**Answers to Referee’s comments**  
Referee #1:  
  
Comment 1: The manuscript by Leeman-Neill et al. dissected the role of AID in lymphomagenesis, with a prestigious collection of paired FL to DHL transformation patient samples. From the WGS data, they found that the aberrant SHM events frequently occurred in the H3K27Ac/H3K4me3 marked regions, which is correlated with the intragenic super-enhancers. By careful examining of the mutated regions/genes, the authors, strikingly, identified an ‘enhancer retargeting’ mechanism in lymphoma transformation. In this context, solid evidence shows the increased ZCCHC7 expression in the PAX5-TSS2 mutated cells. The authors further delineated the downstream events of ZCCHC7 overexpression, and found that disturbed 5.8S rRNA processing could contribute to lymphomagenesis.  
  
The work clearly demonstrated a new form noncoding genomic mutation in cancer genome, involving a deaminase-mediated enhancer retargeting. Although the AID-dependent translocation is well documented in the literature, this new form of alteration and mechanism are novel. The functions of ZCCHC7 in lymphomagenesis is of great interest, as this gene immediately locates in the vicinity of PAX5 and its role(s) could be overlooked in the past. The uncovered functions of PAX5-ZCCHC7 pair could be applied to other gene pairs in other tumors. The logic of the manuscript is clear, although some of figures need to be cited in order. There are a few minor comments should be addressed.

Answer: We thank the referee for appreciating the importance of our observation that noncoding mutations occurring inside enhancer clusters during lymphoma progression and resulting in retargeting of superenhancer activity eventually leads to reworking of a noncoding RNA that alters protein synthesis. The focus on enhancer retargeting, a less appreciated mechanism of altered gene expression in lymphoma (or in cancer in general), was expertly recognized by the referee. The referee found our observations “striking” and “solid” and provided us with objective feedback and suggestions.

Indeed, retargeting enhancers to activate disease gene expression and promote pathophysiology can be wide spread in DHL where promoters located in enhancer clusters are hypermutated by AID. We have strengthened our analytic pipeline for enhancer retargeting and have identified more gene pairs (Extended Fig. 7). Moreover, as suggested by more than one referee, we directly show that a DHL-specific mutation, identified in our cohort, in the PAX5/ZCCHC7 cluster induce enhancer retargeting, causing gene expression change and inducing proteomic alterations, decreasing the expression of tumor suppressor genes associated with lymphoma progression (Fig. 4 and Extended Fig. 8). The pathophysiological mechanisms by which protein synthesis pathways are altered in lymphoma (or cancer, in general) has not been well appreciated or well investigated; our study opens up this field of research. We provide a proof of principle of enhancer retargeting during transformation to DHL and are quite confident that this mechanism is wide spread during lymphoma progression, especially due to aSHM occurring at many promoters embedded inside SEs.

**Comment 2:** The figures should be cited in order. Figure 2 and 3 should be switched.

**Answer:** We appreciate the referee’s insight on how to present our work coherently and we have indeed rearranged the figures, as suggested.

**Comment 3:** In the cohort, Patient 3 developed DHL and FL at the first time point, which may represent an early transformation or for other reasons. This particular case could be removed to avoid further confusion. In the same context, there are several cases involving both IgH-BCL2 and IgH-cMyc translocations. The authors should illustrate the IgH locus as well.

**Answer:** As indicated, we have now removed P3 from all analyses and figures. Additional evaluation of this P3 pair showed that there were two population of FL cells, with one progressing to DHL. It is likely that the P3 represent a proper sample pair and comparable to the others, but we decided to take it out to remove any confusion. In Fig. 1c, we present data for SHM in the IgHVDJ and IgH constant regions. Moreover, we provide in Extended Fig. 1, extensive data about the translocation properties of IgH region to BCL2 or MYC.

DONG (Important): . I THINK THAT THIS REFEREE IS ASKING US TO REPRODUCE FIGURE 1B BUT FOR IGH. WE HAVEN’T DONE THIS YET. (I’VE SHOWN THIS RESULT IN EXTENDED FIGURE 1C. PLEASE FIND IT.)

As for a detailed analyses of IgH-Bcl2 and IgH-Myc translocations, as described in Extended Fig. 1, the IgH-Bcl2 translocations contain insertions that are often associated with RAG-dependent and AID independent breaks, whereas the IgH-Myc translocations are AID mediated and do not have insertions (Extended Fig. 1d). These observations confirm the role of AID in promoting DHL following initial establishment of the IGH-BCL2. During the investigation of BCL2-IgH translocation break points, the presence of insertions via the activity of terminal deoxynucleotidyl transferase (TdT, encoded by *DNTT* gene). Recent reports have pointed out that TdT enzyme levels have been found to increased in DLBCL cells. We thank the referee for encouraging us to look at this in greater detail since we learned more about our sample pairs with respect to BCL2-IgH translocations..

**Comment 4:** *The authors should illustrate the IgH locus*”

Answer: We have plotted and illustrated somatic mutations (including both point mutation and structural variation) of IgH locus in Fig. 1c and extened figure 1c (as following).

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Dong STILL NEED TO FIX 1B, can you provide more presentation of IgH (heavy and light chain). There are two classes of light chain, IgL & IgK. This referee just asked for IgH (heavy chain). And we have shown this part on Fig. 1C and extended Fig. 1C.

**Comment 5:** The authors observed significant changes on chromatin interaction upon deletion of the PAX5-TSS2 region. Since the cases described in this manuscript were substitution rather than deletion. It would be more convincing to perform the 4C experiment in cell lines with equivalent hypermutated regions as in the DHL samples. In the same context, whether the SUDHL10 cell line already gain mutations at the indicated region? Alternatively, the authors could compare the chromatin interactions in cell lines of FL and DHL origin to address the question.

3.1 “*4C experiment in cell lines with equivalent hypermutated regions as in the DHL samples*”

**Answer:**

We thank the reviewer for suggesting these experiments. We have addressed this point using a cell line that does not have the mutation (SUDH10) and that has the mutation ((Toledo), We clearly found the PAX5/ZCCHC7 SE peak neighboring PAX5-TSS2 relocate its interaction to the ZCCHC7 promoter (Fig. X). We then took a cell line where we had initially shown enhancer retargeting via PAX5-TSS2 deletion, SUDH10. We now used CRISPR-Cas9 HDR technique to introduce two recurrent mutations (chr9:37026315 G->A and chr9:37026316 C->T) in the PAX5 first intron (that overlap the TSS2 region) and performed 4C-seq. We observed that the interaction of the adjacent H3K27Ac enhancer peak interacts with the neighboring ZCCHC7 promoter, consistent with what we have seen in TSS2-delta cells, or Toledo where cells where this region is naturally hypermutated. We thank the referee for these comments and we feel that these experiments provide further confidence regarding enhancer retargeting mechanism during progression of lymphoma from FL to DHL.

Comment 6: “*whether the SUDHL10 cell line already gain mutations at the indicated region*”

Answer: We found four DLBCL cell lines that have microindels or hypermutation (at least 5 point mutations within the 3kb hotspot region) in PAX5 TSS2, including two GCB-DLBCL cell line SU-DHL-6, SU-DHL-9, and two ABC-DLBCL cell line NU-DUL-1, OCI-LY-3.

GCB-DLBCL cell lines that are WT are: DB, OCI-LY-19, OCI-LY-1, OCI-LY-7, WSU-DLCL2.

Since SUDH10 WGS was not available, we performed sanger sequencing for CL-01, SU-DHL-6, SU-DHL-4, SU-DHL-10, Ramos, DB, and Toledo cell lines on the PAX5-TSS2 region. The Sanger sequencing results covered chr9:37,025,781-37,026,500 region and verified the SU-DHL-6 chr9:37025927 A->G mutation that observed in its WGS data. In Toledo cell line, we observed four point mutations chr9:37026169 G->A, chr9:37026316 C->T, chr9:37026417 G->C, and chr9:37026418 C->T. For CL-01, SU-DHL-4, SUDHL10, Ramos, and DB cell line, the Sanger sequencing present WT within the chr9:37,025,781-37,026,500 region.

**Comment 7”** *the authors could compare the chromatin interactions in cell lines of FL and DHL origin*”

**Answer:** There is no particular FL cell line that we could culture at this time. Please refer to Fig. 4 and Extended Figure 8 where we compare the chromatin interactions using 4C seq for the PAX5-TSS2 regions (both deletion and point mutation incorporated). As is presented in the data, the local chromatin landscape and interaction with the ZCCHC7 gene is significantly changed.

**Comment 8:** The authors showed data on the effect of ZCCHC7 overexpression in AID proficient and deficient B cells, which could add another layer on AID activity regulation. In the DHL cells, whether AID is expressed or the cell death is linked to lymphomagenesis? The authors should discuss the relevance of observed rRNA processing defect, AID-dependent cell death in the context of lymphoma transformation.

**Answer:** Available RNAseq data demonstrates some DHL cell lines have increased AID expression and/or ZCCHC7 expression. For 13 DHL cell lines, we found increased ZCCHC7 expression. As discussed later, and in Fig. 4, ZCCHC7 over expression can enormously alter overall protein synthesis in lymphoma cells. Thus, the effect of ZCCHC7 over expression on lymphoma cell biology is complex and the inhibition in cell proliferation may relate to a combination of deregulation of AID activity and changes in protein synthesis. We note that in non-neoplastic cells, rRNA processing defect activates the TP53 pathway and triggers apoptosis. AID and MYC deregulation in DHL may relate to the discrepant consequences of ZCCHC7 overexpression in non-DHL versus DHL cells. Understanding these dynamics might require substantial additional studies.

**Comment 9:** In Extended Fig 2C, logo plot could be separated into two strands, as the current panel.

**Answer:** We’ve separated those mutations into sense strand (for mutations on ‘C’ or ‘T’) and antisense strand (for mutations on ‘A’ or ‘G’) respectively, and then draw the logo plot. The new results were updated in figure 3d showing clear WRCY pattern.

**Comment 10:** Fig. 4C, the nucleoli are better to be indicated with a specific marker. The total fluorescence intensity was weaker in the SUDHL6 and SUDHL10 cells when compared to CL01. The relative enrichment of ZCCHC7 in the nucleoli needs further statistical analysis.

Answer: As indicated by the referee, we have now extensive staining experiments with SUDHL6 cell lines, where ZCCHC7 is significantly overexpressed and shown that it spills out of the nucleoli- with the nucleolus marked with different markers.

Comment 11: On page9, line 4, a “C” is missing in “PAX5-ZCCH7 locus” phrase. Extended Figure 4: label is unmatched. BCL2 “Extended Figure 4B, bottom panel” is Extended Figure 4C; PAX5 is Extended Figure 4D. Extended Figure 8C: label is unmatched. There are a few typos in the main text and legends.

Answer: Thank you, we have addressed this point.   
  
  
Referee #2 (Remarks to the Author):  
  
Comment 1: This study by Uttiya Basu and colleagues is focused on the progression of clinically indolent follicular lymphoma to aggressive diffuse large B-cell lymphoma (DLBCL), including DLBCL cases that acquired a MYC-gene rearrangement in addition to BCL2-IGH. From a clinical point of view, the FL to DLBCL transition marks a critical turning point, the underlying mechanisms of which are poorly understood. Hence, this study based on in-depth longitudinal analysis of nine FL patients and subsequent DLBCL transformation could be of very high significance. Based on whole genome sequencing, the authors demonstrate that the FL-DLBCL transition correlates with de novo aberrant somatic hypermutation (ASHM), which was previously described as an oncogenic driver mechanism in DLBCL but not other B-cell malignancies, including FL (Pasqualucci et al., Nature 2000).   
Answer: We thank the referee for reviewing our manuscript and considering the potential of the study to be of “high significance”. We appreciate the points raised and have attempted our best to address them within the limited time frame we have.   
  
Comment 2: The analysis confirms many known targets of ASHM and identified a few novel targets as well. Extending findings from a previous study (Arthur et al. Nat Comm 2018; reference 40), the authors show that some of the targets of ASHM are superenhancer (SE) regions, adding to the emerging concept that non-coding mutations in SE regions can function as critical oncogenic drivers. The study focused on one gene pair, PAX5 and ZCCH7C and demonstrate that SEs for PAX5 and ZCCH7C reside in topologically associated domains (TADs) and that SE mutation of one gene results in enhancer retargeting of the other. Also the concept of “enhancer release and retargeting” is not entirely novel and was previously exemplified for the PAX5-ZCCH7C gene pair (Oh et al., Nature 2021; reference 36). In addition, copy number alterations described here for PAX5-ZCCH7C were previously shown (Hilton et al., Blood 134: 1528–1532, 2019; Figure 1). Functional studies of PAX5-ZCCH7C focused on ZCCH7C, a known  
regulator of ribosomal biogenesis in the nucleus.

Answer: We thank the referee for highlighting the novelty of our study. As appreciated by this referee and the others, there is “high significance” of what we are conveying here- ie that mutations in promoters of genes alter the enhancer activity of SE and alter overall gene expression. We have used ZCCHC7-PAX5 gene pair for this purpose.

1. The referee raise the issue that the mutations identified in this study are not novel given previous studies by other groups (ref. 40, Hinton et al etc). SE associated mutations and DLBCL associated mutations have been identified by many labs including those in Cassellas, Alt, Dalla Favera, Pasqualucci, Schatz, Staudt, Morin, Scott, Pan Hammarstrom, Shipp- and many more. Many of these groups including Cassellas, Alt, our group and others have already studied and characterized the mechanisms that are used by AID to incorporate mutations in these sequences. The goal of our study is to follow the same patients who have advanced from FL to DHL and compare their genome alteration profile. This is potentially one of the first studies to actually do that. The prior identification of locus alteration in the ZCCHC7-PAX5 region, which has been reported by Hinton et al and which we referenced (ref. 40), is very reassuring to us. The cause, effect or importance of this alterations has not been investigated. Moreover, the previously-reported alteration was a translocation with *MYC*, whereas we are reporting a newly recognized recurrent copy number gain of PAX5/ZCCHC7 upon transformation to DHL, as well as ZCCHC7 mutations (SNV) and rearrangements. In summary, although evidence of ZCCHC7 targeting by AID has been reported in isolated contexts, we have identified novel recurrent alterations in this gene in the context of lymphoma transformation.
2. The referee raises concerns regarding the fact that Michael Rosenfeld et al. proposed enhancer retargeting. We again, referenced this study (ref. 36) and are not claiming to have discovered this biology. As a parallel, there studies published in Nature about role of Cohesin loop extrusion in IgH recombination but the loop extrusion biology was solved much earlier. Similarly, aSHM in oncogenes are being published to be important for lymphoma but these mutations were published previously. Thus, we humbly request that the referee consider that fact that we are newly revealing AID-mediated aSHM as a mechanism of enhancer retargeting. Given that SE have been found to be hypermutated in lymphomas by Cassellas, Alt, Basu, Dalla favera, Pan Hamarstrom, Morin and others, the many mechanisms by which SE SHM can affect gene expression during lymphoma progression becomes an important topic of investigation.

Comment 3: *The proposed connection between ZCCH7C-dependent nucleolar dysregulation and malignant progression towards DLBCL is exciting and would be novel, but the concept lacks rigorous functional validation and experimental support underpinning this hypothesis is weak …* “

Answer: In terms of PAX5/ZCCHC7 enhancer retargeting, taking cues from Referee 1 and 2’ suggestions we have now shown that in comparison to unmutated cells, mutated DHL line (Toledo) cells have enhancer retargeting, Moreoover, using CRISPR/Cas9 HDR approach we incorporated the mutation into cell lines that do not have mutations (SUDH10), we recreated the enhancer retargeting phenomenon. We show that in cell lines that over express ZCCHC7- there is change in protein expression levels including gain of expression of oncogenes DID WE SHOW THAT? and loss of expression of tumor suppressors. We hope that these experiments will address the referee’s concern about characterization of the enhancer retargeting and effect of ZCCHC7 on protein expression. We have also identified ZCCHC7 interacting proteins, thereby firmly establishing its interaction with RNA exosome, and shown that its localization in the nucleolus is altered in cell lines that overexpress ZCCHC7.

Comment 4: “…***more examples*** *and* ***unbiased*** *approaches would be needed to validate the concept …* “

Answer: Using RNA-seq with matched WGS and a pipeline that is used to identify enhancer retargeting, we identified more examples of enhancer retargetting genome-wide. The results are shown in Fig. X. The question regarding validating some of the new examples of enhancer retargeting is quite a lot of work and cannot be accomplished inside the limited time of revision.

Comment 5: “*central weaknesses of this study, including lack of conceptual novelty (references 36, 40 and Hilton et al. 2019) and lack of robust experimental support for the ‘nucleolus/ribosomal biogenesis’ hypothesis substantially decrease the influence that this work will have in the field*”

RE: We thank the referee for reading our paper. Humbly, we cannot agree with this statement, as discussed in points a and b addressing comment 2 from this referee. The study draws on novel observations from whole genome analyses of a unique longitudinal cohort to relate SE mutations with enhancer retargeting and altered protein synthesis.

Comment 6: end of abstract “therapeutic targets in B-NHL”. An interesting thought but further developed in the main manuscript.

Answer: In the revised manuscript, we demonstrate ZCCHC7 overexpression in many DHL and DLBCL tumors and demonstrate a relationship between expression and transformation, highlighting the possible use of ZCCHC7 expression as biomarker in lymphoma. We also show that ZCCHC7 over expression changes DHL protein expression, leading to increased expression of oncogenes and decreased expression of tumor suppressors. This observation raises the possibility that ZCCHC7 can be targeted for therapeutic purposes in lymphoma. Thus, a small molecule or a biomolecule that induced increased ZCCHC7 expression could induced AID expressing lymphoma killing. I THINK THAT THIS SUGGESTION IS VERY PROBLEMATIC—WE HAVE OBSERVED THAT OVEREXPRESSION OF ZCCHC7 IN AID-OVEREXPRESSING DHL CELLS DOES NOT CAUSE CELL DEATH AND ACTUALLY MIGHT PROMOTE GROWTH THROUGH DOWNREGULATION OF TUMOR SUPPRESSOR GENES. Out study sets a proof of principle for new drug discovery and use of ZCCHC7 expression as a biomarker for lymphoma transformation risk. The next step of generating a therapeutic compound is a long term goal. We have added a discussion of these opportunities for clinical translation of our findings in the discussion of the paper.

Comment 7: “Regulation of DNA-targeting by AID in models of lymphoma progression has not been investigated”. This is not correct, as multiple aspects (e.g. miR155) underlying aberrant recruitment and activation of AID in B-cell lymphomas have been studied by the Nussenzweig, Casellas, Alt and other laboratories.

Answer: We thank the referee for mentioning this and we have discussed some of these papers in the manuscript, albeit briefly due to limited space. In this regard, we have significantly contributed in these studies as well (ref.). However, despite this progress, AID recruitment to certain regions of the genome and the consequences of these mutations are still not clear. There is evidence that the presence of SE markers, sense/antisense RNAs, RNA polII stalling-pausing etc but these have not unequivocally explained AID targeting. Moreover, and more importantly, the effect of these mutations on B cell biology is only beginning to be understood. We have rewritten this part, athough morecould have been written but we have very restricted space. If desired, this topic can be discussed further in a supplemental discussion.

Comment 8: and throughout: suggest to use UniGene nomenclature “MYC” instead of “CMYC”.

Answer: We thank the referee, and we have done accordingly.

Comment 9: Contamination: what is the evidence suggesting this?

Answer: As suggested by referee 1, P3 has been removed to avoid confusion. The contamination mentioned previously related to microdissection of two lymphomas within the same tissue section.

Comment 10: , 6 of 9 DHL cases: How often are these in primary DLBCL (see Hilton et al., 2019)?

Answer: Two out of 20 DHL have MYC-ZCCHC7 translocation in Hilton et al., 2019. Three out 39 primary DLBCL have ZCCHC7 copy number gain in Morin et al., 2013. We also investigated ZCCHC7 mutations in cell lines. Among 23 DLBCL cell lines, SUDHL6, NUDHL1, and OCILY18 show ZCCHC7 CNV gain and ZCCHC7 overexpression. WE MIGHT NEED TO CLARIFY THE LYMPHOMA TYPES IN EACH OF THESE PAPERS, HILTON ET AL REFERS TO DHL-LIKE LYMPHOMAS. I THINK THE REFEREE IS ASKING ABOUT PRIMARY (DE NOVO) DLBCL.

Comment 11: microdeletions and microinsertions: These are described as phenomena, but their significance is not clear. A general drawback of the often descriptive nature of this manuscript.

Answer: We also observed lots of microdeletions and microinsertions in the PAX5-TSS2 hotspot (including three out of 9 DHL in this study, three out of 12 DLBCL cell line, and two out of 39 primary DLBCL tumor from Morin et al., 2013). These alterations may contribute more to the PAX5-ZCCHC7 enhancer retargeting. We included these examples in extended Fig. 6b. I DON’T THINK THIS ANSWER ADDRESSES THE CONCERN. CAN WE DELETE THE DATA ABOUT MICRODELETIONS AND INSERTIONS (EXT FIG 6a)? I DON’T THINK IT ADDS ANYTHING AND THAT IS THEIR COMPLAINT. Should we provide some implication (related to our PAX5-TSS2 deletion experiment? ) in discussion part?

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Comment 12: 5’ part of PAX5 and 3’ part of ZCCH7C: the two genes are oriented in opposite direction. Is antisense orientation relevant to the observed relationship between the two genes?

Answer: By investigating the expression correlation of 19676 gene pairs on 11 DLBCL cell line, we found little difference of spearman rho distribution of gene expression between identical orientation and reverse orientation. DO WE NEED TO PUT THIS IN THE PAPER?

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Comment 13: PAX5-ZCCH7C represents a frequent gene fusion in B-ALL. How does genomic amplification of this region, combined with SE-mutations in DLBCL related to PAX5-ZCCH7C gene-fusions in B-ALL?

Answer: Thank you for raising this issue. We have investigated the frequency of PAX5-ZCCHC7 fusions in B-ALL and associated expression of ZCCHC7 in the revised manuscript (and extended Fig.12).

Comment 14: Extended Data Figure 4C -related to 4D?

Answer: We have corrected this mistake.

Comment 15: effect of ZCCH7C-overexpression was not observed in AID-deficient B-cells. What is the reason for AID-dependency of these effects?

Answer; ZCCHC7 over expression significantly changes gene expression in DHL cells (Fig. X). This includes genes that affect transcription and secondary DNA structures. Not only does cell cycle progression and gene expression change in ZCCHC7 overexpressing cells, it is also possible that there is increase ssDNA in ZCCHC7 oe cells. Consistent with this observation, alteration of the RNA surveillance pathway or rRNA biogenesis pathway have been related with secondary DNA structure (non B DNA structure) stabilization. These are targets of AID and it is possible that AID hypermutates these regions and induce cellular apoptosis. AID over expression often induces cell death.

Comment 16: CL01 cells: these are EBV-transformed cells and it is not clear whether they are suitable as normal B-cell control.

RE: We have used CLO1 cells as controls since it is impossible to keep primary B cells alive for the experiments that we do here. We also study aSHM in DHL cells lines for CRISPR experiments, and we need a cell line control. CL01 are transformed but not due to alteration in AID (and SE SHM) biology. Thus, we considered this as a control. We also now use untransfected DHL cells as controls in the 4C (enhancer. Retargeting assay). Finally, we also controlled the rRNA processing experiment (for detecting pre-5.8SrRNA) using primary tonsil B cell RNA. We hope this will satisfy the concern raised by the referee.

Comment 17: Figure 4E: ZCCHC7 is a cofactor in the RNA exosome complex, which degrades internal space 2 on pre-rRNA. In addition, 5.8S + 40 pre-rRNA is converted to 5.8S by Exosc10. Therefore, the proposed mechanism appears to be that ZCCHC7 overexpression increases pre-rRNA processing up to the point of 5.8S + 40, where Exosc10 is unable to keep up with increased levels of this pre-rRNA, thus leading to its accumulation. Is this the proposed scenario by the authors? This should be clarified in the text. If this scenario, or another one, is proposed, it should be supported by further evidence. For instance, Exosc10 could be overexpressed in B-cell lymphoma cells to see if this would normalize 5.8S + 40 levels.

Answer: This is a good suggestion and we appreciate the referee for the input. Exosc10 is a very big protein (larger than 100 KDa) and it is almost impossible to introduce it in lymphoma cells. Thus, at this moment it is particularly difficult for us the do this experiment.

Minor points  
Comment 18: As a general observation, it can be difficult to follow the flow of the figures, as they are not presented in logical order in the text I THINK WE STILL NEED TO WORK ON THIS, ESPECIALLY THE EXTENDED FIGURES

Answer: Thank you. We have addressed this issue.

Commnet 19: Bottom of page 8 and top of page 9: “This is likely, as PAX5/ZCCHC7 gene promoters exist in the same TAD in mouse (Extended Figure 7) and humans (Extended Figure 4C)”. Extended Figure 4C shows the TAD for BCL2. Did the authors instead want to refer to Extended Figure 4D?

Answer: Thank you. We addressed the point.

Comment 20: Extended Figure 6B: more detail is needed regarding the bioinformatic approach used to predict zones of enhancer retargeting. It appears that gene pairs at hypermutation loci from the current study and from a prior study (labelled as “Nature2021” in the figure) have been analyzed to find negative correlation between gene expression, which was put in relationship with hypermutation at those regions. Has this method been used or validated before? Furthermore, it seems that the “Nature2021” study is not cited at the corresponding location in the text.

Answer: We have updated this part and put the results on extended figure 7. We have included the mentioned citations similarly as we have used it in other parts of the manuscript. I FEEL THAT WE SHOULD DECRIBE THIS IN METHODS. Will add this part in method.

Comment 21: Extended figure 8C: it is unclear why this experiment was performed, and what the implications are. Does it mean that increased expression of ZCCHC7 is beneficial to lymphoma cells up to a certain point, and more expression becomes detrimental? It is also unclear why the same experiment was performed in AID cells and what the implications of this finding are.

Answer: We are aware of the concern raised by referee here. We wanted to evaluate the effect of ZCCHC7 in survival of B Cells or on its effect on CSR. This is important since it does interact with RNA exosome and RNA exosome has been shown to affect CSR>Surprisingly we find ZCCHC7 is toxic to B cells when it is over expressed (and it is likely) it changes protein expression. Some cells that are able to survive (perhaps due to secondary mutations like Ig-Bcl2/Myc translocations) select themselves for progression to lymphoma. We would have removed this data, precisely due the confusion that is indicated by referee, but we also think this data promotes that fact that ZCCHC7 oe can be used as a therapeutic target for AID expressing lymphoma cells. AGAIN, I DON’T THINK WE SHOULD SUGGEST THIS: I THINK OUR DATA WOULD SUPPORT A THERAPEUTIC ROLE FOR ZCCHC7 INHIBITION AS ITS OVEREXPRESSION DOWNREGULATED TUMOR SUPPRESSOR GENES. MAYBE WE SHOULD TAKE THIS DATA OUT OF THE PAPER  
  
  
  
  
Referee #3 (Remarks to the Author):  
  
Comment 1: The study focuses on the effect of somatic hypermutation on superenhancer status and downstream consequences with regards to transformation of follicular lymphoma (FL) to double hit lymphoma (DHL), an aggressive subtype of non-Hodgkin B cell lymphoma.   
The authors provide a novel important finding on enhancer retargeting leading to activation of ZCCHC7 and defective processing of the 5.8S ribosomal RNA, component of the ribosome, that potential leads to aberrant protein translation. These findings are of interest and the authors need to further investigate a few of the study aspects to validate and solidify their findings.

Answer: We thank the referee for appreciating our study and considering the finding of interest. In response to the referees’ comments, we have now shown changes in protein synthesis in the ZCCHC7 overexpressing cells, elaborated on the enhancer retargeting phenomenon, and addressed other concerns of the referee.   
  
Comment 2: Are there fluctuations in the AID levels and activity in patients during the transition to DHL in the patients used compared to the rest of the FL patient cohort? Was this higher activity (and/or levels) present in the samples before the transition? Although previous studies have suggested aberrant AID activity in DH lymphomas, the authors should take advantage of the longitudinal sample sets and properly address this.  
Answer: This is a terrific suggestion from the referee and we would have really liked to address this question. Unfortunately, these samples were collected over long period of time and we currently do not have the corresponding RNAs or protein samples from these patients. We isolated DNA mostly from formalin fixed paraffin embedded tissue to generate the data we have here.

Comment 3: To what extent the mutations studied affect CTCF motifs and binding? As this insulator protein affects enhancer-promoter interactions and CTCF binding sites can be mutation hotspots in cancer it is important to address how the alterations in question affect CTCF binding. Additionally, motif analysis for binding of main B-cell related transcriptional activators in the mutated areas is needed. Is ZCCHC7 expression impacted by any common transcription factors and by changes in CTCF binding in the locus? Is ZCCHC7 expression sensitive to Bromodomain/BET inhibitors?

Answer: We evaluated TF binding sites due to aSHM during FL to DHL transformation and provide the data here (Fig. X).

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The mutations in the PAX5 locus did affect CTCF binding sites. Most of the aSHM sites in the genome that we were able to find hypermutated during FL-DHL transformation were not overlapping CTCF occupancy sites. Although it is very likely that these mutations alter local DNA topology and eventually affect loop extrusion and genome architecture- we did not find a direct evidence of such. It is still possible that CTCF binding or cohesin movement to induce loop extrusion is affected due to TF binding. We discuss these possibilities.

Comment 4: The location of the mutations with regards to TADs as well as the extent to which they affect TAD boundaries both warrant further investigation. Do the authors observe changes in TAD boundaries in their matched FL-DHL pairs?

Answer: We have provided detailed evaluation of the location of the mutations with respect to the TAD boundaries in extended Fig. 5g. We thanks the referee for encouraging us to do this. Loop extrusion has been proposed to be a potential mechanism for AID recruitment and most mutations were present in active TADs. However, the mutations did not overlap the boundary CTCF sites and were unlikely to disturb the TADs. It is possible that aSHM requires TAD integrity and loop extrusion. Careful molecular genetics of the aSHMs, with overlapping TAD analyses, will be needed to show the effect on TAD integrity. Of note, ZCCHC7 interacts with the RNA exosome that has been shown by us (ref.) and others (Ref) to be important for promoting cohesin binding and loop extrusion by controlling transcription termination of convergent genes (ref.) and/or removal of non-B DNA structures in the genome and allowing cohesin localization (Ref.).

Comment 5: ZCCHC7 controls nucleolus homeostasis and the processing of 5.8S rRNA. This is an exciting finding that requires further investigation. For instance, the exact mechanism of action of ZCCHC7 is not well characterized. Is the processing of other rRNAs unaffected?

RE: These are terrific points. We have now performed in depth analyses of ZCCHC7. First, using proteomic methods we show that it interacts with RNA exosome (a bonafide regulator of ribosome biogenesis), by imaging, we show its localization the nucleolus in B cells but that this localization is disturbed due to ZCCHC7 overexpression in lymphoma cells (ref.). We show that following ZCCHC7 over expression, lymphoma cells show an altered protein expression profile with suppression of tumor suppressor proteins. Finally, we show that the PAX5 aSHM indeed changes enhancer retargeting of PAX5SE and leads to ZCCHC7 overexpression not only via TSS2 deletion but also in cell lines that have PAX5 TSS associated mutations. Taken together, we have done indepth study of how ZCCHC7 can promote lymphomagenic protein synthesis and how enhancer retargeting (besides chromosomal amplification) can cause such outcomes.   
  
e) What is the impact of this observed aberrant 5.8S rRNA processing on translation? Do the authors observe changes in translation efficiency? Are specific proteins aberrantly produced? Could this promote the translation of MYC or other oncogenic targets? Polysome analysis and quantitative proteomics in combination with transcriptome analysis should be performed to properly address these questions.

Answer: We have now addressed this question through overexpression of ZCCHC7 in lymphoma cells. We clearly show that protein synthesis is altered, with a group of proteins that are overexpressed (including B Cell associated oncoproteins) and some tumor suppressors that are under expressed. This observation is not only visible at the loading of mRNAs on polysomes but also at the level of how proteins are made. We established a link between altered expression of ZCCHC7 and protein synthesis alterations of oncoproteins and tumor suppressors in lymphomas.  
  
Referee #4 (Remarks to the Author):  
  
Comment 1: The authors performed whole genome sequence of an interesting cohort of follicular lymphomas (FL) transformed into a particular form of very aggressive lymphoma carrying BCL2 and MYC rearrangements (Double hit lymphomas, DHL). In this study they identify a higher number of non-coding somatic hypermutations (SHM) that accumulate in promoter regions embedded in active superenhancers (SE) included in Topologically Associated Domains (TAD). The study identified many potential genes related to these non-coding mutations that are related to B-cell lymphomagenesis. One of them is PAX5/ZCCHC7 gene pair. The authors use CRISPR/Cas9 approach to show that mutations in the PAX5 regulatory regions may upregulate distant genes such as ZCCHC7 by enhancer re-targeting. Finally, they show that ZCCH7 is upregulated in aggressive lymphomas and its overexpression may interfere with rRNA processing.   
  
The study shows an interesting accumulation of non-coding mutations in promoters embedded in superenhancers in transformed aggressive FL and its potential influence in downstream dysregulation of distant genes. Although the concept is of interest, the data provided requires more precise information and confirmation.

Answer: We thank the referee for reading our manuscript and the feedback. The referee appreciated our proposed mechanism of how enhancer retargeting can explain how a few mutations associated with FL-DHL transformation can have large scale effects on gene expression. The referee has asked for more precise information and confirmation and we have provided changes to address the concerns.   
  
Comment 2:   
1. The study provides whole genome information of the transformation of follicular lymphoma (FL) to a particular form of aggressive high-grade lymphoma carrying BCL2 and MYC rearrangements, also called doble-hit lymphomas. The study is of interest, but I am afraid relevant information is missing or incomplete:  
  
a. I am afraid I do not find the data related to the sequencing results of all the cases included in the study. Am I missing it? This whole information must be provided. There are some findings in the mutations reported in Extended Figure 1 that would require a more specific analysis. E.g. the authors find mutations in SF3B1, that is uncommon in FL. Is the same hot spot than in CLL? Where is the stop mutation in CCND3? Although this information may be secondary for the main goals of the study the mutational catalogue, including the non-coding regions must be provided.

Answer: Referee has asked for more details from our sequencing results. We have expanded on our analyses and it is reflected in the main figures and also in the new Supplemental figures (Fig. X-Y). Specific answers to the referee’s queries are as follows.

a. Data related with this study is uploaded in XXXX.

1. The SF3B1 mutation is at a hotspot and it occurs in only one patient.
2. The stop mutation of CCND3 is at Q280\* and is observed in one case. This mutation has been reported previously in DLBCL (ref)
3. We will provide the whole table of the mutations from these patients. MISSING RAD51B AND PAX5-ZCCHC7 TRANSLOCATIONS

Comment 3: Mutations Extended Figure 1: TP53 and TNFRS14 mutations are frequently associated with deletions of the other allele. Is this not the case in this cohort?

Answer: The answer to this query is - Yes. P1 harbored one allele deletion and one allele mutation in TP53, while P8 harbored two TP53 mutations, with one additional TP53 mutation Y234H gained when progressed to DKL.

P9 harbored one allele deletion and one allele mutation in TNFRSF14, while P6 harbored two mutations in Q148\* and C53R. P7 only has one mutation C111Y but LOH was observed in chr1p.

In the updated Figure S1b, we have added these observations.  
  
COMMENT 4: The authors indicate that MYC translocation was detected at low allelic frequency by WGS in the diagnostic sample of FL in patients 3 and 4. They interpret that in case 3 this alteration may correspond to contamination from the transformed doble-hit lymphoma micro-dissected from the same specimen. It would be easy to perform FISH analysis for MYC translocation of the initial FL and transformed sample in these two patients (patients 3 and 4) to determine whether this translocation was already in the initial sample or was acquired in the transformed lymphoma and the percentage of tumor cells carrying this translocation in both samples.

Answer: Suggested by Referee 1, “Patient 3 developed DHL and FL at the first time point, which may represent an early transformation or for other reasons. This particular case could be removed to avoid further confusion”.

MYC FISH was performed for P3 and P4 and was negative in the FL from P3 (now removed from the study) and positive in the FL from P4, so that this case represents a follicular lymphoma with MYC rearrangement (which is occasionally observed). A summary and some examples of FISH performed on our cohort are provided in Fig. SX.

COMMENT 5: The analysis of aSHM in the different genes shown in Figure 2, Figure 3, Extended Figure 2 and 3 show that most of the mutations detected in the FL are not present in their respective DHL transformation. Similarly, some coding mutations shown in Extended figure 1b are present in the FL component but not in their corresponding transformed DHL (e.g EZH2, H2-1 and FAM102A in patient 5 and PIM1 and KMLD2 in patient 6). Given that the transformed DHL and the initial FL are clonally related, these findings would support that the transformed DHL would derive from a common progenitor cell by a divergent pathway rather than a linear evolution. These findings would support the idea that transformation in FL seems to derive from these common progenitor cells.

ANSWER: We agree with the referee that FL and DHL were derived from a common progenitor. Phylogenetic trees have been constructed and demonstrate divergent evolution (Fig X).

COMMENT 6: The analysis of the active regulatory regions and promoters in the FL and DHL is based on the Encode data of H3K27ac and H3K4me3 marks in SU-DHL-6 and OCI-LY-1 cell lines. The authors should also include the data of H3K4me1 to better delineate the enhancer regions. This may be relevant since one of the major findings of the study is the accumulation of aSHM predominantly in promoters included within SE regions but apparently not in enhancer areas. Therefore, to delineate more precisely these areas may highlight the findings.

ANSWER: we have included the ENCSR184QUS data to address this comment.  
  
COMMENT 7: Related to the previous point, although figure 3A is demonstrative showing the enrichment of aSHM in promoters included in H3K27ac areas, could the authors substantiate this conclusion with statistical analysis of the difference? Is there a difference of aSHM in promoters outside super-enhancers? It is not clear from the figure legend if the middle panel represent the mutations in “all” H3K4me3 or only these regions outside H3K27ac areas?

ANSWER: This analysis is already in hand. We will add two figures to illustrate this point. NEED STATISTICS?

COMMENT 8: In Extended Figure 5B, the comparison of aSHM in CLL and DLBCL of previous studies and DHL in this study show that the accumulation of these mutations occurs in a similar region in CLL and DLBCL but seems different from the region involved in DHL. Do the authors have any suggestion on this observation?

COMMENT 9: The sequencing depth of previous study was very low in the PAX5 TSS2 hotspot region. IT SEEMS LIKE WE NEED TO SAY MORE HERE—NOT ENOUGH TO JUST SUGGEST THAT THE OTHER STUDIES MISSED THESE MUTATIONS. Here is a possible answer: AID is expected to play a greater role in mutagenesis occurring in GC B cell type lymphomas (e.g. FL, DLBCL with GC phenotype, and DHL that harbor BCL2 translocation and orginate from FL) than non-GC type lymphomas (e.g. CLL and DLBCL with ABC phenotype). Cells with mutations affecting this locus might be selected in both GC and non-GC types of lymphomas with mutations occurring through different mechanisms and therefore at different sites. IT MIGHT BE HELPFUL TO KNOW IF THE MUTATIONS IN CLL ETC HAD AN AID SIGNATURE

Graphical user interface

Description automatically generated with low confidence

Chart, box and whisker chart

Description automatically generated

COMMENT 9: I am afraid Extended Figure 6a is not mentioned in the main text or extended information. The reference to Nature 2021 in Extended Figure 6b needs to be clarified. The reference is not in the figure legend or text. The reference for the published 47 DLBCL whole genomes used in this figure is not mentioned in the main text (page 9 line 2) or in this figure.

ANSWER: The figure and the reference are clarified. (Oh, Soohwan, et al. "Enhancer release and retargeting activates disease-susceptibility genes." *Nature* 595.7869 (2021): 735-740.)

*COMMENT 10: “The reference for the published 47 DLBCL whole genomes used in this figure is not mentioned in the main text (page 9 line 2) or in this figure”*

ANSWER: The 48 WGS data (39 DLBCL tumors from Morin, et al + 9 DHL tumors from this study. (Morin, Ryan D., et al. "Mutational and structural analysis of diffuse large B-cell lymphoma using whole-genome sequencing." *Blood, The Journal of the American Society of Hematology* 122.7 (2013): 1256-1265.)

COMMENT 11: I do not find the experiments described in Extended Figure 7 and last paragraph of page 9 very clear. Which type of mouse cells were used to generate the data of this figure? Which kind of “translocation capture sequencing experiments? Where are the DNA/RNA hybrid experiments described?

ANSWER: These are mouse primary B cells grown in culture in vitro via cytokine stimulation. The translocation capture used here is from the manuscript (ref.). The DNA/RNA hybrid assay was described in our previous study (Laffleur 2021) and the data is used here for this locus.   
  
COMMENT 12: The idea that mutations in the enhancer regions neighboring PAX5 interact with the ZCCHC7 promoter and increase the expression of ZCCHC7 shown in Extended Figure 6c is of interest and one of the major conclusions of the study. However, to which extent the reported mutations in this region lead to the overexpression of the gene in cell lines or primary tumors is not clear. Does the CRISPR/Cas9 experiment reproduce the mutations observed in the primary tumors? I understand from the description in page 9 line 5 that the target region is the same, but I am not sure it is reproducing the SNV found or is deleting a broader region. This is relevant because it seems that the idea that mutations in promoters can alter gene expression in neighboring genes is based only in this experiment.

Answer: The Crispr/cas9 experiment deleted a hotspot region. In new experiments we have performed the 4C experiment to evaluate enhancer targeting using (1) cell lines that have these PAX5-TSS2 mutations and (2) In cell lines were we have used CRISPR-HDR to intrduce these mutations. In both cases, we were able to recapitulate the enhancer retaregetting aspects mentioned in the manuscript. We thank the referee for encouraging us to do this experiment since it makes the results much better and gives us elevated confidence in out conclsuisons.

COMMENT 12: *“However, to which extent the reported mutations in this region lead to the overexpression of the gene in cell lines or primary tumors is not clear.”*

ANSWER: We have shown that in cell lines where the TSS2 harbors a mutation observed in our cohort, there is elevated expression of ZCCHC7 and decreased PAX5 expression.

COMMENT 13: Regarding the previous point, the immunohistochemical studies performed to demonstrate the expression of ZCCHC7 in DLBCL, DHL and pair samples of FL and their transformed DLBCL shown in Fig 4B, and Extended Figure 8A-B are of interest and confirm the relevance of the overexpression of this gene in aggressive lymphomas. However, the relationship between the overexpression of this gene and the presence of aSHM or copy number gains in the PAX5/ ZCCHC7 regions is not clear. I assume that the DHL shown in Extended Figure 8a, are the same whose genome has been analyzed in the current study. Comparing the expression score of these cases in Extended Figure 8a with the aSHM and copy number alteration shown in figure 3d, it seems that the cases with the highest protein expression (cases 4 and 8) have no or very few mutations in the highlighted PAX5 region. On the other hand, cases with the highest number of mutations in the regulatory regions and higher gain levels (case 1 and 2) have  
the lowest ZCCHC7 expression levels. Do the authors have any explanation? Are these findings really supporting the role of these aSHM retargeting the superenhancer and leading to the overexpression of the gene? Do the authors have any information on the copy number alterations in this region in the cases studied by immunohistochemistry to confirm the potential role of this type of alteration on the expression?

RE: We apologize for the confusion. The Fig4B and Ex Fig 8A-B staining is from a cohort separate from those that were sequenced for our study. The paired FL/DHL samples were unavailable for staining. However, we created TMAs containing unpaired DLBCL and DHL and also stained longitudinal FL/DLBCL (not DHL) pairs. This has been clarified in the revised manuscript. To further address this concern, we sequenced to DHL from our TMA that demonstrated the highest degree of ZCCHC7 expression. As described in the revised manuscript, one of these cases harbored a ZCCHC7-MYC translocation. The other harbored PAX5 mutations (AT THE TSS? I DON’T THINK THE SECOND PATIENT IS DISCUSSED IN THE CURRENT VERSION).

Minor  
1. References of prior genomic studies of transformation of FL in the introduction are missing the relevant studies by e.g Kridel et al PLoS Med 2016 Dec 13;13(12):e1002197 and reference 34 that must be quoted also here. Reference 27 is old (1993) and it is not related to “genomic” studies since only reports a single gene (TP53). The relationship between high-risk follicular lymphoma and AID induced somatic mutations was also shown in the study by Tsukamoto T et al. Sci Rep. 2017 Oct 25;7(1):14039 that the authors may consider quoting in the introduction.

RE:   
  
2. Extended figure 1B in page 5, last paragraph line 5, probably should be Extended figure 1D. In brackets the authors say “intronic sequences”, but the figure legend says intronic or promoter mutations.

RE:  
  
3. Figure 2c lower panel: The annotation of the different patients is missing. This is missing also in several figures.   
RE:

4. H-score used to evaluate the immunohistochemical studies shown in Extended figure 8 is only described in figure legend b when it is already used in panel a. Perhaps it is sufficient with the description in the methods sections  
RE:

5. Terminology of genes needs to be revised and homogenized according to current standards. For instance, they use MYC, c-MYC, CMYC, C-MYC, Pax5 and PAX5, Bcl6 and BCL6, with and without italics throughout the main text, figure legends and Extended information.   
RE:

6. The authors use the terminology of DHL cell lines in the experiments described in page 10. This is a little confusing because they are also using this terminology for the transformed FL to DHL. In fact, the cell lines used are SU-DHL-6 and 10, not just DHL cell lines. I would suggest to use the proper term of the cell lines.

RE: