Appendix B

Results of sorting Day 14 cells with FACS machine

As explained in Chapter 5 section 5.5.2, CD34+ cord cells at their day 14 of differentiation process, were sorted using a FACSAria IIu flow cytometer (Beckton Dickinson Immunocytometry Systems (BD), UK) running BD FACSDiva v6 Software. Two sets of samples from the same donor are used and both of them are constructed in the same way. The obtained results from gating and sorting the cells are presented in Figs. 1 and 2. In the both figures, the first row consists of three diagrams and all together they are the gating strategy. The first diagram (Figs. B-1 and B-2 (a)) is so-called singlets gating, in which the single cells are separated from the doublets. Once the singlets are gated successfully, the obtained singlets can be plotted in another diagram which is Side-Scatter light (SSC) versus Forwarded-Scattered light (FSC) (Figs. B-1 and B-2 (b)). It gives an extra insight into the cells population and it is easy way to look at the population as it is a function of particles/cells size. Higher the FSC, bigger the particle/cell. In this diagram there is another gate called "Events". This gate includes both cells and nuclei (they can be recognize with the difference in the size). Everything that is outside this gate is considered as cell debris. Once the Events gate generated, cells can plot on CD235a+ versus DRAQ-5 diagram (Figs. B-1 and B-2 (c)). To distinguish between nucleated and enucleated cells, DRAQ-5 stain was used to test them for nucleus presence. The enucleated cells are CD235a+ (positive) and DRAQ-5 (negative) while nucleated cells are CD235a+ (positive) and DRAQ-5 (positive), and finally nuclei are smaller and additionally they express similar features as nucleated cells because they are surrounded by a piece of cell membrane with the CD235a+ markers. Based on that, enucleated, nucleated, nuclei gates are created on the third diagram. Once the gating strategy become ready, FACS machine can be used to sort cells from each gate into separate collection tubes. At the end of sorting process quality control was performed by running sorted sub-populations separately using the same gating strategy, which are presented in the next rows (Figs. B-1 and B-2 (d, e and f)). Underneath each diagram there is a small label showing the name of the displayed sample. It can be seen that for sorted cells, they are presented in the predicted gates and other gates are empty, e.g. for enucleated cells "nucleated" and "nuclei" gates are empty. This can confirm that the sorting is pure.

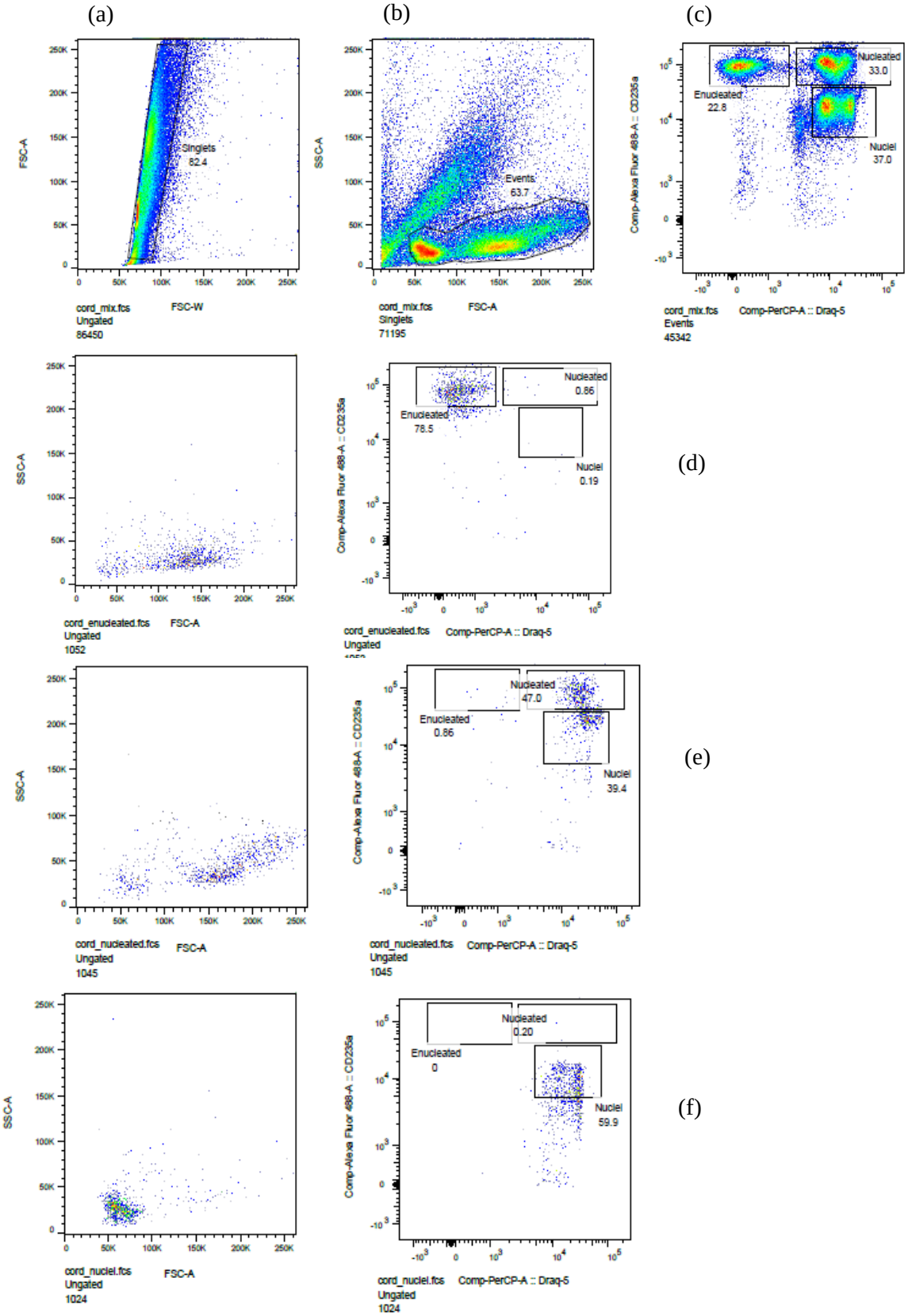

Figure B-1. Results from gating and sorting the first set of CD34+ cells. Diagram (a) shows separating singlets from the rest of the sample. Diagram (b) shows separating the desired cells from the debris. In diagram (c) cells were gated in three groups of enucleated, nucleated and nucleus. Diagrams (d), (e) and (f) are showing the results of quality control.

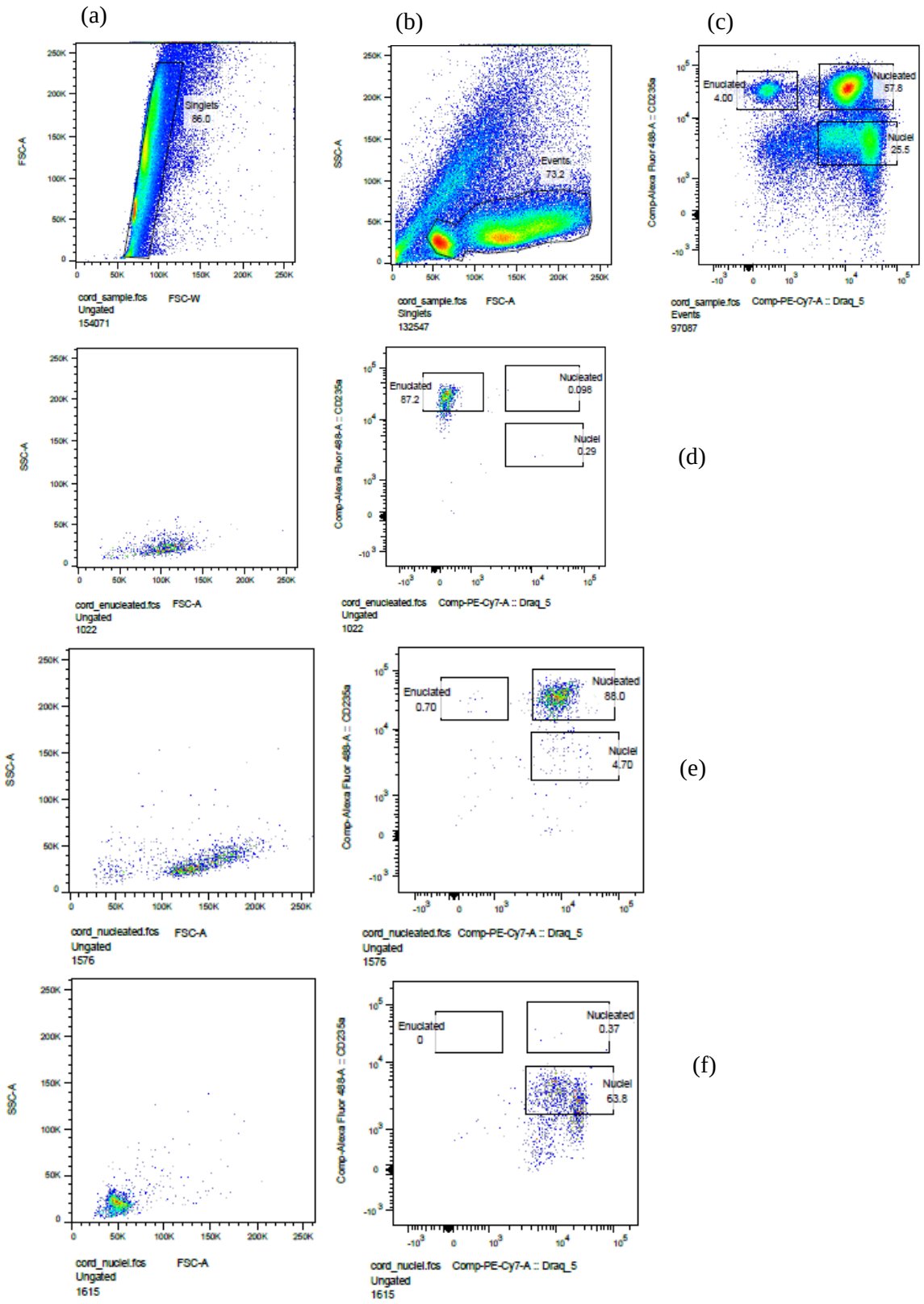

Figure B-2. Results from gating and sorting the second set of CD34+ cells. Diagram (a) shows separating singlets from the rest of the sample. Diagram (b) shows separating the desired cells from the debris. In diagram (c) cells were gated in three groups of enucleated, nucleated and nucleus. Diagrams (d), (e) and (f) are showing the results of quality control.