Peer Review File

Reviewer A

This study used bioinformatics analyses to demonstrate that BEND5 is downregulated in LUAD and can be used as a prognostic biomarker for malignancy. In cell culture studies, BEND5 inhibits the proliferation of LUAD cells. The research is of interesting value in the field. Some comments:

Reply: We appreciate the editor's kind reminder and added the relevant information.

1. Bend5 level is low in LUAD that means it's not likely to be a target for treatment. The value of bend5 in this aspect need to be toned down in the whole manuscript.

1. We have toned down this aspect throughout the manuscript (see Page 2: line 58; Page 17: line 530)

2. In discussion section, please add discussion about this family of proteins, including other prominent members, play in cancer development, and what are the common/uncommon things of bend5 with other members, etc.

2. We have modified our text as advised (see Page 17: line 533-534, 537-542)

3. Current writing language is not perfect nut does not affect communication. In revision, please pay attention to the use of proper scientific terms, for example, the assay in fig 7b is colonogenic assay but not colony formation assay.

3. We have changed the name of the experiment to the correct one（see Figure legend 7 and 8; Page 10: line 317 323; Page 15: line 468 477）

Reviewer B

1. Abstract

UALCAN and TIMER were only mentioned in the method of abstract, but they weren't mentioned in the other sections of the main text, please check.

36	**Methods:** The Cancer Genome Atlas (TCGA) database was used to extensively examine
37	*BEND5* dysregulation and its prognostic significance in pan-cancer data. Databases including
38	TCGA, GEPIA (Gene Expression Profiling Interactive Analysis), UALCAN, and STRING
39	were used to perform analysis of the expression pattern and clinical significance of *BEND5* in
40	patients with LUAD, and the possible regulatory mechanisms responsible for the occurrence
41	and progression of LUAD. The TIMER (Tumor IMmune Estimation Resource) and GEPIA

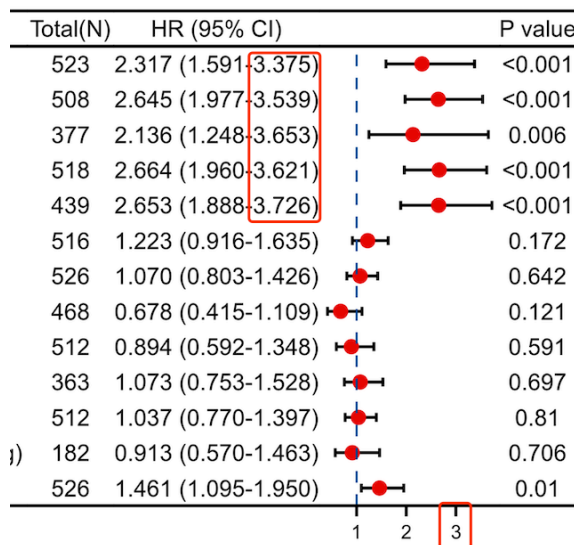| 41 | and progression of LUAD. The TIMER (Tumor IMmune Estimation Resource) and GEPIA |
| 42 | databases were used to explore the relationship between *BEND5* expression and tumor |
| 43 | immunity in LUAD. Finally, transfection experiments using an *in vitro* model were performed |

Reply: We thank the editor for the comment and have revised the manuscript accordingly.

Changes in the text: We have modified our text as advised (see Page 2, line 38, 41)
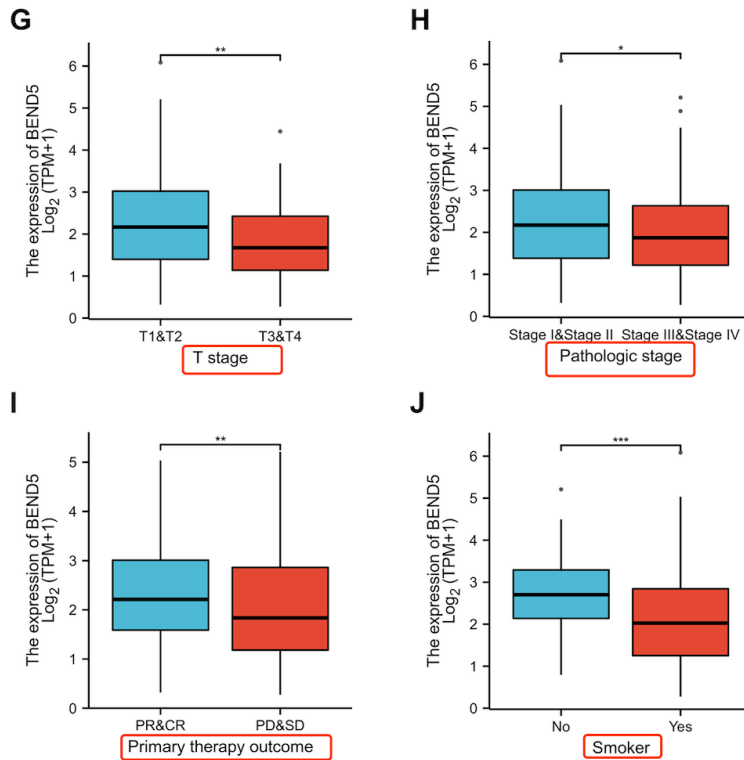
2. Figure 3
a) Please explain OS, DSS in the legend.
b) We suggest to add the scale bar "4" in the x-axis.

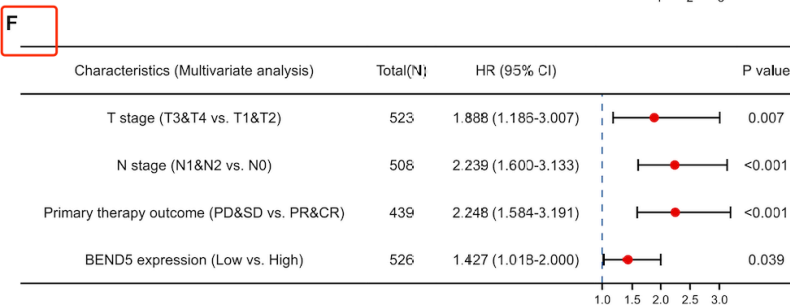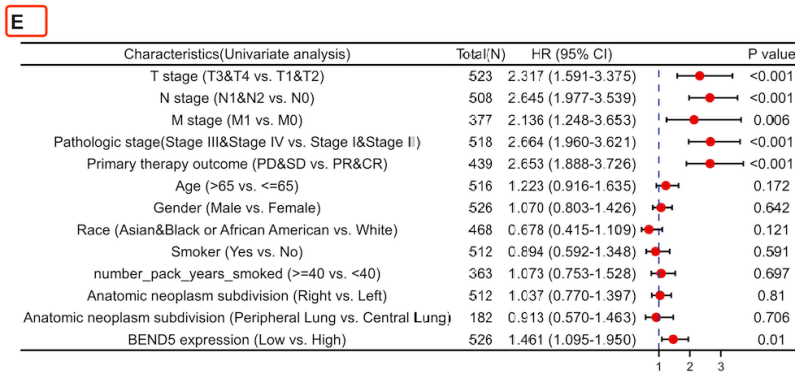| Total(N)	HR (95% CI)		P value
523	2.317 (1.591-3.375)		<0.001
508	2.645 (1.977-3.539)		<0.001
377	2.136 (1.248-3.653)		0.006
518	2.664 (1.960-3.621)		<0.001
439	2.653 (1.888-3.726)		<0.001
516	1.223 (0.916-1.635)		0.172
526	1.070 (0.803-1.426)		0.642
468	0.678 (0.415-1.109)		0.121
512	0.894 (0.592-1.348)		0.591
363	1.073 (0.753-1.528)		0.697
512	1.037 (0.770-1.397)		0.81
182	0.913 (0.570-1.463)		0.706
526	1.461 (1.095-1.950)		0.01

1   2   3

c) The legend didn't match the figure, please revise.

expression and DSS in patients with LUAD. (E-H) The relationships between tumor characteristics (E: T stage; F: pathologic stage; G: primary therapeutic result; H: smoker) and *BEND5* expression in LUAD. (I,J) Forest plots showing the results of univariate

and *BEND5* expression in LUAD (I,J) Forest plots showing the results of univariate

Characteristics(Univariate analysis)	Total(N)	HR (95% CI)		P value
T stage (T3&T4 vs. T1&T2)	523	2.317 (1.591-3.375)		<0.001
N stage (N1&N2 vs. N0)	508	2.645 (1.977-3.539)		<0.001
M stage (M1 vs. M0)	377	2.136 (1.248-3.653)		0.006
Pathologic stage(Stage III&Stage IV vs. Stage I&Stage II)	518	2.664 (1.960-3.621)		<0.001
Primary therapy outcome (PD&SD vs. PR&CR)	439	2.653 (1.888-3.726)		<0.001
Age (>65 vs. <=65)	516	1.223 (0.916-1.635)		0.172
Gender (Male vs. Female)	526	1.070 (0.803-1.426)		0.642
Race (Asian&Black or African American vs. White)	468	0.678 (0.415-1.109)		0.121
Smoker (Yes vs. No)	512	0.894 (0.592-1.348)		0.591
number_pack_years_smoked (>=40 vs. <40)	363	1.073 (0.753-1.528)		0.697
Anatomic neoplasm subdivision (Right vs. Left)	512	1.037 (0.770-1.397)		0.81
Anatomic neoplasm subdivision (Peripheral Lung vs. Central Lung)	182	0.913 (0.570-1.463)		0.706
BEND5 expression (Low vs. High)	526	1.461 (1.095-1.950)		0.01

Characteristics (Multivariate analysis)	Total(N)	HR (95% CI)		P value
T stage (T3&T4 vs. T1&T2)	523	1.888 (1.185-3.007)		0.007
N stage (N1&N2 vs. N0)	508	2.239 (1.600-3.133)		<0.001
Primary therapy outcome (PD&SD vs. PR&CR)	439	2.248 (1.584-3.191)		<0.001
BEND5 expression (Low vs. High)	526	1.427 (1.018-2.000)		0.039

Reply: We appreciate the editor's kind reminder and modified the Figure 3 as suggested.
Changes in the text:
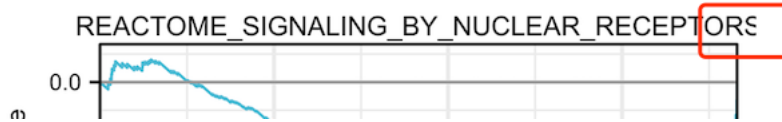a) We have modified our text as advised (see Figure 3 legend)

b) As I am using the software for plotting, the HR value is over "3" but not "4", so the scale bar "4" is not shown on the X axis. I tried to regenerate the diagram based on that comment, but very unfortunately, it was not added successfully.

c) We apologize for this error due to our carelessness and will send the corrected diagram with this document.

3. Figure 6
The word is not complete, please revise.

Reply: We thank the editor for kind reminder and modified the Figure 6 as suggested.
Changes in the text: We have modified the word as advised (see Figure 6 legend)

4. Figure 7
Please provide the magnification in the legend for 7B.
Reply: Thank you for your kind advice. Because the results of the colonogenic assay were observed with microscope and photographed with a camera, the magnification of the camera is not known.

5. Figure 8
Please provide the magnification in the legend for 7B.
Reply: We thank you for this valuable advice. Because the results of the colonogenic assay were observed with microscope and photographed with a camera, the magnification of the camera is not known.