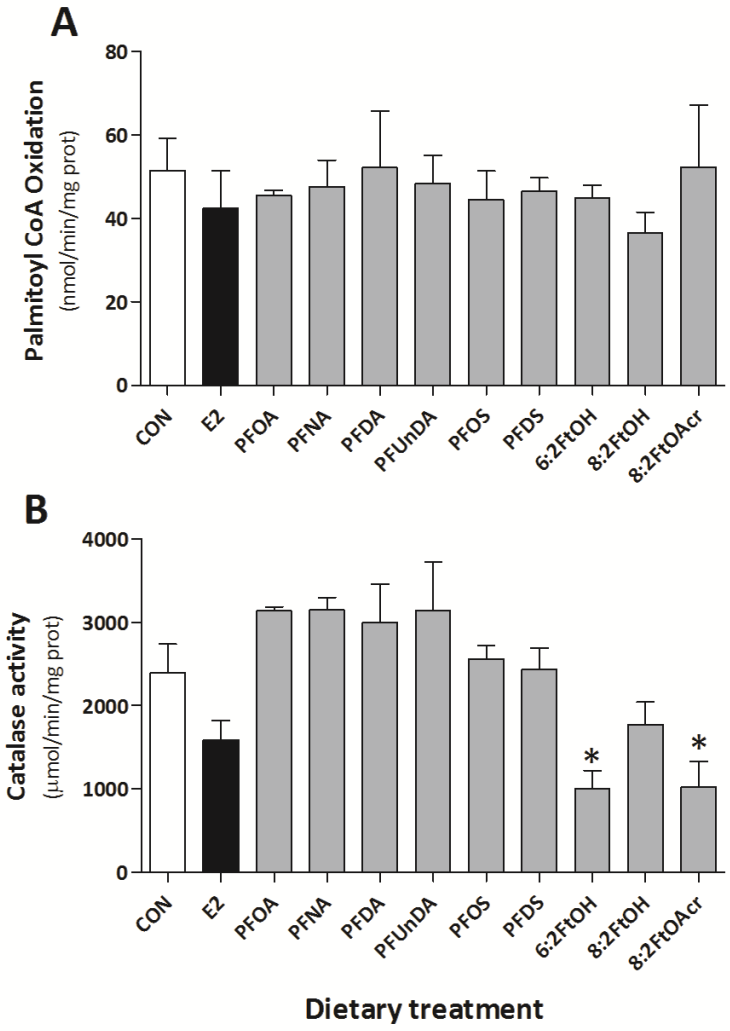
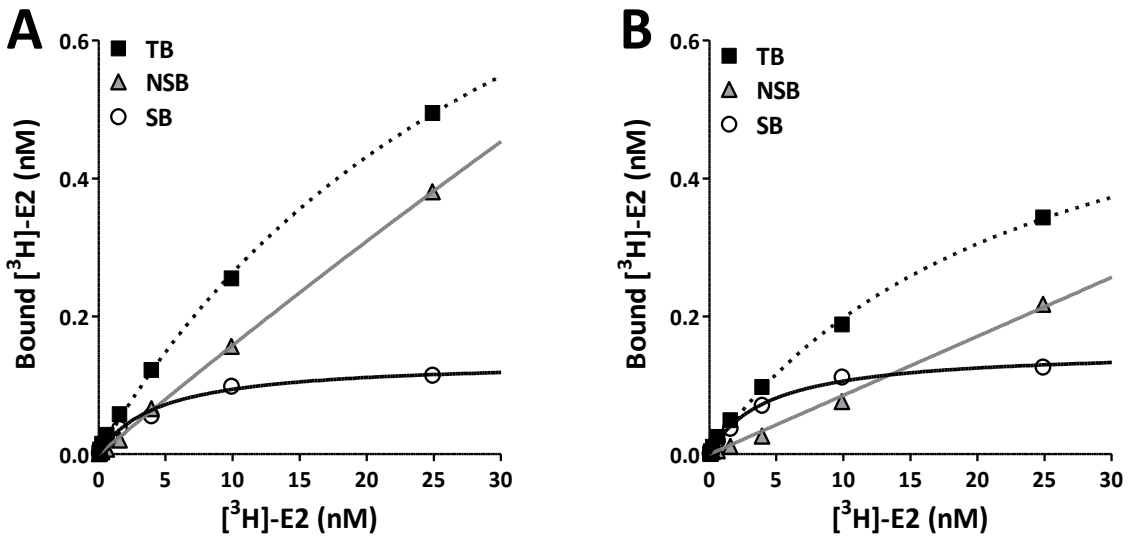
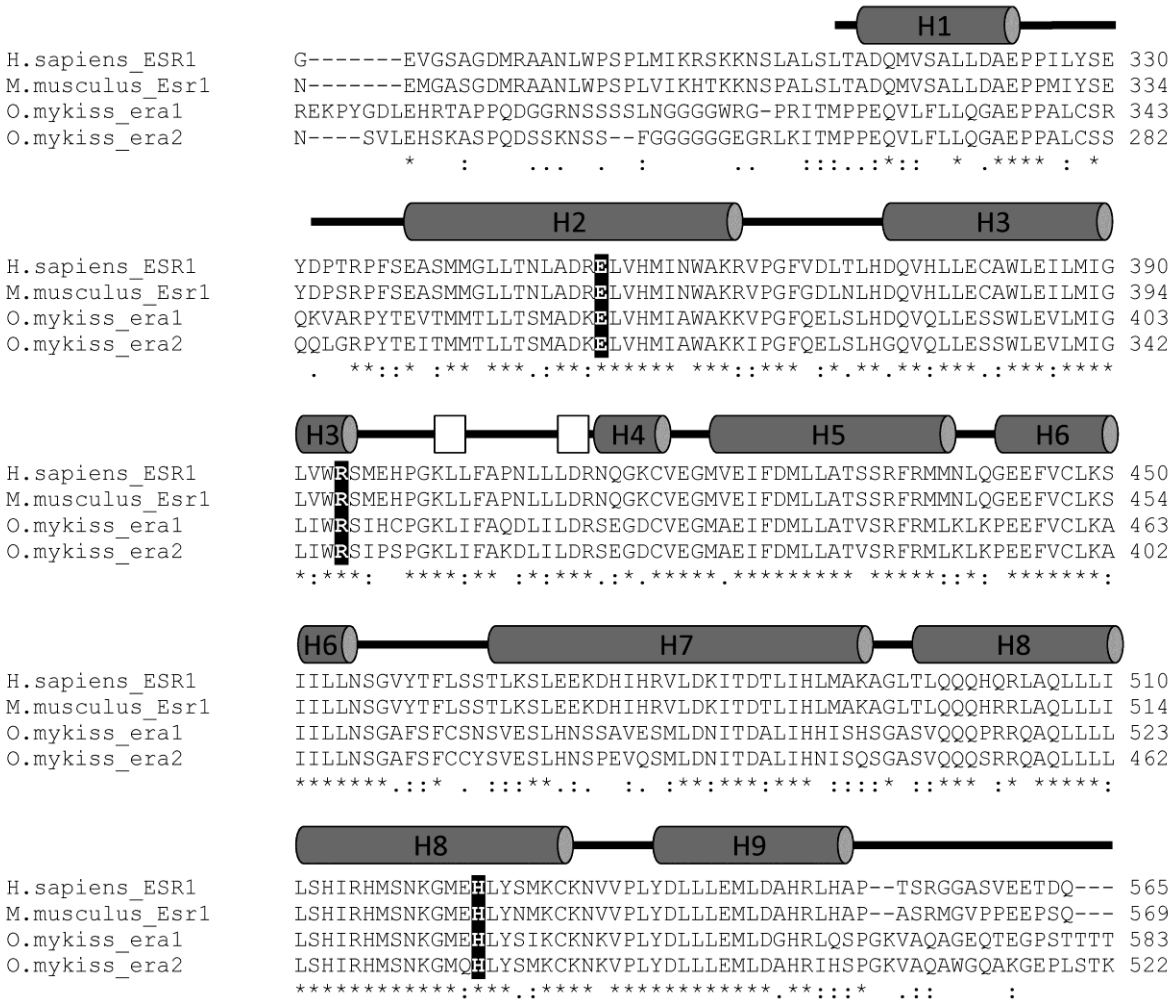
SUPPLEMENTARY FIGURES



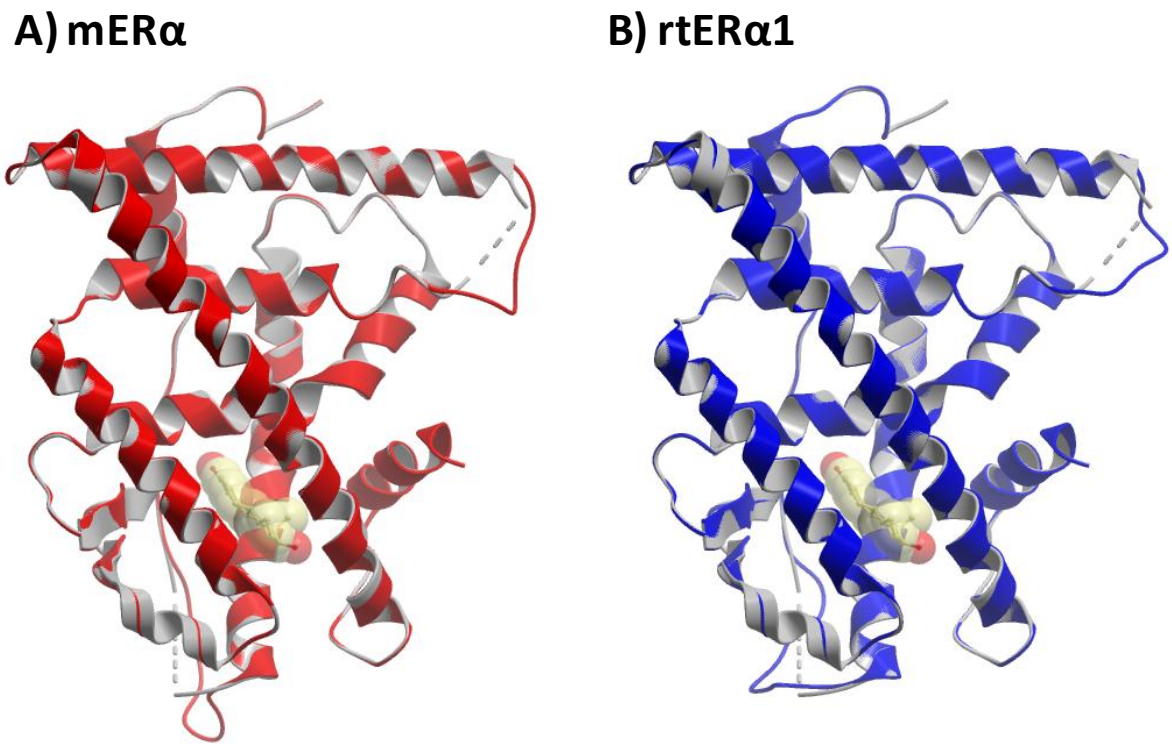
Supplementary Figure 1. Impact of dietary PFCs on enzyme markers of peroxisome proliferation. Mean rates of palmitoyl CoA oxidation (A) and catalase activity (B) are shown + SEM (N = 18 for control, N = 12 for E2, and N = 6 for all other treatments). The diet concentration was 250 ppm (approximately 5 mg/kg bw/day) for all perfluoroalkyl compounds and 5 ppm for E2, the positive control. *, $p < 0.05$ compared to the control treatment group as determined by one-way ANOVA with Dunnett's multiple comparisons post-hoc test.



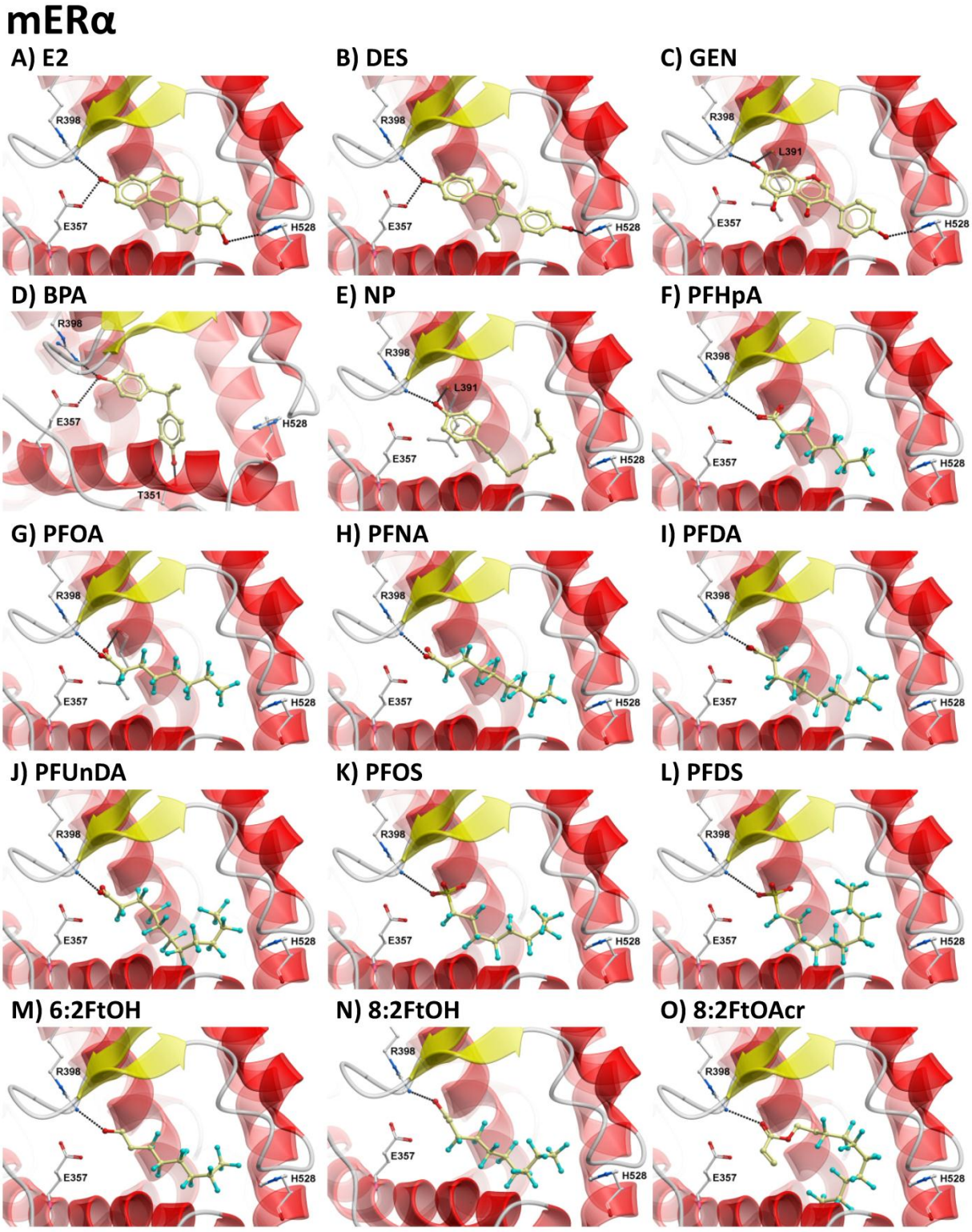
Supplementary Figure 2. Saturation analysis of [^{3}H]-E2 binding in juvenile rainbow trout liver. Binding characteristics of the estrogen receptor were compared in liver cytosol extracts obtained from juvenile rainbow trout fed control diet (A) or trout fed diet supplemented with 5 mg/kg E2 (B) for two weeks. Saturation plots of [^{3}H]-E2 binding in trout liver cytosol extracts are shown, and each symbol represents the mean of triplicate observations. Total binding, TB; specific binding, SB; and non-specific binding, NSB. A non-linear regression model for one-site binding was used to calculate values for the dissociation constant (K_d) and maximum number of binding sites (B_{max}), which are as follows: control liver cytosol, K_d = 4.4 ± 0.7 nM, B_{max} = 136 pM; E2 liver cytosol, K_d = 4.3 ± 0.4 nM, B_{max} = 151 pM.



Supplementary Figure 3. Alignment of the ligand-binding domain amino acid sequences for human, mouse and trout ERα proteins. Protein sequence alignment was performed using ClustalW2 (EMBL-EBI) using the complete amino acid sequences for human ESR1 (accession P03372), mouse Esr1 (P19785), trout era1 (P16058) and trout era2 **(**Q0H7E4). This figure shows the relevant portion of the ligand-binding domain region (*e.g.*, amino acids 311 to 564 for human ESR1) and the putative sites of estrogen hydrogen-bonding at the indicated glutamic acid, arginine and histidine residues (shaded in black). Symbols in the last line indicate the level of similarity of the sequences: the star represents conserved residues, the colon indicates highly similar residues, and the dot indicates weakly similar residues.



Supplementary Figure 4. Superimposition of mouse and trout ERα homology models with the human ERα crystal structure. Overlay plots were created using the SuperImpose algorithm in ICM-Browser (Molsoft). In both panels, hERα is represented in gray, whereas mERα (A) is shown in red and rtERα1 (B) is shown in blue. E2 is shown as a stick/space-filling model in both plots in the orientation determined by the hERα crystal structure (PDB accession 1ERE).


mERα
A) E2
B) DES
C) GEN
D) BPA
E) NP
F) PFHpA
G) PFOA
H) PFNA
I) PFDA
J) PFUnDA
K) PFOS
L) PFDS
M) 6:2FtOH
N) 8:2FtOH
O) 8:2FtOAcr
R398
E357
H528
L391
T351

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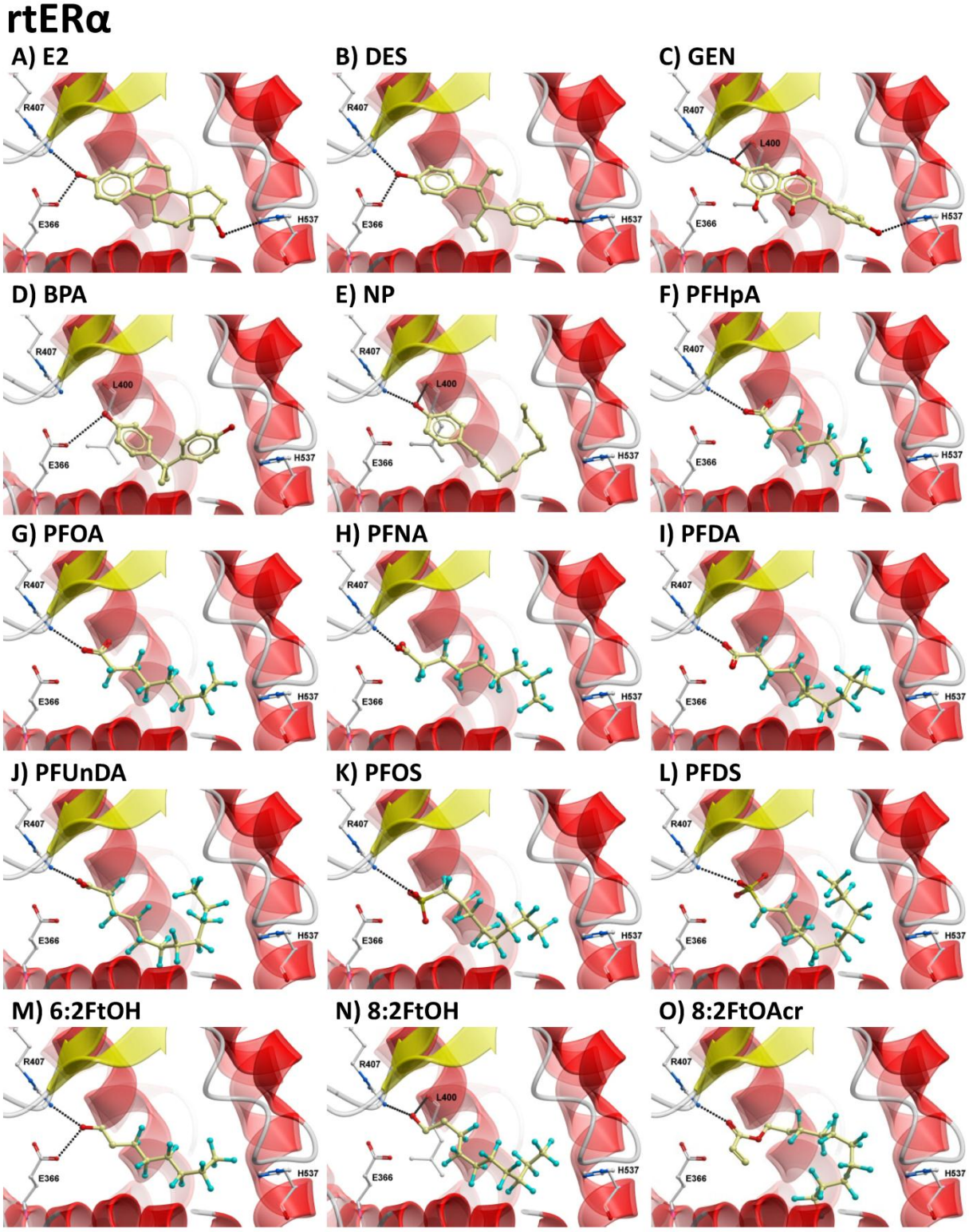
Supplementary Figure 5. *In silico* model showing docking of estrogens and select polyfluorinated chemicals into the mERα ligand-binding domain. Docking of test ligands into the mERα ligand-binding domain was performed using Molsoft ICM, and the lowest ICM-scored poses for each calculated ligand-protein docking are shown. Ligands are colored by atom type (the carbon atoms in tan) and are displayed as ball and sticks. Relevant protein residues are displayed as ball and sticks and colored by atom type: carbon atoms in gray, oxygen in red, sulfur in yellow and fluorine in cyan. Hydrogen bonds are represented by black dotted lines between the donor (*D*) and the acceptor (*A*) and are defined as follows: Distance *D*---*A*: 2.8-3.2 Å; Angle *D*-H---*A*: 140-180˚.


rtERα
A) E2
B) DES
C) GEN
D) BPA
E) NP
F) PFHpA
G) PFOA
H) PFNA
I) PFDA
J) PFUnDA
K) PFOS
L) PFDS
M) 6:2FtOH
N) 8:2FtOH
O) 8:2FtOAcr
R407
L400
E366
H537

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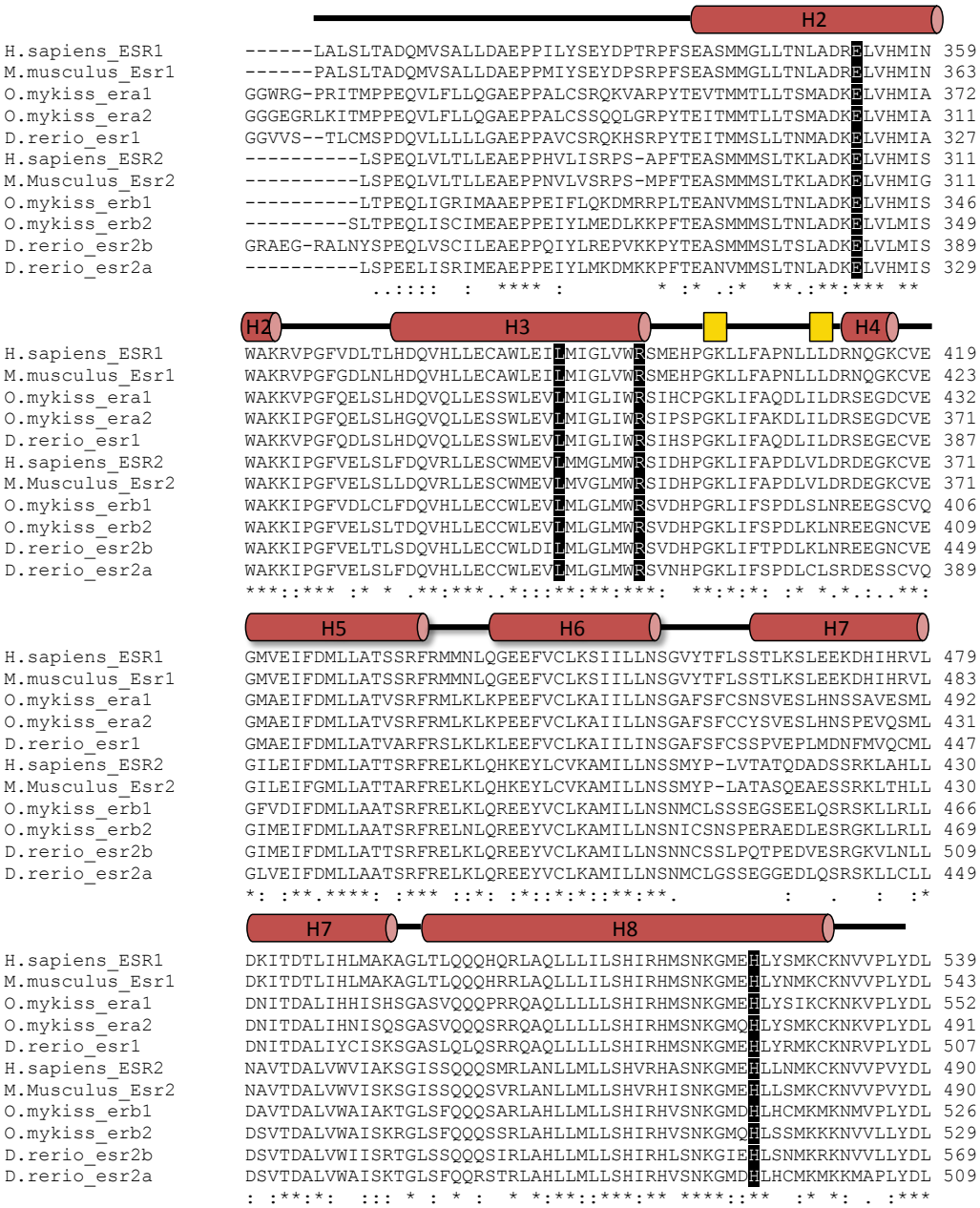
Supplementary Figure 6. *In silico* model showing docking of estrogens and select polyfluorinated chemicals into the rtERα1 ligand-binding domain. Docking of test ligands into the rtERα1 ligand-binding domain was performed using Molsoft ICM, and the lowest ICM-scored poses for each calculated ligand-protein docking are shown. Ligands are colored by atom type (the carbon atoms in tan) and are displayed as ball and sticks. Relevant protein residues are displayed as ball and sticks and colored by atom type: carbon atoms in gray, oxygen in red, sulfur in yellow and fluorine in cyan. Hydrogen bonds are represented by black dotted lines between the donor (*D*) and the acceptor (*A*) and are defined as follows: Distance *D*---*A*: 2.8-3.2 Å; Angle *D*-H---*A*: 140-180˚.



Supplementary Figure 7. Alignment of the ligand-binding domain amino acid sequences for human, mouse, trout and zebrafish ERα and β isoforms. Protein sequence alignment was performed using ClustalW2 (EMBL-EBI) using the complete amino acid sequences for human ESR1 (accession P03372), mouse Esr1 (P19785), trout era1 (P16058), trout era2 **(**Q0H7E4), zebrafish esr1 (P57717), human ESR2 (Q92731), mouse Esr2 (O08537), trout erb1 (Q0H7E3), trout erb2 (Q0H3B4), zebrafish esr2a (Q8AV62) and zebrafish esr2b (Q90WS9). Zebrafish sequences have been included as this fish model is now

commonly used for toxicity testing. This figure shows the relevant portion of the ligand-binding domain region (*e.g.*, amino acids 311 to 564 for human ESR1) and the putative sites of estrogen hydrogen-bonding for ERα and corresponding alignment with ERβ proteins at the indicated glutamic acid, arginine and histidine residues (shaded in black). Symbols in the last line indicate the level of similarity of the sequences: the star represents conserved residues, the colon indicates highly similar residues, and the dot indicates weakly similar residues.