Minimal activation of the p53 DNA damage response by a modular cytosine base editor enables effective multiplexed gene knockout in induced pluripotent stem cells

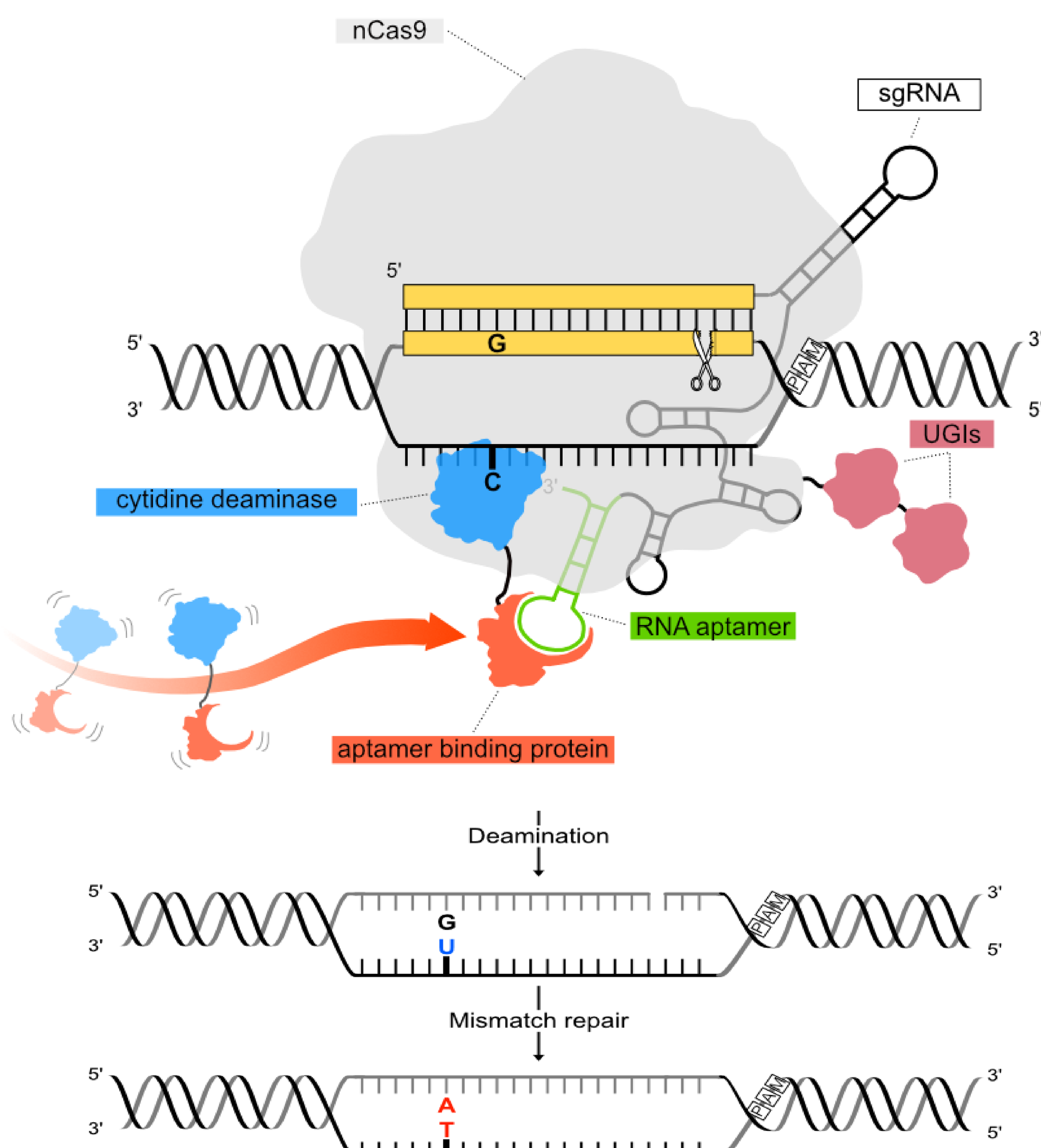
Robert Blassberg, Olga Mielczarek, Jesse Stombaugh, Immacolata Porreca

Visit poster P0625 to learn how we use the Pin-point platform to engineer hypoimmunogenic iPSCs.

1 Abstract

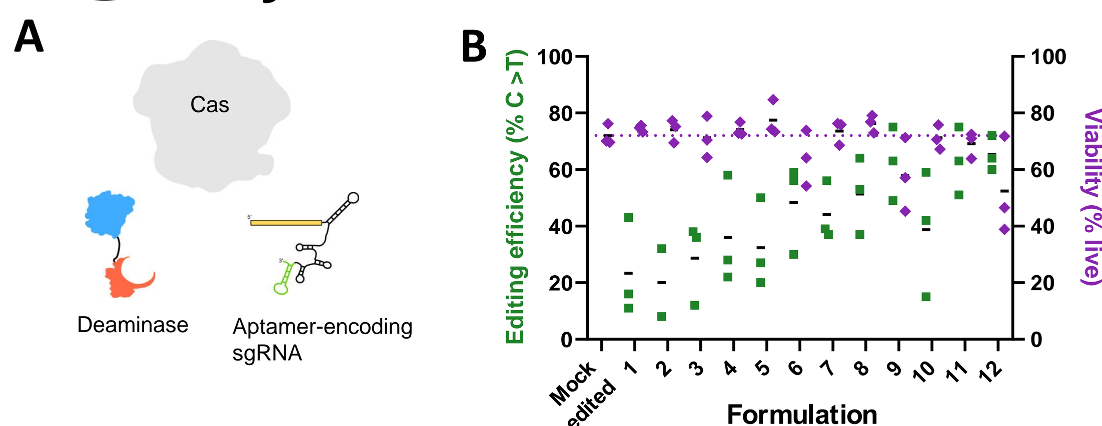
Precise genome editing of induced pluripotent stem cells (iPSC) holds great promise for engineering advanced cell therapies. CRISPR-Cas systems have been widely adopted in genome engineering applications, however their dependence on genotoxic DNA double strand breaks (DSBs) presents challenges in hypersensitive iPSCs, including the selection for defective DNA DSB responses. Base editors are capable of both modifying and ablating gene function without generating DSBs making them an attractive solution in iPSC engineering applications. Here we report efficient and durable target knockout and substantially improved cell viability and expansion with a cytosine base editor assembled using the Pin-point™ platform compared to SpCas9. The cytosine base editor minimally activated p53-mediated DNA damage signalling independently of the number of simultaneous edits installed. By contrast, multiplexed editing with SpCas9 lead to high levels of p53 signalling and an associated reduction in editing efficiency. While transient inhibition of the p53 DNA DSB response enhanced SpCas9 multiplexed gene editing efficiency this was not required to achieve maximal base editing efficiency. Multiplexed editing of iPSCs with Pin-point base editors therefore both enhances the efficiency of genome engineering processes and substantially reduces the risk of selection for defective DNA damage responses inherent to DSB-dependent CRISPR-Cas systems.

2 The modular Pin-point base editing platform



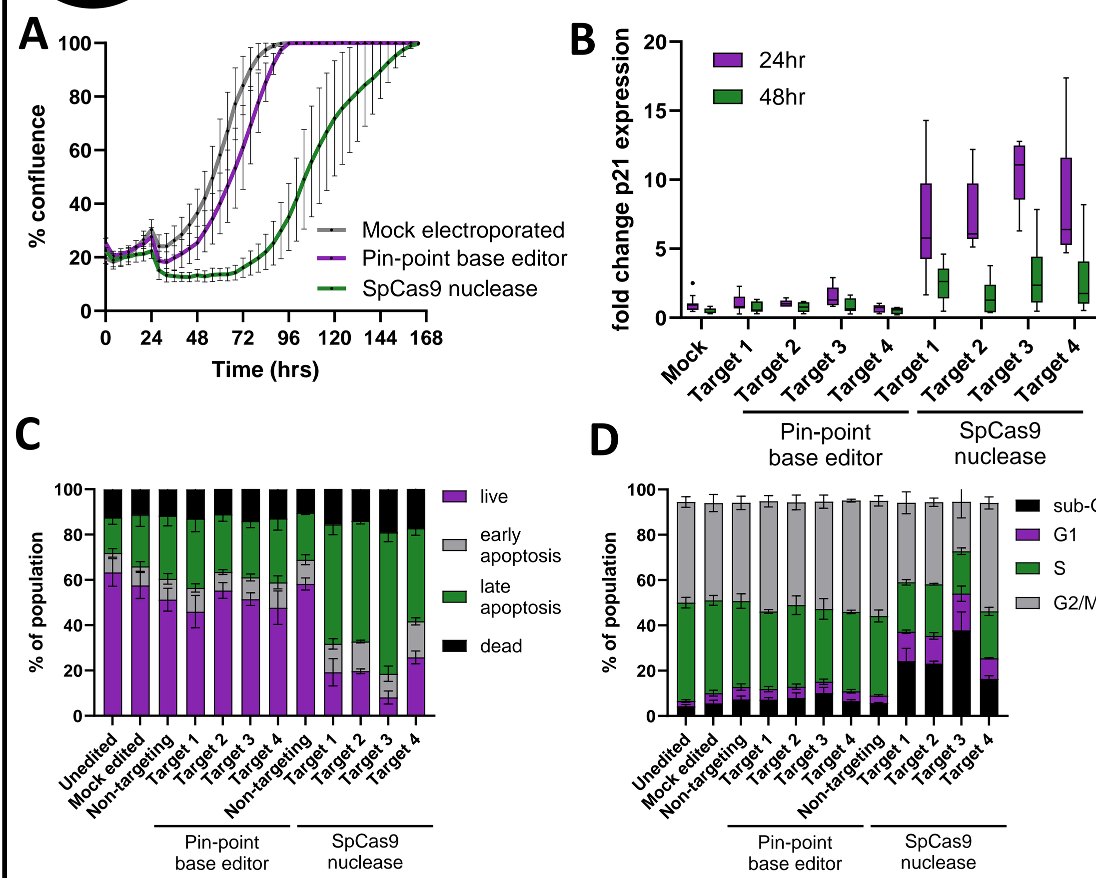
The Cas component is recruited to the DNA target sequence via a single guide RNA (sgRNA) encoding an aptamer in the scaffold region. The aptamer recruits a DNA-modifying deaminase to the DNA target sequence via an aptamer binding protein. The three independent components of the system can be configured according to editing requirements and delivered to cells either as mRNA and synthetic sgRNA.

3 Optimisation of a Pin-point cytidine base editor



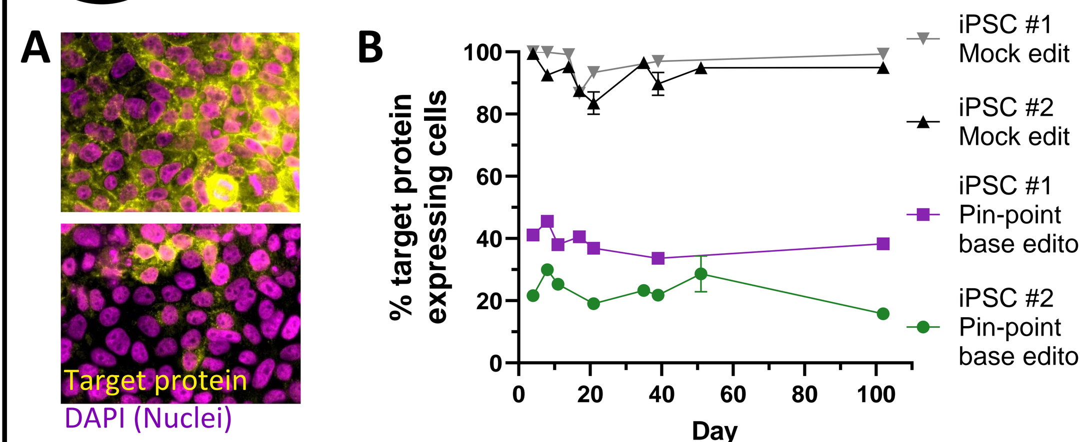
A) The three components of the Pin-point platform can be formulated to obtain base editors with the required properties.
B) The ratios of the nSpCas9-UGI mRNA, Rat APOBEC1 deaminase mRNA, and synthetic sgRNA components were varied to define reagent formulations that optimise base editing efficiency and cell viability in iPSCs. n=3 iPSC donors.

4 Enhanced cell viability and proliferation



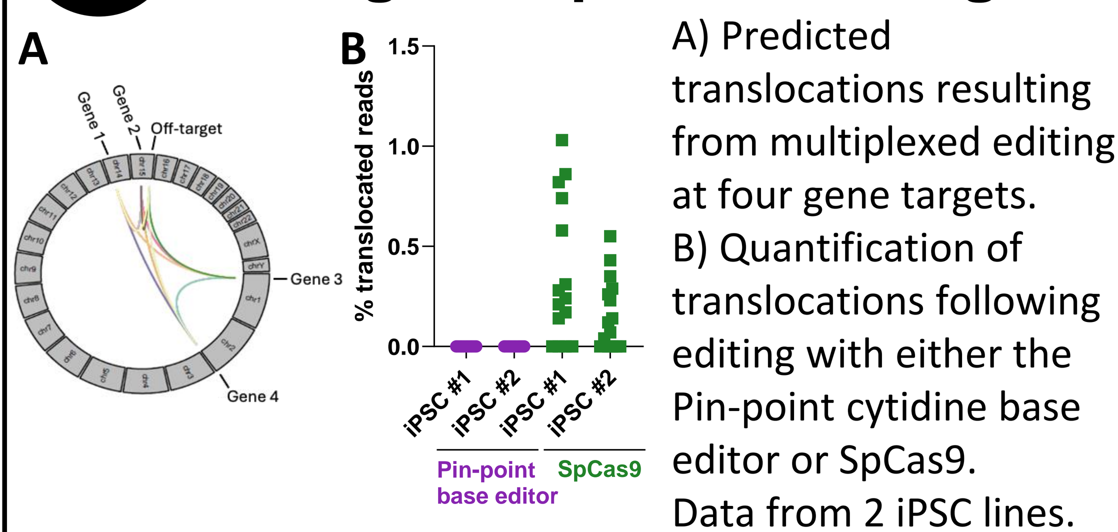
A) iPSCs edited at a single locus with SpCas9 exhibit markedly retarded growth kinetics compared to iPSCs edited with the Pin-point cytidine base editor or mock edited control samples. N=3. B) p53 target gene (p21) expression (N=6-12) and C) Apoptosis (N=3) are markedly elevated following single target editing at 4 loci individually with SpCas9 compared to either the Pin-point cytidine base editor or mock edited control samples. D) Cell cycle progression is retarded in SpCas9 single target edited samples (N=3) compared to either the Pin-point cytidine base editor or mock edited controls.

5 Efficient and durable target knockout



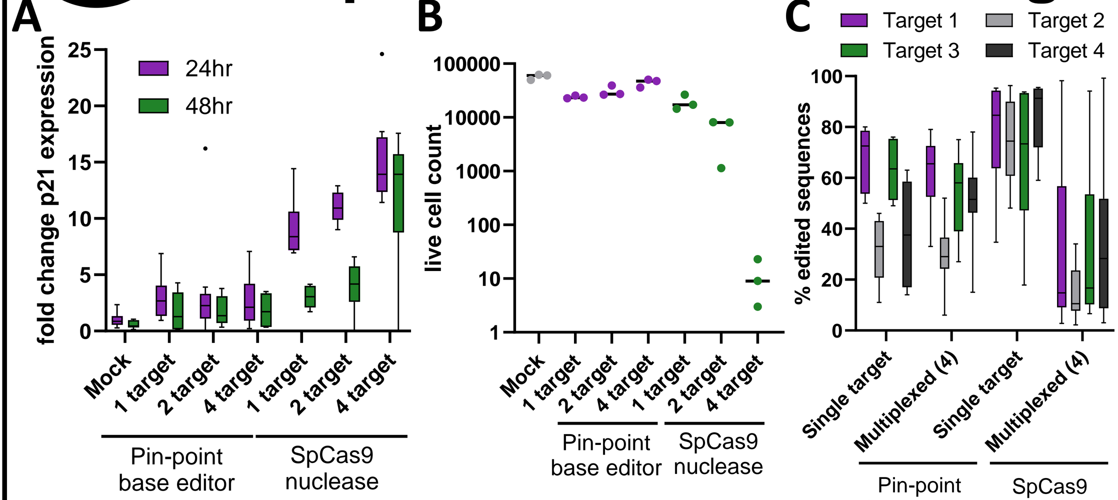
A) Target protein knockout by the optimised cytidine base editor formulation in a pool of edited iPSCs. B) Target protein knockout was stable during extended culture (100 days) of edited pools, indicating no adverse impact on the fitness of cytidine base edited iPSCs. Data from 2 iPSC lines.

6 Genome integrity maintained during multiplexed editing



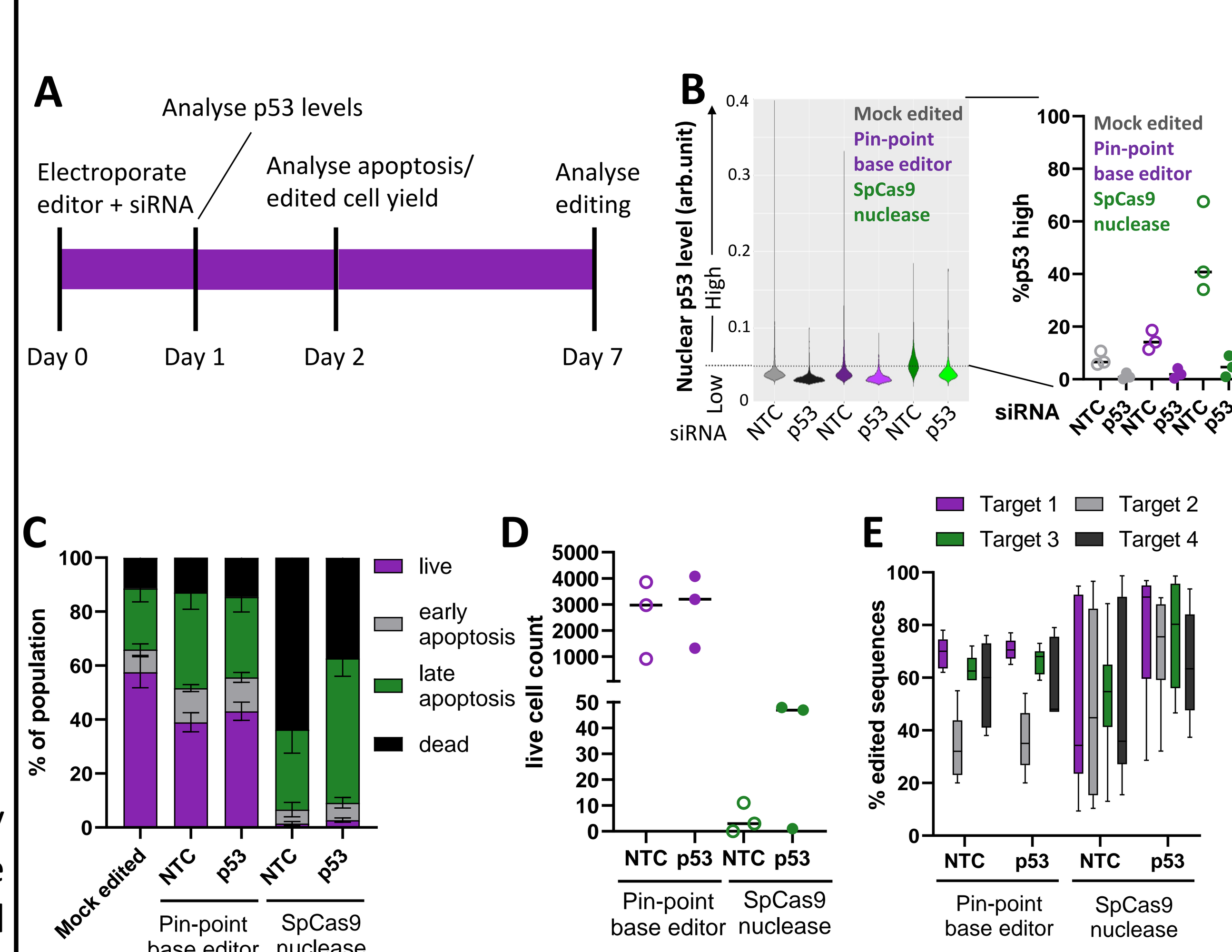
A) Predicted translocations resulting from multiplexed editing at four gene targets. B) Quantification of translocations following editing with either the Pin-point cytidine base editor or SpCas9. Data from 2 iPSC lines.

7 p53 activation selects against multiplexed nuclease editing



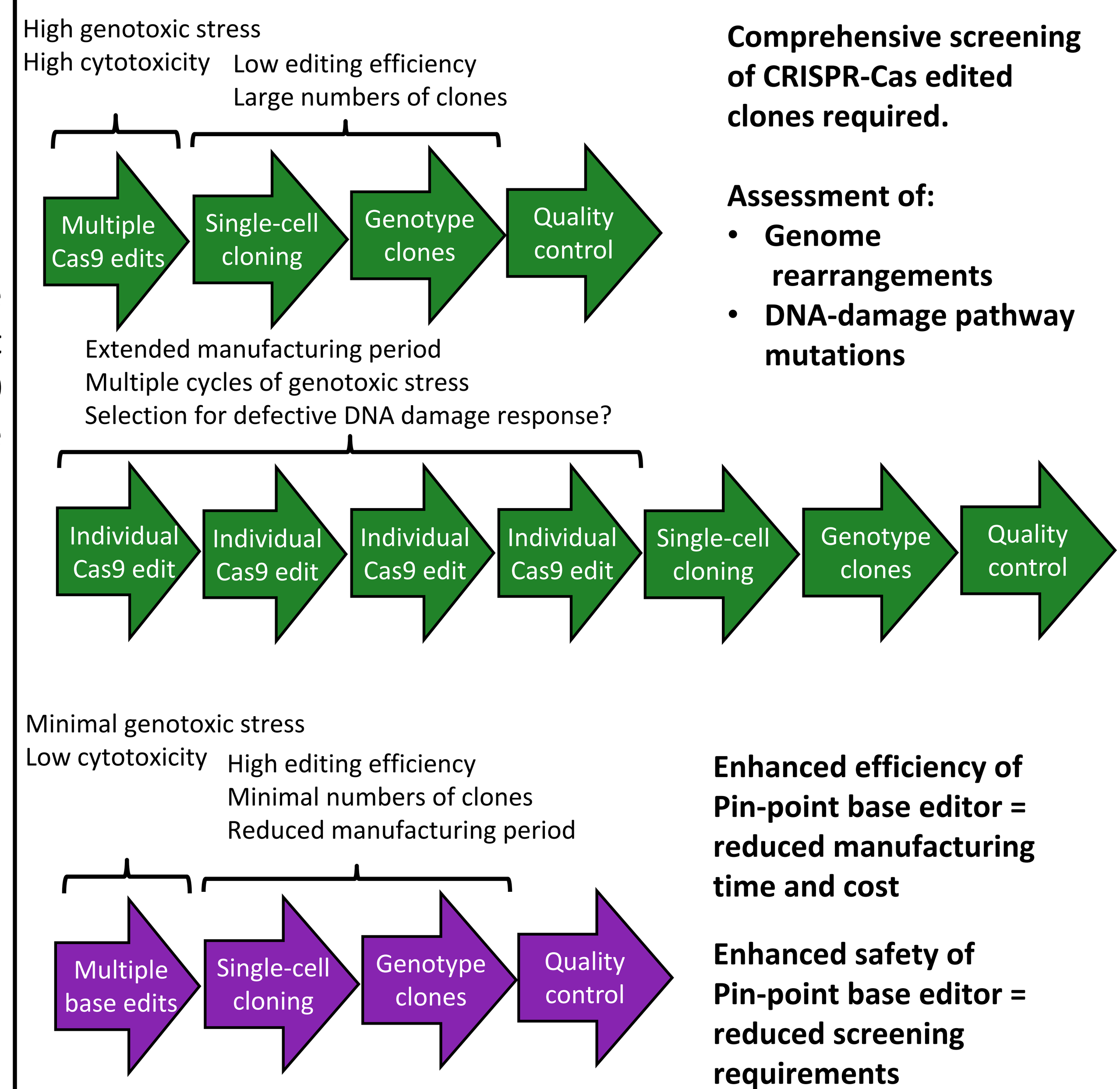
A) p21 expression is markedly elevated following multiplexed editing at 4 loci simultaneously with SpCas9 compared to either single target editing, multiplexed editing with the Pin-point cytidine base editor or mock edited control samples. (N=6-9). B) Live cell count is unaffected by multiplexed base editing with the Pin-point cytidine base editor whereas it is markedly reduced following multiplexed editing with SpCas9. n=3 iPSC donors. C) Multiplex editing with SpCas9 results in reduced editing efficiency following 7 days of culture compared to either single target editing or multiplexed editing with the Pin-point cytidine base editor. n=3 iPSC donors. 2 independent experiments for single target editing; 9 independent experiments for multiplexed editing.

8 p53 activity does not select against editing with the optimised Pin-point cytidine base editor



A) Experimental scheme. B) Inclusion of siRNAs targeting the p53 transcript, but not non-targeting control (NTC) siRNAs, reduced the proportion of edited nuclei with high levels of p53 to below the levels of the mock edited control. Inclusion of siRNAs targeting the p53 transcript C) reduced the proportion of dead cells, D) increased the live cell yield, and E) increased editing efficiency in samples edited with SpCas9 but not the cytidine base editor.

9 Pin-point base editing improves the efficiency & safety of iPSC engineering



Comprehensive screening of CRISPR-Cas edited clones required.

Assessment of:

- **Genome rearrangements**
- **DNA-damage pathway mutations**

Enhanced efficiency of Pin-point base editor = reduced manufacturing time and cost

Enhanced safety of Pin-point base editor = reduced screening requirements

10 Summary

- The modularity of the Pin-point platform offers enhanced flexibility to formulate reagents that optimise editing efficiency and viability of sensitive therapeutic cell types such as iPSCs
- Editing with the optimised Pin-point cytidine base editor results in minimal genotoxic stress
- Minimal p53 pathway activation enables high efficiency multiplexed editing, and reduces the risk of selecting for clones with defective responses to genotoxic stress

11 References

- Collantes et al. The CRISPR Journal. DOI:10.1089/crispr.2020.003
- Porreca et al. Mol Ther DOI: 10.1016/j.ymthe.2024.06.033

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