Response to Reviewer 1

Thank you very much for taking the time to review this manuscript. Please find the detailed responses below and the corresponding revisions/corrections highlighted in the re-submitted file.

Questions for General Evaluation	Reviewer's Evaluation	Response and Revisions
Does the introduction provide sufficient background and include all relevant references?	Can be improved	
Are all the cited references relevant to the research?	Yes	
Is the research design appropriate?	Can be improved	
Are the methods adequately described?	Can be improved	
Are the results clearly presented?	Must be improved	
Are the conclusions supported by the results?	Must be improved	

Point-by-point response to comments and suggestions for authors

Comment 1:
Authors tried to understand the pathophysiology of the Desbuquois dysplasia type 2, which can be caused by heterozygous defects in xylosyltransferase 1 (XYLT1) gene. For this aim, they developed XYLT1 haploinsufficient human fibroblasts by using a CRIPR/Cas9 system and showed that XYLT1 deficiency caused inappropriate induction of alpha-SMA gene, suggesting a differentiation shift into myofibroblasts by XYLT1 insufficiency.
They also showed the effects of CRIPR/Cas9-mediated XYLT2 deficiency (probably due to homozygous XYLT2 defects) and XYLT1/XYLT2 knockdown by siRNA introduction in human fibroblasts. However, XYLT2 is not a causal gene for Desbuquois dysplasia type 2, and therefore, the presentation of those data in main text induces confusion in the mind of readers.

Response 1:
We have tried to analyze the molecular mechanisms of two sydromes. One is Desbuquois dysplasia type 2, which is caused by mutations in the *XYLT1* gene, and the other is spondylo-ocular syndrome, which is caused by mutations in the *XYLT2* gene. Both syndromes belong to the GAG linkeropathies and were mentioned in the abstract as well as in the introduction. The data for both knockout systems is therefore presented in the main text.

Comment 2:
In addition, the data regarding ACAN is confusing: ACAN gene expression was strongly suppressed in both XYLT1- and XYLT2-deficient cells (Figure 3A), while its expression was remarkably upregualted in XYLT1/XYLT2 knockdown cells (Figure 7A).
Due to these confusing points, the reviewer does not recommend the presentation of the data regarding XYLT2-deficient cells and those of XYLT1/XYLT2 knockdown cells in main text.

Response 2:
It is precisely these observations that we find very exciting and important to discuss. The short-term disruption of ECM synthesis by *XYLT1*/*XYLT2* knockdown leads to compensatory *ACAN* gene induction. The long-term lack of functional GAG synthesis, on the other hand, leads to a reduction in *ACAN* expression. It is possible that the permanent stress in the cell and the associated senescence could be responsible for these observations.

Comment 3:
The reason why authors took those "unrelated" approaches may come from the fact that they could not detect the induction of TGFB1 gene in XYLT1 haploinsufficient human fibroblasts. Nevertheless,

they successfully showed TGFB1 gene induction in XYLT1/XYLT2 double knockdown cells. However, XYLT1/XYLT2 double knockdown does nor properly reflect the genetic background of Desbuquois dysplasia type 2 patients.

Response 3:
That is correct. We cannot automatically assume the involvement of the TGFβ signaling pathway in Desbuquois dysplasia type 2. This is not what we wanted to express. We wanted to look at XT deficiency in different "degrees of severity". On the one hand, there are the individual knockouts of *XYLT1* and *XYLT2* and, after the unsuccessful establishment of a complete XT knockout system, an *XYLT1*/*XYLT2* knockdown and its consequences.

Comment 4:
Their hypothesis that "XYLT1 deficiency inappropriately induces myofibroblast differentiation via TGFB1 signaling" is very interesting and has novelty.
It is well known that an activation of TGFB1 signaling is induced not only by its gene induction but also the release of the protein from its latent form, which consist of the LAP/LTBP/TGFB complex. To check the activation of TGFB1, immunostaining study using an anti-phosho-smad2/smad3 antibody is useful. If nuclear translation of phosho-smad2/smad3 is detected, it indicates that TGFB signaling is activated in the cells.
If authors can show the activation of TGFB1 in XYLT1 haploinsufficient human fibroblasts, the point of view will be simplified. In that case, the data regarding XYLT2-deficient and XYLT1/XYLT2 knockdown cells can be translocated to supplemental information to avoid confusion of the readers mind.
To assess the possible activation of TGB signaling by XYLT1 deficiency, please perform immunostaining study using an anti-phosho-smad2/smad3 antibody in XYLT1 haploinsufficient human fibroblasts.

Response 4:
The aim of this paper was to establish model systems for GAG-linkeropathy syndromes. This made it possible to analyze the long-term effects of defective tetrasaccharide linker synthesis. In connection with the knockdown system of *XYLT1* and *XYLT2*, an explanatory approach for the detected changes could be postulated. Whether TGF signaling still plays a major role during the long-term model systems can and will certainly be analyzed in future work. However, it is beyond the scope of this paper.

Comment 5:
In Title, the word "acute" sounds rather bizzar since this term indicates the rapidness in the process of disease development in patients. Please change the title into a proper one.

Response 5:
We change the title to “Xylosyltransferase-deficiency in human dermal fibroblasts induces compensatory myofibroblast differentiation and long-term ECM reduction”

Comment 6:
In line 64, the word "ambylophilia" should be corrected as amblyopia.

Response 6:
Agree. I have changed the word (Page 2, line 64)