Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers

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Technologies that enable targeted manipulation of epigenetic marks could be used to precisely control cell phenotype or interrogate the relationship between the epigenome and transcriptional control. Here we describe a programmable, CRISPR-Cas9-based acetyltransferase consisting of the nuclease-null dCas9 protein fused to the catalytic core of the human acetyltransferase p300. The fusion protein catalyzes acetylation of histone H3 lysine 27 at its target sites, leading to robust transactivation of target genes from promoters and both proximal and distal enhancers. Gene activation by the targeted acetyltransferase was highly specific across the genome. In contrast to previous dCas9-based activators, the acetyltransferase activates genes from enhancer regions and with an individual guide RNA. We also show that the core p300 domain can be fused to other programmable DNA-binding proteins. These results support targeted acetylation as a causal mechanism of transactivation and provide a robust tool for manipulating gene regulation.

A central challenge in functional genomics is to develop technologies for precise manipulation of individual loci. Projects such as ENCODE1 and the Roadmap Epigenomics Project2 have identified millions of epigenetic marks across the human genome for many human cell types and tissues. Studying the function of those marks, however, has been largely limited to determining statistical associations with gene expression. Technologies for targeted direct manipulation of epigenetic marks are needed to transform association-based findings into mechanistic principles of gene regulation. Such advances have the potential to benefit human health by enabling new platforms for disease modeling, drug screening, gene therapy and cell lineage specification. Small-molecule drugs, such as inhibitors of histone deacetylases or DNA methyltransferases, alter the epigenome and transcriptome globally and cannot target individual loci. Fusions of epigenome-modifying enzymes to programmable DNA-binding proteins, such as zinc finger proteins and transcription activator-like effectors (TALEs), are effective at achieving targeted DNA methylation and hydroxymethylation, and histone demethylation, methylation and deacetylation3–8. However, a method for targeted histone acetylation, which is strongly associated with active gene regulatory elements and enhancers, has not been described. In addition, the CRISPR-Cas9 (clustered, regularly interspaced, short palindromic repeat–CRISPR-associated protein) genome engineering tool9,10, which can be readily targeted to loci of interest, has not yet been extensively applied to epigenome editing.

The Cas9 nuclease can be directed to specific genomic loci using complementarity between an engineered guide RNA (gRNA) and the target site11–13. The enzymatic activity of the Cas9 nuclease can be abolished by mutation of the RuvC and HNH domains, generating the nuclease-null deactivated Cas9 (dCas9)12. Fused to repression domains, such as the KRAB domain, or to activation domains, such as oligomers of the herpes simplex viral protein 16 (VP16), dCas9 can function as a synthetic transcriptional regulator14–21. However, dCas9 activators have limitations, including the need for multiple activation domains14,20,22,23 or combinations of gRNAs16,17 to achieve high levels of gene induction by synergistic effects between activation domains24,25. Moreover, the activator domains used in these engineered transcriptional factors, such as the VP16 tetramer VP64 (ref. 26), act as scaffolds for recruiting multiple components of the preinitiation complex27,28 and do not enzymatically modulate the chromatin state directly. This indirect method of epigenetic remodeling does not allow testing of the role of specific epigenetic marks and may not be as potent as direct alteration of epigenetic states. We hypothesized that recruitment of an acetyltransferase by dCas9 to a genomic target site would directly modulate the epigenome and activate nearby gene expression. To this end, we designed a fusion protein of dCas9 with the catalytic histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (ref. 29), a highly conserved acetyltransferase involved in a wide range of cellular processes30,31.
We demonstrate that this easily programmable approach facilitates robust control of the epigenome and downstream gene expression.

RESULTS

A dCas9 fusion to the p300 HAT domain activates target genes

We fused the full-length human p300 protein to dCas9 (dCas9FLp300, Fig. 1a,b) and assayed its capacity for transactivation by transient co-transfection of human HEK293T cells with four gRNAs targeting the endogenous promoters of ILIRN, MYOD (also known as MYOD1) and OCT4 (also known as POU5F1; Fig. 1c). We used a combination of four gRNAs targeting each promoter based on our and others’ previous observations that multiple gRNAs at a single promoter are necessary for robust gene activation14–22. dCas9FLp300 was well-expressed and induced modest activation above background compared to the canonical dCas9 effector fusion targeted to the VP64 acidic activation domain (dCas9VP64, Figs. 1a–c). The full-length p300 protein is a promiscuous acetyltransferase, which interacts with a multitude of endogenous proteins, largely through its termini29,31. To mitigate these interactions we isolated the contiguous region of full-length p300 (2,414 amino acids) solely required for inherent activation domain (dCas9 VP64; Supplementary Figs. 1a,b), which is known as the p300 HAT core domain (p300 Core)29. When fused to the C terminus of dCas9 (dCas9p300 Core, Figs. 1a,b), the p300 Core domain induced high levels of transcription from endogenous gRNA-targeted promoters (Fig. 1c). When targeted to the ILIRN and MYOD promoters, the dCas9p300 Core fusion displayed significantly higher levels of transactivation than dCas9VP64 (P = 0.0124 and 0.0394, respectively; Fig. 1c). These dCas9-effector fusion proteins were expressed at similar levels (Fig. 1b and Supplementary Fig. 1) indicating that the observed differences reflect the distinct transactivation capacity of each construct. In addition, no changes to target gene expression were observed when the effector fusions were transfected without gRNAs (Supplementary Fig. 2). To confirm that the p300 Core acetyltransferase activity was responsible for gene transactivation using the dCas9p300 Core fusion, we screened a panel of dCas9p300 Core HAT-domain mutant fusion proteins (Supplementary Fig. 1)29. A single inactivating amino acid substitution within the HAT core domain (WT residue D1399 of full-length p300) of dCas9p300 Core (dCas9p300 Core (D1399Y); Fig. 1a) abolished the transactivation capacity of the fusion protein (Fig. 1c), demonstrating that intact p300 Core acetyltransferase activity was required for dCas9p300 Core-mediated transactivation.

dCas9p300 Core activates genes from proximal and distal enhancers

Although there are many published examples of genes being activated with engineered transcription factors targeted to promoters, inducing gene expression from other distal regulatory elements has been limited, particularly for dCas9-based activators32–35. Given the role and localization of p300 at endogenous enhancers36,37, we hypothesized that the dCas9p300 Core would effectively induce transcription from distal regulatory regions with appropriately targeted gRNAs. We targeted the distal regulatory region (DRR) and core enhancer (CE) of the human MYOD locus38 through co-transfection of four gRNAs targeted to each region and either dCas9VP64 or dCas9p300 Core (Fig. 2a). Compared to a mock-transfected control, dCas9VP64 did not lead to any induction when targeted to the MYOD DRR or CE region. In contrast, dCas9p300 Core induced significant transcription when targeted to either MYOD regulatory element with corresponding gRNAs (P = 0.0115 and 0.0009 for the CE and DRR regions, respectively). We also targeted the upstream proximal (PE) and distal enhancer (DE) regions of human OCT4 (ref. 39) by co-transfection of six gRNAs and either dCas9VP64 or dCas9p300 Core (Fig. 2b). dCas9p300 Core induced significant transcription from these regions (P ≤ 0.0001 and P = 0.003 for the DE and PE, respectively), whereas dCas9VP64 was unable to activate OCT4 above background levels when targeted to either the PE or DE regions.

The well-characterized mammalian β-globin locus control region (LCR) orchestrates transcription of the downstream hemoglobin genes; hemoglobin epsilon 1 (HBE, also known as HBE1, from ~11 kb), hemoglobin gamma 1 and 2 (HBG, also known as HBG1, from ~30 kb), hemoglobin delta (HBD, from ~46 kb) and hemoglobin beta (HBB, from ~54 kb) (Fig. 2c)35,40. DNase hypersensitive sites within the β-globin LCR serve as docking sites for transcriptional and chromatin modifiers, including p300 (ref. 41), which coordinate distal target gene expression. We designed four gRNAs targeting the DNase hypersensitive site 2 within the LCR enhancer region (HS2 enhancer). These four HS2-targeted gRNAs were co-transfected with dCas9, dCas9VP64, dCas9p300 Core or

**Figure 1** The dCas9p300 Core fusion protein activates transcription of endogenous genes from proximal promoter regions. (a) Schematic of dCas9 fusion proteins dCas9VP64, dCas9FLp300 and dCas9p300 Core. S. pyogenes dCas9 contains nuclease-inactivating mutations D10A and H840A. The D1399 catalytic residue in the p300 HAT domain is indicated. (b) Western blot showing expression of dCas9 fusion proteins and GAPDH in co-transfected cells (full blot shown in Supplementary Fig. 1c). (c) Relative mRNA expression of ILIRN, MYOD and OCT4, determined by qRT-PCR, by the indicated dCas9 fusion protein co-transfected with four gRNAs targeted to each promoter region. (Tukey-test, *P < 0.05, n = 3 independent experiments each; error bars, s.e.m.). Numbers above bars indicate mean relative expression. FLAG, epitope tag; NLS, nuclear localization signal; HA, hemagglutinin epitope tag; CH, cystein histidine-rich region; Bd, bromodomain; HAT, histone acetyltransferase domain.
Figure 2 The dCas9p300 Core fusion protein activates transcription of endogenous genes from distal enhancer regions. (a) Relative MYOD mRNA production in cells co-transfected with a pool of gRNAs targeted to either the proximal or distal regulatory regions and dCas9VP64 or dCas9p300 Core; promoter data from Figure 1c (Tukey test *P < 0.05 compared to mock-transfected cells; Tukey test 1P < 0.05 between dCas9p300 Core and dCas9VP64, n = 3 independent experiments; error bars, s.e.m.). The human MYOD locus is schematically depicted with corresponding gRNA locations in red, CE, Myod core enhancer; DRR, Myod distal regulatory region. (b) Relative OCT4 mRNA production in cells co-transfected with a pool of gRNAs targeted to the proximal and distal regulatory regions and dCas9VP64 or dCas9p300 Core; promoter data from Figure 1c (Tukey test †P < 0.05 compared to mock-transfected cells, Tukey test †P < 0.05 between dCas9p300 Core and dCas9VP64, n = 3 independent experiments; error bars, s.e.m.). The human OCT4 locus is schematically depicted with corresponding gRNA locations in red. DE, Oct4 distal regulatory region. (c) The human β-globin locus is schematically depicted with approximate locations of the hypersensitive site 2 (HS2) enhancer region and downstream genes (HBE, HBG, HBD and HBB). Corresponding HS2 gRNA locations are shown in red. Relative mRNA production from distal genes in cells co-transfected with four gRNAs targeted to the HS2 enhancer and the indicated dCas9 proteins. Numbers above bars indicate mean relative expression. Note logarithmic y-axis and dashed red line indicating background expression (Tukey test among conditions for each β-globin gene, *P < 0.05, n = 3 independent experiments, error bars, s.e.m.). n.s., not significant.

dCas9p300 Core (D1399Y), and the resulting mRNA production from HBE, HBG, HBD and HBB was assayed (Fig. 2c). dCas9, dCas9VP64 and dCas9p300 Core (D1399Y) did not transactivate any downstream genes when targeted to the HS2 enhancer. In contrast, targeting of dCas9p300 Core to the HS2 enhancer led to significant expression of the downstream HBE, HBG and HBD genes (P ≤ 0.0001, 0.0056, and 0.0003 between dCas9p300 Core and mock-transfected cells for HBE, HBG and HBD, respectively). Overall, HBD and HBE appeared relatively less responsive to synthetic p300 Core–mediated activation from the HS2 enhancer; a finding consistent with lower rates of general transcription from these two genes across several cell lines (Supplementary Fig. 3). Nevertheless, with the exception of the most distal HBB gene, dCas9p300 Core activated transcription from downstream genes when targeted to all characterized enhancer regions assayed, a capability not observed for dCas9VP64. Together, these results demonstrate that dCas9p300 Core is a potent programmable transcription factor that can be used to regulate gene expression from a variety of promoter-proximal and promoter-distal locations.

Gene activation by dCas9p300 Core is highly specific

Recent reports indicate that dCas9 may have widespread off-target binding events in mammalian cells in combination with some gRNAs42,43, which could potentially lead to off-target changes in gene expression. To assess the transcriptional specificity of the dCas9p300 Core fusion protein we performed transcriptome profiling by RNA-seq in cells co-transfected with four IL1RN-targeted gRNAs and either dCas9, dCas9VP64, dCas9p300 Core or dCas9p300 Core (D1399Y). Genome-wide transcriptional changes were compared between
dCas9 with no fused effector domain and either dCas9<sup>VP64</sup>, dCas<sup>p300</sup><sub>Core</sub> or dCas<sup>p300</sup><sub>Core</sub> (D1399Y) (Fig. 3). Whereas both dCas9<sup>VP64</sup> and dCas<sup>p300</sup><sub>Core</sub> upregulated all four <i>ILIRN</i> isoforms, only the effects of dCas<sup>p300</sup><sub>Core</sub> reached genome-wide significance (Fig. 3a,b and Supplementary Table 1; <i>P</i> = 4.9 × 10<sup>-4</sup> to 2.7 × 10<sup>-3</sup> for dCas9<sup>VP64</sup>, <i>P</i> = 1.5 × 10<sup>-19</sup> to 4.9 × 10<sup>-17</sup> for dCas<sup>p300</sup><sub>Core</sub>). In contrast, dCas<sup>p300</sup><sub>Core</sub> (D1399Y) did not significantly induce any <i>ILIRN</i> expression (Fig. 3c; <i>P</i> > 0.5 for all four <i>ILIRN</i> isoforms). Comparative analysis to dCas9 revealed limited dCas<sup>p300</sup><sub>Core</sub> off-target gene induction, with only two transcripts induced significantly above background at a false discovery rate (FDR) < 5%; <i>KDR</i> (FDR = 1.4 × 10<sup>-3</sup>) and <i>FAM49A</i> (FDR = 0.04) (Fig. 3b and Supplementary Table 1). We also found increased expression of p300 mRNA in cells transfected with dCas<sup>p300</sup><sub>Core</sub> and dCas<sup>p300</sup><sub>Core</sub> (D1399Y). This finding is most likely explained by RNA-seq reads mapping to mRNA from the transiently transfected p300 core fusion domains. Thus the dCas<sup>p300</sup><sub>Core</sub> fusion displayed high genome-wide targeted transcriptional specificity and robust gene induction of all four targeted <i>ILIRN</i> isoforms.

dCas<sup>p300</sup><sub>Core</sub> acetylates H3K27 at enhancers and promoters

Activity of regulatory elements correlates with covalent histone modifications, such as acetylation and methylation. Of those histone modifications, acetylation of lysine 27 on histone H3 (H3K27ac) is one of the most widely documented indicators of enhancer activity. Acetylation of H3K27 is catalyzed by p300 and is also correlated with endogenous p300 binding profiles. Therefore, we used H3K27ac enrichment as a measurement of relative dCas<sup>p300</sup><sub>Core</sub>-mediated acetylation at the genomic target site. To quantify targeted H3K27 acetylation by dCas<sup>p300</sup><sub>Core</sub>, we performed chromatin immunoprecipitation with an anti-H3K27ac antibody followed by quantitative PCR (ChIP-qPCR) in HEK293T cells co-transfected with four HS2 enhancer–targeted gRNAs and either dCas9, dCas9<sup>VP64</sup>, dCas<sup>p300</sup><sub>Core</sub> or dCas<sup>p300</sup><sub>Core</sub> (D1399Y) (Fig. 4). We analyzed three amplicons at or around the target site in the HS2 enhancer or within the promoter regions of the <i>HBE</i> and <i>HBG</i> genes (Fig. 4a). Notably, H3K27ac is enriched in each of these regions in the human K562 erythroid cell line, which has a high level of globin gene expression (Fig. 4a). We observed significant H3K27ac enrichment at the HS2 enhancer target locus compared to treatment with dCas9 in both the dCas9<sup>VP64</sup> (<i>P</i> = 0.0056 for ChIP Region 1 and <i>P</i> = 0.0029 for ChIP Region 3) and dCas<sup>p300</sup><sub>Core</sub> (<i>P</i> = 0.0013 for ChIP Region 1 and <i>P</i> = 0.0069 for ChIP Region 3) co-transfected samples (Fig. 4b). A similar trend of H3K27ac enrichment was also observed when targeting the <i>ILIRN</i> promoter with dCas9<sup>VP64</sup> or dCas<sup>p300</sup><sub>Core</sub> (Supplementary Fig. 4). In contrast to these increases in H3K27ac at the target sites by both dCas9<sup>VP64</sup> and dCas<sup>p300</sup><sub>Core</sub>, robust enrichment in H3K27ac at the HS2-regulated <i>HBE</i> and <i>HBG</i> promoters was observed only with dCas<sup>p300</sup><sub>Core</sub> treatment (Fig. 4c,d). Together these results demonstrate that dCas<sup>p300</sub><sub>Core</sub> uniquely catalyzes H3K27ac enrichment at gRNA-targeted loci and at enhancer-targeted distal promoters. Therefore, the acetylation established by dCas<sup>p300</sub><sub>Core</sub> at HS2 may catalyze enhancer activity in a manner distinct from direct recruitment of preinitiation complex components by dCas9<sup>VP64</sup> (refs. 27,28), as indicated by the distal activation of the <i>HBE</i>, <i>HBG</i>...
and HBD genes from the HS2 enhancer by dCas9p300 Core but not by dCas9VP64 (Fig. 2c and Supplementary Fig. 3).

dCas9p300 Core activates genes with a single gRNA

Robust transactivation using dCas9-effector fusion proteins currently relies upon the application of multiple gRNAs, multiple effector domains or both. Transcriptional activation could be simplified with the use of single gRNAs in tandem with a single dCas9-effector fusion. This would also facilitate multiplexing distinct target genes and the incorporation of additional functionalities into the system. We compared the transactivation potential of dCas9p300 Core with single gRNAs and four pooled gRNAs targeting the IL1RN, MYOD and OCT4 promoters (Fig. 5a–c). Substantial activation was observed upon co-transfection of the dCas9p300 Core and a single gRNA for each promoter tested. For the IL1RN and MYOD promoters, there was no significant difference between the pooled gRNAs and the best individual gRNA (Fig. 5a–b; IL1RN gRNA “C”, P = 0.78; MYOD gRNA “D”, P = 0.26). Although activation of the OCT4 promoter produced additive effects when four gRNAs were pooled with dCas9p300 Core, the most potent single gRNA (gRNA “D”) induced a statistically equivalent amount of gene expression to that observed upon co-transfection of dCas9VP64 with an equimolar pool of all four promoter gRNAs (P = 0.73; Fig. 5c). Compared to dCas9p300 Core, gene activation with dCas9VP64 and single gRNAs was substantially lower. Also, in contrast to dCas9p300 Core, dCas9VP64 demonstrated synergistic effects with combinations of gRNAs in every case (Fig. 5a–c), as reported previously.

Based on the transactivation ability of dCas9p300 Core at enhancer regions and with single gRNAs at promoter regions, we hypothesized that dCas9p300 Core might also be able to transactivate enhancers by means of a single targeted gRNA. We tested the MYOD (DRR and CE), OCT4 (PE and DE) and HS2 enhancer regions with equimolar concentrations of pooled or individual gRNAs (Fig. 5d–g). For both MYOD enhancer regions, co-transfection of dCas9p300 Core and a single enhancer-targeting gRNA was sufficient to activate gene expression to levels similar to cells co-transfected with dCas9p300 Core and the four pooled enhancer gRNAs (Fig. 5d). Similarly, OCT4 gene expression was activated from the PE through dCas9p300 Core localization with a single gRNA to similar levels as dCas9p300 Core localized with a pool of six PE-targeted gRNAs (Fig. 5e). dCas9p300 Core-mediated induction of OCT4 from the DE (Fig. 5e) and HBE and HBG genes from the HS2 enhancer (Fig. 5f,g) showed increased expression with the pooled gRNAs relative to single gRNAs. Nevertheless, there was activation of target gene expression above control for several single gRNAs at these enhancers (Fig. 5e–g).

The p300 HAT domain is portable to other DNA-binding proteins

The dCas9-gRNA system from Streptococcus pyogenes has been widely adopted due to its robust, versatile and easily programmable properties. However, several other programmable DNA-binding proteins are also under development for various applications and may be preferable for particular applications, including orthogonal dCas9 systems from other species, TALEs and zinc finger proteins. To determine if the p300 Core HAT domain is portable to these other systems, we created fusions to dCas9 from Neisseria meningitidis (Nm-dCas9) and four different TALEs targeting the IL1RN promoter and a zinc finger protein targeting ICAM1 (Fig. 6). Co-transfection of Nm-dCas9p300 Core and five Nm-gRNAs targeted to the HBE or the HBG promoters led
to significant gene induction compared to mock-transfected controls (P = 0.038 and 0.0141 for HBE and HBG, respectively) (Fig. 6b,c).

When co-transfected with five Nm- gRNAs targeted to the HS2 enhancer, Nm-dCas9p300 Core also significantly activated the distal HBE and HBG globin genes compared to mock-transfected controls (P = 0.0192 and P = 0.0323, respectively) (Fig. 6d,e). Similar to dCas9p300 Core, Nm-dCas9p300 Core activated gene expression from promoters and the HS2 enhancer with a single gRNA. Nm-dCas9VP64 displayed negligibly capacity to transactivate HBE or HBG regardless of localization to promoter regions or to the HS2 enhancer either with single or multiple gRNAs (Fig. 6b–e). Transfection of the expression plasmids for a combination of four TALEp300 Core fusion proteins targeted to the IL1RN promoter (IL1RN TALEp300 Core) also activated downstream gene expression, although to a lesser extent than four corresponding TALEVP64 fusions (IL1RN TALEVP64) (Fig. 6f,g). However, single p300 core effectors were much more potent than single VP64 domains when fused to IL1RN TALEs. Interestingly, dCas9p300 Core directed to any single binding site generated comparable IL1RN expression relative to single or pooled IL1RN TALE effectors, and direct comparisons suggest that dCas9 may be a more robust activator than TALEs when fused to the larger p300 Core fusion domain (Supplementary Fig. 5). Finally, the ZFP300 Core fusion targeted to the ICAM1 promoter (ICAM1 ZFP300 Core) also activated its target gene relative to control and at a similar level as ZFVP64 (ICAM1 ZFVP64) (Fig. 6h,i). The versatility of the p300 Core fusion with multiple targeting domains is evidence that this is a robust approach for targeted acetylation and gene regulation. The various p300 core fusion proteins were expressed well, as determined by western blot analysis (Supplementary Fig. 6), but differences in p300 Core activity between different fusion proteins could be attributable to binding affinity or protein folding.

**DISCUSSION**

These results establish the dCas9p300 Core fusion protein as a potent and easily programmable tool to synthetically manipulate acetylation at targeted endogenous loci, leading to regulation of proximal and distal enhancer–regulated genes. The intrinsic p300 Core acetyltransferase activity is crucial for this efficacy, as demonstrated by the consistent lack of chromatin modification and transactivation potential of the dCas9p300 Core (D1399Y) acetyltransferase-null mutant (Figs. 1c, 2c and 4b–d and Supplementary Fig. 1). Fusion of the catalytic core domain of p300 to dCas9 resulted in substantially higher transactivation of downstream genes than the direct fusion of full-length p300 protein despite robust protein expression (Fig. 1b,c). This may be due to differences in protein structure and function or interactions with other cellular proteins, suggesting that isolation of catalytic core regions is a useful strategy for future programmable epigenome editing tools. The dCas9p300 Core fusion protein also had an increased transactivation capacity relative to dCas9VP64 (Figs. 1c, 2, 3 and 5), including in the context of the Nm-dCas9 scaffold (Fig. 6b–e). This was especially evident at distal enhancer regions, at which dCas9VP64 displayed little, if any, measurable downstream transcriptional activity (Figs. 2 and 6d,e). In addition, the dCas9p300 Core displayed precise and robust genome-wide transcriptional specificity (Fig. 3 and Supplementary Fig. 2).

The observation that targeted acetylation is sufficient for gene activation at an endogenous locus and enhancer is a notable finding of our study. Although it is possible that activation or recruitment of other co-factors is involved in the targeted dCas9p300 Core, mediated epigenomic control, only dCas9p300 Core and not VP64 was capable of potent transcriptional activation and co-enrichment of acetylation at promoters targeted by the epigenetically modified enhancer (Figs. 2c and 3c,d and Supplementary Fig. 3). The two
The synthetic control of transcription and chromatin remodeling is a critical component of cellular engineering. dCas9p300 Core takes advantage of the simple programmability of the CRISPR-Cas9 system to target acetyltransferase activity and complements other recently described epigenetic editing tools, including fusions of demethylases, methyltransferases and deacetylases to generate a more complete set of epigenome editing tools.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: GSE66742.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

I.B.H., A.M.D., C.M.V., P.I.T., G.E.C., T.E.R. and C.A.G. analyzed the data. I.B.H. and C.A.G. wrote the manuscript with contributions by all authors.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Cell lines and transfection. HEK293T cells were procured from the American Tissue Collection Center (ATCC, Manassas, VA) through the Duke University Cell Culture Facility. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin and maintained at 37 °C and 5% CO2. Transfections were performed in 24-well plates using 375 ng of respective dCas9 expression vector and 125 ng of equimolar pooled or individual gRNA expression vectors mixed with Lipofectamine 2000 (Life Technologies, cat. #11668019) as per manufacturer’s instruction. For ChIP-qPCR experiments, HEK293T cells were transfected in 15-cm dishes with Lipofectamine 2000 and 30 μg of respective dCas9 expression vector and 10 μg of equimolar pooled gRNA expression vectors as per manufacturer’s instruction.

Plasmid constructs. pDNA-dCas9VP64 (dCas9VP64; Addgene, plasmid #74107) has been described previously16. An HA epitope tag was added to dCas9 (no effector) by removing the VP64 effector domain from dCas9VP64 via Ascl/PacI restriction sites. TALEs were generated by subcloning the HA-tagged p300 Core domain from dCas9p300 Core (Addgene, plasmid #23252)53 in two separate fragments and cloning these fragments into the dCas9VP64 backbone via isothermal assembly. A substitution in the full-length p300 protein (L553M), located outside of the dCas9 Core region, was identified in dCas9FLp300 and in the precursor pDNA3.1-p300 sequence validation. pDNA-dCas9p300 Core (dCas9p300 Core) was generated by first amplifying amino acids 1,048–1,664 of human p300 from cDNA and then subcloning the resulting amplicon into pCR-Blunt (pCR-Blunt Core) (Life Technologies cat. #K2700). An Ascl site, HA-epitope tag, and a Pmel site were added by PCR amplification of the p300 Core from pCR-Blunt Core, and subsequently this amplicon was cloned into pCR-Blunt Core + HA (Life Technologies cat. #K2700). The HA-tagged p300 Core was cloned from pCR-Blunt Core + HA into the dCas9VP64 backbone via shared Ascl/Pmel restriction sites. pDNA-dCas9p300 Core (D1399Y) was generated by amplification of the p300 Core domain from dCas9p300 Core in overlapping fragments with primer sets including the specified nucleic acid mutations, with a subsequent round of linkage PCR and cloning into the dCas9p300 Core backbone using isothermal assembly (NEB cat. #2611). Protein sequences of all dCas9 constructs are shown in Supplementary Table 1.

IL1RN, MYOD and OCT4 promoter gRNA protospacers have been described previously16,53. Neisseria meningitidis IL1RN (Nm-IL1RN) was obtained from Addgene (plasmid #48676)44. Nm-dCas9p300 Core was created by amplifying the HA-tagged p300 Core from dCas9p300 Core with primers to facilitate subcloning into the AelI/AgeI-digested Nm-dCas9p300 Core backbone using isothermal assembly (NEB cat. #2611). ILIRN TALEp300 Core TALEs were generated by subcloning the HA-tagged p300 Core domain from dCas9p300 Core into previously published25 ILIRN TALEp64 constructs via shared Ascl/Pmel restriction sites. ILIRN TALE target sites are shown in Supplementary Table 2. ICAMI ZFp64 and ICAMI ZEp300 Core constructs were constructed by subcloning the ICAMI ZF from pMX-CD54-310Opt-VP64 (ref. 54) into dCas9VP64 and dCas9p300 Core backbones, respectively, using isothermal assembly (NEB cat. #2611). Protein sequences of ICAMI ZF constructs are shown in Supplementary Note 2. Transfection efficiency was routinely above 90% as assayed by co-transfection of PL-SIN-EF1α-EGFP (Addgene plasmid #47108)16 for expression as described previously16 with slight modifications using NEB BsbI and T4 ligase (Cat. # K0259 and M0202). Nm-dCas9 gRNA oligos were rationally designed using published PAM requirements44, and then cloned into pZDonor-Nm-dCas9-gRNA-hU6 (Addgene, plasmid #61366) via BbsI sites. Plasmids are available through Addgene (Supplementary Table 3). All gRNA protospacer targets are listed in Supplementary Table 4.

Western blot analysis. 20 μg of protein was loaded for SDS PAGE and transferred onto a nitrocellulose membrane for western blots. Primary antibodies (α-FLAG; Sigma-Aldrich cat. #F7425 and α-GAPDH; Cell Signaling Technology cat. #41C10) were used at a 1:1,000 dilution in TBSB + 5% milk. Secondary α-Rabbit HRP (Sigma-Aldrich cat. #A6154) was used at a 1:5,000 dilution in TBSB + 5% milk. Membranes were exposed after addition of ECL (Bio-Rad cat. #170-5060).

Quantitative reverse-transcription PCR. RNA was isolated from transfected cells using the RNAeasy Plus mini kit (Qiagen cat. #74136) and 500 ng of purified RNA was used as template for cDNA synthesis (Life Technologies, cat. #11754). Real-time PCR was performed using PerfeCTa SYBR Green FastMix (Quanta Biosciences, cat. #95072) and a CFX96 Real-Time PCR Detection System with a C1000 Thermal Cycler (Bio-Rad). Baselines were subtracted using the baseline subtraction curve fit analysis mode and thresholds were automatically calculated using the Bio-Rad CFX Manager software version 2.1. Results are expressed as fold change above control mock transfected cells (no DNA) after normalization to GAPDH expression using the ∆∆Ct method, as previously described44. All qPCR primers and conditions are listed in Supplementary Table 5.

RNA-seq. RNA-seq was performed using three replicates per experimental condition. RNA was isolated from transfected cells using the RNAeasy Plus mini kit (Qiagen cat. #74136) and 1 μg of purified mRNA was used as template for cDNA synthesis and library construction using the PrepX RNA-Seq Library Kit (Wafergen Biosystems, cat. #400039). Libraries were prepared using the Apollo 324 liquid handling platform, as per manufacturer’s instruction. Indexed libraries were validated for quality and size distribution using the Tapestation 2200 (Agilent) and quantified by qPCR using the KAPA Library Quantification Kit (KAPA Biosystems; KK4835) before multiplex pooling and sequencing at the Duke University Genome Sequencing Shared Resource facility. Libraries were prepared and then 50-bp single-end reads were sequenced on a HiSeq 2500 (Illumina), de-multiplexed and then aligned to the HG19 transcriptome using Bowtie 2 (ref. 57). Transcript abundance was calculated using the SAMTools44 suite, and differential expression was determined in R using the DESeq2 analysis package. Multiple hypothesis correction was performed using the method of Benjamini and Hochberg with a FDR of < 5%.

ChIP-qPCR. HEK293T cells were co-transfected with four HS2 enhancer gRNA constructs and indicated dCas9 fusion expression vectors in 15-cm plates in biological triplicate for each condition tested. Cells were cross-linked with 1% formaldehyde (final concentration; Sigma F8775–25ML) for 10 min at RT and then the reaction was stopped by the addition of glycine to a final concentration of 125 mM. From each plate ~2.5e7 cells were used for H3K27ac ChIP-enrichment. Chromatin was sheared to a median fragment size of 250 bp using a Bioruptor XL (Diagenode). H3K27ac enrichment was performed by incubation with 5 μg of Abcam ab4729 and 200 μl of sheep anti-ribbon IgG magnetic beads (Life Technologies 11201D) for 16 h at 4 °C. Cross-links were reversed via overnight incubation at 65 °C with sodium dodecyl sulfate, and DNA was purified using MinElute DNA purification columns (Qiagen). 10 ng of DNA was used for subsequent qPCR reactions using a CFX96 Real-Time PCR Detection System with a C1000 Thermal Cycler (Bio-Rad). Baselines were subtracted using the baseline subtraction curve fit analysis mode and thresholds were automatically calculated using the Bio-Rad CFX Manager software version 2.1. Results are expressed as fold change above cells co-transfected with dCas9 and four HS2 gRNAs after normalization to β-actin enrichment using the ∆∆Ct method as previously described44. All ChIP-qPCR primers and conditions are listed in Supplementary Table 5.

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