

Supplementary material

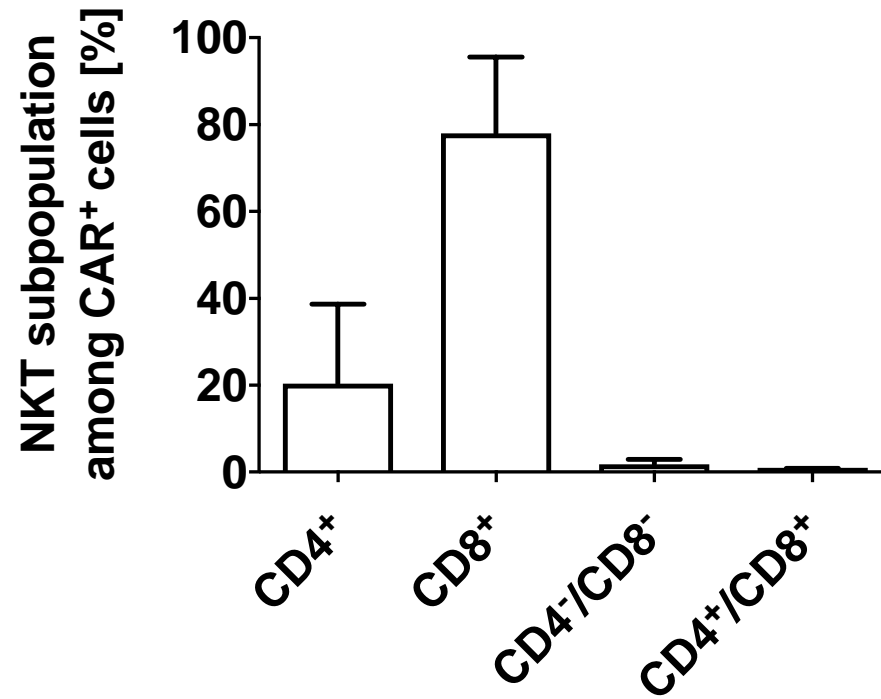
Supplementary Table 1: Activity matrix of TCT process

Day	Activity	Volume [ml]
0	CD4/8 separation	
	Activation (CD3/CD28)	
	01:00 p.m. Start of culture	70
1	10:00 a.m. Transduction with lentiviral vector (2×10^8 viral particles per 100 ml)	+30 → 100
2	05:00 p.m. Culture wash	Complete replacement → 200
	06:00 p.m. Activate shaker type I	
5	10:00 a.m. Feed	+50 → 250
6	02:00 p.m. Medium exchange	125/125 → 250
8	02:00 p.m. Medium exchange	150/150 → 250
	03:00 p.m. Activate shaker type III	
10	02:00 p.m. Medium exchange	180/180 → 250
12	09:00 a.m. Harvest, end of culture	

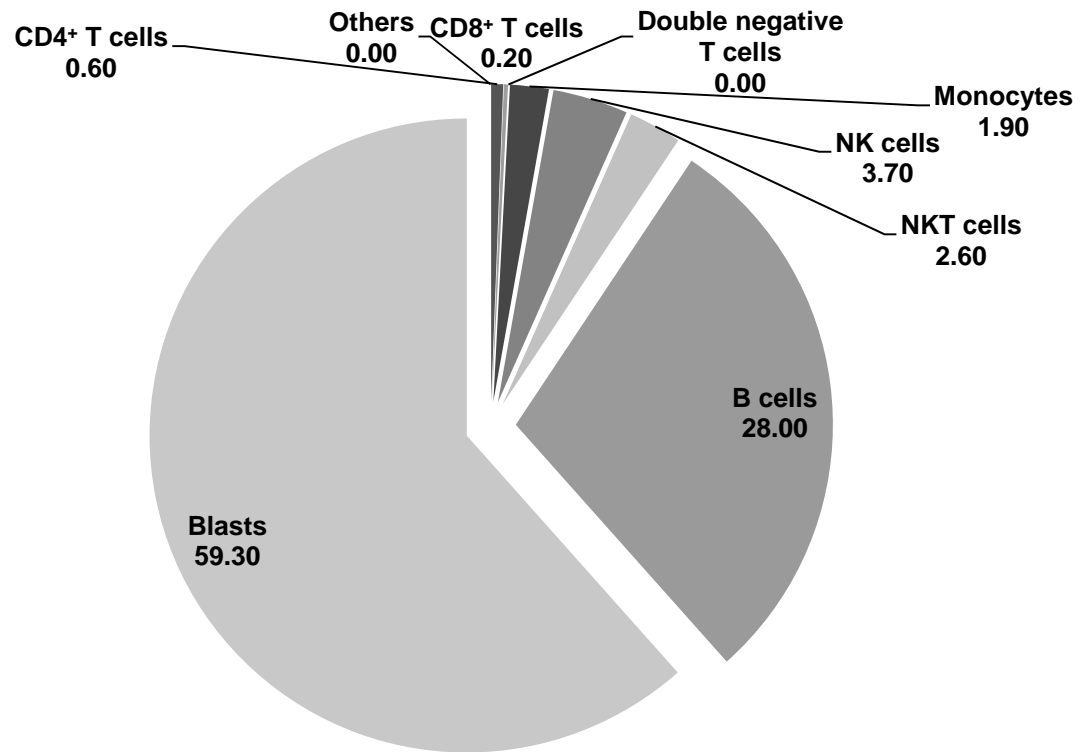
Supplementary Table 2: Viral concentration, MOI and T-cell transduction rate of patient runs CAR001-004

Run	Concentration [viral particles/ml]	MOI [viral particles/ T cell]	T-cell transduction rate day 12 [%]
CAR001	2x10 ⁶	11.49	40.4
CAR002	2x10 ⁶	4.1	19.6
CAR003	2x10 ⁶	5.18	19.3
CAR004	2x10 ⁶	21.37	28.5

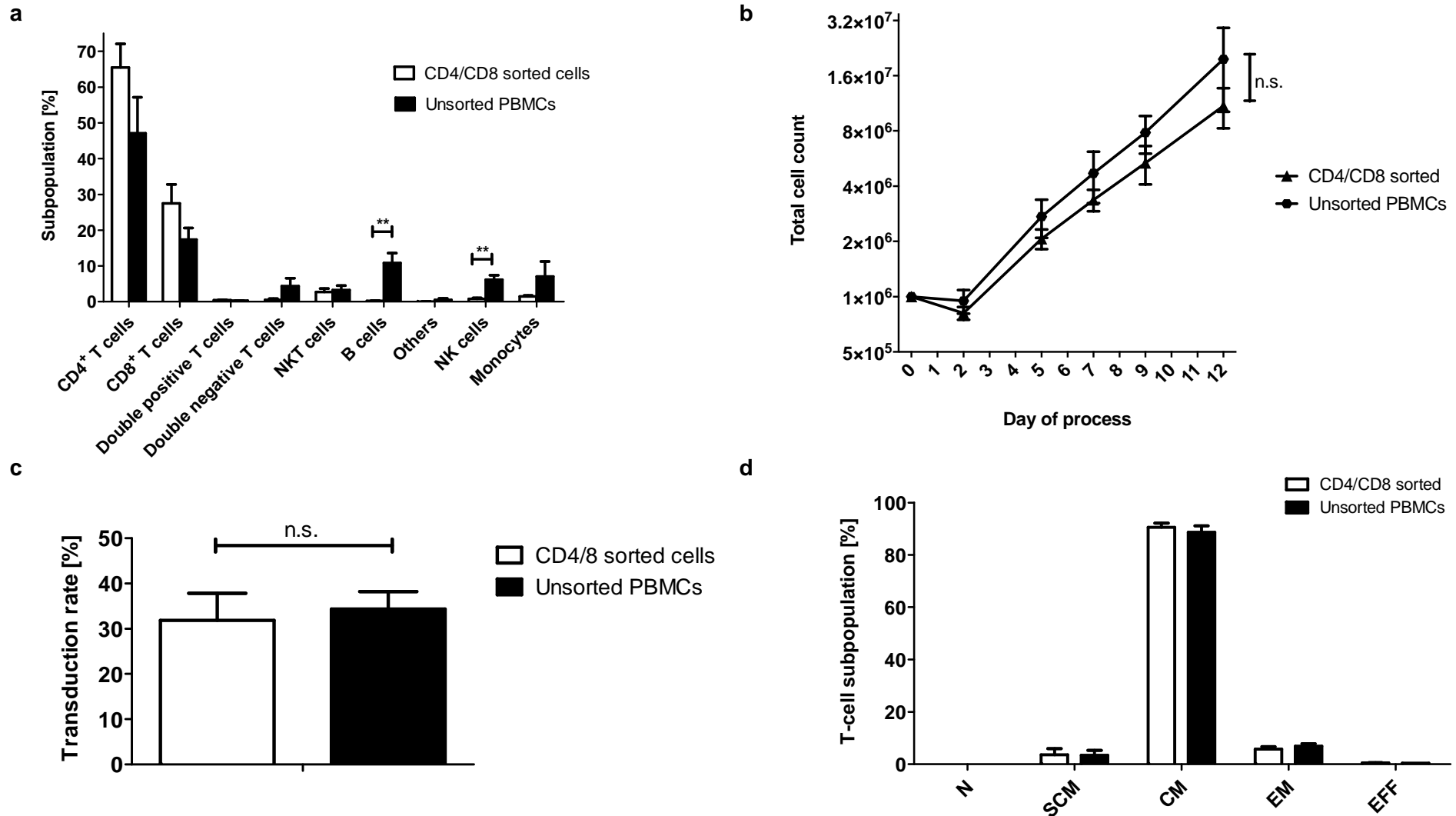
Supplementary Figure 1: CAR⁺ NKT cells were analyzed for their CD4 and CD8 expression by flow cytometry at the end of the process.



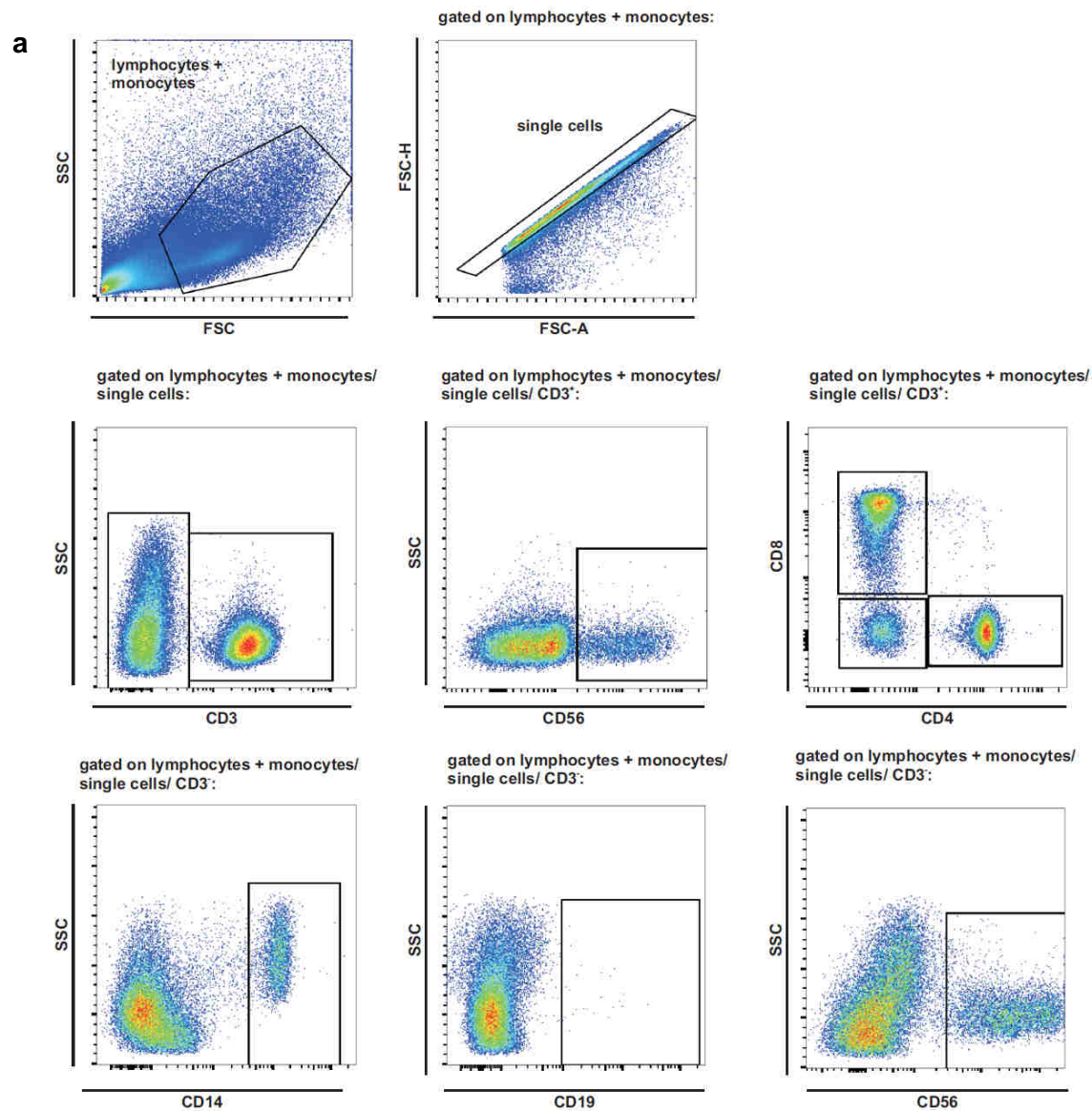
Supplementary Figure 2: The negative fraction after CD4/8 separation of initial blood product CAR003 was analyzed by flow cytometry and then used for the flow cytometry-based cytokine release assay shown in [Figure 6](#).



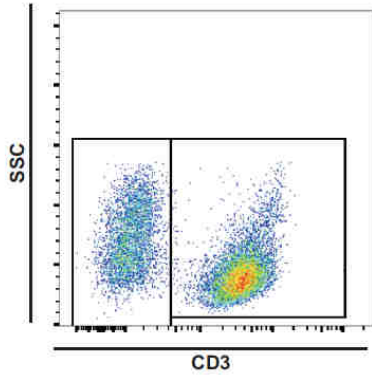
Supplementary Figure 3: Impact of CD4/CD8 separation on cellular composition, expansion, transduction and T-cell phenotype. **a** Cellular composition of unsorted PBMCs and CD4/CD8-sorted cells derived from healthy donors was analyzed by flow cytometry on day 0. **b-c** No significant difference in expansion behavior and transduction rate was found after 12 days of small scale culture. **d** T-cell phenotype on day 12 showed no significant difference. Statistical significance was determined by two-tailed paired T test.



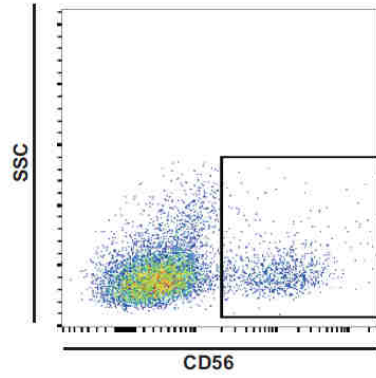
Supplementary Figure 4: **a** Flow cytometry-based gating strategy to determine cellular composition and exemplary plots of the starting fraction, **b** the positive fraction after CD4/CD8 separation, **c** the negative fraction after CD4/CD8 separation and **d** the composition of the final product. For figure part b, c and d, lymphocytes + monocytes and single cells were gated in analogy to part a.



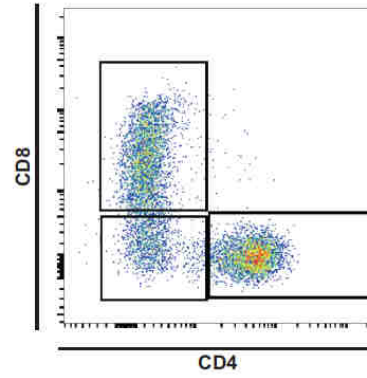
b gated on lymphocytes + monocytes/
single cells:



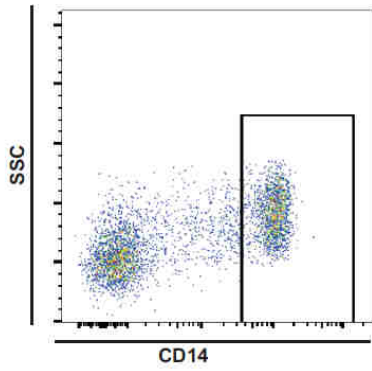
gated on lymphocytes + monocytes/
single cells/ CD3⁺:



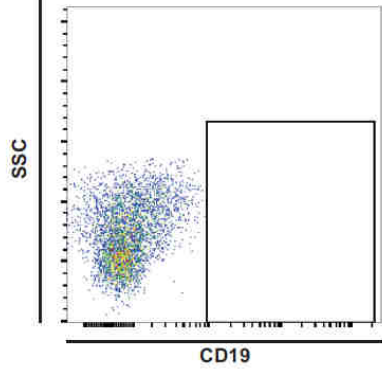
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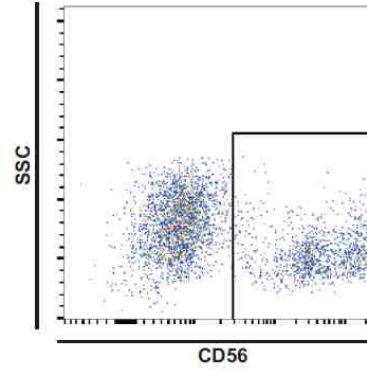
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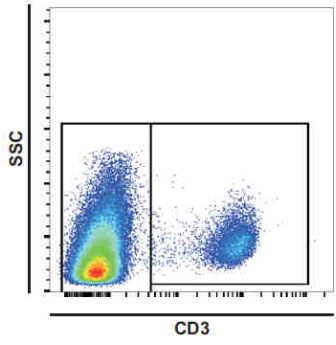


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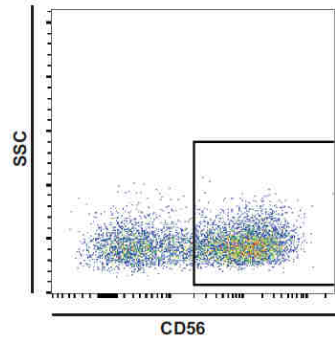


C

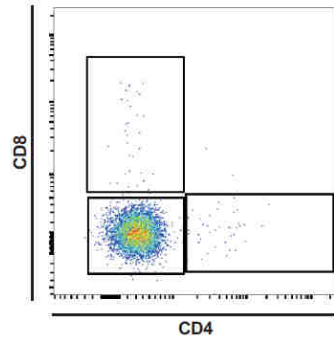
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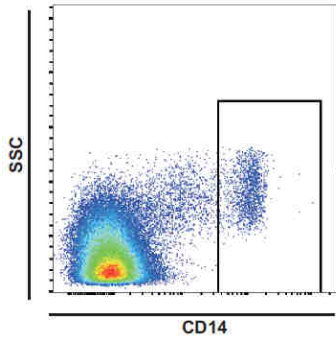
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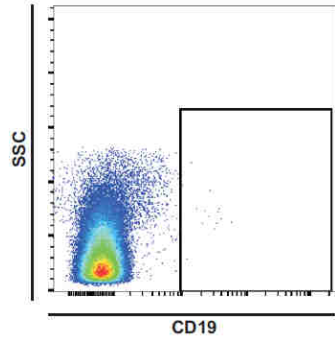
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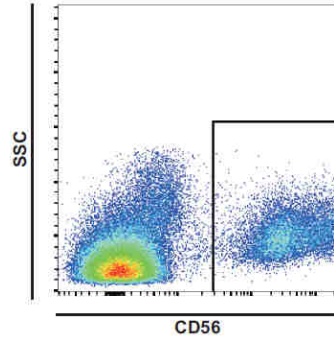
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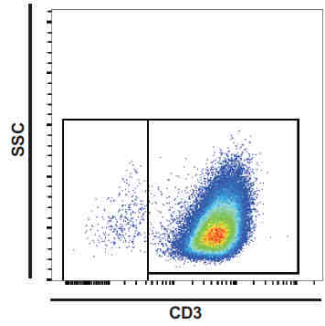


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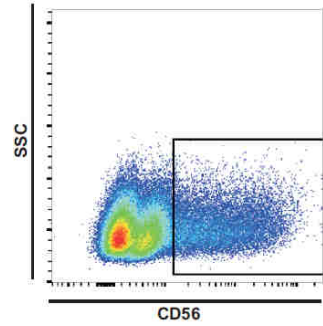


d

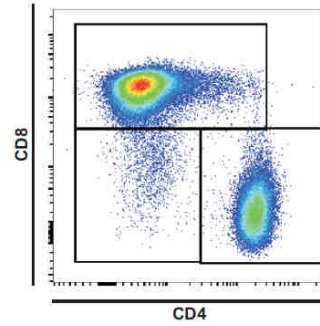
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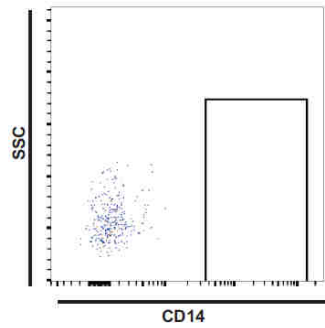
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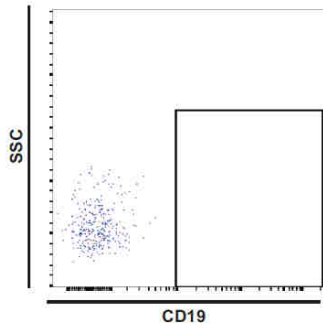
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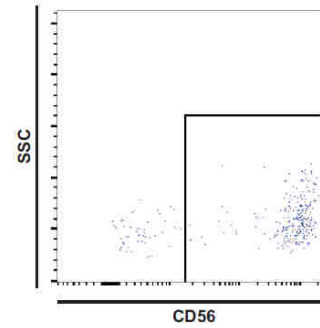
gated on lymphocytes/ monocytes
single cells/ CD3⁺:



gated on lymphocytes/ monocytes
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single cells/ CD3⁺:



Supplementary Figure 5: Flow cytometry-based gating strategy to determine the T-cell phenotype.

