Gene Expression Modulation Systems (GEMs): A CRISPR-Based Epigenome Editing Platform for In vivo therapeutics

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Abstract

Epigenome editing holds immense potential for programmably modulating gene expression, enabling therapeutic applications. However there remain significant barriers to realizing this potential including in delivering bulky and highly active epigenome editing platforms *in vivo*. In this study, we present the development of a highly optimized CRISPR-based epigenome editing platform termed Gene Expression Modulation systems (GEMs).

Through extensive engineering efforts, we have made notable improvements in Cas proteins, guide RNA scaffolds, and added the discovery of novel compact modulator proteins. Our platform integrates these advancements to achieve precise and efficient control over gene expression. Firstly, we have systematically identified and characterized thousands of compact modulators capable of transient and persistent gene regulation.

These compact modulator proteins serve as effective tools for manipulating gene expression, allowing fine-tuning of specific target genes in a programmable manner. Additionally, we have engineered a CRISPR-Cas system to enhance the functionality of the ribonucleoprotein (RNP) complex for more efficient epigenetic editing. Through these modifications, we have achieved a more compact RNP complex that exhibits improved editing activity, thus enhancing the precision and efficacy of our epigenome editing system.

Overall, our GEMs provide a versatile and powerful tool for a broad range of epigenome modulation and gene therapy applications. The optimized CRISPR-based platform, combined with the extensive repertoire of compact modulator proteins, enables precise control over gene expression, opening new avenues for therapeutic interventions and potential cures for genetic disorders *in vivo*.

GEMS

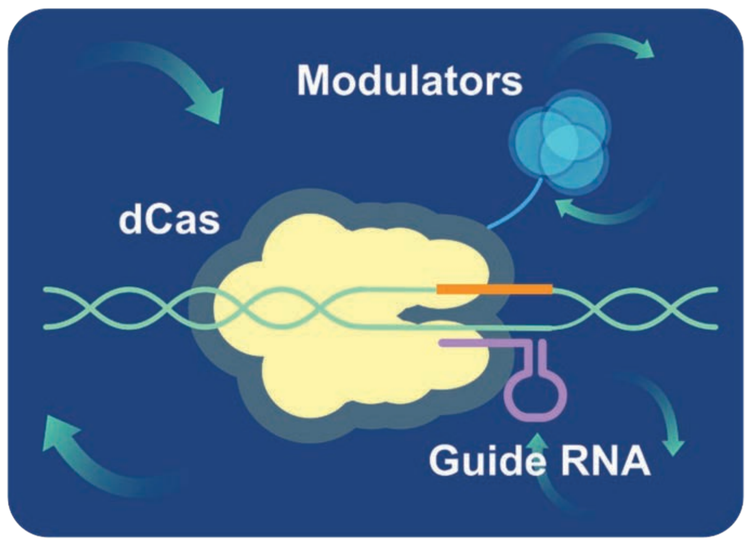


Figure 1. Epic Bio's Gene Expression Modulation System Platform A modular system comprising highly compact "dead" **Cas proteins**, transcriptional and epigenetic **modulators** and engineered compact guide RNAs. GEMS can fit comfortably within the packaging limits of rAAVs (~4.7kb) with approximately 2kb of extra cargo space. As a result GEMS can be delivered to target organs *in vivo* for therapeutic applications.

Compact Cas Proteins

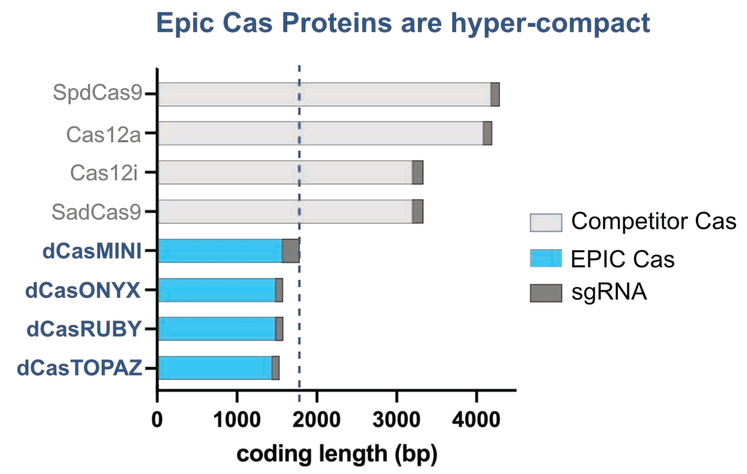


Figure 2. (A) Size comparison of Epicrispr Biotechnogies' Cas proteins to benchmark Cas molecules.

CasONYX, CasRUBY, and CasTOPAZ all fall under 1500 nt in length (less than 500 amino acids). Together with CasMINI, they constitute a suite of compact and active proteins suitable for *in vivo* gene therapeutic applications which can be delivered by rAAVs and other delivery modalities.

Compact Modulators

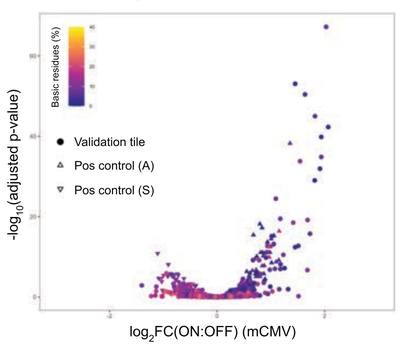


Figure 3. Single Modulators

At Epic Bio we have conducted screens to successfully identify hundreds of Modulator proteins, capable of robust gene activation and suppression. These proteins, some of which out-perform benchmark activators (such as VP64 and VPR) and suppressors (such as Kox1 KRAB) are compact (less than or equal to 85 amino acids in length).

We have successfully identified biophysical features of these molecules that are key to their activity and exploit these features through semi-rational engineering approaches.

Combined Modulators

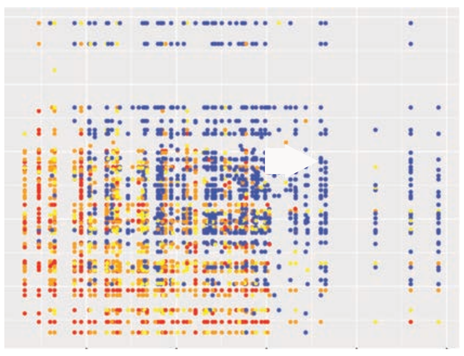


Figure 4. Combined Modulators

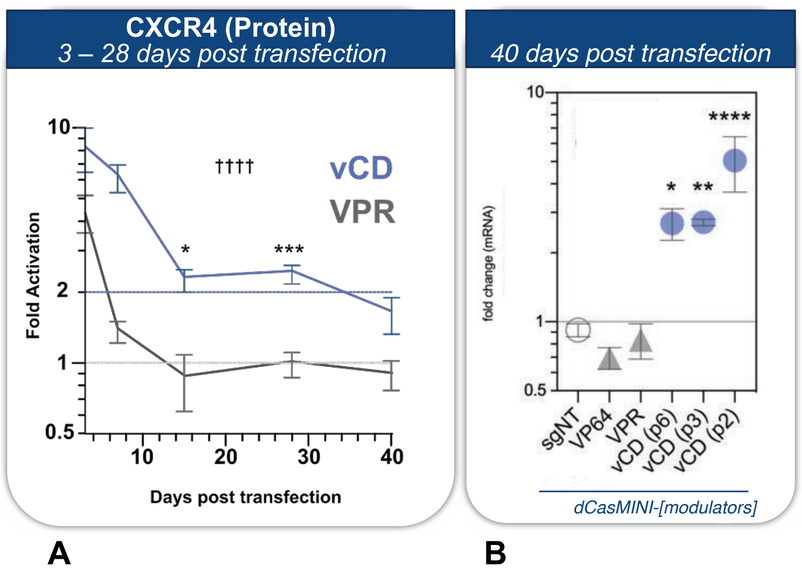
Rational combinations of transcriptional activation domains have long been used to demonstrate synergistic gene activation.
Here we have exploited this approach to combine hundreds of our Modulators in order to identify new activities, and identify synergistic combinations to potentiate target gene activation in human cells.

Synthetic Modulators

Figure 5. Machine Learning-enabled Synthetic Modulators

We have successfully developed a machine learning pipeline for the discovery of novel and active Modulator proteins. This has been accomplished by leveraging the data generated from our Modulator screens. By this approach both the scale of discovery and the rate of hit identification have been significantly enhanced (from 0.5% to 20%).

Epigenetic Modulators



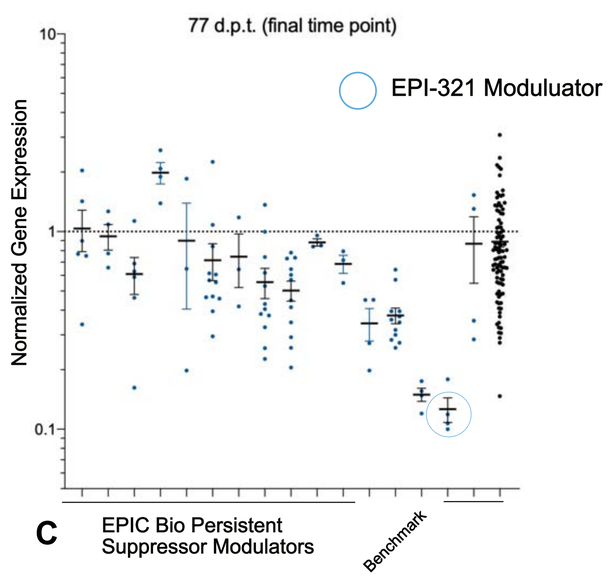


Figure 6.
A). Plot of CXCR4 protein expression in HEK 293 T cells transiently transfected with the indicated constructs. Control activator VPR induces robust activation initially, but this activation decays to background levels by 12 days post-transfection. Epic Bio's Modulator vCD, by contrast, induces robust, and subsequently durable gene activation and protein expression lasting at leas 40 days post-transfection

B). Messenger RNA from the same experiment was monitored at 40 days post-transfection. Corroborating the results from panel (A), transfection of Epic Bio's Modulators resulted in gene activation 40 days post-transient transfection.

(C). We have developed a compact and robust epigenetic silencing Modulator complex at Epic Bio. In this assay, target reporter gene expression is significantly suppressed 77 days post-transient transfection. The Modulators showing the most robust activity (in blue circle) are those used in EPI-321 (described in Figure 8).

A potential cure for FSHD

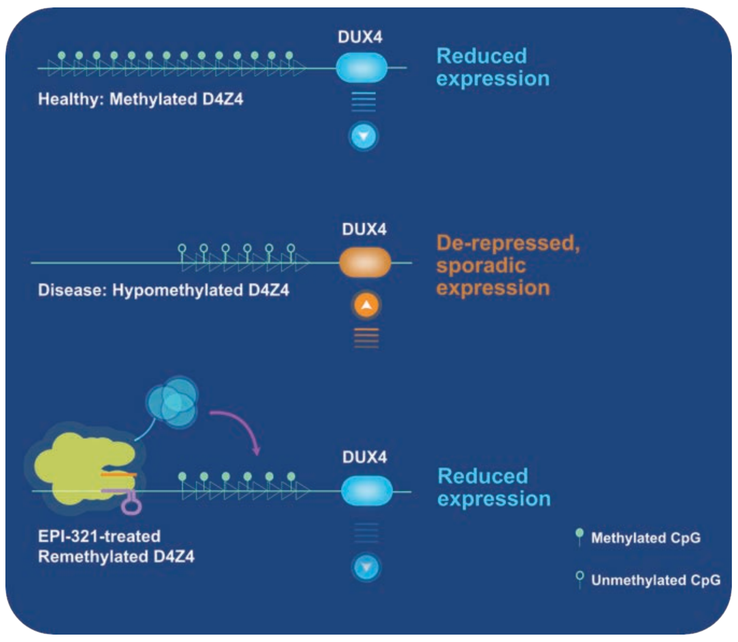


Figure 8. (A). A schematic representation of the epigentic state at the human D4Z4 locus on chromosome 4. The "healthy" locus shows methylated CpGs within the D4Z4 repeat region. Healthy individuals have >10 repeats. In diseased alleles there are typically fewer than 10 D4Z4 repeats and the region is hypomethylated at constituent CpGs. This hypomethylation causes de-repression of the DUX4 gene. DUX4 is a developmental transcription factor whose aberrant expression in adult myocytes triggers a transcriptional cascade resulting in myocyte apoptosis. These molecular hallmarks form the underpinnings of **facioscapulohumeral muscular dystrophy (FSHD).** This is a progressive disease characterized by muscle weakness. There is no cure for this disease.

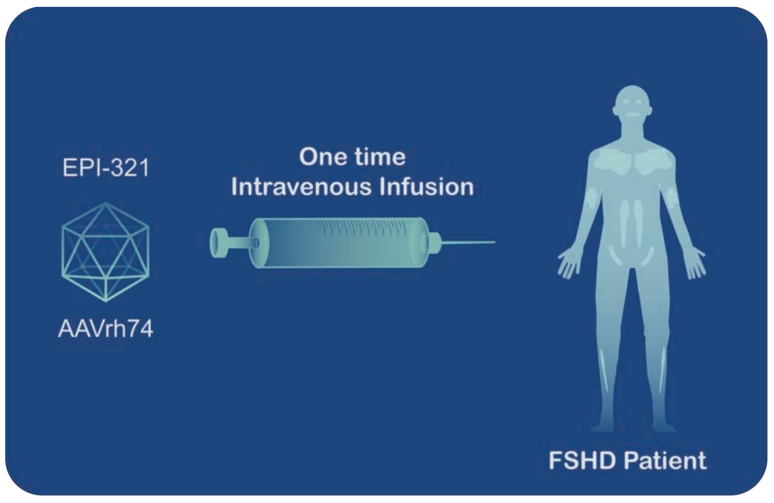


Figure 8. (B) Schematic showing proposal for a one-and-done strategy to treat FSHD. The therapeutic approach is uniquely possible with EPI-321, an epigenome editing technology that is designed to restore methylation to the affected locus and thereby suppress the stochastic and toxic DUX4 gene. Thus suppression will be achieved by epigenetic means, mitotically-stable, and long-lasting.

Conclusions, related posters

Epic Bio GEMS represents a powerful platform for therapeutic editing of the epigenome. Featuring compact component proteins, highly interchangeable, and programmable platform, GEMS offers the prospect of safe and precise epigenome editing both *ex vivo* and *in vivo.*

For more details please view the following posters:

P605: EPI-321: a potential cure for Facioscapulohumeral Muscular Dystrophy (FSHD) targeting D4Z4 epigenome

P632: Discovery and engineering of hypercompact epigenetic modulators for durable activation of therapeutic gene targets

P633: Design and characterization of compact and precise Cas molecules for treating diseases in patients

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