

Supplementary Methods

Plasmids Construction

The DNA sequences of human codon-optimized AsCpf1 and LbCpf1 with NLS and HA tag were synthesized from iGene. DNase deactivated mutants were constructed by mutagenesis with Gibson Assembly. The sequence of human p300core was amplified from HEK293T cells and sub-cloned into dCpf1 expression plasmids. Cpf1, dCpf1 and dCpf1-p300core were sub-cloned into a mammalian expression vector containing a pBlueScript backbone, a CAG promoter and a bGH poly A signal sequence. To generate SunTag system plasmid, the 10xGCN4 sequence amplified from pHRdSV40-dCas9-10xGCN4_v4-P2A-BFP plasmid (Addgene #60903) was fused to dCpf1 expression vector by ligation. And the scFv-GCN4-sfGFP-VP64-GB1-NLS from pHRdSV40-scFv-GCN4-sfGFP-VP64-GB1-NLS plasmid (Addgene #60904) was cloned into a pBlueScript backbone with an EF-1- α core promoter and a SV40 polyA signal sequence. To generate gRNA expression plasmid, pBlueScript SK+ vector and the U6 promoter sequence amplified from pLentiCRISPR v2 plasmid (Addgene #52961) via PCR were fused by Gibson Assembly. Annealed oligos for AsCpf1 or LbCpf1 gRNA scaffold were ligated to the pBlueScript-U6 vector to generate gRNA basic plasmids. gRNA expression plasmid for each target site was constructed by ligating the annealed corresponding oligos to the basic plasmid. gRNA oligos were designed through www.Benchling.com. Primers were listed in Table 1.

Cell lines and transfection

HEK293T and U2OS 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. MCF7 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin and maintained at 37 °C and 5% CO₂. Transfections were performed in 12-well plates using 200ng of respective dCpf1-p300core expression plasmids and 800ng of equimolar pooled gRNA expression vectors with polyethylenimine (PEI) solution. 48 hr later, genomic DNA was harvested for PAGE and T7E1 assays, or total RNA was harvested for gene expression assay.

Polyacrylamide gel electrophoresis (PAGE) assay

Genomic DNA was isolated with sarkosyl lysis buffer (10 mM Tris pH7.6, 10 mM EDTA, 10 mM NaCl, 0.5% Sarkosyl, 0.1 mg/ml proteinase K) and amplified by PCR with primers flanking target sites. After reannealed to form heteroduplexs, PCR amplicons were subjected to 5% polyacrylamide gel electrophoresis and stained in 2xGel-red solution before ultraviolet imaging.

T7 endonuclease I (T7E1) assay

PCR amplicons containing target genomic region were purified with Qiaquick PCR Purification Kit (Qiagen). Purified PCR products were denatured and cooled down to form heteroduplex DNA in NEB buffer 2 using a thermocycler. Annealed PCR amplicons were incubated with T7 endonuclease I (NEB) for 90min at 37 °C and subjected to 2% agarose gel electrophoresis.

Western blotting

The HEK293T cells transiently transfected with dCpf1-p300core plasmids were lysed in 2XSDS loading buffer (100 mM Tris pH 6.8, 4% SDS, 20% glycerol, 200 mM β -mercaptoethanol, 0.05% bromophenol blue) and boiled for 10 min. The lysates were resolved by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk in TBST containing 0.05% Tween 20, and sequentially incubated with the indicated primary antibodies (anti-HA, MBL m180-3; anti- β -tubulin, Proteintech 66240-I-Ig) and HRP-conjugated horse anti-mouse IgG secondary antibody (CST 7076S). The probed proteins were finally detected by chemiluminescence according to manufacturer's instructions (Pierce)

Quantitative real-time PCR

Total RNA was isolated from transfected cells with RNeasy Mini Kit (Qiagen) and reverse transcribed into cDNA using PrimeScript RT Reagent Kit (TAKARA). Quantitative real-time PCR was performed using SYBR Premix Ex Taq II (TAKARA) with a Mx3005p Thermal Cycler (Agilent Technologies). Results are expressed as fold change above mock-transfected cells after normalizing to GAPDH expression using the $\Delta\Delta C_t$ method. All qPCR primers are listed in Table 1.

RNA-seq analysis

Total RNA was isolated from transfected HEK293T cells with RNeasy Mini Kit (Qiagen). Briefly, mRNA was enriched and fragmented. And random hexamers were used for the first strand synthesis, followed by the second strand synthesis. After cleaned up, double strand cDNA was added with adaptors and amplified by PCR to finalize library construction. After verified using Qubit 3.0, Agilent 2100 Bioanalyzer and quantitative RT-PCR, libraries were pooled and sequenced on an Illumina HiSeq X Ten with 150bp paired-end module.. Reads were aligned to the hg19 transcriptome using Tophat2, and gene expression was quantified using Cufflinks 2.2.1. Differential expression was defined by a Benjamini-Hochberg adjusted p value (q value | FDR) of <0.05 and fold change of >2 or <0.5.

Supplementary Figure Legends

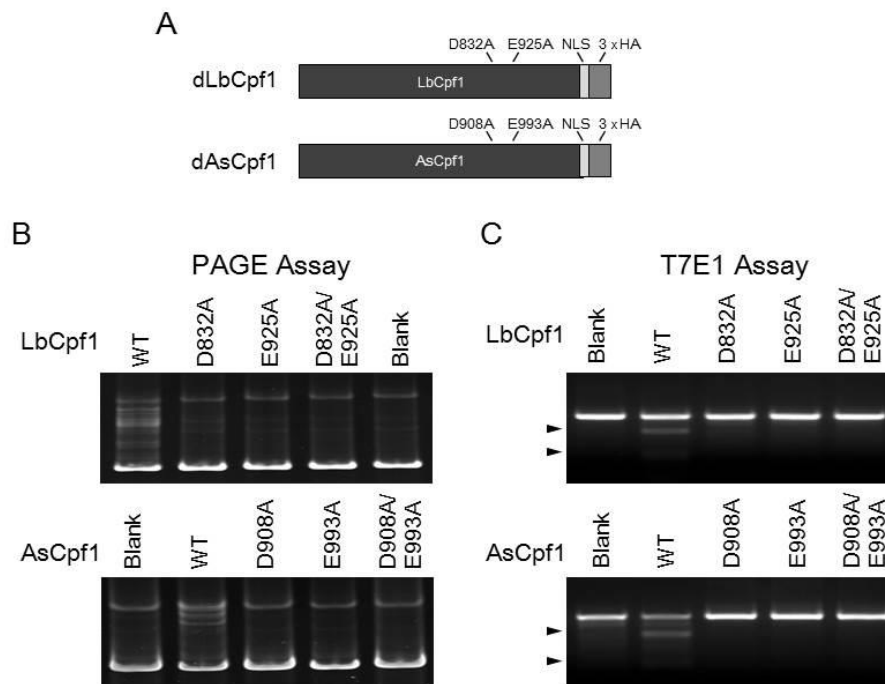


Figure S1

Figure S1. Generation of LbCpf1 and AsCpf1 mutants without DNase activity. (A) Schematic of LbCpf1 and AsCpf1 DNase-dead mutants. dLbCpf1 mutants contain DNase-deactivating mutations D832A, E925A or both, while dAsCpf1 mutants contain D908A, E993A or both. NLS, nuclear localization signal; HA, human hemagglutinin epitope tag. (B and C) PAGE and T7E1 analyses showing the DNase activities of wide-type and mutants of Cpf1 in human cells. DNA samples were harvested from HEK293T cells co-transfected with wide-type or mutants of Cpf1 and DNMT1 site 3 targeted gRNA plasmids.

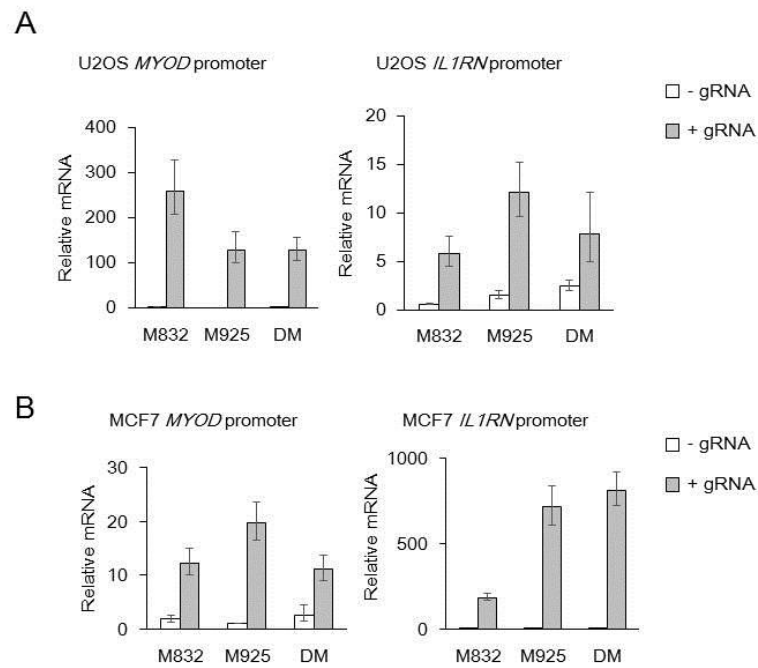


Figure S2

Figure S2. dLbCpf1-p300core proteins activate gene expression in multiple cell lines. (A & B) Relative mRNA expression of *MYOD* and *IL1RN* in U2OS cells (A) and MCF7 cells (B) co-transfected with dCpf1-p300core fusion proteins and four gRNAs targeting each promoter region of target genes. Mean value are presented with S.D. (n = 3). Tukey-test, p < 0.05 compared to cells transfected with dCpf1-p300core only, n = 3 independent experiments.

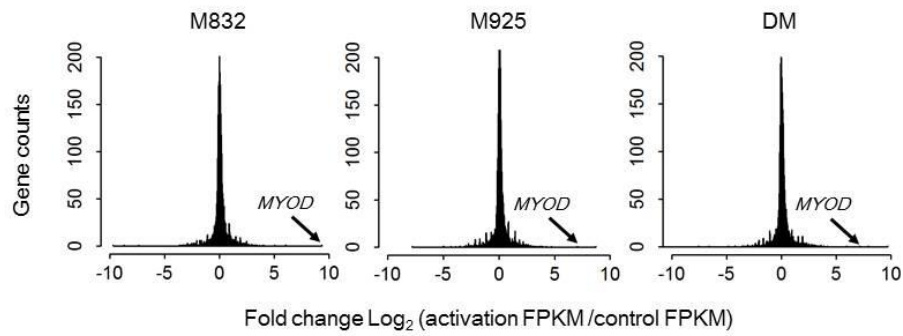


Figure S3

Figure S3. Transcriptional activation by dLbCpf1-p300core fusion proteins is specific and robust. Histograms of the distribution of fold changes in gene expression (Log_2 (activation / control)). The Histograms were generated from RNA-seq data from HEK293T cells transiently co-transfected with dLbCpf1-p300core proteins and four gRNAs targeting *MYOD* promoter. The control samples were transfected with dLbCpf1-p300core plasmids only. Genes with 0 FPKM were excluded. The fold change of *MYOD* is indicated.

Table 1. Primer list

gRNA primers	Sequence (5'-3')
MyoD-P1-F	TGGGCTATTTATCCGTAGTAGCCTTTTTTTC
MyoD-P1-R	CCGGGAAAAAATGGGCTATTTATCCGTAGTAGCC
MyoD-P2-F	TCCGTAGTAGCCTAAACGCCCCGTTTTTTC
MyoD-P2-R	CCGGGAAAAAACCGGGGCGTTTAGGCTACTACGGA
MyoD-P3-F	GAAAGGGCGTGCCGGAGAGCCAATTTTTTTC
MyoD-P3-R	CCGGGAAAAAATTGGCTCTCCGGCACGCCCTTTC
MyoD-P4-F	CCGCGGATACAGCAGTCGGGTGTTTTTTC
MyoD-P4-R	CCGGGAAAAAACACCCGACTGCTGTATCCGCGG
MyoD-DRR1-F	CCGGGAAAAAACACTCTGTTTCAGCAAAGGTTGG
MyoD-DRR1-R	GCGCCCCCACCTCCCGGCCAGATTTTTTTC
MyoD-DRR2-F	TATATATAGCCTCTGGAAACCCATTTTTTTC
MyoD-DRR2-R	CCGGGAAAAAATGGGTTTCCAGAGGCTATATATA
MyoD-DRR3-F	CCAGGGAGCAAGTTTGTGAGGGGTTTTTTC
MyoD-DRR3-R	CCGGGAAAAAACCCTGACAACTTGCTCCCTGG
MyoD-DRR4-F	CAGAGGCCAGCTCTCCATTTATATTTTTTTC
MyoD-DRR4-R	CCGGGAAAAAATATAAATGGAGAGCTGGCCTCTG
IL1RN-P1-F	TTAGAGCGTTGGGGACCTTGTCTTTTTTTC
IL1RN-P1-R	CCGGGAAAAAAGACAAGGTCCCAACGCTCTAA
IL1RN-P2-F	GAATGAATGTGTGCACACATGCATTTTTTTC
IL1RN-P2-R	CCGGGAAAAAATGCATGTGTGCACACATTCATTC
IL1RN-P3-F	CAGGAGGGTGACTCAGGCTAGCATTTTTTTC
IL1RN-P3-R	CCGGGAAAAAATGCTAGCCTGAGTCACCCTCCTG
IL1RN-P4-F	TGCTAGCCTGAGTCACCCTCCTGTTTTTTC
IL1RN-P4-R	CCGGGAAAAAACAGGAGGGTGACTCAGGCTAGCA
OCT4-PE1-F	GGCCCCCTCCACTATGGAACCTGTTTTTTC
OCT4-PE1-R	CCGGGAAAAAACAGGTTCCATAGTGGAGGGGGCC
OCT4-PE2-F	GGGTTAGAGCTGCCCCCTCTGGGTTTTTTC
OCT4-PE2-R	CCGGGAAAAAACCAGAGGGGGCAGCTCTAACCC
OCT4-PE3-F	GCGTCTCTGAAGGGGATTCTGTGTTTTTTC
OCT4-PE3-R	CCGGGAAAAAACACAGAATCCCCTTCAGAGACGC
OCT4-PE4-F	CCAACCTTTGCTGAAACAGAGTGTTTTTTC
OCT4-PE4-R	CCGGGAAAAAACACTCTGTTTCAGCAAAGGTTGG
DNMT1-3-F	CTGATGGTCCATGTCTGTTACTCTTTTT
DNMT1-3-R	GAGTAACAGACATGGACCATCAGATCTA
HBG2-P1-F	TCCTTAGAAACCACTGCTAACTGTTTTTTG
HBG2-P1-R	GATCCAAAAAACAGAAATGTAACAGGAACCTAAG
HBG2-P2-F	AACTACAGGCCTCACTGGAGCTATTTTTTTG
HBG2-P2-R	GATCCAAAAAATAGCTCCAGTGAGGCCTGTAGTT
HBG2-P3-F	CCTTGTCAGGCTATTGGTCAAGTTTTTTTG
HBG2-P3-R	GATCCAAAAAAGTTGACCAATAGCCTTGACAAGG
HBG2-P4-F	ATTCTTCATCCCTAGCCAGCCGCTTTTTTTG

HBG2-P4-R	GATCCAAAAAAGCGGCTGGCTAGGGATGAAGAAT
HS2-1F	CTTAGTTCCTGTTACATTTCTGTTTTTTTTG
HS2-1R	GATCCAAAAAACAGAAATGTAACAGGAACCTAAG
HS2-2F	CTTAGAAGGTTACACAGAACCAGTTTTTTTTG
HS2-2R	GATCCAAAAAACTGGTTCTGTGTAACTTCTAAG
HS2-3F	TGCCATCTGCCCTGTAAGCATCCTTTTTTTG
HS2-3R	GATCCAAAAAAGGATGCTTACAGGGCAGATGGCA
HS2-4F	GAAGCACCTGCTGGATGCTAACTTTTTTTG
HS2-4R	GATCCAAAAAAGTTTAGCATCCAGCAGGTGCTTC
Mutation Primers	Sequence (5'-3')
AsCpf1-Mut-D908A-F	CGACCTGTCTGATGAGGCCAGGGCCCTGC
AsCpf1-Mut-D908A-Rm	CTCTCGCCCCGGGCGATGCCGATGATAGG
AsCpf1-Mut-D908A-Fm	ATCGGCATCGCCCGGGGCGAGAGAAAC
AsCpf1-Mut-D908A- R	AAAGTGGCACCGAGTCGGTGCGGATCC
AsCpf1-Mut-E993A-Rm	ATTCAGGTTGGCTAGCACCACCACGGC
AsCpf1-Mut-E993A-Fm	GGTGGTGCTAGCCAACCTGAATTTTCGG
LbCpf1-Mut-D832A-Fm	AACCCCTATGTGATCGGCATCGCTAGGGGCGAGCG C
LbCpf1-Mut - R	CTTGAAAAAGTGGCACCGAGTCGGTGCGGATCC
LbCpf1-Mut-E925A-F	ACGATAACCCCTATGTGATCGGCATCGATAGG
LbCpf1-Mut-E925A-Rm	AGTTCAGGTCGGCTAGCGCGATCACGGCATCG
LbCpf1-Mut-E925A-Fm	CCGTGATCGCGCTAGCCGACCTGAACTCTGGC
PCR Primers	Sequence (5'-3')
DNMT1-3-PCR-F	CAAGTGCTTAGAGCAGGCGT
DNMT1-3-PCR-R	GTGACGGGAGGGCAGAACTA
p300 PCR Primer-F	GCGCCTCGAGATTTTCAAACCAGAAG
p300 PCR Primer-R	ATATACCGGTGTCCTGGCTCTGCGTGT
10xGCN4 Primer-F	ATTATTACTCGAGAGCCTGGGCAGCGGCT
10xGCN4 Primer-R	GCCGTGTACATTACACCTTGCGCTTCTTC
RT-PCR Primers	Sequence (5'-3')
GAPDH-qPCR-F	AGAAGGCTGGGGCTCATTTG
GAPDH-qPCR-R	AGGGGCCATCCACAGTCTTC
MyoD-qPCR-F	TCCCTCTTTCACGGTCTCAC
MyoD-qPCT-R	AACACCCGACTGCTGTATCC
IL1RN-qPCR-F	GGAATCCATGGAGGGAAGAT
IL1RN-qPCR-R	TGTTCTCGCTCAGGTCAGTG
OCT-4-qPCR-F	CGAAAGAGAAAGCGAACCAGTATCGAGAAC

OCT-4-qPCR-R	CGTTGTGCATAGTCGCTGCTTGATCGC
HBG2-qPCR-F	GCACGTGGATCCTGAGAACT
HBG2-qPCR-R	ATTGGACAGCAAGAAAGCGAG