A single-step process for engineering hypoimmunogenic pluripotent stem cells with the Pin-point™ platform

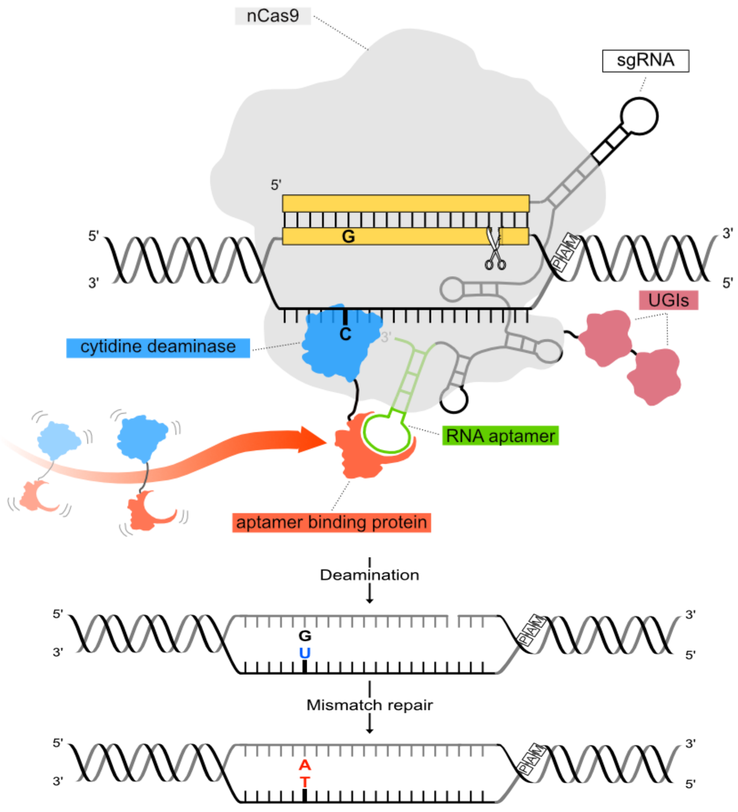
Leigh-Anne Thomas , Olga Mielczarek, Jesse Stombaugh, Robin Loesch, Kevin Hemphill, Immacolata Porreca, Robert Blassberg

Visit poster P0673 to learn how using the Pin-point base editing platform can reduce editing-associated genotoxic stress in iPSCs.

1 Abstract

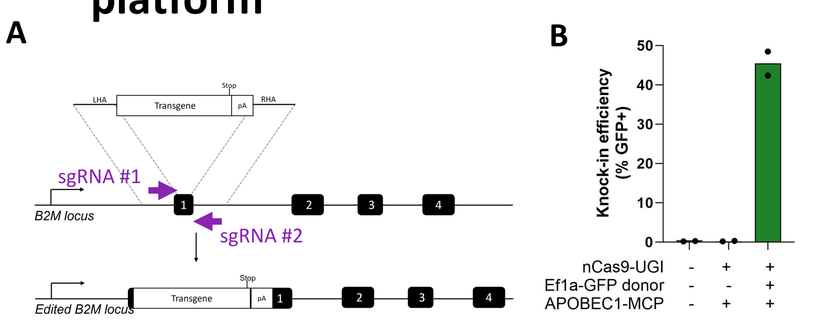
Pluripotent stem cells (PSCs) hold great promise for the manufacturing of numerous advanced cell therapies. Off-the-shelf allogeneic products derived from PSCs engineered to be compatible with large cohorts of patients have the potential to dramatically broaden access to these therapies, however their sensitivity to DNA damage presents challenges for efficiently performing the complex genome editing operations necessary to realise much of their potential. Base editors represent a potential solution to these challenges due to their reduced genotoxicity compared to nuclease-based technologies. We have developed the Pin-point™ platform, which enables the modular assembly of base editors composed of DNA binding Cas and DNA modifying deaminase components associated via an aptamer encoded in the sequence-targeting guide RNA (gRNA). Owing to the aptamer-dependent recruitment of the deaminase component to target DNA sequences, the Pin-point platform uniquely allows multi-purposing of a single Cas nickase component for simultaneous multiplexed base editing and targeted transgene knock-in. Transient delivery of mRNAs encoding a Pin-point base editor composed of Rat APOBEC1 and SpCas9 nickase in combination with synthetic aptamer-encoding gRNAs achieved durable target protein knockout, and substantially improved cell viability, editing efficiency, and genome integrity following multiplexed base editing compared to CRISPR-Cas9 with no adverse impacts on pluripotency. To demonstrate the utility of the Pin-point platform for the engineering of allogeneic PSCs we generated a panel of clonal hypoimmunogenic iPSC lines with a range of genotypes using an automated clone tracking and picking workflow. Hypoimmunogenic iPSC lines generated via both multiplexed base editing and simultaneous base editing with targeted transgene integration retained pluripotency and exhibited the expected human leukocyte antigen (HLA) phenotypes when differentiated to therapeutic cell products. The Pin-point platform therefore represents a safe and efficient solution to simultaneously perform multiple genome engineering operations via a novel single step process compatible with downstream automation, offering the opportunity to dramatically streamline the development of allogeneic iPSC-derived cell therapies.

2 The modular Pin-point base editing platform



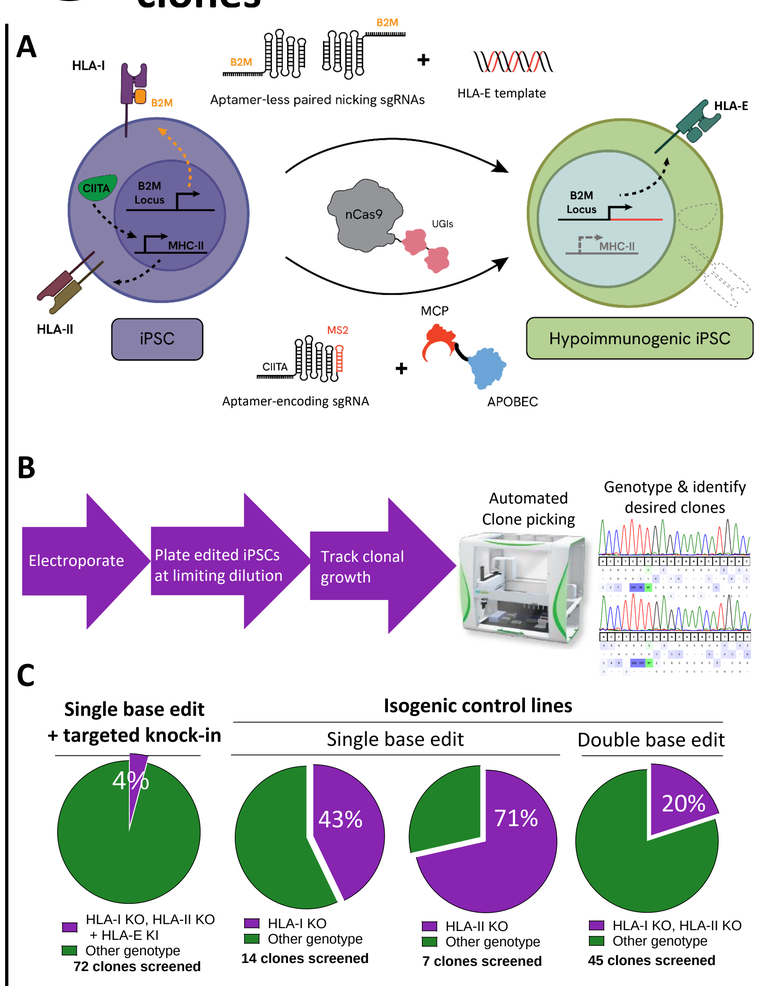
The Cas component is recruited to the DNA target sequence via a single guide RNA (sgRNA) encoding an aptamer in the scaffold region. The aptamer recruits a DNA-modifying deaminase to the DNA target sequence via an aptamer binding protein. The three independent components of the system can be configured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

3 Targeted transgene knock-in is compatible with the Pin-point platform



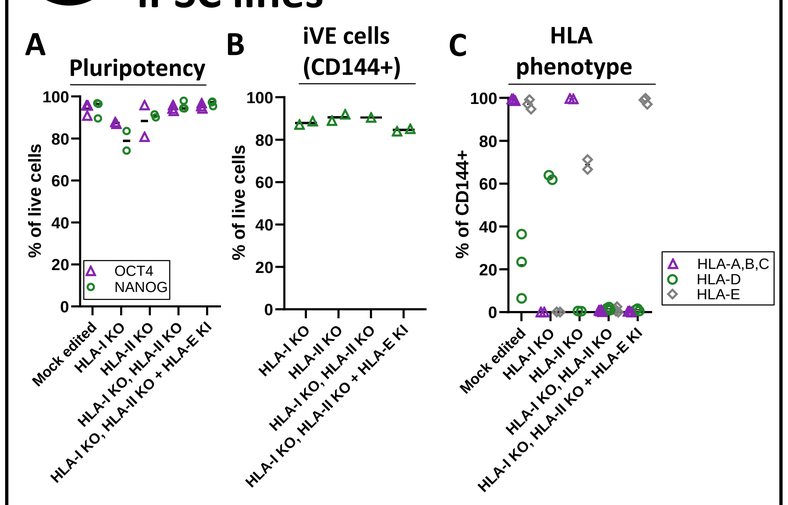
A) Schematic of Ef1a-GFP transgene knock-in using paired sgRNAs to target the nCas9-UGI component of the Pin-point base editor to the B2M locus.
B) Efficiency of Ef1a-GFP transgene integration using optimised component formulation.
B calculated from percentage of GFP positive iPSCs in the edited population

4 Single-step engineering of iPSC clones



A) Schematic of single-step hypoimmunogenic iPSC line engineering by combining aptamer-encoding and aptamer-less sgRNAs to simultaneously knock-out HLA-II expression by base editing of CIITA and to generate an HLA-E targeted transgene knock-in.
B) Schematic of automated clone tracking and picking workflow.
C) Efficiency of single-step engineering of clonal hypoimmunogenic iPSC lines and isogenic control single- and double knockout iPSC lines generated by base editing.

5 Validation of clonal engineered iPSC lines



A) Engineered iPSC lines retained expression of pluripotency markers OCT4 and NANOG.
B) Engineered iPSC lines retained capacity for differentiation to CD144+ induced vascular endothelial (iVE) cells.
C) Engineered iPSC lines differentiated to iVE cells express the expected repertoire of human leukocyte antigens (HLA).
A-C n=2 clonal engineered iPSC lines per genotype.

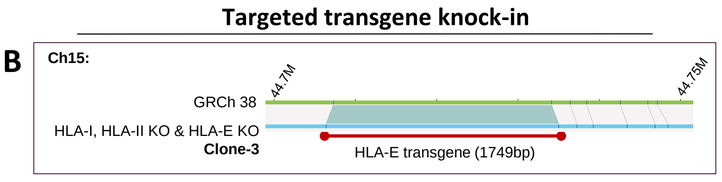
6 Optical Genome Mapping of engineered iPSC clones

Structural variation

A

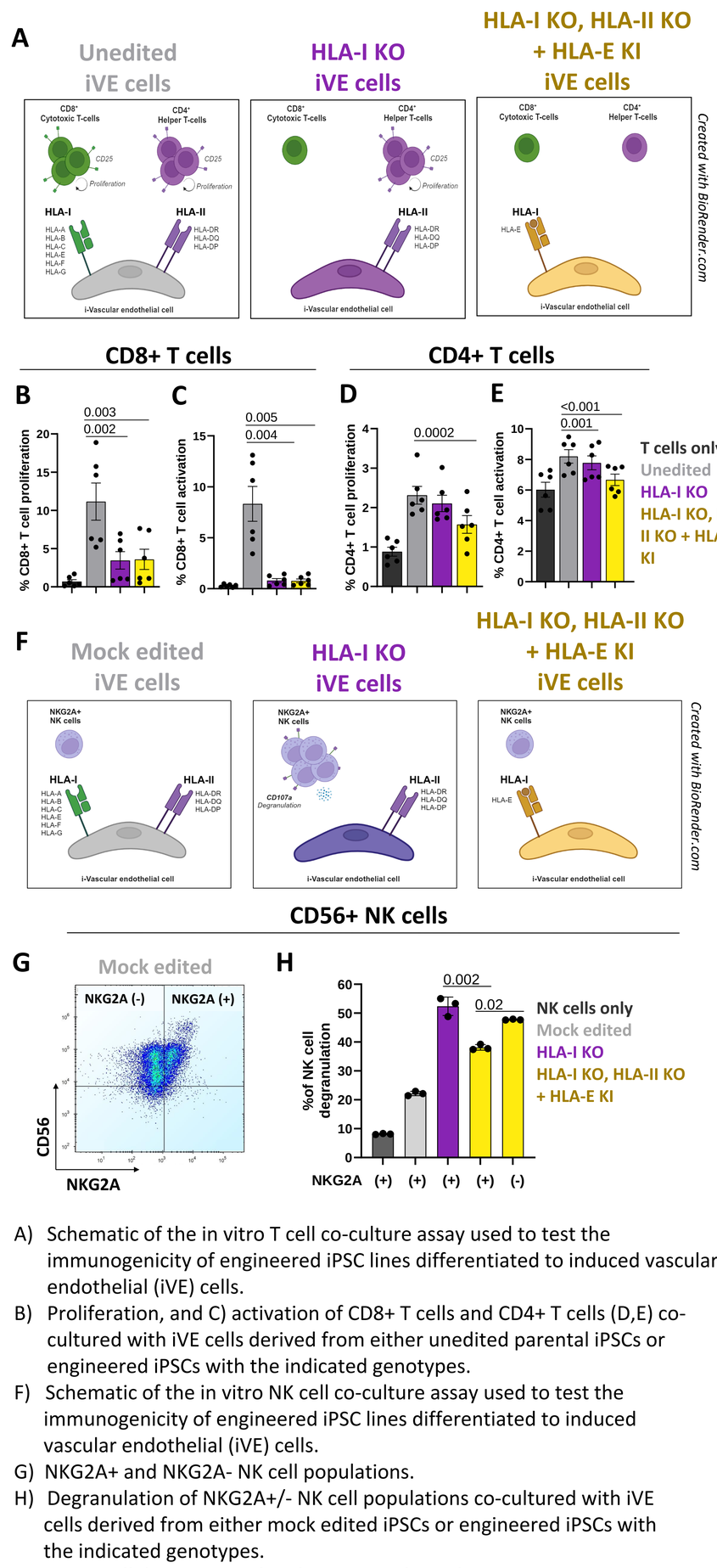
Category		Parental NH50191	Mock		HLA-I & HLA-II KO			HLA-I, HLA-II KO + HLA-E KI		
		De novo SV	Clone-1	Clone-2	Clone-1	Clone-2	Clone-3	Clone-1	Clone-2	Clone-3
Insertion	⊕	16	1	0	0	0	1	1	0	0
Deletion	⊖	18	0	0	0	1	0	0	0	0
Duplication	▷▷	29	0	0	0	0	0	0	0	0
Inversion	⋈	22	0	0	0	0	0	0	0	0
CNV Gain	+	1	0	0	0	0	0	0	0	0
TOTAL		86	1	0	0	1	1	1	0	0

Targeted transgene knock-in



A) Clonal hypoimmunogenic iPSC lines generated by single-step engineering exhibit comparable structural variation to base-edited isogenic and mock edited controls. Structural variants identified by Optical Genome Mapping using the de novo analysis pipeline (FDR < 0.05). Variants undetected in the parental iPSC line are reported for each clone.
B) Optical Genome Mapping demonstrates precise site-specific transgene knock-in in clonal hypoimmunogenic iPSC lines.

7 In vitro immunogenicity of engineered iPSC products



A) Schematic of the in vitro T cell co-culture assay used to test the immunogenicity of engineered iPSC lines differentiated to induced vascular endothelial (iVE) cells.
B) Proliferation, and C) activation of CD8+ T cells and CD4+ T cells (D,E) co-cultured with iVE cells derived from either unedited parental iPSCs or engineered iPSCs with the indicated genotypes.
F) Schematic of the in vitro NK cell co-culture assay used to test the immunogenicity of engineered iPSC lines differentiated to induced vascular endothelial (iVE) cells.
G) NKG2A+ and NKG2A- NK cell populations.
H) Degranulation of NKG2A+/- NK cell populations co-cultured with iVE cells derived from either mock edited iPSCs or engineered iPSCs with the indicated genotypes.
B-E n=2 T cell donors, n=1 clonal engineered iPSC line per genotype.
p-values from paired t-test.
H-I n=1 NK cell donor, n=1 clonal engineered iPSC line per genotype.
p-values from paired t-test.

8 Summary

- Aptamer-dependent recruitment of the deaminase component enables efficient site-specific transgene knock-in in parallel with base editing
- Multiplexed iPSC engineering with the Pin-point platform is compatible with automated manufacturing processes
- Hypoimmunogenic iPSC lines manufactured by single-step engineering with the Pin-point platform can be differentiated to therapeutic cell types with expected immunoreactivity profiles
- Clonal hypoimmunogenic iPSCs engineered with the Pin-Point platform maintain genomic integrity with comparable structural variation to base-edited isogenic and mock edited controls.

9 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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