

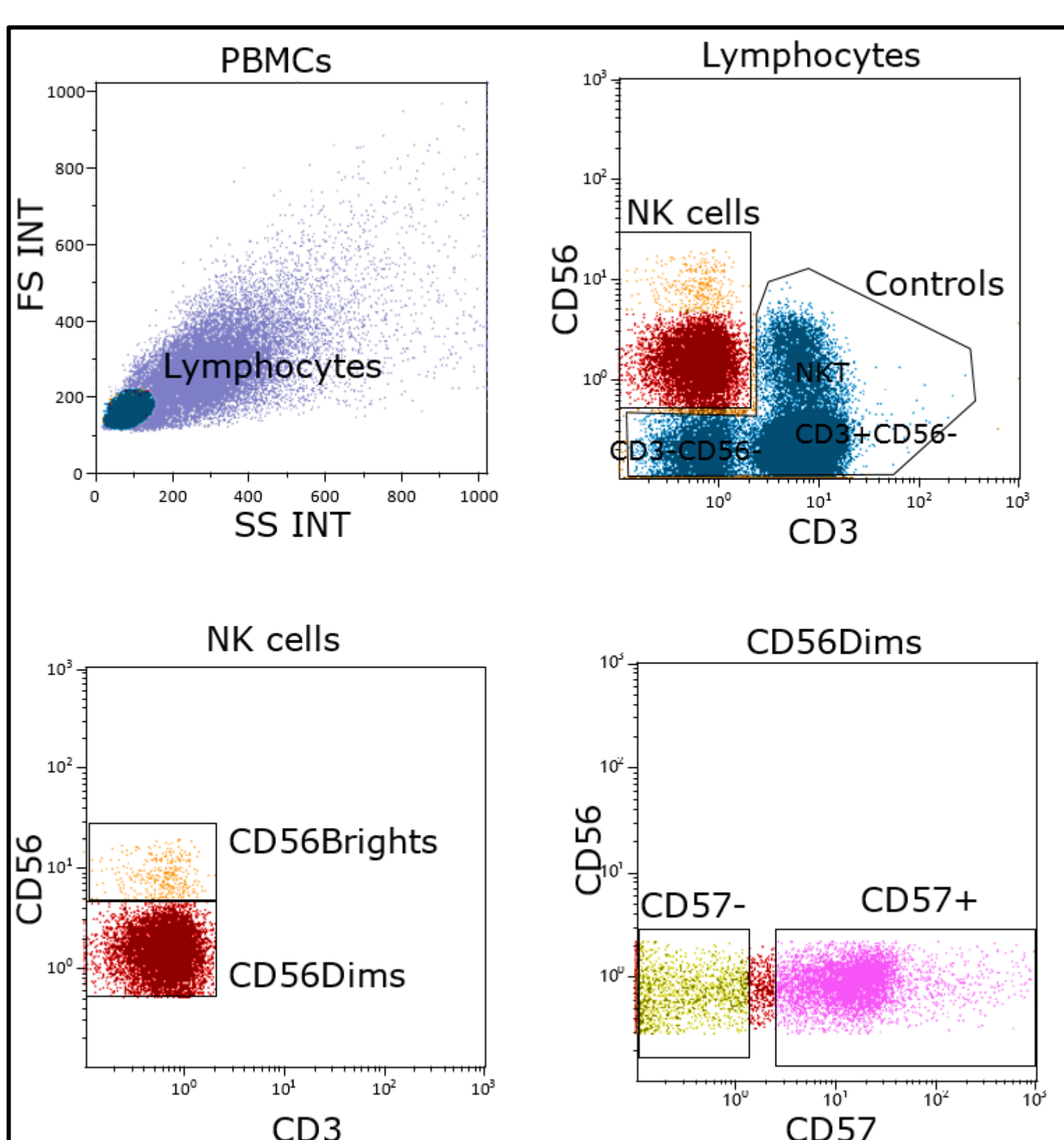
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**Background:** Killer cell immunoglobulin-like receptors (KIR) are expressed on NK cells and subsets of T cells. The *KIR* genes are polymorphic and the gene complex is polygenic with a varying number of inhibitory (iKIRs) and activating (aKIRs) receptors. Several disease association studies indicate that the interaction between the KIR and HLA loci affects the activatory threshold of NK cells and may have a role in infectious diseases, autoimmune disorders and cancer. NK cells can be separated into distinct subsets (based on level of CD56 expression) that have functional differences in relation to the level of cytotoxicity, however the immunological profile of these NK cell subsets and how it relates to function is not clear. In order to address this issue, we examined the expression profile of a panel of known immune-related genes on NK cell subsets using the new nCounter analysis system (NanoString technology). This technology enables the digital quantification of multiplexed target RNA molecules using color-coded molecular barcodes and single-molecule imaging. This system gives discrete counts of RNA transcripts and is capable of providing a high level of precision and sensitivity.

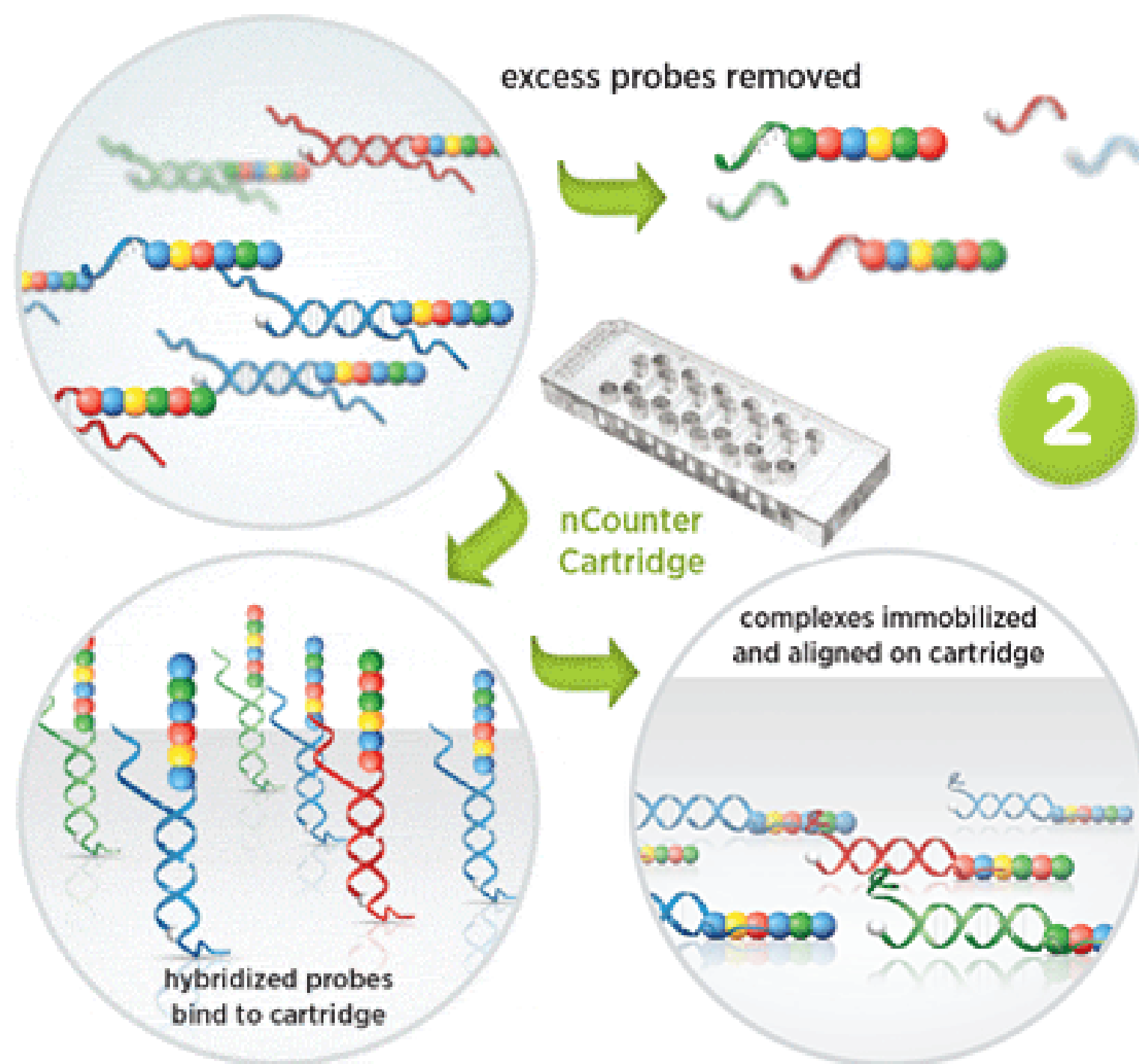
## Workflow

### Step 1: Cell Sorting



PBMC are sorted by flow cytometry to isolate NK cell subsets using the cell surface markers CD3 - T cell, CD56 - NK cell, CD57 - maturation

### Step 2: Nanostring Pipeline



Gene expression analysis performed using the nCounter GX Human Immunology panel of 582 genes on the Nanostring platform with RNA extracted from these sorted cell populations.

### Step 3: Data Analysis

Gene Name	Avg Count	Min Count	Max Count	HCV	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
HLA-A	7008.26	3712.45	11096.21	52.14	7268.89	7597	7568.76	7177.93	6018.91	3712.45
HLA-B	6225.88	4073.87	8293.51	13.35	5556.17	7002.76	6308.21	5488.54	5795.27	4073.87
HLA-C	3307.57	1284.7	5428.44	44.69	5438.44	5272.16	4973.09	4772.66	3482.88	1284.7
KIR3DL1	50.81	15.27	124.54	70.65	31.22	124.54	75.96	56.73	16.66	22.13
KIR3DL2	58.49	9.51	142.33	68.18	69.67	142.33	67.52	98.33	27.77	9.51
KIR3DL3	22.29	3.15	52.31	67.03	29.03	32.02	14.07	30.25	8.33	15.83
KIR-Activating	101.32	15.27	359.39	191.69	46.45	359.39	220.83	185.31	34.71	34.87
KIR-Activating	42.25	7.4	86.98	65.48	52.25	85.4	66.11	86.98	12.5	12.58
KIR-Inhibiting	233.31	18.05	530.81	43.39	545.75	398.51	246.02	226.91	18.05	30.73
KIR-Inhibiting	238.31	30.55	627.03	81.23	627.03	352.27	395.25	234.47	30.55	57.03
KIT	110.86	12.49	679.28	166.15	679.28	49.82	126.59	68.07	18.05	104.62
HLA-DMA	427.98	77.75	1220.57	86.58	203.2	128.1	175.82	112.36	77.75	1220.57
HLA-DMB	477.62	45.82	1756.36	110.81	307.71	81.84	115.34	173.96	45.82	1756.36
HLA-DQB1	141.13	44.89	478.72	86.82	131.53	92.52	90.02	79.42	80.53	478.72
HLA-DPA1	841.15	129.98	1986.92	48.37	232.23	192.12	163.42	253.38	120.98	1887.32
HLA-DPB1	2131.41	451.51	5228.24	92.68	435.18	473.25	451.51	832	452.51	3514.12
HLA-DQA1	557.02	7.48	4070.7	226.86	92.89	39.14	91.43	86.98	29.16	4070.7

The raw count data produced by the nCounter analysis is normalized with internal controls and Housekeeping genes and differential expression analysis is performed on the normalized data using a web based software NanoStrIDE<sup>3</sup>

## Conclusions

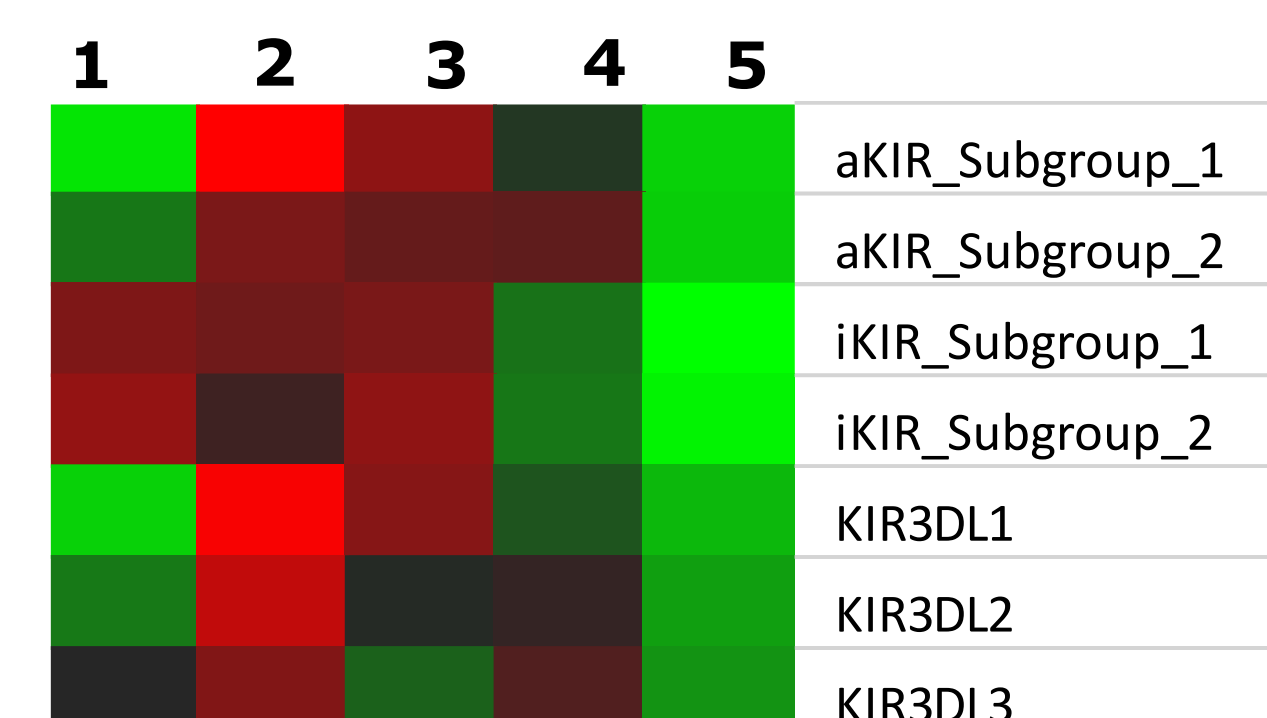
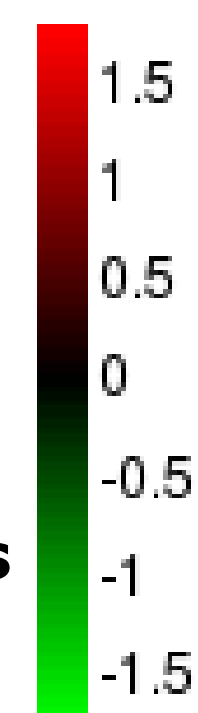
Nanostring technology has enabled us to identify T cell specific and NK cell specific profiles that contain - as expected - genes known to be involved in antigen presentation and NK-cell mediated cytotoxicity, respectively. Future work will generate an immunology signature for the different NK subsets for the other genes that have been indicated by this technique.

## Results

PBMCs from one sample was taken through the workflow and profiles for each subset was normalized. Different patterns were obtained for the subsets including specific profiles for T cells, NKT cells and the NK subsets. Initial analysis revealed CD56<sup>+</sup>Bright cells expressed low aKIR and high iKIR than the CD56<sup>+</sup> dim population as might be expected from known cytotoxic properties of these two subsets as shown in **Fig1**. Expression profiles common between different subset combinations will be further explored to determine potential functional consequences.

### Keys

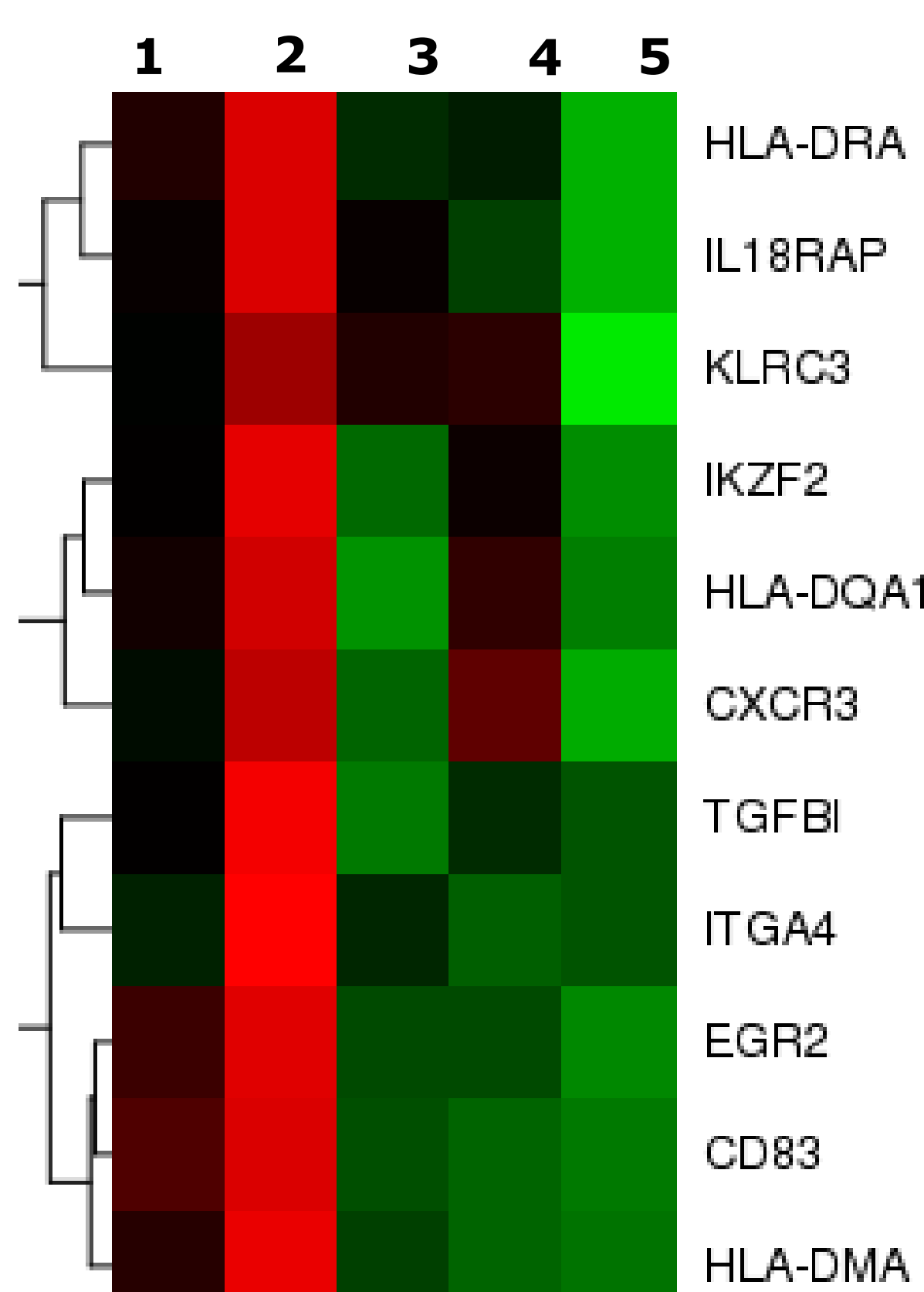
- 1 CD56 Bright
- 2 CD56 Dim 57-
- 3 CD56 Dim 57+
- 4 NKT
- 5 CD56- CD3+ T cells



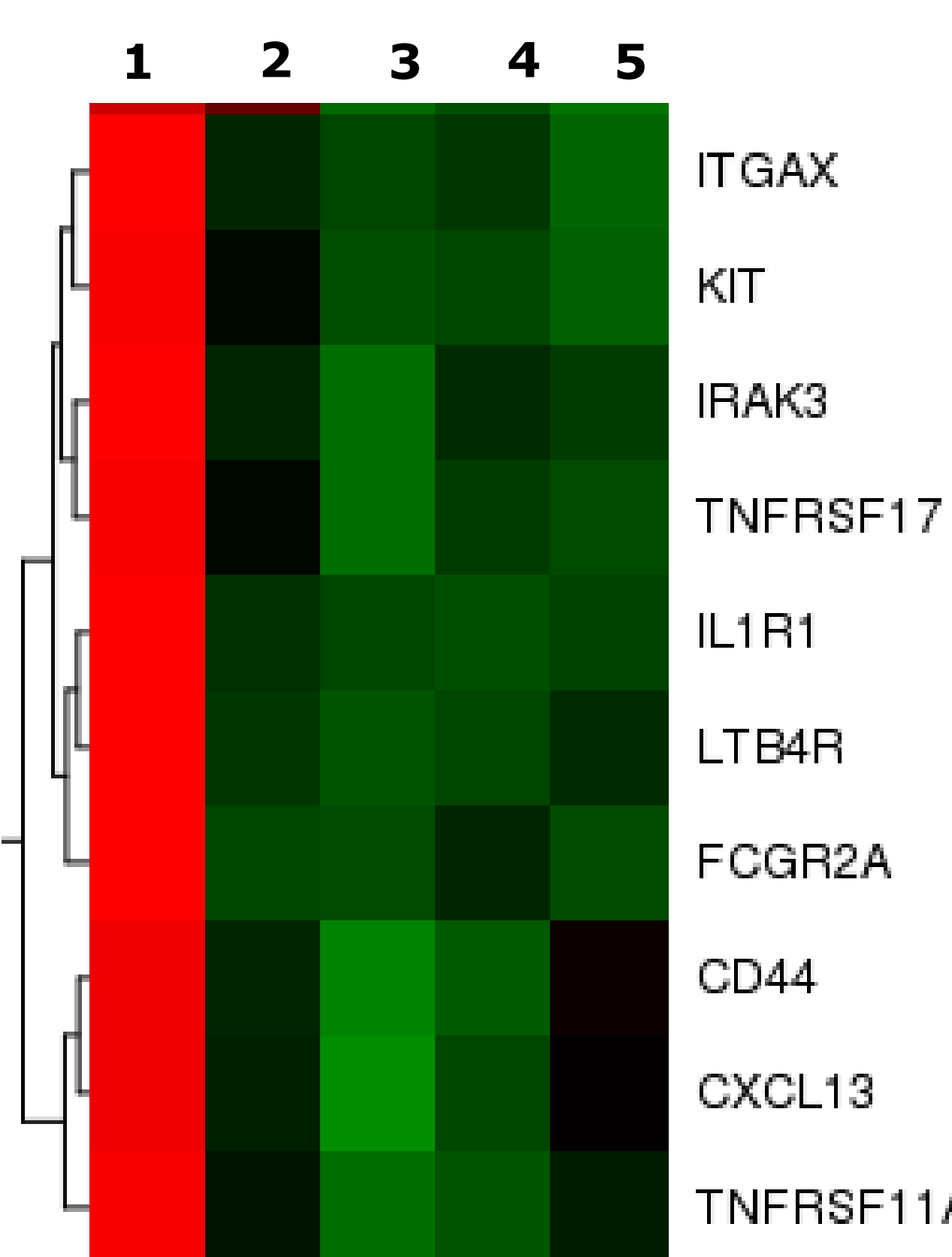
aKIR Subgroup1	KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS4
aKIR Subgroup2	KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5
iKIR Subgroup1	KIR2DL1, KIR2DL2, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL3
iKIR Subgroup2	KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL3, KIR2DL1, KIR2DL2, KIR3DL1, KIR3DL2

Gene content of the KIR Subgroups

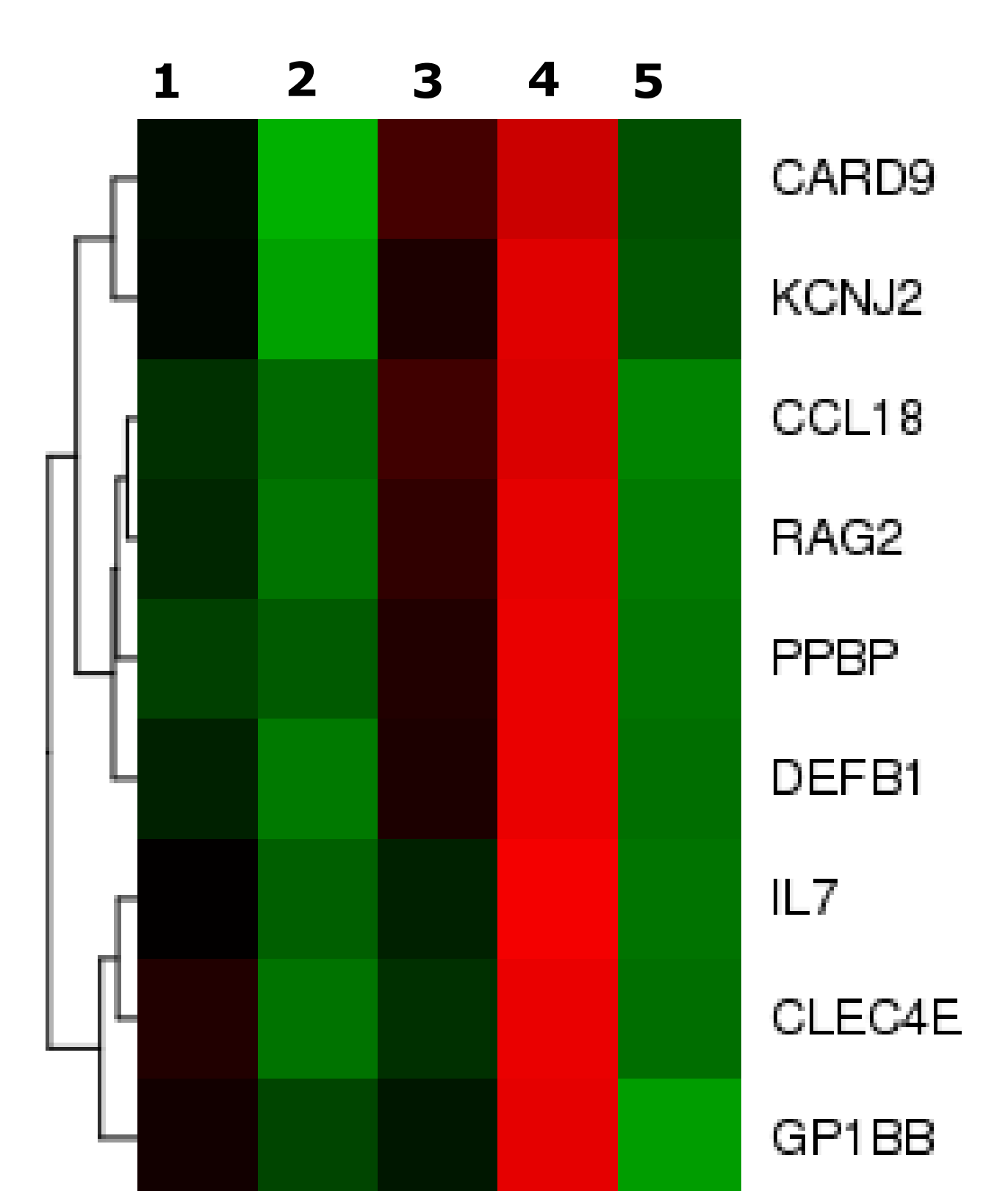
**Fig 1:** CD56 Bright cells express higher levels of inhibitory KIR as compared to the CD56 DIMs which express more activating receptors.



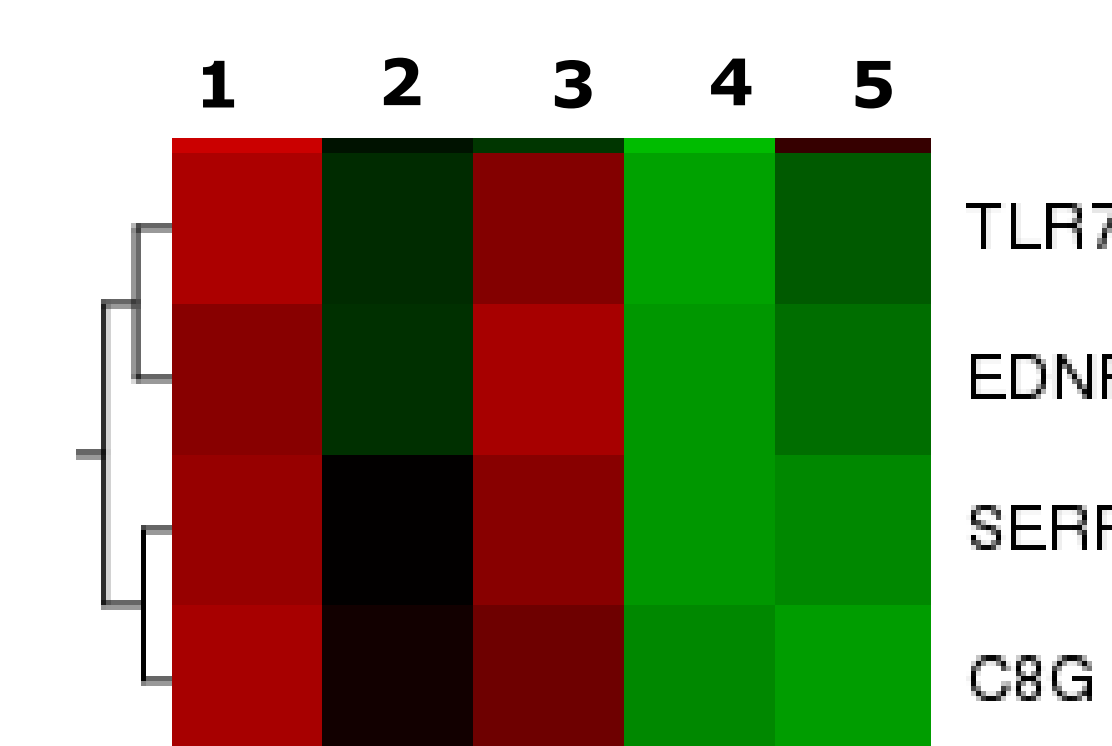
CD56 dim cells express higher levels of Class 2 HLA compared to the non CD56



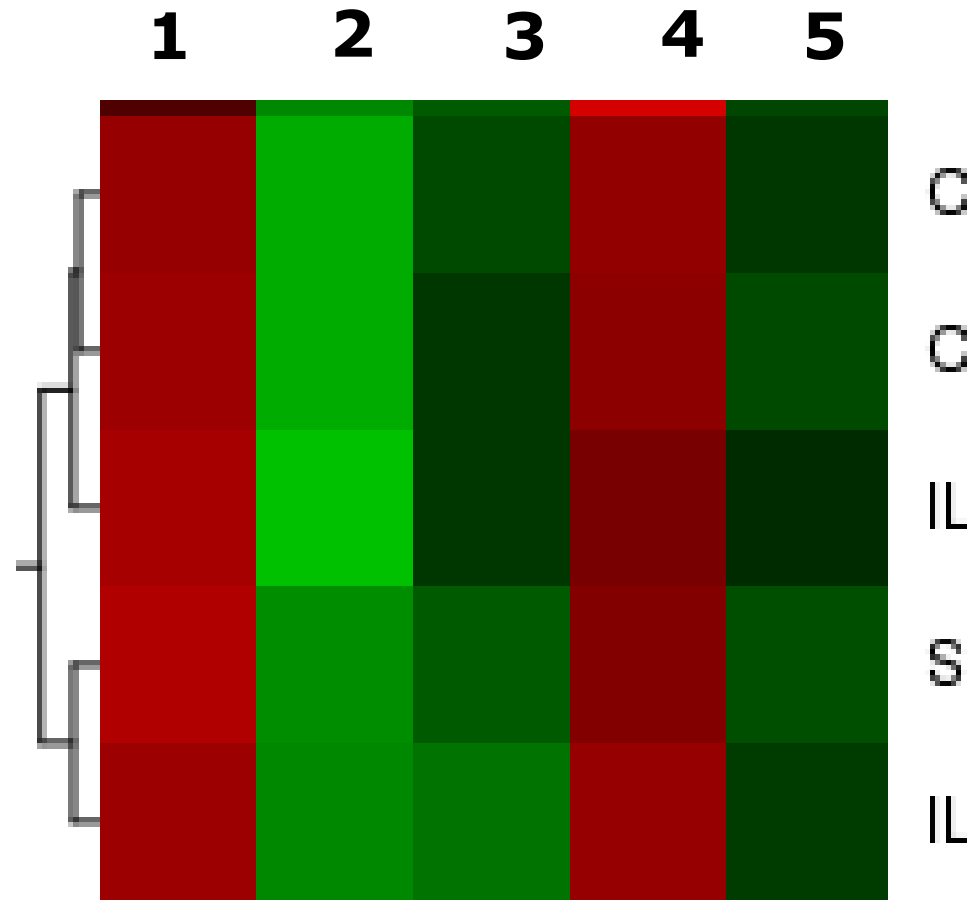
Some more gene profiles that have high expression in CD56+ Bright cells.



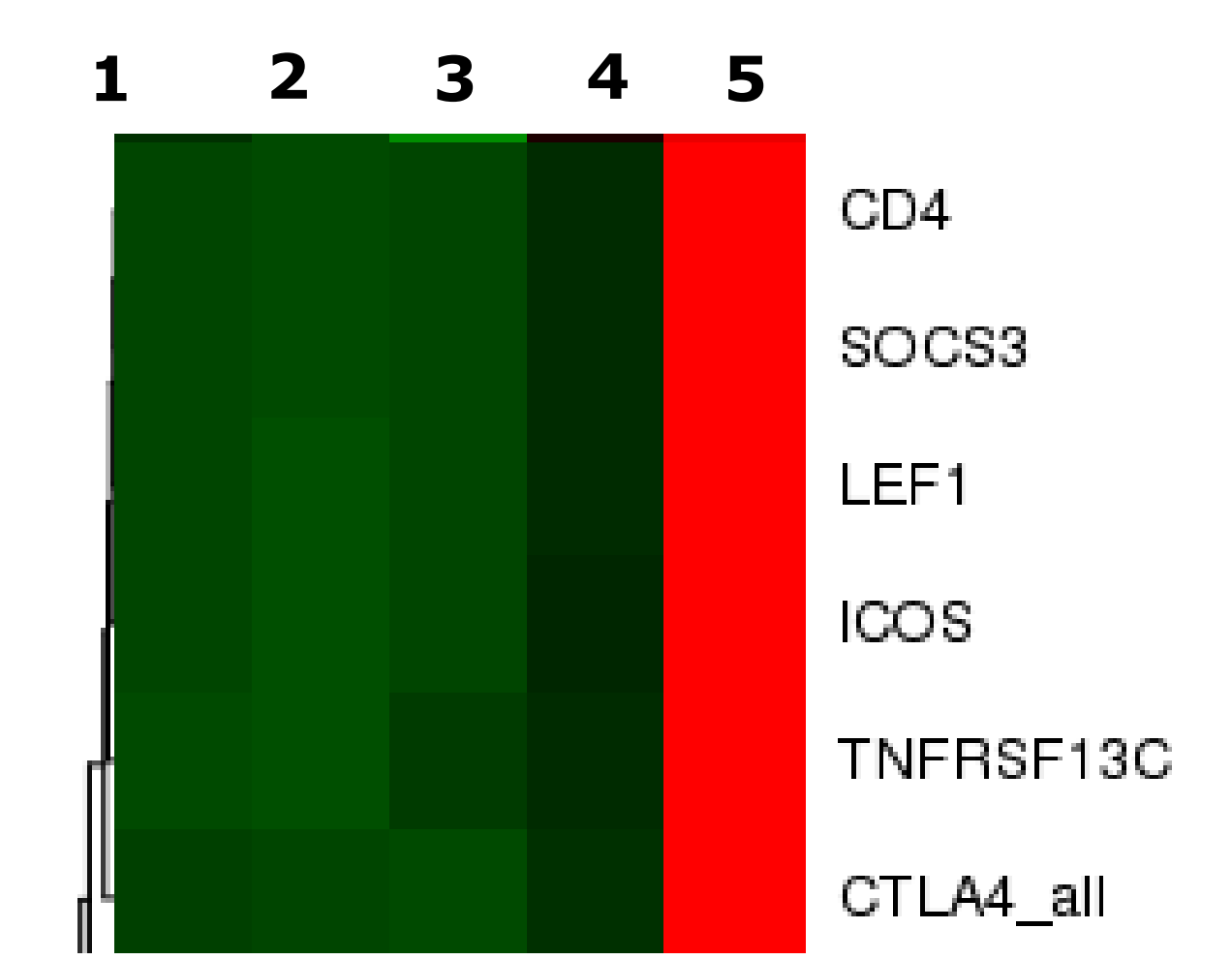
Gene expression in NKT Cells is distinct from the NK cells



CD56+ bright cells share similar expression levels of some genes with CD56 dim CD57+ population these genes are likely involved in antiviral responses.



Expression of genes shared between NKT cells and CD56 bright



Expression profile of signature genes in the T cell population.