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The long non-coding RNA LINC00941 and SPRR5 are novel regulators of human epidermal homeostasis

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Review timeline:

Submission date:	19 June 2018
Editorial Decision:	26 July 2018
Revision received:	24 October 2018
Editorial Decision:	20 November 2018
Revision received:	29 November 2018
Accepted:	4 December 2018

Editor: Esther Schnapp

Transaction Report:

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

1st Editorial Decision 26 July 2018

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. We have now received the full set of referee reports as well as referee cross-comments, pasted below.

As you will see, the referees agree that the findings are potentially interesting. However, they also think that the study needs to be strengthened, and they make several suggestions for how this can be done. I think that all suggestions are sensible and good - several also overlap - and all should therefore be addressed (with the exception of point 5 of referee 3, which is not a strict requirement). Importantly, as referees 1 and 3 note, the co-depletion of both lnc00941 and SPRR5 and the effect on keratinocyte differentiation should be done, and RNA-seq data and statistics must be provided and technical from biological replicates distinguished.

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

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss this further. You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods must be included in the main manuscript file.

Supplementary figures, tables and movies can be provided as Expanded View (EV) files, and we can offer a maximum of 5 EV figures per manuscript. EV figures are embedded in the main manuscript text and expand when clicked in the html version. Additional supplementary figures will need to be included in an Appendix file. Tables can either be provided as regular tables, as EV tables or as Datasets. Please see our guide to authors for more information.

Regarding data quantification, please specify the number "n" for how many independent experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

We strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where in the manuscript the requested information can be found. The completed author checklist will also be part of the RPF (see below).
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution. In order to avoid delays later in the process, please read our figure guidelines before preparing your manuscript figures at: http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf

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

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

REFEREE REPORTS

Referee #1:

The paper by Ziegler and colleagues describe the role of lncRNA linc00941 in regulation of skin homeostasis. This lncRNA act as a repressor of SPRR5 gene, whose function is to positively regulate keratinocyte differentiation.
The authors identified potential regulatory network between linc00941 and SPRR5 gene and suggest that this network is important for epidermal homeostasis. By depleting linc00941, several differentiation genes were upregulated. In contrast, depletion of SPRR5 leads to downregulation of early and late differentiation genes. Using RNA-seq the authors identified "hot spots" of linc00941,

with SPRR5 being a top candidate. The authors provide interesting observations how linc00941 regulate SPRR5, but they did not explore this in details. If SPRR5 acts downstream of linc00941, identifying regulatory network specific for SPRR5 and linc00941 will bring the novelty to this manuscript.
The manuscript is well written, experiments are shown in a convincing manner. Overall, the manuscript could be strengthened if the authors focus on the mechanism how lncRNA regulates SPRR5. This will provide new insights how linc00941 contribute to tissue homeostasis and how is this different from other lncRNAs involved in epidermal homeostasis (eg. ANCR, TINCR).

Major comments:
1) It is necessary to show the genomic locus of linc00941 using different well-curated databases (eg. GENCODE) including its chromatin landscape (Fantom TSS, enhancer marks, poly A, transcription). Characterisation of linc00941 in skin should be expanded with RNA FISH (see my comment No 4). Is linc00941 conserved?
2) What is the expression of Linc00941 in different tissues? Is it skin specific? The authors could use GTEX data. Where is linc00941 localized in organotypic epidermis?
3) No statistical significance in any of the figures. Two biological replicates for all the figures are not acceptable.
4) Where is Linc00941 localized in keratinocytes? Is it cytoplasmic or nuclear lncRNA? Does its localization change during keratinocyte differentiation? That is an important experiment in the term of lncRNA possible function. Linc00941 is present on chr 12 while SPRR5 is on chr 1. Do the authors think that this is trans function of linc00941?
5) How does linc00941 inhibits SPRR5? Transcriptionally or post-transcriptionally? Knowing cellular localization, the authors could speculate about is functional role in regulation of SPRR5. This should be discussed.
6) If linc00941 and SPRR5 are acting in the same pathway, what happens if you co-deplete linc00941 and SPRR5? Can you rescue for example KRT1 levels and the phenotype?
7) How many genes (FIG 5G,H, 69 genes) are specific for linc00941 and SPRR5? Can the authors identify specific set of genes that are dependent on linc00941 and SPRR5?
8) DISCUSSION: more emphasis should be given to the potential regulatory network between lin00941 and SPRR5. What is the "common mode of action" of these two molecules in regulation of skin homeostasis? How is linc00941 different from ANCR or TINCR?
9) any evidence of inverse regulation of linc00941 and SPRR5 in skin diseases?

I suggest major revision if the authors can address my comments.

Referee #2:

In this very well-done and rigorous study, Ziegler et al show that the lncRNA LINC00941 is a crucial regulator of human epidermal homeostasis. This lncRNA is enriched in progenitor keratinocytes and loss of function experiments indicate that it represses keratinocyte differentiation. Furthermore, LINC00941 represses SPRR5, a previously uncharacterized molecule, which functions as an essential positive regulator of keratinocyte differentiation. Interestingly, almost half of the genes repressed in SPRR5 deficient epidermal tissue are upregulated upon LINC00941 knockdown suggesting a common mode of action for these molecules. Overall, this is a very elegant study and only minor revision is required before acceptance.

1. Page numbers in the text are missing. This makes it hard for me to comment on the text. Nevertheless, the first page of introduction "many lncRNAs" could be changed to "most lncRNAs".
2. In the first page of results, it would be good to know the following. (a) which human tissues express LINC00941. The authors can use the tool GTEXPortal (https://www.gtexportal.org/home/) to address this question. This tool has RNAseq data from more than 50 normal human tissues and is very reliable. The graph can be shown in Supplemental material. In addition, please use this tool for SPRR5 and show the data in Supplemental Figure. (b) change calcium induced to calcium-induced. (c) is LINC00941 is localized in the nucleus or cytoplasm or both compartments? qRT-PCR from nuclear and cytoplasmic fractions could be used to answer this question. (d) What is the copy number of LINC00941 per cell? This can be addressed using a synthetic LINC00941 RNA as standard.
3. It would be good to move the data from Fig. EV2B to main Figure.

4. Why are there no error bars in the control (black) bar in Figure 3B and 3C. It is important to show the error bars in the control because it gives a sense of how noisy the control is. Also, please show p-values for all graphs where the experiment was done at least 3 times.
5. Figure 3D: Change Y-axis label to "Fold change relative to undiff. KCs

Referee #3:

The manuscript by Ziegler et al describes a role for the long non-coding RNA LINC00941 in human epidermal differentiation. Knockdown of this lnc RNA results in increased expression of terminal differentiation genes in cultured keratinocytes and organotypic cultures. RNAseq further identifies a previously uncharacterized SPRR gene, SPRR5 as one target of LINC00941. The authors find that SPRR5 is controlled by p63 and promotes expression of terminal differentiation genes.

This is an interesting manuscript that sheds light on the poorly understood functions and mechanisms of lncRNAs in cell physiology. The manuscript is well written and clear and the experiments of good quality.

The mean weakness is that no mechanism of how LINC00941 would regulate keratinocyte differentiation is provided. It is also not clear if SPRR5 is a key target or just one of the many effectors of LINC00941. In addition, it is not clear how p63 fits into the picture as this is a key stem/progenitor gene. Thus it is hard to understand how terminal differentiation of SPRR5 would work if it its expression is positively controlled by the key transcription that maintains stemness. The manuscript would greatly benefit from some clarifications of these aspects.

Specific points

1. The authors claim that LINC00941 prevents "premature differentiation" of progenitors. However, the organotypic cultures of control and LINC00941 siRNA cells look strikingly similar in their thickness and gross architecture. If the progenitors would prematurely differentiate, one would assume that this would negatively affect their ability to self-renew and proliferate, and thereby affect growth and thickness of the organotypic culture. Additional assays for stem cell function, such as colony formation assays are required to support this claim. To this end also p63 staining should be shown and quantified in the organotypic cultures.
2. To demonstrate that LINC00941 acts through SPRR5, the authors should assess if SPRR5 overexpression rescues the differentiation phenotype of LINC00941-depleted cells.
3. Assessing the RNAseq data is impossible as no raw data or gene lists with appropriate statistics and quantifications have been included. These must be included.
4. From the figure legends and methods it is not clear whether the term "replicate" refers to biological replicates/independent experiments or technical replicates. It is also not clear why sometimes only two "replicates" are shown, as this raises questions of the robustness of the effects. Also statistical analysis has been omitted throughout
5. Would be interesting to show what are the main known and predicted differences between SPRR5 and the other SPRR proteins

1st Revision - authors' response 24 October 2018

Reviewer #1:

We thank the reviewer for thoughtful and constructive comments on the article, which have greatly helped in improving our work.

1) Genomic locus of LINC00941 and Characterization of LINC00941 in skin:
Based on the suggestion of reviewer 1, we included further information about the LINC00941 genomic locus, its chromatin landscape in human keratinocytes, conservation, positions of transcription start- and poly A sites, DNAseI hypersensitive sites and transcription factor occupancy data, which can be found in the expanded view file EV1B of the revised manuscript.

We agree with the reviewer, that RNA FISH would be an excellent method to visualize LINC00941 localization in epidermal tissue. We performed single molecule FISH in tissue with fluorochrome-tagged, tiling oligomers specific to LINC00941, but we were not able to convincingly detect signals of sufficient strength above background with the set of probes targeting LINC00941.
Likely, this is due to the high background staining we observe solely with all FISH methods used in epidermis/skin tissue sections, combined with low levels of expression of LINC00941 in keratinocytes (on average approx. 59 ± 14 copies per cell). To circumvent this technical problem, we measured LINC00941 RNA levels throughout different stages of organotypic epidermis regeneration by qRT-PCR analysis and – as expected- found the highest abundance of LINC00941 on day zero. This data is also included in EV1E.

2) Expression of LINC00941 in different tissues vs. skin:
According to the reviewer's suggestion, we now included data about the expression patterns of LINC00941 in different human tissues, according to the GTEX data, in expanded view file EV1A.

3) Statistical significance and number of biological replicates:
We completely agree with the reviewer that statistical tests were missing in several figures and apologize for the oversight. We now included additional statistics, where applicable, and updated the material and methods sections with details on the performed tests. We apologize for having shown only two biological replicates in subfigures 1A-C and 3B in the previous version of the article. We repeated the corresponding experiments with more replicates and updated the respective subfigures.

4) Localization of LINC00941 in keratinocytes and function *in cis* or *in trans*:
In order to address this question, we performed cellular fractionation experiments with human primary keratinocytes and found LINC00941 to be present in both, cytoplasmic and nuclear compartments, with an increased nuclear enrichment compared to mRNAs. This data is now included in expanded figure EV1D. As suggested, we also performed the experiment with keratinocytes at different stages of differentiation and saw similar preliminary results. Nevertheless, due to the inherent property of differentiated keratinocytes as part of the permeability barrier of the epidermis to become strongly adhered and cross-linked together, the fractionation procedure is much less efficient and high levels of carry-over between fractions is unavoidable. We therefore decided to not include subcellular fractionation data of differentiated keratinocytes into this article.
Based on the insightful suggestion of reviewer 1 and the consequent finding that LINC00941 shows increased nuclear enrichment compared to mRNAs, we hypothesize that LINC00941 might act *in trans*, possibly repressing premature activation of gene clusters in the epidermal differentiation complex through recruitment or modulation in activity of gene regulatory machineries. We included this aspect in the revised manuscript (page 12, paragraph 1).

5) Discussion of LINC00941-mediated regulation of SPRR5:
In light of the finding that LINC00941 shows increased nuclear enrichment compared to mRNAs and appears to repress premature activation of LCE- and SPRR- gene clusters, we hypothesize that SPRR5 (which is located directly upstream of the previously known SPRR gene cluster) might be regulated through epigenetic gene regulatory processes or modulation of enhancer activity in respective loci of the epidermal differentiation complex. Nevertheless, we cannot rule out a potential cytoplasmic mode of action of LINC00941 since the fractionation experiments showed significant abundance of the lncRNA in cytoplasmic fractions. As suggested by reviewer 1, these thoughts are now included in the revised version of the manuscript (page 12, paragraph 1).

6) Co-depletion of LINC00941 and SPRR5:
The reviewer poses an important question about the potential interrelationship of LINC00941 and SPRR5 and suggested co-depletion of both molecules to get more detailed information. Correspondingly, we generated LINC00941-/SPRR5- double-depleted human primary keratinocytes and compared abundance of Keratin1 and Filaggrin (all inversely regulated in LINC00941- or SPRR5- single depleted cells). Interestingly, we found that double depletion of both, LINC00941 and SPRR5, led to reduced levels of Keratin1and Filaggrin, resembling the phenotype seen with SPRR5 knockdown. These data suggest that LINC00941-mediated repression of differentiation might indeed, at least in part, be mediated by repression of SPRR5 Additionally, based on these findings SPRR5 appears to be a dominant regulator of differentiation and its abundance might need

to be tightly regulated in early differentiation. This data is included in expanded view file EV5A-B of the revised manuscript.

7) Identification of gene sets dependent on LINC00941 and SPRR5:
We apologize for the lack of clarity concerning the reported gene numbers in the previous version of the manuscript text. We now included the number of total genes regulated by depletion of LINC00941 (223) and the amount of genes solely regulated in SPRR5-deficient organotypic epidermis (126). Additionally, we included the absolute number of genes that are conversely regulated by LINC00941 and SPRR5 (69). To address the question concerning a specific set of genes that is dependent on LINC00941 and SPRR5, we observed that our identified regulators of epidermal tissue homeostasis conversely regulated many LCE genes. We included this observation in the revised version of the manuscript (page 10, paragraph 2).

8) Discussion of the potential regulatory network between LINC00941 and SPRR5:
As requested by reviewer 1, we now discuss a potential common mode of action for LINC00941 and SPRR5 in regulation of epidermal homeostasis. Additionally, we discuss potential differences and similarities between LINC00941 and ANCR as well as TINCR in the revised version of this article (page 12, paragraph 1).

9) Evidence of inverse regulation of LINC00941 and SPRR5 in skin diseases:
To address this interesting point raised by the reviewer, we analyzed publicly available RNA sequencing datasets for basal cell carcinoma, squamous cell carcinoma and psoriasis specimens. Interestingly, for all these skin diseases we found LINC00941 and SPRR5 to be inversely regulated. We described this observation in the revised manuscript (page 11, paragraph 2) and updated the methods section with details of this analysis.

Reviewer #2:

We thank the reviewer for helpful and positive comments, which we believe have significantly improved the paper.

1) Missing page numbers and change of wording:
We apologize for this oversight. We included page numbers in the revised version of the manuscript and changed “many lncRNAs” to “most lncRNAs” in the introduction.

2a) LINC00941 expression pattern in human tissues:
For LINC00941, we included the expression data in human tissues from GTEX in expanded view file EV1A. Unfortunately, for SPRR5 (and for its previous gene identifier RP1-20N18.10), which was only recently annotated, no such data was available in GTEX at the time of this revision.

2b) Changing “calcium induced” to “calcium-induced”:
Thank you for pointing this out. We have corrected the spelling mistake accordingly.

2c) Localization of LINC00941 in keratinocytes:
In order to address this question, we performed cellular fractionation experiments with human primary keratinocytes and found LINC00941 to be present in both, cytoplasmic and nuclear compartments, with an increased nuclear enrichment compared to mRNAs. This data is now included in expanded view file EV1D.

2d) Copy number per cell of LINC00941:
Based on the reviewer’s valuable suggestion, we measured the copy number of LINC00941 and found on average 59 ± 14 copies per non-differentiated, primary human keratinocyte. We included this data as well as a description of the method in the revised version of this article (page 5, paragraph 1).

3) Rearrangement of data from Fig EV2B to into main figure:
As suggested by reviewer 2, we moved expanded view file EV2B to figure 3C in the revised article.

4) Statistical tests and error bars:

We completely agree with the reviewer 2 that statistical tests as well as error bars for control samples were missing and apologize for the oversight. We now included p-values for respective figures and updated the material and methods sections with details of the performed tests. Additionally, we added error bars for the control samples in order to provide a sense for the variation within our biological replicates. Furthermore, we apologize for having shown only two biological replicates in subfigures 1A-C and 3B in the previous version of the article. We repeated the corresponding experiments with more replicates and updated the respective subfigures.

5) Change of Y-axis label in figure 3D:
As suggested by the reviewer, we changed the label on the Y-axis of figure 3D (Fig. 3E in the revised version of this manuscript) accordingly.

Reviewer #3:

We thank the reviewer for very helpful and constructive comments on our article, which have greatly helped us enhance our manuscript.

1) Assessing LINC00941 function on progenitor status of basal keratinocytes:
We apologize for the lack of clarity concerning our postulated function of LINC00941. We did not want to convey the impression that LINC00941 acts as a stemness factor regulating the progenitor status of keratinocytes.
Instead, we believe our data indicates that LINC00941 inhibits premature differentiation in weakly differentiated strata of the human epidermis. To further clarify our message, we carefully went through the text of the revised manuscript and changed respective wording accordingly.
Additionally, we more clearly discussed a possible role of LINC00941 in repression of weakly differentiated keratinocytes and potential differences to ANCR, a lncRNA involved in regulating progenitor maintenance of keratinocytes (page 12, paragraph 1).

2) Co-regulation of LINC00941 and SPRR5:
The reviewer poses an important question about the potential interrelationship of LINC00941 and SPRR5 and suggested forced expression of SPRR5 in combination with LINC00941-depletion to address this point. We completely agree with the reviewer that further experimentation is needed to clarify the mechanism. However, since depletion of LINC00941 led to increase of SPRR5 abundance, additional ectopic expression of SPRR5 might yield a similar phenotype as LINC00941 knockdown alone. Therefore, to address this question, we chose a different strategy to test interrelationship of LINC00941 and SPRR5, which is the double-depletion of both molecules – also suggested by reviewer 1.
For this purpose, we generated LINC00941-/SPRR5- double-depleted human primary keratinocytes and compared abundance of Keratin1 and Filaggrin (all inversely regulated in LINC00941- or SPRR5- single depleted cells). Interestingly, we found that double depletion of both, LINC00941 and SPRR5, led to reduced levels of Keratin1 and Filaggrin, resembling the phenotype seen with SPRR5 knockdown. These data suggest that LINC00941-mediated repression of differentiation might indeed be mediated by repression of SPRR5. Additionally, based on these findings SPRR5 appears to be a dominant regulator of differentiation and its abundance might need to be tightly regulated in early differentiation. This data is included in expanded view file EV5A-B of the revised manuscript.

3) RNA sequencing data:
We completely agree with reviewer 3 that submitting the raw RNA sequencing data as well as gene lists of differentially expressed genes is not negotiable and apologize for the oversight. We added gene lists with differentially expressed genes upon SPRR5 and LINC00941 depletion as supplementary data section to the revised version of this manuscript. Additionally, we submitted the RNA sequencing data generated by us to GEO (GSE118077) and will make this dataset publically available after our manuscript will be published. Until then, you can access the data via this reviewer token: uvyfgikodbqhlad.

4) Clarification on usage of experimental replicates and statistical analyses:
We apologize for this impreciseness. All replicates are biological replicates and we indicated this in the revised Figure legends. We completely agree with the reviewer that statistical tests were missing

in several figures and apologize for the oversight. We now included additional statistics, where applicable, and updated the material and methods sections with details on the performed tests. We apologize for having shown only two biological replicates in subfigures 1A-C and 3B in the previous version of the article. We repeated the corresponding experiments with more replicates and updated the respective subfigures.

5) Known and predicted differences between SPRR5 and the other SPRR proteins:
SPRR5 has only recently been annotated and according to Uniprot it is still uncertain whether the putative small proline-rich protein 5 is indeed expressed. Therefore, the data publically available for SPRR5 is very limited. However, we do know that the SPRR5 gene is in proximity to the small proline-rich protein gene cluster and the putative SPRR5 protein shows similarities in the composition of the protein structure as compared to other SPRR proteins. Regarding the differences, there is a drastic difference in the phenotype, as we report in this manuscript: SPRR5 depletion leads to an impaired differentiation program in epidermal tissue, whereas, to our best knowledge, knockout of other SPRR proteins in the murine system lead to no severe aberrant skin differentiation defect or any other reported disturbance in skin development or appearance. This proposed difference in cellular functions is in agreement with the isolated position of SPRR5 in a phylogenetic tree of all human SPRR proteins, presented in figure 5I of this manuscript. We updated the section about the differences between SPRR5 and the other SPRR proteins to more clearly emphasize and specify their dissimilarities (page 11; paragraph 2).

Additional points raised by reviewer 3:

A) Importance of SPRR5 as a downstream target of LINC00941:
We agree with the reviewer's concern about the centrality and uniqueness of SPRR5 as an effector molecule of LINC00941 function. To address this question, we performed co-depletion of both SPRR5 and LINC00941 to get more detailed information. We generated LINC00941-/SPRR5-double-depleted human primary keratinocytes and compared abundance of Keratin1 and Filaggrin (all inversely regulated in LINC00941- or SPRR5- single depleted cells). Interestingly, we found that double depletion of both, LINC00941 and SPRR5, led to reduced levels of Keratin1 and Filaggrin, resembling the phenotype seen with SPRR5 knockdown. These data suggest that LINC00941-mediated repression of differentiation might indeed, at least in part, be mediated by repression of SPRR5. This data is included in expanded figure EV5A-B of the revised manuscript.

B) Interpretation of p63-mediated control of SPRR5:
The reviewer raises the valid concern, that it is surprising to see that SPRR5 as a regulator of terminal differentiation appears to be controlled by p63, a well-known master regulator of progenitor cell progression. Interestingly, several studies have shown that p63 is an important regulator of epidermal commitment, keratinocyte proliferation and also of differentiation [reviewed in Soares & Zhou. Cell Mol Life Sci. 2018]. We previously showed that inhibition of p53 in p63-deficient human organotypic epidermis rescued the p63 proliferation and stratification defect, but not defective differentiation [Truong et al., Genes Dev. 2006]. These findings suggest a separate role for p63 in both processes. Furthermore, p63 occupancy was observed in regulatory regions controlling not only keratinocyte proliferation, but also differentiation [Kouwenhoven et al., EMBO Rep. 2015; Bao et al. Genome Biol.2015; Cavazza et al., Stem Cell Rep. 2016].
We thank the reviewer for bringing up this issue and addressed the dual role of p63 in epidermal homeostasis in the revised version of this manuscript to avoid confusion on the point noted (page 8; paragraph 2).

C) Analysis of LINC00941 expression in p63-deficient keratinocytes:
In contrast to SPRR5, knockdown of p63 does not affect LINC00941 abundance (shown in figure 3F), suggesting LINC00941 is transcriptionally regulated independently of p63. We clarified this finding in the written text of the revised manuscript. (page 8, paragraph 2 and page 9, paragraph 1).

2nd Editorial Decision 20 November 2018

Thank you for the submission of your revised manuscript to our office. We have now received the enclosed reports from the referees that were asked to assess it. Referee 1 still has a few more suggestions that I would like you to incorporate before we can proceed with the official acceptance of your manuscript.

Figure 3E states n=2, and in this case no error bars can be shown. Please either repeat the experiment one more time, or remove the error bars. You can show the data points from both experiments along with their mean in the figure.

I am also not certain what you mean by "biological replicates". Are these independent experiments? Statistics should only be calculated on data derived from 3 or more independent experiments.

Please add callouts to figures EV2C, EV4B+C and Dataset EV1 in the manuscript text, as all figure panels and data need to be called out. And please add a legend to the first tab of the Dataset EV1.

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

I look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have any questions.

REFEREE REPORTS

Referee #1:

The authors have responded to my previous concerns and therefore I recommend acceptance of the manuscript for publication after the following clarification:

1. It was not clear to me whether 69 genes from Fig 5G include the genes shown in previous figures such as Keratin1/10, Filaggrin. I would suspect so, based on the new results provided in the Supporting Figure EV5, but was not able to find any table of these 69 common genes in the Suppl Table.

2. Did the authors check if p63 is regulated by linc00941? Do levels of p63 change in linc00941 knock down cells?

3. There is no clear division from Results to Discussion.

4.The authors discuss about the converse regulation of linc00941 and SPRR5 in cancer and psoriasis but they don't show these data (page 12). These findings should be included in the Suppl Figures.

5. The authors should check their methods carefully. For example, there is a space in Methods (page 17), the font of some of the words in Methods is not the identical, and I have also found some "typo" mistakes (page 17). In addition, PCR conditions for RACE are missing in Methods. That data should be included.

Referee #2:

The authors have done a great job in revising this manuscript. All concerns have been addressed.

Referee #3:

The authors have succesfully addressed my comments and the manuscript has improved.

2nd Revision - authors' response 29 November 2018

Editor:

1) Number of replicates in figure 3E:

The previous version of our expression time course of SPRR5 in Fig. 3E, and also the expression time course for LINC00941 (Fig. 1A) was originally designed to show separate data points for different batches of primary keratinocytes (KC1-4 and KC1-3 respectively). This was done in order to emphasize that expression changes were very consistent across all batches of primary cells tested – thus strongly indicating a very significant dynamic regulation, independent of the source of the keratinocytes. For each batch, we have done two separate replicate experiments and previously have incorrectly shown statistics solely based on these two replicates per batch.
Additionally, this way of presenting the data was not optimal as it underrepresents the statistical significance of the data presented in both subfigures. To clarify our data in both figures 1A and 3E, we now show the expression time course for all independent biological replicate samples – irrespective of cell batch (eight and six respectively)-, calculated statistics correspondingly and adjusted legends of respective figures.

2) Definition of biological replicates:

By the term “biological replicates” we mean the usage of independent experimental setups.

3) Callouts to several figures and legend to dataset EV1:

We have now included callouts for figures EV2C, EV4B+C, Dataset EV1 and the new Dataset EV2 in the manuscript text. We also included legends in the first row of dataset EV1 (for each tab in the excel file) as well as the new dataset EV2.

4) Synopsis image, short summary and bullet points for key results:

As requested, we have included a synopsis image, a two-sentence summary and three bullet points for the most important results in the manuscript. These data are uploaded as separate files during submission of our revised manuscript.

Reviewer #1:

1) Gene list of the 69 conversely regulated genes from Fig. 5G:
We apologize for the oversight of not including the list of 69 conversely regulated genes into the manuscript. The data is now added into dataset EV1.
All RNAseq analyses were done with SPRR5- or LINC00941-depleted organotypic epidermal tissue. Consequentially, the list of 69 conversely regulated genes is based on epidermal tissue data while co-depletion of both molecules was performed in cultured keratinocytes. While keratin 1/filaggrin was regulated in keratinocyte cultures as well as organotypic cultures, they did not quite reach the stringent statistical cut-off of some of our RNA sequencing analyses in organotypic cultures and were therefore not included into the list of 69 conversely regulated genes.
The co-depletion experiment was done in cultured keratinocytes because of technical limitations when aiming for efficient knockdown of both, SPRR5 and LINC00941 at the same time of culture growth, in combination with finding a suitable time point where both single-depletion phenotypes overlap most profoundly.
Keratin1 and Filaggrin were consistently and conversely regulated in cultured keratinocytes of our single-knockdown assays (Fig. 1C and 4B), and analysis of Keratin1 in co-depletion experiments was
previously suggested by Referee 1 (Point six). Therefore, we selected those for our co-depletion analysis, which showed that levels of Keratin 1 and Filaggrin were reduced to similar levels as with depletion of SPRR5 alone.

2) Regulation of p63 by LINC00941:
To address this interesting suggestion by reviewer 1, we measured p63 abundance in LINC00941-knockdown vs. control tissue by qRT-PCR, and found no significant changes in expression levels. This data can now be found in Fig. EV3E and mentioned on page 9, first paragraph of the manuscript text.

3) Division of results and discussion section:
We have combined Results and Discussion sections to adhere to the guidelines for Scientific Report articles.

4) Converse regulation of LINC00941 and SPRR5 in cancer and psoriasis:
The analysis of converse regulation of LINC00941 and SPRR5 in cancer and psoriasis samples was not based on our own datasets, but extracted from publically available data as described in the methods section. We apologize for not making this clearer in the results/discussion section of the previous version and corrected this mistake in the new manuscript file (page 11, last paragraph and page 12, first paragraph). We additionally included the results of converse regulation in the Supplementary section (Dataset EV2).

5) Formatting mistakes in the methods section and PCR conditions for RACE:
We apologize for the formatting mistakes in the methods section, as well as for the missing PCR conditions of the RACE method. These mistakes are corrected in the new version of the manuscript.

EMBO PRESS

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr. Markus Kretz
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2018-46612

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

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

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample sizes were chosen as large as possible, while keeping the experimental procedures feasible in terms of data collection and handling. Furthermore, representative results shown here are the mean of at least three biological replicates and the observed effect has been reproducible during further experiments.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	The pre-established exclusion criteria was: Samples exhibting a vast difference in their differentiation pattern (as compared to other samples from the same timepoint and to previous experiments) were excluded during the analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Samples were allocated to treamtent or control groups randomly and were treated or harvested in random order.
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	All corresponding samples were treated at the same time and the observed phenotype has been confirmed by at least two different researchers and was reproducible during subsequent experiments. Microscopic analyses were performed using a blinded approach.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes
Is there an estimate of variation within each group of data?	Yes (Standard error bars are provided in the graphs)
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

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http://ClinicalTrials.gov
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http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Antibodies used in this publication are all commercially availabe (Source and ordering information are given in the material and methods section) and their specificity has been shown by the supplier as well as in previous publications.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Pooled primary human keratinocytes from different donors were obtained from PromoCell (Lot.No.: 1020401, 1040101 and 407Z001) or Lonza (Lot.No.: 0000402834) and all passed the mycoplasma contamination test.

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

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

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	We submitted our RNA-Seq data to GEO (GSE118077) and added gene lists with differentially expressed genes upon SPRR5 and LINC00941 depletion in the supplementary data of the manuscript.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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