

## Peer Review File

Article information: <https://dx.doi.org/10.21037/tcr-24-248>

### Reviewer A

Comment 1: This is a new aspects of GC stemness. Is it possible to show IHC of ISL1 overexpressing tissue? Are there any good antibodies?

Reply 1: We thank the reviewers for their careful reading of the manuscript and their constructive remarks. We supplemented the IHC for ISL1 in gastric cancer and paracancerous tissues.(see figure1B-C; Page 6, line 125-136; Page 13, 337-344;) The brand of the antibody we used to detect ISL1 is Thermo Fisher, catalog number: PA5-121000.

Comment 2: Many readers would like to reproduce the results their own cohort and co-existence of CD44 and ISL1 would be interesting. If any efforts were done, please give the information on that for future studies.

Reply 2: We appreciate the thoughtful and constructive comments from the reviewers. We performed immunofluorescent multicolor staining to validate the co-localisation of CD44 and ISL1 in gastric cancer tissues.(see figure6E; Page 8, line 189-191; Page 14, line 380-381).

Comment 3: CK18 would be available and I don't know OCT4. CD44 with its famous aberrant spliced species is notorious for difficulty in IHC.

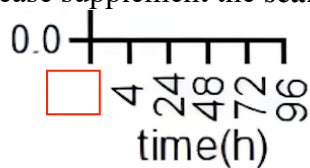
Did you test Anti-ISL1 (HPA048613) by ATLAS antibodies?

Reply 3: Thank you to the reviewers for their questions. OCT4 has been established as a stem cell marker (Semin Cancer Biol. 2023;88:123-137.). We detected CD44 expression in gastric cancer tissues using immunofluorescence (see figure6E; Page 8, line 189-191; Page 14, line 380-381).The brand of the antibody we used to detect ISL1 is Thermo Fisher, catalog number: PA5-121000.

### Reviewer B

#### 1. Figure 2

(1) Please supplement the scale in Figure 2A-2D, 4B-4D.



Reply : Thank you to the editor for reviewing and we have changed the images accordingly.

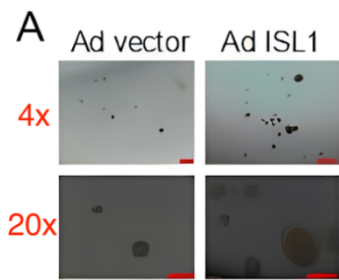
(2) It is suggested to use **different font color** for the scale (magnification) in **Figures 2E-2H** for easier reading.



Reply : Thank you to the editor for reviewing and we have changed the images accordingly.

#### 2. Figure 3

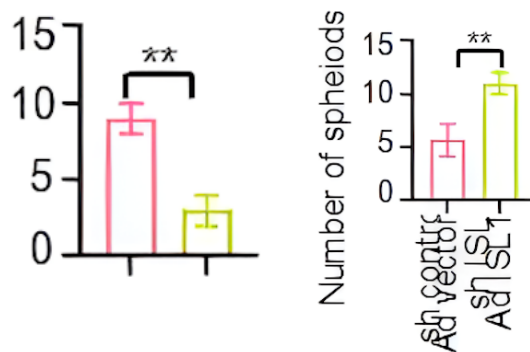
(3) You may add the magnification in **Figure 3A-3D** for better presentation. example:



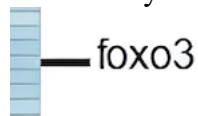
Reply : Thank you to the editor for reviewing and we have changed the images accordingly.

(4) Descriptions for **Figures 3B-3C** need to revise.

Reply : Thanks to the editor's review, we have revised the description of Figure 3A-D in Figure 3 legend and modified Figure 3B-C.



3. Please unify to use “**FOXO3**” in **Figure 6**.



Reply : Thanks to the editor's review, We've changed “foxo3” to “FOXO3”.