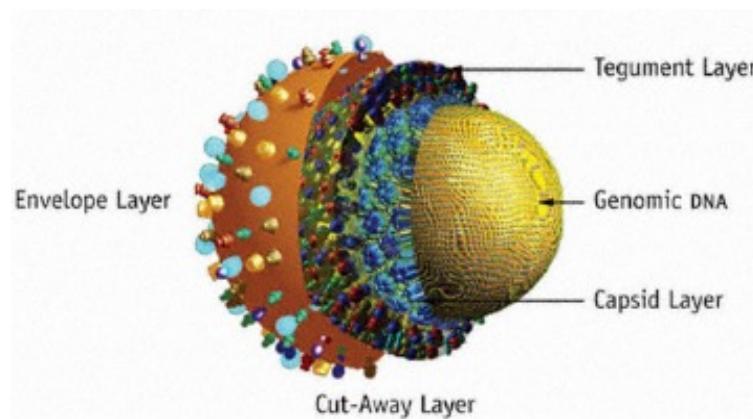
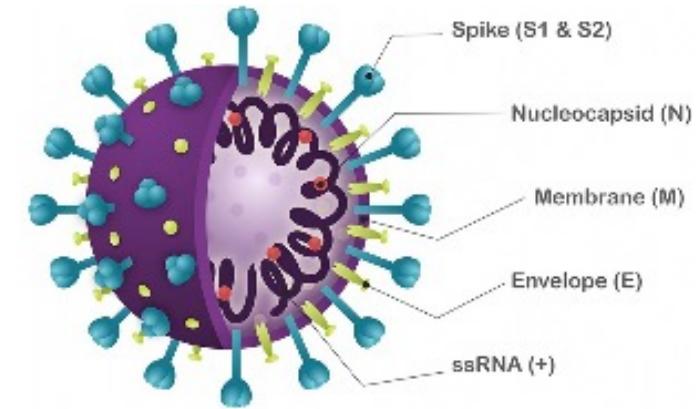


Human Herpesviruses in Augmenting COVID-19 Symptoms



Gammaherpesvirus

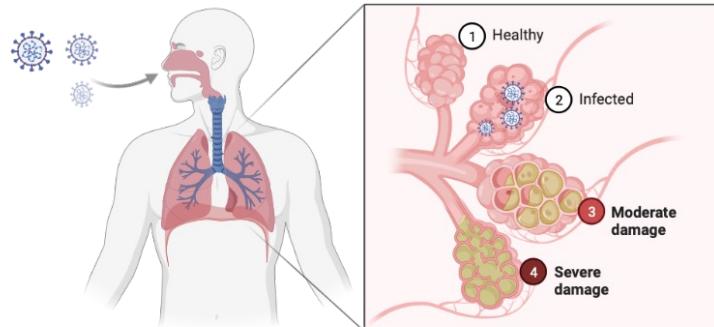


SARS-CoV-2

Subhash C. Verma, Ph.D.
Microbiology and Immunology, UNR School of Medicine

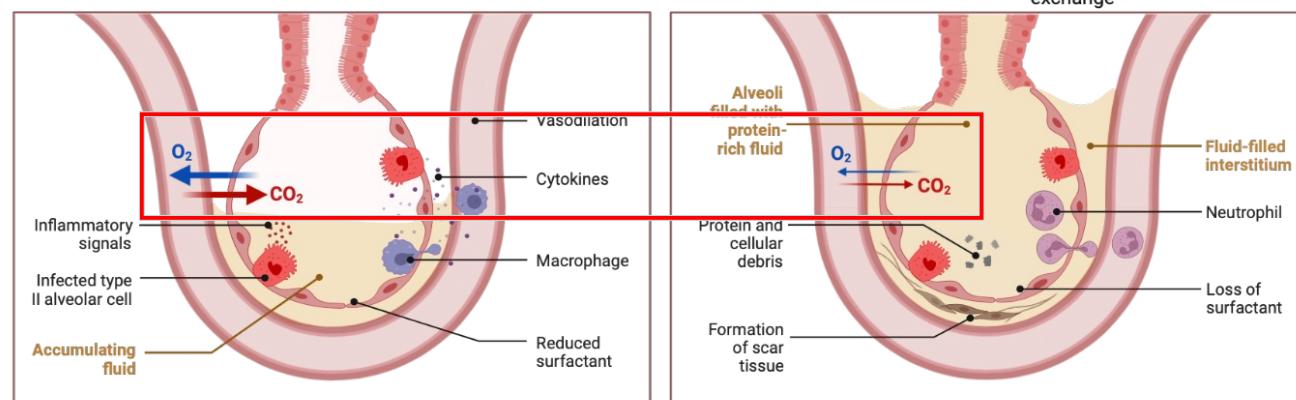
Acute Respiratory Distress Syndrome (ARDS) and COVID-19

COVID-19: ARDS



Moderate damage: Accumulating fluid, reduced gas exchange

Severe damage: Build up of protein-rich fluid, very limited gas exchange



SpO₂ <90% (Req. Hospitalization)

Hypoxia

Activation of Hypoxic Transcription Factors (e.g., HIF-1 α)

Reactivation of HHVs

Herpesviruses (HHVs) Reactivation in COVID-19 Patients

COVID-19

HSV-1 (HHV1) Reactivation



VZV (HHV3) Reactivation



HHV-1 Herpes Simplex Virus 1 (HSV-1)

HHV-2 Herpes Simplex Virus 2 (HSV-2)

HHV-3 Varicella Zoster Virus (VZV)

HHV-4 Epstein-Barr Virus (EBV)

HHV-5 Cytomegalovirus (CMV)

HHV-6 Human Herpes Virus 6 (HHV-6)

HHV-7 Human Herpes Virus 7 (HHV-7)

HHV-8 Kaposi's Sarcoma-associated Herpes Virus (KSHV)

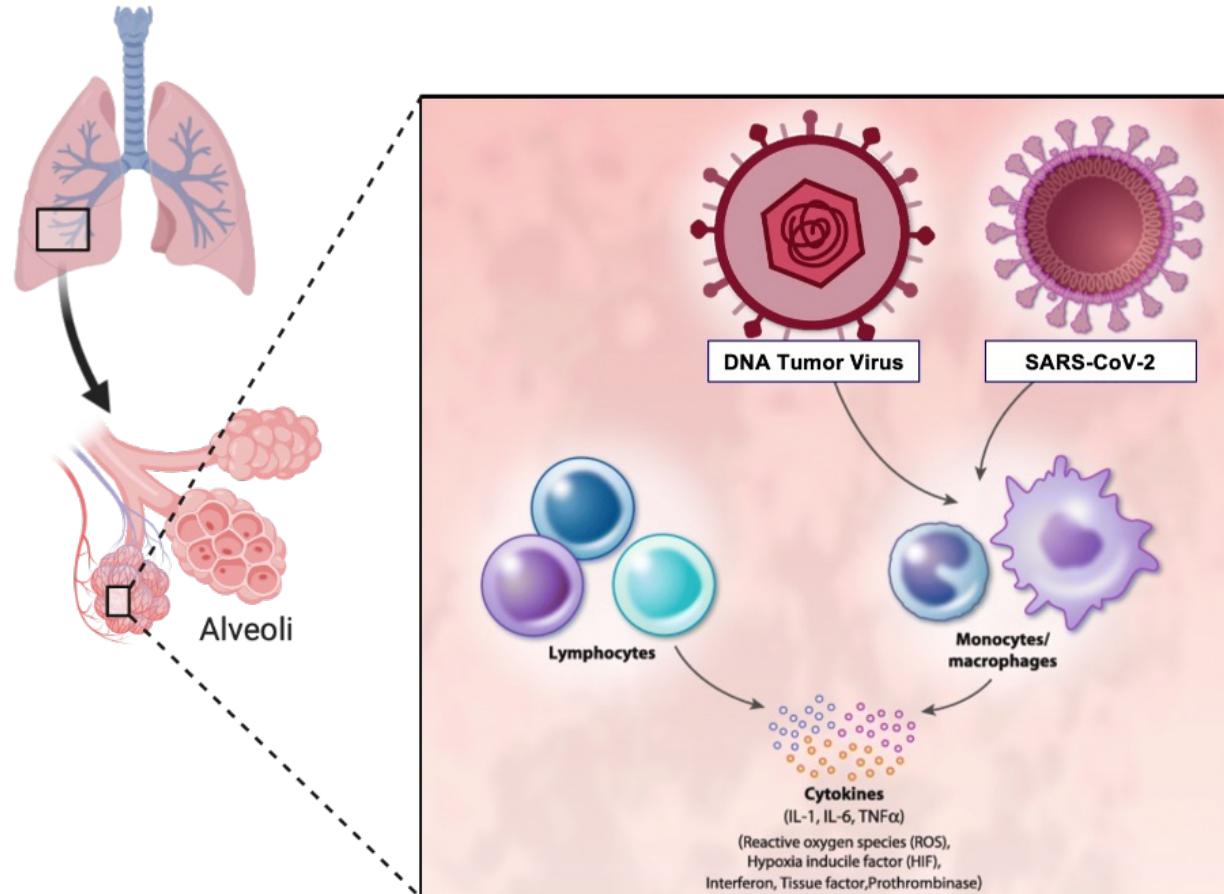
α

γ

β

γ

Cytokine Storm in COVID-19 Patients



1. HHVs/SARS-CoV-2 infect lung epithelial cells
2. Immune cells produce cytokines
3. Cytokines attract more immune cells - *increased* cytokine production and inflammation
4. Damage of lung cells (via fibrin)
5. Weakened blood vessels, fluid sequestration in the lung cavity

COVID-19 Patient Cohort (PBMCs)

(n=57)	COVID-19 Patients, n (%)	Healthy Controls, n (%)
<i>Total number of cases, n (% of total cases)</i>	51 (89.5)	6 (10.5)
<i>SARS-CoV-2 status</i>	51 (100)	-
<i>COVID-19 severity, n (%)</i>		
Asymptomatic	9 (15.8)	-
Mild	25 (43.9)	-
Severe	9 (15.8)	-
Critical	9 (15.8)	-
<i>Gender</i>		
Male	26 (45.6)	3 (50)
Female	28 (49.1)	3 (50)
<i>Age (Male)</i>		
19-64	20 (35.1)	2 (33.3)
65+	6 (10.5)	1 (16.6)
<i>Age (Female)</i>		
19-64	18 (31.6)	3 (50)
65+	10 (17.5)	0 (0)

- Isolated RNA from PBMCs from COVID-19 patients and determined HHV levels
- Metagenomics analysis
- Identified cellular and HHV genes augmenting COVID-19 pathology



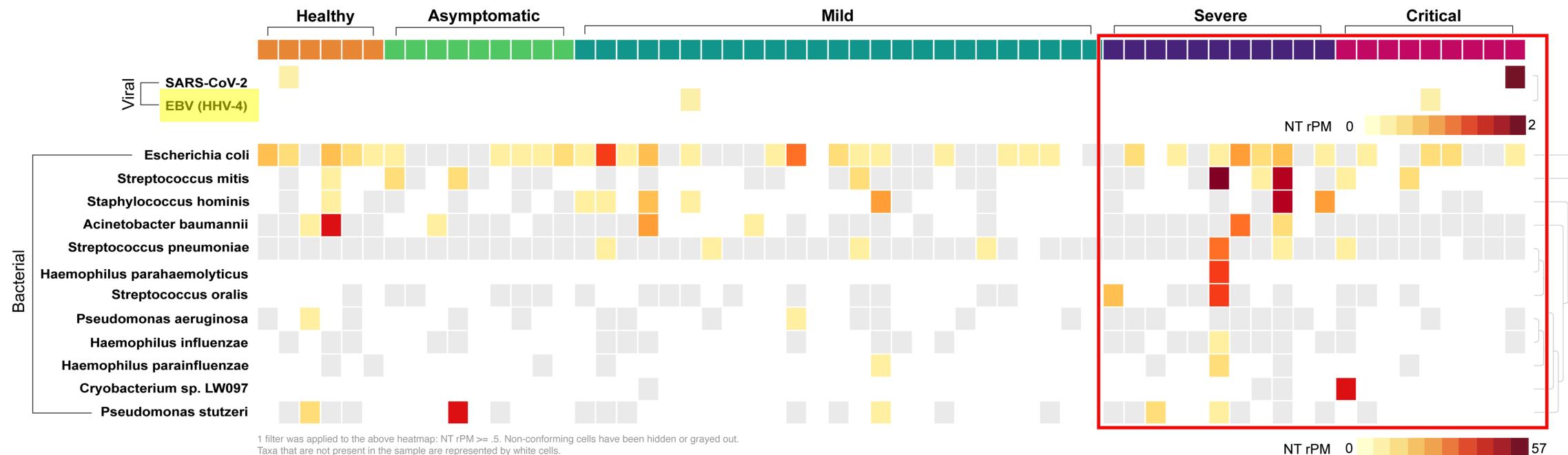
Pathogen Detection in PBMCs – Metagenomics (CZiD.org)

Reactivation of HHVs

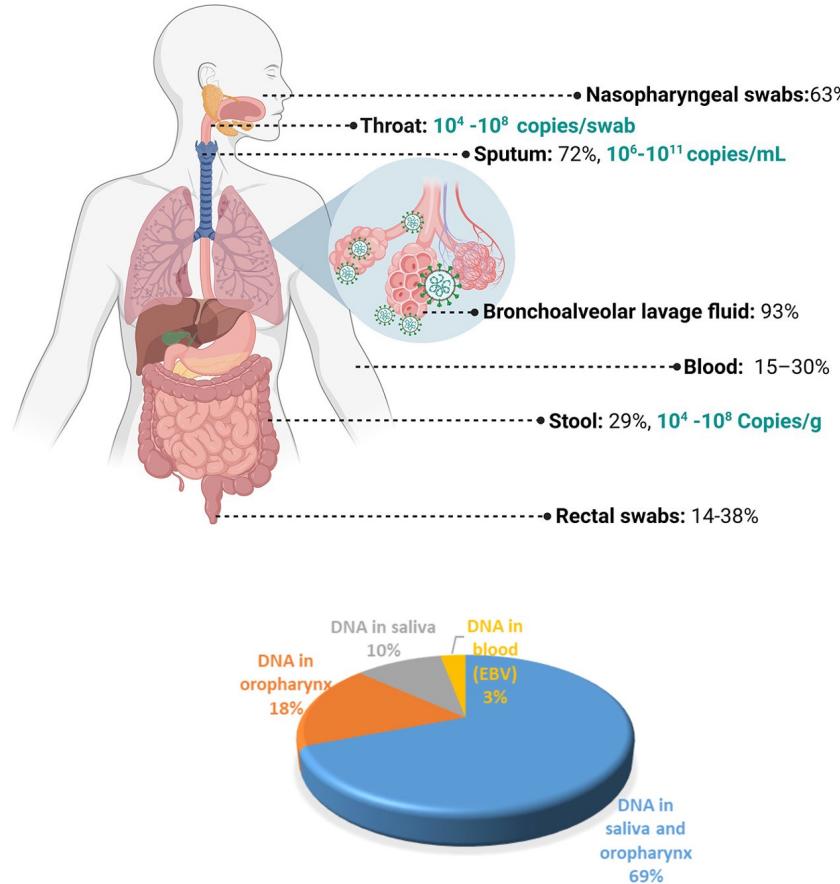
Clinical Severity	EBV n (%)	HCMV n (%)
Healthy	0/6 (0)	0/6 (0)
Asymp.	1/9 (11.1)	0/9 (0)
Mild	1/25 (4)	0/25 (0)
Severe	2/9 (22.2)	0/9 (0)
Critical	2/9 (22.2)	1/9 (11.1)
Total	6/57 (10.52)	1/57 (1.75)

Serum IL-6 Levels

	Healthy	COVID-19
Total (n=80)	10	70
IL-6 (pg/mL/1 µg protein)	4.9 ± 5.1	67.4 ± 9.3



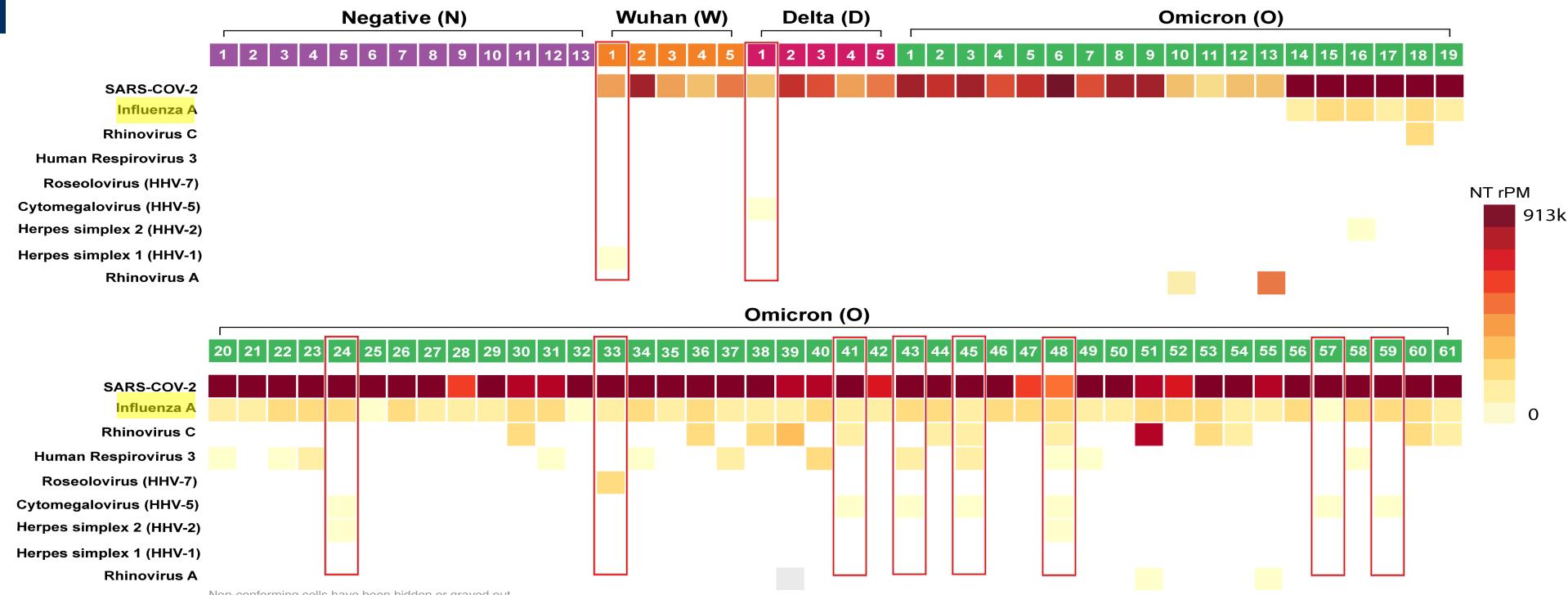
NP Swabs as a Proxy for Lung: COVID-19 Patient Cohort



(n=84)	COVID-19 Patients, n (%)	Healthy Controls, n (%)
Total number of cases, n (% of total cases)	71 (84.5)	13 (15.5)
SARS-CoV-2 status		
Wuhan	5 (6)	-
Delta	5 (6)	-
Omicron	61 (72.6)	-
COVID-19 severity, n (%)		
Asymptomatic	8 (11.27)	-
Mild	15 (21.13)	-
Severe	19 (26.76)	-
Critical	4 (5.63)	-
Gender		
Male	31 (43.6)	4 (30.8)
Female	31 (43.6)	5 (38.5)
Unknown	9 (12.7)	4 (30.8)
Age (Male)		
≤18	5 (7)	0 (0)
19-64	22 (31)	4 (30.8)
65+	4 (5.6)	0 (0)
Age (Female)		
≤18	2 (2.8)	0 (0)
19-64	22 (31)	4 (30.8)
65+	7 (9.9)	1 (7.7)



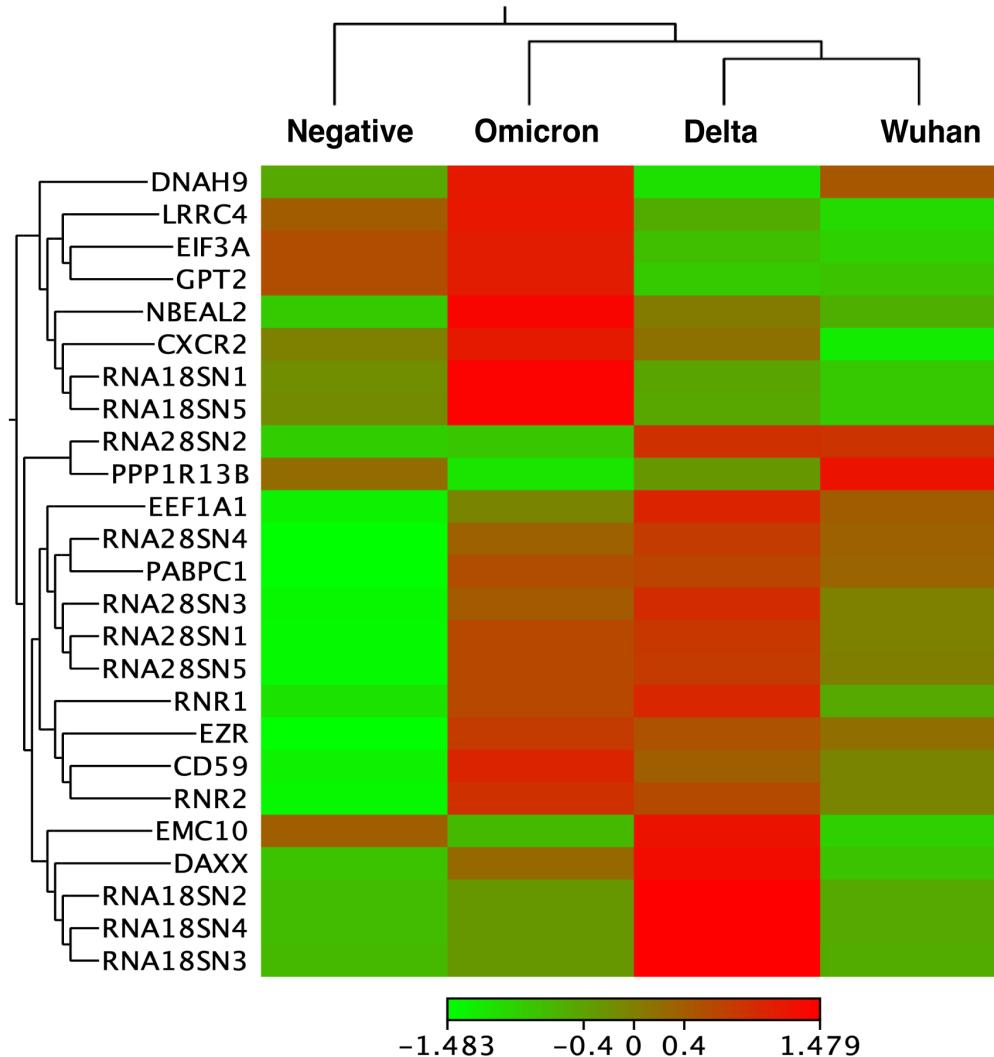
Respiratory pathogens in NP Swabs – Metagenomics (CZiD.org)



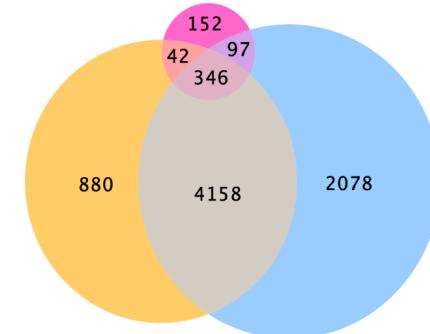
Reactivation of HHVs			
Clinical Severity	EBV n (%)	HCMV n (%)	HSV n (%)
Healthy	0/13 (0)	0/13	0/13
Asymp.	0/8 (0)	0/8 (0)	1/8 (12.5)
Mild	0/3 (0)	3/15 (20)	0/15 (0)
Severe	0/19 (0)	2/19 (10.5)	2/19 (10.5)
Critical	0/4 (0)	3/4 (75)	1/4 (25)
N/A	0/61 (0)	13/61 (21.3)	7/61 (11.5)
Total	0/84 (0)	21/84 (25)	11 (13.1)



Cellular Gene Profiling in COVID-19 Patients (NP Swabs)



Negative vs Delta (637)

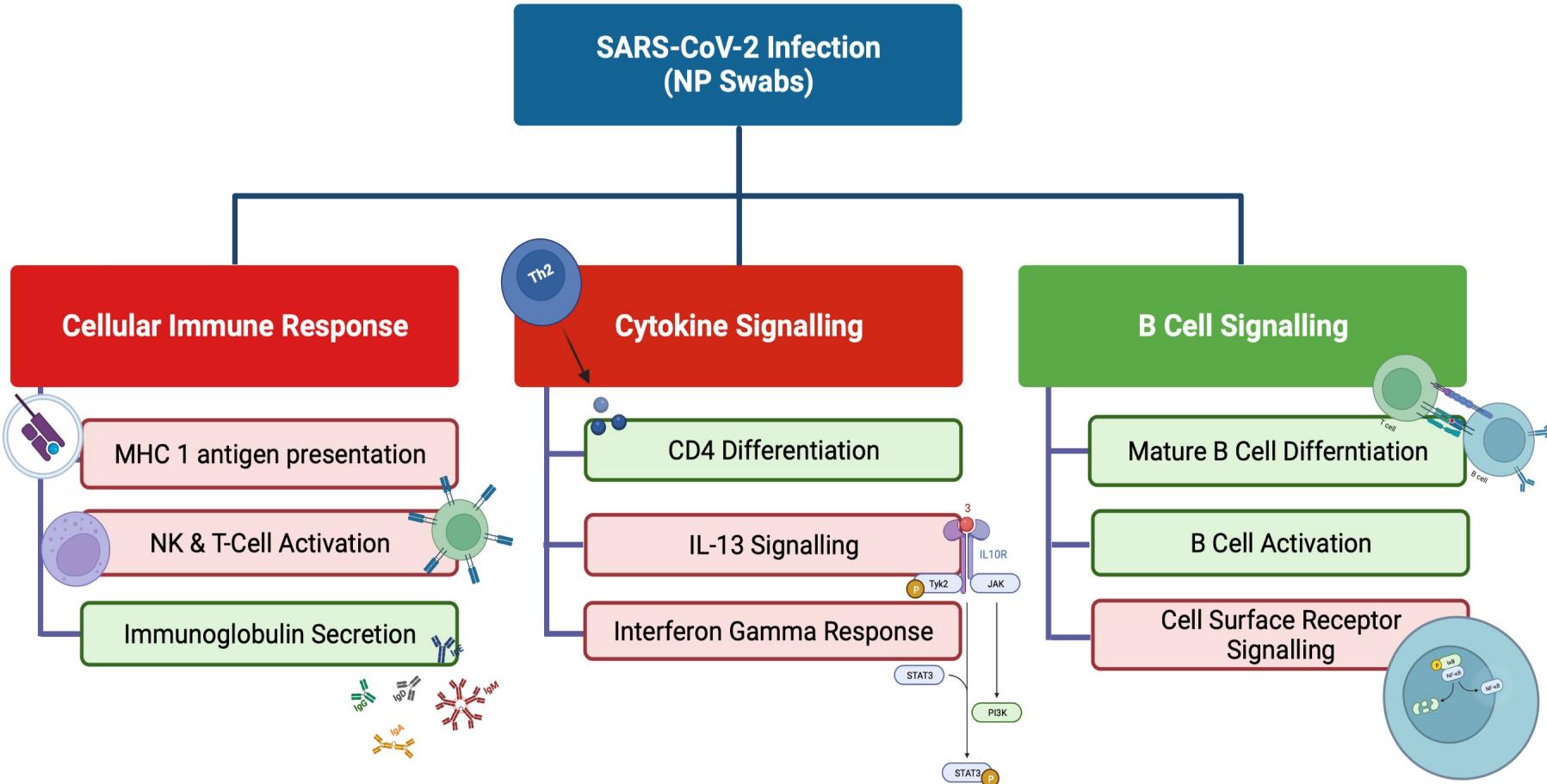
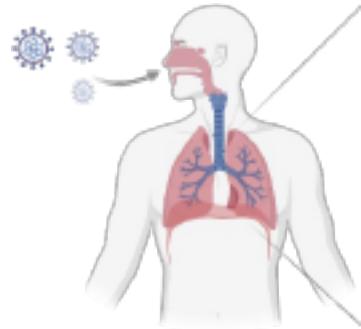


Negative vs Wuhan (5426) Negative vs Omicron (6679)

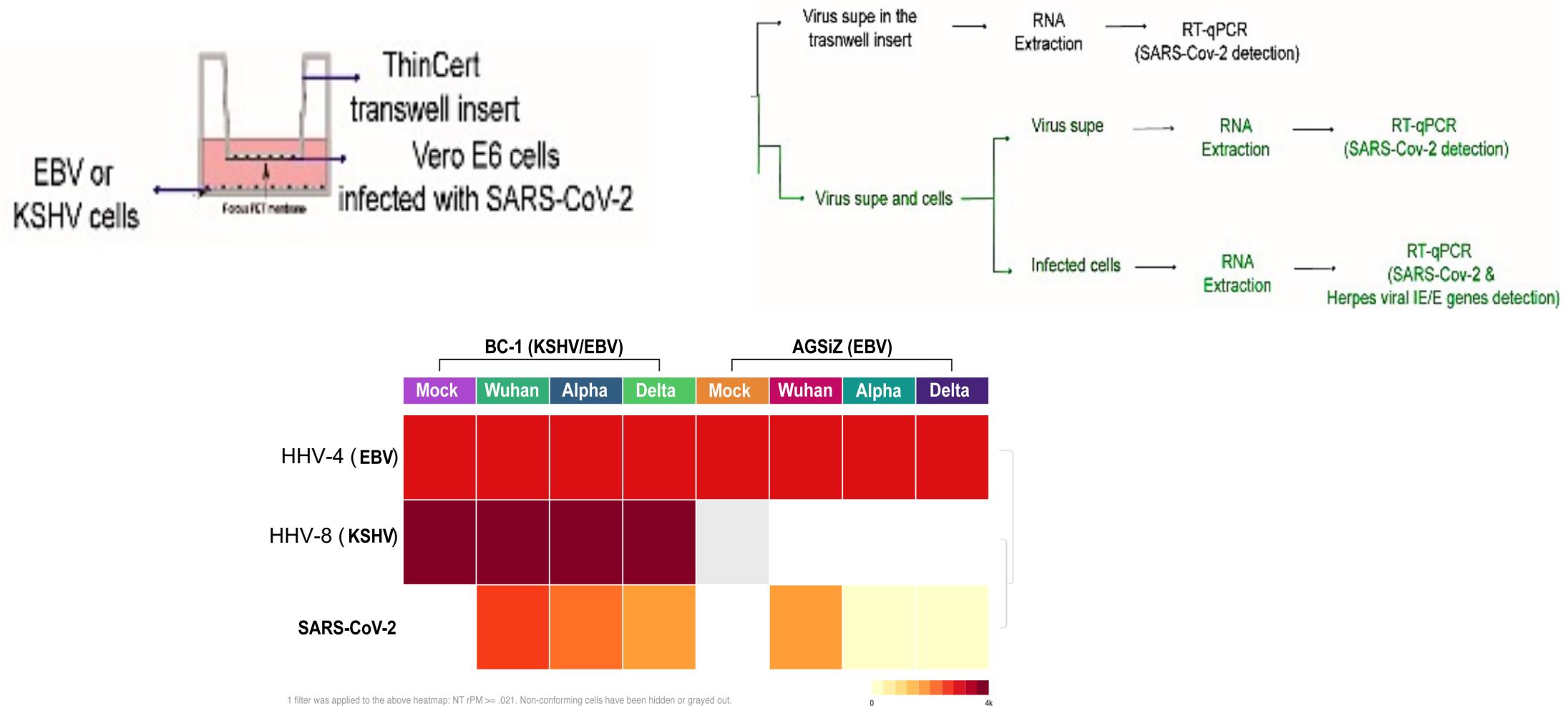
↑ LZTS3, TTC22,
VPS26C, VPS41,
IRAK3, STARD7
↓ ADARB2, SYT15,
AZIN2, EMC10,
ZNF175, KCTD2

*Absolute FC >1.2, p<0.05

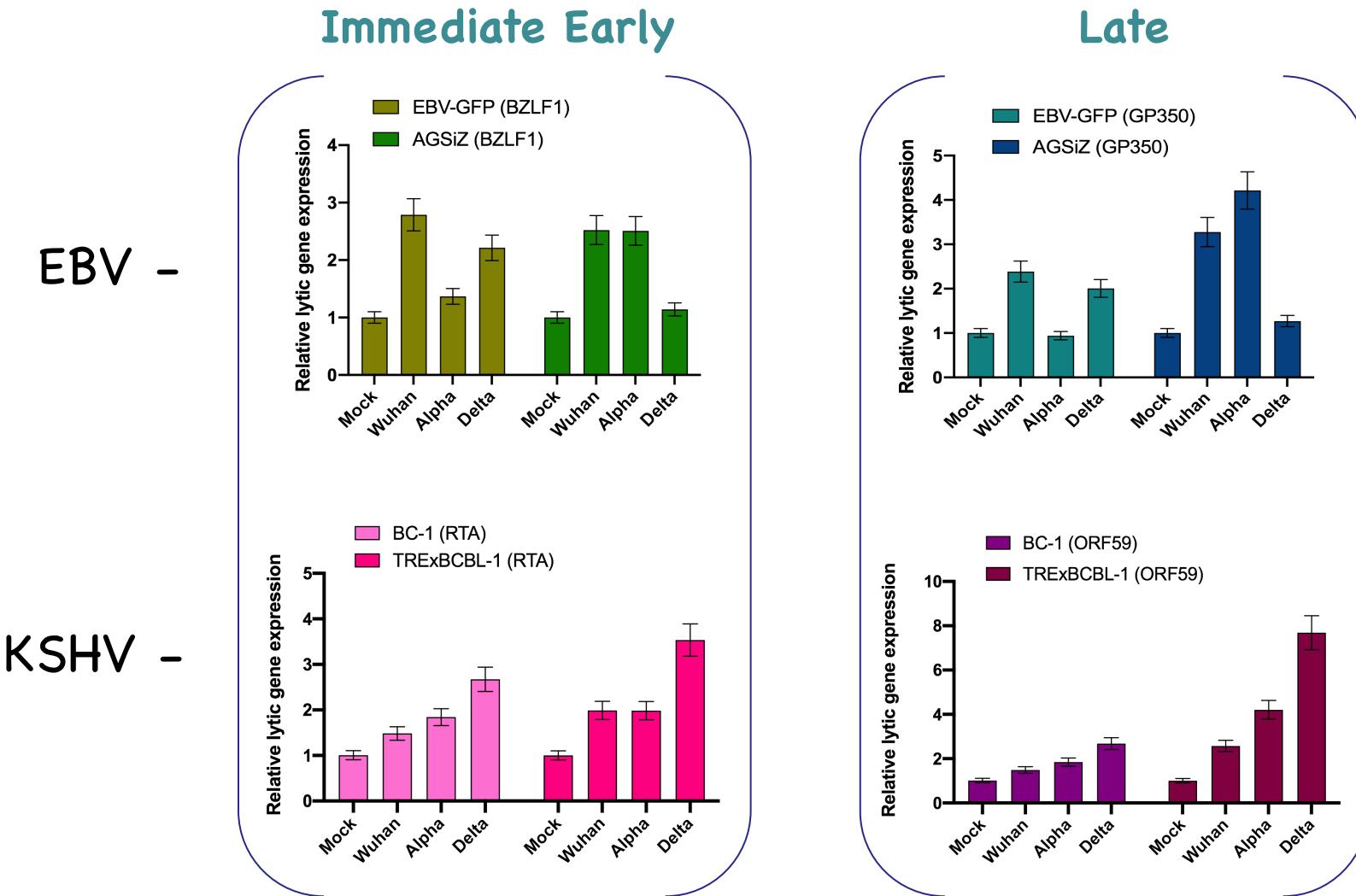
Cellular Gene Pathways in COVID-19 Patients (NP Swabs)



HHVs Reactivation in SARS-CoV-2 Infected Cells: *in-vitro*



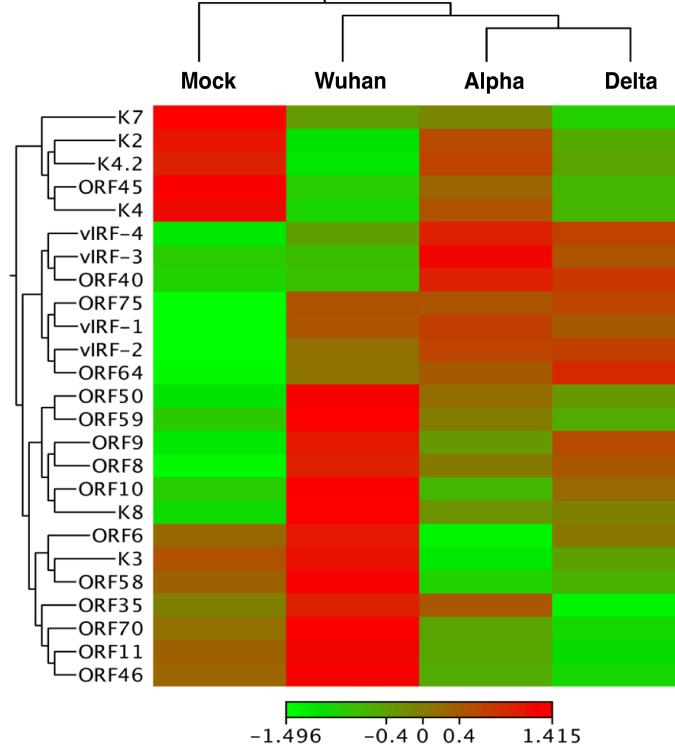
HHVs Reactivation Genes in SARS-CoV-2 Co-Culture Model



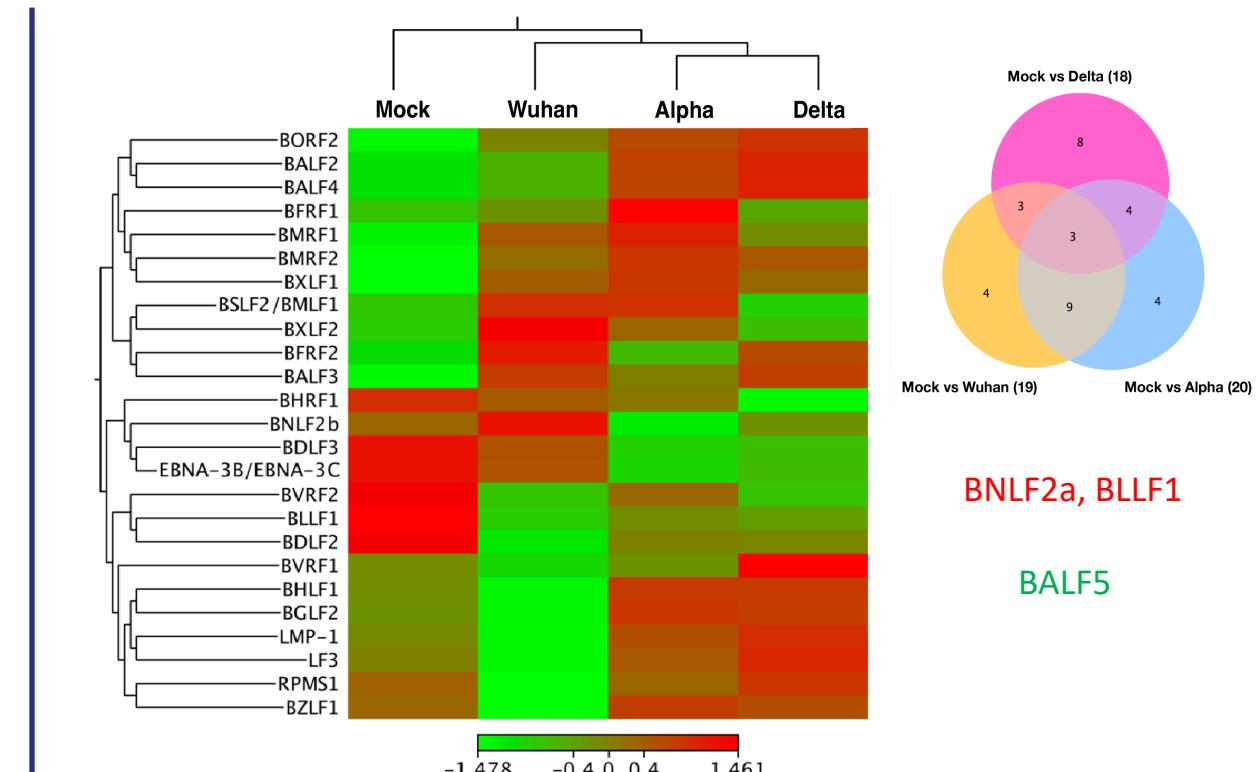


HHVs Reactivation in SARS-CoV-2 Infected Cells (RNA-seq)

KSHV



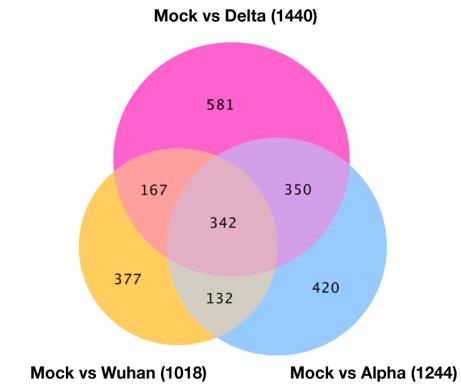
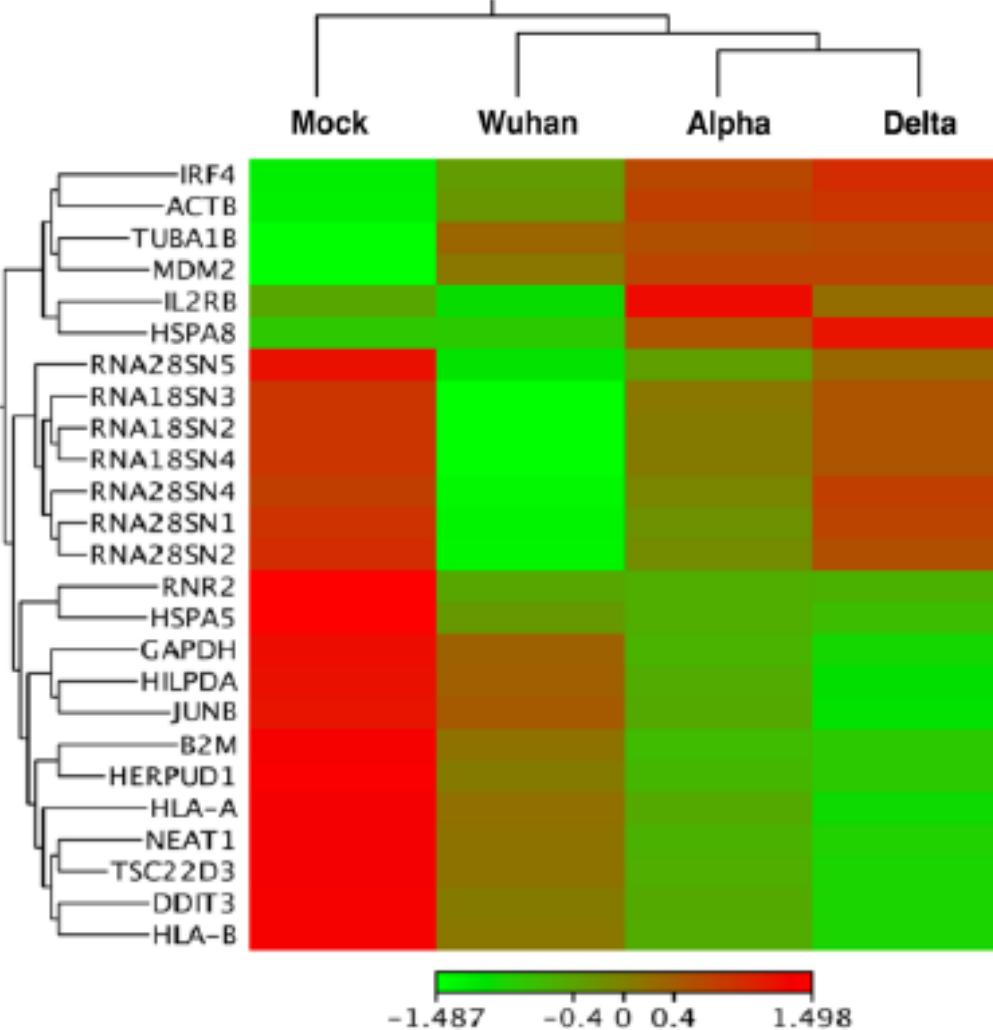
EBV



*Absolute FC >1.2, p<0.05



Cellular Gene Profiling in SARS-CoV-2 Infected Cells



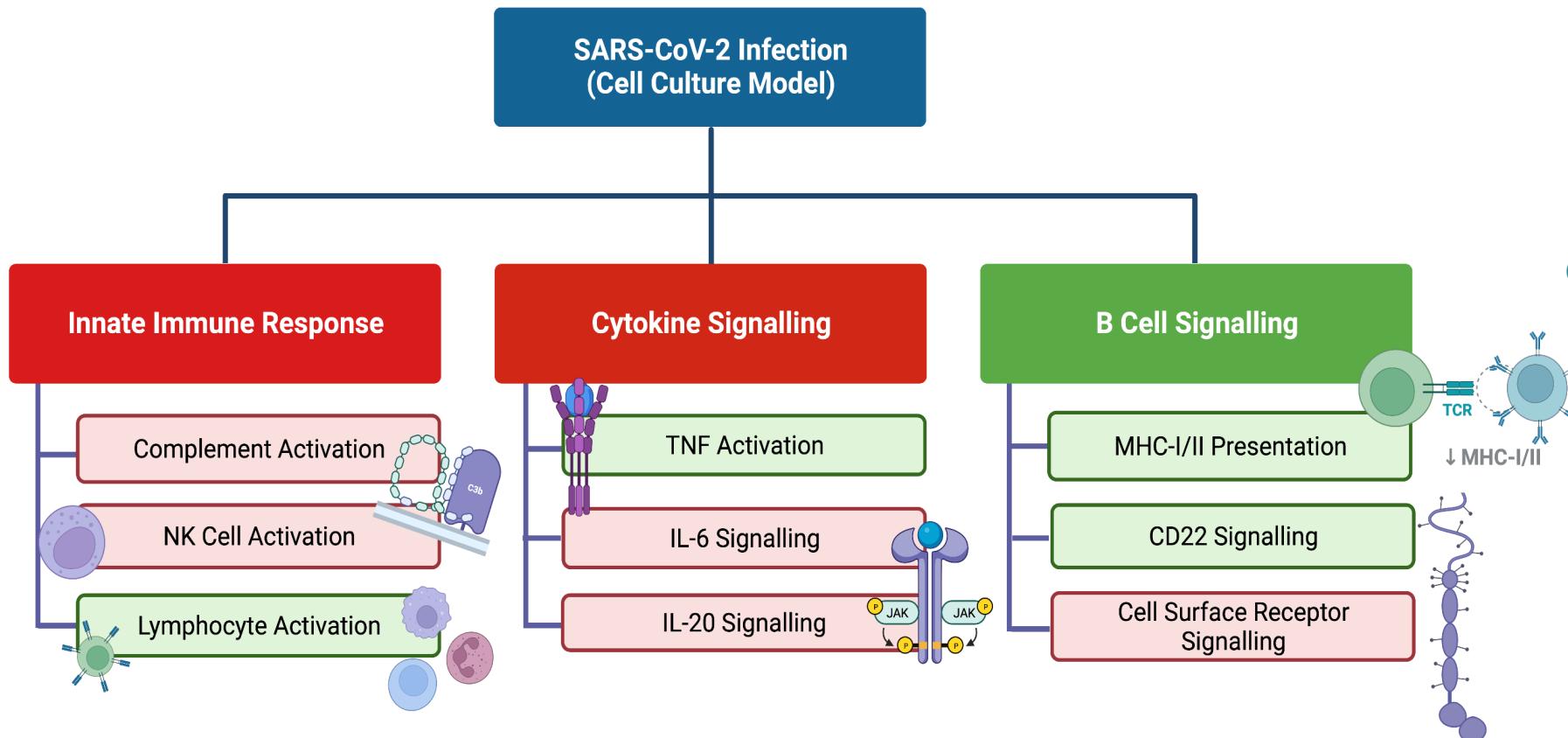
TIGIT, RELN,
SRRM5, FILIP1,
DBIL5P

SMIM11A, ZASP,
RNA5-8SN1, RNU1-
1, RNA5-8SN2

*Absolute FC >2, p<0.05



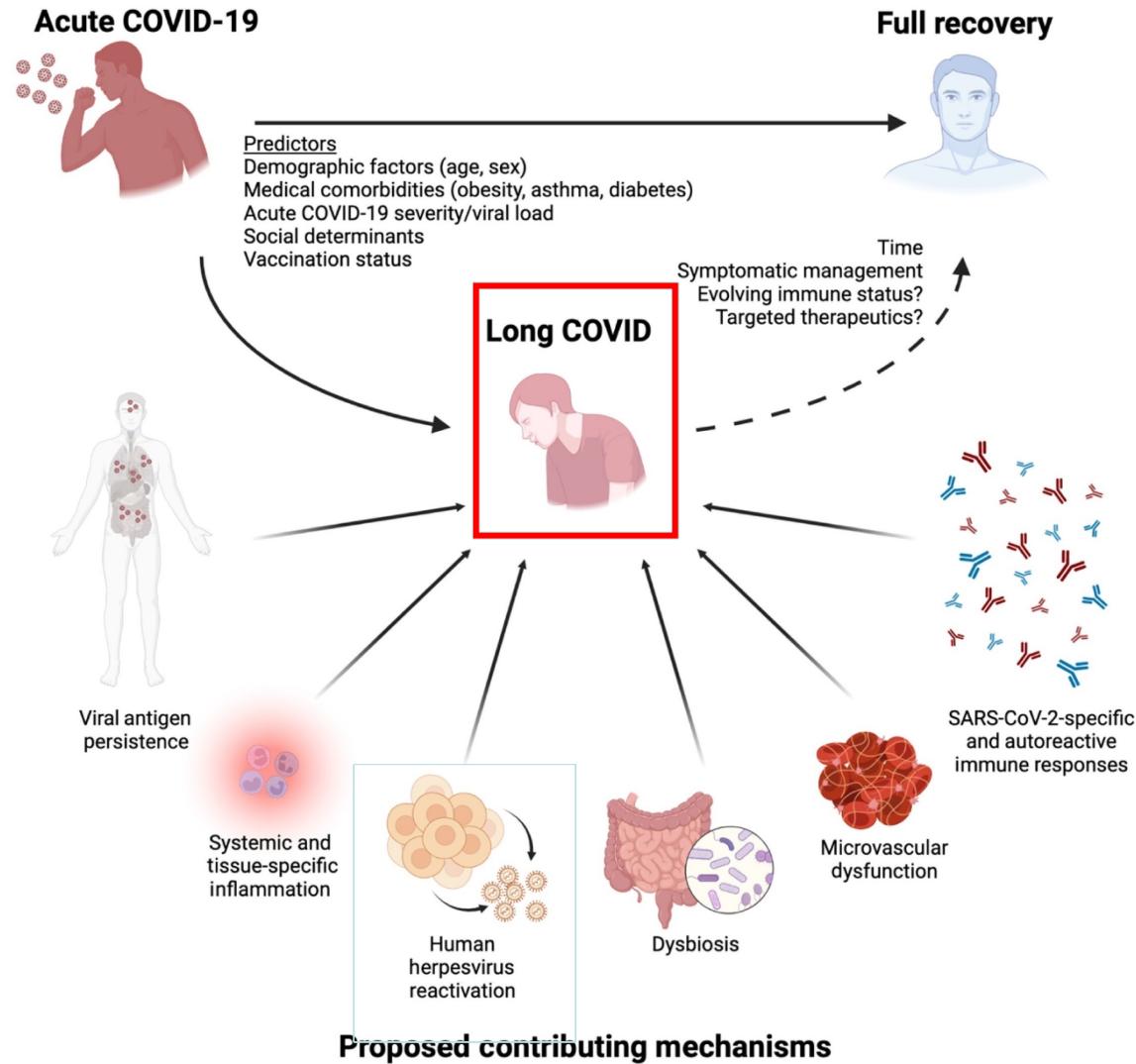
Cellular Gene Pathways in SARS-CoV-2 Co-Culture Model



Shared Pathways in NP Swabs:

Cellular Immune Responses
B Cell Activation/Signaling
Cytokine Signaling
Lymphocyte Activation
IL-6 Signaling
Cellular Proliferation
WBC Proliferation

Future Directions: HHVs in Long-COVID





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University of Nevada, Reno
School of Medicine

