# LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription

**Article** *in* Nature · October 2005

DOI: 10.1038/nature04020 · Source: PubMed

LETTERS

# LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription

Eric Metzger[1], Melanie Wissmann[1]*, Na Yin[1]*, Judith M. Müller[1], Robert Schneider[2], Antoine H. F. M. Peters[3], Thomas Günther[1], Reinhard Buettner[4] & Roland Schüle[1]

**Gene regulation in eukaryotes requires the coordinate interaction of chromatin-modulating proteins with specific transcription factors such as the androgen receptor[1]. Gene activation and repression is specifically regulated by histone methylation status at distinct lysine residues[2]. Here we show that lysine-specific demethylase 1 (LSD1; also known as BHC110)[3] co-localizes with the androgen receptor in normal human prostate and prostate tumour. LSD1 interacts with androgen receptor *in vitro* and *in vivo*, and stimulates androgen-receptor-dependent transcription. Conversely, knockdown of LSD1 protein levels abrogates androgen-induced transcriptional activation and cell proliferation. Chromatin immunoprecipitation analyses demonstrate that androgen receptor and LSD1 form chromatin-associated complexes in a ligand-dependent manner. LSD1 relieves repressive histone marks by demethylation of histone H3 at lysine 9 (H3-K9), thereby leading to de-repression of androgen receptor target genes. Furthermore, we identify pargyline as an inhibitor of LSD1. Pargyline blocks demethylation of H3-K9 by LSD1 and consequently androgen-receptor-dependent transcription. Thus, modulation of LSD1 activity offers a new strategy to regulate androgen receptor functions. Here, we link demethylation of a repressive histone mark with androgen-receptor-dependent gene activation, thus providing a mechanism by which demethylases control specific gene expression.**

Transcriptional regulation by nuclear receptors such as androgen receptor (AR) involves interaction with multiple factors that act in both a sequential and combinatorial manner to reorganize chromatin[1]. Central to this dynamic organization is the modification of core histones. The amino-terminal tails of histones are subject to various covalent modifications such as acetylation, phosphorylation, ubiquitination and methylation by specific chromatin-modifying enzymes[2]. Histone methylation at specific lysine residues is linked to both transcriptional repression and activation[2].

We isolated LSD1 (ref. 3) by searching for new AR-interacting proteins. Endogenous LSD1 and AR associate specifically *in vivo* in androgen-sensitive tissues such as testis (Fig. 1a). LSD1 contains a centrally located SWIRM domain, which functions as a putative protein–protein interaction motif, and a carboxy-terminal amine oxidase domain that harbours the demethylase activity[3] (Fig. 1b). As shown in glutathione *S*-transferase (GST) pull-down analyses, full-length LSD1, as well as the SWIRM domain (LSD1 175–246) and the amine oxidase domain (LSD1 247–852) alone, associate with the N terminus (NTD), the DNA-binding domain (DBD) and the ligand-binding domain (LBD) of AR (Fig. 1b). In contrast, the N terminus of LSD1 (LSD1 1–174) does not interact with AR. Furthermore, neither LSD1 nor the LSD1 mutants associate with GST, GST–Nix1, GST–RORβ or GST–ERβ-NTD, thus demonstrating specificity of interaction with AR.

To examine the expression pattern of LSD1, we performed northern blot analyses. *LSD1* messenger RNA is ubiquitously expressed in human and murine fetal and adult tissue (Fig. 2a and data not shown) as a transcript of 3.3 kilobases (Supplementary Fig. S2a). To investigate LSD1 localization in normal prostate and prostate tumours, we used immunohistochemical analyses of 100 prostate cancer biopsies on tissue microarrays. As shown exemplarily in Fig. 2b, LSD1 is detected in the epithelium of normal prostate and in tumour cells. Importantly, these cells also express AR (Fig. 2b), showing that LSD1 and AR co-localize. The nuclear co-localization of LSD1 and AR was verified further in human LNCaP prostate tumour cells (Supplementary Fig. S2b). Taken together, our data

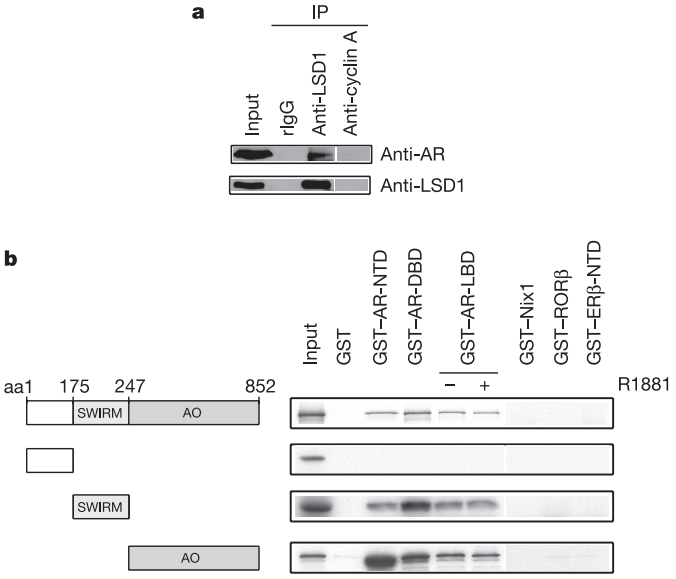


**Figure 1 | LSD1 interacts with AR *in vivo* and *in vitro*. a**, AR co-immunoprecipitates with LSD1. Extracts from mouse testis were immunoprecipitated (IP) with anti-LSD1 or anti-cyclin A antibodies and rabbit IgG as control. Western blots were decorated with anti-AR and anti-LSD1 antibodies. **b**, GST pull-down assays were performed with labelled LSD1 or LSD1 mutants and the corresponding bacterially expressed GST–AR fusion proteins. GST, GST–Nix1, GST–RORβ and GST–ERβ-NTD proteins were used as controls. aa, amino acids; NTD, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain.

[1]Universitäts-Frauenklinik und Zentrum für Klinische Forschung, Klinikum der Universität Freiburg, Breisacherstrasse 66, 79106 Freiburg, Germany. [2]Max-Planck-Institut für Immunbiologie, Stübeweg 51, 79108 Freiburg, Germany. [3]Friedrich Miescher Institute for Biomedical Research, Novartis Research Foundation, Maulbeerstrasse 66, 4058 Basel, Switzerland. [4]Institut für Pathologie, Universitätsklinikum Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany.
*These authors contributed equally to this work.

demonstrate that LSD1 is a nuclear protein that co-localizes with AR in androgen-sensitive tissues such as prostate.

Because LSD1 associates with chromatin and demethylates histone H3 at lysine 4 (H3-K4) *in vitro*[3], we verified that LSD1 binds to core histones, histone H3 and the N-terminal tail of histone H3 *in vitro* (Supplementary Fig. S3). To determine whether LSD1 and AR associate with chromatin *in vivo*, LNCaP cells treated with or without the synthetic AR agonist R1881 were subjected to chromatin immunoprecipitation (ChIP). Genomic DNA corresponding to the androgen response elements ARE I + II and ARE III, located in the promoter and enhancer of the prostate-specific antigen (*PSA*) gene, respectively, was immunoprecipitated in a ligand-dependent manner with anti-AR antibodies (Fig. 3a). DNA from a region between the enhancer and promoter was not enriched, thus demonstrating specificity (Fig. 3a). LSD1 specifically associates with chromatin on the *PSA* promoter both in the presence or absence of ligand (Fig. 3a). Association of LSD1 with the chromatinized *PSA* promoter is specific because DNA from neither exon 4 of the *PSA* gene nor the promoters of the *GAPDH* and *U6* genes is enriched (Fig. 3a). To demonstrate that LSD1 and AR form ligand-dependent complexes on chromatinized AREs, agonist-treated LNCaP cells were subjected to sequential chromatin immunoprecipitation (Re-ChIP), first with an anti-AR antibody and next with either anti-LSD1 antibody or rabbit IgG. Both ARE-containing regions were enriched, demonstrating that LSD1 and AR form a ligand-dependent complex on chromatin (Fig. 3a).

Because AR induces *PSA* gene expression we analysed methylation levels of repressive histone marks such as histone 3 at lysine 9 (H3-K9), histone 3 at lysine 27 (H3-K27) and histone 4 at lysine 20 (H4-K20). Stimulation of LNCaP cells with R1881 results in androgen-induced transcription and is accompanied by a robust decrease in mono-, di- and trimethyl H3-K9 at the *PSA* promoter (Fig. 3b). In addition, we observed a ligand-dependent decrease in dimethyl H4-K20, whereas mono- and trimethyl H4-K20 and

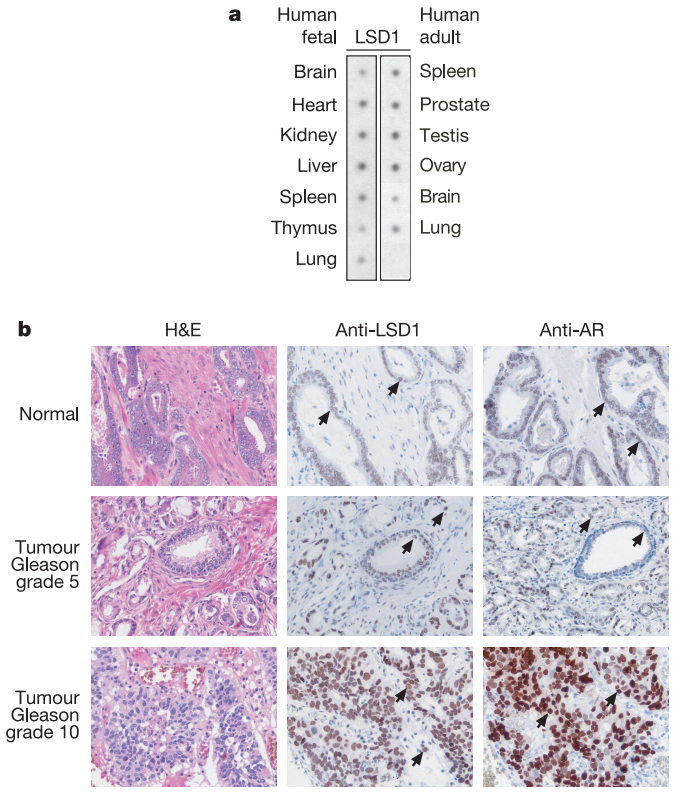


**Figure 2** | **LSD1 expression analyses.** **a**, Expression of *LSD1* mRNA in human tissues was examined by northern blot analyses on a human multiple tissue expression array. **b**, Immunohistochemical staining of LSD1 and AR in human normal prostate and tumour prostate. LSD1 (middle column) and AR (right column) immunoreactivity is detected in the secretory epithelium of normal prostate (arrows in top row) and tumour cells (arrows in middle and bottom rows). Haematoxylin and eosin (H&E)-stained sections are shown (first column). All sections were taken from the same radical prostatectomy specimen. Magnification: × 250.

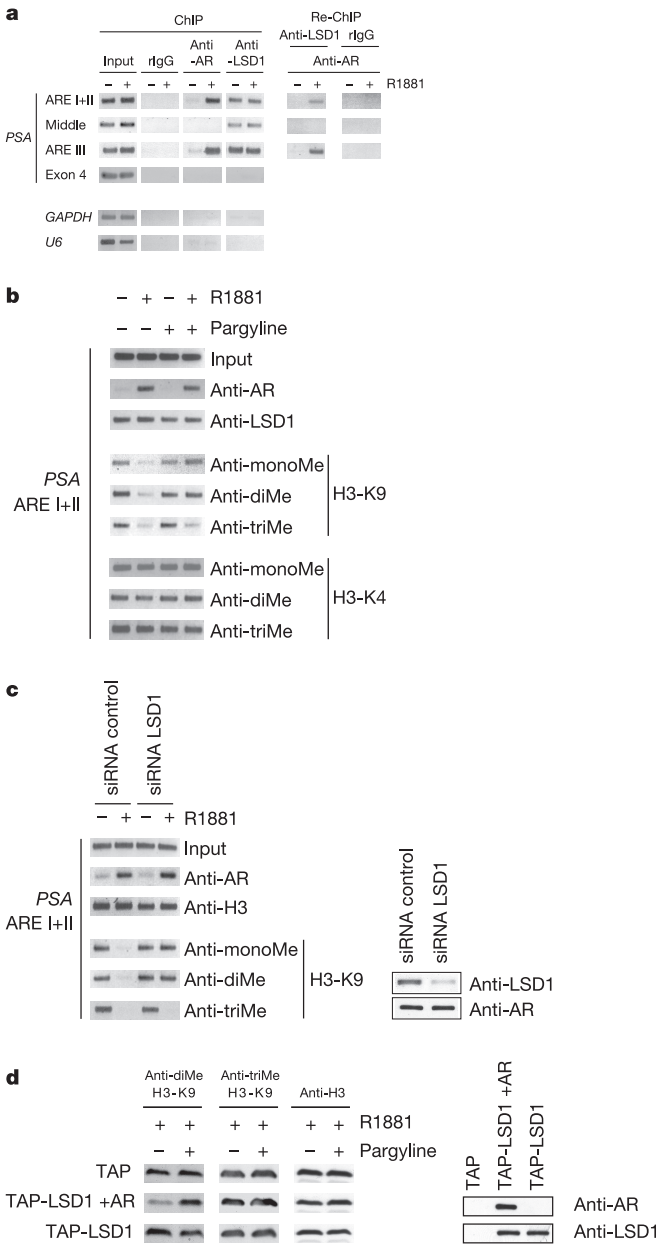


**Figure 3** | **LSD1 interacts with chromatin.** **a**–**c**, LNCaP cells were incubated with or without R1881, treated with or without pargyline (**b**), or transfected with siRNA (**c**). ChIP or Re-ChIP was performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter region (ARE I + II), the middle region (middle), the enhancer region (ARE III), exon 4 of the *PSA* gene, or the promoters of the *GAPDH* and *U6* genes. SiRNA-mediated knockdown of LSD1 is verified by western blot analysis (**c**, right panel) using anti-AR and anti-LSD1 antibodies. **d**, Native nucleosomes from HeLa cells were incubated in the presence of R1881 with either purified TAP, TAP-LSD1–AR, or TAP-LSD1 complexes with or without pargyline. Western blots were decorated with the indicated antibodies (left panel). The presence of LSD1 and AR in the TAP purified protein complexes was verified by western blotting using anti-AR and anti-LSD1 antibodies (right panel).

methylation levels of H3-K27 remain unchanged (Supplementary Fig. S4). Because LSD1 is an amine oxidase that catalyses demethylation, we tested whether monoamine oxidase inhibitors such as pargyline might block demethylation. Pargyline blocks demethylation of mono- and dimethyl H3-K9 during androgen-induced transcription, whereas methylation levels of trimethyl H3-K9 and the methylation status of H4-K20 and H3-K27 remain unchanged (Fig. 3b; see also Supplementary Fig. S4). Interestingly, methylation of histone H3-K4 is not altered in the presence of R1881 and not influenced by pargyline *in vivo* (Fig. 3b). To prove that LSD1 executed the ligand-dependent demethylation of mono- and dimethyl H3-K9, we designed various short interfering (si)RNAs directed against LSD1 or an unrelated control (Supplementary Fig. S5). Transfection of LNCaP cells leads to efficient and specific downregulation of endogenous LSD1 but does not affect the level of endogenous AR (Fig. 3c). LSD1 knockdown blocks ligand-dependent demethylation of mono- and dimethyl H3-K9 but not that of trimethyl H3-K9 (Fig. 3c). The amount of total H3 on the *PSA* promoter is not influenced by LSD1 knockdown (Fig. 3c). To validate further that a LSD1–AR complex removes H3-K9 dimethyl marks in the presence of R1881 we established a demethylation assay *in vitro*. Tandem affinity purified (TAP) LSD1 in the presence or absence of AR (Fig. 3d) was incubated in the presence of R1881, with HeLa nucleosomes as substrate. The TAP-LSD1–AR complex demethylated dimethyl H3-K9 *in vitro*, whereas TAP-LSD1 or the TAP control failed to do so. The methylation status of the trimethyl H3-K9 control is not altered (Fig. 3d). Notably, addition of pargyline blocked demethylation of dimethyl H3-K9 by the TAP-tagged LSD1–AR complex (Fig. 3d). Thus, the *in vitro* assay demonstrates that the LSD1–AR complex directly and specifically demethylates H3-K9 and demethylation is blocked by pargyline. Taken together, these data show the ligand-dependent association of LSD1 and AR on chromatinized AREs at the promoter of the *PSA* gene, and the specific demethylation of the repressive histone marks mono- and dimethyl H3-K9.

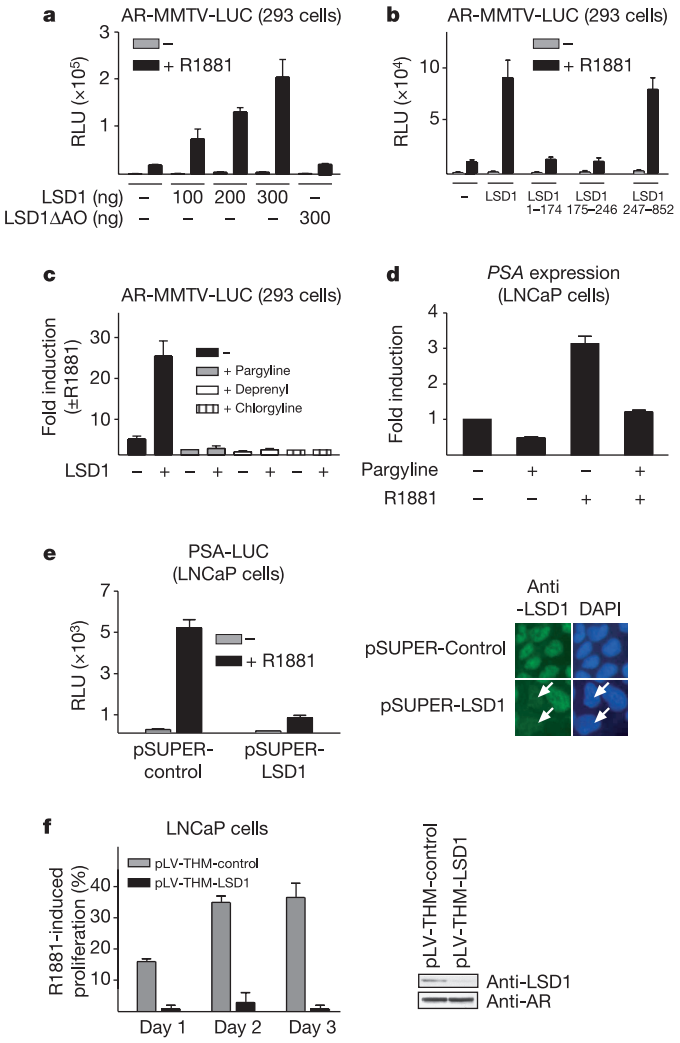


**Figure 4 | LSD1 controls AR-induced transcriptional activity and cell proliferation.** 293 (**a**–**c**) or LNCaP (**e**) cells were transfected with the indicated AR-dependent reporters in the presence of AR expression plasmid (**a**–**c**). Cells were treated with or without R1881, pargyline, deprenyl, or chlorgyline. LSD1-induced ligand-dependent activation of AR (**a**) is mediated by the amine oxidase domain (LSD1 247–852, **b**) and blocked by monoamine oxidase inhibitors (**c**). RLU, relative light units. **d**, Pargyline also reduces endogenous *PSA* gene expression in LNCaP cells as quantified by quantitative RT–PCR. **e**, In LNCaP cells, siRNA-mediated LSD1 knockdown reduces AR activity (left panel). **f**, LSD1 knockdown inhibits R1881-induced LNCaP cell proliferation (left panel). Knockdown of LSD1 is verified by immunofluorescence (**e**, right panel, arrows) and western blot analysis (**f**, right panel) using anti-AR and anti-LSD1 antibodies. Bars represent mean + s.d. ($n \geq 5$).

Next, we performed transient transfection assays to test whether LSD1 modulates the transcriptional activity of AR. Co-expression of LSD1 and AR results in a strong ligand-dependent activation of a mouse mammary tumour virus (MMTV)-luciferase reporter (Fig. 4a), which is not observed with a LSD1 deletion mutant lacking the amine oxidase domain (LSD1ΔAO) or in the absence of either ligand or AR (Fig. 4a; see also Supplementary Fig. S6). Stimulation of AR activity by LSD1 is potent in different cell lines, and AR-responsive minimal, synthetic and complex promoters are activated by LSD1 in a ligand-dependent manner (Supplementary Fig. S6b, c). LSD1 does not affect the transcriptional activity of related steroid hormone receptors, indicating that stimulation of AR is selective (Supplementary Fig. S6h). Furthermore, we demonstrate that the amine oxidase domain (LSD1 247–852) of LSD1 suffices to stimulate AR- and ligand-dependent reporter gene activity (Fig. 4b; see also Supplementary Fig. S6i). Because displacement of repressive histone marks by LSD1 increases AR-dependent gene expression, inhibition of LSD1 should reduce AR activity. Consequently, monoamine oxidase inhibitors such as pargyline, chlorgyline and deprenyl severely impair LSD1-induced activation of AR (Fig. 4c). In LNCaP cells, which express endogenous AR, only androgen-dependent but not unrelated reporters such as TK-LUC are inhibited by pargyline, thus demonstrating specificity (Supplementary Fig. S6j). Pargyline does not influence the activity of other nuclear receptors (Supplementary Fig. S6k). Moreover, quantitative RT–PCR analyses demonstrate that pargyline also blocks the androgen-induced expression of the endogenous *PSA* gene in LNCaP cells (Fig. 4d). Next, we efficiently reduced endogenously expressed LSD1 in LNCaP cells by vector (pSUPER-LSD1)-mediated RNA interference (Fig. 4e). Paralleling LSD1 knockdown, a significant ligand-dependent decrease of PSA-LUC reporter gene expression was observed (Fig. 4e), whereas expression of the unrelated TK-LUC is not influenced (data not shown). To address whether LSD1 governs androgen-dependent cell growth, we infected LNCaP cells with a lentivirus (pLV-THM-LSD1) expressing siRNA directed against LSD1. Infection with pLV-THM-LSD1 causes efficient and specific downregulation of endogenous LSD1 but does not affect the level of endogenous AR (Fig. 4f). When compared to cells transduced with the pLV-THM-control virus, androgen-induced proliferation of LNCaP cells is markedly inhibited by pLV-THM-LSD1-mediated LSD1 knockdown (Fig. 4f). These results show the physiological importance of LSD1 in the control of androgen-induced gene regulation and cell proliferation.

Our data demonstrate that AR function is controlled by the demethylase LSD1. LSD1 and AR associate at chromatinized AREs in a ligand-dependent manner, which results in concomitant specific

demethylation of the repressive histone marks mono- and dimethyl H3-K9. LSD1 has been described as a component of co-repressor complexes[4–7], and a recent model proposes that LSD1 represses transcription of genes silenced by Co-REST due to demethylation of the activating histone marks mono- and dimethyl H3-K4 (ref. 3). However, when in complex with AR, LSD1 demethylates the repressing histone marks mono- and dimethyl H3-K9 and thereby promotes gene activation. Thus, depending on the specific interacting partners, LSD1 action results in either gene silencing or activation. Of importance is our observation that inhibitors such as pargyline control the demethylase activity of LSD1 and thereby regulate AR. Thus, specific modulation of LSD1 activity might be a promising therapeutic target in tissues such as brain, testis and prostate, where AR has a pivotal physiological role.

## METHODS

Full details of the Methods are given in the Supplementary Information.

**Cell culture and transfections.** 293 and CV-1 cells were cultured and transfected as described[8]. LNCaP cells were cultured in phenol-red-free RPMI1640 supplemented with 10% double-stripped fetal calf serum (dsFCS) and transfected with Effectene (Qiagen). The following amounts per well were used: 500 ng each of MMTV-LUC, $ARE_{2\times}$-TATA-LUC, $ARE_{2\times}$-TK-LUC, TK-LUC, TREp-LUC, βRE-LUC, $ERE_{2\times}$-TATA-LUC, PSA-LUC, Slp-ARU-TATA-LUC; 25 ng expression plasmids for AR, PR, ERα, RARα and TRβ; 500–700 ng expression plasmids for LSD1 1–174, LSD1 175–246, LSD1 247–852, LSD1Δ281–360, LSD1ΔAO, pSUPER-control and pSUPER-LSD1; 100–700 ng expression plasmids for LSD1 were transfected per well. Chemicals were obtained as indicated: pargyline (Sigma); deprenyl and chlorgyline (ICN Biomedicals Inc.); R1881, T3, $E_2$, all-*trans* retinoic acid and R5020 (Schering AG). Cells were treated with or without $10^{-10}$ M R1881, $10^{-8}$ M R5020, $10^{-9}$ M $E_2$, $10^{-7}$ M T3, $10^{-6}$ M all-*trans* RA, $3 \times 10^{-3}$ M pargyline, $10^{-3}$ M deprenyl, or $10^{-4}$ M chlorgyline for 18 h, as indicated. Luciferase activity was assayed as described[9]. All experiments were repeated at least five times in duplicate.

**Immunohistochemistry.** Polyclonal rabbit anti-LSD1 antibody was generated according to standard procedures. Stainings were performed using a protocol[9] for antigen retrieval and indirect immunoperoxidase. Anti-AR 441 (Santa Cruz) and anti-LSD1 were used at a dilution of 1:75 and 1:500, rabbit IgG and mouse IgG (1:500; Dako) were used as secondary antibodies, and immunoreactions were visualized with the ABC complex diluted 1:50 in PBS (Vectastain, Vector).

**Chromatin immunoprecipitation.** ChIP experiments were performed essentially as described[10]. LNCaP cells were treated for 18 h with or without pargyline and for 210 min with or without $10^{-8}$ M R1881 as indicated. LNCaP cells were transfected 3 days before harvesting for ChIP with or without siRNA (Qiagen) following the manufacture's instructions. Immunoprecipitation was performed with specific antibodies (anti-monoMeK9H3, anti-diMeK9H3, anti-triMeK9H3, anti-monoMeK4H3, anti-diMeK4H3, anti-triMeK4H3, anti-monoMeK27H3, anti-diMeK27H3, anti-triMeK27H3, anti-monoMeK20H4, anti-diMeK20H4, anti-triMeK20H4, anti-H3 (all obtained from Upstate Biotechnology), anti-LSD1 and anti-AR PG21 (Upstate Biotechnology)) on GammaBind-Sepharose 4B (GE-Healthcare). For PCR, 1–5 μl out of 50 μl DNA extract was used. For Re-ChIP assays, immunoprecipitations were sequentially washed with TSE I, TSE II, buffer III (0,25 mM LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA and 10 mM Tris-HCl pH 8.1) and TE[10]. Complexes were eluted by incubation with 10 mM dithiothreitol at 37 °C for 30 min, diluted 50 times with dilution buffer[10] followed by a second immunoprecipitation with the indicated antibody. Primer sequences were as follows: exon 4, *PSA* (+3909 to +4067) 5′-GTG TGTGGACCTCCATGTTATT-3′ and 5′-CCACTCACCTTTCCCCTCAAG-3′; middle, *PSA* (−2223 to −1951) 5′-TGGGTTGGGTCAGGTTTTGGTT-3′ and 5′-TCTTCCCCTGTTTCTAGTTGAGTG-3′. PCR primers for ARE I + II (*PSA* (−459 to −121)), ARE III (*PSA* (−4288 to −3922)), *GAPDH* and *U6* have been described previously[3,11,12].

**Co-immunoprecipitation assays and western blot analyses.** Experiments were performed essentially as described[13]. Immunoprecipitations from extracts of murine testis were performed in the presence of $1 \times 10^{-9}$ M R1881 with anti-LSD1 antibody, anti-cyclin A[13] antibody, or rabbit IgG. Western blots were decorated as indicated. Anti-AR (N20, Santa Cruz) was used. Ten per cent of testis extract was loaded as input.

**Cell proliferation assay.** pLV-THM-control and pLV-THM-LSD1 were used to produce recombinant lentiviruses to infect LNCaP cells as described[14]. The infected cells were cultured for 72 h in medium supplemented with 10% dsFCS. $0.3 \times 10^4$ cells were plated in a 96-well plate with or without $10^{-7}$ M R1881. The cell proliferation Elisa BrdU Colorimetric Assay (Roche) was performed according to the manufacturer's instructions. The experiments were repeated three times in quadruplet.

**Quantitative RT–PCR and statistical analysis.** DNaseI-treated RNA isolated using RNAwiz (Ambion) was used for reverse transcription. Quantitative PCR was performed in an ABI PRISM 7700 sequence detector. Product formation was detected by incorporation of SYBR green I using ROX as a passive reference (ABgene). The expression ratios of the analysed cDNAs were related to the normalized $C_p$ (crossing point) of the housekeeping gene *GAPDH* in each sample. The following primers were used: *GAPDH* 5′-GAAGGTGAAGGTCG GACTC-3′ and 5′-GAAGATGGTGATGGGATTTC-3′; *PSA* 5′-CACCTGC TCGGGTGATTCTG-3′ and 5′-CCACTTCCGGTAATGCACCA-3′. Statistical analysis for quantitative PCR was performed by group-wise comparison based on PCR efficiencies and the mean crossing point deviation between sample and control group using Relative Expression Software Tool[15]. Experiments were repeated and analysed three times.

**Demethylase assay.** The demethlylation assay was essentially performed as described[3]. TAP-tagged proteins were bound to IgG-sepharose, washed and incubated in buffer 1 (50 mM Tris pH 8.5, 50 mM KCl, 5 mM MgCl, 0.5% BSA and 5% glycerol) supplemented with 10 mM ATP, $10^{-9}$ M R1881 with or without $1 \times 10^{-3}$ M pargyline and 1 μg of nucleosomes purified from HeLa cells[16] for 6 h at 37 °C. The reaction mixture was analysed by SDS–PAGE followed by western blotting using antibodies as indicated.

**Received 27 April; accepted 8 July 2005.**
**Published online 3 August 2005.**

1. Glass, C. K. & Rosenfeld, M. G. The coregulator exchange in transcriptional function of nuclear receptors. *Genes Dev.* **14,** 121–141 (2000).
2. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403,** 41–45 (2000).
3. Shi, Y. *et al.* Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119,** 941–953 (2004).
4. Shi, Y. *et al.* Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* **422,** 735–738 (2003).
5. Hakimi, M. A. *et al.* A candidate X-linked mental retardation gene is a component of a new family of histone deacetylase-containing complexes. *J. Biol. Chem.* **278,** 7234–7239 (2003).
6. Hakimi, M. A. *et al.* A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. *Proc. Natl Acad. Sci. USA* **99,** 7420–7425 (2002).
7. Eimer, S. *et al.* Loss of spr-5 bypasses the requirement for the *C. elegans* presenilin sel-12 by derepressing hop-1. *EMBO J.* **21,** 5787–5796 (2002).
8. Müller, J. M. *et al.* FHL2, a novel tissue-specific coactivator of the androgen receptor. *EMBO J.* **19,** 359–369 (2000).
9. Müller, J. M. *et al.* The transcriptional coactivator FHL2 transmits Rho signals from the cell membrane into the nucleus. *EMBO J.* **21,** 736–748 (2002).
10. Shang, Y., Myers, M. & Brown, M. Formation of the androgen receptor transcription complex. *Mol. Cell* **9,** 601–610 (2002).
11. Shatkina, L. *et al.* The cochaperone Bag-1L enhances androgen receptor action via interaction with the $NH_2$-terminal region of the receptor. *Mol. Cell. Biol.* **23,** 7189–7197 (2003).
12. Kang, Z., Pirskanen, A., Jänne, O. A. & Palvimo, J. J. Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. *J. Biol. Chem.* **277,** 48366–48371 (2002).
13. Metzger, E. *et al.* A novel inducible transactivation domain in the androgen receptor: implications for PRK in prostate cancer. *EMBO J.* **22,** 270–280 (2003).
14. Wiznerowicz, M. & Trono, D. Conditional suppression of cellular genes: lentivirus vector-mediated drug-inducible RNA interference. *J. Virol.* **77,** 8957–8961 (2003).
15. Pfaffl, M. W., Horgan, G. W. & Dempfle, L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res.* **30,** e36 (2002).
16. O'Neill, T. E., Roberge, M. & Bradbury, E. M. Nucleosome arrays inhibit both initiation and elongation of transcripts by bacteriophage T7 RNA polymerase. *J. Mol. Biol.* **223,** 67–78 (1992).

**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

**Acknowledgements** We thank T. Benzing, F. Claessens, T. Jenuwein, Z. Sun and D. Trono for providing reagents. We are obliged to the members of the Schüle laboratory for discussions. We thank K. Fischer, P. Kahl and L. Heukamp for technical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft and Deutsche Krebshilfe to R.S.

**Author Information** Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to R.S. (roland.schuele@uniklinik-freiburg.de).