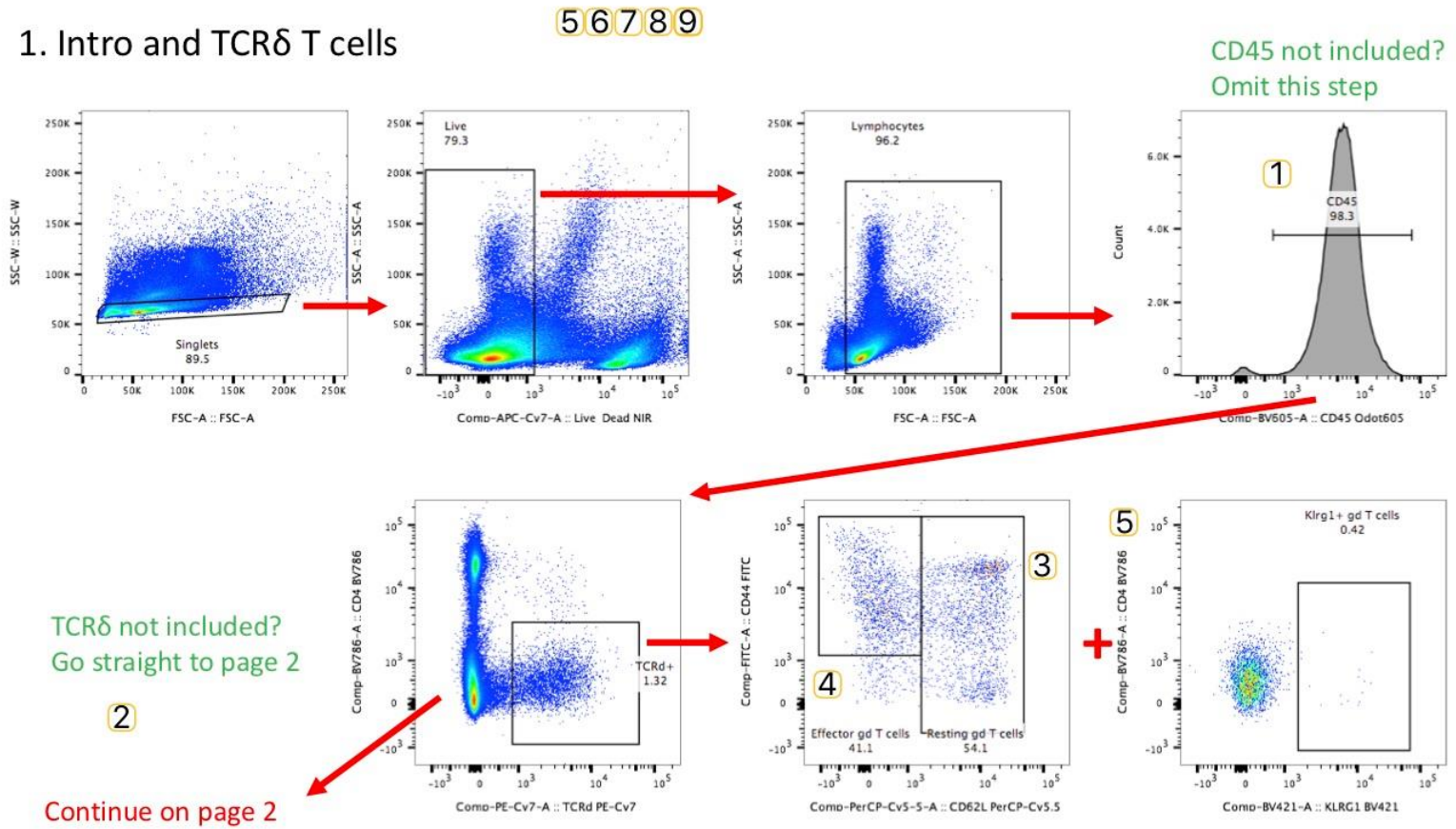
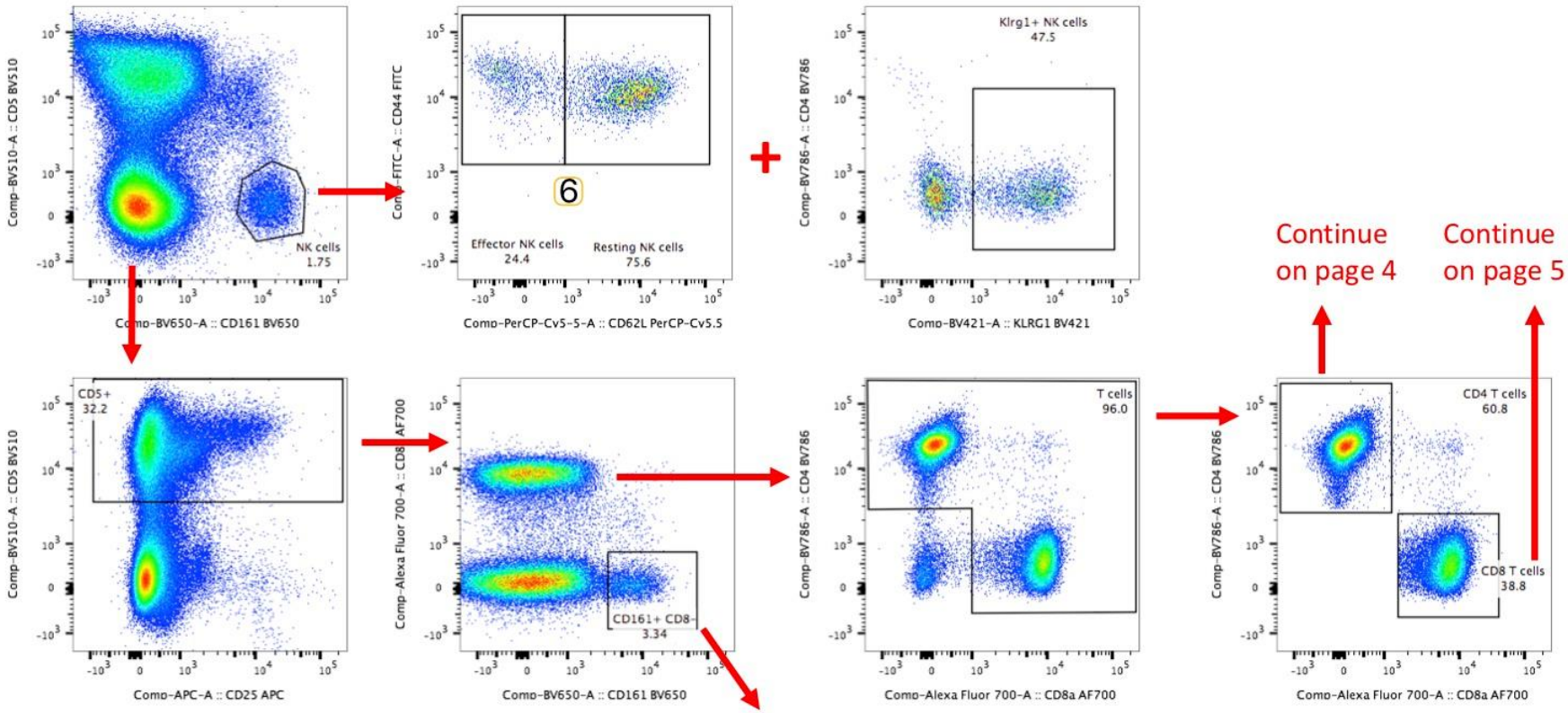
APPENDIX 1. GATING HIERARCHIES

Panel A. Page 1



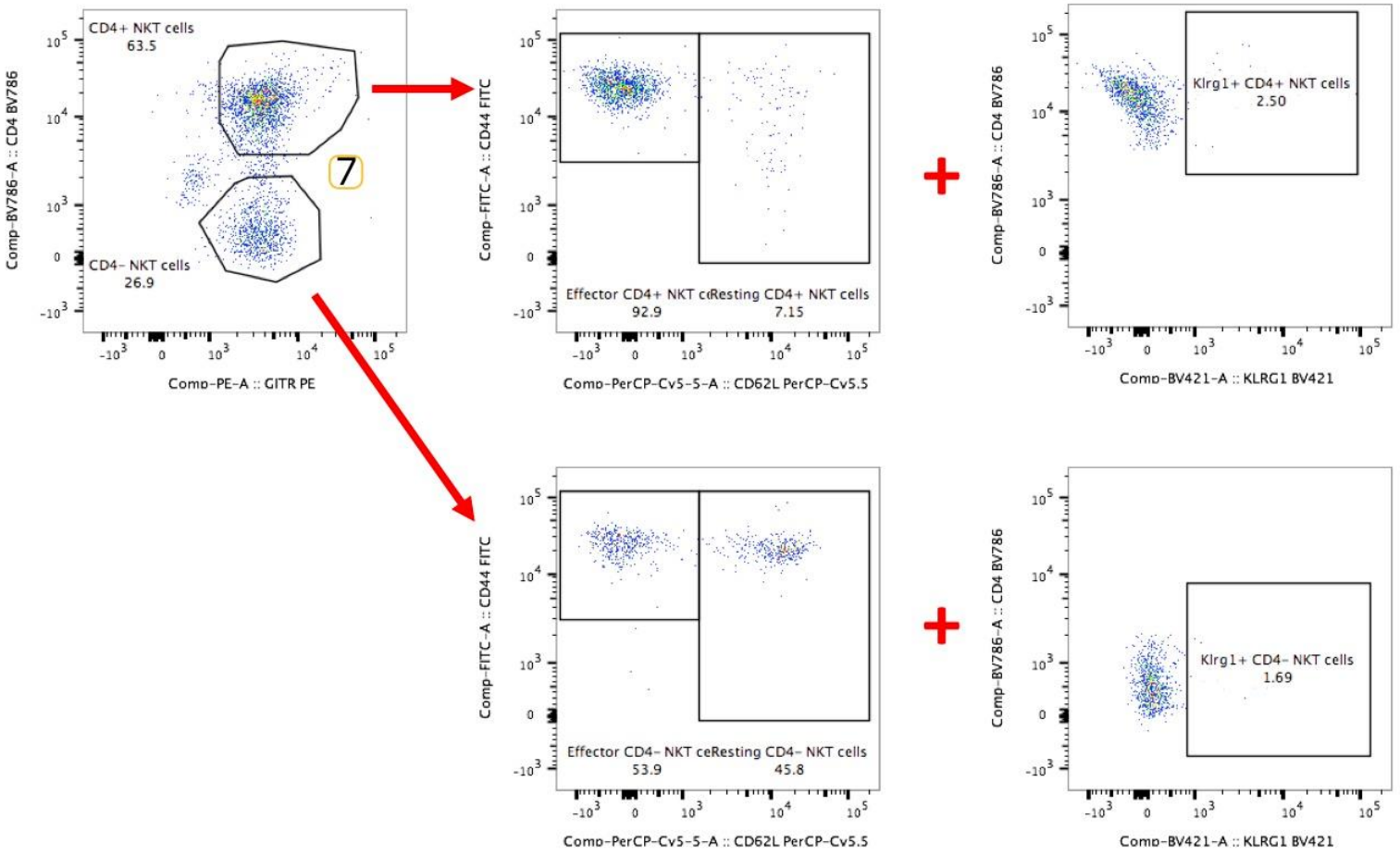
Panel A. Page 2.

2. NK cells and further gating



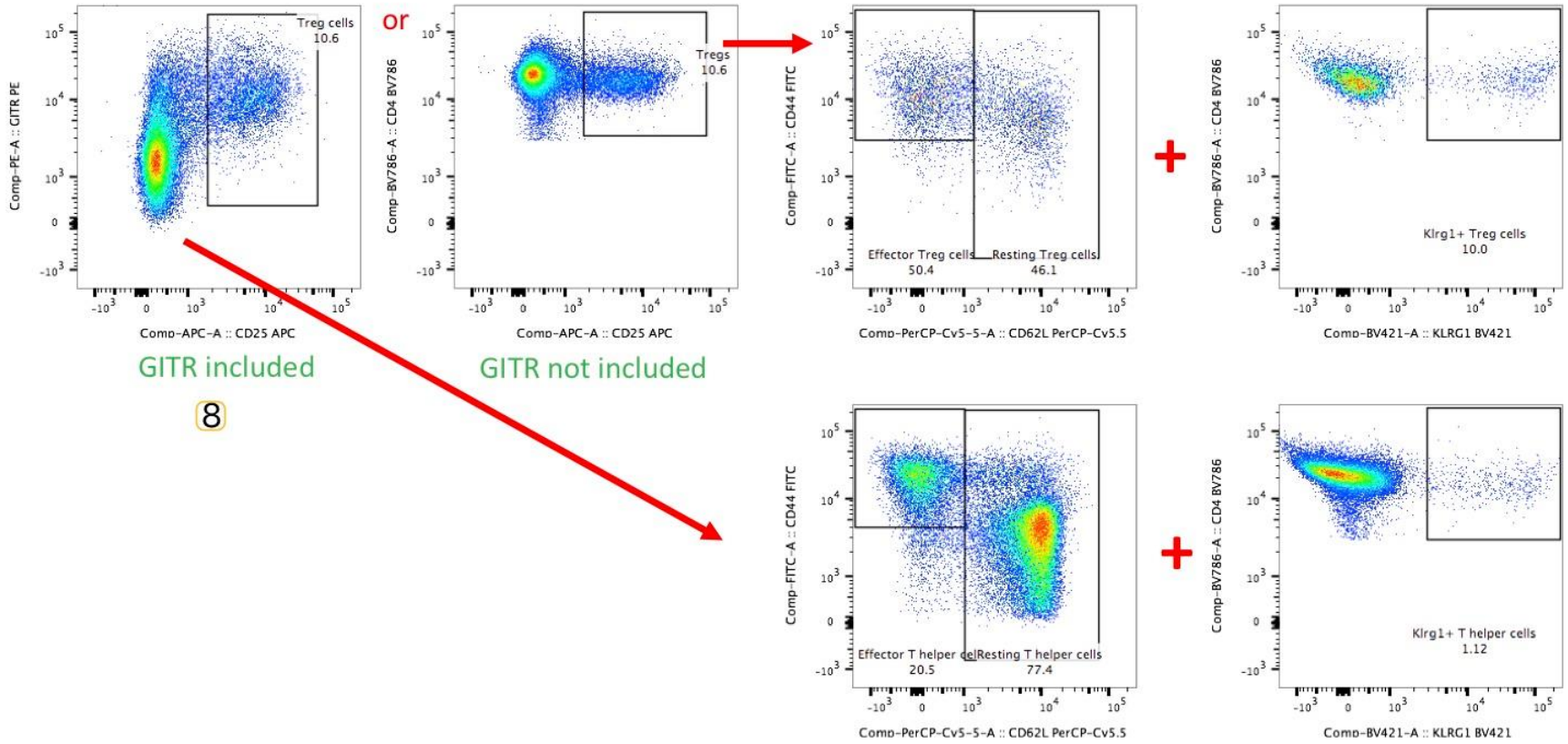
Panel A. Page 3.

3. NKT cells



Panel A. Page 4.

4. Tregs and T helper cells



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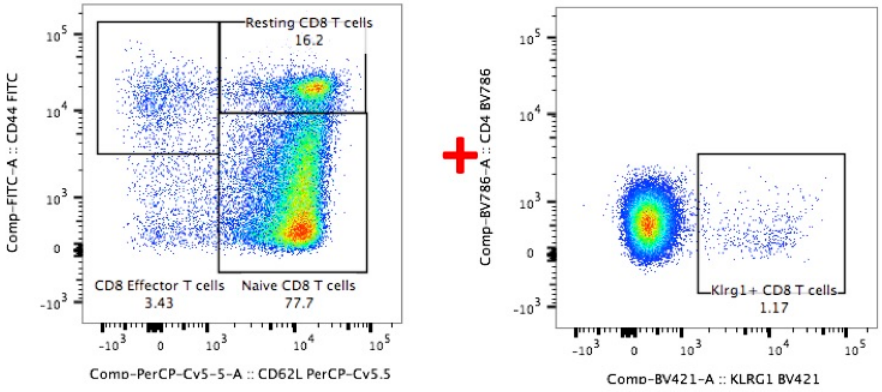
Panel A. Page 5.

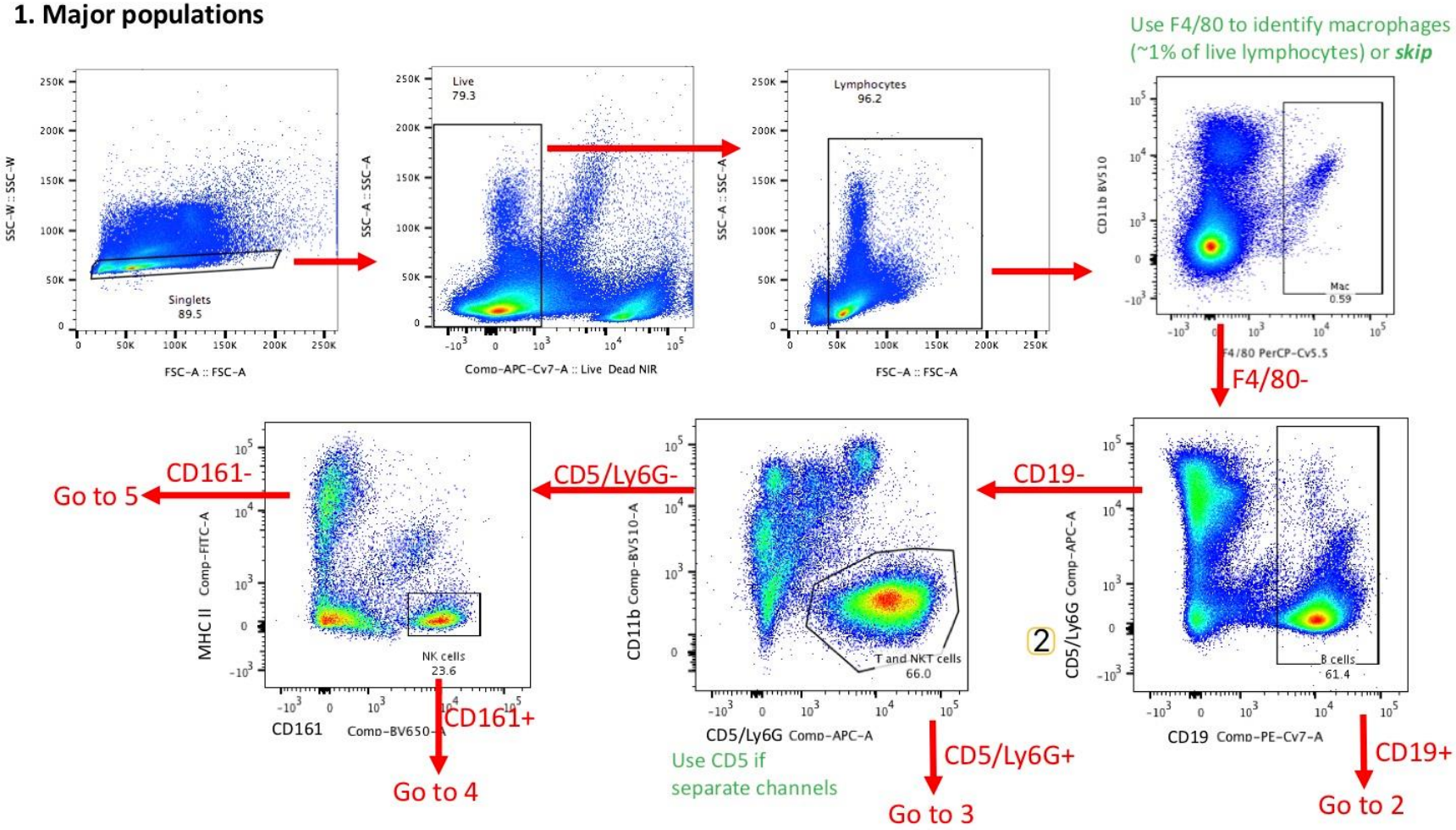
5. CD8 T cells and notes



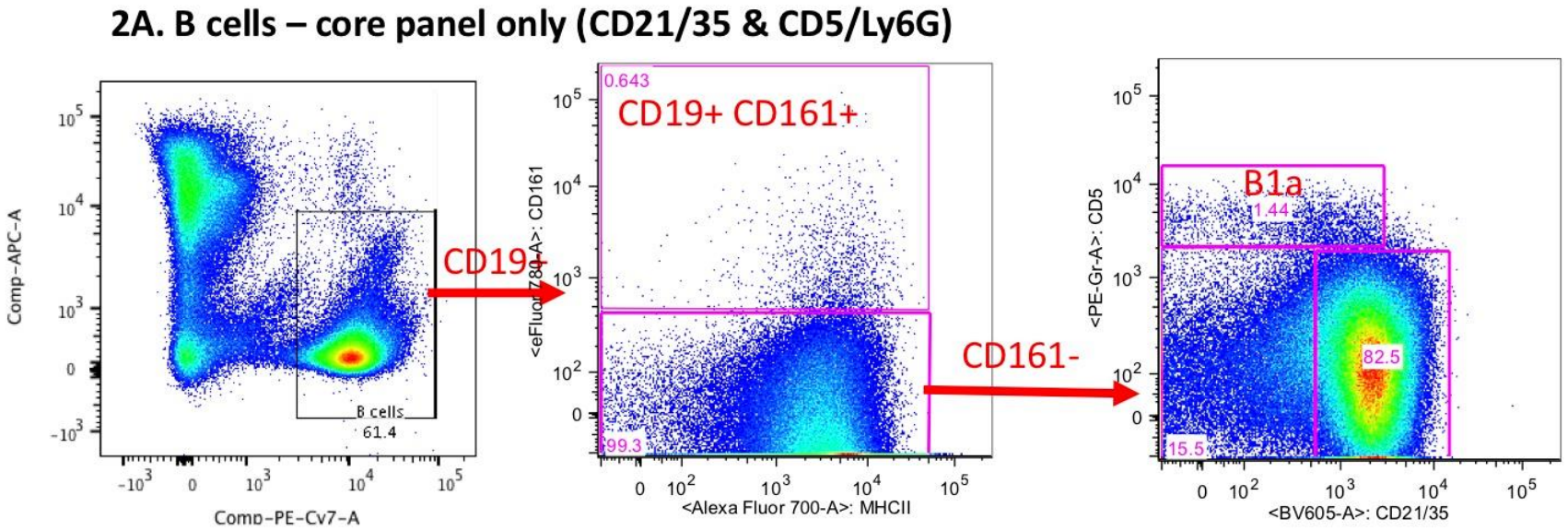
1. If there is no CD45 in the panel, omit this step.
2. Approximately 50% of γδ T cells are CD5-, so they will drop out when gating on CD5 later on. Of the remaining cells, approximately 90% are CD4- CD8- and will drop out of the T cell gate. Only 5% (approximately 0.2% of lymphocytes) will end up in the CD8 T cell gate which is negligible .
3. Please note that each cell type requires different thresholds for both CD44 and CD62L.
4. CD44- CD62L- cells do not occur naturally and show up when CD62L is shed from resting cells during sample preparation.
5. I have chosen CD4 for the y axis because gives a nice compact population for almost all cell types which makes it easy to see the Klrg1+ cells. However, if CD4 doesn't work for you because of your fluorochrome combinations, it can be substituted by any other marker.
6. The name effector is fine for CD4 and CD8 T cells, it is a bit unusual for γδ T cells, NKT cells and NK cells. We settled for this term in the end and also added these population names (with a more detailed description) to the MGI ontology, so MP terms that we use now carry these names.
7. These need to be added up to give the counts of total NKT cells. Use any fluorochrome on the y axis that gates out the non-specific autofluorescent population between the two distinct populations
8. If you don't have GITR, use CD4 on the y axis instead. It works almost as well.

Panel B. Page 1.

1. Major populations

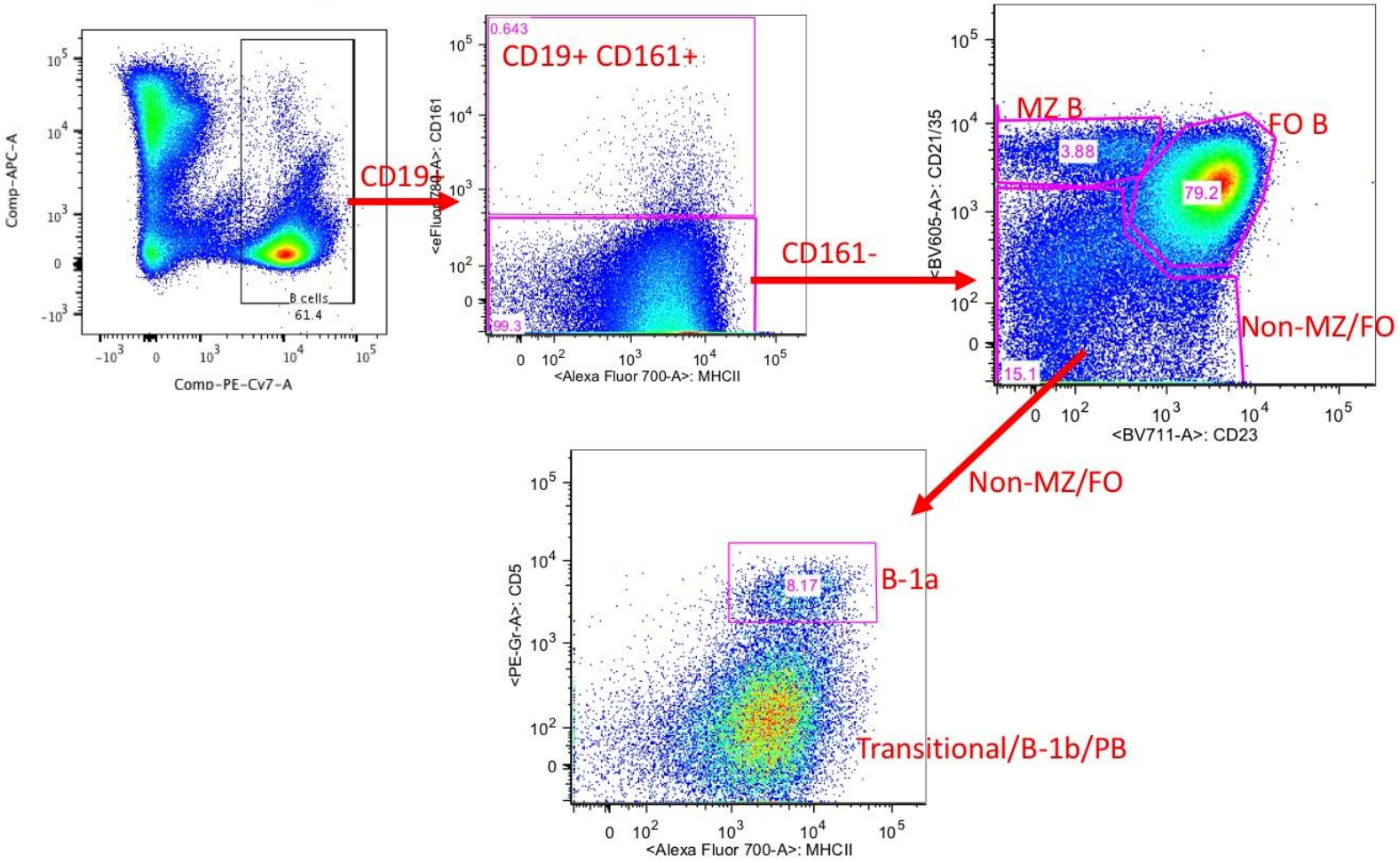


Panel B. Page 2A.

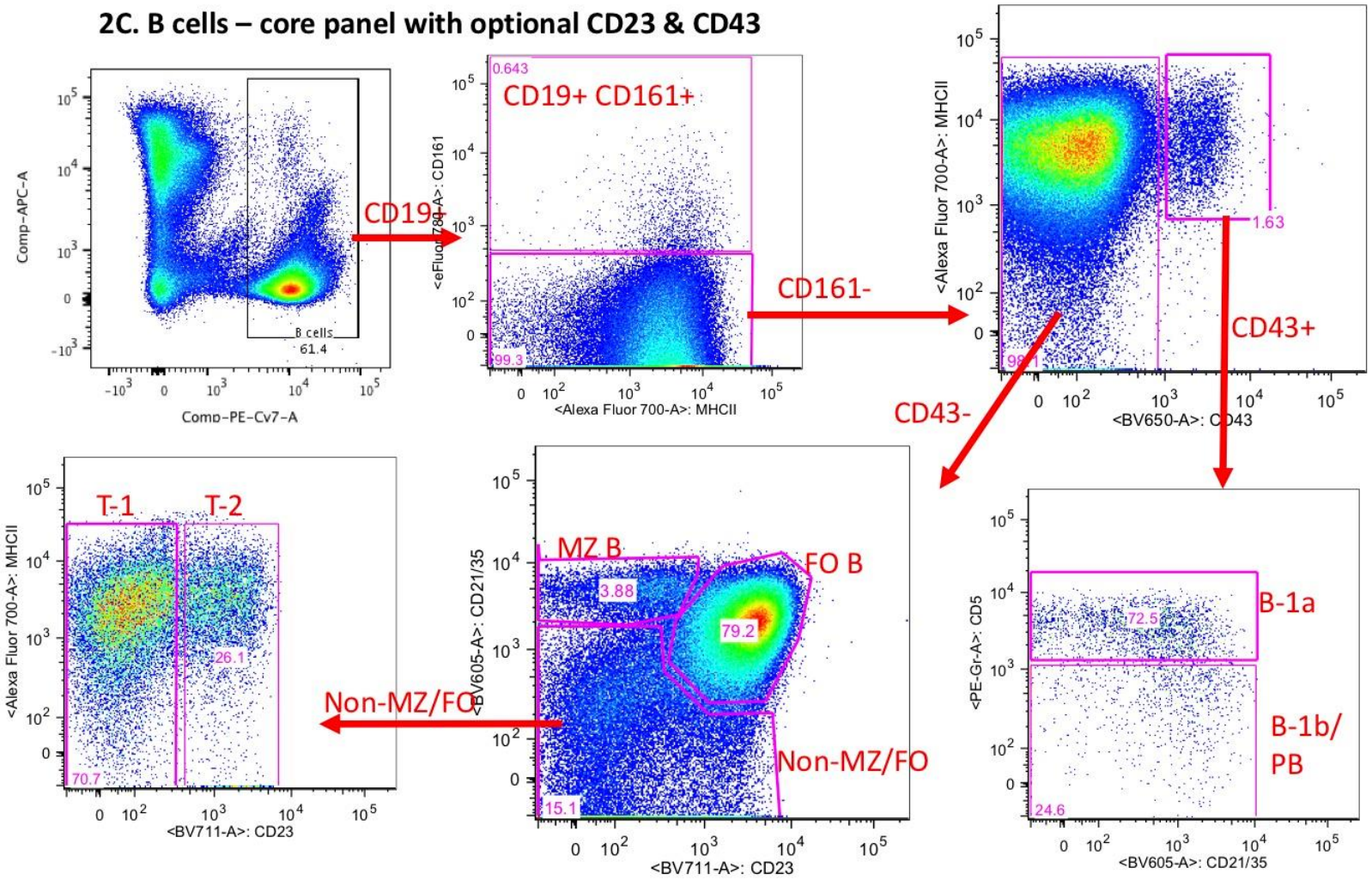


Panel B. Page 2B.

2B. B cells – core panel with optional CD23

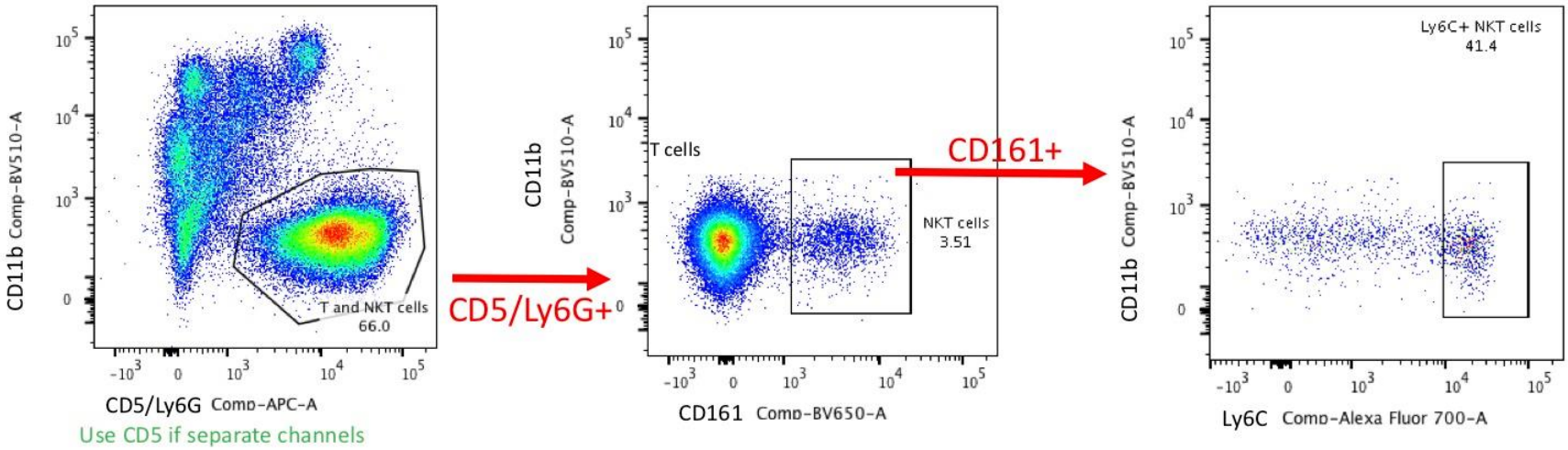


Panel B. Page 2C.



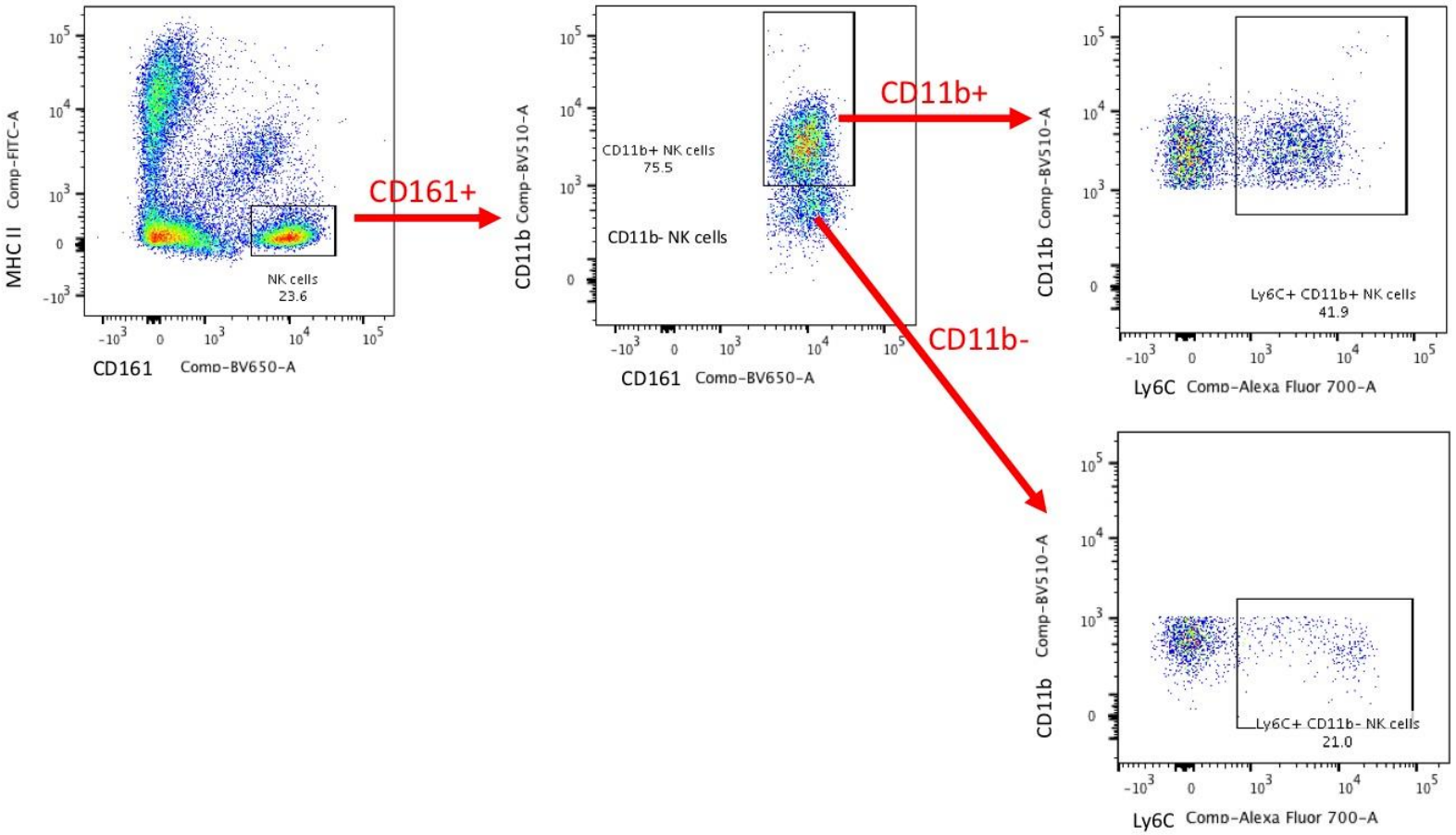
Panel B. Page 3.

3. T cells and NK T cells



Panel B. Page 4.

4. NK cells



Panel B. Page 5.

5. Myeloid cells