

Dermal papillary fibroblasts promote persistent granulation tissue formation in junctional EB

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30th Jul 2025

Dear Dr. Rognoni,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the three reviewers who agreed to evaluate your manuscript.

As you will see from the enclosed reports, the reviewers agree that the findings are of potential interest, but referees #2 and #3 also all raise serious concerns, including, but not limited to, the inadequacy of the model system, the use of a single clone, the over-reliance on immunofluorescence, the absence of quantification, and the unclear rationale and methodology.

Based on the nature of the concerns and the amount of time and work that would be needed to address them, considering also that at EMBO Press we encourage a single round of revisions in a limited time frame, we prefer to return the manuscript to you at this point with the decision that we cannot offer to publish it.

However, given the potential interest of the findings, we would welcome a new manuscript on the same topic if you obtained data that would address the referees' concerns in full.

To be completely clear, however, I would like to stress that if you were to send a new manuscript, this would be treated as a new submission rather than a revision and would be reviewed afresh, in particular with respect to the literature and the novelty of your findings at the time of resubmission. If you decide to follow this route, please make sure you nevertheless upload a letter of response to the referees' comments.

I am sorry that I could not bring better news this time and hope that the referee comments are helpful in your continued work in this area.

With kind regards,

Lise Roth

Lise Roth, Ph.D
Senior Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The manuscript by Khan et al. explores the roles of distinct subsets of dermal fibroblast in the pathogenesis of junctional epidermolysis bullosa (JEB). The primary experiments involve reconstituted JEB organotypic cultures of patient-derived cells and K14-CreER.Lama3-floxed animal model JEB. The manuscript is well-prepared and the findings are novel, revealing papillary fibroblast-derived TGFb as a potential target for JEB treatment.

A few minor comments:

1. It's nice that the cell surface marker-based cell isolation method is well-established and allows lineage selection. Given that these markers are also expressed by other cells, e.g. T-cells, can the authors clarify whether the immunohistological staining data specifically detects fibroblasts or a mix of different cell types (Figure 4)?
2. From the cytokine array, a number of cytokines, including TNF and IL6, were increased in papillary fibroblasts co-cultured with JEB OTs. Can authors comment if these cytokines were normalized after RepSox treatment? Perhaps authors discuss what might be the role of these other cytokines. Also, how might mutant keratinocytes affect papillary fibroblast behavior, e.g to produce more TGFb.
3. Figure 1F Ki67 +ve epidermal cells: please clarify or reiterate what "Ve" means?
4. Figure 2A- Scale bar is unclear.
5. Labeling of DAPI on dark background doesn't show well
6. Please confirm that both Suppl Figure S1 and 2 are thigh skin samples.

Referee #2 (Comments on Novelty/Model System for Author):

There is a complete reliance on immunofluorescence staining as the analytical technique. The disease model used is based on shRNA knockdown of the laminin alpha 3 gene. While this is one gene mutated in laminin-332-deficient junctional epidermolysis bullosa, it cannot be concluded that this model accurately represents the disease. All observations should be confirmed using laminin-332-deficient junctional epidermolysis bullosa models and/or skin. To name the model a junctional epidermolysis bullosa model is, at this stage, not correct.

All experiments with the laminin alpha 3-deficient human keratinocytes are based on one clone. This is a major limitation.

There are many conclusions in the paper that are somewhat illogical-a non-significant change in laminin-332 deposition leading to a significant reduction in proliferation. This is likely due to low sensitivity in the analytical techniques or the use of only three replicates not being sufficient.

The authors conclude from the secretome analyses, performed on an unclear number of samples, that laminin-332 deficiency in keratinocytes does not alter TGF-beta signaling, but that this rather occurs when co-cultured with papillary fibroblasts. Yet, they later proceed to analyze and detect differences in TGF-beta signaling in mono-cultured laminin alpha 3-deficient keratinocytes. Again, this could be explained by their analyses not being sensitive enough or by not including sufficient replicates to detect such changes, but it is nevertheless illogical.

The analyses and stains of the extracellular matrix are of low quality and often appear unspecific.

The analyses of the in vivo model do not specifically assess granulation tissue formation.

The analyses are based on female donor fibroblasts only. There is no reason for this, apart from perhaps practical ones. It must be justified.

Referee #2 (Remarks for Author):

In this paper, the mechanisms behind the observed granulation tissue formation in laminin-332-deficient junctional epidermolysis bullosa (JEB) are studied. The authors identify papillary fibroblasts as mediators of this process, whereas reticular fibroblasts appear to play a minor role. The authors further identify that this activation of dermal papillary fibroblasts and their subsequent enhancement of keratinocyte activity is dependent on transforming growth factor beta. Targeting transforming growth factor beta in an in vivo model of laminin alpha 3 expression deficiency in keratinocytes improves the appearance of skin. In general, the paper presents an interesting story. At this stage, it feels premature, and additional models, analytical techniques, and considerations are needed.

From the Methods section, it appears that all in vitro models are based on a single clone of shLAMA3 keratinocytes and a single clone of shControl keratinocytes. If this is indeed the case, it should be shown that the differences are not dependent on the selected clones. More importantly, it is essential to directly establish the relevance of the models and the observed phenotypic changes in the context of JEB. This includes analyses of JEB donor-derived samples-cells or skin (as shown in Figure 4). Unless such information is included, the models cannot, at this stage, be referred to as JEB models.

Following the previous comment, it should be shown that rete ridges are increased in JEB skin; if not, it should be explained why this is observed in your model. Hyperproliferation of keratinocytes and altered epidermal appearance should be confirmed in JEB donor skin, and if these are not observed, an explanation needs to be provided.

The mechanism of enhanced TGF-beta activity in co-culture of shLAMA3 keratinocytes and papillary fibroblasts is interesting. However, it would be important to understand how shLAMA3 keratinocytes trigger this. Is it through enhanced activation of latent TGF-beta, or through factors that stimulate TGF-beta expression?

The cytokine arrays shown in Supplementary Figure 8 appear diffuse and could be technically improved. This could be one reason to potential pathways not being detected.

The number of samples analyzed in the secretome analysis should be stated. The authors find no significant difference in TGF-beta1 concentration between shC and shLAMA3 keratinocytes when cultured as monocultures (Figure 2C). Yet, they nevertheless use these to test for and detect differences in TGF-beta signaling (pSMAD2) (Figure 2G and Supplemental Figure 4). This appears somewhat illogical and needs to be better justified.

It is my understanding that TGF-beta activity in co-culture models, as used by the authors, reduces keratinocyte proliferation rather than enhances it (e.g., PMID: 7930681). Please explain. Is this due to the keratinocytes used here being somewhat transformed?

It would be important to include a full description of donors and biopsy sites of the skin samples used for Figure 4. Are the

control samples really biopsies from healthy newborns, and were they taken from similar sites as the JEB donors?

In the Methods it is stated that recombinant laminin-332 was added to the cultures presented in Supplemental Figure 3A before polymerization. Consequently, some dermal staining could have been expected. It is somewhat unexpected to see staining in the upper epidermis of the recombinant laminin-332-treated shLAMA3 cultures. Does this imply laminin-332 transport from the dermis up to the other epidermal aspects? Could the authors stain with laminin-332 binding partners integrin alpha 6 beta 4 and type XVII collagen to see if they display similar alterations? This is just to understand the validity of the models and may also perhaps give some insights to the observed epidermal phenotype.

In general, the paper relies solely on immunofluorescence. It would be important to include additional techniques that allow better quantitative analysis of key findings, including western blot analyses of TGF-beta signaling.

The staining for collagen III and tenascin-C is unclear and should be improved. The staining for fibronectin is not convincing and rather appears to show blistered (no-staining) vs. non-blistered skin (staining). Please replace or improve the presentation.

It is not clear how much of the skin in the conditional laminin alpha 3-deficient mice was treated with RepSox. Was the entire mouse covered, or only the skin lesions?

The appearance of granulation tissue in Figure 5D is not clear.

The analyses of immune infiltrates need to be improved and performed in much more detail. TGF-beta is generally considered to be immunosuppressive, so understanding how a reduction in TGF-beta signaling in the context of JEB leads to reduced inflammation is important.

I would recommend that the Discussion section be shortened and focused on JEB. Please also carefully check all references to ensure that they are correct and contain the cited information. Care should be taken regarding losartan and decorin: while both have been reported to reduce TGF-beta activity through different mechanisms, they are not exclusively TGF-beta inhibitors. Targeting other mechanisms might be as, or even more, important than interference with TGF-beta.

Overall, it is a nice piece of work; however, as mentioned, it is my impression that the story is still somewhat premature. I recommend including additional support for the models and further investigating the underlying mechanisms to complete the story.

Referee #3 (Comments on Novelty/Model System for Author):

Please see Major Comment #9 below.

Referee #3 (Remarks for Author):

This manuscript by Khan et al. investigates the role of dermal fibroblast subpopulations in the progression of Junctional Epidermolysis Bullosa (JEB), a severe skin blistering disorder caused by mutations in LAMA3 and loss of laminin-332. Using human 3D organotypic (OT) skin cultures composed of LAMA3-knockdown or wild-type keratinocytes co-cultured with FACS-sorted dermal fibroblasts (papillary, reticular, or mixed), the authors show that papillary fibroblasts uniquely promote keratinocyte hyperproliferation and dermal remodeling. Cytokine profiling reveals elevated TGFβ1 levels (along with other inflammatory mediators) in conditioned media from JEB cultures with papillary fibroblasts. These findings are supported by increased SMAD2 phosphorylation, indicating active TGFβ signaling.

The authors extend these findings in vivo using a tamoxifen-inducible Lama3 flox/flox; K14-CreERT mouse model and JEB patient skin biopsies. Both models exhibit an expansion of CD39+ papillary fibroblasts and enhanced pSMAD2 expression. Topical application of the TGFβ inhibitor RepSox in JEB mice improves skin pathology, reduces inflammation, and mitigates blister formation, suggesting translational potential.

Overall, this study presents compelling in vitro and in vivo data implicating papillary fibroblasts and TGFβ signaling in JEB pathology. However, the mechanistic model remains incomplete. There is a critical need to better explain the logic linking fibroblast-derived signals, TGFβ activation, and keratinocyte behavior. Moreover, several technical aspects - including interpretation of SMAD detection, antibody selection, and cytokine source - require clarification. Manuscript clarity would also be significantly improved by explicitly labeling sample types (epithelial vs fibroblast) and experimental contexts (in vitro vs in vivo) throughout.

Additionally, numerous textual and formatting issues (e.g., duplicated figure legends) detract from the manuscript and should be carefully corrected.

Major Comments

1. Clarify rationale for focusing on TGF β

The cytokine array data show increased levels of multiple factors (e.g., IL-6, TNF- α , FGF-9, VEGF) in JEB OT cultures. The authors should justify the focus on TGF β 1. Was it the most consistently upregulated factor across samples? Were others ruled out experimentally?

2. Demonstrate fibroblast-specific contribution of TGF β in vivo

Although CD39+ fibroblasts and pSMAD2+ cells are increased in JEB skin, direct evidence linking fibroblasts to TGF β production or signaling is lacking. Quantitative co-localization of CD39 and pSMAD2, or lineage-specific manipulation (e.g., CD39-Cre TGF β 1 KO) would help establish causality.

3. Clarify RepSox specificity and off-target effects

RepSox inhibits both SMAD2 and SMAD1/5/8 phosphorylation. As it is primarily a TGF β type I receptor inhibitor, the observed impact on BMP signaling (SMAD1/5/8) needs clarification. Additionally, RepSox appears to reduce proliferation even in WT skin. Were these effects quantified and controlled (e.g., vehicle-treated controls)?

4. Provide detailed explanation of SMAD analysis and TGF β signaling pathway

The manuscript should include a schematic or textual overview of the canonical TGF β pathway, including the role of SMAD2/3 phosphorylation, SMAD4 complexing, and nuclear translocation. It is also unclear why both phospho-specific and general SMAD antibodies were used. Clarify their respective purposes and expected localization (e.g., nuclear vs cytoplasmic). Lastly, SMAD proteins are intracellular; explain how SMAD was detected in conditioned media—could this reflect cell lysis or contamination?

5. Clarify causality: Are fibroblasts initiating or responding to JEB pathology?

The interpretation that papillary fibroblasts drive JEB phenotypes needs more support. Are similar fibroblast phenotypes observed in other inflammatory skin contexts? Additional controls (e.g., wounding or non-laminin inflammatory models) could help determine whether fibroblast activation is a primary driver or a downstream response to epithelial injury.

6. Quantitative analysis of key immunostaining and histological findings

Several figures rely on representative immunostaining (CD39, pSMAD2, ECM markers) without quantification. Please provide quantification of marker expression (e.g., % CD39+ fibroblasts, % pSMAD2+CD39+ cells, immune cell counts) to support conclusions.

7. Clarify sample and model types throughout the manuscript

The manuscript often refers to "samples" or "cells" without specifying whether they are keratinocyte- or fibroblast-derived, or from in vitro cultures vs in vivo tissues. This ambiguity hinders interpretation. All figures, legends, and text should clearly state sample source, cell type, and model system.

8. Discuss therapeutic implications, limitations, and alternatives

RepSox shows promise in reducing disease burden, but TGF β inhibition carries risks due to its broad physiological roles. The authors should address these concerns and consider alternative approaches, such as fibroblast-specific targeting (e.g., CD39 inhibition), downstream effectors (e.g., IL-11 or tenascin-C), or localized delivery strategies.

9. Improve statistical and methodological transparency

The statistical analysis throughout the manuscript requires substantial clarification and improvement. For experiments where both fibroblast subtype (reticular vs. papillary) and keratinocyte manipulation (Ctrl vs. shLama3) are compared simultaneously, a two-way ANOVA should be used instead of a one-way ANOVA to properly assess the interaction effects between these variables. Additionally, the biological replicates and sample sizes (N) are inconsistently reported or appear insufficient. For example, the cytokine array appears to be shown from a single experiment without any replicates, which severely limits the interpretability and robustness of the findings. Please ensure all experiments include appropriate biological replicates and clearly indicate N values in the figure legends and methods. Without proper statistical treatment and adequate replicates, the conclusions drawn are not sufficiently supported.

Minor Comments

1. Thorough proofreading needed: Numerous errors were noted, including duplicated figure legends, inconsistent terminology, and formatting issues. Carefully review all text, legends, labels, and supplementary data for clarity and accuracy.

2. Define fibroblast subtypes and sorting methods: Clearly describe how papillary and reticular fibroblasts were defined and isolated, including markers used for sorting.

3. Clarify cytokine source: Specify whether cytokine data are from fibroblasts, keratinocytes, or whole OT cultures; and whether measurements are from cell lysates or conditioned media.

4. Label immunofluorescence images clearly: Include scale bars, labeled structures, and matched controls. High-resolution images are needed for all key comparisons.
5. Include WT + RepSox controls: These are essential for assessing specificity of treatment effects.
6. Specify fibroblast donor source: Clarify whether fibroblasts were derived from neonatal or adult skin, and whether multiple donors were used.
7. Reorganize Results section: Consider grouping in vitro and in vivo findings separately to improve clarity.
8. Add model summary table: A table summarizing all experimental models (e.g., genotype, fibroblast subtype, treatments) would help orient readers.

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***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The manuscript by Khan et al. explores the roles of distinct subsets of dermal fibroblast in the pathogenesis of junctional epidermolysis bullosa (JEB). The primary experiments involve reconstituted JEB organotypic cultures of patient-derived cells and K14-CreER.Lama3-floxed animal model JEB. The manuscript is well-prepared and the findings are novel, revealing papillary fibroblast-derived TGF β as a potential target for JEB treatment.

A few minor comments:

1. It's nice that the cell surface marker-based cell isolation method is well-established and allows lineage selection. Given that these markers are also expressed by other cells, e.g. T-cells, can the authors clarify whether the immunohistological staining data specifically detects fibroblasts or a mix of different cell types (Figure 4)?

Many thanks for the positive evaluation of our manuscript. We are well aware of the current challenges to identify fibroblast subpopulation specific markers for immunohistology analysis. The used fibroblast markers (CD39, Sca1, CD90, PDGFR α) have been widely used by us and other to label fibroblasts in mouse and human skin (see for example PMID: 34537192, PMID: 34939928, PMID: 27287810, PMID: 29391249). We have now included CD39/CD90 co-staining to better define the papillary fibroblast subpopulations (new Fig EV5) and have clarified it more clearly in the text that fibroblast markers are also expressed by other skin cells.

2. From the cytokine array, a number of cytokines, including TNF and IL6, were increased in papillary fibroblasts co-cultured with JEB OTs. Can authors comment if these cytokines were normalized after RepSox treatment? Perhaps authors discuss what might be the role of these other cytokines. Also, how might mutant keratinocytes affect papillary fibroblast behavior, e.g to produce more TGF β .

We agree with the reviewer that beside TGF β the cytokine array indicates changes in multiple different cytokines including TNF and IL6. We have now added substantial new mechanistic data on TGF β and integrin $\alpha\beta$ 6 signalling and exploring the impact of other cytokines is beyond the scope of this manuscript. We have decided to remove the cytokine array from the revised manuscript and explore the other cytokines in future experiments.

To address the question on how mutant keratinocytes are affecting papillary fibroblast behaviour, we have now included more mechanistic data showing that loss of laminin-332 induces integrin $\alpha\beta$ 6 expression in keratinocytes which is important to regulate

TGF β availability and signalling. Furthermore, we show that papillary fibroblasts secrete more TGF β 1 and increase expression of integrin α β 6 in Lama3 deficient keratinocytes, which promotes aberrant TGF β signalling in a profibrotic feedback loop (PMID: 10025398). The increased expression of these proinflammatory cytokines promote fibroblast activation, fibrotic ECM deposition and proliferation as observed in our JEB in vivo disease model. Importantly, direct inhibition of TGF β with RepSox is ameliorating these JEB disease phenotypes.

3. Figure 1F Ki67 +ve epidermal cells: please clarify or reiterate what "Ve" means?

We apologise for the lack of clarity. "Ve" meant positive cells and we have now simplified this to "+" throughout the text and figures.

4. Figure 2A- Scale bar is unclear.

The scale bar got lost during the pdf conversion process and we have added it back.

5. Labeling of DAPI on dark background doesn't show well

We have now increased the DAPI brightness throughout the figures.

6. Please confirm that both Suppl Figure S1 and 2 are thigh skin samples.

Many thanks and we have corrected this mistake. Suppl Figure S1 (now Fig EV1) referred to breast skin and Suppl Figure 2 (now Fig EV2) to thigh skin samples.

Referee #2 (Comments on Novelty/Model System for Author):

There is a complete reliance on immunofluorescence staining as the analytical technique. The disease model used is based on shRNA knockdown of the laminin alpha 3 gene. While this is one gene mutated in laminin-332-deficient junctional epidermolysis bullosa, it cannot be concluded that this model accurately represents the disease. All observations should be confirmed using laminin-332-deficient junctional epidermolysis bullosa models and/or skin. To name the model a junctional epidermolysis bullosa model is, at this stage, not correct.

All experiments with the laminin alpha 3-deficient human keratinocytes are based on one clone. This is a major limitation.

We disagree with the reviewer's view that our Lama3 deletion models are not representative of JEB disease. Laminin-332 is a heterotrimer, disruption of the heterotrimer is generated by the loss of any of the laminin chains. shLAMA3 was selected as this matches both the available JEB mouse model and the patient samples. We have now expanded our analysis of the deregulated TGF β signalling to our previously generated cell models depleting the LAMB3 or LAMC2 laminin chain, which both show that increased pSMAD2/TGF β signalling is observed when any of the laminin chains is depleted (new Fig 2F,G). Due to the severity of the JEB disease and early lethality, working with patient derived cells in particular keratinocytes is very challenging and where available there are no matched healthy controls from a close sibling, highlighting the need to work with engineered cell models. For our developed JEB in vitro models, we would like to clarify that while a single shRNA was used to generate the NTERT laminin chain knockdown keratinocytes, these are all mixed populations and have not been clonally selected. While we agree that these JEB models are not perfect, we would like to emphasize the excellent correlation of the JEB disease phenotype data obtained from our 2D, 3D in vitro systems, JEB transgenic model and patient skin biopsy staining.

Lastly, we would like to highlight that our study combines a range of state-of-the-art technologies including 2D and 3D JEB in vitro models, in vivo disease models, immunofluorescence imaging, FACS, ELISA and western blots.

There are many conclusions in the paper that are somewhat illogical-a non-significant change in laminin-332 deposition leading to a significant reduction in proliferation. This is likely due to low sensitivity in the analytical techniques or the use of only three replicates not being sufficient.

We assume that the reviewer is referring to the rLAMA3 rescue experiment in Figure EV3. We would like to clarify that this is a rescue experiment where recombinant laminin-332 (rLN332) was added to the top of the collagen gel before adding the keratinocytes leading to a laminin-332 restoration of over 50% at the dermal epidermal junction. We showed that loss of Lama3 leads to an increase in cell proliferation in 2D and 3D culture as well as in vivo (Jones et al., 2024, PMID: 39811761). When we add back rLN332 in JEB OTs we are decreasing the increased cell proliferation, showing that the observed deregulated proliferation is caused by the loss of laminin-332. We have now made this clearer in the text and improved our statistical analysis.

The authors conclude from the secretome analyses, performed on an unclear number of samples, that laminin-332 deficiency in keratinocytes does not alter TGF-beta

signaling, but that this rather occurs when co-cultured with papillary fibroblasts. Yet, they later proceed to analyze and detect differences in TGF-beta signaling in mono-cultured laminin alpha 3-deficient keratinocytes. Again, this could be explained by their analyses not being sensitive enough or by not including sufficient replicates to detect such changes, but it is nevertheless illogical.

As mentioned in the comments above, we have now decided to remove the cytokine array screening and we have added considerable new mechanistic data to clarify the link of deregulated TGF β signalling in JEB (new Fig 2). We now show that loss of Lama3 leads to an increased expression of integrin $\alpha\beta6$ which has a key role in regulating TGF β availability and signalling (shown by increased nuclear pSMAD2 levels). TGF β 1 itself is able to promote $\alpha\beta6$ integrin expression in addition to SMAD signalling. Next, we show that papillary fibroblasts that are enriched in JEB skin, increase $\alpha\beta6$ integrin expression and pSMAD signalling in JEB OTs (new Fig 3A,B). To dissect the individual cellular contribution, we performed TGF β ELISA measurements of conditioned media from different fibroblasts subpopulations as well as shC and shLAMA3 NTERT keratinocytes, revealing that papillary fibroblasts secrete the highest level of TGF β . In line with these observations conditioned media from 2D cultured papillary fibroblasts, significantly increased TGF β /SMAD signalling and cell proliferation in 2D cultured shLAMA3 keratinocytes. Targeting aberrant TGF β /SMAD signalling is able to reduce increased $\alpha\beta6$ integrin expression and improve the JEB phenotype. We substantially revised the manuscript text and have better specified n numbers in the figure legends.

The analyses and stains of the extracellular matrix are of low quality and often appear unspecific.

We have improved our ECM staining and quantification in the revised manuscript.

The analyses of the in vivo model do not specifically assess granulation tissue formation.

We have increased our analysis of the granulation tissue formation in the JEB disease model in the revised Fig 8, showing stainings of key granulation tissue markers and expanded the description in the revised manuscript.

The analyses are based on female donor fibroblasts only. There is no reason for this, apart from perhaps practical ones. It must be justified.

We have added a justification in the method section.

Referee #2 (Remarks for Author):

In this paper, the mechanisms behind the observed granulation tissue formation in laminin-332-deficient junctional epidermolysis bullosa (JEB) are studied. The authors identify papillary fibroblasts as mediators of this process, whereas reticular fibroblasts appear to play a minor role. The authors further identify that this activation of dermal papillary fibroblasts and their subsequent enhancement of keratinocyte activity is dependent on transforming growth factor beta. Targeting transforming growth factor beta in an in vivo model of laminin alpha 3 expression deficiency in keratinocytes improves the appearance of skin. In general, the paper presents an interesting story. At this stage, it feels premature, and additional models, analytical techniques, and considerations are needed.

From the Methods section, it appears that all in vitro models are based on a single clone of shLAMA3 keratinocytes and a single clone of shControl keratinocytes. If this is indeed the case, it should be shown that the differences are not dependent on the selected clones. More importantly, it is essential to directly establish the relevance of the models and the observed phenotypic changes in the context of JEB. This includes analyses of JEB donor-derived samples-cells or skin (as shown in Figure 4). Unless such information is included, the models cannot, at this stage, be referred to as JEB models.

Many thanks for highlighting the novelty of our manuscript. As mentioned above, while our developed JEB in vitro models used a single shRNA to generate the NTERT laminin chain knockdown keratinocytes, these are all mixed populations and have not been clonally selected. We have now included additional data on the TGF β phenotype after depletion of the other laminin 332 chains (Fig 2F,G). Further we would like to emphasize the excellent correlation of the JEB disease phenotype data obtained from our 2D, 3D in vitro systems, JEB transgenic model and patient skin biopsy sections, including changes in cell differentiation, proliferation, actin organisation and deregulation of proinflammatory signalling pathways. Importantly, addition of recombinant laminin-332 is reverting these phenotypes. We have now expanded our analysis of JEB patient skin sections, however we would like to emphasise that due to the JEB disease severity and early lethality, working with patient derived cells in particular keratinocytes is very challenging and where available there are no matched healthy controls from a close sibling, highlighting the need to work with engineered cell models.

Following the previous comment, it should be shown that rete ridges are increased in JEB skin; if not, it should be explained why this is observed in your model. Hyperproliferation of keratinocytes and altered epidermal appearance should be

confirmed in JEB donor skin, and if these are not observed, an explanation needs to be provided.

Many thanks for this comment and we would like to clarify that our 3D in vitro JEB organotypics are models to mimic early skin development and thus only early stages of the JEB disease. The observed rete ridge-like invagination that are particularly pronounced in papillary fibroblast cocultures, are signs of increased epidermal cell activity which is supported by our proliferation and cell differentiation marker analysis and not directly comparable to rete ridges observed in vivo. We have now made this clearer in the text and figure by labelling these as “epidermal corrugation”. Importantly, epidermal thickness and frequency of epidermal corrugation/rete ridge-like invaginations is highly dependent on the skin anatomical site and in extra digit skin these are more pronounced than in other skin anatomical areas. Due to the lack of anatomical site matched control skin, with our current human patient sample set we can not conclude if there are changes in rete ridges in JEB patient skin and we have added additional comments in the discussion.

To get an indication if these might be changed in JEB skin, we generated virtual H&E from our immunofluorescence images (Fig1A) and compared epidermal thickness and corrugation in neonatal healthy skin H&E images from the skin aging Biomas tissue microarray (Fig1B). While there is no difference between healthy extra digit and JEB skin, the neonatal Biomas tissue array shows significant less epidermal thickness and corrugation (Fig1C). Due to the limited amount of JEB patient tissue, we were not able to stain for proliferation markers.

Figure for reviewers removed

*Quantification of epidermal thickness and corrugation. Please note while extra digit epidermal thickness and corrugation is similar to JEB skin, skin samples from Biomax tissue array are significantly less. Scale bar 100um, for Biomax microtissue array images were scaled to epidermal nuclear size. For statistical comparisons, a one-way ANOVA test was used where ns, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NHS, normal human skin, ED, extra digit; Biomax, Biomax tissue samples.*

The mechanism of enhanced TGF-beta activity in co-culture of shLAMA3 keratinocytes and papillary fibroblasts is interesting. However, it would be important to understand how shLAMA3 keratinocytes trigger this. Is it through enhanced activation of latent TGF-beta, or through factors that stimulate TGF-beta expression?

Many thanks for this comment and we have now added substantial new data to clarify these points (revised Fig 2 and 3). As mentioned above, we now show that loss of Lama3 leads to an increase expression of integrin $\alpha\beta6$ in particular in blistered skin areas, which has a key role in regulating TGF β availability and signalling. Persistence of papillary fibroblasts that secrete high levels of TGF β , a known regulator of integrin $\alpha\beta6$ expression, increased epidermal integrin $\alpha\beta6$ expression, which is reduced by TGF β inhibition.

The cytokine arrays shown in Supplementary Figure 8 appear diffuse and could be technically improved. This could be one reason to potential pathways not being detected.

As explained above we have now decided to remove this assay from the revised manuscript to focus more on the new mechanistic TGF β /integrin $\alpha\beta6$ signalling data.

The number of samples analyzed in the secretome analysis should be stated. The authors find no significant difference in TGF-beta1 concentration between shC and shLAMA3 keratinocytes when cultured as monocultures (Figure 2C). Yet, they nevertheless use these to test for and detect differences in TGF-beta signaling (pSMAD2) (Figure 2G and Supplemental Figure 4). This appears somewhat illogical and needs to be better justified.

We have now specified the n numbers for every experiment in the figure legends. Our ELISA data showed that knockdown of Lama3 in NTERT keratinocytes leads to slight increase in TGF β 1 secretion and a significant increase in the downstream nuclear pSMAD signalling, which indicates an increase in cellular TGF β /SMAD signalling activity. In addition, we now show that integrin $\alpha\beta6$ is prominently upregulated upon laminin-332

loss of function, which actively contributes to downstream TGF β signalling activation and is further enhanced by TGF β (revised Fig 2 and 3).

It is my understanding that TGF-beta activity in co-culture models, as used by the authors, reduces keratinocyte proliferation rather than enhances it (e.g., PMID: 7930681). Please explain. Is this due to the keratinocytes used here being somewhat transformed?

The used NTERT cells are immortalised and considered not transformed. Further we would like to point out that the role of TGF β is highly context dependent as it can both promote and inhibit keratinocyte proliferation. As shown in the highlighted publication by the reviewer, while at high concentrations (7ng/ml) in the presented organotypic TGF β inhibits keratinocyte proliferation, at lower concentrations (1-2.5ng/ml) it promotes epithelial regeneration balancing proliferation and migration. The TGF β concentration that we measured in the conditioned media from our organotypic and 2D cultures were all in the lower TGF β concentration range (1-2ng/ml), in agreement with previously published data. We have now made the role of TGF β in skin homeostasis and reepithelialisation clearer in the revised manuscript.

It would be important to include a full description of donors and biopsy sites of the skin samples used for Figure 4. Are the control samples really biopsies from healthy newborns, and were they taken from similar sites as the JEB donors?

We apologise for the lack of clarity and have now additional information about the skin donors. The healthy control skin is not site matched as it is obtained from redundant under 1 year old extra digit, while JEB patient biopsies are usually obtained from lower limb skin regions at the time of diagnosis.

In the Methods it is stated that recombinant laminin-332 was added to the cultures presented in Supplemental Figure 3A before polymerization. Consequently, some dermal staining could have been expected. It is somewhat unexpected to see staining in the upper epidermis of the recombinant laminin-332-treated shLAMA3 cultures. Does this imply laminin-332 transport from the dermis up to the other epidermal aspects? Could the authors stain with laminin-332 binding partners integrin alpha 6 beta 4 and type XVII collagen to see if they display similar alterations? This is just to understand the validity of the models and may also perhaps give some insights to the observed epidermal phenotype.

This is an interesting observation also seen with recombinant type VII collagen. Basement membrane proteins when added to ECM or injected intradermally into patient skin do appear to accumulate in the basement membrane zone (see for example Woodley et al., 2004, Nat Med PMID: 15195089 or Martins et al., 2015, J Natl Cancer Inst. PMID: 26476432). Consistently, we observed that addition of rLN332 in Con OT and JEB OTs does not lead to strong LAMA3 staining in the dermis after 2 weeks of culture. We speculate that this might be due to the intense ECM remodelling during organotypic growth where deposited laminin-332 is actively embedded into the forming basement membrane. Furthermore, we showed that JEB OTs have dysregulated epidermal differentiation and shLAMA3 cells are probably not adapted to embed rLN332 as efficiently as shC keratinocytes, leading to basal cells to may pull rLN332 into the suprabasal layers. Below we have included immunostaining of vehicle and rLN332 treated organotypics for Lama3 and ITGA6, showing increased ITGA6 expression in JEB OTs that colocalises with rLN332 in the basal and suprabasal layers (Fig 2).

Figure for reviewers removed

In general, the paper relies solely on immunofluorescence. It would be important to include additional techniques that allow better quantitative analysis of key findings, including western blot analyses of TGF-beta signaling.

We have now added western blot analysis of the TGFβ signalling in the revised manuscript confirming the results obtained with our immunofluorescence and ELISA analysis (new Fig 2E). We would like to note that we have used well established antibodies to dissect the TGFβ and integrin αvβ6 integrin signalling.

The staining for collagen III and tenascin-C is unclear and should be improved. The

staining for fibronectin is not convincing and rather appears to show blistered (no-staining) vs. non-blistered skin (staining). Please replace or improve the presentation.

We have improved the quality of these images and added qualifications. All granulation tissue staining are now in the revised main Figure 8.

It is not clear how much of the skin in the conditional laminin alpha 3-deficient mice was treated with RepSox. Was the entire mouse covered, or only the skin lesions?

We apologies for the lack of clarity here and have added additional description in the method and result sections. The entire central mouse back skin was treated with RepSOX, however it was very interesting to see that even this local treatment was sufficient to improve the overall health of the treated JEB mice as observed by the increase in body weight (see Fig 6B). We have highlighted this more clearly in the revised manuscript.

The appearance of granulation tissue in Figure 5D is not clear.

We have now improved the analysis of granulation tissue markers (Col3, Tnc, Fn, CD31 and various fibroblast markers) in the revised manuscript, which are now shown in new Fig 8. Also we would like to note that all in vivo images have been reviewed by our clinical skin expert Prof Edel O'Toole.

The analyses of immune infiltrates need to be improved and performed in much more detail. TGF-beta is generally considered to be immunosuppressive, so understanding how a reduction in TGF-beta signaling in the context of JEB leads to reduced inflammation is important.

We have now expanded our immune cell analysis. Beside total immune cells (CD45+ cells), we have quantified infiltration of T cells (CD3+), macrophages (F4/80) and neutrophils (Ly6G+) in Control and JEB (eKO) skin with and without RepSox treatment (Fig 7), showing significant reduction in all assessed immune cell populations. Furthermore, we have expanded the discussion emphasizing the role of TGF β signalling in regulating skin inflammation.

I would recommend that the Discussion section be shortened and focused on JEB.

Due to the substantial new data, we have now significantly revised the current discussion. While JEB is the main disease focus of this manuscript, we would still like to include a broader discussion of how different fibroblast subsets promote disease pathogenesis potentially also in other EB subtypes and beyond.

Please also carefully check all references to ensure that they are correct and contain the cited information. Care should be taken regarding losartan and decorin: while both have been reported to reduce TGF-beta activity through different mechanisms, they are not exclusively TGF-beta inhibitors. Targeting other mechanisms might be as, or even more, important than interference with TGF-beta.

Many thanks for this comment and we are aware of the differential and additional inhibition activities of decorin and Losartan which we have emphasized now more clearly in the manuscript.

Overall, it is a nice piece of work; however, as mentioned, it is my impression that the story is still somewhat premature. I recommend including additional support for the models and further investigating the underlying mechanisms to complete the story.

Many thanks for the positive evaluation of our work and please see our responses to comments above.

Referee #3 (Comments on Novelty/Model System for Author):

Please see Major Comment #9 below.

Referee #3 (Remarks for Author):

This manuscript by Khan et al. investigates the role of dermal fibroblast subpopulations in the progression of Junctional Epidermolysis Bullosa (JEB), a severe skin blistering disorder caused by mutations in LAMA3 and loss of laminin-332. Using human 3D organotypic (OT) skin cultures composed of LAMA3-knockdown or wild-type keratinocytes co-cultured with FACS-sorted dermal fibroblasts (papillary, reticular, or mixed), the authors show that papillary fibroblasts uniquely promote keratinocyte hyperproliferation and dermal remodeling. Cytokine profiling reveals elevated TGFβ1 levels (along with other inflammatory mediators) in conditioned media from JEB cultures with papillary fibroblasts. These findings are supported by increased SMAD2 phosphorylation, indicating active TGFβ signaling.

The authors extend these findings in vivo using a tamoxifen-inducible Lama3 flox/flox; K14-CreERT mouse model and JEB patient skin biopsies. Both models exhibit an expansion of CD39+ papillary fibroblasts and enhanced pSMAD2 expression. Topical application of the TGFβ inhibitor RepSox in JEB mice improves skin pathology, reduces

inflammation, and mitigates blister formation, suggesting translational potential.

Overall, this study presents compelling in vitro and in vivo data implicating papillary fibroblasts and TGF β signaling in JEB pathology. However, the mechanistic model remains incomplete. There is a critical need to better explain the logic linking fibroblast-derived signals, TGF β activation, and keratinocyte behavior. Moreover, several technical aspects - including interpretation of SMAD detection, antibody selection, and cytokine source - require clarification. Manuscript clarity would also be significantly improved by explicitly labeling sample types (epithelial vs fibroblast) and experimental contexts (in vitro vs in vivo) throughout.

Additionally, numerous textual and formatting issues (e.g., duplicated figure legends) detract from the manuscript and should be carefully corrected.

Many thanks for the positive evaluation of our manuscript and we addressed the formatting and labelling issues throughout the manuscript distinguishing 2D culture, 3D organotypic and in vivo experiments. Importantly we have now added substantial new mechanistic data (please see new Fig 2 and 3 with detailed comments above and below).

Major Comments

1. Clarify rationale for focusing on TGF β

The cytokine array data show increased levels of multiple factors (e.g., IL-6, TNF- α , FGF-9, VEGF) in JEB OT cultures. The authors should justify the focus on TGF β 1. Was it the most consistently upregulated factor across samples? Were others ruled out experimentally?

Considering our new mechanistic data shown that papillary fibroblasts lead to an increase in α v β 6 integrin expression and TGF β signalling in JEB skin (see revised Fig 2 and 3), we have strengthened the rational for focusing on TGF β and decided to remove the cytokine array from the revised manuscript. We will publish this in a separate manuscript where we can focus on the different cytokine changes.

2. Demonstrate fibroblast-specific contribution of TGF β in vivo

Although CD39+ fibroblasts and pSMAD2+ cells are increased in JEB skin, direct evidence linking fibroblasts to TGF β production or signaling is lacking. Quantitative co-localization of CD39 and pSMAD2, or lineage-specific manipulation (e.g., CD39-Cre TGF β 1 KO) would help establish causality.

Many thanks for this comment and we have now included an extensive quantification of CD39+/pSMAD2+ cells, showing in addition to epidermal cells a prominent increase in TGF β activity in CD39+ dermal cells. We have included a CD39 and CD90 co staining to confirm the increase in CD39+CD90+ fibroblasts in JEB skin (Fig EV5). Furthermore, we now show in revised Fig 2&3 that co culture of papillary fibroblasts, which secrete high level of TGF β , lead to an increase in integrin $\alpha\beta$ 6 expression and TGF β signalling, strengthening the mechanistic links in the revised manuscript.

3. Clarify RepSox specificity and off-target effects

RepSox inhibits both SMAD2 and SMAD1/5/8 phosphorylation. As it is primarily a TGF β type I receptor inhibitor, the observed impact on BMP signaling (SMAD1/5/8) needs clarification. Additionally, RepSox appears to reduce proliferation even in WT skin. Were these effects quantified and controlled (e.g., vehicle-treated controls)?

We can confirm that all TGF β inhibition experiments were quantified and controlled with vehicle treated controls. We have now improved the method description and labelling in the figures. While RepSox is highly selective for TGF β type I receptor/ALK5, it has been reported that it can also inhibit other kinases including p38 MAPK, JNK1 and GSK3 which influence BMP signalling as well as cell proliferation. Also, there is significant crosstalk between TGF β /Smad2 and BMP/Smad1/5/8 which are both upregulated in inflammatory conditions such as JEB (Dituri F et al., Cells. 2019 PMID: 31547567). Thus, it is not very surprising that RepSox in JEB disease condition also has an effect on pSMAD1/5/8 signalling, as previously observed in myeloma cells (Olsen OE et al., Biomolecules. 2020 PMID: 32235336). In line with our data, it has been previously shown that RepSox is inhibiting cell growth of human epidermal keratinocytes in feeder free/ low serum conditions in a concentration dependent manner (Suzuki et al., 2017, Wound Repair Regen. PMID: 28437853).

4. Provide detailed explanation of SMAD analysis and TGF β signaling pathway

The manuscript should include a schematic or textual overview of the canonical TGF β pathway, including the role of SMAD2/3 phosphorylation, SMAD4 complexing, and nuclear translocation. It is also unclear why both phospho-specific and general SMAD antibodies were used. Clarify their respective purposes and expected localization (e.g., nuclear vs cytoplasmic). Lastly, SMAD proteins are intracellular; explain how SMAD was detected in conditioned media-could this reflect cell lysis or contamination?

We have expanded our explanation of the TGF β /Smad analysis and signal localisation in the manuscript text and included additional experiments. SMAD2 is phosphorylated at several sites and our immunofluorescence and western blot analysis showed that total

Smad2 and Phospho-Smad2 (Ser465/467) are both prominently increased in JEB cells as these are directly linked at multiple levels (see Lönn P et al., Cell Res. 2009 PMID: 19030025). While non-phosphorylated SMAD2 can be found in the cytoplasm and nucleus, phosphorylated SMAD2 is predominantly localised in the nucleus where it complexes with SMAD4 (Liu L et al., Sci Rep. 2016, PMID: 26905010). We have specified it more clearly that in our immunofluorescent total SMAD analysis only nuclear SMAD signal was quantified.

Lastly, we would like to clarify the misconception that we did not detect SMAD in the conditioned media. In our conditioned media experiments secreted TGFβ1 was measured directly with ELISA and SMAD signalling was detected by immunofluorescence of 2D cultured cells that were treated with conditioned media (see Fig 2 and 3).

5. Clarify causality: Are fibroblasts initiating or responding to JEB pathology?

The interpretation that papillary fibroblasts drive JEB phenotypes needs more support.

Are similar fibroblast phenotypes observed in other inflammatory skin contexts?

Additional controls (e.g., wounding or non-laminin inflammatory models) could help determine whether fibroblast activation is a primary driver or a downstream response to epithelial injury.

We would like to highlight that our presented data show that papillary fibroblasts promote and amplify the JEB disease phenotype that is caused by the loss of function of laminin-332 in skin which is only expressed by epithelial cells (Aumailley & Rousselle, 1999, Matrix Biology). The induced blisters/adhesion loss and increased proinflammatory signalling are promoting fibroblast activation and an ongoing tissue repair response in the upper dermis. In the discussion we have highlighted several recent examples where papillary fibroblasts have been shown to promote inflammatory processes in hypertrophic scars, psoriasis or skin aging.

6. Quantitative analysis of key immunostaining and histological findings

Several figures rely on representative immunostaining (CD39, pSMAD2, ECM markers) without quantification. Please provide quantification of marker expression (e.g., % CD39+ fibroblasts, % pSMAD2+CD39+ cells, immune cell counts) to support conclusions.

We have now expanded our analysis and included more quantifications including pSmad2, CD39, Tnc and ColIII (see revised Fig 5, 7 and 8). These are now expressed as percentage positive cells where possible.

7. Clarify sample and model types throughout the manuscript
The manuscript often refers to "samples" or "cells" without specifying whether they are keratinocyte- or fibroblast-derived, or from in vitro cultures vs in vivo tissues. This ambiguity hinders interpretation. All figures, legends, and text should clearly state sample source, cell type, and model system.

We apologise for the lack of clarity and have now substantially improved the description and labelling of individual experiments throughout the manuscript.

8. Discuss therapeutic implications, limitations, and alternatives
RepSox shows promise in reducing disease burden, but TGF β inhibition carries risks due to its broad physiological roles. The authors should address these concerns and consider alternative approaches, such as fibroblast-specific targeting (e.g., CD39 inhibition), downstream effectors (e.g., IL-11 or tenascin-C), or localized delivery strategies.

Due to the significant changes and additional data throughout the manuscript, we have now expanded our discussion of the implications of TGF β signalling inhibition and highlighted potential more targeted approaches including direct inhibition of integrin $\alpha\text{v}\beta 6$.

9. Improve statistical and methodological transparency
The statistical analysis throughout the manuscript requires substantial clarification and improvement. For experiments where both fibroblast subtype (reticular vs. papillary) and keratinocyte manipulation (Ctrl vs. shLama3) are compared simultaneously, a two-way ANOVA should be used instead of a one-way ANOVA to properly assess the interaction effects between these variables. Additionally, the biological replicates and sample sizes (N) are inconsistently reported or appear insufficient. For example, the cytokine array appears to be shown from a single experiment without any replicates, which severely limits the interpretability and robustness of the findings. Please ensure all experiments include appropriate biological replicates and clearly indicate N values in the figure legends and methods. Without proper statistical treatment and adequate replicates, the conclusions drawn are not sufficiently supported.

We thank the reviewer for this comment and have now clarified the used statistics and n numbers in the figure legends and methods.

Minor Comments

1. Thorough proofreading needed: Numerous errors were noted, including duplicated figure legends, inconsistent terminology, and formatting issues. Carefully review all text, legends, labels, and supplementary data for clarity and accuracy.

Many thanks and we have carefully revised the manuscript following the reviewer's suggestions.

2. Define fibroblast subtypes and sorting methods: Clearly describe how papillary and reticular fibroblasts were defined and isolated, including markers used for sorting.

We have expanded our description here. Please note that this information has already been previously published (Philippeos et al., 2018, JID) and we are using the fibroblast cell lines that had been established through this work.

3. Clarify cytokine source: Specify whether cytokine data are from fibroblasts, keratinocytes, or whole OT cultures; and whether measurements are from cell lysates or conditioned media.

We have now made this clearer in the revised manuscript text and figure legends.

4. Label immunofluorescence images clearly: Include scale bars, labeled structures, and matched controls. High-resolution images are needed for all key comparisons.

We have revised the manuscript to the best of our knowledge and improve the images.

5. Include WT + RepSox controls: These are essential for assessing specificity of treatment effects.

We have now added the Control skin with and without RepSox treatment in every shown experiment.

6. Specify fibroblast donor source: Clarify whether fibroblasts were derived from neonatal or adult skin, and whether multiple donors were used.

This information is included in the Supplements Table 1 (now Table EV1) and we have expanded the method section for the JEB and healthy skin tissue section samples.

7. Reorganize Results section: Consider grouping in vitro and in vivo findings separately to improve clarity.

We have significantly revised the manuscript text and figures to improve the clarity here but would like to keep our overall narrative flow.

8. Add model summary table: A table summarizing all experimental models (e.g., genotype, fibroblast subtype, treatments) would help orient readers.

We have already included a table summarizing all experimental source data and we feel that an additional table will create more confusion. Instead, we have highlighted it more clearly in the text what model system have been used for the shown experiments.

15th Jan 2026

Dear Dr. Rognoni,

Thank you for submitting your revised manuscript to EMBO Molecular Medicine, and please accept my apologies for the delay in getting back to you, which is due to the high volume of revisions and new manuscripts submitted to our office over the last month.

The manuscript has been reviewed by the three initial referees. As you will see below, while referees #1 and #3 are satisfied overall (pending minor revisions), referee #2 still raises major concerns relating mainly to the methodology employed (immunofluorescence) and the inadequacy of the immortalized keratinocyte model system.

We have discussed these points further within our editorial team and with the referees, and agreed to invite further revisions to the manuscript as follows:

- Please address the referees' concerns regarding Western blots. In this regard, Referee #1 added: "it is important to have reproducible Western blots of pSMAd2/SMAD2/Actin in shC and shLAMA3 keratinocytes. Ideally, one could also include Western blots of shC and shLAMA3 of fibroblasts, which might indirectly address referee #2's comment about expression of LAMA3 in fibroblasts- re-specificity concerns."
- If you cannot obtain JEP patient-derived cells as requested by Referee #2, please provide additional discussion on the limitations of the model and tone down the conclusions in both your rebuttal letter and the manuscript.
- Address the remaining comments from the referees.

Please note that the revised manuscript will be reviewed again, and we cannot guarantee a positive outcome at this stage.

Should you find that the requested revisions are not feasible within the outlined constraints and therefore prefer to submit your paper elsewhere, we would welcome a message to this effect.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Lise Roth

Lise Roth, Ph.D
Senior Editor
EMBO Molecular Medicine

When preparing your revised manuscript, please refer to our guidelines: <https://link.springer.com/journal/44321/submission-guidelines#cms-Revised-submissions>. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

- 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). For guidance, download the 'Figure Guide PDF': <https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>.
- 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. 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.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public.

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

8) At EMBO Press we ask authors to provide source data for the main manuscript figures. You will receive a separate email with instructions for providing source data with your revised manuscript, including how to upload and organize the files.

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.

10) 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.

- 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.

11) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting

- the medical issue you are addressing,
- the results obtained and
- their clinical impact.

This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

12) Author contributions: You will be asked to provide CRediT (Contributor Role Taxonomy) terms in the submission system. These replace a narrative author contribution section in the manuscript.

13) A Conflict of Interest statement should be provided in the main text.

14) Every published paper includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one-sentences bullet points that summarizes the paper. Please write the bullet points to summarize the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300-600 px high.

15) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during

review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The study utilized in vitro and in vivo models to characterize papillary fibroblast-derived TGF β in pathogenesis of Laminin 3-deficiency-induced JEB. The results of the study may provide guidance for TGF β as a treatment for junctional bullosa.

Referee #1 (Remarks for Author):

Figure 2E-western blot shows total SMAD expression. Please clarify if SMAD2 and pSMAD2 are interchangeable here. Why not blot for pSMAD2. Also the Actin image seems to have lower resolution than that SMAD2.

Referee #2 (Comments on Novelty/Model System for Author):

The data provided still remain limited in supporting the conclusion that the model fully represents laminin-332-deficient junctional epidermolysis bullosa (JEB). The study employs shRNA-mediated knockdown of LAMA3 in immortalised human keratinocytes derived from healthy donors. While reduced LAMA3 expression may reproduce certain features of JEB, this approach alone is unlikely to capture the full complexity of the disease, particularly aspects related to scarring. Other factors, including disease progression, inflammatory responses, and the involvement of immune cells, which are known to influence fibrotic processes, should also be considered. Laminin α 3 expression in additional relevant cell types, such as dermal fibroblasts, may also contribute and warrants attention. Furthermore, patients with laminin-332 deficiency display considerable variability in clinical presentation, including scarring, with differences in the affected laminin chain or specific gene variants likely influencing disease severity. For these reasons, validation using JEB patient-derived cells or tissues would still strengthen the relevance of the model.

The analyses still rely primarily on immunofluorescence staining, which has inherent limitations for quantitative assessment. Including complementary approaches, such as western blotting or other quantitative methods, would help strengthen the conclusions, particularly regarding signalling and fibrosis-related findings.

It is also still unclear why TGF- β does not suppress keratinocyte proliferation in the models used. Context-dependent effects and the general statement that the cells are considered untransformed are not sufficient explanations. Further exploration of this could provide important and potentially unexpected insights.

Referee #2 (Remarks for Author):

The revised manuscript incorporates additional data that further suggest a mechanism involving enhanced TGF- β -associated signalling in laminin α 3 chain-deficient keratinocytes. Phospho-SMAD2 staining in laminin β 3- and laminin γ 2-knockdown keratinocytes is now also included. Overall, however, the quantitative analyses appear more persuasive than the representative images provided. An increase in integrin α V β 6 expression is not demonstrated, and the newly added results continue to rely predominantly on immunofluorescence staining. Although a Western blot and quantification showing increased total SMAD2 protein levels in laminin α 3 chain-deficient keratinocytes are presented, phosphorylated SMAD2 is not examined. On the basis of the data shown, increased total SMAD2 levels alone are insufficient to substantiate enhanced TGF- β activity in this context.

The following comments are intended to be constructive. The principal concerns remain largely unresolved, particularly the limited evidence supporting the relevance of the experimental model to laminin-332-deficient junctional epidermolysis bullosa. It may also be important to consider that, according to previously published data from some of the authors, laminin α 3 is expressed by subpopulations of superficial dermal fibroblasts. To strengthen the conclusions, the inclusion of additional experimental approaches beyond immunostaining and its quantification would be highly valuable. In several instances, the quantified data appear clearer and more convincing than the corresponding representative images.

Furthermore, while the challenges associated with working with junctional epidermolysis bullosa-derived cells are acknowledged, this justification is difficult to reconcile with the use of an immortalised keratinocyte cell line. In principle, immortalisation of

patient-derived keratinocytes may also be achievable.

It would also be helpful to clarify whether observations regarding proliferation are consistent with previous findings in models and JEB skin, such as those reported by De Rosa et al., 2019, or whether they indicate a discrepancy that should be addressed.

Overall, this is an interesting and potentially important study. However, to improve its translational relevance, the conclusions would need to be more robustly supported within a junctional epidermolysis bullosa-relevant framework and through the application of additional experimental methodologies. These issues have not yet been fully addressed in the revised manuscript.

Referee #3 (Comments on Novelty/Model System for Author):

As stated below, this revised manuscript shows considerable improvement compared with the initial submission, with clearer organization, strengthened experimental validation, and improved integration of human, organotypic, and mouse model data. However, the mechanistic relationship between a laminin-332 mutation in basal keratinocytes and TGF β secretion by papillary fibroblasts remains unclear.

Referee #3 (Remarks for Author):

This revised manuscript shows considerable improvement compared with the initial submission, with clearer organization, strengthened experimental validation, and improved integration of human, organotypic, and mouse model data. The authors investigate the roles of distinct dermal fibroblast populations in the progression of Junctional Epidermolysis Bullosa (JEB). Using organotypic cultures incorporating WT or LAMA3-knockdown human keratinocytes together with sorted primary human papillary or reticular fibroblasts, the authors demonstrate that papillary fibroblasts-but not reticular fibroblasts-promote keratinocyte proliferation and dermal invagination in JEB models.

The study further identifies upregulation of integrin $\alpha\beta 6$ and enhanced TGF β signaling in JEB organotypic cultures, mouse models, and human patient specimens. Importantly, topical inhibition of TGF β signaling using RepSox alleviates dermal invagination in a JEB mouse model, suggesting a potential therapeutic strategy. Overall, the revised work provides strong evidence that papillary fibroblasts drive JEB-associated pathology through TGF β -dependent mechanisms.

Major Comments

None.

Minor Comments

1. Please clarify how quantification was performed in Fig. 1A,B. The NHS control image in Fig. 1A does not appear representative of the quantification shown in Fig. 1B (e.g., apparent abundance of CD39⁺ cells in images versus near-zero quantification). Please explain this discrepancy or replace with a more representative image.
2. In Fig. 1J, all control samples show an epidermal corrugation value of 1, despite visible deviations from a straight epidermal-dermal junction in representative images. Please clarify whether the data were pre-normalized to control values. If not, consider adjusting the y-axis range to better display variability.
3. In the Results section discussing basal keratinocyte responses to skin damage and TGF β signaling, please add appropriate citations.
4. Figure legends for Fig. 3F and 3G appear to be reversed.
5. Please define "RM+ growth media" in the Methods section.
6. The mechanistic relationship between laminin-332 mutation in basal keratinocytes and TGF β secretion by papillary fibroblasts remains unclear; additional discussion would strengthen the manuscript.
7. Scale bars are missing in Figures EV2C and EV6.
8. Replace "Close up" with "Zoom" and add a corresponding "Zoom" label in Fig. 1A.
9. Figure formatting: labels are off-center in Figures EV4A and EV4C. Please carefully review all figures for organization and proper alignment.
10. DAPI staining appears overly dark in some images, making labels difficult to read.
11. Please use the standard abbreviation "Krt" for keratin.

12/ Adding dashed lines to demarcate the basement membrane in tissue images (Fig. 1D-F) would improve clarity.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The study utilized *in vitro* and *in vivo* models to characterize papillary fibroblast-derived TGF β in pathogenesis of Laminin 3-deficiency-induced JEB. The results of the study may provide guidance for TGF β as a treatment for junctional bullosa.

Referee #1 (Remarks for Author):

Figure 2E-western blot shows total SMAD expression. Please clarify if SMAD2 and pSMAD2 are interchangeable here. Why not blot for pSMAD2. Also the Actin image seems to have lower resolution than that SMAD2.

Many thanks for this comment and we have now substantially revised the western blot analysis in the manuscript. In addition to the increase in total SMAD2, we can now show that pSMAD2 is significantly increased in shLAMA3 keratinocytes, further strengthening the data supporting increased TGF β signalling upon LAMA3 depletion (new Fig 2F).

We would like to apologise for the low-resolution image for the actin western blot, which was accidentally caused by the figure file PDF conversion process. The uncropped western blot images of the same blot we provided in Supplementary Figure 1 is showing that all western blot images were acquired with a comparable resolution. We have now reimported the image at the correct resolution in the revised manuscript (new Fig 2G).

Referee #2 (Comments on Novelty/Model System for Author):

The data provided still remain limited in supporting the conclusion that the model fully represents laminin-332-deficient junctional epidermolysis bullosa (JEB). The study employs shRNA-mediated knockdown of LAMA3 in immortalised human keratinocytes derived from healthy donors. While reduced LAMA3 expression may reproduce certain features of JEB, this approach alone is unlikely to capture the full complexity of the disease, particularly aspects related to scarring. Other factors, including disease progression, inflammatory responses, and the involvement of immune cells, which are known to influence fibrotic processes, should also be considered. Laminin α 3 expression in additional relevant cell types, such as dermal fibroblasts, may also contribute and warrants attention.

Many thanks for this comment. While we agree that our *in vitro* experimental model systems may not be able to recapitulate the full JEB phenotype spectrum the inducible *Lama3* knockout mouse does capture more of the disease complexity. We would like to emphasize the excellent correlation of key phenotypic observations including aberrant TGF β / α v β 6 integrin signalling, increase in papillary fibroblasts, and granulation tissue formation between our shLAMA3 2D/3D *in vitro* models, *Lama3* *in vivo* disease model, and JEB patient samples with pathogenic LAMA3 variants. We have highlighted key limitations and strengths of our experimental systems more clearly in the revised discussion and toned down the generalizability of our conclusions for all JEB subtypes.

Furthermore, patients with laminin-332 deficiency display considerable variability in clinical presentation, including scarring, with differences in the affected laminin chain or specific gene variants likely influencing disease severity. For these reasons, validation using JEB patient-derived cells or tissues would still strengthen the relevance of the model.

We agree with the reviewer's assessment on the high degree of variability in JEB. We have validated key observations including changes in dermal fibroblasts and expression of $\alpha\beta6$ integrin in three samples from neonatal JEB patients with different pathogenic variants in *LAMA3*. Unfortunately, due to the limited access to patient samples, we could not expand our tissue analysis with further JEB patient biopsies from other JEB subtypes. We are now highlighting this as a limitation of our current study in the discussion, which warrant further investigations.

The analyses still rely primarily on immunofluorescence staining, which has inherent limitations for quantitative assessment. Including complementary approaches, such as western blotting or other quantitative methods, would help strengthen the conclusions, particularly regarding signalling and fibrosis-related findings.

We would like to emphasise again that our study combines a range of methods including 2D and 3D JEB *in vitro* models, an optimized JEB *in vivo* disease model, immunofluorescence imaging, flow cytometry, ELISA, and western blotting. We have now expanded our western blot analysis to TGF β signalling and include flow cytometry and transcriptomic analysis of $\alpha\beta6$ integrin expression. Please see extended responses below.

It is also still unclear why TGF- β does not suppress keratinocyte proliferation in the models used. Context-dependent effects and the general statement that the cells are considered untransformed are not sufficient explanations. Further exploration of this could provide important and potentially unexpected insights.

As stated in our previous response, this is highly likely due to the differential dose response of keratinocytes to TGF β in agreement with previously published data highlighted by the reviewer. We have now performed a TGF β 1 dose response experiment of shC and shLAMA3 NTERT keratinocytes showing that low concentrations of TGF β 1 (1-2.5 ng/ml) TGF β significantly increase proliferation (Ki67-positive cells) in shC and shLAMA3 whereas at higher concentrations (7 ng/ml) the positive effect on proliferation is lost. The TGF β concentrations that we measured in the conditioned media from our organotypic and 2D cultures were all in the lower TGF β concentration range (1-2 ng/ml) which positively influences cell proliferation (Figure 1 for reviewer).

Figure for reviewers removed

Referee #2 (Remarks for Author):

The revised manuscript incorporates additional data that further suggest a mechanism involving enhanced TGF- β -associated signalling in laminin $\alpha 3$ chain-deficient keratinocytes. Phospho-SMAD2 staining in laminin $\beta 3$ - and laminin $\gamma 2$ -knockdown keratinocytes is now also included. Overall, however, the quantitative analyses appear more persuasive than the representative images provided. An increase in integrin $\alpha V\beta 6$ expression is not demonstrated, and the newly added results continue to rely predominantly on immunofluorescence staining. Although a Western blot and quantification showing increased total SMAD2 protein levels in laminin $\alpha 3$ chain-deficient keratinocytes are presented, phosphorylated SMAD2 is not examined. On the basis of the data shown, increased total SMAD2 levels alone are insufficient to substantiate enhanced TGF- β activity in this context.

Many thanks for these constructive comments that will help strengthening our conclusions. In addition to our $\alpha V\beta 6$ integrin immunofluorescence analysis of human patient skin, JEB (eKO) sections, and shLAMA3 cells, we have now included flow cytometry analysis of shC and shLAMA3 keratinocytes showing a strong increase in $\alpha V\beta 6$ integrin expression upon *LAMA3* depletion (new Fig 2E). In addition, reanalysis of published epidermal RNAseq data (Tayem *et al*, 2021), revealed that *Itgb6* (gene for the $\beta 6$ subunit) was the most significantly increased integrin in JEB (eKO) mouse skin compared to Control (Figure 2 for reviewer). This is now revealing with multiple methods that $\alpha V\beta 6$ integrin expression is increased after depletion of *LAMA3*.

Figure for reviewers removed

In line with the comment of Reviewer 1, we have now included pSMAD2 western blot analysis of shC and shLAMA3 keratinocytes, showing a strong increase upon LAMA3 loss that is indicative of an increase in TGF β activity (new Fig 2E).

The following comments are intended to be constructive. The principal concerns remain largely unresolved, particularly the limited evidence supporting the relevance of the experimental model to laminin-332-deficient junctional epidermolysis bullosa. It may also be important to consider that, according to previously published data from some of the authors, laminin α 3 is expressed by subpopulations of superficial dermal fibroblasts.

Many thanks for this comment and we would like to highlight again the excellent correlation we are observing between our 2D and 3D shLAMA3 keratinocyte knockdown models, the conditional JEB *Lama3* knockout in vivo model, and *LAMA3* mutant JEB patient skin biopsy stainings. We have revised the manuscript text to highlight more clearly the advantages and limitations of our used experimental model systems.

We would also like to emphasize again, that the *LAMA3* gene is not expressed by fibroblasts (Aumailley & Rousselle, 1999). Below we have now included a western blot analysis using a Lam α 3-specific antibody (mouse monoclonal anti-Laminin α 3 (clone CL3112; Novus Biologicals, NBP2-42390), used at 1:500 dilution), showing that Lam α 3 protein is not expressed in skin fibroblasts (Figure 3 for reviewer).

In the first western blot (Figure 3A for reviewer) we compared our shC NTERT keratinocytes with a mixed population of fibroblasts (Mixed Fb) cultured at medium density. Cells were either extracted with RIPA buffer after trypsinisation (first two lanes, enriching for intracellular proteins) or lysed directly on the plate with reducing Laemmli buffer (lanes 3 and 4) which will also enrich for deposited extracellular matrix proteins. Please note, while Lam α 3 protein is strongly expressed and deposited by NTERT keratinocytes, it is not detectable in skin fibroblasts in either condition. In a second western blot we compared the effect of low (LD) and high-density culture (HD) on Lam α 3 protein expression in shC and shLAMA3 NTERT keratinocytes with Lam α 3 protein expression in papillary (CD39+) fibroblasts (Figure 3B for reviewer). In this experiment, cells were extracted using RIPA buffer. In high density culture of shC NTERT keratinocytes the unprocessed (~190 kDa) form of LAMA3 (which is associated with keratinocyte migration) disappears when compared to low density culture, and only the mature form (~165 kDa, associated with hemidesmosome formation) remains expressed. The reduced amount

of processed Lam α 3 protein in HD compared to LD culture of is likely due to incorporation of Lam α 3 protein into RIPA-insoluble deposits. Thus, NTERT keratinocytes behave similar to primary keratinocytes in this respect(Hamill *et al*, 2010; Baudoin *et al*, 2005). In contrast to shC keratinocytes, in shLAMA3 keratinocytes Lam α 3 protein is barely detectable in both conditions. Importantly, Lam α 3 protein expression is also not detectable in cultured CD39+ fibroblasts.

Figure for reviewers removed

To strengthen the conclusions, the inclusion of additional experimental approaches beyond immunostaining and its quantification would be highly valuable. In several instances, the quantified data appear clearer and more convincing than the corresponding representative images.

We have now expanded our western blot analysis for pSMAD2 of our 2D JEB in vitro model (shC and shLAMA3 cells), revealing a prominent increase in TGF β signalling after *LAMA3* depletion. The in vivo JEB disease model phenotype has already been extensively characterised by us and others using multiple methods (Western blot analysis, histology, flow cytometry and RNAseq analysis) (Pesch *et al*, 2017; Jones *et al*, 2025; Tayem *et al*, 2021). We have also added flow cytometry analysis (new Fig 2E)

and reanalysed existing RNAseq data (Figure 2 for reviewer) to confirm an increase in $\alpha\text{v}\beta 6$ integrin expression with other independent methods. We have carefully checked our representative images and are making all the source data and quantification available, according to the author checklist guidelines.

Furthermore, while the challenges associated with working with junctional epidermolysis bullosa-derived cells are acknowledged, this justification is difficult to reconcile with the use of an immortalised keratinocyte cell line. In principle, immortalisation of patient-derived keratinocytes may also be achievable.

Many thanks for this comment. Due to the rarity of this disease, we were unable to obtain primary keratinocytes from JEB patients with *LAMA3* mutations. As mentioned above we would like to emphasise again the good correlation between our 2D&3D *in vitro* JEB models, murine JEB *in vivo* disease model, and JEB patient biopsies with pathogenic genetic variants in the *LAMA3* gene. We have now toned down our conclusions of our work regarding JEB pathogenesis and added additional discussion to highlight limitations and advantages of our experimental systems.

It would also be helpful to clarify whether observations regarding proliferation are consistent with previous findings in models and JEB skin, such as those reported by De Rosa et al., 2019, or whether they indicate a discrepancy that should be addressed.

Many thanks for raising this important point. JEB severe caused by *LAMA3* variants is rare and thus understudied. Our work was therefore aimed to fill this knowledge gap. Our data show that depletion of *LAMA3* in keratinocytes *in vitro* and *in vivo* leads to an increase in epidermal cell proliferation and aberrant $\text{TGF}\beta/\alpha\text{v}\beta 6$ integrin signalling, which can be suppressed by $\text{TGF}\beta$ inhibition with RepSox. Our recent work is revealing considerable heterogeneity between JEB patients with pathogenic variants in different Laminin chains. We have included a PCA of bulk RNAseq analysis of EB patient biopsies completed as part of an ongoing study to be published later this year (Figure 4 for reviewers). The PCA clustering shows that JEB patients form two distinct clusters. Patients with variants in *LAMA3* cluster separately to patients with variants in *LAMC2* and patients with variants in *LAMB3* are split between clusters. This may explain some of the patient variability shown in De Rosa et al., 2019 and explains the difference in proliferation seen between this study and our own. While our work has focused on investigating the loss of *LAMA3*, the De Rosa work has focused on JEB patients with genetic variants in *LAMB3* and the isolated patient cells showed a high degree of variability concerning loss of stemness and proliferative capability. Preliminary data using our isogenic NTERT-based JEB models appears to indicate that, consistent with the findings from De Rosa et al., knockdown of *LAMB3* reduces clonal growth, while that of *LAMA3* does not (Figure 5 for reviewer). Thus, besides possible patient to patient variability, we now speculate in the revised discussion that the observed differences to previously reported findings are primarily due to the pathogenic variant in different laminin chains, and this is something that will warrant further investigations in the future.

Figures for reviewers removed

Overall, this is an interesting and potentially important study. However, to improve its translational relevance, the conclusions would need to be more robustly supported within a junctional epidermolysis bullosa-relevant framework and through the application of additional experimental methodologies. These issues have not yet been fully addressed in the revised manuscript.

Many thanks for highlighting the potential importance of our study and we hope that our revisions have strengthened our conclusions.

Referee #3 (Comments on Novelty/Model System for Author):

As stated below, this revised manuscript shows considerable improvement compared with the initial submission, with clearer organization, strengthened experimental validation, and improved integration of human, organotypic, and mouse model data. However, the mechanistic relationship between a laminin-332 mutation in basal keratinocytes and TGF β secretion by papillary fibroblasts remains unclear.

We would like to thank the reviewer for the positive assessment of our revised manuscript. While we agree that a direct link between a laminin-332 mutation in basal keratinocytes and TGF β secretion by papillary fibroblasts remains unclear, our data point to a mechanism where loss of laminin-332 leads to aberrant TGF β signalling in epidermal keratinocytes due to an increase in α v β 6 integrin expression which is induced and further enhanced by TGF β . While α v β 6 integrin is likely to be increased by a loss of cell adhesion in keratinocytes as part of the wound healing response, its expression is also prominently increased by TGF β which is released by α v class integrins from the ECM. In addition to this autoregulatory feedback loop, we show that papillary fibroblasts are able to secrete high levels of TGF β and promote aberrant TGF β / α v β 6 integrin signalling in laminin-332 depleted keratinocytes. Because laminin-332 is not expressed in skin fibroblasts (see Figure 3 for reviewers), it is highly unlikely that variants in laminin 332 directly influence intrinsic functions of papillary fibroblasts. We have made this clearer in the revised manuscript discussion.

Referee #3 (Remarks for Author):

This revised manuscript shows considerable improvement compared with the initial submission, with clearer organization, strengthened experimental validation, and improved integration of human, organotypic, and mouse model data. The authors investigate the roles of distinct dermal fibroblast populations in the progression of Junctional Epidermolysis Bullosa (JEB). Using organotypic cultures incorporating WT or LAMA3-knockdown human keratinocytes together with sorted primary human papillary or reticular fibroblasts, the authors demonstrate that papillary fibroblasts-but not reticular fibroblasts-promote keratinocyte proliferation and dermal invagination in JEB models.

The study further identifies upregulation of integrin α v β 6 and enhanced TGF β signaling in JEB organotypic cultures, mouse models, and human patient specimens. Importantly, topical inhibition of TGF β signaling using RepSox alleviates dermal invagination in a JEB mouse model, suggesting a potential therapeutic strategy. Overall, the revised work provides strong evidence that papillary fibroblasts drive JEB-associated pathology through TGF β -dependent mechanisms.

Major Comments

None.

Minor Comments

1. Please clarify how quantification was performed in Fig. 1A,B. The NHS control image in Fig. 1A does not appear representative of the quantification shown in Fig. 1B (e.g., apparent abundance of CD39⁺ cells in images versus near-zero quantification). Please explain this discrepancy or replace with a more representative image.

Many thanks for this comment and we have revised the HALO quantification in Fig 1B which was set at a too low signal threshold.

2. In Fig. 1J, all control samples show an epidermal corrugation value of 1, despite visible deviations from a straight epidermal-dermal junction in representative images. Please clarify whether the data were pre-normalized to control values. If not, consider adjusting the y-axis range to better display variability.

Many thanks for this suggestion and we have now removed the pre-normalization to control values to also show the corrugation changes in control samples. This has been revised in Fig 1J, Fig 4H, Fig EV1G, Fig EV 2G and Fig EV3G.

3. In the Results section discussing basal keratinocyte responses to skin damage and TGF β signaling, please add appropriate citations.

These have now been added in the result section. Please see Yu et al, 2024; Häkkinen et al, 2004a and Watt, 2002 to underline the role of cell adhesion receptors in basal keratinocytes during wound repair and skin homeostasis.

4. Figure legends for Fig. 3F and 3G appear to be reversed.

Many thanks, and this has now been corrected.

5. Please define "RM+ growth media" in the Methods section.

We have now specified the composition of the RM+ growth media in the method section.

6. The mechanistic relationship between laminin-332 mutation in basal keratinocytes and TGF β secretion by papillary fibroblasts remains unclear; additional discussion would strengthen the manuscript.

We have now expanded the discussion with the following section: *"By establishing a novel JEB-like OT disease model with distinct fibroblast subpopulations from different body sites, we uncovered a key role of papillary fibroblasts in driving JEB pathogenesis and granulation tissue formation through increasing integrin α v β 6 expression and TGF β signalling. While depletion of LAMA3 is sufficient to induce increased α v β 6 integrin expression and SMAD signalling in our 2D JEB models, it remains currently unclear if there is also a direct mechanistic link between laminin-332 loss of function and increased TGF β secretion in papillary fibroblasts. Because laminin-332 is not expressed in fibroblasts (Aumailley & Rousselle, 1999), our data support a model where in response to skin damage papillary fibroblasts that intrinsically secrete higher levels of TGF β 1 accumulate in the upper dermis of JEB skin, especially around blistered skin areas and promote increased α v β 6 integrin expression and TGF β signalling activation in basal JEB keratinocytes, thereby enhancing a profibrotic and inflammatory environment. Inhibition of TGF β signalling activity in JEB keratinocytes and fibroblasts with topical RepSox treatment was able to significantly improve the JEB skin phenotype and enhance skin regeneration."*

7. Scale bars are missing in Figures EV2C and EV6.

Many thanks for flagging this up and these have now been added.

8. Replace "Close up" with "Zoom" and add a corresponding "Zoom" label in Fig. 1A.

This has been done now.

9. Figure formatting: labels are off-center in Figures EV4A and EV4C. Please carefully review all figures for organization and proper alignment.

Many thanks and we have revised the indicated figure panels.

10. DAPI staining appears overly dark in some images, making labels difficult to read.

We have tried to improve this where possible.

11. Please use the standard abbreviation "Krt" for keratin.

We have used "K" for keratin protein abbreviation and "KRT" for keratin gene specification throughout the manuscript, following the current keratin nomenclature convention (Schweizer et al., J Cell Biol. 2006 PMID: 16831889).

12/ Adding dashed lines to demarcate the basement membrane in tissue images (Fig. 1D-F) would improve clarity.

These have been added in the revised figures.

19th May 2026

Dear Dr. Rognoni,

Thank you for submitting your revised study. Referee #1 has evaluated your revised manuscript in response to the referees' concerns and is satisfied with the revisions. We will therefore be able to accept your manuscript once the following minor editorial matters are addressed:

1/ Manuscript text:

- Please remove the red font text and only keep in track changes mode any new modification.
- Please correct the order and headings of the sections in the manuscript text to: Abstract / The Paper Explained / Introduction / Results / Discussion / Methods / Data Availability / Acknowledgements / Disclosure and Competing Interests Statement / References / Figure Legends / Expanded View Figure Legends
- Methods:
 - o Human samples: please include the full statement confirming 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. If collected and within the bounds of privacy constraints, report on age, sex and gender or ethnicity for all study participants.
 - o Cell lines: indicate whether the cells were authenticated.
 - o Statistics: include a statement about sample size and exclusion/inclusion criteria
 - Data Availability: please remove the current text and indicate "this study includes no data that requires deposition in a public database". If your source data are deposited in a public repository, please provide a direct URL in this section (and correct the checklist accordingly).
 - Author contributions: Remove this section from the manuscript, and provide CRediT (Contributor Role Taxonomy) terms in the submission system instead.
 - "Conflict of interest statement" should be renamed "Disclosure and competing interest statement". Fiona Watt should be mentioned as editorial board member.

2/ Figures:

- Please upload main and EV figures as separate, high resolution figure files.
- Appendix: please add a table of contents with page numbers.
- Please address the queries from our data editors in the figure legends:
 1. Please note that the legend for figure EV1 is not provided in the sequential manner. This needs to be rectified.
 2. Please note that the exact p values are not provided in the legends of figures 1B, H, I, J; 2D, F, G, I, K; 3B, D, E, G, I, K; 4B, D, F, H; 5B, D, F; 6E-G; 7A-G; EV1 E-G; EV2 E-G; EV3 B, D, F, G; EV4 B, D; EV5
 3. Please note that information related to n is missing in the legends of figures 1B, H, I, J; 2B, D, F, G, K; 3B, D, E, F, G, K; 4B, D, F, H; 6E-G; 7A-G; 8A-H; EV1 E-G; EV2 E-G; EV3 B, D, F, G; EV4 B, D; EV5
 4. Please note that the error bars are not defined in the legend of figure EV5.
 5. Please note that the white arrow heads are not defined in the legend of figure 2C. This needs to be rectified.

3/ Source data:

Please upload the Source Data as 1 zip file per figure and provide the link to the deposited data.

4/ I introduced minor edits in The paper explained. Please let me know if you agree with the changes or amend as you see fit:
Problem:

Junctional epidermolysis bullosa (JEB) is a rare inherited skin disease caused by defects in basement membrane proteins that anchor the epidermis to the dermis. Patients suffer from extreme skin fragility, chronic wounds and excessive granulation tissue, which severely impair skin healing and quality of life. While JEB is initiated by defects in epidermal cells, the role of fibroblasts - the supporting cells in the dermis - in disease progression remains unclear, limiting the development of effective therapies.

Results:

Using patient skin samples, laboratory grown human skin and an inducible mouse model of JEB, we show that a specific dermal fibroblast subpopulation, the papillary fibroblasts, is strongly expanded in blistered JEB skin. These fibroblasts secrete elevated levels of the signalling molecule TGF β , which drives pathological activation of skin repair pathways, including the α v β 6 integrin-TGF β signalling axis in keratinocytes. This aberrant epithelial-mesenchymal communication promotes skin blistering and excessive granulation tissue formation in JEB. Importantly, pharmacological inhibition of TGF β signalling with the small-molecule inhibitor RepSox normalized abnormal keratinocyte behaviour in human JEB skin models in vitro and markedly improved disease severity in mice in vivo. Treated mice showed reduced granulation tissue, diminished inflammation, fewer and smaller skin blisters, and overall improved skin integrity, despite the continued presence of the underlying genetic defect.

Clinical impact:

Our findings identify papillary fibroblasts as active drivers of JEB pathology and demonstrate that targeting pathological fibroblast-keratinocyte signalling can improve skin health. This work highlights TGF β and α v β 6 integrin signalling as promising therapeutic targets for JEB and potentially other blistering disorders, offering a strategy to complement emerging genetic therapies by improving wound healing and tissue resilience in affected patients.

5/ Synopsis:

I introduced minor edits to your text, please let me know if you agree with the following or amend as you see fit:

"The role of papillary fibroblasts in the progression of the inherited blistering disorder Junctional epidermolysis bullosa (JEB) is investigated, together with the potential of targeting the underlying signalling crosstalk to improve skin health and regeneration.

- Papillary (CD39+) fibroblasts accumulate in JEB granulation tissue of blistered skin and promote JEB pathogenesis.
- Loss of LAMA3 in keratinocytes leads to increased $\alpha\beta6$ integrin expression and TGF β / SMAD signalling.
- Papillary fibroblasts secrete high level of TGF β , promoting increased integrin $\alpha\beta6$ /TGF β signalling in JEB keratinocytes.
- Preclinical testing of TGF β inhibition with RepSox reduces granulation tissue formation and improves JEB symptoms in Lama3 JEB in vivo disease model."

Please resize your visual abstract to a jpeg/tiff/png file 550 px wide x 300 px high, and make sure that the text remains legible.

6/ As part of the EMBO Publications transparent editorial process initiative (see our Editorial at <http://embomolmed.embopress.org/content/2/9/329>), EMBO Molecular Medicine will publish online a Review Process File (RPF) to accompany accepted manuscripts.

This file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. Let us know whether you agree with the publication of the RPF and as here, if you want to remove or not any figures from it prior to publication.

Please note that the Authors checklist will be published at the end of the RPF.

I look forward to receiving your revised manuscript.

Yours sincerely,

Lise Roth

Lise Roth, PhD
Senior Editor
EMBO Molecular Medicine

To submit your manuscript, please follow this link:
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***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The study used reconstructed 3-D skin models to demonstrate the TGF β /Smad signaling pathway as a dominant driver of JEB caused by Laminin 3-deficiency. The results are validated at varying levels with both mouse model JEB and human patient skin samples. Given the rarity of the disease, validation with patient-derived keratinocytes for in vitro skin model could be challenging but is recommended for follow-up studies. Overall, the findings provide basis for potentially applying TGF β receptor inhibitor (RepSox) as novel treatment for a subpopulation of JEB patients.

Referee #1 (Remarks for Author):

Revision has improved the quality of the paper.

The authors addressed the remaining editorial issues.

12th Jun 2026

Dear Dr. Rognoni,

Thank you for submitting your revised files. We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

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If you have any questions, please do not hesitate to contact the Editorial Office.
Thank you for your contribution to EMBO Molecular Medicine!

With kind regards,

Lise Roth

Lise Roth, Ph.D
Senior Editor
EMBO Molecular Medicine

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This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](https://doi.org/10.31222/osf.io/9sm4x)). Please follow the journal's guidelines in preparing your

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Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if $n < 5$, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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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 χ^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.

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Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Data Availability Section
Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For antibodies provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and/or clone number - Non-commercial: RRID or citation	Yes	Materials and Methods and Table EV2
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Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	
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Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Materials and Methods and Table EV1
Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Material and Method section includes statement on authentication and mycoplasma testing
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Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
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Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Information added under acknowledgments.

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been pre-registered , provide DOI in the manuscript. For clinical trials, provide the trial registration number OR cite DOI.	Not Applicable	
Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	
Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if external detailed step-by-step protocols are available.	Not Applicable	
Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about sample size estimate even if no statistical methods were used.	Yes	Material and Methods
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, have they been described?	Yes	Material and Methods
Include a statement about blinding even if no blinding was done.	Yes	Material and Methods
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Yes	JEB eKO mice skin severity inclusion/ exclusion criteria are described under Material and Methods.
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.		
For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Information is included in the Figure legends including the exact p values and under Material and Methods
Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was replicated in laboratory .	Yes	Included in the figures and figure legends
In the figure legends: define whether data describe technical or biological replicates .	Yes	Included in the figures and figure legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving human participants : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	Yes	Study approval under Material and Methods
Studies involving human participants : 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.	Yes	Study approval under Material and Methods
Studies involving human participants : For publication of patient photos , include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental animals : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Study approval under Material and Methods
Studies involving specimen and field samples : State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	
Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of select agents and toxins (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the authority granting approval and reference number for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., ICMJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.	Yes	Material and Methods
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.	Not Applicable	
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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have primary datasets been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Not Applicable	
Were human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Not Applicable	