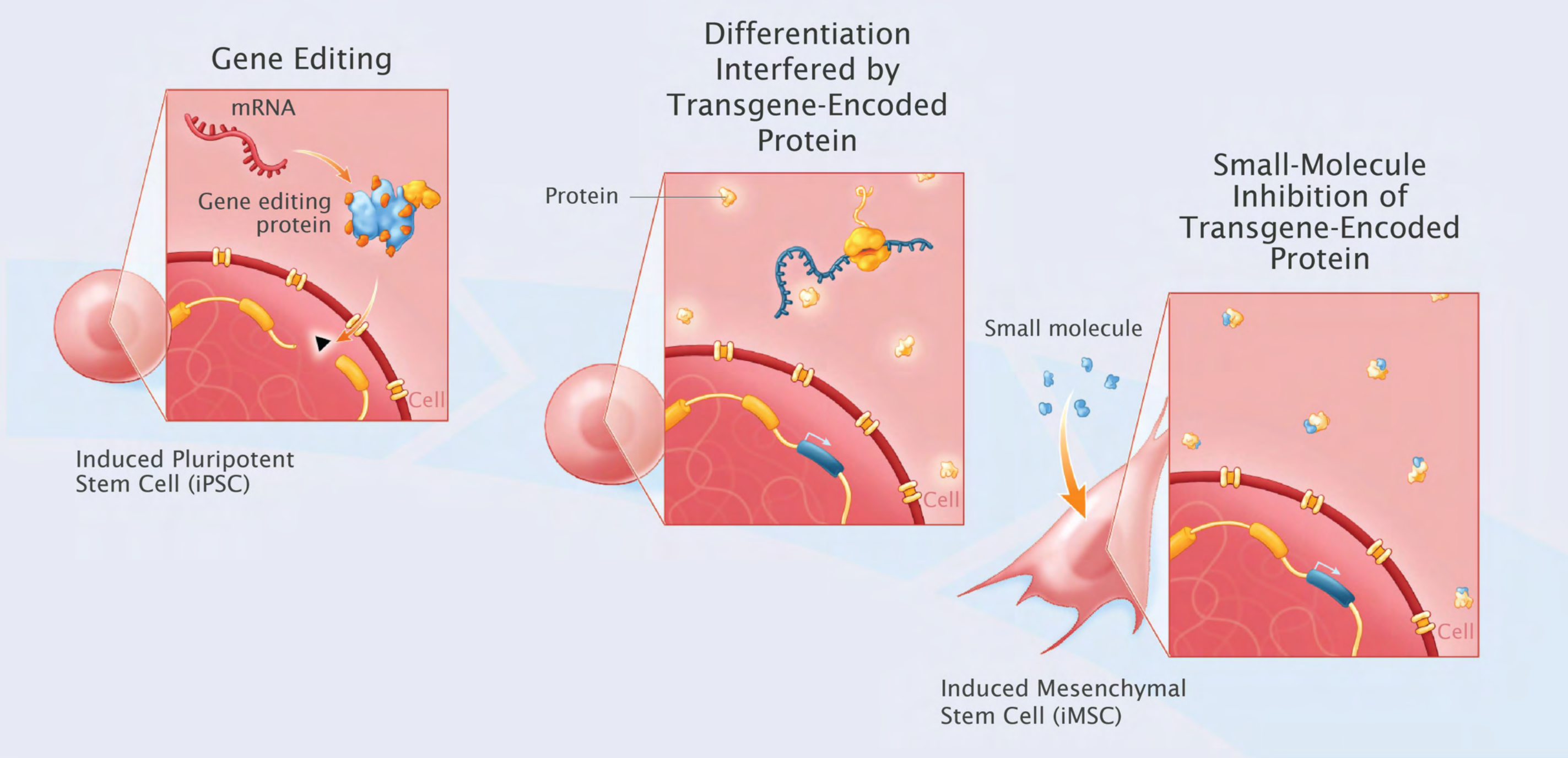


Introduction

Indoleamine 2,3-dioxygenase 1 (IDO1) is an inducible, heme-containing enzyme that is critically involved in tryptophan catabolism and known to be a prominent immune regulator. Cell therapies with increased IDO1 expression are of high interest for a variety of indications, including autoimmune disorders, inflammatory diseases, transplant recovery, and wound healing. In particular, iPSC-derived mesenchymal stem cells (iMSCs) engineered to overexpress IDO1 may be ideal for suppressing dysregulated immune cells while simultaneously promoting expansion of regulatory T lymphocytes and the M1 (pro-inflammatory) to M2 (anti-inflammatory) polarization of macrophages. Here, we report the development of an iMSC cell line containing an IDO1 transgene under the control of a JeT promoter that was inserted into the AAVS1 safe-harbor locus in mRNA-reprogrammed iPSCs. A clonal population of edited cells (i.e. IDO1-iPSCs) was isolated using single-cell sorting. The IDO1-iPSCs were then differentiated to IDO1-iMSCs. During differentiation, we found that the IDO1-Inserted cells showed an unexpected, cuboidal morphology and noticeably decreased proliferation rates relative to control iPSCs, possibly indicating that IDO1 was interfering with the differentiation process. Accordingly, the use of small-molecule IDO1 inhibitors were investigated to determine whether such intervention could be a valid strategy for improving the differentiation.



Conclusions

- Engineered iPS cell lines that contain certain transgenes can exhibit altered growth characteristics and slower proliferation rates during differentiation, which may hinder scale-up and manufacturing process design.
- We demonstrate that small-molecule inhibition of a transgene-encoded protein can enhance the growth rate of such gene-edited iPS cells during differentiation to iMSCs.
- Thus, the use of small-molecule inhibitors represents a promising new strategy to alleviate aberrant differentiation characteristics caused by the presence of a transgene in gene-edited iPSCs, which may prove useful for the generation of engineered iPSC-derived cell therapies.

1 Gene Editing and Cell Line Development



Figure 1. Flow chart illustrating the process of gene-edited iPSC cell line development. The iPSCs are first electroporated using mRNA encoding UltraSlice™, Factor Bioscience's proprietary gene-editing technology, to insert a single-stranded DNA (ssDNA) repair template for a gene of interest. Gene-edited iPSCs are then single-cell sorted using a microfluidic cell sorter. When single-cell derived colonies recover, biallelic insertion is confirmed by agarose gel electrophoresis and amplicon sequencing.

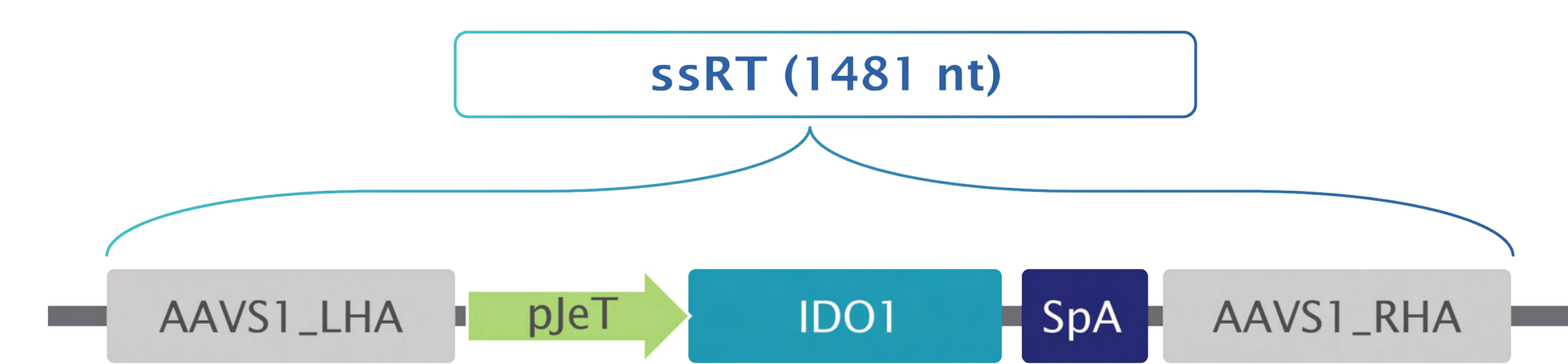


Figure 2. Schematic of the IDO1-encoding single-stranded repair template (1481 nt), which is under control of the JeT promoter, terminated with a synthetic polyadenylation site, and bordered by both the left and right homology arms that are specific for the AAVS1 safe harbor locus.

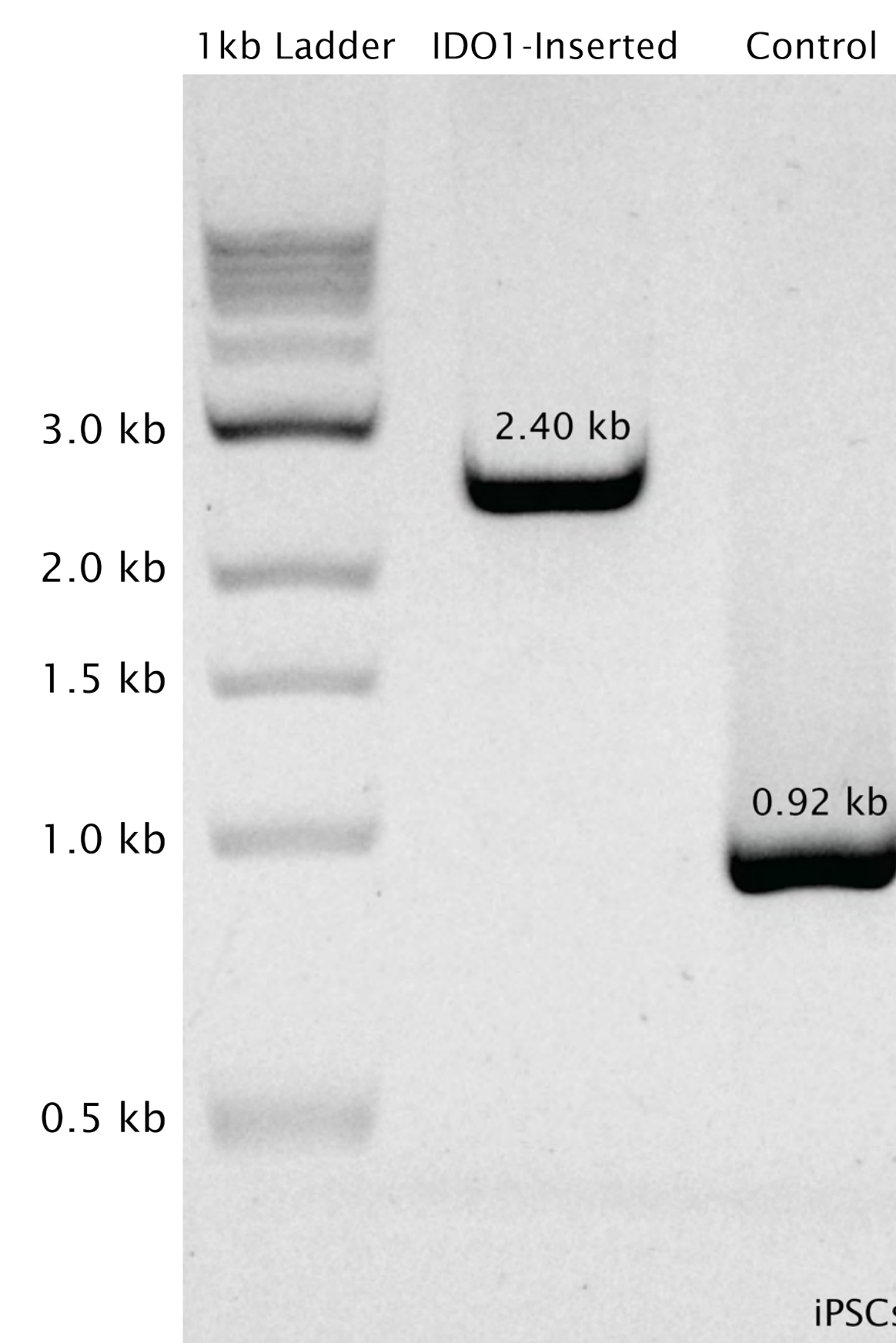


Figure 3. Agarose gel depicting bands of the AAVS1 amplicon for both Control iPSCs and IDO1-Inserted iPSCs, with a 1kb ladder.

2 iMSC Differentiation

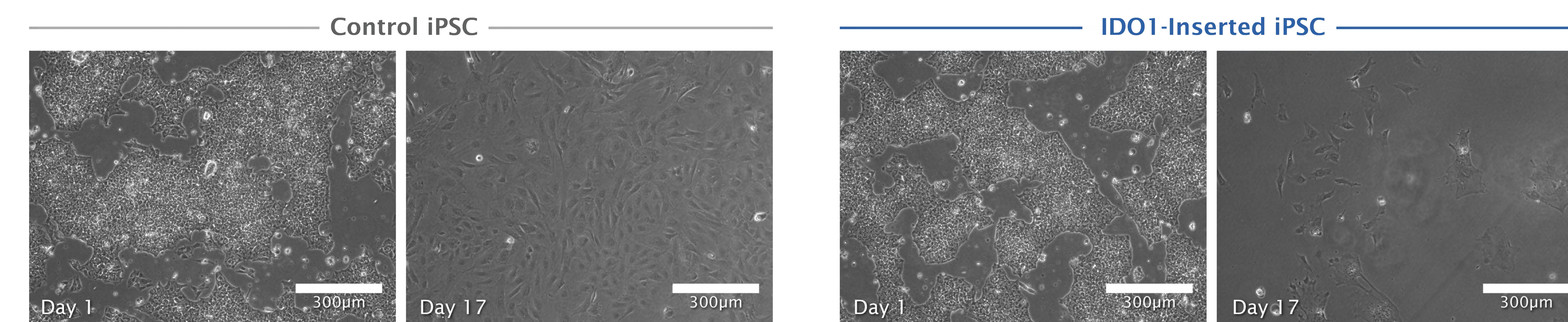
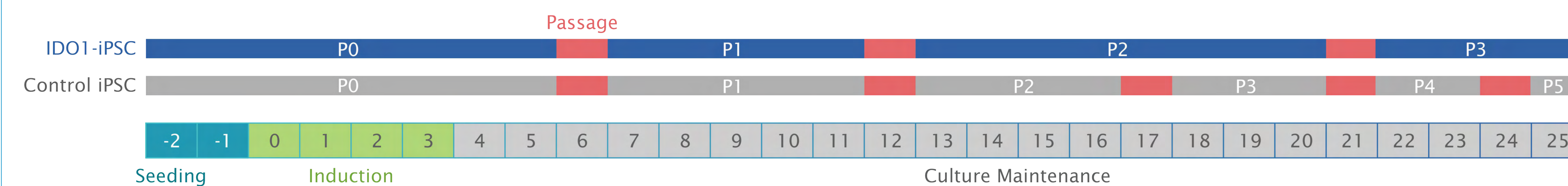


Figure 4. Control and IDO1-Inserted iPSCs were differentiated into iMSCs using a commercially available kit. The cells with the IDO1 transgene proliferated at a slower rate relative to the Control iPSCs and therefore took more time to reach necessary confluence level for passaging. Thus, the IDO1 transgene appeared to negatively affect the iPSC-to-iMSC differentiation.

3 Cell Surface Characterization of Differentiated iMSCs

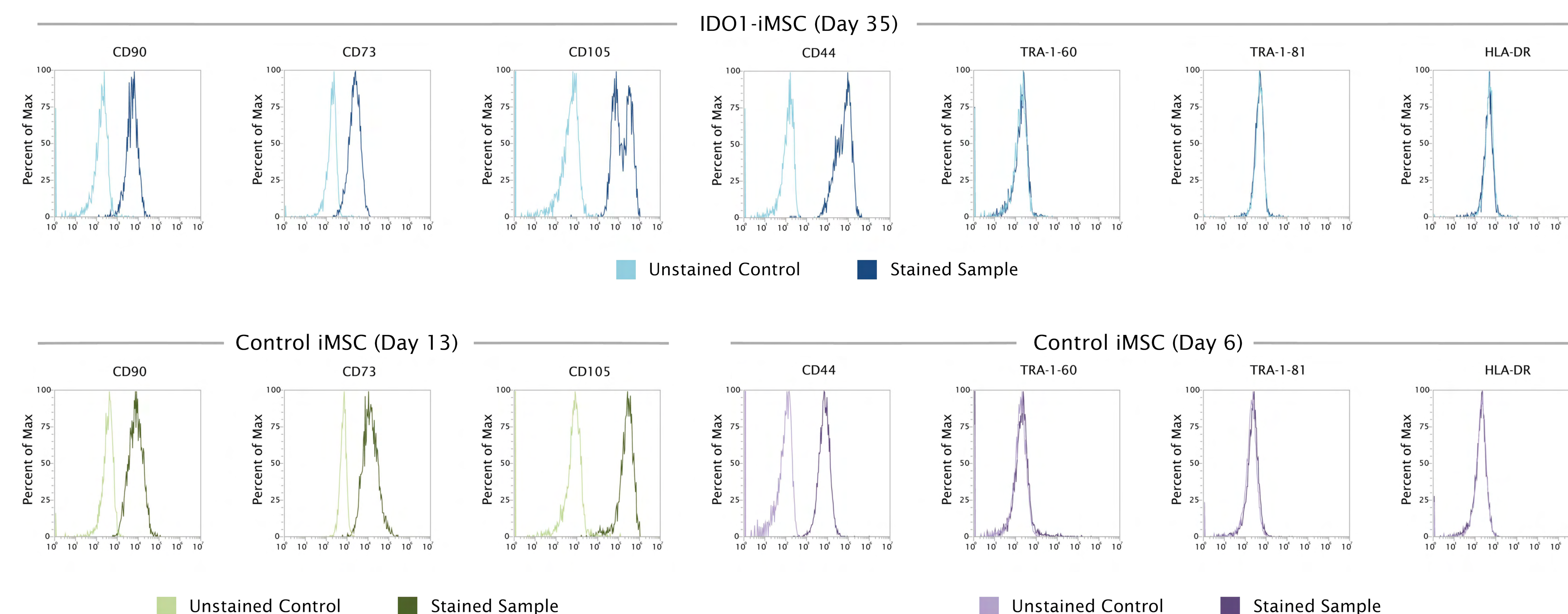


Figure 5. Flow cytometry analysis of IDO1-iMSCs (top row) and Control iMSCs (bottom row). By day 35 of differentiation, IDO1-iMSCs showed phenotypic characteristics consistent with Control iMSCs.

4 Effects of Small-Molecule IDO1 Inhibitors

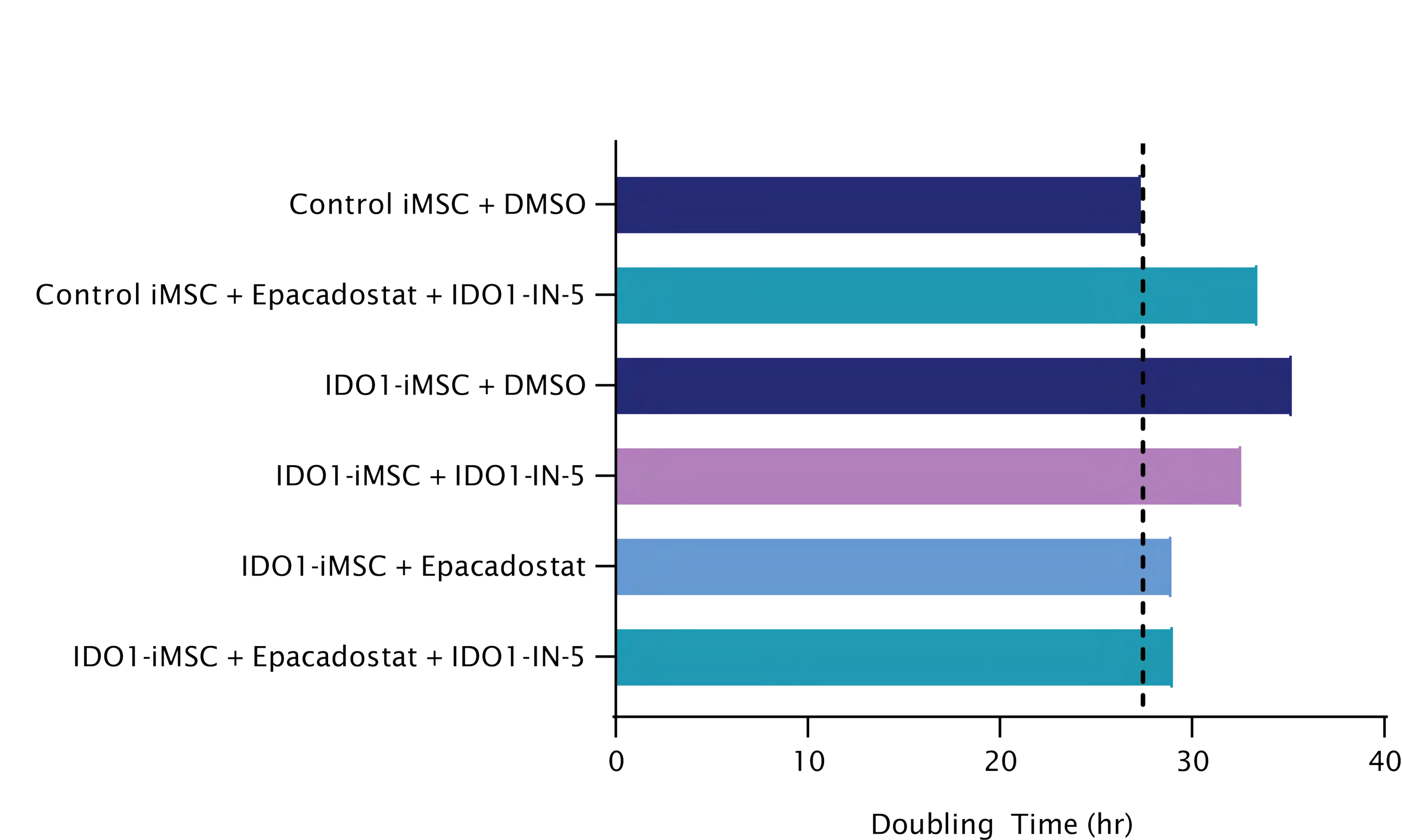


Figure 6. Doubling time of Control or IDO1-Inserted iMSCs after a 5 day incubation with small-molecule IDO1 inhibitors or controls. Dotted line indicates doubling time of Control iMSC + DMSO. Cells were seeded at day 35 post differentiation induction. All inhibitors were used at 10µM concentrations in 0.5% DMSO. IDO1-Inserted iMSCs showed an increased proliferation rate when cultured in an IDO1 inhibitory environment.

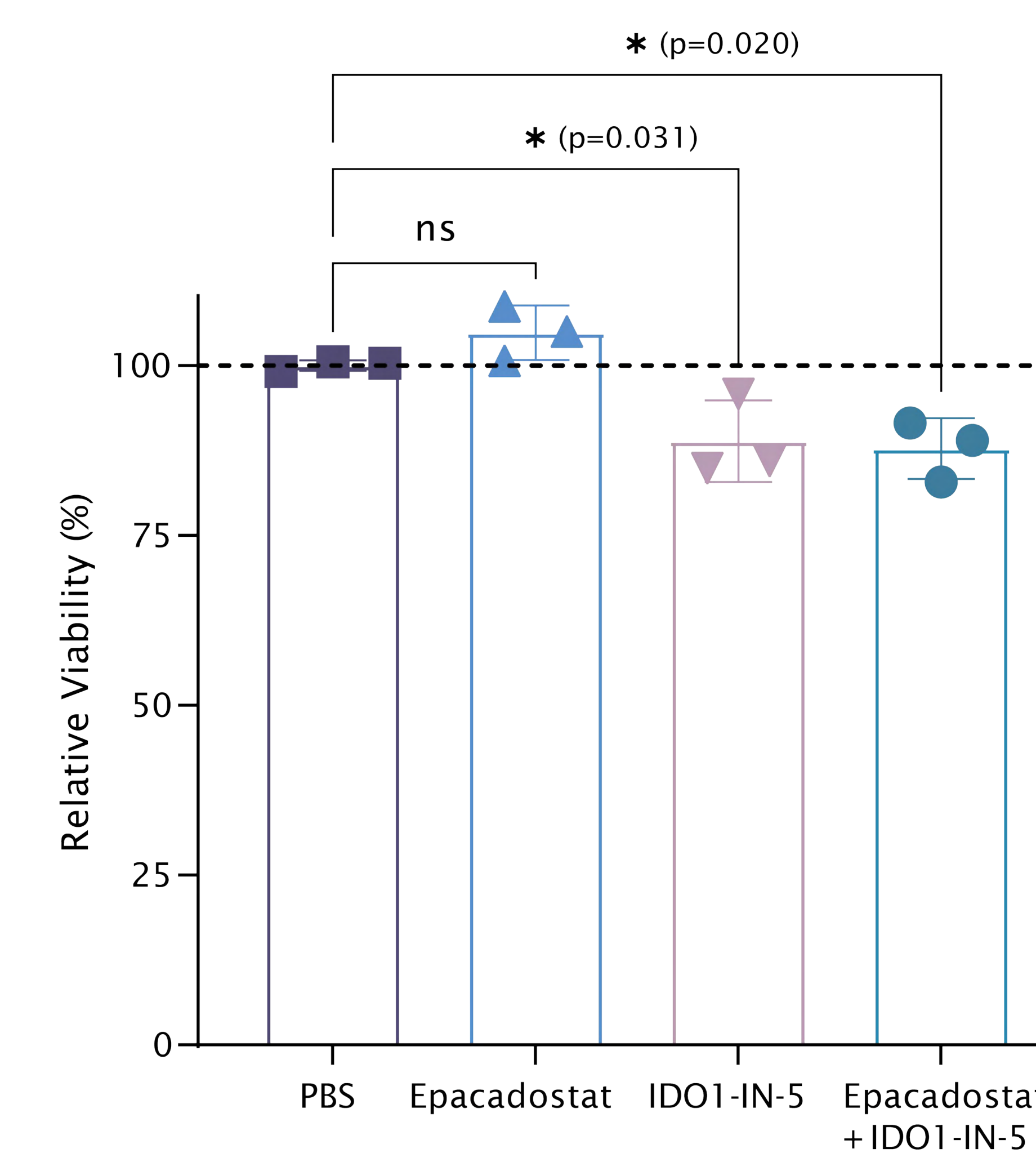


Figure 7. Cytotoxicity of small-molecule IDO1 inhibitors was assessed by flow cytometry after a 48 hour incubation with Control iMSCs. Viability is shown relative to PBS control. Epacadostat showed no significant difference relative to PBS, whereas IDO1-IN-5 and IDO1-IN-5 + Epacadostat showed mild cytotoxicity (p=0.031 and p=0.020, respectively).