Supplementary Information

Characterization of a core region in the A2UCOE that confers effective anti-silencing activity

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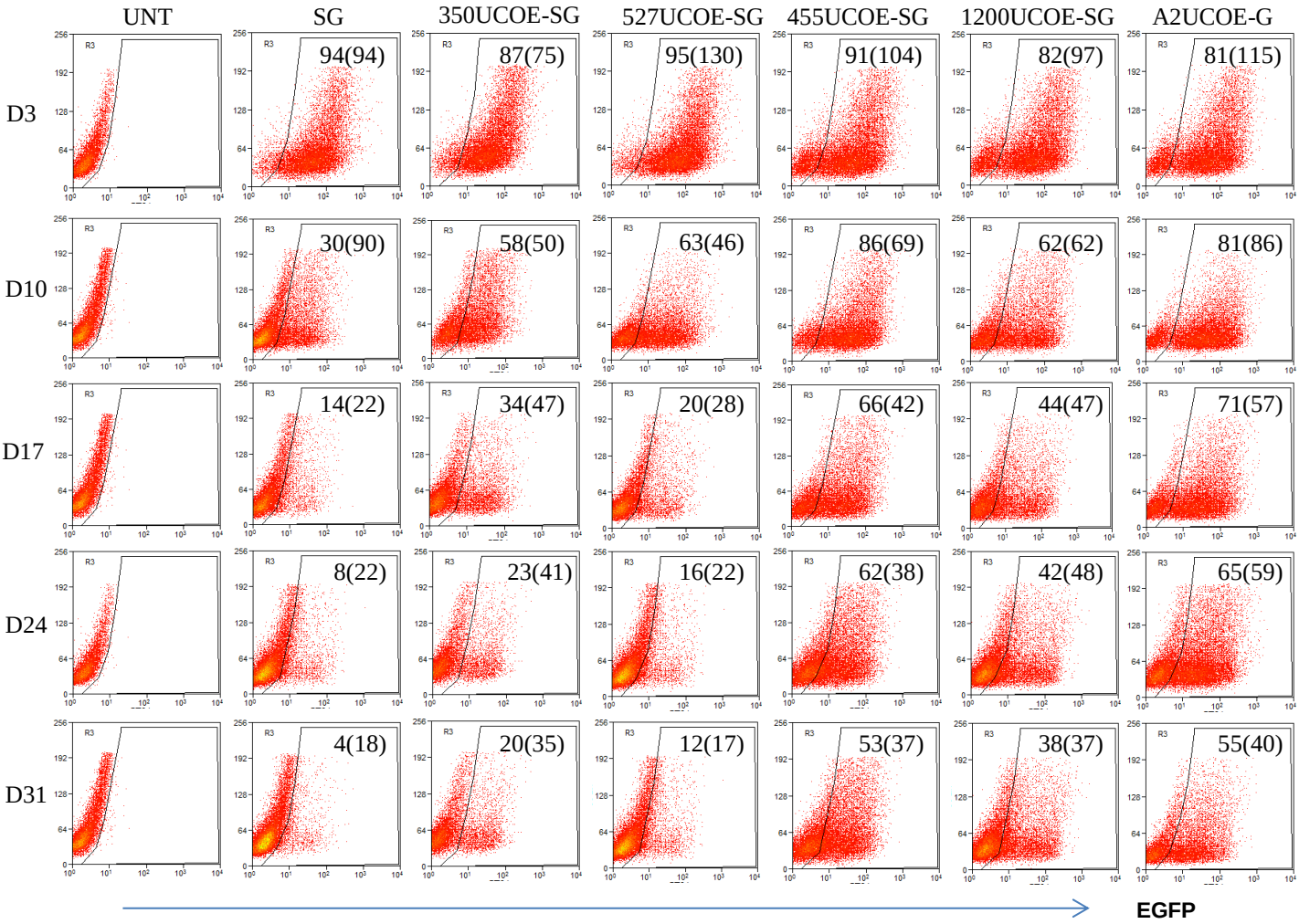
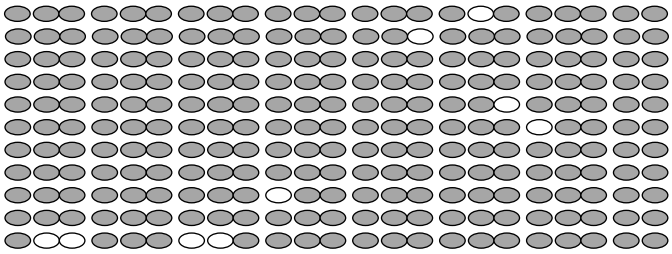
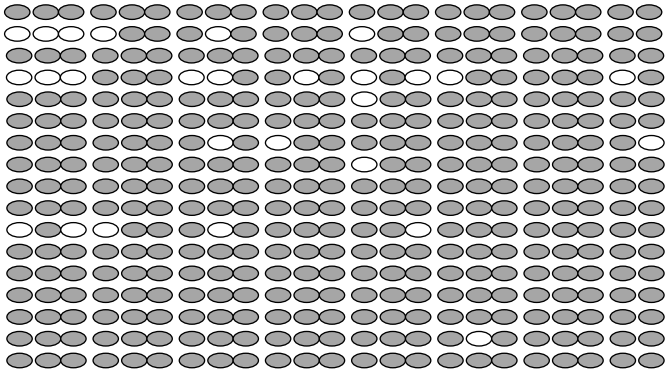


Supplementary Figure S1: The flow cytometry analysis of EGFP expression in P19 cells following the viral transduction with the indicated vectors at different time points. The numbers in each plots represent the percentage of EGFP cells and the mean fluorescence intensity (in brackets). UNT: untransduced

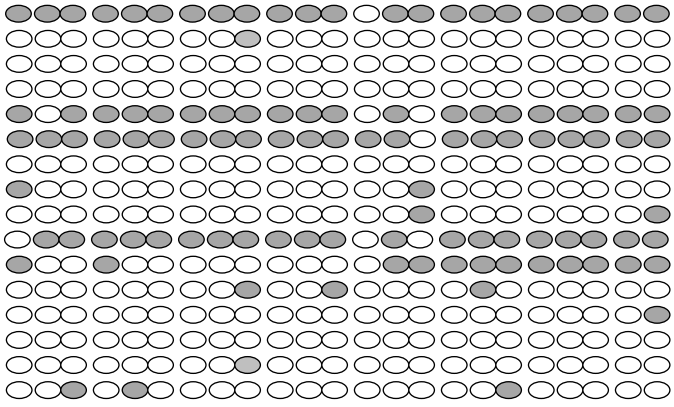
SG



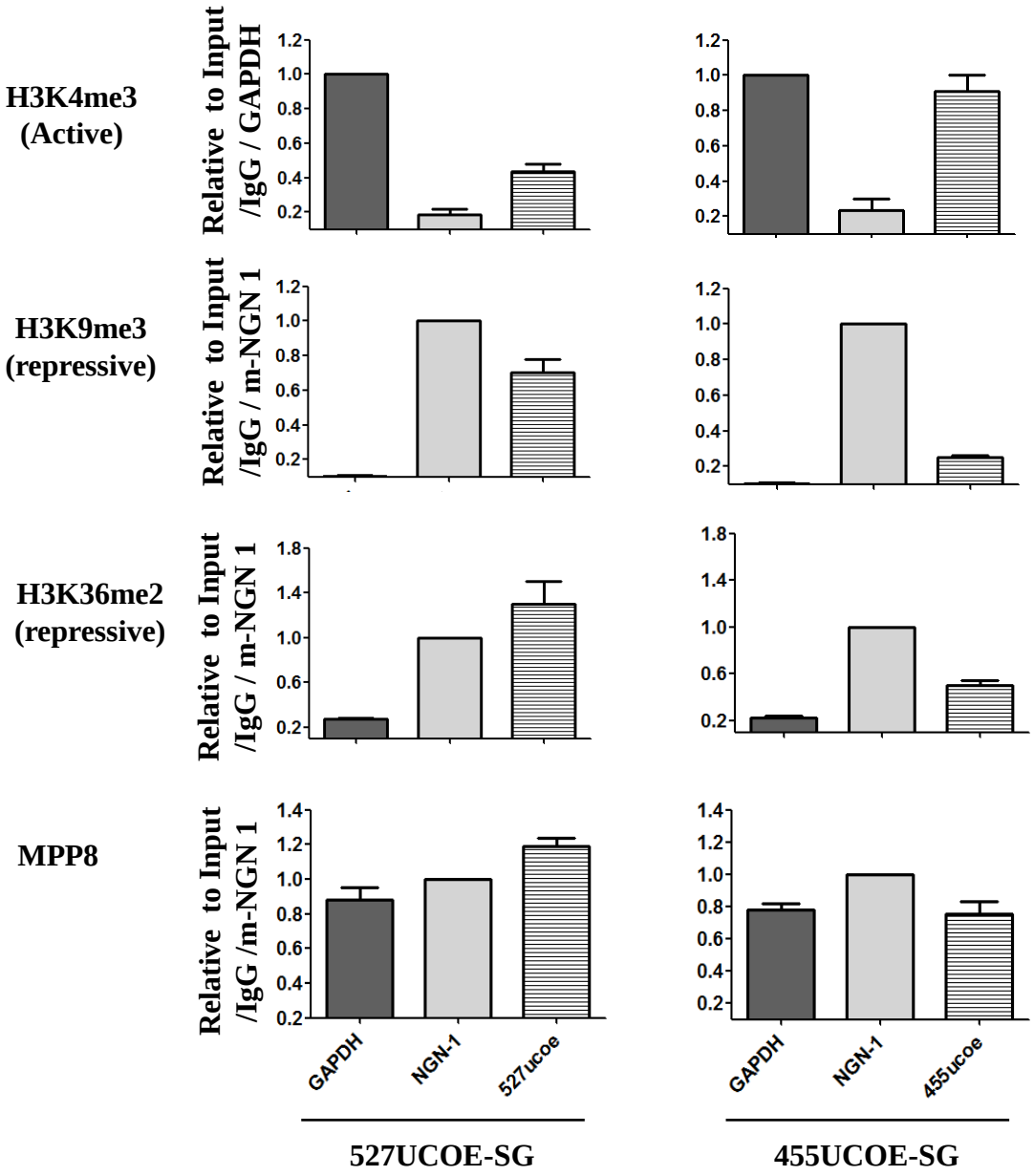
527UCOE-SG



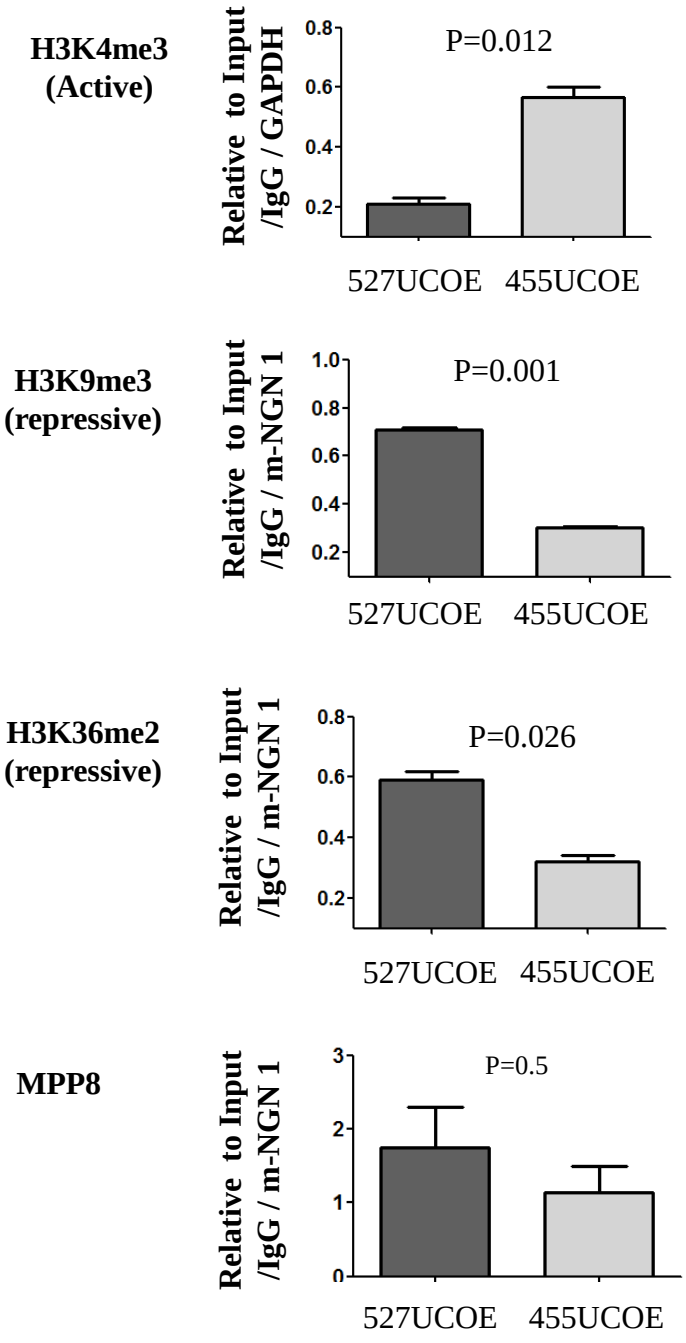
455UCOE-SG



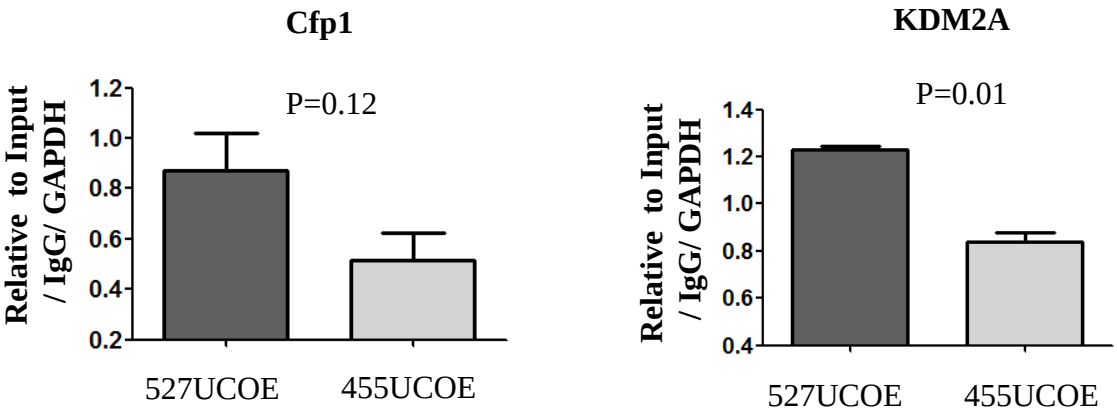
Supplementary Figure S2: **The CpG methylation on the SFFV LTR region in P19 cells following the lentiviral transduction.** Genomic DNA from cells transduced with lentiviruses at day 17 was isolated and subjected to methylation analysis by bisulfite conversion and sequencing. Methylation status of randomly selected PCR colonies is shown. White boxes: unmethylated CpG sites. Black boxes: methylated CpG sites. Upper panel: SFFV-EGFP. Middle panel:: 527UCOE-SG. Lower panel: 455UCOE-SG.



Supplementary Figure S3: **The 455UCOE region remains as an active chromatin domain compared to the 527UCOE region in murine P19 cells**. The ChIP assay was performed in P19 cells at day 10 post transduction. The value of enrichment for each antibody is determined relative to the input, IgG and normalized to the actively transcribed GAPDH or to the repressive mouse NGN1 locus. The data represents 2-3 repeated ChIP assays.



Supplementary Figure S4: **The 455UCOE region remains as an active chromatin domain compared to the 527UCOE region in murine P19 cells.** Enrichment of active and repressive histone marks and the MPP8 on the 455UCOE and the 527UCOE region was assessed by ChIP assay in P19 cells at day 17 post transduction. The value of enrichment for each antibodies is determined relative to the input, IgG and then normalized to the actively transcribed GAPDH or to the repressive mouse NGN1 locus. The normalized values of the enrichment at the 527UCOE, and the 455UCOE are shown. The data represents 2-3 independent ChIP assays. *P* values are calculated by Student's *t*-test.



Supplementary Figure S5: Enrichment of CpG binding proteins Cfp1, KDM2A on the 455UCOE and the 527UCOE regions. The ChIP assay was performed in P19 cells at day 10 post transduction. The value of enrichment for each antibody is determined relative to the input, IgG and then normalized to the actively transcribed GAPDH. The normalized values of the enrichment at the 527UCOE, and the 455UCOE are shown . The data represents three independent ChIP assay. *P* values are calculated by Student's *t*-test.

Supplementary Table S1: Primers used in DNA methylation, Q-PCR and ChIP analysis.

Methylation	**Forward 5'-3'**	**Reverse 5'-3'**
Me-SFFV 1_{st} PCR	TAGAAAAAGGGGGGAATGAAA	AAACAACTCCTCACCCTTACTCA C
Me-SFFV 2_{nd} PCR	GGGGGAATGAAAGATTTTATT TG	ACCCTTACTCACCATAATTTC AA CC
Chimeric	GGAGGAGGGAGTATAGTAGTA	CAAACCAACTCAAACCCAATAC
Q-PCR		
Lenti-PSI	CAGGACTCG GCTTGCTGAAG	TCCCCCGCTTAATACTGACG
	Probe: FAM-CGCACGGCA AGA GGCGAGG TAMRA	
Mouse Titin	AAAACGAGCAGTGACCTGAGG	TTCAGTCATGCTGCTAGCGC
	Probe: FAM TGCACGGAA TCTC GTCTCAGTC TAMRA	
Human Albumin	GCTGCTATCTCTTGTGGGCTGT	ACTCATGGGAGCTGCTGGTTC
	Probe: VIC-CCT GTC ATG CCC ACA CAA ATC TCT CC TAMRA	
ChIP		
527ucoe	GAAATGCGCTTTGTCTCGAA	CCC CCC TTT TTC TGG AGA CTA*
455ucoe	AGTGACCGGAGTCTCCTC A	CCC CCC TTT TTC TGG AGA CTA*
SFFV	AATCAGCCTGCTTCTCGCTTCT	TGA ACA GCT CCT CGC CCT T
h-GAPDH	GCTACTAGCGGTTTTACGGGCG	TGC GGC TGA CTG TCG AAC AGG
h-Chrom.18	ACTCCCCTTTCATGCTTCTG	AGGTCCCAGGACATATCCATT
m-GAPDH	TCC CCT CCC CCT ATC AGT TC	GAC CCG CCT CAT TTT TGA AA
m-NGN-1	TCCGTTTCCTGCGTTTCAA	TGCTCTGGGCTGGCTGTC

***** See the details in the materials and methods.