"Genetic repair of heart failure using base editing"

Our overarching goal is to engineer advanced cellular, microtissue and genetically humanized mouse models and develop a heart failure therapeutic using AAVs and programmable base editing technology.

This project aligns with JAX Cell Modeling initiatives to leverage cellular and biomimetic cardiac tissue engineering platforms (**Hinson** lab), genetic diversity (**Rosenthal** lab), and cross-species cellular engineering (**Reinhardt**, **Pera** and **Skarnes** labs) to develop the first therapeutic targeting *TTN* mutations that are the major genetic risk factor for heart failure.

Background and Significance

Dilated cardiomyopathy (DCM) - DCM affects 1:200 individuals and is defined as reduced cardiac contractility and enlarged chambers (**Fig. 1A**). DCM causes heart failure and sudden cardiac death, and secondary to insufficient therapeutics, most individuals die five years from diagnosis[7]. *Titin* gene (*TTN)* truncation variants ("tvs") are the most frequent cause of not only DCM, but also peripartum cardiomyopathy (PPCM), which is an understudied disorder affecting pregnant or early postpartum females with high morbidity and mortality (**Fig. 1B**)[8]. TTNtvs also exhibit incomplete penetrance due to incompletely defined genetic and environmental factors[9]. Therapeutic development for TTNtvs has been unsuccessful because of TTN's immense size (>30k residues) precluding gene replacement, and functional complexity without small molecule binding sites for drug development.

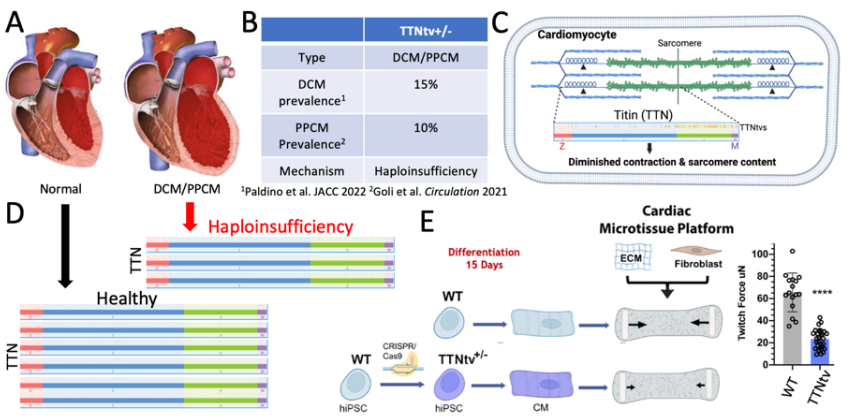

Figure 1. **A)** Dilated and peripartum cardiomyopathies (DCM/PPCM) are diagnosed by cardiac dilation and reduced contractility. **B)** TTNtvs are the most common genetic cause of DCM/PPCM through haploinsufficiency. **C)** *TTN* encodes a giant sarcomere structural protein spanning Z-disk to M-line, and TTNtvs reduce sarcomere contraction and content in parallel with **D)** reduced TTN protein. **E)** Microtissues from iPSCs recapitulate DCM features *in vitro* including reduced contraction (arrow denotes contraction direction and length is proportional to levels) and reduced TTN levels.

TTNtv pathophysiology and DCM models - *TTN* encodes the giant ~3 MDa backbone of the sarcomere, the force-producing organelle of the cardiomyocyte. TTN has multiple structural domains spanning the half sarcomere from Z-disk to M-line (**Fig. 1C**); and TTNtvs have been shown through haploinsufficiency to reduce TTN levels (**Fig. 1D**), sarcomere quantity and contractile functions in induced pluripotent stem cell (iPSC)-derived cardiomyocyte (iCM) models[5, 6] (**Fig. 1E**) as well as in mouse and human heart studies[1, 10]. While Ttntv$^{+/-}$ mice exhibit stress-induced cardiac dysfunction in C57BL/6J[1], the field lacks development of mouse models composed of human *TTN* sequences and variants, TTNtv therapeutics and identification of TTNtv genetic modifiers.

CRISPR adenine base editors (ABEs) – Genetic correction of TTNtvs is now feasible. By fusing adenine base editing enzymes (ABEmax) such as modified TadA to a nuclease-dead dCas9 that when co-expressed with a guide RNA (**Fig. 2A**), enables adenine-to-guanine (A->G) base substitutions without generating double-strand DNA breaks that can be toxic[11]. ABEs are being tested in humans and have been recently shown to be safe and efficacious in non-human primate studies for hypercholesterolemia due to *PCSK9* mutations[12]. Our team will use a toolkit of dCas9 enzymes that exhibit expanded protospacer (PAM) recognition (e.g., dCas9-NG recognizes "NG" PAM motifs; **Fig. 2B**)[11], optimized for delivery in adeno-associated viruses (AAVs) that have enhanced cardiotropism[13], and precise with well-characterized A->G base editing windows (see positions A4-7 in **Fig. 2C**). Recently, the feasibility of targeting a monogenic cardiac condition was demonstrated for hypertrophic cardiomyopathy with similar ABE enzymes delivered by AAVs[14].

Innovation and Impact

Human(ized) TTNtv models – To date, no humanized mouse model has been developed to study TTNtvs, the most prevalent genetic risk factor for heart failure. The combination of a TTNtv engineered into a humanized *Ttn* exon (i.e., "humanized" TTNtv mouse) with TTNtv human and mouse iPS models will enable TTNtv-directed therapeutic development, cross-species (mouse/human) and cross-platform (*in vitro*/*in vivo*) analyses using iCMs and cardiac microtissue assays.

AAVs encoding PAM-flexible ABEs – With this proposal, AAVs encoding a toolkit of Cas9 enzymes (e.g., Cas9-SpRY, -SpG, -NG and -VRQR) with broadened PAM recognition[11] will be developed and shared to the JAX community to enable targeting of a large proportion of genetic variants across campuses and diseases.

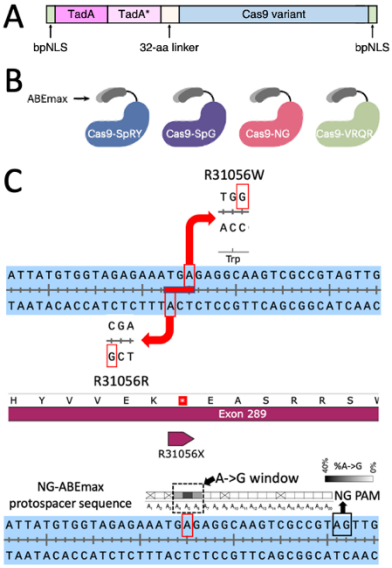

Figure 2. **A)** ABEmax structure shown with tandem TadAs linked to dCas9 that is flanked by nuclear localization sequences (NLS). **B)** dCas9s have been reprogrammed for expanded PAM recognition (dCas9-NG recognizes "NG"). **C)** TTNtv-R31056X will be engineered into mice and iPScs, and X->R or X->W corrected when targeted with NG-ABEmax. Candidate PAM is shown (black box) and editing window (dashed box) overlapping A5 (red box).

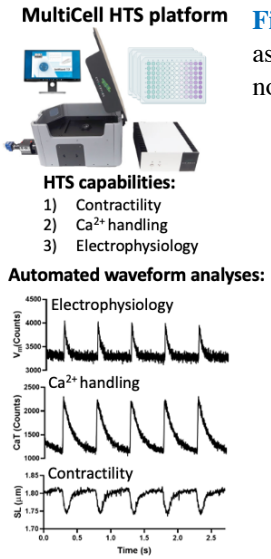

Figure 3. MultiCell HTS platform enables automated physiological assessments of contractility, calcium handling and electrophysiology not currently available at JAX.

TTNtvs in diverse genetic backgrounds – The heart failure field lacks knowledge of genetic modifiers of TTN functions, which would be enabled by our establishment of mouse and iPS models derived from heart failure-sensitized and -resilient strains. Genetic modifiers would also be nominated for additional therapeutic development in future proposals, thus feeding new grant submissions.

MultiCell HTS platform for high-throughput physiology screens (Fig. 3) – Currently, JAX lacks the capacity for high-throughput physiological assessments, which would be enabled by the acquisition of this platform for use across different cell types (see **Varn, Stitzel** and **Lee LOS**)

Preliminary Data

High efficiency AAV gene delivery to the mouse heart – AAVs ("MyoAAV") have been recently capsid-evolved for enhanced mouse and non-human primate cardiac tropism[13]. MyoAAVs have been produced and studied by our group to transduce C57BL6/J cardiomyocytes with up to 97% efficiency after tail or retro-orbital vein injections in adult mice at a dose of 5x10^12- 3x10^13 vg/kg (**Fig. 4**). The high level of transduction enables AAV-directed TTNtv base editing studies.

Recapitulation of DCM in a Ttntv$^{+/-}$ mouse – Demonstrating feasibility, our team has CRISPR-engineered a standard Ttntv$^{+/-}$ C57BL6/J model (JAX Genetic Engineering Technologies; see **Boumil LOS**) using CRISPR technology with a DNA repair template to introduce ~2kb cassette composed of a one nucleotide frameshift Ttntv preceded by a HaloTag[15]. Because CRISPR enzymes cannot correct a frameshift deletion mutation without generating DNA double-strand breaks and thus genotoxicity and the lack of humanized TTN sequences, this model will not be applicable to this proposal. However, using isoproterenol (Iso) stress, we have established stress-induced DCM in this model as reported previously[1].

Biomimetic cardiac microtissue (CMT) assays enabling gene editing optimization and development in vitro (**Fig. 6**)– Our team has developed a biomimetic, 3-diminsional CMT platform composed of iCMs differentiated from iPSCs using well-established Wnt modulation protocols as reported previously to investigate mechanisms of heart failure and fibrosis[3-6, 16]. This model enables advanced and cross-species functional interrogation of TTNtvs in a human cellular and genomic context.

Project Aims

1) Integrative and cross-species functional and therapeutic screening in TTNtv cellular and microtissue models

2) CRISPR base editing and mouse genetic diversity to study and treat humanized TTNtv models of heart failure.

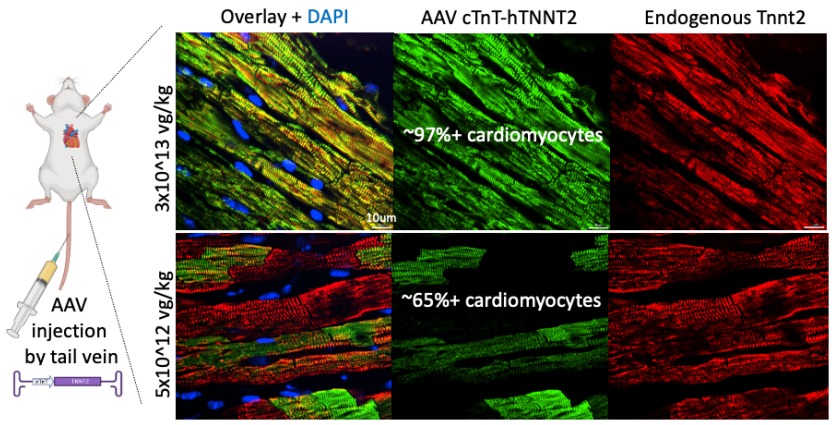

Figure 4. Up to 97% transduction efficiency using MyoAAV delivered by tail vein injection to adult mice as demonstrated by exogenous human AAV-TNNT2 protein (green or G) and co-localized with endogenous Tnnt2 (red or R) in mouse cardiac sections 3-weeks post injection. G+R+/R+ is transduction efficiency.

Aim 1: Integrative and cross-species functional and therapeutic screening in TTNtv cellular and microtissue models.

Rationale: The field lacks a method to repair TTNtvs that are the major risk factor for human heart failure in part due to TTN's immense size and complexity; and until recently, the lack of models to develop such approaches. Our published and preliminary results demonstrate robust models of TTNtv-DCM using iCM and CMT assays that can now enable therapeutic development to repair the underling TTNtv and circumvent previous limitations of TTN studies. We hypothesize that cardiotropic AAVs delivering ABEs can replace *TTN* premature truncation codons with codons reversing haploinsufficiency and TTN protein deficiencies. Here, we will engineer cross-species TTNtv iPSC models to develop DCM iCM and CMTs to enable ABE screening and therapeutic development. Application of the MultiCell HTS platform will enable higher throughput screening of ABE components not previously feasible in standard iCM and highly complex 3-dimensional CMTs.

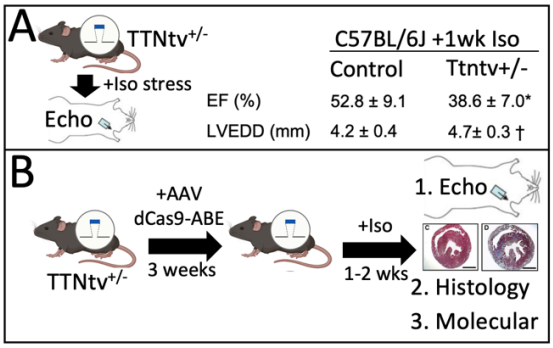

Figure 5. A) Stress-induced DCM model[1] that uses a well-established isoproterenol chronic stress model that induces dilated left ventricular end-diastolic diameter (LVEDD), and reduced ejection fraction (EF). **B)** Experimental overview of AAV delivery of ABEs, stress induction, and functional characterizations to develop a therapeutic for TTNtv-heart failure.

Approach: *Engineering a pathogenic ABE-compatible TTNtv into human and mouse iPSCs*- A patient-derived TTNtv (R31056X) will be generated in TTN N2BA transcript exon 289 (**Fig. 2C**). The single nucleotide substitution TTNtv replaces the cytosine of the arginine-encoding codon with a thymine, thus producing a "TGA" premature truncation codon (PTC). This variant has been identified in several families with DCM[17, 18]. An allelic series of the TTNtv will be generated by standardized CRISPR ribonucleoprotein methods[19], and initially generated in the commonly used KOLF2.1J hiPSC line[20] that has been routinely studied by JAX Cellular Engineering (See **McDonough LOS**) and has previously been optimized for efficient iCM differentiation by our group. The TTNtv will also be engineered into iPSCs derived from C57BL6/J to provide a cross-species analysis.

Production and on-target functional testing of a panel of dCas9-ABEs with diverse PAM specificities for correction of the TTNtv- Leveraging ABEs that replace an adenine with a cytosine, the "TGA" premature truncation codon can be modified to either a "TGG" codon encoding tryptophan or a "CGA" codon encoding back arginine by targeting the – or + strand (**Fig. 2C** and **7B**), respectively. Both editing results would be predicted to restore normal TTN protein levels and eliminate any poison peptide-like effects derived from protein production from the PTC allele. Here, we will first develop lentiviral and AAV vectors expressing a panel of dCas9-ABEs (**Fig. 2B**) such as NG-ABEmax, as well as a panel of TTNtv-specific gRNAs. All vectors will be concentrated and tittered before experimental studies using standard methods.

*Lead ABE identification using iCM assays, MultiCell HTS and biomimetic CMT assays (**Fig. 6**)*- We will differentiate TTNtv$^{+/-}$ and control iPSCs to iCMs using a directed differentiation monolayer protocol of sequential Wnt activation and inhibition by small molecules as described previously[21]. Purified iCMs will be generated by metabolic enrichment in glucose-free media as described previously[22], and their quality will be assessed by morphology and FACS analysis**.** For initial ABE screening, we will quantify on-target TTNtv base editing results using next-generation amplicon sequencing (Illumina) from extracted DNA following ABE lentiviral transduction. Lentivirus transduces iCMs >99% as shown previously[6, 23]. On-target editing analysis will be performed using EditR, a web-based algorithm for base editing assessments[24]. Candidate ABEs that produce *TTN* alleles with X31056R and X31056W edits will be nominated for additional functional testing including TTN protein quantifications using vertical agarose gel electrophoresis as described previously[6]. Next, candidate ABEs will be screened using the MultiCell HTS platform that enables high throughput single cell physiological assessments including automated contractility, calcium handling and electrophysiological measurements using motion detection, Rhod-2/AM and Fluo-Volt dyes, respectively. These parameters are critical for testing efficacy as well as safety of candidate ABEs. Here, we will treat iCMs with ABEs, and use the MultiCell HTS platform including IonWizard (IonOptix) computational analysis to nominate lead ABEs for more complex functional testing in CMT assays.

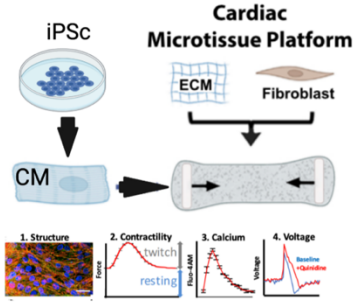

Figure 6. Cardiac microtissue platform composed of iCMs differentiated from iPScs can be functionally interrogated across tissue structure, contractility, calcium handling and electrophysiological studies (Quinidine is an arrhythmia-inducing drug that prolongs QT intervals) as reported[2-6].

Finally, lead ABEs will be tested in CMTs, as previously described[5]. Briefly, dissociated iCMs (ABE treated and controls) and normal human cardiac fibroblasts (Lonza) will be mixed at a ratio of 1:10 in a collagen-fibrinogen slurry and co-seeded onto a microfabricated array of 24 micro-tissue gauges (μTUGs). Each μTUG is molded from cross-linked polydimethylsiloxane and features two elastomeric cantilevers, separated by 1.3 mm, the tips of which are marked with fluorescent microbeads. In this context, the cells will self-organize into CMTs that span the gap between the paired cantilever tips of each μTUG. CMTs will be maintained in high-glucose, serum- and glutamine-supplemented media, and exchanged for Tyrode's solution (+1.2 mM calcium chloride) prior to force measurements. CMTs will be subjected to biomechanical characterization including twitch force, resting tension, and contraction kinetics. CMTs will also be subjected to electrophysiological testing. CMT imaging will be performed on the Andor Dragonfly microscope. We will also perform a comparative analysis of TTNtv and control CMTs generated from mouse iCMs.

Aim 2: CRISPR base editing and mouse genetic diversity to study and treat humanized TTNtv models of heart failure.

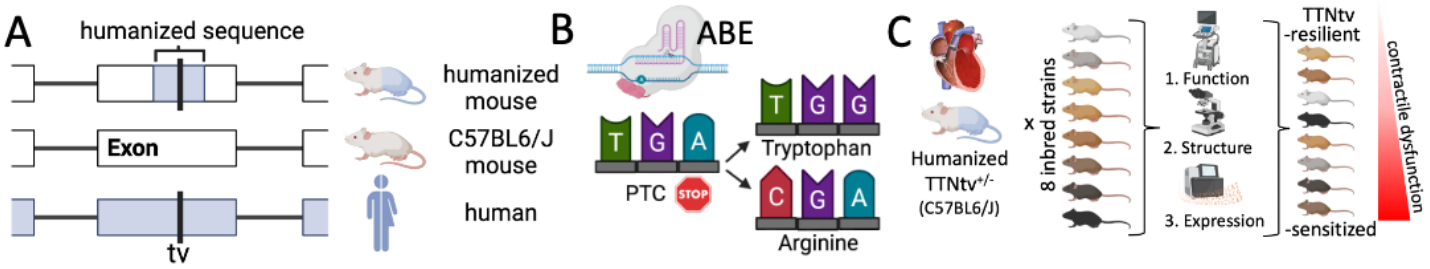

Figure 7. A) A sequence humanized TTNtv will be introduced into the homologous mouse *Ttn* locus (white) in C57BL6/J with flaking human sequences of ~1kb (light blue). **B)** ABEs can convert a premature truncation codon (PTC) to a tryptophan or arginine, thus restoring TTN levels and functions. **C)** General overview of approach to identify TTNtv-sensitized and resilient genetic backgrounds using F1s derived from eight CC inbred strain crosses.

Rationale: Large scale clinical studies of human heart failure and cardiac dysfunction have demonstrated *TTN* variants as the most common genetic risk factor[17, 18, 25], yet neither TTN-directed therapeutics nor genome editors correcting a TTNtv have been developed. Recently, two research groups utilized ABEs to correct a hypertrophic cardiomyopathy associated *MYH7* missense variant (R403Q) in knock-in mouse models that prevented disease progression[14, 26]. With cardiotropic AAVs now in hand such as MyoAAV and TTNtv disease models, we hypothesize that ABEs targeting a TTNtv can prevent heart failure development in mice. Finally, leveraging genetic diversity such as within the eight Collaborative Cross (CC) founder strains[27], we will identify TTNtv-sensitized and -resilient genetic strains for developing new models and studying factors underlying the incomplete penetrance observed in the human population[9]. While the underlying cause of incomplete penetrance has not been fully explained, some loci have been implicated, including LMNA and RBM120, where certain alleles have been associated with oligogenic or polygenic DCM etiology[28], as well as penetrance of TTNtvs[29, 30]. High-impact variants in these genes, as well as others related to DCM, have been identified through whole genome sequencing of the CC founder strains, particularly in CAST/EiJ and PWK/PhJ. Furthermore, CAST/EiJ and 129S loci associated with DCM penetrance have been identified through quantitative trait mapping in muscular dystrophy disease models[31, 32]. These findings support our use of CC founder strains for TTNtv-sensitized and -resilient backgrounds.

Approach: *Engineering the sequence-humanized TTNtv into C57BL6/J (**Fig. 7A**)*- Using CRISPR technology and a homology-directed repair plasmid template as routinely done by JAX G.E.T. (see **Boumil LOS**), we will engineer the first humanized TTNtv DCM model. The patient-derived TTNtv-R31056X, which is also utilized in **Aim 1** studies, will be engineered into the homologous mouse *Ttn* exon 288 (ENSMUST00000111882.8) that is 89% sequence-conserved with human *TTN* exon 289. We will insert human flanking sequences of about ~1kb in length to keep within the ~2kb cassette insertion range shown easily feasible by recently developed models. If needed, we can shorten the flanking sequence size or develop other premature truncation codons from the clinic. The model will be validated by approaches to exclude plasmid backbone insertions and tandem cassette insertions; as well as backcrossing to minimize bi-allelic or off-target edits.

*In vivo development of dCas9-ABEs with diverse PAM specificities for TTNtv correction (**Figs. 5B** **and 7B**)* – Secondary to our cross-species approach utilizing the identical TTNtv in a humanized mouse as well as human iPSCs, the same ABE editors that will be tested *in vivo* as in **Aim 1** studies. To start, we will inject a panel of MyoAAVs or saline controls encoding lead ABEs at a range of dosages (up to 1x10^14 vg/g) into 3-week-old mice. ABEs that restore TTN functions in **Aim 1** studies will be prioritized for *in vivo* validation. We will first quantify adult cardiomyocyte transduction rates using immunofluorescence assays with antibodies recognizing ABE components co-stained with cardiomyocyte markers (α-

cTnT), and on-target editing using amplicon analysis as described in **Aim 1**. Importantly, cardiomyocytes will also be purified using the Langendorff-free method for more accurate cardiomyocyte-specific editing assessments as the ABEs are regulated by cardiomyocyte-specific troponin T promoters[33]. Lead ABEs that produce *Ttn* alleles with X31056R and X31056W edits will be comprehensively studied in a stress-induced DCM model as described previously using chronic isoproterenol delivered by Alzet osmotic pumps inserted into the subcutaneous space (**Fig. 5B**)[1]. To assess therapeutic efficacy, cardiac echocardiography (echo) will be performed at 1- and 2-weeks post isoproterenol treatment, and efficacy will be determined if %EF is significantly greater, and LVEDD is significantly smaller with ABE treatment compared to saline control. We propose to study N=25 mice (1:1 M:F) per lead ABE for 90% confidence to identify an estimated 15% improvement in EF% at P<0.05. We will inject AAV-ABEs by retro-orbital vein using a 29G needle. In addition, additional studies will be performed including cardiac histopathology to assess % fibrosis; and molecular analyses including Ttn expression levels by agarose electrophoresis and quantitative PCR for stress markers including *Nppb* and cardiac fibrosis markers including *Postn*.

Identifying TTNtv-resilient and -sensitized mouse strains –Humanized TTNtv$^{+/-}$ mice will be crossed with the eight CC founder strains to generate at least three F1 litters (**Fig. 7C**). F1s (N=25 mice per CC founder, 1:1 M:F) will undergo chronic isoproterenol stress and will be studied by echocardiography to identify TTNtv-sensitized and -resilient strains if the DCM phenotype is greater or worse, respectively, when compared to C57BL6/J alone. For the identified sensitized and resilient strains that are identified, histopathological analyses and molecular analyses will be assessed to quantify %cardiac fibrosis and stress marker expression levels. Finally, candidate molecular and genetic modifiers will be procured from Cube initiative transcriptomics and genotyping data in candidate modifier genes such as those nominated by published human GWAS studies of heart failure[34] and DCM[35]. This strategy has been in use by our group for other cardiac disorders[36, 37].

Potential Problems and Alternative Strategies

If we have unexpected challenges generating TTNtvs, we can test numerous other *TTN* PTC variants that are amenable to base editing in human DCM. For mouse iPSCs, we can use CRISPR to knock-in the TTNtv into a wildtype background or reprogram iPSCs directly from the humanized mouse. If we have issues with base editing using ABEs, we can test other enzymes that do not generate double strand breaks including cytosine base editors (CBEs) or prime editors[38].

Project Summary, Deliverables and Future directions

1. **Develop the first TTNtv therapeutic for heart failure –** Lead ABEs will be candidates for patent applications and could stimulate the development of genome editing therapeutics for other rare monogenic disorders. For example, base editing *TTN* regulatory elements like enhancers could reverse TTNtv-related haploinsufficiency and could be applicable to many *TTN* variants that result in haploinsufficiency or other forms of heart failure as GWAS studies have implicated variants in the *TTN* locus as a modifier for more common types of heart failure and cardiac dysfunction.
2. **Foundation for building a resource available to other JAX researchers** to utilize mouse and human iPSC-derived cellular models for diseases beyond heart failure such as iPS-derived pancreatic beta cells (see **Stitzel LOS**) from diabetogenic mouse strains, or iCMs exhibiting altered TGF-beta family signaling (see **Lee LOS**). By means of programming a 20-nucleotide gRNA, other JAX researchers (see **Lutz LOS**) could leverage this toolkit of ABEs and delivery systems to target other monogenic disorders. The MultiCell HTS platform fills an unmet need at JAX for higher throughput physiological analyses. This platform can be utilized for studies of other cell types including neurons, skeletal myocytes and adult cardiomyocytes.
3. **Catalyst for integration with research from other JAX labs.** For example, TTNtv-sensitized and -resilient strains will stimulate opportunities leveraging Cube initiative genomics and epigenomics data across the CC strains. The MultiCell HTS platform will be of immediate usage for beta cell testing (new R01-5%ile; see **Stitzel LOS**).
4. **Future grant applications.** This DIF will provide advanced cellular and humanized mouse models of the most common genetic risk factor for heart failure, which could inspire follow-up TTN studies such as focused on putative modifier genes identified from CC F1s. In addition, technical knowledge gained from developing *TTN* base editing could provide essential preliminary data to catalyze new collaborations targeting other disorders within or beyond the heart such as development of validated AAVs for targeting mouse tissues and ABEs that could be reprogrammed for other variants.

Project Timeline and Budget

Aims 1-2 will be executed contemporaneously starting 2023-Q3, and we anticipate completion of TTNtv iPS model and ABE panel production by 2024-Q2, ABE testing in iCMs including MultiCell HTS acquisition and cell analyses by 2024-Q4, and CMT assays by 2025-Q2. We anticipate completion of humanized TTNtv model generation by 2024-Q2, lead ABE functional testing in vivo by 2025-Q2, and TTNtv F1 studies by 2024-Q4. We are requesting a budget for year 1 of $570,100 inclusive of the acquisition cost of the MultiCell HTS platform, and Year 2 of $289,500 for a total of $859,600.

References

1. Gramlich M, Michely B, Krohne C, Heuser A, Erdmann B, Klaassen S, Hudson B, Magarin M, Kirchner F, Todiras M, Granzier H, Labeit S, Thierfelder L, Gerull B. Stress-induced dilated cardiomyopathy in a knock-in mouse model mimicking human titin-based disease. J Mol Cell Cardiol. 2009;47(3):352-8. Epub 20090504. doi: 10.1016/j.yjmcc.2009.04.014. PubMed PMID: 19406126; PMCID: PMC2764556.
2. Bredemeyer AL, Amrute JM, Koenig AL, Idol RA, He L, Luff SA, Dege C, Leid JM, Schilling JD, Hinson JT, Dinauer MC, Sturgeon CM, Lavine KJ. Derivation of extra-embryonic and intra-embryonic macrophage lineages from human pluripotent stem cells. Development. 2022;149(8). Epub 20220503. doi: 10.1242/dev.200016. PubMed PMID: 35178561; PMCID: PMC9124573.
3. Pettinato AM, Ladha FA, Mellert DJ, Legere N, Cohn R, Romano R, Thakar K, Chen YS, Hinson JT. Development of a Cardiac Sarcomere Functional Genomics Platform to Enable Scalable Interrogation of Human TNNT2 Variants. Circulation. 2020;142(23):2262-75. Epub 20201007. doi: 10.1161/CIRCULATIONAHA.120.047999. PubMed PMID: 33025817; PMCID: PMC7722228.
4. Cohn R, Thakar K, Lowe A, Ladha FA, Pettinato AM, Romano R, Meredith E, Chen YS, Atamanuk K, Huey BD, Hinson JT. A Contraction Stress Model of Hypertrophic Cardiomyopathy due to Sarcomere Mutations. Stem Cell Reports. 2019;12(1):71-83. Epub 20181213. doi: 10.1016/j.stemcr.2018.11.015. PubMed PMID: 30554920; PMCID: PMC6335568.
5. Hinson JT, Chopra A, Nafissi N, Polacheck WJ, Benson CC, Swist S, Gorham J, Yang L, Schafer S, Sheng CC, Haghighi A, Homsy J, Hubner N, Church G, Cook SA, Linke WA, Chen CS, Seidman JG, Seidman CE. HEART DISEASE. Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science. 2015;349(6251):982-6. Epub 2015/09/01. doi: 10.1126/science.aaa5458

349/6251/982 [pii]. PubMed PMID: 26315439.
6. Romano R, Ghahremani S, Zimmerman T, Legere N, Thakar K, Ladha FA, Pettinato AM, Hinson JT. Reading Frame Repair of TTN Truncation Variants Restores Titin Quantity and Functions. Circulation. 2022;145(3):194-205. Epub 20211214. doi: 10.1161/CIRCULATIONAHA.120.049997. PubMed PMID: 34905694; PMCID: PMC8766920.
7. Shah KS, Xu H, Matsouaka RA, Bhatt DL, Heidenreich PA, Hernandez AF, Devore AD, Yancy CW, Fonarow GC. Heart Failure With Preserved, Borderline, and Reduced Ejection Fraction: 5-Year Outcomes. J Am Coll Cardiol. 2017;70(20):2476-86. Epub 2017/11/17. doi: 10.1016/j.jacc.2017.08.074. PubMed PMID: 29141781.
8. Irizarry OC, Levine LD, Lewey J, Boyer T, Riis V, Elovitz MA, Arany Z. Comparison of Clinical Characteristics and Outcomes of Peripartum Cardiomyopathy Between African American and Non-African American Women. JAMA Cardiol. 2017;2(11):1256-60. Epub 2017/10/20. doi: 10.1001/jamacardio.2017.3574. PubMed PMID: 29049825; PMCID: PMC5815055 ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Irizarry reported receiving grants unrelated to the present work from FOCUS Medical Student Fellowship in Women's Health supported by the Edna G. Kynett Memorial Foundation. No other disclosures were reported.
9. Schafer S, de Marvao A, Adami E, Fiedler LR, Ng B, Khin E, Rackham OJ, van Heesch S, Pua CJ, Kui M, Walsh R, Tayal U, Prasad SK, Dawes TJ, Ko NS, Sim D, Chan LL, Chin CW, Mazzarotto F, Barton PJ, Kreuchwig F, de Kleijn DP, Totman T, Biffi C, Tee N, Rueckert D, Schneider V, Faber A, Regitz-Zagrosek V, Seidman JG, Seidman CE, Linke WA, Kovalik JP, O'Regan D, Ware JS, Hubner N, Cook SA. Titin-truncating variants affect heart function in disease cohorts and the general population. Nat Genet. 2017;49(1):46-53. Epub 20161121. doi: 10.1038/ng.3719. PubMed PMID: 27869827; PMCID: PMC5201198.
10. McAfee Q, Chen CY, Yang Y, Caporizzo MA, Morley M, Babu A, Jeong S, Brandimarto J, Bedi KC, Jr., Flam E, Cesare J, Cappola TP, Margulies K, Prosser B, Arany Z. Truncated titin proteins in dilated cardiomyopathy. Sci Transl Med. 2021;13(618):eabd7287. Epub 20211103. doi: 10.1126/scitranslmed.abd7287. PubMed PMID: 34731015; PMCID: PMC9236909.
11. Huang TP, Zhao KT, Miller SM, Gaudelli NM, Oakes BL, Fellmann C, Savage DF, Liu DR. Circularly permuted and PAM-modified Cas9 variants broaden the targeting scope of base editors. Nat Biotechnol. 2019;37(6):626-31. Epub 20190520. doi: 10.1038/s41587-019-0134-y. PubMed PMID: 31110355; PMCID: PMC6551276.
12. Lee RG, Mazzola AM, Braun MC, Platt C, Vafai SB, Kathiresan S, Rohde E, Bellinger AM, Khera AV. Efficacy and Safety of an Investigational Single-Course CRISPR Base-Editing Therapy Targeting PCSK9 in Nonhuman Primate and Mouse Models. Circulation. 2023;147(3):242-53. Epub 20221031. doi: 10.1161/CIRCULATIONAHA.122.062132. PubMed PMID: 36314243.

13. Tabebordbar M, Lagerborg KA, Stanton A, King EM, Ye S, Tellez L, Krunnfusz A, Tavakoli S, Widrick JJ, Messemer KA, Troiano EC, Moghadaszadeh B, Peacker BL, Leacock KA, Horwitz N, Beggs AH, Wagers AJ, Sabeti PC. Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across species. Cell. 2021;184(19):4919-38 e22. Epub 20210909. doi: 10.1016/j.cell.2021.08.028. PubMed PMID: 34506722; PMCID: PMC9344975.
14. Chai AC, Cui M, Chemello F, Li H, Chen K, Tan W, Atmanli A, McAnally JR, Zhang Y, Xu L, Liu N, Bassel-Duby R, Olson EN. Base editing correction of hypertrophic cardiomyopathy in human cardiomyocytes and humanized mice. Nat Med. 2023;29(2):401-11. Epub 20230216. doi: 10.1038/s41591-022-02176-5. PubMed PMID: 36797478; PMCID: PMC10053064.
15. Los GV, Encell LP, McDougall MG, Hartzell DD, Karassina N, Zimprich C, Wood MG, Learish R, Ohana RF, Urh M, Simpson D, Mendez J, Zimmerman K, Otto P, Vidugiris G, Zhu J, Darzins A, Klaubert DH, Bulleit RF, Wood KV. HaloTag: a novel protein labeling technology for cell imaging and protein analysis. ACS Chem Biol. 2008;3(6):373-82. doi: 10.1021/cb800025k. PubMed PMID: 18533659.
16. Forte E, Ramialison M, Nim HT, Mara M, Li JY, Cohn R, Daigle SL, Boyd S, Stanley EG, Elefanty AG, Hinson JT, Costa MW, Rosenthal NA, Furtado MB. Adult mouse fibroblasts retain organ-specific transcriptomic identity. Elife. 2022;11. Epub 20220316. doi: 10.7554/eLife.71008. PubMed PMID: 35293863; PMCID: PMC8959603.
17. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, Banner NR, Pennell DJ, Graw S, Merlo M, Di Lenarda A, Sinagra G, Bos JM, Ackerman MJ, Mitchell RN, Murry CE, Lakdawala NK, Ho CY, Barton PJ, Cook SA, Mestroni L, Seidman JG, Seidman CE. Truncations of titin causing dilated cardiomyopathy. N Engl J Med. 2012;366(7):619-28. doi: 10.1056/NEJMoa1110186. PubMed PMID: 22335739; PMCID: PMC3660031.
18. Roberts AM, Ware JS, Herman DS, Schafer S, Baksi J, Bick AG, Buchan RJ, Walsh R, John S, Wilkinson S, Mazzarotto F, Felkin LE, Gong S, MacArthur JA, Cunningham F, Flannick J, Gabriel SB, Altshuler DM, Macdonald PS, Heinig M, Keogh AM, Hayward CS, Banner NR, Pennell DJ, O'Regan DP, San TR, de Marvao A, Dawes TJ, Gulati A, Birks EJ, Yacoub MH, Radke M, Gotthardt M, Wilson JG, O'Donnell CJ, Prasad SK, Barton PJ, Fatkin D, Hubner N, Seidman JG, Seidman CE, Cook SA. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Sci Transl Med. 2015;7(270):270ra6. doi: 10.1126/scitranslmed.3010134. PubMed PMID: 25589632; PMCID: PMC4560092.
19. Skarnes WC, Pellegrino E, McDonough JA. Improving homology-directed repair efficiency in human stem cells. Methods. 2019;164-165:18-28. Epub 2019/06/20. doi: 10.1016/j.ymeth.2019.06.016. PubMed PMID: 31216442.
20. Pantazis CB, Yang A, Lara E, McDonough JA, Blauwendraat C, Peng L, Oguro H, Kanaujiya J, Zou J, Sebesta D, Pratt G, Cross E, Blockwick J, Buxton P, Kinner-Bibeau L, Medura C, Tompkins C, Hughes S, Santiana M, Faghri F, Nalls MA, Vitale D, Ballard S, Qi YA, Ramos DM, Anderson KM, Stadler J, Narayan P, Papademetriou J, Reilly L, Nelson MP, Aggarwal S, Rosen LU, Kirwan P, Pisupati V, Coon SL, Scholz SW, Priebe T, Ottl M, Dong J, Meijer M, Janssen LJM, Lourenco VS, van der Kant R, Crusius D, Paquet D, Raulin AC, Bu G, Held A, Wainger BJ, Gabriele RMC, Casey JM, Wray S, Abu-Bonsrah D, Parish CL, Beccari MS, Cleveland DW, Li E, Rose IVL, Kampmann M, Calatayud Aristoy C, Verstreken P, Heinrich L, Chen MY, Schule B, Dou D, Holzbaur ELF, Zanellati MC, Basundra R, Deshmukh M, Cohen S, Khanna R, Raman M, Nevin ZS, Matia M, Van Lent J, Timmerman V, Conklin BR, Johnson Chase K, Zhang K, Funes S, Bosco DA, Erlebach L, Welzer M, Kronenberg-Versteeg D, Lyu G, Arenas E, Coccia E, Sarrafha L, Ahfeldt T, Marioni JC, Skarnes WC, Cookson MR, Ward ME, Merkle FT. A reference human induced pluripotent stem cell line for large-scale collaborative studies. Cell Stem Cell. 2022;29(12):1685-702 e22. Epub 2022/12/03. doi: 10.1016/j.stem.2022.11.004. PubMed PMID: 36459969; PMCID: PMC9782786.
21. Lian X, Hsiao C, Wilson G, Zhu K, Hazeltine LB, Azarin SM, Raval KK, Zhang J, Kamp TJ, Palecek SP. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. Proc Natl Acad Sci U S A. 2012;109(27):E1848-57. Epub 2012/05/31. doi: 10.1073/pnas.1200250109

1200250109 [pii]. PubMed PMID: 22645348.
22. Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, Hashimoto H, Suzuki T, Yamashita H, Satoh Y, Egashira T, Seki T, Muraoka N, Yamakawa H, Ohgino Y, Tanaka T, Yoichi M, Yuasa S, Murata M, Suematsu M, Fukuda K. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell. 2013;12(1):127-37. Epub 2012/11/22. doi: 10.1016/j.stem.2012.09.013

S1934-5909(12)00579-6 [pii]. PubMed PMID: 23168164.

23. Hinson JT, Chopra A, Lowe A, Sheng CC, Gupta RM, Kuppusamy R, O'Sullivan J, Rowe G, Wakimoto H, Gorham J, Burke MA, Zhang K, Musunuru K, Gerszten RE, Wu SM, Chen CS, Seidman JG, Seidman CE. Integrative Analysis of PRKAG2 Cardiomyopathy iPS and Microtissue Models Identifies AMPK as a Regulator of Metabolism, Survival, and Fibrosis. Cell Rep. 2016;17(12):3292-304. doi: 10.1016/j.celrep.2016.11.066. PubMed PMID: 28009297; PMCID: PMC5193246.
24. Kluesner MG, Nedveck DA, Lahr WS, Garbe JR, Abrahante JE, Webber BR, Moriarity BS. EditR: A Method to Quantify Base Editing from Sanger Sequencing. CRISPR J. 2018;1(3):239-50. doi: 10.1089/crispr.2018.0014. PubMed PMID: 31021262; PMCID: PMC6694769.
25. Pirruccello JP, Bick A, Wang M, Chaffin M, Friedman S, Yao J, Guo X, Venkatesh BA, Taylor KD, Post WS, Rich S, Lima JAC, Rotter JI, Philippakis A, Lubitz SA, Ellinor PT, Khera AV, Kathiresan S, Aragam KG. Analysis of cardiac magnetic resonance imaging in 36,000 individuals yields genetic insights into dilated cardiomyopathy. Nat Commun. 2020;11(1):2254. Epub 20200507. doi: 10.1038/s41467-020-15823-7. PubMed PMID: 32382064; PMCID: PMC7206184.
26. Reichart D, Newby GA, Wakimoto H, Lun M, Gorham JM, Curran JJ, Raguram A, DeLaughter DM, Conner DA, Marsiglia JDC, Kohli S, Chmatal L, Page DC, Zabaleta N, Vandenberghe L, Liu DR, Seidman JG, Seidman C. Efficient in vivo genome editing prevents hypertrophic cardiomyopathy in mice. Nat Med. 2023;29(2):412-21. Epub 20230216. doi: 10.1038/s41591-022-02190-7. PubMed PMID: 36797483; PMCID: PMC9941048.
27. Threadgill DW, Churchill GA. Ten years of the Collaborative Cross. Genetics. 2012;190(2):291-4. doi: 10.1534/genetics.111.138032. PubMed PMID: 22345604; PMCID: PMC3276648.
28. Njoroge JN, Mangena JC, Aribeana C, Parikh VN. Emerging Genotype-Phenotype Associations in Dilated Cardiomyopathy. Curr Cardiol Rep. 2022;24(9):1077-84. Epub 20220728. doi: 10.1007/s11886-022-01727-z. PubMed PMID: 35900642.
29. Gaertner A, Bloebaum J, Brodehl A, Klauke B, Sielemann K, Kassner A, Fox H, Morshuis M, Tiesmeier J, Schulz U, Knoell R, Gummert J, Milting H. The Combined Human Genotype of Truncating TTN and RBM20 Mutations Is Associated with Severe and Early Onset of Dilated Cardiomyopathy. Genes (Basel). 2021;12(6). Epub 20210608. doi: 10.3390/genes12060883. PubMed PMID: 34201072; PMCID: PMC8228627.
30. Roncarati R, Viviani Anselmi C, Krawitz P, Lattanzi G, von Kodolitsch Y, Perrot A, di Pasquale E, Papa L, Portararo P, Columbaro M, Forni A, Faggian G, Condorelli G, Robinson PN. Doubly heterozygous LMNA and TTN mutations revealed by exome sequencing in a severe form of dilated cardiomyopathy. Eur J Hum Genet. 2013;21(10):1105-11. Epub 20130306. doi: 10.1038/ejhg.2013.16. PubMed PMID: 23463027; PMCID: PMC3778353.
31. Maddatu TP, Garvey SM, Schroeder DG, Zhang W, Kim SY, Nicholson AI, Davis CJ, Cox GA. Dilated cardiomyopathy in the nmd mouse: transgenic rescue and QTLs that improve cardiac function and survival. Hum Mol Genet. 2005;14(21):3179-89. Epub 20050920. doi: 10.1093/hmg/ddi349. PubMed PMID: 16174646; PMCID: PMC1350304.
32. Vignier N, Mougenot N, Bonne G, Muchir A. Effect of genetic background on the cardiac phenotype in a mouse model of Emery-Dreifuss muscular dystrophy. Biochem Biophys Rep. 2019;19:100664. Epub 20190712. doi: 10.1016/j.bbrep.2019.100664. PubMed PMID: 31341969; PMCID: PMC6630059.
33. Ackers-Johnson M, Li PY, Holmes AP, O'Brien SM, Pavlovic D, Foo RS. A Simplified, Langendorff-Free Method for Concomitant Isolation of Viable Cardiac Myocytes and Nonmyocytes From the Adult Mouse Heart. Circ Res. 2016;119(8):909-20. Epub 20160808. doi: 10.1161/CIRCRESAHA.116.309202. PubMed PMID: 27502479; PMCID: PMC5965670.
34. Shah S, Henry A, Roselli C, Lin H, Sveinbjornsson G, Fatemifar G, Hedman AK, Wilk JB, Morley MP, Chaffin MD, Helgadottir A, Verweij N, Dehghan A, Almgren P, Andersson C, Aragam KG, Arnlov J, Backman JD, Biggs ML, Bloom HL, Brandimarto J, Brown MR, Buckbinder L, Carey DJ, Chasman DI, Chen X, Chen X, Chung J, Chutkow W, Cook JP, Delgado GE, Denaxas S, Doney AS, Dorr M, Dudley SC, Dunn ME, Engstrom G, Esko T, Felix SB, Finan C, Ford I, Ghanbari M, Ghasemi S, Giedraitis V, Giulianini F, Gottdiener JS, Gross S, Guethbjartsson DF, Gutmann R, Haggerty CM, van der Harst P, Hyde CL, Ingelsson E, Jukema JW, Kavousi M, Khaw KT, Kleber ME, Kober L, Koekemoer A, Langenberg C, Lind L, Lindgren CM, London B, Lotta LA, Lovering RC, Luan J, Magnusson P, Mahajan A, Margulies KB, Marz W, Melander O, Mordi IR, Morgan T, Morris AD, Morris AP, Morrison AC, Nagle MW, Nelson CP, Niessner A, Niiranen T, O'Donoghue ML, Owens AT, Palmer CNA, Parry HM, Perola M, Portilla-Fernandez E, Psaty BM, Regeneron Genetics C, Rice KM, Ridker PM, Romaine SPR, Rotter JI, Salo P, Salomaa V, van Setten J, Shalaby AA, Smelser DT, Smith NL, Stender S, Stott DJ, Svensson P, Tammesoo ML, Taylor KD, Teder-Laving M, Teumer A, Thorgeirsson G, Thorsteinsdottir U, Torp-Pedersen C, Trompet S, Tyl B, Uitterlinden AG, Veluchamy A, Volker U, Voors AA, Wang X, Wareham NJ, Waterworth D, Weeke PE, Weiss R, Wiggins KL, Xing H, Yerges-Armstrong LM, Yu B, Zannad F, Zhao JH, Hemingway H, Samani NJ, McMurray JJV, Yang J, Visscher PM, Newton-

Cheh C, Malarstig A, Holm H, Lubitz SA, Sattar N, Holmes MV, Cappola TP, Asselbergs FW, Hingorani AD, Kuchenbaecker K, Ellinor PT, Lang CC, Stefansson K, Smith JG, Vasan RS, Swerdlow DI, Lumbers RT. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. Nat Commun. 2020;11(1):163. Epub 2020/01/11. doi: 10.1038/s41467-019-13690-5. PubMed PMID: 31919418; PMCID: PMC6952380 employee of Pfizer who may hold Pfizer stock and/or stock options. J.D.B. and J.C. are employees of Regeneron Genetics Center. M.E.D. is an employee of Regeneron Pharmaceuticals. W.M. reports grants and personal fees from Siemens Diagnostics, grants and personal fees from Aegerion Pharmaceuticals, grants and personal fees from AMGEN, grants and personal fees from Astrazeneca, grants and personal fees from Danone Research, personal fees from Hoffmann LaRoche, personal fees from MSD, grants and personal fees from Pfizer, personal fees from Sanofi, personal fees from Synageva, grants and personal fees from BASF, grants from Abbott Diagnostics, grants and personal fees from Numares AG, grants and personal fees from Berlin-Chemie, employment with Synlab Holding Deutschland GmbH, all outside the submitted work. M.L.O. reports grant support from GlaxoSmithKline, Eisai, Janssen, Merck and AstraZeneca. B.M.P. serves on the DSMB of a clinical trial funded by Zoll LifeCor and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. V.S. participated in a conference trip sponsored by Novo Nordisk and received a honorarium from the same source for participating in an advisory board meeting. He also has ongoing research collaboration with Bayer Ltd. B.T. is a full-time employee of Servier. S.A.L. receives sponsored research support from Bristol Myers Squibb/Pfizer, Bayer AG and Boehringer Ingelheim, and has consulted for Abbott, Quest Diagnostics and Bristol Myers Squibb/Pfizer. M.V.H. has collaborated with Boehringer Ingelheim in research, and in accordance with the policy of the The Clinical Trial Service Unit and Epidemiological Studies Unit (University of Oxford), did not accept any personal payment. P.T.E. receives sponsored research support from Bayer AG, and has consulted with Bayer AG, Novartis and Quest Diagnostics. D.I.S. is a full-time employee of BenevolentAI. R.T.L. has received research grants from Pfizer. The remaining authors declare no competing interest.

35. Tadros R, Francis C, Xu X, Vermeer AMC, Harper AR, Huurman R, Kelu Bisabu K, Walsh R, Hoorntje ET, Te Rijdt WP, Buchan RJ, van Velzen HG, van Slegtenhorst MA, Vermeulen JM, Offerhaus JA, Bai W, de Marvao A, Lahrouchi N, Beekman L, Karper JC, Veldink JH, Kayvanpour E, Pantazis A, Baksi AJ, Whiffin N, Mazzarotto F, Sloane G, Suzuki H, Schneider-Luftman D, Elliott P, Richard P, Ader F, Villard E, Lichtner P, Meitinger T, Tanck MWT, van Tintelen JP, Thain A, McCarty D, Hegele RA, Roberts JD, Amyot J, Dube MP, Cadrin-Tourigny J, Giraldeau G, L'Allier PL, Garceau P, Tardif JC, Boekholdt SM, Lumbers RT, Asselbergs FW, Barton PJR, Cook SA, Prasad SK, O'Regan DP, van der Velden J, Verweij KJH, Talajic M, Lettre G, Pinto YM, Meder B, Charron P, de Boer RA, Christiaans I, Michels M, Wilde AAM, Watkins H, Matthews PM, Ware JS, Bezzina CR. Shared genetic pathways contribute to risk of hypertrophic and dilated cardiomyopathies with opposite directions of effect. Nat Genet. 2021;53(2):128-34. Epub 2021/01/27. doi: 10.1038/s41588-020-00762-2. PubMed PMID: 33495596; PMCID: PMC7611259.

36. Forte E, Skelly DA, Chen M, Daigle S, Morelli KA, Hon O, Philip VM, Costa MW, Rosenthal NA, Furtado MB. Dynamic Interstitial Cell Response during Myocardial Infarction Predicts Resilience to Rupture in Genetically Diverse Mice. Cell Rep. 2020;30(9):3149-63 e6. Epub 2020/03/05. doi: 10.1016/j.celrep.2020.02.008. PubMed PMID: 32130914; PMCID: PMC7059115.

37. Salimova E, Nowak KJ, Estrada AC, Furtado MB, McNamara E, Nguyen Q, Balmer L, Preuss C, Holmes JW, Ramialison M, Morahan G, Rosenthal NA. Variable outcomes of human heart attack recapitulated in genetically diverse mice. NPJ Regen Med. 2019;4:5. Epub 2019/03/12. doi: 10.1038/s41536-019-0067-6. PubMed PMID: 30854227; PMCID: PMC6399323.

38. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A, Liu DR. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149-57. Epub 2019/10/22. doi: 10.1038/s41586-019-1711-4. PubMed PMID: 31634902; PMCID: PMC6907074.

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April 6, 2023

Cell Modeling working group
The Jackson Laboratory
10 Discovery Drive
Farmington, CT 06030

Dear Dr. Hinson and the working group,

As Senior Study Director of The Jackson Laboratory's Genetic Engineering Technologies group, I am writing to confirm our full support for the proposed DIF application entitled, "*Genetic repair of heart failure using base editing"*. We pledge our support to this exciting project to develop the first genome editing therapeutic targeting *Titin*. For *Titin*, we have had previous success generating cassette knockins of a like size (~2-5kb) to what is being proposed for the humanized TTNtv mouse as described in Aim 2, using our standard methods. For example, knockin insertions of 4kb are ~80% successful and we have achieved a 9kb knockin at very high frequencies (2.2% of the injected embryos contained this large targeted insertion).

The JAX Genetic Engineering Technology (GET) group is regularly using the CRISPR/Cas9 gene editing system, combined with 1-cell zygote embryo manipulation delivery methods (e.g. microinjection or electroporation), to induce novel genetic changes in a variety of mouse strains. In the past 8 years, we have successfully designed and generated over 1060 new mouse models using CRISPR/Cas9 with ~200-300 active projects at any one time. Our large scale capacity not only enables us to accommodate at least 200-300 comprehensive projects per year, but it easily accommodated the JAX Knockout Mouse Project (KOMP) pipeline which required the production of 400-500 new CRISPR mediated deletion strains annually. We have experience with over 200 different mouse strains including a variety of classical inbreds such as C57BL/6J, C57BL/6NJ, DBA/2J, FVB/NJ, C3H/HeJ, NSG and Balb/cJ as well as the challenging "wild strains" such as *Mus Castaneus* (CAST). We have an ~97% success rate generating knockout mutations (indel or <4kb deletions) and a 90% success rate of knockin alleles using small single stranded oligo donor DNA molecules for the introduction of SNPs and short amino acid tags. We have also made significant strides using dsDNA plasmid donors for large "one step" DNA knockins such as reporter or floxed conditional alleles. Knockin insertions of 4kb are ~80% successful and we have achieved a 9kb knockin at very high frequencies (2.2% of the injected embryos contained this large targeted insertion). The GET group is delivering founder strains, with the above listed induced modifications in ~12-14 weeks for indels in C57BL/6J while more sophisticated models requiring complex donor vector design and cloning typically require 4-6 months.

Please do not hesitate to let me know if I could be of further assistance.

Regards,

Rebecca Boumil, PhD
Senior Study Director, Model Generation Services
Genetic Engineering Technologies

The Jackson Laboratory

Leading the search for tomorrow's cures

Se-Jin Lee, M.D., Ph.D.
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April 6, 2023

Travis Hinson, M.D.
The Jackson Laboratory for Genomic Medicine
10 Discovery Drive
Farmington, CT 06032

Re: MultiCELL HTS for automated cell physiology screening

Dear Travis,

Our laboratory studies the role of TGF-beta related family signaling using molecular genetic approaches. I am excited to know that your proposal entitled, "Genetic repair of heart failure using base editing", may result in the acquisition of an IonOptix MultiCell HTS screening platform. Our group would be very interested in using this platform to screen TGF-beta related family factors, downstream signaling pathways and candidate therapeutics in both cardiomyocytes and other cell types obtained from mice and iPSC models. Previously, JAX has not had a such a platform to functionally screen cells across contraction, Ca^{2+} and electrophysiology particularly at moderate to high throughput. This would be a timely acquisition particularly aligned with JAX Cell Modeling initiatives.

If I can be of any assistance on the MultiCell HTS Platform, please do not hesitate to reach out to me.

Sincerely,

Se-Jin Lee, M.D., Ph.D.

April 7, 2023

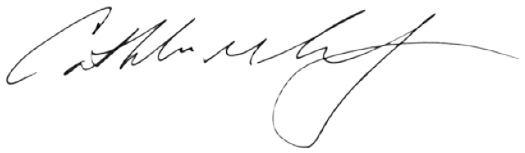
Travis Hinson, M.D.
The Jackson Laboratory
10 Discovery Drive
Farmington, CT 06032

Re: DIF application support

Dear Travis,

I am writing this letter to reflect my enthusiastic support of your new DIF proposal entitled, "**Genetic repair of heart failure using base editing**". Your proposal to address a monogenic form of heart failure due to *Titin* variants is timely and converges well with our group's pursuit of developing therapeutics for other monogenic rare disorders. I also wanted to convey to you what I anticipate as a critical aspect of your proposal- the development and validation of a panel of base editors fused to Cas9 enzymes with broadened genomic recognition. Having validated base editors that can be delivered to mouse tissues would enable an array of future projects addressing important human genetic disorders. This would be an excellent JAX-wide resource. Please let me know if I can be of any assistance as you tackle this important project.

Best,



Cat Lutz, Ph.D, MBA
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The Jackson Laboratory

Leading the search for tomorrow's cures

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J. Travis Hinson, M.D.
Assistant Professor
The Jackson Laboratory for Genomic Medicine
10 Discovery Drive
Farmington, CT 06032

April 5, 2023

Re: Support for DIF application

Dear Travis,

We enjoyed discussing your team's DIF proposal for "Genetic repair of heart failure using base editing" and would be happy to participate as consultants for this project. In Aim 1, you are planning to develop a knock-in hiPSC model of *TTN*-R31056X. From our conversation, this is a technically straightford single nucleotide substitution variant that we can easily engineer with CRISPR ribonucleoproteins (RNPs) and single stranded oligonucleotides as homology-directed repair templates. As you know, we have extensive experience using CRISPR/Cas9 and other genome engineering tools to generate mutations and knock-in constructs, and we are equipped to lend you our expertise in genome editing.

The JAX Cellular Engineering Core was established in 2017 and works hand-in-hand with the Skarnes research lab on several large scale engineering efforts. We are actively using CRISPR/Cas9 and Cas12a technology to generate hundreds of mutations in human iPSCs for the NIH's iPSC Neurodegenerative Disease Initiative (iNDI) and Molecular Phenotypes of Null Alleles in Cells (MorPhiC) project. We have developed improved protocols and workflows for highly efficient and precise CRISPR-based editing of human iPS cells. The allele types we routinely produce include knock-ins similar to those proposed in your study of *TTN*. For example, we deposited a preprint (Skarnes et al. Controlling homology-directed repair outcomes in human stem cells with dCas9. BioRxiv 2021.12.16.472942) that is directly applicable to your study. Our core mission is to share technical protocols, reagents and materials to JAX investigators like yourself, and providing any technical assistance that you may need.

I look forward to working together with you on your TTN study and I wish you the best of luck with your application.

Sincerely,

William C. Skarnes, Ph.D
Professor

Justin A. McDonough, Ph.D.
Associate Director,
Cellular Engineering

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Leading the search for tomorrow's cures

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April 6, 2023

J. Travis Hinson, M.D.
The Jackson Laboratory for Genomic Medicine
10 Discovery Drive
Farmington, CT 06032

Re: Support for MultiCELL HTS acquisition

Dear Travis,

I am writing this letter to reflect my enthusiastic support for JAX to acquire a MultiCELL HTS instrument as part of your DIF project. I enjoyed discussing your proposal and am particularly excited about the applications and benefits of the IonOptix MultiCell HTS screening platform. As you know, my laboratory has a long-standing interest in type 2 diabetes and islet dysfunction in human and mouse islets. Islet insulin-producing beta cells resemble cardiomyocytes in many ways including the dynamic utilization of Ca^{2+}. For beta cells, Ca^{2+} flux and handling is a central regulator of insulin production and secretion, and our laboratory has identified several genes through single cell profiling and CRISPR/Cas9 genome-wide screening approaches that we hypothesize may regulate this pathway for both beneficial/adaptive and detrimental/pathophysiologic effects. As such, *my lab can envision multiple projects,* ***including one described in a recent R01 application that scored 5th percentile in scientific review on 3/10/23,*** *that could utilize and benefit from the MultiCellHTS system* given its capacity to determine Ca^{2+} handling using dyes such as ratiometric Fura-2 in a multi-well format.

I look forward to working with your team to enable expansion of our work to beta cell physiology particularly related to Ca^{2+} handling. If I can be of any assistance on the MultiCellHTS Platform, do not hesitate to reach out to me.

Best,

Michael L. Stitzel, Ph.D

The Jackson Laboratory

Leading the search for tomorrow's cures

Frederick Varn, Ph.D.
Assistant Professor
The Jackson Laboratory for Genomic Medicine
10 Discovery Drive
Farmington, CT 06032
860-837-2243 *t* |Frederick.varn@Jax.org

April 4, 2023

J. Travis Hinson, MD
The Jackson Laboratory for Genomic Medicine
10 Discovery Drive
Farmington, CT 06032

Re: Letter of Support for MultiCell HTS platform

Dear Travis and the Cell Modeling initiative group,

Thank you for sharing with me your team's DIF proposal for "Genetic repair of heart failure using base editing" in which you are developing innovative base editors for heart failure using various cellular and humanized mouse models. As you know, my lab investigates genetic and molecular mechanisms of glioblastoma, and we have an interest in examining the cellular pathophysiology underlying specific tumor cell subsets that we have identified using single cell multi-omics approaches. Relevant to your proposal, we recently identified a cell population that exhibits neuron-like expression activity including expression of transcripts encoding electrophysiological components. As such, we have an emerging interest to explore the electrophysiological functions of these cells, including testing modulation of these pathways, which is similar to how you will be measuring these functions in cardiomyocytes. Thank you for sharing with me information on, and a demo of, the MultiCell HTS platform that automates single cell physiological assessments across various cell types. My laboratory would be interested in using this platform to test tumor cell electrophysiology functions for our ongoing studies.

Good luck with your proposal, and let me know if I can be of any assistance.

Best wishes,

Frederick Varn, Ph.D.
Assistant Professor
The Jackson Laboratory

Quotation Form

IonOptix LLC
396 University Ave.
Westwood, MA 02090
USA
t: +1 617 696-7335
e: info@ionoptix.com
f: +1 617 698-3553
w: www.ionoptix.com

Dr. Travis Hinson
JAX Center for 3D Genomics and Biology
Farmington, CT

Quote Number	**104446**
Creation Date	**3/20/2023**
Salesperson	Hanan Qasim
Valid For	30 days
Currency	USD

Qty	Description	Unit Price	Line Total
	IonOptix Fura-2 Calcium and Contractility MultiCell HTS for Primary and iPSC Cardiomyocytes		
	Hardware		
1	CYCY100 -- CytoCypher HTS Motorized Microscope System - Includes fast x, y, z motorized objective positioning, stable environment temperature control, easy preparation access, perfusion and accessory ports	115,200.00	115,200.00
1	IAC100DM -- Interface and acquisition core - includes system interface with FPGA chip and acquisition-configured workstation with I/O card	11,200.00	11,200.00
1	LED100D -- Dual Excitation LED Light Source. Compact, optimized, stable LED light source for 340nm / 385nm ratiometric Fura-2 fluorescence illumination. Includes light guide, microscope cube and epi-fl adaptor, dye optics, beam combiner and optics.	14,400.00	14,400.00
1	OP-Fura-HS -- Fura-2 Optics Package for HyperSwitch/LED Sys. (not sold separately; requires additional filter holders)	1,900.00	1,900.00
1	**DISCOUNT -- First Optics Included**	-1,900.00	-1,900.00
1	CFA-CY -- Cell framing adapter for CytoCypher MultiCell HTS (requires 1X C-Mount adapter)	2,800.00	2,800.00
1	PMT400 -- Photomultiplier sub-system	4,100.00	4,100.00
1	MCS300 -- MyoCam-S3™ Fast CMOS video system	7,500.00	7,500.00
1	MYP100 -- MyoPacer Field Stimulator	3,900.00	3,900.00
1	CYPAC35 -- Pacing block for 35 mm dish	1,100.00	1,100.00

1	OBJ2-40X0.95 -- Olympus UPLXAPO 40X Objective (NA 0.95, WD 0.18mm) for Ca Photometry	4,700.00	4,700.00
	MultiWell Add-on		
1	MWL100 -- MultiWell Motorized Stage for CyCy microscope (requires upgrade to Series 4 equivalent; concurrent bearing service/tuning recommended)	19,620.00	19,620.00
1	STS-6M-CAB-KIT-S2 -- StimSwitch cabling for CytoCypher HTS series 2 and newer CyCy microscopes	100.00	100.00
1	STS-6M.100 -- StimSwitch-6M six channel manual multiplexer permits routing high voltage stimulation signals	1,500.00	1,500.00
1	STS6M-MYP-CAB -- MyoPacer to StimSwitch adapter cable	200.00	200.00
	Perfusion Pump		
1	CY-FLO50 -- CytoCypher Balanced Flow Continuous Perfusion Pump w dual 10 ml inlet pumps and single 50 ml outlet pump (includes stand-alone control software)	20,700.00	20,700.00
	Software		
1	IONWIZ -- IonWizard Core + Analysis	4,600.00	4,600.00
1	CYTMOT -- CytoMotion Pixel Intensity and Correlation Acquisition add-on	2,300.00	2,300.00
1	SARACQ -- SarcLen™ Sarcomere Length Measurement add-on	2,100.00	2,100.00
1	EDGACQ -- SoftEdge™ Edge-Detection add-on	2,100.00	2,100.00
1	PMTACQ -- PMT Acquisition add-on	800.00	800.00
1	CYCHTA -- CytoCypher CytoSolver Batch Analysis (includes 1 year subscription)	4,800.00	4,800.00
1	CYCCFI -- CytoCypher Mark and Find Automated Cell Finder	14,700.00	14,700.00
1	MULACQ -- MultiCell Acquisition add-on for IonWizard	2,100.00	2,100.00
	Sub-Total		240,520.00
1	SIH -- Shipping, insurance and handling - charged at cost	700.00	700.00
1	TRAV -- Travel Costs - charged at cost	2,000.00	2,000.00
1	INST -- On-Site Installation and Training	1,800.00	1,800.00
	Quote Total USD		**245,020.00**

Terms of Trading: Terms of sale: DAP (quotation excludes Sales Tax and/or VAT as well as taxes or duties that may have to be paid on import). Terms of credit: Net 15 (payment is in advance except for pre-approved accounts where 15 days net credit applies). A 3% transaction fee will be charged for

purchases made by credit card. Goods remain the property of the seller until paid for in full. Interest and debt-recovery charges may be applied to overdue payments. Non-enforcement of any of the sellers rights or terms will not constitute a waiver of the sellers right to invoke and enforce such terms in the future. Warranty: 1 year parts and labor. Please send purchase orders to info@ionoptix.com.

CALCIUM & CONTRACTILITY SYSTEMS

Characterizing Excitation-Contraction Coupling in Cardiomyocytes

Standard C&C
Standard Data Acquisition
(~50 cells/day)
×1

When we add **Motorized Stage & MultiCell Modules** enables auto cell finding and repeated measures

MultiCell Lite
Median Data Acquisition
(~100 – 500 cells/day with repeated measures)
×10

By using **CytoCypher Microscope** Eliminates the need of dark room or temperature control

MultiCell HTS*
High throughput data
(~ 500 - 1000 cells/day with repeated measures)
×20

Motorized MultiWell Stage Accommodates cell culture plates

MultiCell PLUS HTS* (MultiWell HTS)
High throughput data
(> 1000 cells/day with repeated measures)
> ×20

	Standard C&C	MultiCell Lite	MultiCell HTS	MultiCell PLUS HTS
System Hardware Components and Specifications				
Built-in Temperature Control	✗	✗	✓	✓
Automatic Cell Finding & Repeated Measures	✗	✓	✓	✓
Motorized Stage	✗	✓	✗	✓
Motorized Lens	✗	✗	✓	✓
Requires a Dark Room	✓	✓	✗	✗
Requires Vibration Isolated Table	✓	✓	✓	✓
Accommodates Cell Culture Plates	✗	✓	✗	✓
Paced & Perfused Dishes/Well Plate options				
35 mm dish	✓	✓	✓	✓
24-well plate (6 wells paced at a time)	✗	✓	✗	✓

- Both the **MultiCell Lite** system and the **MultiCell PLUS HTS** can accommodate any cell culture plate. However, only 24-well plates can be perfused & electrically stimulated.
- **MultiCell HTS** can also accommodate cell culture plates. However, plates must be moved manually while **MultiCell Lite** and **MultiCell PLUS HTS** automate plate movement.

JOB NO.
THE JACKSON LABORATORY
PROJECT EXPENDITURE REPORT

April 10th, 2023

Sponsor: Alan Sawyer/Charles Lee

Estimated Cost: $245,020.00
Price after negotiations: $245,020.00

Client: Travis Hinson, M.D. – Assistant Professor
Project Manager: Ryan E. Pelkey
TITLE: CytoCypher HTS Motorized Microscope System

Description and Justification:

CytoCypher high-throughput (HTS) cell physiology screening platform enables cellular physiology and screening particularly for contractility and Ca2+ flux/membrane potential analyses. The system has been utilized by scientists studying cardiac and skeletal myocytes, neurons and cancer cells including islet cell physiology (Ca2+ flux is critical to islet insulin regulation) and neurophysiology particularly ion channel flux and all cells that generate motion or cycle ions across membranes). There are multiple JAX PIs whose research could benefit greatly from having access to this system.

Location, space, and oversight TBD, pending results of DIF approval.

Payback:	Payback period (if any)	Cost Savings:	No
Estimated Life:	10 yrs.	Increased Capacity:	Yes
Asset Replaced:	No	Improved Conditions:	Yes
Cube Related:	No	Necessity:	Yes
CPS#:	DIF Submission	Baseline Budget Adjustment	No

Approved by: Ryan E. Pelkey _____date:_____

Alan Sawyer_____date:_____

Andy Greene _____date:_____

Jay Vetelino _____date:_____

Doug Abbott _____date:_____

Catherine Longley _____date:_____

Lon Cardon _____date:_____

Activity Number: TBD

Capital Budget Balance

THE JACKSON LABORATORY
PEP SUPPLEMENT FOR SCIENTIFIC EQUIPMENT

Activity Number: TBD

GENERAL COUNSEL COMPONENT:
To be filled out by General Counsel Services

Approved by: *General Counsel* _____date:_____
Daniel Hoag

BUDGETS AND PLANNING COMPONENT:
To be filled out by Financial Services

Approved by: *Financial Services* _____date:_____
Michael Dearing

SCIENTIFIC INSTRUMENT SERVICES COMPONENT: See Equipment Procurement Checklist. Location, space, and oversight TBD, pending results of DIF approval.

Approved by: *Scientific Instrument Services Manager* _____date:_____
Ryan E. Pelkey

FACILITIES ENGINEERING COMPONENT:
To be filled out by Facilities Engineering - Description of work to be performed, estimated duration, and costs.

Approved by: *Engineering* _____date:_____
Norm Burdzel

IT COMPONENT:
To be filled out by Information Technology - Description of work to be performed, estimated duration, and cost. IT network, data storage requirements, LIMS requirements.

Approved by: *IT Manager* _____date:_____
Brendan Arbuckle

EHSS & RADIATION COMPONENT:
EHSS: To be filled out by EHSS - Description of any EHSS concerns/impacts.

Approved by: *EHSS Manager* _____date:_____
Izabela Puskarz or Mike Lee

Radiation Safety: To be filled out by Radiation - Description of any Radiation or Laser concerns/impacts.

Approved by: *Radiation Safety Officer* _____date:_____
Julie Alderman