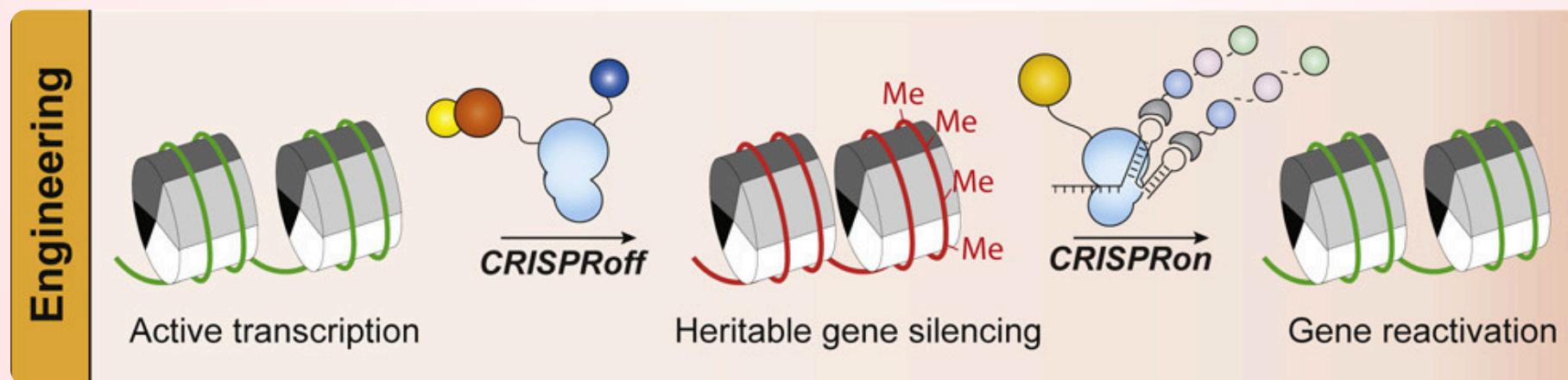
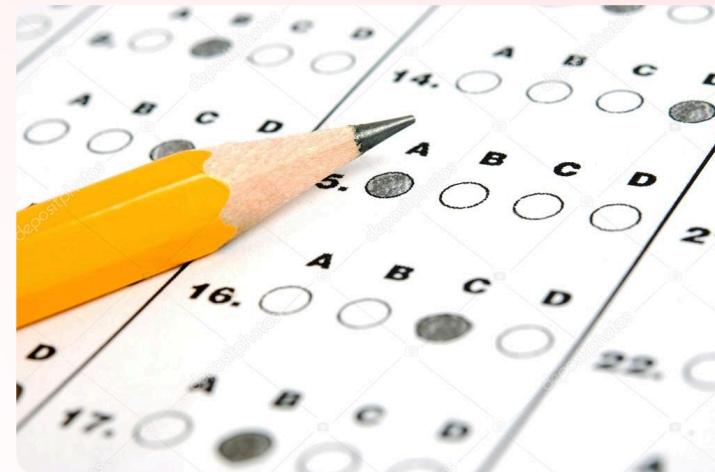


PENCILS AND ERASERS, ON DNA

Genome-wide programmable transcriptional memory by
CRISPR-based epigenome editing¹

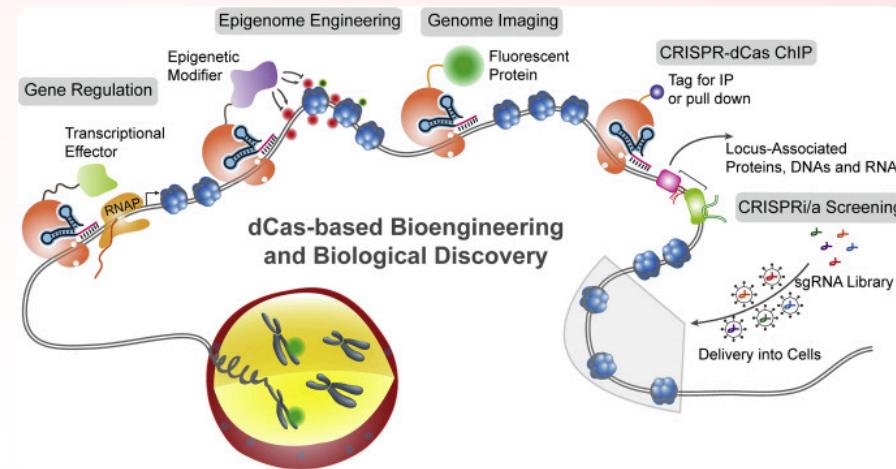
SUMMARY

- * The author presented a novel mechanism for writing heritable programmable epigenetic memory with higher efficiency than previous dCas9-based systems.
- * The memory can be written using dCas9-guided DNA methylation and transcriptional suppression using the CRISPROff system and erased using a transactivation domain as in CRISPRon.



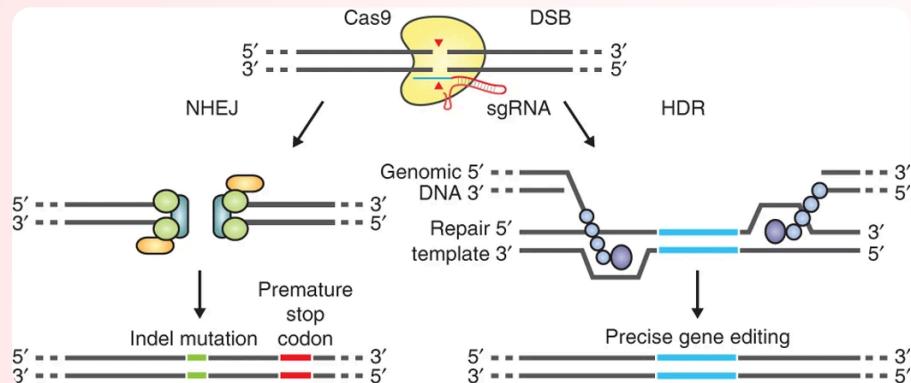
QUESTION

- * How can we “turn off” a gene?
- * Change the DNA sequence?²
- * Blocks transcription?³
- * Introduce methyltransferase?
 - * TALE⁴ ¹
 - * CRISPR-dCas⁹⁵
1. Transcription activator-like effectors



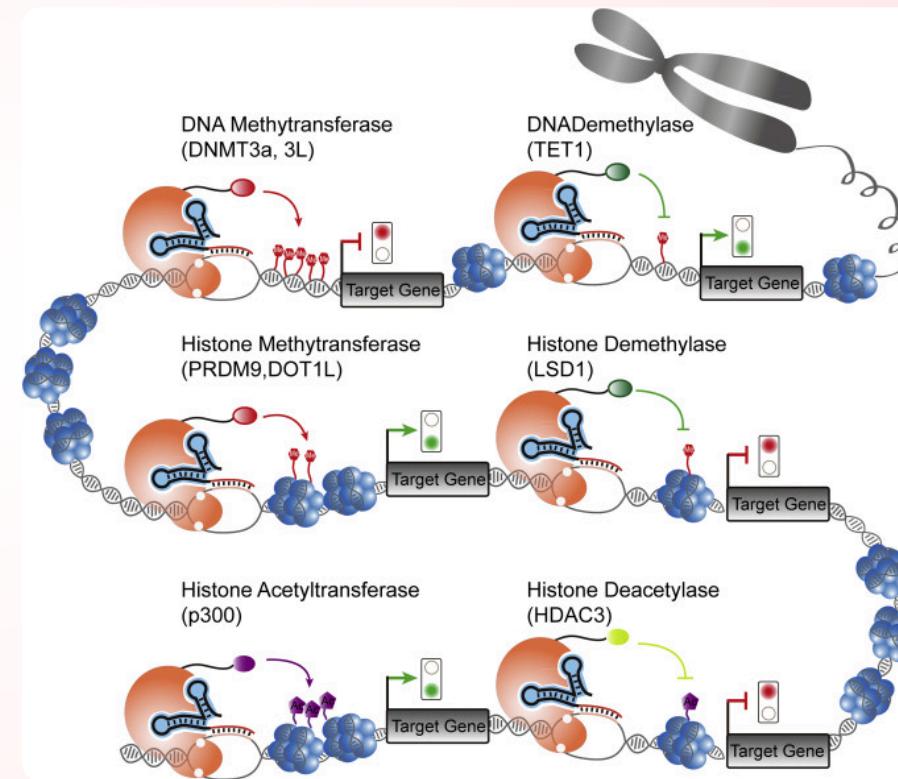
BACKGROUND

CRISPR-(d)Cas9⁶



CRISPR/Cas9 system introduces double-stranded break.

- * A “dead” Cas9 (dCas9) can be used to target specific DNA sequences without introducing double-stranded breaks.

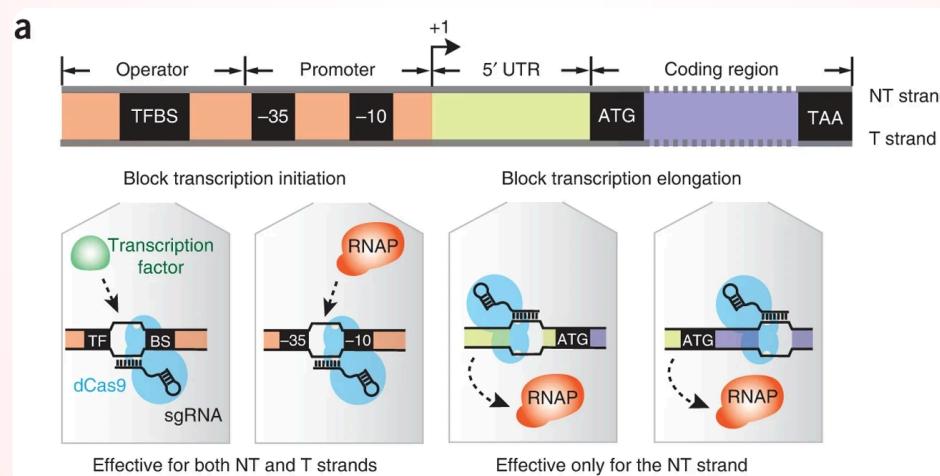


CRISPR-dCas9-based epigenome engineering.⁵

BACKGROUND

CRISPRi (CRISPR interference)³

- ✿ Using dCas9 to bind to DNA and prevent TFs from binding to the promoter region and/or block RNA polymerase from transcribing the gene.

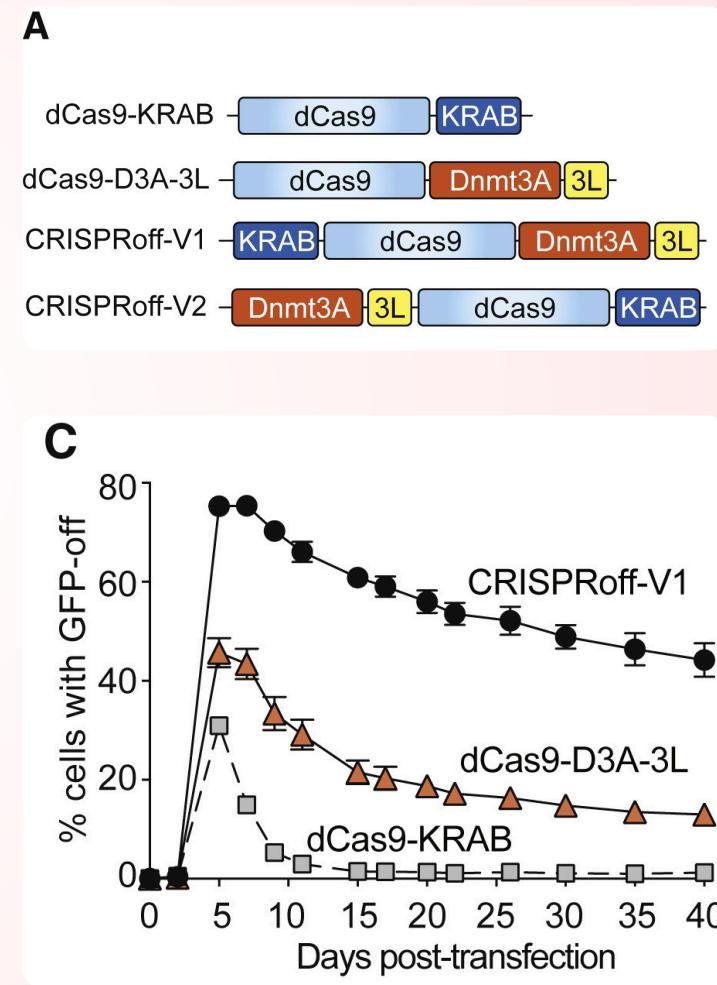


CRISPRi Overview

NEW METHOD - CRISPROFF

dCas9 guided DNA methylation
and transcriptional suppression
(Panel A) to achieve gene silencing
that is:

- ✿ durable (Panel C)
- ✿ with a wide target window
- ✿ independent on the presence of CpG islands.

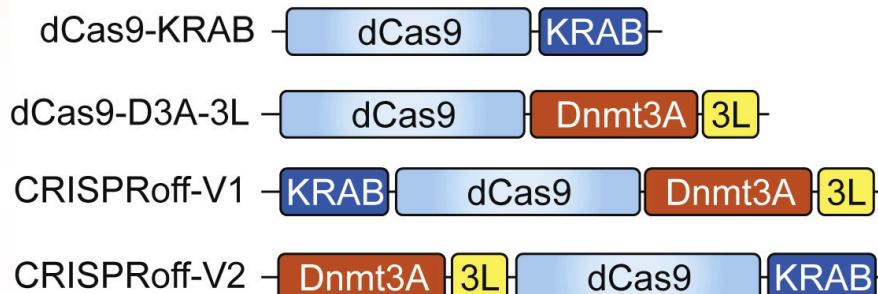


INTRODUCTION

CRISPRoff D3A^{E765A} Mutant

- * A mutation is introduced to the Dnmt3A protein domain to prevent it from methylating DNA.
- * Creates a CRISPRoff system that is incapable of methylating DNA.
- * Used to compare methylation-dependent and methylation-independent gene silencing.

A



INTRODUCTION

CPG ISLANDS (CGI)



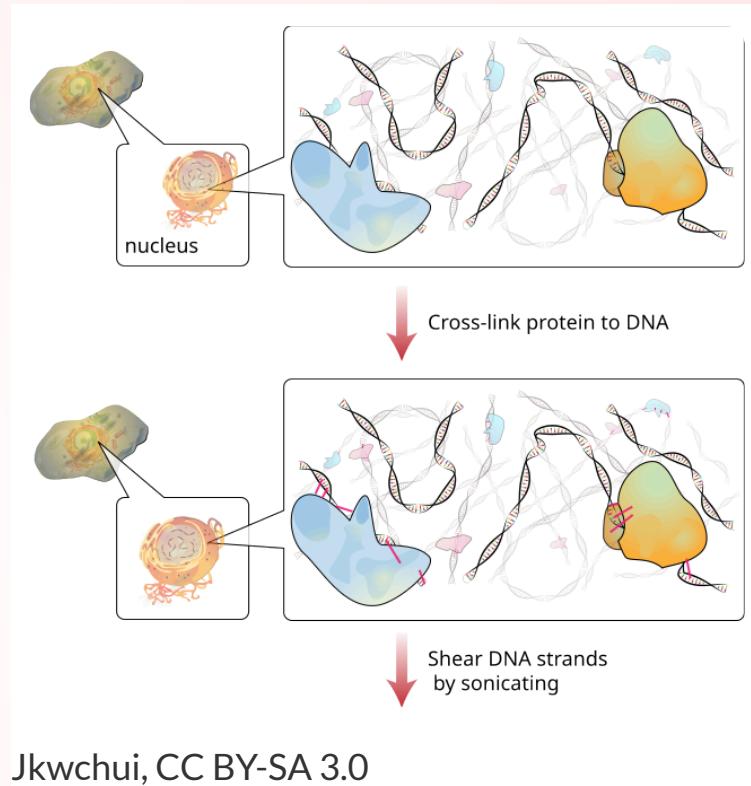
Helixitta, CC BY-SA 4.0

- * CpG sites are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide (separated by a phosphate group) along its 5' -> 3' direction.
- * CpG islands are regions with a high frequency of CpG sites.
 - * Typically occur at or near the transcription start site of genes.
 - * Methylation of CpG islands is associated with gene silencing.

INTRODUCTION

Chromatin immunoprecipitation sequencing (ChIP-seq)⁷

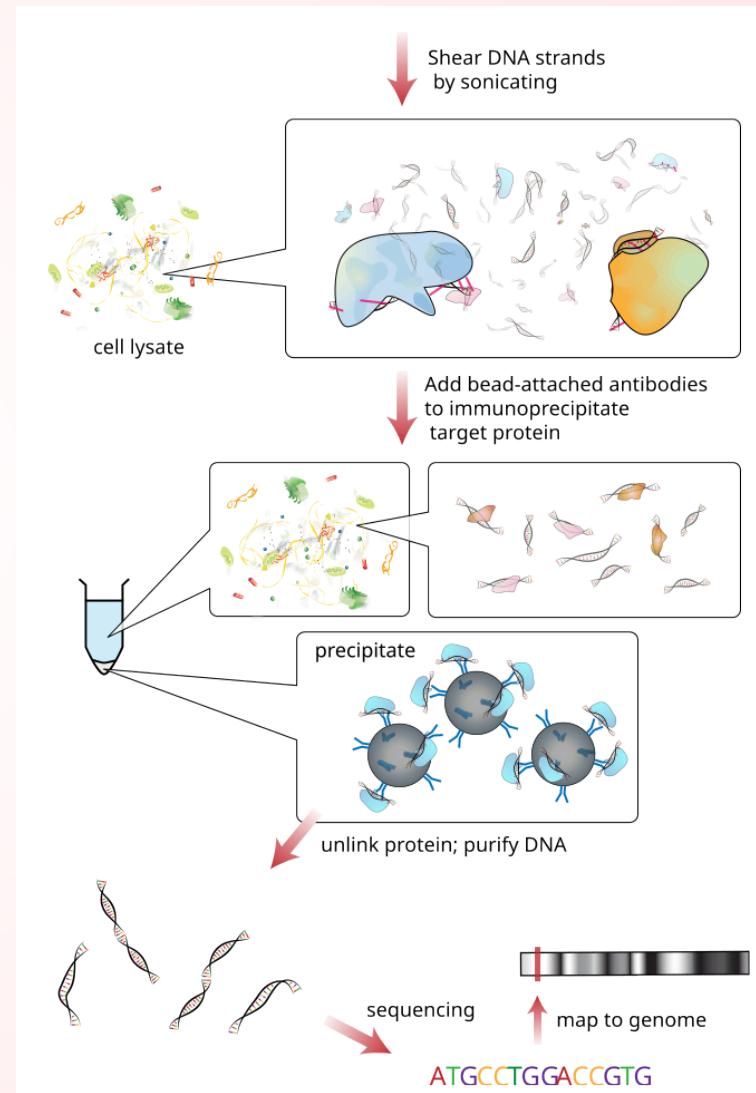
- * ChIP-seq analyzes protein interaction with DNA. It is used to identify binding sites of DNA-associated proteins, such as transcription factors, histones, etc.
- * In this study the author used ChIP-seq to identify epigenetic modifications to Histone H3 (H3K9me3) in the genome.



INTRODUCTION

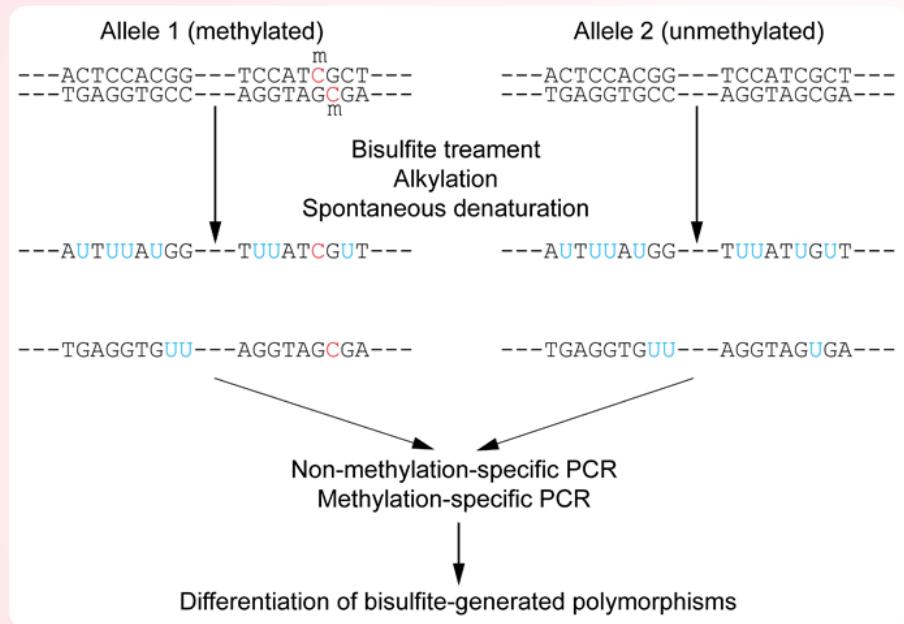
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INTRODUCTION

BISULFITE SEQUENCING PCR⁸



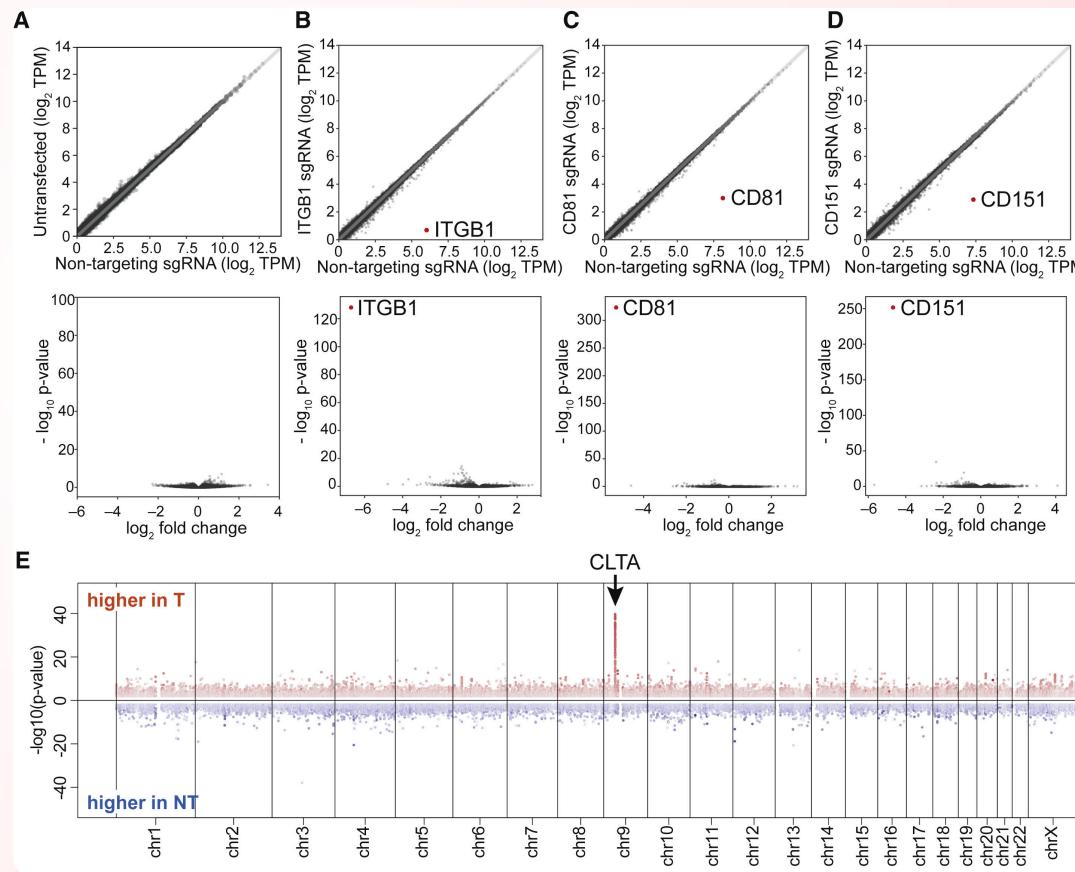
- * The method can be combined with modern NGS technologies to provide a genome-wide view of DNA methylation patterns.

- * Detects 5-methylcytosine residues in DNA.
- * The method utilizes bisulfite-induced modification of genomic DNA, under conditions whereby cytosine is converted to uracil, but 5-methylcytosine remains nonreactive.

KEY FINDINGS

EPIGENOME EDITING USING CRISPROFF IS HIGHLY SPECIFIC

- * Using RNAseq, near complete silencing of target genes are observed in comparison between:
 - * Untransfected cells (Panel A)
 - * Cells transfected with non-targeting sgRNA (Panel A-D)
 - * Cells transfected with targeting sgRNA (Panel B-D)
- * No significant off-target effects were observed (Panel A, E).

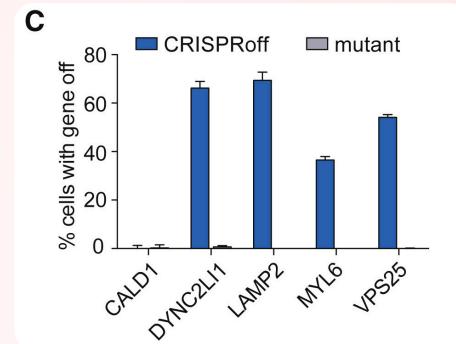
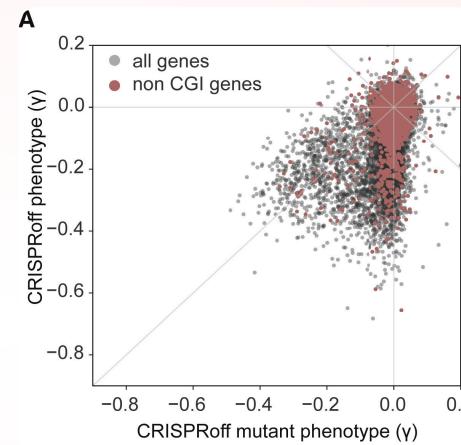


Transcriptome of cells with vs. without CRISPRoff (TPM = Transcripts per million)

KEY FINDINGS

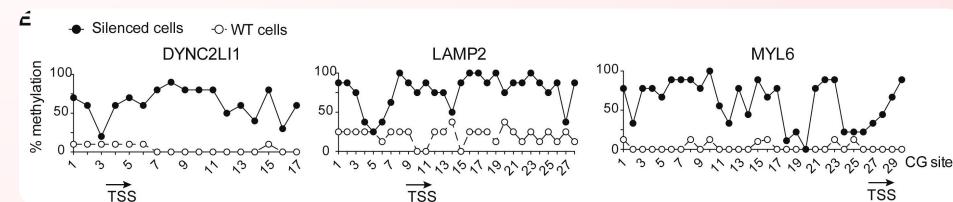
CRISPROFF CAN SILENCE GENES THAT LACK CGI ANNOTATIONS

- * CRISPROff successfully silenced 4 out of 5 non-CGI genes. (Panel C)
- * CRISPROff of genes lacking CGIs lead to a more significant decrease in cell growth compared to CRISPROff mutants in both CGI and non-CGI genes. (Panel A)
- * The silencing of non-CGI genes are also methylation-dependent. (Panel E)
 - * Hypothesis: CALD are not silenced due to complete lack of CpG sites.



Silencing of non-CGI genes

Growth of CRISPROff vs. mutant cells

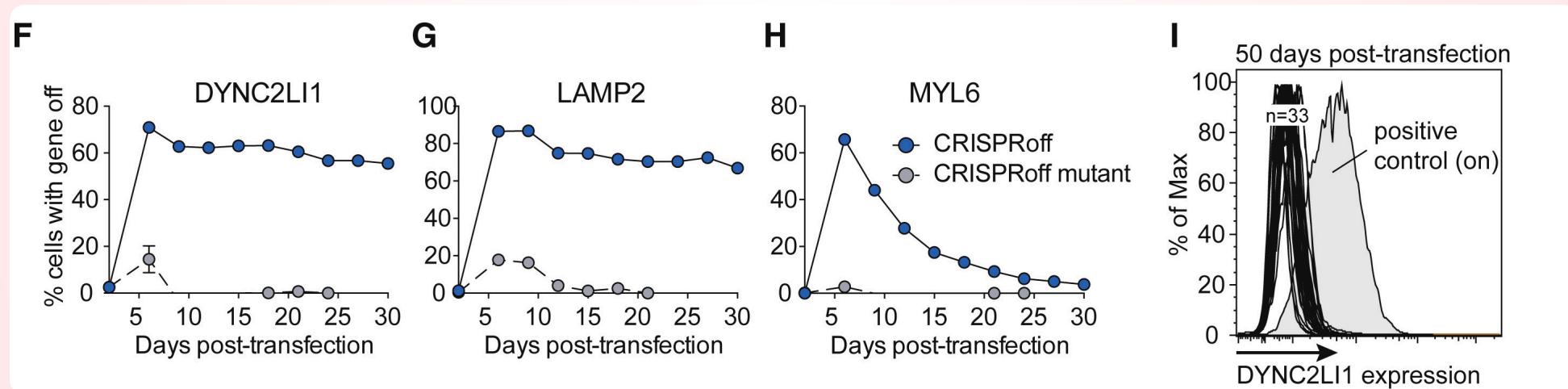


Methylation of non-CGI genes

KEY FINDINGS

CRISPROFF CAN SILENCE GENES THAT LACK CGI ANNOTATIONS (CONT'D)

- * CRISPROff can silence non-CGI genes durably. (Panel F, G, I)
- * Silencing of non-CGI gene MYL6 is metastable. (Panel H)

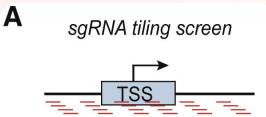


Durability of gene silencing in non-CGI genes

KEY FINDINGS

CRISPROFF TARGETING RULES

- * An sgRNA tiling screen was performed.



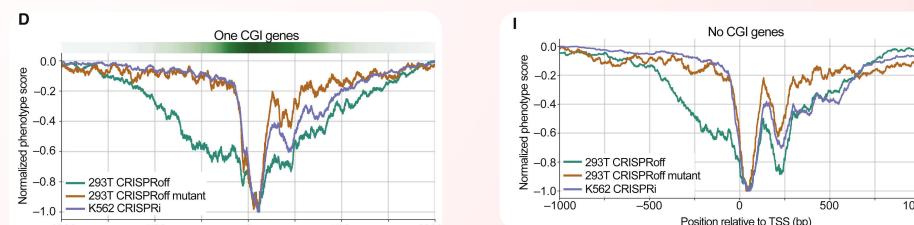
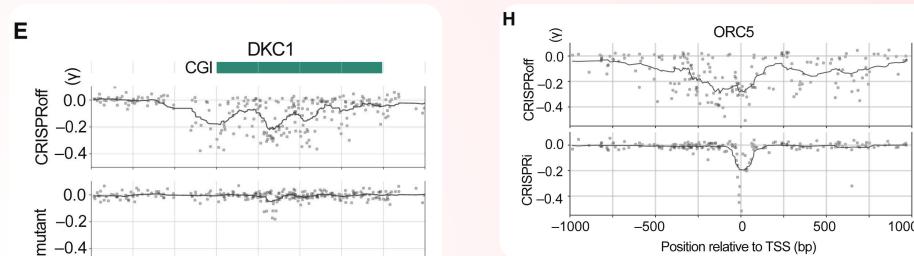
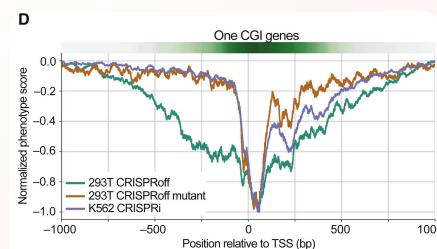
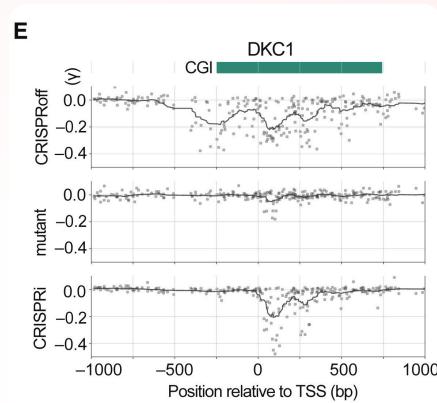
- * CRISPROff has a wider target window (-500 to +500 bp from TSS¹) compared to CRISPRI (-75 to +75 bp from TSS).

(Panel E, H)

- * Active sgRNAs are not evenly distributed but instead appear in a periodic pattern. (Panel D, I)

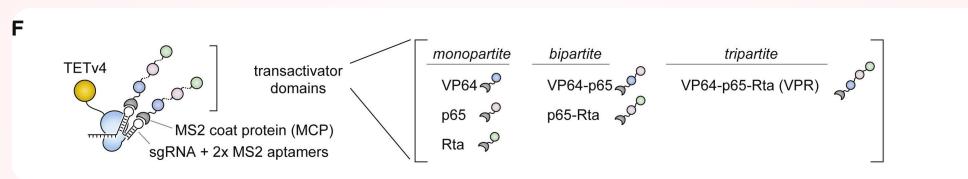
* Hypothesis: The periodic pattern is due to the nucleosome positioning.

1. Transcription start site

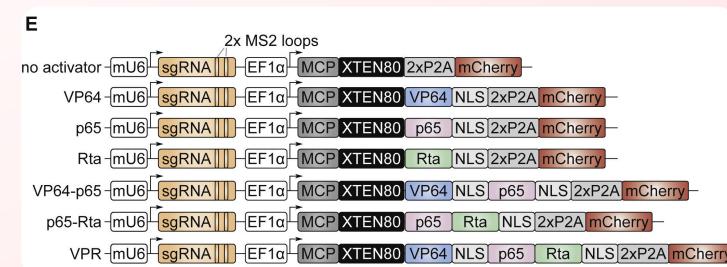
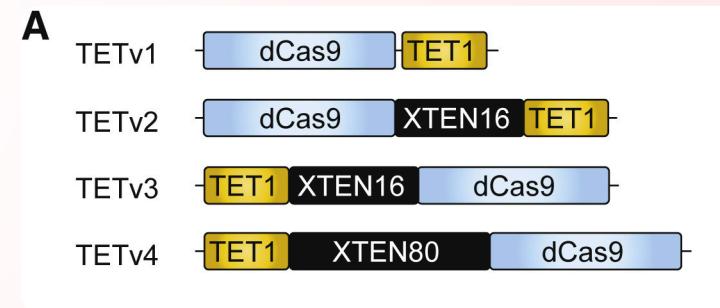


KEY FINDINGS

CRISPRON CAN TURN ON SILENCED GENES

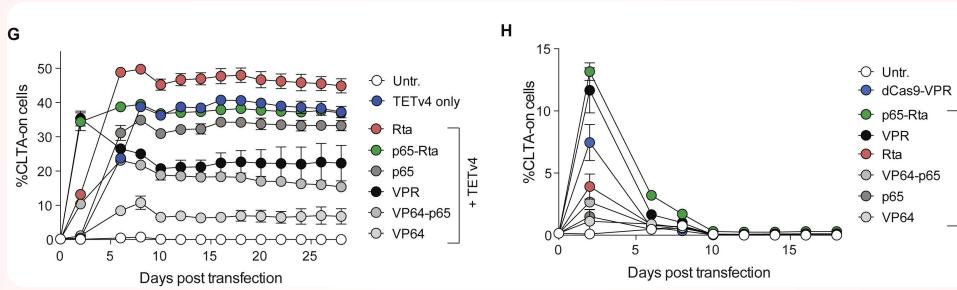


- Two constructs are used to create CRISPRon (Panel F).
 - The first is a dCas9-TET1 fusion protein (TETv4) that demethylates DNA. (Panel A).
 - The second is a sequence of transactivator domains fused with a MS2 coat protein (MCP) (Panel E). MS2 coat protein binds to the MS2 loops on the sgRNA achieving recruitment of the transactivator domains to the target gene (Panel F).

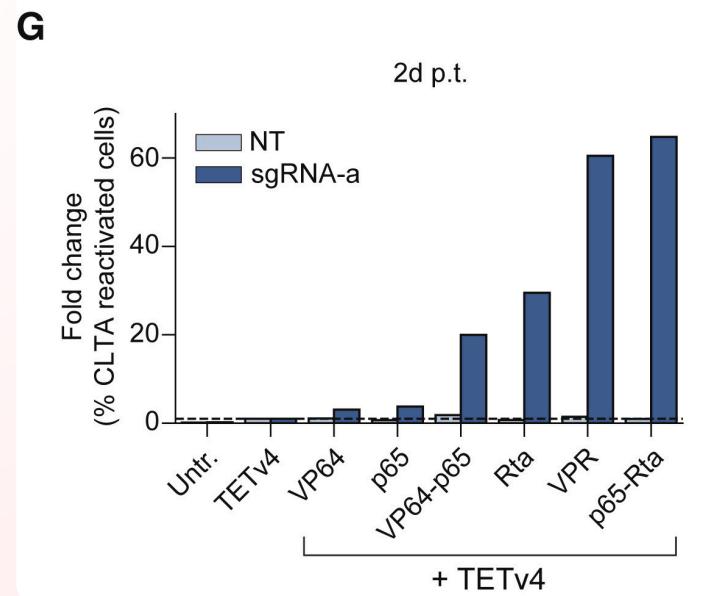


KEY FINDINGS

CRISPRON CAN TURN ON SILENCED GENES (CONT'D)



- * Reactivation of genes is DNA-demethylation dependent (panel G, H, top).
- * TETv4 alone is not effective in reactivating previously silenced genes (Panel G, right).
- * In summary, both TETv4 and transactivator are necessary for most effective reactivation of genes.



NEXT STEPS

- ✿ Optimization of the sgRNA design rules for CRISPRoff.
- ✿ Why are some genes not durably silenced?
- ✿ More research into the functional effects of artificially introduced DNA methylation.

FUTURE DIRECTIONS OF CRISPRON

- * What is the efficacy of CRISPRon on genes that are naturally methylated?
 - * Design sgRNAs to target several genes that are naturally methylated. Use a library tiling screen to help determine the optimal sgRNA design.
 - * Use bisulfite sequencing to determine the methylation status of the genes, before and after CRISPRon, with and without using transactivators.
 - * Measure the differential transcription of the genes using RNAseq.
 - * Measure differential protein production using a tagged protein and Western blotting or a fluorescent protein and flow cytometry.

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