

To read a heatmap, you need  
to know how to build it

S. Granjeaud

CRCM – Inserm – AFC

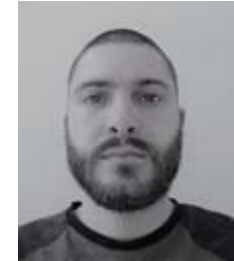
v230510

“What I cannot build. I do not understand.”

Richard Feynman

# Thanks to

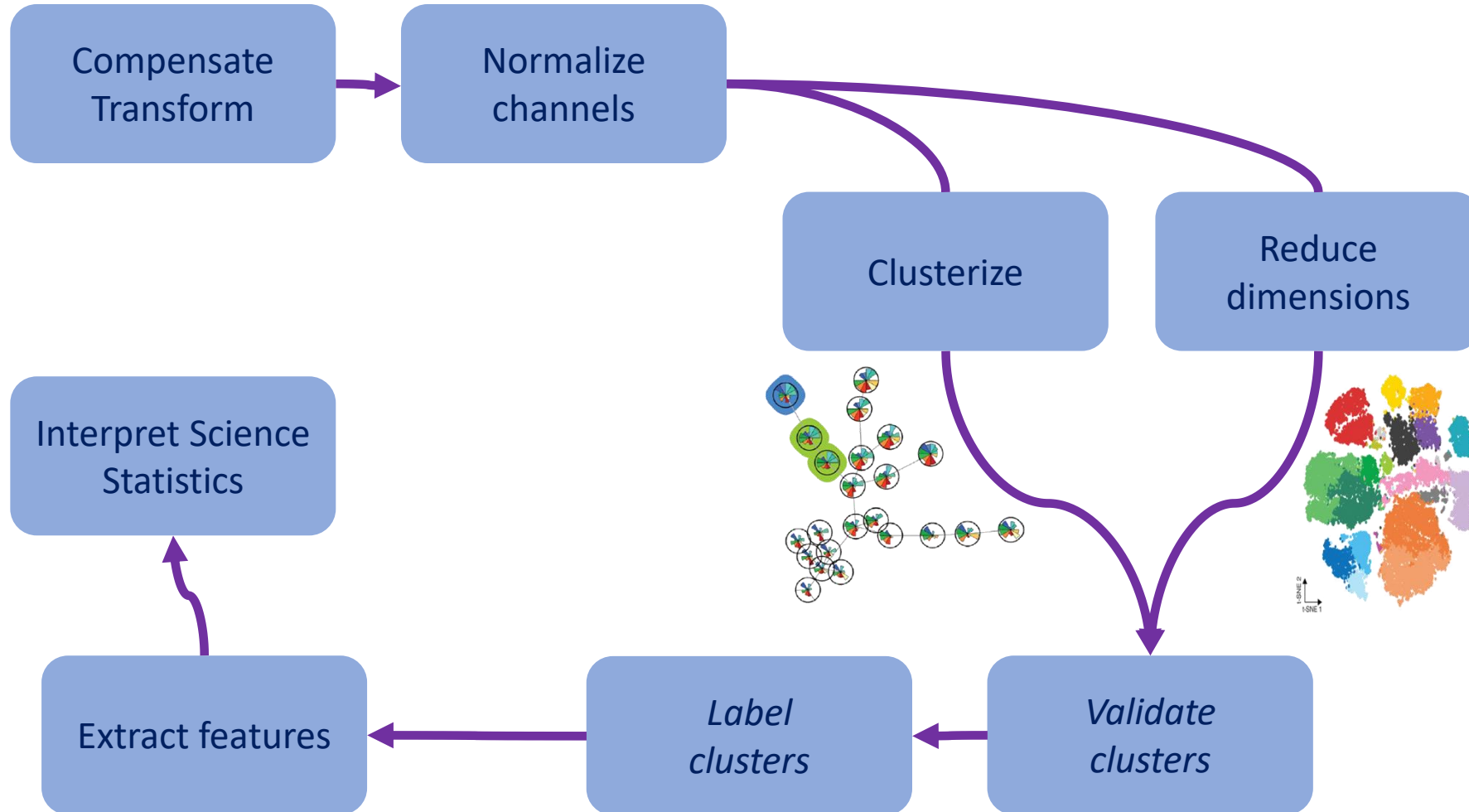
- AFC & Aïda
- Data extraction & analysis
- Phantasus
- Projects building, Support



# Outline

- Pipeline recap
- Dataset and extracted tables
- What is a heatmap?
- MFI: what is the goal?
- Scalings... and results
- Heatmap with Excel
- Heatmap with Phantasus

# Pipeline recap

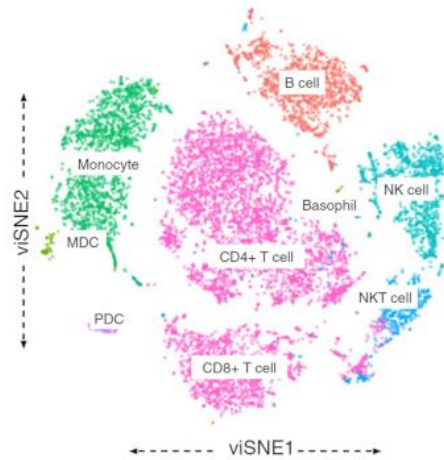


# Dataset







## Article

- [Pubmed](#)

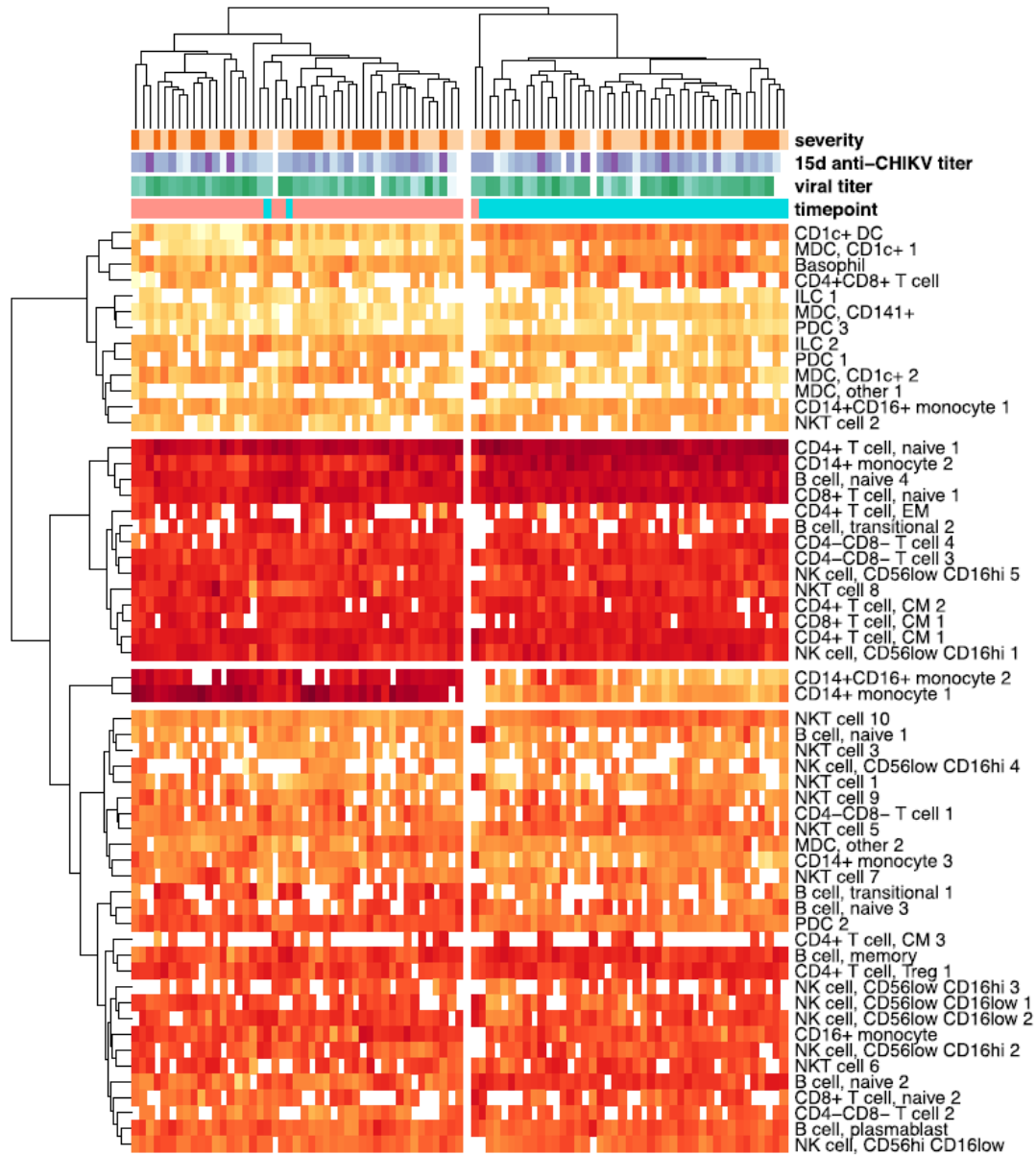


## Comprehensive innate immune profiling of chikungunya virus infection in pediatric cases

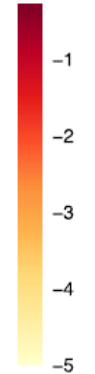
Daniela Michlmayr<sup>1,†</sup>, Theodore R Pak<sup>2,†</sup> , Adeeb H Rahman<sup>2,3</sup>, El-Ad David Amir<sup>2,3</sup>, Eun-Young Kim<sup>4</sup> , Seunghee Kim-Schulze<sup>2,3</sup>, Maria Suprun<sup>5</sup>, Michael G Stewart<sup>4</sup>, Guajira P Thomas<sup>4</sup>, Angel Balmaseda<sup>6</sup>, Li Wang<sup>2</sup>, Jun Zhu<sup>2</sup> , Mayte Suárez-Fariñas<sup>2,5</sup>, Steven M Wolinsky<sup>4</sup>, Andrew Kasarskis<sup>2</sup> & Eva Harris<sup>1,\*</sup> 

- We performed whole-blood RNA-seq, 37-plex **mass cytometry** of peripheral blood mononuclear cells (**PBMCs**), and serum cytokine measurements of **acute- and convalescent-phase** samples obtained from **42 children** naturally infected with CHIKV. Semi-supervised classification and clustering of single-cell events into **57 sub-communities** of canonical leukocyte phenotypes revealed a **monocyte-driven response** to acute infection, with the greatest expansions in “**intermediate**” **CD14++CD16+ monocytes** and an **activated subpopulation of CD14+ monocytes**. **Increases in acute-phase CHIKV** envelope protein E2 expression were highest for **monocytes and dendritic cells**.

# Dataset - Example



log10 frequency



severity

less  
 more

15d anti-CHIKV titer

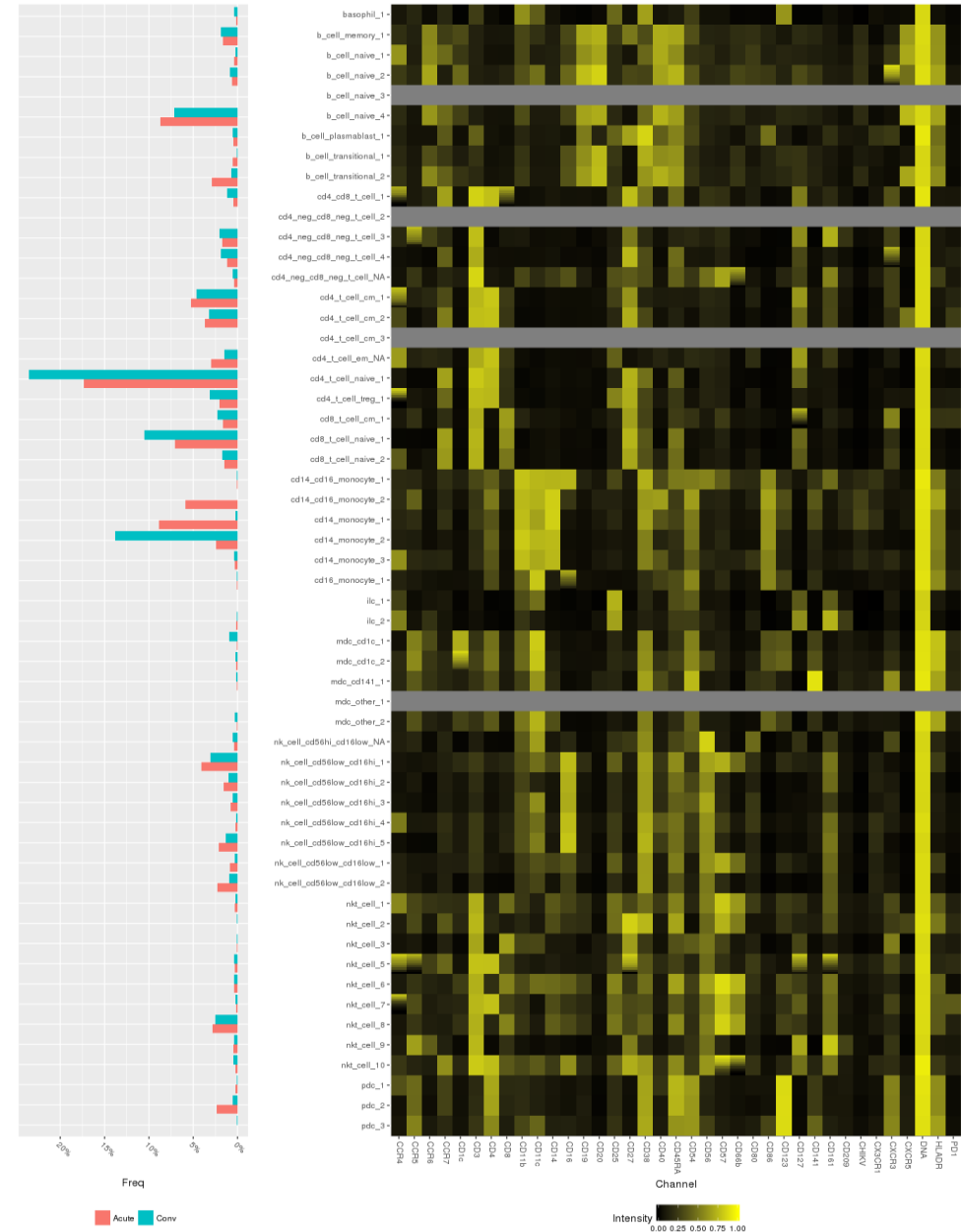
3.8  
 2.4

viral titer

10  
 4

timepoint

Acute  
 Conv



# Heatmap

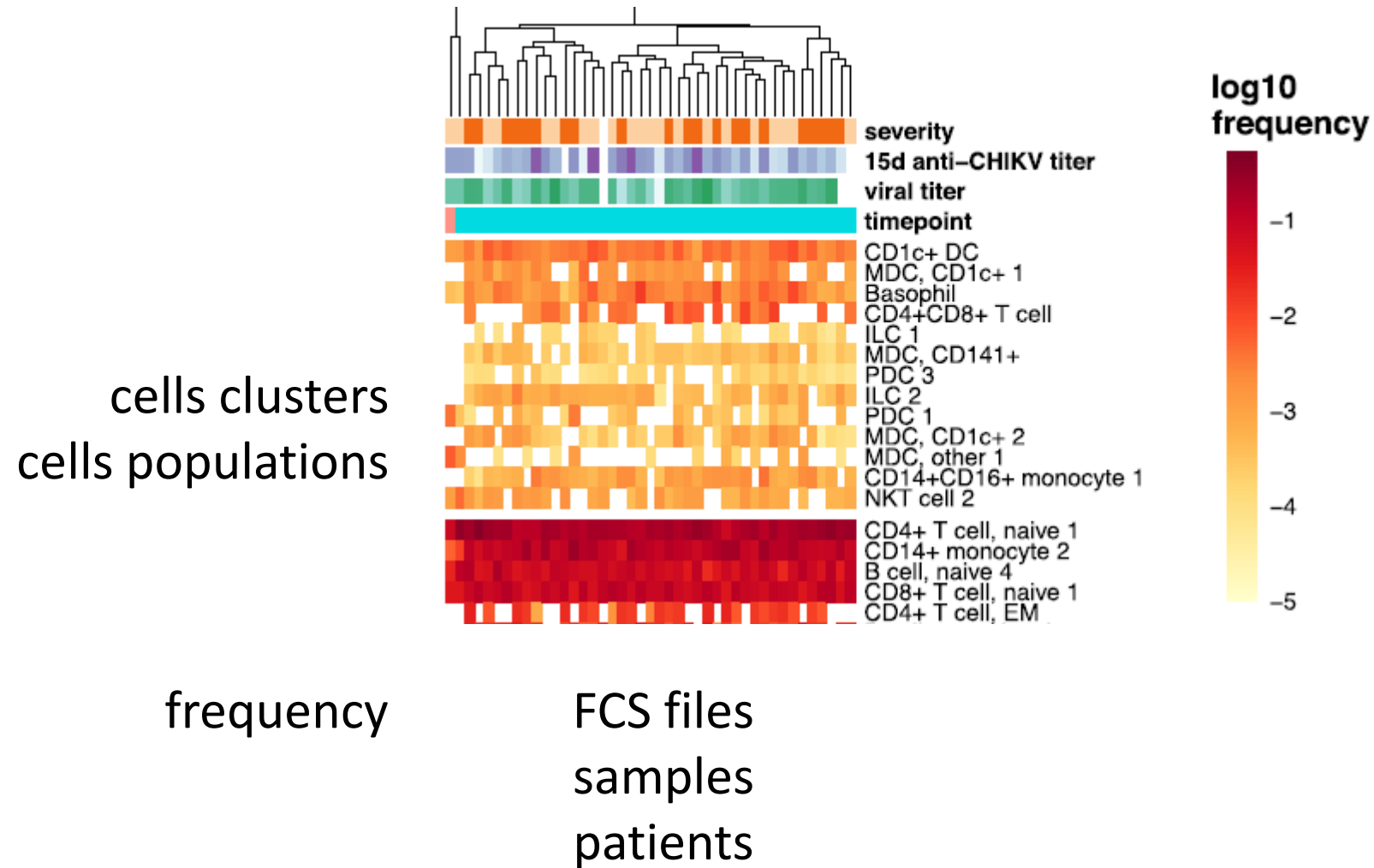
- A heatmap represents a rectangular matrix of data as an image
- A heatmap implies a numeric to color coding
- The color scale is the same for all the table (aka matrix)
  
- A heatmap is a XY plot of rectangular points with a color associated to a measure
- What is the measure?
- What is X axis? Y axis?

# Matrix of data

- Different elements in rows and columns: clusters, markers, patients
- Many contents: count, percent, MFI
- Each content has a large dynamic range
- The dynamic range is larger than the information we look for

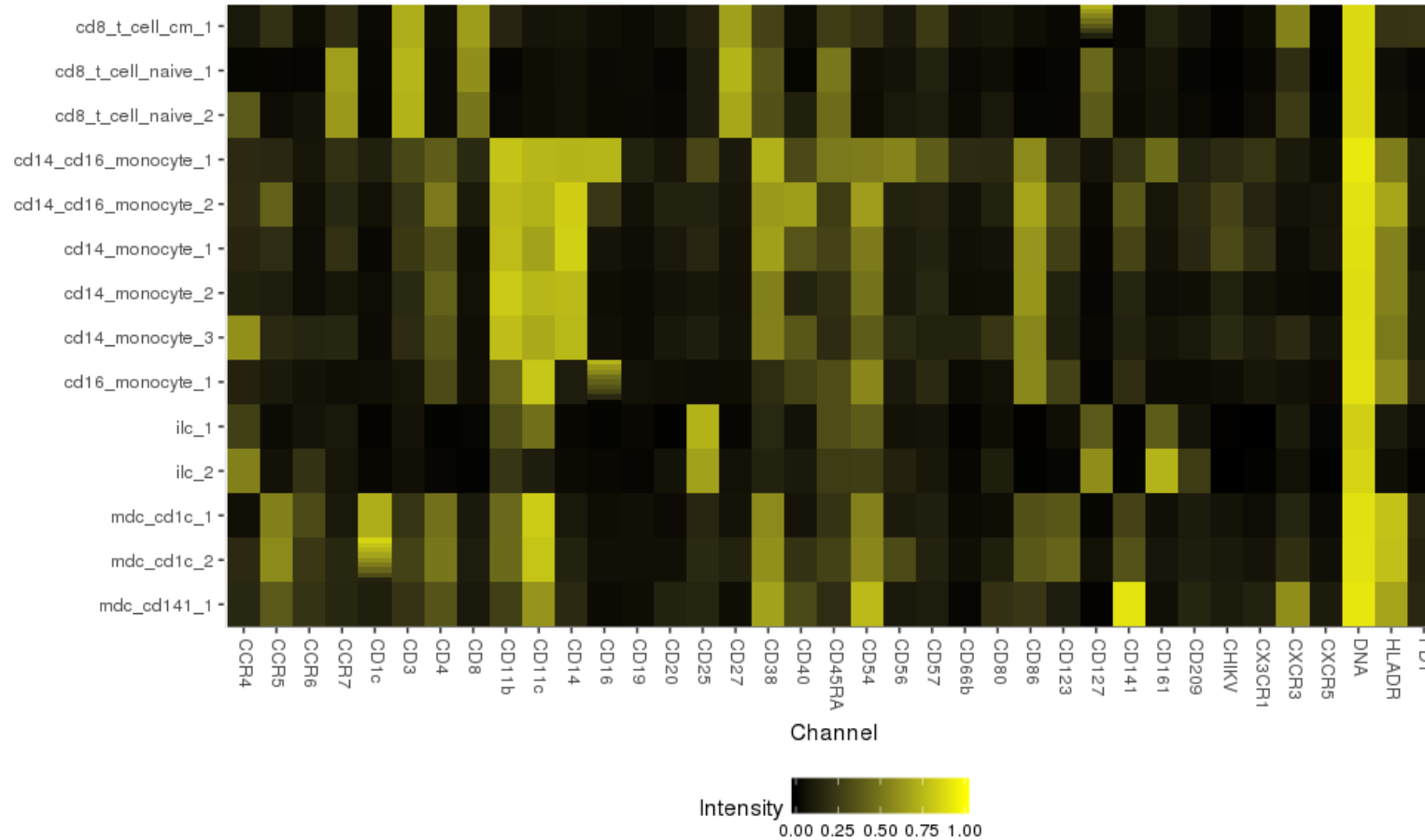


# Heatmap of frequency



# Heatmap of MFI

FCS markers  
proteins



cells clusters  
cells populations

intensity

# Matrix of data

- Different elements in rows and columns: clusters, markers, patients
- Many contents: count, percent, MFI
- Each content has a large dynamic range
  
- What is the information we are looking in the graphics?
- **What is the question?**
- How the content should be processed to become information?
- The content must be scaled in order to show the information
- The scaling depends on the content and the elements in rows and columns

# Methods for scaling

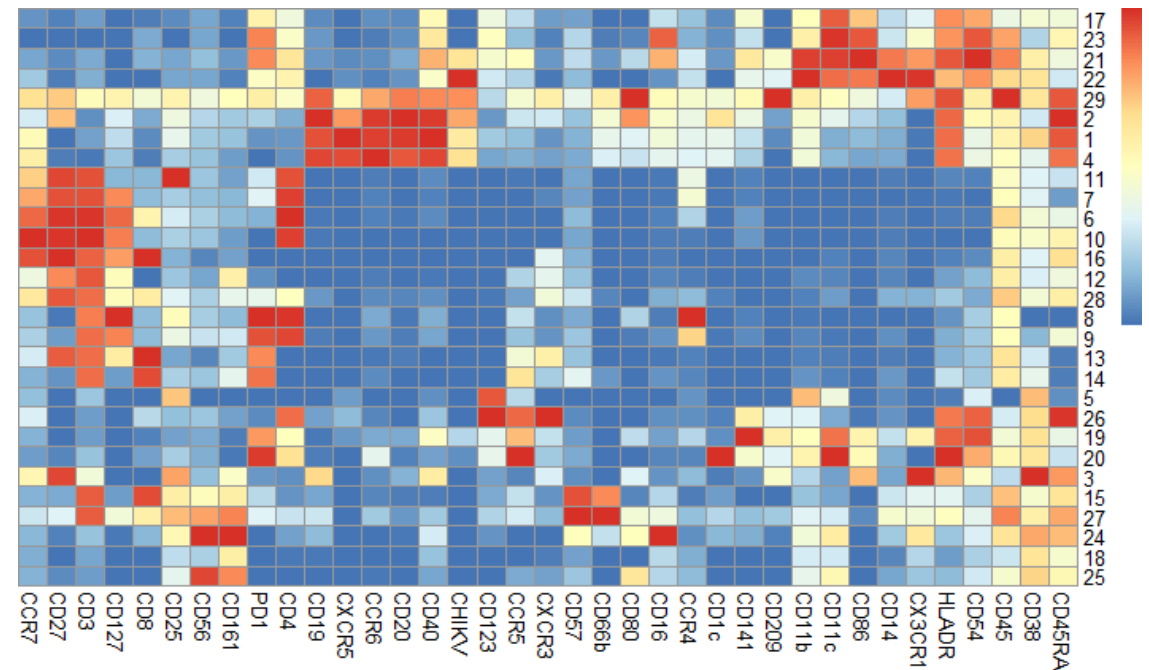
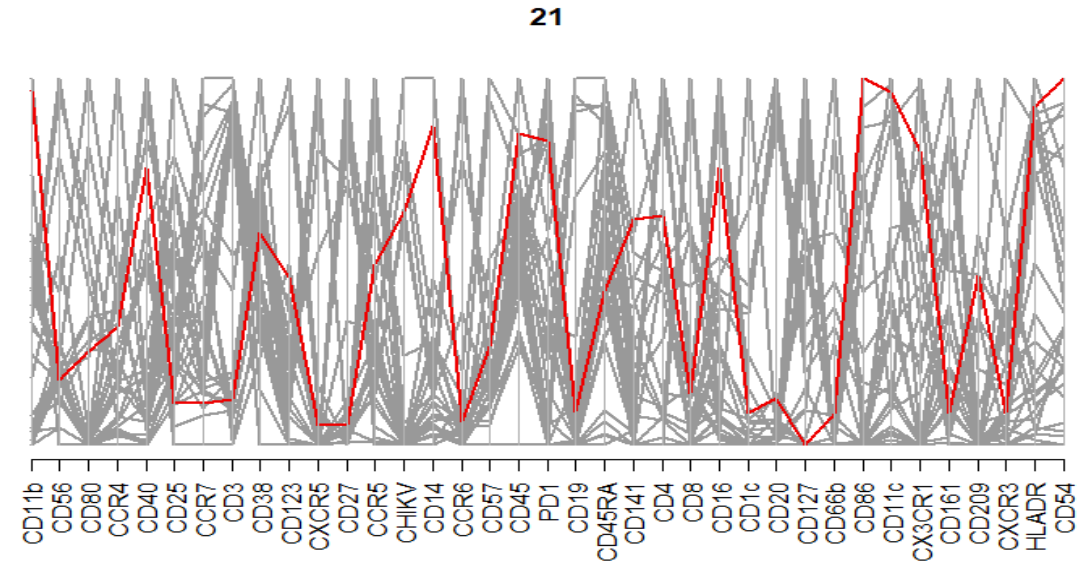
- min to max
  - $\text{min} = 0, \text{max} = 1$
- center
  - $\text{mean} = 0$  or  $\text{median} = 0$
- standardize
  - centering
  - divide by standard deviation
- apply to all data, but determine coefficients on all or a subset of data?

# Data to visualize = Matrix of MFI

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL
1	cluster_i	CD11b	CD56	CD80	CCR4	CD40	CD25	CCR7	CD3	CD38	CD123	CXCR5	CD27	CCR5	CHIKV	CD14	CCR6	CD57	CD45	PD1	CD19	CD45RA	CD141	CD4	CD8	CD16	CD1c	CD20	CD127	CD66b	CD86	CD11c	CX3CR1	CD161	CD209	CXCR3	HLADR	CD54
2	K1	20.64	5.3266	14861	10.153	156.48	7.2641	16.557	7.8572	426	6.0576	108.46	0.2031	2.2559	2.2927	3.7343	65.04	4.5073	103.93	0.3367	84.279	123.92	3.213	1.7888	0.6821	18.039	8.8621	600.55	3.2587	3.396	2.5884	4.6494	0.0535	2.5136	0.1234	1.1209	387.73	11.585
3	K2	23.981	6.2879	3.7006	11.475	138.22	7.9443	7.3281	6.5878	39.229	2.2805	51.6	56.046	3.9683	3.0324	4.951	66.599	5.6596	105.33	1.0379	109.13	164.79	4.8735	3.001	1.6452	21.683	24.581	681.7	4.1348	4.8948	4.014	22.714	0	2.7695	0.2089	4.8724	405.42	21.246
4	K3	7.7701	4.4041	1.4501	3.5945	29.57	23.338	17.675	37.04	2936.8	2.6209	0	151.96	0.6032	0	3.1067	1.0417	2.044	57.547	0.3839	31.809	79.242	0.8648	1.3245	0	1.7107	0.4553	4.4681	0	0	21.552	3.334	3.1216	6.8557	0.9792	5.3602	184.95	16.61
5	K4	19.778	4.7692	1.2052	8.6627	132.27	4.116	20.696	4.621	56.842	3.0394	88.724	0.5824	1.7315	2.4596	3.3021	72.283	3.948	92.339	0.0069	91.8	105.35	2.5665	1.4831	0.3668	13.814	8.4242	440.23	2.3983	3.3481	1.7123	5.4924	0	1.4312	0	1.4893	371.92	11.737
6	K5	96.655	0.8045	0	1.5568	1.325	19.328	3.6643	12.828	571.44	253.6	1.9037	0	3.4056	0	1.4858	0.0563	0.4706	33.321	0	0	4.127	0	0	0	0	0	0	0	0	0	30.79	0	0.3746	0	0	1.6339	8.5792
7	K6	0	5.7129	0	5.3518	1.1559	6.0124	62.26	482.35	87.811	0	0	171.56	0	0	0.7264	0.5924	5.181	135.3	0.6562	0.2501	16.723	1.0459	147.73	9.399	0.7145	0	0.4232	20.183	0	0	0.0514	0	2.1642	0	0.0484	1.7031	0.4854
8	K7	0	4.4829	0	13.981	0.7861	4.0033	40.405	389.65	50.159	0	0	135.17	0	0	0.1052	0.5782	3.1044	94.564	1.5586	0	4.8535	0.3589	129.32	2.0664	0.3464	0	0.1134	17.32	0	0	0	0	2.1263	0	0.6617	1.1962	0
9	K8	0.2649	5.6312	1.0098	131.86	3.0103	11.05	3.8045	269.66	5.0243	0	0	0.2149	3.6419	0	0.1826	2.1832	3.6906	94.843	5.1466	0	2.715	0.4085	138.27	2.1449	0.4925	0	0.505	26.05	0	0	0	0	2.1614	0	0.8755	2.9536	4.9932
10	K9	1.103	7.5616	0.085	36.982	2.4876	7.8136	4.8136	305.59	14.978	0	0	1.9926	2.3941	0	1.6449	1.4983	6.1927	94.924	4.708	0.5509	20.798	0.6483	121.17	2.1874	1.9266	0.3208	0.6179	18.012	0	0	0.3741	0	4.0294	0	1.182	5.131	4.6492
11	K10	0	4.8961	0	0.4984	0.0236	4.1797	89.277	520.01	110.19	0	0	169.38	0	0	0.8001	0	3.3682	95.466	0	0.2233	31.809	0.9149	128.36	2.1175	0.7018	0	0	18.327	0	0	0.0643	0	1.338	0	0	0.8664	0
12	K11	0	5.1009	0	11.665	1.2536	41.047	30.21	380.67	46.686	0	0	150.96	0.2421	0	0.6141	0.4184	3.4444	89.897	1.4097	0	11.222	0.341	114.71	1.9578	0.3657	0	0	1.8429	0	0	0	0	1.4986	0	0	2.2146	0.7448
13	K12	1.4749	3.1938	0	0.4414	0.2309	3.696	10.436	370.32	44.515	0	0	90.194	3.0925	0	1.32	0.3636	5.7177	111.33	0.3007	0	18.835	0	0	0	0.969	0	0	7.7017	0	0	1.5144	0	8.904	0	6.1514	4.2652	3.6683
14	K13	0.8914	1.6804	0	0.7193	0	2.4401	7.3958	301.96	39.075	0	0	123.61	6.6436	0	0.6447	0.1777	5.9175	122.9	4.1285	0	3.351	0	0	30.425	0.2637	0	0.7089	9.4407	0	0	0.7427	0	2.7404	0	12.512	5.6058	4.4999
15	K14	1.9772	5.0121	0	0.8447	0.0836	4.2725	3.1276	306.51	62.611	0	0	1.6778	11.722	0	2.1527	0.9919	14.758	112.7	4.382	0	3.2521	0	0	26.189	1.6843	0	0.0564	1.0847	0.785	0	0.9945	0	4.9928	0	3.3212	13.436	4.8061
16	K15	5.2168	19.618	0.1839	0.4968	0.537	13.44	3.0661	356.89	101.14	3.4204	0	2.6882	3.7888	0	10.105	0.7746	265.39	148.68	1.1549	2.2655	43.008	0	1.541	26.662	7.0244	1.8353	0.7793	1.1442	12.19	0	3.8676	1.058	9.2807	0	1.0146	21.016	5.2546
17	K16	0	1.6608	0	0	0	2.977	70.067	339.47	47.981	0	0	180.43	0	0	1.2631	0	4.4466	110.59	0	0	46.272	0	0	30.176	0.6328	0	0	16.004	0	0	1.067	0	1.51	0	6.0684	1.9466	1.225
18	K17	31.047	3.1074	0	3.989	21.532	1.59	1.7497	7.5903	97.552	18.591	0.003	0.6601	3.5318	0	8.3728	1.1328	3.2357	74.859	2.6652	0.9873	19.593	5.8228	13.25	0.2717	8.361	1.0735	1.5488	0	0	20.382	606.4	1.01	0.6418	0	1.3781	275.15	59.32
19	K18	11.592	5.9401	0	2.9047	4.29	5.051	2.8679	8.7276	297.99	0	0	0.1185	0.0838	0	1.2629	0.0615	3.1041	60.969	0	0.2436	22.347	0	0.3716	0	7.4128	0	0	0	0	14.834	0.712	9.2202	0	0.3041	3.7455	5.1342	
20	K19	29.92	3.2282	1.123	5.5788	18.267	2.4054	3.0615	7.7214	336.59	15.077	1.5831	0.1712	15.262	0.9062	9.2538	2.2271	2.974	81.621	3.9732	0.3086	16.83	21.532	18.944	0.4999	2.4499	1.2525	3.9372	0	0	12.324	468.75	1.671	0.6347	1.2033	4.3182	438.27	111.41
21	K20	38.836	3.5987	0	0.9955	2.3307	4.0923	2.1229	12.368	284.05	15.896	0	0.9322	30.257	0.2226	3.6876	7.687	3.8166	101.42	4.9732	0.4565	11.665	5.7048	34.014	1.3199	1.0288	91.73	0.9203	0	0	11.011	1013.8	0.0179	0.5003	0.7207	3.113	740.88	54.898
22	K21	301.96	4.538	1.0764	8.1465	57.087	2.4652	2.4352	8.9226	217.68	24.409	1.0068	1.1274	7.7818	2.4372	173.88	1.0335	9.5237	191.87	4.1096	1.6966	19.116	8.7956	32.028	1.7915	88.065	1.5536	3.8975	0	0.8154	47.14	816.28	2.4608	1.2649	0.9717	1.255	483.77	145.81
23	K22	356.24	3.1708	0.0405	6.6226	17.704	2.4669	4.3285	9.3024	302.09	10.957	1.2104	0.5318	3.1566	4.0393	327.72	0.5532	5.261	134.36	2.2697	0.1459	12.149	4.5854	23.546	0.0219	1.855	0	2.0975	0	0	31.69	498.15	3.0686	0.7013	0.7217	0.164	168.48	70.028
24	K23	42.418	3.0072	0.2017	2.9354	31.381	0.9108	0.3469	4.2894	26.205	29.01	0.0305	0.1494	2.3182	0	10.112	0.3827	8.253	169.34	4.178	1.6177	30.702	3.1645	16.798	1.5789	179.29	1.1505	1.9426	0	0.1981	37.407	926.95	1.3993	0.2987	0	0.4789	265.91	106.3
25	K24	20.601	120.1	2.2096	1.2567	8.5138	11.547	3.2338	11.992	711.81	1.93	0	0.8022	0.1092	0	5.9754	0.1544	32.608	122.44	0	3.4121	62.341	1.4427	2.1757	1.9968	278.29	2.9114	0.395	0.1806	2.7893	0	86.707	1.8403	24.744	0.082	0	7.7847	8.5648
26	K25	16.043	100.91	2.7413	3.4643	2.3665	6.9362	3.1429	7.8232	419.99	0.1601	0	1.1718	1.6497	0	2.923	0.1951	6.3873	84.441	0	0	29.647	0	0	7.3004	0	0	0	0	0.0009	0	63.526	0.5668	17.078	0	1.4584	6.5403	7.8762
27	K26	12.667	4.9847	0	1.2674	4.4191	3.4543	7.5963	7.6099	377.82	372.22	3.2141	0	23.313	0	2.0377	0.8288	1.8237	63.507	0.233	2.0157	155.35	8.0728	93.893	3.3742	1.4403	0.6458	0	0	0.005	0.152	4.0803	0	1.4579	0.6947	43.2	353.63	97.587
28	K27	13.481	53.605	1.8708	3.6962	4.7178	20.243	6.6171	356.72	209.05	7.5432	0.0216	10.021	4.3629	0	22.534	3.6774	357.98	190.08	1.5423	6.7789	72.37	1.9257	7.3038	9.8684	16.896	4.9433	2.5622	5.822	17.252	0.8035	16.435	1.2797	17.29	0.4327	2.4267	45.128	10.319
29	K28	0.8737	5.855	0	3.5187	1.3289	6.6138	23.102	301.14	87.052	0.3478	0	130.29	1.3643	0	3.8316	0.9664	10.819	143.65	1.708	1.6596	35.758	0.0317	18.581	10.655	3.4538	0.7009	1.2709	7.7155	0.4752	0	3.0414	0.4256	5.2085	0	7.6483	8.5216	2.7553
30	K30	43.232	13.337	4.7273	14.922	78.358	12.284	25.115	57.368	292.87	8.2823	17.744	51.253	6.8674	3.2616	11.486	33.749	16.523	261.37	2.7664	77.509	125.41	6.9998	16.679	6.5089	27.732	11.371	303.16	8.6027	6.8994	7.8429	48.984	2.4408	7.4007	2.1489	12.291	515.86	26.645

# How to visualize?

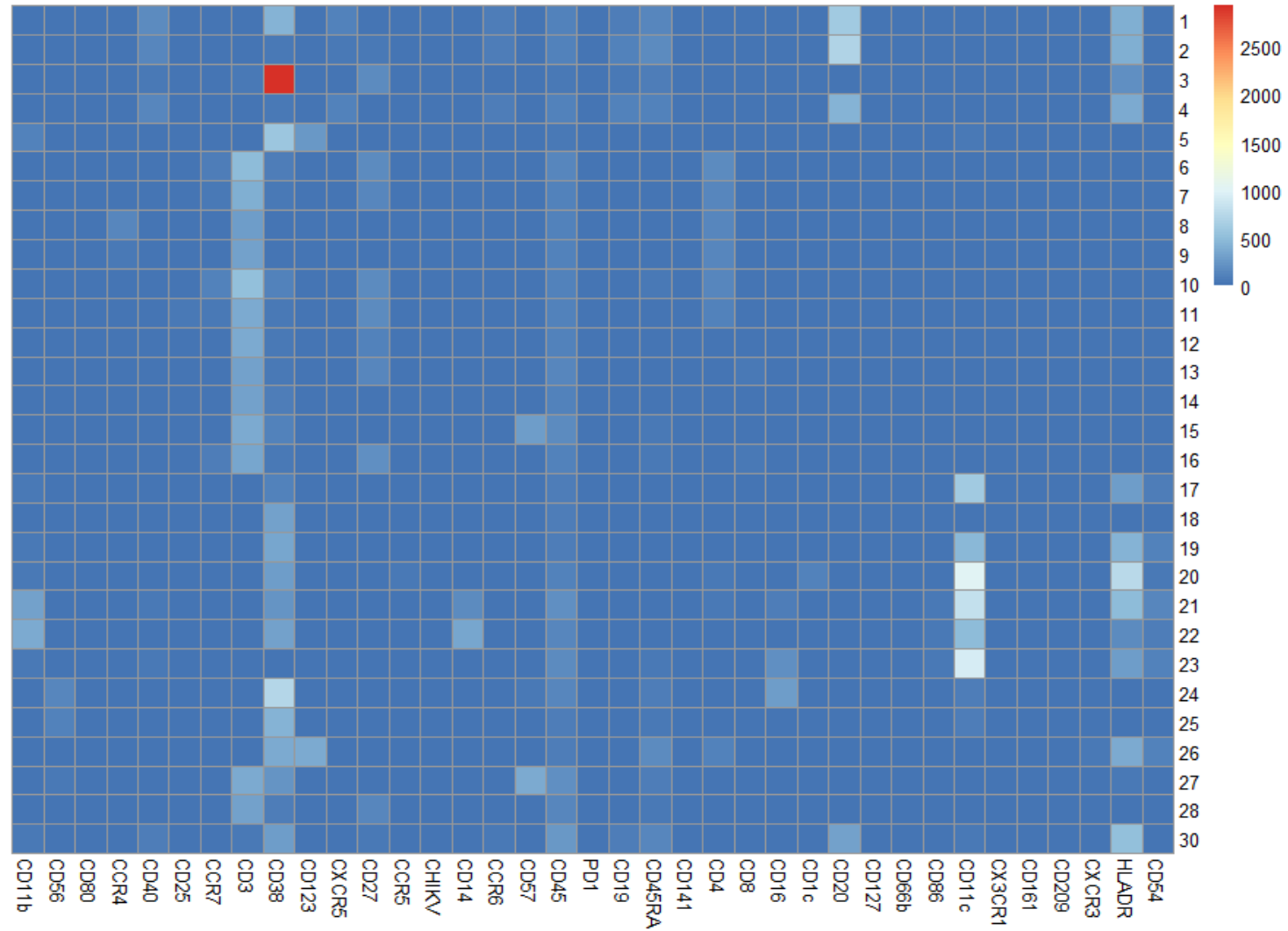
- Parallel coordinates
- Heatmap
- Pros/Cons



# Question = Annotate clusters

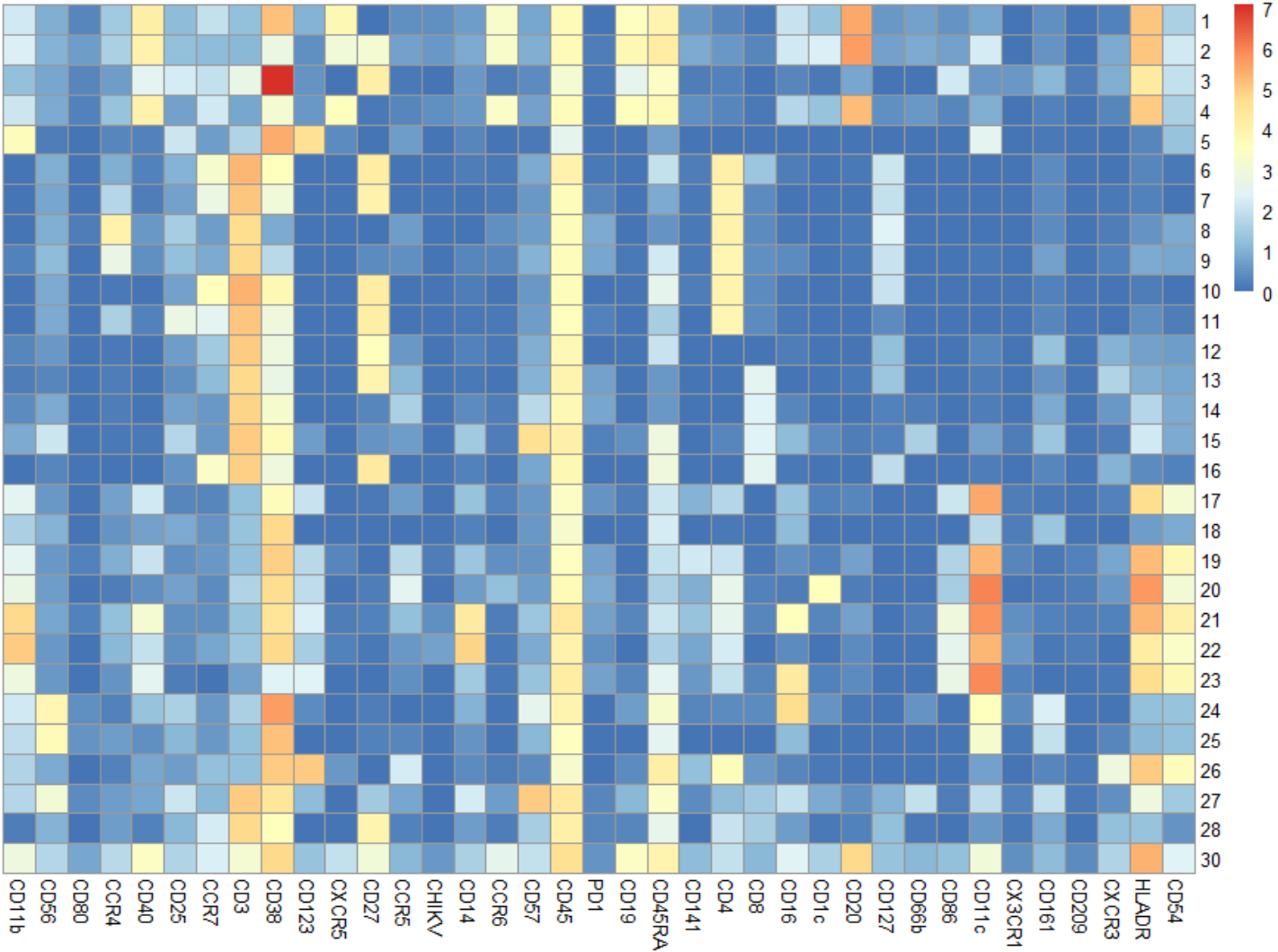
- MFI is based on transformed intensity (usually asinh)
  - MFI of untransformed intensities can be transformed at this stage
- Goal: determine the cell population in a cluster from its expression profile (aka MFI)
  - Determine which marker is high or low in a cluster
- Hypothesis: the expression of a marker ranges from low to high in the set of clusters
- Scaling: min max each marker
$$\min(\text{marker}_i\text{scaled}) = 0, \max(\text{marker}_i\text{scaled}) = 1$$
$$\text{marker}_i\text{scaled} = \frac{(\text{marker}_i - \min(\text{marker}_i))}{(\max(\text{marker}_i) - \min(\text{marker}_i))}$$

# Heatmap of MFI

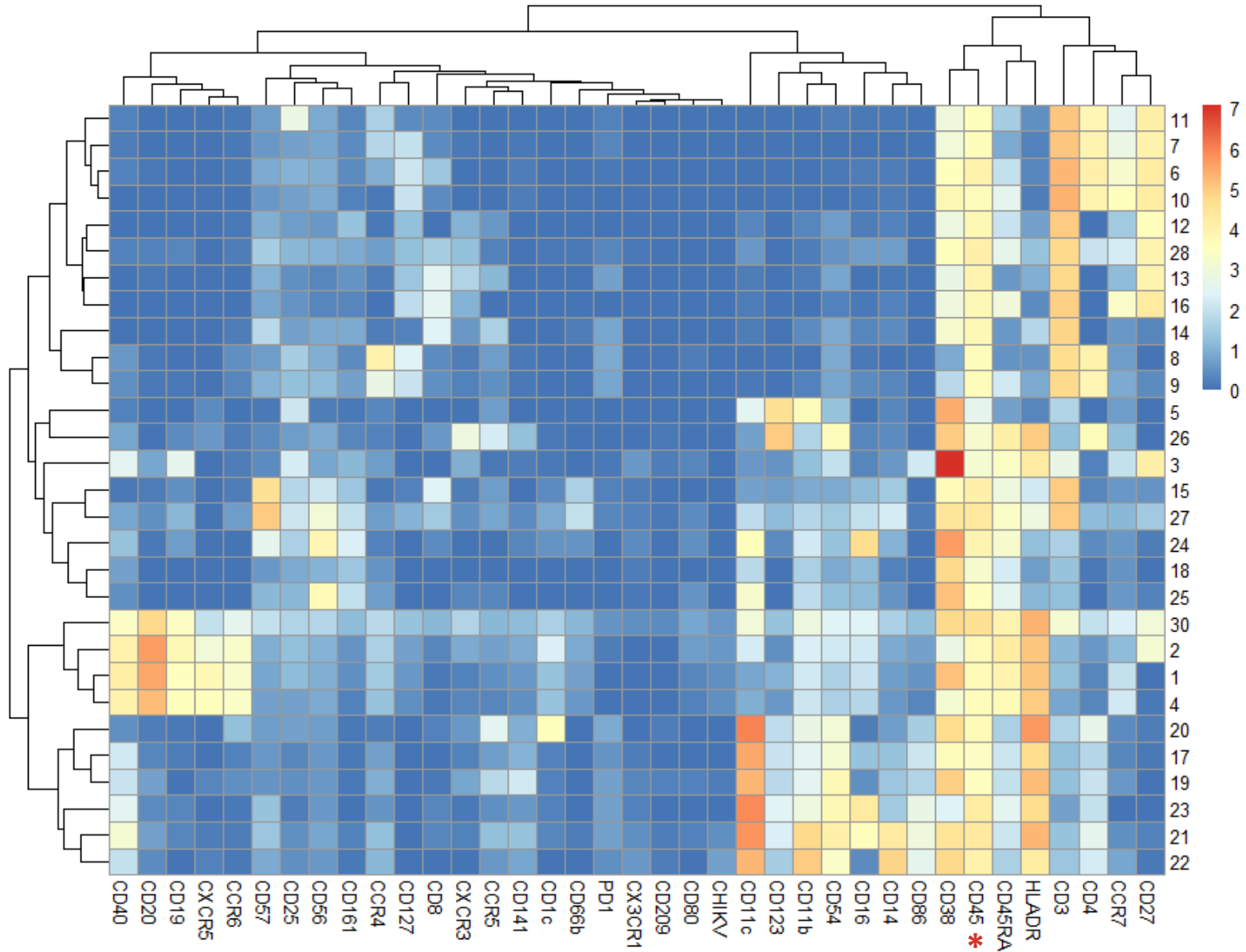




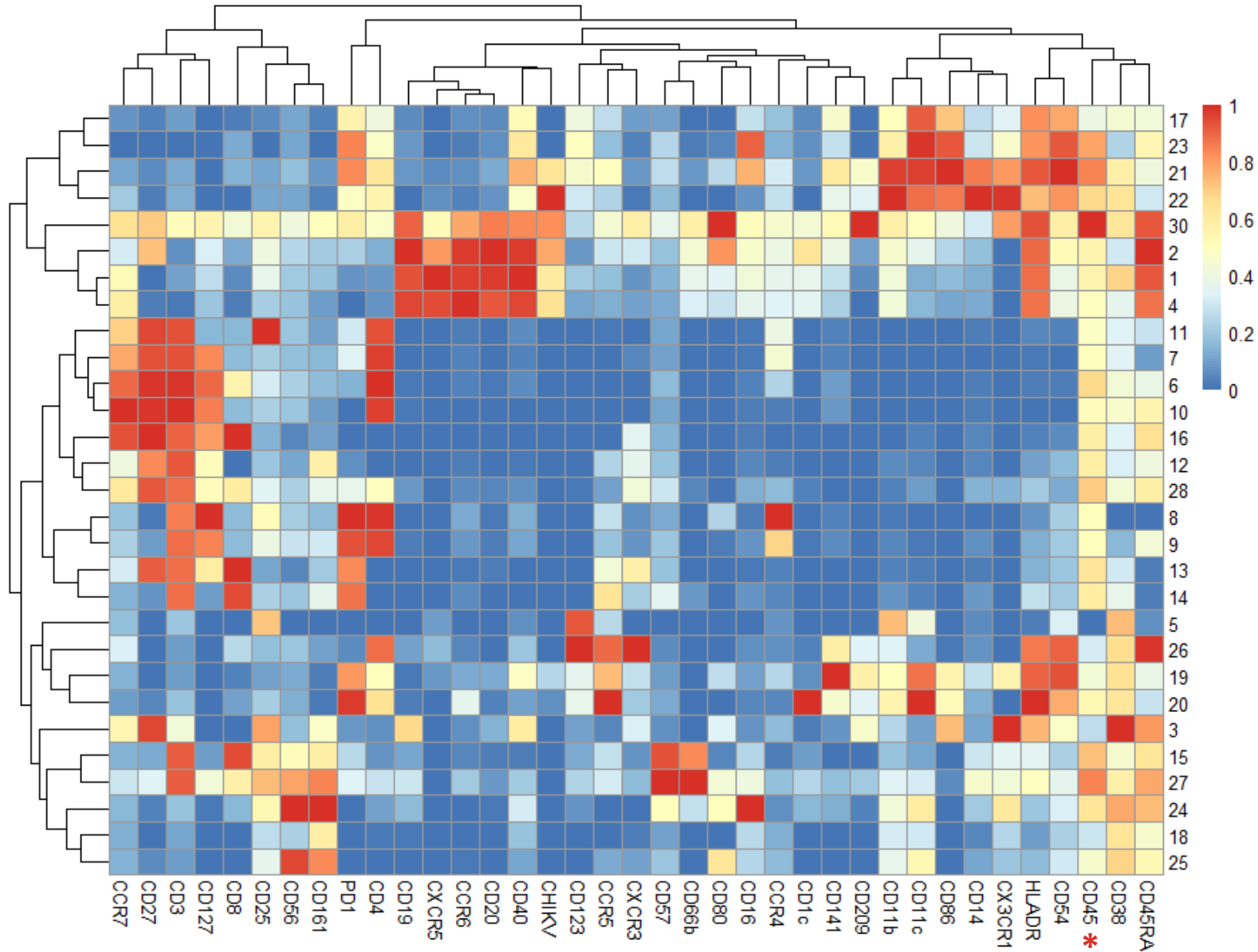
# Transformed MFI



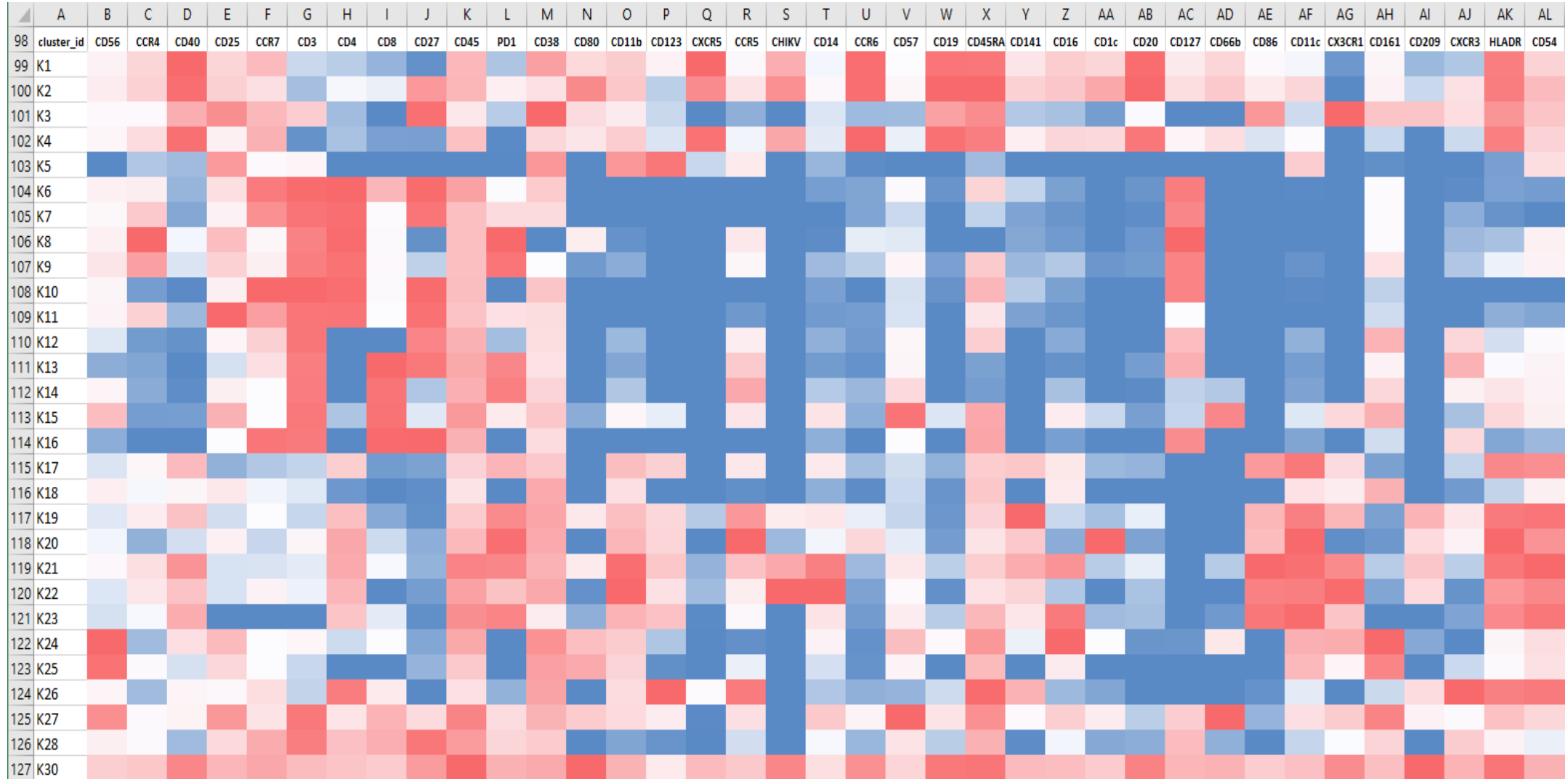
# Transformed MFI with hierar. clustering



# Scaled transformed MFI with hcl.



# Let's do it with Excel!



# Let's view it with Phantasus

The screenshot displays the Phantasus web interface. At the top, there are browser tabs for 'mfis\_cxm\_tr' and 'mfis\_cxm\_tr\_01\_with k'. Below the tabs is a menu bar with 'File', 'Edit', 'View', 'Tools', and 'Help'. A status bar indicates '29 rows by 37 columns' and '0 rows, 0 columns selected'. A toolbar contains icons for search, options, print, and zoom. The main area shows a heatmap with dendrograms on the top and left. The columns are labeled with cluster IDs: CCR4, CD4, PD1, CCR7, CD27, CD3, CD127, CD8, CXCR3, CD25, CD56, CD161, CD57, CD66b, CD45, CD16, CD80, CHIKV, CD40, CXCR5, CCR6, CD20, CD19, CD45RA, CD1c, CD38, CD123, CCR5, CD14, CX3CR1, CD11b, CD11c, CD86, CD141, HLADR, CD54, and CD209. The rows are labeled with cluster IDs: K2, K1, K4, K30, K8, K9, K7, K6, K10, K11, K12, K16, K28, K13, K14, K3, K15, K27, K18, K24, K25, K5, K26, K20, K19, K17, K23, K21, and K22. The heatmap uses a color scale from blue (low) to red (high). On the right, an 'Options' panel is open, showing the 'Color Scheme' tab. It features a color gradient bar and several controls: 'Selected color' (black), 'Selected value' (input field), 'Delete Selected Color Stop' (button), 'Add Color Stop' (button), 'Minimum' (0), 'Maximum' (1), 'Transform values' (None), and 'Missing color' (grey). A red dashed box highlights the 'Relative color scheme' checkbox, which is unchecked. A red annotation next to it says 'unselect for avoiding another transform'.

Options

Annotations Color Scheme Display

Selected color

Selected value

Delete Selected Color Stop

Add Color Stop

Minimum 0

Maximum 1

Transform values None

Missing color

Relative color scheme  
A relative color scheme uses the minimum and maximum values in each row to convert values to colors

*unselect for avoiding another transform*

- <https://artyomovlab.wustl.edu/phantasus/>

# Conclusion

- Heatmap is a classical way to annotate cells clusters
- MFI need to be transformed and scaled from 0 to 1
- Caveats of pre-gated markers
  
- Data available at [i-cyto.github.io](https://i-cyto.github.io)
  
- To be continued...