Flanagan, JM; Munoz-Alegre, M; Henderson, S; Tang, T; Sun, P; Johnson, N; Fletcher, O; Silva, ID; Peto, J; Boshoff, C; Narod, S; Petronis, A (2009) Gene-body hypermethylation of ATM in peripheral blood DNA of bilateral breast cancer patients. Human molecular genetics, 18 (7). pp. 1332-1342. ISSN 0964-6906 DOI: https://doi.org/10.1093/hmg/ddp033

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Supplementary Data

Supplementary Table 1. Gene list and sizes of genes tiled on the custom microarray

Supplementary Table 2. Genomic locations of 181 MVPs within the 17 candidate breast cancer susceptibility genes and 38 peripherally targeted genes.

Supplementary Table 3. Primers used in this study.

Supplementary Table 4. Cell lines used in this study.

Supplementary Figure 1. Methylation microarray analysis of seventeen genes reveals methylation variability in repetitive elements. A) Schematic of each of the genes tiled on the custom microarray. B) Example of an inter-individual methylation variable position (MVP) (black bar above) within the gene-body of the ATM gene showing an association with the repetitive element (grey bars below).

Supplementary Figure 2. Methylation microarray data. A) Methylation microarray data as presented on the UCSC genome web browser via custom .WIG and .BED files online. B) Schematic of Methylation microarray data as presented on the UCSC genome web browser. Each MVP is represented by a vertical pink bar, genes are coloured dark blue (no intragenic MVPs), light blue (very few intragenic MVPs) or pink (containing numerous intragenic MVPs).

Supplementary Figure 3. Supporting statistical analysis of ATM mvp2b in peripheral blood DNA. Methylation values for cases and controls were randomly separated into two groups and the Wilcoxon signed rank test was used to calculate the
test statistic, W. Random sampling was performed 1000 times (median simulated $W = 17935$) compared to the observed $W=14605$. The actual P-value was calculated as $p=0.002$.

**Supplementary Figure 4. Correlation between age and methylation for the ATM mvp2b.** A) Distribution of ages in cases and controls. Methylation was plotted against age at blood draw for controls (B) and cases (C) or age at first (D) or second (E) diagnosis for cases.

**Supplementary Figure 5. Methylation analysis of ATM mvp2b in blood cell fractions reveals no cell-type specific methylation.** B-cells, T-cells and monocytes were isolated from the peripheral blood mononuclear cells (PBMCs) from two unaffected controls and pyrosequencing based methylation analysis of ATM mvp2b was performed. Methylation percentages represent an average of four CpG dinucleotides. As a positive control we have assayed a region, chr13:76462565-76463564, that was previously described as differentially methylated between B cells and T cells, shown as the theoretical value (+) (21).
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### Supplementary Table 3 (primers)

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          (outer)ATM-P5-2R  GAAGTGGTTATGATTTTGGTT
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          (inner)ATM-P5-2R2  gacGGGACACCGCTGATCGTTATTTT

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ATM-mvp7  P7-1  ATM-P7-1F  gacGGGACACCGCTGATCGTTTAcct
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Sequencing Primers

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          ATM-P1-1pos3._4 .5
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          ATM-P1-2SEQ pos 4 5
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          ATM-P2-2POS1_2
          ATM-P2-2pos_3
          ATM-P2-2pos_4
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| ATM-mvp7 | P7-1 | ATM-P7-1-SEQunique | TTTGTTAAATGGGGATAATA |
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<tr>
<td>RH1 Human Ewing sarcoma</td>
<td>Sarcoma</td>
<td></td>
</tr>
<tr>
<td>HT1080 Human fibrosarcoma</td>
<td>Sarcoma</td>
<td></td>
</tr>
<tr>
<td>SW684 Human fibrosarcoma</td>
<td>Sarcoma</td>
<td></td>
</tr>
<tr>
<td>A 431 Human Epidermoid Carcinoma</td>
<td>skin</td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Figure 1.
BRCA1  BRCA2  CHEK2  ESR1
SFN  CDKN2A  HIC1  ATM
GSTP1  CDH13  PGR  SFRP1
HSD17B4  TP53  MLH1  CDH1
RAR
Gene lengths not to scale

Supplementary Figure 2A.
Supplementary Figure 2B.
Supplementary Figure 3

\[ P = 1 - \left( \frac{\text{#W}_{\text{simulated}} > \text{W}_{\text{observed}}}{1000} \right) \]

\[ P = 1 - \left( \frac{998}{1000} \right) \]

\[ P = 0.002 \]
Age at blood draw distributions

Supplementary Figure 4
Supplementary Figure 5

ATM-mvp2b

chr13:76462565-76463564

Methylation (%)

B cell  T cell  Monocytes  PBMCs

Individual A  Individual B  theoretical value