nature portfolio

Peer Review File

Transcriptional coactivator MED15 is required for beta cell maturation

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

Nature Communications NCOMMS-19-25720-T
Kadhim et al, Transcriptional coactivator MED15 is required for beta cell maturation

In this manuscript, Kadhim et al describe a series of experiments to implicate the MED15 subunit of the Mediator coactivator complex in pancreatic beta cell function. Following their observation that MED15 is expressed in developing pancreas and other pancreatic cell derivatives, they find that its reduced expression in diabetic (T2D) patient-derived islets also correlates with expression of glucose-stimulated insulin secretion (GSIS) marker genes. Towards implicating MED15 directly in the beta cell gene expression program, a mouse knock-out model (IM15KO, under the control of beta cell-specific Ins1cre) was generated. Largely consistent with the patient tissue data, extensive characterizations of the phenotypes of IM15KO show impairment in genes responsible for beta cell maturation and glucose uptake functions. The data also suggested defects in normal mitochondrial physiology. Towards understanding how transcription of the relevant genes might be controlled by MED15, the authors performed ChIP-seq with commercial antibodies against MED15 in MIN6 insulinoma cells. Resulting tracks on selected genes are shown along with the data from a MED1 ChIP-seq performed by the authors in the same cell line, as well as tracks from ChIP-seq data on other transcription factors (obtained from public databases). To obtain further clues about MED15 function on target genes, various bioinformatic analyses (including chromatin state segmentation, de novo motif analysis) were undertaken. De novo motif analyses of genomic sites coinciding with MED15 indicated that they are enriched for binding motifs for transcription factors Nkx6-1 and NeuroD1, as well as additional factors, dependent on which input parameters (H3K4me1-containing enhancers or other) were specified. Towards gauging whether the binding motif coincidence results from potential physical interactions between MED15 and the transcription factors, co-immunoprecipitation experiments were also attempted. Finally, overexpression of MED15 in human ES cells programmed to generate islet-like spheroids yielded beta-like cells in which some of the markers that were identified as MED15 targets in the earlier analyses displayed somewhat elevated expression.

This paper represents a tremendous amount of work undertaken in support of the hypothesis that MED15 is a key transcriptional coactivator for beta cells. It is therefore very unfortunate that taken as a whole, the paper ultimately fails to build a convincing case for MED15 function as a master coactivator for beta cells. As this reviewer sees it, and as detailed below, this stems largely from the authors' idiosyncratic view of the Mediator complex, which appears to be at variance from the current thinking in the transcription field. No question that the detailed description of what happens to beta cell function when MED15 is knocked out reflects the importance of MED15 in this cell type. But what the authors' handling of the problem appears to unwittingly ignore is that MED15 works as part of the gigantic Mediator complex. Any mechanistic understanding of its mechanism of action will not be forthcoming until it is viewed as an integral part of this complex. It is true that the authors have looked at another subunit, MED1, in some detail. But in the end the provided data do not allow one to construct in one's head a clear model of action for what might be going on with MED15 and Mediator in the context of beta cells. Moreover, at this stage, it is no longer in question whether a given transcriptional program is reliant on the Mediator or even whether one or the other of its subunits is involved. Rather, the bigger challenge is to describe what specific mechanistic roles

the Mediator (via an identified subunit interaction) might be playing in the context of that transcriptional program. Thus whereas it is clear phenomenologically that these cells require MED15 for their specialized functions, it remains unclear what the underlying mechanism vis a vis the Mediator complex is. This reviewer therefore feels that in this regard at least the current story is somewhat incomplete.

Major points related to establishing MED15 function in the context of the larger Mediator complex:

1. A simple question that remains unanswered relates to tissue-specificity for MED15. The authors' singular focus on pancreatic cells is obviously well justified. But given that MED15 has been implicated in other gene expression programs (lipid metabolism, signaling via STATs), an analysis of expression patterns in other tissues besides the pancreas (Fig.1) would have been extremely instructive. Are the observed expression kinetics unique to pancreatic cells and correlate with the expression kinetics of critical genes?

2. Conversely, and related to another aspect of the specificity of MED15 action, it is not clear whether just MED15 or another Mediator subunit, in particular a tail module subunit, might be equivalently behaved in the context of beta cells. The authors have nicely noted how MED1, a middle module subunit of the Mediator, behaves both similarly in some respects to MED15 yet quite differently in others. The authors were obviously guided in their choice of this subunit as a kind of control for MED15 based on the large number of publications that have used this subunit in ChIP-seq experiments as as a surrogate for the entire Mediator complex. But MED1 is a special case and it would have been really nice to see the authors include some additional subunits in their analyses. With regard to MED1, it is often overlooked by researchers in the field that the MED1 content of the Mediator complex might be variable. Thus MED1 is substoichiometric (relative to the core complex) in some cell types (see Zhang et al Mol Cell 2005) whereas it appears to be overexpressed in some cancer cell types. Could it be that MED15 stoichiometry vis a vis the core complex is also variable? The authors themselves wonder (Discussion, line 324) as to how changing the amount of a single Mediator subunit has the potential to affect function. The authors raise, but do not address, a number of possibilities, among them the issue of how complex stability might be regulated by MED15. It occurs to this reviewer that there is a strong possibility that MED15 might control the association within the core complex of some tail subunits, which might otherwise be labile, consistent with their observed biochemical behavior. Together with point 3 raised further below that the authors' have not credibly demonstrated a direct MED15 interaction with any of their candidate transcription factors (TFs), the possibility remains that it is one or more of these other tail subunits that are the actual physical targets of TFs that regulate the beta cell transcriptional program and that MED15 is providing an ancillary role. Other tail subunits must therefore be incorporated into the analyses, both cell based and those looking at physical interactions (below).

3. A major shortcoming of the paper is that direct physical interactions between MED15 and any of the candidate TFs (Nkx6-1, NeuroD1) have not been convincingly demonstrated. Because of this, the basic premise of the paper and even its title are on shaky grounds. In the co-ip data shown in Fig. 5F, which is the apparent basis of the various claims related to physical interactions between MED15 and TFs, the following issues were noted and must be addressed:

(i) First, by their very nature, co-ips only show what factors exist in association with each other, whether through direct contacts or through intermediary factors. To claim direct interactions, use of purified TFs and coactivators is necessary. Even better, and for a more credible claim, such analyses

should be followed up by mapping of the interacting domains in both the TF and the coactivator, and ideally should be coupled with some cell-based demonstration of the validity of the interaction through use of appropriate mutants.

(ii) Relevant to the issue of MED15 function as part of the larger Mediator complex, the authors need to probe for some additional Mediator subunits. In addition to the various tail subunits, in light of point 2 above, representative subunits from each of the remaining Mediator modules (head, middle, kinase) also need to be probed. Furthermore, a number of unbiased biochemical methods (e.g., cross link-MS) exist to identify Mediator subunits that serve as targets for a given activator. They should be considered.

(iii) The data here for MED1 is very problematic. No controls are shown for the efficacy of the ip. Also, no indication is provided in the Methods on the source (and validation) of the antibody used here in the ip, as well as the one that might have been used in the ChIP-seq analyses (Fig. 5A etc.).

Other points:

1. With all due respect to Ernst & Kellis and other bioinformaticians, this reviewer feels that the chromatin state segmentation analyses on which the present authors rely to contextualize their ChIP-seq findings can be quite misleading. The approach and the resulting terminology collapse two distinct parameters: one relates to spatial location and the other to the transcriptional state (active vs. inactive). Ideally, this should be avoided but if the authors insist on invoking this, sufficient background and explanatory notes need to be provided to the average reader to appreciate why the authors resorted to this analysis.

2. Fig. 4A. The schematic is somewhat confusing. What are the “coregulators” in the cartoon? Why is MED1 highlighted? Also, there is likely a typo in the “Grants et al 2016” reference in the figure legend.

3. The authors use MIN6 cells for some of their analyses (including ChIP-seq). From Fig. 1E (ratios in lane 1 vs. lane 3), it appears that MED15 might be overexpressed. Could this affect the authors’ conclusions about target genes under normal conditions where MED15 levels are not as high?

4. Inconsistent terminology. The authors use “Med15” and “MED15” interchangeably to refer to this protein. Sometimes, the inconsistency occurs in the same sentence (line 317). Whereas the reference to the mouse genes encoding this protein correctly follows the accepted conventions, references to the protein should ideally follow the recommendation of Bourbon et al, Mol Cell 2004. Ditto for Med1/MED1.

5. The Abstract is a little ambiguous. Addressing the following might help to make it crisper: (i) “We sought to understand whether Mediator subunit MED15 acts as a node….” Use of the vaguely defined term “node” has the effect of rendering the stated goals of the study also appear somewhat vague. (ii) For those without a beta cell physiology background, the statement that MED15 loss did not affect insulin expression appears at first contradictory. Although the significance of this becomes absolutely clear as one reads the text, it would be nice to add a clarifying clause. (iii) “… an additional layer of control….” Since no other “layers” have been introduced, the precise meaning is lost on the reader.

Reviewer #2 (Remarks to the Author):

This manuscript by Kadhim et al. examines the roles/mechanisms of Med15 in pancreatic islet beta cells, in both mouse and human beta-cell models. Med15 is a subunit of the Mediator co-regulator, which interacts with transcription factors (TF) and RNA PolII for transcriptional regulation. Based on some studies on Med15 expression, beta-cell specific knockout, and genomic analyses, the authors suggest that Mediator regulates beta-cell maturation. This particular role is likely fulfilled by Med15 fine-tuning transcription regulated by TFs NeuroD1 and Nkx6.1, well-established regulators of beta-cell function/maturation.

Overall, the study is well designed and carried out. The findings sufficiently support a key role of Med15 in postnatal beta-cell function. They further implicate how some seemingly general Mediator subunits can selectively regulate specific transcriptional pathways (likely being recruited via specific TFs, e.g., Nkx6.1 for Med15 while others for Med1). Thus, its publication should be of interest for readers who study either beta-cell biology or transcription regulation.

Yet there is a major concern that needs to be addressed to better support the authors' overall conclusion that "MED15 is required for 1 beta cell maturation". It is generally agreed that beta-cell maturation in mice is completed by roughly two weeks after birth. Thus, the authors' conclusion would suggest that beta-cell defects will become obvious by ~2-weeks after birth. Yet most of the phenotypic analyses in this study were in ~2-months old mice. Thus the current study cannot distinguish if Med15 is required for beta-cell functional maturation and/or maintenance. Thus, the authors should attempt to answer the following key questions:

1) What are the expression levels of Med15 between newly born to 2-months of age (e.g., P14, the stage when beta cells establish proper glucose response and P28, when beta cells establish full function to handle new diet exposure)?.
2) What are the insulin secretion/mitochondrial phenotypes/beta-cell gene expression patterns between newly born to 2-months of age?

Additional questions are, not in particular order:

1) In the introduction, a summary about the known beta-cell maturation process will help (metabolism, insulin vesicle production/maturation, cell-cell communication, stimulus-secretion coupling etc.) to lay down the pathways to be studied later.
2) Line 39, the authors may want to use a different word to replace "lack"---it's more about the expression level instead of presence/absence during maturation.
3) Lines 41-42 "β-cells are not glucose responsive at birth" may also need rewording, see Blum et al., 2012.
4) The introduction (line 74-78) highlighted the potential roles of MDT-15 in hypoxia and oxidative stress. The authors should have some discussion on this topic, based on their gene expression changes detected in Med15 mutant beta-cells.
5) Lines 125-126. Maybe deleting "suggesting no changes in food intake" ?
6) Line 170, it is not clear what "suggesting abnormal mitochondrial physiology" means.
7) Fig. 1B-D, the inclusion of DNA staining is blocking the signals from Med15 staining. It may help to

add a single Med15 channel.
8) “M15betaKO” might be a better way of indicating beta-cell specific Med15 KO, instead of “IM15KO”. This format will be a better match with the latter use of “MED15βOE”.
9) Lines 188-189, “To confirm specific Med15 binding, we performed ChIP-seq on islets from control and IM15KO mice”. What was the estimated beta-cell % in these islets? This info will be helpful for non-islet readers.
10) Some studies used Ins1Cre as controls, while others use Med15F/F? Are there any differences?
11) The presented results are mostly from male mice (line 454). What about the females?
12) Figure 2B: do the IM15KO pancreata maintain Med15 expression in non-beta cells?
13) Lines 216-220, how many other TFs were examined in addition to those listed here?
14) The authors need to examine cell proliferation/death, because of the known effect of NeuroD1 mutation on beta-cell death.
15) Figure 1. Do the authors have insulin GSIS data on those T2D islets?
16) Figure 2 title: removing “hyperglycemia” because the authors have no data on this?
17) Figure 6D, a split channel is needed to show the expression of MED15, which looked like cytoplasmic.
18) Figure S2G, if the insulin content/microgram DNA doesn’t change, how do the authors explain the reduced insulin granules in mutant beta cells (in Figure 3C)?

Reviewer #3 (Remarks to the Author):

Summary
Khadim et al have examined the role of a subunit of the Mediator complex, MED15, in regulating the expression of genes that are important for the maturation of pancreatic β-cells. They show that KO of MED15 causes β-cell dysfunction, in part due to altered mitochondrial respiration, and diabetes. They also show that MED15 is often downregulated in type 2 diabetes islets. They carried out MED15 and MED1 Chip-seq in MIN6 cells and mouse islets, and use IPs to show that MED15 interacts with key β-cell transcription factors (Nkx6-1 and NeuroD1). Finally, they carried out MED15 overexpression studies in stem cell models, suggesting that this could help improve in vitro β-cell differentiation
This work provides interesting insights on how a Mediator complex subunit can regulate cell-type specific transcriptional networks. However, the CHIP analysis and stem cell work is very preliminary, and there are several shortcomings that need to be addressed before publication.

Major points
• The cellular specificity of med15 is unclear. The qPCRs are not very informative, but the immunofluorescence images seem to show that med15 is present in islet beta and alpha cells, but not in exocrine cells. Is this interpretation correct? Given the focus on differentiation, when is Med15 expressed during pancreas development? It is possible that although the function Mediator is ubiquitous, med15 is exclusively expressed in islet cells within the pancreas, and other subunits carry out its role in other cell types. Is this cell-specific expression consistent with what is already known about med15, or is this a novel finding? These points are very relevant to the major thrust of this manuscript, and should be clarified.

• Figure 2B. If the authors used an insulin Cre mouse line, which is supposed to delete in beta cells, why is med15 absent in alpha cells? Figure 1 shows expression in wild type alpha cells.

• Figure 1F. Gene expression studies need to adjust for multiple comparisons.

• Authors have been careful to use a Cre-expressing control for some studies, but the RNA-seq experiments have been done without the Cre, and with normal insulin gene dosage. It is important to validate results with qPCR in Cre-expressing controls.

• Can authors reconcile normal insulin content and normal insulin secretion with KCl and α-ketoglutarate in the KO with reduced granule number and size?

• The analysis of CHIP data is very preliminary. It is not surprising that downregulated or upregulated genes overlap with genes that are located near mediator bound regions. Mediator is meant to bind near many if not most active genes. Is this overlap in some way greater than expected by chance? If not, the interest of this analysis is somewhat limited.

• Following this same logic, are downregulated/upregulated genes specifically enriched in Med15, rather than Med1? In fact, it is unclear if Med15 really binds to different locations than Med1. Most regions seem to show overlap. It is important to analyze Med1 binding in Med15-only peaks and vice versa to show specificity.

• The authors show that 30% Med15 peaks are still there in the Med15 KO. Presumably these are peaks from non beta cells, but this needs to be explained more clearly in the results section (another possibility of course is that there is there cross reactivity with other proteins)

• Figure 4E. This analysis is not explained properly (and probably should be done very differently). There is no information on what data is being used, or how the authors have chosen to center the histone mark data. Mediator is known to bind to the center of enhancers and promoters and this type of representation is not helping to show that.

• Figure S4D shows that mediator peaks are most enriched in regions that lack histone marks, which account for nearly 50% of mediator peaks. This is unexpected since mediator has been studied extensively in a variety of cellular models with clear enrichment in super-enhancers and other regulatory elements. This needs more careful attention to understand and explain

• Overlap with sites bound by all pancreatic islet transcription factors is expected, since both Mediator and transcription factors bind to enhancers and promoters. However, the authors do not seem to show that. Instead, figure 5D shows “Overlap of IM15KO downregulated genes with transcription factor datasets as determined by ARCHS4 datamining”. This is a very indirect comparison.

• Authors have not shown cooperative binding or function. This conclusion does not seem warranted.

• It would be good to show the reverse Co-IP for Nkx6.1 and NeuroD1-MED15 interactions, as well as the whole membrane.

• Overexpression of MED15 in hESCs is interesting, but needs considerable work to provide a more convincing message.

o The differentiation protocol needs to be described and characterized. The number of insulin expressing and MED15 expressing cells is very low in control conditions.

o The genetic modifications need to be characterized and the genome editing strategy needs to be fully described. How have they selected the clones? Is the sequence of alleles that does not carry the intended modification normal? How many different clones were used, and how many independent experiments?

o Most MED15 seems to be in the cytoplasm in the overexpression model, is MED15 in the nucleus in any cells?

o Does MED15 increase the function of beta cells or the number of beta cells? Immunofluorescence studies of maturation markers (e.g. for upregulated genes) are needed.

o Authors suggest that they cannot elicit glucose-stimulated insulin secretion in these cells due to the use of Alk5 inhibitors during the β-cell differentiation protocol. It should be straightforward to remove it and reassess β-cell maturation.

Minor points
• Line 150 ◊ remove “control and”.
• Fig. 4D and E axis definitions are confusing

Rebuttal for Nature Communications NCOMMS-19-25720-T, Transcriptional coactivator MED15 is required for beta cell maturation

Reviewer #1 (Remarks to the Author):

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Kadhim et al, Transcriptional coactivator MED15 is required for beta cell maturation

In this manuscript, Kadhim et al describe a series of experiments to implicate the MED15 subunit of the Mediator coactivator complex in pancreatic beta cell function. Following their observation that MED15 is expressed in developing pancreas and other pancreatic cell derivatives, they find that its reduced expression in diabetic (T2D) patient-derived islets also correlates with expression of glucose-stimulated insulin secretion (GSIS) marker genes. Towards implicating MED15 directly in the beta cell gene expression program, a mouse knock-out model (IM15KO, under the control of beta cell-specific Ins1cre) was generated. Largely consistent with the patient tissue data, extensive characterizations of the phenotypes of IM15KO show impairment in genes responsible for beta cell maturation and glucose uptake functions. The data also suggested defects in normal mitochondrial physiology. Towards understanding how transcription of the relevant genes might be controlled by MED15, the authors performed ChIP-seq with commercial antibodies against MED15 in MIN6 insulinoma cells. Resulting tracks on selected genes are shown along with the data from a MED1 ChIP-seq performed by the authors in the same cell line, as well as tracks from ChIP-seq data on other transcription factors (obtained from public databases). To obtain further clues about MED15 function on target genes, various bioinformatic analyses (including chromatin state segmentation, de novo motif analysis) were undertaken. De novo motif analyses of genomic sites coinciding with MED15 indicated that they are enriched for binding motifs for transcription factors Nkx6-1 and NeuroD1, as well as additional factors, dependent on which input parameters (H3K4me1-containing enhancers or other) were specified. Towards gauging whether the binding motif coincidence results from potential physical interactions between MED15 and the transcription factors, co-immunoprecipitation experiments were also attempted. Finally, overexpression of MED15 in human ES cells programmed to generate islet-like spheroids yielded beta-like cells in which some of the markers that were identified as MED15 targets in the earlier analyses displayed somewhat elevated expression.

This paper represents a tremendous amount of work undertaken in support of the hypothesis that MED15 is a key transcriptional coactivator for beta cells. It is therefore very unfortunate that taken as a whole, the paper ultimately fails to build a convincing case for MED15 function as a master coactivator for beta cells. As this reviewer sees it, and as detailed below, this stems largely from the authors' idiosyncratic view of the Mediator complex, which appears to be at variance from the current thinking in the transcription field. No question that the detailed description of what happens to beta cell function when MED15 is knocked out reflects the importance of MED15 in this cell type. But what the authors' handling of the problem appears to unwittingly ignore is that MED15 works as part of the gigantic Mediator complex. Any mechanistic understanding of its mechanism of action will not be forthcoming until it is viewed as an integral part of this complex. It is true that the authors have looked at another subunit, MED1, in some detail. But in the end the provided data do not allow one to construct in one's head a clear model of action for what might be going on with MED15 and Mediator in the context of beta cells. Moreover, at this stage, it is no longer in question whether a given transcriptional program is reliant on the Mediator or even whether one or the other of its subunits is involved. Rather, the bigger challenge is to describe what specific mechanistic roles the Mediator (via an identified subunit interaction) might be playing in the context of that transcriptional program. Thus, whereas it is clear phenomenologically that these cells require MED15 for their specialized functions, it remains unclear what the

underlying mechanism vis a vis the Mediator complex is. This reviewer therefore feels that in this regard at least the current story is somewhat incomplete.

We thank the reviewer for their thorough assessment of our work and for the acknowledgement that this manuscript represents a "tremendous amount of work".

As the reviewer points out, we did consider Med15 function in the context of Mediator through a careful analysis of MED1. Med1, despite its sub-stoichiometric association with the Mediator complex, is often used as a proxy for Mediator chromatin occupancy (PMIDs: 25644604, 27739523, 23582322, 30672466, 26330467). However, we indeed have not studied all Mediator subunits in beta cells; such an endeavour would be a massive undertaking well beyond the scope of our study; indeed, we are not aware of a study that has systematically investigated the role of all Mediator subunits in one tissue of mice (e.g., in a physiological context). Rather, we have identified here a new role for Mediator (via one its subunits) in the metabolically important pancreatic beta cells, which lays the foundation for follow-up work.

We respectfully disagree with the notion that it is "no longer in question whether a given transcriptional program is reliant on the Mediator or even whether one or the other of its subunits is involved.", and we dispute that such studies lack value. It is correct that, conceptually, it is not in question that a Mediator subunit can regulate a transcriptional program; however, this statement misrepresents the impact and volume of our (and many others') findings. Identifying specific developmental and physiological gene programs that are regulated by individual Mediator subunits is, in our opinion, one of the most important tasks towards a better in vivo and therapeutic understanding of Mediator, and one that is the subject of many papers, including recent ones (PMIDs: 30250136, 28834744, 31805036, 36252121)

Furthermore, in our manuscript we do what the reviewer is asking, to "describe what specific mechanistic roles the Mediator (via an identified subunit interaction) might be playing in the context of that transcriptional program". We demonstrate that Mediator is recruited to promoters and enhancers of key beta cell genes. Furthermore, we demonstrate that MED15 can interact with at least two key beta cell transcription factors. There is certainly mechanistic detail (e.g., adoption of structural conformation, etc) that our study, like most *in vivo* Mediator physiology studies, does not capture. We venture that delineating how exactly Nkx6.1 and/or NeuroD1 influence Mediator structure, composition and/or conformation will require substantial amounts of work using other, specialized systems and approaches that are well outside of the scope of this manuscript, which focused on physiological, cellular, and molecular characterization to reveal an exciting new role for Mediator and MED15 in pancreatic endocrine cells.

We reason that studies on Mediator in physiological settings such as ours are complementary to studies on Mediator's molecular mechanisms (e.g., phase separation studies, structural studies with Cryo-EM, etc). These second type of study will provide detailed insight into Mediator's molecular mechanism but less so on its role and effect on tissue development and physiology, which the first type of study (including ours) can provide. Ultimately, both types of work are essential and, together, contribute to a greater understanding of Mediator dependent regulation of gene expression and biology.

Major points related to establishing MED15 function in the context of the larger Mediator complex:

1. A simple question that remains unanswered relates to tissue-specificity for MED15. The authors' singular focus on pancreatic cells is obviously well justified. But given that MED15 has been implicated in other gene expression programs (lipid metabolism, signaling via STATs), an analysis of expression patterns in other tissues besides the pancreas (Fig.1) would have been extremely

instructive. Are the observed expression kinetics unique to pancreatic cells and correlate with the expression kinetics of critical genes?

We appreciate the reviewer's agreement that our focus on pancreatic cells is well justified. We also thank them for this excellent suggestion and agree that is insightful to delineate Med15 expression in other mouse tissues during embryonic development. In our revised manuscript we have performed gene expression analysis to provide a time course of Med15 expression across metabolically relevant tissues. Specifically at embryonic days (E)12.5, E15.5, E18.5 and postnatal days (P) P1, P7, P14, P21 and 4wks, 6wks, 8wks of age. Please refer to Figure S1 for these data. We note that MED15 peaks in expression in some, but not all, tissues around parturition, suggesting it may play conserved roles in driving cellular maturation in other tissues as well.

2. Conversely, and related to another aspect of the specificity of MED15 action, it is not clear whether just MED15 or another Mediator subunit, in particular a tail module subunit, might be equivalently behaved in the context of beta cells. The authors have nicely noted how MED1, a middle module subunit of the Mediator, behaves both similarly in some respects to MED15 yet quite differently in others. The authors were obviously guided in their choice of this subunit as a kind of control for MED15 based on the large number of publications that have used this subunit in ChIP-seq experiments as a surrogate for the entire Mediator complex. But MED1 is a special case and it would have been really nice to see the authors include some additional subunits in their analyses. With regard to MED1, it is often overlooked by researchers in the field that the MED1 content of the Mediator complex might be variable. Thus, MED1 is substoichiometric (relative to the core complex) in some cell types (see Zhang et al Mol Cell 2005) whereas it appears to be overexpressed in some cancer cell types. Could it be that MED15 stoichiometry vis a vis the core complex is also variable? The authors themselves wonder (Discussion, line 324) as to how changing the amount of a single Mediator subunit has the potential to affect function. The authors raise, but do not address, a number of possibilities, among them the issue of how complex stability might be regulated by MED15. It occurs to this reviewer that there is a strong possibility that MED15 might control the association within the core complex of some tail subunits, which might otherwise be labile, consistent with their observed biochemical behavior. Together with point 3 raised further below that the authors' have not credibly demonstrated a direct MED15 interaction with any of their candidate transcription factors (TFs), the possibility remains that it is one or more of these other tail subunits that are the actual physical targets of TFs that regulate the beta cell transcriptional program and that MED15 is providing an ancillary role. Other tail subunits must therefore be incorporated into the analyses, both cell based and those looking at physical interactions (below).

We thank the reviewer for this multi-part query and for the outstanding recommendations for future experiments. We have responded below to these two lines of questioning. We hope this addresses these concerns:

1) Mediator subunit specificity: In this manuscript we described a completely new role for Med15 in the pancreatic beta cell. Understanding the individual roles of other Mediator subunits in this cell type is certainly an exciting topic for future research. In this context, it is noteworthy that while this paper was being revised, another paper was published describing Cdk8 KO in beta cells (PMID: 31509750). Intriguingly, the described phenotypes are distinct from the ones we describe for Med15, suggesting Mediator subunit non-equivalency in beta cells. In sum, we, like the reviewer, are intrigued to find out more about the roles of other Mediator (tail) module subunits in the beta cells. However, generating and characterizing knockout mice for additional Mediator (tail) subunits, an extensive in vivo endeavour, is clearly well beyond the scope of the present manuscript.

2) Mediator subunit expression and complex stoichiometry: We agree that Mediator subunit expression and precise complex composition may influence physiology and disease, and that it will be important to delineate pertinent mechanisms. We note that, not only Med1, but almost all Mediator subunits are over- or underexpressed in some cancer types (e.g. PMIDs: 18794900, 19790197, 24374838, 24938407, 25791637, 26377566, 27050271, 28367434). What exactly these changes, which may influence Mediator stoichiometry, mean for the function of the complex is, to our knowledge, unclear in virtually all cases (except for CDK8 overexpression in colon cancers PMID: 30185549). However, dissecting what the differential expression/association of a Mediator subunit implies for complex function in various cell types is a topic for a study(s) unto itself and well beyond the scope of a single manuscript.

3. A major shortcoming of the paper is that direct physical interactions between MED15 and any of the candidate TFs (Nkx6-1, NeuroD1) have not been convincingly demonstrated. Because of this, the basic premise of the paper and even its title are on shaky grounds. In the co-ip data shown in Fig. 5F, which is the apparent basis of the various claims related to physical interactions between MED15 and TFs, the following issues were noted and must be addressed:

i. First, by their very nature, co-ips only show what factors exist in association with each other, whether through direct contacts or through intermediary factors. To claim direct interactions, use of purified TFs and coactivators is necessary. Even better, and for a more credible claim, such analyses should be followed up by mapping of the interacting domains in both the TF and the coactivator, and ideally should be coupled with some cell-based demonstration of the validity of the interaction through use of appropriate mutants.

We thank the reviewer for this suggestion. As requested, we have performed assays using purified NKX6-1 and MED15 to replicate this interaction in vitro. Specifically, we expressed tagged HA-Nkx6.1 and FlagMed15 in HEK293 cells via transient transfection, affinity purified them with antibodies, and then assessed binding of eluted, purified HA-Nkx6.1 to Flag beads (negative control) and Flag-MED15 beads. We observed substantial binding of Nkx6.1, in agreement with our Co-IPs. These results can be found in Figure 5.

We also attempted to assess this interaction with GST-pulldown assays; however, in our hands, MED15 proteins expressed poorly, except very short truncated variants, perhaps due to prevalence of intrinsically disorganized regions in Med15 (PMID: 19096501), which may make expression and/or folding challenging in *E. coli*. In addition, we also tried to test this interaction in yeast-two-hybrid assays. Interestingly, we observed interaction above background between some Med15 constructs with Nkx6-1, specifically including the region 2-266 of MED15; however, interaction strength was relatively weak compared to positive controls and hampered by background binding activities, and we therefore opted to not place these data in the manuscript.

Finally, as noted below, we also completely revised our analysis of the ChIP-seq data, and investigated protein-protein interactions of Med15 in silico using Alphafold. Collectively, these new data analyses also strongly implicate Nkx6-1 as a key interacting factor for Med15, supporting the above interaction studies.

ii. Relevant to the issue of MED15 function as part of the larger Mediator complex, the authors need to probe for some additional Mediator subunits. In addition to the various tail subunits, in light of point 2 above, representative subunits from each of the remaining Mediator modules (head, middle, kinase) also need to be probed. Furthermore, a number of unbiased biochemical methods (e.g., cross

link-MS) exist to identify Mediator subunits that serve as targets for a given activator. They should be considered.

We thank the reviewer for this suggestion and agree that it would be useful to comprehensively identify Mediator subunit interactions in beta cells. To move towards, this we have carried out expression profiling for other Mediator factor members in islets (Figure S1) across development. However, we respectfully feel that the experiments suggested by the reviewer here will not answer the question critical to advancing the understanding the biology we discovered here: whether Med15 binds key beta cell TFs in this cell type, and if so, which ones. We venture that, while feasible, identifying additional Mediator subunits in Med15 IPs isn't especially informative as it simply confirms what our Med1 IP and ChIP analysis already suggest: that Mediator subunits interact in this cell type. Vice versa, showing that, for example, Nkx6.1 binds additional Mediator subunits in vivo in CoIP studies is similarly not very informative, in part due to the caveats of in vivo CoIPs outlined by the reviewer.

Thus, we instead focused our further molecular mechanistic studies on more convincingly demonstrating that Med15 binds key beta cell TFs, as outlined above in response to point 3.

We reiterate that the goal of our study was not to comprehensively identify Mediator targets of the many beta cell TFs; but rather to identify a physiological role of Med15 in beta cells, and, if possible, to identify its TF partners. As such, we focused our studies on Med15 and its putative interactions.

The data here for MED1 is very problematic. No controls are shown for the efficacy of the ip. Also, no indication is provided in the Methods on the source (and validation) of the antibody used here in the ip, as well as the one that might have been used in the ChIP-seq analyses (Fig. 5A etc.).

We apologize for this oversight. The antibody we used is the Med1 antibody that is widely used and well established to study Med1 by ChIP (e.g. PMIDs: 25644604, 27739523, 23582322, 30672466, 26330467) and immunoprecipitation (e.g. PMIDs: 31028171, 35394046). We have added this information to the revised manuscript.

Other points:

1. With all due respect to Ernst & Kellis and other bioinformaticians, this reviewer feels that the chromatin state segmentation analyses on which the present authors rely to contextualize their ChIP-seq findings can be quite misleading. The approach and the resulting terminology collapse two distinct parameters: one relates to spatial location and the other to the transcriptional state (active vs. inactive). Ideally, this should be avoided but if the authors insist on invoking this, sufficient background and explanatory notes need to be provided to the average reader to appreciate why the authors resorted to this analysis.

We thank the reviewer for asking us to reconsider this analysis. We agree and, as suggested, we have removed this analysis from the manuscript and completely revised Figure 4.

2. Fig. 4A. The schematic is somewhat confusing. What are the "coregulators" in the cartoon? Why is MED1 highlighted? Also, there is likely a typo in the "Grants et al 2016" reference in the figure legend.

We apologize for the confusion and have removed this panel from the figure. The Grants et al. reference has been corrected to Grants et al 2015.

3. The authors use MIN6 cells for some of their analyses (including ChIP-seq). From Fig. 1E (ratios in lane 1 vs. lane 3), it appears that MED15 might be overexpressed. Could this affect the authors' conclusions about target genes under normal conditions where MED15 levels are not as high?

We thank the reviewer for catching this very interesting observation. While not perfect, mouse MIN6 are a widely-used proxy for beta cells. It is certainly possible that the slightly higher expression of Med15 in mouse MIN6 cells (lane 1; vs. mouse islets, lane 2) reflects an overexpression. Alternatively, it may reflect the fact that the islets in lane 2 also contain non-beta cells, which may express less Med15. Alternatively, these data may simply reflect a difference between differentiated primary tissue and a proliferating cell line.

4. Inconsistent terminology. The authors use "Med15" and "MED15" interchangeably to refer to this protein. Sometimes, the inconsistency occurs in the same sentence (line 317). Whereas the reference to the mouse genes encoding this protein correctly follows the accepted conventions, references to the protein should ideally follow the recommendation of Bourbon et al, Mol Cell 2004. Ditto for Med1/MED1.

We apologize to the reviewer for any inconsistencies they found in our manuscript. During revision we have paid particular attention to make sure our terminology is consistent and to not use "Med15" and "MED15" interchangeably. We have used MED15 for the human *GENE*/PROTEIN and Med15 for the mouse *Gene*/Protein, following widely accepted conventions from HUGO and MGI, respectively. Please note that the indicated Bourbon et al paper only suggests terminology for human Mediator subunits (which we name accordingly), but not mouse subunits.

5. The Abstract is a little ambiguous. Addressing the following might help to make it crisper: (i) "We sought to understand whether Mediator subunit MED15 acts as a node…." Use of the vaguely defined term "node" has the effect of rendering the stated goals of the study also appear somewhat vague. (ii) For those without a beta cell physiology background, the statement that MED15 loss did not affect insulin expression appears at first contradictory. Although the significance of this becomes absolutely clear as one reads the text, it would be nice to add a clarifying clause. (iii) "… an additional layer of control…." Since no other "layers" have been introduced, the precise meaning is lost on the reader.

We thank the reviewer for these excellent suggestions. We have revised the abstract with an eye towards improving crispness and clarity.

"Mediator, a co-regulator complex required for RNA pol II activity, interacts with tissue-specific transcription factors to regulate development and maintain homeostasis. We found reduced Mediator subunit MED15 expression in endocrine hormone-producing pancreatic islets from human T2D and sought to understand how MED15 controls gene expression programs important for the function of insulin-producing β-cells. We found Med15 to be expressed during mouse β-cell development and maturation. Knockout of Med15 in mouse β-cells caused defects in β-cell maturation without affecting β-cell mass or insulin expression. ChIP-seq and co-immunoprecipitation analyses determined that Med15 binds β-cell transcription factors Nkx6-1 and NeuroD1 to regulate key β-cell maturation genes. In support of a conserved role during human development, human embryonic stem cell derived β-like cells, genetically engineered to express high levels of MED15, expressed increased levels of maturation markers. We provide the first evidence of a conserved role for Mediator in β-cell maturation and demonstrate an additional layer of control that tunes transcription factor function."

Reviewer #2 (Remarks to the Author):

This manuscript by Kadhim et al. examines the roles/mechanisms of Med15 in pancreatic islet beta cells, in both mouse and human beta-cell models. Med15 is a subunit of the Mediator co-regulator, which interacts with transcription factors (TF) and RNA PolII for transcriptional regulation. Based on some studies on Med15 expression, beta-cell specific knockout, and genomic analyses, the authors suggest that Mediator regulates beta-cell maturation. This particular role is likely fulfilled by Med15 fine-tuning transcription regulated by TFs NeuroD1 and Nkx6.1, well-established regulators of beta-cell function/maturation.
Overall, the study is well designed and carried out. The findings sufficiently support a key role of Med15 in postnatal beta-cell function. They further implicate how some seemingly general Mediator subunits can selectively regulate specific transcriptional pathways (likely being recruited via specific TFs, e.g., Nkx6.1 for Med15 while others for Med1). Thus, its publication should be of interest for readers who study either beta-cell biology or transcription regulation.

We thank the reviewer for this kind and constructive review and for pointing out the strengths of our study.

Yet there is a major concern that needs to be addressed to better support the authors' overall conclusion that "MED15 is required for 1 beta cell maturation". It is generally agreed that beta-cell maturation in mice is completed by roughly two weeks after birth. Thus, the authors' conclusion would suggest that beta-cell defects will become obvious by ~2-weeks after birth. Yet most of the phenotypic analyses in this study were in ~2-months old mice. Thus, the current study cannot distinguish if Med15 is required for beta-cell functional maturation and/or maintenance. Thus, the authors should attempt to answer the following key questions:

1. What are the expression levels of Med15 between newly born to 2-months of age (e.g., P14, the stage when beta cells establish proper glucose response and P28, when beta cells establish full function to handle new diet exposure)?

We thank the reviewer for this recommendation. As requested, we have profiled Med15 expression in in P1, P7, P14 and 3-, 4-, 6- and 8-week old islets. We note that Med15 expression peaks at P14, a time when pups start eating solid food and slow down their milk consumption, and when beta cells begin their maturation (e.g. PMID: 25662175). These data have been included in Figure S1.

2. What are the insulin secretion/mitochondrial phenotypes/beta-cell gene expression patterns between newly born to 2-months of age?

We thank the reviewer for this outstanding recommendation that has strengthened our manuscript. As requested, we have carried out glucose tolerance tests on 3-week old mice, including measurement of plasma insulin. We observed significant glucose intolerance at this age, although not as profound as in 8-week old animals (Figures 1 and S3). Furthermore, at both stages, we were unable detect glucose-stimulated insulin in the plasma. Collectively, these data suggest that the impairments in beta cell function occur early in life and support defects in beta cell maturation.

To confirm this, we collected tissues from P1, P7, P14, and 3- and 4-week old mice and carried out immunostaining for maturation markers (Figure S4). These data clearly show that none of Mafa, Ucn3 or

Glut-2 are expressed at any stage in the MED15KO mouse islet development, confirming that cells do not mature and then lose maturity over time. This agrees with the functional data and suggests that Med15 is required for the maturation process.

Additional questions are, not in particular order:

1. In the introduction, a summary about the known beta-cell maturation process will help (metabolism, insulin vesicle production/maturation, cell-cell communication, stimulus-secretion coupling etc.) to lay down the pathways to be studied later.

We thank the reviewer for this suggestion. We have revised the first paragraph of the introduction to include a more detailed description of how maturation changes beta cell physiology.

2. Line 39, the authors may want to use a different word to replace "lack"---it's more about the expression level instead of presence/absence during maturation.

We agree with the reviewer, this was a poor word choice and has been revised.

3. Lines 41-42 "β-cells are not glucose responsive at birth" may also need rewording, see Blum et al., 2012.

Again, we thank the reviewer for this suggestion. Blum et al. demonstrated that glucose responsiveness was left-shifted (i.e., to lower glucose levels in immature cells). We have revised our manuscript to reflect this important work.

4. The introduction (line 74-78) highlighted the potential roles of MDT-15 in hypoxia and oxidative stress. The authors should have some discussion on this topic, based on their gene expression changes detected in Med15 mutant beta-cells.

We thank the reviewer for this recommendation. We have de-emphasized this aspect of Med15 in the introduction and so have not included any discussion on this as we feel it does not easily fit with overall message of the manuscript. We feel like this discussion would be more appropriate if we were studying Med15 in the context of beta cell stress, type 2 diabetes or islet transplantation and will likely revisit this in the future as funding allows.

5. Lines 125-126. Maybe deleting "suggesting no changes in food intake"?

We have not measured food intake and we agree with the reviewer that it is not the only way mice can lose weight. We have deleted as suggested.

6. Line 170, it is not clear what "suggesting abnormal mitochondrial physiology" means.

We apologize for our imprecise language here. We have revised to, "*altered mitochondrial dynamics that likely results from β-cell immaturity.*"

7. Fig. 1B-D, the inclusion of DNA staining is blocking the signals from Med15 staining. It may help to add a single Med15 channel.

We apologize for the lack of clarity in these images. As requested, we now show the Med15 single channel images to allow the reader to more easily see the immunostaining.

8. "M15betaKO" might be a better way of indicating beta-cell specific Med15 KO, instead of "IM15KO". This format will be a better match with the latter use of "MED15βOE".

We agree with the reviewer and throughout we have revised IM15KO to M15betaKO as suggested.

9. Lines 188-189, "To confirm specific Med15 binding, we performed ChIP-seq on islets from control and IM15KO mice". What was the estimated beta-cell % in these islets? This info will be helpful for non-islet readers.

In order to focus the manuscript, we have removed the islet ChIP-seq data from this manuscript. However, we have pointed out in our rationale for using MIN6 cells for ChIP-seq data that they represent a more pure population of beta cells than islets and are therefore likely a better tool for these types of analyses than whole islets.

10. Some studies used Ins1Cre as controls, while others use Med15F/F? Are there any differences?

We thank the reviewer for asking for clarification. For all studies except the RNA-seq the mouse genotypes that were used were $Ins1^{Cre/+}$; $Med15^{flox/flox}$ (M15betaKO) and $Ins1^{Cre/+}$; $Med15^{+/+}$ (Control). For the RNA-seq experiments the genotypes used were $Ins1^{Cre/+}$; $Med15^{flox/flox}$ (M15betaKO) and $Ins1^{+/+}$; $Med15^{flox/flox}$. We note that RNA-seq experiments were validated with immunofluorescent staining analyses, which use $Ins1^{Cre/+}$; $Med15^{flox/flox}$ (M15betaKO) and $Ins1^{Cre/+}$; $Med15^{+/+}$ (Control) (Figures 3 and S4). In all cases, littermate controls were used in the experiments. More information regarding mouse genotypes and confirmatory experiments can be found in response 4 to Reviewer 3 below.

11. The presented results are mostly from male mice (line 454). What about the females?

We thank the reviewer for this important request. In response, we have carried out similar experiments using female mice. As can be seen in Fig S3 there is no dramatic sex-specificity to the knockout phenotype.

12. Figure 2B: do the IM15KO pancreata maintain Med15 expression in non-beta cells?

We thank the reviewer for requesting these data. We have included new images that show Med15 immunostaining in non-beta cells in the knockout in Figure 2B.

13. Lines 216-220, how many other TFs were examined in addition to those listed here?

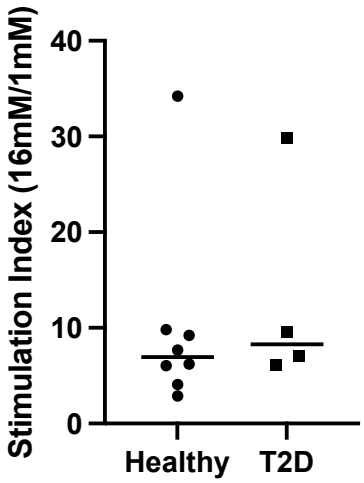
We thank the reviewer for this query. We tested Med15 interaction with a number of transcription factors using co-immunoprecipitation on MIN6 cell lysates, including: Nkx6-1 (+), NeuroD1 (+), Pax6 (-), Pdx1 (+/-), Hnf1a (-), RXRa (+/-) and SREBP-1(-). Amongst these, as indicated in the brackets, we only saw replicable interactions for Nkx6-1 and NeuroD1. This is likely explained by rather weak interactions as predicted by Alphapulldown in our revised manuscript.

14. The authors need to examine cell proliferation/death, because of the known effect of NeuroD1 mutation on beta-cell death.

We thank the reviewer for this suggestion and have carried out EdU staining on islets from Med15 knockout mice. We observed a significant increase in β-cell proliferation in the knockout (Figure S3E). This is in agreement with our previous work that shows that beta cell function and proliferation are inversely related (PMID: 26626461) and this is probably is not related to Neurod1 per se (PMID: 31519700).

15. Figure 1. Do the authors have insulin GSIS data on those T2D islets?

We did not carry out GSIS in these T2D islets; although the University of Alberta Isletcore does routinely carry out these types of studies on islets they isolate. We have included these data below on a subset of the islets we studied below, which we obtained from the Isletcore, and are happy to include in the manuscript if the reviewer feels it would be valuable for the reader to see.



16. Figure 2 title: removing "hyperglycemia" because the authors have no data on this?

We thank the reviewer for this suggestion. While the fasting glycemia is not significantly higher in knockouts, the mice do become significantly glucose intolerant. We have revised the figure legend title to reflect this.

17. Figure 6D, a split channel is needed to show the expression of MED15, which looked like cytoplasmic.

The reviewer is correct, we see significant expression of MED15 in the cytoplasm in both stage 6 stem cell-derived beta cells and in E18.5 beta cells (Figure 1). MED15 is known to shuttle between the cytoplasm and nucleus (e.g. PMID:35035429). We are not yet sure why MED15 seems to be predominantly located in the cytoplasm in immature beta cells but believe it is an interesting observation that warrants additional study. That said, we have removed Figure 6D from the revised manuscript and this interesting shuttling between cytoplasm and nucleus, which is similar to shuttling observed by Stein and colleagues for MAFA and NKX6-1 during diabetes (PMID: 23863625), will be part of a follow-up study.

18. Figure S2G, if the insulin content/microgram DNA doesn't change, how do the authors explain the reduced insulin granules in mutant beta cells (in Figure 3C)?

We appreciate the reviewer's concern that on the surface these seem like disparate observations. We believe that there is not a huge discrepancy here. Insulin granule biogenesis requires the proper movement of insulin from the ER through the Golgi to the secretory granule. During this process, there is processing of insulin by prohormone convertases and carboxy peptidase E and transport of zinc into the granules through ZnT8. As the ZnT8 knockout mice have demonstrated (PMID: 19706465), zinc is needed for proper crystallization of insulin and formation of the dense core granules that we counted. Because there is a reduction in ZnT8 in immature MED15 knockout beta cells (Figure 3A), this crystallization process does not happen as it would in control adult beta cells. As we measured mature, dense core granules in our analysis, any differences we observed do not necessarily indicate a reduction in total insulin content within beta cells, but rather a defect in granule maturation, which is a marker of beta cell immaturity. We have clarified this point in the manuscript as outlined below:

"indicating lack of insulin granule maturation, likely due to reduced Slc30a8 expression and impaired zinc concentration and insulin crystallization within the granules"

Reviewer #3 (Remarks to the Author):
Summary

Khadim et al have examined the role of a subunit of the Mediator complex, MED15, in regulating the expression of genes that are important for the maturation of pancreatic β-cells. They show that KO of MED15 causes β-cell dysfunction, in part due to altered mitochondrial respiration, and diabetes. They also show that MED15 is often downregulated in type 2 diabetes islets. They carried out MED15 and MED1 Chip-seq in MIN6 cells and mouse islets, and use IPs to show that MED15 interacts with key β-cell transcription factors (Nkx6-1 and NeuroD1). Finally, they carried out MED15 overexpression studies in stem cell models, suggesting that this could help improve in vitro β-cell differentiation.
This work provides interesting insights on how a Mediator complex subunit can regulate cell-type specific transcriptional networks. However, the CHIP analysis and stem cell work is very preliminary, and there are several shortcomings that need to be addressed before publication.

We thank the reviewer for their thorough assessment of our work. We appreciate that they feel it provides interesting insights into how Med15 regulates beta cell function. We have spent considerable time revising the ChIP and stem cell work and feel that the manuscript is now strengthened by the reviewer's insights.

Major points

1. The cellular specificity of med15 is unclear. The qPCRs are not very informative, but the immunofluorescence images seem to show that med15 is present in islet beta and alpha cells, but not in exocrine cells. Is this interpretation correct? Given the focus on differentiation, when is Med15 expressed during pancreas development? It is possible that although the function Mediator is ubiquitous, med15 is exclusively expressed in islet cells within the pancreas, and other subunits carry out its role in other cell types. Is this cell-specific expression consistent with what is already known about med15, or is this a novel finding? These points are very relevant to the major thrust of this manuscript, and should be clarified.

We thank the reviewer for requesting clarification. We observed that Med15 is expressed primarily in beta cells early (E13.5) during pancreas development and that as development proceeds it also becomes expressed in the other endocrine cell types at E18.5 and in islets. Furthermore, we demonstrate that it is not expressed in alpha cells prior to the secondary transition (Figure 1). We demonstrate in Figure S1 that Med15 is also expressed in other tissues during development and postnatally.

In support of these observations, we have also deleted Med15 in the entire developing pancreas using Pdx1Cre, which deletes in the pancreatic progenitors at E9.5 (unpublished). In these mice, we observed no changes in exocrine pancreas formation but impairments in islet cell formation. This suggests that, in the pancreas, Med15 plays specific roles in the endocrine compartment, and highlights that Mediator complex subunits are not necessarily equivalent in all tissues but can have cell-type specific regulatory roles.

To our knowledge, we are the first group to study Med15 in the pancreas and the first to knock it out in mice. This manuscript therefore represents a completely novel area of research.

2. Figure 2B. If the authors used an insulin Cre mouse line, which is supposed to delete in beta cells, why is med15 absent in alpha cells? Figure 1 shows expression in wild type alpha cells.

We thank the reviewer for this question and for asking us to reconcile this disparity. In this revision we have included improved images that show Med15 immunostaining in non-beta cells, including in alpha cells, in the knockout in Figure 2B.

3. Figure 1F. Gene expression studies need to adjust for multiple comparisons.

We thank the reviewer for pointing out the deficiencies with Figure 1F. In response, we have revised this figure and rather showed graphs so that gene expression changes could be more easily appreciated. We are not clear why the reviewer feels we have carried out multiple comparisons in this figure as we only compared expression of genes from in islets donated by health controls with those from individuals with Type 2 diabetes. If this is incorrect, it would be helpful if the reviewer could let us know which statistical tests they would like us to carry out on these data.

4. Authors have been careful to use a Cre-expressing control for some studies, but the RNA-seq experiments have been done without the Cre, and with normal insulin gene dosage. It is important to validate results with qPCR in Cre-expressing controls.

We thank the reviewer for raising this point and we acknowledge that it was an oversight that the RNA-seq studies were carried out in mice without a Ins^{Cre} allele. We also agree that as a result, careful validation of the RNA-seq experiment using Cre expressing controls strain is important. In response, we note that in our study, we used immunofluorescence in Ins1Cre/+; Med15+/+ and Ins1Cre/+; Med15flox/flox mice to show reduced expression of Ucn3, MafA, and Glut2 (Figures 3B and S4), suggesting that the downregulation observed following Med15 loss is consistent across control genotypes.

5. Can authors reconcile normal insulin content and normal insulin secretion with KCl and α-ketoglutarate in the KO with reduced granule number and size?

(please see response to reviewer #2, comment 18).

We appreciate the reviewer's concern that on the surface these seem like disparate observations. We believe that there is not a huge discrepancy here though. Insulin granule biogenesis requires the proper movement of insulin from the ER, through the Golgi and to the secretory granule. During this process, there is processing of insulin by prohormone convertases and carboxy peptidase E and transport of zinc into the granules through ZnT8. As the ZnT8 knockout mice have demonstrated, zinc is needed for proper crystallization of insulin and formation of the dense core granules that we counted. Since there

is a reduction in ZnT8 in immature beta cells, this crystallization process does not happen as it would in adult beta cells. As we measured, mature, dense core granules in our analysis any differences we saw do not necessarily indicate a reduction in insulin content within beta cells but rather a defect in beta cell maturation. In response we have clarified this point in the manuscript as outlined below:

"…indicating lack of insulin granule maturation, likely due to reduced Slc30a8 expression and impaired zinc concentration and insulin crystallization within the granules."

6. The analysis of CHIP data is very preliminary. It is not surprising that downregulated or upregulated genes overlap with genes that are located near mediator bound regions. Mediator is meant to bind near many if not most active genes. Is this overlap in some way greater than expected by chance? If not, the interest of this analysis is somewhat limited.

We thank the reviewer for requesting we carry out more robust analysis of our ChIP-seq datasets. We have now done this and present a completely revised analysis of the data presented in Figure 4. We make a number of new observations that have greatly improved our manuscript, including:

1) Both Med15 and Med1 bind to a variety of sites in the genome, and a significant number of these sites are not shared.
2) Med15 and Med1 peaks are enriched for different transcription factor binding motifs, and Med15 peaks more significantly overlap with binding motifs of transcription factors that are important for pancreatic beta cell maturation, including Nkx6-1, NeuroD1, MafA, and important beta cell immediate early genes like Fos, JunB, and Npas4 that have also been implicated more recently in beta cell maturation.
3) We find significant overlap between Med15 and Nkx6-1, NeuroD1, Glis3, and Mafa consensus binding sites at gene promoters in pancreatic beta cells and significant overlap in the transcript expression between genes that are regulated by Med15 and these factors.
4) Finally, to directly answer the reviewer's question, we present data in Figure S6A that show that Med15 and Med1 were observed at islet enhancers and promoters at significantly greater levels than by chance alone, suggesting that Med15 plays key regulatory roles in beta cells.

All of these observations support our central hypothesis that Med15 acts to coordinate the transcriptional network required for beta cell maturation.

7. Following this same logic, are downregulated/upregulated genes specifically enriched in Med15, rather than Med1? In fact, it is unclear if Med15 really binds to different locations than Med1. Most regions seem to show overlap. It is important to analyze Med1 binding in Med15-only peaks and vice versa to show specificity.

We thank the reviewer for this question. As outlined in the previous response we have now completed these analyses; the data may be found in Figures 4E and S6. We find that Med15 and Med1 share binding sites but also have distinct areas of enrichment. Further, Med1 and Med15 peaks are both overrepresented in genes that are downregulated in the Med15betaKO, but underrepresented in genes that are upregulated in the Med15betaKO, in line with the view that Med15KO downregulated genes likely represent direct targets while upregulated genes may also reflect compensatory/indirect changes. Additionally, in Figure 4F we demonstrate that transcription factor motifs enriched within Med1 and Med15 peaks are distinct.

8. The authors show that 30% Med15 peaks are still there in the Med15 KO. Presumably these are peaks from non beta cells, but this needs to be explained more clearly in the results section (another possibility of course is that there is there cross reactivity with other proteins)

We thank the reviewer for this question. We believe that this signal likely reflects the presence of other Med15-expressing (presumably endocrine) cells in isolated islets. In support of this, we have recently knocked out Med15 in alpha cells and show that there is an important role for it in this islet cell type as well (unpublished). To address this concern, we have removed the islet ChIP-seq dataset from the manuscript.

9. Figure 4E. This analysis is not explained properly (and probably should be done very differently). There is no information on what data is being used, or how the authors have chosen to center the histone mark data. Mediator is known to bind to the center of enhancers and promoters and this type of representation is not helping to show that.

We thank the reviewer for this comment. In response to this comment and that from Reviewer 1, we have removed this figure from the manuscript along with the bulk of the chromatin segmentation analyses.

10. Figure S6D shows that mediator peaks are most enriched in regions that lack histone marks, which account for nearly 50% of mediator peaks. This is unexpected since mediator has been studied extensively in a variety of cellular models with clear enrichment in super-enhancers and other regulatory elements. This needs more careful attention to understand and explained

We agree with the reviewer that these data did not provide significant insight into the role of Med15 in beta cells. As highlighted above, we have removed these data from the manuscript and replaced with entirely new versions of Figure 4 and Figure S6.

11. Overlap with sites bound by all pancreatic islet transcription factors is expected, since both Mediator and transcription factors bind to enhancers and promoters. However, the authors do not seem to show that. Instead, figure 5D shows "Overlap of IM15KO downregulated genes with transcription factor datasets as determined by ARCHS4 datamining". This is a very indirect comparison.

We thank the reviewer for this comment. As outlined above, we have addressed this in the revised Figure 4H.

12. Authors have not shown cooperative binding or function. This conclusion does not seem warranted.

It will be very difficult to show this experimentally so we have tempered this conclusion. We thank the reviewer for pointing this out.

13. It would be good to show the reverse Co-IP for Nkx6.1 and NeuroD1-MED15 interactions, as well as the whole membrane

We thank the reviewer for this suggestion, which was similar to a concern Reviewer 1 raised. In response, we have added new data in figure 5 that supports a direct interaction between Nkx6-1 and Med15, including reverse IP for Nkx6-1 as requested. We could not reproducibly pull down NeuroD1 with Med15 antibodies, possibly indicating that the interaction is not able to occur without the

stabilizing effects of DNA binding. However, AlphaPullDown data in Figure S7 do support weak interactions between NEUROD1 and MED15. Furthermore, unpublished mass spec proteomics data carried out following Neurod1 immunoprecipitation on MIN6 cell lysates, shared with us by colleagues at the Barbara Davis Center, also detected Med15 - suggesting a *bone fide* interaction.

14. Overexpression of MED15 in hESCs is interesting, but needs considerable work to provide a more convincing message

We thank the reviewer for these questions. We have spent significant time revising Figure 6 in response and this has improved the robustness of our observations and conclusions.

a. The differentiation protocol needs to be described and characterized. The number of insulin expressing and MED15 expressing cells is very low in control conditions.

We have referenced the now-published differentiation protocol in the revised manuscript. We have improved our efficiency of differentiation since we initially submitted this work, and typically observe at least 30% insulin-expressing cells at the stage described in this work (e.g., Fig 6G)

b. The genetic modifications need to be characterized and the genome editing strategy needs to be fully described. How have they selected the clones? Is the sequence of alleles that does not carry the intended modification normal? How many different clones were used, and how many independent experiments?

We have now described in detail our genome editing strategy, which we have published on extensively (PMIDs: 36801003, 30886088, 30540962, 30012219, 28441428, 25474420; DOI: /10.1101/2023.04.19.537542, /10.1101/2023.04.05.535733). We have carried out these experiments on three well characterized clones (Fig S8) and used at least three biological replicates, which in our group is at least three independent differentiations.

c. Most MED15 seems to be in the cytoplasm in the overexpression model, is MED15 in the nucleus in any cells?

The reviewer rightly points out that the majority of MED15 expression is seen in the cytoplasm during our differentiations. Interestingly, we observed a similar phenotype late in pancreatic development in mice (Figure 1B). We hypothesize that the cellular localization of MED15 in maturing beta cells is important for function. We hope to follow-up on this in future studies to understand if and how MED15 is shuttled between the nucleus and cytoplasm and how this regulates the function of MED15 during development, similar to experiments we carried on NEUROG3 (PMID 28441528). That said, we do see some nuclear expression of Med15 in many nascent beta cells. In the interest of clarity, we have removed this panel from the revised Figure 6.

d. Does MED15 increase the function of beta cells or the number of beta cells? Immunofluorescence studies of maturation markers (e.g., for upregulated genes) are needed.

We have carefully quantified the number of insulin-producing cells in control and MED15 OE cell lines by flow cytometry and, similar to the Med15 knockout experiments in mice, did not detect any dramatic difference in the number of beta cells (Figures 2, S8). So, we do not believe that MED15 overexpression in nascent beta cells changes their differentiation dramatically. As shown in Figure 6,

MED15 overexpression significantly increases the expression of maturation markers at both the mRNA and protein levels.

e. Authors suggest that they cannot elicit glucose-stimulated insulin secretion in these cells due to the use of Alk5 inhibitors during the β-cell differentiation protocol. It should be straightforward to remove it and reassess β-cell maturation

We now routinely remove the Alk5 inhibitor from our differentiations and do not see any immediate impact on glucose-stimulated insulin secretion. Furthermore, we do not see significant glucose-stimulated insulin secretion in early stage 6 when these experiments were carried out, but do see at trend towards significance with MED15 overexpression. As a result, we have removed insulin secretion data from this figure and show expression of beta cell maturation markers, which supports a conserved role for MED15 in regulating human beta cell maturation.

Minor points

- Line 150 ◊ remove “control and”.

Revised as suggested, thank you.

- Fig. 4D and E axis definitions are confusing

Figure 4 has been revised, thank you.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Kadhim et al, NCOMMS-19-25720A-Z

This is a revised version of a manuscript previously critiqued by this reviewer some years ago. Whereas many of the reviewer's ancillary points have been satisfactorily addressed in this version, the main issues relating to insights into the mechanisms whereby MED15 might serve as a preferred coactivator in beta cells remain, for the most part, outstanding. It was recommended to the authors that to move beyond the phenomenological description that their cell- and animal-based studies provide – and thus to make the paper more attractive for audiences outside of the confines of the beta cell/diabetes field – they might want to probe deeper into how MED15 effects play out in the context of the Mediator complex.

In this regard, and among other suggestions, it was recommended that they fortify IP-type data by probing their IPs for other representative Mediator subunits, which could reveal something about whether the interactions reflect autonomous MED15 interactions (unlikely) or entail the entire Mediator complex (more likely). However, the authors misconstrued this to mean that they are being asked to analyze pairwise interactions with the full set of 30 subunits and did not heed the simple suggestion. Furthermore, in response to a related point, and to their credit, the authors attempted to further probe interactions between MED15 and NKX6-1, a putative interacting activator using what they describe as purified proteins (Fig. 5). This was a step in the right direction, but unfortunately the final result is difficult to interpret because HEK293-expressed MED15 is very likely to have picked up the bulk Mediator subunits, one or more of which may be responsible for the interaction result in the figure. It is recommended that the authors repeat the experiment using a different source of recombinant factors (e.g., via baculovirus expression). The authors also tried to buttress this conclusion by including an in silico model. Clearly, a mutational analysis that included mapping of the interactions would have been much more convincing. Another important concern, again related to the issue of how MED15 behaves vis a vis the bulk Mediator complex, was that the MED15 knockout may adversely affect the complex's Tail module and that disruption rather than a MED15 interaction per se may be responsible for the observed biological effects. Once again, the authors evidently misconstrued this as a suggestion to investigate the roles of all the Tail subunits! In the end, the revised version, like the original, does not further our mechanistic understanding of the described phenomenology.

Overall, it appears to this reviewer from the authors' rebuttal and the revisions that they chose to implement that they are more invested in advancing beta cell biology rather than an understanding of the underlying mechanisms for coactivator function. The paper in its current form nicely fulfills that goal and should be suitable for journals catering to that field.

Reviewer #2 (Remarks to the Author):

In this revised manuscript, Kadhim and colleagues examined the roles of Med15, a subunit of co-regulator Mediator, in islet beta cell maturation and function. The most noteworthy result is that although the Mediator complex participates general transcription regulation in most cells, inactivating Med15 in the pancreatic beta cells only produces significant maturation phenotypes. This appears to be mediated by specific recruitment of Med15 by NeuroD1 and/or Nkx6.1, which are essential for islet beta-cell production, function, and survival. In addition, the authors showed that Med15 levels are reduced in islet (including beta) of T2D human subjects. Its over-expression also helps to improve the maturation of human ES cell-derived beta-like cells. Thus, this study is significant in not only revealing a mechanism by which general factors exert cell type/stage specific regulation, but also highlight a factor that can be manipulated to improve hESC-derived beta-like cells for therapy. Therefore, this work will be of significant for readers in transcription regulation and diabetes research.

In the original round of review, this reviewer's opinion lied in the positive side. His main concern is that the authors lacked sufficient evidence to distinguish whether Med15 is required for beta-cell maturation or functon(identity) maintenance. He also had several detail-related problems that need to be addressed.

In this revised version, the authors have added a lot more data to directly address this reviewer's concerns, including more detailed gene expression/function analysis at multiple stages. Thus, this reviewer thinks that the authors conclusion is now fully supported by their findings, which were built upon sound methods.

Therefore, this reviewer no longer has issues.

Reviewer #1 (Remarks to the Author in blue):

We appreciate that Reviewer 1 is an expert in Mediator biology and that they have very strong views on what constitutes a novel and interesting paper on Mediator complex proteins. We hope to convince them that work like ours does provide novelty that others in the Mediator community will be interested in, in addition to researchers in other fields (developmental biology, endocrinology, metabolism).

1) "Whereas many of the reviewer's ancillary points have been satisfactorily addressed in this version…".

We thank the reviewer for this comment. We highlight that the reviewer's report had many points, and they considered many of them important. For example, under "Major Points…", they wrote, "an analysis of expression patterns in other tissues besides the pancreas (Fig.1) would have been extremely instructive." In our revision, we directly addressed this concern and performed an expression analysis of several Mediator subunits and other genes on a detailed time-course in several tissues, as requested.

2) …the main issues relating to insights into the mechanisms whereby MED15 might serve as a preferred coactivator in beta cells remain, for the most part, outstanding.

Again, we thank the reviewer for this comment. The main area of disagreement appears to be based on a misunderstanding of what our research shows and what we concluded. We are not clear why the reviewer claims that we concluded that Med15 was a "preferred coactivator in beta cells". Nowhere in our manuscript do we make this claim. Rather, we provide evidence that Med15 plays a key role at an important time in beta cell ontogeny: during functional maturation.

In response, we have revised our introduction and conclusions to clarify that we are not claiming that Med15 is a preferred co-activator in beta cells but rather suggest that it acts, likely via the Mediator complex, as an essential factor in beta cells during this key developmental time.

For example,

Line 79: *"Because Med15 orthologs participate in these processes and interact with molecular partners relevant to β-cell development and maturation, we hypothesized that Med15—at least partly through its important role in Mediator—may act as a nexus that couples β-cell-specific transcription factors to core promoters to regulate β-cell maturation and function"*

Line 90: *"Collectively, these data identify Mediator family member Med15 as a conserved β-cell co-activator required for efficient maturation."*

Line 270: *"Here, we demonstrate that the transcriptional co-activator Med15, a subunit of the evolutionarily conserved Mediator complex, is essential for β-cell maturation."*

Line 323: *"Taken together, our work demonstrates the importance of Med15 and Mediator in the process of β-cell maturation, likely at least in part through mediating interactions between maturation transcription factors binding sites and core gene promoters."*

3) It was recommended to the authors that to move beyond the phenomenological description that their cell- and animal-based studies provide – and thus to make the paper more attractive for audiences outside of the confines of the beta cell/diabetes field – they might want to probe deeper into how MED15 effects play out in the context of the Mediator complex.

We appreciate that the reviewer would like our work to be attractive to a wide audience. We believe that our manuscript, which includes in vivo animal physiology, cell and developmental biology, molecular genomic, and molecular biology studies, already goes well beyond a phenomenological description and is likely of interest to researchers in many fields, such as developmental biology, islet biology, endocrinology, diabetes, metabolism, and transcriptional regulation (including Mediator biology). To address this comment, we have revised the text to more succinctly highlight the novelty of our observations in the introduction (lines 79-92) and discussion (lines 270-279).

4) In this regard, and among other suggestions, it was recommended that they fortify IP-type data by probing their IPs for other representative Mediator subunits, which could reveal something about whether the interactions reflect autonomous MED15 interactions (unlikely) or entail the entire Mediator complex (more likely).

We thank the reviewer for this suggestion. As noted in our response above, we did not conclude, nor do we believe, that Med15 acts in isolation. Rather, we favour the same model as the reviewer: that Med15 acts in the context of the whole Mediator complex. Our manuscript actually provides substantial support of this model via our ChIP-seq analysis (Fig 4, S5), which shows that many genomic sites are occupied by both Med15 and Med1 (and is likely an underestimation, because as noted by the reviewer Med1 association may be substoichiometric).

While we agree that this does not reflect the whole complex, in our view, it provides compelling evidence that Med15 functions via the Mediator complex. Critically, our data show co-occupancy of key beta cell transcription factors, Med15, and Med1 at genes associated with beta cell maturity, such as IAPP (Fig 4I). In our view, co-occupancy of key factors (Med15, Med1, Nkx6-1, NeuroD1) at key targets provides more relevant insight than overall co-IP of Mediator from beta cells, which merely show that the complex exists in beta cells and/or binds a TFs, but doesn't reveal functional association with key transcriptional targets.

To further to address this comment (and the following comment), we have performed new co-IP experiments for other Mediator subunits as requested and added them as new Figure 5C and S7A. Because our paper primarily implicates Nkx6-1, we have focused these new experiments on Nkx6-1. In line with our hypothesis and other data presented in Fig 5A, B and D, we observed in these experiments the presence of MED15 after Nkx6-1 immobilization; in addition, we also observed the presence of MED6, and of MED12 in 2 of 3 experiments. Notably, both MED6 and MED12 appear to be present in smaller fractional amounts, consistent with the model that MED15 may be the anchoring subunit for Nkx6-1; this also addresses the concern (#7) of the

reviewer, listed below. In contrast, we did not detect Med23. The reasons for this are unclear; it is possible that Med23 association is less stable in the Nkx6-1:Med15 induced conformation of Mediator, or Med23 may dissociate during our purification process. We did not reliably detect Med7.

The copurification of Med15, Med6, and Med12 with immobilized Nkx6-1 strongly suggests that Nkx6-1 recruits not just Med15 but the Mediator complex. To further ascertain that Med15 incorporates into a *bona fide* Mediator complex, we immobilized Med15 and monitored for associated Mediator subunits (new Fig S7B). As expected, we consistently detected the presence of Med12, Med6, and Med23 after Med15 immobilization.

Together with our additional interaction data presented in Fig 5 as well as our ChIP-seq data, we argue that this provides strong support for our model–that Med15 forms a *bona fide* Mediator complex in MIN6 cells and that Nkx6-1 associates with this complex, via Med15 as the anchoring subunit. We hope that the reviewer agrees with this view.

5) However, the authors misconstrued this to mean that they are being asked to analyze pairwise interactions with the full set of 30 subunits and did not heed the simple suggestion.

We hope our response to #4 now addresses this concern.

6) Furthermore, in response to a related point, and to their credit, the authors attempted to further probe interactions between MED15 and NKX6-1, a putative interacting activator using what they describe as purified proteins (Fig. 5). This was a step in the right direction, but unfortunately the final result is difficult to interpret because HEK293-expressed MED15 is very likely to have picked up the bulk Mediator subunits, one or more of which may be responsible for the interaction result in the figure.

We thank the reviewer for acknowledging this work we did to address their concern.

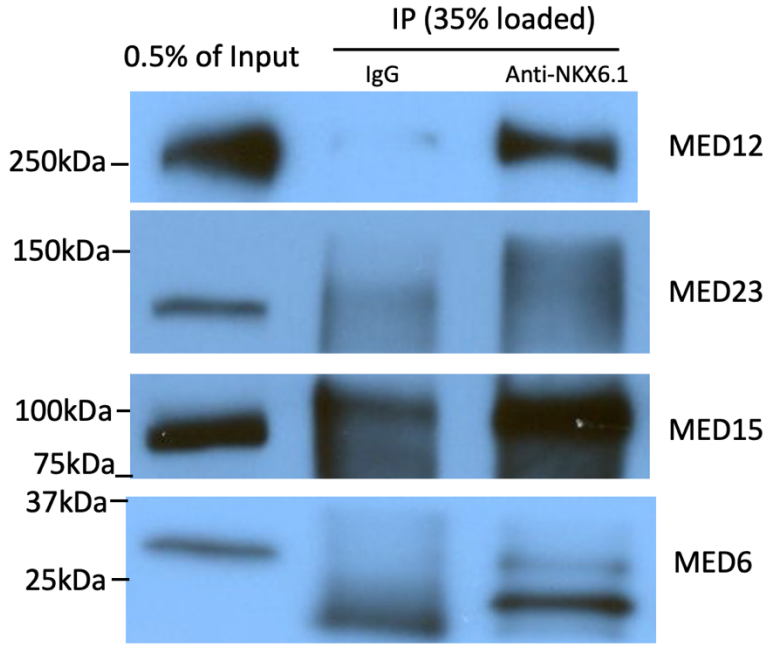
7) It is recommended that the authors repeat the experiment using a different source of recombinant factors (e.g., via baculovirus expression).

We thank the reviewer for this comment. However, as we used an overexpression system, we believe it is highly unlikely that the proteins we isolated from 293 cells were “contaminated” with other Mediator family proteins. We think that any other proteins we did pull down would be in very small amounts and would likely be largely washed away during the purification.

In an effort to demonstrate that Med15 was the component of the Mediator responsible for Nkx6-1 interactions we carried out additional 293 experiments. We transfected both epitope-tagged Nkx6-1 and/or Med15 in various iterations of these experiments. The results we obtained are consistent with the data obtained from the experiments above (response to concern #4): Med15, Med6, and Med12 co-purify when Nkx6-1 is immobilized, whereas Med23 was not detected (see results panel below, using immobilized, epitope-tagged Nkx6-1).

However, while performing these experiments, we also monitored the localization of ectopically expressed, epitope-tagged Med15. We found that a majority of the Med15 signal localized to the cytoplasm, suggesting that ectopically overexpressed Med15 does not reliably recapitulate the function of endogenous Med15. Because of this caveat, we have decided not to include these data in the revised manuscript.

The experiment described in the response to the above reviewer concerns show that Med15 is present at fractionally higher amount in Nkx6-1 purifications than are Med12 and Med6 (and Med23 was not detected), so we feel that this experiment sufficiently and successfully addresses the question as to whether Med15 indeed is an anchoring subunit within the Mediator complex for Nkx6-1.



8) The authors also tried to buttress this conclusion by including an in silico model. Clearly, a mutational analysis that included mapping of the interactions would have been much more convincing.

We thank the reviewer for this suggestion. We have mapped the interactions between MED15 and important beta cell transcription factors using alpha fold. Future mutational scanning analyses will allow us to fully map the interactions and determine which domains in Nkx6-1 and Med15 interact. At this time, we feel experiments like these would be quite supplementary to the key messages of this work.

9) Another important concern, again related to the issue of how MED15 behaves vis a vis the bulk Mediator complex, was that the MED15 knockout may adversely affect the complex's Tail module and that disruption rather than a MED15 interaction per se may be responsible for the observed biological effects. Once again, the authors evidently misconstrued this as a suggestion to investigate the roles of all the Tail subunits!

Again, we are sorry that the reviewer felt we may have "misconstrued" their previous suggestion; however, they did specifically write "Other tail subunits must therefore be incorporated into the analyses, both cell based and those looking at physical interactions".

Cell-based methods to compare Med15 gene function to that of other Mediator genes would require making new animal models (since Med15 KO cells are animal derived). We felt this was well outside the scope of this study no matter how many Tail subunits would be studied.

We would like to add that Mediator complex purification from islets with Med15KO beta cells would be technically extremely challenging because islets are rare, fragile, and enriched for digestive enzymes, necessitating hand-picking for molecular analysis; worse, such islet preparations nevertheless inevitably include non-beta cells (where Med15 is not deleted), which might confound analysis. We therefore feel that detailed analysis of Med15 loss on Mediator complex stoichiometry in Med15KO beta cells is a whole endeavour on its own that is well beyond the scope of this work.

To clarify that we are aware of the fact that Med15 loss may cause loss of other Mediator proteins, which might contribute to the observed phenotypes, we have revised the text in order to acknowledge this caveat and clarify that we are not sure how changing the expression of Med15 may impact the complex (line 314):

"However, it remains unclear how changing the amount of one Mediator subunit would affect the activity of the entire complex."

10) In the end, the revised version, like the original, does not further our mechanistic understanding of the described phenomenology.

Overall, it appears to this reviewer from the authors' rebuttal and the revisions that they chose to implement that they are more invested in advancing beta cell biology rather than an understanding of the underlying mechanisms for coactivator function. The paper in its current form nicely fulfills that goal and should be suitable for journals catering to that field.

We thank the reviewer for this comment and are sorry that they do not feel our work provided sufficient mechanistic details; we appreciate the reviewer's opinion about what type of article is suitable for Nature Communications. We have read a number of papers in this journal that describe advances in beta cell biology and islet biology and are highly cited, e.g. recent examples include PMID: 37813862 and 37777536. Thus, in our estimation a focus on "advancing beta cell biology" does not preclude our work, or anyone else's, from publication in Nature Communications.

Reviewer #2 (Remarks to the Author in blue):

In this revised manuscript, Kadhim and colleagues examined the roles of Med15, a subunit of co-regulator Mediator, in islet beta cell maturation and function. The most noteworthy result is that although the Mediator complex participates general transcription regulation in most cells,

inactivating Med15 in the pancreatic beta cells only produces significant maturation phenotypes. This appears to be mediated by specific recruitment of Med15 by NeuroD1 and/or Nkx6.1, which are essential for islet beta-cell production, function, and survival. In addition, the authors showed that Med15 levels are reduced in islet (including beta) of T2D human subjects. Its over-expression also helps to improve the maturation of human ES cell-derived beta-like cells. Thus, this study is significant in not only revealing a mechanism by which general factors exert cell type/stage specific regulation, but also highlight a factor that can be manipulated to improve hESC-derived beta-like cells for therapy. Therefore, this work will be of significant for readers in transcription regulation and diabetes research.

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Therefore, this reviewer no longer has issues.

We thank the reviewer for their careful assessment and constructive and complimentary review.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

Kadhim et al Nature Comm NCOMM19-25720B

This most recent version of the Kadhim et al manuscript reflects a laudable effort on the part of the authors to address my earlier concerns relating to their implication of the MED15 subunit of the Mediator complex in beta cell ontogeny. Together with the very strong in vivo results, the newly added biochemical data have now made this a quite convincing study. I have no major lingering concerns, just one minor, cosmetic-type suggestion: in the newly added Western blot figures, some of the antibodies (especially anti-MED6) yield multiple (non-specific) bands. For each panel, please either identify the authentic band somehow or, alternatively, mark the non-specific bands with asterisks together with a corresponding note in the figure legend.

Response to reviewer comments

Reviewer #1 (Remarks to the Author):

Kadhim et al Nature Comm NCOMM19-25720B

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We are grateful to the reviewer for all the time they've spent on our work aiming to improve its presentation and we thank the reviewer for their thoughtful review and feedback. As requested, we have labelled the non-specific bands in Figure 5 and Supplemental Figure 7 with asterisks and the predicted molecular weights with arrows. We have also added a note in the figure legend that defines those annotations.