nature research

Peer Review Information

Journal: Nature Genetics
Manuscript Title: The HIF complex recruits the histone methyltransferase SET1B to activate specific hypoxia-inducible genes
Corresponding author name(s): Dr. James Nathan

Reviewer Comments & Decisions:

Decision Letter, initial version:

3rd Nov 2020

Dear James,

Your Article, "SET1B facilitates activation of the hypoxia response through site-specific histone methylation" has now been seen by 4 referees. You will see from their comments copied below that while they find your work of potential interest, they have raised quite substantial concerns that must be fully addressed. In light of these comments, we cannot accept the manuscript for publication, but would be interested in considering a revised version that addresses these serious concerns.

Reviewer #1 highlights that HIF-1 is known to functionally interact with histone modifiers so, from a conceptual perspective, the findings can be seen as incremental. The reviewer has some thoughtful suggestions for improvement, though. The experimental requests are significant but should be doable.
Reviewer #2 is positive about this work overall. He/she has some requests for new data but these are not extensive (apart from one in vivo experiment that entails conditional HIF-1 depletion).
Reviewer #3's comments are mainly about one conceptual criticism, which should be easy to address textually.
Q4 thinks this is a nice contribution to the field, overall. Their comments are constructive and addressable.

We hope you will find the referees' comments useful as you decide how to proceed. If you wish to submit a substantially revised manuscript, please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

If you choose to revise your manuscript taking into account all reviewer comments, please highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript

in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact me if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

If revising your manuscript:

*1) Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

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If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Genetics or published elsewhere. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

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from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

Thank you for the opportunity to review your work.

Sincerely,

Tiago

Tiago Faial, PhD
Senior Editor
Nature Genetics
https://orcid.org/0000-0003-0864-1200

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors describe here a connection between the H3K4 methyltransferase SET1B and HIF-1, showing that SET1B is recruited to chromatin in hypoxia, and regulates HIF-1 transcriptional activity via modulating the expression of HIF-1-responsive angiogenic genes. The concept of HIF-1 recruiting epigenetic modulators on chromatin is per se not new. Even though most of the literature regarding HIF-1 and epigenetic regulators is about the interaction with ep300/CBP, there has been evidence that HIF-1 can also recruit erasers on chromatin, enabling removal of repressive marks and activation of gene expression (e.g.: Proc. Natl Acad. Sci. USA 109(49), E3367–E3376, 2012). Additionally, it has been shown that some methyltransferase activities are important for HIF-1 functionality. In particular, it has been reported that the H3K4 methyltransferase SET9 can bind to HIF-1a and stabilize it. Moreover SET9 is enriched at HIF-1-responsive glycolytic genes to enhance their expression (Biochim Biophys Acta. 2015 May; 1853(5): 881–891). The similar mode of action of SET9 and SET1B, partially compromises the novelty of the presented findings.

The connection between SET1B and HIF-1 is, however, new and sheds light on novel regulatory aspects of HIF-1, whose function is fundamental for cancer cell adaptation and tumor progression. The manuscript has a nice narrative: the involvement of SET1B in hypoxia is well documented, and it is interesting that it is selectively involved in the regulation of a set of genes implicated in a specific response (angiogenesis), rather than in the regulation of every HIF-1 target gene. The used functional genomics approach is elegant, it offers an easy readout, and it has been overall well planned and executed; nevertheless the details of the screen require some clarifications (see Minor points).
The sequencing data analysis is good, and the used statistical tests are correct (additional suggestions addressed in Minor points). However, 3 biological replicates instead than 2 would have make the analysis of the omics statistically more powerful.

The manuscript is overall potentially interesting, but more mechanistic insights are required to fill important gaps in the story. Among them, the following suggested experiments could considerably increase the general impact of the manuscript, refining its trajectory.

Major points:
The authors are claiming that the HIF-1/SET1B interaction is required for the expression of HIF-1 responsive angiogenic genes. However, no mechanism is proposed about how SET1B is recruited on chromatin, neither an explanation is given about why only to specific HIF-1 target loci (angiogenic gene promoters), and the development of these aspects could add a novel spin to the work. To this aim, the authors should:

1) show whether SET1B nuclear and chromatin localization during oxygen deprivation decreases upon HIF-1a/b downregulation
2) investigate whether SET1B nuclear accumulation during hypoxia is mediated by HIF-1 interaction. To do so, the authors should test whether there is any impairment of SET1B nuclear and chromatin recruitment during hypoxia in HIF-1a-depleted cells and reconstituted with the HIF-1a mutant unable to interact with SET1B (PAS-B).
a. regarding the interaction of SET1B with HIF-1 a and b subunits: even though the interaction with HIF-1a seems to be more pronounced, how do the authors explain that in hypoxia it seems that SET1B interaction with HIF-1a decreases, while with HIF-1b considerably increases?
3) investigate whether the sole localisation of SET1B in the nucleus can rescue HIF-1a/b depletion (via checking the expression of selected gene candidates), if needed by fusing SET1B with a nuclear localisation signal to force its nuclear localisation.

The selective recruitment of SET1B to the promoter of HIF-1-responsive angiogenic genes is one of the key aspects of the manuscript, however results are only shown for very few HIF target genes, thus limiting the impact of the results. To strengthen this key finding, the authors should:

1) perform ChIPseq of HIF-1 (ideally a/b subunits), SET1B and H3K4me3 in normoxia and hypoxia, to eventually show whether SET1B binds
a. exclusively to the promoter of HIF-1-responsive angiogenic genes
b. to a subset of those promoters
c. also to additional promoters of HIF-1-target genes involved in different HIF-1 downstream pathways.
Moreover, the analysis should consider all the different HIF-1-target pathways and include, but not be limited to, angiogenesis and glycolysis.
2) Integrate ChIP-seq and gene expression data to understand whether gene promoters bound by SET1B/HIF-1 are solely those defined by the authors as SET1B/HIF-1 dependent (form the RNAseq analysis).

In general, the author should show ChIP-seq data as metagene plots rather than box plots as in figure 7d/e. Similarly, supplementary figure 9 d/e/f should be accompanied by metagene plots showing the distribution of H3K4me3 across different groups of HIF-1 target gene promoters (including, but not limited to, angiogenesis and glycolysis).

Another important aspect of the paper that need to be better explored is the potential in vivo antitumoral effect of targeting SET1B. In particular, the author show that in the Hela and A549 xenografts there is not an effect in tumor growth, but rather in tumor phenotype with an apparent role

of the SET1B-HIF-1 interaction in the establishment of a proper tumor vasculature. Therefore, the author should

1) perform a more detailed characterization of the tumors, e.g.:
a. Are tumors more cystic?
b. Is the present vasculature aberrant (dilated, twisted or anastomotic vessels)?
2) focus on a more angiogenic tumor model. For instance, the clear cell renal carcinoma mentioned in the discussion would have been more physiologically relevant
3) Given the effect on tumor vascularization, test whether SET1B downregulated tumors are less prone to develop distal metastasis

Minor points:
Functional genomics approach: the authors say that they have kept always 150X coverage but they apparently started with 5X10^8, which would rather account for 900X coverage (considering 30% MIO and 176500 sgRNA of the mentioned library). Also, it is not clear how many cells have been sorted and how long those have been in culture prior library preparation and sequencing. The authors should clarify details of the screening approach.

The authors show rescue of CA9 expression with overexpression of SET1B, maybe is worth also showing that SET1B overexpression can rescue the HIF reporter activity.

Fig. 7f. Could the authors perform a statistical test (like Kolmogorov - Smirnov Test) to test if the differences are significant? For EGLN3 and VEGF, it is not completely clear.

Chip-seq analysis: is there any particular reason why the authors chose the common peaks from the 2 peak callers (MACS and T-Pic)? Different peak callers have different algorithms for peak detection, and often times there are discrepancies.

Reviewer #2:
Remarks to the Author:
In the manuscript "SET1B facilitates activation of the hypoxia response through site-specific histone methylation" Ortmann et al. conducted a genome-wide mutagenesis screen and identified SET1B as a required element to activate a HIF transcriptional response to hypoxia. They performed a mechanistic study defining how SET1B is recruited by HIF1a and brought into the nucleus where it binds to chromatin and amplifies transcription of HIF target genes (mostly angiogenic) through methylation and acetylation of histones in the promoters of HIF1 targets. Overall the manuscript is well written and experiments have substantial rigor. However, the following concerns need to be addressed prior to publication.

Major:
1. The authors should provide additional information on the hits identified in their genome-wide mutagenesis screen outside of HIF components, SET1B, UBE2A and PPP4C? How might they be involved in a response?
2. In Figure 2, experiments demonstrate that SET1B is required in both transformed cells and non-transformed skin fibroblasts, suggesting that "normal" cells operate on the basis. More discussion is warranted on this point.

3. The authors assert that SET1B does not show bias between HIF1a/2a isoforms. In supplementary Figure 5, there was a striking difference on the influence of SET1B depletion between the HIF1a and HIF2a reporters. What explains this difference? How can the authors make the claim that there is no isoform bias, when the data in this figure looks different between the two reporters.
4. HIFs have influence on a broad range of cellular processes. In Figure 4/supplementary Figure 7, the authors assess the effects of SET1B loss in vitro on cell proliferation, growth and apoptosis. This analysis would be improved by assessing cell growth over a longer time course, in addition to cell motility and in 3D spheroid cultures.
5. To assess the effects of SET1B loss in vivo, the authors perform constitutive depletion in a subcutaneous xenograft model. No alterations were found in tumor growth, but a delay in tumor initiation was observed. Conditional depletion of SET1B would allow the authors to more directly understand the role of SET1B in tumor angiogenesis, bypassing its potential effect on tumor initiation. Moreover, the data on blood vessel content was rather modest.
6. Although the association between SET1B and HIF2a is less prominent compared to its other isoform, does SET1B bind to HIF2a through the PAS domain? The predominance of either isoform can be tumor type specific. Also, can HIF2a promote SET1B accumulation on chromatin at HIF target genes as observed with HIF1a?

Minor:
1. In Figure 3D, the three groups are represented by red, green and grey. It is difficult to discern between the three groups in this figure. A similar color scheme was utilized in other figures and is problematic for people with color blindness. Different colors for these figures are recommended.

Reviewer #3:
Remarks to the Author:
Ortmann and coworkers have performed a fluorescent reporter-based genome-wide mutagenesis screen in human cancer cells aimed at identifying new genes required for HIF-dependent transcription in hypoxia. They found that the histone methyltransferase SET1B is necessary for transcription of a subset of HIF target genes in hypoxia, which in turn results in largely impaired tumorigenesis in SET1B loss-of-function mouse xenografts. SET1B is recruited to the chromatin in hypoxic conditions in a HIF-dependent manner, resulting in H3K4 trimethylation at promoters of a subset of HIF targets. HIF binding to the chromatin does not depend on SET1B. Interestingly, SET1B knock-out prevents histone acetylation at the promoters where SET1B mediates methylation.
The current study is an elegant and complete work that provides important insights into HIF-dependent gene expression in hypoxia. The data are sound, and the experiments well-conducted and carefully controlled. I don´t feel that additional experiments are needed before the manuscript can be accepted for publication.
I have just a conceptual comment that the authors may want to consider to modify part of the discussion and perhaps, a couple of sentences at the Introduction: Chromatin modifications and their regulators (histone methyltransferases; demethylases, acetyl transferases, deacetylases, etc) are essentially promoter-specific rather than transcription factor-specific. Gene specificity of chromatin regulators is therefore the rule rather than the exception. At the Discussion (lines 353 to 367) the authors attempt to explain or speculate on why only a limited subset of HIF target genes is affected by SET1B loss-of-function, and what is the source of such specificity. Given the current general knowledge on chromatin regulators, a general effect on HIF-dependent gene expression is not expected at all. In line with this, it is surprising that people in the hypoxia field generally consider the

histone acetyl transferase CBP/P300, as well as the dioxygenase FIH, general regulators of HIF-dependent transcription. There is no proof for this, and a histone acetyl transferase (CBP/P300) is not expected to have a global effect on HIF-dependent gene expression. In fact, FIH KO mice have only minor defects on HIF-dependent transcription. The authors might want to take this into consideration, and modify the relevant sentences accordingly (specially at the Discussion).
Minor points and typos:
1- Please move the Venn diagram of Supplementary Fig. 4A to a main figure. It is a good summary of the effect of SET1B on hypoxia-dependent gene expression, and should be in a main figure.

2- Line 266: Supplementary Figure 8i, j instead of 9i, j

3- Line 298: "Figure 7e" is repeated. Please delete.

4- Line 420: Please delete "(a gift from Patrick Maxwell)" PHM is co-author.

Reviewer #4:
Remarks to the Author:
Ortmann et al

This is a well written and well executed paper that clearly shows that SET1B modulates the HIF response and that SET1B is recruited to a subset of HIF targets in a HIF-dependent manner, leading to increased histone methylation and gene expression. It is a very nice contribution to the field.

Major comments:

1. The negative controls could be better described in the text. For example, were the negative controls for the sgRNA experiments (e.g. Fig 1f) untreated cells, cells treated with a non-cutting sgRNA, cells treated with an irrelevant sgRNA? Important when looking at subtle differences. For the tumor xenograft experiments, were the controls untreated cells, cells infected with irrelevant sgRNAs and put through same drug selection protocol, something else?
2. Fig 2i and 2k don't support the claim that WDR82sgRNA inhibited the HIF response "similarly" to SET1b loss. First, the WDR82KD sgRNA is decreasing HIF1A protein (see Fig. 2k), which confounds things. Second, the leftward shift in the rightward peak in Fig 2i is barely perceptible. Finally, in Fig. 2i there is a small subpopulation of cells that don't fire the reporter at all. Are the authors including the second observation as further evidence that WDR82 inhibits the response? I fear it might be technical if you compare the SET1B sgRNA data in Fig 2i to 1f and S1. This is such a minor point to the paper I wonder if the authors wouldn't be better served removing it or softening their conclusions
3. I think Fig S5g is hard to interpret. The HIF2 ko cells look like the parental cells, suggesting HIF1 is the major driver in these cells of the hypoxic response. It looks like in the HIF1 ko cells the major effect is that most of the cells don't induce the reporter in response to DMOG at all but the subpopulation that does seemingly does so as well as the parental cells despite SET1B sgRNA. Are these the cells that escaped editing? Just not sure I agree that SET1B sgRNA is decreasing HIF response in the HIF2 only cells.
4. The mapping in Fig 5 is not very compelling. The binding in Fig 5e largely tracks with the level of abundance in Fig 5d. A worrisome sign."1-396" is comigrating with a background band in Fig 5e and the 1-78 might have been cut off/run off. I would consider removing the mapping data and

strengthening that binding of SET1B to HIF is robust and specific (especially as the signals in Fig S8b and 8d for SET1B and HIF (VHL298 lane) are just at the level of detection). For example, is there a nuclear TF that doesn't coIP with SET1B or CFP1? Can recombinant HIF bind specifically to recombinant SET1B?

Minor Comments:

1. Line 46. If going to cite ref. 3 should probably cite Bruick and McKnight 2001 Science paper and if going to cite ref. 5 should probably cite Jaakkola 2001 Science
2. Line 58. I think the authors are referring to FIH-1
3. Line 67. Why "However, this situation is complex as recent…"? You would expect an increase in histone methylases if the demethylases are inhibited by oxygen. The wording makes it sound like this is unexpected.
4. Line 80. For clarity, should be "through HIF-1alpha's PAS-B domain"
5. Would be useful to know if the mCherry reporter cells used for the screen were clonal or not.
6. In Fig S2a, the CA9 band the SET1B KD cells doesn't align with either the 8 hour or 24 hour lanes. And are all these blots really from the same gel (hence justifying a single loading control blot)?
7. Line 143. S3c, not S3e.
8. For my taste Fig 2i is pretty preliminary with respect to specificity controls.
9. Rather hard to see the different colors for the SET1B and HIF1beta dots in Fig . 3d.
10. In Fig 3f and S4e, there actually does appear to be a possible effect of SET1B knockdown on BAP1 mRNA levels. I suspect is currently "ns" (and should be marked as such), but wonder if would become significant with more replicates. And note that there are similar differences, now "significant", in Fig S7d
11. Line 206. I wouldn't call the downregulation of SET1B protein in Fig 4c and 4d "marked".
12. The increase in cleaved PARP in Fig S7g in SET1B kd cells is underwhelming.
13. Line 226. Clarify is subcutaneous injection.
14. Clarify in text and/or legend that tumors for 4i and 4j were harvested when they reached 1000 mm3. Yes?
15. Line 243. I would be careful saying binding to HIF1A is more prominent than to HIF1B, because do not know the relative binding affinities of the two respective HIF antibodies.
16. Line 266. Supplementary Fig 8i,j not 9i,j
17. Can you indicate p values for Fig 7d and 7e?
18. The differences in H3K4 methylation at the PHD3 and VEGF promoter are subtle, especially for SET1B kd. Can the reader be given a sense of the reproducibility of these patterns?
19. Line 353. Isn't VEGF driven by HIF1 in most cell types? Where were the data on PLIN2 and NDRG.
20. Line 398. Why not target SET1B catalytic activity? And, as indicated above, I think the mapping data are weak.

Author Rebuttal to Initial comments

Response to Referees

Reviewer #1:
Remarks to the Author:
The authors describe here a connection between the H3K4 methyltransferase SET1B and HIF-1, showing that SET1B is recruited to chromatin in hypoxia, and regulates HIF-1 transcriptional activity via modulating the expression of HIF-1-responsive angiogenic genes. The concept of HIF-1 recruiting epigenetic modulators on chromatin is per se

not new. Even though most of the literature regarding HIF-1 and epigenetic regulators is about the interaction with ep300/CBP, there has been evidence that HIF-1 can also recruit erasers on chromatin, enabling removal of repressive marks and activation of gene expression (e.g.: Proc. Natl Acad. Sci. USA 109(49), E3367–E3376, 2012). Additionally, it has been shown that some methyltransferase activities are important for HIF-1 functionality. In particular, it has been reported that the H3K4 methyltransferase SET9 can bind to HIF-1a and stabilize it. Moreover SET9 is enriched at HIF-1-responsive glycolytic genes to enhance their expression (Biochim Biophys Acta. 2015 May; 1853(5): 881–891). The similar mode of action of SET9 and SET1B, partially compromises the novelty of the presented findings.

The connection between SET1B and HIF-1 is, however, new and sheds light on novel regulatory aspects of HIF-1, whose function is fundamental for cancer cell adaptation and tumor progression. The manuscript has a nice narrative: the involvement of SET1B in hypoxia is well documented, and it is interesting that it is selectively involved in the regulation of a set of genes implicated in a specific response (angiogenesis), rather than in the regulation of every HIF-1 target gene. The used functional genomics approach is elegant, it offers an easy readout, and it has been overall well planned and executed; nevertheless the details of the screen require some clarifications (see Minor points).
The sequencing data analysis is good, and the used statistical tests are correct (additional suggestions addressed in Minor points). However, 3 biological replicates instead than 2 would have make the analysis of the omics statistically more powerful.

The manuscript is overall potentially interesting, but more mechanistic insights are required to fill important gaps in the story. Among them, the following suggested experiments could considerably increase the general impact of the manuscript, refining its trajectory.

We are pleased this reviewer found the connection between SET1B and HIFs interesting and novel. We have extended our discussion of the prior literature to address the points raised regarding other epigenetic modulators recruited by HIFs. The recruitment of the demethylase JMJD2C by HIF-1 is less directly relevant to our work but is interesting to discuss in the context of recent studies showing that hypoxia decreases KDM activity (**Discussion, p20**). We disagree with the suggestion that SET1B has a similar mode of action to SET9. SET9 has been reported to stabilise HIF-1α by preventing its degradation, independent of methyltransferase activity (BBA, 2015). SET9 also catalyses monomeric methylation of histones and other substrates, which is again distinct from our findings with SET1B (**Discussion, p19**). We have addressed the other points raised regarding the screen and sequencing data below.

Major points:
The authors are claiming that the HIF-1/SET1B interaction is required for the expression of HIF-1 responsive angiogenic genes. However, no mechanism is proposed about how SET1B is recruited on chromatin, neither an explanation is given about why only to specific HIF-1 target loci (angiogenic gene promoters), and the development of these aspects could add a novel spin to the work. To this aim, the authors should:

1) show whether SET1B nuclear and chromatin localization during oxygen deprivation decreases upon HIF-1a/b downregulation

2) investigate whether SET1B nuclear accumulation during hypoxia is mediated by HIF-1 interaction. To do so, the authors should test whether there is any impairment of SET1B nuclear and chromatin recruitment during hypoxia in HIF-1a-depleted cells and reconstituted with the HIF-1a mutant unable to interact with SET1B (PAS-B).

We thank the reviewer for raising these important points, which we have explored in several ways. Firstly, treatment with DMOG and selective PHD inhibition (Roxadustat) still results in SET1B nuclear accumulation, (**New Figure 5h, Supplementary Figure 10j**). Secondly, to determine if HIF-1 was responsible for the nuclear accumulation we repeated the fractionation experiments in combined HIF-1α/HIF1β deficient cells, and now show that HIF-1 loss impairs the accumulation of SET1B in the chromatin fraction in HeLa cells (**New Figure 5i**).

HIF-2α may still be involved to a lesser extent, as HIF1β loss alone does not prevent the chromatin accumulation of SET1B, and in VHL reconstituted 786-0 cells that just express HIF-2α, we still observe some accumulation of SET1B in hypoxia (**New Supplementary Figure 10k**). Reconstitution experiments of HIF-1α alone would therefore not be informative.

Overall, these findings are consistent with HIF-1 recruiting SET1B, which then accumulates on chromatin. However, other mechanisms may be still be involved, such as prolyl hydroxylation of SET1B or an associated complex member, which may provide an additional stimulus for the cytosolic localisation when oxygen is present (**Discussion, p18**). This is an area that we are keen to follow up in future studies but beyond the scope of the current manuscript. Importantly, our ChIP-PCR studies do confirm that HIF recruitment of SET1B is required for the efficient activation of target gene expression (**Figure 6**).

a. regarding the interaction of SET1B with HIF-1 a and b subunits: even though the interaction with HIF-1a seems to be more pronounced, how do the authors explain that in hypoxia it seems that SET1B interaction with HIF-1a decreases, while with HIF-1b considerably increases?

We disagree that this occurs. **Figure 5b** shows that HIF-1α and HIF1β are only visualised on SET1B in hypoxia, and both HIF components are increased compared to levels in 21% oxygen.

3) investigate whether the sole localisation of SET1B in the nucleus can rescue HIF-1a/b depletion (via checking the expression of selected gene candidates), if needed by fusing SET1B with a nuclear localisation signal to force its nuclear localisation.

The ChIP-PCR data shows that SET1B is not recruited to these target genes when HIF1β is depleted (**Figure 6a**), making it unlikely that SET1B could rescue gene expression. To further clarify this point we now include data which shows that overexpressed SET1B is not able to rescue expression of CA9 in HIF1β null cells during hypoxia (**New Supplementary Figure 10m-o**).

The selective recruitment of SET1B to the promoter of HIF-1-responsive angiogenic genes is one of the key aspects of the manuscript, however results are only shown for very few HIF target genes, thus limiting the impact of the results.

We would emphasise that a key finding of our work is the site-specific involvement of SET1B to a subset of HIF-1 responsive genes, rather than selection to just angiogenic genes. HIF responsive genes such as CA9 and PHD3 require SET1B but are not involved in angiogenesis. We disagree that this limits the impact of our findings, as we provide important insights into the complexity of the HIF response. We have now undertaken new ChIP-seq studies and analysis of the RNA-seq data to address the points raised below.

To strengthen this key finding, the authors should:

1) perform ChIPseq of HIF-1 (ideally a/b subunits), SET1B and H3K4me3 in normoxia and hypoxia, to eventually show whether SET1B binds

a. exclusively to the promoter of HIF-1-responsive angiogenic genes
b. to a subset of those promoters
c. also to additional promoters of HIF-1-target genes involved in different HIF-1 downstream pathways.
Moreover, the analysis should consider all the different HIF-1-target pathways and include, but not be limited to, angiogenesis and glycolysis.

2) Integrate ChIP-seq and gene expression data to understand whether gene promoters bound by SET1B/HIF-1 are solely those defined by the authors as SET1B/HIF-1 dependent (form the RNAseq analysis).

We have now performed ChIP-seq for HIF-1α, HIF-2α and HIF1β in HeLa cells in hypoxia to define the binding regions. SET1B ChIP-seq has not been technically feasible, and the sequencing data shows no specific binding. It is

also worth noting that there is no published data for SET1B ChIP-seq, consistent with this not being technically possible. Changes in H3K4me3 provide the best current evidence for SET1B activity. By integrating the HIF ChIP-seq, H3K4me3 ChIP-seq and RNA-seq data, we now show the following:

- Using gene set enrichment analysis, HIF-bound genes are enriched amongst genes downregulated by SET1B depletion in hypoxia, similarly to HIF1β loss (**New Supplementary Figure 11g, h**).
- H3K4me3 increases at HIF binding sites, and this is for the most part dependent on HIF binding (**New Figure 7d, Supplementary Figure 12**).
- HIF1β dependent genes show a decrease in H3K4me3 at the transcriptional start site (TSS) and across the gene body when HIF1β is depleted (**Figure 7b-d, Supplementary Figure 11f**).
-SET1B dependent genes can show a decrease in H3K4me3 at the TSS, but predominantly show a reduction across the gene body (**Figure 7b-d, Supplementary Figure 11f, i**).
-HIF independent genes that are upregulated in hypoxia also show an increase in H3K4me3, but this is not dependent on HIF1β loss or SET1B loss (**Figure 7b, c**), and likely relates to demethylase inhibition or alternative transcription factor binding in hypoxia (e.g. PMID 30872526, 30872525).

Taken together, these results show that the effect of hypoxic gene expression of SET1B is marked, and consistent between the HIF ChIP-seq and RNA-seq. We observe SET1B altering H3K4me3 across the gene body, consistent with prior studies (PMID 32637595). This can be seen most clearly in the individual gene tracts presented, which now also includes the HIF binding sites (**New Figure 7d, Supplementary Figure 12**).

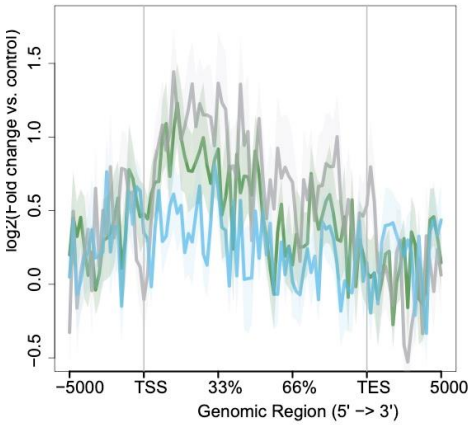
It is important to note that the direct relationship between H3K4me3 and gene transcription remains to be fully resolved, and it is possible that SET1B may have additional functions, aside from H3K4me3, that account for its clear effect on HIF target gene expression. For instance, the effect of SET1B loss on mRNA expression of *VEGF* and PHD3 (*EGLN3)* is more marked than changes in H3K4me3 across the promoter/gene body. This is an area that we plan to pursue in future studies.

An additional suggestion related to the preferential involvement of SET1B in specific HIF pathways. We did not find a significant differential H3K4me3 signal in the integrated ChIP-seq analysis for angiogenesis compared to the HIF target genes, but as mentioned above and noted by Reviewer 3, chromatin regulators can be considered to be promoter/site-specific rather than pathway specific. However, we have noted that hypoxic SET1B dependent genes are those that tend to show intermediate/low levels of expression in 21% oxygen, and mostly exclude highly expressed HIF-dependent genes (**New Figure 3h**). These findings suggest that SET1B may have a role in regulating other chromatin marks linked to gene repression, consistent with recent studies from Shilatifard's group where the COMPASS member MLL2 methylates H3K4 by repelling repression by PRC2 (Douillet et al, Nat Gen 2020). There is also further complexity here, as hypoxia may also be altering demethylase activity (see also new section in the **Discussion, p19/20**).

In general, the author should show ChIP-seq data as metagene plots rather than box plots as in figure 7d/e. Similarly, supplementary figure 9 d/e/f should be accompanied by metagene plots showing the distribution of H3K4me3 across different groups of HIF-1 target gene promoters (including, but not limited to, angiogenesis and glycolysis).

We have now included the metagene plots in addition to the box plots **(New Figure 7b, c**), and extended the analysis of individual glycolysis and angiogenic genes (**New Supplementary Figure 11g-i,12**) The metagene plots of small numbers of HIF target genes or gene subsets are difficult to interpret (see below for example of 15 core HIF target genes), and we do not feel they offer further insights from visualising individual gene tracts already included in the manuscript.

H3K4me3 ChIP-seq for 15 core target HIF genes.



Another important aspect of the paper that need to be better explored is the potential in vivo antitumoral effect of targeting SET1B. In particular, the author show that in the Hela and A549 xenografts there is not an effect in tumor growth, but rather in tumor phenotype with an apparent role of the SET1B-HIF-1 interaction in the establishment of a proper tumor vasculature. Therefore, the author should

1) perform a more detailed characterization of the tumors, e.g.:
a. Are tumors more cystic?
b. Is the present vasculature aberrant (dilated, twisted or anastomotic vessels)?
2) focus on a more angiogenic tumor model. For instance, the clear cell renal carcinoma mentioned in the discussion would have been more physiologically relevant
3) Given the effect on tumor vascularization, test whether SET1B downregulated tumors are less prone to develop distal metastasis

Thank you for raising these important points that we were keen to address.

1a) We have now included specialist histopathological assessment of the tumours. Macroscopically, no cysts were identified in any of the A549 tumours. Microscopically, the tumour is arranged into a predominantly sheet-like architecture, with some areas showing more well-defined acinar and glandular structures (**New Figure 4l**). Where present, glandular structures are irregularly shaped and show a variable degree of dilatation. Some larger gland lumens contain pale eosinophilic mucinous material with interspersed lymphocytes and neutrophils. Occasional mucin pools are present. Tumour cells are densely packed with round – ovoid nuclei. There is an increased nuclear : cytoplasmic ratio, nuclear membrane irregularities, distorted chromatin pattern and moderate pleomorphism.

In summary, no cystic component was identified in these tumours and therefore it was not possible to pursue further investigation in this area.

1b) We have performed IHC for CD31, and a more detailed analysis using angiogenesis quantification software developed specifically for this purpose. We now show that not only is vessel density in tumours reduced in the HIF 1β KO and SET 1B KO, but vessels in these models are also smaller. Data highlighting differences in vessel morphology between the different groups is now shown (**New Figure 4l, m, Supplementary Figure 9c**), providing strong evidence that SET1B depletion decreases angiogenesis, similarly to HIF1β loss.

Analysing the degree of 'twistiness' and the number of anastomotic vessels would require imaging techniques involving 3-dimensional reconstruction from serial scanned sections, and is beyond the scope of the current investigation. We have however discussed this line of enquiry with individuals with expertise in this area, and plan to interrogate the 3-dimensional vasculature architecture in these mouse models in our future work.

2) We established the 786-0 renal cell carcinoma xenograft model, as suggested. This recapitulates the effects we see in the A549 and HeLa xenograft models (**New Figure 4n, o**). HIF1β loss prevents 786-0 xenografts from establishing, and SET1B loss shows an intermediate effect compared to the control and HIF1β deficient 786-0 tumours, with 3/7 failing to establish any tumours and delayed tumour growth overall (**New Figure 4n, o**).

3) Examining the effect on distal metastases is an area we are exploring, but really is a distinct question and is beyond the scope of this manuscript, where we have already examined three xenograft models in support of our findings with SET1B. We plan to undertake this metastasis work in conjunction with how SET1B may be regulating the immune compartment, as both the angiogenic studies and immune function are required to interpret distant spread.

Minor points:
Functional genomics approach: the authors say that they have kept always 150X coverage but they apparently started with 5X10^8, which would rather account for 900X coverage (considering 30% MIO and 176500 sgRNA of the mentioned library). Also, it is not clear how many cells have been sorted and how long those have been in culture prior library preparation and sequencing. The authors should clarify details of the screening approach.

We apologise for the typographical error and lack of clarity. We started with $1x10^8$ cells (170x initial coverage) for the transduction, and maintained the library at a minimum of $5x10^7$ cells. This is in line with our prior studies. Cells were sorted at day 9 after transduction, and incubated in 1% oxygen for 24 hr. Cells were then harvested at day 17 or 21 from the initial transduction for the library preparation. We did attempt DNA extraction immediately after the sorts but the quality of the DNA was poor, likely due to hypoxia induced apoptosis. We therefore opted to culture the post-sorted cells for longer, to allow recovery and to ensure sufficient quality DNA for extraction. The hypoxia sorted libraries were compared to corresponding control libraries taken at the same time intervals. We have clarified this in the text (**Methods, Discussion (p17), Figure 1 legend**).

The authors show rescue of CA9 expression with overexpression of SET1B, maybe is worth also showing that SET1B overexpression can rescue the HIF reporter activity.
We have now included this data (**New Supplementary Figure 2h**).
Fig. 7f. Could the authors perform a statistical test (like Kolmogorov - Smirnov Test) to test if the differences are significant? For EGLN3 and VEGF, it is not completely clear.
We do not think statistics are of value on an individual gene tract, and KS is generally not applicable to this type of data, and may be misleading.
Chip-seq analysis: is there any particular reason why the authors chose the common peaks from the 2 peak callers (MACS and T-Pic)? Different peak callers have different algorithms for peak detection, and often times there are discrepancies.
The reason for using the two peak callers is to increase the confidence in the peaks we are using. There will be discrepancies, but we are only using those peaks common to both callers.

Reviewer #2:
Remarks to the Author:
In the manuscript “SET1B facilitates activation of the hypoxia response through site-specific histone methylation” Ortmann et al. conducted a genome-wide mutagenesis screen and identified SET1B as a required element to activate a HIF transcriptional response to hypoxia. They performed a mechanistic study defining how SET1B is recruited by HIF1a and brought into the nucleus where it binds to chromatin and amplifies transcription of HIF target genes (mostly angiogenic) through methylation and acetylation of histones in the promoters of HIF1 targets. Overall the manuscript is well written and experiments have substantial rigor. However, the following concerns need to be addressed prior to publication.

We are pleased that this reviewer appreciated our studies and have addressed their additional concerns.

Major:
1. The authors should provide additional information on the hits identified in their genome-wide mutagenesis screen outside of HIF components, SET1B, UBE2A and PPP4C? How might they be involved in a response?

We have now included a table of the other hits identified above the threshold (>3 -log10(p value)) (New **Supplemental Table 1**), totalling 17 genes. We selected all those with a -log10 p value > 4 for validation in alternate cell types (HIF1β, ABHD2, HIF-2α, SET1B and UBE2A). ABHD2 did not validate and has therefore not been pursued further. Most of the other top candidate genes encode LIM or Zn finger domains, and may be involved in transcriptional regulation. Two proteins are putatively involved in apoptosis (ATCAY and CYFIP2) and may be enriched due to decreased apoptosis following hypoxia. The remainder are involved in signalling or have unknown functions. We have included a discussion of these findings (**Results, p5, and Discussion, p17**), and their putative involvement is the subject of our ongoing studies.

2. In Figure 2, experiments demonstrate that SET1B is required in both transformed cells and non-transformed skin fibroblasts, suggesting that "normal" cells operate on the basis. More discussion is warranted on this point.

This is an important point, that we have now emphasised (**Discussion, p18**).

3. The authors assert that SET1B does not show bias between HIF1a/2a isoforms. In supplementary Figure 5, there was a striking difference on the influence of SET1B depletion between the HIF1a and HIF2a reporters. What explains this difference? How can the authors make the claim that there is no isoform bias, when the data in this figure looks different between the two reporters.

The difference in the activation of the HIF isoform reporters relates to lower levels of HIF-2 in HeLa cells, rather than isoform specificity. We have clarified this in the text (**Results, p9**). We have also included data using the renal cell carcinoma 786-0 cells, which do not express HIF-1α and have constitutive HIF-2α expression due to a VHL mutation. SET1B loss prevents full activation of HIF-2α target genes in these cells, indicative of no isoform bias (**New Supplementary Figure 5h, i**).

4. HIFs have influence on a broad range of cellular processes. In Figure 4/supplementary Figure 7, the authors assess the effects of SET1B loss in vitro on cell proliferation, growth and apoptosis. This analysis would be improved by assessing cell growth over a longer time course, in addition to cell motility and in 3D spheroid cultures.

Thank you for these helpful suggestions. We have now included a longer time course in hypoxia, but could not proceed beyond 4 days as the cells die in the control conditions (**New Figure 4a**). We have not found cell motility assays a particularly useful model in hypoxia but we now include spheroid studies in the HeLa HIF-reporter cells. These demonstrate loss of integrity of the spheroids following SET1B depletion, consistent with our findings with HIF loss in these 3D cultures (**New Figure 4c-e, Supplemental Figure 7k**). Together, these results further support the involvement of SET1B in the HIF response.

5. To assess the effects of SET1B loss in vivo, the authors perform constitutive depletion in a subcutaneous xenograft model. No alterations were found in tumor growth, but a delay in tumor initiation was observed. Conditional depletion of SET1B would allow the authors to more directly understand the role of SET1B in tumor angiogenesis, bypassing its potential effect on tumor initiation. Moreover, the data on blood vessel content was rather modest.

We have now undertaken further studies on the blood vessel content in the A549 cells. These studies show decreased vessel density and size in the SET1B and HIF1β deficient tumours compared to the controls (**New Figure 4l, m, Supplementary Figure 9c**). We also established the 786-0 xenograft model as a further measure of an angiogenic HIF dependency, which recapitulates the effects seen with the HeLa and A549 xenografts (**New Figure 4n, o**) (please also see comments to Reviewer 1).

Whilst the idea of conditional depletion is attractive, it is complex to establish and interpret due to the timing and extent of the depletion, which is difficult to control in an *in vivo* setting. However, this is an area we are actively pursuing for our future work. We would also emphasise that we are not suggesting that SET1B is only involved in HIF

mediated angiogenesis, but that this is one pathway that has a requirement for SET1B.

6. Although the association between SET1B and HIF2a is less prominent compared to its other isoform, does SET1B bind to HIF2a through the PAS domain? The predominance of either isoform can be tumor type specific. Also, can HIF2a promote SET1B accumulation on chromatin at HIF target genes as observed with HIF1a?

The 786-0 studies confirm that SET1B is still required for a HIF-2α response and we still see accumulation of SET1B on chromatin in these cells when they are reconstituted with VHL (**New Supplementary Figure 10l**). How HIF-2α binds SET1B forms a major part of our ongoing studies, and we are exploring this also in relation to HIF-2α inhibition. This requires a structural and biochemical approach and is beyond the scope of this manuscript.

Minor:
1. In Figure 3D, the three groups are represented by red, green and grey. It is difficult to discern between the three groups in this figure. A similar color scheme was utilized in other figures and is problematic for people with color blindness. Different colors for these figures are recommended.

We did consider colour-blindness and used a colour palette that was meant to distinguish between the type of red/green colour used, but we agree that they were still problematic, and we have altered the colour scheme throughout. We have also displayed Figure 3D (**now Figure 3e**) in a clearer format.

Reviewer #3:
Remarks to the Author:
Ortmann and coworkers have performed a fluorescent reporter-based genome-wide mutagenesis screen in human cancer cells aimed at identifying new genes required for HIF-dependent transcription in hypoxia. They found that the histone methyltransferase SET1B is necessary for transcription of a subset of HIF target genes in hypoxia, which in turn results in largely impaired tumorigenesis in SET1B loss-of-function mouse xenografts. SET1B is recruited to the chromatin in hypoxic conditions in a HIF-dependent manner, resulting in H3K4 trimethylation at promoters of a subset of HIF targets. HIF binding to the chromatin does not depend on SET1B. Interestingly, SET1B knock-out prevents histone acetylation at the promoters where SET1B mediates methylation.
The current study is an elegant and complete work that provides important insights into HIF-dependent gene expression in hypoxia. The data are sound, and the experiments well-conducted and carefully controlled. I don´t feel that additional experiments are needed before the manuscript can be accepted for publication.
I have just a conceptual comment that the authors may want to consider to modify part of the discussion and perhaps, a couple of sentences at the Introduction: Chromatin modifications and their regulators (histone methyltransferases; demethylases, acetyl transferases, deacetylases, etc) are essentially promoter-specific rather than transcription factor-specific. Gene specificity of chromatin regulators is therefore the rule rather than the exception. At the Discussion (lines 353 to 367) the authors attempt to explain or speculate on why only a limited subset of HIF target genes is affected by SET1B loss-of-function, and what is the source of such specificity. Given the current general knowledge on chromatin regulators, a general effect on HIF-dependent gene expression is not expected at all. In line with this, it is surprising that people in the hypoxia field generally consider the histone acetyl transferase CBP/P300, as well as the dioxygenase FIH, general regulators of
HIF-dependent transcription. There is no proof for this, and a histone acetyl transferase (CBP/P300) is not expected to have a global effect on HIF-dependent gene expression. In fact, FIH KO mice have only minor defects on HIF-dependent transcription. The authors might want to take this into consideration, and modify the relevant sentences accordingly (specially at the Discussion).

We thank this reviewer for their appreciation of our work and their helpful comments. We agree that this notion of gene-specificity is important and helpful to discuss, and emphasise the role of chromatin regulators in terms of being promoter-specific, rather than just being reliant on transcription factors (**Introduction, p4** and **Discussion, p19**).

Minor points and typos:
1- Please move the Venn diagram of Supplementary Fig. 4A to a main figure. It is a good summary of the effect of SET1B on hypoxia-dependent gene expression, and should be in a main figure.

2- Line 266: Supplementary Figure 8i, j instead of 9i, j

3- Line 298: “Figure 7e” is repeated. Please delete.

4- Line 420: Please delete “(a gift from Patrick Maxwell)” PHM is co-author.

We have moved the Venn diagram to the main figure and corrected the other points.

Reviewer #4:
Remarks to the Author:
Ortmann et al

This is a well written and well executed paper that clearly shows that SET1B modulates the HIF response and that SET1B is recruited to a subset of HIF targets in a HIF-dependent manner, leading to increased histone methylation and gene expression. It is a very nice contribution to the field.

We are pleased this reviewer recognised the contribution our work makes to the field.

Major comments:

1. The negative controls could be better described in the text. For example, were the negative controls for the sgRNA experiments (e.g. Fig 1f) untreated cells, cells treated with a non-cutting sgRNA, cells treated with an irrelevant sgRNA? Important when looking at subtle differences. For the tumor xenograft experiments, were the controls untreated cells, cells infected with irrelevant sgRNAs and put through same drug selection protocol, something else?

We have included a clearer discussion of the negative controls. We always use both negative and positive controls for our sgRNA validation experiments, and show that sgRNA targeting β2m (MHC Class I component) does not alter HIF activity in hypoxia or with DMOG treatment (**New Supplementary Figure 1b-d**). The tumour xenograft experiments used control untreated cells, as we did not observe any change in growth assays with the sgRNA control (β2m) (**New Supplementary Figure 8c**).

2. Fig 2i and 2k don’t support the claim that WDR82sgRNA inhibited the HIF response “similarly” to SET1b loss. First, the WDR82KD sgRNA is decreasing HIF1A protein (see Fig. 2k), which confounds things. Second, the leftward shift in the rightward peak in Fig 2i is barely perceptible. Finally, in Fig. 2i there is a small subpopulation of cells that don’t fire the reporter at all. Are the authors including the second observation as further evidence that WDR82 inhibits the response? I fear it might be technical if you compare the SET1B sgRNA data in Fig 2i to 1f and S1. This is such a minor point to the paper I wonder if the authors wouldn’t be better served removing it or softening their conclusions

WDR82 depletion is quite toxic to the cells, which has made these experiments more challenging, and HIF-1α is reduced in line with a reduction in SET1A activity (**Supplemental Figure 7d**). The HIF reporter often shows a bimodal distribution, and we do see a dual effect as observed with WDR2 loss, where there is shift of the main population and a separate GFP low group. This is not a technical issue, but is observed with PHD inhibition with DMOG, likely due to the reporter also encoding part of the HIF-1α ODD, which can dimerise. This also occurs with SET1B depletion (**Supplementary Figure 3a)** and is more pronounced in the isoform specific reporters (**Supplemental Figure 5g**). We have moderated our conclusions with respect to WDR82 (**Results, p8**).

3. I think Fig S5g is hard to interpret. The HIF2 ko cells look like the parental cells, suggesting HIF1 is the major driver in these cells of the hypoxic response. It looks like in the HIF1 ko cells the major effect is that most of the cells don’t induce the reporter in response to DMOG at all but the subpopulation that does seemingly does so as well as the parental cells despite SET1B sgRNA. Are these the cells that escaped editing? Just not sure I agree that SET1B sgRNA is decreasing HIF response in the HIF2 only cells.

We agree that HIF-1α is the major driver in these cells, but they do express HIF-2α and it does contribute to the activation of the reporter (**Supplementary Figure 5g**). The bimodal activation of the reporter is particularly evident with the HIF-2α reporter (HIF-1α KO), which is clonal and has not escaped editing. We now also include experiments in 786-0 cells that only express HIF-2α, and still observe the involvement of SET1B (**New Figure 4n, o, Supplementary Figure 5h, i**)(please also see earlier comments to Reviewers on 786-0 cells and HIF-2α).

4. The mapping in Fig 5 is not very compelling. The binding in Fig 5e largely tracks with the level of abundance in Fig 5d. A worrisome sign."1-396" is comigrating with a background band in Fig 5e and the 1-78 might have been cut off/run off. I would consider removing the mapping data and strengthening that binding of SET1B to HIF is robust and specific (especially as the signals in Fig S8b and 8d for SET1B and HIF (VHL298 lane) are just at the level of detection). For example, is there a nuclear TF that doesn't coIP with SET1B or CFP1? Can recombinant HIF bind specifically to recombinant SET1B?

We agree that the binding assays could have been clearer, and we now include further images of the initial experiments, and new binding assays to delineate the involvement of the PAS domain region (**New Figure 5d, e**). The association with the PAS region does not track with the protein levels of the HIF-α truncation mutants, and the 1-78 construct has not been cut off (full uncropped images are also included in the **Supplementary Figures**). Further truncation constructs show that SET1B only associates when both PAS-A and PAS-B are present and not to PAS-A alone (**New Figure 5e**). It has not been technically possible to obtain a PAS-B domain only construct and we have moderated our conclusions in line with our findings. We agree that recombinant studies may be helpful, and these biochemical and structural studies form part of our ongoing work.

We have also included a chromatin bound complex that does not interact with SET1B (**New Supplementary Figure 10d**).

Minor Comments:

1. Line 46. If going to cite ref. 3 should probably cite Bruick and McKnight 2001 Science paper and if going to cite ref. 5 should probably cite Jaakkola 2001 Science
These have been added.
2. Line 58. I think the authors are referring to FIH-1
This has been corrected.
3. Line 67. Why "However, this situation is complex as recent…"? You would expect an increase in histone methylases if the demethylases are inhibited by oxygen. The wording makes it sound like this is unexpected.
Agreed. this has been rephrased.
4. Line 80. For clarity, should be "through HIF-1alpha's PAS-B domain"
Corrected.
5. Would be useful to know if the mCherry reporter cells used for the screen were clonal or not.
The mCherry reporter is clonal prior to Cas9 being transduced, but we do not generally find the need to use Cas9 clones. This has been included in the **Methods**.
6. In Fig S2a, the CA9 band the SET1B KD cells doesn't align with either the 8 hour or 24 hour lanes. And are all these blots really from the same gel (hence justifying a single loading control blot)?
These are all from the same blot and correctly aligned. The uncropped images are now included in the **Supplementary Figures**.
7. Line 143. S3c, not S3e.
Corrected.
8. For my taste Fig 2i is pretty preliminary with respect to specificity controls.
The association of SET1B with WDR82 and CFP1 is well substantiated in the literature (e.g. PMID 17355966).
9. Rather hard to see the different colors for the SET1B and HIF1beta dots in Fig . 3d.
We agree and have changed the colour scheme.
10. In Fig 3f and S4e, there actually does appear to be a possible effect of SET1B knockdown on BAP1 mRNA levels. I suspect is currently "ns" (and should be marked as such), but wonder if would become significant with more replicates. And note that there are similar differences, now "significant", in Fig S7d

I think the confusion here relates to the significant changes in Figure S7d (now **Supplementary Figure 8g**) being SET1A and not SET1B. We have not observed any change in BAP1 mRNA with SET1B loss but SET1A loss decreases BAP1, consistent with SET1A having more general role in transcriptional regulation.

11. Line 206. I wouldn't call the downregulation of SET1B protein in Fig 4c and 4d "marked".
This has been amended.

12. The increase in cleaved PARP in Fig S7g in SET1B kd cells is underwhelming.
Along with the more quantitative measure of Caspase activity (**Now Supplementary Figure 8i**) this is consistent with an increase in apoptosis.

13. Line 226. Clarify is subcutaneous injection.
Correct. This has been amended.

14. Clarify in text and/or legend that tumors for 4i and 4j were harvested when they reached 1000 mm3. Yes?
Yes, and this has been included in the figure legend.

15. Line 243. I would be careful saying binding to HIF1A is more prominent than to HIF1B, because do not know the relative binding affinities of the two respective HIF antibodies.
This statement has been modified.

16. Line 266. Supplementary Fig 8i,j not 9i,j
This has been corrected.

17. Can you indicate p values for Fig 7d and 7e?
These have been included.

18. The differences in H3K4 methylation at the PHD3 and VEGF promoter are subtle, especially for SET1B kd. Can the reader be given a sense of the reproducibility of these patterns?
The H3K4me3 changes are very similar between the two biological ChIP-seq replicates, and the predominant effect of SET1B loss is the decreased extension of H3K4me3 across the gene body (**New Figure 7d, Supplementary Figure 12a**). We now also demonstrate that this occurs across multiple sites (**New Supplementary Figure 11i**). It is interesting that a relatively small change in H3K4me3 can result in larger changes in gene transcription, and it is possible that SET1B has additional functions that are yet to be fully determined (**Discussion, p19**).

19. Line 353. Isn't VEGF driven by HIF1 in most cell types? Where were the data on PLIN2 and NDRG.
We actually find that VEGF can be driven by both HIF-1 and HIF-2, and this is shown with the 786-0 cells (**New Figure 3i, Supplementary Figure 5i**). PLIN2 and NDRG1 are downregulated by either HIF1β or SET1B loss in hypoxia (**New Figure 3f**).

20. Line 398. Why not target SET1B catalytic activity? And, as indicated above, I think the mapping data are weak.
Targeting SET1B catalytic activity is challenging, as catalytic activity of this family of SET proteins is dependent on the association with other complex subunits (e.g. WDR5–ASH2L) (PMID: 26886794, 28160335) and these are present in both MLLs and SET1A/B. We have strengthened the mapping data and we plan to explore in further biochemical and structural studies how SET1B interacts with the PAS domains.

Decision Letter, first revision:

Dear James,

Your Article, "SET1B facilitates activation of the hypoxia response through site-specific histone methylation" has now been seen by the original 4 referees. You will see from their comments below that while reviewers #1 and #3 are now satisfied, some important points are raised by reviewers #2 and #4. As much as possible, we encourage you to address these comments experimentally.

We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We therefore invite you to revise your manuscript taking into account all reviewer comments. Please

highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact me if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome. I would be happy to discuss the reviewers' comments in detail, if you wish.

When revising your manuscript:

*1) Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

Please use the link below to submit your revised manuscript and related files:

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Genetics is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Tiago

Tiago Faial, PhD
Senior Editor
Nature Genetics
https://orcid.org/0000-0003-0864-1200

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
I would like to congratulate the authors for the thorough revision they have performed. The manuscript has been considerably improved, both in its experimental part and in the discussion, and reports novel, clear and robust findings. I, hereby, believe that the manuscript now meets Nature Genetics' high standards.

Reviewer #2:
Remarks to the Author:
In the paper, "SET1B facilitates activation of the hypoxia response through site-specific histone methylation", the authors show that SET1B is important for HIF transcriptional activation. While the authors did address our concerns in "Major" revision suggestions 1-3, significant portions of revisions 4-6 were not completed. Bellow we have outlined our initial revision request (e.g. "R4"), the response of the authors (e.g. "A4", authors response) and experiments we feel need to be completed. Unfortunately, after reviewing the authors response to suggested revisions we don't feel that the paper is acceptable for publication at this time.

R4: HIFs have influence on a broad range of cellular processes. In figure 4/supplementary figure 7, the authors assess the effects of SET1B loss in vitro on cell proliferation, growth and apoptosis. This analysis would be improved by assessing cell growth over a longer time course, cell motility and in spheroid culture.

A4: "We have not found cell motility assays a particularly useful model in hypoxia, but we now include spheroid studies in the HeLa HIF-reporter cells."

My response to the authors: The authors failed to assess the influence of SET1B on cell motility. One

of the major readouts of HIF transcription is the regulation of genes involved in metastasis; therefore, we wanted the authors to determine if SET1B can influence HIF's control of cell motility in functional assays such as a scratch assay or a Boyden chamber assay. This should be well within the capabilities of their experimental setup.

R5: To assess the effects of SET1B loss in vivo, by constitutive depletion in a subcutaneous xenograft model. No alterations were found in tumor growth, but a delay in xenograft tumor initiation was observed. Conditional depletion of SET1B would allow the authors to more directly understand the role of SET1B in tumor angiogenesis, bypassing its potential effect on tumor initiation.

A5: "While the idea of conditional depletion is attractive, it is complex to establish and interpret due to the timing and extent of depletion, which is difficult to control in an in vivo setting. However, this is an area we are actively pursuing in our future work. We would also like to emphasize that we are not suggesting that SET1B is only involved in HIF mediated angiogenesis, but that this one pathway that has a requirement for SET1B."

My response to the authors: HIFs are critical in transcriptionally controlling angiogenesis during tumor development. The authors utilized a subcutaneous xenograft model of ccRCC with constitutive K/O of SET1B by CRISPR. While subcutaneous xenografts are important, it is difficult to assess tumor initiation with these models. To help differentiate between the effects of SET1B on tumor initiation and angiogenesis we asked the authors to perform a subcutaneous xenograft with conditional depletion of SET1B (e.g. shRNA under the control of doxycycline) to allow tumors to establish and then deplete them of SET1B. Instead, the authors provided an additional xenograft with 786-0 cells. Unfortunately, we feel that this falls short at helping to differentiate the influence of SET1B on tumor initiation and angiogenesis.

R6: Although the association between SET1B and HIF2α is less prominent than it's other isoform, does SET1B bind to HIF2α through the PAS domain? The prominence of either isoform can be tumor type specific; do the authors observe a predominance of HIF1α in multiple cancer types? Also, can HIF2α promote SET1B accumulation on chromatin at HIF target genes as observed with HIF1α?

A6: "The 786-0 studies confirm that SET1B is still required for a HIF-2α response and we still see accumulation of SET1B on chromatin in these cells when they are reconstituted with VHL (New Supplemental Figure. 10l). How HIF-2α binds SET1B forms a major part of our ongoing studies, and we are exploring this also in relation to HIF-2α inhibition. This requires a structural and biochemical approach and is beyond the scope of this manuscript"

My response to the authors: The authors failed to address suggested revisions above: how do SET1B and HIF2α physically interact; and can SET1B promote HIF-2α accumulation at HIF target genes? A few simple experiments could begin to demonstrate these points and then more in-depth analyses saved for future studies. Could the authors at least comment/speculate on whether the prominence of HIF-1α over HIF-2α isoform with SET1B is seen in other cancer types in the Discussion?

Reviewer #3:

Remarks to the Author:
My suggestions have been very well addressed, so I am now happy to recommend publication.

Reviewer #4:
Remarks to the Author:
The authors have satisfactorily addressed my earlier comments. As before, I think this paper is a very nice contribution to the field. My heart goes out to them, because I am not sure the review process in this case (and the substantial amount of additional work required) substantially improved the paper and, I would argue, in some cases might have detracted from it. Some minor comments for them to consider:

1. The authors can do what they want but I still think the WDR82 data adds very little in Fig 2. First, it doesn't speak to the SET1A vs SET1B specificity issue since it is shared by both (the heart of the relevant paragraph). Secondly, it is confounded by the lower HIF1 protein levels, which as the authors indicate might reflect toxicity. I might keep the CFP1 data, not because it speaks to A vs B, but because it supports that the SET1B result is real and that regulation of HIF by SET1B reflects its canonical function.
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9. For future reference, I think it is a bit dangerous to use parental tumor cells as controls in tumor xenograft experiments when comparing to genetically engineered derivatives. The latter cells have already been through a form of "selection" and the associated manipulations, including introduction of drug-selection markers, can affect their fitness and immunogenicity in vivo.

Author Rebuttal, first revision:

Response to Referees

Reviewer #1:
Remarks to the Author:
I would like to congratulate the authors for the thorough revision they have performed. The manuscript has been

considerably improved, both in its experimental part and in the discussion, and reports novel, clear and robust findings. I, hereby, believe that the manuscript now meets Nature Genetics' high standards.

We thank this reviewer for their appreciation of our work.

Reviewer #2:
Remarks to the Author:
In the paper, “SET1B facilitates activation of the hypoxia response through site-specific histone methylation”, the authors show that SET1B is important for HIF transcriptional activation. While the authors did address our concerns in “Major” revision suggestions 1-3, significant portions of revisions 4-6 were not completed. Bellow we have outlined our initial revision request (e.g. “R4”), the response of the authors (e.g. “A4”, authors response) and experiments we feel need to be completed. Unfortunately, after reviewing the authors response to suggested revisions we don’t feel that the paper is acceptable for publication at this time.

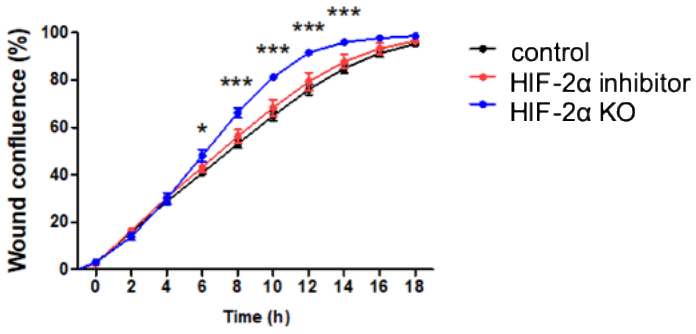
R4: HIFs have influence on a broad range of cellular processes. In figure 4/supplementary figure 7, the authors assess the effects of SET1B loss in vitro on cell proliferation, growth and apoptosis. This analysis would be improved by assessing cell growth over a longer time course, cell motility and in spheroid culture.

A4: “We have not found cell motility assays a particularly useful model in hypoxia, but we now include spheroid studies in the HeLa HIF-reporter cells.”

My response to the authors: The authors failed to assess the influence of SET1B on cell motility. One of the major readouts of HIF transcription is the regulation of genes involved in metastasis; therefore, we wanted the authors to determine if SET1B can influence HIF’s control of cell motility in functional assays such as a scratch assay or a Boyden chamber assay. This should be well within the capabilities of their experimental setup.

We should have been clearer as to why we do not find scratch assays helpful. Prolonged hypoxia in HeLa or A549 cells (55+ hr) is required to see any effect on wound closure, and we have only observed minor effects with HIF1B loss, therefore it is not a robust model.

In 786-0 cells, counterintuitive results have been observed, with HIF-2α loss resulting in slightly faster wound closure, and no effect observed with HIF-2α inhibition (illustrative figure from collaborator included (under revision), **Rebuttal Figure 1**). These changes also do not correlate with metastatic invasion or the anti-tumour effect of HIF-2α inhibitors in tumour models (e.g. PMID 27595393). Therefore, we do not agree that these studies would add any meaningful data to our manuscript.



Rebuttal Figure 1: Assay performed using the WoundMaker and IncuCyte ZOOM from Essen Bioscience. 18 wells per conditions per biological replicate, 3 biological replicates (figure courtesy of collaborator, under revision).

We already addressed all other points raised in R4, including the helpful suggestion of the spheroid cultures.

R5: To assess the effects of SET1B loss in vivo, by constitutive depletion in a subcutaneous xenograft model. No alterations were found in tumor growth, but a delay in xenograft tumor initiation was observed. Conditional depletion of SET1B would allow the authors to more directly understand the role of SET1B in tumor angiogenesis, bypassing its potential effect on tumor initiation.

A5: "While the idea of conditional depletion is attractive, it is complex to establish and interpret due to the timing and extent of depletion, which is difficult to control in an in vivo setting. However, this is an area we are actively pursuing in our future work. We would also like to emphasize that we are not suggesting that SET1B is only involved in HIF mediated angiogenesis, but that this one pathway that has a requirement for SET1B."

My response to the authors: HIFs are critical in transcriptionally controlling angiogenesis during tumor development. The authors utilized a subcutaneous xenograft model of ccRCC with constitutive K/O of SET1B by CRISPR. While subcutaneous xenografts are important, it is difficult to assess tumor initiation with these models. To help differentiate between the effects of SET1B on tumor initiation and angiogenesis we asked the authors to perform a subcutaneous xenograft with conditional depletion of SET1B (e.g. shRNA under the control of doxycycline) to allow tumors to establish and then deplete them of SET1B. Instead, the authors provided an additional xenograft with 786-0 cells. Unfortunately, we feel that this falls short at helping to differentiate the influence of SET1B on tumor initiation and angiogenesis.

We agree that further studies will be required to fully dissect the role of SET1B in tumour initiation versus angiogenesis, but this is not the focus of our manuscript. The 786-0 model was chosen as a well-established HIF dependent xenograft model (note original comments by Reviewer 1), and strengthens the involvement of SET1B with HIF-2. The HIF dependence of this model is evident with HIF1B loss preventing tumours establishing (**Figure 4n, o**). SET1B loss has an intermediate phenotype, which is entirely consistent with our cell based assays. We did extend all our analysis of the angiogenesis data, as suggested by this reviewer (original point R5), which demonstrates the involvement of SET1B in blood vessel formation in the tumours (**Figure 4l, m, Supplementary Figure 9c**).

We agree that the conditional model suggested would be a helpful future addition to explore the role of SET1B in cancer, but we reiterate that it is not straightforward, and would take a considerable amount of time. The full extent of how SET1B is involved in tumour metastases is a distinct question from our current studies.

R6: Although the association between SET1B and HIF2α is less prominent than it's other isoform, does SET1B bind to HIF2α through the PAS domain? The prominence of either isoform can be tumor type specific; do the authors observe a predominance of HIF1α in multiple cancer types? Also, can HIF2α promote SET1B accumulation on chromatin at HIF target genes as observed with HIF1α?

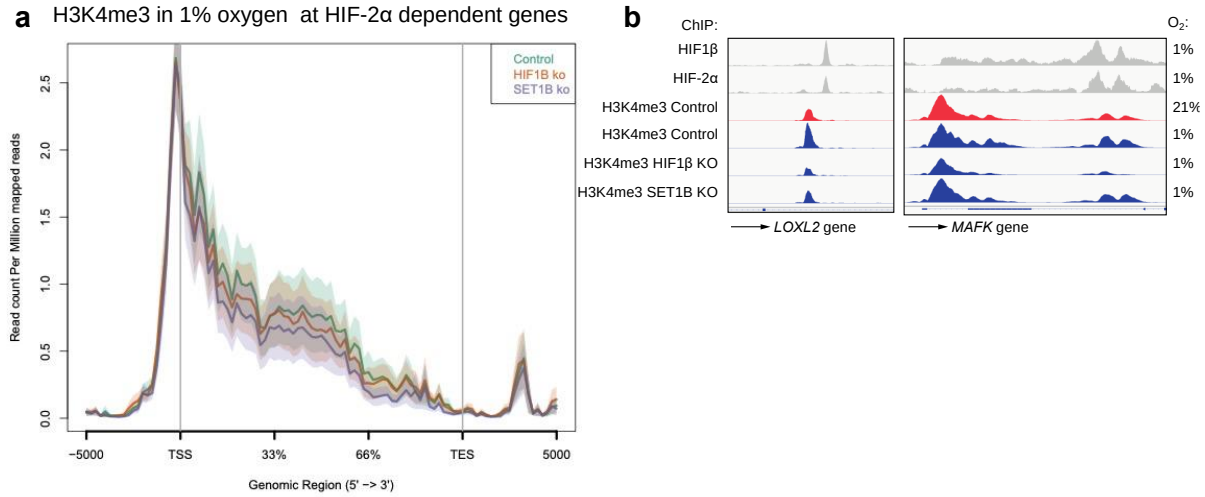
A6: "The 786-0 studies confirm that SET1B is still required for a HIF-2α response and we still see accumulation of SET1B on chromatin in these cells when they are reconstituted with VHL (New Supplemental Figure. 10l). How HIF-2α binds SET1B forms a major part of our ongoing studies, and we are exploring this also in relation to HIF-2α inhibition. This requires a structural and biochemical approach and is beyond the scope of this manuscript"

My response to the authors: The authors failed to address suggested revisions above: how do SET1B and HIF2α physically interact; and can SET1B promote HIF-2α accumulation at HIF target genes? A few simple experiments could begin to demonstrate these points and then more in-depth analyses saved for future studies. Could the authors at least comment/speculate on whether the prominence of HIF-1α over HIF-2α isoform with SET1B is seen in other cancer types in the Discussion?

The key point regarding SET1B was whether it was HIF isoform specific, and our data confirms that SET1B alters the activation of both HIF-1 and HIF-2 target genes (**Supplementary Figure 5**). We do not agree that it would be helpful to include preliminary/early studies on the HIF-2α/SET1B interaction, which are not directly related to our main findings. For interest though, our initial studies suggest that the interaction may be somewhat different to HIF-1α, and partially involve binding to HIF1B. SET1B loss also alters HIF-2α target gene expression in the context of HIF-2α

inhibition (which targets the PAS-B domain of HIF-2α). However, these are preliminary findings, and as we stipulated, are not central to the manuscript, and are the subject of ongoing work.

We assume that the authors meant whether HIF-2α can promote SET1B accumulation at HIF target genes (as in the original point R6)? Using HIF-2 only target genes (**Supplementary Figure 11g**) we find that SET1B loss decreases H3K4me3 across the gene body in HIF-2 only target genes, similarly to HIF1B loss (**Rebuttal Figure 1a**), in keeping with HIF-2α recruitment of SET1B (**Rebuttal Figure 2a**). This is entirely in keeping with the data already shown in the manuscript. We can also see this at individual HIF-2α target genes (**Rebuttal Figure 2b**). We have included this point in the revised text, indicating that the changes in H3K4me3 (i.e. SET1B function) are not HIF-α isoform specific (**page 16, and 19**).



Rebuttal Figure 2: (a) Metagene plot of H3K4me3 across the gene body in HIF-2α only dependent genes. **(b)** Representative analysis of ChIP-seq for HIF-2α, HIF1β and H3K4me3 using the IVG genome browser.

We have now also included a discussion regarding the prominence of the HIF-α isoforms and how their relative abundance may differentially recruit SET1B, allowing selective target gene activation (**page 19**).

Reviewer #3:
Remarks to the Author:
My suggestions have been very well addressed, so I am now happy to recommend publication.

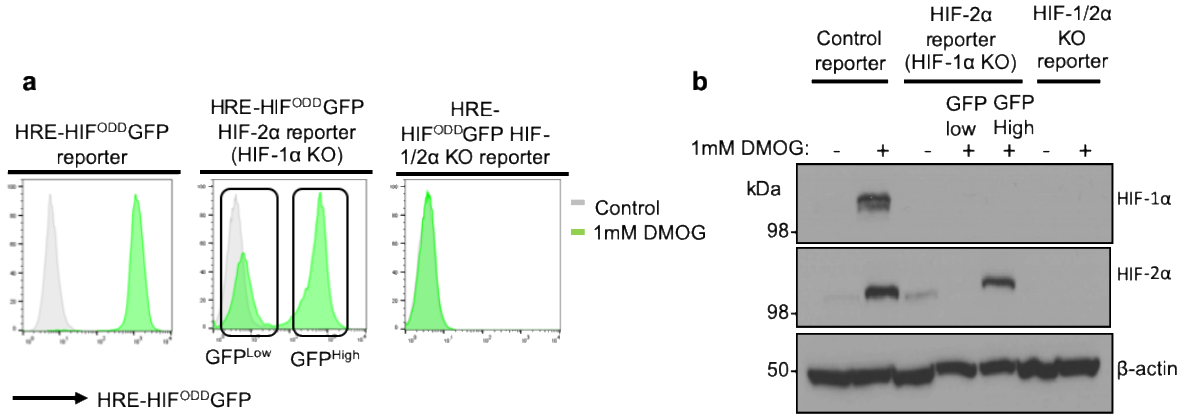
We thank this reviewer for the appreciation of our work and for their recommendation to publish.

Reviewer #4:
Remarks to the Author:
The authors have satisfactorily addressed my earlier comments. As before, I think this paper is a very nice contribution to the field. My heart goes out to them, because I am not sure the review process in this case (and the substantial amount of additional work required) substantially improved the paper and, I would argue, in some cases might have detracted from it. Some minor comments for them to consider:

We are pleased that this reviewer recognises the contribution this work makes to the field. We agree that it is important to maintain the central message of the paper and avoid tangential studies that detract from our main findings. We have addressed their additional helpful comments.

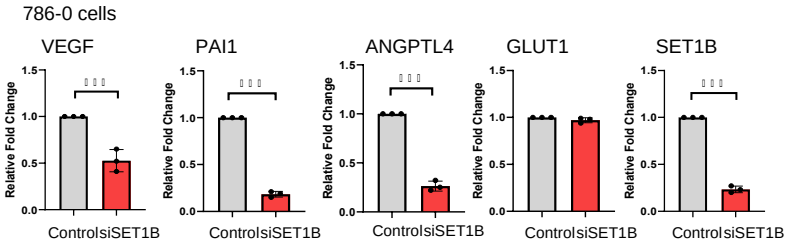
1. The authors can do what they want but I still think the WDR82 data adds very little in Fig 2. First, it doesn't speak to the SET1A vs SET1B specificity issue since it is shared by both (the heart of the relevant paragraph). Secondly, it is confounded by the lower HIF1 protein levels, which as the authors indicate might reflect toxicity. I might keep the CFP1 data, not because it speaks to A vs B, but because it supports that the SET1B result is real and that regulation of HIF by SET1B reflects its canonical function.
In light of the toxicity of WDR82 depletion, we have removed the WDR82 data, as suggested. However, we still include the reference to WDR82 toxicity in the text (**page 7, 8**).

2. I still don't understand the bimodal response if Fig. S5G in the HIF-1alpha KO cells. In the response the authors indicate the cells are "clonal", but have they sorted for the cells that induce the reporter and confirmed that HIF1 was edited? And I would still argue you can't say HIF2 is present at lower levels than HIF1 (at least not based on S5E) because that depends on antibody binding affinities and exposure times (among other things).
We can confirm that they are clonal and that there is no 'escape' as the HIF-2α KO clones were generated from the HIF-1α KO clones. The combined KO clearly shows that there is no activity of the reporter following HIF-2α loss in the HIF-1α KO clone (**Supplementary Figure 5f**). As suggested, we also confirm that cells sorted for GFP induction (GFP^{High}) by FACS do not show any detectable HIF-1α in the HIF-1α KO clone, but HIF-2α is stabilised (**Rebuttal Figure 3**). Therefore, HIF-1α has been edited, and the weaker induction of GFP compared to the HIF-2α KO cells (HIF-1α reporter) is consistent with HIF-2α being present at lower levels (**Supplementary Figure 5g**).



Rebuttal Figure 3: (a, b) HeLa HRE-HIF^{ODD}GFP reporter cells, clonal HRE-HIF^{ODD}GFP HIF-1α KO reporter, or clonal HRE-HIF^{ODD}GFP HIF-1/2α KO reporter cells were treated with 1 mM DMOG for 24 hr. The HIF-1α reporter cells were then sorted by FACS into GFP^{Low} or GFP^{High} populations **(a)**. HIF-1α and HIF-2α levels were measured by immunoblot in the reporter cells, and sorted populations **(b)**.

3. S5I ok but on thin ice here. "VEGF" is repressed but "GLUT1" not. VEGF is decreased less than 50% and GLUT1 looks like decreased 20% (realize not significant with this number of replicates).
We have also undertaken SET1B depletion using siRNA in 786-0 cells, which reproducibly shows that GLUT1 expression is not altered by SET1B loss (**Rebuttal Figure 4**). We would prefer to include the CRIPSR studies for continuity with the rest of our studies, as the change in GLUT1 is not significant in either method.



Rebuttal Figure 4: siRNA depletion of SET1B in 786-0 cells, showing no effect of SET1B loss on GLUT1 expression.

4. Minor point: in Fig S7C, doesn't look like drugs are increasing acidification unless I am misreading.
The predominant effect of HIF activation is an increase in glycolytic rates, and this is measured by the glycoPER assay, which uses both the OCR and ECAR to determine the glycolytic proton efflux rate (a real-time measurement of glycolytic rate). We have included the OCR, ECAR and glycoPER in **Supplementary Figure 7** for completeness, but acidification alone cannot be used to determine glycolysis. The key point is that HIF1B prevents the increase in glycoPER following PHD inhibition, but this is not observed with SET1B depletion, consistent with SET1B loss not altering glycolysis. We have clarified this in the text (**page 9, 10**).

5. Fig S10E is very weak- the recovery of HIF1 mirrors its total abundance so this experiment really needed more specificity controls (first time using VH298 in the paper). I think S10E/F are rather tangential to the story anyway.
We agree that these experiments are tangential and have removed the data. Other data already presented in the manuscript shows that prolyl hydroxylation is not involved in SET1B and HIF-1α binding, as binding occurs through the PAS domain and not through the oxygen degradation domain (**Figure 5c-e**).

6. Fig S10J really pushing the envelope of credulity (subtle differences) and again this is tangential anyway.
We would prefer to still include this data, and now provide a further replicate of the Roxadustat experiment and quantification of SET1B localisation (now **Supplemental Figure 10i, j**). These findings show that PHD inhibition is sufficient to result in SET1B accumulation on chromatin, and are consistent with the changes observed following DMOG treatment (**Figure 5h**).

7. Line 363. Should be “from the promoter region to the gene body in SET1B KO cells”. Yes?
Yes, this has been corrected.

8. Line 411. Do the authors mean “this relocalization also occurs” rather than “this relocalization still occurs”. As written, sounds like test hypoxia plus PHD inhibitors in combination.
We mean ‘also occurs’, and this has been corrected.

9. For future reference, I think it is a bit dangerous to use parental tumor cells as controls in tumor xenograft experiments when comparing to genetically engineered derivatives. The latter cells have already been through a form of “selection” and the associated manipulations, including introduction of drug-selection markers, can affect their fitness and immunogenicity in vivo.
We note this point. We did not observe any difference using control sgRNA against β2m or with or without Cas9 in assays of growth, spheroid culture or activation of HIF target genes. The parental tumour cells have been through the Cas9 selection, similarly to the mixed KO cell lines.

Decision Letter, second revision:

Our ref: NG-A55978R1

22nd Apr 2021

Dear James,

Thank you for submitting your revised manuscript "SET1B facilitates activation of the hypoxia response through site-specific histone methylation" (NG-A55978R1).

My colleagues and I find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Genetics, pending minor revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics. Please do not hesitate to contact me if you have any questions.

Congratulations!

Sincerely,

Tiago

Tiago Faial, PhD
Senior Editor
Nature Genetics
https://orcid.org/0000-0003-0864-1200

Final Decision Letter:

In reply please quote: NG-A55978R2 Nathan

14th May 2021

Dear James,

I am delighted to say that your manuscript "The HIF complex recruits the histone methyltransferase SET1B to activate specific hypoxia-inducible genes" has been accepted for publication in an upcoming issue of Nature Genetics.

Prior to setting your manuscript, we may make minor changes to enhance the lucidity of the text and with reference to our house style. We therefore ask that you examine the proofs most carefully to ensure that we have not inadvertently altered the sense of your text in any way.

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Sincerely,

Tiago

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