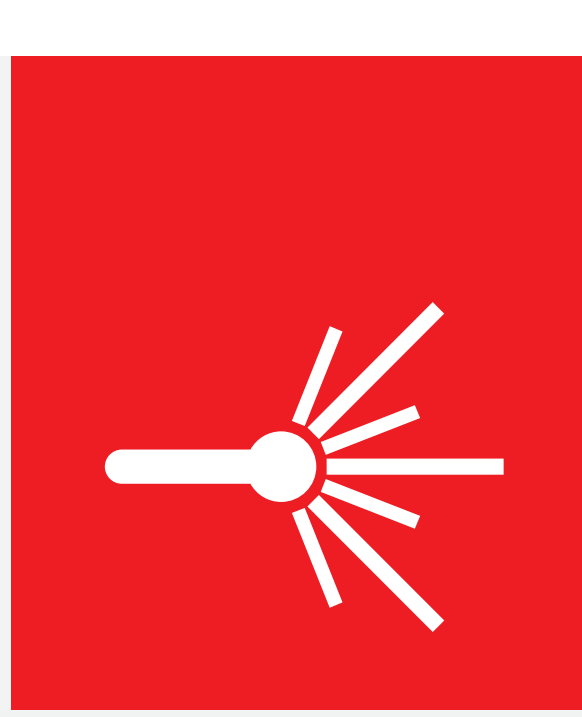




PE-IR and APC-IR Tandems

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INTRODUCTION

Recent advancements in flow cytometry allow users to simultaneously detect and analyze cell markers using multiplex panels. Flow cytometers equipped with an avalanche photodiode (APD) detection system, like the CytoFLEX LX instrument, are capable of detecting fluorescence emission beyond 820 nm. However, there are only a limited number of dyes with peak emission signals beyond 820 nm. Here, we introduce new phycoerythrin-infrared (PE-IR) and allophycocyanin-infrared (APC-IR) tandems designed to expand the IR spectrum using Yellow-Green (YG) and Red (R) laser excitation.

METHODS

The new YG and R laser-excitable IR tandems were generated based on the fluorescence resonance energy transfer (FRET) principle. We used PE and APC phycobiliproteins as donor molecules and selected CF*820, CF*850, and CF*870 dyes that emit in the IR region as acceptor molecules to make the tandems (designated as PE-IR and APC-IR tandems for PE and APC, respectively). The acceptor-to-donor ratio for each tandem dye was selected for the most balanced performance. The tandems were then conjugated to various monoclonal antibodies with different specificities (CD4, CD8, CD38, CD45RA, HLA-DR, etc.) and purified using a size exclusion column (ÄKTA™ pure, GE Healthcare). To test the prepared conjugates, we used a whole blood staining procedure, followed by lysis (VersaLyse) and fixation (IOTest 3). We tested the prepared conjugates using two flow cytometry instruments. The first was a modified CytoFLEX LX Flow Cytometer, where the red laser configuration was adjusted to detect emissions from the tandem conjugates in the yellow-green (for PE-IR conjugates) or red spectra (for APC-IR conjugates). The second was the CytoFLEX mosaic 88 Spectral Detection Module connected to the CytoFLEX LX Flow Cytometer. For the modified CytoFLEX LX instrument, data was compensated and analyzed using CytExpert or Kaluza software. Data acquired on the CytoFLEX mosaic 88 Spectral Detection Module was unmixing using a Poisson hybrid algorithm and analyzed with CytExpert for Spectral software and Kaluza software.

RESULTS

Figure 1: CD8-PE-CF*820 tandem conjugate performance and spectral flow cytometry analysis

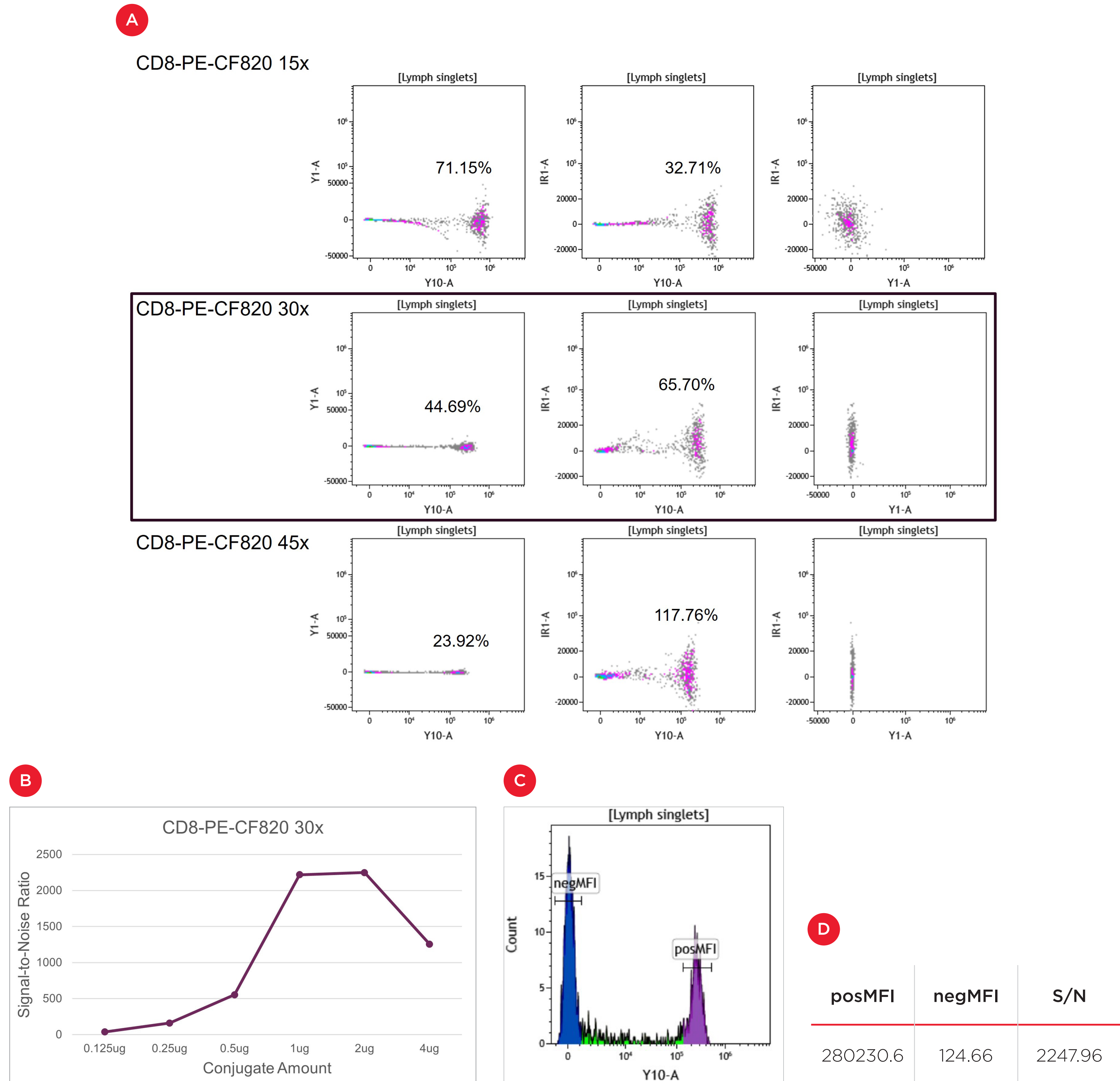


Figure 1. A: Three CD8-PE-CF*820 tandem conjugates with different molar ratios (15x, 30x, and 45x) of acceptor (CF820) molecule to donor (PE) molecule. Based on the most balanced spreading of the tandem conjugate into the donor channel (Y1) and the IR channel (IR1), CD8-PE-CF*820 30x was chosen for titration. **B:** Titration plot of the CD8-PE-CF*820 conjugate. Whole blood was stained with 2-fold dilutions, and the signal-to-noise ratio (S/N) of each dilution was calculated and plotted. **C:** Histogram plot of 2 µg CD8-PE-CF*820. **D:** Table with measured positive (pos) and negative (neg) MFI and calculated S/N. Data acquisition was performed on the CytoFLEX mosaic 88 Spectral Detection Module connected to the CytoFLEX LX Flow Cytometer.

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Figure 2: 3-color panel with CD38-PE-CF*850

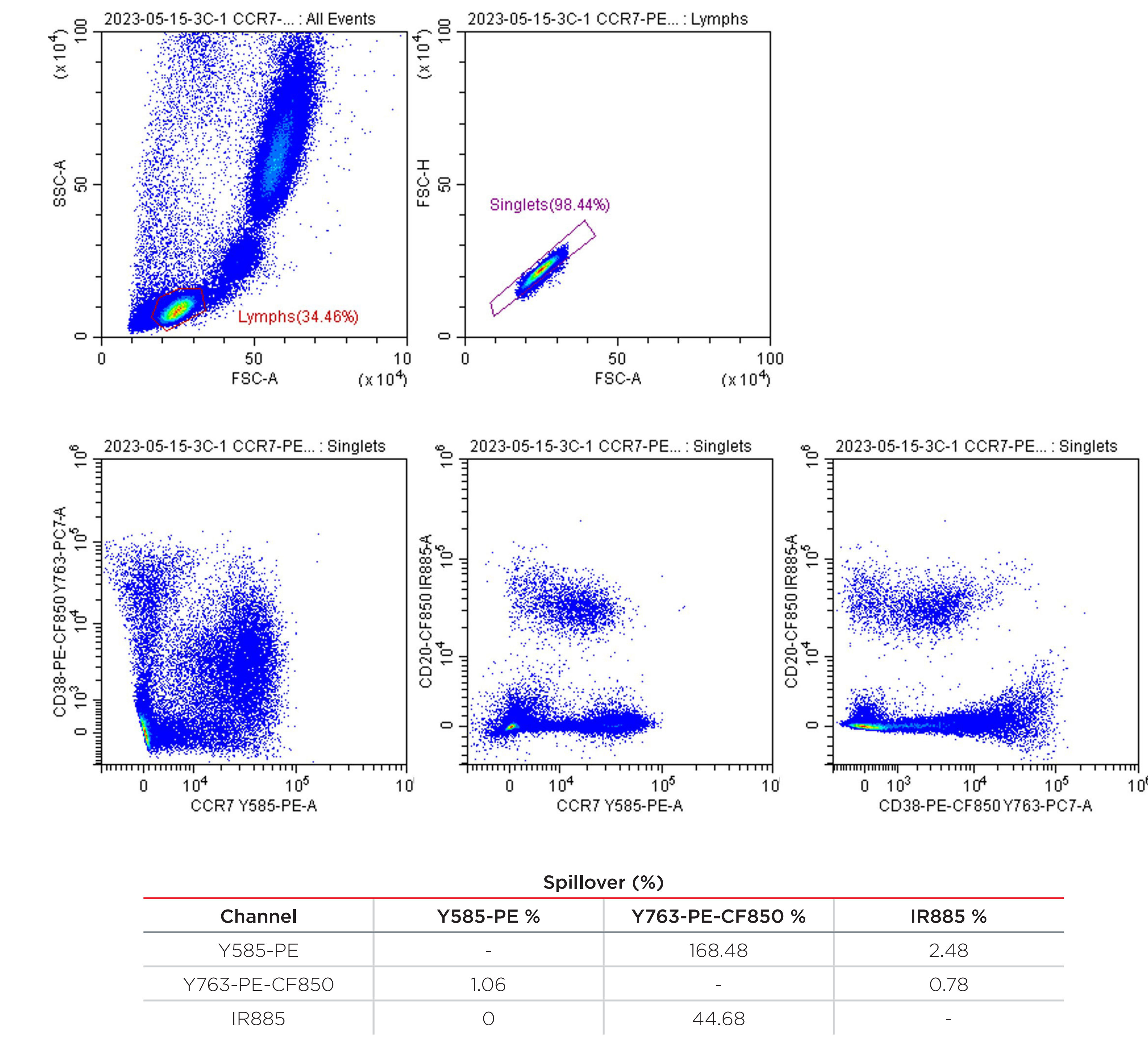


Figure 2. Acquisition was performed on a modified CytoFLEX LX Flow Cytometer, where the fluorescent detector Y763 (763/43) was replaced with an 885/40 bandpass filter. Compensation and analysis were done using CytExpert software. Although spreading was observed between CCR7-PE and CD38-PE-CF*850, and between CD38-PE-CF*850 and CD20-CF*850, the data was compensated properly, and we observed correct biological phenotypes.

Figure 3: Immunophenotyping using APC-IR tandems

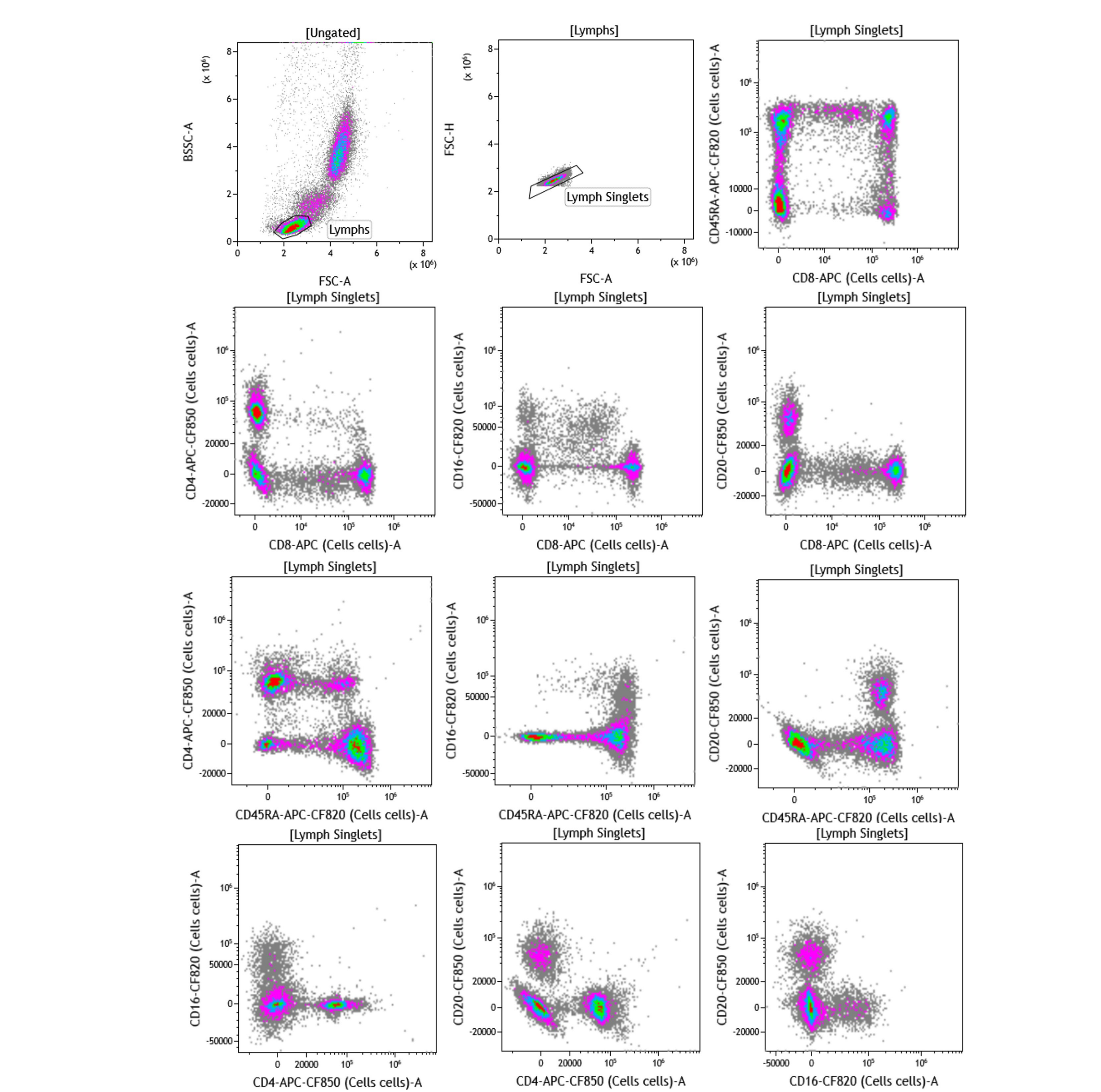


Figure 3. The panel consists of APC-CF*820 and APC-CF*850 tandem conjugates, together with the APC, CF*820, and CF*850 non-tandem conjugates. Data acquisition was performed on a CytoFLEX mosaic 88 Spectral Detection Module attached to the CytoFLEX LX Flow Cytometer. Unmixing was performed using the Poisson hybrid unmixing algorithm in CytExpert for Spectral software. The data was properly unmixed, and all biological phenotypes based on the analyzed biomarkers were correct.

Figure 4: Immunophenotyping using PE-IR and APC-IR tandems

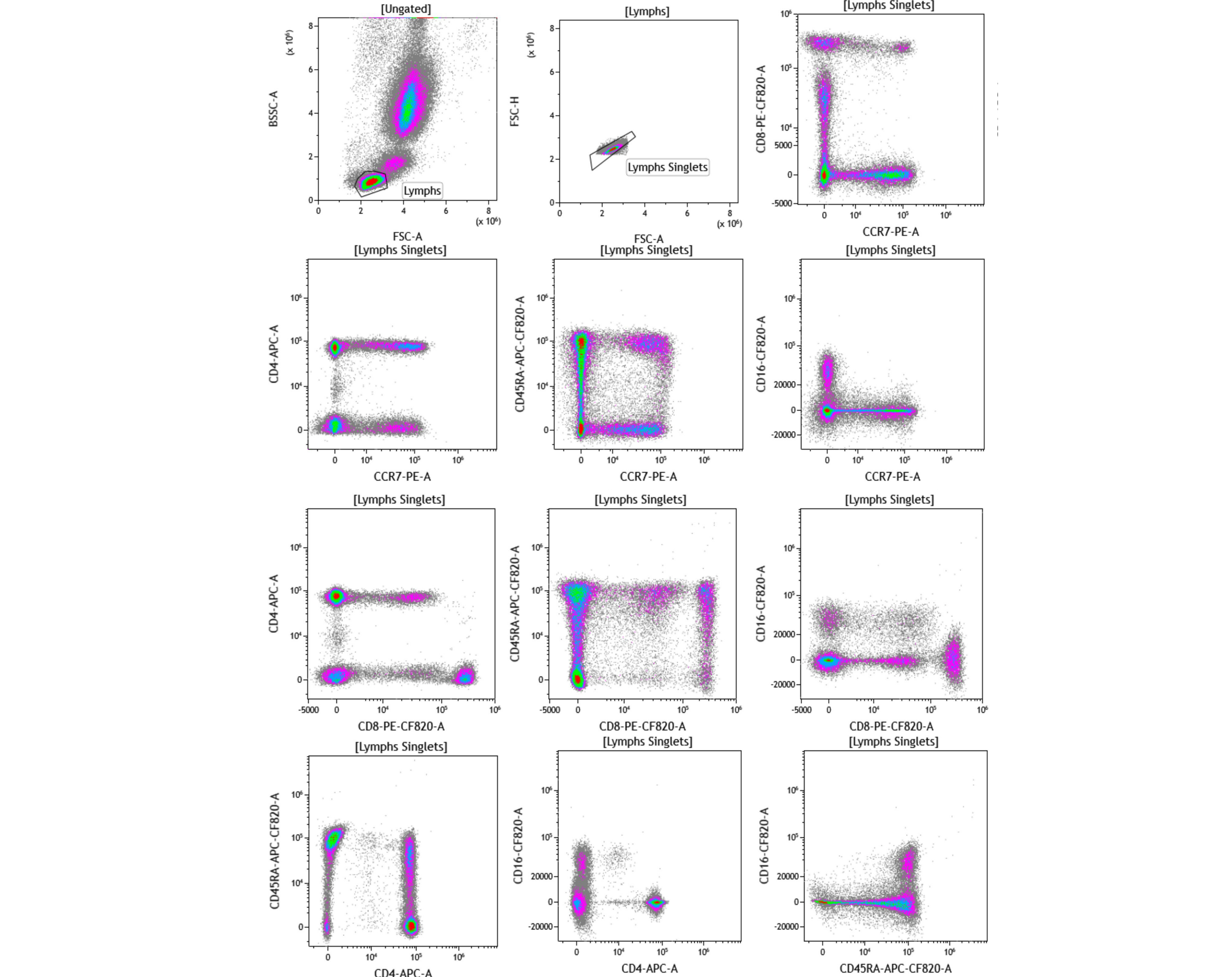


Figure 4. Immunophenotyping using PE-IR and APC-IR tandems. The panel consists of PE-CF*820 and APC-CF*820 tandem conjugates, together with APC, PE, and CF*820 non-tandem conjugates. Data acquisition was performed on the CytoFLEX mosaic 88 Spectral Detection Module attached to the CytoFLEX LX Flow Cytometer. Unmixing was performed using the Poisson hybrid unmixing algorithm in CytExpert for Spectral software. The data was properly unmixed, and all biological phenotypes based on the analyzed biomarkers were correct.

Figure 5: APC-IR tandems on modified CytoFLEX LX instrument

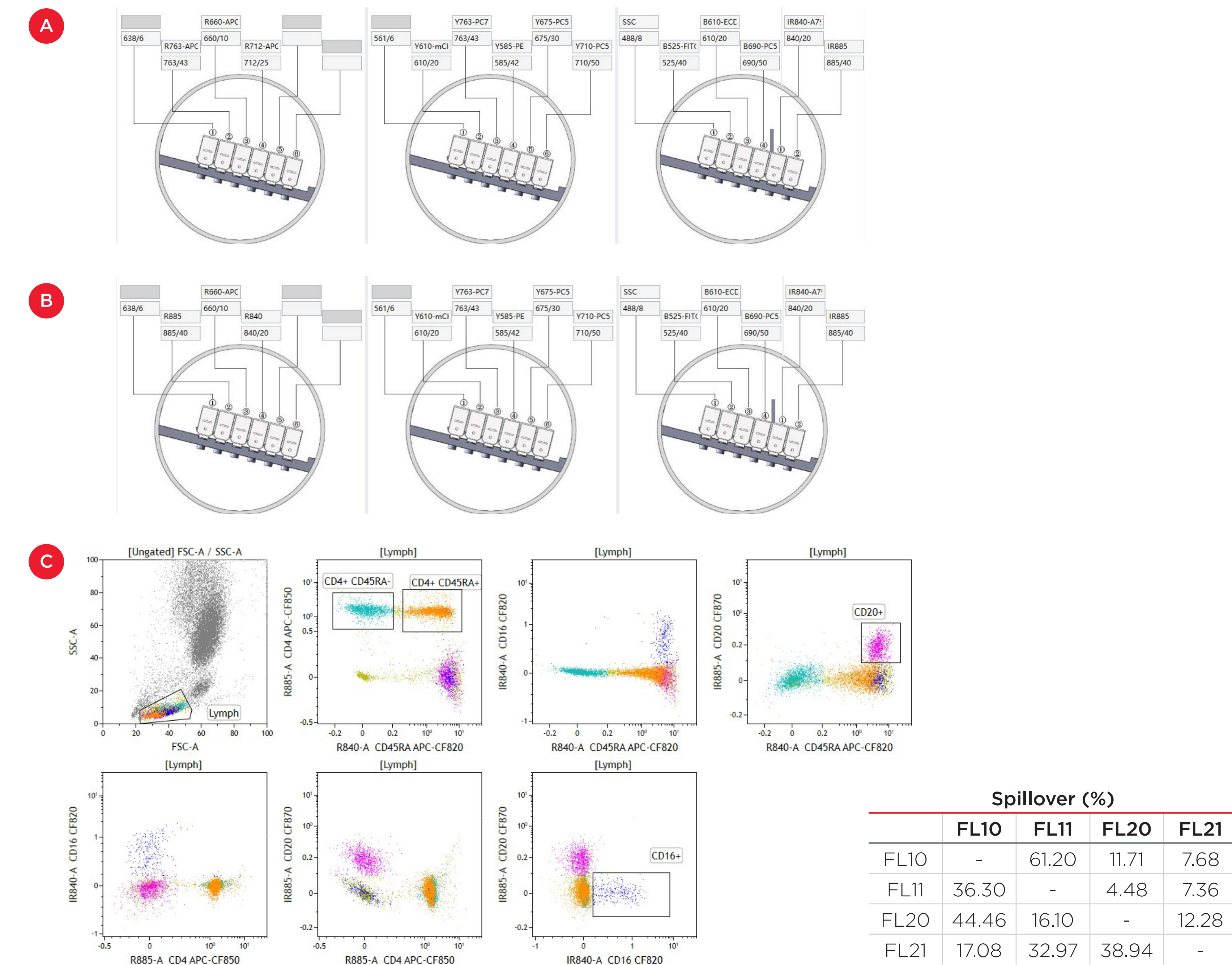


Figure 5. A: Default detector configuration. **B:** Modified detector configuration. **C:** The panel consists of APC-CF*820 and APC-CF*850 tandem conjugates and CF*820 and CF*850 non-tandem conjugates. Data acquisition was performed on the CytoFLEX LX Flow Cytometer. Compensation and analysis were done using Kaluza software. The data was properly compensated (post-compensation adjustment was not needed), and all biological phenotypes based on the analyzed biomarkers were correct.

CONCLUSION

PE-IR and APC-IR tandems were created and successfully conjugated to monoclonal antibodies. The results demonstrated effective compensation and unmixing of all conjugates in multicolor experiments, showing that PE-IR and APC-IR tandems can effectively fill the existing gap in the IR spectral region using YG or R laser excitation. These new tandems offer the potential to design even more complex multicolor panels.