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

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CONTRACTING ORGANIZATION: The Jackson Laboratory

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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 stressors and to understand how naturally occurring genetic variants (particularly T2D-associated SNPs) alter these responses to contribute to islet dysfunction and T2D pathogenesis. To this end, we are applying innovative sequencing and genome-editing technologies to study how islets behave after exposure to oxidative and inflammatory stressors. We will measure changes in the genome that result from these two stress conditions in human islet samples from 100 non-diabetic organ donors. We will determine how DNA sequence variants alter 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).*

   Human islets, ATAC-seq, RNA-seq, inflammatory cytokines, interleukin-1 beta (IL-1ß), interferon gamma, chromatin accessibility, quantitative trait locus (QTL), response expression QTL (reQTL), oxidative stress, peroxide, single cell RNA-seq (scRNA-seq), single nucleus ATAC-seq (snATAC-seq), CRISPR/Cas9, genetics

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:
   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 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 Task 1, Subtask 2:** Generated RNA-seq libraries from 50 non-diabetic islet donors under steady state conditions or after exposure to oxidative (peroxide) or inflammatory cytokine (IL-1ß, IFN-gamma) stressors (total 150 libraries; 3 for each individual) (*Mr. Khetan*); **(b) Major Task 1, Subtask 3:** Processed and analyzed RNA-seq libraries of islets from 20 individuals exposed to inflammatory cytokine and oxidative stressors *(Dr. Ucar, Mr. Lawlor)*; **(c) Major Task 3, Subtask 2:** Develop algorithms for better analyses of single cell datasets (Dr. Ucar and Mr. Karakaslar, Mr. Sharma)

   Aim 2: Elucidate genetic effects on stress response regulatory element (RE) use in human islets. **(a) Major Task 1, Subtask 1:** Generated ATAC-seq libraries from 50 non-diabetic islet donors under steady state conditions or after exposure to oxidative and inflammatory cytokine stressors (150 total; 3 libraries for each individual) (*Dr. Ucar, Mr. Khetan*); **Major Task 1, Subtask 2:** Analyses of ATAC-seq datasets (*Dr. Ucar, Mr. Lawlor, Mr. Karakaslar*) **Major Task 3, Subtask 2:** Completed amplification and quality control of MPRA 2.0 SNP library (*Dr. Ucar, Mr. Khetan)*; **Major Task 3, Subtask 3:** Optimized nucleofection conditions to deliver MPRA 2.0 SNP library into EndoC-ßH3 cells with >80% cell survival and >70% transfection efficiency (*Dr. Ucar, Mr. Khetan).*

   Aim 3: Identify genes modulating beta cell oxidative stress responses using CRISPR/Cas9.
   (a) Major Task 1, Subtask 2: Engineered Cas9 and dCas9-KRAB EndoC-ßH3. Currently testing (epi)genome editing efficacy of clones with targeted edits of *INS* and *PAXIP1* loci *(Dr. Ucar, Mr. Khetan).*

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

2) **Specific Objectives** in this reporting period were to:
   - Determine how oxidative and inflammatory cytokine stresses alter gene expression and gene regulation in 50 individuals using RNA-seq and ATAC-seq of islets.
   - Assess common and stress response-specific epigenetic and gene expression alterations in human islets
   - Identify putative T2D target/effector genes that are induced or repressed by cytokines or oxidative stress in islets
   - Identify stress-responsive regulatory elements that contain T2D SNPs and assess their effects on regulatory element use and/or activity.

3) Significant Results and Key Outcomes:

Identification of islet stress response genes (Aim 1):

To date, we have exposed islets from 50 different individuals to cytokines and peroxide. RNA-seq has been completed for 20 individuals. Comparison of these RNA-seq profiles revealed that approximately 12% (n=1370) of islet-expressed genes were ≥2-fold induced or repressed at FDR<5% by cytokines (**Table 1**; FDR<5%). In contrast, <1% (n=96) were differentially expressed

Table 1. Genes induced and repressed by cytokine, oxidative, and ER stressors

Comparison	Genes Tested	# Up	# Down	%_Up	%_Down	T2D genes up	T2D loci up	T2D genes down	T2D loci down
Cytokine.24hr.vs.Cytokine.4hr	10942	264	149	2.41	1.36	38	20	16	16
Cytokine.24hr.vs.Untreated.0hr	11110	669	253	6.02	2.28	72	40	25	23
Cytokine.24hr.vs.Untreated.24hr	11177	528	148	4.72	1.32	59	33	12	14
Cytokine.4hr.vs.Untreated.0hr	11101	658	169	5.93	1.52	72	42	27	28
H2O2.24hr.vs.H2O2.2hr	11294	7	60	0.06	0.53	1	1	6	6
H2O2.24hr.vs.Untreated.0hr	11245	25	19	0.22	0.17	1	1	0	0
H2O2.24hr.vs.Untreated.24hr	11292	0	0	0	0	0	0	0	0
H2O2.2hr.vs.Untreated.0hr	11301	81	15	0.72	0.13	9	9	1	1
Thapsigargin.24hr.vs.DMSO.24hr	11176	158	131	1.41	1.17	11	12	9	10
Untreated.24hr.vs.Untreated.0hr	11296	53	23	0.47	0.2	5	5	1	1

in response to oxidative stress. To understand if these stressors altered the expression of genes implicated in type 2 diabetes risk and progression, we overlapped the list of differentially expressed genes with our list of putative type 2 diabetes target/effector genes constructed from recent eQTL and 3-D epigenome mapping studies by us and others in human islets and EndoC beta cells. Cytokines modulated the expression of 153 putative T2D effector genes (110 up, 43 down), while oxidative stress altered the expression of 18 putative T2D effector genes (11 up, 7 down). These analyses link islet stress responses to T2D genetic risk and nominate specific T2D effector genes whose manipulation may modulate T2D risk and progression. We are designing guide RNA libraries in the upcoming period to target each of these genes and assess their phenotypic effects in

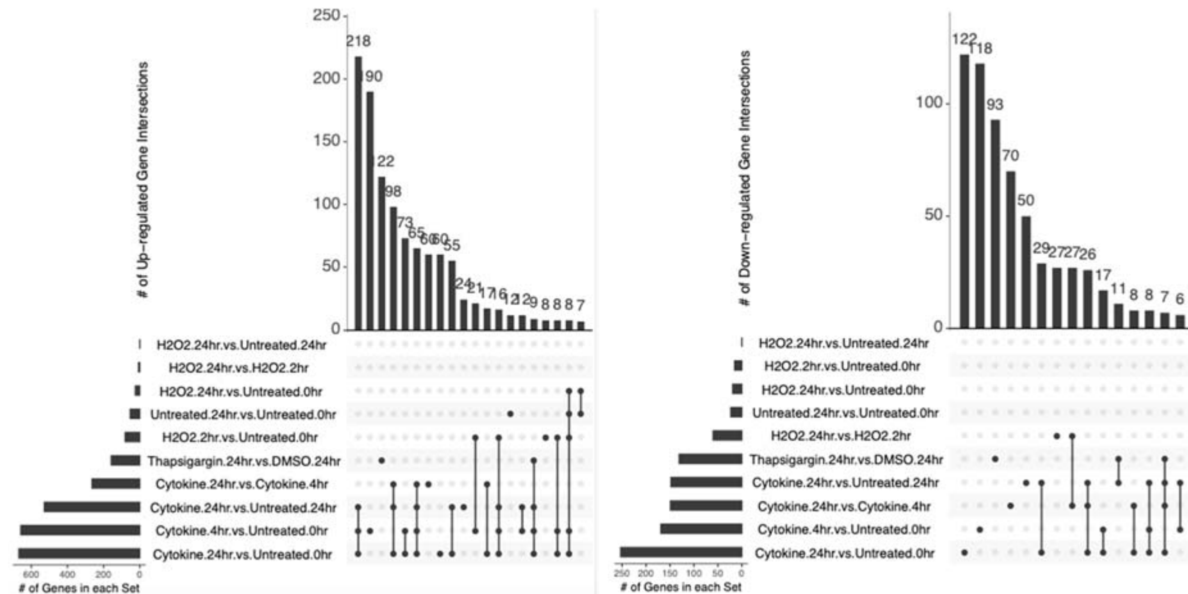

Figure 1. Majority of genes induced (left) or repressed (right) in islets are unique to each stressor. Upset plots showing the number of differentially expressed genes in islets in response to one or more condition. H2O2=peroxide; thapsigargin= ER stress agent.

human EndoC-ßH3 beta cells.

The overwhelming majority of genes induced (**Figure 1, left**) or repressed (**Figure 1, right)** by these two stressors and the diabetes-related endoplasmic reticulum (ER) stress response are not shared between responses, suggesting that these stress responses do not engage a core stress response program but rather induce unique gene sets that may offer specific and/or combinatorial targeting opportunities. This was also reflected in gene ontology and pathway analysis of the differentially expressed genes. Cytokine exposure induced genes with functions related to cytokine and chemokine receptor binding and signaling and KEGG pathways related to cellular responses to viral infection and autoimmune processes. Peroxide induced the expression of genes associated with oxidoreductase and oxygen binding activity and TGF-beta signaling, and ER stress induced genes to resolve protein misfolding as anticipated. Cytokines repressed genes with glycosaminoglycan binding functions and KEGG pathways related to pancreatic secretion. We will assess single cell time course analysis of these responses recently completed in this project period to understand how each islet cell type responds to these proinflammatory cytokines and determine if these largely immune-related pathways are induced in the endocrine cells or may be attributed to resident immune cells that remain in the isolated islets.

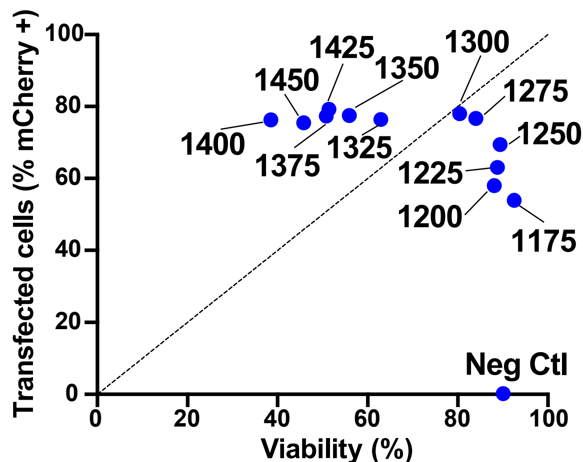

Figure 2. Optimization of EndoC-ßH3 transfection conditions. FACS was used to measure cell survival (x-axis) and transfection efficiency (y-axis) of nucleofected EndoC-ßH3 cells. Numbers indicate the voltage used. Neg Ctk= untreated cells

To determine if these differentially expressed genes affect important ß cell phenotypes, we queried their loss-of-function effects on ß cell viability and/or insulin content in MIN6 ß cells from the genome-wide CRISPR knockout screen described in the previous reporting period. In total, 102 of the differentially expressed genes exhibited a loss-of-function phenotype in MIN6. 84 genes altered MIN6 ß cell viability at FDR<10%, including *Nkx6.1, Isl1,* and *NeuroD1* islet transcription factors and putative T2D effector genes *Socs3, Celsr2,* and *C2cd4a*. 18 additional genes independently altered insulin content. We are planning targeted follow-up of these genes in human EndoC-ßH3 cells in the upcoming period.

Table 2. Islet regulatory elements altered by cytokine or oxidative stress

	Inflammatory cytokines	**Oxidative Stress (H2O2)**
Islet Donors (n)	20	20
Closing/opening REs	ATAC-seq	ATAC-seq
All	10,031 (5162 up, 4869 down)	9291 (2226 up, 7065 down)
T2D SNP-containing	112 (95 up, 17 down)	94 (29 up, 65 down)

Epigenetic signatures of islet stress responses:

To date, we have completed transposition of islets from 50 individuals under steady state or after cytokine or peroxide exposures (150 total ATAC-seq libraries; 3 per individual). We have sequenced ATAC-seq libraries from 20 individuals to date. To identify chromatin-modulating

effects of cytokine and oxidative stress on islets, we compared open chromatin profiles (ATAC-seq peaks) of cytokine- or peroxide-treated cells to their corresponding steady state/untreated profiles. We identified ~10,000 differentially accessible peaks in cytokine- or oxidative stress-treated cells (**Table 2**). Approximately 2000 opening and 650 closing peaks were shared between each stress treatment. Shared opening peaks were enriched for binding motifs of general stress response factors, such as ARNT, ELF1/ETS, YY1 and E-box binding motifs, while shared closing peaks were enriched for NFAT and SMAD3 binding sequences. Together with an observed enrichment of SOX2 and OCT4 pluripotency factor binding motifs, these data may suggest that islet stressors elicit or poise islet cells to dedifferentiate in response to pathophysiologic signals. We are planning to test this provocative hypothesis using single nucleus ATAC-seq analyses of islets exposed to stressors in the upcoming period to determine if specific cell types (most notably beta cells) exhibit stress-induced epigenetic signatures of de-differentiation.

To determine if T2D SNPs could potentially alter the use or activity of these putative stress-responsive regulatory elements, we overlapped the locations of T2D 99% credible set SNPs reported by Mahajan and colleagues in 2018 (*Nature Genetics*). As shown in **Table 2**, approximately 100 differentially accessible sites (i.e., putative stress-responsive regulatory elements) in islets harbored T2D SNPs. In the upcoming period, we will complete sequencing of the ATAC-seq libraries from the 30 additional individuals and begin allelic analyses to test the hypothesis that these SNPs alter cytokine or oxidative stress-responsive regulatory elements. In addition, we will build upon the critical improvements we have made in this period to increase EndoC-ßH3 transfection efficiency (**Figure 2)** from by almost four-fold (from 15% using lipofection to ~80% using nucleofection) to test these SNPs for their transcription-modulating effects using the massively parallel reporter assay (MPRA) 2.0 library in cells exposed to cytokines and oxidative stress. Together, these complementary *in vivo* and *in vitro* assays and analyses should provide us comprehensive knowledge of the chromatin and sequence features of stress-responsive regulatory elements and identify T2D SNP effects on their use and/or activity.

New algorithms for single nucleus ATAC-seq analyses: As part of this proposal, we have been generating single cell RNA-seq. Based on observations from Year 1 and recent single cell RNA-seq analyses we are currently completing, we anticipate that single nucleus ATAC-seq (snATAC-seq) data from islet samples will be critical to increase resolution of epigenomic maps to study T2D related regulatory changes. However, similar to other single cell technologies, snATAC-seq harbors doublet/multiplet cells that confound downstream analyses. Doublet detection is

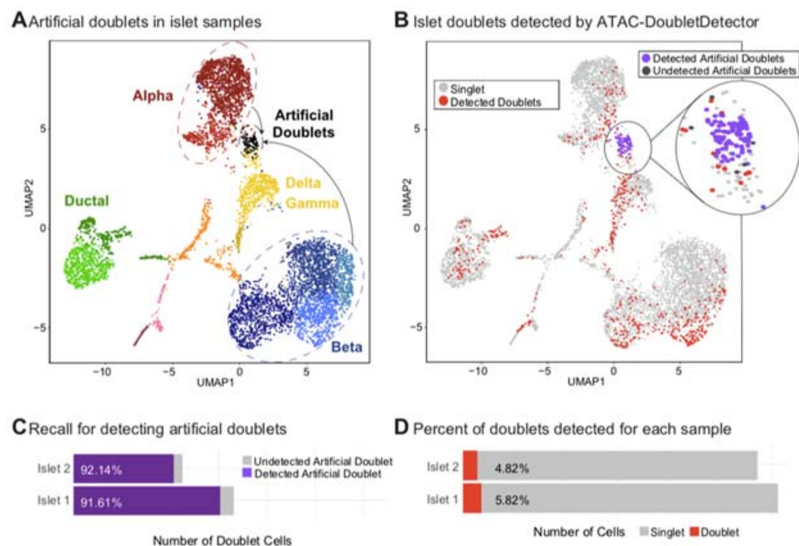

Figure 3. Doublet detection from snATAC-seq samples in islets. A) snATAC-seq map generated from a human islet sample including artificially generated doublets **B)** Doublets detected from the same islet sample. **C)** Recall rate for doublet detection based on artificially generated doublets in two human islet samples. **D)** Doublet rates detected by our method in two human islet snATAC-seq samples.

particularly challenging in snATAC-seq due to the sparsity of chromatin accessibility levels in a single cell (closed - 0 reads, open in one allele - 1 read, open in two alleles - 2 reads). To better analyze snATAC-seq maps from human islet samples, we developed a novel count based method, ATAC-DoubletDetector. ATAC-DoubletDetector was effective in capturing artificial doublets derived from high read count cells in human islet (n=2) samples with high recall which we quantified using artificially simulated doublet cells (>0.9 for all samples) (**Figure 3**). Our method captured around 5% doublets in both islet samples (**Figure 3D**), which are filtered out from the downstream analyses. As part of this computational pipeline, we also developed a novel pipeline to annotate the cell type origin(s) of these detected doublets. This annotation pipeline was effective in identifying cell origins for artificially created doublets (84% accuracy for islet samples). Identifying cell origins may provide insights into the frequency of specific cell-cell interactions. These novel computational methods will be utilized to better study dynamics of islet epigenomes.

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."

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.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

1) After completing JAX and HRPO IRB review/approval, subscribe to procure islets from University of Alberta Diabetes Center; complete RNA-seq and ATAC-seq of steady state, oxidative stress, and inflammatory cytokine stress-treated islets from 40 individuals (yielding 90 individuals total) (**Aims 1, 2**).
2) Analyze single cell transcriptomes of cytokine- and peroxide-treated islets to determine cell type-specificity of these islet stress responses (**Aim 1**)

3) Genotype additional islets collected and profiled to enable allelic, eQTL, and caQTL analyses (**Aims 1,2**)
4) Sequence ATAC-seq libraries from additional individuals and complete allelic and caQTL analyses **(Aim 2)**
5) Complete MPRA 2.0 experiments in cytokine- and peroxide-treated human EndoC-ßH3 cells to define stress-responsive transcriptional activating sequences and features and to identify T2D SNPs that alter their activity (**Aim 2)**
6) Targeted and broad-based (custom gRNA library) (epi)genome editing of differentially expressed genes to determine their effects on human EndoC-ßH3 survival and insulin content.

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).

Genome-wide association studies (GWAS) have linked DNA sequence variants at >250 loci in the human genome to type 2 diabetes risk (T2D SNP alleles). For each locus, identifying the functional variant(s) among multiple SNPs in high linkage disequilibrium is critical to understand molecular mechanisms underlying T2D genetic risk. Using massively parallel reporter assays (MPRA), we tested the transcriptional regulatory effects of T2D SNPs and those associated with altered *in vivo* human islet chromatin accessibility in insulin-producing MIN6 β cells under normal and stress-responsive conditions. In total, 29.9% of SNP-containing elements (n=1,983/6,628) activated transcription in MIN6. SNP alleles in 44.3% of these element (n=879/1,983) altered MPRA activity. Surprisingly, multiple T2D SNPs altered the activity of specific repetitive sequence-containing elements that were strongly induced by cellular stress. We identified 220 functional variants at 104 T2D association signals, narrowing 54 signals to a single candidate SNP and providing new mechanistic insights in specific T2D-associated regions, such as the *PEX5L, LARP6, RNF6,* and *SLC35D3* loci. Together, this study identified elements driving β cell steady state and stress-responsive transcriptional activation, nominated causal T2D SNPs, and uncovered potential roles for repetitive elements in β cell stress response and T2D genetics. The insights and data from this study will contribute substantially to ongoing efforts to uncover and categorize the function of the "dark matter" of the human genome and new variant-to-function (V2F) initiatives launched worldwide.

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.

We have provided these datasets to colleagues at NIH to inform machine learning-based approaches to define the key, predictive features of functional, regulatory DNA sequences and to predict SNP alleles that significantly disrupt these regulatory element use and/or activity in human islets and other type 2 diabetes-relevant metabolic cell types.

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:

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.

The Covid-19 pandemic has significantly and adversely affected our progress in two major ways:

1) Reduced productivity of personnel due to state-mandated 3-month lockdown (March-June 2020) to mitigate SARS-CoV2 community spread and institutional policies set in place to reduce personnel density in accordance with CDC and CT-mandated guidelines. This has delayed our progress (i) generating and testing (d)Cas9 (epi)genome editing-engineered EndoC-ßH3 human ß cells; (ii) creating the custom gRNA libraries, and (iii) optimizing EndoC-ßH3 transfection conditions to complete MPRA 2.0 experiments.
   Actions and plans to resolve problems: (i) Dr. Kursawe and Mr. Bhuiyan are coordinating efforts to create and test (d)Cas9 engineered cells; (ii) we are investigating the cost and logistics of ordering prepared, amplified gRNA libraries instead of generating the gRNA libraries ourselves from synthesized oligos to save time; and (iii) we have switched from lipid-based transfection to nucleofection to increase efficiency of MPRA library delivery and reduce the number of cells needed to complete the MPRA2.0 experiments. EndoC-ßH3 take approximately 7-10 days to double, so we expect this 3-4x reduction in the number of cells needed per replicate and treatment condition will significantly reduce the time required to complete these experiments.
2) Complete shut-down of islet transplant centers, ProdoLabs, and the Integrated Islet Distribution Program (IIDP) from March through mid-June, 2020, which prevented us from procuring any human islets during this 3-month period, reducing the number of donor samples we were able to procure from that expected.
   Actions and plans to resolve problems: We have implemented shift/weekend work to expand our available times to receive islets to include the weekend. Additionally, we are registering both this and another grant project as High Demand to increase our opportunity to receive multiple offers per week since operations resumed with IIDP and the member transplant centers. Finally, we have reached out to Dr. MacDonald and his team at the Alberta Diabetes Institute at University of Alberta in Edmonton, Canada about opportunities to procure islets from his center. He has

recently agreed to ship a portion of the islets isolated at his center to the IIDP phenotyping center at Vanderbilt, so these islets will now have the same standard phenotyping performed and metrics reported as the other islet samples we procure from IIDP. We expect this to increase our procurement rate by 25-50% and are beginning IRB review and approval at JAX-GM and will contact HRPO urgently to initiate review and approval.

We are also addressing personnel changes necessitated by transitions of two team members. First, Mr. Khetan earned his Ph.D. in July, 2020 and has left the team for a postdoctoral position at Harvard Medical School. As mentioned in the previous report, we had begun training Mr. Redwan Bhuiyan, a new MD/PhD student on the project to facilitate a smooth transition. In addition, Mr. Nathan Lawlor accepted a position with Sema4 at the end of August 2020. We immediately initiated a search for a new Bioinformatics Analyst II to fill this void and have identified a strong candidate, whom we anticipate hiring in the next week or two, to complete analyses assigned to Mr. Lawlor.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

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 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).*

  Shubham Khetan, Susan Kales, Romy Kursawe, Alexandria Jillette, Steven K. Reilly, Duygu Ucar, Ryan Tewhey, and Michael L. Stitzel. Functional characterization of type 2 diabetes-associated SNP effects on steady state and endoplasmic reticulum stress-responsive ß cell transcriptional activation. *In revision, Nature Communications*

  Acknowledgement of federal support: Yes

  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.*

Nothing to Report

- **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.

Nothing to Report

- **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.*

Nothing to Report

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: No change

Name: Nathan Lawlor
Project Role: Data analyst
Researcher Identifier: http://orcid.org/0000-0003-3263-6057
Nearest person month worked: 3 CM
Contribution to Project: No change.
Name: Emin Onur Karakaslar
Project Role: Research Associate (Computational)
Nearest person month worked: 12 CM
Contribution to Project: Computational data analyses focusing on epigenomic datasets. Mr. Karakaslar replaced Dr. Youn's role in this project, since Dr. Youn left the institute.

Name: Siddhartha Sharma
Project Role: Research Associate (Computational)
Nearest person month worked: 2 CM
Contribution to Project: Providing help with the analyses of single cell data. Mr. Sharma is assisting Mr. Karakaslar in the analyses of epigenomic maps as well.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Changes in Active OS

Supporting Agency:	NIH/NIA 1 R13 AG069519-01	PI:	(Ucar)
Project Title:	Systems Immunology in Aging and Chronic Diseases of Aging		
Role:	PD/PI	Effort:	0.18 CM
Entire Project:	08/01/2020 - 07/31/2023		
Project Goals:	The overall goal of this interdisciplinary conference is to bring together immunologists, computational biologists, gerontologists and geriatricians in the aging field and through this meeting, we aim to bring together scientists from these disciplines while also training the next generation of scientists in the aging field in advanced computational skills.		
Specific Aims:	Aim 1: Organize an interdisciplinary meeting and hands-on workshop focused on aging and age associated diseases. Aim 2: Promote interactions to foster collaborative research and career advancement. Aim 3: Recruit diverse attendees.		
Overlap:	None		
Contract/Grants Officer:	Max Guo	qmguo@mail.nih.gov	

Supporting Agency:	Jackson Laboratory, The JAX-DIF-FY20-DU-Vaccine	PI:	(Ucar)

Project Title:	Vaccine responsiveness in older adults		
Role:	PD/PI	Effort:	0.01 CM
Entire Project:	04/03/2020 - 03/04/2021		
Project Goals:	Our goal is to study the effects of long COVID-19 in context of vaccine responsiveness.		
Specific Aims:	In this project, we will study influenza vaccine responsiveness of COVID-19 convalescent patients (n=10) using single cell transcriptome and epigenome profiling.		
Overlap:	None		
Contract/Grants Officer:	Deborah Shurberg	Deborah.Shurberg@jax.org	

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

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)
- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*

Nothing to Report

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.*