

Cytometry by time of flight sample preparation and analysis

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Cryopreserved cell suspensions were thawed and labeled with Rh103 intercalator as a viability marker. Cells from each tissue were barcoded using CD45 Abs conjugated with monoisotopic cisplatin, pooled, and stained with a panel of Abs (Supplemental Table II). Samples were then incubated in 0.125-nM Cell-ID Intercalator-Ir and acquired on a CyTOF2 (Fluidigm). The data were deconvolved for each tissue by Boolean gating on CD45 barcodes, leaving DNA+CD45+Rh103⁺ singlets for analysis. Data were visualized using principal component analysis (PCA) and viSNE (Amir, 2013) and implemented using FCS Express v6 (De Novo Software, CA). For heatmaps, samples were clustered by unsupervised hierarchical clustering with the R function hclust.

R code and the data used in the analysis are available on <https://michellemiron.github.io/Human-T-cell-CyTOF/>.

References

- Amir el-AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, Krishnaswamy S, Nolan GP, Pe'er D. (2013) viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol.* Jun;31(6):545-52. doi: 10.1038/nbt.2594. PMID: 23685480; PMCID: PMC4076922.
- Miron M, Kumar BV, Meng W, Granot T, Carpenter DJ, Senda T, Chen D, Rosenfeld AM, Zhang B, Lerner H, Friedman AL, Hershberg U, Shen Y, Rahman A, Luning Prak ET, Farber DL. (2018) Human Lymph Nodes Maintain TCF-1(hi) Memory T Cells with High Functional Potential and Clonal Diversity throughout Life. *J Immunol.* **201**(7):2132-2140. doi: 10.4049/jimmunol.1800716. PMID: 30111633; PMCID: PMC6143415.