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Re: DISC0 18038 "Base Editing, Single-Cell Multiomics, and Cardiac Organoids to Decode Genetic Variants"

Dear Members of the CIRM Board,

I am writing regarding our DISC0 application (18038), "Base Editing, Single-Cell Multiomics, and Cardiac Organoids to Decode Genetic Variants", which received a **score of 85** from the GWG review. We are grateful for the reviewers' recognition of the proposal's strengths and have carefully considered their constructive critiques. We systematically address each comment with targeted refinements to the study design and contingency planning. We respectfully submit this response to assist the Board in its deliberations and funding decision.

Study Goal and Research Design: This DISC0 project aims to develop a scalable human platform to functionally interpret hypertrophic cardiomyopathy (**HCM**)-associated missense variants. The *central innovation* is the integration of dual-reporter iPSC-derived cardiac organoids, CRISPR base editing, and single-cell multiomics in genetically diverse donor backgrounds. The workflow includes: **(1)** generation of dual-reporter iPSCs; **(2)** optimization of 3D cardiac organoid culture for base editing screens; **(3)** delivery of a lentiviral sgRNA library targeting HCM variants; **(4)** sorting and sequencing of edited organoids by reporter activity to quantify variant function; **(5)** validation of top variants in individual organoids; and **(6)** application of single-cell multiomics to delineate gene networks across diverse genetic contexts. This project will establish a *clinical trial in-a-dish* platform for precision interpretation of HCM variants with broad relevance to inherited cardiovascular disorders.

Response to Major Critiques: The GWG reviewers endorsed our proposal's *"high qualified team," "highly innovative approach,"* and *"outstanding preliminary data".* While acknowledging these strengths, they identified opportunities for further enhancement in key areas: **(1)** base editing efficiency, **(2)** correlation with clinical phenotypes, **(3)** accounting for paracrine effects, **(4)** feasibility and scope of the organoid village approach, and **(5)** overall risk and contingency planning. We address each below with targeted refinements.

(1) Base editing efficiency and identification of true hits. Reviewers noted that locus-specific variability in base editing efficiency could complicate pooled screen interpretation. **In response**, we will leverage co-PI Le Cong's recently developed CRISPR-GPT agentic automation framework (***Nat Biomed Eng*** 2025), which integrates large language models with experimental feedback loops to automate guide RNA design, predict locus-specific editing efficiencies, recommend delivery strategies, and adapt protocols in real time. This system has been shown to outperform conventional approaches in selecting sgRNAs with higher on-target activity and lower off-target risk, thereby providing a powerful solution to variability across genomic loci. Complementing this,

all variants identified in pooled base editing screens will undergo orthogonal validation in individual cardiac organoids, ensuring that only reproducible and biologically meaningful hits are advanced

(2) Correlation with clinical phenotypes. Reviewers raised concerns that the study design does not directly link variant function to patient disease severity or progression. **In response**, we will integrate genotype-phenotype metadata from institutional biobank resources to prioritize variants with linked clinical data whenever available. Importantly, we will leverage PI Joseph Wu's recent development of human gastruloid systems, which have been shown to recapitulate the early cardiac and hepatic vascularization (***Science*** 2025). Incorporating these multicellular developmental contexts will enhance our ability to capture clinically relevant variant effects.

(3) Accounting for paracrine effects. Reviewers suggested that paracrine signaling should be better addressed in organoid readouts. **In response**, we will incorporate secretome profiling and computational network analysis to systematically characterize paracrine mediators and their contribution to variant-associated phenotypes. Furthermore, we will adapt methodologies from co-PI Sarah Heilshorn's recent work (***Nat Comm*** *2025*), where engineered hydrogel was shown to modulate cell-cell interactions and signaling. By integrating similar matrix-engineering strategies into our cardiac organoid, we will create controlled microenvironments that enhance the detection and quantification of paracrine effects, thereby directly addressing this critique

(4) Feasibility and scope of "cell villages". Some reviewers questioned the feasibility and population design of the village approach. **In response**, the SCVI iPSC biobank contains over 2,500 genotyped and phenotyped lines, providing an unparalleled resource for population-scale modeling. We have already identified 240 qualified lines that meet all experimental requirements. Each village will be assembled with a balanced representation of five ethnic populations, ensuring both genetic diversity and reproducibility. By leveraging these existing resources, we can efficiently construct organoid villages in a cost-effective and scalable manner.

(5) Overall risk and contingency planning. While the proposal was considered comprehensive, reviewers highlighted Aims 1 and 3 as relatively high risk. **In response**, we have adopted tiered milestones: ***(i)*** reproducibility benchmarks for dual-reporter assays prior to screening, ***(ii)*** validation of candidate variants in multiple donor lines, and ***(iii)*** pilot village studies prior to larger-scale analyses. This phased approach ensures that only well-validated systems and variants progress to the next stage.

Together, these refinements ensure a rigorous platform to decode genetic variants underlying hypertrophic cardiomyopathy. Since our DISC0 submission, we have also published pivotal studies demonstrating the project's *feasibility, productivity,* and *scientific rigor*, including: mechanosensing in cardiac fibrosis (***Nature*** 2025), vascularized organoid development (***Science*** 2025), engineered hydrogel to modulate cell-cell interactions (***Nat Comm*** 2025), and CRISPR-GPT agentic automation framework (***Nat Biomed Eng*** 2025). In summary, we thank the Board for its careful consideration and remain available for any additional information or clarification.

Sincerely,

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