Modulation of the DNA damage response to enhance targeted integration at CRISPR/Cas9-mediated DNA double strand breaks

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Abstract

The low efficiency of gene knock-in (KI) events at CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-associated protein 9 (Cas9) induced DNA Double Strand Breaks (DSBs) through Homology Directed Repair (HDR) represents a major obstacle in precise genome engineering. The interplay between CRISPR/Cas9 systems and eukaryotic DNA Damage Repair pathways (DDR) is poorly understood.

We studied the interaction between Cas9 and the cellular DNA repair machinery to improve the efficiency of Cas9-dependent KIs in eukaryotic cells. The study contributes to our understanding of target-engagement of compounds interacting with the DDR pathways. The findings have a number of important applications for cell lines generation and cell & gene therapy where high KI efficiencies are needed.

Introduction

- We evaluated the potential 19,516 compounds to enhance the efficiency of genome editing
- Effective compounds were further evaluated using deep targeted sequencing followed by analysis with Rational Indel Meta-Analysis (RIMA)[1] *for knock-in*

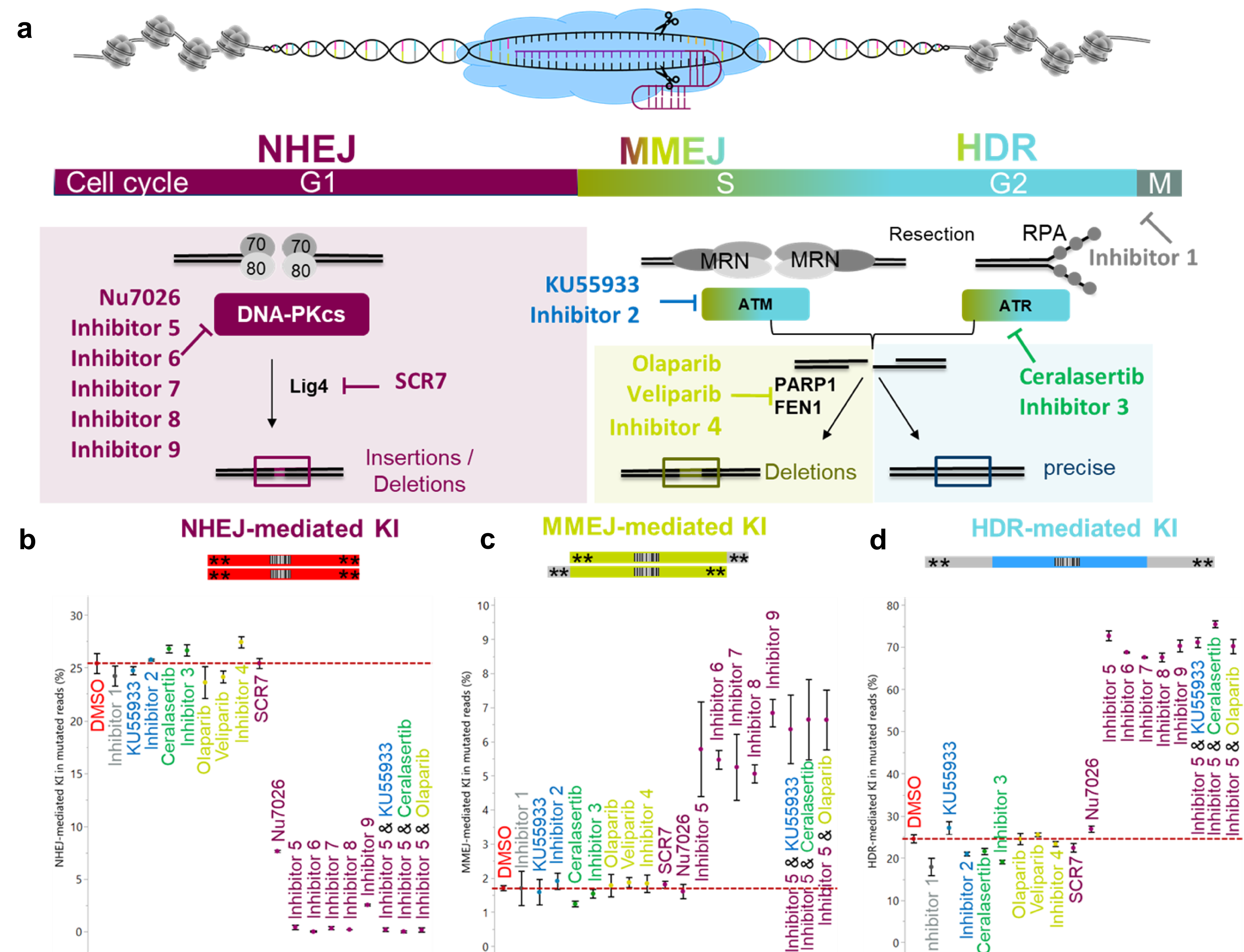

Figure 1. Major mammalian DNA damage repair pathways on Cas9-induced DSBs and *RIMA for knock-in* analysis of deep targeted amplicon sequencing data to characterize and quantify DNA repair pathways at Cas9-induced DSBs.
a) Non-Homologous End Joining (NHEJ), Microhomology-mediated End Joining (MMEJ), Homology Directed Repair (HDR). **b,c,d)** Cells were treated with different inhibitors targeting DDR enzymes and DMSO control. Points represent average NHEJ-mediated, MMEJ-mediated & HDR-mediated knock-in efficiency in fraction of modified reads. ± SD (n=3).

Methods

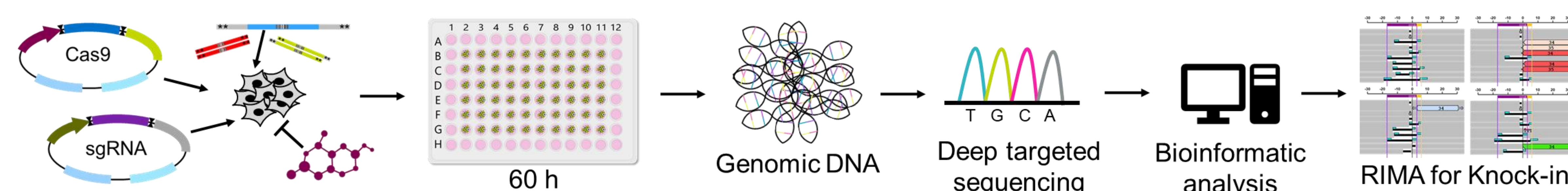

Figure 2. Experimental procedure to study DNA repair profiles following Cas9 cleavage.

Results

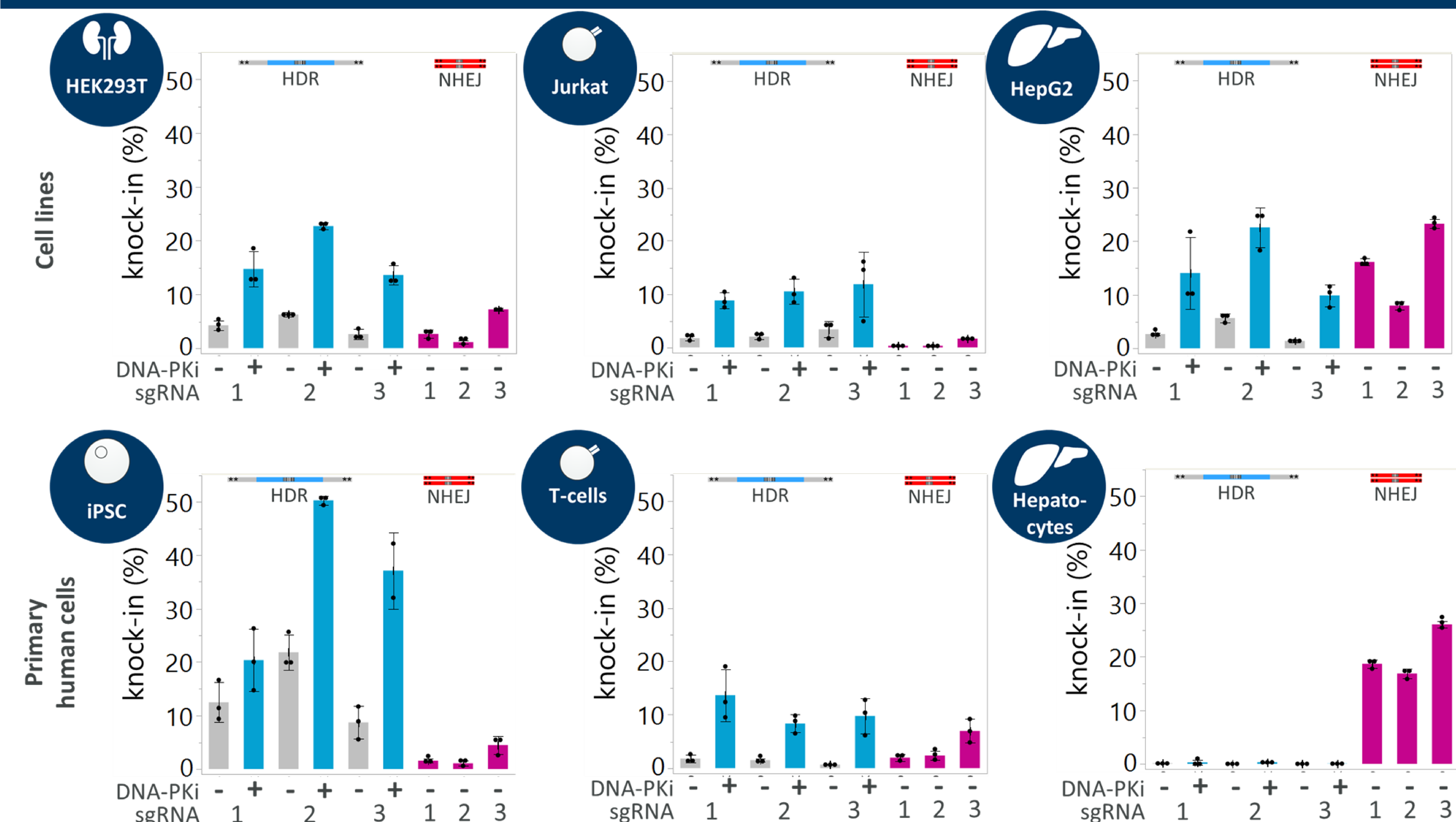

Figure 3. RIMA *for knock-in* analysis of amplicon-sequencing data to quantify different KI strategies in several cell types.
Cells were treated with different DNA-PK inhibitor and DMSO control. Bar graphs represent average NHEJ-mediated and HDR-mediated knock-in efficiency in mapped reads. ± SD (n=3). Induced Pluripotent Stem Cells (iPSC).

Conclusion

- We developed a new tool "RIMA for knock-in" to study the effects of compounds on DNA repair pathways and Cas9-mediated knock-ins
- Among the drugs tested, DNA-PK inhibitors showed the most profound effect on the preference of the DNA repair pathway: nearly no NHEJ-mediated knock-ins were detected, while MMEJ- and HDR-mediated knock-ins were increased up to 15.1-fold
- Careful evaluation of different KI strategies in the cell type of interest will maximize KI efficiencies: high levels of HDR-mediated KI was measured in iPSC, whereas in postmitotic primary hepatocytes only NHEJ-mediated KI was detected

References

1. Taheri-Ghahfarokhi, A. et al. Decoding non-random mutational signatures at Cas9 targeted sites. Nucleic Acids Res (2018).

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