Supplementary data

CDKN2A-sequence after bisulfite conversion (498 bp):

GTAGTATGGAGTTTTTGGTTGATTGGTTGGTTATGGTTGTGGTTTGGGGTTGGGTAGAGGAGG
TGTGGGTGTTGTTGGAGGTGGGGGTGTTGTTTAATGTATTGAATAGTTATGGTTGGAGGTTGAT
TTAGGTGGGTAGAGGGTTTGTAGTGGGAGTAGGGGATGGTGGGTGATTTTGGAGGATGAAGT
TTGTAGGGGAATTGGAATTAGGTAGTGTTTTGATTTTTTGGAAAAAGGGGAGGTTTTTTGGGGA
GTTTTTAGAAGGGGTTTGTAATTATAGATTTTTTTTTGGTGATGTTTTGGGGGTTTGGGAAGTTA
AGGAAGAGGAATGAGGAGTTATGTGTGTATAGATTTTTTGAATGTTGAGAAGATTTGAAGGGGG
GAATATATTTGTATTAGATGGAAGTATGTTTTTTATTAGATATAAAATTTATGAATGTTTGGGATA
AAAAGGGAGTTTTAAAGAAATGTAAGATGTGTTGGGATTATTTAGTTTTT

Col1A2-sequence after bisulfite conversion (295 bp):

GGAGGTATTTTAGGGTTAGGGAAATTTTTGTTGTATAAATAGGGTAGATTTGGGTTTTATTATTT
TAGTATTATGGTAGTAGGAGGTTTTGGTTAAGTTGGAGGTATTGGTTATGATTGTATGTTTGTGT
TTGTTAGGTGATATTTTTGTTGGTGATTTAGGGGTTTTGTGATATAAGGAGTTTGTATGTTTAAG
TGTTAGATATGTTTAGTTTTGTGGATATGTGGATTTTGTTGTTGTTTGTAGTAATTTTATGTTTAG
TAATATGTTAATGTAAGTGTTTTTAGTTTGTTTG

Pyrosequencing primers

Table S1:

Primer	Name	Sequence 5'-3'
PCR-Forward	CDKN2A-F	GATTTAGGTGGGTAGAGGGTTTG
PCR-Reverse	CDKN2A-R	[Biotin]CCCCTTCAAATCTTCTCAACATT
Sequencing	CDKN2A-S	TTGTAGGGGAATTGGA

Supplementary Figures

Supplementary Figure 1:

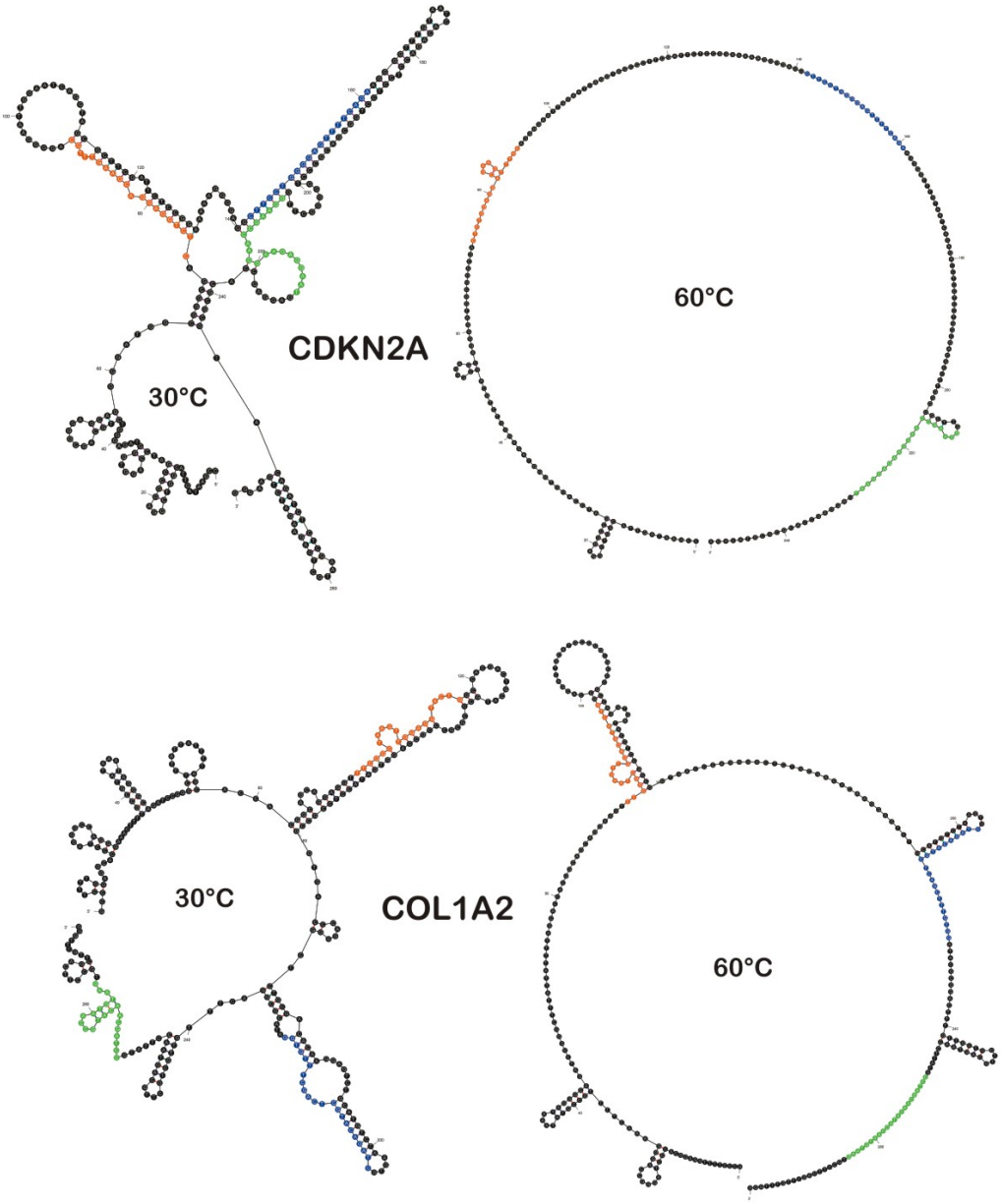

Fig. S1: Primer design for single-cell PCR. The promoter region to be analyzed may form hairpin-structures and loops than can interfere with primer binding. Primer sequences (F1 = orange, F2 = Blue, R = Green) should be placed outside of hairpin-structures as much as possible. The folding structures shown are the lowest energy configurations for 30°C and 60°C (annealing temperature) predicted by Mfold [1].

Supplementary Figure 2:

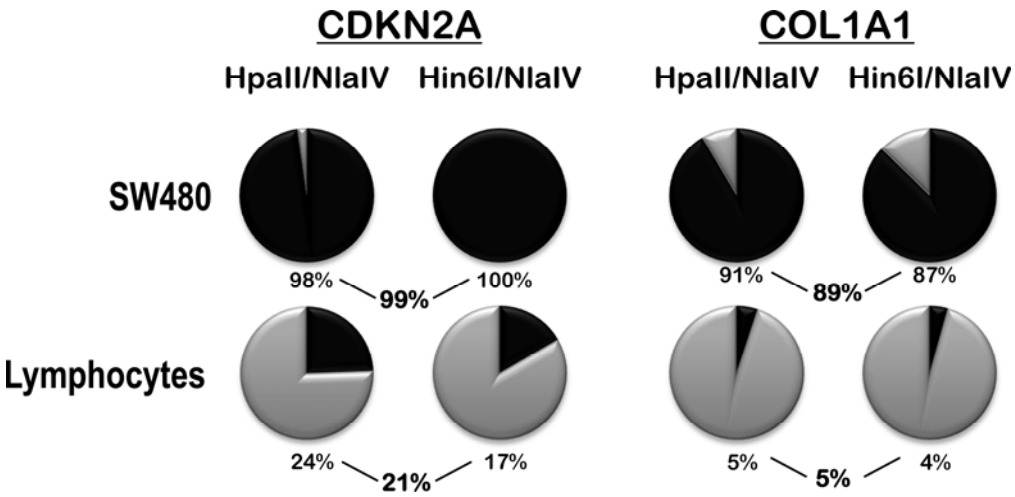

Fig. S2: Overall methylation levels of CpG dinucleotides within the promoters of the CDKN2A and COL1A1 genes in the colorectal cancer cell line SW480 and in lymphocytes, measured with RSMA in single-cells. In agreement with published data and our bisulfite sequencing results, both genes were categorized as significantly hypermethylated in SW480 cells, but hypomethylated in normal lymphocytes. Methylation levels for the HpaII and Hin6I sites were highly concordant, indicating that measurements of single CpG dinucleotides may suffice to establish the methylation level of most CpG islands. Interindividual variation of methylation levels between cells from the same tissue underscore the need for high-throughput profiling capabilities to perform population statistics. Since previous studies reported no significant methylation levels in the CDKN2A CpG island in lymphocytes, we tried to establish if the residual methylation levels measured in our samples at the 3'-end of the CpG island can be reproduced. Hence, for the HpaII restriction site we repeated our experiment on a batch of commercially available lymphocytes (n=73) and also on freshly prepared lymphocytes (n=33). In those cases as well, we found a methylation of 32% and 24% respectively, indicating that the data from the first round of experiments reflects real residual methylation at the upstream CpG island border.

Supplementary Figure 3:

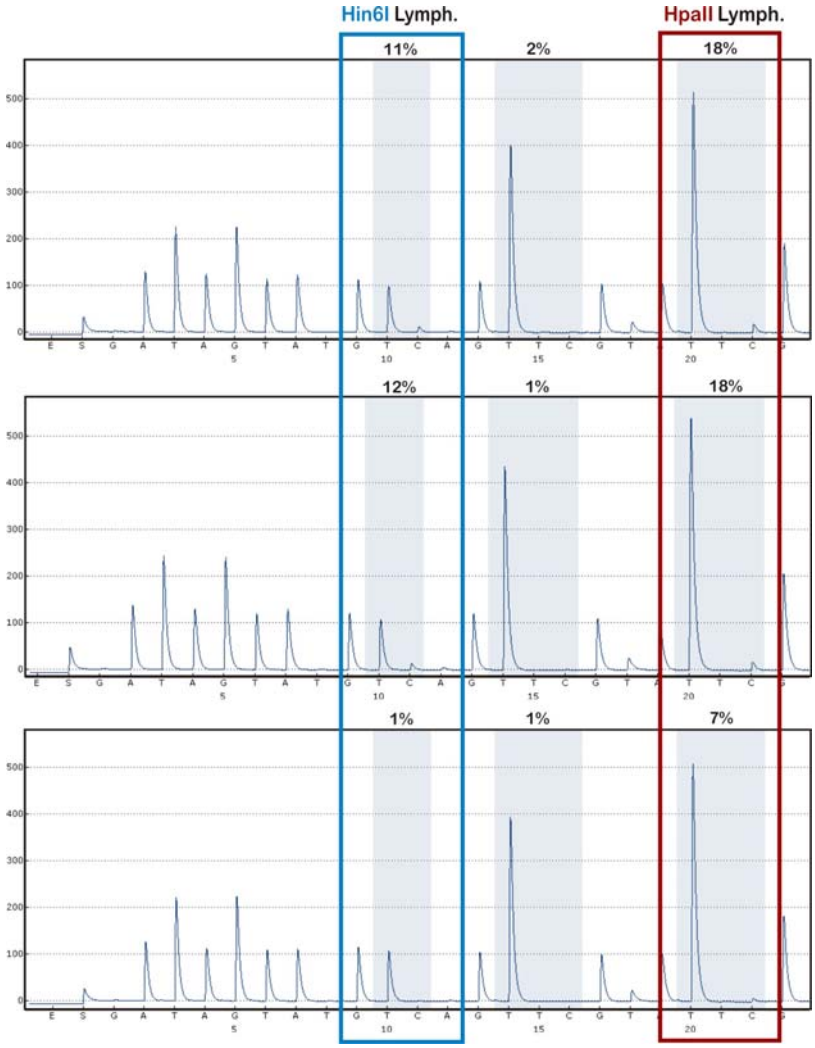

Fig. S3: Pyrogram trace of a quantitative DNA methylation analysis of three CpGs at the distal border of the CDKN2A CpG island in lymphocytes of 3 individuals. Highlighted areas indicate variable CpG positions. The methylation level of the Hin6I and HpaII sites demonstrate interindividual variance of methylation, ranging up to 18% (+-5%). Those data verify the results from the RSMA single-cell measurements; indicating that partial methylation at the border of the CDKN2A CpG island exists in human lymphocytes.

Supplementary references

1. Zuker, M., *Mfold web server for nucleic acid folding and hybridization prediction.* Nucleic Acids Res, 2003. **31**(13): p. 3406-15.