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Dear Members of the ICOC Application Review Subcommittee:

First, we would like to thank the members of the Grants Working Group (GWG) very much for their favorable review and for their unanimous recommendation to fund our DISC2 (Quest) Application **DISC2-09635**.

Second, we wanted to provide some additional information in response to the reviewers' comments and questions.

1. There was a comment that Aim 1.1 would produce results that are redundant with published literature.

We agree that there have been several studies that have begun investigating human pancreatic islet architecture, as well as differences between human and mouse islets, and we have cited some of those papers in our proposal accordingly. As we noted, however, analyses of human islets have led to conflicting reports, and thus we believe that there is still a lack of consensus in the field. In addition, while the recent existing studies focused most on the endocrine cells that comprise the human islet, there was relatively much less attention given to the cells that comprise the niche, or microenvironment, such as smooth muscle cells, mesenchymal cells, pericytes, neuronal cells, and endothelial cells. Given the strength of our preliminary data demonstrating the importance of these niche cells in inducing and maintaining pancreatic beta cell function, we believe that a more definitive characterization of human islet architecture – one that includes a strong focus on the niche cells – is warranted, as it will provide novel information that is likely to be relevant to beta cell function.

2. Reviewers suggested that "care needs to be taken not to get lost in the complexities of adding various niche components to partially differentiated beta cells."

We appreciate this comment and agree that it will be important to move forward swiftly and efficiently on this front, without becoming mired in an overly complex cellular system. Given the strength of our existing data on human embryonic stem cell (hESC)-derived islet-like spheres incorporating endothelium, mesenchyme, and pericytes, we believe that we are already in a strong position to begin with this experimental condition as our launching off point.

3. There was the suggestion to include more discussion about the need for immunoisolation devices or for immunosuppression to prevent autoimmunity.

We certainly appreciate the importance of this point – particularly in the case of cell therapy approaches aimed at curing automimmune diseases such as type I diabetes. In fact, as another reviewer noted, we did include this as an important future direction, noting that "In future work, engineered islets may be encapsulated to protect them from autoimmune attack." We regret that a more detailed discussion was not included in this proposal due to space constraints; instead, in this particular proposal we focused our attention first on the cellular side of the challenge – i.e., on producing the best possible material for glucose homeostatic control. Our rationale is that while immunoisolation and/or immunosuppression will be important, solving this problem is outside of the scope of our proposal and instead represents an important future direction that we will pursue.

4. While our microwell aggregation was described as a "key innovation," there was some question regarding the potential scalability of this approach for translation to human therapy.

We thank the reviewers for this comment and agree that scalability is, indeed, going to be critical for translation. As our approach has already demonstrated great success on the scale of tens to hundreds of millions of cells' worth of aggregates, we have already begun to collaborate with a bioengineering lab here at UCSF with the capability to customize the production of the materials necessary for substantial scale-up. In short, we appreciate the importance of scalability and will continue pushing forward with this approach. Once we have identified the key parameters (cell-wise and engineering-wise) in our system using small animals, we do hope to address this question of scale-up in a future proposal.

5. A reviewer commented that sorting other islet niche cells for aggregation with IBCs from cadaveric pancreatic islets is not feasible for producing a product for transplantation, due to the poor availability of cadaveric pancreata.

We apologize if we were not clear in our proposal about our intent regarding such experiments. While we completely agree that sorting niche cells from cadaveric pancreata is **not** a feasible approach for producing hESC-derived islet-like spheres at scale, we **do** propose that it could be a useful approach for understanding the underlying biology. That is, in the case that additional primary niche cell lines (such as smooth muscle cells) do not incorporate well in our aggregations, we could try to understand why by testing whether freshly-isolated human islet niche cells perform better. If so, then we would aim to compare the primary cell line to the freshly-isolated cells (for instance, on a transcriptional level) and use that information to provide insight into sourcing cells that best mimic the freshly-sorted cells and that do incorporate successfully.

6. There were some concerns regarding the "delay" in diabetes reversal, that signals in the mouse that drive IBC full differentiation may be different in humans, and that cells may be "changing" during the course of implantation.

We believe that this is an important point for any cellular therapy. In fact, this is a major motivator of our proposal. Indeed, while existing protocols for producing hESC-derived beta-like cells suffer from lack of phenotypic stability (and rapidly lose function *in vitro*), with our hESC-derived islet-like spheres we demonstrate phenotypic stability even after several weeks *in vitro*. Thus, we believe that recapitulation of the islet niche allows for implantation of a cellular product that shows superior stability to existing products. Please also note that the delay in human insulin production shown in Figure 6 was with an older method of producing beta-like cells. We have since repeated this experiment with our newest protocol for beta cell differentiation, and as a consequence are able to detect human insulin considerably earlier.

7. Reviewers suggested the use of mammary fat pad in female mice or the epididymal fat pad in male mice, rather than the omentum, given the less vascularized nature of the latter.

We appreciate this suggestion and thank the reviewer for sharing his or her expertise. We will modify our experimental plan accordingly.

In summary, we would like to thank the reviewers once again for their thoughtful consideration of our proposal and for their unanimous recommendation for funding. We believe that the optimal therapeutic benefit for type I diabetes will only be achieved once whole functional islet-like clusters are transplanted into patients, rather than beta cells alone. Further, we believe that our proposed studies would address key bottlenecks in the field and as such would represent an important contribution to the types of stem cell replacement therapies that are so critical to CIRM's core mission.

Sincerely,

Julie B. Sneddon, Ph.D.