

Reviewer Report

Title: Genome-wide determination of on-target and off-target characteristics for RNA-guided DNA Methylation by dCas9 methyltransferases

Version: Revision 1 **Date:** 07 Aug 2017

Reviewer name: Tamir Chandra

Reviewer Comments to Author:

Ln 400:

I am still unsure as how representative selected loci to study off-target effects are. The authors could maybe calculate the number of loci in the genome that have similar number of mismatches as the selected loci and compare them in their genome-wide data.

Ln 469:

The authors mention fluctuations of methylation states in cancer cells. The study they are citing follows cells over many generations (up to 300) and is therefore not a suitable comparison. I am not clear how stochastic changes of methylation, as the authors claim, would be seen in a population of cells within the time scales the experiments were conducted. I still think we cannot exclude that the potential off-target effects are an expression of noise between experiments and with only one replicate, there is no way that the authors can back up their claims. Although the number of hypomethylated regions has gone down, it is still significant, at a similar level to the hypermethylated regions, weakening the argument that the hypermethylated regions observed are results of CRISPR targeting. I believe that the authors are looking at noise/technical variation between two data-sets.

495 onwards

Correlating gain of methylation to open chromatin regions is very descriptive and often subject to confounding factors.

I believe that the study does not provide enough evidence nor is it conducted with the required statistical power to quantify the extent of off-target effects of CRISPR targeting.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? No

Conclusions

Are the conclusions adequately supported by the data shown? No

Reporting Standards

Does the manuscript adhere to the journal's guidelines on [minimum standards of reporting](#)? Yes

Statistics

Are you able to assess all statistics in the manuscript, including the appropriateness of statistical tests used? No, I do not feel adequately qualified to assess the statistics.

Quality of Written English

Please indicate the quality of language in the manuscript: Acceptable

Declaration of Competing Interests

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