Science Advances
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Supplementary Materials for

Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm

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Published 13 November 2020, *Sci. Adv.* **6**, eabe3024 (2020)
DOI: 10.1126/sciadv.abe3024

This PDF file includes:

Figs. S1 to S13

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/sciadv.abe3024/DC1)

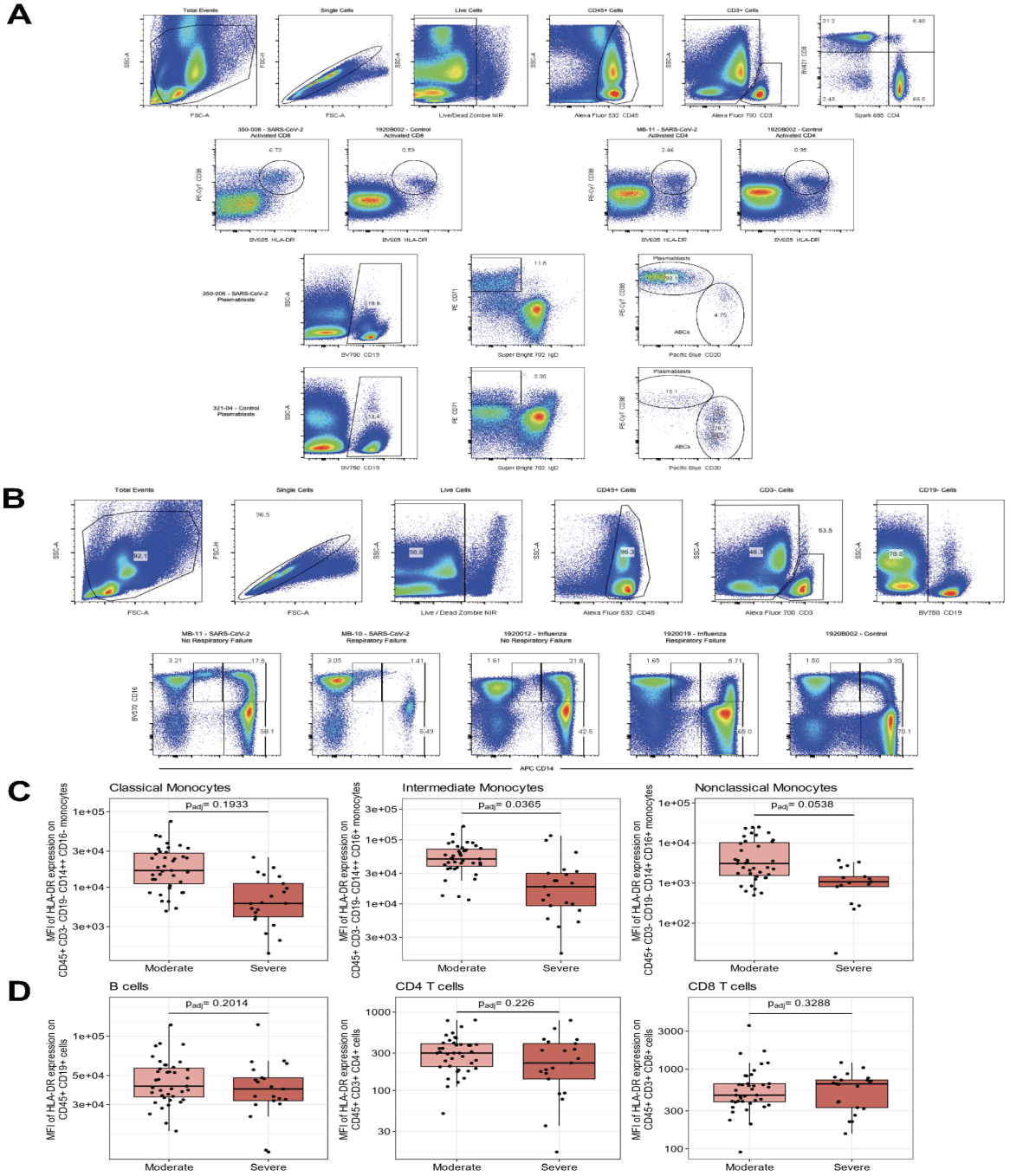
Tables S1 to S2

Supplementary Tables are available for download separately.

Supplementary Table 1. Flow cytometry, cytokine, and metadata for all study subjects. In the "Data" tab, columns A-Y contain metadata (e.g., age, ethnicity), Z-BH are cytokines levels (in pg/mL), and BI-CI contain flow cytometry results. Descriptions of metadata variables are located in the "Dictionary" tab.

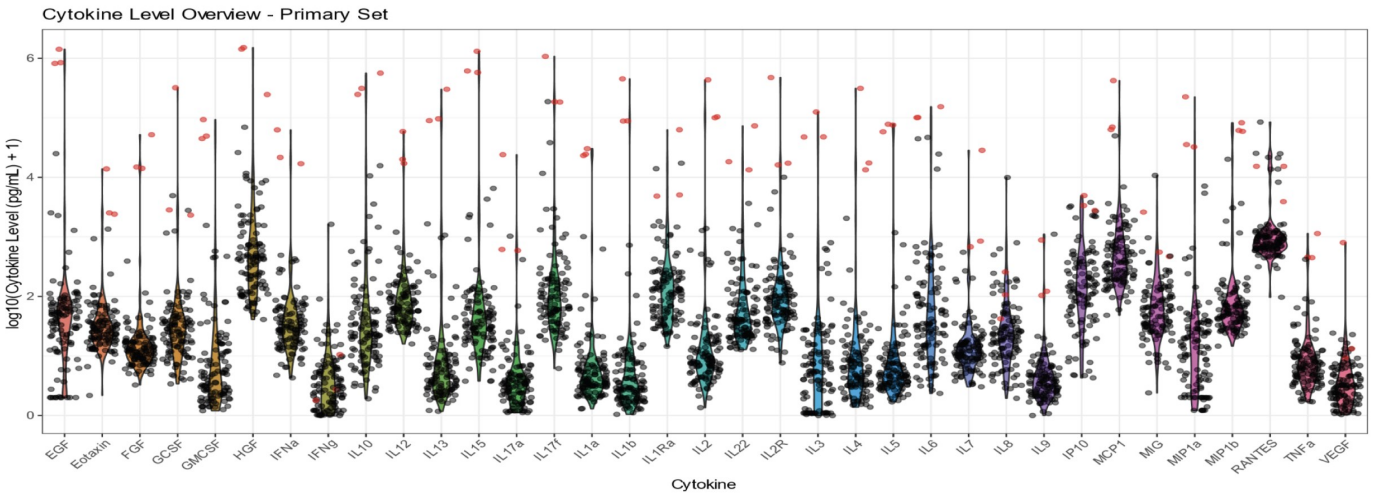
Supplementary Table 2. COVID-19 versus influenza differential gene expression results by major cell subsets. Differential gene expression analysis was conducted in Seurat, comparing cells from COVID-19-infected patients to those from influenza-infected patients for each major subset annotated in clustering analyses. A positive average log fold change (avg logFC) indicates an upregulation among COVID-19 relative to influenza. Pct.1 corresponds to the percentage of cells from COVID-19 subjects within a given subset expressing a given gene, whereas Pct.2 corresponds to the percentage of cells from influenza subjects within a given subset expressing a given gene. The statistical test utilized was "MAST", with no log fold change or minimum percent threshold. P-values were adjusted for multiple comparisons using Bonferroni correction.

Supplementary Figure 1:

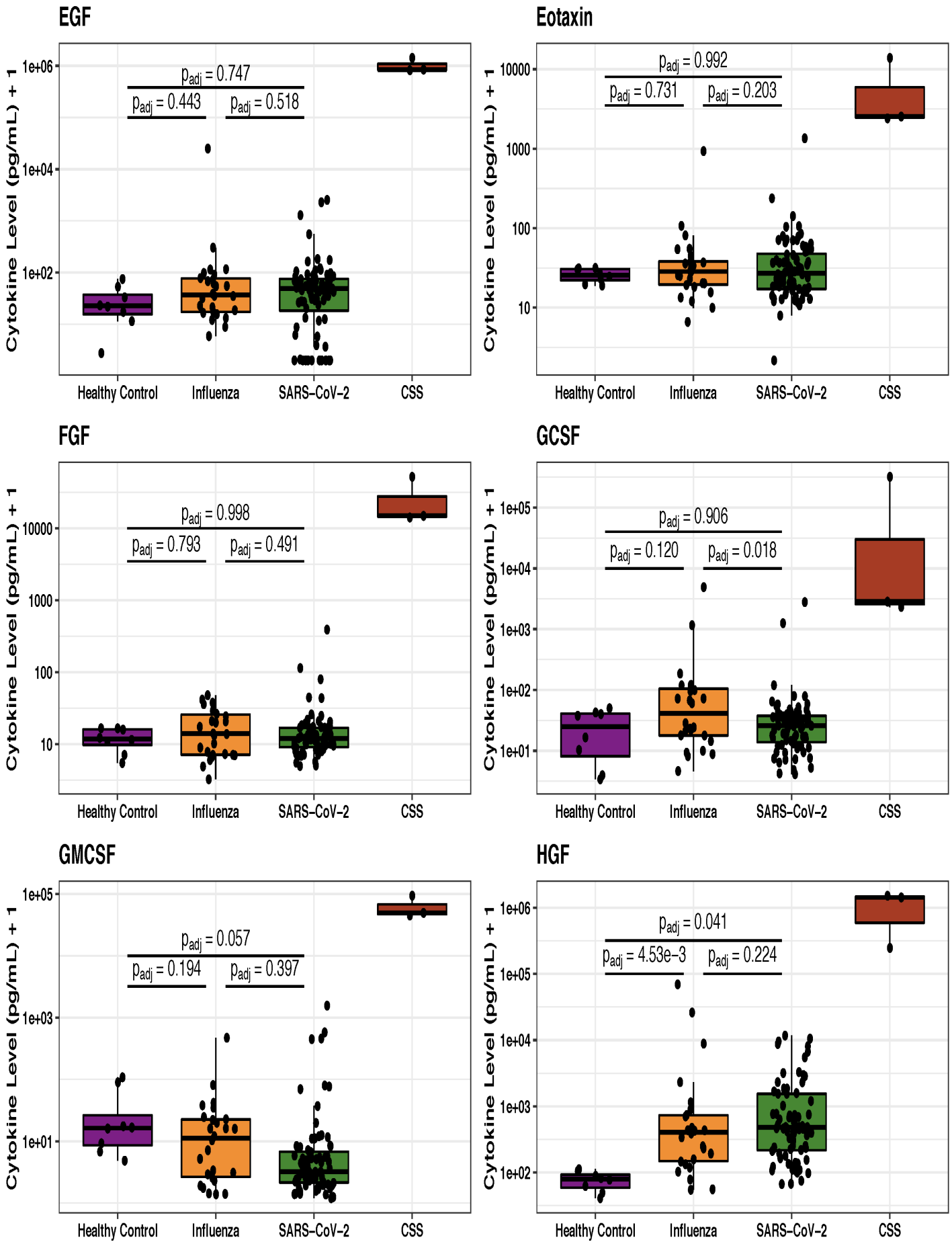


Supplementary Figure 1. Gating strategy for flow cytometry analyses. Gating strategy and representative plots for lymphocyte **(A)** and monocyte **(B)** subsets. Evaluation of HLA-DR abundance as a function of disease severity on monocyte **(C)** and lymphocyte **(D)** subpopulations in select SARS-CoV-2-infected subjects (N=22) as measured by geometric mean fluorescence intensity (MFI) using flow cytometry. Severe patients were those who required intubation and mechanical ventilation or who expired as a result of their illness, whereas moderately ill patients were all others who sought hospital care for their illness. Presented p-values are from pairwise comparisons of estimated marginal means of linear regression models that adjust for ethnicity, sex, age, days of symptom duration at study enrollment, and all comorbidities (immunocompromised, end stage renal disease, chronic lung disease, chronic heart failure, and diabetes mellitus). P-values were adjusted for multiple comparisons using Tukey's method.

Supplemental Figure 2.



Supplementary Figure 2. Overview of cytokine levels across all primary cohort subjects. Data points from the 3 COVID-19 patients with extremely high cytokine concentrations (deemed cytokine storm profiles) are shown in red.


EGF
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1e+06
1e+04
1e+02
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padj = 0.518
Healthy Control
Influenza
SARS-CoV-2
CSS
Eotaxin
Cytokine Level (pg/mL) + 1
10000
1000
100
10
padj = 0.992
padj = 0.731
padj = 0.203
Healthy Control
Influenza
SARS-CoV-2
CSS
FGF
Cytokine Level (pg/mL) + 1
10000
1000
100
10
padj = 0.998
padj = 0.793
padj = 0.491
Healthy Control
Influenza
SARS-CoV-2
CSS
GCSF
Cytokine Level (pg/mL) + 1
1e+05
1e+04
1e+03
1e+02
1e+01
padj = 0.906
padj = 0.120
padj = 0.018
Healthy Control
Influenza
SARS-CoV-2
CSS
GMCSF
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
padj = 0.057
padj = 0.194
padj = 0.397
Healthy Control
Influenza
SARS-CoV-2
CSS
HGF
Cytokine Level (pg/mL) + 1
1e+06
1e+05
1e+04
1e+03
1e+02
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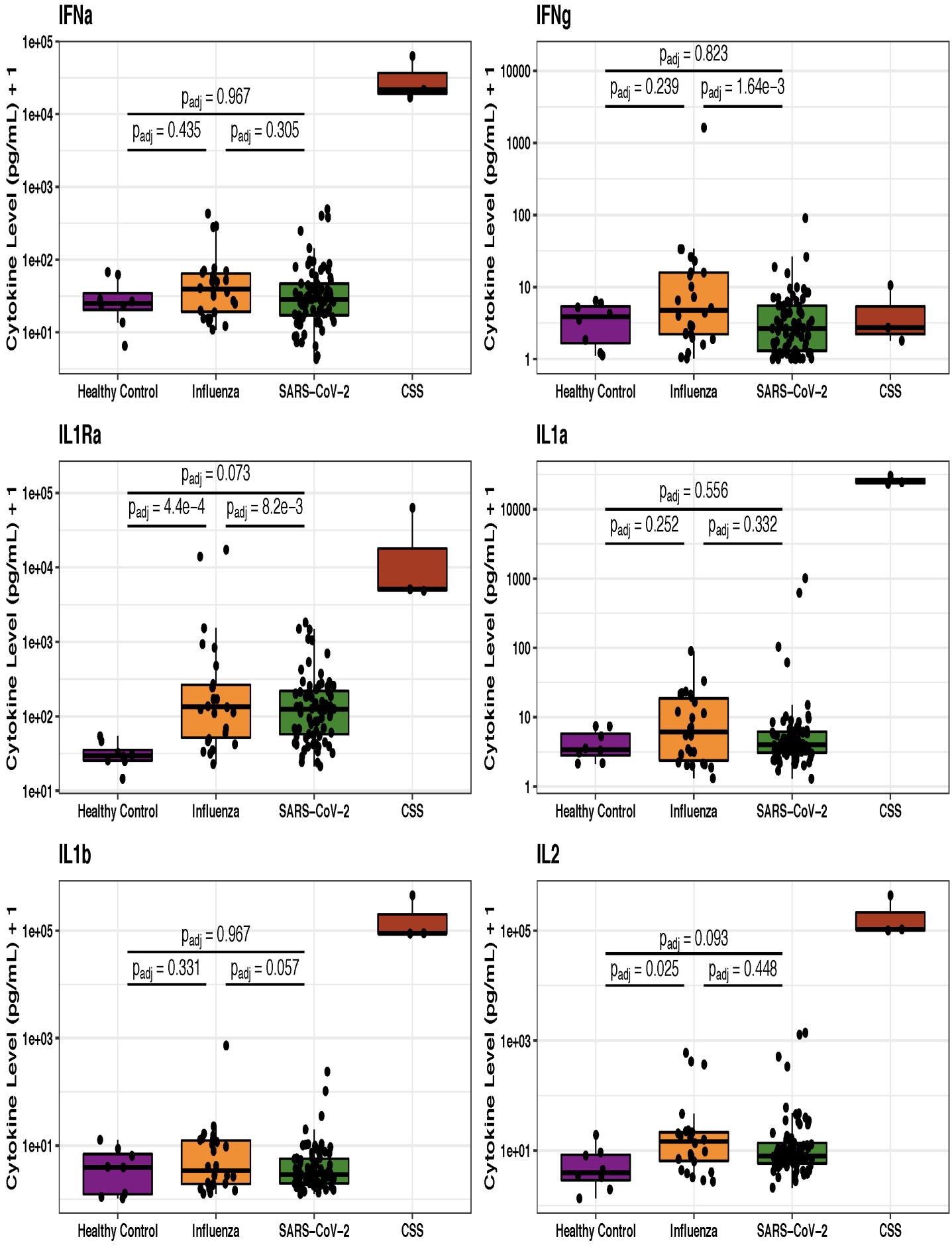
padj = 0.224
Healthy Control
Influenza
SARS-CoV-2
CSS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1e+03
1e+02
1e+01
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padj = 0.305
Healthy Control
Influenza
SARS-CoV-2
CSS
IFNg
Cytokine Level (pg/mL) + 1
10000
1000
100
10
1
padj = 0.823
padj = 0.239
padj = 1.64e-3
Healthy Control
Influenza
SARS-CoV-2
CSS
IL1Ra
Cytokine Level (pg/mL) + 1
1e+05
1e+04
1e+03
1e+02
1e+01
padj = 0.073
padj = 4.4e-4
padj = 8.2e-3
Healthy Control
Influenza
SARS-CoV-2
CSS
IL1a
Cytokine Level (pg/mL) + 1
10000
1000
100
10
1
padj = 0.556
padj = 0.252
padj = 0.332
Healthy Control
Influenza
SARS-CoV-2
CSS
IL1b
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
padj = 0.967
padj = 0.331
padj = 0.057
Healthy Control
Influenza
SARS-CoV-2
CSS
IL2
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
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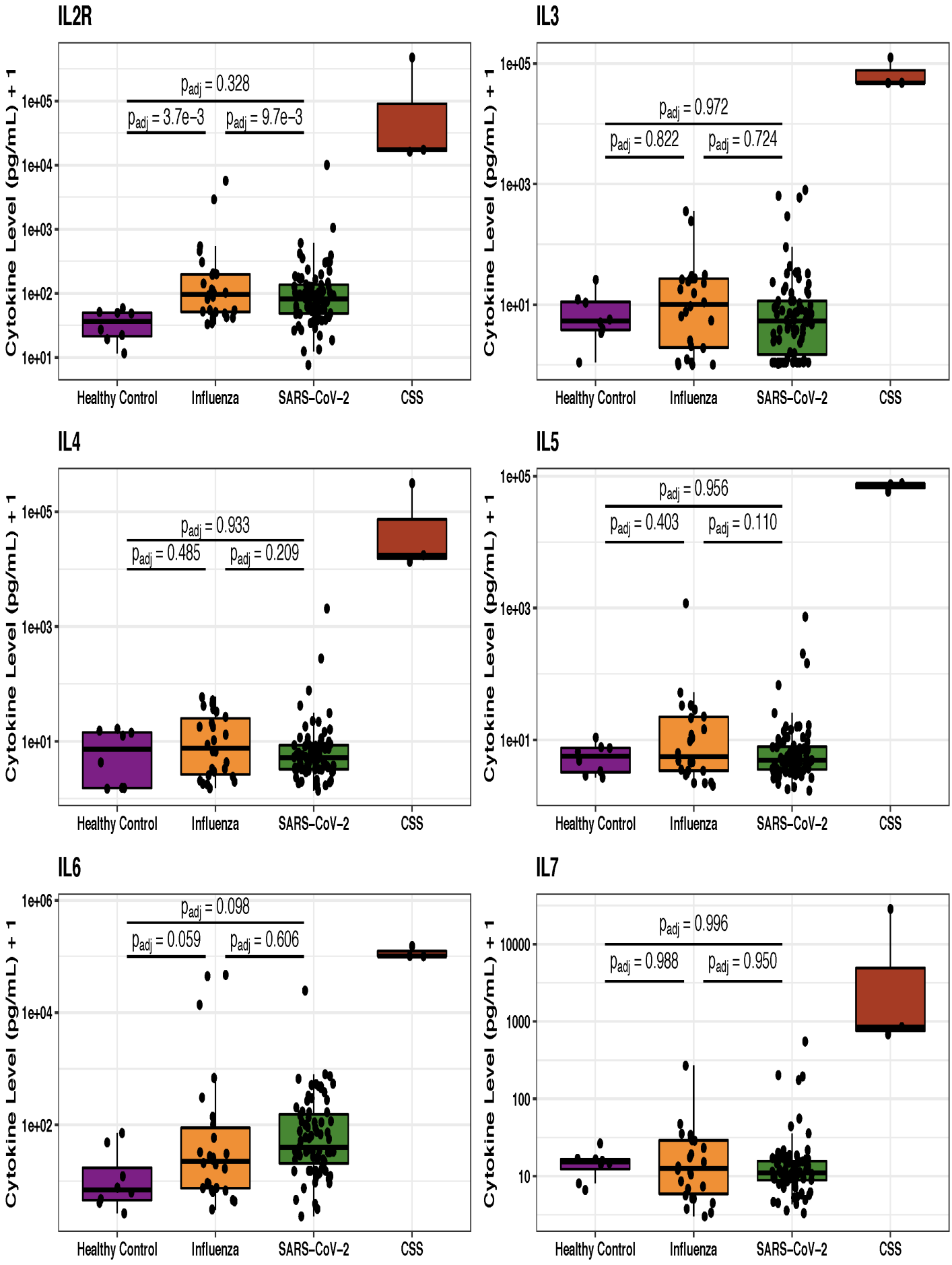
Healthy Control
Influenza
SARS-CoV-2
CSS


IL2R
Cytokine Level (pg/mL) + 1
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1e+04
1e+03
1e+02
1e+01
padj = 0.328
padj = 3.7e−3
padj = 9.7e−3
Healthy Control
Influenza
SARS−CoV−2
CSS
IL3
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
padj = 0.972
padj = 0.822
padj = 0.724
Healthy Control
Influenza
SARS−CoV−2
CSS
IL4
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
padj = 0.933
padj = 0.485
padj = 0.209
Healthy Control
Influenza
SARS−CoV−2
CSS
IL5
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
padj = 0.956
padj = 0.403
padj = 0.110
Healthy Control
Influenza
SARS−CoV−2
CSS
IL6
Cytokine Level (pg/mL) + 1
1e+06
1e+04
1e+02
padj = 0.098
padj = 0.059
padj = 0.606
Healthy Control
Influenza
SARS−CoV−2
CSS
IL7
Cytokine Level (pg/mL) + 1
10000
1000
100
10
padj = 0.996
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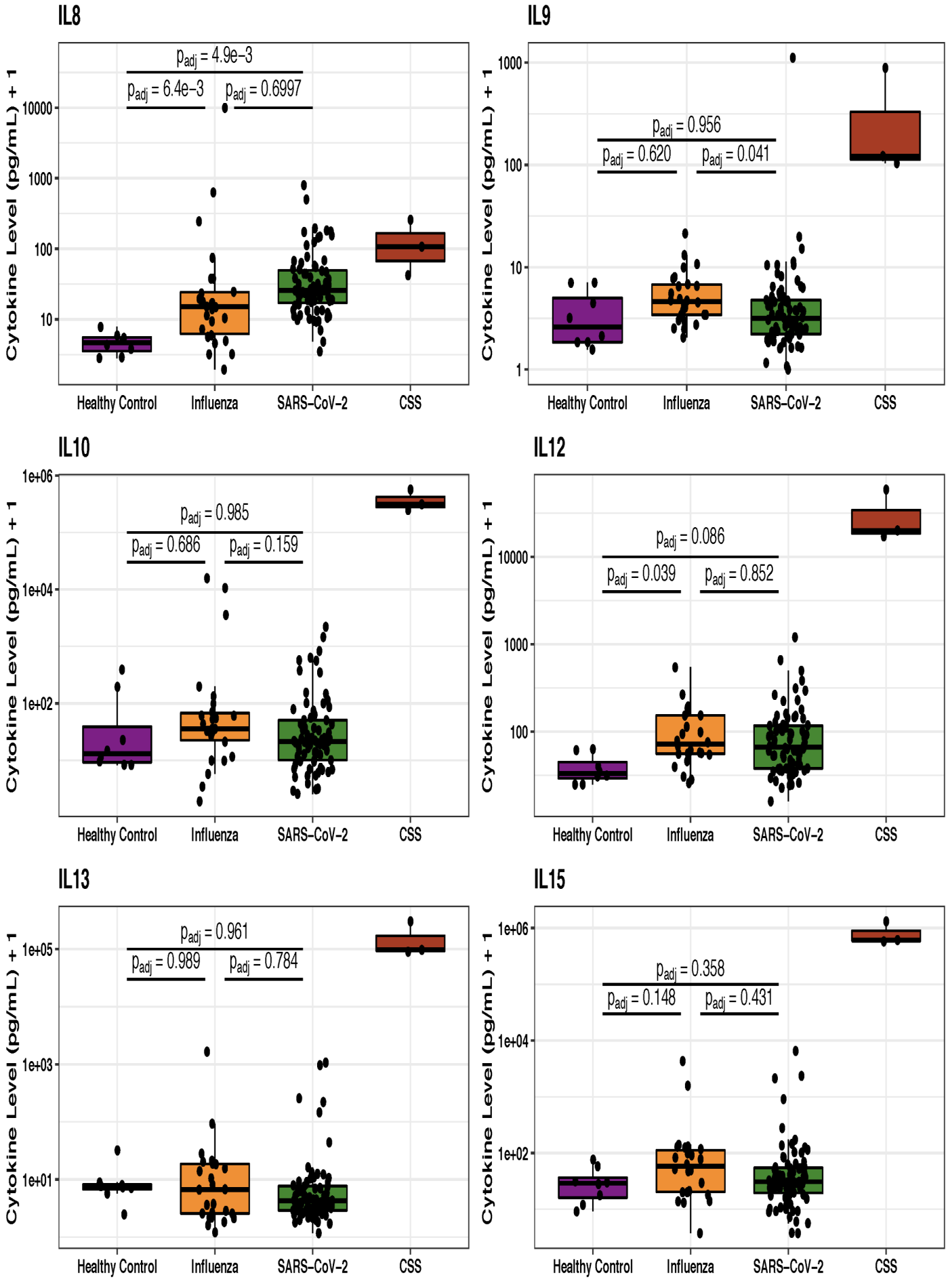
padj = 0.950
Healthy Control
Influenza
SARS−CoV−2
CSS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padj = 0.6997
IL9
padj = 0.956
padj = 0.620
padj = 0.041
IL10
padj = 0.985
padj = 0.686
padj = 0.159
IL12
padj = 0.086
padj = 0.039
padj = 0.852
IL13
padj = 0.961
padj = 0.989
padj = 0.784
IL15
padj = 0.358
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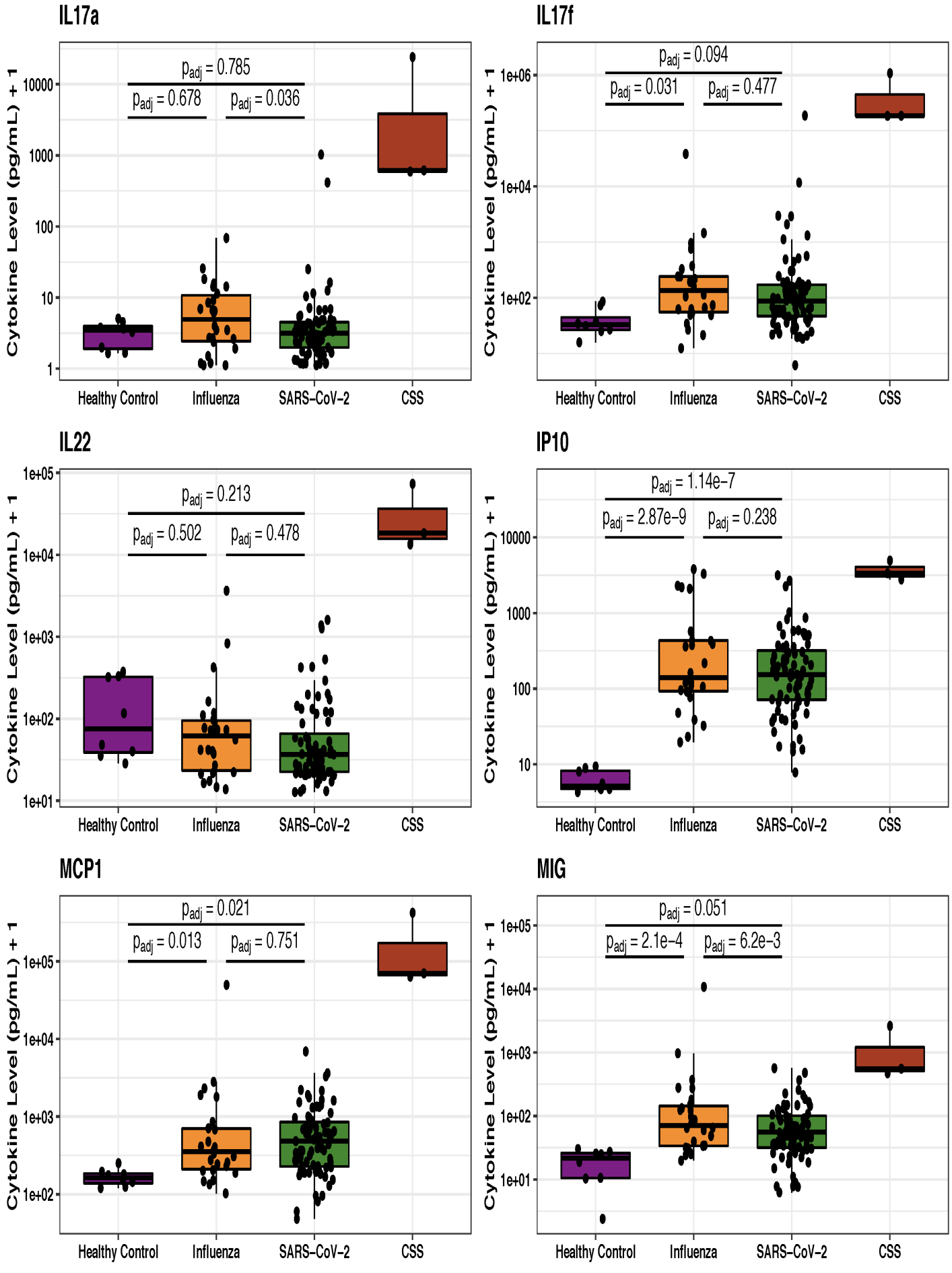
Cytokine Level (pg/mL) + 1
Healthy Control
Influenza
SARS−CoV−2
CSS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Cytokine Level (pg/mL) + 1
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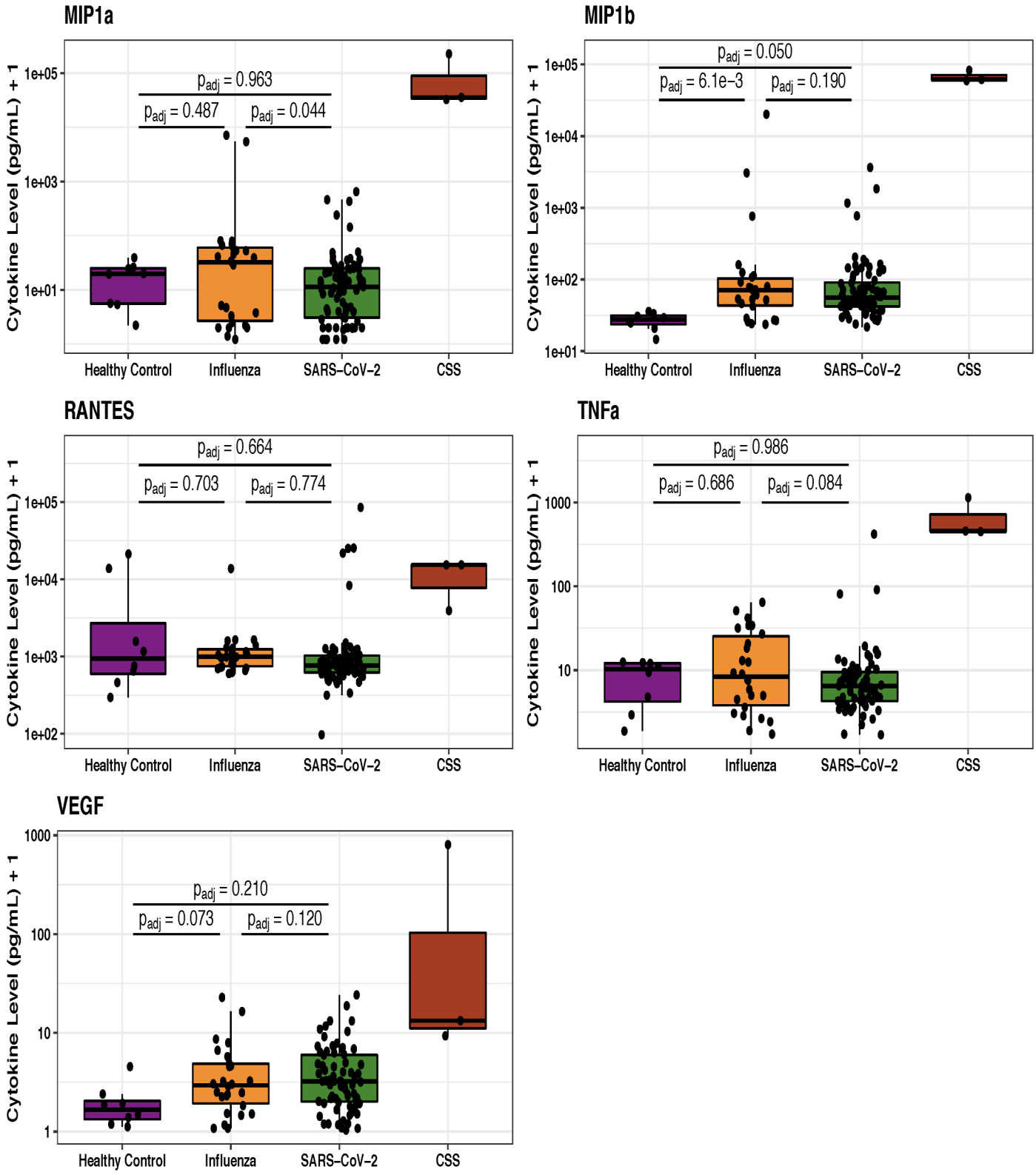
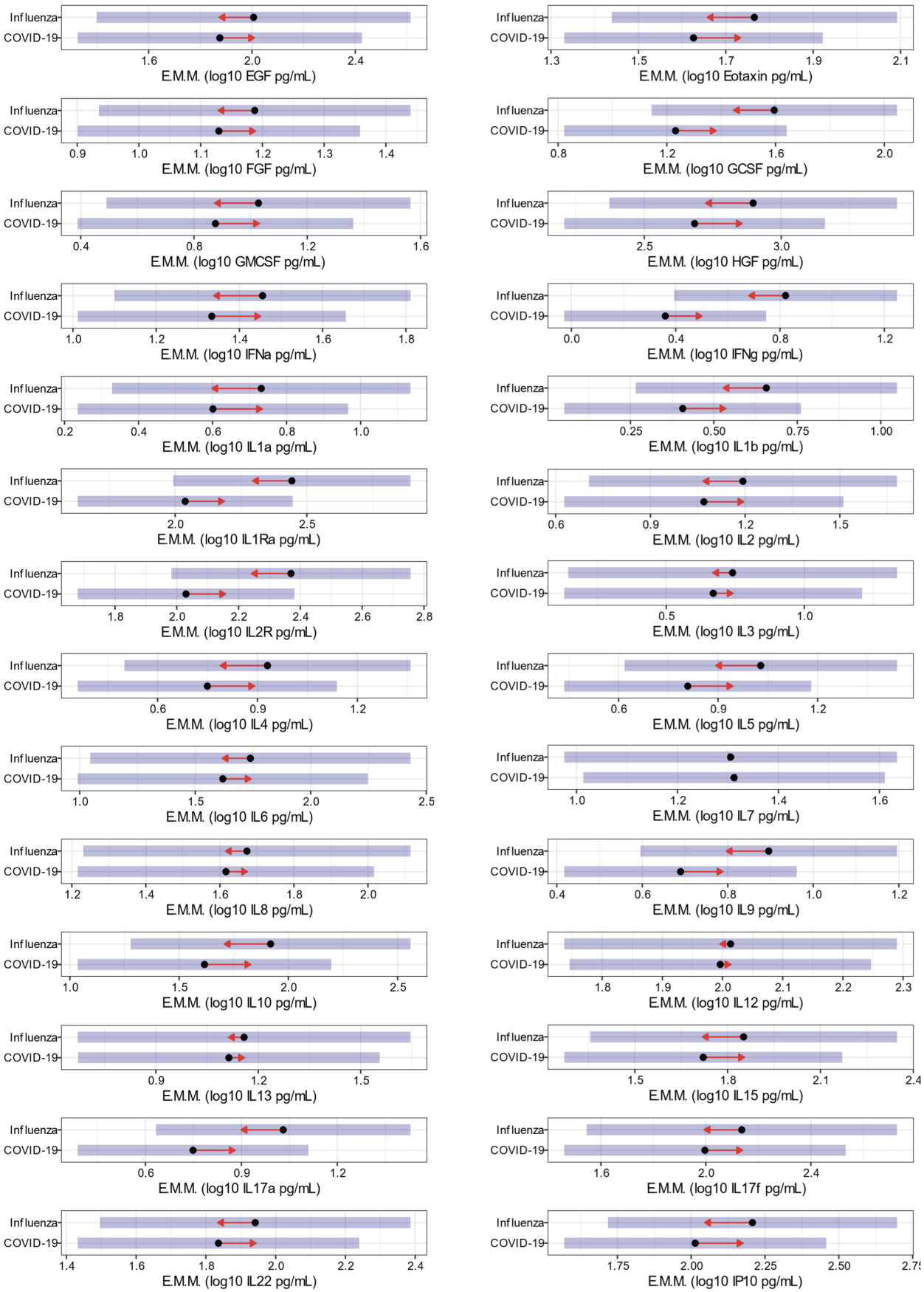
padj = 0.678
padj = 0.036
Healthy Control
Influenza
SARS-CoV-2
CSS
IL17f
padj = 0.094
padj = 0.031
padj = 0.477
IL22
padj = 0.213
padj = 0.502
padj = 0.478
IP10
padj = 1.14e-7
padj = 2.87e-9
padj = 0.238
MCP1
padj = 0.021
padj = 0.013
padj = 0.751
MIG
padj = 0.051
padj = 2.1e-4
padj = 6.2e-3

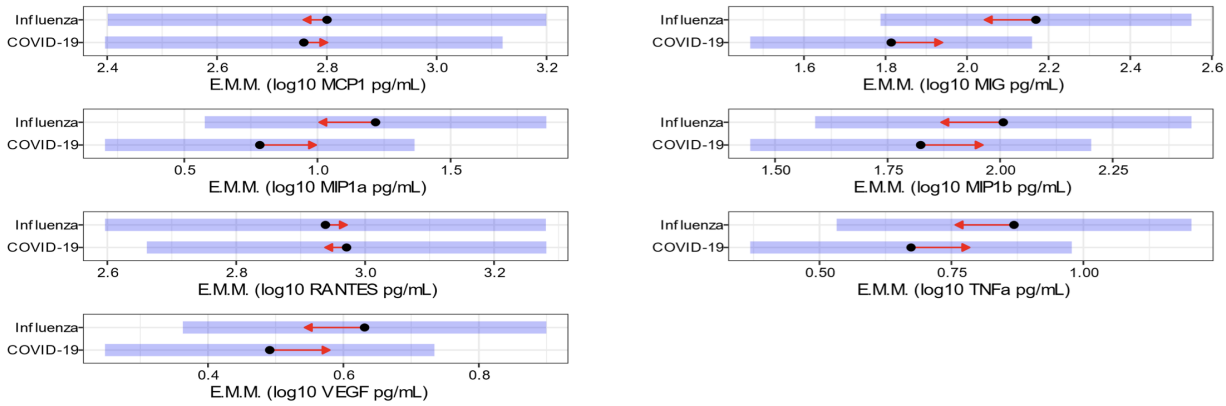


Supplementary Figure 3. Box plots show cytokine concentrations in healthy, influenza, COVID-19, and CSS (cytokine storm syndrome) subjects for all 35 cytokines measured, with raw values plotted on the log10 scale. Presented p-values are from estimated marginal means (EMM) comparisons, averaging over all demographic and clinical factors that were included as covariates and p-values adjusted for multiple comparisons.

Supplementary Figure 4: Primary Flu-Covid EMM Plots

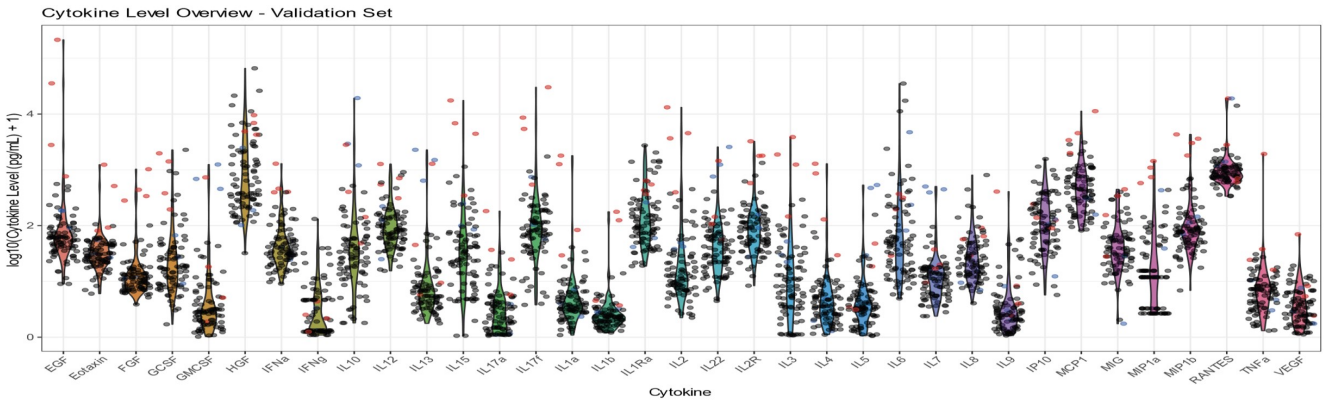


Supplementary Figure 4 part 2

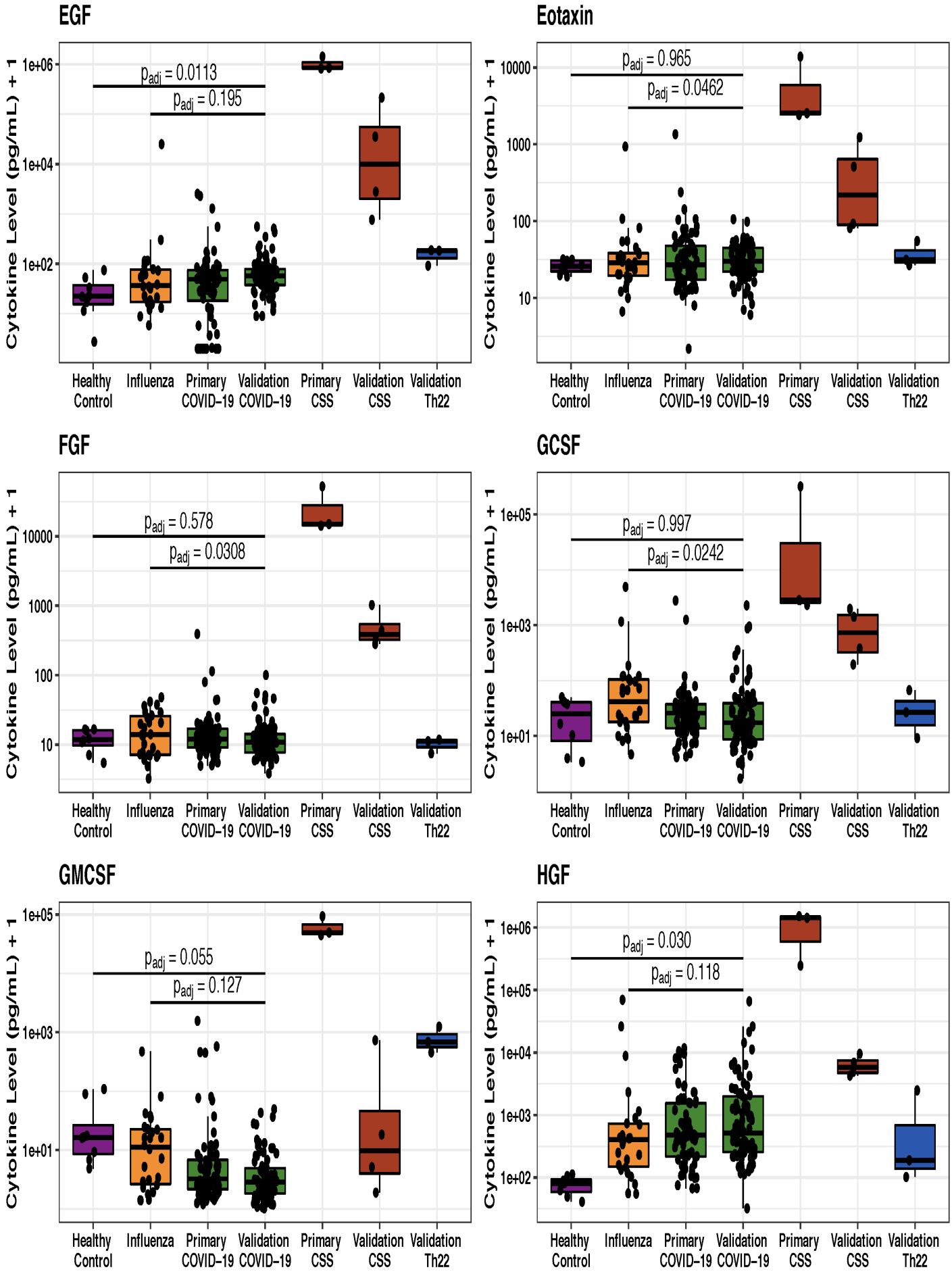


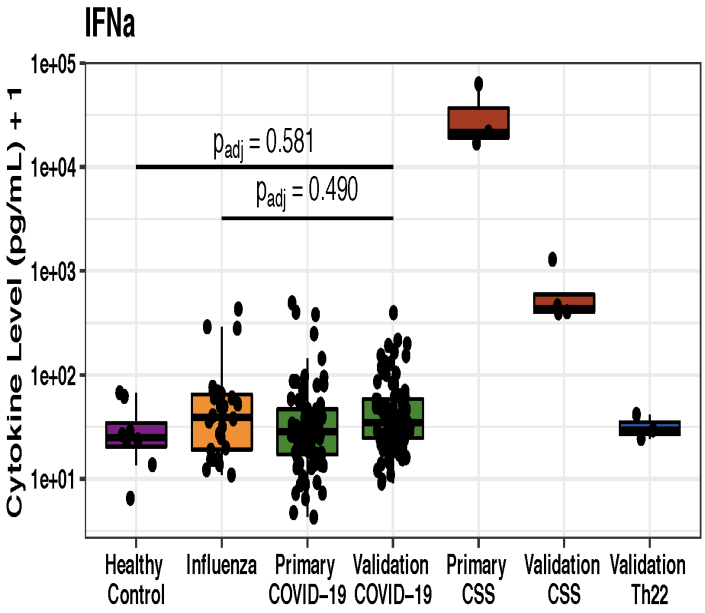
Supplementary Figure 4. Estimated marginal means (EMM) plots comparing cytokine expression between influenza and primary COVID-19 patients. The black dot represents the estimated marginal mean for the log10 concentration of the cytokine for a given condition, averaged over the levels of all other covariates (e.g. age, sex, ethnicity), and the blue shading represents the corresponding 95% confidence interval. The red arrows represent the standard error (SE) in one direction. Overlapping SE arrows indicate no significant difference between the EMM of a given cytokine in influenza subjects versus COVID-19 subjects.

Supplemental Figure 5.



Supplementary Figure 5. Overview of cytokine levels across all validation cohort subjects (all of which were positive for COVID-19). Data points from the 4 patients with CSS (cytokine storm syndrome) profiles are shown in red, and data points from the 3 patients with Th22 cytokine profiles are shown in blue.


EGF
Eotaxin
FGF
GCSF
GMCSF
HGF
Cytokine Level (pg/mL) + 1
padj = 0.0113
padj = 0.195
padj = 0.965
padj = 0.0462
padj = 0.578
padj = 0.0308
padj = 0.997
padj = 0.0242
padj = 0.055
padj = 0.127
padj = 0.030
padj = 0.118
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22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Cytokine Level (pg/mL) + 1
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1e+04
1e+03
1e+02
1e+01
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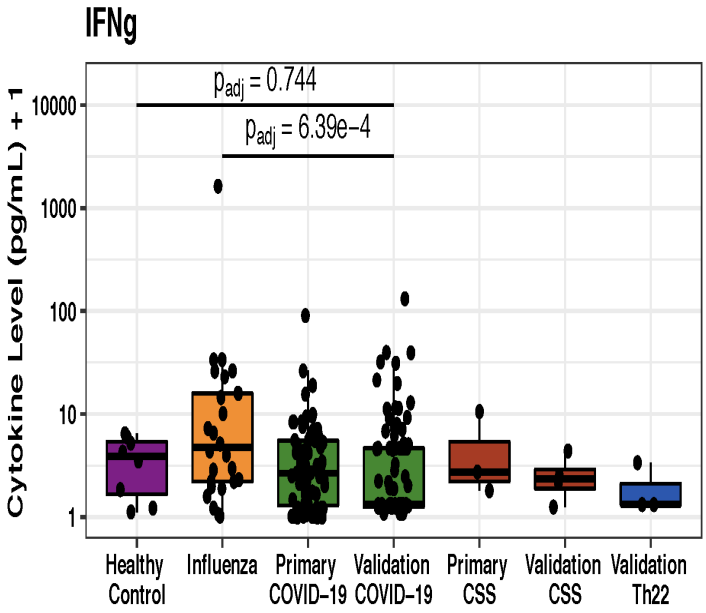
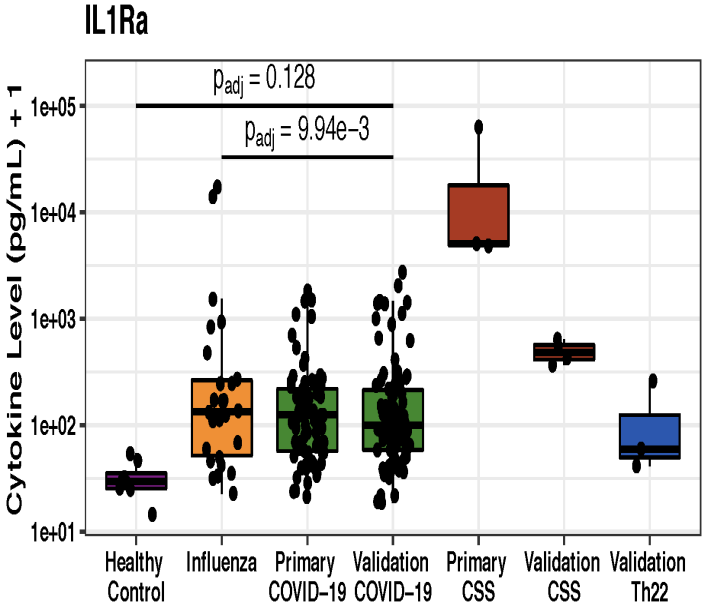
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22


IFNg
Cytokine Level (pg/mL) + 1
10000
1000
100
10
1
padj = 0.744
padj = 6.39e−4
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22


IL1Ra
Cytokine Level (pg/mL) + 1
1e+05
1e+04
1e+03
1e+02
1e+01
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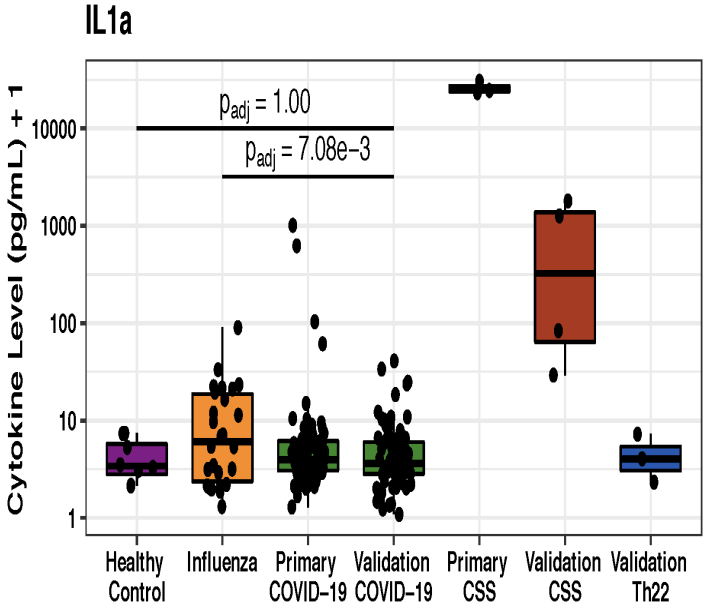
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22


IL1a
Cytokine Level (pg/mL) + 1
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1000
100
10
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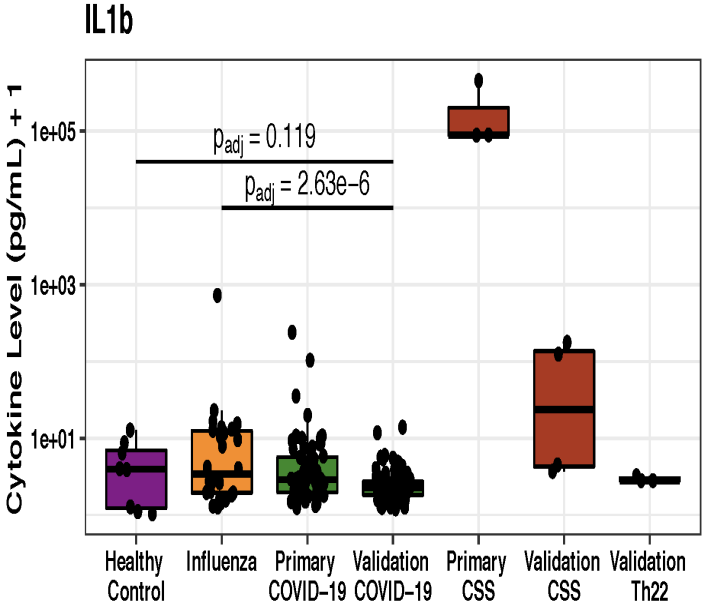
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22


IL1b
Cytokine Level (pg/mL) + 1
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1e+03
1e+01
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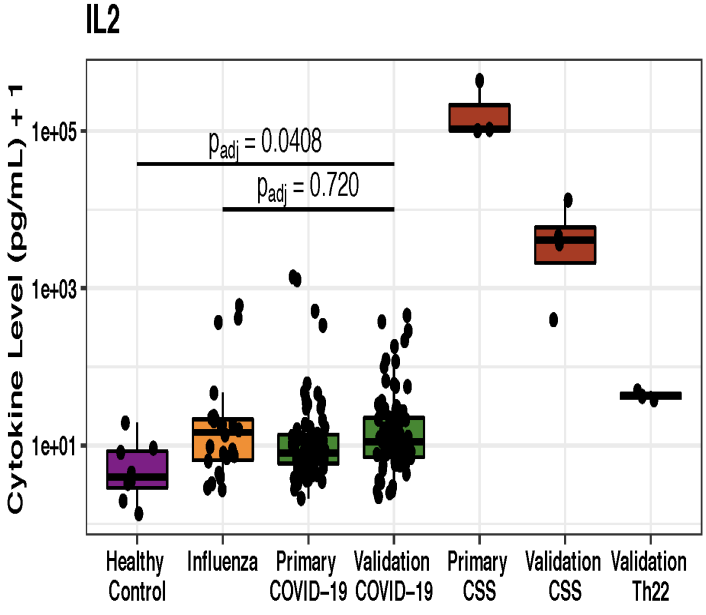
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22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1e+03
1e+01
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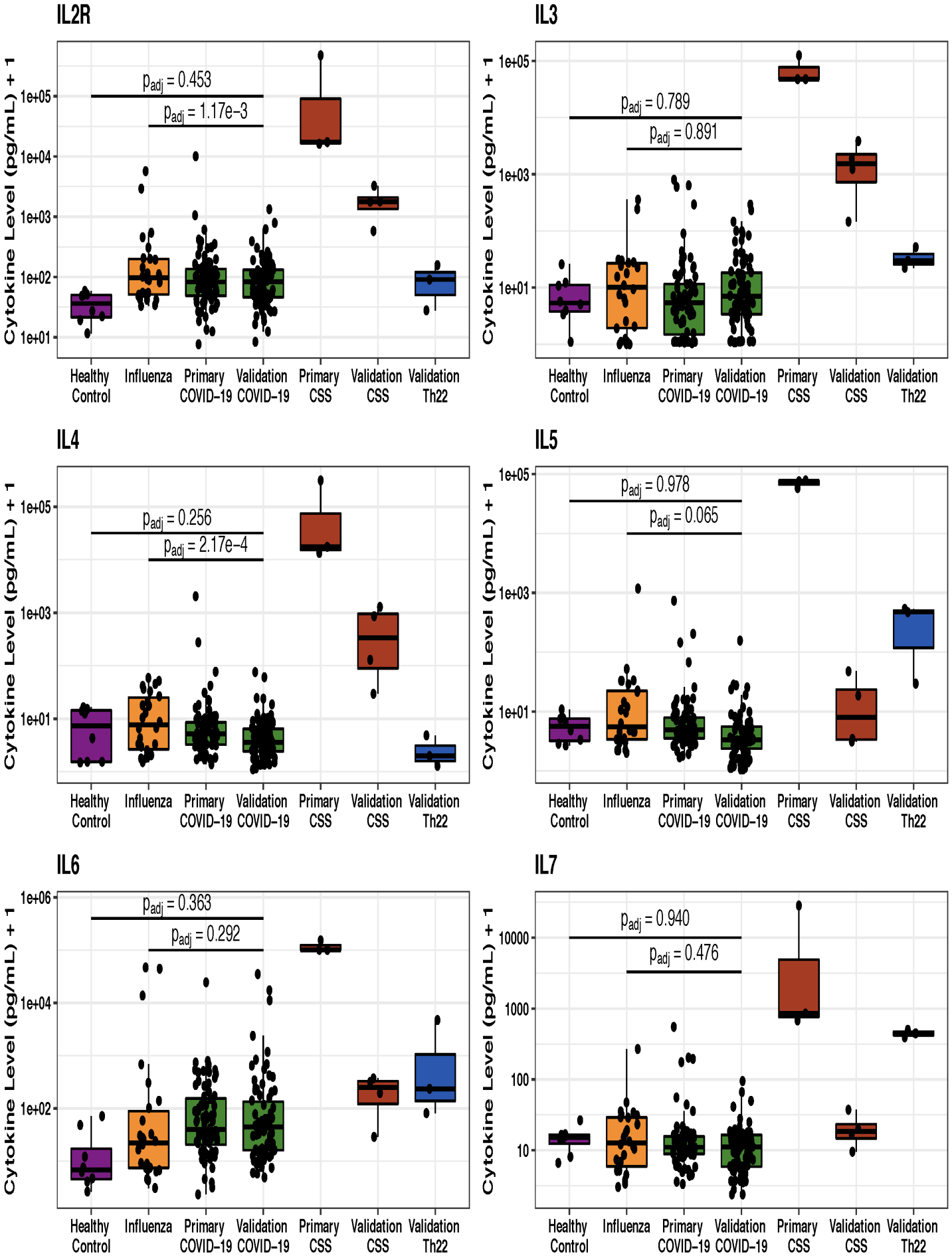
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22


IL2R
Cytokine Level (pg/mL) + 1
1e+05
1e+04
1e+03
1e+02
1e+01
p_{adj} = 0.453
p_{adj} = 1.17e−3
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22
IL3
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
p_{adj} = 0.789
p_{adj} = 0.891
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22
IL4
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
p_{adj} = 0.256
p_{adj} = 2.17e−4
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22
IL5
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
p_{adj} = 0.978
p_{adj} = 0.065
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22
IL6
Cytokine Level (pg/mL) + 1
1e+06
1e+04
1e+02
p_{adj} = 0.363
p_{adj} = 0.292
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22
IL7
Cytokine Level (pg/mL) + 1
10000
1000
100
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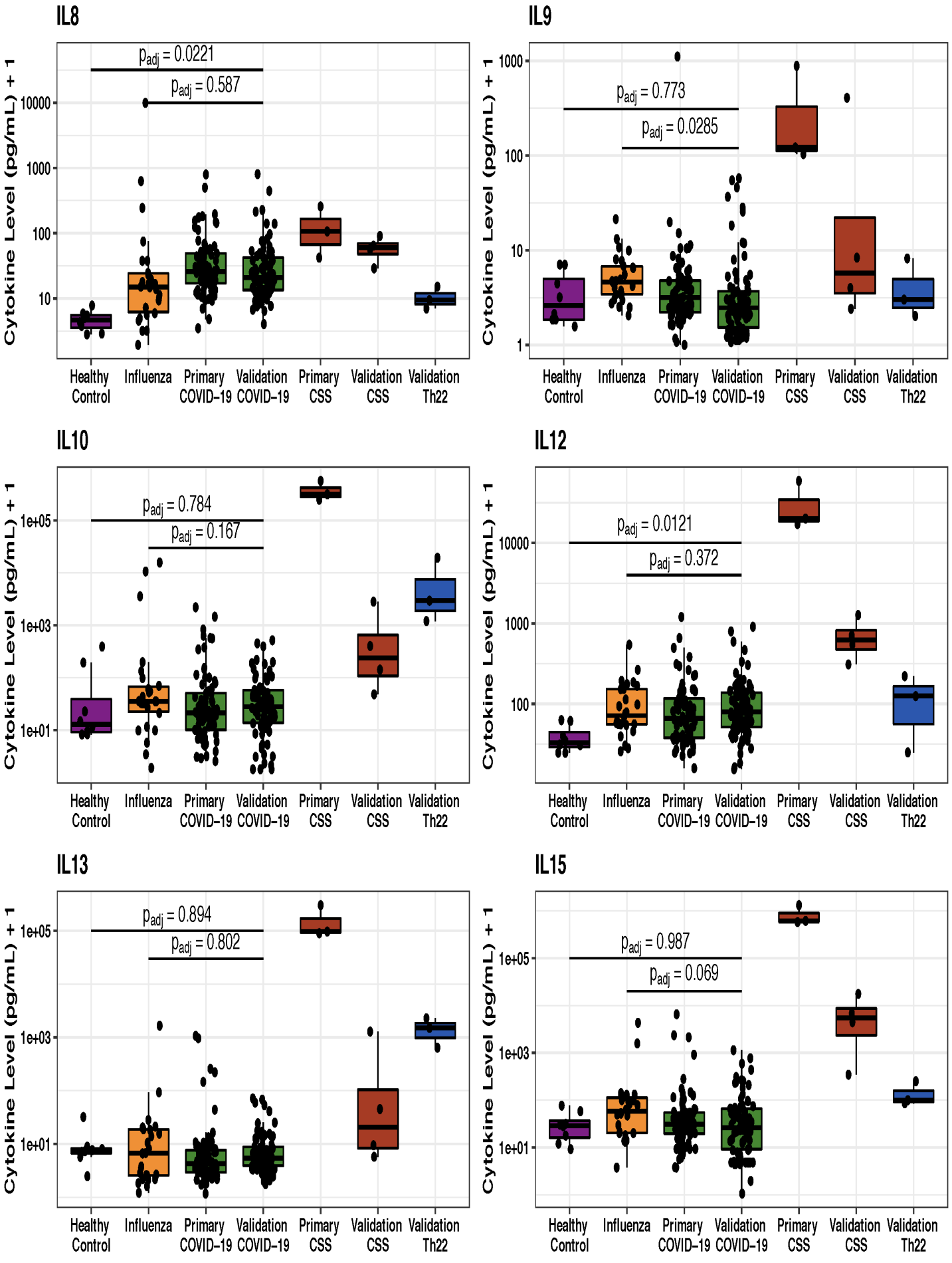
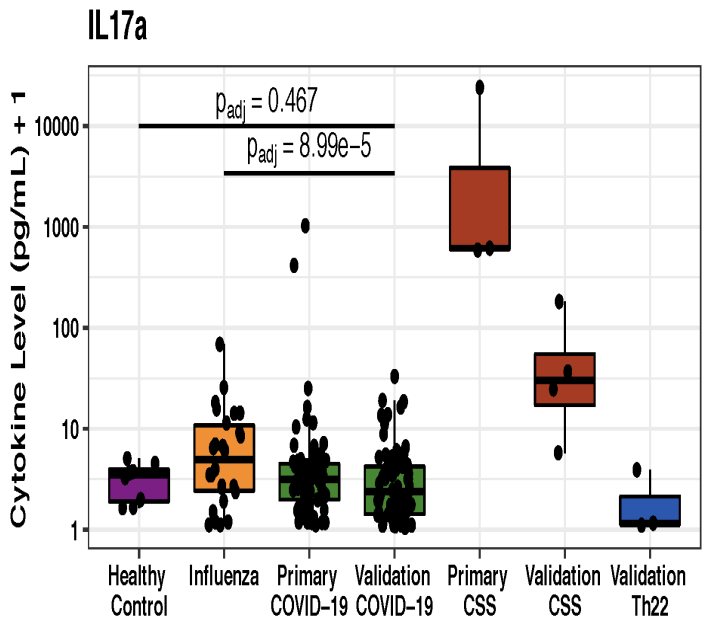
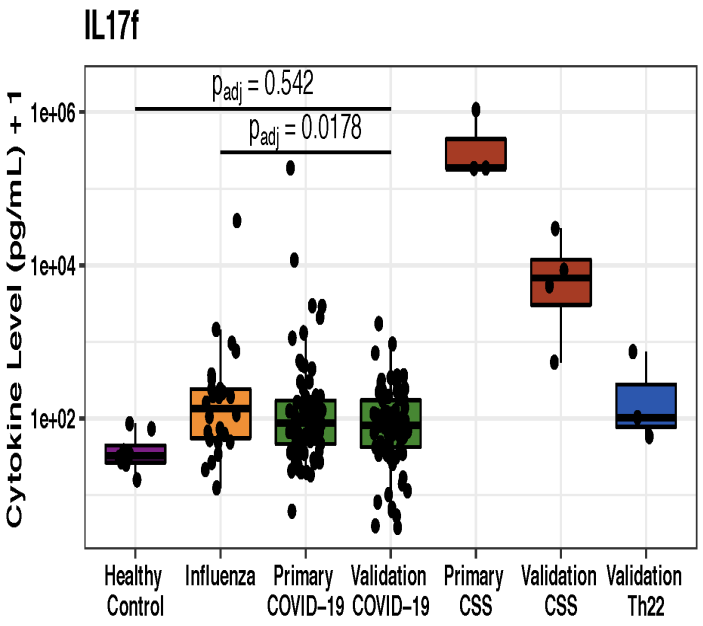
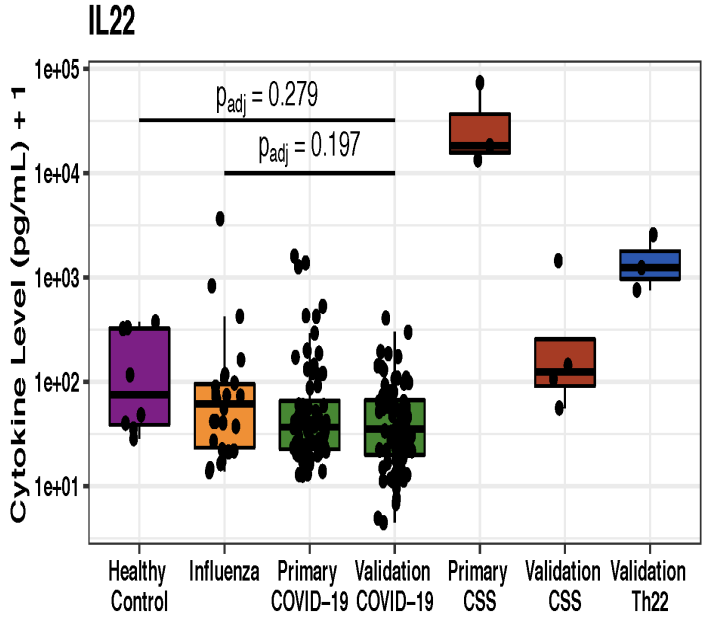
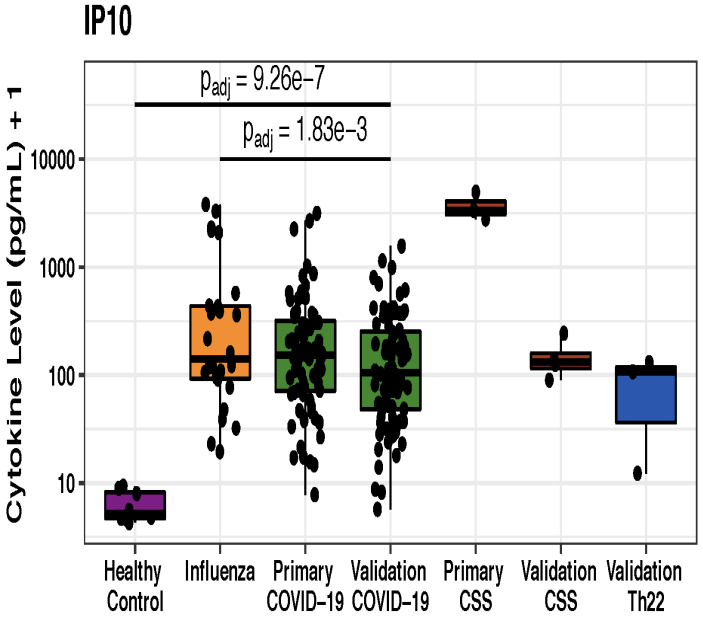
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22


IL8
IL9
IL10
IL12
IL13
IL15
Cytokine Level (pg/mL) + 1
padj = 0.0221
padj = 0.587
padj = 0.773
padj = 0.0285
padj = 0.784
padj = 0.167
padj = 0.0121
padj = 0.372
padj = 0.894
padj = 0.802
padj = 0.987
padj = 0.069
Healthy Control
Influenza
Primary COVID-19
Validation COVID-19
Primary CSS
Validation CSS
Validation Th22


IL17a
p_{adj} = 0.467
p_{adj} = 8.99e−5
Cytokine Level (pg/mL) + 1
10000
1000
100
10
1
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22


IL17f
p_{adj} = 0.542
p_{adj} = 0.0178
Cytokine Level (pg/mL) + 1
1e+06
1e+04
1e+02
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22


IL22
p_{adj} = 0.279
p_{adj} = 0.197
Cytokine Level (pg/mL) + 1
1e+05
1e+04
1e+03
1e+02
1e+01
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22


IP10
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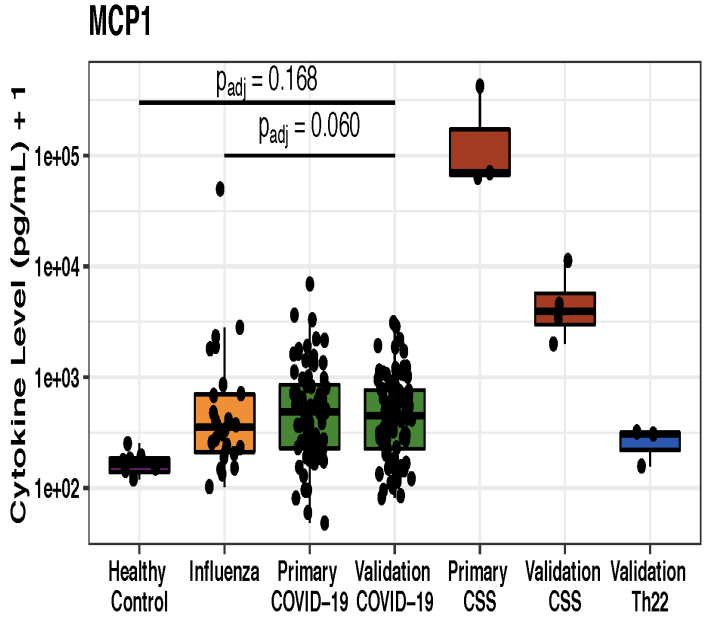
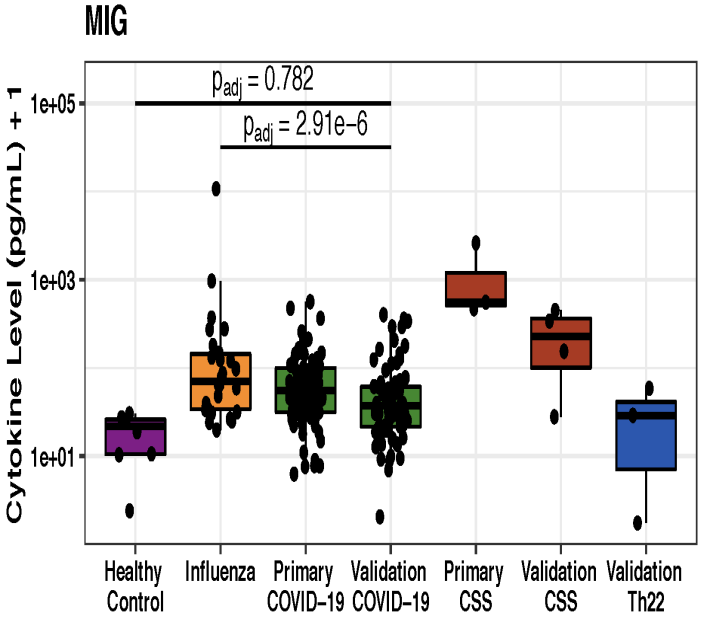
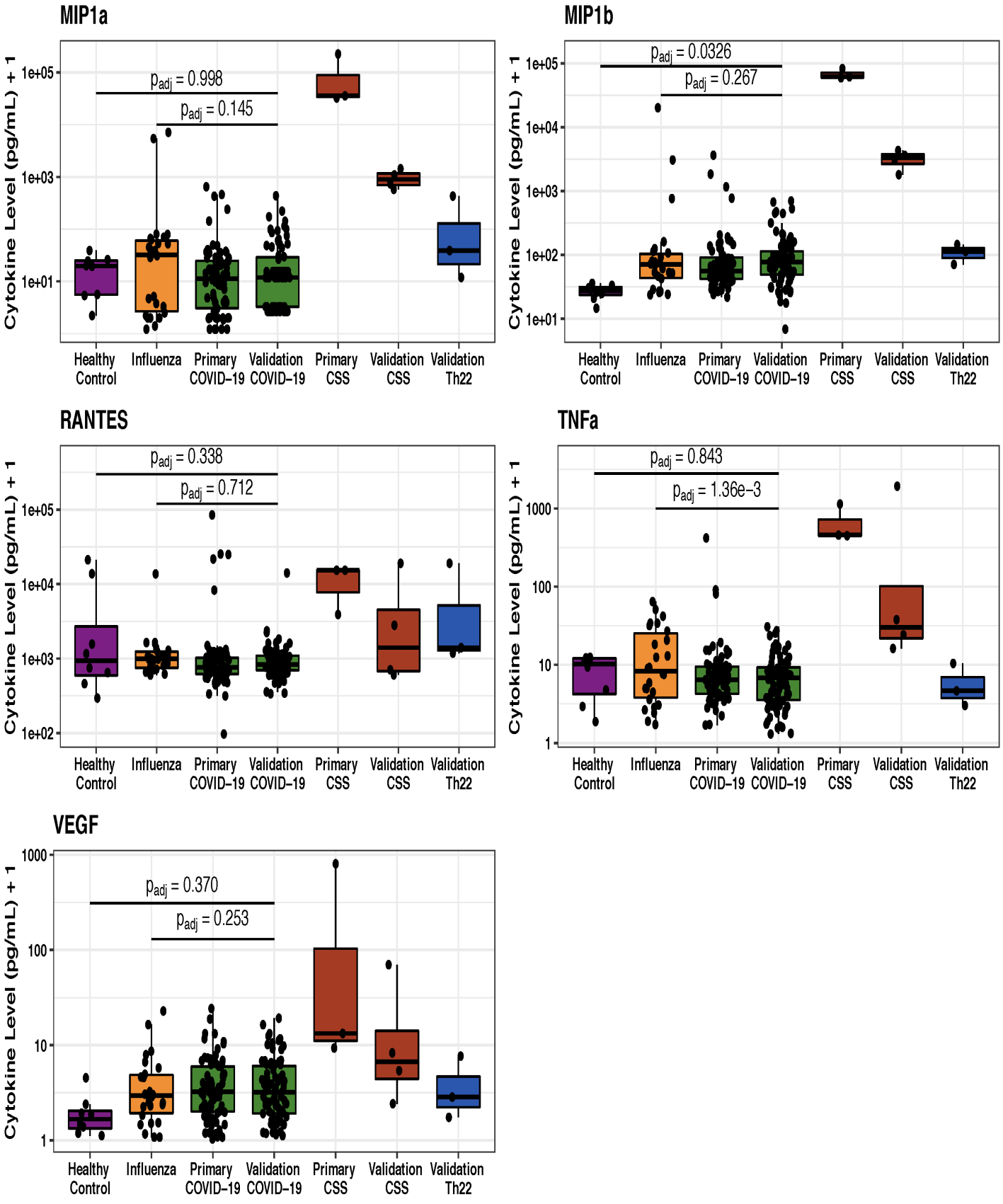
p_{adj} = 1.83e−3
Cytokine Level (pg/mL) + 1
10000
1000
100
10
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22

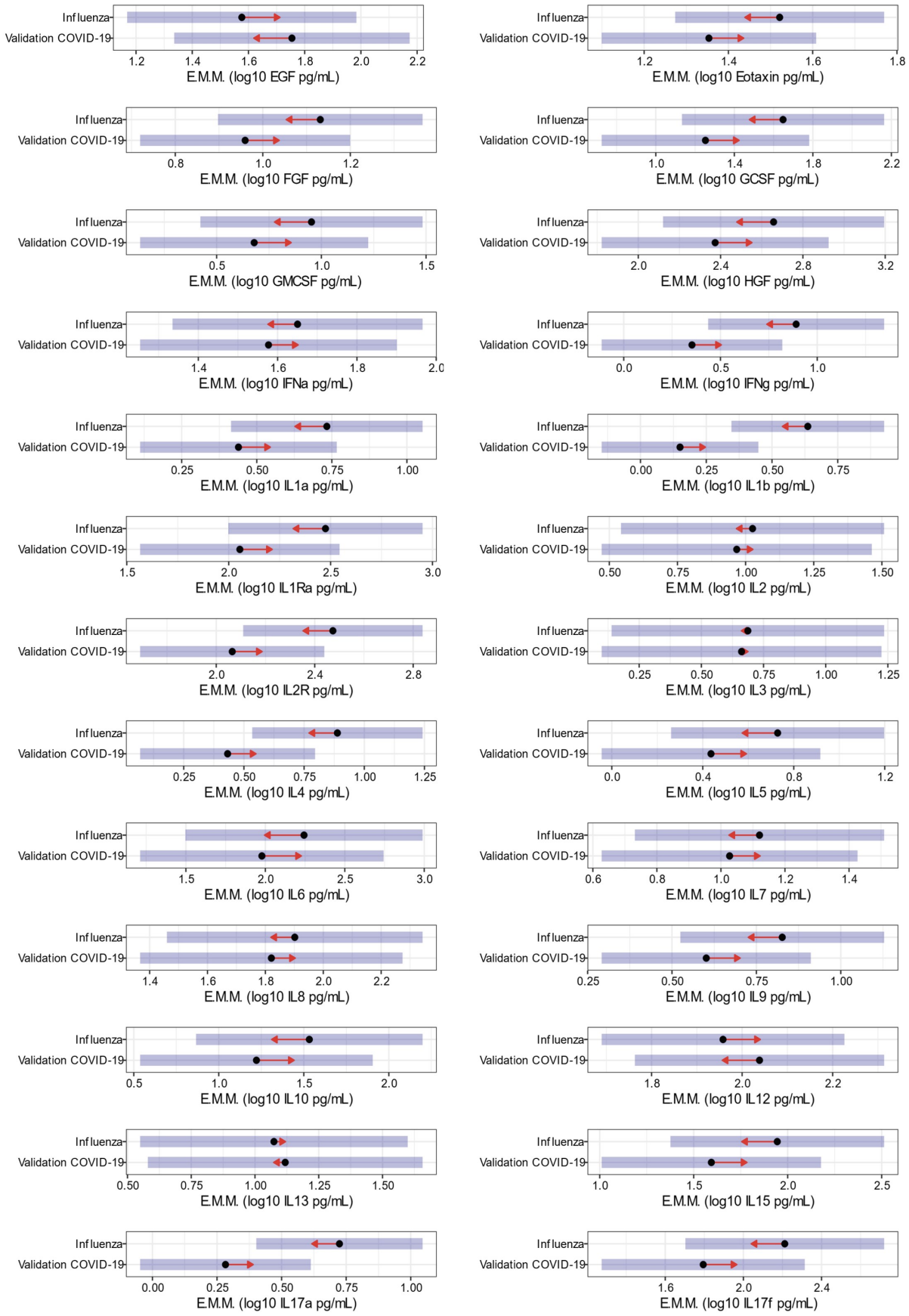

MCP1
p_{adj} = 0.168
p_{adj} = 0.060
Cytokine Level (pg/mL) + 1
1e+05
1e+04
1e+03
1e+02
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22


MIG
p_{adj} = 0.782
p_{adj} = 2.91e−6
Cytokine Level (pg/mL) + 1
1e+05
1e+03
1e+01
Healthy Control
Influenza
Primary COVID−19
Validation COVID−19
Primary CSS
Validation CSS
Validation Th22

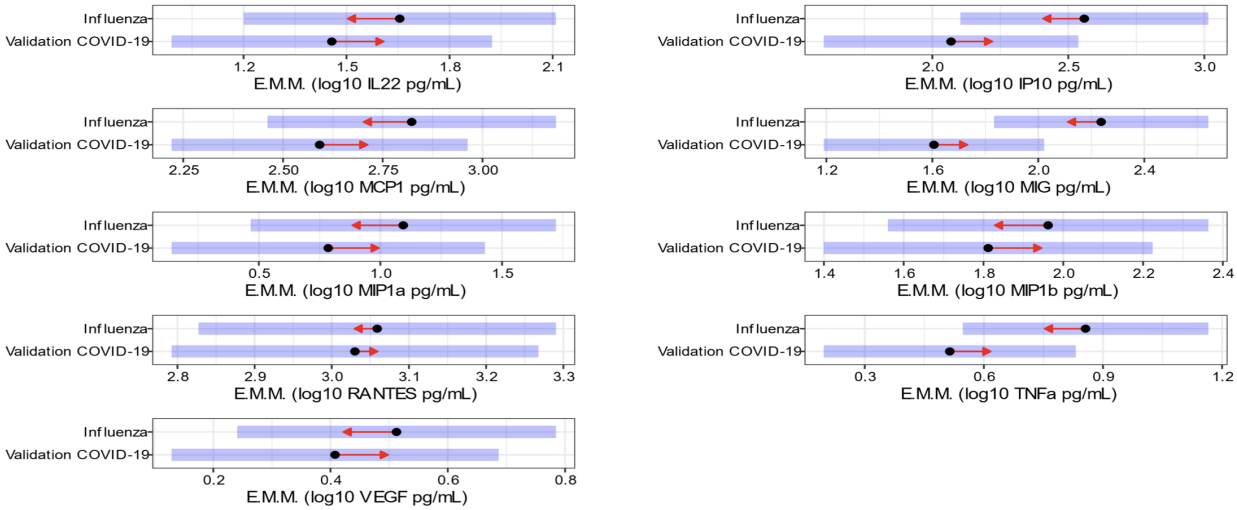


Supplementary Figure 6. Box plots show cytokine concentrations in healthy, influenza, primary COVID-19, validation COVID-19, primary CSS, validation CSS, and validation Th22 groups for all 35 cytokines measured, with raw values plotted on the log10 scale. Presented p-values are from estimated marginal means (EMM) comparisons, averaging over all demographic and clinical factors that were included as covariates and p-values adjusted for multiple comparisons. Although Th22 samples were visualized separately, they were included as validation COVID-19 samples in the underlying statistical analyses.

Sup Fig 7-EMM plots of Flu vs Validation

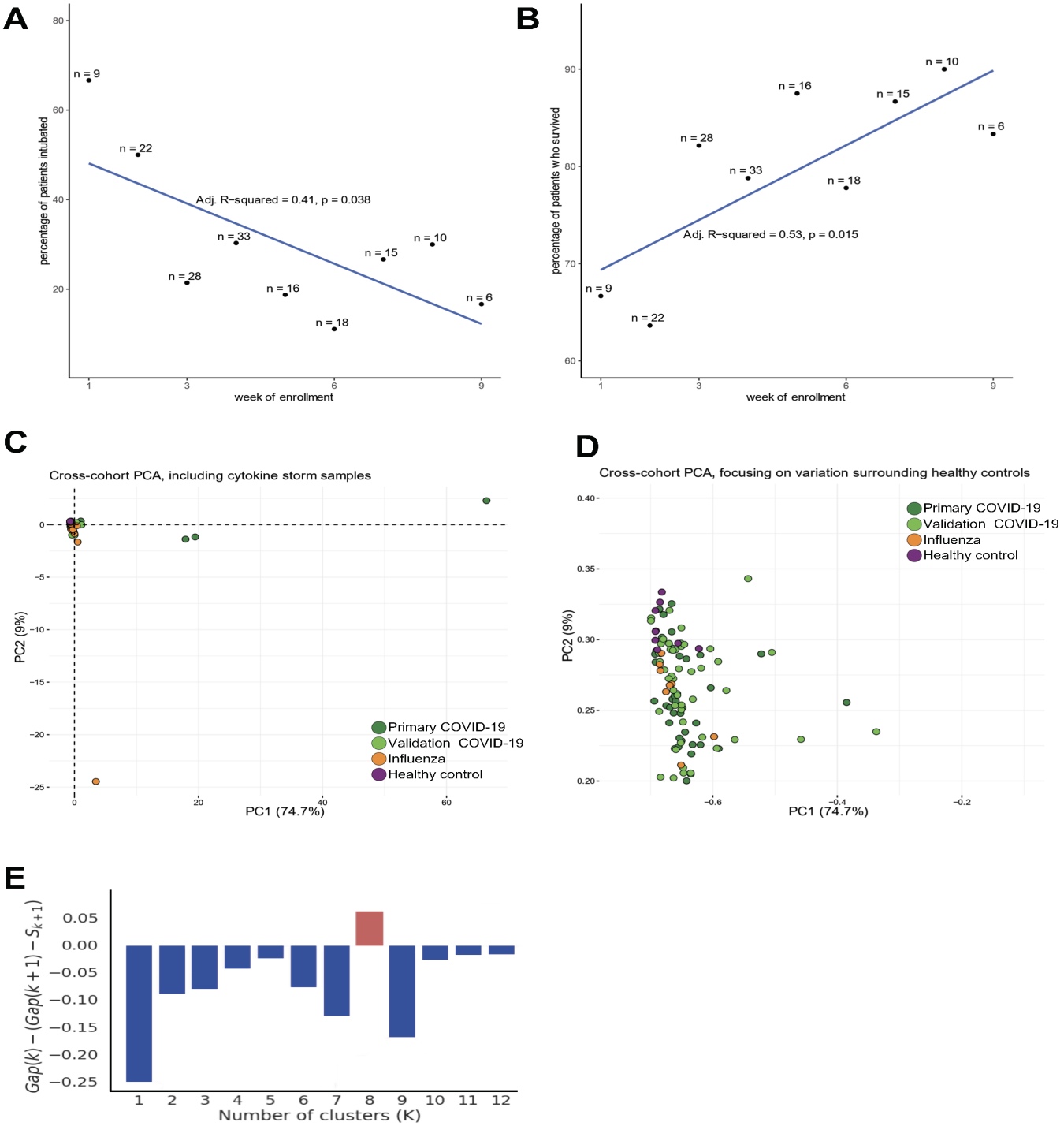


Sup Fig 7 part 2



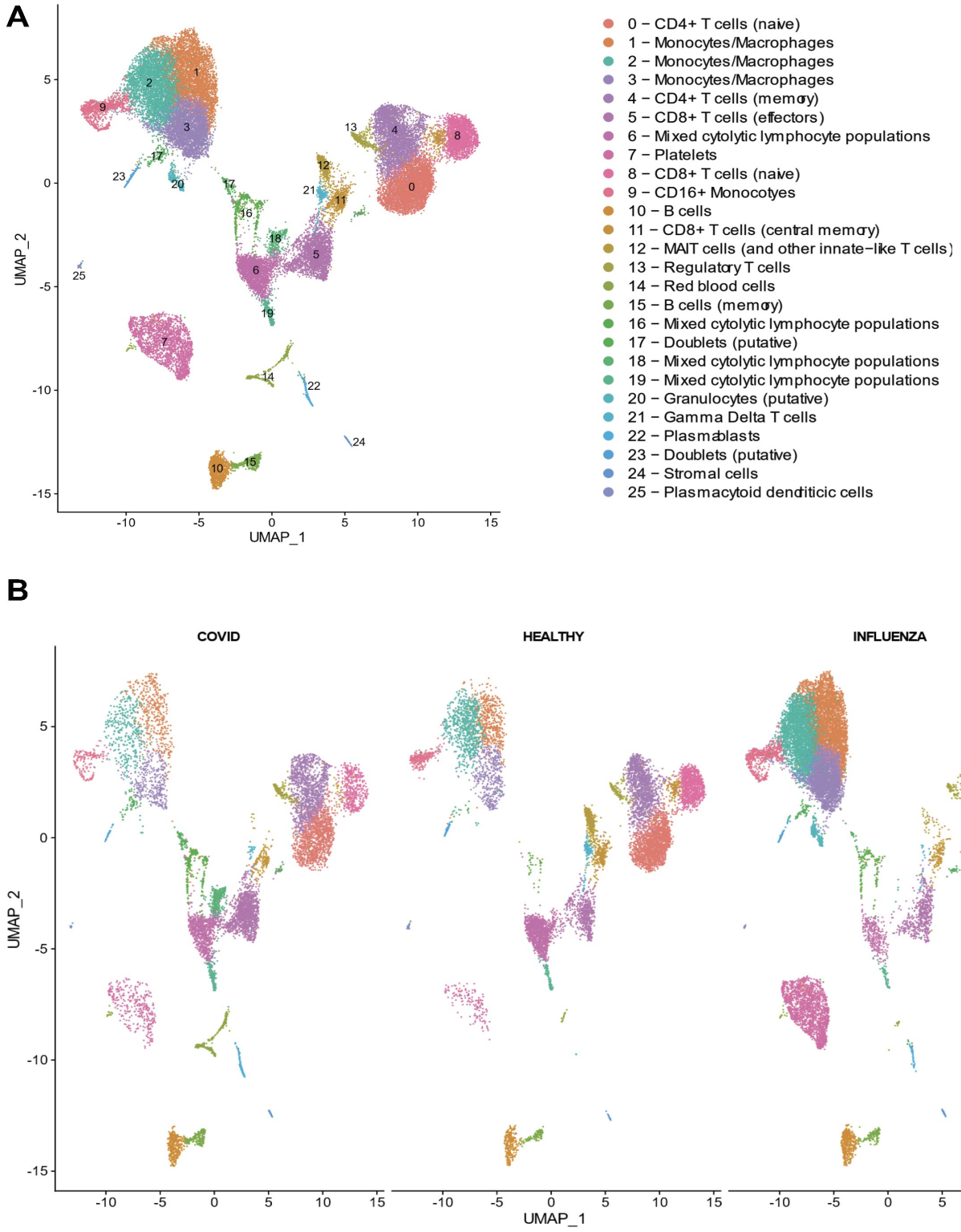
Supplementary Figure 7. Estimated marginal means (EMM) plots comparing cytokine expression between influenza and validation COVID-19 patients. The black dot represents the estimated marginal mean for the log10 concentration of the cytokine for a given condition, averaged over the levels of all other covariates (e.g. age, sex, ethnicity), and the blue shading represents the corresponding 95% confidence Interval. The red arrows represent the standard error (SE) in one direction. Overlapping SE arrows indicate no significant difference between the EMM of a given cytokine in influenza subjects versus COVID-19 subjects.

Supplemental Figure 8.

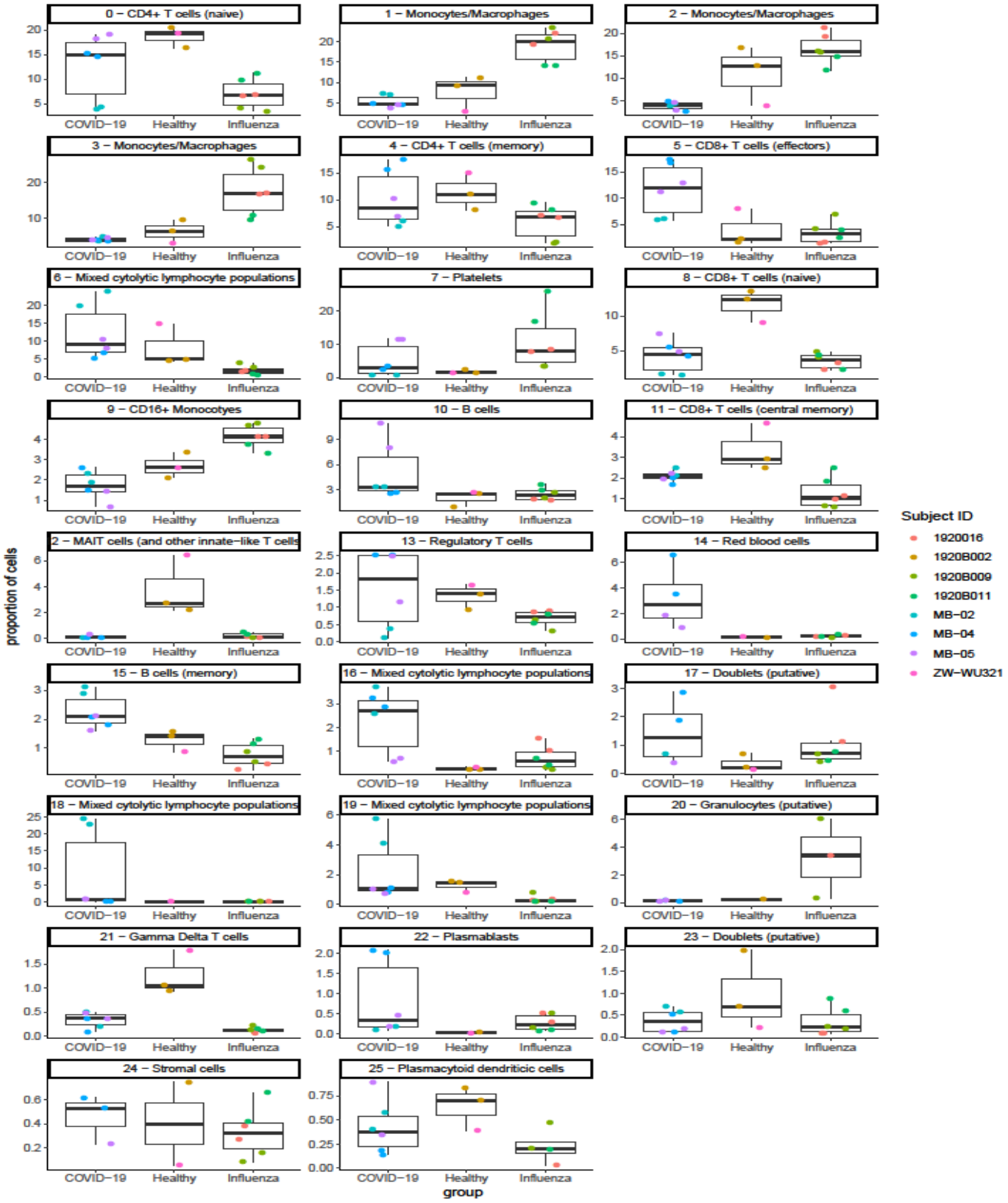


Supplementary Figure 8. Additional cross-cohort comparisons and supporting figures. **(A)** Linear regression of intubation rates binned by week of enrollment shows a significant negative correlation between intubation and time. **(B)** Linear regression of survival rates binned by week of enrollment shows a significant positive correlation between survival and time. **(C)** Principal component analysis (PCA) of all 35 cytokines measured across all patient groups in both the primary and validation cohorts, visualizing all samples with complete cytokine data. **(D)** The same PCA shown in (C) but focused on the variation immediately surrounding healthy controls (i.e., variation owing more extreme values are beyond the bounds of the plot). Magenta circles represent samples from healthy controls, orange circles represent samples from influenza-infected patients, dark green circles represent COVID-19-infected patients from the primary cohort, and light green circles represent COVID-19-infected patients from the validation cohort. **(E)** Change in gap statistic across numbers of clusters (k) for CytoMod module analysis demonstrates an optimal value of k=8.

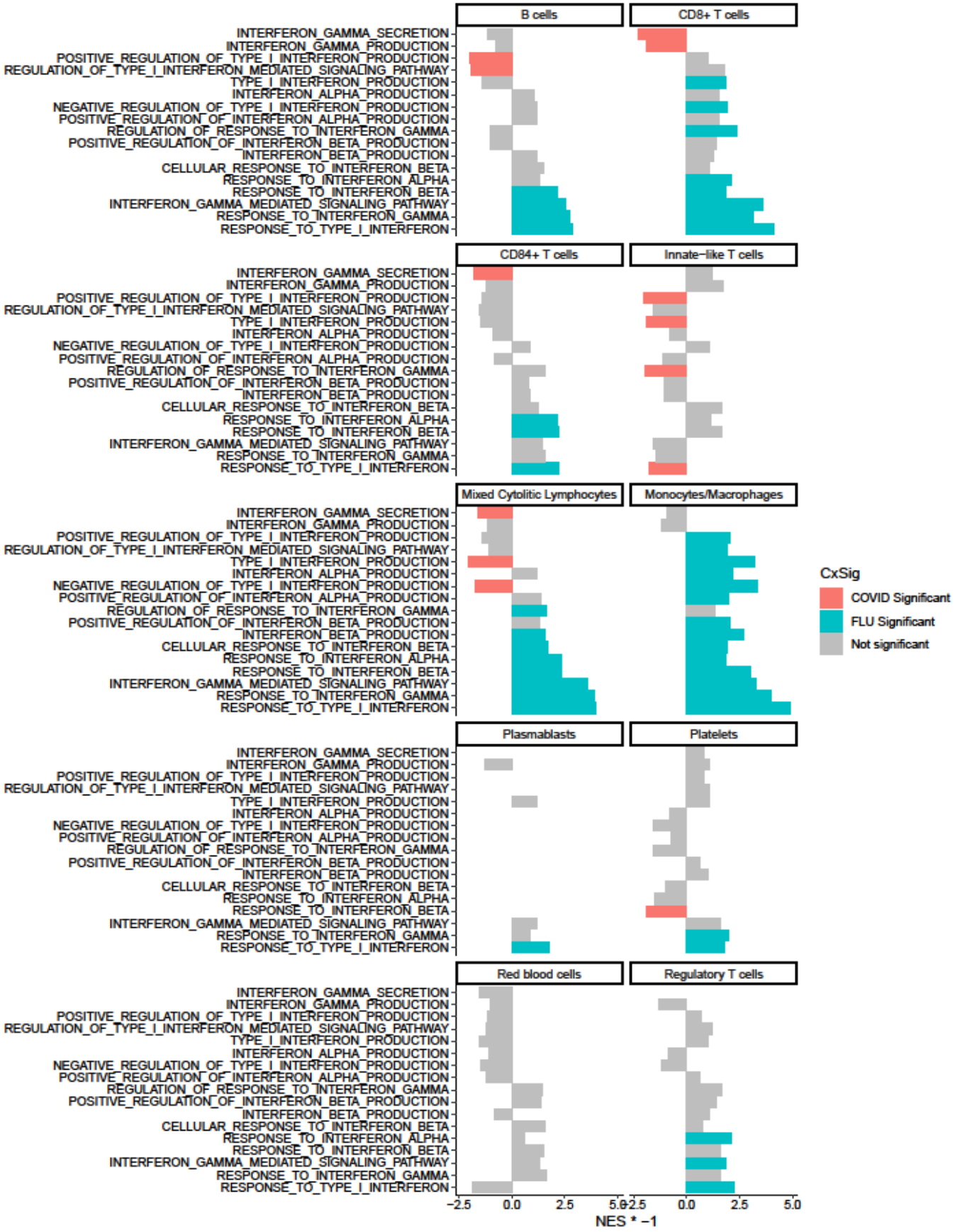
Supplementary Figure 9



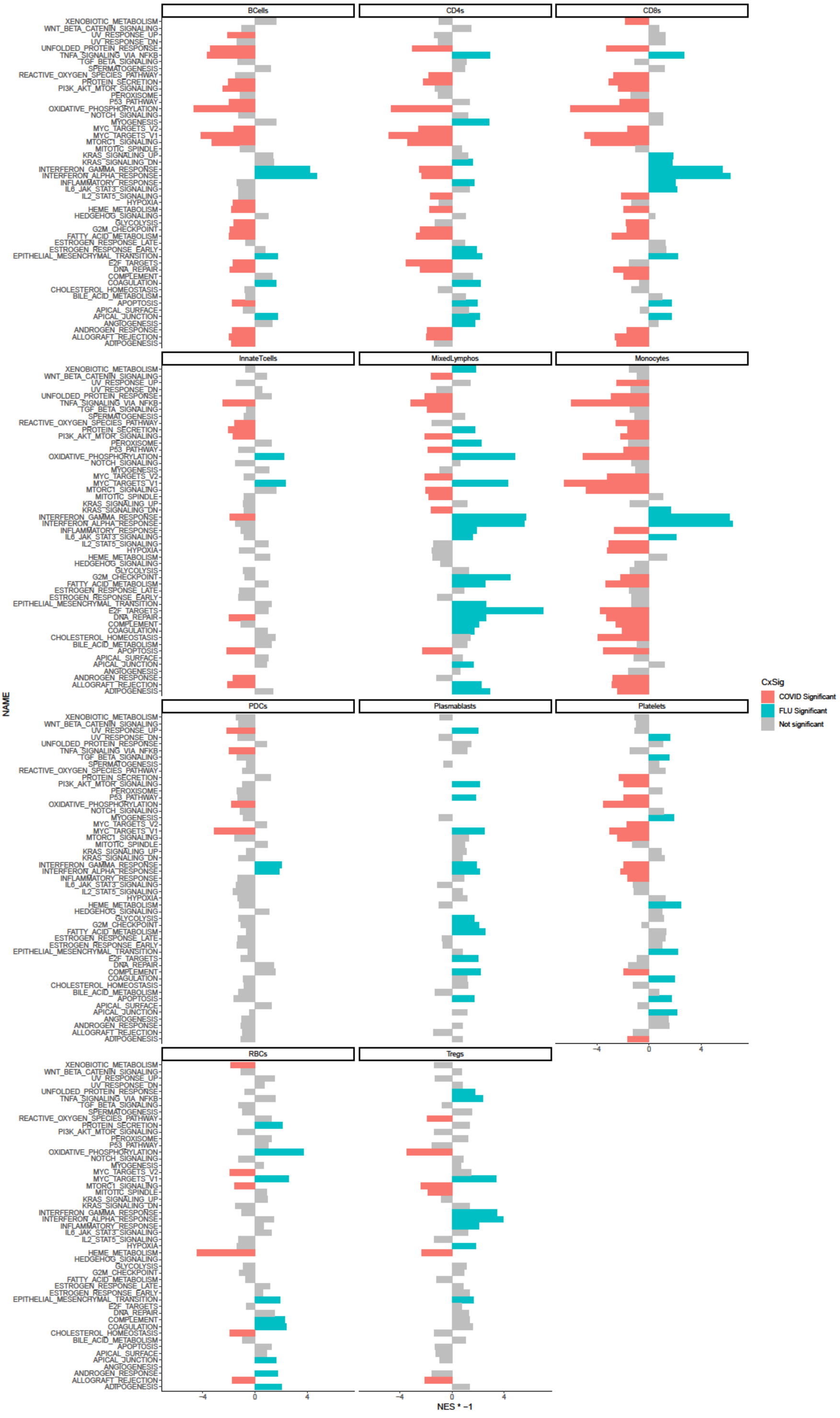
Supplementary Figure 9. Single-cell gene expression analyses of PBMCs from COVID-19-infected, influenza-infected, and healthy subjects demonstrate consistency in major cell subsets despite underlying transcriptional differences across conditions. **(A)** UMAP (uniform Manifold Approximation and Projection) plots depict transcriptional clusters used to define cell subsets. In this case, any differences between subjects has been obscured by merging the data sets with integration anchors via canonical correlation analysis. **(B)** UMAPs split by condition demonstrate the consistency transcriptional cluster identity and presence across conditions.



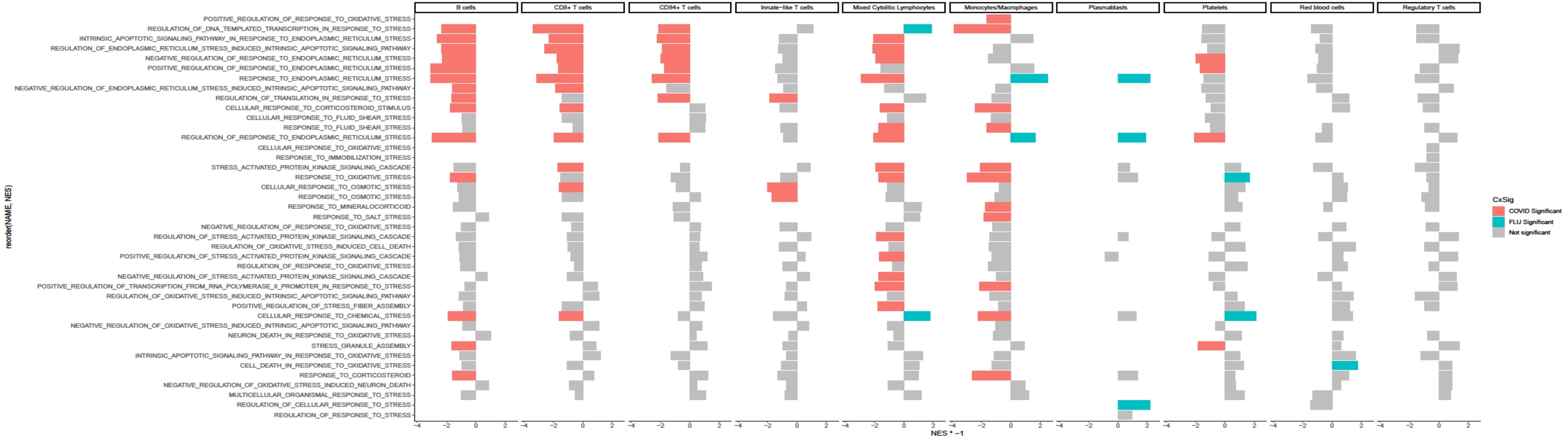
Supplementary Figure 10. For each transcriptionally identified cell subset, the proportion of cells from each single-cell gene expression library that were assigned to that subset is presented. Colors correspond to the patient from which the sample was obtained, with each patient except ZW-WU321 represented by two independent gene expression libraries constructed from distinct cell aliquots from the same PBMC sample (i.e., colors indicate the linked nature of the underlying biological samples). Omission of a point for a cluster indicates failure to detect that cluster in that library.



Supplementary Figure 11. Graphs depicting selected results from Preranked Gene Set Enrichment Analysis of Gene Ontology gene sets. Gene Ontology Biological Processes gene sets that passed default GSEA thresholds were tested, with all results with the term "interferon" in the gene set name that were significant in either direction for at least one cell subset plotted. Red: significantly enriched in cells from COVID-19-infected patients; blue: significantly enriched in cells from influenza-infected patients; grey: not statistically enriched in either condition.



Supplementary Figure 12. Graphs depicting all results of Preranked Gene Set Enrichment Analysis of Hallmark gene sets when comparing COVID-19-infected and influenza-infected subjects on a per-cell-subset basis. Red: significantly enriched in cells from COVID-19-infected patients; blue: significantly enriched in cells from influenza-infected patients; grey: not statistically enriched in either condition.



Supplementary Figure 13. Graphs depicting selected results from Preranked Gene Set Enrichment Analysis of Gene Ontology gene sets. Gene Ontology Biological Processes gene sets that passed default GSEA thresholds were tested, and all results with the terms "cortico", "cortisol", or "stress" in the gene set name that were significant in either direction for at least one cell subset plotted. Red: significantly enriched in cells from COVID-19-infected patients; blue: significantly enriched in cells from influenza-infected patients; grey: not statistically enriched in either condition.