

Introduction

The preparation of cellular products for clinical settings typically comprises several independent processing steps including density gradient centrifugation, cell separation, cell washing, and cell formulation. In the case of Mo-DC vaccines, isolated monocytes additionally need to be cultured at controlled temperature and pH for several days and Mo-DCs often need to be electroporated for efficient antigen presentation. Cultivation also requires a number of manual handling steps, such as media exchange and supplementation of additives, thus generating the risk of contamination. To meet the increasing regulatory requirements for cell-based therapeutics, we integrated all manufacturing steps in a closed system operated by an automated cell processing instrument, the CliniMACS Prodigy® (fig. 1). Here we show the detailed phenotypic and functional characterization of Mo-DCs from several independent production batches derived from apheresis samples obtained from healthy donors. Moreover, we show the successful electroporation of the generated Mo-DCs with the CliniMACS® Electroporator.



Figure 1

Results

1 Performance of the automated CD14⁺ cell separation and Mo-DC generation process on the CliniMACS Prodigy®

Using the CliniMACS CD14 Reagent monocytes were routinely enriched to a purity of 97% and a recovery of 81%. Viability of the cells was 98% on average (fig. 2A). Isolated monocytes were cultured for 6 days in the presence of GM-CSF and IL-4, yielding immature Mo-DCs (imMo-DCs), which were subsequently matured for an additional 24 hours in the presence of IL-1 β , IL-6,

TNF- α , and PGE₂. Mature Mo-DCs (mMo-DCs) were generated with recoveries of 20% on average, calculated from the number of initially seeded monocytes. The viability of mMo-DCs was evaluated by flow cytometry, i.e., exclusion of PI⁺ dead cells, and amounted to 97% (fig. 2B).

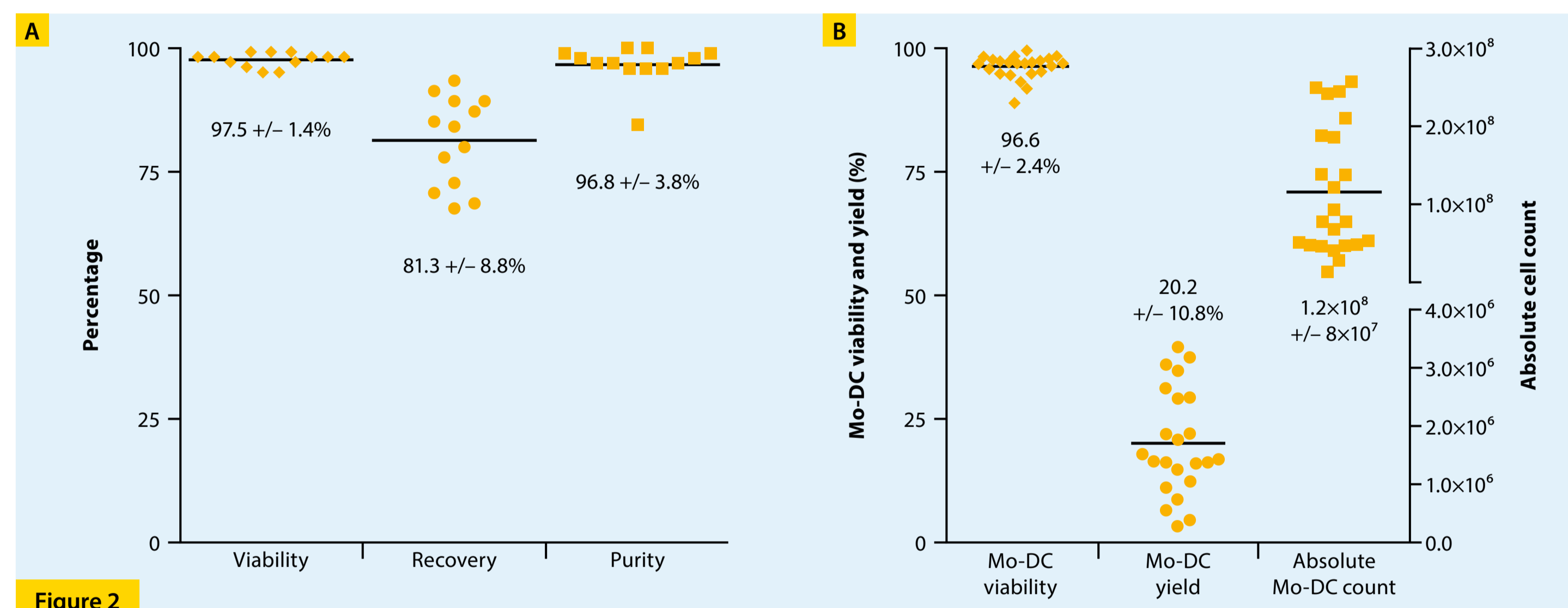


Figure 2

2 Mo-DCs show typical phenotypic DC characteristics

During differentiation monocytes down-regulated the expression of CD14 (fig. 3). In contrast, mMo-DCs expressed various DC markers that are involved in the formation of immunological synapse between DCs and naive T cells, including CD80, CD86, CD54, MHC I (HLA-ABC), and MHC II (HLA-DR).

Mature Mo-DCs also expressed DC activation markers CD83, CD25, and CD40, which were up-regulated accordingly. Expression of CCR7, which is required for migration of DCs to draining lymph nodes, was also up-regulated. MFI denotes the mean fluorescence intensity. Black: isotype controls; red: specific antibodies.

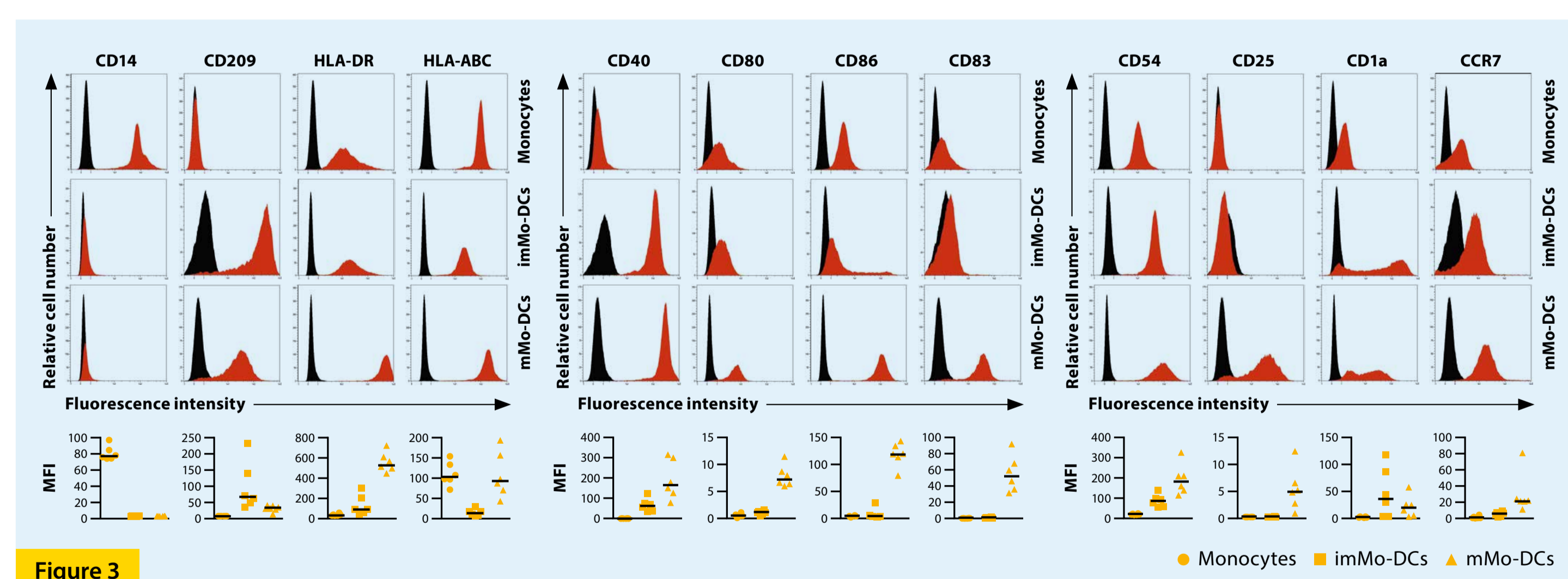


Figure 3

3 mMo-DCs migrate in a CCL19-dependent manner

Upon activation in the periphery, DCs migrate to the draining lymph nodes (LN) where they encounter naive T cells. Migration through the endothelial layer and within the LN, along the CCL19 gradient towards the T cell area, depends on the expression of CCR7 on the DC surface. CCR7 expression was up-regulated during Mo-DC maturation (fig. 3), and mMo-DCs migrated towards CCL19 in a dose-dependent manner, as determined using Transwell® Plates (fig. 4).

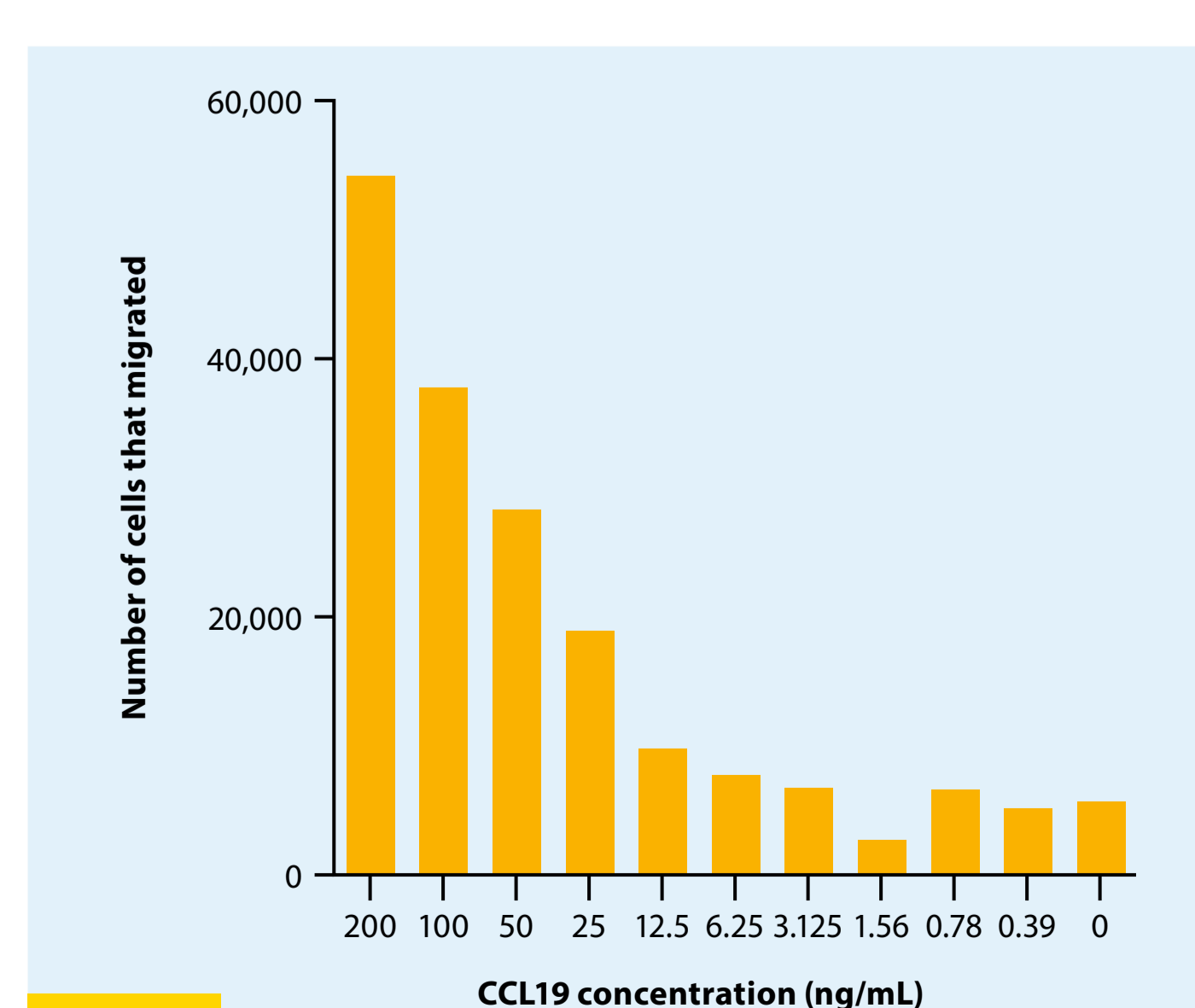


Figure 4

4 mMo-DCs are strong inducers of T cell proliferation

T cell priming capacity of monocytes and Mo-DCs was assessed in a mixed lymphocyte reaction. To this end, CellTrace™ Violet-labeled, allogeneic, naive CD45RA⁺CD45RO⁻ T cells (fig. 5A,B) were cocultured with monocytes or Mo-DCs. After 7 days cell numbers and the CellTrace Violet staining intensity of the T cells were analyzed (fig. 5C). In contrast to monocytes, both imMo-DCs and mMo-DCs induced T cell proliferation as

indicated by the cell counts and by the reduction of CellTrace Violet staining. T cell proliferation was highest when mMo-DCs were used as antigen-presenting cells (fig. 5D). This observation was in line with the aforementioned result (fig. 3) showing that receptors involved in T cell priming are expressed on mMo-DCs at the highest level.

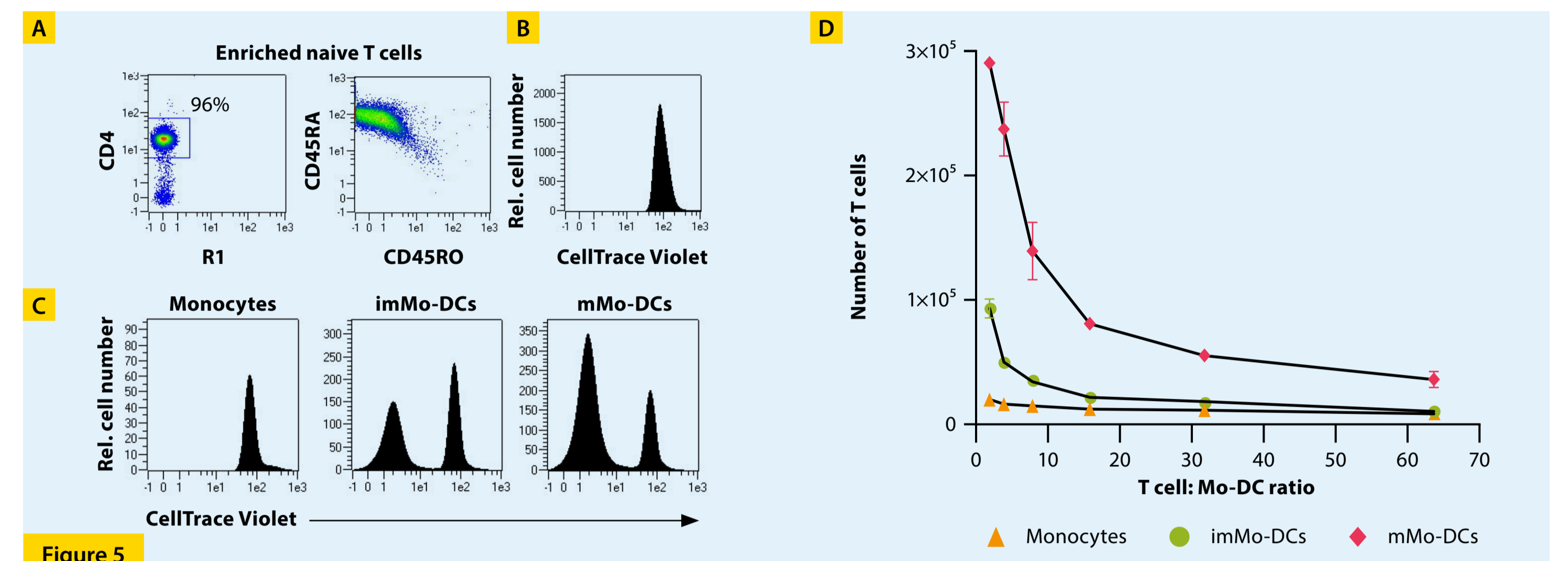


Figure 5

5 Electroporation of mMo-DCs with the CliniMACS® Electroporator

The CliniMACS® Electroporator is powered and controlled by the CliniMACS Prodigy. A closed tubing system ensures sterile processing during the fully automated cell electroporation procedure. A cyclic process enables electroporation of multiple sample aliquots (500–1000 μ L) in a row, from a cell suspension with a total volume of up to 50 mL. For each cycle, the cells and DNA/RNA are mixed just before electroporation. The CliniMACS Electroporator provides variable protocols enabling different pulsing parameters. For example, the first pulse can be powered with up to 1000 V, the second pulse with up to 500 V.

The generated mMo-DCs were electroporated with or without GFP-mRNA or left non-electroporated. Subsequently the cells were cultured in cell culture dishes for 17 h. Figure 6 shows microscopic images of the cells captured at 400x magnification. Non-electroporated cells (A), cells electroporated without addition of RNA (B), and cells electroporated with GFP-RNA (C) displayed the typical DC morphology with cytoplasmic protrusions.

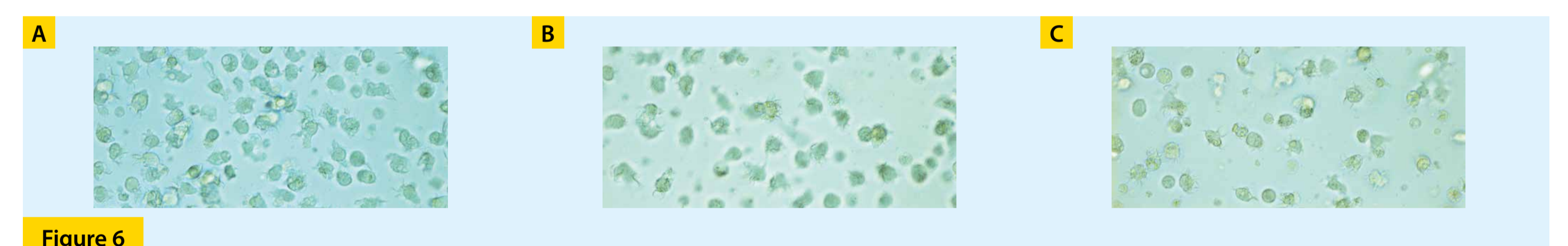


Figure 6

6 Transfection efficiency and viability of mMo-DCs electroporated with the CliniMACS® Electroporator

Electroporated cells and control cells cultured for 17 h were also analyzed by flow cytometry to check for transfection efficiency and viability (fig. 7). The forward/side scatter plots (left column) showed the typical appearance of Mo-DCs with all samples. As expected a slight increase in the percentage of debris could be seen in the electroporated samples (middle left and lower left). The intact cells gated in P1 showed good viability in all samples (middle column). The Mo-DCs that were electroporated with GFP-RNA (lower right), showed strong GFP expression and a high percentage of GFP-positive cells (85.3%).

Figure 8 shows the viability and percentage of GFP-expressing mMo-DCs after electroporation with GFP-mRNA followed by the 17-h culture period. Three different electroporation methods have been tested: i) electroporation in a single-use cuvette (Bio-Rad® Gene Pulser® Cuvette) with the decay-pulse electroporator Bio-Rad Gene Pulser II; ii) electroporation in the same type of single-use cuvette using the CliniMACS® Electroporator (square-wave pulse); iii) electroporation of 12 aliquots in a row, using the CliniMACS Prodigy Tubing Set and its integral cuvette with the CliniMACS Electroporator (pulse 1–12). After a culture period of 17 h the viability of the cells was greater than 90% in every sample except for the control sample treated with GFP-RNA and electroporated using the decay pulse. The percentage of GFP-expressing cells amounted to 57% after pulsing with GFP-RNA using the decay pulse and – as expected – no GFP signal was observed without the addition of RNA. After electroporation with the square wave pulse of the CliniMACS Electroporator and GFP-RNA about 80% of Mo-DCs expressed GFP, regardless of which cuvette type had been used. Twelve samples in a row were successfully electroporated with the new system.

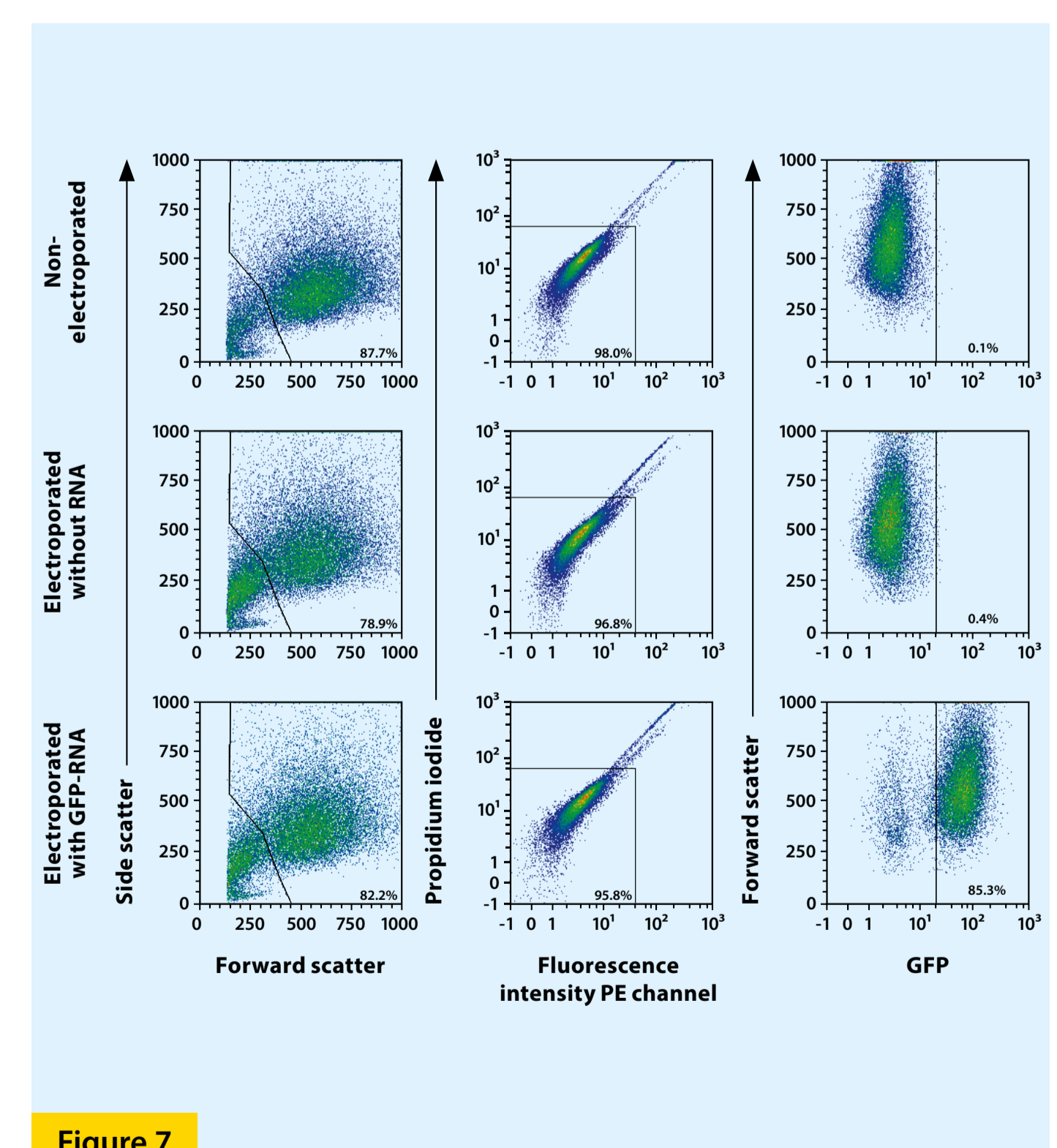


Figure 7

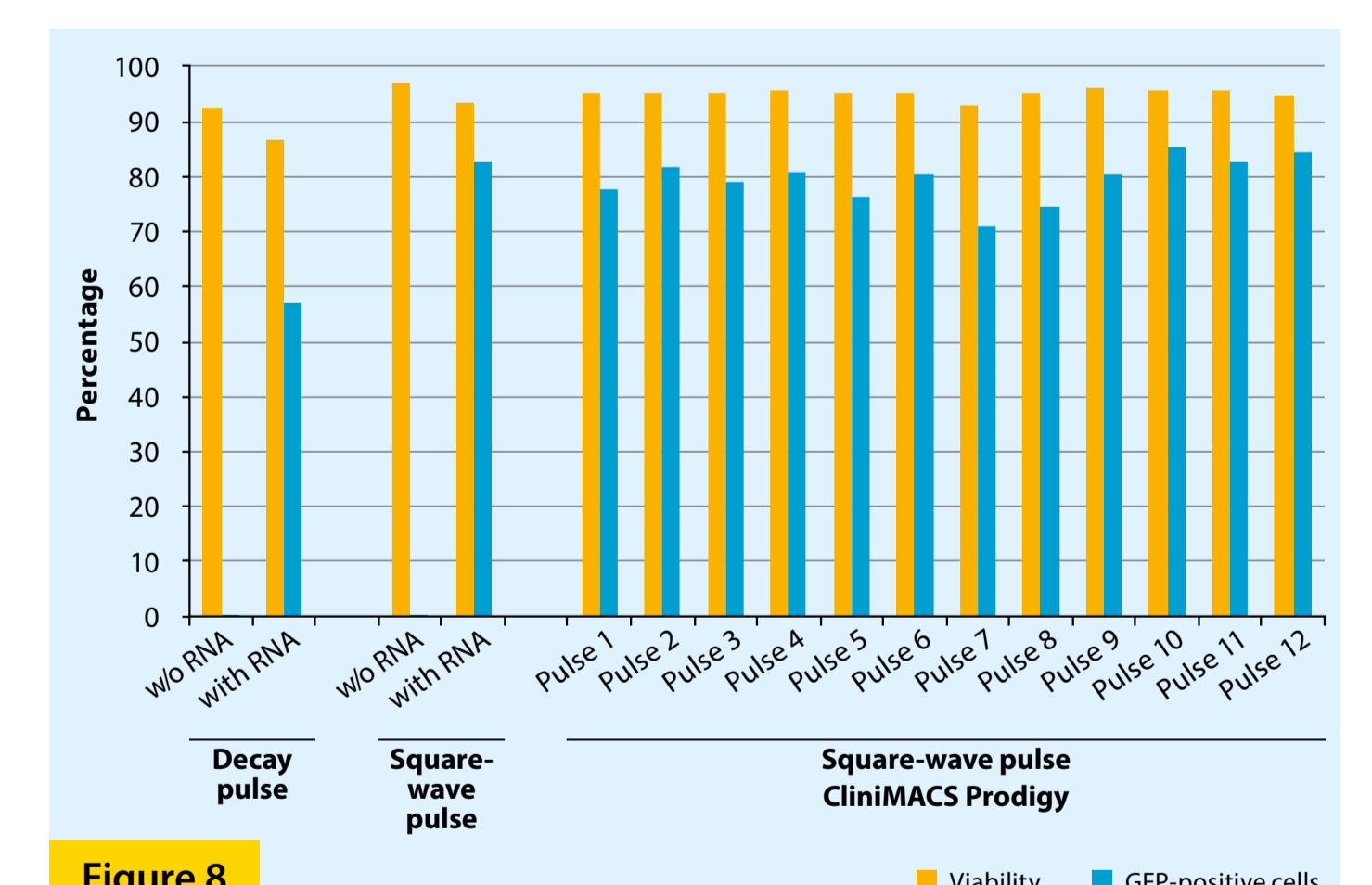


Figure 8

Conclusion

- The CliniMACS Prodigy enables fully automated isolation of monocytes with high purities and recoveries, and Mo-DC generation yielding recoveries of 20% on average.
- In vitro* generated imMo-DCs and mMo-DCs possess the anticipated characteristics of DCs in terms of i) morphology, ii) surface marker expression, iii) migration towards CCL19, and iv) induction of T cell proliferation.
- With the CliniMACS Electroporator mMo-DCs can be easily electroporated with mRNA in an automated fashion. The system enables efficient and reproducible transfection of Mo-DCs.

