Methods

Xenograft animal model and experimental therapy

22RV1 (2 x 10^6 cells/site) mixed with Matrigel (Thermo Fisher Scientific) at 1:1 (v/v) was subcutaneously injected into each flank of NOD-SCID animal. Once tumor became palpable and reached around 75 mm^3, tumor-bearing animals were randomized into treatment group (TST-D, 300 μg/kg/day) or control group (DMSO) that were delivered using ALZET osmotic pumps (DURECT Corporation, Cupertino, CA) by subcutaneous implantation. Tumor volume was measured by using caliper prior-injection and every 2 days post-injection. Due to extensive tumor necrosis, experiment was terminated 4 days after treatment. Each tumor was excised and weighted then either snap frozen or fixed with formalin.

Table S1 List of Antibodies

Name	Clone	Company (CAT#)
β-actin		Sigma-Aldrich(A5441)
AR	N-20	Santa Cruz (sc-816)
AR-V7		Presion Antibody (AG10008)
AKR1C3		Sigma-Aldrich (A6229)
SAP155	16	MBL (D221-3)
U2AF65	MC3	Santa Cruz (sc-53942)
U2AF35	N-16	Santa Cruz (sc-19961)
Cleaved caspase-3^{Asp175} (IHC)	D3E9	Cell Signaling (9579S)
Ki67	B56	BD (550609)

Table S2 List of Primers

Name	Primer sequence	Location
AR-V7 F	5'-CCATCTTGTCGTCTTCGGAAATGTTATGAAGC-3'	
AR-V7 R	5'-TTTGAATGAGGCAAGTCAGCCTTTCT-3'	
AR-FL F	5'-ACATCAAGGAACTCGATCGTATCATTGC-3'	
AR-FL R	5'-TTGGGCACTTGCACAGAGAT-3'	
AR-V7 mini F	5'-CAGGGATGACTCTGGGAGAA-3'	Exon3/3b
AR-V7 mini R	5'-GCCCTCTAGAGCCCTCATTT-3'	3' UTR
AR mini F	5'-TCTTGTCGTCTTCGGAAATGT-3'	Exon 3
AR mini R	5'-AAGCCTCTCCTTCCTCCTGTA-3'	Exon 4
PSA F	5'-GACCAAGTTCATGCTGTGTG-3'	
PSA R	5'-ACTAGGGAGCCATGGAGGAC-3'	
18s rRNA F	5′-GGAATTGACGGAAGGGCACCACC-3′	
18s rRNA R	5′-GTGCAGCCCCGGACATCTAAGG-3'	
MDM2-FL F	5'-GCAGTGAATCTACAGGGACGC-3'	
MDM2-FL R	5′-ATCCTGATCCAACCAATCACC-3'	
MDM2-C F	5′-GAAAGAGGATCTTGATGCTGGTGTA-3'	
MDM2-C R	5′-GGGGGATTCATTTCATTGCATG-3'	
CD44-FL F	5′-AGCAACCAAGAGGCAAGAAA-3'	
CD44-FL R	5′-GTGTGGTTGAAATGGTGCTG-3'	
CD44-S F	5′-TCCAACACCTCCCAGTATGACA-3'	
CD44-S R	5′-CCCACATGCCATCTGTTGCC-3'	
CD44-V6 F	5′-GGAACAGTGGTTTGGCAACAG-3'	
CD44-V6 R	5′-TTGGGTGTTTGGCGATATCC-3'	
$VEGFA_{8a}$ F	5′-CACCGCCTCGGCTTGTCACAT-3'	
$VEGFA_{8a}$ R	5′-GAGATGAGCTTCCTACAGCAC-3'	
$VEGFA_{8b7}$ F	5′-GAGATGAGCTTCCTACAGCAC-3'	
$VEGFA_{8b7}$ R	5′-TTAAGCTTTCAGTCTTTCCTGGTGAGAGATCTGCA-3'	

Table S3 MS analyses of the association of splicing factor(s) with AR-V7 ISE. 22RV1 and VCaP cell lysates were incubated with RNA oligos containing ISE sequence for pull-down and the associated factors were subjected to LC-MS/MS analysis.

					SPECTRAL COUNTS				SPECTRAL INDEX (MIC SIn)			
22RV1	VCaP				22RV1		VCaP		22RV1		VCaP	
TST/DMSO	TST/DMSO	PSMs	eptide Seq	% Coverage	DMSO	TST-D	DMSO	TST-D	DMSO	TST-D	DMSO	TST-D
HNRPU_HUMAN Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6												
0.75	1.44	443	56	53.6	67.5	54.5	58.5	64	1E-05	1E-05	1E-05	2E-05
HNRPR_HUMAN Heterogeneous nuclear ribonucleoprotein R OS=Homo sapiens GN=HNRNPR PE=1 SV=1												
0.72	1.20	396	38	53.7	114.09	97.36	76.73	103.24	4E-05	3E-05	2E-05	3E-05
SF3B3_HUMAN Splicing factor 3B subunit 3 OS=Homo sapiens GN=SF3B3 PE=1 SV=4												
0.46	0.44	349	49	43	110.86	75.89	100.88	60.92	8E-06	4E-06	8E-06	4E-06
SF3B1_HUMAN Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3												
0.38	0.29	309	58	50.7	111	70	92	37	6E-06	2E-06	5E-06	1E-06
HNRPQ_HUMAN Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2												
1.06	1.79	265	39	63.9	89.13	89.13	35.64	48.51	2E-05	2E-05	4E-06	8E-06
SF3B2_HUMAN Splicing factor 3B subunit 2 OS=Homo sapiens GN=SF3B2 PE=1 SV=2												
0.35	0.10	213	40	50.4	84.23	50.54	60.43	15.91	6E-06	2E-06	4E-06	4E-07
G8JLB6_HUMAN Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=2 SV=1												
1.18	1.20	159	19	57	39.68	44.55	31.97	37.75	7E-06	8E-06	5E-06	6E-06
HNRPF_HUMAN Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens GN=HNRNPF PE=1 SV=3												
1.17	0.98	155	17	57.1	36	47	37	35	7E-06	9E-06	7E-06	6E-06
PTBP2_HUMAN Polypyrimidine tract-binding protein 2 OS=Homo sapiens GN=PTBP2 PE=1 SV=1												
0.94	2.35	114	19	43.9	32.67	32.67	20.79	26.73	7E-06	6E-06	2E-06	5E-06
U2AF2_HUMAN Splicing factor U2AF 65 kDa subunit OS=Homo sapiens GN=U2AF2 PE=1 SV=4												
1.06	0.73	101	16	38.6	27	27	28	20	4E-06	4E-06	5E-06	4E-06
SF3A1_HUMAN Splicing factor 3A subunit 1 OS=Homo sapiens GN=SF3A1 PE=1 SV=1												
0.17	0.16	94	26	37.1	42.86	12.95	30.89	7.99	2E-06	3E-07	1E-06	2E-07
U2AF1_HUMAN Splicing factor U2AF 35 kDa subunit OS=Homo sapiens GN=U2AF1 PE=1 SV=3												
0.53	0.66	66	10	51.2	16.83	13.87	17.82	16.84	4E-06	2E-06	5E-06	3E-06
SF3A3_HUMAN Splicing factor 3A subunit 3 OS=Homo sapiens GN=SF3A3 PE=1 SV=1												
0.10	0.21	59	16	32.1	20	7	24	9	1E-06	1E-07	2E-06	4E-07
*only PSMs >50 is counted												
	<0.8											
	0.8-1.2											
	>1.2											

Supplemental Figure Legends

Figure S1. (**A**) Chemical structure of TST-A and TST-D. (**B**) The effect of TST-A or TST-D on the growth of 22RV1 stimulated by DHT. **(C)** LAPC-4 and C4-2 were treated with TST-A or TST-D at different concentrations and relative cell number was determined by crystal violet assay 24 hours after treatment.

Figure S2. (**A**) Cells were treated with TST-A at 0, 0.5, 2, 5 nM or TST-D at 0, 5, 20, 50 nM for 4 hours. (**B**) Cells were treated with 5 nM TST-A or 50 nM TST-D at the indicated time. Total cellular RNA was prepared and subjected to qRT-PCR analyses. After normalizing with 18S rRNA, the relative levels of AR-FL mRNA from each sample were calculated using control (=1). (**C**) Cells were treated with 5 nM TST-A or 50 nM TST-D at the indicated time. Total cellular RNA was prepared and subjected to qRT-PCR analyses. After normalizing with 18S rRNA, the relative levels of AKR1C3 mRNA from each sample were calculated using control (=1).

Figure S3. (**A**) Immunostaining of AR-V7 protein in 22RV1 cells treated with TST-D. (**B**) Immunostaining of AR-V7 protein in 22RV1 cells treated with TST-D.

Figure S4. The effect of 20 nM TST-D on the expression of different gene splicing variants in AR-V7-expressing cells. The relative levels of mRNA expression from each gene were calculated using control (=1).

Figure S5. 22RV1 were transfected with GFP-vec or GFP-AR-FL cDNA for 24 hours; cells were treated with different concentrations of TST-D. Endogenous or exogenous AR-FL protein expression was determined by western blot from cells 4 hours after treatment (lower panel). Cell growth was determined 24 hours after treatment (upper panel). * $P<0.05$, ** $P<0.01$.

Figure S6. 22RV1, VCaP and HEK293 cells were transiently transfected with mock vector or ARV7 minigene plasmid. Twenty-four hours after transfection, cells were treated with TST-A at 0, 0.5, 2, 5 nM or TST-D at 0, 5, 20, 50 nM for 4 hours. After normalizing with 18S rRNA, relative levels of AR-FL mRNA from each sample were calculated using control (=1).

Figure S7. 22RV1 was pre-treated with 50 nM TST-D for 4 hours, total cell lysate were immunoprecipitated with U2AF35 antibodies plus protein G beads. Bound proteins were eluted and analyzed by western blot

Figure S8. (**A**) Mice bearing 22RV1 xenografts were treated with DMSO or 300 μg/kg/day TST-D and tumor volumes were measured every other day. Tumors were excised and photographed at Day 4. (**B**) 22RV1 xenografts were treated with DMSO or different dosages ranging from 50 μg/kg/day to 30 mg/kg/day, western blot was used to determine the expression of AR-V7 and AR-FL. * $P<0.05$ ** $P<0.01$.

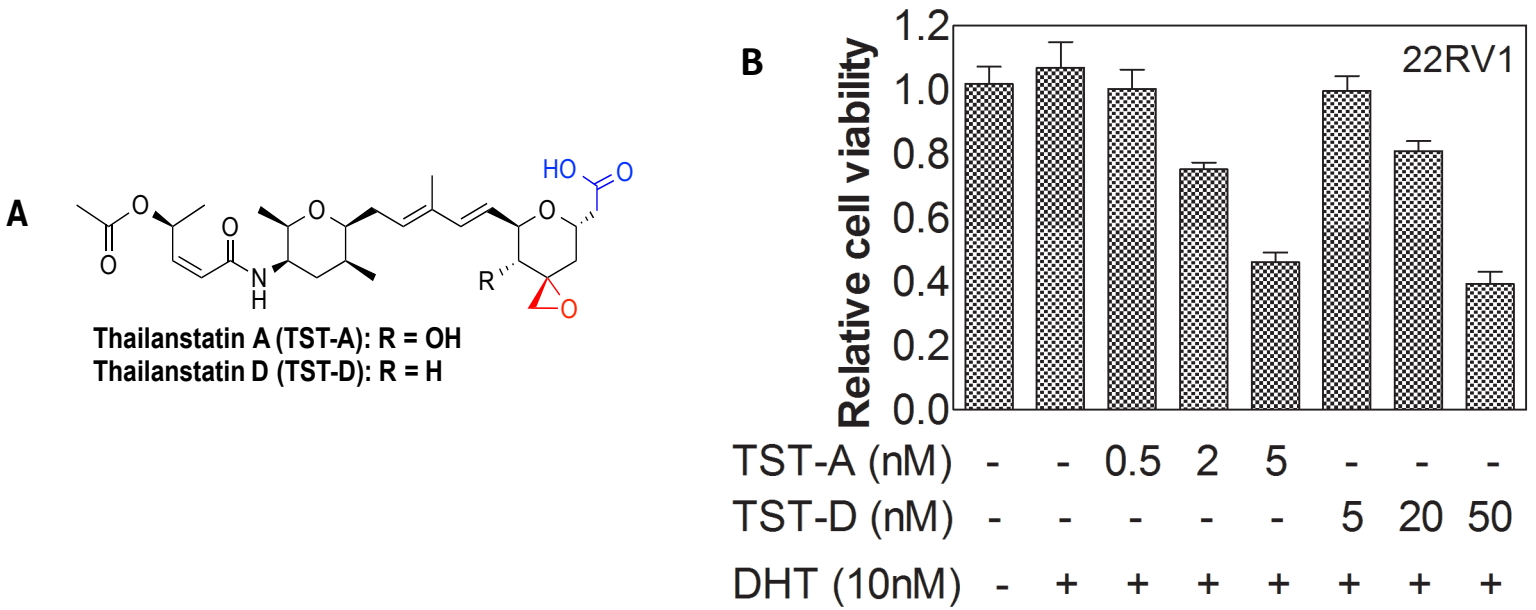

A
HO
O
O
O
O
O
N
H
R
O
Thailanstatin A (TST-A): R = OH
Thailanstatin D (TST-D): R = H
B
22RV1
Relative cell viability
1.2
1.0
0.8
0.6
0.4
0.2
0.0
TST-A (nM) - - 0.5 2 5 - - -
TST-D (nM) - - - - - 5 20 50
DHT (10nM) - + + + + + + +
C
TST-A
0nM
0.5nM
2nM
5nM
Relative cell viability
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LAPC-4
C4-2
TST-D
0nM
5nM
20nM
50nM
Relative cell viability
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LAPC-4
C4-2

Figure S1

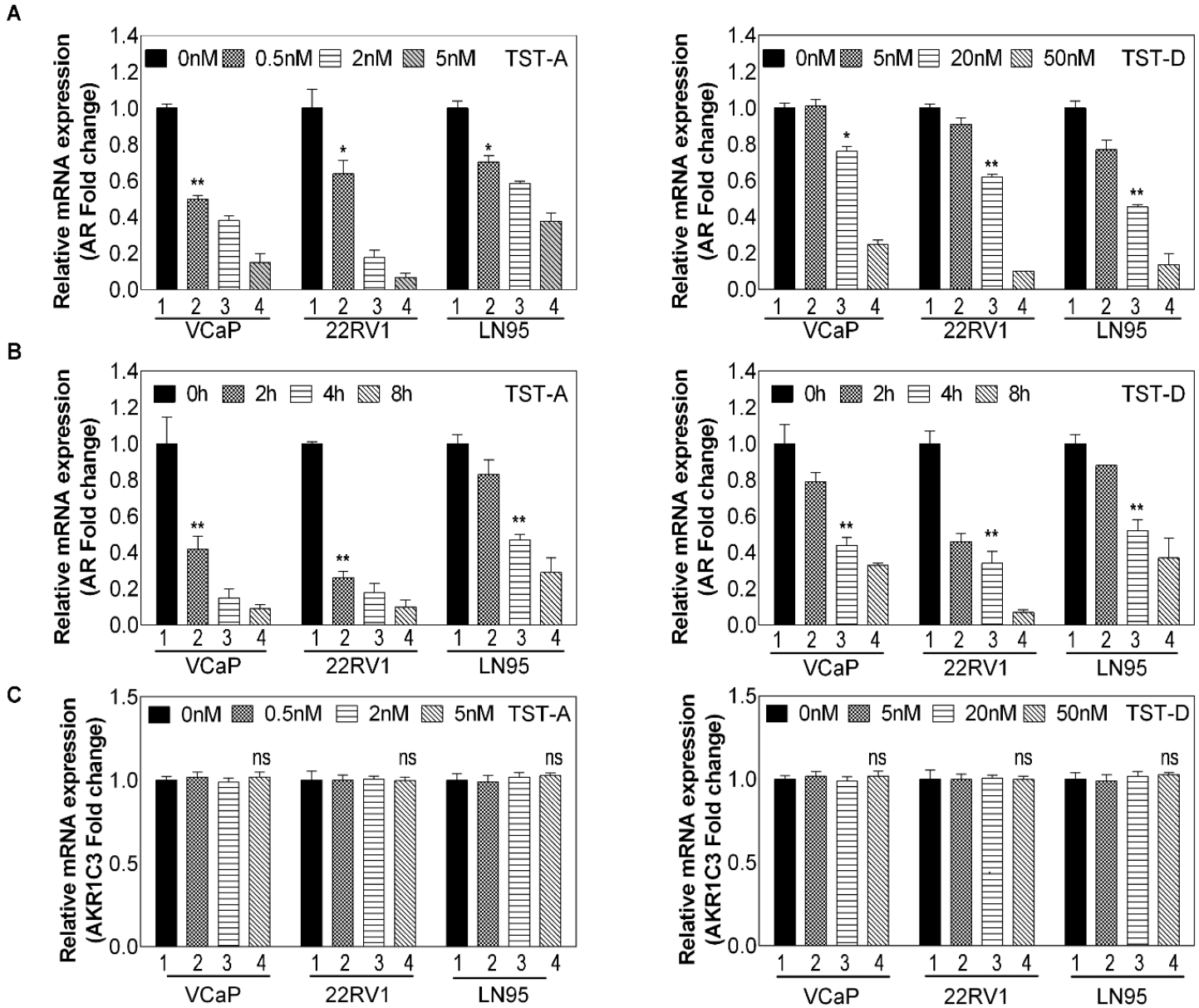

A
Relative mRNA expression (AR Fold change)
0nM 0.5nM 2nM 5nM TST-A
0nM 5nM 20nM 50nM TST-D
VCaP 22RV1 LN95
B
0h 2h 4h 8h TST-A
0h 2h 4h 8h TST-D
C
Relative mRNA expression (AKR1C3 Fold change)
ns

Figure S2

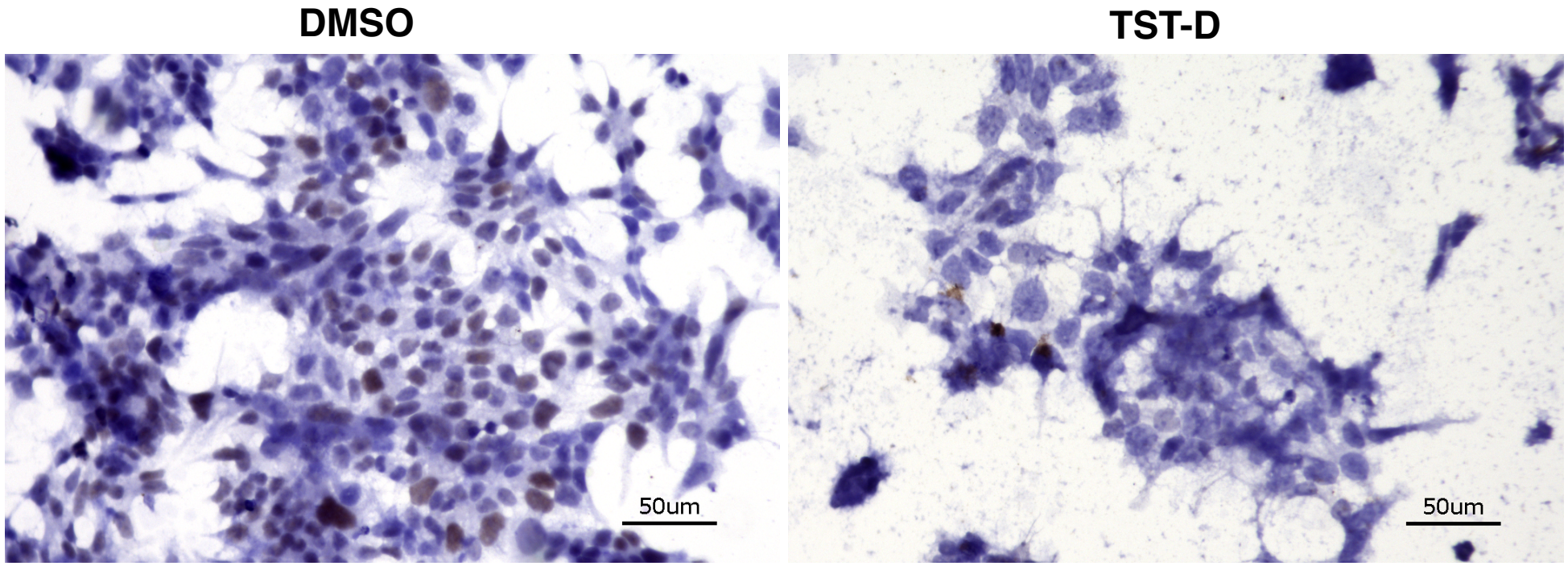


Figure S3

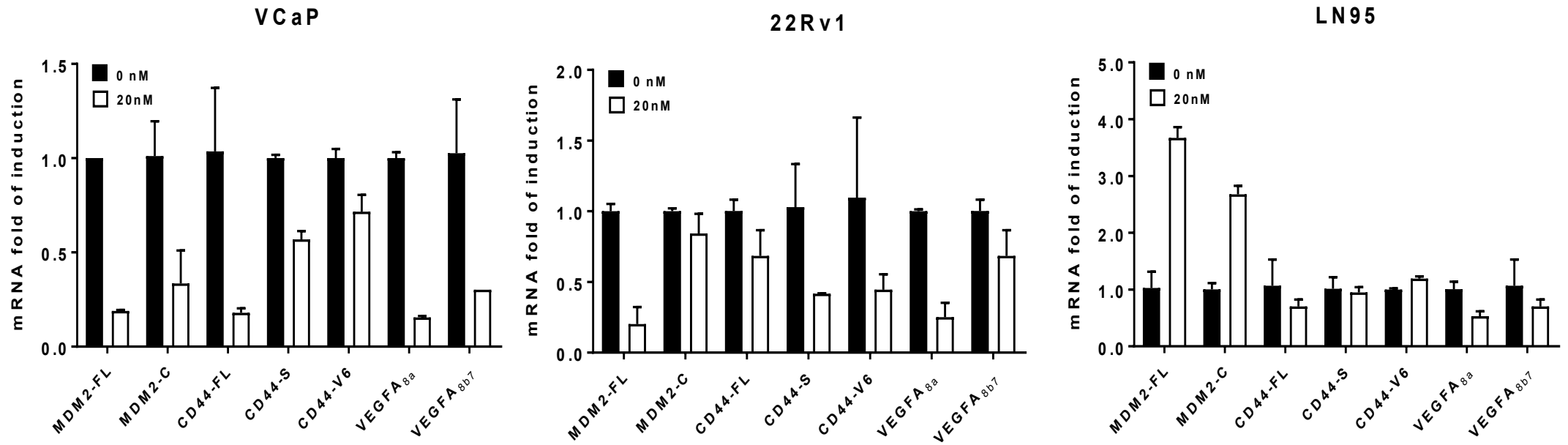

VCaP
22Rv1
LN95
mRNA fold of induction
0 nM
20nM
MDM2-FL
MDM2-C
CD44-FL
CD44-S
CD44-V6
VEGFA8a
VEGFA8b7

Figure S4

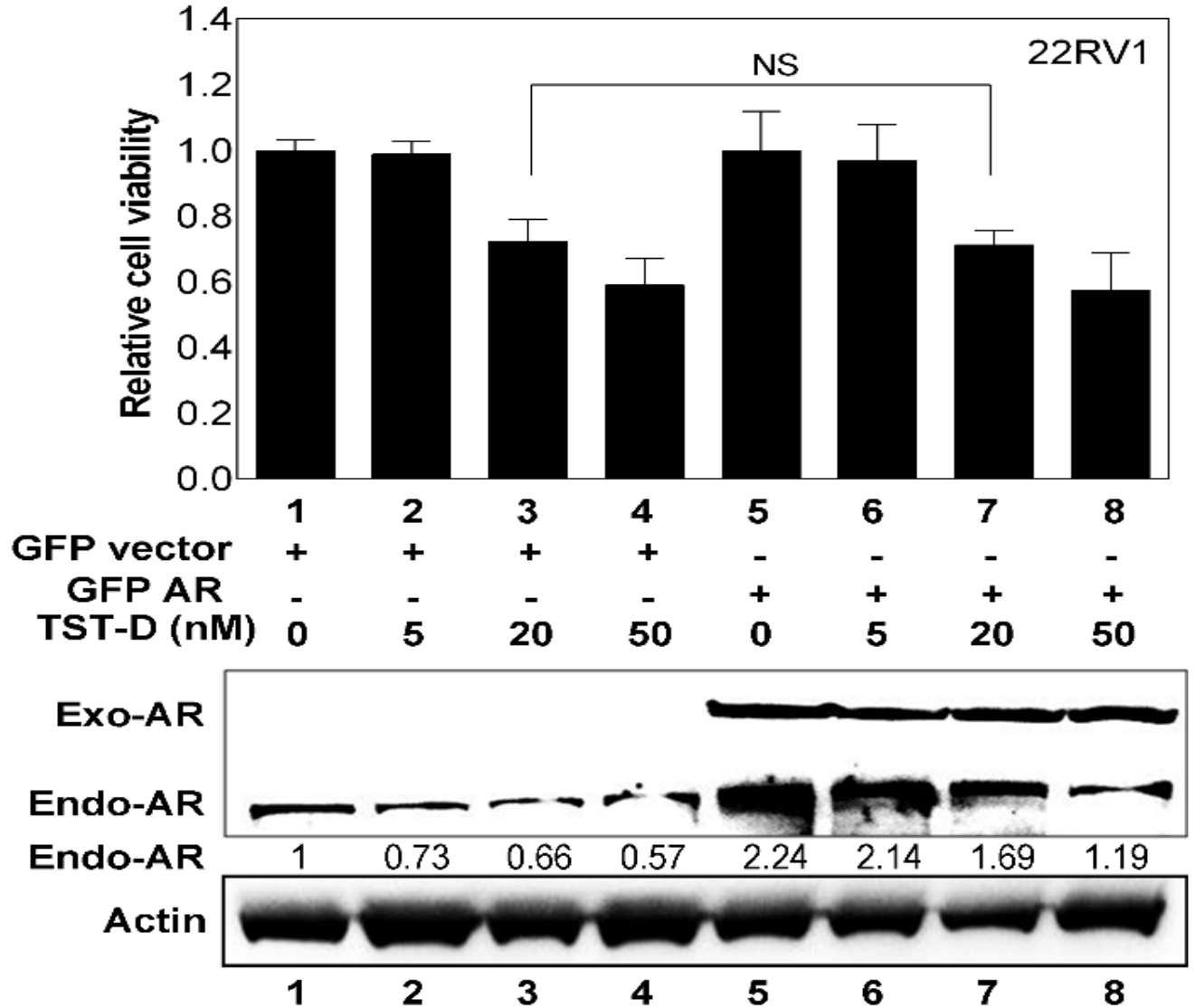

22RV1
NS
Relative cell viability
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8
GFP vector + + + + - - - -
GFP AR - - - - + + + +
TST-D (nM) 0 5 20 50 0 5 20 50
Exo-AR
Endo-AR
Endo-AR 1 0.73 0.66 0.57 2.24 2.14 1.69 1.19
Actin
1 2 3 4 5 6 7 8

Figure S5

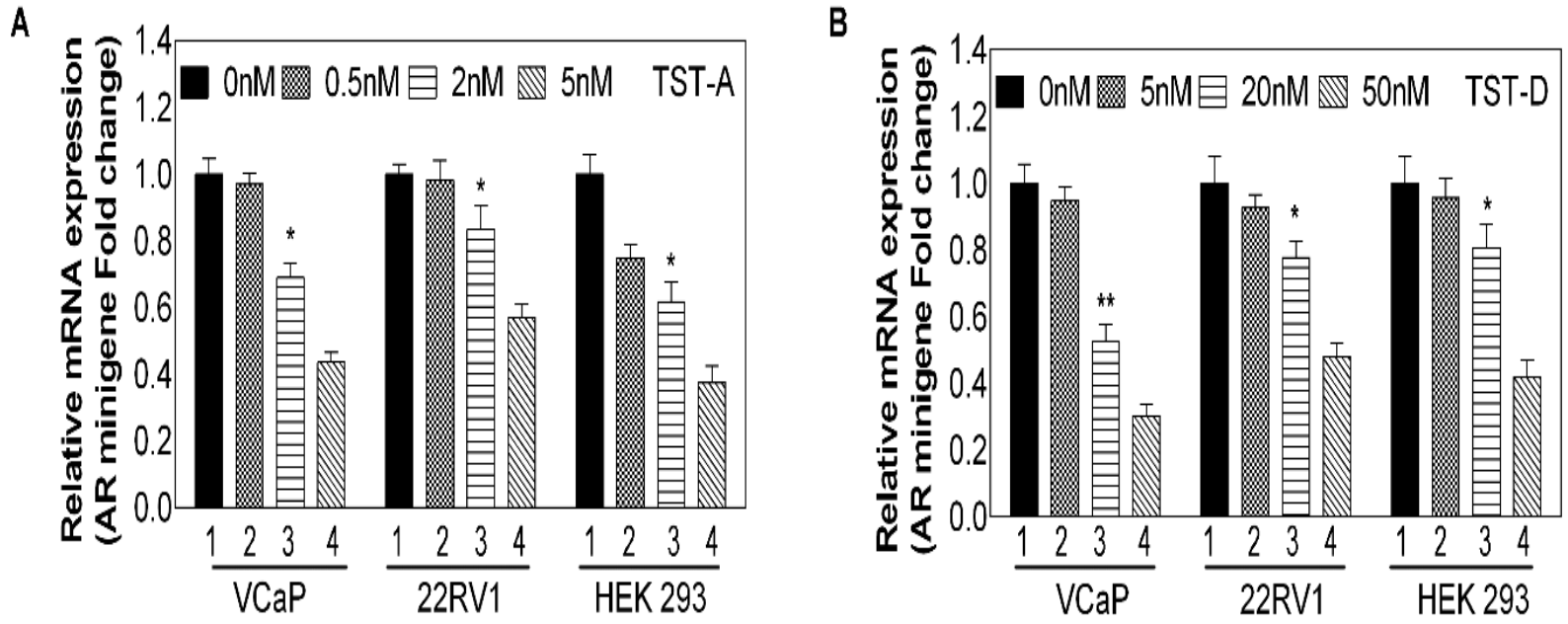

A
Relative mRNA expression
(AR minigene Fold change)
0nM
0.5nM
2nM
5nM
TST-A
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4
VCaP
22RV1
HEK 293
B
Relative mRNA expression
(AR minigene Fold change)
0nM
5nM
20nM
50nM
TST-D
VCaP
22RV1
HEK 293

Figure S6

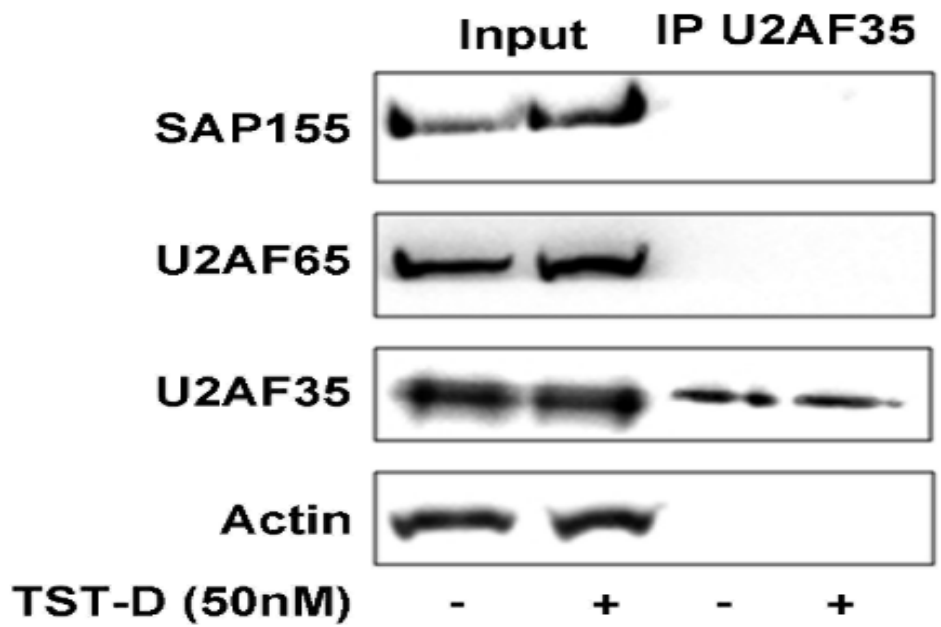
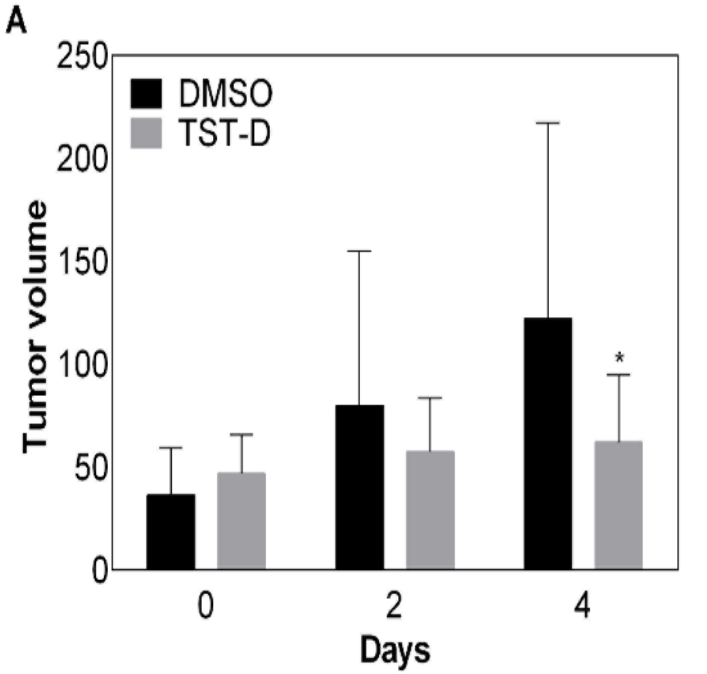
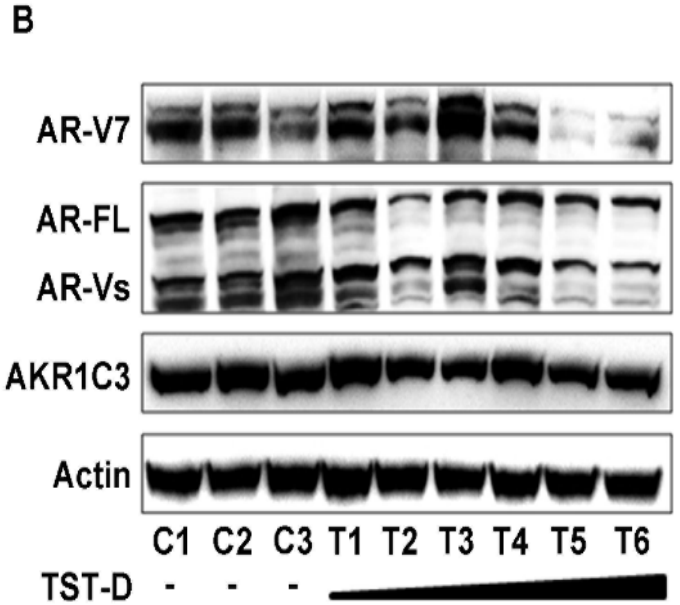


Figure S7


A
250
200
150
100
50
0
Tumor volume
DMSO
TST-D
*
0
2
4
Days


B
AR-V7
AR-FL
AR-Vs
AKR1C3
Actin
C1 C2 C3 T1 T2 T3 T4 T5 T6
TST-D - - -

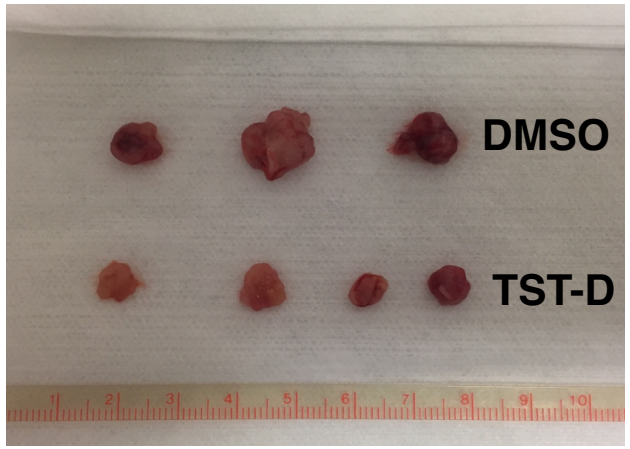

DMSO
TST-D

Figure S8