Supplemental Data

Fig. S1: Alignment of annelid ERs with mollusk and human ERs. Only the DBD and LBD are included. A mutation in the activation function surface AF-2 that disrupts coactivators recruitment and transcriptional activation is boxed.

DBD

```
platynereisER    CQICSDLASGFHYGVWSCEGCKAFFKRSLQ----GPVDYVCPATNNCTIDKHRRKSCQAC
capitellaER      CQICDDAASGFHYGVWSCEGCKAFFKRSLQAMSSGPVDYVCPATQNCTIDRQRRKSCQAC
octopusER        CQVCDDNASGFHYGVWSCEGCKAFFKRSIQ----GPVDYVCPATNSCTIDKHRRKSCQAC
aplysiaER        CQVCSDNASGFHYGVWSCEGCKAFFKRSIQ----GPVDYICPATNTCTIDKHRRKSCQAC
humanERa         CAVCNDYASGYHYGVWSCEGCKAFFKRSIQ----GHNDYMCPATNQCTIDKNRRKSCQAC
```

```
platynereisER    RFRKCLEVGMMKRRERRTTKKVKSPGSPEEKITKKVRTPNTKPNPKEKGPPVENNGQSRR
capitellaER      RLNKCIQMGMSRGNCRRERERPGK-----ANRKKGDDAQTSPP----------------
octopusER        RLRKCYEVGMNKGSQRKERKNSSN----QTKVKRSSAD--------------------
aplysiaER        RLRRCYEVGMNKGSQRKEKRNSGN--TSSLKGKRCRAD--------------------
humanERa         RLRKCYEVGMMKGGIRKDRRGGRM-----LKHKRQRDDGEGR----------------
```

```
platynereisER    VLQLEETVAVESVKTEAVSPQECSPSGHTSQEDLSVPMTSASQSFNLTPPPSTSSSTSSP
capitellaER      ----KVRIKEEKDGNRSPVAGSSSTAAAAAAAAAAAAVVATPPPAPQAPPAAASNEPVP
octopusER        ---------------------------FSDSTVNSTSGN--------QPAKSQRLSK
aplysiaER        ---------------------------SSDSAVNSTNNG-------ASSSKSSKRSR
humanERa         ------------------------GEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLT
```

LBD

```
platynereisER    SNGTSSLQIDASTFKIDPGLPLDPPHPLVVILEENDLPPKICKEPLTAESEETEYSLLHR
capitellaER      IH----------------------TLVAHLMRVDPP--VRHANHDHSLPDTWENLTAS
octopusER        SS----------------------SLVEELSKNDFA--VPECKLNPSIPLTKNYILQL
aplysiaER        SA----------------------SILEALQKADLP--VMDSYHNHNLPATRVHLLNT
humanERa         AD----------------------QMVSALLDAEPP--ILYSEYDPTRPFSEASMMGL
```

```
platynereisER    LIGLADMELVDVVNWAKVLPGFSGLELRDRIAILESCWMELLCIGAAGRSR-LNTTFQVN
capitellaER      LFKLADFELMDVITWAKNIPGYSALSLKLRIHLLEACWMEVLIIGLLWRSQ-NHKD-CLM
octopusER        LIQVADKDLVQLINWAKHIPGYADLSLSDQVHLIECCWMELVLLNCAYRSM-EYEGKRLA
aplysiaER        LIKLADRELVYLINWAKHVPGYTCLTLGDQVHLIECCWMELLLLNCAFRSM-EHEGRTLV
humanERa         LTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLVWRSM-EHPG-KLL
```

```
platynereisER    FAENLHFN-EETAKKAKMSSIVGEIWQISQQFRYLELSNHEFMLLRVVTMLNA-------
capitellaER      FAPDLEFD-RTRIRIAELESISTPILRLSQLFTRLHVTREEMVLLRVLALINS------D
octopusER        FASNLILE-KHHWEILGMTQILEQVAAVSEQLLQFGINREELLLLEATILVNA-------
aplysiaER        FAPDFHLE-RQQWALTGMGDVLEQVSAVSEQMLLHGLNKEELLLLQATVLVNA-------
humanERa         FAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLS
```

```
platynereisER    -ESIRLSCLDAMHKIRQQYLEALHFECGRTLGKISESSCVRMAQILCILPFARQVSLKAI
capitellaER      ICGDNDEERSLQEDLQQSVHEAFEYTVIRRQRQ----PLSRLLNLLSLLPHVRMAAMLSL
octopusER        -EVRRLAGFSKIDDIRQIILNALIDTAQKYHPD----NPRHVPSALLLLSHVRQASDRSI
aplysiaER        -EVRPLDSFLKIQEMRQLILDVFMEVAGRHQGFG---NWRHAPSILLLLTHIRQAGERGI
humanERa         STLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQ-QQHQRLAQLLLILSHIRHMSNKGM
```

```
platynereisER    THLFNMHNQHAVPVGDLVAEMLVAQKELMTNEDSAKIIT--------------------
capitellaER      QQISEIKSSLRLPLEDLLNEMMEGSTKASRTMTP------------------------
octopusER        IYLQKQKDEGHVTFCELITEMLEAQNSSNDIVAPRADVIGMGT---------------
aplysiaER        TYFQKLKMEGCVTFCDLLTEMLDAHNSSGERRRLQQQQQQPQQQSHAHHHHPPPPLPHHH
humanERa         EHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEETDQSHLATAGSTSSHSLQKY
```

Fig. S2: Complete steroid receptor phylogeny inferred using Bayesian MCMC. Node labels show inferred posterior probabilities for each clade.
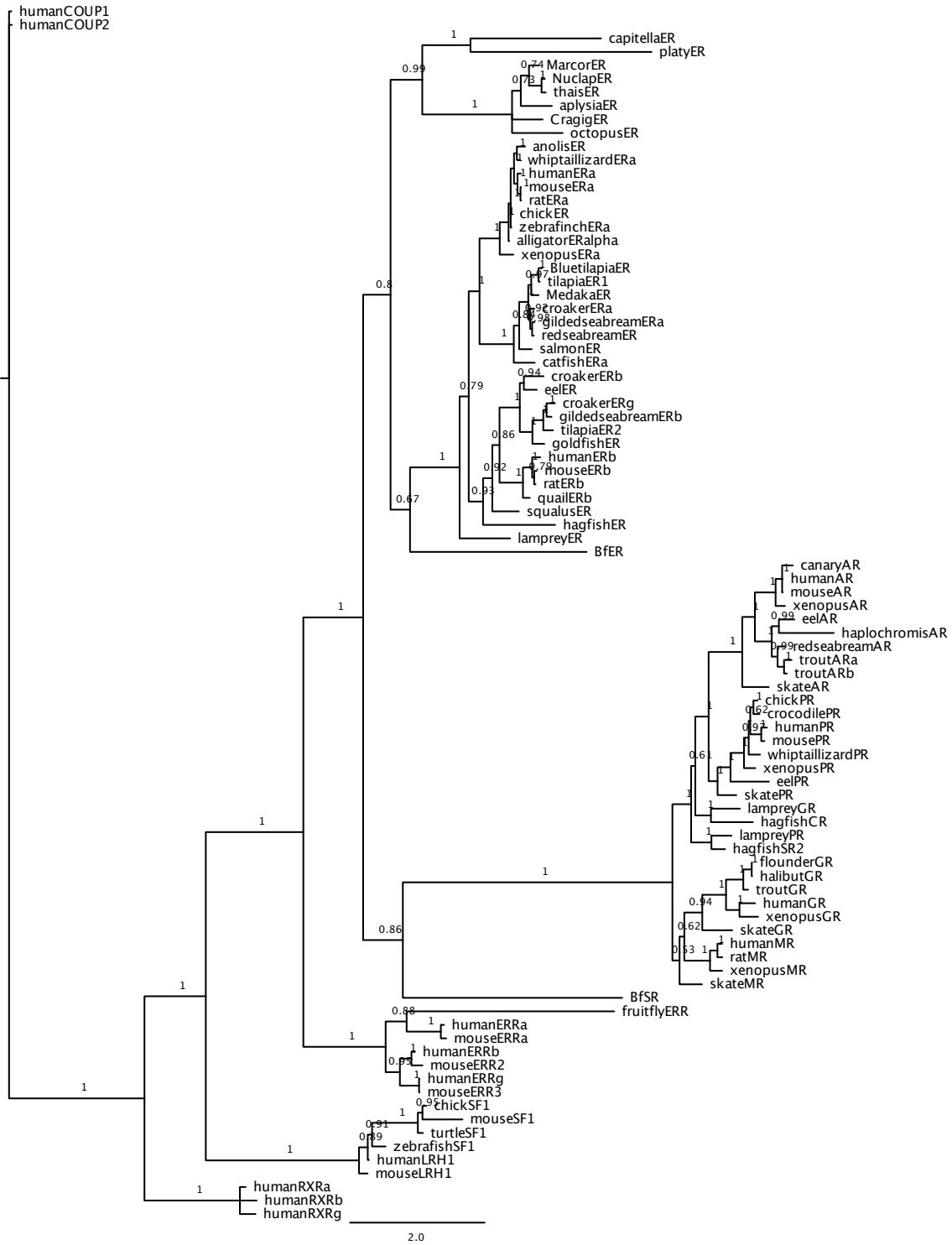
Fig. S3: Steroid receptor phylogeny inferred using maximum likelihood, with likelihood ratio statistics. Node labels show approximate likelihood ratio statistics (LRS = 2ln(likelihood ratio). The likelihood ratio compares the likelihood of the maximum likelihood phylogeny to the likelihood of the highest-likelihood phylogeny missing the node of interest.
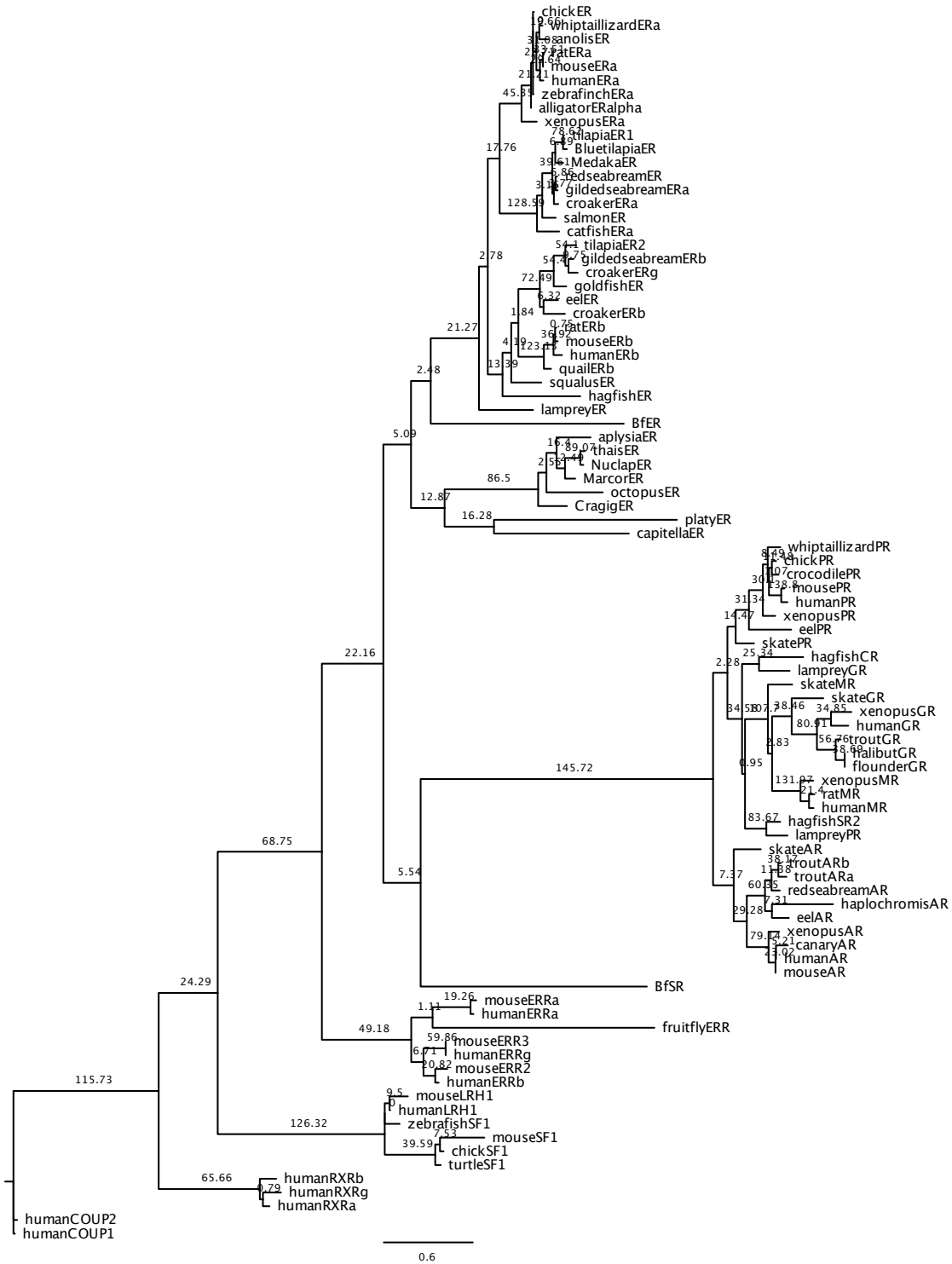
Fig. S4: Steroid receptor phylogeny inferred using maximum likelihood, with chi-square support statistics. Chi-square statistics represent 1 minus the probability that the likelihood ratio statistic observed for each node would have occurred by chance if the node is in fact false.
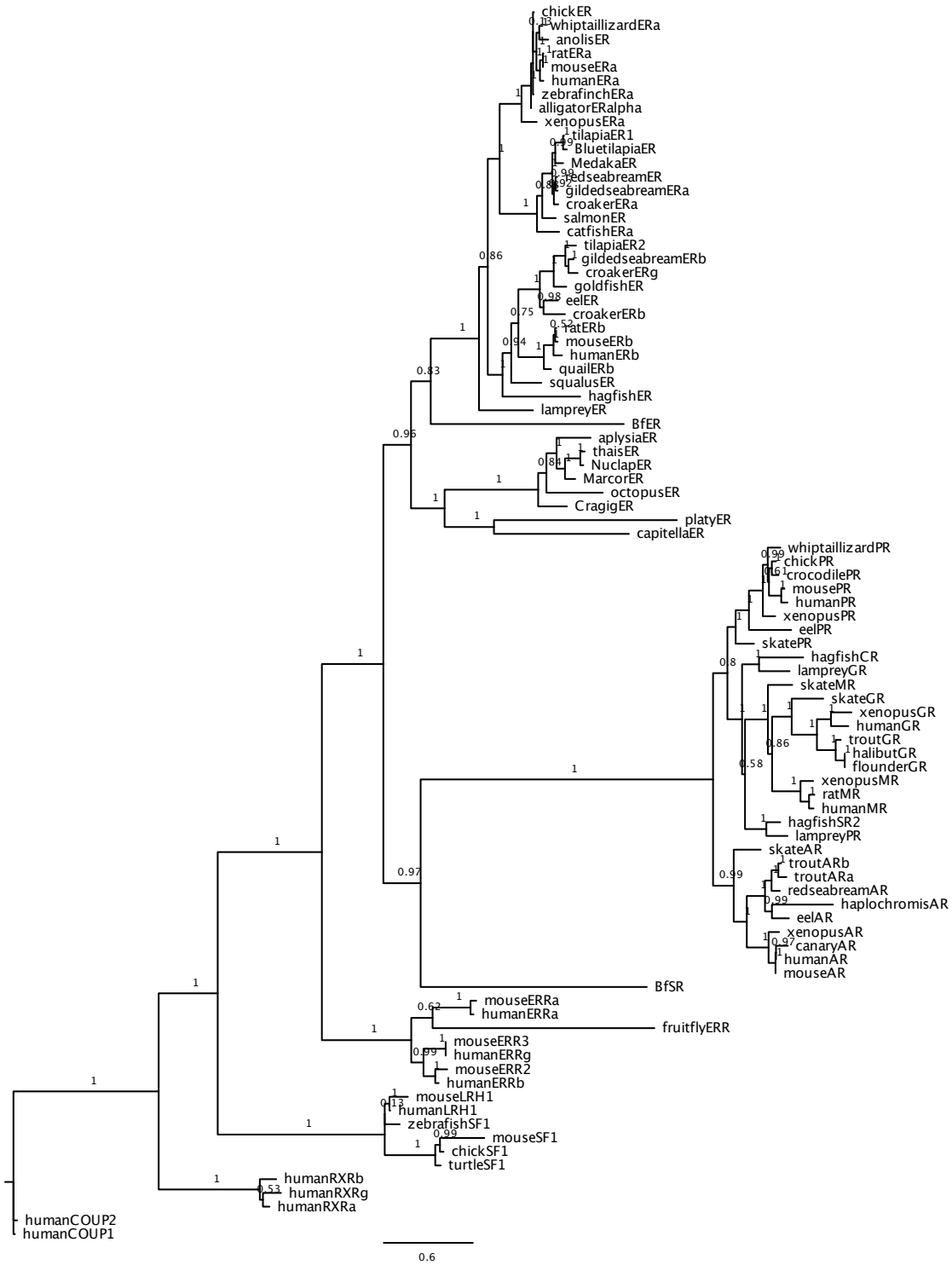
Fig. S5: Steroid receptor phylogeny excluding cephalochordate receptors. Maximum likelihood topology for the same alignment in Figs. S2-S4 but with ER and SR from Branchiostoma floridae excluded. Node labels show approximate likelihood ratio, the chi-square statistic for the approximate likelihood ratio, and the Bayesian posterior probability.
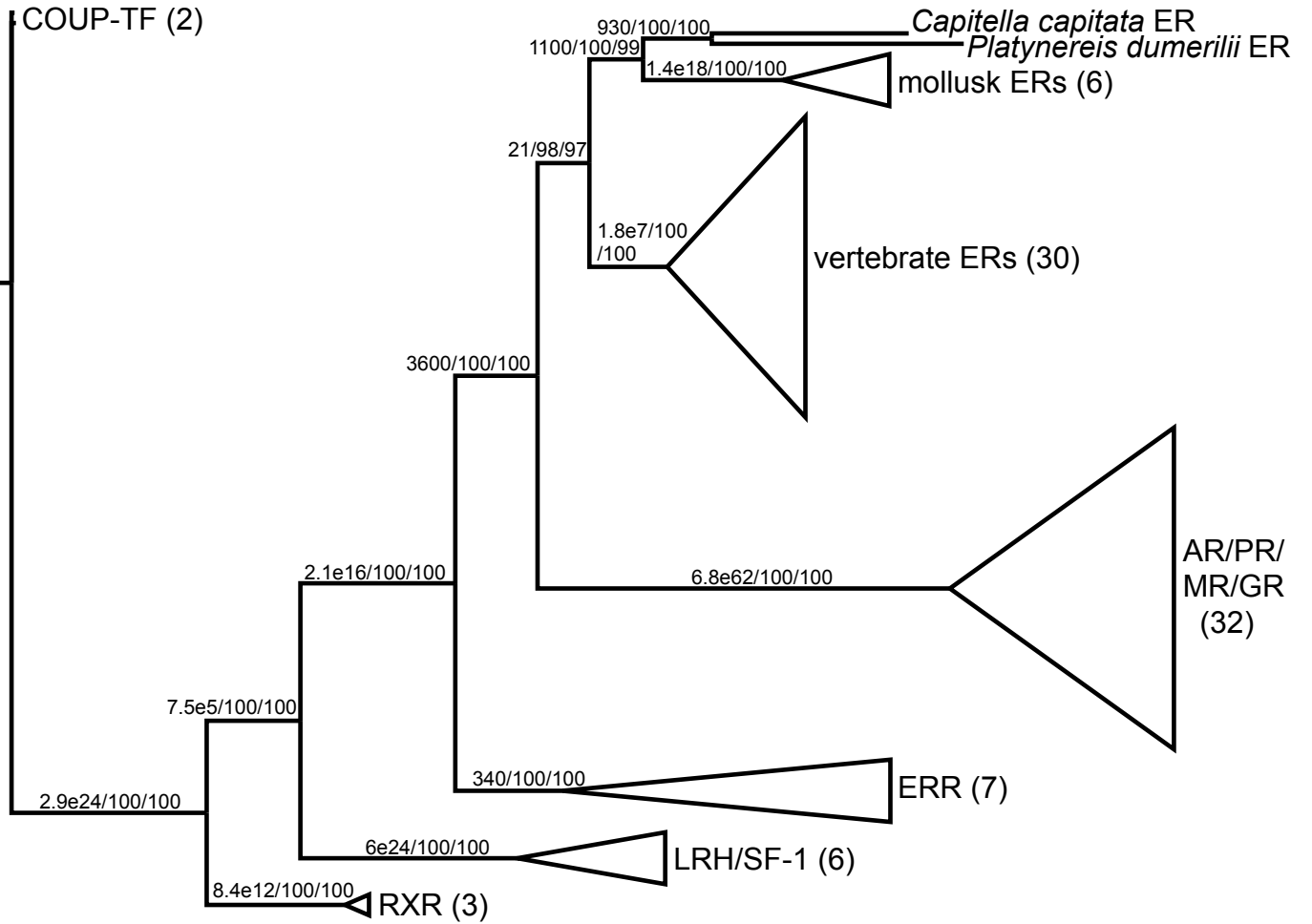
Fig. S6: Hormone and xenobiotic sensitivity of full-length PdER and human ER. Fold-activation of an ERE-driven luciferase reporter over vector-only control by full-length receptors is shown. DES, diethyl-stilbestrol; 4-OP, 4-octylphenol; BPA, Bisphenol A.
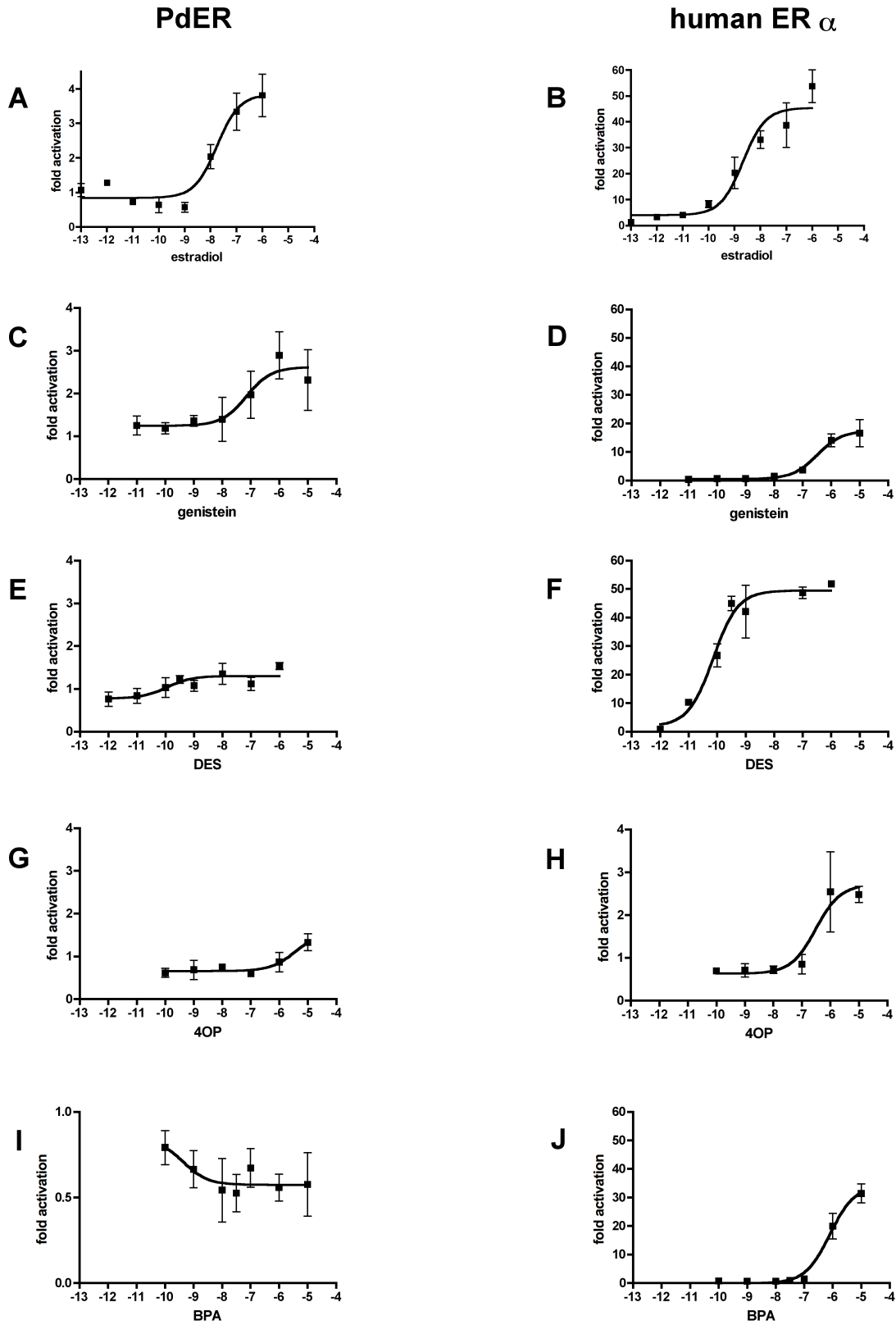
Fig. S7: Estrogen-specificity of CcER. Fold activation of a luciferase reporter (relative to vehicle-only control) by a Gal4DBD-CcERLBD fusion protein is shown in the presence of various hormones, each at 1 M.