JCB

Synergistic anti-cancer effect by targeting CDK2 and EGFR-ERK signaling

Jinhuan Wu, Yuping Chen, Rui Li, Ya Guan, Mu Chen, Hui Yin, Xiaoning Yang, Ming Jin, Bing Huang, Xin Ding, Jie Yang, Zhe Wang, Yi He, Qian Wang, Jian Luo, Ping Wang, Zhi Mao, Michael Huen, Zhenkun Lou, Jian Yuan, and Fanghua Gong

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April 14, 2022

Re: JCB manuscript #202203005

Prof. Jian Yuan
Tongji University
tongji university, siping road no.1239, yangpu district
shanghai, State... 200120
China

Dear Prof. Yuan,

Thank you for submitting your manuscript entitled "Synergistic anticancer effect acquired by targeting CDK2 and EGFR-ERK signaling". The manuscript has been evaluated by expert reviewers, whose reports are appended below. Unfortunately, after an assessment of the reviewer feedback, our editorial decision is against publication in JCB.

As you will see, while the reviewers recognize that the ERK regulation by CDK2 is a new mechanism, they find that this major finding is not sufficiently developed for a general cell biology journal like JCB -this overlapping concern was also shared by us, the editors, at the initial evaluation stage. In particular, the reviewers note that more direct evidence and mechanistic insights are needed to implicate the cell cycle regulated CDK2-mediated phosphorylation (and activation) of USP37 in the regulation of ERK levels and to firmly demonstrate the requirement of the activation of the USP37 DUB activity by CDK2 for ERK accumulation. In addition, reviewer #2 thinks that the cancer significance of this regulatory mechanism needs to be strengthen. The reviewers also raise a significant number of technical concerns.

As we do not have the level of reviewer enthusiasm necessary to move forward and based on the extent of revisions that would be necessary to address the reviewers' concerns, we cannot consider your manuscript for publication in JCB at this time. Thus, if you wish to expedite publication of the current data, it may be best to pursue publication at another journal -our office would be happy to assist you with the transfer to any journal of your choice.

However, given interest in the topic, we would be open to an appeal of this decision and resubmission to JCB of a significantly revised and extended manuscript that completely addresses each of the reviewers' concerns in full. We would like to insist on the need of addressing in full all the reviewers' criticisms for a successful appeal, except for point 5 of reviewer #2 of including additional pharmacological experiments in mice -these experiments in cell lines would suffice for us. If you decide to file an appeal, we encourage you to submit a detailed revision plan before embarking yourself in a lengthy revision, so we can give you feedback on its suitability -we may need to seek reviewer input before approving your revision plan. Please note that priority and novelty would be reassessed at resubmission and the paper would, of course, be subject to re-review by the same reviewers (if possible).

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

Pier Paolo Di Fiore, MD, PhD
Editor
The Journal of Cell Biology

Lucia Morgado-Palacin, PhD
Scientific Editor
Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In this manuscript, Wu et al. describe a novel mechanism by which CDK2 upregulates ERK signaling in cancer and identify USP37 as the intermediate that stabilizes ERK through deubiquitinylation. The authors go on to demonstrate that combined inhibition of CDK2 and EGFR signaling synergizes in reducing the growth of cancer cells in vitro and in vivo, the latter using also PDX.
The data are mostly convincing, but the manuscript needs attention in terms of language and form. For instance, the discussion

should not reiterate concepts already mentioned in the introduction
Specific points are listed below:

• In some of the Figures, for instance Figure 2A, the effects of the CDK inhibitor are rather small, and in others, such as in Fig. 2F they are not completely reversed by MG132 - there clearly is some fluctuation, a quantification of 3 experiments would be good to get an idea of the standard deviation.
• The interaction between ERK and USP37 should be shown with endogenous proteins.
• In Figure 5A, there is hardly any effect of the shRNA on its target USP37, but a comparatively large effect on ERK. This is inconsistent.
• Figure 6B, there is a huge difference between the efficiency with which ERK is reduce by inhibitor treatment and by USP37 shRNA. Under these experimental conditions, it would be very difficult to discern any synergy.
• The experiment in Figure 6L is both important and interesting. However, it should be shown that the increase in ERK and USP37 observed under these conditions is post-translational.
• In addition, the authors should carry out this experiment in cells treated with USP37 shRNA to make a convincing case that USP37 is responsible for CDK2-mediated ERK accumulation.
• Figure 7B, RNA data are necessary to back up the assumption that these increases are post-translational in nature.
• Figure 7, the USP37/ERK/pERK plots shown in Supplementary Figure 6 E-G should be moved here.
• Supplementary Figure 6D, a USP37 immunoblot is missing here.

Reviewer #2 (Comments to the Authors (Required)):

In current study, Wu et al found that combination of CDK1/2 inhibitor and EGFR inhibitor synergistically induced tumor cell death and inhibited tumor cell growth in vitro. They further found that inhibition or knockdown of CDK2 led to degradation and ubiquitination of ERK1/2. Mechanistically, CDK2 induced phosphorylation and activation of USP37 to deubiquitinate and stabilize ERK1/2, thereby promoting cell proliferation and survival. Although these findings are of potential interest, there are several missing pieces of the puzzle that should be provided.

Major points:
1 Most experiments were performed with cell lines and overexpressed plasmids. The authors should examine the endogenous ubiquitination of ERK1/2 in at least two types of cell lines and primary cells in the presence of CDK1/2 inhibitor and USP37 shRNA.

2 The types of ubiquitin chains on ERK1/2 that were cleaved by USP37 should be investigated. The antibodies to detect the ubiquitin linkages and TUBEs to pulldown different types of ubiquitin chains are commercially available.

3 Because USP37 also targets Cyclin A that binds to CDK2 to promote cell cycle, it is expected that knockdown of USP37 inhibited tumor cell growth in the presence or absence of ERK1/2 shRNA. The authors should examine whether reconstitution of Cyclin A or ERK1/2 into USP37-knockdown cells could rescue the cell growth and CDK1/2i-induced cell death.

4 Similarly, the authors should examine whether knockdown of CDK2 or CDK1/2i has any effect on the ubiquitination and stability of USP37 and whether reconstitution of USP37 abolishes the inhibitory effect of CDK1/2i or CKD2 shRNA on ERK1/2 degradation and tumor cell growth/survival.

5 AZD9291 is a specific inhibitor for EGFR E19del and L858R/T790M mutations. However, the authors used a lot of EGFR WT cells, such as A549, HCT116 and NCI-H460 that bare KRAS mutations and respond poorly to EGFR inhibitors. However, the EGFR inhibitors are not recommended for EGFR WT patients in clinic. It would be better if the authors examine whether the combo treatment has a synergistic effect on EGFR mut cells in cultures and in mice.

6 In Fig S1, 1 uM AZD9291 treatment did not result in obvious cell death. However, in Fig 1, 1 uM AZD9291 treatment led to 40-90% cell death. The authors should explain the discrepancies.

7 The authors claimed that CDK2-mediated phosphorylation of USP37 activated the DUB activity. They should examine USP37SA, SD and CS mutations rescue CDK1/2i-induced degradation of ERK1/2 in cells.

8 The GST-USP37 should be purified as there were too many non-specific bands in Fig 3E. In addition, results from Fig 3E could not support the direct association between USP37 and ERK1/2. They should use purified proteins and preform GST or His pulldown assays.

Minor points:
1 Markers were missing in Fig 3E.

2 The authors should clearly describe the criteria of high and low expression of USP37 in M&M.

3 The information of PDX tumor is missing. What type is the tumor? What are the genetic mutations? KRAS mutant? EGFR mutant? The resolution of Fig 7F needs to be improved.

Reviewer #3 (Comments to the Authors (Required)):

This paper describes a relationship between the kinase CDK2 and the DUB USP37 by which CDK2 activation of USP37 leads to stabilisation of the signaling molecules ERK1/2 which themselves lie downstream of EGFR They go on to claim synergy between CDK2 and EGFR inhibition for cancer cell growth. To my mind there are interesting elements to this paper, but I am not finding enough "cell biology" for it to be appropriate for JCB. I think it fits much better with a more specialised oncology journal.

The phosphorylation at S628and activation of USP37 by CDK2 have been shown previously DOI 10.1016/j.molcel.2011.03.027.

The novelty and strength lies in the links between CDK2/USP37 and ERK1/2 stability, which on the whole is well made and newsworthy. Their interpretation that USP37 requirement for cell growth is via ERK1/2 stabilisation is highly plausible.

Figure 1: This I found confusing. The X-axis needs to be more clearly labelled that this represents EGFRi concentration. They present a wide range of cell types but there is little attempt to link the magnitude of any effect to mutational status- e.g. A549 have activating KRAS and H1975 have EGFR activation, which affects the IC50 of the inhibitor.

Fig. 3A,B need MW markers.

Fig. 4F CHX chase. I am a little bit uncomfortable about- this. I would have adjusted the loading so that the 0 time point was equal across conditions.

Fig. 5O- the labelling is confusing.

Fig. 7A I do not think this data is enough to establish a correlation between USP37 and ERK1/2 levels. What do transcriptomics from the cancer cell line encyclopedia say? Recently the Gygi group has extended this to proteomics doi: 10.1016/j.cell.2019.12.023.

Overall I find the experiments showing synergy between EGFRi and CDK2i a bit distracting from the main message of USP37 regulation of ERK1/2- particularly at JCB.

Response to Reviewers

Reviewer comments:

Reviewer: 1

In this manuscript, Wu et al. describe a novel mechanism by which CDK2 upregulates ERK signaling in cancer and identify USP37 as the intermediate that stabilizes ERK through deubiquitinylation. The authors go on to demonstrate that combined inhibition of CDK2 and EGFR signaling synergizes in reducing the growth of cancer cells in vitro and in vivo, the latter using also PDX.

The data are mostly convincing, but the manuscript needs attention in terms of language and form. For instance, the discussion should not reiterate concepts already mentioned in the introduction

We thank the reviewer for the positive and constructive comments. We have enlisted a native English speaker to polish the manuscript and rewrite the discussion.

1. In some of the Figures, for instance Figure 2A, the effects of the CDK inhibitor are rather small, and in others, such as in Fig. 2F they are not completely reversed by MG132 - there clearly is some fluctuation, a quantification of 3 experiments would be good to get an idea of the standard deviation.

According to the reviewer's suggestion, we performed two additional experiments as in Figures 2A–2B and S2A–S2B. We then quantified and analyzed the results of the 3 experiments. These results provide clear evidence that ERK1/2 proteins are almost completely reversed by MG132; for instance, the MG132 rescued protein levels reached approximately 90% and 80% in Figures 2A and Fig. S2A, respectively.

We also performed two additional experiments as in Figure S2H–S2K and quantified and analyzed the results of the 3 experiments. These results provide additional evidence that ERK1/2 proteins are almost completely reversed by MG132.

2. The interaction between ERK and USP37 should be shown with endogenous proteins.

According to the reviewer's suggestion, we performed an endogenous co-immunoprecipitation (CO-IP) assay to detect the interaction between USP37 and ERK1/2 using various cancer cell lines. As shown in Figure 3C–3D, endogenous USP37 and ERK1/2 interact with each other in HEPG2 and A549 cells.

3. In Figure 5A, there is hardly any effect of the shRNA on its target USP37, but a comparatively large effect on ERK. This is inconsistent.

According to the reviewer's suggestion, we re-generated several USP37-depleted stable cancer cell lines by shRNA, such as SK-MES-1 and NCI-H460. As shown in Figures 5A and S4A, about 90% USP37 protein levels were decreased in USP37 depletion cells, while the ERK1/2 protein levels were about 50% decreased in cells depleted of USP37.

4. Figure 6B, there is a huge difference between the efficiency with which ERK is reduce by inhibitor treatment and by USP37 shRNA. Under these experimental conditions, it would be very difficult to discern any synergy?

There may be a misunderstanding regarding our model that CDK2 regulates ERK1/2 stability via USP37. We hypothesized that CDK2 modulates ERK1/2 protein levels through phosphorylating and activating USP37, so CDK1/2 inhibition may not show as strong effect as USP37 shRNA in ERK reduction, which is consistent with our result in Figure 6B. When tested, we found that CDK1/2 inhibitor can only decrease ERK1/2 protein levels in control cells, but not in USP37-depleted cells (Figure 6B). To further confirm these results, we performed two additional experiments and quantified and analyzed the results of 3 experiments (Figure S5B–S5E).

5. The experiment in Figure 6L is both important and interesting. However, it should be shown that the increase in ERK and USP37 observed under these conditions is post-translational.

According to the reviewer's suggestion, we performed a qPCR assay to detect the mRNA levels of USP37 and ERK1/2. As shown in Figure S6C, we did not see a dramatic increase in the mRNA levels of USP37 or ERK1/2 following a double thymidine block release but we observed the increase in the protein level (Figure 6L). To further confirm the increase is post-translational, we determined the poly-ubiquitination of USP37 and ERK1/2 following double thymidine block release. As shown in Figure S6E–S6F, we found that poly-ubiquitination of ERK1/2 and USP37 decreased following double thymidine block release. Altogether, these results suggest that the downregulation of poly-ubiquitination contributes to the increased protein levels of USP37 and ERK1/2.

6. In addition, the authors should carry out this experiment in cells treated with USP37 shRNA to make a convincing case that USP37 is responsible for CDK2-mediated ERK accumulation.

We appreciate the reviewer's suggestion. We constructed a stable USP37-depleted NCI-A549 cell line, then determined ERK1/2 protein levels following a double thymidine block release. As shown in Figure S6D, the ERK1/2 protein levels did not show increase in USP37 depletion cells following double thymidine block release, but in control cells, we observed increased ERK1/2 protein levels (Figure 6L).These results suggest that USP37 is responsible for CDK2-mediated ERK1/2 accumulation.

7. Figure 7B, RNA data are necessary to back up the assumption that these increases are post-translational in nature.

We appreciate the reviewer's suggestion. Since the commercial lung tissue microarray slides are utilized for Figure 7A, which cannot be used to extract RNA. To answer the reviewer's question, we collected 10 pairs of lung cancer and lung cancer-adjacent normal tissues and examined the protein and mRNA levels of USP37 and ERK1/2. We found that the protein levels but not mRNA levels of USP37 and ERK1/2 were correlated with each other and higher in cancer tissue compared to adjacent normal tissues (Figure S7C–S7D).

8. Figure 7, the USP37/ERK/pERK plots shown in Supplementary Figure 6 E-G should be moved here.

We appreciate the reviewer's suggestion. We moved the USP37/ERK/pERK plots shown in Supplementary Figure 6E–6G to Figure 7K–7M.

9. Supplementary Figure 6D, a USP37 immunoblot is missing here.

We appreciate the reviewer's suggestion. We blotted USP37 using the same sample and show it in Supplementary Figure 8G.

Reviewer: 2

In current study, Wu et al found that combination of CDK1/2 inhibitor and EGFR inhibitor synergistically induced tumor cell death and inhibited tumor cell growth in vitro. They further found that inhibition or knockdown of CDK2 led to degradation and ubiquitination of ERK1/2. Mechanistically, CDK2 induced phosphorylation and activation of USP37 to deubiquitinate and stabilize ERK1/2, thereby promoting cell proliferation and survival. Although these findings are of potential interest, there are several missing pieces of the puzzle that should be provided.

We thank the reviewer for the positive and constructive comments.

Major points

1. Most experiments were performed with cell lines and overexpressed plasmids. The authors should examine the endogenous ubiquitination of ERK1/2 in at least two types of cell lines and primary cells in the presence of CDK1/2 inhibitor and USP37 shRNA.

We appreciate the reviewer's suggestion. We examined endogenous ubiquitination of ERK1/2 in USP37-depleted HEPG2 or NCI-H460 cancer cells and in CDK1/2i-treated HEPG2 and HEK293T cells. As shown in Figures S2L–S2M and S3C–S3D, the endogenous ubiquitination of ERK1/2 significantly increased in CDK1/2i-treated or USP37-depleted cells.

In addition, the endogenous ubiquitination of ERK1/2 significantly increased in USP37 siRNA transfected primary cells and CDK1/2i treated primary cells (Figures S3E and Figures 2J).

2. The types of ubiquitin chains on ERK1/2 that were cleaved by USP37 should be investigated. The antibodies to detect the ubiquitin linkages and TUBEs to pulldown different types of ubiquitin chains are commercially available.

According to the reviewer's suggestion, HA-tagged ubiquitin wild type and various HA-tagged ubiquitin mutants (Ub-K6 only, Ub-K11 only, Ub-K27 only, Ub-K29 only, Ub-K33 only, Ub-K48 only or Ub-K63 only) were utilized to perform ubiquitination assays. We found that USP37 decreased the K48-linked ubiquitination of ERK1/2 (Figure S3F).

Moreover, we also confirmed the endogenous poly-ubiquitination link to ERK1/2 in USP37-overexpressed cells by using anti-K48 or anti-K63 Ub antibodies. We found that the K48-linked, but not the K63-linked, poly-ubiquitin chains of ERK1/2 were decreased in cells overexpressed with USP37 (Figure S3G).

3. Because USP37 also targets Cyclin A that binds to CDK2 to promote cell cycle, it is expected that knockdown of USP37 inhibited tumor cell growth in the presence or absence of ERK1/2 shRNA. The authors should examine whether reconstitution of Cyclin A or ERK1/2 into USP37-knockdown cells could rescue the cell growth and CDK1/2i-induced cell death.

We appreciate the reviewer's suggestion. We reconstituted cyclin A or ERK1/2 into USP37-knockdown cells and examined cell growth by colony formation assay. As shown in Figure S6G–S6H, we found that reconstitution of ERK1/2 rescued the cell growth from 20% to 60% . However, the rescue phenotype of Cyclin A reconstitution was mild (about 20% to 30%) (Figure S6H). In addition, we found that overexpression of ERK1/2 but not Cyclin A in USP37-shRNA-depleted cancer cells led cells resistance to the CDK1/2 inhibitor (Figure S6I).

4. Similarly, the authors should examine whether knockdown of CDK2 or CDK1/2i has any effect on the ubiquitination and stability of USP37 and whether reconstitution of USP37 abolishes the inhibitory effect of CDK1/2i or CDK2 shRNA on ERK1/2 degradation and tumor cell growth/survival.

We appreciate the reviewer's suggestion. In fact, we have detected poly-ubiquitination and stability of USP37 in CDK1/2i (JNJ-7706621)-treated cells.

We found that inhibition of CDK1/2 significantly decreased the USP37 protein level (Figures 6A–6B and S5A–S5E) and increased poly-ubiquitination of USP37 (Supplementary Figure 5I). To further confirm these results, we examined USP37 poly-ubiquitination by ubiquitination assay and USP37 stability by CHX-chasing assay in CDK2-depleted cells. As shown in Figure S5J–S5L, the CDK2 knockdown increased USP37 ubiquitination but decreased USP37 protein stability. And we think the mechanism is because that CDK2 phosphorylated and activated USP37, which induced USP37 auto-deubiquitination (Figure 6I and S5I).

In addition, we reconstituted USP37 in CDK2-knockdown cells or cells treated with CDK1/2 inhibitor, then examined cell growth by colony formation assay. As shown in Figure S6J–S6M, we found that USP37 reconstitution partially rescue the ERK1/2 degradation and tumor cell growth in CDK2-knockdown or CDK1/2i-treated cells.

5. AZD9291 is a specific inhibitor for EGFR E19del and L858R/T790M mutations. However, the authors used a lot of EGFR WT cells, such as A549, HCT116 and NCI-H460 that bare KRAS mutations and respond poorly to EGFR inhibitors. However, the EGFR inhibitors are not recommended for EGFR WT patients in clinic. It would be better if the authors examine whether the combo treatment has a synergistic effect on EGFR mut cells in cultures and in mice.

We appreciate the reviewer's suggestion. We performed colony formation assays with cells treated with CDK1/2i and EGFRi using H1975 cells, which carry EGFR L858R and T790M mutations, or PC-9 cells, which harbor an exon 19 deletion mutation. As shown in Figure S8A–S8B, the combination of CDK1/2 and EGFR inhibitors produced synergetic killing effects in H1975 and PC-9 cells with EGFR mutations.

In addition, we performed a CDX xenograft assay using the NCI-H1975 cell line, which carries the EGFR L858R and T790M mutations. In these cells, CDK1/2 or EGFR inhibitors alone showed mild killing effect, while the combination of CDK1/2 and EGFR inhibitors produced synergetic killing effects in vivo (Figure S8C–S8E).

6. In Fig S1, 1 uM AZD9291 treatment did not result in obvious cell death. However, in Fig 1, 1 uM AZD9291 treatment led to 40-90% cell death. The authors should explain the discrepancies.

We appreciate the reviewer's suggestion. After inspection of our raw data, we found a mistake in the Figure S1 X-axis label regarding the concentration of AZD9291. The concentration needs to be divided by 100 to be correct. We have revised this in the manuscript (Figure S1).

7. The authors claimed that CDK2-mediated phosphorylation of USP37 activated the DUB activity. They should examine USP37SA, SD and CS mutations rescue CDK1/2i-induced degradation of ERK1/2 in cells.

We overexpressed USP37 WT or USP37 SA, SD or CS mutations in CDK1/2i-treated cells and examined the ERK1/2 level. We found that USP37 WT or the SD mutant rescued the ERK1/2 protein levels in CDK1/2i treated cells, but the CS or SA mutants did not (Figure S6B).

Minor points

1. Markers were missing in Fig 3E.

Thanks for the review's kind reminder. We re-labeled the MW markers in Figure 3E.

2. The authors should clearly describe the criteria of high and low expression of USP37 in M&M.

As suggested by the reviewer, we clearly described the criteria for high and low expression of USP37 in IHC staining in the Materials and Methods section.

3. The information of PDX tumor is missing. What type is the tumor? What are the genetic mutations? KRAS mutant? EGFR mutant? The resolution of Fig 7F needs to be improved?

We appreciate the reviewer's suggestion. The PDX tumor is from a patient with colon cancer. Genetic sequencing showed that the PDX sample had wild-type EGFR and KRAS genes. As shown in Figure 7E, we also replaced Figure 7F with a high-resolution image.

Reviewer: 3

This paper describes a relationship between the kinase CDK2 and the DUB USP37 by which CDK2 activation of USP37 leads to stabilisation of the signaling molecules ERK1/2 which themselves lie downstream of EGFR They go on to claim synergy between CDK2 and EGFR inhibition for cancer cell growth. To my mind there are interesting elements to this paper, but I am not finding enough "cell biology" for it to be appropriate for JCB. I think it fits much better with a more specialised oncology journal.

The phosphorylation at S628 and activation of USP37 by CDK2 have been shown previously DOI 10.1016/j.molcel.2011.03.027.

The novelty and strength lie in the links between CDK2/USP37 and ERK1/2 stability, which on the whole is well-made and newsworthy. Their interpretation that the USP37 requirement for cell growth via ERK1/2 stabilization is highly plausible.

We thank the reviewer for the positive and constructive comments.

1. This I found confusing. The X-axis needs to be more clearly labelled that this represents EGFRi concentration. They present a wide range of cell types but there is little attempt to link the magnitude of any effect to mutational status- e.g. A549 have activating KRAS and H1975 have EGFR activation, which affects the IC50 of the inhibitor.

We thank the reviewer for their kind reminder. There is a mistake on the X-axis. The X-axis should be labeled for EGFRi (AZD9291) concentration, which has now been fixed (Figure 1A-1C).

To further confirm the killing effect of EGFRi in cancer cells with different EGFR status, we examined and compared the IC50 of EGFRi (AZD9291) via a cell counting Kit-8 assay using different EGFR status cancer cell lines. As shown in Figure S8H–S8J, lung cancer cell lines with EGFR mutation have lower EGFR inhibitor IC50s. As shown in Figure S8H , the IC50 of EGFRi was 4.758 uM (PC-9) and 10.91 uM (NCI-H1975) in EGFR mutant cells. The IC50 of EGFRi was 23.77 uM

(NCI-A549), 33.23 uM (SKMES-1), and 37.78 uM (NCI-H1299) in EGFR wild type cells. In addition,the IC50 of EGFRi was 34.93 uM (NCI-H460), 23.41 uM (HCT116), 18.14 uM (SW480), and 37.02 uM (SW620) in KRAS mutant cells (Figure S8H-S8I).

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2. Fig. 3A, B need MW markers.

Thank you for the review's kind reminder. We re-labeled the MW markers in Figures 3A and 3B.

3. Fig. 4F CHX chase. I am a little bit uncomfortable about- this. I would have adjusted the loading so that the 0 time point was equal across conditions.

According to the reviewer's suggestion, we repeated the experiments and adjusted the loading so that the 0-time point was equal across conditions. As shown in Figure S3A–S3B, the depletion of USP37 significantly decreased the ERK1/2 protein stability.

4. Fig. 5O- the labelling is confusing.

Thank you for the reviewer's kind reminder. We re-labeled the figure to make it clearer (Figure 5O).

5. Fig. 7A I do not think this data is enough to establish a correlation between USP37 and ERK1/2 levels. What do transcriptomics from the cancer cell line encyclopedia say? Recently the Gygi group has extended this to proteomics doi: 10.1016/j.cell.2019.12.023.

We found the proteins, but not mRNAs expression of ERK1/2 and USP37 were highly correlated and much higher in lung cancer cell lines compared with normal lung cell lines (Figure S7A–S7B). Furthermore, the expression of USP37 and ERK1/2 was higher in lung cancer tissue compared to corresponding normal lung tissues (Figure S7C–S7D).

According to reviewer's suggestion, the data from the Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle/) was utilized to analyze the correlation between USP37 and ERK1/2 in mRNA or protein expression levels. As shown in Figure S7G–S7H, there is a higher correlation between USP37 and ERK1/2

in protein levels than mRNA expression in cancer cell lines, which is consistent with our findings (Figure S7H).

May 30, 2023

RE: JCB Manuscript #202203005R-A

Prof. Jian Yuan
Tongji University
tongji university, siping road no.1239, yangpu district
shanghai, State... 200120
China

Dear Prof. Yuan:

Thank you for submitting your revised manuscript entitled "Synergistic anti-cancer effect acquired by targeting CDK2 and EGFR-ERK signaling". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below) and one request by Reviewer 3. This Reviewer felt the cycloheximide chase assay should examine timpoints earlier than 6 hours, and we agree these data should be in place in a final manuscript. We will assess this and other formatting revisions without further reviewer input.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, http://jcb.rupress.org/submission-guidelines#revised. Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.

1) Text limits: Character count for Articles is < 40,000, not including spaces. Count includes abstract, introduction, results, discussion, and acknowledgments. Count does not include title page, figure legends, materials and methods, references, tables, or supplemental legends.

2) Figures limits: Articles may have up to 10 main figures and 5 supplemental figures/tables.
** Please arrange figures to meet this requirement by eliminating data in supplemental figures that are not essential, by making supplemental figures into main figures, or both.

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.
** Please include molecular weight markers to the blot in Supplemental Figure 6C.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (either in the figure legend itself or in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Abstract and title: The abstract should be no longer than 160 words and should communicate the significance of the paper for a general audience. The title should be less than 100 characters including spaces. Make the title concise but accessible to a general readership.
** While the current title is accurate, it would be improved by eliminating the word "acquired" which is not needed. We recommend: "Synergistic anti-cancer effects by targeting CDK2 and EGFR-ERK signaling"
** In the abstract, the use of the term "chemotherapeutic" may not be appropriate here, and the use of the term "clinical" may be redundant. We suggest changing this sentence to: "Indeed, our patient-derived xenograft (PDX) results suggest that targeting both ERK1/2 stability and activity kills cancer cells more efficiently even at lower doses of these two inhibitors, which may reduce their associated side effects and indicate a potential new combination strategy for cancer therapy."

6) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts. We also provide a report from SciScore and an associate score, which we encourage you to use as a means of evaluating and improving the methods section.

7) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies. Please also indicate the acquisition and quantification methods for immunoblotting/western blots.

8) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. Imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

9) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

10) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

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Sincerely,

Pier Paolo Di Fiore, MD, PhD
Editor
The Journal of Cell Biology

Tim Fessenden
Scientific Editor
Journal of Cell Biology

Reviewer #2 (Comments to the Authors (Required)):

In this revised manuscript, the authors have provided additional evidence that USP37 is a substrate of CDK2 and that phosphorylated USP37 deubiquitinates ERK1/2 to maintain their stability and to support tumor cell growth. Consequently, combo of CDK2 and EGFR inhibitors synergistically inhibit tumor growth. These findings highlight potential therapeutic strategies for treatment of ERK-driven cancers. I thus recommend its publication in JCB.

Reviewer #3 (Comments to the Authors (Required)):

I remain of the opinion that this is better suited to a more specialist journal, not really enough cell biology for my liking. I do though find the central finding interesting and well supported by the data. There are over 70 data panels in the main text and similar number in supplementary. It is incumbent on the authors to design well controlled and better quantitated experiments that distill down their essential message. As it stands I think it is not communicating well with the prospective reader/reviewer. My specific previous comments have been partially addressed. I still think the cycloheximide chase data is weak and the authors would have been better getting more accurate and time resolved data over the first 6 hours, which is the time window over which the principal differences seem to occur. Supplementary 7G and H are hard to follow and not fully explained in the legend.