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**Absence of Runx3 expression in normal gastrointestinal epithelium calls into question its tumor suppressor function**

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*Corresponding author: Yoram Groner, The Weizmann Inst. of Science***Review timeline:**

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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

10 June 2011

Dear Prof. Groner,

Thank you for the submission of your manuscript "Absence of Runx3 expression in normal gastrointestinal epithelium calls into question its tumor suppressor function" to EMBO Molecular Medicine. We have now heard back from the three reviewers whom we asked to evaluate your manuscript. You will see that they find the topic of your manuscript potentially interesting.

However, they also raise significant concerns on the study, which should be addressed in a major revision of the manuscript.

In particular, all reviewers highlight that it is essential to tone down the conclusions regarding the exclusion of a tumor suppressor function of Runx3 in the GI epithelium in absence of data directly refuting the tumor suppressor effect of Runx3. In addition, reviewer #2 points to some technical concerns regarding the expression analyses, which should be addressed to strengthen the study.

Given the balance of these evaluations, we feel that we can consider a revision of your manuscript if you can convincingly address the issues that have been raised within the time constraints outlined below.

Revised manuscripts should be submitted within three months of a request for revision. They will otherwise be treated as new submissions, unless arranged differently with the editor.

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

Yours sincerely,

Editor  
EMBO Molecular Medicine

#### REFEREE REPORTS:

Referee #1:

The authors exhaustively scrutinized the expression of Runx3 in mice with emphasis on the gastrointestinal-epithelial tract, using several knockin reporters and a range of antibodies. Their data convincingly show that the expression of Runx3 in this compartment is below the detection level with the methods employed. In this regard the observations are in contrast to an earlier report (Li et al 2002) in which expression of Runx3 in gastrointestinal epithelium was reported and loss of expression causally related to tumor development. The expression data between the two studies are very difficult to reconcile. The methods used in this report seem to guarantee a specificity and sensitivity of detection that is rather higher than lower than that reported in Li et al. and therefore the current study raises serious doubts about the correctness of the expression of Runx3 in the intestinal epithelium as reported by Li et al.

The work of Li et al has led to many subsequent studies in which a tumor suppressor function of Runx3 was further explored. In many of these studies lack of expression of Runx3 was associated with poor prognosis or it enhanced tumorigenesis in experimental settings. The authors now challenge the tumor suppressor function of Runx3 in epithelial tumors based on the lack of expression of Runx3 in gastrointestinal epithelium. Evidently, a tumor suppressor protein needs to be expressed to exert its tumor suppressing function. Although I subscribe that statement it does not permit the conclusion that Runx3 can not have a tumor suppressing role in gastrointestinal epithelium and other epithelial tissues. Firstly, expression of Runx3 might only be expressed when cells sense changes that are part of the tumorigenic process. There are ample examples for this, such as the induction of p16Ink4a, leading to senescence or induction of p14Arf as a consequence of oncogenic stress. These genes are not normally expressed in cells but nevertheless have been shown to act as very prominent potent tumor suppressors. Secondly, the tumor suppressing function could be exerted in only a small subset of the cells, e.g. those with self renewal capacity, and therefore the lack of expression in the epithelium does not exclude expression in a small progenitor compartment that serve as the driver of tumorigenesis. Thirdly, other cells in the stromal compartment, such as the Runx3 positive leukocytes, could influence tumorigenesis by paracrine mechanisms and loss of Runx3 expression could therefore also change the microenvironment in which tumor cell growth can flourish. Therefore, a tumor suppressor function of Runx3 is not refuted by the extensive expression analyses shown. Functional studies using Cre mediated deletion of Runx3 in the various cell compartments will be needed to prove that Runx3 expression does or does not exert a tumor suppressing function. The authors now jump to the conclusion that Runx3 does not fulfill that role based only on the lack of expression in the gastro-intestinal epithelium.

Therefore, on the basis of the data shown the authors need to be much more modest in their claims. Their conclusion based on the work reported in this manuscript cannot go further than the statement that in contrast to the report of Li et al. Runx3 is undetectable in the GI epithelium, indicating that the expression data of Li et al are likely not correct. Such statement is better communicated to "Cell" with the underlying evidence, hopefully leading to further scrutiny of the expression results in Li et al. In order to justify an separate paper as intended by this submission, more evidence should be provided that directly challenge the tumor suppressor effect of Runx3 in the GI tract.

Minor point.

- How was the experiment shown in fig. 5H precisely performed? It seems as if only a relatively small fraction of the CD45 positive cells express dTomato? The authors need to explain this more clearly, especially since one might argue that a high background staining might obscure a low level of expression.

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

This is summarized in the "remarks to be sent to the author" section.

Referee #2 (Other Remarks):

This study by Levanon et al. is important. Runx3 is believed to be expressed in the healthy GIT epithelium, and its loss is believed to play a role in GIT tumorigenesis. Here, the authors report the complete absence of Runx3 expression in the mouse healthy GI tract epithelium and question its role as a tumor suppressor. The data are of high technical quality, and the conjunction of ISH and IHC, as well as of LacZ and GFP reporter mouse strains, makes the conclusion that Runx3 is not expressed in the GIT epithelium rather convincing. There are, however, two points that absolutely need to be addressed before definitive conclusions can be drawn and before this study can be published.

1) Tissue expression data should be confirmed using the most conventional methods, i.e. RT-PCR, Northern blotting or Western blotting. As reported here, GIT epithelial cells seem devoid of Runx3 expression whereas intra-epithelial lymphocytes (IEL) express it. Extracts from whole intestinal tissue may thus not apply but, since the authors are able to sort EpCam+ epithelial cells versus CD45+ IEL, they should use such sorting, followed by real-time PCR to confirm lack of expression in the epithelial compartment.

In addition, the GI tract is an organ with a long rostro-caudal axis, along which gene expression may vary. Data should always consider several sites along this axis (small intestine and colon is a minimum; the small intestine segment should be indicated) and, when early lethality does not preclude it, the adult expression patterns should always be provided.

The authors should include data on Runx3 expression in a panel of mouse/human tumors and their adjacent healthy tissue to make the analysis more complete. Several of the literature references provided by the authors studied human tissues/tumors. This study should also consider the human situation that may differ from that of the mouse.

2) The authors are too hasty in their exclusion of a tumor suppressing function of Runx3 in the GIT epithelium, based on its lack of expression in this compartment. Previous studies have reported gene alterations in stromal cells (in fibroblasts: Bhowmick et al., Science 2004 ; Katajisto et al, Nat Genet 2008 ; in T lymphocytes: Kim et al., Nature 2006 ; in glial cells: Neunlist et al., Am J Physiol Gastrointest Liver Physiol 2006) that result in epithelial tumorigenesis. The conclusions of this study should take in account such scenarios.

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

please see review/remarks to the authors.

Referee #3 (Other Remarks):

This manuscript describes an interesting series of findings that undermines the logical/ conceptual foundation of an area of cancer biology, specifically the extensive research that describes the involvement of the Runx3 gene and more specifically its inactivation in the pathogenesis of a variety of tumors. This work traces its roots to the paper by Li et al published in 2002, in which was first demonstrated the expression of Runx3 in the gastric epithelium and its inactivation in stomach carcinomas. A critical element of this paper was, of course, the demonstrated expression of the Runx3 gene and protein in normal gastric epithelium. Indeed, if it is never expressed in such an epithelium, then its loss cannot be invoked to explain the runaway proliferation of gastric carcinoma cells. In the decade that followed almost 300 papers have been published in which the loss of Runx3 has been invoked to explain the pathogenesis of one or another type of human cancer, with the correctness of the 2002 report always being assumed. All the while, there have been those who have questioned the data of the original report, which is now reported in the manuscript under review, in which the current authors examine in great and rigorous detail the question of whether the results of the Li et al paper could have been correct and conclude with very persuasive data that it could not have been. Correctives such as this one are critical to science, which is, after all, a self-correcting discipline. (Unfortunately, negative results such as this one are not often reported, which leads, as is apparently in the present case, to significant parts of experimental research being built on

foundations of sand.) This ms. is definitely worthy of publication, with several minor editorial changes (see below). It is well done and will attract wide attention.

p.4. Top paragraph. The authors need to be more explicit here in stating that if Runx3 is never expressed in a cell type, loss of its expression in such a tissue cannot be invoked to explain mechanistically the pathogenesis of tumors in that tissue. While this idea is widely embedded in the thinking of many, this needs to be stated here clearly and explicitly (as well as in the Abstract).

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p.7 top ""by 35S-RISH" .. This is an abbreviation that few if any readers will be familiar with and should be spelled out.

The authors then proceed to use a series of rigorous and sensitive procedures that together constitute a robust body of evidence that Runx3 is not expressed in gastrointestinal epithelial cells. These include a series of immunohistochemistry approaches and yet others involving transgenic knock-in into the Runx3 locus. Taken together, one cannot fault the authors' basic conclusion; indeed the authors have gone far beyond the normal standard of proof to demonstrate the lack of expression of Runx3 in the initially claimed tissue. As their final sentence, the authors note that the present results "call into question" whether Runx3 functions as a Tumor Suppressor Gene (TSG) in other carcinomas. While it is certainly not essential for the publication of the present ms., it would be interesting if the authors could mention their own results here concerning their surveys of the tissues of embryonic and adult mice, more specifically whether they were or were not able to detect Runx3 expression in other normal epithelia beyond the GI tract.

1st Revision - Authors' Response

07 July 2011

Attached is our revised manuscript. We highly appreciate the constructive nature of the referees' comments and suggestions that we feel have much improved the manuscript. We have addressed every point raised by the three referees by providing explanations, new experimental data and/or making changes in the text as indicated in this letter. Specifically, we have substantially toned-down and qualified the conclusions regarding the exclusion of tumor suppressor function of Runx3, include and discuss the potential other scenarios mentioned by referees #1 and #2, performed the additional RT-qPCR analysis and IHC of human gastric tissues requested by referee #2 and incorporated all the corrections suggested by referee #3.

The following addressed each of the referees' comments.

Referee #1:

"The authors exhaustively scrutinized the expression of Runx3 in mice with emphasis on the gastrointestinal-epithelial tract, using several knockin reporters and a range of antibodies. Their data convincingly show that the expression of Runx3 in this compartment is below the detection level with the methods employed. In this regard the observations are in contrast to an earlier report (Li et al 2002) in which expression of Runx3 in gastrointestinal epithelium was reported and loss of expression causally related to tumor development. The expression data between the two studies are very difficult to reconcile. The methods used in this report seem to guarantee a specificity and sensitivity of detection that is rather higher than lower than that reported in Li et al. and therefore the current study raises serious doubts about the correctness of the expression of Runx3 in the intestinal epithelium as reported by Li et al.

The work of Li et al has led to many subsequent studies in which a tumor suppressor function of Runx3 was further explored. In many of these studies lack of expression of Runx3 was associated with poor prognosis or it enhanced tumorigenesis in experimental settings.

The authors now challenge the tumor suppressor function of Runx3 in epithelial tumors based on the lack of expression of Runx3 in gastrointestinal epithelium.

Evidently, a tumor suppressor protein needs to be expressed to exert its tumor suppressing function. Although I subscribe that statement it does not permit the conclusion that Runx3 cannot have a tumor suppressing role in gastrointestinal epithelium and other epithelial tissues.

Firstly, expression of Runx3 might only be expressed when cells sense changes that are part of the tumorigenic process. There are ample examples for this, such as the induction of p16Ink4a, leading to senescence or induction of p14Arf as a consequence of oncogenic stress. These genes are not normally expressed in cells but nevertheless have been shown to act as very prominent potent tumor suppressors.”

*This point is well taken. The idea that Runx3 acts as TSG via different mechanisms is interesting and was also raised by referee #2. We certainly agree that a scenario of TSG induction by an oncogenic stress is possible. That is to say, Runx3 is not expressed in normal GIT epithelium and acts as TSG upon its induction. However, as also pointed out by the referee, this possibility was not considered by Li et al who attributed their tumor suppressor claim to their finding of loss of pronounced Runx3 expression in normal GIT epithelium. Equally significant, this scenario was not implicated by any, not even a single one, of the 286 published papers that based their research on the correctness of Li et al findings and went on to postulate loss of Runx3 expression in normal GIT epithelium to explain the pathogenesis of various types of cancer.*

*We have now included and discussed this issue in the revised MS on page number 12.*

“Secondly, the tumor suppressing function could be exerted in only a small subset of the cells, e.g. those with self renewal capacity, and therefore the lack of expression in the epithelium does not exclude expression in a small progenitor compartment that serve as the driver of tumorigenesis.”

*The occurrence of this scenario is less likely for the following reasons: 1) Expression of LacZ and TdTomato by the Rosa26 locus is known to be highly efficient. 2) The R26-LacZ or R26-TdTomato expression is launched by of the cell specific Runx3-driven Cre. 3) Once set on, these reporter genes are constitutively expressed and permanently mark the progenitor/stem cell precursor populations throughout cell lineage development. 4) Nevertheless, we show that Runx3 expression is undetectable in embryonic and adult GIT epithelium even when the highly sensitive combination of enhanced expression of R26-LacZ or R26-TdTomato and FACS analysis is used. This issue is now further clarified on page number 9 of the revised MS.*

“Thirdly, other cells in the stromal compartment, such as the Runx3 positive leukocytes, could influence tumorigenesis by paracrine mechanisms and loss of Runx3 expression could therefore also change the microenvironment in which tumor cell growth can flourish”.

*This scenario suggests a non-cell autonomous function of Runx3 as TSG, which is certainly an interesting possibility. However, once again it was neither considered by Li et al nor by the numerous publications that based their research on the Li et al findings. Moreover, we (Brenner et al., 2004) and others (Naoe et al., 2007; Sugai et al., 2011) have previously shown that Runx3 is highly expressed in GIT leukocytes including CD8<sup>+</sup> T cells, DC and NK cells. Thus, the possibility that loss of Runx3 expression in leukocytes and/or in other stromal cells might change the microenvironment and promote tumor cell growth is well taken. However, as now explained in the revised manuscript on page number 13, loss of Runx3 in GIT leukocytes was not associated with any increase in the incidence of tumor development in these mice.*

“Therefore, a tumor suppressor function of Runx3 is not refuted by the extensive expression analyses shown. Functional studies using Cre mediated deletion of Runx3 in the various cell compartments will be needed to prove that Runx3 expression does or does not exert a tumor suppressing function. The authors now jump to the conclusion that Runx3 does not fulfill that role based only on the lack of expression in the gastro-intestinal epithelium.

*Experiments along the line suggested by the referee have been done. Several strains of mice lacking Runx3 activity in all cellular compartments, due to either germline ablation of Runx3 or due to inactivation of its essential partner protein Cbfb, were created and studied during past years (Brenner et al., 2004; Naoe et al., 2007) While Runx3<sup>-/-</sup> mice developed GIT hyperplasia and inflammation, none of these mice displayed increased tumorigenesis. Thus, the notion that Runx3 is a bona fide TSG was based on the claim that it is highly expressed in the normal healthy tissue.*

Therefore, on the basis of the data shown the authors need to be much more modest in their claims. Their conclusion based on the work reported in this manuscript cannot go further than the statement that in contrast to the report of Li et al. Runx3 is undetectable in the GI epithelium, indicating that the expression data of Li et al are likely not correct. Such statement is better communicated to "Cell" with the underlying evidence, hopefully leading to further scrutiny of the expression results in Li et al. In order to justify an separate paper as intended by this submission, more evidence should be provided that directly challenge the tumor suppressor effect of Runx3 in the GI tract."

*Point well taken. As already mentioned we have substantially toned-down and qualified the conclusions regarding the exclusion of tumor suppressor function of Runx3 and included in the text (page number 13) the potential other scenarios mentioned by the referee.*

"minor point.

- How was the experiment shown in fig. 5H precisely performed? It seems as if only a relatively small fraction of the CD45 positive cells express dTomato? The authors need to explain this more clearly, especially since one might argue that a high background staining might obscure a low level of expression."

*Our thanks for bringing this up. The referee is right, only a fraction of the GIT intraepithelial leukocytes express Runx3 as reflected in the low percentage of double positive CD45<sup>+</sup>/tdTomato<sup>+</sup> cells. Crossing of Runx3<sup>Cre</sup> mice onto R26-stop<sup>Floxed</sup>-tdTomato mice activates the reporter specifically in Runx3 expressing cells giving rise to constitutive steady-state levels of reporter expression solely determined by the activity of the R26 locus. Thus, expression of Runx3 in EpCAM<sup>+</sup> cells would have generated a tdTomato signal with intensity similar to that of CD45<sup>+</sup>/tdTomato<sup>+</sup> cells (i.e. 10<sup>4</sup> to 10<sup>5</sup>) well separated from the background staining of EpCAM<sup>+</sup> cells. The experiment in Figure 5H is now explained in more details in the text (page number 10).*

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

This is summarized in the "remarks to be sent to the author" section.

Referee #2 (Other Remarks):

This study by Levanon et al. is important. Runx3 is believed to be expressed in the healthy GIT epithelium, and its loss is believed to play a role in GIT tumorigenesis. Here, the authors report the complete absence of Runx3 expression in the mouse healthy GI tract epithelium and question its role as a tumor suppressor. The data are of high technical quality, and the conjunction of ISH and IHC, as well as of LacZ and GFP reporter mouse strains, makes the conclusion that Runx3 is not expressed in the GIT epithelium rather convincing. There are, however, two points that absolutely need to be addressed before definitive conclusions can be drawn and before this study can be published.

"1) Tissue expression data should be confirmed using the most conventional methods, i.e. RT-PCR, Northern blotting or Western blotting. As reported here, GIT epithelial cells seem devoid of Runx3 expression whereas intra-epithelial lymphocytes (IEL) express it. Extracts from whole intestinal tissue may thus not apply but, since the authors are able to sort EpCam<sup>+</sup> epithelial cells versus CD45<sup>+</sup> IEL, they should use such sorting, followed by real-time PCR to confirm lack of expression in the epithelial compartment."

*Point well taken. To confirm lack of expression in the GIT epithelial compartment as suggested, we have conducted TaqMan RT-qPCR experiments using RNA of FACS sorted adult GIT EpCam<sup>+</sup>*

*epithelial cells versus CD45<sup>+</sup> NK cells. The new data is presented in the revised Figure 5I and on page 11 and RT-qPCR procedure described under Material and Methods. The RT-qPCR analysis clearly shows that while Runx3 is readily detected in NK cells its expression is undetectable in GIT epithelium.*

In addition, the GI tract is an organ with a long rostro-caudal axis, along which gene expression may vary. Data should always consider several sites along this axis (small intestine and colon is a minimum; the small intestine segment should be indicated) and, when early lethality does not preclude it, the adult expression patterns should always be provided.

*We have recognized the importance of analyzing several sites along the rostro-caudal axis, as suggested by the referee, and have thus conducted the analysis using embryo (whole mount or sections of the complete GIT) and sections of adult GIT (both small intestine and colon). Detailed indications of the regions analyzed are specified in the relevant Figures' legends.*

“The authors should include data on Runx3 expression in a panel of mouse/human tumors and their adjacent healthy tissue to make the analysis more complete. Several of the literature references provided by the authors studied human tissues/tumors. This study should also consider the human situation that may differ from that of the mouse.”

*We certainly agree that analysis of tumors could be interesting. However, this falls outside the scope of this report, which specifically aimed to examine whether Runx3 is indeed highly expressed in normal GIT epithelium. In addition, as already mentioned above (answer to referee #1), while Runx3<sup>-/-</sup> mice spontaneously develop GIT hyperplasia due to inflammation the mice do not develop tumors. Therefore, as suggested by the referee; -making the analysis more complete-, we have included a new panel, (Fig S2 of Supporting Information), documenting the expression of RUNX3 in human gastric tissues. Using two different anti-RUNX3 antibodies the protein is clearly detected in the tissue embedded leukocytes, but not in the epithelium (Fig S2). Thus, similar to mouse RUNX3 is undetectable in human gastric epithelium.*

2) The authors are too hasty in their exclusion of a tumor suppressing function of Runx3 in the GIT epithelium, based on its lack of expression in this compartment. Previous studies have reported gene alterations in stromal cells (in fibroblasts: Bhowmick et al., Science 2004 ; Katajisto et al, Nat Genet 2008 ; in T lymphocytes: Kim et al., Nature 2006 ; in glial cells: Neunlist et al., Am J Physiol Gastrointest Liver Physiol 2006) that result in epithelial tumorigenesis. The conclusions of this study should take in account such scenarios.

*Point well taken. As suggested, we have now included and discuss these scenarios in the conclusions of the revised MS on page number 12 (please see also response to point #3 of referee No. 1).*

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

please see review/remarks to the authors.

Referee #3 (Other Remarks):

“This manuscript describes an interesting series of findings that undermines the logical/ conceptual foundation of an area of cancer biology, specifically the extensive research that describes the involvement of the Runx3 gene and more specifically its inactivation in the pathogenesis of a variety of tumors. This work traces its roots to the paper by Li et al published in 2002, in which was first demonstrated the expression of Runx3 in the gastric epithelium and its inactivation in stomach carcinomas. A critical element of this paper was, of course, the demonstrated expression of the Runx3 gene and protein in normal gastric epithelium. Indeed, if it is never expressed in such an epithelium, then its loss cannot be invoked to explain the runaway proliferation of gastric carcinoma cells. In the decade that followed almost 300 papers have been published in which the loss of Runx3 has been invoked to explain the pathogenesis of one or another type of human cancer, with the correctness of the 2002 report always being assumed. All the while, there have been those who have questioned the data of the original report, which is now reported in the manuscript under review, in which the current authors examine in great and rigorous detail the question of whether the results of

the Li et al paper could have been correct and conclude with very persuasive data that it could not have been. Correctives such as this one are critical to science, which is, after all, a self-correcting discipline. (Unfortunately, negative results such as this one are not often reported, which leads, as is apparently in the present case, to significant parts of experimental research being built on foundations of sand.) This ms. is definitely worthy of publication, with several minor editorial changes (see below). It is well done and will attract wide attention”.

“p.4. Top paragraph. The authors need to be more explicit here in stating that if Runx3 is never expressed in a cell type, loss of its expression in such a tissue cannot be invoked to explain mechanistically the pathogenesis of tumors in that tissue. While this idea is widely embedded in the thinking of many, this needs to be stated here clearly and explicitly (as well as in the Abstract)”.

*Our thanks for bringing this up. This notion is now more explicitly emphasized on page number 5 of the revised MS, but not in the abstract, due to space limitations.*

“p.5 It is not as clear as it should be that poly-SA represents an anti-Runx3 polyclonal antibody (which it apparently does)”

*The point that Poly-SA represents an anti-Runx3 polyclonal antibody is now clarified on page number 6.*

“p.6. "the group of Yoshiaki Ito.." It is not clear whether or not this represents that same group as the one that published the original Li et al paper (i.e., it is not obvious from the text up to this point). Was this indeed the case?”

*This point is now clarified on page number 6.*

“p.6 bottom "of the 7-anti-Runx3 Abs ... that we have tested" Why is there a hyphen after the "7"? what is meant here? “

*Was corrected*

p6. bottom "This conclusion is strongly supported ... " It's not clear precisely what conclusion is meant here by the authors.

*This is clarified on page number 6.*

p.7 top ""by 35S-RISH" .. This is an abbreviation that few if any readers will be familiar with and should be spelled out.

*<sup>35</sup>S-RISH is now abbreviated on page number 5 where it is first mentioned.*

“The authors then proceed to use a series of rigorous and sensitive procedures that together constitute a robust body of evidence that Runx3 is not expressed in gastrointestinal epithelial cells. These include a series of immunohistochemistry approaches and yet others involving transgenic knock-in into the Runx3 locus. Taken together, one cannot fault the authors' basic conclusion; indeed the authors have gone far beyond the normal standard of proof to demonstrate the lack of expression of Runx3 in the initially claimed tissue. As their final sentence, the authors note that the present results "call into question" whether Runx3 functions as a Tumor Suppressor Gene (TSG) in other carcinomas. While it is certainly not essential for the publication of the present ms., it would be interesting if the authors could mention their own results here concerning their surveys of the tissues of embryonic and adult mice, more specifically whether they were or were not able to detect Runx3 expression in other normal epithelia beyond the GI tract.

*We thank the referee for the constructive suggestion. Significantly, our detailed analysis have demonstrated that of the two family members Runx1 and Runx3 the expression of Runx1 is readily detected in a variety of epithelia including mucosa of the esophagus and stomach, the respiratory and olfactory epithelium and the salivary glands ducts and bronchi mucosa, whereas Runx3 was undetectable in any of these epithelia. These findings were incorporated with the relevant reference into the revised MS introduction on page number 3.*



Dear Prof. Groner,

Please find enclosed the final report on your manuscript. We are pleased to inform you that your manuscript is accepted for publication and will be sent to our publisher to be included in the next available issue of EMBO Molecular Medicine if or once we have received your licenses (see below).

Please see below for additional IMPORTANT information and instructions regarding your article, its publication, and the production process.

Congratulations on your interesting work.

Yours sincerely,

Editor  
EMBO Molecular Medicine

REFEREE REPORTS:

Referee #2 :

This is an excellent study that clarifies an important point in the field of oncology. The authors significantly improved their manuscript, which is now, in my opinion, suitable for publication.