ISG report

Patient ID: ISG-6

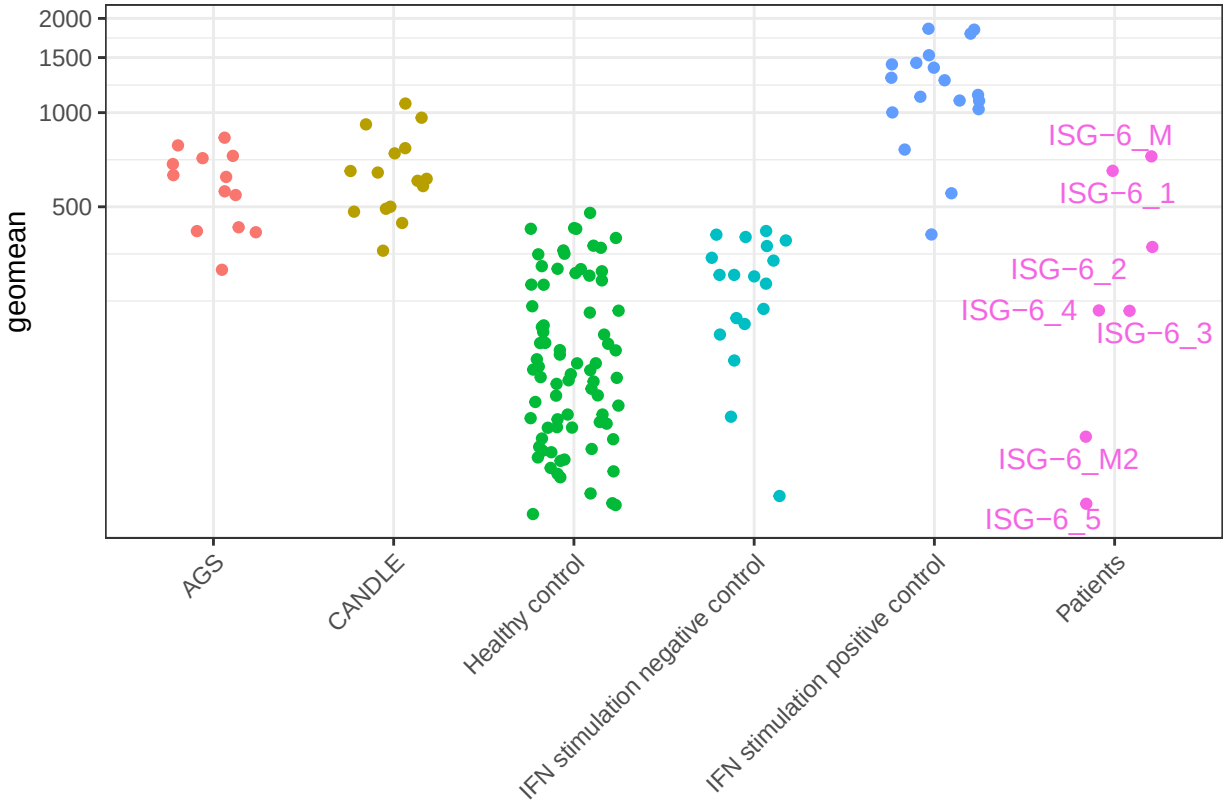
Contact: petter.brodin@ki.se, annacarin.horne@ki.se

Patient ID	ISG-6	ISG-6	ISG-6
Visit	1	2	3
Referring physician	Petter Brodin	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna	Karolinska - Solna
Date of sampling	2021-06-23	2022-12-22	2023-03-02
Date of RNA extraction	2021-07-19	2023-01-30	2023-04-03
Date of experiment	2021-07-23	2023-02-03	2023-04-05
Experimental probes lot	RC7448X2 and CP8129X2	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-21-DN5209	EXP-22-run21	EXP-23-DN5231
Sample ID	11070	12262	12354
Panel version	v1	v2	v2

Patient ID	ISG-6	ISG-6
Visit	4	5
Referring physician	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna
Date of sampling	2023-03-10	2023-04-05
Date of RNA extraction	2023-04-03	2023-04-11
Date of experiment	2023-04-05	2023-04-12
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-23-DN5231	EXP-23-DN5230
Sample ID	12362	12374
Panel version	v2	v2

Patient ID	ISG-6	ISG-6
Visit	M	M2
Referring physician	AnnaCarin Horne	AnnaCarin Horne
Sample collection location	Karolinska - Solna	Karolinska - Solna
Date of sampling	2023-01-26	2023-09-04
Date of RNA extraction	2023-01-30	2023-11-07
Date of experiment	2023-02-03	2023-11-08
Experimental probes lot	RC9753X2 and CP9753X2	RC9753X2 and CP9753X2
Experiment batch	EXP-22-run21	EXP-23-DN5234
Sample ID	12304	12881
Panel version	v2	v2

Results (ISG)


2000
1500
1000
500
geomean
ISG−6_M
ISG−6_1
ISG−6_2
ISG−6_4
ISG−6_3
ISG−6_M2
ISG−6_5
AGS
CANDLE
Healthy control
IFN stimulation negative control
IFN stimulation positive control
Patients

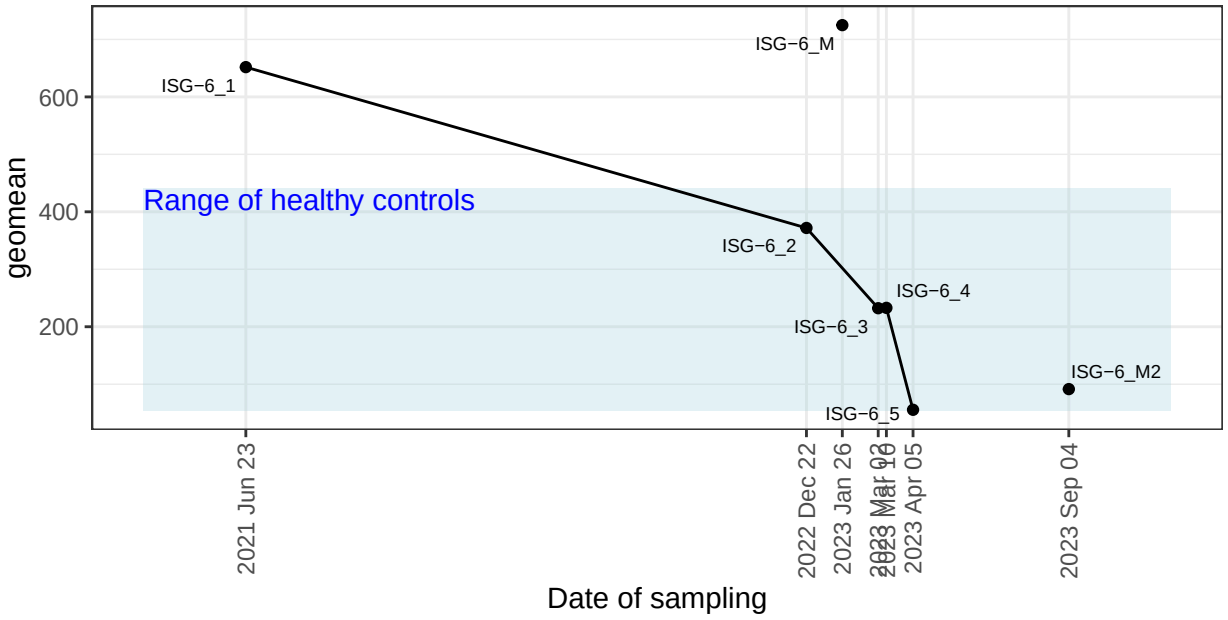

600
400
200
geomean
ISG−6_M
ISG−6_1
Range of healthy controls
ISG−6_2
ISG−6_4
ISG−6_3
ISG−6_M2
ISG−6_5
2021 Jun 23
2022 Dec 22
2023 Jan 26
2023 Apr 05
2023 Sep 04
Date of sampling

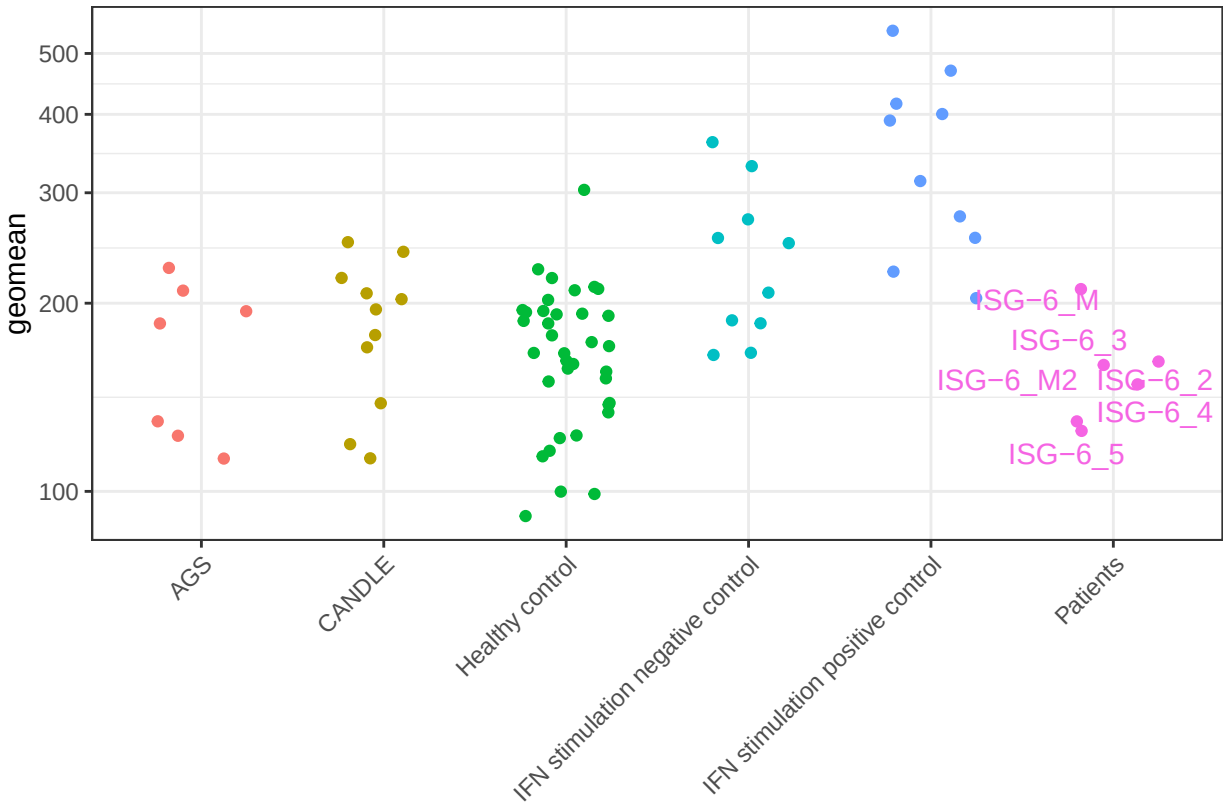
Table 1: ISG scores and the panel

	ISG-6_Visit1	ISG-6_Visit2	ISG-6_Visit3	ISG-6_Visit4
geomean	652	372	232	233
zscore	111	31	1	3
CEACAM6	18	29	55	23
CMPK2	1321	447	210	194
CRISP3	38	78	56	47
DDX60	3358	1571	723	551
DEFA4	80	52	76	36
EPSTI1	736	980	515	655
FBXO39	59	50	37	39
HERC5	3005	1127	508	421
HES4	57	23	15	19
IFI27	18494	2590	5353	6541
IFI44	6692	1577	844	1405
IFI44L	16843	5480	1886	3258
IFI6	1407	613	446	371
IFIH1	1087	389	215	213
IFIT1	5694	1461	740	407
IRF7	900	501	170	196
ISG15	5231	2864	1353	1783
LAMP3	115	62	17	36
LCN2	66	141	116	91
LTF	16	108	73	94
LY6E	6255	3664	2339	1325
MMP8	19	20	20	62
MX1	11178	6620	2555	2526
NRIR	542	222	197	223
OAS1	6685	2303	1087	1014
OASL	750	328	193	98
OTOF	37	13	10	7
RSAD2	11169	3368	2026	2066
SIGLEC1	183	173	92	112
SPATS2L	388	137	70	106

Table 2: ISG scores and the panel

	ISG-6_Visit5	ISG-6_VisitM2	ISG-6_VisitM
geomean	55	91	725
zscore	-23	-16	163
CEACAM6	27	34	22
CMPK2	30	70	1143
CRISP3	38	27	39
DDX60	342	428	712
DEFA4	44	45	57
EPSTI1	115	159	1262
FBXO39	14	8	96
HERC5	129	306	7769
HES4	4	31	265
IFI27	18	39	5341
IFI44	181	261	6341
IFI44L	174	341	6214
IFI6	48	134	1029
IFIH1	145	159	3722
IFIT1	30	278	3678
IRF7	112	182	875
ISG15	295	485	11384
LAMP3	20	27	122
LCN2	88	92	154
LTF	52	30	94
LY6E	432	323	12402
MMP8	8	3	17
MX1	404	1126	7340
NRIR	53	175	1047
OAS1	269	403	1861
OASL	26	130	2851
OTOF	5	4	35
RSAD2	82	354	8554
SIGLEC1	5	27	194
SPATS2L	38	32	198

Results (NFkb)



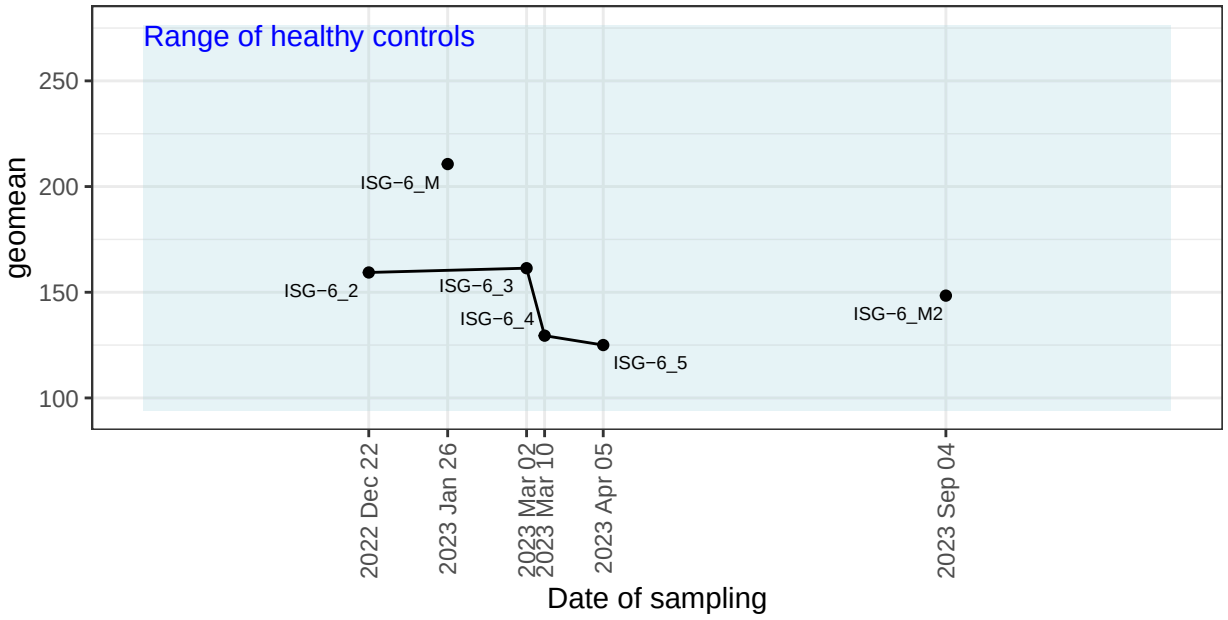


Table 3: NFkb scores and the panel

	ISG-6_Visit2	ISG-6_Visit3	ISG-6_Visit4
geomean	159	161	129
zscore	0	0	-6
AICDA	17	18	9
CCND2	281	191	242
EBI3	6	8	7
GZMB	457	751	254
IFNG	5	8	13
MSR1	66	51	110
SELL	7505	6582	2947
SELP	79	66	65
TANK	1168	1129	1454
TLR2	671	1328	330
XIAP	496	268	213

Table 4: NFkb scores and the panel

	ISG-6_Visit5	ISG-6_VisitM2	ISG-6_VisitM
geomean	125	148	211
zscore	-6	1	10
AICDA	13	11	15
CCND2	263	249	183
EBI3	8	1	7
GZMB	381	666	821
IFNG	7	3	35
MSR1	25	49	31
SELL	4867	7452	5816
SELP	63	68	96
TANK	1094	1974	2828
TLR2	453	1784	3176
XIAP	280	448	302

Results (IFNg)

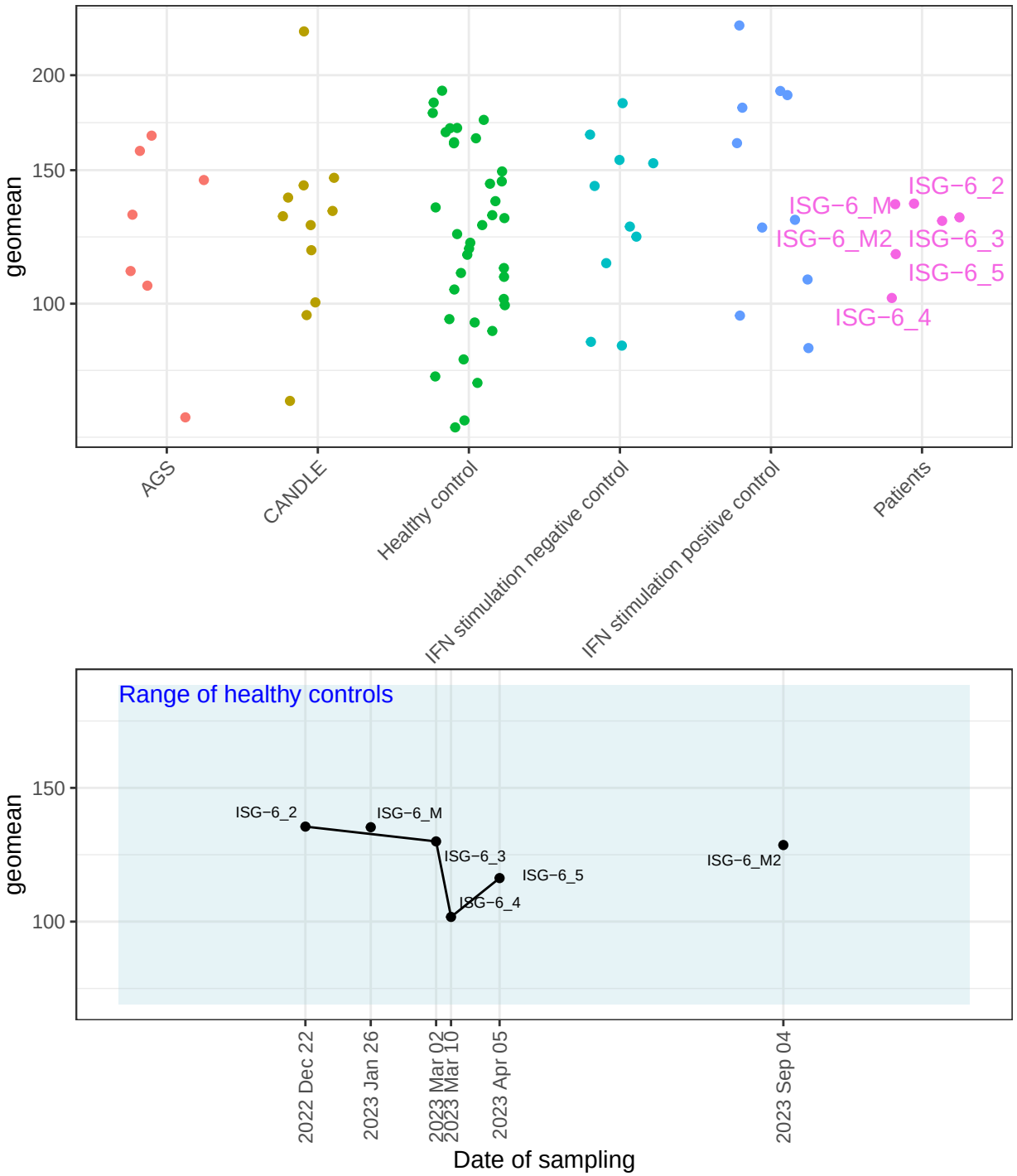


Table 5: IFNg scores and the panel

	ISG-6_Visit2	ISG-6_Visit3	ISG-6_Visit4
geomean	136	130	102
zscore	6	0	-8
AHR	814	914	929
B4GALT3	411	506	324
BMF	184	133	70
CXCL9	3	7	5
DNA2	37	32	30
FBXL5	2641	2116	1568
GPR146	445	867	828
GPR18	427	248	165
IFITM1	9783	8774	6918
PKDCC	0	1	1
RAPGEF6	214	153	101
SELP	79	66	65
SLC5A6	97	63	47
TRIB3	25	20	13
TRIM16	50	28	25

Table 6: IFNg scores and the panel

	ISG-6_Visit5	ISG-6_VisitM2	ISG-6_VisitM
geomean	116	129	135
zscore	-3	0	11
AHR	800	1262	3002
B4GALT3	408	358	281
BMF	155	114	688
CXCL9	2	6	7
DNA2	24	31	28
FBXL5	2382	4190	2716
GPR146	542	537	433
GPR18	216	117	80
IFITM1	5687	8471	21647
PKDCC	3	1	0
RAPGEF6	143	286	152
SELP	63	68	96
SLC5A6	66	52	50
TRIB3	19	20	26
TRIM16	35	32	15

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).