Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation

Enhancer RNAs Participate in Androgen Receptor-Driven Looping that Selectively Enhances Gene Activation

Chen-Lin Hsieh[1], Teng Fei[1,2], Yiwen Chen[2,3], Tiantian Li[1], Yanfei Gao[4], Xiaodong Wang[1], Tong Sun[1], Christopher J. Sweeney[1], Gwo-Shu Mary Lee[1], Shaoyong Chen[4], Steven P. Balk[4], X. Shirley Liu[2,3], Myles Brown[1,2] and Philip W. Kantoff[1*]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115, USA

[2]Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute and Harvard School of Public Health, Boston, MA 02115, USA

[4]Hematology-Oncology Division, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA

*To whom correspondence should be addressed: Philip W. Kantoff, MD, email: philip_kantoff@dfci.harvard.edu

The authors declare no conflict of interest.

Classification: Biological Sciences (Cell Biology)

Short Title: Androgen-stimulated eRNA enhances transcription

Keyword: Enhancer RNAs, KLK3e/AR/Med1 complex, Chromosomal looping, RNA-dependent enhancer activity

Abstract:

The androgen receptor (AR) is a key factor that regulates the behavior and fate of prostate cancer cells. The AR-regulated network is activated when AR binds enhancer elements and modulates specific enhancer-promoter looping. Kallikrein-related peptidase 3 (KLK3), which codes for prostate specific antigen (PSA) is a well-known AR-regulated gene and its upstream enhancers produce bidirectional enhancer RNAs (eRNAs), termed KLK3e. Here, we demonstrate that KLK3e facilitates the spatial interaction of the KLK3 enhancer and the KLK2 promoter and enhances long-distance KLK2 transcriptional activation. KLK3e carries the core enhancer element derived from the androgen response element III (ARE III) which is required for the interaction of AR and Mediator 1 (Med1). Furthermore, we show that KLK3e processes RNA-dependent enhancer activity depending on the integrity of core enhancer elements. The transcription of KLK3e was detectable and its expression is significantly correlated with KLK3 (R^2=0.6213, p<5x10^{-11}) and KLK2 (R^2=0.5893, p<5x10^{-10}) in human prostate tissues. Interestingly, RNAi silencing of KLK3e resulted in a modest negative effect on prostate cancer cell proliferation. Accordingly, we report that an androgen-induced eRNA scaffolds the AR-associated protein complex that modulates chromosomal architecture and selectively enhances AR-dependent gene expression.

Significance Statement:

We report that eRNAs, a class of long noncoding RNAs (lncRNAs), participate in the AR-dependent looping complex that enhances spatial communication of distal enhancers and target promoters, leading to transcriptional activation events. Furthermore, our data show that KLK3 eRNA (KLK3e) selectively enhances the gene expression of AR-regulated genes, and provide evidence for a positive regulatory loop in which AR-dependent transcription is modulated by an intermediate eRNA. These findings may translate into improved RNA-based therapy (eRNA suppression) to enhance the durability of androgen deprivation therapy (ADT) and prediction the efficacy of ADT by measuring the enhancer-derived activity (eRNA expression) in prostate tumors.

Introduction:

Androgens and the androgen receptor (AR) play pivotal roles not only in the development of the normal prostate gland but also in the growth and progression of prostate cancer (PCa) (1-4). Androgens exert their biological and physiological effects through activating AR transcriptional activity. AR, a

transcription factor, recruits ligand-dependent transcriptional machinery that activates a tissue-specific transcriptional program involved in development and differentiation (4-6). In PCa cells, AR occupies numerous gene loci to activate transcripts mediating critical cellular activities, such as energy and metabolism (5). AR activation and reactivation are essential for PCa development and tumor recurrence (7).

One unanticipated observation that emerged from multiple global studies of RNA Polymerase II (Pol II) occupancy was that Pol II binds to a large number of intergenic AR-bound enhancers marked by histone H3 lysine 4 mono-methylation (H3K4me1) and lysine 27 acetylation (H3K27ac) and produces enhancer-derived long non-coding RNAs (eRNAs) (8-11). While eRNAs are bidirectional and non-polyadenylated, their induction correlates with the induction of adjacent exon-coding genes (9). The activity of eRNAs was shown to augment the expression of not only neighboring but also intra-chromosomally distant genes to convey enhancer-dependent transcription (12, 13). Recently, the 5'-GRO-seq data have revealed that sequence-specific transcription factors and eRNA transcripts are required for transfer of full enhancer activity to a target promoter (14). Moreover, eRNAs have been shown to configure a chromatin state that facilitate transcription factors binding on the target promoters at defined genomic loci (15).

Mechanistically, eRNAs have been shown to associate with the cohesin complex at estrogen receptor binding sites (ERBSs) and contribute to gene activation by stabilizing estrogen receptor-α (ER-α)/eRNA-induced enhancer-promoter looping (16, 17). In addition, ncRNA-activating (ncRNA-a), a class of enhancer-like RNAs, has been shown to interact with the Mediator complex that enhances transcription in a cis-mediated mechanism (18), highlighting an RNA-dependent functional effect. Therefore, we hypothesized that eRNAs facilitates spatial communication of enhancers and promoters, thereby enhancing transcriptional activation events. Here, we report that the Kallikrein-related peptidase 3 (KLK3) enhancer, one of the strongest AR-bound enhancers in prostate cancer cells, produces KLK3 eRNA (KLK3e) which impacts androgen-induced gene activation. This work reveals that KLK3e selectively enhances AR-regulated gene expression through its collaboration with the AR-dependent looping complex. Thus, our findings provide evidence for a positive regulatory loop in which the AR-regulated network is modulated by an intermediate eRNA.

Results:

The KLK3 enhancer produces enhancer RNA (KLK3e)

We compiled recently established genomic data sets (19) and showed that the KLK3 enhancer, also defined as the androgen response element III (ARE III) (20) was marked by AR, H3K27ac, and H3K4me1, implying that this region is transcriptionally active (Fig. 1A). In fact, the transcription of KLK3 eRNA (KLK3e) was demonstrated by GRO-seq data (10) and confirmed by our reverse transcription quantitative polymerase chain reaction (RT-qPCR) analyses in androgen-dependent LNCaP, VCaP and androgen-independent LNCaP-abl cells (21) (Fig. 1B, S1A, S1B). The expression of KLK3e was induced within the first hour of dihydrotestosterone (DHT) exposure and its expression level gradually increased in a time-dependent manner, resembling the expression of mature KLK3 in both LNCaP and VCaP cells; in contrast, while transcription at the KLK3 locus remains active in androgen-independent LNCaP-abl, androgens (DHT) did not stimulate their mRNA expression to a comparable magnitude as those in parental LNCaP cells (Fig. S1B). Next, we investigated the impact of AR antagonist, bicalutamide on the expression of KLK3e. The expression of KLK3e and KLK3 was induced upon DHT stimulation and blocked by bicalutamide (Fig. S1C), revealing that ligand-dependent eRNA synthesis takes place in association with target mRNA transcription. Taken together, our findings are consistent with the previous report that there is a high correlation of activity-dependent induction between eRNAs and adjacent coding mRNAs (9).

Although multiple studies have identified different eRNA species from mammalian enhancers, the precise mapping of eRNAs has not been described. Since eRNAs produced from these intergenic enhancers are low at steady state (9) and estimated at fewer than 100 copies per cell (17), we enriched cellular RNAs by depleting ribosomal RNAs (rRNAs) prior to reverse transcription (RT). PCR primer sets against the *antisense* (AS1-AS4) and *sense* (S1-S7) of KLK3e were designed based on the GRO-seq and genomic data sets (10, 19). As shown in Fig. 1C, the transcription of bidirectional KLK3e ~~as~~ was captured; whereas, no robust DHT-induced transcription was detectable beyond AS4 and S7. We performed primer-extension PCR assay to connect shorter fragments (Fig. S1D) and further showed PCR products for the *antisense* (2230bp) and *sense* (2170bp) of KLK3e (Fig. 1C and cDNA sequences available in SI). To distinguish whether the eRNA at KLK3 enhancer are polyadenylated or not, we performed reverse transcription using random hexamer primers and 3' RACE (Poly-T) adapter from the same RNA materials. As shown in S1E, the expression of AS4 and S7 was substantially induced by DHT treatment, suggesting that the poly-A tail was added to the 3'-end of *antisense* and *sense* KLK3e. In contrast, S1 showed less DHT-stimulated expression in the Poly-T priming as compared to random hexamer primed cDNA. These data suggest that this locus produces a mixture of RNA species; some of

them are polyadenylated while others may be actively undergoing synthesis of polyadenylation, but we cannot totally exclude the existence of those species without poly-A tails. To detect the size of the mature eRNA at this loci, we customized double DIG-labeled probes using Locked Nucleic Acids (LNA™) (Exiqon). Northern analysis revealed that a major form (~2.5kb) and a minor form (~1.7kb) of *sense* KLK3e were produced upon DHT induction; whereas no signal was detected at the *antisense* strand (Fig. S1F), suggesting that the expression of antisense strand may be lower than the levels that can be detected by the LNA probe. (Fig. S1F). Collectively, this suggests that the *sense* strand (S1-S7) KLK3e, a ~2.2 kb transcript, is polyadenylated and yields a ~2.5 kb transcript shown in Northern analysis. Although the *antisense* KLK3e can be polyadenylated and detected by qPCR, its expression may be substantially lower than the *sense* strand and cannot be detected by Northern analysis under the same experimental settings, indicating that the *sense* strand KLK3e is the dominate species.

The regulatory function of KLK3e on the Kallikrein Locus

To investigate the potential function of eRNAs in transcriptional regulation, short interfering RNAs (siRNAs) targeting KLK3e (siKLK3e) were designed on the basis of KLK3e mapping. Results obtained from distinct sets of siRNAs against the *sense* strand of KLK3e showed that siKLK3e specifically targeted *sense* KLK3e and significantly impeded DHT-induced KLK3 expression in both LNCaP and VCaP cells but had a minimal impact on LNCaP-abl cells (Fig. 2B and S2A, B, C, D, and E), consistent with the notion that ligand-activated eRNAs are important for nearby gene activation (13, 17). Considering that KLK15, 2, and 4 were adjacent to KLK3 and androgen-responsive (Fig. 2A), we examined whether KLK3e impacted transcription of these KLKs. Interestingly, knocking down of *sense* KLK3e significantly inhibited the expression of KLK2; whereas, other KLKs remained unchanged (Fig. 2B). We also investigated the effect of *antisense* KLK3e on the regulation of target gene expression. RNAi silencing of the *antisense* of KLK3e showed a marginal inhibitory effect on KLK3 and KLK2 gene expression (Fig. S2F). Despite the presence of bidirectional KLK3e transcription, data from siRNA to KLK3e and Northern analysis showed that the *sense* strand of KLK3e is the dominant transcript with functional effects on the expression of KLK3 and distant KLK2. Thus, we chose the *sense* KLK3e and continued to study its RNA-dependent enhancer activity.

Since the induction of eRNA is strongly correlated with the enhancer looping to target gene promoters (13, 16, 17), it is possible that the KLK3 enhancer element is able to interact with the KLK2 promoter. To address this, we utilized the chromosome conformation capture (3C) assay to study the

spatial interaction of the KLK3/2 loci. It should be noted that the KLK2 promoter contains an ARE I which shares over 80% sequence homology with the ARE I in the KLK3 promoter (20). Indeed, DHT induces KLK2 transcription and the interaction between the anchor and fragment D and E by 2~3 fold (Fig. 2C). Amplified PCR of anchor-fragment D (105bp) and anchor-fragment E (115bp) was confirmed by DNA sequencing (Fig. S2G and H), suggesting that the KLK3 enhancer interacts with the KLK2 promoter to mediate distal KLK2 gene activation.

To study the mechanism of KLK3e-mediated transcriptional events, we looked for KLK3e interacting partners by asking whether KLK3e interacts with AR, since KLK3e carries the core enhancer element. We conducted AR-directed RNA immunoprecipitation (RIP) coupled with qPCR using primer sets targeting the core enhancer element at 5'end (S1), middle segment (S4) and 3' end (S7) of the *sense* KLK3e (Fig. 1C). Our results show a specific DHT-induced interaction between AR and S1 (Fig. S2I), suggesting that AR relies on the core enhancer element of KLK3e. Since Med1 has been shown to directly interact with AR and is involved in the chromosomal interaction of enhancer-promoter looping (22, 23), we next asked whether Med1 participates in the observed eRNA functions. RIP analysis revealed a strong androgen-stimulated interaction of S1 and Med1 (Fig. S2J), comparable to the interaction of KLK3e and AR. Conversely, the *antisense* KLK3e (AS1) exhibited modest interaction with AR and Med1 (Fig. S2K), implying that the enhancer function of these two KLK3e species is determined by the levels of interaction with the AR-Med1 complex.

To investigate the potential role of KLK3e in the KLK3/2 loci, we next used 3C-qPCR to assess the interaction of the anchor-fragment D/E regions in KLK3e depleted cells. Silencing of KLK3e impaired KLK2 gene expression and the interaction of the KLK2 promoter and the KLK3 enhancer (Fig. 2D). Suppression of Med1 also substantially reduced the interaction of the KLK3/2 loci (Fig. 2D, S2L), supporting a pivotal role of Med1 in mediating this long range chromatin looping (18, 22). Hence, our results demonstrate that both KLK3e and Med1 are involved in maintaining the spatial interaction between an enhancer and a target promoter.

The KLK3e/AR/Med1 ribonucleoprotein complex transcriptionally regulates target promoters

To ensure that the endogenous KLK3e plays a functional role on its target promoters, we performed chromatin immunoprecipitation (ChIP) using antibodies against AR and activated RNA Polymerase II (Pol II S5p) in KLK3e depleted cells. These analyses demonstrated that depletion of KLK3e inhibited AR binding and Pol II activation at the KLK2 promoter, consistent with the reduction

of gene expression of KLK2 (Fig. 3A). Next, we examined the regulatory effect of Med1 on the target promoters. As expected, knockdown of Med1 significantly reduced AR occupancy and Pol II activation at the KLK2 promoter (Fig. 3B). Whether KLK3e collaborates with Med1 and functionally affects transcription of the KLK2 promoter is unclear. Depletion KLK3e resulted in decrease of Med1 binding to the promoters of KLK2 (Fig. 3C). In addition, the effect of siKLK3e and siMed1 on the gene expression of KLK2 and Med1 was shown in Fig. S3A. Altogether our data suggest that KLK3e cooperates with Med1 to enhance KLK2 gene expression. It should be noted that the actions of AR, Med1 and Pol II at the KLK3 promoter were comparable to those at the KLK2 promoter (Fig.S3B, C and D), implying that the regulatory function of KLK3e at the KLK3/2 promoters may act through the same AR-dependent ribonucleoprotein complex.

Interestingly, silencing of KLK3e did not affect the action of AR and Pol II on the KLK3 enhancer (Fig S3E), suggesting that the biogenesis of KLK3e takes place after ligand-activated AR was recruited to the enhancer. While depletion of Med1 blocked AR binding and Pol II activation on the KLK3 enhancer, the expression of KLK3e was not significantly affected, implying that residual Med1-associated module may be sufficient for KLK3e production (Fig.S3F). Collectively, our data show the mechanism by which KLK3e forms a functional complex with AR and Med1 to promote transcription, supporting the findings that transfer of full enhancer activity to a target promoter depends on enhancer-bound transcription factors and enhancer-derived RNA transcripts (14).

The core enhancer element renders KLK3e RNA-dependent enhancer activity.

To validate the enhancer function of KLK3e, we utilized a luciferase reporter assay to quantitatively measure AR transcriptional activity. While inclusion of KLK3e (S1-S7) or flipped (S7-S1) sequences markedly increased KLK2 promoter activity by 4~5 fold, this effect was abolished when ARE III sequences were deleted (S2-S7) (Fig. 4A), confirming the importance of the enhancer integrity and their orientational independency (17, 24).

The expression of the KLK3e from the luciferase reporter was validated by RT-PCR (Figure S4A), indicating that AREs have the capacity to produce eRNAs. We next asked whether the KLK3e transcript alone is adequate for this transcriptional enhancement. For this purpose, we performed a co-transfection study based on reporters together with CMV-driven *sense* KLK3e constructs. As shown, the full-length (S1-S7) and flipped (S7-S1) KLK3e increased luciferase expression by 2~2.5 fold; whereas, ARE deletion (S2-S7) failed to enhance promoter activity (Fig. 4B). Furthermore, *antisense* KLK3e

(AS1-AS4) was unable to induce luciferase expression as *sense* KLK3e (S1-S7) did (Fig. S4B and C), again arguing that the *sense* KLK3e is the functional lncRNA rather than the *antisense* strand. To examine whether AR is required for the action of KLK3e, we conducted experiments in AR-negative COS-7 cells and confirmed that the activity of KLK3e depends on the availability of ligand-activated AR (Fig. S4D and E). Altogether, these data show that the core enhancer elements are essential for the enhancer activity of KLK3e and that ectopic KLK3e induces the promoter activity on a luciferase reporter, implying a trans-regulatory activation mediated by KLK3e.

As a complementary approach, we also addressed whether the observed luciferase expression is mediated through endogenous KLK3e. RNAi silencing of KLK3e significantly reduced luciferase activity in the reporters with or without KLK3e insertion (Fig. 4C, left), showing that KLK3e is required for the enhancement of DHT-induced activation. When KLK3e was ectopically expressed, it restored the luciferase expression inhibited by KLK3e suppression (Fig. S4F). Most importantly, the luciferase activity in the reporter with KLK3e insertion was reduced by ~50% in Med1 depleted cells (Fig. 4C, right), suggesting that Med1 facilitates KLK3e-dependent transcription. Notably, ncRNA-a has been shown to mediate long-range transcriptional activation through its association with the Med1 complex (18). In summary, our results strongly suggest that KLK3e forms a functional complex with AR and Med1 which facilitates the association of AR-bound enhancers with promoters of target genes, resulting in transcriptional activation.

KLK3 eRNA selectively enhances AR-regulated gene expression

To explore the potential effect of KLK3e beyond the KLK locus, we measured the expression of canonical androgen-regulated genes in KLK3e depleted cells. Suppression of KLK3e decreased the expression of NKX3.1, FKBP5 and PLZF; whereas, the expression of TMPRSS2, PDE9A and GUCY1A3 was not affected (Fig. 5A). Additionally, we observed a comparable KLK3e-mediated inhibition of NXK3.1 gene expression in VCaP cells (Fig. S2D). Moreover, we showed the expression eRNAs at upstream of NKX3.1, FKBP5, PLZF was induced by DHT but not affected by KLK3e siRNA knockdown (Fig. S5A), suggesting that the KLK3e siRNA does not interfere the transcription of eRNAs found at the enhancers of NKX3.1, FKBP5 and PLZF. Although eRNA-mediated trans effects are likely to be relatively infrequent or quantitatively small (17), our data show that KLK3e selectively regulates the expression of AR-regulated genes which do not reside on the same chromosome, implying a trans-regulatory function of KLK3e on AR-dependent gene expression.

We further assessed the regulatory role of KLK3e and Med1 on the promoter of NKX3.1 and TMPRSS2 to mechanistically validate our above findings in the KLK loci. While depletion of KLK3e significantly reduced the enrichment of AR and Pol II S5p at the NKX3.1 promoter (Fig. S5B, upper panel), the action of these factors at the TMPRSS2 promoter was unaffected (Fig. S5B, lower panel). In contrast, Med1 knockdown considerably blocked both AR occupancy and Pol II activity at both the NKX3.1 and TMPRSS2 promoters (Fig. S5C). These results demonstrate that KLK3e selectively enhances gene expression, while Med1 has a more general effect on the regulation of AR-dependent transcription. Most importantly, KLK3e depletion reduced Med1 recruitment to the promoter of NKX3.1, not TMPRSS2 (Fig. S5D and E), revealing that this eRNA/AR/Med1 complex may possess a degree of specificity in the AR-dependent transcriptional machinery. Furthermore, we evaluated the gene expression from the normal human prostate gland (n=4), primary (n=11) and metastatic (n=32) human prostate tumors (25) and showed that the correlation (R^2) of KLK3e (S1) with KLK3, KLK2, NKX3.1 and TMPRSS2 was 0.6213 ($p<5x10^{-11}$), 0.5893 ($p<5x10^{-10}$), 0.4624 ($p<2x10^{-7}$) and 0.3787 ($p<5x10^{-6}$), respectively. In addition, the expression the KLK3 and KLK2 was the most correlated ($R^2=0.8361$, $p<3x10^{-16}$) (Fig. 5B and S5F). Collectively, these *in vivo* data indicate that the transcription of KLK3e was detectable and its presence correlates with KLK3/2 gene expression. We noted less of a correlation between KLK3e expression and those genes in trans, and the correlation was stronger with NKX3.1 than TMPRSS2, consistent with our *in vitro* data, although these data do not prove a direct effect *in vivo* of KLK3e on the transcription of these genes. Next, we investigated the biological effect of KLK3e siRNA on LNCaP cells and showed that KLK3e depletion significantly compromised cell growth in the presence and absence of exogenous androgens (Fig. 5C). While this negative effect is modest, we postulate that suppression of multiple eRNAs produced from distinct enhancer elements may synergetically block AR transcriptional program. Thus, substantial efforts are needed to fully characterize the transcriptional and biological functions of other significant androgen-induced eRNAs in the future.

Discussion

We demonstrate that bidirectional KLK3e was produced from one of the strongest AR-bound enhancers (ARE III) and its expression pattern resemble KLK3 in prostate cancer cell lines (Figure 1, S1), in agreement with the previous report that a high correlation of activity-dependent induction exists between eRNAs and adjacent coding mRNAs (9). Suppression of KLK3e exclusively reduces gene

expression of KLK3 and KLK2, not other KLK family members and that KLK3e facilitates the assembly of the transcriptional apparatus at the promoters during gene activation events. This classifies KLK3e as a family of functional lncRNA involved in enhancing long distance gene transcription.

Whether eRNAs function as molecular bridges that mediate spatial interactions of distal enhancers and target promoters, or directly configure a chromatin state that facilitate transcription factor binding at the target promoters is unclear. Recently, chromatin isolation by RNA purification (ChIRP) coupled with deep sequencing was deployed to interrogate the nature of RNA and DNA interaction in a genome wide scale (26). Rosenfeld and colleagues used this methodology to identify potential sites where estrogen-induced FOXC1 eRNA localizes in the genome (17). While FOXC1 eRNA is specifically enriched in a small fraction of genomic loci, there were no known estrogen-regulated genes found adjacent to these RNA/DNA binding sites (17), implying that the functional effect of eRNAs on target genes may not completely rely on nucleotide base pairing. In this report, we demonstrate that KLK3e mediates chromosomal looping of KLK3/2 loci and confirmed in a heterologous reporter assay that KLK3e cooperates with AR and Med1 to enhance KLK2 promoter activity through that the core enhancer element (ARE III). The state of KLK3e and KLK3/2 was further validated in human prostate tissues (n=47) and showed that the expression of KLK3e significantly correlates with KLK3 (R^2=0.6213, p<5x10-11) and KLK2 (R^2=0.5893, p<5x10-10), implying that KLK3e may coordinate the transcription of KLK3/2 (Fig. 5B). However, we do not completely understand how KLK-associated eRNAs are regulated in castration resistant prostate cancer (CRPC), although it is well-accepted that the AR-mediated transcriptional program remains active. This observation can be explained by the activity of other nuclear receptors, since the core sequence of ARE can be also be recognized by glucocorticoid receptor (GR), progesterone receptor (PR), and mineralocorticoid receptor (MR) (27). More importantly, it has been shown that AR inhibition resulted in GR upregulation in a subset of prostate cancer cells supporting a mechanism of bypassing AR blockade via an alternative nuclear receptor in CRPC (28). Thus, it is possible that GR, PR or MR may mediate the expression of KLK3e, KLK3 and KLK2, attributing to adaptive or compensatory effects generated during CRPC development.

In view of the regulatory potential of lncRNAs on gene regulation in trans (29-31), eRNAs may be part of co-activator complexes and regulatory factors that participate in the transcriptional regulatory network. Although the entire circuitry of AR-dependent genes is unlikely controlled by a single eRNA, presumably, there are more androgen-induced eRNAs, and there could be redundancy in their enhancer activity. It is conceivable that multiple eRNAs produced from distinct enhancer elements tether with

their own transcriptional modules that operate simultaneously to initiate an accurate and a rapid transcriptional program. Therefore, we propose that an eRNA may function as a scaffold that guides an AR-associated protein complex to target chromatin and selectively enhances DHT-stimulated transcription either intra-chromosomally (cis activity) or inter-chromosomally (trans activity). This positive regulatory loop may provide new insight into RNA-dependent functional effects on the regulation of lineage- or tissue-specific gene expression.

While androgen-deprivation therapy (ADT) is designed to block the androgen signaling pathway and is the standard treatment for advanced prostate cancer, this study may help us to understand how androgen ADT influences the activity of AR-bound enhancers through altered expression of eRNAs. In addition, the differential eRNA expression patterns observed may be predicative markers of ADT efficacy for individual prostate cancer patients. More importantly, eRNAs may represent new targets that when suppressed may improve the therapeutic durability of ADT.

Experimental Procedures:

Chromatin Immunoprecipitation (ChIP) assay

ChIP experiments were performed as previously described (19) with some modifications. Briefly,
10 million cells were used per IP. Cells were fixed with 1% formaldehyde solution. DNA was sonicated and subjected into immunoprecipitated with antibodies against androgen receptor (N-20X, Santa Cruz biotechnology), Med1/TRAP220 (A300-793A, Bethyl Laboratories), RNA Polymerase II (p-Ser5) (ab5131, abcam), or nonspecific IgG. DNA was purified and analyzed by qPCR. The primers for qPCR are provided in the Supplemental Information

RNA Immunoprecipitation (RIP) assay

RIP experiments were performed as described previously with some modification (31). Briefly, 10 million cells were used per IP. Cells were fixed in 0.3 % formaldehyde solution. Total chromatin and RNAs were sonicated and subjected into immunoprecipitation with the same AR and Med1 antibodies used in ChIP experiments. Immunoprecipitated RNAs were isolated using TRIzol LS reagent, followed by cDNA synthesis. The primers for qPCR are provided in the Supplemental Information.

Chromosome Conformation capture (3C) assay

3C assay was performed as previously described with some modifications (32). After DHT (10nM) treatment, LNCaP cells were fixed with 1% formaldehyde. Cell pellets were lysed and resuspended in restriction buffer for BstY1 and 0.1% SDS for 10 min at 65^0C. Trion X-100 was added to a final concentration of 1.8% followed by overnight digestion of BstY1 (1500 U per 10^7 cells) at 37°C. DNA ligation was performed for 4 days at 16°C. The ligated samples were reverse-crosslinked with proteinase K at 65^0C overnight, followed by Phenol/Chloroform extraction, EtOH precipitation. The primers for qPCR are provided in the Supplemental Information.

Additional experimental procedures and methods are listed in the Supplementary Information.

Acknowledgment:

We would like to extend our gratitude to Dr. Changmeng Cai for his contributions in experimental and technical assistance, suggestions and critical reading of the manuscript. The work from Kantoff P.W. laboratory was supported by the Prostate SPORE (P50CA090381) and DoD post-doctoral training award (W81XWH-13-1-0383). Balk S.P. was supported by NIH P01 CA163227 and DOD W81XWH-11-1-0295.

References:

1. Heinlein CA (2004) Androgen Receptor in Prostate Cancer. *Endocrine Reviews* 25(2):276-308.
2. Knudsen KE & Penning TM (2010) Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. *Trends in Endocrinology & Metabolism* 21(5):315-324.
3. Nelson PS (2012) Molecular States Underlying Androgen Receptor Activation: A Framework for Therapeutics Targeting Androgen Signaling in Prostate Cancer. *JOURNAL OF CLINICAL ONCOLOGY*.
4. Hendriksen PJM (2006) Evolution of the Androgen Receptor Pathway during Progression of Prostate Cancer. *Cancer Research* 66(10):5012-5020.
5. Massie CE, *et al.* (2011) The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. *The EMBO Journal* 30(13):2719-2733.
6. Baek SH (2006) Ligand-specific allosteric regulation of coactivator functions of androgen receptor in prostate cancer cells. *Proceedings of the National Academy of Sciences* 103(9):3100-3105.
7. Yuan X & Balk SP (2009) Mechanisms mediating androgen receptor reactivation after castration. *Urologic Oncology: Seminars and Original Investigations* 27(1):36-41.
8. Mattick JS, *et al.* (2010) A Large Fraction of Extragenic RNA Pol II Transcription Sites Overlap Enhancers. *PLoS Biology* 8(5):e1000384.

9. Kim T-K, *et al.* (2010) Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465(7295):182-187.
10. Wang D, *et al.* (2011) Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature* 474(7351):390-394.
11. Orom UA & Shiekhattar R (2013) Long Noncoding RNAs Usher In a New Era in the Biology of Enhancers. *Cell* 154(6):1190-1193.
12. Ørom UA, *et al.* (2010) Long Noncoding RNAs with Enhancer-like Function in Human Cells. *Cell* 143(1):46-58.
13. Melo Carlos A, *et al.* (2013) eRNAs Are Required for p53-Dependent Enhancer Activity and Gene Transcription. *Molecular Cell* 49(3):524-535.
14. Lam MT, *et al.* (2013) Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. *Nature* 498(7455):511-515.
15. Mousavi K, *et al.* (2013) eRNAs Promote Transcription by Establishing Chromatin Accessibility at Defined Genomic Loci. *Mol Cell* 51(5):606-617.
16. Hah N, Murakami S, Nagari A, Danko C, & Kraus WL (2013) Enhancer Transcripts Mark Active Estrogen Receptor Binding Sites. *Genome Research*.
17. Li W, *et al.* (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature*.
18. Lai F, *et al.* (2013) Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. *Nature* 494(7438):497-501.
19. Yu J, *et al.* (2010) An Integrated Network of Androgen Receptor, Polycomb, and TMPRSS2-ERG Gene Fusions in Prostate Cancer Progression. *Cancer Cell* 17(5):443-454.
20. Lawrence MG, Lai J, & Clements JA (2010) Kallikreins on Steroids: Structure, Function, and Hormonal Regulation of Prostate-Specific Antigen and the Extended Kallikrein Locus. *Endocrine Reviews* 31(4):407-446.
21. Wang Q, *et al.* (2009) Androgen Receptor Regulates a Distinct Transcription Program in Androgen-Independent Prostate Cancer. *Cell* 138(2):245-256.
22. Chen Z, *et al.* (2011) Phospho-MED1-enhanced UBE2C locus looping drives castration-resistant prostate cancer growth. *The EMBO Journal* 30(12):2405-2419.
23. Jin F, Claessens F, & Fondell JD (2011) Regulation of Androgen Receptor-dependent Transcription by Coactivator MED1 Is Mediated through a Newly Discovered Noncanonical Binding Motif. *Journal of Biological Chemistry* 287(2):858-870.
24. Kong S, Bohl D, Li C, & Tuan D (1997) Transcription of the HS2 Enhancer toward a cis-Linked Gene Is Independent of the Orientation, Position, and Distance of the Enhancer Relative to the Gene. *MOLECULAR AND CELLULAR BIOLOGY* 17(7):3955-3965.
25. Stanbrough M (2006) Increased Expression of Genes Converting Adrenal Androgens to Testosterone in Androgen-Independent Prostate Cancer. *Cancer Research* 66(5):2815-2825.
26. Chu C, Qu K, Zhong Franklin L, Artandi Steven E, & Chang Howard Y (2011) Genomic Maps of Long Noncoding RNA Occupancy Reveal Principles of RNA-Chromatin Interactions. *Molecular Cell* 44(4):667-678.
27. Kim H-J, Park YI, & Dong M-S (2006) Comparison of prostate cancer cell lines for androgen receptor-mediated reporter gene assays. *Toxicology in Vitro* 20(7):1159-1167.
28. Arora VK, *et al.* (2013) Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 155(6):1309-1322.
29. Rinn JL, *et al.* (2007) Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs. *Cell* 129(7):1311-1323.

30. Prensner JR, *et al.* (2011) Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. *Nature Biotechnology* 29(8):742-749.
31. Yang L, *et al.* (2013) lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. *Nature* 500(7464):598-602.
32. Li Q, *et al.* (2013) Integrative eQTL-Based Analyses Reveal the Biology of Breast Cancer Risk Loci. *Cell* 152(3):633-641.

Figure Legends:

Figure 1. The KLK3 enhancer produces long non-coding RNAs

(A) The chromatin status in the vicinity of KLK3. ChIP-seq data sets of AR, H3K27ac, and H3K4me1 (19).

(B) RT-qPCR analyses of expression of *antisense* KLK3 eRNA (red bar), *sense* KLK3 eRNA (green bar), and mature KLK3 (blue bar) upon DHT (10nM) treatment in a time-dependent manner in LNCaP cells.

(C) Top panel: A diagram of functional ARE positions that are relative to the KLK3 transcription starting site (TSS) adapted from (20);

Bottom panel: RT-qPCR analyses of the expression of segments of KLK3e with DHT (10nM) treatment in LNCaP cells. The regions of LNA probes against the *antisense* and *sense* KLK3e were as indicated. Primer sets for qPCR were designed to specifically target either *antisense* (primers AS1-AS4) or *sense* strand (primer S1-S7) of KLK3e as showed. The right electrophoresis images show the PCR products of *antisense* (2230 bp) and *sense* (2170 bp) KLK3e.

All data analyzed by RT-qPCR were normalized to the expression level in vehicle (-). Data were shown as mean ± S.D. ($n \geq 3$).

Figure 2. The regulatory function of KLK3e on the KLK locus

(A) A heatmap of the KLK transcripts upon 4hr and 16 hr of DHT exposure in LNCaP cells (21). Genes are listed as their order in the kallikrein loci from centromere (top) to telomere (bottom).

(B) Top panel: Schematic drawing indicates the relative position of AR-regulated KLK genes and KLK3e on the chromosome 19;

Bottom panel: LNCaP cells were treated with vehicle (-) or DHT (10nM) and 50 nM of non-silencing control (siCtrl) or KLK3e siRNA (siKLK3e); lower case 'e' denoted as eRNA. Efficacy and effects of KLK3e depletion on KLK genes were assessed by RT-qPCR.

(C) Top panel: Schematic graph shows the KLK3/2 gene loci;

Bottom panel: LNCaP cells were treated with vehicle or DHT for 4 hrs. KLK2 mRNA expression was accessed by RT-qPCR (Insert). The relative cross-linking frequency between the anchor region (ARE III) and distal fragments (shaded bars) was determined by qPCR and normalized to the control region (Fragment A).

(D) The relative cross-linking frequency between the anchor region-D and -E region was determined by qPCR and normalized to the A region.

All data analyzed by RT-qPCR or qPCR were normalized to the expression level in vehicle and shown as mean ± S.D. (n≥3) and * $p<0.05$.

Figure 3. The KLK3e/AR/Med1 ribonucleoprotein complex transcriptionally regulates target promoters

Chromatin immunoprecipitation (ChIP) of AR or Med1 or activated Pol II (S5p) was performed in KLK3e or Med1 depleted LNCaP cells with or without DHT (10nM) for 2 or 8 hrs.

(A, B, C) qPCR was conducted to measure the action of AR, Pol II (S5p) and Med1 at the KLK2 promoter. Data were shown as mean ± S.D. (n≥3) and * $p<0.05$.

Figure 4. The core enhancer element renders KLK3e RNA-dependent enhancer activity

(A)Top panel: Schematic representation shows the insertion in KLK2 promoter driven luciferase reporter.

Bottom panel: Reporter assays analyses were performed with or without insertion of full-length (S1-S7), flipped (S7-S1) and ARE deleted (S2-S7) of *sense* KLK3e.

(B) Reporter analysis of co-transfecting the reporter with independent overexpressing vector as indicated.

(C) The effects of siKLK3e (left) or siMed1 (right) on the KLK2 promoter-driven Luciferase activity.

Luciferase activity was measured in the presence of DHT (10nM) for 24 hrs. All data shown are mean ±S.D. of at least three independent experiments. * $p<0.05$; firefly luciferase/ Renilla luciferase (FL/RL).

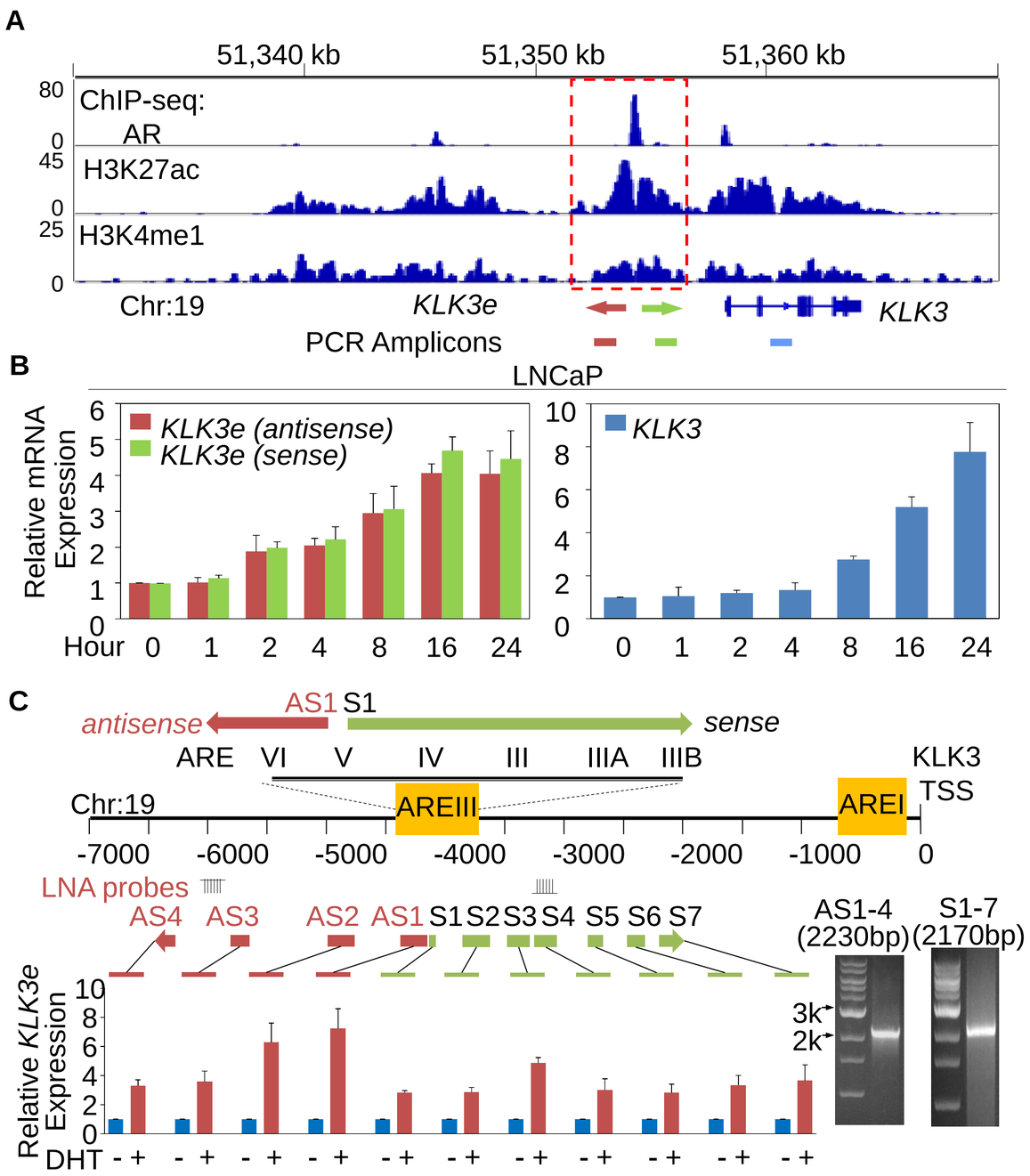
Figure 5. KLK3e selectively enhances AR-regulated gene expression

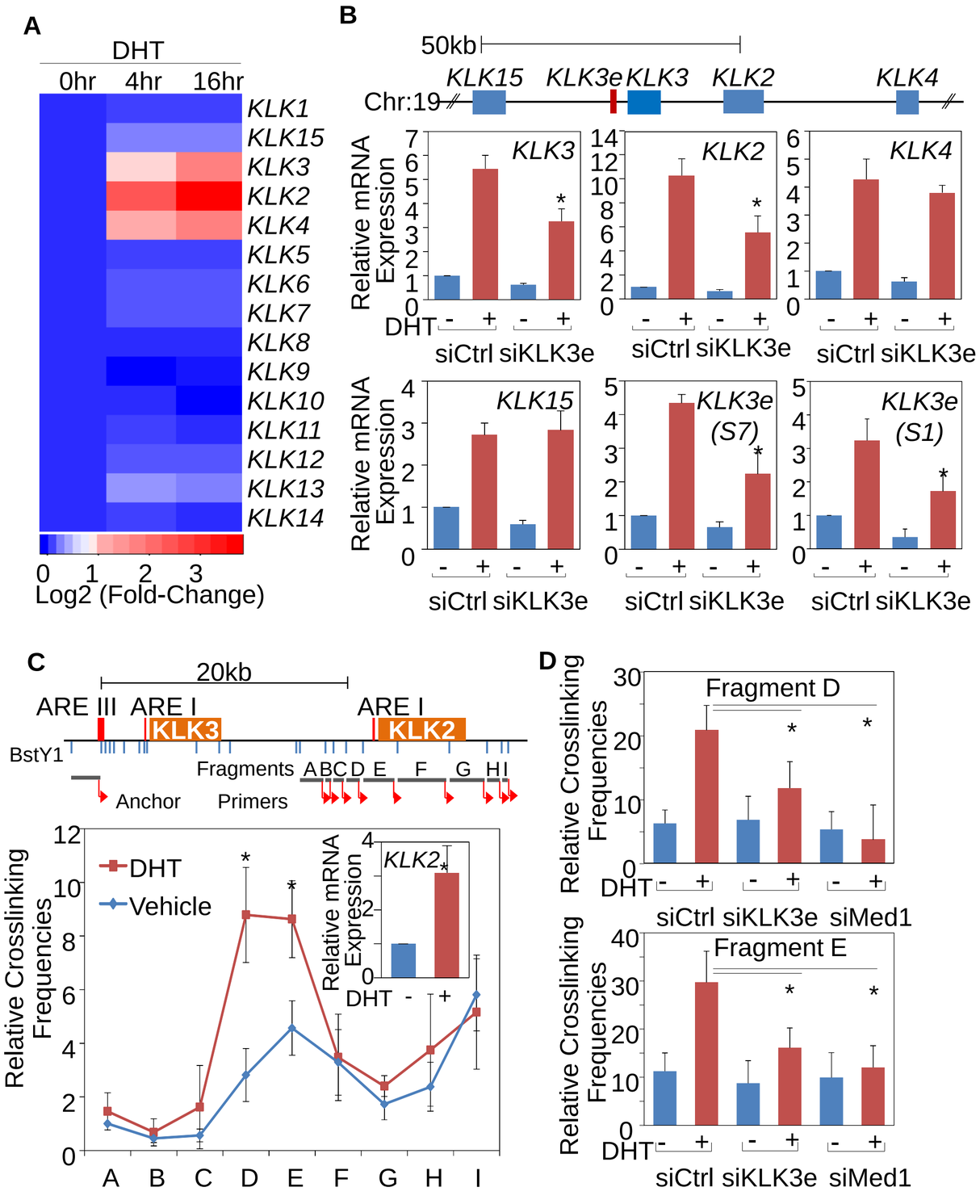
(A) LNCaP cells were treated with vehicle or DHT (10nM) and/or siCtrl or siKLK3e. The effect of siKLK3e on AR-regulated gene expression was determined by RT-qPCR.

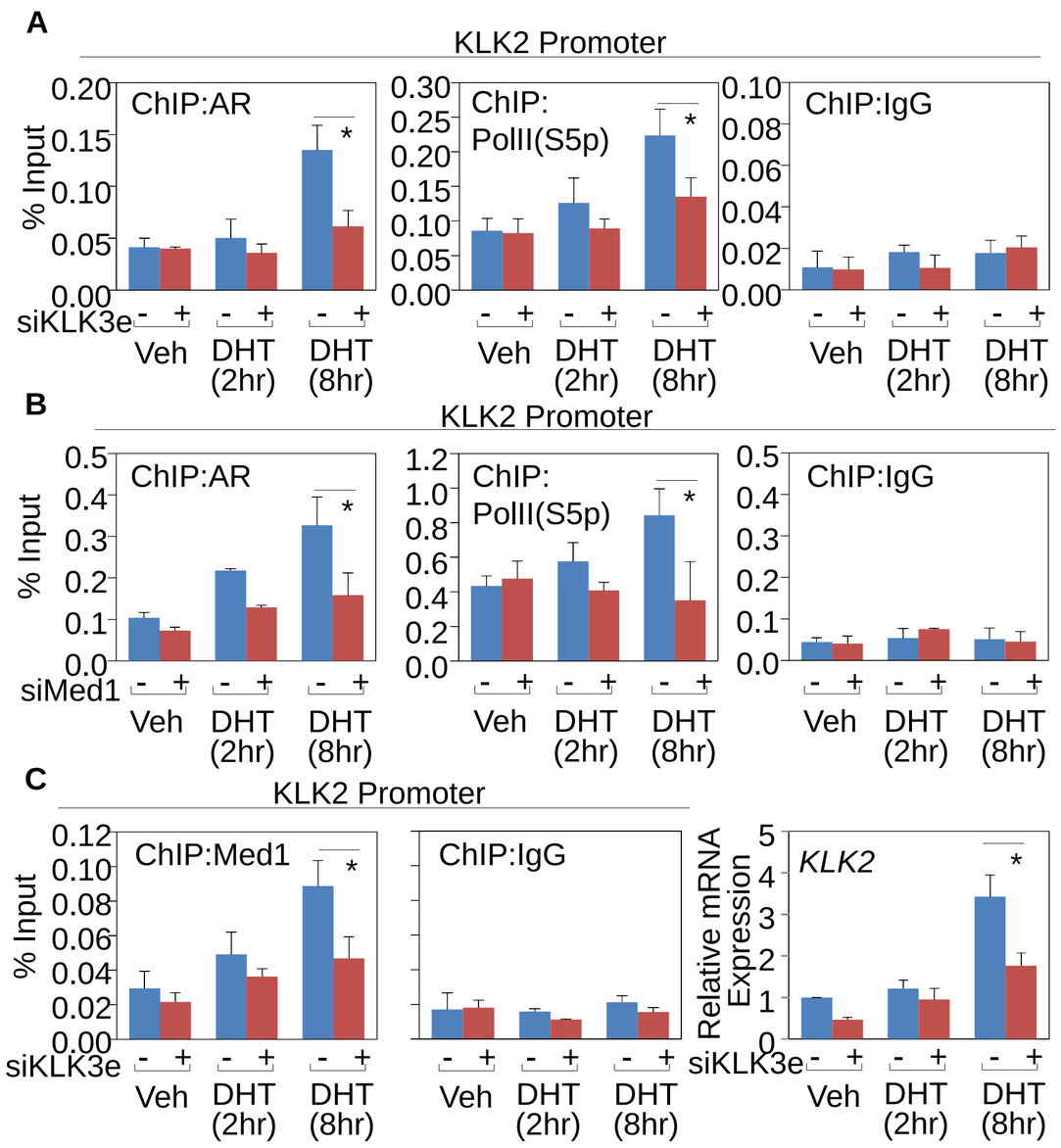
(B) Correlation between KLK3e (S1) and target gene expression *in vivo*. All data were normalized for GAPDH by the differences in threshold cycles (ΔC_T) from the normal human prostate gland (n=4), primary (n=11) and metastatic (n=32) prostate tumors (25).

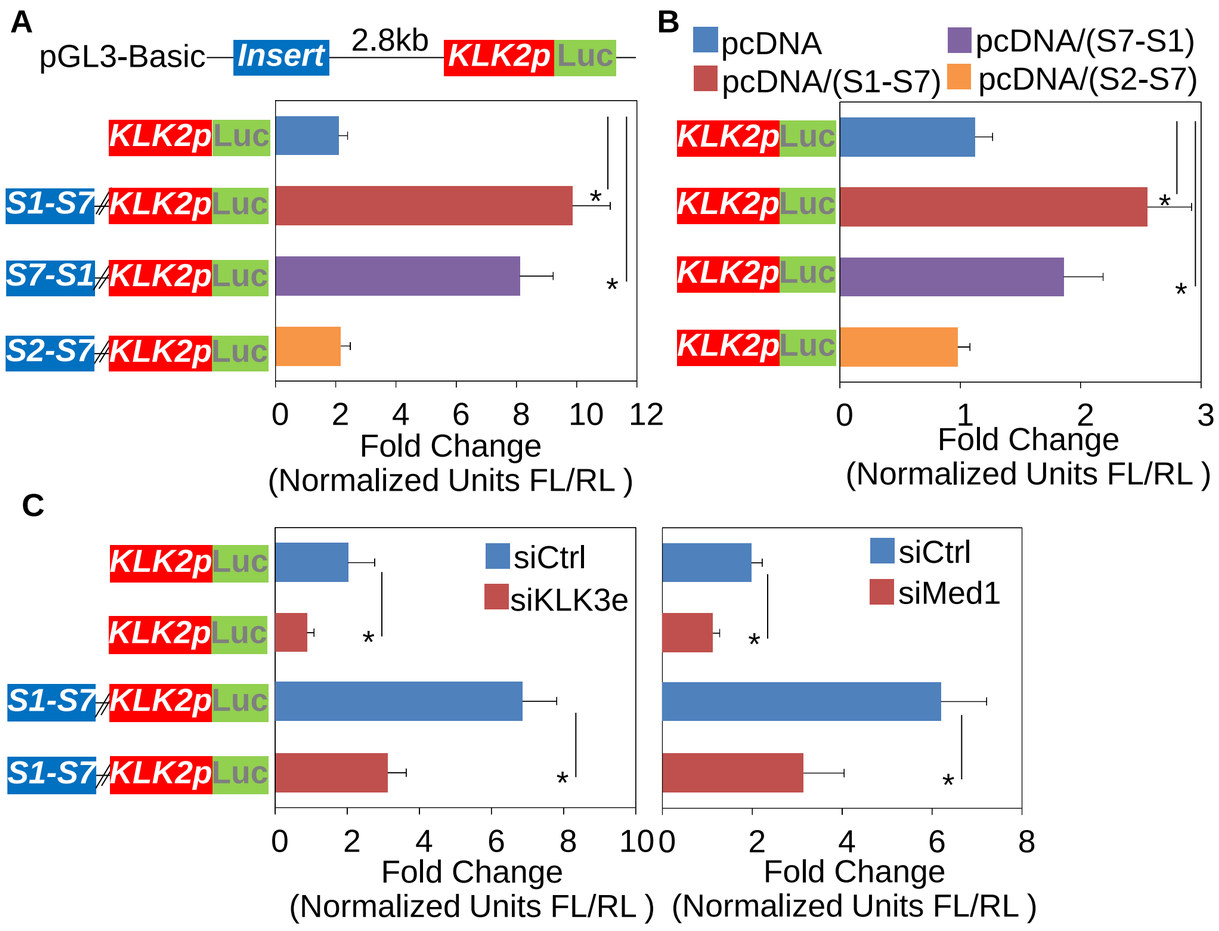
(C) LNCaP cells were cultured in 5% charcoal/dextran treated FBS (C-FBS) medium, followed by siRNA knockdown of scrambled control (siCtrl) or KLK3e (siKLK3e) with ethanol (veh) or DHT (10nM) treatment. Cell growth was measured by WST1 assay. Data represent mean ± S.D. ($n\geq3$) and * $p<0.05$.

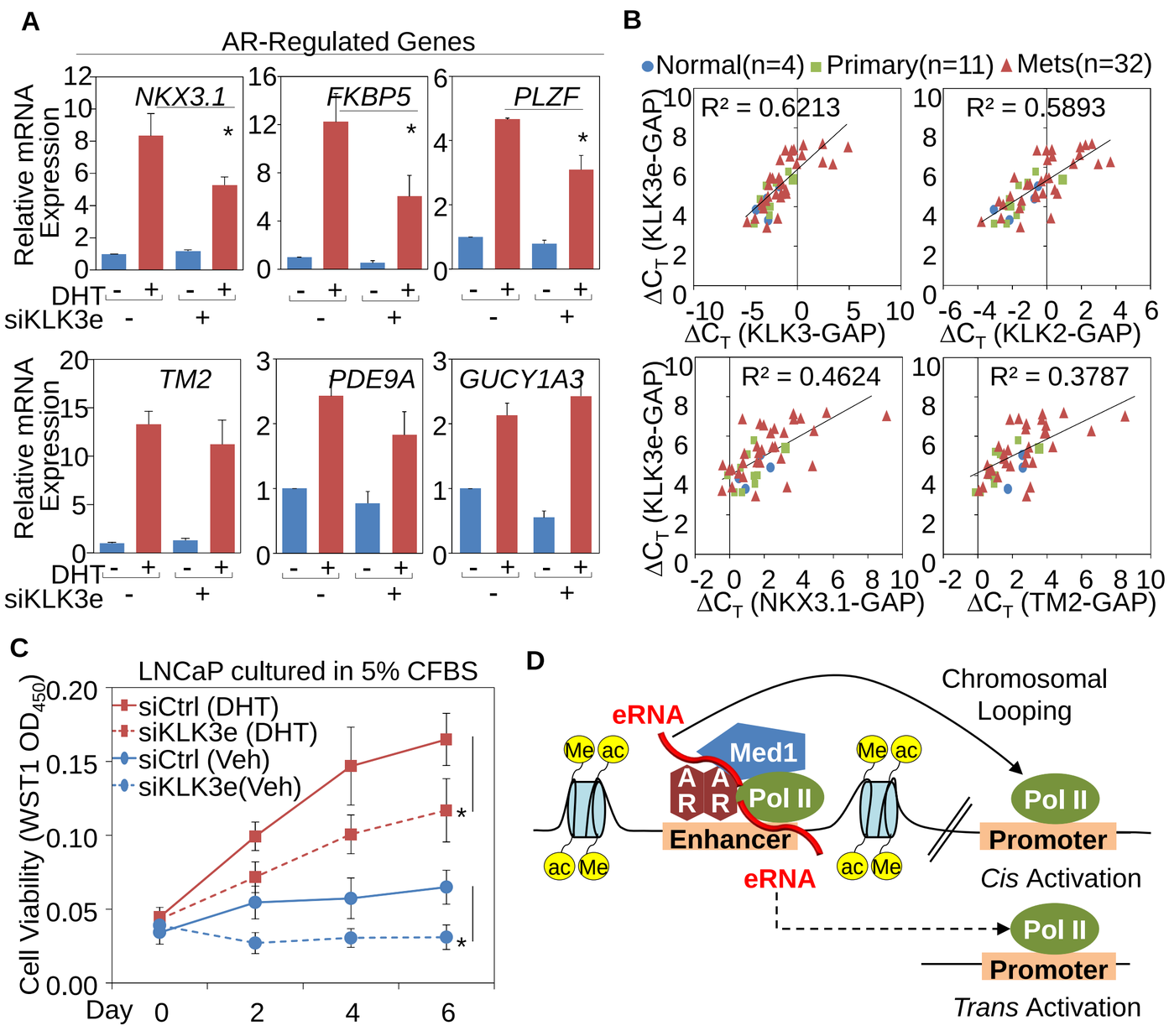
(D) A proposed model for functional activities of the eRNA/AR/Med1 complex in DHT-induced cis (solid line) and potential trans (dish line) gene activation events.


A
51,340 kb
51,350 kb
51,360 kb
80
0
ChIP-seq:
AR
45
0
H3K27ac
25
0
H3K4me1
Chr:19
KLK3e
KLK3
PCR Amplicons
B
LNCaP
Relative mRNA Expression
KLK3e (antisense)
KLK3e (sense)
KLK3
Hour 0 1 2 4 8 16 24
C
antisense
AS1
S1
sense
ARE VI V IV III IIIA IIIB
KLK3
TSS
Chr:19
AREIII
AREI
-7000 -6000 -5000 -4000 -3000 -2000 -1000 0
LNA probes
AS4 AS3 AS2 AS1 S1 S2 S3 S4 S5 S6 S7
AS1-4 (2230bp)
S1-7 (2170bp)
3k
2k
Relative KLK3e Expression
DHT - + - + - + - + - + - + - + - + - + - + - +


A
DHT
0hr
4hr
16hr
KLK1
KLK15
KLK3
KLK2
KLK4
KLK5
KLK6
KLK7
KLK8
KLK9
KLK10
KLK11
KLK12
KLK13
KLK14
0
1
2
3
Log2 (Fold-Change)
B
50kb
KLK15
KLK3e
KLK3
KLK2
KLK4
Chr:19
Relative mRNA Expression
KLK3
KLK2
KLK4
DHT
siCtrl
siKLK3e
KLK15
KLK3e (S7)
KLK3e (S1)
C
20kb
ARE III
ARE I
ARE I
KLK3
KLK2
BstY1
Fragments
A
B
C
D
E
F
G
H
I
Anchor
Primers
Relative Crosslinking Frequencies
DHT
Vehicle
Relative mRNA Expression
KLK2
D
Fragment D
Fragment E
siMed1


A
KLK2 Promoter
ChIP:AR
ChIP:
PolII(S5p)
ChIP:IgG
% Input
0.20
0.15
0.10
0.05
0.00
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.10
0.08
0.06
0.04
0.02
0.00
*
siKLK3e
- + - + - +
Veh
DHT (2hr)
DHT (8hr)
B
KLK2 Promoter
ChIP:AR
ChIP:
PolII(S5p)
ChIP:IgG
% Input
0.5
0.4
0.3
0.2
0.1
0.0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
siMed1
- + - + - +
Veh
DHT (2hr)
DHT (8hr)
C
KLK2 Promoter
ChIP:Med1
ChIP:IgG
KLK2
% Input
Relative mRNA Expression
0.12
0.10
0.08
0.06
0.04
0.02
0.00
5
4
3
2
1
0
*
siKLK3e
- + - + - +
Veh
DHT (2hr)
DHT (8hr)


A
pGL3-Basic
Insert
2.8kb
KLK2p
Luc
KLK2pLuc
S1-S7KLK2pLuc
S7-S1KLK2pLuc
S2-S7KLK2pLuc
0 2 4 6 8 10 12
Fold Change
(Normalized Units FL/RL)
B
pcDNA
pcDNA/(S7-S1)
pcDNA/(S1-S7)
pcDNA/(S2-S7)
KLK2pLuc
KLK2pLuc
KLK2pLuc
KLK2pLuc
0 1 2 3
Fold Change
(Normalized Units FL/RL)
C
KLK2pLuc
KLK2pLuc
S1-S7KLK2pLuc
S1-S7KLK2pLuc
siCtrl
siKLK3e
0 2 4 6 8 10
Fold Change
(Normalized Units FL/RL)
siCtrl
siMed1
0 2 4 6 8
Fold Change
(Normalized Units FL/RL)


A
AR-Regulated Genes
Relative mRNA Expression
NKX3.1
FKBP5
PLZF
TM2
PDE9A
GUCY1A3
DHT
siKLK3e
B
Normal(n=4) Primary(n=11) Mets(n=32)
R² = 0.6213
R² = 0.5893
R² = 0.4624
R² = 0.3787
ΔC_T (KLK3e-GAP)
ΔC_T (KLK3-GAP)
ΔC_T (KLK2-GAP)
ΔC_T (NKX3.1-GAP)
ΔC_T (TM2-GAP)
C
LNCaP cultured in 5% CFBS
siCtrl (DHT)
siKLK3e (DHT)
siCtrl (Veh)
siKLK3e(Veh)
Cell Viability (WST1 OD450)
Day
D
eRNA
Chromosomal Looping
Me
ac
Med1
A R
Pol II
Enhancer
Promoter
Cis Activation
Trans Activation

Supplemental Information:

Figure S1. The KLK3 enhancer produces long non-coding RNAs, related to Figure 1.

(A, B) VCaP and LNCaP-abl cells were treated with DHT (10nM) in a time dependent manner. The expression of antisense (red bar), sense (green bar), or mature KLK3 (blue bar) was determined by RT-qPCR.

(C) LNCaP cells were treated with or without 10 nM of DHT and 10μM of bicalutamide (bic). The expression of KLK3e and KLK3 was measure by RT-qPCR.

(D) Electrophoresis images show that the PCR products from fragments of *antisense* and *sense* KLK3e upon DHT (10nM) stimulation for 24 hr.

(E) Reverse transcription was performed using random hexamer primers and 3' RACE (Poly-T) adapter from the same RNA materials isolated from LNCaP cells treated with or without DHT (10 nM). The expression of KLK3e and KLK3 was measure by RT-qPCR.

(F) RNA was isolated and purified from LNCaP cells treated without (-) or with (+) DHT. 2 μg or 40μg of total RNA was subjected to electrophoresis, followed by hybridization using the LNA probes against KLK3, actin, *antisense* and *sense* KLK3e whose targeting regions are shown in Fig. C.

All data analyzed by RT-qPCR were normalized to individual expression in the absence of DHT exposure. Data represent mean ± S.D. ($n \geq 3$) and * $p<0.05$.

Figure S2. The regulatory function of KLK3e on the KLK locus, related to Figure 2.

Suppression of *antisense* (AS) and *sense* (S) KLK3e using distinct siRNA sets (1 construct targeting AS; whereas 2 constructs targeting S7 and 1 construct targeting S1) in LNCaP cells.

(A, B, E) RT-qPCR was used to determine the effect of KLK3e siRNAs on target mRNA expression. Transfection of siKLK3 and/or pcDNA/KLK3 in an AR-negative COS-7 cells.

(C) The gel image shows the PCR product of KLK3e (S1-S7) that is diminished in the presence of siKLK3e; RT, reverse transcription.

(D, E) Efficacy and effects of siKLK3e on KLK3e and AR-regulated genes in VCaP and LNCaP-abl cells were assessed by RT-qPCR.

(F) Efficacy and effects of siRNA against the *antisense* or *sense* KLK3e on KLK3e and AR-regulated genes in LNCaP cells were assessed by RT-qPCR.

(G) Electrophoresis images show the PCR products of anchor-fragment D (105bp) and anchor-fragment E (115bp) with or without DNA ligation.

(H) DNA sequencing results from the PCR products of the KLK3 enhancer and KLK2 promoter using the primer sets of anchor-fragment D and -fragment E.
(I, J) Fragments of *sense* or (K) *antisense* KLK3e interact with the AR and Med1. RNA immunoprecipitation (RIP) of AR or MED1 was performed using LNCaP cells treated with vehicle or DHT (10nM) for 4 hrs.
(L) The effect and efficacy of siKLK3e or siMed1 with or without DHT on KLK2 gene expression was measured by RT-qPCR.
All data analyzed by RT-qPCR or qPCR were normalized to the expression level in vehicle. Data were shown as mean ± S.D. (n≥3) and * $p<0.05$.

Figure S3. The KLK3e/AR/Med1 ribonucleoprotein complex transcriptionally regulates target promoters, related to Figure 3.

Chromatin immunoprecipitation (ChIP) of AR or Med1 or activated Pol II (S5p) was performed in KLK3e or Med1 depleted LNCaP cells with or without DHT (10nM) for 2 or 8 hrs.
qPCR was conducted to measure the activity on the (B, C, D) KLK3 promoter and (E, F) KLK3 enhancer. Also accessed was the mRNA expression of (A) KLK2, and Med1 as well as (B, C, E, F) KLK3e and KLK3 by RT-qPCR analysis. Data represent mean ± S.D. (n≥3) and * $p<0.05$.

Figure S4. KLK3e harbors sequence-specific enhancer activity, related to Figure 4.

LNCaP cells were transfected with expression plasmids as indicated. (A, C) The gel image shows PCR product of *sense* (S7) or *antisense* (AS4) KLK3e.Co-transfection of constructed reporters and/or overexpressing plasmids as indicated in (B) LNCaP and (D, E, F) COS-7 cells were treated with DHT for 24 hrs for Luc reporter analysis. All data shown are mean ±S.D. of three or more independent experiments. * $p<0.05$; firefly luciferase/Renilla luciferase (FL/RL).

Figure S5. KLK3e selectively regulates the promoter activity of target gene, related to Figure 5.

(A) The chromatin status at the upstream enhancers of FKBP5, NKX3.1 and PLZF. ChIP-seq data sets of AR, H3K27ac, and H3K4me1 (28). RT-qPCR analyses of the expression of eRNAs with or without KLK3e silencing and/or DHT (10nM) treatment in LNCaP cells.

ChIP of AR or Med1 or activated Pol II (S5p) was performed using LNCaP cells transfected with (B, D, E) siKLK3e or (C) siMed and treated with or without DHT (10nM) for 2 or 8 hrs. qPCR analysis was used to access the promoter activity of NKX3.1 and TMPRSS2. mRNA expression levels of NKX3.1 versus TMPRSS2 in KLK3e and Med1 depleted LNCaP cells were determined by RT-qPCR.
(F) Correlation between KLK3 and KLK2 gene expression *in vivo*. All data were normalized for GAPDH by the differences in threshold cycles (ΔC_T) from the normal human prostate gland (n=4), primary (n=11) and metastatic (n=32) prostate tumors (33).
All data shown are mean ±S.D. of three or more independent experiments, * $p<0.05$.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Cell Culture and siRNA Transfection

LNCaP cells were cultured in RPMI1640 medium with 10% FBS. VCaP and COS-7cells were cultured in DMEM medium with 10% FBS. Prior to androgen induction or transfection, cells were grown to 50%–60% confluence in 5% charcoal/dextran treated FBS (C-FBS) medium for 3 days and then treated with 10 nM of DHT or siRNA (50nM).

RNA, Isolation, Purification, and cDNA Synthesis

Prostate cancer cells and human prostate tissue frozen sections (25) were harvested and resuspended in TRIzol (Invitrogen) and RNAs were extracted according to the manufacturer's protocol. Total RNAs were purified by RNeasy Kit (QIAgene). Purified RNA from LNCaP cells was subsequently washed by RiboMinus Eukaryote Kit (Invitrogen). The ribosomal RNA depleted RNA fraction was subject to cDNA synthesis was performed using High Capacity cDNA Reverse Transcription Kit (Life Technology) or 3'RACE poly-T adaptor (Ambion).

Reagents used for siRNA-Mediated Knockdown experiments

siRNA sequences targeting the *sense* strand of KLK3e: 3'-end (S7) #1, 5'-gaggaugaguugaggauauuu-3'; #2, 5'-ggguuggcuuguuagguuauu-3'; 5'-end (S1): 5'-ggaacauauuguaucgauuuu-3' and the *antisense* strand (siAS): 5'-gcaaaaggauacagaugaauu-3'. siRNA targeting Med1 and non-targeting siRNA were ON-TARGETplus Human MED1 siRNA and ON-TARGETplus Non-Targeting siRNA#1 from Thermo Scientific. Transfection of LNCaP and COS-7 cells was performed with Lipofectimane 2000 or Lipofectimane RNAiMax (Invitrogen) according to the manufacturer's protocol.

Plasmid Constructs for Luciferase Assay

Full-length (S1-S7), flipped (S7-S1), ARE deleted (S2-S7), and *antisense* (AS1-AS4) of KLK3e were subcloned into pcDNA3 (Invitrogen) using EcoRI and XbaI sites. pGL3-Basic (Promega) was digested with SacI and XhoI and KLK2 promoter (-407~+143) promoter was inserted into these sites. Additionally, TK promoter sequences from pRL-TK (Promega) were subcloned into pGL3/KLK2 promoter using BglII and HindIII. Full length (S1-S7), flipped (S7-S1) and ARE deleted (S2-S7) KLK3e were inserted into downstream of SV40 late poly(A) signal of luciferase reporter. Luciferase experiments were conducted in 96-well white plates using Dual-Glo (Promega) according to the manufacturer's protocol.

Northern blot analysis

Northern blot assay was performed using DIG Northern Starter Kit (Roche) with some modifications. Total RNA was isolated and purified from LNCaP treated with or without DHT (10 nM). 2μg or 40μg of RNA were subjected to electrophoresis, followed by hybridization using the 10 nM of LNA probes (Exiqon) against KLK3: /5DigN/tcaggaggctcatatcgtaga/3DigN/, actin (Exiqon), *sense* KLK3e: /5DigN/atgctggatgatgagtggatga/3DigN/ and *antisense* KLK3e: /5DigN/agtgacctaacatctctgtga/3DigN/.

Cell Proliferation assay

Cell growth was examined using the WST1 assay (Roche) following the manufacturer's protocol. Briefly, 3,000 LNCaP cells were grown in a 96-well-plate in the RPMI-1640 medium containing 5% C-FBS. After 2-day incubation, cells were transfected with 50 nM of siRNAs to non-silencing control (siCtrl) or KLK3e (siKLK3e). After 24 hours of incubation, cells were treated with ethanol (Veh) or DHT (10nM). Then, the WST1 assay was performed after Day 0-6.

Supplemental Information:

***sense* and *antisense* KLK3e sequences:**

sense (S1-S7):

TGGGACAACTTGCAAACCTGCTCAGCCTTTGTCTCTGATGAAGATATTATCTTCATGATCTT
GGATTGAAAACAGACCTACTCTGGAGGAACATATTGTATCGATTGTCCTTGACAGTAAACA
AATCTGTTGTAAGAGACATTATCTTTATTATCTAGGACAGTAAGCAAGCCTGGATCTGAGA

GAGATATCATCTTGCAAGGATGCCTGCTTTACAAACATCCTTGAAACAACAATCCAGAAAA
AAAAAGGTGTTGCTGTCTTTGCTCAGAAGACACACAGATACGTGACAGAACCATGGAGAAT
TGCCTCCCAACACTGTTCAGCCAGAGCCTTCCACCCTTGTCTGCAGGACAGTCTCAACGTTC
CACCATTAAATACTTCTTCTGTCACATCCTGCTTATTTATGCCTAACCAAGGTTCTAGGTCCC
GATCGACTGTGTCTGGCAGCACTCCACTGCCAAACCCAGAATAAGGCAGCGCTCAGGATCC
CGAAGGGGCATGGCTGGGGATCAGAACTTCTGGGTTTGAGTGAGGAGTGGGTCCACCCTCT
TGAATTTCAAAGGAGGAAGAGGCTGGATGTGAAGGAACTGGGGGAGGGAAAGTGTCAGTT
CCGAACTCTTAGGTCAATGAGGGAGGAGACTGGTAAGGTCCCAGCTCCCGAGGTACTGATG
TGGGAATGGCCTAAGAATCTCATATCCTCAGGAAGAAGGTGCTGGAATCCTGAGGGGTAG
AGTTCTGGGTATATTTGTGGCTTAAGGCTCTTTGGCCCCTGAAGGGCAGAGGCTGGAACCA
TTAGGTCCAGGGTTTGGGGTGATAGTAATGGGATCTCTTGATTCCTCAAGAGTCTGAGGAT
CGAGGGTTGCCCATTCTTCCATCTTGCCACCTAATCCTTACTCCACTTGAGGGTATCACCAG
CCCTTCTAGCTCCATGAAGGTGCCCCTGGGCAAGCACAATCTGAGCATGAAAGATGCCCCA
GAGGCCTTGGGTGTCATCCACTCATCATCCAGCATCCACACTCTGAGGGTGTGGCCAGCAC
CATGACGTCATGTTGCTGTGACTATCCCTGCAGCGTGCCTCTCCAGCCACCTGCCAACCGTA
GAGCTGCCGACATCCTCCTCTGGTGGGAGTGGCCTGCATGGTGCCAGGCTGAGGCCTAGTG
TCAGACAGGGAGCCTGGAATCATAGGGATCCAGGACTCAAAAGTGCTAGAGAATGGCCAT
ATGTCACCATCCATGAAATCTCAAGGGCTTCTGGGTGGAGGGCACAGGGACCTGAACTTAT
GGGTTTTCCCCAAGTCTATTGCTCTCCCAAGTGAGTCTCCCAGATACGAGGCACTGTGCCAG
CATCAGCCTTATCTCCACCACATCTTGTAAAAGGGACTACCCAGGGCCCTGATGAACACCA
TGGTGTGTACAGGAGTAGGGGGTGGAGGCACGGACTCCTGTGAGGTCACAGCCAAGGGAG
CATCATCATGGGTGGGGAGGAGGCAATGGACAGGCTTGAGAACGGGGATGTGGTTGTATTT
GGTTTTCTTTGGTTAGATAAAGTGCTGGGTATAGGATTGAGAGTGGAGTATGAAGACCAGT
TAGGATGGAGGATCAGATTGGAGTTGGGTTAGAGATGGGGTAAAATTGTGCTTCGGATGAG
TTTGGGATTGACACTGTGGAGGTGGTTTGGGATGGCATGGCTTTGGGATGGAAATAGATTT
GTTTTGATGTTGGCTCAGACATCCTTGGGGATTGAACTGGGGATGAAGCTGGGTTTGATTTT
GGAGGTAGAAGACGTGGAAGTAGCTGTCAGATTTGACAGTGGCCATGAGTTTTGTTTGATG
GGGAATCAAACAATGGGGGAAGACATAAGGGTTGGCTTGTTAGGTTAAGTTGCGTTGGGTT
GATGGGGTCGGGGCTGTGTATAATGCAGTTGGATTGGTTTGTATTAAATTGGGTTGGGTCA
GGTTTTGGTTGAGGATGAGTTGAGGATATGCTTGGGGACACCGGATCCATGAGGTTCTCAC
TGGAGTGGAGACAAACTTCCTTTCCAGGATGAATCCGGGGAAGCCTTAATTCACGTGTAGG

GGAGGTCAGGCCACTGGCTAAGTATATCCTTCCACTCCAGCTCTAAGATGGTCTTAAATTGT
GATTATCTATATCCACCTCTGTCTCCCTCA

antisense (AS1-AS4):

TCCGGAACACAGATCTGACATGCTCATCTGCTGCGTGGGGTTTACTATCTGCCTTGAATCCT
AAAGTCTGCCCCTGAGGAGTCTGGAATCTGGAATCTATTATCTTTAGTTGAACACCTGCAGT
CTGCGTTCTGCTTTGCAGAGCTGGATTCTTTACCCGTGATCCGTTCTGGCATTTCAACTTCAC
ACAGAATAGGCCACTTCATGGTACTGTTTTCAACCAATCAAGCAAATTAATCGTTAGAGCC
CTTTCTAATTCCTCTAGTTGCAATGTTAAACCCAATATTTCTGTGTAGTGGGTCCATATTAAT
AAATTCAGCCTGATCTAACCTTATATTCCTTCCACCATTATTCCATACCAACCCTTAATATG
CATTCCCACATATATTTCCTGGATTTCTGTCTATATAATGGGCAATCCCGGCTTTGCCAGTTT
CTAGTGACCTAACATCTCTGTGACTCATGTTATTGTACTTTAAAGTGGAAATAATAACAGTT
CCTGTACAGTTTAGGGCTTCCCAAGATGACCCTCCATTGACATCAGTTGAGATTCCAGGAG
AGTCCAAAAGATCATCCTTAAGGTTCAGTCGTTTGCTAGGATGCTCATGGTTGTGGTTTATT
ACAGCAAAAGGATACAGATGAAAATCTGGCAAGGGAGAGGTGAATGAGGCAAAGACAAG
GAGAACTCAAAGTGCGAAGCTTCTAGTTTTCTTTTCCCGGTGACATCGTGGAAAGCACTAG
CATCTCTAAGCAATGATCTGTGACAATATTCACAGTGTATTGCCATCCAGGGAACTCAACT
GAGCCTTGATGTCCAGAGATTTTTGTGTTTTTTTCTGAGACTGAGTCTCGCTCTGTGCCCAG
GCTGGAGTGCAGTGGTGCAACCTTGGCTCACTGCAAGCTCCGCCTCCTGGGTTCACGCCAT
TCTCCTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGGCACCCGCCACCACGCCTGGCTAA
TTTTTTTGTATTTTTAGTAGAGATGGGGTTTCACTGTGTTAGCCAGGATGGTCTCAGTCTCCT
GACCTCGTGATCTGCCCACCTTGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACTG
CGCCTGGCCGATATCCAGAGATTTTTTGGGGGGCTCCATCACACAGACATGTTGACTGTCTT
CATGGTTGACTTTTAGTATCCAGCCCCTCTAGAAATCTAGCTGATATAGTGTGGCTCAAAAC
CTTCAGCACAAATCACACCGTTAGACTATCTGGTGTGGCCCAAACCTTCAGGTGAACAAAG
GCACTCTAAACTGGCAGGATATTCCAAAGCATTAGAGATGACCTCTTGCAAAGAAAAAGA
AATGGAAAAGAAAAAGAAAGAAAGGAAAAAAAAAAAAAAAAAGAGATGACCTCTCAGGC
TCTGAGGGGAAACGCCTGAGGTCTTTGAGCAAGGTCAGTCCTCTGTTGCACAGTCTCCCTC
ACAGGGTCATTGTGACGATCAAATGTGGTCACGTGTATGAGGCACCAGCACATGCCTGGCT
CTGGGGAGTGCCGTGTAAGTGTATGCTTGCACTGCTGAATGGCTGGGATGTGTCAGGGATT
ATCTTCAGCACTTACAGATGCTCATCTCATCCTCACAGCATCACTATGGGATGGGTATTACT

GGCCTCATTTGATGGAGAAACTGGCTGTGGCTCAGAAAGGGGGGACCACTAGACCAGGGA
CACTCTGGATGCTGGGGACTCCAGAGACCATGACCACTCACCAACTGCAGAGAAATTAATT
GTGGCCTGATGTCCCTGTCCTGGAGAGGGTGGAGGTGGACCTTCACTAACCTCCTACCTTG
ACCCTCTCTTTTAGGGCTCTTTCTGACCTCCACCATGATACTAGGACCCCATTGTATTCTGTA
CCCTCTTGACTCTATGACCCCCACTGCCCACTGCATCCAGCTGGGTCCCCTCCTATCTCTATT
CCCAGCTGGCCAGTGCAGTCTCAGTGCCCACCTGTTTGTCAGTAACTCTGAAGGGGCTGAC
ATTTTACTGACTTGCAAACAAATAAGCTAACTTTCCAGAGTTTTGTGAATGCTGGCAGAGTC
CATGAGACTCCTGAGTCAGAGGCAAAGGCTTTTACTGCTCACAGCTTAGCAGACAGCATGA
GGTTCATGTTCACATTAGTACACCTTGCCCCCCCCCAAATCTTGTAGGGTGACCAGAGCAGTC
TAGGTGGATGCTGTGCA

Real time RT-PCR primers:

KLK3 Forward; GATGAAACAGGCTGTGCCG

KLK3 Reverse; CCTCACAGCTACCCACTGCA

KLK3e S1 Forward; TGGGACAACTTGCAAACCTG

KLK3e S1 Reverse; CCAGAGTAGGTCTGTTTTCAATCCA

KLK3e S2 Forward; AGAATTGCCTCCCAACACTG

KLK3e S2 Reverse; GGACCCACTCCTCACTCAAA

KLK3e S3 Forward; GATGTGGGAATGGCCTAAGA

KLK3e S3 Reverse; GGATTAGGTGGCAAGATGGA

KLK3e S4 Forward; AGGGTATCACCAGCCCTTCT

KLK3e S4 Reverse; GAGGATGTCGGCAGCTCTAC

KLK3e S5 Forward; GCCAGCATCAGCCTTATCTC

KLK3e S5 Reverse; TACAACCACATCCCCGTTCT

KLK3e S6 Forward; TTGTGCTTCGGATGAGTTTG

KLK3e S6 Reverse; CCATTGTTTGATTCCCCATC

KLK3e S7 Forward; GGTCAGGTTTTGGTTGAGGA

KLK3e S7 Reverse; TGAGGGAGACAGAGGTGGAT

KLK3e AS4 Forward; TCCGGAACACAGATCTGACA

KLK3e AS4 Reverse; GAACGGATCACGGGTAAAGA

KLK3e AS3 Forward; AAATCTGGCAAGGGAGAGGT

KLK3e AS3 Reverse; GAGTTCCCTGGATGGCAATA

KLK3e AS2 Forward; GACGATCAAATGTGGTCACG

KLK3e AS2 Reverse; CTCCATCAAATGAGGCCAGT

KLK3e AS1 Forward; TTTTGTGAATGCTGGCAGAG

KLK3e AS1 Reverse; TGCACAGCATCCACCTAGAC

KLK2 Forward; GCTGCCCATTGCCTAAAGAAG

KLK2 Reverse; TGGGAAGCTGTGGCTGACA

KLK15 Forward; TAACTGTGGCGCTTCCCTCATC

KLK15 Reverse; AGACGTGGTCCGTAGTTGCTCT

KLK4 Forward; GGCACTGGTCATGGAAAACGA

KLK4 Reverse; TCAAGACTGTGCAGGCCCAGCC

NKX31 Forward; CGCAGAACGACCAGCTGAGCA

NKX31 Reverse; CCTGAAGTGTTTTCAGAGTCCAAC

NKX31e Forward; GCCCAGTTCATTCTTTGGAA

NKX31e Reverse; CCAAGTCAAAGGGGAATCCT

TMPRSS2 Forward; CCTGTGTGCCAAGACGACTG

TMPRSS2 Reverse; TTATAGCCCATGTCCCTGCAG

FKBP5 Forward; TGCCAAGACCTTCCGAGGCGTC

FKBP5 Reverse; GGCTTCCGCTCTGCCCAAAGA

FKBP5e Forward; GCGGTGTCTTTTCGTAGAGG

FKBP5e Reverse; TAAGGCCCCTTCCTCATTCT

PLZF Forward; AGCGGGTACTGCGAACTGC

PLZF Reverse; ATCGCACAAAGTCCCGGCCAG

PLZFe Forward; AGACCAGGCCAAAATGTCAG

PLZFe Reverse; TTTGTTTCCCCTCCTCCTCT

PDE9A Forward; GATCCCAATGTTTGAAACAGTGAC

PDE9A Reverse; TCCCAAAGTGGCTGCAGC

GUCY1A3 Forward; AGCAGTGTGGAGAGCTGGAT

GUCY1A3 Reverse; CTGATCCAGAGTGCAGTCCA

GAPDH Forward; CCTGTTCGACAGTCAGCCGCATC

GAPDH Reverse; GGTGACCAGGCGCCCAATACG

PCR primers for constructing plasmids:

KLK2 promoter luc forward; GATCGAGCTCGAGTTCAAGGCTACAGGGAGC

KLK2 promoter luc reverse; GATCCTCGAGGGGCTTCAGCATAAGAGTCAG

KLK2 promoter (-407~+143) was subcloned into pGL3 basic using SacI and XhoI

KLK3e S1_promoter luc; GATCGGATCCGAATTCTGGGACAACTTGCAAACCTGC

KLK3e S7_ promoter luc; GATCGTCGACTGAGGGAGACAGAGGTGGATA

KLK3e S7_promoter luc; GATCGTCGACTGGGACAACTTGCAAACCTGC

KLK3e S1_ promoter luc; GATCGGATCCGAATTCTGAGGGAGACAGAGGTGGATA

KLK3e_S2 promoter luc; GATCGAATTCAGAATTGCCTCCCAACACTGT

KLK3e_S7 promoter luc; GATCGTCGACTGAGGGAGACAGAGGTGGATA

Full-length (S1-S7), flipped (S7-S1) and ARE deleted (S2-S7) KLK3e were subcloned into KLK2 promoter (-407~+143) luc using BamHI+EcoRI and SalI.

KLK3e_S1; GATCGAATTCTGGGACAACTTGCAAACCTGC

KLK3e_S7; GATCTCTAGATGAGGGAGACAGAGGTGGATA

KLK3e_S7; GATCTCTAGATGGGACAACTTGCAAACCTGC

KLK3e_S1; GATCGAATTCTGAGGGAGACAGAGGTGGATA

KLK3e_S2; GATCGAATTCAGAATTGCCTCCCAACACTGT

KLK3e_S7; GATCTCTAGATGAGGGAGACAGAGGTGGATA

Full-length (S1-S7), flipped (S7-S1) and ARE deleted (S2-S7) KLK3e were subcloned into pcDNA3 using EcoRI and XbaI.

KLK3e_AS1; GATCCTCGAGTGCACAGCATCCACCTAGAC

KLK3e_AS4; GATCGAATTCTCCGGAACACAGATCTGACA

antisense KLK3e (AS4-AS1) was subcloned into pcDNA3 using EcoRI and XhoI.

ChIP/RIP real-time PCR primers

KLK3 Enhancer Forward; TGGGACAACTTGCAAACCTG

KLK3 Enhancer Reverse; CCAGAGTAGGTCTGTTTTCAATCCA

KLK3 promoter forward; CCTAGATGAAGTCTCCATGAGCTACA

KLK3 promoter reverse; GGGAGGGAGAGCTAGCACTTG

KLK2 promoter forward; CTCCAGACTGATCTAGTATG

KLK2 promoter reverse; TTGGCACCTAGATGCTGACC

NKX31 promoter ChIP forward; GCAGATCTGAGTTTGCACCA

NKX31 promoter ChIP reverse; TGGGACGATCAAGACAAACA

TMPRSS2 Promoter forward; CGCCCCAGAGTCCCTTAT

TMPRSS2 Promoter reverse; TAATCTCAGGAGGCGGTGTC

U1 Forward; ATACTTACCTGGCAGGGGAG

U1 Reverse; CAGGGGGAAAGCGCGAACGCA

PCR primers for 3C assays:

Anchor; TCGATTGTCCTTGACAGTAAACA

A; GGCTCCAGAGGACCATGATT

B; GGTACCAGCAAACTTGTCCAG

C; GACGTCATGTAGCTGCGACT

D; ACCCTTGACCTCCTGGACTT

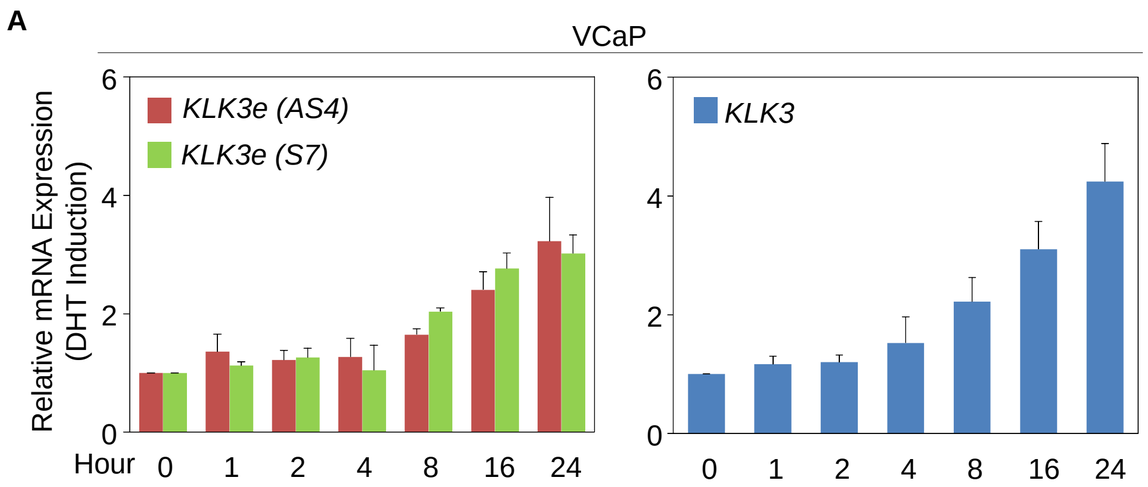
E; TGCCCATTGCCTAAAGAAGT

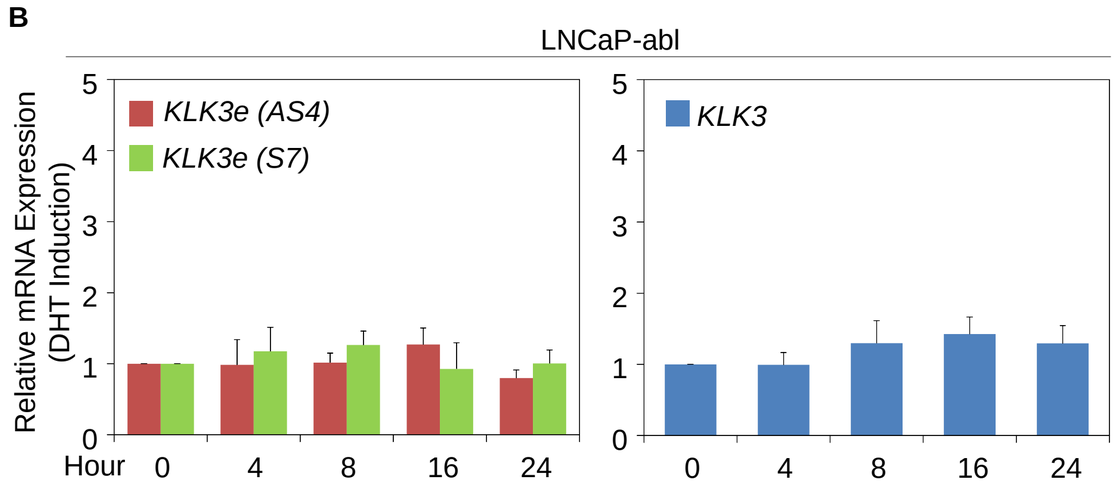
F; AGATAAGGCCGTGAGCAGAA

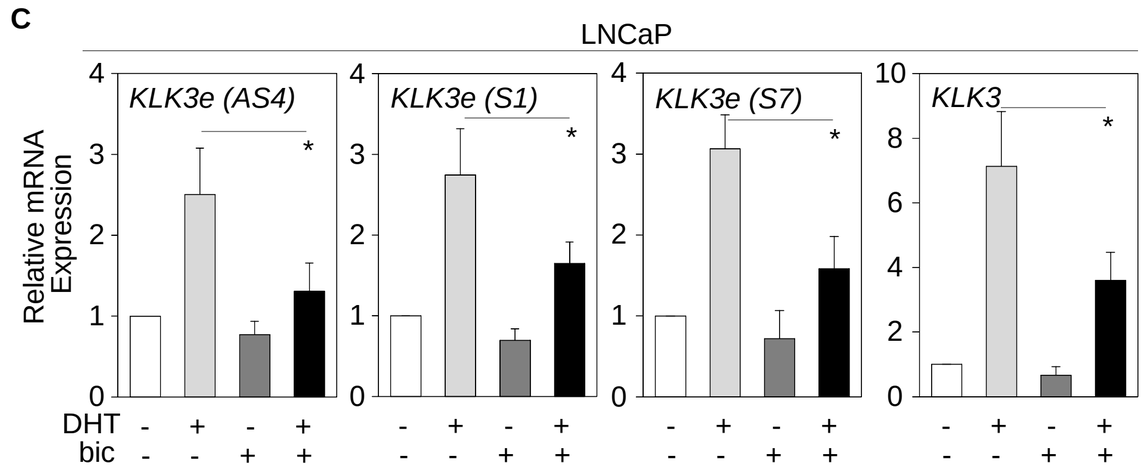
G; CCCCTCGTGGTTAACATCAC

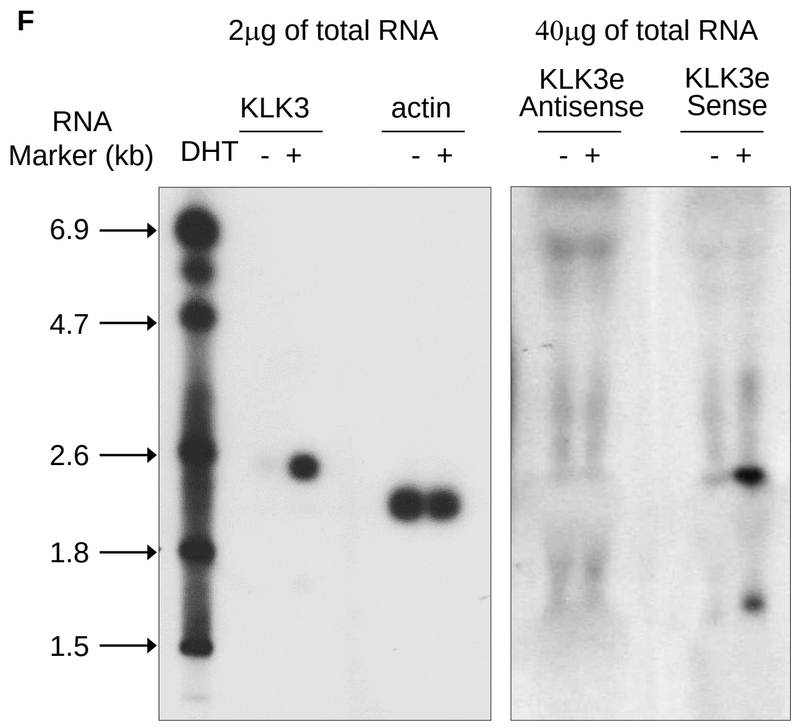
H; TGGTTCCCACTACATCCACA

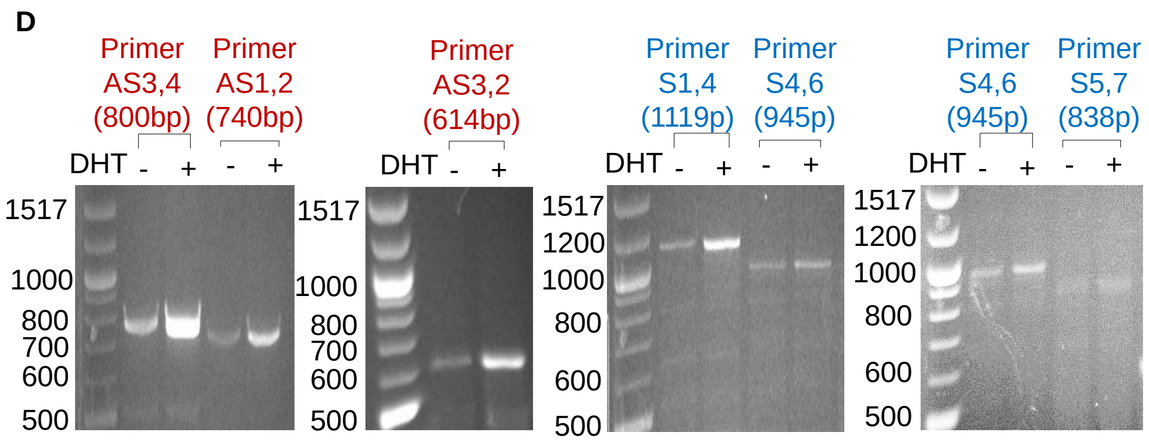
I; GCTGGAGGTGAGTTCTTTGG

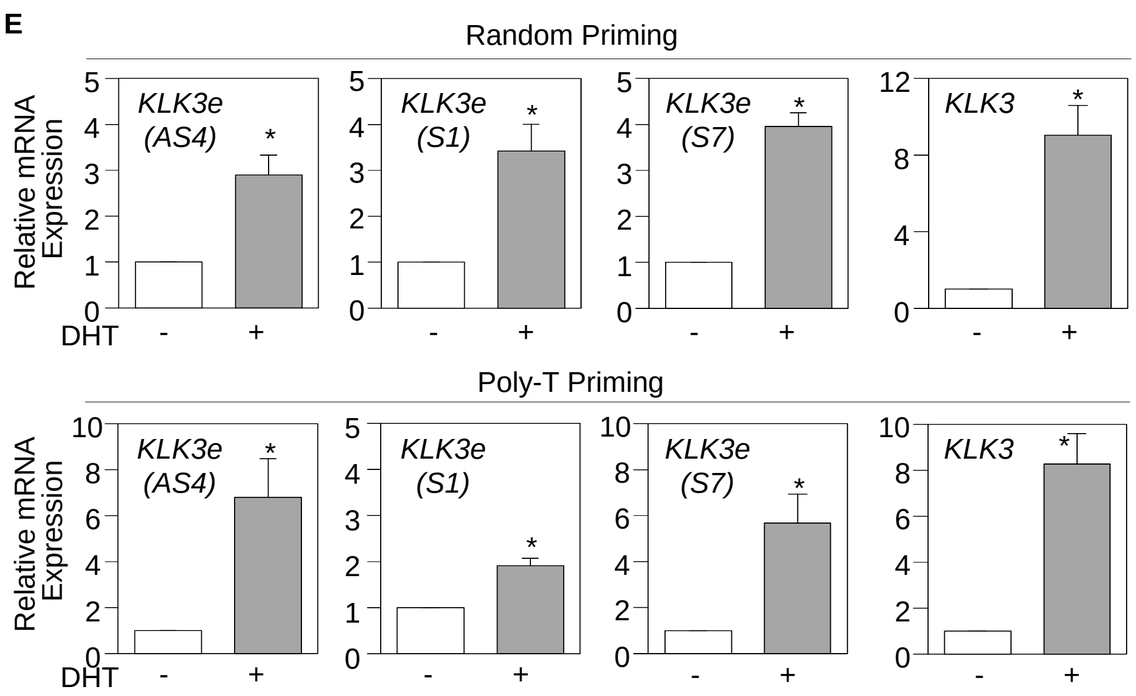

A
VCaP
Relative mRNA Expression (DHT Induction)
KLK3e (AS4)
KLK3e (S7)
KLK3
Hour 0 1 2 4 8 16 24


B
LNCaP-abl
Relative mRNA Expression (DHT Induction)
KLK3e (AS4)
KLK3e (S7)
KLK3
Hour 0 4 8 16 24

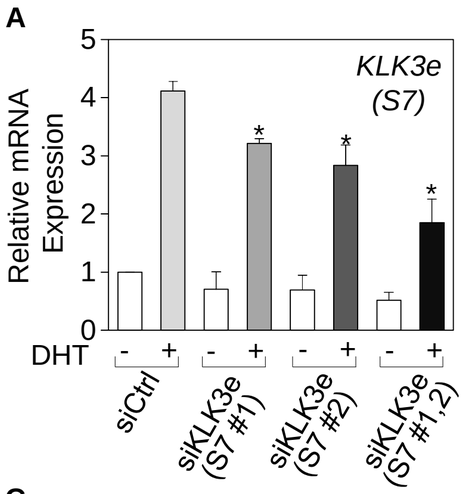

C
LNCaP
KLK3e (AS4)
KLK3e (S1)
KLK3e (S7)
KLK3
Relative mRNA Expression
DHT
bic


F
2μg of total RNA
40μg of total RNA
KLK3
actin
KLK3e Antisense
KLK3e Sense
RNA Marker (kb)
DHT
6.9
4.7
2.6
1.8
1.5

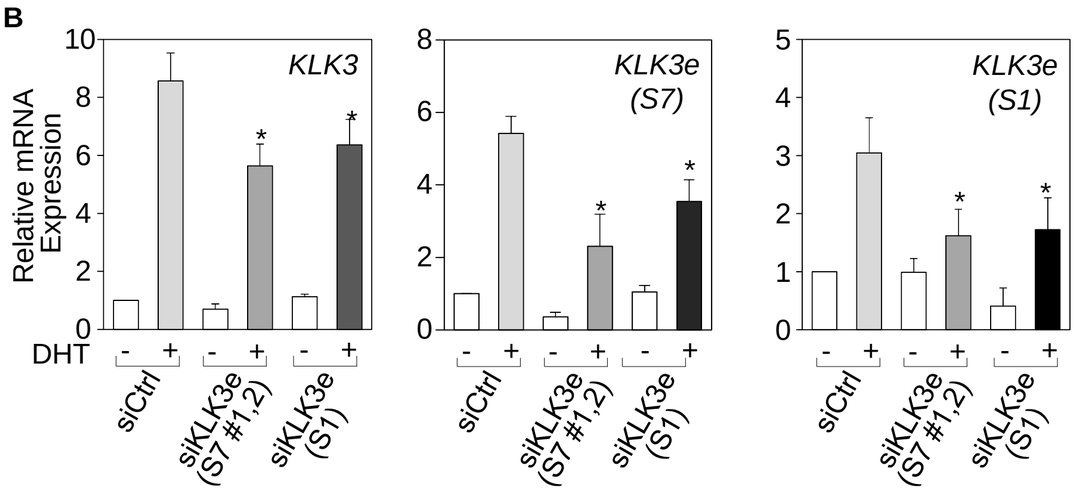

D
Primer AS3,4 (800bp)
Primer AS1,2 (740bp)
Primer AS3,2 (614bp)
Primer S1,4 (1119p)
Primer S4,6 (945p)
Primer S4,6 (945p)
Primer S5,7 (838p)
DHT
1517
1000
800
700
600
500
1200


E
Random Priming
Poly-T Priming
KLK3e (AS4)
KLK3e (S1)
KLK3e (S7)
KLK3
Relative mRNA Expression
DHT

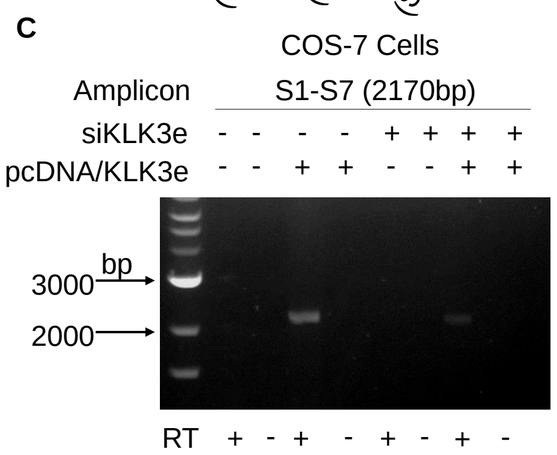
A



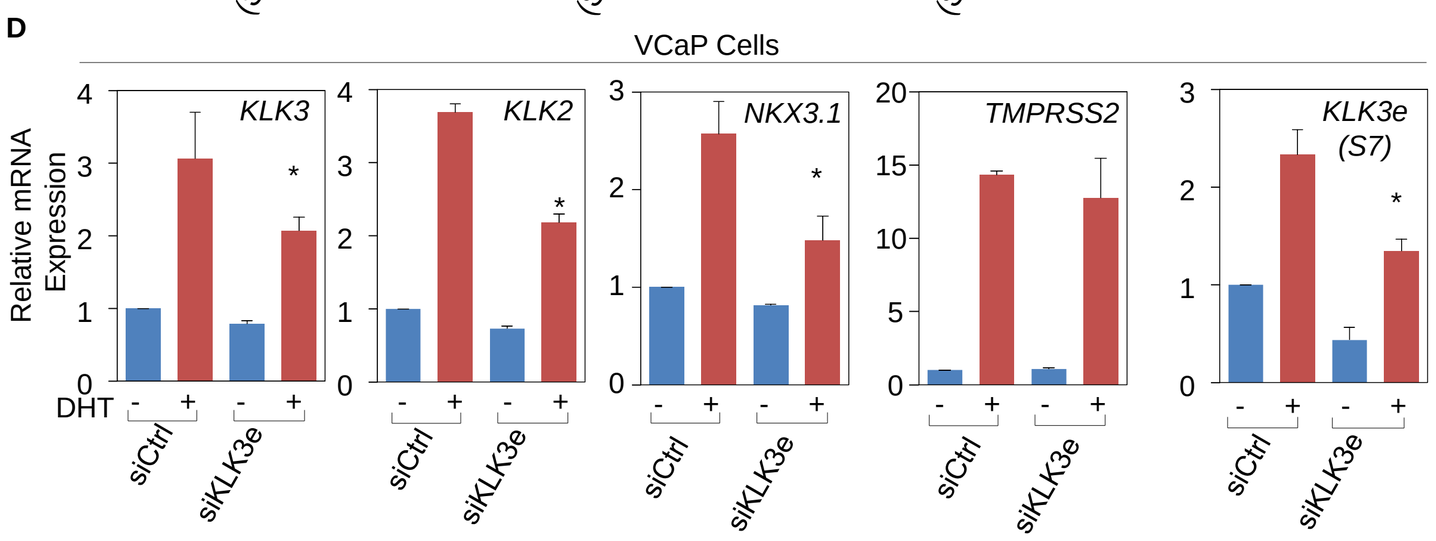
B



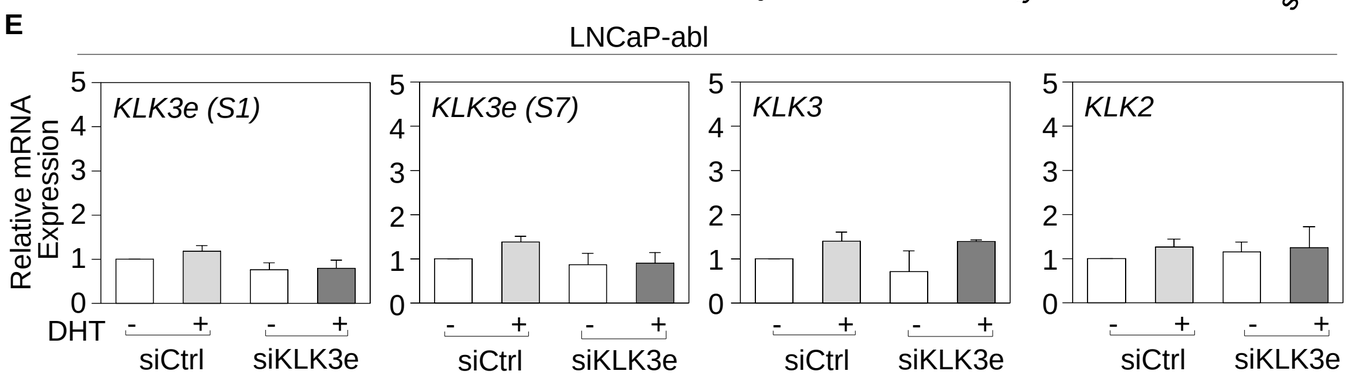
C



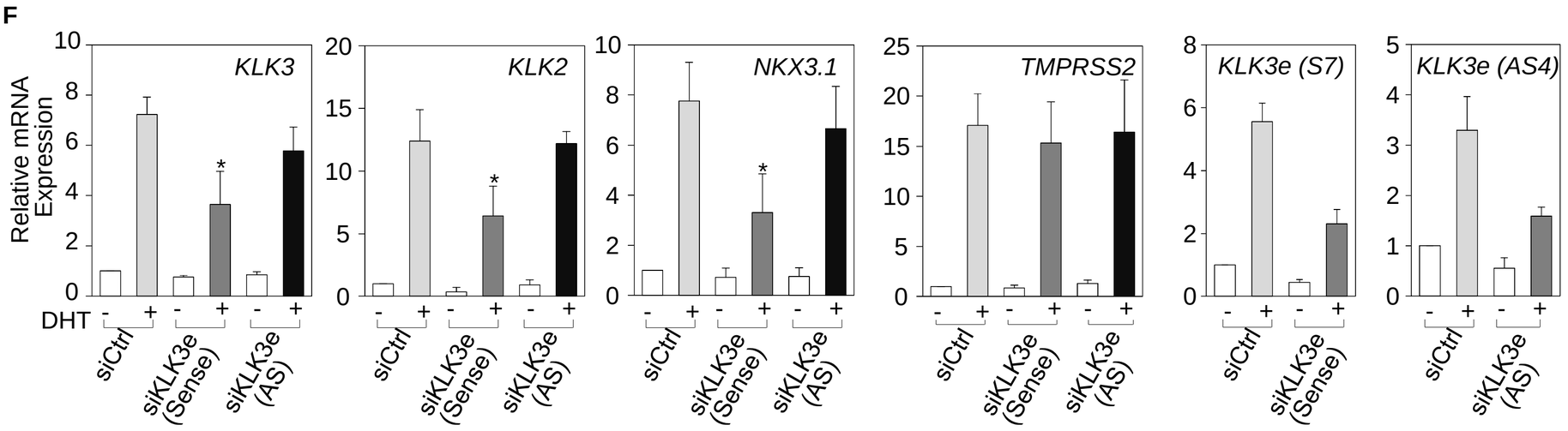
D



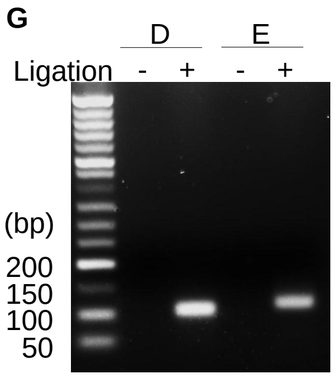
E



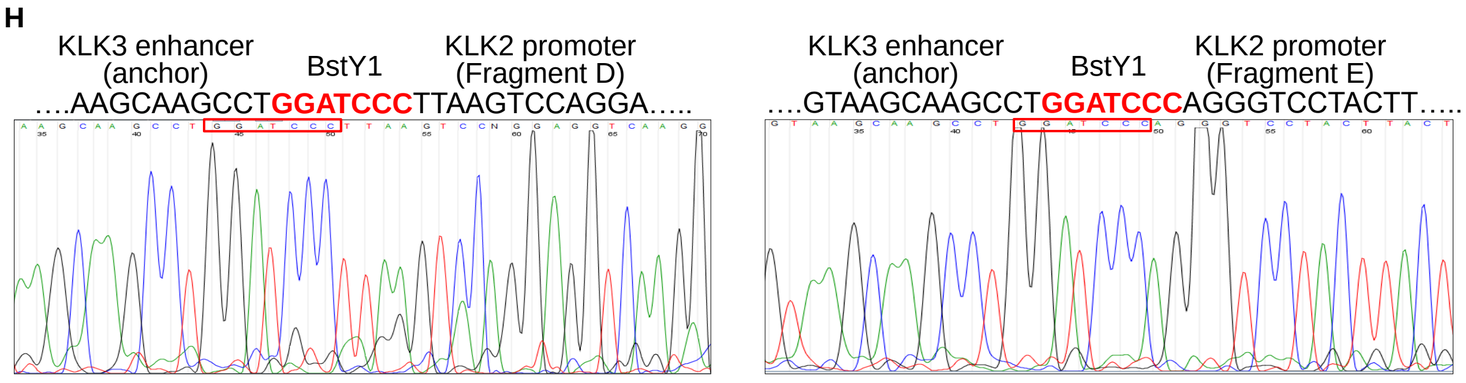
F



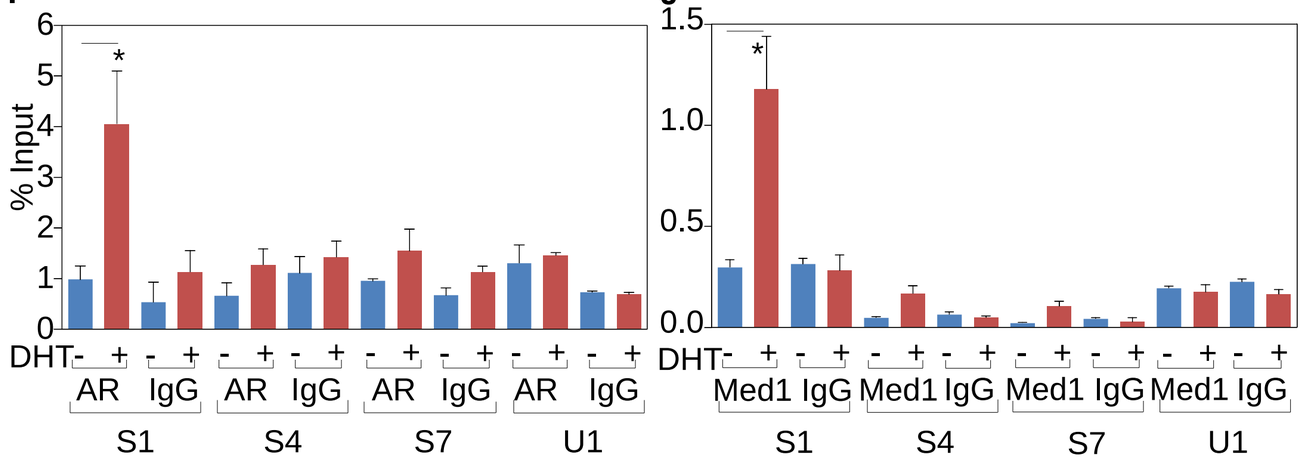
G



H

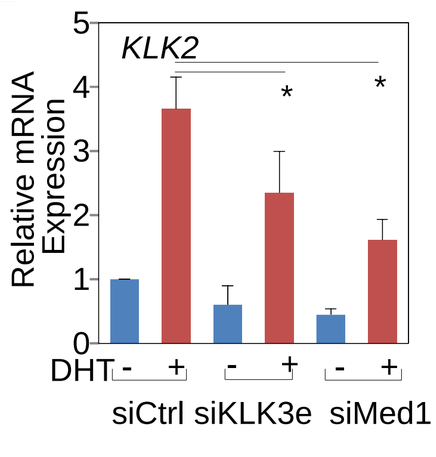


I

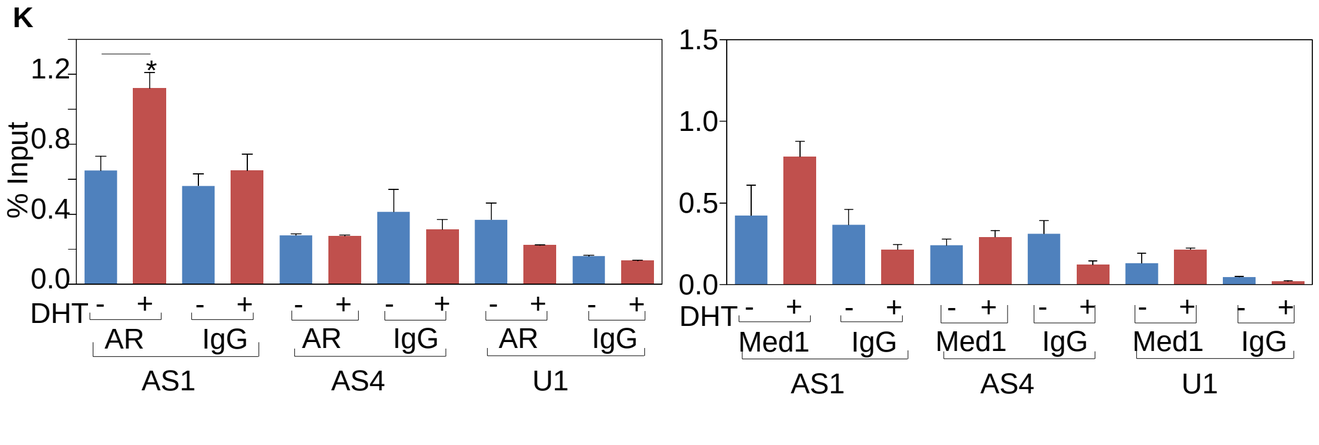


J

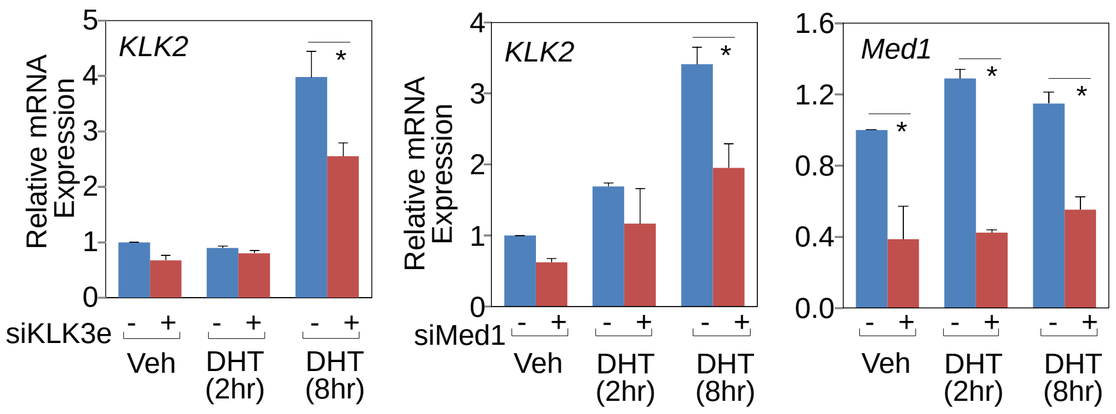
L



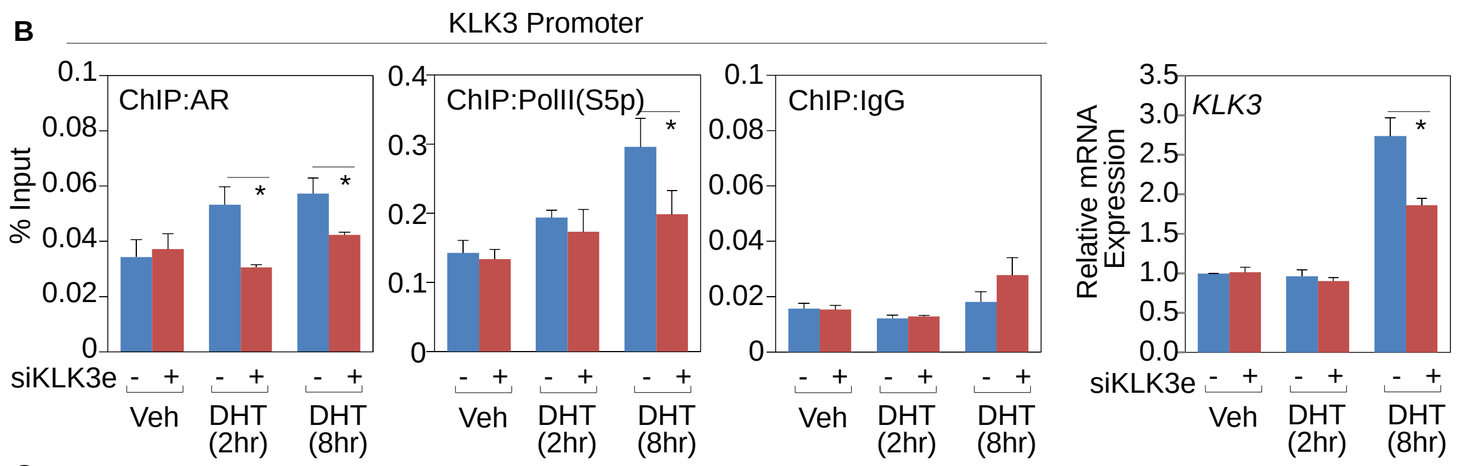
K



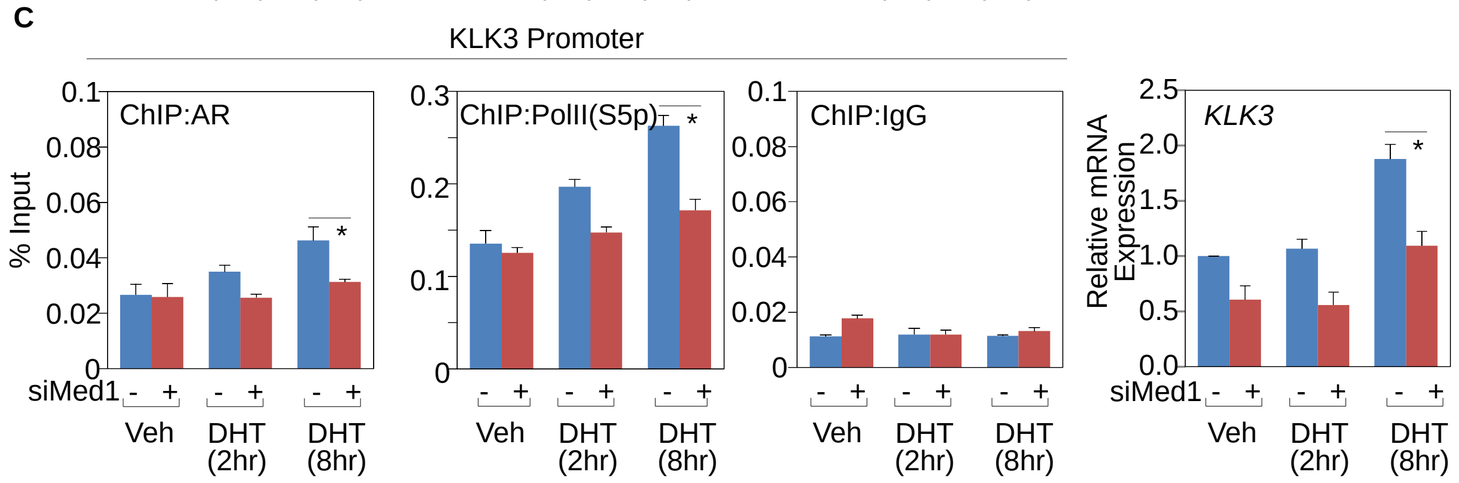
A


KLK2
Relative mRNA Expression
siKLK3e
Veh
DHT (2hr)
DHT (8hr)
KLK2
siMed1
Med1

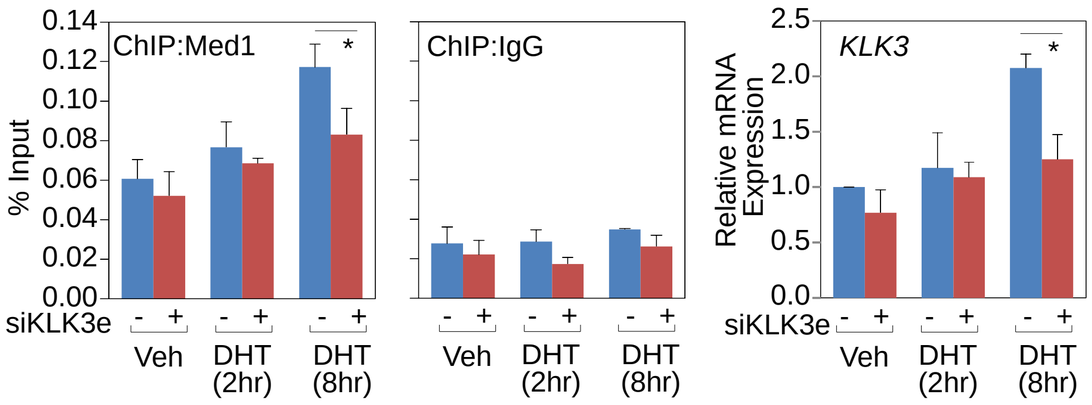
B


KLK3 Promoter
ChIP:AR
ChIP:PolII(S5p)
ChIP:IgG
% Input
KLK3
Relative mRNA Expression
siKLK3e
Veh
DHT (2hr)
DHT (8hr)

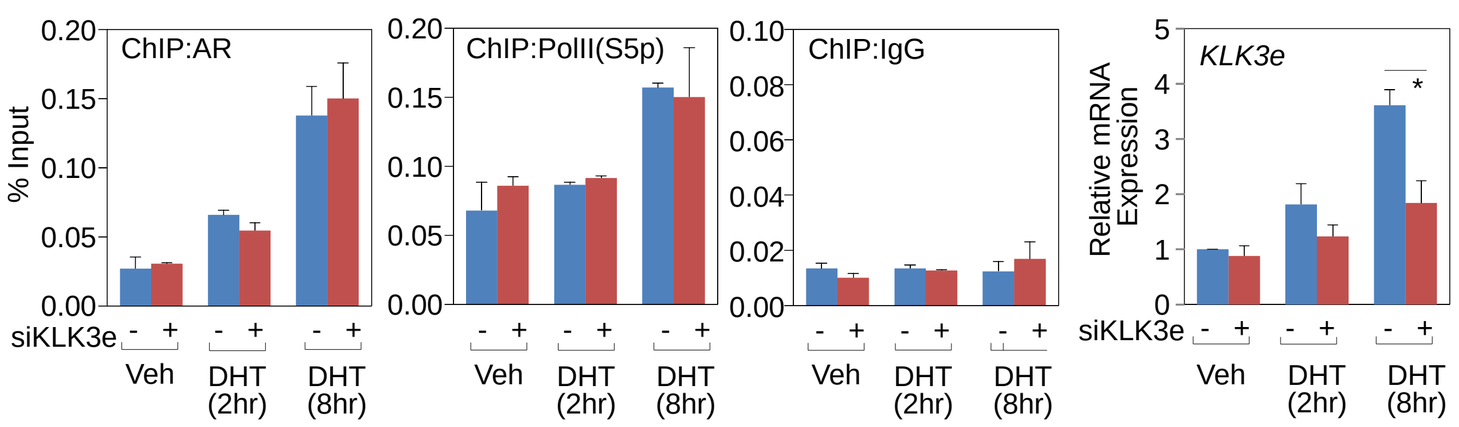
C


KLK3 Promoter
ChIP:AR
ChIP:PolII(S5p)
ChIP:IgG
% Input
KLK3
Relative mRNA Expression
siMed1
Veh
DHT (2hr)
DHT (8hr)

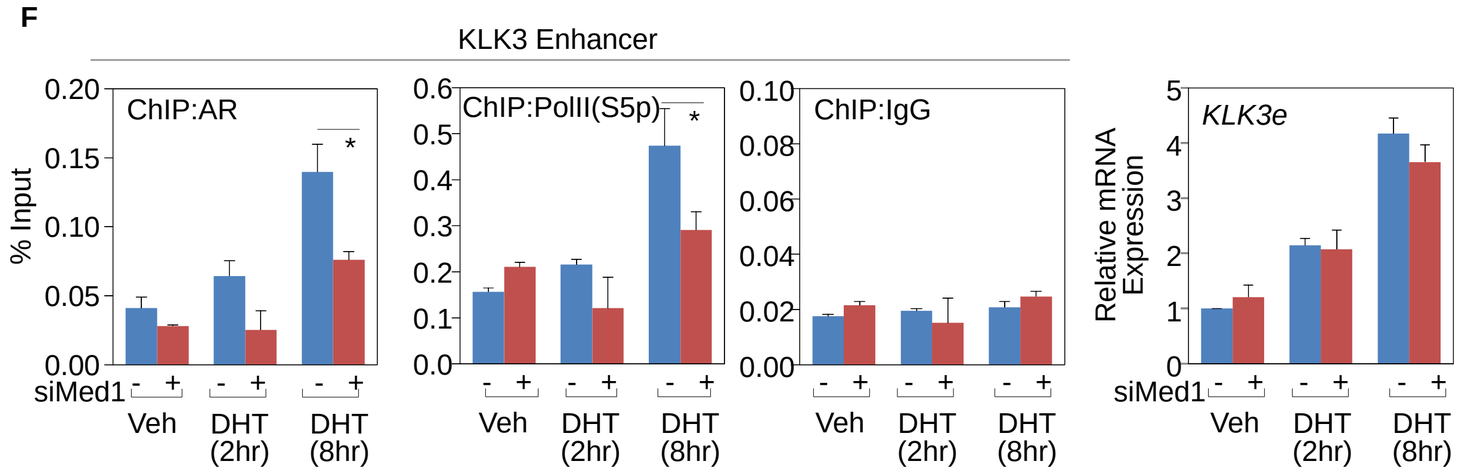
D


KLK3 Promoter
ChIP:Med1
ChIP:IgG
% Input
KLK3
Relative mRNA Expression
siKLK3e
Veh
DHT (2hr)
DHT (8hr)

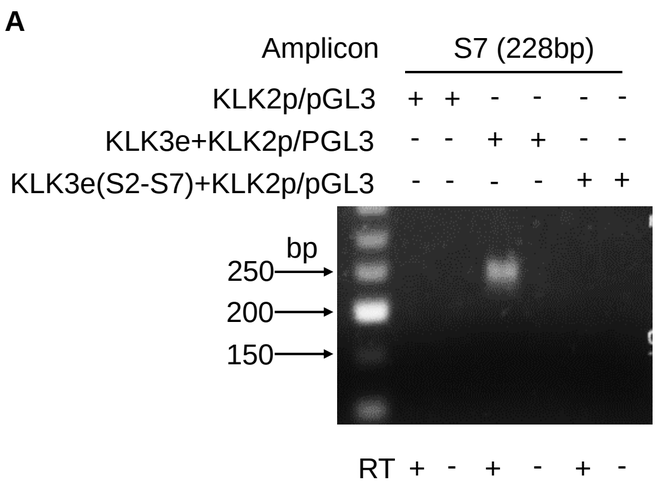
E


KLK3 Enhancer
ChIP:AR
ChIP:PolII(S5p)
ChIP:IgG
% Input
KLK3e
Relative mRNA Expression
siKLK3e
Veh
DHT (2hr)
DHT (8hr)

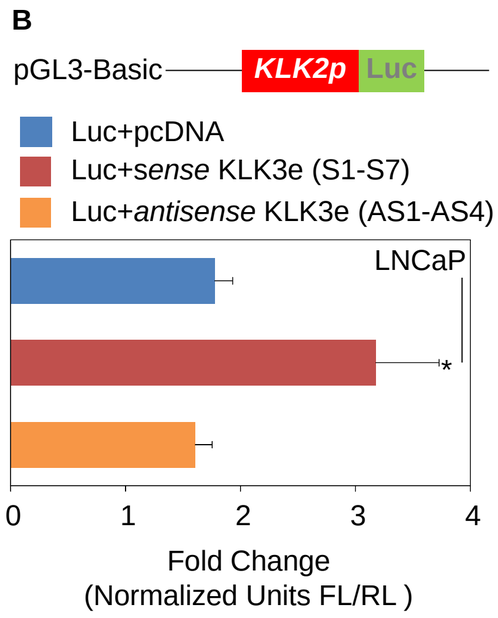
F


KLK3 Enhancer
ChIP:AR
ChIP:PolII(S5p)
ChIP:IgG
% Input
KLK3e
Relative mRNA Expression
siMed1
Veh
DHT (2hr)
DHT (8hr)

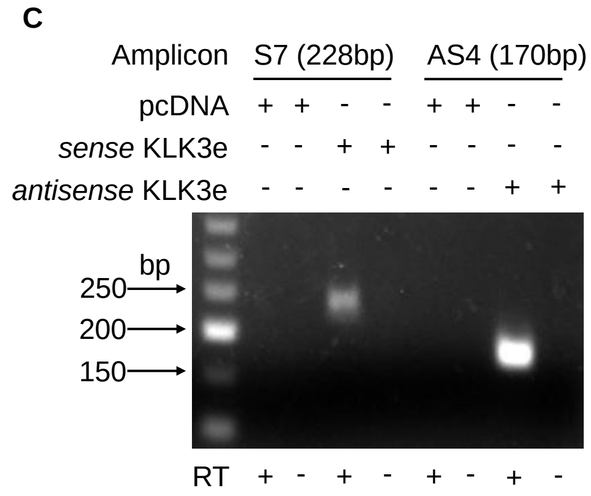
A


Amplicon
S7 (228bp)
KLK2p/pGL3 + + - - - -
KLK3e+KLK2p/PGL3 - - + + - -
KLK3e(S2-S7)+KLK2p/pGL3 - - - - + +
bp
250
200
150
RT + - + - + -

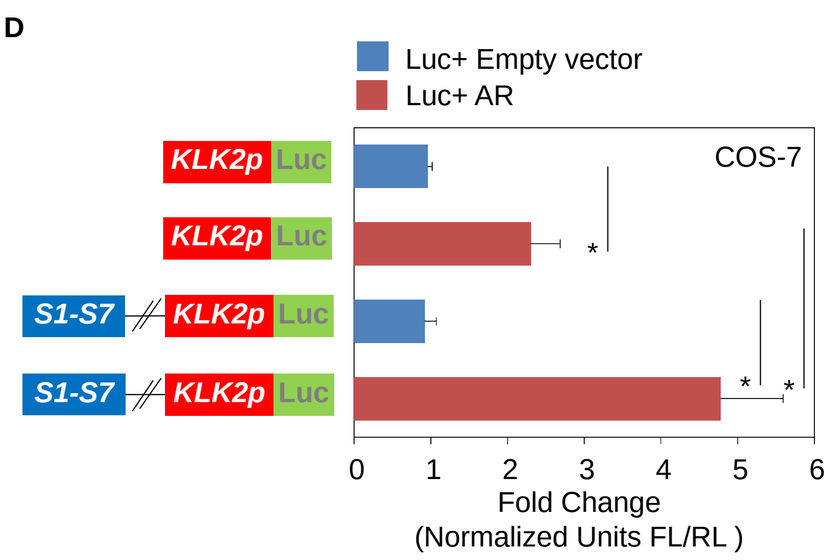
B


pGL3-Basic
KLK2p
Luc
Luc+pcDNA
Luc+sense KLK3e (S1-S7)
Luc+antisense KLK3e (AS1-AS4)
LNCaP
*
0
1
2
3
4
Fold Change
(Normalized Units FL/RL)

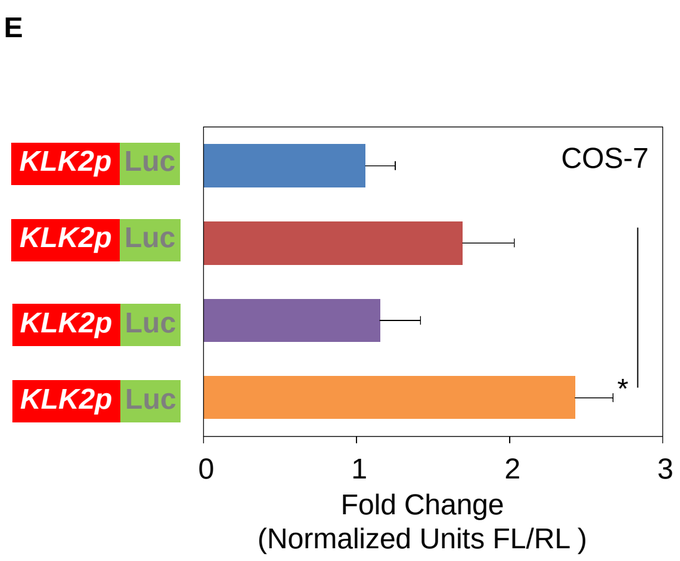
C


Amplicon
S7 (228bp)
AS4 (170bp)
pcDNA + + - - + + - -
sense KLK3e - - + + - - - -
antisense KLK3e - - - - - - + +
bp
250
200
150
RT + - + - + - + -

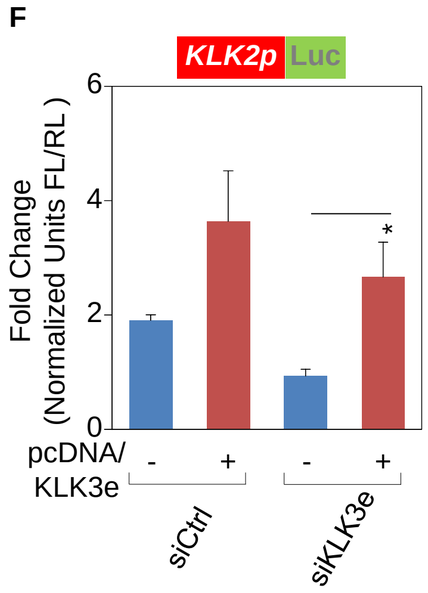
D

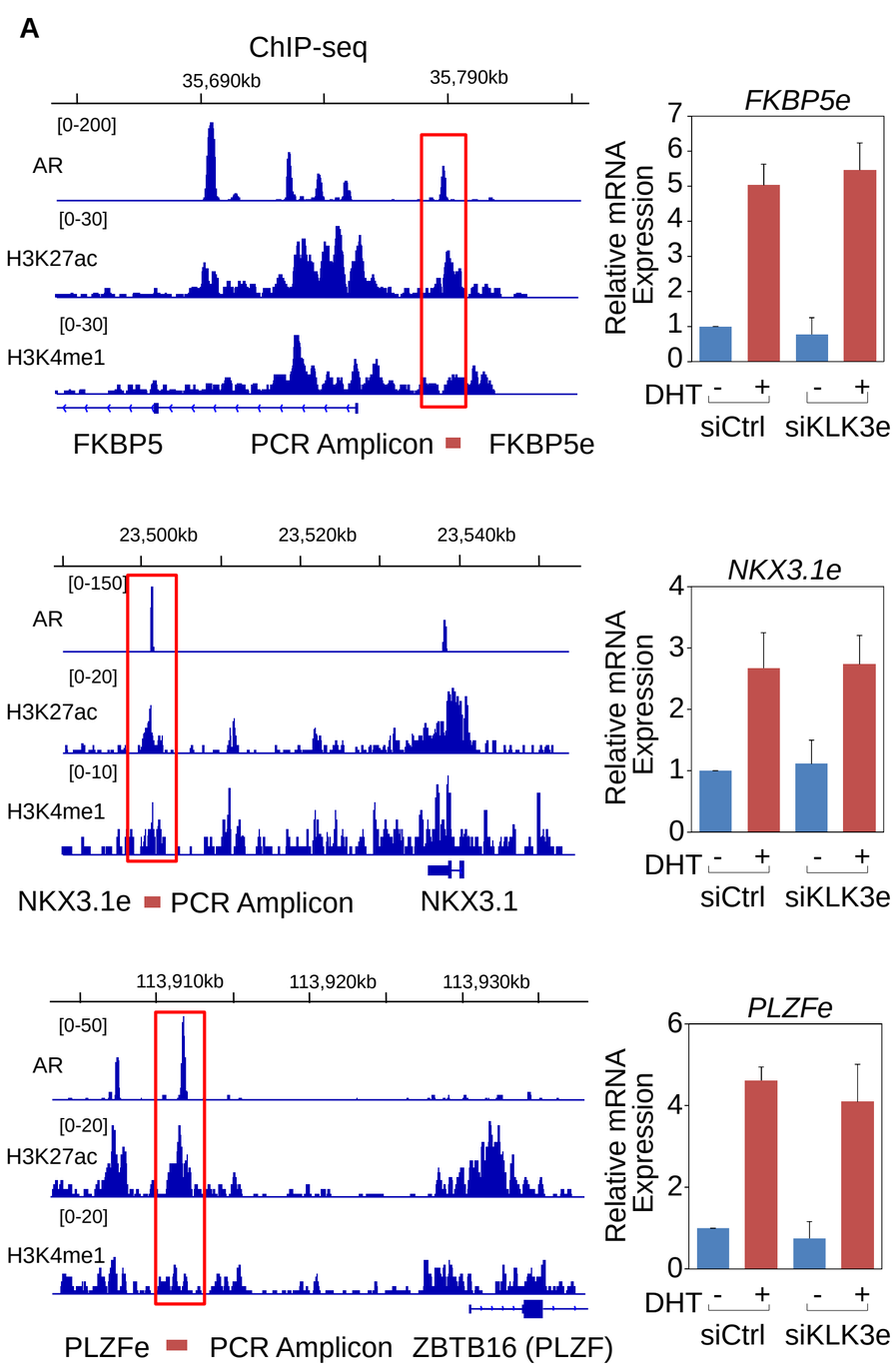

Luc+ Empty vector
Luc+ AR
KLK2p
Luc
S1-S7
COS-7
*
0
1
2
3
4
5
6
Fold Change
(Normalized Units FL/RL)

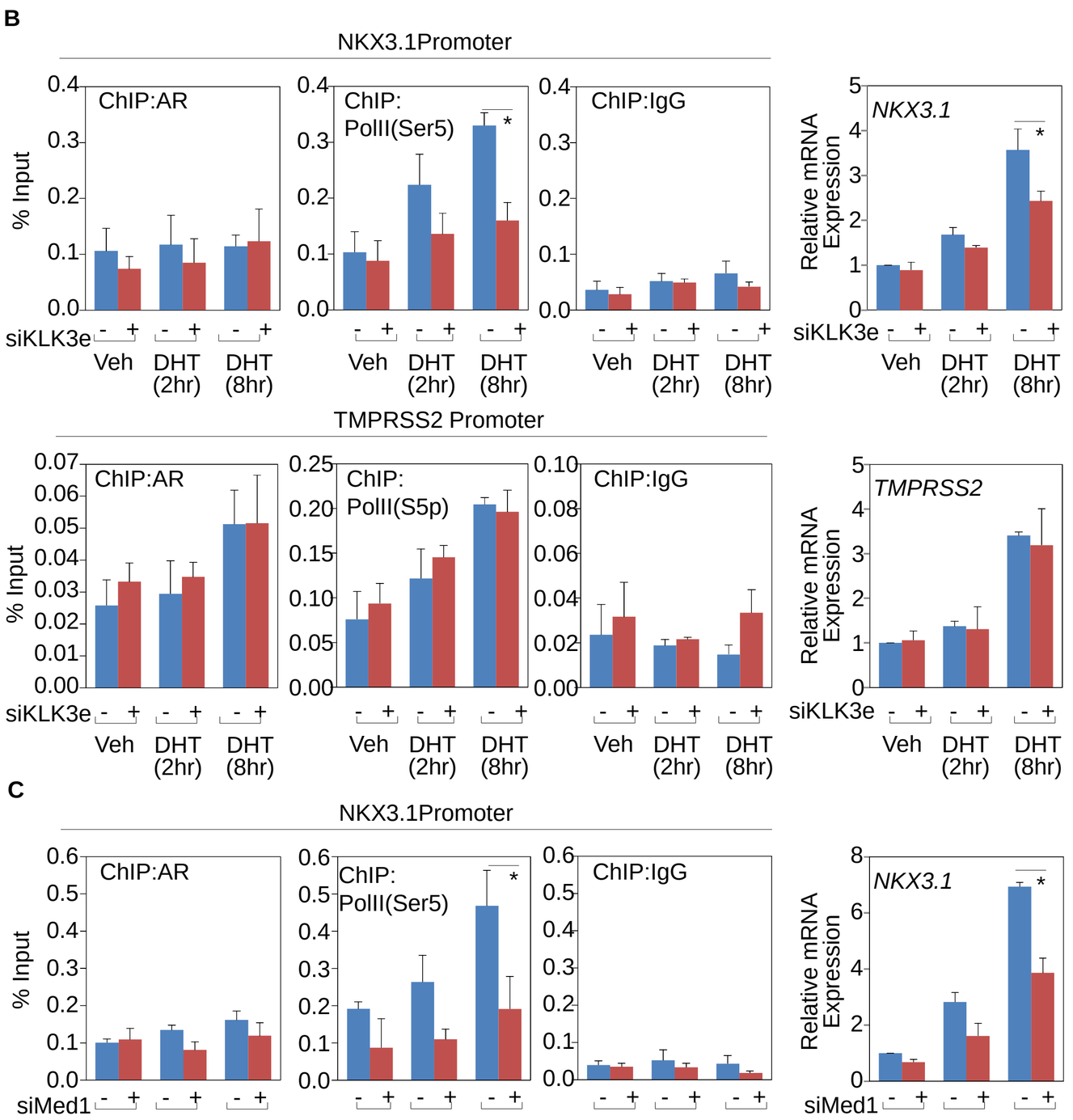
E


Co-Expressing with
AR
KLK3e
- -
+ -
- +
+ +
KLK2p
Luc
COS-7
*
0
1
2
3
Fold Change
(Normalized Units FL/RL)

F


KLK2p
Luc
Fold Change
(Normalized Units FL/RL)
6
4
2
0
*
pcDNA/
KLK3e
- + - +
siCtrl
siKLK3e


A
ChIP-seq
35,690kb
35,790kb
[0-200]
AR
[0-30]
H3K27ac
[0-30]
H3K4me1
FKBP5
PCR Amplicon
FKBP5e
23,500kb
23,520kb
23,540kb
FKBP5e
Relative mRNA Expression
DHT
siCtrl
siKLK3e
[0-150]
AR
[0-20]
H3K27ac
[0-10]
H3K4me1
NKX3.1e
PCR Amplicon
NKX3.1
NKX3.1e
113,910kb
113,920kb
113,930kb
[0-50]
AR
[0-20]
H3K27ac
[0-20]
H3K4me1
PLZFe
PCR Amplicon
ZBTB16 (PLZF)
PLZFe


B
NKX3.1Promoter
ChIP:AR
ChIP: PolII(Ser5)
ChIP:IgG
% Input
siKLK3e
Veh
DHT (2hr)
DHT (8hr)
NKX3.1
Relative mRNA Expression
TMPRSS2 Promoter
TMPRSS2
C
NKX3.1Promoter
ChIP:AR
ChIP: PolII(Ser5)
ChIP:IgG
% Input
siMed1
NKX3.1
TMPRSS2 Promoter
TMPRSS2
D
NKX3.1 Promoter
ChIP:Med1
ChIP:IgG
% Input
siKLK3e
NKX3.1
Relative mRNA Expression
E
TMPRSS2 Promoter
ChIP:Med1
ChIP:IgG
% Input
siKLK3e
TMPRSS2
Relative mRNA Expression
F
R² = 0.8361
ΔC_T (KLK2-GAPDH)
ΔC_T (KLK3-GAPDH)