Reviewer’s report

**Title:** Short hairpin RNA targeting 2B gene of coxsackievirus B3 exhibits potential antiviral effects both in vitro and in vivo

**Version:** 3  **Date:** 12 June 2012

**Reviewer:** Andreas Henke

**Reviewer’s report:**

Minor essential revisions:
1. Page 3, line 8: Do you really think that CVB3 “is among the most common and significant agents of infectious illness in humans”? What about HIV, HCV, HEV, influenza virus, Mycobacterium tuberculosis, denguevirus etc. etc.
2. Please look carefully at your text again, especially in view of missing blanks (e.g. page 5 line 9) or unnecessary words (e.g. page 21, line 11 “CVB3”).
3. Please provide a reference for the virus strain (CVB3M), which you received from Dr. Huber.
4. Page 6, line 14: The headline of this paragraph does not really fit the text. Here, you describe how the mice were treated and when the samples were taken. You are not describing the myocarditis model.
5. Page 7 line 6: Here it is better to write: “In the supernatants, infectious virus were analyzed by.....”
6. Page 7, line 11: From where did you receive you VP1 antibody?
7. In general, please state in the text how often each experiment was repeated and what is demonstrated at each figure (mean +/- SD of all experiments or a representative result?).
8. Figure 3 A and B: Here a better labeling of the y-axis would be “Surviving animals [%]”, right now you are using “percent” twice.
9. Page 20, line 18 and page 21, 3: Here, I guess, you mean “weighted” instead of “weighed”.

Major compulsory revisions:
1. Figure 1 A and B: Please provide mean +/- SD concerning these experiments. Figure 1B: Here, the distance on the x-axis between 18-24 h (6 h) and 24-36 h (12 h) is identical. Please adjust the diagram. Figure 1 A and B: Please provide a precise description how the transfection rate was determined. Figure 1E pGCL-NC: Please state that the identical picture is demonstrated via LM and FM. It seems that not the same section is demonstrated (also Figure 2B Lenti-NC). Figure 1 and 2: How was the transfection/transduction rate between your 2B expressing systems and the controls? Any differences?
2. Results section, page 10, line 9 starting “Inhibition of CVB3 replication in coxsackievirus-induced myocarditis model”. Dose-response analyses would be
much better. In your response to my comment during the first review process you mentioned that you tried other dosages as well. Why did you omit this in the revised manuscript? If you don’t get any protection if CVB3 is applied prior or at the same time as the treatment started what is the meaning of the experiments at all? Nobody is going to inject people with lentiviruses prior any infection.

3. Figure 6: Here, please give an exact description what “untreated control” means (which sample, why only IHC-CVB3).

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.