To *International Journal of Molecular Sciences* Journal

Dear Editors-In-Chief,

We want to thank the Reviewer for their constructive comments and efforts toward improving our manuscript. Without these efforts, maintaining a peer-reviewed journal's high standard would be challenging. In the following, we highlight the general concerns of the Reviewer and our efforts to address these concerns. Please find below a detailed point-by-point response to the comments of the Reviewer. The main corrections made in the manuscript text have been highlighted in yellow.

Reviewer 1

Comments and Suggestions for Authors

1. **Mention the main findings of your previous study in a separate paragraph and explain the differences in the methodology and design and how this study can further your cause, (reference 20)**

   Response: Thanks to the reviewer for pointing this out. The main findings of our previous work have been included in the revised version of the manuscript, emphasizing the distinctions from the present work. The main difference between the previous and the present work is that previously we have performed genome-wide methylated DNA analysis in the cancerous and non-cancerous renal tissue samples and investigated the suitability of the selected genes *ZNF677*, *PCDH8*, *FLRT2*, *FBN2*, *TAC1*, and *TFAP2B* for non-invasive urine-based detection of renal clear cell carcinoma by performing discovery and validation analysis in the two independent cohorts. These cohorts consisted of patients, diagnosed with 57 mm and 49 mm size tumors on average respectively and about half (54% and 46% respectively) were pT3-pT4 stage tumors. Meanwhile, in the present study, we sought to evaluate the suitability of these biomarkers for extremely early detection of small mass renal tumors (average tumor size 23 mm; pT1a stage) and assess their applicability for tumor growth prediction during the active surveillance of these patients.

2. **Please separate the methods that were used in the previous study and mention the necessary parts for the current study**

   Response: The description of Quantitative DNA methylation analysis was abbreviated by providing a link to the previous study (now reference 22).

3. **Please justify using non-parametric tests instead of parametric tests in the statistical analysis**

   Response: The clarification of the usage of the non-parametric tests was included in the "Statistical analysis" section of the manuscript. All quantitative variables were tested for normality by the Shapiro-Wilk test and since the data significantly deviate from a normal distribution ($P < 0.001$), nonparametric tests were applied for the following statistical analysis.

4. **Please explain more about the p-value within the group in Figure 2**

   Response: Addressing the reviewer's concern, in the revised version of the manuscript, we have explained in slightly more detail the meaning of "significant differences within the group". In Figures 2 C and D, the double asterisk (**) depict significant differences in tumor size between the first scan and the subsequent scans within the group (when only non-progressing or only progressing cases are compared), while the single asterisk (*) depicts significant differences among the corresponding scans between the groups (Non-progressing *vs.* Progressing).

5. **Please arrange tables in Figure 3 based on the AUC (increasing/decreasing)**

   Response: Thanks to the reviewer for the suggestion. In the revised version of the manuscript, we have arranged the provided genes according to the decreasing AUC values in Figure 3C, while in the D part of the same figure, the combinations of the biomarkers are arranged according to the increasing number of genes in the panel.

6. **What kind of correlation was used in fig 4? Pearson's correlation is a parametric test.**

   Response: Because of non-normal data distribution, Spearman's rank (R_S) correlation coefficients were calculated to test the associations between two continuous variables.

7. **First paragraph in discussion should be dedicated to the main findings of the current study and not the previous one (ref 20)**

   Response: We agree with the reviewer on this point. The main findings provided in the first paragraph of the discussion are based on the results obtained in the present study. The previous work (now reference 22) is mentioned only to advert the repeatability of the higher methylation levels of *TFAP2B, TAC1, PCDH8, ZNF677,* and *FLRT2* in the urine samples from patients, diagnosed with renal cell carcinoma.