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Epigenome profiling and editing of neocortical progenitor cells during development

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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.)

1st Editorial Decision 21 March 2017

Thank you for submitting your resource manuscript for consideration by the EMBO Journal. It has now been seen by three referees and their comments are shown below.

As you will see from the reports, our three referees all express interest in the data set presented in your manuscript but they also raise a number of technical and conceptual concerns that you will have to address before they can recommend publication here. In particular, ref #1 finds that additional replicas of the genome-wide ChIPseq experiments are necessary for the manuscript to reach the level of conclusiveness expected of a resource paper. At the same time ref #3 suggests the inclusion of additional histone marks in the analysis. I realize that these are demanding experiments but at the same time I agree with the referees that a resource paper rides on the conclusiveness and breadth of the data it presents. While you may not be able to include both additional histone marks and more replicas I would be interested in discussing what type of additional experimental data you could include in a revised manuscript.
In addition to these underlying concerns about the experimental setup, the referees point to a number of minor issues regarding description, interpretation and analysis of the existing data that should be possible to address.

Given the referees' overall positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

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

revision.

REFEREE REPORTS

Referee #1:

The article by Albert and colleagues presents the epigenomic profiles for 5 different neural cell types. Different cortical cellular populations were isolated at specific time points during development and neurogenesis and the profiles for two histone methylation modifications, H3K4me3 and H3K27me3 (associated with active transcription and repression, respectively), were determined using a ChIP-seq protocol for small samples. In addition, in the last part of the manuscript, the authors used CRISPR/dCas9 technology to manipulate the H3K27 methylation status of the Eomes locus. This manipulation
resulted in reduced Tbr2 expression and progenitor abundance.

I found the study novel and potentially interesting. The progress in understanding the role of epigenetic mechanisms in neuronal differentiation and function, particularly in a heterogeneous tissue like the mammalian brain, requires the use of cell type-specific information. In that sense, I agree with the authors in the value as a resource of the datasets contributed by their study. The epigenetic editing experiment at the Eomes locus is also interesting and adds significant value to the study. Few studies in the nervous system have still achieved this kind of functional insight using epi-editing techniques. Unfortunately, the study also presents some important weaknesses that need to be corrected.

Main criticisms:
1. If I am not mistaken, the authors only obtained replicates for two conditions: input and NECs. All other conclusions are based on a single sample per cell type and histone mark, which is a serious concern. Although this was not uncommon in seminal papers using ChIPseq, current standards recommend including at least replicates. This is particularly critical in a study like this completely based on the quality and novelty of the genomic profiles. The confidence in the annotation of bivalent genes and cell type-specific peaks would be much higher if the authors obtain and analyse at least a replicate per condition (this would be a minimum requirement). Increasing the "n" of the experiment would also allow a better statistical treatment of the data (for example, by running one-way ANOVA of all the samples for each mark and performing post hoc analyses to identify cell type-specific peaks with a confidence and reliability much higher than in the current situation).
2. The authors produced genomic profiles for two important histone PTMs, H3K4me3 and H3K27me3, in 5 populations of mouse neocortex cells (including different types of progenitor cells and mature neurons), but they did not produce the corresponding transcription profiles. The only transcriptome data generated in the context of this study is in NECs (they also compared with neuron and RG data generated in the context of a previous study), which greatly limits the "resource" value of the study and constrains the interpretation of epigenome data.
3. The description of supplementary material files is very poor. The excel files should contain a header or legend that clearly explain their content. Surprisingly, none of the excel files contains any numerical value or statistic associated with gene names. Therefore, the usefulness and reliability of these gene lists is very limited. Particularly, considering that the authors argue that this study has a "Resource" value.

Other criticisms:
4. The thresholds for peak detection and classification should be better explained (in fact, they should likely be modified by applying more rigorous statistical criteria once the authors obtain duplicates). Significance cut-off was based on p-values or FDR corrected p-values? What particular reference was used to call TSSs and other gene features? etc.
5. I did not find information on how the authors performed the Gene ontology analyses that constitute a very significant part of their study. What is "enrichment score"? Is it a direct measure of enrichment on terms of fold change or is it related to the p-value of the enrichment.
6. The authors often over-interpret the results of the GO analysis. For example, in page 9 the authors indicate "genes with these shared broad domains were characterized by general GO terms like..."

when the analysis just indicates that among the genes with broad domain there are more genes than expected by chance related to these GO terms. Statements such as this or the section header "Broad H3K4me3 domains mark neural regulators" should be based on concrete numbers and percentages. For example: What percentage of "broad domain" genes belongs to the "neurogenesis" category? How many genes are responsible for the modest enrichment in this category? Some conclusions should be rephrased to reflect more accurately the data.
7. In general, the explanation of thresholds and filtering criteria to generate the gene list lack essential information to interpret the result. This is a consistent weakness through the manuscript. For example:
• Figure 2, 3 and others present lists of "selected" genes. How were these genes selected? Why were they selected and what is the significance of these gene lists?
• What percentage is presented in Figure 3D? Percentage of total number of H3K4me3 peaks or only those in the proximity of an annotated gene? Percentage of H3K4me3 peaks in which cell type?
• Figure 3: was domain breadth corrected by gene length or intron content? These factors are likely to contribute to peak breadth regardless of gene function.
• Figure 5A presents a line plot for 50 genes presenting "dynamic" changes in H3K27me3. The authors report the identification of thousands of H3K27me3 peaks. How were these 50 genes selected? Did the authors use any statistical criteria? Figure 5C also refers to "selected" genes. Again, what means "selected"? According to which (statistical) criteria were these genes selected? Etc.
8. Figure 7G should include data corresponding to the catalytic dead control (dCas9-Ezh2*).
9. The authors suggested that bivalent genes across the analysis are poised for activation during later stages of neuronal maturation. However, whether the two "opposing" histone marks providing the "bivalent" status, occur in the same cell or in the same nucleosome is unknown. The authors should consider this when discussing their results.
Minor:
10. Panels D and E of Figure EV4 are confusing. A heatmap would show the same information in a more visual and compact manner.
11. The authors could discuss more the importance of the fine regulation of the epigenome by enzymes like Ezh2 and Mll2 in neurodevelopmental disorders.
12. The manuscript contains some typos:
• Abstract: The last sentence seems incomplete. The authors likely meant "histone methylation data" or "histone methylation profiles".
• Page 10: "which is line"; "strongest enrichment"
• Page 11: "populatiosn"
• Page 38: "number such genes".

Referee #2:

In this manuscript, Albert et al. describe the genome-wide analysis of H3K4me3 and H3K27me3 deposition in distinct neural progenitor cell (NPC) subtypes in the developing mouse neocortex. They not only provide a comprehensive and useful resource regarding histone methylation patterns in NPCs, but also make important discoveries concerning epigenetic regulation of NPC fate. They found that genes with both H3K4me3 and H3K27me3 marks in E14.5 neurons tend to increase their expression levels by P1, indicating that such "bivalent" genes are indeed poised for activation in the context of neuronal maturation. They also identified genes with a broad H3K4me3 domain (breath) at the TSS in each NPC subtype, again providing a rich resource for future studies on genes related to cell identity. Interestingly, they detected dynamic changes in H3K27me3 profiles that appear to facilitate cell fate transitions. Namely, they describe (1) two major transitions in H3K27me3 patterns in which genes that lose H3K27me3 are expressed at higher levels globally (E9.5 NECs to E14.5 aRG-P, and E14.5 aRG-N to E14.5 bRG), and (2) transient loss of H3K27me3 in aRG-N at key neural genes such as Eomes. In addition to these valuable observations, Albert et al. went on to examine the causal relation among H3K27me3 deposition, repression of gene expression, and cell fate transition through artificial recruitment of Ezh2 to the Eomes locus with the dCas9 system. It is impressive that they succeeded in suppressing Eomes (Tbr2) expression as well as in reducing basal cell division in vivo by such epigenome editing.

Overall, the study is well executed and the manuscript is well written. The results contribute to a better understanding of epigenetic regulation of NPCs and are of great value to the field of neural

development. I have no particular criticisms of this manuscript.

Referee #3:

In the manuscript "Epigenome profiling and editing of neocortical progenitor cell during development", Albert and colleagues present a data resource with ChIP-Seq data for H3K4me3 and H3K27me3 for specific populations of embryonic neural progenitors. The authors provide evidence of the role of resolution of bivalency and loss of H3K27me3 as important events in the regulation of transcriptional states of these progenitors. The conclusions are not entirely novel, given that similar studies have been perform in neural progenitors and neurons derived from ES cells (Mikkelsen et al. 207, Mohn et al. 2008, but also Burney at el., Stem Cells 2013). Moreover, the study would benefit from the inclusion of other histone marks that are deposited in enhancers (H3K27ac, for instance), at repressed genes (H3K9me3), RNA Polymerase II, or H3K27me3 methyltransferases (Ezh2) or demethylases. Nevertheless, the study does provide unique H3K4me3/H3K27me3 datasets in specific in vivo neural progenitors, which will be undoubtfully useful resources for the scientific community. It is also not trivial to perform ChIP-Seq with such low numbers of cells, which strengths the resource. The authors also confirm the role of H3K27me3 deposition, using elegant experiments with dCas9 where Ezh2 is recruited specifically to the Eomes regulatory region, deposits H3K27me3 and represses expression of Tbr2. Overall, I find the study interesting and I deem it suitable for publication in EMBO Journal. Nevertheless, the following points should be taken in consideration:

1) The authors present an excel file as supplementary table with a list of genes where the histone marks are present. This is not satisfactory for a resource. At least the exact location of the peaks should be given and ideally BED files that the readers can upload into genome browsers and directly explore the dataset. The authors mention a GEO accession number (GSE90694) in the methods in which raw data and bigwig files are provided, but I could not find it in GEO or access it.

2) The authors present RNA-Seq bar plots in supplementary figures. It would be better to add genome browser screenshots of the RNA-Seq data also in the main figures, so the reader can do a direct comparison between the ChIP-Seq data and the RNA-Seq data.

3) In page 7, the authors refer that in general in the neural lineage, H3K27me3 marks genes critical to the development of other organs. The authors do no mention glial lineages (astrocytes and oligodendrocytes), which one would expect to be repressed within the neuronal lineage, what is their profile?

4) Figure 2:

a. Figure 2b - It is not clear why the authors use for analysis previously identified bivalent genes present in ESCs (2491), rather than NEC bivalent genes (4986)? Would the category of genes where the resolution of bivalency occurs be different if NEC bivalent genes are used?
b. Figure 2c - Are all GO categories presented statistical significant? Otherwise, it would be better to present in the x-axis the P-value (or another measure of significance) and superimpose the number of genes per category.

5) The findings of the H3K4m3 broad domains are interesting, but could be explored in more detail. In the original publication (Benayoun et l., 2014), these domains were associated with transcriptional consistency. Since the authors have performed RNA-Seq, they should also investigate whether this is the case in their datasets.

6) Figure 4

a. The use of PCA is quite informative. It would be good also to include a PCA analysis of the cell populations referring to bivalency. Would such analysis confirm the dramatic resolving of bivalency between NEC and aRG-P (Figure 2)?
b. What is driving the differences observed in PC2 in Fig. 4a and 4b, which have a similar scale of variance as PC1?
c. As mentioned before, it would be good to complement Figs. 4D,E,G,H with genome browser

screenshots of the RNA-Seq data instead in the main figures, so the reader can do a direct comparison between the ChIP-Seq data and the RNA-Seq data

7) Figure 5

a. Page 12, second paragraph - The paragraph is not very clear. Were the authors looking for transient changes in general and found the transient decrease between aRG-P and aRG-N, or did were they specifically looking for this transient decrease, as the text implies?
b. Page 12, third paragraph - for how many genes the transient decrease in H3K27me3 translated in increased expression levels?

8) Editing of H3K27me3

a. Fig. EV7 - It is not clear how should one interpret the agarose gel. The authors should add where the PCR primers were designed and the expected size of the PCR products

b. Fig. 7C and D
i. Judging from Figure 7C, there are several cells that have Cas9 (RFP+) that do not have Tbr2 in gLacZ-dCas9-Ezh2 (and also in gEomes dCas9-Ezh2*). However, in the quantification in Figure 7D, the % of Tbr2+ dCas9+/dCas9+ is around 100%. How was the quantification performed, was it restricted to Tbr2+ regions? This needs to be clarified in the text and figures.
ii. The efficiency of gEomes dCas9-Ezh2 transfection seems lower than gLacZ-dCas9-Ezh2 and gEomes dCas9-Ezh2*. Was this the case? It would be good that the authors present as supplementary figure that counts for RFP and Tbr2 separate, so the reader can better access the functional effects of Ezh2 recruitment. The same applies to Figure 7F, the authors should present in supplementary the number of mitoses without normalizing to control.

c. Fig. 7G - The ChIP-qPCR experiments indicate that H3K27me3 is indeed increased upon recruiting of H3K27me3. However:
i. Does the mutated Ezh2 affect H3K27me3?
ii. The experiments presented should not have statistics, since the error bars are from technical replicates (which should be stated clearly in the figure legend, not only in the methods). I understand that the baseline variability of ChIP-qPCR experiments makes it sometimes difficult to do averages between independent experiments. Nevertheless, presenting representative experiments is not ideal, and the reader can be mislead to think that the error bars reflect independent experiments. At least, the authors should present in supplementary the data of all the replicates performed (a similar situation happens with the qRT-PCR data in Figure EV1b, the average data of all the biological replicates should be presented, instead of a representative experiment)

9) Methods:

a. ChIP-Seq analysis:
i. When peaks are called using MACS, the authors refer that H3K4me3 are called using "default" parameters, while H3K27me3 with the "broad" option. The authors should explain why these settings were choosen.
ii. When dynamic changes of H3K27me3 at E14.5 was measured, the authors used raw reads instead of reads belonging to the peaks. The authors should explain why these settings were choosen.

b. ChIP-qPCR and qRT-PCR: which method of quantification was used, standard curve or delta-delta-Ct? In the latter case, was the efficiency of the primers tested?

c. qRT-PCR: Is GAPDH an appropriate housekeeping gene for normalization? Is its expression non-variable between samples? This should be at least stated.

10) The paper has many abbreviations (in particular of the neural progenitors states), I would recommend not to use them and spell out the names of the different neural progenitors throughout the text. This would make the text easier to follow by the readers not familiar with the neural progenitors specific abbreviations.

1st Revision - authors' response 23 May 2017

Response to Reviewers – Overview of Revision

Item	Panel	Contents	Reviewer(s)
Fig 1	**NEW** Panel C	RNA-seq data added	# 3
Fig 2	Panel C, D	Fold enrichment, number of genes per category and p-values added	# 1, 3
Fig 3	**NEW** Panel C	RNA-seq data added	# 3
	Panel D	previous panel C; Fold enrichment, number of genes per category and p-values added	# 1, 3
	Panel E	previous panel D	
	NEW Panel F	Analysis of expression variance added	# 3
Fig 4	**NEW** Panel D, G	RNA-seq data added	# 3
	Panel E	previous panel D, E	
	Panel H	previous panel G, H	
Fig 5	Panel A	Filtering criteria described in detail	# 1, 3
	Panel C	Statistical significance test added	# 1, 3
	Panel D	Fold enrichment, number of genes per category and p-values added	# 1, 3
Fig 7	Panel F	Absolute numbers presented	# 3
	Panel G	Data corresponding to g*Eomes* dCas9-Ezh2* added; data presented as fold change relative to control; error bars changed to represent biological replicates	# 1, 3
Fig S1		RNA-seq data moved to Fig 1C; additional biological replicate added for RT-qPCR	# 3
Fig S2	Panel B	moved to NEW Fig S3	
NEW Fig S3		previous Fig EV2B; genome browser tracks for replicates of all samples presented	# 1
Fig S4		previous Fig EV3	
Fig S5	Panel A, B	previous Fig EV4B, C; Fold enrichment, number of genes per category and p-values added	# 1, 3
NEW Fig S6		ChIP-seq and RNA-seq data of glial genes presented	# 3
Fig S7	NEW Panel C	previous Fig EV5; RNA-seq data added	# 3
Fig S8		previous Fig EV6	
Fig S9	NEW Panel B	Scheme of PCR template sizes and gRNA locations added	# 3

NEW Panel D	Quantification of individual IUE experiments (n=7) without setting control to 100% added	# 3
NEW Table EV2	List of H3K4me3 peaks with chromosomal locations and q-values added	# 1, 3
NEW Table EV3	List of H3K27me3 peaks with chromosomal locations and q-values added	# 1, 3
Table EV4	previous Table EV2	
Table EV5	previous Table EV3	
Table EV6	previous Table EV4	
Table EV7	previous Table EV5; qPCR primer efficiencies added	# 3

Response to Reviewers

Reviewer #1

Reviewer's Comment:
The article by Albert and colleagues presents the epigenomic profiles for 5 different neural cell types. Different cortical cellular populations were isolated at specific time points during development and neurogenesis and the profiles for two histone methylation modifications, H3K4me3 and H3K27me3 (associated with active transcription and repression, respectively), were determined using a ChIP-seq protocol for small samples. In addition, in the last part of the manuscript, the authors used CRISPR/dCas9 technology to manipulate the H3K27 methylation status of the Eomes locus. This manipulation resulted in reduced Tbr2 expression and progenitor abundance.

I found the study novel and potentially interesting. The progress in understanding the role of epigenetic mechanisms in neuronal differentiation and function, particularly in a heterogeneous tissue like the mammalian brain, requires the use of cell type-specific information. In that sense, I agree with the authors in the value as a resource of the datasets contributed by their study. The epigenetic editing experiment at the Eomes locus is also interesting and adds significant value to the study. Few studies in the nervous system have still achieved this kind of functional insight using epi-editing techniques.

Authors' Response:
We thank the reviewer for his/her positive comments and for appreciating the novelty of our study. In particular, we are grateful that the reviewer acknowledges the "*value as a resource*" and appreciates that "*few studies in the nervous system have still achieved this kind of functional insight using epi-editing techniques"*.

Reviewer's Comment:
Unfortunately, the study also presents some important weaknesses that need to be corrected.
Main criticisms:
1. If I am not mistaken, the authors only obtained replicates for two conditions: input and NECs. All other conclusions are based on a single sample per cell type and histone mark, which is a serious concern. Although this was not uncommon in seminal papers using ChIPseq, current standards recommend including at least replicates. This is particularly critical in a study like this completely based on the quality and novelty of the genomic profiles. The confidence in the annotation of bivalent genes and cell type-specific peaks would be much higher if the authors obtain and analyse at least a replicate per condition (this would be a minimum requirement). Increasing the "n" of the experiment would also allow a better statistical treatment of the data (for example, by running one-way ANOVA of all the samples for each mark and performing post hoc analyses to identify cell type-specific peaks with a confidence and reliability much higher than in the current situation).

Authors' Response:
There appears to be a misunderstanding here. In contrary to the reviewer's impression, the data presented in this study are based on two replicates per sample, and thus follows the recommendations of the ENCODE consortium presented in the "*ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia*" (Landt *et al*., *Genome Res*, 2012). The misunderstanding about the number of replicates likely arose from the example replicate ChIP-seq tracks presented in the previous Figure EV2B, which only included NEC and Input. We apologize for this misleading presentation and have now included example ChIP-seq tracks for H3K4me3 and H3K27me3 for all cell types in the new Figure S3. In addition, we have added the information about replicates to the main text (see p. 7). The replicates represent biological replicates of batches of 50,000 cells sorted from different pools of embryos for all samples, except for H3K27me3 of aRG-N where technical replicates are shown. The raw data for all replicates have been deposited at the GEO database and are available under the accession number GSE90694.

To detect significant peaks, we have used the software package MACS2, which is a commonly used tool and which is among the peak calling softwares listed in the "*ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia*" (Landt *et al*., *Genome Res*, 2012). For replicate samples, we proceeded as recommended in the MACS manual (see https://github.com/taoliu/MACS): "*For the experiment with several replicates, it is recommended to concatenate several ChIP-seq treatment files into a single file.*" Therefore, we have pooled replicates before calling peaks with MACS2. This is now stated in the method section (p. 23). The cutoff to call significant regions was based on a Q-value of 0.05 for both modifications. The information about Q-values for each gene is now available in the new Tables EV2 (H3K4me3) and EV3 (H3K27me3). We hope that by adding this additional information about the replicates, we can increase this reviewer's confidence in the quality of our data.

Reviewer's Comment:
2. The authors produced genomic profiles for two important histone PTMs, H3K4me3 and H3K27me3, in 5 populations of mouse neocortex cells (including different types of progenitor cells and mature neurons), but they did not produce the corresponding transcription profiles. The only transcriptome data generated in the context of this study is in NECs (they also compared with neuron and RG data generated in the context of a previous study), which greatly limits the "resource" value of the study and constrains the interpretation of epigenome data.

Authors' Response:
The reviewer correctly points out that we have generated genomic profiles for two histone methylation states in five neocortical cell populations in this study. As to the reviewer's comment "*but they did not produce the corresponding transcription profiles"*, it should be noted that we have previously generated transcriptome data for four of the populations using the same purification strategy used here, so the cell populations are analogous and the data sets directly comparable. These four data sets have been published in *Science* in 2015 (Florio *et al*.; please note that the first, second and last author of the previous study are co-authors on the present manuscript) and have been deposited in the GEO database for download (GSE65000). Since these four transcriptome data sets are already available, we saw no reason to repeat the analysis here. For one of the cell types included in the current study (NEC), the transcriptome had not previously been analyzed. We have therefore performed this analysis as part of the current study, using the same method for RNA-seq and data analysis as described in our previous paper to facilitate comparison of the data sets. Taken together, we provide a complete resource, including histone methylation data for H3K4me3 and H3K27me3 (this study) and transcriptome data for the five neocortical cell populations (previous study, supplemented with NEC data in this study) to support the interpretation of epigenome data.

Reviewer's Comment:
3. The description of supplementary material files is very poor. The excel files should contain a header or legend that clearly explain their content. Surprisingly, none of the excel files contains any numerical value or statistic associated with gene names. Therefore, the usefulness and reliability of these gene lists is very limited. Particularly, considering that the authors argue that this study has a "Resource" value.

Authors' Response:
The legends of the supplementary tables have already been provided at the end of the manuscript text document. However, we have now provided additional details in the table legends to make their contents even more clear. In addition, we now provide detailed information for all peaks called within each data set, including chromosomal locations of peaks, reference to nearby genes and statistical values (see new Tables EV2 and EV3). We thank the reviewer for pointing this out and hope that the additional information will increase the resource value of our study. Moreover, the raw data and bigwig files for upload into genome browsers are available under GEO accession number (GSE90694). In addition, we have now also included the bed files with peak locations under the same GEO entry.

Reviewer's Comment:
Other criticisms:
4. The thresholds for peak detection and classification should be better explained (in fact, they should likely be modified by applying more rigorous statistical criteria once the authors obtain duplicates). Significance cut-off was based on p-values or FDR corrected p-values? What particular reference was used to call TSSs and other gene features? etc.

Authors' Response:
To detect significant peaks, we have used the software package MACS2 (see response to point 1). The cutoff to call significant regions was based on a Q-value of 0.05 for both modifications. Q-values were calculated from p-values using the Benjamini-Hochberg procedure. The Q-values are now included in the new Tables EV2 and EV3. Annotation of significant peaks to genomic features including TSSs was performed with ChIPpeakAnno R/Bioconductor (Zhu et al., 2010) using Ensembl genes v67 as reference. This additional information is now included in the methods section of the manuscript.

Reviewer's Comment:
5. I did not find information on how the authors performed the Gene ontology analyses that constitute a very significant part of their study. What is "enrichment score"? Is it a direct measure of enrichment on terms of fold change or is it related to the p-value of the enrichment.

Authors' Response:
The gene ontology analysis was performed using the DAVID tool, which employs functional annotation clustering and provides a group enrichment score (the geometric mean (in –log scale) of member's p-values in a corresponding annotation cluster). We have used this method as it reduces redundancy among the functional annotations. Since both this reviewer and reviewer 3 seem to prefer the traditional display of fold change or p-value, we have re-run the GO term analysis using the PANTHER tool which provides all of these values. We have used the 'PANTHER GO-slim' for 'biological process' option which is based on a selected set of terms from the Gene Ontology TM and also reduces redundancy. Due to different subsets of GO terms used in each program, the individual terms have changed slightly, but support the same conclusions. We now present the data as 'Fold enrichment' over expected (see revised Figures 2C/D, 3D, 5D and S5A/B). In addition, we provide the number of genes from the tested list over the number of genes in the reference gene set (numbers within the bars). The individual p-values are now also shown next to each bar.

Reviewer's Comment:
6. The authors often over-interpret the results of the GO analysis. For example, in page 9 the authors indicate "genes with these shared broad domains were characterized by general GO terms like..." when the analysis just indicates that among the genes with broad domain there are more genes than expected by chance related to these GO terms. Statements such as this or the section header "Broad H3K4me3 domains mark neural regulators" should be based on concrete numbers and percentages. For example: What percentage of "broad domain" genes belongs to the "neurogenesis" category? How many genes are responsible for the modest enrichment in this category? Some conclusions should be rephrased to reflect more accurately the data.

Authors' Response:
As requested by this reviewer, we have rephrased the results section pertaining to the GO term enrichment analysis to now read "genes showed enrichment for the GO term categories" instead of "were characterized by" (see p. 10, 11 and 13). We hope that the reviewer will find this phrasing

more accurate. In addition, the GO term analysis now includes concrete numbers, fold changes and p-values. Regarding the section header *"Broad H3K4me3 domains mark neural regulators"*, we would like to respectfully point out that this conclusion is based not only on the GO term analysis presented in Figure 3D. Figure 3E shows that the genes with the broadest H3K4me3 domains are enriched for 'NPC regulators' and even more significantly for 'neuron differentiation' genes (with a –log10(p-value) of >30), whereas gene lists related to other organs or cell types, like 'ESC regulators', 'muscle' and 'spermatogenesis' were not strongly enriched. Moreover, 7 of the 11 known induced neural stem cell reprogramming factors (Figure S7B; Table EV6) were found to be marked by broad H3K4me3 domains in the developing neocortex. We have, however, re-phrased the section header to "*Genes with broad H3K4me3 domains are enriched for neural cell type regulators*".

Reviewer's Comment:
7. In general, the explanation of thresholds and filtering criteria to generate the gene list lack essential information to interpret the result. This is a consistent weakness through the manuscript. For example:
Figure 2, 3 and others present lists of "selected" genes. How were these genes selected? Why were they selected and what is the significance of these gene lists?

Authors' Response:
The presented lists of "selected" genes in Figures 2C/D and 3A were (and are) meant to give some examples of genes that are present in the described categories. Apparently the choice of the word "selected" was misleading. We have replaced it with "example genes" to make clear that these gene lists are just meant to provide the names of a few well-known interesting genes that may be meaningful to the reader.

Reviewer's Comment:
What percentage is presented in Figure 3D? Percentage of total number of H3K4me3 peaks or only those in the proximity of an annotated gene? Percentage of H3K4me3 peaks in which cell type?

Authors' Response:
In Figure 3E (previous Figure 3D), the H3K4me3 peaks were binned into 5% quantiles based on their width, with the top 5% broadest H3K4me3 peaks represented in the last bin (95%). For the H3K4me3 breadth analysis, only the peaks overlapping the TSS +/–2kb were taken into account (see legend to Figure 3A). The cell type is indicated above each of the five squares.

Reviewer's Comment:
Figure 3: was domain breadth corrected by gene length or intron content? These factors are likely to contribute to peak breadth regardless of gene function.

Authors' Response:
We respectfully disagree with the reviewer on this point. The promotor or gene structure has previously been shown to not affect the H3K4me3 domain breadth (see original publication by Benayoun *et al*. in *Cell* 2014, Figure S1). Specifically, broader H3K4me3 domains were not found to mark gene cluster regions nor to correlate with gene length or the number of used TSS (alternative splicing). In light of these results, we have not corrected the H3K4me3 domain breadth for any of these gene features.

Reviewer's Comment:
Figure 5A presents a line plot for 50 genes presenting "dynamic" changes in H3K27me3. The authors report the identification of thousands of H3K27me3 peaks. How were these 50 genes selected? Did the authors use any statistical criteria? Figure 5C also refers to "selected" genes. Again, what means "selected"? According to which (statistical) criteria were these genes selected? Etc.

Authors' Response:
The "dynamic" genes were chosen based on several criteria, which we have now explained in the results section (see p. 12/13) and in the methods section (see p. 24/25). The aim was to identify genes that are marked by H3K27me3 in the closely related E14.5 cell population but undergo a transient decrease in H3K27me3, potentially involved in subpopulation-specific gene expression.

Firstly, we have filtered for genes that show a significant H3K27me3 peak (called by MACS2, significance cutoff was based on a Q-value of 0.05) in proliferative progenitors (aRG-P) and in neurons (N). To compare the differences in H3K27me3 more quantitatively, not just with regard to the presence/absence of a significant peak, we have calculated an enrichment score for each gene (based on a previously described method (see Pataskar *et al*., *EMBO* 2016)). Secondly, using this score we have filtered for genes with an enrichment >0.5 in aRG-P and N to select genes with high levels of H3K27me3. Lastly, we have filtered for genes that show a >1.5fold change from proliferative to neurogenic aRG (aRG-P vs aRG-N). Using these criteria, we ended up with 50 genes which are shown in Figure 5A. These genes were further analyzed for significant gene expression changes ($p < 0.01$) between aRG-P and aRG-N. We apologize for the word "selected" here, which was misleading. Figure 5C shows all genes with dynamic H3K27me3 (Figure 5A) that undergo concomitant differences in gene expression (6 of the 50 genes). We hope that the revised result and method section presents the data more clearly.

Reviewer's Comment:
8. Figure 7G should include data corresponding to the catalytic dead control (dCas9-Ezh2).*

Authors' Response:
To address the reviewer's point, we have performed an additional set of IUE experiments (n=4) that now include three conditions: g*LacZ* dCas9-Ezh2; g*Eomes* dCas9-Ezh2 and g*Eomes* dCas9-Ezh2*. The data are presented in revised Figure 7G. The ChIP-qPCR data show that H3K27me3 levels are increased at the *Eomes* locus following g*Eomes* dCas9-Ezh2 IUE compared to g*LacZ* dCas9-Ezh2 control, but not upon IUE of the catalytically dead control (g*Eomes* dCas9-Ezh2*).

Reviewer's Comment:
9. The authors suggested that bivalent genes across the analysis are poised for activation during later stages of neuronal maturation. However, whether the two "opposing" histone marks providing the "bivalent" status, occur in the same cell or in the same nucleosome is unknown. The authors should consider this when discussing their results.

Authors' Response:
We have considered this point in our discussion and have added one additional remark (see p. 17/18): "*As ChIP-seq is currently performed on cell populations rather than single cells, the existence of true bivalent domains at single alleles has been questioned. Using different strategies, the presence of bivalent domains has recently been confirmed at individual alleles of key regulatory genes in different cell types (Kinkley et al., 2016, Lorzadeh et al., 2016, Weiner et al., 2016). Although we cannot rule out the presence of H3K4me3 and H3K27me3 in different cells of the population, we have isolated different NPC subpopulations to minimize cellular heterogeneity. We find that a substantial number of genes is marked by bivalent modifications in cortical NPCs and, more strikingly, also in neurons of the developing neocortex.*" To the best of our knowledge, the H3K4me3 and H3K27me3 marks do not need to be present on the same nucleosome for a gene to be considered bivalent. The above cited papers, however, do establish that these modifications do indeed occur at individual alleles (with the ChIP-fragments potentially carrying multiple nucleosomes).

Reviewer's Comment:
10. Panels D and E of Figure EV4 are confusing. A heatmap would show the same information in a more visual and compact manner.

Authors' Response:
In these two panels (previous Figure EV4D/E, now Figure S5D/E), the expression of neuron bivalent genes is explored in previously published expression data sets of different pyramidal neuron subtypes at two developmental stages (Molyneaux *et al*., *Neuron* 2015). The authors have made their data available on the following resource platform: The Developing Cortical Neuron Transcriptome Resource (DeCoN: http://decon.fas.harvard.edu/). They have chosen to display the gene expression data as dot plots. In respecting the authors' choice, we would prefer to stick with this way of presenting the data, and hope that the reviewer will agree. Moreover, in contrast to a heat map, each dot conveys information about the magnitude of expression (size of the dot) and neuron sub-type specificity (color code). This information is provided in the legend of Figure S5D/E.

Reviewer's Comment:
11. The authors could discuss more the importance of the fine regulation of the epigenome by enzymes like Ezh2 and Mll2 in neurodevelopmental disorders.

Authors' Response:
We have followed the reviewer's suggestion and included a sentence in the discussion of the manuscript: *"The importance of the regulation of the epigenome by enzymes modifying H3K27me3 (like EZH2) and H3K4me3 (like MLL2, UTX and SETD1A) is also highlighted by mutations in the related genes in human patients with neurodevelopmental disorders (Mastrototaro et al., 2017)."*

Reviewer's Comment:
12. The manuscript contains some typos:
• Abstract: The last sentence seems incomplete. The authors likely meant "histone methylation data" or "histone methylation profiles".
• Page 10: "which is line"; "strongest enrichment"
• Page 11: "populatiosn"
• Page 38: "number such genes".

Authors' Response:
We thank the reviewer for pointing out these typos. They have been corrected in the revised version.

Response to Reviewers

Reviewer #2

Reviewer's Comment:
In this manuscript, Albert et al. describe the genome-wide analysis of H3K4me3 and H3K27me3 deposition in distinct neural progenitor cell (NPC) subtypes in the developing mouse neocortex. They not only provide a comprehensive and useful resource regarding histone methylation patterns in NPCs, but also make important discoveries concerning epigenetic regulation of NPC fate. They found that genes with both H3K4me3 and H3K27me3 marks in E14.5 neurons tend to increase their expression levels by P1, indicating that such "bivalent" genes are indeed poised for activation in the context of neuronal maturation. They also identified genes with a broad H3K4me3 domain (breath) at the TSS in each NPC subtype, again providing a rich resource for future studies on genes related to cell identity. Interestingly, they detected dynamic changes in H3K27me3 profiles that appear to facilitate cell fate transitions. Namely, they describe (1) two major transitions in H3K27me3 patterns in which genes that lose H3K27me3 are expressed at higher levels globally (E9.5 NECs to E14.5 aRG-P, and E14.5 aRG-N to E14.5 bRG), and (2) transient loss of H3K27me3 in aRG-N at key neural genes such as Eomes. In addition to these valuable observations, Albert et al. went on to examine the causal relation among H3K27me3 deposition, repression of gene expression, and cell fate transition through artificial recruitment of Ezh2 to the Eomes locus with the dCas9 system. It is impressive that they succeeded in suppressing Eomes (Tbr2) expression as well as in reducing basal cell division in vivo by such epigenome editing.

Overall, the study is well executed and the manuscript is well written. The results contribute to a better understanding of epigenetic regulation of NPCs and are of great value to the field of neural development. I have no particular criticisms of this manuscript.

Authors' Response:
We would like to thank the reviewer for his/her positive evaluation and his/her strong support of the manuscript.

Response to Reviewers

Reviewer #3

Reviewer's Comment:
In the manuscript "Epigenome profiling and editing of neocortical progenitor cell during development", Albert and colleagues present a data resource with ChIP-Seq data for H3K4me3 and H3K27me3 for specific populations of embryonic neural progenitors. The authors provide evidence of the role of resolution of bivalency and loss of H3K27me3 as important events in the regulation of transcriptional states of these progenitors. The conclusions are not entirely novel, given that similar

studies have been perform in neural progenitors and neurons derived from ES cells (Mikkelsen et al. 207, Mohn et al. 2008, but also Burney at el., Stem Cells 2013). Moreover, the study would benefit from the inclusion of other histone marks that are deposited in enhancers (H3K27ac, for instance), at repressed genes (H3K9me3), RNA Polymerase II, or H3K27me3 methyltransferases (Ezh2) or demethylases. Nevertheless, the study does provide unique H3K4me3/H3K27me3 datasets in specific in vivo neural progenitors, which will be undoubtfully useful resources for the scientific community. It is also not trivial to perform ChIP-Seq with such low numbers of cells, which strengths the resource. The authors also confirm the role of H3K27me3 deposition, using elegant experiments with dCas9 where Ezh2 is recruited specifically to the Eomes regulatory region, deposits H3K27me3 and represses expression of Tbr2. Overall, I find the study interesting and I deem it suitable for publication in EMBO Journal.

Authors' Response:
We thank the reviewer for his/her positive feedback on our manuscript and his/her appreciation of the resource value of our "*unique H3K4me3/H3K27me3 datasets in specific in vivo neural progenitors* […] *for the scientific community"*. Even though, the reviewer finds that "*the conclusions are not entirely novel, given that similar studies have been perform in neural progenitors and neurons derived from ES cells"*, we would like to emphasize that our study analyses different *in vivo* neural progenitor subtypes from the developing neocortex most of which are not present in the *in vitro* cultures. Moreover, the reviewer suggests that "*the study would benefit"* from the inclusion of additional histone marks, histone modifying enzymes and RNAPII. While certainly interesting, we do feel that this goes beyond the scope of the present manuscript. As the reviewer correctly points out, "*it is also not trivial to perform ChIP-Seq with such low numbers of cells*" and the ChIP method would need to be optimized individually for each of the suggested targets. It may not even be feasible for the chromatin-associated proteins, which tend to have lower ChIP efficiencies than histone modifications. Moreover, even though the number of cells used for ChIP here is low compared to conventional ChIP studies, it is still high in terms of progenitor numbers that one can collect with sufficient homogeneity from the complex tissue of the developing mouse neocortex. We hope that the reviewer will find the H3K4me3 and H3K27me3 data sets sufficient, especially since we provide *in vivo* functional insights for the H3K27me3 modification. We apologize for neglecting the reference to Burney *at el*., *Stem Cells* 2013, which is now included in the revised version of the manuscript (p. 8/9).

Reviewer's Comment:
Nevertheless, the following points should be taken in consideration:
1) The authors present an excel file as supplementary table with a list of genes where the histone marks are present. This is not satisfactory for a resource. At least the exact location of the peaks should be given and ideally BED files that the readers can upload into genome browsers and directly explore the dataset. The authors mention a GEO accession number (GSE90694) in the methods in which raw data and bigwig files are provided, but I could not find it in GEO or access it.

Authors' Response:
We agree with the reviewer that a resource should include the requested information. We therefore now provide new tables (Tables EV2 and EV3) listing H3K4me3 and H3K27me3 peaks with chromosomal locations, peak width, q-values and reference to nearby genes. Moreover, we have included the BED files into the GEO entry (GSE90694) for download. The GEO is currently private and will be released for public viewing upon acceptance of the manuscript. Please contact the editor for reviewer access to the GEO entry. We hope that these additional files will facilitate exploration of our datasets.

Reviewer's Comment:
2) The authors present RNA-Seq bar plots in supplementary figures. It would be better to add genome browser screenshots of the RNA-Seq data also in the main figures, so the reader can do a direct comparison between the ChIP-Seq data and the RNA-Seq data.

Authors' Response:
We thank the reviewer for his/her suggestion to include the RNA-seq data in the main figures along with the ChIP-seq data. We agree that this is useful to allow direct comparison. However, we feel that genome browser screenshots of the 2kb window around the TSS are not the best choice for representation of the RNA-seq data. Firstly, especially for longer genes, the region around the TSS

is not always representative of full-length transcript levels due to the described 3' bias of RNA-seq data using polyA tail-based methods. Secondly, depending on the lengths and location of the first exon(s), the small window around the TSS may not be very informative. Instead we have included the bar plots from the previous Figure S1 into the main Figure 1C. In addition, we have included bar plots of RNA-seq data along with all other genome browser views of ChIP-seq data (see new Figures 3C, 4D, 4G, S6B and S7C).

Reviewer's Comment:
3) In page 7, the authors refer that in general in the neural lineage, H3K27me3 marks genes critical to the development of other organs. The authors do no mention glial lineages (astrocytes and oligodendrocytes), which one would expect to be repressed within the neuronal lineage, what is their profile?

Authors' Response:
Glial genes do not show up in our GO term analysis. We have therefore looked at the histone methylation profiles of several genes that represent known glial markers (Rowitch & Kriegstein, *Nature* 2010) or were identified to be specifically expressed in glial cells by transcriptome analysis (Cahoy *et al*., *J Neurosci* 2008). We found that several genes expressed in the oligodendrocytic lineage are marked by H3K27me3 in our neural cell populations. In contrast, genes characteristic for astrocytes do not carry H3K27me3 (see new Figure S6), which is in agreement with previous reports indicating that several astrocyte-specific genes are regulated by DNA methylation in NPCs (Hatada *et al*., *PlosOne* 2008). This is now stated in the revised manuscript (see p. 8).

Reviewer's Comment:
4) Figure 2:
a. Figure 2b - It is not clear why the authors use for analysis previously identified bivalent genes present in ESCs (2491), rather than NEC bivalent genes (4986)? Would the category of genes where the resolution of bivalency occurs be different if NEC bivalent genes are used?

Authors' Response:
We have used ESC bivalent genes, as they are thought to encode TFs of developmental importance (Bernstein *et al*., *Cell* 2006; Mikkelsen *et al*., *Nature* 2007; Azuara *et al*., *Nat Cell Biol* 2006 and many others). Moreover, the resolution of genes that are bivalent in ESCs to an H3K4me3-positive state in ESC-derived differentiated cells has been previously exploited to identify novel regulators in cell lineages of interest (Lien *et al*., *Stem Cell* 2011). But in fact, the enriched categories would not change much if NEC bivalent genes had been used. The top five categories for NEC-bivalent genes resolved to H3K27me3 are 'skeletal system development', 'muscle organ development', 'mesoderm development', 'ectoderm development' and 'nervous system development', which is very similar to the GO terms reported for H3K27me3 in Figure 2C (right panel). Moreover, the top five categories for NEC-bivalent genes resolved to H3K4me3 are 'synaptic transmission', 'locomotion', heart development', 'transmembrane signaling' and 'nervous system development', which is also overlapping with the GO terms reported for H3K4me3 in Figure 2C (right panel).

Reviewer's Comment:
b. Figure 2c - Are all GO categories presented statistical significant? Otherwise, it would be better to present in the x-axis the P-value (or another measure of significance) and superimpose the number of genes per category.

Authors' Response:
All the presented GO categories are statistically significant. The gene ontology analysis was performed using the DAVID tool, which employs functional annotation clustering and provides a group enrichment score (the geometric mean (in –log scale) of member's p-values in a corresponding annotation cluster), which was plotted on the x-axis. We have, however, re-run the GO analyses using a different tool (see response to reviewer 1, point 5) and now present the data as 'Fold enrichment' over expected (see revised Figures 2C/D, 3D, 5D and S5A/B). In addition, we provide the number of genes from the tested list over the number of genes in the reference gene set (numbers within the bars). The individual p-values are now also shown next to each bar.

Reviewer's Comment:
5) The findings of the H3K4m3 broad domains are interesting, but could be explored in more detail.

In the original publication (Benayoun et l., 2014), these domains were associated with transcriptional consistency. Since the authors have performed RNA-Seq, they should also investigate whether this is the case in their datasets.

Authors' Response:
As suggested by the reviewer, we have analyzed transcriptional consistency for the genes with broad H3K4me3 domains in the neocortex. Indeed, the genes with the top 5% broadest H3K4me3 domains show significantly reduced variance in gene expression compared to the remaining genes in all five neural cell populations ($p < 2.2e{-}16$ for all cell types; see new Figure 3F, the legend for Figure 3F (p. 40) and the results section (p. 11)). Thus, our data support the original finding by Benayoun *et al*., *Cell* 2014.

Reviewer's Comment:
6) Figure 4
a. The use of PCA is quite informative. It would be good also to include a PCA analysis of the cell populations referring to bivalency. Would such analysis confirm the dramatic resolving of bivalency between NEC and aRG-P (Figure 2)?

Authors' Response:
The PCA analysis for H3K4me3 and H3K27me3 was performed with the spearman correlation coefficients obtained from the bamcorrelate program (Ramirez et al., 2014) using stats::prcomp in R. The tool works on BAM files that contain read-related information and is based on the comparison of read coverages within defined genomic regions (bins). In contrast, bivalency is defined by the presence of both, H3K4me3 and H3K27me3. In our study, all genes that have a significant H3K4me3 or H3K27me3 peak associated (called using MACS2) were considered to be bivalent, resulting in a binary file with bivalency displayed as either "1" (present) or "0" (absent) lacking a quantitative measure (see Table EV1). Similar presentation of data was used in many key publications related to bivalency (for example in Mikkelsen *et al*., *Nature* 2007; Meissner *et al*., *Nature* 2008, Mohn *et al*., *Mol Cell* 2008, Bilodeau *et al*., *Genes Dev* 2009). As PCA is trying to capture the total variance in the set of variables, it does not work on a binary data set. We are not aware of any tool that would allow to "quantify" the degree of bivalency. However, the dramatic resolving of bivalency from NEC to aRG-P can be seen in several analyses: 1) in the absolute number of bivalent genes (Figure 2A), 2) the percentage of H3K27me3-positive genes (Figure S5C), and 3) among ESC bivalent genes (Figure 2B), suggesting that bivalent domains are indeed resolved with progression of neocortical development from E9.5 to E14.5. This is in line with the proposed role of these domains (Voigt *et al*., *Genes Dev* 2013).

Reviewer's Comment:
b. What is driving the differences observed in PC2 in Fig. 4a and 4b, which have a similar scale of variance as PC1?

Authors' Response:
As explained above, the PCA was performed using the bamcorrelate program, which is based on read coverage distribution within defined bins covering the entire genome. Thus, the observed differences cannot be easily attributed to concrete genes or pathways. The aim of the PCA was to visualize the genome-wide data set in a 2D space.

Reviewer's Comment:
c. As mentioned before, it would be good to complement Figs. 4D,E,G,H with genome browser screenshots of the RNA-Seq data instead in the main figures, so the reader can do a direct comparison between the ChIP-Seq data and the RNA-Seq data.

Authors' Response:
As mentioned above (see Authors' Response to point 2), we prefer to present the RNA-seq data as bar plots instead of genome browser screen shots. The ChIP-seq data in Figure 4C and 4F as well as for all other genome browser views of ChIP-seq data are now complemented with RNA-seq data, which we hope will aid direct comparisons. The box plots in previous Figures 4D, E, G, H (now Figure 4E/H) represent mRNA expression of several hundred to thousand genes (see figure legend). We do not think that it is feasible to present RNA-seq data for all of these genes individually.

Reviewer's Comment:
7) Figure 5
a. Page 12, second paragraph - The paragraph is not very clear. Were the authors looking for transient changes in general and found the transient decrease between aRG-P and aRG-N, or did were they specifically looking for this transient decrease, as the text implies?

Authors' Response:
We have specifically looked for genes that show a transient decrease in H3K27me3 in the closely related neural cell populations as such decrease may potentially be related to a subpopulation-specific induction of gene expression. This is now stated more clearly in the result section.

Reviewer's Comment:
b. Page 12, third paragraph - for how many genes the transient decrease in H3K27me3 translated in increased expression levels?

Authors' Response:
The transient decrease in H3K27me3 is accompanied by significant changes in gene expression ($p<0.01$) for 6 of the 50 genes. This is also stated more clearly in the results section now (p. 13).

Reviewer's Comment:
8) Editing of H3K27me3
a. Fig. EV7 - It is not clear how should one interpret the agarose gel. The authors should add where the PCR primers were designed and the expected size of the PCR products.

Authors' Response:
As requested by the reviewer, to facilitate interpretation of the agarose gel, we have now included a scheme in the new Figure S9B that shows the sizes of PCR products, location of *Eomes* guide RNA binding sides and expected sizes of cut fragments.

Reviewer's Comment:
b. Fig. 7C and D
i. Judging from Figure 7C, there are several cells that have Cas9 (RFP+) that do not have Tbr2 in gLacZ-dCas9-Ezh2 (and also in gEomes dCas9-Ezh2). However, in the quantification in Figure 7D, the % of Tbr2+ dCas9+/dCas9+ is around 100%. How was the quantification performed, was it restricted to Tbr2+ regions? This needs to be clarified in the text and figures.*

Authors' Response:
As stated in the figure legend, the "*control was set to 100%*", which means that the number of Tbr2+ dCas9+ cells in the control was set to 100%. All ratios are presented as percent of control as indicated on the y-axis of the graph (Figure 7D). To show the ratio of Tbr2+ dCas9+ / dCas9+ without setting the control to 100%, we have now included an additional graph in the supplementary section (see new Figure S9D). This graph shows that roughly half of the dCas9+ (RFP+) cells express Tbr2 in the control, which is consistent with previous publications (see for example Arai *et al*., *Nat Comm* 2011; Kalebic *et al*., *EMBO Rep* 2016). Please also see our response to the next comment for additional information about quantification of IUE data.

Reviewer's Comment:
ii. The efficiency of gEomes dCas9-Ezh2 transfection seems lower than gLacZ-dCas9-Ezh2 and gEomes dCas9-Ezh2. Was this the case? It would be good that the authors present as supplementary figure that counts for RFP and Tbr2 separate, so the reader can better access the functional effects of Ezh2 recruitment. The same applies to Figure 7F, the authors should present in supplementary the number of mitoses without normalizing to control.*

Authors' Response:
The constructs encoding the guide RNAs and dCas9-Ezh2(*) were delivered into the brain of developing mouse embryos by in utero electroporation (IUE). As the method involves manual placement of the injection needle as well as manual placement of the electrodes for electroporation, the method is inherently prone to some variability. However, the experiments were performed by a highly-experienced scientist to ensure reproducibility of results. Great care was taken to only include embryos for experimental evaluation in which comparable areas of the neocortex were

electroporated. IUE efficiency varies greatly among different size plasmids; however, for all conditions compared here, the plasmids were identical in size. Moreover, for all of the IUE experiments the number of independent biological experiments (i.e. independent IUE experiments) was large (n=4-7 for Figure 7C/D; n=9 for Figure 7E/F; n=4 for Figure 7G) and in many cases each experiment included more than one embryo, which together is expected to average out the noise introduced by the IUE technique. Finally, the quantification was performed as fraction of electroporated (PaprikaRFP+) cells, both in control and experimental conditions, and thus is not affected by IUE efficiency. This is also the rationale for presenting the data in Figure 7D as ratio of Tbr2+ dCas9+ / dCas9+. We do, however, now provide a new supplementary figure (Figure S9D) showing the individual experiments without setting the control to 100% but still as ratio of Tbr2+ dCas9+ / dCas9+. Regarding Figure 7F, we have changed the graph in the main figure to present absolute numbers (dCas9+ mitoses/microscopic field) instead of percentage of control as requested by the reviewer. In this case, we have performed additional replicates (now n=9 independent IUEs), which were of comparable efficiencies, and therefore the absolute numbers should not be affected by individual variations in IUE. Moreover, we have confirmed that the results are reproduced when normalized to RFP+ cells.

Reviewer's Comment:
c. Fig. 7G - The ChIP-qPCR experiments indicate that H3K27me3 is indeed increased upon recruiting of H3K27me3. However:
i. Does the mutated Ezh2 affect H3K27me3?

Authors' Response:
To address this question, we have performed additional IUE experiments now including three conditions: g*LacZ* dCas9-Ezh2; g*Eomes* dCas9-Ezh2 and g*Eomes* catalytically dead dCas9-Ezh2*. The data are presented in the revised Figure 7G. The ChIP-qPCR data show that H3K27me3 levels are increased at the *Eomes* locus following g*Eomes* dCas9-Ezh2 IUE compared to g*LacZ* dCas9-Ezh2control, but not upon IUE of g*Eomes* dCas9-Ezh2*.

Reviewer's Comment:
ii. The experiments presented should not have statistics, since the error bars are from technical replicates (which should be stated clearly in the figure legend, not only in the methods). I understand that the baseline variability of ChIP-qPCR experiments makes it sometimes difficult to do averages between independent experiments. Nevertheless, presenting representative experiments is not ideal, and the reader can be mislead to think that the error bars reflect independent experiments. At least, the authors should present in supplementary the data of all the replicates performed (a similar situation happens with the qRT-PCR data in Figure EV1b, the average data of all the biological replicates should be presented, instead of a representative experiment).

Authors' Response:
We have now performed additional independent sets of IUE experiments to increase the number of biological replicates for the ChIP-qPCR data. As the reviewer notes, the variability of qPCR data makes it difficult to perform comparisons between independent experiments. We have therefore presented the data as fold change relative to respective controls in the revised Figure 7G. The error bars now represent SD of biological replicates (n=4 independent IUE experiments). Significance was calculated using a paired Student's t-test. Regarding the RT-qPCR data presented in the previous Figure EV1B, we have now included a second biological replicate (see revised Figure S1). In addition, gene expression data for the same genes obtained by RNA-seq are shown in the new Figure 1C, which is presented as average +/– SD of four to five biological replicates. Thus, the gene expression data were verified by two different methods analyzing a total of at least six biological replicates.

Reviewer's Comment:
9) Methods:
a. ChIP-Seq analysis:
i. When peaks are called using MACS, the authors refer that H3K4me3 are called using "default" parameters, while H3K27me3 with the "broad" option. The authors should explain why these settings were choosen.

Authors' Response:

The two types of histone modifications studied here have very different peak profiles. While H3K4me3 is enriched at highly expressed genes with the known bimodal peak shape around the TSS, H3K27me3 is enriched broadly across silent or lowly expressed genes (Figure S2). Most peak calling algorithms, including MACS, are able to detect significant regions of enrichment for factors and modifications with discrete ("narrow") peaks, like H3K4me3, and such modifications can thus be called using the 'default' option. In contrast, calling discrete regions of enrichment for Broad-source factors or Mixed-source factors, like H3K27me3, is more challenging (Landt *et al*., *Genome Res*, 2012). MACS2, which is an updated version of MACS (Zhang et al., 2008), was specifically designed to process broad signal types using the 'broad' peak option (see https://github.com/taoliu/MACS). The reference to the MACS manual is now included in the methods section of the paper.

Reviewer's Comment:
ii. When dynamic changes of H3K27me3 at E14.5 was measured, the authors used raw reads instead of reads belonging to the peaks. The authors should explain why these settings were choosen.

Authors' Response:
We have chosen to analyze a defined region around the TSS (+/– 2kb), as this is the region where the average peak maximum is located (see Figure S2). One could have also chosen to analyze peak regions instead. The difficulty with peaks is: how to deal with genes that have multiple peaks? In that case, one can define criteria for merging peaks or for considering multiple peaks. In our opinion, both methods are valid (see also Pataskar *et al*., *EMBO* 2016 in which promoters, enhancers or peaks were used depending on the context). For our analysis, we preferred to use a defined region which is identical for all genes.

Reviewer's Comment:
b. ChIP-qPCR and qRT-PCR: which method of quantification was used, standard curve or delta-delta-Ct? In the latter case, was the efficiency of the primers tested?

Authors' Response:
The delta-delta-Ct method was used for quantification of qPCR. The efficiency of all primers was tested prior to use in RT-qPCR and ChIP-qPCR. The efficiencies of primers are now indicated in Table EV7.

Reviewer's Comment:
c. qRT-PCR: Is GAPDH an appropriate housekeeping gene for normalization? Is its expression non-variable between samples? This should be at least stated.

Authors' Response:
Based on our previous study (Florio *et al.* 2015), we have chosen *Gapdh* as housekeeping gene for normalization of RT-qPCR data as this gene was found to be expressed at comparable levels in the isolated cell populations as shown by RNA-seq (GSE65000). We have now included a statement in the method section of the revised manuscript.

Reviewer's Comment:
10) The paper has many abbreviations (in particular of the neural progenitors states), I would recommend not to use them and spell out the names of the different neural progenitors throughout the text. This would make the text easier to follow by the readers not familiar with the neural progenitors specific abbreviations.

Authors' Response:
We acknowledge that abbreviations may appear complicated at first sight, and have seriously considered to implement the reviewer's suggestion. However, using the full names of the different progenitor subtypes throughout would make the text very convoluted and difficult to follow. Therefore, we have provided an explanation and a definition of each of the progenitor subtypes in the second paragraph of the introduction (p. 3). In analogy, we also introduce all the epigenetic terms in the introduction section. We hope that by introducing all abbreviations, we make the manuscript accessible to a broad audience.

2nd Editorial Decision 16 June 2017

Thank you for submitting a revised version of your manuscript. It has now been seen by two of the original referees whose comments are shown below.

As you will see they both find that all major criticisms have been sufficiently addressed and recommend the manuscript for publication, pending minor revision for a few points as outlined in their reports. The remaining criticisms largely relate to additional clarification and suggestions for data presentation and additional analysis. I would encourage you to include the depiction of replica variation requested by ref #1. For the last two points from ref #3, you're welcome to include this additional analysis but it is not a strict demand from our side, although you should comment on the points raised.

Given the positive recommendations from the referees I would like to invite you to submit a final revision in which you address these remaining referee concerns as well as the following editorial/formatting issues.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to receiving your final revision.

REFEREE REPORTS

Referee #1:

The revised version of the manuscript includes several new analyses and a few new experiments. It also addresses important technical concerns by providing additional details concerning the number of samples and the methods and thresholds used in the genomic screens. Overall, the article has been very much improved, but the authors should still consider the following addition:

Regarding the response to point #1:
This clarification is very important and it certainly increased my confidence in the quality of the study. The misunderstanding about the number of replicates did not only arise from Figure EV2B, but also from the lack of detail regarding the number of samples (this has been now corrected in page 6 and Fig. S3) and from the poor use of replicate information. Although I agree with the authors that pooling the replicates before calling peaks should increase the number of detected peaks (thereby reducing the number of false negative regions), the authors could also use the information in each replicate to gain confidence in their analyses (i.e., to reduce the number of false positives). For example, the authors could report how many of the peaks detected in the pooled samples were also detected in each one of the two replicates, as an estimation of the most reliable peaks. At a minimum, the authors should include data from both replicate in Figure 4A (PCA graphs) to demonstrate that the replicates clustered together and the separation was primarily caused by cell type.

Referee #3:

The authors' response to my concerns is satisfactory and I therefore recommend the publication of the manuscript "Epigenome profiling and editing of neocortical progenitor cell during development" in EMBO Journal. There are nevertheless some points that I would advice the authors to revise in the manuscript:

- Figure 1c - instead of bar plots, I would advice the authors to use box plots, to shown the distribution of FPKMs.
- Regarding the new Appendix Figure S6, could the authors comment on Pdgfra being expressed in

NEC, despite the absence of H3K4me3 marks (also for Plp1 in all populations)?
- I could not find the description of the transcriptional consistency analysis
- Regarding Figures 7D and F, giving the author's comments, I would still advice to present counts for RFP and Tbr2 separate, and not only the ratio of Tbr2+ dCas9+ / dCas9+ in supplementary. Regarding Figure 7D, if the control is set to 100%, what is the variance SD of the control referring to? Regarding Figure 7G, was paired t-test statistics performed on normalized data? By normalizing data, the authors are eliminating the variance of the control sample. The authors should add in the methods a paragraph justifying the statistical methods applied.
- Regarding the response on PCA and bivalency, PCA analysis could be informative to compare the distributions of both H3K27me3 and H3K4me3 together in the defined cell populations. As the authors mentioned, the input data as a binary file will not make possible to perform a PCA. However, a different treatment in the processing of the data, by for example, performing a PCA analysis with the read coverages of both H3K4me3 and H3K27me3 in the same regions as an enrichment value instead of the Spearman correlation could help to confirm the resolving of bivalency between the cell populations. In addition, following the same approach the authors have used, a PCA of the Spearman correlations from bamcorrelate of the bivalent genes in the different cell populations, will probably show the differences of the distributions of the histone modifications across the entire genome for these bivalent regions in the different cell types.

2nd Revision - authors' response 27 June 2017

Response to Reviewers – Overview of Revision

Item	Panel	Contents	Reviewer(s)
Fig 7	Panel D	Information added to legend	# 3
	Panel G	Statistical analysis changed	# 3
Fig S6	Panel C	Published RNA-seq data for oligodendrocytes and astrocytes added	# 3
NEW Fig S8	Panel A and B	PCA of H3K4me3 and H3K27me3 replicates added	# 1
Fig S9		previous Fig S8	
Fig S10	NEW Panel E	Number of electroporated cells per condition added	# 3
	NEW Panel F	Log-transformed fold changes of ChIP-qPCR data added for statistical analysis	# 3

Response to Reviewers

Reviewer #1

Reviewer's Comment:
The revised version of the manuscript includes several new analyses and a few new experiments. It also addresses important technical concerns by providing additional details concerning the number of samples and the methods and thresholds used in the genomic screens. Overall, the article has been very much improved, but the authors should still consider the following addition:

Regarding the response to point #1:
This clarification is very important and it certainly increased my confidence in the quality of the study. The misunderstanding about the number of replicates did not only arise from Figure EV2B, but also from the lack of detail regarding the number of samples (this has been now corrected in page 6 and Fig. S3) and from the poor use of replicate information. Although I agree with the authors that pooling the replicates before calling peaks should increase the number of detected peaks (thereby reducing the number of false negative regions), the authors could also use the information in each replicate to gain confidence in their analyses (i.e., to reduce the number of false positives). For example, the authors could report how many of the peaks detected in the pooled samples were also detected in each one of the two replicates, as an estimation of the most reliable

peaks. At a minimum, the authors should include data from both replicate in Figure 4A (PCA graphs) to demonstrate that the replicates clustered together and the separation was primarily caused by cell type.

Authors' Response:
We would like to thank the reviewer for his/her appreciation of the revised version of the manuscript. Following the reviewer's recommendation, we have performed a principle component analysis (PCA) of the replicates of H3K4me3 and H3K27me3 ChIP-seq data, which is presented in new Appendix Fig S8 (rather than Fig 4A). Regarding H3K4me3, the PCA of pooled replicates indicated that the five cell populations are positioned along PC1 according to the known progression of the neural lineage (Fig 4A). The H3K4me3 replicates largely follow this trend along principle component 1 (PC1), which explains 27% of the variation among samples (new Appendix Fig S8A). In contrast, PCA for H3K27me3 revealed three different groups: NECs were separate from aRG-P and aRG-N, which clustered together, and those three populations were separate from bRG and neurons, which were also close together (Fig 4B). The new PCA analysis of H3K27me3 replicates supports this conclusion (new Appendix Fig S8B).

Response to Reviewers

Reviewer #3

Reviewer's Comment:
The authors' response to my concerns is satisfactory and I therefore recommend the publication of the manuscript "Epigenome profiling and editing of neocortical progenitor cell during development" in EMBO Journal.

Authors' Response:
We are pleased that the reviewer is satisfied with our responses and thank the reviewer for recommending the publication of our manuscript.

Reviewer's Comment:
There are nevertheless some points that I would advice the authors to revise in the manuscript: Figure 1c - instead of bar plots, I would advice the authors to use box plots, to shown the distribution of FPKMs.

Authors' Response:
We prefer to stick with the representation of RNA-seq data as bar graphs as this is a widely-used way to display gene expression data, especially since the data is based on four or five data points (replicates) per condition. Box plots are beneficial for the presentation of a large number of data points (as for example obtained from single cell gene expression analysis), but this is not the case here. The error bars representing the standard deviation are indicative of the distribution of FPKMs.

Reviewer's Comment:
Regarding the new Appendix Figure S6, could the authors comment on Pdgfra being expressed in NEC, despite the absence of H3K4me3 marks (also for Plp1 in all populations)?

Authors' Response:
Please note that the FPKM values for *Pdgfra* and *Plp1* are relatively low in NEC and the other neural progenitor populations included in our study. For comparison, we have now included RNA-seq data from Zhang *et al.* (*J Neurosci* 2014) for all the oligodendrocyte and astrocyte genes in new Appendix Figure S6C. Even though the FPKM values are not directly comparable, the order of magnitude of expression is very different. Moreover, after normalization of expression values to the house keeping gene *Hprt*, the *Pdgfra* gene is still expressed ≈400-fold higher in OPC compared to NEC and the *Plp1* gene more than 100-fold higher in oligodendrocytes compared to NEC. This also likely explains the absence of H3K4me3 at the *Pdgfra* and *Plp1* promoters. It is well documented in the literature that genes positive for H3K27me3 might still be expressed at low levels, as opposed to genes silenced by H3K9me3 and DNA methylation which tend to be shut down completely.

Reviewer's Comment:
I could not find the description of the transcriptional consistency analysis

Authors' Response:
The analysis of transcriptional consistency is described in the Methods section as follows: *"Transcriptional consistency was assessed as previously described (Benayoun et al., 2014), using RNA-seq data sets with four or five replicates. To eliminate biases in the magnitude of variance due to differences in absolute expression levels, gene expression was scaled to mean expression levels."* It may have escaped the reviewer's attention as is was termed *"transcriptional variability"* in the previous revised version of the manuscript.

Reviewer's Comment:
Regarding Figures 7D and F, giving the author's comments, I would still advice to present counts for RFP and Tbr2 separate, and not only the ratio of Tbr2+ dCas9+ / dCas9+ in supplementary.

Authors' Response:
As we have explained in the first response-to-reviewers, quantification of IUE experiments should be performed as proportion of electroporated cells to account for differences in IUE efficiency between experiments and is the standard in the field. For references, please see publications from leading labs in cortical development, including the Rakic lab (Ishii *et al*., *Nat Commun* 2017), Goetz and Borrell lab (Stahl *et al*., *Cell* 2013), Kiegstein lab (Rani *et al*., *Neuron* 2016; Ramos *et al*., *Cell Stem Cell* 2015), Walsh lab (Johnson *et al.*, *Nat Neurosci* 2015), Calegari and Tiwari lab (Pataskar *et al*., *EMBO J* 2016) and many others. However, to convince the reviewer that the differences in Tbr2 and PH3 are not due to differences in IUE efficiencies, we have now plotted the number of Cas9+ cells (revealed by RFP) per microscopic field for all three conditions tested (shown in the new Appendix Fig S10E, rather than Fig 7). Indeed, there are no statistically significant changes between the average IUE efficiencies of different tested conditions.

Reviewer's Comment:
Regarding Figure 7D, if the control is set to 100%, what is the variance SD of the control referring to?

Authors' Response:
The average of all control samples is set to 100%. The SD of the control sample refers to the variance of the individual control values relative to the control average. This is now stated more clearly in the figure legend.

Reviewer's Comment:
Regarding Figure 7G, was paired t-test statistics performed on normalized data? By normalizing data, the authors are eliminating the variance of the control sample. The authors should add in the methods a paragraph justifying the statistical methods applied.

Authors' Response:
Based on the reviewer's criticism, we have changed the statistical test for this type of normalized data. We have expressed the fold changes as logFC (resulting a log-normal distribution of values) and used a 'One sample t-test', which is more appropriate for the experimental data. The corresponding graph displaying logFC is shown in new Appendix Fig S10G (rather than Fig 7G).

Reviewer's Comment:
Regarding the response on PCA and bivalency, PCA analysis could be informative to compare the distributions of both H3K27me3 and H3K4me3 together in the defined cell populations. As the authors mentioned, the input data as a binary file will not make possible to perform a PCA. However, a different treatment in the processing of the data, by for example, performing a PCA analysis with the read coverages of both H3K4me3 and H3K27me3 in the same regions as an enrichment value instead of the Spearman correlation could help to confirm the resolving of bivalency between the cell populations. In addition, following the same approach the authors have used, a PCA of the Spearman correlations from bamcorrelate of the bivalent genes in the different cell populations, will probably show the differences of the distributions of the histone modifications across the entire genome for these bivalent regions in the different cell types.

Authors' Response:
A PCA could be performed with read coverages of both H3K4me3 and H3K27me3 in the regions that are scored as bivalent in a given cell type, however, it is not clear to us how this would show the resolution of bivalent domains. That would require a calculation of an enrichment score with prior definition of a ratio of H3K4me3 to H3K27me3 that should be considered as bivalent. Since no such measure has been established in the field, we feel that this goes beyond the scope of the current manuscript.

3rd Editorial Decision 03 July 2017

Thank you for submitting the final revision of your manuscript, I am pleased to inform you that your study has now been accepted for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Mareike Albert and Wieland B. Huttner
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2017-96764

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

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The ChIP-seq experiments were performed in duplicates (p. 7) as recommended by the ENCODE consortium. RNA-seq experiments were perfomred with 5 replicates (methods, p 26). The sample size for IUE experiments is described in the figure legends (p. 43/44) and was determined by experience.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For ChIP and RNA, the number of animals depended on the minimum number of cells required to perform each method (see Methods, p. 22, 25-27). For IUE experiments, at least four different litters were used for each experiment. From each litter, several embryos were analyzed and data pooled together (see Methods, p.29; figure legend, p. 43/44).
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For IUE experiments, only embryos with comparable IUE efficiencies were included. Embryos with very low IUE efficiency were not included in the quantifications.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Randomization was done when possible, such as when electroporating embryos in utero.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization was used for animal studies.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Yes, blinding of the investigator was done for some experiments, such as microscopy and data quantification, when possible.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding was done for some experiments.
5. For every figure, are statistical tests justified as appropriate?	Yes (see Methods and figure legends).
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. Normality tests were performed and if normal ditribution was not given, appropriate alternative tests (like Kruskal-Wallis or Mann–Whitney) were used.
Is there an estimate of variation within each group of data?	The variation is shown with error bars (standard deviation) in bar graphs. The distribution of larger data sets is presented as box plots. For average read counts, the SEM is shown as shading.
Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	The catalog numbers of the primary antibodies used for ChIP and immunofluorescence are provided (see Methods, p. 22, 29).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	The requested information is described in the Method section (p. 21). The gender of mouse embryos was not assessed. The embryonic age is indicated (see Methods and figure legends).
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The requested information is included in the Method section (p. 21). "All experimental procedures were designed and conducted in agreement with the German Animal Welfare Legislation after approval by the Landesdirektion Sachsen. "
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA.

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	As indicated in the method section, ChIP-seq raw data, bigwig files and bed files with peak locations have been deposited with the Gene Expression Omnibus under the accession code GSE90694. RNA-seq data for NECs has been deposited with the Gene Expression Omnibus under accession code GSE90447.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA.
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA.
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA.

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA.