THE EMBO JOURNAL

Manuscript EMBO-2012-82931

YY1 Controls Igκ Repertoire and B Cell Development, and Localizes with Condensin on the Igκ Locus

Xuan Pan, Madhusudahan Papasani, Yi Hao, Marco Calamito, Fang Wei, William Quinn III, Arindam Basu, Junwen Wang, Suchita Hodawadekar, Kristina Zaprazna, Huifei Liu, Yang Shi, David Allman, Michael Cancro and Michael L Atchison

Corresponding author: Michael Atchison, University of Pennsylvania

Review timeline:

Submission date:	09 August 2012
Editorial Decision:	26 September 2012
Revision received:	23 December 2012
Accepted:	11 February 2013

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: Karin Dumstrei

1st Editorial Decision 26 September 2012

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

As you can see the referees appreciate the link between YY1 and the condensin complex. However, they also raise a number of specific concerns that would have to be addressed in order for consideration here. Some molecular insight into why you get skewed Vk utilization is also needed. Should you be able to address the raised concerns in full then we would consider a revised manuscript. I should add that it is EMBO Journal policy to allow a single major round of revision only and that it is therefore important to resolve the raised concerns at this stage.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1

Conditional deletion of YY1 in early pro-B cells was shown to block B cell development at the pro-B cell stage. In this manuscript, Yan et al started by showing that retroviral transduction of full length YY1 into YY1fl/fl x mb-1 cre hematopoietic stem cells rescued B cell development, but a deletion mutant of YY1 (ΔREPO) that abolishes polycomb function did not (Fig. 1). YY1- and ΔREPO-expressing cells had comparable VH gene rearrangements, but differed in the distribution of VK rearrangements (Fig. 2,3). To investigate the role of REPO, they used a GST-REPO fusion protein as an affinity matrix to identify condensin components SMC 1,4 as REPO-interacting proteins (Fig. 4). ChIP assays demonstrated SMC 2,4 recruitment to parts of the κ locus that bound YY1 (Fig. 5,6) and knock-down of SMC4 in IL-7-expanded primary pro-B cells altered VK gene rearrangements (Fig. 7). The authors conclude that YY1 binding to the κ locus permits chromosomal compaction via condensin components to facilitate VK rearrangements.

Identification of condensin components as YY1-associated proteins, and the demonstration of a role for condensins in Igκ chain rearrangements is novel and interesting. In the context of earlier work from the Atchinson lab that the REPO domain of YY1 mediates polycomb functions, it is likely that these observations will apply to a wider array of developmental processes and therefore be of interest to readers of EMBO Journal. The only major shortcoming of the current manuscript is that it does not provide even a hint of a mechanism for the profound skewing of VK utilization towards the most distal VK gene segment in ΔREPO reconstituted pro-B cells. Specifically, the biggest difference in VK utilization between YY1 reconstituted and ΔREPO reconstituted pro-B cells is a huge increase in VK 24-140 utilization in the latter. What could be the basis for this? Additional points that should be clarified are:

1. The pattern of VK utilization in full length YY1 reconstituted pro-B cells looks very different from Bl6 cells?
2. The choice of VK segments used to study the effects of SMC knock-down in Fig. 7 is not clear. For example, 38-93 is noted to be a gene segment that "fails to rearrange in a ΔREPO background" whereas 12-44 is one that "continues to rearrange in a ΔREPO background". However, comparison of Figs. 2C and D show that 1) 38-93 rearranges not all in WT pro-B cells, poorly in YY1 reconstituted cells, and not at all in ΔREPO reconstituted cells. Thus, 38-93 does not seem to be a good choice to discriminate between YY1 and REPO. The case for 12-44 is even more puzzling since, in contradiction to the statement quoted above (from page 18 of the manuscript), 12-44 rearranges at high levels in WT and YY1 reconstituted pro-B cells, but not at all in ΔREPO cells. In other words, 12-44 is actually a good example of a REPO-requiring gene while 38-93 is not. Yet, 12-44 rearrangements are not affected in SMC4 KD (Fig. 7B). A good gene segment to test would be 24-140 which has the opposite response to removal of the REPO domain; it goes from being rearranged at very low levels in WT or YY1-reconstituted pro-B cells to being highly recombined in ΔREPO cells.

Overall, the connection between polycomb and condensin is an interesting one. With consideration of the points raised above, the paper will be appropriate for publication in the EMBO Journal.

Referee #2

In this manuscript, Pan et al. describe the analysis of a domain of YY1 that mediates the polycomb Group (PcG)-mediated repression. The authors performed a retroviral complementation assay of YY1-deficient bone marrow cells with wild type YY1 or with a mutant form of YY1 that lacks the REPO domain. In YY1deltaREPO-transduced B lineage cells, the authors observed a defect in differentiation and VJ rearrangements of the immunoglobulin (Ig) kappa locus. By biochemical purification of proteins that differentially associate with YY1 and YY1deltaREPO, the authors identified components of the condensin complex. The interaction could be confirmed in co-immunoprecipitations with endogenous proteins and knockdown of SMC4 resulted in a defect in VJ kappa rearrangement similar to the knockdown of YY1.

The identification of a condensin complex as a partner of YY1 that mediates the PcG function in B cell differentiation and Igkappa locus rearrangement is interesting and novel. In general, the data are convincing and well presented. However, the authors should address the following comments.

Specific comments:

1) The authors identified SMC1 and SMC4 by mass spec analysis of proteins that are co-immunoprecipitated with GAL or GAL-REPO. To assess the extent of the differential protein enrichment, It would be useful to show the SDS-PAGE in a Supplemental Figure. It would also be interesting to present the list of other identified proteins.

2) I appreciate that the authors performed a co-immunoprecipitation experiment with endogenous proteins. However, no lane of immunoprecipitated YY1 is included and the overall quality of the data shown in Figure 4B could be improved. Is the association also detected in a reciprocal co-immunoprecipitation experiment?

3) In Figure 5, the authors show ChIP experiments to examine the in vivo binding of YY1, SMC4 and EZH2 to multiple sites in the Ig kappa locus. For this experiment, the authors use IL7-cultured B lineage cells, and it would be interesting to examine whether the recruitment of SMC4 to these sites is impaired in IL7-cultured B cells in which endogenous YY1 has been replaced with YY1deltaREPO.

4) The quantitative ChIP data shown in Figure 5D should be presented as percent input, rather than relative enrichment.
5) The bands in the control lanes of Figure 7B are rather weak, which makes it difficult to assess the effects of the YY1 knockdown.

Referee #3

YY1 Controls Igκ Repertoire and B Cell Development, and Localizes with Condensin on the Igκ Locus
Xuan Pan et. al.

The manuscript from Michaeil L Atchison group reports the function of transcription factor and polycomb group(PcG) protein YY1 in B cell development and Igk recombination. Earlier work from this group demonstrated that the conditional knock-out of YY1 results in pro-B cell arrest with reduced IgH locus contraction. The authors now show very convincingly that the same YY1 has selective impact at the Igk locus resulting skewing of the expressed Igk repertoire. This functional effect is shown to be mediated by the YY1 REPO domain that is necessary for YY1 PcG funtion. The interaction of the REPO domain of YY1 with condensin and cohesin complexes and colocalization of YY1 with histone methyl ransferase Ezh2 at several sites of Igk locus are suggested to be involved in Igk locus contraction and rearrangement.

The data presented in this paper are very convincing and interesting although the data do not support some of the conclusions derived. The authors should consider the following points.

1) The YY1 REPO domain and PcG function appeared to be largely dispensable for heavy chain rearrangement. Then why the YY1ΔREPO reconstituted mice show decreased pro-B and pre-B compartments that have not started Igk rearrangement? Previous work (Liu H et. al. 2007) indicated that loss of YY1 did not interfere with the initial chromatin opening of the IgH locus. Additionally loss of YY1 did not appear to affect the expression of genes previously shown to be critical for early B-cell development. Therefore, the author should address if the observed defect in YY1ΔREPO reconstituted mice is due to decreased proliferation and survival of Igμ+ pre-B cells.

2) A recent study (Aoki-Ota M et. al. J Immunol. 2012 Mar 1;188(5):2305-15) analyzing Vk repertoire usage described that seven Igk V genes 1-135, 9-120, 10-96, 19-93, 6-23 6-17 and 6-15 were each found to be used at a frequency of 5-7% in the B6 WT bone marrow. Interestingly, none of these Vk genes were found to be rearranged frequently in the present study of B6 WT bone marrow. The author should comment about these and other discrepancies between the papers.

3) The authors show that condensin molecules SMC4 and BRNN1 physically interact with YY1 by co-immunoprecipitation experiments (Fig 4B) using IL-7 cultured bone marrow B cells. Later they described co-localization of SMC4, SMC2, BRNN1 and Ezh2 by chromatin immunoprecipation using same cells. But direct evidence that YY1 recruits the condensin molecules SMC2 and Ezh2 is lacking. An additional co-immunoprecipitation experiments are needed.

4) The information regarding the peptide sequencing experiments in Aim 4 are incomplete. More peptides must have been observed, what were they? What was the negative control IP? The investigators need to show the raw gels from which the bands were extracted, comment on the molecular weights, etc. For panel 4B, something that YY1 does not interact with, a negative, needs to be provided. Are the YY1 immunoprecipitations specific?

5) The strongest evidence of YY1 mediated Igk regulation is provided by the SMC4 and YY1 knockdown experiments. Additionally a direct effect of YY1 on Igk locus structure is demonstrated. However, the possibility of developmental block prior to Igk rearrangement is not ruled out. As a supporting evidence the authors describes "It should be noted that RNA expression profiles of PcG protein EZH2, and condensin subunit proteins SMC4, SMC2, CAP-G, CAP-H, and CAP-D2 peak during B cell development at the pre-B cell stage (www.immgen.org) . Expression levels are also high in pro-B cells, but peak in pre-B cells, then drop in immature B cell stages. This expression pattern is coincident with the timing of Ig rearrangement and is consistent with a role in Ig locus contraction and rearrangement." The statement is true but not the whole truth. The expression of the above mentioned PcG protein Ezh2 and condensin molecules described above are peaked at cycling pre-B cells where the clonal expansion of the pre-B cells expressing successfully rearranged heavy chain take place. Interestingly, there are multiple reports that describe the role of all the condensin molecules mentioned above in chromosomal condensation during mitosis of cell cycle. According to the www.immgen.org the expression of the above proteins starts to drop from the small pre-B cells at which stage the cells initiate the Igk rearrangement. Therefore to rule out the second possibility, the authors should check the cell cycle of the pro-B and pre-B cells isolated from YY1 and YY1ΔREPO reconstituted mice. The discussion section has to be modified accordingly.

6) The discussion needs to be shortened and focused.

Minor Comments:

1) Avoid using word like "Fortunately" while describing a mutant YY1 in first paragraph of result section.

2) Supplemental figure S1 should be introduced in the main figure as Figure 1A.

3) The official gene name for BRNN1 should be used as in most of the publications it is described as BRRN1.

4) Provide a reference that describes the YY1 binding site as GCCATNTT (in the section explaining "Identification of YY1 binding sites across the Igk locus").

1st Revision - authors' response 23 December 2012

Reviewer #1

The reviewer noted that “Identification of condensin components as YY1-associated proteins, and the demonstration of a role for condensins in Igκ chain rearrangements is novel and interesting.” In addition the reviewer states “it is likely that these observations will apply to a wider array of developmental processes and therefore be of interest to readers of EMBO Journal”. However, the reviewer noted that a mechanism for the profound skewing of V kappa utilization in the YY1deltaREPO background was not provided. We have addressed this comment as follows:
Response
We have developed a model that proposes the YY1 REPO domain maintains the Ig kappa locus in a way to position V kappa genes in appropriate contexts for V-J rearrangement. In the absence of

YY1 REPO function, these loops are not maintained eliminating rearrangement to most V kappa genes. The only loops remaining would be maintained by other factors. We hypothesize that Pax5 or E47 are involved in maintaining these remaining loops as these factors were previously suggested to play roles in Ig rearrangement. To test the feasibility of this model, we examined whether Pax5 and E47 bound to regions nearby the most active V kappa genes in a YY1 delta REPO background (V kappa genes 24-140 and dv-36). Indeed, by ChIP we found both Pax5 and E47 bound strongly to these DNA regions. These new data are presented in new Figure 8A. In addition, we have prepared a figure to describe our model and this model is presented in new Figure 8B.

Other points by Reviewer #1

1. The reviewer asks for clarification of differences in V kappa utilization in our YY1 reconstituted mice compared to wild type C57BL/6 mice.

Response

Although there are many similarities between the two repertoires, there are differences. Similarities include Vk gene usage spanning the entire Vk locus in both systems. However, the precise Vk genes used are not identical between wild type YY1 reconstituted mice and C57BL/6 mice. This may relate to comparison between wild-type mice and bone marrow transfer mice, or perhaps due to retroviral YY1 expression in the bone marrow transplants which may not completely recapitulate normal endogenous YY1 expression. None the less, the patterns are similar in that many Vk genes are rearranged in YY1 mice that span the full breadth of the Igk locus, similar to C57BL/6 mice, and these patterns are very distinct from the V kappa usage pattern in YY1 delta REPO mice. We have included the above discussion of C57BL/6 and YY1 repertoire differences on page 13 of the manuscript.

2. The reviewer questions the choice of V kappa gene primers for rearrangement assays after SMC4 or YY1 knock-down.

Response

The reviewer is correctly confused by a misstatement in the original manuscript stating that Vkappa 12-44 continues to rearrange in a YY1deltaREPO background. This misstatement is corrected in the current manuscript. Our original strategy was to use primers that would detect V kappa rearrangements that are similar in YY1 and YY1delta REPO mice compared to primers that detect rearrangements that occur in YY1 mice, but not YY1deltaREPO mice. However our choice quickly became more limited due to difficulty finding primers that efficiently and reproducibly detected V-J rearrangements in the IL7 withdrawal bone marrow culture system. The reviewer correctly points out that Vkappa 24-140 primers would be an excellent choice. We agree. We labored extensively to get these primers to work through many primer pairs and many conditions, but ultimately failed. Therefore, we chose to use the adjacent V kappa 2-139 gene which also rearranges well in both YY1 and YY1deltaREPO mice. Other primers detected V genes active in YY1 mice (38-93, 21-4) but not YY1deltaREPO, or that rearranged well in YY1deltaREPO (8-27) but not YY1. Ultimately we were not able to discern a distinct pattern with these primers other than most of them were impacted by either SMC4 or YY1 knock-down, a significant result suggesting an effect of YY1 and SMC4 on rearrangement.

Reviewer #2

The reviewer states, “The identification of a condensin complex as a partner of YY1 that mediates the PcG function in B cell differentiation and Ig kappa locus rearrangement is interesting and novel. In general, the data are convincing and well presented. However, the authors should address the following comments.”

1. The authors identified SMC1 and SMC4 by mass spec analysis of proteins that are co-immunoprecipitated with GAL or GAL-REPO. To assess the extent of the differential protein enrichment, it would be useful to show the SDS-PAGE in a Supplemental Figure. It would also be interesting to present the list of other identified proteins.

Response

We now provide a supplementary figure (Supplementary Figure S4) that shows an example of an SDS polyacrylamide gel of proteins co-immunoprecipitated from GAL or GAL-REPO transfected 293 cells. Arrows point to bands present in the GAL-REPO samples that were excised and

evaluated by MALDI-TOF mass spec. The specific proteins and peptides detected in this analysis are now shown in a new Table, Supplementary Table I as requested by the reviewer.

2. The reviewer apprecitates the effort required to co-immunoprecipitate endogenous proteins showing YY1 and condensin complex interactions, but requests higher quality data to be presented in Figure 4B.
Response
We have repeated these co-immuniprecipitation experiments using a number of approaches. First we used retroviral expressed BRRN1 and YY1 to detect coimmunoprecipitation in 38B9 pro-B cells. We also performed additional co-immunprecitation experiments from IL7 bone marrow cultures assessing YY1 co-immunprecipitation with endogenous BRRN1, SMC4, EZH2, and SuZ12. Finally we performed co-immunoprecipitation experiments with lysates from primary pro-B cells isolated from mice to detect interactions between YY1 and SMC4. Negative controls showing no interaction with lamin B or beta-actin are also now included. These new data are presented in a revised Figure 4B and 4C.

3. The reviewer asks for ChIP studies in IL7 cultured cells in which YY1 is replaced with YY1deltaREPO.
Response
This is an excellent suggestion and one which we have attempted to accomplished for over one year. Thus far we have been unable to delete the endogenous YY1 gene in IL7 cultures to provide a system where we can replace YY1 with YY1deltaREPO. We are actively pursuing a number of strategies to accomplish this experiment with the hopes that one approach will be successful. Thus, we would like to perform the experiment requested but technical difficulties have made that impossible thus far. We hope the current body of work will be sufficient and this experiment will be hopefully included in future studies beyond the scope of the current manuscript.

4. The reviewer asks that quantitative ChIP date in Figure 5D be presented as percent input.
Response
This has been done.

5. The reviewer notes that the control bands in Figure 7 are weak making the YY1 knock-down more difficult to assess.
Response
We agree with the reviewer. We have repeated these experiments to provide data with stronger control lanes. This new data is presented in revised Figure 7C.

Reviewer #3

The reviewer notes that, "The data presented in this paper are very convincing and interesting". The reviewer requests that a number of points be addressed.

1. The reviewer notes that YY1delta REPO mice show decreased pro-B and pre-B compartments which precede the timing of the V kappa gene rearrangement defect. The reviewer asks for an explanation and wonders if decreased proliferation or survival might be caused by the YY1deltaREPO protein.
Response
The reviewer is correct that this defect caused by YY1deltaREPO was unexpected. Some possibilities include a dominant negative effect of YY1deltaREPO that might impact cell growth or survival. We have performed experiments to explore the growth and apoptotic properties of YY1 compared to YY1deltaREPO. Previously (Pan et al, 2012) we showed that overexpression of YY1 can reduce B cell growth and increase B cell apoptosis. We have performed additional experiments here (presented in supplementary figure S2) to compare the impacts of YY1 compared to YY1deltaREPO on cell growth and apoptosis. These experiments did not reveal any difference between YY1 and YY1deltaREPO suggesting that the reduction in pro-B and pre-B cells in YY1deltaREPO mice is due to another mechanism. However, we cannot conclusively eliminate a role for proliferation effects and we have included discussion of these points in the Discussion section of the revised manuscript. We will continue to explore this potential mechanism but believe it is beyond the scope of the current manuscript.

2. The reviewer requests that we comment on differences between our results with Vk repertoires in wild type and YY1 mice to those in C57BL/6 mice observed by Nemazee et al in an October, 2012 publication.

Response

There are many similarities in repertoire between our two studies, but we agree with the reviewer that there are also many differences. The reasons for these differences are not clear, but may relate to methodological differences in V gene amplification or housing and environmental differences of our mice. We have included a more expanded discussion of the differences in our studies on page 22 in the Discussion section of the revised manuscript. At any rate, the differences observed do not impact our conclusions regarding the skewed repertoire in YY1deltaREPO mice compared to YY1 mice.

3. The reviewer asks for additional co-immunoprecipitation experiments to show interactions between condensin and PcG proteins.

Response

We have performed additional co-immuniprecipitation experiments using a number of approaches. First we used retroviral expressed BRRN1 and YY1 to detect coimmunoprecipitation in 38B9 pro-B cells. We also performed additional co-immunprecitation experiments from IL7 bone marrow cultures assessing YY1 co-immunprecipitation with endogenous BRRN1, SMC4, EZH2, and SuZ12. Finally we performed co-immunoprecipitation experiments with lysates from primary pro-B cells isolated from mice to detect interactions between YY1 and SMC4. Negative controls showing no interaction with lamin B or beta-actin are also now included. These new data are presented in a revised Figure 4B and 4C.

4. The reviewer asks for gel data and peptides indentified in our GAL vs GALREPO co-immunoprecipitation studies.

Response

We now provide a supplementary figure (Supplementary Figure S4) that shows an example of an SDS polyacrylamide gel of proteins co-immunoprecipitated from GAL or GAL-REPO transfected 293 cells. Arrows point to bands present in the GAL-REPO samples that were excised and evaluated by MALDI-TOF mass spec. The specific proteins and peptides detected in this analysis are now shown in new Supplementary Table I as requested by the reviewer.

5. The reviewer points out that expression of the PcG protein Ezh2 and condensin molecules peak in cycling pre-B cells where the clonal expansion of the pre-B cells expressing successfully rearranged heavy chain take place. Thus the high level expression of these factors may relate to their effect on proliferation in addition to their impact on V kappa rearrangement.

Response

The reviewer is correct that high expression of PcG and condensin proteins correspond to the timing of the pre-B cell proliferative burst as well as to Ig kappa rearrangement. We have expanded upon this in the Discussion section of the revised manuscript as advised by the reviewer. The reviewer also suggests studies on cell proliferation in a YY1 delta REPO background. We have performed experiments to explore the growth and apoptotic properties of YY1 compared to YY1deltaREPO. Previously (Pan et al, 2012) we showed that overexpression of YY1 can reduce B cell growth and increase B cell apoptosis. We have performed additional experiments here (presented in supplementary figure S2) to compare the impacts of YY1 compared to YY1deltaREPO on cell growth and apoptosis. These experiments did not reveal any difference between YY1 and YY1deltaREPO. To completely rule out proliferation we will need to more thoroughly evaluate pro-B and pre-B cells isolated from YY1 and YY1ΔREPO reconstituted mice. As these studies require generation of additional mice and require many controls we believe they are very important but beyond the scope of the current manuscript. However, we have modified the discussion to include the possibility of defects in proliferation caused by YY1deltaREPO.

Minor Comments

1) Avoid using word like "Fortunately" while describing a mutant YY1 in first paragraph of result section.

Response

This word is now deleted.

2) Supplemental figure S1 should be introduced in the main figure as Figure 1A.
Response
This has been done according to the reviewer's suggestion.

3) The official gene name for BRNN1 should be used as in most of the publications it is described as BRRN1.
Response
BRRN1 is now used throughout the manuscript.

4) Provide a reference that describes the YY1 binding site as GCCATNTT (in the section explaining "Identification of YY1 binding sites across the Igk locus").
Response
A reference for the consensus binding site is now included.

We thank the reviewers for their careful analyses and comments on our manuscript. We believe the work we have done to address their comments has resulted in an improved manuscript. We hope that it is now acceptable for publication in EMBO J.

Acceptance Letter 11 February 2013

Thank you for submitting your revised manuscript to the EMBO Journal. Your manuscript has now been re-reviewed by the referees and as you can see below the referees appreciate the introduced changes. I am therefore very pleased to proceed with the acceptance of the paper for publication in The EMBO Journal.

A few remaining editorial points:

-) Please incorporate the materials and methods from the supplemental file into the main manuscript file.

-) We also now encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. Would you be willing to provide a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files. If you have any questions regarding this just contact me.

You can send us the modified MS and supplemental file plus source data to my email and we will upload it for you.

Please also see below for important information on how to proceed. Make sure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

Thank you for contributing to the EMBO Journal

REFEREE REPORTS

Referee #1

Re-Review of Pan et al

In the revised manuscript, Pan et al have made good-faith effort to address many points of concerns raised by the previous reviews. Specifically, in response to my comments they have done the following:

1. Proposed a model for the control of K gene rearrangements by YY1. The model is shown in a new Fig. 8B and some experimental evidence in support of the model is provided in a new Fig. 8A. The crux of the model is that Pax5 and E47 maintain YY1-independent loops that skew VK recombination in YY1-delta REPO background. I was not aware that Pax5 had been implicated in VK recombination; the authors should include a brief justification for the proposed roles of Pax5 and E47 in the K locus looping.

2. Added additional discussion of BL/6 and YY1 repertoire differences, as requested.

3. Made a serious effort to use different sets of VK primers to assay rearrangements in delta-REPO expression cells.

With these changes, the manuscript is suitable for publication in the EMBO J.

Referee #2

In the revised manuscript, the authors have adequately addressed most of my previous concerns. In particular, the authors provide higher quality data showing YY1 and condensin complex interactions (new figures 4B and 4C) and include new data with more convincing control lanes (Figure 7C). The authors also include the SDS-PAGE of proteins co-immunoprecipitated from GAL and GAL-REPO transfected cells, and they provide a list of identified proteins. Finally, the authors attempted ChIP studies in cells in which YY1 has been replaced with YY1dleta REPO. Unfortunately, the authors encountered technical problems. Given the comprehensive analysis and careful revisions, the manuscript is now ready for publication.

Referee #3

The revised manuscript is now suitable for publication.

