Answers to reviewers comments

We are thankful to the reviewer for their critical comments. This has helped us to enhance the quality of the manuscript. The point wise response to each comment is appended below. We hope that the edited manuscript will now be suitable for publication in your esteemed journal.

Reviewer #1:

1.Please explain the distinguish of LHRH(6leu)-LTB, LHRH(6leu)-LTB proteins, LHRH and LHRH fusion protein. It really confused us.

The terms **LHRH(6leu)-LTB, LHRH(6leu)-LTB proteins, LHRH and LHRH fusion protein** mean the same thing. We have now changed to LHRH(6leu)-LTB protein at all places in the manuscript.

2.From fig.1, we found that the volume of tumor was significantly suppressed by survivin at a does of 20 ug. While, increase in vaccine dose to 50 μg did not lead to any significant inhibition in tumor volume. Please explain which is dose-inhibition related effects and the potential mechanism.

The mechanism of dose inhibition has not been elucidated yet. However, it is already known from literature that increasing the dose after an optimum level reduces the bioefficacy of the drug/ antigen. Relevant studies have been quoted as Reference: 29-31 of the manuscript.

3. What's more, there are only two groups of survivin in fig.1. Thus, we could not get the conclusion that there is a dose-inhibition related effects. We think there is at least 5 group of different does of survivin.

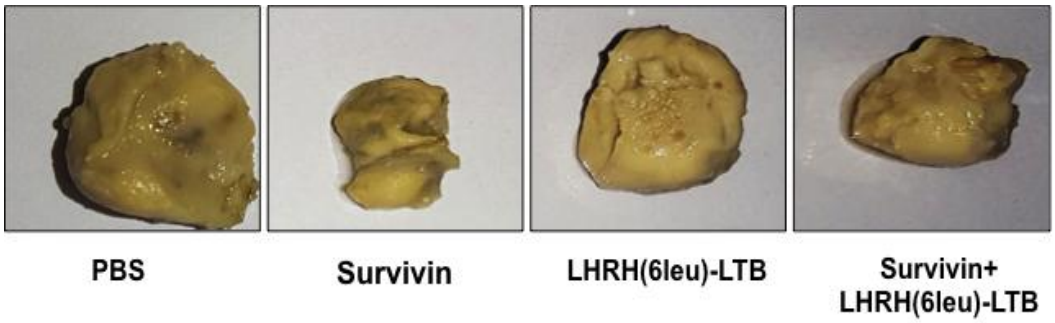
Two other doses, 5ug and 10ug, have already been tested for their tumor suppressive effects in 4T-1 model and results have been previously published by authors in another journal (Reference: 16 of the manuscript; Garg H, Gupta JC, Talwar GP, Dubey S. Immunotherapy approach with recombinant survivin adjuvanted with alum and MIP suppresses tumor growth in murine model of breast cancer. Preparative biochemistry & biotechnology. 2018:1-6. Epub 2018/01/23).

4.From the figure 2, we confused that which take the main role in suppressing tumor volume. Please explain the relationship among svrvivin, anti-survivin antibodies and IFN- levels.

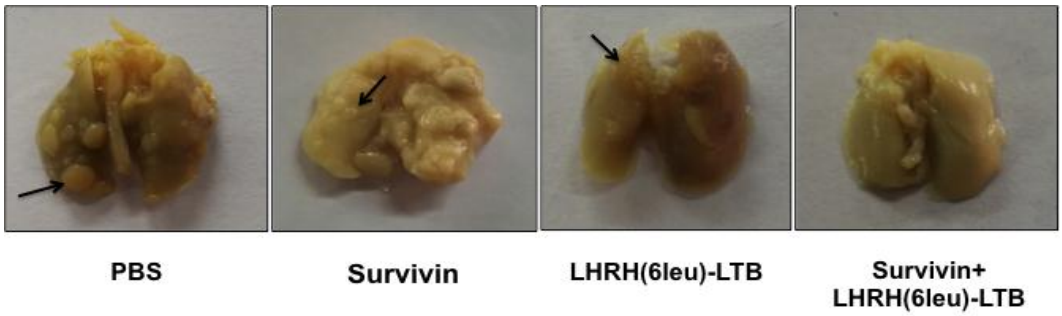
The molecular mechanism of tumor supression has not been elucidated yet. We hypothesize that both anti-survivin humoral response and Th1 cytokine response indued after immunization has a role in tumor suppression.

5.Please provided the figure of gross tumor and metastatic lung tumor.

a) Representative images of primary tumor in each immunization group



b) Representative images of metastatic lung tumor in each group of mice



Reviewer #2: Dear editor, The manuscript is well written and deserves publication. Below is some minor issues: -

Abstract section: In methods the authors should give details of the study groups.

Necessary correction has been done.

Abstract section: In results the authors should give their important findings with p values.

Necessary correction has been done.

Abstract section: Comments in results should be transferred to conclusion.

Necessary correction has been done.

Core tip: The authors should provide their important findings

Necessary correction has been done.

Discussion section: The authors should mention about the limitations of the study.

Necessary correction has been done.

Reviewer #3: My pleasure to review the article by Garg et al. The manuscript is well-written. I have the following suggestions for further improvement of the paper:

#1. The term, LTB, should be introduced as a heat-labile enterotoxin of E. coli at the first appearance in the main text.

The term has been introduced as suggested by the reviewer.

#2. The statement, 'We reported previously that the recombinant Survivin vaccine is efficacious in preventing the growth of 4T-1 based murine model of breast cancer (13).' was tagged with a wrong citation. Reference #13 is an article written by none of the current authors.

The reference has now been corrected.

#3. In view of the above, all other references should be checked for appropriateness and correctness.

All references have been checked.

#4. Page 11. Second paragraph, Line 3. Please add premenopausal before breast cancers. In the human model, LHRH agonists are used in premenopausal women only.

Necessary correction has been done in the manuscript.

#5. Figure 2. The legend. Interferon-gamma, not interferon-y.

Necessary correction has been done in the manuscript.

#6. Table 1. In the first row of Table, Right column, please add 'lung' before nodules.

Necessary correction has been done in the manuscript.

#7. Because the authors have previously published several papers regarding the basic science innovative research using two recombinant proteins, Survivin and luteinizing hormone-releasing hormone fusion protein [LHRH(6leu)-LTB] for immunotherapy of breast cancer, I would challenge the authors in this paper to draw a colorful diagram showing the mechanistic steps from reconstruction of the recombinant proteins to act as a vaccine targeting what pathway in breast cancer. This figure can be put into the Discussion section to enrich the entire article.

We have added a figure showing the steps involved in the approach used in the study and added it as Figure 5 in discussion section of the manuscript.

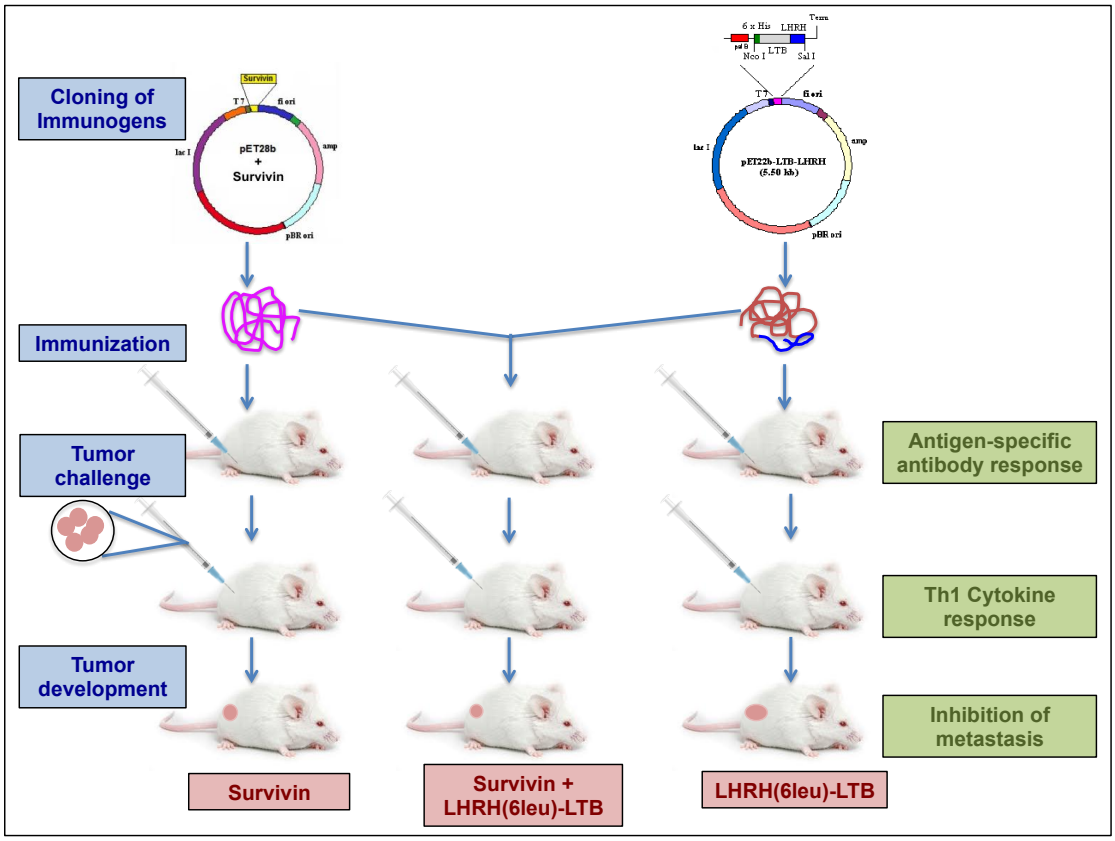


Figure 5: Schematic representation of the study of anti-tumor effect of Survivin, LHRH fusion protein individually and in combination. The study was conducted on four groups of mice: Untreated or control, Survivin alone, LHRH(6leu)LTB alone and Survivin+LHRH(6leu)LTB. Individual recombinant proteins were purified and used as immunogen along with MIP as an immunomodulator in

murine model. The mice from all the groups were further challenged with tumor cells, 4T1 and tumor development was observed in each group. LHRH fusion protein was not effective in suppressing tumor alone. However, the combination of both Survivin and LHRH(6leu)LTB inhibited tumor growth substantially followed by Survivin alone.