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# DYRK1A phoshorylates histone H3 to differentially regulate the binding of HP1 isoforms and antagonize HP1-mediated transcriptional repression

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**Review timeline:**

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## Transaction Report:

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

*Editor: Esther Schnapp*

1st Editorial Decision 17 January 2014

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. I apologize for the delay in getting back to you, which is due to the difficulty to find referees over the Christmas break. We have now received the full set of referee reports that is copied below.

As you will see, all referees acknowledge that the findings are interesting and the data overall convincing. Their main concerns are the missing quantifications and statistical analyses, the in some parts missing discussion of the data, and the insufficient description of the methods and experiments. Referee 2 also points out some overstatements that either need to be toned down or supported by experimental evidence.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as mentioned above and in their reports) must be fully addressed and their suggestions taken on board. 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.

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 tests used to calculate p-values in all

 

the relevant figure legends? This information must be provided in the figure legends. Please also add clearly visible scale bars to all microscope images.

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

REFEREE REPORTS:

Referee #1:

Summary:

1. Does this manuscript report a single key finding? YES
The manuscript reports on the antagonistic effect of histone H3 phosphorylation by the DYRK1A kinase on HP1 binding to H3 and on widespread transcriptional activation by DYRK1A.

2. Is the reported work of significance? YES

3. Is it of general interest to the molecular biology community? YES
The large number of genes which this study shows as being affected by DYRK1A-mediated histone phosphorylation, as well as its apparent relation to chromatin remodeling, should in my view merit general interest.

4. Is the single major finding robustly documented using independent lines of experimental evidence? YES

Report:

In this study, the authors report that phosphorylation of histone H3 at positions T45 and S57 interferes with binding of heterochromatin protein 1 (HP1) isoforms, that phosphorylation at these positions is mediated by the kinase DYRK1A, and that this kinase upregulates expression of numerous genes, notably ones involved in immune response. While I am not an expert in the specific field of this study, the reported findings seem to me to be of sufficient novelty and broad significance (e.g. in view of the widespread regulatory effects of DYRK1A on transcription) to merit publication in EMBO Reports. The manuscript is well written overall, the amount of experimental data in vitro and in vivo is impressive, the data are described well and generally support the conclusions of the authors. However, a few points would in my view justify further explanation (or experiments), which leads me to suggest acceptance of the manuscript after minor revision.

Specific points:

1. I have some doubts about the quantitative nature of the binding data of HP1 proteins to rH3 constructs shown in Fig. 1. It is stated that HP1 isoforms and Brg1 bind full-length H3 with "comparable efficiencies" (p.5), yet the signals show very different intensities, especially comparing lanes 4 and 5 in Fig. 1B. This is probably not much of an issue, since only the binding per se is really interesting at this point, but then one should not claim "comparable efficiencies". Also, I find it difficult to confirm that a H3S57E mutation reduces HP1gamma binding by "approximately 50%" (p.6 and Fig. 1C, lanes 7,8) - it clearly reduces the binding, but where do the 50% come from? Was the binding properly quantified? - and the weak band in lane 8 suggests that the binding may not be abolished as "completely" as the authors claim. In addition, there seems to be some spillover into lane 8 in the HP1beta binding experiment of Fig. 1C, and there may be an image artifact at the bottom of lane 2 in the test of Brg1 binding to H3V46A in the same figure. These issues would merit some comments of the authors, discussion in/rewording of the manuscript, or a re-run of gels in question.

2. A rather crucial point concerns Fig. 1E. Clearly binding of HP1 isoforms to a rH3 construct is less affected by incubation of rH3 with kinase-defective DYRK1A than with active DYRK1A. However,

 

the bands after incubation with mutant DYRK1A nevertheless consistently seem somewhat weaker - visually, the difference in the HP1gamma binding experiment between lanes 1 and 3 in Fig. 1E does not seem less pronounced than the difference between lanes 6 and 7 for HP1gamma binding to WT and S57E rH3 in Fig. 1C, yet in the latter case the authors claim "little or no effect", in the former "50% reduction". Also, the effect of DYRK1A-mediated phosphorylation of rH3 appears different from the one observed with phosphorylation mimic mutants. In the latter, already T45E abolished HP1alpha and -beta binding and an additional S57E mutant was required to also abolish HP1gamma binding, while with "real" phosphorylation at (presumably - in that specific experiment, only T45 phosphorylation was controlled) both sites, HP1alpha and beta still seem to bind to some extent, while HP1gamma seems more affected. Is there any explanation for this?

3. I do not see much connection between the experiments investigating global impact of DYRK1A on gene expression using exon arrays (Fig. S3) and using RT-qPCR (Tab. S1). The authors claim that the latter validate the former, but no quantitative data from the exon array analysis on individual genes are given. Couldn't they be added to Table S1 at least for those genes that were investigated by both methods (if there were any, which in my view is implied by the word "validation")? Do they agree? It is difficult from the current presentation to make connections, but I found that e.g. in Fig. S3C, FAS is marked in green, indicating no regulation by DYRK1A, while the results in Table S1 do indicate FAS to be regulated by DYRK1A. This apparent conflict should be addressed by the authors. As I stated, "validation" implies that a different analysis was carried out on the same target, so its results should either be shown if it was done (i.e. results of exon array and RT-qPCR on the same gene), or done if not.

4. The authors state that ChIP "established the presence of [DYRK1A] at the promotors of ..., but not TGFbeta" (p. 10 and Fig. S4A). However, as far as I see it, Fig. S4A shows that TGFbeta DNA could indeed be detected after ChIP with DYRK1A. Also, it does not seem to be significantly affected by DYRK1A depletion. This could be explained, for example, if the absolute levels of detected TGFbeta DNA were much lower than those of the other DNAs, but only relative levels are given in Fig. S4A. This should be addressed.

5. p. 12 / Fig. 4A: The authors state that they found elevated levels of DYRK1A mRNA in spleen, but the corresponding bar is clearly below average. Did they mean to write "thymus" instead, for which eleveated levels are clearly seen?

6. The point mentioned at the bottom of p. 11 that neutralization of ShaDock binding activity of HP1 proteins might also affect HP1 binding to H3K9me is interesting and might merit further discussion. Reading the in vitro data about the effect of ShaDock phosphorylation on HP1 binding to it I was wondering whether that made any difference since at least HP1alpha should still bind to H3K9me. The in vivo data address this issue, but the question remains why this might be and how this might work. I do realize that this point is likely beyond the scope of this article, and detailed mechanistic insight is not required for EMBO Reports, but maybe a more detailed mention of this point in the conclusions and avenues toward its investigation would be merited.

Minor points:

7. write "latter" instead of "later" (abstract and p.11, 2nd paragraph)

8. Scale bars and their labels in Figs. S1 and S2 are barely visible.

9. Fig. S2C: row 1 is not labeled (presumably EGFP), and data for the DYRK1A mutant mentioned in the text are missing.

10. p.8, beginning of 2nd paragraph: write "in addition to being excluded" instead of "be excluded"

11. Table 1: please explain what KD and the p-value mean in this context, it is not evident to non-specialists (who might think of KD only as a binding constant).

 

Referee #2:

This paper examines the role of the kinase DYRK1A in regulating the binding of human HP1 isoforms alpha, beta and gamma to histone H3. Their approach is mainly in vitro using peptides. The authors first show using pull-down experiments and mutagenized peptides that the HP1 isoforms bind specific regions within the H3 peptide centered around a PXXVXL motif. They then go on to show that phosphorylation of 2 key residues both within (T45) and outside (S57) this motif can inhibit binding of HP1 isoforms in vitro. This data was complemented using mutations that mimic phosphorylation. Thus, a region of H3 which does not contain the H3K9 residue (known to be an important binding site for HP1 when methylated) nevertheless binds HP1 isoforms and this binding can be modulated by phosphorylation by DYRK1A in vitro. This data presented largely support these conclusions but there are some issues that need to be addressed (see below). They went on to show that overexpression of DYRK1A resulted in increased phosphorylation of the T45 residue in the nucleus NIH 3T3 cells and that this distribution was similar to the kinase. Using a knockdown of DYRK1A in Hela cells they performed microarray transcriptomic analysis and identified 1068 genes are downregulated and only 28 upregulated. Focusing on a subset of downregulated genes that had previously been shown to be regulated by or bind to HP1 at their promoters they went on to show using chromatin immunoprecipitation that knockdown resulted in less binding of DYRK1A and reduced phosphorylation of T45 and S57 accompanied by decreased binding of HP1 whilst leaving H3ser10 and H3K9me3 largely unaffected. This result suggests that the HP1 binding is dependent not only H3K9me3 but also the phosphorylation state of the Shadoc domain. The authors then investigate a subset of cytokine genes in the Downs Syndrome related acute megakaryoblastic leukaemia which was previously been shown in a mouse model to be promoted by an extra copy of DYRK1A using a cell line in which they inhibit DYRK1A with L41 and find a dose-dependent decrease in IL-8 and IL1alpha expression and increase in BTG2 and ID2. The authors confirm some of the results by knockdown in HL60 cells. They conclude that inhibiting DYRK1A in this form of leukaemia might reduce autocrine cytokine signaling however, the relevance of this effect is not discussed.

The paper's strength lies in the identification of an additional layer in regulating HP1 binding which might have important consequence for regulating heterochromatin as well as genes that are silenced by HP1. This is a novel finding that will be of interest to a wide readership. It is doubtful as to whether the potentially interesting preliminary findings in the Down's Syndrome associated leukaemia add much to this study at this stage, there is insufficient provided in paper as it stands.

Major issues:

1) Transcriptome with exon array (also Table S1)
Please indicate number of biological and technical replicates of the RT-PCR confirmation experiments.
Please include statistical analysis to show significance of effects observed.
Please provide a more detailed version of methods for RTPCR and its analysis e.g. indicate any endogenous control used.
Please explain the reason for overexpression of DYRK1A in HEK cells rather than performing a "rescue" in HeLa cells which were used in the original siRNA knockdown experiment

2) Result and Discussion (page 9)
"To investigate a potential antagonism between DYRK1A and HP1 proteins in transcriptional regulation, we focused on a series of NF-κB-regulated genes including IL8, TNFα, IL6, IL1α, IFI44, TNFAIP6, and IL7R. These genes were either previously described as recruiting HP1 proteins to their promoters [5,9-11,33] or appeared in an earlier array analysis as regulated by HP1γ [34]."
Please explain how these genes were selected. It would be interesting to know what the total number of genes identified that were NF-kB regulated genes as well as being regulated by or recruiting HP1 proteins?

3) Conclusion (page 13)

 

The authors state: "Its phosphorylation at H3S57, observed only at target promoters in interphase, weakens HP1γ binding while not affecting HP1α and HP1β."

This statement is too strong and imprecise. It has not been shown directly that H3S57P weakens HP1γ interaction with H3 only by use of a phospho-mimic - this could be done using the anti-H3S57p antibody on a western blot as was done for H3t45p in Figure 1E. Also, immunofluoresence of H3S57P showed strong signal only in mitosis but not interphase (Supplementary Figure 2B) although the authors suggest the lack of signal in interphase is due to accessibility problems it seems odd to now claim that the expression is restricted to interphase as the sentence implies. Moreover, chromatin immunprecipitation was not performed in cell cycle sorted cells so the authors cannot be sure that the targetting is restricted to interphase.
Please rewrite this section or perform more experiments to substantiate this claim.

4) Figure 1C
The BRG1 data is incomplete with no explanation. Please either provide data for the pull-down assay of GST-Brg1 with WT, S57E and T45E/S57E mutant peptide or an explanation.

5) Figure 1C
In the text (page 6) it is stated that "dual H3T45/H3S57 phosphorylation functions as a master regulator of all HP1-H3 interaction"
Although mutant T45E abolished binding of HP1β, dual mutation H3 T45E/H3 S57E appears to show a band in the HP1β region, it is not really possible to decide whether this band is an extension of the signal from lane 7 or not. It would be better to show an ambiguous blot for this important piece of data.

6) Figure 1D
Please include a JAK2 kinase or any other positive control for the anti-H3Y41P antibody.
Alternatively, immunoblot for anti-H3Y41P antibody could be left out as Fig. 1D provides clear-cut data showing DYRK1A is functioning specifically.

7) Supplementary Fig 1A
Please provide merged image that overlays DYRK1A and DAPI signal
Please put the scale bar in sensible place - it looks odd in the middle of the figure.
Please zoom in to provide better images for looking at localisation of the proteins (similar to Supplementary Fig. 1C)

8) Statistical analysis
Please include statistical analysis for all qRTPCR results to show what is statistically significant or not

9) Supplementary figure S3B
Please include statistical analysis for GO terms analysis
Please indicate if the data has been filtered according to probability or any other criteria used.

10) Figure 2B, 2D and 2F
Loading control missing

11) Supplementary Figure S4A
Results and Discussion (page 10):
"Chromatin immunoprecipitation (ChIP) experiments with anti-DYRK1A antibodies established the presence of this protein at the promoters of IL8, TNFα, IL6, IL1α, IFI44, and TNFAIP6, but not TGFβ"
This data appeared to have normalized to samples transfected with non-targeting siRNA. Please clarify this in the text.
Also, it is a bit unusual to normalize to background signal (IgG Mouse) as it is usually very low. Was the signal for DYRK1A very low?

12) Figure 4A

 

Please explain the reason for normalizing the DYRK1A mRNA level in different tissues to prostate - I don't get a feel for the overall abdundance of this RNA relative to other genes - perhaps the authors can indicate this.
Please also explain why the Y axis of figure 4A was labeled as "relative accumulation" - surely we're looking at steady state levels.

13) Proposed model
Perhaps a diagram showing the hypothesized model would make the findings more easily accessible.

Minor issues:
14) Introduction (page3) - second paragraph
"HP1 proteins are archetypical archetypal examples of transcriptional regulator functioning in both stable "silencing" and transient repression of euchromatic genes."

15) Introduction (page3) - reference 4
"likewise, the ....."
HP1γ has not been studied in reference 4 for the induction of heterochromatin formation as implied in the preceding sentence. Please re-write the sentence

16) Results and Discussion (page 5)
"We also note that earlier studies used H3 fragments shorter than the rH3(25-66) in their interaction experiments [21], possibly explaining why we do not see H3-binding restricted to HP1α whereas in those experiments this was only HP1 isoform that interacted with the peptide."

I have added the underlined section to try and make this sentence understandable to someone who has not read reference 21 - please check this is what you meant and modify the sentence accordingly.

17) Result and Discussion (page 6)
"These data suggested that H3T45 phosphorylation controls binding of the HP1α and HP1β isoforms, while dual H3T45/H3S57 phosphorylation functions as a master regulator of all HP1-H3 interactions."

I think the term 'master' regulator is a bit mis-leading as this is an in vitro experiment using only a fragment of H3 - I suggest deleting the word 'master'.

18) Result and Discussion (page 7)
"As anticipated from the H3 phospho-mimic mutants, rH3(25-66) phosphorylated by GST-DYRK1A in vitro displayed a reduced affinity for HP1α, HP1β, and HP1γ, but not for Brg1 (Fig 1E, lanes 1 and 2)."
Binding of Brg1 was obviously enhanced with GST-DYRK1A. Please make this clear in the text.

19) Result and Discussion (page 7)
"Incubation of rH3(25- 66) with the GST-DYRK1A(K188R) kinase-dead mutant had little or no effect on its ability to bind its molecular partners (Fig 1E, lane 3)."
When taking the loading control into account, all of the three HP1 isoforms show reduced signal with GST-DYRK1A(K188R) kinase-dead mutant.

In fact all the signals appear slightly decreased taking into account the loading control - the sentence should be modified for accuracy.

20) Result and Discussion (page 7)
"Together, these observations showed that the H3 ShaDock functions as a hub for histone modifications controlling the binding of HP1 proteins and that DYRK1A plays a potential role in discriminating between HP1 and Brg1 binding in this region"
This statement is speculative, this should be made clear. It is too broad and overstates the findings. The experiments were done in the absence of histone modifications apart from phosphorylation and

 

were in vitro - this sentence should be re-written taking these points into account so as not to mislead the reader.

21) Result and Discussion (page 8)
"Global increase in levels of H3S57p was associated with mitosis but was not observed upon EGFP-DYRK1A8 overexpression, possibly reflecting the limited accessibility of this very intranucleosomal position in interphase (supplementary FIgS2B,C online)."

In FigS2B and S2C the chromosomes were ill-defined making it difficult to conclude that these were mitotic. Please provide better images and possibly include staining of an alternative marker to show cells are in M phase. The treated control in metaphase is missing.
The use of the word 'very' seems inappropriate it's either intranucleosomal or not, maybe this could be rephrased.

22) Result and Discussion (page 8)
"In that sense, it showed a distribution similar to that of the chromatin remodeler Brg1, while it was excluded from regions enriched in HP1α (supplementary Fig S1C,D online). This sub-nuclear localization was independent of its kinase activity."
Although HP1α was concentrated in DAPI dense regions that DYRK1A appeared not co-localise with, there was a diffuse pattern of HP1α staining that does in fact co-localise with DYRK1A signal. A sentence to describe this diffuse staining pattern should be included.

23) Result and Discussion (page 8)
"These data that were validated by RTqPCR on a large series of genes with two different DYRK1A siRNAs (supplementary Table S1 online) strongly suggested that DYRK1A predominantly functions a transcriptional activator."

The more accurate statement would be that DYRK1A's function is to prevent repression rather than activate itself - the sentence should be modified.

24) Result and Discussion (page 9)
"Altogether, these observations suggested that DYRK1A has a major might have a function in the response to extracellular stressors."
This statement should be toned down as suggested above (underlined) or something similar.
25) Result and Discussion (page 11)
"Importantly, upon DYRK1A depletion, we detected a very systematic increase in recruitment of the three HP1 proteins to the investigated promoters (Fig 3D,F)."
The use of the word 'very' is again in appropriate - you could simply delete it.

26) Result and Discussion (page 11)
"At all promoters except that of TGFβ, we observed decreased levels of histone acetylation as asserted ascertained by ChIP with a pan anti-acetyl histone antibody (supplementary Fig S4B online)."

Wrong use of the word 'asserted' perhaps you meant 'ascertained'.

27) Result and Discussion (page 11)
"In contrast, levels of the repressive H3K9 tri-methylation (H3K9me3) mark were either mostly unchanged or but in one case up-regulated (supplementary Fig S4C online). This was interesting because it suggested the hypothesis that neutralization of the ShaDock binding activity of the HP1 proteins impacts their H3K9me-binding activity"
I have included a suggested edit (underlined) to this sentence above to make it more accurate.

28) Result and Discussion (page 11-12)
"Finally, we noted a moderate but systematic reduction in the recruitment of Brg1, indicating that all the HP1 and DYRK1A target genes we put under scrutiny also are are also Brg1 target genes, and that Brg1 is dependent on DYRK1A activity for optimal recruitment to the promoter of these genes (supplementary Fig S4D online)."
Corrected text underlined.

 

29) Result and Discussion (page 12)
"Consistent with this, we found DYRK1A mRNA to be abundant in spleen, peripheral blood mononuclear cells (PBMCs), T cell-derived Jurkat cells, and promyelocytic leukemia HL60 cells (Fig 4A)."
Corrected text underlined.

30) Result and Discussion (page 12)
"Children with Down's syndrome (DS) are at increased risk to of developing acute megakaryoblastic leukemia"
Corrected text underlined.

31) Result and Discussion (page 13)
"These observations suggest that targeting DYRK1A may allow reducing reduce autocrine inflammatory signaling in myeloid leukemias."

32) Conclusion (page 13)
"When phosphorylated at H3T45, the ShaDock binds exclusively Brg1 and HP1γ"

Strictly speaking this has not been shown but was shown for the phosphomimic Fig1C T45E - this needs clarification in the sentence shown above.

33) Supplementary Material and Methods
Culture condition for NIH3T3 cells is missing

34) Material and Methods
Please include the methods of Microarray analysis in the material and methods section but not in the figure legend.
Please provide methods of the analysis in more detail particularly for the GO terms and pathways. Please also include any statistical analysis that has been used.

35) Figure 1A
Numbering of amino acid residues of H3 appears not agree with the conventional labelling of histone modifications in figure 1A. Instead of having the first amino acid of the peptide residue 25, it should be 23 - perhaps there is another convention that I am not aware of? Please explain.

36) Supplementary Fig 1C
Please add scale bar and label "DYRK1A" and "EGFP-DYRK1A"

37) Figure S2B
"S" should be "M" which stands for mitosis

38) Figure 2
Please explain what "RPL0" stands for.

39) Figure 3 and Supplementary Fig S4
Please provide clear labeling for the bars representing control and DYRK1A KD samples

Referee #3:

In this manuscript the authors describe the identification of two residues within the histone fold motif of histone H3 (T45 and S57) that can be modified resulting in the regulation of the association between the Heterochromatin Protein 1 (HP1) family members as well as the chromatin remodeling factor BRG1 with histone H3. They further show that the kinase DYRK1A phosphorylates these residues in vitro and provide evidence that this kinase is involved in the regulation of gene expression, most likely through regulation of HP1 binding to the promoter regions of these genes. Lastly, they provide evidence that DYRK1A may be involved in myeloid leukemia by allowing the expression of genes involved in autocrine inflammatory signaling. The data presented in this

 

manuscript are of high quality and the conclusions are convincing. The strength of this manuscript is that the authors confirm, extend and make the link between findings coming from different studies as well as new data that finally allow proposing a general mechanism of gene regulation through modification of HP1 association to chromatin.
These findings are of major interest in the field of molecular biology and more specifically in elucidating the mechanisms and functions of the regulation of chromatin organization on gene expression.

Specific comments:
Figure 1B: Can the authors explain what is ΔBRG1? Because the authors claim for an interplay between HP1 and BRG1 binding, it would be interesting to show the binding of BRG1 to the mutants S57E and T45E/S57E
Figure 1E: In the manuscript, the authors claim that the mutant DYRK1A (K188R) has no or little effect on the ability of rH3(25-66) to bind to the different HP1 isoforms. This effect does not look that limited and not that different from the binding in presence of the WT DYRK1A protein on the figure. Can the authors quantify the binding and/or comment on this aspect?
Figure 2: the statistical analysis of the data is missing to conclude whether the expression of the various genes is altered in the different conditions. This is particularly important for the gene expression profile in response to HP1 depletion that is only mildly affected.
Figure 3: the code for the colors of the bars should be added to the legend.
Figure 4B: it would be more informative to show the absolute level of expression rather that the relative level, to see that indeed there is an undetectable expression of the cytokine genes that are not sensitive to DYRK1A inhibitor.

In conclusion I find this manuscript of major interest and the data of very good quality and would therefore recommend it for publication following minor revisions as suggested above.

1st Revision - authors' response 06 March 2014

Referee #1:
*1. I have some doubts about the quantitative nature of the binding data of HP1 proteins to rH3 constructs shown in Fig. 1. It is stated that HP1 isoforms and Brg1 bind full-length H3 with "comparable efficiencies" (p.5), yet the signals show very different intensities, especially comparing lanes 4 and 5 in Fig. 1B. This is probably not much of an issue, since only the binding per se is really interesting at this point, but then one should not claim "comparable efficiencies".*

We agree that it is difficult to compare the affinity of proteins that are at similar but not identical levels of purity as is the case in panel B. "comparable efficiencies" has been removed from the text.

*Also, I find it difficult to confirm that a H3S57E mutation reduces HP1gamma binding by "approximately 50%" (p.6 and Fig. 1C, lanes 7,8) - it clearly reduces the binding, but where do the 50% come from? Was the binding properly quantified? - and the weak band in lane 8 suggests that the binding may not be abolished as "completely" as the authors claim.*

This section has been rephrased to: “A H3S57E phospho-mimic mutation reduced HP1γ binding to an intermediate level, while a combined H3S57E/H3T45E mutation had a much stronger affect”.

*In addition, there seems to be some spillover into lane 8 in the HP1beta binding experiment of Fig. 1C, and there may be an image artifact at the bottom of lane 2 in the test of Brg1 binding to H3V46A in the same figure. These issues would merit some comments of the authors, discussion in/rewording of the manuscript, or a rerun of gels in question.*

We re-ran the gels as requested and the 2 panels have been replaced.

*2. A rather crucial point concerns Fig. 1E. Clearly binding of HP1 isoforms to a rH3 construct is less affected by incubation of rH3 with kinase-defective DYRK1A than with active DYRK1A. However, the bands after incubation with mutant DYRK1A nevertheless consistently seem somewhat*

 

*weaker - visually, the difference in the HP1gamma binding experiment between lanes 1 and 3 in Fig. 1E does not seem*
*less pronounced than the difference between lanes 6 and 7 for HP1gamma binding to WT and S57E rH3 in Fig. 1C, yet in the latter case the authors claim "little or no effect", in the former "50% reduction". Also, the effect of DYRK1Amediated phosphorylation of rH3 appears different from the one observed with phosphorylation mimic mutants. In the latter, already T45E abolished HP1alpha and -beta binding and an additional S57E mutant was required to also abolish HP1gamma binding, while with "real" phosphorylation at (presumably - in that specific experiment, only T45 phosphorylation was controlled) both sites, HP1alpha and beta still seem to bind to some extent, while HP1gamma seems more affected. Is there any explanation for this?*
During the revision, we investigated the causes behind the effect of the DYRK1A mutant on HP1 binding: as it turns out, this mutant has moderate but clear residual kinase activity in our *in vitro* assay. This observation that complicates the interpretation of the 2 panel 1E is shown in a Figure "for the referees only" at the end of the rebuttal.

In addition, in the experiment of panel 1E, because the *in vitro* phosphorylation of rH3(25-66) was incomplete, we are in fact testing the binding of a mixture of nonphosphorylated, mono-phosphorylated, and di-phosphorylated rH3(25-66). This heterogeneity most likely explains the differences noted by the reviewer between the apparent effects of "real" phosphorylation *vs*. phospho-mimics.Because of these complications, we have removed panel 1E in the new version of the paper.

*3. I do not see much connection between the experiments investigating global impact of DYRK1A on gene expression using exon arrays (Fig. S3) and using RTqPCR (Tab. S1). The authors claim that the latter validate the former, but no quantitative data from the exon array analysis on individual genes are given. Couldn't they be added to Table S1 at least for those genes that were investigated by both methods (if there were any, which in my view is implied by the word "validation")? Do they agree? It is difficult from the current presentation to make connections, but I found that e.g. in Fig. S3C, FAS is marked in green, indicating no regulation by DYRK1A, while the results in Table S1 do indicate FAS to be regulated by DYRK1A. This apparent conflict should be addressed by the authors. As I stated, "validation" implies that a different analysis was carried out on the same target, so its results should either be shown if it was done (i.e. results of exon array and RT-qPCR on the same gene), or done if not.*

The data from the exon arrays have now been added to Supplemental Table S2 (former Table 1). This data is also available on GEO (code: GSE 43259). FAS was forgotten when we entered the gene names to perform the pathway analysis. We have now corrected this mistake.

*4. The authors state that ChIP "established the presence of [DYRK1A] at the promotors of …, but not TGFbeta" (p. 10 and Fig. S4A). However, as far as I see it, Fig. S4A shows that TGFbeta DNA could indeed be detected after ChIP with DYRK1A. Also, it does not seem to be significantly affected by DYRK1A depletion. This could be explained, for example, if the absolute levels of detected TGFbeta DNA were much lower than those of the other DNAs, but only relative levels are given in Fig. S4A. This should be addressed.*

Theoretically, the reviewer is right: representing absolute levels would be the best way to show the accumulation of DYRK1A on the cytokine genes. However, technically, our experience is that absolute levels from ChIP experiments are not reliable and that only ratios between easily comparable conditions (same antibody, same qPCR primers) are trustworthy. This is the reason why we show only relative ChIP data in the paper.

That depletion of DYRK1A causes reduces recruitment of DYRK1A at the NFkappaB target promoters but not at the TGFbeta promoter is clearly visualized when we normalize the anti-DYRK1A ChIP signal to that obtained with non immune mouse IgGs (without setting the siNT value to 1). A panel showing this is provided at the end of this document. Yet, in the interest of homogeneity between panels, we would prefer keeping the panel in the manuscript in its current form. Instead, to address the reviewers concern, statistics based on the three independent experiments used to produce the data have now been added to Panel S4A. These calculations show that depleting the cells from DYRK1A significantly reduces the anti-DYRK1A ChIP signal on the IL8, TNF, IL6, IL1A, IFI44, and TNFAIP6 promoters, while variations on the TGFbeta promoter are not significant.

 

*5. p. 12 / Fig. 4A: The authors state that they found elevated levels of DYRK1A mRNA in spleen, but the corresponding bar is clearly below average. Did they mean to write "thymus" instead, for which eleveated levels are clearly seen?*

We thank the reviewer for making us aware of this mistake. It has now been corrected.

*6. The point mentioned at the bottom of p. 11 that neutralization of ShaDock binding activity of HP1 proteins might also affect HP1 binding to H3K9me is interesting and might merit further discussion. Reading the in vitro data about the effect of ShaDock phosphorylation on HP1 binding to it I was wondering whether that made any difference since at least HP1alpha should still bind to H3K9me. The in vivo data address this issue, but the question remains why this might be and how this might work. I do realize that this point is likely beyond the scope of this article, and detailed mechanistic insight is not required for EMBO Reports, but maybe a more detailed mention of this point in the conclusions and avenues toward its investigation would be merited.*

The cross talk between HP1 binding to H3K9me and its binding to the ShaDock is now mentioned in the conclusions.

Minor points:

*7. write "latter" instead of "later" (abstract and p.11, 2nd paragraph)*

This has been corrected.

*8. Scale bars and their labels in Figs. S1 and S2 are barely visible.*

Larger scale bars have been inserted.

*9. Fig. S2C: row 1 is not labeled (presumably EGFP), and data for the DYRK1A mutant mentioned in the text are missing.*

The panel has been relabeled.

*10. p.8, beginning of 2nd paragraph: write "in addition to being excluded" instead of "be excluded"*

This was corrected.

*11. Table 1: please explain what KD and the p-value mean in this context, it is not evident to non-specialists (who might think of KD only as a binding constant).*

The columns in the table have been relabeled.

Referee #2:

First, we would like to thank this reviewer for his very thorough reading of the manuscript and for the time spend on suggesting alternative sentences to improve the manuscript.

Major issues:

*1) Transcriptome with exon array (also Table S1)*
*Please indicate number of biological and technical replicates of the RT-PCR confirmation experiments.*
*Please include statistical analysis to show significance of effects observed.*
*Please provide a more detailed version of methods for RTPCR and its analysis e.g. indicate any endogenous control used.*

Table S1 has now been reformatted to contain microarray and RT-qPCR data with associated p-values. The required information has been added to the Material and Methods section dealing with the RT-qPCR reaction.

*Please explain the reason for overexpression of DYRK1A in HEK cells rather than performing a "rescue" in HeLa cells which were used in the original siRNA knockdown experiment.*

For this experiment, because overexpression of DYRK1A was reported to inhibit cell proliferation, we sought out to find a very tightly regulated inducible system. In our hand, the less leaky and most inducible system turned out to be the one based on the ponasterone/ecdysone receptor, that unfortunately is not available in HeLa cells. We agree that an experiment overexpressing DYRK1A in HeLa cells would have made good sense; but we also believe that

 

observing an effect in two different cell lines makes it more reliable.

*2) Result and Discussion (page 9)*
*"To investigate a potential antagonism between DYRK1A and HP1 proteins in transcriptional regulation, we focused on a series of NF-κB-regulated genes including IL8, TNFα, IL6, IL1α, IFI44, TNFAIP6, and IL7R. These genes were either previously described as recruiting HP1 proteins to their promoters [5,9-11,33] or appeared in an earlier array analysis as regulated by HP1γ [34]."*
*Please explain how these genes were selected. It would be interesting to know what the total number of genes identified that were NF-kB regulated genes as well as being regulated by or recruiting HP1 proteins?*

The genes regulated by both DYRK1A and NFkappa-B were selected by crossing the hits from the DYRK1A depletion experiment with data from an on-line resource providing a list of NFkappa-B target genes (http://bioinfo.lifl.fr/NF-KB/). After filtering away some genes for which the evidence for regulation by NFkappa-B seemed insufficient, we generated Supplementary Table S2 (former Table 1), that we consider as an exhaustive list of genes suspected to be both DYRK1A and NFkappa-B targets. This is now described in the Materials and Methods section.

As described in the text, within this list, we selected genes that in the literature were proposed as HP1 targets, namely IL8, IL6, IL1α, IFI44, TNFAIP6, and IL7R. To this list, we added TNFα, an NFkappa-B- and HP1-regulated gene that seemed a good candidate for a regulation by DYRK1A.

To identify more systematically the NFkappa-B target genes also regulated by HP1 proteins is an important issue, but we believe that it is beyond the scope of this manuscript.

*3) Conclusion (page 13)*
*The authors state: "Its phosphorylation at H3S57, observed only at target promoters in interphase, weakens HP1γ binding while not affecting HP1α and HP1β."*
*This statement is too strong and imprecise. It has not been shown directly that H3S57P weakens HP1γ interaction with H3 only by use of a phospho-mimic - this could be done using the anti-H3S57p antibody on a western blot as was done for H3t45p in Figure 1E.*
*Also, immunofluoresence of H3S57P showed strong signal only in mitosis but not interphase (Supplementary Figure 2B) although the authors suggest the lack of signal in interphase is due to accessibility problems it seems odd to now claim that the expression is restricted to interphase as the sentence implies. Moreover, chromatin immunprecipitation was not performed in cell cycle sorted cells so the authors cannot be sure that the targetting is restricted to interphase. Please rewrite this section or perform more experiments to substantiate this claim.*

We agree that this was an overstatement. The section has been rewritten and conclusions made less peremptory.

4) Figure 1C
The BRG1 data is incomplete with no explanation. Please either provide data for the pull-down assay of GST-Brg1 with WT, S57E and T45E/S57E mutant peptide or an explanation.

The data has been inserted.

*5) Figure 1C*
*In the text (page 6) it is stated that "dual H3T45/H3S57 phosphorylation functions as a master regulator of all HP1-H3 interaction"*
*Although mutant T45E abolished binding of HP1β, dual mutation H3 T45E/H3 S57E appears to show a band in the HP1β region, it is not really possible to decide whether this band is an extension of the signal from lane 7 or not. It would be better to show an ambiguous blot for this important piece of data.*

We re-ran the gel as requested and the panel has been replaced.

*6) Figure 1D*
*Please include a JAK2 kinase or any other positive control for the anti-H3Y41P antibody. Alternatively, immunoblot for anti-H3Y41P antibody could be left out as Fig. 1D provides clear-cut data showing DYRK1A is functioning specifically.*

The immunoblot for anti-H3Y41P antibody was left out. This panel is now presented in Fig. S2B.

 

*7) Supplementary Fig 1A*
*Please provide merged image that overlays DYRK1A and DAPI signal*
*Please put the scale bar in sensible place - it looks odd in the middle of the figure.*
*Please zoom in to provide better images for looking at localisation of the proteins (similar to Supplementary Fig. 1C)*

The requested panels were inserted.

*8) Statistical analysis*
*Please include statistical analysis for all qRTPCR results to show what is statistically significant or not.*

Statistical analysis is now present on each panel involving RT-qPCR.

*9) Supplementary figure S3B*
*Please include statistical analysis for GO terms analysis*
*Please indicate if the data has been filtered according to probability or any other criteria used.*
Statistics for the GO terms analysis are now given in the legend of Figure S3.

*10) Figure 2B, 2D and 2F*
*Loading control missing*

The loading controls have been added.

*11) Supplementary Figure S4A*
*Results and Discussion (page 10):*
*"Chromatin immunoprecipitation (ChIP) experiments with anti-DYRK1A antibodies established the presence of this protein at the promoters of IL8, TNFα, IL6, IL1α, IFI44, and TNFAIP6, but not TGFβ" This data appeared to have normalized to samples transfected with non-targeting siRNA. Please clarify this in the text.*
*Also, it is a bit unusual to normalize to background signal (IgG Mouse) as it is usually very low. Was the signal for DYRK1A very low?*
The missing labels mentioning the non-targeting siRNA used as control have been added to the figure.

It is not clear to us why the reviewer consider normalizing to IgGs of matched isotype as unusual. It is one of the very common ways of defining background signal.
The DYRK1A signal in the ChIP assays was comparable to the signals we got with the anti-HP1 antibodies (around 23 CTs).

*12) Figure 4A*
*Please explain the reason for normalizing the DYRK1A mRNA level in different tissues to prostate - I don't get a feel for the overall abdundance of this RNA relative to other genes - perhaps the authors can indicate this.*
*Please also explain why the Y axis of figure 4A was labeled as "relative accumulation" - surely we're looking at steady state levels.*

We use mRNA level in prostate to normalize the other values because this level was very close to the average level in all tissues. This is now indicated in the Figure legend. The reviewer is right; the figure is presenting steady state levels. Yet, the level in each organ is compared to a reference (level in prostate) and is therefore “relative”. We have now taken these arguments into consideration and corrected the label of the Y axis that now reads “relative levels”.

*13) Proposed model*
*Perhaps a diagram showing the hypothesized model would make the findings more easily accessible.*

A model has been included in a novel panel S4E.

Minor issues:
*14) Introduction (page3) - second paragraph*
*"HP1 proteins are archetypical archetypal examples of transcriptional regulator functioning in both stable "silencing" and transient repression of euchromatic genes."*
Archetypical was changed to archetypal

 

*15) Introduction (page3) - reference 4*
*"likewise, the ....."*
*HP1γ has not been studied in reference 4 for the induction of heterochromatin formation as implied in the preceding sentence. Please re-write the sentence*
The paragraph has been remodeled and the mistake eliminated

*16) Results and Discussion (page 5)*
*"We also note that earlier studies used H3 fragments shorter than the rH3(25-66) in their interaction experiments [21], possibly explaining why we do not see H3-binding restricted to HP1α whereas in those experiments this was only HP1 isoform that interacted with the peptide."*
*I have added the underlined section to try and make this sentence understandable to someone who has not read reference 21 - please check this is what you meant and modify the sentence accordingly.*
The sentence has been remodeled to make it easier to understand.

*17) Result and Discussion (page 6)*
*"These data suggested that H3T45 phosphorylation controls binding of the HP1α and HP1β isoforms, while dual H3T45/H3S57 phosphorylation functions as a master regulator of all HP1-H3 interactions."*
*I think the term 'master' regulator is a bit mis-leading as this is an in vitro experiment using only a fragment of H3 - I suggest deleting the word 'master'.*
The word “master” was removed.

*18) Result and Discussion (page 7)*
*"As anticipated from the H3 phospho-mimic mutants, rH3(25-66) phosphorylated by GST-DYRK1A in vitro displayed a reduced affinity for HP1α, HP1β, and HP1γ, but not for Brg1 (Fig 1E, lanes 1 and 2)."*
*Binding of Brg1 was obviously enhanced with GST-DYRK1A. Please make this clear in the text.*
Fig 1E was removed as we discovered during revision a residual kinase activity of the DYRK1A (K188R) mutant (data is shown below).

*19) Result and Discussion (page 7)*
*"Incubation of rH3(25- 66) with the GST-DYRK1A(K188R) kinase-dead mutant had little or no effect on its ability to bind its molecular partners (Fig 1E, lane 3)."*
*When taking the loading control into account, all of the three HP1 isoforms show reduced signal with GST-DYRK1A(K188R) kinase-dead mutant.*
*In fact all the signals appear slightly decreased taking into account the loading control - the sentence should be modified for accuracy.*
As mentioned above, Fig 1E was removed as we discovered during revision a residual kinase activity of the DYRK1A (K188R) mutant (data is shown below).

*20) Result and Discussion (page 7)*
*"Together, these observations showed that the H3 ShaDock functions as a hub for histone modifications controlling the binding of HP1 proteins and that DYRK1A plays a potential role in discriminating between HP1 and Brg1 binding in this region"*
*This statement is speculative, this should be made clear. It is too broad and overstates the findings. The experiments were done in the absence of histone modifications apart from phosphorylation and were in vitro - this sentence should be re-written taking these points into account so as not to mislead the reader.*
The text now makes clear that the statement is speculative.

*21) Result and Discussion (page 8)*
*"Global increase in levels of H3S57p was associated with mitosis but was not observed upon EGFP-DYRK1A8 overexpression, possibly reflecting the limited accessibility of this very intranucleosomal position in interphase (supplementary FIgS2B,C online)."*
*In FigS2B and S2C the chromosomes were ill-defined making it difficult to conclude that these were mitotic. Please provide better images and possibly include staining of an alternative marker to show cells are in M phase. The treated control in metaphase is*

 

*missing.*
*The use of the word 'very' seems inappropriate it's either intranucleosomal or not, maybe this could be rephrased.*
• Histone H3 serine 10 phosphorylation (presented in row 2 of S2B) is a common mitosis marker frequently used in histology to count mitotic cells. This has now been made clearer in the text.
• It is not clear to us what the reviewer means by “treated control”.
• The word “very” was removed.

*22) Result and Discussion (page 8)*
*"In that sense, it showed a distribution similar to that of the chromatin remodeler Brg1, while it was excluded from regions enriched in HP1α (supplementary Fig S1C,D online). This sub-nuclear localization was independent of its kinase activity."*
*Although HP1α was concentrated in DAPI dense regions that DYRK1A appeared not colocalise with, there was a diffuse pattern of HP1α staining that does in fact co-localise with DYRK1A signal. A sentence to describe this diffuse staining pattern should be included.*
The sentence mentioned by the reviewer clearly state that DYRK1A is excluded from regions where HP1 is enriched; it does not claim that DYRK1A is excluded from all nuclear regions where HP1 is present.
However, to comply with the reviewer’s request, we are now mentioning the diffused staining of HP1 in the text.

*23) Result and Discussion (page 8)*
*"These data that were validated by RTqPCR on a large series of genes with two different DYRK1A siRNAs (supplementary Table S1 online) strongly suggested that DYRK1A predominantly functions a transcriptional activator."*
*The more accurate statement would be that DYRK1A's function is to prevent repression rather than activate itself - the sentence should be modified.*
The sentence has been modified to introduce the “anti-repressor” effect of DYRK1A.

*24) Result and Discussion (page 9)*
*"Altogether, these observations suggested that DYRK1A has a major might have a function in the response to extracellular stressors."*
*This statement should be toned down as suggested above (underlined) or something similar.*
The sentence has been corrected as suggested by the referee.

*25) Result and Discussion (page 11)*
*"Importantly, upon DYRK1A depletion, we detected a very systematic increase in recruitment of the three HP1 proteins to the investigated promoters (Fig 3D,F)."*
*The use of the word 'very' is again in appropriate - you could simply delete it.*
The word “very” has been deleted.

*26) Result and Discussion (page 11)*
*"At all promoters except that of TGFβ, we observed decreased levels of histone acetylation as asserted ascertained by ChIP with a pan anti-acetyl histone antibody (supplementary Fig S4B online)."*
*Wrong use of the word 'asserted' perhaps you meant 'ascertained'.*
The mistake was corrected.

*27) Result and Discussion (page 11)*
*"In contrast, levels of the repressive H3K9 tri-methylation (H3K9me3) mark were either mostly unchanged or but in one case up-regulated (supplementary Fig S4C online). This was interesting because it suggested the hypothesis that neutralization of the ShaDock binding activity of the HP1 proteins impacts their H3K9me-binding activity"*
*I have included a suggested edit (underlined) to this sentence above to make it more accurate.*
The sentences were corrected according to the reviewer’s suggestions.

 

*28) Result and Discussion (page 11-12)*
*"Finally, we noted a moderate but systematic reduction in the recruitment of Brg1, indicating that all the HP1 and DYRK1A target genes we put under scrutiny also are are also Brg1 target genes, and that Brg1 is dependent on DYRK1A activity for optimal recruitment to the promoter of these genes (supplementary Fig S4D online)."*
*Corrected text underlined.*
Corrected

*29) Result and Discussion (page 12)*
*"Consistent with this, we found DYRK1A mRNA to be abundant in spleen, peripheral blood mononuclear cells (PBMCs), T cell-derived Jurkat cells, and promyelocytic leukemia HL60 cells (Fig 4A)."*
*Corrected text underlined.*
Corrected

*30) Result and Discussion (page 12)*
*"Children with Down's syndrome (DS) are at increased risk to of developing acute megakaryoblastic leukemia"*
*Corrected text underlined.*
Corrected

*31) Result and Discussion (page 13)*
*"These observations suggest that targeting DYRK1A may allow reducing reduce autocrine inflammatory signaling in myeloid leukemias."*
Corrected

*32) Conclusion (page 13)*
*"When phosphorylated at H3T45, the ShaDock binds exclusively Brg1 and HP1γ"*
*Strictly speaking this has not been shown but was shown for the phosphomimic Fig1C T45E - this needs clarification in the sentence shown above.*
The sentence has been removed.

*33) Supplementary Material and Methods*
*Culture condition for NIH3T3 cells is missing*
Culture conditions for NIH3T3 cells are now described in the Material and Methods.

*34) Material and Methods*
*Please include the methods of Microarray analysis in the material and methods section but not in the figure legend.*
*Please provide methods of the analysis in more detail particularly for the GO terms and pathways. Please also include any statistical analysis that has been used.*
The Material and Method section has been made more complete on these issues.

*35) Figure 1A*
*Numbering of amino acid residues of H3 appears not agree with the conventional labelling of histone modifications in figure 1A. Instead of having the first amino acid of the peptide residue 25, it should be 23 - perhaps there is another convention that I am not aware of? Please explain.*
The mistake has been corrected.

*36) Supplementary Fig 1C*
*Please add scale bar and label "DYRK1A" and "EGFP-DYRK1A*"
The figure has been relabeled

*37) Figure S2B*
*"S" should be "M" which stands for mitosis*
The mistake has been corrected

38) Figure 2
Please explain what "RPL0" stands for.

 

"RPL0" has been corrected to "RPLP0". The name of this reference gene has now been spelled out in the legend of figure 2 (Ribosomal Protein, Large, P0).

*39) Figure 3 and Supplementary Fig S4*
*Please provide clear labeling for the bars representing control and DYRK1A KD samples*
The figure has been relabeled.

Referee #3:

Specific comments:
*Figure 1B: Can the authors explain what is ΔBRG1? Because the authors claim for an interplay between HP1 and BRG1 binding, it would be interesting to show the binding of BRG1 to the mutants S57E and T45E/S57E*

ΔBRG1 is a fragment of Brg1 spanning from AA 1223 to 1420. The construct was described in the Material and Methods and previously in Lavigne et al. 2009. To comply with the reviewer's request, the construct is now described in the text.
The requested panel has been added to Figure 1B.

*Figure 1E: In the manuscript, the authors claim that the mutant DYRK1A (K188R) has no or little effect on the ability of rH3(25-66) to bind to the different HP1 isoforms. This effect does not look that limited and not that different from the binding in presence of the WT DYRK1A protein on the figure. Can the authors quantify the binding and/or comment on this aspect?*

We have now investigated the causes behind the effect of the mutant DYRK1A on HP1 binding: as it turns out, this mutant has moderate but clear residual kinase activity in our in vitro assay. This is now shown in a Figure for the referees only (see below), and we have chosen to remove Figure 1E.

*Figure 2: the statistical analysis of the data is missing to conclude whether the expression of the various genes is altered in the different conditions. This is particularly important for the gene expression profile in response to HP1 depletion that is only mildly affected.*

The statistical analysis has been added to the Figures.

*Figure 3: the code for the colors of the bars should be added to the legend.*

The missing color codes have been added.

*Figure 4B: it would be more informative to show the absolute level of expression rather that the relative level, to see that indeed there is an undetectable expression of the cytokine genes that are not sensitive to DYRK1A inhibitor.*

We agree that this figure does not allow comparing the expression levels of the different cytokines in CMKs. The purpose of the Figure is to show variations in expression of these cytokines in the presence of L41. In addition, and as mentioned in the text, cytokine genes expressed at undetectable levels in the CMK cells were not included in the Figure. Thus, to maintain the readability of the Figure, we would like to keep it in its present form.

In conclusion I find this manuscript of major interest and the data of very good quality and would therefore recommend it for publication following minor revisions as suggested above.

 

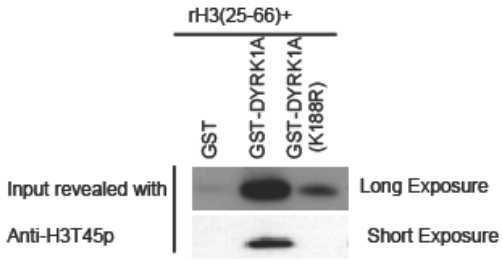


**Figure 1 for Referees:** rH3(25–66) peptide was incubated with either GST, GST-DYRK1A, or mutant GST-DYRK1A(K188R) in kinase buffer with ATP as described in the material and Methods. At the end of the reaction (30 min.), reactions were resolved by SDS-PAGE and revealed by Western blotting with anti-H3T45p antibody. Top and bottom panels were exposed on x-ray film for 15 and 1 min. respectively

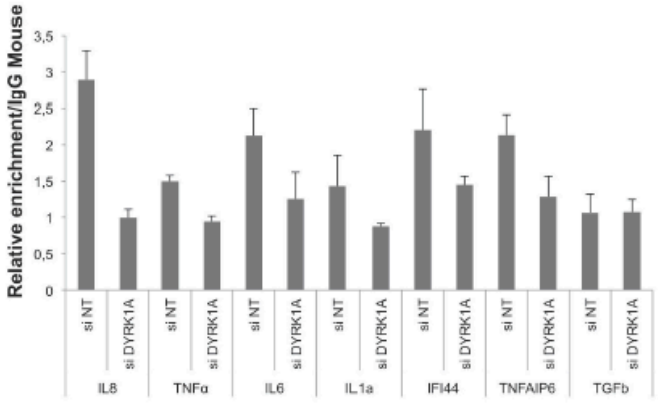


**Figure 2 for Referees:** Alternative version of Figure S4A. HeLa cells were transfected with either non-targeted or DYRK1A siRNAs. Accumulation of the indicated proteins or histone modifications at the promoter of the list genes was evaluated by ChIP analysis followed by qPCR. Data are normalized to the values obtained with either non-immune IgGs. Data shown are means +/- SEM from three independent experiments.

2nd Editorial Decision 20 March 2014

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the comments from the two referees who were asked to assess it, and both support publication of the study in our journal now. Referee 1 has only some minor suggestions that I would like you to address before we can proceed with the official acceptance of your manuscript.

Regarding the statistics, the legend for figure 3 does not define the * and specify the test used to calculate p-values, can you please add this information?

I have noticed that the manuscript does not contain a materials and methods section. Basic materials and methods essential to the understanding of the experiments must be described in the main body of the manuscript and may not be presented as supplementary information. Please add at least a brief

 

description of the materials and methods crucial for the understanding of the main experiments to the main materials and methods sections.

I also would like to suggest to add the word "transcriptional" before "repression" in the last sentence of your abstract and add this information also to the manuscript title:

DYRK1A phosphorylates histone H3 to differentially regulate the binding of HP1 isoforms and antagonize HP1-mediated transcriptional repression

Please let me know whether you agree with these changes.

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

REFEREE REPORTS:

Referee #1:

The authors have adequately and quickly responded to my concerns and those of referee #2, so I recommend their manuscript for publication in EMBO Reports.

There are just a few small issues (typos etc.) that should be corrected in the course of the further editorial process:

- Abstract:
"...that antagonizes HP1-mediated repression and participate in abnormal activation...": please write "participates" instead of "participate"

- referee #2, point 12:
Y axis of Fig 4A still reads "relative accumulation", not "relative levels"

- referee #2, point 22:
on p.6, write "diffuse" rather than "diffused" (HP1alpha signal)

- referee #2, point 27:
the sentence now is actually less accurate. Please write "were mostly unchanged and, in one case, up-regulated".

- referee #2, point 31:
actually the sentence should read "...may allow to reduce..."

- referee #2, point 33:
I did not find culture conditions for NIH3T3 cells in the Materials and Methods; instead, HeLa cells are mentioned twice ("HeLa, HEK293, and HeLa cells were cultured in Dulbecco's modified Eagle's medium..."). I suppose one of the HeLa mentions should read NIH3T3?

- referee #2, point 38:
for clarity, please also include the abbreviation RPLP0 in the legend of Figure 2 (e.g. as "...levels of Ribosomal Protein, Large, P0 (RPLP0) mRNA...")

Referee #3:

In this revised version, the authors have answered most of my concerns and the answer to the other referees is also very satisfactory. The overall quality of the manuscript has been greatly improved, in particular the expression data look more reliable and some overstatements within the text have been corrected. Altogether, I believe that this manuscript is suitable for publication in EMBOrep.

 

2nd Revision - authors' response 24 March 2014

Thank you for you comments. Accordingly, we have brought the following changes to the manuscript:

- The title was changed and now reads: “DYRK1A phoshorylates histone H3 to differentially regulate the binding of HP1 isoforms and antagonize HP1-mediated transcriptional repression”.
- The first paragraph of the introduction was removed to leave space for a “Material and Methods” section.
- A new “Material and Methods” section now describes essential experiments.
- Data from Figure S1 and Panel B of Figure S2 that are important for the understanding of the paper are now presented in a main Figure 2.
- The list of references was updated to include a 2014 review on HP1 from the S. Elgin lab.
- The typos pointed out by Referee #1 have been corrected.

3rd Editorial Decision 26 March 2014

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.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

 