Review of: "Altered DNA repair pathway engagement by engineered CRISPR-Cas9 nucleases"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

“Altered DNA repair pathway engagement by engineered CRISPR-Cas9 nuclease” by Chauhan et al is a very interesting (and timely) study that aims to reduce in/del frequency in CRISPR editing by identifying Cas9 mutant variants. This work will help improve gene targeting and clinical trials of gene therapy.

1. Analysis of the structure of a DNA double strand break induced by vCas9 will be very helpful.
If authors identify the types of the DNA double strand break induced by vCas9, it will help deduce how vCas9 cause more HR and microhomology repair over NHEJ. Because the insertion events are not decreased by vCas9, but small deletions are decreased among the product, the 5’ end of DNA might be more protected.

2. HEK293T cells with MDR templates clearly increase precise repair. However, in non-dividing primary fibroblasts, the precise editing is not decreased albeit the Indels are decreased. The increase in the precise editing by microhomology is not clear.
I wonder if the resection at S and G2 phase might increase to produce much more microhomology mediated repair by vCas9. It might explain why vCas9 decreases indel at non-dividing cells.

3. PolQ siRNA treatment for Fig4b experiment with dividing HEK293T cells will be needed to validate MMEJ mediated repair.