

## **Response to the reviewer's comments.**

### **Reviewer 1**

The review “Role of RUNX3 in Restriction Point Regulation” reports the role of R-point participates in oncogenic surveillance. Here are the comments for the author.

1. The abstract should be modified because authors have started the abstract using this line “Throughout the cell cycle, cells monitor their cumulative exposure to specific signals over time and make the critical decision to pass through the restriction (R)-point.”. Here, authors should start with cell cycle and then discuss about the R-point. So that any reader can understand easily.

**Answer:** As the reviewer suggested, we changed abstract as follows. “A cell cycle is a series of events that takes place in a cell as it grows and divides. At G<sub>1</sub> phase of cell cycle, cells monitor their cumulative exposure to specific signals and make the critical decision to pass through the restriction (R)-point.”

2. The sentence “Deregulation of this machinery is markedly associated with tumorigenesis, making the identity of the molecular mechanisms that govern R-point decision making one of the fundamental issues in tumor biology” should be rephrases and splited into two sentences as it is hard to understand in current format.

**Answer:** As the reviewer suggested, we changed abstract as follows. “Deregulation of this machinery is markedly associated with tumorigenesis. Therefore, identification of the molecular mechanisms that govern the R-point decision is one of the fundamental issues in tumor biology.”

3. Are authors sure “*RUNX3* is frequently inactivated in tumors by epigenetic alterations.” That only one transcription factor is responsible for that? What about other transcription factors?

**Answer:** We don't think that only one transcription factor is responsible for that, therefore, we changed the sentence as follows. “*RUNX3* is one of the genes frequently inactivated in tumors by epigenetic alterations.”

4. Please use the consistent cases throughout the manuscript like here is one example check “*Runx3*”, “*K-Ras*” in the sentence “In particular, *RUNX3* is downregulated in most *K-RAS*-activated human and mouse lung adenocarcinomas (ADCs). Targeted inactivation of *Runx3* in mouse lung induces adenomas (ADs) and markedly shortens the latency of ADC formation induced by oncogenic *K-Ras*.”

**Answer:** We followed general rules for the capitalization of human and mouse genes.

*Human genes:* all letters are in upper case (*RUNX3* and *K-RAS*)

*Mouse genes:* first letter upper case all the rest lower case (*Runx3* and *K-Ras*)

5. Authors have used keywords “ARF”, and “p21” both have not discussed in abstract.

**Answer:** We deleted “ARF”, and “p21” in the keywords.

6. The sentence “In normal cells, reductions in growth factor levels, such as by the removal of serum, prevent the onset of S-phase.” Do not have any reference even it is not appropriate to use word “normal cells” this prevention does with the cell lines also. Here are the link of studies which have shown the addition/ removal of growth factors affect the cell cycle. In addition, serum deprived media and other factors like hydroxyurea/ 2DG arrest the cell cycle at G1 stage. These studies must be discussed here.

<https://doi.org/10.1016/j.lfs.2018.09.055>

<https://doi.org/10.1371/journal.pone.0206364>

**Answer:** We removed “normal” from the sentence and attached appropriate reference as follows. “Reductions in growth factor levels in cells, such as by the removal of serum, prevent the onset of S-phase (Pardee, 1974)”.

7. The statement is misleading “*RUNX3*, which plays pivotal roles in lineage determination and functions as a tumor suppressor, is frequently inactivated in various types of human cancers, including stomach and lung cancers”. However, PU.1 is a transcription factor that also a tumor suppressor in acute myeloid leukemias. PU.1 have also important role in lineage determination, wherein when interact with GATA-1 it leads to erythropoiesis and when it interacts with c-Jun it leads to myelopoiesis. So, both studies should be discussed important to know it in addition to *RUNX3*.

<https://doi.org/10.1038/sj.onc.121100410.2147/IJN.S303235>

**Answer:** We aware of the importance of PU.1 in hematopoietic lineage determination. However, we could not find any paper reporting relationship between PU.1 and R-point. Since this review focuses on the role of Runx3 in R-point regulation, we did not mention about the PU.1.

8. It seems that Figure 2, and Figure 6a are taken from any other paper, then authors should insert the reference in the figure caption.

**Answer:** We removed the Figure 2, because it is not essential.  
We inserted reference for Figure 6a.

9. Figure 3 is very hard to follow. So, authors should provide the heading at the top of each section and mention clearly what they wanted to convey in the figure. The figure should be self-explanatory.

**Answer:** we provide subheadings for the Figures as follows.

Figure 3A: In response to mitogenic stimulation, *RUNX3* opens target loci.

Figure 3B: RUNX3 forms R-point-associated RUNX3-containing Activator (Rpa-RX3-AC) complex at the R-point.

Figure 3C: Rpa-RX3-AC complex is converted to Rpa-RX3-RE after the R-point.

10. The heading “Mechanism for the Induction of R-Point-Associated Genes at the R-Point” is confusing, so it should be changed.

**Answer:** The R-point-associated genes are transiently induced at the R-point and then, quickly suppressed. We explained this phenomenon into two sections with the headings “4. Mechanism for the Induction of R-Point-Associated Genes at the R-Point” and “5. Mechanism for the Suppression of R-Point-Associated Genes after the R-Point”. We are sorry, but we think these headings are appropriate for these sections.