# SINGLE NUCLEOTIDE POLYMORPHISMS INSIDE microRNA TARGET SITES INFLUENCE TUMOR SUSCEPTIBILITY

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## SUPPLEMENTAL MATERIAL

## Supplemental Material and Methods

**Patient Information.** Cases were 335 female patients affected with invasive BC. Familial BC cases were ascertained through the Medical Genetics Unit of the INT Milan, as eligible for mutation testing in *BRCA1* and *BRCA2* genes, based on criteria including family history and age at cancer diagnosis (1). Mutation analysis was carried out as previously described (1). Only individuals who tested negative for deleterious mutations in coding sequences of both genes were included in the study. This group included 169 women with BC (median age at diagnosis: 44; range: 21- 77). Sporadic BC cases included 166 consecutive women at first diagnosis of BC, surgically treated at INT Milan between November 2004 and August 2005 and unselected for family history of cancer (median age at diagnosis: 56; range: 23-97). Controls were 186 Italian female blood donors recruited through the Immunohematology and Transfusion Medicine Service of INT Milan (median age: 56; range: 48-71).

**Statistical Analysis.** For multivariate analysis, initially a full model including SNPs with p value less than 0.1 in the univariate analysis and age were fitted, then a backward selection procedure was used for model selection until all variables retained in the model were significant at the 0.05 significance level. The univariate and multivariate logistic regression analyses were performed for the following comparisons: Familiar + Sporadic vs. Control, Familiar vs. Control, Sporadic vs. control and Familiar vs. **Sporadic.**

**RNA extraction, Retrotranscription and Realtime PCR.** Cells total RNA was isolated from using TRIzol reagent (Invitrogen). For quantification of transfected and/or endogenous mature miRNA levels (data not shown) we used TaqMan® MicroRNA Reverse Transcription Kit, TaqMan® MicroRNA assays together with TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems). We employed the 2-Delta Ct method to calculate the relative abundance of microRNA compared with RNU6B expression (2). Realtime PCR reaction and analyses were carried out in 96-well optical reaction plates using iQ5 MultiColor Detection system (Biorad).

## Supplemental Figures

**Figure S1.** Realtime analyses of endogenous miRNA levels in breast samples and cell lines. miRNAs tested for their interaction with BC associated SNPs in the main text are expressed in breast samples (normal and tumor) from an independent set of patients. Samples number 3, 6, 9 are paired (normal and tumor). MM231, MBMDA231.

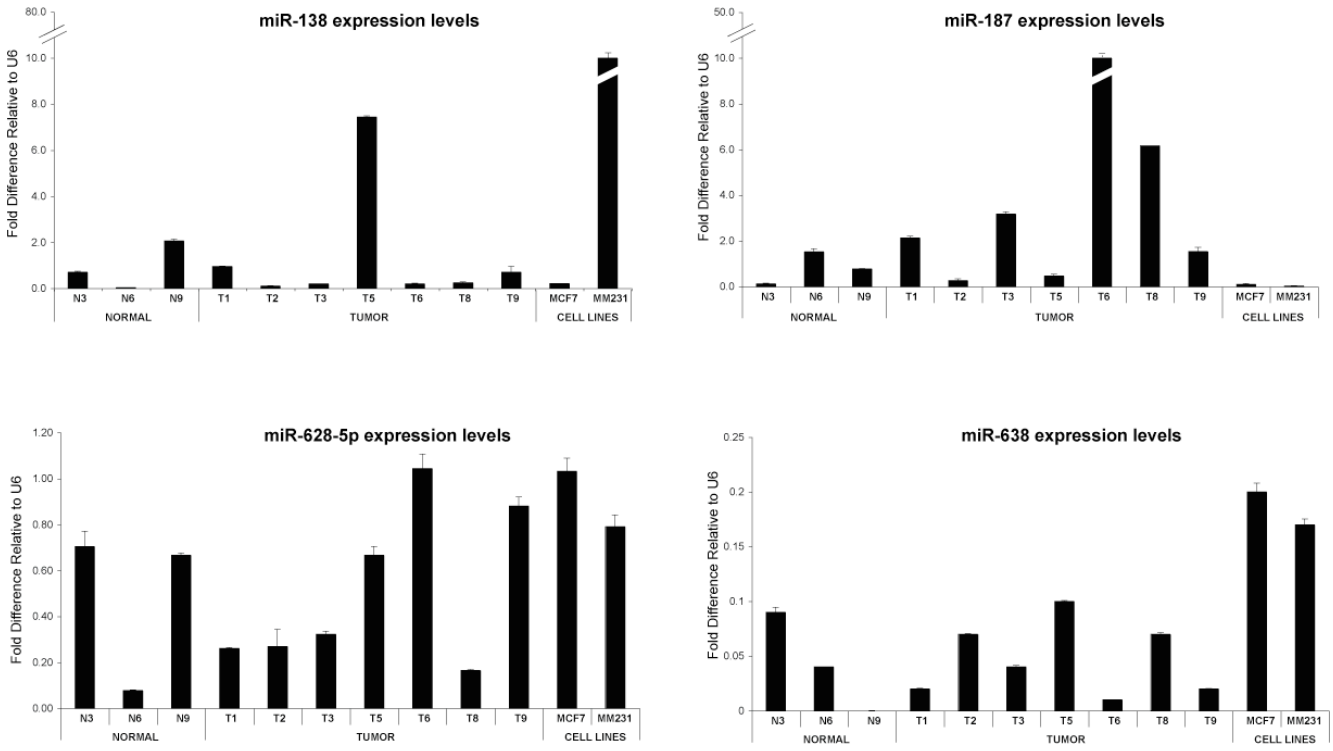


**Figure S2.** MFE change distribution for SNPs located either in 3' UTRs, CDS or 5' UTRs. In red is marked the ± 8% threshold in MFE change, which corresponds for all three distributions to the peaks of the bimodal distribution and to the 20 and 80 percentiles.

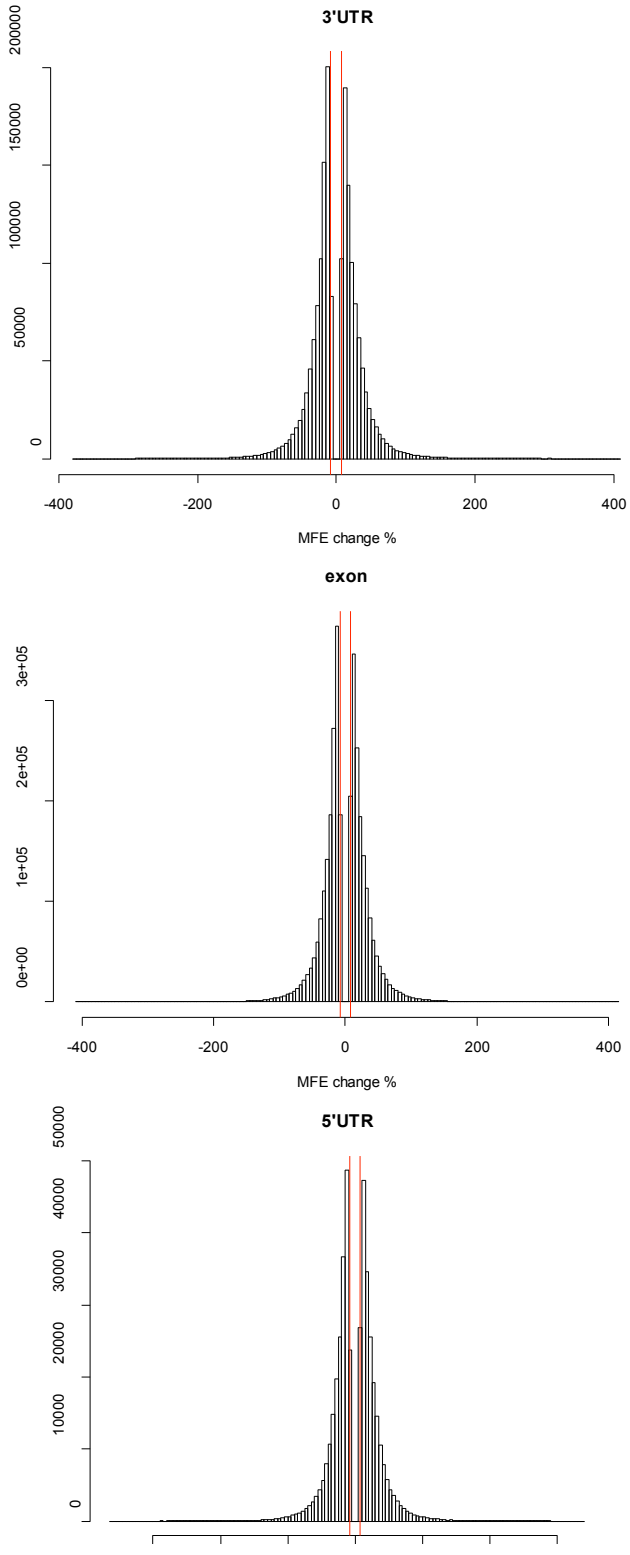


**Figure S3.** Luciferase reporter assay for pGL3-rs28382751-XIAP. **A)** miRNA::mRNA duplex for rs28382751-XIAP::*miR-542-5p* interaction is shown, with the active allele (green) or the non-active allele (red) highlighted. **B)** Luciferase reporter assay for pGL3-rs28382751 co-transfected either with scrambled negative control and *miR-542-5p*. Luciferase activity is expressed relative to scrambled negative control (=1); values represent the average +/- standard deviation of 3 independent experiments performed in six replicates.

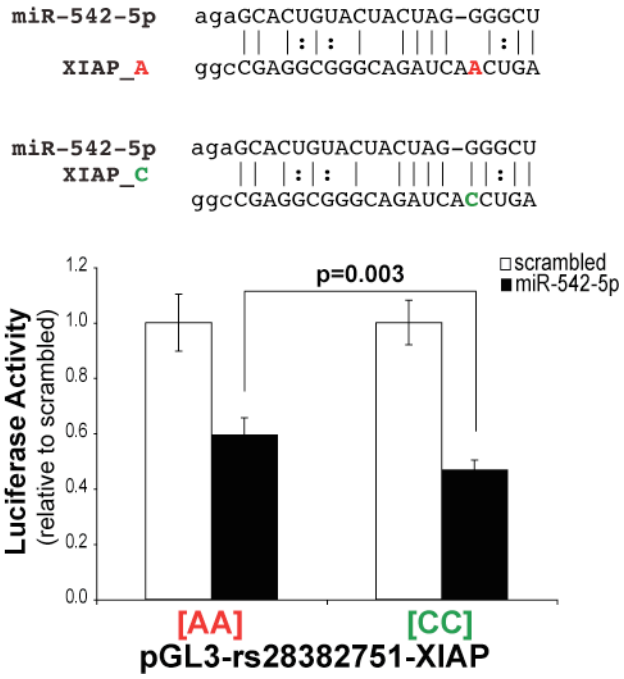
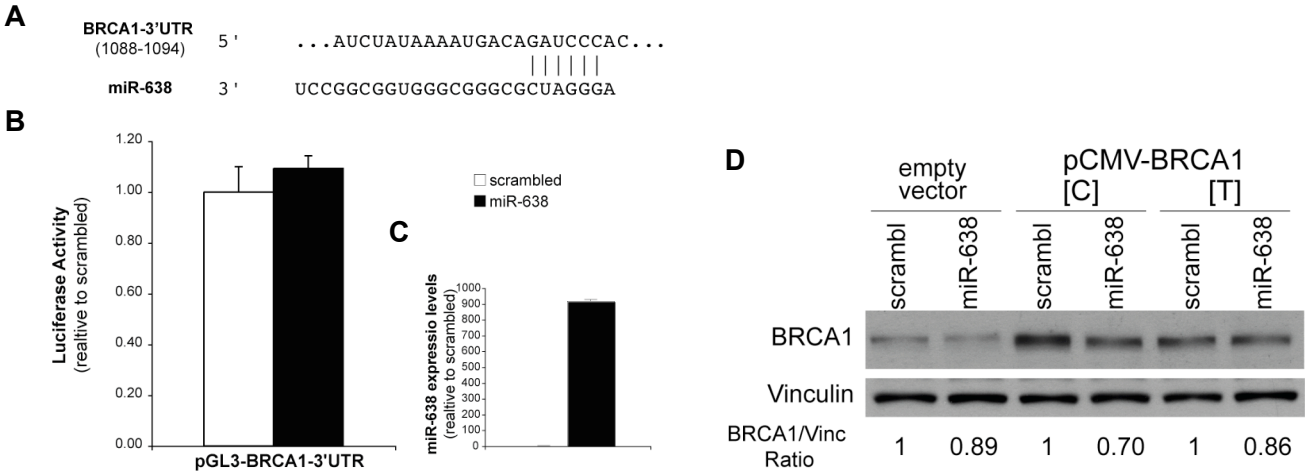


**Figure S4.** Effects on luciferase activity and protein expression of a conserved miR-638 target inside BRCA1 3' UTR. **A)** Targetscan prediction for a *miR-638* conserved target site inside BRCA1 3' UTR. **B)** Luciferase reporter assay for pGL3-BRCA1-3' UTR co-transfected either with scrambled negative control or *miR-638*. Luciferase activity is expressed relative to scrambled negative control (=1); values represent the average +/- standard deviation of 3 independent experiments performed in six replicates. miR-638 does not repress the activity of the luciferase construct containing BRCA1 3' UTR. **C)** Over-expression of miR-638 was validated by realtime PCR. **D)** WB for BRCA1 in MCF7 cells co-transfected with scrambled negative control or *miR-638* together with a pCMV empty vector or pCMV BRCA1 expressing vector without its 3' UTR. BRCA1 CDS was mutagenized (QuikChange Site-Directed Mutagenesis Kit, Stratagene) to obtain two different pCMV-BRCA1 vectors encoding the rs777917 [C] and [T] alleles. Suppression of BRCA1 protein levels was achieved by *miR-638* in the absence of BRCA1 3' UTR and was greater with rs777917 [C] active allele.



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