Cell Genomics Transparent Peer Review Record

Type 1 diabetes risk genes mediate pancreatic beta cell survival in response to proinflammatory cytokines

AUTHOR LIST AND AFFILIATIONS

Paola Benaglio[1,10], Han Zhu[1,2,10], Mei-Lin Okino[1,2,10], Jian Yan[3,4,5,6], Ruth Elgamal[1], Naoki Nariai[1], Elisha Beebe[1], Katha Korgaonkar[1], Yunjiang Qiu[2], Margaret Donovan[1], Joshua Chiou[1], Gaowei Wang[1,2], Jacklyn Newsome[1], Jaspreet Kaur[1], Michael Miller[7], Sebastian Preissl[7], Sierra Corban[1], Anthony Aylward[1], Jussi Taipale[6,8,9], Bing Ren[2,3,7], Kelly A Frazer[1], Maike Sander[1,2,11], Kyle J Gaulton[1,11,12,*]

Summary

Initial submission:	Received : 12/27/2021
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Second round of review:	Number of reviewers: 2 Accepted : 10/14/2022
Data freely available:	Yes
Code freely available:	Yes

Referees' reports, first round of review

Reviewer #1: The authors carry out a comprehensive genomic assessment of pancreatic beta cell survival in response to proinflammatory cytokines, including placing the observations in the context of type 1 diabetes GWAS reports. This study represents a very large amount of work and is executed well, revealing some key findings for the field. In particular, the magnitude of evidence for SOCS1 is impressive. My comments are principally to help strengthen the manuscript further:

1. The comprehensive loss-of-function CRISPR screen generated some key findings. However, after reading that section in the Results, one is left with the unmet need to see those observations intersected with the implicated genes in the prior sections i.e. some form of Venn diagram would help the reader in this regard.

2. The authors switch from using the Human ENDOC-BH1 cells in their experiments to the murine MIN6 line later in the paper - this shift is hard to follow in the narrative. The authors are obligated to clarify their rationale for the cell line elections for the various experiments.

3. The Hi-ChIP is limited to H3K27ac marks - but we know there are other types of marks for enhancers and repressors - do the authors believe they will have missed some contacts between their CREs and promoters employing this method?

4. The authors state: "Together these results reveal the target genes of cytokine-responsive distal cCRE activity in beta cells." This is one example of where the authors use definitive language - I believe it would be more accurate to say 'implicate' than 'reveal' until subsequent and full functional validation is carried out.

Reviewer #2: The manuscript by Benaglio and colleagues from the Kyle Gaulton laboratory at UCSD represents an enormous research effort with significant support from leaders in diverse areas of experimentation, analysis and computation (Ren, Frazer, Sander). The information presented, including the Supplementary material, will provide a valuable resource for those in the field of beta cell (and islet) biology as well as those interested in potential pathways related to cytokine stimulation and potential target genes related to type 1 diabetes. The authors are to be commended in provide such an extensive array of experiments, approaches and data; however, it also makes for a difficult manuscript to read, ingest, evaluate and make critical comments. In essence, the manuscript could benefit from a more focused and targeted description of experiments and results, rather than an almost "diary of a student" feeling, going from one thing to the next. In particular, one could focus only on the high cytokine loading and time course experiments in the main text, given this is the focus of downstream results, and provide the information on the numerous other conditions to the Supplementary Material. This would reduce the size of the manuscript, make it easier to follow, and provide a more 'digestible' paper for the readership.

With respect to the content of the manuscript, the focus on beta cells is appropriate, as the destruction of the beta cells and absence of insulin as a result is the hallmark of type 1 diabetes; at the same time, the disease is autoimmune, and the immune system and its role in the response to foreign antigen(s), development of islet autoimmunity, and T cell-mediated destruction of the beta cells, is given limited attention; thus, there should be the connection and rationale for studying cytokine stimulation of different cell types in the islet as a viable model for type 1 diabetes.

Specific points for consideration, clarification and justification (numbered for convenience, not for priority):
1) The number of donors is small (n=4) and include 3 males and 1 female. How much does the high-dose cytokine in tissue culture reflect disease-related biology? Are differences due to sex/age of the donors? Are they of the same genetic ancestry? It is important to note that the results are limited by the diversity in the donors and their underlying genomes.

2) There is a mix of p-values and FDR throughout the manuscript, and even the Supplementary tables have p-values and "adjusted" p-values, although it is not clear what is considered "significant"; for example, with ~165,000 sites, a Bonferroni corrected p-value ~ 3x10-7 (likely conservative) yet at other times a p ~ 10-5 is used, or an FDR < 0.10. Given the limited number of subjects (n=4) with many conditions tested and multiple 'omics approaches, a more stringent significance threshold should be considered - especially when strong claims are made (see below) regarding the findings.

3) Are any of the recently published risk variants for type 1 diabetes (not including type 2 diabetes) from credible sets of SNPs as published in Chiou [Gaulton lab] and Robertson [Rich lab] known to have eQTL effect in islets? In particular, are there effects for genes in which highly-induced expression is mapped to known loci such as PTPN2, EPSTI1, SOCS1, PSMB2, PP1R11, LPIN1, and LMO7?

4) It is not clear why mouse-derived MIN6 cells were used when other experiments were conducted with human-derived EndoC-bH1 cells; this seems to be a significant limitation, especially for the EMSA assays in which nuclear extracts are used (no transfection?)

5) There are a number of statements that appear to be overly strong and conclusive, given the data:
- "These results suggest that beta cell chromatin is more responsive to pro-inflammatory stress than chromatin in other islet cell types"; not clear to me, and certainly not all pro-inflammatory cytokines were tested in all cell types under all conditions, in large numbers of donors or different cell culture conditions
- "Compared to alpha cells, there were substantially more cCREs with cytokine-responsive activity in beta cells (2,412 vs. 226) despite having similar total numbers of cells (Supplementary Figure 3c)"; while a striking difference, can part of this be due to beta cells being located more internal in the islet compared to alpha cells (more on the outer side of the islet and thereby allowing them to be oxygenated and less stressed?) Can the measured response be driven by in vitro conditions - both high cytokine treatment and/or hypoxic conditions as you survey cells more internal in the islet?
- other strong claims are made that, in my opinion, need to be moderated

6) The promotion of SOCS1 as a strong candidate gene involved in type 1 diabetes has historically been considered as a strong candidate, yet eQTL analyses in multiple immune cell types prioritized DEXI over SOCS1; the authors note that there are (known) animal models illustrating the role of SOCS1 in beta cell survival upon cytokine stimulation. Thus, while the authors' experimental work is impressive, the results are incremental in that it adds to (and replicates) results in the literature.

7) In Figure 2F, it is not clear why there are four clusters for beta cells? It would seem that the chromatin landscape cannot be "that different" in beta cells (although high and low insulin-producing cells might have some difference but not that much); the four clusters are not representing the four donors?

Minor:
- Two typographical errors in Figure 6 legend - "EndoC-bH1 respectively. G) ... targeting SOCS1 in untreated ang high-cytokine treated Endob-CH1"

Authors' response to the first round of review

We thank the reviewers for the highly positive comments on our study. We have revised the manuscript and responses to reviewer comments and corresponding revisions are detailed in line.

Reviewer #1:
The authors carry out a comprehensive genomic assessment of pancreatic beta cell survival in response to proinflammatory cytokines, including placing the observations in the context of type 1 diabetes GWAS reports. This study represents a very large amount of work and is executed well, revealing some key findings for the field. In particular, the magnitude of evidence for SOCS1 is impressive.
My comments are principally to help strengthen the manuscript further:
1. The comprehensive loss-of-function CRISPR screen generated some key findings. However, after reading that section in the Results, one is left with the unmet need to see those observations intersected with the implicated genes in the prior sections i.e. some form of Venn diagram would help the reader in this regard.

We agree with the reviewer comment. In the manuscript we intersected the genes affecting beta cell survival from the CRISPR screen with genes mapping to T1D GWAS loci. We now show in Supplementary Figure 4 the proportion of genes affecting beta cell survival from the CRISPR screen linked to cytokine-responsive cCREs and with cytokine-responsive changes in expression. We also included these results in the revised manuscript:

Line 271: "More than half of genes affecting beta cell loss (57% pro-death, 60% pro-survival) were linked to a cytokine-responsive cCRE, and a quarter of genes affecting beta cell loss (20% pro-death, 27% of prosurvival) also had cytokine-induced changes in expression (Supplementary Figure 4h)."

2. The authors switch from using the Human ENDOC-BH1 cells in their experiments to the murine MIN6 line later in the paper - this shift is hard to follow in the narrative. The authors are obligated to clarify their rationale for the cell line elections for the various experiments.

We agree that for consistency it would be ideal to use EndoC-bh1 cells for experiments throughout the manuscript. As SOCS1 has not been shown previously to affect beta cell survival in humans and the goal of the SOCS1 knock-down was to validate the effects observed in the CRISPR screen, we felt the SOCS1 knock-down was the critical experiment to re-do in EndoC-Bh1 cells. Therefore, we repeated these experiments using shRNA knockdown in EndoC-bh1 cells. The results of these experiments confirmed that decreased SOCS1 expression increased beta cell apoptosis compared to control, and this effect was specific to cytokine stimulated cells. We have included these results in the revised manuscript and in Figure 6i and Supplementary Figure 7.

Line 444: "In the CRISPR screen SOCS1 promoted beta cell survival after cytokine exposure, and SOCS1 had significant increase in cytokine-induced expression (Figure 6g-h). We determined the effects of SOCS1 on cytokine-induced beta cell survival using an independent assay that measures apoptosis using a fluorogenic probe (see Methods). We performed knockdown of SOCS1 via lentiviral transduction of shRNA in EndoC-βH1cell line cultured in untreated or high-dose IL-1b, IFNg and TNFa treated conditions and measured staining for apoptosis using flow cytometry (Supplementary Figure 7a-c). We observed a significant increase in apoptosis in SOCS1 shRNA compared to scramble control shRNA and that had a stronger effect in cytokine-treated cells (shRNA P=0.018, shRNA-treatment interaction P=0.018, two-way ANOVA; Figure 6i)."

Line 1082: "Flow cytometry analysis of EndoC-βH1 apoptosis. Lentivirus construct expressing SOCS1 shRNA (shSOCS1) was obtained from Sigma-Aldrich (TRC# TRCN0000356244, TTTCGCCCTTAGCGTGAAGAT). A none-targeting scramble shRNA (Scramble, CCTAAGGTTAAGTCGCCCTCG) construct in the same vector was used as control. Lentivirus expressing shSOCS1 and Scramble were packaged in 293T cells and introduced into

EndoC-βH1 cells using the spininoculation protocol described above. Transduced EndoC-βH1 cells were cultured for two days before a 72-hour treatment of vehicle (0.1%BSA) and cytokine (10 ng/mL IFN-γ, 0.5 ng/mL IL-1β, 1 ng/mL TNF-α). Treated EndoC-βH1 cells were then dissociated into single cell suspension using 0.25% Trypsin-EDTA. Cells were washed with 1 mL ice-cold flow buffer comprised of 0.2% BSA in PBS and centrifuged at 200 x g for 5 min. Cells were then resuspended in flow buffer containing ApotrackerTM-Green (Biolegend) and Propidium iodide and stained following manufacture's instruction. Cells were washed twice with 1 mL icecold flow buffer and centrifuged at 4 °C and 200 x g for 5 min. Cell pellets were resuspended in 300 µL icecold flow buffer and analyzed in a FACS LSRFortessa™ system (BD Biosciences). To confirm SOCS1 knock-down, approximately 1.2 million EndoC-βH1 cells were collected and washed before RNA isolation using the RNeasy Micro kit (QIAGEN) according to the manufacturer's instructions and RT-qPCR was performed. 500 ng for total RNA was converted to cDNA using iScript™ cDNA Synthesis Kit (Bio-Rad). Gene expression was quantified with iQ™ SYBR® Green Supermix (Bio-Rad), using the following primers:
SOCS1_1_FWD_qPCR CACGCACTTCCGCACATTC
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For the gel shift reporter experiments, we used MIN6 cells as they are a well-established model that both our group and others have previously used to study regulatory variant effects in multiple studies, and we now provide this rationale in the revised manuscript.

Line 385: "Electrophoretic mobility shift assay (EMSA) using nuclear extract from the beta cell line MIN6, a rodent line which has been used extensively for reporter assays of variant activity in previous studies32– 35, demonstrated protein binding to the T1D risk allele (Figure 5f)."

3. The Hi-ChIP is limited to H3K27ac marks - but we know there are other types of marks for enhancers and repressors - do the authors believe they will have missed some contacts between their CREs and promoters employing this method?

We appreciate the reviewer comment. The HiChIP assay is performed using a single histone modification, and we selected H3K27ac for these assays as it is a marker of both active enhancer and promoter elements. We appreciate that this mark will not capture interactions involving all classes of distal cCREs; for example, poised enhancers that are not yet active will have H3K4me1 but not H3K27ac. Therefore, we modified the text to highlight that our HiChIP results don't capture all classes of regulatory elements and therefore some cCREs may not be annotated.

Line 208: "We used an H3K27ac antibody for the HiChIP assays to identify interactions involving active regulatory elements such as enhancers and promoters, and thus likely did not effectively capture interactions between other classes of regulatory elements."

4. The authors state: "Together these results reveal the target genes of cytokine-responsive distal cCRE activity in beta cells." This is one example of where the authors use definitive language - I believe it would be more accurate to say 'implicate' than 'reveal' until subsequent and full functional validation is carried out. We appreciate the reviewer comment and agree that co-accessibility and 3D chromatin interactions alone do not prove that these genes are regulated by cCRE activity. We therefore modified the sentence as suggested:

Line 246: "Together these results implicate target genes of cytokine-responsive distal cCRE activity in beta cells."

Reviewer #2:
The manuscript by Benaglio and colleagues from the Kyle Gaulton laboratory at UCSD represents an enormous research effort with significant support from leaders in diverse areas of experimentation, analysis and computation (Ren, Frazer, Sander). The information presented, including the Supplementary material, will provide a valuable resource for those in the field of beta cell (and islet) biology as well as those interested in potential pathways related to cytokine stimulation and potential target genes related to type 1 diabetes. The authors are to be commended in provide such an extensive array of experiments, approaches and data; however, it also makes for a difficult manuscript to read, ingest, evaluate and make critical comments. In essence, the manuscript could benefit from a more focused and targeted description of experiments and results, rather than an almost "diary of a student" feeling, going from one thing to the next. In particular, one could focus only on the high cytokine loading and time course

experiments in the main text, given this is the focus of downstream results, and provide the information on the numerous other conditions to the Supplementary Material. This would reduce the size of the manuscript, make it easier to follow, and provide a more 'digestible' paper for the readership. With respect to the content of the manuscript, the focus on beta cells is appropriate, as the destruction of the beta cells and absence of insulin as a result is the hallmark of type 1 diabetes; at the same time, the disease is autoimmune, and the immune system and its role in the response to foreign antigen(s), development of islet autoimmunity, and T cell-mediated destruction of the beta cells, is given limited attention; thus, there should be the connection and rationale for studying cytokine stimulation of different cell types in the islet as a viable model for type 1 diabetes.

We appreciate the positive comments on our manuscript from the reviewer. We have revised the manuscript to address several points highlighted by the reviewer.

First, for clarity we substantially condensed the description of the bulk ATAC-seq data and moved many of these results for low-dose and other treatment dimensions to the supplement.

Line 121: “To identify epigenomic changes in pancreatic islets in response to cytokine exposure, we first performed bulk ATAC-seq in a total of 7 primary islet donors cultured in vitro with the cytokines IL-1b, IFNy, and TNFa as well as in untreated conditions (Supplementary Table 1). We performed these assays across multiple dimensions (35 assays in total), including treatment doses (high-dose: 0.5 ng/mL IL-1β, 10 ng/mL IFNγ, 1 ng/mL TNFα; low-dose: 0.01 ng/mL IL-1β, 0.2 ng/mL IFNγ, 0.02 ng/mL TNFα), duration (6, 24, 48, 72hr), and cytokines used (IL-1β, IFNγ and TNFa; or just IL-1β and IFNγ). We determined the effects of cytokine signaling on islet accessible chromatin broadly by performing principal component analysis (PCA) of normalized read counts (Supplementary Figure 1a), which revealed highly reproducible changes in cytokine treatment compared to control as well as patterns across treatment dimensions such as an intermediate effect of low-dose compared to high-dose treatment.

We next identified specific islet cCREs responsive to cytokine stimulation. We first defined a canonical set of 165,884 genome-wide cCREs in islets, and then identified cCREs with differential accessibility in cytokine treatment compared to control using DESeq213. There were 22,877 cCREs with increased activity in any cytokine treatment and 22,092 cCREs with decreased activity in any cytokine treatment (FDR<0.1, Figure 2a, Supplementary Figure 1b, Supplementary Table 2). Consistent with previous reports7, cCREs with increased activity in cytokines were strongly enriched for IRF (IRF1 P<10-300), STAT (STAT1 P=2.8x10-130) and NFkB (NFKB-P65-REL P=2.1x10-279) motifs whereas cCREs with decreased activity were enriched for FOXA (P=5.8x10-63), NKX6.1 (P=1.1x10-28), and NFAT (P=3.8x10-29) motifs (Figure 2b-c, Supplementary Table 3). We observed marked differences in cCREs that respond to cytokines across dose and duration (Supplementary Figure 1a-e), as well as stronger effects on cCRE activity when including TNFa (Supplementary Figure 1c). Sequence motifs also showed variable enrichment across dimensions of cytokine treatment, for example SMAD family TFs were more enriched at longer treatment durations (SMAD2 6hr P =0.24, 24hr P =0.03, 48hr P =8.6x10-4, 72hr P =2.2x10-6) (Supplementary Figure 1f).

The effects of cytokine exposure on individual islet cell types are obscured from assays of bulk tissue. Therefore, we next performed single nuclear ATAC-seq (snATAC-seq) in cytokine-treated and untreated islets from four donors at 24 hours post-treatment. We used high-dose of all three cytokines IL-1b, IFNy, and TNFa as the treatment for these assays, as this produced the strongest effects in bulk assays. After extensive quality control, which included removal of low quality and doublet cells (see Methods), we performed UMAP dimensionality reduction and clustering on a total of 7,829 nuclei (Figure 2d). Each of the resulting clusters contained cells from all four donors and was represented by untreated (total nuclei = 3,947) and cytokine-treated (total nuclei = 3,882) cells (Figure 2e, Supplementary Figure 2a). We assigned clusters cell type identity based on accessibility at the promoters of known marker genes (Supplementary Figure 2c-d), which revealed endocrine alpha, beta, and delta cells as well as exocrine, endothelial, and stellate cells. In addition, we identified two clusters of beta cells which were enriched for gene sets related to hormone production and stress response, respectively, suggesting the clusters represent distinct beta cell states in line with previous findings14 (Supplementary Figure 2b).”

Second, we provided greater context to the role of the immune system and T cells in type 1 diabetes as well as a stronger rationale for cytokine stimulation of islet cells as an appropriate model of type 1 diabetes.

Line 68: “Type 1 diabetes (T1D) is a complex disease caused by autoimmune destruction of the insulinproducing beta cells in the pancreas. The pathophysiology of T1D is characterized by aberrant immune response to antigens leading to the development of islet autoimmunity and eventually T cell-mediated destruction of beta cells1. As part of the progression to T1D, immune cell infiltration and inflammation occurs in the local environment around islets, through which beta cells and other islet cell types are directly exposed to external stimuli such as proinflammatory cytokines produced by immune cells2. Beta cells themselves have been argued to intrinsically contribute to T1D in response to these stimuli, for example by promoting cell death. Studying beta cell function during T1D progression directly is challenging, however, due to the limited availability of samples and the difficulty in capturing the precise window in which beta cells are the target of immune attack. An alternate strategy is to model T1D progression in vitro, for example by culturing islets or beta cells with interleukin 1β (IL-1β), interferon γ (IFNγ), and tumor necrosis factor α (TNFα)3–7 which are pro-inflammatory cytokines produced by antigen-producing cells and T cells during
T1D that signal to beta cells. Previous studies using this in vitro model has revealed widespread effects on beta cell gene regulation, function and survival3,5–8; however, the genes and processes in beta cells that may directly influence T1D progression in response to pro-inflammatory cytokines are poorly defined.”

Specific points for consideration, clarification and justification (numbered for convenience, not for priority):

1. The number of donors is small (n=4) and include 3 males and 1 female. How much does the high-dose cytokine in tissue culture reflect disease-related biology? Are differences due to sex/age of the donors? Are they of the same genetic ancestry? It is important to note that the results are limited by the diversity in the donors and their underlying genomes.

We appreciate the reviewer comment and agree that it is important to note limitations in the islet donors being profiled. The design of our study involved comparing the effects of cytokine treatment to no treatment within the same donor by including the sample ID as a blocking factor. As such, the changes in chromatin and gene expression that we observed are those that are consistent across samples and not due to sample-specific effects. We acknowledge, however, that our findings come from a limited number of donors and profiling larger sample sizes will both give greater power for these analyses as well as enable other analyses for example to identify cytokine responsive effects dependent on phenotype, such as sex and age, or genotype. We have included these results in the discussion of the revised manuscript.

Line 556: “Finally, as we profiled a relatively small number of donors, larger sample sizes will enable detecting more subtle changes in gene regulation in response to cytokine exposure as well as identifying cytokine responses that interact with phenotype, such as age and sex, or genotype.”

2. There is a mix of p-values and FDR throughout the manuscript, and even the Supplementary tables have p-values and "adjusted" p-values, although it is not clear what is considered "significant"; for example, with ~165,000 sites, a Bonferroni corrected p-value ~ 3x10-7 (likely conservative) yet at other times a p ~ 10-5 is used, or an FDR < 0.10. Given the limited number of subjects (n=4) with many conditions tested and multiple 'omics approaches, a more stringent significance threshold should be considered - especially when strong claims are made (see below) regarding the findings.

We apologize for the confusion over the significance thresholds used in the manuscript. Almost all the genome-wide analyses in the manuscript used an FDR threshold of .10, which is widely used for family-wise error rate correction in genomics, including cCREs with differential activity in ATACseq and snATAC-seq, genes with differential expression in RNA-seq, genes affecting beta cell survival in the CRISPR screen, and chromatin interactions with HiChIP.

In the handful of cases where we used a different threshold, for example in the SELEX-seq analysis, we highlighted the rationale for this choice in the revised manuscript. We also clarified several definitions in the main text and tables, one of which was incorrect in the submitted manuscript where the threshold was used was FDR<1x10-5 and not un-corrected P<1x10-5:

Line 303: “This enrichment was stronger when considering only genes with the most significant increases (FDR<1x10-5) in cytokine-induced expression (++exp OR=3.28, 95% CI=1.33,7.37 P=5.1x10-3).”

3. Are any of the recently published risk variants for type 1 diabetes (not including type 2 diabetes) from credible sets of SNPs as published in Chiou [Gaulton lab] and Robertson [Rich lab] known to have eQTL effect in islets? In particular, are there effects for genes in which highly-induced expression is mapped to known loci such as PTPN2, EPSTI1, SOCS1, PSMB2, PP1R11, LPIN1, and LMO7?

We appreciate the reviewer comment. We investigated islet eQTLs for the 84 T1D-enriched genes with up-regulated expression and promoting beta cell survival in cytokines using data from the INSPIRE consortium with 420 islet donors (Vinuela et al Nat. Comm). While 5 of these genes were eGenes in this study, meaning there was at least one variant with significant effects on its expression, we did not observe evidence that any fine-mapped T1D variants themselves had nominal QTL association (P<.01) to any of these genes.

In our view, however, it may not be particularly surprising that we did not find evidence for islet eQTLs for these T1D variants. The islet eQTL data are from non-diabetic individuals in baseline conditions, and therefore variants affecting islet regulation in cytokine stimulated conditions are unlikely to be captured in current eQTL data. Generating RNA-seq data in islets exposed to cytokines from many donors and mapping eQTLs in cytokine-exposed islets will likely help to clarify the genes regulated by these variants, although this is of course outside the scope of the current study. We have included this point in the main text as well as discussion of the revised manuscript.

Line 413: “We did not find evidence that credible set variants were islet expression QTLs (eQTLs) for these genes40, although these maps were generated from islets in baseline conditions and not under cytokine stimulation.”

Line 537: “While we did not identify evidence that T1D risk variants affected the expression of target genes of cytokine-responsive cCREs, existing eQTL maps in islets are from baseline conditions and may not capture effects that depend on cytokine exposure. Mapping eQTLs from large numbers of islet donors exposed to cytokines will therefore help uncover these variant effects.”

4. It is not clear why mouse-derived MIN6 cells were used when other experiments were conducted with human-derived EndoC-bH1 cells; this seems to be a significant limitation, especially for the EMSA assays in which nuclear extracts are used (no transfection?)

We agree that for consistency it would be ideal to use EndoC-bh1 cells for experiments throughout the manuscript. As SOCS1 has not been shown previously to affect beta cell survival in humans and the goal of the SOCS1 knock-down was to validate the effects observed in the CRISPR screen, we felt the SOCS1 knock-down was the critical experiment to re-do in EndoC-Bh1 cells. Therefore, we repeated these experiments using shRNA knockdown in EndoC-bh1 cells. The results of these experiments confirmed that decreased SOCS1 expression increased beta cell apoptosis compared to control, and this effect was specific to cytokine stimulated cells. We have included these results in the revised manuscript and in Figure 6 and Supplementary Figure 7.

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Line 1082: “Flow cytometry analysis of EndoC-βH1 apoptosis. Lentivirus construct expressing SOCS1 shRNA (shSOCS1) was obtained from Sigma-Aldrich (TRC# TRCN0000356244, TTTCGCCCTTAGCGTGAAGAT). A none-targeting scramble shRNA (Scramble, CCTAAGGTTAAGTCGCCCTCG) construct in the same vector was used as control. Lentivirus expressing shSOCS1 and Scramble were packaged in 293T cells and introduced into EndoC-βH1 cells using the spininoculation protocol described above. Transduced EndoC-βH1 cells were cultured for two days before a 72-hour treatment of vehicle (0.1%BSA) and cytokine (10 ng/mL IFN-γ, 0.5 ng/mL IL-1β, 1

ng/mL TNF-α). Treated EndoC-βH1 cells were then dissociated into single cell suspension using 0.25% Trypsin-EDTA. Cells were washed with 1 mL ice-cold flow buffer comprised of 0.2% BSA in PBS and centrifuged at 200 x g for 5 min. Cells were then resuspended in flow buffer containing ApotrackerTM-Green (Biolegend) and Propidium iodide and stained following manufacture's instruction. Cells were washed twice with 1 mL icecold flow buffer and centrifuged at 4 °C and 200 x g for 5 min. Cell pellets were resuspended in 300 µL icecold flow buffer and analyzed in a FACS LSRFortessa™ system (BD Biosciences). To confirm SOCS1 knock-down, approximately 1.2 million EndoC-βH1 cells were collected and washed before RNA isolation using the RNeasy Micro kit (QIAGEN) according to the manufacturer's instructions and RT-qPCR was performed. 500 ng for total RNA was converted to cDNA using iScript™ cDNA Synthesis Kit (Bio-Rad). Gene expression was quantified with iQ™ SYBR® Green Supermix (Bio-Rad), using the following primers:
SOCS1_1_FWD_qPCR CACGCACTTCCGCACATTC
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5. There are a number of statements that appear to be overly strong and conclusive, given the data:

We have the modified the statements listed by the reviewer below-

"These results suggest that beta cell chromatin is more responsive to pro-inflammatory stress than chromatin in other islet cell types"; not clear to me, and certainly not all pro-inflammatory cytokines were tested in all cell types under all conditions, in large numbers of donors or different cell culture conditions

We modified this sentence in the revised manuscript to tone down the conclusions.

Line 178: "These results suggest that beta cell chromatin may be more responsive to the cytokines IL-1β, IFNγ and TNFα compared to alpha cells."

- "Compared to alpha cells, there were substantially more cCREs with cytokine-responsive activity in beta cells (2,412 vs. 226) despite having similar total numbers of cells (Supplementary Figure 3c)"; while a striking difference, can part of this be due to beta cells being located more internal in the islet compared to alpha cells (more on the outer side of the islet and thereby allowing them to be oxygenated and less stressed?) Can the measured response be driven by in vitro conditions - both high cytokine treatment and/or hypoxic conditions as you survey cells more internal in the islet?

To the reviewer comment, in mice the islets are structured with alpha cells on the periphery and beta cells on the inside but in human islets the alpha and beta cells are more distributed throughout the islet and evenly aligned along blood vessels (PMID 31983511, 16461897, 20657742).

However, the reviewer is generally correct that one of the limitations of the in vitro culture models is that the cells are taken out of their natural context where islet cells may have differential exposure to environmental stimuli such as cytokines. We have therefore included this point in the revised discussion.

Line 553: "As in vitro models only partially re-capitulate disease biology, mapping regulatory programs in beta cells from individuals in pre-T1D or the early stages of T1D will also help in interpreting disease risk. In addition, cells within the islet may have heterogeneity in their exposure to environmental stimuli that is not fully captured by in vitro models."

- Other strong claims are made that, in my opinion, need to be moderated

We have tempered the description of results and conclusions throughout our study, for example:
Line 459: "Combining functional genomics and genetic association implicated genes and molecular processes in risk of T1D in beta cells."

Line 454: "These results reveal that the induction of SOCS1 activity in response to cytokine exposure promotes human beta cell survival and may play a causal role in risk of T1D."

Line 477: "Furthermore, based on direct links to a fine-mapped T1D risk variant, the regulation of SOCS1 activity in beta cells after cytokine exposure potentially plays a causal role in T1D."

Line 466: "As a result, risk of T1D may be explained in part by reduced induction of pro-survival genes in beta cells in response to proinflammatory cytokines during disease progression."

6. The promotion of SOCS1 as a strong candidate gene involved in type 1 diabetes has historically been considered as a strong candidate, yet eQTL analyses in multiple immune cell types prioritized DEXI over SOCS1; the authors note that there are (known) animal models illustrating the role of SOCS1 in beta cell survival upon cytokine stimulation. Thus, while the authors' experimental work is impressive, the results are incremental in that it adds to (and replicates) results in the literature.

We appreciate the reviewer comment. While SOCS1 affects beta cell survival in animal models and is therefore a natural candidate gene at this locus, our study adds several key pieces including that (i) SOCS1 affects survival in human beta cells in cytokine exposure, (ii) SOCS1 is linked directly to T1D risk variants in cytokine-responsive cCREs, and (iii) SOCS1 is part of a larger set of cytokineinduced beta cell pro-survival genes that are broadly enriched at T1D loci.

Many loci will contain candidate genes with compelling disease-relevant biology, yet the key question and challenge is which of these genes are affected by risk variant activity directly and in what context. This is relevant to the 16p13 locus as there are multiple strong candidate genes: SOCS1 and DEXI - highlighted by the reviewer - as well as other genes such as CLEC16A and CIITA. Despite SOCS1 being a strong biological candidate, however, no previous studies to our knowledge have linked T1D risk to this gene directly. In our study we show that SOCS1 both physically interacts with cytokine-induced chromatin sites harboring T1D risk variants and is in a class of cytokine-induced genes broadly enriched at T1D risk loci.

As the reviewer notes, previous studies have also identified links between T1D risk variants and DEXI in immune cells as well as in islets, and it is certainly possible that multiple genes affecting multiple cell types contribute to disease risk at this locus, although this remains to be determined.

7. In Figure 2F, it is not clear why there are four clusters for beta cells? It would seem that the chromatin landscape cannot be "that different" in beta cells (although high and low insulin-producing cells might have some difference but not that much); the four clusters are not representing the four donors?

We apologize for the confusion in the clustering results. The beta cell clusters we identified each contained cells from all four donors, and therefore the clusters don't just represent the four different donors. The breakdown of each beta cell cluster by sample was provided in Supplementary Figure 2A in the submitted manuscript.

Given the reviewer comment, however, we investigated the beta cell clusters in more detail. We first performed re-clustering, which revealed two distinct beta cells clusters that each had representation from all four donors. We next identified gene sets enriched among promoters with differential activity across these two clusters, which revealed enrichment of hormone producing genes in one cluster and stress response and signaling genes in the other cluster. These results are in line with our previous findings described in Chiou et al 2019 which identified distinct hormone
producing and signal responsive states in the beta cell epigenome. We have updated the manuscript to include this updated clustering and sample breakdown in Figure 1d and Supplementary Figure 2a and included gene sets up-regulated in each beta cell cluster in Supplementary Figure 2b.

Line 160: “In addition, we identified two clusters of beta cells which were enriched for gene sets related to hormone production and stress response, respectively, suggesting the clusters represent distinct beta cell states in line with previous findings14 (Supplementary Figure 2b).”

Line 153: “After extensive quality control, which included removal of low quality and doublet cells (see Methods), we performed UMAP dimensionality reduction and clustering on a total of 7,829 nuclei (Figure 2d). Each of the resulting clusters contained cells from all four donors and was represented by untreated (total nuclei = 3,947) and cytokine-treated (total nuclei = 3,882) cells (Figure 2e, Supplementary Figure 2a).”

Minor:
Two typographical errors in Figure 6 legend - "EndoC-bH1 respectively. G) ... targeting SOCS1 in untreated ang high-cytokine treated Endob-CH1""

We thank the reviewer for highlighting these errors which we fixed

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Referees’ report, second round of review

Reviewer #1: The authors have satisfied the concerns of this reviewer.

Reviewer #2: The authors have been greatly responsive to the concerns of this (and the other) reviewer. The changes made (and reduction due to movement of materials to the Supplement) have made the revised manuscript much improved. No additional concerns/comments.

Authors’ response to the second round of review