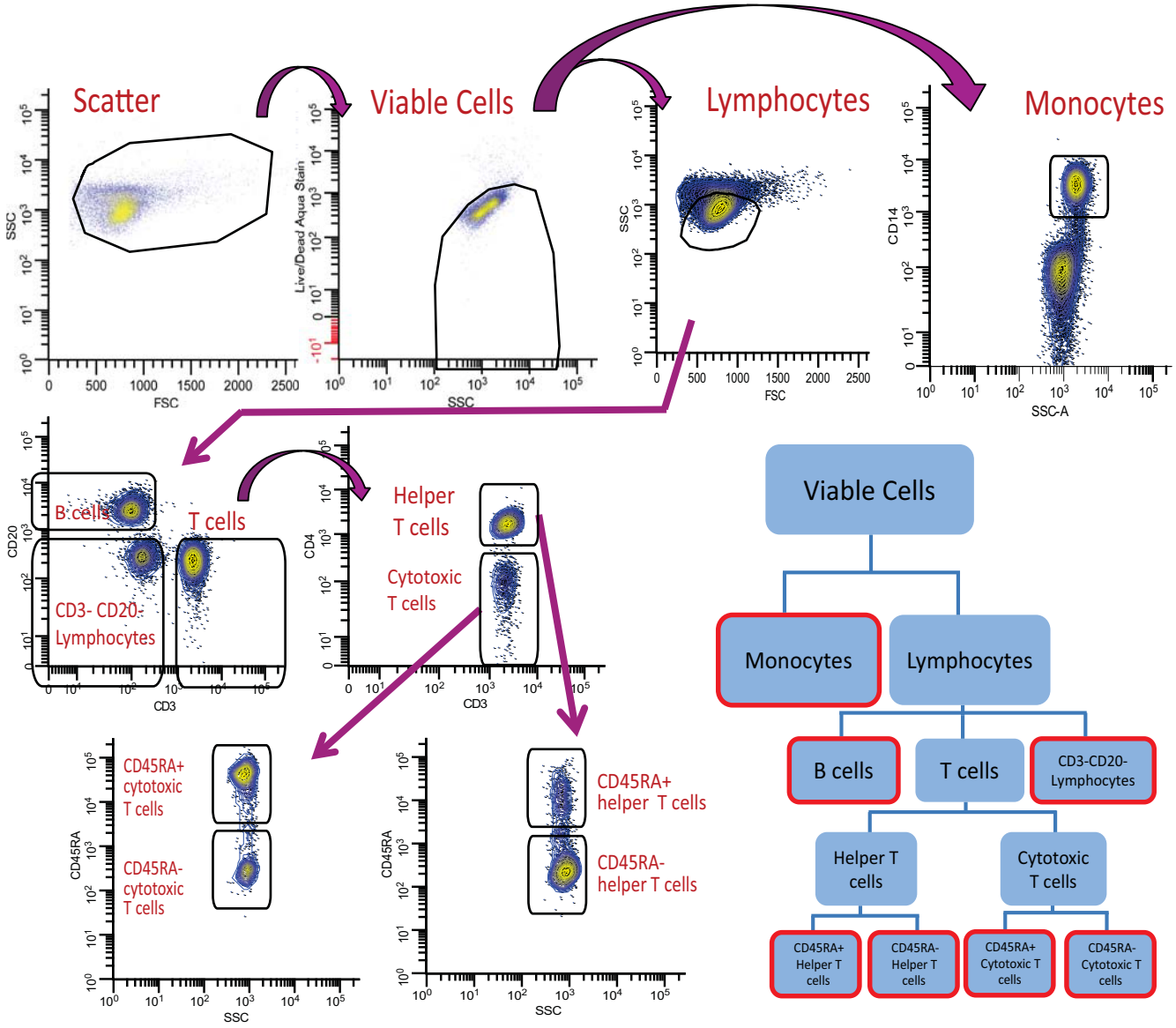
Supplemental Legends

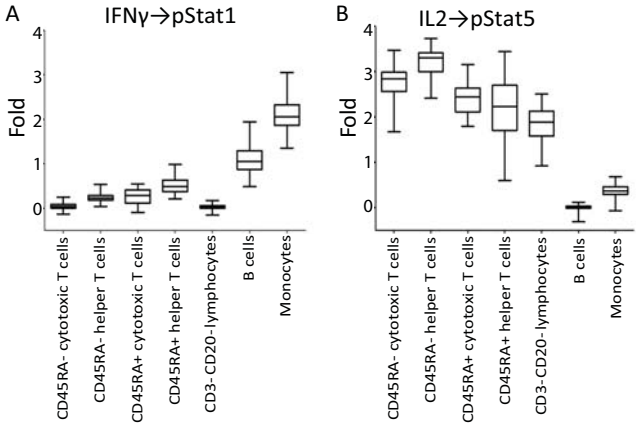
Fig. S1. Gating strategy used to delineate immune cell subpopulations within PBMCs. The dendrogram shows the gating hierarchy for the 12 cell subpopulations with the seven distinct immune cell subpopulations outlined in red.

Fig. S2. Differential activation of cell subpopulations. *A*, IFNγ→pStat1 responses in the seven distinct cell subpopulations for all 60 donors. *B*, IL2→pStat5 responses in the seven distinct cell subpopulations for all 60 donors.

Fig S3. Healthy immune signaling network map with responsive nodes only. A heatmap representing the magnitude of the Pearson correlation coefficients between pairs of nodes both within and between all seven distinct cell subpopulations. Signaling nodes that did not show a response (Fold values greater than 0.25, see Fig. 1) are excluded from the figure. Increasing positive correlations are shown by increasing green intensity and increasing negative correlations by increasing purple intensity.

Table S1. Thirty-eight signaling nodes measured in the study. All signaling nodes were measured in each immune cell subpopulation.


Scatter
Viable Cells
Lymphocytes
Monocytes
SSC
FSC
Live/Dead Aqua Stain
SSC
FSC
SSC
CD14
SSC-A
B cells
T cells
CD3- CD20-
Lymphocytes
CD20
CD3
Helper
T cells
Cytotoxic
T cells
CD4
CD3
CD45RA+
cytotoxic
T cells
CD45RA-
cytotoxic
T cells
CD45RA
SSC
CD45RA+
helper T cells
CD45RA-
helper T cells
CD45RA
SSC
Viable Cells
Monocytes
Lymphocytes
B cells
T cells
CD3-CD20-
Lymphocytes
Helper T
cells
Cytotoxic
T cells
CD45RA+
Helper T
cells
CD45RA-
Helper T
cells
CD45RA+
Cytotoxic T
cells
CD45RA-
Cytotoxic T
cells


A
IFNγ→pStat1
Fold
4
3
2
1
0
CD45RA- cytotoxic T cells
CD45RA- helper T cells
CD45RA+ cytotoxic T cells
CD45RA+ helper T cells
CD3-CD20- lymphocytes
B cells
Monocytes
B
IL2→pStat5
Fold
4
3
2
1
0
CD45RA- cytotoxic T cells
CD45RA- helper T cells
CD45RA+ cytotoxic T cells
CD45RA+ helper T cells
CD3-CD20- lymphocytes
B cells
Monocytes

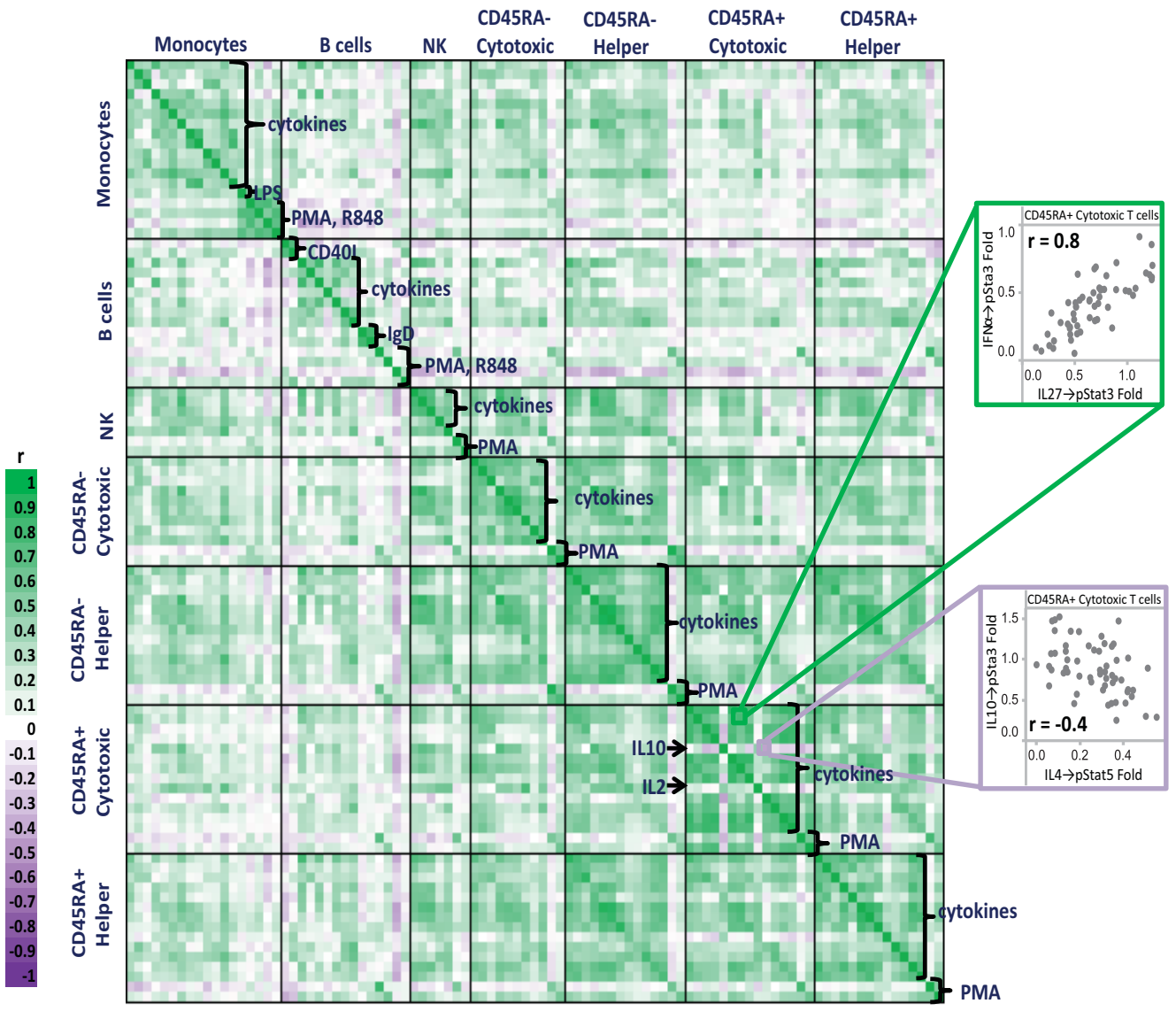

Monocytes
B cells
NK
CD45RA- Cytotoxic
CD45RA- Helper
CD45RA+ Cytotoxic
CD45RA+ Helper
cytokines
LPS
PMA, R848
CD40L
cytokines
IgD
PMA, R848
cytokines
PMA
cytokines
PMA
cytokines
PMA
IL10
IL2
cytokines
PMA
cytokines
PMA
r
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1
CD45RA+ Cytotoxic T cells
r = 0.8
IFNα→pStat3 Fold
IL27→pStat3 Fold
CD45RA+ Cytotoxic T cells
r = -0.4
IL10→pStat3 Fold
IL4→pStat5 Fold

Table S1. Thirty-eight signaling nodes measured in the study. All signaling nodes were measured in each immune cell subpopulation.

	Signaling Node
1	IFNα → pStat1
2	IFNα → pStat3
3	IFNα → pStat5
4	IFNα → pStat6
5	IFNγ → pStat1
6	IFNγ → pStat3
7	IFNγ → pStat5
8	IFNγ → pStat6
9	IL2 → pStat5
10	IL2 → pStat6
11	IL4 → pStat5
12	IL4 → pStat6
13	IL6 → pStat1
14	IL6 → pStat3
15	IL10 → pStat1
16	IL10 → pStat3
17	IL27 → pStat1
18	IL27 → pStat3
19	IL27 → pStat5
20	IL27 → pStat6
21	α-IgD/LPS → pS6
22	α-IgD/LPS → pAkt
23	R848 → pErk
24	R848 → pNFκB
25	CD40L → pErk
26	CD40L → pNFκB
27	PMA → pS6
28	PMA → pErk
29	Unmodulated → pStat1
30	Unmodulated → pStat3
31	Unmodulated → pStat5
32	Unmodulated → pStat6
33	Unmodulated → pS6
34	Unmodulated → pAkt
35	Unmodulated → pErk
36	Unmodulated → pNFκB
37	Unmodulated (DMSO) → pS6
38	Unmodulated (DMSO) → pErk