Supplementary Information

Figure legends

Supplementary Figure S1

a, b miRNA-mediated PRK1 knockdown (**a**) or the PRK1 inhibitor Ro318220 (**b**) does not reduce expression of the *HPRT*, *PS2*, or *GAPDH* genes. LNCaP cells were cultivated in the presence or absence of the AR agonist R1881. For quantitative RT-PCR, the following primers were used: *HPRT* 5'-CCTGGCGTCGTGATTAGTGAT-3' and 5'-AGACGTTCAGTCCTGTCCATAA-3'; *PS2* 5'-CTGGCTACCCAGGGGAGGAG-3' and 5'-GTGATGGAGATGGGGAGGGC-3'. Primers for *GAPDH* have been described previously[3]. **c, d** PRK1 controls AR-dependent gene expression. LNCaP cells were cultivated in the presence or absence of the AR agonist R1881. miRNA-mediated PRK1 knockdown (**c**) or the inhibitor Ro318220 (**d**) severely reduces AR-dependent reporter activity. Bars represent mean +SD ($n \geq 3$). **e** PRK1 interacts neither with a region between the enhancer and promoter nor with exon 4 of the *PSA* gene. Cells were cultivated in the presence or absence of the AR agonist R1881 for 45 minutes. ChIP analyses were performed with α-PRK1 antibody. The precipitated chromatin was amplified by PCR using primers flanking a region between the enhancer and promoter of the *PSA* gene or exon 4 of the *PSA* gene[3]. **f** R1881 does not alter AR or PRK1 protein levels. LNCaP cells were cultivated in the presence or absence of 1×10^{-8} M R1881 for 210 minutes. Western blots were decorated with the indicated antibodies. LSD1 was used as a control. **g, h** In 293 cell lysates, the presence of PRK1 proteins used for the kinase assay was verified by Western blot analysis using α-myc (**g**) or α-flag (**h**) antibody. LSD1 was used as loading control.

Supplementary Figure S2

a, b, c, d The α-H3T11ph antibodies used for Western blot analysis or ChIP assays specifically recognize H3T11ph. The indicated amounts of peptides were spotted onto nitrocellulose (Protran BA 79, Schleicher & Schuell). H3 (**a**, **b**, **c**, **d**), H3K9me1T11ph (**d**), H3K9me2T11ph (**d**), and H3K9me3T11ph (**d**) peptides were obtained from Peptides & Elephants. H3S10ph (**a**, **b**), H3T11ph (**a**, **b**, **c**, **d**), and H3T6ph (**c**) peptides were obtained from Abcam. Western blots were decorated as indicated. Controls show the amounts of Ponceau red stained peptides (**a**, **b**, **c**, **d**, lower panels). **e** R1881 specifically induces phosphorylation of H3T11, which is blocked by Ro318220. LNCaP cells cultivated in 10% double-stripped FCS for 72 hours were pre-treated for one hour with or without 1 x 10^{-5} M Ro318220 followed by incubation for 30 minutes in presence or absence of 1 x 10^{-8} M R1881. Core histones isolated from LNCaP cells were analysed in Western blots with the indicated antibodies. **f, g** R1881 does not induce phosphorylation of H3T11 at promoters of non AR-regulated genes. Cells were cultivated in the presence or absence of the AR agonist R1881 and the inhibitor Ro318220 (**g**), or transfected with siRNA (**f**). ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter regions of the *GAPDH* and *HSP70* genes[5]. **h** The specificity of α-H3K9me3/2/1 antibodies is not altered by phosphorylation of H3T11. Equal amounts of either untreated nucleosomes (lane1) or nucleosomes phosphorylated at H3T11 *in vitro* (lane 2) were used for Western blot analysis. Western blots were decorated with the indicated antibodies.

Supplementary Figure S3

a, b PRK1 knockdown by RNAi or inhibition by Ro318220 does not alter H3K9me3/2/1 and H3K9/K14ac levels at non AR-regulated control promoters. Cells were cultivated in the presence or absence of the AR agonist R1881 and the inhibitor Ro318220 (**b**) or transfected

with siRNA (**a**). ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter regions of the *GAPDH* and *HSP70* genes[5]. **c-h** Quantitative PCR analysis of ChIP demonstrate that R1881-induced demethylation of H3K9me3 (**c, d**), H3K9me2 (**e**, **f**), and H3K9me1 (**g**, **h**) is significantly reduced by PRK1 knockdown (**c**, **d**, **e**, **f**, **g**, **h**). Cells were transfected with the indicated siRNA and cultivated in the presence or absence of the AR agonist R1881. ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter regions of the *PSA* (5'-CCCCTGTTTCTGTTTCATCC-3' and 5'-CCCTTGTAGCTCATGGAGACTT-3') and *KLK2* (5'-CCTGTTGCTGTTCATCCTGA-3' and 5'-CCTATGGATCATGGAGATGTGA-3') genes. The amounts of immunoprecipitated chromatin obtained from cells cultivated in absence of R1881 were set to 100%. **i-n** Quantitative PCR analysis of ChIP demonstrate that R1881-induced demethylation of H3K9me3 (**i, j**), H3K9me2 (**k**, **l**), and H3K9me1 (**m**, **n**) is blocked by the PRK1 inhibitor Ro318220 (**i**, **j**, **k**, **l**, **m**, **n**). Cells were cultivated with or without the inhibitor Ro318220 in the presence or absence of the AR agonist R1881. ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter regions of the *PSA* (5'-CCCCTGTTTCTGTTTCATCC-3' and 5'-CCCTTGTAGCTCATGGAGACTT-3') and *KLK2* (5'-CCTGTTGCTGTTCATCCTGA-3' and 5'-CCTATGGATCATGGAGATGTGA-3') genes. The amounts of immunoprecipitated chromatin obtained from cells cultivated in absence of R1881 were set to 100%.

Supplementary Figure S4

a, b, c AR, PRK1 K644E, JMJD2C, and LSD1 do not influence the transcriptional activity of the TK-LUC control reporter. CV1 (**a**, **c**) or 293 cells (**b**) were co-transfected with expression plasmids and the TK-LUC reporter in the presence or absence of R1881 and Ro318220, as

indicated. Bars represent mean +SD (n=4). **d, e** PRK1 depletion by RNAi or inhibition by Ro318220 does not affect the presence of S5-P CTD pol II at promoters of non AR-regulated control genes. Cells were cultivated in the presence or absence of the AR agonist R1881 and the inhibitor Ro318220 (**e**) or transfected with siRNA (**d**). ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter regions of the *GAPDH* and *HSP70* genes[5]. **f** PRK1 does not phosphorylate JMJD2C. Bacterially expressed GST, GST-H3 1-44, MBP, and MBP-JMJD2C were incubated for 5 minutes with recombinantly expressed and purified PRK1. Coomassie blue staining shows the amounts of GST and MBP fusion proteins, marked with an asterisk, that were used for the assay. **g** PRK1 does not phosphorylate LSD1. Bacterially expressed GST, GST-H3 1-44, and GST-LSD1 were incubated for 5 minutes with recombinantly expressed and purified PRK1. Coomassie blue staining shows the amounts of GST fusion proteins, marked with an asterisk, that were used for the assay. Bars represent mean +SD (n=4).

Supplementary Figure S5

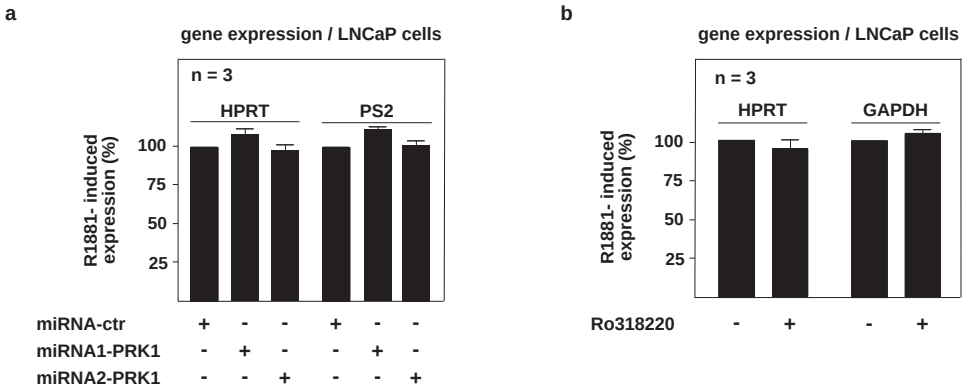
a PRK1 does not phosphorylate AR. Bacterially expressed GST, GST-AR-NTD, GST-AR-DBD, GST-AR-LBD and GST-H3 1-44 proteins were incubated for 5 minutes with recombinantly expressed and purified PRK1 in the presence or absence of 1 x 10^{-8} M R1881. Coomassie blue staining shows the amounts of GST fusion proteins, marked with an asterisk, that were used for the assay. **b** Inhibition of PRK1 by Ro318220 blocks ligand-induced recruitment of p300. LNCaP cells were cultivated in the presence or absence of the AR agonist R1881 and the inhibitor Ro318220 as indicated. ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking the promoter regions of the *PSA* and *KLK2* genes[3]. **c, d, e** Neither JMJD2C (**c**) nor LSD1 (**d**) nor TIF2 (**e**) knockdown affects R1881-induced H3T11 phosphorylation in the

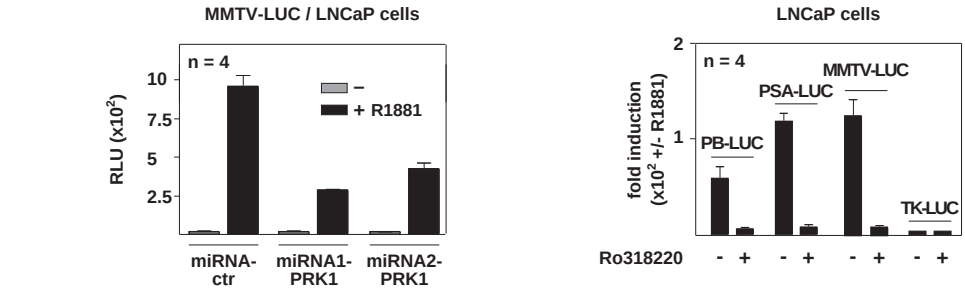
promoter region of the *PSA* and *KLK2* genes. For ChIP (**c**, **d**, **e**, left panel), LNCaP cells were transfected with siRNA and cultivated in the presence or absence of the AR agonist R1881 for 45 minutes as indicated. ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking AREs in the promoter region of the *PSA* and *KLK2* genes. Knockdown of JMJD2C (**c**, right panel), LSD1 (**d**, right panel) and TIF2 (**e**, right panel) is verified by Western blot analyses using the indicated antibodies. **f** The PRK1 inhibitor Ro318220 severely reduces R1881-induced cell proliferation. LNCaP cells cultivated in 10% double-stripped FCS were treated with 1 x 10^{-8} M R1881 or 3 x 10^{-6} M Ro318220 as indicated. **g, h** Dlk/ZIP does not influence the transcriptional activity of AR. 293 cells (**g**) were co-transfected with AR, LSD1, or Dlk/ZIP expression plasmids and the MMTV-LUC reporter in the presence or absence of R1881. LNCaP cells (**h**) were co-transfected Dlk/ZIP expression plasmids and the MMTV-LUC reporter in the presence or absence of R1881. Bars represent mean +SD ($n \geq 4$).

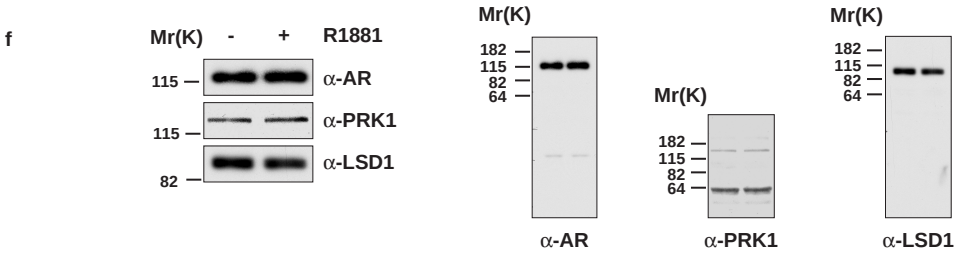
Supplementary Figure S6

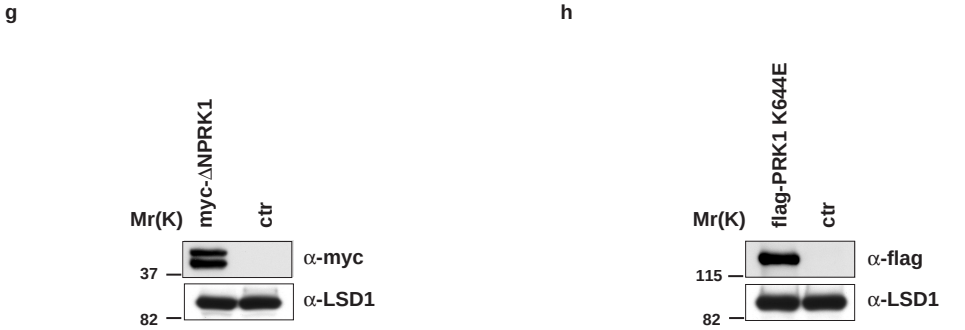
Fulls scans of gel/western/kinase assay data for Figure 1a, Figure 2a-d, Figure 3i, Supplementary Information Figure S1g, h, Supplementary Information Figure S2e, and Supplementary Information Figure S5c-e.

Schuele_Supplementary Figure S1









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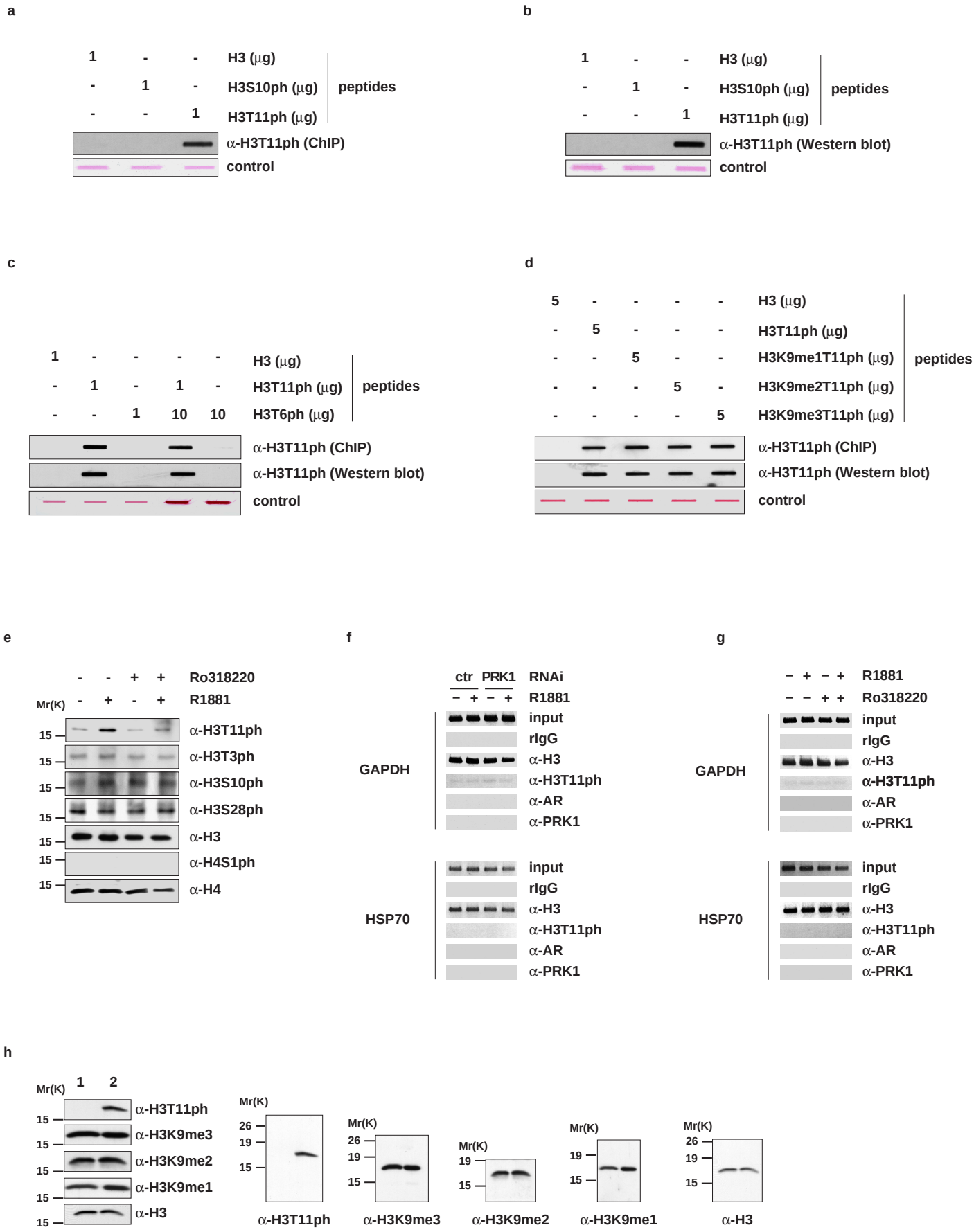

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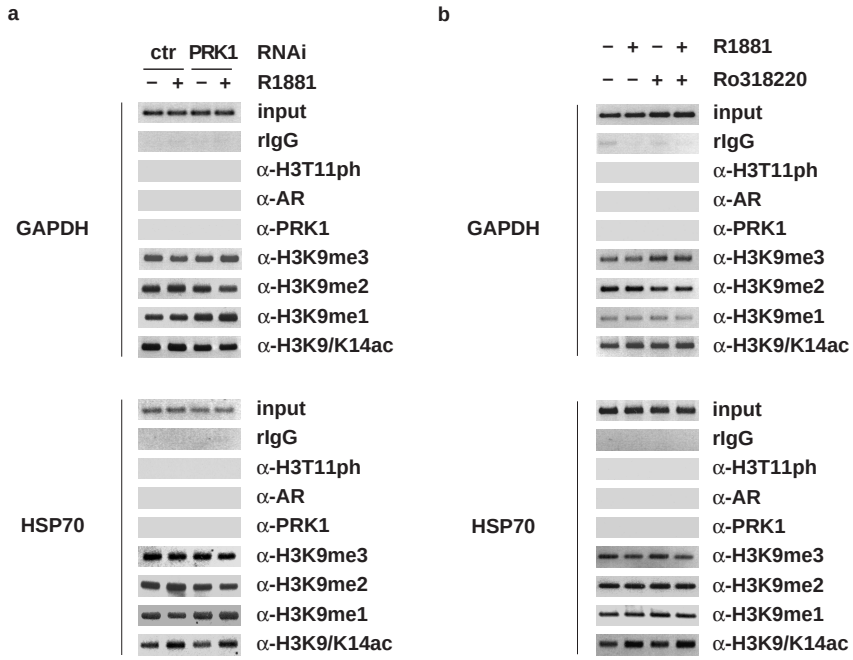

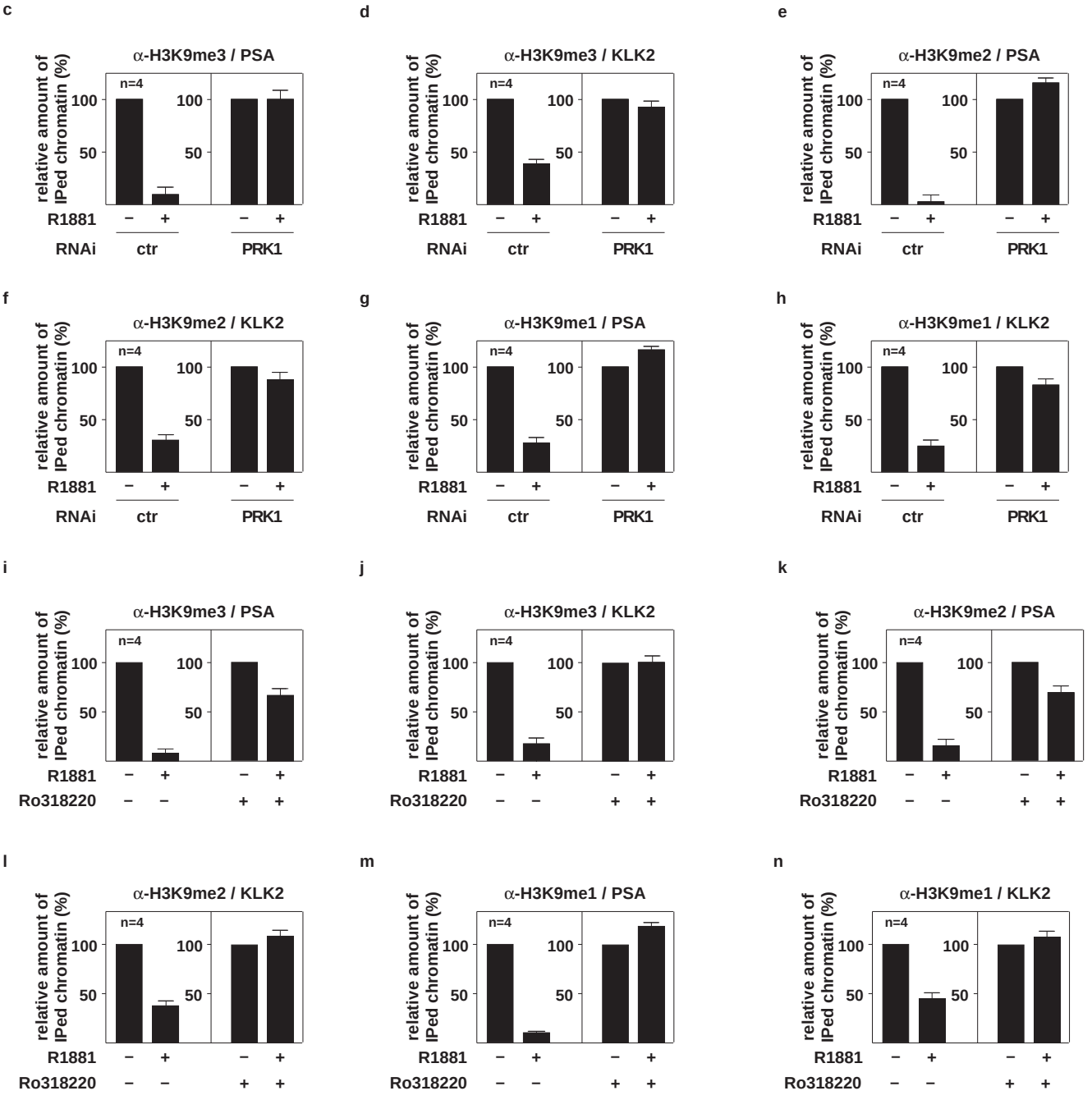

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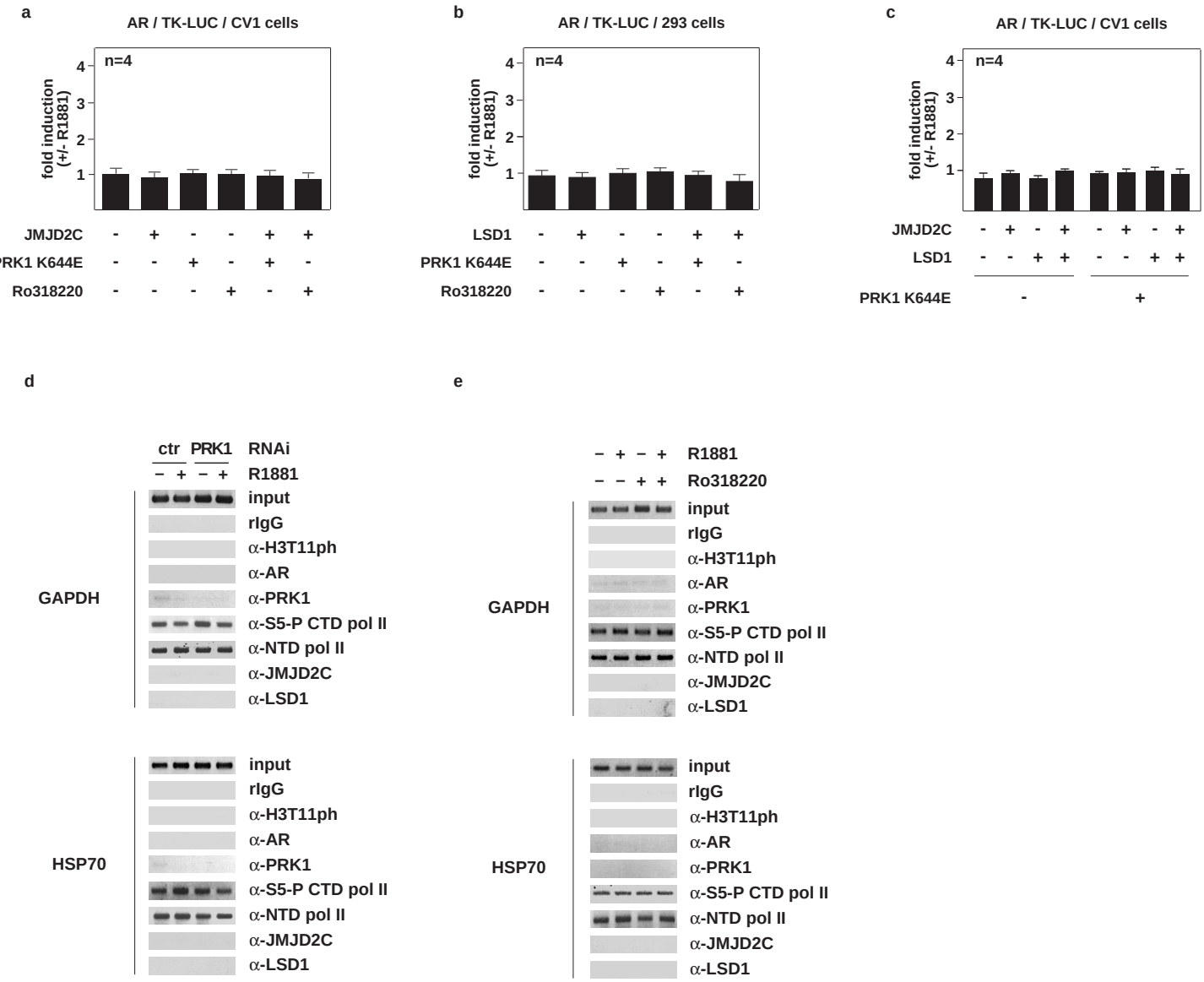

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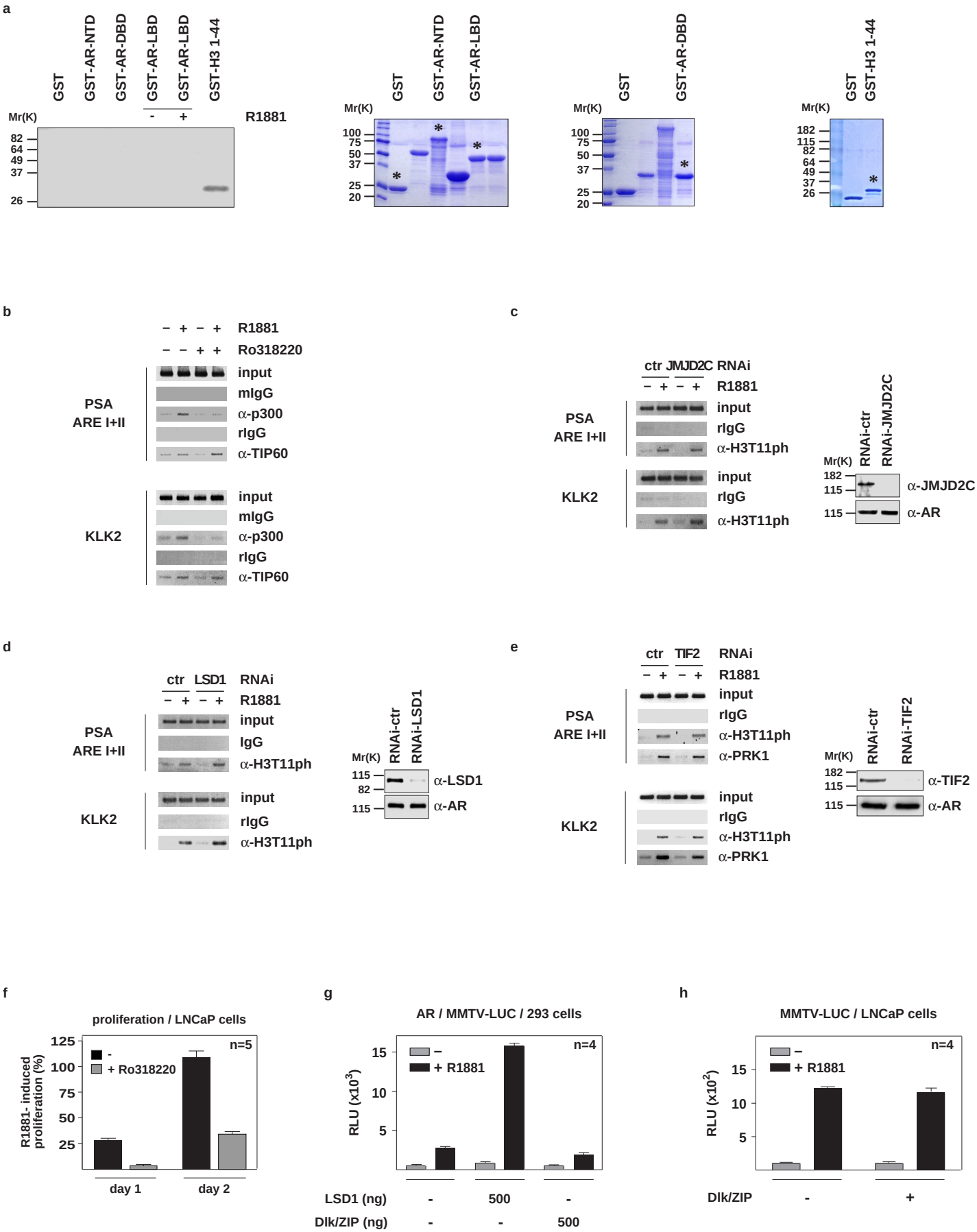


Schüle_Supplementary Figure S6

Figure 1a

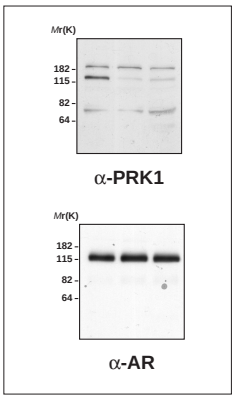
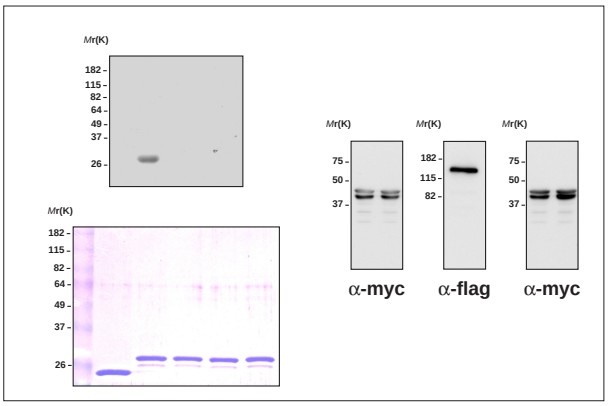


Figure 2a



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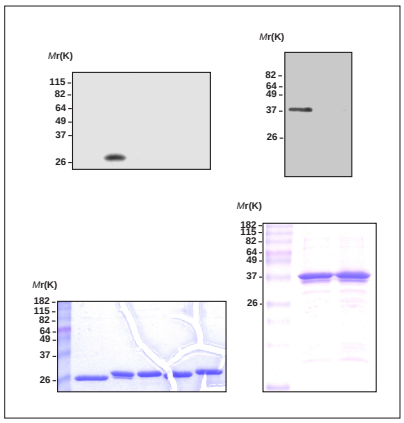

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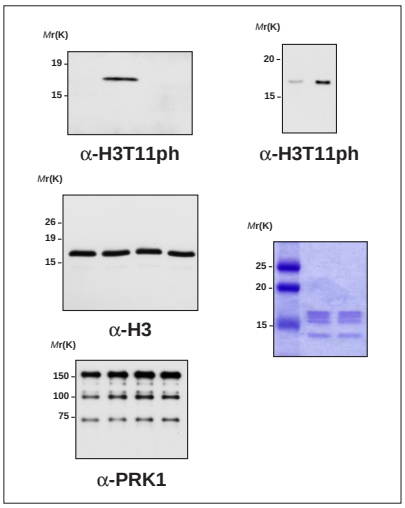


Figure 2d

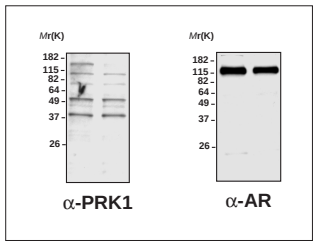
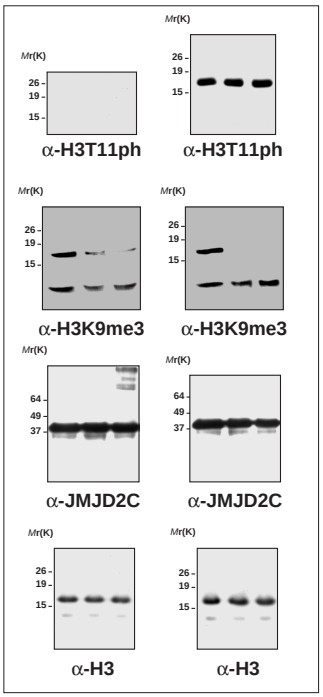
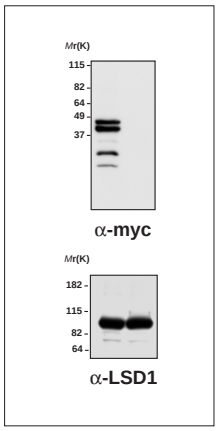


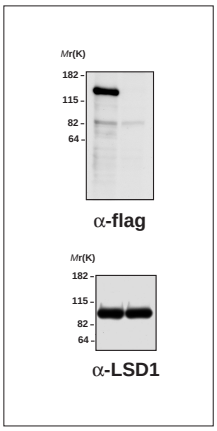
Figure 3i



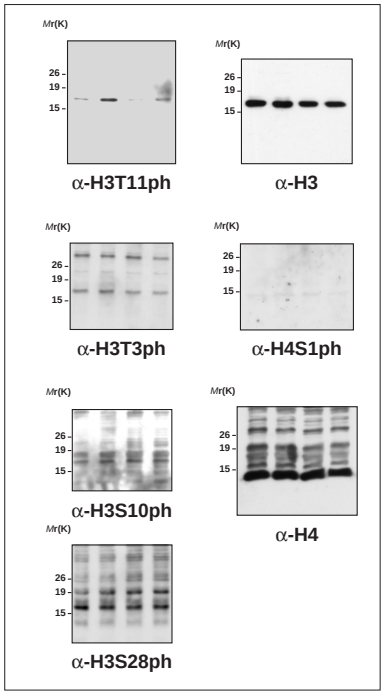
SI Figure S1g



SI Figure S1h



SI Figure S2e



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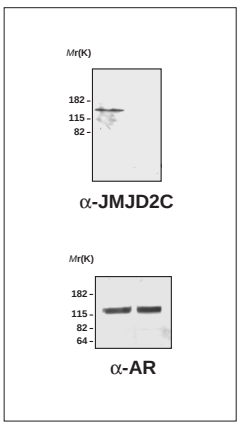

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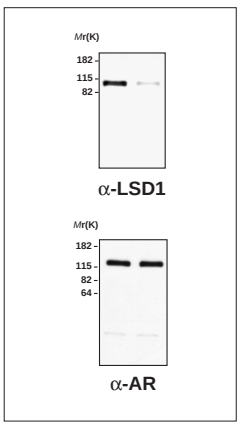

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