AWARD NUMBER: W81XWH-18-1-0402

TITLE: Dissecting the Heterogeneity of Human Islet Stress Responses in Type 2 Diabetes

PRINCIPAL INVESTIGATOR: Duygu Ucar

CONTRACTING ORGANIZATION: The Jackson Laboratory
Farmington, CT 06032

REPORT DATE: Sept 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2019	Annual	1 Sept 2018-31 Aug 2019

4. TITLE AND SUBTITLE
Dissecting the Heterogeneity of Human Islet Stress Responses in Type 2 Diabetes

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0402

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Duygu Ucar

E-Mail: Duygu.Ucar@jax.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Jackson Laboratory for Genomic Medicine
10 Discovery Drive
Farmington, CT 06032-2374

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall objective of this project is to dissect the genetic regulation of islet stress responses and to determine how genetic variants, including those associated with type 2 diabetes (T2D SNPs), modulate these responses to contribute to islet dysfunction and T2D pathogenesis. This project will address significant gaps in our knowledge of the genetic and cellular heterogeneity of T2D by 1) defining the precise transcriptomic and epigenomic alterations in islets associated with diabetogenic oxidative/metabolic and inflammatory stressors; 2) determining how each islet cell type responds to each stressor; 3) identifying individual genetic variants, particularly T2D SNPs, that modulate these responses to increase or decrease diabetes susceptibility; and 4) testing whether the genes and pathways induced by these stressors are compensatory/protective or pathogenic. In the first year of this project, we have completed longitudinal profiling of islets from six individuals and identified 1,382 islet genes that are consistently induced (n=967) or repressed (n=415) by oxidative and/or inflammatory cytokine stresses. This gene set included two genes, *C2CD4A* and *C2CD4B,* which we have recently identified as putative T2D GWAS effector genes, as well as 142 additional putative T2D GWAS effector genes based on reported positional candidate scores. Preliminary single cell transcriptome analyses have revealed provocative differences in both the dynamics and features of transcriptional stress responses in islet insulin-secreting beta *vs.* glucagon-secreting alpha cells. CRISPR/Cas9 gene knockout/inactivation links approximately 5% of the stress-repressed genes (n=20/415) to impaired viability/proliferation or reduced insulin content in MIN6 beta cells, suggesting their repression by oxidative or inflammatory cytokine stressors harmful to beta cells. Efforts are ongoing to define cytokine and oxidative stress-responsive transcriptomes and epigenomes from 100 individuals and link naturally occurring genetic variation, including T2D SNPs to enhanced or impaired responses to these stressors. Together, these encouraging Year 1 results demonstrate the early insights generated and potential power of the integrated (epi)genomic profiling and (epi)genome editing approaches to identify modulators of islet cell resilience or failure and to contextualize the genetic and cellular heterogeneity underlying T2D risk and progression.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
		Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

	Page
1. Introduction	1
2. Keywords	1
3. Accomplishments	1
4. Impact	9
5. Changes/Problems	10
6. Products	11
7. Participants & Other Collaborating Organizations	13
8. Special Reporting Requirements	17
9. Appendices	18

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The *overall objective* of this project is to determine how islet cells respond to type 2 diabetes (T2D)-provoking stress conditions and to understand how naturally occurring genetic variants (particularly T2D-associated SNPs) perturb these responses to contribute to islet dysfunction and T2D pathogenesis. To this end, we will use cutting-edge sequencing and genome editing technologies to study how islet cells behave after exposure to oxidative and inflammatory stress. We will measure changes in the genome as a result of these two stress conditions in human islet samples from 100 non-diabetic organ donors. We will uncover how DNA sequence variation alters these stress responses. Finally, we will assess how the stress-induced or repressed genes affect 1) beta cell viability, 2) insulin content, and 3) exacerbation or amelioration of the stress responses.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Islet, ATAC-seq, RNA-seq, cytokines, chromatin accessibility, quantitative trait locus (QTL), response expression (reQTL), oxidative stress, interleukin-1beta (IL-1ß), interferon gamma, inflammatory cytokines, massively parallel reporter assay (MPRA), CRISPR/Cas9, genetics, single cell RNA-seq (scRNA-seq), peroxide

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major goals for this project are as follows:
AIM 1: Identify genetic variants altering human islet oxidative and inflammatory transcriptional stress responses
AIM 2: Elucidate genetic effects on stress response regulatory element (RE) use in human islets
AIM 3: Identify genes modulating beta cell oxidative stress responses using CRISPR/Cas9

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) **Major activities** in this reporting period are reported by Aim below:

Aim 1: Identify genetic variants altering human islet oxidative and inflammatory transcriptional stress responses. **a) Major Task1, Subtask 2:** Generated RNA-seq libraries from 20 non-diabetic islet donors under steady state conditions or after exposure to oxidative and inflammatory cytokine stressors (60 total; 3 libraries for each individual) *(Mr. Khetan)*; **b) Major Task 1, Subtask 3:** Processed and analyzed RNA-seq libraries of islets from six individuals exposed to peroxide or inflammatory cytokines for different times (see **Table 1**) to identify differentially expressed genes; initial pathway, GO, and GSEA analyses and overlap with T2D-associated genes (*Drs Ucar and Youn, Mr. Lawlor)*; **c) Major Task 2, Subtask 2:** Begin comparison of transcriptional differences between two stressors *(Drs. Ucar and Youn, Mr. Lawlor);* **d) Major Task 3, Subtask 2:** Analysis of time course single cell transcriptome profiling data of islets from one donor under steady state conditions and after exposure to oxidative (2 hours) and inflammatory cytokine (4 and 24 hours) stressors *(Dr. Ucar, Mr. Lawlor)*;

Aim 2: Elucidate genetic effects on stress response regulatory element (RE) use in human islets. **a) Major Task1, Subtask 1:** Generated ATAC-seq libraries from 20 non-diabetic islet donors under steady state conditions or after exposure to oxidative and inflammatory cytokine stressors (60 total; 3 libraries for each individual) (*Dr. Ucar and Mr. Khetan)*; **b) Major Task 3, Subtasks 1 and 2:** We have tested an initial set of T2D-associated SNPs and those that alter *in vivo* chromatin accessibility in human islets for their effects on transcriptional activity using MPRA and have designed a new panel of T2D 99% credible set SNPs based on the most recent meta-analyses by Anubha Mahajan and colleagues in the DIAMANTE Consortium, published in *Nature Genetics* in August 2018 (*Dr. Ucar and Mr. Khetan).*

Aim 3: **Identify genes modulating beta cell oxidative stress responses using CRISPR/Cas9. a) Major Task 1, Subtask 3:** We have analyzed phenotype data from the MIN6 GeCKO CRISPR screens to identify inactivated genes that 1) alter beta cell viability/proliferation and 2) modulate insulin content *(Dr. Ucar and Mr. Khetan)*

All activities were completed at JAX-GM in collaboration with Dr. Stitzel (Initiating PI)

2) **Specific objectives** in this reporting period were to:
   - To determine how oxidative and inflammatory cytokine stressors alter gene expression and gene regulation in 30 individuals/donors using RNA-seq and ATAC-seq of islets.
   - To complete longitudinal profiling of these responses and identify a comprehensive set of oxidative and inflammatory cytokine stress-responsive genes.
   - To identify common and cell type-specific transcriptional responses of each islet cell type using single cell transcriptome profiling.
   - To identify putative transcriptional regulatory sequences and determine T2D SNP effects on their activity using massively parallel reporter assays (MPRA).

3) **Significant results/Key outcomes**

Identification of oxidative and inflammatory cytokine stress-responsive genes in islets:

Longitudinal RNA-seq profiling of islets from six different individuals exposed to cytokine and oxidative stress agents (**Table 1**) identified approximately 12% (1382/11,536) of expressed genes as stress-responsive, with 967 induced and 415 repressed. The 97 and 13 genes induced or repressed, respectively, by oxidative stress extensively overlap those modulated by inflammatory cytokines. The set of differentially expressed genes included two genes *C2CD4A* and *C2CD4B*, which we have recently identified as putative T2D effector genes and 142 additional genes that were recently reported as putative type 2 diabetes positional candidate effector/target genes, suggesting that genetic variants that modulate expression of these genes upon stress in islets may contribute to genetic risk for islet dysfunction and T2D. To determine the potential functional effects of the differentially expressed genes identified, we compared the set of repressed genes to those whose knockout altered cell viability or insulin content in the genome-scale CRISPR Knockout (GeCKO) screen in mouse MIN6 beta cells. Approximately 5% (20/415) of the repressed genes significantly reduced beta cell viability/proliferation (n=14) or insulin content (n=6; *FOXA2, SOX4, GPR116, RAMP2, SEMA5A, and MIS18BP1*) reduced insulin content under steady state conditions. In the upcoming period, we will expand upon these encouraging preliminary hits and insights to test, in a targeted fashion, if the 1382 differentially expressed genes identified exacerbate or ameliorate inflammatory and oxidative stress responses.

Table 1: RNA-seq islet stress response time points

Treatment	Time points (hrs)	Total # of measurements
Baseline	0, 2, 24	3
H2O2	1, 1.5, 2	3
IL1B + IFN-γ	1, 2, 4, 16, 24	5

X 6 Biological replicates

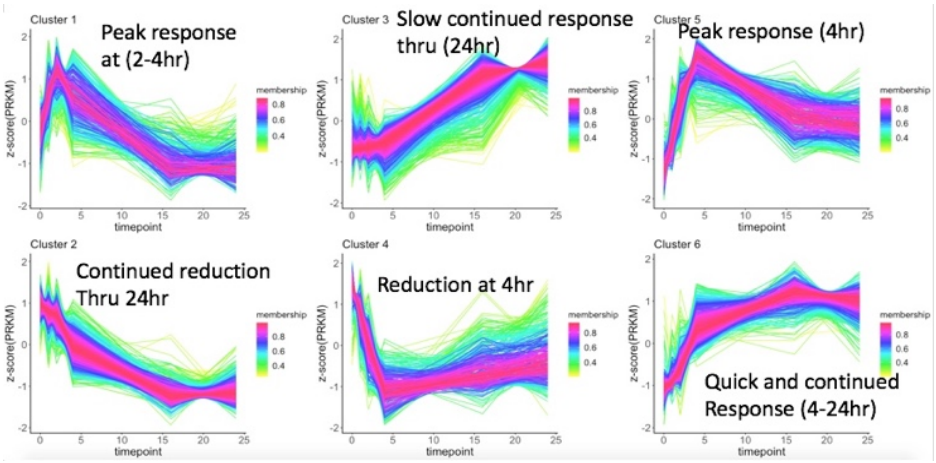

Figure 1. Distinct temporal expression patterns of cytokine-responsive islet genes

The majority of cytokine-responsive islet genes were detected 4 and 16 hours after treatment, leading us to hypothesize that longer exposures to peroxide may similarly identify more genes and perhaps more divergent gene sets that mediate islet oxidative stress responses. To test this hypothesis and identify both 1) an inclusive set of response genes to functionally assess using CRISPR in Aim 3 and 2) additional genes wherein regulatory variants may modulate this islet stress response, we will complete RNA-seq from islets exposed to peroxide for ≥16 hours. As shown in **Figure 1**, cytokine-responsive islet genes grouped into 6 gene clusters with different temporal patterns of cytokine-induced and repressed gene expression and are completing gene set enrichment analysis (GSEA), gene ontology (GO) and KEGG pathway analyses to determine the processes and pathways that are modulated and pathway analyses to identify the role(s) of the genes exhibiting these distinct patterns of gene expression, suggesting there are early, intermediate, and late phases of islet stress responses. We are currently completing analysis to determine the KEGG pathways and gene ontology (GO) processes enriched in each of these gene clusters. As expected, preliminary

analyses suggest that early induced genes mediating antigen processing and presentation are rapidly induced, while genes facilitating insulin production, processing, and secretion are repressed by cytokine treatment.

Cell type specificity of islet oxidative and inflammatory cytokine stress responses

We have completed our first round of single cell profiling to determine how oxidative and inflammatory cytokine stressors affect each islet cell type and identify potential cell type-specific responses to these conditions. Recently, a cell hashing approach has been developed that uses antibodies to widely expressed proteins (B2M and CD298) to label different groups of cells with distinct nucleotide barcodes (HHTO). HHTO-labeled cells can then be pooled and sequenced together, and the HHTOs can be used to assign each single cell transcriptome profile to the specific condition/timepoint. As shown in **Figure 2A**, we have successfully applied cell hashing to assess islet cell type-specific responses to oxidative and cytokine stress responses. Preliminary principal component/dimension reductionality analyses suggest that both qualitative and dynamic aspects of cytokine-induced alpha and beta cell response programs differ. For example, **Figure 2B** shows that alpha cells in the 4-hour exposure group (red) cluster farther from the untreated alpha

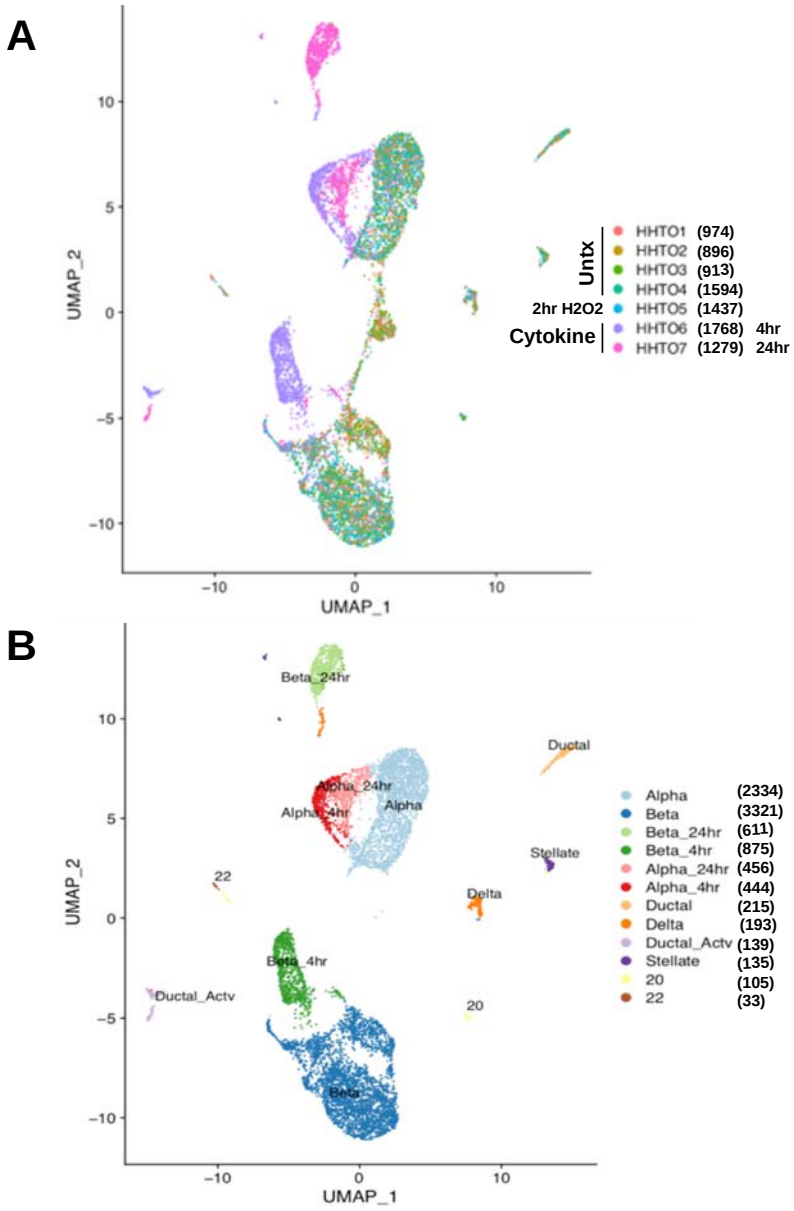

Figure 2. Islet cell type-specific differences in extent and dynamics of stress responses. (A) Distinct clustering of cells with hashtag oligos (HHTOs) corresponding to steady state (untx) or stress agent-treated conditions as indicated. Number of cells per HHTO (condition) are indicated in parentheses. **(B)** Single cell transcriptome analyses detect distinct clustering of steady state and stressed cells, particularly cytokine-treated alpha and beta cells treated with cytokines. Islet cell types were assigned by hormone marker gene expression (e.g., *INS* for beta cells, *GCG* for alpha cells, etc.)

TABLE 1. MPRA Designs and targets		
MPRA features	**MPRA 1.0**	**MPRA 2.0**
T2D-associated loci covered	207	342
T2D SNPs (best-associated or genetically-linked)	2547	27,551
Chromatin-accessibility modulating (caQTL) SNPs	2020	0
Positive controls (SNPs overlapping NFE2L2, DDIT3, GLIS3, HIF1A, XBP1 sequence motifs)	156	1000
Negative controls (non-islet-caQTL; no OCR overlap)	2150	1449
Total elements (Total alleles) tested	6873	30,000

cells (light blue) than those in the 24-hour exposure group (pink), suggesting that alpha cell cytokine responses are rapidly induced by 4 hours and begin to resolve by 24 hours after exposure. In contrast, beta cells appear to execute a distinct, and perhaps irreversible, cytokine response, as 24 hour-exposed beta cells (light green) cluster more distantly from untreated beta cells (dark blue) than 4 hour-exposed beta cells (dark green). We will complete pseudo-time analyses of these data in the upcoming project period to formally test this model and are in the process of completing comparative analyses of beta and alpha cell gene expression at each time point to identify the specific genes, gene sets, and pathways that are common and unique to each cell type's response.

Functional identification of transcriptional cis- regulatory element sequences and type 2 diabetes-associated variant effects on their activity.

One key goal of this study was to test putative islet/β cell regulatory sequences for enhancer activity, and identify SNPs that alter their regulatory activity. As a step toward this goal and to test the system, we created and tested a massively parallel reporter assay (MPRA) library (**Figure 3A and Table 1, MPRA1.0**) comprising 13,764 200-base pair sequences overlapping i) SNPs associated with altered human islet chromatin accessibility (caQTLs); ii) SNPs overlapping human islet ATAC-seq peaks, but not associated with altered chromatin accessibility (not-caQTLs); and iii) a set of 2500 SNPs in LD (r2>0.8) with T2D-associated index SNPs from the NHGRI/EBI GWAS catalog (T2D-associated SNPs/indels).

To test these putative islet/β cell regulatory sequences for enhancer activity, we transfected the MPRA plasmid library into five independent cultures of MIN6 mouse β cells. The transfected cells were harvested 30 hours later for RNA isolation, *GFP* mRNA capture, and Illumina sequencing of barcodes in the captured mRNAs (**Figure 3B)**. 2224 out of 13,764 sequences (16.2%) produced significantly higher counts (FDR<1%) after transfection compared to the plasmid library input, and were thus identified as enhancers (**Figure 3C**). As anticipated, sequences with enhancer activity were

significantly enriched for *in vivo* binding by islet-specific TFs (PDX1, NKX6.1 and FOXA2; ChIP-seq in human islets; **Figure 3D**).

In addition to testing putative islet/β cell regulatory sequences for enhancer activity, another major objective was to identify SNPs that modulate regulatory element activity. For elements with one or both alleles showing significant enhancer activity, we therefore assessed allelic differences in enhancer activity (see schema in Figure 3B) under each of the three experimental conditions tested. In total, 879 elements exhibited allelic differences in enhancer activity at FDR <10%. After identifying SNPs with an allelic skew in enhancer activity, we investigated the correlation between SNP effects on *in vivo* islet chromatin accessibility (caQTLs) and *in vitro* MPRA enhancer activity. 297/1829 caQTLs were characterized by a significant difference in MPRA enhancer activity. As shown in **Figure 4**, caQTL and MPRA directions-of-effect for these 297 SNPs was significantly correlated (Pearson R = 0.526), i.e., alleles associated with higher chromatin accessibility had higher MPRA activity.

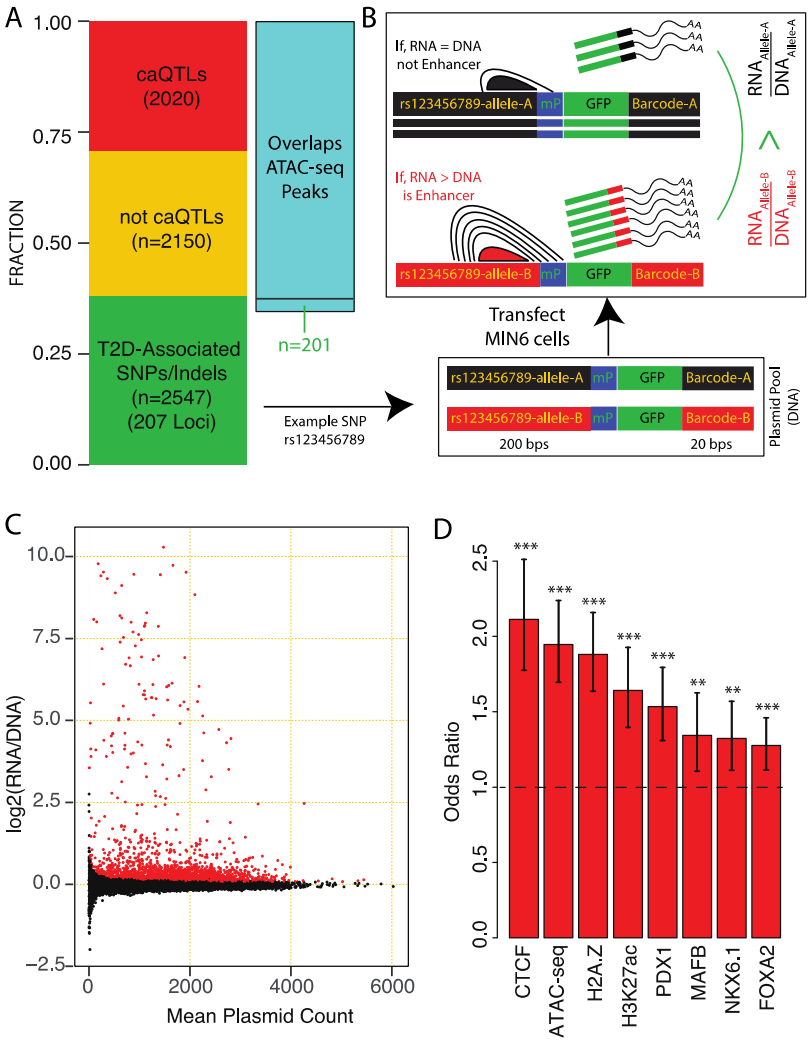

Figure 3. Massively parallel reporter assays identify active transcriptional regulatory sequences. (A) Characteristics of selected SNP-containing sequences. **(B)** Schematic of experimental approach. **(C)** Scatter plot of RNA-seq (y-axis) vs. plasmid library sequencing (x-axis) reads. Red dots denote sequences exhibiting significant (FDR<1%) enhancer activity in MIN6 beta cells. **(D)** MPRA active sequences are enriched for empiric *in vivo* measures of regulatory elements.

One key challenge to translate T2D GWAS associations into molecular understanding is to determine which SNPs in LD with the reported index SNPs are the functional/causal variants. Here, we tested 2500 T2D-associated index or linked (r^2>0.8) SNPs at 259 loci reported in the NHGRI/EBI GWAS Catalog for MPRA enhancer activity in MIN6 beta cells. One or both alleles at 492/2500 T2D-associated elements were identified as enhancers. Out of 492 elements, SNPs within 220 elements mapping to 104 distinct T2D-associated loci also had a significant allelic skew in enhancer activity.

For ~50% of T2D-associated loci (54/104), only one SNP was found to have an allelic skew among all the SNPs tested, and therefore, represents a putative causal SNP with strong potential for additional exploration (**Figure 5**). For example, out of 49 SNPs LD with the T2D-associated index SNP rs73230612 at the *ZBTB20* locus, only 1 SNP (rs987964) was observed to show allelic skew in enhancer activity. However, the number of SNPs with allelic skew in enhancer activity was found to generally scale with the number of SNPs tested per loci. For example, out of 45 SNPs in LD with the index SNP rs12304921 at the *HIGD1C* locus, 10 had an allelic skew in enhancer activity, not all of which were in the same direction. While we cannot rule out multiple causal alleles, and such loci are of notable interest; these regions underscore the use of MPRA as a preliminary screen where additional follow-up is critical. We expect that MPRA will be a key screening tool in the upcoming project period to identify SNPs of particular interest for their potential effects on *in vivo* chromatin accessibility and stress-responsive gene expression. We will screen a new MPRA library (**Table 1, MPRA 2.0**) containing recently reported T2D 99% credible set SNPs to identify sequences, and T2D SNP alleles, that mediate and modulate oxidative and inflammatory cytokine stress-induced transcriptional responses.

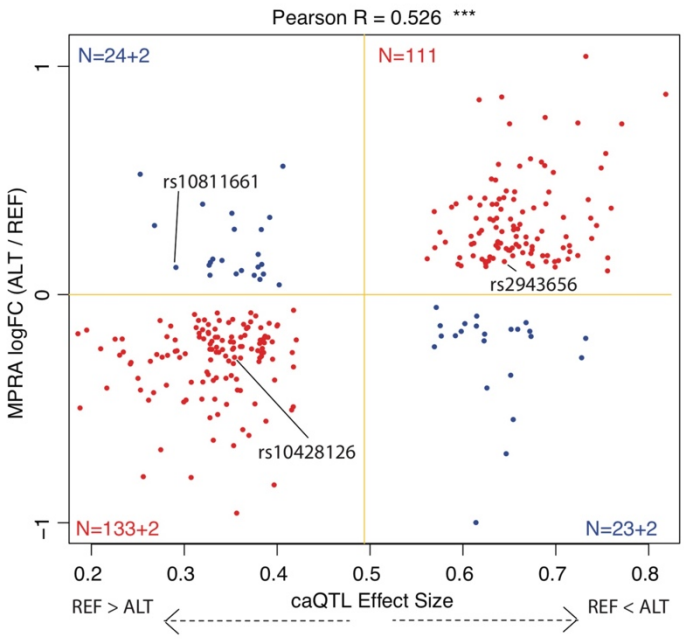

Figure 4. SNP allele effects on *in vitro* MPRA activity and *in vivo* islet chromatin accessibility are correlated. Scatterplot indicating the relative MPRA activity (y-axis) and *in vivo* chromatin accessibility (x-axis) of the alternate and reference alleles for 297 previously reported islet caQTLs.

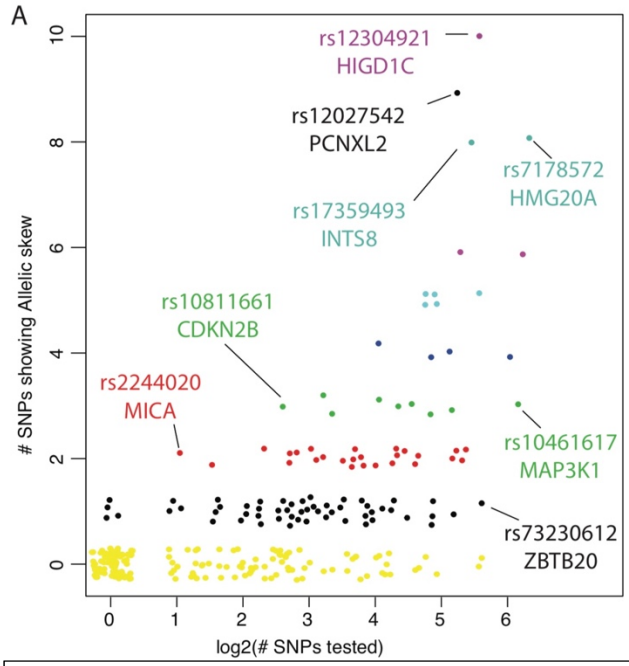

Figure 5. MPRA nominates putative expression-modulating variants among genetically-linked T2D-associated SNPs. The scatterplot shows the number of SNPs showing allelic MPRA effects (y-axis) compared to the number of putative functional variants (x-axis, log2 scale) in each of 207 T2D-associated loci tested.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We are currently preparing a manuscript describing the identification of transcriptional enhancer sequences and type 2 diabetes-associated SNP alleles that alter this enhancer activity using massively parallel reporter assays (MPRA) in MIN6 beta cells. We anticipate submitting this manuscript to *Nature Communications* or *Genome Research* and depositing it in *bioRxiv* in October 2019.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

1) RNA-seq of steady state, oxidative stress, and inflammatory cytokine stress treated islets from ≥50 individuals (yielding 80 individuals, 240 libraries total) and compare differential expression patterns between stressors. (**Aim 1)**
2) Complete and analyze single cell transcriptome profiling of untreated and treated islets (as shown for **Figure 2**) from five additional individuals to identify islet cell-specific patterns and responses. Write and submit a manuscript describing this study and results **(Aim 1)**
3) Chromatin accessibility (ATAC-seq) profiling of steady state, oxidative stress, and inflammatory cytokine stress treated islets from ≥50 individuals (yielding 80 individuals, 240 libraries total). Begin differential chromatin accessibility analyses and TF footprinting/motif analyses. **(Aim 2)**

4) Complete MPRA 2.0 library experiments and analyses in EndoC-ßH3 cells exposed to peroxide and inflammatory cytokines. Begin comparing MPRA results to *in vivo* islet chromatin accessibility and gene expression data generated in Aims 1 and 2. **(Aim 2)**
5) Phenotyping of EndoC cells transduced with GeCKO and SAM gRNA libraries. (**Aim 3**)
6) Design, synthesize, and test custom gRNA libraries for the 1382 stress-responsive genes identified in Aim 1. (**Aim 3**)

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We have procured islets from 20 out of the 30 proposed donors (66%) for Year 1 of this project. Islet availability and frequency varies seasonally, so we anticipate Fall and Winter of this upcoming will yield more samples. Dr. Stitzel has also sought alternative sources of human islets and has reached out to Dr. Patrick MacDonald, Professor, at the University of Alberta Diabetes Center. Dr. MacDonald's diabetes center isolates pancreatic islets from Canadian organ donors, which is a separate source of donors Integrated Islet Distribution Program (IIDP) and ProdoLab. Importantly, his group has shipped islets to distant research sites in Europe, and he now provides a portion of each islet isolation to the IIDP's human islet pancreas program (HIPP) for their analyses, so these islets will have the same valuable QC and physiologic data as current islets we are procuring from IIDP and Prodo. We will discuss this additional potential source of islets with the program officer to obtain approval and ensure all HRPO human tissue criteria are satisfied prior to arranging for procurement.

We have not had major changes in this reporting period, but we do anticipate adding a portion of a new MD/PhD student's (Mr. Redwan Bhuiyan) effort to this project in Year 2 and up to 100% effort in year 3. Dr. Ucar and I anticipate Mr. Khetan will be writing his PhD thesis and graduating in May/June of 2020 (Year 2), and believe adding Mr. Bhuiyan will be critical to ensure a smooth transition and successful completion of this project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report/Not Applicable

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Cell line: Engineered MIN6 mouse beta cell line containing 1) sfGFP integrated into the C-peptide-encoding portion of the endogenous *Ins2* locus; 2) Cas9 effector protein; and 3) mCherry.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Duygu Ucar
Project Role: Partnering PI
Researcher Identifier: https://orcid.org/0000-0002-9772-3066
Nearest person month worked: 3 CM
Contribution to Project: Design and implementation of analytical pipelines; managing and coordinating personnel and the analyses they complete; discussing results and writing reports; supervising manuscript preparation for MPRA study

Name: Shubham Khetan
Project Role: Graduate student
Researcher Identifier: N/A
Nearest person month worked: 12 CM
Contribution to Project: Design, completion, and analysis of MPRA1.0 experiments in MIN6; writing MPRA manuscript; design, completion, and analysis of GeCKO experiments in MIN6

Name: Nathan Lawlor
Project Role: Data analyst
Researcher Identifier: http://orcid.org/0000-0003-3263-6057
Nearest person month worked: 4 CM
Contribution to Project: Implementation of RNA-seq and ATAC-seq pipelines; analyze RNA-seq data, differential expression analyses; complete single cell RNA-seq HHTO oligo deconvolution and transcriptome analyses; assist in MPRA and GeCKO data analyses

Name: Ahrim Youn
Project Role: Computational biologist
Researcher Identifier: https://orcid.org/0000-0002-2158-9307
Nearest person month worked: 4 CM
Contribution to Project: Establishment of statistical pipelines for upcoming data analyses including caQTL analyses, transcription factor footprinting analyses from ATAC-seq data, and differential analyses.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Changes in Active OS

Supporting Agency:	*NIH/NIAID 1 R01 AI141609-01*	***PI:***	*Williams*
Project Title:	*lncRNA Control of Airway Epithelial Cell Responses to Type 2 Inflammation*		
Role:	*Co-Investigator*	***Effort:***	*0.60 CM*
Entire Project:	*01/01/2019 – 12/31/2022*	*$2,530,196*	
Current Year:	*01/01/2019 – 12/31/2020*	*$597,025*	
Project Goals:	*The goal of the proposal is to determine the mechanism by which the long non-coding RNA (lncRNA) WFDC21P controls response of the airway epithelium to IL13.*		
Specific Aims:	*Aim 1, Determine which cell types upregulate WFDC21P following IL13 exposure, and identify which transcription factors (TFs) control its expression; Aim 2, Determine which cell types require WFDC21P to drive IL13-mediated asthma pathology; Aim 3, Identify the mechanism used by WFDC21P to regulate epithelial STAT signaling.*		
Overlap:	*None*		

Supporting Agency:	*NIH/NIAID DK117137-01A1*	***PI:***	*Stitzel*
Project Title:	*Regulation and Function of the Type 2 Diabetes-Associated C2CD4A/B Locus*		
Role:	*Asst. Professor*	***Effort:***	*0.30 CM*
Entire Project:	*01/01/2019 – 11/30/2023*	*$2,101,309*	
Current Year:	*01/02/2019 – 11/30/2019*	*$597,025*	
Project Goals:	*Our overall goal is to understand the islet/beta cell regulation and function of the C2CD4A/B locus in physiologic and diabetogenic states.*		
Specific Aims:	*Aim 1, Determine the regulation of the C2CD4A/B locus; Aim 2, Elucidate the roles of C2cd4a and C2cd4b in beta cell function and GSIS; Aim 3, Establish the roles of the C2cd4a/b locus in diabetes pathogenesis.*		
Overlap:	*None*		

Supporting Agency:	*NIH/NIA 1 R13 AG064968-01A1*	***PI:***	*Ucar*
Project Title:	*Systems Biology of Aging: Data-science meets Gero-science*		
Role:	*Principal Investigator*	***Effort:***	*0.60 CM*
Entire Project:	*08/27/2019 – 07/31/2020*	*$38,566*	
Current Year:	*08/27/2019 – 07/31/2020*	*$38,566*	
Project Goals:	*The funds requested in this R13 application are for partial support of "Systems Biology of Aging: Data-science meets Gero-science" annual meetings to be offered each August/September from 2019 through 2022 at The Jackson Laboratory for Genomic Medicine (JAX-GM) in Farmington, Connecticut. This meeting will bring together up to 150*		

	interdisciplinary scientists including molecular biologists, immunologists, computational biologists, and geriatricians, who share a common interest in understanding aging and aging-associated disease at the systems level.
Specific Aims:	*Aim 1, Organize an interdisciplinary meeting and hands-on workshop focused on aging and aging-related diseases; Aim 2: Promote interactions to foster collaborative research and career advancement; Aim 3: Recruit diverse attendees..*
Overlap:	*None*

Supporting Agency:	*NIH/NIA 5 UH3 AG056925-03*	***PI:***	*Banchereau*
Project Title:	*Physical Resilience: Indicators and Mechanisms in the Elderly (PRIME Collaborative)*		
Role:	*Consortium-Investigator*	***Effort:***	*0.60 CM*
Entire Project:	*09/30/2017 – 05/31/2022*	*$1,153,949*	
Current Year:	*06/01/2019 – 03/31/2020*	*$255,864*	
Project Goals:	*The overarching objectives of this project are to characterize specific resilience phenotypes, elucidate biological mechanisms, and validate clinically valuable predictive tools and measures of physical resilience.*		
Specific Aims:			
Overlap:	*None*		

Supporting Agency:	*Dept. of Defense W81XWH-18-1-0402*	***PI:***	*Ucar*
Project Title:	*Dissecting the Heterogeneity of Human Islet Stress Responses in Type 2 Diabetes*		
Role:	*Principal Investigator*	***Effort:***	*3.00 CM*
Entire Project:	*09/01/2018 – 08/31/2021*	*$849,888*	
Current Year:	*09/01/2019 – 08/31/2020*	*$278,229*	
Project Goals:	*The overall objective of this proposal is to dissect the genetic regulation of islet stress responses and to determine how genetic variants, including T2D-associated SNVs, modulate these responses to contribute to islet dysfunction and T2D pathogenesis.*		
Specific Aims:	*1. Identify genetic variants altering human islet oxidative and inflammatory transcriptional stress responses; 2. Elucidate genetic effects on stress response regulatory element (RE) use in human islets; 3. Identify genes modulating beta cell oxidative stress responses using CRISPR/Cas9.*		
Overlap:	*None*		

Supporting Agency:	*NIH/NIAID 1 U19 AI142733-01*	***PI:***	*Palucka*
Project Title:	*Modulation of Lung Immune Responses to Viral Infection*		
Role:	*Co-Investigator*	***Effort:***	*0.30 CM*
Entire Project:	*03/05/2019 -02/29/2024*	*$622,562*	
Current Year:	*03/05/2019 – 03/04/2020*	*$622,562*	

Project Goals:	*Project 1 will elucidate how the networks of antigen presenting cells (APCs) in the human lung regulate immunity to respiratory viruses. The goal is to also explain how microbiome-driven lung inflammation or inflammation that is linked with neoplastic processes affects such responses.*
Specific Aims:	*1) Test the hypothesis that steady state cellular and molecular networks in human lung tissue regulate the early response to respiratory viruses; 2) test the hypothesis that the generation of anti-viral T-cell immunity is modulated by lung epithelial cell (EC)-DC crosstalk and that this crosstalk is further modulated by commensal bacteria; 3) test the hypothesis that the lung microenvironment modulates the cross-presentation capacity of lung-resident APCs thereby dictating the fate of viral antigen-specific CD8+ T cells.*
Overlap:	*None*

Completed OS

Dept. of Defense *W81XWH-16-1-0130* *PI: Stitzel*
Single-Cell Dissection of Human Pancreatic Islet Dysfunction in Diabetes
12/01/2017 – 11/30/2018 *$350,000*
Role: Co-Investigator

Chan Zuckerberg Initiative *CZI 2018-182753* *PI: Ucar*
Single cell RNA-Seq analysis of immune activation using iteratively adjusted surrogate variable analysis
04/01/2018 – 09/30/2019 *$250,000*
Role: Principal Investigator

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report. All experiments and analyses were performed at JAX-GM.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*