Reviewer 1

We thank the reviewer for the through revision of our manuscript. Thanks to the helpful suggestions of both reviewers, we believe that the manuscript is much improved.

Modifications made to the main text are highlighted in red.

In this research article the authors evaluate a workflow for label-free quantification of protein isoforms. With the workflow at hand they were subsequently able to determine protein abundance of histone H1 isoforms from breast cancer tissue. Although label-free quantification with MaxQuant (MQ) is a common approach in proteomics studies, this manuscript has a lot of strengths. I detail these are: i) the application of the MQ-workflow to quantify protein isoforms, ii) the evaluation of the suitability of differently treated patient samples for high-throughput scale screening, iii) the determination of sensitivity limits in terms of necessary sample amount and iv) the option to extend the workflow to characterization of relevant PTMs.

There is really not much to improve at this paper. Still, I have two major concerns: First concerns data availability. Here the authors forgot to provide the reviewers login to the datasets, which makes it impossible to judge on the completeness of the dataset.

> We thank the reviewer for the positive evaluation of our manuscript and we apologize for not providing the reviewer login credential for the PRIDE datasets, which are:
> Dataset PXD020537:
> Username: reviewer80261@ebi.ac.uk; Password: nvhlBB9n
> Dataset PXD020524:
> Username: reviewer07707@ebi.ac.uk, Password: OAACBzye

Secondly, the authors should discuss the applicability of spectral library searches in comparison to the match-between-run feature in MQ for detection and quantification of protein isoforms from low amounts of sample (see line 220).

> We added a discussion on spectral library searches in comparison to the match between run approach in the Discussion, on pages 9-10. We thank the reviewer for the useful suggestion.

I strongly believe that the current work will be of interest for a broad range of readers. In addition to my major concern some improvements should be made in the manuscript text, which I listed below.

- line 95 and throughout the manuscript: it should be kDa. Please check for spaces between numbers and units.
- line 133: please specify the abbreviation MEF also here in the figure caption as this abbreviation is introduced in the main text after the reference to the figure.
- line 158: Figure 2 B is not fitting the text before the reference. Please refer to the right Figure (2C-D?) and add a reference for figure 2B where appropriate.
- Line 328 f. Something with punctuation is wrong here.
- Line 336: CO_2
- Line 353: It should be: Samples with infiltrating carcinoma….
- Line 366: pH 8.0 (space is missing)

> We thank the reviewer for pointing out all these mistakes, which we fixed in the revised version of the manuscript.

- Figure 1C, line 138 f.. I can't find the number of proteins identified in this image.

We added the number of proteins to panel 1C. We thank the reviewer for pointing out its absence.

- line 170: It is not clear which is the basis for these “expectations”. Please explain.

  We added an explanation on how the expected fold changes were calculated in Figure S1 legend.

- Figure 3B: Here it states 2 unique peptides for variant H1.3 in sample WT 1. How can this be as Fig. 1 A shows only one unique peptide in H1.3?

  In Figure 1A we showed only peptides identified in at least 3 samples. Because histone H1.3 peptide SETAPAAPAAPAPVEKTPVKK was identified only in two samples, it was not listed in this figure. It was however counted in figure 3B. The complete list of peptides identified from MEFs can now be found in Figure S2.

- Line 525: The figure does not match the sentence.

  Line 525 is not present in the manuscript. However, we moved the reference to Figure 5B, which we think was misplaced in the original text.

- Figure 5: What is the difference between dataset 1 and 2 whose results are shown in Fig. 5 C-D and 5 E-F? Why there are two datasets?

  As mentioned on pages 8-9, Dataset 1 includes 37 triple negative breast cancer samples, stored in OCT and processed specifically for the analysis of histone H1 variant. Dataset 2 includes 9 FFPE samples, for which whole proteomes were acquired. Because the samples were processed through different protocols and acquired separately, they are shown as separate datasets.

- Please improve the resolution of images. Especially axis-labeling is very small and hence sometimes blurred.

  We included in the new version of the manuscript higher resolution images.

- Table S1: Please provide a table caption explaining the columns listed.

  We added a caption to Table S1.

- Figure S3B: How can the number of peptides for each sequence be higher than 1?

  The numbers indicate the number of times a peptide with a certain sequence was identified. This includes multiple identifications during the chromatographic run, as well as identifications of peptides with different charges and with/without methionine oxidation or N-terminal acetylation, as now mentioned in the legend of the figure.