

3.5.3 Gating Strategy

Figure 1: FSC-A v SSC-A

Debris is identified as SSC^{low}FSC^{low}, gated accordingly and removed from further analysis by inverting the Debris gate to obtain a NOT(Debris) population.

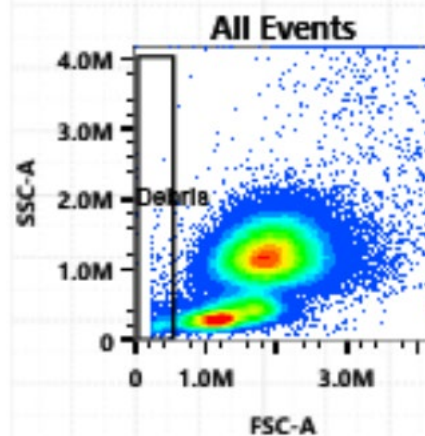


Figure 2: FSC-W v FSC-H

From the NOT(Debris) population, FSC Singlets are identified as shown.

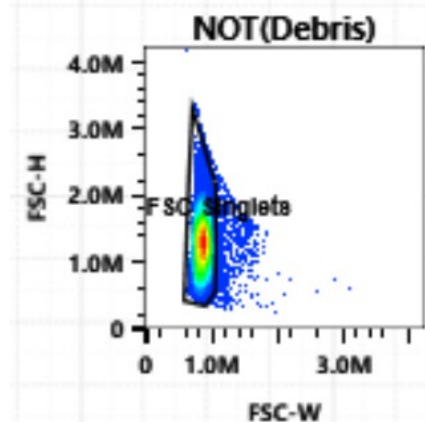


Figure 3: SSC-W v SSC-H

From the FSC Singlets population, the SSC Singlets are identified as shown.

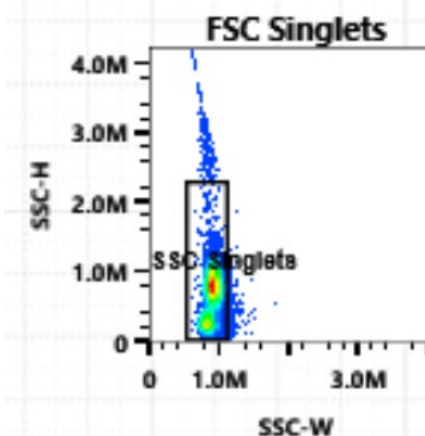


Figure 4: CD45 Alexa Fluor 532 v SSC-H
From the SSC Singlets population, CD45+ cells are identified as shown.

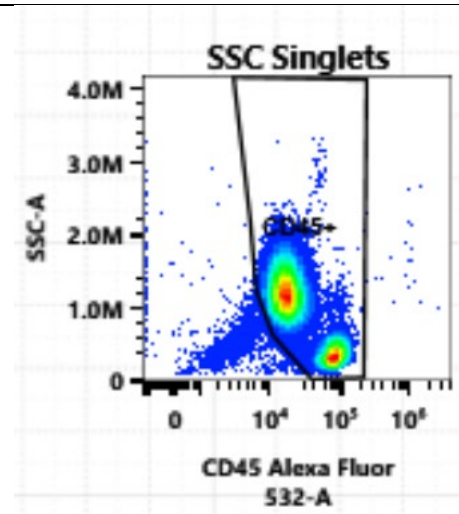


Figure 5: CD66b Alexa Fluor 700 v SSC-A
From the CD45+ population, the Grans CD66b+ population and Lymph Mono population are identified as shown.

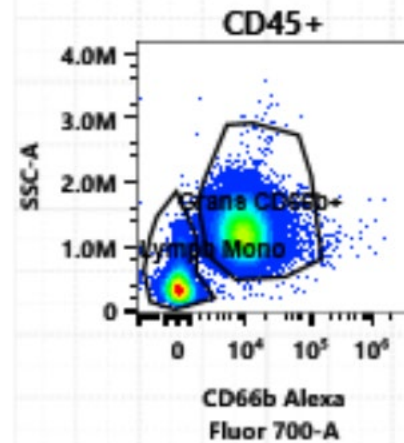


Figure 6: CD45 Alexa Fluor 532 v SSC-A
From the Lymph Mono population, the Lymphs population is identified as shown.

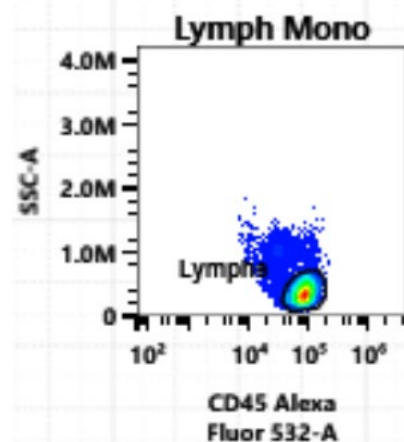


Figure 7: CD14 BUV615 v SSC-A

From the Lymph Mono population, the CD14+ population is identified as shown.

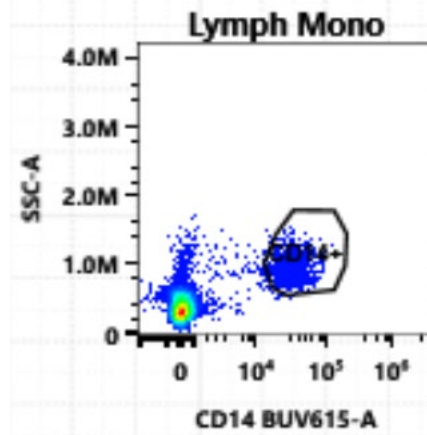


Figure 8: CD3 BV785 v CD56 BV605

From the Lymphs population, CD3-CD56+ and CD3+CD56- events are identified as shown.

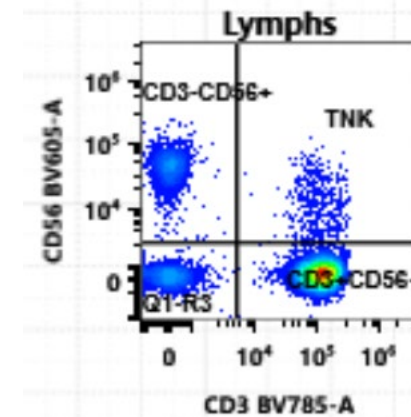


Figure 9: CD20 BUV496 v CD45 Alexa Fluor 532

From the Lymphs population, the CD20+ population is identified as shown.

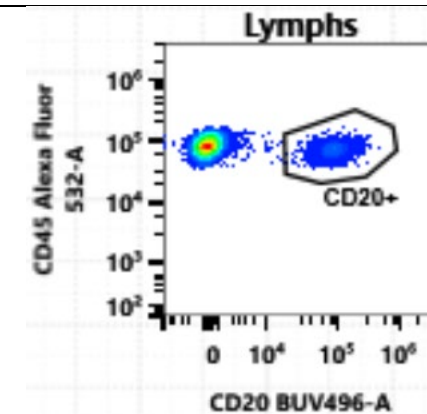


Figure 10: CD3 BV785 v CD45 Alexa Fluor 532

From the Lymphs population, the CD3+ population is identified as shown.

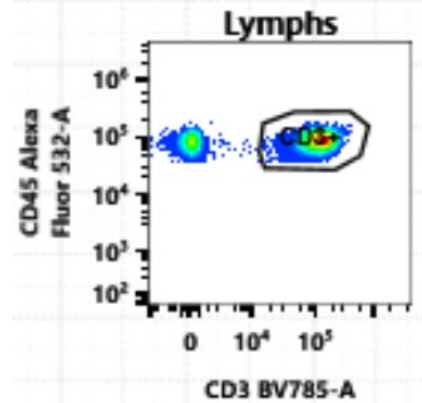


Figure 11: CD2 PE v CD3 BV785

From the CD3+ population the CD3+CD2+ gate is set to include 0.5% (± 0.3) of the negative population in Tube 1 (A) to be carried over to Tube 2 (B).

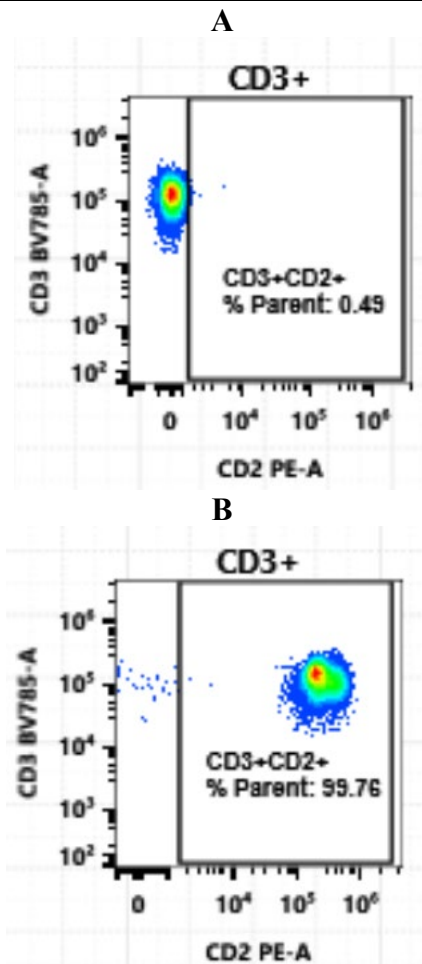


Figure 12: CD45RA Alexa Fluor 488 v CD28 BV711

From the CD3+ population, the CD3+CD28+ population is identified as shown.

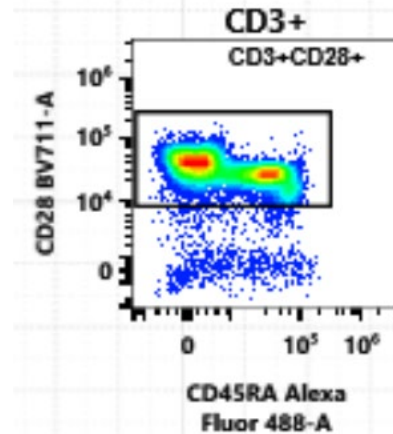


Figure 13: CD58 BV421 v CD45RA Alexa Fluor 488

From the CD3+ population the CD3+CD58+ gate is set to include 0.5% (± 0.3) of the negative population in Tube 1 (A) and is then adjusted to the edge of the observed negative population in Tube 2 (B).

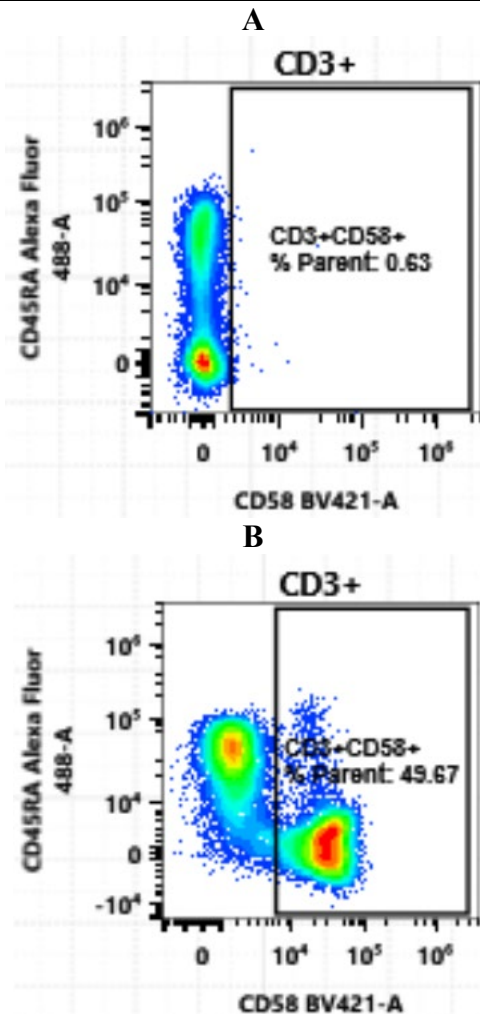


Figure 14: CD4 APC-H7 v CD8 BUV395
From the CD3+ population, the CD8+ and CD4+ populations are identified as shown.

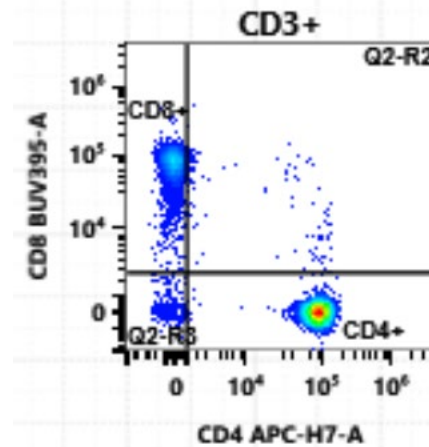


Figure 15: CD197 PE-Cy7 v CD45RA Alexa Fluor 488

From the CD4+ population, the quadrant gate is set to divide the population into the following subsets:

- CD4+ Central Memory (CM4)
- CD4+ Naïve Memory (Naïve4)
- CD4+ Effective Memory (EM4)
- CD4+ Temporary Memory (TEMRA4)

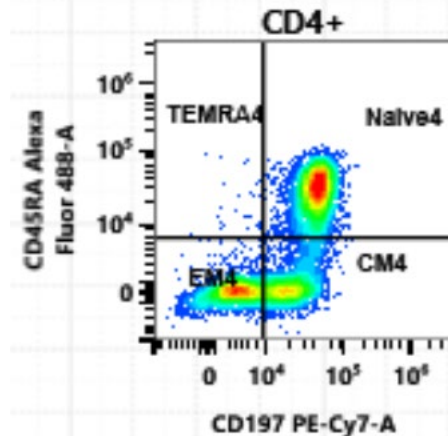
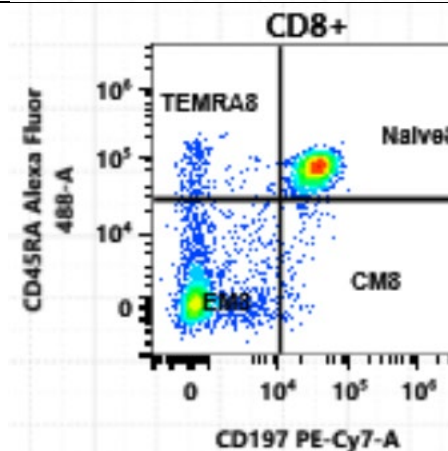


Figure 16: CD197 PE-Cy7 v CD45RA Alexa Fluor 488

From the CD8+ population, the quadrant gate is set to divide the population into the following subsets:

- CD8+ Central Memory (CM8)
- CD8+ Naïve Memory (Naïve8)
- CD8+ Effective Memory (EM8)
- CD8+ Temporary Memory (TEMRA8)



**Figure 17: CD58 BV421 v CD45RA Alexa
Fluor 488**

From the CD8+ population, the CD8+CD58+
+ gate is set to include 0.5% (± 0.3) of the
negative population in Tube 1 (A) then
adjusted to the edge of the observed
negative population in Tube 2 (B) as shown.

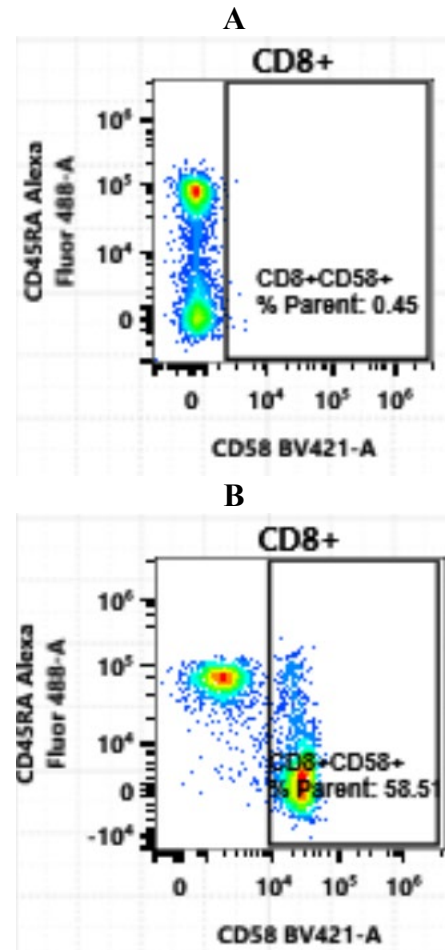


Figure 18: MsiG1 AF700 v CD45RA BV510

From the CD4+ population, the CD4+CD58+ + gate is set to include 0.5% (± 0.3) of the negative population in Tube 1 (A) then adjusted to the edge of the observed negative population in Tube 2 (B) as shown.

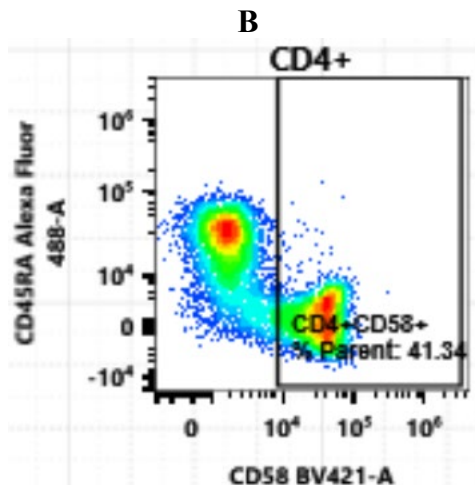
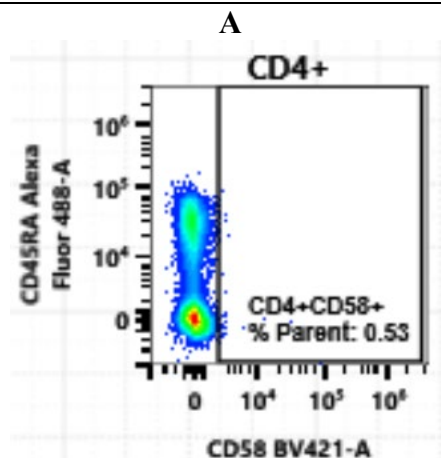


Figure 19: CD127 BUV737 v CD25 BV480

In Tube 2, from the CD4+ population, the CD25+CD127lo population is identified as shown.

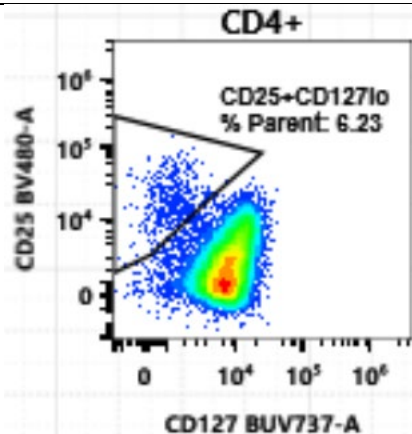


Figure 20: FoxP3 Alexa Fluor 647 v CD25 BV480

In Tube 2, from the CD4+ population, the FoxP3+ population is identified as shown.

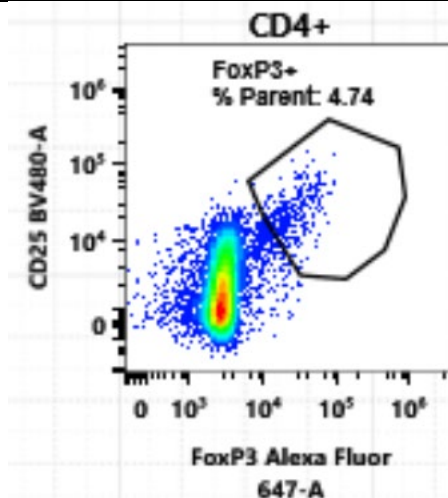


Figure 21: CD2 PE v CD45RA Alexa Fluor 488

From the CD3-CD56+ population, the CD56+CD2+ gate is set to include 0.5% (± 0.3) of the negative population in Tube 1 (A) then adjusted to the edge of the observed negative population in Tube 2 (B) as shown.

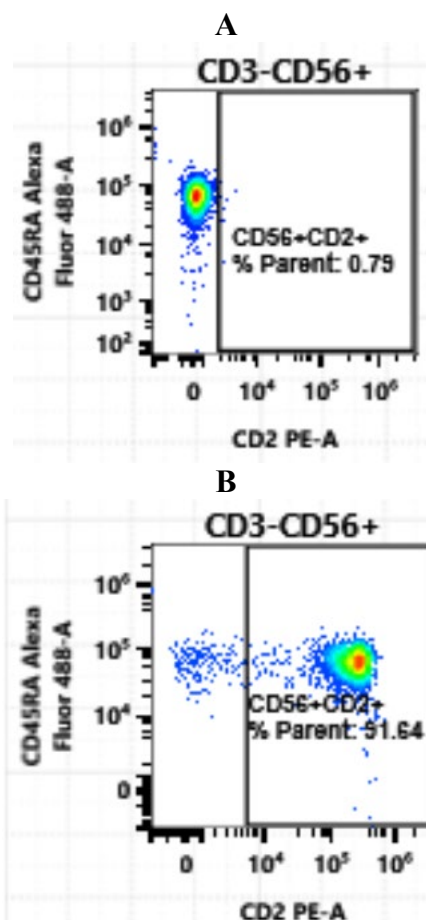


Figure 22: CD28 BV711 v CD45RA Alexa Fluor 488

From the CD3-CD56+ population, the CD56+CD28+ is set to the edge of the negative population in Tube 1 (A) to be carried over to Tube 2 (B).

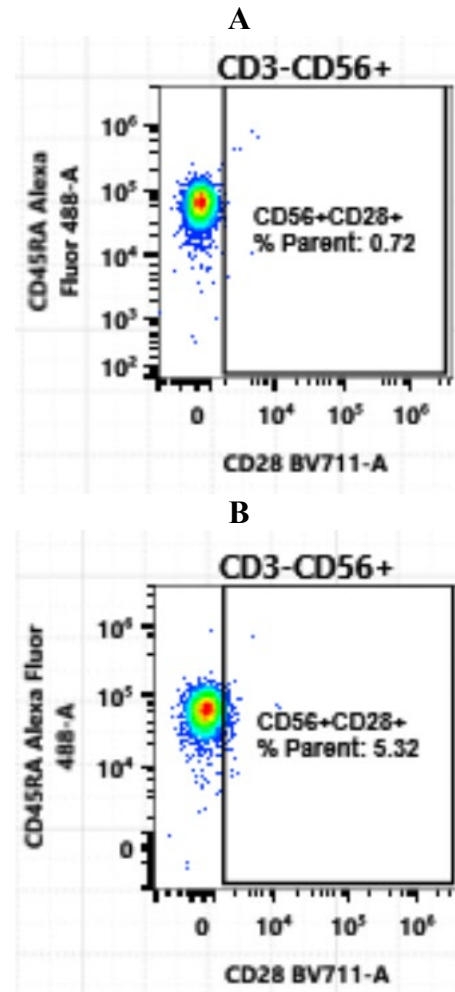


Figure 23: CD58 BV421 v CD45RA Alexa Fluor 488

From the CD3-CD56+ population the CD56+CD58+ gate is set to the edge of the negative population in Tube 1 (A) to be carried over to Tube 2 (B).

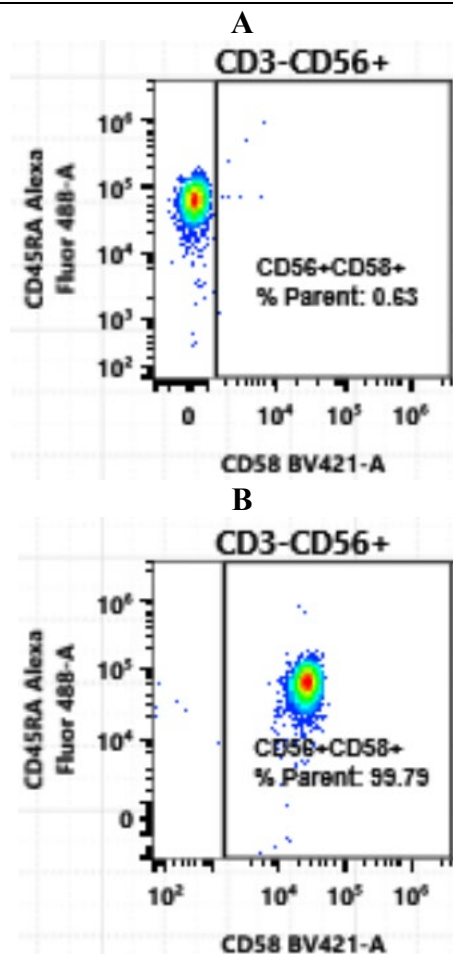


Figure 24: CD2 PE v CD45RA Alexa Fluor 488

From the CD20+ population, CD20+CD2+ gate is set to the edge of the negative population in Tube 1 (A) to be carried over to Tube 2 (B).

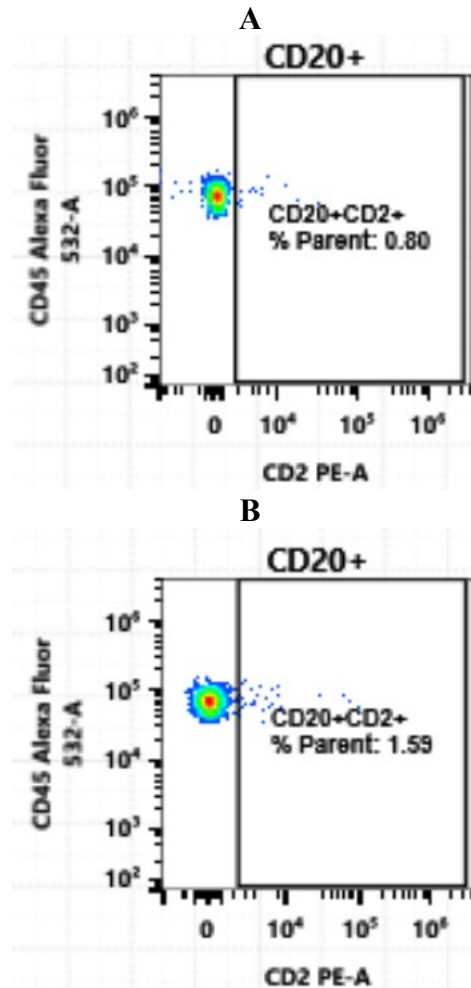


Figure 25: CD28 BV711 v CD45RA Alexa Fluor 532

From the CD20+ population, CD20+CD28+ gate is set to the edge of the negative population in Tube 1 (A) to be carried over to Tube 2 (B).

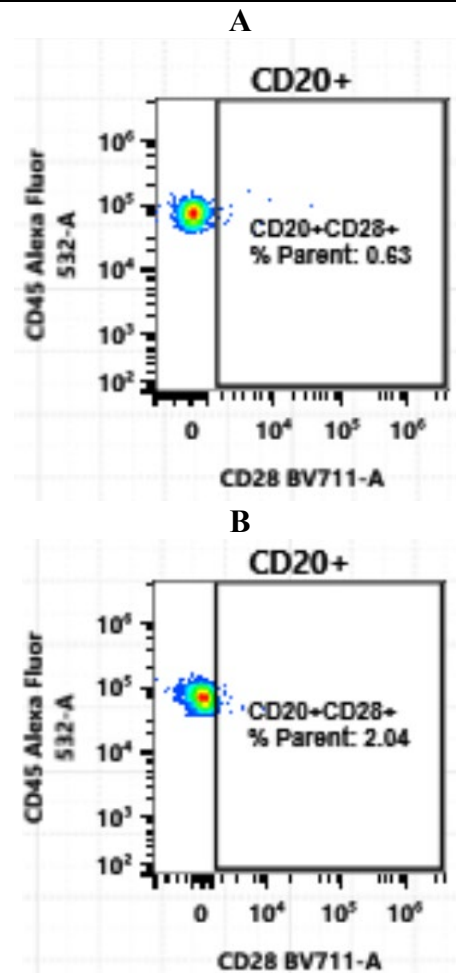
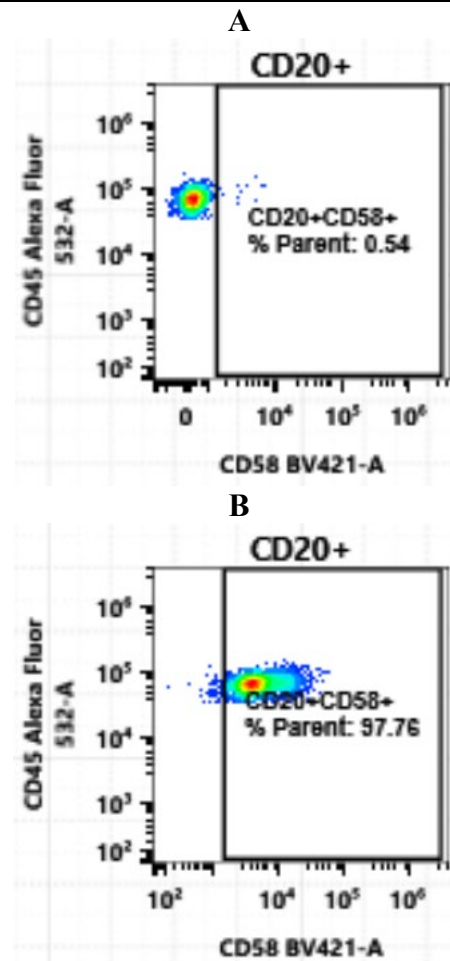


Figure 26: CD58 BV421 v CD45RA Alexa Fluor 488

From the CD20+ population, CD20+CD58+ gate is set to the edge of the negative population in Tube 1 (A) to be carried over to Tube 2 (B).



**Figure 27: HLA-DR PE-Cy5.5 v CD3
BUV785**

From the CD3+ population, CD3+HLA-DR+ gate is set to the edge of the negative population (0.5% ±0.3) in Tube 1 (A) then adjusted to the edge of the observed negative population in the event of a shift in Tube 2 (B) as shown.

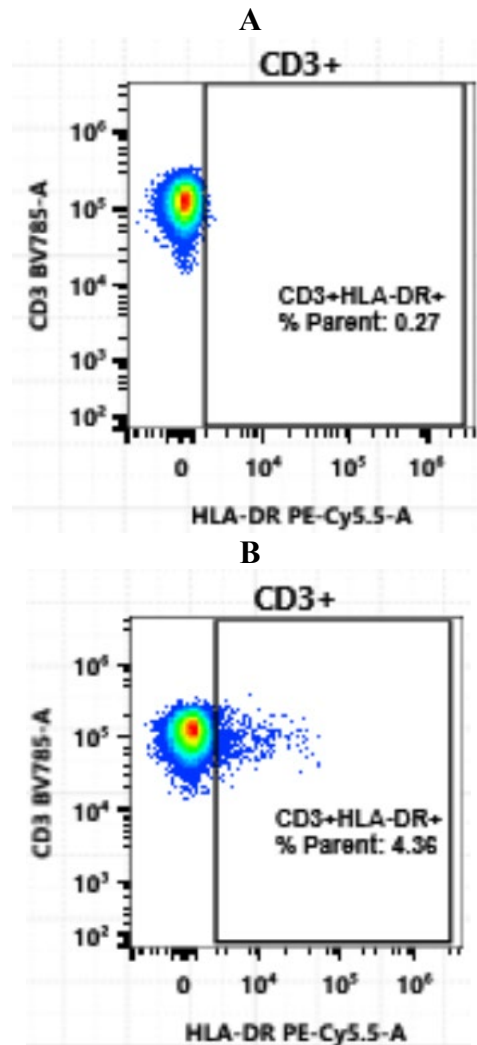


Figure 28: HLA-DR PE-Cy5.5 v CD4 APC-H7

From the CD4+ population, CD4+HLA-DR+ gate is set to the edge of the negative population (0.5% ±0.3) in Tube 1 (A) then adjusted to the edge of the observed negative population in the event of a shift in Tube 2 (B) as shown.

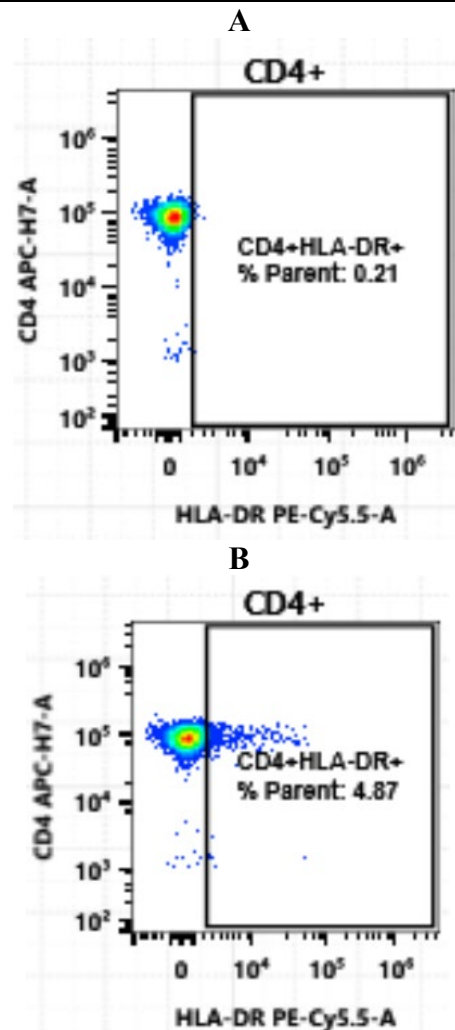


Figure 29: HLA-DR PE-Cy5.5 v CD8 BUV395

From the CD8+ population, CD8+HLA-DR+ gate is set to the edge of the negative population ($0.5\% \pm 0.3$) in Tube 1 (A) then adjusted to the edge of the observed negative population in the event of a shift in Tube 2 (B) as shown.

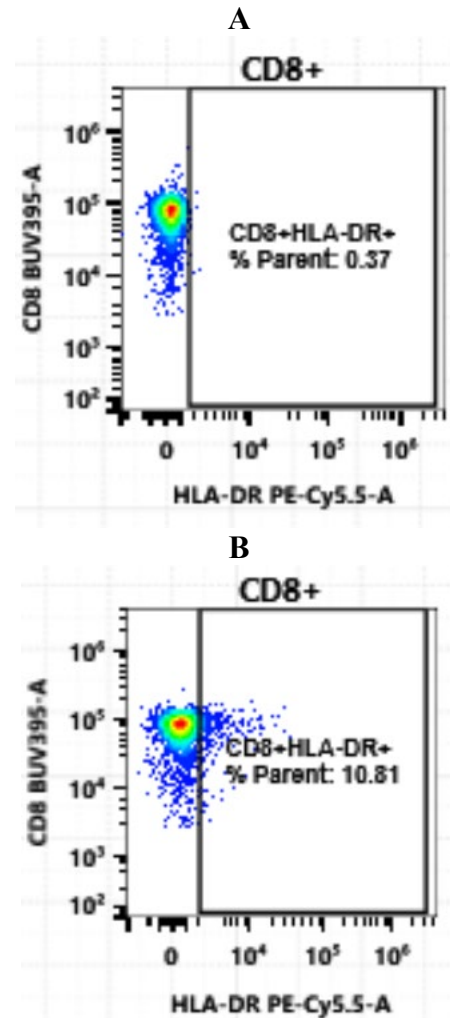


Figure 15: Hierarchy

Population Hierarchy A752 Validation w/ 08Feb2021-Day0-Group_001-B1 D3 1		
Population	% Parent	Count
▼ All Events	100.00	115,576
▼ Detrits	2.22	2,508
▼ NOT(Detrits)	97.78	113,067
▼ FSC Singlets	97.83	110,555
▼ SSC Singlets	95.99	109,433
▼ CD45+	95.89	109,033
▼ Gran CD66+	67.44	71,505
▼ Lymph Mono	31.89	33,514
▼ Lymphs	87.59	29,819
▼ CD3-CD28+	14.02	4,156
CD28+CD2+	0.79	33
CD28+CD28+	0.72	30
CD28+CD58+	0.63	20
TKC	3.49	1,036
Q1-R3	14.82	4,329
CD3+CD56-	67.87	20,102
▼ CD20+	14.35	4,250
CD20+CD2+	0.80	36
CD20+CD28+	0.63	27
CD20+CD58+	0.64	22
▼ CD3+	71.29	21,114
CD3+CD2+	0.49	104
CD3+CD28+	0.63	134
▼ CD8+	24.98	5,951
TEMRA6	7.59	364
Naive6	35.58	1,806
EM6	52.71	2,678
CM6	4.15	211
CD8+CD58+	0.45	22
CD8+HLA-DR+	0.37	19
TEMRA6 AND CD8+CD56+	0.02	1