Dear Editor,

Thank you very much for decision letter along with the reviewer’s comments for our manuscript No.: ijms-414213

The reviewers requested additional data modification, and more clarification. They also gave us several excellent suggestions which would strengthen our manuscript. We thank for reviewer’s constructive criticisms.

Please find some of our changes in revised MS. as highlighted in red in marked version.

Our point by point response to the comments of the reviewer is as follows:

Reviewer 1:

Comments and Suggestions for Authors Authors did not perform any further experiments based on comment 1 and 2. Lacking evidences are for conclusions.

Comment 1, In general, the mechanistic study of this MS is not deep despite many data. There are lacking evidences for title. How about effect of mTOR/p70S6K/4E-BP1 and Raf/MEK/ERK inhibitor or siRNA transfection on proliferation and HIF1 alpha in HCT116 cells under hypoxia?

Thanks to the reviewer’s suggestions. We have used the mTOR inhibitor (rapamycin) and MEK inhibitor (PD98059) to examine HIF-1α expression level and clonogenicity in HCT116 cells. The results showed that the vanillic acid (30 μM), or rapamycin (100 nM) suppressed the expression of HIF-1α, which was further inhibited by the combination of vanillic acid and rapamycin (Fig. 4C and 4G). Comparable results were obtained in Fig. 4D and 4H, vanillic acid (30 μM) or PD98059 (50 μM)
suppressed the expression of HIF-1α, which was further inhibited by the combination of vanillic acid and PD98059.

Fig. 6F results showed that vanillic acid (30 μM) or rapamycin (100 nM) suppressed clonogenicity of HCT116 cells, which was further inhibited by the combination of vanillic acid and rapamycin. Comparable results were obtained in Fig. 6G, vanillic acid (30 μM) or PD98059 (50 μM) suppressed clonogenicity of HCT116 cells, which was further inhibited by the combination of vanillic acid and PD98059. And the results are as follows.
Comment 2. Show antiproliferative effect of vanillic acid in other colon cancer lines under hypoxia. You just showed its effect only on HCT116 cells?

We thank the reviewer’s suggestions. We have examined the effect of vanillic acid on proliferative in other colon cancer lines by performed western blot assay and clonogenic assay. The results showed that vanillic acid dose-dependently reduced HIF-1α protein expression induced by 1% O₂ in SW620 cells (Fig. 2C and Fig. 2F). Fig. 6E results showed that vanillic acid significantly inhibited the clonogenicity of SW620 cells. And the results are as follows.
**Figure E**

Images showing different conditions:
- 20% O₂
- 1% O₂
- 1% O₂ + Van (3 µM)
- 1% O₂ + Van (10 µM)
- 1% O₂ + Van (30 µM)

**Figure F**

Graph showing relative density of cells under hypoxia control conditions.