**Editorial comments:**  
  
The manuscript has been modified and the updated manuscript, **58614\_R1.docx**, is attached and located in your Editorial Manager account. **Please use the updated version to make your revisions.**  
  
**1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.**

*Author response: We have proofread the manuscript and fixed spelling and grammatical mistakes.*

**2. Please remove all headers from Representative Results.**

*Author response: The headers from Representative Results have been removed from the manuscript as suggested.*

**3. JoVE cannot publish manuscripts containing commercial language. This includes company names of an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. Examples of commercial language in your manuscript include SYBR Lo-ROX, Trajan Scientific and Medical, etc.**

*Author response: Generic terms have been adopted in the manuscript and all commercial language has been moved to the Table of Materials and Reagents.*

**4. Please provide the corresponding author and email of all authors in the manuscript.**  
*Author response: Email addresses have been provided for each author and the corresponding author has been added at the end of the section.*

**5. Step 5.7: Calculation steps without graphical user interface cannot be filmed. For steps that are done using software with graphical user interface, a step-wise description of software usage must be included in the step. Please mention what button is clicked on in the software, or which menu items need to be selected to perform the step.**

*Author response: A step-wise description of software usage has been provided under step 5.7 in the Protocol section (see below)*

*‘5.7. Calculate the RNA concentration of samples by interpolating from a standard curve prepared from templates with known concentrations.*

*5.7.1. Create a new experiment in the software.*

*5.7.2. Click ‘Define and Set Up Standards’ to set up serial dilution for plates.*

*5.7.3. Select and arrange the wells for standards and samples. Click ‘Apply’.*

*5.7.4. Click ‘Analyze’ in the Result tab to assess the standard curve. Confirm the slope, R2 value, amplification efficiency and error meet the experimental criteria.*

*5.7.5. Check the quantities of unknown samples visually on the standard curve based on their Ct values.*

*Note: The construction of standard curve, including choice of dilution factor, is performed according to published protocols11–13.’*

**6. 5.8: Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.**

*Author response: A step-wise description has been provided to detail how step 5.8 is performed, and references to published materials about the approach have been added (see below).*

*‘5.8. Perform gene expression analysis of mRNA by using the ΔCt method, Ct that is normalized to a refence gene.*

*5.8.1. Subtract the Ct value of target gene from the Ct value of reference gene to obtain the ΔCt value.*

*5.8.2. Average the ΔCt values of technical replicates.*

*5.8.3. Plot and analyze gene expression with statistics software11,14 (see Table of Materials).*

*Note: Alternatively, the gene expression analysis is performed according to published protocols13.’*

**7. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.**  
*Author response: All trademark and registered symbols have been removed from the Table of Equipment and Materials.*

**8. Please revise the table of materials to include all essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.**

*Author response: The Materials and Reagents table has been revised to contain the above information in an xls file (see attachment ‘Lei et al JoVE\_Materials\_09-08-2018.xls’).*