Written comments by the applicant to the ICOC

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Thank you very much for the opportunity to comment to our proposal. We appreciate the reviewers' comments and great suggestions, and we would like to briefly address them to emphasize the importance and clinical relevance of our proposal:

The reviewers identified as main weakness of our approach "a lack of consideration of NK cells and their potential role in rejection," and we were "encouraged to also provide another fetal-maternal interaction to the proposed work." We want to note that we studied the efficacy of 12 different fetomaternal molecules that are important for the fetal-maternal interaction in pregnancy in the last 8 years in our lab. These studies revealed that the proposed combination of beta2-microglobulin-knockout, C2TA knockout, and CD47 overexpression resulted in true hypo-immunogeneic induced pluripotent stem cells (iPSCs), which survive long term after allogeneic transplantation in mice. Since knocking out beta2-microglobulin and C2TA results in HLA-I and II elimination, our cells are most likely susceptible for NK cell killing, according to the "missing-self" hypothesis that cells expressing HLA-I molecules are protected from NK cells, but those that lack this self-marker are eliminated by NK cells. Therefore, our approach includes the overexpression of the special identifier protein (integrin-associated protein) CD47 which is recognized by SIRP-α and SIRP-β2 on NK cells. CD47 is known as the "don't eat me" molecule that suppresses phagocytic innate immune surveillance and elimination. HLA-devoid cells are otherwise susceptible to innate killing. In a proof-of-concept study, we demonstrated, that our hypo-immunogenic iPSCs are not rejected by NK cells.

Another concern was raised regarding our proposed PATCHs; we have developed the technology to generate these patches, and have recently published the method (Science Translational Medicine 2016: 8 (363), 363ra148). We also demonstrated cell survival of transplanted PATCHs for more than 100 days in a syngeneic rat model (Stem Cells Transl Med. 2015; 4(6):625-31). The follow-up period in our proposal is 28 days, and therefore, we are therefore expecting survival of the hypo-PATCHs at this time point.

To address the final critique, using the heterotopic heart transplantation model is an elegant way to ensure robust and standardized transplantation of the PATCHs on infarcted mouse hearts. PATCH transplantations onto beating mouse hearts might be feasible, but this approach is not reproducible due to the high beating frequency of mouse hearts. Another advantage of our model is the possibility to create large infarcts without jeopardizing the hemodynamically stability of the humanized mice. Again, this technique is established in and published by our lab (Transpl

Immunol. 2010;23(1-2):65-70), and functional analyses of heterotopic transplanted hearts have been described before (Cell Transplant. 2009;18(3):275-82; J Thorac Cardiovasc Surg. 2004 Oct;128(4):571-8).

We want to emphasize that hypoimmunogenic iPSCs would have significant impact by enabling allogeneic therapies, which are more scalable and cost effective than autologous therapies, and that generating off-the-shelf, hypo-immunogenic cardiomyocyte would have a significant impact on wide range of cardiovascular diseases. Our proposal meets the mission of CIRM to accelerate stem cell treatments to patients with unmet medical needs; our approach holds the potential for treatment of not only for heart regeneration, but for various other diseases, such as diabetes, neurological, lung, or vascular diseases. Our preliminary data indicate that this approach is feasible, and in keeping with California's leadership in clinical research developments, we ask for CIRM support to pave the way for an approach that could overcome the challenges of immune rejection and create safe and effective transplantation therapies on a scale not previously achieved.