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Multiplexed editing without chromosomal rearrangements using epigenetic editors

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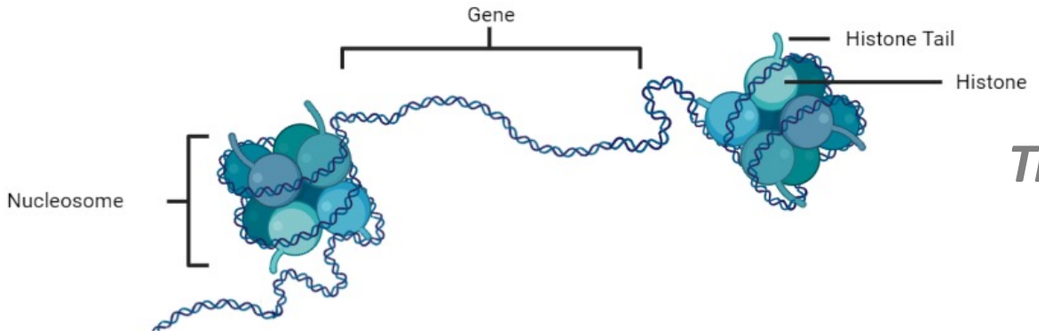
@chromamedicine

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

Durable change in phenotype without a change in genotype



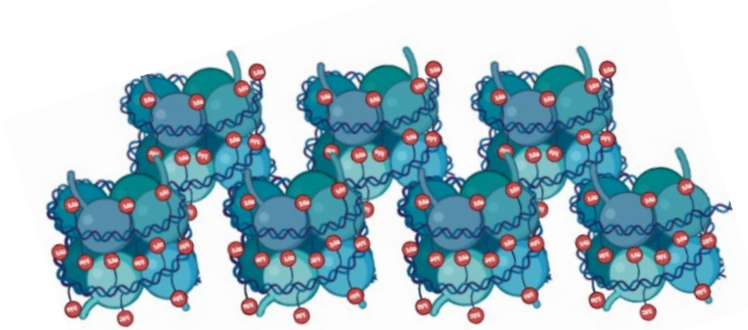
Epigenetic Repressor
Methylates Targets

Transient Application

Epigenetic Activator
Demethylates Targets

Gene is Active
DNA is Open and Accessible

Gene is Inactive
DNA is Closed and Inaccessible



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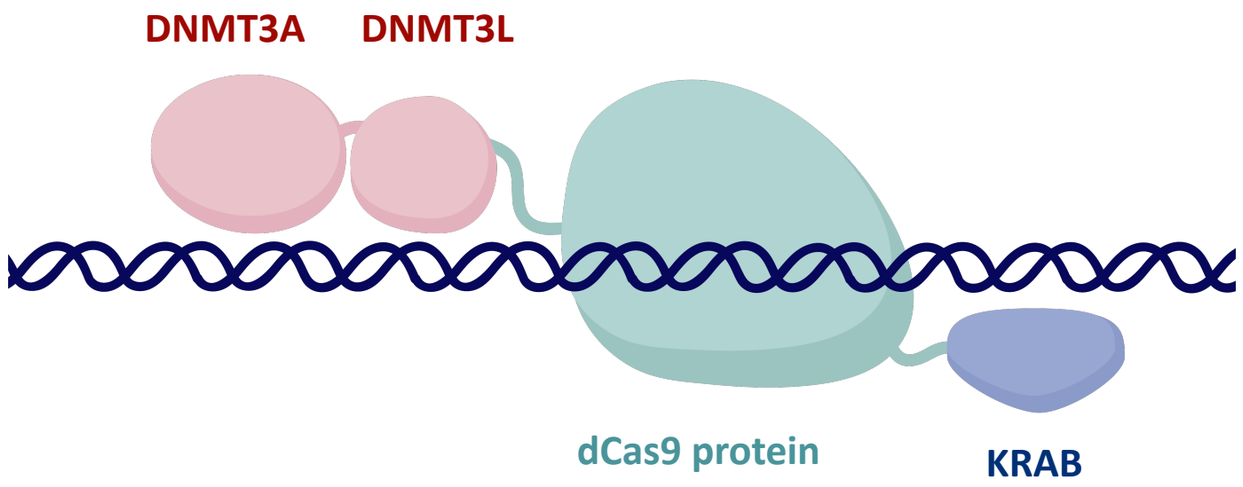
Chroma's epigenetic editors are single fusion proteins with three functional domains

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, Cogan JZ, Replogle JM, Adriaens C, Ramadoss GN, Shi Q, Hung KL, Samelson AJ, Pogson AN, Kim JYS, Chung A, Leonetti MD, Chang HY, Kampmann M, Bernstein BE, Hovestadt V, Gilbert LA, Weissman JS. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. Cell. 2021

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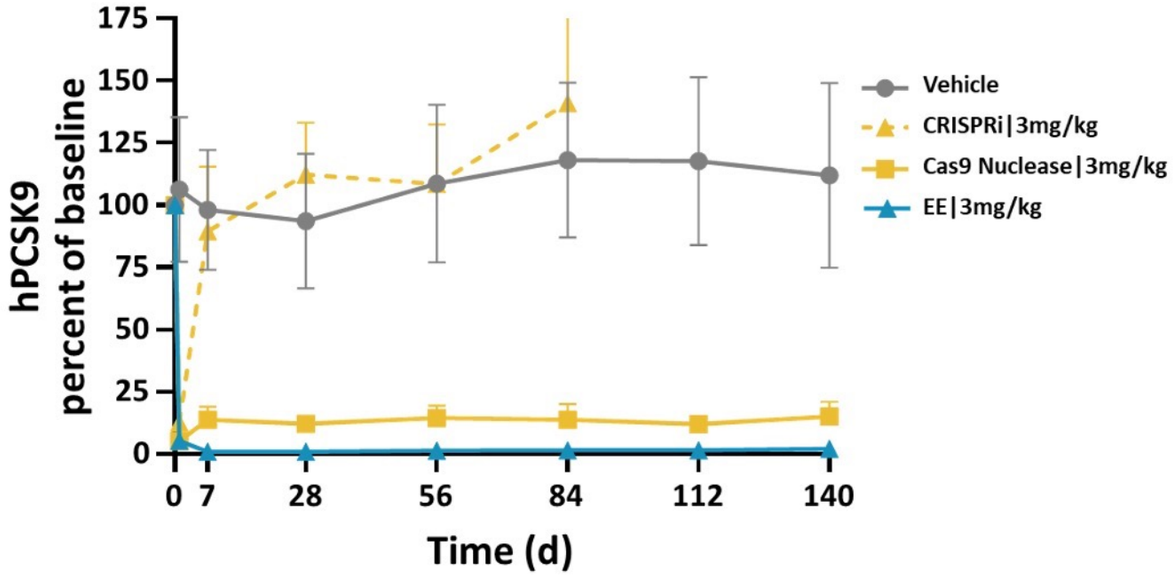
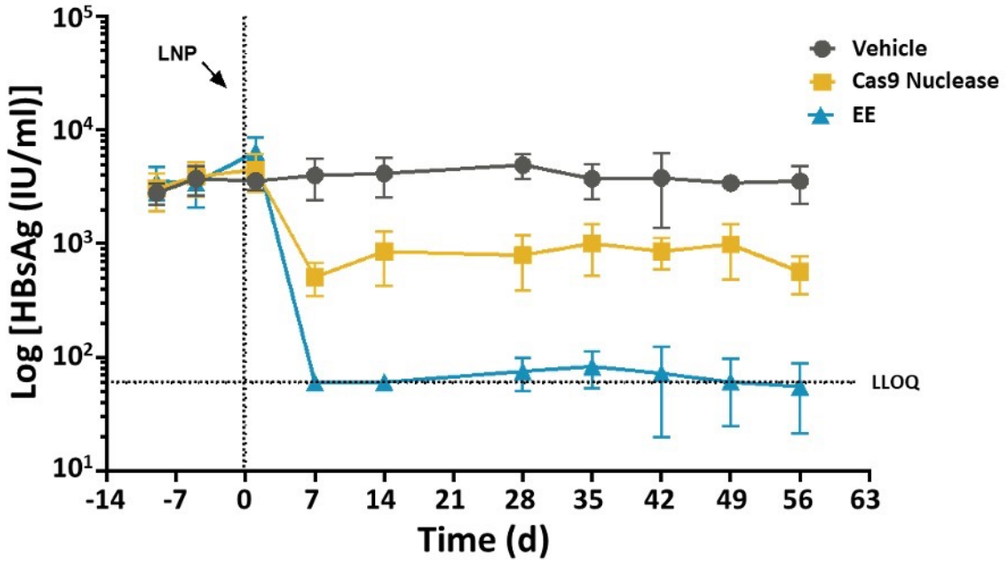
Chroma epigenetic editors effectively and durable silence in vivo at multiple targets

99% PCSK9 Silencing Achieved 140 Days Post-dose



Reduction of HBsAg Below LLOQ 56 Days Post-dose



Aron Jaffe, Development of a human PCSK9-targeting epigenetic editor with durable, near-complete in vivo silencing, ASGCT 2023

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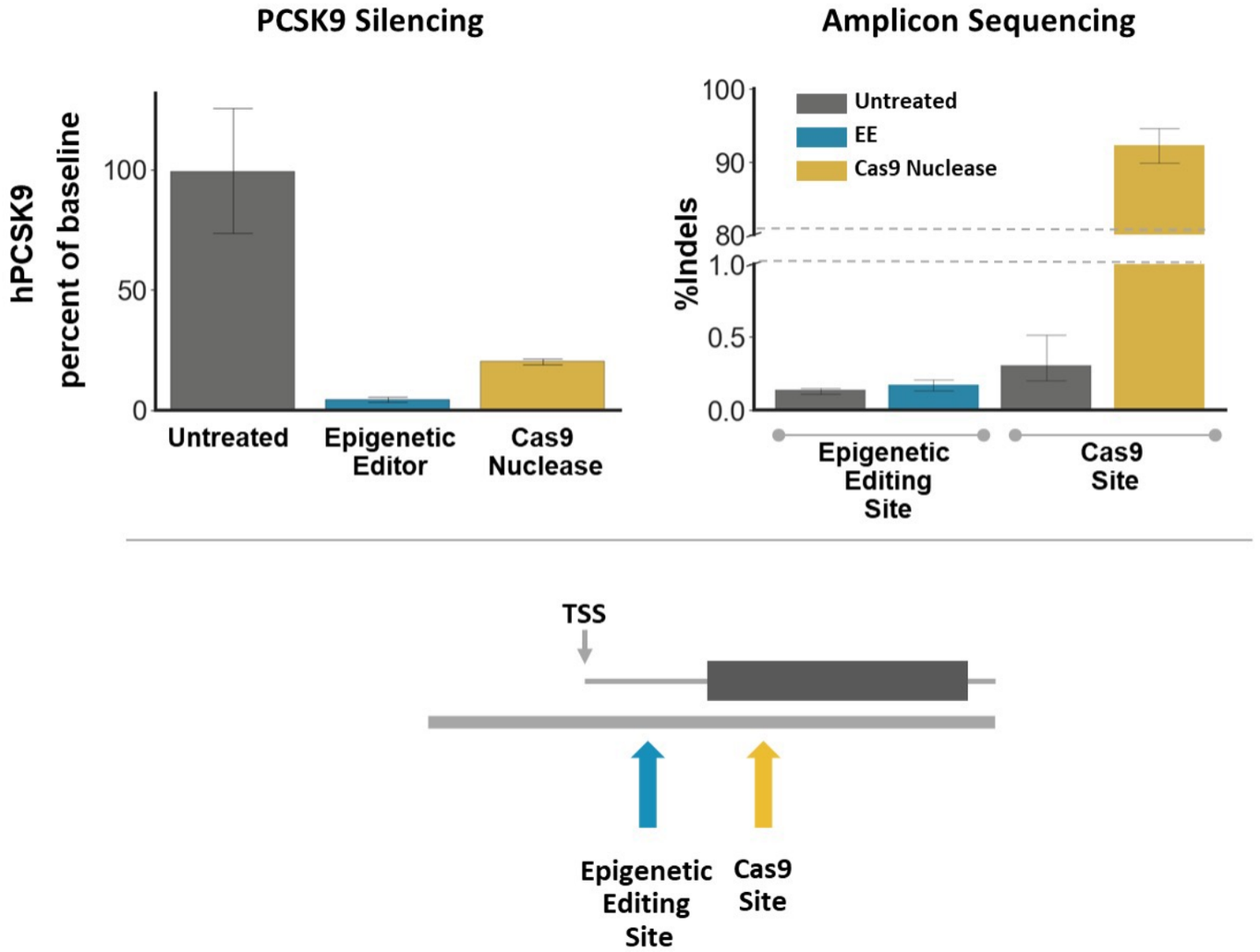
Epigenetic editor shows no indels at PCSK9

- Reduction of PCSK9 = 80% for Cas9 and 95% for epigenetic editor
- Amplicon sequencing assay measures indels at the Cas9 and epigenetic editing sites
- Epigenetic editing shows no indels, at the level of untreated
- Indels observed with Cas9



*CRISPRESSO: Pinello, L., Canver, M., Hoban, M. et al. Analyzing CRISPR genome-editing experiments with CRISPResso. Nat Biotechnol **34**, 695–697 (2016)*

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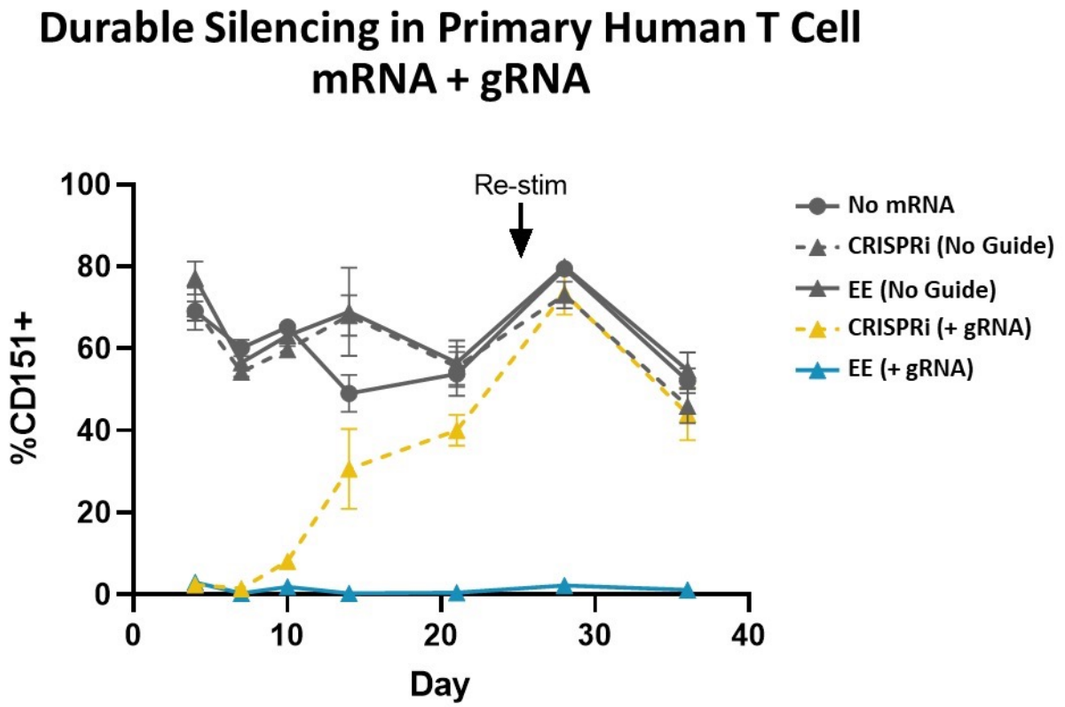
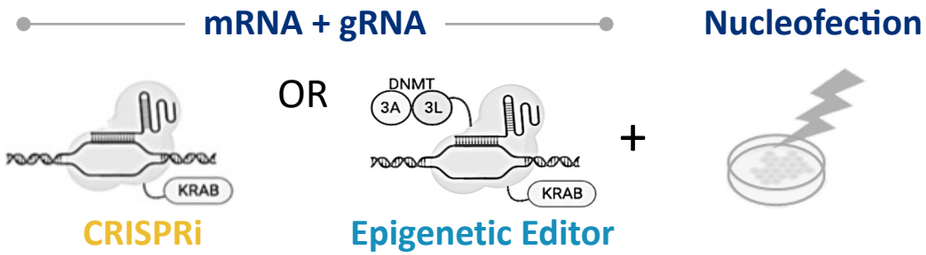
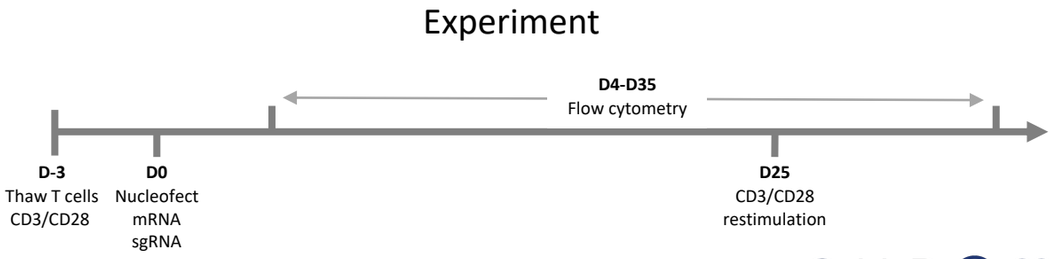
Epigenetic editing shows durable silencing in primary human T cells

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







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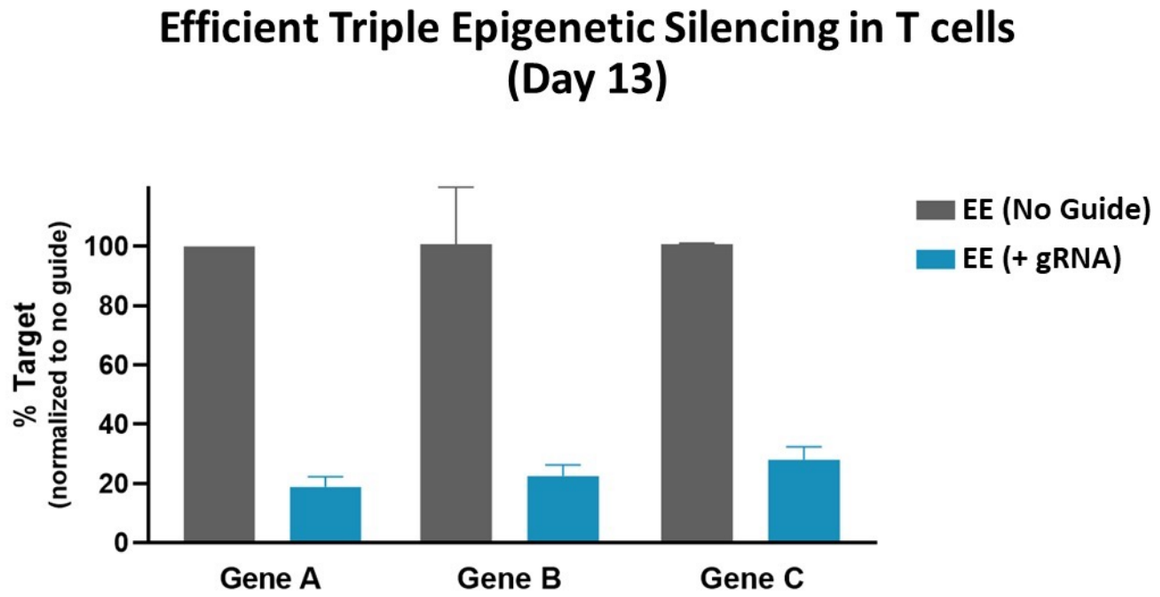
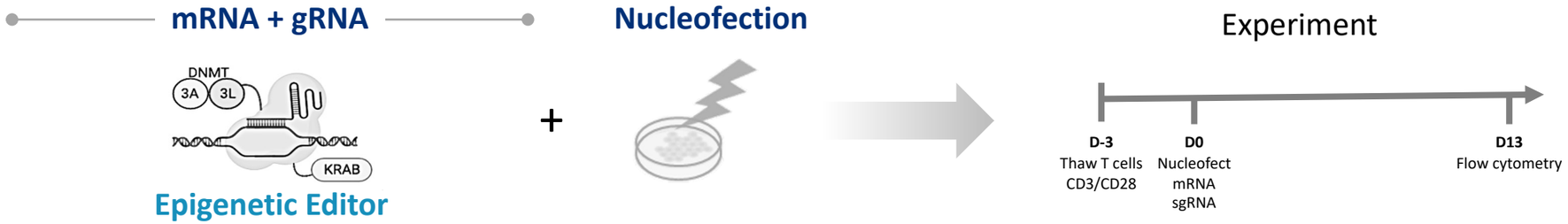
Efficient multiplex silencing with epigenetic editor

- 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





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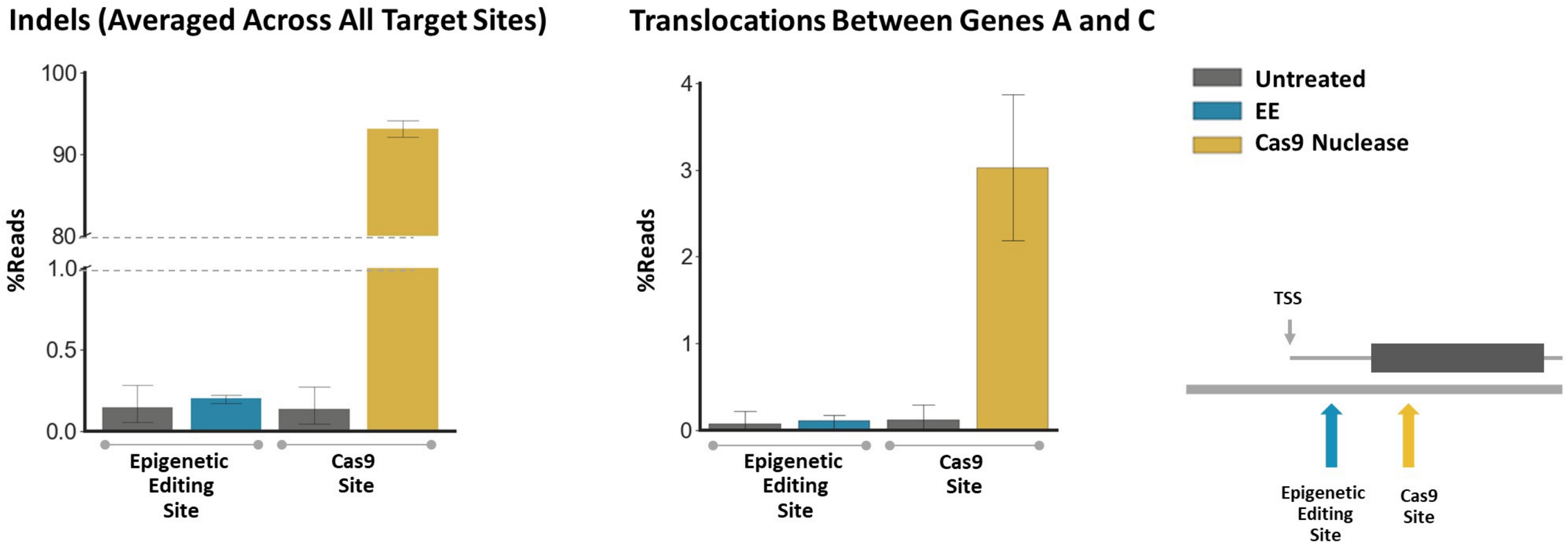
Multiplex epigenetic editing shows no indels or translocations



Using unidirectional sequencing, we identified

- **~93%** indels and **~7%** total on-target translocations with Cas9
- **<1%** indels and **<0.2%** on-target translocations with epigenetic editor (similar to untreated)

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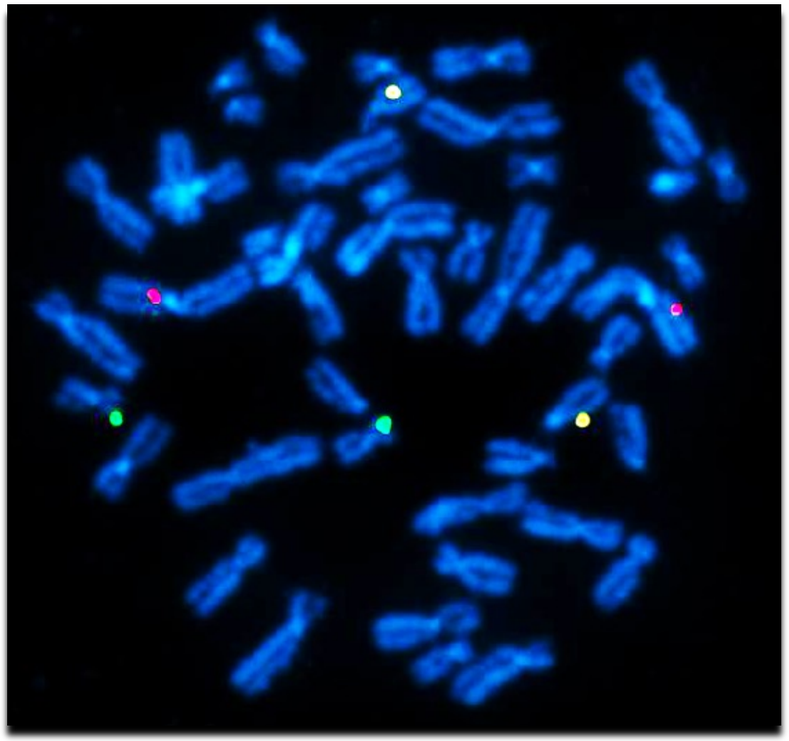
Single-cell imaging approach to visualize genomic rearrangements

- 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

KromaTiD

Robinson, E. et al. Directional Genomic Hybridization (dGH) for Detection of Intrachromosomal Rearrangements. In: Kato, T., Wilson, P. (eds) Radiation Cytogenetics. Methods in Molecular Biology, vol 1984. Humana, New York, NY (2019)

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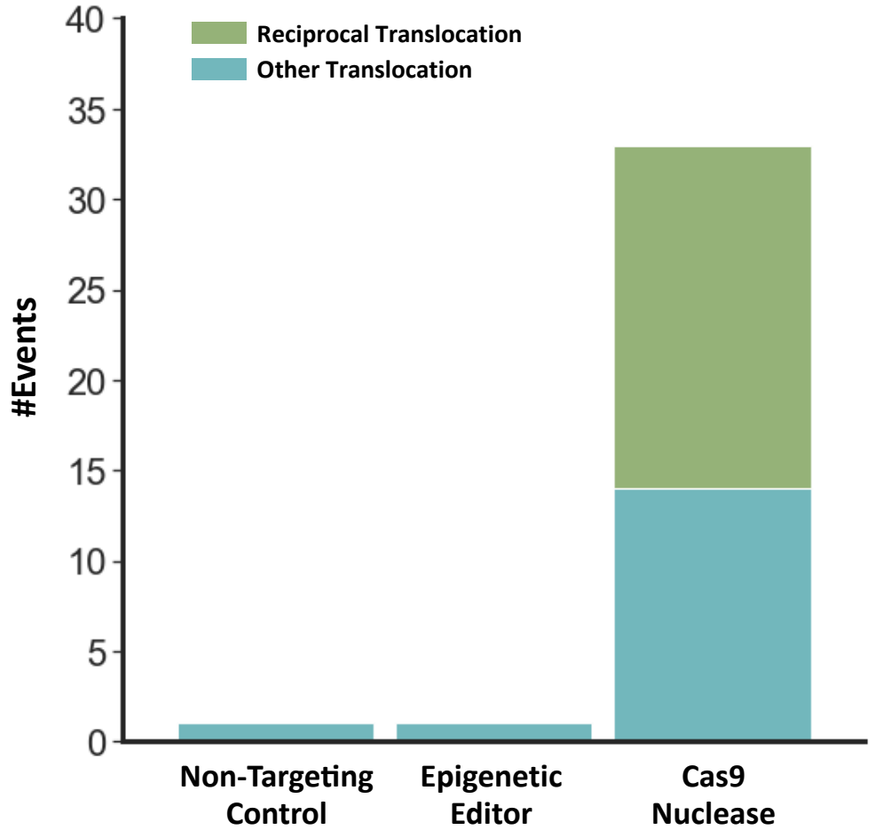
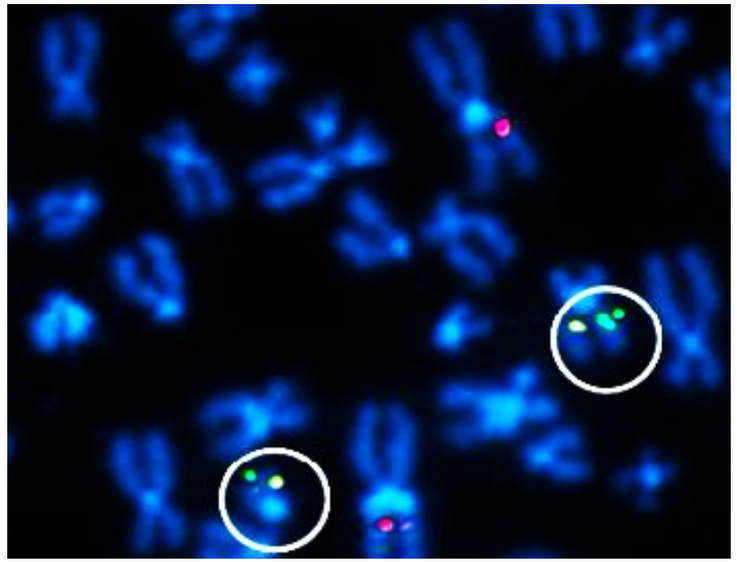
Multiplexing with epigenetic editor does not result in translocations

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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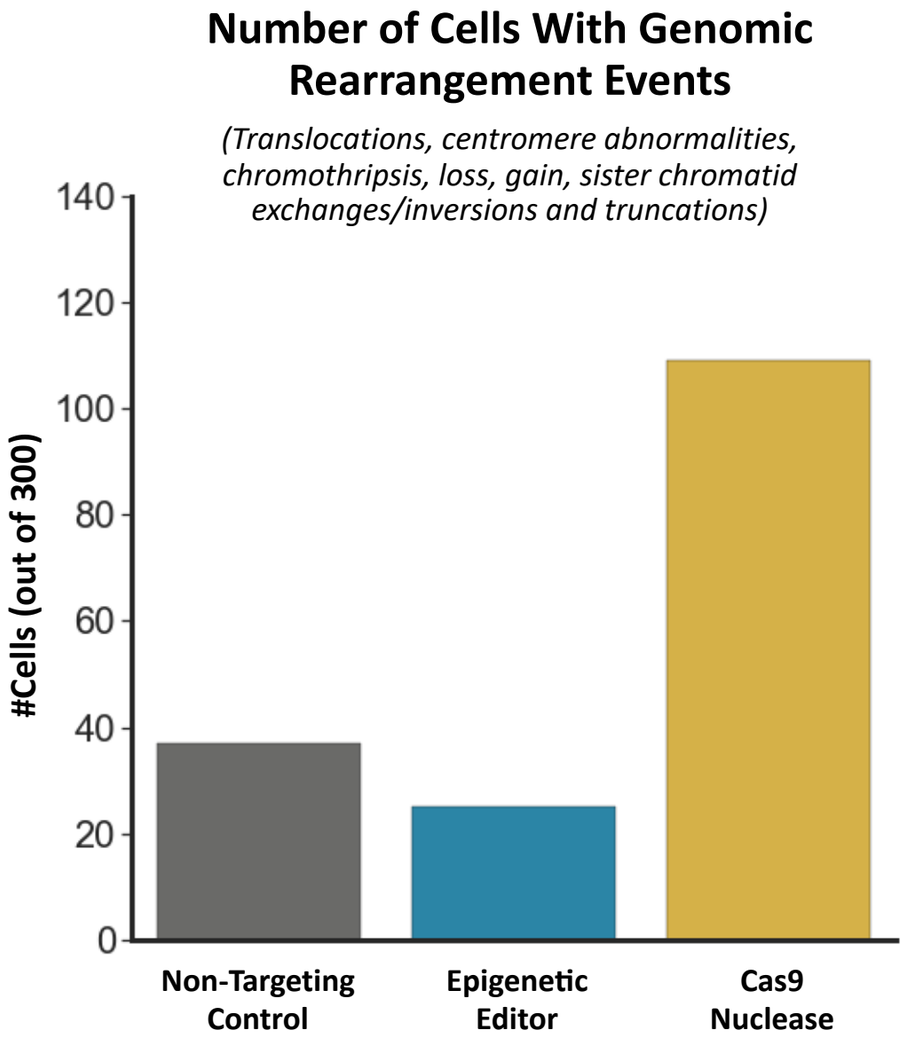
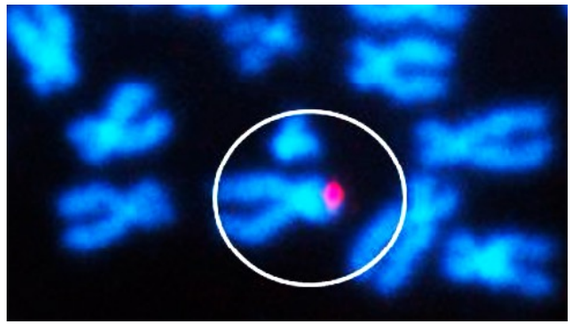
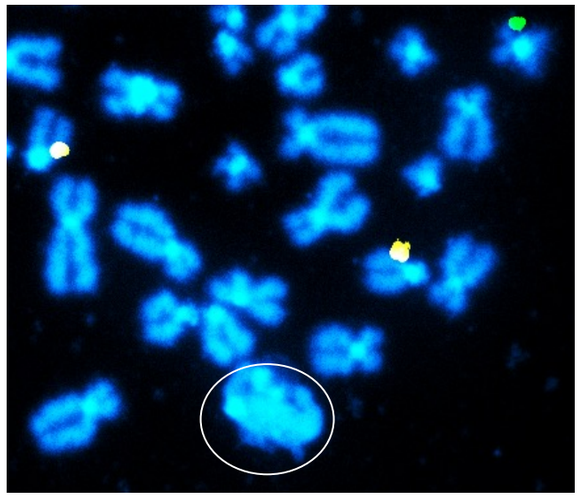
Multiplexing with epigenetic editor does not induce genomic rearrangement events





Edit Site Truncation with Cas9 nuclease



Chromothripsis with Cas9 nuclease

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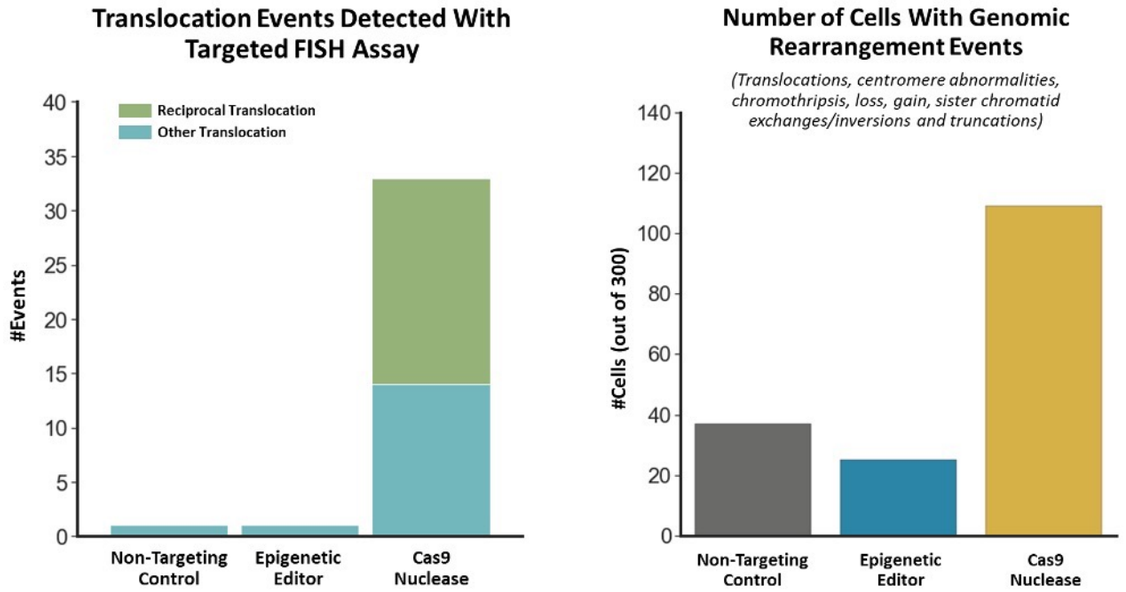
Summary

- Epigenetic editing leverages an endogenous mechanism for regulating gene expression and enables durable modulation of gene expression without altering the DNA sequence
- Chroma epigenetic editors can efficiently multiplex without inducing indels, translocations, and other genomic rearrangements
- The lack of genomic alterations make Chroma's epigenetic editors extremely well-suited for simultaneous multiplexing

Multiplexing with epigenetic editor does not induce translocations or genomic rearrangement events



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Acknowledgements

Thank you to the entire Chroma team and collaborators!

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