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The role of epigenetics and long non-coding RNA MIAT in neuroendocrine prostate cancer

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ABSTRACT: Neuroendocrine prostate cancer (NEPC) is the most lethal prostatic neoplasm. NEPC is thought to originate from the trans-differentiation of AR-positive adenocarcinoma cells. We have previously shown that an epigenetic/non-coding interactome (ENI) orchestrates cancer cells' plasticity, thereby allowing the emergence of metastatic, drug-resistant neoplasms. The primary objective of this manuscript is to discuss evidence indicating that some components of the ENI (Polycomb genes, microRNAs) play a key role in NEPC initiation and progression. Long non-coding RNAs (lncRNAs) represent vast and largely unexplored component of the ENI. Their role in NEPC has not been investigated. We show preliminary evidence indicating that a lncRNA (MIAT) is selectively up-regulated in NEPCs and might interact with Polycomb genes. Our results indicate that lncRNAs can be exploited as new biomarkers and therapeutic targets for NEPC.

Keywords: Neuroendocrine prostate cancer; MIAT; Long non-coding RNAs; Polycomb; Epigenetic/non-coding interactome; Trans-differentiation.

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1. Neuroendocrine Prostate Cancer: Clinical and Molecular Features

In adult males, the prostate is a small acorn-shaped tissue with ductal-acinar histology surrounding the urethra at the base of the bladder. Its main function is to contribute secretory proteins to the seminal fluid [1]. The adult prostate is a pseudo-stratified epithelium composed of three main cell lineages (Fig.1, left panel):

1) secretory luminal cells are the predominant cell type; these cells express keratins (K8, K18), the androgen receptor (AR) and secretory proteins such as prostate-specific antigen (PSA) and prostatic specific acid phosphatase (PSAP);
2) basal cells expressing K5 and K14 keratins and p63 are the second major cell type;
3) neuroendocrine cells (NEC) expressing chromogranin A (CHGA), synaptophysin (SYP), and neuropeptides are scattered throughout the basal layer and comprise less than 1% of normal prostatic glandular epithelium [1-3].

Prostate cancer (PCa) represents the second most frequently diagnosed neoplasm and is the sixth leading cause of cancer-related deaths in males worldwide [4,5]. In keeping with the composition of prostate epithelium, more than 95% of PCas are classified as adenocarcinomas, which show luminal phenotype and AR expression [6] (Fig.1, middle panel). Endogenous androgens, mainly produced by the testis, bind to the AR and fuel prostate adenocarcinoma proliferation [7]. For this reason, androgen-deprivation therapy (a.k.a. castration) is an effective therapeutic strategy for this disease. However, patients invariably relapse despite castrate androgen levels (castration-resistant PCa, CRPC) mainly via genetic and epigenetic alterations that facilitate ligand-independent AR activation, amplify the AR-dependent signaling, or trigger different proliferative pathways [7]. CRPCs are characterized by substantially worse prognoses, but chemotherapeutics and newly approved hormonal treatments (e.g., Enzalutamide [8] and Abiraterone [9]) are still effective in prolonging patients' survival at this stage.
Between 0.5-2% of newly diagnosed prostatic neoplasms are classified as neuroendocrine PCa (NEPC), which is insensitive to all forms of hormonal treatment [10]. The neuroendocrine phenotype is significantly associated with lower AR expression [11]. However, some reports indicate that AR expression might be retained in a relevant fraction of NEPCs [12,13]. NEPC is characterized by positive immunohistochemical (IHC) staining for CHGA, SYP, and neuron-specific enolase. However, sparse NEPC cells are not immediately identifiable on IHC sections [3,14,15]. Furthermore, NEPC patients do not present elevated circulating PSA and PSAP levels. These two markers are important indexes to assess the potential presence, or monitor the progression of PCa [6,16]. As a result of these peculiarities, NEPC is often diagnosed at a metastatic stage [17]. In addition, no treatment has demonstrated efficacy in extending the survival of NEPC patients. While median prostate adenocarcinoma survival is

125 months, median NEPC survival is only 7 months [18,19]. Hence, the phenotypic distinction between NEPC and adenocarcinoma is extremely important from a clinical perspective.

There are two prevalent theories regarding the cellular origin of NEPC. One model hypothesizes that NEPCs originate from the transformation of prostate NECs that share a common origin with the luminal and basal prostatic cells. This model is based on the observation that PCa is often multifocal and that NECs are a normal component of the prostatic epithelium [10,20,21]. According to this model, environmental stress (e.g. androgen deprivation) favors the survival of these more proliferative and AR-negative NEPC cells. In keeping with this hypothesis, SV40 T-Ag expressing NECs are able to generate NEPCs in a murine transgenic model [22]. Despite this convincing rationale, the experimental and clinical evidence in support of this model is still limited [23]. Currently available evidence seems to favor another paradigm: under specific conditions, adenocarcinoma cells acquire NEC markers and lose AR expression thereby trans-differentiating into NEPC cells (Fig. 1, right panel). The mechanism by which adenocarcinoma cells acquire the NEPC phenotype is still not fully elucidated [24]. One model suggests that NEPC could originate from luminal cells expressing NE genes, potentially due to input from surrounding NECs. NE trans-differentiation is primarily a mechanism of adaptive response/tumor resistance [10,25]. *In vitro* data demonstrate that LNCaP cells can be induced to trans-differentiate into NEPC cells by various stimuli such as androgen depletion, or supplementation with cAMP, cytokines, or growth factors [21]. More recently, we reported that a patient-derived prostate adenocarcinoma xenograft model (LTL331) developed complete NEPC relapse (LTL331R) upon castration. Notably, the original hormone-sensitive adenocarcinoma and the derived NEPC exhibited matching genetic profiles [26]. Furthermore, an analysis of *ERG* rearrangement and *TP53* status in clinical samples with mixed NEPC/adenocarcinoma phenotype suggested a single clonal origin for the two PCa subtypes, thereby supporting the trans-differentiation model [13,27-31].

Regardless of its cellular origin, NEPC will likely become a major clinical issue in the near future. Although the diagnosis of *de novo* NEPC is rare, sparse NEPC clones often coexist with more abundant adenocarcinoma cells. Upon repeated cycles of hormonal therapy, NEPC clones can become the dominant population [10,26,32]. AR-negative NEPC cells easily adapt to androgen deprivation and are highly proliferative (more than 50% of tumor cells in NEPCs are positive for Ki67 IHC staining) [1,21,26]. It is therefore not surprising that NEPC cells replace adenocarcinoma cells after prolonged AR signaling suppression. It has been suggested that the emergence of more potent hormonal therapies (enzalutamide, abiraterone) might increase NEPC incidence [33]. Hence, a priority of future research will be to identify the molecular mechanisms underlying NEPC emergence, and to identify viable therapeutic targets to prevent or at least delay the development of this incurable disease.

2. The Epigenetic/Non-coding Interactome and its implication in the initiation and progression of Neuroendocrine Trans-differentiation

As highlighted in the previous paragraph, obtaining a more unified understanding of the molecular mechanisms that drive NEPC progression will enable us to identify novel therapeutic tools for this lethal disease. NEPC progression is often associated with genetic alterations including AR inactivation, the loss of specific tumor suppressors (*RB1, PTEN, TP53*), *TMPRSS2-ERG* rearrangement, and amplification of *MYCN* and *AURKA* oncogenes [21,34,35]. Although the roles of these irreversible genetic events have been well characterized, no effective targeted treatment has been developed so far. Emerging evidence indicates that an epigenetic/non-coding interactome (ENI) could play a more fundamental function in NEPC initiation and progression. We have previously proposed that the ENI confers unique plasticity to cancer cells, thereby allowing them to become metastatic and drug-resistant [28,36]. Notably, these two deadly features are hallmarks of NEPC. Here, we will discuss initial evidence suggesting that the ENI is implicated in NEPC initiation.

The ENI is comprised of two major components: epigenetic effectors (proteins) and non-coding RNAs (ncRNAs) [36]. Polycomb group (PcG) proteins are epigenetic effectors organized in multimeric complexes known as the Polycomb Repressive Complexes (PRCs) [37]. The two main PRCs (PRC1 and PRC2) act in concert to silence gene transcription. PRC2 functions to trimethylate histone H3 lysine 27 (H3K27me3) in the promoter region of a target gene, thus creating a repressive chromatin mark [38]. This histone modification is subsequently recognized by the chromodomain of the CBX polycomb proteins (CBX2,4,6,7,8) [39] which facilitate the recruitment of the PRC1 to the chromatin [40]. PRC1 then monoubiquitylates lysine 119 of histone H2A (H2AK119ub1) via its catalytic ligases RING1a and RING1b [41], thereby silencing transcription at target sites [42]. To date, many studies have shown that over-expression of the PcG proteins EZH2 and BMI1 facilitates metastasis in several cancers [43-45]. In addition, upstream and downstream miRNAs interact with EZH2 function to promote drug resistance [46-48]. Taken together, these findings indicate the involvement of PcG proteins in two hallmarks of NEPC: metastasis and drug resistance. PcG proteins are also known to regulate stem cell differentiation and neurogenesis [49,50]. Loss of EZH2 in neuronal progenitor cells led to reduced proliferation and survival [51]. This evidence indicates that dysregulated PcG-mediated repression could play a role in neuroendocrine trans-differentiation (Fig. 1, right panel).

Recently, we successfully developed the first-in-field patient tissue-derived xenograft model of complete NEPC trans-differentiation from prostate adenocarcinoma [36,52]. To identify the mechanisms of NEPC initiation, we conducted transcriptomic and genomic analyses on our ADT-induced NEPC model (LTL-331R) and on its hormone-sensitive predecessor (LTL-331). We found that the two models share identical genetic profiles, suggesting that genetic alterations may not exclusively drive NEPC trans-differentiation [36].

Interestingly, our analysis revealed that CBX2 and EZH2 (PcG members) were significantly up-regulated in NEPC pre-clinical models and clinical samples [26]. This study also identified 185 PcG target genes that were significantly down-regulated indicating a relevant role of PcG complexes in NEPC. This 'neuroendocrine-associated repression signature' (NEARS) is associated with higher-grade neoplasms, metastatic progression, and poor outcome in multiple clinical datasets [26]. In line with this model, we also found that the chromatin modifier DEK is up-regulated in NEPC cells, and that targeting this gene reduces NEPC proliferation and migration [53]. Notably, PcG-targeting drugs are being developed and have been successfully tested in PCa pre-clinical models [45]. Hence, the deregulated expression of epigenetic effectors may offer viable drug targets for NEPC.

While some epigenetic effectors (e.g. PcGs) are hyper-activated during NEPC progression, others might be suppressed. The loss of the REST gene in CRPC promotes NEPC development [12]. REST is part of the KDM1A-coREST-REST (Lysine-specific histone demethylase 1A-REST Corepressor 1-RE1-silencing transcription factor) histone modifying complex which is bound by HOTAIR, a long intergenic ncRNA that coordinates histone H3 lysine 27 methylation and lysine 4 demethylation [54]. Given that REST is commonly inactivated in NEPC and is responsible for repressing neuronal genes [55], aberrant silencing of this gene could trigger neuronal differentiation programs in trans-differentiating cells [56]. Notably, epigenetic modifications are known to precede genetic alterations in human neoplasms [57]. Therefore, we believe that the ENI plays a significant role in the initiating stages of NEPC trans-differentiation via epigenetic modification of downstream gene targets. These reversible epigenetic changes can in turn promote cellular plasticity and allow for more flexible adaptation to extreme conditions, including those associated with drug resistance and metastatic potential of NEPC.

The second crucial component of the ENI is represented by ncRNAs [36]. Recent advancements in transcriptome analysis support the notion that although approximately 90% of the genome is actively transcribed, only 2% of it encodes for proteins [58,59]. The remaining RNA molecules produced by the cells have been long considered transcriptional noise, which lacks relevant cellular functions [60,61]. More recently, a multitude of experimental studies have identified regulatory ncRNAs that play functional roles in mammalian cells [62-64] and in cancer progression [65]. The category of regulatory ncRNAs includes long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), both of which have been implicated in facilitating cancer metastasis [66,67] and drug resistance [68-70]. Emerging evidence suggests that ncRNAs might interact with epigenetic effectors to drive NEPC initiation and progression (Fig.1, right panel).

MiRNAs are small ncRNAs (<200bp) ~22nt in length, and are produced by two RNase III proteins known as Drosha and Dicer [71]. The main function of miRNAs is to repress the translation of proteins by binding to the 3' untranslated region (UTR) of their complementary (target) messenger RNA molecules. This action is completed via integration of mature

miRNAs into the microRNA-induced silencing complex (miRISC or RISC) [72]. The role of miRNAs in cancer progression has been described before [73]. Initial evidence suggests that miRNAs interact with epigenetic effectors to drive NEPC initiation. For example, miR-124 is up-regulated in response to loss of REST gene [55], a phenomenon observed in 50% of NEPC tumors [74]. Notably, miR-124 represses BAF53a, a chromatin remodeling protein that is essential for suppressing neuronal differentiation [75]. Therefore, loss of REST and subsequent up-regulation of miR-124 could facilitate the activation of pro-neural genes in NEPC trans-differentiating cells. Other miRNAs could play a multi-faceted role in NEPC initiation and progression. Let-7b targets the nuclear receptor TLX, thereby promoting neuronal differentiation programs [76]. On the other hand, TLX is over-expressed in high-grade PCa tissues and PCa cell lines, thereby promoting cell growth and inhibiting *PTEN*-induced senescence [77]. In addition, let-7b down-regulation has been shown to facilitate the metastatic activity of established neuroendocrine tumors [78]. Taken together, these data indicate that let-7b-mediated TLX silencing may constitute an initiating event of NE trans-differentiation, and that subsequent down-regulation of this miRNA could trigger further NEPC development. Therefore, activation of neuronal genes potentially involved in NEPC trans-differentiation may be orchestrated by reversible and plastic mechanisms, which ensure timely activation and de-activation of specific genetic programs.

Long ncRNAs (lncRNAs) are often described as non-coding transcripts longer than 200bp. Alike protein-coding genes, lncRNA expression is regulated by histone-post translational modifications and DNA methylation [79]. LncRNAs have been shown to modulate transcriptional programs by functioning as molecular scaffolds that target histone-modifying complexes to specific *loci* [54]. In keeping with this model, lncRNAs regulate histone methylation, interact with chromatin modifying proteins, and influence local gene expression via DNA-binding [80-82]. Examples of lncRNAs associated with chromatin modifying complexes include HOTAIR, AIR, and Kcnq1ot1 [83-85].

As a result of their properties, lncRNAs can act in concert with the histone modifying complexes to repress transcription of potentially onco-suppressive genes (Fig.1, right panel). The lncRNAs PTENP1 and GAS5 have been identified as regulators of the tumor suppressor PTEN [86,87], whose loss is commonly implicated in NEPC. In addition, the lncRNA H19 was previously shown to down-regulate expression of pRb in colorectal cancer cells [88]. Although the aforementioned lncRNAs have not been studied in NEPC, it is conceivable that some of them are implicated in the silencing of specific onco-suppressors during NEPC trans-differentiation.

Another key characteristic of NEPC is its elevated metastatic potential. There is mounting evidence to support the involvement of lncRNAs (e.g. SChLAP1, PCAT1, and MALAT1) in PCa invasion and metastasis [89-91]. In light of this evidence, it is likely that lncRNAs contribute to NEPC progression by promoting metastatic dissemination. Henceforth, lncRNAs may play a valuable and largely unexplored role in NE trans-differentiation and evolution. No direct

evidence so far has supported this hypothesis. In the next paragraph, we will discuss preliminary data on a lncRNA that seems to be specifically expressed in NEPC cells.

3. Discovery of MIAT as a NEPC-associated lncRNA

In the previous section, we have shown evidence suggesting that the ENI plays a key role in NEPC initiation and progression. Since the ENI mediates reversible changes in gene expression, its components are ideal drug targets [28,92]. While the importance of epigenetic effectors in NEPC is emerging, the role of ncRNAs in this disease is still largely unknown. LncRNAs represent a vast portion of our transcriptome (more than 50,000 unique sequences [93]) and have been described as a “gold mine” for the discovery of new biomarkers and therapeutic targets [65]. To gain insights into the role of lncRNAs in NEPC, we analyzed our collection of patient-derived PCa xenografts, searching for lncRNAs associated with NEPC. The parental adenocarcinoma (LTL331) and the relapsed NEPC line (LTL331R) described in the previous section, have been profiled though Agilent one-color microarrays. This platform includes 2497 lncRNA probes. The same platform has been used to profile our unique collection of patient-derived PCa xenografts, which includes androgen-dependent and independent adenocarcinomas as well as additional NEPC models [36] (Suppl. Fig.1).

In order to discover potentially relevant lncRNAs, we first identified transcripts showing a greater than 2-fold up-regulation in LTL331R (NEPC) versus LTL331 (adenocarcinoma), and then cross-validated these against the lncRNAs specifically up-regulated in a previously described cohort of NEPC cases [28]. We ranked those NEPC-specific transcripts based on their differential expression. The most highly up-regulated transcript in this list was MIAT-Gomafu, a lncRNA previously described for its role in neural cell activation [94]. Microarray analysis revealed that MIAT is exclusively expressed in NEPC patient-derived models (Fig. 2A, NEPC vs. Adenocarcinoma, difference between means = 6871±1783, p=0.001). Notably, the expression pattern of MIAT is unique when compared to other PCa-associated lncRNAs represented in the array (Fig. 2A). Dramatic MIAT up-regulation in LTL331R vs. LTL331 was confirmed by qPCR (Suppl. Fig.2A). RNA fractionation experiments revealed that MIAT expression is restricted to the nucleus of NEPC cells (Suppl. Fig. 2B). Unsupervised hierarchical clustering demonstrated that MIAT expression can efficiently discriminate NEPC and adenocarcinoma samples in a clinical cohort (Fig. 2B).

According to the Ensembl database (http://uswest.ensembl.org/, annotation release 62), the human MIAT locus maps on chromosome 22-q12.1 and it can be spliced into 21 different isoforms. In order to investigate the clinical relevance of MIAT, we interrogated different publically available gene expression databases. Microarray profiling of 12 normal human tissues indicated that, in physiological conditions, MIAT is significantly up-regulated in neural cells (Fig. 2C), thus confirming previous findings [94]. Oncomine database analyses revealed

that MIAT is significantly up-regulated in prostate cancer metastatic lesions (Fig. 2D) and positively associated with Rb mutation (Fig. 2E). Of note, a recent study reported higher Rb mutation rates in NEPC versus prostatic adenocarcinoma [95].

We then performed significance analysis of microarray (SAM) to identify transcripts positively and negatively associated with MIAT in prostate cancer samples. This analysis was performed on a publically available database including 131 primary and 19 metastatic PCa tissues [96]. In this dataset, MIAT was over-expressed in 6/131 (4.8%) primary and 2/19 (10.5%) metastatic samples (Z score>2.0 vs. non-neoplastic prostate tissue). This consistent up-regulation of MIAT in metastatic lesions is intriguing, but not fully in accordance with the NEPC-specificity of this gene (metastatic lesions often do not express NEPC markers). However, samples showing MIAT up-regulation also showed higher expression of the NEPC marker SYP (synaptophysin; odds ratio: 61.5, 95% Confidence Interval: 7.3-514.6; p=0.000527). Moreover, genes positively associated with MIAT were highly enriched for transcripts associated with poorer prognosis and with genomic alterations found in metastatic disease (Fig. 2F). Interestingly, transcripts negatively associated with MIAT included androgen dependent genes, genes silenced in embryonic stem cells and HIF1 (hypoxia-inducible factor 1) targets (Suppl. Tab. 1). As noted before, PcGs are epigenetic silencers that often interact with nuclear lncRNAs. PcGs are crucial for PCa stem cell proliferation and metastatic dissemination [45]. In addition, PcGs are known to interact with HIF1 [97]. For these reasons, we directly investigated the correlation between MIAT and PcG expression, finding that this lncRNA is significantly associated with CBX2 (linear regression R^2=0.45 p<0.0001, Suppl. Fig. 3A), a PcG member that our group identified as implicated in NEPC. According to our predictions, MIAT was also negatively associated with Rb expression (linear regression R^2=0.41 p<0.0001, Suppl. Fig. 3B). While our results indicate that MIAT variation is associated with CBX2 and Rb, they also suggest that MIAT is not primarily regulated by these proteins (R^2<0.5). Further experimental studies are needed to dissect the molecular mechanisms of MIAT/CBX/Rb interaction.

Taken together, these data are the first demonstration of a lncRNA specifically expressed in NEPC, and possibly implicated in this disease.

Conclusions and Future Perspectives

NEPC is an incurable disease. For this reason, the identification of viable therapeutic targets is of paramount importance. We have shown evidence suggesting that the ENI plays a crucial role in NEPC development and progression. While evidence on the role of epigenetic effectors was available in the literature, the role of ncRNAs (and particularly lncRNAs) has been overlooked so far. We postulated that some lncRNAs are implicated in NEPC initiation

(onco-suppressor gene silencing) and progression (acquisition of metastatic and drug resistance potential). In line with our predictions, we found that MIAT expression is restricted to a small percentage of PCas, with high metastatic potential, poor prognosis and frequent Rb mutations. Notably, all those are hallmarks of NEPC [95,98]. In addition, we find strong indications that MIAT transcripts in NEPC are restricted to the nucleus. Our data suggest that MIAT can interact with Polycomb and Rb pathways, which may explain the association of MIAT expression with an aggressive PCa phenotype. Interestingly, previous data support an interaction between MIAT and epigenetic modifiers in neural cells [99]. Future studies will investigate the molecular mechanisms by which MIAT mediates an aggressive phenotype, and the utility of MIAT and other lncRNAs as NEPC-specific therapeutic targets.

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Executive summary

- Neuroendocrine prostate cancer (NEPC) is an androgen receptor- (AR) negative neoplasm, which is resistant to any available treatment
- NEPC originates from the trans-differentiation of AR-positive adenocarcinoma cells. The molecular mechanisms underpinning this phenomenon are still largely unknown.
- NEPC is an incurable disease. As a result of increasingly more aggressive hormonal treatments, NEPC incidence is sharply rising.
- The epigenetic/non-coding interactome (ENI) is composed of epigenetic effectors, microRNAs and long non-coding RNAs (lncRNAs).
- Current evidence indicates that some ENI components (miRNAs, Polycomb genes) are implicated in NEPC initiation and progression
- By analyzing our patient-derived prostate cancer xenografts, we find evidence suggesting that at least one lncRNA (MIAT) is specifically expressed in NEPCs and might interact with key oncogenic pathways (Polycomb).
- Our results indicate that the ENI (and particularly lncRNAs) are a potential “gold mine” that will enable us to discover effective therapies for NEPC

Figure 1. Evolution of prostatic neoplasms. Left panel: the normal prostate epithelium and its components. Middle panel: emergence of AR^+ PCa (red nuclei, neoplastic cells; blue nuclei, normal cells). Right panel: NEPC trans-differentiation. The putative role of PcGs, miRNAs and lncRNAs in NEPC trans-differentiation is summarized.

Figure 2. Identification of MIAT as a NEPC-specific lncRNA. A, Expression of MIAT and 4 other prostate cancer-associated lncRNA in 18 prostatic adenocarcinoma and 3 NEPC models established and maintained at the Living Tumor Lab (www.livingtumorlab.com). Each transplantable model has been characterized by clinico-pathological examination. The expression of adenocarcinoma- (PSA, AR) and NEPC- (chromogranin A and synaptophysin) associated genes was assessed by immunohistochemistry (as described in suppl. Fig. 1 and in ref. 29. Gene expression is calculated based on microarray data described in ref. 6. **p=0.001 (unpaired, 2-tailed T test). **B**, Unsupervised hierarchical clustering discriminates 6 NEPC from all other prostate cancer samples, based on gene expression profiles (data from ref. 1). MIAT was included in the list of genes that discriminate the 2 prostate cancer subtypes (1886 genes up-regulated in NEPC and 1010 genes up-regulated in Adenocarcinoma). The 6 NEPC samples are on the right side of the dendrogram. Red means up-regulation, blue means down-regulation. **C**, Expression of MIAT in a collection of non-neoplastic human tissues (Gene Expression Ominubus profile ID: 2896847, 2 samples per tissue). ****p<0.0001 vs. all other tissues (ANOVA and Dunnet's post-test). **D**, MIAT expression in Primary (7) vs. Metastatic (6) prostate cancer samples. Fold change: 7.9, ***p<0.001. **E**, MIAT expression in Rb wild-type (27) vs. Rb mutated (2) prostate cancer samples. Fold change: 109.8, ****p<0.0001. **F,** Genes significantly associated with MIAT were uploaded to the Oncomine database to identify clinically relevant correlations in prostate cancer samples. SAM was performed as described in ref. 5 Genes were considered positively associated with MIAT if they displayed fold change (FC)>2.0 and q<0.001. We ranked the positively associated genes based on FC and uploaded the first 1000 to the Oncomine database. Oncomine software (Life Technology) was used for analysis and visualization in Figures 1 D, E, F.

Supplementary Material

Suppl. Fig. 1: Immunohistochemical and hematoxylin-eosin (H-E) staining, representative of 4 LTL models employed in this study. LTL331R, LTL352 and LTL370 are androgen-receptor (AR) and prostate specific agent (PSA) negative lines, which are able to grow in castrate animals and express high levels of chromogranin A (CHGA) and synaptophysin (SYP). For this reason, they are designated as NEPC lines. LTL556B is an androgen-dependent line with typical adenocarcinoma phenotype, which stains negative for all NEPC markers, including CD56.

Suppl. Fig. 2: A, qPCR analysis of MIAT expression in LTL331 (adenocarcinoma) and LTL331R (NEPC). B, Sub-cellular localization of MIAT, GAPDH mRNA, and small nucleolar RNA 55 (snoRNA55) in LTL331R cells. RNA extraction, reverse-transcription, fractionation and qPCR were performed as described in ref. 38. The Applied Biosystems TaqMan

probes Hs00978815_m1, Hs03298696_s1, Hs02800695_m1, and Hs02758991_g1 were used for MIAT, snoRNA55, HPRT1 and GAPDH, respectively. HPRT1 was used as reference gene

Suppl. Fig. 3: Linear regression analysis of MIAT vs. CBX2 (A) and MIAT vs. RB1 (B) in the Taylor prostate cancer cohort (131 primary and 19 metastatic prostate cancer samples).

Suppl. Tab. 1: Genes negatively associated with MIAT (SAM analysis, same thresholds as indicated in Fig 2 D) were queried in the Oncomine database for “literature-defined concepts”. SAM was performed as described in ref. 5Here we show significantly associated concepts that are relevant for prostate cancer biology.

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The following references are of interest (*)

1) Lin D, Wyatt AW, Xue H *et al*. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. *Cancer Res.* 74(4), 1272-1283 (2014).

   Comment: this manuscript describes the generation of the first-in-field in vivo model of trans-differentiation to NEPC

2) Khalil AM, Guttman M, Huarte M *et al*. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc. Natl. Acad. Sci. USA* 106(28), 11667-11672 (2009).

   Comment: this is an elegant demonstration of the interaction btween ncRNAs and epigenetics

The following reference is of considerable interest (**)

1) Prensner JR, Iyer MK, Sahu A *et al*. The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. *Nat. Genet.* 45(11), 1392-1398 (2013).

   Comment: this is a seminal paper on epigenetic/non-coding mechanisms of prostate cancer progression.

Figure 1

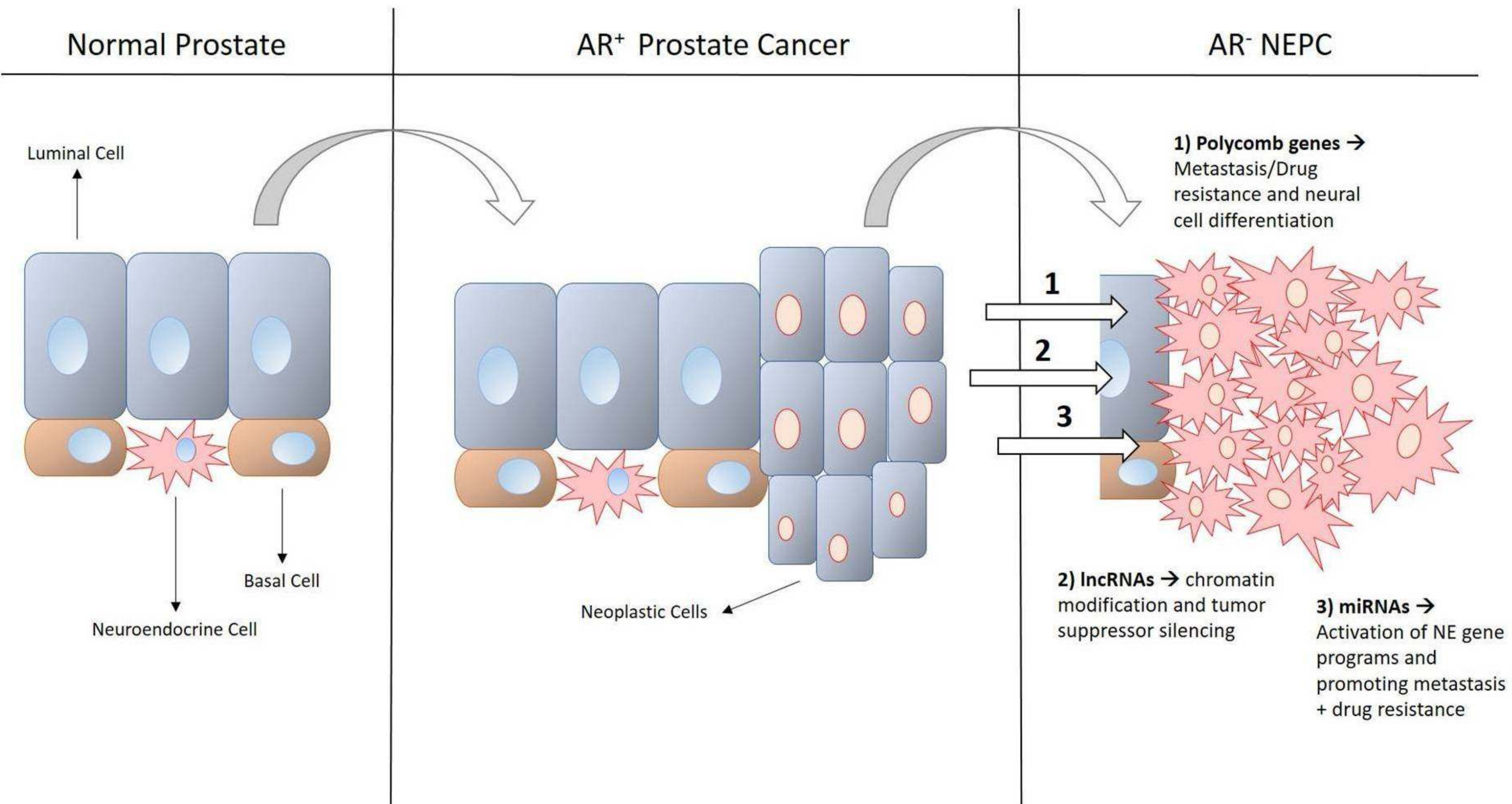
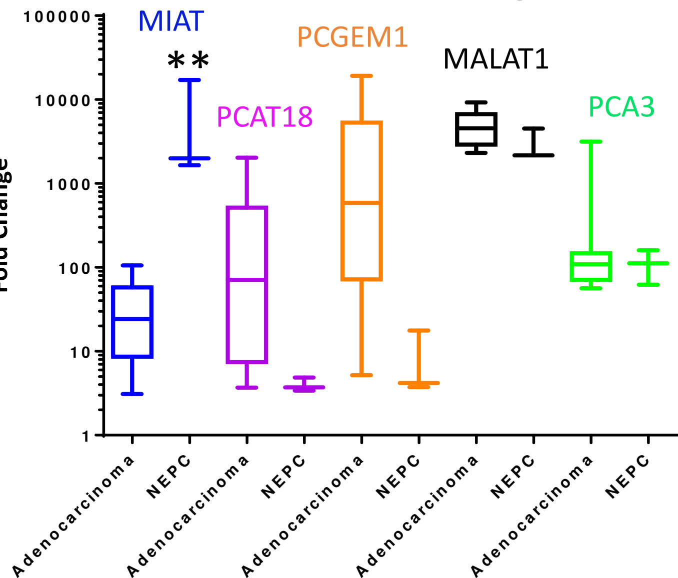


Figure 2



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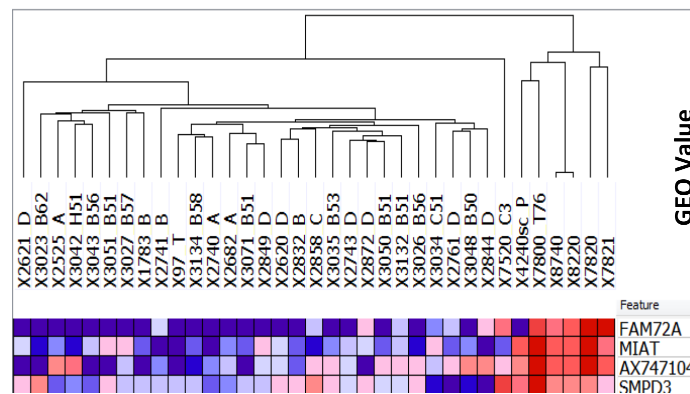

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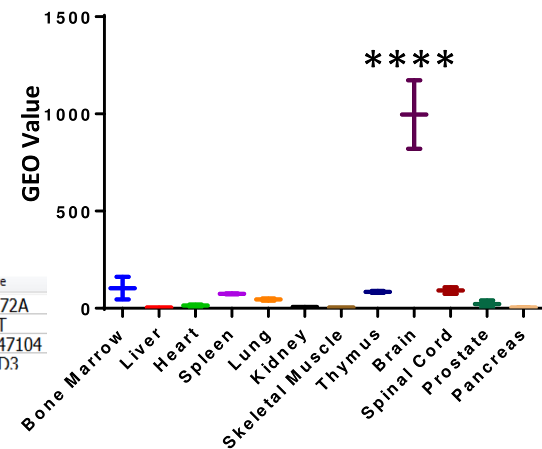
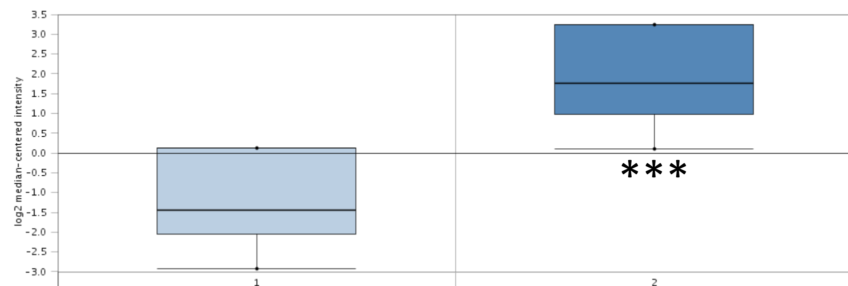
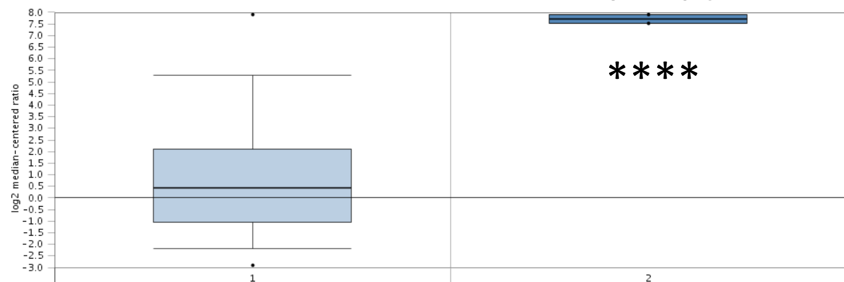


D Primary Metastatic



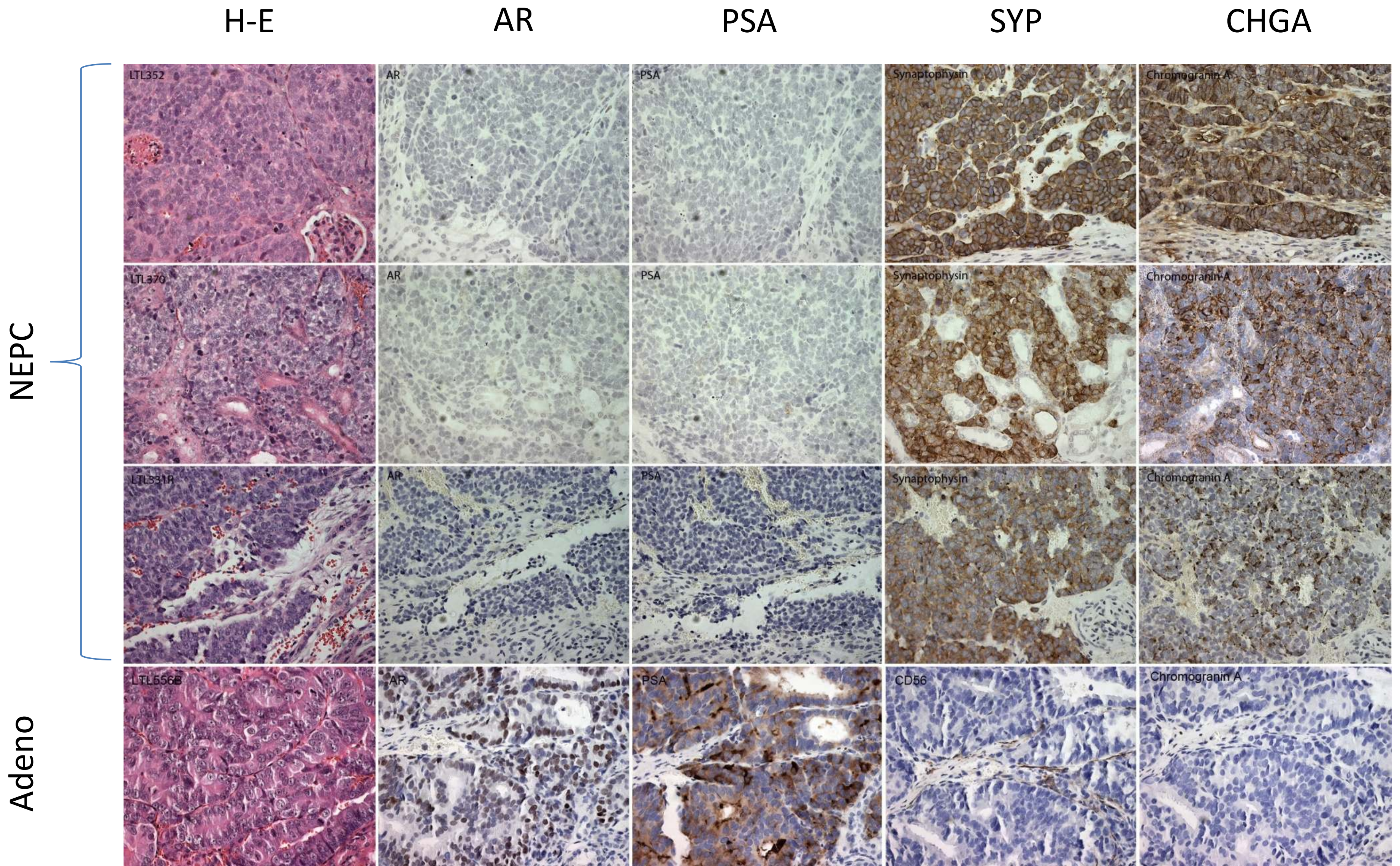
E Rb w.t. Rb mut.



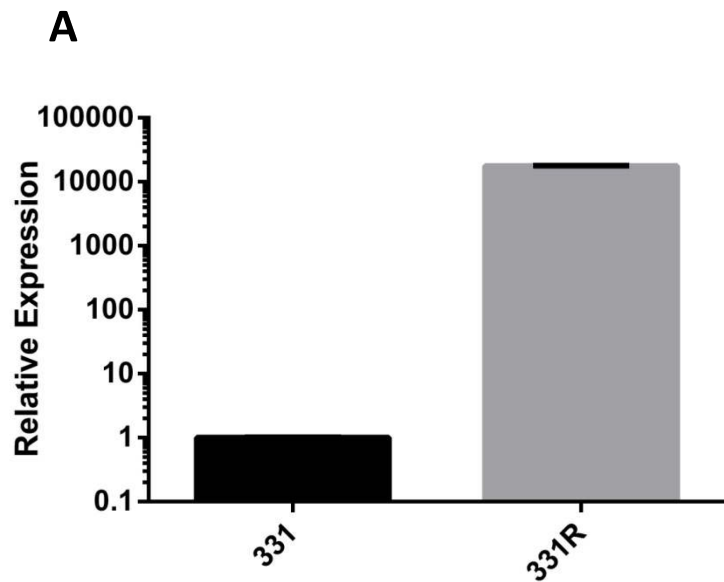
F

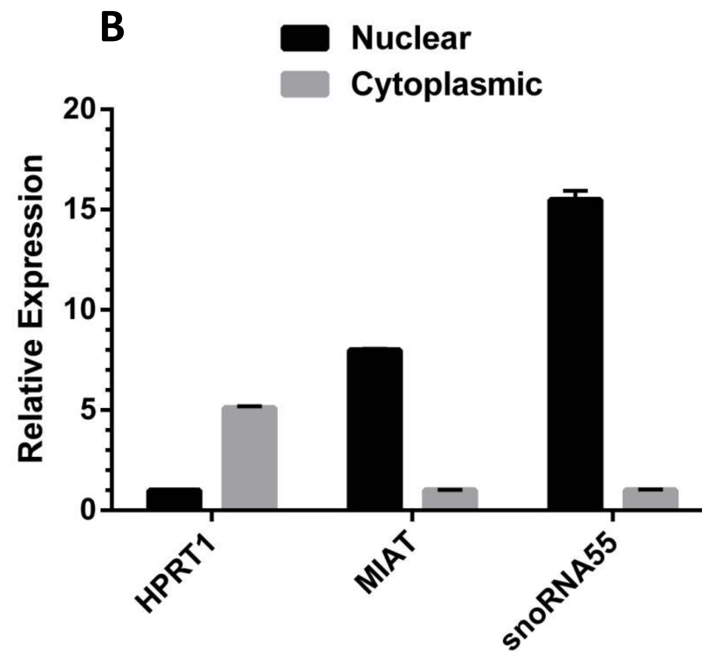
Concept	P value	Odds Ratio	Patients
Recurrence at 5 years	1.0E-13	2.2	61
Recurrence at 1 year	6.5E-4	3.2	173
ETS Family Fusion	1.6 E-8	2.2	58
Metastatic PCa, AR amplification	1.6E-8	5.5	31
Metastatic PCa, ETS2 deletion	1.6E-8	5.5	35
Metastatic PCa, ERG rearrangment	6.2E-4	3.3	35

Suppl. Fig. 1



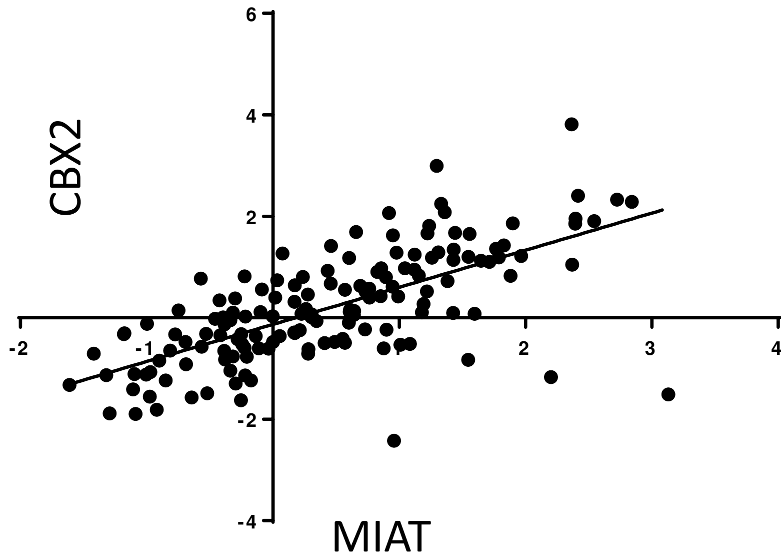
Suppl. Fig. 2





Suppl. Fig. 3

A



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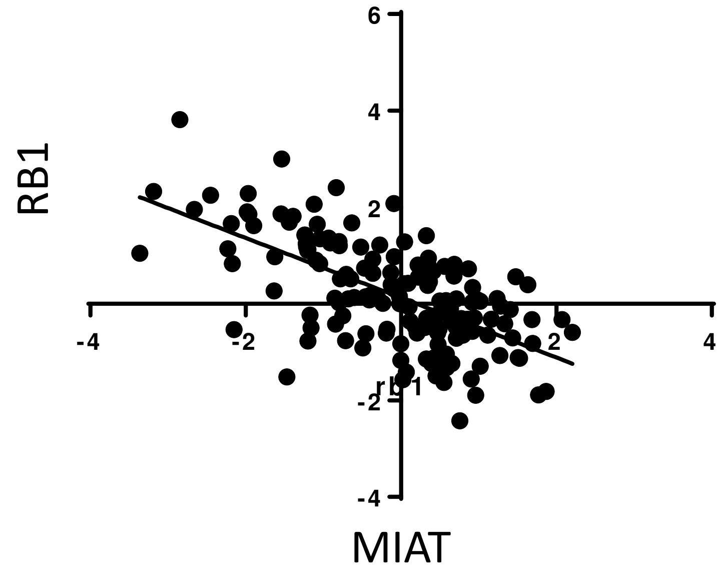

Suppl tab. 1

Concept	P value	Odds Ratio
Genes up-regulated in prostate cancer cells in response to synthetic androgen R1881	4.04E-22	7.6
Genes up-regulated in prostate cancer in response to androgen	7.36E-18	7.3
Genes down-regulated In human embryonic stem cells vs. differentiated counterparts	5.07 E-15	3.2
Genes down-regulated in response to hypoxia and to HIF1 expression	0.008	2.2