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Jarid2 regulates mouse epidermal stem cell activation and differentiation

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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 April 2011

Thank you very much for submitting your research manuscript on Jarid2 in mouse epidermal development for consideration to The EMBO Journal editorial office.
I did receive consistent comments from three experts attached to this message. As you will recognize, all three are interested in the study and offer constructive criticisms to overcome the relative preliminary state of the work. Ref#1 demands further elucidation of potential Jarid2/PRC2 or potential other interacting partners that might gain further insight into Jarid's molecular contribution. Further investigations into expression of Jarid2 itself as well as controlling the level of depletion might increase the confidence in the data. I also recognize that particularly the requests of ref#1 (points 2 and 3) are rather demanding and would thus not insist on the proposed in-vitro studies (point 3) if you were able to satisfy the ChIP-requests in his/her second point.

Overall, I am happy to offer you the chance to amend the manuscript according to central requests from the referees. I also have to remind you that it is EMBO_J policy to allow a single round of major revisions and that the final decision on your manuscript entirely depends on content and strength of the ultimate version.

Thank you for the opportunity to consider your work for publication. I look forward to your revisions.

Yours truly,

Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1:

In this manuscript Mejetta et al. analyze the role of Jarid2 in epidermis and hair formation at three different developmental stages (embryonic development, postnatal morphogenesis and adult homeostasis) using a conditional system that allows for tissue-specific deletion of Jarid2 in skin during late mouse development (E13.5) (Jarid2cKO). The authors compare Jarid2cKO and wild-type mice using immunohistology analysis and conclude that 1) during embryonic development Jarid2 is not required for the formation of either epidermis or hair follicle placodes. 2) During neonatal development of Jarid2cKO, the proliferation of basal progenitors ex vivo is decreased and associated with an accumulation of differentiated cells in epidermis, however no effect is detected in hair or hair-follicle formation. 3) In adulthood, Jarid2cKO show normal formation of epidermis but display delayed, yet eventually successful, onset of adult hair formation (anagen). This is associated with decreased number of proliferating cells in several cell types in the hair follicle. 4) ChIP analysis shows reduced marking of H3K27me3 associated with increased mRNA expression of a subset of differentiation-associated genes in newborn epidermis and p16 in adults.

The topic of the manuscript is interesting and careful analysis of the role of Jarid2 in adult tissue homeostasis would be very beneficial to the field. The data is well analyzed and presented. However, at its current form the manuscript does not represent a significant enough advance in the understanding of Jarid2 function to merit publication in the EMBO journal.

The present study suggests that Jarid2 is required for the binding of Suz12, for high levels of H3K27me3 marking and for full PRC2-mediated repression of target genes in keratinocytes. However, as discussed by the authors in the manuscript, mice lacking Ezh2 or Jarid2 in skin cells show different phenotypes suggesting that the phenotype observed in Jarid2cKO could be PRC2-independent. The role of Jarid2 in PRC2 function in embryonic stem cells is not yet completely understood and there is evidence to suggest that Jarid2 may associate with other complexes in other cell types. Therefore, further experimentation should be carried out to confirm that Jarid2 acts in association with PRC2 during skin development and homeostasis and also to provide some insight as to whether Jarid2 plays a positive or negative role on PRC2 function in this cell type.

1. Jarid2 has been shown to interact with PRC2 in embryonic stem cells, however several reports suggest that Jarid2 can interact with other proteins (i.e. G9a, GLP, Rb protein) in other cell types. Analysis of biochemical interactions between Jarid2 and PRC2 subunits in skin cells would be needed. Protein IP followed by Westerns for PRC2 subunits could be carried out.

2. ChIP analysis and gene expression analysis of PRC2 targets should be extended. ChIP analysis of the binding of Jarid2, Ezh2, Suz12 and H3K27me3 in Jarid2cKO should be carried out in a systematic way in adult and newborn skin for a representative subset of PRC2 target genes and compared to gene expression data in both tissues. This dataset should be discussed and compared to published data of Ezh2cKO. Are the different phenotypes in both tissues a consequence of misregulation of different subset of genes? Plotting different genes in figures 6C and 7A on the same plot with the same scale and putting together negative controls would facilitate interpreting the data.

3. Double depletion of Jarid2 and Ezh2 in skin cells in vitro should be carried out to address the functional relationship between Jarid2 and PRC2 at both the molecular and cellular level.

4. At which developmental stages and in which cell types is Jarid2 expressed in skin tissue (in situ RNA and/or IF)? How does this compare to Ezh2 and/or other PRC2 subunits? How does the depletion of Jarid2 affect the cells in which it is expressed?

5. The extend of depletion of Jarid2 expression in Jarid2cKO should be checked by qRT-PCR as well as at the protein level.

6. Is proliferation of basal interfollicular progenitors affected in neonatal epidermis in vivo in

Jarid2cKO?

7. Why do the authors interpret increased numbers of filaggrin-positive cells in the epidermis of newborn Jarid2cKO mice as increased differentiation instead of decreased cell death?

8. The manuscript would benefit from reducing the number of main figures by combining some figures and maybe taking some pieces to supplementary material (merging figures 2 + 3, figures 4 + 5 and figures 6 + 7).

Referee #2:

Mejetta et al. describe the effects of epidermal deletion of Jarid2, a member of the Jumonji/JmjC protein family that recruits Polycomb Repressive Complex 2 to promoters in ES cells. The authors show that Jarid2 is enriched in postnatal basal epidermal cells relative to more differentiated suprabasal cells. Deletion of Jarid2 does not appear to affect embryonic development of the epidermis and hair follicles, but postnatally results in increased epidermal differentiation, decreased proliferation, and delayed entry of hair follicles into a new growth phase. Expression of PRC2 repressed genes, including p16/INK4a, is de-repressed in Jarid2-depleted epidermis. While the phenotype of these mutants is relatively subtle, the data appear to be reproducible and provide an interesting and important addition to our understanding of the functions of PRC2 complexes in the skin. Several points should be addressed before publication:

1. The authors should show (using immunostaining, in situ hybridization, or an alternate technique) whether, where and when Jarid2 is expressed in embryonic skin.
2. It would be helpful to use immunostaining to compare the localization of expression of p16/INK4a in control and mutant postnatal skin and hair follicles.
3. Figure 2A, F and Figure 4A - these graphs are too small and it is not possible to read the lettering at normal magnification. Please enlarge these.
4. Figure 3 legend: panel (B) is incorrectly referred to as (E).
5. The timing of entry into the natural postnatal anagen growth phase is quite variable between different strains, and between individual mice maintained on a mixed strain background. The authors should therefore provide the strain background of the mice used in this study. They indicate that 4 mice were analyzed at each time point for Figure 4C. Does this mean 4 mice total, or 4 controls and 4 mutants? If the former, this is an insufficient number to obtain reliable data even on an inbred background - at least 4 mutants and 4 controls should be analyzed for each time point. If the strain background is mixed, this number should be increased to at least 6 mutants and 6 controls.
6. Figure 4D: hair follicles indicated as being in telogen (resting phase) are in fact in early anagen. (Telogen stage follicles show negligible proliferation). This should be corrected.
7. Figure 5: the figure legend does not correspond with the figure panels.

Referee #3:

Mejetta et al. have investigated the function of Jarid2 in mouse epidermis.
They report that Jarid2 has a partially overlapping function with PRC2. Interestingly, some aspects of mouse epidermal biology are selectively influenced by Jarid2, not by PRC2, and viceversa.
Overall, this study provides interesting observations and will be certainly of interest to the field of mouse skin biology.
However, there are several points that need to be addressed before considering the manuscript for publication.

COMMENTS:

1. Jarid2 expression in the epidermis of developing embryos, neonates, and adult animals has to be investigated to fully understand how Jarid2 contributes to epidermis biology. This could be pursued by in situ hybridization or, even better, by Jarid2 antibody staining.

2. The extent of Jarid2 floxed-alleles deletion should be documented by RNA and protein assays.

3. Similarly, Jarid2 protein and H3K27me3 should be evaluated in FACS-isolated cells- every time the cells are employed for experimental manipulation.

4. Fig.2. C. Filaggrin expression should be documented by immunoblot.
Fig.2E. Cells should be immunostained with at least one differentiation marker.

5. Fig.6. In Jarid2 KO cells, Suz12 recruitment is reduced at the p16 promoter. Since Jarid2 doe not seem to influence neonatal epidermis before P21-30, it would be interesting to evaluate whether Jarid2 deletion affects Suz12 chromatin recruitment at P8-10.

1st Revision - authors' response 05 July 2011

Referee #1:

In this manuscript Mejetta et al. analyze the role of Jarid2 in epidermis and hair formation at three different developmental stages (embryonic development, postnatal morphogenesis and adult homeostasis) using a conditional system that allows for tissue-∞© specific deletion of Jarid2 in skin during late mouse development (E13.5) (Jarid2cKO). The authors compare Jarid2cKO and wild-∞© type mice using immunohistology analysis and conclude that 1) during embryonic development Jarid2 is not required for the formation of either epidermis or hair follicle placodes. 2) During neonatal development of Jarid2cKO, the proliferation of basal progenitors ex vivo is decreased and associated with an accumulation of differentiated cells in epidermis, however no effect is detected in hair or hair-∞© follicle formation. 3) In adulthood, Jarid2cKO show normal formation of epidermis but display delayed, yet eventually successful, onset of adult hair formation (anagen). This is associated with decreased number of proliferating cells in several cell types in the hair follicle. 4) ChIP analysis shows reduced marking of H3K27me3 associated with increased mRNA expression of a subset of differentiation-∞© associated genes in newborn epidermis and p16 in adults. The topic of the manuscript is interesting and careful analysis of the role of Jarid2 in adult tissue homeostasis would be very beneficial to the field. The data is well analyzed and presented. However, at its current form the manuscript does not represent a significant enough advance in the understanding of Jarid2 function to merit publication in the EMBO journal.

We thank reviewer 1 for her/his positive comments on our work.

The present study suggests that Jarid2 is required for the binding of Suz12, for high levels of H3K27me3 marking and for full PRC2-∞© mediated repression of target genes in keratinocytes. However, as discussed by the authors in the manuscript, mice lacking Ezh2 or Jarid2 in skin cells show different phenotypes suggesting that the phenotype observed in Jarid2cKO could be PRC2-∞© independent. The role of Jarid2 in PRC2 function in embryonic stem cells is not yet completely understood and there is evidence to suggest that Jarid2 may associate with other complexes in other cell types. Therefore, further experimentation should be carried out to confirm that Jarid2 acts in association with PRC2 during skin development and homeostasis and also to provide some insight as to whether Jarid2 plays a positive or negative role on PRC2 function in this cell type.

We fully agree with Reviewer 1 that the precise role of Jarid2 in regulating the activity of PRC2 in ES cells is still not fully understood. Although all reports to date on this matter unequivocally show that Jarid2 is required for the genomic occupancy of PRC2, the effect of Jarid2 on the enzymatic activity of PRC2 is less clear. We now show that endogenous Jarid2 co-∞©immunoprecipitates with endogenous Suz12 and Ezh2 (two major components of the PRC2 complex) in mouse keratinocytes (Fig 5A). We also show by ChIP analysis that Jarid2 binds to the promoters of PRC2-∞©target genes in newborn keratinocytes (Figure 5B). In addition we have extended our initial analysis on the effect of the deletion of Jarid2 over the genomic occupancy of PRC2 and deposition of H3K27me3 in epidermal specific and non-∞©epidermal specific targets of PRC2 (Fig. 5, 6 and 7). To this end we have analyzed most of the genes reported in Ezhkova et al, 2009 to be PRC2 targets in mouse keratinocytes. We show that deletion of Jarid2 results in a reduction of PRC2 occupancy and H3K27me3 levels in these genes. Moreover we show that deletion of Jarid2 enhances the expression of terminal differentiation genes, as well as p16, both of which account for the phenotype of the

Jarid2cKO mice (Fig. 6 and 7).

1. Jarid2 has been shown to interact with PRC2 in embryonic stem cells, however several reports suggest that Jarid2 can interact with other proteins (i.e. G9a, GLP, Rb protein) in other cell types. Analysis of biochemical interactions between Jarid2 and PRC2 subunits in skin cells would be needed. Protein IP followed by Westerns for PRC2 subunits could be carried out.

We now include in Figure 5A the experiment suggested by reviewer 1 and show that endogenous Jarid2 co-∞©immunoprecipitates with endogenous Suz12 and Ezh2 in primary mouse keratinocytes.

2. ChIP analysis and gene expression analysis of PRC2 targets should be extended. ChIP analysis of the binding of Jarid2, Ezh2, Suz12 and H3K27me3 in Jarid2cKO should be carried out in a systematic way in adult and newborn skin for a representative subset of PRC2 target genes and compared to gene expression data in both tissues. This dataset should be discussed and compared to published data of Ezh2cKO. Are the different phenotypes in both tissues a consequence of misregulation of different subset of genes? Plotting different genes in figures 6C and 7A on the same plot with the same scale and putting together negative controls would facilitate interpreting the data.

We have now extended our analysis as suggested by Reviewer 1. We show that Jarid2 binds to the promoters of epidermal differentiation genes previously shown to be targets of PRC2 in Ezhova et al., 2009 (Figure 5B). In addition, we have analyzed the occupancy of Suz12 and the deposition of H3K27me3 in virtually all the genes described in Ezhova et al. (2009) in Jarid2 and WT newborn keratinocytes (Figure 5C-∞© E, Figure 6 and Figure 7). We show that occupancy of Suz12 and the levels of H3K27me3 are reduced in these same genes upon deletion of Jarid2 (Figure 5), and that this translates into higher transcript levels of these same genes (Figure 6). Interestingly, in adult keratinocytes the same type of analysis revealed that Jarid2 and PRC2 are no longer responsible for regulating the expression of terminal differentiation genes (which is exactly the same as what was shown by Ezhova et al., 2011), but still necessary to repress the INK4a locus (p16). Therefore, Jarid2 is required for regulating the scheduled proliferation and differentiation of basal epidermal progenitors in neonatal morphogenesis, but not in adult keratinocytes. However, Jarid2 regulates the expression of p16 both in neonatal and adult epidermis.

3. Double depletion of Jarid2 and Ezh2 in skin cells in vitro should be carried out to address the functional relationship between Jarid2 and PRC2 at both the molecular and cellular level.

We thank the reviewer for this suggestion. It would be necessary to obtain Ezh2 conditional KO mice and cross them with our Jarid2KO mice in order to perform these experiments properly, specially with respect to their role in hair follicle and epidermal morphogenesis. However, obtaining the mice, placing the crosses and analyzing the phenotype is a long process and we think it is therefore beyond the scope of the present work.

4. At which developmental stages and in which cell types is Jarid2 expressed in skin tissue (in situ RNA and/or IF)? How does this compare to Ezh2 and/or other PRC2 subunits? How does the depletion of Jarid2 affect the cells in which it is expressed?

We have now studied the expression of Jarid2 in embryonic epidermis. To this end, we have FACS sorted basal undifferentiated (6bright) and suprabasal differentiated (6dim) keratinocytes from the skin of E16.5 and E18.5 embryos, and have analyzed the expression of Jarid2 in these two populations of cells (Supplementary Figure 2).We chose these two time points, since they are those previously analyzed for the expression of Ezh2 in the developing murine epidermis (Ezhkova et al. 2009). Interestingly, the expression of Jarid2 does not change between undifferentiated and differentiated embryonic keratinocytes, as it does in neonatal and adult epidermis (6 integrin and Loricrin were used a positive controls for the sorting strategy). Moreover, this is different to the expression of Ezh2. Therefore, the expression pattern of Jarid2 and Ezh2 is different during embryonic development. These results also suggest that there must be functional redundancy between Jarid2 and some other factor (yet to be identified), since deletion of Jarid2 does not cause any effect over the embryonic development of the epidermis (Figure 1).

5. The extend of depletion of Jarid2 expression in Jarid2cKO should be checked by qRT-∞© PCR as

well as at the protein level.

As suggested, we have now included in supplementary Figure S1C and S1D the PCR, RT-∞©qPCR and Western blot that shows the complete deletion of Jarid2 in keratinocytes isolated from Jarid2cKO mice.

6. Is proliferation of basal interfollicular progenitors affected in neonatal epidermis in vivo in Jarid2cKO?

We have counted the number of ki67+ basal interfollicular cells in neonatal Jarid2cKO and control mice and show that there is a statistically significant reduction in the number of proliferative cells in the KO mice (Figure 1H). In addition, we have sorted by FACS the 6bright population from control and Jarid2cKO mice and show that KO cells significantly express lower mRNA levels of ki67 than control cells (Figure 1H).

7. Why do the authors interpret increased numbers of filaggrin-∞© positive cells in the epidermis of newborn Jarid2cKO mice as increased differentiation instead of decreased cell death?

This is an interesting point raised by reviewer 1. Certainly, a decreased capability to shed cells from the cornified layer could result in a somewhat similar phenotype (although it would not explain the increased thickness of the granular-∞©filaggrin positive-∞© layer in KO mice). We have concluded that deletion of Jarid2 enhances differentiation since Jarid2cKO cells express higher levels of terminal differentiation genes (Figure 6), Jarid2 cells form abortive differentiated colonies in culture (Figure 2), and the epidermis of Jarid2cKO mice has a thicker granular and cornified layers (Figure 2). We have performed tunnel assay and we could not score any apoptotic cells neither in wild type nor KO mice (data not shown). It will be interesting in the future though to study whether Jarid2 might be regulating cell death.

8. The manuscript would benefit from reducing the number of main figures by combining some figures and maybe taking some pieces to supplementary material (merging figures 2 + 3, figures 4 + 5 and figures 6 + 7).

We thank this reviewer for these suggestions. We have now added several new results in the revised version of the manuscript, and therefore the overall layout has been substantially modified.

Referee #2:

Mejetta et al. describe the effects of epidermal deletion of Jarid2, a member of the Jumonji/JmjC protein family that recruits Polycomb Repressive Complex 2 to promoters in ES cells. The authors show that Jarid2 is enriched in postnatal basal epidermal cells relative to more differentiated suprabasal cells. Deletion of Jarid2 does not appear to affect embryonic development of the epidermis and hair follicles, but postnatally results in increased epidermal differentiation, decreased proliferation, and delayed entry of hair follicles into a new growth phase. Expression of PRC2 repressed genes, including p16/INK4a, is de-∞© repressed in Jarid2-∞© depleted epidermis. While the phenotype of these mutants is relatively subtle, the data appear to be reproducible and provide an interesting and important addition to our understanding of the functions of PRC2 complexes in the skin. Several points should be addressed before publication:

We thank reviewer 2 for her/his positive comments on our findings.

1. The authors should show (using immunostaining, in situ hybridization, or an alternate technique) whether, where and when Jarid2 is expressed in embryonic skin.

We now include as Supplementary Figure 2 the pattern of expression of Jarid2 in embryonic epidermis (E16.5 and E18.5). Our RT-∞©qPCR results show that Jarid2 is expressed in embryonic epidermis, but that contrary to newborn and adult epidermis, its levels do not decrease upon differentiation. This intriguing difference may account for the differences in the embryonic phenotypes of Jarid2 and Ezh2 conditional epidermal knockout mice.

2. It would be helpful to use immunostaining to compare the localization of expression of p16/INK4a

in control and mutant postnatal skin and hair follicles.

We have performed the suggested experiment. We now show that p16 is expressed in interfollicular epidermis (but not hair follicles) of neonatal Jarid2cKO mice compared to control mice (in fact, we could not observe any p16 staining in any of the control mice analyzed) (Figure 6C). We also observed expression of p16 in the interfollicular epidermis of adult Jarid2cKO mice (Figure 7B). However, in the adult skin the HF of Jarid2cKO mice showed a clear increase in the expression of p16, compared to control mice, which is well in accordance with the delayed entry into anagen observed in these mice (Figure 7B).

3. Figure 2A, F and Figure 4A -∞© these graphs are too small and it is not possible to read the lettering at normal magnification. Please enlarge these.

We apologize for this. We have now changed the size of the letters to make the figure clearer.

4. Figure 3 legend: panel (B) is incorrectly referred to as (E).

The layout of the figures in the revised version is different and therefore this has been now corrected.

5. The timing of entry into the natural postnatal anagen growth phase is quite variable between different strains, and between individual mice maintained on a mixed strain background. The authors should therefore provide the strain background of the mice used in this study. They indicate that 4 mice were analyzed at each time point for Figure 4C. Does this mean 4 mice total, or 4 controls and 4 mutants? If the former, this is an insufficient number to obtain reliable data even on an inbred background -∞© at least 4 mutants and 4 controls should be analyzed for each time point. If the strain background is mixed, this number should be increased to at least 6 mutants and 6 controls.

We fully agree with this issue raised by reviewer 1. The mice are kept in the C57Bl6/FvBN mixed background (we state this in the materials and methods section). We have now analysed several additional mice (7 controls and 8 KO for each time point). This is now stated in the figure legend of Figure 2.

6. Figure 4D: hair follicles indicated as being in telogen (resting phase) are in fact in early anagen. (Telogen stage follicles show negligible proliferation). This should be corrected.

This has been now corrected in the figure legend and main text of the manuscript.

7. Figure 5: the figure legend does not correspond with the figure panels.

We apologize for this mistake. This has been corrected in the revised version of the manuscript.

Referee #3:

Mejetta et al. have investigated the function of Jarid2 in mouse epidermis. They report that Jarid2 has a partially overlapping function with PRC2. Interestingly, some aspects of mouse epidermal biology are selectively influenced by Jarid2, not by PRC2, and vice versa. Overall, this study provides interesting observations and will be certainly of interest to the field of mouse skin biology. However, there are several points that need to be addressed before considering the manuscript for publication.

We thank reviewer 3 for her/his positive comments on our work.

COMMENTS: 1. Jarid2 expression in the epidermis of developing embryos, neonates, and adult animals has to be investigated to fully understand how Jarid2 contributes to epidermis biology. This could be pursued by in situ hybridization or, even better, by Jarid2 antibody staining.

We have attempted to study the expression of Jarid2 by immunohistochemistry in embryonic, neonatal and adult epidermis. However, we have tested all commercially available antibodies (plus

an antibody generated in the laboratory of Youngsook Lee) with many different conditions (paraffin embedded tissue, OCT frozen, and formaldehyde-∞©fixed OCT frozen; different antigen retrieval procedures, blocking solutions, etc) and none have worked. We have contacted people in the field working with Jarid2 and all have encountered the same problem (which explains why none of the works published so far describing the function of Jarid2 in ES cells showed any immunostaining of the protein). However, we have studied the expression of Jarid2 by RT-∞©qPCR of sorted basal/suprabasal cells from embryonic, neonatal and adult epidermis. These analysis have revealed that Jarid2 is expressed predominantly (but not uniquely) in basal interfollicular progenitors in neonatal and adult epidermis, and that its expression decreases as the cells become differentiated. On the other hand, Jarid2 expression is high in the developing epidermis, but its expression is unchanged between progenitors and differentiated cells.

2. The extent of Jarid2 floxed-∞© alleles deletion should be documented by RNA and protein assays.

We now include in the supplementary Figure S1 the extent of the deletion of Jarid2, as measured by RT-∞©PCR, RT-∞©qPCR and western blot.

3. Similarly, Jarid2 protein and H3K27me3 should be evaluated in FACS-∞© isolated cells-∞© every time the cells are employed for experimental manipulation.

Before performing any experiment shown we have genotyped the cells isolated from Jarid2cKO and control mice to confirm the full deletion of Jarid2. As mentioned above we now include the western blot analysis showing the complete deletion of Jarid2 in our experimental cells (Supplementary Figure S2). In addition, we now have extended the analysis of the impact of deletion of Jarid2 over the levels of H3K27me3 (Figure 5). Of note, it should be mentioned that in all the FACS sorting experiments used for isolating epidermal keratinocytes from KO and WT skin a cocktail of antibodies (CD31, CD140, and CD45) was used to exclude endothelial, mesenchymal and hematopoietic lineages from our preparations. It cannot be excluded that some minor contamination might still be present in our cell preparations, but certainly it would be negligible as shown by the western of Jarid2 and RT-∞©qPCR to measure the extent of the deletion (Supplementary Figure S1).

4. Fig.2. C. Filaggrin expression should be documented by immunoblot. Fig.2E. Cells should be immunostained with at least one differentiation marker.

As suggested we now show that Jarid2cKO cells express higher levels of filaggrin by western immunobloting (Figure 2F), and that the colonies formed by Jarid2cKO cells contain a significant increase in the number of cells positive for filaggrin and loricrin (Figure 2E).

5. Fig.6. In Jarid2 KO cells, Suz12 recruitment is reduced at the p16 promoter. Since Jarid2 does not seem to influence neonatal epidermis before P21-∞© 30, it would be interesting to evaluate whether Jarid2 deletion affects Suz12 chromatin recruitment at P8-∞© 10.

Deletion Jarid2 does indeed have an impact over neonatal epidermis before P21-∞©P30. As we show in figure 2, the epidermis is less proliferative, contains a higher proportion of filaggrin positive granular layer cells, and is hyperkeratotic. We compared the skin of control and Jarid2cKO mice between P0 and P8, and the increased differentiation was evident at every time point. We have nevertheless analyzed the expression of p16 and terminal differentiation markers in basal (6bright) keratinocytes isolated from control and Jarid2cKO P8 mice, and show that Jarid2cKO cells express higher levels of all these genes, similar to what we observed in P0 (Supplementary Figure S5). We also show that deletion of Jarid2 reduces the genomic occupancy of Suz12, and the deposition of the H3K27me3 repressive mark in the promoters of these genes. On the other hand, in adult epidermis Jarid2 is no longer responsible for repressing epidermal differentiation genes (we have analyzed a large cohort of mice and there are no statistical differences; data now shown), but is still required for modulating the expression of p16.

