACG
	R: GGCATTTTGGACAAAKCGTCTA
ACE2-long isoform	F: CAAGAGCAAACGGTTGAACAC
	R: CCAGAGCCTCTCATTGTAGTCT
ACE2-short isoform	F: GTGAGAGCCTTAGGTTGGATTC
	R: TAAGGATCCTCCCTCCTTTGT
IFN-α	F: AGAAGGCTCCAGCCATCTCTGT
	R: TGCTGGTAGAGTTCGGTGCAGA
IFN-β	F: CAACTTGCTTGGATTCCTACAAAG
	R: TGCCACAGGAGCTTCTGACA
IFN-λ1	F: GCCCCCAAAAAGGAGTCCG
	R: AGGTTCCCATCGGCCACATA
IFN-λ2/3	F: TTTAAGAGGGCCAAAGATGC
	R: TGGGCTGAGGCTGGATACAG
IL-1β	F: CACGATGCACCTGTACGATCA
	R: GTTGCTCCATATCCTGTCCCT
IL-6	F: GCATGGGCACCTCAGATTGT
	R: TGCCCAGTGGACAGGTTTCT
IL-8	F: CACCGGAAGGAACCATCTCA
	R: AGAGCCACGGCCAGCTT
IP-10	F: ATTATTCCTGCAAGCCAATTTTG
	R: TCACCCTTCTTTTTCATTGTAGCA
ISG15	F: CAAATGCGACGAACCTCTGA
	R: CCGCTCACTTGCTGCTTCA
MCP-1	F: CAAGCAGAAGTGGGTTCAGGAT
	R: TCTTCGGAGTTTGGGTTTGC
MDA-5	F: TCACAAGTTGATGGTCCTCAAGT
	R: CCTTCTCCAGATTTGGCTGAAC
TNF-α	F: GCAGGTCTACTTTGGGATCATTG
	R: GCGTTTGGGAAGGTTGGA



Supplementary Figure S1. Replication kinetics of influenza A virus and SARS-CoV-2 co-infection in ex vivo culture of human respiratory tract tissues. The area-under-curve (AUC) depicted from the viral replication of SARS-CoV-2 Omicron (SCoV2/BA.1) and influenza A viruses in human bronchus **(a)** and lung **(b)** tissue explants. The viral replications were determined by infectious titres using $TCID_{50}$ assays or SARS-CoV-2 ORF1b gene copies using quantitative PCR. Data are presented as the mean values ± SD from 4 individual donors, analyzed with one-way ANOVA followed by Tukey's test. $^{*}p < 0.05$.

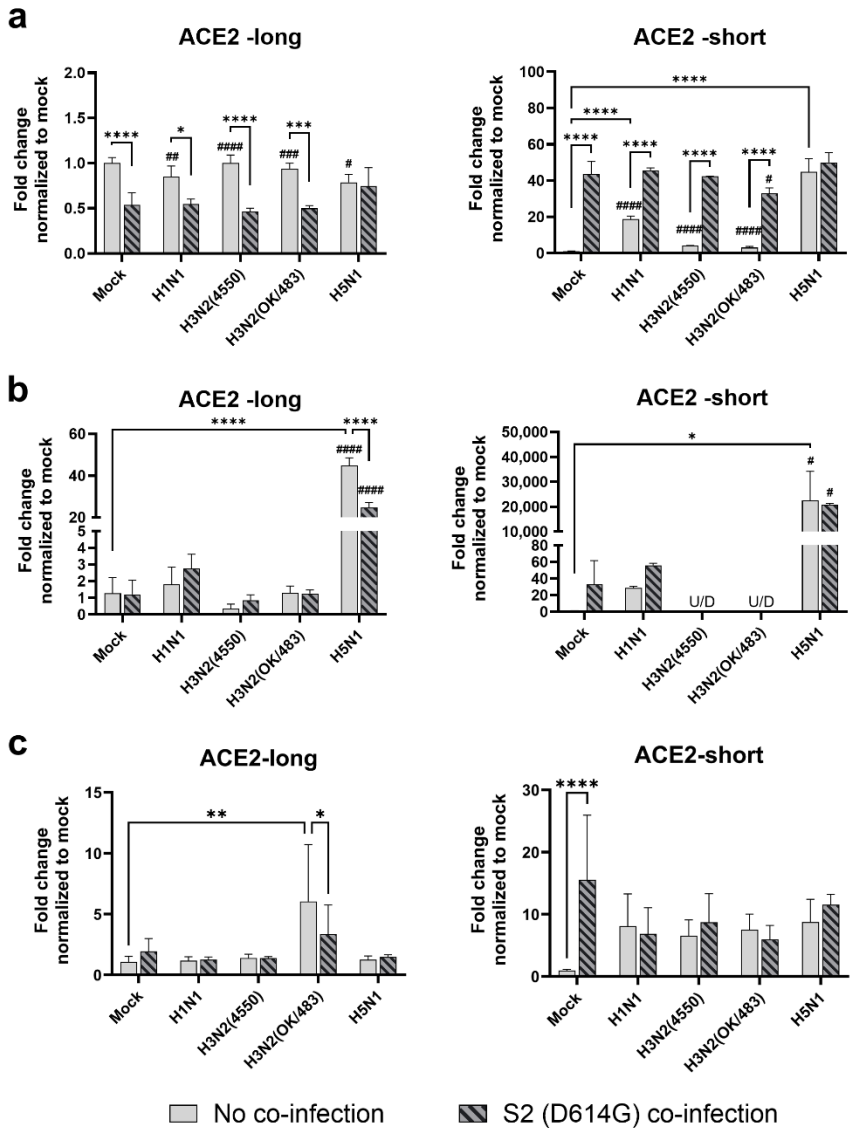


Supplementary Figure S2. Replication kinetics of influenza A virus and SARS-CoV-2 co-infection in human airway and alveolar epithelial cells. The area-under-curve (AUC) depicted from the viral replications of SCoV2/D614G and influenza A viruses in human airway epithelial cells Calu-3 **(a and b)** and alveolar epithelial cells A549/ACE2 **(c and d)** from simultaneous co-infection; in human airway organoid culture **(e and f)** sequentially infected with SCoV2/D614G followed by influenza A viruses, and in A549/ACE2 cells **(g and h)** sequentially infected with influenza H5N1 followed by SCoV2/D614G. Data are presented as the mean values ± SD (N ≥3), analyzed with one-way ANOVA followed by Tukey's test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.



Supplementary Figure S3. Cytokine productions in human airway and alveolar epithelial cells upon co-infection of SARS-CoV-2 and influenza A virus. The protein levels of cytokines and chemokines in cell culture of Calu-3 **(a)** and A549/ACE2 **(b)** infected with SCoV2/D614G and/or

influenza A virus strains, measured with Cytometric Bead Array at 72 h post-infection. Data are presented as the mean values ± SD (N ≥ 3), analyzed with two-way ANOVA followed by Tukey's test. Hashtags # denote the statistical significance in comparison to SCoV2/D614G single infection. *# p <0.05; ## p <0.01; ### p <0.001; #### p <0.0001.* * *p <0.05;* ** *p <0.01;* *** *p <0.001;* **** *p <0.0001.*



Supplementary Figure S4. ACE2 expression in human airway and alveolar epithelial cells infected with SARS-CoV-2 and influenza A virus. The mRNA expressions of long and short isoforms of ACE2 gene in Calu-3 cells **(a)**, A549/ACE2 cells **(b)**, and human airway organoid culture **(c)** infected with SCoV2/D614G and/or influenza A virus strains, measured with quantitative PCR at 72 h post-infection. Simultaneous co-infection **(a and b)** of SCoV2/D614G and influenza viruses, and sequential infection **(c)** with SCoV2/D614G followed by influenza A viruses at 24 hpi. Data are presented as the mean values ± SD (N ≥ 3), analyzed with two-way ANOVA followed by Tukey's test. Hashtags # denote the statistical significance in comparison to SCoV2/D614G single infection. *# p <0.05; ## p<0.01; ### p <0.001; #### p <0.0001. * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.*