Supporting Information

Evidence that HDAC7 Acts as an Epigenetic "Reader" of AR Acetylation through NCoR-HDAC3 Dissociation

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A.

Name*	Sequence
HDAC4-(H976Y)-F	5’-ctcgagggaggctacgacctgaccg-3’
HDAC4-(H976Y)-R	3’-gagctccctccgatgctggactggc-5’
HDAC5-(H1006Y)-F	5’-gcggtcaagtcatagcctccctccagg-3'
HDAC5-(H1006Y)-R	5’-cctggagggaggctatgacttgaccgc-3'
HDAC7-(H842Y)-F	5'-ccttggagggtggctatgacctcacagcc-3'
HDAC7-(H842Y)-R	5’-ggctgtgaggtcatagccaccctccaagg-3'
HDAC9-(H956Y)-F	5’-tggctctagaaggaggatatgatctcacagccatc-3’
HDAC9-(H956)-R	5’-gatggctgtgagatcatatcctccttctagagcca-3'

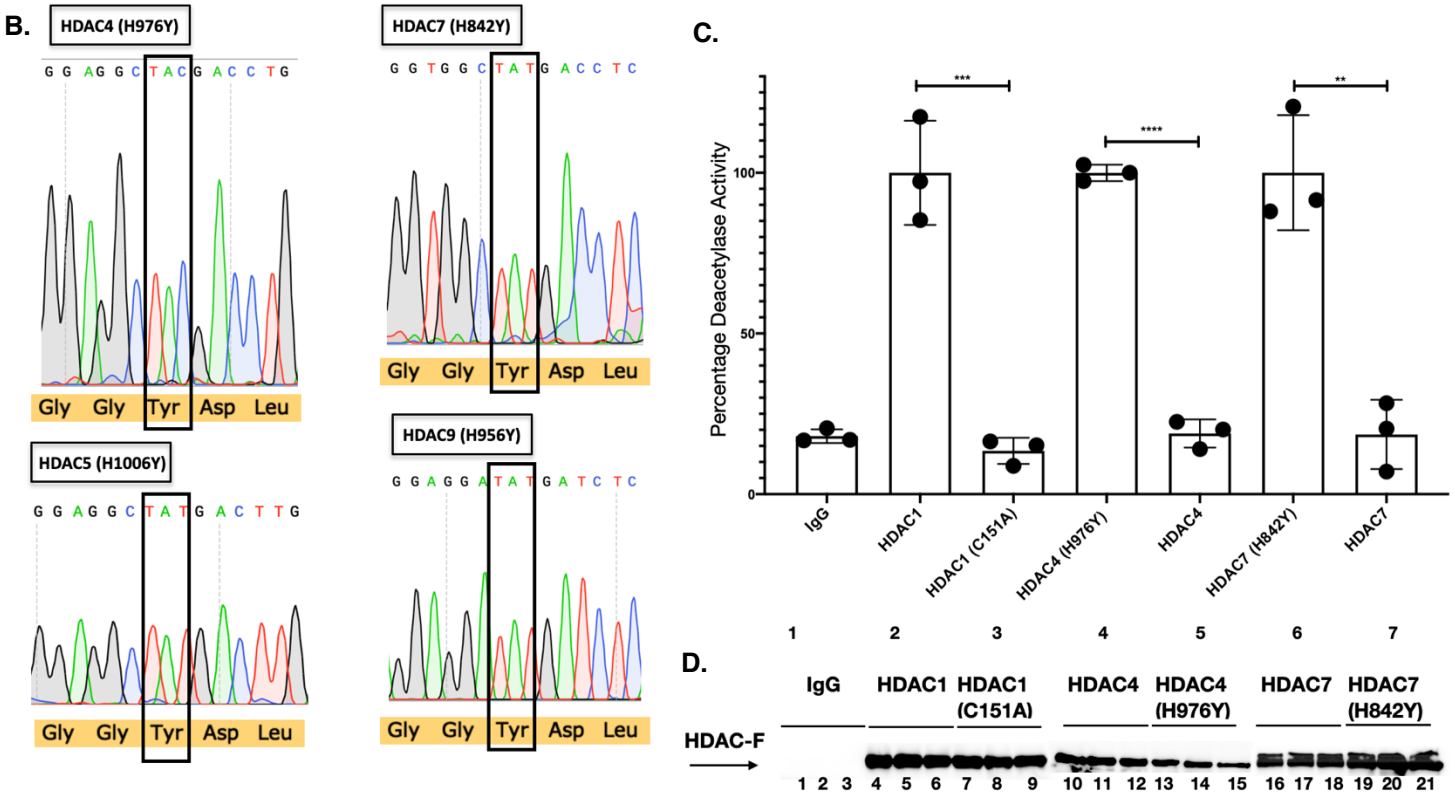


Figure S1. Deacetylase activity assay of HDAC1, HDAC4 and HDAC7, related to Figures 1. (A) Primers used for Mutagenesis. **(**B) DNA sequencing after mutagenesis showed successful restoration of the critical Tyr residue in all class IIa HDAC proteins to create a gain-of-function (GOF) mutants. **(**C) WT or C151A HDAC1 (as a positive control), WT or H976Y HDAC4, and WT or H842Y HDAC7 were expressed as Flag-tagged proteins in HEK293 cells. After 48 hr of cell growth, HDAC1, HDAC4 and HDAC7 were immunoprecipitated via the Flag tag from the lysate. HDAC deacetylase activity assay was carried out using the HDAC-Glo^{TM} Assay (Promega), monitoring luciferase induced light signal according to manufacturer procedure to generate the histogram. The light signal from each sample was normalized as the percentage of the activity of the active form of each individual HDAC protein (wild type HDAC1 or GOF HDAC4 and HDAC7, set as 100%). The IgG sample was normalized to GOF HDAC4. The results from three independent trials are shown with mean and standard deviation. ** = $p < 0.01$, ***= $p < 0.001$, and ***= $p < 0.0001$. (D) Bound proteins after immunoprecipitation were eluted and analyzed by western blot for Flag levels to assure equal amounts in the assay. Immunoprecipitation data from the three independent trials used in the HDAC-Glo^{TM} assay are shown.

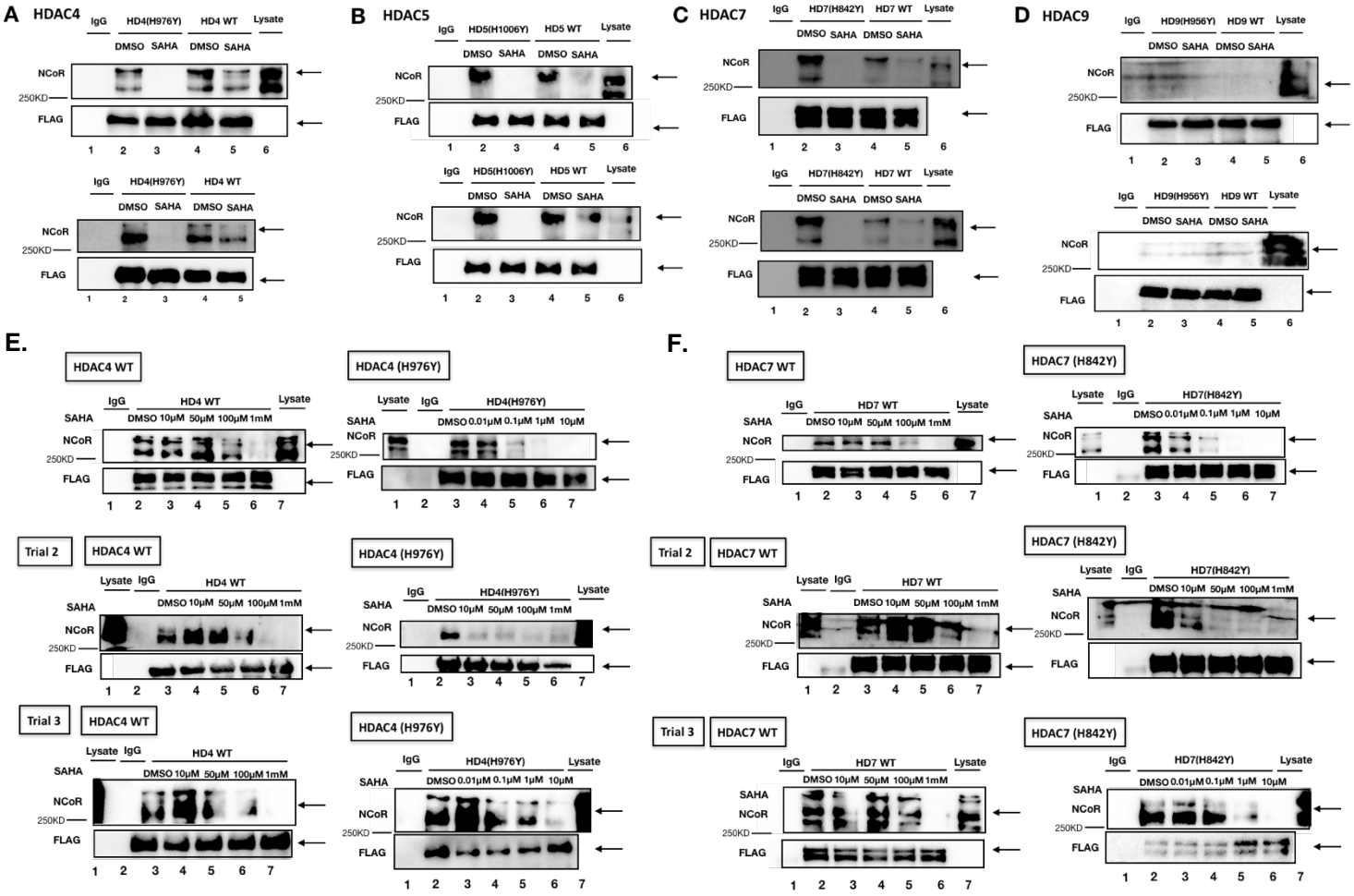
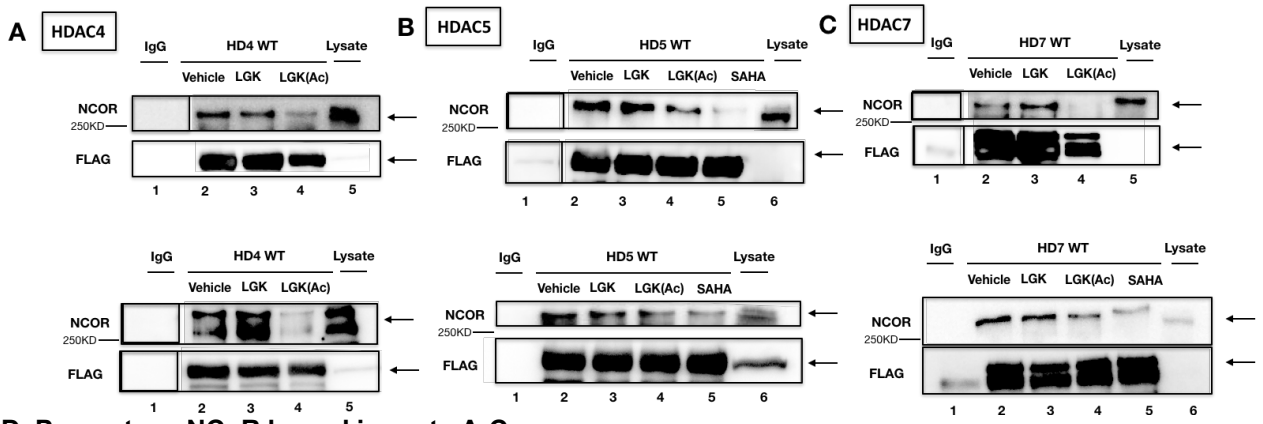
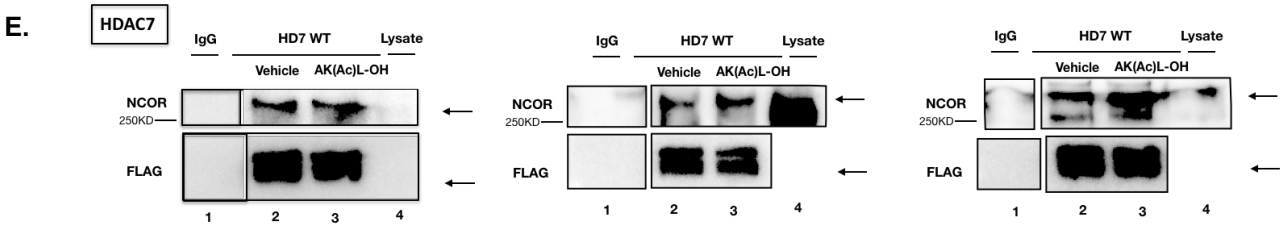


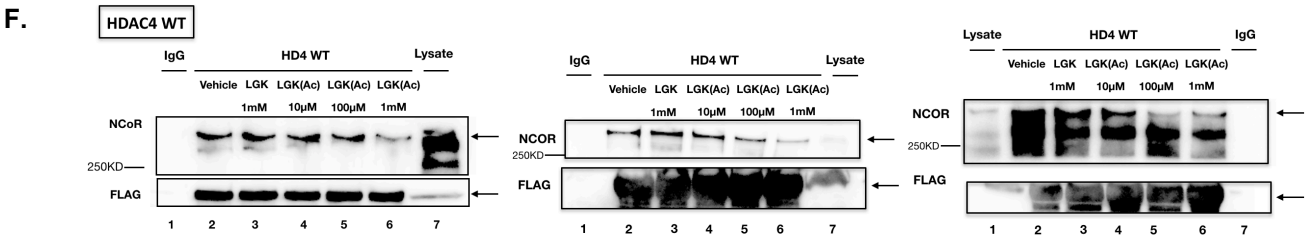
Figure S2. Repetitive trials of SAHA-dependent NCOR-Class IIa HDAC binding, related to Figure 1. Wild type (WT) and GOF mutants of HDAC4 (A and E), HDAC5 (B), HDAC7 (C and F) and HDAC9 (D) were expressed as Flag-tagged proteins in HEK293 cells. After 24h, cells were treated with SAHA (10 μM) for another 24 h to induce robust acetylation. After lysis, WT and GOF mutants of HDAC4, HDAC5, HDAC7 and HDAC9 were immunoprecipitated via the Flag beads in the absence (2% DMSO) or presence of SAHA (100 μM or concentration shown in 2% DMSO) and bound proteins were eluted. The eluate was separated by SDS-PAGE and subjected to western blotting with NCoR and FLAG antibodies. In parts A-D, two independent trials are shown here for each HDAC isoform, with the third trial for each isoform shown in Figure 1. For parts E-F, three independent trials are shown for each wild type and GOF mutant. The arrows indicate either NCoR or HDAC-Flag bands.



D. Percentage NCoR bound in parts A-C

Isoform	Treatment	Trial 1	Trial 2	Trial 3	Mean	Standard Error
HDAC4	Untreated	100	100	100	100	0
	LGK	90	96	105	97	4
	LGK(Ac)	15	15	7	12.	3
HDAC5	Untreated	100	100	100	100	0
	LGK	121	105	68	98	16
	LGK(Ac)	10	12	20	14	3
HDAC7	Untreated	100	100	100	100	0
	LGK	111	140	105	119	11
	LGK(Ac)	15	6	20	14	4





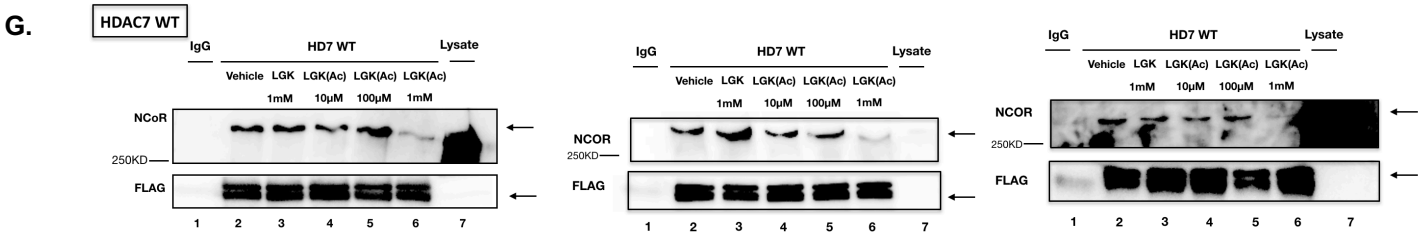
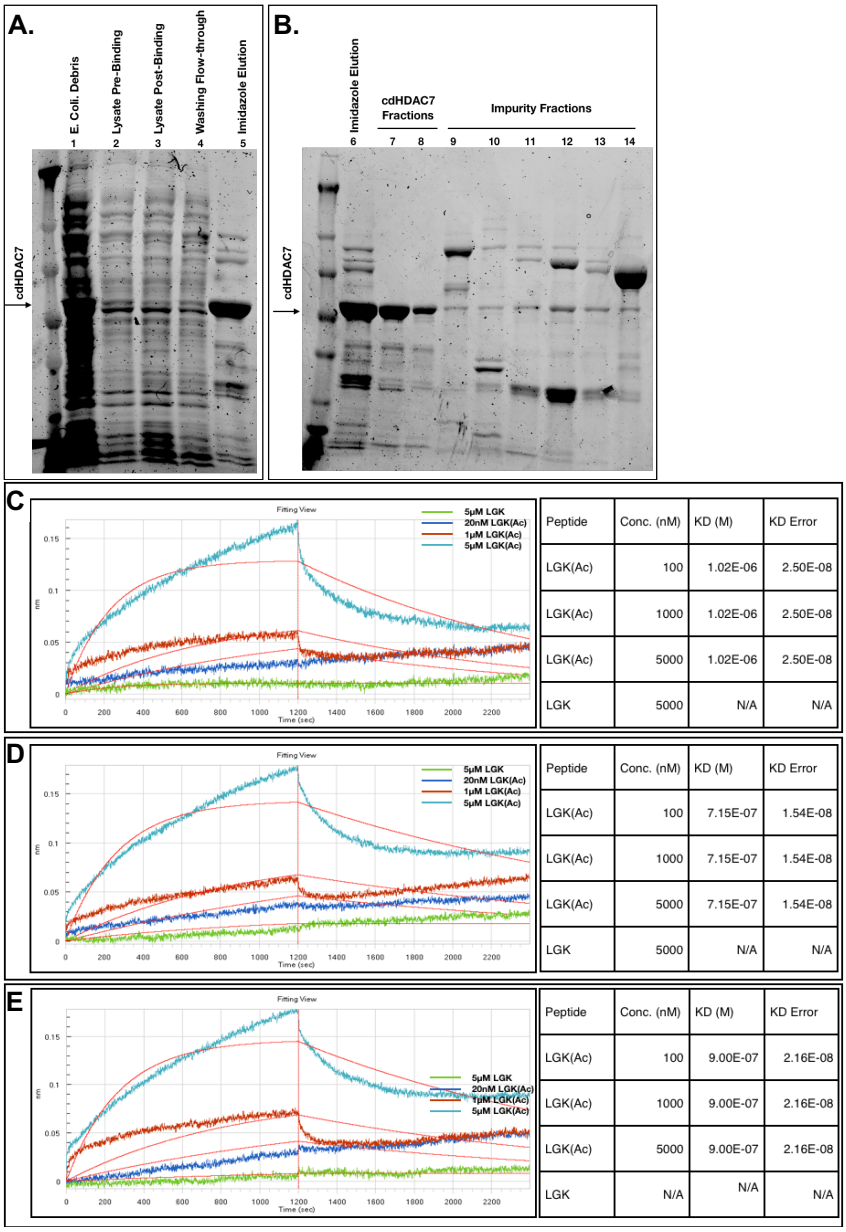


Figure S3. Repetitive trials of acetyllysine-dependent NCOR-Class IIa HDAC binding, related to Figure 2. WT HDAC4 (A and F), HDAC5 (B) or HDAC7 (C, D, and G) were expressed as Flag-tagged proteins in HEK293 cells, and after 24h, cells were treated with SAHA (10 μM) for another 24h. WT HDAC4, HDAC5 or HDAC7 were immunoprecipitated in the absence (vehicle, H_2O) or presence of LGK, LGKAc, SAHA, or AK(Ac)L-OH (1 mM or concentration indicated). Bound proteins were eluted, separated by SDS-PAGE, and subjected to western blotting using NCoR and Flag antibodies. The arrows indicate either NCoR or HDAC-Flag bands. For parts A-C, two independent trials are shown, with the third trial shown in Figure 2A-C. (D) The band intensities from the NCoR in parts A-C of this Figure and Figure 2A-C of the manuscript were quantified and normalized to the untreated (vehicle, H_2O) control to calculate percentage mean and standard error values, which are reported as a histogram in Figure 2D. For parts F-G, three independent trials are shown.

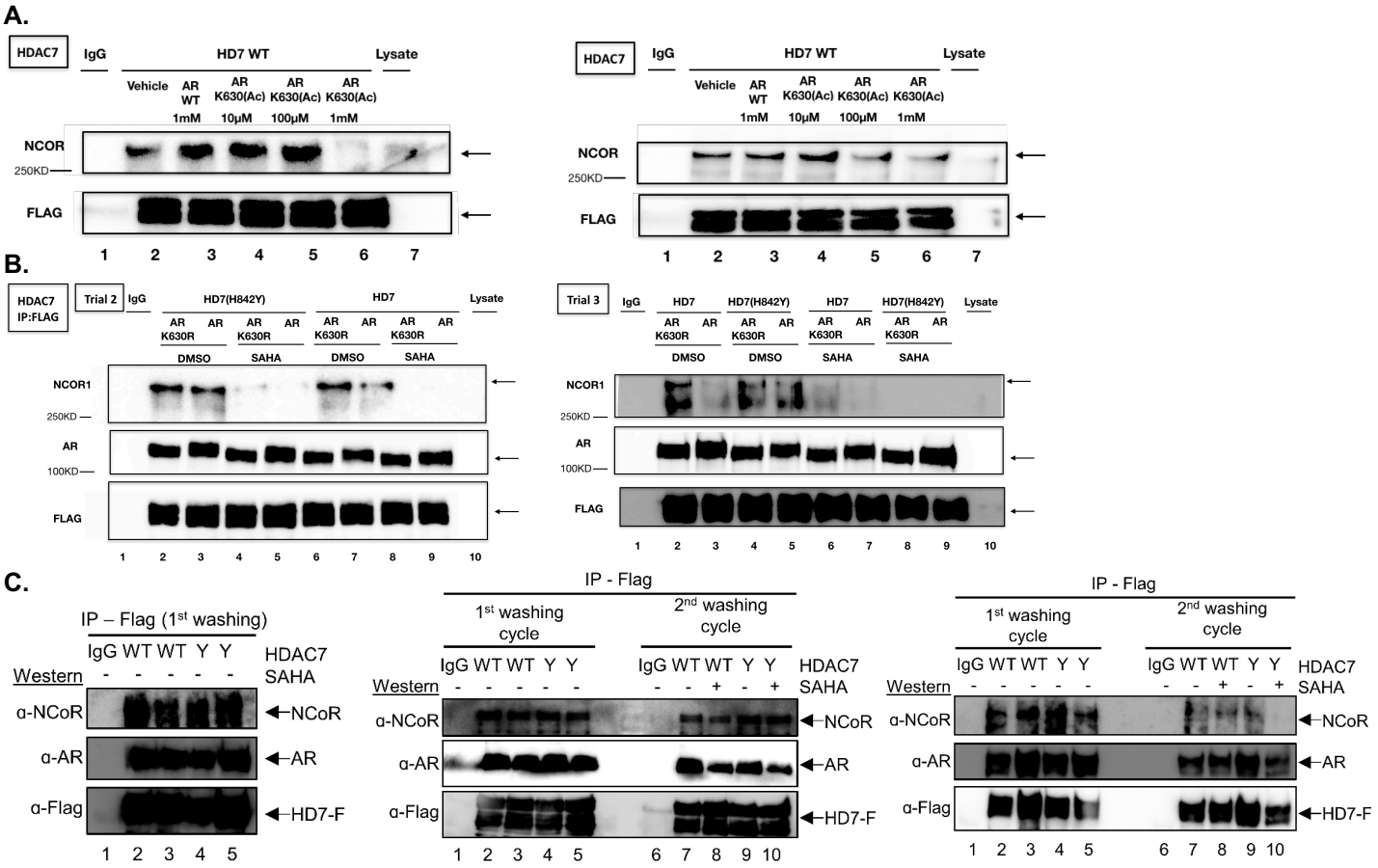


Peptide	Conc. (nM)	KD (M)	KD Error
LGK(Ac)	100	1.02E-06	2.50E-08
LGK(Ac)	1000	1.02E-06	2.50E-08
LGK(Ac)	5000	1.02E-06	2.50E-08
LGK	5000	N/A	N/A

Peptide	Conc. (nM)	KD (M)	KD Error
LGK(Ac)	100	7.15E-07	1.54E-08
LGK(Ac)	1000	7.15E-07	1.54E-08
LGK(Ac)	5000	7.15E-07	1.54E-08
LGK	5000	N/A	N/A

Peptide	Conc. (nM)	KD (M)	KD Error
LGK(Ac)	100	9.00E-07	2.16E-08
LGK(Ac)	1000	9.00E-07	2.16E-08
LGK(Ac)	5000	9.00E-07	2.16E-08
LGK	N/A	N/A	N/A

Figure S4. Binding Assessment of the cdHDAC7-LGK(Ac) interaction, related to Figure 2. (A-B) The pET28a-LIC-cdHDAC7-6XHis expression plasmid was transformed into bacteria, followed by induction of protein expression. Cell pellet before lysis (lane 1), and the soluble fraction of the lysate containing expressed cdHDAC7-6XHis (lane 2) was subjected to Ni-NTA column purification, with the unbound proteins in the lysate (lane 3), the washing (lane 4), and the eluted proteins (lane 5) shown in part A. The Ni-NTA purified cdHDAC7 protein (lane 6) was further purified using a HiTrap Q HP anion exchange column, with the two fractions containing purified cdHDAC7 (which were later combined, lanes 7 and 8) and the six fractions containing impurities (lanes 9-14) shown in part B. (C-E) Purified cdHDAC7 protein was biotinylated, loaded onto super streptavidin coated sensors, and subjected to binding to LGK(Ac) or LGK peptides using Bio-Layer Interferometry (BLI). Three repetitive trials are shown. Tables on right side for each trial show analyzed binding data by curve fitting to obtain binding affinities (dissociation contanst, K_D) in Molar units.



D.

Sample	Trial 1	Trial 2	Trial 3	Mean	Standard Error	p value
WT	100%	100%	100%	100%	-	
WT + SAHA	65%	89%	26%	60%	19%	0.0470
H842Y	100%	100%	100%	100%	-	
H842Y + SAHA	13%	65%	19%	32%	16%	0.0070

Figure S5. Repetitive trials of HDAC7-NCoR complex disruption by AR, related to Figure 3. (A) WT HDAC7 was expressed as Flag-tagged proteins in HEK293 cells. After 24h, cells were treated with SAHA (10 μM) for another 24h. WT HDAC7 were immunoprecipitated in the presence of AR K630 WT peptide or different concentration of AR K630(Ac) peptides. Bound proteins were eluted, separated by SDS-PAGE and subjected to western blotting using NCoR and Flag antibodies. Two independent trials are shown here with the third trial shown in Figure 3A. (B) Flag-tagged wild type (WT) or GOF mutant of HDAC7 were co-expressed with AR WT or AR K630R in HEK293 cells. After 24h, cells were treated with SAHA for another 24h to induce robust acetylation. WT and GOF mutant of HDAC7 were immunoprecipitated via their Flag tags in the absence or presence of SAHA (100 μM). Bound proteins were eluted, separated by SDS-PAGE, and subjected to western blot analysis using NCoR, AR, or Flag antibodies. Two independent trials are shown here with the third trial shown in Figure 3B. (C) Flag-tagged wild type (WT) or GOF mutant (Y) HDAC7 were co-expressed with AR WT in HEK293 cells. After 24 hr recovery, cells were treated with SAHA (10 μM) for another 24 hours, followed by lysis and immunoprecipitation (IP) via Flag. Beads after IP were first washed three times without inhibitor treatment (1st washing cycle), and then another three times (2nd washing cycle) with SAHA (100 μM in 2% DMSO) or DMSO carrier. All trials include a bead binding control using lysates without expression of HDAC7-Flag (IgG). Two independent trials are shown here, with the 2nd washing cycle of the third trial shown in Figure 3C. (D) The NCoR proteins levels after the second washing from three independent trials (Figure 3C and part C) were quantified, and SAHA treated samples were normalized to samples without SAHA (set to 100%). Raw percentage values of each individual trial and mean and standard error are shown. A histogram with this data is shown in in Figure 3D of the main manuscript.

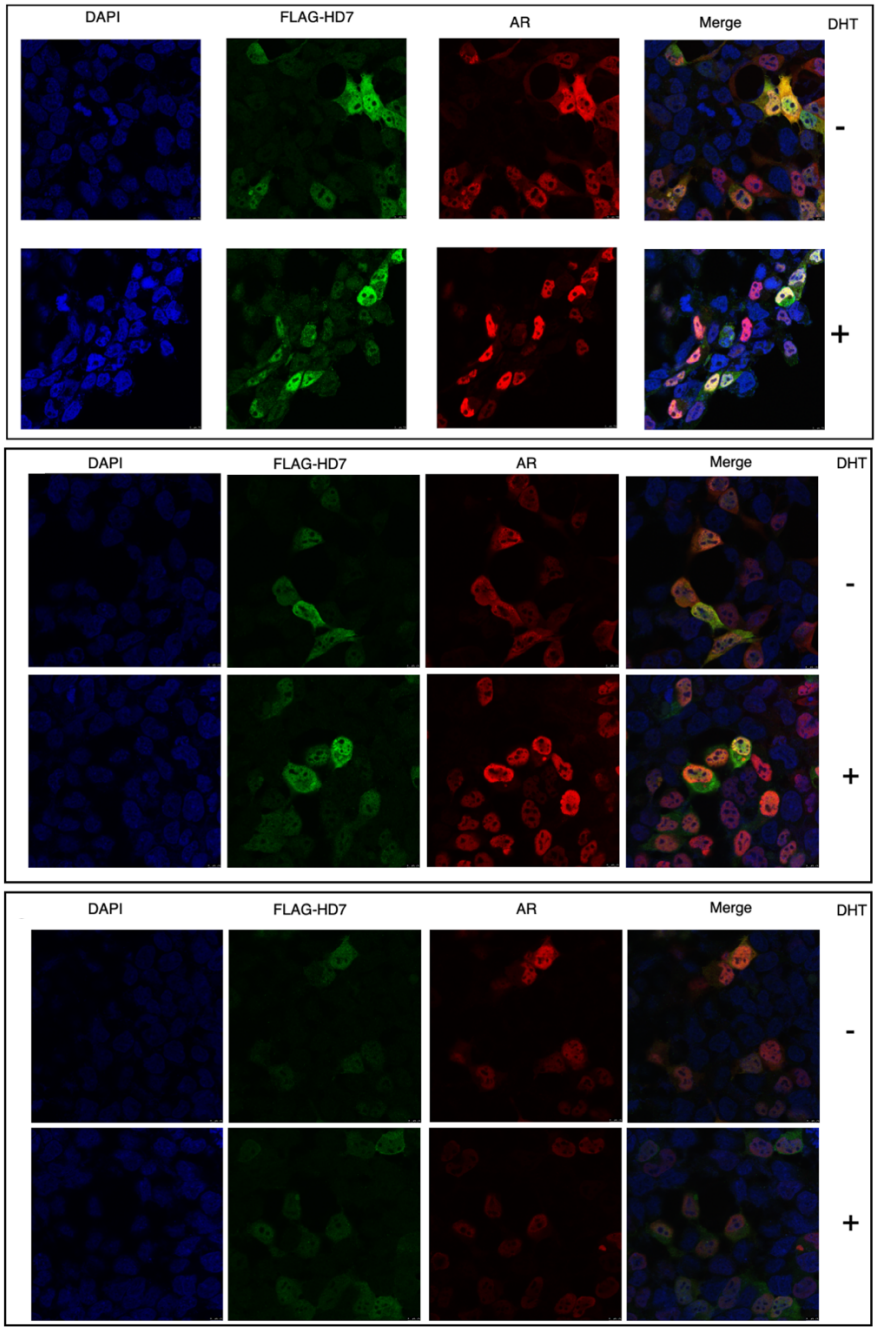


Figure S6. AR and HDAC7 colocalization, related to Figure 3. WT AR and Flag-tagged HDAC7 were overexpressed in HEK293 cells and incubated with DHT ligand (1 nM in 0.1% v/v of DMSO) or 0.1% v/v of DMSO carrier. After DHT or DMSO incubation, cells were fixed and immunostained using anti-Flag and anti-AR antibodies, counterstained with DAPI, and visualized using a fluorescence confocal microscope. Three independent trials are shown here.

A.

	AR WT						AR					
			HDAC7		HDAC9	HDAC4			HDAC7		HDAC9	HDAC4
	No DHT	DHT	WT	GOF	GOF	WT	No DHT	DHT	WT	GOF	GOF	WT
Trial 1	10%	100%	101%	58%	99%	46%	11%	9.8%	8.9%	7.7%	8.4%	3.7%
Trial 2	11%	100%	98%	55%	88%	44%	10%	9.7%	7.6%	7.4%	7.3%	4.1%
Trial 3	9.9%	100%	94%	53%	91%	40%	9.8%	9.7%	7.7%	7.0%	7.7%	3.6%
Mean	10%	100%	98%	56%	93%	43%	10%	9.7%	8.1%	7.4%	7.8%	3.8%
Stnd Err	0.3%	2%	2%	2%	3%	2%	0.4%	0.1%	0.4%	0.2%	0.3%	0.2%

B.

			HDAC4		HDAC5		HDAC7		HDAC9	
	No DHT	DHT	WT	GOF	WT	GOF	WT	GOF	WT	GOF
Trial 1	12%	100%	30%	39%	79%	101%	98%	69%	96%	99%
Trial 2	20%	100%	48%	20%	68%	85%	85%	50%	93%	88%
Trial 3	17%	100%	40%	32%	103%	99%	85%	58%	88%	83%
Mean	17%	100%	40%	31%	83%	95%	89%	59%	92%	90%
Stnd Err	2%	2%	5%	6%	11%	5%	4%	5%	2%	5%

C.

			HDAC7			
			WT	GOF	GOF	GOF
	No DHT	DHT	-	-	SHI	RGFP
Trial 1	20%	100	80%	53%	48%	106%
Trial 2	17%	100	90%	55%	47%	112%
Trial 3	11%	100	82%	52%	43%	104%
Mean	16%	100	84%	53%	46%	107%
Stnd Err	3%	3%	3%	1%	1%	2%

Figure S7. Quantified data from transcriptional gene reporter assays, related to Figure 4. Mean light intensity data were normalized as a percentage of the DHT sample (lane 2, set to 100%) from each of the three independent trials. Data is each part corresponds to the data reported in each histogram in each part of Figure 4.

A. Primers used in RT-PCR studies

Name*	Sequence
SFRP5-F	5'-cagatgtgctccagtgactttg-3'
SFRP5-R	5'-agaagaaagggtagtagagggag-3'
Wnt16-F	5'-ccaaggaaactggatgtggt-3'
Wnt16-R	5'-tcatgcagttccatctctcg-3'
GPDH-F	5'-ccatcaccatcttccaggagcg-3'
GPDH-R	5'-agagatgatgacccttttggc-3'

B. SPRF5

	nontreated	DHT	HDAC7 WT	HDAC7 GOF
Trial 1	37%	106.4%	73.3%	44.2%
Trial 2	26%	104.7%	71.1%	43.1%
Trial 3	26%	88.9%	65.1%	45.7%
Mean	29%	100%	70%	44%
Stnd Err	4%	6%	2%	1%

C. Wnt16

	nontreated	DHT	HDAC7 WT	HDAC7 GOF
Trial 1	20.2%	102.6%	97.0%	39.9%
Trial 2	21.7%	96.5%	85.9%	40.8%
Trial 3	20.6%	100.9%	82.6%	40.7%
Mean	21%	100%	86%	41%
Stnd Err	1%	2%	4%	1%

Figure S8. Primers used and quantified data from RT-PCR assays, related to Figure 5. (A) Primers used in RT-PCR studies. (B-C) Mean light intensity data were normalized as a percentage of the DHT sample (lane 2, set to 100%) from each of the three independent trials. Data is each part corresponds to the data reported in each histogram in each part of Figure 5B and 5C.

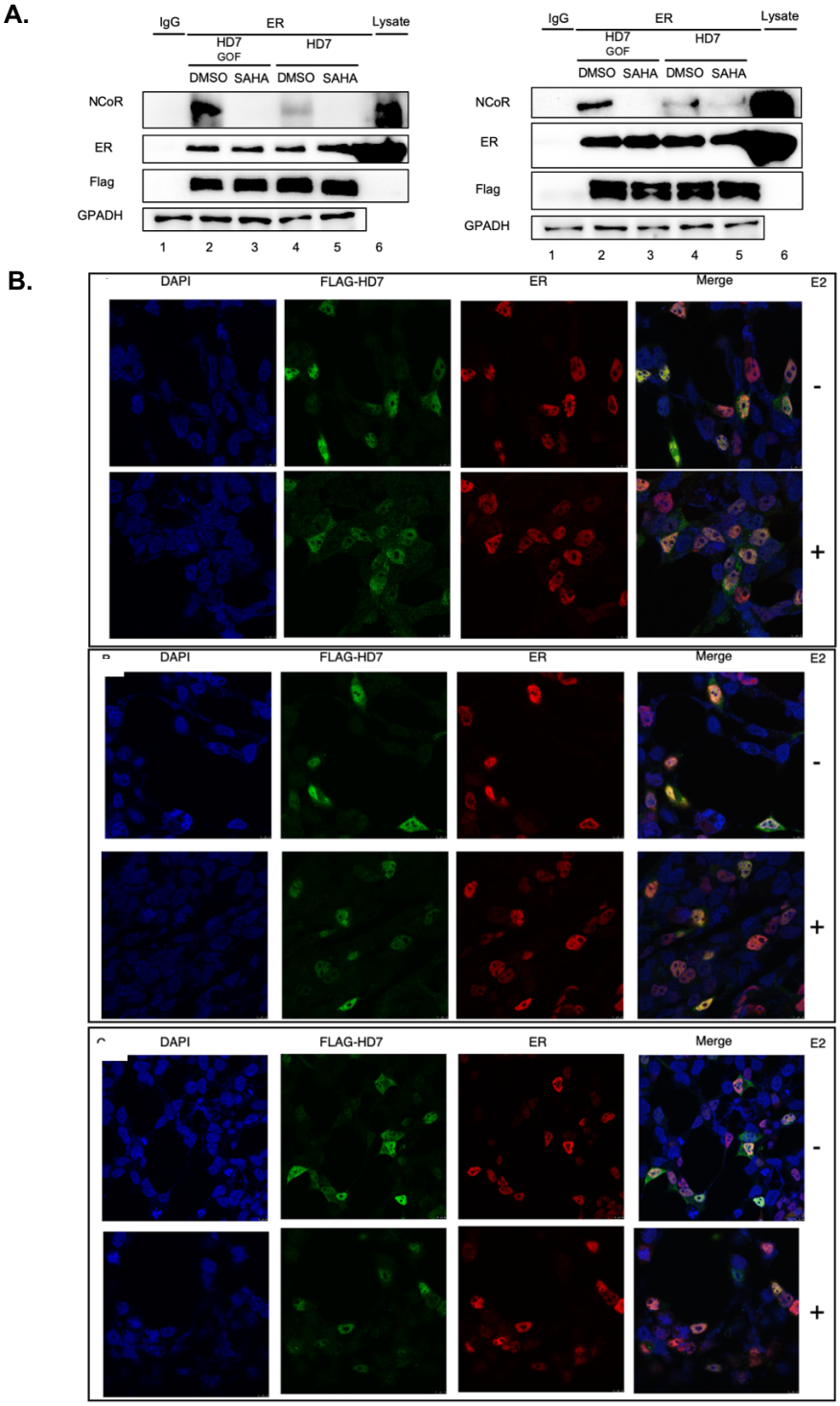


Figure S9. Disruption of HDAC7-NCoR in the presence of full-length ER, related to Figure 6. (A) Flag-tagged fusion proteins of wild type (WT) or GOF mutant HDAC7 were co-expressed with ER WT in HEK293 cells with supplement of E2 ER ligand. After 24h, cells were treated with SAHA (10 μM) for another 24h to induce robust acetylation. HDAC7 was immunoprecipitated via its Flag tag in the absence or presence of SAHA (100 μM). Bound proteins were eluted, separated by SDS-PAGE, and subjected to western blot analysis using NCoR, ER, or Flag antibodies. Two independent trials are shown with the third trial in the main text (Figure 6). (B) WT ER and Flag-tagged HDAC7 were overexpressed in HEK293 cells and incubated with 10 pM of E2 ligand or with 0.1% v/v of ethanol carrier. After E2 or ethanol incubation, cells were fixed and immuno-stained using anti-Flag and anti-ER antibodies, counterstained with DAPI, and visualized using a fluorescence confocal microscope. Three independent trials are shown.

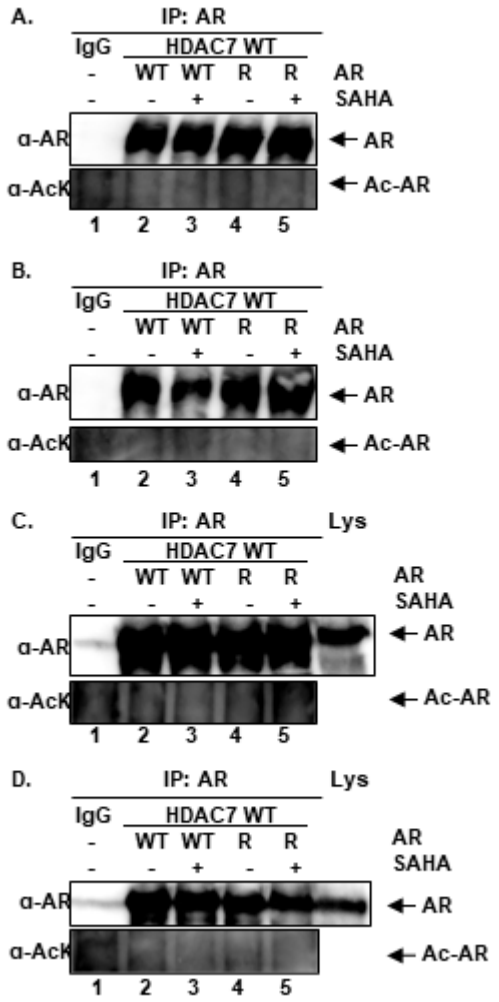


Figure S10. Acetylation levels of overexpressed AR, related to Figure 7. Flag-tagged HDAC7 was co-expressed with AR WT or AR K630R in HEK293 cells. After 24 hours, cells were incubated with SAHA (10 μM) for another 24 hours to induce robust acetylation. Cells were then harvested, lysed and AR was immunoprecipitated overnight either in the presence of SAHA (100 μM in 2% DMSO) or 0.2% DMSO carrier. Bound proteins were eluted, resolved by 10% SDS-PAGE, transferred to a PVDF membrane and visualized via Western Blot using either AR or Acetylated Lysine antibodies. The lysate lane (Lys) represents 50 μg of total protein input from the AR WT sample prior to immunoprecipitation. Four independent trials are shown here.