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Standardized generation, purification, and quality control of human pluripotent stem cell–derived cardiomyocytes

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Introduction

Fast and highly efficient generation of pure pluripotent stem cell–derived cardiomyocytes (PSC-CMs) is a prerequisite for the development of clinical applications, drug development, and several research applications, e.g., heart disease modeling. In recent years, various protocols to differentiate PSCs into cardiovascular cells such as CMs have been published. However, cardiovascular differentiation of human PSC cultures does not result in a homogeneous cell population, but rather gives rise to a variety of CMs and non-CMs including different CM subtypes or subpopulations. The cell composition after each differentiation currently depends on the stem cell clone, passage, differentiation protocol used, and other experimental parameters. To circumvent these experimental variations and prepare for standardized processes suitable for automation and clinical scale-up, we have established a new workflow ranging from controlled cardiac differentiation to CM harvesting, purification, analysis, replating, and freezing (fig. 1A).

Results

1 Culture and cardiovascular differentiation of hPSCs

hPSCs were maintained under xeno-free conditions in StemMACS™ iPS-Brew XF medium (fig. 1B, left) and differentiated into CMs using an optimized differentiation protocol that reproducibly resulted in up to 70% cardiac differentiation efficiency within less than 10 days of differentiation (fig. 1B, right). Differentiated CMs were characterized using our newly generated antibodies against cardiac-specific cytoskeletal markers. Typical staining patterns for α-Actinin, cardiac Troponin T, myosin light chain 2a (MLC2a), and myosin heavy chain (MHC) were observed in IF experiments (fig. 1C). Similar results were obtained when using flow cytometry.

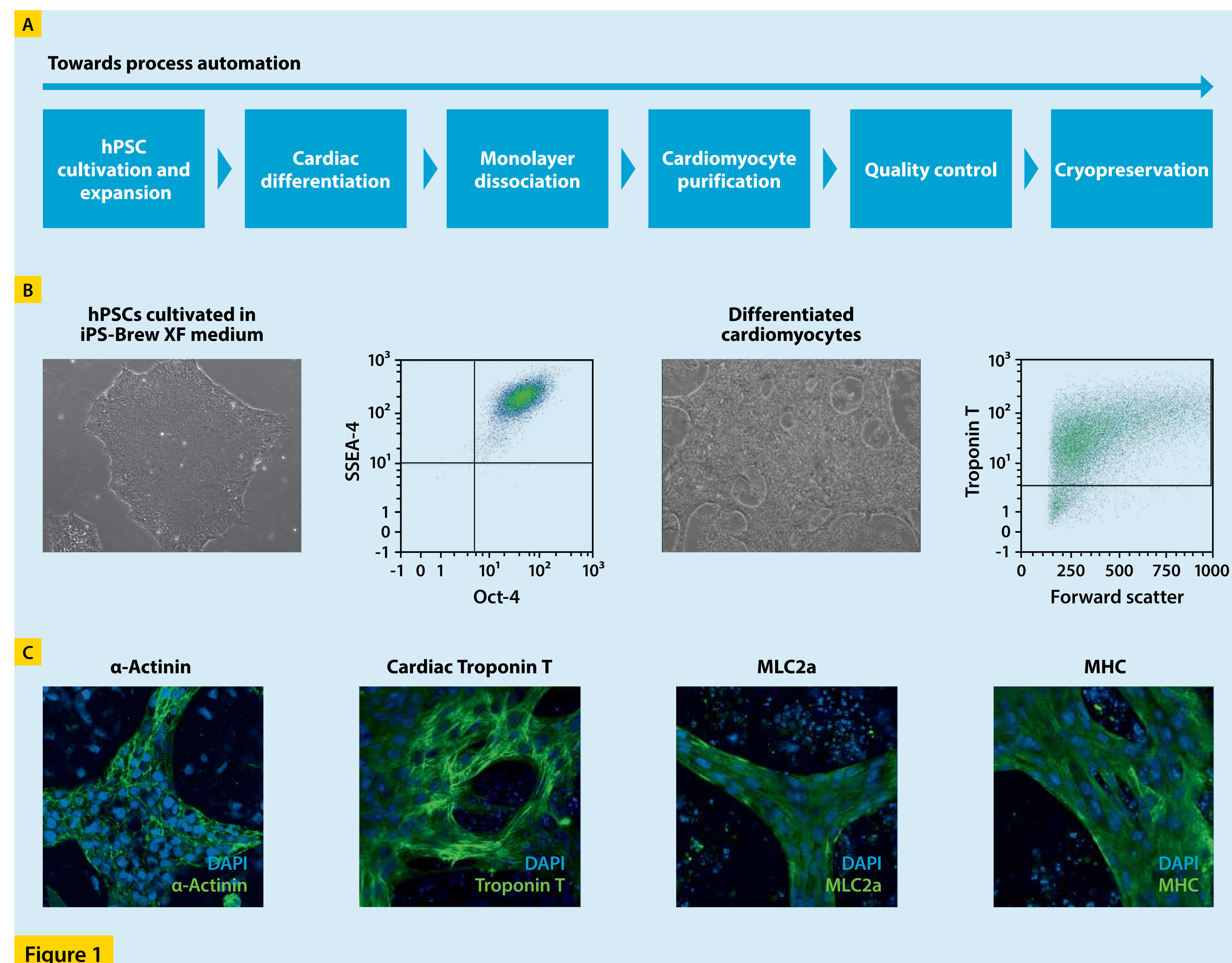

Figure 1

2 Purification of hPSC-derived CMs

As shown in figure 1 differentiated CM cultures did not contain a homogeneous cell population, but were rather composed of a mixture of CMs and non-CMs. The ratio of these populations can differ from clone to clone and experiment to experiment. To purify PSC-derived CMs a selection of specific cell surface markers labeling either CMs or non-CMs, previously identified by screening an antibody library of >400 cell surface markers, were used. Based on this marker combination we developed a highly standardized magnetic cell separation procedure that reproducibly delivered CM purities of >90%, regardless of the differentiation protocol, hPSC line used, time point and efficiency of differentiation. Depending on the hPSC differentiation efficiency different magnetic cell sorting strategies for the enrichment of hPSC-derived CMs were applicable. For cell populations with low differentiation efficiencies, e.g., at CM ratios of <50%, strategy A (depletion of non-myocytes) could be combined with strategy B (CM enrichment). For samples showing a higher differentiation efficiency, strategy A alone was sufficient. The cell separation process could be easily scaled up for the use of 5×10^7 cells per separation, thus making it suitable for large cell numbers and therefore interesting for automated processes.

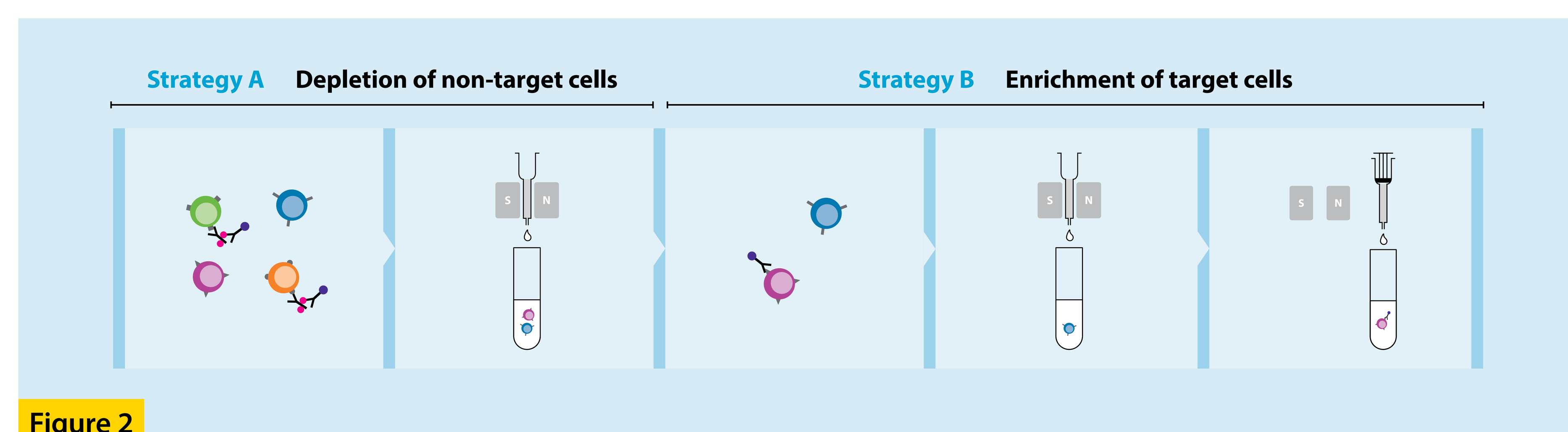

Figure 2

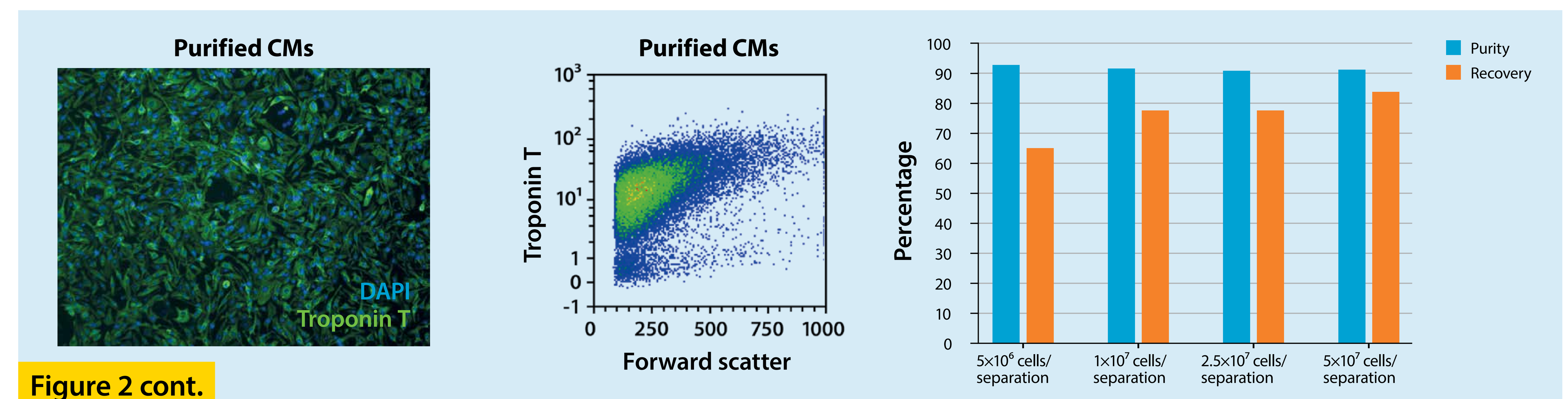

Figure 2 cont.

3 Purified CMs are functional and express CM-specific markers

Regardless of the strategy chosen enriched CMs attached well, initiated contractions, and could be stably maintained in culture. They showed a typical morphology and sarcomeric structure. Moreover, flow cytometry analysis demonstrated the expression of CM-specific markers, such as α-Actinin, MHC, MLC2v, MLC2a, and Troponin T, indicating that the newly developed cell separation strategy is suitable for enrichment of CMs and different CM subtypes. Electrophysiological analysis revealed that the purified CMs showed normal action potentials, while in the non-CM fraction action potentials were missing. These data prove that CMs isolated by depletion of non-CMs (Strategy A) are functional and possess the appropriate phenotype.

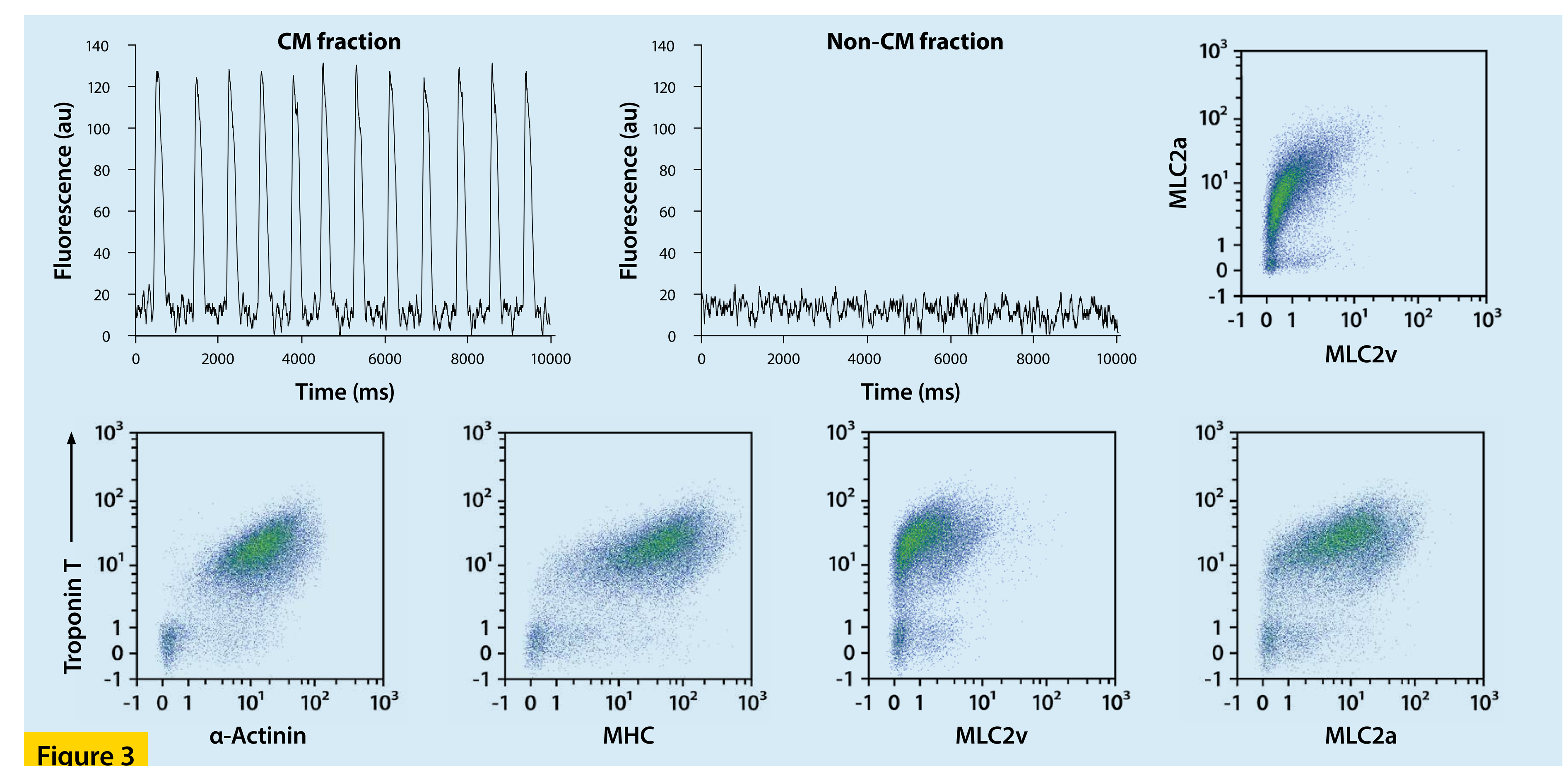

Figure 3

4 Microchip technology allows fluorescence-based sorting of highly pure and viable CMs

The surface marker combination chosen for magnetic separation by MACS® Technology could also be used for microchip-based flow sorting using the MACSQuant® Tyto™. This technique allows for high-speed fluorescence-based cell sorting resulting in gentle CM purification. The method yielded highly pure and viable CMs that attached well and initiated contractions after 24–48 h in culture. Moreover, purified CMs showed typical morphological features and the expression of cardiac markers.

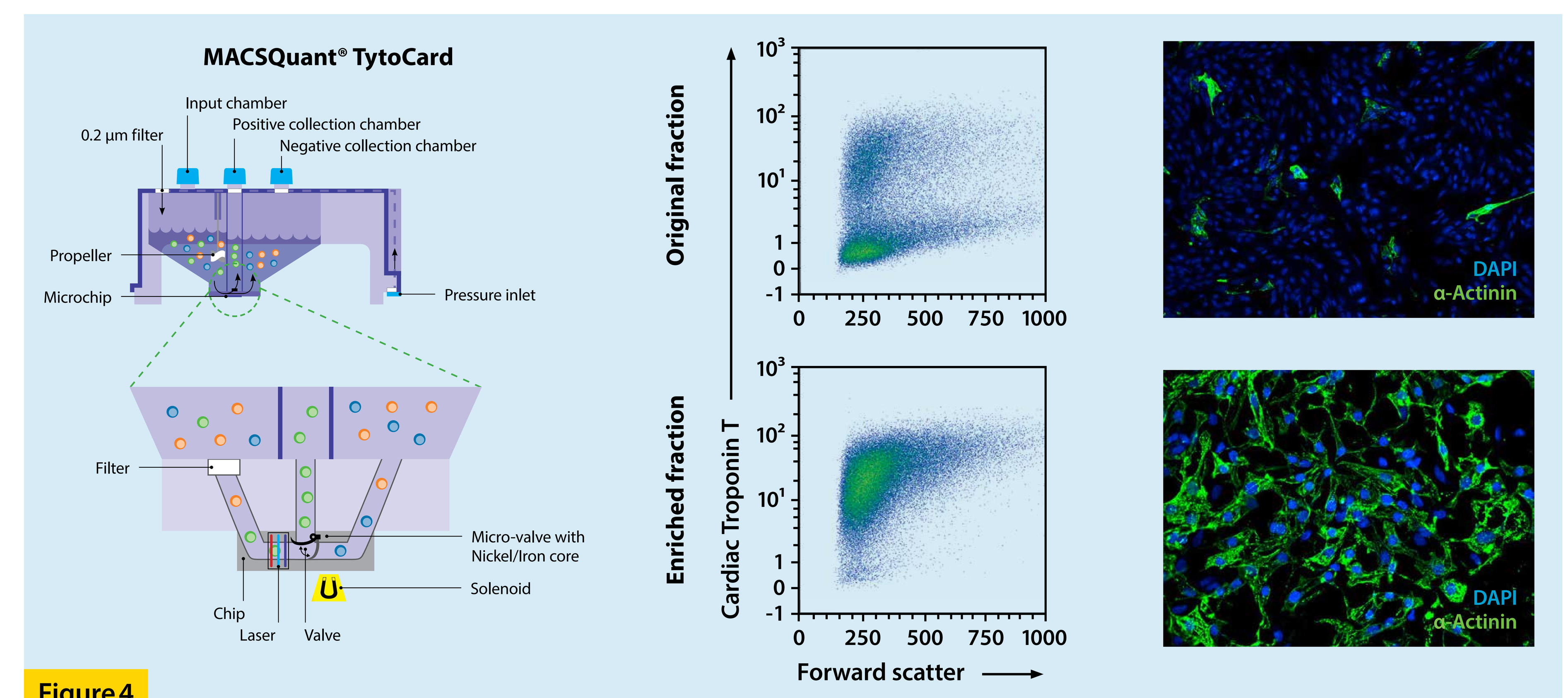

Figure 4

Conclusion

We developed tools that allow for the standardized, scalable magnetic and microchip-based purification, flow cytometry– or immunofluorescence-based quality control, and cryopreservation of hPSC-derived CMs. This workflow is a prerequisite for clinical scale-up and process automation.

- MACS Technology enables the scalable enrichment of hPSC-derived CMs to high purities regardless of the differentiation efficiency, stem cell clone, differentiation time point, or differentiation protocol used.
- Magnetically purified and flow-sorted CMs attach well, start rhythmic contractions 24 h after plating, and can be stably maintained in culture.
- CMs were quality controlled using our newly developed recombinant antibody conjugates labeling intracellular cardiac muscle proteins, such as α-Actinin, Troponin T, MHC, MLC2a, and MLC2v for precise detection of CMs and distinction between CM subtypes.

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