Supplementary information

Simultaneous Profiling of DNA Mutation and Methylation by Melting Analysis Using Magnetoresistive Biosensor Array

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Table S1: List of ssDNA probes used for mutation and methylation profiling.

GENE	Site	T_m [°C]*	Sequence**
BRAF Exon 11	c.1391G>A	44.7	NH2-C6-5'-(9xT)AAATGATCCAGAT**C**CAATTCTTTGTCC-3'
NM_004333.4	WT	44.7	NH2-C6-5'-(9xT) AATGATCCAGATTCAATTCTTTGTCCC-3'
BRAF Exon 15	c.1799T>A	46.3	NH2-C6-5'-(9xT) CTCCATCGAGATTTC**T**CTGTAGCTAGAC-3'
NM_004333.4	c.1798GT>AA	46.6	NH2-C6-5'-(9xT) TCCATCGAGATTTC**TT**TGTAGCTAGACC-3'
	WT	46.4	NH2-C6-5'-(9xT) TCCATCGAGATTTCACTGTAGCTAGAC-3'
NRAS Exon 2	c.181C>A	45.3	NH2-C6-5'-(9xT) ACTGTACTCTTCTTT**T**TCCAGCTGT-3'
NM_002524.4	c.182A>T	45.5	NH2-C6-5'-(9xT) ACTGTACTCTTCT**A**GTCCAGCTGTA-3'
	WT	45.5	NH2-C6-5'-(9xT) CTGTACTCTTCTTGTCCAGCTGT-3'
KIT Promoter	P1 Meth	46.5	NH2-C6-5'-(9xT) CCCAAAACC**G**C**G**AAC**G**AC-3'
	P1 uMeth	46.4	NH2-C6-5'-(9xT)CCCCCAAAACCACAAACAACAA-3'
KIT Promoter	P2 Meth	46.4	NH2-C6-5'-(9xT) **G**AAC**G**C**G**ACAAAACC**G**AACC-3'
	P2 uMeth	46.5	NH2-C6-5'-(9xT) ACAAACACAACAAAACCAAACCCC-3'
RARB Promoter	P1 Meth	44.0	NH2-C6-5'-(9xT) ATCCTCAAACAACTC**G**CATAAAAAAATTC-3'
	P1 uMeth	43.8	NH2-C6-5'-(9xT)AATCCTCAAACAACTCACATAAAAAAATTCT-3'
RARB Promoter	P2 Meth	45.6	NH2-C6-5'-(9xT) **G**AATCCTACCCC**G**AC**G**ATACC -3'
	P2 uMeth	45.7	NH2-C6-5'-(9xT) AAATCCTACCCCAACAATACCCA -3'
Reference	Positive		NH2-C6-5'-(9xT) TGC GAG CTT CGT ATT ATG GCG -3' TEG Biotin
	Negative		NH2-C6-5'-(9xT) GTGGGGCTAGGTG -3'

*Theoretical melting temperatures (T_m) were calculated with nearest neighbour (NN) model for 10 mM Na^+ ionic concentration. Probes were designed to have matched T_m.

**All probes are amino-labelled to bind to GMR sensor surfaces.

Table S2: PCR primers for amplification of EST cell line genomic DNA.

GENE	Sequence	Product length
BRAF Exon 11	fw: biotin-C6-5'-TTGACTTTTTTACTGTTTTTATC-3'	167bp
NM_004333.4	bw: 5'-ATGTCACCACATTACATACTTAC-3'	
BRAF Exon 15	fw: biotin- C6-5'- TTTTCCTTTACTTACTACACCTC -3'	167bp
NM_004333.4	bw: 5'- GGAAAAATAGCCTCAATTCT -3'	
NRAS Exon 2	fw: biotin- C6-5'- CAAGTGGTTATAGATGGTGA -3'	110bp
NM_002524.4	bw: 5'- AGGAAGCCTTCGCCTGTCCT -3'	
KIT Promoter*	fw: biotin- C6-5'- GGGAGGAGGGGTTGTTGTT -3'	82bp
	bw: 5'- TTCCAACTCTCCCCCAAATACAAC -3'	
RARB Promoter*	fw: biotin- C6-5'- GGTTTATTTTTTGTTAAAGGGG -3'	179bp
	bw: 5'- AAAAATCCCAAATTCTCCTTC -3'	

* *KIT* and *RARB* primers were designed to amplify bisulphite converted promoter region.

Table S3: Primers for pyrosequencing *KIT* and *RARB* promoter regions of bisulphite converted DNA from EST cell lines.

Gene	Sequence
KIT Promoter	fw: 5'- GTGGAAAGGTGGAGAGAGAAA -3' bw: biotin-5'- TTCCAACTCTCCCCCAAATACAAC -3' S1: 5'- GAGGAGGGGTTGTTG -3'
RARB promoter	fw: biotin- C6-5'- GGTTTATTTTTTGTTAAAGGGG -3' bw: 5'- AAAAATCCCAAATTCTCCTTC -3' S1: 5'- ACATCCCAATCCTCA -3' S2: 5'- ATACTTACAAAAAACCTTCC -3'

Table S4: Parameters from linear fitting of ΔT_m vs. methylation density by pyrosequencing (Figure 5). Numbers in parenthesis are standard errors on the last digits from the fitting routine.

Location	Slope [°C/%]	Intercept [°C]	R^2
KIT p1	0.22(1)	−9(1)	0.97
KIT p2	0.25(1)	−8.8(7)	0.98
RARB p1	0.075(5)	−5.1(2)	0.97
RARB p2	0.22(2)	−9.3(7)	0.94

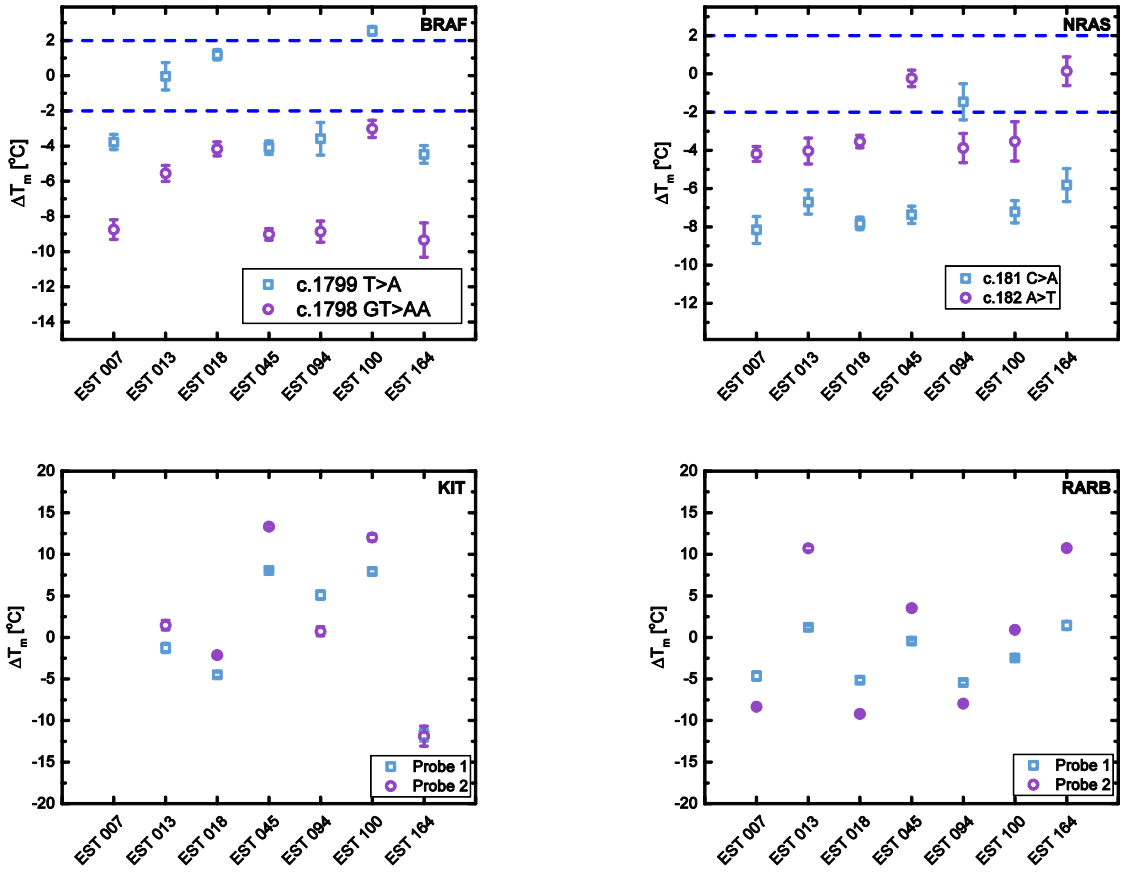

Figure S1: ΔT_m measured for all investigated probes for the seven investigated cell lines. Error bars are one standard deviation (n = 4-6).

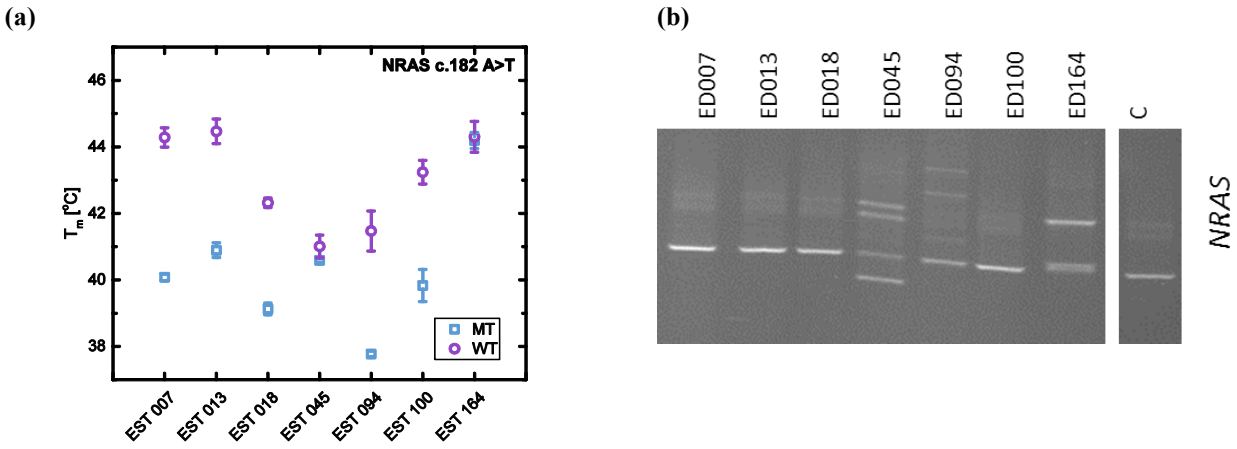

Figure S2: (a) Absolute melting temperatures (T_m) measured for WT and MT probes targeting *NRAS* c.182 A>T mutation. T_m (WT) and T_m (MT) for EST045 showed approximately the same value of about 41°C. For both probes, the melting temperature was significantly lower than the maximum one (about 44°C). This can be explained by the target having a mutation different from the one targeted by the MT probe. **(b)** Denaturing gradient gel electrophoresis of *NRAS* exon 2 PCR products. EST045 is heterozygous mutant for a different mutation than the one present in EST164.

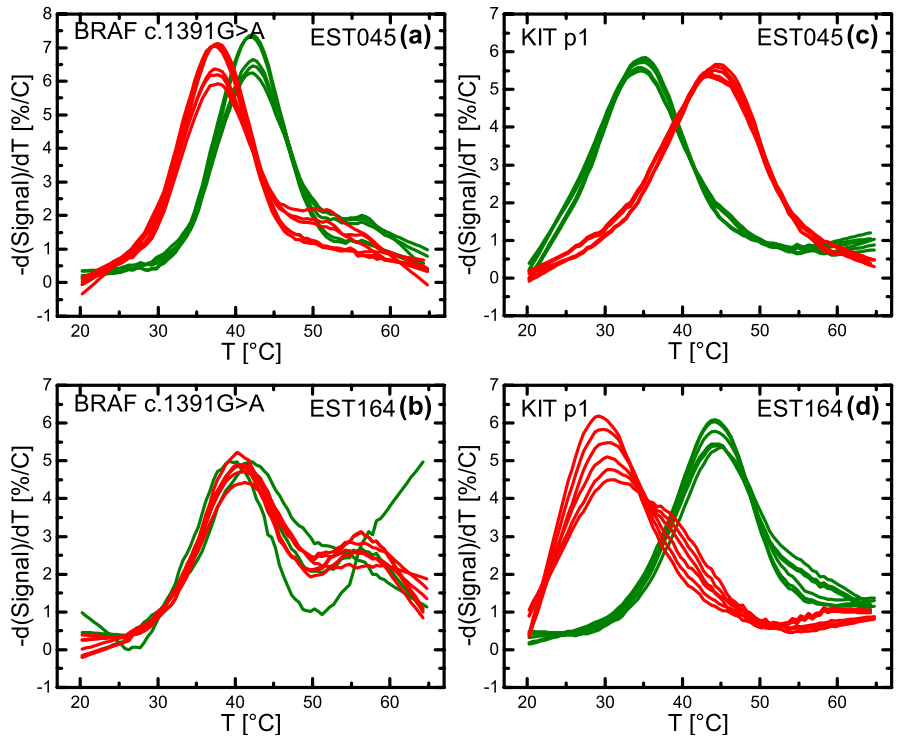

Figure S3: First temperature derivatives of the melting curves of Figures 2-3. Melting curves measured for **(a,b)** *BRAF* c.1391G>A mutation and **(c,d)** *KIT* p1 methylation sites. The curve were measured for **(a,c)** EST045 and **(b,d)** EST164 cell lines respectively. The curves show reproducible single peaks in most cases. In panel **(b)** two peaks are clearly visible for both WT (green) and MT (red) probes. This is compatible with the expectation for a heterozygous mutant cell line.

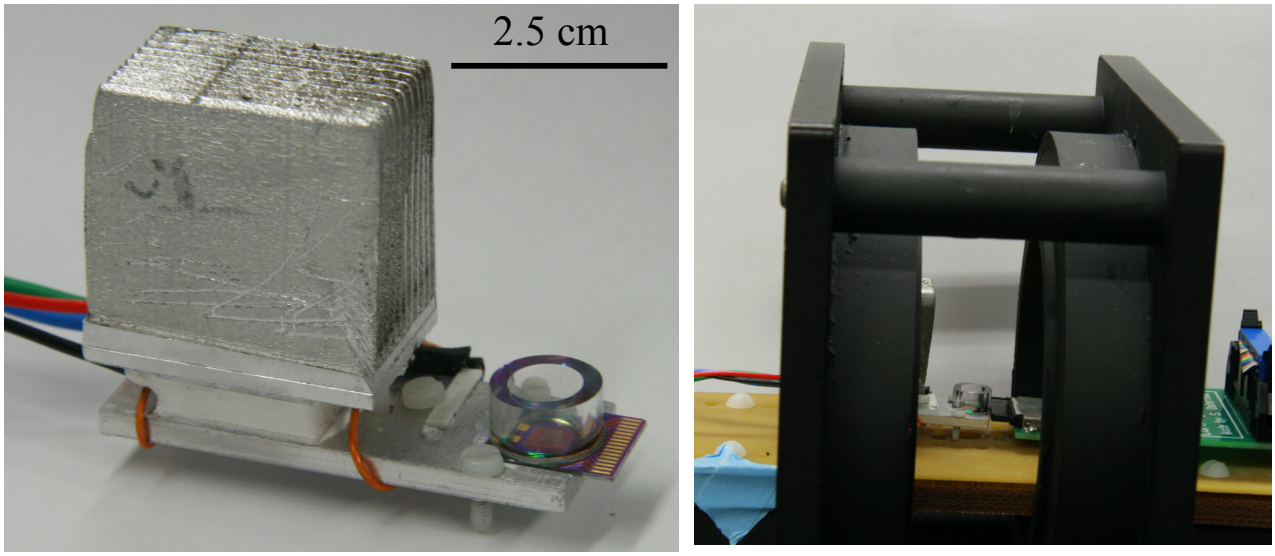

Figure S4: (Left) GMR biosensor array mounted in the temperature controlled holder. The temperature of the aluminium holder is controlled by a Peltier element and a Pt1000 thermometer. **(Right)** Chip and temperature control system mounted in the Helmholtz coils for magnetic measurements.

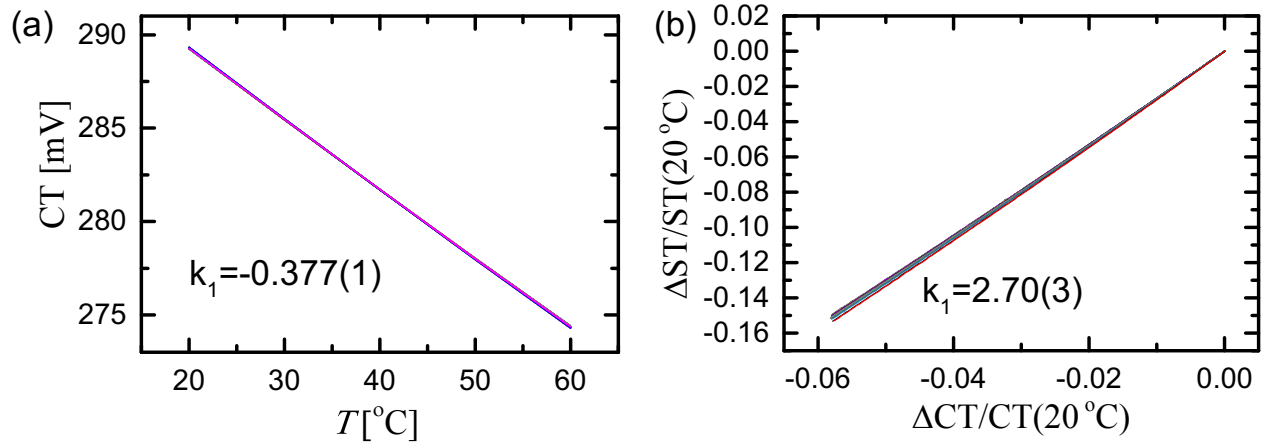

Figure S5: (a) Measured temperature dependence of sensor center tone (CT). The signal was measured at 20,40,50,60°C. All sensors showed a linear temperature dependence and a similar first order polynomial coefficient k_1. **(b)** Temperature dependence of the side tone (ST) signal. Due to the results of panel (a), the dependence is plotted against CT. The curve was measured during a downward temperature ramp from 65 °C to 20 °C. In only one chip (data not shown) the temperature dependence was found to be non-linear at high temperature (T>45 °C) and a 5th order polynomial fitting was used to correct the data.