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### **Supplemental Material**

#### **Screening of Organophosphate Flame Retardants with Placentation-Disrupting Effects in Human Trophoblast Organoid Model and Characterization of Adverse Pregnancy Outcomes in Mice**

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**Figure S1. Induced and Characterized Trophoblast Organoids and Optimized Protocol.** (A) Human trophoblast organoid; (B) Immunofluorescence of villus markers KRT7 (green), GATA3 (red), and DAPI (blue) in trophoblast organoids following previous protocol (Sheridan, 2020); (C) Previous digestion (Sheridan, 2020) and optimized digestion (3 min in 37°C and pipetted gently); (D) Relative Ki67 intensity (means  $\pm$  SDs) of organoids after 2-day culture in previous digestion (Sheridan, 2020) and optimized digestion (3 min in 37°C and pipetted gently); (E) Relative Ki67 intensity (means  $\pm$  SDs) of organoids after 2-day culture in previous and optimized TOM (Table S5). Data in (C) and (D) are expressed relative to the levels of previous digestion and previous TOM, respectively, which were set to 1.  $n = 3$ . All organoids in (C) and (D) were from a single donor. Data were analyzed using an unpaired two-tailed Student's t-test. Indicated values are significantly different from control value.  $*p < 0.05$ . KRT7, keratin 7; GATA3, GATA binding protein 3; SD, standard deviation; TOM, trophoblast organoid medium. Scale bars, 60  $\mu\text{m}$ . Numeric data in (D)-(E) were listed in Table S16.

**Figure S2. Additional measures of short- and long-term toxicity of EHDPP in organoids.** (A) Relative fluorescence intensity (means  $\pm$  SDs) of Sytox Green (green) in trophoblast organoids in 2-day EHDPP exposure; (B) Relative fluorescence intensity (means  $\pm$  SDs) of Sytox Green (green) in trophoblast organoids in 10-day EHDPP exposure; (C) Western blotting of PD in control and 2-day EHDPP exposure groups; (D) Relative protein levels of PD (means  $\pm$  SDs) in western blotting in control and 2-day EHDPP exposure groups; (E) CD71 (green) and DAPI (grey) in control and 2-day EHDPP (10,000 nM) exposure groups; (F) Relative intensity (means  $\pm$  SDs) of CD71 in (E); (G) HLA-G (red), F-actin (blue) and DAPI (grey) in 4-day culture. Data in (A)-(G) are expressed relative to the levels of DMSO-treated organoids, which were set to 1.  $n = 3$ . All organoids in (A)-(G) were from a single donor. Analyzed by the unpaired two-tailed Student's t-test. Indicated values are significantly different from control value.  $*p < 0.05$ .  $**p < 0.01$ . Scale bars, 20  $\mu\text{m}$ . EHDPP, 2-ethylhexyl-diphenyl phosphate; PD, pyruvate dehydrogenase complex; CD71, transferrin receptor; HLA-G, human leucocyte antigen protein-G; DAPI, 4',6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide. Numeric data in (A), (B), (D) and (F) were listed in Table S16.

**Figure S3. Mechanism of placentation disruption.** (A) Relative expression (means  $\pm$  SDs) of IGF1 in 2-day EHDPP exposure; (B) Relative expression (means  $\pm$  SDs) of IGF2 exposure in 2-day EHDPP exposure; (C) Western blotting of IGF1R in HA-IGF1R and HA-GFP overexpression groups in 293T; (D) Relative intensity (means  $\pm$  SDs) of Ki67 in control and 2-day OSI-906 (50 nM) exposure groups; (E) Relative intensity (means  $\pm$  SDs) of TP63 in control and 2-day OSI-906 (50 nM) exposure groups; (F) CD71 (green) and DAPI (grey) in control and 2-day OSI-906 (50 nM) exposure groups; (G) Relative intensity (means  $\pm$  SDs) of CD71 in (F); (H) Relative intensity (means  $\pm$  SDs) of Ki67 in control and 10-day OSI-906 (50 nM) exposure groups; (I) Relative intensity (means  $\pm$  SDs) of TP63 in control and 10-day OSI-906 (50 nM) exposure groups; (J) Relative intensity (means  $\pm$  SDs) of CD71 in control and 10-day OSI-906 (50 nM) exposure groups; (K) Relative intensity (means  $\pm$  SDs) of HLA-G in control and 10-day OSI-906 (50 nM) exposure groups. Data in (A) and (B) and (D) and (E) and (G) and (K) are expressed relative to the levels of DMSO-treated organoids, which were set to 1.  $n = 3$ . All organoids in (A)-(K) were from one single donor. Analyzed by the unpaired two-tailed Student's t-test. Indicated values are significantly different from control value.  $*p < 0.05$ .  $**p < 0.01$ . Scale bars, 20  $\mu\text{m}$ . IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IGF1R, insulin-like growth factor 1 receptor; TP63, tumor protein 63; CD71, transferrin receptor; HLA-G, human leucocyte antigen protein-G; OSI-906, Linsitinib; DAPI, 4',6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide. Numeric data in (A), (B), (D), (E), (G), (H), (I), (J) and (K) were listed in Table S17.

**Figure S4. Effects of EHDPP on placental structure and pregnancy in female mice.** (A) HAND1 (red) and DAPI (blue) in E19.5 placentas in control and EHDPP (10 mg/kg/day) exposure group and scale bars in , 500  $\mu\text{m}$  in  $\times 2$  and 50  $\mu\text{m}$  in  $\times 40$ .; (B) Relative intensity (means  $\pm$  SDs) of TPBPA; (C) Relative intensity (means  $\pm$  SDs) of TFAP2C; (D) Relative intensity (means  $\pm$  SDs) of HAND1 (B)-(D) were detected in control and EHDPP (10 mg/kg/day) exposure groups in E19.5 placentas; (E) Relative protein level (means  $\pm$  SDs) of p-IGF1R (Y1135)/IGF1R in E19.5 fetal placentas; (F) p-IGF1R (green) in control and EHDPP exposure groups in E19.5 placentas; (G) Relative protein level (means  $\pm$  SDs) of PD in western blotting in control and EHDPP exposure groups in E19.5 placentas; (H) Relative protein level (means  $\pm$  SDs) of cytochrome C in western blotting in control and EHDPP exposure groups in E19.5 placentas; (I) GSEA analysis between placentas of Control and 10 mg/kg EHDPP exposure group in E19.5 placentas; (J) FGR incidence rates after EHDPP exposure. Data in (B) and (C) and (D) and (E) and (G) and (H) are expressed relative to the levels of control group, which were set to 1. (B)-(E),  $n = 5$ ; (G)-(I),  $n = 3$ ; (J),  $n = 10$ . (B) and (C) and (D) and (E) and (G) and (H) was analyzed by the unpaired two-tailed Student's t-test. (J) was analyzed by  $\chi^2$  test. Indicated values are significantly different from control value. \* $p < 0.05$  and \*\* $p < 0.01$ . (A): Scale bars, 500  $\mu\text{m}$  in  $\times 2$  and 50  $\mu\text{m}$  in  $\times 40$ . HAND1, crest derivatives-expressed protein 1; TPBPA, trophoblast-specific protein  $\alpha$ ; TFAP2C, transcription factor AP-2  $\gamma$ ; IGF1R, insulin-like growth factor 1 receptor; p-IGF1R, phosphorylated-IGF1R; EHDPP, 2-ethylhexyl-diphenyl phosphate; PD, pyruvate dehydrogenase complex; FGR, fetal growth restriction;  $\chi^2$ , chi-square. Numeric data in (B), (C), (D), (E), (G) and (H) were listed in Table S17.

**Table S1.** OPFRs list for screening.

**Table S2.** Geographical information of exposure in 16 OPFRs.

**Table S3.** Chemicals used in this paper.

**Table S4.** Information of donors.

**Table S5.** Trophoblast organoid medium (TOM).

**Table S6.** Primer sequences in RT-qPCR.

**Table S7.** Antibodies and DAPI.

**Table S8.** Screening of OPFRs.

**Table S9.** Numeric data for Fig 3B, 3D, 3E, 3G, 3I, 3K, 3L, 3M, 3O, 3P (means  $\pm$  SDs).

**Table S10.** Numeric data for Fig 3C (oxygen consumption rate; pmol/min).

**Table S11.** Numeric data for Fig 3Q (means  $\pm$  SDs).

**Table S12.** Numeric data for Fig 4C, 4D, 4E.

**Table S13.** Numeric data for Fig 4F (% inhibition).

**Table S14.** Numeric data for Fig 5D, 5E, 5J, 5L, 5M.

**Table S15.** Numeric data for Fig 5K (means  $\pm$  SDs).

**Table S16.** Numeric data for Fig S1D, S1E, S2A, S2B, S2F (means  $\pm$  SDs), and S2D.

**Table S17.** Numeric data for Fig S3A, S3B, S3D, S3E, S3G, S3H, S3I, S3J, S3K (mean  $\pm$  SDs).

**Table S18.** Numeric data for Fig S4B, S4C, S4D, S4E, S4G, S4H.

### **Sequence of HA-GFP**

### **References**

**Additional File-** Excel Document