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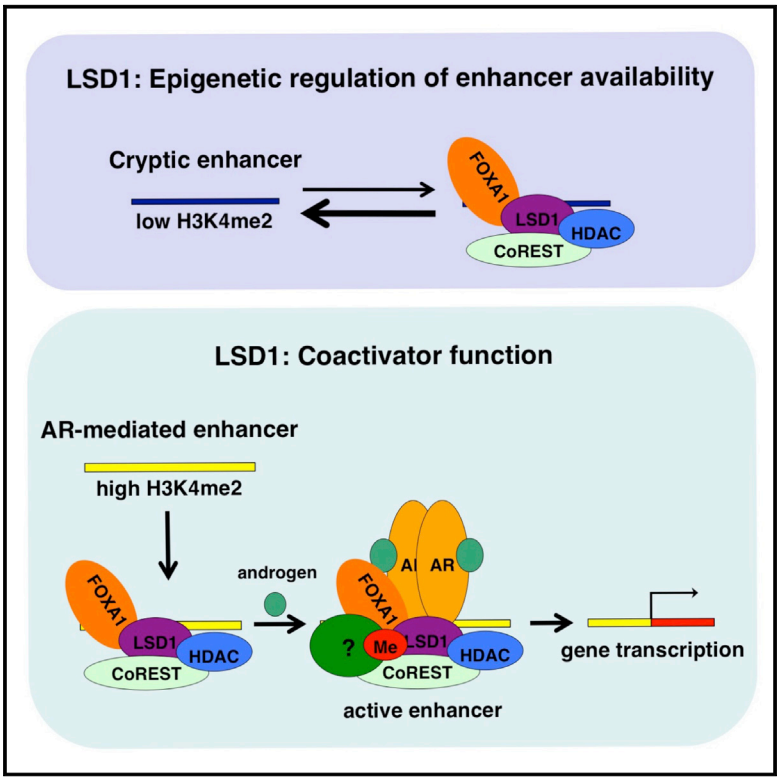
Lysine-Specific Demethylase 1 Has Dual Functions as a Major Regulator of Androgen Receptor Transcriptional Activity

Graphical Abstract



Highlights

LSD1 is broadly associated with AR-regulated enhancers, and a subset is H3T6ph marked

LSD1 is associated with CoREST at these sites and interacts with FOXA1

Despite coactivator function at these sites, LSD1 mediates their H3K4 demethylation

LSD1 coactivation is through demethylation of novel histone or nonhistone substrates

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In Brief

Cai et al. show that lysine-specific demethylase 1 (LSD1), although generally a transcriptional corepressor through H3K4 demethylation, functions broadly as a coactivator for androgen receptor and interacts with FOXA1 on androgen-stimulated genes. LSD1-mediated H3K4 demethylation persists at these sites, indicating a distinct coactivator function mediated by demethylation of other substrates.

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Lysine-Specific Demethylase 1 Has Dual Functions as a Major Regulator of Androgen Receptor Transcriptional Activity

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SUMMARY

Lysine-Specific Demethylase 1 (LSD1, KDM1A) functions as a transcriptional corepressor through demethylation of histone 3 lysine 4 (H3K4) but has a coactivator function on some genes through mechanisms that are unclear. We show that LSD1, interacting with CoREST, associates with and coactivates androgen receptor (AR) on a large fraction of androgen-stimulated genes. A subset of these AR/LSD1-associated enhancer sites have histone 3 threonine 6 phosphorylation (H3T6ph), and these sites are further enriched for androgen-stimulated genes. Significantly, despite its coactivator activity, LSD1 still mediates H3K4me2 demethylation at these androgen-stimulated enhancers. FOXA1 is also associated with LSD1 at AR-regulated enhancer sites, and a FOXA1 interaction with LSD1 enhances binding of both proteins at these sites. These findings show that LSD1 functions broadly as a regulator of AR function, that it maintains a transcriptional repression function at AR-regulated enhancers through H3K4 demethylation, and that it has a distinct AR-linked coactivator function mediated by demethylation of other substrates.

INTRODUCTION

Androgen receptor (AR) is highly expressed in prostate cancer (PCa) cells and plays a pivotal role in PCa through transactivation of multiple genes (Green et al., 2012; Yuan et al., 2014). Patients with metastatic PCa are treated with androgen deprivation therapy (ADT) to block AR activity, but the tumors invariably relapse (castration-resistant prostate cancer [CRPC]). Significantly, AR expression is increased in CRPC and most AR-stimulated genes are highly expressed, indicating that AR transcriptional activity has been substantially restored (Yuan et al., 2014). The recent clinical success of abiraterone (CYP17A1 inhibitor that further suppresses androgen synthesis) and enzalutamide (AR antagonist) has confirmed that AR, stimulated by residual androgens, is a driver of tumor growth in CRPC (de Bono et al., 2011; Ryan et al., 2013; Scher et al., 2012). However, patients treated with these agents still generally relapse within 1–2 years, and high levels of AR and of AR-regulated genes in many of these relapsed tumors indicate that AR activity has again been restored. Therefore, there is still a pressing need to better understand AR transcriptional mechanisms in order to develop further approaches for blocking or modulating its activity.

AR also has a transcriptional repression function that is dependent on lysine-specific demethylase 1 (LSD1, KDM1A) (Cai et al., 2011). Best characterized as a transcriptional repressor, LSD1 associates tightly with CoREST and demethylates enhancer-associated H3K4me1,2 (Lee et al., 2005; Shi et al., 2004, 2005; You et al., 2001). The histone deacetylases HDAC1 and 2 are usually associated with the LSD1-CoREST complex and can further suppress gene transcription. Nonetheless, in contrast to its well-established corepressor function, LSD1 has been found to coactivate several transcription factors including AR on a small set of genes (Garcia-Bassets et al., 2007; Metzger et al., 2005; Wang et al., 2007a; Wissmann et al., 2007; Yatim et al.,

2012), where phosphorylation of H3T6 and H3T11 may switch LSD1 substrate specificity from H3K4me1,2 to H3K9me1,2 (Metzger et al., 2008, 2010). However, the extent to which LSD1 functions as a general regulator of AR transcriptional activities and the roles of histone phosphorylation and demethylation in mediating its AR coactivator function remain to be established. In this study, to systematically assess the role of LSD1 in regulating AR functions, we performed an integrated analysis with LSD1, AR, and FOXA1 chromatin immunoprecipitation sequencing (ChIP-seq), and with gene expression arrays. Our results demonstrate that LSD1 functions broadly as a coactivator at AR-stimulated enhancers but retains its H3K4me1,2 demethylase activity at these sites. This activity may provide negative feedback to suppress gene expression in the absence of androgen and prevent the aberrant activation of cryptic AR enhancers. Moreover, it indicates that androgen-dependent LSD1 coactivator function is mediated by demethylation of other histone or nonhistone substrates.

RESULTS

LSD1-Positive AR Binding Sites Are Associated with Androgen-Stimulated Genes

Using ChIP-seq for LSD1 in androgen-treated PCa cells (LNCaP), in combination with previous ChIP-seq data for AR and gene expression arrays (Cai et al., 2011; Lupien et al., 2008; Wang et al., 2007b, 2009b; Yu et al., 2010), we found that ~20% of LSD1 and AR sites were overlapping (Figure 1A). AR binding sites were enriched ~3-fold among genes that were increased after 4 or 16 hr of dihydrotestosterone (DHT) treatment, with ~56% of DHT-stimulated genes having AR binding sites (Figure 1B). Similarly, LSD1 binding sites were enriched ~2-fold among DHT-stimulated genes, with ~42% having LSD1 binding sites (Figure 1C). AR binding was not clearly enriched among DHT-repressed genes (Figure 1B), whereas LSD1 binding was enriched ~1.5-fold on these genes, and ~29% had LSD1 binding sites (versus 20% background) (Figure 1C). However, direct examination of ChIP-seq data for LSD1-occupied sites in many DHT-repressed genes showed that AR was binding but was below the peak threshold (Figure S1A), possibly reflecting weaker AR binding or lower affinity of the antibodies for AR at these sites.

LSD1$^+$ as compared to LSD1$^-$ AR binding sites were further enriched among DHT-stimulated genes (~5- to 7-fold versus ~2-fold) (Figure 1D), and Binding and Expression Target Analysis (BETA) (Wang et al., 2013) confirmed that LSD1$^+$ AR sites were significantly enriched for these genes (Figure S1B). Overall, among the genes stimulated by DHT after 4 hr that had AR binding sites (~56%), LSD1 was associated with the AR site at ~39% (22%/56%). As expected, AR$^+$/LSD1$^+$ sites were enriched for AR and FOXA1 binding motifs, whereas AR$^-$/LSD1$^+$ sites were enriched for repressors such as REST or NF1, as well as for FOXA1 (Figure 1D; Table S1). LSD1 binding intensity was similar between AR-positive and -negative sites (Figures S1C and S1D), but AR-negative LSD1 sites were enriched in promoter regions (Figure S1E). Overall these findings showed that LSD1 was broadly associated with AR at androgen-stimulated genes.

LSD1 Functions as a Coactivator on a Large Fraction of AR-Stimulated Genes

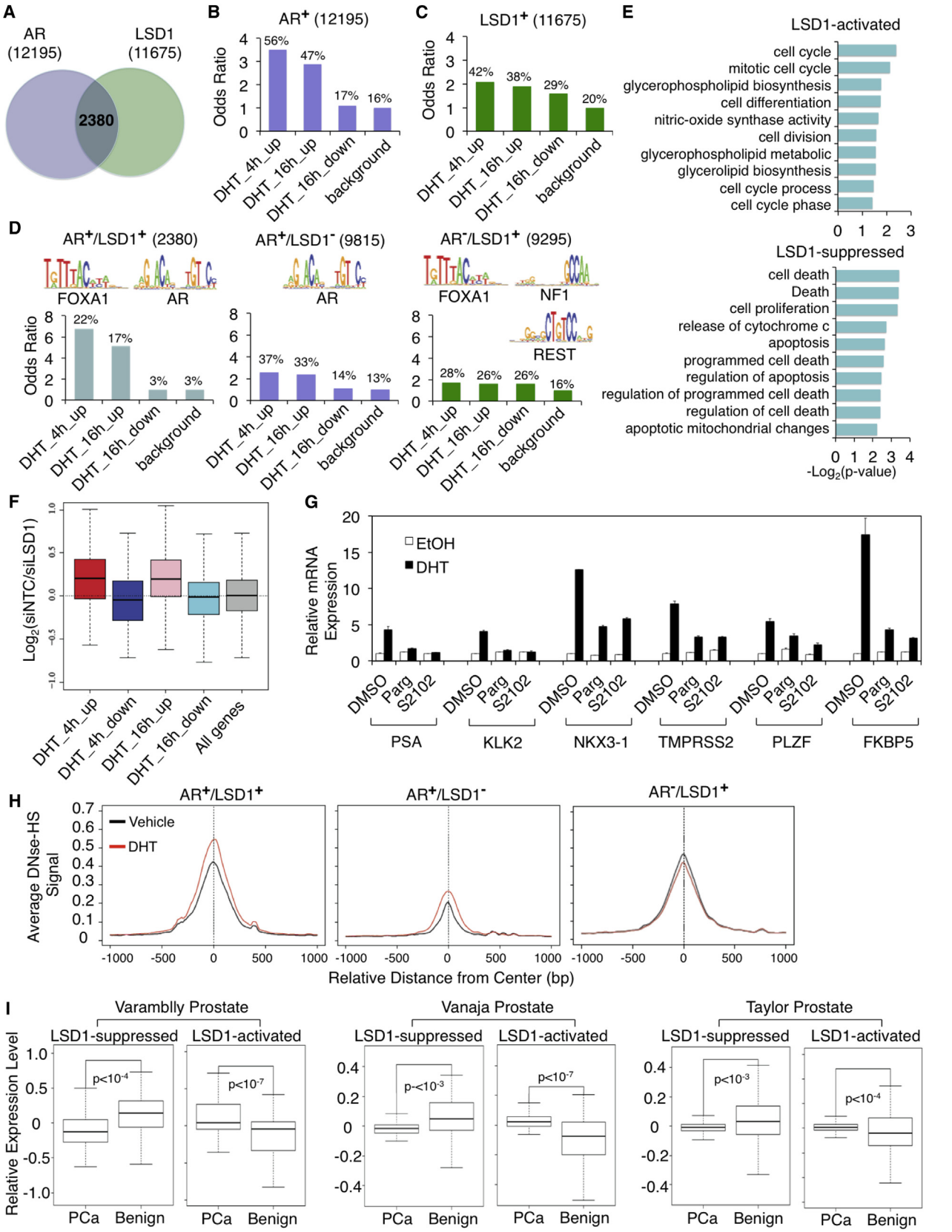
Transcript profiling in LSD1 versus control small interfering RNA (siRNA)-treated cells next showed that LSD1-activated genes were enriched for cell-cycle and lipid synthesis pathways, whereas LSD1-suppressed genes were enriched for apoptosis (Figure 1E; Table S1). Significantly, androgen-stimulated genes were broadly decreased by LSD1 siRNA (Figure 1F). AR-positive LSD1 binding sites were also enriched for LSD1-activated genes, whereas AR-negative LSD1 binding sites were enriched for LSD1-suppressed genes (Figure S1F), consistent with LSD1 functioning generally as a corepressor at AR-negative sites, and indicating that its coactivation function is linked to AR. LSD1 inhibitors (pargyline and S2101) also impaired, but did not totally eliminate, androgen-stimulated expression of multiple AR-activated genes (Figure 1G), further indicating that LSD1 contributes to AR transcriptional activity but may not be absolutely required.

AR$^+$/LSD1$^+$ sites in VCaP cells similarly showed enrichment for AR-stimulated genes, and LSD1 RNAi and inhibitors similarly decreased androgen-stimulated expression of these genes (Figures S1G–S1M). Finally, DNase sequencing (DNase-seq) (He et al., 2012) showed that AR$^+$/LSD1$^+$ sites were DNase hypersensitive (DNase-HS) and were more sensitive than AR$^+$/LSD1$^-$ sites (Figure 1H), further indicating these sites are AR-regulated transcriptional enhancers. DNase-HS was increased by DHT at AR binding sites (Figures 1H and S1N), consistent with AR-mediated loss of a central nucleosome overlapping the AR binding site (Andreu-Vieyra et al., 2011; He et al., 2010). Together these data indicated that whereas LSD1 is a corepressor at most sites, it functions broadly as a coactivator on AR-regulated transcriptional enhancers.

Because the LSD1 transcription profiling indicated that LSD1 might both stimulate PCa growth through its AR coactivator functions and suppress apoptosis through AR-independent mechanisms, we examined whether LSD1 activity was increased in PCa. In three cohorts, expression of LSD1-activated genes (genes with LSD1 binding sites that were decreased by LSD1 siRNA) was increased in PCa versus normal prostate, whereas LSD1-suppressed genes were decreased (Figure 1I). Higher LSD1 mRNA also was significantly associated with lower survival in one patient cohort, whereas there was a similar trend in two other cohorts that did not reach statistical significance (Figure S1O).

LSD1 Mediates H3K4 Demethylation on AR-Stimulated Genes

To assess LSD1 effects on H3K4 and K9 methylation, we next examined AR$^+$/LSD1$^+$ binding sites in a series of AR-stimulated genes (Figure S2A). As expected, FOXA1 was bound to these sites prior to DHT treatment, and AR binding was stimulated by DHT (Figure S2B). FOXA1 and DHT-stimulated AR binding were also observed at AR$^+$/LSD1$^-$ sites (*CREG1* and *KIAA0040* genes), but not at AR$^-$/LSD1$^+$ sites in three other genes that were not androgen-regulated (*HMGB2*, *HHLA3*, *SH2B1*) (Figure S2C). DHT decreased H3K9me1,2 at each of the AR$^+$/LSD1$^+$ sites (Figure S2D), consistent with previous data indicating that LSD1 may switch from H3K4 to H3K9 demethylation at AR-regulated enhancers (Metzger et al., 2005, 2008,



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2010; Wissmann et al., 2007). However, H3K4me1,2 was also decreased by DHT at each AR$^+$/LSD1$^+$ site (Figure S2D), but not at control AR$^-$/LSD1$^+$ or AR$^+$/LSD1$^-$ sites (Figure S2C, right panel), suggesting that LSD1 still had H3K4 demethylation activity at AR$^+$/LSD1$^+$ sites.

Consistent with previous data (Andreu-Vieyra et al., 2011; He et al., 2010), ChIP showed that histone 3 (H3) binding was also decreased by DHT at each AR binding site (Figure S2D), indicating that the decrease in H3K4 and H3K9 methylation at AR$^+$/LSD1$^+$ sites may in part reflect loss of the central nucleosome rather than H3 demethylation. Therefore, to determine if decreased methylation reflects nucleosome loss, we next examined the effects of LSD1 inhibitors on AR and H3 binding, and on H3 methylation. Blocking LSD1 with pargyline prevented DHT-stimulated H3K4me2 loss at all sites examined except the *PLZF*-ARE (Figure 2A, upper-left panel) but did not impair DHT-stimulated AR binding or H3 loss (Figure 2A, upper-right and lower-left panels). This result indicates that nucleosome loss does not substantially contribute to the overall decrease in H3K4me2 methylation, perhaps reflecting preferential loss of unmethylated central nucleosomes.

LSD1 inhibition similarly impaired the DHT-stimulated decrease in H3K9me1, although it had varying effects on basal H3K9me1 (Figure 2A, lower right panel). Significantly, pargyline also increased H3K9me1 at AR-negative LSD1 sites, including REST sites where LSD1 functions as a corepressor, indicating that H3K9 demethylation by LSD1 may not be unique to AR-stimulated genes (Figure S2E). Another LSD1 inhibitor (S2101) (Figure S2F) or LSD1 siRNA (Figure S2G) similarly did not impair AR binding, and comparable results for AR, H3, H3K4me2, and H3K9me1 were also obtained in VCaP cells with transient or stable knockdown of LSD1 (Figures S2H and S2I). Collectively, these results show that LSD1 retains its H3K4 demethylation activity at these AR-regulated enhancers.

We next used ChIP-seq to globally assess H3K4me2 in response to DHT (He et al., 2010). AR$^+$/LSD1$^+$ sites had higher basal H3K4me2 compared to AR$^+$/LSD1$^-$ sites (Figure 2B), consistent with the AR$^+$/LSD1$^+$ sites being enhancers. DHT stimulated an increase in H3K4me2 at flanking nucleosomes at AR$^+$/LSD1$^-$ sites, indicating AR recruitment of a histone methyltransferase (Gaughan et al., 2011; Ko et al., 2011). Significantly, in addition to lower basal H3K4me2, the AR$^+$/LSD1$^-$ sites have lower DNase-HS and are less enriched for androgen-stimulated genes (see Figures 1D and 1H), indicating they may be cryptic AR-regulated enhancers. In contrast, DHT treatment at AR$^+$/LSD1$^+$ sites did not increase H3K4me2 marks over the positions of the flanking nucleosomes and caused a loss of H3K4me2 over the central nucleosome (Figures 2B and S2J). Collectively, these results showed that LSD1, despite functioning as a coactivator at AR-regulated enhancers, retains its H3K4 demethylation activity that is associated with repression.

LSD1-CoREST Complex Is Associated with LSD1 Coactivator Function

Because LSD1 H3K4 demethylation activity is enhanced by CoREST (Lee et al., 2005; Shi et al., 2005), we next asked whether LSD1 was associated with the CoREST repressor complex at AR enhancers. Coimmunoprecipitation confirmed the strong association of LSD1 with CoREST, HDAC1, and HDAC2 in LNCaP (Figure 2C) and VCaP (Figures S2K and S2L) cells, and by ChIP we found that CoREST binding to a series of AR-regulated enhancers correlated with LSD1 binding (Figure 2D). Interestingly, DHT caused a reduction of LSD1-CoREST binding at many of these sites, possibly reflecting subsequent chromatin remodeling. Significantly, CoREST siRNA mimicked the effects of LSD1 siRNA in decreasing expression of AR-stimulated genes (Figure 2E), further supporting a coactivator function for the LSD1-CoREST complex.

H3T6ph-Positive AR/LSD1 Sites Are Markedly Enriched for Androgen-Stimulated Genes

Although these data indicated that LSD1 generally retains its H3K4 demethylase activity at AR-regulated enhancers, this activity may be impaired by H3T6ph at a subset of sites (Metzger et al., 2005, 2008, 2010). Therefore, we next performed H3T6ph ChIP-seq to globally assess the association of H3T6ph with AR and LSD1 binding, with gene expression, and with H3K4 methylation. Approximately 12% of total AR binding sites and 20% of AR$^+$/LSD1$^+$ sites overlapped with H3T6ph sites (Figures 3A and 3B). Significantly, although only a minority of DHT-stimulated genes had H3T6ph-positive AR sites (14%–20%), these H3T6-positive sites were markedly enriched (~12-fold) for DHT-stimulated genes (Figure 3C). Moreover, H3T6ph-positive AR$^+$/LSD1$^+$ sites were even further enriched for DHT-stimulated genes (~18-fold) (Figure 3D). Finally, consistent with a previous report that androgen stimulates H3T6 phosphorylation through activating PKCβ1 (Metzger et al., 2010), treating cells with a

Figure 1. LSD1 Functions Broadly as an AR Coactivator

(A) Overlap between AR and LSD1 ChIP-seq in LNCaP cells treated for 4 hours (hr) with 10 nM DHT.

(B) AR binding site enrichment for genes increased after 4 or 16 hr DHT stimulation (DHT_4h_up, DHT_16h_up) or suppressed genes after 16 hr DHT (DHT_16h_down).

(C) LSD1 binding site enrichment for expression of androgen-regulated genes.

(D) Enrichment analysis of AR$^+$/LSD1$^+$, AR$^+$/LSD1$^-$, or AR$^-$/LSD1$^+$ sites for androgen-regulated genes. High-frequency motifs and corresponding proteins on each subset of binding sites are shown. Note: the percentage labeled on the bar graph of (B)–(D) indicates the percentage of DHT-induced or -repressed genes that have the respective binding site.

(E) LNCaP cells transfected with nontarget control (siNTC) or LSD1 siRNA (siLSD1) in presence of DHT were subjected to RNA-seq. Gene ontology analysis was done on 284 LSD1-activated genes and 223 LSD1-suppressed genes (cutoff 2-fold).

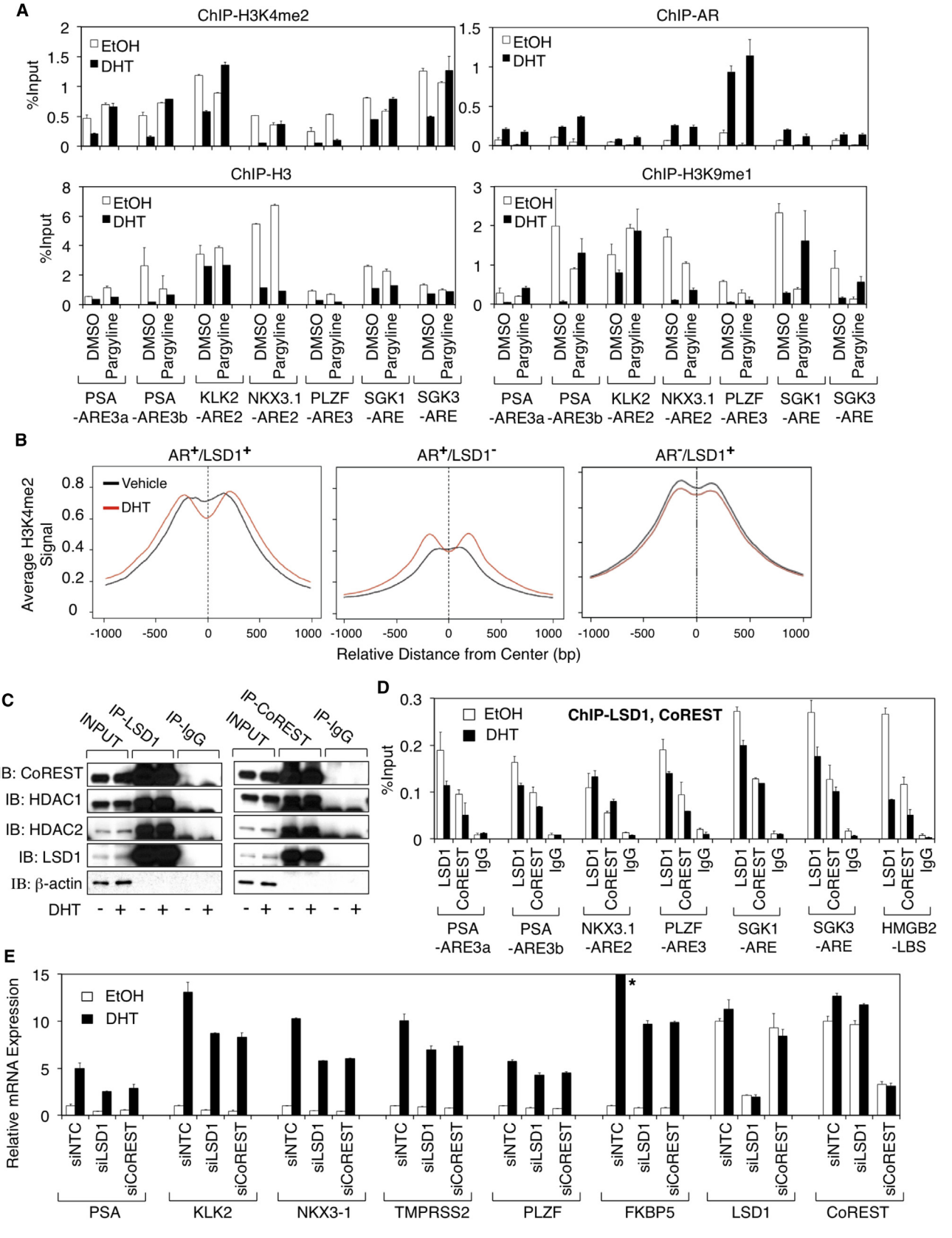
(F) Ratio of expression in siNTC versus in siLSD1-treated cells (log scale) is plotted for DHT-regulated genes.

(G) qRT-PCR in LNCaP cells treated with LSD1 inhibitor pargyline (1 mM) or S2101 (100 μM) for 24 hr with/out DHT.

(H) Mean DNase-HS signals in DHT-stimulated versus vehicle-treated LNCaP cells at the indicated sites.

(I) Box plot for expression of direct LSD1-regulated genes in PCa versus benign samples in three independent cohorts from Oncomine database.

Data in bar graphs represent means ± SD of at least three biological repeats. See also Figure S1 and Table S1.



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PKCβ inhibitor (BIM1) blocked the DHT-stimulated increase in H3T6ph and impaired the DHT stimulation of a panel of AR-regulated genes (Figures S3A and S3B). Together these findings indicate that H3T6 phosphorylation is linked to LSD1 coactivation of AR transcriptional activity.

DNase-HS also was increased at H3T6ph-positive versus -negative $AR^+/LSD1^+$ sites (Figure 3E), further linking H3T6ph to transcriptional activation. In contrast, DNase-HS at H3T6-positive $AR^+/LSD1^-$ sites was low (Figure 3E, right panel), whereas DNase-HS at H3T6ph-positive versus -negative LSD1 sites was increased (Figure S3C), further supporting a link between LSD1 and H3T6ph. Similarly to DNase-HS, basal (prior to DHT stimulation) H3K4me2 was increased at H3T6ph-positive versus -negative $AR^+/LSD1^+$ sites (Figure 3F). However, DHT still caused a decrease in H3K4me2 methylation at these sites, versus an increase at LSD1-negative $AR^+/H3T6ph^+$ sites (Figure 3F). The DNase-HS and H3K4me2 ChIP-seq data are quantified in Figures S3D and S3E. We also assessed H3T6ph and H3K4me2 demethylation at a series of individual $AR^+/LSD1^+$ sites. These sites in four of six genes had high H3T6ph (*PSA*, *NKX3.1*, *PLZF*, and *SGK3*) (Figure S3F), but all underwent H3K4me2 demethylation in response to DHT (see Figure 2A). Finally, inhibition of H3T6 phosphorylation with BIM1 did not enhance the androgen-stimulated demethylation of H3K4 or impair the demethylation H3K9 (Figure S3G). Together, these findings indicate that H3T6ph does not abrogate H3K4 demethylation by LSD1.

FOXA1 Associates with LSD1 and Mediates Its Recruitment to AR-Dependent Enhancers

As FOXA1 is a pioneer factor for AR-regulated enhancers, we next performed FOXA1 ChIP-seq and found substantial overlap (~42%) between LSD1 and FOXA1 sites (Figure 4A). Significantly, $AR^+/LSD1^+/FOXA1^+$ sites were more enriched (~9-fold) among AR-activated genes than $AR^+/LSD1^+/FOXA1^-$ (~5-fold) or $AR^+/LSD1^-/FOXA1^+$ (~5-fold) sites (Figure 4B), indicating that LSD1 and FOXA1 may collaborate to regulate AR activity. Similarly, $AR^+/LSD1^+/FOXA1^+$ sites were also more enriched for LSD1-activated genes than $AR^+/LSD1^+/FOXA1^-$ or $AR^+/LSD1^-/FOXA1^+$ sites (Figure S4A). Finally, FOXA1-positive $AR^+/LSD1^+$ sites had increased basal DNase-HS versus FOXA1-negative $AR^+/LSD1^+$ sites (Figure 4C), consistent with the FOXA1-positive sites being enhancers, although DHT increased DNase-HS at all sites (Figure S4B).

Coimmunoprecipitations in LNCaP cells indicated an association between FOXA1 and the LSD1-CoREST complex (Figure 4D). This was not mediated by AR, as we could also coprecipitate FOXA1 with transfected FLAG-LSD1 in AR-negative PC3 cells, although the interaction could still be indirect and through another protein or long noncoding RNA (Figure 4E). We next used FOXA1 siRNA to determine whether basal (prior to DHT treatment) LSD1 binding to $AR^+/FOXA1^+$ sites in AR-regulated enhancers was FOXA1 dependent. Immunoblotting confirmed that FOXA1 siRNA markedly decreased FOXA1, but not LSD1 or CoREST (Figure 4F). Significantly, ChIP then showed that FOXA1 siRNA decreased basal LSD1 binding to a similar extent as CoREST siRNA at a series of AR/LSD1-regulated enhancers (Figure 4G). In the converse experiment, we treated cells with LSD1 siRNA and found a marked decrease in FOXA1 binding at these enhancers (Figure 4H). Collectively, these results indicate that FOXA1 may initially recruit the LSD1-CoREST complex to androgen-regulated enhancers, where FOXA1-LSD1 interactions may then stabilize the binding of both proteins to chromatin. The LSD1-CoREST complex may then function through HDACs to suppress basal transcription, and by H3K4 demethylation as a negative-feedback mechanism to close any aberrantly opened cryptic enhancers. Indeed, we found that HDAC1 and 2, which are components of the LSD1-CoREST complex, are associated with AR-regulated enhancers even in the absence of AR (Figure S4C), and that HDAC inhibition increased basal expression of AR-stimulated genes (Figure S4D).

Two recent studies found that FOXA1 RNAi caused a marked redistribution of AR, with loss of AR binding at many sites and generation of new binding sites (Sahu et al., 2011; Wang et al., 2011). Using these data (Wang et al., 2011) in conjunction with our AR and LSD1 ChIP-seq data, we found that ~60% of AR binding sites were lost after FOXA1 knockdown, but that this loss was only 39% for the LSD1-positive AR binding sites (Figure S4E). Moreover, AR binding prior to FOXA1 siRNA was higher at the 61% of LSD1-positive AR sites that were retained versus the 39% that were lost, and AR binding at these 61% retained sites increased after FOXA1 siRNA (Figure S4F). H3K4me1 and H3K4me2 were also decreased after FOXA1 siRNA at these 61% of sites with retained/increased AR binding (Figure S4F). In conjunction with the above results, these findings suggest that LSD1 may stabilize AR binding directly, or indirectly through enhanced binding of residual FOXA1. Although we found that short-term inhibition or depletion of LSD1 did not impair AR binding (see Figures 2 and S2), we are currently exploring the long-term effect of LSD1 silencing on AR binding and enhancer activity through FOXA1-dependent or -independent mechanisms.

DISCUSSION

The H3K4me1,2 demethylase activity of LSD1 in LSD1-CoREST repressor complexes is well established, but LSD1 also can mediate H3K9me1,2 demethylation and function as a coactivator

Figure 2. LSD1 Demethylates H3K4me2 on AR-Regulated Enhancers

(A) ChIP-qPCR for AR, H3, H3K4me2, or H3K9me1 in LNCaP cells pretreated with pargyline for 6 hr and then treated with/out DHT for 4 hr.

(B) Mean of H3K4me2 ChIP-seq signals in DHT-stimulated versus vehicle-treated LNCaP cells. The center of curve was aligned with the center of AR peak or LSD1 peak for AR-negative LSD1 sites.

(C) Co-IP of LSD1 or CoREST in LNCaP cells treated with/out DHT for 6 hr.

(D) ChIP-qPCR for LSD1 or CoREST at androgen-stimulated enhancer sites and an $AR^-/LSD1^+$ site in the *HMGB2* gene in LNCaP cells treated with/out DHT for 4 hr.

(E) qRT-PCR in LNCaP cells transfected with nontargeting control (NTC), LSD1, or CoREST siRNA with/out DHT (*~18-fold).

Data in bar graphs represent means ± SD of at least three biological repeats. See also Figure S2.

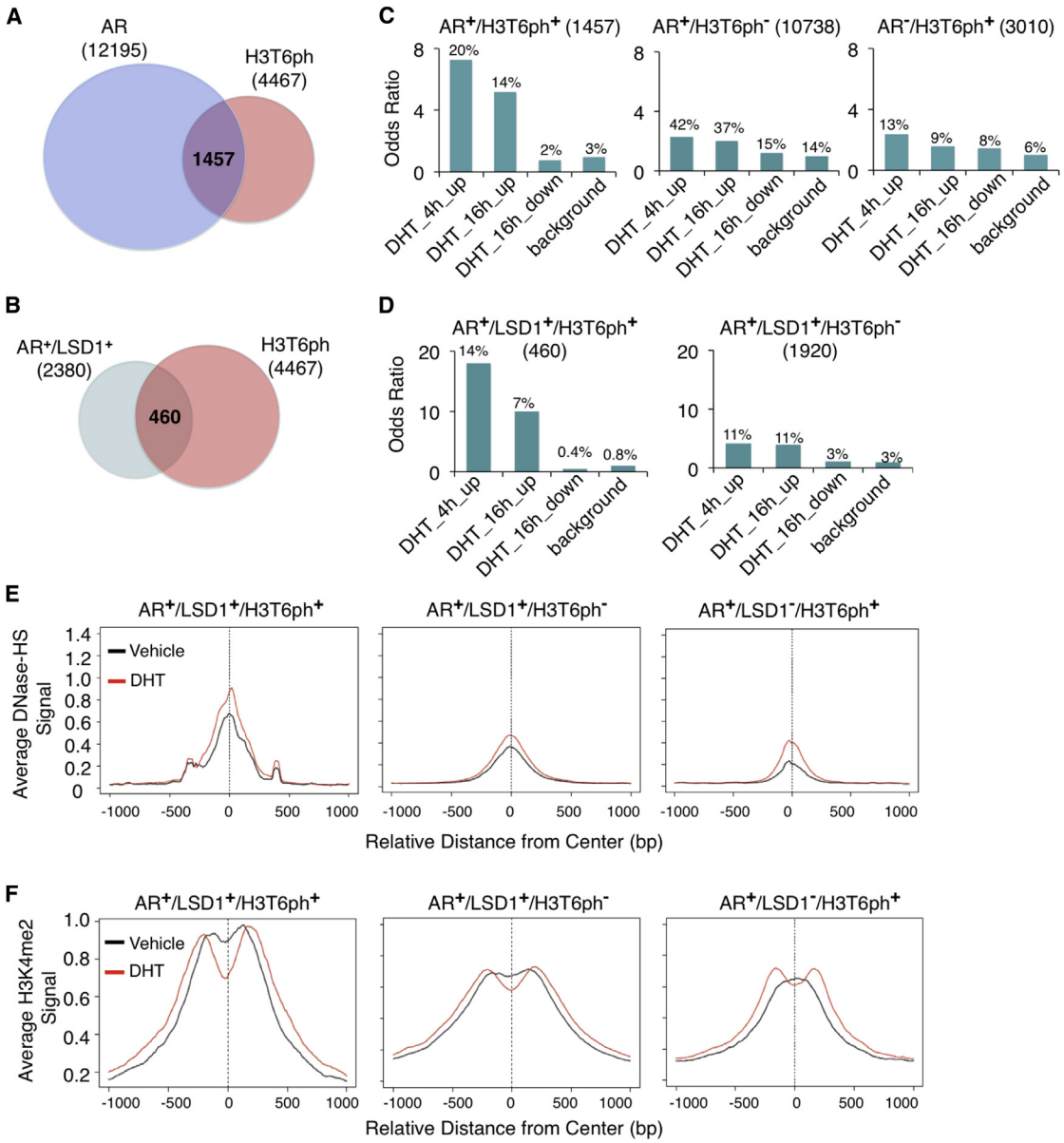

Figure 3. LSD1 Mediates H3K4me2 Demethylation at H3T6ph-Positive AR and LSD1 Sites

(A) Overlap of AR and H3T6ph ChIP-seq in LNCaP cells treated with DHT.

(B) Overlap between AR$^+$/LSD1$^+$ sites and H3T6ph peaks.

(C and D) Enrichment for androgen-stimulated genes at AR and H3T6ph overlapping sites (C) and at AR, LSD1, and H3T6ph overlapping sites (D).

(E and F) Mean DNase-HS signals (E) or mean H3K4me2 ChIP-seq signals (F) at indicated sites in LNCaP cells stimulated with DHT or vehicle.

See also Figure S3.

for several AR-stimulated genes (Metzger et al., 2005; Wissmann et al., 2007), and an H3T6 and T11 phosphorylation-mediated switch from H3K4 to K9 demethylation may contribute to this LSD1 coactivator function (Metzger et al., 2008, 2010). This study shows that LSD1 is broadly associated with AR-regulated enhancers and functions as a coactivator for AR at these sites, and that H3T6ph-positive AR$^+$/LSD1$^+$ sites are further enriched for AR-activated genes. However, H3K4me2 ChIP studies showed that androgen-stimulated H3K4 demethylation persisted at AR$^+$/LSD1$^+$ sites, including H3T6ph-positive AR$^+$/LSD1$^+$ sites. Moreover, LSD1-mediated H3K9 demethylation could also be observed at AR-independent LSD1 sites. Therefore, whereas some bias in favor of H3K9 versus H3K4 demethylation may contribute to LSD1 coactivator function, these findings indicate that LSD1 has dual corepressor and coactivator functions at androgen-stimulated genes, with the latter coactivator function being mediated by demethylation of additional nonhistone substrates.

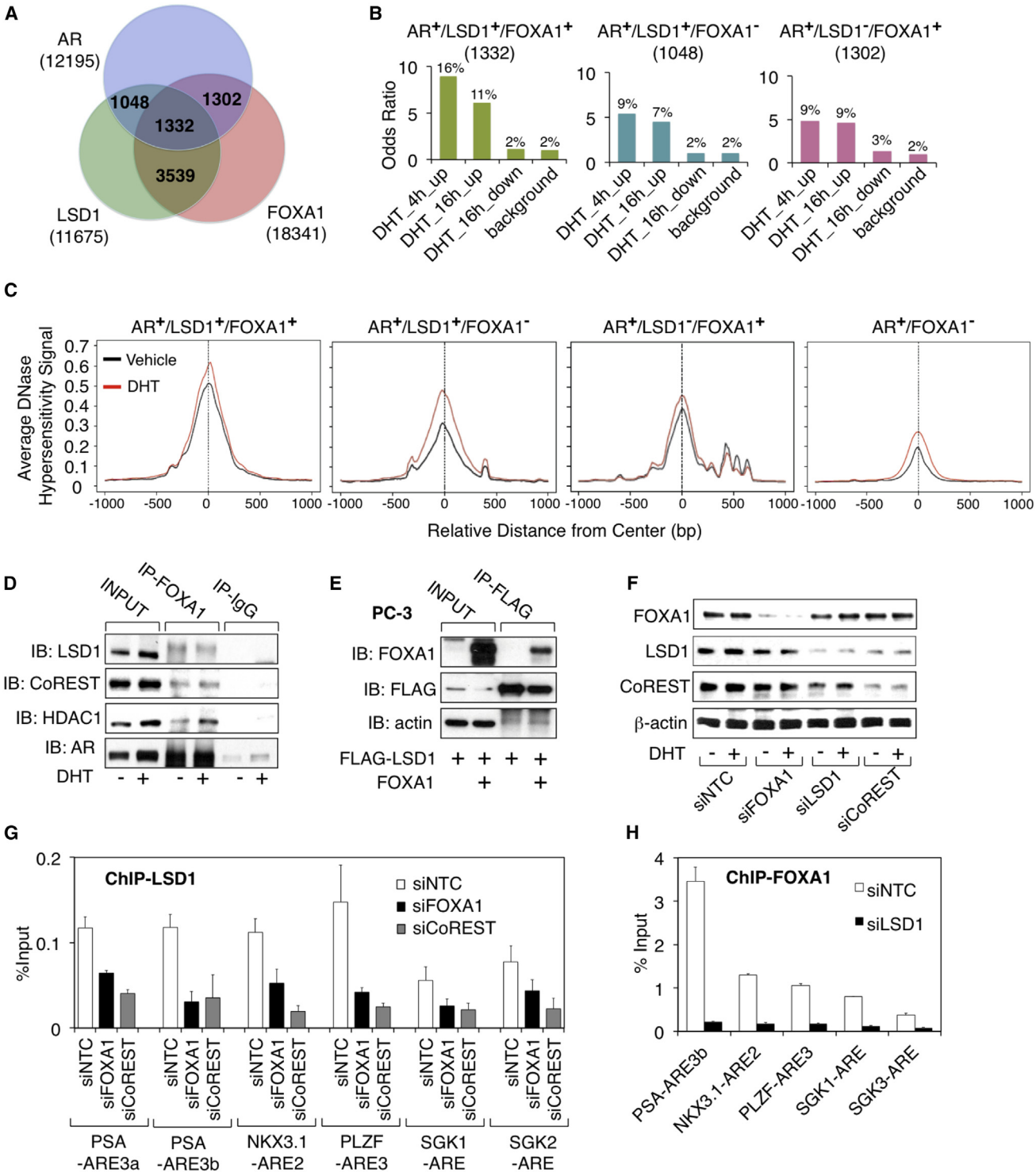

Figure 4. FOXA1 Associates with LSD1-CoREST Complex at AR-Stimulated Enhancers

(A) Overlap between AR, FOXA1, and LSD1 binding sites in LNCaP cells stimulated with DHT or vehicle.

(B) Enrichment analysis of AR$^+$/LSD1$^+$/FOXA1$^+$, AR$^+$/LSD1$^+$/FOXA1$^-$, or AR$^+$/LSD1$^-$/FOXA1$^+$ sites for androgen-regulated genes.

(C) Mean DNase-HS signals at indicated sites in LNCaP cells treated with DHT or vehicle.

(D) Anti-FOXA1 immunoprecipitated proteins from LNCaP cells treated with/out DHT for 6 hr were immunoblotted as indicated.

(E) FOXA1 coimmunoprecipitation with anti-FLAG from FLAG-LSD1- and FOXA1-transfected PC-3 cells.

(legend continued on next page)

H3K4 methylation and FOXA1 binding serve to initially expose enhancer binding sites prior to ligand-stimulated binding of steroid receptors (Carroll et al., 2005; Lupien et al., 2008). This study shows that FOXA1 interacts with the LSD1-CoREST complex and may mediate its initial recruitment to AR-regulated enhancers. Conversely, LSD1 binding at these sites can then stabilize FOXA1 binding. LSD1 may then mediate demethylation of H3K4me2 at these sites as a negative-feedback mechanism to close enhancers if their H3K4 methylation and FOXA1 binding is not reinforced by AR, thereby preventing aberrant enhancer activation. AR may reinforce FOXA1 binding through a direct interaction, and indirectly by recruitment of H3K4 methyltransferases and HATs, the latter which may impair LSD1 activity through H3 acetylation. Finally, HDACs associated with the LSD1-CoREST complex may also serve to suppress gene expression prior to androgen stimulation and AR binding.

We hypothesize that the AR coactivator function of LSD1 reflects demethylation of one or more nonhistone substrates, but the identity of any novel LSD1 substrates and the functional consequences of their demethylation remain unclear. Mass spectrometry has identified methylated lysines in AR, but there are no data showing that they are regulated by LSD1 (Gaughan et al., 2011; Ko et al., 2011). Recent studies have identified nonhistone LSD1 substrates, including transcription factors, but none are known to have roles in AR-mediated transcription (Cho et al., 2011; Huang et al., 2007; Kontaki and Talianidis, 2010; Wang et al., 2009a). LSD1, possibly through interactions with long noncoding RNA, may also contribute to the targeting of AR-stimulated genes to specific nuclear domains that are enriched in transcriptional cofactors (Hu et al., 2008). Importantly, our data show that inhibiting or depleting LSD1 acutely does not prevent AR binding to chromatin or displacement of the central nucleosome overlapping the AR binding site, indicating that any critical LSD1 substrates are functioning downstream of these initial steps. Identification of these substrates may allow for the selective abrogation of LSD1 coactivator function and provide a novel approach for PCa therapy.

EXPERIMENTAL PROCEDURES

RT-PCR, Immunoblotting, and Coimmunoprecipitation

Quantitative real-time RT-PCR amplification was done with TaqMan one-step RT-PCR reagents and results were normalized to coamplified 18S RNA or GAPDH. For immunoblotting, cells were lysed with RIPA buffer with protease inhibitors. For immunoprecipitation assay, equal amounts of protein (1–5 mg) were mixed with 20–50 μl of antibody-conjugated agarose beads. Antibodies, primers, and probes are listed in Supplemental Experimental Procedures section. Gels shown are representative of at least three independent experiments.

Chromatin Immunoprecipitation

Cells were formalin fixed, lysed, and sonicated to break the chromatin into ~500 bp fragments. ChIP-grade antibodies were used to precipitate chromatin fragments from cell extracts. We used real-time quantitative PCR (SYBR green) to amplify the DNA fragment in the antibody precipitated DNA and the unprecipitated input DNA to calculate the percentage. ChIP-seq was done with higher intensity sonication of cell lysates and sequenced with Illumina Genome Analyzer or High-seq. Significantly ($p < 1 \times 10^{-5}$) enriched regions were detected using MACS. For all integrated analyses, we searched binding sites around 20 kb from transcriptional starting site.

Statistical Analyses

Data in bar graphs represent means ± SD of at least three biological repeats. Statistical analysis was performed by Student's t test. A p value < 0.05 was considered to be statistically significant.

ACCESSION NUMBERS

The GEO accession for LSD1, H3T6ph, and FOXA1 ChIP-seq and LSD1 siRNA RNA-seq data is GSE52201.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.11.008.

AUTHOR CONTRIBUTIONS

C.C., H.H.H., X.S.L., M.B., and S.P.B., designed the study. C.C., H.H.H., S.G., Sen Chen, Z.Y., Y.G., M.W.C., J.Z., and M.A. performed experiments and analyzed data. Shaoyong Chen, Y.W., E.M., and R.S. provided advice and helped plan experiments. C.C., H.H.H., and S.P. wrote the manuscript. All authors discussed the results and commented on the manuscript.

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REFERENCES

Andreu-Vieyra, C., Lai, J., Berman, B.P., Frenkel, B., Jia, L., Jones, P.A., and Coetzee, G.A. (2011). Dynamic nucleosome-depleted regions at androgen receptor enhancers in the absence of ligand in prostate cancer cells. Mol. Cell. Biol. *31*, 4648–4662.

Cai, C., He, H.H., Chen, S., Coleman, I., Wang, H., Fang, Z., Chen, S., Nelson, P.S., Liu, X.S., Brown, M., and Balk, S.P. (2011). Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. Cancer Cell *20*, 457–471.

Carroll, J.S., Liu, X.S., Brodsky, A.S., Li, W., Meyer, C.A., Szary, A.J., Eeckhoute, J., Shao, W., Hestermann, E.V., Geistlinger, T.R., et al. (2005). Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell *122*, 33–43.

(F) LNCaP cells transfected with siNTC or siRNA against FOXA1 or CoREST were treated with/out DHT for 24 hr and then immunoblotted as indicated.
(G and H) LNCaP cells were transfected with siNTC or siRNA against FOXA1 or CoREST (G) or LSD1 (H) for 3 days in hormone-depleted medium, followed by ChIP-qPCR for LSD1 (G) or FOXA1 (H) at androgen-stimulated enhancers.
Data in bar graphs represent means ± SD of at least three biological repeats. See also Figure S4.

Cho, H.S., Suzuki, T., Dohmae, N., Hayami, S., Unoki, M., Yoshimatsu, M., Toyokawa, G., Takawa, M., Chen, T., Kurash, J.K., et al. (2011). Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. Cancer Res. *71*, 655–660.

de Bono, J.S., Logothetis, C.J., Molina, A., Fizazi, K., North, S., Chu, L., Chi, K.N., Jones, R.J., Goodman, O.B., Jr., Saad, F., et al.; COU-AA-301 Investigators (2011). Abiraterone and increased survival in metastatic prostate cancer. N. Engl. J. Med. *364*, 1995–2005.

Garcia-Bassets, I., Kwon, Y.S., Telese, F., Prefontaine, G.G., Hutt, K.R., Cheng, C.S., Ju, B.G., Ohgi, K.A., Wang, J., Escoubet-Lozach, L., et al. (2007). Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. Cell *128*, 505–518.

Gaughan, L., Stockley, J., Wang, N., McCracken, S.R., Treumann, A., Armstrong, K., Shaheen, F., Watt, K., McEwan, I.J., Wang, C., et al. (2011). Regulation of the androgen receptor by SET9-mediated methylation. Nucleic Acids Res. *39*, 1266–1279.

Green, S.M., Mostaghel, E.A., and Nelson, P.S. (2012). Androgen action and metabolism in prostate cancer. Mol. Cell. Endocrinol. *360*, 3–13.

He, H.H., Meyer, C.A., Shin, H., Bailey, S.T., Wei, G., Wang, Q., Zhang, Y., Xu, K., Ni, M., Lupien, M., et al. (2010). Nucleosome dynamics define transcriptional enhancers. Nat. Genet. *42*, 343–347.

He, H.H., Meyer, C.A., Chen, M.W., Jordan, V.C., Brown, M., and Liu, X.S. (2012). Differential DNase I hypersensitivity reveals factor-dependent chromatin dynamics. Genome Res. *22*, 1015–1025.

Hu, Q., Kwon, Y.S., Nunez, E., Cardamone, M.D., Hutt, K.R., Ohgi, K.A., Garcia-Bassets, I., Rose, D.W., Glass, C.K., Rosenfeld, M.G., and Fu, X.D. (2008). Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules. Proc. Natl. Acad. Sci. USA *105*, 19199–19204.

Huang, J., Sengupta, R., Espejo, A.B., Lee, M.G., Dorsey, J.A., Richter, M., Opravil, S., Shiekhattar, R., Bedford, M.T., Jenuwein, T., and Berger, S.L. (2007). p53 is regulated by the lysine demethylase LSD1. Nature *449*, 105–108.

Ko, S., Ahn, J., Song, C.S., Kim, S., Knapczyk-Stwora, K., and Chatterjee, B. (2011). Lysine methylation and functional modulation of androgen receptor by Set9 methyltransferase. Mol. Endocrinol. *25*, 433–444.

Kontaki, H., and Talianidis, I. (2010). Lysine methylation regulates E2F1-induced cell death. Mol. Cell *39*, 152–160.

Lee, M.G., Wynder, C., Cooch, N., and Shiekhattar, R. (2005). An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. Nature *437*, 432–435.

Lupien, M., Eeckhoute, J., Meyer, C.A., Wang, Q., Zhang, Y., Li, W., Carroll, J.S., Liu, X.S., and Brown, M. (2008). FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. Cell *132*, 958–970.

Metzger, E., Wissmann, M., Yin, N., Müller, J.M., Schneider, R., Peters, A.H., Günther, T., Buettner, R., and Schüle, R. (2005). LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. Nature *437*, 436–439.

Metzger, E., Yin, N., Wissmann, M., Kunowska, N., Fischer, K., Friedrichs, N., Patnaik, D., Higgins, J.M., Potier, N., Scheidtmann, K.H., et al. (2008). Phosphorylation of histone H3 at threonine 11 establishes a novel chromatin mark for transcriptional regulation. Nat. Cell Biol. *10*, 53–60.

Metzger, E., Imhof, A., Patel, D., Kahl, P., Hoffmeyer, K., Friedrichs, N., Müller, J.M., Greschik, H., Kirfel, J., Ji, S., et al. (2010). Phosphorylation of histone H3T6 by PKCbeta(I) controls demethylation at histone H3K4. Nature *464*, 792–796.

Ryan, C.J., Smith, M.R., de Bono, J.S., Molina, A., Logothetis, C.J., de Souza, P., Fizazi, K., Mainwaring, P., Piulats, J.M., Ng, S., et al.; COU-AA-302 Investigators (2013). Abiraterone in metastatic prostate cancer without previous chemotherapy. N. Engl. J. Med. *368*, 138–148.

Sahu, B., Laakso, M., Ovaska, K., Mirtti, T., Lundin, J., Rannikko, A., Sankila, A., Turunen, J.P., Lundin, M., Konsti, J., et al. (2011). Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. EMBO J. *30*, 3962–3976.

Scher, H.I., Fizazi, K., Saad, F., Taplin, M.E., Sternberg, C.N., Miller, K., de Wit, R., Mulders, P., Chi, K.N., Shore, N.D., et al.; AFFIRM Investigators (2012). Increased survival with enzalutamide in prostate cancer after chemotherapy. N. Engl. J. Med. *367*, 1187–1197.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., Casero, R.A., and Shi, Y. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell *119*, 941–953.

Shi, Y.J., Matson, C., Lan, F., Iwase, S., Baba, T., and Shi, Y. (2005). Regulation of LSD1 histone demethylase activity by its associated factors. Mol. Cell *19*, 857–864.

Wang, J., Scully, K., Zhu, X., Cai, L., Zhang, J., Prefontaine, G.G., Krones, A., Ohgi, K.A., Zhu, P., Garcia-Bassets, I., et al. (2007a). Opposing LSD1 complexes function in developmental gene activation and repression programmes. Nature *446*, 882–887.

Wang, Q., Li, W., Liu, X.S., Carroll, J.S., Jänne, O.A., Keeton, E.K., Chinnaiyan, A.M., Pienta, K.J., and Brown, M. (2007b). A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. Mol. Cell *27*, 380–392.

Wang, J., Hevi, S., Kurash, J.K., Lei, H., Gay, F., Bajko, J., Su, H., Sun, W., Chang, H., Xu, G., et al. (2009a). The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat. Genet. *41*, 125–129.

Wang, Q., Li, W., Zhang, Y., Yuan, X., Xu, K., Yu, J., Chen, Z., Beroukhim, R., Wang, H., Lupien, M., et al. (2009b). Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell *138*, 245–256.

Wang, D., Garcia-Bassets, I., Benner, C., Li, W., Su, X., Zhou, Y., Qiu, J., Liu, W., Kaikkonen, M.U., Ohgi, K.A., et al. (2011). Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature *474*, 390–394.

Wang, S., Sun, H., Ma, J., Zang, C., Wang, C., Wang, J., Tang, Q., Meyer, C.A., Zhang, Y., and Liu, X.S. (2013). Target analysis by integration of transcriptome and ChIP-seq data with BETA. Nat. Protoc. *8*, 2502–2515.

Wissmann, M., Yin, N., Müller, J.M., Greschik, H., Fodor, B.D., Jenuwein, T., Vogler, C., Schneider, R., Günther, T., Buettner, R., et al. (2007). Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. Nat. Cell Biol. *9*, 347–353.

Yatim, A., Benne, C., Sobhian, B., Laurent-Chabalier, S., Deas, O., Judde, J.G., Lelievre, J.D., Levy, Y., and Benkirane, M. (2012). NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function. Mol. Cell *48*, 445–458.

You, A., Tong, J.K., Grozinger, C.M., and Schreiber, S.L. (2001). CoREST is an integral component of the CoREST- human histone deacetylase complex. Proc. Natl. Acad. Sci. USA *98*, 1454–1458.

Yu, J., Yu, J., Mani, R.S., Cao, Q., Brenner, C.J., Cao, X., Wang, X., Wu, L., Li, J., Hu, M., et al. (2010). An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. Cancer Cell *17*, 443–454.

Yuan, X., Cai, C., Chen, S., Yu, Z., and Balk, S.P. (2014). Androgen receptor functions in castration-resistant prostate cancer and mechanisms of resistance to new agents targeting the androgen axis. Oncogene *33*, 2815–2825.