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Resetting cancer stem cell regulatory nodes upon MYC inhibition

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1st Editorial Decision, Part 1 04 December 2015

Thank you for the submission of your research manuscript to EMBO reports. It has been sent to three referees, and so far we have received reports from two of them, which I copy below. As both referees feel that the manuscript is interesting and recommend that you should be given a chance to revise it, I would like to ask you to begin revising your manuscript according to the referees' comments.

Please note that this is a preliminary decision made in the interest of time, and that it is subject to change should the third referee offer very strong and convincing reasons for this.

As you will see, both referees acknowledge the potential interest of the findings. However, referees 1 and 2 also point out several technical concerns and have a number of suggestions for how the study should be strengthened, and I think that all of them should be addressed.

Given the present referee comments and the potential interest of your study, we would like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete

point-by-point response. 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; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

I apologize again for all the delays and 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, "Resetting cancer stem cell regulatory nodes upon MYC inhibition" Galardi, Savino and colleagues utilize an inducible Omomyc miniprotein that interferes with normal MYC function, in the setting of patient derived glioblastoma stem cells (GSCs) to interrogate the impact of MYC inhibition in mediating the tumorigenic function of these cancer stem cells.

Evidence is presented to demonstrate that induction of the Omomyc protein reverses, both in vitro and in vivo, many of the hallmarks of the tumor status of several of these cells lines, including the ability to migrate and differentiate and the expression of several molecular markers of neural stem cell self-renewal and proliferation. Further, in the mouse transplant studies, expression of Omomyc lead to the suppression of both cell intrinsic and tumor microenvironment functions typical of glioblastoma.
This study ultimately identifies and validates MYC target genes, specifically upregulated microRNAs of the miR-146b and the miR-200a-200b-429 clusters, shown in vitro to impact cell proliferation and migratory potential. Importantly, as referenced, microRNA's are known to play a role in mediating MYC's tumorigenic influence in glioblastoma.

Overall this study provides important insights towards understanding the complexity behind the role of Omomyc in mitigating MYC's oncogenic function, specifically in the setting of glioblastoma where it already has preclinical implications. One of the potential, key novel aspects of this work stems from the genome-wide changes in MYC and Pol II occupancy that occur upon Omomyc induction. However, the data supporting these findings is incomplete and in some instances less convincing. The ultimate aim of identifying the important functional gene targets in the MYC GSC transcriptome that occur upon Omomyc induction could theoretically be achieved by examining the MYC ChIP-seq and RNA-seq data. The authors state that they focus on the genes whose expression is most significantly modulated upon Omomyc induction and that also represent those bound by MYC (genes associated with a MYC peak and likely containing the E-box MYC/MAX binding motif). Such a gene set, while unique for different cancer cell types as stated, also represents the genes predicted to be functionally important for MYC function, at least in part, based on previous studies.

One potentially interesting aspect of this work is the observation of minimal global effects on Pol II loading upon Omomyc induction. However, this would be greatly enhanced by the inclusion of ChIP-seq studies that also looked at potential changes in Pol II function, i.e., Pol II phosphorylated at Ser 5 and Ser 2 (of the CTD), representing marks of initiating and elongating Pol II, respectively (and while it is not expected that this study should comprehensively examine, in the context of Omomyc, all potential histone marks that are associated with different aspects of functional MYC, i.e. enhancers, promoters, etc., this would also be enlightening for Omomyc function).

Also, the percentage of MYC target genes identified in this study whose Pol II loading DOES change upon Omomyc expression is not clearly stated and/or depicted. This would be an interesting addition to the analysis and would potentially correlate with the genes whose expression significantly changes when Omomyc is expressed. As the data is presented, it is not clear how the Pol II ChIP-seq data contributes to the overall selection of genes that are functionally critical to the ability of Omomyc to mitigate the tumourigenic effects of MYC. Finally, this section would be

improved by the inclusion of several validating gene specific qChIP assays (against MYC, Omomyc, Pol II, etc.), quantitatively comparing and validating the qualitatively different MYC targets represented by, for example, NCL and HDAC1.

The data that is the subject of Figure 6 is interesting and correlative but does not entirely support the conclusions that are drawn. If the hypothesis is that there are key MYC targets (i.e. master transcriptional regulators) that themselves regulate gene programs that are important for the GSC cancer phenotype, ultimately it seems necessary to examine this kinetically, measuring gene expression at earlier time points, using a system of rapid Omomyc induction or by forcing expression of a limited set of these master transcription factors to recapitulate, at least in part, the oncogenic MYC transcriptome in these cells. While these experiments might be outside the scope of this work, these concepts are not discussed. Further complicating the analysis of data represented in this section is other, unknown/uncharacterized effects of Omomyc expression that may fall outside of simply replacing MYC. Importantly these concepts are touched on in the Discussion.

Lastly, the Discussion section is well written and brings to the forefront many of the key, current questions that MYC field needs to address to reconcile MYC's role as a genome-wide multi-functional transcription factor/chromatin regulator and its oncogenic function, in different cancer type settings. However these points are not related back to the data presented and no Figures are referenced in the Discussion, linking them in to their wider implications.

Minor points:

• In some instances, the references cited are incomplete. For example, in the Introduction, the sentence "this view is supported by studies showing the potential therapeutic efficacy of drugs impairing MYC transcription" is followed by the sole citation of Delmore et al., 2011 but leaves out the contemporary BET protein inhibitor studies including Zuber et al., Nature, 2011 and Dawson et al., Nature 2011. Similarly, the statement "current views consider MYC as either a universal non-linear amplifier or a gene-specific modulator" leaves out important primary references including Nie et al., Cell, 2012 and Sabo et al., Nature 2014, even though reviews are also cited.

• Figure 6A: it is not clear how the DUSP family genes act as a control set, this is not explained well in the text or in the methods/Figure legend. All except one appear to be up or downregulated in response to Omomyc expression and are expressed at baseline, while some are enriched for MYC binding. What is this gene family controlling for?

• At the end of the Results section titled "Impact on cancer stem cell transcriptome" the authors do not reference the Figure/Table where these gene sets/terms are presented.

• Some of the methods and/or Figure legends are incomplete, e.g. for the ChIP-seq experiments how many biological replicates were conducted/analyzed and what specific antibodies were used. Also, a better description of how the analyses depicted in Figures 5 and 6 were carried out. Minor: there is no specific reference to the Dang and Kim datasets in the Figure 6 legends (nor are these references cited in the main text). The methods for the migration assays are minimal - the Figure shows a bar graph of "cells per field" yet the methods don't say how many fields were counted per assay (presumably performed as 3 independent biological replicate experiments). Also, is the assay used for Figure 7F the same as for Figure 1B? If so, one is measured as cell number while the latter is in units of Absorbance. They are both referred to as growth curves in the Figure legends and from the text appear to be assaying the same thing - this is confusing. Generally speaking, the methods and Figure legends could be improved with more detail.

• Minor grammatical errors, etc.:

1. Results, p.2, first sentence: "...we resorted to inducible Omomyc". Given that the word "resorted" is often used in the context of "turn to and adopt a course of action, especially an extreme or undesirable one" an alternate word choice might better reflect the meaning intended.
2. Results, p.3, section heading: "impact" should be capitalized.
3. Results, p.4, top paragraph: "...concordantly with published data...consistently with the view..." should read "...concordant with published data...consistent with the view...". Also in the Discussion, p.8, "Consistently with this possibility..." should be "Consistent with this possibility...".

4. Throughout the text (including Figure legends) and in some Figure panels, Pol II is incorrectly written without a space, as "PolII".
5. All Figures are lacking Figure numbers; Figure 6 is lacking in labels (A, B?)

Referee #2:

Galardi et al. investigate the function and mechanistic effect of a Myc inhibitor (OmoMyc) on 3 glioblastoma cell lines. They state that "Omomyc bridles the key cancer stem cell features and affects tumour microenvironment, inhibiting angiogenesis". At a molecular level they report the global changes in Myc binding and OmoMyc binding using ChIP-seq, as well as report RNA and protein expression changes on these targets. This large body of data are nicely presented and for the most part the experiments are generally well controlled. Issues associated with this manuscript are important and need to be addressed to increase clarity:

1. The use of "cancer stem cell (CSC)" throughout, including the title, is inappropriate and must be changed. The authors define the CSC as neurosphere-derived cells. Evidence is not provided to show that the authors' neurospheres fulfill the criteria of a glioblastoma cancer stem cell.

2. The authors grow the cell lines as neurospheres prior to injecting single cells derived from these neurospheres for xenograft formation. However, it remains unclear whether these cell lines are grown as neurospheres for all other assays or whether they are grown as traditional 2D populations on tissue culture plastic. This is an important detail that must be included in the methods and body of the manuscript. This detail also speaks to the authors claim that the analyses they perform are relevant to the CSC. Was the ChIP-seq conducted from neurospheres? If not then the functional and molecular analyses have been performed under different conditions. This needs to be made clear in both the methods and in the text.

3. Does OmoMyc bind E-boxes in partnership with Max? Max ChIP-seq would address this important mechanistic question. However, conducting OmoMyc and Max ChIP-qPCR on specific target genes would be sufficient to answer this question in this manuscript.

4. Table 1: This table shows that the motif analysis of Myc-bound target genes is significantly altered in response to dox alone. Without dox, motif analysis shows Myc binds to motifs that resemble sites associated with Myc, Mycn, MYC:MAX, etc. However when cells are treated with dox, the motif analysis shows Myc binds to motifs that resemble sites associated with TBP, MEF2A, MEF2C, FOXL1, etc. This means that the motifs associated with OmoMyc binding are also influenced by the treatment with dox. This needs to be made clear in the text as a caveat to this experiment.

5. Miniprotein: This is lab jargon that must be removed from the manuscript as it may confuse some readers. It is better to define OmoMyc and then refer to the inhibitor by name.

6. Fig 1G and EV1E & F: It would add weight to the manuscript to have the micrographs quantified.

7. Legend 1E and EV1: "qRT-PCR of relevant markers ..". Figures should be understandable without having to read the text. Define relevant markers in the legend.

8. Fig 5D: targes should be targets

9. Page 6, half way down the page: This sentence needs to be corrected, "A similar distribution was observed in not target genes as well (Fig 5C)."

1st Editorial Decision, Part 2 — 23 December 2015

As indicated in my previous letter I have - in the interest of time - made a preliminary decision concerning your manuscript based on two out of three referee reports. I also pointed out that this decision can be subject to change should the third referee offer very strong and convincing reasons for this. We have meanwhile received the report of referee 3 that is copied below.

You will see from the report that referee 3 raises significant concerns regarding the conclusiveness

of your data, in particular the data that analyze the biological effect. The concerns partially overlap with the comments from the other two referees. We will not revise our original decision but I want to point out that the concerns of referee 3 have to be addressed in the revised manuscript.

After further discussion with the referees I would like to add some suggestions.
Major comments, point 5 of referee 3 could be assessed by providing quantitative qChIP as validation as also suggested by referee 1.
Point 7 of referee 3 would be addressed by the inclusion of Pol II ChIP-seq studies (Pol II phosphorylated at Ser 5 and Ser 2 of the CTD) as suggested by referee 1.

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 #3:

This manuscript meanders through a large number of diverse observations that try to shed light on the function of a dominant negative allele of MYC termed OmoMYC. The authors report on cellular effects of OmoMYC, on in vivo effects, on its DNA binding properties, on binding of RNA polymerase, on transcriptome effects and on effects on microRNAs.

One general issue with the manuscript is lack of novelty; this relate to the biological effects. Previously published work (Annibali et al., 2014) showed that OmoMYC has significant effects in glioblastoma development, both in mouse models and using orthotopic xenografts. Some of the published data are of significantly higher quality than the ones reported here.

The central criticism is, however, that much of the data is not convincing and does not sufficiently support the central claims made by the authors. In particular the data that analyze the underlying the biological effects and aim to establish the mechanism of OmoMYC action are largely unclear, in part due to low data quality. As a result, the central questions of how OmoMYC acts to inhibit MYC, whether this is specific, how it exerts the effects on expression of stem cell markers and whether these effects are relevant for its in vivo action remain unresolved.

Major comments:

Despite the striking title, no data are presented that the cells studied are functional glioblastoma stem cells or that OmoMYC affects stem cell characteristics. Rather, effects on the proliferation of the bulk cell population are reported. Glioblastoma stem cells have been identified in transplantation assays and the authors need to use such assays to support the claim that stem cells are affected.

In Figure 1G, the data are based on very small and variable numbers of cells and no statistics are given. For example the critical claim that nestin by OmoMYC is suppressed is based on two (!) cells that remain positive after prolonged incubation in differentiation-inducing medium. Whether these are significant effects remains completely open, in particular since the observation that nestin is not suppressed in control cells after 7 days of incubation is highly unusual.

Figure 1 reports a major decrease in proliferation of the bulk cell population as the major read out of OmoMyc action. Yet the histology of xenografts shown in Figure 2 shows at best a very small decrease in Ki67 staining; taking the density of nuclei into account, there is none. So are cells highly proliferative although they differentiate? Figure 2 also shows that only a subset of cells expresses OmoMYC and it is not clear whether this is the same population of cells that shows an increase in cifferentiation.

The critical two survival curves (Figure EV2A) are really presented in an inappropriate way: In both curves, the x-axis is massively spread out and on a normal scale starting at 0 there would be virtually no visible difference for one of the cell lines.

The ChIP results shown in Figure 3 are highly unusual and untypical of many published ChIP sequencing results for Myc, raising doubts about what they actually show. In the heatmaps, the Myc

peak are unusually broad; one possible reason is shown in the panels below where the OLIG2, HDAC1 and DUSP10 "peaks" are not real peaks, arguing that the overall quality of the ChIPs is very low.

Strikingly, the authors claim that "Myc is replaced by OmoMyc", a central claim of the figure and the abstract, but the data shown in the heatmap in Figure 3A show unequivocally that MYC binding upon doxycycline addition collapses even when now OmoMYC is bound to a promoter (bottom 1000 or so promoters).

Furthermore, the authors have previously shown that OmoMYC does not bind to DNA in gel shift assays, so how is it targeted to chromatin?

In Figure 4, the authors show variable effects on RNA polymerase loading on some genes and it is not clear why these genes are selected and what conclusions a more general analysis would allow. No attempt is made to correlate this to MYC or OmoMYC binding.
To rationalize this presentation, the authors state: "We did not observe the strong and generalized change of PolII density around TSSs of MYC promoter target genes that might have been expected upon MYC binding inhibition (Fig 4A).", but there is no model in the current literature that claims a generalized change of loading at the TSS (the general amplifier model of Young claims a general effect on elongation, which was confirmed by others.

The description of Figure 5A ("the correlation disappeared") is wrong. It is not also not clear how the plot is generated. Similarly, it is unclear what Figure 5B shows, The legends claims that the dots are single genes, but then the selection is very unclear.

Importantly (and related to the point about ChIP above), the gene expression analysis shown in Figure 5C shows and the text explicitly states that the effects on non-MYC bound genes are exactly the same as on MYC bound genes. But then how does OMOMYC act here? And what is the relationship to the ChIP data?

Table II shows no primary data on miRNAs. The relevance of this is unclear.

Reference:

Annibali, D., Whitfield, J. R., Favuzzi, E., Jauset, T., Serrano, E., Cuartas, I., Redondo-Campos, S., Folch, G., Gonzalez-Junca, A., Sodir, N. M., et al. (2014). Myc inhibition is effective against glioma and reveals a role for Myc in proficient mitosis. Nat Commun 5, 4632.

1st Revision - authors' response 05 June 2016

Referee #1:

In this manuscript, "Resetting cancer stem cell regulatory nodes upon MYC inhibition" Galardi, Savino and colleagues utilize an inducible Omomyc miniprotein that interferes with normal MYC function, in the setting of patient derived glioblastoma stem cells (GSCs) to interrogate the impact of MYC inhibition in mediating the tumorigenic function of these cancer stem cells.

Evidence is presented to demonstrate that induction of the Omomyc protein reverses, both in vitro and in vivo, many of the hallmarks of the tumor status of several of these cells lines, including the ability to migrate and differentiate and the expression of several molecular markers of neural stem cell self-renewal and proliferation. Further, in the mouse transplant studies, expression of Omomyc lead to the suppression of both cell intrinsic and tumor microenvironment functions typical of glioblastoma.
This study ultimately identifies and validates MYC target genes, specifically upregulated microRNAs of the miR-146b and the miR-200a-200b-429 clusters, shown in vitro to impact cell proliferation and migratory potential. Importantly, as referenced, microRNA's are known to play a role in mediating MYC's tumorigenic influence in glioblastoma.

1.) Overall this study provides important insights towards understanding the complexity behind the role of Omomyc in mitigating MYC's oncogenic function, specifically in the setting of glioblastoma

where it already has preclinical implications. One of the potential, key novel aspects of this work stems from the genome-wide changes in MYC and Pol II occupancy that occur upon Omomyc induction. However, the data supporting these findings is incomplete and in some instances less convincing. The ultimate aim of identifying the important functional gene targets in the MYC GSC transcriptome that occur upon Omomyc induction could theoretically be achieved by examining the MYC ChIP-seq and RNA-seq data. The authors state that they focus on the genes whose expression is most significantly modulated upon Omomyc induction and that also represent those bound by MYC (genes associated with a MYC peak and likely containing the E-box MYC/MAX binding motif). Such a gene set, while unique for different cancer cell types as stated, also represents the genes predicted to be functionally important for MYC function, at least in part, based on previous studies.

Answer: We thank the reviewer for having appreciated our work. We agree with the remark that at least part of the gene set most significantly modulated by Omomyc was shown by previous studies to be functionally important for MYC. This is expected because part of the set of "MYC-important" genes - like those involved in conserved, basic pathways - is conserved among different cell types and supports our view of the MYC-specificity of Omomyc action. As the reviewer says, this is true for only part of genes modulated by Omomyc. Therefore, our work adds novel information about the MYC-relevant but glioblastoma stem-like specific genes.

2.) One potentially interesting aspect of this work is the observation of minimal global effects on Pol II loading upon Omomyc induction. However, this would be greatly enhanced by the inclusion of ChIP-seq studies that also looked at potential changes in Pol II function, i.e., Pol II phosphorylated at Ser 5 and Ser 2 (of the CTD), representing marks of initiating and elongating Pol II, respectively (and while it is not expected that this study should comprehensively examine, in the context of Omomyc, all potential histone marks that are associated with different aspects of functional MYC, i.e. enhancers, promoters, etc., this would also be enlightening for Omomyc function).

Answer: We agree that data on ser2 and ser5 phosphorylation of Pol II CTD in the context of Omomyc may clarify some aspects of the manuscript. Notwithstanding, we found the request of ChIP-seq for both markers a bit out of the focus of this work and too demanding, considering that - to our knowledge - only few ChIP-seq studies of Pol II ser2 and ser5 phosphorylation have been reported so far (Rahl et al., Cell. 2010; Brookes et al., Cell Stem Cell. 2012; Walz et al., Nature 2014). We have now included in the manuscript data of a Pol II phospho-ser2 ChIP-seq in U87FO (see results and Fig. EV4). Basically, the result is similar to the Pol II ChIP-seq in the same cells: no significant global changes upon MYC inhibition. This evidence may suggest that ser5 phosphorylation as well would not globally change upon Omomyc expression, since we expect that a change in phospho-ser5 distribution would entail a change in either Pol II or Pol II-ser2p distribution, which are instead unaffected. We also show results from single gene qChIPs of Pol II ser2 and ser5 phosphorylation on MYC target genes to support this evidence. Although minor changes in Pol II-ser2p and -ser5p levels can be found in these single genes, their relative ratios remain constant upon Omomyc expression (Fig.4D). As a further indication that Omomyc does not compromise transcriptional pause release, we measured, from the Pol II ChIP-seqs, the RNA Polymerase II traveling ratio - defined as in Rahl et al., 2010 - in the presence or absence of Omomyc. We find the same traveling ratio distribution reported by Rahl et al. (Cell. 2010) - which validates our data - but minimal changes upon Omomyc expression (Fig. 4E). We think that the ChIP-seqs, together single gene qChIPs and the traveling ratio analysis are sufficient to infer that at a genomic level pause release and elongation are only marginally - or not at all - affected by Omomyc.

The histone marks associated with MYC and affected by Omomyc are an interesting issue. We have previously shown (Savino et al., PLoS One 2011) that Omomyc - oppositely to MYC - leads to decreased acetylation and increased dimethylation at H3 lysine 9. More recently (Mongiardi et al., 2015) we found that the histone mark H4R3me2s - a modification specifically carried out by PRMT5 (Protein Arginine Methyltransferase 5) - is induced by both Omomyc and MYC, and that both Omomyc and MYC interact with a PRMT5-MEP50 complex. This suggests that H4R3 symmetric dimethylation may be a mediator of MYC activity at the epigenetic level. This point is now touched in the discussion.

3.) Also, the percentage of MYC target genes identified in this study whose Pol II loading DOES change upon Omomyc expression is not clearly stated and/or depicted. This would be an interesting addition to the analysis and would potentially correlate with the genes whose expression significantly changes when Omomyc is expressed. As the data is presented, it is not clear how the Pol II ChIP-seq data contributes to the overall selection of genes that are functionally critical to the ability of Omomyc to mitigate the tumourigenic effects of MYC. Finally, this section would be improved by the inclusion of several validating gene specific qChIP assays (against MYC, Omomyc, Pol II, etc.), quantitatively comparing and validating the qualitatively different MYC targets represented by, for example, NCL and HDAC1.

Answer: We followed the suggestion and measured the percentage of MYC target genes whose Pol II loading changes upon Omomyc expression. At the genomic level, we did not find any relevant change on Pol II and Pol II-ser2. We tried different approaches, but only a negligible fraction of all genes showed significant change in Pol II enrichment at TSS (2% of all genes and 4% of MYC target genes) or Pol II-ser2p at the TTS (1%). Also, the change was more or less equally split between genes with greater enrichment in -DOX and those with more enrichment in +DOX cells. All in all, these data do not allow us to conjecture any relevant change of Pol II loading upon Omomyc induction. As suggested, we have included several validating, gene specific qChIP assays with MYC, FLAG (Omomyc), and MAX antibodies on genes like NCL, HDAC1, miR-17-92 and DUSP10. qChIP data - included in figure EV3 - are in agreement with MYC and Omomyc ChIP-seqs. Moreover they demonstrate that MAX binding to DNA is affected by Omomyc. This last finding indicates that Omomyc does not bind to DNA in a complex with MAX, but rather as homodimer.

4.) The data that is the subject of Figure 6 is interesting and correlative but does not entirely support the conclusions that are drawn. If the hypothesis is that there are key MYC targets (i.e. master transcriptional regulators) that themselves regulate gene programs that are important for the GSC cancer phenotype, ultimately it seems necessary to examine this kinetically, measuring gene expression at earlier time points, using a system of rapid Omomyc induction or by forcing expression of a limited set of these master transcription factors to recapitulate, at least in part, the oncogenic MYC transcriptome in these cells. While these experiments might be outside the scope of this work, these concepts are not discussed. Further complicating the analysis of data represented in this section is other, unknown/uncharacterized effects of Omomyc expression that may fall outside of simply replacing MYC. Importantly these concepts are touched on in the Discussion.
Answer: We agree that a kinetic analysis may be the answer to the relevant question of whether MYC acts hierarchically (by first targeting a limited set of master transcription factors) or horizontally, by acting on a large number of genes, in parallel. We performed some new RNA-seq focusing on shorter time points (4 and 8 h) as well: data are reported in datasets EV2 and EV3, and Figure EV5, included in the results. Data are compatible with the idea of a small set of key MYC targets that are rapidly hit and able to regulate important gene programs for the GSC phenotype, although we probably do not have a rapid enough Omomyc induction to clearly assess this point. We also touch - for example regarding the interaction with the PRMT5 / methylosome complex - the issue of Omomyc effects falling outside simply inhibiting MYC.

5.) Lastly, the Discussion section is well written and brings to the forefront many of the key, current questions that MYC field needs to address to reconcile MYC's role as a genome-wide multi-functional transcription factor/chromatin regulator and its oncogenic function, in different cancer type settings. However these points are not related back to the data presented and no Figures are referenced in the Discussion, linking them into their wider implications.

Answer: We thank the reviewer for appreciating our discussion, which we have tried to improve, also by better relating to the data and figures presented.

Minor points:

In some instances, the references cited are incomplete. For example, in the Introduction, the sentence "this view is supported by studies showing the potential therapeutic efficacy of drugs impairing MYC transcription" is followed by the sole citation of Delmore et al., 2011 but leaves out the contemporary BET protein inhibitor studies including Zuber et al., Nature, 2011 and Dawson et al., Nature 2011. Similarly, the statement "current views consider MYC as either a universal non-

linear amplifier or a gene-specific modulator" leaves out important primary references including Nie et al., Cell, 2012 and Sabo et al., Nature 2014, even though reviews are also cited.

Answer: We have tried to be synthetic about references, but the reviewer is right: we should have cared more about completeness. To this end, we have added the following new references:

Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature. 2011 Aug 3;478(7370):524-8.

Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirguet O, Doehner K, Delwel R, Burnett AK, Jeffrey P, Drewes G, Lee K, Huntly BJ, Kouzarides T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature. 2011 Oct 2;478(7370):529-33.

Nie Z, Hu G, Wei G, Cui K, Yamane A, Resch W, Wang R, Green DR, Tessarollo L, Casellas R, Zhao K, Levens D. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell. 2012 Sep 28;151(1):68-79.

Sabò A, Kress TR, Pelizzola M, de Pretis S, Gorski MM, Tesi A, Morelli MJ, Bora P, Doni M, Verrecchia A, Tonelli C, Fagà G, Bianchi V, Ronchi A, Low D, Müller H, Guccione E, Campaner S, Amati B. Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. Nature. 2014 Jul 24;511(7510).

Figure 6A: it is not clear how the DUSP family genes act as a control set, this is not explained well in the text or in the methods/Figure legend. All except one appear to be up or downregulated in response to Omomyc expression and are expressed at baseline, while some are enriched for MYC binding. What is this gene family controlling for?

Answer: While the genes represented in the last two groups (colums) in Fig 6 have been selected by a literature-supervised choice as genes known to have a role in Myc response, tumorigenesis or tumor suppression in a variety of tumors, or in GSC stemness, the choice of the DUSP family was a not supervised one. It should be controlling the fact that a gene family is affected by Omomyc in either direction, and that is not preferentially down regulated, as might be expected according to the MYC amplifier model. DUSPs are interesting because they mediate signaling specificity and we ignored whether single DUSPs were associated to GBM. It is interesting that Omomyc modulates the expression of those DUSP, like DUSPs 4, 5, 6 that were reported to be involved in GBM.

At the end of the Results section titled "Impact on cancer stem cell transcriptome" the authors do not reference the Figure/Table where these gene sets/terms are presented.

Answer: We thank the reviewer for pointing this out. We have added dataset EV3 and Fig. EV5 - related to gene set analysis - to the manuscript, and improved the reference to figures and dataset where the gene sets / terms are presented.

Some of the methods and/or Figure legends are incomplete, e.g. for the ChIP-seq experiments how many biological replicates were conducted/analyzed and what specific antibodies were used. Also, a better description of how the analyses depicted in Figures 5 and 6 were carried out. Minor: there is no specific reference to the Dang and Kim datasets in the Figure 6 legends (nor are these references cited in the main text). The methods for the migration assays are minimal - the Figure shows a bar graph of "cells per field" yet the methods don't say how many fields were counted per assay (presumably performed as 3 independent biological replicate experiments). Also, is the assay used for Figure 7F the same as for Figure 1B? If so, one is measured as cell number while the latter is in units of Absorbance. They are both referred to as growth curves in the Figure legends and from the text appear to be assaying the same thing - this is confusing. Generally speaking, the methods and Figure legends could be improved with more detail.

Answer: We have now enriched methods and figure legends to take into account these remarks. We have improved the explanations of Figs 5 and 6, added references, and clarified the source of datasets employed in Figure 6. These datasets, all belonging to MSigDB (http://www.broadinstitute.org/gsea/msigdb), were chosen as examples of MYC-regulated gene sets. All references - when not listed - can be found in the MSigDB. For what concerns the criticism about the cell growth assays in Fig1B and Fig7F, the one in Fig.1B is a proliferation assay based on counting cells daily, while that one used for Fig.7F is a MTS assay; we clarified this in the methods and legends.

Minor grammatical errors, etc.:
1. Results, p.2, first sentence: "...we resorted to inducible Omomyc." Given that the word "resorted" is often used in the context of "turn to and adopt a course of action, especially an extreme or undesirable one" an alternate word choice might better reflect the meaning intended.
2. Results, p.3, section heading: "impact" should be capitalized.
3. Results, p.4, top paragraph: "...concordantly with published data...consistently with the view..." should read "...concordant with published data...consistent with the view...". Also in the Discussion, p.8, "Consistently with this possibility..." should be "Consistent with this possibility...".
4. Throughout the text (including Figure legends) and in some Figure panels, Pol II is incorrectly written without a space, as "PolII".
5. All Figures are lacking Figure numbers; Figure 6 is lacking in labels (A, B?)

Answer: We thank the reviewer for the suggestions: we have corrected the grammatical errors and inserted the figure numbers to all figures.

Referee #2:

Galardi et al. investigate the function and mechanistic effect of a Myc inhibitor (OmoMyc) on 3 glioblastoma cell lines. They state that "Omomyc bridles the key cancer stem cell features and affects tumour microenvironment, inhibiting angiogenesis". At a molecular level they report the global changes in Myc binding and OmoMyc binding using ChIP-seq, as well as report RNA and protein expression changes on these targets. This large body of data are nicely presented and for the most part the experiments are generally well controlled. Issues associated with this manuscript are important and need to be addressed to increase clarity:

1.The use of "cancer stem cell (CSC)" throughout, including the title, is inappropriate and must be changed. The authors define the CSC as neurosphere-derived cells. Evidence is not provided to show that the authors' neurospheres fulfill the criteria of a glioblastoma cancer stem cell.

Answer: Although the term cancer stem cells is found in many articles to indicate cells similar to the ones we have employed, we realize that it may seem inappropriate. In agreement with the observation of the reviewer we will define the GBM-derived neurospheres (NS) as glioblastoma–stem like cells (GSCs), a widely used term in the literature. The two co-authors in the Molecular Neuro-Oncology laboratory at Istituto Neurologico Besta in Milan, (Serena Pellegatta and Gaetano Finocchiaro) have quite strong expertise in producing and characterizing NS from specimens of human GBM that dates back to 2004 (Tunici et al., 2004). From then on, their lab has been systematically deriving GBM primary cell lines from specimens obtained from the Department of Neurosurgery of the Istituto C. Besta. After mechanical and enzymatic dissociation of specimens, cell suspensions are cultured under specific conditions, favoring the growth of neurospheres (NS) in the presence of EGF and bFGF and absence of serum. Such NS mirror the actual biology of GBM much more closely than serum-based glioma cell lines (e.g. U87MG), they are always tumorigenic in immunodeficient hosts and the tumors they form in these hosts are much more representative of the clinical presentation of human GBM (Finocchiaro & Pellegatta, 2015; Tunici et al., 2004).

Recent data from the Molecular Neuro-Oncology lab demonstrate that GBM-NS maintain in vitro many features of their original sub-classification, defined in agreement to Verhaak and Brennan (De Bacco et al., 2012). In fact, it was demonstrated that GBM neurospheres harbor genetic lesions specific of glioblastoma subtypes and can be classified as classical, mesenchymal, or proneural according to their gene expression profile; moreover, the same mutations of primary glioblastomas are found in their matched neurospheres. This confirms that GBM neurospheres are a faithful in

vitro model of the original tumor, useful to dissect the relationship between genetics and biology, and also to predict the therapeutical response.

Thus, it is soundly established that they closely represent glioblastoma initiating cells. It is clear, from our data, that these cells own stemness features, as they express stemness markers, are able to self-renew, can differentiate when placed in appropriate conditions, and form tumors very efficiently when injected into immunodeficient mice. Moreover, our data show that Omomyc does affect these same stem cell characteristics, as its induction leads to the inhibition of stemness maintenance factors, enhances differentiation in vitro and in vivo, and impairs self-renewal in vitro.

2. The authors grow the cell lines as neurospheres prior to injecting single cells derived from these neurospheres for xenograft formation. However, it remains unclear whether these cell lines are grown as neurospheres for all other assays or whether they are grown as traditional 2D populations on tissue culture plastic. This is an important detail that must be included in the methods and body of the manuscript. This detail also speaks to the authors claim that the analyses they perform are relevant to the CSC. Was the ChIP-seq conducted from neurospheres? If not then the functional and molecular analyses have been performed under different conditions. This needs to be made clear in both the methods and in the text.

Answer: We have always grown the glioblastoma stem-like cells (BT168, BT275 and BT308) as neurospheres - in neurosphere (neural stem cell) medium - with the exception of the "differentiation" experiments, in which cells are grown in the presence of a small percentage of serum and attached to tissue culture dishes, as required by the protocol. U87MG - the most widely used glioblastoma cells - are not stem-like and are grown in standard growth medium (10% serum) in 2D. These details are included in methods and main text. ChIP-seqs and RNA-seqs from BT168 cells have always been conducted from neurospheres.

3. Does OmoMyc bind E-boxes in partnership with Max? Max ChIP-seq would address this important mechanistic question. However, conducting OmoMyc and Max ChIP-qPCR on specific target genes would be sufficient to answer this question in this manuscript.

Answer: We have performed single gene MAX qChIPs on nucleolin (NCL), miR-17-92, HDAC1, and DUSP10 (data shown in supplementary fig. EV3) as well as a MAX ChIP-seq (not shown). Both demonstrate a strong attenuation of MAX signal in the presence of Omomyc, accompanied by a parallel increase in Omomyc binding. This indicates that Omomyc does not bind DNA in partnership with MAX, but rather as homodimer. This conclusion is supported by immunoprecipitation experiments (in U87FO and BT168FO cells, not shown) demonstrating that, intracellularly, Omomyc dimers are much more abundant than heterodimers with MAX and even more than heterodimers with MYC. Omomyc capacity to affect MAX binding to DNA also indicates that the whole MYC/MAX networks would be affected by Omomyc since DNA binding of MAX complexes with other partners like the MADs and other, would be inhibited.

4. Table 1: This table shows that the motif analysis of Myc-bound target genes is significantly altered in response to dox alone. Without dox, motif analysis shows Myc binds to motifs that resemble sites associated with Myc, Mycn, MYC:MAX, etc. However when cells are treated with dox, the motif analysis shows Myc binds to motifs that resemble sites associated with TBP, MEF2A, MEF2C, FOXL1, etc. This means that the motifs associated with OmoMyc binding are also influenced by the treatment with dox. This needs to be made clear in the text as a caveat to this experiment.

Answer: Maybe we have not been clear enough and this caused a misunderstanding. The cells in Table 1 are those harboring dox-inducible Omomyc: BT168/FO and U87/FO. The term DOX treated cells refers to cells that express Omomyc. The figure therefore shows that the motifs of MYC-bound genes are altered in the presence Omomyc and that Omomyc binds to the same motifs preferentially bound by MYC. It also shows that, in the presence of Omomyc, there is residual MYC binding to motifs like MEF2A, FOXL1, which are associated to differentiation, and were not represented among the preferred Myc-bound motifs.

5. Miniprotein: This is lab jargon that must be removed from the manuscript as it may confuse some readers. It is better to define OmoMyc and then refer to the inhibitor by name.

Answer: Although we do not share the reviewer's opinion on the term miniprotein (we have already employed this term in other articles; this term is also currently employed in the scientific literature to denote peptides, as a matter of fact smaller than Omomyc, that fold into a stable protein-like structure) we will not use it since the reviewer finds it may be confusing. We point out that the real name of our molecule is Omomyc (Soucek et al., Oncogene 1998) and not OmoMyc. We have now used this name as much as possible throughout our manuscript, avoiding diminutive terms like miniprotein.

6. Fig 1G and EV1E & F: It would add weight to the manuscript to have the micrographs quantified.

Answer: This has been done in the revised version of Fig 1G, by taking into account fields with a higher cell number.

7. Legend 1E and EV1: "qRT-PCR of relevant markers ...". Figures should be understandable without having to read the text. Define relevant markers in the legend.

Answer: We have followed the reviewer's suggestion, and defined specific markers in the figure legends.

8. Fig 5D: targes should be targets

Answer: We have changed this in the text.

9. Page 6, half way down the page: This sentence needs to be corrected, "A similar distribution was observed in not target genes as well (Fig 5C)."

Answer: We have made this change in the text.

Referee #3:

This manuscript meanders through a large number of diverse observations that try to shed light on the function of a dominant negative allele of MYC termed OmoMYC. The authors report on cellular effects of OmoMYC, on in vivo effects, on its DNA binding properties, on binding of RNA polymerase, on transcriptome effects and on effects on microRNAs.

One general issue with the manuscript is lack of novelty; this relate to the biological effects. Previously published work (Annibali et al., 2014) showed that OmoMYC has significant effects in glioblastoma development, both in mouse models and using orthotopic xenografts. Some of the published data are of significantly higher quality than the ones reported here.

Answer: We do not understand why the reviewer is so aggressive and we disagree with this comment. The study by Annibali and co-authors concerns human GSCs only in the final part of the article: Figure 8. We think that our work with GSCs has been much more accurate. In the Annibali paper, GSCs from one single patient were tested, against the three we have employed and described. Regarding the in vitro characterization, only neurosphere growth and self-renewal were assayed: no differentiation studies, no migration studies, no molecular, and no genomic analyses are shown. As to the histology in vivo, only a nestin staining is shown, against the many markers we have analysed to address key features of glioblastoma like angiogenesis, stemness, migration, differentiation. Our work did not aim at demonstrating the tumor suppressive properties of Omomyc, which had been already tested in other transgenic cancer model cancer models, but was more focused on molecular and genomic mechanisms, particularly in cancer stem-like cells, which had not been investigated previously.

The central criticism is, however, that much of the data is not convincing and does not sufficiently support the central claims made by the authors. In particular the data that analyze the underlying biological effects and aim to establish the mechanism of OmoMYC action are largely unclear, in part due to low data quality. As a result, the central questions of how OmoMYC acts to inhibit MYC, whether this is specific, how it exerts the effects on expression of stem cell markers and whether these effects are relevant for its in vivo action remain unresolved.

Answer: The answer to this criticism can be found in the answers to the single major comments, which detail this wide criticism.

Major comments

1.Despite the striking title, no data are presented that the cells studied are functional glioblastoma stem cells or that OmoMYC affects stem cell characteristics. Rather, effects on the proliferation of the bulk cell population are reported. Glioblastoma stem cells have been identified in transplantation assays and the authors need to use such assays to support the claim that stem cells are affected.

Answer: This issue has been thoroughly discussed in the answer to reviewer n. 2, which is summarized here. As explained in that answer, it is firmly established that the BT (Brain Tumor) cells studied (BT168, 275, 308) closely represent glioblastoma initiating cells (De Bacco et al (2012). Cancer Research, 72(17), 4537–4550. Finocchiaro, G., & Pellegatta, S. (2015). Cancer Immunology, Immunotherapy : CII. doi:10.1007/s00262-015-1754-9). The author that provided the cells and performed the in vivo assays (Serena Pellegatta) is quite skilled and expert in the glioblastoma stem cell field and works in one of the pioneering lab in GSCs (the Neuro-Oncology lab at Istituto Besta in Milan), with an expertise dating back to 2004 (Tunici et al., 2004). From our data as well it is clear that these cells own stemness features, as they express stemness markers, are able to self-renew, can differentiate when placed in appropriate conditions, and form tumors efficiently when injected into immunodeficient mice. Moreover, our data show that Omomyc does affect these same stem cell characteristics, as its induction leads to the inhibition of stemness maintenance factors, enhances differentiation in vitro and in vivo, and impairs self-renewal in vitro. We do not understand the remark about the bulk cell population. U87MG cells can be considered an example of bulk cell population, whereas BT cells - as explained above and in the answer to reviewer 2 - are bona fide glioblastoma stem-like cells: no more and no less than glioblastoma stem-like cells used by other authors.

2. In Figure 1G, the data are based on very small and variable numbers of cells and no statistics are given. For example the critical claim that nestin by OmoMYC is suppressed is based on two (!) cells that remain positive after prolonged incubation in differentiation-inducing medium. Whether these are significant effects remains completely open, in particular since the observation that nestin is not suppressed in control cells after 7 days of incubation is highly unusual.

Answer: This right remark has been addressed in the revised version of Figure 1G and Figure 1G legend, by taking into account several fields with a higher cell number.

3. Figure 1 reports a major decrease in proliferation of the bulk cell population as the major read out of OmoMyc action. Yet the histology of xenografts shown in Figure 2 shows at best a very small decrease in Ki6 staining; taking the density of nuclei into account, there is none. So are cells highly proliferative although they differentiate? Figure 2 also shows that only a subset of cells expresses OmoMYC and it is not clear whether this is the same population of cells that shows an increase in differentiation.

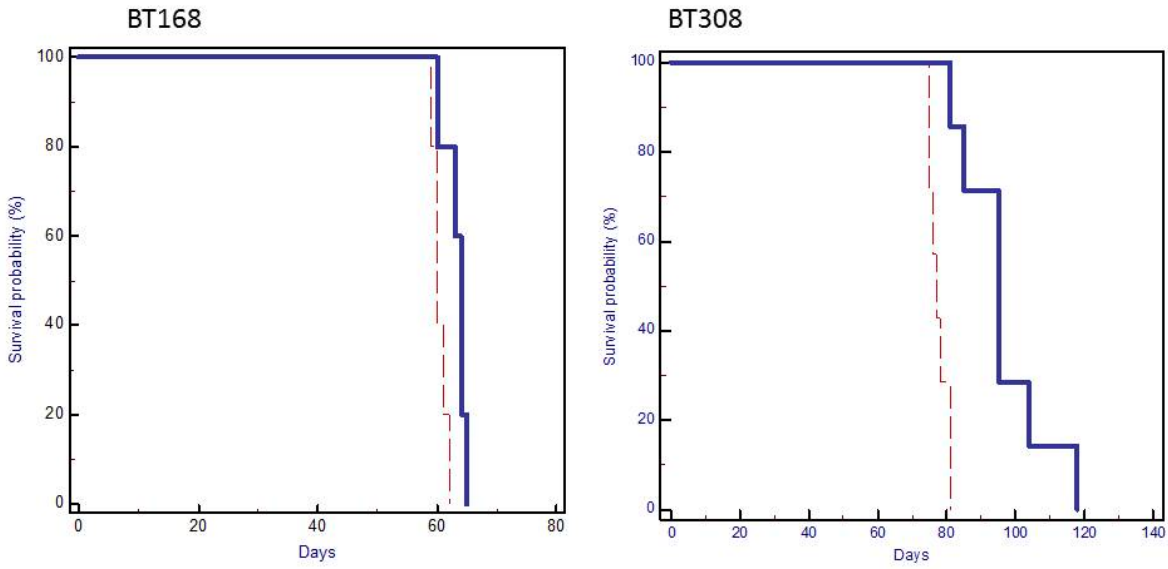
Answer: We do not understand what the reviewer means when talking about "bulk cell population". Maybe there is a misunderstanding. Data of figure 1 are not related to a bulk cell population, but to glioblastoma stem-like cells BT168 (described by De Bacco et al., 2012) growing as neurospheres in neural stem cell, serum free, medium. This is presumed to be a homogenous population of GSCs, similarly to BT275 and BT308 shown in Figures EV1 and 2. These three GSC lines differ for some features, as described by De Bacco et al.

We point out in the manuscript that the cell density in the Omomyc xenografts is lower than controls, indicative of an impaired proliferation of the Omomyc xenografts. Since the fraction of proliferating cells is similar to controls, as indicated by the Ki67 staining, the finding that cell density in Omomyc xenografts is lower than controls suggests that may be due to a slower cell cycle (as observed for instance in myc null Rat fibroblasts). Moreover, as stated in the manuscript and illustrated in Figure 2A, a large subset of the cells in the xenografts had lost Omomyc expression at the time of sacrifice, probably because of epigenetic silencing of the CMV promoter, guiding Omomyc expression. So, it is possible that the Omomyc xenografts experienced a decreased

proliferation before the histological sections were taken. It should also be considered that finding that a major decrease in proliferation of GSCs is a major readout for Omomyc in vitro, does not necessarily imply that the same thing should occur in the orthotopic xenograft. Another explanation of the IHC data is that proliferating and differentiating cells belong to two different populations, the former having lost Omomyc and the latter still expressing it. Another possible and interesting explanation is that Omomyc expressing cells may have not cell autonomous effects, influencing differentiation of surrounding cells.

4.The critical two survival curves (Figure EV2A) are really presented in an inappropriate way: In both curves, the x-axis is massively spread out and on a normal scale starting at 0 there would be virtually no visible difference for one of the cell lines.

Answer: We chose to present KM survival curves this way, to focus onto the differences in survival that are visible only at late times after injection. All previous time-points do not show any difference and were in fact excluded automatically by the algorithm designing the survival curves (MedCalc 12.7). This way of representing KM curves is frequent in the literature: see for instance the article "HDAC and PI3K Antagonists Cooperate to Inhibit Growth of MYC-Driven Medulloblastoma." (Cancer Cell Volume 29, Issue 3, 14 March 2016). The differences shown, though very tiny especially in BT168, are anyway statistically significant (see p-values). For the sake of clarity, however, we are here including in our response the complete forms of both survival curves.



5. The ChIP results shown in Figure 3 are highly unusual and untypical of many published ChIP sequencing results for Myc, raising doubts about what they actually show. In the heatmaps, the Myc peak are unusually broad; one possible reason is shown in the panels below where the OLIG2, HDAC1 and DUSP10 "peaks" are not real peaks, arguing that the overall quality of the ChIPs is very low.

Answer: We think it is quite hard to figure out how "broad" peaks are from the heatmaps alone. Perhaps the reviewer is referring to the "peak" plots shown next to heatmaps. However, these are not enrichment maps for MYC ChIP-Seq signal around peak summits, but around TSSs of promoters

bound by MYC ("0" is the transcription start site). Hence, the "unusual broadness" noticed by the referee - mainly in Fig. 3A, top left plot, since the other heat map "peaks" seem narrow enough to us - is due to the fact that MYC does not bind exactly at the same distance from the TSS in all promoters, and hence the region where the plot reaches the maximum (just upstream of the TSS) is broader, highlighting the fact that MYC binds at different positions in the region, with a corresponding overall enrichment from the ChIP-Seq. We have now tried to write more clearly this point in the manuscript.

We agree that the peaks shown in the below panels (C and D) of Fig. 3 - which the reviewer view as not representing real peaks - are a bit ugly: the reason is similar to what we just said about heatmap peaks. We prefer to leave these "peaks" as they are in the below panels, and to rename them from peaks to enrichment areas. If the reviewer considers this a critical point we may remove the low panel plots.

As to the referee's ungenerous remark that our ChIPs (they are ChIP-seqs as a matter of fact) are low quality we answer that they respect the standards. For data processing we employed well-established workflows, which confirmed significant and non-random enrichment for the peak regions. Also, motif analysis clearly showed that the E-box is the binding motif associated with the peaks, appearing centered around the peak summit in nearly all of them. Finally, we ourselves have performed several ChIP-seq with a given antibody in the same cell type, and discarded experiments that were below standard.

6. Strikingly, the authors claim that "Myc is replaced by OmoMyc", a central claim of the figure and the abstract, but the data shown in the heatmap in Figure 3A show unequivocally that MYC binding upon doxycycline addition collapses even when now OmoMYC is bound to a promoter (bottom 1000 or so promoters).

Answer: We think Figure 3A illustrates the collapse of MYC binding caused by Omomyc, and clearly shows that the Omomyc signal in both U87 and BT168 cells seems to correlate very well with the MYC signal in the large majority of genes. What the reviewer remarks for the "bottom promoters" - where MYC enrichment is weaker - is especially true for U87MG cells (Fig 3B, actually), and we have highlighted this in the binding profile shown at the right of the "bottom promoter" cluster of the heatmap in Fig. 3B. In BT168 cells, instead, there still is a Omomyc signal in the bottom promoters, although weaker than MYC signal (Fig 3A). We do not know whether the lower signal for Omomyc in U87 cells may be due to technical reasons or represent something more significant. Overall, we find that our claim that "Myc is replaced by Omomyc" is valid for the large majority - if not all - of the genes. We have detailed this point in the manuscript, taking into account the reviewer's remark about the bottom genes in U87MG cells.

7. Furthermore, the authors have previously shown that OmoMYC does not bind to DNA in gel shift assays, so how is it targeted to chromatin?

Answer: In the original manuscript describing Omomyc (Soucek et al., Oncogene, 1998), we measured by gel shift the binding of Omomyc, Myc, and Max GST-fusion proteins to the CACGTG E-box. We showed (Fig 4 A and B) that Omomyc dimers do indeed specifically bind to this E-box sequence, albeit with lower efficiency than Myc/Max and Max/Max dimers. We also showed that Omomyc can bind to DNA as Omomyc/MAX heterodimer but not as a Omomyc/MYC heterodimer. However, we think that Omomyc binding to DNA in partnership with MAX is an event quite infrequent if not absent within cells (see answer to reviewer 1) since we see very little Omomyc bound to MAX in immunoprecipitation experiments (not shown) and show in the new Figure EV3 that MAX binding to DNA of MYC target genes is strongly affected by Omomyc (also confirmed by a MAX ChIP-seq, not shown), similarly to MYC binding.

Moreover, as shown by the Eilers group for MYC recruitment to some (repressed) targets via its interaction with MIZ1, Omomyc might as well be brought to chromatin via protein interactions with MIZ1 or other interactors. Our feeling, however, is that Omomyc binds DNA as a dimer tout court.

8. In Figure 4, the authors show variable effects on RNA polymerase loading on some genes and it is not clear why these genes are selected and what conclusions a more general analysis would allow. No attempt is made to correlate this to MYC or OmoMYC binding.

To rationalize this presentation, the authors state: "We did not observe the strong and generalized change of PolII density around TSSs of MYC promoter target genes that might have been expected upon MYC binding inhibition (Fig 4A).", but there is no model in the current literature that claims a generalized change of loading at the TSS (the general amplifier model of Young claims a general effect on elongation, which was confirmed by others).

Answer: The text now explains more clearly why the genes plotted in Figure 4 were selected. They were chosen because they all present MYC binding at their promoter, show loss of MYC binding and appearance of Omomyc binding upon Dox treatment, but their transcriptional response is different, some being modulated by Omomyc and some others not. The correlation with MYC or Omomyc binding can be found both in Figure and in the text. We have performed the more general analysis suggested by the reviewer regarding Pol II occupancy, MYC and Omomyc binding, and transcript levels. This is described in the main text. We agree only in part with the reviewer's remark that "there is no model in the current literature that claims a generalized change of loading at the TSS". The article by Walz et al. (Nature 511, 483–487, 24 July 2014) shows in Figure 2 that MYC regulates Pol II recruitment. Anyhow we have now rephrased this sentence.

9. The description of Figure 5A ("the correlation disappeared") is wrong. It is not also not clear how the plot is generated. Similarly, it is unclear what Figure 5B shows. The legends claims that the dots are single genes, but then the selection is very unclear.

Answer: The scatter plot shown in Figure 5A shows the relationship between the amount of promoter occupancy by MYC determined by ChIP-seq (y axis) and transcript levels, defined by FPKM values of RNA-seq data (x axis). We realized that the x axis of Figure 5A, which represents FPKM values like in the other panels, was not specified. We have now added the missing x axis caption. The same considerations can be made for figure 5B: FPKMs of single genes are shown and the large majority cluster near the origin, at relatively moderate transcript expression. The description of Figure 5A in the text was probably not clear enough and we have rephrased it; we removed the statement "the correlation disappeared", to comply with the reviewer's criticism. Accordingly, we have modified the Figure legend as well.

10. Importantly (and related to the point about ChIP above), the gene expression analysis shown in Figure 5C shows and the text explicitly states that the effects on non-MYC bound genes are exactly the same as on MYC bound genes. But then how does OMOMYC act here? And what is the relationship to the ChIP data?

Answer: We also were surprised by this finding, which led us to reconsider MYC action. The expression data in Figure 5C refer to 24 h of Dox treatment. One consideration is that having MYC bound to an E-box is not sufficient for a gene to be regulated by MYC, so that the global pattern of MYC-binding only partially overlaps with the pattern of genes affected by the inhibition of MYC binding to DNA. Reciprocally, the same thing may occur with Omomyc: Omomyc binding per se may not be sufficient for gene modulation. One can envisage that a difference of Omomyc action on MYC-bound vs not MYC-bound gene may be strong at early time points only, and may flatten at later time points because of indirect effects. For example Omomyc might quickly and directly impact the expression of a few MYC-bound genes encoding masters regulators that, in turn, will affect the expression of many genes, either MYC target or not. Moreover, cells might be endowed with homeostatic mechanisms to globally balance gene expression gene expression changes.

11.Table II shows no primary data on miRNAs. The relevance of this is unclear.

Answer: Primary data have now been added in an expanded view dataset, EV4.

2nd Editorial Decision 25 July 2016

Thank you for the submission of your revised manuscript to EMBO reports. Please accept again my apologies for the delayed response. We have only now received the comments from all three referees that were asked to assess it and I have also contacted an advisor concerning your manuscript, as I had indicated in my earlier mail.

I am sorry to say that the evaluation of your manuscript is not a positive one. As you will see, while

referee 1 and 2 support publication of the revised manuscript, referee 3 remains skeptical and is not convinced by the data presented. In particular, this referee is concerned about the quality of the ChIP-seq data and its analysis and is not convinced of the in vivo data.

Given these contrasting referee reports and the important nature of the concerns of referee 3, we contacted an Editorial Advisor with expertise in ChIP-seq and cancer to evaluate these points. The advisor shares the concerns of referee 3 regarding the quality of the ChIP data and considers panels C and D not convincing. He/she appreciates that you have performed ChIP-qPCR to validate the ChIP-seq results but points out that the enrichment over background appears to be very low, indicating a low quality of the ChIP data. Moreover, the advisor also conforms to the concerns regarding the in vivo/xenograft data and the survival curves, which show little effect. Overall, in the opinion of the advisor, the experiments in Fig EV2 also rather address if Myc is required for tumor initiation than GBM maintenance.

Given these substantial concerns, the fact that you already had a chance to significantly revise the study and that EMBO reports allows a single round of revision only, I am afraid that we cannot offer to publish the manuscript at this point.

I am sorry that this decision emerges as the outcome of a lengthy review process but given these substantial concerns, I have no other option but to reject your manuscript.

I am sorry that I could not bring better news this time and wish you success with publication of your work elsewhere.

Appeal 26 July 2016

Thank you for the email.

I confess that we find your decision very unjust. We had exhaustively answered to all comments raised by all referees and complied to the requests you had made about the revision of our manuscript. For these reasons we were confident of a positive outcome. We observe instead that reviewer 3's opinion had greater influence than the opinion of the other two reviewers, who were in favour of publication.

As a matter of fact, we have serious difficulties in understanding the process of management of our work and we want to underline some of the several steps of our story:

2. December 4, 2015: you sent us a preliminary decision where you asked us to begin revising our manuscript according to the comments of referee 1 and referee 2, as both acknowledged the potential interest of the findings.

3. December 23, 2015: you sent us an email with the report of referee 3 where you stated that, even if he/she raised significant concerns regarding the conclusiveness of our data, you have not revised your original decision.

In particular you wrote: "After further discussion with the referees I would like to add some suggestions. Major comments, point 5 of referee 3 could be assessed by providing quantitative qChIP as validation as also suggested by referee 1. Point 7 of referee 3 would be addressed by the inclusion of Pol II ChIPseq studies (Pol II phosphorylated at Ser 5 and Ser 2 of the CTD) as suggested by referee 1."

In our interpretation, by performing the experiments that you and referee 1 were suggesting, we would have answered to the key points raised. In fact, we were aware that referee 3 attitude against our manuscript was totally and unexpectedly negative and we focused on these two points because we considered them, following your suggestion, as the essential ones for our manuscript to be accepted. Moreover, to enrich the manuscript and increase its clarity, we performed several other experiments that were not strictly requested.

The new experiments presented in the revised manuscript satisfied both referees 1 and 2; in fact they suggested to accept it. Only the third one expressed a negative comment. Based on this, we wonder

why the opinion of ONE reviewer weighs more than those of TWO, equally qualified ones.

Specifically regarding referee 3's concerns about the quality of our ChiP-seq data, we observe that, while referee 3 and the Advisor agree on this issue, the two other Referees do not raise any doubt about this technical aspect. You ought as well take into account the answer to this objection in our rebuttal letter and consider that we performed several independent ChIP-seqs with similar results. The bioinformatician, and ChIP-seq expert, who examined the data did not find them to be of such a low quality. If the problem is the absence of a scale bar in Fig. 3 panels C and D, this can be easily done (as a matter of fact, we had made the scale bar and I was convinced of having included it in the revised Figure 3). Again, we do not see why you should take into account the negative opinions instead of the positive ones, which are greater in number. Moreover, we have validated the ChIP-seq data by performing single gene ChIPs as suggested. Furthermore, while the Advisor agrees with referee 3 on essentially two weaknesses (quality of ChiP data and in vivo/xenograft data), the Advisor doesn't express all the other concerns still raised by referee 3. Also, the in vivo data did not appear to represent a problem in the first review process, as you did not mention it as a critical topic to address. Reviewer 3 observes that "The survival curves in EV2 remain presented in an unusual and somewhat misleading way". We believe that we have represented all the in vivo data very correctly, that we did not make unjustified claims from the survival curves, and we did not attempt to mislead anybody. In the rebuttal letter, we showed that our way of representing the survival curves is not so unusual. However, since we understand that other ways of representing such curves are equally valid or preferred by someones, in the rebuttal letter we included the survival curves presented as the third reviewer prefers: there would be no problem in inserting this representation in the manuscript. Therefore, this is quite a weak criticism, but, as above, what results is that the minor negative aspects seen by referee 3 are taken into largely more account that the positive evaluations done by all other referees. Similar considerations can be made regarding other reviewer 3's issues.

By looking carefully at his/her comments, it appears that the reviewer recognises that the majority of his/her comments have been addressed. For the rest, it is a matter of different interpretation / opinion of the data between us and the reviewer and I do not think one can reject a different opinion. In conclusion, it seems that the manuscript is being rejected mostly for panels C and D of figure 3. Sincerely, I find difficult to accept this, given the explanations provided to the reviewer in the rebuttal letter and considering that the two panels are not so crucial for the manuscript given all the other data presented, which support our interpretation.

In my personal opinion what the third reviewer does not like in our manuscript is the criticism of the view of Myc as universal amplifier. This was already stated in his/her first review and is evident in the response to our revision "Figure 5C shows that in total approximately 15000 transcript levels change in response to OmoMYC, regardless whether the promoters are bound by OmoMYC or not. This may be in line with the view that MYC is an universal transcription factor. But the entire claim of the paper is that OmoMYC leads to "selective repression of master transcription factors for glioblastoma stem-like cell identity" (Abstract) and does so since it "broadly replaces MYC on the genome" and Figure 5C is at odds with both statements." In the results and the discussion we take into serious account this issue and give our explanation, which does not coincide with the reviewer's but is not for this less worthy.

For all these reasons, and also because we had to wait THREE MONTHS for the first revision and FIFTY DAYS for the second one - this of course negatively affecting the novelty of our data - we are now strongly asking you to reconsider your decision about our work and give us the opportunity of publishing it in EMBO reports.

I also wish to communicate another consideration regarding the signal intensity and the reliability of our Myc ChIP-seqs. Various articles have shown that Myc binding intensity progressively increases with Myc protein level. The amount of endogenous Myc in glioblastoma stem like cells like T168 may not be so high as in cells over expressing Myc through an expression plasmid or in some other tumour cell lines, explaining a lower signal intensity. Anyhow, the signals are bona fide Myc signals. This is clearly shown by the motif-enrichment analyses of transcription factor DNA binding motifs (Pscan-ChIP) in the MYC bound regions (Table I) - reporting that the Myc binding detected by ChIP-seq is strongly and significantly enriched where it should be enriched, like at E-boxes and some other motifs - and by the single gene qChIPs that, similarly to ChIP-seqs, show significant enrichment at the expected position in genes like *nucleolin* and *miR-17-92* that represent well

known and universal Myc binding targets.

3rd Editorial Decision 01 August 2016

Thank you again for your letter asking us to reconsider our decision on your manuscript and for your patience. I have now had the opportunity to carefully read and consider your arguments and also re-read the manuscript and the reviewer's and the advisors' reports.

Since the decision was based on two positive and one negative referee report, it appeared that more importance was attached to the opinion of referee 3, as you mention in your letter. However, looking at the crucial points of this referee shows that one major concern was the quality of the ChIP data and its analysis and the second pertained to the in vivo data. You might agree that the ChIP data are very much at the core of the claims raised in your manuscript and hence their quality is crucial in supporting the conclusions drawn. It is for this reason that the concerns were particularly important in the final decision. Two independent expert advisors in fact, who are world experts in this approach, agreed that the enrichment appeared to be very low and that the in vivo data are not convincing.

However, on re-evaluation of your manuscript we think that it could be published if the figures are made transparent (add scale and enrichment, modify survival curves) and the text is toned down so that the limitations of the data are disclosed.

- Please label the screenshots shown in Figure 3C and D to illustrate enrichment and peakwidth (basepairs).

- Please change the survival curves shown in Figure EV2A and B, and please indicate also the number of mice analyzed in addition to the % survival probability.

- The limitations of the data, i.e., the low enrichment and potential reasons for this (like the low Myc expression levels in glioblastoma stem cells you mention) should be discussed.

- Moreover, the following three points that were highlighted by the two advisors we had contacted have to be incorporated in the discussion:

1) One advisor pointed out that the Omomyc peaks appeared broader: "Clearly there is less Myc and more Omomyc although the peaks are more diffuse. So while it is not certain that Omomyc replaces Myc, rather I would say that it does appear to interfere with proper Myc localization. "

2) In addition, this advisor indicated that your data is in accord with the amplifier model, contrary to your statement in the discussion:
" 'Omomyc modulated transcripts present either a linear attenuation or amplification and only the targets that are more highly expressed tend to be downregulated, indicating that the amplification effect of MYC on transcription is not universal.' This is in fact not a proper statement of the amplifier model. The model says that MYC is a non-linear amplifier-it is non-linear precisely because it operates better on highly expressed than upon weakly expressed genes. So Omomyc's effect is exactly in accord with the amplifier model."

3) As I had noted before, the second advisor indicated that the in vivo data are rather consistent with Myc being required for tumor formation instead of being required for GBM maintenance, because the expression of Omomyc was induced before the cells were xenografted.
Please incorporate these points in your discussion.

From an editorial point of view there are also some things we need before we can proceed with the official acceptance of your study:

- It is a precondition for publication in EMBO reports that authors agree to make all data freely available, where possible in an appropriate public database. Functional genomics data these should be deposited in the ArrayExpress (http://www.ebi.ac.uk/arrayexpress/), GEO (http://www.ncbi.nlm.nih.gov/geo/) or CIBEX (http://cibex.nig.ac.jp/index.jsp) databases in compliance to the MIAME (http://www.mged.org/Workgroups/MIAME/miame.html) standards and

the MINSEQE (http://www.mged.org/minseqe/) draft proposal.

Please deposit your data in one of these databases and provide the reference number in the manuscript.

- Please add scale bars to the microscopy images in Figures EV1 and EV2.

- Please update the table references to the numbered EMBO reports style.

Please contact me any time of you have any questions.
I look forward to seeing a revised form of your manuscript when it is ready.

3rd Revision - authors' response 07 September 2016

We are now submitting the revised version of our manuscript “Resetting cancer stem cell regulatory nodes upon MYC inhibition.”

We have taken into account your last observations and requests. We have accordingly modified the part in Results concerning MYC and Omomyc ChIP-seq, and the discussion regarding the MYC amplification model and the interpretation of our ChIP-seq data.

We have modified the figures as requested. We display the survival curves as required by referee #3 and added Figure EV3 to illustrate ChIP-seq gene tracks gene with normalized binding enrichments and peak-width.

We think the manuscript has been improved. In particular:

- we have labelled the screenshots shown in Figure 3C and D to illustrate enrichment and peakwidth (basepairs); they definitely look better. As a matter of fact, the screenshots were very badly visible if inserted in Figure 3, so we created a new EV Figure (Fig EV3) to display the screenshots focused on three esablished MYC targets. I hope they will be more clear now. The old Figures EV3 and EV4 have so become Fig EV4 and EV5. The old Figure EV5 is now Appendix S1.

- we have changed the display of the survival curves shown in Figure EV2A and B according to referee #3 request. We have inserted in the figure legend the number of mice analysed. Previously it was only mentioned in Methods. We feel that even when the enrichments are not very high, it is still emerges a DNA binding pattern showing that attenuation of MYC is accompanied by enrichment of Omomyc in the same region.

- we have indicated the limitations of the ChIP-seq data pointed out by referee and advisor, and briefly mentioned a possible explanation. This point is also touched in the discussion. We think that a more extended discussion of this issue is probably unnecessary and would be uninteresting to the reader. Although some ChIP-seqs may technically be improved by obtaining a higher number of reads, we find that the message they convey is already clear enough.

- the three points that were highlighted by the two advisors you had contacted have been incorporated in the discussion and results:

1) peak broadness has been mentioned in results and discussion. We have followed the advisor suggestion about the interference with MYC localisation and the uncertainty regarding the debated "replacement" of MYC by Omomyc. We also accepted referee #3 remark and modified the sentence in the abstract about Omomyc replacing MYC.

2) the part of the discussion regarding the amplifier model has been revised and extended. We have now represented the model more properly, as correctly pointed out, and included the advisors's observation, with which we agree, on our data being at least in part in accord with the amplifier model. I have extended the discussion about this topic and indicated points that may not be in accord or may need to be clarified.

3) I have incorporated in the discussion the observation made by the second advisor about the role of MYC in formation versus maintenance of GBM.

- we have deposited the -seq data in GEO and provided the accession reference in the manuscript, at the end of Methods

- scale bars to the microscopy images in Figures EV1 and EV2 have been added

- we have updated the table references to the EMBO reports style

We are convinced that this revised version is most clear and convincing and strongly hope that you will now consider it acceptable for publication.

4th Editorial Decision 27 September 2016

Thank you again for your patience while the final version of your manuscript was seen by an arbitrator, who is an expert in the field and whose opinion we trust. He/she confirmed that the ChIP data appear to be of sufficient quality to support the conclusions made and that the manuscript is suitable for publication in EMBO reports. I am therefore 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, as follows.

- Regarding data quantification, can you please specify the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends? This information is currently incomplete and must be provided in the figure legends.

- Could you please provide a higher quality Appendix figure? Moreover, the Appendix requires a table of content, even if it displays only one image.

If all remaining corrections have been attended to, you will then receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Thank you for your contribution to EMBO reports.

5th Editorial Decision 07 October 2016

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding author: Sergio Nasi
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2015-41489V2

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 χ^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.

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).

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?	NA
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.	Page 12
For animal studies, include a statement about randomization even if no randomization was used.	Page 12
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.	Pages 12 and 13
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5. For every figure, are statistical tests justified as appropriate?	Page 13
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

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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).	Page 14
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Page 12

* 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.	Page 13
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Page 13
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.	Page 13

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
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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

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. 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 e. Proteomics and molecular interactions	Complete next generation sequencing data are being submitted to the NCBI Short Read Archive (SRA).
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).	Complete next generation sequencing data are being submitted to the NCBI Short Read Archive (SRA). Datasets which are central for this study have been included as Expanded View datasets
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. Please state whether you have included this 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	NA
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.	NA