THE EMBO JOURNAL

GRASP55 regulates intra-Golgi localization of glycoslyation enzymes to control glycosphingolipid synthesis

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DOI: 10.15252/embj.2021107766

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Review Timeline:

Submission Date:	18th Jan 21
Editorial Decision:	11th Feb 21
Revision Received:	14th Apr 21
Editorial Decision:	28th Apr 21
Revision Received:	26th Jul 21
Accepted:	6th Aug 21

Editor: Elisabetta Argenzio

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 11th Feb 2021

Thank you for submitting your manuscript entitled "GRASP55 regulates intra-Golgi localization of glycan enzymes to control glycosphingolipid synthesis" [EMBOJ-2021-107766] to The EMBO Journal. Your study has now been assessed by three reviewers, whose reports are enclosed below for your information.

As you can see, the referees find the study novel and interesting but also raise several issues that need to be addressed before they can support publication in The EMBO Journal.

Given the overall interest of your study, we have decided to invite you to submit a new version of the manuscript revised according to the referees' requests. I should add that it is The EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in the revised version.

We generally grant three months as standard revision time. As we are aware that many laboratories cannot function at full capacity owing to the COVID-19 pandemic, we may relax this deadline. Also, we have decided to apply our 'scooping protection policy' to the time span required for you to fully revise your manuscript and address the experimental issues highlighted herein. Nevertheless, please inform us as soon as a paper with related content is published elsewhere.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File and will therefore be made available online. For more details on our Transparent Editorial Process, please visit our website: http://emboj.embopress.org/about#Transparent_Process

Before submitting your revised manuscript, deposit any primary datasets and computer code produced in this study in an appropriate public database (see http://msb.embopress.org/authorguide#dataavailability). Please remember to provide a reviewer password, in case such datasets are not yet public. The accession numbers and database names should be listed in a formal "Data Availability" section (placed after Materials & Method). Provide a "Data availability" section even if there are no primary datasets produced in the study.

Feel free to contact me if you have any questions about the submission of the revised manuscript to The EMBO Journal. I thank you again for the opportunity to consider this work for publication and look forward to your revision.

Referee #1:

Pothukuchi et al. address the question whether localization of biosynthetic enzymes to different compartments in the Golgi regulates the ratio at which different glycosphingolipid classes are synthesized. The authors show that enzymes for sphingolipid synthesis are not uniformly distributed in the Golgi and identify the Golgi-associated protein Grasp55 as a factor required both for proper sphingolipid synthesis and localization of corresponding enzymes (GCS, LCS). On a

mechanistic level, the authors demonstrate interaction of Grasp55 with GCS and LCS and provide evidence that this interaction serves to prevent their retrograde flow through the Golgi. Intriguingly, altering the sub-Golgi localization of GCS and LCS is sufficient to alter the relative proportions of synthesized glycosphingolipids.

How cells produce a defined set of glycosphingolipids through consecutive action of biosynthetic enzymes that compete for substrates is an important and poorly understood question. Through a series of well-controlled experiments, the authors show that sub-Golgi localization of biosynthetic enzymes is an important determinant and identify a role for Grasp55 in regulating this process. The manuscript is well-written and findings are discussed conclusively. The combination of cell biological experiments addressing the regulation of enzyme trafficking with lipid analyses to establish consequences for biosynthetic output make this study of interest for a broad readership. A few, mostly minor experiments to strengthen the author's conclusions are outlined below:

1. Fig. 5: The authors provide evidence that Grasp55 KO increases the localization of GCS and LCS to peri-Golgi vesicles, suggesting increased retrograde transport. However, Grasp55 KO also causes Golgi fragmentation (Fig. S4). Can the authors explain how they distinguish these two phenotypes in the electron microscopy images? Along the same lines, is it possible in the in vitro COPI vesicle formation assay (Fig. 5D) that enzymes show increased abundance in supernatants of Grasp55 KO, because they reside in a more fragmented Golgi, which might fractionate similarly? In this regard, it would also be helpful to show Western blots for organelle / vesicle markers.

2. Fig. 5: The authors propose that Grasp55 functions as retainer of sphingolipid biosynthetic enzymes in the trans Golgi. According to this model, Grasp55 antagonizes the function of Golph3, which mediates retrograde transport of GCS and LCS. It would be interesting to now where GLC and LCS localize in Grasp55 / Golph3 DKO cells. Would the DKO rescue the opposing phenotypes observed in the single KOs?

3. Fig. 6: In elegant experiments, the authors express mutant variants of GCS and LCS that mislocalize in the Golgi / ER and demonstrate that this is sufficient to alter the ratio at which different sphingolipid classes are synthesized. For interpretation of the results, it would be important to show the relative levels at which the different enzyme variants are expressed. Moreover, while the different GCS variants clearly show perturbed subcellular localization (Fig. S11), co-staining with the relevant organelle markers would help interpret their localization patterns.

Minor points:
1. Fig 3C: Please indicate which image is control / KO and which clone was used.
2. Fig. S5: I understand the kinetics of some of lipid species (e.g. decrease of Cer over time), but interpretation of others is less obvious (e.g. why is SM already maximal at the beginning of the chase? Why are the kinetics of GM3 and Gb3 so different in Grasp55 KO vs control)? I consider interpretation of all of observed changes beyond the scope of this study, but it would be helpful if the authors could discuss the data a bit more in detail.

3. Fig. S8: It would be helpful to show representative images as in Fig. 3A - C.

Referee #2:

Review of the manuscript entitled " GRASP55 regulates intra-Golgi localization of glycosylation enzymes to control glycosphingolipid biosynthesis" authored by Prathyush Pothukuchi et al.
In this work, it is demonstrated the role of GRASP55 in the compartmentalization and localization of key enzymes involved in the glycosphingolipid (GSL) biosynthesis. Mechanistically, GRASP55 binds and concentrate key enzymes of the glycosphingolipid (GSL) biosynthesis in the trans-Golgi by preventing their entry to COPI derived retrograde transport.
By mean of an ample set of experiments, including GRASP55 knock down or cells expressing mutant enzymes lacking GRASP55 binding site, it is demonstrated the functional role of GRASP55 in this biosynthetic pathway.
The results of this study are new and do not overlap with other studies. Importantly, it provides relevant new information about the molecular players implicated in the Golgi biology, affecting key metabolic pathways with biological relevance.
Interestingly, authors also indicate the potential novel functions of GRASP55 by mean of the putative interactions with enzymes containing PDZ domain binding motifs.
The study is well conducted and easy to follow. The manuscript is well written and organized.
There are performed an ample set of experimental approaches (CRISPR-CAS9, expression constructs, Immunoprecipitation, peptide pull down assay, reconstitution of COPI vesicles, immunofluorescence and confocal microscopy,Flow cytometry analysis, Electron microscopy, Cryo-immuno EM, lipid Analysis -HPLC-Mass Spectrometry, MALDI-MS Ceramide Transport assay, bodipy-Ceramide in the Golgi...) to demonstrate the biological relevance and molecular mechanism of action of GRASP55 in the control of the biosynthetic pathways of GSLs.
There is a balanced number of figures and illustrations to clarify the results.
The conclusions are supported by the experiments performed.
In general, I find the findings very interesting and that could be relevant and valuable in a clinical setting.
Overall, the results are clear, and the manuscript is well organized and well written, nevertheless there are minor concerns that should be addressed.
Minor comments:
- Discussion will be definitively benefit if it is included additional information about described GRASP55 alterations, for example in cancer cell lines such as amplifications, mutations and/or expression levels (http://www.cbioportal.org/), to highlight the consequences in the area of the sphingolipid metabolism and the therapeutic relevance in tumours in cancer patients.

Referee #3:

This manuscript explores the role of the Golgi protein GRASP55 in the localization of sphingolipid glycosylation enzymes. It addresses a relatively small mystery, namely, the function of GRASP55, and a large mystery, namely, the mechanism by which Golgi enzymes are compartmentalized to specific parts of the stack. GRASP55 is found to be important for compartmentalizing two key enzymes in sphingolipid glycosylation, GlcCer synthase and LacCer synthase. Direct interaction of GRASP55 with the C-terminus of GlcCer synthase is demonstrated, and functional tests indicate that GRASP55 is important to localize both enzymes toward the trans side of the stack. The interpretation is that GRASP55 binds to GlcCer synthase and prevents its retrograde traffic in COPI vesicles, consequently ensuring that the enzyme remains in the trans portion of the Golgi. A less well characterized, potentially indirect interaction may confer trans-Golgi localization on LacCer synthase.

The analysis is thorough, careful, and imaginative. There are a lot of interpretations, but they are

reasonable and consistent with the data. Overall, I find this work to be a novel and important contribution. I have only minor comments.

1) The citation format with two authors followed by "et al." is distracting.

2) GRASP55 seems to be a recent invention in evolutionary terms. In yeast and Drosophila, there is a single GRASP that is present early in the secretory pathway. Yet polarized glycosphingolipid synthesis is presumably conserved, so the mechanisms described here are probably animal-specific. This consideration does not diminish the value of the findings, but it should be discussed to provide context.

3) In Fig. 2C, the GM/Gb ratio is not reproducibly changed in the different clones. The authors say the effect is present in 2 out of 3 clones, but only 1 out of 3 shows a strong effect. Yet Fig. 6B seems to show a reproducibly strong effect. Why the discrepancy?

4) In Fig. S6A, SMS1 levels are reproducibly increased by knocking out GRASP55. This increase is comparable to other changes that the authors view as noteworthy, yet they simply state that there is no reduction in SMS1 levels. This omission should be corrected.

5) In Fig. 5F, the graph presumably represents gold particles marking GCS rather than total gold particles. That fact should be indicated somehow in the figure.

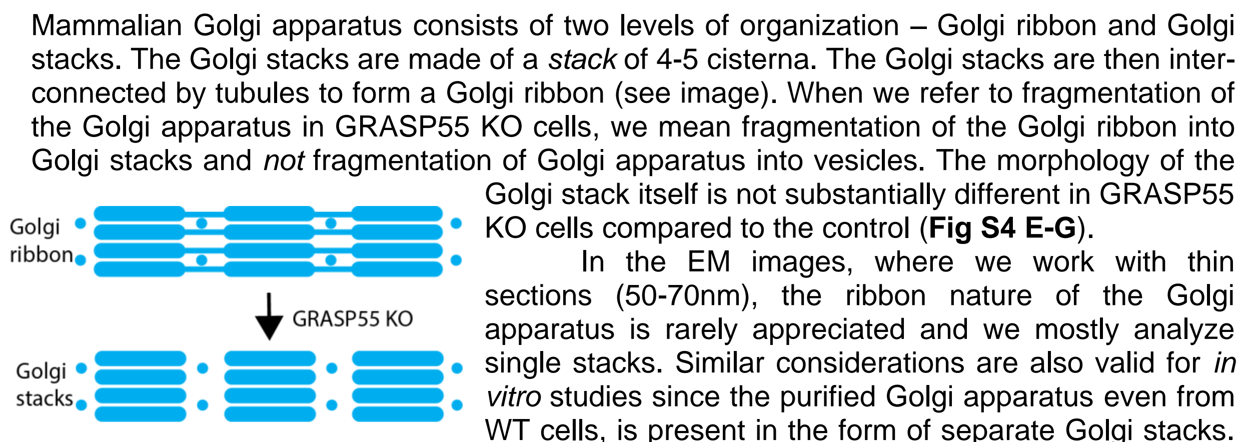
6) In Fig. 5G, are the black structures supposed to be arrows? They are so stylized that for a while I thought they represented something like coats. A simpler and more standard arrow representation is recommended.

7) Coatomer is depicted in Fig. 5G as acting at the trans-most Golgi cisterna, but tomographic reconstructions have indicated that COPI vesicles do not bud from the trans-most cisterna. Please comment on how the distribution of coatomer factors into the model.

We thank the reviewers for their encouraging and enthusiastic comments. Below is our response to the critical comments of the reviewers:

1. Fig. 5: The authors provide evidence that Grasp55 KO increases the localization of GCS and LCS to peri-Golgi vesicles, suggesting increased retrograde transport. However, Grasp55 KO also causes Golgi fragmentation (Fig. S4). Can the authors explain how they distinguish these two phenotypes in the electron microscopy images? Along the same lines, is it possible in the in vitro COPI vesicle formation assay (Fig. 5D) that enzymes show increased abundance in supernatants of Grasp55 KO, because they reside in a more fragmented Golgi, which might fractionate similarly? In this regard, it would also be helpful to show Western blots for organelle / vesicle markers.

Mammalian Golgi apparatus consists of two levels of organization – Golgi ribbon and Golgi stacks. The Golgi stacks are made of a *stack* of 4-5 cisterna. The Golgi stacks are then inter-connected by tubules to form a Golgi ribbon (see image). When we refer to fragmentation of the Golgi apparatus in GRASP55 KO cells, we mean fragmentation of the Golgi ribbon into Golgi stacks and *not* fragmentation of Golgi apparatus into vesicles. The morphology of the Golgi stack itself is not substantially different in GRASP55 KO cells compared to the control (**Fig S4 E-G**).



In the EM images, where we work with thin sections (50-70nm), the ribbon nature of the Golgi apparatus is rarely appreciated and we mostly analyze single stacks. Similar considerations are also valid for *in vitro* studies since the purified Golgi apparatus even from WT cells, is present in the form of separate Golgi stacks. Thus, in both the cases we are dealing with Golgi stacks and not Golgi ribbon and so our results were not likely influenced by the effect of GRASP55 KO on Golgi ribbon architecture. Nevertheless, to answer the concern raised by the reviewer we present the following data:

a. We have now included data on morphological evaluation of the Golgi stacks which show that length of the Golgi cisternae was not reduced or were the number vesicles increased in GRASP55 KO conditions suggesting that the *Golgi stack* had not fragmented into vesicles under our experimental conditions (**new Fig. S4E-G).**
b. Data presented in **Fig.S10A** shows that in the *in vitro* assay, the formation of vesicles depended on the presence of ARF1, ARFGAP and BARS suggesting that the vesicles were indeed genuine COPI vesicles and are not likely to be fragmented Golgi. Western blotting for β-COP (a vesicle marker) shows what we purify is indeed COPI dependent vesicles (**Fig.S10A**).
c. Further we also show an organelle marker (Transferrin receptor, a cargo molecule that does not enter vesicles) is not present in the purified vesicles but remains with the Golgi fraction. This shows that the purified vesicle fraction were genuine vesicles and not fragmented Golgi.

These considerations and data together provide convincing evidence that the vesicles analyzed by EM or *in vitro* assays were genuine COPI dependent vesicles and not fragmented Golgi apparatus.

2. Fig. 5: The authors propose that Grasp55 functions as retainer of sphingolipid biosynthetic

enzymes in the trans Golgi. According to this model, Grasp55 antagonizes the function of Golph3, which mediates retrograde transport of GCS and LCS. It would be interesting to now where GLC and LCS localize in Grasp55 / Golph3 DKO cells. Would the DKO rescue the opposing phenotypes observed in the single KOs?

GOLPH3 is involved in the retrograde transport of LCS. As for GCS, we do not know if there is an adaptor involved. Our *in vitro* studies have not shown an interaction between GCS cytoplasmic tail and GOLPH3. So, the proposed experiment will be meaningful only in the case of LCS, which we have now done. These data are presented in (**Fig. S10C)**. We have used double KDs by siRNA rather than double KOs since preparation and characterization of KO clones takes a lot of time. We find that eliminating both GOLPH3 and GRASP55 phenocopies GOLPH3 KD. GRASP55 KD promotes retrograde transport of LCS by moving it towards *cis*-Golgi (**Fig. 3B,E)** as well as to the ER (**Fig. S10C**). Removal of GOLPH3, a filter that prevents their transport out of the Golgi, under conditions of GRASP55 KD leads to leakage of enzymes out of the Golgi similar to what happens when only GOLPH3 is downregulated (**Fig. S10C)**. Thus, we conclude that GRASP55 and GOLPH3 act sequentially with GRASP55 promoting transport of LCS towards trans-Golgi and GOLPH3 acting to prevent exit from trans-Golgi. Since GOLPH3 acts downstream of GRASP55 the effect of GOLPH3 KD dominates over the effect of GRASP55 reduction on LCS localization.

3. Fig. 6: In elegant experiments, the authors express mutant variants of GCS and LCS that mislocalize in the Golgi / ER and demonstrate that this is sufficient to alter the ratio at which different sphingolipid classes are synthesized. For interpretation of the results, it would be important to show the relative levels at which the different enzyme variants are expressed. Moreover, while the different GCS variants clearly show perturbed subcellular localization (Fig. S11), co-staining with the relevant organelle markers would help interpret their localization patterns.

We have now included western blotting data to show the relative expression levels of the enzymes (**Fig. S11B**). We also show that indeed the mutant GCS proteins localize to the proposed organelles by co-staining with appropriate compartment markers. $GCS_{N\text{-term}}$ localizes exclusively to ER (labelled with Calnexin) and $GCS_{C\text{-term}}$ localizes to ER (labelled with Calnexin) and Golgi (labelled with GM130) (**Fig. S11A**).

Minor points:

1. Fig 3C: Please indicate which image is control / KO and which clone was used.

We have now indicated which image belongs to control and KO.

2. Fig. S5: I understand the kinetics of some of lipid species (e.g. decrease of Cer over time), but interpretation of others is less obvious (e.g. why is SM already maximal at the beginning of the chase? Why are the kinetics of GM3 and Gb3 so different in Grasp55 KO vs control)? I consider interpretation of all of observed changes beyond the scope of this study, but it would be helpful if the authors could discuss the data a bit more in detail.

Biosynthesis of SM depends on faster, CERT-mediated transport of ceramide to the Golgi apparatus. This could explain the faster biosynthesis of SM already at the beginning of the chase. As for the differences between Gb3 and GM3 biosynthetic rates in control *vs* GRASP55 KO cells, Gb3 is consistently more in GRASP55 KO cells across all time points but a large increase in GM levels in GRASP55 KO cells was observed only at the final 24h time point. This is likely due to the very low levels of GM in HeLa cells that it requires 24h of chase to accumulate substantial amounts of lipid before the difference can be seen. We have now discussed these issues in the manuscript. (Line 222-230).

3. Fig. S8: It would be helpful to show representative images as in Fig. 3A - C.

We have included this data in **Fig. S8C-E**

Referee #2:

- Discussion will be definitively benefit if it is included additional information about described GRASP55 alterations, for example in cancer cell lines such as amplifications, mutations and/or expression levels (http://www.cbioportal.org/), to highlight the consequences in the area of the sphingolipid metabolism and the therapeutic relevance in tumours in cancer patients.

Analysis of the alterations in *GRASP55* gene (mutations or copy number alterations; data from cBioportal) did not reveal any big changes in cancers but expression levels of *GRASP55* was moderately low in acute myeloid leukemia, kidney cancers and thymoma compared to other cancer types (www.cbioportal.org). Acute myeloid leukemic cells are known to have increased levels of GSLs compared to non-transformed lymphocytes (Wang et al., 2012). Whether GRASP55 contributes to this phenomenon is worthy of exploration. Further, mouse insertional mutagenesis studies have found that the inactivation of GRASP55 promotes tumor transformation and/or growth in liver and colorectal cancer models (Ref: Candidate Cancer Gene Database). Whether this is related to the effect of GRASP55 on GSLs will be interesting to study. We have now included this in the discussion (Line 616-626).

Referee #3:

1) The citation format with two authors followed by "et al." is distracting.

We have changed it.

2) GRASP55 seems to be a recent invention in evolutionary terms. In yeast and Drosophila, there is a single GRASP that is present early in the secretory pathway. Yet polarized glycosphingolipid synthesis is presumably conserved, so the mechanisms described here are probably animal-specific. This consideration does not diminish the value of the findings, but it should be discussed to provide context.

Recent studies have unequivocally shown that the absence of GRASP proteins does not affect Golgi stacking. So, the function of GRASP proteins at the Golgi remains unclear. In this light, we propose a new role for GRASP55 at the Golgi where it regulates localization of glycosylation enzyme(s). We find that C-terminal PDZ interacting motif in GCS is highly conserved in animals from zebrafish to humans along with the presence of GRASP55, as noted by the reviewer. Thus, the evolution of the binding motif in GCS parallels the evolution of GRASP55, further strengthening the link between GRASP55 and regulation of GSL biosynthesis in vertebrates. The absence of classical PDZ binding motif in other organisms, may likely reflect the absence of such regulation in these organisms. We have now included this in the discussion (Line 607-615). It is an interesting aspect and we thank the reviewer for pointing us in this direction.

3) In Fig. 2C, the GM/Gb ratio is not reproducibly changed in the different clones. The authors say the effect is present in 2 out of 3 clones, but only 1 out of 3 shows a strong effect. Yet Fig. 6B seems to show a reproducibly strong effect. Why the discrepancy?

We agree with the reviewer, while 2 out of 3 clones show a statistically significant difference in Gm/Gb ratio, only 1 out of 3 shows a strong effect. In addition, GRASP55 siRNA treatment also increased the Gm/Gb ratio. We have now added a note about this in the manuscript (Line 220-223).

As for the discrepancy between Fig.2C and Fig.6B, it is probably due to the difference is chase time. The data in Fig.2C were obtained following 24h of chase. In this

case, the final results depend not only on the biosynthesis of GSLs but perhaps also on their degradation. While the data in Fig.6B were obtained after 8h of chase since BFA treatment was toxic to cells at longer time points. Given the very slow biosynthesis of GSLs, the 8h timepoint is likely to be less influenced by factors other than biosynthesis. This may be the reason for the apparent discrepancy. Nevertheless, in both cases the GM/Gb ratio showed similar tendency with G55KO #1 > G55KO #2 > G55KO #3. While we had mentioned this time difference earlier in Figure legend, we have now added a note also in the main text to avoid confusion (Line 476-481).

4) In Fig. S6A, SMS1 levels are reproducibly increased by knocking out GRASP55. This increase is comparable to other changes that the authors view as noteworthy, yet they simply state that there is no reduction in SMS1 levels. This omission should be corrected.

We apologize for the omission, and this has now been corrected (Line 253).

5) In Fig. 5F, the graph presumably represents gold particles marking GCS rather than total gold particles. That fact should be indicated somehow in the figure.

We have made the corrections.

6) In Fig. 5G, are the black structures supposed to be arrows? They are so stylized that for a while I thought they represented something like coats. A simpler and more standard arrow representation is recommended.

We have modified the figure using simpler arrow representation.

7) Coatomer is depicted in Fig. 5G as acting at the trans-most Golgi cisterna, but tomographic reconstructions have indicated that COPI vesicles do not bud from the trans-most cisterna. Please comment on how the distribution of coatomer factors into the model.

Tomographic reconstructions have shown that the "*trans*-most cisternae" contain only clathrin coated buds (Ladinsky et al., 1999), while immunogold localization of β-COP subunit has shown that a significant amount of it is also localized to the *trans*-Golgi (Oprins et al., 1993). These two studies can be reconciled if the "*trans*-most cisterna" referred to in the tomographic reconstructions is actually a part of the TGN and not *trans*-Golgi proper. Since the last two *trans* cisternae in the tomogram also had close contacts with the ER (Ladinsky et al., 1999), it is very likely that they were TGN, and so are expected to have clathrin buds.

Our model depends on the action of coatomer at the Golgi stack (from cis to trans-Golgi, distinct from TGN), where the enzymes of our interest, GCS and LCS are mainly localized. So, we have modified the figure to distinguish *trans*-cisterna from TGN. We have also added a note about this in the results section where the model is described (Line 464-467).

References:

Ladinsky, M.S., D.N. Mastronarde, J.R. McIntosh, K.E. Howell, and L.A. Staehelin. 1999. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J Cell Biol*. 144:1135-1149.

Oprins, A., R. Duden, T.E. Kreis, H.J. Geuze, and J.W. Slot. 1993. Beta-COP localizes mainly to the cis-Golgi side in exocrine pancreas. *J Cell Biol*. 121:49-59.

Wang, Z., L. Wen, X. Ma, Z. Chen, Y. Yu, J. Zhu, Y. Wang, Z. Liu, H. Liu, D. Wu, D. Zhou, and Y. Li. 2012. High expression of lactotriaosylceramide, a differentiation-associated glycosphingolipid, in the bone marrow of acute myeloid leukemia patients. *Glycobiology*. 22:930-938.

1st Revision - Editorial Decision 28th Apr 2021

Thank you for submitting your revised study. The manuscript has now been sent back to the original referee #1 and #3, whose comments are appended below.

As you will see, the referees find that their criticisms have been adequately addressed and recommend the study for publication here.

However, there are few editorial issues concerning the text and the figures that I need you to address before we can officially accept your manuscript.

Referee #1:

The authors have fully addressed my concerns. This is an important study that will be of high interest for the broad readership of The EMBO Journal.

Referee #3:

The authors have done a nice job of responding to the suggestions from me and the other reviewers. I have no further comments.

2nd Revision - Editorial Decision 6th Aug 2021

I am pleased to inform you that your manuscript has been accepted for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr. Seetharaman Parashuraman

Journal Submitted to: the EMBO Journal

Manuscript Number: EMBOJ-2021-107766R

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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 χ2 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)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was chosen based on previous experiments and publications PMID:23913272, PMID:23775191.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data were excluded.
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.	Confocal microscopy analysis, random fields were chosen by observing the samples on channels which are not relevant for the analysis (i.e., DAPI). Images were then acquired in all the channels and were subjected to software based automated analysis. For electron microscopy analysis, Golgi fields where morphology was preserved and labelling was satisfactory were chosen for
For animal studies, include a statement about randomization even if no randomization was used.	NA
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.	The investigators were not blinded during data collection and analysis
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	All the statistical evaluations were done using GraphPad Prism built-in tests (unpaired two-tailed Student's t-tests).
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	No specific methods were used to assess distribution.

Is there an estimate of variation within each group of data?	All the statistical evaluations performed assumed unequal variance within a group/dataset. Thus, significance was evaulated on stringent conditions.
Is the variance similar between the groups that are being statistically compared?	All the statistical evaluations performed assumed unequal variance between groups. Thus, significance was evaulated on stringent conditions.

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).	Commercially available antibodies were used in the study. Detailed description which includes the species, catalog/clone number and the identifier is provided in (Appendix Table EV4).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All the cell lines used (along with source) are mentioned in Methods section. Mycoplasma contamination was not observed in cell cultures as observed by DAPI staining.

* 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.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
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.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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.	NA
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.	NA

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	NA
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).	All the numerical raw data will provided by the corresponding author upon reasonable request.
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).	NA
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.	NA

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.	We do not see room for dual use in our research