CHROMA MEDICINE

Epigenetic Editors: A New Class of Genomic Medicines

Vic Myer, President and CSO

ep·i·ge·net·ics

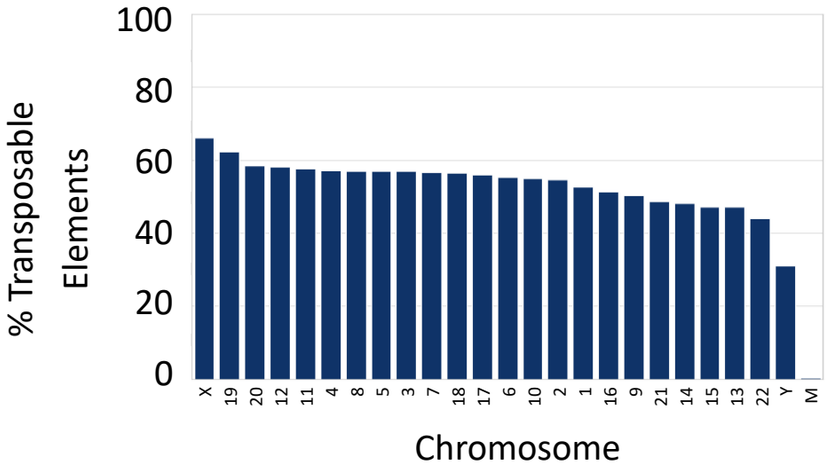
/ˌepəjəˈnediks/

noun **BIOLOGY**

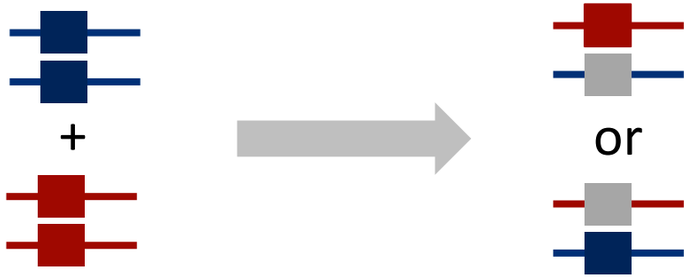
the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.
"epigenetics has transformed the way we think about genomes"

Epigenetics plays several critical roles

Silencing of transposable elements



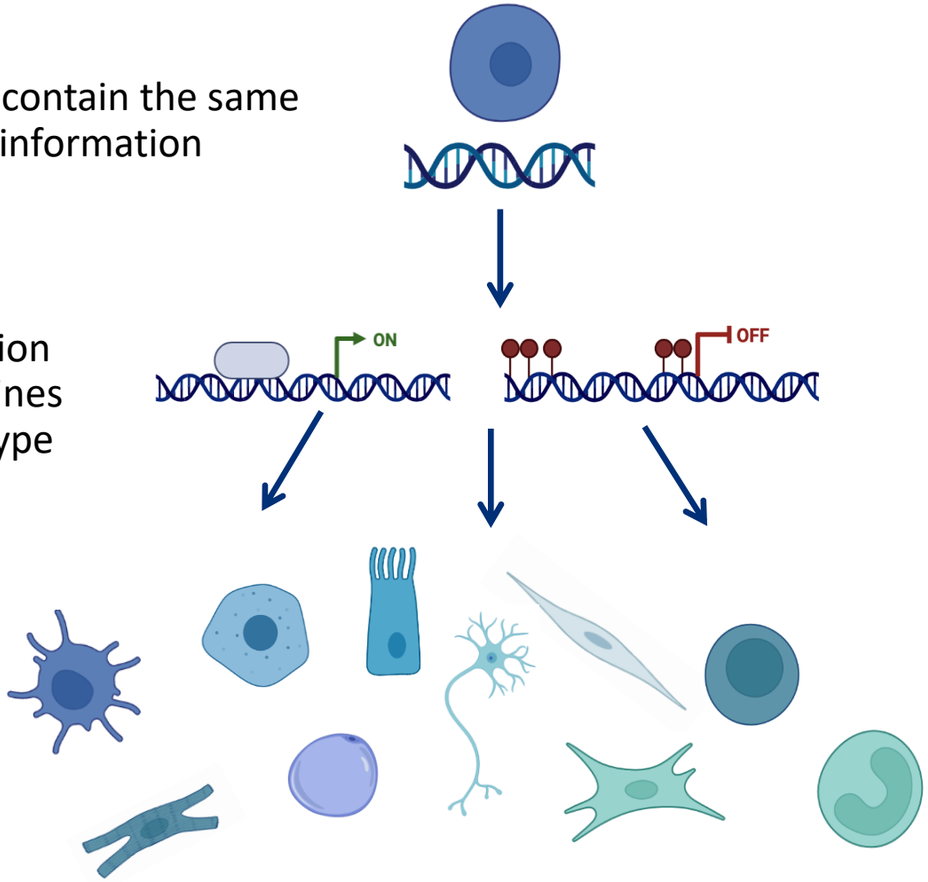
Imprinting / X-inactivation



Regulation of gene expression to determine cell fate

All cells contain the same genetic information

Expression determines phenotype



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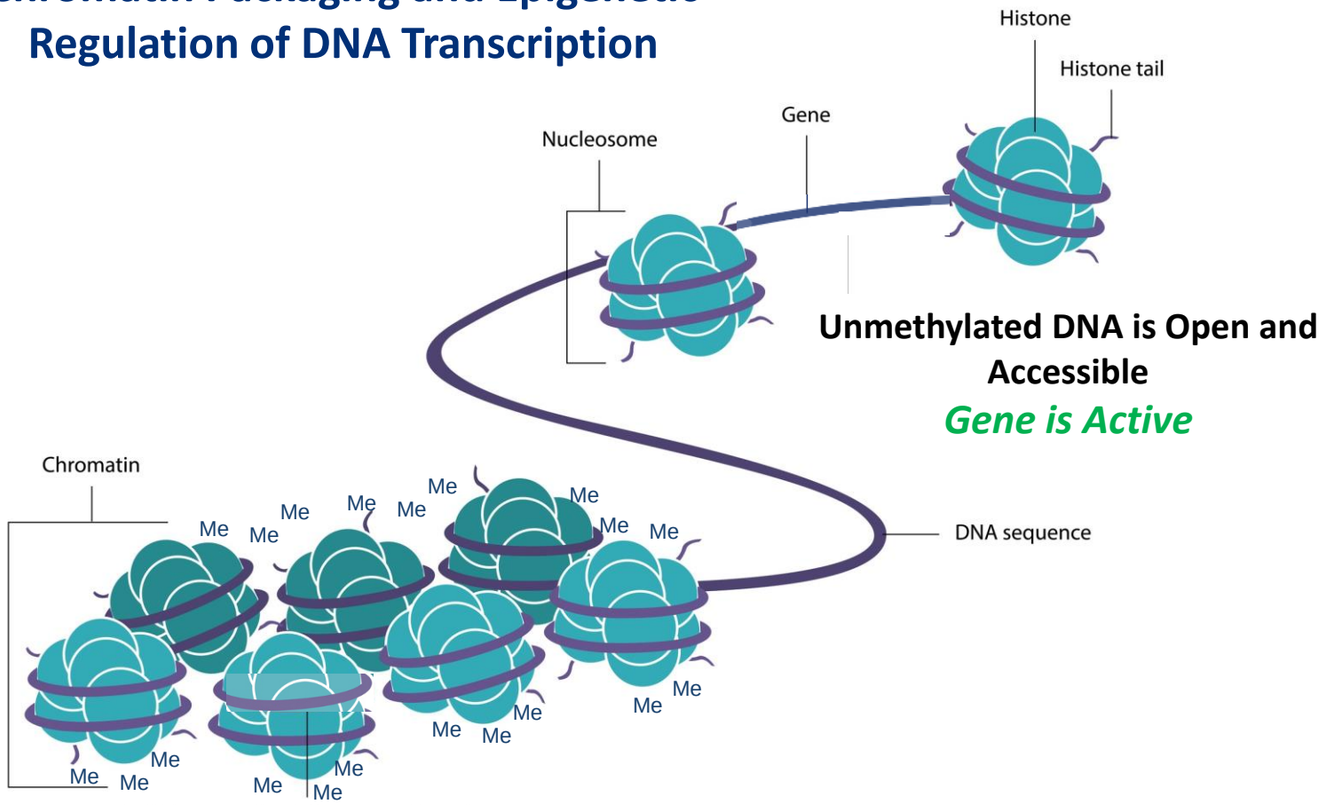
Epigenetics: The central regulator of gene expression

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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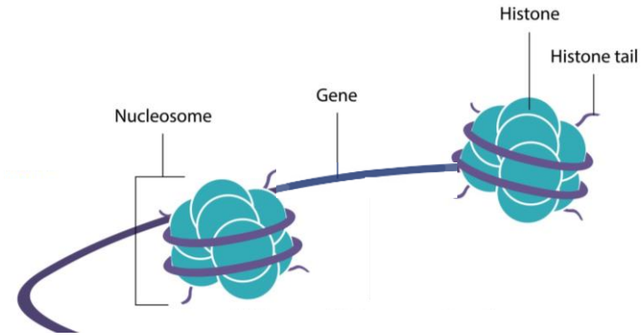
Gene expression is controlled by epigenetic state

Expressed genes are characterized by promoter hypo-methylation and open chromatin



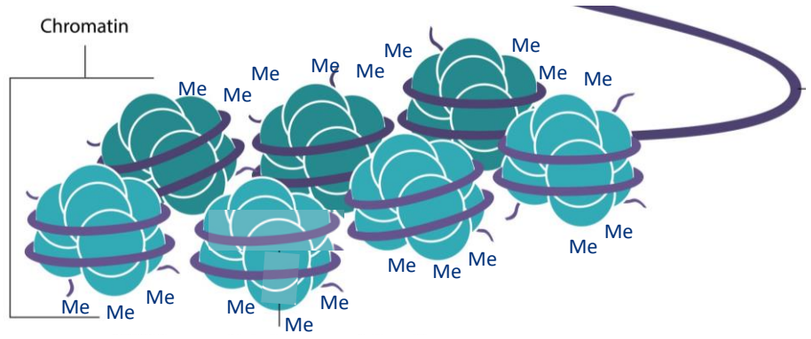
Gene is Active

DNA is Open and Accessible

DNA Methylation

DNA De-methylation

Silenced genes are characterized by promoter hyper-methylation and closed chromatin



Gene is Inactive

DNA is Closed and Inaccessible

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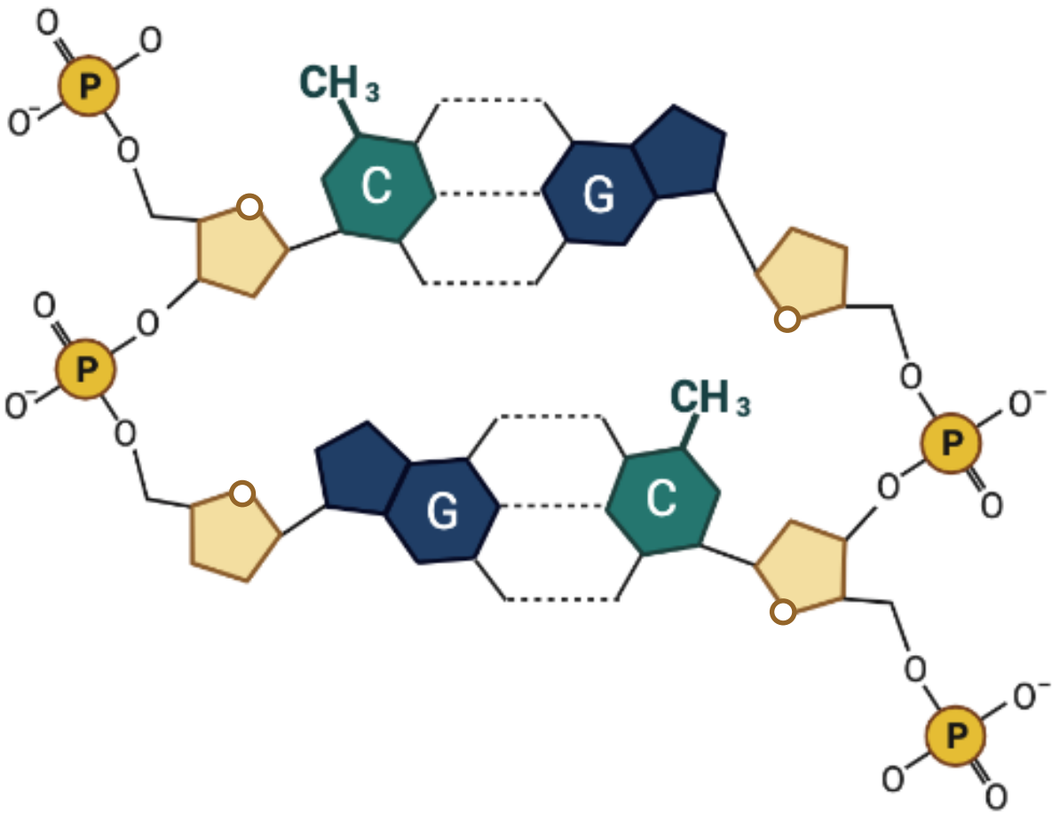
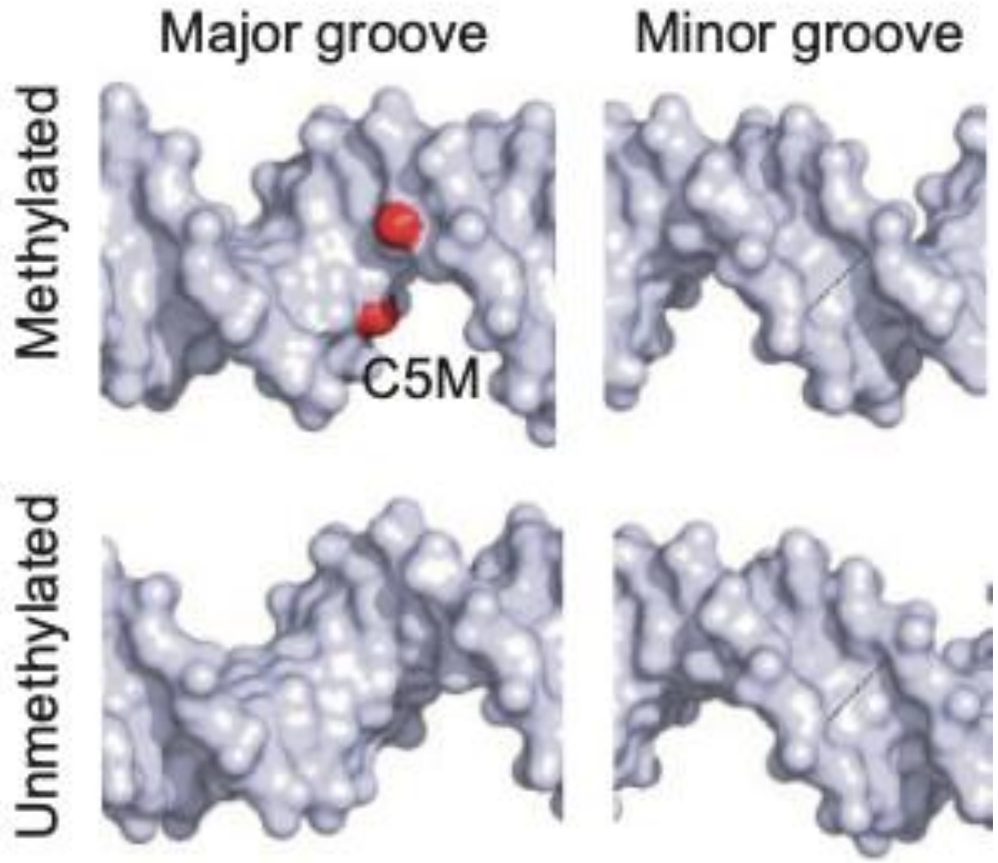
CpG methylation effects major groove contacts and α-helix structure





Dantas Machado et al., "Evolving insights on how cytosine methylation affects protein-DNA binding" (2014) Briefings in Functional Genomics, V14, 61-73.

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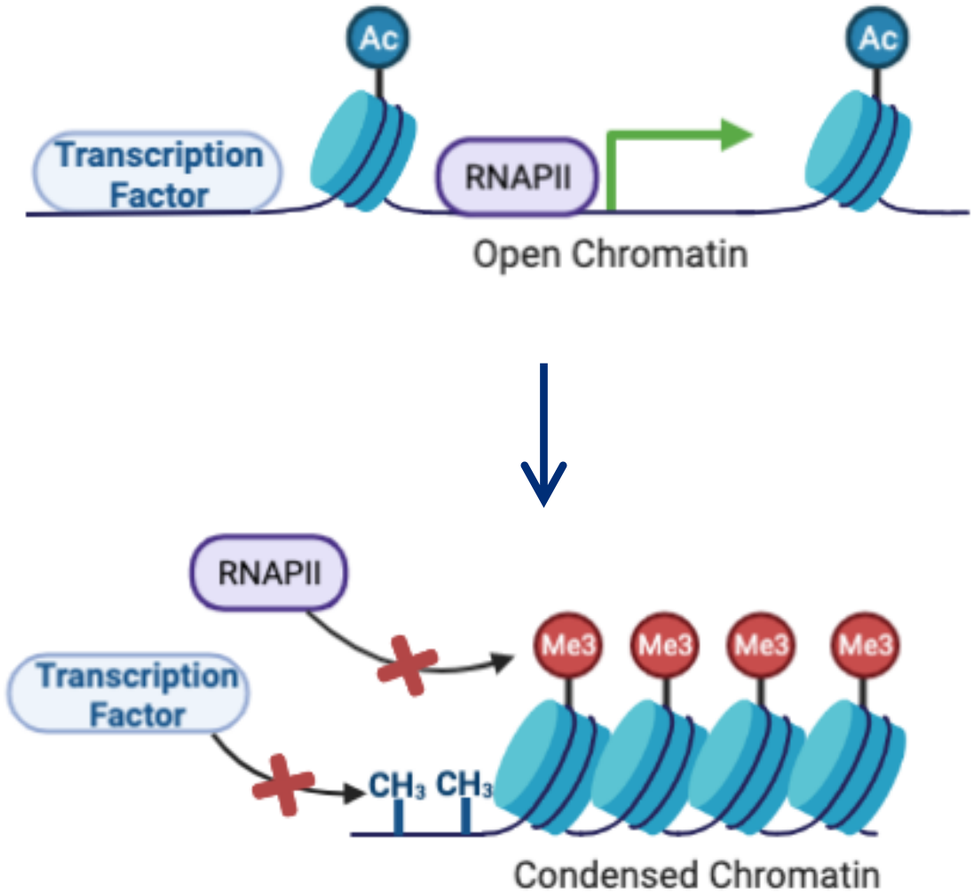
DNA methylation catalyzes and stabilizes a repressed gene state

ON

OFF



- DNA methylation effects protein binding (e.g. transcription factors) and thereby may remove positive expression signals
- Specific classes of proteins (e.g. methyl binding proteins, DNMTs) recognize methylated DNA and interact with histone deacetylases and histone methyltransferases which mediate repressive chromatin marks
- Methyl binding proteins, themselves, also contain repression domains

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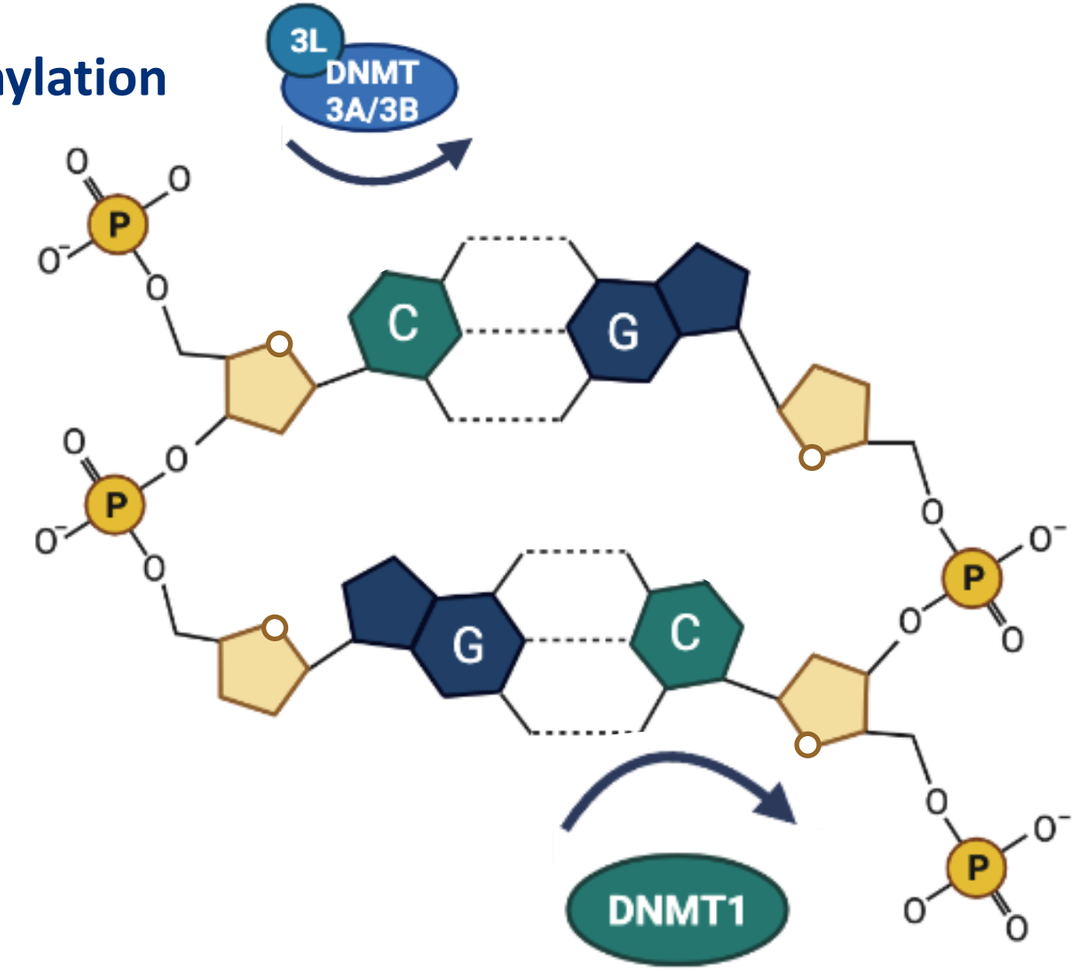
DNA methylation is a heritable epigenetic mark

De novo methylation



- DNA methylation patterns are established very early in development
- DNA methylation is heritable as there are specific systems evolved to recognize, and methylate hemi-methylated CpGs
- In mammals, methylation occurs predominantly at CpG dinucleotides

Maintenance methylation

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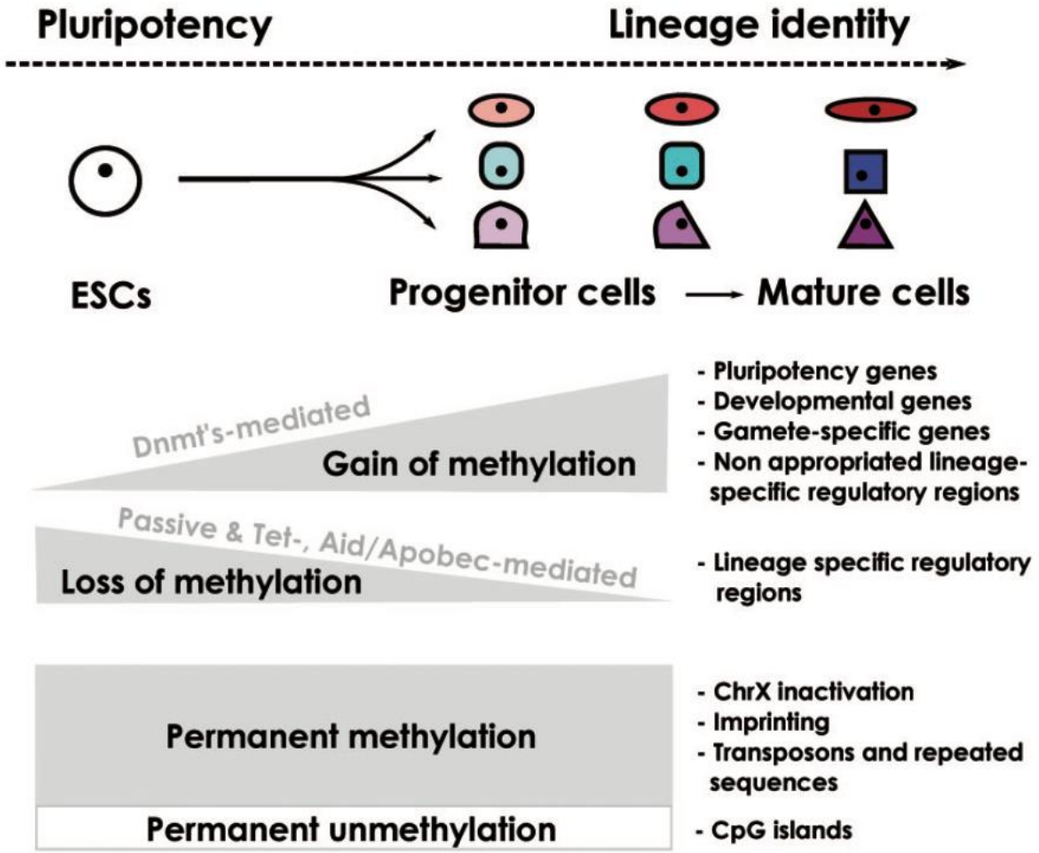
Changes in methylation correlate with differences in cell state



- Permanent DNA methylation is involved in X-chromosome inactivation, imprinting and silencing of transposon and repeat elements
- During development and differentiation the overall CpG methylome pluripotency genes gain methylation and lineage-specific genes lose methylation
- These cell-specific changes in methylation are restricted to narrow windows of promoter and enhancer CpGs

Suelves et al., Brief Funct Genomics 2016

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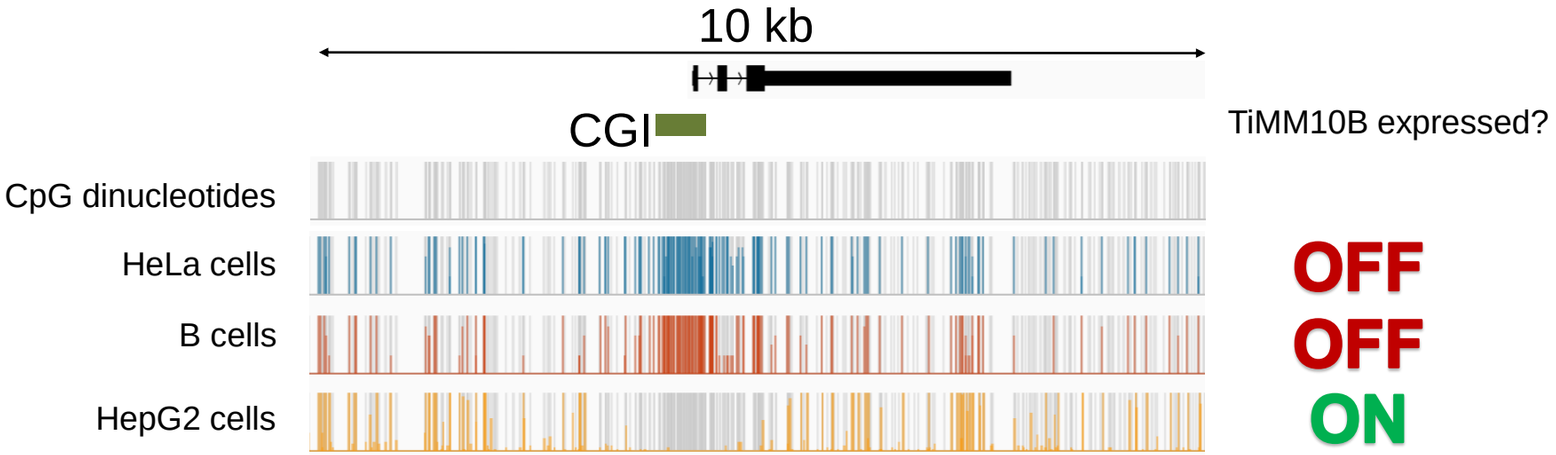
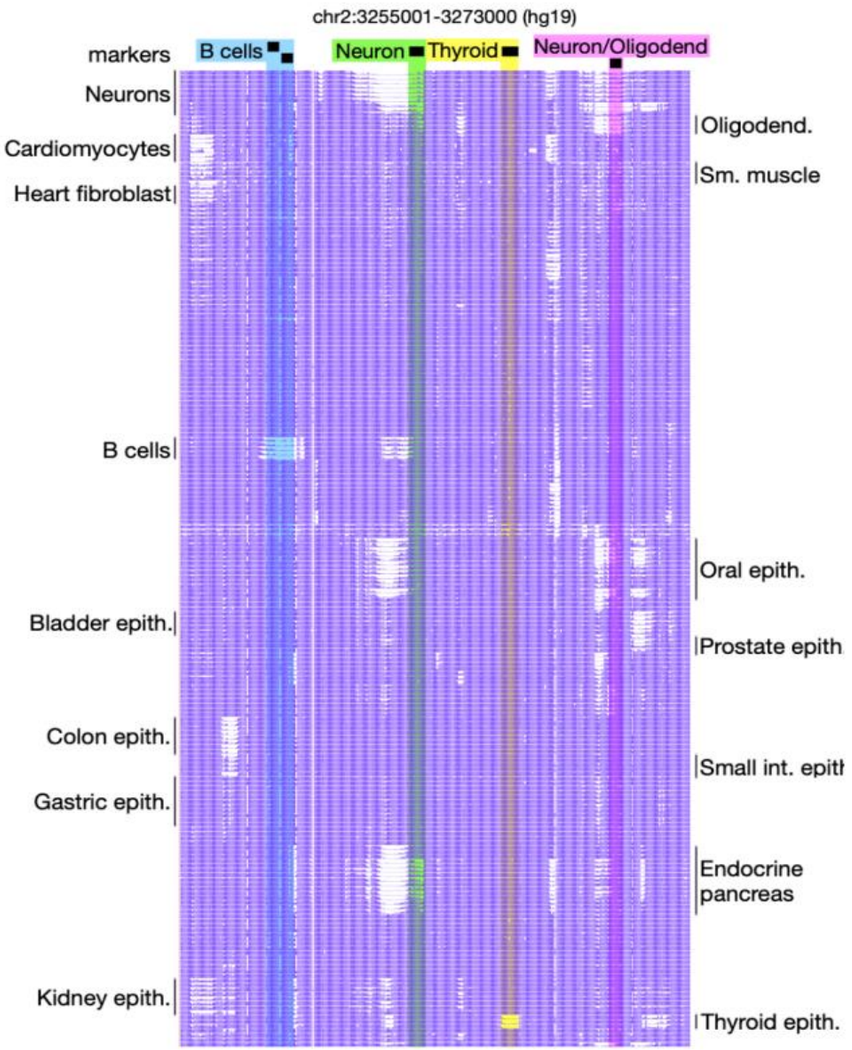
Methylation patterns are highly conserved with differences contained to small regions





- WGBS analysis of 207 methylomes across 77 different cell types from 137 donors
- The majority of CpGs are methylated (~ 70% across the genome)
- The patterns are very consistent across individuals within a cell type (>99.5% identical), and across cell types within an individual (>95% identical)
- Focal patterns around promoters and enhancers are the sites of differences across cell types and determine gene expression

Loyfer et al., BioRxiv 2022

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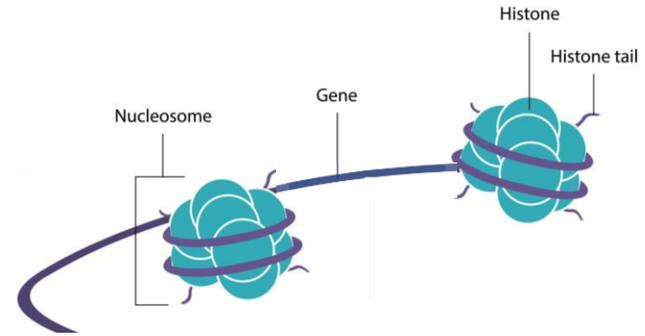
Epigenetic editing leverages the endogenous system to precisely control gene expression

Durable Change in Phenotype Without a Change in Genotype



Gene is Active

DNA is Open and Accessible

Epigenetic Repressor

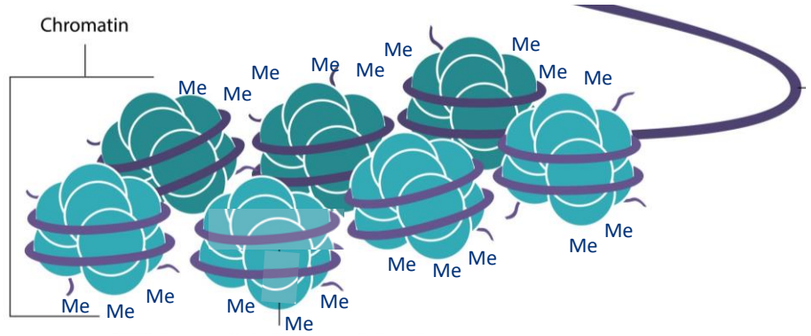
Methylates Targets

Epigenetic Activator

Demethylates Targets

Gene is Inactive

DNA is Closed and Inaccessible



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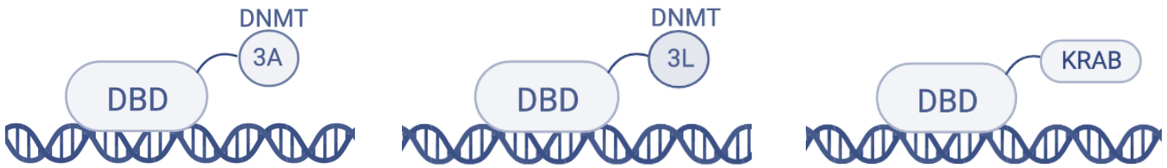
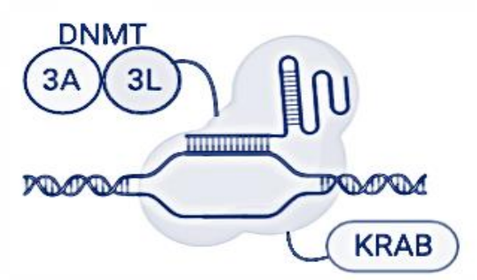
Chroma epigenetic editors: modular and versatile

Proprietary Epigenetic Editors

Triple ETRs



CRISPR-Off



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

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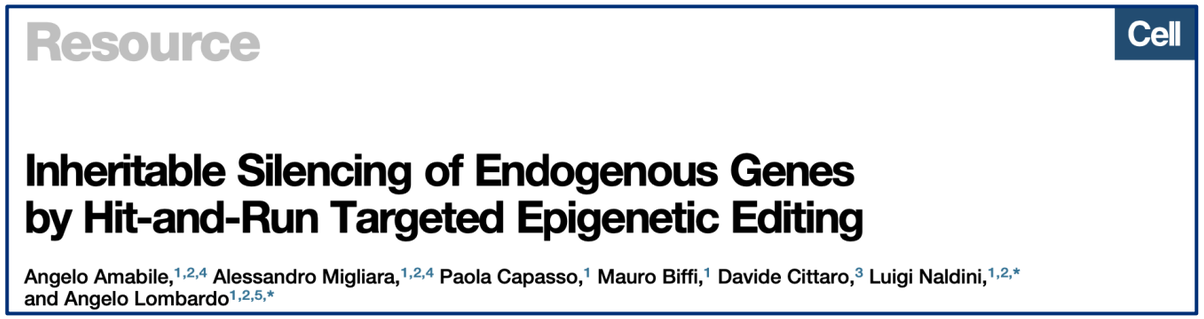
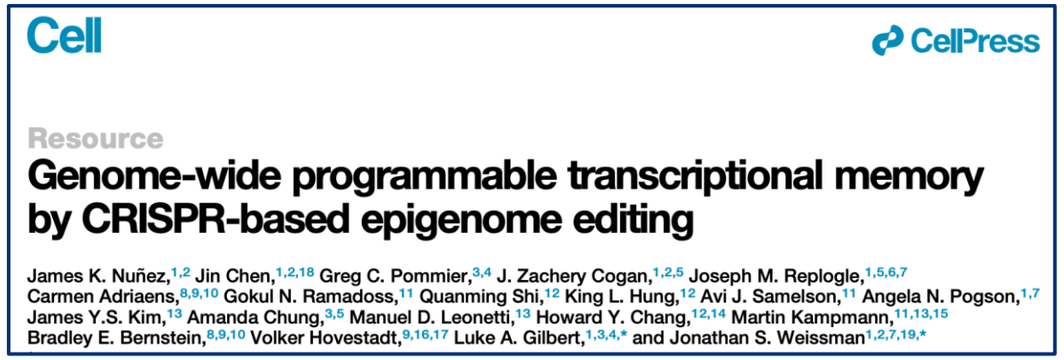
Platform enabled by breakthroughs from Chroma founders


Resource

Cell

Inheritable Silencing of Endogenous Genes by Hit-and-Run Targeted Epigenetic Editing

Angelo Amabile,[1,2,4] Alessandro Migliara,[1,2,4] Paola Capasso,[1] Mauro Biffi,[1] Davide Cittaro,[3] Luigi Naldini,[1,2,*] and Angelo Lombardo[1,2,5,*]


Cell

CellPress

Resource

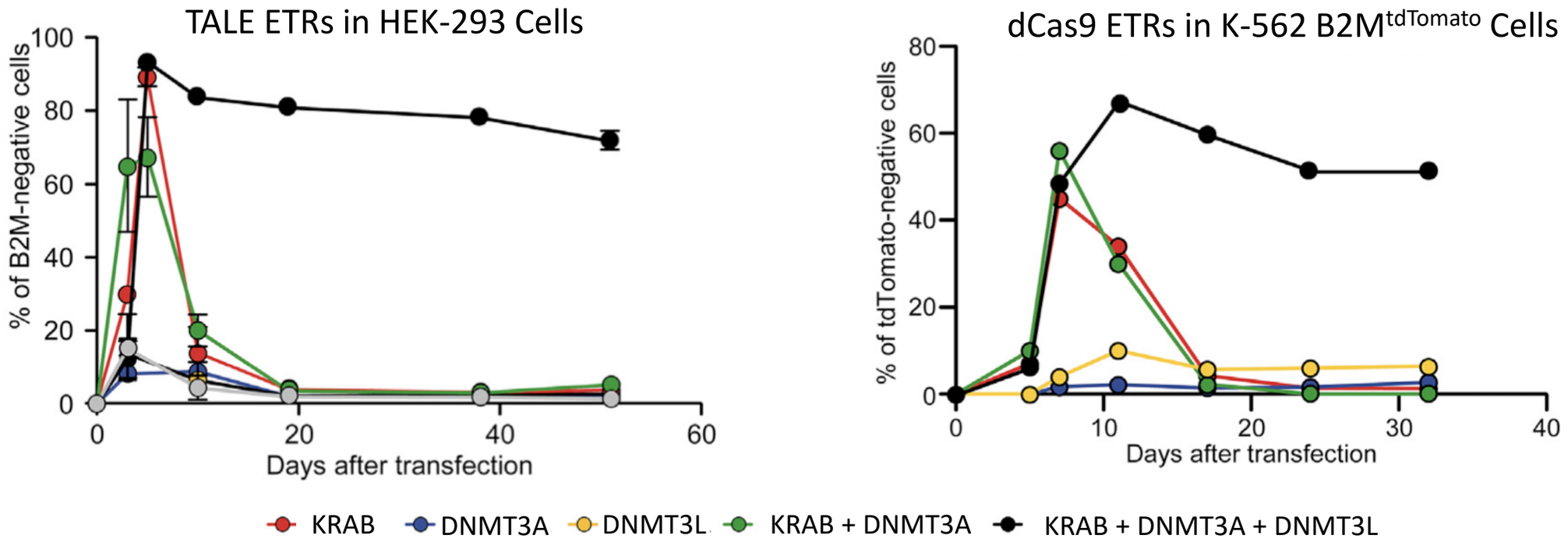
Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing

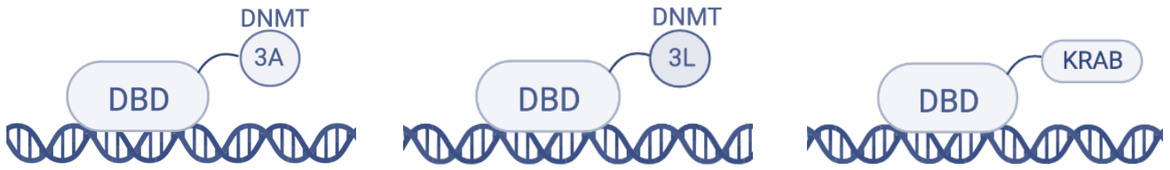
James K. Nuñez,[1,2] Jin Chen,[1,2,18] Greg C. Pommier,[3,4] J. Zachery Cogan,[1,2,5] Joseph M. Replogle,[1,5,6,7] Carmen Adriaens,[8,9,10] Gokul N. Ramadoss,[11] Quanming Shi,[12] King L. Hung,[12] Avi J. Samelson,[11] Angela N. Pogson,[1,7] James Y.S. Kim,[13] Amanda Chung,[3,5] Manuel D. Leonetti,[13] Howard Y. Chang,[12,14] Martin Kampmann,[11,13,15] Bradley E. Bernstein,[8,9,10] Volker Hovestadt,[9,16,17] Luke A. Gilbert,[1,3,4,*] and Jonathan S. Weissman[1,2,7,19,*]

- **Epigenetic Editing** can induce **durable gene silencing** upon **transient treatment**
  - Broadly applicable and not restricted to genes with CpG islands
  - Heritable through 100s of cell divisions and during cell differentiation
- A **single fusion construct** can efficiently drive epigenetic editing
- **Specific** and **multiplexable** and **reversible** via targeted de-methylation
- CRISPR-On can ‘reset’ endogenous expression at a **tunable** level
- Amenable to **genome-scale approaches** for target discovery and lead optimization

Amabile et al identified effector combination for stable epigenetic silencing

Targeted co-recruitment of KRAB, DNMT3A and DNMT3L is sufficient to induce stable epigenetic silencing





Amabile et al., Cell 2016

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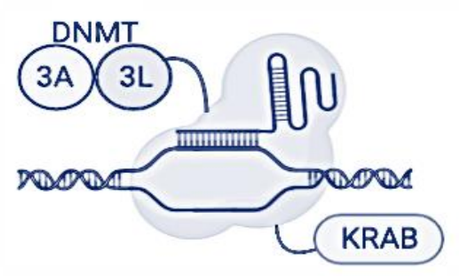
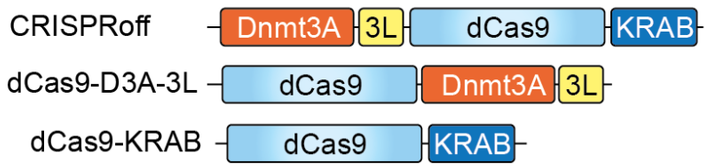
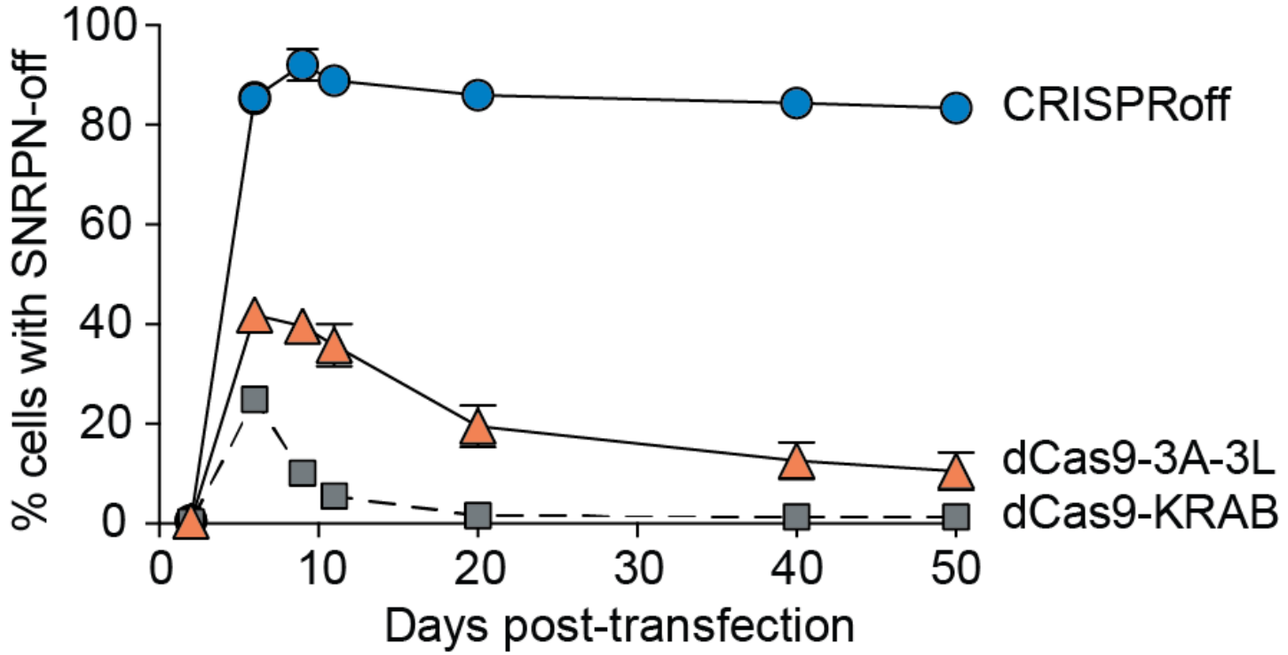
Nunez et al developed CRISPR-Off: a single, highly efficient construct

Optimization of linkers and domain architecture leads to a single, highly effective fusion protein

CRISPR-Off







Nuñez et al., Cell 2021

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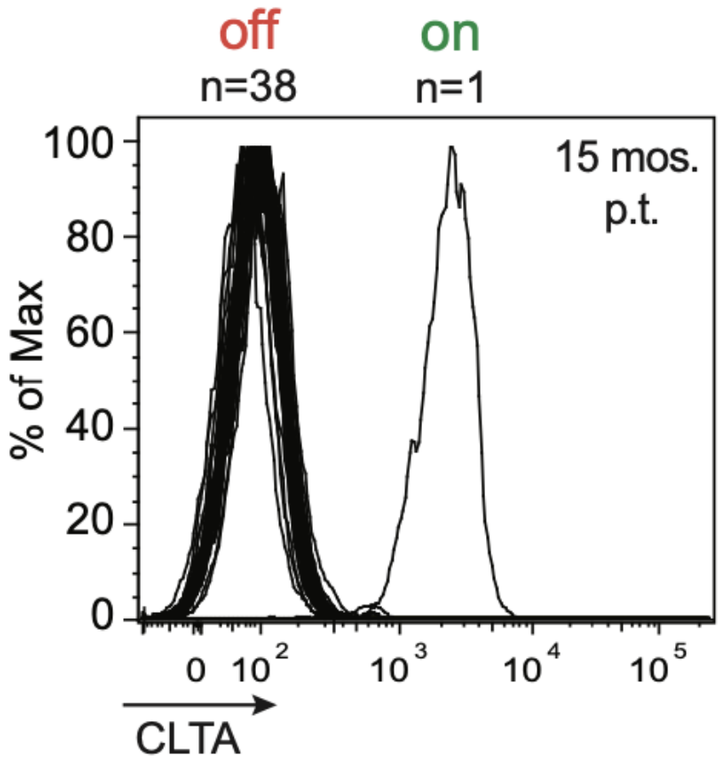
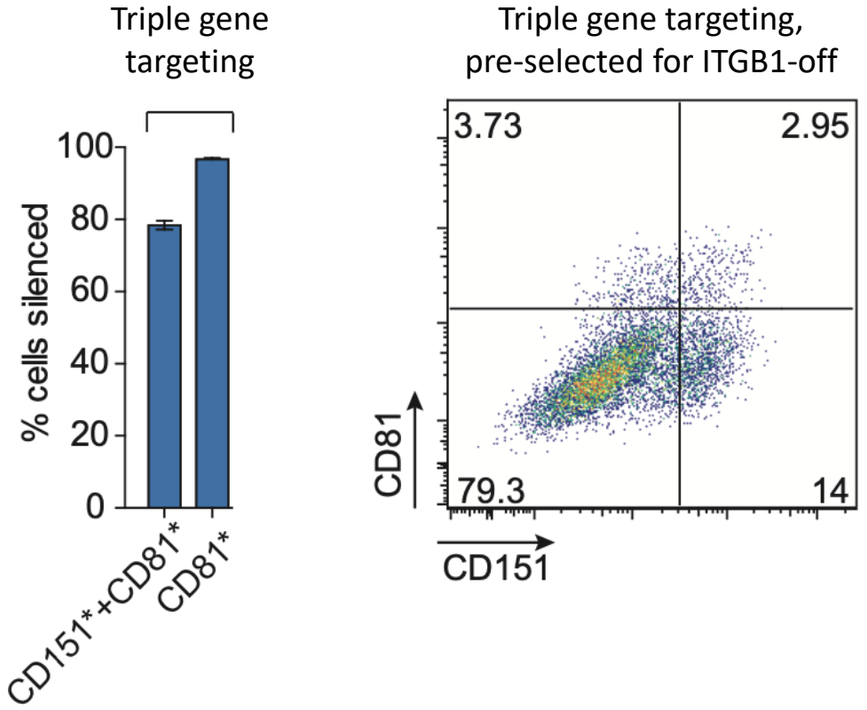
CRISPR-Off silencing is durable, heritable, and multiplexable

Cells retain memory after 15 months in continuous culture after transient treatment



Nuñez et al., Cell 2021

Multiplexing of CRISPR-Off gene silencing is achievable with high efficiency (~80%+)



Triple silencing of CD81, CD151 and ITGB1

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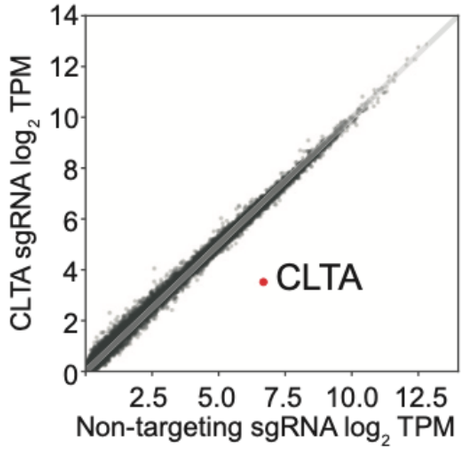
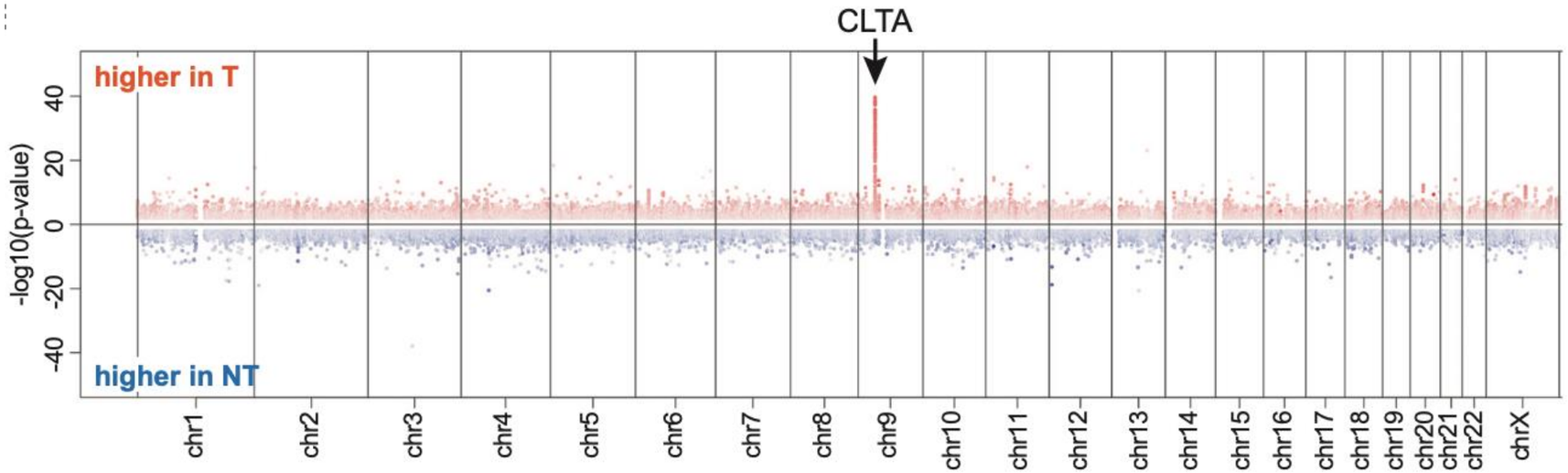
CRISPR-Off is highly specific

By both whole transcriptome profiling & whole genome bisulfite sequencing the approach is specific





Nuñez et al., Cell 2021

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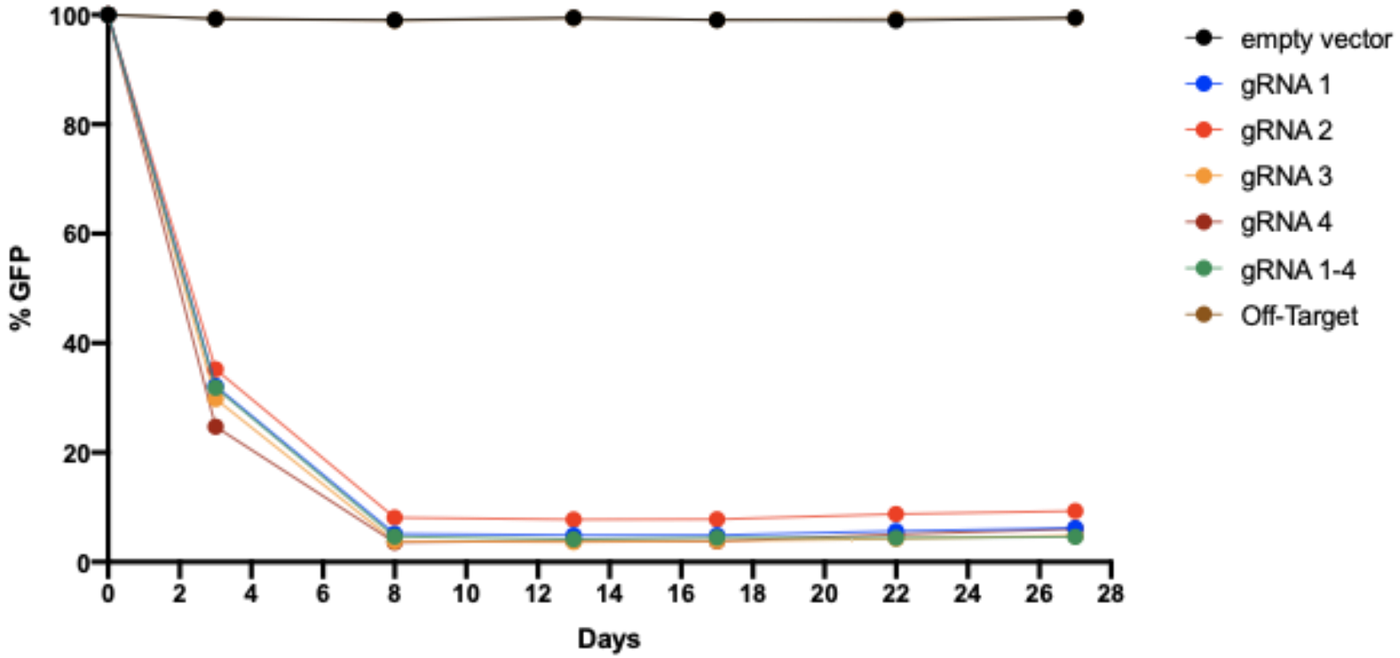
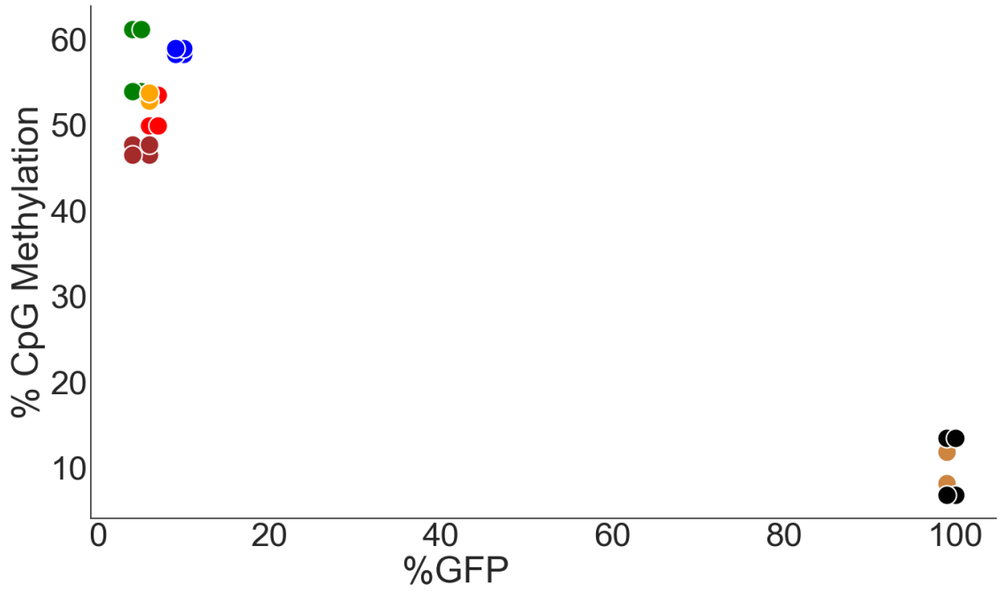
CRISPR-Off deployed robustly at Chroma

Methylation changes for single and multi-guide CRISPR-Off are consistent with decrease in expression of on-target gene



Time course of on-target gene expression after CRISPR-Off



Methylation vs expression at day 35

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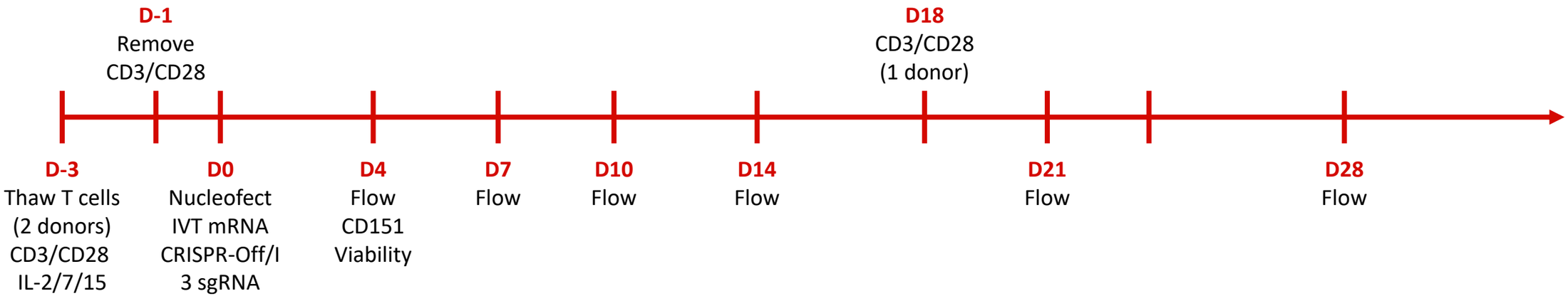
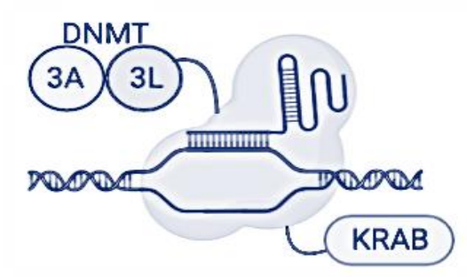
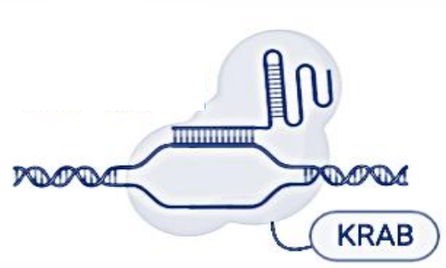
Experimental overview: Primary T cell silencing at a demo locus



CRISPR-Off



CRISPR-i



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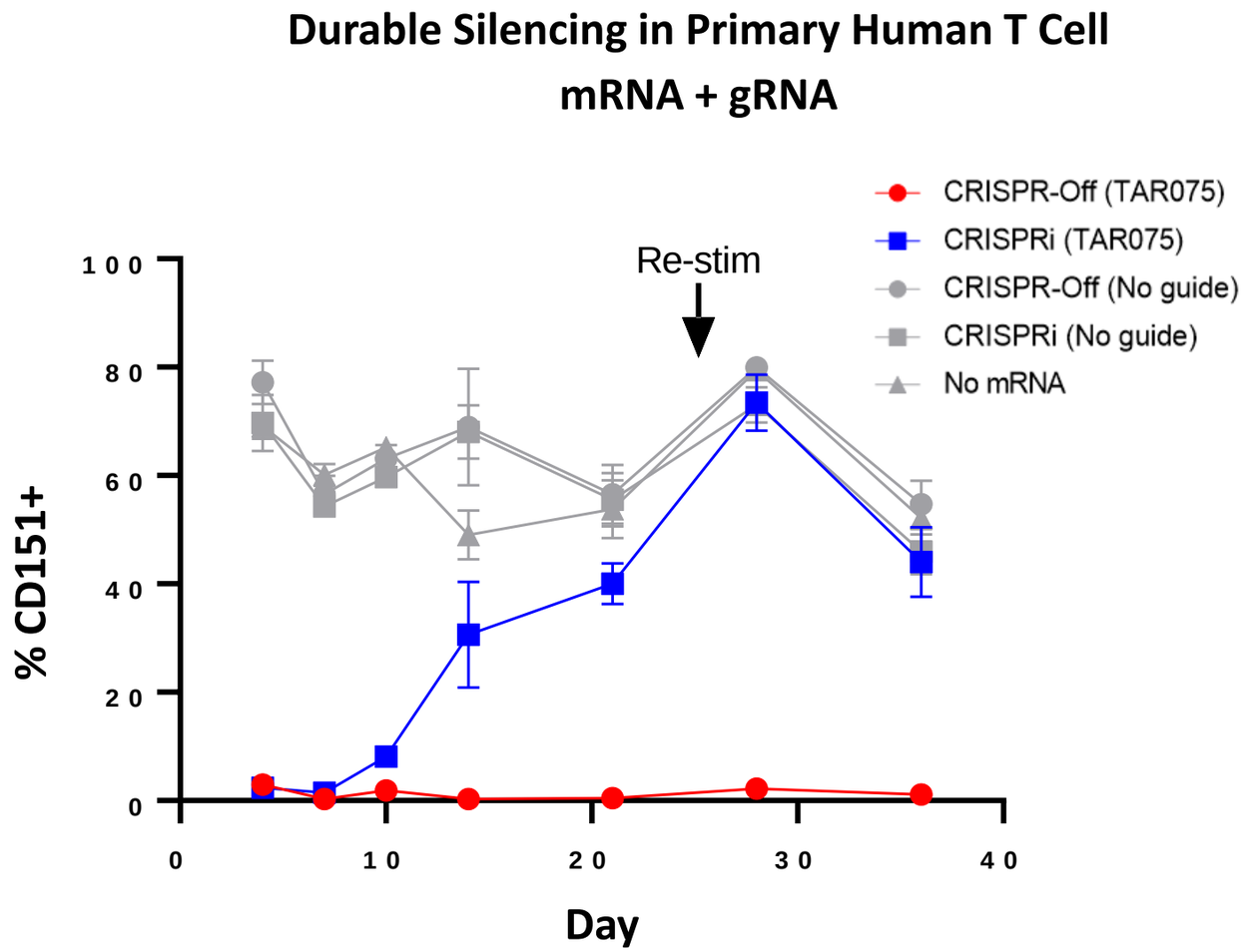
Durable silencing in primary human T cells with CRISPR-Off

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



Epigenetic editing has a fundamental mechanistic advantage

Provides control of drug target

- **No aberrant** RNA or truncated / mutant proteins produced
- **Uniform on target editing:** no break-induced mutations, translocations, rearrangements or chromothripsis

Unlocks large indication space

- Drug targets **difficult and/or intractable for existing modalities** (e.g. RNA dominant diseases, or non-coding RNAs)
- Positioned to **rapidly address** coming wave of novel epigenetic targets

Building the epigenetic editing leader

Building the leading epigenetic editing company focused on delivering precision cures for patients suffering from serious diseases

New class of genomic medicines harnessing nature's innate mechanism for gene regulation

Step change advance enabling durable gene regulation without consequences of cutting

Broad therapeutic potential to silence, activate, multiplex, and address targets unreachable for existing modalities

PoC for platform demonstrated by durable silencing in multiple primary cell types

World-class team and investors with track record of building genomic medicine platforms

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