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PIKE-A is Required for Prolactin-mediated STAT5a Activation in Mammary Gland Development

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

1st Editorial Decision 14 May 2009

Thank you for submitting your manuscript to the EMBO Journal. Your study has now been seen by three referees and their comments to the authors are provide below. As you can see, there is an interest in the work and referee #3 is quite supportive of the study as it is. However, both referees #1 and 2 also raise significant concerns with core parts of the study and it is uncertain if these concerns can be fully resolved. I will not repeat all of the raised concerns here, but the referees find that further understanding of why the observed phenotype is restricted to lacation only as Stat5 is important in late gestation as well is needed. Referee #2 also finds that much further work is needed to clarify the role of PIKE-A in gland development. While I appreciate that there is an interest in the findings reported, it is also clear that much further work would be needed in order to further understand the function of PIKE-A and it is uncertain if these issues can be fully resolved. Given this, I am afraid that I cannot offer to commit to a revised version at this stage. However as there is an interest in the study, I can offer that should you be able to thoroughly extend the analysis along the lines suggested by the referees that I am not opposed to consider a re-submission of the study. However, I should point that for resubmissions that we consider the novelty of data at the time of resubmission and may, if needed, bring in new referee(s).

I thank you in any case for the opportunity to consider this manuscript. I am sorry we cannot be more positive on this occasion, but we hope nevertheless that you will find our referees' comments helpful.

Yours sincerely,

Editor
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REFEREE REPORTS

Referee #1 (Remarks to the Author):

This manuscript describes a role for PIKE-A, a GTPase involved in the activation of PI3K signaling, in mammary gland development during pregnancy and early lactation. Mice that are deficient in PIKE-A have been used to show that lobuloalveolar development is perturbed in the absence of PIKE-A and that consequently, lactation fails resulting in the death of newborn pups. The authors then show that cyclin D1 is downregulated in the absence of PIKE-A both in vivo and in cell culture using knockdown of PIKE-A. Importantly, the PIKE-A phenotype can be rescued by overexpressing cyclinD1.

Using a range of deletion constructs, the authors show that this defect in development post-partum is due to the essential interaction of Stat5 with PIKE-A and suggest that PIKE-A acts as an adaptor for Stat5 to bring it to the PRLR where Stat5 is then phosphorylated by Jak2 in response to PRL signaling. It is clearly shown that PIKE-A binds to Stat5 thru its amino-terminal domain and that this mediates the recruitment of the Stat5/PIKE-A complex to the PRL-bound PRL receptor. pStat5 then dissociates from the PRLR leaving PIKE-A bound to the receptor. The precise domains that mediate the interaction between PIKE-A and Stat5 have not been identified. Does PIKE-A also bind to Stat5b or Stat3?

An interesting question that has not been addressed is why this phenotype is restricted to lactation as Stat5 is important in late gestation for full differentiation. This would suggest that the adaptor function of PIKE-A is not required during gestation. Is there another adaptor that carries out this function? This is all the more curious since loss of CyclinD1 results in impaired mammary gland development during pregnancy.

Figure 5 does not show data from the 18.5dpc samples. This is important to exclude the possibility that cells are failing to differentiate at this time and are undergoing apoptosis. The defect in lactation seems to be a failure to expand the alveolar lumen. It would be interesting to compare global gene expression profiles between control and PIKE-A deficient mammary glands at both d18dpc and 1dpp to show that PIKE-A has a function in lactation and not during gestation.

In order to confirm that development is normal at 18.5dpc, and that the phenotype is restricted to early lactation, an immunoblot analysis for the markers shown in Figure 4F and Figure 5E should be carried out. This can be addressed by carrying out immunoblot analysis for 18.5dpc samples for the same markers as shown in Figure 1F. This is an important point since Stat5 is active during late gestation and milk protein genes such as beta-casein and WAP are expressed. It would be very interesting if PIKE only has a role in lactation and the proposed adaptor function for PIKE is not required for Stat5 function during gestation. This needs to be conclusively addressed.

The cyclinD1 data demonstrate quite convincingly that overexpression of cyclinD1 can compensate for the loss of PIKE-A. However, the data in Figure 7 are restricted to a whole mount analysis. Some analysis of molecular markers is essential, including cyclin E1, which is a downstream target of cyclin D1.

Data are presented which show that the interaction between PIKE-A and PRLR is transient. The model in Figure 7 does not illustrate this. What is the mechanism of PIKE-A dissociation from the PRLR?

The concluding paragraph of the discussion is not strictly correct as it has been shown that Stat5 can be phosphorylated in the absence of PIKE-A during gestation.

Referee #2 (Remarks to the Author):

The authors provide evidence that PIKE-A, a novel binding partners of the prolactin receptor (PrlR) and STAT5A, is essential for the phosphorylation and transcriptional activity of STAT5A in mammary tissue. They demonstrated that PIKE-A mediated expression of the cyclin D1 gene and cell proliferation by regulating prolactin/STAT5 signaling pathway both in vitro and in vivo. The

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study has been well done. However, the authors need to address some fundamental questions needed to clarify the roles of PIKE-A in the mammary gland development in vivo.

The authors demonstrate convincingly that PIKE-A is required for the lactational process. However, the results allow other interpretations. In judging Figure 4 in its entity, proliferation seems to be quite normal in the ko mice, as the number of alveoli per field is similar in ko and control mice (e.g. 4F). To me it appears that the paucity of proliferation is not the culprit per se, but rather the differentiation of epithelial cells. Increased apoptosis could simply be the default of a lack of differentiation. In order to demonstrate that increased cell death is partly responsible for the observed defect, the authors would need to attempt to rescue the ko cells using a lentivirus expressing e.g. bcl2.

The author showed that, in the absence of PIKE-A, phosphorylation of STAT5 was not detectable. However, the defect of mammary gland development in PIKE-A knockout mice seemed to be much more moderate than that of PRLR, STAT5A, cyclinD1 or Akt-null mice (Ormandy CJ et al., Genes Dev 1997 (11) 167-178, Liu X et al., Genes Dev 1997 (11) 179-186, Sicinski LP et al., Cell 1995 (82) 621-630, Maroulakou IG et al., J Cell Physiol 2008 (217) 468-477). The PIKE-A knockout mice had the defect only after parturition, while the other knockout mice displayed defects during pregnancy. This is all quite puzzling and it is essential that the authors provide results (western blots) on the levels of PIKE-A in mammary tissue of virgin, 7.5 dpc, 13.5 dpc, 18.5 dpc and 1 day postpartum control and pike-a knockout mice (part of Fig. 6). Moreover, it is necessary to provide phospho- and total-STAT5 immunostaining of mammary tissue at 13.5 or 18.5 dpc (include into Figure 6B). The data must be discussed to relate PIKE-A expression to STAT5 phosphorylation.

In PIKE-A knockout mice, the impaired lactogenesis was rescued by exogenous induction of only one proliferation factor, cyclin D1, while not only impaired proliferation but also increase of apoptosis (that occurred before parturition) had been seen. Is it possible that the overexpression of cyclin D1 (i.e. hyperproliferation) overcomes increased apoptosis seen in the ko mice? Ki67 and TUNEL staining on the mammary tissue section of PIKE-A -/- MMTV-cyclin D1 transgenic mice needs to be included. As stated earlier, to this reviewer the defect appears to be on the level of differentiation and I wonder how cyclin D1 can rescue differentiation. Expression of the MMTV-LTR is dependent on the presence of activated STAT5. Since STAT5 activity is very low in PIKE-A ko mammary tissue the authors need to demonstrate activity of the MMTV-cyclinD1 transgene. They alos need to demonstrate differentiation of these cells.

Is the defect of lactogenesis in pike-a knockout mice due to epithelial cell autonomous or interaction with stromal cells? PIKE-A immunostaining of mammary tissue from virgin, 7.5 dpc, 13.5 dpc, 18.5 dpc and 1 day postpartum of wild-type and pike-a knockout mice. If PIKE-A is expressed also in stromal cells, transplantation of wild-type and PIKE-A knockout mammary epithelium into cleared fat pad of wild-type and PIKE-A knockout mice needs to be performed.

Can pike-a knockout mice nurse their pups? The authors showed PIKE-A knockout mice had a defect of mammary gland development and milk protein production. Show percentage of surviving pups born by PIKE-A wild-type and knockout mothers.

Does PIKE-A bind to JAK2? The author stated that the prolactin receptor/PIKE-A/STAT5 complex was disrupted by JAK2 phosphorylation on STAT5, leading to STAT5 nuclear translocation. JAK2 is the critical mediator of prolactin-STAT5 signaling pathway. Is there any interaction between PIKE-A and JAK2? Do the authors observe binding of PIKE-A to the prolactin receptor and STAT5 in the presence of a JAK2 inhibitor?

Lastly, since non-functional mammary tissue tends to undergo involution following parturition, all biochemical studies (such as apoptosis studies) need to be performed prior to parturition. Otherwise artifacts will be generated.

Referee #3 (Remarks to the Author):

This is an excellent paper that adds a new signaling molecule to the Jak-Stat pathway downstream of the prolactin receptor. I have a number of suggestions to improve the manuscript.

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Signaling aspects

p6- the data suggests interaction between 486-608 not 398-608.
p7- data in figure 2 does not exclude phosphorylation of PikeA, what is the effect of a Jak inhibitor?
p7- what are the kinetics of activation of PikeA relative to Stat5 and Prlr?
p7- does PikeA knockdown alter the association of Stat5 with Prlr?
Fig1E loweer panel lane 1 looks under loaded?
Fig2C panel 4 ERK expression reduced in lanes 3 and 4. Needs a loading control like tubulin or actin. Is there a non specific effect of the shRNAs?

Developmental aspects

PikeA knockout- epithelial transplantation to the cleared fat pad would convince the reader of the mammary cell autonomous action of PikeA. Rescue by MMTV driven Cyclin D1 suggest so, but transplants would settle the argument.

Source of the anti prolactin receptor antibody must be stated. Use of the Prlr knockout mouse to prove specificity should be done as there is very widespread skepticism regarding specificity and affinity of anti Prlr antibodies.

Cyclin D1 rescue of PikeA knockout is not sufficiently well described. Functional aspects such as lactation should be measured by comparison of standardised litter (8pups) weight gain. Needs IHC sections and examination of milk proteins indicative of developmental stage. Morphology by whole mount alone is not sufficient to support the conclusion of rescue. In fact the rescue looks partial by the data provided. This new data must be provided if full rescue is claimed. Current data supports an at-least partial rescue to the secretory initiation or activation stage.

Do compound PikeA/Prlr heterozygous mice show an exacerbated lactation phenotype over Prlr+/- alone? Not essential but an interesting experiment for the future.

New Submission Received 11 June 2009

Referee #1

1. The reviewer questioned that “the precise domains that mediate the interaction between PIKE-A and STAT5 have not been identified. Does PIKE-A also bind STAT5b or STAT3”?

We have shown the N-terminal 1-72 aa of PIKE-A interacts with the DNA binding domain of STAT5a in Fig 1C and 1E respectively. We have also shown that PIKE-A binds to STAT5a only as no interaction was detected when PIKE-A and STAT5b or STAT1 was co-transfected in HEK293 cells (Fig 1A).

2. The reviewer questioned why the specific phenotype is restricted to lactation as STAT5 is important in late gestation for full differentiation.

We hypothesized that the lactation-specific defect of PIKE KO mammary gland might relate to the specific temporal expression pattern of PIKE-A. If PIKE-A is only functional during lactation, it might provide a reasonable answer to the above question. We thus performed a Western blot analysis on the expression of PIKE-A during different stage of pregnancy. We found that expression of PIKE-A was highly increased during lactation but not other gestational stages (Fig 6, 7th panel). Therefore, deletion of PIKE-A in mammary gland will only affect the signal transduction during lactation but not gestation. As a result, normal mammary gland developmental was found in early and mid gestation of PIKE KO mice. This is further supported by the fact that STAT5 phosphorylation was not altered in PIKE KO mammary during gestation (Fig 6A, 5th panels), indicating STAT5 phosphorylation was only affected by PIKE-A during lactation but not other gestation stages. Also, the PRLR +/- mammary gland develops normally to late gestation then fails, indicating that increased PRLR signaling is required for lactation. Loss of PIKE may not sufficiently

decrease PRLR signaling until the really big boost is required post partum, provided by increase serum PRL but also increased PIKE-A.

3. The reviewer commented that “Fig 5 does not show data on 18.5 dpc samples”.

We have included TUNEL and Western blot analysis of mammary gland samples from 18.5 d.p.c. in Fig 5C and 5E respectively. Our results suggested that mammary gland apoptosis could be detected on 18.5 d.p.c.

4. The reviewer suggested to “compare global gene expression profiles between control and PIKE-A deficient glands at both 18dpc and 1dpp”.

We have performed a microarray analysis on PIKE-A deficient glands at both 18.5 d.p.c. and 1 day postpartum. We found more than 50 genes are affected in lactating PIKE-null tissues ranging including metabolism, cell cycle, transcription, etc. Projects are now under progress to validate if such expression changes are a result of defective lobuloalveogenesis or a direct consequence of PIKE deficiency. Nevertheless, expression of genes such as cyclin D1, casein, Bcl-xL, etc. were found to be significantly decreased in lactating PIKE-null tissues, which further confirmed our observations in the manuscript. Since the results from the microarray are preliminary and beyond the scope of the current studies, we have not included them in the revised manuscript.

5. The reviewer suggested that “an immunoblott analysis for 18.5dpc sample for the same marker as shown in Fig 4F and 5E”.

As suggested, we have examined the molecular markers in Fig 4F in 18.5 dpc mammary gland. As expected, expression of milk proteins (-casein, WAP), signaling protein phosphorylation (STAT5 and ERK) and cyclin D1 expression were comparable between wild-type and PIKE KO tissues, suggesting that mammary gland development in PIKE KO mammary is normal during gestation.

6. The reviewer suggested “some analysis of molecular marker is essential” in PIKE-/-cyclin D1-Tg samples.

As suggested, we have examined a variety of markers including milk protein expression (-casein, WAP), signaling protein phosphorylation (STAT5, ERK and Akt), apoptotic marker (Bcl-2, Bcl-XL and PARP cleavage) and cyclin E1 expression as shown in Fig 7E. The phosphorylation of STAT5 and Akt (3rd and 4th panels) was diminished in PIKE-/-cyclin D1-Tg tissues. The reduced STAT5 phosphorylation thus results in a reduction of of -casein and WAP expression (1st and 2nd panels). On the other hand, Bcl-2 expression and PARP cleavage was rescued (7th and 8th panels), suggesting cyclin D1 overexpression reduced apoptosis in PIKE null mammary. Cylin E1 level was comparable between wild-type and PIKE null tissues when cyclin D1 is overexpressed (9th panel).

7. The reviewer suggested a modification of the proposed model on Fig 7 as “the model in Fig 7 does not illustrate that the interaction between PIKE-A and PRLR is transient”. Moreover, s/he thinks the “concluding paragraph of the discussion is not strictly correct as it has been shown that Stat5 can be phosphorylated in the absence of PIKE-A during gestation”.

We have amended our model of PIKE function in PRL signaling in Fig 7F showing the event is transient. We have also discussed the dissociation mechanism of PIKE-A from PRLR (p.16). Since PIKE-A/PRLR association depends on the phosphorylation status of PRLR (Fig 2B and C), we suggested that dephosphorylation PRLR by yet unknown phosphatase or the binding of SOCS proteins after PRL association represents a molecular signal for the receptor-bound PIKE-A to dissociate from the receptor. We have also revised the concluding paragraph in the discussion to state that STAT5 phosphorylation was only affected during lactation in PIKE KO mice.

Referee #2

1. The reviewer suggested “to attempt to rescue the KO cells using a lentivirus expressing Bcl2” to demonstrate that increased cell death is partly responsible for the observed defect.

As suggested, we have injected adenovirus overexpresisng Bcl-2 into the mammary gland of PIKE KO at 12.5 dpc. It was shown that caspase 3 and PARP cleavage (Fig 5F, 2nd and 3rd panels) were reduced when Bcl-2 was overexpressed, indicating the reduction of apoptosis. Moreover, the milk protein expression was increased, suggesting that the defective PIKE KO cells are, or partly, rescued when apoptosis is reduced.

2. The reviewer suggested to "provide results (western blot) on the levels of PIKE-A in mammary tissues of virgin, 7.5 dpc, 13.5 dpc, 18.5 dpc and 1 day postpartum control and PIKE-A knockout mice" and "phosphor- and total-STAT5 immunostaining of mammary tissues at 13.5 or 18.5 dpc".

The expression profile of PIKE-A in mammary gland during different gestation and lactation time were shown in Fig 6A (7th panel). Total and phosphor-STAT5 immunostainings on mammary tissues collected from 13.5 and 18.5 dpc were also provided in Fig 6B. Expression of PIKE-A increased only at 1d postapartum, suggesting PIKE-A might function exclusively during lactation. This expression pattern provides a possible explanation to the distinct phenotype of PIKE KO mice as mammary gland defect was only seen in 1 day postpartum but not other gestational stages. This result was further confirmed by the STAT5 phosphoryaltion as a down-regulated STAT5 phosphoryaltion was observed in 1d postpartum by both Western blot and immunohistostaining. All these results point to a conclusion that PIKE-A exert a temporal specific role in mammary gland function. A discussion about these observations is included in the revised manuscript (p.16-17).

3. The reviewer questioned "if overexpression of cyclin D1 overcomes increased apoptosis seen in the PIKE KO mice".

To address this issue, Ki67 staining and TUNEL assay were performed on PIKE-/-cyclin D1-Tg mammary tissues. It was found that the number of Ki67 positive cells was comparable between Wild-type and PIKE KO mammary when cyclin D1 is overexpressed (Sup Fig 6 C). Moreover, the apoptosis, as revealed by positive TUNEL results, in PIKE KO mammary was abolished when cyclin D1 is over-expressed (Sup Fig 6F). These results suggested that overexpression of cylin D1 could overcome the apoptosis triggered by PIKE-A ablation. The conclusion is further supported by the comparable Bcl-2 expression, and reduced PARP cleavage shown in Fig 7E (7th and 8th panels). However, differentiation of PIKE null mammary epithelial cells could only be partial rescued as expression of WAP and -casein were less than the wildtype mice even the cyclin D1 is overexpressed (Fig 7D, 1st and 2nd panels and Sup Fig 6E).

4. The reviewer suggested "to demonstrate the activity of the MMTV-cyclin D1 transgene and the differentiation of these cells".

We have examined the cyclin D1 expression in the PIKE-/-cyclin D1-Tg mammary gland. As shown in Fig 7C, cyclin D1 expression was drastically increased in the transgenic lines, indicating the successful overexpression of the protein. We have also examined the differentiation of the PIKE-/-cyclin D1-Tg cells by determining the expression of milk proteins (Fig 7E and Sup Fig 6E). These results suggested the successful development of cyclin D1 transgene in PIKE knockout mice and the overexpression of cyclin D1 can partially rescue the differentiation of the mammary epithelia cells as revealed by the increased WAP expression.

5. The reviewer questioned if the defective lactogenesis in PIKE-A knockout mice is due to autonomous defect of epithelial cells and suggested an immunostaining of PIKE-A in different stages of mammary gland development.

As suggested, we have examined the PIKE-A localization in different gestation time by *immunostaining (Sup Fig 1D). Positive signals were found in epithelial cell of the mammary gland as well as some surrounding adipocytes. The expression is increased dramatically during lactation, which fits with the observation from Western blot analysis (Fig 6A, 7th panel). We have also performed the mammary gland transplantations in clear fat pad between wild-type and PIKE KO mice. Mammary gland network could be observed 8 weeks after transplantation in wild-type recipient transplanted with PIKE-null mammary tissues, suggesting PIKE-A is dispensable for pubertal mammary gland development. However, defective alvelolobulagenesis was observed during lactation in the wild-type recipient transplanted with PIKE null mammary tissues. In*

contrast, normal development of mammary gland was observed in PIKE KO recipient transplanted with wildtype mammary tissues mammary tissues (Fig7 A). These results strongly suggested the defective lactogenesis in PIKE-A knockout mice is epithelial cell autonomous.

6. The reviewer questioned if PIKE KO mice can nurse their pups and suggested to show the percentage of surviving pups born by PIKE-A wild-type and KO mothers.

As suggested, we have stated in the supplemental data that PIKE KO mothers were able to nurse the young as maternal behavior including pups licking, crouching and nest building were seen. We have also shown the pup surviving rate in Sup Fig 1E by dams of both genotypes.

7. The reviewer asked if PIKE-A interact with JAK2.

We have performed an immunoprecipitation in HEK293 cells overexpressing mGST-PIKE-A and JAK2 but we did not observed any detectable interaction between the two proteins (p.6). We have also performed the experiments suggested by the reviewer to test if JAK2 inhibitor affects the PIKE-A/PRLR/STAT5 binding. As shown in Fig 2C, pretreating HC11 cells with AG490 diminished the PRL-induced PIKE-A/PRLR interaction and the PIKE-A/STAT5 dissociation.

8. The reviewer suggested to perform all biochemical studies on mammary samples collected prior to parturition.

As suggested, results of biochemical analysis including Western blot analysis, Ki67 staining, TUNEL assay and immunostaining on wild-type and PIKE KO mammary tissues on 13.5 dpc and 18.5 dpc were included in the revised figures (Fig 4F, 5C, 5E and 6B). All the results point to the fact that enhanced apoptosis of occurs during the mid-gestation but differentiation defects present during lactation in PIKE KO mammary glands.

Referee #3

1. The reviewer point out an error that PIKE-A interacts with PRLR aa 486-608 but not aa398-608.

The error has been corrected.

2. The reviewer questioned about the effect of JAK2 on PIKE-A phosphorylation.

As stated in the manuscript (p.6), we do not detect any interaction between JAK2 and PIKE-A. Conceivably, JAK2 plays no detectable role, at least in the current study, on PIKE-A phosphorylation. However, the presence of JAK2 inhibitor inhibits PIKE-A/PRLR interaction, suggesting phosphorylation of PRLR by JAK2 is critical for PIKE-A association (Fig 2C).

3. The reviewer asked if PIKE-A knockodown affect the association of STAT5 with PRLR.

As shown in Fig 2D (1st panel), ablation of PIKE-A in HC11 reduces the PRL-induced STAT5/PRLR association. This results is further supported by PIKE-A KO mammary glands in which association of STAT5 and PRLR is reduced during lactation (Fig 6D). These results suggest that PIKE-A is critical for PRLR/STAT5 interaction.

4. Bottom panel on Fig 1E is replaced with a better gel picture showing equal expression of various myc-STAT5 truncates.

5. A loading control of β-tubulin expression is now included in Fig 2D. We have not observed any non-specific effect on PIKE-A knockdown using shRNA as expression of proteins like PRLR, STAT5 and tubulin were not changed.

6. The reviewer suggested an epithelial transplantation experiment to cleared fat pad to convince the reader of the mammary cell autonomous action of PIKE-A.

As suggested, we have performed the mammary gland transplantations in clear fat pad between wild-type and PIKE KO mice. Mammary gland network could be regenerated 8 weeks after transplantation in wild-type recipient transplanted with PIKE-null mammary epithelial cells, suggesting a normal development in non-pregnant status. However, defective alvelolobulagenesis was observed during lactation in the wild-type recipient transplanted with PIKE-null mammary epithelial cells. In contrast, normal development of mammary gland was observed in PIKE KO recipient transplanted with wildtype mammary tissues mammary tissues (Fig 7A). These results strongly suggest the defective lactogenesis in PIKE-A knockout mice is epithelial cell autonomous.

7. The reviewer suggested to state the source of anti-PRPR used and validate its specificity.

We have included the source of anti-PRLR used in the ìMaterial and Methodsî section. We have also tested the specificity of the antibody using PRLR knockout mammary tissues. As shown in Sup Fig 4, the antibody could recognize PRLR at correct size in wild-type but not PRLR KO tissues, suggesting the antibody indeed recognizes the PRLR in tissues.

8. The reviewer criticized the “cyclin D1 rescue of PIKE-A Knockout is not sufficiently well described”.

We have now extended our discussion on the effect of cyclin D1 overexpression on PIKE KO mammary tissues by performing experiments including IHC staining of milk proteins (-casein and WAP, Fig Sup Fig 6 E), measurement of pup weight gain (Sup Fig 6A), histological analysis (Fig Sup Fig 6B), biochemical analysis on various molecular markers (Fig 7E), proliferation (Fig Sup Fig 6C) and apoptotic analysis (Sup Fig 6F). These results suggested that overexpression of cyclin D1 overcomes the apoptosis induced by PIKE deficiency. However, the differentiation of mammary epithelial could only be partially rescued expression of milk protein (e.g. WAP) is still lower in PIKE null mammary.

2nd Editorial Decision 14 December 2009

Thank you for submitting your manuscript to the EMBO Journal. This is an invited resubmission of manuscript # 71083 that was rejected after review earlier this year. I asked the original referee #1 to review the resubmission and I have now heard back from this referee. As you can see below, this referee finds the manuscript improved. While the referee still has some concerns regarding why the phenotype is restricted to lactation, the referee also finds the manuscript overall interesting and is supportive of publication in the EMBO Journal. I am therefore pleased to proceed with the acceptance of the paper. You will receive the formal acceptance letter shortly.

Sincerely

Editor
The EMBO Journal

REFEREE REPORT

Referee #1:

This manuscript is considerably improved by the inclusion of additional data. I am still a little concerned that the phenotype is not evident until lactation while there is clearly an increase in cell death during gestation in the absence of PIKEA which is over-ridden by expression of CyclinD1.