Supplementary information

Targeted DNA demethylation of the *Fgf21* promoter by CRISPR/dCas9-mediated epigenome editing

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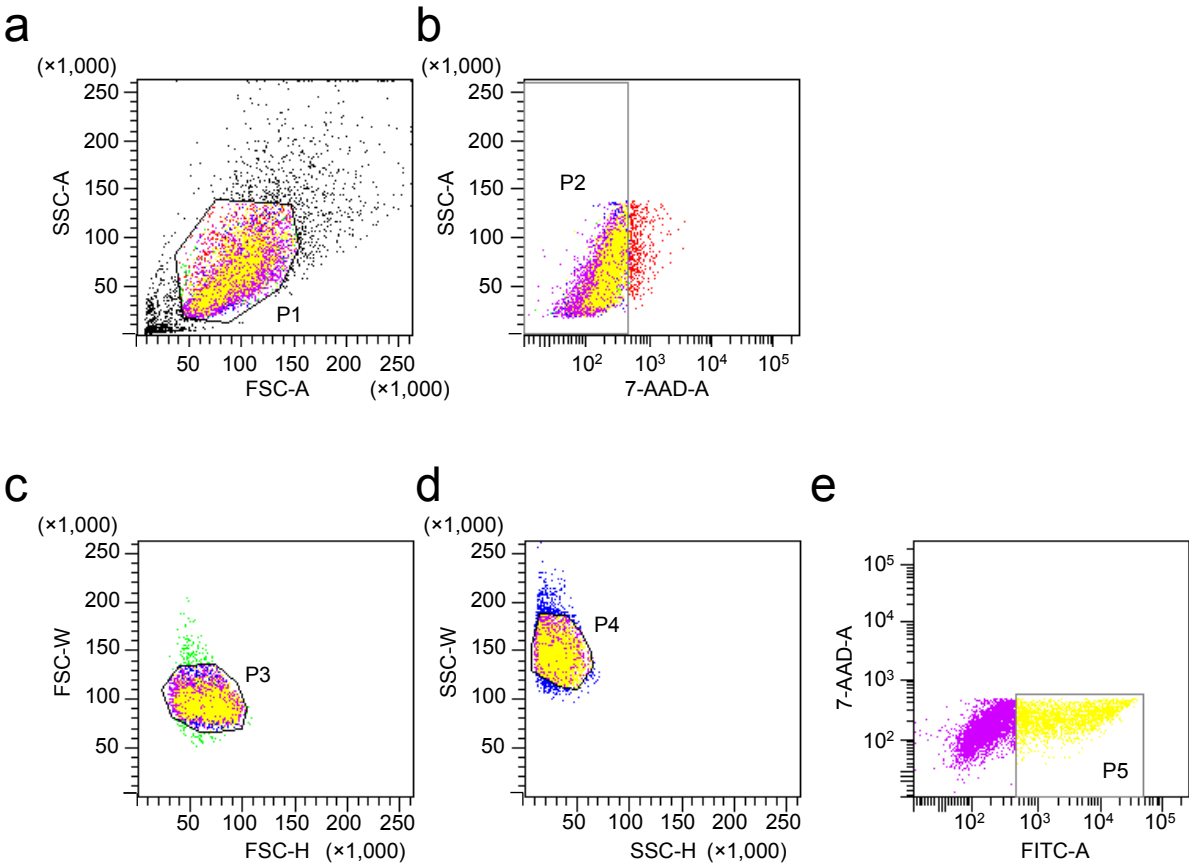
Supplementary Figure 1

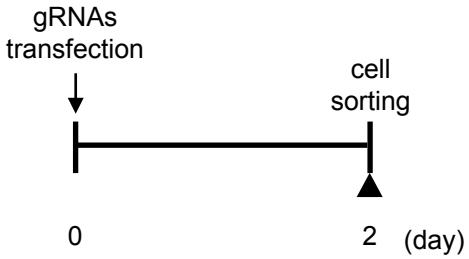


f

Population	#Events	%Parent	%Total
All Events	10,000	-	100.0
P1	8,396	84.0	84.0
P2	7,974	95.0	79.7
P3	7,782	97.6	77.8
P4	7,177	92.2	71.8
P5	1936	27.0	19.4

Supplementary Figure 2

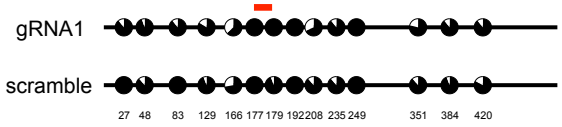
a



b

gRNA1 off target

CCACGAGAATATCACGCGTCAGG



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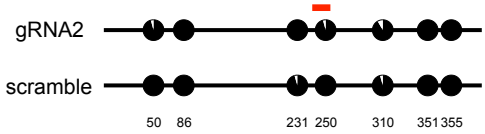
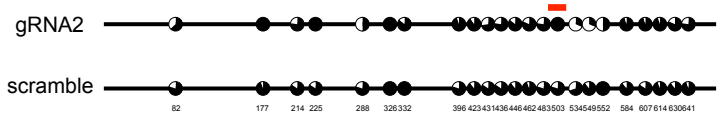
gRNA2 off target

ATAAACAGCAAGTCCGGCAGTGG



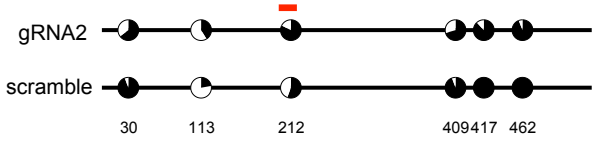
P=0.74

CCTGAGTCCAAGTCCGGCAGTGG



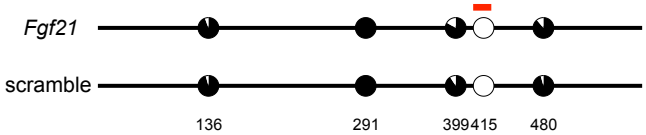
P=0.01

AAAGTGTCCAAGTCCGGCAGTGG



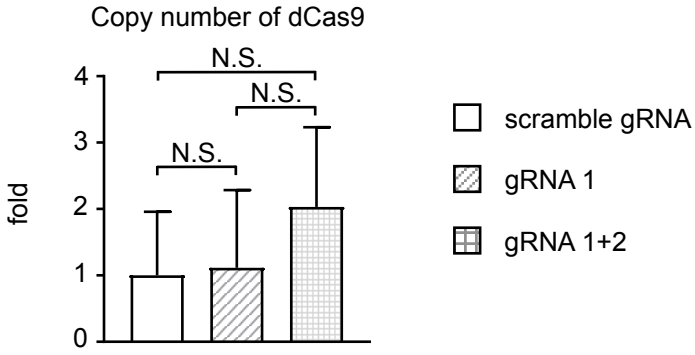
P=0.46

AACAACAACAAGTCCGGCAGTGG

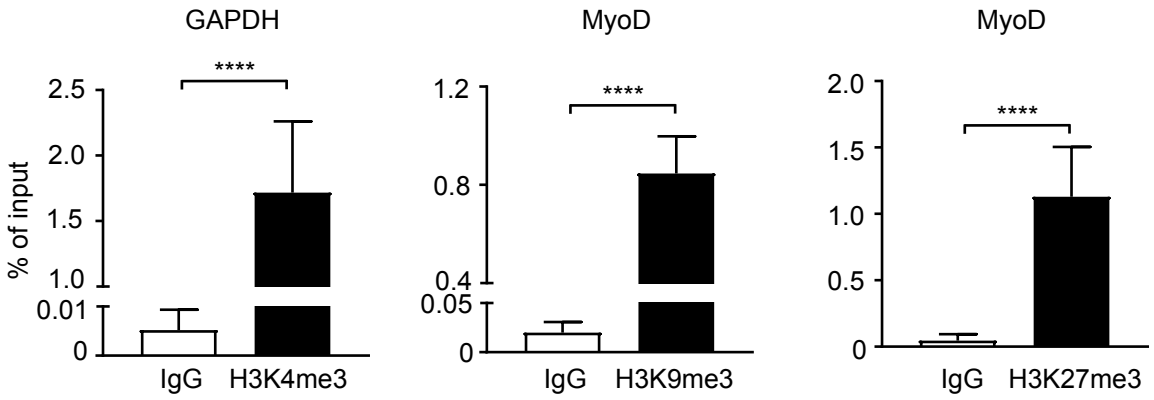


P=0.70

Supplementary Figure 3

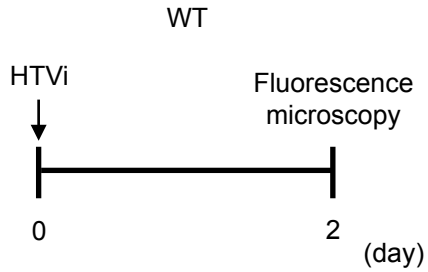


Supplementary Figure 4



Supplementary Figure 5

a



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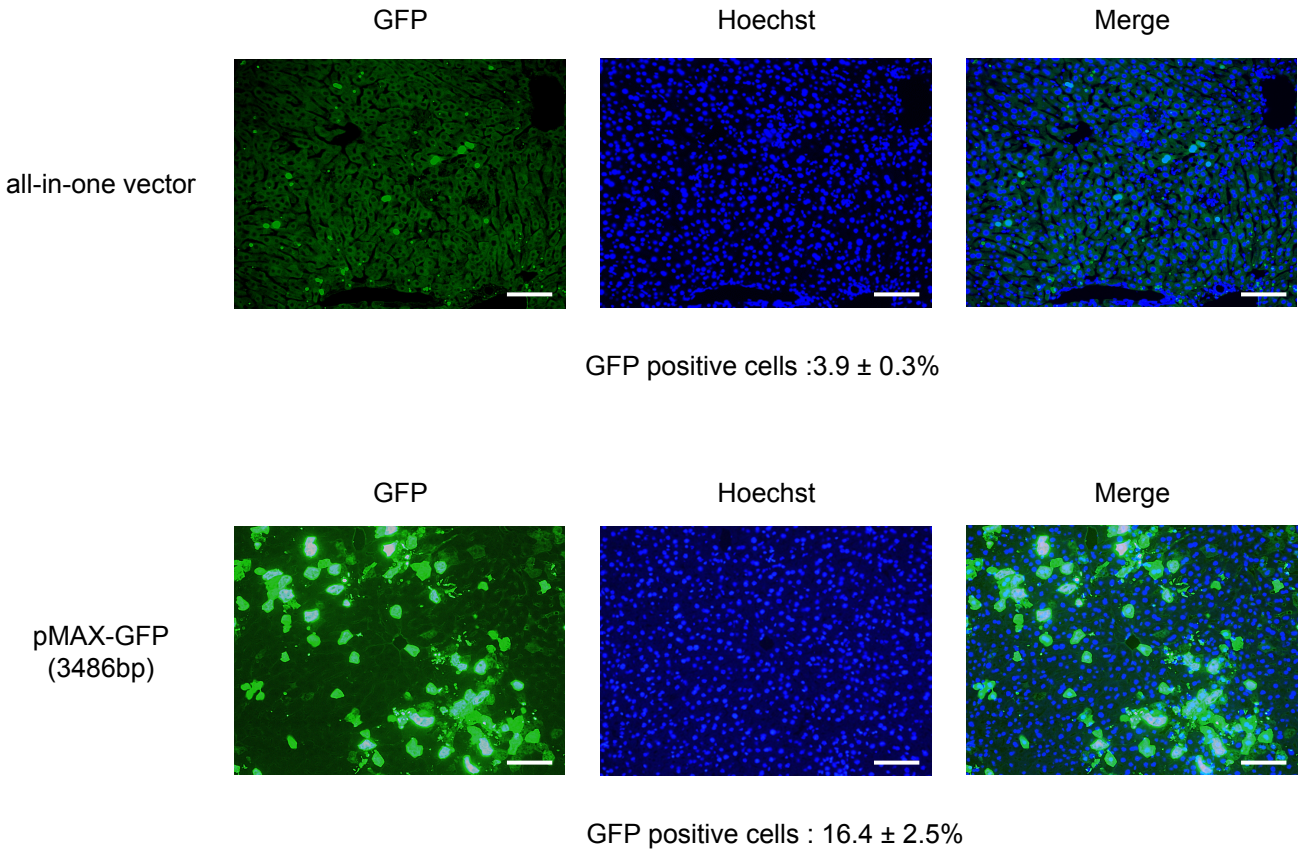
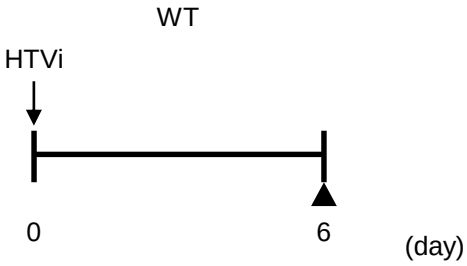


Supplementary Figure 6

a



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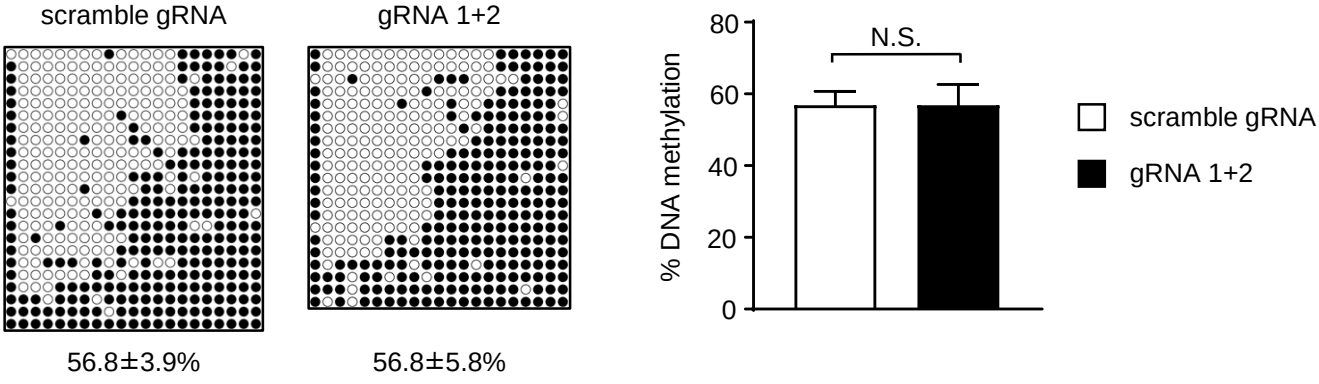

Supplementary table 1

a gRNA target sequences

Target name	Target Sequence
scramble	GCACTACCAGAGCTAACTCA
1	TGTGTCAAATATCACGCGTCAGG
2	TTGGGGGTCAAGTCCGGCAGAGG

b Primer sequences for RT-qPCR

Gene	Forward	Reverse
m36B4	GGCCCTGCACTCTCGCTTTC	TGCCAGGACGCGCTTGT
mFgf21	CCTCTAGGTTTCTTTGCCAACAG	AAGCTGCAGGCCTCAGGAT
mDnmt1	GGCTTCAGTGGCATGAAC	CTGCAGCCAAGATGATGG
mDnmt3a	CATGAACAGGCCTTTGGCA	TCTTGCAGCTCCAGCTTATC
mDnmt3b	CCAAAAGGAGGCCCATTAGAG	GTACCCCGTTGCAATTCCAT
sfGFP	TTCTTTGTTTGGTCCGGCAG	ATCCCACACCATGATGAGCA
TET1CD	GATCCGAACTGCCCACCTG	TCCTGCCCCAAGGTGTGTAT
dCas9	TGCCCCAAGTGAATATCGTG	GACTTGCCCTTTTCCACTTTG

c Primer sequences for copy number of dCas9

Gene	Forward	Reverse
dCas9	TGCCCCAAGTGAATATCGTG	GACTTGCCCTTTTCCACTTTG
Ndufv1	CTTCCCCACTGGCCTCAAG	CCAAAACCCAGTGATCCAGC

Supplementary table 2

a Antibodies used in ChIP assay

Antibody	Manufacturer	Dilution rate
anti-trimethyl-histone H3 (Lys4)	Merck Millipore, 07-473	1:267
anti-trimethyl-histone H3 (Lys27)	Abcam, ab6002	1:267
anti-trimethyl-histone H3 (Lys9)	Abcam, ab8898	1:267
anti-DNA methyltransferase (Dnmt)1	Abcam, ab13537	1:267
anti-DNA methyltransferase (Dnmt)3a	Abcam, ab2850	1:267
anti-DNA methyltransferase (Dnmt)3b	Abcam, ab2851	1:267
anti-RNA polymerase (pol) II	Abcam, ab5408	1:267
anti-rabbit IgG	Abcam, ab171870	1:300
anti-mouse IgG	Abcam, ab18413	1:300

b Primer sequences for ChIP assay

Gene	Forward	Reverse
mFgf21 (-997/-923bp)	AGGCCCGAATGCTAAGC	AGCCCAGCAGGTGGAAGTCT
mFgf21 (-106/+21bp)	TGGAATTCAGGTTCCTGCCAA	GAGAAGACACTAAGGCTGTC
Gapdh	ACCAGGGAGGGCTGCAGTCC	TCAGTTCGGAGCCCACACGC
MyoD	GCCCGCAGTAGCAAAGTAAG	GAAACCGGATCCAACTAGCA

c Primer sequences for bisulfite sequencing

Gene	Forward	Reverse
mFgf21	AGTGGTGAGAATTTAGGAGTTTTTTT	TCCTCCAAATTTAAAAATACAAACC

d Primer sequences for bisulfite sequencing (off target)

Gene	Forward	Reverse
gRNA 1 off target	TATATTTTTTTAAAGAAAGTTGTAGT	ACAACACTAATCAAATTAATATTTTT
gRNA 2 off target 1	TAAGGGAGAAAGAGGAGAAAGTTTTAT	CAAACTAACCTTAAACTCAAAAATCTACC
gRNA 2 off target 2	TTAGGAATTTTGGTTTTTTAAAGTAGGGAT	CAAATAAAACTCCATAAAAATAAAACCC
gRNA 2 off target 3	ATTTTTATTTTTTTGATGGTTAAGGAT	TTCTTTTATACTCTTTATAACCCAAACTAA
gRNA 2 off target 4	TGGGATTTGAATTTAGGATTTTTAG	CAATTCCATCACCTTTTTAATTATATTATC