The Pin-point™ base editing platform streamlines the generation of hypoimmunogenic iPSCs for allogeneic cell therapy

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The Pin-point platform enables the modular assembly of base editors composed of a DNA modifying deaminase and a DNA binding Cas component via an aptamer encoded in the sequence-targeting guide RNA (gRNA). We optimized conditions for the delivery of synthetic gRNAs and mRNAs encoding a Pin-point base editor composed of Rat APOBEC1 and SpCas9 nickase to iPSCs, and demonstrated that simultaneous multiplex editing with this editor configuration was significantly more efficient, and resulted in substantially enhanced cell viability and genome integrity compared to CRISPR-Cas9.

To apply the Pin-point base editing technology to the engineering of hypoimmunogenic allogeneic iPSC lines we screened sgRNAs for efficient knock-out of class 1 and class 2 HLA antigens. Clonal engineered hypoimmunogenic iPSC lines retained pluripotency and could be differentiated to products with potential in a range of cell replacement therapies. To expand the utility of the Pin-point platform we optimised conditions for the co-delivery of reagents for targeted transgene integration and base editing. Combining aptamer containing Pin-point base editing gRNAs with aptamer-less gRNAs and a donor DNA template enabled simultaneous multi-gene knock-out and transgene knock-in. The Pin-point platform therefore provides the capability to safely, efficiently, and precisely perform multiple genome engineering operations in a one-step process, offering the opportunity to dramatically streamline the development of allogeneic iPSC-derived cell therapies.

The Pin-point platform

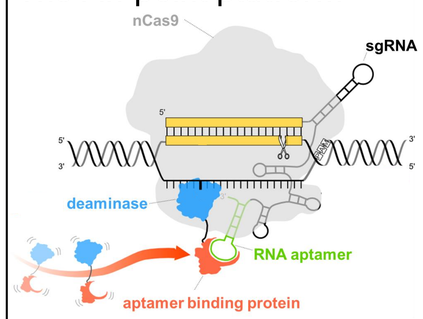

Fig 1. Schematic of the modular Pin-point base editor system
A Cas nickase (e.g. nCas9) is recruited to the DNA target site via a single guide RNA (sgRNA) encoding an aptamer in the scaffold region. The aptamer recruits a DNA-modifying deaminase to the DNA target site via an aptamer binding protein. The three independent components of the system can be configured according to editing requirements and delivered to cells either as mRNA and synthetic sgRNA, or packaged in viral particles.

Enhanced efficiency and safety of multiplex editing

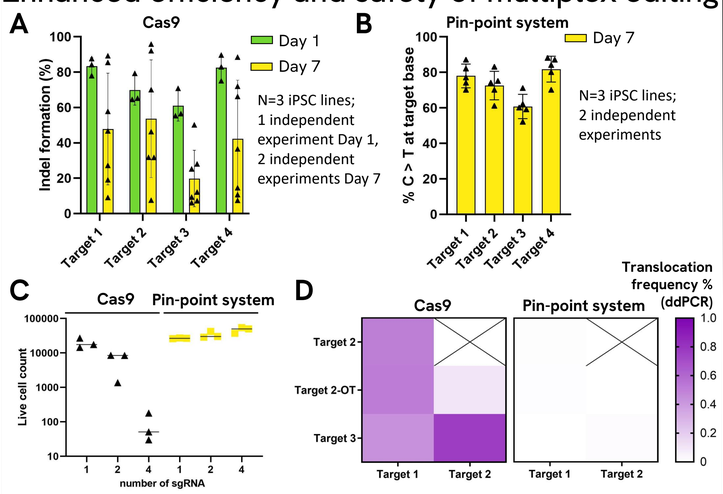

Fig 3. Multiplex editing with Cas9 and the Pin-point base editor system
A) Four gene multiplex editing with Cas9 results in increased variability and reduced editing efficiency (TIDE) following 7 days of culture. B) Efficient C>T conversion following four gene multiplex base editing with the Pin-point system. C) Live cell count is dramatically reduced following multiplex editing with Cas9. D) Multiplex editing with Cas9 leads to translocations between guide RNA targets; translocations are undetectable in iPSCs multiplex edited with the Pin-point system .

Engineering and differentiation of multiplex edited clonal hypoimmunogenic iPSC lines

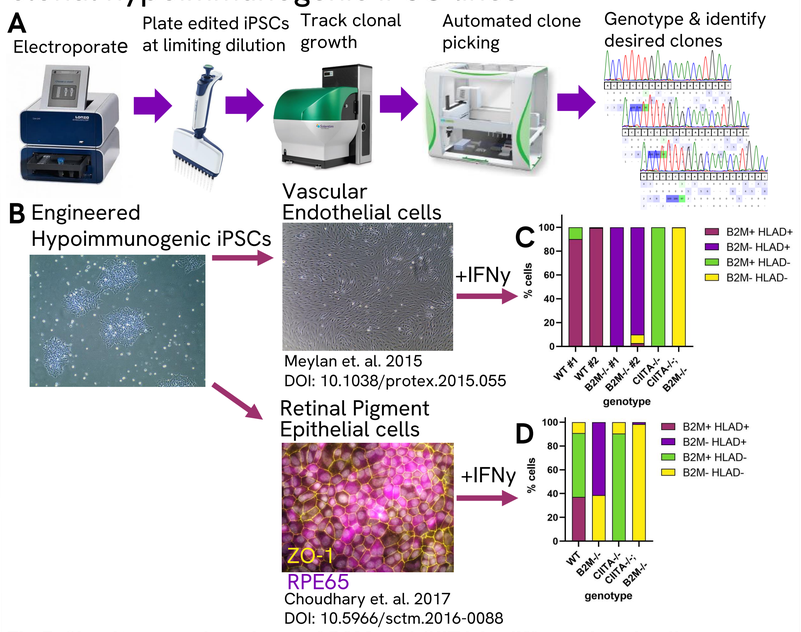

Fig 5. Simultaneous knockout of B2M and CIITA in differentiated cells
A) iPSC clonal line derivation workflow. B) Differentiation of homozygous single- and double edited clonal iPSCs to therapeutic cell types. Expression of B2M and HLA class II (HLAD) by vascular endothelial (C), and retinal pigment epithelial cells (D) derived from single and double knock-out iPSCs in response to inflammatory cytokine interferon gamma (IFNγ).

iPSCs edited with the Pin-point system retain pluripotency

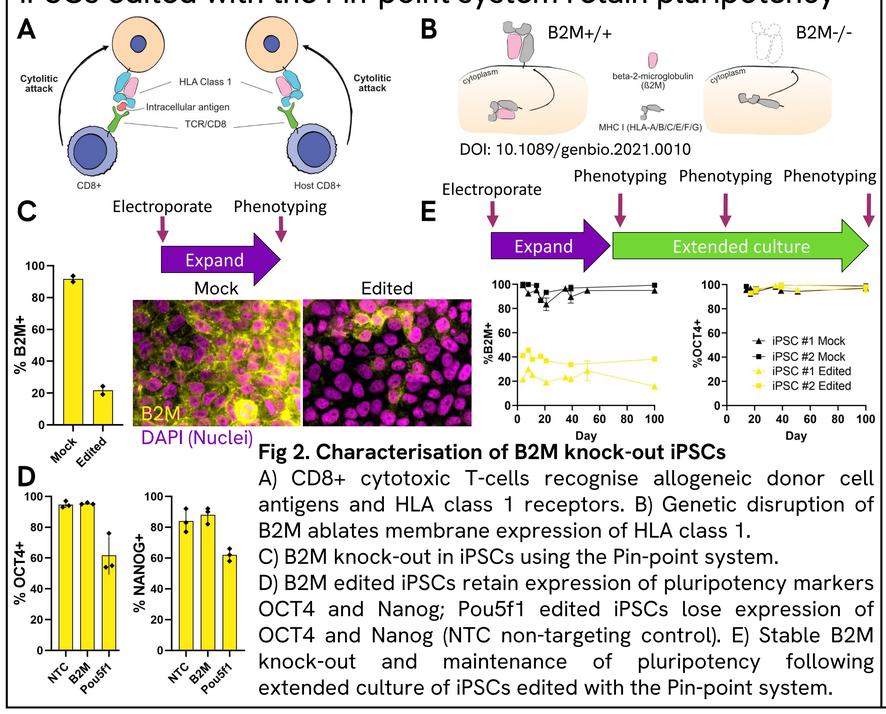

Fig 2. Characterisation of B2M knock-out iPSCs
A) CD8+ cytotoxic T-cells recognise allogeneic donor cell antigens and HLA class 1 receptors. B) Genetic disruption of B2M ablates membrane expression of HLA class 1.
C) B2M knock-out in iPSCs using the Pin-point system.
D) B2M edited iPSCs retain expression of pluripotency markers OCT4 and Nanog; Pou5f1 edited iPSCs lose expression of OCT4 and Nanog (NTC non-targeting control). E) Stable B2M knock-out and maintenance of pluripotency following extended culture of iPSCs edited with the Pin-point system.

Cell models for guide RNA screening

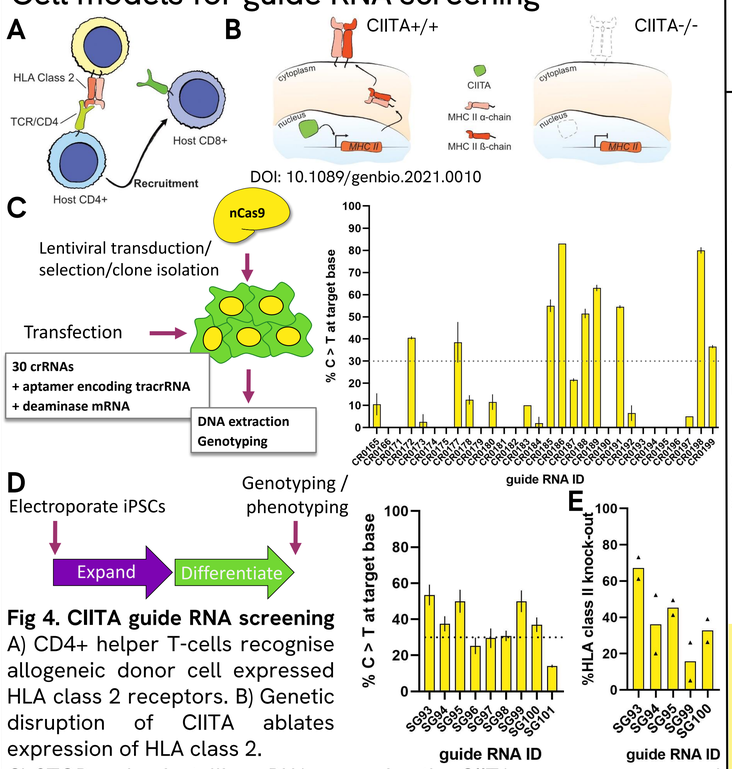

Fig 4. CIITA guide RNA screening
A) CD4+ helper T-cells recognise allogeneic donor cell expressed HLA class 2 receptors. B) Genetic disruption of CIITA ablates expression of HLA class 2.
C) STOP-codon installing gRNAs spanning the CIITA gene were screened for C >T conversion efficiency in an nCas9 stable HEK cell line. D) gRNA sequences with >30% editing efficiency were synthesised as sgRNAs and screened for C >T conversion efficiency in iPSCs. E) sgRNA sequences with >30% editing efficiency were assessed for HLA class II knock-out in differentiated iPSCs.

One-step knock-in and multiplex knock-out in iPSCs

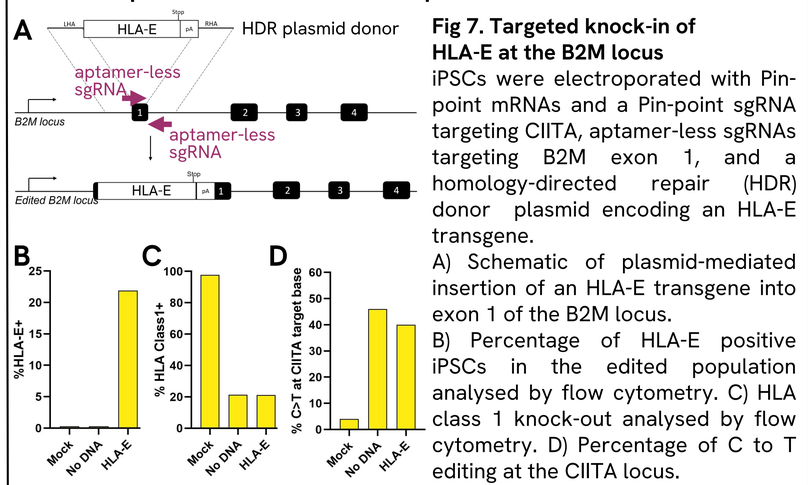

Fig 7. Targeted knock-in of HLA-E at the B2M locus
iPSCs were electroporated with Pin-point mRNAs and a Pin-point sgRNA targeting CIITA, aptamer-less sgRNAs targeting B2M exon 1, and a homology-directed repair (HDR) donor plasmid encoding an HLA-E transgene.
A) Schematic of plasmid-mediated insertion of an HLA-E transgene into exon 1 of the B2M locus.
B) Percentage of HLA-E positive iPSCs in the edited population analysed by flow cytometry. C) HLA class 1 knock-out analysed by flow cytometry. D) Percentage of C to T editing at the CIITA locus.

Conclusions

- iPSCs edited with the Pin-point system retain pluripotency and can be differentiated to therapeutic cell types.
- Greatly improved editing efficiency, viability, and genome stability of iPSCs multi-gene edited with the Pin-point system compared to Cas9.
- The Pin-point platform enables efficient simultaneous site specific transgene knock-in and multi-gene knock-out in iPSCs.

References

Collantes et al. The CRISPR Journal. Feb 2021.58-68

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