Point by point response to the reviewers' comments

We thank both reviewers for the time they spent and for their valuable comments! Our reply to the remaining minor points is appended below.

Reviewer 1

COMMENTS FOR THE AUTHOR:

The new experiment that the authors performed at my request further supports their primary claim. Also, the new text about existing method for controlling FDR is also very helpful in clarifying how the proposed method differs from these existing approaches.

We thank the reviewer for the support.

1. The text or supplement should state how the FDR estimation is done in the context of the cleaning procedure. I assume that the standard Elias & Gygi approach is used, but this should be stated explicitly.

We would like to draw the attention of the reviewer to the bottom of page 9 in our manuscript (section "Identification of conflicting PSMs"). We write:

"*Spectra were searched against a concatenated database, which contains all candidate proteins plus control proteins with pseudo-reverted sequences as previously described (23).*"

The cited reference (Cox and Mann, 2008) contains a detailed description of how pseudo-reversed decoy sequences are generated:

"This decoy approach allows for a straightforward assessment of the likelihood of false positive identifications. However, a problem with the reverse database approach is that it creates peptides that have precisely the same composition as the peptides in the forward database. This happens in half the cases with tryptic peptides and almost all peptides in the case of LysC peptides. Thus the reverse database overestimates the number of random hits, especially for very high mass accuracy data. Several remedies have been suggested, for example randomizing the sequences. However, these procedures change the local relationships between amino acids and may lead to a different length distribution. Here we avoid both problems by constructing the decoy database in two steps. First we reverse all sequences as before. In a second step, we swap each arginine and each lysine with the preceding amino acids (or only each lysine in the case of LysC). The decoy peptide database constructed in this way has the same mass and amino acid distribution but avoids the spurious repetition of the exact same mass values that are in the forward database."

2. One minor question arose as I looked over this "cleaned" approach. In figure 5a, I don't understand what the three different rectangles with removed upper-left corners represent. Are those supposed to be three different .RAW files, each containing spectra? I'm not sure what the

purpose is of representing this three-fold structure in the figure. This should either be mentioned and explained in the caption or removed from the figure.

The reviewer is right: the rectangles in the upper left hand corner represent different raw files. Otherwise, the three-fold structure has no specific meaning. We therefore changed the figure as suggested. We added text to the figure indicating that we are referring to different raw files. For all other lists we removed the three-fold structure.

3. Also, the final step in Figure 5 ("FDR adjustment") should probably say something like "FDR estimation."

We changed the text in the figure to “FDR filtering”. We think this is more adequate than “FDR estimation” because here the lists are filtered to yield results at the desired FDR level.

Reviewer 3

COMMENTS FOR THE AUTHOR:

This revised manuscript continues to be well written, describes thoughtfully executed examinations of the core issue, provides significant examples, and demonstrates a viable strategy for addressing the core issue. The authors have done an excellent job addressing all of the issues I had with the previous revision. I have also read and understand the very thorough response to issues raised by reviewer 1. I believe the authors have addressed those issues in a reasonable manner. In the event that reviewer 1 is unsatisfied, I encourage the editor to require no more than minor edits to the text of the manuscript.

Thank you very much!