Supplementary Material

Supplementary Figures

Figure S1

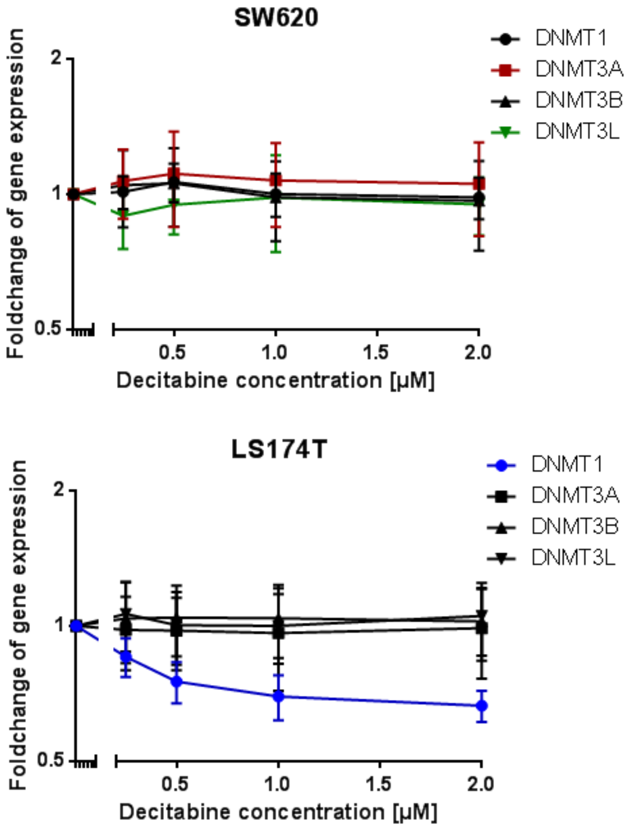

Figure S1: Modulation of DNA methyltransferases (*DNMT1*, *DNMT2*, *DNMT3a*, and *DNMT3b*) after exposure to three daily doses of incrementing decitabine concentrations (0.5, 1.0, 1.5, 2.0 µM) in two human (SW620 and LS174T) colorectal cancer cell lines as observed by microarray analysis.
a) Fold-change of gene expression of endothelin system members in SW620 cells.
b) Fold-change of gene expression of endothelin system members in LS174T cells.
The values represent the fold change in gene expression as determined by micro-array in colorectal cancer cells that were exposed to decitabine. The x-axis gives the concentration of decitabine (µM). The y-axis gives the mRNA expression of the studied gene relative to that level found in control cells.

Supplementary Materials and Methods

Synthesis of complementary DNA (cDNA)

A reaction mixture was used to synthesize cDNA from isolated RNA. 1000-1500 ng of sample total RNA was mixed with RNase free water, initial heating at 65 °C was done for 5min. Afterwards the following reagents were added per sample: revertAid reverse transcriptase enzyme (2 µl), ribolock RNase inhibitor (1µl), oligo(dt) primer 5´-d(TTTT)-3` (2 µl), dNTP Mix (5 mM each dNTP) (4 µl), 5× buffer (8 µl) and RNAse free water to yield a total volume of 40 µl/sample. Then the mixture was incubated at 42 °C for 60min, followed by a cooling step at 4 °C forever. All reaction materials were supplied from Thermo Scientific.

DNA band expression by agarose gel

2 µl of cDNA samples were amplified using a Mastercycler® nexus (Eppendorf) by using red taq DNA polymerase (Genaxxon), then the DNA products were separated by electrophoresis in a 2% agarose gel

2 µl cDNA was mixed with a mixture of magnesium chloride ($MgCl_2$) (2 µl, MBI fermentas), dNTPs (1 µl, Thermoscientic), buffer 10× (5µl, Genaxxon), red taq DNA polymerase (0.3 µl, Genaxxon), the selected primer pairs right and left (1µl each), and the remaining amount is double-distilled water (ddH2O) to form total volume of 50µl/sample. Then cDNA was amplified by using the program in table 6 in a Mastercycler® nexus (Eppendorf). Primers used are presented in suppl Table 1. Then the products were analyzed by electrophoresis in a 2% agarose gel.

2% agarose gels were prepared as follows:1X tris-acetate-EDTA (TAE) with 2% agarose were heated at 600 W, then Gel Stain was added (3 µL) after cooling, and the mixture were left to solidify & cast. Samples were run in an electrophoresis chamber at 150 V for 45 min. After electrophoresis, the gel was visualized under ultraviolet light (Gel Doc XR, Bio-Rad, Munich, Germany).

Table S1: PCR program used in a Mastercycler® nexus (Eppendorf).

Steps	**Temperature (°C)**	**Time**	
Initial heating	95	3 min	
Denaturation	95	30 sec	Repeated for 35 cycles
Annealing	58	1 min	
Elongation	72	45 sec	
Final elongation	72	5 min	
Final hold	4	for ever	

Table S2: Endothelin primer sequences and UPL probe numbers (Roche) used for real-time PCR

	UPL number	**Primer Sequence**		**Amplicon**
Human primers				
endothelin 1 (*EDN1*) NM_001955.4	87	Left	gctcgtccctgatggataaa	88 nt
		Right	ccatacggaacaacgtgct	
endothelin 2 (*EDN2*) NM_001956.3	1	Left	ctatggtctccgtgcctacc	123 nt
		Right	cttgggcatgagatgaggac	

endothelin 3 (*EDN3*) NM_000114.2	22	Left	acaaagaagaggaagggaaggt	120 nt
		Right	gggggcaggtagatggag	
endothelin converting enzyme 1 (*ECE1*) NM_001397.2	54	Left	actaccgtgcgtgcatga	102 nt
		Right	agggacctgtgatgttccag	
endothelin converting enzyme 2 (*ECE2*) NM_014693.3	3	Left	aacagaactgccaatgagaaagt	105 nt
		Right	ctgcatctgctccctcgt	
endothelin receptor type A (*EDNRA*) NM_001957.3	20	Left	atgtggtcattgatctccctatc	90 nt
		Right	tgcaaagaaatacgccaaagt	
endothelin receptor type B (*EDNRB*) NM_003991.3	62	Left	caatatcttgatcgccagctt	103 nt
		Right	tccaaatggccagtcctct	
Rat primers				
endothelin 1 (*Edn1*) NM_012548.2	63	Left	caggtccaagcgttgctc	114 nt
		Right	cctagtccatacgggacgac	
endothelin 2 (*Edn2*) NM_012549.2	29	Left	ctggggactctgcctgtg	76 nt
		Right	ccctggctacttgaggtgac	
endothelin 3 (*Edn3*) NM_001077650.1	64	Left	tccttctcgggctcacagt	112 nt
		Right	accctcttttcctcctggtc	
endothelin converting enzyme 1 (*Ece1*) NM_053596.2	58	Left	cctgaacaagacggaaaatga	121 nt
		Right	caggatctgctgcatctgg	
endothelin-converting enzyme 2 (*Ece2*) NM_001002815.2	3	Left	cctaaatagaactgccaatgagaaa	135 nt
		Right	agttgtatctccagttccagcac	
endothelin receptor type A (*Ednra*) NM_012550.2	78	Left	aagtttcctccagccgagac	143 nt
		Right	ttggtctcacacctttccttc	
endothelin receptor type B (Ednrb) NM_017333.1	18	Left	ctgttggcttccccttcac	107 nt
		Right	tgtagtccaaaaccagcaaaaa	

Quantitative real-time PCR (qRt-PCR)

Real-time PCR was performed using Light Cycler® 480 Probes Master 2X Conc (Roche, Germany), and the human Universal Probe Library kit (Roche, Mannheim, Germany, for probes and primers used see suppl Table 2) was used following the manufacturer's protocol, each well contained (5 µl Master 2× conc, 2.5 µl ddH2O, 2 µl sample DNA, right and left primers (10 µM) 0.2 µl each, universal library probe 0.1 µl, final volume10 µl). Each sample was pipetted in triplicate into 384-well-plates and amplified according to the program in suppl. Table 3. For analysis of the results, the comparative CT method (ΔΔCT Method) was used (Livak and Schmittgen 2001).

Table S3: Quantitative real-time PCR program

	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp rate (C/s)
Pre-incubation 1 cycle	95	-	00:10:00	4.8
Amplification 50 cycles, Quantification	95	-	00:00:10	4.8
	59	single	00:00:30	2.5
	72	-	00:00:01	4.8

Cooling 1 cycle	40	-	00:00:30	2.5

Western blotting.

After treatment, cells were harvested, transferred to 1.5-ml micro-centrifuge tubes, washed in PBS and pellets were lysed in RIPA buffer (150 mM sodium chloride, 1.0 % NP-40, 0.5 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate, 50 mM Tris, pH 8.0), and supplemented with complete protease inhibitor cocktail tablets (proteinase inhibitor (25× stock), phosphostop (10× stock), Na_2VO_3 (100× stock)) (Roche, Mannheim, Germany). Then the appropriate volume of this solution was added onto samples under continuous agitation by a shaker (30min at -4 °C on ice), then the mixture was centrifuged at 14,000 rpm, 20 min, 4 °C and the supernatant fluid was collected.

Then the protein concentration was determined using the Pierce Protein Assay, for which a relation was used to serial dilutions of standard bovine serum albumin (BSA), and the absorption was read at 450 & 595 nm.

Afterwards each probe containing 40µg sample protein was mixed with 4× LDS sample Buffer NuPAGE (NOVEX Life technology) and 1µl dithiothreitol (DTT; 1M). The probes were mixed by the thermomixer for 5 min at 99 degree at 300 rpm.

Proteins were separated on prefabricated gels (Serva) by electrophoresis at 150 V for 90 minutes in 1× Laemmli buffer. Spectra multicolor broad range protein ladder was used as a size standard.

After that the separated proteins were blotted onto a polyvinylidenfloride (PVDF) western blotting membrane using the A wet-blotting system with transfer buffer (35 V, 60 min).

Then the membrane was blocked with skim milk for 60 min, and the primary antibody was added and left at shaker at 4 °C overnight, afterwards washed with a washing buffer and the appropriate secondary antibody was added for 60 min with continuous shaking at room temperature, and then the membrane was washed again three times with TPBS and then incubated in a mixture of enhanced luminal reagent plus and oxidizing reagent plus for 1 min.

Development of the film was done in the dark in a developing machine (AGFA CURIX 60, Agfa HealthCare). When the membrane was to be reused for another primary antibody, the membrane was stripped by stripping solution for 30 min at 80 °C with continuous shaking, and then the membrane was washed, blocked and re-incubated with the respective primary antibody.

Knockdown by Small interfering RNA siRNA

Rat siRNA Edn1-465 (sense: GCGAUCCUUGAAAGACUUA) and human siRNA EDN1-498 (sense: GCUCGUCCCUGAUGGAUAA) (Invitrogen) were used for endothelin 1 knockdown with Xtreme gene or lipofectamine RNAiMAX transfection reagents according to the manufacturer protocols. In short, cells were seeded for 24h in 6 well plates, then the siRNA was added to the cells in combination with one of the transfection reagents.

Two methods for transfection by siRNA were used

- **Xtreme gene transfection reagent protocol**

The colorectal cancer cells were cultured in six well plates in the appropriate number, and then left for 24h before start of transfection. Then, the appropriate amount of siRNA was added to Opti-MEM media to reach a final volume of 100 µl, the appropriate amounts of siRNA are around 10 µl to 20 µl from siRNA stock of 20 nM to reach final concentration at the cells 100 nM or 200 nM. The transfection reagent Xtreme gene transfection reagent (Roche) was added to opti-media in ratio of two or three in relation to amount of siRNA, according to manufacture protocols, the final volume of solution is 100 µl, then after 5 min incubation in room temperature, the siRNA solution was added to transfection reagent solution, and the mixture was incubated in room temperature for 20 min, then the mixture was added to the cells. Then after 24h, 48h or 72h the cells were collected for assessment of transfection by PCR and western blot.

- **Lipofectamine RNAiMAX reagent protocol**

The colorectal cancer cells were cultivated in 12 well plate in the appropriate number 24h before transfection start, then lipofectamine reagent (Invitrogen) was diluted in Opti-MEM media 6 µl and 100 µl, and siRNA was diluted in opti-Medium 2 µl and 100 µl. After that, the diluted siRNA was added to diluted lipofectamine reagent (1:1 ratio) and incubated for 5 min in room temperature. Then the siRNA-lipid complex was added to cells. Then after 24h, 48h or 72h the cells were collected for assessment of transfection by PCR and western blot.

Infinium Methylation Assay

Genome-wide screening of DNA methylation patterns was performed by using the Infinium MethylationEPIC BeadChips (Illumina, San Diego, US), allowing the simultaneous quantitative measurement of the methylation status at 865,918 CpG sites. By combining Infinium I and Infinium II assay chemistry technologies, the BeadChip provides coverage of 99% of RefSeq genes and 96 % of CpG islands (see supplementary file Materials and Methods for more detail).

DNA concentrations were determined using PicoGreen (Molecular Probes Inc., Eugene, USA). The quality of genomic DNA samples was checked by agarose-gel analysis, and samples with an average fragment size > 3kb were selected for methylation analysis. The laboratory work was done in the Genomics and Proteomics Core Facility at the German Cancer Research Center, Heidelberg, Germany (DKFZ).

500 ng genomic DNA from each sample was bisulfite converted using the EZ-96 DNA Methylation Kit (Zymo Research Corporation, Orange, US) according to the manufacturer recommendations. Bisulfite treatment leads to the deamination of non-methylated cytosines to uracils, while methylated cytosines are refractory to the effects of bisulfite and remain cytosine.

Each sample was whole genome amplified and enzymatically fragmented following the instructions in the Illumina Infinium HD Assay Methylation Protocol Guide (genomic DNA). The DNA was applied to Infinium MethylationEPIC BeadChip and hybridization is performed for 16-24h at 48°C. During hybridization, the DNA molecules anneal to locus-specific DNA oligomers linked to individual bead types. One or two probes are used to interrogate CpG locus, depending on the probe design for a particular CpG site.

Allele-specific primer annealing is followed by single-base extension using DNP- and Biotin-labeled ddNTPs. For Infinium I assay design, both bead types (one each for the methylated and unmethylated states) for the same CpG locus incorporate the same type of labeled nucleotide, determined by the base preceding the interrogated “C” in the CpG locus, and therefore are detected in the same color channel. Infinium II uses only one bead type with a unique type of probe allowing detection of both alleles. The methylated and unmethylated signals are generated respectively in the green and the red channels.

After extension, the array is fluorescently stained, scanned, and the intensities at each CpGs were measured. Microarray scanning was done using an iScan array scanner (Illumina). DNA methylation values, described as beta values, are recorded for each locus in each sample. DNA methylation beta values are continuous variables between 0 and 1, representing the percentage of methylation of a given cytosine corresponding to the ratio of the methylated signal over the sum of the methylated and unmethylated signals. Data analysis was performed with the Illumina's GenomeStudio 2011.1 (Modul M Version 1.9.0).

Table S4: Methylation pattern of some endothelin system members in decitabine-treated (T) and control (C) human LS174T colorectal cancer cells.

Gene[a)]	cgID[b)]	Site[c)]	AVG_Beta (T)[d)]	AVG_Beta (C)[e)]
Edn1	cg08291302	TSS200;TSS200	0,1071455	0,1311027
	cg11215644	3'UTR;3'UTR	0,07255715	0,08927538
	cg14244360	TSS200;TSS200	0,1253476	0,146307
	cg16537245	TSS1500;TSS1500	0,08132226	0,1113849
	cg24244437	TSS1500;TSS1500	0,09560417	0,1348058
	cg25559625	TSS1500;TSS1500	0,09778348	0,1405008
	cg27565517	Body;Body	0,0586364	0,07348236
	cg20376899	1stExon;5'UTR;5'UTR;1stExon	0,145763	0,1775706
Edn2	cg02634222	3'UTR	0,3708056	0,5562898
	cg08460875	TSS1500;TSS200;TSS200	0,2470948	0,3064442
	cg14202907	TSS1500;TSS1500;TSS1500	0,3990755	0,7638527
	cg14978637	TSS1500;TSS1500;TSS1500	0,5827832	0,827497
	cg16736826	TSS1500;TSS1500	0,1373331	0,1939932
	cg20367961	3'UTR;TSS1500;1stExon;1stExon	0,5385646	0,7219612
	cg20700731	TSS1500;TSS1500	0,1343819	0,2146148
	cg21862529	3'UTR;3'UTR	0,4142498	0,5216267
	cg27238470	3'UTR;TSS1500;1stExon;1stExon;5'UTR	0,3428136	0,4381104
	cg07563342	3'UTR;TSS200	0,5107244	0,7484288
	cg08686022	3'UTR;3'UTR	0,6207902	0,9151602
	cg15807154	TSS1500;TSS200;TSS200	0,1840817	0,2137354
	cg19874515	TSS1500;TSS1500	0,4230038	0,833869
Edn3	cg00633740	Body;Body;Body;Body	0,3918477	0,6529219
	cg04048259	TSS200;TSS200;TSS200;TSS200	0,6427182	0,9673312
	cg07341690	TSS1500;TSS1500;TSS1500;TSS1500	0,1412242	0,2355442
	cg08318212	TSS200;TSS200;TSS200;TSS200	0,4999388	0,9439076
	cg09005679	TSS200;TSS200;TSS200;TSS200	0,5764251	0,7804127
	cg13919285	Body;Body;Body;Body	0,6017595	0,8572922
	cg16205854	TSS200;TSS200;TSS200;TSS200	0,604432	0,8450123
	cg17146570	TSS200;TSS200;TSS200;TSS200	0,5349287	0,951803
	cg17952468	TSS1500;TSS1500;TSS1500;TSS1500	0,2010071	0,2560778
	cg20910807	TSS200;TSS200;TSS200;TSS200	0,6396294	0,747744
	cg21163415	Body;Body;Body;Body	0,2758578	0,4018897
	cg01665123	TSS1500;TSS1500;TSS1500;TSS1500;TSS1500	0,2029526	0,3093193
	cg04296743	Body;Body;Body;Body;Body	0,1016442	0,1361438
	cg04477502	Body;Body;Body;Body;Body	0,3530108	0,5296436
	cg07814082	3'UTR;3'UTR;3'UTR;3'UTR;3'UTR	0,2168905	0,3979693
	cg12718049	Body;Body;Body;Body;Body	0,1621816	0,2371006
	cg14770089	3'UTR;3'UTR;3'UTR;3'UTR;3'UTR	0,3139811	0,4902204
	cg14809794	Body;Body;Body;Body;Body	0,1010172	0,1382381
	cg19905109	Body;Body;Body;Body;Body	0,07661048	0,1312846
	cg22024589	Body;Body;Body;Body;Body	0,08357058	0,1145039
	cg19194373	5'UTR;1stExon;1stExon;5'UTR;5'UTR;1stExon;1stExon;5'UTR	0,6344967	0,9438744
	cg20671059	5'UTR;5'UTR;5'UTR;5'UTR;5'UTR;1stExon;1stExon;1stExon;1stExon;1stExon	0,6426538	0,7966031
	cg23710795	5'UTR;5'UTR;5'UTR;5'UTR;5'UTR;1stExon;1stExon;1stExon;1stExon;1stExon	0,4879484	0,5395017

Ednra	cg00379467	TSS200;TSS200;TSS200	0,5429557	0,7054018
	cg00974629	TSS200;TSS200;TSS200	0,5828776	0,8930468
	cg03573792	Body;3'UTR;3'UTR	0,3631145	0,6595846
	cg03580568	Body;5'UTR;5'UTR	0,300923	0,3564449
	cg05102394	Body;5'UTR;5'UTR	0,5389909	0,804934
	cg05618426	TSS200;TSS200;TSS200	0,3216511	0,5353364
	cg10112943	TSS1500;TSS1500;TSS1500	0,2969109	0,4885649
	cg14948448	TSS1500;TSS1500;TSS1500	0,6130844	0,8631078
	cg16264526	TSS1500;TSS1500;TSS1500	0,2218601	0,4902192
	cg17073859	TSS1500;TSS1500;TSS1500	0,599831	0,8537132
	cg22930773	Body;Body;Body	0,2103172	0,4094081
	cg23865342	Body;5'UTR;5'UTR	0,2647553	0,4454416
	cg24789447	Body;5'UTR;5'UTR	0,1878639	0,245694
	cg01421256	5'UTR;Body;Body;Body	0,510362	0,8965383
	cg05857597	5'UTR;5'UTR;5'UTR;Body	0,151121	0,3279359
	cg12831879	5'UTR;5'UTR;5'UTR;Body	0,3657451	0,746397
	cg13257535	5'UTR;Body;Body;Body	0,2925659	0,7008027
	cg14449534	5'UTR;5'UTR;5'UTR;Body	0,5703558	0,910007
	cg15500141	5'UTR;Body;Body;Body	0,09199282	0,1576776
	cg18057932	5'UTR;Body;Body;Body	0,1178647	0,2567794
	cg19207921	5'UTR;Body;Body;Body	0,3392339	0,7809858
	cg20402736	TSS1500;TSS1500;TSS1500;TSS1500	0,2271679	0,2936738
	cg23718823	5'UTR;Body;Body;Body	0,2246386	0,4653105
	cg24782857	5'UTR;Body;Body;Body	0,1664288	0,3213757
	cg25705272	5'UTR;Body;Body;Body	0,4723865	0,6547812
	cg25984941	5'UTR;Body;Body;Body	0,2075505	0,3442599
	cg04045079	Body;1stExon;5'UTR;1stExon;5'UTR	0,2998205	0,3772426
	cg12009023	Body;1stExon;5'UTR;1stExon;5'UTR	0,3712821	0,5259702
	cg20557687	Body;1stExon;5'UTR;1stExon;5'UTR	0,3106749	0,3614736
	cg14986746	1stExon;1stExon;1stExon;5'UTR;5'UTR;5'UTR;Body	0,3352302	0,4374267
Ednrb	cg06106063	TSS1500	0,1563893	0,2345513
	cg10042288	TSS200	0,4951259	0,696665
	cg10389399	5'UTR	0,2281593	0,4736207
	cg10792120	TSS1500	0,1178066	0,139707
	cg12321193	TSS1500	0,1144078	0,1877395
	cg13432560	5'UTR	0,1990677	0,3446122
	cg16203262	5'UTR	0,09030718	0,1202725
	cg22154447	TSS1500	0,2621025	0,3643319
	cg24588582	TSS200	0,4372807	0,8789999
	cg27647038	5'UTR	0,07295255	0,1200555
	cg01239435	TSS1500;5'UTR	0,1705173	0,2125463
	cg08983760	5'UTR;1stExon	0,3546112	0,5327781
	cg18003135	5'UTR;1stExon	0,3102905	0,4056658
	cg01910869	TSS1500;5'UTR;TSS1500	0,5020744	0,5858602
	cg02147695	TSS1500;5'UTR;TSS1500	0,3750089	0,604161
	cg03086857	1stExon;1stExon;Body	0,2730309	0,3019888
	cg04390523	5'UTR;TSS200;TSS200	0,5963429	0,8213792
	cg06057566	TSS1500;5'UTR;TSS1500	0,5586634	0,8603113

	cg06179060	5'UTR;TSS200;TSS200	0,1884483	0,336491
	cg07035515	TSS1500;5'UTR;TSS1500	0,2089058	0,3681335
	cg07495027	Body;Body;Body	0,09866967	0,1560619
	cg07974719	TSS1500;5'UTR;TSS1500	0,6184321	0,9066217
	cg08321129	TSS1500;5'UTR;TSS1500	0,21271	0,322584
	cg08634041	TSS1500;5'UTR;TSS1500	0,5380208	0,8907503
	cg09152886	TSS1500;5'UTR;TSS1500	0,2147224	0,3738428
	cg09786383	TSS1500;5'UTR;TSS1500	0,1716985	0,2120599
	cg10016380	TSS1500;5'UTR;TSS1500	0,6209602	0,8392283
	cg10764762	TSS1500;5'UTR;TSS1500	0,2681316	0,4464264
	cg11074192	TSS1500;5'UTR;TSS1500	0,5480265	0,8842228
	cg12120741	1stExon;1stExon;Body	0,5023271	0,594822
	cg12602112	TSS1500;5'UTR;TSS1500	0,7159739	0,8916239
	cg12847373	TSS1500;5'UTR;TSS1500	0,5023698	0,6537069
	cg12935136	TSS1500;5'UTR;TSS1500	0,5983497	0,8512115
	cg12983394	TSS1500;5'UTR;TSS1500	0,4464557	0,6029329
	cg13434989	TSS1500;5'UTR;TSS1500	0,6253272	0,9260656
	cg13818654	5'UTR;TSS200;TSS200	0,4407227	0,5079631
	cg13866767	Body;3'UTR;3'UTR	0,3302879	0,6709737
	cg15699226	1stExon;1stExon;Body	0,5571828	0,9578596
	cg15836660	5'UTR;TSS200;TSS200	0,404382	0,4920833
	cg16739796	TSS1500;5'UTR;TSS1500	0,1499697	0,1944652
	cg18032190	TSS1500;5'UTR;TSS1500	0,5793512	0,8843021
	cg18568990	TSS1500;5'UTR;TSS1500	0,3548283	0,4873351
	cg19650157	TSS1500;5'UTR;TSS1500	0,6906412	0,9113404
	cg19742055	TSS1500;5'UTR;TSS1500	0,5685055	0,719283
	cg19759502	TSS1500;5'UTR;TSS1500	0,5742196	0,8186762
	cg19916212	TSS1500;5'UTR;TSS1500	0,6720599	0,8941883
	cg21364111	TSS1500;5'UTR;TSS1500	0,1201939	0,1827052
	cg21675115	TSS1500;5'UTR;TSS1500	0,5600346	0,8018074
	cg22310279	TSS1500;5'UTR;TSS1500	0,5220866	0,821213
	cg22541679	TSS1500;5'UTR;TSS1500	0,3746499	0,642967
	cg23326536	Body;Body;Body	0,06866998	0,08492734
	cg23494140	TSS1500;5'UTR;TSS1500	0,4315396	0,6579756
	cg23702615	TSS1500;5'UTR;TSS1500	0,6251598	0,9502311
	cg24745738	TSS1500;5'UTR;TSS1500	0,5338176	0,851151
	cg24785726	Body;Body;Body	0,2138873	0,4372701
	cg24942919	TSS1500;5'UTR;TSS1500	0,4123036	0,7379285
	cg25717994	TSS1500;5'UTR;TSS1500	0,2159612	0,2971753
	cg26022015	TSS1500;5'UTR;TSS1500	0,594691	0,8487691
	cg16571983	5'UTR;1stExon;5'UTR;1stExon;5'UTR	0,5824263	0,9418846
	cg23316360	5'UTR;1stExon;5'UTR;1stExon;5'UTR	0,5154684	0,6827052
Ece1	cg00054372	Body;Body;Body;Body	0,5160899	0,8922374
	cg00228796	Body	0,4971438	0,8751765
	cg00323385	Body;Body;Body;Body	0,4523316	0,8462206
	cg00344655	Body;Body;Body;Body	0,5024834	0,8404501
	cg00831171	Body	0,4591194	0,9005086
	cg00964400	Body	0,3552727	0,5424841

	cg01080480	TSS1500	0,5598758	0,8840505
	cg01306962	Body;Body;Body;Body	0,4136099	0,8976192
	cg01383475	Body;Body;Body;Body	0,6219062	0,9246137
	cg01478827	Body	0,4750775	0,8693681
	cg01753382	Body;Body;Body;Body	0,5447907	0,8879702
	cg01758046	Body;Body;Body;Body	0,5674443	0,9028005
	cg01780990	Body;Body;Body;Body	0,5570714	0,8008551
	cg01922918	TSS1500;Body;Body;Body	0,432688	0,7751598
	cg01977520	Body	0,5430368	0,7648504
	cg02492946	TSS1500;TSS1500;Body	0,4034836	0,5901228
	cg02582404	Body	0,3074535	0,3508659
	cg02994246	Body;Body;Body;Body	0,4577986	0,8621366
	cg03056614	Body;Body;Body;Body	0,567679	0,8766845
	cg03123991	Body;Body;Body;Body	0,5355924	0,8959425
	cg03432181	Body;Body;Body;Body	0,4442818	0,8440905
	cg04114429	Body;Body;Body;Body	0,4578052	0,8706138
	cg04236915	Body;Body;Body;Body	0,4869596	0,7770153
	cg04621069	3'UTR;3'UTR;3'UTR;3'UTR	0,5148234	0,8569035
	cg05364829	1stExon;5'UTR	0,1250674	0,09883196
	cg05370193	Body;Body;Body;Body	0,6609569	0,9070401
	cg05610701	Body	0,4061956	0,6938559
	cg05954918	Body	0,397916	0,6852275
	cg06405255	Body;Body;Body;Body	0,6224656	0,9286655
	cg06705062	Body	0,5913404	0,9210311
	cg06819200	1stExon;Body;Body	0,06468166	0,08827946
	cg06878749	Body;Body;Body;Body	0,5638185	0,8984544
	cg07094113	Body;Body;Body;Body	0,4114555	0,864806
	cg07114751	Body;Body;Body;Body	0,5973849	0,7964132
	cg07133269	Body;Body;Body;Body	0,5847712	0,9028306
	cg07157016	Body	0,427033	0,8564727
	cg07996532	Body	0,5330157	0,8941814
	cg08146932	Body;Body;Body;Body	0,6863397	0,8954595
	cg08421373	Body	0,4702829	0,8894932
	cg08574008	Body	0,4864087	0,795975
	cg10326339	Body;Body;Body;Body	0,5024337	0,8619369
	cg10639743	Body;Body;Body;Body	0,4887088	0,8703957
	cg10699884	Body	0,3389252	0,448399
	cg10761088	TSS1500	0,0589516	0,07407764
	cg10820600	Body	0,5625433	0,908385
	cg10869378	Body	0,461891	0,8372829
	cg10984417	Body;Body;Body;Body	0,3143419	0,4980228
	cg11834658	Body;Body;Body	0,6891918	0,9075647
	cg12037641	Body;Body;Body;Body	0,5285005	0,8821381
	cg12216986	TSS1500;TSS200;Body	0,03209314	0,04916658
	cg12460254	Body	0,4492529	0,7966434
	cg12771446	Body	0,4157157	0,8379029
	cg12841412	Body;Body;Body;Body	0,580312	0,8584121
	cg13086983	Body	0,4570371	0,865887

	cg13151223	Body;Body;Body;Body	0,5197842	0,8891437
	cg13212362	Body	0,4495982	0,7742246
	cg13447099	Body;Body;5'UTR;Body;1stExon	0,5565451	0,8638147
	cg13551754	Body	0,5132991	0,7200916
	cg13562122	Body;Body;Body;Body	0,5412646	0,8924241
	cg14557415	Body	0,3368974	0,5458655
	cg14649592	Body	0,5126345	0,8932124
	cg14783091	Body;Body;Body;Body	0,5702599	0,8852898
	cg15041744	TSS200;Body;Body;Body	0,5637949	0,9007236
	cg15462862	Body	0,1309985	0,1850792
	cg15598754	Body;Body;Body;Body	0,6587689	0,9484772
	cg15883633	Body;Body;Body;Body	0,546877	0,8845945
	cg16033503	Body	0,5780666	0,9294233
	cg16357113	Body	0,5754561	0,8943019
	cg16385763	Body;Body;Body;Body	0,5492553	0,9429329
	cg16416158	Body	0,4582991	0,7328736
	cg16461781	Body;Body;Body;Body	0,1397837	0,2666322
	cg16514473	Body	0,2562492	0,4113524
	cg16603916	Body;Body;Body	0,6202773	0,8185524
	cg16829306	Body	0,08215101	0,08268329
	cg16910448	TSS1500;TSS1500;Body	0,5867798	0,9004042
	cg17031944	Body;Body;Body	0,5278974	0,9112751
	cg17445490	Body	0,4861459	0,9003378
	cg17446661	Body	0,5209528	0,8869022
	cg17850498	Body;Body;Body;Body	0,4816263	0,8463635
	cg18116670	Body	0,5158193	0,8182775
	cg18574822	Body;Body;Body;Body	0,5056469	0,8240592
	cg19165640	Body	0,5408223	0,6947109
	cg19215233	Body	0,5577188	0,9045973
	cg19273683	Body	0,1708786	0,2762079
	cg19363242	Body;Body;Body;Body	0,6764126	0,8584427
	cg19827182	Body	0,3922957	0,853984
	cg19838586	Body	0,4116277	0,8486367
	cg19953129	Body;Body;Body;Body	0,5592375	0,9287599
	cg19987248	Body;Body;Body;Body	0,519192	0,8675829
	cg20015787	TSS1500;TSS1500;Body	0,4710129	0,8060876
	cg20554838	Body;Body;Body;Body	0,5568978	0,8585842
	cg21005416	TSS1500;Body;Body;Body	0,2746885	0,3444025
	cg21022327	Body;Body;Body;Body	0,4989291	0,8997188
	cg22040535	Body;Body;Body;Body	0,4314674	0,8917283
	cg22054164	TSS1500;TSS1500;Body	0,5392867	0,6966603
	cg22501927	Body;Body;Body;Body	0,6117615	0,911074
	cg22501992	Body	0,5175881	0,8991973
	cg22887668	TSS200;Body;Body;Body	0,4827111	0,7850565
	cg23072086	TSS1500;Body;Body;Body	0,4876537	0,7971941
	cg23092576	TSS1500	0,06413924	0,08903753
	cg23347052	Body	0,4975078	0,8973305
	cg23470779	Body;Body;Body;Body	0,5348771	0,9342144

	cg23764158	1stExon;5'UTR;Body;Body;Body	0,5695956	0,9053636
	cg25007705	Body;Body;Body;Body	0,3046945	0,4447208
	cg25033646	Body	0,4682641	0,7097588
	cg26348226	TSS1500;TSS1500;Body	0,1468259	0,2198202
	cg26467313	Body;Body;Body;Body	0,4030575	0,69133
Ece2	cg05951558	Body;TSS1500;TSS1500;TSS1500	0,6173308	0,8767751
	cg13527631	Body;Body;Body;Body	0,5295414	0,8296077
	cg22980150	Body;Body;Body;Body	0,1115364	0,1390877
	cg23872669	TSS200;Body;TSS200;TSS200	0,7095958	0,9134879
	cg03835095	Body;Body	0,4206189	0,8643024
	cg04414966	TSS200;Body;TSS200;TSS200	0,7316712	0,8617532
	cg05088549	Body;Body;Body;Body	0,2333519	0,3095841
	cg07460919	Body;TSS1500	0,3884878	0,6252621
	cg07864323	Body;3'UTR	0,5289074	0,8472652
	cg09970348	Body;Body	0,4783616	0,6872687
	cg22700017	TSS200;Body;TSS200;TSS200	0,7541537	0,8799804
	cg23527621	Body	0,5395114	0,8535576
	cg23749739	Body;Body;Body;Body	0,3414487	0,5381204
	cg08770580	Body;TSS1500	0,4936361	0,8445451
	cg12232835	Body;Body	0,1617173	0,2217056
	cg01876619	Body;Body;Body;Body	0,7905389	0,9514056
	cg14653325	Body;5'UTR;1stExon	0,07258382	0,08507334
	cg15112081	Body;TSS1500;TSS1500;TSS1500	0,360003	0,4987566
	cg01179696	Body;Body;Body;Body	0,6599057	0,9012217
	cg01189038	Body;Body	0,3692151	0,4527996
	cg01333080	Body;TSS1500;TSS1500;TSS1500	0,4175735	0,7864716
	cg01596834	Body;5'UTR;1stExon	0,05489216	0,06608124
	cg02791331	Body;Body;Body;Body	0,183118	0,3064053
	cg03766859	Body	0,1144155	0,1783217
	cg05118764	Body;Body	0,5248614	0,9086988
	cg07131336	Body;Body	0,4330482	0,6673376
	cg08154107	1stExon;Body;5'UTR;5'UTR;5'UTR;1stExon;1stExon	0,6864698	0,963538
	cg08832414	TSS200;Body;TSS200;TSS200	0,7140014	0,9430322
	cg09405238	Body;Body;Body;Body	0,5858308	0,5961924
	cg11755372	Body;TSS1500;TSS1500;TSS1500	0,5233252	0,8624272
	cg12942257	Body;Body;Body;Body	0,4987124	0,8522331
	cg19098796	Body;TSS1500	0,08640636	0,0973777
	cg19100138	Body	0,6091952	0,8492848
	cg19141258	Body;3'UTR	0,63787	0,8882393
	cg22113788	Body;TSS200	0,07880112	0,07746631
	cg24976089	Body	0,08134003	0,1560419
	cg16953297	ExonBnd;ExonBnd;ExonBnd;ExonBnd;Body;Body;Body;Body	0,09962696	0,2193574
	cg24218885	Body;Body;Body;Body	0,4277208	0,4676185

[a)] Gene abbreviation; [b)] Illumina assay probe identification; **[c)]** Site(s) of methylation; [d),e)] An average β-value (AVG_Beta) for each CpG locus ranging from 0 (unmethylated) to 1 (completely methylated) was extracted utilizing the GenomeStudio program (Illumina) for DNA extracted from control (C) and treated (T) LS174 cells.