Lieberman et al. Supplemental Figure legends

Figure e-1: *Gating strategy for %CD62L analysis.* Doublet cells were excluded from the analysis. A gate on total lymphocytes was set followed by a subgate on the live lymphocytes. Live lymphocytes were then gated for CD3+ T cells and subsequently gated to separate the CD4+ and CD8+ cells. The CD4+ (Q3) cells were then plotted as CD45RA vs CD62L to assess the maturity of the T cells. A histogram of total %CD62L+ can be seen on the final histogram.

Figure e-2 Low forward scatter lymphocytes are predominately Annexin V+ and CD62L-, high forward scatter lymphoctyes are predominately Annexin V- and CD62L+. 10 controls frozen PBMCs were for lymphocyte Annexin V, 7-AAD and CD62L staining in the CD4+ population.

Figure e-3 %live lymphocytes did not differ in natalizumab-treated non-PML and pre-PML samples (p=.6517).

Figure e-4 Technical replicates are excellent in the modified %CD62L assay. Frozen PBMCs from 2 healthy donors were assessed in each of 5 runs in which the study data was collected as a measure of staining quality, FACS machine set up and general reproducibility.

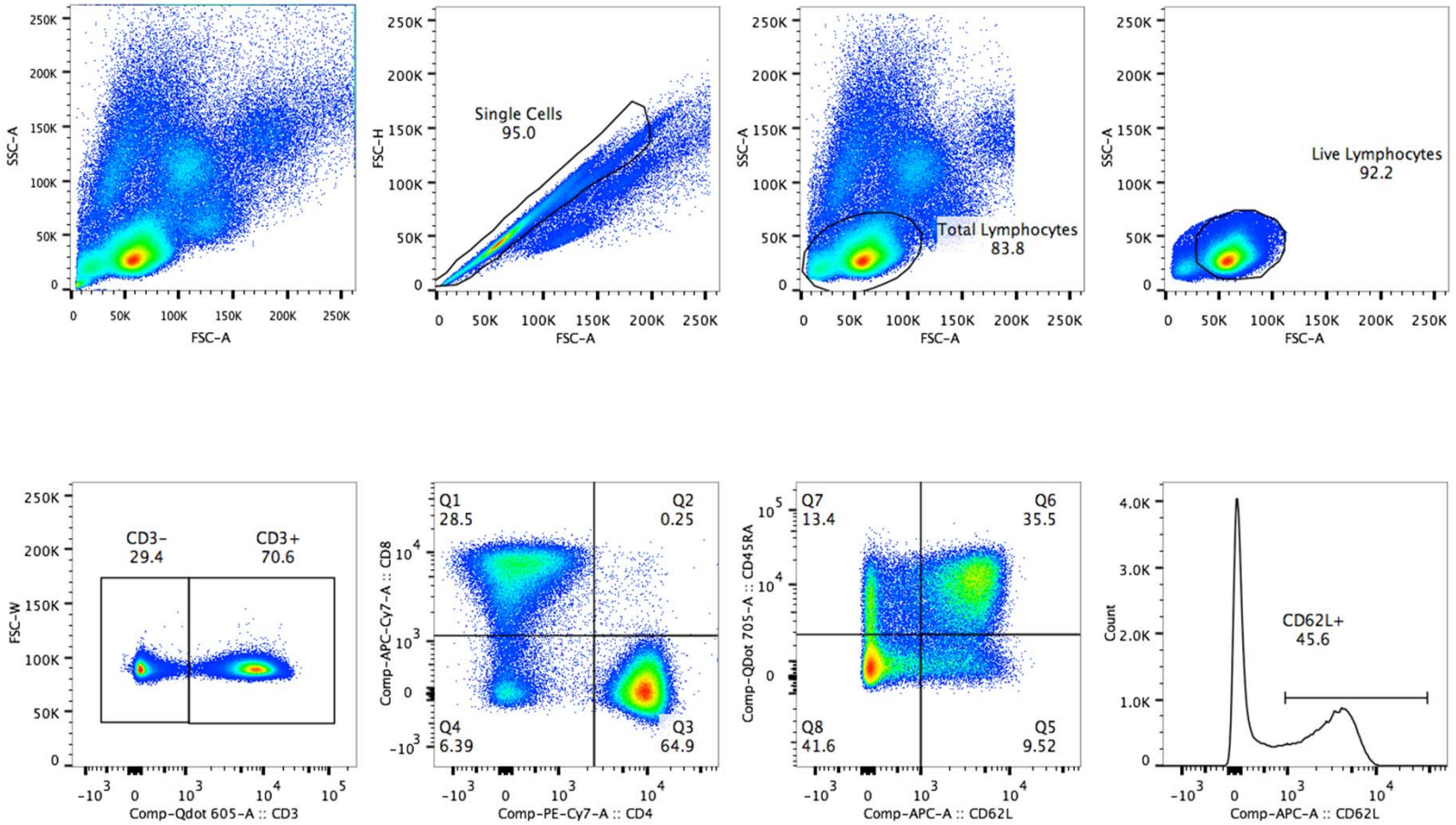

SSC-A
FSC-A
Single Cells
95.0
FSC-H
Total Lymphocytes
83.8
Live Lymphocytes
92.2
CD3-
29.4
CD3+
70.6
FSC-W
Comp-Qdot 605-A :: CD3
Q1
28.5
Q2
0.25
Q4
6.39
Q3
64.9
Comp-APC-Cy7-A :: CD8
Comp-PE-Cy7-A :: CD4
Q7
13.4
Q6
35.5
Q8
41.6
Q5
9.52
Comp-QDot 705-A :: CD45RA
Comp-APC-A :: CD62L
CD62L+
45.6
Count

Figure e-1

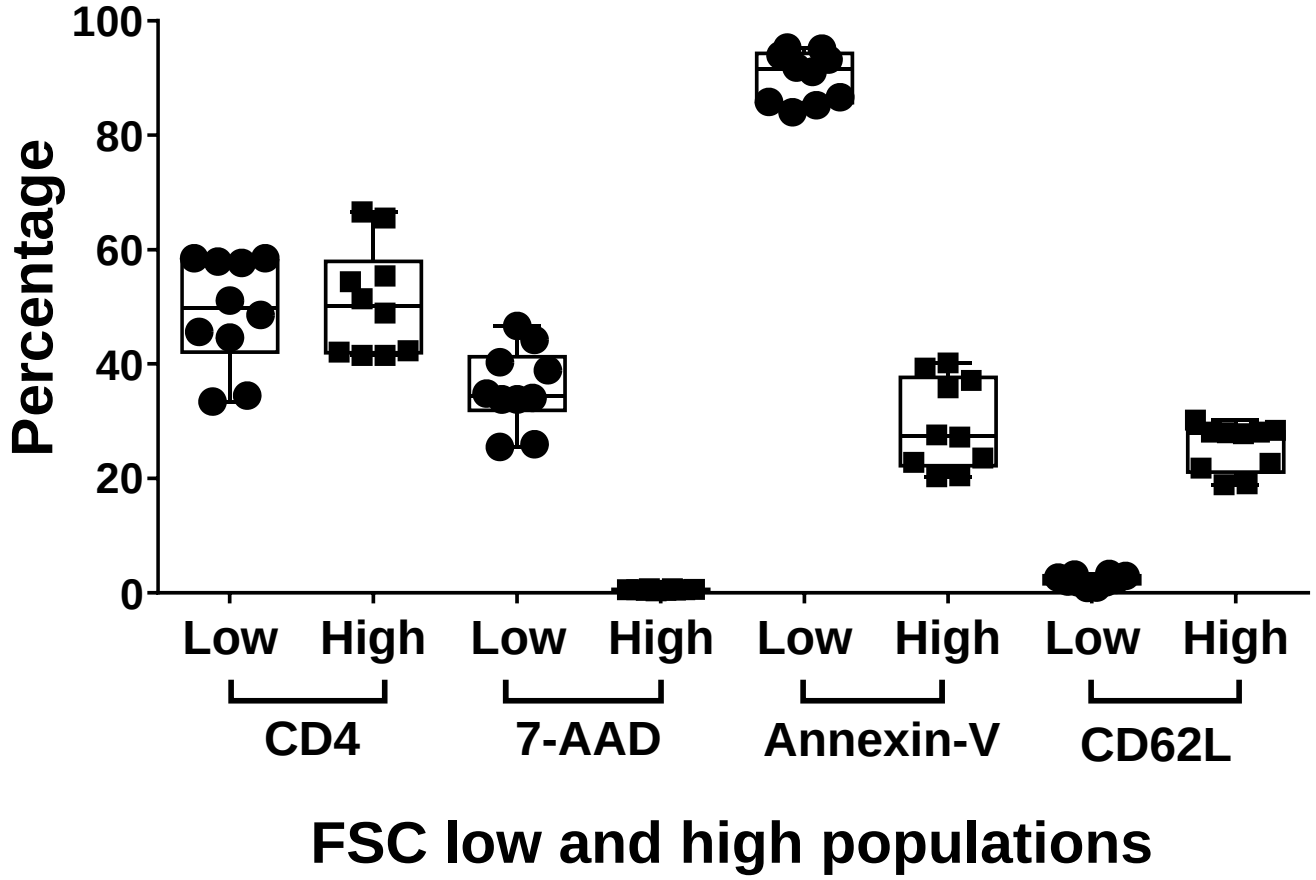
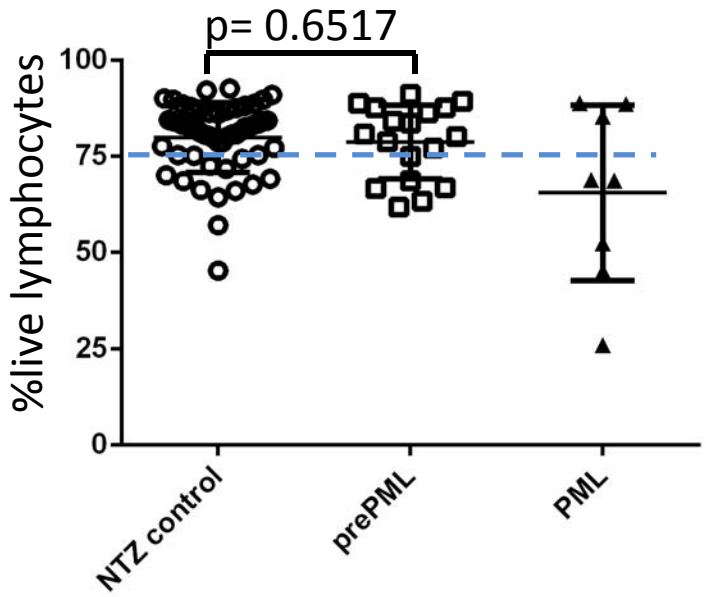

Percentage
100
80
60
40
20
0
Low
High
Low
High
Low
High
Low
High
CD4
7-AAD
Annexin-V
CD62L
FSC low and high populations

Figure e-2

No difference in lymphocyte viability in non-PML natalizumab (NTZ) control or pre-PML samples



STRATA samples

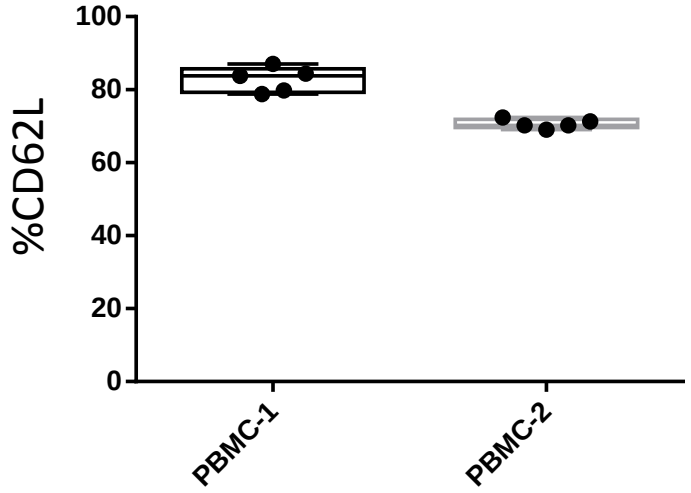
Figure e-3

Parameters	Muenster	Biogen	Notes
NTZ treated patients	9 sites	case-control clinical trials	Biogen matched controls for age, gender, NTZ doses, location
Samples	frozen PBMCs	frozen PBMCs	sample processing has impact, BIIB trial samples processed at CRO
Anti-coagulant	EDTA	Heparin	No Change to CD62L staining
Thaw conditions	PBS	PBS + Benzonase	No Change to CD62L staining
Staining Buffer	1XPBS,0.5%BSA, 2mM EDTA	1XPBS,2%FBS, 0.1% Azide	No Change to CD62L staining
Staining Conditions	10 min. at RT	30 min. on ice	No Change to CD62L staining
Antibody	DREG-56	DREG-56	identical clone
Wash	1x	3x	Less variable background with 3x wash
Fixative	none	2% paraformaldehyde	Fix provides better reproducibility and stability, Biogen assay is not time sensitive to measurement

Biogen successfully modified the %CD62L assay to standardize the assay, improve reproducibility and eliminate the necessity to analyze the samples within 10 minutes of staining. The impact of changes in the assay on %CD62L, in direct comparisons to that published (Schwab et al. 2013), was minimal and is outlined above.

Table e-1

Reproducibility of Biogen Assay



Frozen PBMCs from 2 healthy donors assayed on 5 different days

Figure e-4