Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum

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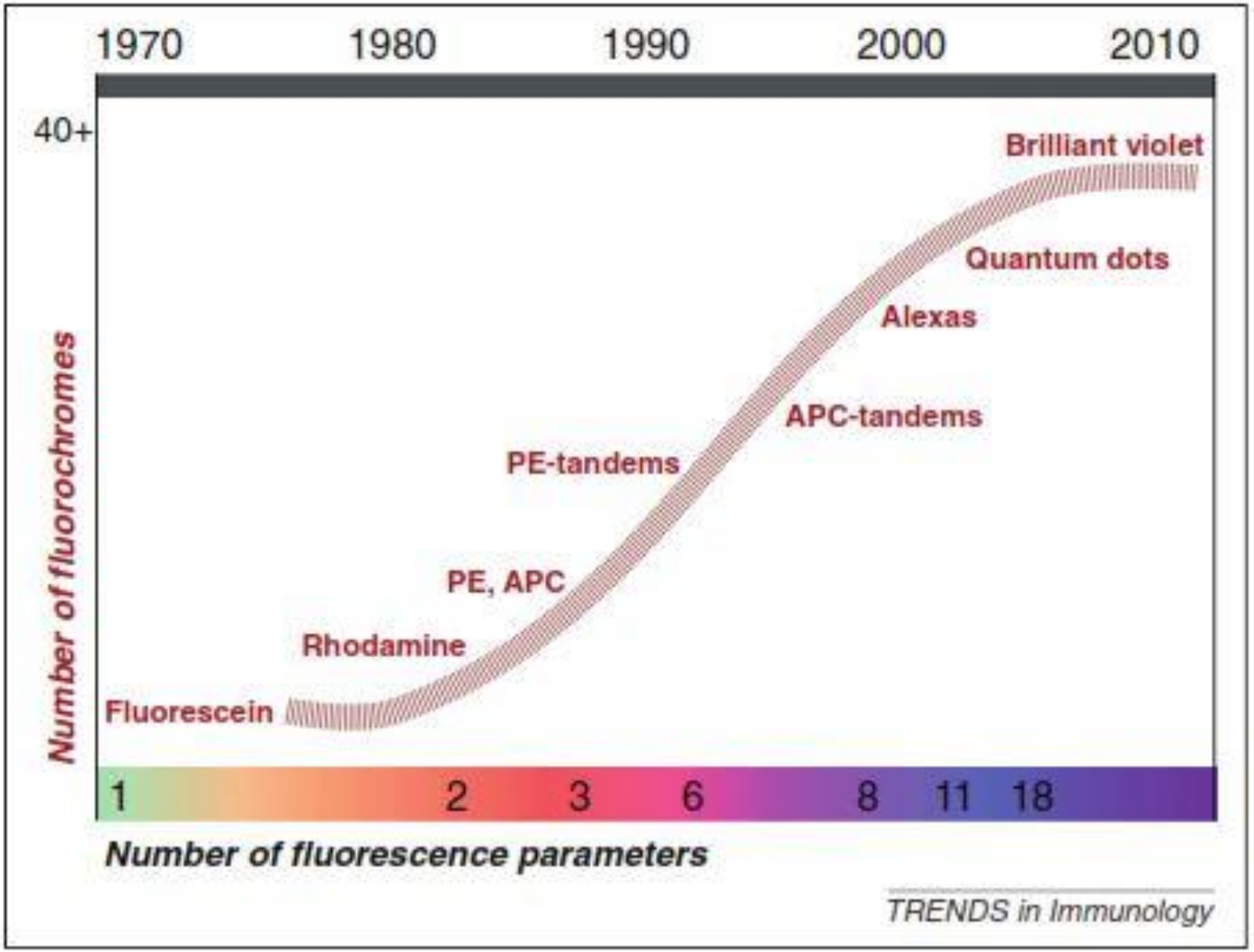
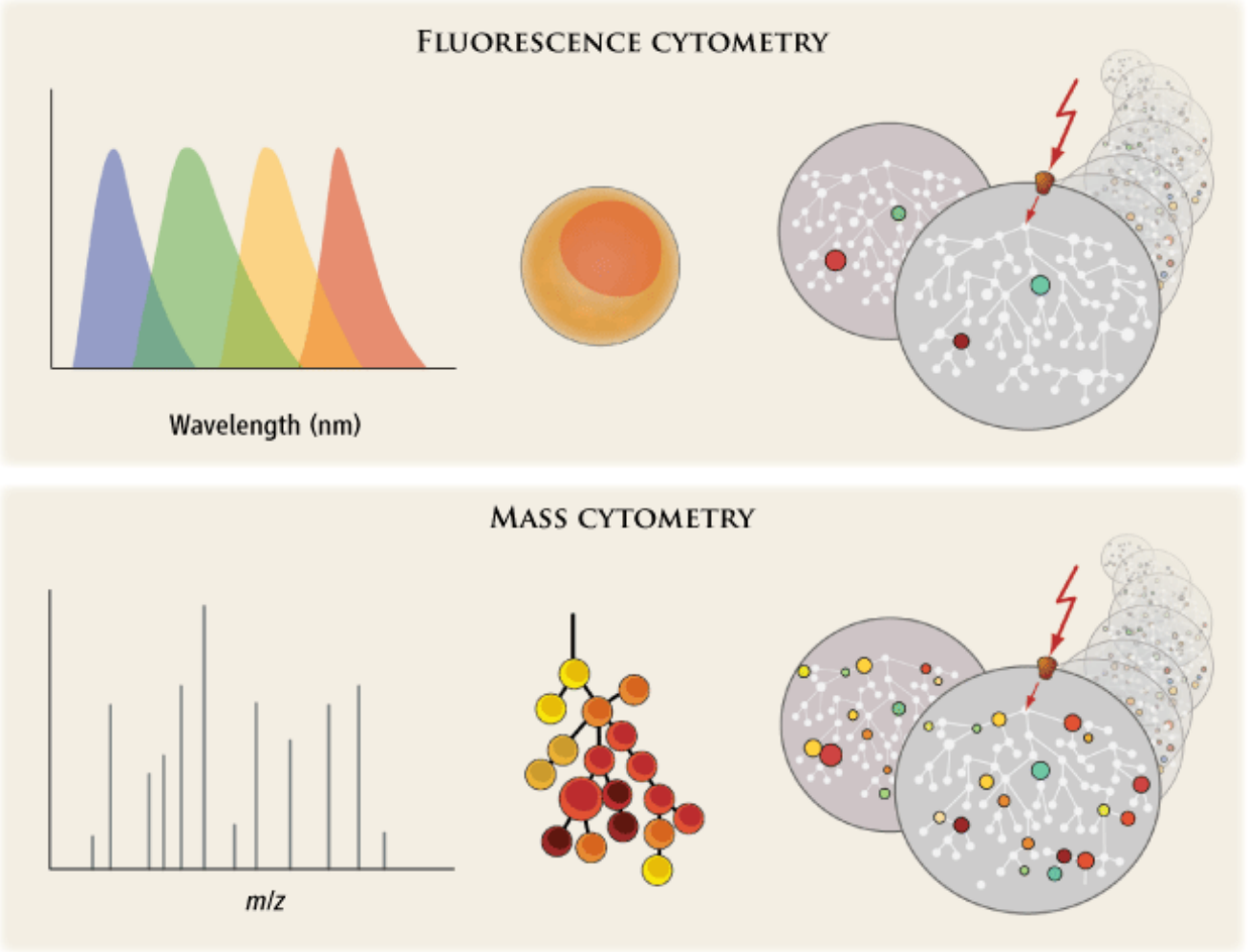


Bendall *et al. Trends in Immunology* **33**: 323 (2012)



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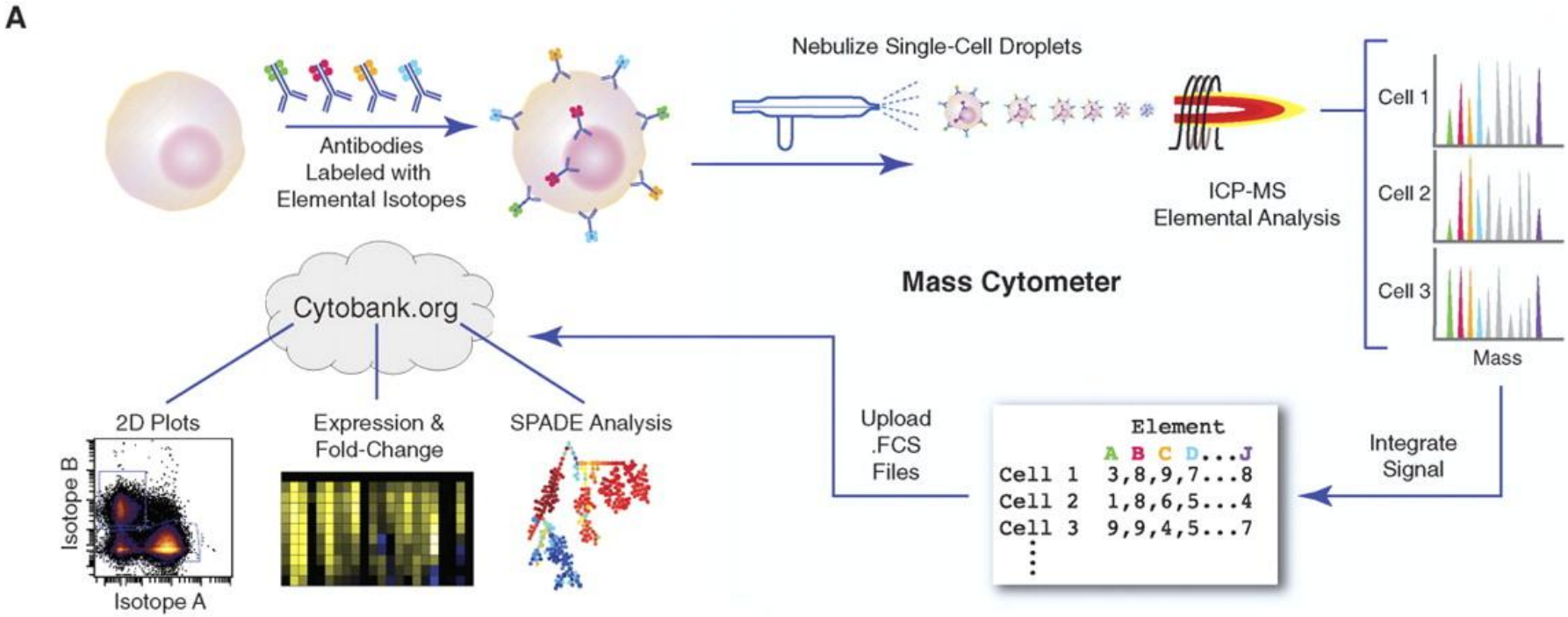
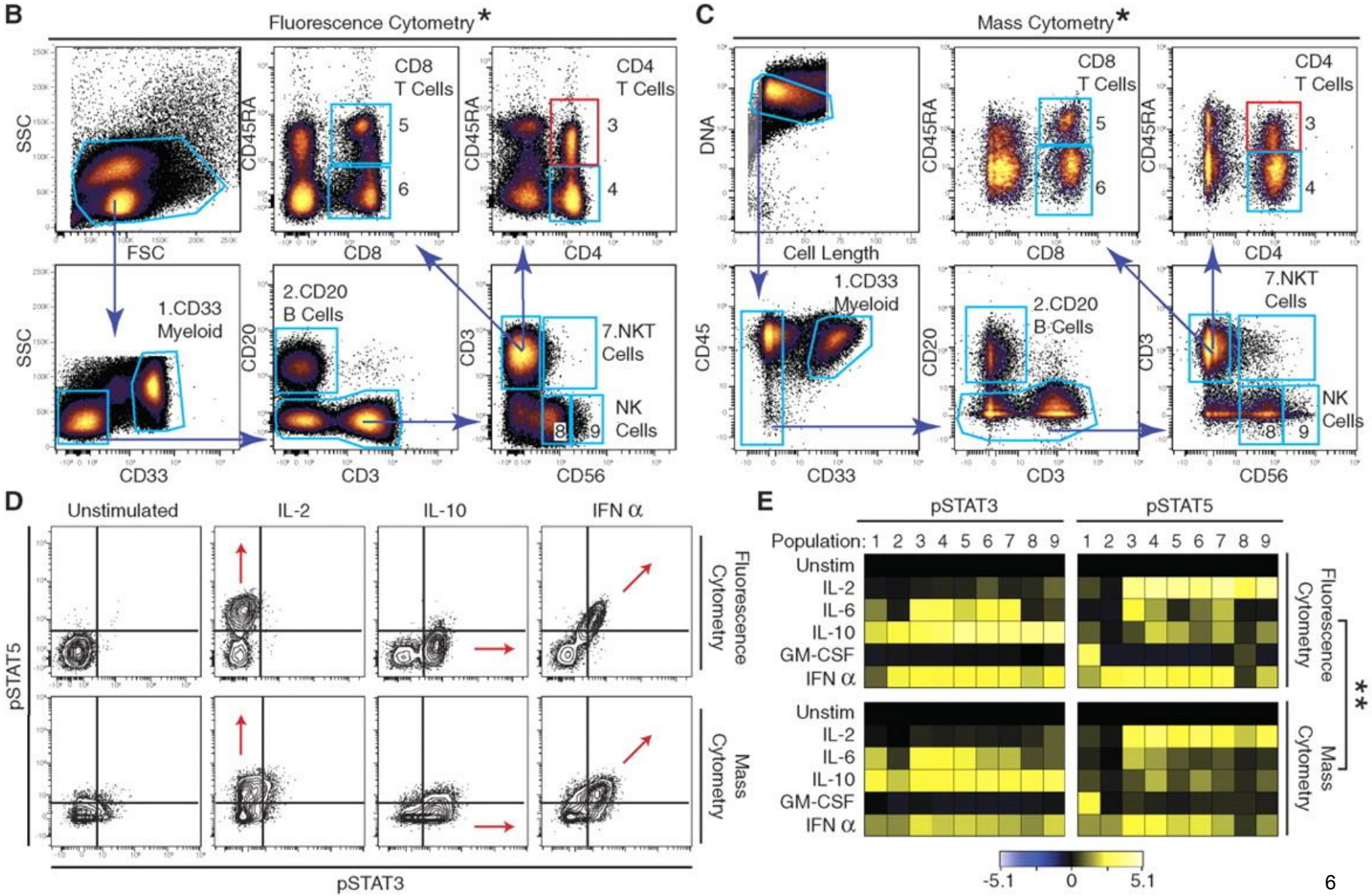
Random samples

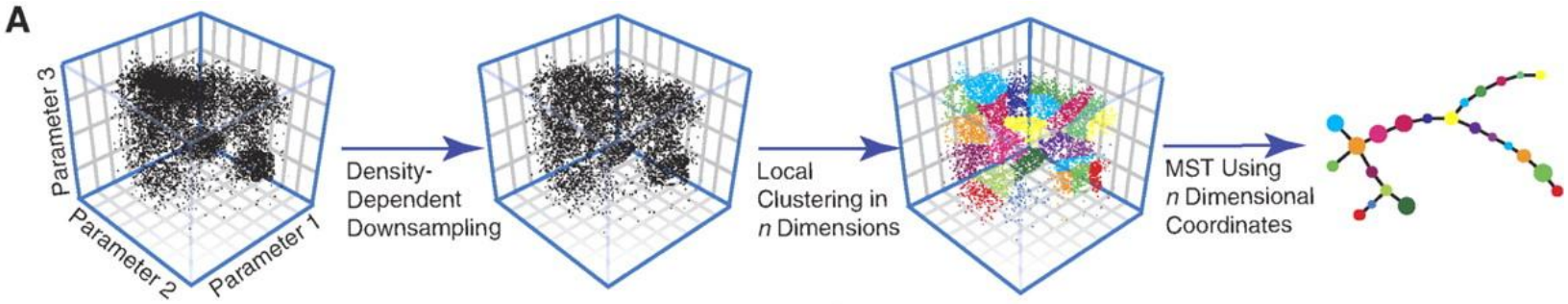
- transition element isotopes not normally found in biological systems as chelated antibody tags
- this technology can reasonably allow for a as many as 100 “tags” per cell
- currently TOF sampling resolution enables up to 1000 cells per second (~400,000 events per run)
- workflow for mass cytometry is comparable with that of fluorescence flow cytometry


A
Antibodies
Labeled with
Elemental Isotopes
Nebulize Single-Cell Droplets
ICP-MS
Elemental Analysis
Cell 1
Cell 2
Cell 3
Mass
Mass Cytometer
Integrate
Signal
Element
A B C D...J
Cell 1 3,8,9,7...8
Cell 2 1,8,6,5...4
Cell 3 9,9,4,5...7
Upload
.FCS
Files
Cytobank.org
2D Plots
Isotope B
Isotope A
Expression &
Fold-Change
SPADE Analysis

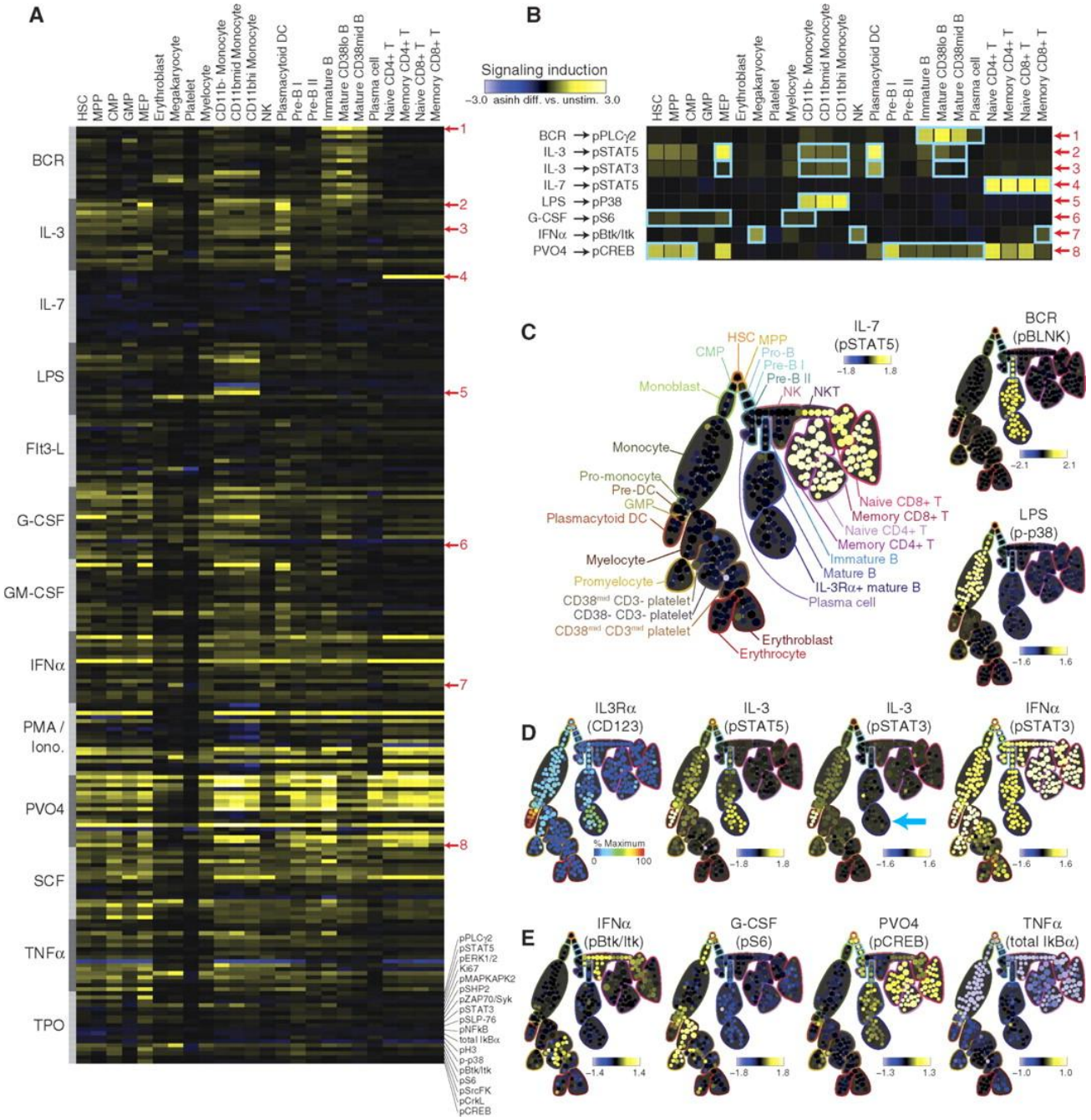

B
Fluorescence Cytometry*
SSC
FSC
CD45RA
CD8
CD8 T Cells
5
6
CD4
CD4 T Cells
3
4
1.CD33 Myeloid
CD33
2.CD20 B Cells
CD20
CD3
7.NKT Cells
NK Cells
8
9
CD56
C
Mass Cytometry*
DNA
Cell Length
CD45
D
Unstimulated
IL-2
IL-10
IFN α
pSTAT5
pSTAT3
Fluorescence Cytometry
Mass Cytometry
E
pSTAT3
pSTAT5
Population: 1 2 3 4 5 6 7 8 9
Unstim
IL-2
IL-6
IL-10
GM-CSF
IFN α
**
-5.1
0
5.1

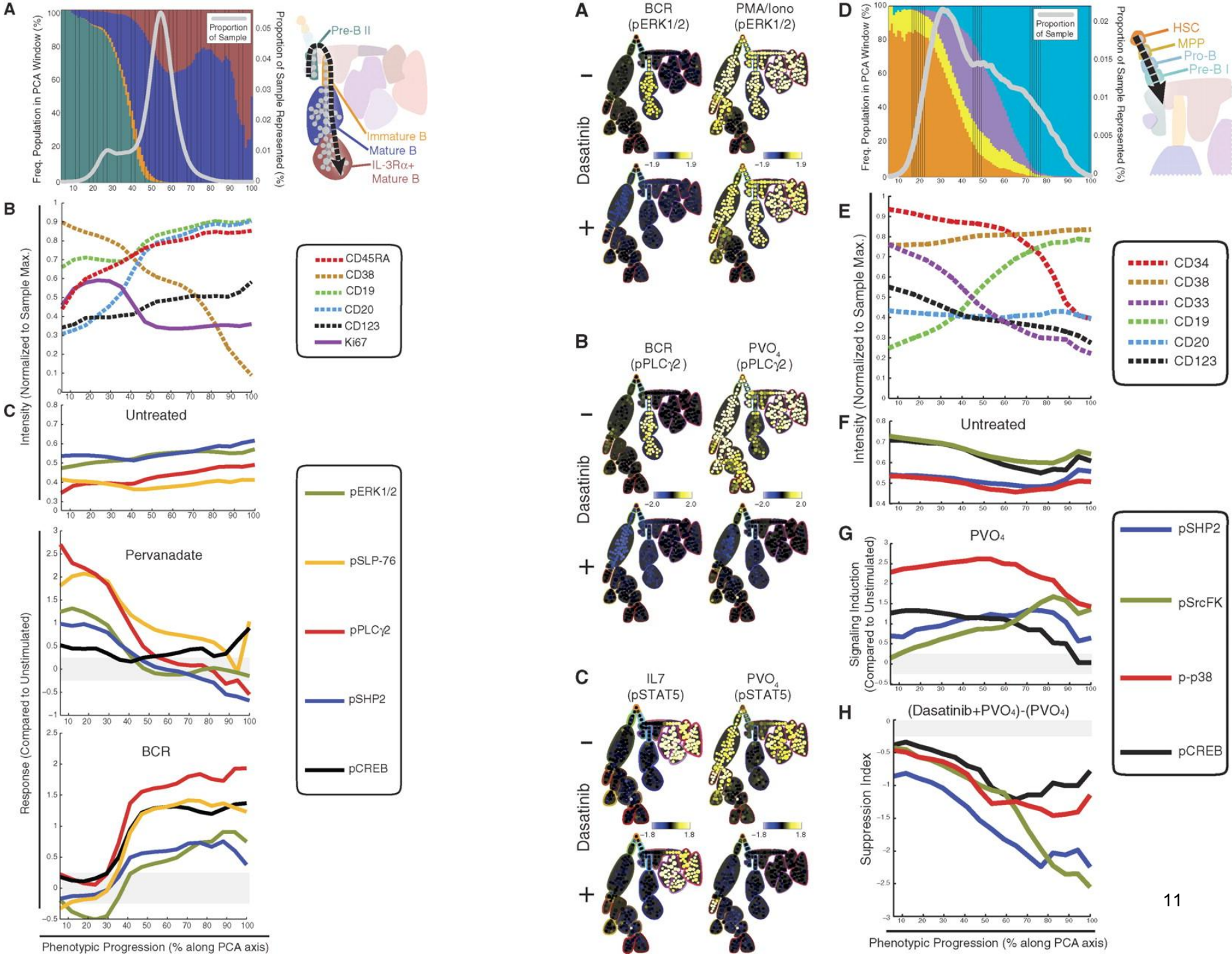
Two panels

- An “immunophenotyping” panel was designed that monitored 13 “core” surface markers and 18 subset-specific cell-surface markers to allow identification of human hematologic cell types.
- A “functional” panel contained the 13 core surface markers and also 18 intracellular epitopes that reflect intracellular signaling states, such as phosphorylation status of kinase substrates.
- Analyses by clustering, heatmaps and “spanning-tree progression analysis of density-normalized events”


A
Parameter 3
Parameter 2
Parameter 1
Density-
Dependent
Downsampling
Local
Clustering in
n Dimensions
MST Using
n Dimensional
Coordinates

- The unsupervised organization of phenotypically related cell types into adjacent branches, such as CD4 and CD8 T cells (**Fig. 2C**), mature and immature B cells (**Fig. 2D**), and different clusters of myeloid cells (**Fig. 2E**) collectively illustrates that the algorithmic ordering of surface marker similarity can objectively organize cell types into physiologically relevant compartments.


A
HSC
MPP
CMP
GMP
MEP
Erythroblast
Megakaryocyte
Platelet
Myelocyte
CD11b- Monocyte
CD11bmid Monocyte
CD11bhi Monocyte
NK
Plasmacytoid DC
Pre-B I
Pre-B II
Immature B
Mature CD38lo B
Mature CD38mid B
Plasma cell
Naive CD4+ T
Memory CD4+ T
Naive CD8+ T
Memory CD8+ T
BCR
IL-3
IL-7
LPS
Flt3-L
G-CSF
GM-CSF
IFNα
PMA / Iono.
PVO4
SCF
TNFα
TPO
pPLCγ2
pSTAT5
pERK1/2
Ki67
pMAPKAPK2
pSHP2
pZAP70/Syk
pSTAT3
pSLP-76
pNFkB
total IkBα
pH3
p-p38
pBtk/Itk
pS6
pSrcFK
pCrkL
pCREB
B
Signaling induction
−3.0 asinh diff. vs. unstim. 3.0
BCR → pPLCγ2
IL-3 → pSTAT5
IL-3 → pSTAT3
IL-7 → pSTAT5
LPS → pP38
G-CSF → pS6
IFNα → pBtk/Itk
PVO4 → pCREB
C
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
NK
NKT
Monoblast
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
IL-7 (pSTAT5)
−1.8
1.8
BCR (pBLNK)
−2.1
2.1
LPS (p-p38)
−1.6
1.6
D
IL3Rα (CD123)
% Maximum
0
100
IL-3 (pSTAT5)
−1.8
1.8
IL-3 (pSTAT3)
−1.6
1.6
IFNα (pSTAT3)
−1.6
1.6
E
IFNα (pBtk/Itk)
−1.4
1.4
G-CSF (pS6)
−1.8
1.8
PVO4 (pCREB)
−1.3
1.3
TNFα (total IkBα)
−1.0
1.0


A
Freq. Population in PCA Window (%)
Proportion of Sample
Proportion of Sample Represented (%)
Pre-B II
Immature B
Mature B
IL-3Rα+ Mature B
B
Intensity (Normalized to Sample Max.)
CD45RA
CD38
CD19
CD20
CD123
Ki67
C
Untreated
Pervanadate
BCR
Response (Compared to Unstimulated)
pERK1/2
pSLP-76
pPLCγ2
pSHP2
pCREB
Phenotypic Progression (% along PCA axis)
A
BCR (pERK1/2)
PMA/Iono (pERK1/2)
Dasatinib
B
BCR (pPLCγ2)
PVO4 (pPLCγ2)
C
IL7 (pSTAT5)
PVO4 (pSTAT5)
D
Freq. Population in PCA Window (%)
Proportion of Sample
Proportion of Sample Represented (%)
HSC
MPP
Pro-B
Pre-B I
E
Intensity (Normalized to Sample Max.)
CD34
CD38
CD33
CD19
CD20
CD123
F
Untreated
G
PVO4
Signaling Induction (Compared to Unstimulated)
pSHP2
pSrcFK
p-p38
pCREB
H
(Dasatinib+PVO4)-(PVO4)
Suppression Index
Phenotypic Progression (% along PCA axis)

- “A central dogma of immunology is that cells at different stages of maturation can be characterized by the expression of unique sets of proteins on the cell surface.”
- “The number of nodes and ultimately their boundaries is driven by a user-definable value (21).”