



Additional file 2 – Conservation of VDR and PXR ligand-binding residues across species. The ligand-binding residues in VDRs are from x-ray crystallographic structures of human [1-3], rat [4, 5], and zebrafish VDRs [6, 7]. (A) Of the 22 amino acids residues shown to interact with ligands at human, rat, and/or zebrafish VDRs, only 6 residues show any sequence variation across vertebrate species (including the sea lamprey). (B) Only 2 of 22 ligand-binding residues show any variation across mammalian VDRs. (C) In contrast to VDRs, PXR ligand-binding residues, as determined from multiple crystal structures of human PXR [8-12], are quite variable across species. Only 4 of 23 positions are conserved throughout the vertebrate PXRs, with a number of the positions showing extensive sequence variation across species. (D) Even within mammalian species, only 10 of 23 ligand-binding residues are conserved. VDR analysis was based on 27 total species (8 mammals) (Ensembl sequences can be found at <http://www.ensembl.org>): human [GenBank:NM\_00376], chimpanzee [Ensembl:ENSPTRT00000009010], cotton-top tamarin monkey [Genbank:AF354232], rhesus monkey [Ensembl:ENSMMUT00000009414], cow [Ensembl:ENSBTAT00000021832], dog [Ensembl:ENCAFT00000014497], mouse [GenBank:NM\_008504], rat [Genbank:NM\_017058], chicken

[GenBank:AF011356], Japanese quail [GenBank:U12641], *Xenopus laevis* [GenBank:U91849], *Xenopus tropicalis* [Ensembl:ENSXETT00000023339], Nile crocodile [Genbank:AJ011391], snake (*Elaphe* sp.) [Genbank:AJ286866], red-eared slider turtle [Genbank:AJ286870], giant toad (*Bufo marinus*) [Genbank:AY268062], tokay (*Gekko gecko*) [Genbank:AY254096], Asiatic carp [Genbank:AJ784084], fugu VDR $\alpha$  [Ensembl:SINFRUT00000174563], fugu VDR $\alpha$  [Ensembl:SINFRUT00000165525], Japanese flounder VDR $\alpha$  [GenBank:AB037674], Japanese flounder VDR $\beta$  [GenBank:AB037673], zebrafish [GenBank:AF164512], medaka [Ensembl:ENSORLT00000001311], salmon [Genbank"AJ780914], sea bass [Genbank:AM040727], and sea lamprey [GenBank:AY249863]. Reliable sequence was not available for *Xenopus tropicalis* for R158; sequences were not available for horse, crocodile, gecko, toad, snake, turtle, carp, and salmon for H397; sequences were not available for dog, horse, crocodile, gecko, toad, snake, turtle, carp, and salmon for L414, V418, and F422. PXR data was based on analysis of 17 total species (10 mammalian species): human [GenBank:AF061056], chimpanzee [Ensembl:ENPTRT00000028510], rhesus monkey [GenBank:AF454671], cow [Ensembl:ENSBTAT00000026059], dog [Genbank:AF454670], pig [Genbank:AF454672], mouse [AF031814], rat [Genbank:AF151377], rabbit [GenBank:AF188476], opossum [Ensembl:ENSMODT00000023109], chicken [GenBank:AF276753], *Xenopus laevis* BXR $\alpha$  [GenBank:BC041187], *Xenopus tropicalis* [Ensembl:ENSXETT00000039109], fugu [Ensembl:NEWSINFRUT00000171584], medaka [Ensembl:ENSORLT00000022473], *Tetraodon nigriviridis* [Ensembl:GSTENT00026021001], and zebrafish [GenBank:AF454674, GenBank:AF502918].

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