Response to reviewer comments on JoVE50523R3: “**Metabolic labeling of leucine rich repeat kinases 1 and 2 with radioactive phosphate**” by Jean-Marc Taymans, Fangye Gao, Veerle Baekelandt. A detailed account of the comments and action taken is given below.

**Reviewers' comments:**

**Reviewer #1:**

*Minor Concerns:*

The authors should list the equipment used, for example it isn't clear what transfer apparatus they are using and steps such as the transfer top do will vary considerably between different semi dry transfer modules. The authors should also take into account that people carrying out the protocol may not have access to exactly the same equipment and make the protocol as generalisable as possible.

**Response**: The authors agree that the protocol should be as generalisable as possible and several brand names of products have for this reason already been removed from the manuscript text. The authors have also now removed the mention of the NuPAGE brand of SDS PAGE gels as gels with the given compositions can be obtained from several different suppliers. The name of the used semi-dry apparatus has now been mentioned, as well as further information on compatibility with other semi-dry blotters as well as tank blotters.

**Reviewer #2:**

*Minor Concerns:*

I think the authors need to add the necessary caveats of the meaningfulness of total phosphorylation vs site specific phosphorylation. Total phosphorylation may tell little of LRRK2 function activity whereas specific sites may be more important. In addition, the authors have selected Flag tagged LRRk2 and M2 resin as it could be applicable to other tagged proteins, however, if they were to also describe a LRRK2-specific antibody pulldown, then at least the method would apply to endogenous LRRK2. Without that, this method is only useful for a crude measure of the total phosphorylation of over-expression LRRK2 (or other protein), limiting physiological relevance. Perhaps total phosphorylation only reports proper folding of the protein when compared to various mutants, for example.

**Response**: The authors agree with these points. There are now several reports on phosphosites of LRRK2 and clues are beginning to emerge indicating different functional roles for different phosphosites. Mention has been made of this in the text. The mention of a LRRK2-specific antibody pulldown is indeed a good idea and has already been mentioned in the discussion text. In this revision, we now also include a recent publication comparing 10 different monoclonal anti-LRRK2 antibodies including for immunoprecipitation of endogenous LRRK2. We would find no mention of whether total phosphorylation is a marker for protein folding, however as mentioned in the text, this method has the advantage over phospho-antibody based methods to detect phosphorylation in that different proteins can be quantitatively compared for their total phosphorylation levels given that incorporated radioactive phosphates show the same activity from one protein to another. The discussion text has been modified to take these points into account.

**Reviewer #3:**

*Minor Concerns:*

1) Section 1.1.4.2: It is not clear how much lentivirus was used for transduction experiments.

**Response**: Twice as many transducing units, ie functional vector particles, should be added relative to the number of cells in the culture vessel. This information has been added to point 1.1.4.2.

2) Section 1.2.13: It could be nice if authors used the kinase inhibitor (for example IN1 for LRRK2) and demonstrated decrease in overall phosphorylation as an example/control.

**Response**: The authors agree that this would be useful to show, however this result has currently been included in another manuscript in submission and we are therefore not at liberty to include this result here.

3) Section 2.1.6. It is important to use phosphatase inhibitors not only in lysis buffer but also in washing buffers during IP procedure, since absence of phosphatase inhibitors may result in protein dephosporylation by phosphatases. It is possible that this might not be critical for LRRK, but might be critical for other proteins assayed using this technique.

**Response**: The authors agree that this is a useful extra measure to increase sensitivity of the detection. The same holds true for protease inhibitors. This information has been added to section 2.1.6.

4) Lines 416, 432: 3xflag is not labeled consistently throughout the manuscript (lowercase vs. uppercase letters)

**Response**: Where needed, the text was modified for the 3xflag to be consistently labelled throughout the manuscript.

5) Punctuation is not consistently used throughout the article. Please consider whether full stop should be used for the following lines: 116, 121, 123, 126, 131, 140, 186, 188, 203, 211, 225, 244, 246, 249, 253, 259,268, 282, 284, 286, 289, 291, 298, 311, 313, 316, 339, 344, 349, 354, 356, 367, 373, 378, 381, 383, 385, 387, 394, 398, 405. Please check 324 for a possibly missing comma.

**Response**: The punctuation has been reviewed for consistency. In particular, full stops have been included in protocol steps, as well as a comma at line 324.

6) Typo in figure 1, line 497, "comparative". Consider changing for "comparative" or another equally powerful word.

**Response**: This typo has been corrected.