1 Supplementary Information

1.1 Sequence alignments and phylogenetic analysis

GenBank accession numbers for VDR proteins used for phylogenetic analyses: Mexican tetra (*Astyanax mexicanus*) Vdra (XP_007240874), Vdrb (XP_022531506.1), elephant shark (*Callorhinchus milii*) Vdr (XP_007908698.1), tongue sole (*Cynoglossus semilaevis*) Vdra (XP_008316613.1), Vdrb (XP_008318450.1), sheepshead minnow (*Cyprinodon variegatus*) Vdra (XP_015248871.1), Vdrb (XP_015258624.1), zebrafish (*Danio rerio*) Vdra (Q9PTN2.2), Vdrb (NP_001153457.1), mummichog (*Fundulus heteroclitus*) Vdra (XP_012717416.1), Vdrb (XP_012725968.2), chicken (*Gallus gallus*) VDR (XP_015128089.1), stickleback (*Gasterosteus aculeatus*) Vdra (AIX95267.1), Vdrb (AIX95268.1), human (*Homo sapiens*) VDR (NP_000367.1), ballan wrasse (*Labris bergylta*) Vdra (XP_020491649.1), Vdrb (XP_020502555.1), mouse (*Mus musculus*) VDR (NP_033530.2), rainbow trout (*Oncorhynchus mykiss*) Vdra1 (XP_021471952.1), Vdra2 (XP_021421982.1), Vdrb1 (XP_021466275.1), Vdrb2 (XP_021424248.1), Japanese medaka (*Oryzias latipes*) Vdra (NP_001121988.1), Vdrb (NP_001121989.1), lamprey (*Petromyzon marinus*) Vdr (XP_032821301.1), rat (*Rattus norvegicus*) VDR (EDL87095.1), Atlantic salmon (*Salmo salar*) Vdra1 (XP_014001846.1), Vdra2 (XP_013990932.1), Vdrb1 (XP_014023471.1), Vdrb2 (XP_013987594.1), river trout (*Salmo trutta*) Vdra1 (XP_029575114.1), Vdra2 (XP_029579034.1), Vdrb1 (XP_029549000.1), Vdrb2 (XP_029632121.1), turbot (*Scophthalmus maximus*) Vdra (AWP09263.1), Vdrb (AWP03123.1), greater amberjack (*Seriola dumerili*) (XP_022613231.1) Vdra (XP_022613231.1), Vdrb (XP_022600922.1), gilthead seabream (*Sparus aurata*) Vdra (XP_030279396.1), Vdrb (XP_030276225.1), frog (*Xenopus laevis*) VDR (NP_001079288.1). Vdra and Vdrb sequences for haddock

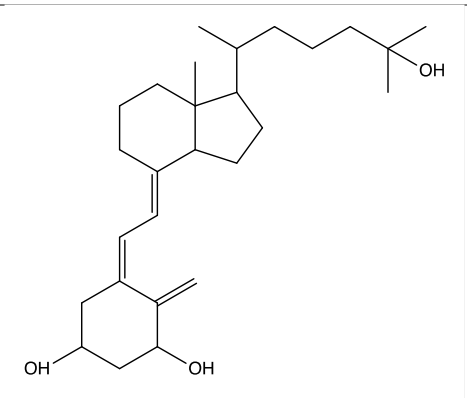
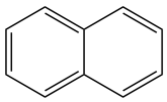
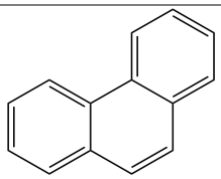
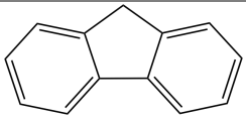
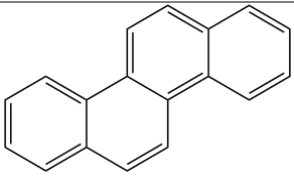
(*Melanogrammus aeglefinus*) was retrieved by blasting the genome (GenBank ID: OLKM01000001.1) to Atlantic cod sequences.

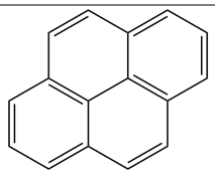
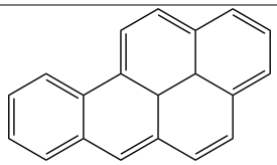
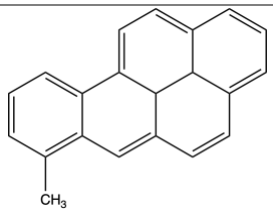
1.2 Phylogenetic three analysis

Phylogenetic tree was constructed in MrBayes 3.2.6 with 300000 generations for each 1000 samples with a 25% burn-in Med Markov Chain Monte Carlo (MCMC) with mixed models.

1.3 Chemicals

Table S1. Name, CAS-number and structure of calcitriol and PAHs used in the study.

Compound	CAS-number	Structure
Calcitriol		
Naphthalene	91-20-3	
Phenanthrene	85-01-8	
Fluorene	86-73-7	
Chrysene	218-01-9	

Pyrene	129-00-0	
Benzo(a)pyrene	50-32-8	
7-methylbenzo(a)pyrene	63041-77-0	

1.4 qPCR and cloning details

For *gmvdra* and *gmvdrb*, 5 µl of cDNA (diluted 1:5), 500 nM of forward and reverse primers, and 10 µl of SYBR Green Master I (Roche Applied Sciences, Basel, Switzerland) constituting a total of 20 µl reaction mix, was added to the qPCR plate. For the reference genes (*uba52* and *rplp1*), the cDNA was diluted 1:50 in the qPCR reactions due to higher abundances of these genes in the tissue samples. The reactions were run in a CFX96 Touch Real-Time PCR Detection System (BioRad) following these conditions: 3 min 95°C, 40 cycles (15 sec 95°C, 30 sec 55°C, 30 sec 72°C), 30 sec 72°C, and a melt curve were recorded at the end of the run. A no reverse transcriptase control (NRT) and a no template control (NTC) were included for each primer combination. The qPCR was performed with technical triplicates for each fish. Amplification efficiency was calculated for each primer set from a standard curve prepared with gel-purified qPCR products as templates.

Table S2. Overview of primers used for amplification of Atlantic cod *vdr* sequences from Atlantic cod cDNA.

Primer name	Primer sequence (5´ - 3´)	Target
gmVdr fwd	GTGGACATCGGCATGATGAAGG	Atlantic cod Vdr
gmVdr rev	CTAGGAGACCTCGCTGCCGAAC	
gmVdr EcoRI fwd	GGAATTCGTGGACATCGGCATGATGAAGG	pCMX-Gal4
gmVdr BamHI rev	GGATCCCTAGGAGACCTCGCTGCCGAAC	pCMX-Gal4

Table S3. Overview of the different primers used in tissue-specific quantitative real time PCR analysis in Atlantic cod.

Gene	Accession number (GenBank)	Forward primer (5´ - 3´)	Reverse primer (5´ - 3´)	Amplicon size (bp)	PCR Eff.
gmvdra	MT344110	CCGACTTCAAGTACTGCGTCAA	CCGGACGGTCAGGGGAGA	162	1.98
gmvdrb	MT344111	CACAAGACCTTCGACGACTCC	GACAGAGAGTGGAGGGAAGCA	110	1.96
uba52	EX735613	GGCCGCAAAGATGCAGAT	CTGGGCTCGACCTCAAGAGT	69	1.91
rplp1	EX741373	TGATCCTCCACGACGATGAG	CAGGGCCTTGGCGAAGA	113	1.94

1.5 VDR sequence comparison

Table S4. Amino acid similarities and identities of full-length Atlantic cod (gm), zebrafish (dr), and human (hs) vitamin D receptor protein sequences.

	Sequence similarity		Sequence identity	
	gmVdra	gmVdrb	gmVdra	gmVdrb
gmVdra	-	91%	-	87.0%
gmVdrb	91%	-	87.0%	-
drVdra	88%	86.2%	84%	-
drVdrb	-	93%	-	89%
hsVDR	81%	79%	71%	70%

1.6 Tissue specific expression of *gmvdra* and *gmvdrb* in male Atlantic cod

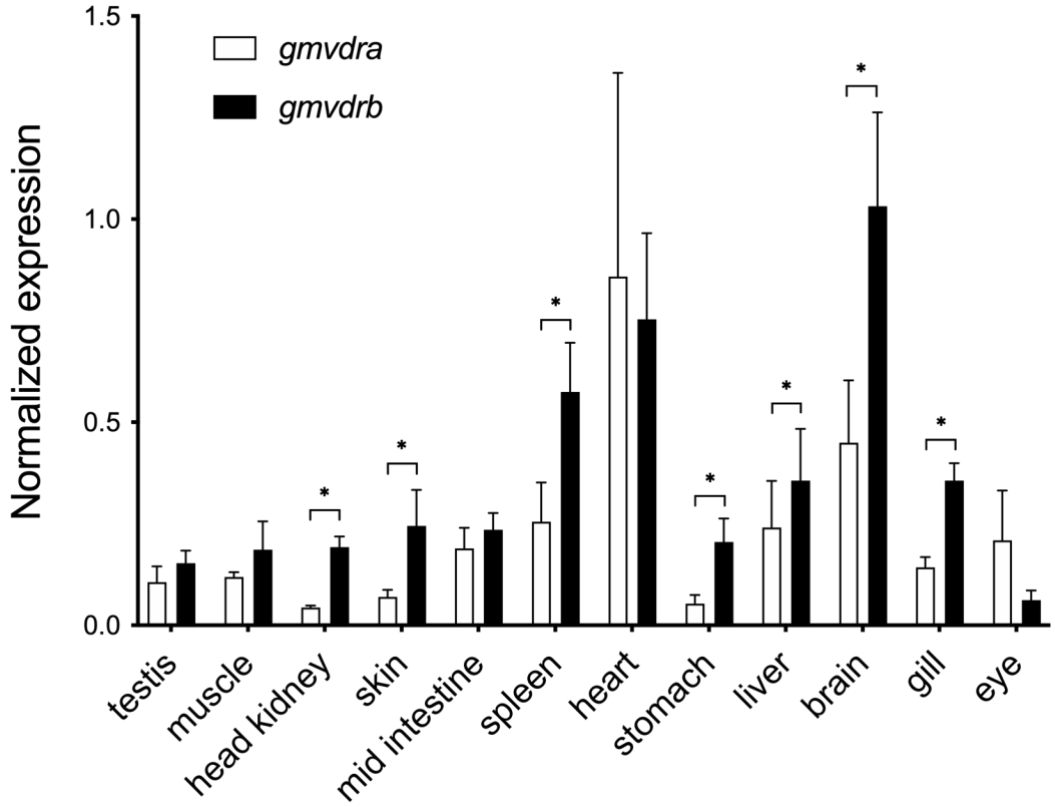


Figure S1. Tissue specific distribution of *gmvdra* and *gmvdrb* transcripts in mature Atlantic cod males. qPCR was used to assess the expression levels of *gmvdr* paralogs in testis, muscle, head kidney, skin, mid intestine, spleen, heart, stomach, liver, brain, gill, and eye obtained from mature Atlantic cod males (n=4). Expression levels were normalized against the reference genes *ubi* and *arp*. Data is presented as normalized expression ± SEM. Statistical differences (* = $p < 0.05$) in expression levels between *gmvdra* and *gmvdrb* were assessed using paired t-test on log2-transformed data.

1.7 Luciferase reporter gene assay, agonistic test

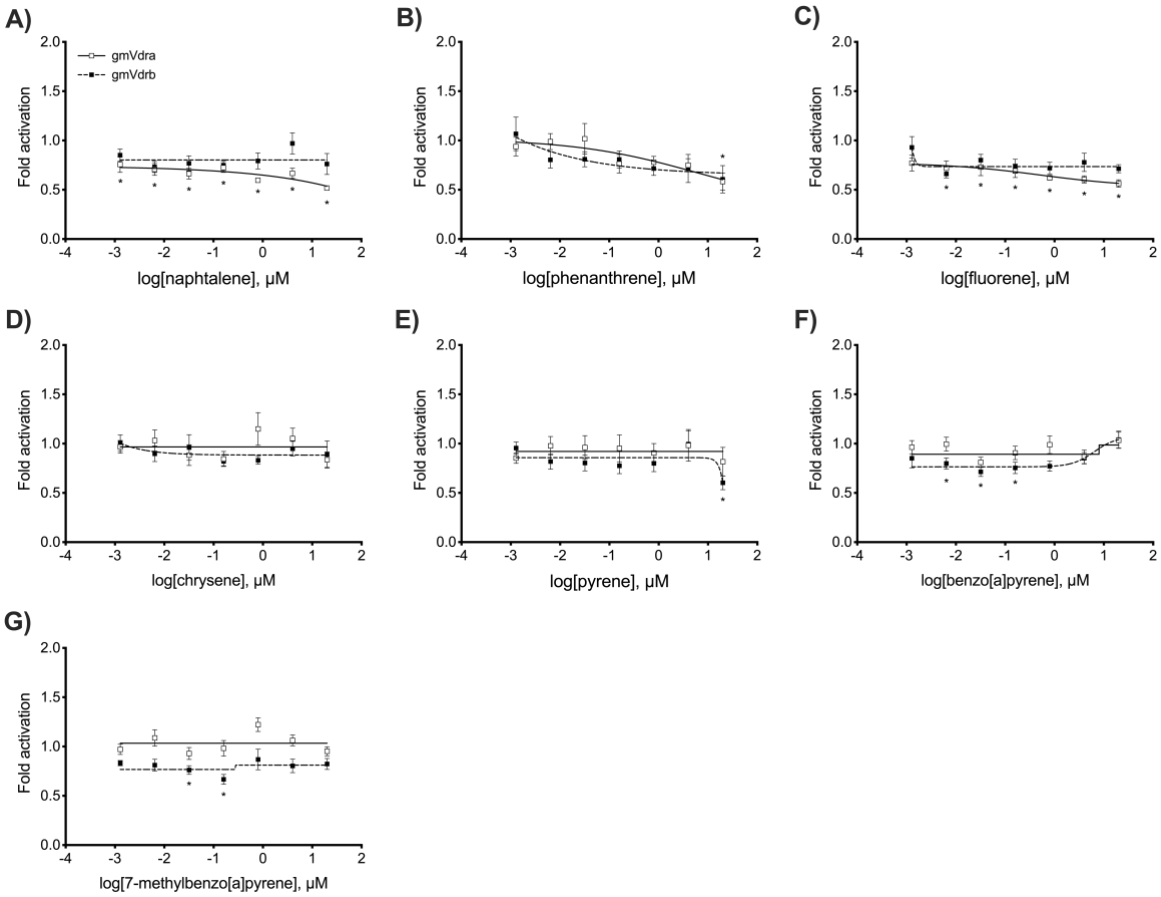


Figure S2. Luciferase reporter gene assay with PAHs and COS-7 cells transiently expressing gmVdra and gmVdrb. COS-7 cells transiently expressing gmVdra-LBD (white) or gmVdrb-LBD (black) were exposed to naphthalene, phenanthrene, fluorene, chrysene, pyrene, BaP or 7mBaP (0.001-20 µM). Responses are presented as mean fold activation relative to solvent exposed cells ± SEM from n =3 independent experiments with 3 technical replicates. Dose-response curves were fitted by nonlinear regression (GraphPad Prism). Statistical differences were assessed by ANOVA and Dunnett's multiple comparisons test. An asterisk (*) indicates a significant difference ($p<0.05$) between PAH exposed cells and solvent exposed cells.

1.8 Viability assay

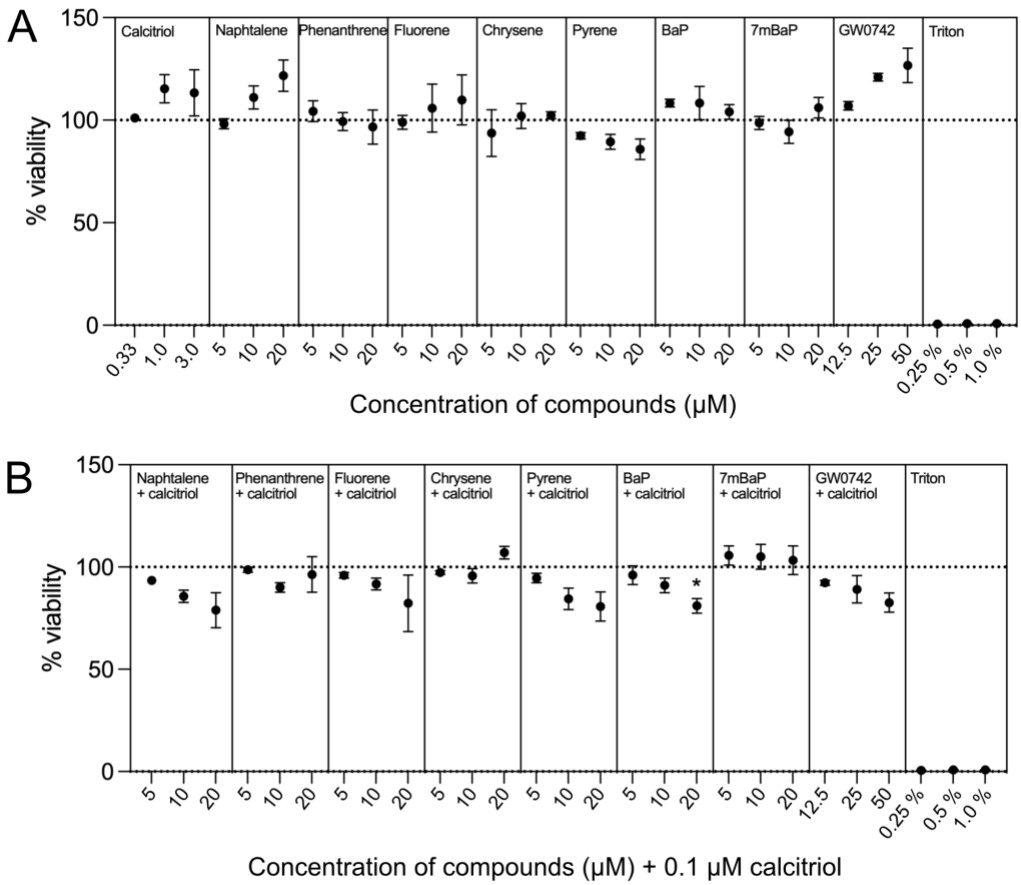


Figure S3. Viability assay of COS-7 cells exposed to calcitriol, PAHs, GW0742, and Triton X-100. COS-7 cells were assayed for changes in viability by measuring mitochondrial metabolic activity using the resazurin reduction method essentially as described by Pérez-Albaladejo (Pérez-Albaladejo et al., 2016). **A)** Calcitriol (0.33, 1, and 3 µM), naphthalene (5, 10, and 20 µM), phenanthrene (5, 10, and 20 µM), fluorene (5, 10, and 20 µM), chrysene (5, 10, and 20 µM), pyrene (5, 10, and 20 µM), BaP (5, 10, and 20 µM), 7mBaP (5, 10, and 20 µM), GW0742 (12.5, 25, and 50 µM), Triton X-100 (0.25, 0.5, and 1%). **B)** Calcitriol (fixed concentration of 0.1 µM) co-exposed with 5, 10, and 20 µM naphthalene, phenanthrene, fluorene, chrysene, pyrene, BaP, 7mBaP, and GW0742 (12.5, 25, and 50 µM). Viability in exposed cells is presented relative to responses in DMSO exposed cells (**A**) or DMSO + 0.1 µM calcitriol exposed cells (**B**) (adjusted to 100%) ± SEM from 2-3 independent experiments (n=2 or 3), where each experiment included 3 technical replicates. Statistical differences to control were assessed using Kruskal-Wallis non-parametric testing in GraphPad Prism ver. 8. An asterisk (*) indicates a significant difference ($p<0.05$).

1.9 Solvent mapping of gmVdrb

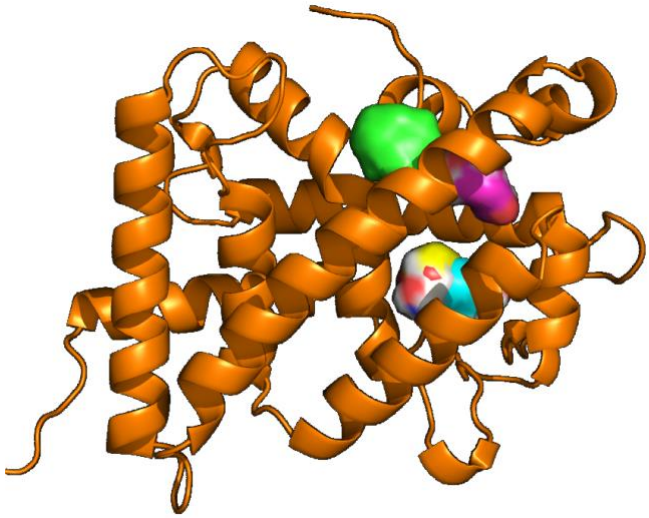


Figure S4. Solvent mapping of gmVdrb. Solvent mapping of Atlantic cod Vdrb predicted protein structure. Solvent mapping of small molecular fragments shows hot spots of ligand affinity both in the LBD and in an exposed surface cleft of gmVdrb. The solvent fragments docking into the LBD are variously colored, while the cleft hot spot is colored green.