THE
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JOURNAL

Translational offsetting as a mode of estrogen receptor α-dependent regulation of gene expression

Julie Lorent, Eric P. Kusnadi, Vincent van Hoef, Richard J. Rebello, Matthew Leibovitch, Johannes Ristau, Shan Chen, Mitchell G. Lawrence, Krzysztof J. Szkop, Baila Samreen, Preetika Balanathan, Francesca Rapino, Pierre Close, Patricia Bukczynska, Karin Scharmann, Itsuhiro Takizawa, Gail P. Risbridger, Luke A. Selth, Sebastian A. Leidel, Qishan Lin, Ivan Topisirovic, Ola Larsson, Luc Furic

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Editor: Karin Dumstrei

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14th Jan 2019

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by two referees and their comments are provided below.

As you can see from these comments there is an interest in the study, but the referees also raise major concerns with the analysis. Lots of further work is needed to substantiate and extend the key findings. Should you be willing and able to embark on a major revision then I am open to consider a revised manuscript. If you find your self in a position not to be able to respond to the major concerns raised then it is in your best interest to go elsewhere at this stage.

REFEREE REPORTS:

Referee #1:

This is a potentially interesting and original study in which the impacts of removing a transcription factor on mRNA levels and on corresponding protein levels are compared. There are sets of mRNAs that fall into particularly intriguing categories. There are both upregulated and downregulated mRNAs whose protein outputs appear not to change. The authors go on to characterize them and notably discover that tRNA levels may affet outcome. For the upregulated mRNA, the authors come up with the hypothesis that the transcription factor under study regulates the translational offset of its target genes through regulating the expression of some tRNA-modifying enzymes. In its current form, the manuscripts suffers from being too cryptic for a general readership and from providing insufficient support for the claims made.

Major comments:

(1) Technology choices: It could be (and has been) argued that the isolation of polysomes is more prone to artefacts than ribosome profiling. This is a key issue and must at least be appropriately discussed. Moreover, the choice of microarrays for polysome profiling seems strange considering that RNA-seq would yield much more quantitative data.
(2) Pharmacological tools: It isn't clear why the pharmacological tools (estrogens and anti-estrogens) are only used in MCF7 cells and not for some (or most) of the experiments in BM67. Although there is ERβ in BM67, there are some reasonably good ERα-specific drugs that are commercially available.
(3) DEK: The DEK story is confusing. In BM67 cells it seems to be one of those mRNAs, whose levels go up upon ERα KD but where translational offset keeps protein levels more or less the same (Fig. 1F and G). However, in MCF7 cells, as judged by the immunoblot of Fig. 6E, E2-induced ERα activity may induce DEK protein expression (and the ERα antagonist ICI leaves it untouched). While there may well be cell line-specific differences, it makes it impossible to compare these experiments. In any case, multiple immunoblots would have to be quantitated to draw firm conclusions about relatively minor effects.
(4) ELP3/ALKBH8/CTU2 rescue experiment: this is definitely an original way of testing the hypothesis. Unfortunately, the protein data of Fig. 6F is not sufficiently clear. Incidently, ERα levels may be slightly higher in the sample where ALKBH8 is overexpressed. Multiple experiments would have to be quantitated with proper standardization to actin and/or GAPDH.
(5) Does the U34 connection mediate the translational offsetting of ERα target mRNAs? The data are insufficient to make this claim. Since only ALKBH8 seems to work in the experiment of Fig. 6F, one cannot conclude anything about ELP3 and CTU1/2. More direct experiments such knocking down or overexpressing these enzymes in the ERα KD BM65 cells might allow one to draw this conclusion.
(6) Density graphs (of Fig. 1B, D, S1C-D, ...) must be explained more intelligibly. Neither the legends nor the supplementary methods provide a clear explanation of what the two axes are and how the graphs were generated. Similarly, it must be explained how "translation" can be computed and plotted (using the same units on both axes as for the other two plots?).
(7) Fig. 4F-J are also much too cryptic despite some explanations in the supplementary methods. Fig. 5C-D is similarly cryptic.
(8) Fig. 6D: the differences, despite being apparently statistically significant, are minimal. The Y-axis not starting at 0 makes the graph rather deceiving.
(9) Title: the title makes too sweeping a statement. ERα may regulate the protein output of a small subset of its target genes by controlling the expression of enzymes involved in regulating translational offset. Indeed, the "reverse" statement ("Translational offset controls expression of some ERα target genes") would be more accurate.

Minor comments:
(1) mRNA quantitation: for the 86 quantitated by nanostring, it would be good to have a separate Table of the fold changes. That would facilitate reading figures like 1F/G.
(2) Impact of ERα KD on expression of ELP3 (and ALKBH8 and CTU1/2), page 12: it isn't stated where the mRNA data are shown or from.

Referee #2:

In their manuscript, Lorent et al describe results that indicate the down regulation of U34-tRNA modifying enzymes following ERa-depletion leads to translational offsetting of upregulated translational targets. While the concept is attractive and interesting, the manuscript falls short in demonstrating direct effects and the biological importance of the pathway. It remains on a very descriptive level.

Major comments:

1.The authors used only one shRNA targeting ERa. At the minimum, another independent vector should be used to confirm the results. In particular, the offsetting phenomenon.

2. The authors used DNA-microarrays to quantify gene expression in polysome profilings. A much

superior analysis would have been to perform RNAseq. Knowledge on change in isoform expression and a better window of differential expression would have been obtained. I think this has to be done to confirm that the effects are indeed due to offset.

3. Figure 1G and 6C show western blot analyses. The changes shown are not very convincing, and it is not clear whether they are reproducible. The blots should be quantified and statistically evaluated in multiple biological replicates.

4. The authors have not ruled out changes in protein stability. Can it be that some of the effects they observed as offset is actually coordinated regulation at the protein level? This should be experimentally examined on a couple of selected targets.

5. The authors pinpoint association of the upregulated offset group following ERα KD with reduced tRNA modification. However, a direct connection is missing. This can be obtained first by quantification of the tRNA modification, and second phenotypically by measuring the expression of a reporter gene with altered coding usage.

6. Figure 6A. the authors state that no significant change in tRNA expression was observed (page 11). Looking at the figure, I just cannot figure out how they reached this conclusion.

7. The authors perform few validation experiments in the human MCF7 cells (while the rest of the studies were in BM67 mouse cells). While this adds some confidence in the regulation of the tRNA modification enzyme, the basic offset phenomenon was not reproduced in this cell line. This is important.

8. The suggestion that ELP3 is a target of ERa can be used to examine biological effects. In the context of ERa KD in BM67 cells, does the expression of ELP3 has biological consequences to cell response.

1st Revision - authors' response 20th Jul 2019

Dear Dr. Dumstrei,
We would like to thank you and the reviewers for insightful comments and suggestions to improve our manuscript. Both reviewers raised questions regarding the use of DNA-microarrays to quantify translatomes and suggested additional experiments to strengthen the link between ERα and tRNA U34 modifications. These issues were addressed in the revised version of the manuscript as follows: i) we complemented DNA-microarray results with RNA sequencing-based quantification of translatomes which further substantiated that ERα depletion causes translational offsetting. ii) we now provide further evidence that inhibition or depletion of ERα or ELP3 reduces levels of U34 tRNA-modifications. This further corroborates the tenet that ERα-dependent translational offsetting is a consequence of the reduction in tRNA U34 modifications. iii) we excluded a major role of alterations in protein stability in observed DEK mRNA/protein discrepancy. iv) we identified a biological role of ERα-dependent translational offsetting by showing that attenuation of mcm5s2U tRNA-modification via ELP3 knockout diminishes the effects of estradiol and fulvestrant on stimulating and inhibiting proliferation of BM67 cells, respectively.
Results of the experiments which were performed during revision are now included in 18 figure panels. We have also incorporated text modifications as suggested by the Reviewers.
Our detailed point-by-point response to reviewers' comments is outlined below:

Referee #1:

This is a potentially interesting and original study in which the impacts of removing a transcription factor on mRNA levels and corresponding protein levels are compared. There are sets of mRNAs that fall into particularly intriguing categories. There are both upregulated and downregulated mRNAs whose protein outputs appear not to change. The authors go on to characterize them and notably discover that tRNA levels may affect outcome. For the upregulated mRNA, the authors come up with the hypothesis that the transcription factor under study regulates the translational offset of its target genes through regulating the expression of some tRNA-modifying enzymes. In its

current form, the manuscripts suffers from being too cryptic for a general readership and from providing insufficient support for the claims made.

We would like to thank the reviewer for finding our study interesting and original. We believe that addressing the comments raised by the reviewers have substantially improved the study. We have also, to the best of our ability, re-written parts of the manuscript to render it more easily accessible.

Major comments:

(1) Technology choices: It could be (and has been) argued that the isolation of polysomes is more prone to artefacts than ribosome profiling. This is a key issue and must at least be appropriately discussed. Moreover, the choice of microarrays for polysome profiling seems strange considering that RNA-seq would yield much more quantitative data.

We agree with the reviewer and have performed RNA sequencing to complement DNA-microarray studies. Of note, DNA-microarrays were applied as this was the method commonly used when this study was initiated (it has taken almost 8 years to complete this project). Importantly, our new RNA sequencing-based quantification replicated results obtained using microarrays (**Fig EV1**). Moreover, we validated 86 genes from DNA-microarray/RNAseq data sets using nanostring codesets (**Fig 2**).

Of note, we showed that notwithstanding that ribosome profiling has many advantages over polysome profiling, the latter method has superior performance in capturing changes in translational efficiency (Gandin et al 2016, Genome Res; Masvidal et al RNA Biology 2017). Nonetheless, we are well aware that polysome profiling does not provide information regarding the ribosome positioning or the part of mRNA that is being translated. We are therefore planning to perform ribosome profiling studies in the future to explore the effects of ERα depletion on e.g. alternative ORF usage, elongation pausing etc. However, considering time constraints and the volume of this work, we trust that ribosome profiling studies are out of the scope of the present manuscript.

(2) Pharmacological tools: It isn't clear why the pharmacological tools (estrogens and anti-estrogens) are only used in MCF7 cells and not for some (or most) of the experiments in BM67. Although there is ERβ in BM67, there are some reasonably good ERα-specific drugs that are commercially available.

As the reviewer pointed out, we did not use pharmacological tools because of the presence of ERβ and AR in BM67 cells. To circumvent this, we opted for depletion of ERα by shRNA to attenuate ERα signalling without altering levels of estrogens/testosterone in serum or compete with ERβ and AR. Conversely, fulvestrant was used in MCF7 because of the difficulties we and others have experienced in trying to generate MCF7 clones with strong ERα knockdown and/or complete knockout (Fu et al. 2006). Moreover, we now provide new data showing that BM67 cells are responsive to E2 (~30% increase in proliferation) and fulvestrant (20% decrease in proliferation). Importantly, the effects of E2 and fulvestrant on BM67 cell proliferation were abolished when the mcm^5s^2 modification of U34 was impeded by ELP3 knockout. These new data establish a link between the effects of estrogen signalling on proliferation and U34 tRNA-modification machinery (**new Fig EV5**).

(3) DEK: The DEK story is confusing. In BM67 cells it seems to be one of those mRNAs, whose levels go up upon ERα KD but where translational offset keeps protein levels more or less the same (Fig. 1F and G). However, in MCF7 cells, as judged by the immunoblot of Fig. 6E, E2-induced ERα activity may induce DEK protein expression (and the ERα antagonist ICI leaves it untouched). While there may well be cell line-specific differences, it makes it impossible to compare these experiments. In any case, multiple immunoblots would have to be quantitated to draw firm conclusions about relatively minor effects.

We agree that the BM67 and MCF7 cells may show cell line specific differences regarding how they respond to ERα depletion/inhibition (indeed, MCF7 cells appear to be more dependent on ERα and E2 than BM67 cells). This may also be related to a chronic (BM67) vs. acute (MCF7) reduction in ERα signaling. Nevertheless, we now provide quantification of JAG1, AR, DEK, DCXR and CHK1 proteins in former **Fig 1G** (new **Fig 2B**; n=3-5). Indeed, whereas the level of JAG1 and AR are decreased in BM67-shERα cells as compared to control cells, levels of DCXR, DEK and CHK1 proteins are not significantly altered. We also performed quantification of protein levels in former **Fig 6E** (new **Fig 7B**) which confirmed that E2 and fulvestrant modulate ELP3 levels in MCF7 cells while not altering DEK protein abundance. Moreover, to directly establish the relationship between ERα and ELP3 expression, we performed CHIP-seq for ERα binding to the ELP3 locus upon E2 simulation. These experiments demonstrate that ELP3 is a direct ERα-regulated gene (new **Fig 7C**).

(4) ELP3/ALKBH8/CTU2 rescue experiment: this is definitely an original way of testing the hypothesis. Unfortunately, the protein data of Fig. 6F is not sufficiently clear. Incidently, ERα levels

may be slightly higher in the sample where ALKBH8 is overexpressed. Multiple experiments would have to be quantitated with proper standardization to actin and/or GAPDH.

The original submission included 2 independent experiments of this rescue experiment. For this revision we performed one additional experiment. Although the directionality is consistent, the effects are as pointed out small (<2-fold) and the variability relatively high. Therefore, when performing statistical analysis on the 3 replicates we only obtain a marginal p-value for rescuing DEK expression when overexpressing ALKBH8. A power analysis indicated that to observe a significant difference ($p<0.05$) we would need to perform >6 experiments. As this experiment is technically very challenging and has in total already taken us >6 months, we decided to employ an alternative strategy. To this end, we used CRISPR/Cas9 to silence ELP3 (as ELP3 is the most upstream protein in the pathway) in BM67-cells and rescued ELP3 expression using a construct which is not targeted by gRNA. Consistent with translational offsetting, loss of ELP3 in BM67 cells, resulted in no alterations in DEK protein despite increased levels of the DEK mRNA (**Fig 6C**). Moreover, upon rescue with gRNA-resistant ELP3, offsetting was reversed as mRNA levels were restored while DEK protein level remained constant (**Fig 6D**). These data suggest that the ELP3/ALKBH8/CTU2 pathway mediates translational offsetting in BM67 cells.

(5) Does the U34 connection mediate the translational offsetting of ERα target mRNAs? The data are insufficient to make this claim. Since only ALKBH8 seems to work in the experiment of Fig. 6F, one cannot conclude anything about ELP3 and CTU1/2. More direct experiments such knocking down or overexpressing these enzymes in the ERα KD BM65 cells might allow one to draw this conclusion.
We agree with the reviewer. As indicated above we changed strategy to link activity of the U34-modification pathway to translational offsetting. We selected to target ELP3 as this is the most upstream factor in the pathway and now show that ELP3 null BM67 cells show the expected translational offsetting of the DEK mRNA, which can be rescued by reintroducing a gRNA-resistant version of ELP3.
(6) Density graphs (of Fig. 1B, D, S1C-D, ...) must be explained more intelligibly. Neither the legends nor the supplementary methods provide a clear explanation of what the two axes are and how the graphs were generated. Similarly, it must be explained how "translation" can be computed and plotted (using the same units on both axes as for the other two plots?).

As requested we have re-written the figure legends explaining the density graphs. Also, we now better explain that translation is the estimate for changes in polysome-associated mRNA which are not paralleled by changes in total mRNA as quantified by anota2seq (**page 6 lines 11-12**).
(7) Fig. 4F-J are also much too cryptic despite some explanations in the supplementary methods. Fig. 5C-D is similarly cryptic.
We now provide a more detailed explanation of the analysis in the legend to figure 4 and 5.
(8) Fig. 6D: the differences, despite being apparently statistically significant, are minimal. The Y-axis not starting at 0 makes the graph rather deceiving.
These data (former **Fig 6D**, now new **Fig 7A**) originate from DNA-microarray based analysis of E2 dependent gene expression which can we reversed with fulvestrant. Fold-change differences in DNA-microarray experiments are affected by multiple factors including probe-characteristics. Therefore, the fold-change effect may not fully reflect the change in mRNA expression. As the background for DNA-microarray analysis is not 0 and varies across transcripts it is hard to know where the “true” 0-signal is. We therefore now plot the effects as log2 fold-changes as compared to a vehicle (**Fig 7A**). Moreover, in **Fig 7E** we now show that fulvestrant treatment of MCF7 cells leads to a ~95% reduction in cm5U, ~65% reduction in mcm5U and ~40% reduction in the mcm5s2U modifications. Moreover, as discussed above, we now provide strong evidence that ELP3 is a direct ERα-target using CHIP-seq (**Fig 7C**) and we also found evidence for ERα binding to the CTU1 loci (**Appendix Fig S5**).
(9) Title: the title makes too sweeping a statement. ERα may regulate the protein output of a small subset of its target genes by controlling the expression of enzymes involved in regulating translational offset. Indeed, the "reverse" statement ("Translational offset controls expression of some ERα target genes") would be more accurate.
We concur with the reviewer and have adopted what we hope will be considered a more accurate title: “Translational offsetting as a mode of estrogen receptor α-dependent regulation of gene expression”

Minor comments:
(1) mRNA quantitation: for the 86 quantitated by nanostring, it would be good to have a separate Table of the fold changes. That would facilitate reading figures like 1F/G.
We now provide a table detailing fold change for the nanostring codesets (**Table EV2**).
(2) Impact of ERα KD on expression of ELP3 (and ALKBH8 and CTU1/2), page 12: it isn't stated where the mRNA data are shown or from.
We have clarified that this originates from the polysome-profiling experiment in **Fig 1** on **page 12 line 11**. Notably the results were comparable for both DNA microarray- and RNA sequencing-based quantification.

Referee #2:

In their manuscript, Lorent et al describe results that indicate the down regulation of U34-tRNA modifying enzymes following ERa-depletion leads to translational offsetting of upregulated translational targets. While the concept is attractive and interesting, the manuscript falls short in demonstrating direct effects and the biological importance of the pathway. It remains on a very descriptive level.
We would like to thank the reviewer for finding our study interesting. To address reviewer's concerns, we performed a significant number of experiments to address mechanistic and functional aspects of ERα-dependent translational offsetting.

Major comments:
1.The authors used only one shRNA targeting ERα. At the minimum, another independent vector should be used to confirm the results. In particular, the offsetting phenomenon.
To address this important concern, we have generated 5 ERα knockout BM67 lines using CRISPR/Cas9. As expected, this resulted in reduction of ELP3 protein expression (**Fig 6B upper left**). Consistently with translational offsetting of DEK gene expression following ERα silencing using CRISPR/Cas9, although DEK protein levels were on average unchanged across the 5 cell lines as compared to control, mRNA levels were on average increased (**Fig 6B lower left**; 4 out of 5 cell lines showed the expected offsetting between protein and mRNA levels).
2. The authors used DNA-microarrays to quantify gene expression in polysome profilings. A much superior analysis would have been to perform RNAseq. Knowledge on change in isoform expression and a better window of differential expression would have been obtained. I think this has to be done to confirm that the effects are indeed due to offset.
We agree with the reviewer. As noted in the response to the Reviewer # 1's (comment 1), we have performed RNA sequencing to complement DNA-microarray studies. Of note, DNA-microarrays were applied as this was the method commonly used when this study was initiated (it has taken almost 8 years to complete this project). Importantly, our new RNA sequencing-based quantification replicated results obtained using microarrays (**Fig EV1**). Moreover, we validated 86 genes using nanostring codesets (**Fig 2**).
3. Figure 1G and 6C show western blot analyses. The changes shown are not very convincing, and it is not clear whether they are reproducible. The blots should be quantified and statistically evaluated in multiple biological replicates.
To address reviewer's concerns, we quantified the blots from formed figure 1G (**new Fig 2B**) and performed statistical analysis (**new Fig 2B**). Similarly, we have now quantified ELP3 protein expression in 3 biological replicates (**Fig 6A**). Furthermore, due to the concern in comment #1, we have added ELP3 western blotting images and their quantification across 5 ERα knock out cell lines (**Fig 6B**; see discussion above under comment #1)

4. The authors have not ruled out changes in protein stability. Can it be that some of the effects they observed as offset is actually coordinated regulation at the protein level? This should be experimentally examined on a couple of selected targets.
This is a very important point that we addressed by performing cycloheximide chase experiments. These experiments are now shown in **Fig 2C**. We did not observe differences in protein stability for DEK and CHK1 between shERα and control BM67 cells. We therefore conclude that changes in protein stability does not play a major role in translational offsetting of DEK and CHK1.

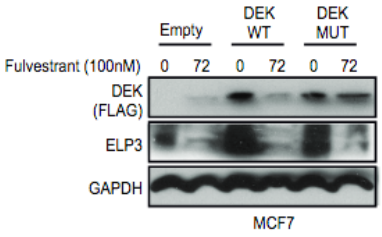
5. The authors pinpoint association of the upregulated offset group following ERá KD with reduced tRNA modification. However, a direct connection is missing. This can be obtained first by

quantification of the tRNA modification, and second phenotypically by measuring the expression of a reporter gene with altered coding usage.
In the original submission we used APM-PAGE to quantify differences in levels of thiolated $tRNA_{UUC}$ (**Appendix Fig S6A**). We now quantified cm5U, mcm5U and mcm5s2U using liquid chromatography coupled-tandem mass spectrometry (LC-MS/MS). Consistent with APM-PAGE data and a recent report (Rapino et al, Nature 2018), we did not observe major differences in accumulation of thiolated $tRNA_{UUC}$ between stably ERα depleted and parental cells (**Appendix Fig S6A**). We reasoned that this may be a consequence of accumulation of thiol-modified tRNAs during chronic ERα depletion (e.g. due to reduction in global protein synthesis). To investigate this in a more acute setup, we performed experiments under conditions wherein ERα was inhibited by fulvestant in MCF7 cells for a shorter period of time, followed by cm5U, mcm5U and mcm5s2U quantification by LC-MS/MS. As a positive control, we abolished ELP3 expression in MCF7 cells. These results are now presented in **Fig 7E** and show that similarly to ELP3 loss, fulvestrant dramatically decreases tRNA U34 modifications as compared to control cells. Indeed, in **Fig 7E** we now show that fulvestrant treatment of MCF7 cells leads to a ~95% reduction in cm5U, ~65% reduction in mcm5U and ~40% reduction in the mcm5s2U modifications. Considering that cm5 addition to U34 is the first step in the synthesis of mcm5s2U it is not surprising that it shows the largest decrease after fulvestrant treatment. It is noteworthy that we could not detect cm5U in BM67 cells due to limit of detection and that it is therefore possible that cm5U would be decrease in BM67 cells lacking ERα. These data provide further evidence that ERα regulates mcm5s2u tRNA modifications.
Regarding the use of a reporter with altered codon composition, although we recognise this would add support to our model, we prioritised the LC-MS/MS approach and did not have time to generate stable clones expressing "recoded" reporter genes.

6. Figure 6A. the authors state that no significant change in tRNA expression was observed (page 11). Looking at the figure, I just cannot figure out how they reached this conclusion.
To make these plots easier to interpret, we have now added a line at FDR = 0.05 on former Fig 6A (new **Fig EV4A**) to show that no change in tRNA abundance passes this threshold.

7. The authors perform few validation experiments in the human MCF7 cells (while the rest of the studies were in BM67 mouse cells). While this adds some confidence in the regulation of the tRNA modification enzyme, the basic offset phenomenon was not reproduced in this cell line. This is important.
As suggested by reviewer #1's comment #3, BM67 cells and MCF7 cells may show cell line specific differences regarding how they respond to ERα depletion/inhibition (indeed MCF7 cells appear to be more dependent on ERα and E2 than BM67 cells). Accordingly it has not been possible to generate a set of ERα knockout MCF7 cells (i.e. similar to BM67 cells in Fig. 6B). Instead we have been forced to suppress ERα activity using inhibitors. This experiment has revealed that under conditions when ERα is modulated using E2 and/or fulvestrant, although ELP3 levels parallel ERα activity, DEK levels were constant. Moreover, in ELP3 knockout MCF7 cells, DEK levels were unchanged (**Fig 7D**).

8. The suggestion that ELP3 is a target of ERa can be used to examine biological effects. In the context of ERa KD in BM67 cells, does the expression of ELP3 has biological consequences to cell response.
To answer this, we employed CRISPR/Cas9-generated ELP3 KO BM67 cells and monitored the effects of fulvestrant and E2 on their proliferation (new **Fig EV5**). These experiments revealed that ELP3 plays a major role in regulating cell proliferation as abrogating its expression significantly reduced proliferation of BM67 cells. Moreover, whereas fulvestrant reduced and E2 induced proliferation of control cells, these effects were abolished in ELP3 KO cells. Thus, in BM67 cells, ERα dependent modulation of proliferation requires expression of ELP3.



2nd Editorial Decision 20th Aug 2019

Thanks for submitting your revised manuscript to The EMBO Journal. Your study has now been re-reviewed by the two referees who appreciate the introduced changes. I am therefore pleased to let you know that we are happy to accept the manuscript for publication here. Before sending you the formal acceptance letter there are just a few last things that needs to be sorted out.

REFEREE REPORTS:

Referee #1:

The manuscript has been substantially complemented with new data/experiments and revised according to the reviewers' comments. It now reads more easily and the data support the claims more convincingly.

Referee #2:

I have studied the response of the authors to referees comments and I think the manuscript is greatly improved and presents a coherent research, and therefore is ready for publication.

2nd Revision - authors' response 28th Aug 2019

The authors performed the requested editorial changes.

3rd Editorial Decision 30th Aug 2019

Thanks for sending me the revised version. I have now had a chance to take a look at everything and all looks good.

I am therefore very pleased to accept the manuscript for publication here.

THE EMBO JOURNAL

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

Corresponding Author Name: Luc Furic
Manusript Number: EMBOJ-2018-101323

Reporting Checklist For Life Sciences Articles

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript (see link list at top right).

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- è the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- è figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- è graphs include clearly labeled error bars only for independent experiments and sample sizes where the application of statistical tests is warranted (error bars should not be shown for technical replicates)
- è when n is small (n < 5), the individual data points from each experiment should be plotted alongside an error bar.
- è Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation (see link list at top right).

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- è a specification of the experimental system investigated (eg cell line, species name).
- è the assay(s) and method(s) used to carry out the reported observations and measurements
- è an explicit mention of the biological and chemical entity(ies) that are being measured.
- è an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- è the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- è a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- è a statement of how many times the experiment shown was independently replicated in the laboratory.
- è definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html	Biosecurity Documents from NIH
http://www.selectagents.gov/	List of Select Agents

B- Statistics and general methods

Please fill out these boxes

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	To our knowledge, this is the first study of the role of estrogen receptor alpha in regulating mRNA translation genome-wide in prostate cancer. Thus, no good estimation of pre-specified effect sizes were available at the start of the study. However, similar studies in other model systems indicate that at least three replicates are needed. Therefore, experiments were performed on cell lines on at least 3 replicates.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Used statistical tests are described in figure legends. Details of more advanced statistical analysis are explained in the methods.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normal distribution was not tested and is difficult to formally assess when there is a limited number of data points.
Is there an estimate of variation within each group of data?	Standard deviation are visualized using error bars.
Is the variance similar between the groups that are being statistically compared?	When two-sample Student t-test were performed, equality of variances was not assumed (Welch t-tests)

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	The following antibodies were used: CHEK1 (56 kDa; CST 2360S) - reactivity: human, mouse - 1:1000 - secondary: mouse; CTU1 (ab136083) Rabbit - reactivity: human and mouse - 1:2000 - 36 kDa; CTU2 (ab177160) - Rabbit, reactivity: human and mouse - 1:2000 - 56 kDa; DCXR (28 kDa) - Proteintech 15188-1-AP - reactivity: human, mouse - secondary: rabbit - 1:1000; DEK (50 kDa; CST 13962S) - reactivity: human, mouse - secondary:rabbit - 1:1000; ELP3 (62 kDa) - reactivity: human, mouse - secondary:rabbit - 1:1000; ERalpha (66 kDa) - NCL-L-ER-6F11 Leica - reactivity:human, mouse - secondary: mouse - 1:1000; Jag1 (CST #2620) - reactivity:human, mouse - 180 kDa - secondary: rabbit - 1:1000; c-MYC (abcam Y69 -ab32072 rabbit) - 57 kDa - 1:1000; AR (Sigma A9853) - rabbit, 110 kDa - 1:5000
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All cell lines were routinely tested for mycoplasma (in house service, Peter MacCallum Cancer Centre). MCF-7 and HEK293T cells which were used to make virus for CRISPR experiments were specifically tested negative for mycoplasma on 2019-03-28 and 2019-04-18 respectively. MCF7 cells were purchased from ATCC and used at low passage for less than 2 months before thawing a new vial. BM67 cells have been described previously (Takizawa et al., 2015) and used at low passage.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines' (see link list at top right). See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. 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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. 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' (see link list at top right).	NA
17. 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' (see link list at top right).	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition' (see link list at top right). Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data	The DNA-microarrays, RNA-seq of full length or small RNAs and nanoCAGE data have been deposited on NCBI GEO repository under accession number GSE120917. The data will be made public upon manuscript acceptance.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	The raw data and pre-processed data are deposited to GEO (see above) while the analysis results tables (list of significantly regulated genes, list of significantly enriched Gene Ontology) are provided as Expanded View tables.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section: Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	We performed re-analysis on 5 public data sets in this study. They are cited both as references and data references (Data Ref)
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No