Supplementary Information

Structural studies on the binding mode of bisphenols to PPARγ

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Table S1. Alignment of human PPARγ and human ERRγ

PPARγ HUMAN	sp|P37231-2|PPARG_HUMAN Isoform 1 of Peroxisome proliferator-activated receptor gamma OS=Homo sapiens OX=9606 GN=PPARG Query ID: lcl|Query_7251043 Length: 272	
		Sequence identity to hPPARγ (%)
ERRγ HUMAN	sp|P62508|ERR3_HUMAN Estrogen-related receptor gamma OS=Homo sapiens OX=9606 GN=ESRRG PE=1 SV=1 Sequence ID: Query_7251045 Length: 237 Range 1: 48 to 232	26

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hPPARg 80   CQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVHEI-----IYTMLA---SLMNK  131
ERRg   48   .DLADR.L.V-.IGW..H....ST.S.A..MS..QSAWM..LILGVV.RS.SFEDE.VYA  106

hPPARg 132  DGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGD  191
ERRg   107  .DYIMD.D.SKLAG---------LL.LNNAILQLVK.YKSMK.EKEEFVTLK.IALANS.  157

hPPARg 192  RPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQVI  251
ERRg   158  SMHIED.EAVQKL..V.HE..QDYEAGQ.M.DPRRAG.M.MTLPL...TS.KA..HFYN.  217

hPPARg 252  KKTETDMSLHPLLQEI  267
ERRg   218  -.L.GKVPM.K.FL.M  232
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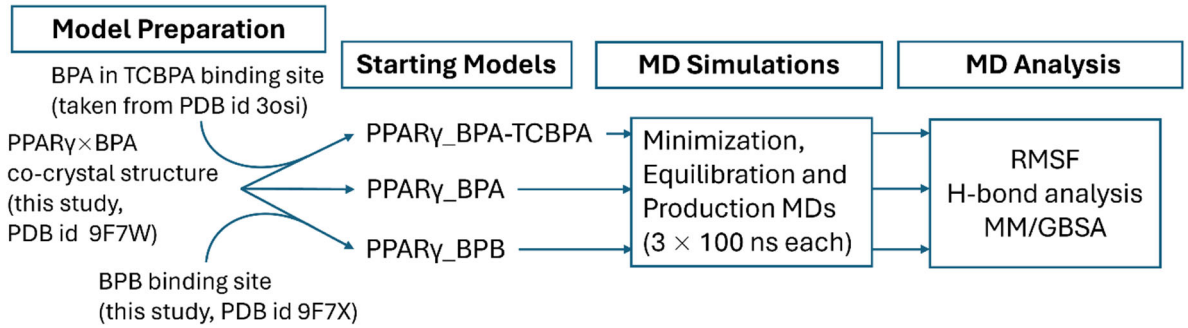
Table S2. Diffraction data and refinement statistics

Compound	**PPARγ×BPA**	**PPARγ×BPB**
PDB entry ID	9F7W	9F7X
Final buffer before cryocooling	20% w/v PEG 3350, 0.2 M Mg-acetate, 137 mM NaCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 1 mM peptide, 15 mM BPA, 1.5 % DMSO	20% w/v PEG 3350, 0.337 M NaCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 1 mM peptide, 15 mM BPB, 1.5 % DMSO
Data collection		
Wavelength (Å)	0.9762	0.9755
Resolution (Å)	41.88-1.25 (1.37-1.25)	42.23-1.63 (1.80-1.63)
Resolution aniso (Å)	1.470, 1.253, 1.331	3.503, 1.749, 1.545
Space group	$P2_1$	$P2_1$
Unit cell dimensions (Å;°)	43.80, 54.19, 66.47; 90.000, 107.035 90.000	40.36, 54.62, 66.72; 90.000, 93.598 90.000
Unique reflections	60529 (3027)	14591 (731)
Multiplicity	6.9 (7.1)	6.8 (6.5)
Completeness (%)* spherical/ellipsoidal	74.1 (16.2) / 91.5 (51.3)	40.3 (8.1) / 88.2 (57.6)
Mean I/s(I)	12.8 (1.5)	10.3 (1.4)
R-meas	0.066 (1.318)	0.103 (1.281)
R-merge	0.061 (1.221)	0.095 (1.178)
R-pim	0.025 (0.491)	0.039 (0.497)
$CC_{1/2}$	0.999 (0.615)	0.999 (0.437)
Wilson B ($Å^2$)	16.96	17.89
Refinement		
Resolution (Å)	41.88-1.25 (1.27-1.25)	42.23-1.63 (1.65-1.63)
R-work	0.1622 (0.2613)	0.2493 (0.4080)
R-free	0.1889 (0.4057)	0.2899 (0.2864)
Number of non-hydrogen atoms, B-value ($Å^2$)		
Protein	2307, 33.14	2223, 29.11
Heterogen	34, 39.69	18, 35.62
Solvent	339, 37.43	119, 20.91
Ligand occupancy	0.58 (BPA1), 0.61 (BPA2)	0.89
Rmsd bonds (Å), angles (°)	0.008, 0.905	0.004, 0.531
Ramachandran favored, allowed, outliers (%)	99.27, 0.73, 0.00	96.67, 3.33, 0.00
Rotamer outliers (%)	0.38	1.20
MolProbity clashscore	1.05	5.48

*Anisotropic truncation has been used. The first line refers to the spherical and the second line to the ellipsoidal completeness.

Table S3. Sequence IDs used for the alignment analyses shown in Figure S4.

PPARγ_HUMAN	sp|P37231-2|PPARG_HUMAN Isoform 1 of Peroxisome proliferator-activated receptor gamma OS=Homo sapiens OX=9606 GN=PPARG Query ID: lcl|Query_1789 Length: 477	
		Sequence identity to hPPARγ (%)
PPARγ_DOG	>sp|Q4U3Q4|PPARG_CANLF Peroxisome proliferator-activated receptor gamma OS=Canis lupus familiaris OX=9615 GN=PPARG PE=2 SV=1	99.16
PPARγ_MOUSE	sp|P37238|PPARG_MOUSE Peroxisome proliferator-activated receptor gamma OS=Mus musculus OX=10090 GN=Pparg PE=1 SV=3	97.90
PPARγ_PIG	sp|O62807|PPARG_PIG Peroxisome proliferator-activated receptor gamma OS=Sus scrofa OX=9823 GN=PPARG PE=2 SV=1	98.32
PPARγ_RAT	sp|O88275|PPARG_RAT Peroxisome proliferator-activated receptor gamma OS=Rattus norvegicus OX=10116 GN=Pparg PE=1 SV=2	97.90
PPARγ_WHALE	tr|A0A2Y9M8F2|A0A2Y9M8F2_DELLE Peroxisome proliferator-activated receptor gamma OS=Delphinapterus leucas OX=9749 GN=LOC111168952 PE=3 SV=1	98.74
PPARγ_CHICK	tr|A0A0S1NES2|A0A0S1NES2_CHICK Peroxisome proliferator-activated receptor gamma OS=Gallus gallus OX=9031 GN=PPARgamma PE=2 SV=1	92.63
PPARγ_LIZARD	>tr|A0A670J836|A0A670J836_PODMU Peroxisome proliferator-activated receptor gamma OS=Podarcis muralis OX=64176 GN=PPARG PE=3 SV=1	90.99
PPARγ_ ZEBRAFISH	>tr|A6XMH6|A6XMH6_DANRE Peroxisome proliferator-activated receptor gamma OS=Danio rerio OX=7955 GN=pparg PE=2 SV=1	63.94
PPARγ_KRYMA	>tr|A0A3Q3ACA3|A0A3Q3ACA3_KRYMA Peroxisome proliferator-activated receptor gamma OS=Kryptolebias marmoratus OX=37003 GN=PPARG PE=3 SV=1	66.39



Scheme S1: Overview of the methodology used for the MD simulations.

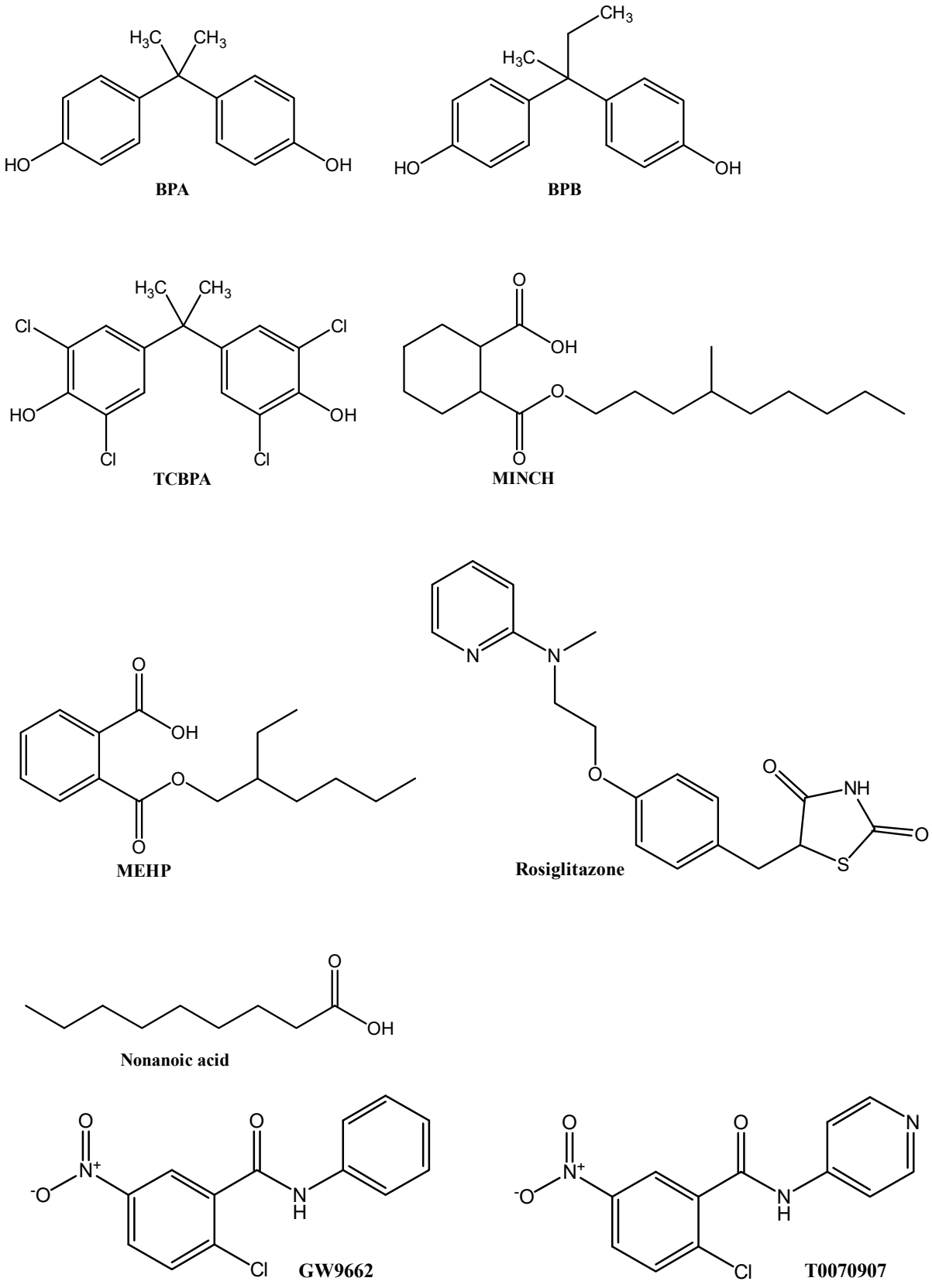


Figure S1. Schematic representation of BPA, BPB, TCBPA, MEHP, MINCH, endogenous (nonanoic acid) and synthetic (rosiglitazone, GW9662, T007090) PPARγ ligands.

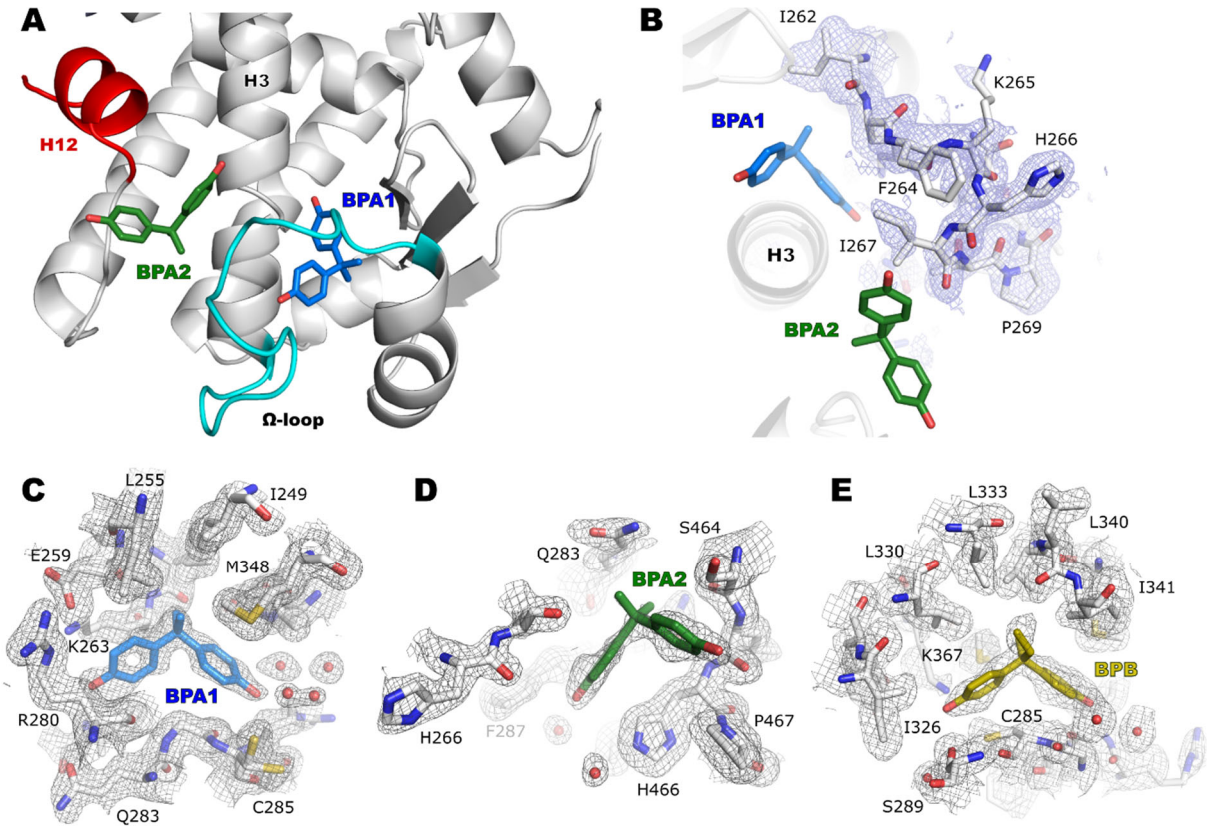


Figure S2. Electron density of the Ω loop and the ligand binding sites. (A) Zoom of the BPA binding sites and the Ω loop. (B) (2Fo-Fc)-type electron density of the Ω-loop at a contour level of 0.5 σ_{rms}. (C-E) (2Fo-Fc)-type electron density maps of the BPA1, BPA2 and BPB binding sites, respectively. The contour level of these maps is 0.7 σ_{rms}.

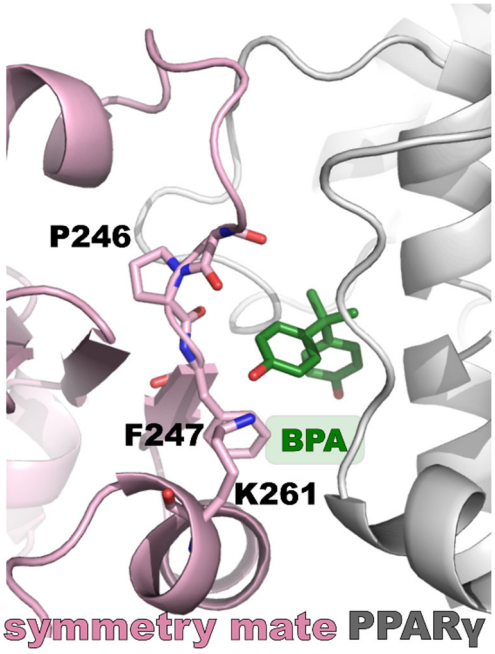


Figure S3. **BPA2 binds at a crystal contact in the PPARγ×BPA structure.** The protein molecule in the asymmetric unit is shown in grey and a symmetry related molecule in pink.

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    HUMAN   1    MTMVDTE-MPFWPTNFGISSVDLSVMEDHSHSFDIKPFTTVDFSSISTPHYEDIP-FTRT  58
      DOG   30    ......-.................D.............................-.S.A  86
      PIG   28   V......-.................D.............................-.P.A  85
    MOUSE   29   I......-.....................................A.......-...A  86
      RAT   29   I......-.................D...................A.......-...A  86
    WHALE   1      .....-.................D...................A.......-.P.A  56
    CHICK   1      .....-.....V.....P....A.D..M...............S........-LG.A  56
   LIZARD   78    ......-.....M.....P..F.G.DE................L.SQ....L.PL...  135
ZEBRAFISH   17     ....Q-TFG..VG..L.ALE.EEL..DT..L.....S.L.Y....GID..NN.--.QN  71
    KRYMA   1      ....QQLLA..VG.SL.T...PELD.S..CL.M.HLL.S.YA...S-----------S  47

    HUMAN   59   DP------VVADYKY-------------------------DLKLQEYQSAIKVEPASPPY  87
      DOG   87   ..------M.....-------------------------.....................  115
      PIG   86   ..------M.....-------------------------.....D.........V....  114
    MOUSE   87   ..------M.....-------------------------.....................  115
      RAT   87   ..------M.....-------------------------.....................  115
    WHALE   57   ..------M.....-------------------------.....................  85
    CHICK   57   .Q------TSI....-------------------------.I...DC.....M..P....  85
   LIZARD   136  .Q------MTV....-------------------------...F..C.NE..I..P....  164
ZEBRAFISH   72   ..TPHMMDLTHM.S.RTQENYRTHEPIYRPEHSSYSPEENTYRA.QI.NS..L..E...Q  131
    KRYMA   48   SI------PSSF.RT-----------------------EQDTHSKPNS..L..E...Q  76

    HUMAN   88   YSEKTQLYNKPHEEPSNSLMAIECRVCGDKASGFHYGVHACEGCKGFFRRTIRLKLIYDR  147
      DOG   116  ..........................................................  175
      PIG   115  ..........................................................  174
    MOUSE   116  .........R................................................  175
      RAT   116  .........R................................................  175
    WHALE   86   ..........................................................  145
    CHICK   86   F...V.........S.....V.....................................  145
   LIZARD   165  F...V.M.......T.....................................  224
ZEBRAFISH   132  FA.NSVSFS.TP.D..S.SLN.....................................V..H  191
    KRYMA   77   ..D-SPVFS.FPDDT.ATTLN.....................................V..H  135

    HUMAN   148  CDLNCRIHKKSRNKCQYCRFQKCLAVGMSHNAIRFGRMPQAEKEKLLAEISSDIDQLNPE  207
      DOG   176  ..........................................................  235
      PIG   175  ..........................................................  234
    MOUSE   176  ..........................................................  235
      RAT   176  ..........................................................  235
    WHALE   146  ..........................................................  205
    CHICK   146  ..........................................................  205
   LIZARD   225  ..........................................................  284
ZEBRAFISH   192  ...H.....................M.....................F...VNHMH..  251
    KRYMA   136  .E.H.....................N.....................F...MEH.H..  195

    HUMAN   208  SADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKIKFKHI  267
      DOG   236  ..........................................................  295
      PIG   235  ..........................................................  294
    MOUSE   236  ..........................................................  295
      RAT   236  ..........................................................  295
    WHALE   206  ..........................................................  265
    CHICK   206  ..................................................R....Q..C..A  265
   LIZARD   285  .........L.....................A.............K....Q..C..L  344
ZEBRAFISH   252  ........R...E..L.Y...........S...S.NA....H..K..VE..QM.NCRYM  311
    KRYMA   196  A.......R...EA.L.Y...........S...GE.V....H..K...E..QF.NCRQ.  255
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Figure continued on next page

Figure S4. Amino acid sequence alignment of selected species for mammalian orthologs (human, dog, pig, mouse, rat, and whale), for birds (chick), for fish (zebrafish and kryma), and for reptiles (lizard). The alignment was done using BLAST program (Altschul et al. 1997). Residues involved in binding sites of BPA and BPB are highlighted in red.

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    HUMAN  268  TPLQEQSK-------EVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKY  320
      DOG  296  ........-------.............................................  348
      PIG  295  ........-------.........................N...................  347
    MOUSE  296  ........-------.........................N....I..............  348
      RAT  296  ........-------.........................N....I..............  348
    WHALE  266  .....P..-------.............................................  318
    CHICK  266  S.....N.-------........R..............F..N...................  318
   LIZARD  345  ..M...N.-------........R..............F..N...................  397
ZEBRAFISH  312  -..H.HRRSDLGIMH..EL.F.HSY.S..A..IS.V..F.......I..............  370
    KRYMA  256  PS.DH.QQ-------VSTL.F.HS..S..A...R.V..F.......ID.............  308

    HUMAN  321  GVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNALELD  380
      DOG  349  ............................................................  408
      PIG  348  ............................................................  407
    MOUSE  349  ....................................N.......................  408
      RAT  349  ............................................................  408
    WHALE  319  ............................................................  378
    CHICK  319  ........L..............D..................C..................  378
   LIZARD  398  ........L...M.........N..................C..................  457
ZEBRAFISH  371  ..I.VMII.ISP......T...Y..I..............CEM.......SI...M....  430
    KRYMA  309  ..I.VLII.MSP......T...Y..I..............CQM.......S....T....  368

    HUMAN  381  DSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMT  440
      DOG  409  ............................................................  468
      PIG  408  ............................................................  467
    MOUSE  409  ...........................................................V.....  468
      RAT  409  ...........................................................V.....  468
    WHALE  379  ............................................................  438
    CHICK  379  ............................................................  438
   LIZARD  458  ......................I.....................................  517
ZEBRAFISH  431  .C.M.L.L.............D......L.ETV.HS......I...D.L.....V.....  490
    KRYMA  369  ...M.L.L...................QL.ETV.HS.........D.L............  428

    HUMAN  441  DLRQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLY  477
      DOG  469  ....................................  505
      PIG  468  ....................................  504
    MOUSE  469  .............H......................  505
      RAT  469  .............H......................  505
    WHALE  439  ....................................  475
    CHICK  439  ..............I.....................  475
   LIZARD  518  ..............I.....................  554
ZEBRAFISH  491  ....L..D....I.MM.E..A.W.........MR...  527
    KRYMA  429  .......D..H.IELL....V..F........M....  465
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Figure S4 (continued)