**Reply to Reviewers and Editorial comments:**

**Changes to be made by the author(s) regarding the manuscript:**

1. What is the ‘SETUP METHOD’ section for? It is not referenced anywhere else. Please either continue the protocol with these steps or provide as a separate supplemental section (in a different file).

*The “setup method” is thought to be performed to find the best working condition. It is not necessary to repeat these steps if EV type and antibodies do not change. However we think that are important before to start analysis, so we changed the numeration and included this part in the main protocol.*

2. It is unclear which samples NTA quantification are done for. Right now, you direct users to possibly skip to sample prep (the current step 4) if an ultracentrifuge is not available.

*It is expected to skip the ultracentrifuge step but not the total number quantification by NTA (or alternatively by protein quantification). This was not clear enough before, now we edited the text accordingly.*

3. Figure 2A: You show gating for both beads and singlets here, but only mention gating for singlets in the legend; please clarify.

*Legend has been now revised accordingly.*

4. Figures 3E,F and 4: Please define the error bars in the legends. Also, what is N in Figure 4?

*Legend has been now revised accordingly.*

5. Tables 2-3: Please use US standards for decimals here (periods, not commas). Also, what are the errors?

*Tables has been now revised accordingly.*