Dear Editor,

We thank you and the reviewers for the constructive comments on our manuscript. We are pleased to submit a revised version of this manuscript with modifications to the manuscript, as suggested by the reviewers.

Response to Reviewers' Comments

Reviewer #1:

I would appreciate some more references in the discussion. And I suggest to put data in suppl. Table 1 into a database, as very few readers actually will be interested in the raw data, which they can look up from a deposit.

We have added some more references in the discussion. We will link the suppl. Table 1 to proteomics database website (http://lifecenter.sgst.cn/dbdepc/index.do). [He Y, et al., dbDEPC 2.0: updated database of differentially expressed proteins in human cancers. Nucleic Acids Res, 2012. **40**: p. D964-71.]

Reviewer #2:

1. **A better explanation of the iTRAQ technique in the introduction would help the reader understand how the technique provide quantitative information. This is a critical point about the technique vs other mass spectrometry approaches.**

We have added an explanation of the iTRAQ in the introduction section.

2. Figure 1 and 2 in the paper should probably be moved to the supplemental figures and the description should be moved to the methods. It is sufficient to discuss the results of the two repeat experiments in the methods as it is not experimental data per se.

We have moved original Figures 1 and 2 to the supplemental figures in the revised version, and moved the description to the methods.

3. Figure 1 should really be the Venn diagrams of comparisons of the different proteins from the different stages. It would be informative to have all the permutations of the comparisons between the different stages presented (i.e. NC vs AP, NC vs CIS, NC vs ICC, etc.) as Venn diagrams which is originally what I thought that Figure 1 was.

A new Figure 1 has been made following reviewer's suggestion.

4. I think that the paper could benefit highly from presenting a table of the top 5-10 different proteins between the different stages. Though this information could be obtained from the supplemental, it would be great to have the top candidates highlighted in the main paper.

A new Table 2 has been made following reviewer's suggestion.

5. **Figure 3 should label the Y axis even though they are percentages it is not clear to the reader what percentages they represent.**

We labeled the Y axis and the original Figure 3 is now Figure 2.

6. Figure 4 has very poor resolution and no axis labels.

We made revision following reviewer's comment. The original Figure 4 is now Figure 3.

7. **There needs to be a better explanation of the clustering and grouping from the reactome analysis. Figure 5 has little in the way of explanation, what is being compared? How are the items clustered? What does this tell us about the proteins expressed at the different stages?**

We added more explanation following reviewer's comment. The original Figure 5 is now Figure 4.

8. Figure 6 also should have the rows/columns of pictures labeled.

We made the revision following reviewer's comment. The original Figure 6 is now Figure 5.

9. The first paragraph of the discussion (as well as the remaining discussion) is difficult to understand and would benefit greatly from separating in the explanations into different paragraphs. The discussion should be reorganized to address a) the limitations of the techniques, b) the top most significant findings and c) what does their data say in the context of what is known.

We have revised the discussion following reviewer's comments.

10. Otherwise, there are small grammatical errors throughout the manuscript and the overall paper would benefit greatly from a careful edit.

We have edited the manuscript carefully and corrected grammatical errors.

Reviewer #3:

1. In defining groups NC, AP, CIS, ICC.
AP – Adenomatous polyp – This wording is scientifically not correct because by definition a 'polyp' is scientifically adenoma. I am wondering whether authors mean they included only pedunculated polyps in their study excluding flat polyps. Please specify the size of the polyps included and nature of the polyps included.
ICC- I am little bit concerned authors combined both local invasion carcinoma and metastatic carcinoma into one category. Because DEP's in Locally invasive carcinoma differs from the DEP's in early metastatic carcinoma without local invasion.

We agree with the reviewer that adenomatous polyp (AP) mentioned in our original manuscript should be scientifically adenoma (AD). AP has been replaced by AD in the text and figures accordingly. The AD tissue specimens obtained from colorectal endoscopic surgery were verified by histopathology. The sizes of AD specimens were ranging from 5mm to 20mm.

The reviewer gave us very good comments on local invasion carcinoma and metastatic carcinoma concerning the ICC category. In our experiments, the tissue samples in ICC group were from colorectal cancer patients with lymph node metastasis. We have added a description in tissue sample preparation section.

2. I would like to suggest to the authors that please represent the all the DEP's in the form graph (Protein – Protein Interaction network analysis) between the specified groups.

It is a very good suggestion. Bioinformatics analysis of protein – protein interaction network will be performed in our next work, and significant proteins will be selected for the further functional study.

3. **Finally I would like to advice that ICC group should have been divided into two separate groups (Local invasive carcinoma and Metastatic carcinoma)**

In our experiments, the tissue samples in ICC group were from colorectal cancer patients with lymph node metastasis, not a combination of samples of Local invasive carcinoma and Metastatic carcinoma.

We thank you and the Reviewers again for your considerable efforts in reviewing our manuscript.

Sincerely,

Yongheng Chen, Ph.D.