Human iPS cell-derived beta cells for drug screening and diabetes research

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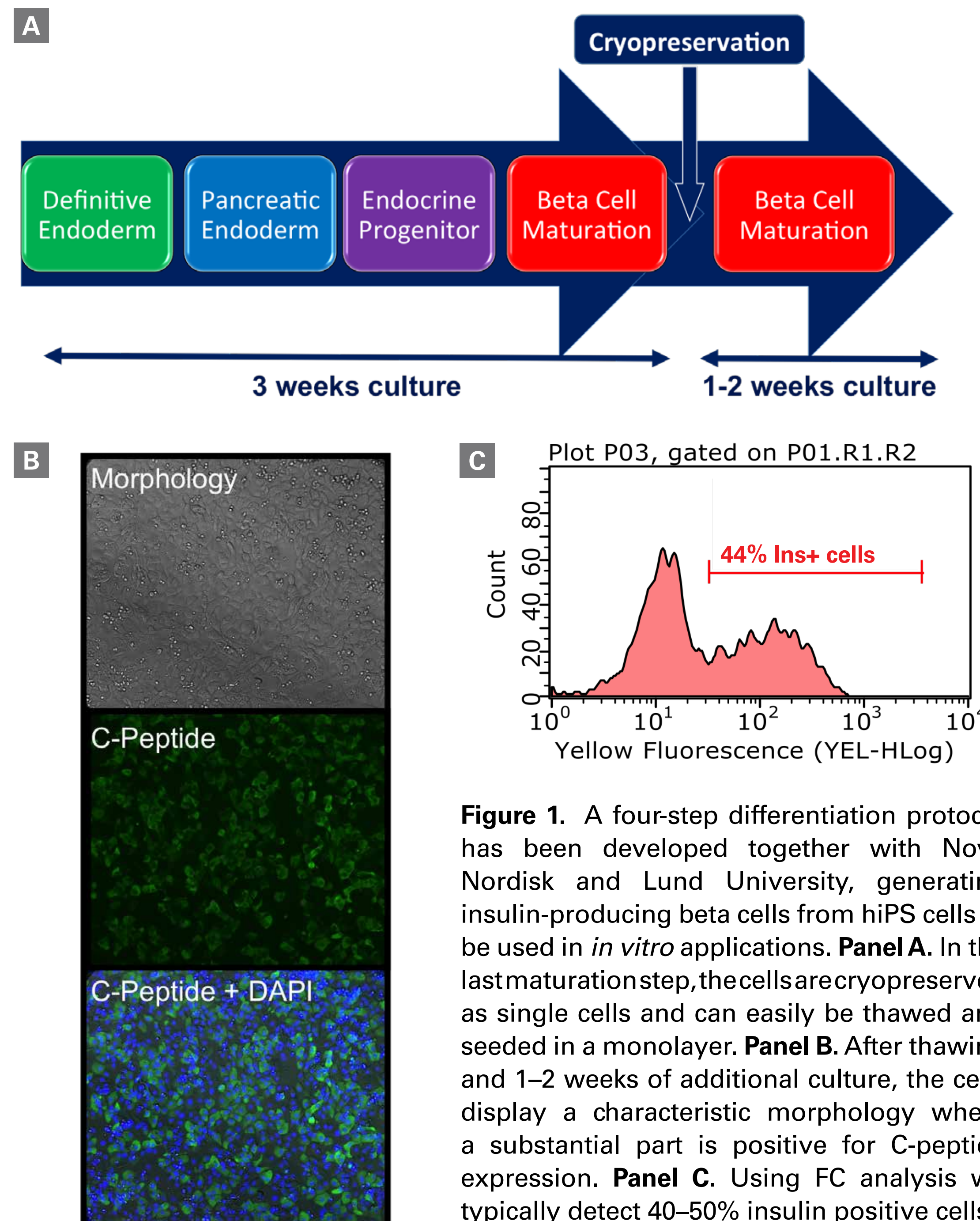
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Abstract

By 2030, diabetes is predicted to be the seventh leading cause of death globally. Human induced pluripotent stem (iPS) cell-derived beta cells have tremendous potential to advance the treatment of diabetes. Insulin-secreting beta cells could serve as a renewable, *in vitro* model system for toxicity testing, vaccine development, and drug discovery. Drug discovery requires large numbers of beta cells with consistent characteristics from batch to batch, and until recently, cell production methods have been unable to deliver. To address this need, we have developed a standardized four-step differentiation protocol, mimicking embryonic development, which can generate industrial-scale quantities of insulin-producing beta cells for *in vitro* applications. Now commercially available, Cellartis® hiPS Beta Cells allow vastly easier access to human beta cells and reduced variability compared to primary islet beta cells, and can be used as a predictive cellular assay for screening compounds that regulate insulin secretion.

Insulin production is a hallmark of beta cells, and is a desirable phenotype when determining functional differences between healthy and various disease states. The expression of PDX1, NKX6.1, and MAFA transcription factors are additional hallmarks of beta-cell development: they regulate the production of preproinsulin, the precursor to proinsulin. In addition to insulin, C-peptide, and MAFA, Cellartis® beta cells express NKX6.1, PDX1, and UCN3 mRNAs, and the cells retain functionality after cryopreservation, including insulin secretion in response to various stimuli. For example, glucose induces beta-cell secretion of insulin (and C-peptide). In addition, members of the incretin hormone family also regulate the secretion of insulin (and C-peptide), and the functionality of this pathway can be tested via incretin stimulation assay. In Cellartis® beta cells, C-peptide secretion increased by 75% in response to incretin stimulation. Taken together, these characteristics indicate the cells' suitability for drug discovery and for the study of beta-cell function, mechanisms of insulin processing and secretion, autoimmune beta-cell destruction, pancreatitis, and transdifferentiation.

Differentiation process

Figure 1. A four-step differentiation protocol has been developed together with Novo Nordisk and Lund University, generating insulin-producing beta cells from hiPS cells to be used in *in vitro* applications. **Panel A.** In the last maturation step, the cells are cryopreserved as single cells and can easily be thawed and seeded in a monolayer. **Panel B.** After thawing and 1–2 weeks of additional culture, the cells display a characteristic morphology where a substantial part is positive for C-peptide expression. **Panel C.** Using FC analysis we typically detect 40–50% insulin positive cells.

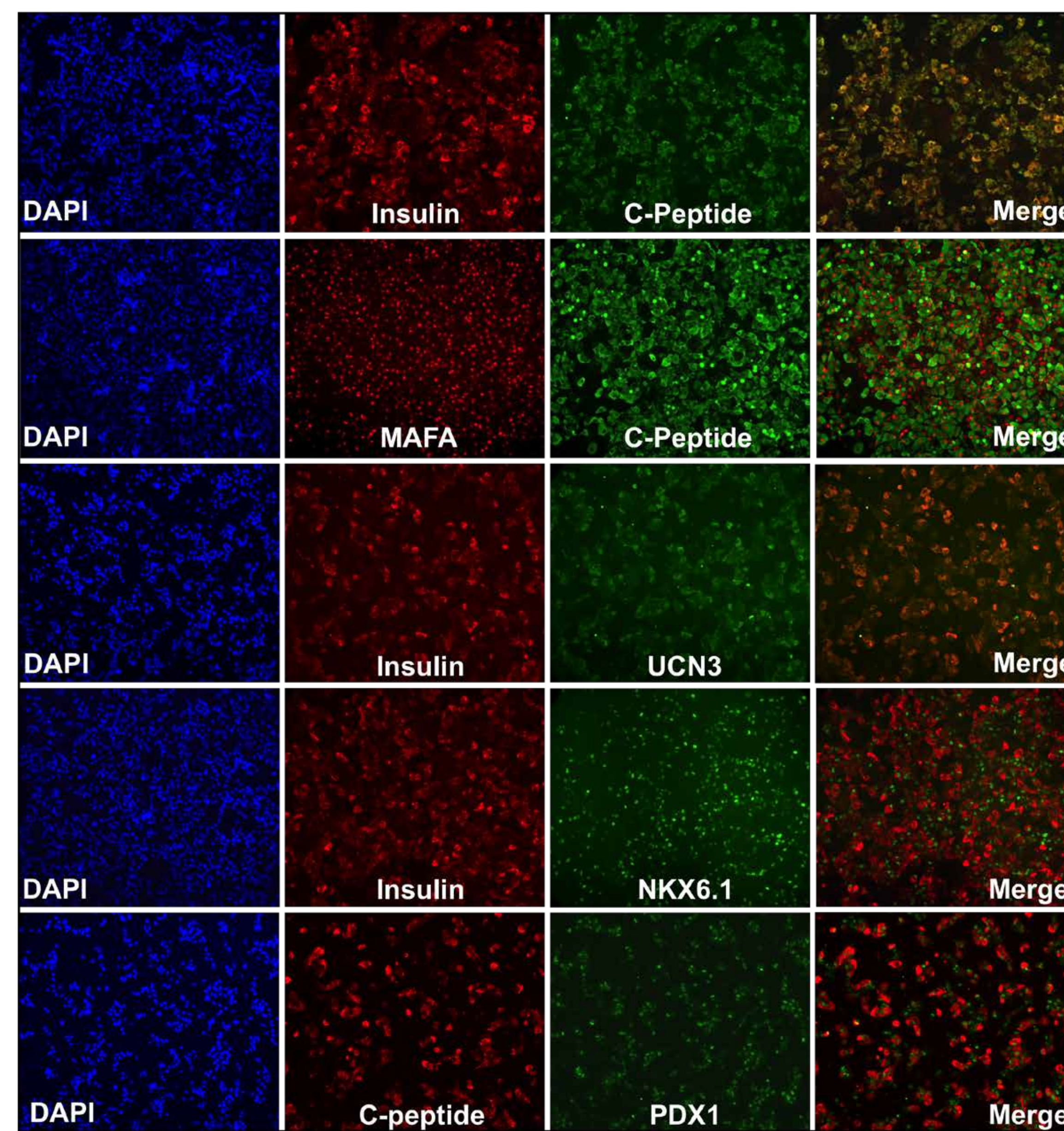## Characteristics of Cellartis® beta cells

Figure 2. Cellartis beta cells were fixed and stained for different maturation markers two weeks after plating. Cells were fixed and co-stained with insulin ab (Abcam, ab7842), C-peptide ab (Cederlane Laboratories, #CLX133AP), MAFA ab (Abcam, ab26405), UCN3 ab (Abcam ab77433), NKX6.1 ab (DSHB, F55A12), and PDX1 ab (Abcam, ab47383).
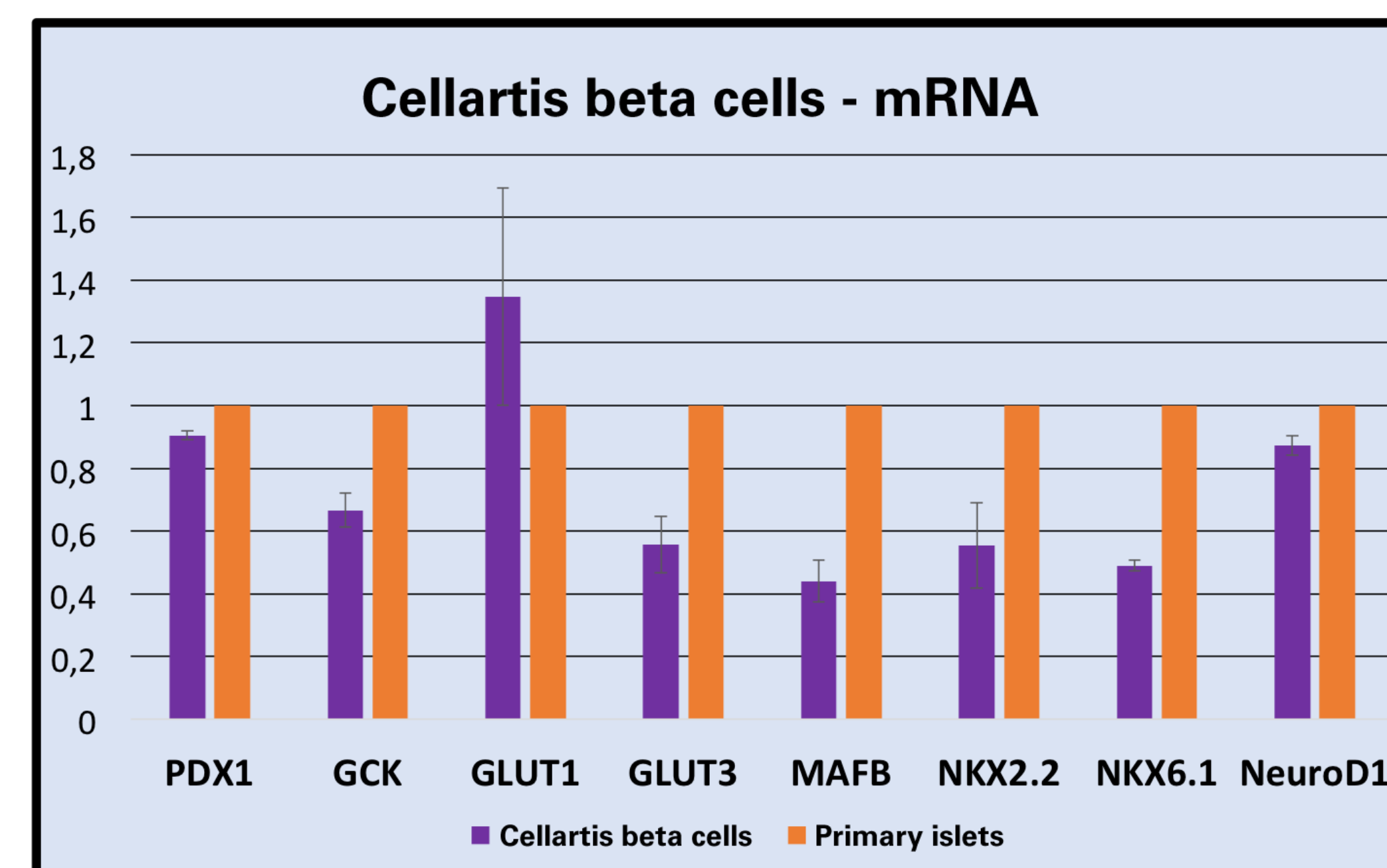

Figure 3. qPCR analysis of mRNA expression comparing Cellartis beta cells to human primary islets. Error bars indicate SEM, n=4.
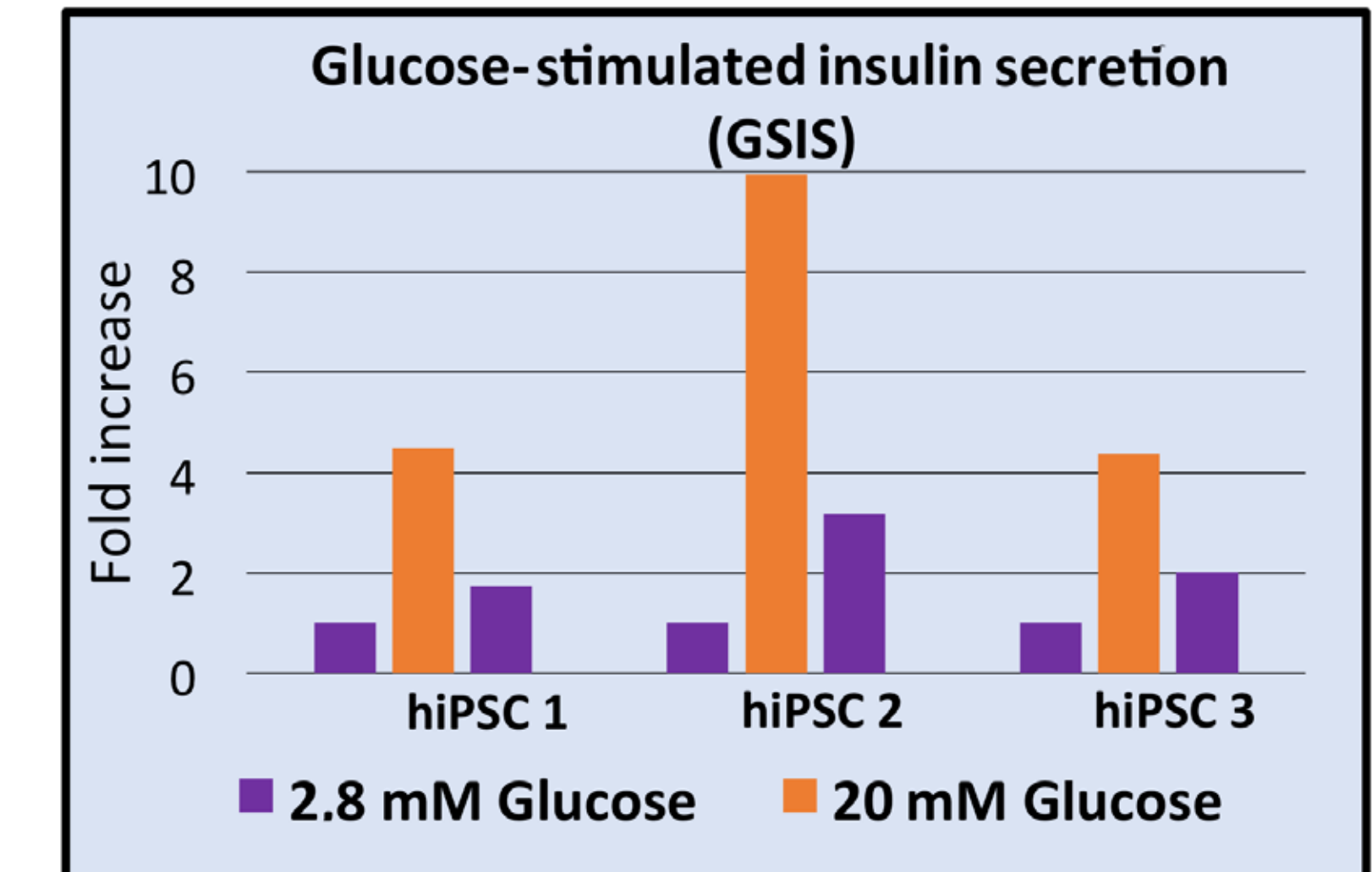

Figure 4. Beta cells differentiated from three different hiPSC lines were subjected to a three-step GSIS protocol, wherein cells were exposed to different concentrations of glucose. Each incubation step lasted for 45 min and the content of C-peptide was analyzed using the Mercodia C-peptide ELISA kit. C-peptide secretion was up-regulated 4.4–9.9 fold upon glucose stimulation, demonstrating beta-cell functionality.
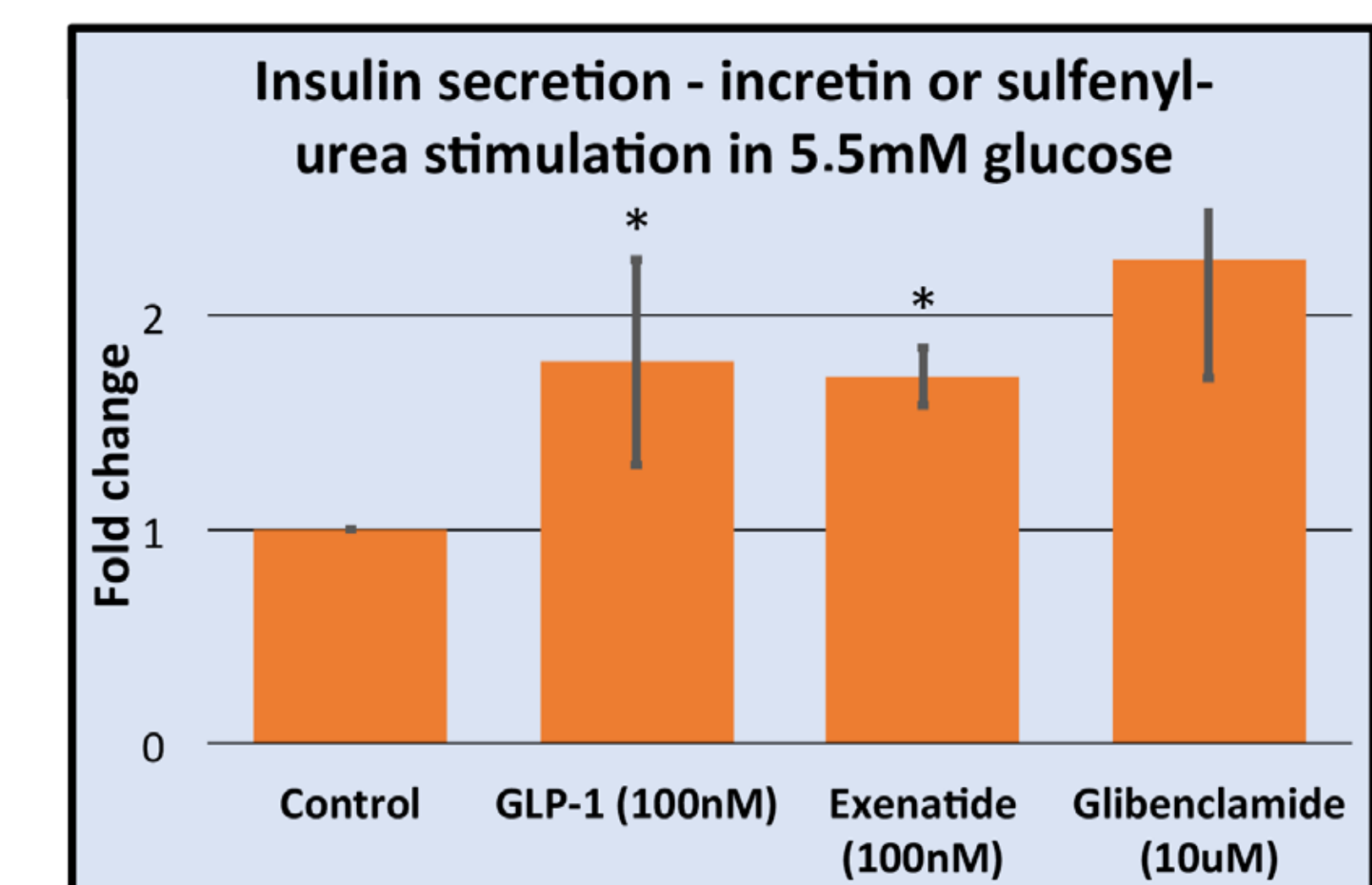

Figure 5. Cellartis beta cells exposed to incretin (GLP-1 and Exenatide) for 45 min in 5.5 mM glucose. The content of secreted C-peptide was analyzed using the Mercodia C-peptide ELISA kit. The results are mean ± SEM (n=5, GLP-1; n=4, Exenatide; n=2, Glibenclamide). *$p < 0.05$.

Conclusions

- Here we present a standardized method to generate a high amount of cryopreserved beta cells derived from hiPSCs to be used in *in vitro* applications

- Cellartis beta cells show high expression of insulin, C-peptide, MAFA, NKX6.1, PDX1 and UCN3. The cells also display GSIS functionality and response to incretin stimulation

- Cellartis beta cells provide better access to beta cells and reduced batch variability compared to human primary islets

We believe this product will be an excellent research tool in the field of diabetes and for other beta-cell-related studies, such as:

- Beta-cell function
- Screening compounds for regulating insulin secretion
- Insulin protein processing and secretion
- GSIS (glucose-stimulated insulin secretion) studies and incretin response
- Autoimmune beta-cell destruction
- Pancreatitis and beta-cell function
- Transdifferentiation