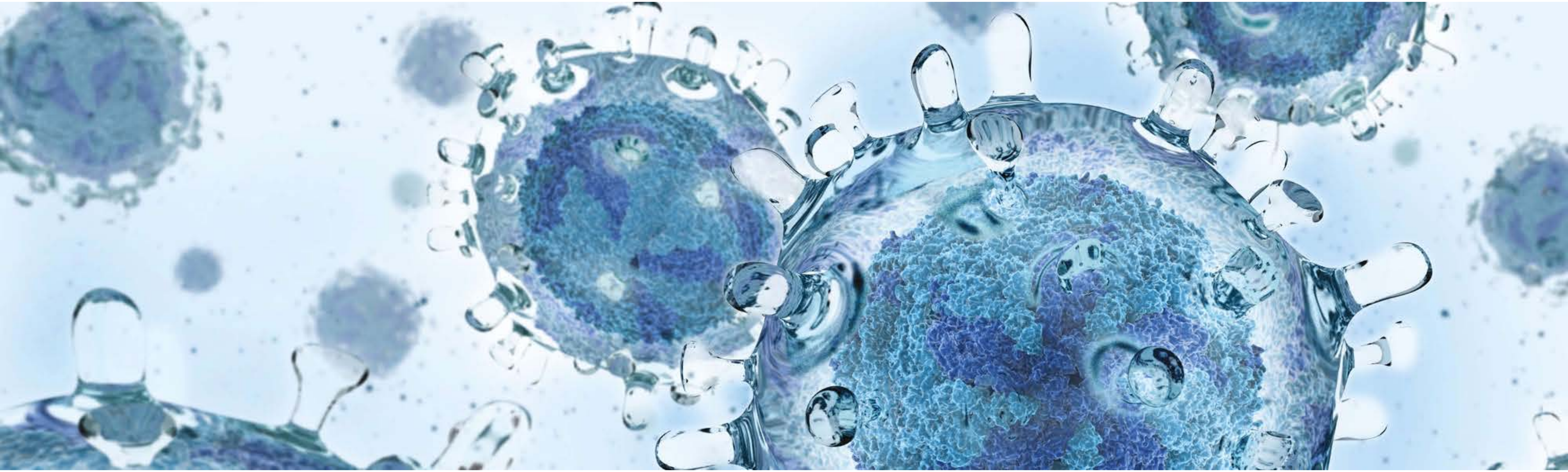


Transcriptomic analyses of in vitro flavivirus- or coronavirus-infected cells

Discovery Team, QIAGEN Digital Insights

Legal disclaimer

Agenda

1 Brief overview on flavivirus and coronavirus

2 QIAGEN Sample to Insight

3 Transcriptomic analyses of in vitro flavivirus- or coronavirus-infected cells using QIAGEN CLC Genomics Workbench and Genomics Cloud Engine

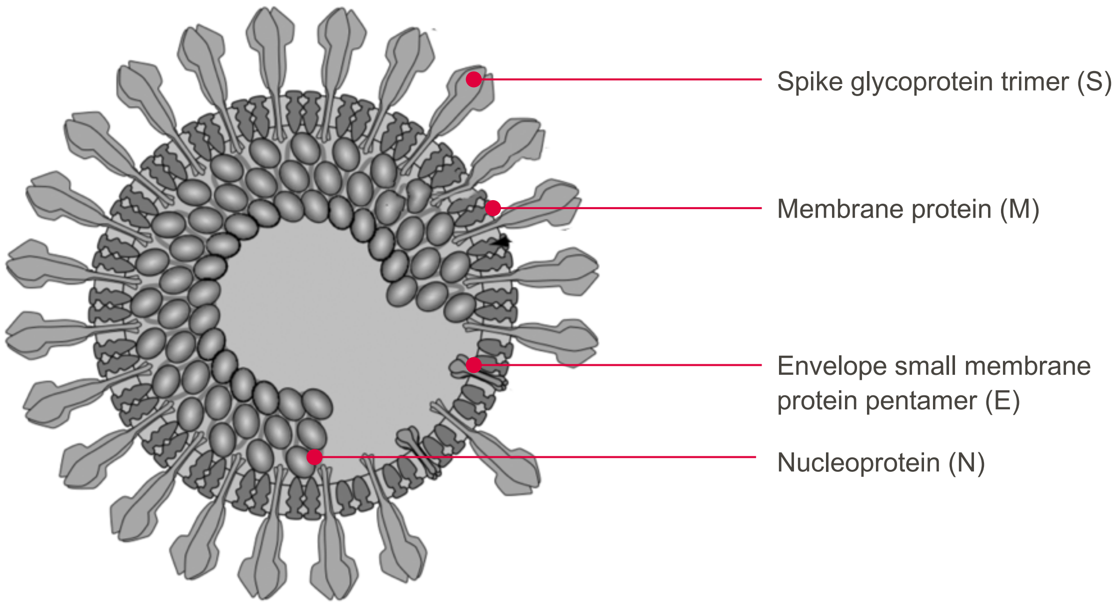
4 From QIAGEN CLC Genomics Workbench to QIAGEN IPA: Highlight the biology of flavivirus or coronavirus infection in vitro

5 Discover hidden biology

6 Conclusions

Coronavirus (SARS-CoV-1, SARS-CoV-2) and Flavivirus (DENV, WNV) virions

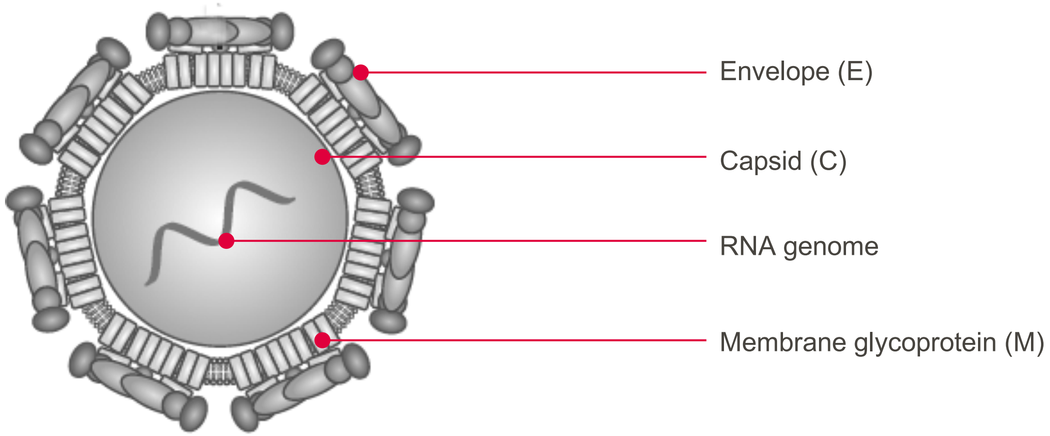
SARS-CoV-1, SARS-CoV-2



Positive SS-RNA, enveloped, 80–160 nm in size, 27–32 Kb,
4 S, 16 NS and 9 accessory proteins (29 proteins in total)

Adapted from ViralZone 2020 SIB Swiss Institute of Bioinformatics:
https://www.viprbrc.org/brc/home.spg?decorator=corona_ncov

Dengue virus, West Nile virus



Positive SS-RNA, enveloped, 40–50 nm in size, 10 Kb,
3 S and 7 NS proteins (10 proteins in total)

Adapted from Pierson, T.C. and Diamond, M.S. (2020) The continued threat of emerging flaviviruses. Nat. Microbiol. 5, 796–812.

Background on SARS-CoV-1 and SARS-CoV-2 coronaviruses

SARS-CoV-1, SARS-CoV-2 (Coronavirus, Coronaviridae)

Worldwide COVID-19 pandemic (>22 million cases, >780,000 deaths) caused by SARS-CoV-2 (as of August 19, 2020)*

- Highly pathogenic, fatality rate: 1–3%
- Infection of airway epithelium cells mainly by airborne droplets
- Hypercytokinemia, severe inflammation, etc.
- Cases with mild symptoms: 80–85%
- Severe cases: 10–15% of cases exhibit severe pneumonia and respiratory failure requiring ICU hospitalization and mechanical ventilation; death rate up to 25%
- Severity correlates with many factors (age, gender, race and co-morbidities such as heart diseases, lung diseases, kidney diseases, type II diabetes and obesity)

Biomedical research response:

- Extremely intensive area of research, some progress, but no highly effective therapies or vaccines for COVID-19 yet.
    - August 19, 2020: 2103 trials, 8 completed with results (https://www.covid19-trials.com)
    - August 19, 2020: 34 vaccine trials in phase I to III, (https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/)

*Johns Hopkins University, Coronavirus Resource Center: https://coronavirus.jhu.edu/map.html

Background on Dengue virus and West Nile arboviruses

DENV and WNV (Flavivirus, Flaviviridae)

Global spread, epidemic transmission

- Both are arboviruses (arthropods are the vectors: mosquitoes)
- Infection of myeloid cells

DENV

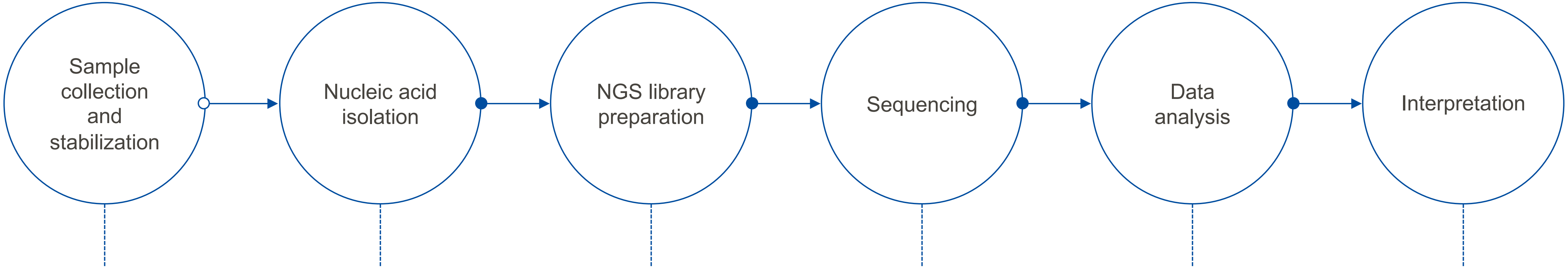
- Mild to severe disease, (390 million total infections), 100 million clinically apparent cases and 500,000/year severe dengue cases, 22,000 deaths and 2.5 billion at risk
- Visceral and hemorrhagic diseases (vascular leakage, thrombocytopenia, shock syndrome, etc.)
- Denvaxia – the only live attenuated vaccine, partially effective against DENV

WNV

- Induces neurological diseases (encephalitis, meningitis, cognitive impairment, seizure disorders and paralysis)
- 80% asymptomatic, but fatality rate for symptomatic cases is 10%
- Severity correlate with many factors (age, co-morbidities, genes polymorphisms, immune status, etc.)
- Currently, no WNV vaccines or antiviral treatments are approved for human use

QIAGEN Sample to Insight

QIAGEN Sample to Insight solution



Sample collection and stabilization

- PAXgene systems

Nucleic acid isolation

- QIAcube Connect
- QIAsymphony
- EZ1 Advanced XL

- QIAamp Kits
- RNeasy Kits
- miRNeasy Kits
- exoRNeasy Kits

NGS library preparation

- QIAseq RNA
- QIAseq DNA

Sequencing

- Illumina and Thermo Fisher sequencers

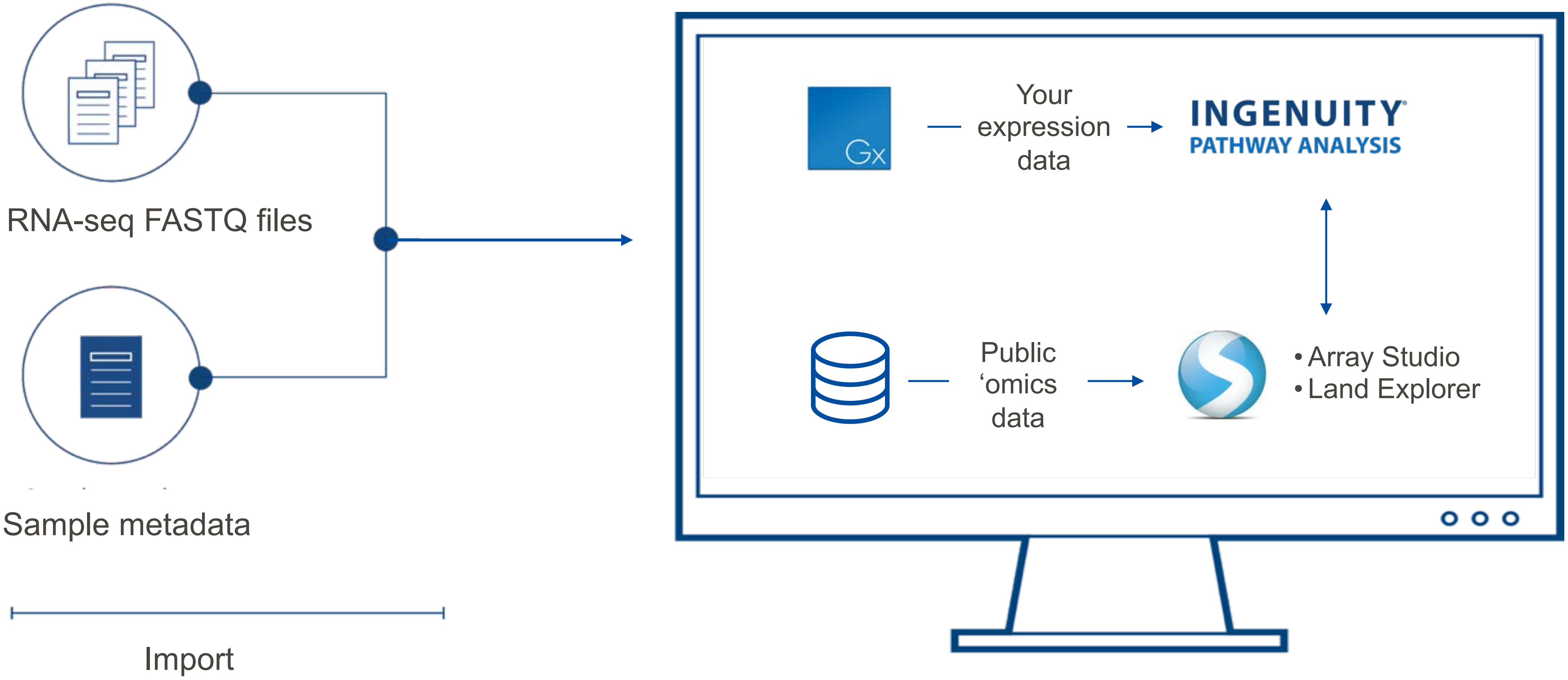
Data analysis

- QIAGEN OmicSoft Array Suite
- QIAGEN CLC Genomics Workbench
- QIAGEN Microbial Genomics Module
- QIAGEN CLC Main Workbench
- QIAGEN CLC Genome Finishing Module

Interpretation

- QIAGEN Ingenuity Pathway Analysis
- QIAGEN Ingenuity Variant Analysis
- HGMD
- QIAGEN OmicSoft Land Explorer
- QIAGEN OmicSoft DiseaseLand
- QIAGEN OmicSoft OncoLand
- QIAGEN OmicSoft GeneticsLand

QIAGEN CLC Genomics Workbench to QIAGEN IPA & QIAGEN OmicSoft Land Explorer



RNA-seq FASTQ files

Sample metadata

Import

Your expression data

INGENUITY PATHWAY ANALYSIS

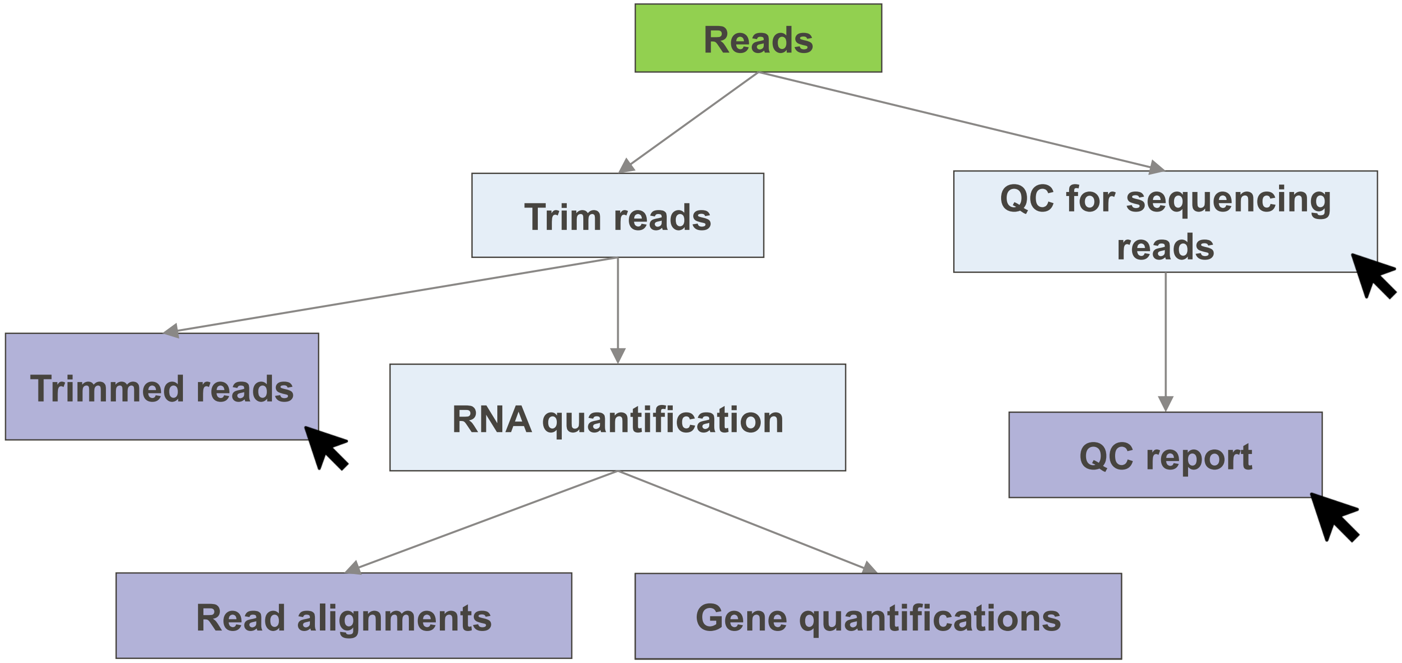
Public 'omics data

- Array Studio
- Land Explorer

An example workflow for RNA-seq

Customizable drag-and-drop workflow

- It can adapt to simple or sophisticated workflows
- It can produce intermediate files



Reads

Trim reads

QC for sequencing reads

Trimmed reads

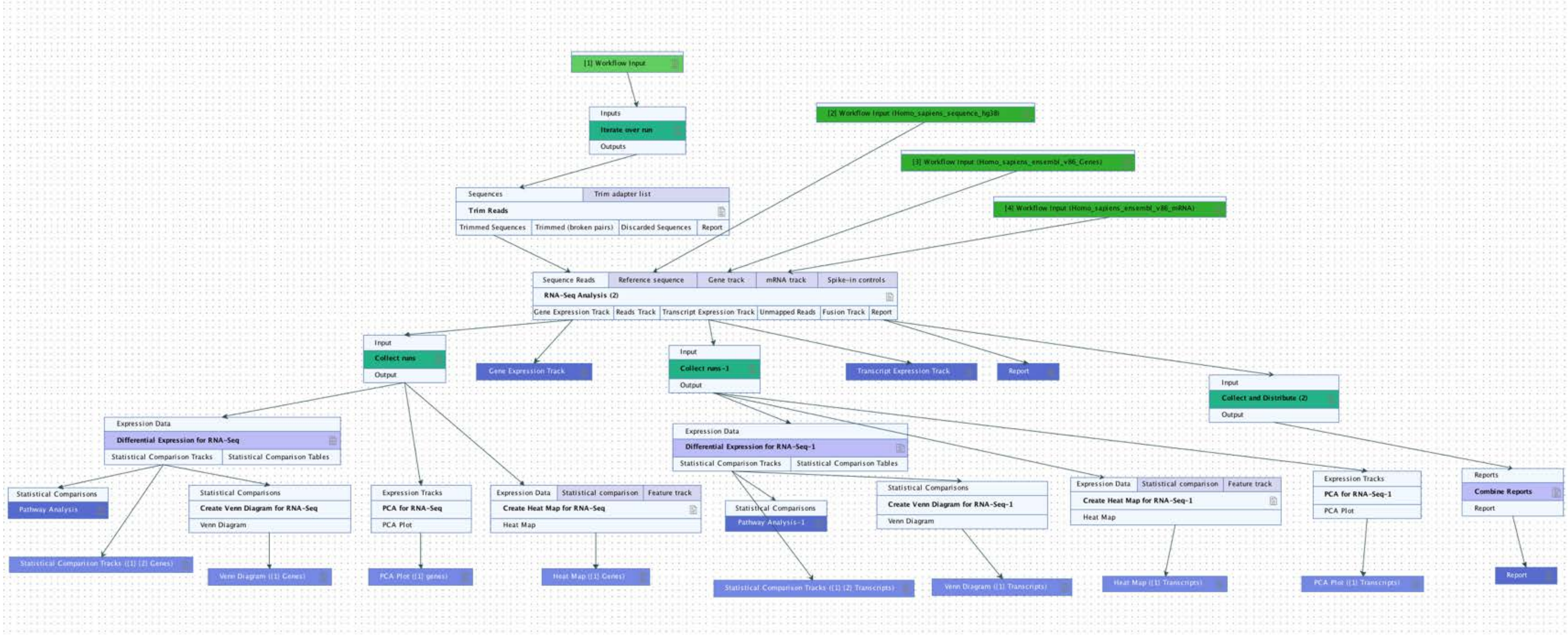
RNA quantification

QC report

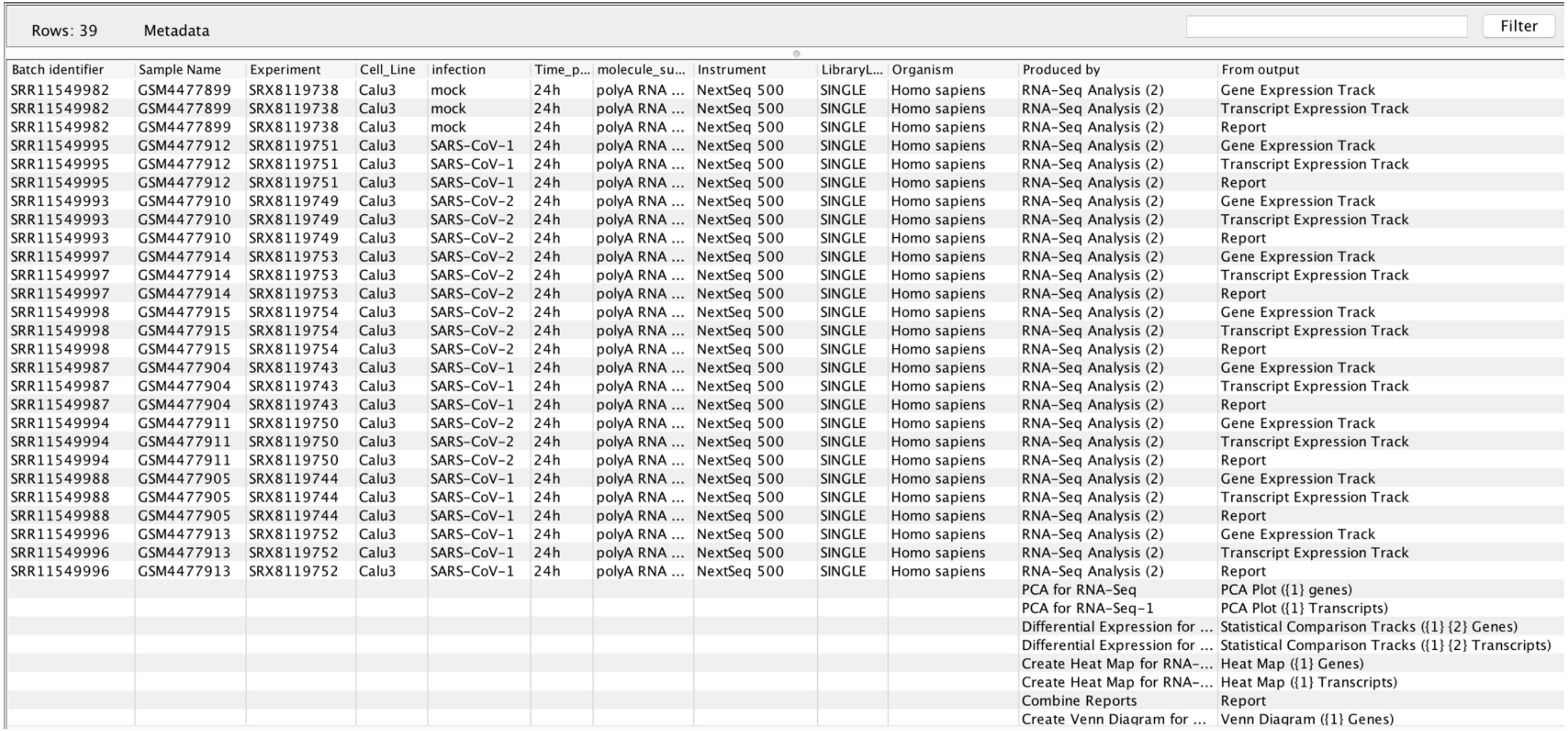
Read alignments

Gene quantifications

Analysis of host response in QIAGEN CLC Genomics Workbench/Genomics Cloud Engine



Workflow metadata



Rows: 39 Metadata

Batch identifier	Sample Name	Experiment	Cell_Line	infection	Time_p...	molecule_su...	Instrument	LibraryL...	Organism	Produced by	From output
SRR11549982	GSM4477899	SRX8119738	Calu3	mock	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
SRR11549982	GSM4477899	SRX8119738	Calu3	mock	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Transcript Expression Track
SRR11549982	GSM4477899	SRX8119738	Calu3	mock	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549995	GSM4477912	SRX8119751	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
SRR11549995	GSM4477912	SRX8119751	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Transcript Expression Track
SRR11549995	GSM4477912	SRX8119751	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549993	GSM4477910	SRX8119749	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
SRR11549993	GSM4477910	SRX8119749	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Transcript Expression Track
SRR11549993	GSM4477910	SRX8119749	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549997	GSM4477914	SRX8119753	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
SRR11549997	GSM4477914	SRX8119753	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Transcript Expression Track
SRR11549997	GSM4477914	SRX8119753	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549998	GSM4477915	SRX8119754	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
SRR11549998	GSM4477915	SRX8119754	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Transcript Expression Track
SRR11549998	GSM4477915	SRX8119754	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549987	GSM4477904	SRX8119743	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
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SRR11549987	GSM4477904	SRX8119743	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549994	GSM4477911	SRX8119750	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
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SRR11549994	GSM4477911	SRX8119750	Calu3	SARS-CoV-2	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549988	GSM4477905	SRX8119744	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
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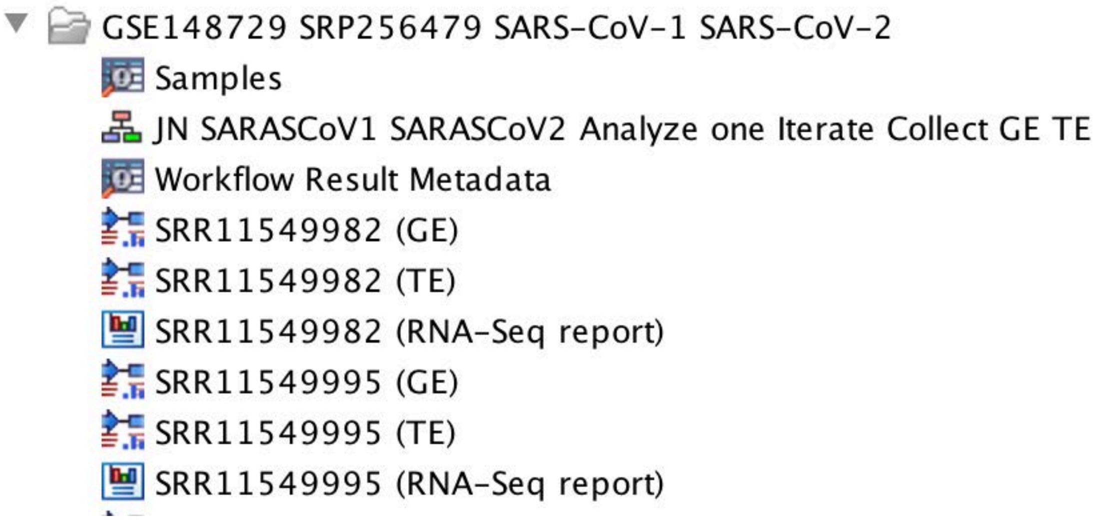
SRR11549988	GSM4477905	SRX8119744	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
SRR11549996	GSM4477913	SRX8119752	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Gene Expression Track
SRR11549996	GSM4477913	SRX8119752	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Transcript Expression Track
SRR11549996	GSM4477913	SRX8119752	Calu3	SARS-CoV-1	24h	polyA RNA ...	NextSeq 500	SINGLE	Homo sapiens	RNA-Seq Analysis (2)	Report
										PCA for RNA-Seq	PCA Plot ({1} genes)
										PCA for RNA-Seq-1	PCA Plot ({1} Transcripts)
										Differential Expression for ...	Statistical Comparison Tracks ({1} {2} Genes)
										Differential Expression for ...	Statistical Comparison Tracks ({1} {2} Transcripts)
										Create Heat Map for RNA-...	Heat Map ({1} Genes)
										Create Heat Map for RNA-...	Heat Map ({1} Transcripts)
										Combine Reports	Report
										Create Venn Diagram for ...	Venn Diagram ({1} Genes)

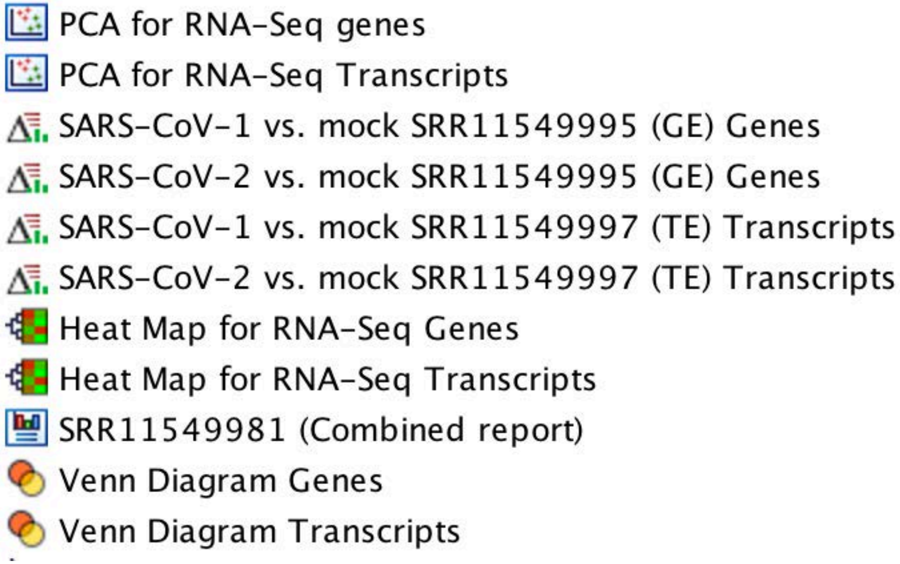
Output from QIAGEN CLC Genomics Workbench

Expression tracking at gene or transcript level



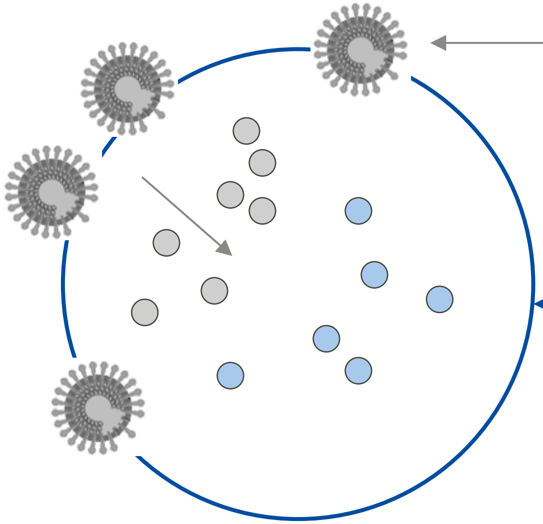
- GSE148729 SRP256479 SARS-CoV-1 SARS-CoV-2
  - Samples
  - JN SARASCoV1 SARASCoV2 Analyze one Iterate Collect GE TE
  - Workflow Result Metadata
  - SRR11549982 (GE)
  - SRR11549982 (TE)
  - SRR11549982 (RNA-Seq report)
  - SRR11549995 (GE)
  - SRR11549995 (TE)
  - SRR11549995 (RNA-Seq report)

Differential expression profiling at gene and transcript level: SARS-CoV-1/-2 vs. mock



- PCA for RNA-Seq genes
- PCA for RNA-Seq Transcripts
- SARS-CoV-1 vs. mock SRR11549995 (GE) Genes
- SARS-CoV-2 vs. mock SRR11549995 (GE) Genes
- SARS-CoV-1 vs. mock SRR11549997 (TE) Transcripts
- SARS-CoV-2 vs. mock SRR11549997 (TE) Transcripts
- Heat Map for RNA-Seq Genes
- Heat Map for RNA-Seq Transcripts
- SRR11549981 (Combined report)
- Venn Diagram Genes
- Venn Diagram Transcripts

The battle of virus versus host



Virus is trying to

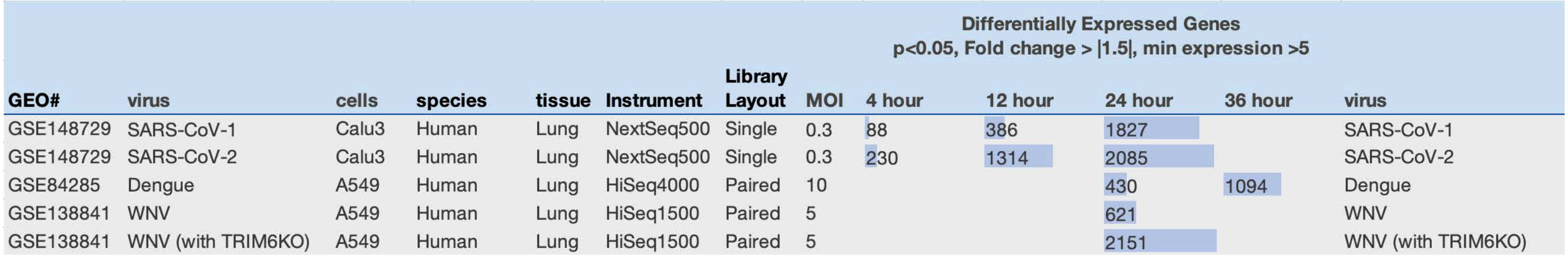
- Shut down the host’s anti-viral activities
- Boost host activities needed for replication

Host is trying to

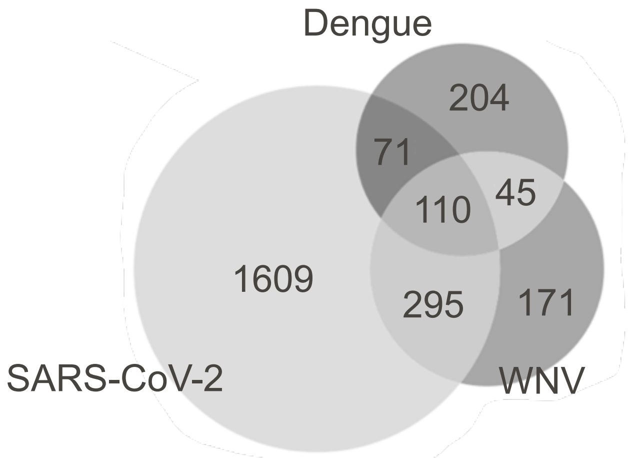
- Increase anti-viral responses
- Starve virus of resources

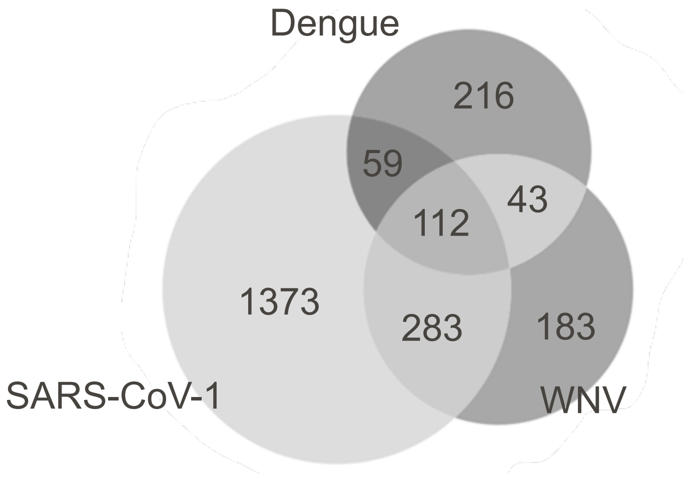
The transcriptome is a snapshot of these opposing efforts at a point in time.

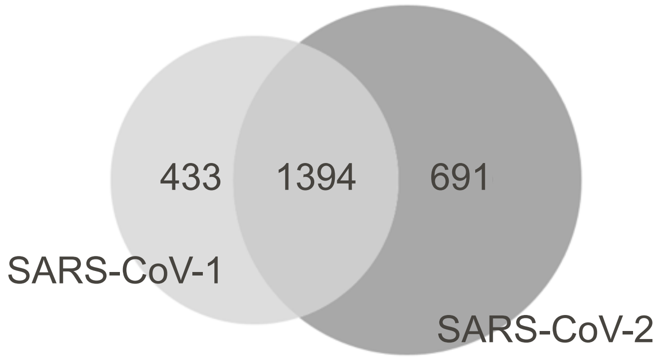
Impact on differential gene expression by each virus



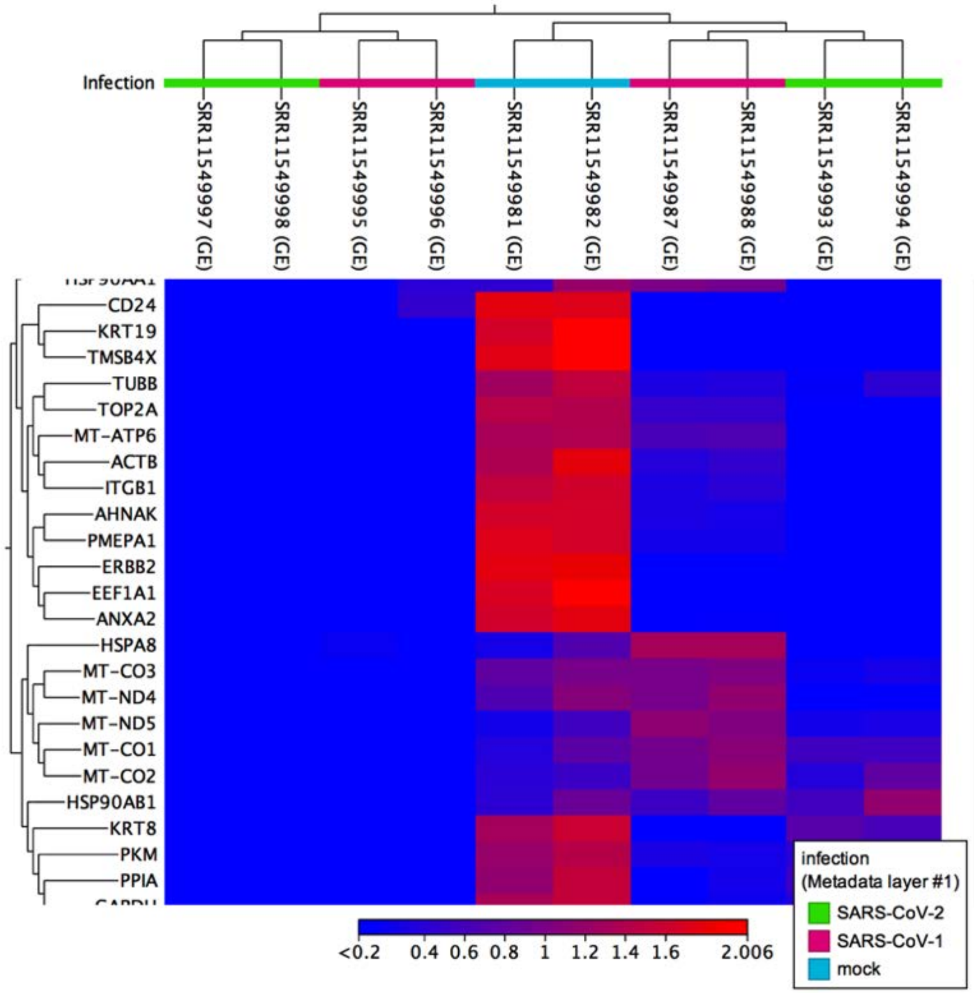
GEO#	virus	cells	species	tissue	Instrument	Library Layout	MOI	Differentially Expressed Genes p<0.05, Fold change > |1.5|, min expression >5 4 hour	12 hour	24 hour	36 hour	virus
GSE148729	SARS-CoV-1	Calu3	Human	Lung	NextSeq500	Single	0.3	88	386	1827		SARS-CoV-1
GSE148729	SARS-CoV-2	Calu3	Human	Lung	NextSeq500	Single	0.3	230	1314	2085		SARS-CoV-2
GSE84285	Dengue	A549	Human	Lung	HiSeq4000	Paired	10			430	1094	Dengue
GSE138841	WNV	A549	Human	Lung	HiSeq1500	Paired	5			621		WNV
GSE138841	WNV (with TRIM6KO)	A549	Human	Lung	HiSeq1500	Paired	5			2151		WNV (with TRIM6KO)

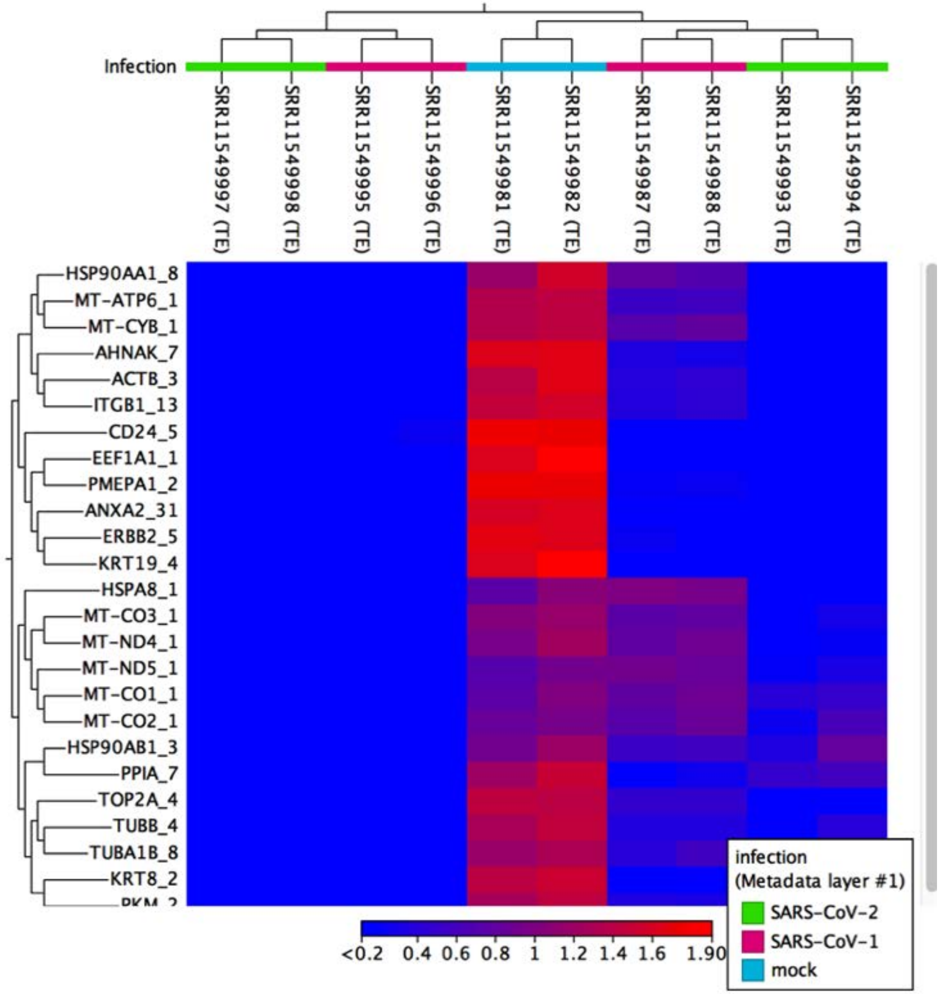






Heatmap of RNA-seq genes: SARS-CoV-1 or SARS-CoV-2 vs. mock

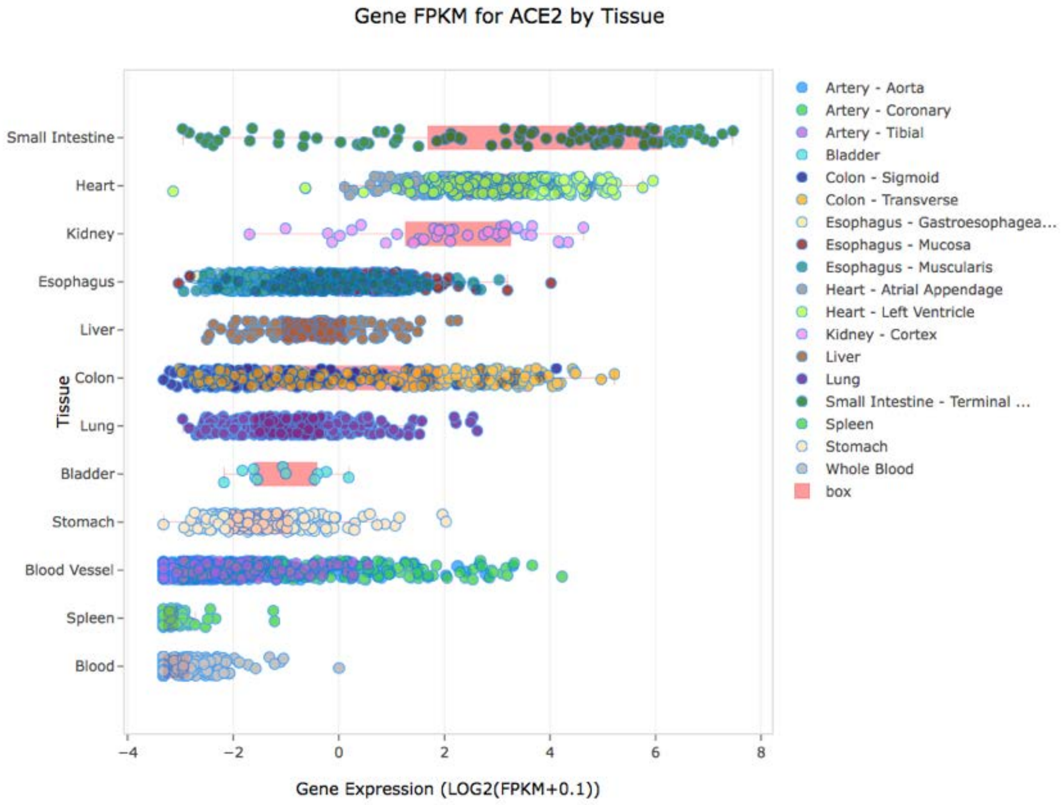




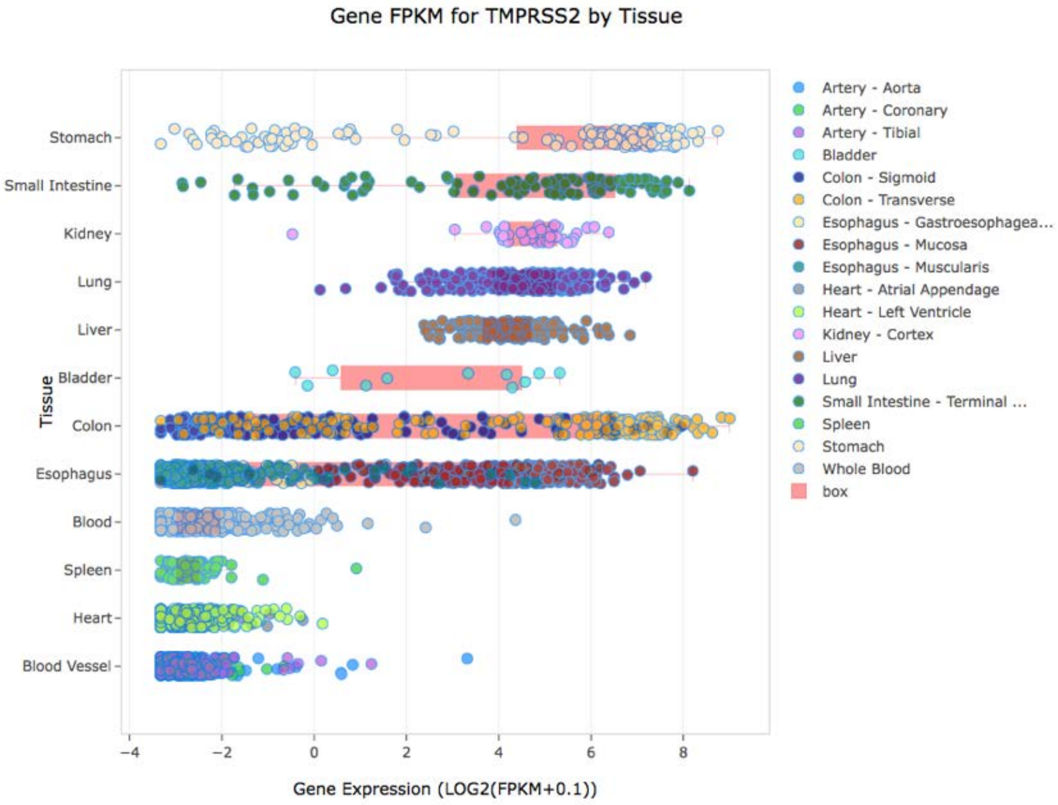
Similarities of molecular patterns after in vitro infection with flavivirus or coronavirus

Expression in lung, kidney, gut and heart tissues and blood vessels (target organs in COVID-19)

ACE2 is receptor, expression in tissues (GTEx-B38)



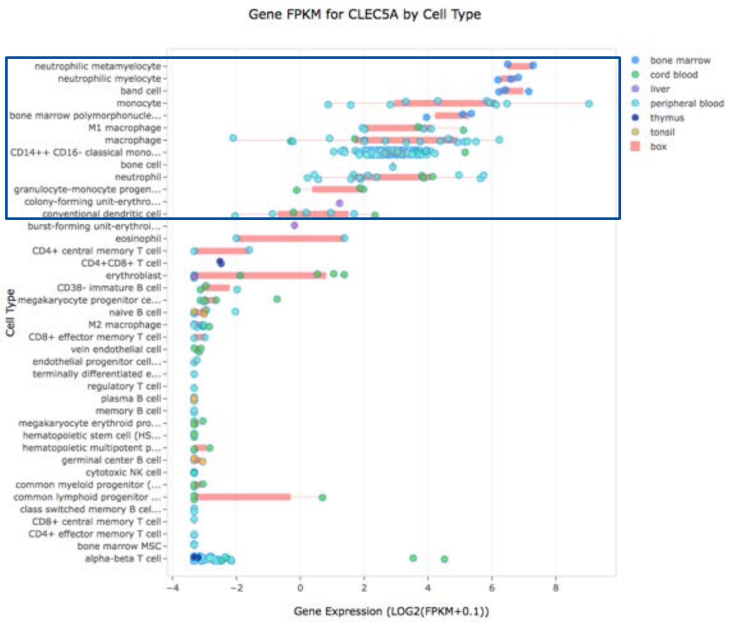
TMPRSS2 is the priming protease, expression in tissues (GTEx-B38)



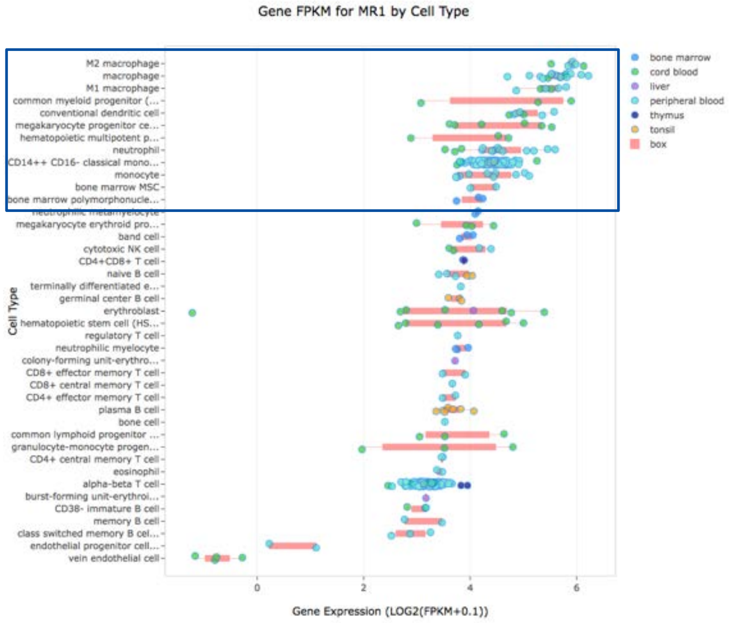
Potential receptors for Dengue virus (3) and West Nile virus (2)

Expression in myeloid cells (Blueprint-B38)

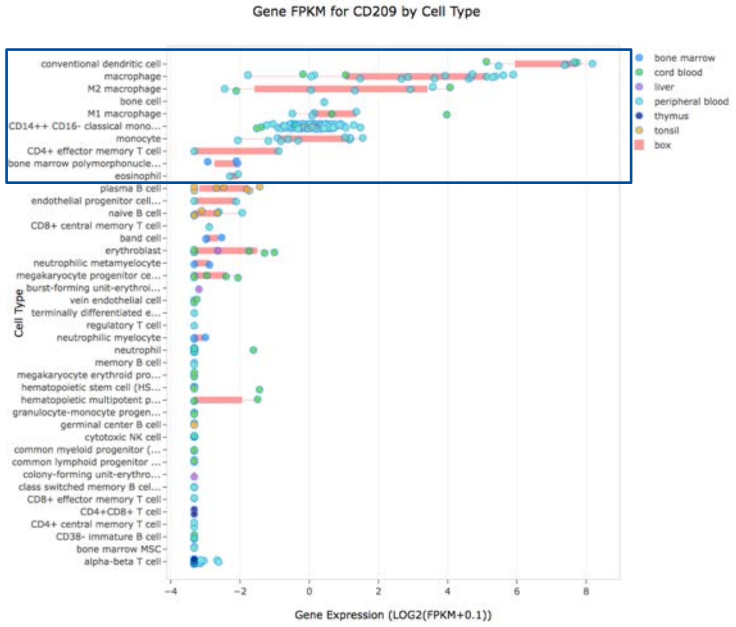
CLEC5A (C-type lectin)



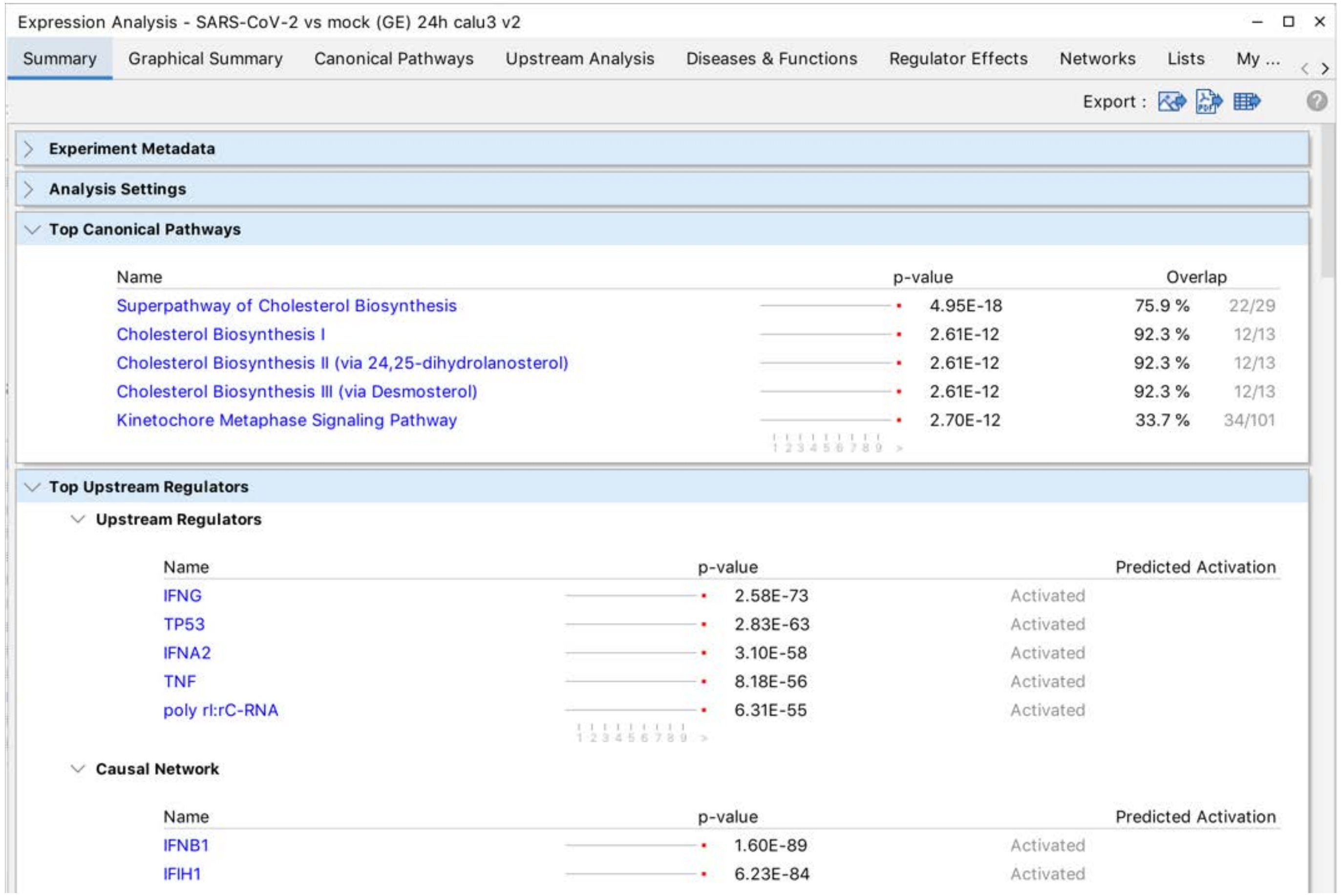
MR1 (Mannose receptor)



CD209 (DC-SIGN)



Summary of a QIAGEN IPA core analysis



Top Canonical Pathways

Name	p-value	Overlap	
Superpathway of Cholesterol Biosynthesis	4.95E-18	75.9 %	22/29
Cholesterol Biosynthesis I	2.61E-12	92.3 %	12/13
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)	2.61E-12	92.3 %	12/13
Cholesterol Biosynthesis III (via Desmosterol)	2.61E-12	92.3 %	12/13
Kinetochore Metaphase Signaling Pathway	2.70E-12	33.7 %	34/101

Top Upstream Regulators

Upstream Regulators

Name	p-value	Predicted Activation
IFNG	2.58E-73	Activated
TP53	2.83E-63	Activated
IFNA2	3.10E-58	Activated
TNF	8.18E-56	Activated
poly rI:rC-RNA	6.31E-55	Activated

Causal Network

Name	p-value	Predicted Activation
IFNB1	1.60E-89	Activated
IFIH1	6.23E-84	Activated

IPA analysis of Calu-3 transcriptome (gene level) 1 day after infection

Cutoffs in IPA:

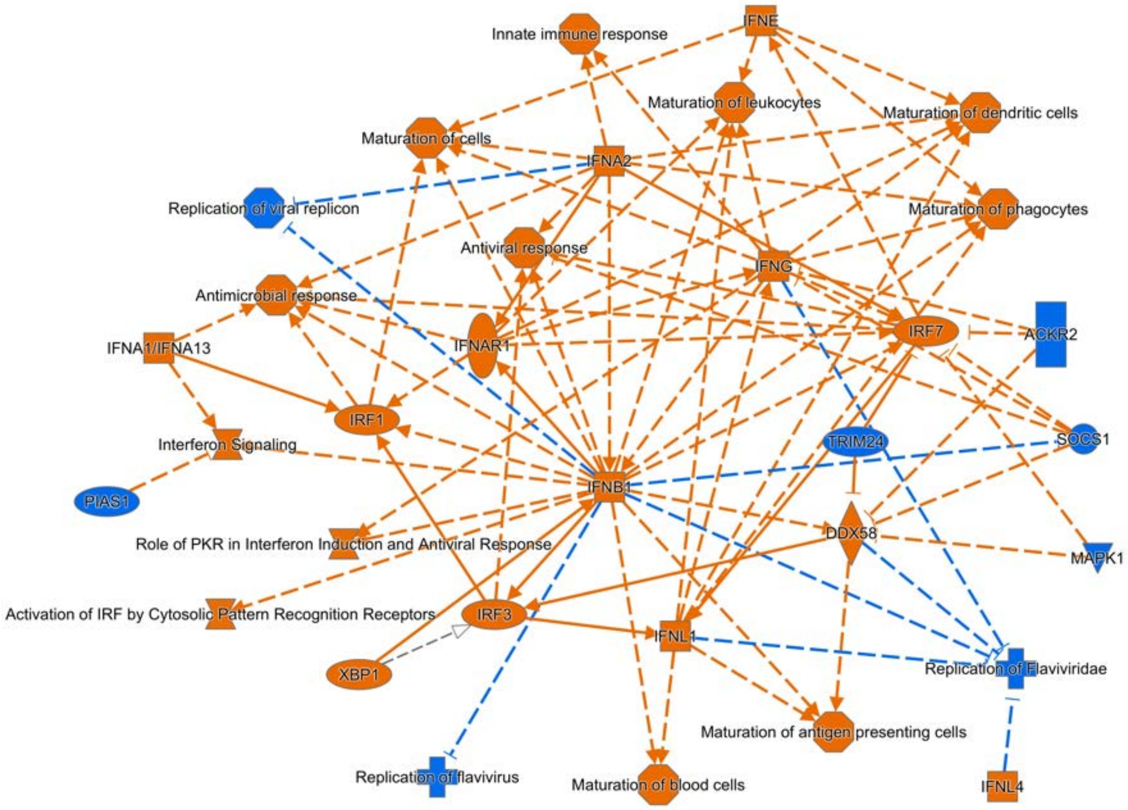
- Absolute fold change >1.5
- p-value < 0.05
- Min. counts (SARS-CoV-2 or mock) ≥ 5

Comparison for this analysis:

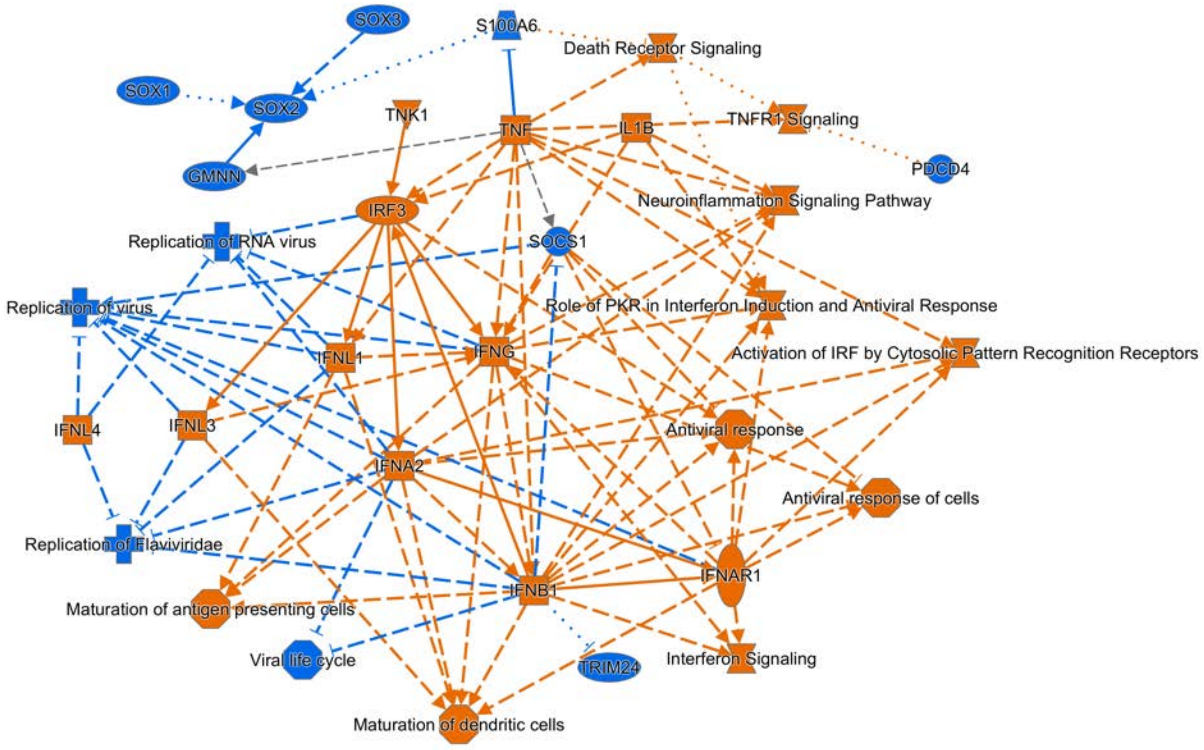
$$\frac{\text{SARS-CoV-2 infected Calu-3}}{\text{Mock-infected Calu-3}}$$

Graphical summary of Dengue virus- and West Nile virus-infected A549 cells

DENV vs. mock (GE) 24 hours, A549

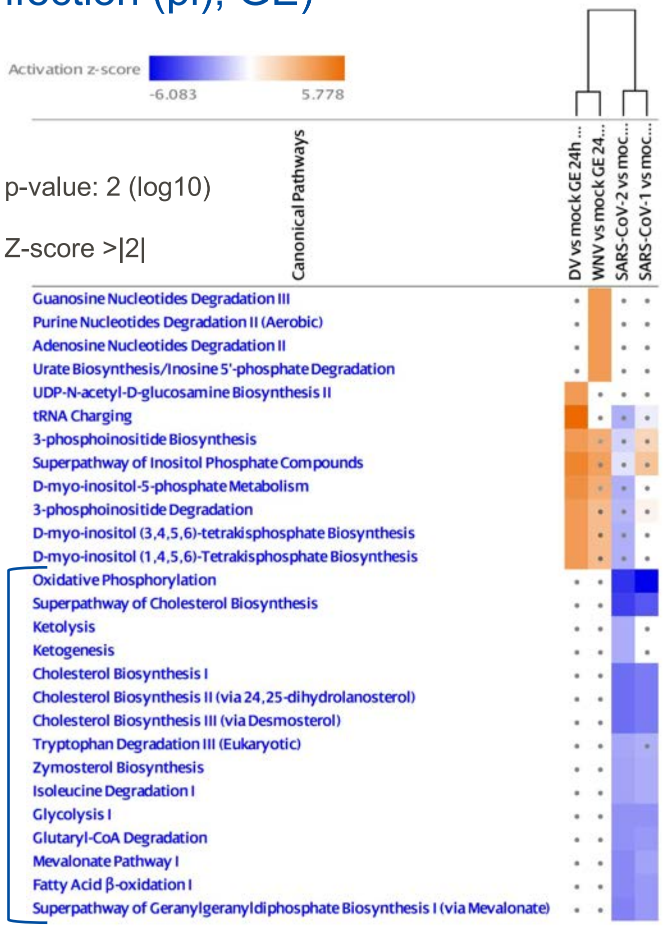


West Nile virus vs. mock (GE) 24 hours, A549



Comparison of Canonical Pathways of the 4 virus-infected cells vs. mock

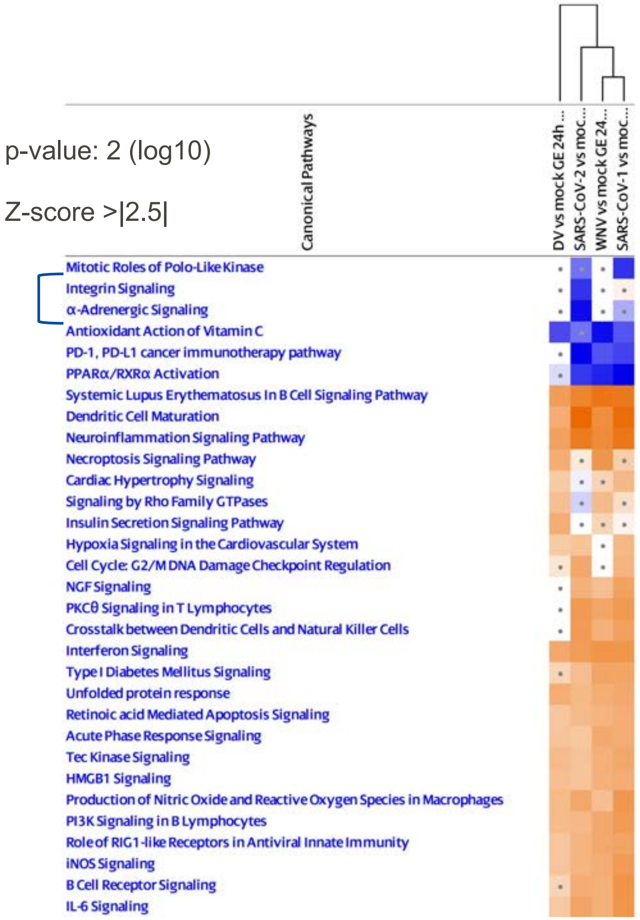
Metabolic Pathways (24 h post infection (pi), GE)



p-value: 2 (log10)

Z-score >|2|

Signaling Pathways (24 h pi, GE)



p-value: 2 (log10)

Z-score >|2.5|

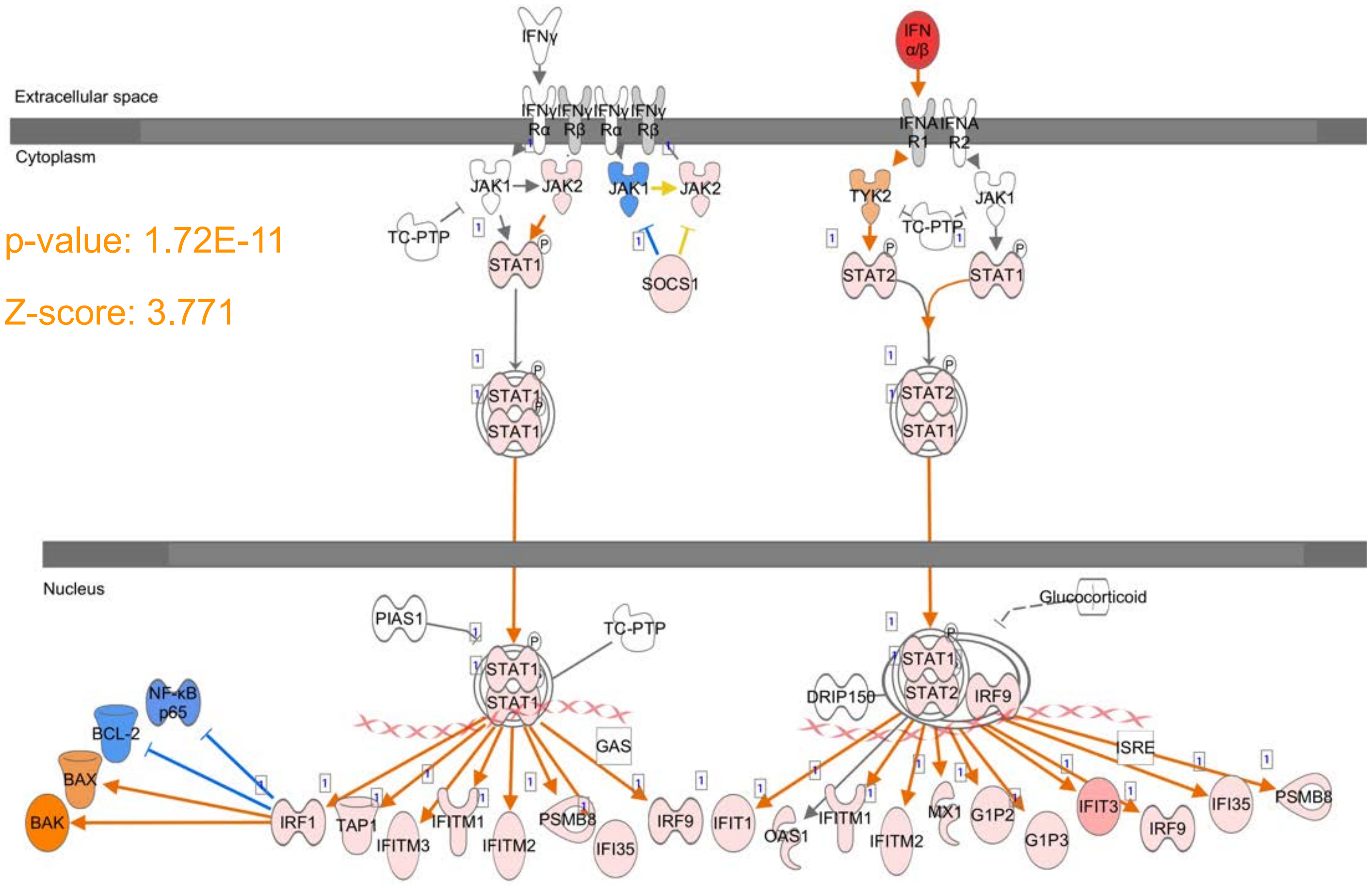
Signaling Pathways (continued)

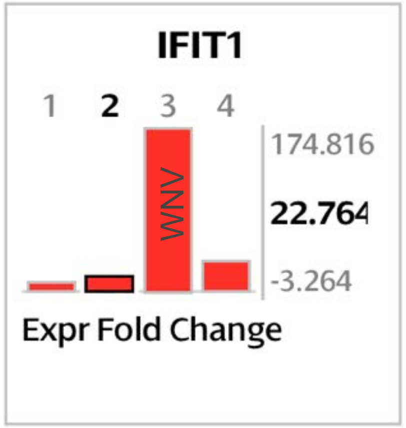
Interferon signaling is activated in SARS-CoV-2-infected Calu-3 cells at 24 h pi (GE)

One of the hallmarks of antiviral defense is activated across all four (not shown) in vitro virus-infected cells

p-value: 1.72E-11

Z-score: 3.771



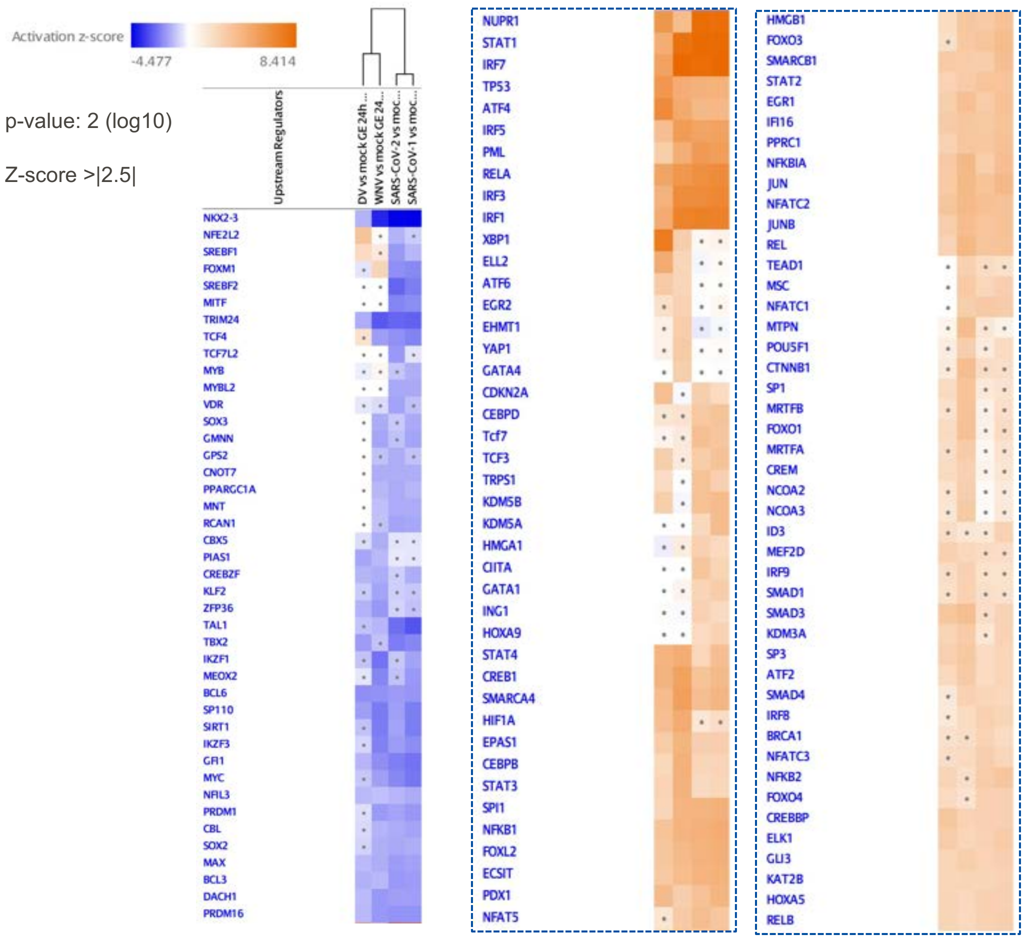


Comparing Upstream Regulators of the 4 virus-infected cells vs. mock at 24 h pi (GE)

Similar Transcription Regulator program is induced

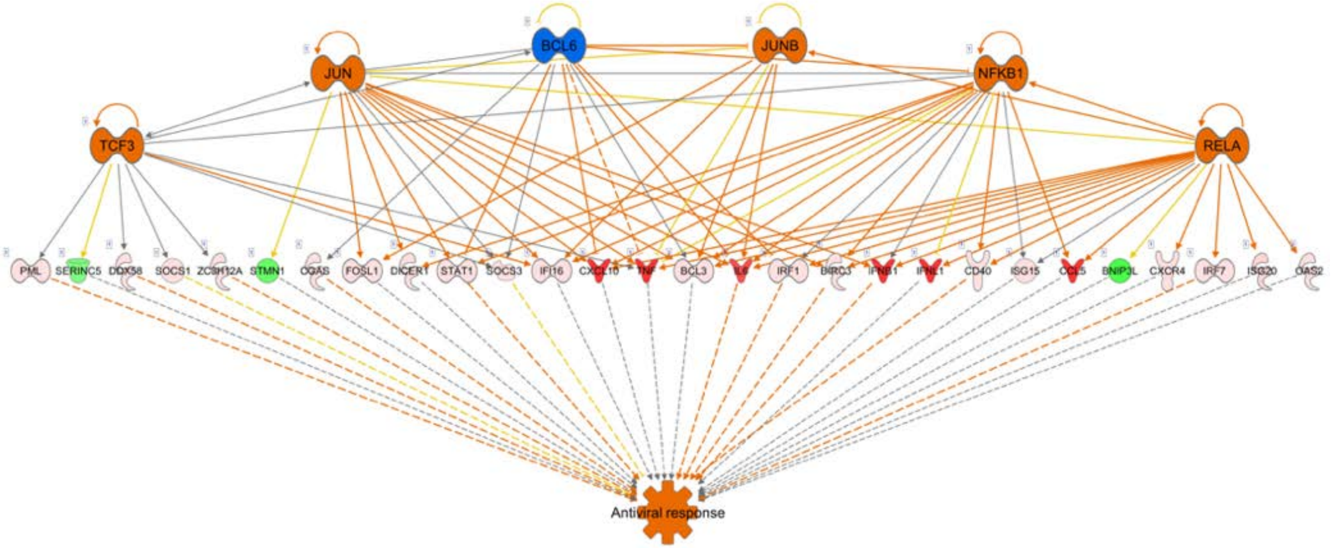
p-value: 2 (log10)

Z-score >|2.5|



Antiviral response is increased (Regulator Effect)

SARS-CoV-2 vs. mock

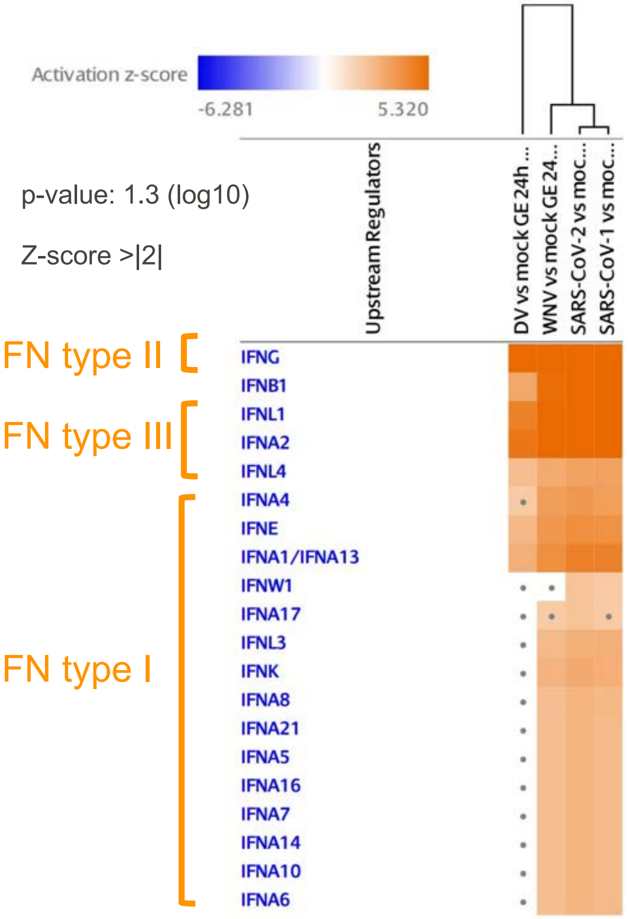


Comparing Upstream Regulators of the 4 virus-infected cells vs. mock at 24 h pi (GE)

Interferons

p-value: 1.3 (log10)

Z-score >|2|



IFN type II

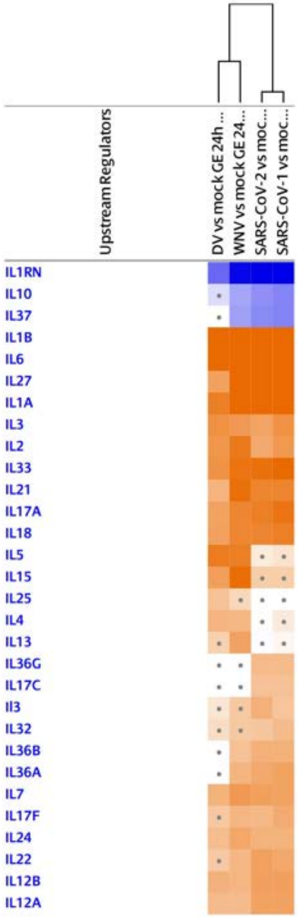
IFN type III

IFN type I

Interleukins

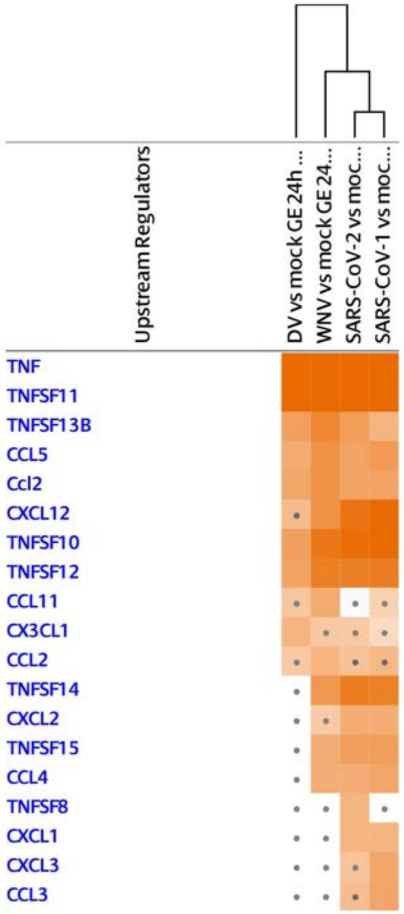
Anti-inflammatory

Pro-inflammatory



(Chemokines and TNF family)

Chemotaxis factors

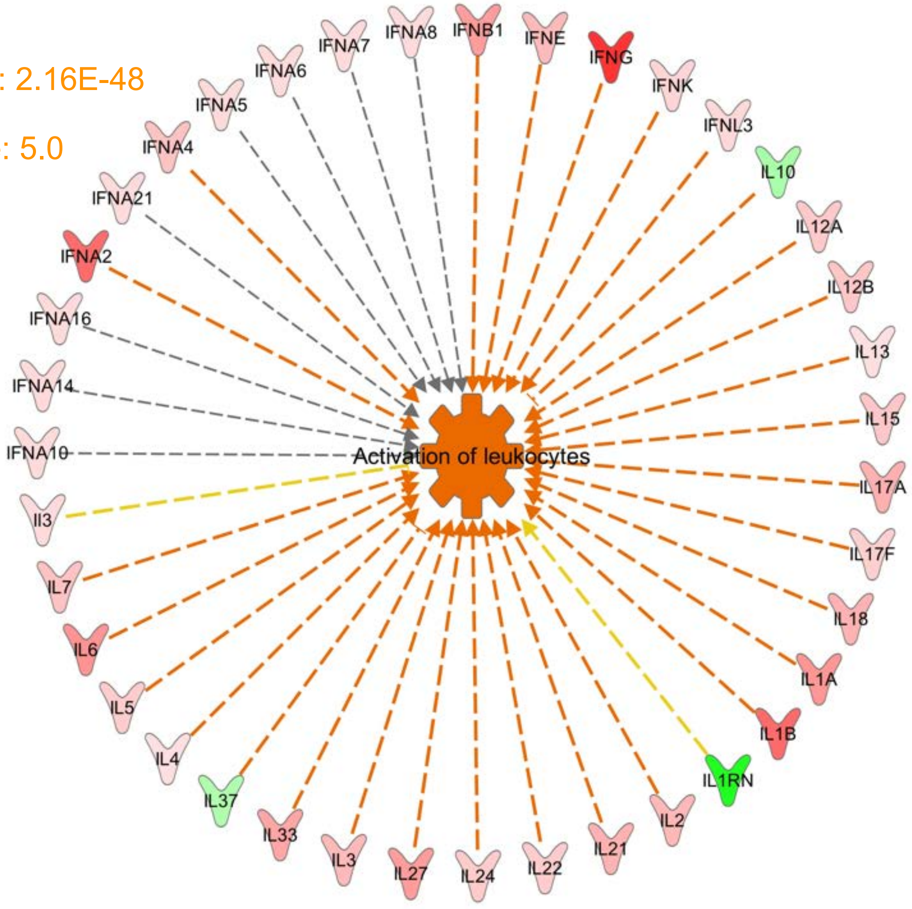


Biological processes induced 24 h pi when IFN and IL pro-inflammatory are activated (GE)

Activation of leukocytes is increased, as well inflammatory response (not shown)

p-value: 2.16E-48

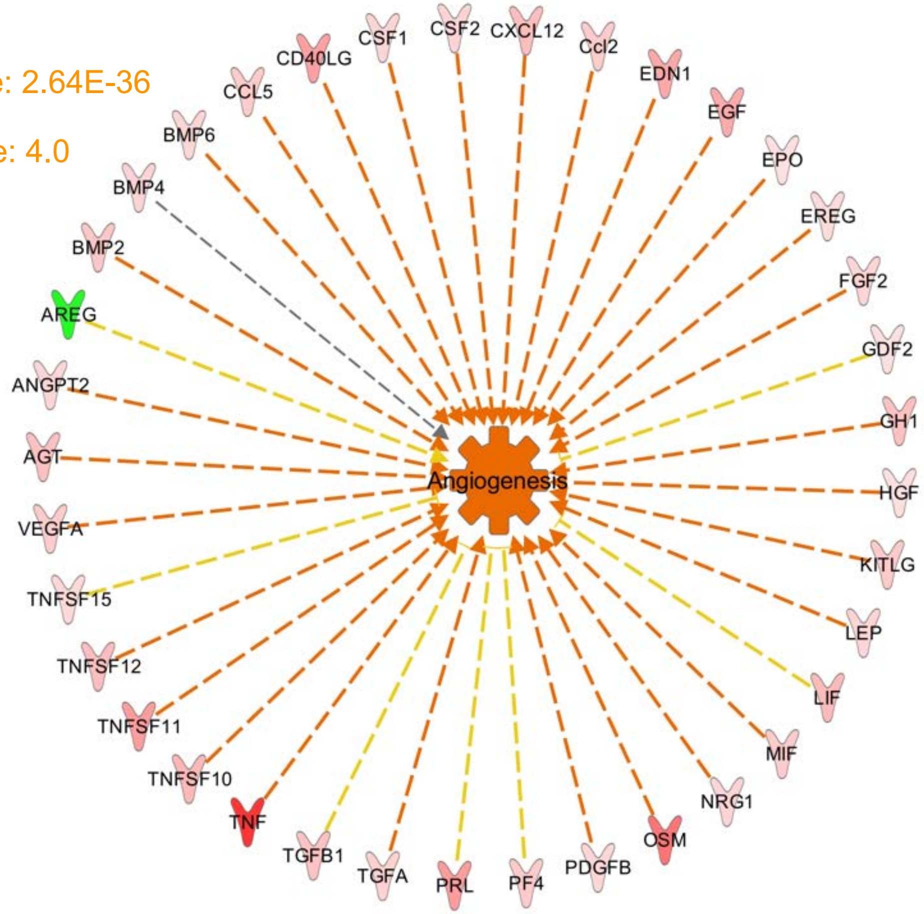
Z-score: 5.0



Angiogenesis is increased in virus-infected cells 24 h pi, as well chemotaxis, cell cycle progression (not shown)

p-value: 2.64E-36

Z-score: 4.0

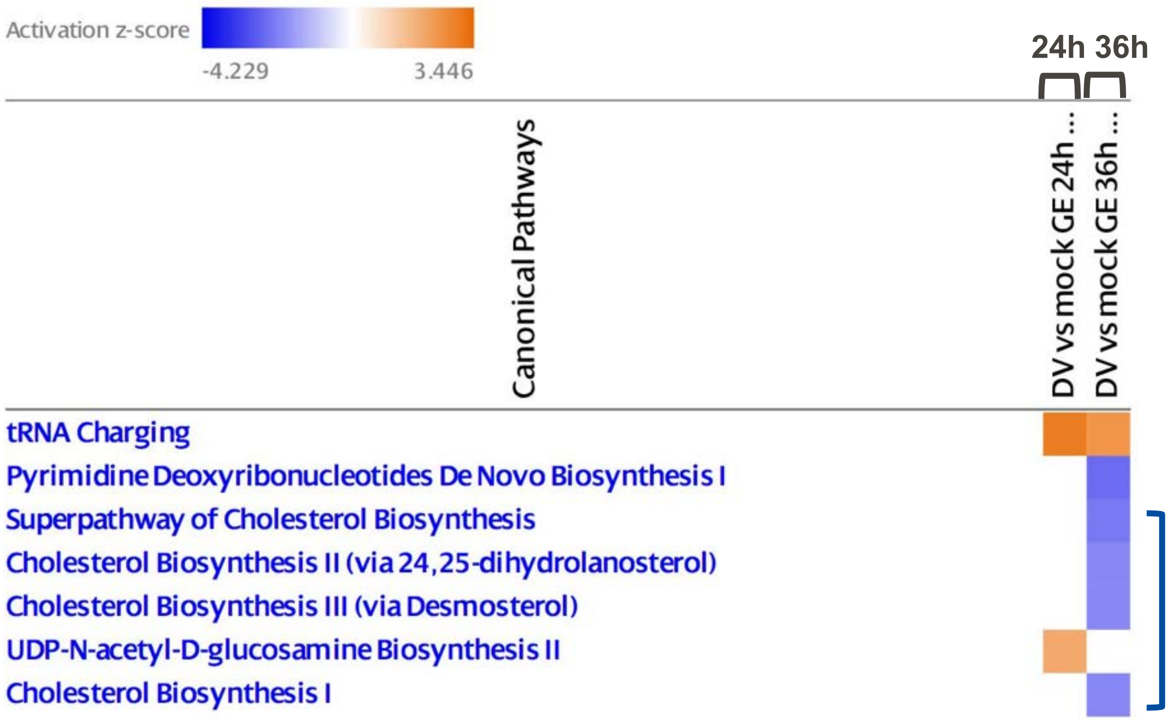


Transcriptomic analyses of time-dependent in vitro infection of A549 cells by Dengue virus

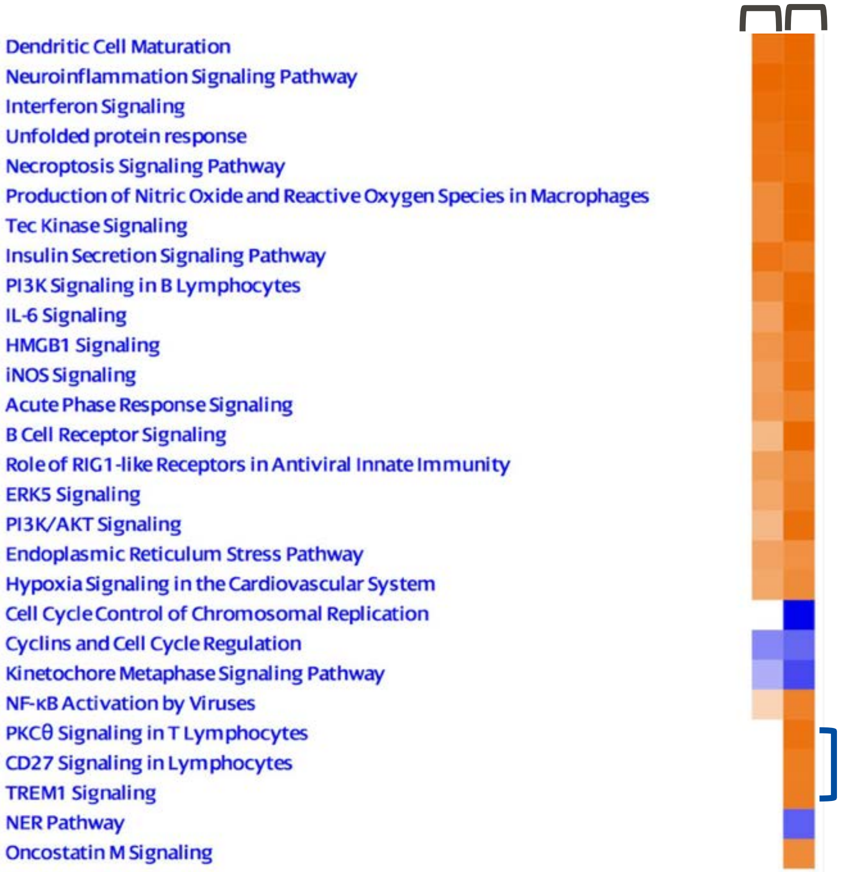
Kinetics of DENV in vitro infection at 36 h pi

Indicates inhibition of cholesterol biosynthesis and activation of T lymphocytes and myeloid specific pathways

Metabolic pathways

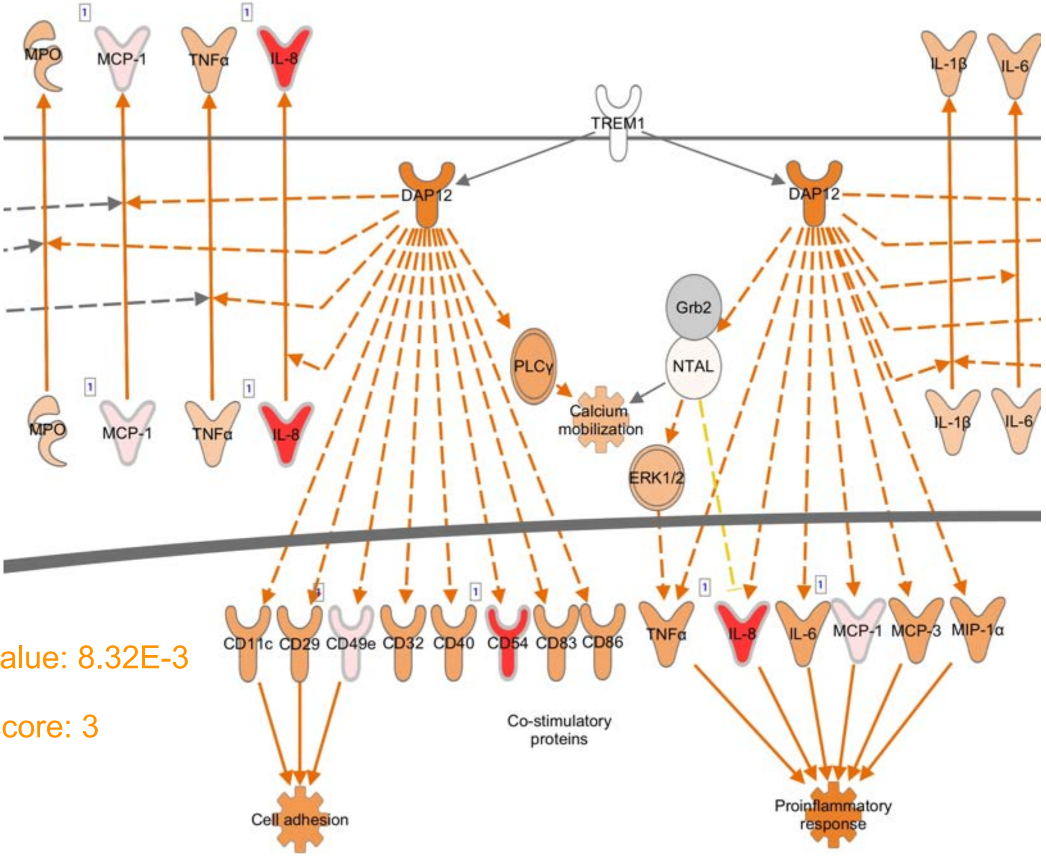


Signaling pathways



TREM1 signaling is predicted to be activated in DENV vs. mock at 36 h pi

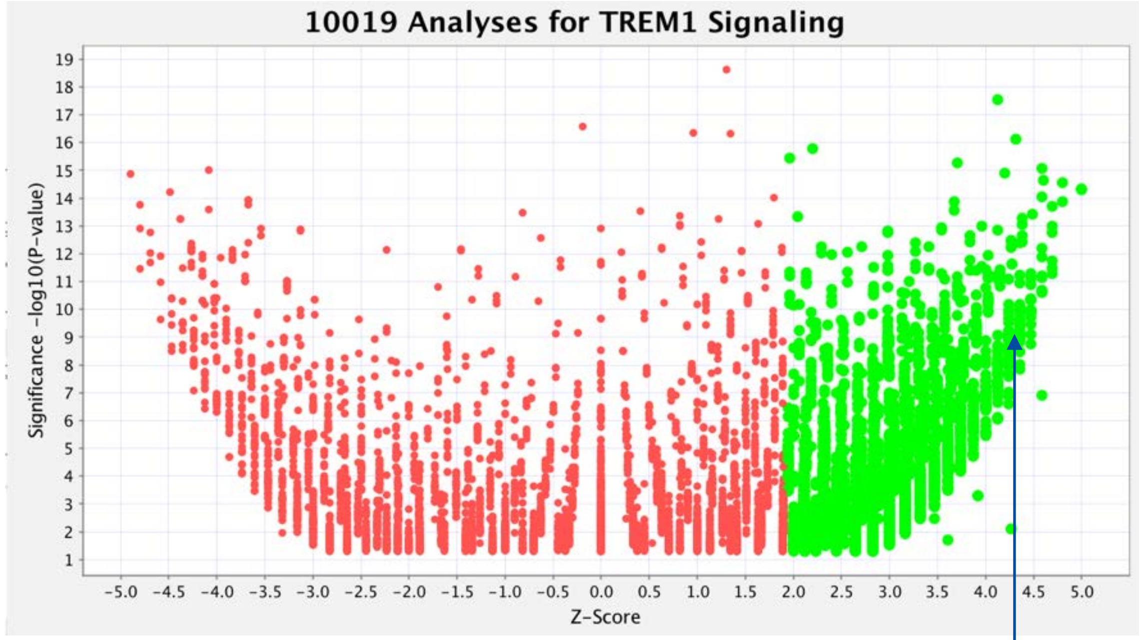
Cell adhesion and proinflammatory response are activated

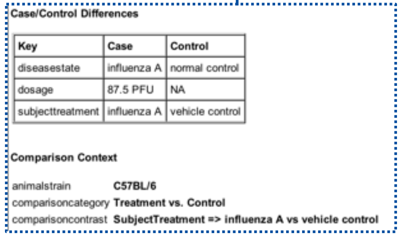


p-value: 8.32E-3

Z-score: 3

TREM1 signaling activity plot across 12,000 analyses





Case/Control Differences

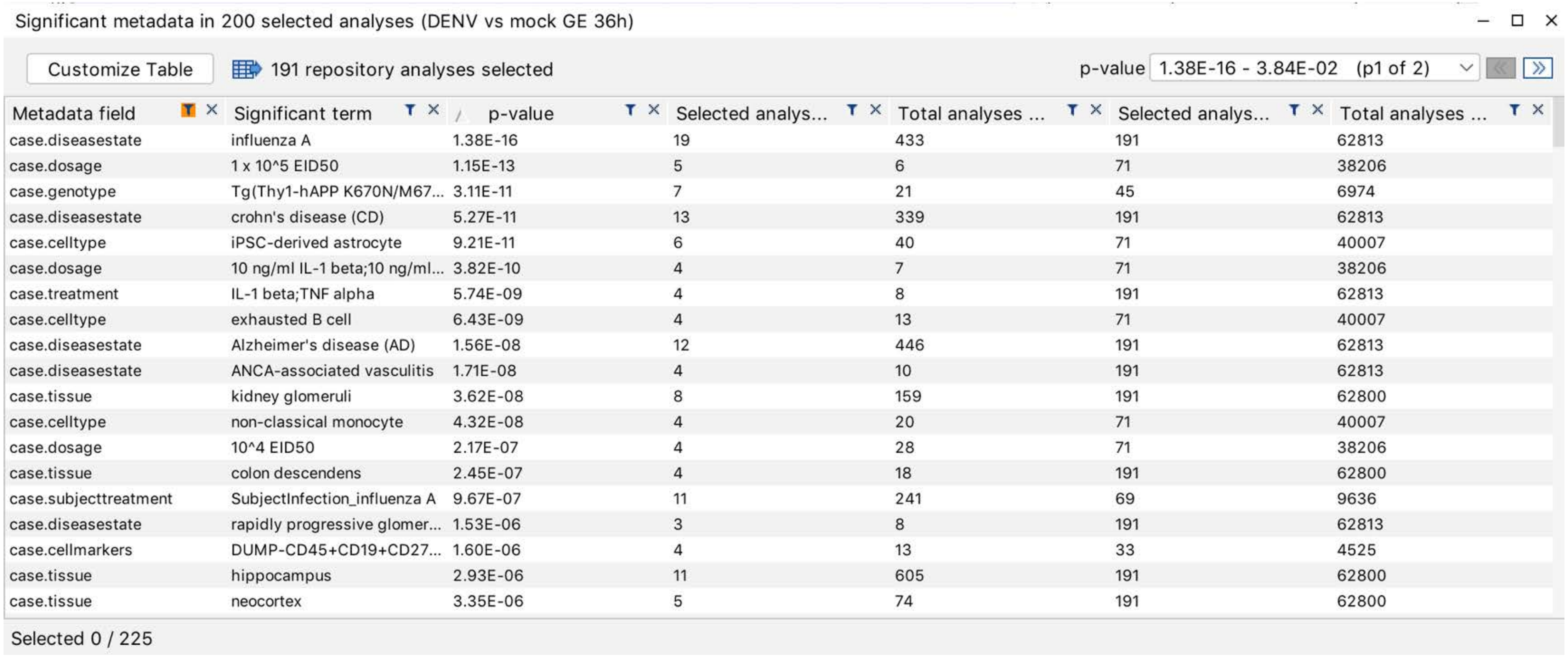
Key	Case	Control
diseasestate	influenza A	normal control
dosage	87.5 PFU	NA
subjecttreatment	influenza A	vehicle control

Comparison Context

animalstrain **C57BL/6**
comparisoncategory **Treatment vs. Control**
comparisoncontrast **SubjectTreatment => influenza A vs vehicle control**

Evaluation of metadata of TREM1 signaling predicted to be activated

TREM1 is activated during viral infection and IL-1 and TNF treatment, and in neutrophil-driven ANCA vasculitis



Significant metadata in 200 selected analyses (DENV vs mock GE 36h)

191 repository analyses selected

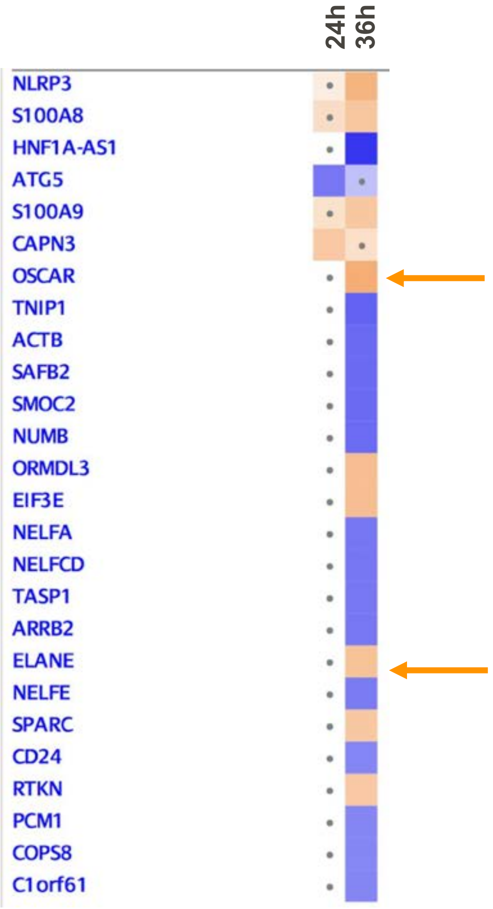
p-value 1.38E-16 - 3.84E-02 (p1 of 2)

Metadata field	Significant term	p-value	Selected analys...	Total analyses ...	Selected analys...	Total analyses ...
case.diseasestate	influenza A	1.38E-16	19	433	191	62813
case.dosage	1 x 10^5 EID50	1.15E-13	5	6	71	38206
case.genotype	Tg(Thy1-hAPP K670N/M67...	3.11E-11	7	21	45	6974
case.diseasestate	crohn's disease (CD)	5.27E-11	13	339	191	62813
case.celltype	iPSC-derived astrocyte	9.21E-11	6	40	71	40007
case.dosage	10 ng/ml IL-1 beta;10 ng/ml...	3.82E-10	4	7	71	38206
case.treatment	IL-1 beta;TNF alpha	5.74E-09	4	8	191	62813
case.celltype	exhausted B cell	6.43E-09	4	13	71	40007
case.diseasestate	Alzheimer's disease (AD)	1.56E-08	12	446	191	62813
case.diseasestate	ANCA-associated vasculitis	1.71E-08	4	10	191	62813
case.tissue	kidney glomeruli	3.62E-08	8	159	191	62800
case.celltype	non-classical monocyte	4.32E-08	4	20	71	40007
case.dosage	10^4 EID50	2.17E-07	4	28	71	38206
case.tissue	colon descendens	2.45E-07	4	18	191	62800
case.subjecttreatment	SubjectInfection_influenza A	9.67E-07	11	241	69	9636
case.diseasestate	rapidly progressive glomer...	1.53E-06	3	8	191	62813
case.cellmarkers	DUMP-CD45+CD19+CD27...	1.60E-06	4	13	33	4525
case.tissue	hippocampus	2.93E-06	11	605	191	62800
case.tissue	neocortex	3.35E-06	5	74	191	62800

Selected 0 / 225

Comparison of Upstream Regulators of DENV vs. mock at 24 h or 36 h pi

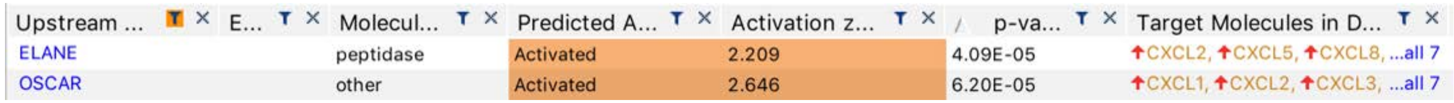
Upstream Regulator: Other and peptidase



p-value: 3 (log10)

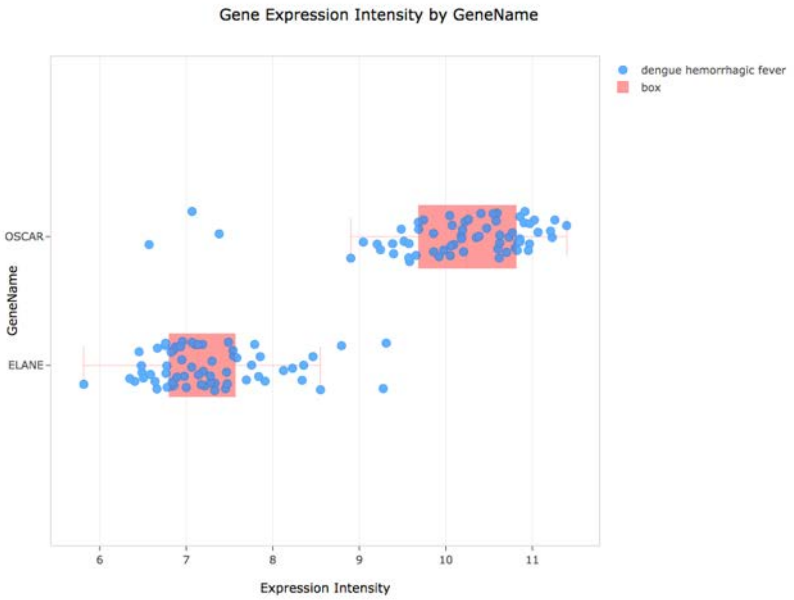
Z-score >|2|

ELANE and OSCAR are predicted to be activated at 36 h pi



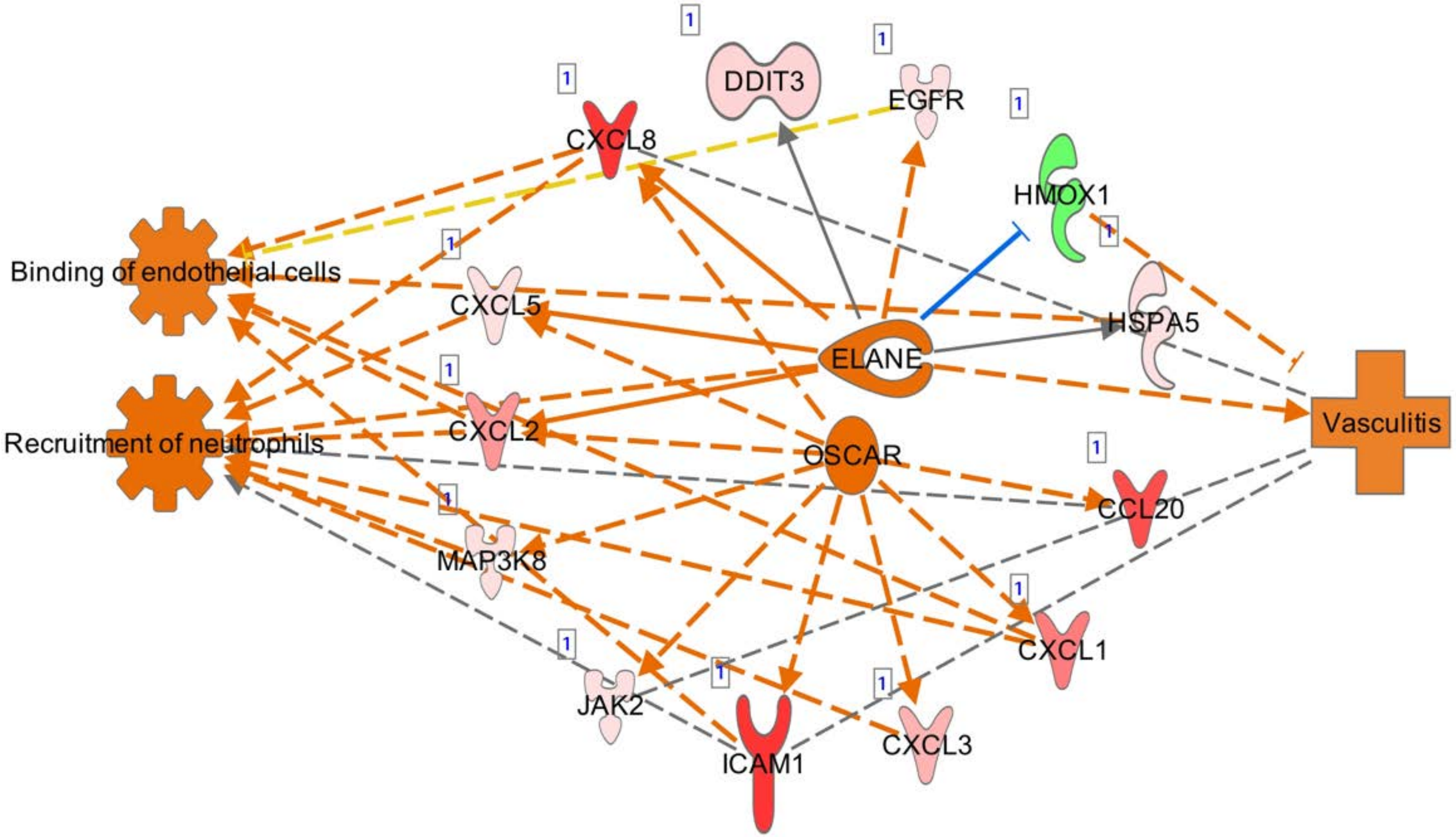
Upstream ...	E...	Molecul...	Predicted A...	Activation z...	p-va...	Target Molecules in D...
ELANE		peptidase	Activated	2.209	4.09E-05	↑CXCL2, ↑CXCL5, ↑CXCL8, ...all 7
OSCAR		other	Activated	2.646	6.20E-05	↑CXCL1, ↑CXCL2, ↑CXCL3, ...all 7

ELANE and OSCAR are highly expressed in Dengue Hemorrhagic fever, Human Disease B37



ELANE and OSCAR are upstream regulators predicted to be activated in DENV at 36 h pi

ELANE and OSCAR are increasing chemotaxis of neutrophil, binding of endothelial cells and vasculitis



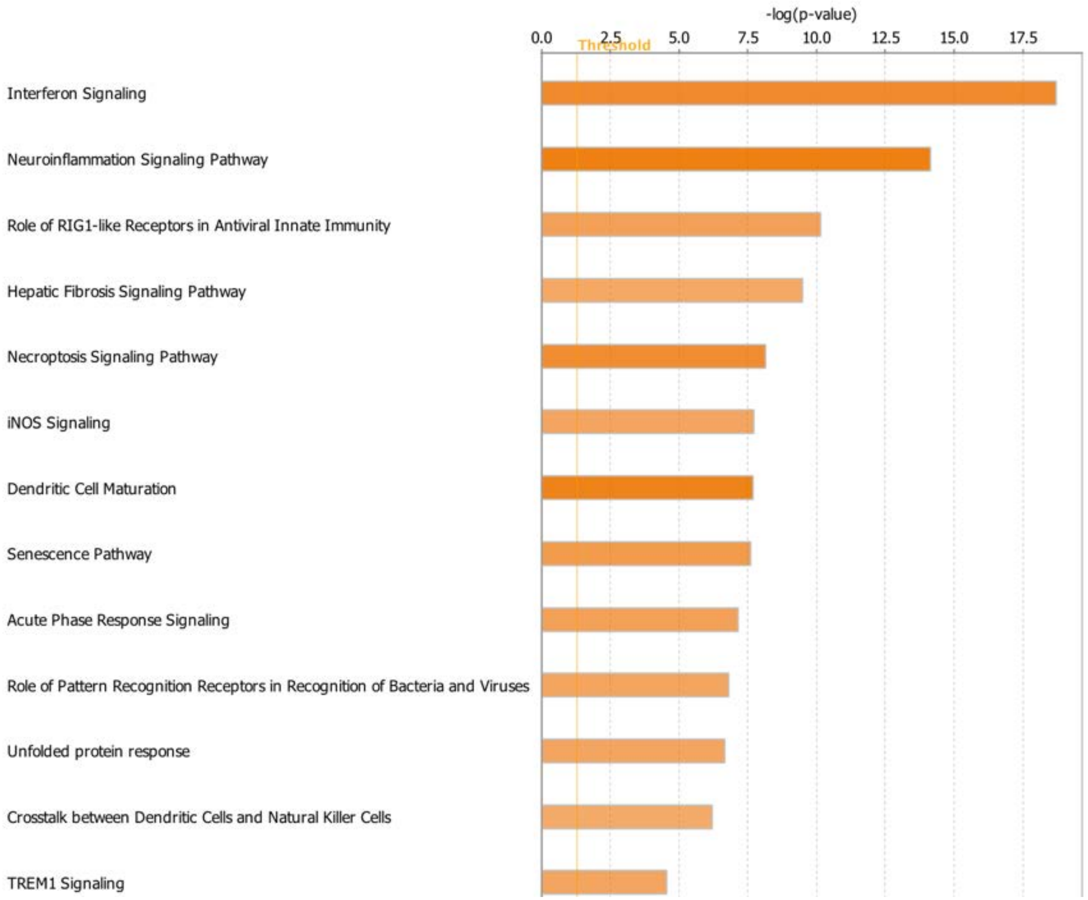
- Neutrophils may have a detrimental role in DENV infection via NET formation, by increasing vascular damages
- ELANE is involved in NET formation and antimicrobial response
- ELANE and OSCAR may contribute to amplify vascular permeability seen in DENV hemorrhagic fever

Source: Sung, P. S., Huang, T. F. and Hsieh, S. L. (2019). Extracellular vesicles from CLEC2-activated platelets enhance dengue virus-induced lethality via CLEC5A/TLR2. Nature communications, **10**, 2402.

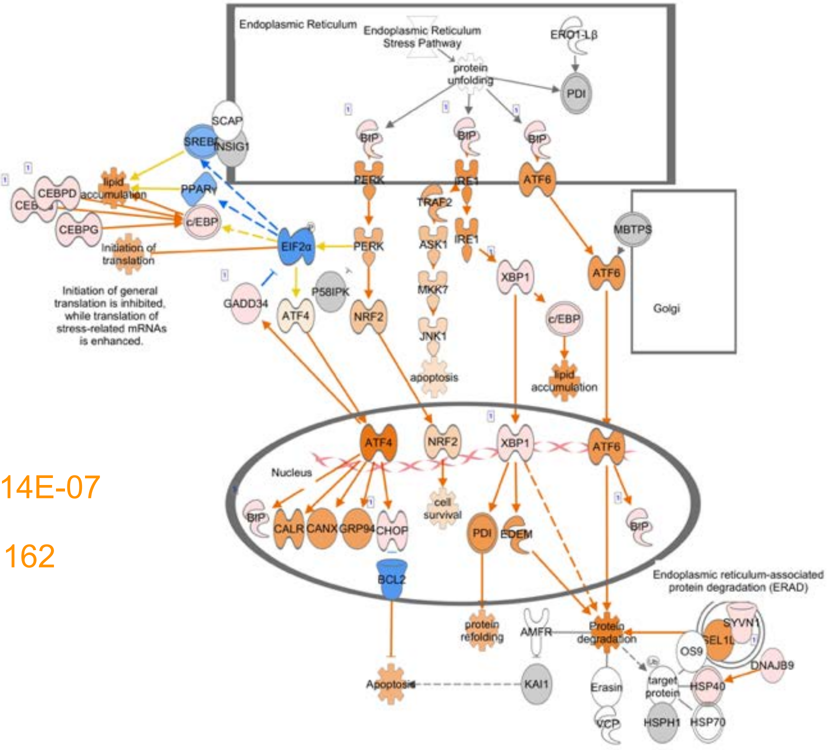
Transcriptomic analyses of in vitro infection of A549 cells by West Nile virus

WNV infection activates immune-related pathways and unfolded protein response 24 h pi

Activated signaling pathways



Unfolded proteins response is activated



p-value: 2.14E-07

Z-score: 3.162

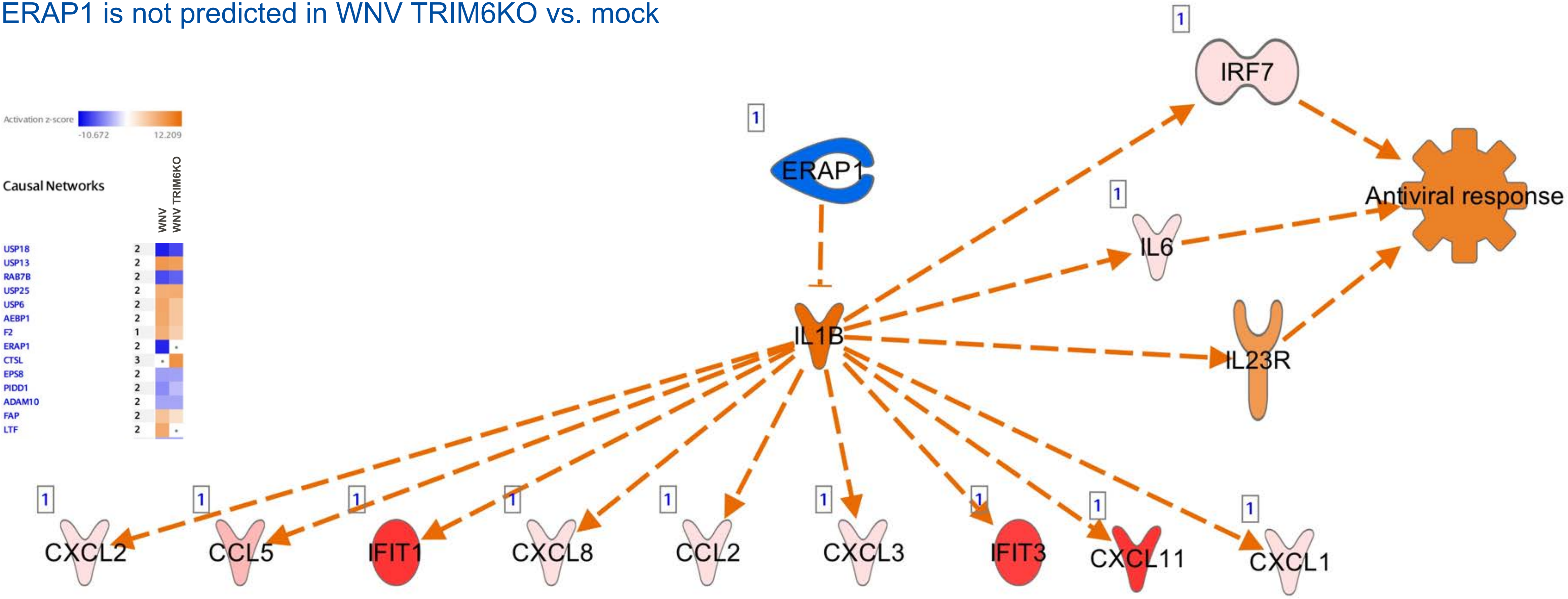
BiP/HSPA5 is upregulated and may play a major role in the UPR during flavivirus infection by promoting protein folding and virion assembly.

Source: Lewy, T. G., Grabowski, J. M., and Bloom, M. E. (2017). BiP: Master regulator of the unfolded protein response and crucial factor in flavivirus biology . The Yale journal of biology and medicine, 90, 291–300.

Inhibition of ERAP1 promotes the antiviral response in WNV-infected cells at 24 h pi (GE)

Some major mediators of antiviral response against WNV are shown here

ERAP1 is not predicted in WNV TRIM6KO vs. mock



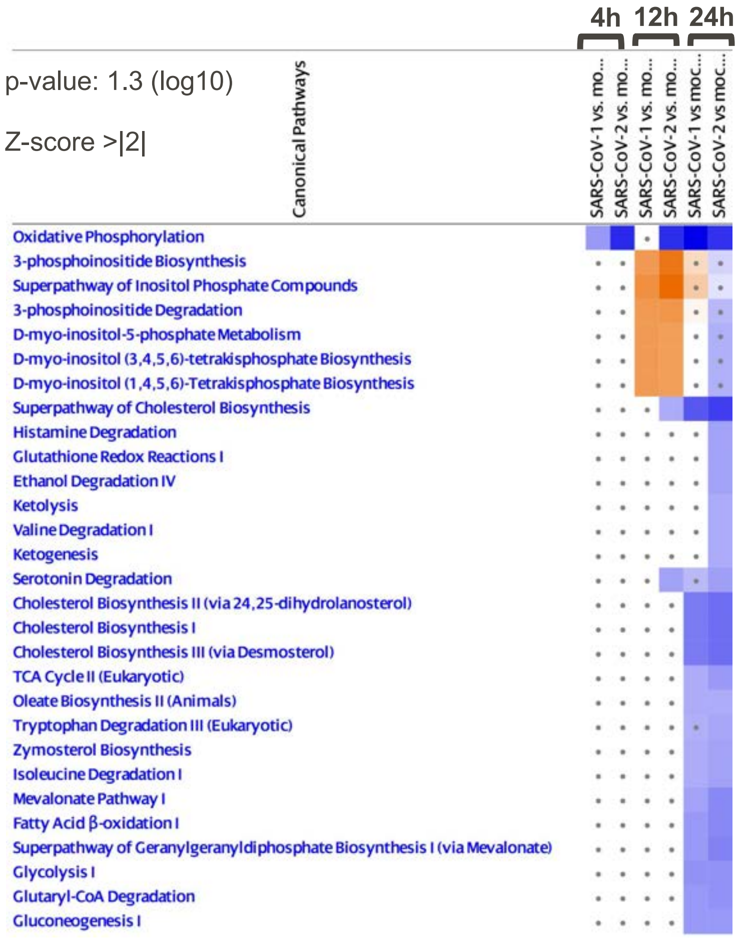
Transcriptomic analyses of SARS-CoV-1 and SARS-CoV-2 in vitro infection of Calu-3 cells

Transcriptomic analyses of SARS-CoV-1 and SARS-CoV-2 at 4,12 and 24 h pi (GE)

Metabolic Pathways

p-value: 1.3 (log10)

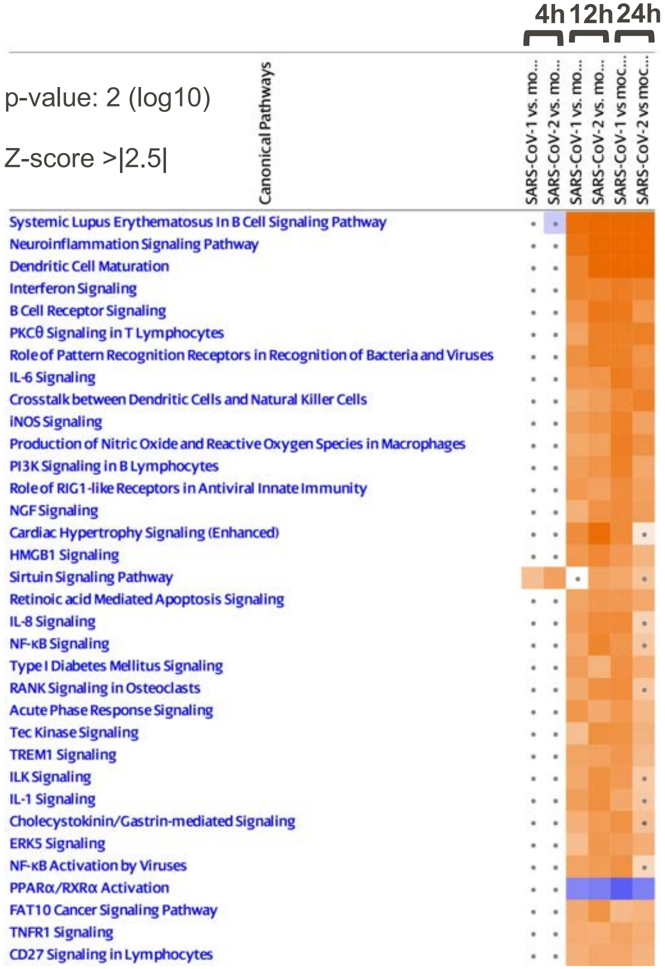
Z-score >|2|



Signaling Pathways

p-value: 2 (log10)

Z-score >|2.5|



Signaling Pathways (continued)

Transcriptomic analyses of SARS-CoV-1 and SARS-CoV-2 at 4,12 and 24 h pi (GE)

Metabolic Pathways

- Oxidative phosphorylation
- Glucose metabolism
- Cholesterol metabolism
- Fatty acid metabolism
- Alcohol metabolism
- Ketones metabolism
- BCAA degradation
- Production of ATP, NADH

Signaling Pathways

- Innate immune responses triggered
- DC, B, T, NK cells engaged
- Interferon response increased
- TH1, TH17 induced
- Pro-inflammatory signaling
- UPR activated

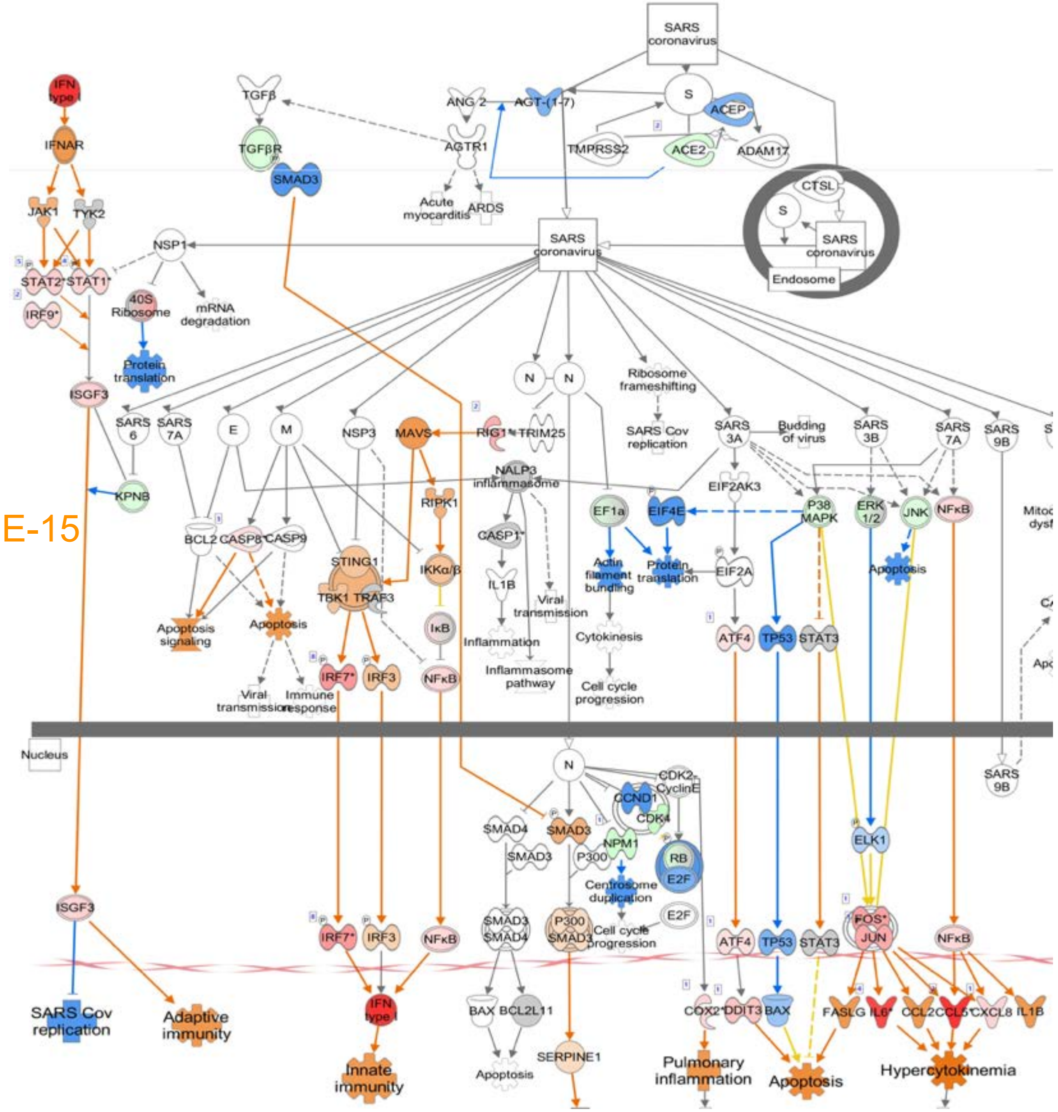
Signaling Pathways (continued)

Coronavirus pathogenesis pathway is activated

SARS-CoV-2 vs. mock 24 h pi (TE)

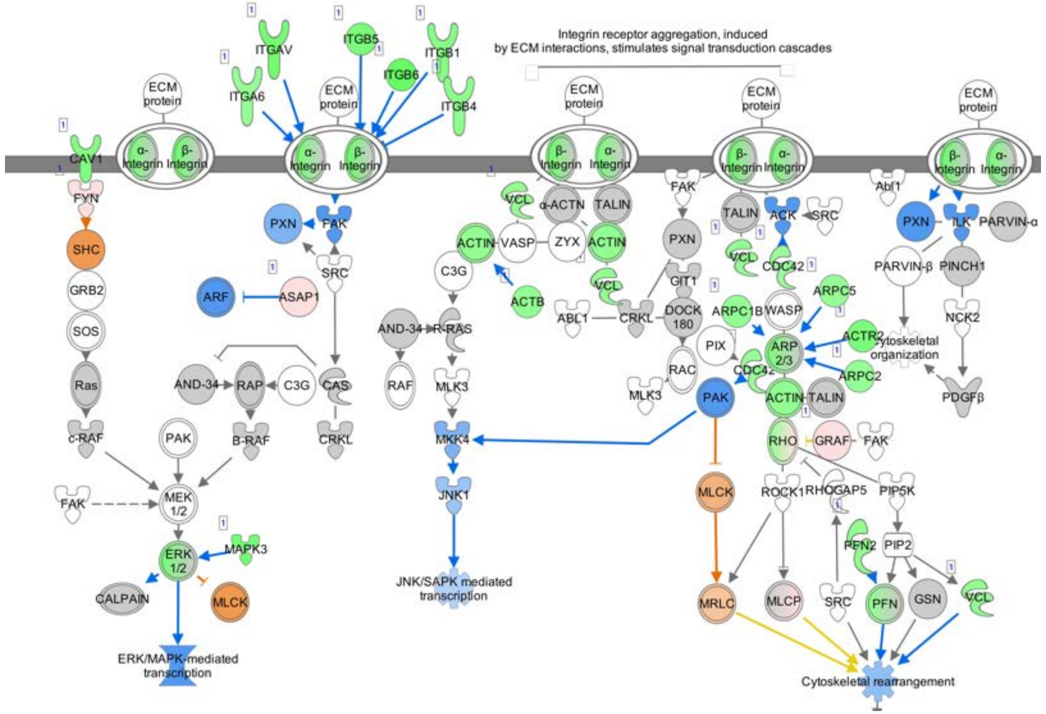
p-value: 1.78E-15

Z-score: 2.7



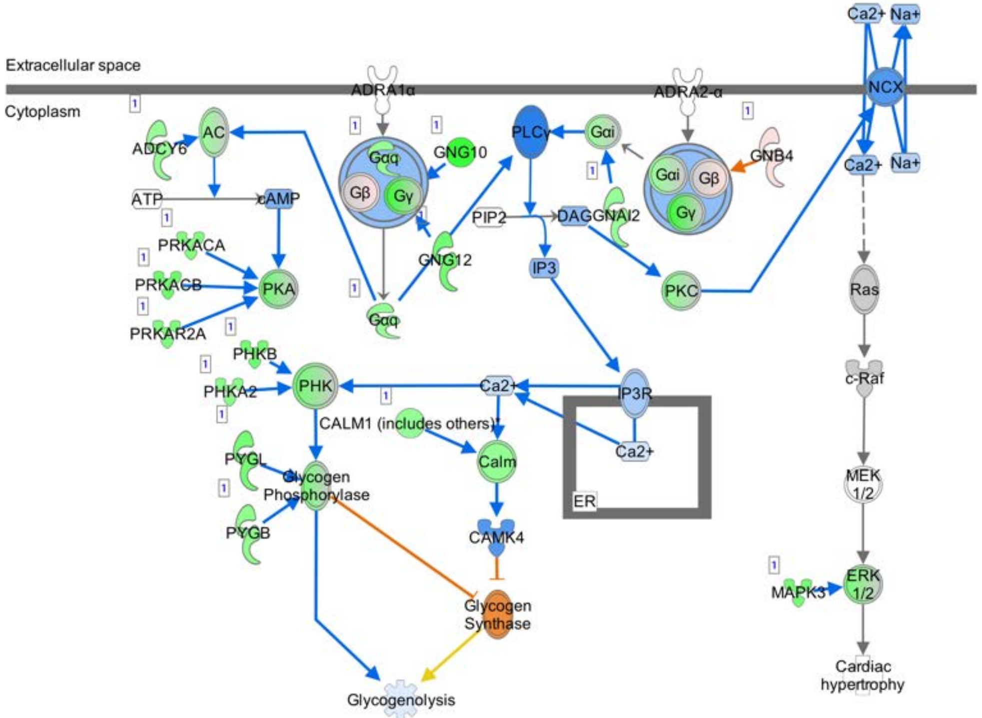
Integrin and α-adrenergic signaling are significantly inhibited in SARS-CoV-2 infection

Integrin signaling is inhibited at 24 h pi (GE)



- SARS-CoV-2 S protein is hypothesized to bind via its RGD/KGD sequence to integrin (e.g., $\alpha5\beta1$); this sequence may facilitate the binding
- Integrin and integrin receptors may be down-regulated after binding (as seen for others viruses)

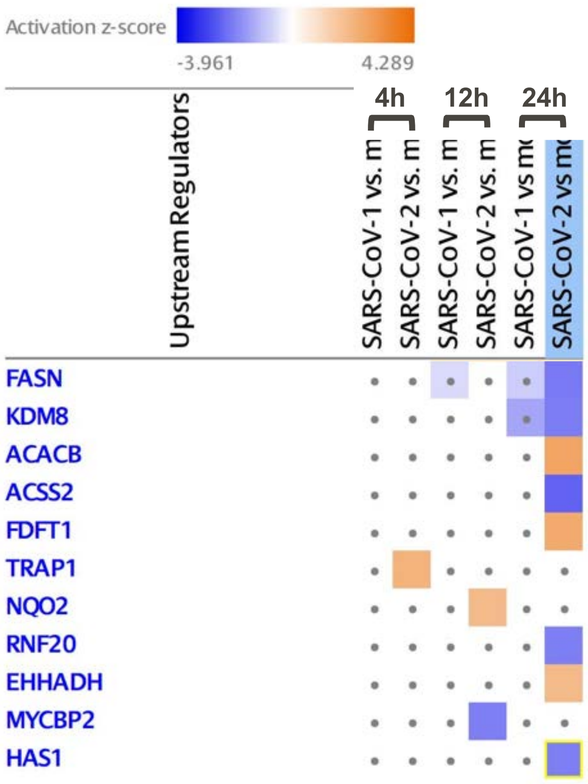
α-adrenergic signaling is inhibited at 24 h pi (GE)

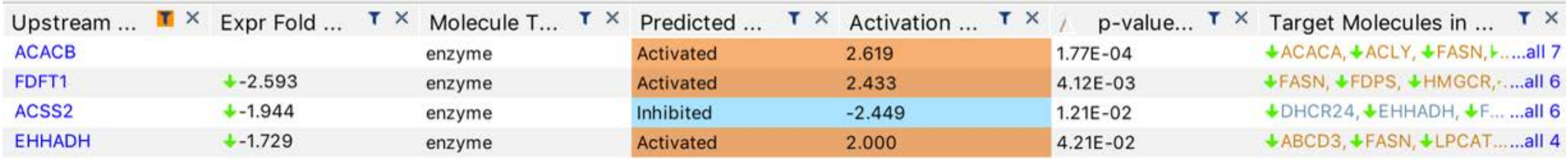


- COVID-19 induces cardiovascular consequences (myocardial injury, myocarditis, stroke, arrhythmias, heart failure, cardiogenic shock, etc.) and the deregulation of this pathway may play a role

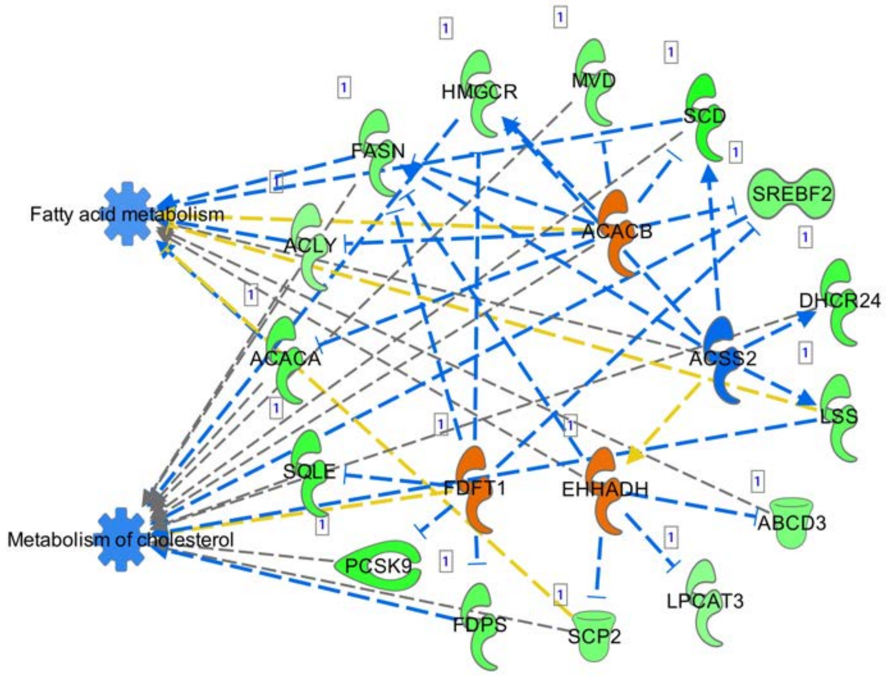
SARS-CoV-2 decreases fatty acid and cholesterol metabolism significantly at 24 h pi

Heatmap of Upstream Regulator Analysis (enzyme, GE)





Upstream ...	Expr Fold ...	Molecule T...	Predicted ...	Activation ...	p-value...	Target Molecules in ...
ACACB		enzyme	Activated	2.619	1.77E-04	↓ACACA, ↓ACLY, ↓FASN, ...all 7
FDFT1	↓-2.593	enzyme	Activated	2.433	4.12E-03	↓FASN, ↓FDPS, ↓HMGCR, ...all 6
ACSS2	↓-1.944	enzyme	Inhibited	-2.449	1.21E-02	↓DHCR24, ↓EHHADH, ↓F... ...all 6
EHHADH	↓-1.729	enzyme	Activated	2.000	4.21E-02	↓ABCD3, ↓FASN, ↓LPCAT... ...all 4

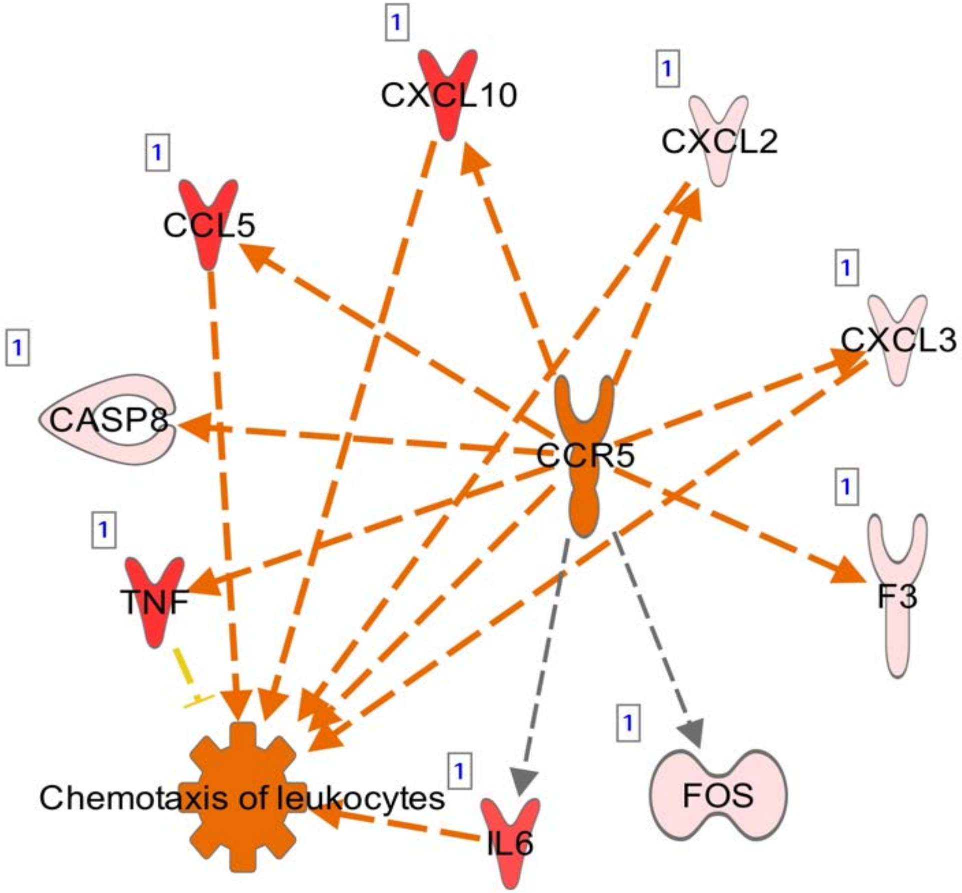


CCR5 is activated allowing chemotaxis of leukocytes

Upstream Regulator Analysis, cytokines, SARS-CoV-2 vs. mock, 24 h pi GE (as soon as 12 h pi)

p-value: 8.90E-3

Z-score: 2.6



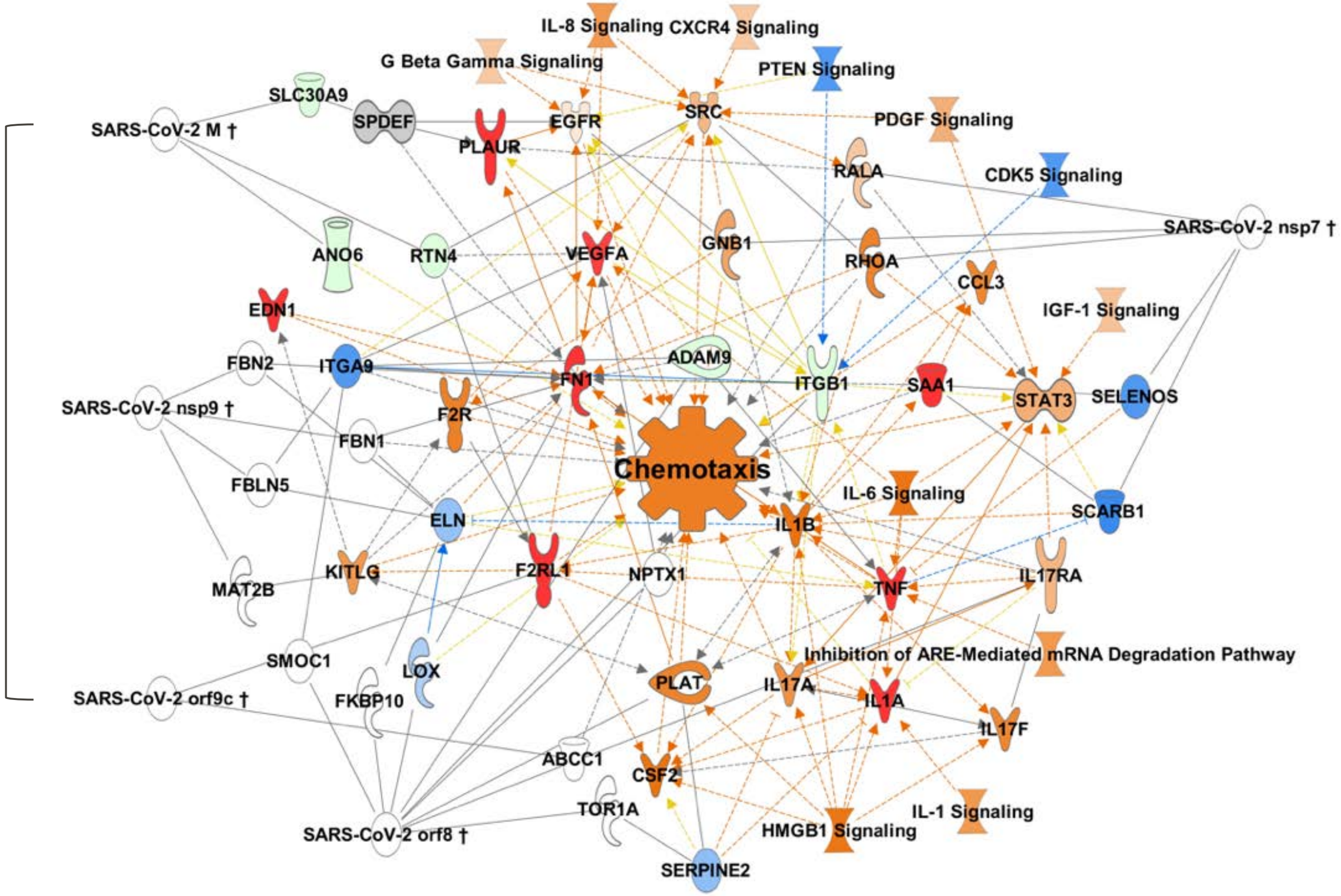
CCR5 may be a potential therapeutic target against SARS-CoV-2 infection

Chua, R.L. et al. (2020) COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis Nat. Biotechnol. [published online ahead of print].

Coronavirus Network Explorer: Chemotaxis

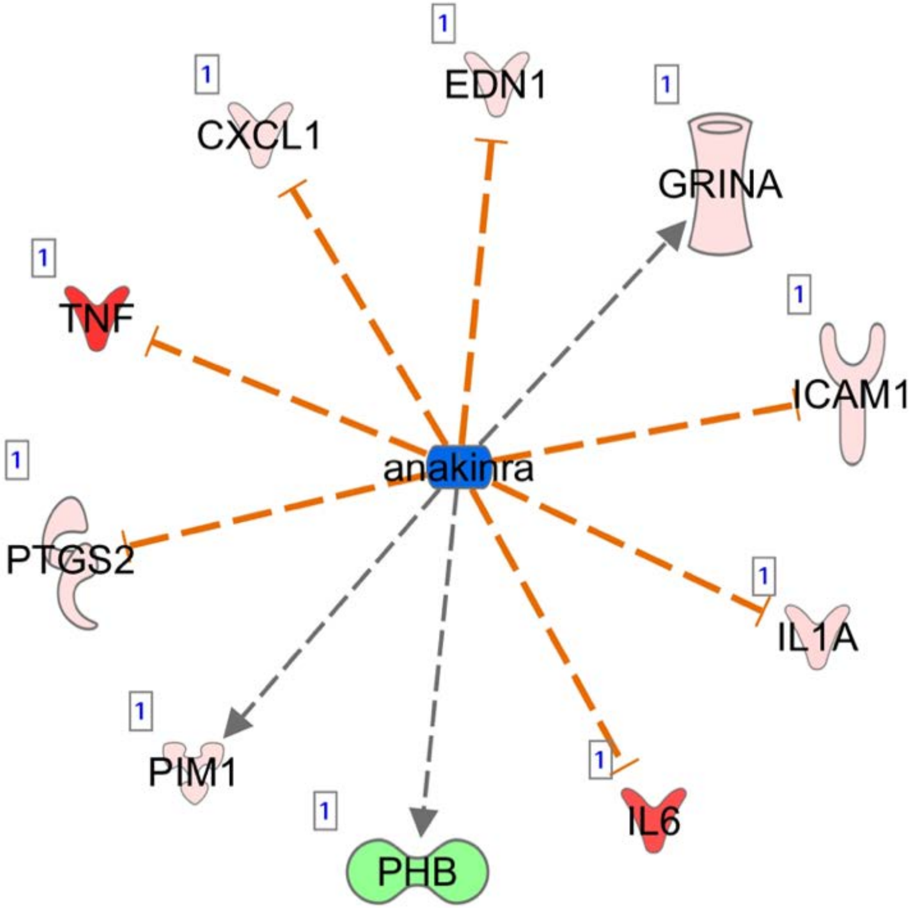
SARS-CoV-2 induces a strong chemotaxis in COVID-19 patients (24 h pi, GE)

SARS-CoV-2 viral proteins



Anakinra could be used to decrease SARS-CoV-2 infection (24 h pi, GE)

Upstream Regulator Analysis predicted that Anakinra (anti-IL1R1) is “inhibited”



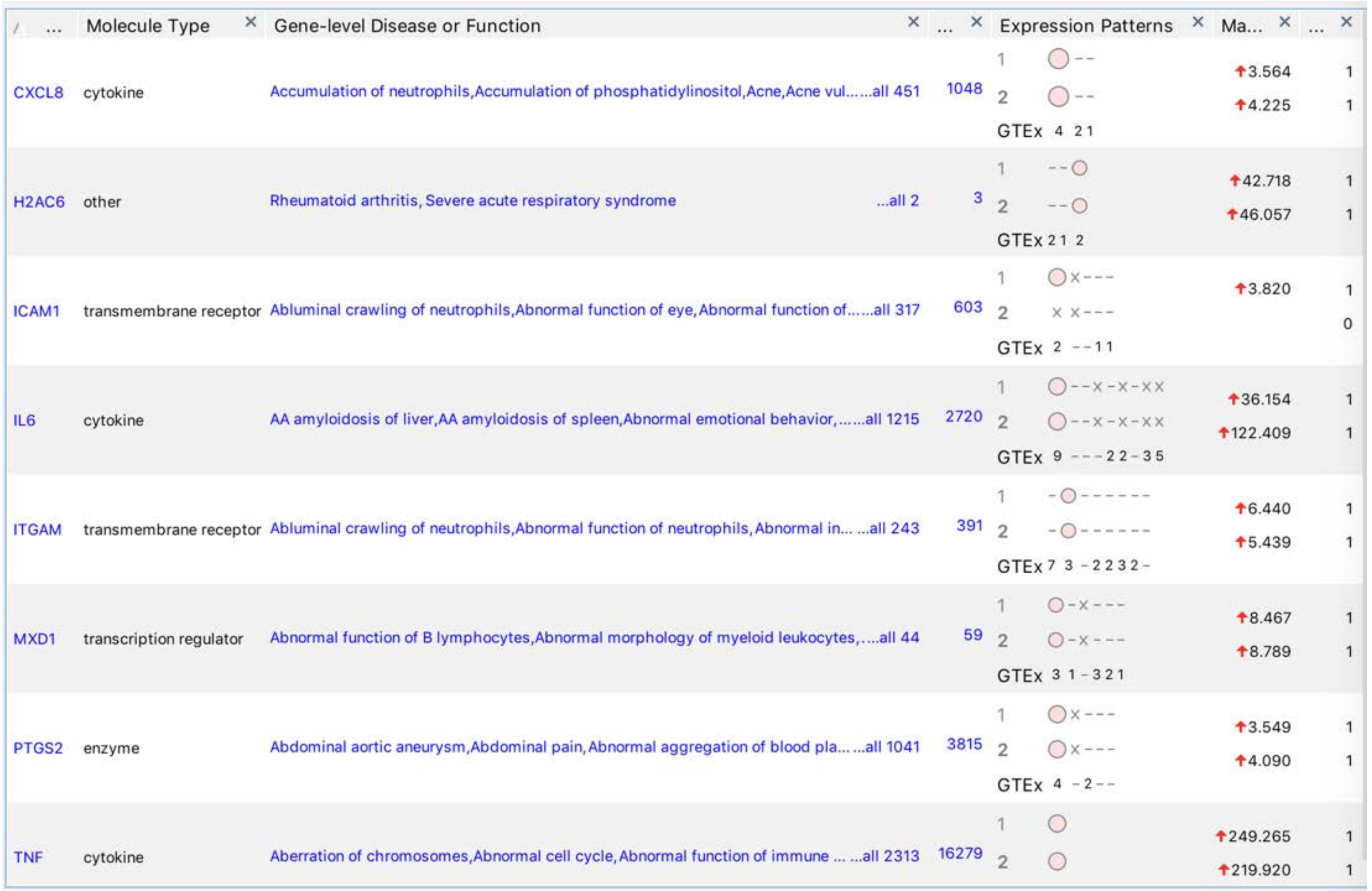
A phase III clinical trial is underway investigating whether blocking IL-1 activity improves the outcome of COVID-19 patients with cytokine release syndrome

Sources:

- Calabrese, L.H. (2020) Cytokine storm and the prospects for immunotherapy with COVID-19. Cleve Clin. J. Med. 87, 389–393.
- Treatment of COVID-19 Patients With Anti-interleukin Drugs (COV-AID), NCT04330638, phase 3, recruiting.

Upregulated transcript variants linked to acute respiratory distress syndrome (ARDS)

IsoProfiler for SARS-CoV-1 vs. mock and SARS-CoV-2 vs. mock (TE) at 24 h pi

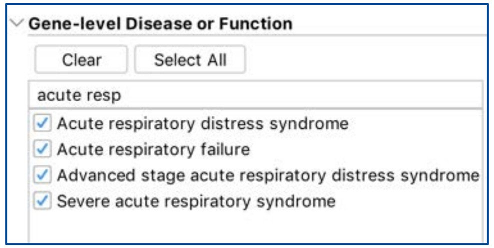


...	Molecule Type	Gene-level Disease or Function	...	Expression Patterns	Ma...	...
CXCL8	cytokine	Accumulation of neutrophils,Accumulation of phosphatidylinositol,Acne,Acne vul......all 451	1048	1 ○-- 2 ○-- GTEx 4 21	↑3.564 ↑4.225	1 1
H2AC6	other	Rheumatoid arthritis, Severe acute respiratory syndrome ...all 2	3	1 --○ 2 --○ GTEx 21 2	↑42.718 ↑46.057	1 1
ICAM1	transmembrane receptor	Abluminal crawling of neutrophils,Abnormal function of eye,Abnormal function of......all 317	603	1 ○x--- 2 x x--- GTEx 2 --11	↑3.820	1 0
IL6	cytokine	AA amyloidosis of liver,AA amyloidosis of spleen,Abnormal emotional behavior,......all 1215	2720	1 ○--x-x-xx 2 ○--x-x-xx GTEx 9 ---22-35	↑36.154 ↑122.409	1 1
ITGAM	transmembrane receptor	Abluminal crawling of neutrophils,Abnormal function of neutrophils,Abnormal in... ...all 243	391	1 -○------ 2 -○------ GTEx 7 3 -2232-	↑6.440 ↑5.439	1 1
MXD1	transcription regulator	Abnormal function of B lymphocytes,Abnormal morphology of myeloid leukocytes,....all 44	59	1 ○-x--- 2 ○-x--- GTEx 3 1-321	↑8.467 ↑8.789	1 1
PTGS2	enzyme	Abdominal aortic aneurysm,Abdominal pain,Abnormal aggregation of blood pla... ...all 1041	3815	1 ○x--- 2 ○x--- GTEx 4 -2--	↑3.549 ↑4.090	1 1
TNF	cytokine	Aberration of chromosomes,Abnormal cell cycle,Abnormal function of immune ... ...all 2313	16279	1 ○ 2 ○	↑249.265 ↑219.920	1 1

Biotype: Protein coding

Transcript Level: TSL1

GTEx Enrichment: Lung, whole blood



Gene-level Disease or Function

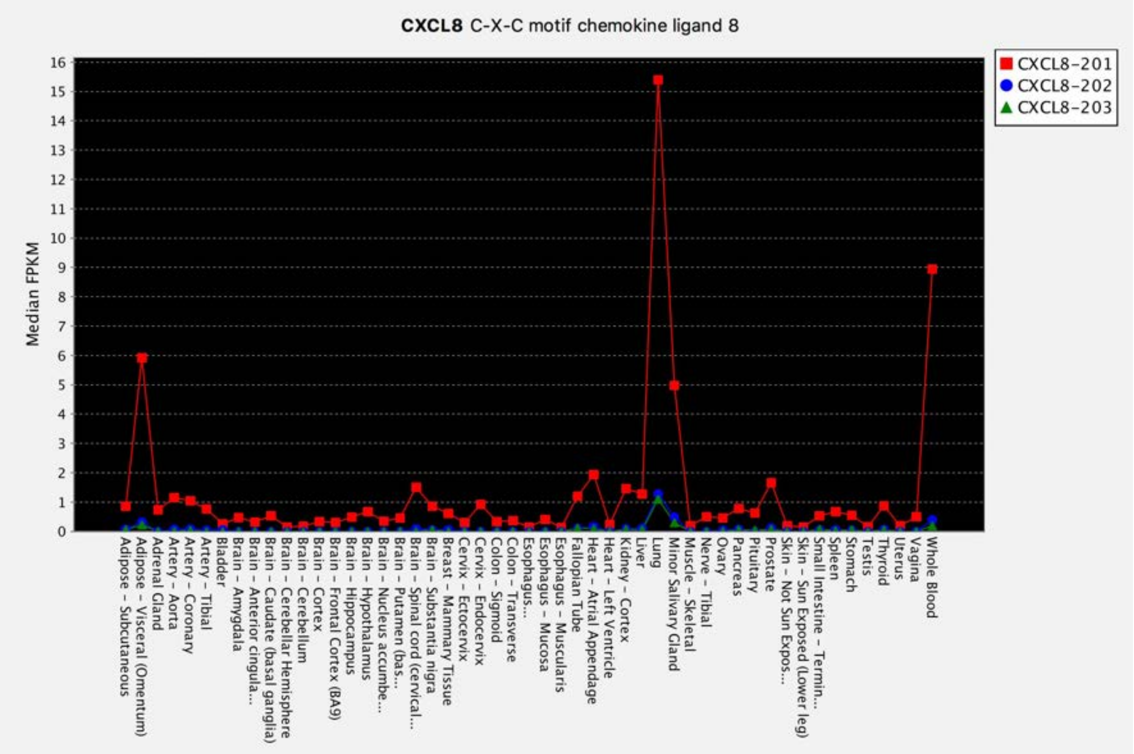
Clear | Select All

acute resp

- ☑ Acute respiratory distress syndrome
- ☑ Acute respiratory failure
- ☑ Advanced stage acute respiratory distress syndrome
- ☑ Severe acute respiratory syndrome

CXCL8-201 as biomarker for prognosis of acute respiratory distress syndrome

Tissue expression enrichment of CXCL8 transcripts

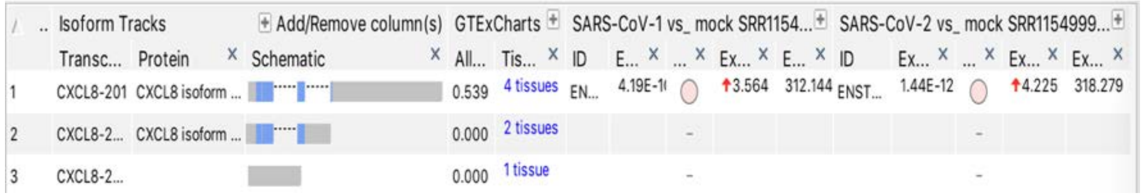


Schematic of CXCL8 transcripts and Findings

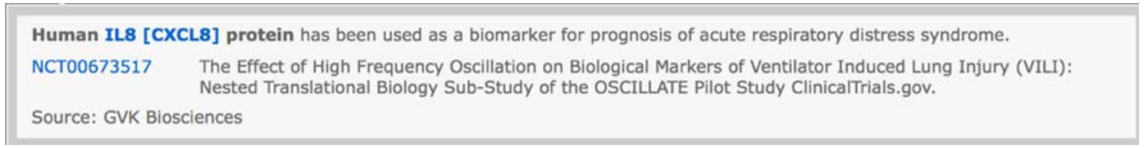
Neutrophil populations is significantly increased in COVID-19 patients with ARDS

CXCL8 is the major neutrophil chemotactic factor in humans

Source: Wilk, A. J. et al. (2020). A single-cell atlas of the peripheral immune response in patients with severe COVID-19. Nature medicine, 26(7), 1070–1076.



..	Isoform Tracks		Add/Remove column(s)	GTExCharts		SARS-CoV-1 vs_ mock SRR1154...					SARS-CoV-2 vs_ mock SRR1154999...				
	Transc...	Protein	Schematic	All...	Tis...	ID	E...	...	Ex...	E...	ID	Ex...	...	Ex...	Ex...
1	CXCL8-201	CXCL8 isoform ...		0.539	4 tissues	EN...	4.19E-1(	○	↑3.564	312.144	ENST...	1.44E-12	○	↑4.225	318.279
2	CXCL8-2...	CXCL8 isoform ...		0.000	2 tissues			-					-		
3	CXCL8-2...			0.000	1 tissue			-					-		



Human IL8 [CXCL8] protein has been used as a biomarker for prognosis of acute respiratory distress syndrome.

NCT00673517	The Effect of High Frequency Oscillation on Biological Markers of Ventilator Induced Lung Injury (VILI): Nested Translational Biology Sub-Study of the OSCILLATE Pilot Study ClinicalTrials.gov.

Source: GVK Biosciences

Analysis Match combines knowledge with data

ArrayExpress, GEO, TCGA, SRA, LINCS, etc.

Reprocessed using the same pipeline

QIAGEN OmicSoft Array Studio

73,000+ expression comparison datasets

Built from >375,000 samples

Journal articles and databases such as Clinical Trials, COSMIC, MGD,OMIM , etc.

Curation and QA

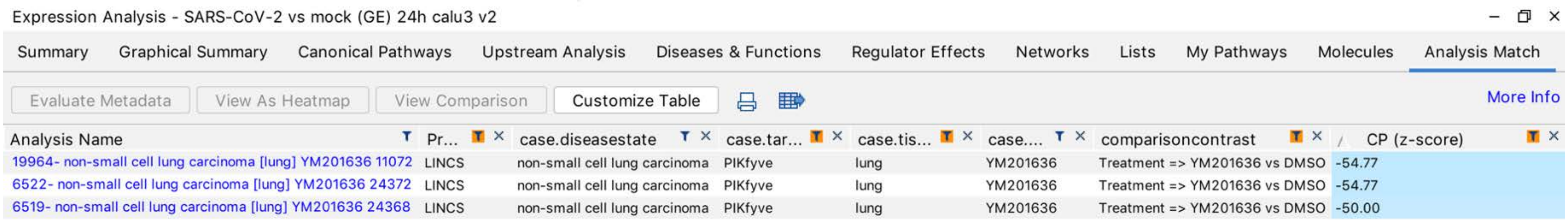
>7.2 million curated Findings

QIAGEN Ingenuity Pathway Analysis

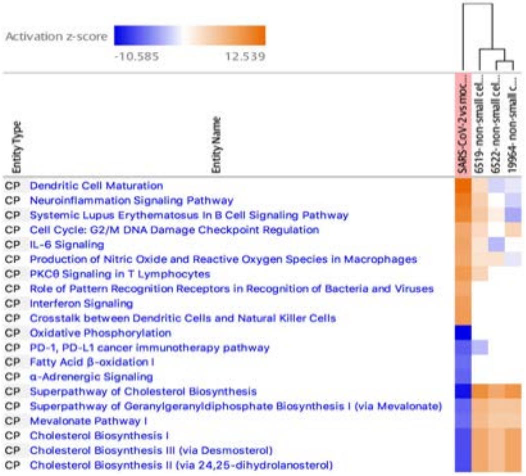
- Biological analyses of each dataset
- Compare your analysis to all QIAGEN OmicSoft analyses

Discovery of a potential inhibitor of SARS-CoV-2 infection using Analysis Match

SARS-CoV-2 vs. mock, 24 h pi, GE, filtering on tissue, CP z-score negative



Analysis Name	Pr...	case.diseasestate	case.tar...	case.tis...	case....	comparisoncontrast	CP (z-score)
19964- non-small cell lung carcinoma [lung] YM201636 11072	LINCS	non-small cell lung carcinoma	PIKfyve	lung	YM201636	Treatment => YM201636 vs DMSO	-54.77
6522- non-small cell lung carcinoma [lung] YM201636 24372	LINCS	non-small cell lung carcinoma	PIKfyve	lung	YM201636	Treatment => YM201636 vs DMSO	-54.77
6519- non-small cell lung carcinoma [lung] YM201636 24368	LINCS	non-small cell lung carcinoma	PIKfyve	lung	YM201636	Treatment => YM201636 vs DMSO	-50.00



PIKfyve (lipid/protein kinase) plays an important role in endocytic mechanism and has been proposed as a potential therapeutic target against SARS-CoV-2 infection

Sources:

- Bouhaddou, M. et al. (2020). The global phosphorylation landscape of SARS-CoV-2 infection. Cell [Advance online publication].
- Ou, X. at al. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature communications, **11**, 1620.

Conclusions

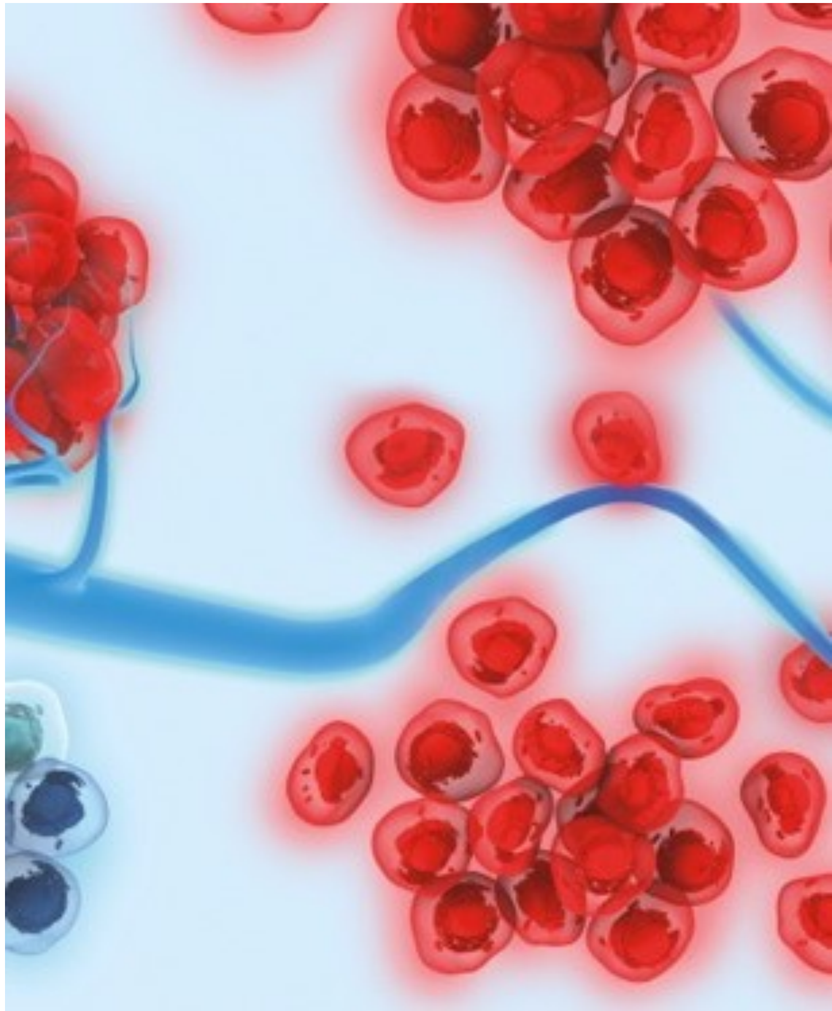
Transcriptomic analyses of in vitro flavivirus- or coronavirus-infected cells

1 Antiviral response is similar across the 4 viruses at 24 h post infection

2 Potential key upstream regulators and biological processes have been identified in these infections

3 Differentially expressed isoforms could be used as biomarker for key pathological endpoints

4 Discovery of potential therapeutic targets or compounds to inhibit these infections by comparing to unrelated thousands of datasets



Resources

QIAGEN IPA

- IPA product info: https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa
- IPA Analysis Match: https://tv.qiagenbioinformatics.com/video/37242337/exploring-ipas-analysis-match-an
- Land Explorer: https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/content-exploration-and-databases/qiagen-omicsoft-land-explorer/
- Coronavirus Network Explorer: https://digitalinsights.qiagen.com/coronavirus-network-explorer/

QIAGEN OmicSoft:

- Product Info: https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/qiagen-omicsoft/

QIAGEN CLC Genomics

- Product info: https://digitalinsights.qiagen.com/products-overview/analysis-and-visualization/qiagen-clc-genomics-workbench/

QIAGEN expands integrated coronavirus NGS and software solutions to accelerate COVID-19 research

- QIAseq SARS-CoV-2 Primer Panel converts viral RNA samples into libraries ready for sequencing
- QIAGEN Digital Insights solutions support COVID-19 drug, vaccine and epidemiology research
- For an overview of QIAGEN’s coronavirus testing solutions, please visit http://www.qiagen.com/coronavirus.
- To explore QIAGEN’s NGS-specific solutions for COVID-19 research, please visit https://go.qiagen.com/CoronavirusNGS
- For details of QIAGEN’s SARS-CoV-2 Whole Genome Sequencing Service, please visit https://www.qiagen.com/applications/genomic-services/sars-cov-2-whole-genome-sequencing-services

Customer support and additional resources

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