

SUPPLEMENTAL TABLES

Supplemental Table 1. Effect of EPI-001 stereoisomers on AR promoters

Compound	IC ₅₀ (uM)	PSA-Luc activity (%)	PB-Luc activity (%)	ARR3-Luc activity (%)
	mean±SD	mean±SD	mean±SD	mean±SD
EPI-001	12.63±4.33	36.91±16.46	63.96±13.70	59.56±12.58
EPI-002	7.40±1.46 *	24.33±13.63	41.58±6.63	61.00±23.48
EPI-003	17.08±3.49	33.55±11.31	54.72±7.83	66.74±22.07
EPI-004	13.78±0.66	45.16±29.13	66.44±12.47	55.93±22.06
EPI-005	10.95±4.79	24.49±13.20	43.70±9.98	37.75±10.60

*EPI-002 vs EPI-003 $P=0.00571$; and EPI-002 vs EPI-004 $P=0.00116$. Ratio Student's 2- tailed t test.

Supplemental Table 2. Effect of EPI-001 stereoisomers on proliferation of LNCaP cells

Compound	G0/G1 (%) mean \pm SD	S (%) mean \pm SD	G2/M (%) mean \pm SD
DMSO	61.85 \pm 1.98	21.65 \pm 0.70	16.09 \pm 1.37
EPI-001	75.94 \pm 2.55	9.85 \pm 2.91	13.75 \pm 0.29
EPI-002	74.44 \pm 6.74	11.72 \pm 0.43	15.06 \pm 4.18
EPI-003	76.76 \pm 7.78	9.16 \pm 4.17	13.73 \pm 3.67
EPI-004	74.09 \pm 8.24	10.48 \pm 3.66	16.43 \pm 2.37
EPI-005	74.81 \pm 5.89	11.82 \pm 3.84	12.98 \pm 2.03
Bicalutamide	77.86 \pm 3.46	9.72 \pm 2.11	12.04 \pm 1.28

n=3 separate experiments

Supplemental Table 3. IC₅₀ values of EPI analogs/probes (PSA-Luc reporter)

Analogue	IC₅₀ (uM) mean ± SD
BADGE.2H ₂ O	74.92 ± 10.41
EPI-046	3.16 ± 1.91
EPI-047	4.10 ± 2.67
EPI-051	12.60 ± 3.98
EPI-052	7.82 ± 1.11
EPI-053	6.70 ± 0.44
EPI-054	6.50 ± 0.76
EPI-055	4.17 ± 2.03
EPI-063	19.94 ± 2.66
EPI-064	7.42 ± 2.62

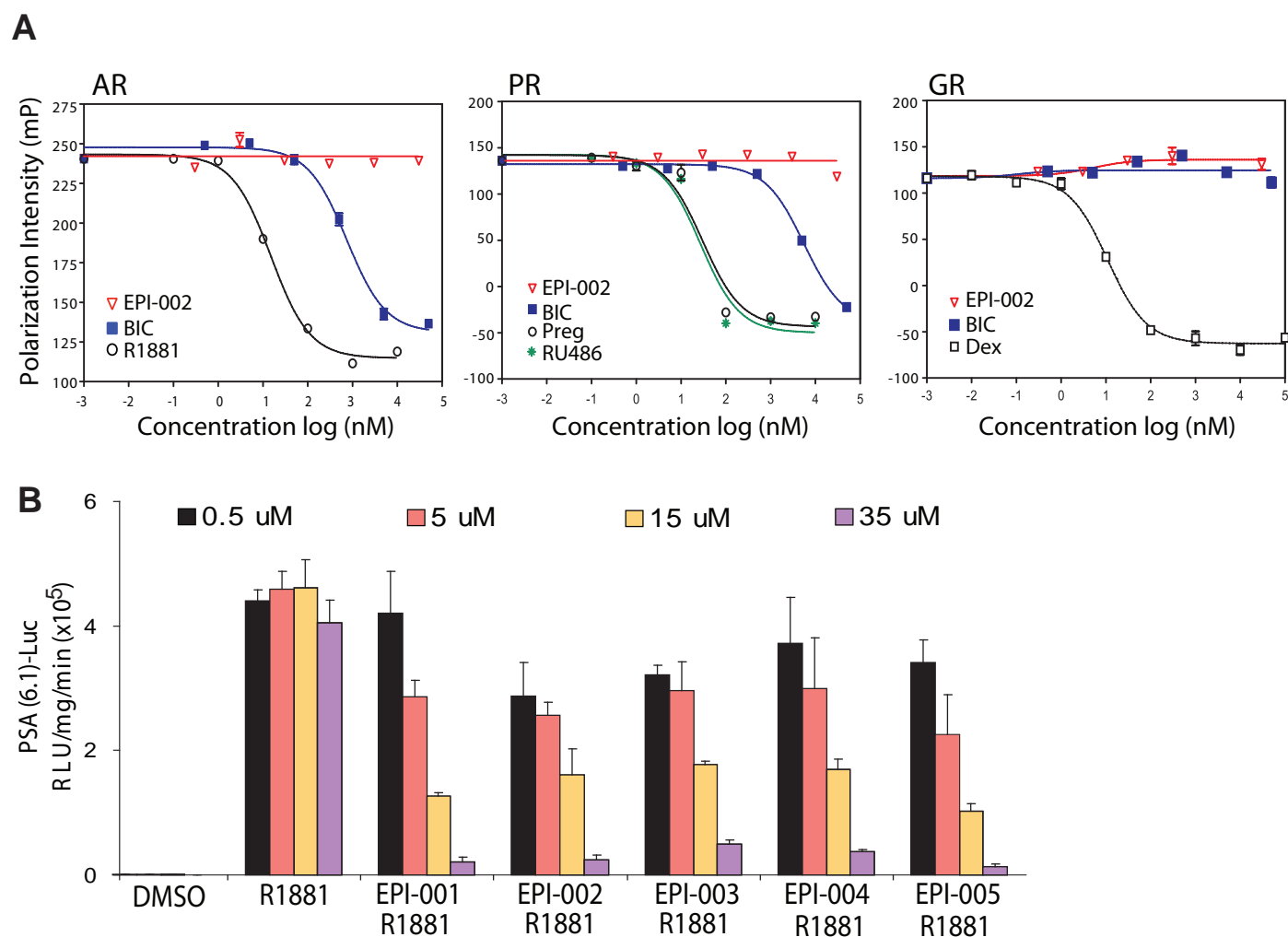
n≥3 separate experiments

Supplemental Table 4. Pharmacokinetic parameters of EPI-001

PK parameters	Units	i.v. (50mg/kg)	Oral (100mg/kg)
		mean \pm SD	mean \pm SD
AUC _{INF}	Hr*mg/L	33.65 \pm 5.15	57.71 \pm 7.53
C _{max}	mg/L	24.25 \pm 3.79	11.93 \pm 2.75
Clearance (CL)	L/hr/kg	1.51 \pm 0.23	-
Cl/F	L/hr/kg	-	1.75 \pm 0.23
Half Life	hr	3.52 \pm 1.95	3.27 \pm 0.16
Vd	L/kg	7.23 \pm 3.03	-
Vd/F	L/kg	-	8.22 \pm 0.68

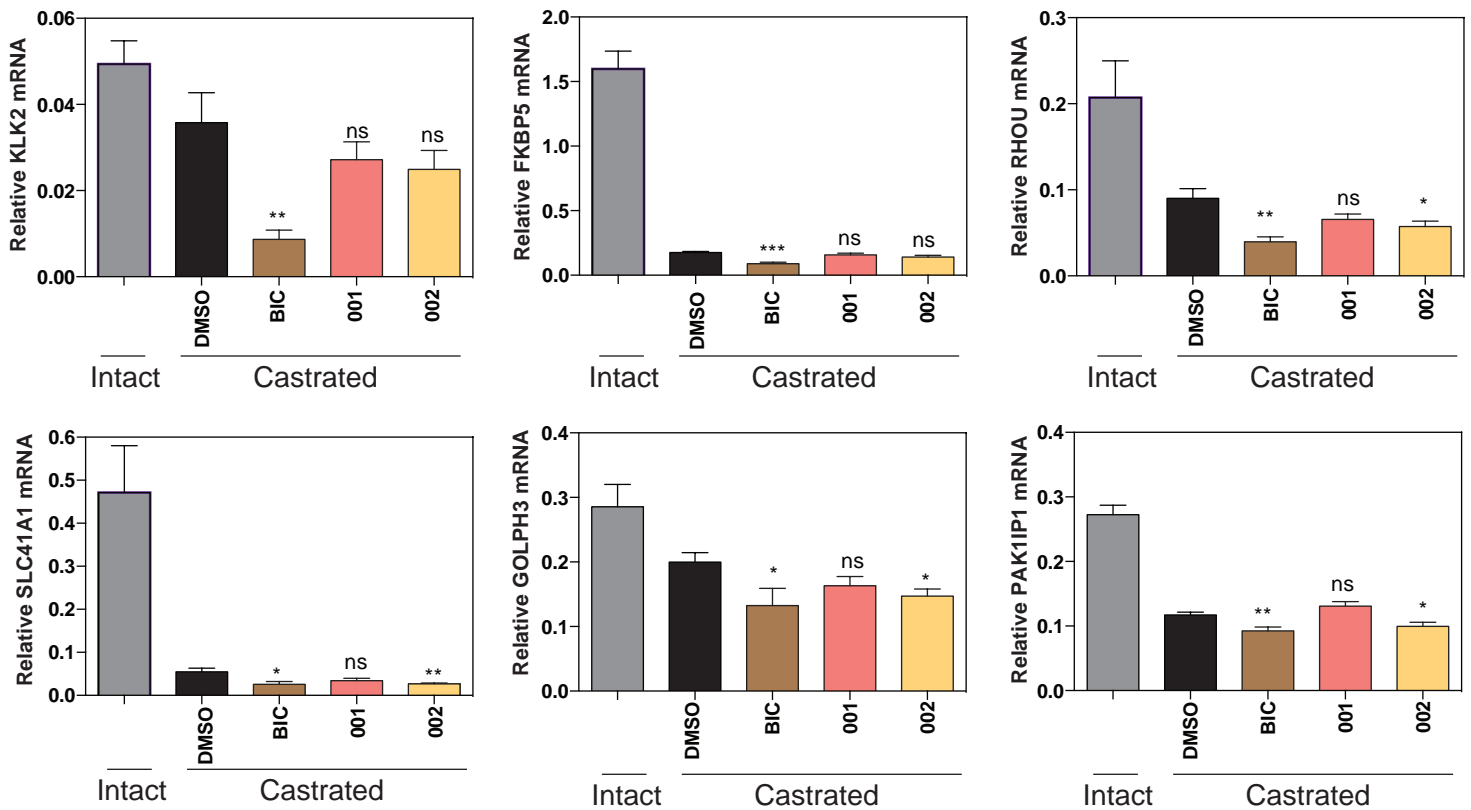
* Non-compartmental analysis using WinNonline, (Pharsight Corporation); linear trapezoidal method used. C_{max}, peak concentration; Vd, volume of distribution; AUC, area under the plasma concentration-time curve.

Supplemental Figure 1



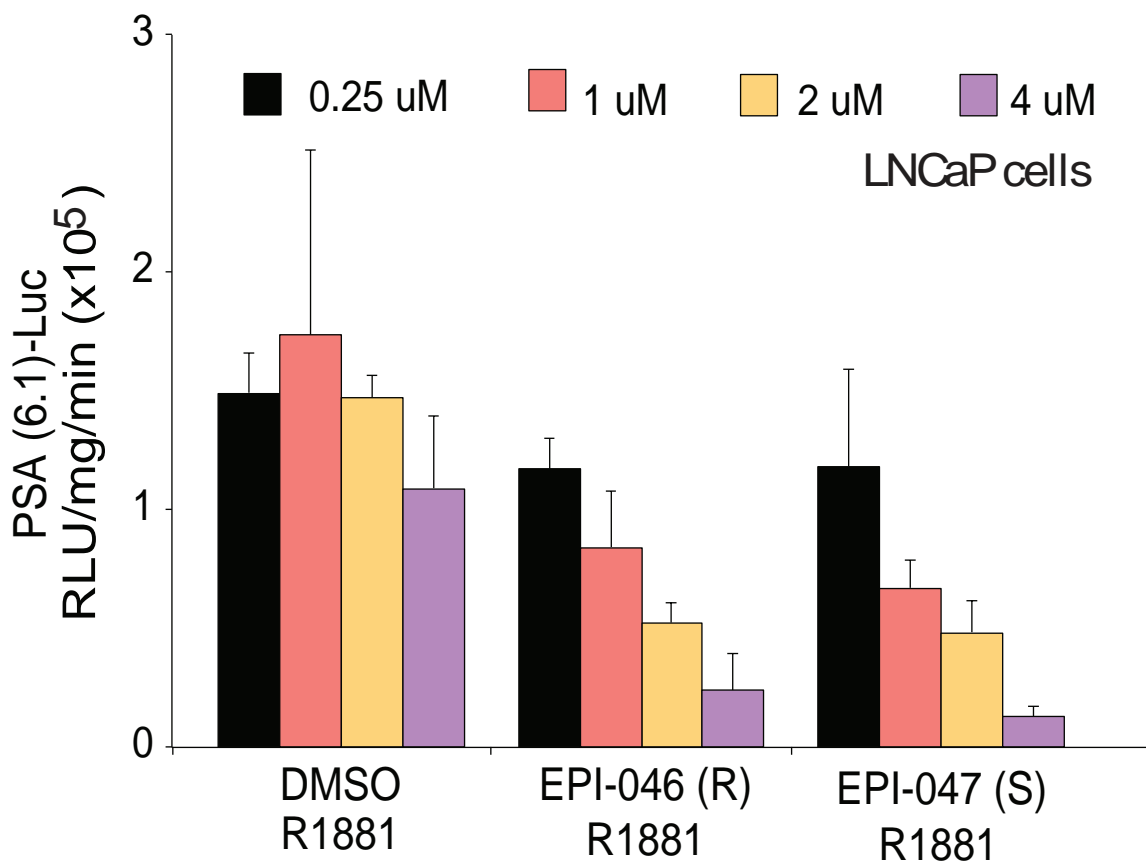
Supplemental Figure 1 (A) Competitive ligand binding curve showing the displacement of 1nM fluorescently labeled ligand from recombinant LBDs of AR, PR and GR by R1881, BIC, 4-pregnene-3,20 dione (Preg), RU486, dexamethasone (Dex), or EPI-002 using fluorescent polarization (mP) with excitation at 470 nm and emission wavelength at 535 nm. Serial dilution was performed for each test compound. Data represents the mean \pm SEM. **(B)** Dose-dependent response to EPI stereoisomers on PSA-luciferase activity in LNCaP cells treated with R1881 (1nM) and the indicated concentrations of EPI stereoisomers for 48 hours. Bars represent mean \pm SD.

Supplemental Figure 2



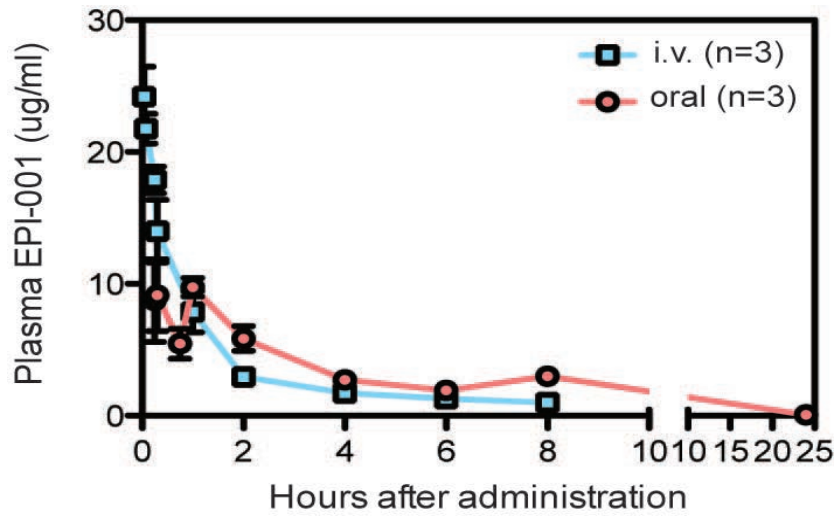
Supplemental Figure 2 Transcript levels of androgen-regulated genes expressed in LNCaP xenografts from castrated hosts employed in Figure 2E to 2H. Non-castrated controls are included (intact, n = 3). Levels of transcript of each gene were measured by qRT-PCR and normalized to *RPL13A*. Student's *t* test was performed to compare drug-treated groups to DMSO-treated in castrated mice. Bars represent the mean \pm SEM. Student's 2-tailed *t* test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) ns= not significant.

Supplemental Figure 3



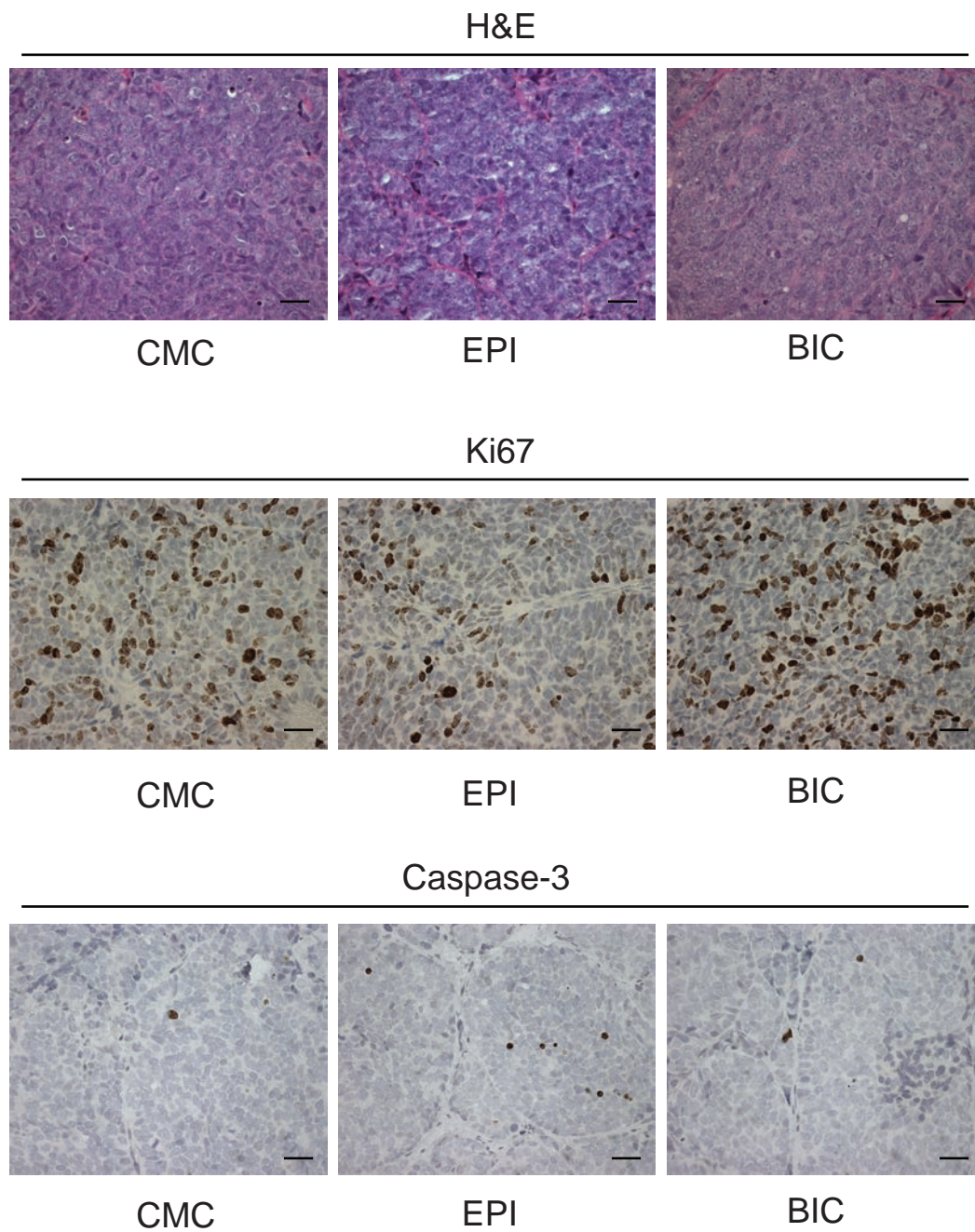
Supplemental Figure 3 Inhibition of AR transcriptional activity by modified stereoisomers of EPI-001 analogues used in Click reactions. LNCaP cells transfected with PSA (6.1kb)-luciferase reporter were treated with increasing concentrations of each probe for 1 hour, before the addition of R1881 (1nM) for an additional 48 hours. Data represent the mean \pm SD.

Supplemental Figure 4



Supplemental Figure 4 Pharmacokinetics of EPI-001. Plasma concentrations of EPI-001 after a single intravenous dose of 50 mg/kg body weight or oral dose of 100 mg/kg body weight. Values represent the mean \pm SEM from 3 mice.

Supplemental Figure 5



Supplemental Figure 5 Immunohistochemistry of representative sections of VCaP xenografts harvested at the duration of the experiment and stained for hematoxylin and eosin (H&E), Ki67 and caspase-3. Black bars = 20 μ m.