EMBO reports

# E2F1 interacts with BCL-xL and regulates its subcellular localization dynamics to trigger cell death

Céline Vuillier, Steven Lohard, Aurélie Fétiveau, Jennifer Allègre, Cémile Kayaci, Louise E. King, Frédérique Braun, Sophie Barillé-Nion, Fabien Gautier, Laurence Dubrez, Andrew P. Gilmore, Philippe P. Juin and Laurent Maillet

**Review timeline:**

| | |
|---|---|
| Submission date: | 7 February 2017 |
| Editorial Decision: | 14 March 2017 |
| Revision received: | 13 July 2017 |
| Editorial Decision: | 6 August 2017 |
| Revision received: | 12 October 2017 |
| Editorial Decision: | 25 October 2017 |
| Revision received: | 6 November 2017 |
| Accepted: | 16 November 2017 |

Editor: Martina Rembold

## 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 14 March 2017

Thank you for the submission of your research manuscript to EMBO reports. I apologize for my delayed response but your manuscript was sent to three referees and I was hoping to receive also the third referee report. However, as we have not received it until now, despite many reminders, I have decided to proceed with the existing reports, which are very much in agreement with each other. Please find the full reports copied below.

As you will see, while the referees acknowledge that the findings are potentially interesting, they all point out that significant revisions are required and that the data need to be strengthened before the study can be considered for publication here. The referees raise partially overlapping concerns regarding the subcellular fractionation experiments, the BH-groove independent interaction between BCL-xL and E2F1, and the mitochondrial localization of endogenous E2F1.
Both referees indicate that the study is currently based on an E2F1 construct that is artificially targeted to mitochondria and that the study would greatly benefit from E2F1 mutants that cannot bind BCL-xL and mitochondria.

From these comments it is clear that publication of the manuscript in our journal cannot be considered at this stage. On the other hand, given the potential interest of your findings, I would like to give you the opportunity to address the concerns and would be willing to consider a revised manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Upon further discussion with the referee 1 it becomes clear that it will be essential to address also the last point mentioned above and to show that a mutant E2F1 that cannot bind BCL-xL or translocate to mitochondria is compromised in pro-apoptotic activity, to substantiate the physiological relevance of your findings. Given the amount of work and the

 

uncertain outcome of these experiments I would understand if you decide to seek rapid publication elsewhere. Should you however decide to embark on such a revision, acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure or per figure panel.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

**********
REFEREE REPORTS

Referee #1:

In this manuscript Vullier and colleagues describe a new pro-apoptotic function for E2F1, in short mitochondrial translocation of a pool of E2F1 it inhibits BCL-xL motility, thereby impeding its anti-apoptotic function. As proposed, this is a novel and very interesting mode of cell death regulation. However, the study suffers from significant shortcomings that need to be addressed in order to support the authors' conclusions.

- Many conclusions are based on overexpression of an E2F1 construct that is artificially targeted to the mitochondria, as such it is completely unclear whether endogenous E2F1 apoptotic effects occur through a similar manner, this needs to be addressed. Given that BCL-xL and E2F1 can be shown to co-ip (Fig 3), it should be possible to map areas of interactions between both proteins to generate endogenous E2F1 mutants that can no longer effect BCL-xL mobility to determine if this (mitochondrial targeting) impacts on E2F1 pro-apoptotic function.

- the rationale that endogenous E2F1 is targeted to mitochondria (Fig 1) needs to be better supported. The lamin signal (nuclear) is significantly weaker than the E2F1 blot signal, yet ratio wise there looks to be a similar amount of lamin signal in the mitochondrial fraction. Secondly, it is unclear how pure the mitochondrial fractions are, probing for ER as well as cytosolic proteins would address this.

- The data supporting a BH-groove independent interaction between BCL-xL/E2F1 is strong (Fig 3), however its key to determine whether E2F1 binding to BCL-xL affects BH3-only proteins or BAK binding to XL - it seems entirely plausible that interaction of E2F1 to BCL-xL could cause conformational change in XL or sterically hinder BAK/BH3 interactions with BCL-xL, thereby promoting apoptosis.

Referee #2:

The authors show that E2F1 has a proapoptotic role that seems independent of its DNA binding ability (see below). They also show that this proapoptotic function can be inhibited by Bcl-xL overexpression and that Bcl-xL interacts with E2F1. As the presence of E2F1 decreases the retrotranslocation rate of Bcl-xL, they conclude that the proapoptotic role of E2F1 is due to the

 

accumulation of Bcl-xL at the MOM, which is less able to block Bak. The study is interesting as it reports a new modulator of Bcl-xL that acts on the shuttling between cytosol and MOM to modulate apoptosis. However, a number of issues should be addressed, which are important to support several of the author's conclusions.

In Fig 1C the authors detect E2F1 in the mitochondrial fraction of control and etoposide-treated cells. However this is a small part of the total E2F1 amount. To judge if the protein detected at mitochondria is significant and specific, the authors should show the results of this experiment in a general fractionation experiment, including nuclear and cytosolic fractions, as well as other cellular membranes like ER and plasma membrane, and the distribution of control proteins of the different fractions.

In Fig 1E, the authors claim that wt and OTC E2F1 transfected cells have decreased levels of OMI-Cherry, but this not so clear specially in the case of OTC-GFP-E2F1, and also seems to be the case of the control OTC-GFP. To properly demonstrate their interpretation of the data, the authors should show better images and quantification of the Cherry levels in a significant number of cells.

The authors should refer to data, if they exist, demonstrating that the E132 mutant does not bind to DNA or demonstrate it themselves.

In Fig 2B, the authors should show the results with ectopic expression of Bax as a control.

Figure EV3 B needs the negative control as a reference for the saturation curves. If Bak and Bcl-xL interact mainly via the BH3 domain/hydrophobic groove, how do the authors interpret the high BRET signal between Bak and the R139D mutant of Bcl-xL. Although the amount of Bcl-xL - R139Dneeded for interaction is slightly higher than the wt, the BRET signal reaches the same saturation levels, indicating interaction. That would invalidate the conclusions from the data in EV3 B and the hypothesis that interactions are not mediated by the BH3 domain.

If E2F1 acts exclusively via Bcl-xL, and Bcl-xL is an inhibitor of both Bax and Bak, how come the effect of E2F1 is only on Bak? In Figure 4A, the levels of Bak-mediated apoptosis in cells transfected with YFP-Bax are already basically reduced to cero with Bcl-xL wild type, so there is no range to detect if the mobile Bcl-xL mutants would have an additional protective effect. The authors should solve this problem, maybe performing the same analysis in Bax-dependent cell death.

The study would greatly benefit of using a E2F1 mutant that does not associate with mitochondria.

Minor comments:
-The authors should explain in the text what OTC stands for.
-The authors should show the data about E2F1 not binding to BAK at least in the supplementary information, as this information is important to understand the mechanism of E2F1.
-The authors should avoid the term "mobile" or "mobility" to refer to Bcl-xL variants that have higher retrotranslocation rates compared with the wild type protein as specify precisely what they refer to. The terms associated with mobility are confusing as they are easily interpreted in the sense of diffusion of the protein at the MOM or in the cytosol.

1st Revision - authors' response 13 July 2017

*Referee #1:*

*In this manuscript Vullier and colleagues describe a new pro-apoptotic function for E2F1, in short mitochondrial translocation of a pool of E2F1 it inhibits BCL-xL motility, thereby impeding its anti-apoptotic function. As proposed, this is a novel and very interesting mode of cell death regulation. However, the study suffers from significant shortcomings that need to be addressed in order to support the authors' conclusions.*

We thank this referee for his evaluation of our work. As discussed below, we tried to address most of the concerns raised in this revised version of the manuscript.

 

*- Many conclusions are based on overexpression of an E2F1 construct that is artificially targeted to the mitochondria, as such it is completely unclear whether endogenous E2F1 apoptotic effects occur through a similar manner, this needs to be addressed. Given that BCL-xL and E2F1 can be shown to co-ip (Fig 3), it should be possible to map areas of interactions between both proteins to generate endogenous E2F1 mutants that can no longer effect BCL-xL mobility to determine if this (mitochondrial targeting) impacts on E2F1 pro-apoptotic function.*

Thank you for this suggestion. We have performed the structure activity relationship analysis demanded and we could identify that the DNA Binding Domain of E2F1 is sufficient *per se* to interact with BCL-xL (new Figure 3B-C, plus EV3C) and trigger cell death and MOMP (new Figure 3D, EV3D), an observation consistent with previous results (Hsieh et al., Genes Dev., 1997). Conversely, an E2F1 form deleted in its N-terminal end (and thus of the DBD) shows significantly reduced interaction and killing activty (Figure 3B-D, EV3C).

*- the rationale that endogenous E2F1 is targeted to mitochondria (Fig 1) needs to be better supported. The lamin signal (nuclear) is significantly weaker than the E2F1 blot signal, yet ratio wise there looks to be a similar amount of lamin signal in the mitochondrial fraction. Secondly, it is unclear how pure the mitochondrial fractions are, probing for ER as well as cytosolic proteins would address this.*

Thank you for raising the lack of clarity in this approach of ours. Our main initial point is that E2F1 is not only nuclear and that it can be found in the cytoplasm of untreated or genotoxic treated cells. We performed a gross subcellular fractionation assay based on differential centrifugations of the cytoplasmic fraction and obtained a heavy membrane fraction (pelleting at 12 000 *g*) that includes mitochondrial markers (COX IV) but also ER markers indeed (KTN1- see new Figure EV1). We made explicit in this new version of the text that E2F1 is detected by western blot in a "heavy membrane fraction (HMF)" that includes mitochondria (instead of a wrongly named "mitochondrial fraction" ). Changes were made to the text accordingly. Regarding the concern about contamination of nuclear components (detected by Lamin A/C western blotting), we have performed densitometric analysis and evaluated ratios between the HM and the total fractions. This evaluation shows that the percentage of E2F1 detected by western blotting at intracellular membranes systematically exceeds contamination rates (detected by Lamin western blotting) (new Figure 1C and EV1A).

*- The data supporting a BH-groove independent interaction between BCL-xL/E2F1 is strong (Fig 3), however its key to determine whether E2F1 binding to BCL-xL affects BH3-only proteins or BAK binding to XL - it seems entirely plausible that interaction of E2F1 to BCL-xL could cause conformational change in XL or sterically hinder BAK/BH3 interactions with BCL-xL, thereby promoting apoptosis.*

Thank you for pointing out that E2F1 might indirectly impact on BAK/BCL-xL molecular interactions. Our point is that the interaction between E2F1 and BCL-xL does not rely on the BH3 binding site of BCL-xL, and thus that E2F1 cannot act as a competitive inhibitor of BH3 binding. However, we do not rule out that E2F1 might influence BAK/BCL-xL interactions indirectly. Since, as mentioned in our manuscript, we detected no effect of recombinant E2F1 on BAK mediated MOMP (or on the protective effect of recombinant BCL-xL) in cell free assays, we inferred that this indirect effect would have to be patent in whole cells, and that dynamic processes needed to be investigated. The effects of E2F1 on BCL-xL localization dynamics, and the consequence of these dynamics on control over BAK (as reported here) do argue that E2F1 impact on the BAK/BCL-xL interplay. Changes were made to the text to clarify this point.

*Referee #2:*

*The authors show that E2F1 has a proapoptotic role that seems independent of its DNA binding ability (see below). They also show that this proapoptotic function can be inhibited by Bcl-xL overexpression and that Bcl-xL interacts with E2F1. As the presence of E2F1 decreases the retrotranslocation rate of Bcl-xL, they conclude that the proapoptotic role of E2F1 is due to the accumulation of Bcl-xL at the MOM, which is less able to block Bak. The study is interesting as it reports a new modulator of Bcl-xL that acts on the shuttling between cytosol and MOM to modulate*

 

*apoptosis. However, a number of issues should be addressed, which are important to support several of the author's conclusions.*
We thank this referee for his positive comments about our manuscript.

*In Fig 1C the authors detect E2F1 in the mitochondrial fraction of control and etoposide-treated cells. However this is a small part of the total E2F1 amount. To judge if the protein detected at mitochondria is significant and specific, the authors should show the results of this experiment in a general fractionation experiment, including nuclear and cytosolic fractions, as well as other cellular membranes like ER and plasma membrane, and the distribution of control proteins of the different fractions.*
As argued in our reply to Referee 1, our gross subcellular fractionation isolated a heavy membrane fraction (rather than a mitochondrial one) with mitochondrial (COXIV) and ER (KTN) markers where E2F1 was detected at levels above nuclear contamination rates. This is now clarified in the text and argues that BCl-xL E2F1 interactions occur in the cytoplasm and more specifically at intracellular membranes, where BCL-xL also is detected (Figure 1C). Figure EV1A shows that E2F1 could also be detected in the cytosolic fraction.

*In Fig 1E, the authors claim that wt and OTC E2F1 transfected cells have decreased levels of OMI-Cherry, but this not so clear specially in the case of OTC-GFP-E2F1, and also seems to be the case of the control OTC-GFP. To properly demonstrate their interpretation of the data, the authors should show better images and quantification of the Cherry levels in a significant number of cells.*
Thank you for raising this point. Firstly, there had been a mistake in our legends of Figure 1E, with the OTC-GFP image corresponded in fact to E132, OTC-GFP-E2F1 to OTC-GFP and E132 to OTC-GFP-E2F1 (mitochondrial). The new Figure EV1H now shows the right legends. Secondly, we performed the experiments demanded and quantified by FACS the Cherry levels in numerous green fluorescent cells expressing variants of GFP fused E2F1. These important new results are shown in Figure 1E, Figure 3D and EV1I.

*The authors should refer to data, if they exist, demonstrating that the E132 mutant does not bind to DNA or demonstrate it themselves.*
This mutant was initially described and shown to be deficient for transcription in Cress et al, Mol Cell Biol. 1993. The DNA binding deficiency for this mutant was shown later in Bell et al., Oncogene 2006 and we have quoted the latter article in our new version of the manuscript.

*In Fig 2B, the authors should show the results with ectopic expression of Bax as a control.*
Thank you for this suggestion. The requested experiment shows that E2F1 can also amplify the pro-apoptotic effects of BAX (new Figure 2C). This lead us to modulate our interpretation of the effects of E2F1: while our data in Saos-2 cells (BAK knock down and lack of effect of BCL-2) show that E2F1 would preferentially impact on BAK activity (justifying our specific analysis of the influence of E2F1 on the BAK/BCL-xL interplay ) this might in fact not be exclusive, and E2F1 might also impact on BAX-induced cell death under some circumstances. We have modified the text accordingly.

*Figure EV3 B needs the negative control as a reference for the saturation curves.*
We now show in Figure 3B the (lack of) BRET signals obtained between Luciferase fused E2F1 and YFP solely fused to the C-terminal end of BCL-xL (a negative control that shares with BCL-xL its subcellular localization but lacks most of its sequence).

*If Bak and Bcl-xL interact mainly via the BH3 domain/hydrophobic groove, how do the authors interpret the high BRET signal between Bak and the R139D mutant of Bcl-xL. Although the amount of Bcl-xL -R139Dneeded for interaction is slightly higher than the wt, the BRET signal reaches the same saturation levels, indicating interaction. That would invalidate the conclusions from the data in EV3 B and the hypothesis that interactions are not mediated by the BH3 domain.*
Comparison of BRET saturation curves between BAK, wild type or R139D BCL-xL shows that alteration of R139 impacts on the amount of acceptor required to reach saturation, and thus, in line with established practice in resonance energy transfer analysis, on the apparent affinity between BAK and BCL-xL. To completely nail down the point you raised, we used, in this new version of the manuscript, a triple mutant of BCL-xL (G138E R139L I140N). As shown on the new Figure EV 3E-F, this mutant shows greatlty reduced BRET signals with BAK, yet essentially unaltered signals with E2F1.

 

*If E2F1 acts exclusively via Bcl-xL, and Bcl-xL is an inhibitor of both Bax and Bak, how come the effect of E2F1 is only on Bak? In Figure 4A, the levels of Bak-mediated apoptosis in cells transfected with YFP-Bax are already basically reduced to cero with Bcl-xL wild type, so there is no range to detect if the mobile Bcl-xL mutants would have an additional protective effect. The authors should solve this problem, maybe performing the same analysis in Bax-dependent cell death.*
The point you address here evokes the one about BAX overexpression you raised above. Our new data indicate that the link between E2F-1 and BAK may not be exclusive and that BAX also may be influenced. The fact that the wild type and D2 mutant of BCL-xL have comparable effects on BAX mediated cell death in Figure 4A may either be due to the fact that BAX (which has higher translocation rates than BAK) is less subject to changes in BCL-xL retrotranslocation than BAK (as we initially suggested) or to the fact that the cell death rates induced by BAX in these assays are too low for us to detect an additional level of regulation by BCL-xL shuttling. We have modified our interpretation of the data in the text.

*The study would greatly benefit of using a E2F1 mutant that does not associate with mitochondria.*
We could not identify a variant of E2F1 that would not associate with intracellular membranes, as subcellular fractionation of cells after their transient (and pro-apoptotic) transfection with E2F-1 proved technically challenging. However, our identification of the DBD of E2F1 as a domain critical for the interaction with BCL-xL has lead us to identify a variant (DN) with significantly diminished interaction and killing activity.

*Minor comments:*
*-The authors should explain in the text what OTC stands for.*
We have now added to the text that OTC stands for the mitochondrial targeting sequence of ornithine carbamoyltransferase

*-The authors should show the data about E2F1 not binding to BAK at least in the supplementary information, as this information is important to understand the mechanism of E2F1.*
This is now shown in Figure EV3B.

*-The authors should avoid the term "mobile" or "mobility" to refer to Bcl-xL variants that have higher retrotranslocation rates compared with the wild type protein as specify precisely what they refer to. The terms associated with mobility are confusing as they are easily interpreted in the sense of diffusion of the protein at the MOM or in the cytosol.*
To follow you r recommendation, we have replaced these terms by “retrotranslocation” or “shuttling”. We describe FRAP assays as a mean to measure “BCL-xL localization dynamics.

2nd Editorial Decision 6 August 2017

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see both referees are now positive about the study but referee 1 indicates that further proof of the mitochondrial localization of endogenous E2F, as requested earlier, would be important and should be added prior to publication, in particular since the conclusions otherwise mostly rely on overexpressed proteins. Upon further discussion with referee 2, this referee agrees that these experiments would increase the quality and impact of the manuscript and should be added.

I look forward to seeing a final version of your manuscript as soon as possible.

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REFEREE REPORTS

Referee #1:

Besides the point detailed below, the authors have done an excellent job in addressing all my

comments.

Reiterating an earlier point I raised, since the vast majority of conclusions are derived from over expression expts. to provide some support that endogenous E2F carries out a similar function at the mitochondria, I still think its important to demonstrate that endogenous E2F is actually targeted to mitochondria be it through a cleaner fractionation protocol/imaging or another approach.

Referee #2:

The authors have addressed all the reviewers' concerns adequately.

2nd Revision - authors' response 12 October 2017

Referee #1:

*Besides the point detailed below, the authors have done an excellent job in addressing all my comments.*

We thank this referee for his positive appreciation.

*Reiterating an earlier point I raised, since the vast majority of conclusions are derived from over expression expts. to provide some support that endogenous E2F carries out a similar function at the mitochondria, I still think its important to demonstrate that* ***endogenous E2F is actually targeted to mitochondria be it through a cleaner fractionation protocol/imaging or another approach.***

We have worked in both directions. Firstly, we have worked on improving our subcellular fractionations based on differential centrifugations of mechanically lysed cells and obtained a heavy membrane fraction with no detectable nuclear markers where there is evidence of E2F-1. Secondly, we used another approach using the MACS Technology and superparamagnetic microbeads conjugated to anti-TOM22 antibody. As judged by the significantly lower amount of endoplasmic reticulum marker in the resulting fraction, this separates better mitochondria from endoplasmic reticulum – even though this task might be difficult to achieve fully due to the numerous contacts between these two organelles. We show that this fraction shows expression of E2F1. In all cases, BCL-xL is also found in fractions where E2F1 is detected. These new data are shown in a new Figure 1C, from which we subtracted the preceding, less convincing fractionation results.

Referee #2:

*The authors have addressed all the reviewers' concerns adequately.*

We thank this referee for his positive evaluation of our revisions.

3rd Editorial Decision 25 October 2017

Thank you for submitting your revised manuscript to EMBO reports. I have meanwhile gone through all the new files and am now writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed.

 

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

| |
|---|
| Corresponding Author Name: JUIN Philippe |
| Journal Submitted to: EMBO report |
| Manuscript Number: EMBOR-2017-44046V1 |

**Reporting Checklist For Life Sciences Articles (Rev. July 2015)**

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.

## A- Figures

### 1. Data

**The data shown in figures should satisfy the following conditions:**

- 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.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- 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.

### 2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- 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.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - 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.

**In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.**

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
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http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

## B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

| | |
|---|---|
| 1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? | No statistical mechods were used to determine sample size in advance. We chose the sample size according to the study of similar fields performed by other researchers. |
| 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? | No samples were excluded from the analysis in the experiments. |
| 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. | Experiments/data were randomly selected without bias. |
| 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? | NA |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | No statistical methods were used to assess it. |
| Is there an estimate of variation within each group of data? | Yes, we provided tha data as means +/- SEM. |
| Is the variance similar between the groups that are being statistically compared? | We hypothesized that the variance is similar between the groups. |

## 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). | ACTIN (MAB1501R) and BIM (AB17003) from Millipore, E2F1 (3742), COXIV (4850), PUMA (4976), BID (2002S) and BAK (3814) from Cell Signaling, BAX (A3533) and BCL-2 (M0887) from Dako, BCL-xL ([E18] Ab32370) and GFP (Ab290) from Abcam, MCL-1 (sc-819) and LAMIN A/C (sc-376248) from Santa Cruz, PARP (#AM30) |

| | |
|---|---|
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | ATCC. Mycoplasma contamination was tetsed on a regular basis. |

* 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 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'. Please confirm you have submitted this list. | 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'. Please confirm you have followed these guidelines. | NA |

## F- Data Accessibility

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| 18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>e. Proteomics and molecular interactions | NA |
| 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). | NA |
| 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. 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

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| 22. 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. | We do not anticipate that our study fall under dual use research restrictions. |