ISG report

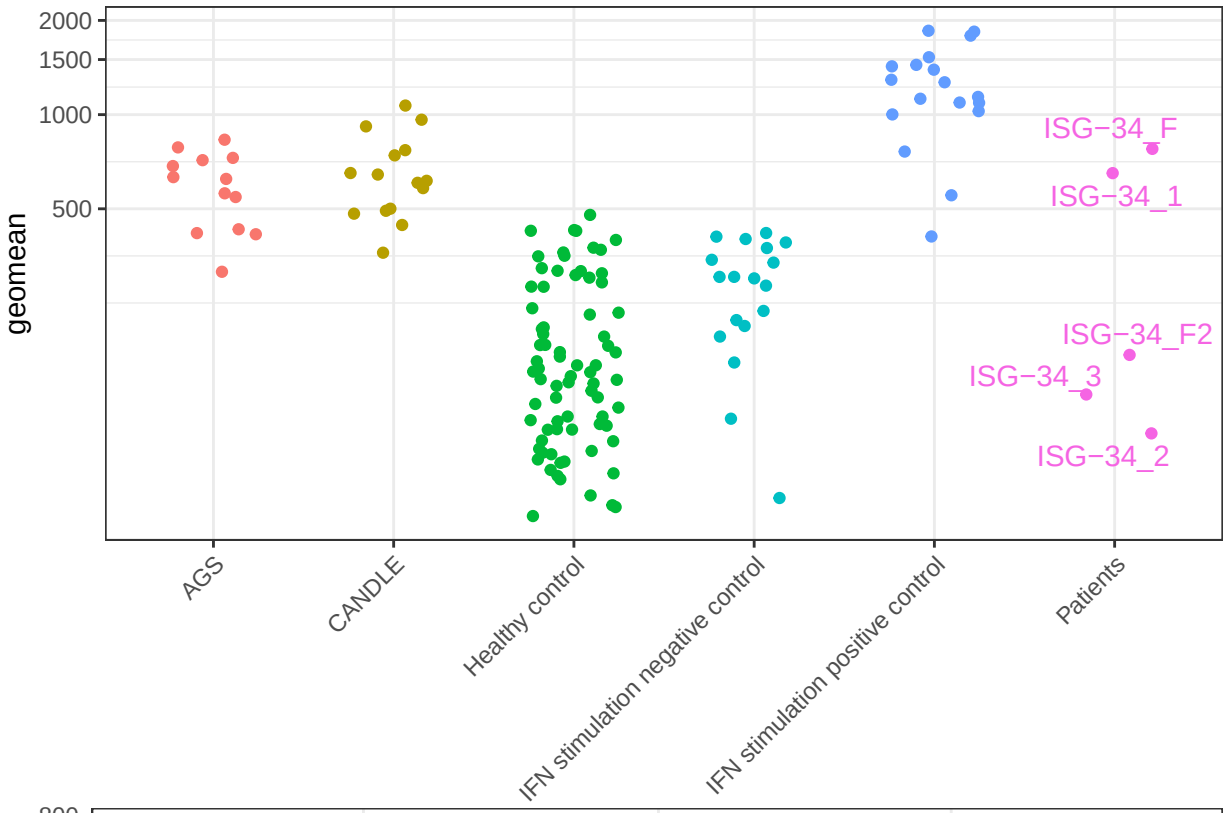
Patient ID: ISG-34

Contact: karin.palmblad@ki.se

Patient ID	ISG-34	ISG-34	ISG-34
Visit	1	2	3
Referring physician	Karin Palmblad	Karin Palmblad	Karin Palmblad
Sample collection location	Karolinska - Solna	Karolinska - Solna	Karolinska - Solna
Date of sampling	2023-02-07	2023-06-21	2023-11-01
Date of RNA extraction	2023-04-03	2023-10-11	2023-11-07
Date of experiment	2023-04-05	2023-10-12	2023-11-08
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-23-DN5231	EXP-23-DN5233	EXP-23-DN5234
Sample ID	12334	012847	12933
Panel version	v2	v2	v2

Patient ID	ISG-34	ISG-34
Visit	F	F2
Referring physician	Karin Palmblad	Karin Palmblad
Sample collection location	Karolinska - Solna	Karolinska - Solna
Date of sampling	2023-02-07	2023-11-01
Date of RNA extraction	2023-04-03	2023-11-07
Date of experiment	2023-04-05	2023-11-08
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-23-DN5231	EXP-23-DN5234
Sample ID	12337	12936
Panel version	v2	v2

Results (ISG)


2000
1500
1000
500
geomean
ISG−34_F
ISG−34_1
ISG−34_F2
ISG−34_3
ISG−34_2
AGS
CANDLE
Healthy control
IFN stimulation negative control
IFN stimulation positive control
Patients

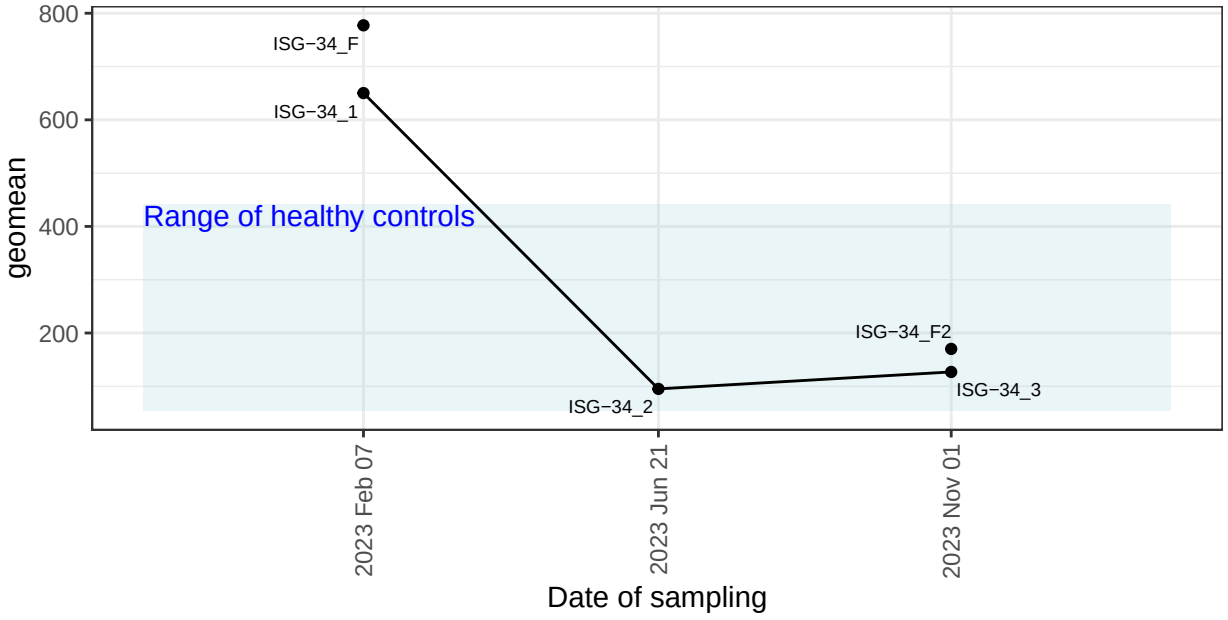

800
600
400
200
geomean
ISG−34_F
ISG−34_1
Range of healthy controls
ISG−34_F2
ISG−34_3
ISG−34_2
2023 Feb 07
2023 Jun 21
2023 Nov 01
Date of sampling

Table 1: ISG scores and the panel

	ISG-34_Visit1	ISG-34_Visit2	ISG-34_Visit3
geomean	650	95	127
zscore	75	-9	6
CEACAM6	300	107	191
CMPK2	370	45	50
CRISP3	546	207	486
DDX60	1022	460	580
DEFA4	591	251	323
EPSTI1	1031	231	282
FBXO39	74	18	6
HERC5	1636	150	196
HES4	41	16	8
IFI27	2270	29	77
IFI44	1798	199	232
IFI44L	3918	190	261
IFI6	868	56	66
IFIH1	314	124	183
IFIT1	2107	79	167
IRF7	313	139	143
ISG15	4355	320	489
LAMP3	54	21	21
LCN2	1557	384	868
LTF	1593	566	1148
LY6E	4854	434	430
MMP8	434	90	225
MX1	5666	386	774
NRIR	313	119	164
OAS1	1784	245	347
OASL	359	57	52
OTOF	20	5	3
RSAD2	5680	97	232
SIGLEC1	163	1	3
SPATS2L	156	34	35

Table 2: ISG scores and the panel

	ISG-34_VisitF2	ISG-34_VisitF
geomean	170	777
zscore	23	93
CEACAM6	438	559
CMPK2	81	626
CRISP3	513	326
DDX60	584	1353
DEFA4	1041	1374
EPSTI1	402	1357
FBXO39	20	76
HERC5	232	1521
HES4	10	40
IFI27	54	4232
IFI44	288	2604
IFI44L	286	5397
IFI6	91	921
IFIH1	233	409
IFIT1	154	2466
IRF7	143	318
ISG15	536	4425
LAMP3	31	63
LCN2	1065	1089
LTF	1372	1236
LY6E	383	5800
MMP8	279	318
MX1	614	5486
NRIR	204	639
OAS1	421	2428
OASL	83	442
OTOF	8	58
RSAD2	345	7007
SIGLEC1	9	86
SPATS2L	39	223

Results (NFkb)

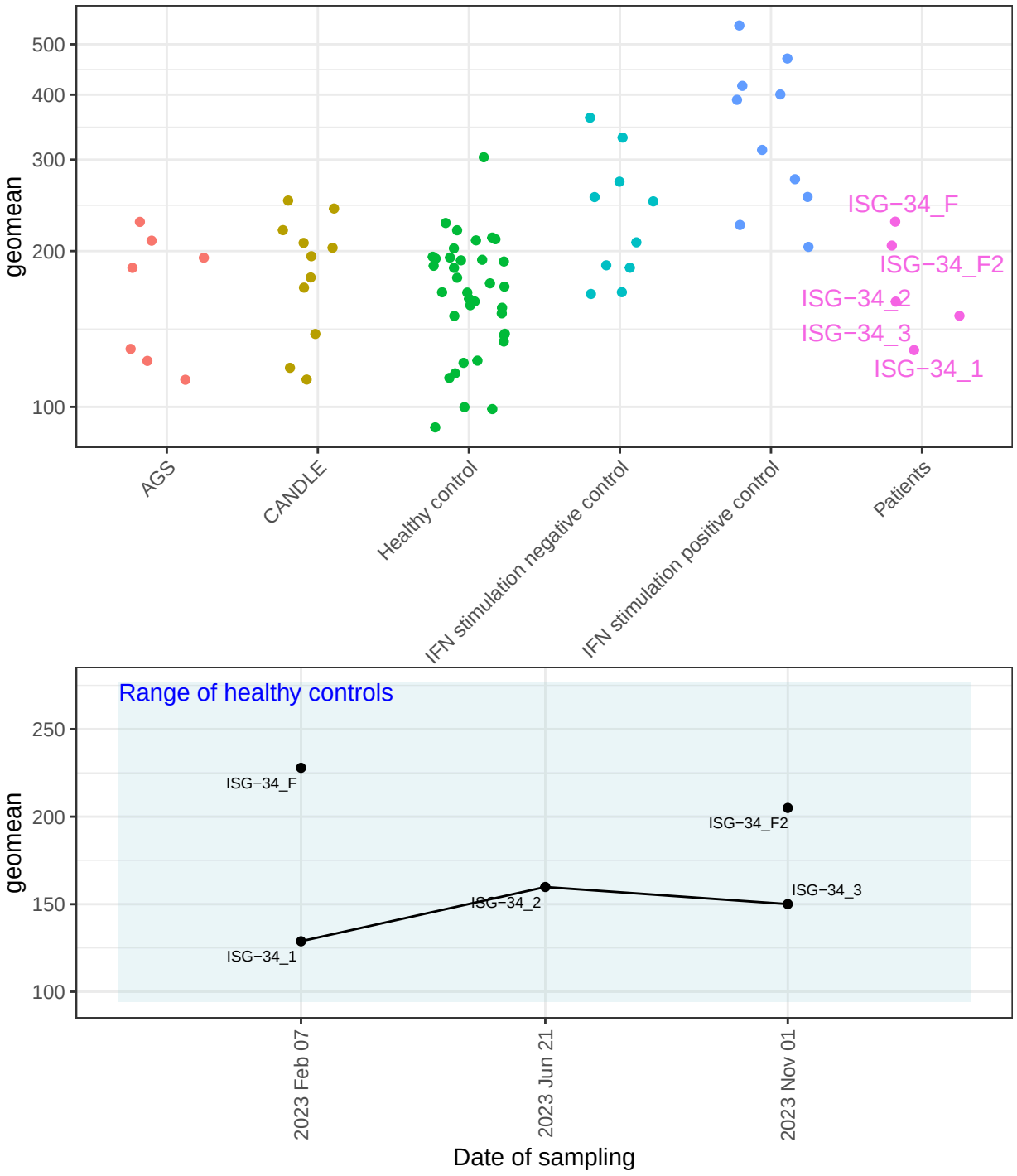


Table 3: NFkb scores and the panel

	ISG-34_Visit1	ISG-34_Visit2	ISG-34_Visit3
geomean	129	160	150
zscore	-3	-1	-1
AICDA	11	19	13
CCND2	105	173	235
EBI3	5	3	2
GZMB	393	801	483
IFNG	6	15	10
MSR1	35	28	35
SELL	8884	7097	8222
SELP	73	72	97
TANK	1057	2439	1988
TLR2	1132	932	786
XIAP	250	300	389

Table 4: NFkb scores and the panel

	ISG-34_VisitF2	ISG-34_VisitF
geomean	205	228
zscore	4	11
AICDA	8	21
CCND2	176	137
EBI3	4	12
GZMB	1355	1802
IFNG	33	27
MSR1	101	102
SELL	7102	9884
SELP	87	42
TANK	2594	1446
TLR2	977	2237
XIAP	414	308

Results (IFNg)

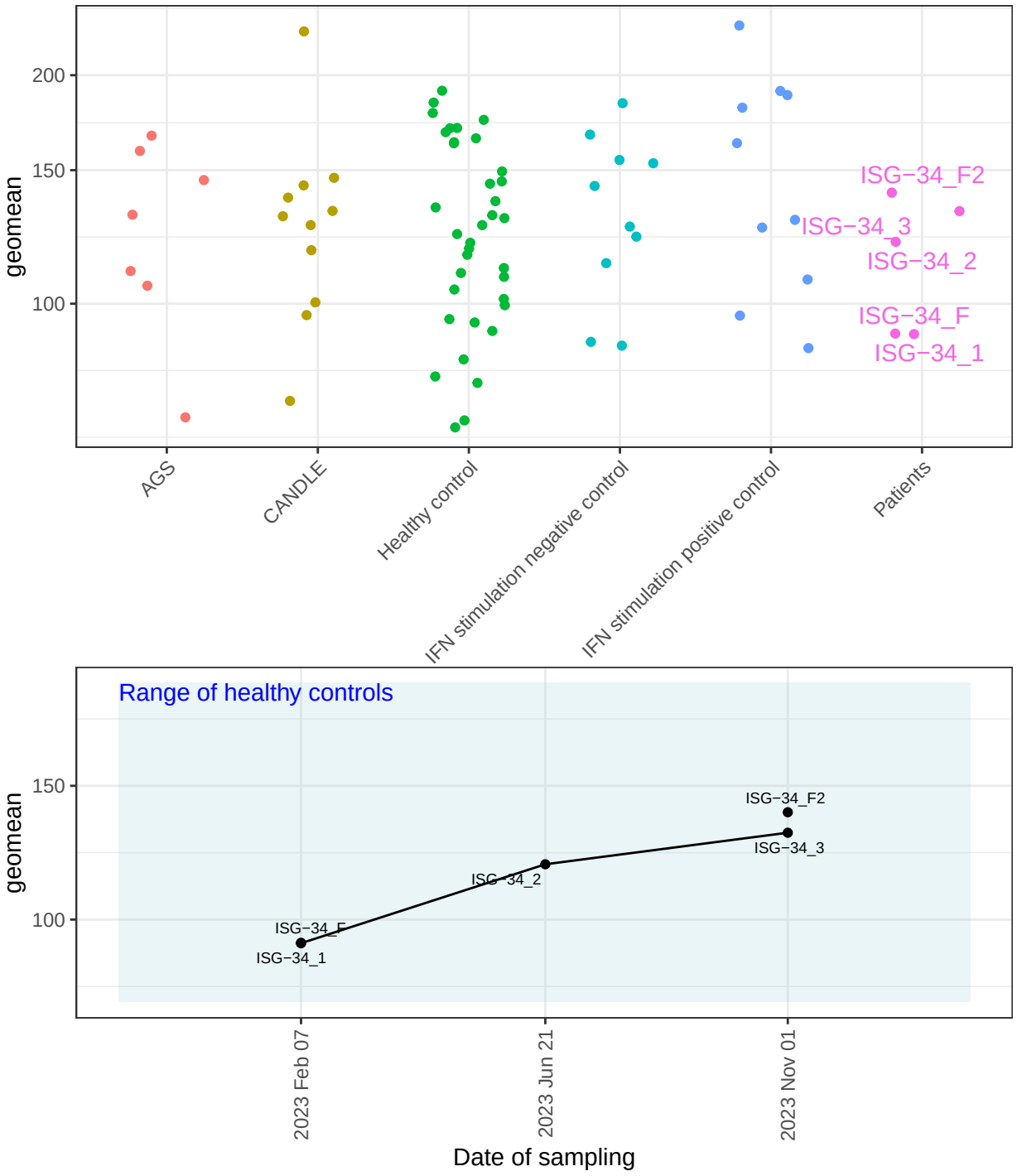


Table 5: IFNg scores and the panel

	ISG-34_Visit1	ISG-34_Visit2	ISG-34_Visit3
geomean	91	121	132
zscore	-8	-1	1
AHR	596	719	818
B4GALT3	172	359	413
BMF	58	119	115
CXCL9	5	4	4
DNA2	16	43	39
FBXL5	3657	3001	3084
GPR146	249	528	746
GPR18	70	180	227
IFITM1	12718	7368	8810
PKDCC	2	1	1
RAPGEF6	98	138	207
SELP	73	72	97
SLC5A6	39	53	52
TRIB3	20	23	16
TRIM16	27	48	48

Table 6: IFNg scores and the panel

	ISG-34_VisitF2	ISG-34_VisitF
geomean	140	91
zscore	4	-6
AHR	864	368
B4GALT3	461	206
BMF	146	64
CXCL9	26	9
DNA2	56	37
FBXL5	3785	3335
GPR146	632	193
GPR18	71	45
IFITM1	10596	20430
PKDCC	1	2
RAPGEF6	141	109
SELP	87	42
SLC5A6	39	23
TRIB3	23	26
TRIM16	44	27

Protocol

Sample processing

Blood drawn in an EDTA tube is, as soon as possible, aliquoted and mixed with PAXgene buffer in a 100:276 blood to PAXgene ratio, i.e. for 1 ml of blood, 2.76 ml of PAXgene buffer are used. After thoroughly mixing, the PAXgene sample is left at RT for a minimum of 1h to ensure blood cell lysis and then frozen at -80ºC until its use for RNA isolation.

RNA isolation

The PAXgene sample previously frozen at -80ºC is thawed and equilibrated at RT for 30 min - 1h. Then, the protocol from PreAnalytix for the automated RNA purification from PAXgene samples is followed. Briefly, the sample is centrifuged twice, and the cell pellet resuspended in a buffer provided in the PAXgene kit. The following column-based purification steps for RNA isolation are performed automatically in the QIAcube (liquid handling platform from QIAGEN). Two aliquots of the eluted RNA are taken for the subsequent concentration measurement and integrity check. The remaining sample is frozen at -80ºC until its use for gene expression analyses.

Gene expression analyses

The gene expression levels of 56 immune-related genes and 3 housekeeping genes are measured using NanoString Technologies. For this, a hybridization reaction between the mRNA molecules in the sample and a set of oligonucleotide probes, designed to capture the specific genes of interest, is carried out following NanoString's recommendations. Briefly, around 5 µl of RNA sample are mixed with the oligonucleotide probes and incubated in a thermocycler at 65ºC, with a heated lid at 70ºC, for 20h. Once the reaction time is completed, the sample is loaded into a cartridge designed to be read by NanoString's nCounter instrument. The cartridge is then placed inside the nCounter and the gene expression assay is carried out within the instrument, which in the end provides a readout with raw mRNA counts of the genes of study.

Clinical samples, along with healthy donor reference samples, are run in the nCounter in batches of 12.

Gene expression data analysis

First, a quality check of the data is done by the nSolver software provided by NanoString. Then, as recommended by Nanostring, the data is pre-processed in two different normalization steps: 1. Internal positive control normalization. 2. Housekeeping genes normalization.

After normalization, two different scores (Z-score and geomean score) are calculated to provide a summary of the expression levels of type I IFN–stimulated genes (ISG scores), NF-kB–regulated genes (NF-kB scores) and type II IFN–regulated genes (IFN-gamma scores)1,2. Of note, NanoString's products, along with any assays developed with its components are intended for research purposes only.

References

1. Kim, H., de Jesus, A. A., Brooks, S. R., Liu, Y., Huang, Y., VanTries, R., … & Goldbach-Mansky, R. (2018). Development of a validated interferon score using NanoString technology. Journal of Interferon & Cytokine Research, 38(4), 171-185.
2. Abers, M. S., Delmonte, O. M., Ricotta, E. E., Fintzi, J., Fink, D. L., de Jesus, A. A. A., … & NIAID COVID-19 Consortium. (2021). An immune-based biomarker signature is associated with mortality in COVID-19 patients. JCI insight, 6(1).