Dear Dr. Dsouza,

We would like to thank you for your effort in reviewing our manuscript entitled “Establishing of Cell Lines Overexpressing DR3 to Assess the Apoptotic Response to Anti-mitotic Therapeutics” (JOVE-58705).

We are grateful for your comments that has helped us make a better revision. Below please see the point-by-point response to your editorial comments. We have also made changes in the manuscript accordingly and highlighted the major changes.

We hope that you will find the revised manuscript acceptable for publication in *JOVE*.

Sincerely yours,

Gelin Wang, Principal Investigator

School of Pharmaceutical Sciences

Tsinghua University

Beijing 100084, China

Tel: (86) 10-62798919

E-mail: gelinwang@tsinghua.edu.cn

Below is the point-by-point response to the editor’s comments.

*1 About the grammatical errors:* A native English speaker has thoroughly revised the manuscript for grammatical errors.

*2 About the license agreement:* The license agreement has been signed and uploaded.

*3 About the reference:* We added the references as requested.

*4 In vivo experiment:* We did test the response of HT29 and HT29-DR3 xenograft models to paclitaxel in our previous work, and the results are referred to *Qi, et al. 2018* (reference 14).

*5 About the molecular mechanisms:* The HT29-DR3 cells system offered us an important tool to study the mechanisms by which anti-mitotic drugs induce apoptosis.

*6 About the MOI:* We did not measure the MOI in this protocol.

*7* The note after step 1.6 was changed to step 1.7.

*8 The size of the cloning cylinder:* The diameter and height of the cylinder are both 8 mm.

*9 Step 1.1:* The details of constructing pMXs-IRES-Blasticidin-DR3 were added.

*10 Step 1.2:* The transfection procedure and the reference were added.

*11 Step 1.6:* The information of blasticidin has been added to Table of Materials.

*12 Step 1.9:* To inactivate trypsin, add 70 µL DMEM to each cylinder.

*13 Step 2.1:* The cell lysates are transferred from the 24-well plate to 1.5 mL tubes before boiling.

*14 Step 2.6:* The information of anti-mouse secondary antibody was added to Table of Materials.

*15 add Step 2.9:* The positive clones are frozen and stored at -80 ℃.

*16 The note before Step 3.1:* The drugs were dissolved in DMSO to make 10 mM stocks and then further diluted to different concentrations: 30 nM,100 nM, 300 nM,1000 nM, 3000 nM and 10000 nM in DMSO.

*17 Step 3.1:* The cells were counted using an automatic cell counter (Table of Materials).

The cell morphology was observed under a microscope at 10X magnification.

*18 Step 3.3:* The cell viability represents the relative luminescence intensity of each well to that of the control well treated with 1% DMSO.

*19 Please add results to demonstrate the outcomes from the in vivo paclitaxel treatment. What was the tumor implantation success rate for HT29 and HT29-DR3? What are the observations from the paclitazel treatment in these groups? Please discuss the significance.*

Instead we only add a note for the in vivo paclitaxel treatment, as the results have already been published in our previous paper (Qi et al., 2018). The tumor implantation success rate for both HT29 and HT29-DR3 are 100%. The xenograft tumors progressed similarly in the vehicle control, however, the HT29-DR3 xenografts displayed faster tumor regression than the HT29 xenografts when treated with paclitaxel at a dose of 20 mg/kg. Our observation of a better response of HT29-DR3 tumors to paclitaxel than that of HT29 tumors *in vivo* further confirmed that ectopic expression of DR3 renders tumor cells more sensitive to anti-mitotics.

*21 Step 3.4.1:* The procedures how to establish xenograft models were described in detail and the reference was added.

*22 Step 3.4.2:* The tumor volumes were measured by calipers.

*23 Step 3.4.4:* In the animal experiments, the dose of paclitaxel was 20 mg/kg. We euthanized the animals after injection of paclitaxel for two weeks, otherwise, terminate the experiment and euthanize the animals if the body weight decreases by 20% or the tumor volumes reach 2000 mm3.

*24* We added our funding sources in the Acknowledgements part.