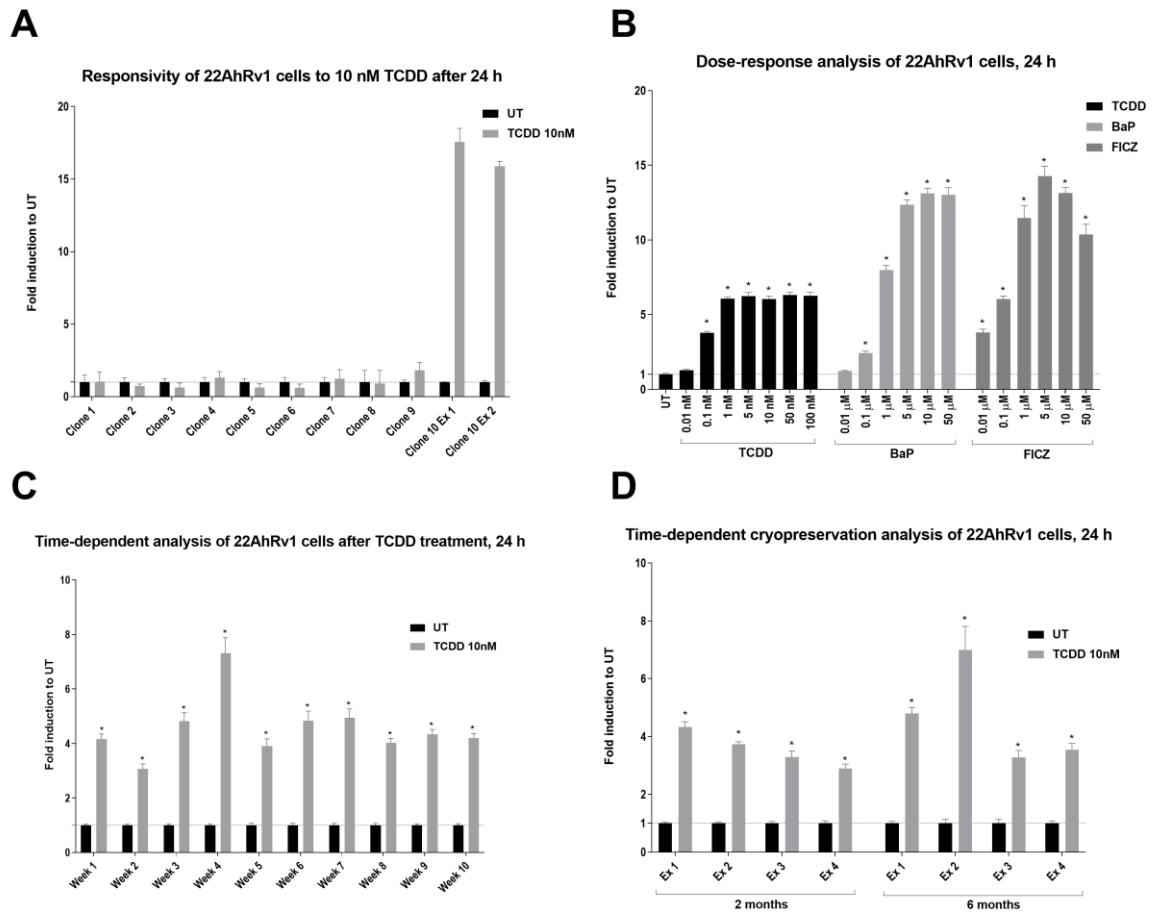
Supplement S1. Stable transfection of 22Rv1 cell line

Initially, a cellular system was established to monitor AhR transcriptional activity in the 22Rv1 cell line. Therefore, a stably transfected 22AhRv1 cell line with the luciferase reporter gene was constructed.

22Rv1 cells were transfected with the plasmid pGL4.27 [luc2P/minP/Hygro] designed by Novotna *et al.* 2011 [1]. Cells were seeded at the density 5 x 10^4 cells in 1 well of 12-well plate in 1 ml of the RPMI medium and transfected with pGL4.27-DRE reporter plasmid (200 ng per well) using FuGENE® Transfection Kit (Promega), according to protocol. Cells were maintained in transfection medium in a humidified incubator for 48 h. Subsequently, the culture medium was replaced with selection medium containing Hygromycin B (Hyg B) inhibitory concentration 25 for 4 days (IC_{25} = 60 μg/ml). The selection pressure was then increased by adding selection medium with 50 % inhibitory concentration (IC_{50} = 110 μg/ml) of HygB. The IC_{50} selection medium was renewed every 3-4 days for 4 weeks, when a polyclonal population was selected.

Subsequently, cells of the polyclonal population were seeded in 10 mm culture plates at the density of 1000 cells per plate. Cells were cultured for another 3 weeks in selection medium (IC_{50}), until colonies of the monoclonal population were visible. Monoclonal colonies were collected, labeled and tested with the Reporter gene assay (RGA) for response to AhR ligand TCDD. Ten different monoclonal populations were isolated for the 22Rv1 transfected cell line. For further characterization, clone 10 of 22Rv1 transfected cell line (titled as **22AhRv1**) was selected.

The novel prostate-specific stably transfected cell line 22AhRv1 was subjected to further characterization, such as dose-dependent response for endogenous and exogenous AhR ligands (TCDD, FICZ, B[a]P), time-dependent analysis of luciferase inducibility, and freeze-thaw cycle monitoring. The results were evaluated with Infinite M200.

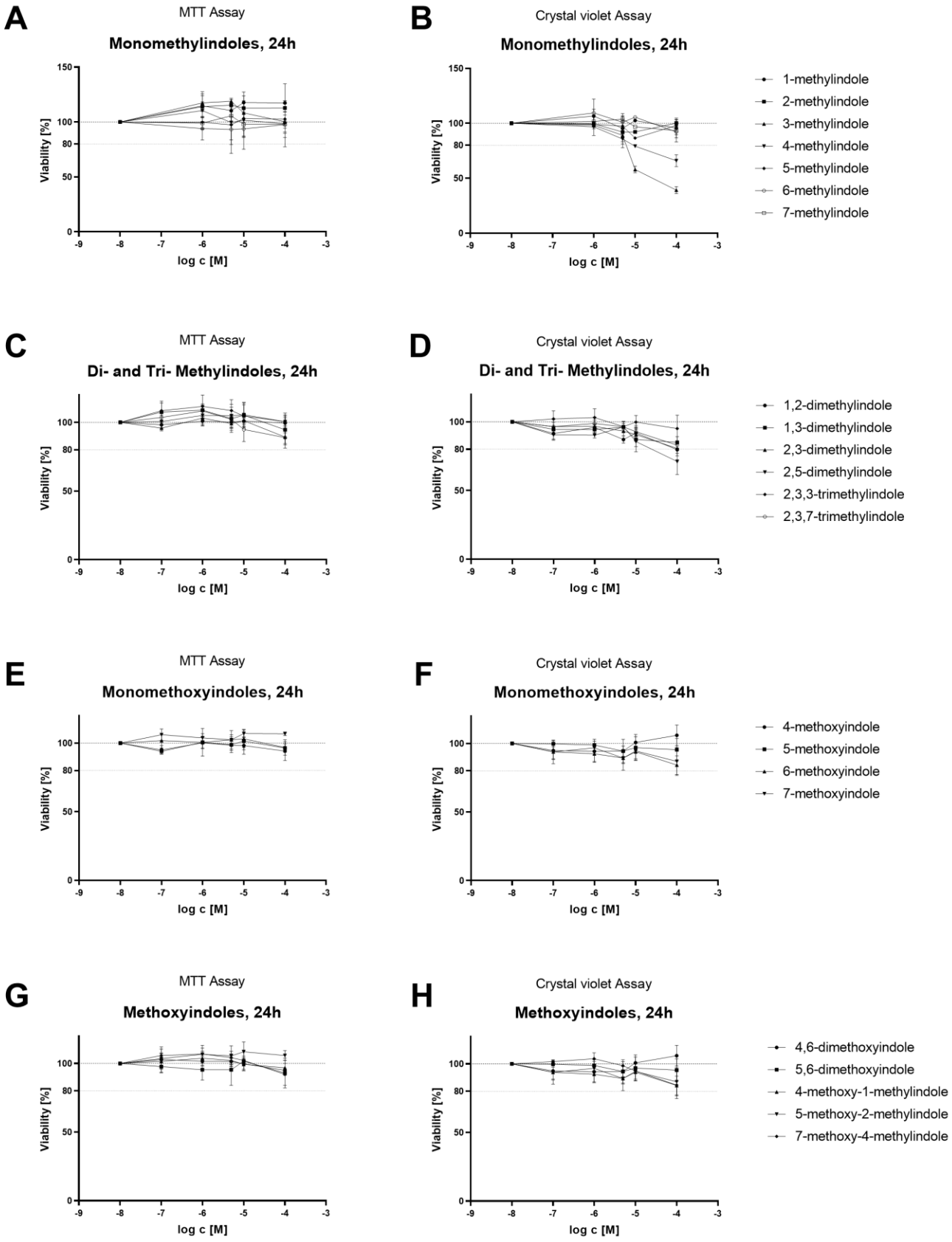


Supplement S1: **Characterization of the newly developed prostate-specific AhR reporter cell line**. 22Rv1 cells were transfected with the reporter plasmid pGL4.27 [luc2P/minP/Hygro]. Monoclonal population was isolated, labelled and tested for response to TCDD (10 nM) by RGA (A). For further characterization, Clone 10 was selected and titled as **22AhRv1** and tested for dose-dependent response of endogenous and exogenous ligands (TCDD, FICZ, B[a]P) of AhR (B). 22AhRv1 cell line was further evaluated for long-term stability of luciferase inducibility (C) and freeze-thaw cycles (D). The results are expressed as fold induction to untreated (UT; DMSO-treated cells).

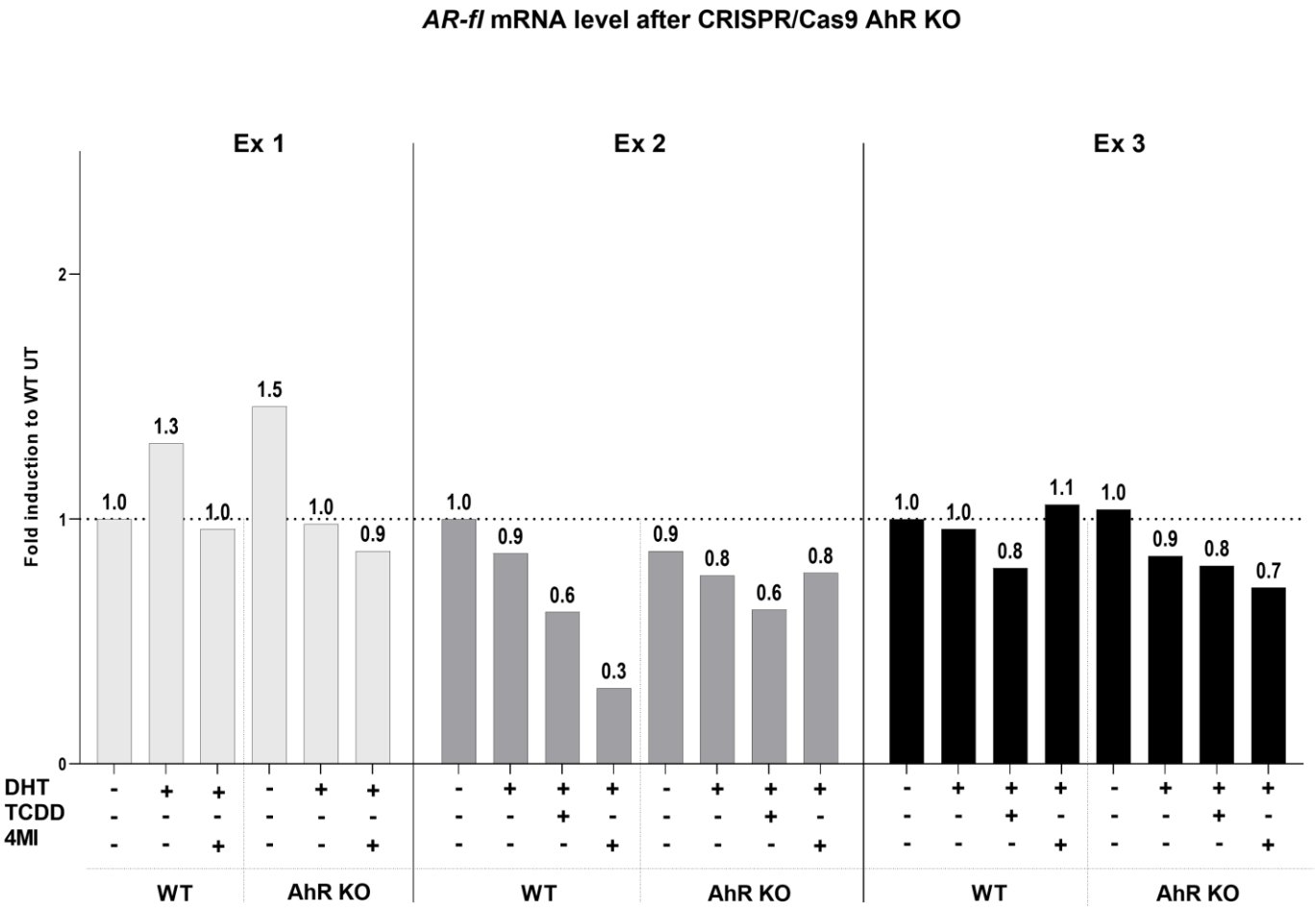
Supplement S2. Cytotoxycity and proliferation assays

22Rv1 cells were seeded in 96-well plates at a density of 2.5 x 10^4 cells per well in volume 200 μl and stabilized until the next day. Subsequently, cells were treated with increasing concentrations (final concentration 1-100 μM) of the tested indoles (***Table 2***) and controls – DMSO (untreated/UT; 0.1 %; v/v) or doxorubicin (positive control; final concentration 20 μM) for 24 h.

a) Then, an MTT cytotoxicity assay was performed. MTT in RPMI medium in final concentration 0.3 mg/ml was used. The formazan crystals were diluted in DMSO after 30-40 min of incubation. Then, the absorbance was measured at 595 nm with Infinite M200 (TECAN, Austria).
b) Thereafter, a Crystal violet proliferation assay was performed. The medium was replaced with Crystal violet solution (0.5 %; v/v) and incubated on Rocker-Shaker (Biosan) for 1 h. After that, the aqueous solution was removed and the cells were washed five times with 1x PBS. The dye was dissolved in 200 μl of methanol and measured at 595 nm with Infinite M200 (TECAN, Austria).

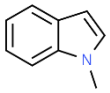
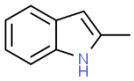
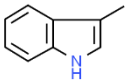
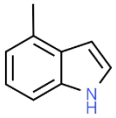
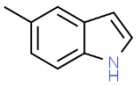
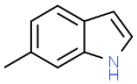
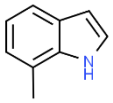
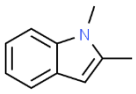
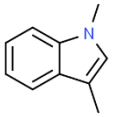
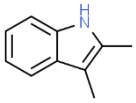
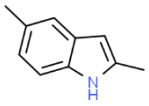
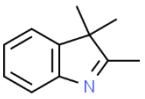


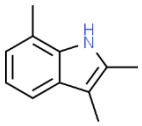
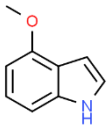
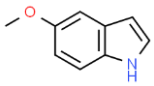
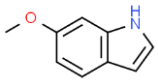
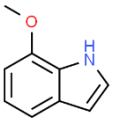
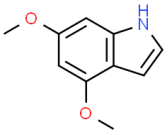
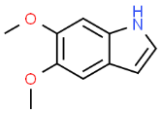
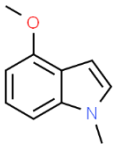
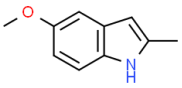
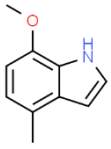
Supplement S2: Cytotoxicity of tested indoles. 22Rv1 cells were incubated for 24 with indoles in the concentration range from 0.1 μM to 100 μM or controls (UT; doxorubicin 20 μM). The cytotoxic effects were then evaluated using MTT or crystal violet assays. All 22 tested indoles were divided into groups titled as Monomethylindoles (A, B), Di- and Tri-Methylindoles (C, D), Monomethoxyindoles (E, F), and Methoxyindoles (G, H). The MTT assay results are given in the left column and the crystal violet assay results are given in the right column. Results are expressed in % of untreated (UT; DMSO-treated) cells.



Supplement S3. Expression of *AR-fl* mRNA CRISPR/Cas9 AhR knockout. 22Rv1 cells were transiently transfected with the CRISPR/Cas 9 AhR knockout plasmid or the Control CRISPR/Cas9 plasmid. After 48 h, transfected cells were treated with controls (UT; TCDD 10 nM; DHT 10 nM) and 4 MI 100 μM. The induction of *AR-fl* was determined by RT-qPCR. The data obtained were normalized per housekeeping gene *GAPDH*. The results are expressed in fold induction to wild-type UT. In total, 3 independent experiments were analysed.

Supplement S4: Key structures of tested indoles

Compound	Molecular formula	Structure
1-methylindole	C_9H_9N	
2-methylindole	C_9H_9N	
3-methylindole	C_9H_9N	
4-methylindole	C_9H_9N	
5-methylindole	C_9H_9N	
6-methylindole	C_9H_9N	
7-methylindole	C_9H_9N	
1,2-methylindole	$C_{10}H_{11}N$	
1,3-methylindole	$C_{10}H_{11}N$	
2,3-methylindole	$C_{10}H_{11}N$	
2,5-methylindole	$C_{10}H_{11}N$	
2,3,3-trimethylindolenine	$C_{11}H_{13}N$	

2,3,7-trimethylindole	$C_{11}H_{13}N$	
4-methoxyindole	C_9H_9NO	
5-methoxyindole	C_9H_9NO	
6-methoxyindole	C_9H_9NO	
7-methoxyindole	C_9H_9NO	
4,6-dimethoxyindole	$C_{10}H_{11}NO_2$	
5,6-dimethoxyindole	$C_{10}H_{11}NO_2$	
4-methoxy-1-methylindole	$C_{10}H_{11}NO$	
5-methoxy-2-methylindole	$C_{10}H_{11}NO$	
7-methoxy-4-methylindole	$C_{10}H_{11}NO$	

Supplement S5: List of used chemicals.

Product	Manufacturer	Catalog #	Lot / Batch #
DMSO	Sigma	D4540	BCCB6907
DMSO pure for MTT	Lach-Ner	20022-CT0	PP/2020/01579
Dulbecco's PBS	Serana	BDL-001	01070619BDL, 01071721BDL, 01041720BDL
Ethyl Alcohol, 99,8%	Lach-Ner	20025-A99	PP/2017/09915
Fetal Bovine Serum	Sigma Aldrich	F7524	1640522, 1638262
Hygromycin B	Santa cruz Biotechnology	sc-29067	H2319
L-Glutamine	Serana	RGL-001	01061019RGL, 13100619RGL
MEM Non-essential amino acid solution	Sigma Aldrich	M7145	RNBJ0369, RBNK8956
Methyl Alcohol	Penta Chemicals	21210-20005	2505130520
MycoAlert Mycoplasma Detection Kit	Lonza	LT07-118	921989
RPMI-1640	Sigma Aldrich	R0883	RNBH9726, RNBK9935, RNBL0672, RNBK0723
Trypan Blue cell culture tested	Sigma	T9650	SLBV4976
Trypsin 0.25% - EDTA in HBSS	Biosera	MS00CY100B	LM-T1720/500
Water, Nuclease-free	Sigma Aldrich	W4502	1003379140
Reporter Gene Assay	**Manufacturer**	**Catalog #**	**Lot / Batch #**
Adenosine 5'-triphoshpate disodium salt hydrate	Sigma Aldrich	A2383-5G	SLBD2725V
Coenzyme A sodium salt hydrate	Sigma Aldrich	C4780-100MG	SLCB8868
D-Luciferin	Sigma Aldrich	L9504-50MG	SLBS2450V
DTT	Sigma Aldrich	43819	BCCB7858
Ethylenediaminetetraacetic Acid	Sigma Aldrich	E9884	BCBN2979V
MgSO4 x 7 H2O	LACHEMA		403
Reporter Lysis 5x Buffer	Promega	E3971	329638, 413170, 398845
Tris-Acetate-EDTA Buffer	Sigma Aldrich	T9650	SLBV4976
Cytotoxicity Assays	**Manufacturer**	**Catalog #**	**Lot / Batch #**
Crystal Violet 1%	Sigma Aldrich	V5265-500ML	SCLF5128
Doxorubicin	PromoCell GmbH	PK-CA577-K329	452PO98-1
Thiazolyl Blue Tetrazolium Bromide (MTT)	Sigma Aldrich	MT2128	MKCD8033
RNA Isolation and Reverse Transcription	**Manufacturer**	**Catalog #**	**Lot / Batch #**
10x M-MuLV Reaction Buffer	BioLabs ® Inc.	B0253S	10058397, 10104588
dATP (2'-deoxyadenosine 5'-triphosphate)	TaKaRa	4026	B7701A
dCTP (2'-deoxycytidine 5'-triphosphate)	TaKaRa	4028	B7901A
dGTP (2'-deoxyguanosine 5'-triphosphate)	TaKaRa	4027	B8001A
dTTP (thymidine 5'-triphosphate)	TaKaRa	4029	B7501A
Chloroform	Sigma Aldrich	C2432	SHBD5858V

Isopropyl Alcohol	Lach-Ner	20037-AT0	PP/2017/03711
M-MuLV (reverse transcriptase)	BioLabs ® Inc.	M0253L	10099097, 10111985
Random Primers 6	BioLabs ® Inc.	S1230S	10058397, 1014737, 10102123
RNAse inhibitor	BioLabs ® Inc.	M0307L	10115397
Tri Reagent ® - RNA/DNA/Protein isolation reagent	Molecular Research Center, inc	TR118	7111
RT-qPCR	**Manufacturer**	**Catalog #**	**Lot / Batch #**
AhR Forward Primer (FH1_AhR)	Sigma Aldrich	8815864959-210/0	ST04825127-001
AhR Probe (PH1_AhR)	Sigma Aldrich	8815864959-210/2	ST04825129-002
AhR Reverse Primer (RH1_AhR)	Sigma Aldrich	8815864959-210/1	ST04825128-001
AhRR Forward Primer (FH1_AhRR)	Sigma Aldrich	8815864959-260/0	ST04825142-001
AhRR Probe (PH1_AhRR)	Sigma Aldrich	8815864959-260/2	ST04825144-002
AhRR Reverse Primer (RH1_AhRR)	Sigma Aldrich	8815864959-260/1	ST04825143-001
AR Forward Primer (FH1_AR)	Sigma Aldrich	8815864959-150/0	ST04825109-001
AR Probe (PH1_AR)	Sigma Aldrich	8815864959-150/2	ST04825111-002
AR Reverse Primer (RH1_AR)	Sigma Aldrich	8815864959-150/1	ST04825110-001
AR v7 Forward Primer (FH1_ARv7)	Sigma Aldrich	8816914473-000010	SY210541259-025
ARv7 Reverse Primer (RH1_ARv7)	Sigma Aldrich	8816914473-000020	SY210541259-026
CYP1A1 Forward Primer (FH1_CYP1A1)	Sigma Aldrich	8815746405-10/0	ST04795193-004
CYP1A1 Probe (PH1_CYP1A1)	Sigma Aldrich	8815746405-10/2	ST04795194-004
CYP1A1 Reverse Primer (RH1_CYP1A1)	Sigma Aldrich	8815746405-10/1	ST04795192-004
FKBP5 Forward Primer (FH1_FKBP5)	Sigma Aldrich	8815864959-280/0	ST04825148-001
FKBP5 Probe (PH1_FKBP5)	Sigma Aldrich	8815864959-280/2	ST04825150-002
FKBP5 Reverse Primer (RH1_FKBP5)	Sigma Aldrich	8815864959-280/1	ST04825149-001
GAPDH Forward Primer (FH1_GAPDH)	Sigma Aldrich	8817264953-40/0	ST04780378-001
GAPDH Probe (PH1_GAPDH)	Sigma Aldrich	8817264953-40/2	ST04780380-002
GAPDH Reverse Primer (RH1_GAPDH)	Sigma Aldrich	8817264953-40/1	ST04780379-001
KiCqStart Probe qPCR ReadyMix	Sigma Aldrich	KCQS04	LN Q66191473, LN Q66184201
KLK3 Forward Primer (FH1_KLK3)	Sigma Aldrich	8815864959-240/0	ST04825136-001
KLK3 Probe (PH1_KLK3)	Sigma Aldrich	8815864959-240/2	ST04825138-002
KLK3 Reverse Primer (RH1_KLK3)	Sigma Aldrich	8815864959-240/1	ST04825137-001
LightCycler ® 480 SYBR® Green I Master	Roche	4707516001	
UBE2C Forward Primer (FH1_UBE2C)	Sigma Aldrich	8815864959-270/0	ST04825145-001
UBE2C Probe (PH1_UBE2C)	Sigma Aldrich	8815864959-270/2	ST04825147-002
UBE2C Reverse Primer (RH1_UBE2C)	Sigma Aldrich	8815864959-270/1	ST04825146-001
Protein Isolation	**Manufacturer**	**Catalog #**	**Lot / Batch #**
HEPES	Sigma Aldrich	H3375	BLBX2657

Bradford Reagent	Sigma Aldrich	B6916	SLCK1933
Halt Protease Inhibitor Single-Use Cocktail	ThermoFisher Scientific	78430	VG297201
PhosSTOP EASYpack	Roche	4906837001	62344300
Sodium chloride	LachNer	30093-AP0	PP/2014/02150
Triton X-100	Sigma Aldrich	T9284	1003426861
Western Blotting	**Manufacturer**	**Catalog #**	**Lot / Batch #**
Re-Blot Plus Strong Solution (10x)	Sigma Aldrich	2504	3286474, 3614274
10x Tris/Glycine Buffer	BioRad	1610771	64337786
10x Tris/Glycine/SDS Buffer	BioRad	1610732	64467353
20x TBS Buffer	ThermoFisher Scientific	28358	WD322613
Acetic acid, 99.8%	Penta Chemicals	607-002-00-6	1812061213
Acrylamide/Bis-acrylamide	Sigma Aldrich	A2792	SLCJ1949
Ah Receptor antibody (A-3)	Santa cruz Biotechnology	sc-133088	K0920
Amonium Persulfate, for electrophoresis	Sigma Aldrich	A3678	MKBC7986
Anti-mouse HRP-linked antibody	Cell Signaling	7076S	36
AR antibody (441)	Santa cruz Biotechnology	sc-7305	A2020
Bovine Serum Albumin	Sigma Aldrich	A2153	SCJ7629
Bromphenol Blue	Sigma Aldrich	B0126	79696HJV
Glycerol	Lach-Ner	40058-A50	PP/2017/02241
Immobilon® -P Transfer Membrane	Sigma Aldrich	IPVH00010	180320
Non-fat dried milk Laktino	ARTIFEX Instant s.r.o.		CZ 17847 ES
Ponceau S. solution	Santa cruz Biotechnology	sc-301558	A0421
Running buffer	BioRad	1610798	L004167A
Sodium Dodecyl Sulfate	Sigma Aldrich	L3771	SLBH870V
Stacking buffer	BioRad	1610799	L004168A
TEMED	Sigma Aldrich	110732	K51567332.928
Tween 20	Sigma Aldrich	P1379	SZBC1240V
WesternSure ® PREMIUM Chemiluminescent Substrate	Li-Cor	926-95000	VH311910, VI311909, UH293688, UI293689
WesternSure® Pre-Stained Chemiluminiscent Protein Ladder	Li-Cor	926-98000	D10827-01, D00227-01
Chromatin Immunoprecipitation	**Manufacturer**	**Catalog #**	**Lot / Batch #**
100bp DNA Ladder	Promega	G210A	456865
Agarose	Serva	11380.02	170138
Agarose, LMP	Promega	V3841	336732
Androgen Receptor D6F1I (rabbit mAb)	Cell Signaling	5153S	9
Anti-rabbit IgG	Cell Signaling	7074P2	29

Blue/Orange 6x Loading Dye	Promega	G190A	455992
GelRed® Nucleic Acid Stain	Biotium	41003	21G1020
ChIP-Grade Protein G Magnetic Beads	Cell Signaling	9006	25
Micrococcal Nuclease	Cell Signaling	10011S	21
Proteinase K	ThermoFisher Scientific	4333793	10860221
SimpleChIP® Plus Enzymatic Chromatin IP Kit	Cell Signaling	9005	
SimpleChIP® KLK3 Promoter Primers	Cell Signaling	32784S	1
Ultra Low Range DNA Ladder	Invitrogen	10597012	538912
Transfection and CRISPR/Cas9 reagents	**Manufacturer**	**Catalog #**	**Lot / Batch #**
FuGENE ® HD - transfection reagent	Promega	E231A	216863
Ah Receptor CRISPR/Cas9 KO Plasmid (h)	Santa Cruz Biotechnology	sc-400297	F2619
Control CRISPR/Cas9 Plasmid	Santa Cruz Biotechnology	sc-418922	J2120
Plasmid Transfection Medium	Santa Cruz Biotechnology	sc-108062	J1920
UltraCruz® Transfection Reagent	Santa Cruz Biotechnology	sc-395739	H1220

Compound	**Manufacturer**	**Catalog #**	**Lot / Batch #**
1-methylindole	Sigma Aldrich	193984	BGBC4251V
2-methylindole	Sigma Aldrich	M51407	BCBF7494V
3-methylindole	Sigma Aldrich	M51458	STBK2264
4-methylindole	Energy Chemical	E020483	AH160074
5-methylindole	Sigma Aldrich	222410	STBF3756V
6-methylindole	Sigma Aldrich	246328	MKBPJ2514V
7-methylindole	Sigma Aldrich	M51490	MKBP5287V
1,2-di-methylindole	Sigma Aldrich	D165603	S43286V
1,3-di-methylindole	Shanghai SINKH Pharmaceuticals Tech. Co., Ltd.	875-30-9	
2,3-di-methylindole	Sigma Aldrich	120812	STBF0465V
2,5-di-methylindole	Sigma Aldrich	D166006	1408950V
2,3,3-tri-methylindolenine	Sigma Aldrich	T76805	STBF9768V
2,3,7-tri-methylindole	Sigma Aldrich	CDS014194	B02665235
4-methoxyindole	Sigma Aldrich	246298	STBF5169V
5-methoxyindole	Sigma Aldrich	M14900	10206LHV
6-methoxyindole	Sigma Aldrich	139858	MKBD1228V
7-methoxyindole	Sigma Aldrich	SC254908	1430903V
4,6-di-methoxyindole	Sigma Aldrich	75066-1G-F	BCBF8458V
5,6-di-methoxyindole	Sigma Aldrich	246255	MKD30455V
4-methoxy-1-methylindole	Sigma Aldrich	259055	08827AD

5-methoxy-2-methylindole	Sigma Aldrich	M15451	STBD7007V
7-methoxy-4-methylindole	1ClickChemistry Inc	1C72757	55484A
BaP (Beno[a]pyrene)	Sigma Aldrich	B1760	
DHT (5α-Androstan-17β-ol-3-one)	Sigma Aldrich	A8380	
ENZ (MDV3100)	Santa cruz Biotechnology	sc-364354	I1021
FICZ (6-Formylindolo[3,2-b]carbazole)	Sigma Aldrich	SML1489	26019
TCDD	Ultra Scientific	RPE-029	

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