

## **SUPPLEMENT**

**Measuring host immune response status by simultaneous and quantitative measurement of activity of the signal transduction pathways that coordinate functional activity of immune cells from the innate and adaptive immune system**

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### **Contains:**

- I. STP activity analysis results, additional datasets for primary immune cell types
- II. Comparison of Affymetrix dataset analysis results between STP analysis (this publication) and analysis performed as described by the investigators who originally generated the dataset

**I. Supplementary figures (STP activity analysis results, additional datasets for primary immune cell types)**

Group	Annotation per sample	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
1. Monocytes	pool 1	-13.2	-6.9	11.1	-4.9	-4.5	-6.6	-6.9	-9.4	-3.1
	pool 2	-14.7	-7.1	13.7	-4.6	6.5	-7.3	-6.1	-7.4	-0.1
	pool 3	-14.0	-6.2	13.7	-4.2	10.4	-6.4	-4.5	-8.2	2.2
	pool 4	-13.8	-7.1	14.2	-5.5	5.8	-7.1	-5.7	-8.6	0.2
	pool 5	-12.7	-5.2	16.6	-4.9	8.1	-7.4	-5.3	-8.5	-0.7
	pool 6	-12.7	-6.0	17.0	-5.0	7.2	-8.4	-4.9	-9.0	-0.2
	pool 7	-12.3	-6.9	10.6	-6.4	0.6	-6.5	-7.1	-12.1	-2.5
	pool 8	-12.6	-8.9	16.9	-5.3	-2.1	-7.1	-7.4	-11.1	-4.7
	pool 9	-11.6	-9.5	18.2	-5.5	-4.2	-7.0	-7.0	-10.2	-4.0
	pool 10	-11.6	-6.6	18.5	-4.5	-4.7	-7.2	-7.0	-9.6	-1.8
2. B- cells	pool 1	-17.4	-16.0	0.6	-9.9	-12.5	-4.4	-8.2	-18.5	-3.9
	pool 2	-18.1	-16.7	0.5	-9.2	-14.6	-4.8	-11.7	-18.0	-4.4
	pool 3	-17.4	-16.1	-0.1	-8.6	-13.8	-3.1	-12.3	-18.3	-6.0
	pool 4	-17.8	-16.1	-0.1	-9.5	-13.9	-4.3	-10.8	-19.4	-7.1
	pool 5	-16.8	-15.8	2.4	-11.4	-12.2	-3.4	-8.4	-17.9	-2.9
3. CD4+T cells	pool 1	-12.5	-16.5	-4.7	-13.0	-6.2	-7.1	-8.6	-18.9	-7.5
	pool 2	-11.2	-14.3	10.9	-8.4	1.7	-8.4	-3.8	-11.8	-3.1
	pool 3	-13.1	-16.6	-0.6	-13.3	-4.7	-7.4	-6.5	-17.4	-6.6
	pool 4	-12.0	-16.1	2.7	-15.0	-5.5	-7.8	-5.7	-18.0	-6.3
	pool 5	-12.1	-17.0	-0.7	-13.2	-8.1	-7.2	-6.6	-19.2	-6.9
4. CD8+T cells	pool 1	-11.5	-16.8	0.9	-11.2	-4.1	-8.4	-6.5	-13.1	-4.7
	pool 2	-12.2	-16.8	0.3	-11.7	-3.7	-8.1	-5.2	-14.0	-5.9
	pool 3	-11.5	-16.8	-2.0	-10.5	-4.3	-8.5	-7.7	-14.2	-6.2
	pool 4	-16.1	-17.1	-6.9	-12.8	-6.2	-7.1	-9.7	-18.3	-5.7
	pool 5	-14.9	-17.6	-4.3	-13.8	-5.6	-7.9	-7.9	-18.3	-5.5
5. NK cells	pool 1	-13.0	-14.4	-1.8	-9.0	-3.9	-7.7	-5.7	-7.8	-4.4
	pool 2	-15.0	-15.8	-5.9	-11.5	-1.4	-7.0	-8.4	-11.4	-5.3
	pool 3	-17.3	-16.2	-8.6	-12.5	-0.6	-6.5	-11.1	-15.2	-4.2
	pool 4	-15.5	-15.6	-6.5	-12.6	-1.4	-7.1	-8.3	-10.9	-5.5
	pool 5	-15.5	-15.6	-4.1	-10.8	-2.6	-3.8	-6.3	-7.1	-2.9
6. mDC	pool 1	-13.3	-10.9	-4.9	-6.2	5.6	-5.7	-7.4	-15.9	-7.3
	pool 2	-13.1	-9.6	-2.7	-7.3	0.3	-5.7	-7.7	-18.5	-6.3
	pool 3	-14.3	-11.8	-9.0	-12.5	0.8	-5.0	-7.5	-17.5	-8.0
	pool 4	-13.1	-8.7	-4.9	-7.8	3.6	-6.0	-5.1	-17.8	-6.2
	pool 5	-13.5	-11.3	-4.5	-8.4	-0.8	-6.8	-6.5	-18.7	-7.2
7. pDC	pool 1	-8.6	-14.8	-4.3	-9.6	-11.7	0.5	-8.7	-18.1	-9.0
	pool 2	-8.3	-14.9	-3.6	-10.2	-12.4	1.8	-8.5	-19.4	-8.1
	pool 3	-8.0	-13.3	-5.8	-12.1	-7.7	0.5	-9.7	-19.2	-8.3
	pool 4	-7.4	-14.0	-2.3	-12.5	-14.7	0.0	-8.7	-18.7	-8.6
	pool 5	-7.5	-13.4	-2.6	-12.2	-13.1	-0.9	-9.2	-19.0	-8.1
1,6,7 vs. 2-5	delta mean	-2.7	-6.5	-6.7	-3.9	-4.8	-1.6	-0.9	-1.6	-0.7
	t-test p-value	0.001085	1.13E-08	0.012667	2.33E-05	0.022999	0.065072	0.137261	0.262859	0.448545
1 vs. 6,7	delta mean	2.2	-5.2	-19.5	-4.8	-7.3	4.4	-1.7	-8.9	-6.2
	t-test p-value	0.046827	9.11E-06	4.9E-12	9.41E-05	0.028843	0.002592	0.005826	1.84E-11	1.58E-06
2 vs. 3,4,5	delta mean	3.9	-0.1	-2.7	-2.2	9.6	-3.3	3.1	4.1	-0.5
	t-test p-value	1.35E-06	0.776482	0.054636	0.005041	8.33E-10	6.92E-06	0.016062	0.001554	0.55408
3 vs. 4	delta mean	-1.1	-0.9	-3.9	0.6	-0.2	-0.4	-1.2	1.5	0.5
	t-test p-value	0.337958	0.125923	0.240733	0.645094	0.906057	0.267641	0.316722	0.419832	0.579261
5 vs. 2,3,4	delta mean	1.0	-0.9	5.3	-0.2	-5.6	-0.1	0.0	-6.5	-1.1
	t-test p-value	0.341245	0.034323	0.005395	0.859121	0.000664	0.903656	0.992236	0.00715	0.099683
2 vs. 3,4,5	delta mean	3.9	-0.1	-2.7	-2.2	9.6	-3.3	3.1	4.1	-0.5
	t-test p-value	1.35E-06	0.776482	0.054636	0.005041	8.33E-10	6.92E-06	0.016062	0.001554	0.55408
6 vs. 7	delta mean	5.5	-3.6	1.5	-2.9	-13.8	6.2	-2.1	-1.2	-1.4
	t-test p-value	1.29E-07	0.001052	0.259737	0.055395	3.1E-05	7.09E-06	0.008227	0.07156	0.00948

**S1. GSE28490, (Allantaz et al., 2012). Immune blood cell types (neutrophils, eosinophils, monocytes, B cells, NK cells, CD4 T cells, CD8 T cells, mDCs and pDCs) isolated from peripheral blood from healthy volunteers. Pathway activity scores are depicted in log2odds values. Color coding visualizes pathway activity scores ranging from blue (lowest) to red (highest pathway activity scores). P<0.01, green; p<0.5 orange.**

Group	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
Resting NK	-7.4	-11.0	7.8	-9.5	8.5	-6.8	0.2	-2.9	0.3
NK 2 hrs IL-2 activation	-8.4	-11.4	1.5	-6.8	2.5	-6.9	-3.0	-1.0	-1.6
NK 8 hrs IL-2 activation	-16.1	-10.1	-6.8	-8.9	-0.4	-7.1	-5.1	-8.4	-4.5
NK 24 hrs IL-2 activation	-13.3	-9.6	-4.9	-11.3	7.6	-7.4	3.8	-7.1	-5.1

A

Group	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
stimulated_24hr_donor_1	-14.0	-13.9	8.0	-14.0	8.2	-4.2	10.3	-7.6	-4.5
stimulated_24hr_donor_2	-13.8	-13.3	7.3	-14.1	9.7	-6.0	12.6	-8.3	-4.3
unstimulated_freshly_isolated_donor_1	-14.4	-16.4	4.1	-14.2	0.6	-6.7	-7.4	-9.6	-3.2
unstimulated_freshly_isolated_donor_2	-8.9	-15.6	4.7	-14.4	0.3	-6.3	-7.3	-10.3	-3.6

B

**S2. Natural killer cells,**

A) GSE8059, (Dybkaer et al., 2007). NK-cells resting and activated with IL-2 (100 IU/ml) for 2, 8 and 24h.

B) GSE22919,(Smith et al., 2010). NK cells unstimulated and stimulated with IL-2 (100U/mL) + IL-12 (10ng/mL) and IL-18 (100ng/mL).

Group	Annotation per sample	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
1. Saline	subject 1	-6.1	-11.8	0.1	-3.2	6.7	-4.3	4.2	-7.1	-3.3
	subject 2	-5.3	-11.9	-0.5	-4.2	-3.1	-4.5	-1.3	-8.9	-4.5
	subject 3	-6.3	-10.5	5.7	-5.0	6.1	-4.2	3.3	-6.7	-4.1
	subject 5	-6.1	-11.1	4.2	-4.4	11.8	-5.2	3.5	-4.2	-1.0
	subject 6	-6.1	-10.1	4.7	-5.0	6.1	-6.3	-0.6	-6.3	-4.9
	subject 7	-6.5	-11.8	2.2	-4.1	9.3	-5.2	3.7	-5.7	-1.6
	2. LPS	subject 1	-5.3	-12.5	1.2	-3.0	18.9	-2.6	5.6	-4.2
subject 3		-6.4	-12.1	1.7	-3.5	23.1	2.3	9.2	-5.1	-2.5
subject 4		-6.2	-12.0	1.1	-4.3	21.0	-2.1	6.1	-4.8	-2.0
subject 5		-5.8	-12.0	1.0	-0.3	22.7	-1.1	9.5	-1.4	0.5
subject 6		-6.2	-12.0	0.7	-2.3	22.3	-0.8	8.7	-4.1	-0.5
subject 7		-6.1	-13.4	0.8	-1.7	23.1	-0.5	9.9	-3.8	-0.7
1 vs. 2		delta mean	0.0	1.4	2.0	-1.5	-15.1	-4.2	-5.4	-2.3
	t-test p-value	0.985001	0.002802	0.068968	0.031379	3.56E-05	0.000156	0.000493	0.008023	0.029465

**S3.** GSE40885, (Reynier et al., 2012). Alveolar macrophages obtained from a contralateral lung segment challenged with LPS (4 ng/kg body weight) or saline (control).

Group	Annotation per sample	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
1. CD4+ naïve	Replicate 2	-11.5	-20.4	5.2	-17.7	4.0	-8.1	-8.0	-13.1	-2.3
	Replicate 3	-11.8	-20.3	0.4	-18.1	-1.5	-6.9	-8.5	-13.0	-2.8
2. CD4+ activated	Replicate 1	-9.4	-19.8	-7.0	-18.0	0.1	-1.6	-5.1	-16.1	-2.6
	Replicate 2	-9.5	-19.8	-6.8	-16.9	-0.1	-1.7	-6.6	-16.4	-2.1
	Replicate 3	-12.6	-19.6	-6.1	-15.4	-1.6	-1.5	-9.3	-14.8	-0.9
3. CD4+ activated + IL12 (Th1)	Replicate 1	-8.3	-19.6	-5.3	-19.4	-0.1	-2.7	-2.5	-15.5	-3.6
	Replicate 2	-7.4	-19.5	-3.3	-18.4	-0.5	-2.1	-2.4	-15.8	-2.4
	Replicate 3	-8.8	-19.4	-3.7	-15.9	-1.7	-3.3	-6.6	-13.1	-2.8
4. CD4+ activated + IL4 (Th2)	Replicate 1	-10.4	-19.5	-3.1	-16.8	-7.1	-6.0	-6.7	-19.7	-3.0
	Replicate 2	-11.0	-20.2	-5.9	-15.7	-2.0	-4.5	-8.4	-18.8	-2.9
	Replicate 3	-12.7	-19.5	1.0	-16.1	-3.0	-6.0	-9.6	-16.2	-2.2
1 vs. 2	delta mean	1.2	0.6	-9.5	1.1	-1.8	5.9	1.3	-2.7	0.7
	t-test p-value	0.387111	0.009662	0.155687	0.266907	0.631143	0.066395	0.410624	0.030814	0.295629
2 vs. 3,4	delta mean	0.7	0.1	3.2	-0.3	-1.9	-2.5	1.0	-0.7	-0.9
	t-test p-value	0.617402	0.354089	0.021409	0.773915	0.157354	0.014281	0.601685	0.519215	0.18578
2 vs. 3	delta mean	2.3	0.3	2.5	-1.1	-0.2	-1.1	3.2	1.0	-1.0
	t-test p-value	0.149679	0.032405	0.040751	0.431792	0.768251	0.085709	0.168605	0.390881	0.167513
2 vs. 4	delta mean	-0.9	0.0	4.0	0.5	-3.5	-3.9	-1.2	-2.5	-0.8
	t-test p-value	0.525597	0.96902	0.184881	0.553179	0.146009	0.016446	0.470216	0.12637	0.227599
1 vs. 3,4	delta mean	1.9	0.7	-6.2	0.8	-3.6	3.4	2.3	-3.5	-0.2
	t-test p-value	0.064911	0.001644	0.197569	0.243846	0.392127	0.024193	0.12428	0.016961	0.528564
1 vs. 3	delta mean	3.5	0.9	-6.9	0.0	-2.0	4.8	4.4	-1.7	-0.3
	t-test p-value	0.008654	0.002995	0.195501	0.99647	0.595507	0.03472	0.081299	0.182828	0.493579
1 vs. 4	delta mean	0.3	0.6	-5.5	1.7	-5.3	2.0	0.1	-5.2	-0.1
	t-test p-value	0.717226	0.118896	0.206303	0.020263	0.259857	0.115904	0.93643	0.038183	0.739914
3 vs. 4	delta mean	-3.2	-0.2	1.4	1.7	-3.3	-2.8	-4.4	-3.5	0.2
	t-test p-value	0.02134	0.401222	0.552599	0.244772	0.165828	0.014521	0.067632	0.065249	0.674315

**S4.** GSE32959, (Aijö et al., 2012). Naïve CD4+ cells, CD4+ activated (antiCD3 500 ng/well+ antiCD28 (500 ng/ml), CD4+ activated + Th1 polarization (IL-12 (2.5ng/ml)) and CD4+ activated + Th2 polarization (IL-4 (10ng/ml and anti-IL-12 (10µg/ml)). For induction of Th1 cell polarization, IL-12 (2.5 ng/ml) was added to the cultures. At 48h after activation, IL-2 was added (17 ng/ml) to all the cells and the polarizing conditions were maintained throughout the culture.

Group	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
Primary peripheral blood B cell - Resting	-8.95	-14.50	8.57	-10.31	0.24	-4.53	-2.65	-6.73	2.43
Primary peripheral blood B cell - Resting	-9.19	-15.22	8.42	-10.51	0.54	-4.81	-3.76	-6.61	1.38
Primary peripheral blood B cell + antiIgM -1hr	-6.20	-12.73	6.35	-10.14	5.82	-2.53	5.58	-2.92	4.62
Primary peripheral blood B cell + antiIgM -3hr	-6.90	-13.38	1.70	-9.17	3.30	-6.92	2.59	-6.68	5.08

**S5. GSE9119, (Shaffer et al., 2008). B-cells resting and activated with anti IgM (25ug/ml) for 1hr and 3hrs.**

*Note: 3 samples failed our Affymetrix QC, on Cutoff criterium.*

infection	Donor	Time (h)	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH
AF	Donor 1, replicate 1	0	-7.5	-9.4	1.1	-8.9	12.8	-11.5	0.7	-15.4	-3.3
AF	Donor 1, replicate 1	0	-9.5	-11.3	-0.9	-9.5	12.1	-11.3	-4.0	-14.4	-3.6
AF	Donor 1, replicate 2	0	-12.4	-13.4	-1.3	-7.1	14.7	-11.0	-3.6	-15.9	-5.1
AF	Donor 2, replicate 2	0	-9.3	-10.4	-2.5	-8.8	9.6	-11.3	-0.9	-14.2	-4.2
AF	Donor 2, replicate 1	1	-10.0	-11.8	-1.9	-9.4	13.3	-11.0	-2.6	-14.3	-4.2
AF	Donor 1, replicate 2	1	-12.4	-13.9	-0.4	-8.1	14.2	-10.1	-3.2	-16.1	-2.9
NDV	Donor 1, replicate 1	1	-7.3	-10.1	3.3	-8.0	13.6	-11.4	1.6	-14.2	-3.2
NDV	Donor 2, replicate 1	1	-9.9	-11.0	-0.8	-9.8	12.2	-10.9	-3.9	-14.5	-4.5
NDV	Donor 1, replicate 2	1	-11.6	-13.5	-0.3	-8.5	15.8	-10.5	-3.0	-16.0	-2.4
NDV	Donor 2, replicate 2	1	-9.1	-11.5	3.1	-9.2	15.0	-11.2	-1.6	-14.3	-2.5
AF	Donor 2, replicate 1	2	-9.6	-12.1	-0.2	-10.3	12.9	-11.3	-2.9	-14.4	-4.2
AF	Donor 1, replicate 2	2	-11.4	-13.8	0.1	-6.9	12.0	-10.4	-2.4	-14.4	-4.6
NDV	Donor 1, replicate 1	2	-7.2	-9.3	3.0	-8.5	16.7	-10.5	2.4	-10.1	-3.6
NDV	Donor 2, replicate 1	2	-11.5	-11.7	-2.9	-11.5	10.5	-10.9	-4.6	-13.4	-3.8
NDV	Donor 1, replicate 2	2	-11.5	-13.5	0.2	-8.5	16.3	-10.5	-2.2	-14.0	-4.7
NDV	Donor 2, replicate 2	2	-9.0	-11.8	5.3	-9.1	15.8	-10.6	-1.5	-12.5	-2.6
NDV	Donor 1, replicate 1	4	-6.8	-11.1	8.9	-9.1	20.1	-0.9	2.4	-12.0	-3.5
NDV	Donor 2, replicate 1	4	-10.2	-11.4	-0.2	-11.5	15.0	-7.4	-4.3	-12.8	-3.4
NDV	Donor 1, replicate 2	4	-12.4	-13.9	2.4	-8.6	19.6	-4.7	-2.0	-13.3	-2.9
NDV	Donor 2, replicate 2	4	-9.0	-13.8	10.3	-10.3	22.1	-0.6	-0.7	-11.5	-2.2
AF	Donor 1, replicate 1	6	-6.4	-9.8	4.3	-8.2	14.7	-10.0	0.3	-11.1	-3.2
AF	Donor 1, replicate 2	6	-12.5	-13.5	2.7	-6.0	12.0	-9.0	-1.2	-12.9	-4.6
AF	Donor 2, replicate 2	6	-8.1	-12.3	11.1	-6.3	15.2	-9.6	-0.8	-10.8	-3.1
NDV	Donor 1, replicate 1	6	-6.6	-10.9	8.6	-9.8	21.8	2.5	2.5	-11.7	-1.4
NDV	Donor 1, replicate 2	6	-10.0	-15.8	6.9	-8.2	28.1	1.4	-1.8	-14.2	-2.3
NDV	Donor 2, replicate 2	6	-7.9	-13.9	12.1	-10.0	27.3	2.0	0.9	-12.0	-2.7
NDV	Donor 1, replicate 1	8	-6.5	-11.1	9.4	-10.8	23.6	4.7	2.7	-10.6	-2.1
NDV	Donor 2, replicate 1	8	-11.2	-13.0	5.1	-12.7	23.5	-0.4	-2.3	-13.4	-4.0
NDV	Donor 1, replicate 2	8	-12.1	-14.5	8.6	-10.5	28.6	2.6	-0.6	-10.4	-3.7
NDV	Donor 2, replicate 2	8	-7.0	-15.4	11.1	-7.8	29.9	4.5	1.4	-5.8	-2.5
AF	Donor 1, replicate 1	10	-6.5	-10.2	6.7	-6.6	17.1	-10.4	1.7	-9.3	-3.3
AF	Donor 2, replicate 1	10	-10.2	-12.1	0.2	-11.0	12.4	-10.1	-3.8	-12.8	-3.8
AF	Donor 1, replicate 2	10	-9.7	-11.3	5.0	-5.9	14.5	-9.7	0.4	-9.9	-3.8
AF	Donor 2, replicate 2	10	-7.7	-12.5	10.2	-7.2	14.3	-9.5	-0.4	-8.6	-3.2
NDV	Donor 1, replicate 1	10	-6.8	-12.3	11.0	-11.4	25.9	5.5	5.7	-4.2	-2.2
NDV	Donor 1, replicate 2	10	-9.7	-13.5	10.9	-9.0	31.1	5.5	1.0	-4.6	-4.0
NDV	Donor 2, replicate 2	10	-7.8	-14.0	10.7	-11.7	30.2	6.7	2.5	-6.8	-3.1
NDV	Donor 1, replicate 1	12	-6.9	-10.7	13.6	-11.0	23.8	7.4	6.7	-2.2	-1.4
NDV	Donor 1, replicate 2	12	-8.5	-12.9	10.9	-10.8	31.6	6.4	1.3	-3.1	-0.5
NDV	Donor 2, replicate 2	12	-7.1	-11.2	9.6	-8.7	32.1	9.1	3.2	-3.3	-2.5
NDV	Donor 1, replicate 1	14	-6.7	-11.0	13.0	-11.2	23.9	8.4	6.7	-1.6	-1.7
NDV	Donor 2, replicate 1	14	-11.1	-14.5	2.4	-11.5	21.5	0.8	-2.6	-8.7	-0.9
NDV	Donor 1, replicate 2	14	-8.7	-12.7	12.8	-10.8	33.0	7.7	2.4	-1.3	-3.3
NDV	Donor 2, replicate 2	14	-8.1	-11.9	9.7	-8.3	32.0	9.3	2.9	-3.2	-1.6
NDV	Donor 1, replicate 1	16	-6.7	-11.0	15.9	-10.6	25.0	9.1	5.9	-2.5	-1.7
NDV	Donor 2, replicate 1	16	-11.3	-14.9	6.1	-12.5	21.9	2.2	-3.1	-8.1	-1.5
NDV	Donor 1, replicate 2	16	-8.3	-11.6	13.9	-10.3	32.8	6.7	1.6	-1.2	-1.6
NDV	Donor 2, replicate 2	16	-9.1	-15.0	7.0	-9.4	28.2	6.7	3.3	-6.2	-2.3
AF	Donor 2, replicate 1	18	-10.0	-12.6	0.7	-12.2	10.6	-10.0	-4.4	-11.4	-3.6
AF	Donor 1, replicate 2	18	-8.7	-12.2	9.5	-6.2	17.9	-9.2	-0.6	-11.7	-4.3
NDV	Donor 1, replicate 2	18	-8.6	-12.9	13.4	-10.4	32.3	7.8	1.7	-1.7	-2.1
NDV	Donor 2, replicate 2	18	-8.7	-14.3	11.0	-11.2	27.9	8.2	3.4	-4.9	-2.2

A

Group	Annotation per sample	AR	ER	FOXO	AP1	NFKB	STAT1-2	STAT3	TGFB	NOTCH	
1. Immature DCs	replicate 1	-7.6	-6.6	5.2	-9.4	14.2	-10.6	6.0	-12.3	0.7	
	replicate 2	-7.6	-6.2	6.2	-8.9	14.2	-10.6	7.5	-10.9	0.5	
	replicate 3	-7.4	-6.1	6.0	-7.3	13.7	-10.5	7.2	-11.6	0.5	
2. Maturing DCs	replicate 1	-7.4	-9.5	16.4	-0.7	27.6	6.3	10.2	0.9	3.0	
	replicate 2	-7.2	-9.0	18.0	-0.7	24.9	6.9	10.7	2.4	3.4	
	replicate 3	-6.9	-9.3	17.3	-0.8	26.5	7.6	10.7	0.5	2.8	
3. Tolerogenic DCs	replicate 1	-7.0	-8.3	10.2	-6.3	10.1	-6.5	3.7	-13.0	0.5	
	replicate 2	-7.1	-8.0	11.1	-5.3	12.2	-6.8	5.2	-11.9	0.3	
	replicate 3	-7.0	-8.1	10.2	-6.0	10.2	-6.7	4.7	-11.8	0.9	
4. Activated tolerogenic DCs	replicate 1	-6.9	-9.2	14.6	-2.0	28.1	3.4	12.1	-2.8	3.6	
	replicate 2	-6.5	-9.0	15.9	-1.1	28.2	3.2	11.8	-0.9	5.0	
	replicate 3	-6.4	-8.5	16.6	-1.0	28.1	3.0	10.9	-1.3	4.4	
1 vs 2	delta mean	-0.4	3.0	-1.4	-11.5	-7.8	-12.3	-17.5	-3.6	-12.9	-2.5
	t-test p-value	0.1220310	0.0001430	0.024902	7.41E-050	0.0062250	0.0030890	0.0004130	0.0092350	0.0001060	0.002872
3 vs 4	delta mean	-0.4	0.8	-4.0	-5.2	-4.5	-17.3	-9.9	-7.0	-10.6	-3.7
	t-test p-value	0.0815110	0.051512	1.41E-05	0.0045960	0.0004970	0.001583	1.59E-06	0.0003240	0.0002460	0.004037

B

### S6. Dendritic cells

A. GSE18791, (Zaslavsky et al., 2010a). All STP PAS from this study. Dendritic cells (Monocyte-derived DCs) from two donors, resting and activated by infection with Newcastle disease virus (NDV), at 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 hours after infection versus control.

B) GSE23371, (Jansen et al., 2011); DCs immature (IL4 and GM-CSF), mature (IL4 and GM-CSF + 6h LPS), tolerogenic (IL4 and GM-CSF + 24 hr IL10/dexamethasone), activated tolerogenic (IL4 and GM-CSF + IL10/dexamethasone 24 hr, then LPS 6 hr).



## **II. Comparison of Affymetrix dataset analysis results between STP analysis (this publication) and analysis performed as described by the investigators who originally generated the dataset**

GSE72642, (Du et al., 2006)

Data analysis: Differential gene expression. No Pathway analysis

No signaling pathway activity identified.

GSE15743, (Stegmann et al., 2010)

Data analysis: Differential expression of TRAIL gene. No pathway analysis.

No signaling pathway activity identified.

GSE22103, (Kotz et al., 2010)

Data analysis: Ingenuity Pathway analysis.

Identified cellular processes (“pathways”) (top 10):

- Upregulated
  - Oxidative phosphorylation
  - Mitochondrial dysfunction
  - Ubiquinone biosynthesis
  - Polyamine regulation in colon cancer
  - Protein ubiquitination pathway
  - Inositol metabolism
  - Pyrimidine metabolism
  - RAN signaling
  - Mitotic roles of polo-like kinases

- N-glycan biosynthesis
- Downregulated:
  - Antigen presentation pathway
  - IL-4 signaling
  - GNRH signaling
  - Retinoic acid-mediated apoptosis signaling
  - Interferon signaling
  - Corticotropin releasing hormone signaling
  - Death receptor signaling
  - B cell receptor signaling
  - Huntington's disease signaling
  - IL-3 signaling

GSE38351 (Smiljanovic et al., 2012)

Data analysis: Ingenuity Pathway Analysis

JAK-STAT1/2 pathway identified, no information on activity of the pathway.

GSE43596, (Lowe et al., 2014).

Data analysis: differential gene expression.

P53/NFκB transcription factor coregulation of immune response genes. No information on activity of the NFκB pathway.

GSE71566, (Kanduri et al., 2015)

Data analysis: differential gene expression. No Pathway analysis.

No signaling pathways identified.

GSE63129, (Maeda et al., 2014)

Data analysis: differential expression. No pathway analysis.

No signaling pathways identified.

GSE39411, (Vallat et al., 2013)

Data analysis: various methods(differential expression, regression, gene networks). No pathway analysis.

No signaling pathways identified for activation of B cells.

GSE18791, (Zaslavsky et al., 2010b)

Data analysis: TRANSFAC transcription factor heatmap construction.

Identification of transcriptional cascade: Interferon response factors (IRF), STATs and NFκB (STAT activity preceded NFκB in the TRANSFAC network).

No identification of signal transduction pathway activity.

GSE65010 (Walter et al., 2016).

Data analysis: Qlucore Omics Explorer software, version 3.0.

Result: no statistically significant differences between RA patients and healthy controls.

Conclusion, cited from abstract: *“Our findings indicate that there is no global defect in either CD45RO1 or CD45RA1 Treg cells in the PB of patients with chronic RA”.*

GSE93272, (Tasaki et al., 2018)

## Data analysis: Differential expression and ssGSEA for pathway analysis

Identified cellular processes (“pathways”):

- Regulation of complement cascade
- Coagulation
- COMP pathway
- Initial triggering of complement
- Complement cascade
- Telomerase pathway
- CXCR3 pathway

No signal transduction pathways identified.

## References

- Aijö, T., Edelman, S.M., Lönnberg, T., Larjo, A., Kallionpää, H., Tuomela, S., Engström, E., Lahesmaa, R., Lähdesmäki, H., 2012. An integrative computational systems biology approach identifies differentially regulated dynamic transcriptome signatures which drive the initiation of human T helper cell differentiation. *BMC Genomics* 13, 572. <https://doi.org/10.1186/1471-2164-13-572>
- Allantaz, F., Cheng, D.T., Bergauer, T., Ravindran, P., Rossier, M.F., Ebeling, M., Badi, L., Reis, B., Bitter, H., D’Asaro, M., Chiappe, A., Sridhar, S., Pacheco, G.D., Burczynski, M.E., Hochstrasser, D., Vonderscher, J., Matthes, T., 2012. Expression profiling of human immune cell subsets identifies miRNA-mRNA regulatory relationships correlated with cell type specific expression. *PLoS One* 7, e29979. <https://doi.org/10.1371/journal.pone.0029979>
- Du, X., Tang, Y., Xu, H., Lit, L., Walker, W., Ashwood, P., Gregg, J.P., Sharp, F.R., 2006. Genomic profiles for human peripheral blood T cells, B cells, natural killer cells, monocytes, and polymorphonuclear cells: comparisons to ischemic stroke, migraine, and Tourette syndrome. *Genomics* 87, 693–703. <https://doi.org/10.1016/j.ygeno.2006.02.003>
- Dybkaer, K., Iqbal, J., Zhou, G., Geng, H., Xiao, L., Schmitz, A., d’Amore, F., Chan, W.C., 2007. Genome wide transcriptional analysis of resting and IL2 activated human natural killer cells: gene expression signatures indicative of novel molecular signaling pathways. *BMC Genomics* 8, 230. <https://doi.org/10.1186/1471-2164-8-230>
- Jansen, B.J., Sama, I.E., Eleveld-Trancikova, D., van Hout-Kuijper, M.A., Jansen, J.H., Huynen, M.A., Adema, G.J., 2011. MicroRNA genes preferentially expressed in dendritic cells contain sites for conserved transcription factor binding motifs in their promoters. *BMC Genomics* 12, 330. <https://doi.org/10.1186/1471-2164-12-330>

- Kanduri, K., Tripathi, S., Larjo, A., Mannerström, H., Ullah, U., Lund, R., Hawkins, R.D., Ren, B., Lähdesmäki, H., Lahesmaa, R., 2015. Identification of global regulators of T-helper cell lineage specification. *Genome Med* 7, 122. <https://doi.org/10.1186/s13073-015-0237-0>
- Kotz, K.T., Xiao, W., Miller-Graziano, C., Qian, W.-J., Russom, A., Warner, E.A., Moldawer, L.L., De, A., Bankey, P.E., Petritis, B.O., Camp, D.G., Rosenbach, A.E., Goverman, J., Fagan, S.P., Brownstein, B.H., Irimia, D., Xu, W., Wilhelmy, J., Mindrinos, M.N., Smith, R.D., Davis, R.W., Tompkins, R.G., Toner, M., Inflammation and the Host Response to Injury Collaborative Research Program, 2010. Clinical microfluidics for neutrophil genomics and proteomics. *Nat Med* 16, 1042–1047. <https://doi.org/10.1038/nm.2205>
- Lowe, J.M., Menendez, D., Bushel, P.R., Shatz, M., Kirk, E.L., Troester, M.A., Garantzotis, S., Fessler, M.B., Resnick, M.A., 2014. p53 and NF- $\kappa$ B Co-regulate Pro-inflammatory Gene Responses in Human Macrophages. *Cancer Res* 74, 2182–2192. <https://doi.org/10.1158/0008-5472.CAN-13-1070>
- Maeda, Y., Nishikawa, H., Sugiyama, D., Ha, D., Hamaguchi, M., Saito, T., Nishioka, M., Wing, J.B., Adeegbe, D., Katayama, I., Sakaguchi, S., 2014. Detection of self-reactive CD8<sup>+</sup> T cells with an anergic phenotype in healthy individuals. *Science* 346, 1536–1540. <https://doi.org/10.1126/science.aaa1292>
- Reynier, F., de Vos, A.F., Hoogerwerf, J.J., Bresser, P., van der Zee, J.S., Paye, M., Pachot, A., Mouglin, B., van der Poll, T., 2012. Gene expression profiles in alveolar macrophages induced by lipopolysaccharide in humans. *Mol Med* 18, 1303–1311. <https://doi.org/10.2119/molmed.2012.00230>
- Shaffer, A.L., Emre, N.C.T., Lamy, L., Ngo, V.N., Wright, G., Xiao, W., Powell, J., Dave, S., Yu, X., Zhao, H., Zeng, Y., Chen, B., Epstein, J., Staudt, L.M., 2008. IRF4 addiction in multiple myeloma. *Nature* 454, 226–231. <https://doi.org/10.1038/nature07064>
- Smiljanovic, B., Grün, J.R., Biesen, R., Schulte-Wrede, U., Baumgrass, R., Stuhlmüller, B., Maslinski, W., Hiepe, F., Burmester, G.-R., Radbruch, A., Häupl, T., Grützkau, A., 2012. The multifaceted balance of TNF- $\alpha$  and type I/II interferon responses in SLE and RA: how monocytes manage the impact of cytokines. *J Mol Med (Berl)* 90, 1295–1309. <https://doi.org/10.1007/s00109-012-0907-y>
- Smith, M.A., Maurin, M., Cho, H.I., Becknell, B., Freud, A.G., Yu, J., Wei, S., Djeu, J., Celis, E., Caligiuri, M.A., Wright, K.L., 2010. PRDM1/Blimp-1 controls effector cytokine production in human NK cells. *J Immunol* 185, 6058–6067. <https://doi.org/10.4049/jimmunol.1001682>
- Stegmann, K.A., Björkström, N.K., Veber, H., Ciesek, S., Riese, P., Wiegand, J., Hadem, J., Suneetha, P.V., Jaroszewicz, J., Wang, C., Schlaphoff, V., Fytily, P., Cornberg, M., Manns, M.P., Geffers, R., Pietschmann, T., Guzmán, C.A., Ljunggren, H.-G., Wedemeyer, H., 2010. Interferon-alpha-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. *Gastroenterology* 138, 1885–1897. <https://doi.org/10.1053/j.gastro.2010.01.051>
- Tasaki, S., Suzuki, K., Kassai, Y., Takeshita, M., Murota, A., Kondo, Y., Ando, T., Nakayama, Y., Okuzono, Y., Takiguchi, M., Kurisu, R., Miyazaki, T., Yoshimoto, K., Yasuoka, H., Yamaoka, K., Morita, R., Yoshimura, A., Toyoshiba, H., Takeuchi, T., 2018. Multi-omics monitoring of drug response in rheumatoid arthritis in pursuit of molecular remission. *Nat Commun* 9, 2755. <https://doi.org/10.1038/s41467-018-05044-4>
- Vallat, L., Kemper, C.A., Jung, N., Maumy-Bertrand, M., Bertrand, F., Meyer, N., Pocheville, A., Fisher, J.W., Gribben, J.G., Bahram, S., 2013. Reverse-engineering the genetic circuitry of a cancer cell with predicted intervention in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 110, 459–464. <https://doi.org/10.1073/pnas.1211130110>

- Walter, G.J., Fleskens, V., Frederiksen, K.S., Rajasekhar, M., Menon, B., Gerwien, J.G., Evans, H.G., Taams, L.S., 2016. Phenotypic, Functional, and Gene Expression Profiling of Peripheral CD45RA+ and CD45RO+ CD4+CD25+CD127(low) Treg Cells in Patients With Chronic Rheumatoid Arthritis. *Arthritis Rheumatol* 68, 103–116.  
<https://doi.org/10.1002/art.39408>
- Zaslavsky, E., Hershberg, U., Seto, J., Pham, A.M., Marquez, S., Duke, J.L., Wetmur, J.G., Tenover, B.R., Sealfon, S.C., Kleinstein, S.H., 2010a. Antiviral response dictated by choreographed cascade of transcription factors. *J. Immunol.* 184, 2908–2917.  
<https://doi.org/10.4049/jimmunol.0903453>
- Zaslavsky, E., Hershberg, U., Seto, J., Pham, A.M., Marquez, S., Duke, J.L., Wetmur, J.G., Tenover, B.R., Sealfon, S.C., Kleinstein, S.H., 2010b. Antiviral response dictated by choreographed cascade of transcription factors. *J. Immunol.* 184, 2908–2917.  
<https://doi.org/10.4049/jimmunol.0903453>