A.

```
                          Helix-1
                          B B          B
PXR_human      141 QGLTEEQRMMIRELMDAQMKTFDTTFSHFKNFRLPGVLSSGCELPESLQAP-SREEA-AK
PXR_chimpanzee     QGLTEEQRMMIRELMDAQMKTFDTTFSHFKNFRLPGVLSSGCELPESLQAP-SREEA-AK
PXR_rhesus         QGLTEEQRMMIRELMDAQMKTFDTTFSHFKNFRLPGVLSSGCEMPESLQAP-SREEA-AK
PXR_dog            KGLSEEQQTMIRELMDAQMKTFDTTFSNFKDFRLPAACSSGREVPGAAHTP-VGEEA-AK
PXR_pig            KGLTEEQRTMISELMNAQMKTFDTTFTHFKNFRLPEVLSSSLEIPECLQTPSSREEA-AK
PXR_rabbit         QGLTGEQRMIIEELMDAQMKTFDTTFSHFKNFRLPEVLGSGCEIPESLQAL-TEEEA-GR
PXR_mouse          QGLTEEQQALIQELMDAQMQTFDTTFSHFKDFRLPAVFHSGCELPEFLQAS-LLEDP-AT
PXR_rat            QGLTEEQQALIQELMDAQMQTFDTTFSHFKDFRLPAVFHSDCELPEVLQAS-LLEDP-AT
PXR_chicken        GGLTAEQQELISILIAAHKRTFDSSFSQFQHYQPAVRLC----IPGPCSQS-PPGPG-VP
BXR-alpha          ASLTPEQQHFLTQLVGAHTKTFDFNFTFSKNFRPIRRSSDPTQEPQAT------------
BXR-beta           ASLTPEQQHFITELVEAHTKTFDFNFTFFKNFRPIRRSPDPTQDPQAT------------
PXR_zebrafish      VTLTPQQEAVIQELLNAHKKTFDMTCAHFSQFRPLDRDQKSVSESSPLTNG-SWIDH-RP
PXR_fugu           IHLSSQQEETIRELLYGHRKTFDLEFYRFSSFRVRTSTTLFDLSKSLSERL-NIFAV-RG
VDR_human      122 PKLSEEQQRIIAILLDAHHKTYDPTYSDFCQFRPPVRVNDGGGSHPSRPNSRHTPSF-SG
VDR_tamarin        PKLSEEQQRIIAILLDAHHKTYDPTYSDFCQFRPPVRVNDGGGSHPSRPNSRHTPSF-SG
VDR_mouse          PKLSEEQQHIIAILLDAHHKTYDPTYADFRDFRPPIRADVSTGSYSPRP----TLSF-SG
VDR_rat            PKLSEEQQHIIAILLDAHHKTYDPTYADFRDFRPPVRMDGSTGSYSPRP----TLSF-SG
VDR_quail          PKLSEEQQKVINILLEAHHKTFDTTYSDFNKFRPPVRSKFSSSTATHSSSVVSQDFS-SE
VDR_chicken        PKLSEEQQKVIDTLLEAHHKTFDTTYSDFNKFRPPVRSKFSSRMATHSSSVVSQDFS-SE
VDR_xenopus        PKISDEQQKMIDILLEAHRKTFDTTYSDFNKFRPPVRENVDPFRRITRSSSVHTQGSPSE
VDR_zebrafish      PRLSDEQMQIINSLVEAHHKTYDDSYSDFVRFRPPVREGPVTRSASRAASLHSLSDA-SS
VDR_halibut        PRLNEEQARMISSLVEAHHKTYDASYSDFSRFRPPVREGPVTRSASRAASLHSLSDA-SS
VDR_fugu           PRLTDEQSQVIATLVEAHHKTYDESYSDFRRFRPPVREGPVTRSASRAASLHSLSDA-SS
VDR_lamprey        PQLLEEQERLIATLIEAHRKTYDASYSDFSQFRPPKRGDGSPECRNATNPFLMSLLN-SD

                                                                Helix-3    B
                       # #*                                   *  *  #*    #
PXR_human      199 WSQVRKDLCSLKVSLQL-RGEDGSVWNYKPP-----ADSGGKEIFSLLPHMADMSTYMFK
PXR_chimpanzee     WSQVRKDLCSLKVSLQL-RGEDGSVWNYKPP-----ADNGGKEIFSLLPHMADMSTYMFK
PXR_rhesus         WNQVRKDLWSVKVSVQL-RGEDGSVWNYKPP-----ADNGGKEIFSLLPHMADMSTYMFK
PXR_dog            WSQVREDLCSLKVCLRL-RGEDGSVQNYTPQ-----ADRSGAEIFSLLPHMADMSTYMFK
PXR_pig            WSKLREDLCSVKLSLQL-RGEDGSVWNYKPP-----ADNSGKEIFSLLPHIADMSTYMFK
PXR_rabbit         WRQIQEELGTMKLSLQL-RGEDGSVWNYTPP-----ADRSGKKLFSLLPHLADMSTYMFK
PXR_mouse          WSQIMKDRVPMKISLQL-RGEDGSIWNYQPP-----SKSDGKEIIPLLPHLADVSTYMFK
PXR_rat            WSQIMKDSVPMKISVQL-RGEDGSIWNYQPP-----SKSDGKEIIPLLPHLADVSTYMFK
PXR_chicken        SASLSPQLDCLDEDVL-------------------------PDVFSILPHFADLSTFMIQ
BXR-alpha          ----------------------------------------SSEAFLMLPHISDLVTYMIK
BXR-beta           ----------------------------------------SSEAFLMLPHISDLFTYMLK
PXR_zebrafish      IAEDPMQWVFNPTSLSS-SSSSYQSLDNKEK-----KHFKSGN-FSSLPHFTDLTTYMIK
PXR_fugu           SSPSGPASSDV--SSLS-TSARLRGRPETPQTQGGENARRGC-VFTALPHVTDLATCMIH
VDR_human      181 DSSSSCSDHCITSSDM-MDSSSFSNLDLSEEDSDDPSVTLELSQLSMLPHLADLVSYSIQ
VDR_tamarin        DSSSCCSDHYITSPDM-MDSSSFSNLDLSEEDSDDPSLTLELSQLSMLPHLADLVSYSIQ
VDR_mouse          DSSSNS-DLYTPSLDM-MEPASFSTMDLNEEGSDDPSVTLDLSPLSMLPHLADLVSYSIQ
VDR_rat            NSSSSSSDLYTTSLDM-MEPSGFSNLDLNGEDSDDPSVTLDLSPLSMLPHLADLVSYSIQ
VDR_quail          DSNDVFGSDAFGAFPEPMEPQMFSNLDLSEESDESPSMNIELPHLPMLPHLADLVSYSIQ
VDR_chicken        DSNDVFGSDAFAAFPEPMEPQMFSNLDLSEESDESPSMNIELPHLPMLPHLADLVSYSIQ
VDR_xenopus        DSDVFTSSPDSSEHGFFSASLFGQFEYSSMGGKSGELS--------MLPHIADLVSYSIQ
VDR_zebrafish      DSFNHSPESVDTKLNFSNLLMMYQDSG-SPDSSEEDQQS----RLSMLPHLADLVSYSIQ
VDR_halibut        DSFNHSPESVDTKMNFSNLLMMYQDGASSPDSSEEN------TKLSMLPHLADLVSYSIQ
VDR_fugu           DSFSHSPESVDNKLNFNNLLMMYHEQGSSPESSEEEA-----SSFSMLPHLADLVSYSIQ
VDR_lamprey        MD-----------------------ELPKASASGAEAAAGDELSMLPHLADLVSYSIQ
```

```
                        Helix-3          Helix-4    Helix-5
                                                    B  B
                                 B                  #  #*  *  B        *
PXR_human          253 GIISFAKVISYFRDLPIEDQISLLKGAAFELCQLRFNTVFNAETGTWECGR--LSY---C
PXR_chimpanzee         GIINFAKVISYFRDLPIEDQIALLKGAAFELCQLRFNTVFNAETGTWECGR--LSY---C
PXR_rhesus             GIINFAKVISYFRDLPIEDQISLLKGATFELCQLRFNTVFNAETGTWECGR--LSY---C
PXR_dog                GVINFAKVISHFRELPIEDQISLLKGATFEVCQLRFNTVFNAETGTWECGR--LSY---C
PXR_pig                GIINFAKVISYFRDLPIEDQISLLKGATFELCQLRFNTVFNAETGTWECGR--LSY---S
PXR_rabbit             GIINFAKVISYFRDLPIEDQISLLKGATLELCLLRFNTVFNAETGTWECGR--LSY---C
PXR_mouse              GVINFAKVISYFRDLPIEDQISLLKGATFEMCILRFNTMFDTETGTWECGR--LAY---C
PXR_rat                GVINFAKVISHFRELPIEDQISLLKGATFEMCILRFNTMFDTETGTWECGR--LAY---C
PXR_chicken            QVIKFAKEIPAFRGLPIDDQISLLKGATLGICQIQFNTVFNEETNAWECGQ--HCF---T
BXR-alpha              GIISFAKMLPYFKSLDIEDQIALLKGSVAEVSVIRFNTVFNPDTNTWECGP--FTY---D
BXR-beta               GVISFAKMLPYFRSLAIEDQIALLKGSVLEVCVIRFNRMFNPKTNTWECGA--FTY---N
PXR_zebrafish          NVINFGKTLTMFRALVMEDQISLLKGATFEIILIHFNMFFNEVTGIWECGP--LQY---C
PXR_fugu               DIIAFSKSLTDFKSLLIGDQIALLKGATFEVMEIRFNMVFNTKTGLWECGH--ATY---C
VDR_human          240 KVIGFAKMIPGFRDLTSEDQIVLLKSSAIEVIMLRSNESFTMDDMSWTCGNQDYKY---R
VDR_tamarin            KVIGFAKMIPGFRDLTSEDQIVLLKSSAIEVIMLRSNESFTMDDMSWTCGNPDYKY---R
VDR_mouse              KVIGFAKMIPGFRDLTSDDQIVLLKSSAIEVIMLRSNQSFTMDDMSWDCGSQDYKY---D
VDR_rat                KVIGFAKMIPGFRDLTSDDQIVLLKSSAIEVIMLRSNQSFTMDDMSWDCGSQDYKY---D
VDR_quail              KVIGFAKMIPGFRDLTAEDQIALLKSSAIEVIMLRSNQSFTMEDMSWTCGSNDFKYKVSD
VDR_chicken            KVIGFAKMIPGFRDLTAEDQIALLKSSAIEVIMLRSNQSFTMEDMSWTCGSNDFKYKVSD
VDR_xenopus            KIIGFAKMIPGFRDLIAEDQIALLKSSVIEVIMLRSNQSFSLDDMSWTCGSEDFKYKVDD
VDR_zebrafish          KVIGFAKMIPGFRDLTAEDQIALLKSSAIEIIMLRSNQSFSLEDMSWSCGGPDFKYCIND
VDR_halibut            KVIGFAKMIPGFRDLTAEDQIALLKSSAIEIIMLRSNQSFSLEDMSWSCGGPDFKYCIND
VDR_fugu               KVIGFAKMIPGFRELTAEDQIALLKSSAIEVIMLRSNQSFNLEDMSWSCGGPDFKYRISD
VDR_lamprey            KVIGFAKMIPGFKELCTEDQISLLKASAIEIIILRSNESFTMEDNSWTCGSNEFKYQIGD

                                      Helix-7              Helix-8              Helix-9
                        B           B       *#       B      B  B  B  BB B
PXR_human          308 LEDT-AGGF----QQLLLEPMLKFHYMLKKLQLHEEEYVLMQAISLFSPDRPGVLQHRVV
PXR_chimpanzee         LEDT-AGGF----QQLLLEPMLKFHYMLKKLQLHEEEYVLMQAISLFSPDRPGVVQHRVV
PXR_rhesus             LEDP-AGGF----QQLLLEPMLKFHYMLKKLQLHEEEYVLMQAISLFSPDRPGVVQHRVV
PXR_dog                LEDP-AGGF----QQLLLEPVLKFHYRLKRLQLHKEEYVLMQAISLFSPDRPGVVQRSVV
PXR_pig                LEDP-SGGF----QQLLLQPMLKFHYMLKKLQLHKEEYVLMQAISLFSPDRPGVVQRQVV
PXR_rabbit             VEDP-EGGF----QQLLVDPLLKFHYMLKKLQLHKEEYVLMQAISLFSPDRPGVVQREVV
PXR_mouse              FEDP-NGGF----QKLLLDPLMKFHCMLKKLQLHKEEYVLMQAISLFSPDRPGVVQRSVV
PXR_rat                FEDP-NGGF----QKLLLDPLMKFHCMLKKLQLREEEYVLMQAISLFSPDRPGVVQRSVV
PXR_chicken            IKDGALAGF----QQIYLEPLLKFHISLKKLRLHEAEYVLLVAMLLFSPDHASVTQRDFI
BXR-alpha              TEDMFLAGF----RQLFLEPLVRIHRMMRKLNLQSEEYAMMAALSIFASDRPGVCDWEKI
BXR-beta               ADDMTMAGF----SQQFLEPLLRIHCMMTKLNLESEAYALMATMALFSSDRPGVSDCEKI
PXR_zebrafish          MDDAFRAGF----QHHLLDPMMNFHYTLRKLRLHEEEYVLMQALSLFSPDRPGVTDHKVI
PXR_fugu               IEDAVRAGF----QPLFLEPLLKFHHTLRNLGLEEEEYVLMQALSLFSPDRPGVQQHSVI
VDR_human          297 VSDVTKAGH----SLELIEPLIKFQVGLKKLNLHEEEHVLLMAICIVSPDRPGVQDAALI
VDR_tamarin            ISDVTKAGH----NLELIEPLIKFQVGLKKLNLHEEEHVLLMAICIVSPDRPGVQDAALI
VDR_mouse              ITDVSRAGH----TLELIEPLIKFQVGLKKLNLHEEEHVLLMAICIVSPDRPGVQDAKLV
VDR_rat                VTDVSKAGH----TLELIEPLIKFQVGLKKLNLHEEEHVLLMAICIVSPDRPGVQDAKLV
VDR_quail              VTQ---AGH----SMDLLEPLVKFQVGLKKLNLHEEEHVLLMAICILSPDRPGVQDTSLV
VDR_chicken            VTQ---AGH----SMDLLEPLVKFQVGLKKLNLHEEEHVLLMAICILSPDRPGVQDTSLV
VDR_xenopus            VTQ---AGH----NMELLEPLVKFQVGLKKLDLHEEEHVLLMAICILSPDRPGLQDKALV
VDR_zebrafish          VTK---AGH----TLELLEPLVKFQVGLKKLKLHEEEHVLLMAICLLSPDRPGVQDHVRI
VDR_halibut            VTK---AGH----TLELLEPLVKFQVGLKKLNLHEEEHVLLMGICLLSPDRPGVQDHARV
VDR_fugu               VTK---AGH----TLELLEPLVKFQVGLKKLNLQEEEHVLLMAICLLSP---GTVDHARV
VDR_lamprey            VMQ---AGH----KLELLEPLVKFQVNMKKLDLHEAEHVLLMAICLFSPDRPGVQDRCRV

                          Helix-9                    Helix-10
                                B     B     B                 B  B*    B +B    *
```

```
PXR_human      363 DQLQEQFAITLKSYIECNR-PQPAHRFLFLKIMAMLTELRSINAQHTQRLLRIQDIHPFA
PXR_chimpanzee     DQLQEKFAITLKSYIECNR-PQPAHRFLFLKIMAMLTELRSINAQHTQRLLRIQDIHPFA
PXR_rhesus         DQLQEQYAITLKSYIECNR-PQPAHRFLFLKIMAMLTELRSINAQHTQRLLRIQDIHPFA
PXR_dog            DQLQERFAIALKAYIECNR-PQPAHRFLFLKIMAMLTELRSINAQHTQKLLRIQDIHPFA
PXR_pig            DQLQERFAITLKAYIECNR-PQPAHRFLFLKIMAMLTELRSINAQHTQRLLRIQDIHPFA
PXR_rabbit         DQLQERFAITLKAYIECSR-PQPTHRFLFLKIMAVLTELRTINAQHTQRLLRIQDTHPFA
PXR_mouse          DQLQERFALTLKAYIECSR-PYPAHRFLFLKIMAVLTELRSINAQQTQQLLRIQDSHPFA
PXR_rat            DQLQERFALTLKAYIECSR-PYPAHRFLFLKIMAVLTELRSINAQQTQQLLRIQDTHPFA
PXR_chicken        DQLQEKVALTLKSYIDHRH-PMPEGRFLYAKLLLLLTELQTLKMENTRQILHIQDLSSM-
BXR-alpha          QKLQEHIALTLKDFIDSQRPPSLQNRLLYPKIMECLTELRTVNDIHSKQLLEIWDIQPDA
BXR-beta           QNLQEHIALMLKAFIESHRPPSPQNRLLYPKIMECLTELRTINDIHSKQLMEIWDIQPDV
PXR_zebrafish      DRNQETLALTLKTYIEAKR-NGPEKHLLFPKIMGCLTEMRSMNEEYTKQVLKIQDMQPEV
PXR_fugu           DKIHENLALALKTRIELKR-TGPEKHMLYPKVLSCLTEMRTMNEEYSKQVLQIQDIQPNV
VDR_human      353 EAIQDRLSNTLQTYIRCRH-PPPGSHLLYAKMIQKLADLRSLNEEHSKQYRCLSFQPECS
VDR_tamarin        EAIQDRLSNTLQTYIRCRH-PPPGSHLLYAKMIQKLADLRSLNEEHSKQYRCLSFQPESS
VDR_mouse          EAIQDRLSNTLQTYIRCRH-PPPGSHQLYAKMIQKLADLRSLNEEHSKQYRSLSFQPENS
VDR_rat            EAIQDRLSNTLQTYIRCRH-PPPGSHQLYAKMIQKLADLRSLNEEHSKQYRSLSFQPENS
VDR_quail          ESIQDRLSDTLQTYIRCRH-PPPGSRLLYAKMIQKLADLRSLNEEHSKQYRCLSFQPEHS
VDR_chicken        ESIQDRLSDILQTYIRCRH-PPPGSRLLYAKMIQKLADLRSLNEEHSKQYRCLSFQPEHS
VDR_xenopus        ESIQDRLSSTLQTYILCKH-PPPGSRLLYAKMIQKLADLRSLNEEHSKQYRSISFLPEHS
VDR_zebrafish      EALQDRLCDVLQAYIRIQH-P--GGRLLYAKMIQKLADLRSLNEEHSKQYRSLSFQPEHS
VDR_halibut        EQLQDRLPEALQAYIRINH-P--GGRLLYAKMIQKLADLRSLNEEHSKQYRSLSFQPEHS
VDR_fugu           EALQDRLSETLQAYIRLHH-P--GGRLLYAKMIQKLADLRSLNEEHSKQYRSLSFQPEHS
VDR_lamprey        EEVQEHLTETLRAYIACRH-PLSCKHMLYTKMVEKLTELRSLNEEHSKQYLQISQDAVNK

                        Helix-AF
                         _______
PXR_human      421 ---TPLMQELFGITGS----
PXR_chimpanzee     ---TPLMQELFGITGS----
PXR_rhesus         ---TPLMQELFGITGS----
PXR_dog            ---SPLMQELFSITDG----
PXR_pig            ---TPLMQELFSITES----
PXR_rabbit         ---TPLMRELFSTTDD----
PXR_mouse          ---TPLMQELFSSTDG----
PXR_rat            ---TPLMQELFSSTDG----
PXR_chicken        ---TPLLSEIIS--------
BXR-alpha          ---TPLMREVFGSPE-----
BXR-beta           ---TPLMREVFGSLNE----
PXR_zebrafish      ---SPLWLEIISKDT-----
PXR_fugu           ---IPPLLMEM---------
VDR_human      412 MKLTPLVLEVFGNEIS----
VDR_tamarin        MKLTPLVLEVFGNEIS----
VDR_mouse          MKLTPLVLEVFGNEIS----
VDR_rat            MKLTPLVLEVFGNEIS----
VDR_quail          MQLTPLVLEVFGNEIS----
VDR_chicken        MQLTPLVLEVFGNEIS----
VDR_xenopus        MKLTPLMLEVFSDEIP----
VDR_zebrafish      MQLTPLVLEVFGSEVS----
VDR_halibut        MQLTPLVLEVFGSEVS----
VDR_fugu           MQLTPLVLEVFGSEVSPRLT
VDR_lamprey        EDLPPLLLEVFGNPTA----
```

B.

```
                   2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 4 4 4
                   0 0 0 4 4 4 4 5 8 8 8 8 9 2 2 0 1 2
```

	6	8	9	0	3	6	7	1	1	4	5	8	9	3	4	7	4	0
PXR_human	L	S	L	**L**	M	M	S	F	F	C	Q	**F**	**W**	M	L	H	I	F
PXR_chicken	L	C	L	**L**	F	L	S	I	L	C	Q	**F**	**W**	L	L	N	I	M
BXR-alpha	-	-	-	**L**	I	L	V	I	A	S	V	**F**	**W**	L	V	H	I	D
BXR-beta	-	-	-	**L**	I	L	F	L	L	C	V	**F**	**W**	L	L	H	I	D
PXR_zebrafish	W	F	N	**L**	F	L	T	I	F	I	L	**F**	**W**	M	M	Y	I	E

LEGEND TO SUPPLEMENTARY FIGURE 1

Supplementary Figure 1. Alignment of the ligand-binding domains of PXR and VDR genes highlighting residues important in ligand binding for human PXR. *(A)* Alignment of the ligand-binding domains of PXR and VDR genes used in the phylogenetic analysis. Accession numbers for the genes are given in the Materials and Methods section. Numbering of genes and assignment of helices is according to Moore et al. (1). The symbols above the human PXR sequences indicate amino acid residues shown to interact directly with ligand in the two crystallographic structures of the human PXR ligand-binding domain complexed with the ligands SR12813 (2) or hyperforin (3): # indicates residues that interact with SR12813 only, + indicates a residue that interacts with hyperforin only, and * indicates residues that interact with both SR12813 and hyperforin. *(B)* Alignment of human PXR, chicken PXR, frog BXRα and BXRβ, and zebrafish PXR sequences for the 18 amino acid residue positions in human PXR shown to interact directly with SR12813 and/or hyperforin. As can be seen, there is substantial diversity at these positions, consistent with the marked cross-species differences in ligands that activate these PXRs. Only 6 of the 18 positions shown are conserved between human and zebrafish PXR and only 3 of 18 between human PXR and either BXRα or BXRβ. The numbers for each position are for human PXR.

<u>References for supplementary figure 1:</u>

1. **Moore LB, Maglich JM, McKee DD, Wisely B, Willson TM, Kliewer SA, Lambert MH, Moore JT** 2002 Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. Mol Endocrinol 16:977-86
2. **Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Williams SP, Willson TM, Kliewer SA, Redinbo MR** 2001 The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. Science 292:2329-33
3. **Watkins RE, Maglich JM, Moore LB, Wisely GB, Noble SM, Davis-Searles PR, Lambert MH, Kliewer SA, Redinbo MR** 2003 2.1 Å crystal structure of human PXR in complex with the St. John's wort compound hyperforin. Biochemistry 42:1430-8