The Golgi-associated retrograde protein (GARP) complex plays an essential role in the maintenance of the Golgi glycosylation machinery

Amrita Khakurel, Tetyana Kudlyk, Juan Bonifacino, and Vladimir Lupashin

Corresponding author(s): Vladimir Lupashin, UAMS

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(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.)

RE: Manuscript #E21-04-0169
TITLE: The Golgi-associated retrograde protein (GARP) complex plays an essential role in the maintenance of the Golgi glycosylation machinery

Dear Vladimir,

Two reviewers have returned comments on your submission, and I am pleased to report that both of them are generally positive about the quality and value of the study. But as usual, they have a number of suggestions for improving the manuscript.

Please pay careful attention to these comments, using your judgement about how best to address the concerns. I will then ask the same reviewers to take a quick look at the revised manuscript.

Best regards,
Ben

Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Lupashin,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

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Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

This paper revisits the role of the GARP tether in mammalian cells by performing targeted knockouts of GARP subunits. Earlier work had implicated GARP in recycling from endosomes. The new results suggest a broader role for GARP in regulating the localizations and stabilities of Golgi glycosylation enzymes. One of the conclusions is that certain glycosylation enzymes may cycle between the Golgi and endosomes, rather than recycling within the Golgi as has been assumed.

This story is presented clearly, and it is logical and thorough, with generally rigorous methods. The authors make a compelling case that GARP has a broader role than has been appreciated. A caveat is that some effects of the knockouts might be indirect due to general perturbation of the Golgi, but the interpretations in this regard are suitably cautious.

The most significant concern is the experiments involving transient transfection to express tagged ST6Gal1 and B4GalT1. Overexpression of type II Golgi enzymes can lead to "spillover" localization to other compartments. My own lab struggles with this issue, and we address it by restricting the analysis to cells with moderate expression levels. It seems possible that high expression is exaggerating traffic in pathways that would normally be minor. The authors should address this point, perhaps by comparing data obtained at different expression levels.

Another significant concern is the interpretation of results showing accumulation of Golgi enzymes in endolysosomes after knockouts of GARP subunits. If GARP is a tether that captures vesicular carriers, shouldn't a GARP knockout cause the trafficked proteins to show a cytoplasmic haze rather than mislocalization to endolysosomes? The logic here needs to be justified better.

The following minor issues could also be addressed.

1. In Figure 1E and 1F, what are the units? If they are arbitrary units, it should be stated.

2. On p. 11 it is suggested that the reduced levels of GPP130, TMEM165, and TGN46 are due to reduced glycosylation, but those reductions could equally be ascribed to GARP-dependent mislocalization that leads to accelerated turnover. Perhaps a nonglycosylated Golgi protein could be examined as a control.

3. In Figure 3E, the ST6GalT1 blot is hard to read. There seems to be an electrophoretic shift in the VSP53 KO that is not apparent in the VPS54 KO, and the authors don't comment on what would cause that difference if GARP is inactivated similarly in both KOs.

4. In Figure 3F, the data for MGAT1 are not terribly convincing, particularly because the VPS54 KO seems to have no effect.

5. In Figure 4, the pattern for LAMP2-GFP is oddly heterogeneous-for example, it looks very different in the VPS53 KO and VPS54 KO images. It would be preferable to see images with a more consistent LAMP2-GFP signal.

Moreover, for the quantification of this figure, the line scanning method is quite crude. Perhaps a better approach would be to make a mask using LAMP2-GFP and then quantify the amount of ST-RFP that overlaps the mask.

6. In Figure 5, some of the images seem to be saturated and/or overly processed.

7. In Figure 7, 3 hours seems like a long time for CQ treatment. If B4GalT1 localizes by cycling through endosomal compartments, what is the expected half-time for this pathway? I would expect something on the order of 15 minutes. How do the kinetics compare for proteins such as GPP130, TGN46, and MPR? If CQ treatment traps those proteins more quickly in endosomal compartments, then perhaps B4GalT1 leaks only occasionally out of the Golgi.

Reviewer #2 (Remarks to the Author):

The resident enzymes of the Golgi apparatus are responsible for a wide range of glycosylation processes. It is likely that they maintain their localization by recycling in vesicles, with various mechanism proposed for the sorting of enzymes into such recycling pathways.

This paper investigates the role of the GARP complex in Golgi enzyme retention. The GARP complex is a vesicle tethering complex involved in recycling from endosomes to the TGN. The authors use CRISPR-Cas9 to knockout GARP subunits from two different human cell lines and find that glycosylation is perturbed and a subset of Golgi enzymes is destabilized, apparently because they traffic beyond the Golgi and get degraded.

Overall, the data to support the main conclusions are clear and convincing. There are thorough and careful phenotypic analyses of knockouts of different cell lines with rescues at near endogenous levels. As such, this seems a useful addition to the literature on understanding the trafficking of glycosylation machinery. However, the mechanism behind the effect is not clear (as the authors acknowledge), and more possible interpretations need to be discussed, and this point generally made clearer. In addition, several aspects of the figures need to be improved, and one experiment has already been reported in the literature. These points are described in detail below. If addressed the paper would seem suitable for publication in Molecular Biology of the Cell.

1) Mechanism.
As the authors note, there are several ways that removal of GARP could have the observed effects. In addition to those listed, the authors should also discuss the possibility that the Golgi itself is perturbed by the failure to recycle from endosomes trafficking machinery that acts in the late Golgi to direct vesicles to earlier compartments. This is important, as it might be that Golgi enzymes do not normally recycle to and from endosomes in significant amounts, but rather they traffic beyond the Golgi more rapidly once the Golgi is perturbed by removing GARP. Indeed, a recent paper has suggested that normally glycosylation enzymes do not recycle back to the Golgi from later compartments (Sun et al BioRxiv, 2021.02.15.431224v1). It should also be noted that some of the protein destabilization observed could be an indirect effect of reduced glycosylation.

2) Chloroquine experiment reported previously.
In Figure 7, the authors show that treatment with chloroquine results in B4GalT1 accumulating in endosomes. This has already been reported by others (eg PubMed ID 19277980).

3) Figures.
a) It was sometimes difficult to follow which cell line had been used in blots and micrographs, and the figures would benefit from in-figure labels to indicate the cell line in addition to explaining it in the figure legends.

b) Please use magenta and green instead of red and green for color blind readers, particularly where only channel overlays of micrographs are shown eg. Figure 3D, 5 and Supplementary Figure 4A.

c) Some key blots would benefit from loading controls (eg. β-actin blots or total protein stains) - eg Figure 1, Supplementary Figures 1, 2 and 3.

d) Bar graphs should show individual data points as well as mean and error.

Figure 1.
Please note how binding was quantified in Fig 1E,F legend - pixel intensity? Y-axis units? Not immediately clear from Material and Methods. Missing legend for I,J.

Figure 3
What about GALNT2 and ST6GAL1 in HeLa cells? Are they not affected - if this is the case, it should be shown somewhere. Please comment on the appearance of the intense lower band in E for VPS53 KO - Is it a cleavage product or hypoglycosylation as suggested in S3C? The lower band is stronger than the WT band but this is not reflected in the quantification.

Figure 4.
Colocalization of ST-RFP with LAMP2 in KOs not entirely convincing with figures in current form. Would benefit from a Pearson's correlation analysis and/or increasing the size of the individual channels with zoom insets on individual lysosomes.

Figure 5.
Same comments for Figure 4 apply to 5D. Legend mentions "biotin mix" - please specify components - biotin-free medium supplemented with biotin (40 μM) and cycloheximide (50 μM)

Figure 6
Legend states: "Following overnight" - does this mean after overnight incubation? following

overnight transfection?

Figure 8
The authors have no evidence that any Golgi enzymes are trafficking to endosomes in normal, untreated cells. Note, COPI-coated vesicles are labelled as clathrin-coated vesicles

Supplementary Figure 5.
Wild-type control lacking. There is a huge difference between the Golgi size and morphology (giantin stain) between the two micrographs.

Supplementary Figure 6.
This figure is missing?

Supplementary Figure 7.
Incorrect capitalization of 'giantin, 'paired', 'top' and 'bottom'. Please indicate what *** means in bar graphs.

Minor Comments and Errors.
P3. 21
Fails to mention or allude to other sugars eg. fucose, GalNac, xylose, glucuronic acid etc (https://doi.org/10.1038/s41580-020-00294-x)

P13. 12
B4GALT1 exists in multiple isoforms which have been reported to differ in their trafficking. In the text, please specify the isoform used for the RUSH experiments (PMID: 1714903).

P14. 5
"where these B4GalT1 in VPS54-KOs localize to" seems wrong.

P18. 16; P19. 21,23; P20. 36,37
correlation instead of "co-relation"

Methods section.
Lectin blotting protocol lacks detail - buffer? blocking agent?, concentration of lectin? wash steps? blocking time?

Dear Editors,

We appreciate the careful review of our manuscript and the positive comments from both reviewers: "*This story is presented clearly, and it is logical and thorough, with generally rigorous methods. The authors make a compelling case that GARP has a broader role than has been appreciated* (Reviewer 1); *Overall, the data to support the main conclusions are clear and convincing* (Reviewer 2)." In response to the reviewers' constructive critique, we updated our data figures and the text to incorporate missing controls, requested experiments and additional discussion.

Point-by-point response:

Reviewer 1.

1. *The most significant concern is the experiments involving transient transfection to express tagged ST6Gal1 and B4GalT1. Overexpression of type II Golgi enzymes can lead to "spillover" localization to other compartments. My own lab struggles with this issue, and we address it by restricting the analysis to cells with moderate expression levels. It seems possible that high expression is exaggerating traffic in pathways that would normally be minor. The authors should address this point, perhaps by comparing data obtained at different expression levels.*

We agree with the reviewer's comment and in our studies we always restricted the analysis to cells with moderate expression levels of transiently expressed tagged proteins. To address the reviewer's point we have repeated the B4GalT1 RUSH experiment in RPE1 cells and compared data obtained at different expression levels. This comparison revealed decreased colocalization of B4GalT1-mCherry with the Golgi marker GM130 in VPS54-KO cells at 6 hours for cells with both high and low/moderate expression of the Golgi enzyme. These data are now shown in Supplementary Figure 5A.

2. *Another significant concern is the interpretation of results showing accumulation of Golgi enzymes in endolysosomes after knockouts of GARP subunits. If GARP is a tether that captures vesicular carriers, shouldn't a GARP knockout cause the trafficked proteins to show a cytoplasmic haze rather than mislocalization to endolysosomes? The logic here needs to be justified better.*

The GARP complex is predicted to work as a tether for vesicle docking and fusion at the *trans*-Golgi/TGN. If this prediction is correct, one would indeed expect accumulation of non-tethered vesicles in cells deficient for GARP complex subunits. Our data demonstrates that in cells completely depleted of VPS54 and VPS53, the stability of Golgi enzymes is significantly compromised, presumably by degradation of mis-targeted vesicles and/or other Golgi physiology changes (altered lipid composition, pH, ionic balance, etc.). We also found transient accumulation of mCherry-tagged Golgi enzymes in the endolysosomal compartment (Rab9-, Lamp2- and CD63-positive) in GARP-KO cells. We have interpreted this result as inefficient retrieval of Golgi enzymes from an endosomal compartment in GARP-deficient cells. In addition, we demonstrated that B4GalT1 can be reversibly relocalized from an endolysosomal compartment in CQ treated/washed-out cells, indicating that the endosome-Golgi retrieval pathway exist at

least for this enzyme. In support to this hypothesis, data in a recent BioRxiv manuscript (Sun et al BioRxiv, 2021.02.15.431224v1) showed that four other tested overexpressed Golgi enzymes (MGAT2, B4GalT7, B3GalT6 and POMGNT1) are retrieved to the Golgi even from the plasma membrane. Our previously published work (PMID: 16420527) that investigated cells depleted of another Golgi vesicular tether, the COG complex, demonstrated that CCD (COG-complex dependent) vesicle accumulation (cytoplasmic haze, detection in glycerol velocity gradient) occurred transiently only after acute (2-6 days) but not prolonged (9 days) depletion of COG complex subunits, indicating a transient nature of vesicle accumulation. In our future studies, we plan to utilize mAid/Tir1 rapid degradation strategy to investigate vesicle accumulation of recycling Golgi enzymes in cells acutely depleted of the GARP complex.

3. *In Figure 1E and 1F, what are the units? If they are arbitrary units, it should be stated.*

Relative binding of Alexa647-labeled lectins to the surface of wt and mutant RPE1 cells was represented in arbitrary units.

4. *On p. 11 it is suggested that the reduced levels of GPP130, TMEM165, and TGN46 are due to reduced glycosylation, but those reductions could equally be ascribed to GARP-dependent mislocalization that leads to accelerated turnover. Perhaps a nonglycosylated Golgi protein could be examined as a control*.

Thank you for your comment. We agree that the reduced levels of GPP130, TMEM165, and TGN46 could be due to reduced glycosylation. This possibility is added to the text. We also added data on a nonglycosylated Golgi protein, GS15/Bet1L (Figure 2E).

5. *In Figure 3E, the ST6GalT1 blot is hard to read. There seems to be an electrophoretic shift in the VSP53 KO that is not apparent in the VPS54 KO, and the authors don't comment on what would cause that difference if GARP is inactivated similarly in both KOs.*

Figure 3E is updated to show a better ST6GalT1 blot. The identity of the additional low molecular band in VPS53KO cells is unknown; therefore, we quantified only the band that corresponds to the full-length protein. One possibility is that the extra band in VPS53KO corresponds to partially degraded ST6GalT1. VPS53 belongs to two tethering complexes, GARP and EARP, and therefore VPS53 KO can inactivate both Golgi-endosomal and endosomal-lysosomal recycling/degradation pathways. We have added this possibility to the Discussion.

6. *In Figure 3F, the data for MGAT1 are not terribly convincing, particularly because the VPS54 KO seems to have no effect.*

Figure 3F is updated to show a better MGAT1 blot. Quantification of three independent blots revealed reduction of MGAT1 in HeLa VPS54-KO cells.

7. *In Figure 4, the pattern for LAMP2-GFP is oddly heterogeneous-for example, it looks very different in the VPS53 KO and VPS54 KO images. It would be preferable to see images with a more consistent LAMP2-GFP signal. Moreover, for the quantification of this figure, the line scanning method is quite crude.*

Figure 4B is updated to show more consistent LAMP2-GFP signal. Colocalization of ST-RFP and LAMP2-GFP transfected for 20 hours was quantified using the Pearson's correlation coefficient.

8. *In Figure 5, some of the images seem to be saturated and/or overly processed.*

To the best of our knowledge not a single image presented in this manuscript has been saturated or overly processed. Possibly a conversion from Adobe Illustrator to PDF format caused some image distortions. We have changed the red color to magenta in Figure 5 to accommodate color-blind readers.

9. *In Figure 7, 3 hours seems like a long time for CQ treatment. If B4GalT1 localizes by cycling through endosomal compartments, what is the expected half-time for this pathway? I would expect something on the order of 15 minutes.*

We have performed a shorter, 90-minute treatment for CQ in both HeLa and RPE1 cells and now report the result of that experiment in Supplementary Figure 6. The shorter treatment was sufficient to significantly mislocalize endogenous Ba4GalT1 in both HeLa and RPE1 cells, but was not long enough to mislocalize GPP130, a known recycling protein, indicating that B4GalT1 recycles faster than GPP130. Current approaches are not precise enough to estimate the exact half-life of B4GalT1 in the Golgi-endolysosomal pathway, but we believe that it takes longer than 15 minutes.

Reviewer 2

1. *As the authors note, there are several ways that removal of GARP could have the observed effects. In addition to those listed, the authors should also discuss the possibility that the Golgi itself is perturbed by the failure to recycle from endosomes trafficking machinery that acts in the late Golgi to direct vesicles to earlier compartments. This is important, as it might be that Golgi enzymes do not normally recycle to and from endosomes in significant amounts, but rather they traffic beyond the Golgi more rapidly once the Golgi is perturbed by removing GARP. Indeed, a recent paper has suggested that normally glycosylation enzymes do not recycle back to the Golgi from later compartments (Sun et al BioRxiv, 2021.02.15.431224v1). It should also be noted that some of the protein destabilization observed could be an indirect effect of reduced glycosylation.*

Thank you for your comment. We agree that GARP KO may not only affect endosomal-Golgi recycling directly, but also can alter Golgi physiology indirectly (lipid composition, pH, ionic balance, etc.). These alterations may certainly change the trafficking pattern of Golgi enzymes. We now acknowledge these possibilities in the Discussion. We do not believe that the essence of the recent BioRxiv manuscript is related to our studies – that manuscript is exclusively dealing with overexpressed Golgi proteins and their possible trafficking to the plasma membrane. Please note that in our studies we are discussing endosome-Golgi recycling, not PM-Golgi recycling. It is interesting, however, that the BioRxiv manuscript showed that four tested overexpressed Golgi enzymes (MGAT2, B4GalT7, B3GalT6 and POMGNT1) are retrieved to the Golgi even from the plasma membrane, indicating the existence of an extensive endocytic retrieval pathway that is utilized by a subset of Golgi enzymes.

2. *In Figure 7, the authors show that treatment with chloroquine results in B4GalT1 accumulating in endosomes. This has already been reported by others (eg PubMed ID 19277980).*

We agree that the mentioned paper already reported that a long, 24 hours CQ treatment alters B4GalT1 localization. The exact effect was not quantified in that paper, but we now mention this published result in our Discussion. We believe that our results showing quantitative mislocalization of B4GalT1 after 90 min of CQ treatment (Supplementary Figure 6) and a significant return of B4GalT1 to the Golgi upon CQ wash-out (Supplementary Figure 7) are in a good agreement with the proposed model for recycling of subset of Golgi enzymes via endosomal compartment (Figure 6).

3. *It was sometimes difficult to follow which cell line had been used in blots and micrographs, and the figures would benefit from in-figure labels to indicate the cell line in addition to explaining it in the figure legends*.

We have updated all blots and micrographs to indicate which cell line was used in the experiment shown.

4. *Please use magenta and green instead of red and green for color blind readers, particularly where only channel overlays of micrographs are shown eg. Figure 3D, 5 and Supplementary Figure 4A.*

We have changed to magenta and green instead of red and green for color blind readers as recommended.

5. *Some key blots would benefit from loading controls (eg. β-actin blots or total protein stains) - eg Figure 1, Supplementary Figures 1, 2 and 3.*

Loading controls were added to key blots as requested.

6. *Bar graphs should show individual data points as well as mean and error.*

We now show individual data points as well as mean and error for all bar graphs. Please note that for confocal images the individual data point indicate one single slice confocal image. Depending on the cell type, density and transfection efficiency, we have quantified from 4 to 30 individual images (1 to 10 cells per image) to account for at least 30 cells per sample.

7. *Please note how binding was quantified in Fig 1E,F legend - pixel intensity? Y-axis units? Not immediately clear from Material and Methods. Missing legend for I,J.*

Relative binding of Alexa647-labeled lectins to the surface of WT and mutant RPE1 cells was depicted in arbitrary units. Missing legend was added.

8. *What about GALNT2 and ST6GAL1 in HeLa cells? Are they not affected - if this is the case, it should be shown somewhere. Please comment on the appearance of the intense lower band in E for VPS53 KO - Is it a cleavage product or hypoglycosylation as suggested in S3C? The lower band is stronger than the WT band but this is not reflected in the quantification*.

As requested, we have now quantified both GALNT2 and ST6GAL1 in HeLa cells (Supplementary Figure 3 D, E) – protein levels of these enzymes were not significantly changed in GARP-KO cells, possibly indicating that trafficking of some Golgi enzymes is different between normal (RPE1, HEK293T) and cancer cell lines.
Figure 3E is updated to show a better ST6GalT1 blot. The identity of the additional low molecular band in VPS53-KO cells is unknown; therefore, we quantified only the band

that corresponds to the full-length protein. One possibility is that the extra band in VPS53-KO corresponds to partially degraded ST6GalT1. VPS53 belongs to two tethering complexes, GARP and EARP and therefore VPS53 KO can inactivate both Golgi-endosomal and endosomal-lysosomal recycling/degradation pathways. We have added this possibility to the Discussion.

9. *Colocalization of ST-RFP with LAMP2 in KOs not entirely convincing with figures in current form. Would benefit from a Pearson's correlation analysis and/or increasing the size of the individual channels with zoom insets on individual lysosomes.*

Figure 4B is updated to show more consistent LAMP2-GFP signal. Colocalization of ST-RFP and LAMP2-GFP in cells transfected for 20 hours was quantified using the Pearson's correlation coefficient.

10. *Same comments for Figure 4 apply to 5D. Legend mentions "biotin mix" - please specify components - biotin-free medium supplemented with biotin (40 μM) and cycloheximide (50 μM).*

Figure legend was updated as recommended.

11. *Legend states: "Following overnight" - does this mean after overnight incubation? following overnight transfection?*

Figure legend was updated as recommended.

12. *The authors have no evidence that any Golgi enzymes are trafficking to endosomes in normal, untreated cells. Note, COPI-coated vesicles are labelled as clathrin-coated vesicles.*

The Supplementary movie shows that in HeLa cells the endogenous GFP-tagged B4GalT1 is visiting a tubulating membrane compartment that could indicate trafficking to endosomes in normal, untreated cells. We also demonstrated that B4GalT1 can be retrieved from an endosomal compartment upon CQ wash-out, indicating the existence of an endosome-Golgi recycling pathway that is utilized by the Golgi enzyme. We believe that the normal endosomal recycling of Golgi enzymes is very transient and can be revealed only by imaging of endogenously tagged Golgi enzymes in cells acutely depleted for the GARP complex.
As suggested, we have changed the labelling of intra-Golgi vesicles as COPI-coated vesicles.

13. *Supplementary Figure 5. Wild-type control lacking. There is a huge difference between the Golgi size and morphology (giantin stain) between the two micrographs.*

We have added wild-type control as recommended.

14. *Supplementary Figure 6. This figure is missing?*

Supplementary Figure 6 is now added.

15. *Supplementary Figure 7. Incorrect capitalization of 'giantin, 'paired', 'top' and 'bottom'. Please indicate what *** means in bar graphs.*

Thank you for your comments. We have fixed the mistakes as recommended.

16. *P3. 21. Fails to mention or allude to other sugars eg. fucose, GalNac, xylose, glucuronic acid etc (https://doi.org/10.1038/s41580-020-00294-x).*

We acknowledge reviewer's comment. We have now included it in the introduction.

17. *P13. 12. B4GALT1 exists in multiple isoforms which have been reported to differ in their trafficking. In the text, please specify the isoform used for the RUSH experiments (PMID: 1714903).*

We have used B4GalT1 isoform 1 in our RUSH studies. This detail is now added to the Methods section.

18. *P14. 5 "where these B4GalT1 in VPS54-KOs localize to" seems wrong*.

Thank you for your comments. We have fixed the mistakes as recommended.

19. *P18. 16; P19. 21,23; P20. 36,37 correlation instead of "co-relation"*

Thank you for your comments. We have fixed the mistakes as recommended.

20. *Lectin blotting protocol lacks detail - buffer? blocking agent?, concentration of lectin? wash steps? blocking time*?

Thank you for your comments. We have added the missing details to the lectin blotting protocol as recommended.

RE: Manuscript #E21-04-0169R
TITLE: "The Golgi-associated retrograde protein (GARP) complex plays an essential role in the maintenance of the Golgi glycosylation machinery"

Dear Vladimir,

I am pleased to report that both of the reviewers are satisfied with the revisions. The manuscript is now accepted for publication.

Thanks to you and your colleagues for sending this nice work to MBoC.

Sincerely,
Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Lupashin:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office

mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The authors have done a satisfactory job of addressing my previous comments.

Reviewer #2 (Remarks to the Author):

The authors have done a good job of addressing my comments about the text and figures. I hope that they feel that the paper has been improved as a result. I am now happy to recommend acceptance.