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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Zhu X, Gebo KA, Abraham AG, et al. Dynamics of inflammatory responses after SARS-CoV-2 infection by vaccination status in the USA: a prospective cohort study. *Lancet Microbe* 2023; published online Aug 7. https://doi.org/10.1016/S2666-5247(23)00171-4.

SUPPMENTAL APPENDIX

Dynamics of inflammatory responses after SARS-CoV-2 infection by vaccination status: a prospective cohort study

Xianming Zhu, et al.

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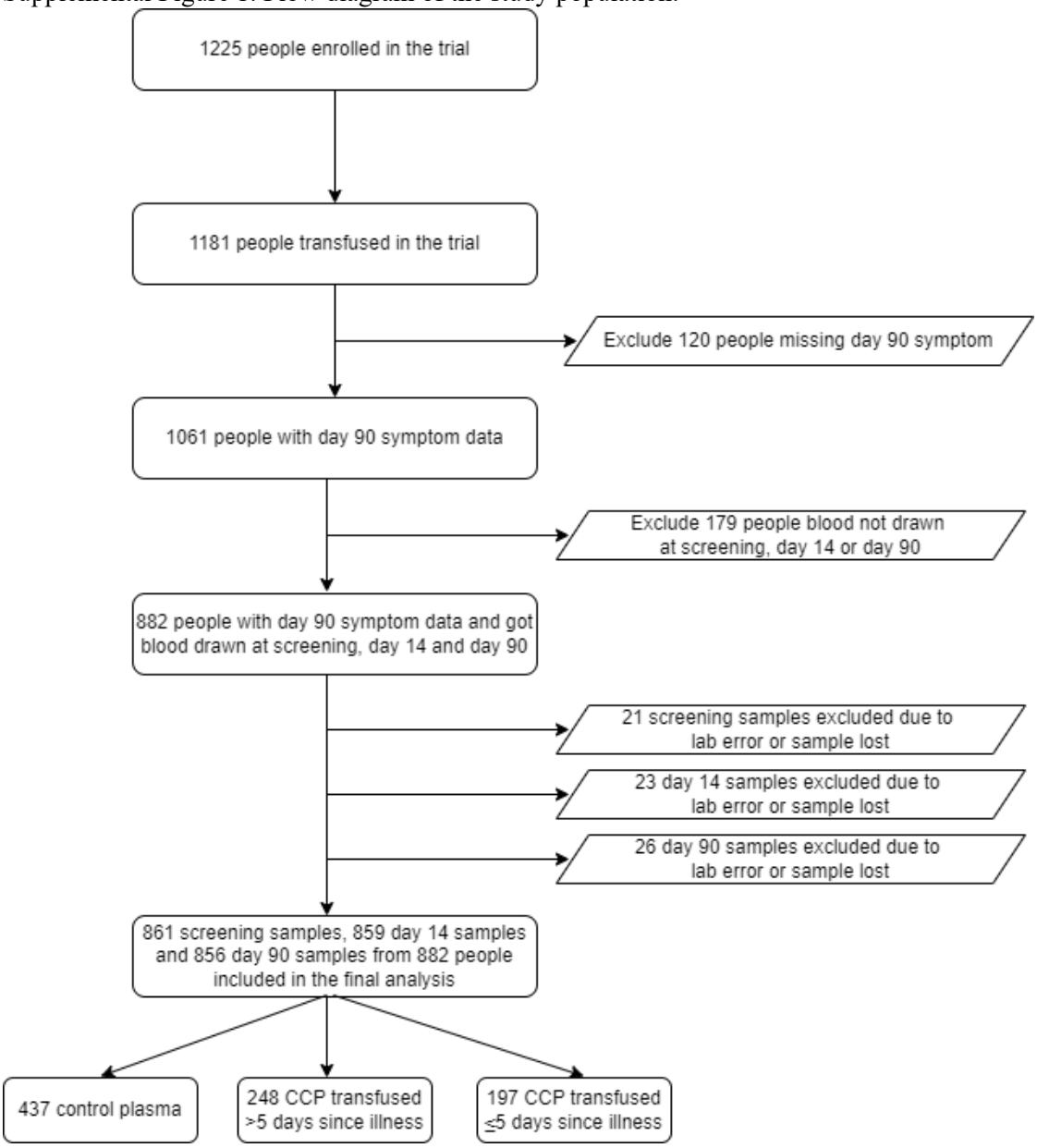
Supplemental Figure 1. Flow diagram of the study population.



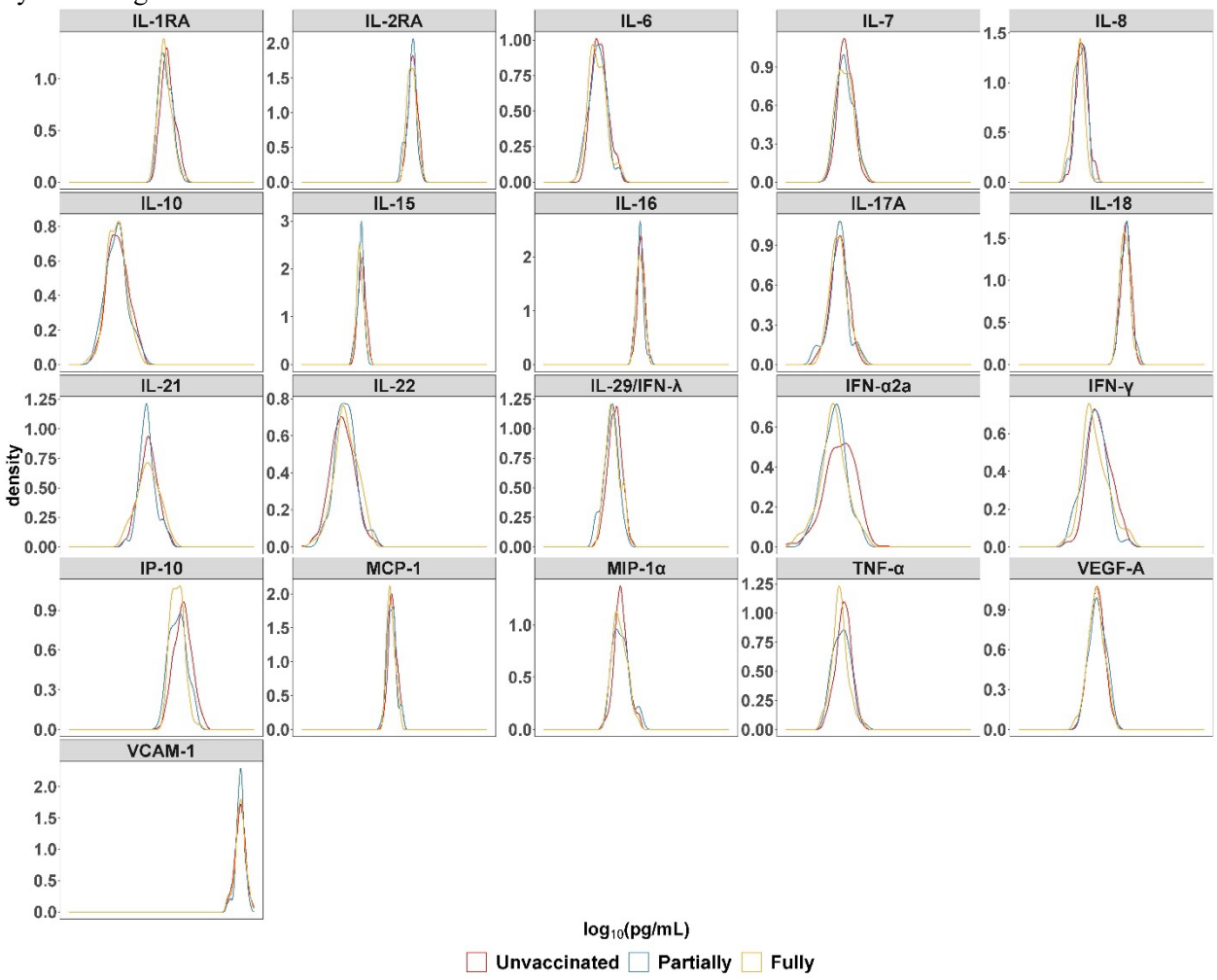
Note: this figure has already been published on https://www.medrxiv.org/content/10.1101/2023.02.13.23285855v1

Supplemental Table 1. Prevalence of sample imputed values and outlier values that were excluded.

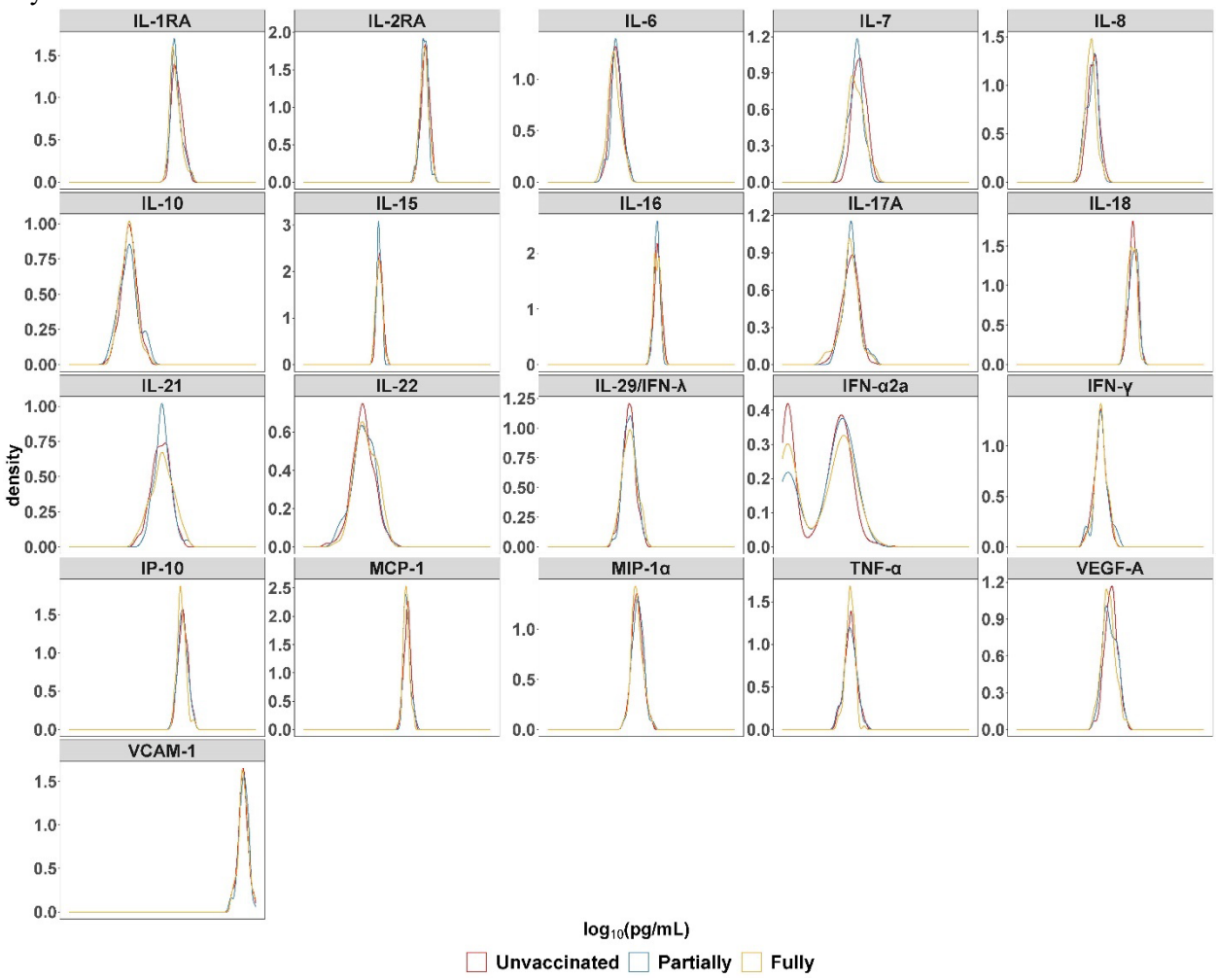
Analyte	Prevalence of imputed values (n=2646 person-visits)	Prevalence of outliers (n=2646 person-visits)
IL-1RA	0.1%	2.2%
IL-2RA	0.0%	1.9%
IL-6	0.9%	3.4%
IL-7	0.2%	0.5%
IL-8	0.6%	6.1%
IL-10	7.6%	6.5%
IL-15	0.0%	2.7%
IL-16	0.0%	2.2%
IL-17A	19.8%	12.0%
IL-18	0.4%	7.1%
IL-21	5.3%	2.4%
IL-22	25.6%	16.5%
IL-29/IFN-λ	2.1%	4.0%
IFN-α2a	40.4%	5.4%
IFN-γ	4.3%	8.8%
IP-10	0.4%	3.7%
MCP-1	0.6%	3.4%
MIP-1α	9.0%	7.9%
TNF-α	2.5%	7.6%
VEGF-A	0.6%	2.6%
VCAM-1	1.5%	5.3%

Note: Missing values were the cytokine levels outside of the assay fit curve range (specific to each plate and analyte) and were imputed. Outliers were the cytokine levels above Q3+1.5×IQR or below Q1-1.5×IQR for each cytokine (specific to each visit and each analyte) and were excluded.

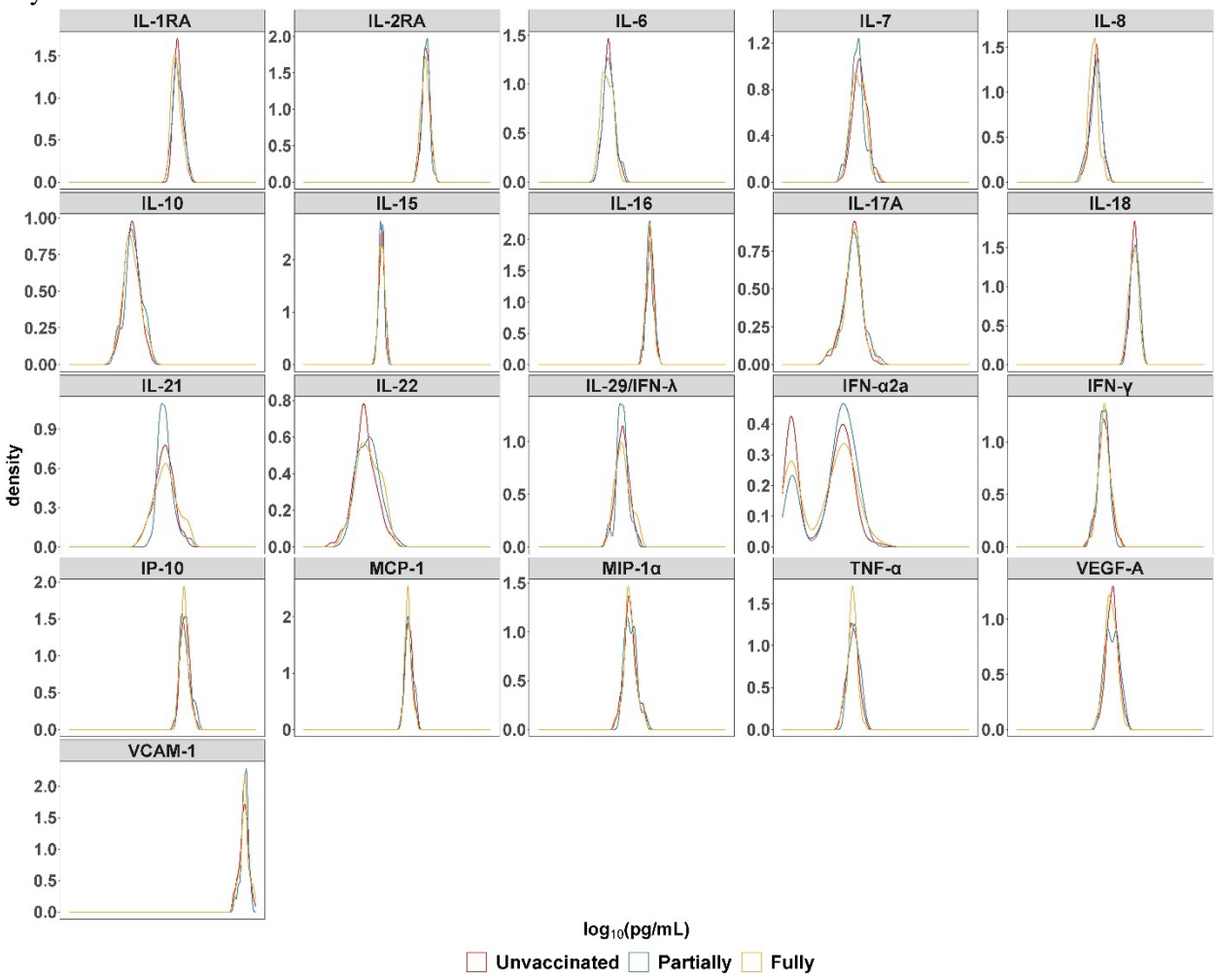
Supplemental Figure 2. The distribution of $\log_{10}$ transformed cytokine and chemokine levels at screening by screening vaccination status.



Supplemental Figure 3. The distribution of $\log_{10}$ transformed cytokine and chemokine levels at day 14 by day 14 vaccination status.

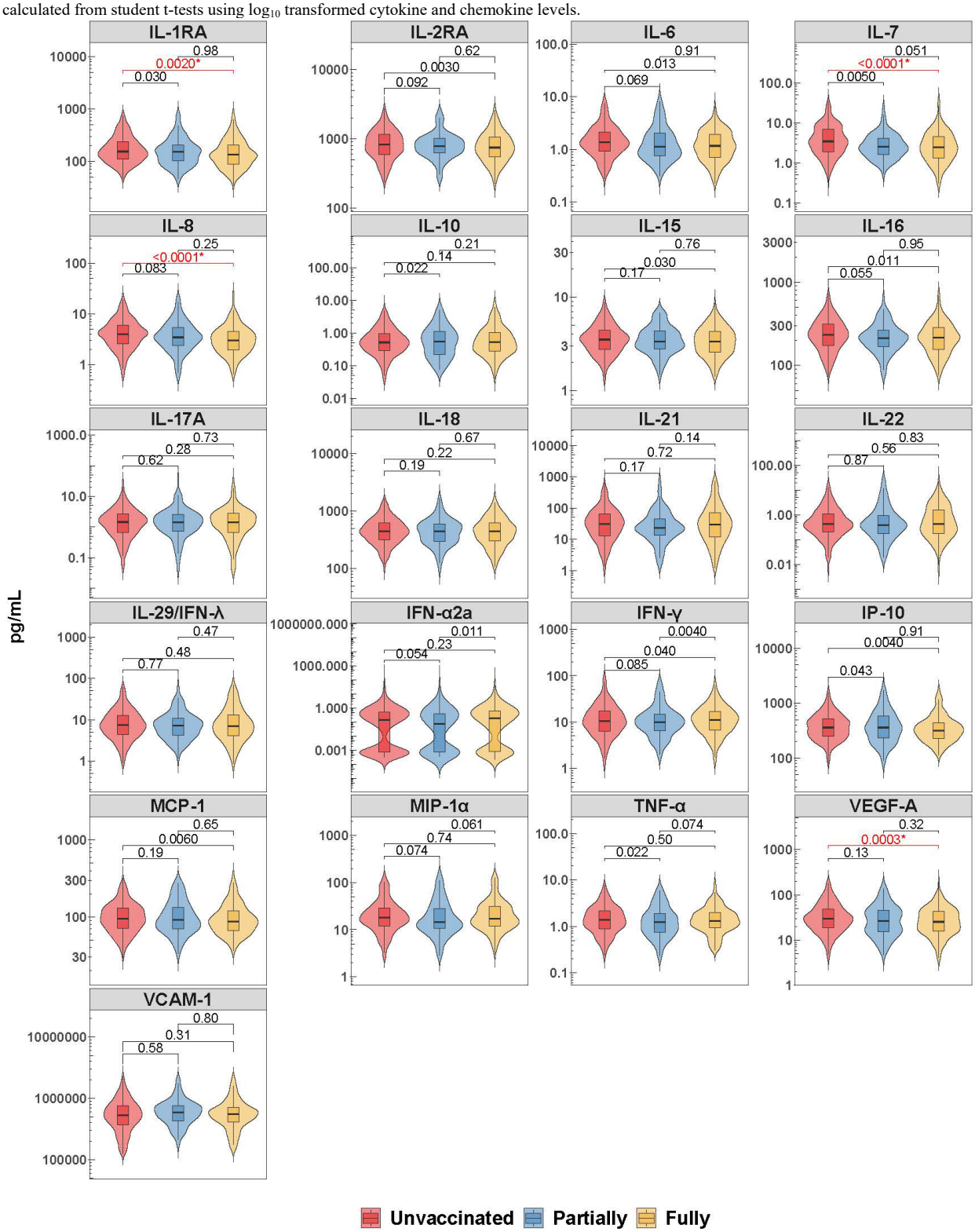


Supplemental Figure 4. The distribution of $\log_{10}$ transformed cytokine and chemokine levels at day 90 by day 90 vaccination status.



Supplemental Figure 5. Day 14 cytokine levels stratified by day 14 vaccination status

Note: *Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024. P values were calculated from student t-tests using log_{10} transformed cytokine and chemokine levels.



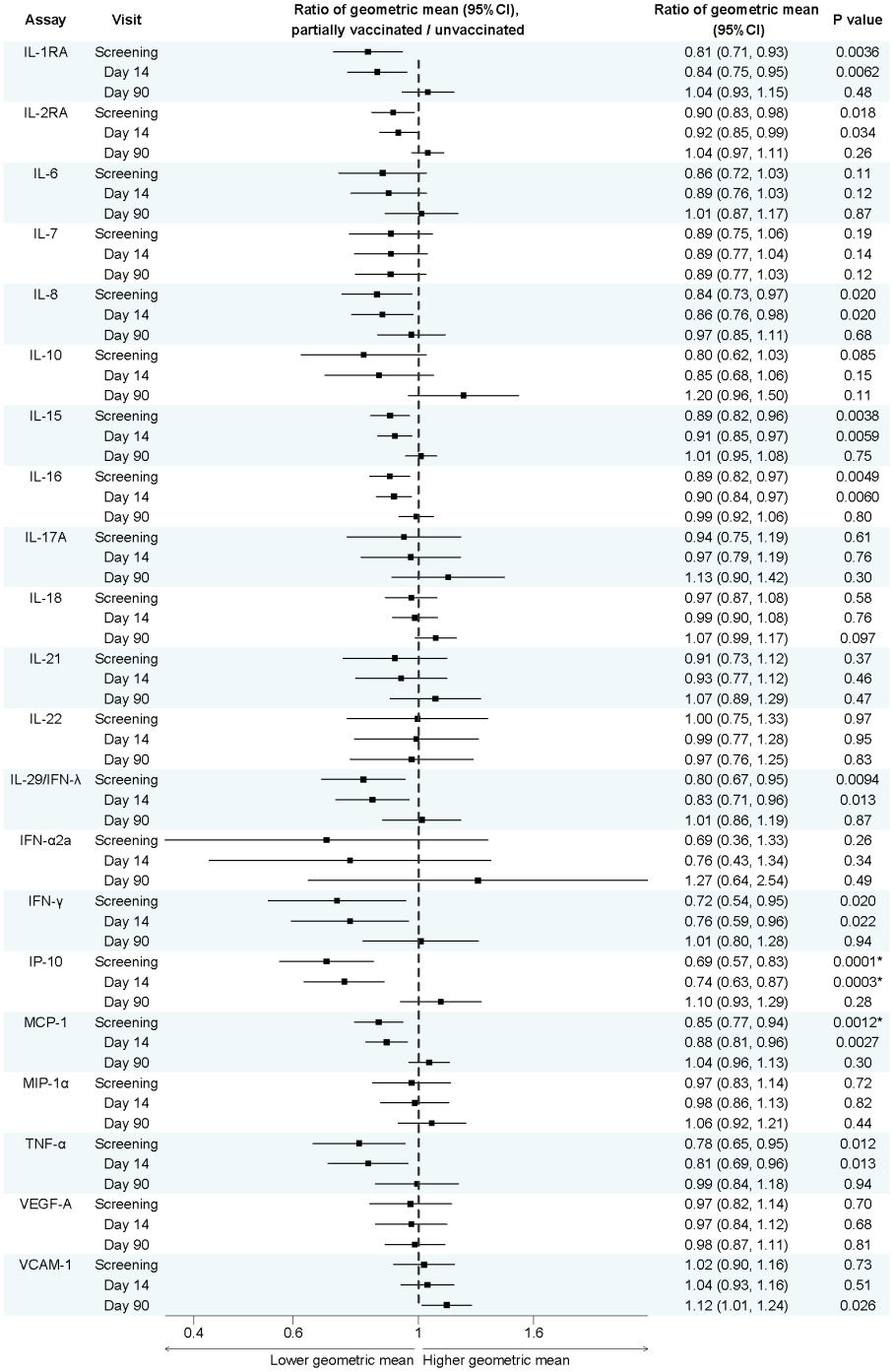
Supplemental Figure 6. Differences of the cytokine levels between unvaccinated and partially vaccinated participants.

Note: ratio (95% CI) of geometric mean between the unvaccinated (denominator) and partially vaccinated (numerator) in pg/mL of each cytokine and chemokine are presented.

Each analyte had a separate mixed effect model and all the models were adjusted for age, sex, BMI, hypertension, diabetes, treatment arms, and COVID-19 waves. Treatment arms were not adjusted at screening, since participants were not transfused at screening.

Among the covariates, only BMI have missing data, and the prevalence of missingness was 5%. Available case method was used to exclude missing values.

*Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024.



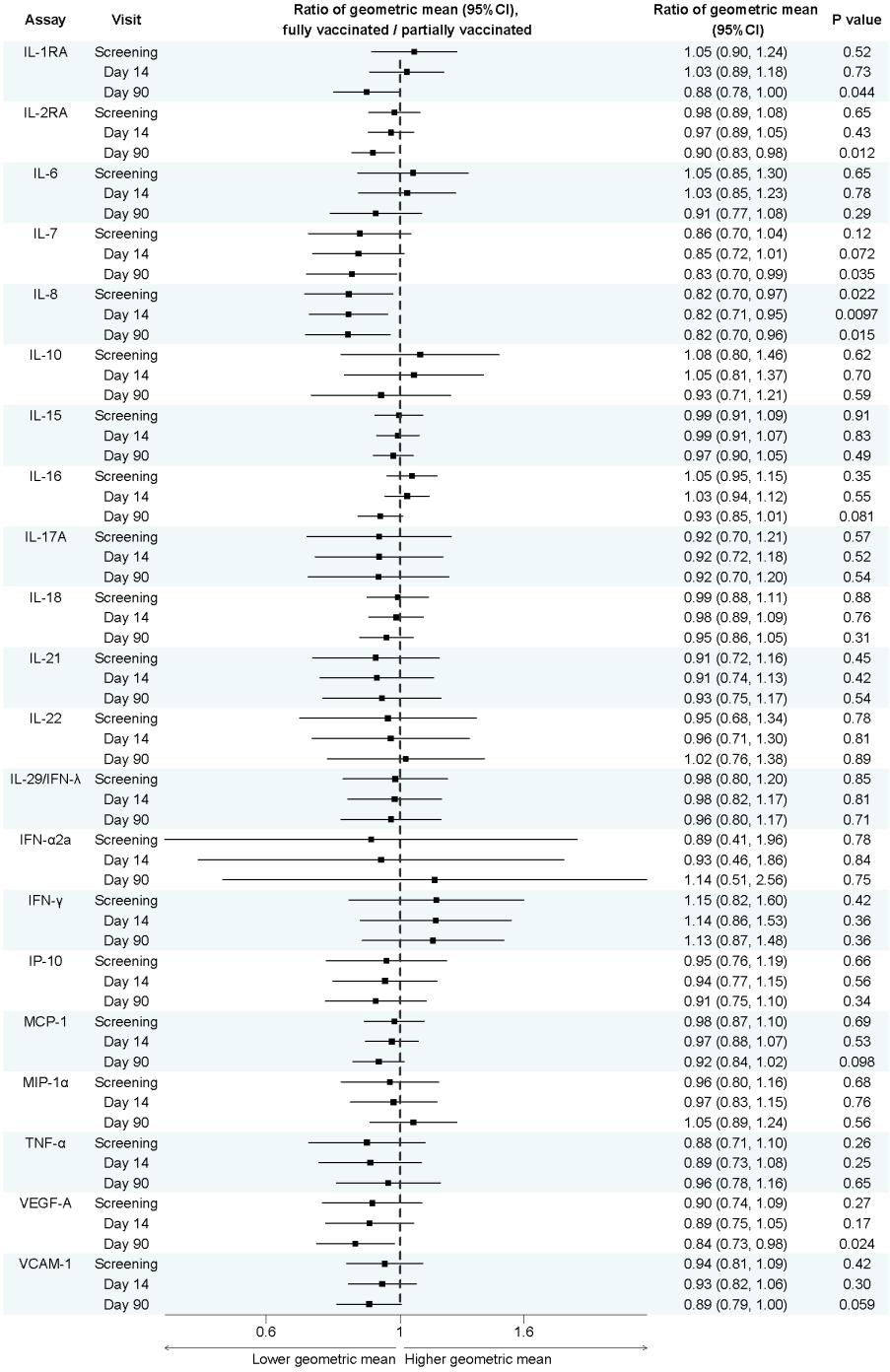
Supplemental Figure 7. Differences of the cytokine levels between partially and fully vaccinated participants.

Note: ratio (95% CI) of geometric mean between the partially (denominator) and fully vaccinated (numerator) in pg/mL of each cytokine and chemokine are presented.

Each analyte had a separate mixed effect model and all the models were adjusted for age, sex, BMI, hypertension, diabetes, treatment arms, and COVID-19 waves. Treatment arms were not adjusted at screening, since participants were not transfused at screening.

Among the covariates, only BMI have missing data, and the prevalence of missingness was 5%. Available case method was used to exclude missing values.

*Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024.



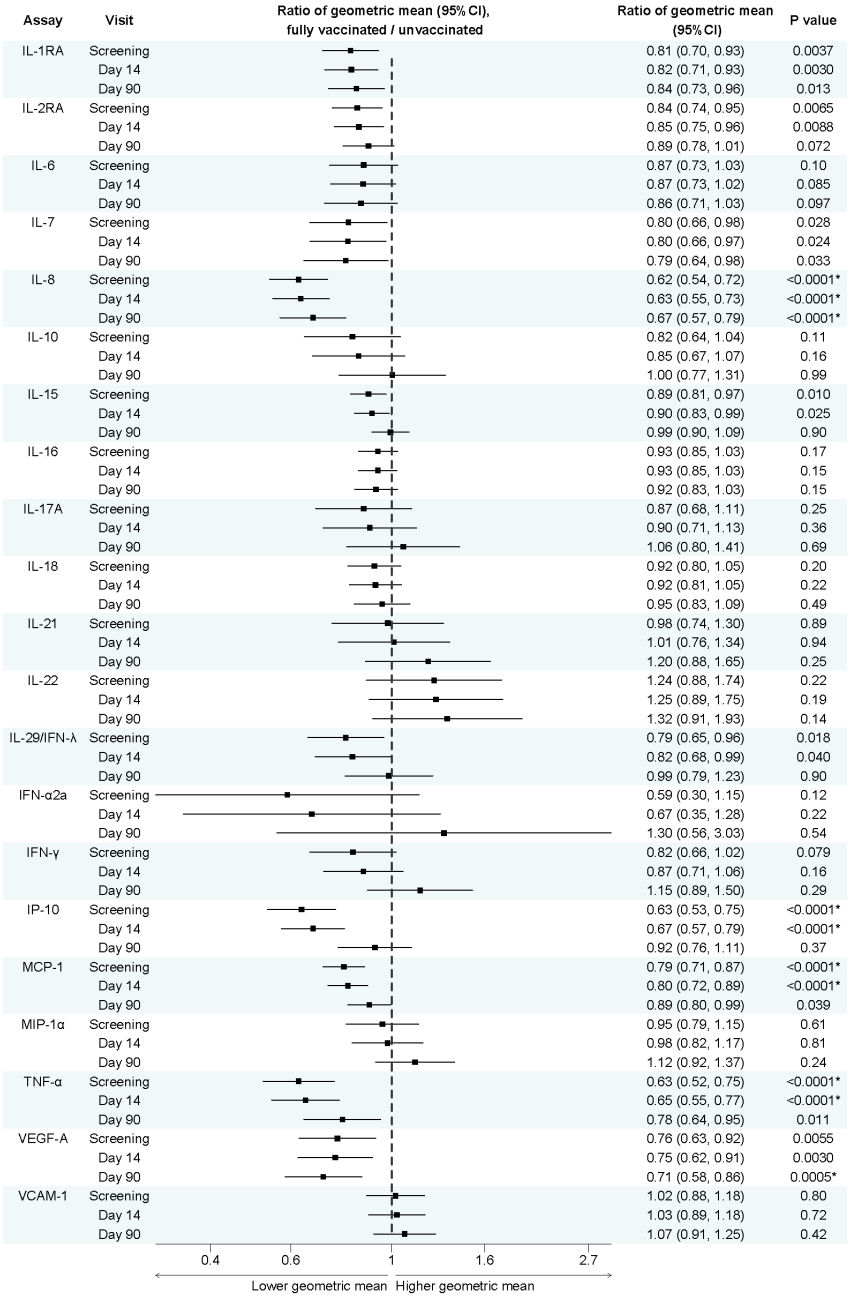
Supplemental Figure 8. Sensitivity analysis to compare the cytokine and chemokine levels between unvaccinated and fully vaccinated participants after censoring the visits among individuals with a change in vaccination status.

Note: ratio (95% CI) of geometric mean between the unvaccinated (denominator) and fully vaccinated (numerator) in pg/mL of each cytokine and chemokine are presented.

Each analyte had a separate mixed effect model and all the models were adjusted for age, sex, BMI, hypertension, diabetes, treatment arms, and COVID-19 waves. Treatment arms were not adjusted at screening, since participants were not transfused at screening.

Among the covariates, only BMI have missing data, and the prevalence of missingness was 5%. Available case method was used to exclude missing values.

*Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024.



Supplemental Figure 9. Sensitivity analysis to compare the cytokine and chemokine levels between unvaccinated and fully vaccinated participants stratified by age.

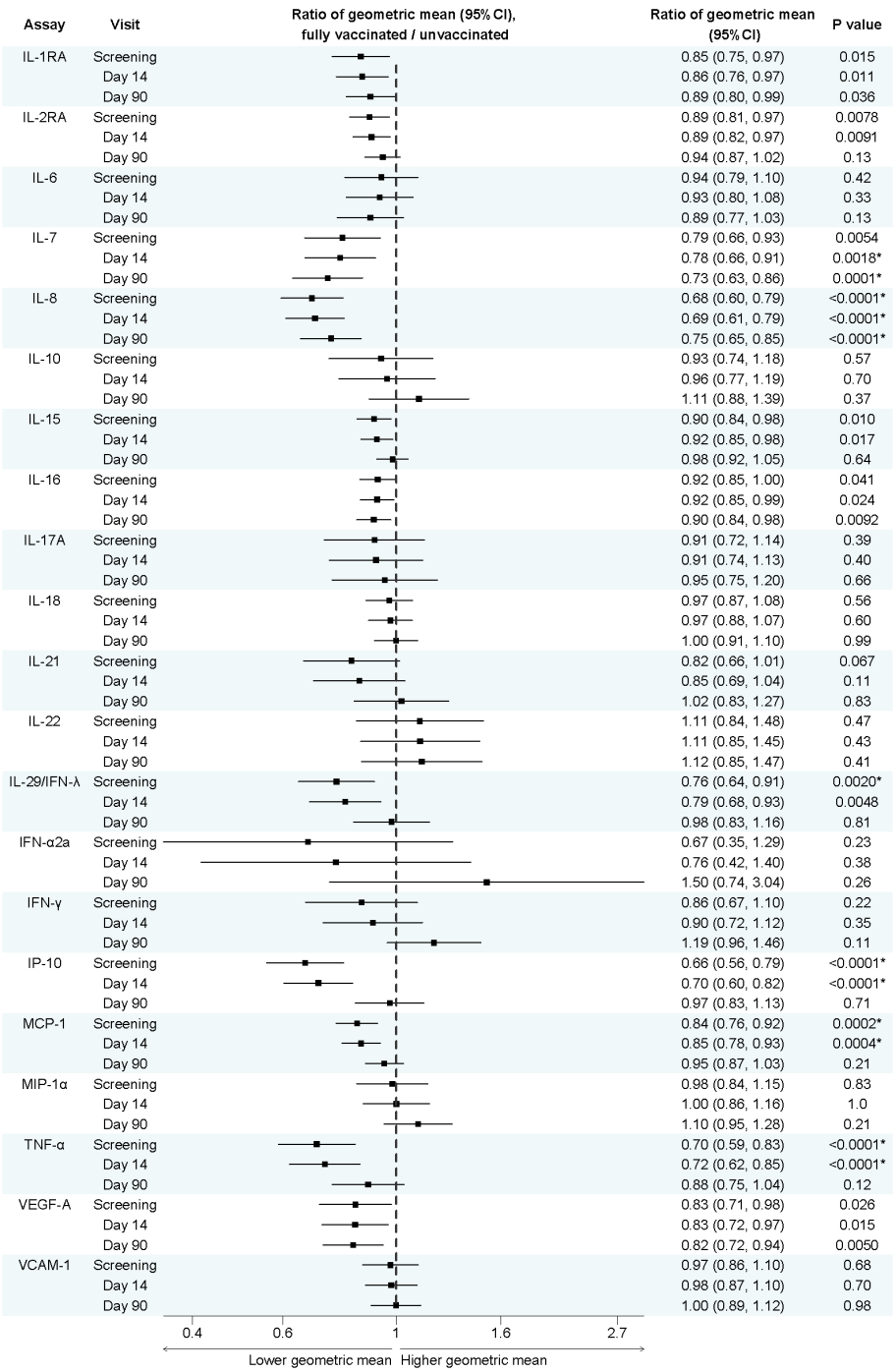
Note: ratio (95% CI) of geometric mean between the unvaccinated (denominator) and fully vaccinated (numerator) in pg/mL of each cytokine and chemokine are presented.

Each analyte had a separate mixed effect model and all the models were adjusted for age, sex, BMI, hypertension, diabetes, treatment arms, and COVID-19 waves. Treatment arms were not adjusted at screening, since participants were not transfused at screening.

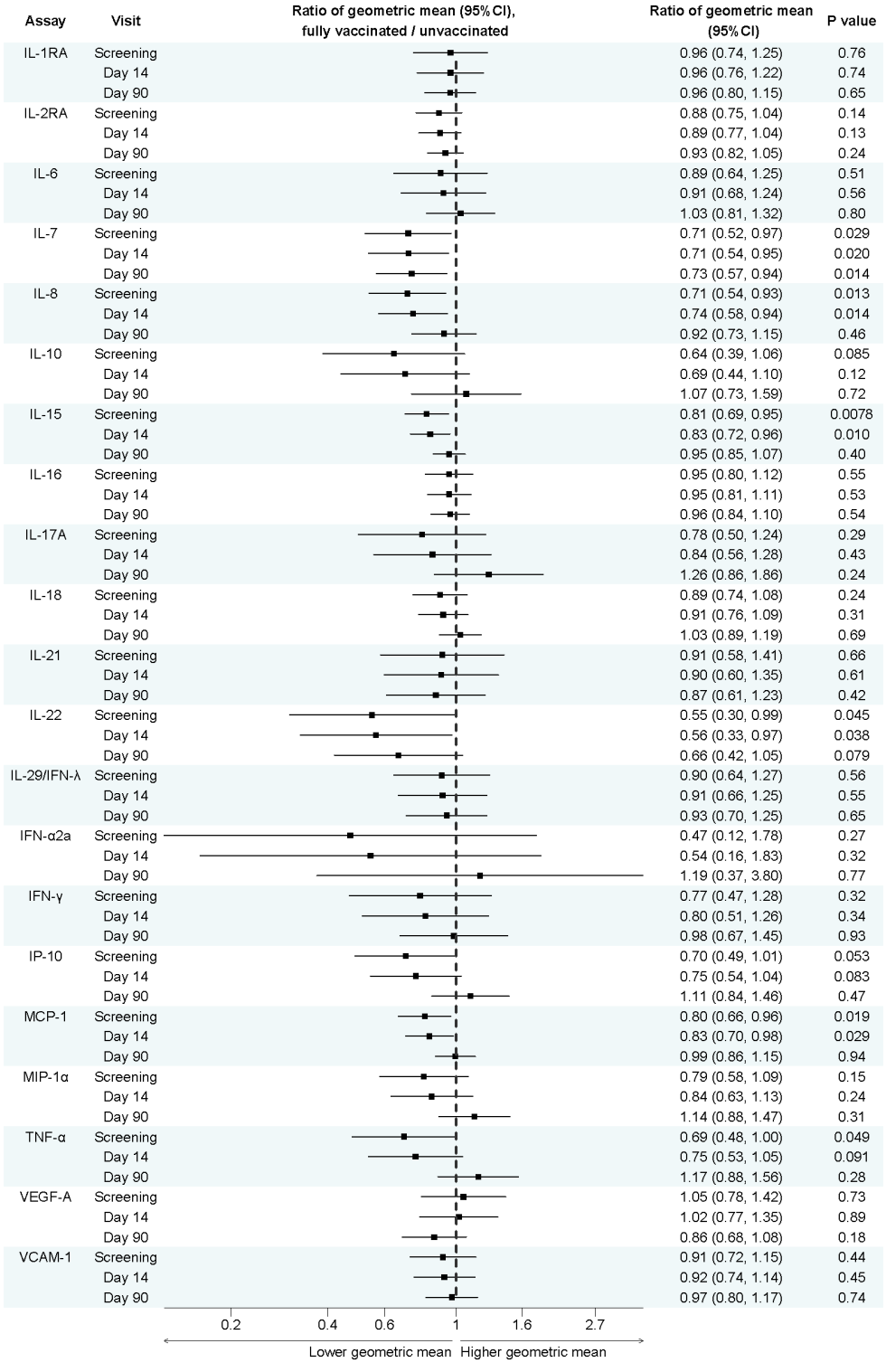
Among the covariates, only BMI have missing data, and the prevalence of missingness was 5%. Available case method was used to exclude missing values.

*Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024.

9.A. age<50



9.B. age≥50


Assay
Visit
Ratio of geometric mean (95% CI), fully vaccinated / unvaccinated
Ratio of geometric mean (95% CI)
P value
IL-1RA Screening 0.96 (0.74, 1.25) 0.76
Day 14 0.96 (0.76, 1.22) 0.74
Day 90 0.96 (0.80, 1.15) 0.65
IL-2RA Screening 0.88 (0.75, 1.04) 0.14
Day 14 0.89 (0.77, 1.04) 0.13
Day 90 0.93 (0.82, 1.05) 0.24
IL-6 Screening 0.89 (0.64, 1.25) 0.51
Day 14 0.91 (0.68, 1.24) 0.56
Day 90 1.03 (0.81, 1.32) 0.80
IL-7 Screening 0.71 (0.52, 0.97) 0.029
Day 14 0.71 (0.54, 0.95) 0.020
Day 90 0.73 (0.57, 0.94) 0.014
IL-8 Screening 0.71 (0.54, 0.93) 0.013
Day 14 0.74 (0.58, 0.94) 0.014
Day 90 0.92 (0.73, 1.15) 0.46
IL-10 Screening 0.64 (0.39, 1.06) 0.085
Day 14 0.69 (0.44, 1.10) 0.12
Day 90 1.07 (0.73, 1.59) 0.72
IL-15 Screening 0.81 (0.69, 0.95) 0.0078
Day 14 0.83 (0.72, 0.96) 0.010
Day 90 0.95 (0.85, 1.07) 0.40
IL-16 Screening 0.95 (0.80, 1.12) 0.55
Day 14 0.95 (0.81, 1.11) 0.53
Day 90 0.96 (0.84, 1.10) 0.54
IL-17A Screening 0.78 (0.50, 1.24) 0.29
Day 14 0.84 (0.56, 1.28) 0.43
Day 90 1.26 (0.86, 1.86) 0.24
IL-18 Screening 0.89 (0.74, 1.08) 0.24
Day 14 0.91 (0.76, 1.09) 0.31
Day 90 1.03 (0.89, 1.19) 0.69
IL-21 Screening 0.91 (0.58, 1.41) 0.66
Day 14 0.90 (0.60, 1.35) 0.61
Day 90 0.87 (0.61, 1.23) 0.42
IL-22 Screening 0.55 (0.30, 0.99) 0.045
Day 14 0.56 (0.33, 0.97) 0.038
Day 90 0.66 (0.42, 1.05) 0.079
IL-29/IFN-λ Screening 0.90 (0.64, 1.27) 0.56
Day 14 0.91 (0.66, 1.25) 0.55
Day 90 0.93 (0.70, 1.25) 0.65
IFN-α2a Screening 0.47 (0.12, 1.78) 0.27
Day 14 0.54 (0.16, 1.83) 0.32
Day 90 1.19 (0.37, 3.80) 0.77
IFN-γ Screening 0.77 (0.47, 1.28) 0.32
Day 14 0.80 (0.51, 1.26) 0.34
Day 90 0.98 (0.67, 1.45) 0.93
IP-10 Screening 0.70 (0.49, 1.01) 0.053
Day 14 0.75 (0.54, 1.04) 0.083
Day 90 1.11 (0.84, 1.46) 0.47
MCP-1 Screening 0.80 (0.66, 0.96) 0.019
Day 14 0.83 (0.70, 0.98) 0.029
Day 90 0.99 (0.86, 1.15) 0.94
MIP-1α Screening 0.79 (0.58, 1.09) 0.15
Day 14 0.84 (0.63, 1.13) 0.24
Day 90 1.14 (0.88, 1.47) 0.31
TNF-α Screening 0.69 (0.48, 1.00) 0.049
Day 14 0.75 (0.53, 1.05) 0.091
Day 90 1.17 (0.88, 1.56) 0.28
VEGF-A Screening 1.05 (0.78, 1.42) 0.73
Day 14 1.02 (0.77, 1.35) 0.89
Day 90 0.86 (0.68, 1.08) 0.18
VCAM-1 Screening 0.91 (0.72, 1.15) 0.44
Day 14 0.92 (0.74, 1.14) 0.45
Day 90 0.97 (0.80, 1.17) 0.74
0.2
0.4
0.6
1
1.6
2.7
Lower geometric mean
Higher geometric mean

Supplemental Figure 10. Sensitivity analysis to compare the cytokine and chemokine levels between unvaccinated and fully vaccinated participants stratified by sex.

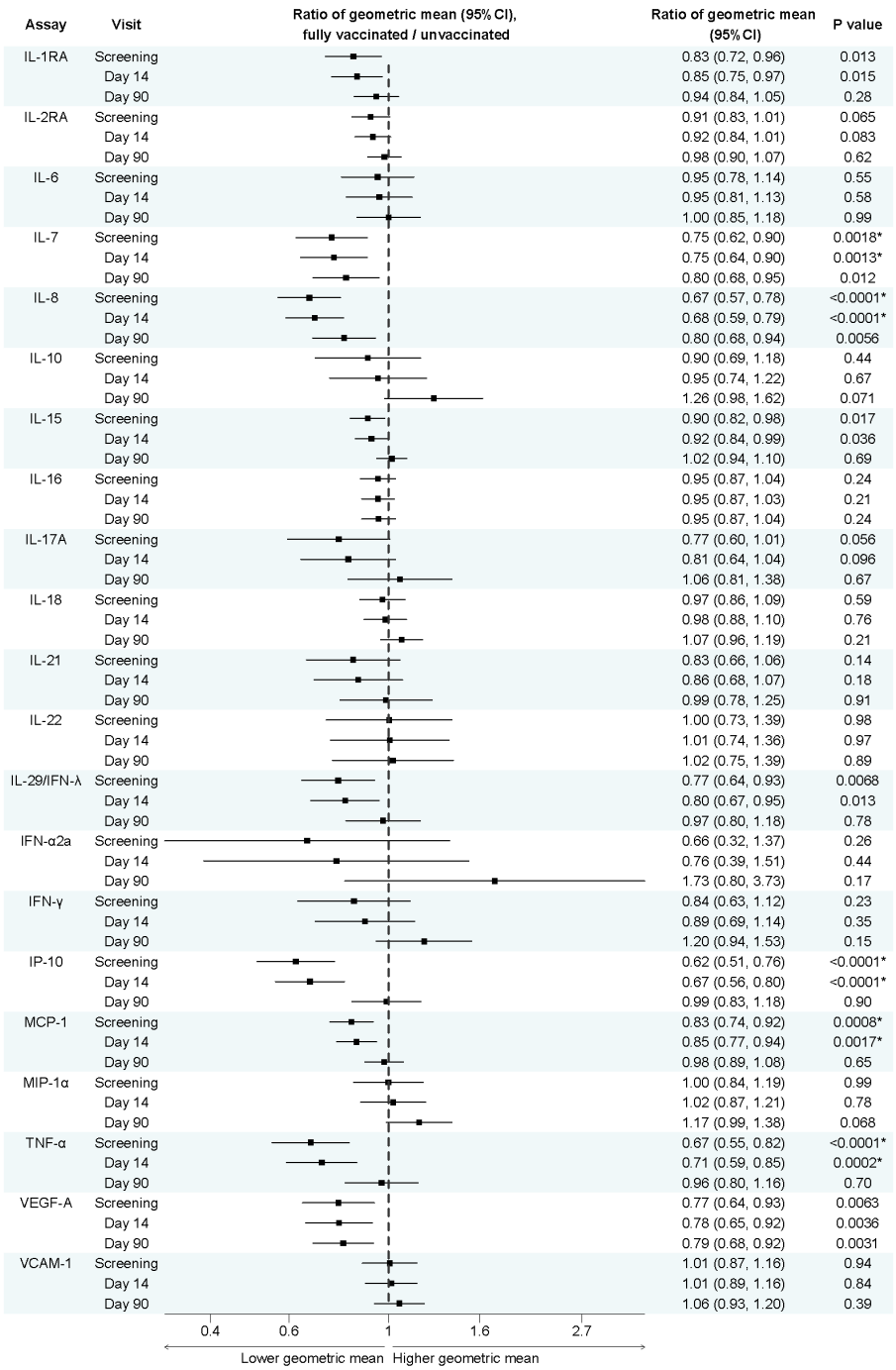
Note: ratio (95% CI) of geometric mean between the unvaccinated (denominator) and fully vaccinated (numerator) in pg/mL of each cytokine and chemokine are presented.

Each analyte had a separate mixed effect model and all the models were adjusted for age, BMI, hypertension, diabetes, treatment arms, and COVID-19 waves. Treatment arms were not adjusted at screening, since participants were not transfused at screening.

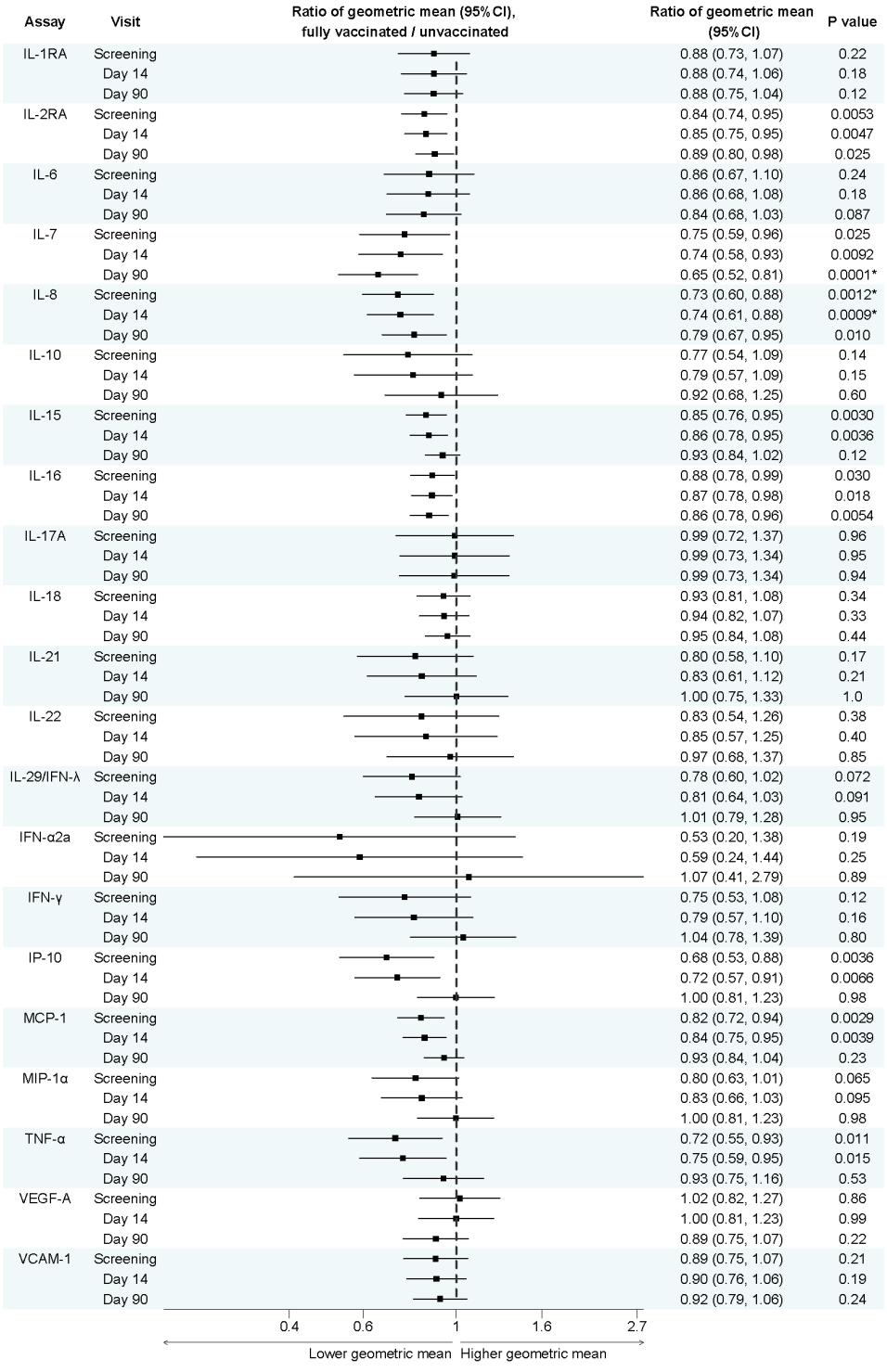
Among the covariates, only BMI have missing data, and the prevalence of missingness was 5%. Available case method was used to exclude missing values.

*Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024.

10A. Female



10B. Male


Assay
Visit
Ratio of geometric mean (95% CI), fully vaccinated / unvaccinated
Ratio of geometric mean (95% CI)
P value
IL-1RA Screening 0.88 (0.73, 1.07) 0.22
Day 14 0.88 (0.74, 1.06) 0.18
Day 90 0.88 (0.75, 1.04) 0.12
IL-2RA Screening 0.84 (0.74, 0.95) 0.0053
Day 14 0.85 (0.75, 0.95) 0.0047
Day 90 0.89 (0.80, 0.98) 0.025
IL-6 Screening 0.86 (0.67, 1.10) 0.24
Day 14 0.86 (0.68, 1.08) 0.18
Day 90 0.84 (0.68, 1.03) 0.087
IL-7 Screening 0.75 (0.59, 0.96) 0.025
Day 14 0.74 (0.58, 0.93) 0.0092
Day 90 0.65 (0.52, 0.81) 0.0001*
IL-8 Screening 0.73 (0.60, 0.88) 0.0012*
Day 14 0.74 (0.61, 0.88) 0.0009*
Day 90 0.79 (0.67, 0.95) 0.010
IL-10 Screening 0.77 (0.54, 1.09) 0.14
Day 14 0.79 (0.57, 1.09) 0.15
Day 90 0.92 (0.68, 1.25) 0.60
IL-15 Screening 0.85 (0.76, 0.95) 0.0030
Day 14 0.86 (0.78, 0.95) 0.0036
Day 90 0.93 (0.84, 1.02) 0.12
IL-16 Screening 0.88 (0.78, 0.99) 0.030
Day 14 0.87 (0.78, 0.98) 0.018
Day 90 0.86 (0.78, 0.96) 0.0054
IL-17A Screening 0.99 (0.72, 1.37) 0.96
Day 14 0.99 (0.73, 1.34) 0.95
Day 90 0.99 (0.73, 1.34) 0.94
IL-18 Screening 0.93 (0.81, 1.08) 0.34
Day 14 0.94 (0.82, 1.07) 0.33
Day 90 0.95 (0.84, 1.08) 0.44
IL-21 Screening 0.80 (0.58, 1.10) 0.17
Day 14 0.83 (0.61, 1.12) 0.21
Day 90 1.00 (0.75, 1.33) 1.0
IL-22 Screening 0.83 (0.54, 1.26) 0.38
Day 14 0.85 (0.57, 1.25) 0.40
Day 90 0.97 (0.68, 1.37) 0.85
IL-29/IFN-λ Screening 0.78 (0.60, 1.02) 0.072
Day 14 0.81 (0.64, 1.03) 0.091
Day 90 1.01 (0.79, 1.28) 0.95
IFN-α2a Screening 0.53 (0.20, 1.38) 0.19
Day 14 0.59 (0.24, 1.44) 0.25
Day 90 1.07 (0.41, 2.79) 0.89
IFN-γ Screening 0.75 (0.53, 1.08) 0.12
Day 14 0.79 (0.57, 1.10) 0.16
Day 90 1.04 (0.78, 1.39) 0.80
IP-10 Screening 0.68 (0.53, 0.88) 0.0036
Day 14 0.72 (0.57, 0.91) 0.0066
Day 90 1.00 (0.81, 1.23) 0.98
MCP-1 Screening 0.82 (0.72, 0.94) 0.0029
Day 14 0.84 (0.75, 0.95) 0.0039
Day 90 0.93 (0.84, 1.04) 0.23
MIP-1α Screening 0.80 (0.63, 1.01) 0.065
Day 14 0.83 (0.66, 1.03) 0.095
Day 90 1.00 (0.81, 1.23) 0.98
TNF-α Screening 0.72 (0.55, 0.93) 0.011
Day 14 0.75 (0.59, 0.95) 0.015
Day 90 0.93 (0.75, 1.16) 0.53
VEGF-A Screening 1.02 (0.82, 1.27) 0.86
Day 14 1.00 (0.81, 1.23) 0.99
Day 90 0.89 (0.75, 1.07) 0.22
VCAM-1 Screening 0.89 (0.75, 1.07) 0.21
Day 14 0.90 (0.76, 1.06) 0.19
Day 90 0.92 (0.79, 1.06) 0.24
0.4
0.6
1
1.6
2.7
Lower geometric mean
Higher geometric mean

Supplemental Table 2. Comparison of the slope of the daily changes of cytokine levels from screening to day 90 between vaccination groups.

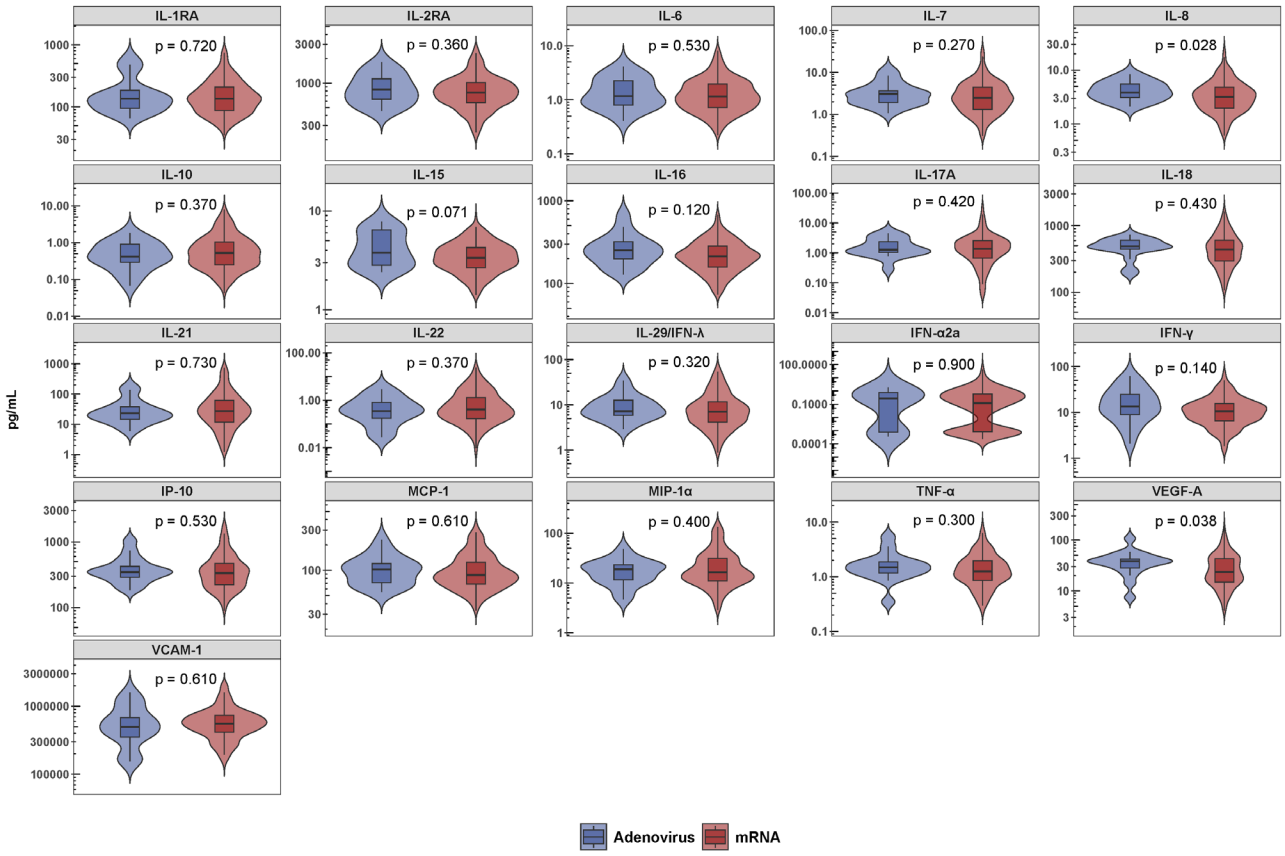
Analyte	Difference between un- and partially vaccinated (partially minus unvaccinated)	Difference between partially and fully (fully minus partially)	Difference between un- and fully vaccinated (fully minus unvaccinated)
IL-1RA	0.0012 (0.0004, 0.0020)	-0.0009 (-0.0018, 0.0000)	0.0003 (-0.0002, 0.0008)
IL-2RA	0.0007 (0.0002, 0.0012)	-0.0004 (-0.0009, 0.0001)	0.0003 (0.0000, 0.0006)
IL-6	0.0008 (-0.0003, 0.0018)	-0.0007 (-0.0019, 0.0005)	0.0001 (-0.0006, 0.0008)
IL-7	-0.0000 (-0.0010, 0.0010)	-0.0001 (-0.0012, 0.0009)	-0.0002 (-0.0008, 0.0005)
IL-8	0.0007 (-0.0002, 0.0016)	-0.0000 (-0.0010, 0.0010)	0.0007 (0.0001, 0.0012)
IL-10	0.0020 (0.0004, 0.0035)	-0.0007 (-0.0025, 0.0010)	0.0013 (0.0002, 0.0023)
IL-15	0.0006 (0.0002, 0.0011)	-0.0001 (-0.0006, 0.0004)	0.0005 (0.0002, 0.0008)*
IL-16	0.0005 (0.0000, 0.0010)	-0.0006 (-0.0011, -0.0001)	-0.0001 (-0.0004, 0.0002)
IL-17A	0.0009 (-0.0006, 0.0024)	-0.0000 (-0.0017, 0.0016)	0.0009 (-0.0001, 0.0018)
IL-18	0.0005 (-0.0001, 0.0011)	-0.0002 (-0.0009, 0.0004)	0.0003 (-0.0001, 0.0006)
IL-21	0.0008 (-0.0004, 0.0020)	0.0001 (-0.0012, 0.0015)	0.0009 (0.0001, 0.0017)
IL-22	-0.0001 (-0.0018, 0.0016)	0.0003 (-0.0016, 0.0022)	0.0002 (-0.0009, 0.0013)
IL-29/IFN-λ	0.0012 (0.0001, 0.0022)	-0.0001 (-0.0012, 0.0011)	0.0011 (0.0004, 0.0018)*
IFN-α2a	0.0030 (-0.0015, 0.0074)	0.0012 (-0.0038, 0.0061)	0.0041 (0.0012, 0.0071)
IFN-γ	0.0017 (-0.0001, 0.0034)	-0.0001 (-0.0020, 0.0019)	0.0016 (0.0004, 0.0028)
IP-10	0.0023 (0.0010, 0.0035)*	-0.0002 (-0.0015, 0.0012)	0.0021 (0.0012, 0.0029)*
MCP-1	0.0010 (0.0004, 0.0016)*	-0.0003 (-0.0009, 0.0004)	0.0007 (0.0003, 0.0011)*
MIP-1α	0.0004 (-0.0005, 0.0013)	0.0004 (-0.0006, 0.0015)	0.0008 (0.0002, 0.0014)
TNF-α	0.0011 (-0.0000, 0.0023)	0.0004 (-0.0009, 0.0017)	0.0015 (0.0008, 0.0023)*
VEGF-A	0.0001 (-0.0008, 0.0010)	-0.0003 (-0.0013, 0.0007)	-0.0002 (-0.0008, 0.0004)
VCAM-1	0.0004 (-0.0003, 0.0012)	-0.0003 (-0.0011, 0.0005)	0.0002 (-0.0003, 0.0006)

Note: the difference in slope (95% CI) of the daily changes of $\log_{10}$ pg/mL values of each analyte during follow up are presented. Each analyte had a separate mixed effect model and all the models were adjusted for age, sex, BMI, hypertension, diabetes, treatment arms, and COVID-19 waves. Treatment arms were not adjusted at screening, since participants were not transfused at screening. Among the covariates, only BMI have missing data, and the prevalence of missingness was 5%. Available case method was used to handle missing values.

*Statistically significant after adjusting for multiple comparison using Bonferroni correction with a p-value cut off of 0.0024.

Supplemental Figure 11. Cytokine levels at day 90 between mRNA COVID vaccine (n=127) and adenovirus-vector COVID vaccine (n=12) among pre-enrollment fully vaccinated participants.

Note: p values were estimated from student t-tests, using $\log_{10}$ transformed cytokine and chemokine levels.



Supplemental Figure 12. The correlation of cytokine levels at screening and days since fully vaccination among pre-enrollment fully vaccinated participants

Note: Correlation coefficient (R) were estimated using spearman rank correlation. P values were computed using asymptotic t approximation. Each dot represents the cytokine level of an individual. Blue line represents the LOESS curve.