Supplementary Data

Supplementary Material and Methods

Fluorescent Immunostaining

Immunofluoresence of polytene chromosome squashes was performed as previously described (1,2). Wandering third instar larvae of *D. melanogaster* were collected, and salivary glands were dissected and fixed in 3.7% formaldehyde, 50% acetic acid for 2 min and squashed in 45% acetic acid on subbed slides. The slides were frozen in liquid nitrogen and blocked in blocking buffer (1×PBS, 5% non-fat dry milk, 0.1% Tween 20), and were then incubated in blocking buffer containing primary antibodies at 4°C overnight. Primary antibodies used were: rabbit polyclonal anti-FLAG (SIGMA F7425, 1:80), mouse polyclonal anti-HP1 C1A9 (University of Iowa, 1:70), or rabbit anti-trimethyl H3-K27 (Upstate Biotechnology, 1:100) antibodies. Incubation was followed by washing in wash buffer (1×PBS, 0.1% Tween 20) three times. The slides were then incubated with secondary FITC-, Cy3-, or Cy5- conjugated antibodies (Jackson Immunoresearch Laboratories Inc.; 1:1000) together with DAPI (Roche, 1:5000 in PBS) for 2 h at room temperature. Slides were again washed in wash buffer three times and mounted in Vectashield anti-fate mounting medium (Vector Laboratories). Immunofluoresence staining was visualized using Zeiss confocal laser scanning system 510.

For immunofluoresence staining of HEK293T cells, the cells were divided into 4-well Chamber Slides (Nalgene Nunc International), and then co-transfected with AR and FLAG-MDC1 expression vectors. 4 h post-transfection, the cultured medium were replaced with a fresh DMEM containing 10^{-8} M DHT as described previously (3). And for immunofluoresence of normal human testis

samples, which were purchased from Biochain Institute Company. The primary antibodies used were: mouse anti-AR (441) (Thermo, 1:100), rabbit polyclonal anti-FLAG (SIGMA F7425, 1:80) antibodies. Incubation was followed by washing in wash buffer, the slides were then incubated with secondary FITC-, or Cy3- conjugated antibodies as above.

Luciferase Assay

Cells were co-transfected the reporter gene carrying MMTV promoter by using superfect (Qiagen) and pRL-TK as the internal control, with other expression plasmids together. At 3 h post-transfection, the cells were rinsed and incubated in DMEM supplemented with 5% coal treated fetal bovine serum in the absence or presence of AR ligand (DHT at 10^{-8} M). After an additional 21 h, the cells were harvested and assayed for luciferase activities using the Dual-Luciferase Reporter Assay System (Promega) as described.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed as previously described. Cells were treated for 4 hr in the presence or absence of DHT (10^{-8} M) and then cross-linked with 1% formaldehyde at room temperature for 10 min, resuspended in lysis buffer with 1× protease inhibitor cocktail (Roche Molecular Biochemicals) and sonicated three times for 10 s each at the maximum setting (Handy Sonic, Model UR-20P) followed by centrifugation for 10 min. Supernatants were collected and diluted in buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1)

followed by immunoclearing with 2 μg sheared salmon sperm DNA-20 μl preimmune serum and protein A-Sepharose (45 μl of 50% slurry in 10 mM Tris-HCl, pH 8.1, 1 mM EDTA) for 2 hr at 4^{o}C. Immunoprecipitations were performed overnight at 4^{o}C with specific antibodies. Protein A-Sepharose beads were added and then washed sequentially with low-salt buffer, high-salt buffer, LiCl buffer, and TE buffer. The protein-DNA complexes were eluted and the crosslinking was reversed at 65 ^{o}C for 6 hr. DNA fragments were purified with a DNA purification kit (DIAquick, Qiagen) and analyzed by regular PCR. Primer sequences for AREI/II、AREIII、*KLK2* promoter regions (-343 to -90) and p21 promoter regions were previously described (4). For PCR, 2 μl from 50μl DNA extraction and 25-28 cycles of amplification were used.

Supplementary references

1. Sawatsubashi, S., Murata, T., Lim, J., Fujiki, R., Ito, S., Suzuki, E., Tanabe, M., Zhao, Y., Kimura, S., Fujiyama, S. *et al.* (2010) A histone chaperone, DEK, transcriptionally coactivates a nuclear receptor. *Genes Dev*, **24**, 159-170.
2. Zhao, Y., Takeyama, K., Sawatsubashi, S., Ito, S., Suzuki, E., Yamagata, K., Tanabe, M., Kimura, S., Fujiyama, S., Ueda, T. *et al.* (2009) Corepressive action of CBP on androgen receptor transactivation in pericentric heterochromatin in a Drosophila experimental model system. *Mol Cell Biol*, **29**, 1017-1034.
3. Zhao, Y., Goto, K., Saitoh, M., Yanase, T., Nomura, M., Okabe, T., Takayanagi, R. and Nawata, H. (2002) Activation function-1 domain of androgen receptor contributes to the interaction between subnuclear splicing factor compartment and nuclear receptor compartment. Identification of the p102 U5 small nuclear ribonucleoprotein particle-binding protein as a coactivator for the receptor. *J Biol Chem*, **277**, 30031-30039.
4. Metzger, E., Wissmann, M., Yin, N., Muller, J.M., Schneider, R., Peters, A.H., Gunther, T., Buettner, R. and Schule, R. (2005) LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature*, **437**, 436-439.

Supplemental Table 1.

Real-time quantitative PCR primers for androgen-responsive genes

Name	sense(5'-3')	anti-sense(5'-3')
Vinculin	GAGGTGGTGGAGACTATGGA	AATGAGAACTGGCAGCAACT
p21	GGCAGACCAGCATGACAGATT	GCGGATTAGGGCTTCCTCTT
NKX3.1	TCTGGTGGTTCTGCTGTTACG	CGCTGTGTTCTTCCTCTGTGA
PMEPA1	GTGGTGGTGATGATGGTGATG	TTCCGTTGCCTGACACTGT
FKBP5	CGCAGGATATACGCCAACAT	CTTGCCCATTGCTTTATTGG
TMPRSS2	TGAAAGCGGGTGTGAGGAGC	TGGTGGTGACCCTGAGTTCAA
PSA	CACCTGCTCGGGTGATTCTG	CCACTTCCGGTAATGCACCA
SLC45H31	GAGCCGAGACGAAGCAGTT	TTAGCAGGTTGACCAGCAAGA
JAG1	CGGCCTCTGAAGAACAGAAC	ACCAAGCAACAGATCCAAGC
HUS1	AAACACTTTCCCTGCCTCAC	GATGTCATGGGTCACAATGC
KRT18	CCACCTTCTCCACCAACTACC	GTCTCCAGGCTCCTCACTCT
FASN	TTGTGGTCTTCTCCTCTGTGA	CGTTGGTGCTCATCGTCTC
KLK4	GTACCACCCCAGCATGTTCT	AGAGTCACCGTTGCAGGAGT
ITGAV	ACTTCTTGGTGGTCCTGGTAG	CAGTCCGAGTTGCTAATTGGTT
BMPRIB	TGAGGGAGATTGTGTGCATC	TGAGTTTTCCCATCTGCCTT
B4GALT1	GGGAGGAGAAGATGATGACATT	TTGGGCGAGATATAGACATGC
ALDH1A3	TGACATTGACCGTGAGATTCG	CTATGCTGTTGTGGCGTTAGA
β-Actin	ATCATGTTTGAGACCTTCAACA	CATCTCTTGCTCGAAGTCCA

Supplemental Table 2.

Real-time quantitative PCR primers for ChIP

Name	sense(5'-3')	anti-sense(5'-3')
PSA AREI/II	GCCAAGACATCTATTTCAGGAGC	CCCACACCCAGAGCTGTGGAAGG
PSA AREIII	GGGGTTTGTGCCACTGGTGAG	GGGAGGCAATTCTCCATGGTT
KLK2 promoter	ACCCCTGTTGCTGTTCATCCTG	CCGCCCTTGCCCTGTTGG
p21 ARE1/2	CTGGGACGTGAAACTGTGG	ACCTACCTGCCTGCTCTGG
p21 ARE3	TCACCTAGTAAAACCTGGAC	AAAGAGCATGGCTACAGAC
GAPDH	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA

Supplementary Figure S1. Localization of mu2 protein in vivo

(A) Mu2 accumulation in polytene chromosome. Polytene chromosomes from the third instar larvae of fly lines carrying *UAS-mu2* expression plasmid and *GMR-GAL4* driver gene were dissected and stained with DAPI to visualize DNA (blue), mouse polyclonal anti-HP1 (red), rabbit polyclonal anti-FLAG (green) antibodies, and the merged images as indicated. (B) Mu2 is necessary for chromosome integrity. Polytene chromosomes from the third instar larvae of fly lines carrying *UAS-mu2* expression plasmid or mu2 mutation ($mu2^1$/+) together with *GMR-GAL4* driver gene were dissected and stained with DAPI to visualize DNA (blue), mouse polyclonal anti-HP1 (red), rabbit polyclonal anti-H3K27 met3 (green) and the merged images as indicated. Scale bar, 5 μm.

Supplementary Figure S2. Localization of MDC1 and AR in human tesitis tissue

Immunohistochemical analyses of serial sections of normal testis are shown. The region in the left image showing AR and MDC1 is enlarged in the right image. Scale bar, 100 μm.

Supplementary Figure S3. NBS1 interacts with N-terminus of MDC1

Flag-MDC1 N1-N3, Flag-MDC1 M1-M2, Flag-MDC1 C1 expression plasmids were transfected into HEK293 cells. Cell lysates were analyzed by immunoprecipitation using anti-FLAG antibody. Precipitated proteins and the input of cell lysates were analyzed by immunoblotting with the indicated antibodies.

Supplementary Figure S4. MDC1 enhances AR-V7-mediated transactivation

HEK293 cells were cotransfected with ARE-luc and pRL-TK, together with the indicated expression plasmids in the absence or presence of DHT. After 24 hrs of DHT treatment, cells were collected and

assayed for luciferase activity. Relative luciferase units shown are the mean value at least three times. Error bars represent mean ± SD. *, $P < 0.05$; **, $P < 0.01$.

Supplementary Figure S5. MDC1 depletion impairs the recruiments of GCN5 to androgen response elements of AR target genes

CWR22Rv1 cells were incubated with or without DHT, transfected with MDC1 expression plasmid or siRNA against MDC1 (siMDC1). ChIP assay was performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter region of *PSA* gene (ARE I + II) (A), *KLK2* promoter (B) and the enhancer region (ARE III) of *PSA* gene (C).

Supplementary Figure S6. MDC1-induced ligand-dependent AR transactivation is cooperated with GCN5

CWR22Rv1 cells were cotransfected with the indicated expression plasmids. Cells were treated with or without DHT. After 24 hrs of DHT treatment, cells were collected and assayed for luciferase activity. Relative luciferase units shown are the mean value at least three times. Error bars represent mean ± SD. *, $P < 0.05$; **, $P < 0.01$.

Supplementary Figure S7. Identification of the interaction domains in AR and MDC1 for association with GCN5

(A) For GST pull-down assay, GST and GST-AR truncated mutants as indicated were incubated with synthesized GCN5 protein in vitro. Bound proteins were detected with anti-GCN5 antibodies. (B) For Co-IP, Flag-MDC1 N1-N3, Flag-MDC1 M1-M2, Flag-MDC1 C1 expression plasmids were transfected into HEK293 cells. Cell lysates were analyzed by immunoprecipitation using anti-FLAG

antibody. Precipitated proteins and the input of cell lysates were analyzed by immunoblotting with the indicated antibodies.

Supplementary Figure S8. The efficiency of siRNA against AR or shRNA against MDC1

CWR22Rv1 cells with shRNA against MDC1 or control shRNA were transfected with control siRNA or siRNA against AR as indicated. Cells were collected for Western Blotting after transfection 24 h.

Supplementary Figure S9. MDC1 overexpression upregulates p21 and Vinculin expression in CWR22Rv1 cells

CWR22Rv1 cells were transfected with the increasing amount of MDC1 expression plasmids in the presence or absence of ligand (DHT 10^{-8}M). The expression levels of Vinculin or p21 were analyzed by immunoblotting with anti-Vinculin or anti-p21 antibody. Levels of GAPDH were used as loading control.

Figure S1

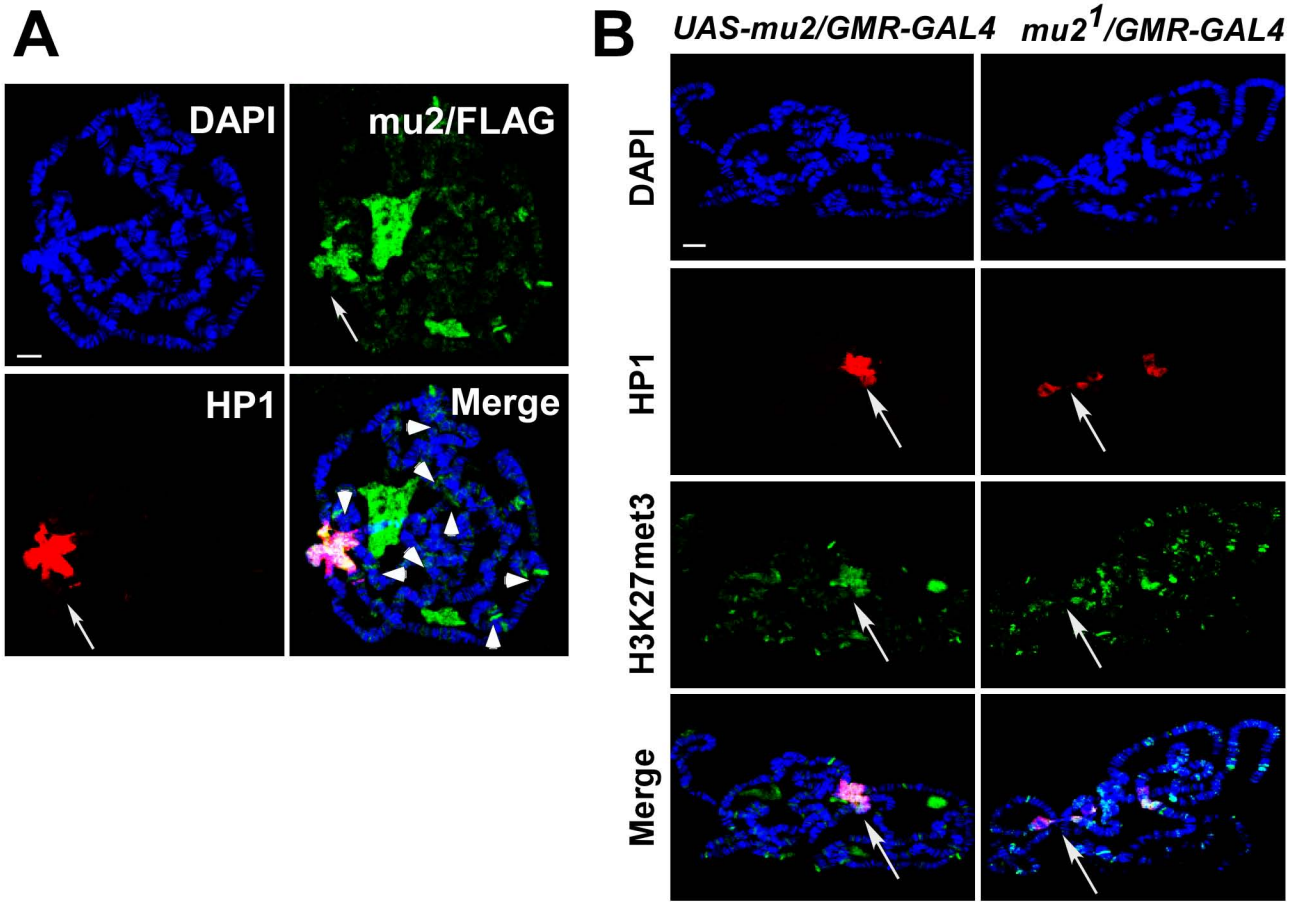

Figure S2

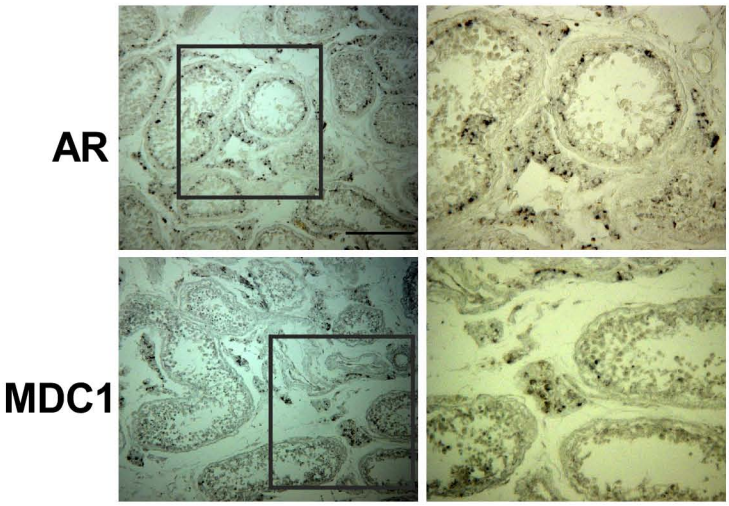

Figure S3

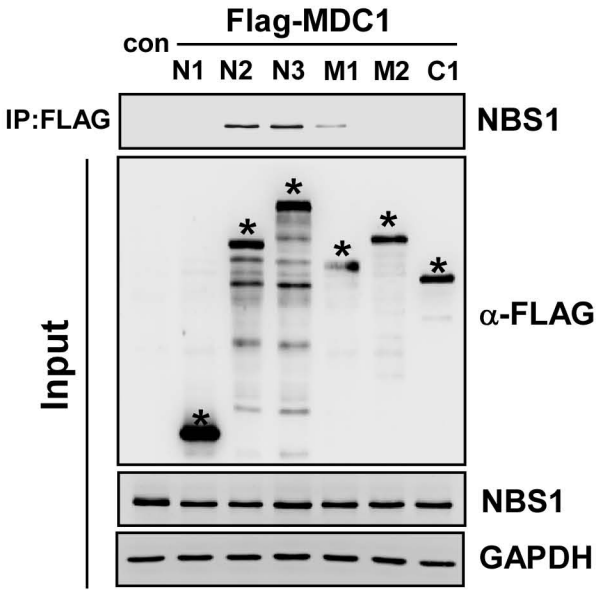

Figure S4

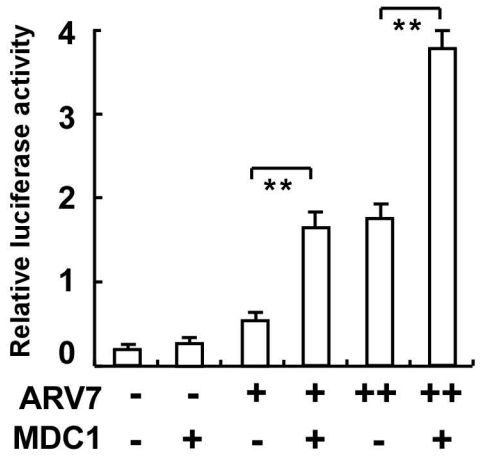

Figure S6

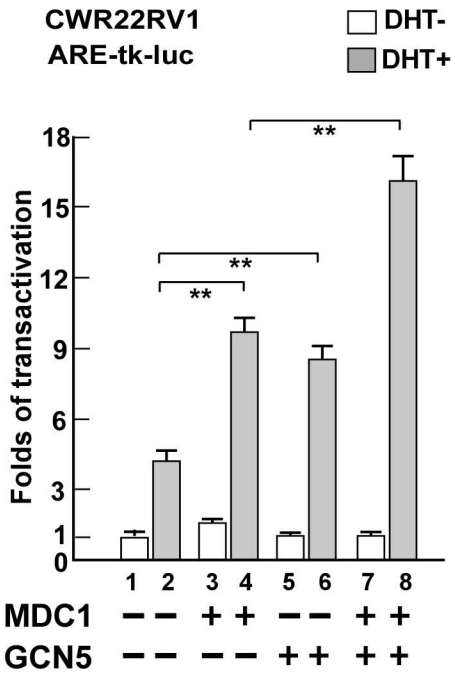

Figure S5

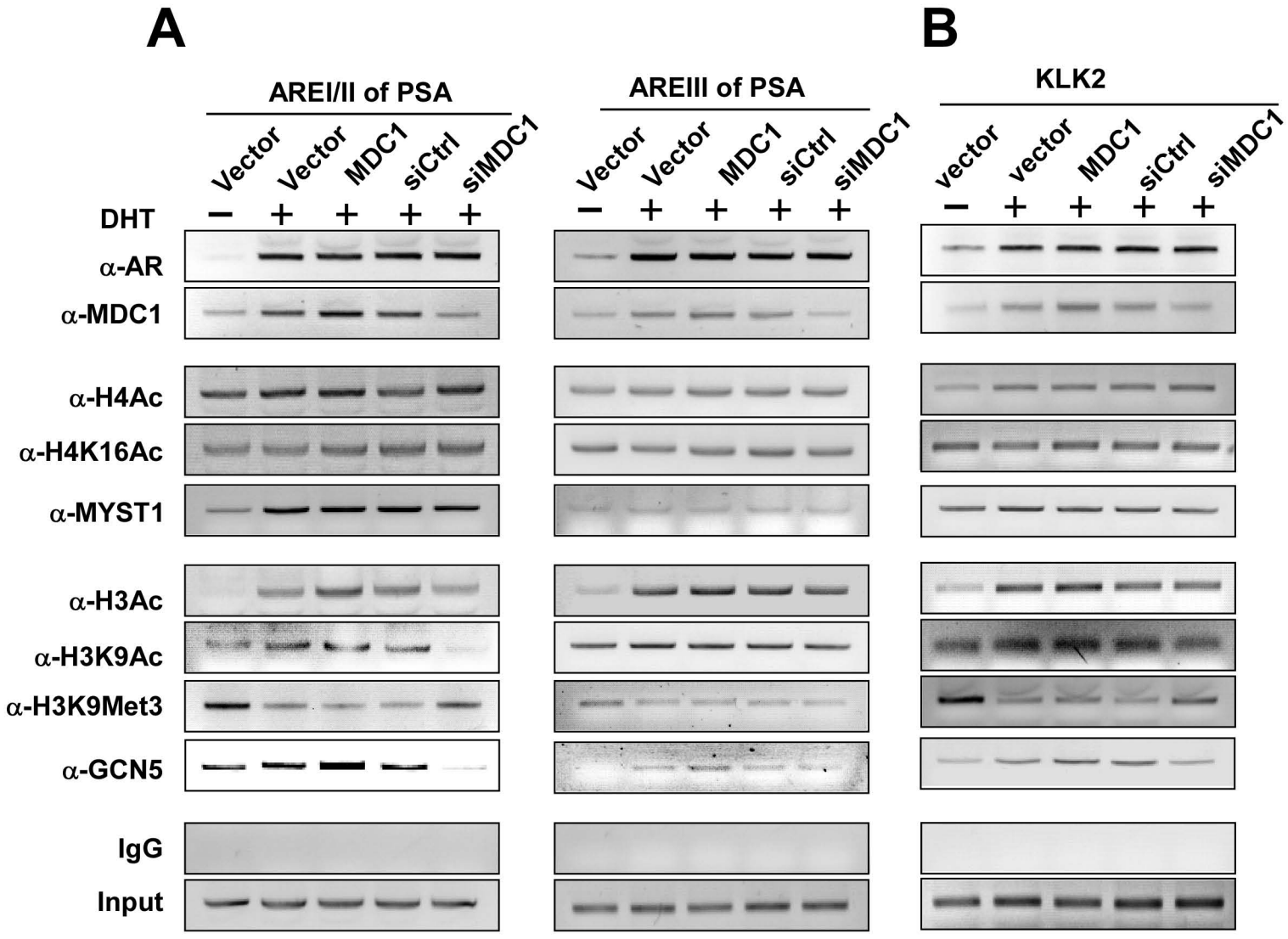

Figure S7

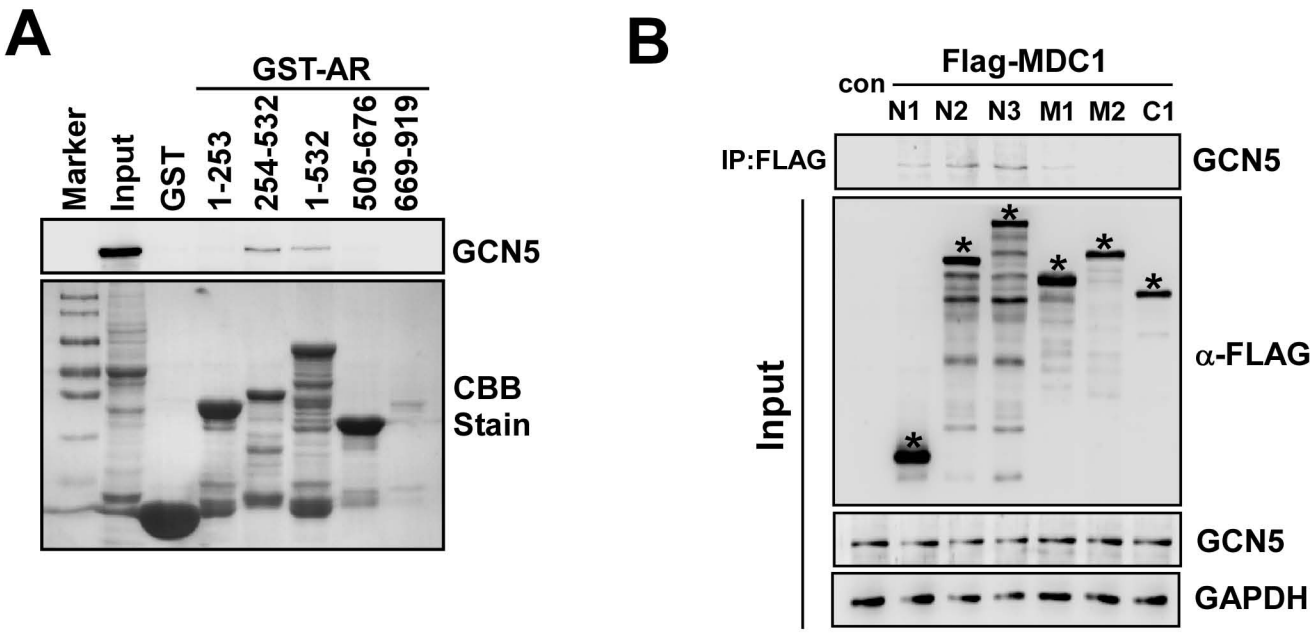

Figure S8

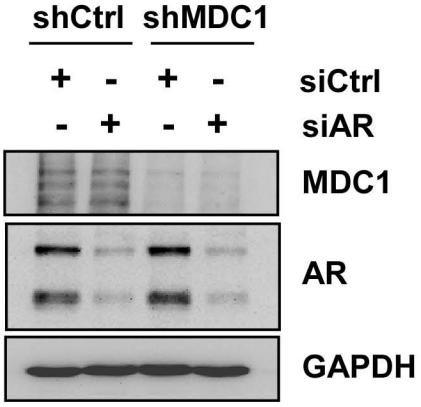

Figure S9