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Durable epigenetic editing for generation of multiplex-edited T cells without chromosomal rearrangements

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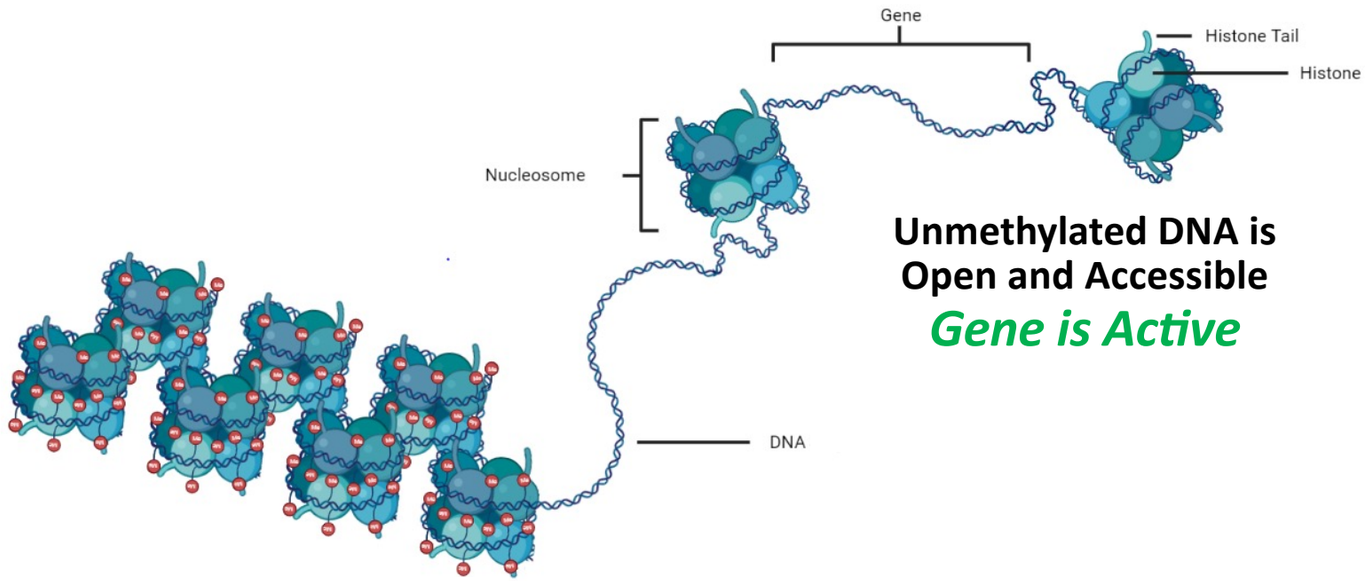
@chromamedicine

Epigenetics: The central regulator of gene expression

Chromatin packaging and epigenetic regulation of DNA transcription



Unmethylated DNA is Open and Accessible

Gene is Active

Methylated DNA Is Closed and Inaccessible

Gene is Inactive

- Conserved mechanism that durably sets the gene expression pattern, defining cell phenotype
- DNA is packaged into chromatin
- Chromatin conformation dictates whether a gene is active or inactive
- DNA methylation and histone modification are central mechanisms governing this conformation

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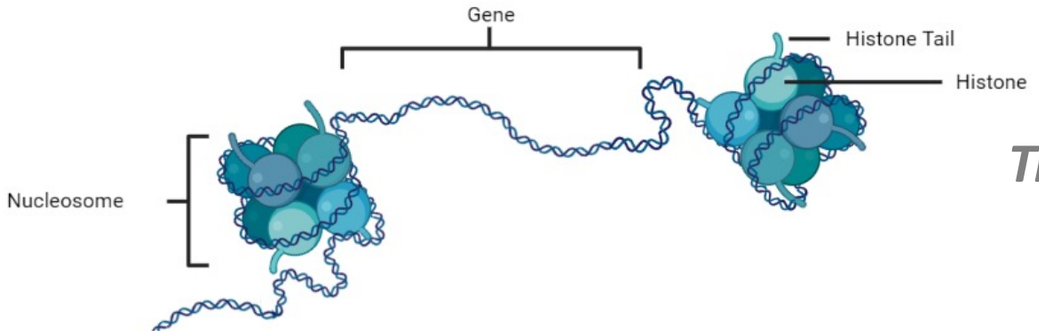
Epigenetic editing leverages the cell's endogenous system to precisely control gene expression

Durable change in phenotype without a change in genotype



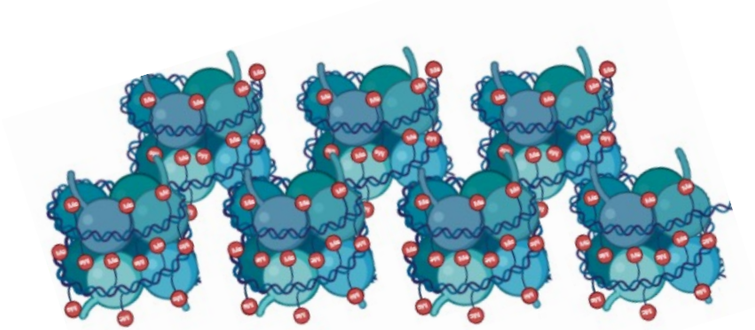
Gene is Active
DNA is Open and Accessible

Epigenetic Repressor
Methylates Targets

Transient Application

Epigenetic Activator
Demethylates Targets

Gene is Inactive
DNA is Closed and Inaccessible



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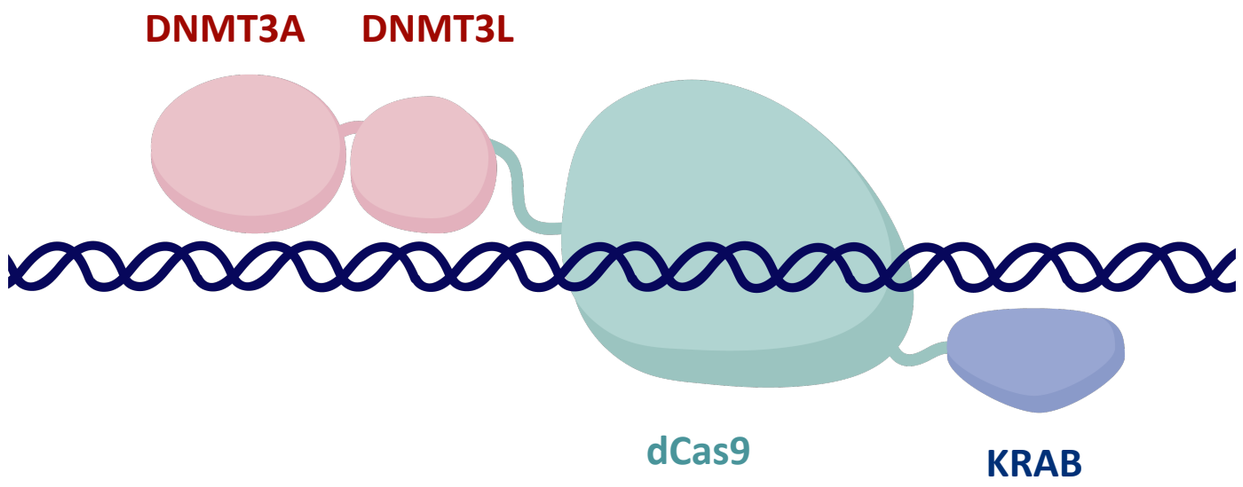
Chroma’s epigenetic editors are single fusion proteins with three functional domains

Chroma Epigenetic Repressor



- **DNA binding domain** precisely localizes effector domains to target sequence
- **Transcription effector domain** transiently represses target gene
- **Methylation effector domain** durably silences target gene

Nuñez JK, Chen J, Pommier GC, et al. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell*. 2021;184(9):2503-2519.e17. doi:10.1016/j.cell.2021.03.025

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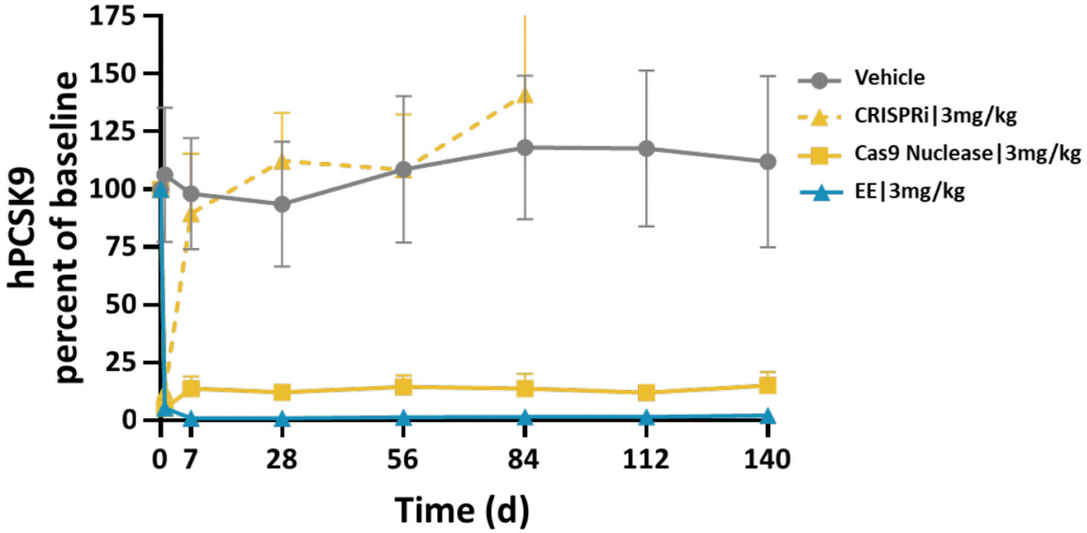
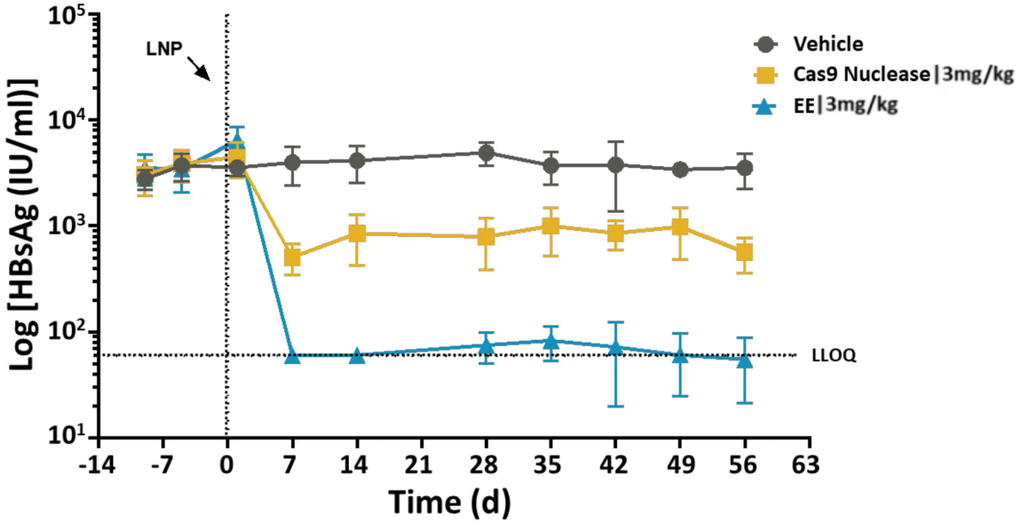
Chroma epigenetic editors effectively and durably silence in vivo at multiple targets

99% PCSK9 Silencing Achieved 140 Days Post-dose



Reduction of HBsAg Below LLOQ 56 Days Post-dose



EE = epigenetic editor

Tremblay F, Kelly K, Shah S, Development of a human PCSK9-targeting epigenetic editor with durable, near-complete in vivo silencing, ASGCT 2023 (presented by Jaffe A)

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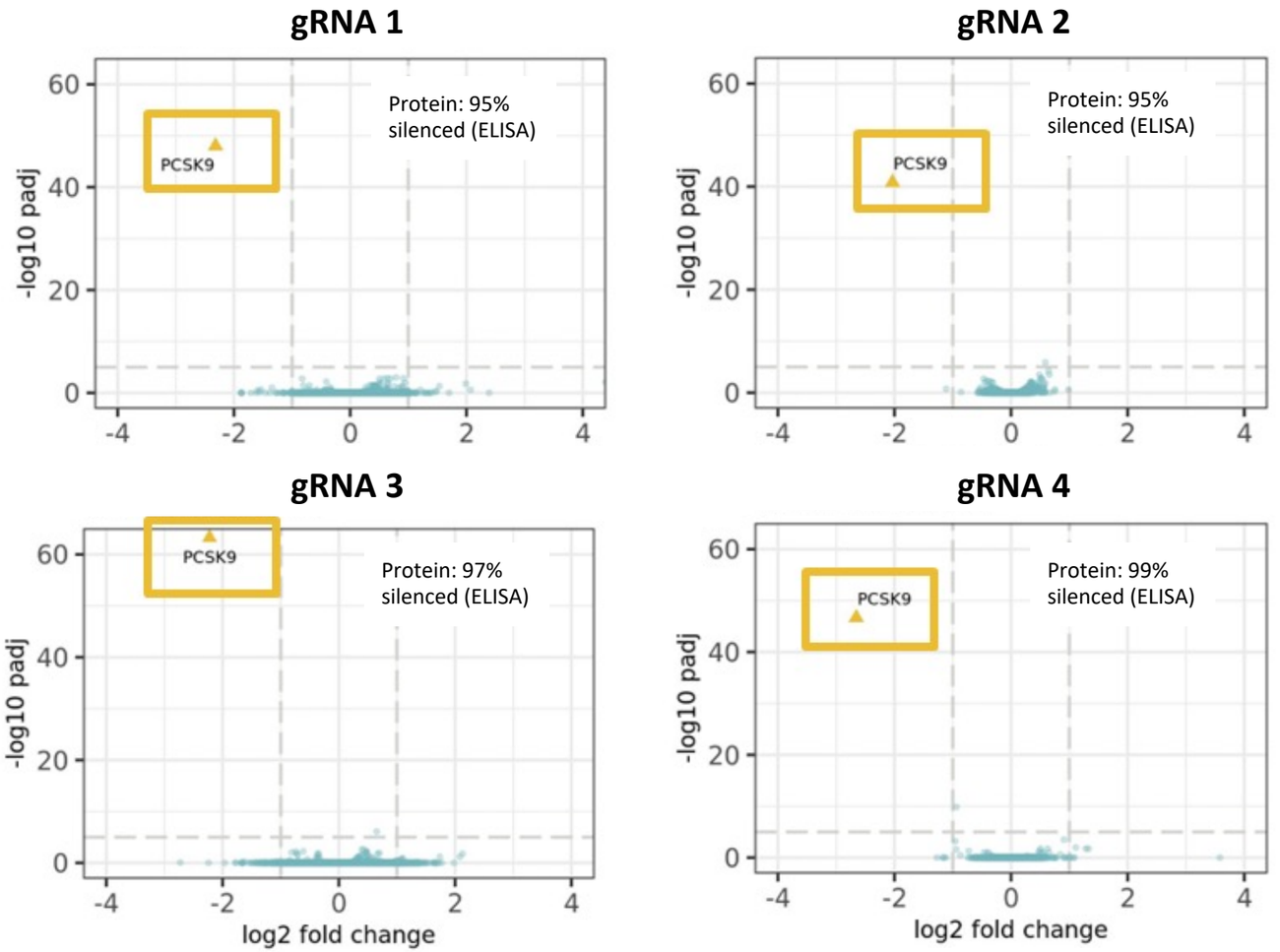
Epigenetic silencing of PCSK9 is highly specific with no off-target changes in expression or methylation

- Lead gRNAs demonstrated high specificity with epigenetic repressor in primary human hepatocytes *in vitro*
  - ✓ No off-target changes in expression (RNA-seq)
  - ✓ No off-target methylation (Illumina Methylation Array, not shown)

Epigenetic Repressor Specificity Analysis: Expression of Targeted vs. Control



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Advantages of epigenetic editing for highly engineered cell therapies

Epigenetic gene regulation is highly efficient, specific, and durable without any cuts, nicks, or changes to the underlying DNA

1 **Enables multiplexing without genotoxic risk**

- Simultaneous silencing of a large number of targets without introducing DNA damage

2 **Streamlines manufacturing**

- Accomplish a high number of multiplex edits in a single step; eliminates need for sequential administration required with nuclease editing
- Reduces need for in-depth characterization of edited T cells for translocations and chromosomal rearrangements

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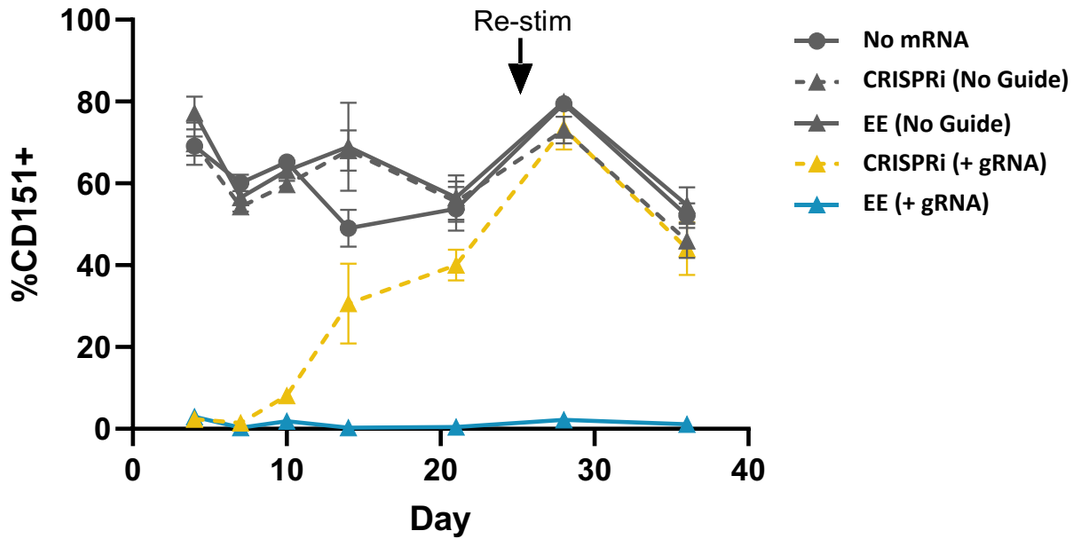
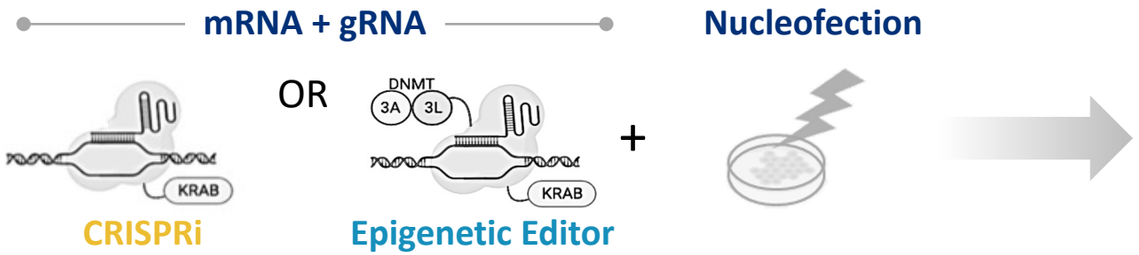
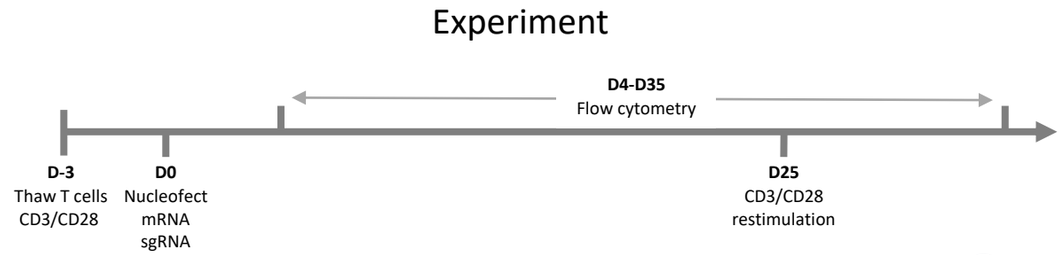
Epigenetic editing shows durable silencing in primary human T cells

- Durable silencing observed in primary human T cells at multiple targets
- Maintained through strong restimulation
- Provides ex vivo PoC for approach

Durable Silencing in Primary Human T Cell
mRNA + gRNA







Abubucker S, Collins M, Hildebrand E, Multiplexed editing without chromosomal rearrangements using epigenetic editors, ASGCT 2023 (presented by Abubucker S)

EE = epigenetic editor

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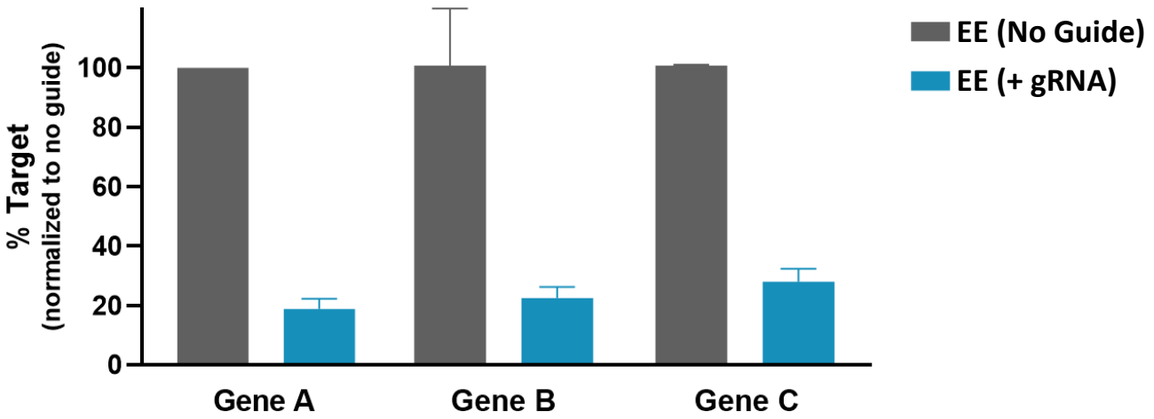
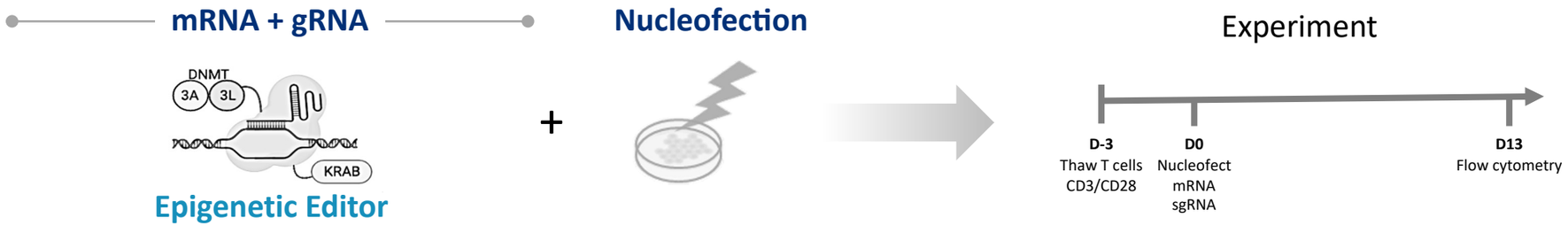
Efficient multiplex silencing with epigenetic editor

- Durable multiplex silencing through day 13
- Efficiency of single-target silencing maintained under multiplex conditions
- Unidirectional sequencing and imaging assays used to assess chromosomal changes in sorted cells

Efficient Triple Epigenetic Silencing in T cells (Day 13)





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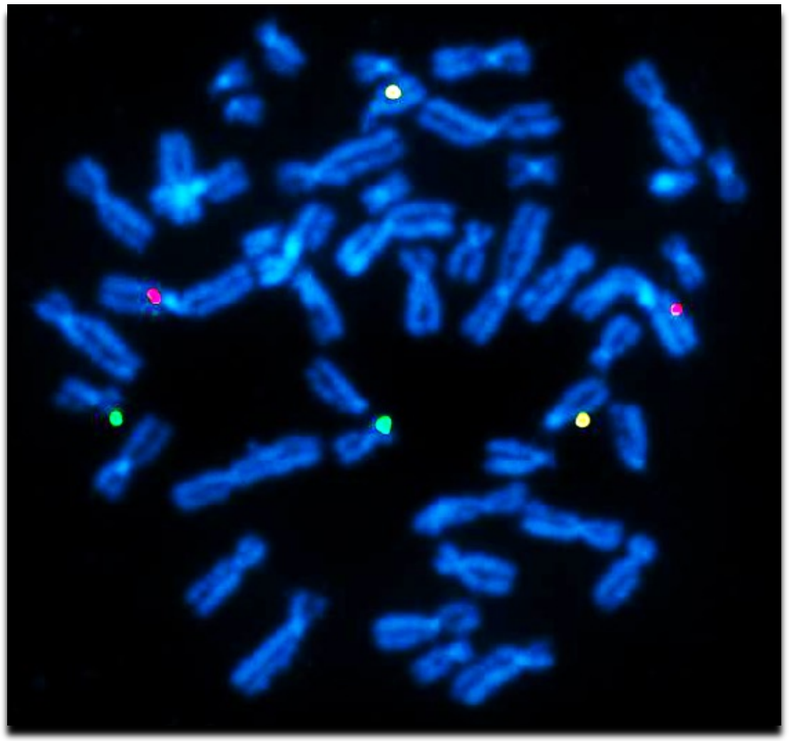
Single-cell imaging approach to visualize genomic rearrangements

- Sequencing will miss many potential outcomes
- Single-cell imaging-based approach captures:
  - Translocations, centromere abnormalities, chromothripsis, loss, gain, and truncations
- KromaTiD in-Site™ : Targeted FISH assay

in-Site Cell Image

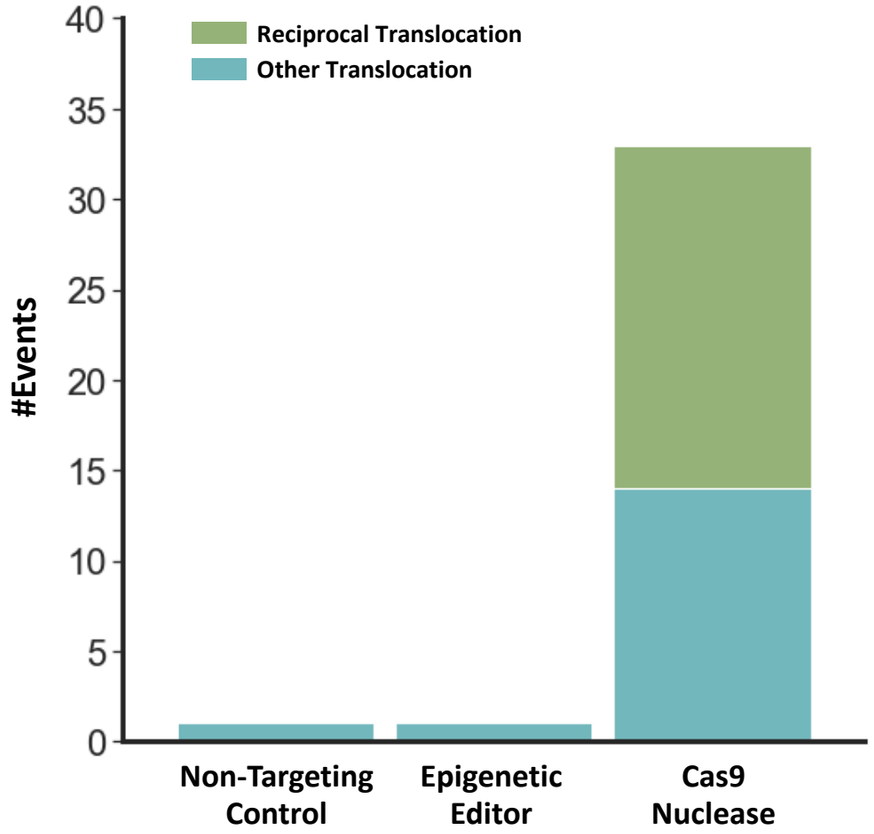


Gene A: pink, Gene B: yellow, Gene C: green

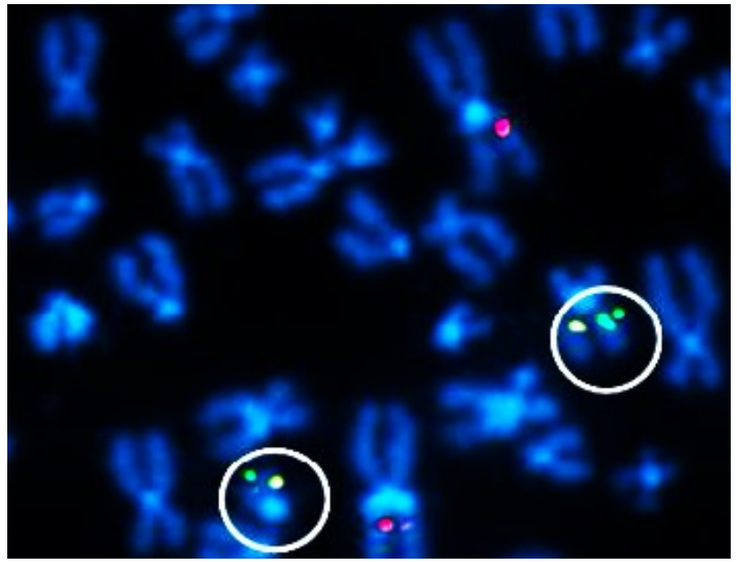
Robinson E, McKenna MJ, Bedford JS, et al. Directional Genomic Hybridization (dGH) for Detection of Intrachromosomal Rearrangements. *Methods Mol Biol*. 2019;1984:107-116. doi:10.1007/978-1-4939-9432-8_13

Multiplexing with epigenetic editor does not result in translocations

Translocation Events Detected With Targeted FISH Assay



Translocations in Cas9 Multiplexed Cells



Targeted FISH Detects Additional Translocation Events

	Sequencing	FISH
Cas9 Nuclease	7%	9%
Epigenetic Editor	<0.1%	<0.3%
Controls	<0.1%	<0.3%

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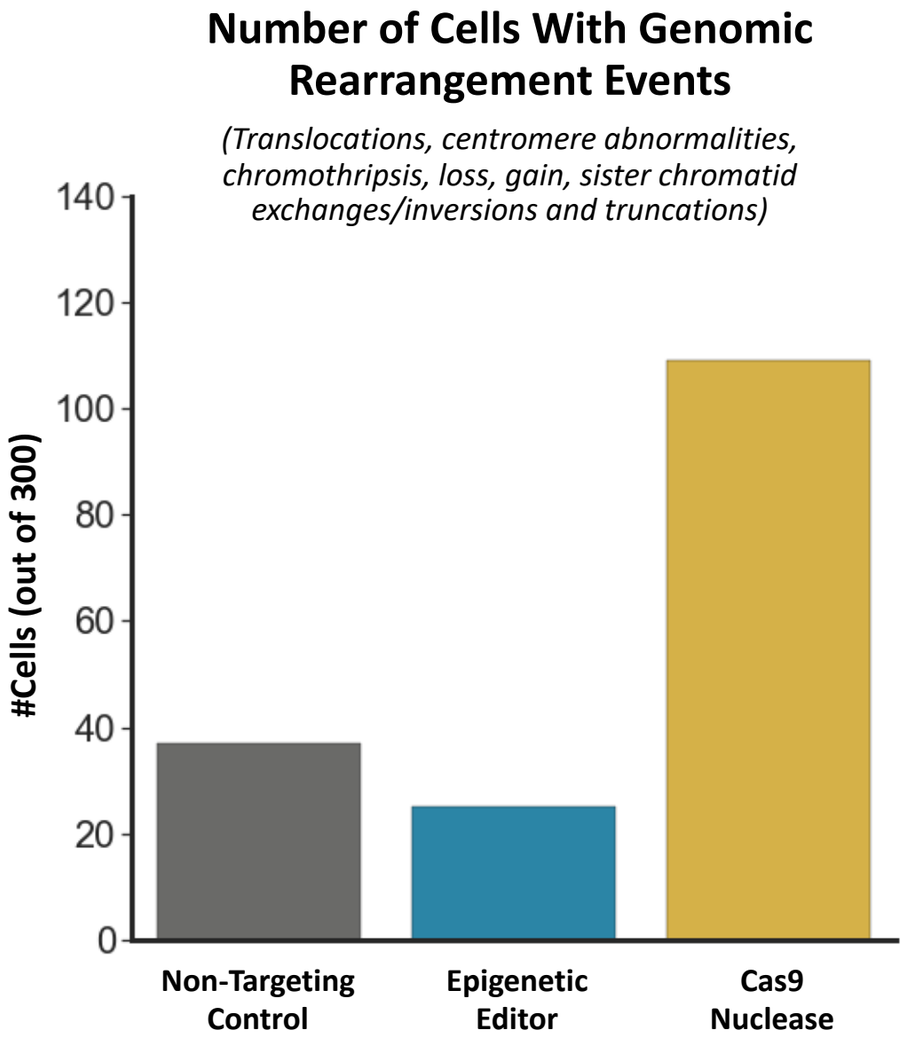
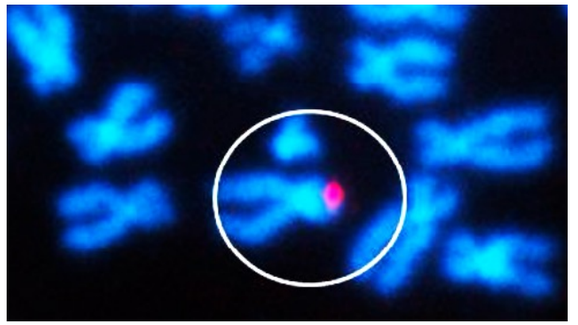
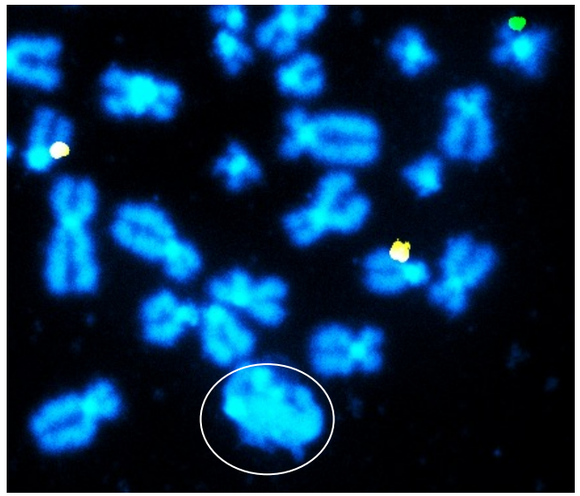
Multiplexing with epigenetic editor does not induce genomic rearrangement events





Edit Site Truncation with Cas9 nuclease



Chromothripsis with Cas9 nuclease

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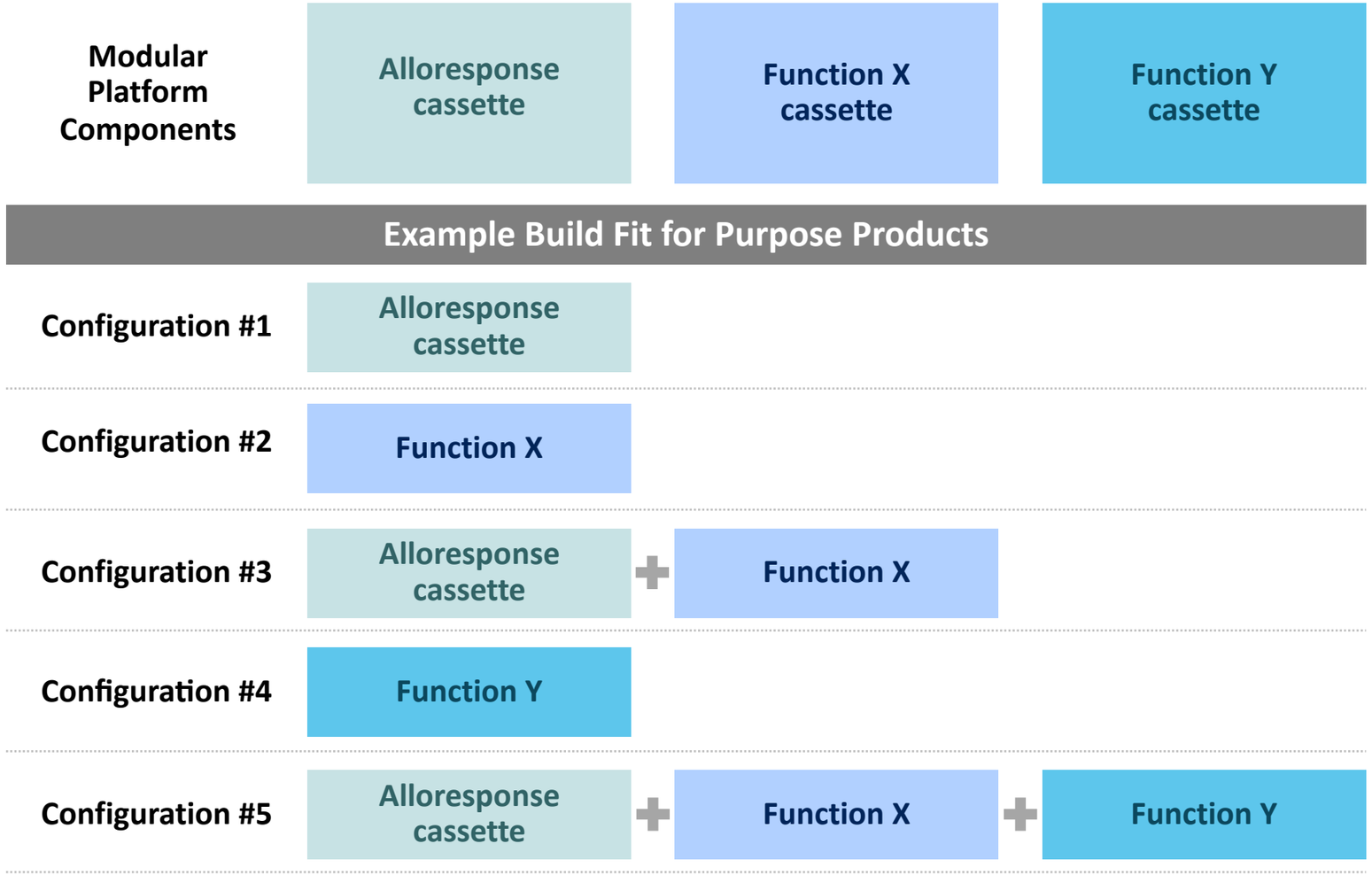
Epigenetic editing enables build of complex edited T cell therapies



- Epigenetic editing allows extensive multiplexing without genotoxic risk
- Modular cassette approach groups edits addressing different aspects of CAR T engineering
- Potential for alloresponse cassette to be combined with other cassettes to improve both function and allo-persistence

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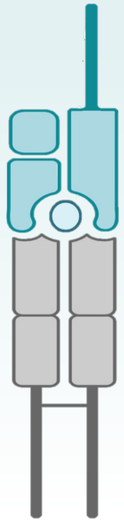
Chroma technology enables a multiplexed allogeneic cassette in T cells to eliminate GVHD and reduce CD8+ and CD4+ T cell responses

CD8+ T resistance

- Reduce MHC class I surface expression
- Reduce CD8+ T cell alloresponse

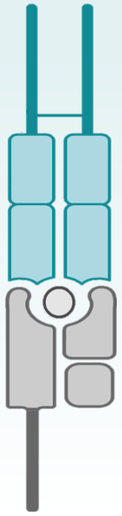


CD4+ T resistance

- Reduce MHC class II expression
- Reduce CD4+ T cell alloresponse

Eliminate GVHD

- Reduce expression of TCR
- Reduce risk of ex vivo T cell graft-vs-host



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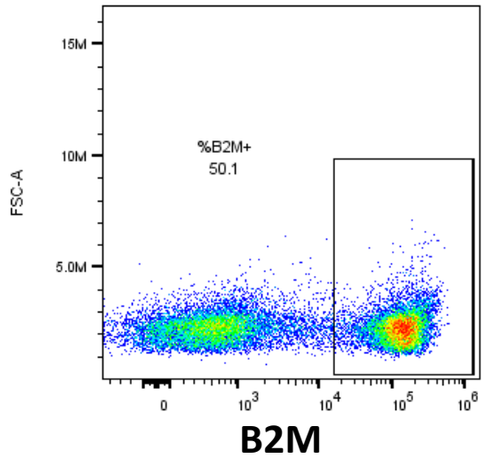
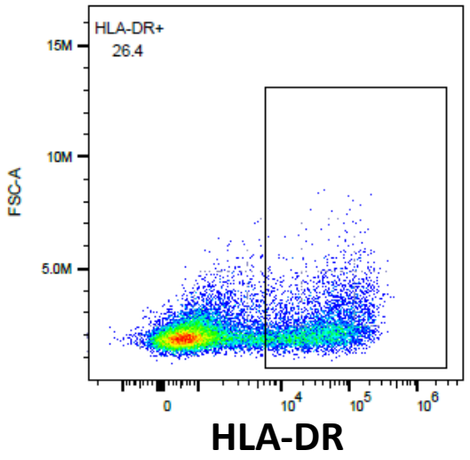
Silencing results in distinct negative population for three allo targets in primary T cell guide screens

CD8+ T resistance



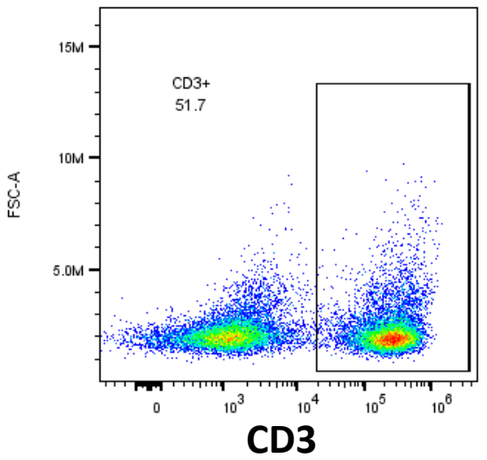
B2M

CD4+ T resistance



HLA-DR

Eliminate GVHD



CD3

- Low efficiency guide conditions shown to demonstrate distinct positive and negative populations

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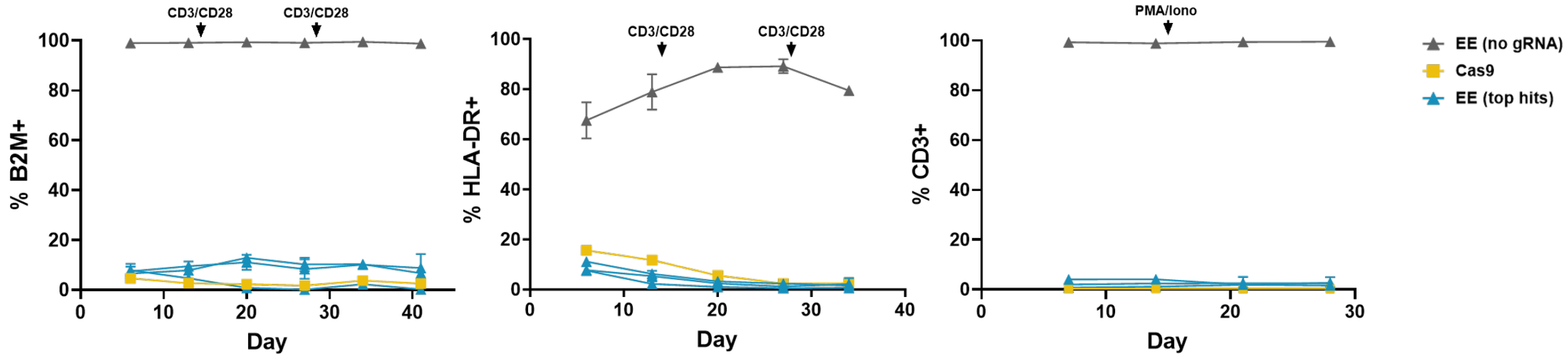
Durable silencing of three allo targets achieved in primary T cells

CD8+ T resistance | **CD4+ T resistance** | **Eliminate GVHD**



- Efficient and durable silencing (90%-99% reduction in expression) for allo-cassette
- Silencing is maintained through restimulation

EE = epigenetic editor

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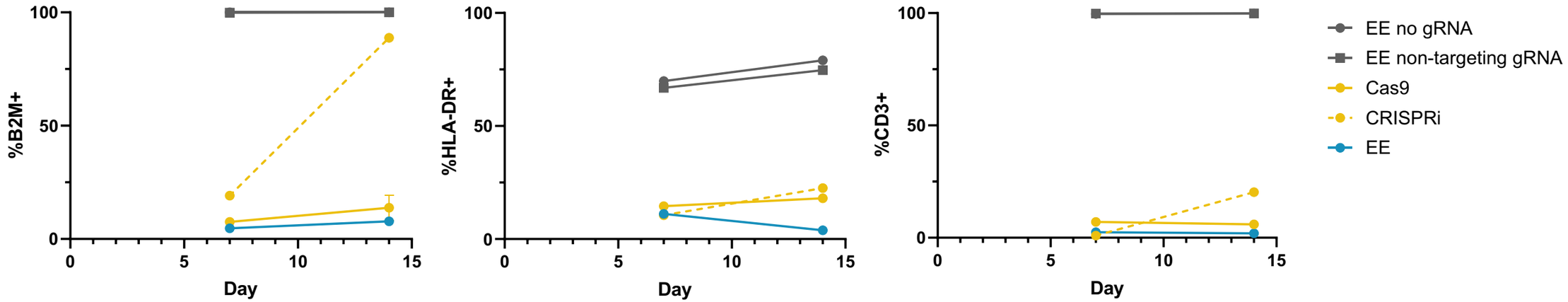
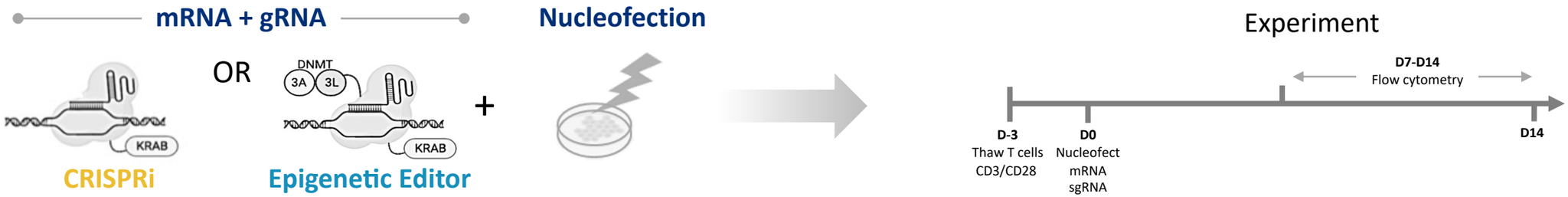
Efficient multiplex silencing of three allo targets in primary T cells

CD8+ T resistance | **CD4+ T resistance** | **Eliminate GVHD**



- Efficient silencing (90%-99% reduction in expression) achieved for all three allo targets silenced simultaneously



EE = epigenetic editor

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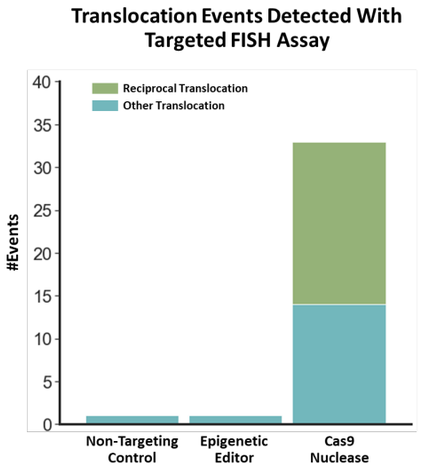
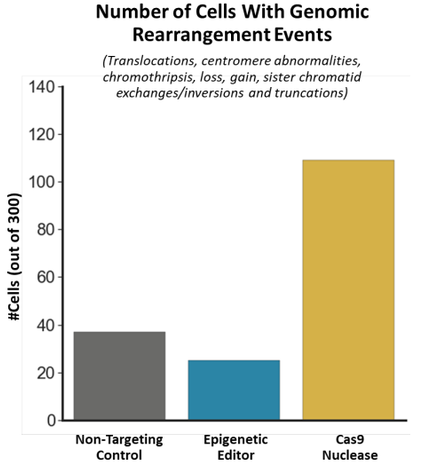
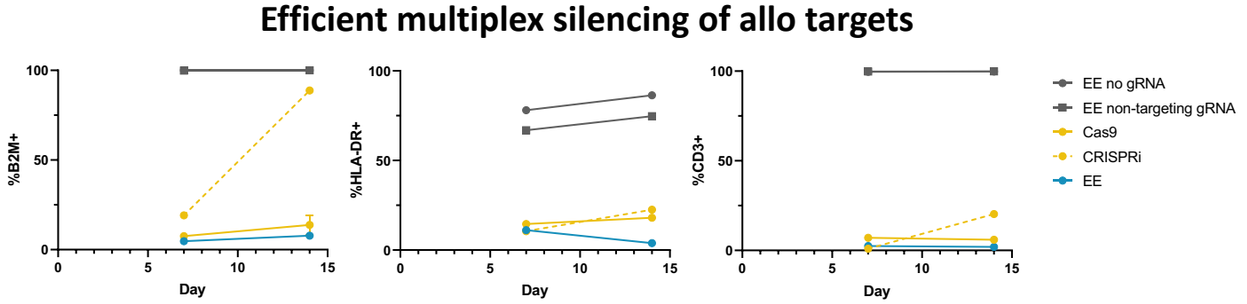
Summary

- Epigenetic editing enables durable modulation of gene expression without genomic alterations
- Chroma's epigenetic editors are therefore extremely well-suited for engineering highly-multiplexed ex vivo cell therapies
- Efficient and durable multiplex silencing in T cells achieved for three allogeneic cassette targets to eliminate GVHD and reduce CD8+ and CD4+ T cell alloresponses







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Acknowledgements

Thank you to the entire Chroma team and collaborators!

KromaTiD

ACUITAS THERAPEUTICS

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