Supplemental Material to:

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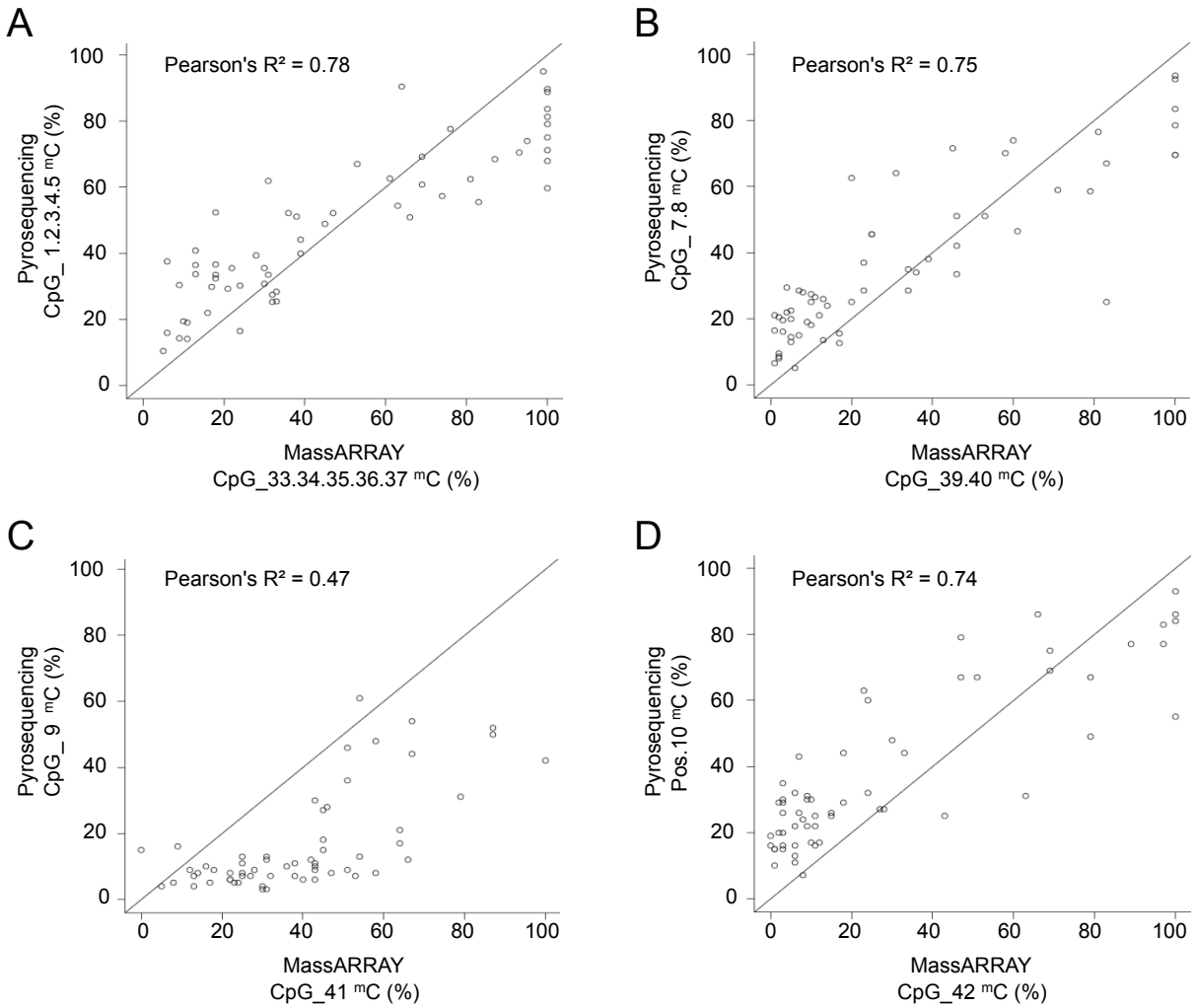
Supplemental Table

MassARRAY	Amplicon name		Primer sequence	Position relative to TSS	Length [bp]	CpGs
	ID4*	F	aggaagagagAATGGAGTGTTTTTTTTATTGGTT	-295/+106	401	50
		R	cagtaatacgactcactatagggagaaggctAATATCCTAATCACTCCCTTC			
	SFRP1	F	aggaagagagGTTTTATTTTGGGGTTTGGAGGTTT	+585/ +863	279	24
		R	cagtaatacgactcactatagggagaaggctACAACAAACCAACAACTAAATACCCCTACT			
	SFRP2	F	aggaagagagGGTTAAGAAAATTTTGGTTGTG	-28/+438	466	46
		R	cagtaatacgactcactatagggagaaggctTAACCCAACAAATTAAACAACC			
	SFRP4	F	aggaagagagATTGGTTAGATTAAAAAGGAGGGA	-112/+273	385	36
		R	cagtaatacgactcactatagggagaaggctAATTTCTTCCCCCAAACTCCAAT			
	SFRP5	F	aggaagagagTAGAGAGATAAGTAAGTGAAGT	-344/+39	383	28
		R	cagtaatacgactcactatagggagaaggctCCCAAATAAATAACAACCTAC			

MSP	Amplicon name		Primer sequence	Position relative to TSS	Reference
	***ID4* U**	F	GGTAGTTGGATTTTTTGTTTTTTAGTATT	-194/ -33	16
	***ID4* U**	R	AACTATATTTATAAAACCATACACCCCA		
	***ID4* M**	F	TAGTCGGATTTTTCGTTTTTTAGTATC	-192/ -35	
	***ID4* M**	R	CTATATTTATAAAACCGTACGCCCCG		
	SFRP1 M	F	TGTAGTTTTCGGAGTTAGTGTCGCGC	+13/+138	26
	SFRP1 M	R	CCTACGATCGAAAACGACGCGAACG		
	SFRP1 U	F	GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT	+9 /+143	
	SFRP1 U	R	CTCAACCTACAATCAAAAACAACACAAACA		
	SFRP2 M	F	GGGTCGGAGTTTTTCGGAGTTGCGC	+23/ +160	26
	SFRP2 M	R	CCGCTCTCTTCGCTAAATACGACTCG		
	SFRP2 U	F	TTTTGGGTTGGAGTTTTTTGGAGTTGTGT	+19/ +163	
	SFRP2 U	R	AACCCACTCTCTTCACTAAATACAACTCA		
	SFRP4 M	F	GGGTGATGTTATCGTTTTTGTATCGAC	-42/ +70	26
	SFRP4 M	R	CCTCCCCTAACGTAAACTCGAAACG		
	SFRP4 U	F	GGGGGTGATGTTATTGTTTTTGTATTGAT	-44/ +72	
	SFRP4 U	R	CACCTCCCCTAACATAAACTCAAAACA		

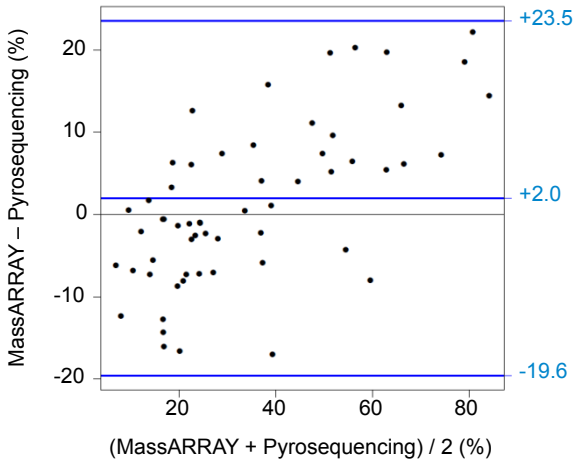
SFRP5 M	F	AAGATTTGGCGTTGGGCGGGACGTTC	-160/ -25	26
SFRP5 M	R	ACTCCAACCCGAACCTCGCCGTACG		
SFRP5 U	F	GTAAGATTTGGTGTTGGGTGGGATGTTT	-162/ -22	
SFRP5 U	R	AAAACTCCAACCCAAACCTCACCATACA		

Abbreviations: F = forward; R = reverse; M = methylated; U = unmethylated, TSS = transcriptional start site.
* This set of primers was used for MassARRAY- and bisulifte sequencing-based DNA methylation analysis.



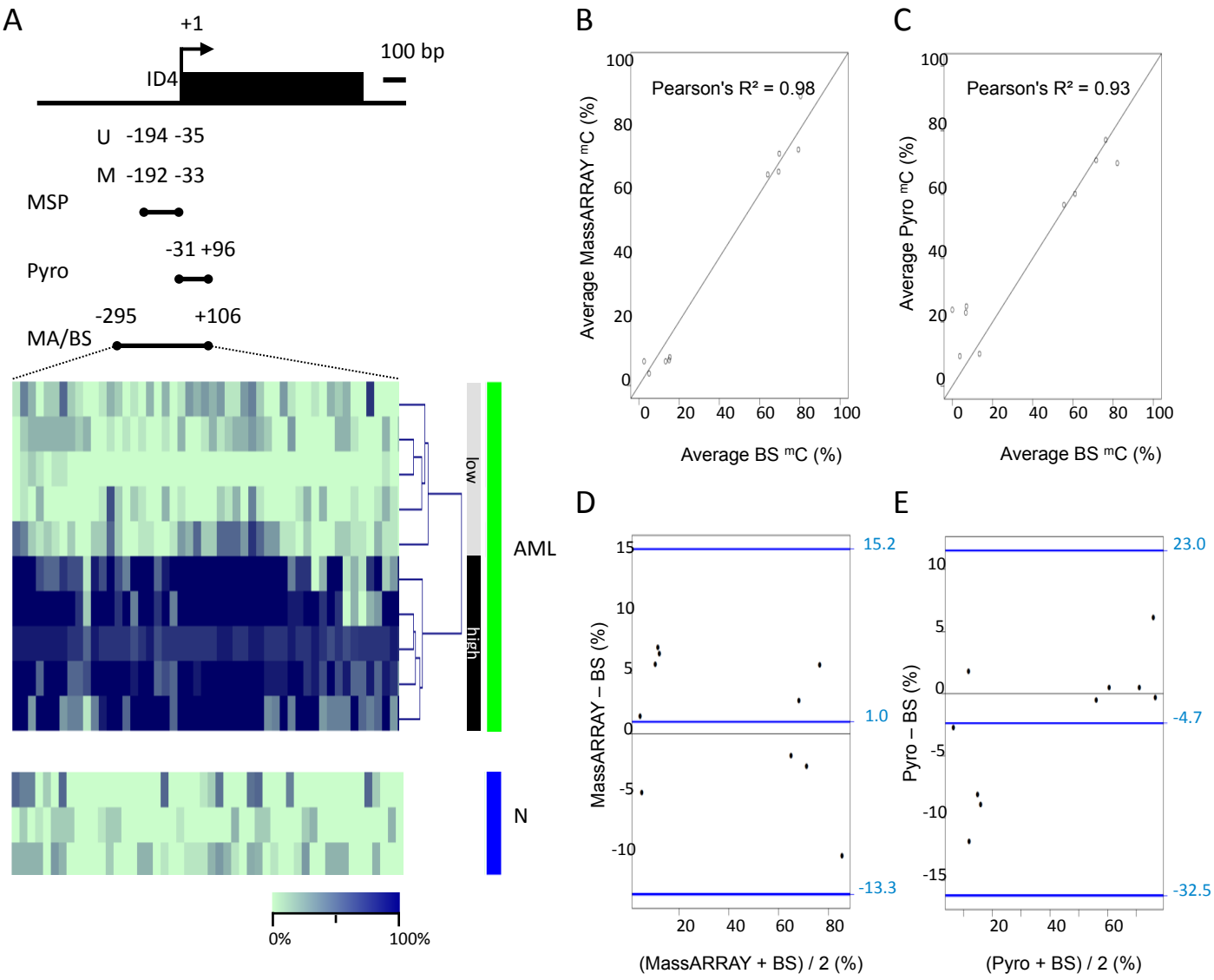
Supplemental Figure 1. Correlation of pyrosequencing- and MassARRAY-derived methylation data

(A-D) Scatterplots displaying the correlation of methylation levels for single CpG units as generated by the MassARRAY assay and corresponding CpGs from the pyrosequencing data. The correlation is calculated as Pearson's R².



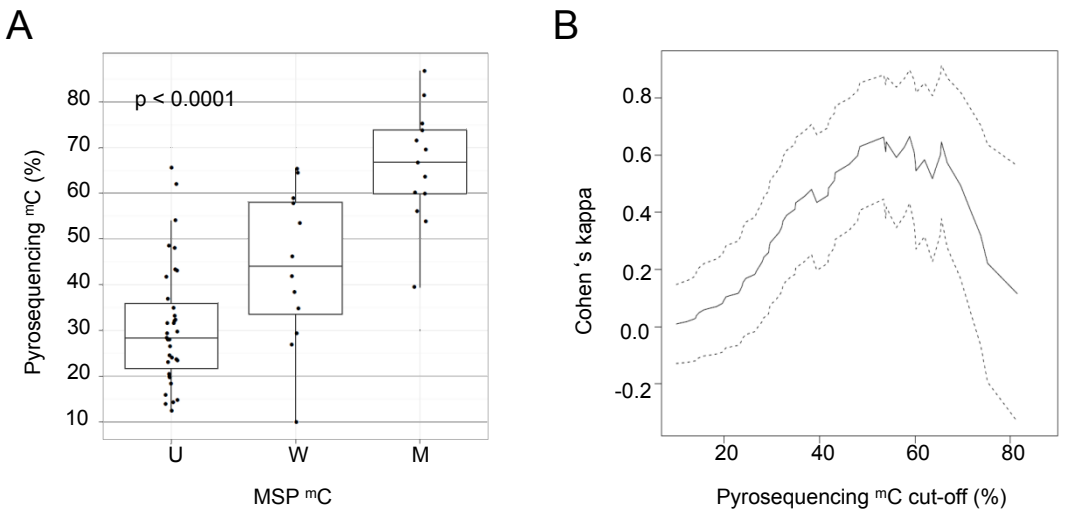
Supplemental Figure 2. Concordance of MassARRAY- and pyrosequencing-derived *ID4* methylation data

Bland−Altman plot displaying the difference between average MassARRAY- and pyrosequencing-derived *ID4* methylation data versus the mean value. Blue lines indicate the limits of agreement.



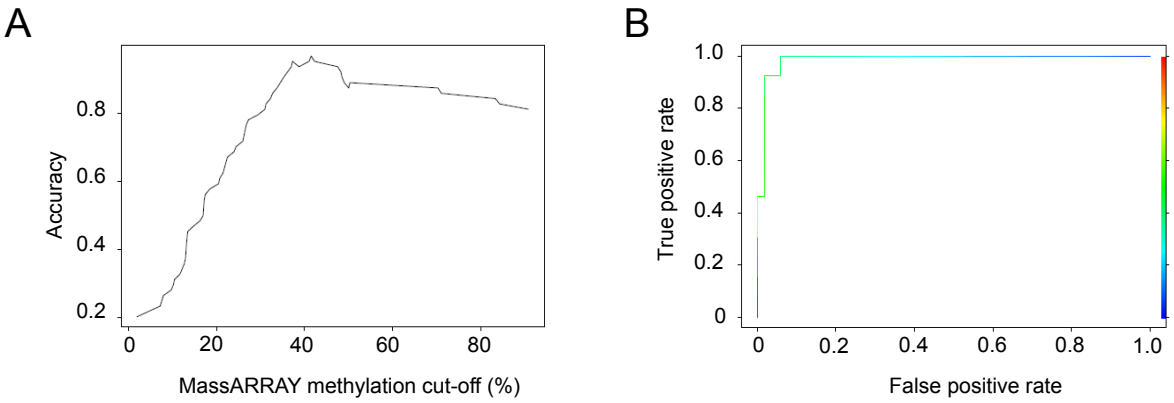
Supplemental Figure 3. Bisulfite sequencing of the *ID4* 5' region

(A) Schematic representation of the *ID4* 5' regulatory region. The amplicon analyzed by the MassARRAY assay (MA) and by bisulfite sequencing (BS) is depicted as black bar including the position relative to the transcriptional start site (TSS). The regions analyzed by pyrosequencing and methylation-specific PCR are labeled Pyro and MSP, respectively. For bisulfite sequencing-based DNA methylation analysis, ten clones were analyzed for each sample. Ten AML samples (AML, green), five of them being highly (high, black bar) and five being lowly methylated (low, bright grey bar) according to the previous MassARRAY-based analysis, as well as three healthy controls (N, blue) were chosen from the initial sample set. AML samples were ordered by unsupervised hierarchical clustering and clearly separated according to the previous MassARRAY-based methylation analysis. The heat map displays the 50 analyzed CpG dinucleotides in columns and the samples in rows. Bright green encodes for low methylation levels, dark blue for high methylation levels. (B, C) Scatterplots and correlations for average methylation levels of CpG dinucleotides asessed by both MassARRAY versus bisulfite sequencing (B) and pyrosequencing versus bisulfite sequencing (C) are shown. The correlation is calculated as Pearson's R^2. (D, E) Bland–Altman plots display the difference between average MassARRAY/pyrosequencing and bisulfite sequencing-derived *ID4* methylation data versus the mean value. Blue lines indicate the limits of agreement.

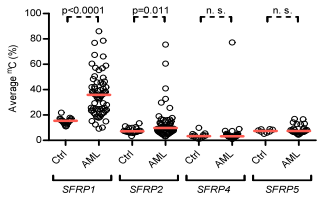


Supplemental Figure 4. Direct comparison of quantitative pyrosequencing and MSP-based methylation data at the *ID4* 5’ region

(A) Distribution of pyrosequencing-derived DNA methylation values for the MSP categories U, W and M. All three MSP groups are tested for difference of location using the non-parametric Kruskal-Wallis test. (B) Agreement between both methods (inter-rater reliability) by Cohen’s kappa including confidence interval depending on quantitative pyrosequencing data.

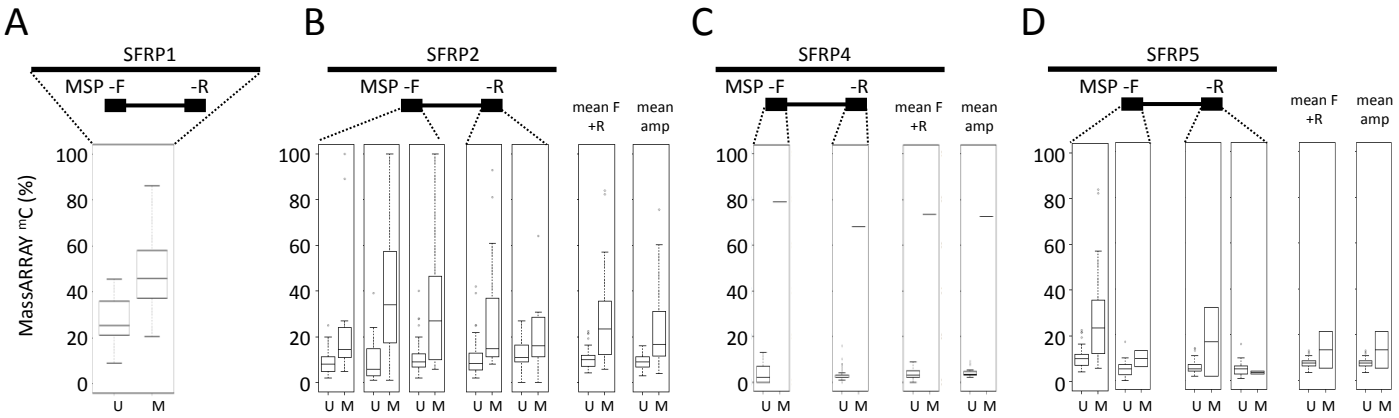


Supplemental Figure 5. Classification of MassARRAY-derived *ID4* methylation data
(A, B) Classification of quantitative MassARRAY-derived methylation data into categories "unmethylated" and "methylated" was evaluated using prediction accuracy plots (A) and receiver operating characteristic (ROC) curves (B). The highest accuracy of 0.97 was reached for the cut-off of 42% methylation at the investigated *ID4* 5' region. Accuracy of prediction is defined as [(true positives+true negatives) / (positives+negatives)].



Supplemental Figure 6. Quantitative MassARRAY-based DNA methylation assessment of *SFRP* gene family members

Distribution of average amplicon methylation values (^{m}C) in AML samples (AML) and pooled healthy controls (Ctrl) for the 5' region of the genes *SFRP1, -2, -4* and *-5.* Significance was assessed by two-sided non-parametric Wilcoxon Mann-Whitney test, n. s. stands for not significant, red bars represent the median.



Supplemental Figure 7. Detailed characterization of the relationship of quantitative DNA methylation and MSP for the *SFRP* family members

(A-D) Distribution of MassARRAY-derived DNA methylation values (^{m}C) for the MSP categories U (unmethylated) and M (methylated) for *SFRP1* (A), *2* (B), *4* (C) and *5* (D). Distributions of samples categorized as either U or M by MSP were assessed for single CpG units directly located in the MSP primer sequences as well as for the average of all CpG units located in MSP primer sequences (indicated as mean F+R) and for the entire MassARRAY amplicons (mean amp). For *SFRP1*, MSP primers are located in direct vicinity outside of the MassARRAY amplicon, therefore, only the mean of the entire MassARRAY amplicon was used for comparison.