Discovery and engineering of hypercompact epigenetic modulators for durable gene activation

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Abstract

Transcriptional and epigenetic regulators shape the chromatin microenvironment and corresponding gene expression during cellular differentiation and homeostasis. Programmable modulators of transcription provide a powerful toolkit for controlling gene dosage in therapeutic applications, but a limited catalog of functional domains constrains their robustness and durability profiles, and large cargo sizes impede clinical delivery.

To address these limitations, here we perform high-throughput screening to discover novel classes of transcriptional modulators among human, viral, and archaeal proteomes and characterize their functions in a multitude of endogenous human contexts. We identify compact, potent activators from viral proteomes with exceptional robustness across silent and expressed genes in varied cell types using distinct dCas systems. Insights from predicted 3-dimensional structures and machine learning models enabled us to rationally engineer improved activators, both in potency and persistence. Notably, engineered activators achieved mitotically durable gene activation following transient delivery.

Our discovery pipeline provides a predictive rubric for the systematic development of hypercompact activators from unannotated proteomes, yielding superior efficiency and kinetics profiles that broadly expand the epigenetic editing toolkit for therapeutic applications.

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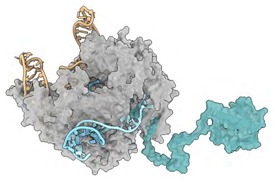
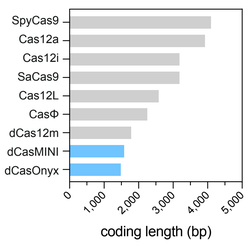
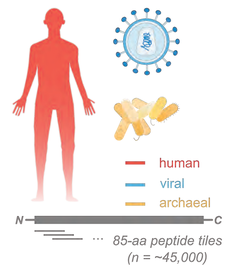
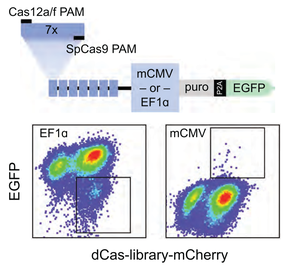
Gene Expression Modulation System (GEMS) for epigenetic engineering

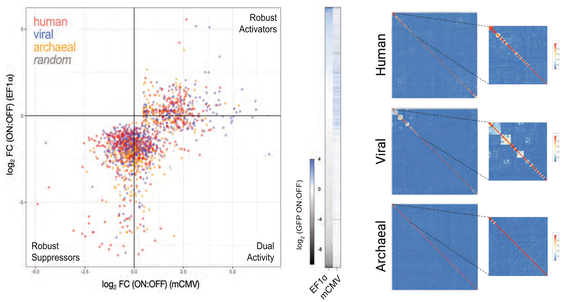




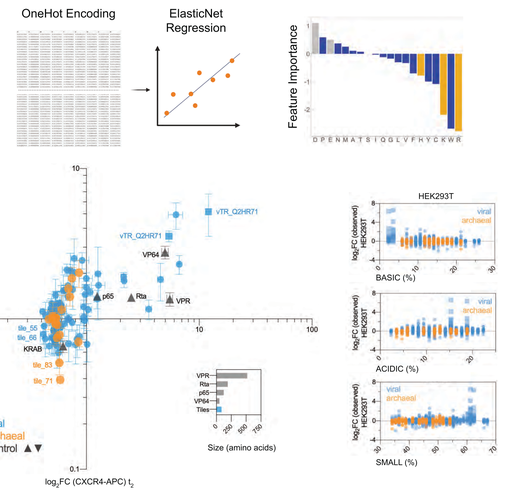
Identification of transcriptional modulators by high-throughput screening



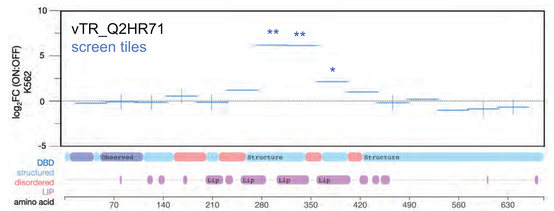


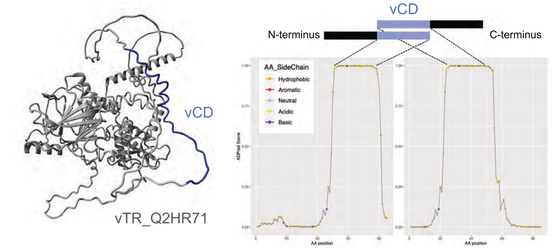


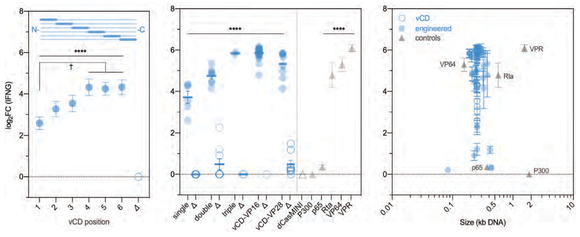
Biochemical feature importance: prediction and validation

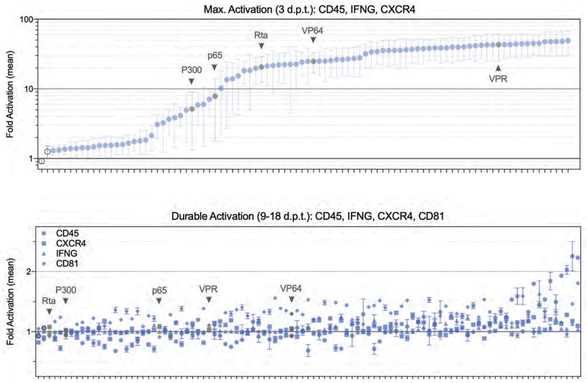


Deep learning identifies minimal domains as a basis for engineered potency

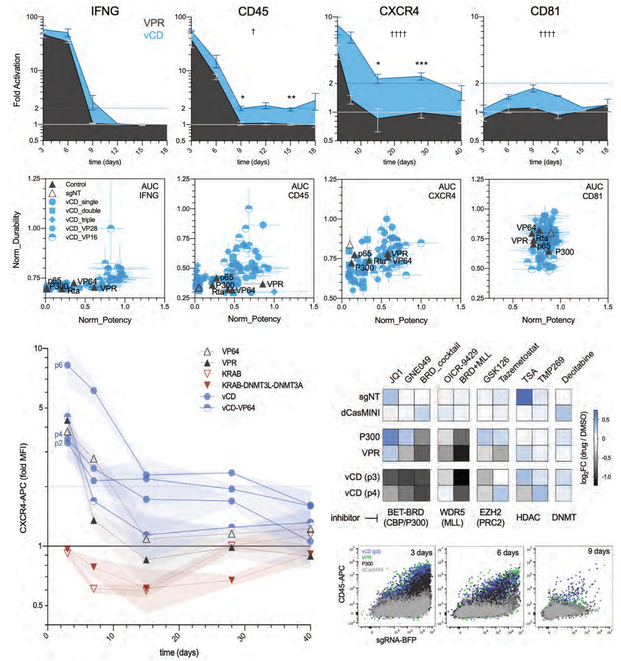








Durable activation by GEMS activators



Conclusions

High-throughput screening identifies known and novel transcriptional modulators from diverse species.

ML-enabled biochemical analysis provides a predictive rubric for modulator functions.

Novel activators outperform larger benchmarks in potency, context-independent robustness, and durability of activity

References

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