Supporting Information for

Nasal administration of anti-CD3 mAb (Foralumab) downregulates *NKG7* and increases *TGFb1* and *GIMAP7* expression in T cells in subjects with COVID-19.

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Tables S1 and S2

Other supporting materials for this manuscript include the following:

Datasets S1 to S8

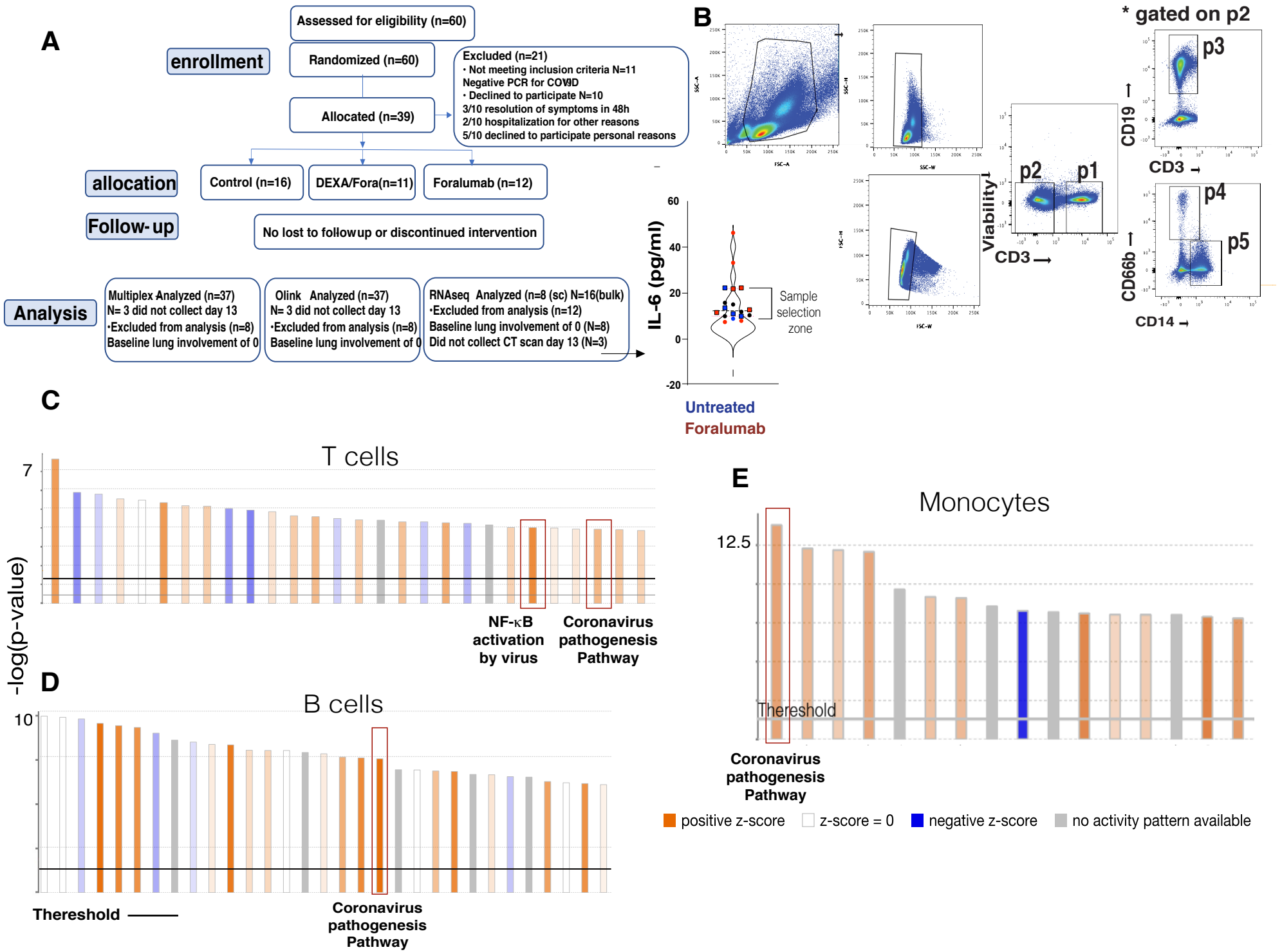
A
enrollment
Assessed for eligibility (n=60)
Randomized (n=60)
Excluded (n=21)
• Not meeting inclusion criteria N=11
Negative PCR for COVID
• Declined to participate N=10
3/10 resolution of symptoms in 48h
2/10 hospitalization for other reasons
5/10 declined to participate personal reasons
Allocated (n=39)
allocation
Control (n=16)
DEXA/Fora(n=11)
Foralumab (n=12)
Follow-up
No lost to followup or discontinued intervention
Analysis
Multiplex Analyzed (n=37)
N= 3 did not collect day 13
•Excluded from analysis (n=8)
Baseline lung involvement of 0
Olink Analyzed (n=37)
N= 3 did not collect day 13
•Excluded from analysis (n=8)
Baseline lung involvement of 0
RNAseq Analyzed (n=8 (sc) N=16(bulk)
•Excluded from analysis (n=12)
Baseline lung involvement of 0 (N=8)
Did not collect CT scan day 13 (N=3)
B
* gated on p2
IL-6 (pg/ml)
60
40
20
0
-20
Sample selection zone
Untreated
Foralumab
SSC-A
FSC-A
SSC-H
SSC-W
FSC-H
FSC-W
Viability
CD3
p1
p2
CD19
CD3
p3
CD66b
CD14
p4
p5
C
T cells
7
-log(p-value)
NF-κB activation by virus
Coronavirus pathogenesis Pathway
D
B cells
10
Thereshold
Coronavirus pathogenesis Pathway
E
Monocytes
12.5
Thereshold
Coronavirus pathogenesis Pathway
positive z-score
z-score = 0
negative z-score
no activity pattern available

Figure S1. Gating strategy for RNA-sequencing and Ingenuity Pathway Analysis A) Sample selection for RNA-sequencing. 39/60 patients were allocated. Samples from Fora/Dexa group were not elected for this study and thus 28/39 patients were selected. Further, 12/28 samples were removed because patients did present lung involvement or did not collect CT scan on endpoint and thus could not be evaluated. We then used IL-6 serum levels at baseline to define a sample selection zone that excluded skewed IL-6 serum levels. Samples within median and interquartile range were used for single cell RNA-seq analysis and it is shown in squared shape. Bulk RNA-seq **B)** Gating strategy for cell sorting: bulk RNA-seq (Smart-Seq2) and 10x single cell RNA-seq**.** PBMCs were stained with viability dye, CD3, CD19, CD66b and CD14 and FACS-sorted. Cells were gated on singlet+ Live+ cells. P1 ($CD3^+$), P3 ($CD19^+$) and P5 ($CD14^+$) were gated on P2, $CD3^-$. **C-E)** Ingenuity Pathways Analysis on DEG genes ($p<0.05$) of comparisons between healthy volunteers and COVID-19 subjects in $CD3^+$ T cells, $CD19^+$ B cells and CD14+ monocytes . N=7 healthy volunteers (HV), N=8 untreated controls and N=8 Foralumab treated

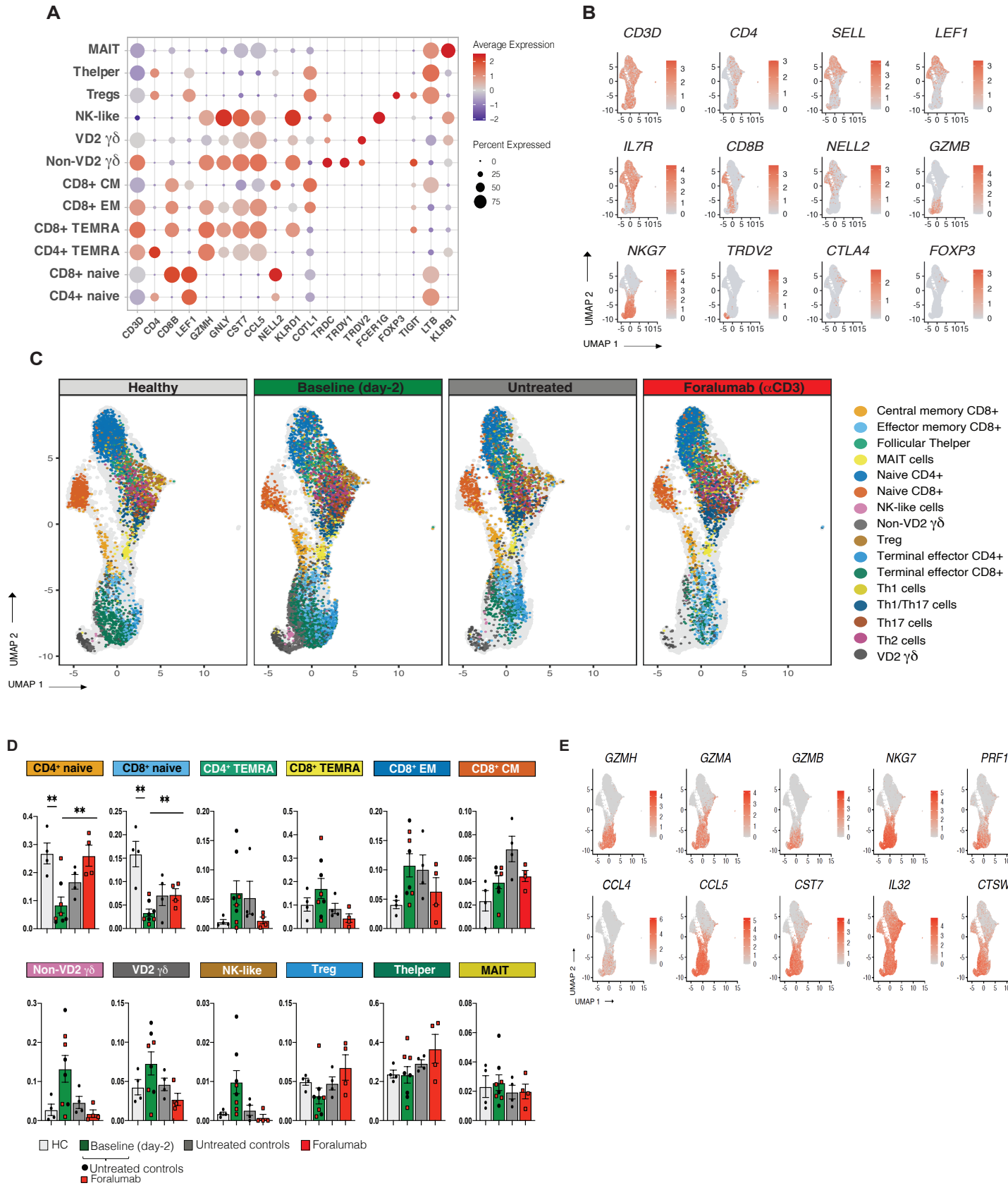
A
MAIT
Thelper
Tregs
NK-like
VD2 γδ
Non-VD2 γδ
CD8+ CM
CD8+ EM
CD8+ TEMRA
CD4+ TEMRA
CD8+ naive
CD4+ naive
CD3D CD4 CD8B LEF1 GZMH GNLY CST7 CCL5 NELL2 KLRD1 COTL1 TRDC TRDV1 TRDV2 FCER1G FOXP3 TIGIT LTB KLRB1
Average Expression
Percent Expressed
B
CD3D
CD4
SELL
LEF1
IL7R
CD8B
NELL2
GZMB
NKG7
TRDV2
CTLA4
FOXP3
UMAP 2
UMAP 1
C
Healthy
Baseline (day-2)
Untreated
Foralumab (αCD3)
UMAP 2
UMAP 1
Central memory CD8+
Effector memory CD8+
Follicular Thelper
MAIT cells
Naive CD4+
Naive CD8+
NK-like cells
Non-VD2 γδ
Treg
Terminal effector CD4+
Terminal effector CD8+
Th1 cells
Th1/Th17 cells
Th17 cells
Th2 cells
VD2 γδ
D
CD4+ naive
CD8+ naive
CD4+ TEMRA
CD8+ TEMRA
CD8+ EM
CD8+ CM
Non-VD2 γδ
VD2 γδ
NK-like
Treg
Thelper
MAIT
HC
Baseline (day-2)
Untreated controls
Foralumab
Untreated controls
Foralumab
E
GZMH
GZMA
GZMB
NKG7
PRF1
CCL4
CCL5
CST7
IL32
CTSW
UMAP 2
UMAP 1

Figure S2. T cell subtyping. A) Canonical cell markers for T cell subsets. **B)** UMAP plots of T cells showing localization of $CD3^+$ cell subset markers. **C)** CD3+ subsets including T helper stratification into Th1, Th2 and Th17 subsets. **D)** Graphic representation T cell subset distribution in healthy controls, untreated and Foralumab treated COVID-19 subjects at baseline (day-2) and at day 10 shown in Fig1E. Bars represent mean±SEM. One-way ANOVA followed by Tukey *post hoc* analysis ** $p<0.01$. **E)** UMAP plots of T cells showing localization of effector genes.

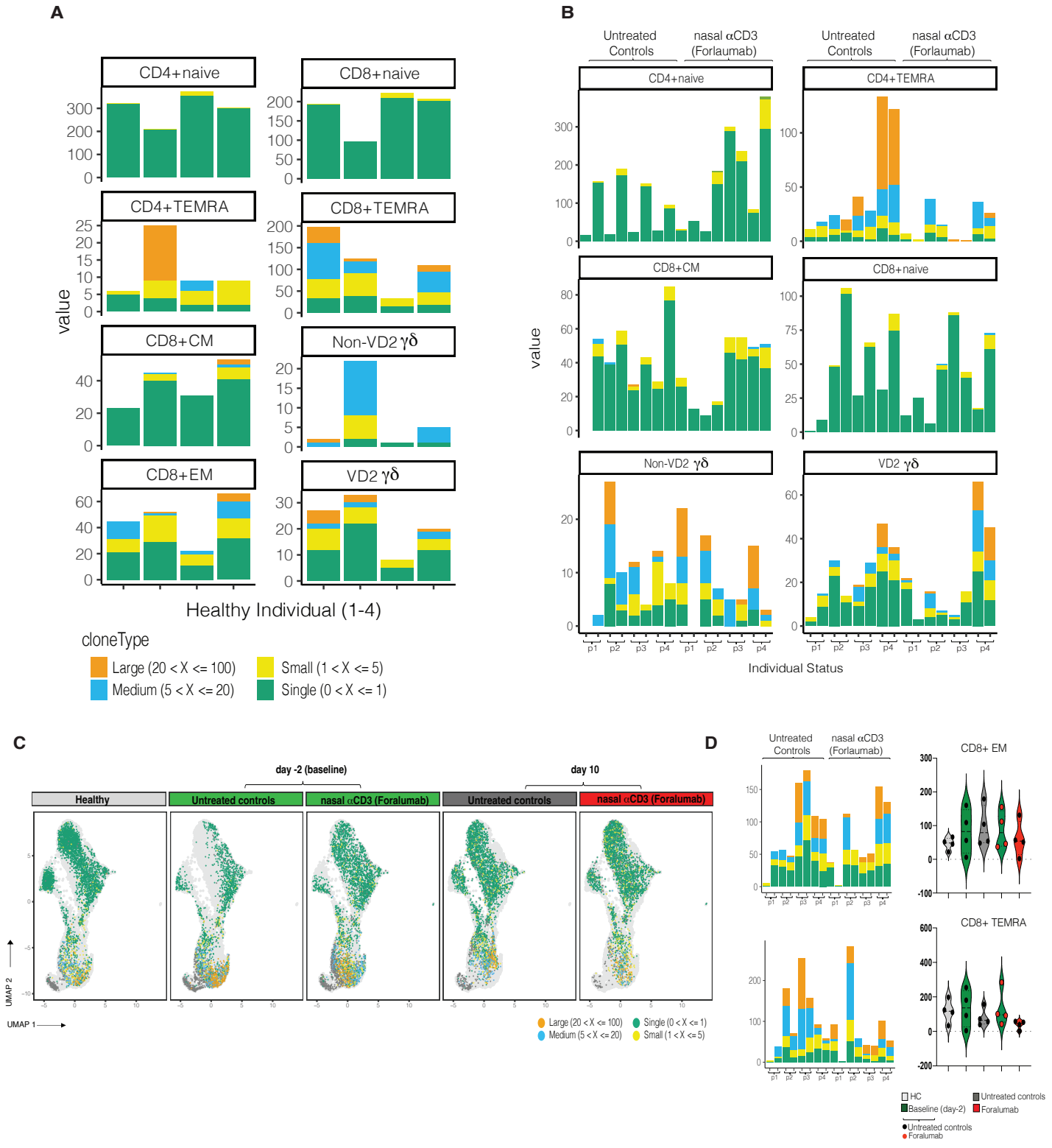

Figure S3. TCR sequencing in Foralumab treated subjects. A, B) TCR sharing patterns of specific T cell subsets by comparing usage of V(D)J genes on healthy controls **(A)**, untreated controls vs. Foralumab treated subjects **(B)**. **C)** Distribution within UMAP plots of T cells subsets. **D)** Violin plots showing the frequency of clonal T cells in $CD8^+$ TEMRA and $CD8^+$ CM cells (Median±IQR). Graphs show percentage of each clonal type and individual status. 4 healthy volunteers, 4 untreated controls (before and after) and 4 Foralumab treated subjects (before and after) were studied.

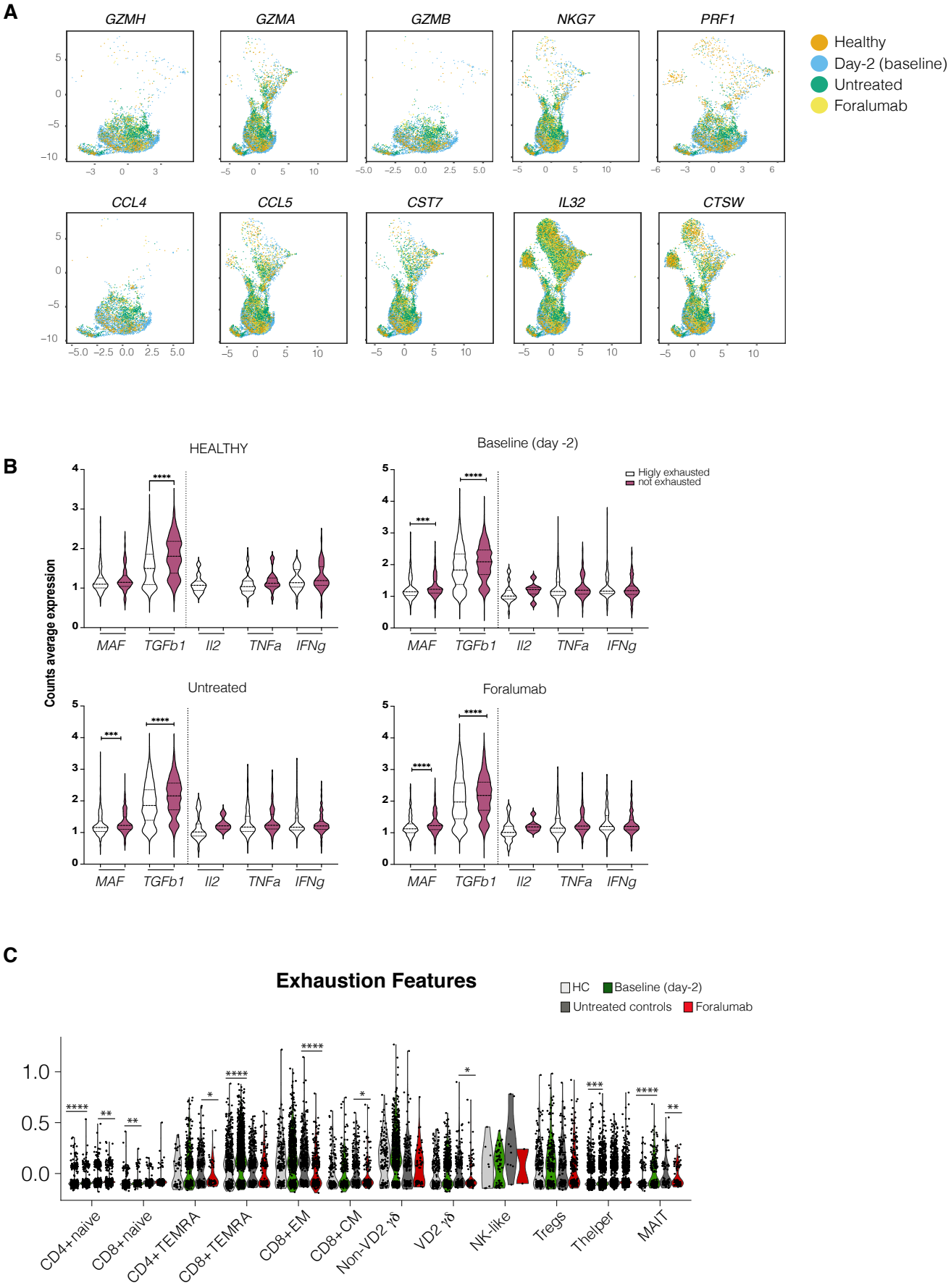
A
GZMH
GZMA
GZMB
NKG7
PRF1
CCL4
CCL5
CST7
IL32
CTSW
Healthy
Day-2 (baseline)
Untreated
Foralumab
B
HEALTHY
Baseline (day -2)
Untreated
Foralumab
Higly exhausted
not exhausted
Counts average expression
MAF
TGFb1
Il2
TNFa
IFNg
C
Exhaustion Features
HC
Baseline (day-2)
Untreated controls
Foralumab
CD4+naive
CD8+naive
CD4+TEMRA
CD8+TEMRA
CD8+EM
CD8+CM
Non-VD2 γδ
VD2 γδ
NK-like
Tregs
Thelper
MAIT

Figure S4. **Effector gene cluster and exhaustion modules in CD3+ T cells. A)** UMAP plots of T cells showing localization of effector genes in CD3+ cells across treatment. **B)** Exhausted score of >0.01 was used to determinate highly exhausted cells and compared to low/not exhausted cells (<0.01). *MAF, TGFB1, IL2, TNFa* and *IFNg* gene expression in exhausted and not exhausted cells is compared and shown in Violin-plots (Median±IQR). **C)** Distribution of exhaustion score within UMAP plots of CD3+ T cells subsets in in healthy controls, untreated COVID19 subjects and Foralumab treated subjects at baseline (day-2) and at day 10. Violin plots (Median±IQR) with individual points is shown. HC= healthy controls. One-way ANOVA, followed by Tukey *post hoc* analysis was performed and comparison between healthy vs. day-2 and untreated vs Foralumab is shown. * $P < 0.05$, ** $P < 0.01$, *** $p<0.001$, **** $P < 0.001$.

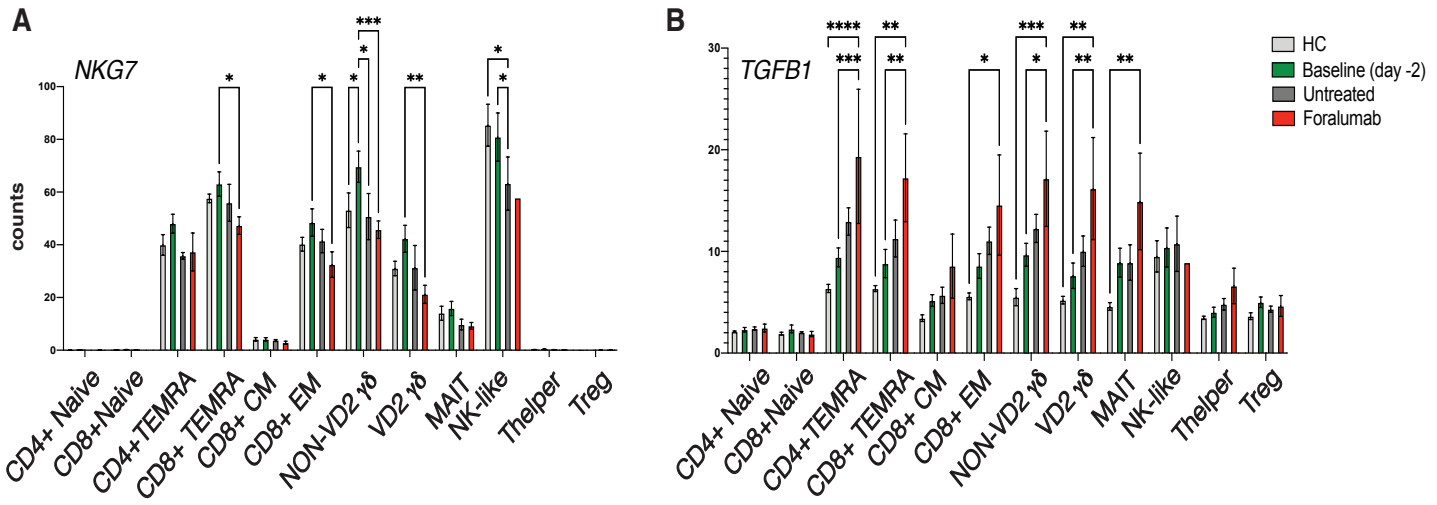

Figure S5. A, B) *NKG7* and *TGFB1* counts in CD3+ subsets in healthy controls, untreated and Foralumab treated COVID-19 subjects at baseline (day-2) and at day 10. *NKG7* **(A)** and *TGFB1* **(B)**. Bars represent mean±SEM. One-way ANOVA followed by Tukey *post hoc* analysis ** p<0.01. * P < 0.05, ** P < 0.01, **** P < 0.001.

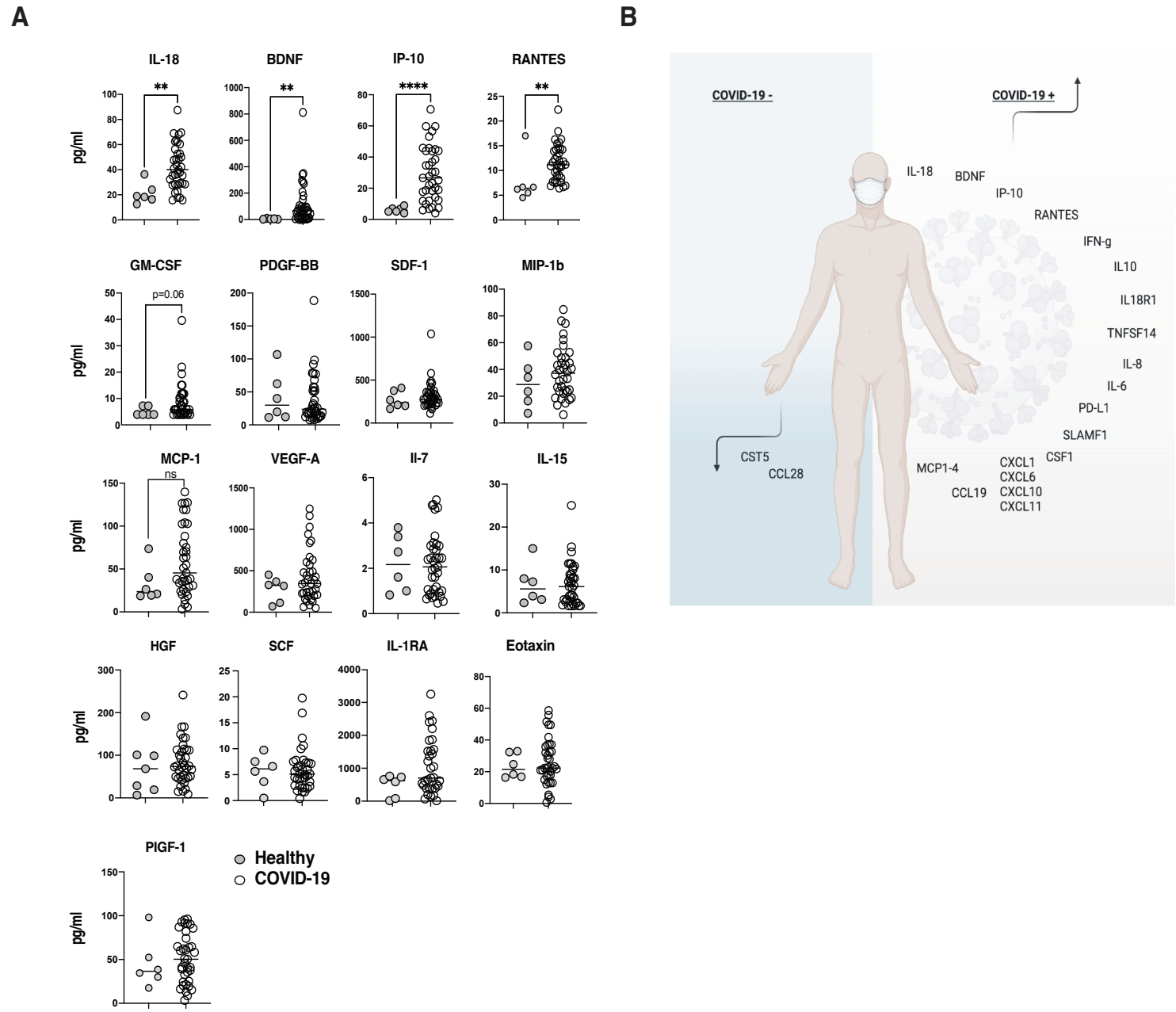

Figure S6. Serum markers altered by COVID-19 detected by proteomics. **A)** Comparison between healthy volunteers and COVID-19 subjects. Dots are individual values, bars represent mean±SEM. Student's t test. Healthy = healthy controls. COVID-19 refers to all COVID-19 subjects at baseline (day-2). **p < 0.01, **** P < 0.001. **B)** Resume of up and downregulated serum markers obtained by Olink.

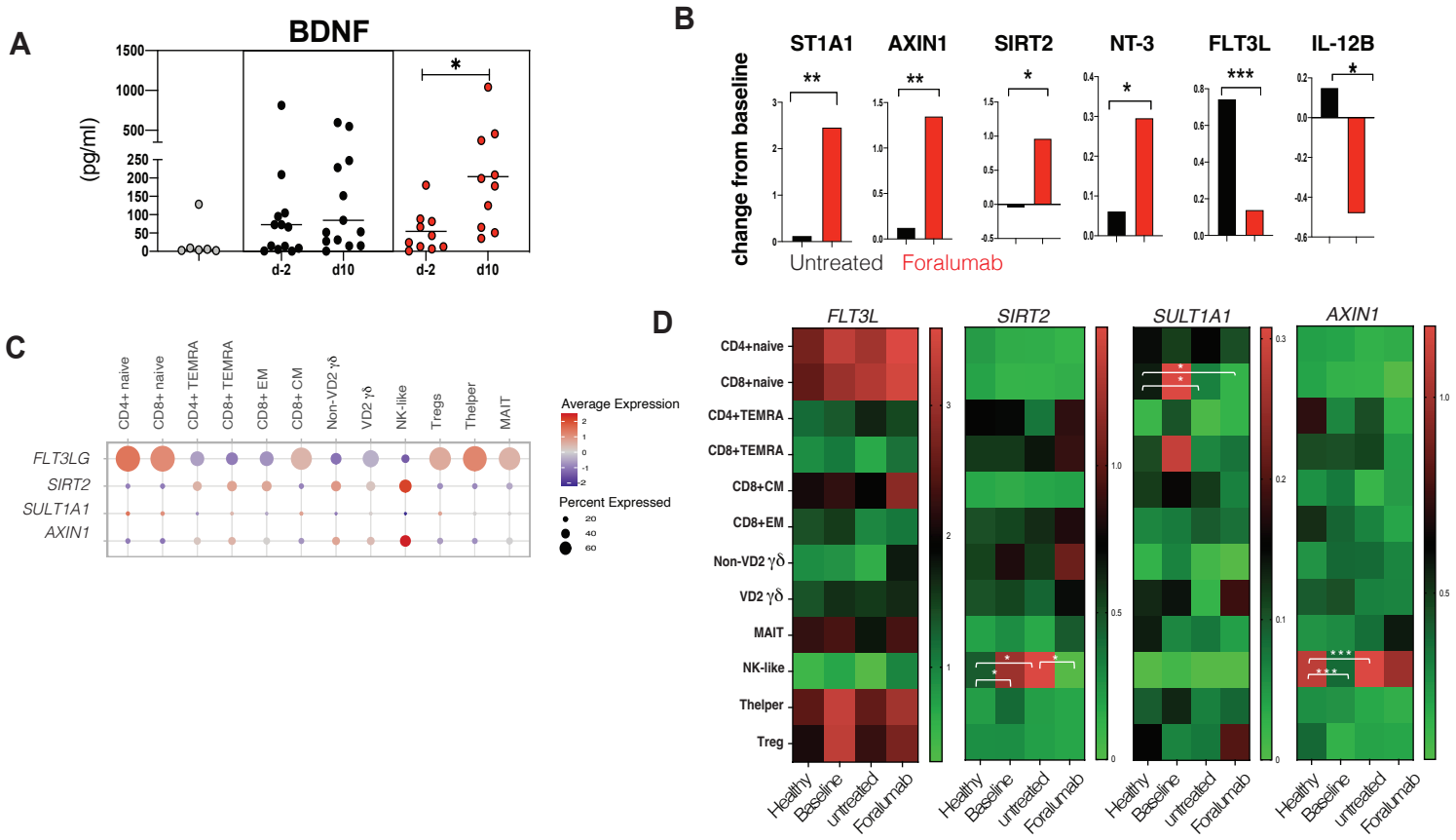

Figure S7. A) Serum BDNF before and after treatment measured by Multiplex. **B)** Serum ST1A1, AXIN1, SIRT2, NT-3, FLT3L and IL-12B change (before and after treatment) measured by Olink. Bars represent change from baseline. Student's t test. **C)** Dot plot showing average expression and percent of *FLT3G*, *SIRT2, SULT1A1* and *AXIN1* in CD3^{+} cell subsets. **D)** Heat maps showing gene expression of the corresponding proteins found to be modulated by Foralumab treatment in sera. One-way ANOVA, followed by Tukey *post hoc* analysis was used for analysis in D. * $P < 0.05$, ** $P < 0.01$, ***$P = 0.001$

Table S1. Subject demographics

		Foralumab		Untreated		Healthy controls	
	Total	Gender (F/M)	Age (yrs)	Gender (F/M)	Age (yrs)	Gender (F/M)	Age (yrs)
Proteomics	n=33	12 (10/2)	44.5 ± 11	15 (9/6)	31 ± 18.9	6 (5/1)	42.4 ± 7
Bulk-RNA	n=23	8 (7/1)	47.6 ± 9.1	8 (6/2)	39.8 ± 19.1	7 (2/5)	31.9 ± 5.1
scRNAseq	n=12	4 (3/1)	46.7± 8.3	4 (3/1)	48.7 ± 24.5	4 (2/2)	35.6 ± 2.5

Proteomics: Multiplex and Olink
Bulk-RNA: Smart2-seq
scRNAseq: 10X genomics
Age ± Standard Deviation

Table S2: Serum Biomarkers before and after treatment measured by Multiplex

	Control					Foralumab				
	Day -2	Day 10				Day -2	Day 10			
	Mean (±SD) #		Change		p-value*	Mean (±SD)		change		p-value
IL.18	34.8 (±18.9)	32.2 (±17.7)	-2.6; 95% CI: -9.6, 4.3	- Ψ	0.429	46.9 (±15.5)	37.6 (±12.6)	-9.3; 95% CI: -18.9, 0.2	- Ψ	0.054
BDNF	144.4 (±217.6)	174.3 (±191)	29.9; 95% CI: -109.2, 169	-	0.651	91.5 (±105.6)	279.6 (±274.6)	188.1; 95% CI: 50.8, 325.4	↑	0.012
VEGF-A	455.6 (±391.8)	525.1 (±359.6)	69.5; 95% CI: -98.1, 237.2	-	0.389	362.1 (±184.7)	610.4 (±310.2)	248.3; 95% CI: 103.8, 392.9	↑	0.003
PIGF-1	49.2 (±29)	67.4 (±38.6)	18.1; 95% CI: -0.8, 37.1	-	0.059	55.8 (±28.7)	87.1 (±41)	31.3; 95% CI: 12.2, 50.5	↑	0.004
SCF	6.3 (±4.6)	6.8 (±3.7)	0.5; 95% CI: -0.6, 1.6	-	0.316	5.5 (±3.2)	6.6 (±4.1)	1.1; 95% CI: 0.2, 2	↑	0.023
HGF	73 (±43.9)	125.5 (±72.4)	52.5; 95% CI: 23.9, 81.1	↑	0.001	89.6 (±41.1)	187.2 (±89.5)	97.6; 95% CI: 63.7, 131.6	↑	<0.001
PDGF-BB	34.6 (±31)	77.5 (±78.4)	42.9; 95% CI: 0.3, 85.5	↑	0.048	48 (±49.5)	159.2 (±219.4)	111.1; 95% CI: -1, 223.3	-	0.052
IP-10	28.2 (±16.5)	12.3 (±8)	-15.9; 95% CI: -23.1, -8.7	↓	<0.001	30.9 (±20.2)	13.4 (±6.7)	-17.5; 95% CI: -29, -6	↓	0.006

Standard Deviation (SD)

*: P-value = difference within groups before (day-2) and after treatment (day 10) and controls. CI= confidence intervals

Ψ Increase (↑) Decrease (↓) No change (-)

IL= Interleukin; Brain Derived Neutrophic Factor (BNDF); Interferon gamma inducible protein-10 (IP-10); Placental Growth Factor (PIGF); Stem cell Factor (SCF); Hepatocyte Growth Factor (HGF); Vascular Endothelial Growth Factor A (VEGF-A); Platelet Derived Growth Factor- (PDGF).