molecular systems biology

Proteomics of colorectal tumors identifies the role of CAVIN1 in tumor relapse

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This manuscript was transferred to Molecular Systems Biology following peer review at another journal.

16th Jan 2025

Manuscript Number: MSB-2025-12851P
Title: Proteomics of colorectal tumors identifies the role of CAVIN1 in tumor relapse
Author: Jesper Olsen

Dear Jesper,

Thank you for submitting your presubmission inquiry. I have now had the opportunity to review the revised manuscript and your point-by-point response. Overall, we believe the study has the potential to make an interesting contribution to the field.

Regarding the remaining comments from reviewers at the other journal, we consider the in vivo animal experiments to validate CAVIN1's role in tumorigenesis, as well as the further mechanistic investigation into how CAVIN1 modulates the mTOR pathway, to be beyond the scope of this study for publishing in Molecular Systems Biology. However, we suggest acknowledging these aspects as limitations and proposing them as potential directions for future research. Further, in response to the comments from Reviewers #1 and Reviewer #5 regarding the previous studies of CAVIN1 in tumor progression, we recommend more clearly highlighting the novelty and unique contributions of this study. Additionally, we think the value of proteome-based subtyping and the clinical relevance of the study could be emphasized more prominently. Lastly, the other concerns raised by Reviewer #5 should be carefully addressed in the revised manuscript. Please feel free to contact me in case you would like to discuss in further detail any of the issues raised by the reviewers.

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- Please provide individual production quality figure files as .eps, .tif, .jpg (one file per figure).

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The accession numbers and database should be listed in a formal "Data Availability" section (placed after Materials & Method) that follows the model below (see also https://www.embopress.org/page/journal/17444292/authorguide#dataavailability). Please note that the Data Availability Section is restricted to new primary data that are part of this study.
Data availability
The datasets (and computer code) produced in this study are available in the following databases:
- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
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I look forward to receiving your revised manuscript soon.

Jingyi

Jingyi Hou, PhD
Senior Editor
Molecular Systems Biology

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3. three to four 'bullet points' highlighting the main findings of your study
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Point-by-point rebuttal letter to REVIEWER COMMENTS

Manuscript Number: **MSB-2025-12851P**

"*Proteomics of colorectal tumors identifies the role of CAVIN1 in tumor relapse*"
by Martinez-Val et al.

Please, find below our responses to reviewers' requests and questions highlighted in blue.

Reviewer #1 (Remarks to the Author):
The authors have performed more experiments to address most of my concerns. The quality of manuscript is largely improved. However, it's suggested to perform additional in vivo experiments to validate the roles of CAVIN1 in tumor invasiveness. For example, nude mice were employed to analyze the invasive ability of CAVIN1 KD cells.

RESPONSE: We appreciate the positive feedback from this reviewer. Although we agree that performing additional in vivo experiments will be of great interest, we consider that such costly and laborious experiments are beyond the scope of the current work, as we have indicated in the Discussion of the manuscript (line 521 to 528), which reads:
"The strength of this study lies in the fact that hypotheses were derived directly from patient samples. However, validation was conducted solely in in vitro cell culture models, such as spheroids, which do not fully represent the observations made from patient biopsies. Further research to validate our findings must be carried out in in vivo models of this disease or in alternative cohorts. Moreover, evaluation on whether the cause of the relapse is due to remnant tumor cells not excised at the time of surgery or due to presence of metastasis that has not been yet diagnosed. If it is due to resistance to therapy, as it has been suggested in previous publications[63,64], cannot be evaluated in this work."

Reviewer #3 (Remarks to the Author):
The authors have addressed all my questions. Thanks.
RESPONSE: We thank you.

Reviewer #5 (Remarks to the Author):
In response to Reviewer 4's concerns, the authors' answers need further clarification. They found that the PROT subtype and the biomarker CAVIN1 are linked to recurrence, but it's unclear whether they are associated with local recurrence or distant metastasis. Additionally, did the patients receive any adjuvant chemotherapy or radiation therapy after surgery? Are the PROT subtype and CAVIN1 related to the treatments the patients received, such as chemotherapy or targeted therapy?

RESPONSE: The primary treatment for all patients included in our cohort was curative intent surgery. Patients were treatment-naïve prior to surgery. Accordingly, any relapse event must mean that residual disease persisted in the patient after surgery. Moreover, in our original discovery cohort there was no local recurrence cases, therefore, in case of distant relapse, it can be argued that surgical removal of the primary tumor was complete, but that occult metastatic spreading (undetectable by diagnostic CT imaging) had already occurred at time of surgery, and that this spread later manifested as detectable lesions.

In relation to the reviewer's comment regarding response to treatment, we do acknowledge, that a minor fraction of patients, mainly patients diagnosed with stage III disease, received adjuvant chemotherapy after surgery, in accordance with Danish national guidelines. Specifically, there are different recommendations for adjuvant chemotherapy for stage III colon cancer, stage III rectal cancer, and stage II colon cancer and rectal cancer. Patients are typically treated with 5-fluorouracil (5-FU) as standard therapy, and in high-risk cases, oxaliplatin is added, unless the patient is over 70 years old, in which case they only receive 5-FU. Treatment durations range from 3 to 6 months, and patients generally do not receive treatment if they are over 75 years (for stage II) or 80 years (for stage III colon and rectal cancer). Moreover, dMMR patients typically do not respond to 5-FU and are therefore not treated.
Taking all this into consideration, for a patient relapsing despite both surgery and adjuvant therapy, the relapsing cells are likely resistant to adjuvant chemotherapy. However, we attempted to gather treatment information from patients in our cohort; but the data were insufficient to derive meaningful insights regarding the relapse risk associated with treatment resistance, and therefore to connect adjuvant treatment effect to CAVIN1 levels.

The authors used different digestion methods, resulting in multiple batches of data. Although they applied batch effect correction twice, it's important to analyze whether these different batch effects have any correlation with recurrence.

RESPONSE: We understand the concern of the reviewer regarding the experimental variability introduced due to different processing batches and digestion strategies. To ascertain that sample allocation to experimental batches did not introduce a bias into the analysis, we performed Cox Multivariate Analysis to evaluate whether any particular batch correlated with relapse in a significant manner (Figure A). As observed in Figure A below, none of the experimental batches (1 to 8) showed significant correlation with recurrence. As a reference, on Figure B, we have represented the sample distribution of patients with and without recurrence among the 8 batches analysed. In this regard, we also did not observe any batch effect on the CAVIN1 levels (Figure C).

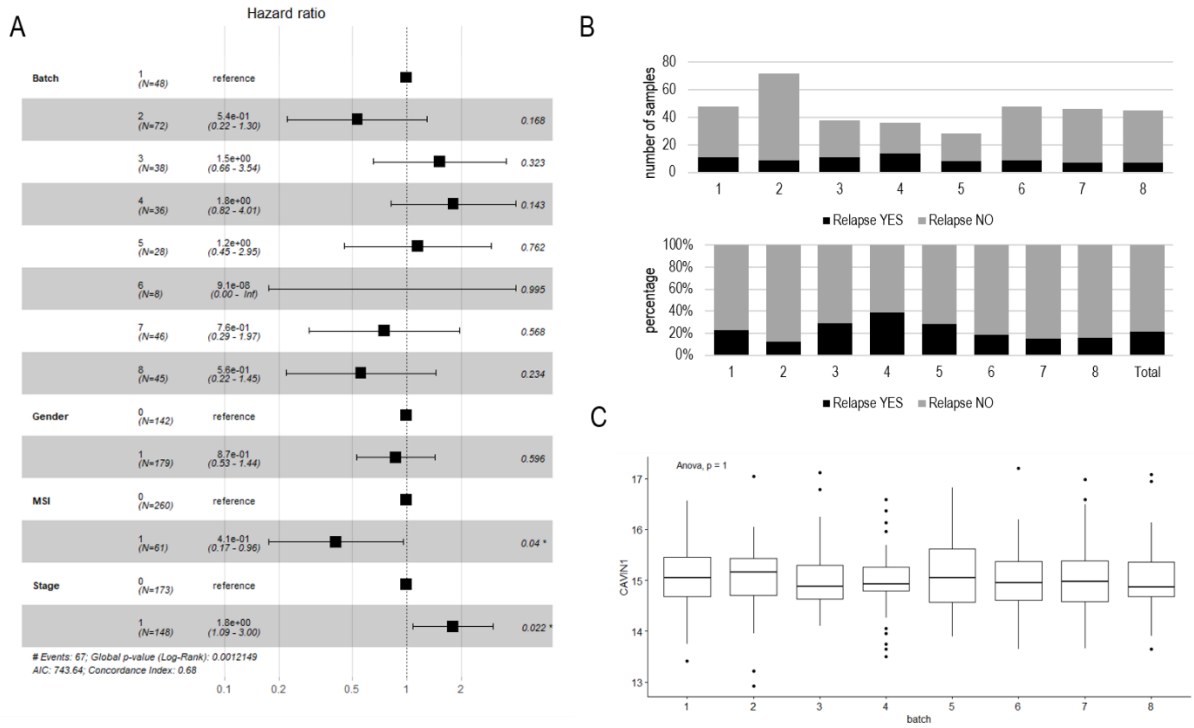

Figure 1. (A) Cox multivariate analysis of relapse in the colorectal cancer patient experimental cohort to assess correlation between different traits: processing batch, gender, MSI status (0=MSS, 1=MSI) and Stage (0=II, 1=III). (B) Distribution of samples across batches, where the height of the bar indicates the total number of samples. Each bar is divided between number of patients that suffered relapsed (in black) and those that did not (grey). Top graph represents total number of samples and bottom graph represent the same data as percentages. (C) Distribution of measured CAVIN1 protein levels across the different experimental batches. Protein distribution is represented as a boxplot. Anova t-test indicates that distribution of CAVIN1 protein intensities is not different between batches.

Moreover, this study analyzed tissue samples from 361 CRC patients using proteomics and identified the role of CAVIN1 in colorectal cancer. However, the authors only conducted simple in vitro experiments to show that CAVIN1 promotes cell migration. More in vivo animal experiments are needed to provide further insight into CAVIN1's function in CRC. Previous research has already established a connection between CAVIN1 and tumor progression, so what is the novel contribution of this study?

On the mechanistic side, the authors have merely indicated an association between CAVIN1 and the mTOR pathway through omics approaches. Additional experiments, like Western Blotting and functional rescue assays, are necessary to confirm how CAVIN1 regulates the mTOR signaling pathway and influences cell migration. The specific mechanism by which CAVIN1 modulates the mTOR pathway has not yet been explained.

RESPONSE: We appreciate the suggestion and we acknowledge that further in vivo experiments would be very valuable to validate the hypothesis derived from our systematic evaluation of colorectal cancer signatures. Moreover, we are aware that CAVIN1 has been previously linked to tumour progression, as we specified in the current manuscript (lines 95 to 101).

Our work focuses on the use of proteomics to define signatures that allow to stratify colorectal cancer patients, and how we can shortlist potential biomarkers from such data, as CAVIN1 in this case. Our data reflects a link between CAVIN1 and EMT molecular phenotype in colorectal cancer by means of proteomics, which, to our knowledge have not previously been described. Finally, we consider that additional in vivo experiments to be out of scope of the present work but we have acknowledged such limitations in the Discussion of the revised paper (lines 521 to 525), which reads:

"The strength of this study lies in the fact that hypotheses were derived directly from patient samples. However, validation was conducted solely in in vitro cell culture models, such as spheroids, which do not fully represent the observations made from patient biopsies. Further research to validate our findings must be carried out in in vivo models of this disease or in alternative cohorts."

While the authors included an additional cohort to show that CAVIN1 has higher recurrence rates in the EMT subtype, this cohort consists of only 50 cases, and they need to justify the validity of this sample size.

RESPONSE: We understand the concern regarding the sample size of our validation cohort, and we agree that the size might limit the interpretation of the results. We have acknowledged this limitation in the revised work. However, to strengthen the validation of our data, we also have performed cross-validation of our findings in additional dataset, such from Li, Wang, and colleagues (PMID: 38086380), which is shown in Supplementary Figure 5C and 5D.

Clinically, the authors claim that CAVIN1 is associated with tumor recurrence. However, previous proteomic studies which have identified many biomarkers in serval independent cohorts related to tumor recurrence. What sets CAVIN1 apart from these other markers? Furthermore, the authors should confirm the expression of CAVIN1 associate with tumor recurrence using immunohistochemistry (IHC) across multiple cohorts.

Additionally, since this biomarker is only associated with recurrence in the EMT subtype, how can it be applied clinically?

RESPONSE: Previous studies reporting alternative biomarkers are highly valuable, as we do not consider that certain biomarkers need to be set apart from others, but, on the contrary, biomarkers need to be considered as complementary to each other to derive panels that best reflect the tumour recurrence in each patient. In this work, we focused on CAVIN1 since it has not previously been identified as a biomarker of Colorectal Cancer survival by ATCG data, but there was prior evidence of the relevance of this protein in tumour progression as previously stated by this reviewer. Altogether we decided to follow up of this protein, which can potentially complement the information from other publications in the field.

Although we consider essential to validate CAVIN1 using IHC, recruiting enough samples from multiple cohorts as suggest by this reviewer, is currently out of the scope of the present manuscript.

Regarding clinical application of CAVIN1 being limited to EMT subtype, our data shows that colorectal cancer is a highly heterogenous disease, where EMT subtype differs greatly from the other three molecular subtypes identified in our cohort. Therefore, we consider it important to identify specific subtype biomarkers, to evaluate the relapse risk as a function of its molecular subtype.

7th Mar 2025

Manuscript Number: MSB-2025-12851
Title: Proteomics of colorectal tumors identifies the role of CAVIN1 in tumor relapse
Author: Ana Martinez-Val
Leander Van der Hoeven
Dorte Bekker-Jensen
Margarita Jørgensen
Jesper Nors
Giulia Franciosa
Claus Andersen
Jesper Bramsen
Jesper Olsen

Dear Jesper,

Thank you for the submission of your revised manuscript to Molecular Systems Biology. We have now had the chance to go through the revised manuscript and your point-by-point response. Overall, we think the concerns of the reviewers at the other journal have been satisfactorily addressed, and the study is now suited for publication in Molecular Systems Biology. Before we can formally accept the study for publication, we would ask you to address the following editorial-level points:

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Thank you for submitting this paper to Molecular Systems Biology.

Yours sincerely,
Jingyi

Jingyi Hou, PhD
Senior Editor
Molecular Systems Biology

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All editorial and formatting issues were resolved by the authors.

2nd Apr 2025
Manuscript number: MSB-2025-12851R
Title: Proteomics of colorectal tumors identifies the role of CAVIN1 in tumor relapse

Dear Jesper,

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If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to Molecular Systems Biology.

Sincerely,
Jingyi

Jingyi Hou, PhD
Senior Editor
Molecular Systems Biology

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Manuscript Number: MSB-2025-12851P	EMBO Reports - Author Guidelines
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DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Reagents and Tool table
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Reagents and Tool table

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible**.**	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Yes	Ethics declarations

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Not Applicable	

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Materials and Methods
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Materials and Methods
Include a statement about **blinding** even if no blinding was done.	Yes	Materials and Methods
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Material and Methods
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figures
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figures

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Yes	Ethics declarations
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Yes	Ethics declarations
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data Availability
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	References