Supplemental Tables and Figures: Scalable Analysis of Flow Cytometry Data using R/Bioconductor

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Supplemental Tables and Figures

	Files	Total.Cells	Live.Cells	Percent
1	Pre-sort Unstained	10000	6784	67.84
2	FITC-CD4 Single	10000	6355	63.55
3	PE-CD44 Single	10000	6508	65.08
4	APC-CD62L Single	10000	6301	63.01
5	Pre-sort Population	10000	6090	60.90
6	CD4+ Subset	10000	7500	75.00
7	CD4- Subset	10000	6150	61.50
8	CD4+CD62L+ Subset	10000	7143	71.43
9	CD4+CD62L- Subset	10000	7165	71.65

Table S-1: Number of cells retained for each sample following gating

Table S-2: Median fluorescence in each channel shown for each sample.

	Files	FITC	PE	APC
		Area	Area	Area
1	Pre-sort Unstained	62.40	51.48	68.88
2	FITC-CD4 Single	68.64	95.16	29.52
3	PE-CD44 Single	71.76	762.84	19.68
4	APC-CD62L Single	46.80	12.48	266.91
5	Pre-sort Population	100.62	837.72	227.55
6	CD4+ Subset	1837.68	1184.04	838.86
7	CD4- Subset	84.24	533.52	172.20
8	CD4+CD62L+ Subset	1723.80	946.92	632.22
9	CD4+CD62L-Subset	1698.84	1463.28	573.18



Figure S-1: Gating on Forward and Side Scatter to identify live splenocytes that were included in subsequent analysis: Pre-sort fraction (A), $CD4^+$ fraction (B), $CD4^-$ fraction (C), and $CD4^+CD62L^+$ fraction (D). The live cells are shown in blue. Rejected cells appear as red dots.



Figure S-2: Pairwise density plots for all three channels - CD4, CD44, and CD62L - shown separately for an unstained aliquot of the pre-sort population and singly stained controls: FITC-CD4, PE-CD44, and APC-CD62L. Each panel corresponds to a particular pair: CD44 versus CD4 (left panel), CD62L versus CD4 (center panel), and CD44 versus CD62L (right panel).



Figure S-3: Pairwise density plots for CD4, CD62L, and CD44 expression shown separately for aliquots obtained from pre-sort, CD4⁺, and CD⁻ fractions. Each panel corresponds to a particular pair: CD44 versus CD4 (left panel) and CD62L versus CD4 (right panel). The solid lines indicate the expression threshold for a cell to be associated with positive expression. Ninety five percent of the unstained cell fraction was contained below the threshold. The dotted line indicates the upper limit of CD44 expression for 95% of the CD4⁺CD62L⁺ fraction.