

### **Data analysis of RNA-Seq data.**

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Sequencing data are available online through Gene Expression Omnibus at accession no. GSE106420. Analysis was done using R programming language package DESeq2 (Love, 2014). For multidimensional scaling analysis, we used the cmdscale and dist functions in R. For heatmaps, Z-score of rlog-normalized values were plotted using pheatmap. For analysis in Fig. 2, CD69+ and CD692 RNA sequencing (RNA-seq) samples were analyzed together by calculating the average of the counts for each gene, normalized using DESeq2, to examine all CD45RO+ cells and analyzed separately in Supplemental Fig. 1.

### **References**

Love MI, Huber W, Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**(12):550. PMID: 25516281; PMCID: PMC4302049.

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.