EMBO *reports*

ABCA12 regulates insulin secretion from β-cells

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 26 July 2019

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees acknowledge that the findings are potentially interesting. However, referees 1 and 2 also point out several concerns and have a number of suggestions for how the study should be strengthened. The specific expression of ABCA12 in pancreatic beta cells, the role of CDC42 and the claim that glucose sensing is intact should be substantiated. Moreover, referee 2 also indicates that a more detailed description of the experiments and data is required.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>)

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Before submitting your revision, primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see <https://www.embopress.org/page/journal/14693178/authorguide#datadeposition>).
Specifically, we would kindly ask you to provide public access to the following datasets:
- Dataset #1: RNA seq analysis
- Dataset #2: mass spectrometry analysis/lipidomics

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Method) that follows the model below (see also <https://www.embopress.org/page/journal/14693178/authorguide#datadeposition>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available <https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

9) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

10) Regarding data quantification:
- Please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.
IMPORTANT: Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates. If the data rely on a smaller number of replicates, scatter blots showing individual data points are recommended.
- Graphs must include a description of the bars and the error bars (s.d., s.e.m.).
- Please also include scale bars in all microscopy images and define their size in the figure legend.

11) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

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REFEREE REPORTS

Referee #1:

In the manuscript by Ursino et al, entitled "ABCA12 regulates insulin secretion from beta cells", the authors test the hypothesis that ABCA12 is a novel regulator of insulin secretion linking cholesterol homeostasis to insulin secretion defects observed during T2D. Through the use of beta cell specific Abca12 KO mice and the min6 cell line, the authors demonstrate that Abca12 depletion leads to decreased expression of Abca1, Abcg1 and LXRb. Altogether, this leads to increased amounts of lipid rafts, probably causing insulin secretion defects. In this manuscript, the authors have

established a new role for Abca12 in regulating glucose homeostasis by controlling insulin secretion. Rescuing Abca1, Abcg1 and LXRb expression does not rescue the insulin secretion defects, suggesting a specific role of Abca12 in glucose stimulated insulin secretion. In addition, they provide evidence that, during ageing, Abca12-mediated inflammation can contribute to beta cell dysfunctions. In general, the experiments are convincing, but some controls are missing (see below). The authors have used the Ins2Cre from Magnusson lab to knock-out Abca12 in pancreatic beta cells. This model has several metabolic effects per se. In the manuscript, the authors have well controlled the potential side effects related to the use of this model, in particular a leak of Cre activity in the hypothalamus, as well as the GH minigene.

Although this is an interesting manuscript for the beta cell biologists, there are several major issues that should be addressed.

Specificity of antibodies is key in demonstrating specific staining. Although the immunofluorescence (IF) experiments demonstrate expression of ABCA12 in beta cells, IF analysis as presented in figure 1 are not fully convincing. Each labeling should be shown (i.e., insulin alone, AbcA12 alone, DAPI alone, as well as costaining). In addition, co-staining with glucagon should be shown, to demonstrate specific expression of ABCA12 in pancreatic beta cells. In addition, western blots should be shown in addition to their quantifications (figure 1M) to demonstrate the specific expression of ABCA12.
Abca12 knock-down in Min6 demonstrate that Abca12 KD impairs glucose-stimulated insulin secretion. Is this also the case in mouse islets isolated from Abca12+/+ and Abca12tm1d mice?
What are the relative ratio of Abca1, Abcg1, LXRb compared to Abca12 in mouse islets?

Are CDC42 and F-actin levels also impacted in old, 24 week old pancreatic islets? What about lipid rafts in this context?
The rescue experiment with constitutive active CDC42 is convincing. Although the molecular mechanisms between abca12, abca1 and cdc42 are discussed, some investigations related the regulation of such cross-talks should be performed.

Finally, is ABCA12 expressed in human islets? As well as in T2D mouse islets (Db/Db, HFD)? The reviewer is aware that having access to human islet is complicated, but data mining using published RNA-seq data could help to demonstrate whether AbcA12 is expressed or not in human islets, as well as during T2D progression in mice.

Referee #2:

In this manuscript, Ursino et al. described a novel mouse model with a lipid transporter ABCA12 specifically deleted in the pancreatic beta cells. Genetically ablating ABCA12 in beta cells led to impaired glucose tolerance in mice (older than 8 wks) and this is due to the impaired glucose-stimulated insulin secretion found in these mice. This highlighted a role of ABCA12 in the regulation of glucose-stimulated insulin secretion. The authors then used the knock out mouse model as well siRNA knock down in MIN6 cell line to understand the link between ABCA12 ablation and insulin secretory defect. They found that ABCA12 deletion led to: 1) changes in granule morphological changes; 2) increase in lipid rafts; 3) reduction in actin and 4). Increase in islet inflammation in older mice. Whereas the inflammatory phenotype was attributed to the progressive accumulation of proinflammatory lipids in the islets; using a range of different rescue strategies, they found low CDC42 activity represented the causal link between ABCA12 deletion and beta cell exocytotic defects. The study provided a wealth of data from detailed phenotyping of the animal models and attempted to bridge islet lipid dynamics and beta cell function. This is an interesting study and can potentially bring further insights to the understanding of the regulation of GSIS.

My specific comments are as follows:

Major:

1. From the data provided, I could not see an obvious link between CDC42 activity and granule morphology, actin network as well as the lipid raft accumulation. The only functional evidence

provided here is GSIS in ABCA12 KD MIN6 cells. This makes the statement that 'ABCB12 deficiency causes impedes activation of CDC42, resulting in reduced actin polymerization and increased abundance of lipid rafts' rather speculative. Evidence of the role of CDC42 activation in beta cell granules, actin and lipid rafts should be provided and the authors have the cell models and techniques to do so.

2. It is not apparent to me that the ABCA12 KO beta cells had intact glucose sensing. Similarity in glucose and high K+ triggered granule fusion events in Abca12tm1d islets could be due to many factors. For example the changes in Ca2+ signalling pathways (one that was also identified by transcriptomic study). It would be more convincing if Ca2+ imaging experiments in islets, which can reflect more beta cell electrical activity and glucose responsiveness, can be performed. Also, glucose sensing in beta cells is not only dependent on Gluts but also many other proteins. KATP-channels and Glucokinase are also critical in beta cell glucose sensing.

Minor:
1. In general the figure legends are overly simplified. There is no annotation of significant levels in all the figs.
2. Page 7, ' ... no effect on the basal insulin secretion at low concentrations of glucose ...' there is no data supporting this. Data in fig. 2K were normalised to basal.
3. MicroRNA assay: miR 158 in text but 758 in the figure.
4. Fig. 3J-N. the GSIS is very variable. Just the control experiments already showing a wide range from 1.2 folds to 10 folds. And it is not clear what the open bars stand for. Are they the control with transfection of the indicated constructs/treatments? If that is the case, overexpression of ABCG1 and LXRbeta seemed to have an effect on GSIS already.
5. Page 10. I could not find table S1.
6. Two photon imaging was not described in the methods.
7. Data are not consistent between Fig. 5 K and Fig. 2I. There was hardly any stimulation (rather a reduction) in insulin secretion at 15 mins in Fig. 2I and a good 2-fold increase in Fig. 5K.
8. Fig. 2 J, lacking quantification. Annotation for the colours should be added.
9. Fig. 6 C-E. Is the CTB binding assay the same as in Fig. 6A? If that is the case, the spectra of CTB and the Fluorescein-488 would overlap. This will introduce significant noise.
10. Fig. 6I. middle panels are too dim to see anything clearly.
11. Fig. 7D, no quantification.

1st Revision - authors' response 20 November 2019

Response to Reviewers

Reviewer 1

Specificity of antibodies is key in demonstrating specific staining. Although the immunofluorescence (IF) experiments demonstrate expression of ABCA12 in beta cells, IF analysis as presented in figure 1 are not fully convincing. Each labeling should be shown (i.e., insulin alone, AbcA12 alone, DAPI alone, as well as costaining).
Response:
We thank the Reviewer for their comments and agree that as originally presented these findings weren't clear. We have now included individual panels for Figure 1 A&B showing single channel images for ABCA12 and Insulin staining as suggested by the Reviewer. In doing so we have also clarified the extend of Abca12 staining in the islet. We had not meant to imply that ABCA12 is exclusively expressed in b-cells in the islets. The new images show clearly that the protein is expressed in all other cell types (albeit at slightly lower levels). In revising this Figure we have taken the opportunity to highlight this (see new Figure 1A). In the process of including these individual channels, we have moved the informative (but not essential) panel showing the conditional allele structure to Appendix Figure 1B. We also include new data from human islets showing broad expression of ABCA12 (see response below r.e. human expression and the new data in Appendix Figure 1A).

In addition, co-staining with glucagon should be shown, to demonstrate specific expression of ABCA12 in pancreatic beta cells.
Response:
With reference to the Reviewer's first comment we are not claiming that ABCA12 is exclusively expressed in b-cells – in fact that is clearly not the case. This has now been made clear in the

revised Figure 1A and also with respect to staining for ABCA12 in human pancreas sections (see new Figure S1A; in response to the Reviewers further comments below). Most importantly there is no question that ABCA12 is expressed in b-cells and we trust that the Reviewer will agree based on the revised images now shown in Figure 1A & B.

In addition, western blots should be shown in addition to their quantifications (figure 1M) to demonstrate the specific expression of ABCA12.
Response:
This has now been included as a sub-panel in the new Figure 1L.

Abca12 knock-down in Min6 demonstrate that Abca12 KD impairs glucose-stimulated insulin secretion. Is this also the case in mouse islets isolated from Abca12+/+ and Abca12tm1d mice?
Response:
This is an excellent question and one which has frustrated us no end. We made several attempts to investigate GSIS from isolated islet but the results were equivocal. Consequently, we elected not to include them. There could be numerous reasons why such a phenotype might not be strongly evident – we suspect it's a function of the subtlety and progressive nature of the phenotype, the limitations of doing such experiments *ex vivo* (particularly in our older mice) and the interaction of the islet with the circulatory system *in vivo*. However, the combined weight of evidence from MIN6 cells, from the mice themselves and from the granule fusion measurements shows that there is a direct effect on insulin secretion. We've refrained from speculation in the manuscript because the phenotype in the mice is so clear. However, we are willing to reconsider and include this data and the associated conjecture if the Reviewers/Editor felt strongly that we should do so.

What are the relative ratio of Abca1, Abcg1, LXRb compared to Abca12 in mouse islets?
Response:
Given that the affinity of the different antibodies we are using to detect these proteins likely differs widely, making any sort of meaningful comparison between them is fraught, even when standardised to a second protein. Even then, the more important question would be the relative ratios at different subcellular locations. Although the total abundance could theoretically be addressed by absolute quantitation (MS, for example), this is beyond the scope of the current study and in any event doesn't address the localisation question. Comparison of gene expression levels is possible, but would likely also be misleading given the key contribution of posttranslational regulation in determining the levels of many of these proteins (see our recent study Fu *et al.* Cell Metabolism 2013).

Are CDC42 and F-actin levels also impacted in old, 24 week old pancreatic islets? What about lipid rafts in this context?
Response:
We thank the reviewer for raising this question and agree that this is an interesting one. The harvesting and analysis of sufficient islets from aged cohorts of mice is technically challenging (given the reduction in size we have noted in the manuscript) and this has limited our capacity for analysis, particularly of lipid rafts by FACS. In response, we assessed whether changes in F-actin abundance are a persistent feature associated with defects in insulin secretion in 24 week old pancreata. Our new study finds this to be the case and we have included these data as updated subfigures 6L and 6M which are also described in the text in p.14, paragraph 1:
"….β-cells isolated from *Abca12*^{tm1d} mice at 8 weeks of age and also much later during disease progression at 24 weeks of age (Figure 6L, M)."
and are also noted in the discussion of late onset phenotypes on page 15
"As has previously been noted, these changes are associated with reduced abundance of F-actin (Figure 6L, M)."

The rescue experiment with constitutive active CDC42 is convincing. Although the molecular mechanisms between abca12, abca1 and cdc42 are discussed, some investigations related the regulation of such cross-talks should be performed.
Response:
We agree and have now performed additional experiments to further support the proposed pathway. In these new studies we have used Jasplakinolide to reverse decline of F-actin abundance due to ABCA12 deficiency. In so doing we demonstrate that restoration of F-actin abundance is able to rescue the effects of ABCA12 deficiency on GSIS. These findings are now presented in Fig. 6 Q,R and described on p.14, paragraph 1 :
"Furthermore, restoration of F-actin abundance using Jasplakinolide to stabilize it (Posey and Bierer, 1999) also resulted in full restoration of impairment of insulin secretion on the background of ABCA12 deficiency (Figure 6 Q, R)."

Finally, is ABCA12 expressed in human islets? As well as in T2D mouse islets (Db/Db, HFD)? The reviewer is aware that having access to human islet is complicated, but data mining using published RNA-seq data could help to demonstrate whether AbcA12 is expressed or not in human islets, as well as during T2D progression in mice.

Response:
These are interesting questions. In response we have mined of a number of datasets for Type 1 and 2 diabetes in both human and mouse. While ABCA12 is expressed in normal and diseased human and mouse pancreas there is no clear correlation between expression and disease in T1D mouse models (Calderon *et al.* PNAS 2011), Human T2D samples (Marselli *et al.* PLoS One 2010) or a mouse T2D model (Stewart *et al.* BMC Genomics 2010). In addition, although we have been limited by tissue availability, we have been able to profile ABCA12 expression in 3 different samples each of human pancreatic tissue from normal individuals and patients affected by T2D. While no correlation was noted in ABCA12 expression between the two groups, these experiments have served to address the Reviewers first question regarding expression. Consistent with our findings in mice, we find that ABCA12 is broadly expressed in the human pancreatic islets in both b and a cells - we had never meant to imply that this wasn't the case. The expression data is now included in Appendix Figure 1A. Given the limited sample size and lack of correlation in expression datasets we haven't included the data from T2D patients in the revised manuscript but could do so if the Reviewer/Editor considered it worthwhile.
We have changed the text in the manuscript to reflect these changes as follows:
Page 5, paragraph 1
"To examine the expression of ABCA12 in the pancreas, we performed immunofluorescent staining of ABCA12 in islets isolated from wild type mice (Figure 1A), the MIN6 mouse b-cell line (Figure 1B) and in sections of human pancreas (Appendix Figure 1A)."
and
Page 7, paragraph 1
"Analysis of previous reports of gene expression in mouse models and patients with T1D and T2D found that while Abca12 was expressed in the pancreas, there was no correlation between the levels of gene expression and disease state."
References accompanying this statement are included in the manuscript file.

Reviewer 2

1. From the data provided, I could not see an obvious link between CDC42 activity and granule morphology, actin network as well as the lipid raft accumulation. The only functional evidence provided here is GSIS in ABCA12 KD MIN6 cells. This makes the statement that 'ABCB12 deficiency causes impedes activation of CDC42, resulting in reduced actin polymerization and increased abundance of lipid rafts' rather speculative. Evidence of the role of CDC42 activation in beta cell granules, actin and lipid rafts should be provided and the authors have the cell models and techniques to do so.
Response:
A connection between CDC42, actin cytoskeleton and lipid rafts in general is well established in the literature (for example in Chadda R. *et al.* Traffic 8: 702-17, 2007 and in reviews including Heasman S.J. & Ridley A.J. Nat Rev Mol Cell Biol 9: 690-701, 2008 and Chichili G.R., & Rodgers W. Cell Mol Life Sci 66: 2319-28, 2009). In this study, we investigated this connection in relation to insulin secretion from β cells and this has been evidenced by reversal of GSIS impairment with restoration of CDC42 activation impairment and of lipid rafts abundance. To address this in more detail in the revised manuscript we include new data on the restoration of GSIS with restoration of defective in actin polymerization (see new Fig. 6 Q,R and associated text changes as detailed in the previous response to Reviewers). A connection between CDC42, cytoskeleton, lipid rafts on the one hand and secretory granule morphology on the other was neither demonstrated nor implied in our study.

2. It is not apparent to me that the ABCA12 KO beta cells had intact glucose sensing. Similarity in glucose and high K+ triggered granule fusion events in Abca12tm1d islets could be due to many factors. For example the changes in Ca2+ signalling pathways (one that was also identified by transcriptomic study). It would be more convincing if Ca2+ imaging experiments in islets, which can reflect more beta cell electrical activity and glucose responsiveness, can be performed. Also, glucose sensing in beta cells is not only dependent on Gluts but also many other proteins. KATP-channels and Glucokinase are also critical in beta cell glucose sensing.
Response:
We thank the Reviewer for their comment and accept the critique. To provide greater clarity with respect to the capacity of these cells to sense glucose, we have performed new calcium imaging experiments which have measured calcium flux in isolated b-cells from wild type and *Abca12*^{tm1d} mice at 11 weeks of age in response to glucose and potassium. We find no differences in the glucose responsiveness of these cells. The provides further evidence that the defects apparent upon *Abca12* deletion are not a consequence of altered glucose sensing. This new data has been included in an additional panel (Figure 4F) and reference to this work made in the manuscript as indicated below.
Page 11, Paragraph 1

"To confirm this was the case, we profiled calcium flux using Fluo-4 in response to either glucose or potassium stimulation in isolated b-cells from *Abca12*^{tm1d} and control mice at 11 weeks of age but found no difference in F/Fo ratios for either treatment (Figure 4F)."
In addition, the Materials and Methods section of the manuscript has been updated to provide details of these experiments and a further author, Prof Helena Parkington, has been added to the manuscript.

Minor Comments:

Reviewer 2 - Minor:

1. In general the figure legends are overly simplified. There is no annotation of significant levels in all the figs.
We apologise for the simplification which was limited somewhat by the number of panels in many of the Figures. The annotation of significance levels was actually covered by a blanket statement in the materials and methods but in the revised manuscript we have added them into the figures, as well as providing more details as suggested.

2. Page 7, '... no effect on the basal insulin secretion at low concentrations of glucose ...' there is no data supporting this. Data in fig. 2K were normalised to basal.
Response:
The data presented here was normalised to account for variation in basal insulin secretion and to control for experimental variability associated with transfection of siRNA reagents. If required we can provide examples of individual experiments, though we note this this is now included in the revised manuscript as part of our examination of the effects of Jasplakinolide – see new Figure 6R.

3. MicroRNA assay: miR 158 in text but 758 in the figure.
Response:
Our apologies – this has been corrected.

4. Fig. 3J-N. the GSIS is very variable. Just the control experiments already showing a wide range from 1.2 folds to 10 folds. And it is not clear what the open bars stand for. Are they the control with transfection of the indicated constructs/treatments? If that is the case, overexpression of ABCG1 and LXRbeta seemed to have an effect on GSIS already.
Response:
Yes, GSIS is variable depending on numerous factors that are difficult to control, such as passage number, cell density etc and even small variations in the basal level of secretion (at low glucose) result in large variability of the GSIS. Open bars do stand for transfections with the indicated constructs and the effects noted by the reviewer are now mentioned on p.9.

"It was noted that transfections with ABCG1 or LXRβ alone also reduced GSIS"

5. Page 10. I could not find table S1.
Response:
As part of the preliminary submission this part of the Supplementary Materials was not uploaded. In this revised version the entire RNA analysis dataset has been submitted to GEO (GSE140379). To review this go to:
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE140379
and enter token "sxwnueogjfutnwp" into the box. In addition, the differential gene expression list now accompanies the manuscript as Appendix Table 1.
In this revision we have also provided a full summary of MS based analysis of lipid levels in different mice examined during the study. This information is now available in the Appendix accompanying the manuscript in Appendix Tables 2-10.

6. Two photon imaging was not described in the methods.
Response:
We apologise for this oversight. A new section detailing these experiments has been added to the Materials and Methods section of the revised manuscript.

7. Data are not consistent between Fig. 5 K and Fig. 2I. There was hardly any stimulation (rather a reduction) in insulin secretion at 15 mins in Fig. 2I and a good 2-fold increase in Fig. 5K.
Response:
The two experiments raised by the Reviewer are not directly comparable. The mice in Figure 2I are on a standard mouse chow where as those in Figure 5K are on a defined chow especially formulated to match the HCD diet of the test animals. Given the significant impact of cholesterol noted in these and associated experiments, making a comparison between the two is inappropriate. Moreover, the mice are of different ages (one having been on a diet for 4 weeks). The more important finding in both cases is that of the relative responses of cells. As a further note, we also point out that the

respective values for tm1d mice at 15' are actually not statistically different from time zero in either of these figures.

8. Fig. 2 J, lacking quantification. Annotation for the colours should be added.
Response:
Quantitation is given in the text (p.6 paragraph 3). Annotation of the different staining has now been added to Figure J.

9. Fig. 6 C-E. Is the CTB binding assay the same as in Fig. 6A? If that is the case, the spectra of CTB and the Fluorescein-488 would overlap. This will introduce significant noise.
Response:
In these experiments an CTB-Alexa Fluor 647 conjugate was used. Consequently, the emission spectra is distant from that of fluorescein-488, ensuring that there is no overlap. We apologise for not making this clear in the original manuscript - this is now specified in the Methods section and Figure 6 legend.

10. Fig. 6I. middle panels are too dim to see anything clearly.
Response:
Our apologies, these images have not converted well. We have improved the brightness of these images in the revised manuscript.

11. Fig. 7D, no quantification.
Response:
Quantification of the differences in IL1b fluorescent signal have now been included as an additional panel in Figure 7 (E) following the images of the samples. This confirms an increase in IL1b expression specifically in tm1d mice.

2nd Editorial Decision 10 December 2019

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, all referees are very positive about the study and request only minor changes to clarify and discuss certain issues. Moreover, the conclusions in the abstract should be toned down and presented in the most appropriate manner. Please also note that the abstract should be written in present tense.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

1) Appendix: please note the nomenclature "Appendix Figure S1" and "Appendix Table S1" etc. Please update the figure and table labels and all callouts in the text.

2) Appendix Figure S1: the last sentence of the legend is incomplete ('All blood samples for cortisol")

3) Appendix figure legends: Please define the nature of the bars and error bars, the number of replicates and the statistical test used (if applicable) for S1C, D, S2A, S3

4) I suggest to submit Appendix Table 1 as Dataset EV1 - in the format of an Excel file with the legend in the first tab of the file.

5) Legend for Figure 6M: the number and nature of the replicates used has not been specified. Please add this information.

6) Fig 5B and B': the magnification image shown in 5B' does not match the box in 5B. Please correct the placement of the box.

7) Thank you for supplying a Data availability section. Just a short reminder to update it before resubmission (reviewer password).

8) Methods: Is the Primer ID specified for the Taqman microRNA assay sufficient to define the

sequence?

9) The contributing author Helena C. Parkington was not entered in the online submission system. We have done this for you but please double-check whether the credit we entered (contribution) is correctly specified in the system.

10) Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (width x height). You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

We look forward to seeing a final version of your manuscript as soon as possible.

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REFEREE REPORTS

Referee #1:

The authors have responded to my major comments. They have performed key additional new experiments, which improve the quality of the manuscript.

Referee #2:

The authors have done additional experiments and revised their manuscript. It is now improved and I only have a few minor comments as listed below.
1. Authors pointed out that 'A connection between CDC42, cytoskeleton, lipid rafts on the one hand and secretory granule morphology on the other was neither demonstrated nor implied in our study.'. This is different from the abstract, which suggested all the defects following depletion of ABCA12 was mediated through dysregulation of CDC42.
2. It is very interesting that lacking abca12 in beta cells resulted larger granules and authors concluded this is due to lacking cholesterol. Can authors please also discuss how low granule cholesterol leads to this phenotype?
3. In the revised manuscript, the authors acknowledged that 'It was noted that transfections with ABCG1 or LXRbeta alone also reduced GSIS'. Can authors also discuss what the possible reasons are for this? Also, the data showing the effects of TO901317 (Fig. 2M) is somehow strange. GSIS in control is very low to start with and KD ABCA12 resulted a negative stimulatory index.
4. Ablating ABCA12 seemed to have multiple effects in beta cells through different pathways. I think a diagram depicting the ABCA12 mediated effects would be beneficial.

2nd Revision - authors' response 12 December 2019

Referee #1:

The authors have responded to my major comments. They have performed key additional new experiments, which improve the quality of the manuscript.

We thank the Reviewer for their suggestions and agree that the manuscript is considerably improved thanks to their input.

Referee #2:

The authors have done additional experiments and revised their manuscript. It is now improved and I only have a few minor comments as listed below.

1. Authors pointed out that 'A connection between CDC42, cytoskeleton, lipid rafts on the one hand

and secretory granule morphology on the other was neither demonstrated nor implied in our study.'. This is different from the abstract, which suggested all the defects following depletion of ABCA12 was mediated through dysregulation of CDC42.

The abstract has been modified to remove the suggestion of causality and instead now reports the observation independently.

2. It is very interesting that lacking abca12 in beta cells resulted larger granules and authors concluded this is due to lacking cholesterol. Can authors please also discuss how low granule cholesterol leads to this phenotype?

These results are consistent with similar observations made in mice lacking ABCG1 (See Sturek et al JCI 2010) and with differences in granule morphology noted in mice lacking factors required for cholesterol biogenesis (Gondre-Lewis et al. J Cell Sci 2006). The reasons for the change in granule morphology are unclear but could be associated with impacts on the regulation of cellular secretory pathways, differences in membrane fusion, changes in SNARE mediated exocytosis or impaired membrane sorting downstream of changes in microdomain composition. All of this is highly speculative and we would prefer not to engage in this in greater detail - though we would point out that most of these possibilities are canvassed already in the manuscript on pages 18 and 19.

3. In the revised manuscript, the authors acknowledged that 'It was noted that transfections with ABCG1 or LXRbeta alone also reduced GSIS'. Can authors also discuss what the possible reasons are for this?
Put simply - we don't know and would prefer not to engage in speculation. LXR beta is the major regulator of ABCG1 and hence the effect is likely to be mediated by the latter protein (i.e. the mechanism underpinning the actions of both proteins are likely to be the same). We've acknowledged this in the revised revision and also the lack of clarity about why this effect is observed.
Also, the data showing the effects of TO901317 (Fig. 2M) is somehow strange. GSIS in control is very low to start with and KD ABCA12 resulted a negative stimulatory index.
This derives from variability of basal (low glucose) insulin levels which has masked, to an extent, the effects of ABCA12 manipulations. None-the-less there is stimulation in controls and no stimulation upon ABCA12 silencing and this is consistent with the significant in vivo experiments undertaken in mice lacking ABCA12 in beta cells.

4. Ablating ABCA12 seemed to have multiple effects in beta cells through different pathways. I think a diagram depicting the ABCA12 mediated effects would be beneficial.

We agree and have now included a Synopsis image in line with the Journal format.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Ian Smyth
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-48692

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was chosen depending on assay undertaken to detect a statisitically sifgnificiant difference of 20%. In most cases sample size was based on previous studies (e.g. for glucose tolerance) in comparable animal models (i.e. deletion of Abca1 or Abcg1).
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The minimum sample size for each tested condition was n=3 but depending on assay and animal availability was as much as 9 per group.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals or samples were excluded from analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Animals were not randomised to treatment groups as genotype information was required prior to undertaking experiments.
For animal studies, include a statement about randomization even if no randomization was used.	Because individual assays required mice of a sepcific genotype animals were not randomised to different treatment groups, however, animals were co-housed to minimise environmental contributions to variance in experiments.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Analysis of biological samples (tissue sections, TEM etc) were performed using double blind approaches.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Animals were not randomised to treatment groups as genotype information was required prior to undertaking experiments.
5. For every figure, are statistical tests justified as appropriate?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	Yes, presented as SEM or SD as indicated in each Figure.
Is the variance similar between the groups that are being statistically compared?	Yes, most comparisions are direct and any experimental variance would not be expected to differ between groups.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Antibodies used were generated against ABCA1 (Abcam, ab7360), ABCG1 (Abcam, ab52617), SR-B1 (Abcam, ab106572), LXRβ (Abcam ab28479), GLUT2 (Abcam, ab54460), GAPDH (Abcam, ab8245) and CDC42 (BD Transduction Laboratories, #610928).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	MIN6 cells were sourced from AddexBio Technologies (San Diego, CA 92117 USA) with permission from Jun-ichi Miyazaki, Division of Stem Cell Regulation Research, Osaka University Medical School, JAPAN, and were routinely tested for mycoplamsa contamination.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	All husbandry conditions were monitored by Monash University Ethics framework and complied with the Australian code for the care and use of animals for scientific purposes (the Code) which promotes the ethical, humane and responsible care and use of animals used for scientific purposes. All mouse strains were maintained on an inbred C57BL6/J background using stocks replaced triannually from those at the Jackson Laboraotories. ABCA12 conditional mouse alleles were generated from ES cell stocks from EUCOMM. We generated β-cell specific Abca12 knockout mice and controls by crossing floxed Abca12 mice with, B6.Cg-Tg (Ins2-cre)25Mgn/J mice expressing Cre under the Rat Insulin Promoter 1 (Rip1Cre) (Jackson Laboratories).
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures complied with standards set under Australian guidelines for animal welfare and experiments were subject to Monash University animal welfare ethics review (Approval #MARP/2016/164).
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance with these Guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	n/a
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	n/a
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	n/a
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	n/a
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	n/a
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	n/a
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	n/a

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	This has been included. RNAseq data is available through GEO and MS analysis of lipid levels in Appendix Tables 2-10.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	n/a
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	n/a
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	n/a

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	n/a