

## **Identification of BAF57 mutations in human breast cancer cell lines**

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## Summary

Accumulating genetic and biochemical evidences support a role for the SWI/SNF chromatin remodeling complex in cancer development and multiple core subunits of these complexes have been found to function as tumor suppressor genes. The core SWI/SNF subunit BAF57 mediates direct interactions with estrogen and androgen receptors (ER and AR) regulating their transcriptional activity. *BAF57* gene maps to chromosome band 17q21 in close proximity to the *BRCAl* gene. This locus has been associated with frequent loss of heterozygosity (LOH) and allelic imbalance in breast cancers; however *BRCAl* mutations are rare events in sporadic breast cancer with LOH in the region, suggesting that another tumor suppressor gene resides in this area. All these reasons prompted us to screen for mutations in the *BAF57* gene using a panel of the most commonly used human breast cancer cell lines. All cell lines analyzed contain wild-type copies of BAF57 gene with the only exception of the breast ductal carcinoma cell line BT549. Sequencing of genomic DNA and cDNA generated from BT549 mRNA demonstrated the presence of a CA dinucleotide insertion in exon 5 of *BAF57*. The absence of wild-type *BAF57* alleles indicates that this is a biallelic inactivating mutation that causes a frameshift and as a consequence a premature stop codon leading to a truncated BAF57 protein. A functional characterization of the truncated BAF57 showed that it has lost the ability to bind to ER but still binds to the nuclear receptor coactivator SRC1e. Furthermore, we observed that the expression of the truncated BAF57 increased the ability of SRC1e to potentiate transcriptional activation by ER $\alpha$ , suggesting that mutations in BAF57 could contribute to the oncogenic transformation in breast cancer cells.

## **Introduction**

Estrogens play a central role in mammary gland development and morphogenesis but they also are important in the proliferation and progression of breast cancer (reviewed in [1]). Although there is growing evidence that estrogens can act through nongenomic pathways most described estrogen actions are mediated by the estrogen receptor alpha ( $ER\alpha$ ) and beta ( $ER\beta$ ) acting as ligand-dependent transcription factors. Estrogen receptors belong to the nuclear receptor superfamily [2] and they regulate many physiological processes in response to its natural ligand,  $17\beta$ -estradiol (E2). Endocrine therapy with ER antagonists, and specially tamoxifen, is the most effective systemic treatment for all stages of ER-positive breast cancer. However, many patients present primary resistance to endocrine therapy despite expressing high levels of ER and all patients with advanced breast cancer eventually develop resistance to therapy ([3] and references therein). To understand the basis of the appearance of this resistance we need to elucidate the detailed molecular mechanisms that regulate ER activity.

Upon ligand binding, ERs bind to specific DNA sequences (EREs) present in the promoters of estrogen-dependent genes, triggering the recruitment of the enzymatic activities responsible for the regulation of gene expression. To overcome the repressive environment that represents the tightly packed chromatin fiber, ERs recruit many chromatin remodeling enzymes that can be divided into two distinct classes; first, ATP-dependent chromatin remodeling complexes that use the energy of ATP hydrolysis to modify the location and association of nucleosomes with DNA, and second, enzymes that catalyze covalent posttranslational modifications in histones. These proteins are named collectively

nuclear receptor coregulators and they act in a concerted manner contributing to the reorganization of the chromatin templates and the recruitment of basal transcription factors and RNA polymerase II [4].

Several studies have demonstrated that mammalian SWI/SNF, an ATP-dependent chromatin remodeling complex, can enhance the transcriptional activity of the ER and several other nuclear receptors [5-10]. The SWI/SNF family comprises a number of large multiprotein complexes containing between eight to ten subunits. These complexes always contain a central catalytic subunit, BRM/SNF $\alpha$  or BRG1/SNF $\beta$ , and several other variable BRG1-associated factors (BAFs) that contribute to the enzymatic activity of the complex and facilitate the recruitment to specific transcription factors although the detailed functions of each individual subunit remain to be determined. Growing evidence indicates that SWI/SNF has a role in cancer development and several subunits either act as tumor suppressors or are required for the regulation of other tumor suppressor genes (Reviewed in [11, 12]). For instance, the SWI/SNF complex has been shown to associate with BRCA1 through a direct interaction with BRG1/SNF $\beta$  subunit and mutations in BRG1/SNF $\beta$  abrogated stimulation of transcription by BRCA1 [13]. Mutations in the *BRCA1* gene confer an increased risk for estrogen- and androgen-responsive cancers [14]. *BRCA1* maps to chromosome band 17q21, a locus associated with frequent loss of heterozygosity (LOH) and allelic imbalance in breast cancers [15]. In sporadic breast cancer the *BRCA1* gene is rarely mutated in samples with LOH in the locus [16], suggesting that other genes in this region might act as tumor suppressors. Interestingly, the gene that encodes BAF57, another SWI/SNF subunit, also maps to chromosome band 17q21. We have previously

demonstrated that BAF57 interacts directly with ER and also with all members of the p160 family of nuclear receptor coactivators [10]. The interaction between BAF57 and ER is regulated by estrogen and BAF57 was shown to be essential for the coactivation of ER carried out by the p160 coactivators using a human breast ductal carcinoma cell line (BT549) reported to be negative for the BAF57 protein [17]. Moreover, BAF57 was recruited to estrogen-responsive promoters in a ligand-dependent manner, suggesting that the *in vivo* functions of ER as transcription factor might depend on the recruitment of SWI/SNF complexes by means of an interaction with BAF57. A previous report analyzed the BAF57 protein status in a number of human cell lines commonly used as *in vitro* models in breast cancer research [17]. In light of the new data reinforcing a role for BAF57 in the control of ER activity, a key regulator of cell proliferation in estrogen-responsive tumors, we have extended those studies by sequencing the BAF57 gene in a panel of human breast cancer cell lines, looking for possible mutations that could have occurred during tumor progression and/or the establishment of the cell line that might affect normal ER-dependent signaling. Here we report our findings describing the first mutation characterized in the human *BAF57* gene and discuss its possible implications in the development of breast cancer.

## **Materials and methods**

### *Cell culture*

Carcinoma-derived cells were grown as a monolayer on 75cm<sup>2</sup> tissue culture flasks at 37°C in a humid atmosphere maintained at 10% (v/v) CO<sub>2</sub>. Cells were maintained in either DMEM (ZR-75-1, MCF-7, T47D, MDA-MB-361, MDA-MB-231, PC-3) or RPMI 1640 (BT549 and LNCaP) supplemented with 10% (v/v) fetal bovine serum.

### *Plasmids*

The mutated BAF57 open reading frame found in BT549 cells was amplified by PCR and subcloned into pSG5 (pSG5-BT-BAF57) or pGEX-6P-1 (Amersham Pharmacia Biotech, GST-BT-BAF57).

The following plasmids have been described previously: pMT2-MOR, pSG5-SRC1e, pGL3-2ERE-PS2-LUC, pSG5-ΔN-BAF57, GST-BAF57 [10].

### *Genomic Extraction*

The DNeasy tissue kit (Qiagen) was used for rapid isolation of total genomic DNA from cultured cells. Cells were grown to 50% confluency in a 9cm petri-dish (maximum number of cells 5×10<sup>6</sup>), scraped off from the bottom of the petri dish, centrifuged at 1000 rpm for 5 min and resuspended in 200μl PBS. Total genomic DNA was isolated according to the manufacturer's protocol using two elution steps to yield DNA concentrations between 60-180 ng/μl.

### *DNA sequencing*

DNA was sequenced by a linear PCR method using dye-labelled terminators from Applied Biosystems. Typically, sequencing reactions were set-up consisting of 1µl DNA, 8µl of Big Dye Terminator Ready Mix 3 (containing Taq polymerase and dye-labelled terminators) and 3µl (10ng/µl) of primer in a 20µl reaction. PCR reactions typically consisted of 25 thermo-cycles of: 94°C for 30 seconds, 50°C for 15 seconds and 60°C for 4 minutes. Following the PCR, the DNA was precipitated, washed and then processed using the ABI 3100 capillary sequencer and the collection data using the ABI 3100 data collection software.

#### *Real-Time RT-PCR*

The Invitrogen ready-to-use TRIZOL reagent was used to isolate total RNA from cultured cells ( $>10^7$ ). Precipitation and concentration of total RNA was achieved by the addition of isopropyl alcohol, centrifugation, and a subsequent 75% ethanol wash. Purified RNA was briefly air dried and re-dissolved in DEPC-treated water (Ambion). The RNA concentration and purity were determined by measurement of the OD<sub>260</sub> and OD<sub>280</sub> on a spectrophotometer. To obtain first strand cDNA, 2µg total RNA were treated with DNase I, and cDNA was prepared using the Superscript First-Strand synthesis System for RT-PCR according to the manufacturer's instructions (Life Technologies, Inc.).

Analysis of gene expression was performed by Real-time PCR using the ABI PRISM 7700 Sequence detector following PE Applied Biosystems' Taqman SYBR Green Master Mix protocol (Applied Biosystems, Foster City, CA). Reactions were carried out using specific primers for hBAF57 and the constitutively expressed L19 ribosomal coding gene

used as an internal control. BAF57 expression levels were normalized to L19 levels. Primer sequences may be obtained on request.

#### *GST pull-down assays*

Expression vectors for ER $\alpha$  or SRC1e-PAS were transcribed and translated *in vitro* with [<sup>35</sup>S]-methionine in reticulocyte lysate (Promega) according to the manufacturer's protocol. GST fusion proteins of full-length BAF57 and truncated BT-BAF57 were induced, purified, bound to Sepharose beads (Amersham), and incubated with *in vitro* translated proteins (ER $\alpha$  or SRC1e-PAS) as described previously [10].

#### *Transient transfections*

COS-1 cells were cultured in DMEM supplemented with 10% fetal bovine serum. Twenty-four hours before transfection, cells were plated in 24-well plates in phenol red-free medium, supplemented with 5% dextran charcoal-stripped serum. Cells were transfected using a modified calcium phosphate protocol [18]. The transfected DNA included a pRL-CMV (Promega) control plasmid (5ng/well), the luciferase reporter plasmid pGL3-2XERE-PS2 (100 ng/well), pMT2-MOR (25 ng/well), pSG5-SRC1e (100 ng/well), pSG5- $\Delta$ N-BAF57 (250 ng/well) or pSG5-BT-BAF57 (250 ng/well). Empty vectors were used to normalize the amounts of DNA. After incubation for 16 hours with the DNAs, the cells were washed and incubated in fresh medium in the presence of 10 nM 17 $\beta$ -estradiol for 24 h. Subsequently, cells were harvested and extracts were assayed as described previously [18].



## RESULTS

### *Screening cancer cell lines for mutations in the BAF57 gene*

In order to investigate the possibility that mutations in *BAF57* occur in human breast cancer cell lines that might affect ER transcriptional activity, we have screened a panel of ER positive and ER negative breast cancer cell lines for mutations in the *BAF57* gene that might affect estrogen signaling. In addition we also analyzed the sequence of *BAF57* gene in two of the most commonly used human prostate cancer cell lines, LNCaP and PC-3 (Table I). The *BAF57* gene was sequenced directly from cDNA generated using RNA extracted from the cell lines MDA-MB-231, PC-3 and LNCaP and no mutations were found affecting *BAF57* sequence. Genomic DNA was extracted from the other five cell lines studied and eleven sets of primers (forward and reverse) were used to both PCR-amplify and sequence all eleven exons of *BAF57*. From all the cell lines sequenced, we found a CA dinucleotide insertion located in exon 5 of *BAF57* derived from the BT549 breast cancer cell line. The 5'-CA-3' insertion was detected in two sequencing reactions with both primers from two independent PCR-amplification reactions of exon 5 (Figure 1a). The presence of single peaks and no overlaps in the DNA sequencing suggests that the CA insertion is either a homozygous mutation or only one mutated allele is present.

A restriction enzyme map analysis of *BAF57* exon 5 showed that the CA mutation disrupted a unique *Rsa* I restriction endonuclease site (GT/AC). Digestion of the wild-type sequence with this enzyme generates two fragments of approximately equal length (Figure 1b). In order to confirm the presence of the CA mutation, exon 5 amplicons were PCR-amplified using genomic DNA from BT549 *and* from MCF-7 (reference control with two

wild-type copies of *BAF57* gene) and were digested with the *Rsa* I endonuclease. The sizes of the products obtained in the digestion reactions were analyzed by agarose gel electrophoresis (Figure 1c). Clearly, the presence of the CA mutation in the DNA derived from BT549 cells disrupted the *Rsa* I restriction site, resulting in the appearance of a single 512-bp band in the gel corresponding to the undigested exon 5 amplicon, while DNA from MCF-7 only containing wild-type *BAF57* copies gave rise to two fragments of 265 and 247-bp that run as a single band in this 1.2% gel. The detection of only a single 512-bp band in the BT549-derived amplicon confirmed that BT549 do not have any wild-type *BAF57* allele.

#### *Characterization of the novel BAF57 mutation found in BT549 cells*

We next investigated the consequences of the BT549 mutation at the protein level. Using the DNA Strider software we analyzed how the CA insertion at exon 5 affects the open reading frame (ORF) of *BAF57* sequence and consequently the translation of the protein (Figure 2). The wild-type protein consists of 411 amino acids (Figure 2a), while as a result of the CA dinucleotide insertion a premature stop codon is introduced at nucleotide 250 giving rise to a truncated protein that contains the first 75 amino acids of *BAF57*, followed by 9 random amino acids (Figure 2b). Furthermore this provides with an explanation to why in a previous study DeCristofaro *et al.* failed to detect full-length *BAF57* protein in BT549 cells by Western blot analysis [17], since the mutation that we have identified in this breast cancer cell line generates a truncated *BAF57* protein.

In order to examine whether the breast ductal carcinoma cell line BT549 expressed the mutated *BAF57* mRNA encoding the truncated protein, we isolated total RNA, generated

cDNA and mRNA levels were monitored by Real-Time RT-PCR (Figure 3). The relative expression levels of BAF57 were analyzed in a number of breast and prostate carcinoma-derived cell lines (BT549, ZR-75-1, MCF-7, T47D, LNCaP and PC-3) and we found detectable BAF57 mRNA expression in BT549 cells. However, it is noteworthy that BT549 cells express significantly lower BAF57 mRNA levels compared to the rest of cell lines examined. The presence of the CA mutation was also confirmed by sequencing the cDNA generated from BT549 cells (data not shown).

In an attempt to characterize the functional effects of the BT549-derived BAF57 (BT-BAF57) truncated protein, we subcloned the mutated ORF into the bacterial expression vector pGEX-6P1 and the mammalian expression vector pSG5, to be used in GST pull-down assays and transient transfection experiments respectively.

We have previously demonstrated that BAF57 directly interacts with both ER $\alpha$  and p160 coactivators and this association is required for the ability of p160 coactivators to potentiate ER-mediated transcription in transient transfection systems [10]. The CA insertion leads to a truncated BAF57 protein what could have important consequences on the functionality of this protein. We carried out GST pull-down assays to investigate the ability of the truncated BT-BAF57 protein to interact *in vitro* with both ER $\alpha$  and the p160 coactivator SRC1e, as compared to wild-type full-length BAF57. In a set of GST pull-down experiments *in vitro* translated <sup>35</sup>S-labelled ER $\alpha$  was incubated with GST-BAF57 (wild-type full-length BAF57) or GST-BT-BAF57 (truncated BAF57) fusion proteins (Figure 4a). According to our previous data, we observed that ER $\alpha$  directly binds to GST-BAF57 in a ligand-dependent manner with a strong interaction detected in the presence of estrogen

(E2) (Figure 4a). However, the *in vitro* binding of ER $\alpha$  to GST-BT-BAF57 was dramatically impaired (Figure 4a). No interaction was observed in the presence of GST alone, as a proof of the specificity of BAF57-ER $\alpha$  interaction. In another set of experiments we analyzed the *in vitro* interaction between the bHLH-PAS domain (amino acids 1-361) of SRC1e (responsible for mediating the interaction with BAF57) and GST-BAF57 or GST-BT-BAF57 fusion proteins (Figure 4b). *In vitro* translated <sup>35</sup>S-labelled PAS-SRC1e interacted in the same weak but specific manner with both full-length BAF57 and BT-BAF57. Collectively the data from the *in vitro* pull-down assays indicate that although the BT-BAF57 still retains its ability to interact with p160 coactivators similar to that of full-length BAF57, this truncated protein lacks capability of binding ER $\alpha$ .

Next we investigated the possible functional consequences of the expression of the truncated BAF57 protein in ER $\alpha$  transcriptional activity. We and others have demonstrated that several truncated BAF57 proteins act as dominant-negative forms on steroid receptor dependent transcriptional activity when expressed in cells containing endogenous BAF57 [10, 20]. To test whether the truncated BT-BAF57 was able to modulate the transcriptional activity of ER $\alpha$  we examined transcription from the 2ERE-PS2-LUC reporter in the presence of the ER $\alpha$ , SRC1e and either the previously characterized dominant negative  $\Delta$ N-BAF57 or the truncated BT-BAF57 cloned from BT549 cells. As previously reported  $\Delta$ N-BAF57 dramatically inhibited the ability of SRC1e to potentiate transcriptional activity of ER $\alpha$  (Figure 4c). Surprisingly, the expression of BT-BAF57 did not reduce SRC1e coactivation potential; on the contrary, we observed an increase in the SRC1e coactivation of ER $\alpha$  in the presence of BT-BAF57 (Figure 4c).



## Discussion

The considerable genetic and biochemical evidence linking BAF57 to cancer prompted us to screen a number of breast and prostate cancer cell lines for mutations in the *BAF57* gene. The human *BAF57* gene maps to chromosome band 17q21, in close proximity to the *BRCA1* gene, a locus associated with frequent LOH and allelic imbalance in breast, ovarian and prostate cancer and it has previously been suggested that BAF57, based on its involvement in DNA transcription and repair events, could function as a tumor suppressor [17]. Moreover the involvement of BAF57 in the regulation of transcriptional activity by ER $\alpha$  [10], which is a key regulator of cell proliferation in normal as well as in cancerous breast and ovarian tissues, further reinforces this notion. In this study we address the characterization of BAF57 gene in the most widely used human cancer cell lines exploited as *in vitro* models in breast cancer research [21]. The experimental approach chosen was preferentially sequencing genomic DNA, because this allows us to detect mutations in *BAF57* even in cases where expression of the gene could be lost during cancer development or cell line establishment. Five out of six breast cancer cell lines studied do not contain mutations in *BAF57* affecting the expressed protein or the DNA sequence. However, a CA dinucleotide insertion in *BAF57* was found in the BT549 breast cancer cell line. The observation of single peaks and no overlaps in the electropherogram in the DNA sequence suggests that the wild-type *BAF57* allele is not present in BT549 cells. A restriction digestion analysis of *BAF57* exon 5 further confirmed that these cells only contain mutated copies of *BAF57*. Therefore, either the CA insertion occurs in homozygosis or, more likely, according to the Knudson's two-hit model [22], the acquisition of this mutation in one copy

of *BAF57* was followed by the subsequent loss of the remaining wild-type copy of the gene. Although we do not have conclusive evidence several observations support this notion. During the PCR-amplification reactions we observed that for BT549 almost double the amount of genomic DNA was required, relative to that of other cell lines, in order to generate the same amount of amplified product. In addition, the results of the Real-Time RT-PCR, where it was shown that BT549 cells express significantly lower levels of *BAF57* mRNA than the other cell lines further suggest the presence of a single *BAF57* allele in this cell line, although we cannot rule out the possibility that other mechanisms, like promoter methylation, could also be responsible for this low expression. As mentioned above, *BAF57* maps to a chromosome area that has been associated with LOH in breast cancer and our data suggest that this is the case in BT549 cells. The CA insertion located in *BAF57* exon 5 causes a frameshift in the ORF and introduces a premature stop codon, leading to a truncated protein (BT-BAF57) consisting of eighty-five amino acids, of which only the first seventy-five correspond to wild-type *BAF57* protein.

In view of the critical role of *BAF57*, acting through SWI/SNF complexes, in the regulation of gene transcription mediated by ER $\alpha$  and AR, the confirmation that all other breast and prostate cancer cell lines analyzed express wild-type *BAF57* might be relevant in future studies performed using those cellular models addressing the molecular mechanisms of action of SWI/SNF complexes, ER or AR.

BT549 is a breast-ductal carcinoma cell line that does not express ER and has a truncated retinoblastoma (pRB) transcript ([23] and references therein). A previous study had also shown that the *BAF57* protein was undetectable by Western blotting in this cell

line [17] and the mutation we identified in the *BAF57* gene that results in the generation of a truncated protein explains why. We have also shown that BT549 cells express *BAF57* mRNA encoding the truncated protein. BT-*BAF57* does not interact with ER but it is still able to bind *in vitro* to the SRC1e coactivator in the same manner as the full-length wild-type protein raising the possibility that the truncated protein may interfere not only with ER $\alpha$  regulation, but also with a number of other nuclear receptor-dependent gene regulation pathways. Alterations on AR transcriptional activity might also be relevant in the generation of the transformed phenotype in breast cancer cells because its activity requires both functional p160 coactivators and *BAF57*, and AR can mediate testosterone antiproliferative and proapoptotic effects in mouse breast cancer models (reviewed in [24])

In most cases cancer arises after an accumulation of mutations that may affect different cell proliferation-regulatory pathways. In the case of BT549, at least three mutations are present, each potentially linked to generation of breast cancer. The pRB protein is a major tumor suppressor and transfection studies have shown that the SWI/SNF complex associates with pRB to inhibit cell proliferation [25]. BT549 cells are also ER negative and estrogen is a major regulator of cell proliferation in normal as well as in breast cancer tissues. Finally, *BAF57* is a core subunit of all human SWI/SNF complexes and a truncated *BAF57* protein may potentially interfere with both pRB-mediated and ER $\alpha$ -mediated pathways. We have observed that expression of BT-*BAF57*, in contrast to the reported dominant-negative action of other artificial *BAF57* deletion mutants, is able to increase the ability of SRC1e to potentiate transcriptional activation by ER $\alpha$ . Therefore, if this mutation occurred prior to the loss of ER expression, it could have contributed to the aberrant



cellular proliferation through excessive estrogen-dependent signaling. It is also possible that the *BAF57* mutation occurred first and pleiotropic effects mediated by deficient SWI/SNF complexes led to the generation of additional mutations. BAF57, acting through SWI/SNF complexes, participates in the regulation of gene expression carried out by many types of transcription factors other than ER and AR. A recent study has shown that stably expression of full-length wild-type BAF57 in BT549 cells reduced the transformed characteristics of this breast cancer cell line, inducing cell cycle arrest and apoptosis and restoring contact inhibition [26], strongly supporting a role for BAF57 as tumor suppressor. To address the possible contribution of this mutation in BAF57 to aberrant ER-dependent signaling it will be necessary to stably express BT-BAF57 mutant in cell lines expressing both wild-type BAF57 and ER in order to analyze how BT-BAF57 affects estrogen dependent gene expression and cell proliferation.

The finding of a mutation in *BAF57* in one breast cancer cell line out of just six that were screened strengthens our view that BAF57 may be associated with tumor generation in the mammary gland and it suggests that it will be worthwhile to initiate studies to screen primary human breast cancer samples for potential mutations in the *BAF57* gene.

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## Figure legends

**Figure 1. Identification of a CA dinucleotide insertion in exon 5 of the *BAF57* gene in the human BT549 breast cancer cell line.** (a) The mutation was detected in two independent sequencing reactions using both the forward (F) and the reverse (R) primers. The wild-type (WT) sequence of exon 5 is given in the bottom line. The data from the sequencing reactions were processed by using the Seqman software (Macintosh). (b) Schematic representation of the restriction digestion strategy used. The *Rsa* I restriction site (GTAC) is located in the middle part of *BAF57* exon 5. Digestion of the PCR-amplified wild-type exon 5 amplicon generates two DNA fragments of similar size (~250bp), while in the BT549-derived amplicon the CA insertion disrupts the *Rsa* I restriction site and the original fragment remains undigested (~500bp). (c) Restriction digestion analysis of *BAF57* exon 5 with *Rsa* I. The restriction digestion reactions were terminated by addition of DNA loading buffer and the sizes of the digestion products were analyzed by agarose gel (1.2%) electrophoresis. Two independently generated PCR amplicons were analyzed in each case.

**Figure 2. The CA insertion present in BT549 cells generates a truncated *BAF57* protein.** (a) Wild-type *BAF57* open reading frame. The wild-type human *BAF57* protein consists of 411 amino acids. (b) The CA insertion identified in BT549 cells (indicated in capital letters) causes a frameshift leading to a premature stop codon at position 85 (indicated by an asterisk) which generates a truncated *BAF57* protein consisting of the first 75 residues of *BAF57* protein followed by 9 random amino acids (highlighted inside a rectangle).

**Figure 3. Comparison of the relative BAF57 expression levels in a panel of breast and prostate cancer cell lines by Real-Time RT-PCR.** Total RNA was extracted from a number of breast and prostate cancer cell lines (BT549, ZR-75-1, MCF-7, T47D, LNCaP and PC-3). cDNA was generated and BAF57 mRNA expression analysed by Real-Time RT-PCR using L19 as control gene. The values are expressed relative to the BAF57 mRNA levels in BT549 cells assigned as 1.

**Figure 4. Functional characterization of the BT-BAF57 truncated mutant.** (a) *In vitro* interaction of BAF57 and BT-BAF57 with ER $\alpha$ . Binding of GST fusion proteins of wild type BAF57 (GST-BAF57) or truncated BT-BAF57 (GST-BT-BAF57) to <sup>35</sup>S-labelled ER $\alpha$  in the presence of 100nM 17 $\beta$ -estradiol (E2) or vehicle (-). (b) *In vitro* interaction of BAF57 and BT-BAF57 with SRC1e. Binding of GST fusion proteins of wild type BAF57 (GST-BAF57) or BT-BAF57 (GST-BT-BAF57) to <sup>35</sup>S-labelled SRC1 bHLH-PAS domain (amino acids 1-361). In each panel, the input lane represents 10% of the total volume of lysate used in each reaction. (c) Effect of BT-BAF57 expression on transcriptional activation by ER $\alpha$  in cells expressing endogenous BAF57. COS-1 cells were transiently transfected with expression vectors for ER $\alpha$  and SRC1e, the 2XERE-pS2 luciferase vector, the dominant negative  $\Delta$ N-BAF57 or BT-BAF57. In each case, after transfection, cells were washed and incubated with vehicle (white bars) or 17 $\beta$ -estradiol (back bars) at 10<sup>-8</sup> for 24 h. Subsequently, cell lysates were assayed using a dual luciferase reporter system. Normalized values are expressed relative to the activity of ER $\alpha$  alone in the presence of ligand. The results shown represent the average of two independent experiments assayed in duplicate.

*Table 1.* Summary of human cancer cell lines screened

Breast cancer cell lines	Genomic DNA	cDNA	ER status	BAF57 mutations
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MCF-7	+	-	+	-
ZR-75-1	+	-	+	-
BT549	+	+	-	+
T47D	+	-	+	-
MDA-MB-361	+	-	+	-
MDA-MB-231	-	+	-	-
Prostate cancer cell lines				
PC-3	-	+	+/-	-
LNCaP	-	+	+/-	-



Figure 1

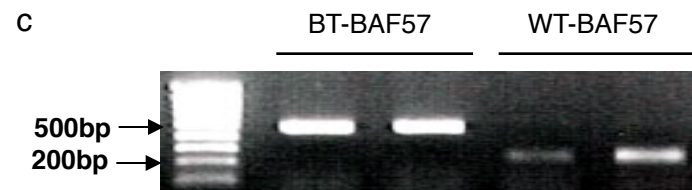
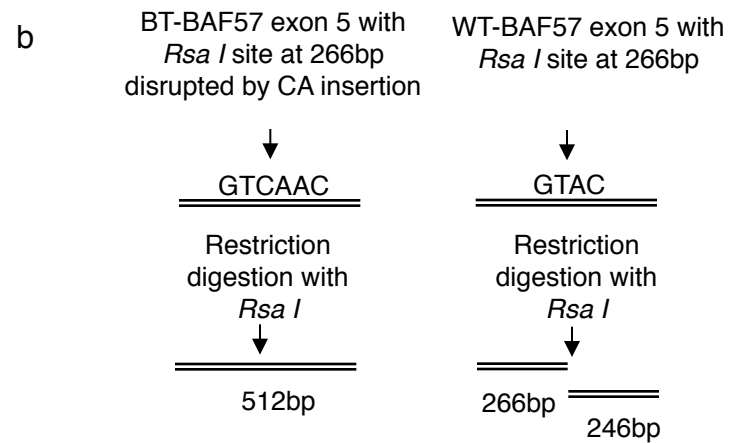
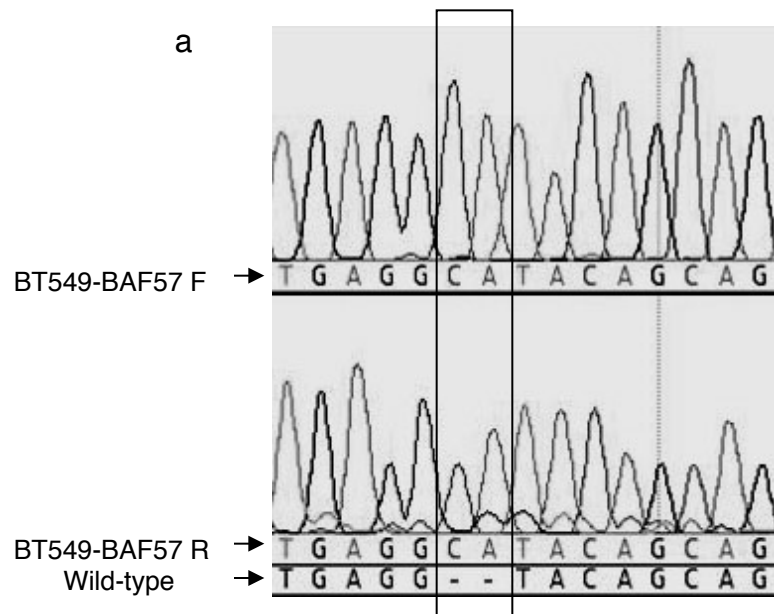


Figure 2

a

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1/1                               31/11                               61/21
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M S K R P S Y A P P P T P A P A T Q M P S T P G F V G Y N P
91/31                               121/41                               151/51
tac agt cat ctc gcc tac aac aac tac agg ctg gga ggg aac ccg ggc acc aac agc cgg gtc acg gca tcc tct ggt atc acg att cca
Y S H L A Y N N Y R L G G N P G T N S R V T A S S G I T I P
181/61                               211/71                               241/81
aaa ccc cca aag cca cca gat aag ccg ctg atg ccc tac atg agg tac agc aga aag gtc tgg gac caa gta aag gct tcc aac cct gac
K P P K P P D K P L M P Y M R Y S R K V W D Q V K A S N P D
271/91                               301/101                               331/111
cta aag ttg tgg gag att ggc aag att att ggt ggc atg tgg cga gat ctc act gat gaa gaa aaa caa gaa tat tta aac gaa tac gaa
L K L W E I G K I I G G M W R D L T D E E K Q E Y L N E Y E
361/121                               391/131                               421/141
gca gaa aag ata gag tac aat gaa tct atg aag gcc tat cat aat tcc ccc gcg tac ctt gct tac ata aat gca aaa agt cgt gca gaa
A E K I E Y N E S M K A Y H N S P A Y L A Y I N A K S R A E
451/151                               481/161                               511/171
gct gct tta gag gaa gaa agt cga cag aga caa tct cgc atg gag aaa gga gaa ccg tac atg agc att cag cct gct gaa gat cca gat
A A L E E E S R Q R Q S R M E K G E P Y M S I Q P A E D P D
541/181                               571/191                               601/201
gat tat gat gat ggc ttt tca atg aag cat aca gcc acc gcc cgt ttc cag aga aac cac cgc ctc atc agt gaa att ctt agt gag agt
D Y D D G F S M K H T A T A R F Q R N H R L I S E I L S E S
631/211                               661/221                               691/231
gtg gtg cca gac gtt cgg tca gtt gtc aca aca gct aga atg cag gtc ctc aaa cgg cag gtc cag tcc tta atg gtt cat cag cga aaa
V V P D V R S V V T T A R M Q V L K R Q V Q S L M V H Q R K
721/241                               751/251                               781/261
cta gaa gct gaa ctt ctt caa ata gag gaa cga cac cag gag aag aag agg aaa ttc ctg gaa agc aca gat tca ttt aac aat gaa ctt
L E A E L L Q I E E R H Q E K K R K F L E S T D S F N N E L
811/271                               841/281                               871/291
aaa agg ttg tgc ggt ctg aaa gta gaa gtg gat atg gag aaa att gca gct gag att gca cag gca gag gaa cag gcc cgc aaa agg cag
K R L C G L K V E V D M E K I A A E I A Q A E E Q A R K R Q
901/301                               931/311                               961/321
gag gaa agg gag aag gag gcc gca gag caa gct gag cgc agt cag agc agc atc gtt cct gag gaa gaa caa gca gct aac aaa ggc gag
E E R E K E A A E Q A E R S Q S S I V P E E E Q A A N K G E
991/331                               1021/341                               1051/351
gag aag aaa gac gac gag aac att ccg atg gag aca gag gag aca cac ctt gaa gaa aca aca gag agc caa cag aat ggt gaa gaa ggc
E K K D D E N I P M E T E E T H L E E T T E S Q Q N G E E G
1081/361                               1111/371                               1141/381
acg tct act cct gag gac aag gag agt ggg cag gag ggg gtc gac agt atg gca gag gaa gga acc agt gat agt aac act ggc tcg gag
T S T P E D K E S G Q E G V D S M A E E G T S D S N T G S E
1171/391                               1201/401                               1231/411
agc aac agt gca aca gtg gag gag cca cca aca gat ccc ata cca gaa gat gag aaa aaa gaa taa
S N S A T V E E P P T D P I P E D E K K E *

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b

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1/1                               31/11                               61/21
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M S K R P S Y A P P P T P A P A T Q M P S T P G F V G Y N P
91/31                               121/41                               151/51
tac agt cat ctc gcc tac aac aac tac agg ctg gga ggg aac ccg ggc acc aac agc cgg gtc acg gca tcc tct ggt atc acg att cca
Y S H L A Y N N Y R L G G N P G T N S R V T A S S G I T I P
181/61                               211/71                               241/81
aaa ccc cca aag cca cca gat aag ccg ctg atg ccc tac atg agg CAt aca gca gaa agg tct ggg acc aag taa
K P P K P P D K P L M P Y M R H T A E R S G T K *

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Figure 3

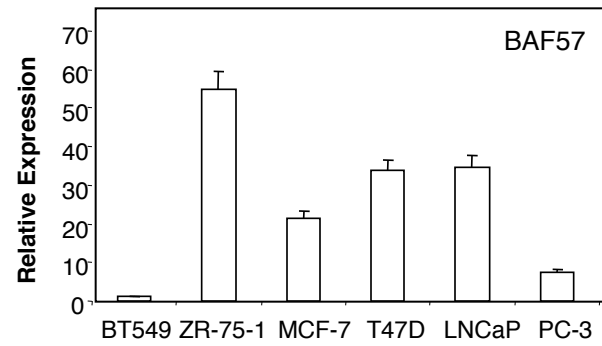


Figure 4

