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Supplementary Materials for

Small-molecule inhibitor targeting orphan nuclear receptor COUP-TFII for prostate cancer treatment

Leiming Wang, Chiang-Min Cheng, Jun Qin, Mafei Xu, Chung-Yang Kao, Jingjing Shi, Erli You, Wanchun Gong, Laura Pedro Rosa, Peter Chase, Louis Scampavia, Franck Madoux, Timothy Spicer, Peter Hodder, H. Eric Xu, Sophia Y. Tsai*, Ming-Jer Tsai*

*Corresponding author. Email: stsai@bcm.edu (S.Y.T.); mtsai@bcm.edu (M.-J.T.)

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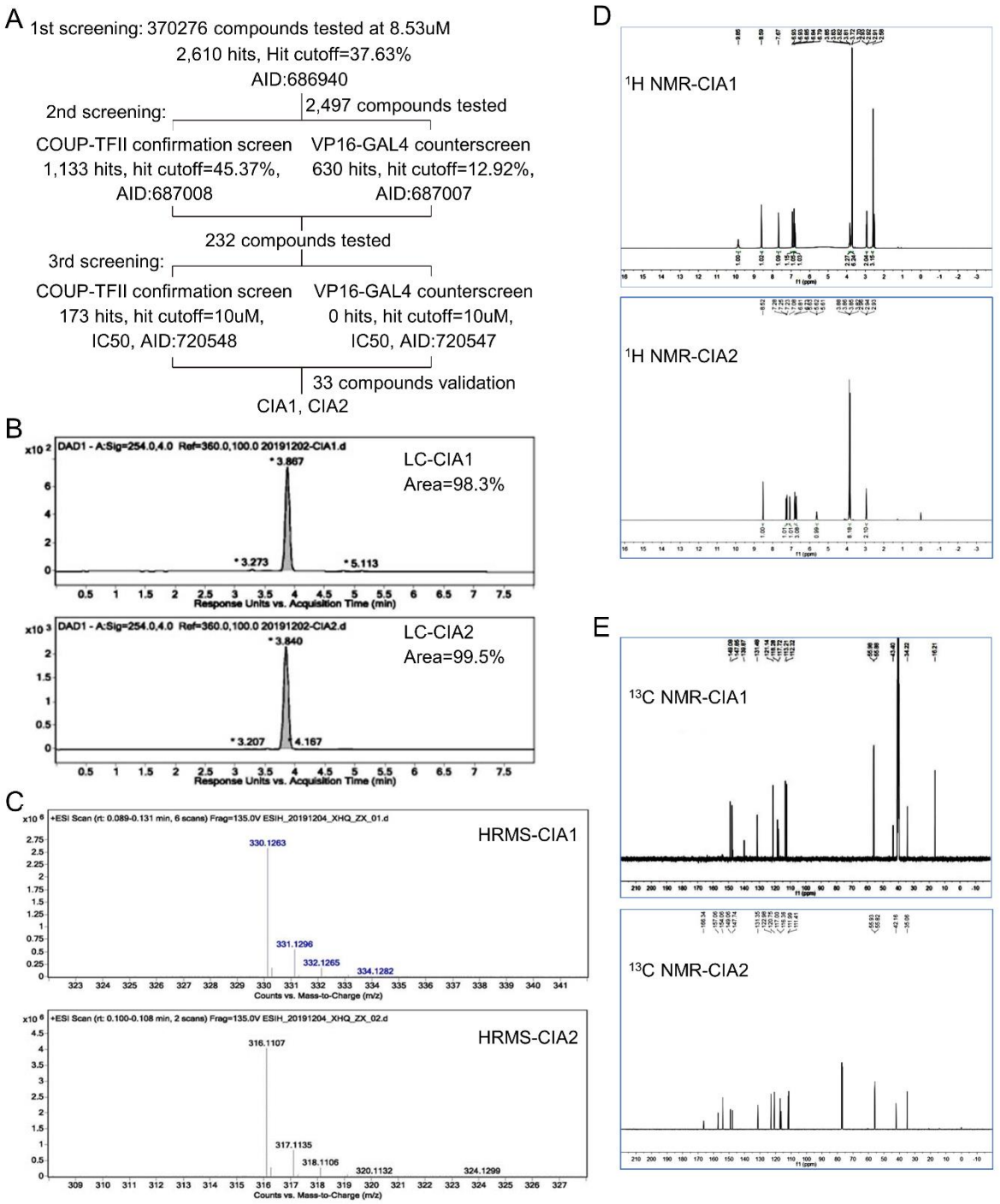

Fig. S1. Identification of COUP-TFII small molecule inhibitors. (A) Schematic of screening process. Each individual assay (AID) was deposited to the PubChem database. LC (**B**), HRMS (**C**), ^{1}H NMR (**D**) and ^{13}C NMR (**E**) analysis of CIA1 and CIA2.

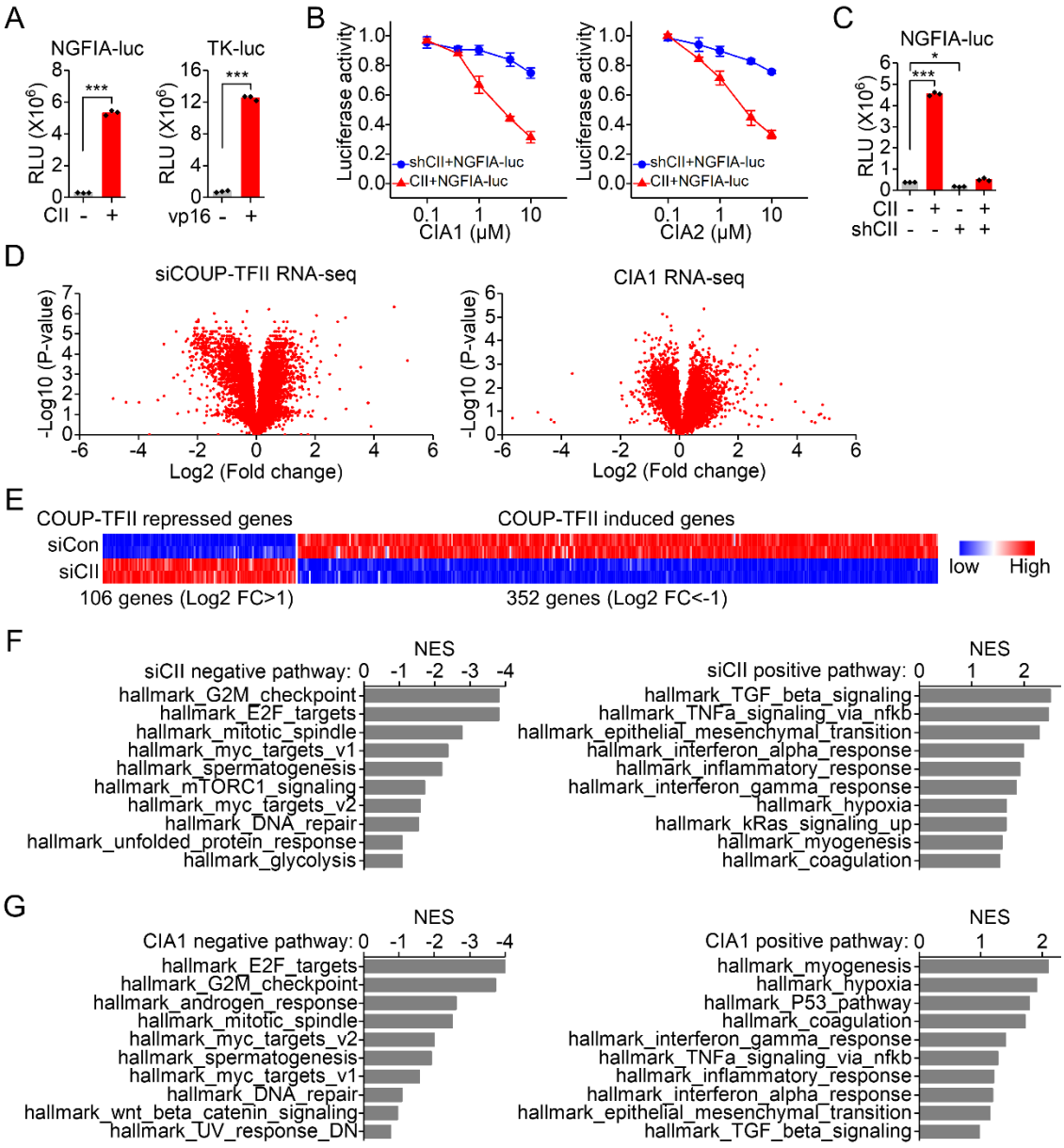

Fig. S2. Targeting COUP-TFII by the inhibitors. (**A**) RLU (relative luminescence unit) of luciferase assay in Fig. 1D. n=3 per group. t test. (**B**) 293T cells that transfected with NGFIA-Luc and COUP-TFII (CII) or shCOUP-TFII (shCII) were treated with CIA1 or CIA2 for 18 hours. Luciferase assay were performed. RUL of luciferase assay was shown in (**C**). n=3 per group. Two-way ANOVA. (**D**) Volcano plot of dysregulated genes in LNCaP cells that transfected with siCOUP-TFII for 72 hours, or treated with 1 μM CIA1 for 18 hours. RNA sequencing was performed. (**E**) COUP-TFII repressed and induced gene signatures were generated by dysregulated genes after COUP-TFII knockdown. GSEA showed the top Hallmark pathways regulated by COUP-TFII (**F**) or CIA1 (**G**). *P<0.05, ***P<0.001.

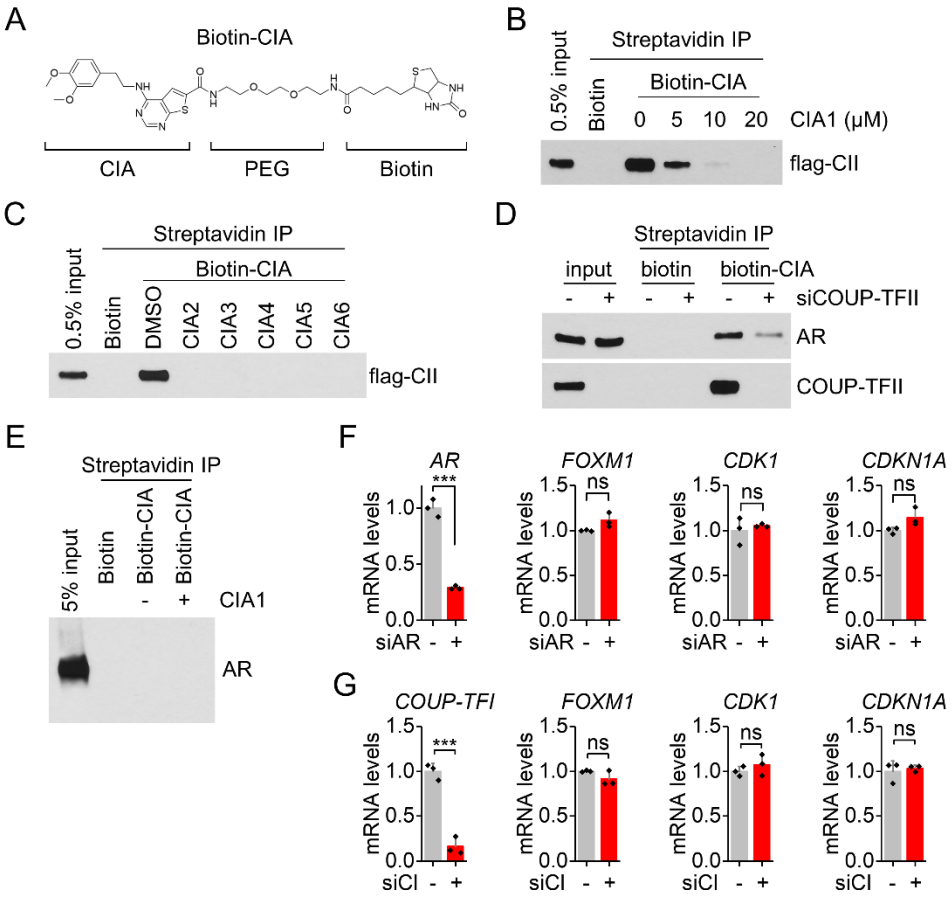

Fig. S3. The direct interaction between the inhibitor and COUP-TFII protein. (**A**) Structure of biotinylated inhibitor. (**B**) Biotinylated inhibitor pulldown assay using overexpressed COUP-TFII in 293T cells. Different doses of CIA1 was used as competitor. (**C**) Biotinylated inhibitor pulldown assay using overexpressed COUP-TFII in 293T cells. 20 μM different active CIA1 analogues were used as competitor. (**D**) LNCaP cells were transfected with siCOUP-TFII for 72 hours. Biotinylated inhibitor pulldown assay was performed using the cell lysate. (**E**) Biotinylated inhibitor pulldown assay using purified AR protein. 20 μM CIA1 was used as competitor. qPCR analysis of indicated genes in LNCaP cells at 72 hours after siAR treatment (**F**) or siCOUP-TFI (siCI) treatment (**G**). n=3 per group. t test, ns=P>0.05, ***P<0.001.

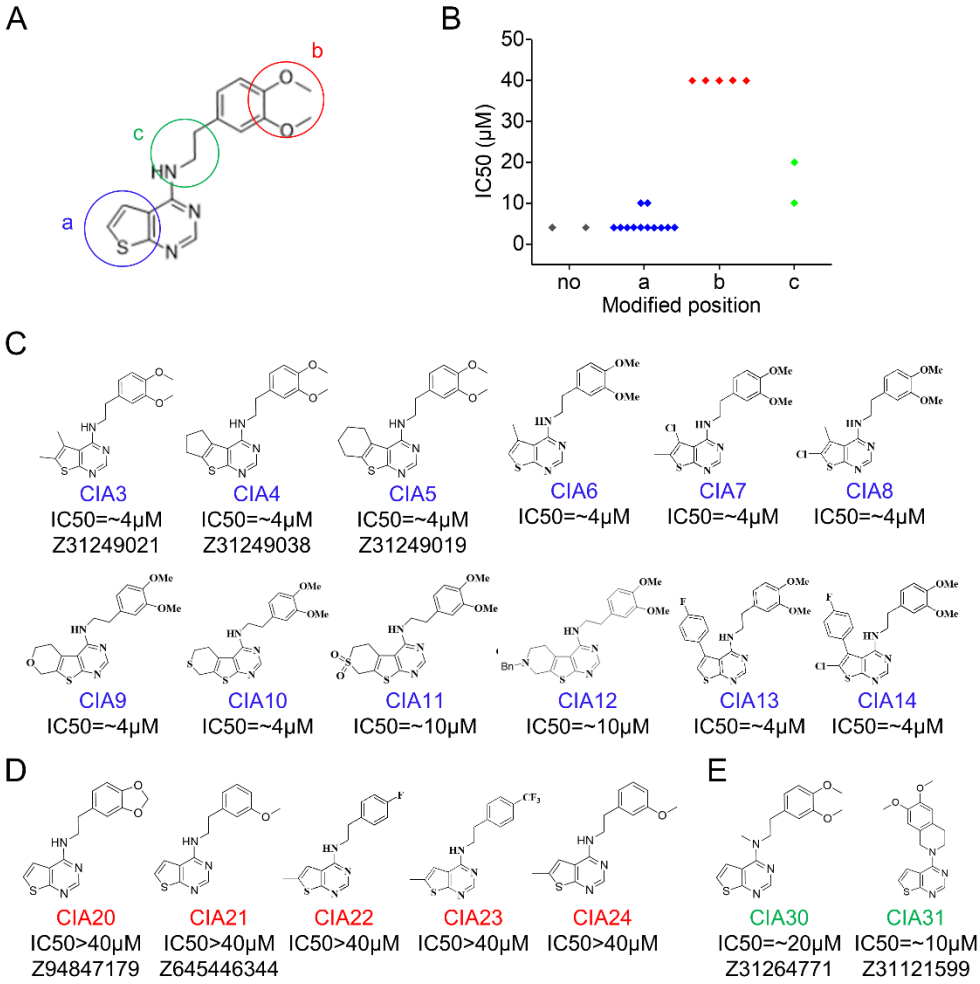

Fig. S4. Screening of CIA1 and CIA2 analogues. (**A**) Modified position as shown on CIA2. a: on the thienopyrimidine ring; b: on the dimethoxybenzene ring; c: on the linker chain. (**B**) IC50 value distribution of compounds by luciferase assay. no: no modification (CIA1 and CIA2). 293T cells that transfected with COUP-TFII and NGFIA-Luc were treated with compound for 18 hours. Luciferase assay was performed to calculate IC50. Detailed structure and IC50 of test compounds with modification on the thienopyrimidine ring (**C**), the dimethoxybenzene ring (**D**), the linker chain (**E**) were shown.

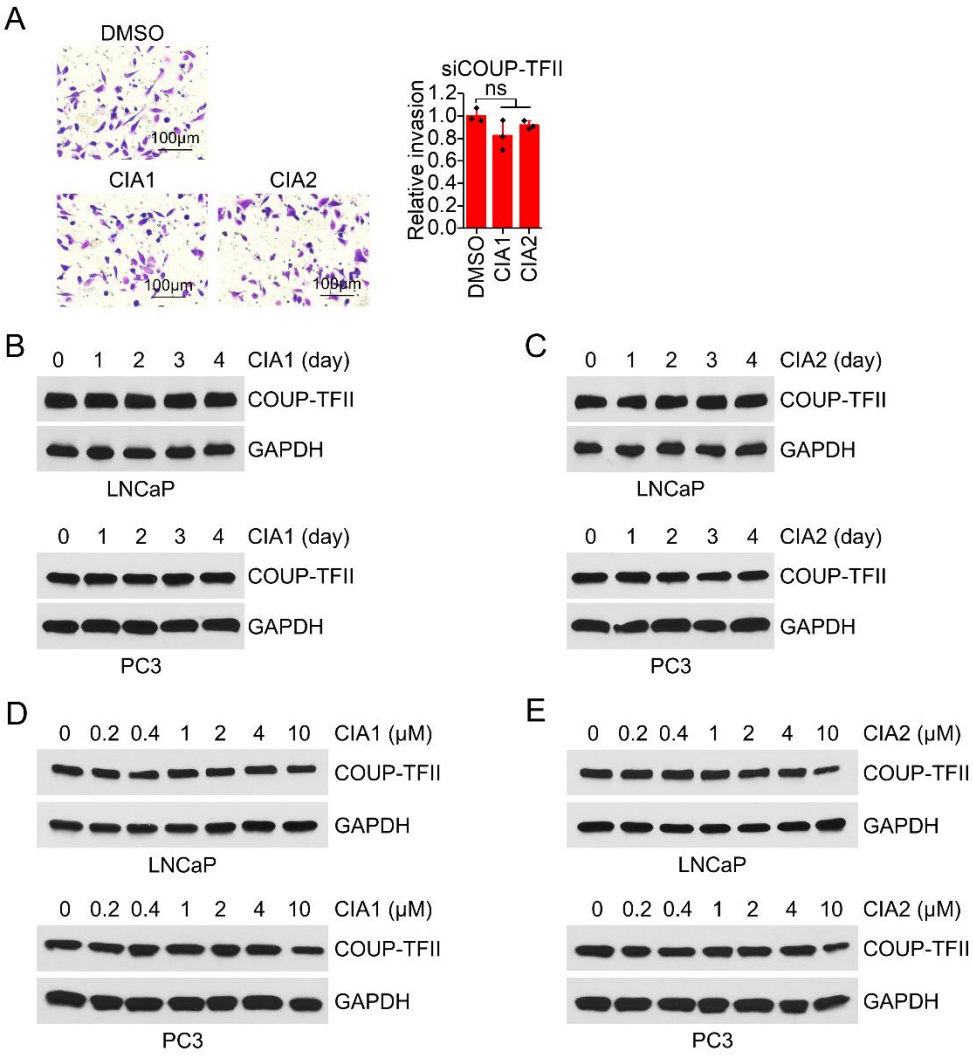

Fig. S5. Related to Fig. 3. (**A**) PC3 cells were transfected with siCOUP-TFII for 24 hours, then treated with 1 μM CIA1 or CIA2 for 48 hours. Invasion was measured by transwell assay. n=3 per group. One-way ANOVA. ns=P>0.05. Time course: No apparent changes in levels of COUP-TFII protein to 1 μM CIA1 (**B**) or 1 μM CIA2 (**C**) treatments in cells for indicated time periods as shown by western blot analysis of COUP-TFII. Dose response: No obvious changes in COUP-TFII protein levels upon treatments with indicated concentrations of CIA1 (**D**) or CIA2 (**E**) in cells for 4 days as shown by western blot analysis of COUP-TFII.

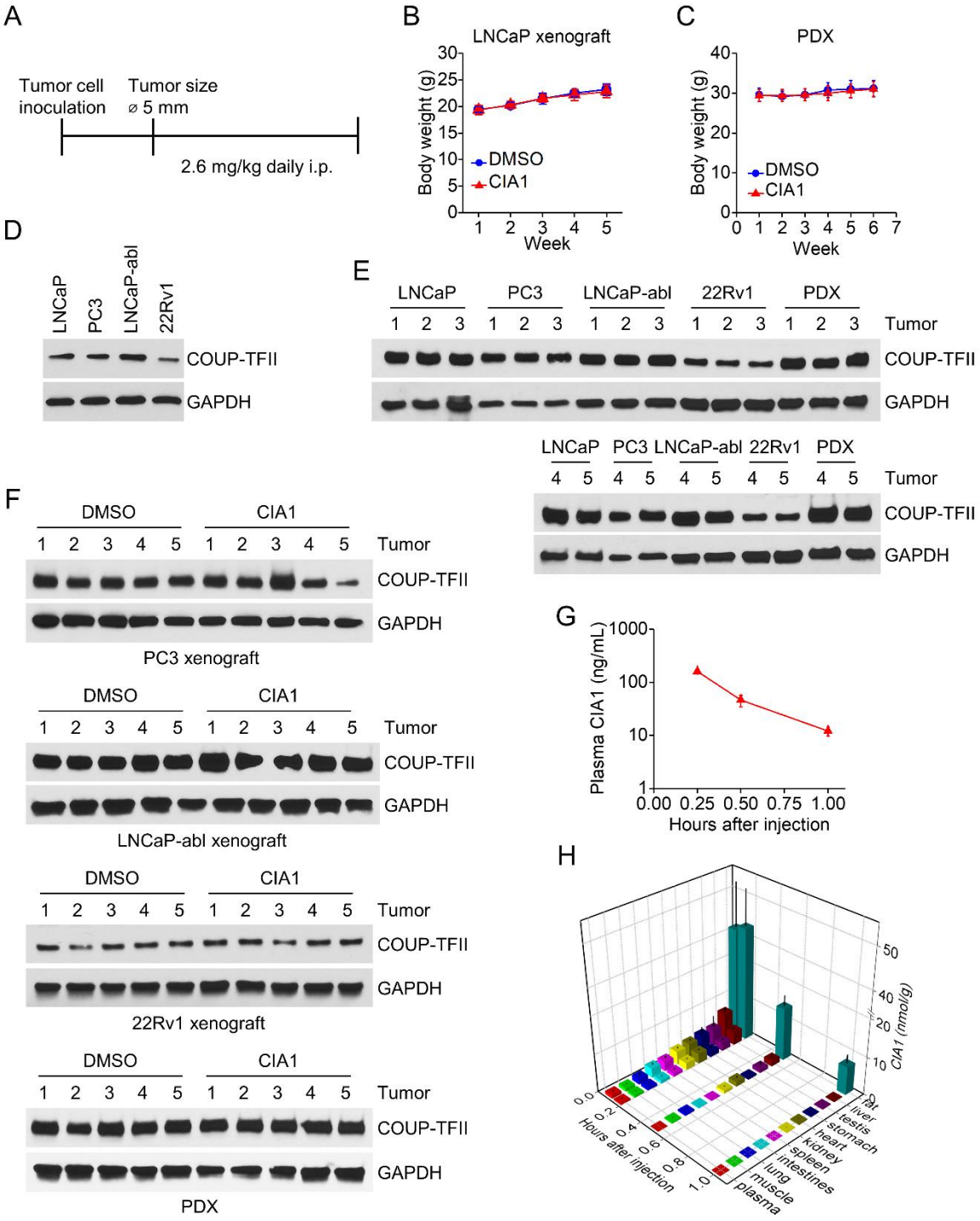

Fig. S6. CIA1 treatment in mice. (**A**) Treatment diagram for xenograft mice. The body weight of LNCaP xenograft bearing mice (**B**) and PDX mice (**C**) after CIA1 treatment. n=5 per group. Western blot analysis of COUP-TFII protein levels in prostate cancer cell lines (**D**) and xenograft tumors that treated with DMSO (**E**). (**F**) Western blot analysis of COUP-TFII protein levels in xenograft tumors after CIA1 treatment. (**G**) DMPK assay of CIA1 in mouse plasma after intraperitoneal injection. (**H**) Tissue distribution of CIA1 in mouse after intraperitoneal injection.

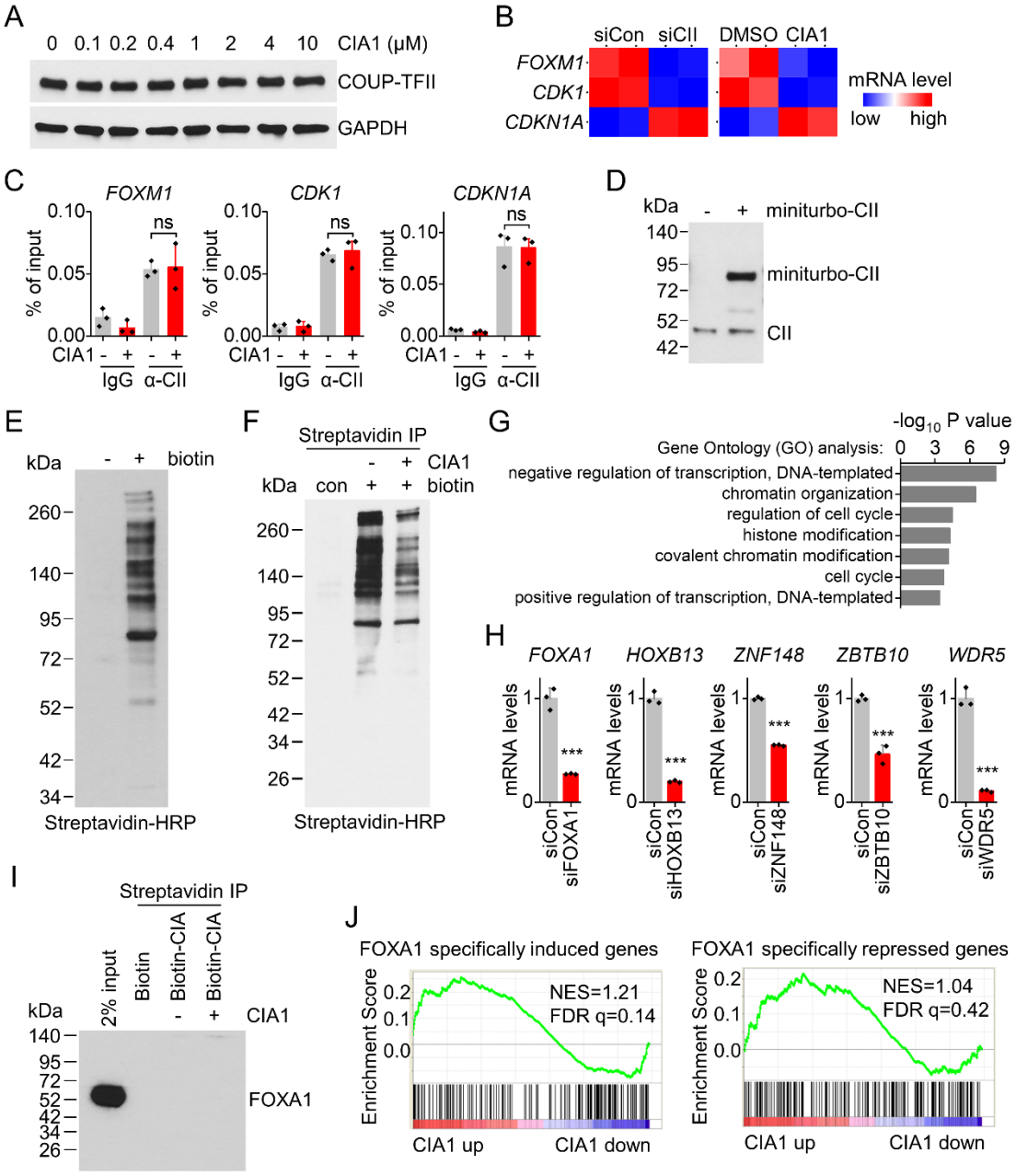

Fig. S7. Disrupted COUP-TFII binding to FOXA1 by CIA1. (**A**) LNCaP cells were treated with CIA1 for 18 hours. Western blot was performed. (**B**) Heatmap of CIA1 regulated COUP-TFII target genes as indicated in RNA sequencing. (**C**) LNCaP cells were treated with 4 μM CIA1 for 12 hours. ChIP-qPCR were performed. n=3 per group. Two-way ANOVA. (**D**) Western blot analysis of overexpressed miniturbo-COUP-TFII in LNCaP cells. (**E**) LNCaP/miniturbo-COUP-TFII cells were treated with 400 μM biotin for 4 hours. Western blot was performed with streptavidin-HRP antibody. (**F**) LNCaP/miniturbo-COUP-TFII cells were treated with 4 μM CIA1 for 3 hours, then 400 μM biotin for 4 hours. Cell lysate was immunoprecipitated with streptavidin beads. (**G**) Gene ontology (GO) analysis of CIA1 reduced COUP-TFII binding proteins. (**H**) Knockdown efficacy of siRNA in Fig. 6C. n=3 per group. t test. (**I**) FOXA1 purified protein was immunoprecipitated with biotinylated inhibitor. 20 μM CIA1 was used as competitor. (**J**) GSEA of FOXA1 specifically regulated signature with CIA1 regulated genes. FOXA1 specifically regulated genes were generated by removing COUP-TFII regulated genes from FOXA1 gene signature (GSE37314).

Table S1. Reduced COUP-TFII binding proteins by CIA1 treatment.

		negative control		DMSO		CIA1	
Gene ID	**Gene Symbol**	**SAF**	**IBAQ**	**SAF**	**IBAQ**	**SAF**	**IBAQ**
25873	RPL36	0	0	667	24	0	0
3169	FOXA1	0	0	357	15.8	0	0
90861	JPT2	0	0	333	11.9	0	0
6159	RPL29	0	0	250	10.2	0	0
10481	HOXB13	0	0	273	9.3	0	0
7707	ZNF148	0	0	179	6.1	0	0
65986	ZBTB10	0	0	116	4.8	0	0
3066	HDAC2	0	0	87	3.9	43.5	0.25
283431	GAS2L3	0	0	103	2.2	0	0
5173	PDYN	0	0	76.9	2.1	0	0
10726	NUDC	0	0	90.9	1.7	0	0
4781	NFIB	87	0	217	1.5	130	0
5335	PLCG1	0	0	14.5	1.4	0	0
8649	LAMTOR3	0	0	143	1.3	0	0
4129	MAOB	69	0	69	1.2	34.5	0
5304	PIP	0	0	111	1.2	0	0
80895	ILKAP	0	0	83.3	1	0	0
200010	SLC5A9	0	0	36.4	0.94	0	0
5987	TRIM27	0	0	35.7	0.84	0	0
4774	NFIA	79.2	0	317	0.82	119	0
202559	KHDRBS2	0	0	66.7	0.78	66.7	0
3936	LCP1	26.3	0	52.6	0.77	0	0
1108	CHD4	10.3	0.05	41.2	0.77	0	0
9667	SAFB2	0	0	97.6	0.65	73.2	0
3054	HCFC1	11.2	0	44.9	0.63	0	0
5621	PRNP	0	0	71.4	0.61	0	0
11091	WDR5	0	0	62.5	0.6	0	0
80347	COASY	0	0	42.7	0.57	0	0
6643	SNX2	0	0	35.7	0.56	0	0
5496	PPM1G	0	0	33.3	0.43	0	0
10009	ZBTB33	0	0	26.3	0.43	0	0
6015	RING1	0	0	55.6	0.37	0	0
6045	RNF2	0	0	55.6	0.37	0	0
58490	RPRD1B	0	0	58.8	0.36	0	0
84277	DNAJC30	0	0	83.3	0.31	0	0
84549	MAK16	0	0	66.7	0.31	0	0
79977	GRHL2	0	0	30.3	0.26	0	0
8841	HDAC3	0	0	38.5	0.24	0	0
6640	SNTA1	0	0	37	0.24	0	0
23011	RAB21	0	0	71.4	0.23	0	0

5770	PTPN1	0	0	34.5	0.21	0	0
6641	SNTB1	0	0	30.3	0.19	0	0
79009	DDX50	25.6	0	25.6	0.17	0	0
8971	H1-10	0	0	83.3	0.16	0	0
10250	SRRM1	0	0	23.8	0.14	0	0
5777	PTPN6	0	0	27.5	0.13	0	0
7874	USP7	0	0	14.9	0.13	0	0
4673	NAP1L1	0	0	62.5	0.12	0	0
1759	DNM1	21.1	0	21.1	0.12	0	0
2961	GTF2E2	0	0	55.6	0.11	0	0
1503	CTPS1	0	0	30.3	0.11	0	0
55746	NUP133	0	0	16.1	0.11	0	0
9945	GFPT2	0	0	23.3	0.1	23.3	0
254048	UBN2	0	0	15.6	0.09	0	0
64324	NSD1	0	0	7.2	0.06	0	0
7402	UTRN	0	0	5.5	0.03	0	0
63922	CHTF18	0	0	20.8	0.01	0	0

SAF: spectral abundance factor; IBAQ: intensity based absolute quantification.

Table S2. siRNAs and primers for qRT-PCR and ChIP-qPCR.

siRNA
universal negative control siRNA: Sigma (SIC001)
siCOUP-TFII-1: GGCCGUAUAUGGCAAUUCA
siCOUP-TFII-2: GUACCUGUCCGGAUAUAUU
siFOXA1-1: GAGAGAAAAAAUCAACAGC
siFOXA1-2: CCAGACGGGUUUCAUUAUU
siCOUP-TFI-1: CAAUCCAGGCCAGUACGCA
siCOUP-TFI-2: CCAACAACAUUAUGGGCAU
siAR-1: GACCUACCGAGGAGCUUUC
siAR-2: UCAAGGAACUCGAUCGUAU
siHOXB13: SASI Hs01 00032419 from Sigma
siZNF148: SASI Hs01 00093930 from Sigma
siZNF148: SASI Hs01 00093935 from Sigma
siZBTB10: SASI Hs02 00324282 from Sigma
siZBTB10: SASI Hs02 00324284 from Sigma
siWDR5: SASI Hs01 00046875 from Sigma
qRT-PCR primer
ACTB-F: TGGCATTGCCGACAGGAT
ACTB-R: GCTCAGGAGGAGCAATGATCT
FOXM1-F: GGAGCAGCGACAGGTTAAGG
FOXM1-R: GTTGATGGCGAATT GTATCATGG
CDK1-F: GCGCGGATCTACCATACCC
CDK1-R: CATGGCTAC CACTTGACCTGT
CDKN1A-F: ACCTCCTCTAAGGTTGGGCA
CDKN1A-R: TGCCTTCACAAGACAGAGGG
FOXA1-F: CTACTACGCAGACACGCAGG
FOXA1-R: CCGCTCGTAGTCATGGTGTT
HOXB13-F: GCGAGCTGGGAGCGATTTA
HOXB13-R: CTCCCAGCAAGCCTTCGATA
ZNF148-F: GAAACGTAGTGGGAGTGCGA
ZNF148-R: CTCAAGACTCCTCCACAGCC
ZBTB10-F: GGGGAGCCCACATAGCATTA
ZBTB10-R: TGGTCCCAATCATGACCCCT
WDR5-F: TGTGAAGTTCTCCCCGAACG
WDR5-R: GTGGCCAGTGTACGTCTTCA
AR-F: CAGTGGATGGGCTGAAAAAT
AR-R: GGAGCTTGGTGAGCTGGTAG
COUP-TFI-F: ATCGTGCTGTTCACGTCAGAC
COUP-TFI-R: TGGCTCCTCACGTACTCCTC
ChIP-qPCR primer
CII-FOXM1-F: CGACTGTGGCTGAGATGAAG

CII-FOXM1-F: GTAAGATGGAGGCGGTGTTG
CII-CDK1-F: GGCGGTCTTTTGAGTTTTCCA
CII-CDK1-R: ACATCGAGATTCCATTACTTTCACT
CII-CDKN1A-F: GCTGTATGACTCAGGGGCAA
CII-CDKN1A-R: CTCACCCCACTGCTTGTGAT