Supplementary Materials and Methods

Tissue culture. LNCaP and C4-2 cells were maintained in 5% FBS and phenol-red supplemented IMEM. For culture in steroid free conditions, cells were switched to phenol-red free 5% charcoal dextran treated (CDT) IMEM.

Plasmids and Reagents. Constructs have been previously described: the pcDNA3.1- BAF57 FLAG construct was a kind gift from Dr. T.K. Archer (NIEHS, NIH, Research Triangle Park, NC) (Chen et al., 2005). Plasmids encoding pBabe-puro and p-Babe-puro- BAF57-FLAG and H2B-GFP have been previously described (Link et al., 2005). Lipofectin, TRIzol and SYBR green were obtained from Life Technologies and X-treme Gene HP DNA transfection reagent from Roche. DHT was purchased from Sigma Aldrich, bicalutamide from Astra Zeneca, MDV3100 from Selleckchem, Calpain inhibitor from Santa Cruz and BrdU labeling reagent from GE Healthcare.

Microarray profiling of gene expression. LNCaP cells were transfected and treated as described in the preceding section. Prior to microarray hybridization, samples were validated by quantitative PCR for induction of BAF57 transcript (Fig. S1C). Gene expression analysis was performed on biological duplicates using the Affymetrix GeneChip Human Gene 1.0 ST Array. Samples were prepared and hybridized as per manufacturer's instructions. Raw data files from the Affymetrix GeneChip Human Gene 1.0 ST arrays were processed using Affymetrix Expression Console version 1.1. Gene-level expression measurements were computed using the iterPLIER algorithm on the "core" probesets and exported with annotation release 30, dated January 22nd, 2010. Additional array preprocessing was performed in Matlab version 7.10 (R2010a), where expression values were converted to iterPLIER+16 by adding 16, and then transformed to log2 scale. Microarray data thus normalized were analyzed for differential gene expression using significance analysis of microarrays (Tusher et al., 2001). Genes with absolute fold change $\geq$ 2 fold and an estimated false discovery rate $\leq$ 25% were identified as differentially expressed. Genes differentially regulated by DHT were then clustered based on expression

profiles across control, DHT and BAF57 overexpressing samples using K-means clustering (K=4). The microarray data have been submitted to the Gene Expression Omnibus (GEO) public database at NCBI (accession number GSE44418).

Transcript analyses. Transcripts harvested using TRIzol were subject to cDNA synthesis using the reverse transcriptase system (Life Technologies) and subsequent q-PCR analyses with SYBR Green. For the detection of *ITGA2* expression, LNCaP cells were cultured for 72h in 5% CDT to deplete hormone and then treated for 16h with 1nM DHT and RNA harvested for transcript analyses.

Anti-androgen blockade analyses. LNCaP cells were transfected as previously described. 24 hour following transfection, cells were treated with either 10μM MDV3100 or 1nM DHT and then harvested 12h post-treatment for RNA, cDNA synthesized and subjected to q-PCR for transcript analyses

Chromatin immunoprecipitation. To examine SWI/SNF occupancy in the presence and absence of BAF57 elevation, C4-2 cells were plated at $1.4x10^6$ density on poly-L-lysine coated 10 cm dishes in 5% CDT supplemented IMEM. Cells were transfected as described with 14μg of pcDNA3.1 3X FLAG or pcDNA3.1-BAF57 FLAG and 2μg of H2B-GFP as transfection marker in serum free medium. After 24h, medium was changed to steroid-depleted IMEM containing 5% CDT. Transfection efficiencies were approximately in the range of 70% as judged by the number of cells scoring positive for GFP. 36h post-transfection, cells were harvested and processed for ChIP analyses using antibodies directed against AR (N-20, Santa Cruz), Brg1 (G-7, Santa Cruz) and Brm (#15597, Abcam). The primers used for ChIP analyses are indicated in Supplementary Table S1. Subsequent q-PCR analyses were conducted using Express SYBR Green from Life Technologies.

Immunoblotting and antibodies. The matched non-neoplastic and tumor tissue analyses of BAF57 expression were performed on samples obtained from radical prostatectomy at Thomas Jefferson University Hospital. For the detection of endogenous BAF57 protein expression in a panel of prostate cancer cell lines, $1.5x10^6$ cells were plated on 10cm dishes and cultured for 72h in either 5% FBS or 5% CDT IMEM. Cells were harvested at the end of 72h, by trypsinization and lysed in radioimmunoprecipitation (RIPA) assay buffer [(150mM sodium chloride, 1%NP-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50mM Tris (pH8.0), supplemented with protease inhibitors and phenylmethylsulfonyl fluoride (PMSF)]. After brief sonication, the lysate was quantitated and subjected to SDS-polyacrylamide gel electrophoresis (PAGE), transferred to an immobilon-P membrane (Milipore). The membrane was then probed with the indicated antibodies: BAF57 (291-310, Bethyl labs), AR (N-20, Santa Cruz), Brg1 (G-7), GAPDH (FL-335, Santa Cruz) and CDK4 (H-22, Santa Cruz). For integrin lysis, cells were not subjected to trypsinization, but washed twice with PBS before being treated with integrin lysis buffer (50mM Tris, 1%Triton, 150mM sodium chloride, 1mM sodium orthovanadate, 10mM sodium pyrophosphate, 50mM sodium fluoride, 1mM calcium chloride, 1mM magnesium chloride) supplemented with 1mM Calpain inhibitor and other protease inhibitors as previously described. After 5 minutes of lysis, cells were gently scraped off the plate, left on ice for 15 minutes and subsequently quantitated before being subject to standard SDS-PAGE and immunoblotting. The membrane was then probed with antibodies directed against α2 integrin (# 55340, Abcam) and ß1 integrin (# 610467, BD Transduction labs).

Migration assays. LNCaP cells were seeded at $3x10^5$ cells per 6-well dish and transfected using pBabe-puro- FLAG or pBabe-puro-BAF57 FLAG and H2B-GFP as transfection marker in steroid-free medium. Following transfection, cells were supplemented with 5% FBS supplemented IMEM and allowed to attain confluency (48h) before the initial wounding. Immediately following wounding, the area was imaged and the timepoint designated 0h. The same area was imaged for GFP-positive cells at the end of 24h and 48h post wounding. C4-2 cells were seeded at a density of $2x10^5$ cells and transfected as already described. Following transfection, cells were

replenished with phenol-red free 5% CDT supplemented IMEM and subjected to the same procedures described above for LNCaP cells.

Migration using integrin antibody blockade in LNCaP. LNCaP cells were seeded at a density of $3x10^5$ cells and transfected with BAF57 as previously described. Following transfection, cells were supplemented with 5% FBS-supplemented IMEM and allowed to attain confluency (48h) before the first scratch was made. Following initial wounding, P1E6 mouse monoclonal antibody (MAB 1950, Millipore) or IgG (MAB002, R&D Biosystems) at a concentration of 10µg/ml was added and the area was imaged with a timepoint designation of 0h. 24h following wounding, antibody or IgG re-additions were made at the designated concentrations and the same area was imaged for GFP-positive cells. The subsequent imaging of the same area 24h following this re-addition of antibodies was designated as the 48h timepoint.

Migration using MDV3100 or DHT treatments in C4-2. C4-2 were seeded at a density of $2x10^5$ and transfected with BAF57 as previously described. Following transfection, cells were supplemented with 5% CDT IMEM and allowed to attain confluency (48h) before the first scratch was made. Following initial wounding, 10µM MDV3100 or 1nM DHT was added at the start of the experiment and the area was imaged with a time point designation of 0h. 24h following wounding, the same area was imaged for GFP-positive cells. The subsequent images of the same area 24h later were designated as the 48h time point.

Matrigel invasion chamber assays. LNCaP cells were transfected using pBabe-puro -FLAG or pBabe-puro- BAF57-FLAG and H2BGFP as transfection marker in OPTI-MEM (GIbco) using X-treme Gene HP DNA transfection reagent (Roche) for 24h. Post-transfection medium was changed to 5% FBS supplemented IMEM for 24h, followed by culturing for 48h in serum free phenol-red free medium. A total of 40,000 cells were plated in the upper chamber of an invasion chamber (BD Biosciences, #354483) in serum-free medium. A chemoattractant gradient was established in the lower chamber with 5% FBS and cells were allowed to invade through the

matrigel matrix for 24hours. Individual chambers were then washed 3x times with PBS and DAPI stained. Total invading cells were counted under a fluorescent microscope at 20X magnification.

BrdU incorporation analysis. LNCaP were seeded at a density of $3x10^5$ cells and transfected using pBabe puro FLAG or pBabe-puro-BAF57 FLAG and H2B-GFP as transfection marker in steroid-free medium. Following transfection, cells were supplemented with 5% CDT supplemented IMEM and allowed to attain confluency (48h) and then incubated with bromodeoxyuridine (BrdU) for 16h. Standard indirect immunofluorescence was performed and the results plotted as percent BrdU incorporation relative to vector control.

Transient transfection of Ishikawa cell lines. Ishikawa cells were seeded at $1.0x10^6$ density per 10cm dish in 5% CDT supplemented phenol-red free MEM and transfected the following day with either vector or BAF57 encoding plasmids along with H2B-GFP using the Xtreme Gene HP DNA transfection reagent (Roche) as per manufacturer's instructions, in OptiMEM. Fresh CDT supplemented MEM was replenished 24h following transfection and cells harvested for mRNA analyses 48h post transfection.

Supplementary Figure Legends

Figure S1. Strategy for mimicking tumor-derived BAF57 elevation *in vitro* and subsequent characterization. **(A)** Representative immunoblot showing expression profiles of BAF57 with Lamin B as loading control in LAPC4, LNCaP, C4-2, 22RV1 and PC3 cell lines in both hormone proficient (left panel) and castrate conditions (right panel). The quantification of BAF57 expression, normalized to loading control Lamin B and set relative to 1 for LNCaP, is expressed as numbers below the respective lanes. **(B)** Schematic of the microarray set up using transient ectopic overexpression of BAF57 in hormone therapy sensitive LNCaP cells. **(C)** q-PCR validation of *SMARCE1* (encoding BAF57) transcript upon ectopic BAF57 overexpression in LNCaP and C4-2 cells. Average of three independent experiments (mean $\pm$ SD). **(D)** Representative immunoblots showing expression profiles of Brg1 and AR upon BAF57 overexpression in the presence and absence of ligand treatment in LNCaP and C4-2 cells.

Figure S2. Complete analyses of genes that are significantly altered as a result of BAF57 elevation (A) K-means cluster analyses clustered transcripts into different groups on the basis of similarities in expression under different treatment conditions resulting in four main clusters.
Group I represents transcripts that are upregulated by both DHT treatment and BAF57 upregulation; Group II comprises transcripts that are upregulated by DHT treatment and downregulated by BAF57; Group III consists of transcripts that are downregulated by DHT treatment and upregulated by BAF57; Group IV consists of transcripts that are downregulated under both conditions **(B)** The Venn diagram represents a comparison of differentially upregulated transcripts in the vector+DHT/vector+vehicle versus the BAF57+vehicle/vector+ vehicle cohorts using a 2-fold cut-off and FDR of 25%. 1323 transcripts are regulated by DHT treatment, while 28 transcripts are uniquely regulated by aberrant BAF57 expression. The comparison encompassing the 558 transcripts represents those normally induced by DHT

treatment that can also be induced by BAF57 overexpression. **(C)** Gene Ontology analysis listing the individual transcripts listed under the designated functional category for the 558 common transcripts regulated by BAF57 overexpression or DHT treatment derived from Panel B. **(D)** Tabulation of the uniquely BAF57 regulated transcripts. **(E)** *MALT1* induction profile with BAF57 overexpression in either LNCaP or C4-2 cells. **(F)** *SMARCE1* and *ITGA2* expression profiles evaluated in the Ishikawa cells, a model of endometrial cancer, with transient ectopic overexpression of BAF57. Average of three independent experiments (mean ± SD). **(G)** *VEGF, HIF1A* and *DPP4* induction profiles relative to the housekeeping gene *GAPDH* in LNCaP and C4-2 cells, with transient BAF57 overexpression. Average of three independent experiments (mean ± SD).

Figure S3. BAF57 deregulation modulates integrin signaling and does not inhibit the action of AR antagonist. (A) Representative immunoblot analyses of ß1 integrin upon BAF57 deregulation in LNCaP cells and C4-2 cells. **(B)** The expression of *ITGA2* transcript was determined in 72 hr hormone depleted LNCaP cells treated with or without 1nM DHT stimulation for 16h. The AR target gene transcript *KLK3/*PSA served as the positive control. Expression was evaluated relative to the housekeeping gene *GAPDH,* and the data represent an average of three independent experiments (mean ± SD) by qPCR. Statistical significance was determined using the t-test, with **=$p<0.01$*. **(C)** Immunoblot analyses of ß1 integrin expression in C4-2 cells upon BAF57 attenuation. **(D)** Effect of BAF57 attenuation upon *ROCK2* transcripts in LNCaP and C4-2 cells. *=$p<0.05$ by t-test. **(E)** UCSC Genome Browser snapshot of the *ITGA2* locus (left panel) of AR ChIP-Seq data in LNCaP and VCaP cells. Dark bars illustrate AR Occupied Regions (ARORs), with the AROR selected in this study being highlighted by a red box. Brm ChIP enrichment (right panel) by q-PCR at the *ITGA2* AR binding locus in C4-2 cells. Results are an average of two independent experiments (mean ± SD). **(F)** Representative experiment evaluating the effect of AR blockade using MDV3100 in LNCaP cells. The expression of *SMARCE1*, AR target gene transcripts *KLK3/*PSA*, TMPRSS2* and *FKBP5* relative to housekeeping gene *18S,* in BAF57-transfected LNCaP cells was evaluated. Following a 24-hour period post-transfection,

cells were treated with vehicle or AR antagonist 10μM MDV3100 or 1nM DHT for 12h. Statistical significance was determined with technical replicates using ANOVA with ****=p<0.001, **=p<0.01 and *=p<0.05*.

Figure S4. BAF57 aberrations govern phenotypes predisposing to metastasis. (A & B) Wound healing assays in C4-2 and LNCaP cells (Panels A and B, respectively). The same wound area was monitored from the time of first wounding (designated time point 0h) up to 48h after. Representative images (left panel) and (right panel) quantification of GFP-positive cells, relative to vector control, migrating into the wound area, computed as average of three independent experiments for C4-2 (mean $\pm$ SD) and two independent experiments for LNCaP. ***=p<0.001, **=p<0.01 *=p<0.05 using ANOVA. **(C)** Wound healing assays in C4-2 cells following 1nM DHT treatment. Transfected C4-2 cells were treated with either vehicle or 1nM DHT, at the start of the wounding experiment, designated the 0h time point and the wound area was monitored for 48h subsequently for migrating cells. Representative experiment is depicted here. Untreated vector control at 48hr is set to 1. **(D)** Vector or BAF57 transfected LNCaP cells co-transfected with GFP were allowed to invade a Boyden chamber matrix towards an androgen proficient gradient for 24hours, after which they were washed and DAPI stained. GFP-positive cells were counted. Representative fields (left panel) of GFP-positive cells at original magnification of 20X and quantification (right panel) of the average of two independent experiments, set relative to vector. **=p<0.01 using the t-test. **(E)** Proliferation in LNCaP cells upon ectopic overexpression of BAF57 in steroid free conditions measured by BrdU incorporation**. (F)** Quantification of the α2 integrin antibody blockade wound healing assays in LNCaP cells. Transfected LNCaP cells were treated with either control IgG or 10μg/ml functional blocking antibody to α2 integrin at the 0h time point and again at the end of 24h, and the wound area was monitored 24h subsequently for migrating cells. Migration was quantified using three independent experiments for LNCaP, comprising several technical replicates (mean $\pm$ S.D.). ***=p<0.001, **=p<0.01 *=p<0.05 using ANOVA. **(G)** Representative images from the α2 integrin antibody blockade wound healing assays performed

in transfected C4-2 cells, with GFP transfected IgG controls (upper panel) and α2 integrin antibody treated transfected cells (lower panels).

Figure S5. Metastatic loci from individual patients display differences in BAF57 elevation patterns. (A) The data plots represent the variations in the average BAF57 immunohistochemical score for liver, lung, lymph node, dura, adrenal or soft tissue metastasis from nine patients displaying concurrent metastases to different loci. BAF57 IHC scores reported as zero are also recorded and not all patients have reported metastases at the same sites. **(B)** The data represent an average of BAF57 IHC scores by individual metastases sites obtained from the TMA. **(C)** Data plots of average BAF57 IHC staining in metastatic tissue relative to primary disease with the lowest combined Gleason score.

Supplementary Table 1. Oligonucleotide sequences used for q-PCR mRNA and ChIP q-PCR analyses.

Supplementary Table 2. Principal Kegg pathway summary of 558 transcripts upregulated by BAF57 overexpression supplanting the requirement for hormone.