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Supplemental Data

DNA Methylome of Familial Breast Cancer Identifies Distinct Profiles Defined by Mutation Status

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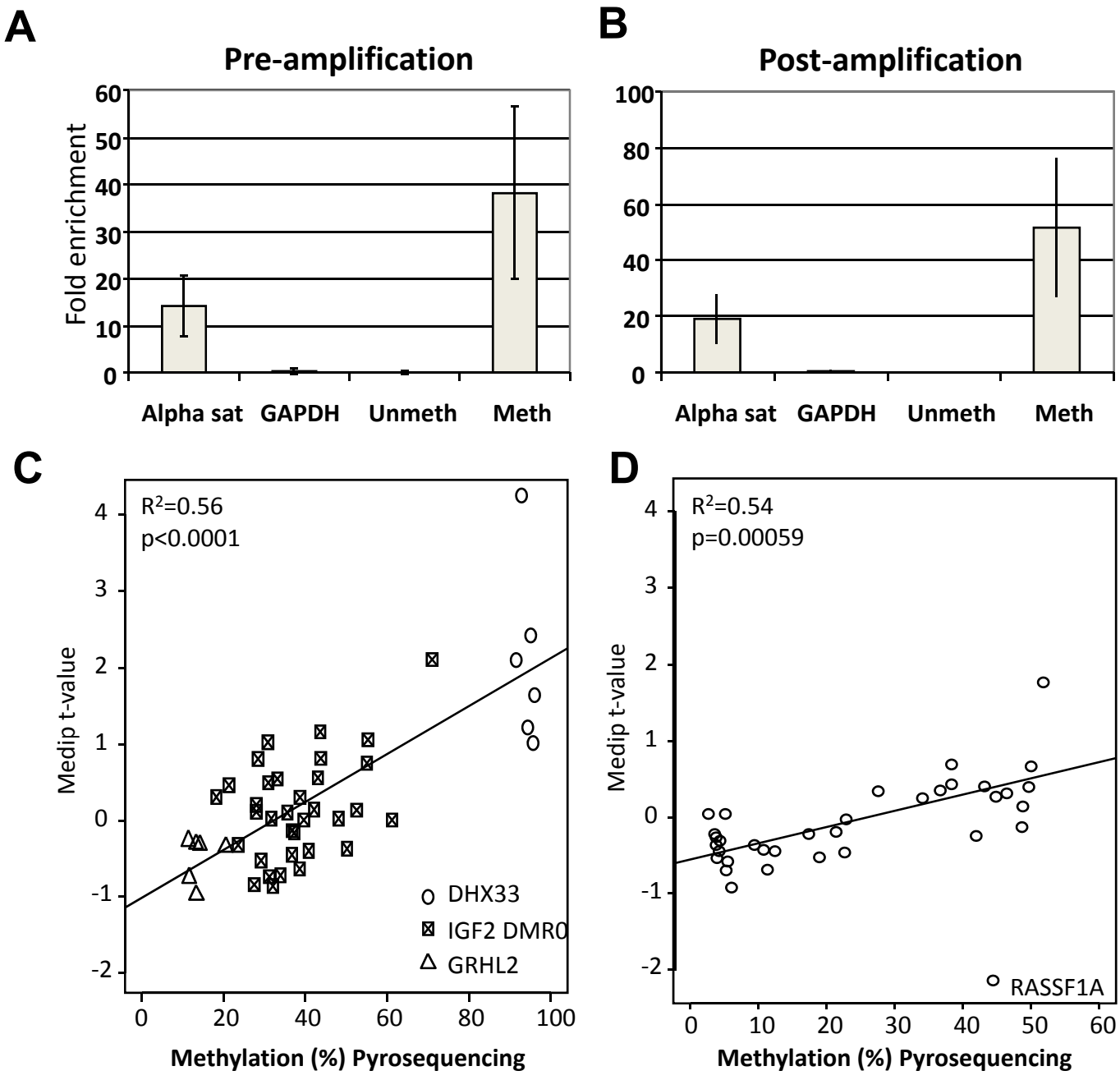
A
Pre-amplification
Fold enrichment
60
50
40
30
20
10
0
Alpha sat
GAPDH
Unmeth
Meth
B
Post-amplification
100
80
60
40
20
0
Alpha sat
GAPDH
Unmeth
Meth
C
R2=0.56
p<0.0001
Medip t-value
4
3
2
1
0
-1
-2
0
20
40
60
80
100
Methylation (%) Pyrosequencing
DHX33
IGF2 DMR0
GRHL2
D
R2=0.54
p=0.00059
Medip t-value
4
3
2
1
0
-1
-2
0
10
20
30
40
50
60
Methylation (%) Pyrosequencing
RASSF1A

Figure S1. Validation of meDIP microarray analysis. A) Pre-amplification analysis of meDIP enrichment by quantitative PCR for known methylated sequences (Alpha Sat repeats and spiked in methylated control) and known unmethylated sequences (GAPDH promoter and spiked in unmethylated control). Y-axis represents fold-change over total input. **B)** Post-amplification analysis of meDIP enrichment by quantitative PCR. **C)** Pyrosequencing-based methylation analysis of an unmethylated gene *GRHL2* (n=6), a methylated gene *DHX33* (n=6) and the *IGF2* imprinted region DMR0 (n=33) showing strong correlation with the meDIP microarray t-values (y-axis). **D)** Pyrosequencing-based methylation analysis of the *RASSF1A* promoter shows strong correlation with the microarray t-values in 33 tumor samples.

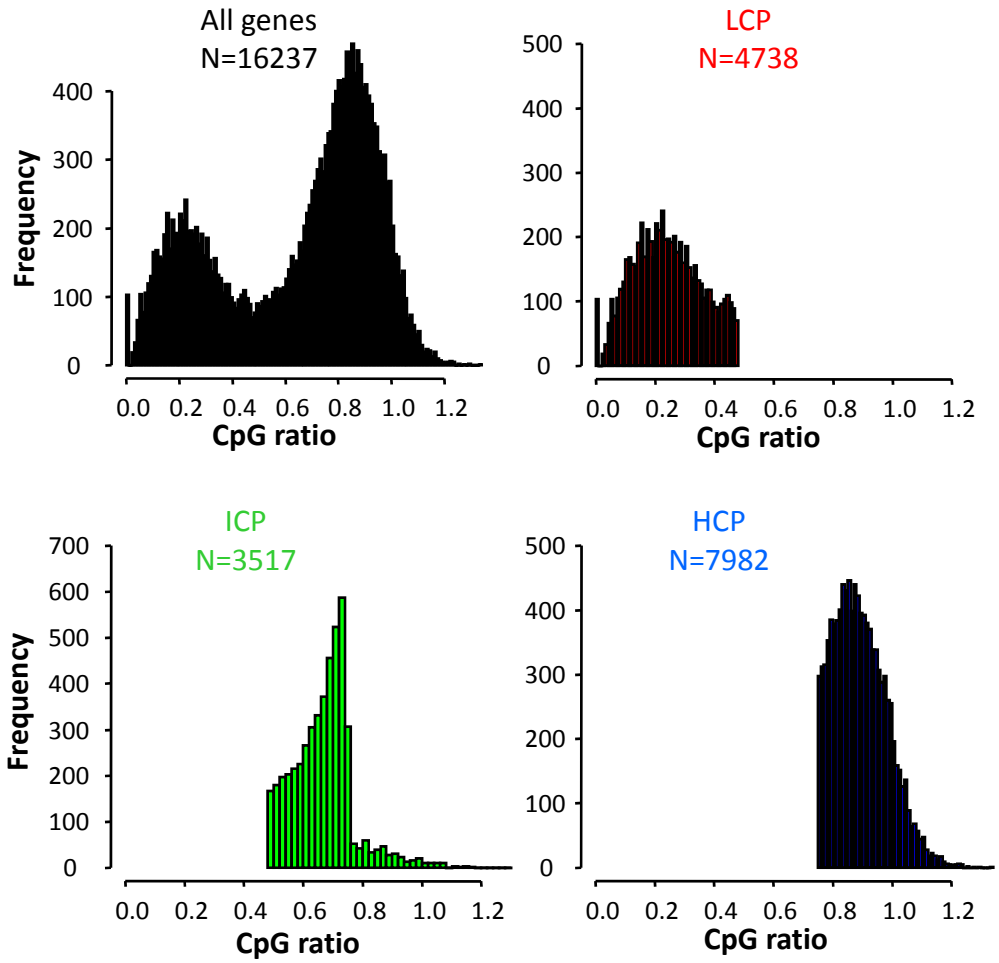
All genes
N=16237
LCP
N=4738
ICP
N=3517
HCP
N=7982
Frequency
CpG ratio
0
100
200
300
400
500
600
700
0.0
0.2
0.4
0.6
0.8
1.0
1.2

Figure S2. Separation of genes by CpG ratio. All transcription start sites were divided into low-CpG content promoters (LCP), intermediate-CpG content promoters (ICP) and high-CpG content promoters (HCP) as previously described [16]. X-axis represents CpG ratio.

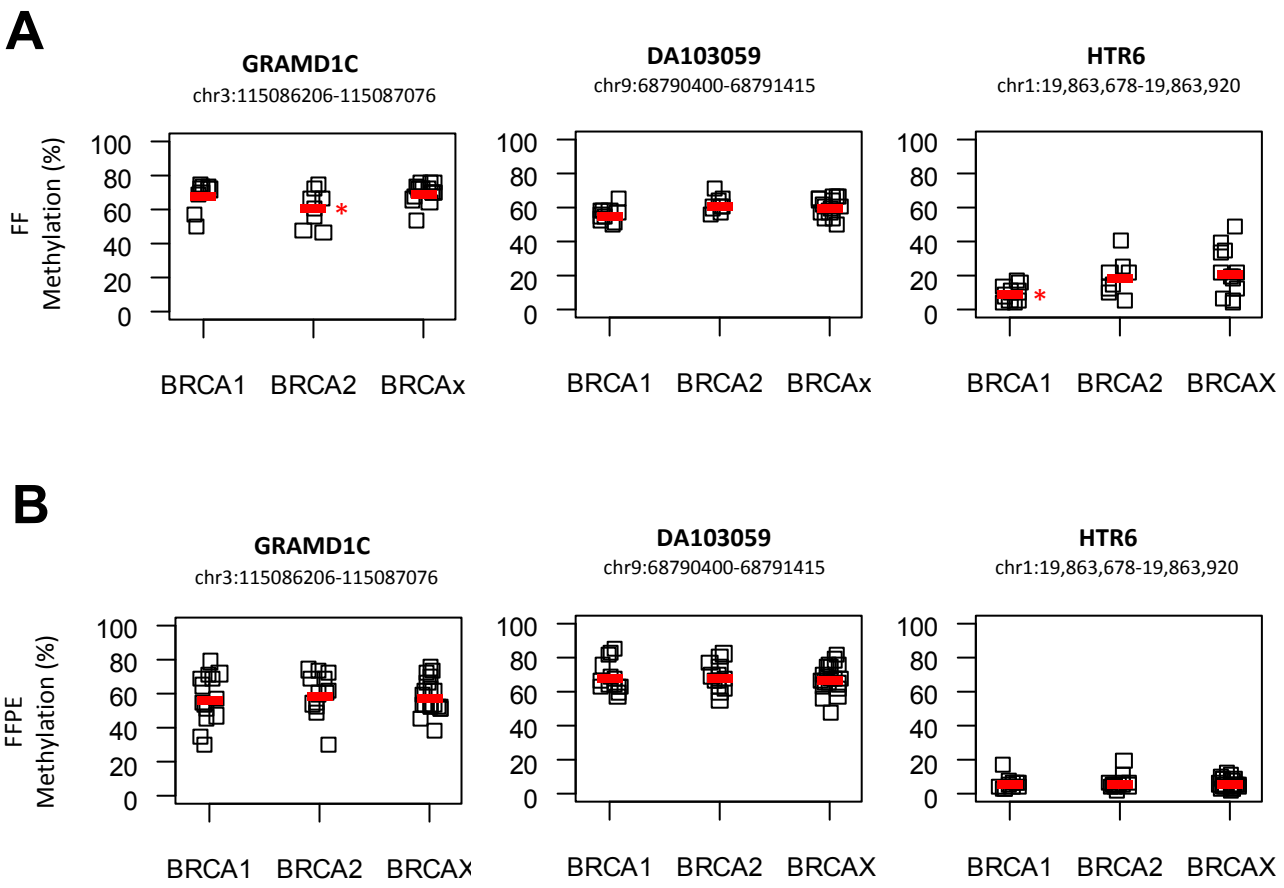
A
GRAMD1C
chr3:115086206-115087076
DA103059
chr9:68790400-68791415
HTR6
chr1:19,863,678-19,863,920
FF
Methylation (%)
100
80
60
40
20
0
BRCA1
BRCA2
BRCAx
BRCAX
B
FFPE
Methylation (%)

Figure S3. DNA methylation profiles of *BRCA1*, *BRCA2* and BRCAx tumors. A) Pyrosequencing-based methylation analysis of *RASSF1A, SGK1, DA103059, GRAMD1C, LRRC55* and *LHCGR* in 33 tumors. The median methylation levels are indicated by the red line. Genomic locations for the regions presented are from Human Mar. 2006 Assembly (hg18). Stars indicate statistically significant differences between groups at $p<0.05$ and $p<0.01$ as indicated, wilcoxon signed rank sum test. **B)** Pyrosequencing-based methylation analysis in 47 FFPE tumor DNA samples.

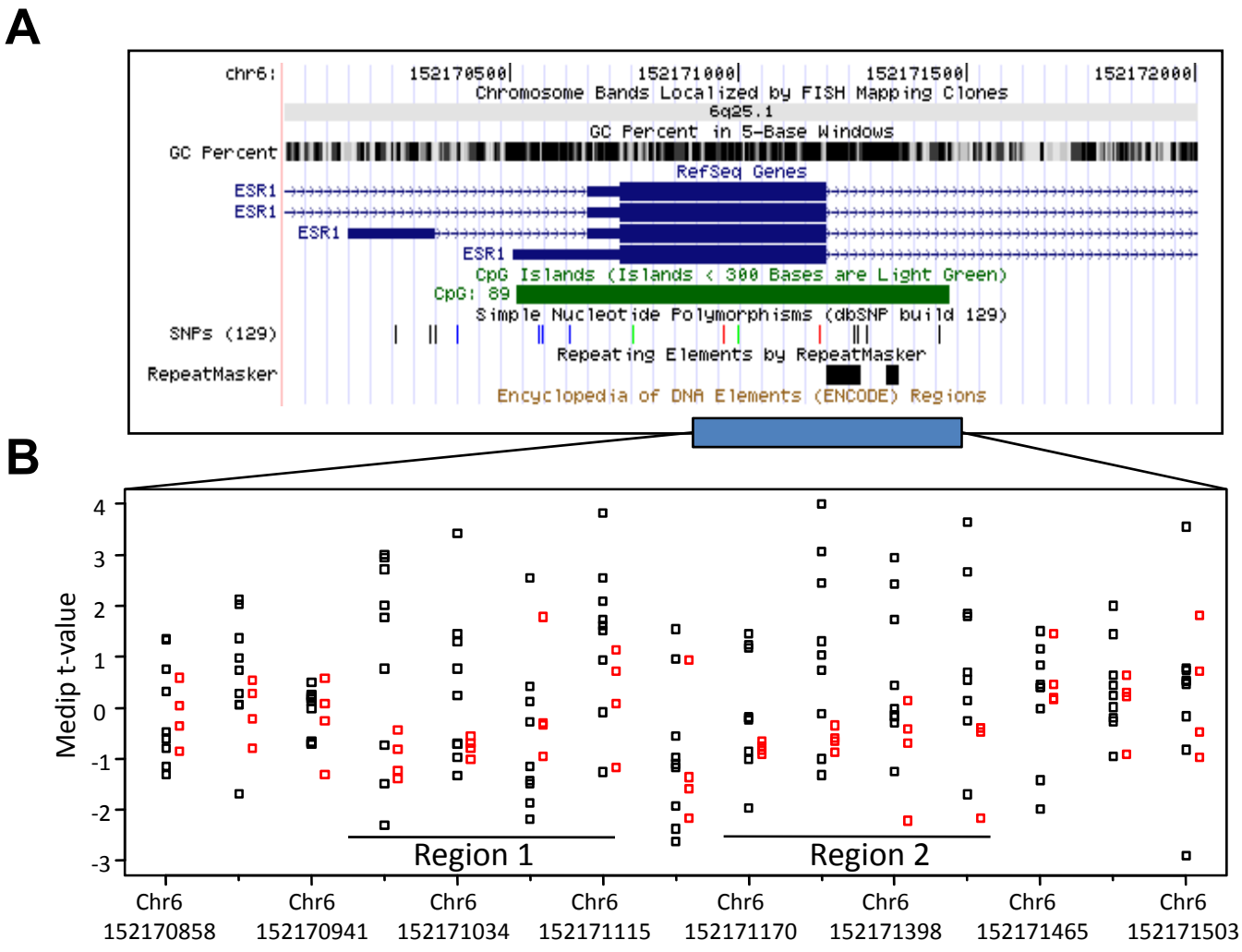
A
chr6:
152170500
152171000
152171500
152172000
Chromosome Bands Localized by FISH Mapping Clones
6q25.1
GC Percent in 5-Base Windows
GC Percent
RefSeq Genes
ESR1
ESR1
ESR1
ESR1
CpG Islands (Islands < 300 Bases are Light Green)
CpG: 89
Simple Nucleotide Polymorphisms (dbSNP build 129)
SNPs (129)
Repeating Elements by RepeatMasker
RepeatMasker
Encyclopedia of DNA Elements (ENCODE) Regions
B
Medip t-value
4
3
2
1
0
-1
-2
-3
Region 1
Region 2
Chr6
152170858
Chr6
152170941
Chr6
152171034
Chr6
152171115
Chr6
152171170
Chr6
152171398
Chr6
152171465
Chr6
152171503
C
Region 1
Region 2
Methylation (%)
20
15
10
5
0
BRCA1
BRCA2
BRCAx
*
P=0.0056

Figure S4. Fine mapping of increased methylation of *ESR1* in ER negative BRCA1 mutation carriers. A) UCSC genome browser image of *ESR1* CpG island and flanking regions. **B)** Probe-wise analysis of DNA methylation across the *ESR1* CpG island identifies two candidate regions of increased methylation in ER negative *BRCA1* mutation carriers (white boxes, n=9) compared to ER negative non-BRCA1 mutation carriers (red boxes, n=4). **C)** Pyrosequencing-based methylation analysis of region 1 (chr6:152171022-152171141) and region 2 (chr6:152171256-152171402) validates the significant increase in methylation of region 2 in ER negative tumors from *BRCA1* mutation carriers compared to ER negative tumors from non-BRCA1 mutation carriers.

Sup Fig.5

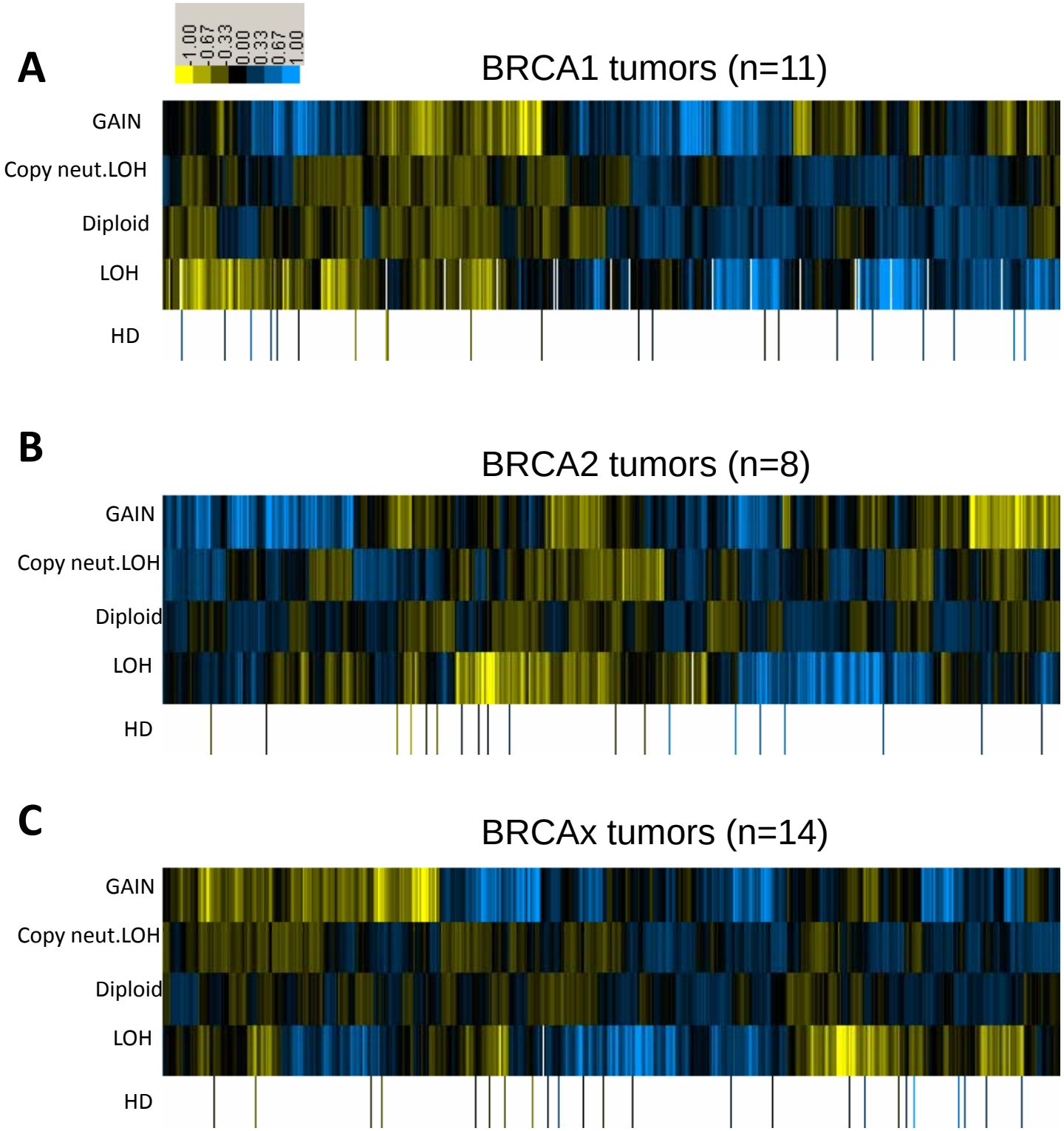
A
-1.00
-0.67
-0.33
0.00
0.33
0.67
1.00
BRCA1 tumors (n=11)
GAIN
Copy neut.LOH
Diploid
LOH
HD
B
BRCA2 tumors (n=8)
GAIN
Copy neut.LOH
Diploid
LOH
HD
C
BRCAx tumors (n=14)
GAIN
Copy neut.LOH
Diploid
LOH
HD

Figure S5. Copy Number versus Methylation in BRCA1, BRCA2 and BRCAx tumors. Heatmap of median methylation levels (medip t-values) for each gene (n=16237) broken down into copy number groups (y-axis) of tumors showing homozygous deletion, loss of heterozygosity, normal diploidy, copy neutral LOH or gains in BRCA1 **(A)**, BRCA2 **(B)** or BRCAx tumors **(C)**.

Supplementary Tables (see attached Excel spreadsheets)

Table S1: Tumor Information

Table S2: Primers used in this study

Table S3: List of 156 genes that differentiate BRCAx subgroups

Table S4: List of frequently hypermethylated genes in breast cancer

Table S5: Chi squared analysis of methylation versus copy number

	LOH	**Copy neutral LOH**	**GAIN**
Higher Methylation (vs diploid)	**600**	**125**	**1033**
Lower Methylation (vs diploid)	**39**	**112**	**48**
$\chi^2 = 407.3$; $p<2.2$ e-16			

Table S6: List of all genes correlation between methylation level and gene expression level.