ISG report

Patient ID: ISG-1

Contact: petter.brodin@ki.se, annacarin.horne@ki.se

Patient ID	ISG-1	ISG-1	ISG-1
Visit	1	2	3
Referring physician	Petter Brodin	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna	Karolinska - Solna
Date of sampling	2021-03-25	2022-02-03	2023-01-13
Date of RNA extraction	2021-03-26	2022-03-30	2023-01-30
Date of experiment	2021-03-30	2022-04-01	2023-02-03
Experimental probes lot	RC7448X2 and CP8129X2	RC9753X1 and CP9753X1	RC9753X2 and CP9753X2
Experiment batch	EXP-21-DN5206	EXP-22-DN5218	EXP-22-run21
Sample ID	100219	11413	12312
Panel version	v1	v2	v2

Patient ID	ISG-1	ISG-1	ISG-1
Visit	4	5	6
Referring physician	AnnaCarin Horne	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna	Karolinska - Solna
Date of sampling	2023-03-09	2023-04-05	2023-04-21
Date of RNA extraction	2023-04-03	2023-04-11	2023-04-24
Date of experiment	2023-04-05	2023-04-12	2023-04-25
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-23-DN5231	EXP-23-DN5230	EXP-23-DN5232
Sample ID	12357	12380	12396
Panel version	v2	v2	v2

Patient ID	ISG-1	ISG-1
Visit	7	8
Referring physician	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna
Date of sampling	2023-06-05	2023-06-27
Date of RNA extraction	2023-10-11	2023-10-11
Date of experiment	2023-10-12	2023-10-12
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-23-DN5233	EXP-23-DN5233
Sample ID	012762	12851
Panel version	v2	v2

Patient ID	ISG-1	ISG-1
Visit	F	M
Referring physician	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna
Date of sampling	2021-03-25	2021-03-25
Date of RNA extraction	2021-03-26	2021-03-26
Date of experiment	2021-03-30	2021-03-30
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-21-DN5206	EXP-21-DN5206
Sample ID	100220	100221
Panel version	v1	v1

Results (ISG)

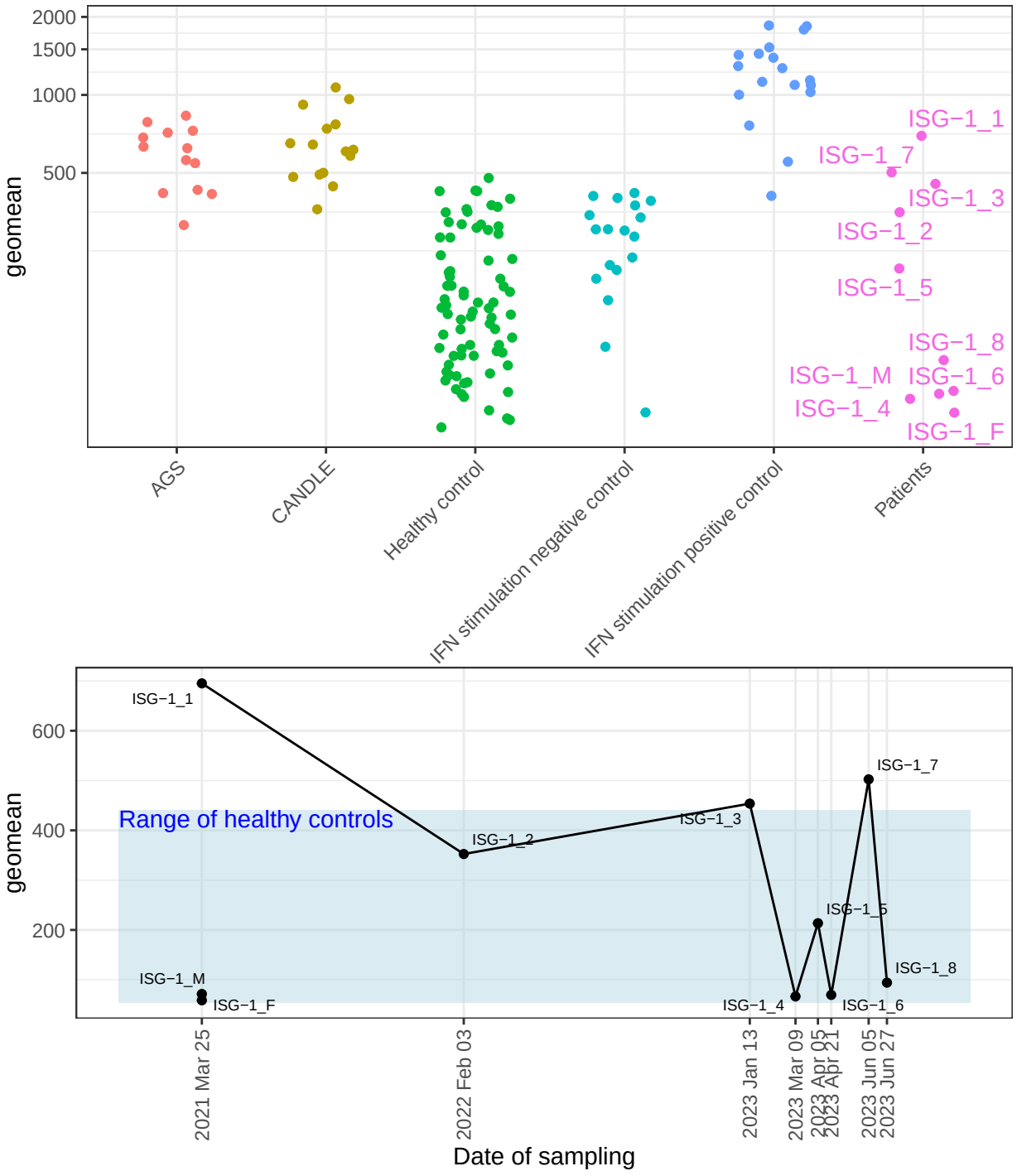

2000
1500
1000
500
geomean
ISG−1_1
ISG−1_7
ISG−1_3
ISG−1_2
ISG−1_5
ISG−1_8
ISG−1_M
ISG−1_6
ISG−1_4
ISG−1_F
AGS
CANDLE
Healthy control
IFN stimulation negative control
IFN stimulation positive control
Patients
600
400
200
geomean
Range of healthy controls
ISG−1_1
ISG−1_2
ISG−1_3
ISG−1_7
ISG−1_5
ISG−1_8
ISG−1_M
ISG−1_F
ISG−1_4
ISG−1_6
2021 Mar 25
2022 Feb 03
2023 Jan 13
2023 Mar 09
2023 Apr 05
2023 Apr 21
2023 Jun 05
2023 Jun 27
Date of sampling

Table 1: ISG scores and the panel

	ISG-1_Visit1	ISG-1_Visit2	ISG-1_Visit3	ISG-1_Visit4
geomean	695	352	454	66
zscore	117	27	40	-20
CEACAM6	96	107	31	44
CMPK2	889	279	441	29
CRISP3	86	263	118	82
DDX60	1408	329	1128	319
DEFA4	234	350	43	101
EPSTI1	550	493	1322	109
FBXO39	35	34	30	18
HERC5	1062	509	855	179
HES4	41	30	32	6
IFI27	336020	26020	7769	18
IFI44	2515	1778	2612	218
IFI44L	6175	4417	6475	208
IFI6	1352	552	822	48
IFIH1	657	319	460	130
IFIT1	1603	432	1439	18
IRF7	410	174	452	91
ISG15	7319	2550	4040	224
LAMP3	79	18	85	26
LCN2	356	752	192	198
LTF	787	1934	271	156
LY6E	2589	1964	2421	500
MMP8	306	210	112	14
MX1	6565	1475	5470	422
NRIR	558	305	317	83
OAS1	3240	691	2421	195
OASL	280	97	244	32
OTOF	44	6	13	5
RSAD2	8747	2247	4411	77
SIGLEC1	72	51	207	6
SPATS2L	214	51	238	43

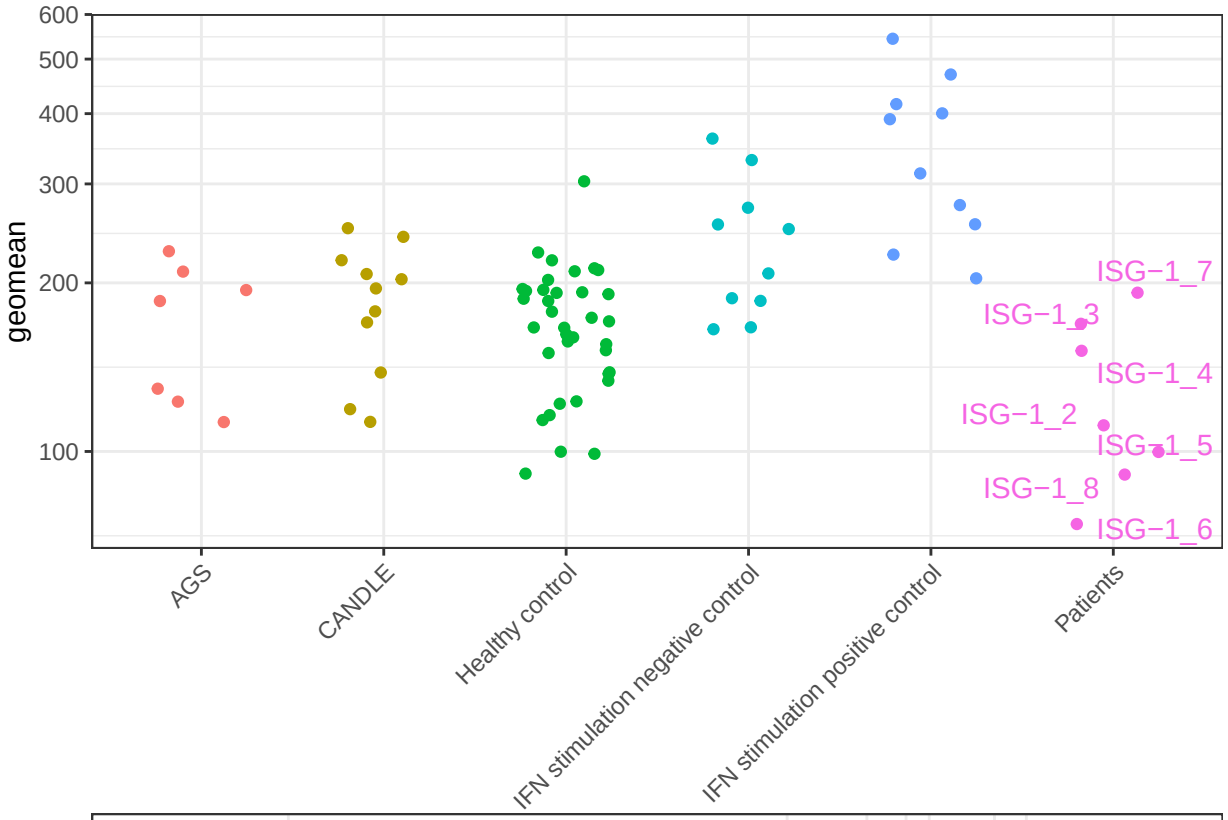
Table 2: ISG scores and the panel

	ISG-1_Visit5	ISG-1_Visit6	ISG-1_Visit7
geomean	213	70	502
zscore	14	-16	49
CEACAM6	68	58	61
CMPK2	278	84	355
CRISP3	167	135	158
DDX60	248	132	1519
DEFA4	200	124	118
EPSTI1	414	177	1449
FBXO39	30	11	46
HERC5	84	36	653
HES4	6	13	84
IFI27	71981	293	24658
IFI44	374	93	2340
IFI44L	792	81	7272
IFI6	488	28	803
IFIH1	112	72	329
IFIT1	242	28	800
IRF7	62	41	294
ISG15	2437	232	4045
LAMP3	13	7	54
LCN2	262	264	234
LTF	573	371	510
LY6E	515	201	2354
MMP8	333	184	150
MX1	680	191	3939
NRIR	538	268	331
OAS1	401	225	1685
OASL	62	15	185
OTOF	9	1	25
RSAD2	3626	710	3112
SIGLEC1	38	2	238
SPATS2L	27	35	225

Table 3: ISG scores and the panel

	ISG-1_Visit8	ISG-1_VisitF	ISG-1_VisitM
geomean	94	59	71
zscore	-2	-24	-21
CEACAM6	137	15	6
CMPK2	231	47	73
CRISP3	166	20	15
DDX60	153	328	467
DEFA4	331	37	40
EPSTI1	110	54	59
FBXO39	19	8	7
HERC5	29	147	246
HES4	6	10	4
IFI27	634	55	55
IFI44	121	122	176
IFI44L	108	123	250
IFI6	24	74	98
IFIH1	94	192	233
IFIT1	35	101	187
IRF7	43	178	226
ISG15	187	139	301
LAMP3	10	9	21
LCN2	523	49	72
LTF	1298	37	50
LY6E	150	384	462
MMP8	386	7	8
MX1	238	1209	1576
NRIR	435	46	50
OAS1	269	715	574
OASL	19	83	132
OTOF	6	1	1
RSAD2	1373	129	241
SIGLEC1	3	10	16
SPATS2L	24	35	47

Results (NFkb)



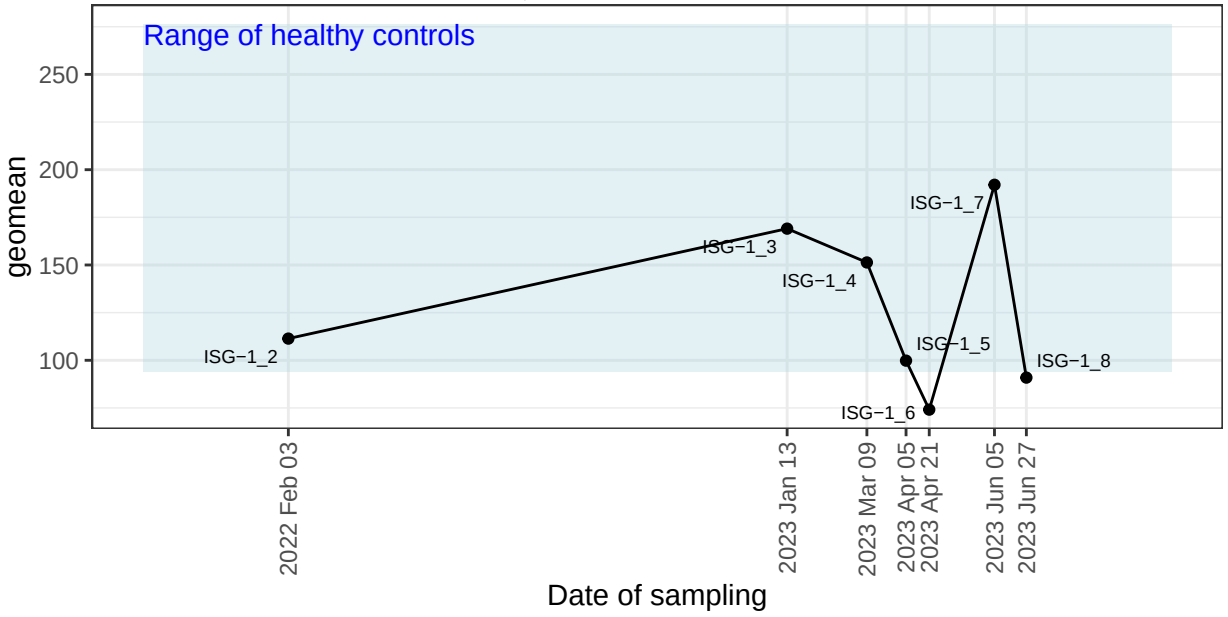


Table 4: NFkb scores and the panel

	ISG-1_Visit2	ISG-1_Visit3	ISG-1_Visit4	ISG-1_Visit5
geomean	111	169	151	100
zscore	-7	4	-1	-6
AICDA	8	8	8	21
CCND2	75	274	189	77
EBI3	2	7	6	6
GZMB	321	376	642	131
IFNG	12	5	9	41
MSR1	46	230	32	28
SELL	3470	5228	5399	1446
SELP	77	122	158	88
TANK	1978	2300	1042	2441
TLR2	1132	613	1331	120
XIAP	137	363	274	126

Table 5: NFkb scores and the panel

	ISG-1_Visit6	ISG-1_Visit7	ISG-1_Visit8
geomean	74	192	91
zscore	-14	3	-11
AICDA	6	12	6
CCND2	125	279	161
EBI3	1	13	4
GZMB	304	633	125
IFNG	2	21	4
MSR1	41	172	35
SELL	2819	3990	2198
SELP	44	93	82
TANK	1353	2191	2147
TLR2	190	433	311
XIAP	155	304	203

Results (IFNg)

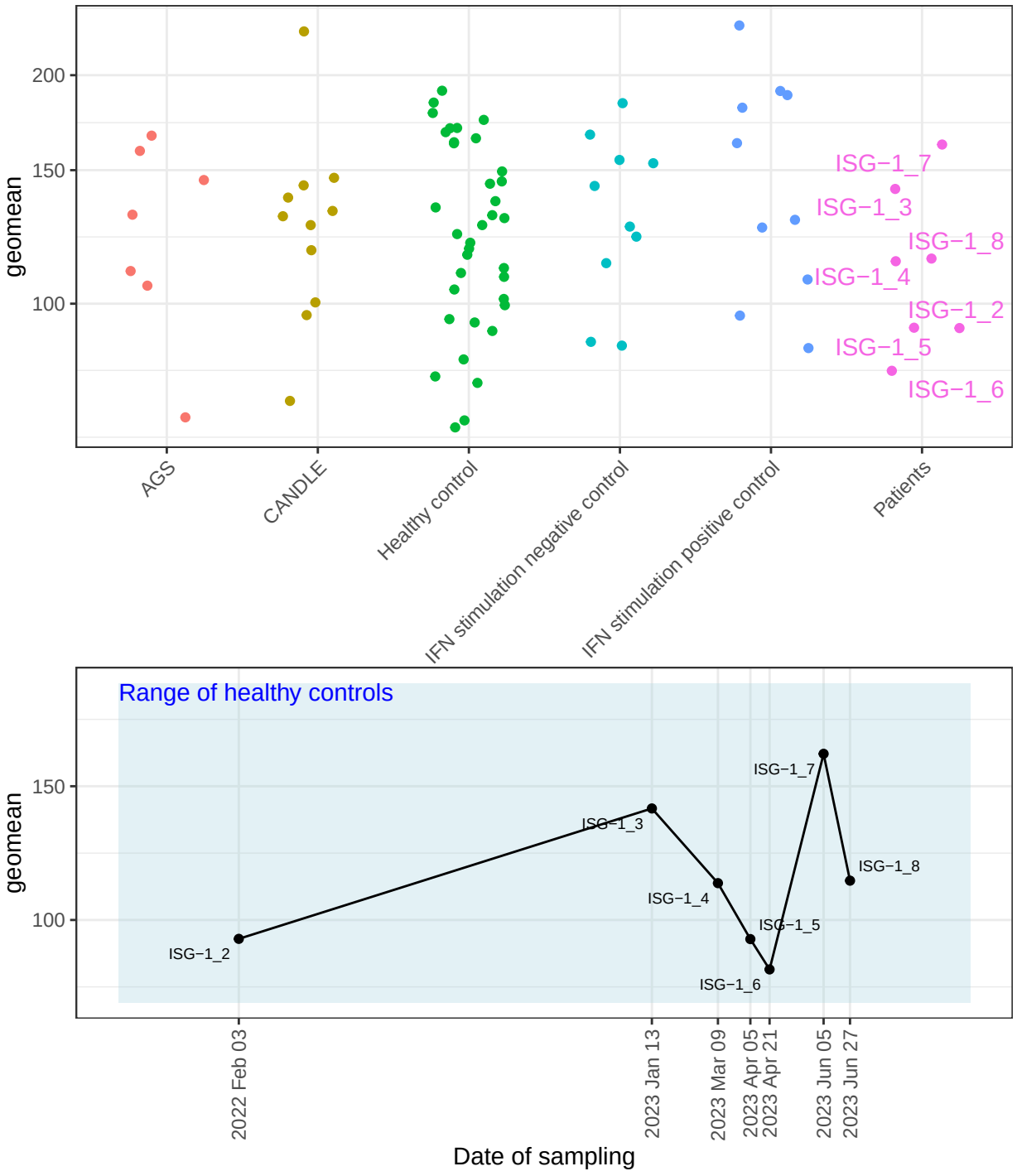


Table 6: IFNg scores and the panel

	ISG-1_Visit2	ISG-1_Visit3	ISG-1_Visit4	ISG-1_Visit5
geomean	93	142	114	93
zscore	-5	5	0	-1
AHR	1194	1468	684	387
B4GALT3	564	466	422	1157
BMF	145	96	146	49
CXCL9	2	8	1	3
DNA2	24	49	21	55
FBXL5	1336	2801	1685	706
GPR146	1926	1161	632	1869
GPR18	77	205	182	60
IFITM1	11609	11674	4044	2477
PKDCC	2	0	1	8
RAPGEF6	32	147	146	39
SELP	77	122	158	88
SLC5A6	57	55	64	30
TRIB3	4	21	25	13
TRIM16	8	36	55	22

Table 7: IFNg scores and the panel

	ISG-1_Visit6	ISG-1_Visit7	ISG-1_Visit8
geomean	82	162	115
zscore	-10	7	3
AHR	832	1020	847
B4GALT3	622	432	1247
BMF	64	160	69
CXCL9	2	8	4
DNA2	40	42	56
FBXL5	1391	2581	1510
GPR146	1429	1050	2041
GPR18	69	256	69
IFITM1	3194	14228	2441
PKDCC	2	4	6
RAPGEF6	70	158	99
SELP	44	93	82
SLC5A6	28	62	24
TRIB3	4	21	16
TRIM16	19	48	26

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).