

Figure S1. Caspase-3/7 activation by 5AZA and decitabine. Fluorescence microscopy (IncuCyte S3) showing caspase-3/7 activation (green cells) in both cell lines treated with either 5AZA or decitabine after 24 h of incubation (n=3). 5AZA, 5'-aza-cytidine; decitabine, 5'-aza-2-deoxycytidine; NT, not treated.

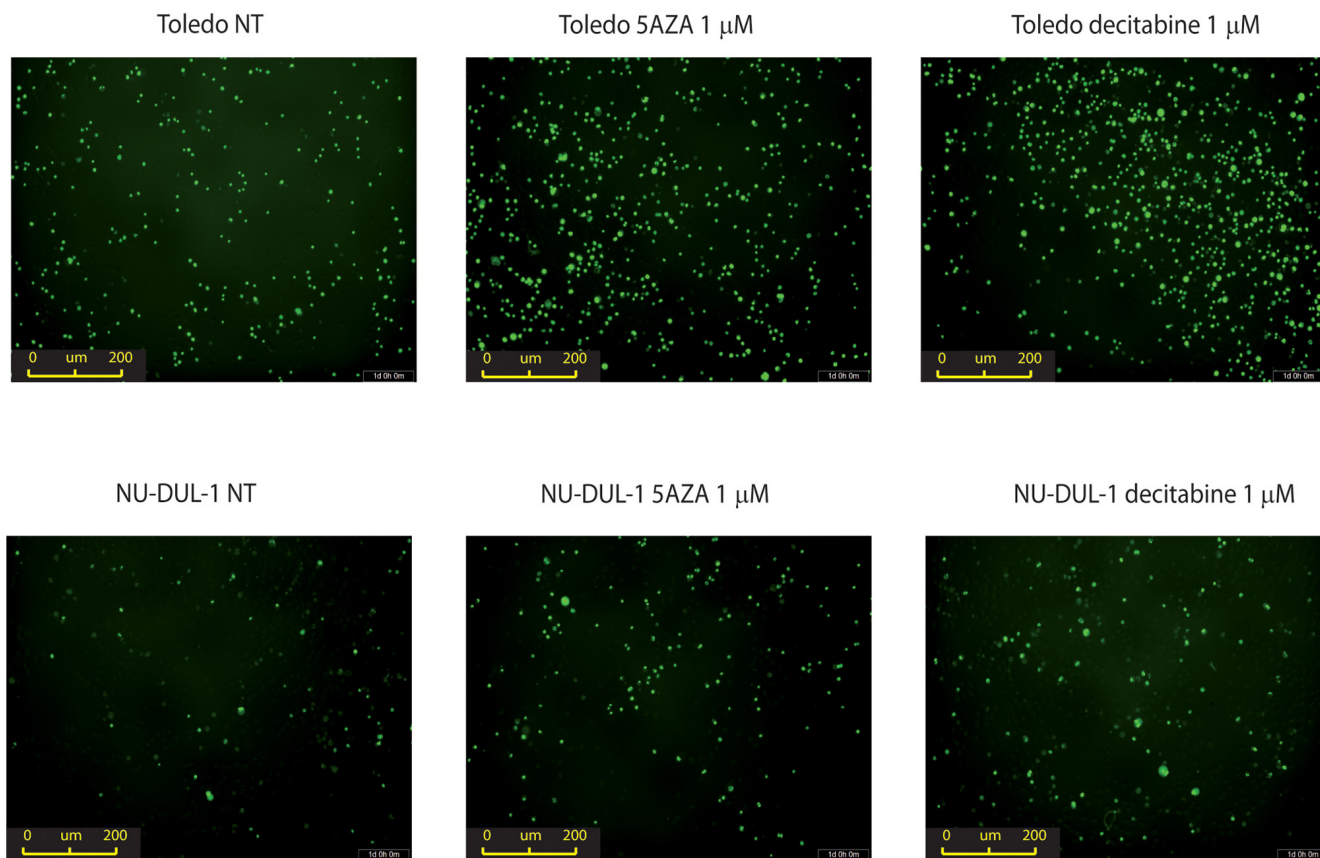


Figure S2. Analysis of the  $\beta$ -values for *SPG20* locus determined by HumanMethylation 27 BeadChip. Data available on 'ArrayExpress', reference 14. The heatmap represents data mining for HCT116 cells either untreated or treated with 5AZA or decitabine.  $\beta$ -values are comprised in the range of 0.7-0.96. 5AZA, 5'-aza-cytidine; decitabine, 5'-aza-2-deoxycytidine; SPG20, spastic paraplegia 20.

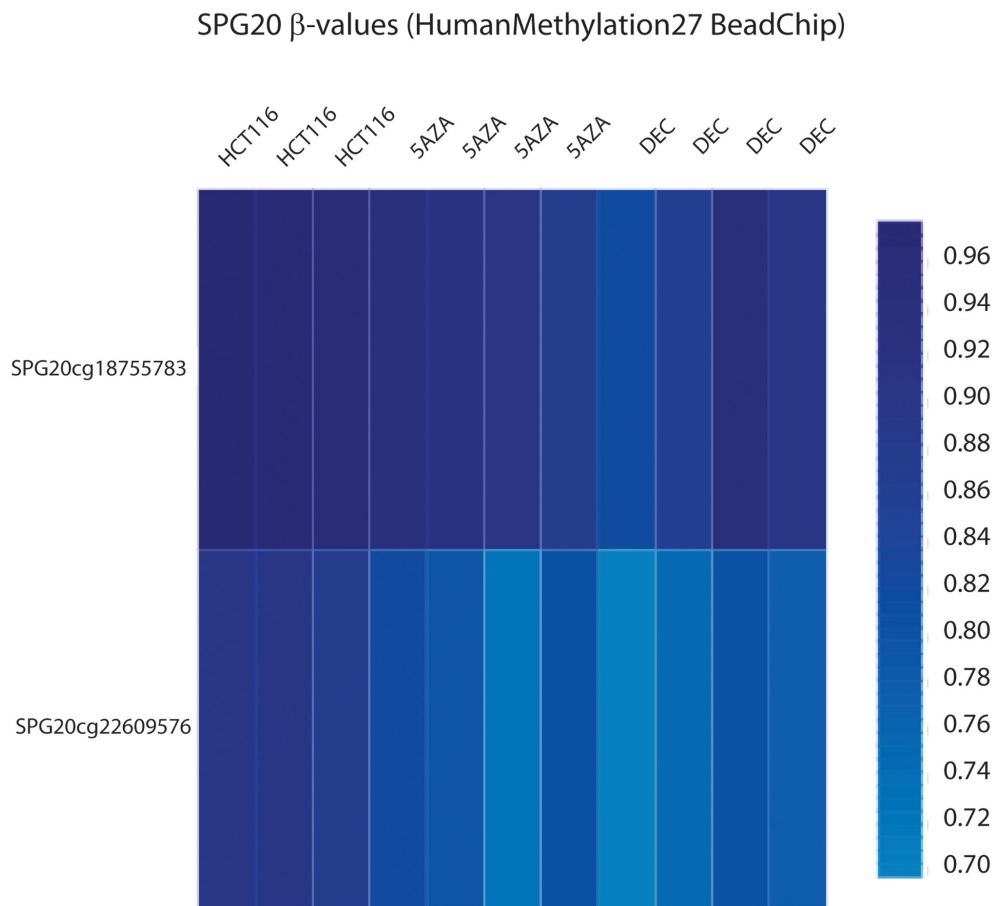


Table SI. Primer sequences for MS-PCR, RT-qPCR and silencing experiments.

A, MS-PCR primers	
Target	Sequences
cg15754752	CTGGCAAAGGCAGCCTTGCAGCTGGGGCCAGGGTCTGTGGTGC TGATCCATCTGCTGGGC[CG]TCACCTCCCGAGAGAGCGTGTGTT CCTCCAACCACGCGTGTCTTAGCACCAGTCTCTGGC
M-specific primers (80 bp)	F: 5'-TTGATTTATTTGTTGGGTC-3'
	R: 5'-ACTAATACTAAAACACGCG-3'
U-specific primers (80 bp)	F: 5'-TTGATTTATTTGTTGGGTT-3'
	R: 5'-ACTAATACTAAAACACACA-3'
B, RT-qPCR primers	
Target	Sequences
DNMT1	F: 5'-ATGGGCTATCAGTGCACCTT-3'
	R: 5'-CAGAGGGAGCTTCTCTCCAG-3'
DNMT3a	F: 5'-AAGGAGGAGCGCCAAGAG-3'
	R: 5'-GGATGGGGACTTGGAGATCA-3'
KLF4	F: 5'-GTTCCCATCTCAAGGCACAC-3'
	R: 5'-CCCCGTGTGTTTACGGTAGT-3'
DAPK1	F: 5'-GCAGGAAAACGTGGATGATT-3'
	R: 5'-CTCACGGCATTCTTCACAA-3'
SPG20	F: 5'-TCCTGGGTACCTTCGAATTG-3'
	R: 5'-TAGGCTCCCGCAGTACATT-3'
RPS7	F: 5'-TTCTGCCTAAGCCAACCTCGAAAA-3'
	R: 5'-CGGATTCTCTTGCCCAACAATTTC-3'
C, siRNA	
Target	Sequences
DNMT1	Sense: 5'-GGUGUGCAUUGAUGCGGAAtt-3'
	Antisense: 5'-UUCCGCAUCAAUUGCACACctt-3'
DNMT1	Sense: 5'-GGAUGAGAAGAGACGUAGAtt-3'

	Antisense: 5'-UCUACGUCUCUUCUCAUCCTg-3'
DNMT3a	Sense: 5'-CCUCAGAGCUAUAACCCAAAtt-3'
	Antisense: 5'-UUGGGUAAUAGCUCUGAGGcg-3'

F, forward; R, reverse; MS-PCR, methylation-specific PCR; RT-qPCR, reverse transcription-quantitative PCR; M, methylated; U, unmethylated; DNMT, DNA methyltransferase; KLF4, Krüppel-like factor 4; DAPK1, death associated protein 1; SPG20, spastic paraplegia 20; RPS7, ribosomal protein S7; siRNA, small interfering RNA.

Table SII. Amplification conditions for MS-PCR and RT-qPCR.

A, MS-PCR conditions		
Step	Temperature, °C	Time
1	95	5 min
2 <sup>a</sup>	94	30 sec
3	51	1 min
4	72	1 min
5	72	5 min
6	4	30 min
B, qPCR conditions		
Step	Temperature, °C	Time
1	98	2 min
2 <sup>b</sup>	98	2 sec
3	56	5 sec
4	70-95	melt curve, 0.5°C

<sup>a</sup>Steps 2-4 were repeated 34 times; <sup>b</sup>steps 2-3 were repeated 39 times. MS-PCR, methylation-specific PCR; qPCR, quantitative PCR.

Table SIII. CpG island positions of the EpiTect Methyl II qPCR used for quantitative methylation.

PCR Primer Assay Kit	CpG Island	Chr position
PCR Primer Assay for Human KLF4	114531	Chr9:110249748-110252660
PCR Primer Assay for Human DAPK1	114427	Chr9:90112514-90113817
PCR Primer Assay for Human SPG20	103708	Chr13:36919737-36921004

Assays were designed by Qiagen, Inc., on NCBI Homo sapiens Build Number:37 version 2. Chr, chromosome.

Table SIV. Cq values of DNMT3a expression as determined by reverse transcription-quantitative PCR.

A, NU-DUL-1 cells				
Mock	Scramble siRNA	siDNMT1	siDNMT3a	siDNMT1 + siDNMT3a
34.36	34.64	34.77	35.16	34.72
34.61	34.67	34.57	35.02	34.47
34.54	34.91	35.01	35.43	34.58
33.47	34.19	35.06	35.08	32.58
33.54	34.07	34.95	34.82	32.71
33.92	34.45	35.12	34.52	32.95
	33.30	35.57	34.89	36.19
	33.35		35.13	36.14
	33.55		34.95	35.99
B, Toledo cells				
Mock	Scramble siRNA	siDNMT1	siDNMT3a	siDNMT1 + siDNMT3a
36.85	34.91	35.08	36.79	35.37
36.54	34.93	35.38	35.81	35.48
36.09	35.50	35.29	36.21	35.55
34.53	35.01	35.00	35.72	35.23
34.38	34.76	35.00	35.59	35.14
34.02	35.24	34.93	36.11	35.45
	33.78	33.65	35.18	34.12
	33.49	33.47	34.32	34.34
	33.57	33.67	34.93	34.73

The results of the experiments after 48 h of incubation from transfection are shown. DNMT, DNA methyltransferase; si/siRNA, small interfering RNA.