Supporting Information

Investigation of binding and activity of perfluoroalkyl substances to human peroxisome proliferator-activated receptor β/δ

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Methods

Chemicals. Seventeen perfluoroalkyl substances (PFASs) were used in this study, including twelve perfluorinated alkyl acids, [perfluorobutyric acid (PFBA, 98%), perfluorohexanoic acid (PFHxA, ≥ 97%), perfluoroheptanoic acid (PFHpA, 99%), perfluorooctanoic acid (PFOA, 96%), perfluorononanoic acid (PFNA, 97%), perfluorodecanoic acid (PFDA, 98%), perfluoroundecanoic acid (PFUnA, 95%), perfluorododecanoc acid (PFDoA, 96%), perfluorotridecanoic acid (PFTrDA, 97%), perfluorotetradecanoic acid (PFTeDA, 97%), perfluorohexadecanoic acid (PFHxDA, 95%), perfluorooctadecanoic acid (PFOcDA, 97%)], three perfluorinated alkyl sulfonates [perfluorobutane sulfonate (PFBS, 97%), perfluorohexane sulfonate (PFHxS, ≥ 98%), perfluorooctane sulfonate (PFOS, ≥ 98%)], and two perfluorinated telomere alcohols [6:2-fluorotelomer alcohol (6:2 FTOH, 97%) and 8:2-fluorotelomer alcohol (8:2 FTOH, 97%)]. PFHxDA, PFOcDA and PFOS were purchased from Alfa Aesar (Ward Hill, MA, USA). Others 14 PFASs were all obtained from Sigma-Aldrich (St. Louis, MO, USA). Human PPARβ-LBD (with purity higher than 85%) was prepared by Zhongding Biotechnology Co. Ltd. (Nanjing, China). 4,4-Difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (C1-BODIPY-C12) probe was purchased from Life Technologies (Carlsbad, CA, USA). GW501516 and arachidonic acid (AA, ≥ 98.5%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Cell Culture and Cytotoxicity Assay. Human HEK 293 embryonal kidney cells were cultured in high-glucose Dulbecco's minimal essential medium (DMEM)

supplemented with 10% fetal bovine serum (Life Technologies), 100 units/mL penicillin and 100 μg/mL streptomycin (GIBCO, Grand Island, NY) in a humidified 5% CO_2 incubator at 37 ℃. The cytotoxicity of HEK 293 cells was determined by WST-1 assay. Briefly, 2×10^4 cells per well were seeded into 96-well plates. After 24 h, the cells were incubated with different concentration of PFASs (up to 500 μM) for 24 h, and then incubation with cell proliferation reagent WST-1 (1:10 dilution) (Roche Applied Science, Penzberg, Germany) at 37 ℃ for two hours. The absorbance was measured at 480 nm using SpectraMax i3x Multi-mode detection platform (Molecular Devices, Sunnyvale, CA). The relative cell viability was presented using 0.1% DMSO as vehicle control.

PPARβ/δ Mediated Luciferase Reporter Assay. A cell-based human PPARβ/δ-driven luciferase reporter assay was performed by co-transfect pBIND-PPARβ/δ vector, pGL4.35[luc2P/9XGAL4UAS/Hygro] vector and PRL-TK vector. The pBIND-PPARβ/δ vector containing yeast Gal4 DNA-binding domain and PPARβ/δ-ligand binding domain fusion genes were provided by GeneChem (Shanghai, China). The pBIND-PPARβ/δ vector can induce the transcription of pGL4.35[luc2P/9XGAL4UAS/Hygro] vector (Promega, Madison, WI, USA) containing an upstream Gal4 upstream activator sequence (UAS) when activated by a ligand. A PRL-TK vector (Promega, Madison, WI, USA) was used as an internal control reporter. HEK 293 cells were seeded at a density of 2×10^5 cells/well in 24-well plates and cultured until 90% confluence. Then they were transiently transfected with the pBIND-PPARβ/δ vector (300 ng), pGL4.35[luc2P/9XGAL4UAS/Hygro]

vector (300 ng) and PRL-TK vector (300 ng). Twenty-four hours later, the wells were replaced with fresh medium containing tested compounds in different concentrations for another 24 h. The cells were then harvested and measured their luciferase activity using a dual-luciferase reporter assay kit (Promega) and normalized to the Renilla luciferase activities and then calculated as relative luciferase activities. All data points were performed at least three independent experiments.

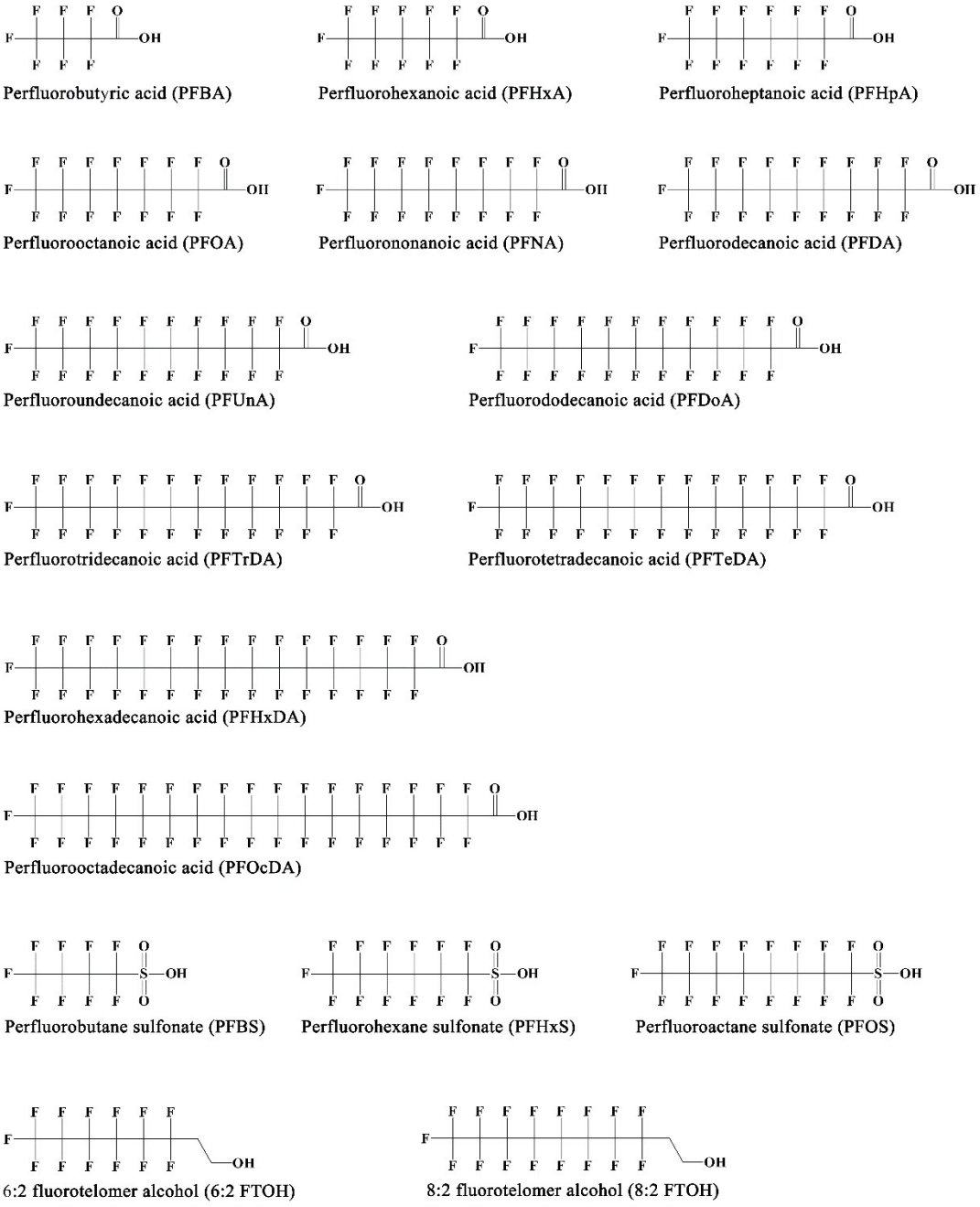

Figure S1. Structures of 17 PFASs used in this study.

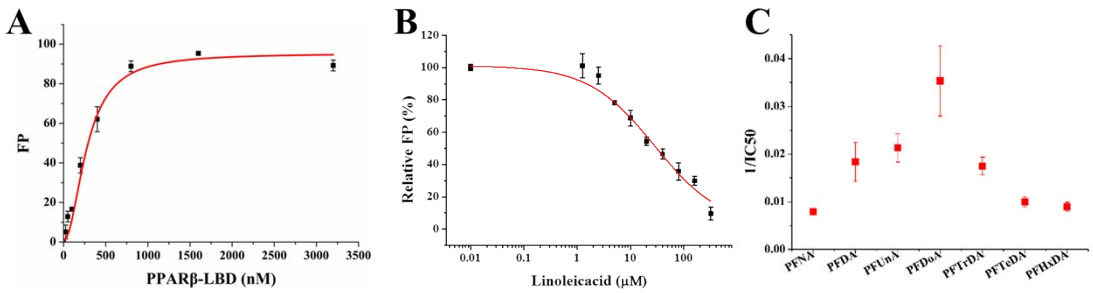

Figure S2. Direct fluorescence polarization binding curve of C1-BODIPY-C12 to human PPARβ/δ-LBD (A), and competitive binding curves of linoleic acid (B), and the relationship between human PPARβ/δ-LBD binding potency (showed as $1/IC_{50}$) and carbon chain length of PFCAs (C). The error bars represent the standard deviation of three measurements.

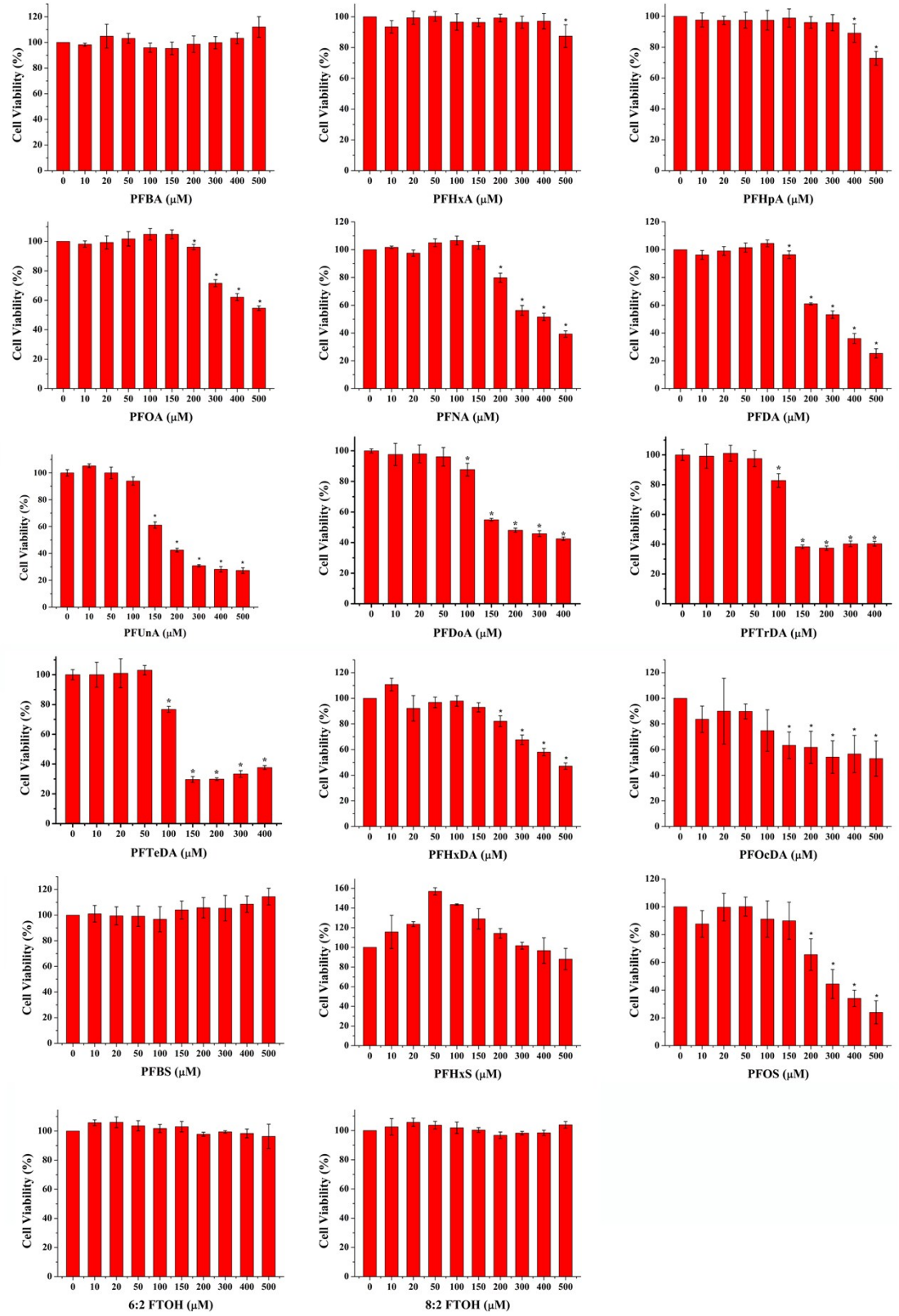

Figure S3. The cytotoxicity of 17 PFASs on HEK 293 cells determined by WST-1 assay. *P < 0.05, compare with the control group (0.1% DMSO).

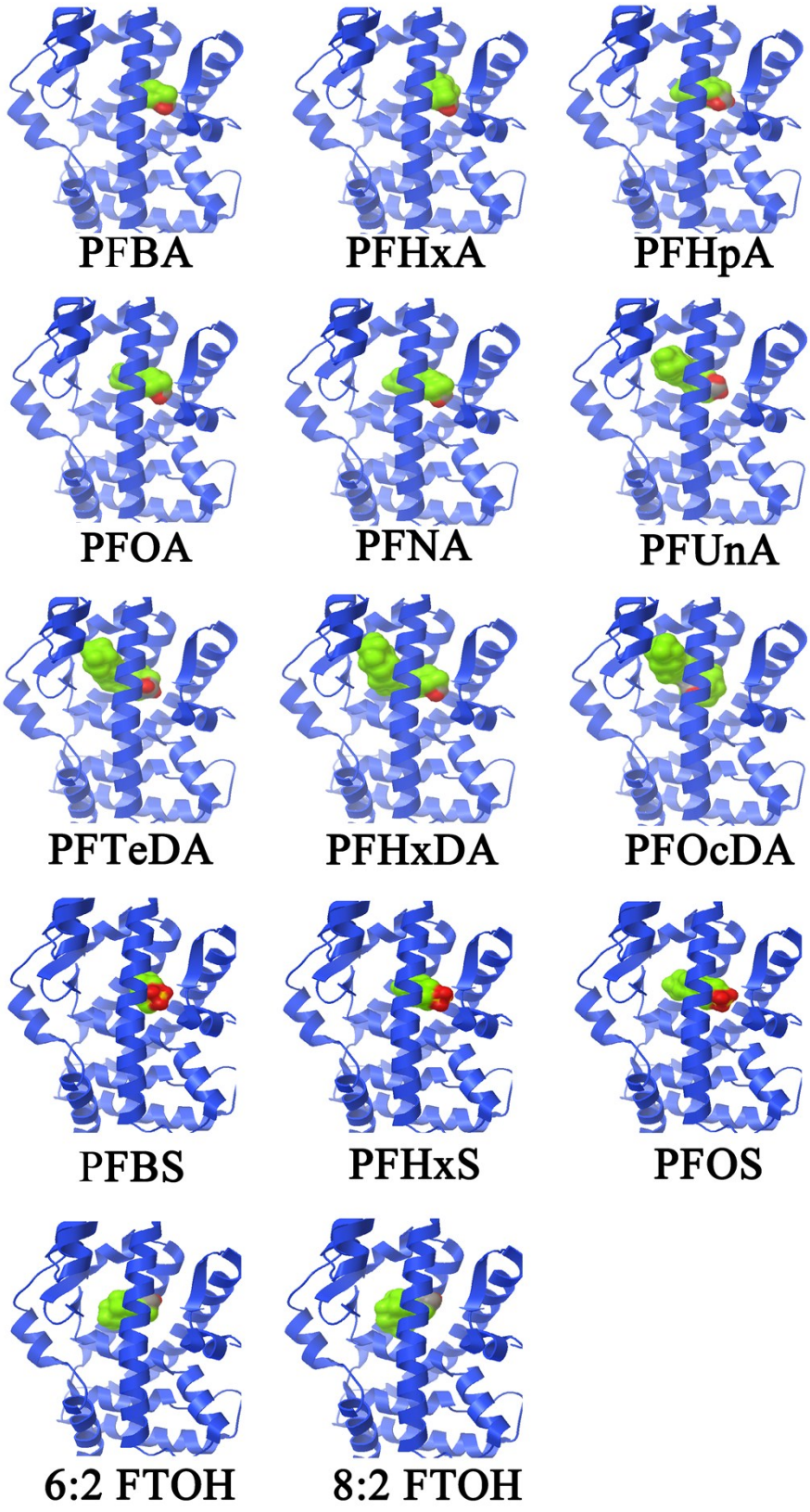

Figure S4. Molecular docking results of PFASs with human PPARβ/δ. PPARβ/δ are represented in blue, and the chemicals are colored by atom type (carbon in gray, oxygen in red, fluorine in green and sulfur in yellow).

Table S1. List of protein sequence used for human PPARβ/δ ligand binding domains protein synthesis.

Number	Species	Amino Acids	Protein Sequence
NP_001165289.1	Human	165-441	meqpqeeape vreeeekeev aeaegapeln ggpqhalpss sytdlsrsss ppslldqlqm gcdgascgsl nmecrvcgdk asgfhygvha cegckgffrr tirmkleyek cersckiqkk nrnkcqycrf qkclalgmsh nairfgrmpe aekrklvagl tanegsqynp qvadlkafsk hiynaylknf nmtkkkarsi ltgkashtap fvihdietlw qaekglvwkq lvnglppyke isvhvfyrcq cttvetvrel tefaksipsf sslflndqvt llkygvheai famlasivnk dgllvangsg fvtreflrsl rkpfsdiiep kfefavkfna lelddsdlal fiaaiilcgd rpglmnvprv eaiqdtilra lefhlqanhp daqylfpkll qkmadlrqlv tehaqmmqri kktetetslh pllqeiykdm y