a System 1

pCAG-dCas9TET1CD

CAG dCas9 TET1CD

gRNA cloning vector

U6 gRNA

b

Supplementary Figure 1

Design and sequence of system 1 for targeted demethylation.

(a) Design of system 1 for targeted demethylation. TET1CD was fused to a catalytic inactive Cas9 nuclease (dCas9) and expressed using the CAG promoter (pCAG-dCas9TET1CD). Each gRNA vector was generated by incorporating the target sequence into the gRNA cloning vector with the U6 promoter (Addgene, 41824) using Gibson assembly (New England BioLabs). (b) Full sequence of pCAG-dCas9TET1CD.

a **System 2**

pCAG-dCas9-10xGCN4_v4

CAG | dCas9 | multi GCN4 (5aa Linker)

gRNA cloning vector

U6 | gRNA

pCAG-scFvGCN4sfGFPTET1CD

CAG | scFv | GFP | TET1CD

b

c

Supplementary Figure 2

Design and sequence of system 2 for targeted demethylation.

(a) Design of system 2 for targeted demethylation. dCas9 with ten copies of the GCN4 peptide was expressed using the CAG promoter (pCAG-dCas9-10xGCN4_v4). The length of the linker separating each 19 aa GCN4 peptide unit of the array was 5 aa. An anti-GCN4 peptide antibody (scFv)-sfGFP-TET1CD fusion protein was expressed using the CAG promoter (pCAG-scFvGCN4sfGFPTET1CD). Each gRNA vector was generated by incorporating the target sequence into the gRNA cloning vector with the U6 promoter (Addgene, 41824) using Gibson assembly (New England BioLabs). (b) Full sequence of pCAG-dCas9-10xGCN4_v4. (c) Full sequence of scFvGCN4sfGFPTET1CD.

a System 3

pCAG-dCas9-5xPlat2AflD

CAG dCas9 multi GCN4 (22aa Linker)

gRNA cloning vector

U6 gRNA

pCAG-scFvGCN4sfGFPTET1CD

CAG scFv GFP TET1CD

b

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Supplementary Figure 3

Design and sequence of system 3 for targeted demethylation.

(a) Design of system 3 for targeted demethylation. dCas9 with five copies of the GCN4 peptide was expressed using the CAG promoter (pCAG-dCas9-5xPlat2AflD). The length of the linker separating each 19 aa GCN4 peptide unit of the array was 22 aa. An anti-GCN4 peptide antibody (scFv)-sfGFP-TET1CD fusion protein was expressed using the CAG promoter (pCAG-scFvGCN4sfGFPTET1CD). Each gRNA vector was generated by incorporating the target sequence into the gRNA cloning vector with the U6 promoter (Addgene: 41824) using Gibson assembly (New England BioLabs). (b) Full sequence of pCAG-dCas9-5xPlat2AflD.

a System 4

pCAG-dCas9-3.5xSuper

CAG | dCas9 | multi GCN4 (43aa Linker)

gRNA cloning vector

U6 | gRNA

pCAG-scFvGCN4sfGFPTET1CD

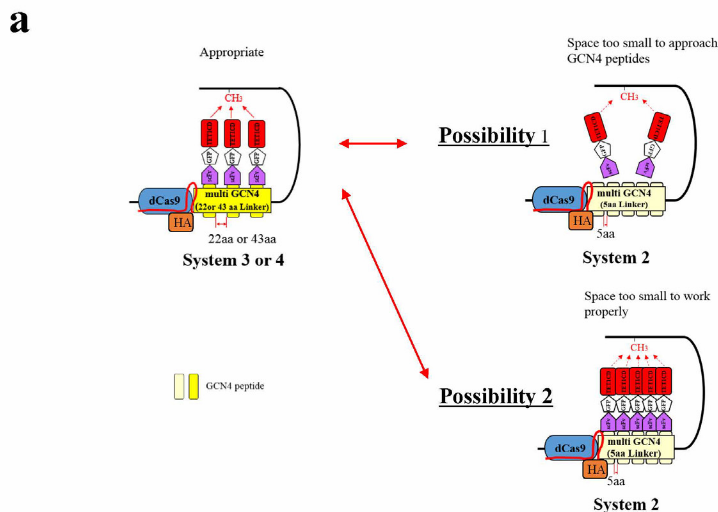
CAG | scFv | GFP | TET1CD

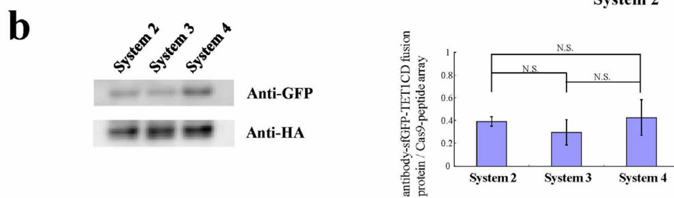
b

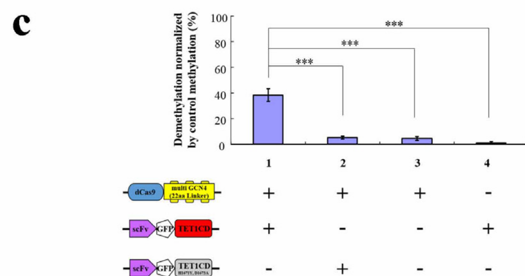
Supplementary Figure 4

Design and sequence of system 4 for targeted demethylation.

(a) Design of system 4 for targeted demethylation. dCas9 with four copies of the GCN4 peptide was expressed using the CAG promoter (pCAG-dCas9-3.5xSuper). The length of the linker separating each 19 aa GCN4 peptide unit of the array was 43 aa. An anti-GCN4 peptide antibody (scFv)-sfGFP-TET1CD fusion protein was expressed using the CAG promoter (pCAG-scFvGCN4sfGFPTET1CD). Each gRNA vector was generated by incorporating the target sequence into the gRNA cloning vector with the U6 promoter (Addgene: 41824) using Gibson assembly (New England BioLabs). (b) Full sequence of pCAG-dCas9-3.5xSuper.





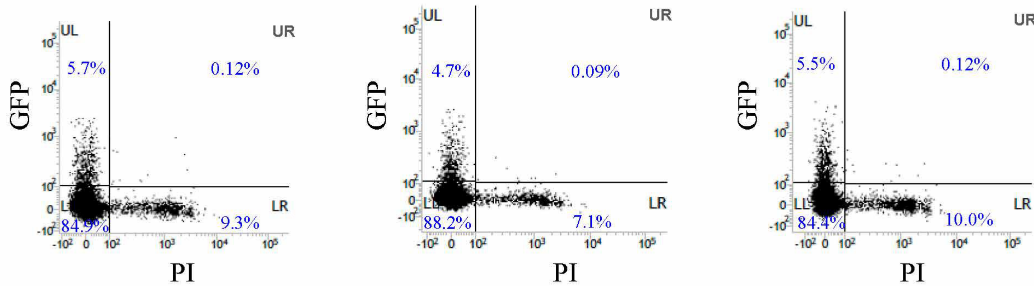


Supplementary Figure 5

Validation of linker length and specificity of demethylation.

(a) The length of the linker separating each GCN4 peptide unit of the array fused to dCas9 is important for maximization of the demethylation activity. There are two possibilities to explain this. If the linker is too short, there is only a small amount of space for antibody-TET1CD fusion proteins to approach and bind to the GCN4 peptide array, resulting in poor demethylation activity (possibility 1). Alternatively, the amount of space is too small for antibody-TET1CD fusion proteins to work properly, resulting in poor demethylation activity (possibility 2). (b) Co-IP of the Cas9-peptide array and antibody-sfGFP-TET1CD fusion protein (systems 2–4). The Cas9-peptide array (with a HA tag) was immunoprecipitated with anti-HA magnetic beads and subjected to western blot analysis. The amount of co-immunoprecipitated antibody-sfGFP-TET1CD fusion protein (vector lacking the HA tag was used in this case) was quantified by western blotting with an anti-GFP antibody and normalized by the amount of the Cas9-peptide array quantified by western blotting with an anti-HA antibody. Normalized values are shown in the bar graph. Data are shown as the mean ± s.e.m. Statistical analyses were performed using an ANOVA with Tukey's post-hoc test (N.S., not significant ($p > 0.05$)). (c) Demethylation activities of system 3 (dCas9-GCN4 and scFV-GFP-TET1CD), that with catalytic inactive TET1 (H1671Y, D1673A), that without scFv-GFP-TET1CD, and that without dCas9-GCN4. Demethylation of the STAT3-binding site was analyzed by COBRA. The gRNA used was target 2 of *Gfap*. Demethylation was analyzed as in Figure 1c. Data are shown as the mean ± s.e.m. ($n = 3$ from two independent experiments). The two-sided Student's t-test was performed. ***$P < 0.005$.

a



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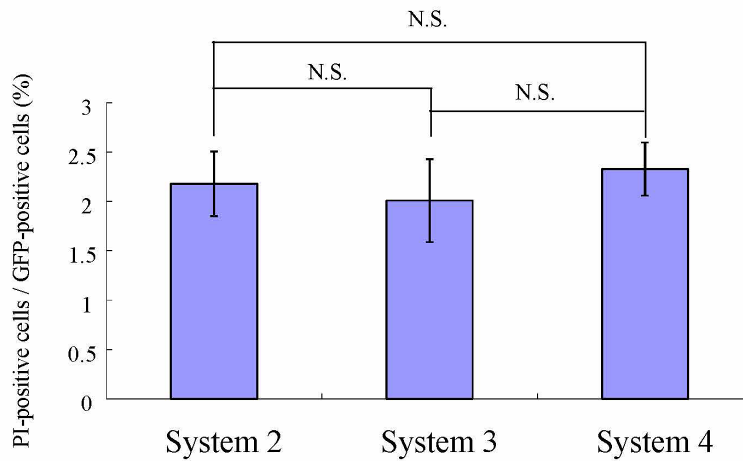


Supplementary Figure 6

Quantification of the viability of ESCs into which systems 2, 3, and 4 were introduced.

(a) ESCs were transfected with the Gfap_2 gRNA using systems 2, 3, and 4. Two days after transfection, ESCs were stained with PI, and cell viability was quantified using a FACSVerse flow cytometer (BD Biosciences) with a 488-nm blue laser. Cells were categorized into four groups based on dye uptake and GFP signals. Quadrant UL shows PI-negative/GFP-positive cells, quadrant UR shows PI/GFP-positive cells, quadrant LL shows PI/GFP-negative cells, and quadrant LR shows PI-positive/GFP-negative cells. (b) The population of PI-positive cells among GFP-positive cells was compared among the systems. Data are shown as the mean ± s.e.m (n = 3 from two independent experiments). Statistical analyses were performed using an ANOVA with Tukey's post-hoc test (N.S., not significant ($p > 0.05$)).

Supplementary Figure 7

a

All-in-one

pPlatTET-gRNA2

CAG dCas9 multi GCN4 (22aa Linker) 2A scFv GFP TET1CD U6 gRNA

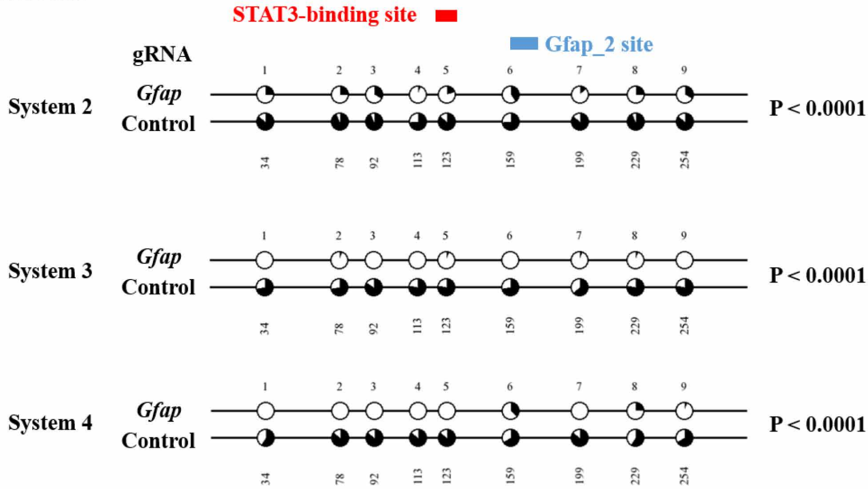
b

Supplementary Figure 7

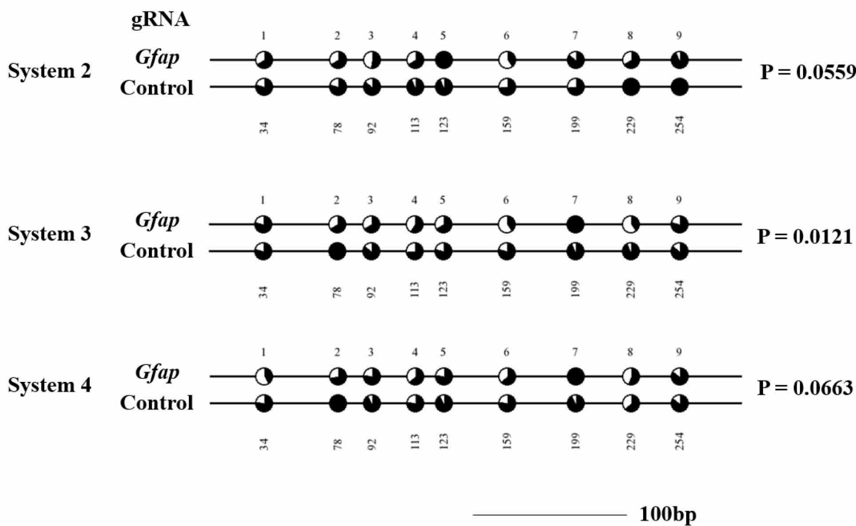
Design and sequence of the all-in-one system for targeted demethylation.

(a) Design of the all-in-one system for targeted demethylation. This vector included the gRNA under the control of the U6 promoter, dCas9 with the GCN4 array (system 3), and the antibody-TET1CD fusion protein. Cloning was performed by linearization of an *Afl* II site and Gibson assembly-mediated incorporation of the gRNA insert fragment. (b) Full sequence of pPlatTET-gRNA2.

TET1CD



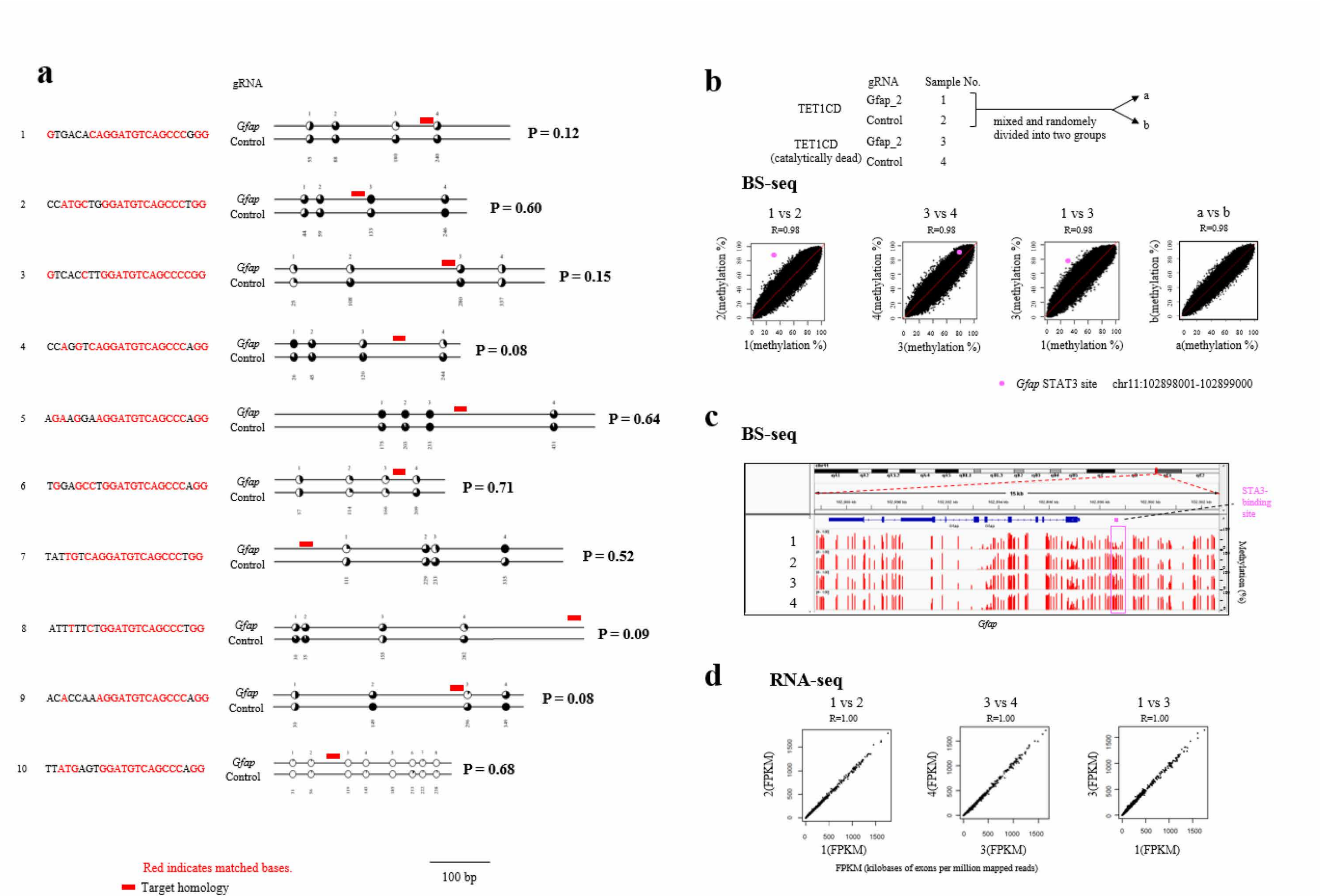
TET1CD (catalytically dead)



Supplementary Figure 8

Methylation surrounding the target site.

ESCs transfected with *Gfap* gRNA (Gfap_2) or a control using systems 2, 3 (same as Figure 1f), and 4 were sorted to isolate GFP-expressing cells, and methylation in the surrounding area was analyzed using bisulfite sequencing. Methylation for active and catalytically-dead TET1 is shown. Black/white circles indicate the percentage of methylation in each CpG site. Black indicates the methylation percentage. Each number beneath the circles indicates the position. The red bar indicates the STAT3-binding site. The blue bar indicates the target site. A scale is provided at the bottom. For each group, at least 14 randomly selected clones were sequenced and analyzed. The statistical significance between the two groups of the entire set of CpG sites was evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test).

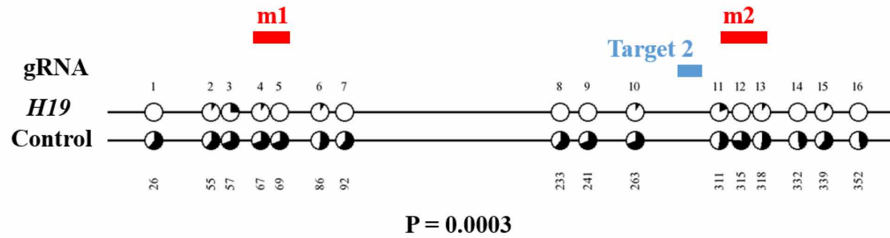


Supplementary Figure 9

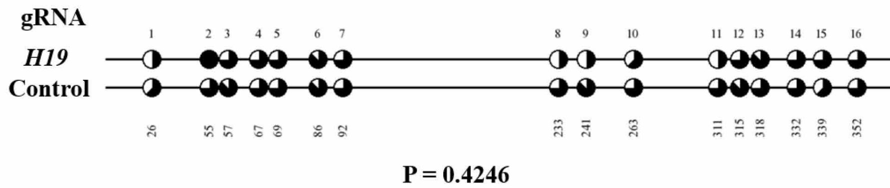
Off-target analysis and genome-wide analysis of DNA methylation and gene expression.

(a) Methylation surrounding the off-target sites of the Gfap_2 gRNA. ESCs were transfected with this gRNA or a control using system 3, sorted, and analyzed for methylation in the area surrounding off-target sites by bisulfite sequencing. Matched sequences are written in red and mismatched sequences are written in black. Red bars indicate the homologous sequence to the *Gfap* target sites. Black/white circles indicate the percentage of methylation in each CpG site. Black indicates the methylation percentage. Each number beneath the circles indicates the position. A scale is provided at the bottom. For each group, at least 14 randomly selected clones were sequenced and analyzed. The statistical significance between the two groups of the entire set of CpG sites was evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test). (b) BS-seq analysis of ESCs that were transfected with the all-in-one vector targeting *Gfap* (samples 1 and 3) or control (samples 2 and 4). Vectors with active (samples 1 and 2) or catalytically-dead (samples 3 and 4) TET1 were used. Sample 1 and 2 was mixed and randomly divided in half to generate samples a and b. Scatter plots of 1 vs. 2, 3 vs. 4, 1 vs. 3, and a vs. b are shown, along with the correlation coefficients. Each red line indicates the regression line of each sample. (c) BS-seq methylation landscape of the *Gfap* gene in samples 1–4. The STAT3-binding site and corresponding methylation are indicated by a pink bar and a pink square, respectively. (d) RNA-seq analysis of the same samples as in Supplementary Figure 9b. Scatter plots of 1 vs. 2, 3 vs. 4, and 1 vs. 3 are shown, along with the correlation coefficients.

TET1CD



TET1CD (catalytically dead)

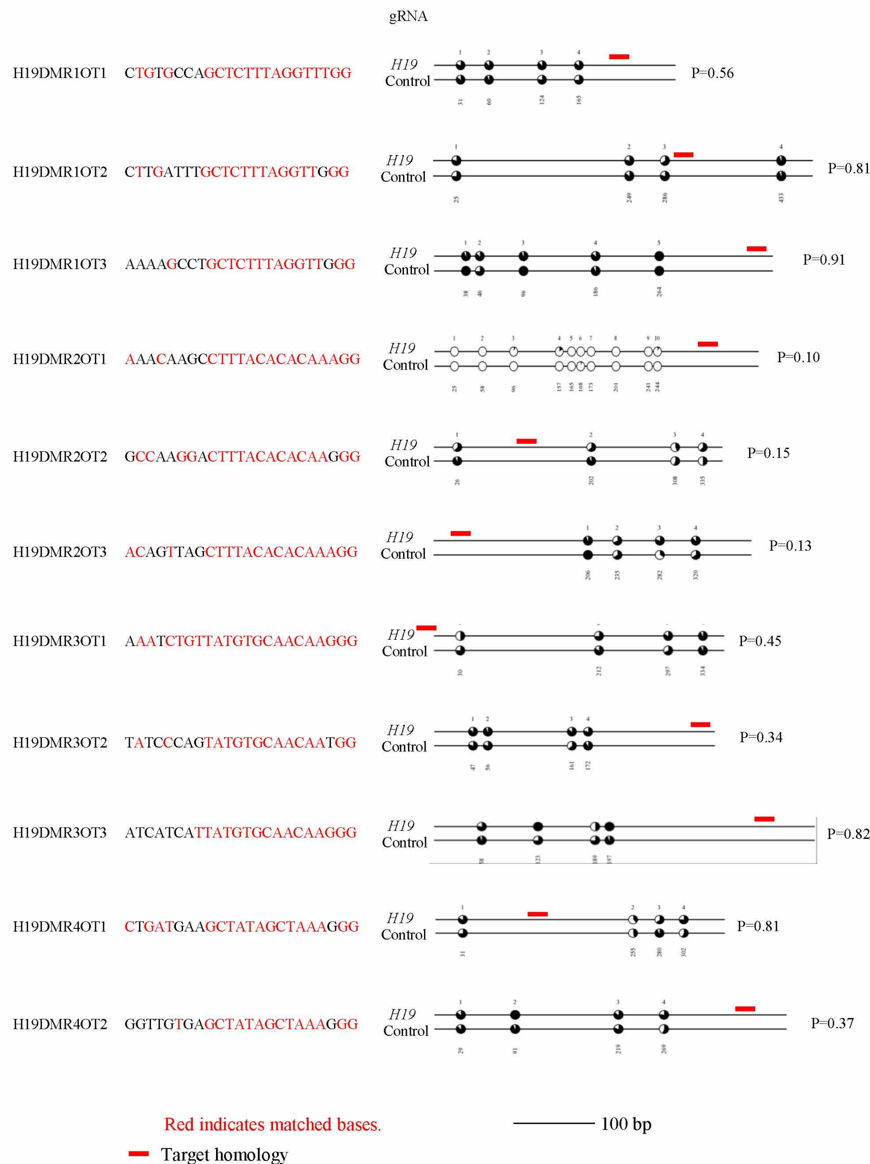


100bp

Supplementary Figure 10

Methylation surrounding the *H19* DMR CTCF-binding sites (m1 and m2).

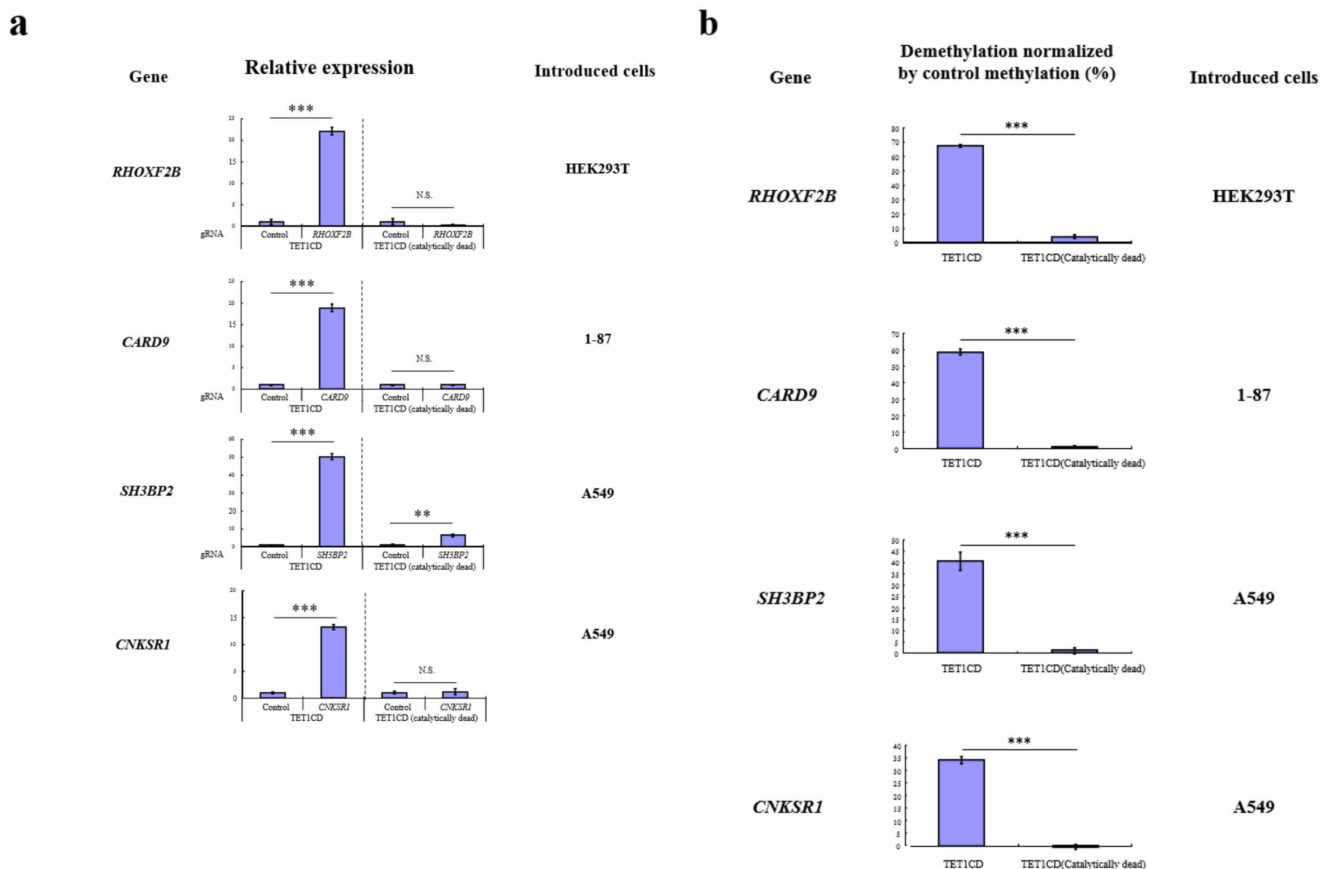
ESCs were transfected with H19 DMR_2 gRNA using system 3, sorted, and analyzed for methylation in the area surrounding target site 2 by bisulfite sequencing. Methylation for active and catalytically-dead TET1 is shown. Black/white circles indicate the percentage of methylation in each CpG site. Black indicates the methylation percentage. Each number beneath the circles indicates the position. Red bars indicate the CTCF-binding sites (m1 and m2). The blue bar indicates the position of target 2. A scale is provided at the bottom. For each group, at least 14 randomly selected clones were sequenced and analyzed. The statistical significance between the two groups of the entire set of CpG sites was evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test).



Supplementary Figure 11

Methylation surrounding the off-target sites of the H19DMR 1–4 gRNAs.

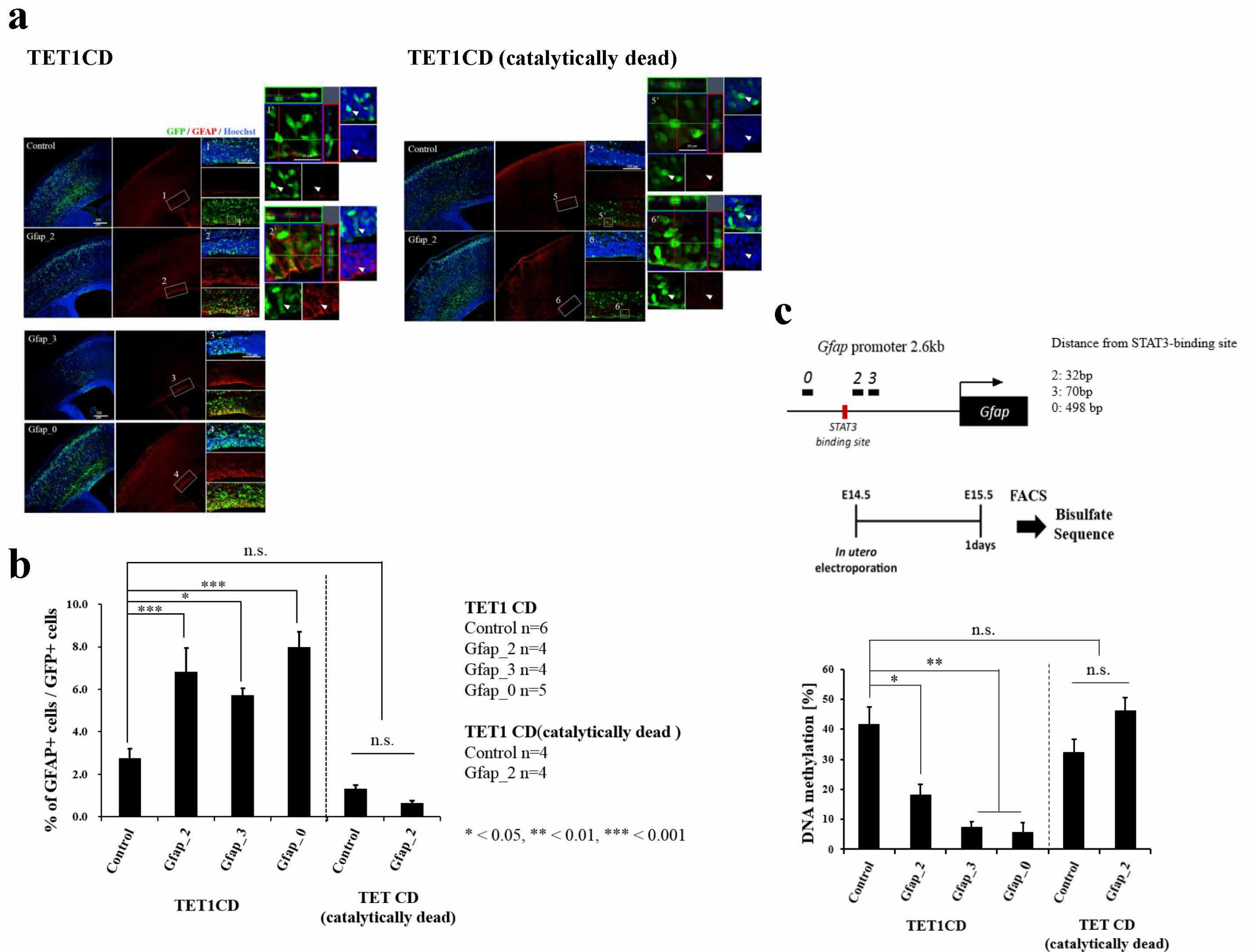
ESCs were transfected with these gRNAs using system 3, sorted, and analyzed for methylation in the area surrounding off-target sites by bisulfite sequencing. Matched sequences are written in red and mismatched sequences are written in black. Red bars indicate the homologous sequence to the *H19* target sites. Black/white circles indicate the percentage of methylation in each CpG site. Black indicates the methylation percentage. Each number beneath the circles indicates the position. A scale is provided at the bottom. For each group, at least 14 randomly selected clones were sequenced and analyzed. The statistical significance between the two groups of the entire set of CpG sites was evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test).



Supplementary Figure 12

Expression and methylation analysis of methylation-edited cells.

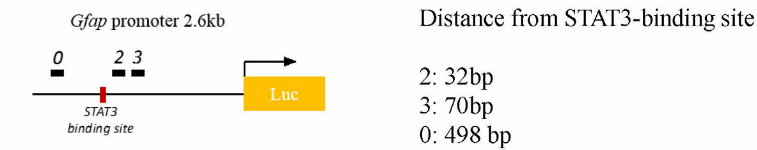
(a) Expression analysis of methylation-edited cells. Cells were transfected with gRNAs for *RHOXF2B*, *CARD9*, *SH3BP2*, or *CNKSR1* using system 3 with active or catalytically-dead TET1CD. The cells were sorted and analyzed for expression by quantitative PCR. Data are shown as the mean ± s.e.m. (n = 3 from two independent experiments). The two-sided Student's t-test was performed. N.S., not significant; $**P < 0.01$; $***P < 0.005$. (b) Demethylation of the *RHOXF2B*, *CARD9*, *SH3BP2*, and *CNKSR1* genes in human cells using system 3 with sorting. Demethylation activities for active and catalytically-dead TET1 are shown. Demethylation was analyzed as in Figure 1c. Data are shown as the mean ± s.e.m. (n = 3 from two independent experiments). The two-sided Student's t-test was performed. N.S., not significant; $***P < 0.005$.

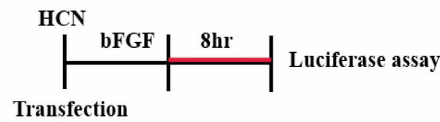


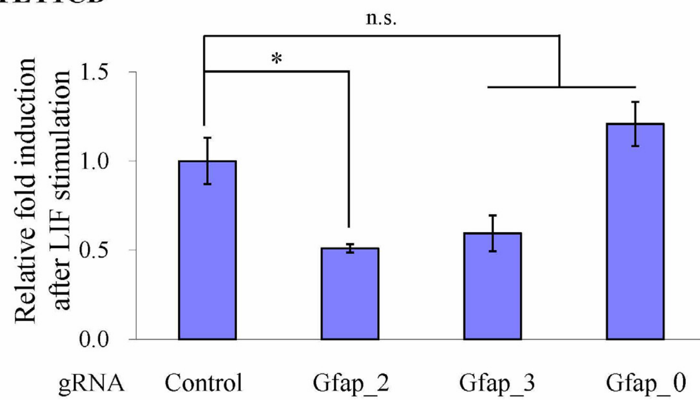
Supplementary Figure 13

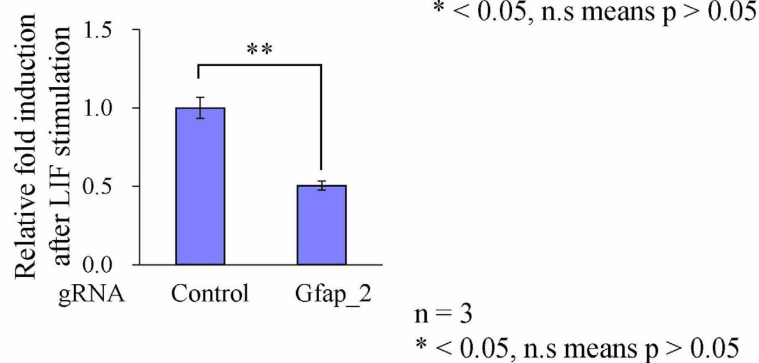
E18 brain sections that were electroporated with the vector targeting *Gfap* or the control vector at E14.

(a) E18 brain sections that were electroporated with the vector targeting *Gfap* or the control vector at E14. Brain sections obtained using catalytically-dead TET1 are also shown. Green, red, and blue indicate GFP, GFAP, and Hoechst, respectively. Magnified images of the boxed areas indicated are also shown in 1, 2, 1', and 2', respectively. The population of GFAP-positive cells among GFP-positive cells was significantly increased compared to the control. A scale bar is provided in the image. (b) The percentage of GFAP-positive cells among GFP-positive cells. The results obtained using catalytically-dead TET1 are also shown. Cortical sections at the same anatomical level were analyzed, and confocal images were taken with a confocal microscope. To assess astrocyte differentiation, at least 300 GFP-positive cells per sample (n=4–6 brains per group) were counted. GFAP-positive cells among GFP-positive cells were counted in high-magnification images, and each GFAP-positive cell was identified by GFAP staining around the nucleus, as indicated by both GFP and Hoechst. Data are shown as the mean ± s.e.m. Statistical analyses were performed using an ANOVA with Tukey's post-hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (c) DNA methylation of the *Gfap* locus in GFP-positive cells in fetal brains that were electroporated with the vector targeting *Gfap* (Gfap_2, Gfap_3, and Gfap_0) or the control vector. The results obtained with catalytically-dead TET1 are also shown. GFP-positive cells were electroporated with the vectors at E14. At 24 h after electroporation, GFP-positive cells were sorted by FACS and used for DNA methylation analysis. The average methylation of CpGs at the *Gfap* locus analyzed by bisulfite sequencing is presented. At least three samples were used for analysis. Data are shown as the mean ± s.e.m. Statistical analyses were performed using an ANOVA with Tukey's post-hoc test (*$p < 0.05$).









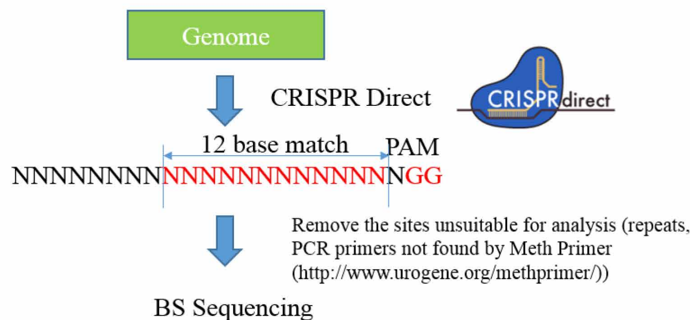
Supplementary Figure 14

Luciferase reporter assay of the *Gfap* promoter.

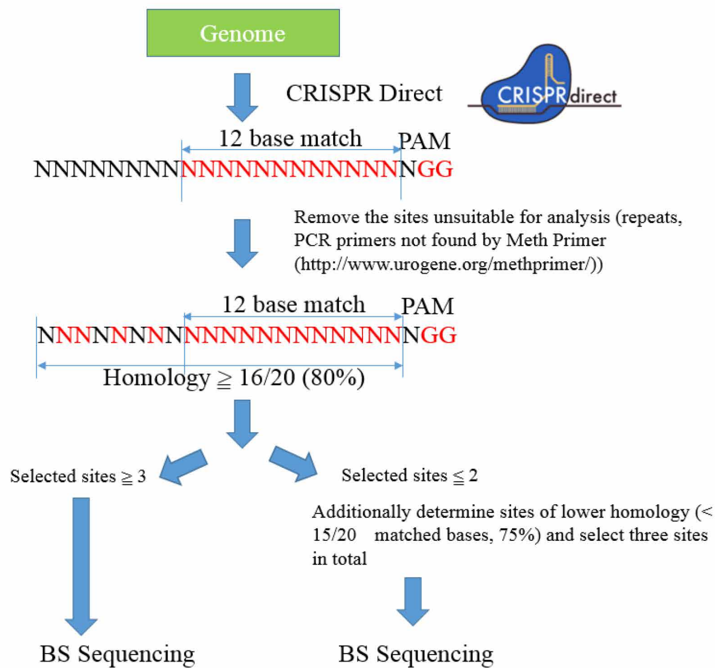
A neural progenitor cell line from adult rat hippocampus (HCN cells) was co-transfected with the all-in-one vector (expressing gRNA for the control or *Gfap* locus) and a *Gfap* promoter-reporter plasmid, which expresses firefly luciferase under the regulation of the 2.6 kb *Gfap* promoter. The reporter assay using catalytically-dead TET1 is also shown. As an internal control, the sea pansy luciferase-expressing vector under the control of the human elongation factor-1a promoter was also co-transfected. One day after transfection, cells were stimulated with LIF (50 ng/ml) for 8 h and used for luciferase analysis. Firefly luciferase activities were determined by three independent transfections and normalized by comparison with Renilla luciferase activities as the internal control. Data are shown as the mean ± s.e.m. Statistical analyses were performed using an ANOVA with Tukey's post-hoc test (*$p < 0.05$). ns, not significant ($p > 0.05$).

Flowchart of selection of off-targets for BS-Seq analysis

Gfap



H19



Supplementary Figure 15

A flowchart of the selection of off-target sites for bisulfite sequencing analysis.

Off-targets sites were searched for using a web tool called CRISPR direct (http://crispr.dbcls.jp/). By using this tool, the 12 bases in the 3' region of the target sequence adjacent to the PAM were searched against the genome because this region contains critical residues determining target specificity. Next, the sites unsuitable for analysis (sequences containing repeats and those giving no PCR primers by Meth Primer (http://www.urogene.org/methprimer/) in the default condition except for the product size) were removed. As for the off-target analysis of *Gfap*, all these sites were selected and subjected to off-target analysis. As for the off-targets of *H19*, at least all the off-targets in which more than 16 of 20 bases match were selected. If there were fewer than three selected sites, sites of lower homology were selected.

Target sequences

Target name	Target sequence	Methylation-sensitive site near the targets
Gfap_0	GTGAGACCACCTTCTGCCTCTGG	*Gfap* STAT3-binding site
Gfap_1	ATAGACATAATGGTCAGGGGTGG	*Gfap* STAT3-binding site
Gfap_2	GGATGCCAGGATGTCAGCCCCGG	*Gfap* STAT3-binding site
Gfap_3	ATATGGCAAGGGCAGCCCCGTGG	*Gfap* STAT3-binding site
H19DMR_1	GTGGGGGGCTCTTTAGGTTTGG	*H19* DMR CTCF-binding site 1
H19DMR_2	ACCCTGGTCTTTACACACAAAGG	*H19* DMR CTCF-binding site 2
H19DMR_3	GAAGCTGTTATGTGCAACAAGGG	*H19* DMR CTCF-binding site 3
H19DMR_4	CAGATTTGGCTATAGCTAAATGG	*H19* DMR CTCF-binding site 4
RHOXF2B	GCTTGGCCTTGCCCGGATGAGGG	RHOXF2B promoter
CARD9	TGGGAGCAGCTTTCCTCTGGAGG	CARD9 promoter
SH3BP2	TGAGGTCCTGAAAGCTGCCTGGG	SH3BP2 promoter
CNKSR1	TGTGAGCCCAGGTATGCAGTAGG	CNKSR1 promoter

PAM sequences are indicated in red.

Sequences of unrelated gRNAs

Target name	gRNA sequence
UR_1	CCATTATTGCATTAATCTGA
UR_2	TAATGCAGCCAGAAAATGAC
UR_3	TCAGGGATCAAATTCTGAGC

COBRA primer sequences

Primer name	Primer sequence	Restriction enzyme	Methylation-sensitive site near the targets
GfapSTAT3-B1	GTTGAAGATTTGGTAGTGTTGAGTT	Hpy188III	*Gfap* STAT3-binding site
GfapSTAT3-B2	TAAAACATATAACAAAAACAACCCC		
H19DMR-B1	AAGGAGATTATGTTTTATTTTTGGA	BstUI	*H19* DMR CTCF-binding site 1
H19DMR-B2	AAAAAAACTCAATCAATTACAATCC		
H19DMR-B1	AAGGAGATTATGTTTTATTTTTGGA	RsaI	*H19* DMR CTCF-binding site 2
H19DMR-B2	AAAAAAACTCAATCAATTACAATCC		
H19DMR-B3	GGGTTTTTTTGGTTATTGAATTTTAA	BstUI	*H19* DMR CTCF-binding site 3
H19DMR-B4	AATACACACATCTTACCACCCCTATA		
H19DMR-B5	TTTTTGGGTAGTTTTTTTAGTTTTG	BstUI	*H19* DMR CTCF-binding site 4
H19DMR-B6	ACACAAATACCTAATCCCTTTATTAAAC		
RHOXF2B-B1	GTTATAAAATGGGTTTGTATAATTTAGTAT	BstUI	RHOXF2B promoter
RHOXF2B-B2	AAAACCTCCTCTCTTACTTTTCTACTTC		
CARD9-B3	GGTTATTAGGGATTGTTTTTTTGTG	Aci I	CARD9 promoter
CARD9-B4	ATCTTCCAAAAACCACCTACACTAC		
SH3BP2-B1	TTATAGGGTAGAAGGTAGGAAGTGT	Aci I	SH3BP2 promoter
SH3BP2-B2	ATCTCCCAAACATATAAAACCTAAC		
CNKSR1-B1	TTTTTTTAGGTTTGGGTTTTGG	Taq I	CNKSR1 promoter
CNKSR1-B2	AATAACCCACCCACCTTAACTTC		

Bisulfite sequencing PCR primer sequences

Primer name	Primer sequence	Methylation-sensitive site near the targets
GfapSTAT3-B3	TTGGTTAGTTTTTAGGATTTTTTTT	*Gfap* STAT3-binding site (ES)
GfapSTAT3-B4	AAAACTTCAAACCCATCTATCTCTTC	
GFmS	GGGATTTATTAGGAGAATTTTAGTAAGTAG	*Gfap* STAT3-binding site (primary culture)
GFmAS	TCTACCCATACTTAAACTTCTAA TATCTAC	
H19DMR-B1	AAGGAGATTATGTTTTATTTTTGGA	*H19* DMR CTCF-binding site 1
H19DMR-B2	AAAAAAACTCAATCAATTACAATCC	
Gfap_O1B1	TTGTAAAGGTAGGATTAATAAGGGAATT	*Gfap* off-target site 1
Gfap_O1B2	AAAAAAAACCCTTCAAAAAAAATCTA	
Gfap_O2B1	TTATTATTTATATTTGGAGGGAGGG	*Gfap* off-target site 2
Gfap_O2B2	ATTACACCAAAAAAATTTTAAAAAC	
Gfap_O3B1	TTTAAATTTTTTTATGTGAATATGG	*Gfap* off-target site 3
Gfap_O3B2	AAACATTTAATTCATTAATACACAC	
Gfap_O4B1	TTTTAAGTTTTTAGGATGAGAAAGA	*Gfap* off-target site 4
Gfap_O4B2	AAAATTATTTCCAATAAACTACCCC	
Gfap_O5B1	ATTATTTTTGTGGATTGTTTTAGGG	*Gfap* off-target site 5
Gfap_O5B2	AACCACCAAAAATTACATAAACTCC	
Gfap_O6B1	TGGGAGAAGTTTTTAGGAGTATGAG	*Gfap* off-target site 6
Gfap_O6B2	ACAAATAAAAAAACCACAAAAAAACA	
Gfap_O7B1	TTAGTTTGGAATTTTGAGGTTAGTAGTTT	*Gfap* off-target site 7
Gfap_O7B2	AAACATCTTACAATACAATAAACATTTACA	
Gfap_O8B1	TGTTTATTTTAAGGTAATAAAGATTTAGT	*Gfap* off-target site 8
Gfap_O8B2	ATAAAATTATCAAATCTCCATATATTACTT	
Gfap_O9B1	TGTTGTTGTAAGTTAGGGGTAGGTT	*Gfap* off-target site 9
Gfap_O9B2	ATTTTCCCACCACACTAAAAATTAC	
Gfap_O10B1	TTGTATTTTTTTAGGGTTGTTTTTTAATT	*Gfap* off-target site 10
Gfap_O10B2	CACACATACCAAAATATACCAATCAC	
H19DMR1OT1-B1	GAGGGTATTGTATATTTGAAGGAGTTT	H19DMR1 off-target site 1
H19DMR1OT1-B2	AATTCTAAAAAACAACAAACTTATCTCACT	
H19DMR1OT2-B1	TTTTTTTTGTTAAAGGTAAGGAAA	H19DMR1 off-target site 2
H19DMR1OT2-B2	CAACAAAACCATCATAACCTACAAA	
H19DMR1OT3-B1	TGTTATTTTTGAGTTTTAATAGTTTTAGAA	H19DMR1 off-target site 3
H19DMR1OT3-B2	ATAAACCCCAACCTAAAAAACAAAC	
H19DMR2OT1-B1	TTTTTTTATTATGTGGTTTAAGTT	H19DMR2 off-target site 1
H19DMR2OT1-B2	ATACAAATTCAAAAAACATTTC	
H19DMR2OT2-B1	GATGTTATTGTTTGTTTTTTAAGAT	H19DMR2 off-target site 2
H19DMR2OT2-B2	TTTCCAAAATTAATTTAAACCTC	
H19DMR2OT3-B1	TTAATAGATTATTTTTTGAATTAAAT	H19DMR2 off-target site 3
H19DMR2OT3-B2	CTAATCACTATACTATATCTCACTAAAATT	
H19DMR3OT1-B1	GGATATTATTATTAATGTTTTAAGTATAA	H19DMR3 off-target site 1
H19DMR3OT1-B2	CATTATATTACTTATCTATTTCCCC	
H19DMR3OT2-B1	TATTAAAGTTTAGTTGGTTTTTTTT	H19DMR3 off-target site 2
H19DMR3OT2-B2	ACACACCATTATTACACATACTAAA	
H19DMR3OT3-B1	TTTGTTTTAGGAATTTTTGTGAAAAAT	H19DMR3 off-target site 3
H19DMR3OT3-B2	TAATACCAAACAACCAAAATATCCC	
H19DMR4OT1-B1	AAATTAGTTTTATTTTGTTTTAAGTTT	H19DMR3 off-target site 1
H19DMR4OT1-B2	TTATATTAAAATTCAATACTCTTAACAATT	
H19DMR4OT2-B1	ATAGGGTTTGGGATGAAATATTATG	H19DMR3 off-target site 2
H19DMR4OT2-B2	ATCTCATTACTACTCACACATCAAAA	

qPCR primer sequences

Primer name	Primer sequence	Analysis
GfapEx1S-1	GGAGAGGGACAACTTTGCAC	*Gfap* expression analysis
GfapEx2AS-1	ATACGCAGCCAGGTTGTTCT	
RHOXF2B-3	GGCAAGAAGCATGAATGTGA	RHOXF2B expression analysis
RHOXF2B-4	TGTCTCCTCCATTTGGCTCT	
H19Ex4,5-S1	TACCTGCCTCAGGAATCTGC	H19 expression analysis
H19Ex4,5-AS1	GTTGGCCATGAAGATGGATT	
Mus18S-S1	CCCGAAGCGTTTACTTTGAA	Normalization for expression in ESCs
Mus18S-AS1	CCCTCTTAATCATGGCCTCA	
ACTB sense	GATGCAGAAGGAGATCACTGC	Normalization for expression in HEK293
ACTB antisense	GTACTTGCGCTCAGGAGGAG	
CARD9-1	CAGGCTCCTGGTGTGTCTG	CARD9 expression analysis
CARD9-2	CTCCAGCACTCGTCATCGT	
SH3BP2-1	ATGTGTTGGGTCAGCACCA	SH3BP2 expression analysis
SH3BP2-2	CAGGCATGGTTAGCAGGTTC	
CNKSR1-1	GGCAAAACAGGAGCTGATTC	CNKSR1 expression analysis
CNKSR1-2	TAGTCCTGCAGGGAGTCGTC	

Nature Biotechnology: doi:10.1038/nbt.3658

Gfap off-targets
Total number: 20
Analyzable site for BS-seq

Name	chr	start	end	Target homology sequence (red indicates matched sequence)	Matched bases	Homology (%)
1	3	150428404	150428426	GTGACACAGGATGTCAGCCCGGG	15	75
2	4	140805328	140805350	CCATGCTGGGATGTCAGCCCTGG	16	80
3	6	119524707	119524729	GTCACCTTGGATGTCAGCCCCGG	14	70
4	7	115312494	115312516	CCAGGTCAGGATGTCAGCCCAGG	16	80
5	9	28791399	28791421	AGAAGGAAGGATGTCAGCCCAGG	16	80
6	12	110252461	110252483	TGGAGCCTGGATGTCAGCCCAGG	16	80
7	15	46277591	46277613	TATTGTCAGGATGTCAGCCCTGG	16	80
8	10	108920123	108920145	ATTTTTCTGGATGTCAGCCCTGG	14	70
9	14	75098476	75098498	ACACCAAAGGATGTCAGCCCAGG	14	70
10	14	118137632	118137654	TTATGAGTGGATGTCAGCCCAGG	15	75

Nature Biotechnology: doi:10.1038/nbt.3658

H19DMR1 off-targets
Total number: 10
Selected for BS-seq

Name	chr	start	end	Target homology sequence (red indicates matched sequence)	Matched bases	Homology (%)
H19DMR1OT1	chr3	144227273	144227295	CTGTGCCAGCTCTTTAGGTTTGG	15	75
H19DMR1OT2	chr12	117985145	117985167	CTTGATTTGCTCTTTAGGTTGGG	14	70
H19DMR1OT3	chr2	78025434	78025456	AAAAGCCTGCTCTTTAGGTTGGG	13	65

H19DMR2 off-targets
Total number: 23
Selected for BS-seq

Name	chr	start	end	Target homology sequence (red indicates matched sequence)	Matched bases	Homology (%)
H19DMR2OT1	chr2	59697881	59697903	AAACAAGCCTTTACACACAAAGG	15	75
H19DMR2OT2	chr1	119309953	119309975	GCCAAGGACTTTACACACAAGGG	16	80
H19DMR2OT3	chr3	103987311	103987333	ACAGTTAGCTTTACACACAAAGG	15	75

H19DMR3 off-targets
Total number: 19
Selected for BS-seq

Name	chr	start	end	Target homology sequence (red indicates matched sequence)	Matched bases	Homology (%)
H19DMR3OT1	chr10	116061270	116061292	AAATCTGTTATGTGCAACAAGGG	18	90
H19DMR3OT2	chr1	38175983	38176005	TATCCCAGTATGTGCAACAATGG	14	70
H19DMR3OT3	chr17	55592675	55592697	ATCATCATTATGTGCAACAAGGG	13	65

H19DMR4 off-targets
Total number: 15
Selected for BS-seq		No primers were found suitable to be designed on other off-targets.

Name	chr	start	end	Target homology sequence (red indicates matched sequence)	Matched bases	Homology (%)
H19DMR4OT1	chr10	28388331	28388353	CTGATGAAGCTATAGCTAAAGGG	16	80
H19DMR4OT2	chr4	101710864	101710886	GGTTGTGAGCTATAGCTAAAGGG	13	65

Nature Biotechnology: doi:10.1038/nbt.3658