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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

Chenke Xu, Haojia Ma, Fumei Gao, Chenhao Zhang, Wenxin Hu, Yingting Jia, Jun Xu, and Jianying Hu

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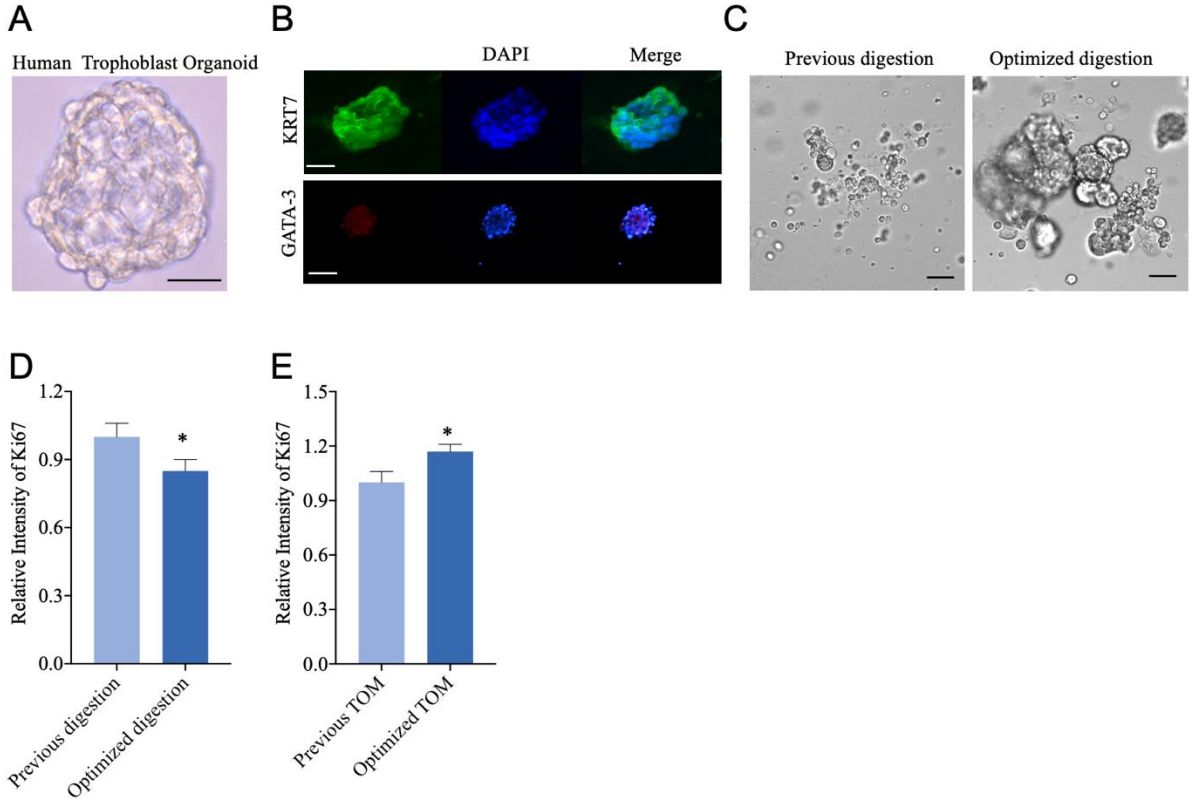
References

Additional File- Excel Document

Supplementary Text



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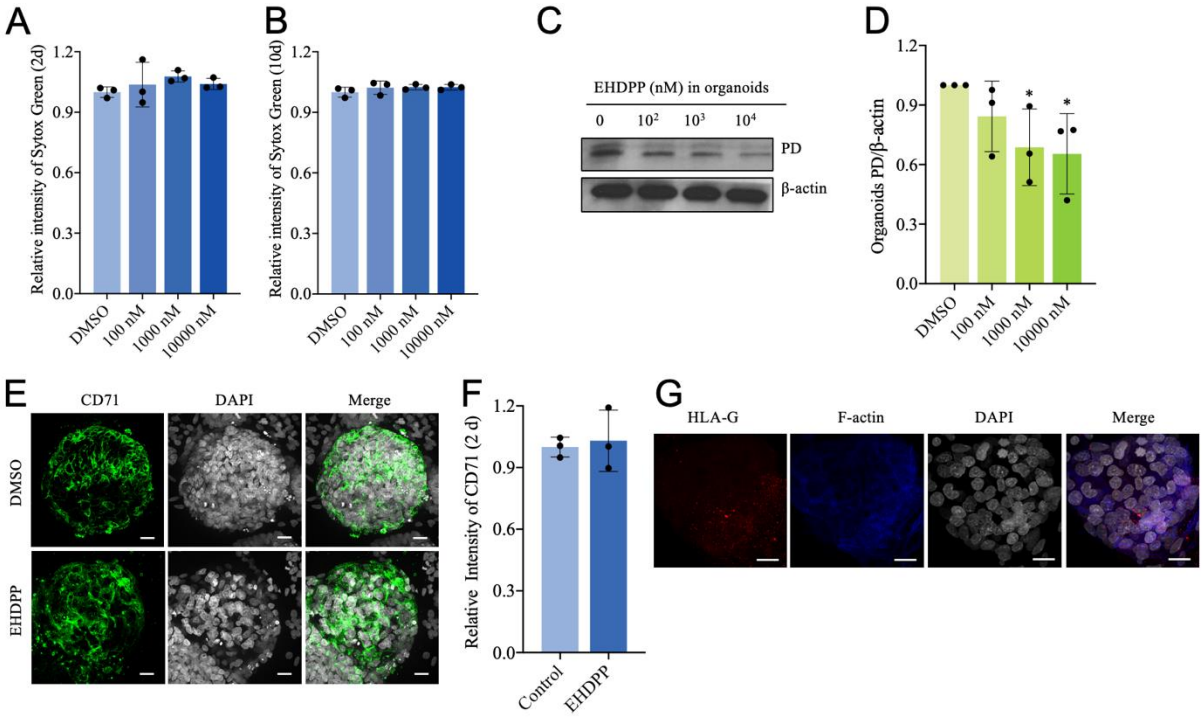

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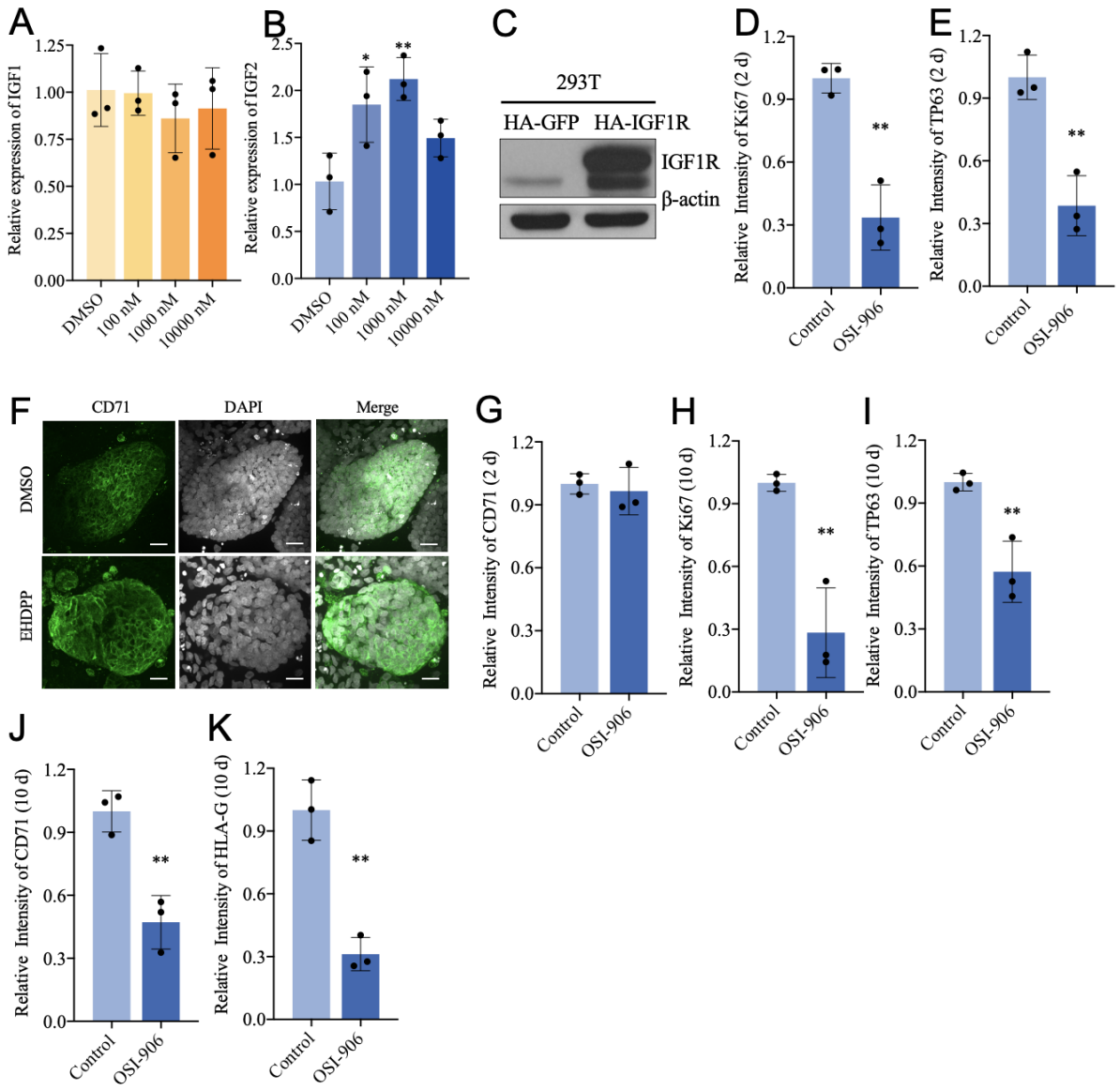

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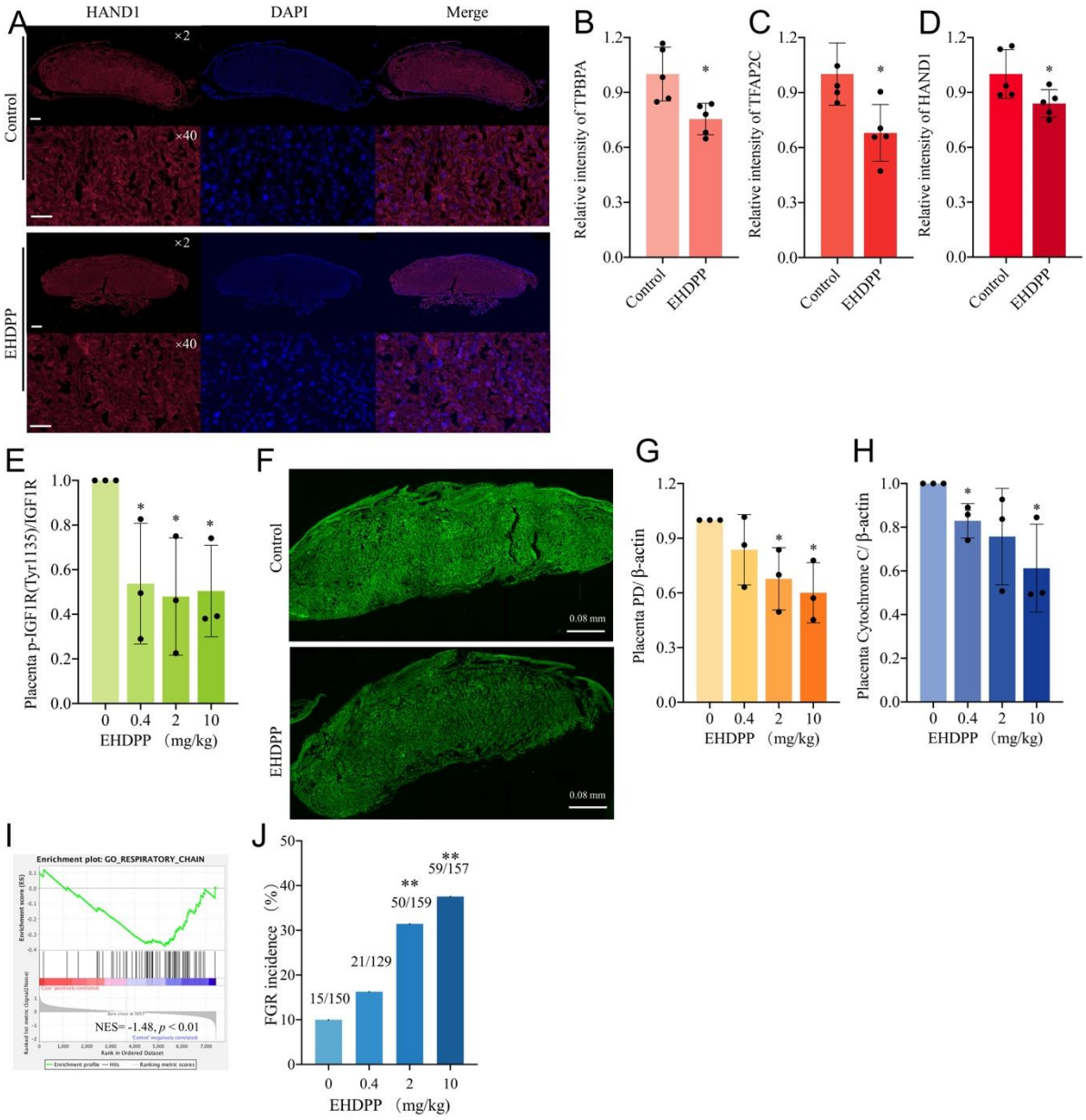

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Classification	Full name (Abbreviation)	CAS	Company
Alkyl-OPFRs	Triethyl phosphate (TEP)	78-40-0	TCI Corp., P0270
	Triisopropyl phosphate (TiPP)	513-02-0	Sigma, 554669
	Tributyl phosphate (TBP)	126-73-8	Sigma, 8186040100
	Tripropyl phosphate (TPrP)	513-08-6	Macklin, T819409
	Triisobutyl phosphate (TiBP)	126-71-6	Macklin, T834115
	Tris(2-ethylhexyl) phosphate (TEHP)	78-42-2	TCI Corp., P1022
	Dimethyl hydrogen phosphate (DMP)	813-78-5	Macklin, D885090
	Diethyl hydrogen phosphate (DEP)	598-02-7	Macklin, D839196
	Dibutyl phosphate (DBP) *(He et al. 2021)	107-66-4	Macklin, D835976
	Bis(2-ethylhexyl) hydrogen phosphate (BEHP) *(Huo et al. 2020)	298-07-7	Macklin, B802752
	Trihexyl phosphate (T6CP) *(Wang, 2020)	2528-39-4	Macklin, T908781
Aryl-OPFRs	Isopropylphenyl Phenyl Phosphate (ip-PPP)	68782-95-6	Macklin, I860749
	2-Ethylhexyl diphenyl phosphate (EHDPP)	1241-94-7	TCI Corp., P1021
	Isodecyl diphenyl phosphate (IDDPP)	29761-21-5	AccuStandard, PFRS-008S
	Triphenyl phosphate (TPhP)	115-86-6	TCI Corp., P0272
	Tris(3,5-xylenyl) phosphate (TXP)	25155-23-1	Synthesized
	Tris(4-tert-butylphenyl) phosphate (T4tBPPP)	78-33-1	Synthesized
	Tricresyl phosphate (TCrP)	1330-78-5	Macklin, T819410
	Diisodecyl phenyl phosphate (DIDPP)	51363-64-5	Synthesized
	Tri-O-cresyl phosphate (o-TCrP)	78-30-8	Macklin, T820120
	Tricresyl phosphate (p-TCrP)	78-32-0	Macklin, T862850
	Tri-M-cresyl phosphate (m-TCrP)	563-04-2	Macklin, T867695
	Tris(4-isopropylphenyl) phosphate (T4IPPP)	2502-15-0	Wellington Laboratories
	Bis(p-tert-butylphenyl) phenyl phosphate (B4tBPPP)	115-87-7	Wellington Laboratories
	Resorcinol bis(diphenyl phosphate) (RDP)	57583-54-7	Macklin, R860747
	Cresyl diphenyl phosphate (CDP)	26444-49-5	Macklin, C8341114
	Diphenyl phosphate (DPP) *(Funk et al. 2019)	838-85-7	Macklin, D831185
	Tris(nonylphenyl) phosphate (TNPP) *(Wang et al. 2020)	26569-53-9	Synthesized
	Isopropylphenyl diphenyl phosphate (2IPPDPP) *(Wang et al. 2020)	64532-94-1	Wellington Laboratories
	Bis(2-isopropylphenyl) phenyl phosphate (B2IPPPP) *(Wang et al. 2020)	69500-29-4	Wellington Laboratories
	4-tert-butylphenyl diphenyl phosphate (4tBPDPP) *(Wang et al. 2020)	981-40-8	Wellington Laboratories
	Bis(2-ethylhexyl) phenyl phosphate (BEHPP) *(Wang et al. 2020)	16368-97-1	Macklin, B905605
	Bisphenol A bis(diphenyl phosphate) (BPADP) *(Wang et al. 2020)	5945-33-5	Aladdin, B304092
	Tris(2,4-ditert-butylphenyl) phosphate (T4DtBPP) *(Wang et al. 2020)	95906-11-9	Wellington Laboratories
	Bis(2-methylphenyl) hydrogen phosphate (DoCP) *(Gao, 2020)	35787-74-7	TRC, D494575

Most of OPFRs were picked from Toxicity Forecaster (Toxcast) list (https://comptox.epa.gov/dashboard/chemical-lists/FLAMERETARD). * Chemicals that are not on the Toxicity Forecaster (Toxcast)

Continued

Classification	Full name (Abbreviation)	CAS	Company
Halogenated-OPFRs	Tris(1,3-dichloro-2-propyl)phosphate (TDCIPP)	13674-87-8	TCI Corp., P0269
	Tris(2-chloroethyl) phosphate (TCEP)	115-96-8	Sigma-Aldrich, 119660
	tert-Butylphenyl diphenyl phosphate (BPDPP)	56803-37-3	Johnlong, CFRT111308
	Bis(2,3-dibromopropyl) hydrogen phosphate (BDBPP)	5412-25-9	Bioruler, RH132419
	Tris(1-chloro-2-propyl) phosphate (TCIPP)	13674-84-5	Rowan, R010190
	2,2-Bis-(bromomethyl)-3-bromo-1-propanol phosphate (TTBNPP)	19186-97-1	Macklin, T859201
	Tris(2,3-dibromopropyl) phosphate (TDBPP)	126-72-7	AccuStandard, PFS-008N
	Oxydi-2,1-Ethanediyl-Phosphoric Acid Tetrakis(2-Chloro-1-Methylethyl) Ester (RDT905)* (Wang et al. 2021)	52186-00-2	Omnistab, FR-RDT 9
	Phosphoric acid, 2,2-bis(chloromethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester (V6)* (Wang et al. 2021)	38051-10-4	AccuStandard, PFS-020S
	3,9-Bis(2,4-di-tert-butylphenoxy)-2,4,8,10-tetraoxa-3,9-diphosphaspiro[5.5]undecane (AO626=O2)*(Wang et al. 2021)	26741-53-7	Macklin, A832583
	Bis(2-chloroethyl) phosphate (BCEP)*(He et al. 2021)	3040-56-0	Johnlong, CCHM701085

Most of OPFRs were picked from Toxicity Forecaster (Toxcast) list (https://comptox.epa.gov/dashboard/chemical-lists/FLAMERETARD). * Chemicals that are not on the Toxicity Forecaster (Toxcast)

Table S2. Geographical information of exposure in 16 OPFRs

Abbreviation	Geographical information	Reference
DBP	the USA	(He et al. 2021)
BEHP	China	(Huo et al. 2020)
T6CP	China	(Gao et al. 2020)
DPP	China; the USA; Canada	(Gao et al. 2020; Carignan et al. 2017; Funk et al. 2019)
TNPP	Canada; China	(Liu et al. 2019; Wang et al. 2020)
2IPPDPP	China	(Wang et al. 2020)
B2IPPPP	China	(Wang et al. 2020)
4tBPDPP	China	(Wang et al. 2020)
BEHPP	China	(Wang et al. 2020)
BPADP	China	(Wang et al. 2020)
T4DtBPP	China	(Wang et al. 2020)
DoCP	China	(Gao et al. 2020)
RDT905	China	(Wang et al. 2021)
V6	China; the USA	(Wang et al. 2021, Stapleton et al. 2011)
AO626=O2	China	(Wang et al. 2021)
BCEP	the USA	(He et al. 2021)

Note: For definition of abbreviations, see Table S1.

Table S3. Chemicals used in this paper

Chemicals	Company	Final concentration
OSI-906 (Linsitinib)	MCE, HY-10191	50 nM
Testosterone	NOVUS, NBP2-45187	40 μM

Table S4. Information of donors

Donor Number	Age	Gestational age	Karyotype
1#	24	7 weeks	44+XX
2#	28	8 weeks	44+XY
3#	30	7 weeks	44+XX
4#	39	7 weeks	44+XY
5#	28	8 weeks	44+XY

Table S5. Trophoblast organoid medium (TOM)

Product	Company and Product Number	Optimized (Previous) concentration
Advanced DMEM/F12	Life Technologies,12634010	1X
N2 supplement	Life Technologies, 17502048	1X
B27 supplement	Life Technologies, 12587010	1X
L-glutamine	Life Technologies, 25030	2 mM
N-acetyl-L-cysteine	Sigma, A9165	1.25 mM
ALK-4, -5, -7 inhibitor, A83-0.1	Tocris, 2939	500 nM
CHIR99021	Tocris, 4423	5 μM (1.5 μM)
Recombinant human EGF	Peprotech, AF-100-15	50 ng/mL
Recombinant human R-spondin 1	BioTechne, 4645-RS	80 ng/mL
Recombinant human FGF2	Peprotech, 100-18C	100 ng/mL
Recombinant human HGF	Peprotech, 100-39	50 ng/mL
Y27632	Tocris, 1254	10 μM (2 μM)
PGE2	Sigma, P0409	10 μM (2.5 μM)
Penicillin Streptomycin	Gibco, 15140122	100X

Table S6. Primer sequences in RT-qPCR

Primer	Forward	Reverse
Human IGF1	GCTCTTCAGTTCGTGTGTGGA	GCCTCCTTAGATCACAGCTCC
Human IGF2	GTGGCATCGTTGAGGAGTG	CACGTCCCTCTCGGACTTG
Human IGF1R	TCGACATCCGCAACGACTATC	CCAGGGCGTAGTTGTAGAAGAG
Human β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

Table S7. Antibodies and DAPI

Antibody	Company	Product number	Dilution
anti-Ki67	CST	12075S	1:100 (IF)
anti-HLA-G	Abcam	ab52454	1:100 (IF)
anti-p63	Abcam	ab124762	1:100 (IF)
anti-CD71	Abcam	ab38171	1:100 (IF)
anti-Cdx2	Abcam	ab576541	1:50 (IF)
anti-rabbit lgG H&L	Abcam	ab150075	1:100 (IF)
anti-mouse lgG H&L	CST	8890S	1:100 (IF)
anti-trophoblast specific protein α	Abcam	ab104401	1:30 (IF)
anti-HAND1	Bioss	bs-9459R	1:30 (IF)
anti-transcription factor AP-2γ	Santa Cruz	sc-8977	1:30 (IF)
DAPI	SIGMA	D9542	1:5000 (IF)
Alexa fluor 488 Phallo	Molecular Probes	A12379	1:200 (IF)
anti-pyruvate dehydrogenase	CST	3250S	1:1000 (WB)
anti-cytochrome c	CST	4280S	1:1000 (WB)
anti-β-Actin	CST	3700S	1:2000 (WB)
anti-mouse IgG HRP-linked	CST	7076S	1:5000 (WB)
anti-rabbit IgG HRP-linked	CST	14708S	1:1000 (WB)
anti-phospho-Akt (Ser_{473})	CST	4060S	1:1000 (WB)
anti-Akt (pan)	CST	7076S	1:5000 (WB)
anti-phospho-IGF1Rβ (Tyr1135)	CST	3918S	1:1000 (WB)
anti-IGF1Rβ	CST	9750S	1:1000 (WB)
anti-KRT7	CST	4465S	1:50 (IF)
anti-GATA3	Abcam	ab100428	1:50 (IF)

* IF, immunofluorescence; WB, western blotting.

Table S8. Screening of OPFRs

Chemicals	Average Intensity of Ki67	Average Intensity of Sytox Green
DMSO	7,832.3 ± 896.1	2,336.7 ± 29.1
TEP	7,129.2 ± 1,478.2	2,381.1 ± 127.4
TiPP	8,214.9 ± 1,151.1	2,280.8 ± 72.5
TBP	7,168.4 ± 1,726.7	2,383.9 ± 20.6
TPrP	7,560.9 ± 392.4	2,422.3 ± 131.7
TiBP	7,168.4 ± 889.5	2,348.1 ± 38.6
TEHP	7,390.8 ± 13.1	2,312.1 ± 35.0
DMP	8,057.9 ± 1268.9	2,339.5 ± 65.7
DEP	7,822.5 ± 1046.5	2,265.2 ± 25.1
DBP	7,220.8 ± 758.7	2,316.5 ± 31.9
BEHP	7,142.3 ± 444.8	2,384.7 ± 27.3
T6CP	7,338.5 ± 1517.4	2,342.0 ± 56.2
ip-PPP	6,370.5 ± 130.8	2,431.7 ± 13.0
EHDPP	5,598.7 ± 601.7	2,622.7 ± 159.5
IDDPP	5,808.0 ± 1,216.5	2,568.9 ± 2.0
TPhP	7,966.4 ± 366.3	2,506.6 ± 56.3
TXP	6,906.8 ± 797.9	2,526.0 ± 35.4
T4tBPPP	7,534.7 ± 745.6	2,590.1 ± 240.0
TCrP	7,403.9 ± 222.4	2,557.9 ± 45.0
DIDPP	6,148.1 ± 954.9	2,407.0 ± 92.2
o-TCrP	5,232.4 ± 811.0	2,391.9 ± 102.7
p-TCrP	8,633.5 ± 366.3	2,409.9 ± 89.7
m-TCrP	6,645.2 ± 994.2	2,388.4 ± 93.6
T4IPPP	6,434.3 ± 497.1	2,469.0 ± 131.1
B4tBPPP	5,546.4 ± 484.0	2,534.9 ± 58.0
RDP	9222.2 ± 457.8	2,303.1 ± 18.6
CDP	6,370.5 ± 143.9	2,422.5 ± 76.8
DPP	7,521.6 ± 706.4	2,403.1 ± 137.2
TNPP	11,092.8 ± 1,420.7	2,302.5 ± 41.2

Continued

Chemicals	Average Intensity of Ki67 (AU)	Average Intensity of Sytox Green (AU)
2IPPDPP	7,168.4 ± 1,530.5	2,299.6 ± 64.5
B2IPPPP	6,684.4 ± 26.2	2,612.4 ± 26.0
4tBPDPP	7,430.1 ± 2,106.1	2,115.2 ± 230.8
BEHPP	7,050.7 ± 797.9	2,334.5 ± 115.3
BPADP	7,142.3 ± 1,151.1	2,365.4 ± 92.7
T4DtBPP	6,815.2 ± 706.4	2,524.9 ± 63.0
DoCP	7,273.1 ± 876.4	2,362.6 ± 39.6
TDCIPP	7,757.1 ± 1,203.5	2,401.5 ± 31.4
TCEP	8,829.7 ± 706.4	2,289.7 ± 41.4
BPDPP	8,254.2 ± 1,412.8	2,361.0 ± 115.7
BDBPP	7,076.9 ± 1,177.3	2,384.2 ± 73.9
TCIPP	6,933.0 ± 811.0	2,354.0 ± 60.4
TTBNPP	6,370.5 ± 196.2	2,461.6 ± 84.5
TDBPP	7,377.7 ± 2,145.3	2,511.9 ±86.0
RDT905	7,678.6 ± 627.9	2,487.8 ± 73.5
V6	6,854.5 ± 340.1	2,566.0 ± 41.3
AO626=O2	8,097.2 ± 1,151.1	2,543.0 ± 113.4
BCEP	7,704.8 ± 1,007.2	2,424.8 ± 271.4

Note: numerical data for Figure 2C, D (means ± SDs); AU, arbitrary units.

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

Experiment	Dose	Numeric data
Average intensity of Ki67 (2 d; AU)	DMSO	187.3 ± 15.0
	100 nM EHDPP	166.7 ± 18.7
	1,000 nM EHDPP	148.0 ± 18.7
	10,000 nM EHDPP	89.9 ± 24.3
Basic Metabolism (pmol/min)	DMSO	3,018.1± 740.3
	100 nM EHDPP	2,042.5 ± 560.4
	1,000 nM EHDPP	1,344.5 ± 380.9
	10,000 nM EHDPP	1,269.1 ± 252.0
Respiratory Capacity (pmol/min)	DMSO	3,012.2± 477.8
	100 nM EHDPP	2,383.6 ± 790.2
	1,000 nM EHDPP	1,643.9 ± 655.9
	10,000 nM EHDPP	1,528.9 ± 383.4
Average intensity of TP63 (2 d; AU)	DMSO	103.3 ± 16.5
	100 nM EHDPP	90.9 ± 13.4
	1,000 nM EHDPP	67.1 ± 12.4
	10,000 nM EHDPP	59.9 ± 18.6
Average intensity of Ki67 (10 d; (AU)	DMSO	172.9 ± 8.6
	100 nM EHDPP	164.3 ± 1.7
	1,000 nM EHDPP	138.3 ± 19.0
	10,000 nM EHDPP	115.9 ± 20.8
Average intensity of TP63 (10 d; AU)	DMSO	97.4 ± 6.8
	100 nM EHDPP	84.7 ± 26.7
	1,000 nM EHDPP	69.1 ± 15.9
	10,000 nM EHDPP	63.8 ± 11.4
Average intensity of CD71 (10 d; AU)	DMSO	100.8 ± 15.1
	100 nM EHDPP	67.5 ± 4.0
	1,000 nM EHDPP	55.4 ± 16.1
	10,000 nM EHDPP	51.4 ± 11.1
Average intensity of HLA-G (10 d; AU)	DMSO	105.7 ± 12.7
	100 nM EHDPP	94.1 ± 22.2
	1,000 nM EHDPP	75.0 ± 16.9
	10,000 nM EHDPP	68.7 ± 12.7
Concentration of hCG (mIU/mL)	DMSO	10,188.3 ± 611.3
	100 nM EHDPP	10,780.0 ± 305.6
	1,000 nM EHDPP	8,456.3 ± 203.8
	10,000 nM EHDPP	7946.9 ± 101.9
Concentration of E2 (pg/mL)	DMSO	24,585.7 ± 245.9
	100 nM EHDPP	22,864.7 ±2,704.4
	1,000 nM EHDPP	19,422.7 ± 1966.9
	10,000 nM EHDPP	17701.7 ± 2950.3

AU, arbitrary units; TP63, tumor protein 63; CD71, transferrin receptor; HLA-G, human leucocyte antigen protein-G; hCG, human chorionic gonadotropin; E2, estradiol

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

Dose	Time (min)	Numeric data (means ± SDs)	Dose	Time (min)	Numeric data (means ± SDs)
DMSO	1.40	615.06 ± 132.10	EHDPP 100 nM	1.40	420.45 ± 119.32
	9.97	588.07 ± 149.15		9.97	401.99 ± 105.11
	18.55	582.39 ± 153.41		18.55	393.47 ± 98.01
	27.21	394.89 ± 123.58		27.21	295.45 ± 80.97
	35.78	306.82 ± 98.01		35.78	248.58 ± 85.23
	44.31	257.10 ± 86.65		44.31	207.39 ± 66.76
	52.99	640.63 ± 83.81		52.99	549.72 ± 150.57
	61.55	602.27 ± 98.01		61.55	480.11 ± 180.40
	70.12	627.84 ± 132.10		70.12	453.13 ± 149.15
	78.80	197.44 ± 89.49		78.80	176.14 ± 39.77
	87.39	130.68 ± 36.93		87.39	132.10 ± 36.93
	95.93	123.58 ± 22.73		95.93	126.42 ± 39.77
EHDPP 1,000 nM	1.40	278.41 ± 82.39	EHDPP 10,000 nM	1.40	262.78 ± 38.35
	9.97	264.20 ± 78.13		9.97	264.20 ± 45.45
	18.55	257.10 ± 79.55		18.55	258.52 ± 49.72
	27.21	184.66 ± 61.08		27.21	213.07 ± 39.77
	35.78	139.20 ± 58.24		35.78	178.98 ± 22.73
	44.31	122.16 ± 42.61		44.31	153.41 ± 24.15
	52.99	356.53 ± 133.64		52.99	365.06 ± 88.07
	61.55	338.07 ± 153.41		61.55	318.18 ± 69.06
	70.12	321.02 ± 154.83		70.12	296.88 ± 62.50
	78.80	142.05 ± 8.52		78.80	125.00 ± 39.77
	87.39	93.75 ± 7.10		87.39	90.91 ± 8.52
	95.93	79.55 ± 2.84		95.93	78.13 ± 5.68

Table S11. Numeric data for Fig 3Q (means ± SDs)

Experiment	Strain of organoids	Group	Numeric data (AU)
Average intensity of Ki67	1#	DMSO	459.5 ± 41.2
		EHDPP	354.4 ± 16.0
	2#	DMSO	383.5 ± 24.7
		EHDPP	287.9 ± 12.3
	3#	DMSO	388.5 ± 42.8
		EHDPP	282.4 ± 14.5
	4#	DMSO	402.2 ± 12.6
		EHDPP	332.7 ± 24.7
	5#	DMSO	349.7 ± 25.9
		EHDPP	281.8 ±14.0

AU, arbitrary units

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

Experiment	Dose	Numeric data		
Gray value of p-Akt (Ser473; Intden/Area, AU)		1#	2#	3#
	DMSO	153.1	149.7	150.5
	100 nM EHDPP	142.7	141.9	147.8
	1,000 nM EHDPP	136.9	144.5	142.2
	10,000 nM EHDPP	142.7	140.2	147.2
	Background	128.6	128.9	136.4
Gray value of Akt (Intden/Area; AU)		1#	2#	3#
	DMSO	201.2	201.8	209.5
	100 nM EHDPP	196.0	202.9	207.2
	1,000 nM EHDPP	190.0	192.0	210.0
	10,000 nM EHDPP	194.7	206.9	219.0
	Background	123.3	125.1	122.5
Gray value of p-IGF1R (Intden/Area; AU)		1#	2#	3#
	DMSO	194.1	205.0	211.1
	100 nM EHDPP	177.8	178.1	180.0
	1,000 nM EHDPP	168.5	169.5	163.6
	10,000 nM EHDPP	174.3	172.1	167.2
	Background	141.5	144.8	131.5
Gray value of IGF1R (Intden/Area; AU)		1#	2#	3#
	DMSO	211.6	202.8	202.4
	100 nM EHDPP	205.0	209.3	194.2
	1,000 nM EHDPP	206.8	200.8	183.8
	10,000 nM EHDPP	200.3	224.6	217.6
	Background	144.5	155.9	158.7
EHDPP (ng/mL; means ± SDs)	HA-GFP transfection (1,000 nM EHDPP)	0.418 ± 0.034		
	HA-IGF1R transfection (1,000 nM EHDPP)	1.162 ± 0.384		
	HA-GFP transfection (10,000 nM EHDPP)	3.177 ± 0.062		
	HA-IGF1R transfection (10,000 nM EHDPP)	10.166 ± 5.433		

AU, arbitrary units

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

Chemical	Dose	Numeric data (means ± SDs)
EHDPP	0.1 nM	116.4 ± 8.5%
	1 nM	120.3 ± 4.2%
	10 nM	95.6 ± 5.0%
	100 nM	70.2 ± 7.7%
	1,000 nM	50.1 ± 11.1%
	10,000 nM	10.5 ± 3.5%
OSI-906	0.001 nM	108.5 ± 5.2%
	0.01 nM	102.4 ± 14.2%
	0.1 nM	76.1 ± 5.3%
	1 nM	62.3 ± 4.2%
	10 nM	50.1 ± 6.3%
	100 nM	15.7 ± 0.4%

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

Experiments	Dose	Numeric data (means ± SDs)
Average intensity of Cdx2 (E7.5; AU)	Control	343.8 ± 58.4
	10 mg/kg EHDPP	240.7 ± 75.64
Average intensity of Ki67 (E7.5; AU)	Control	572.6 ± 40.3
	10 mg/kg EHDPP	503.9 ± 40.1
Body weight (g)	Control	1.495 ± 0.105
	0.4 mg/kg EHDPP	1.490 ± 0.145
	2 mg/kg EHDPP	1.429 ± 0.142
	10 mg/kg EHDPP	1.401 ± 0.146
Implanted embryonic number (Total (Mean ± SD))	Control	156 (15.6 ± 1.58)
	0.4 mg/kg EHDPP	152 (15.2 ± 1.99)
	2 mg/kg EHDPP	142 (14.2 ± 2.10)
	10 mg/kg EHDPP	137 (13.7 ± 1.77)
Surviving embryonic number (Total (Mean ± SD))	Control	152 (15.2 ± 1.87)
	0.4 mg/kg EHDPP	143 (14.3 ± 1.70)
	2 mg/kg EHDPP	128 (12.8 ± 2.62)
	10 mg/kg EHDPP	123 (12.3 ± 2.54)

AU, arbitrary units; Cdx2, caudal-type homeobox 2

Table S15. Numeric data for Fig 5K (means ± SDs)

Dose	Time (min)	Blood glucose (mmol/L)
Control	0	2.46 ± 0.70
	15	11.54 ± 2.77
	30	14.83 ± 2.57
	60	12.04 ± 3.02
	90	6.82 ± 3.18
0.4 mg/kg EHDPP	0	2.77 ± 1.03
	15	12.29 ± 1.15
	30	13.60 ± 2.29
	60	12.09 ± 3.24
	90	8.97± 3.72
2 mg/kg EHDPP	0	1.93 ± 0.95
	15	9.22 ± 3.55
	30	11.93 ± 4.19
	60	11.98 ± 5.06
	90	8.24 ± 3.94
10 mg/kg EHDPP	0	2.07 ± 0.53
	15	13.04 ± 1.12
	30	14.19 ± 2.21
	60	15.25 ± 1.93
	90	11.73 ± 2.91

Table S16. Numeric data for Fig S1D, S1E, S2A, S2B, S2F (means ± SDs), and S2D

Experiments	Group	Numeric data		
Average intensity of Ki67 (AU)	Previous digestion	478.3 ± 29.2		
	Optimized digestion	406.6 ± 28.7		
Average intensity of Ki67 (AU)	Previous TOM	347.7 ± 13.9		
	Optimized TOM	406.8 ± 13.4		
Average intensity of Sytox Green (2 d; AU)	DMSO	1579.1 ± 41.2		
	100 nM EHDPP	1637.7 ± 175.3		
	1,000 nM EHDPP	1701.5 ± 44.0		
	10,000 nM EHDPP	1642.9 ± 43.7		
Average intensity of Sytox Green (2 d; AU)	DMSO	1582.4 ± 38.4		
	100 nM EHDPP	1617.3 ± 52.7		
	1,000 nM EHDPP	1621.6 ± 21.1		
	10,000 nM EHDPP	1621.0 ± 21.9		
Gray value of PD (Intden/Area; AU)		1#	2#	3#
	DMSO	189.5	152.9	148.4
	100 nM EHDPP	170.2	152.6	146.3
	1,000 nM EHDPP	161.6	146.8	145.8
	10,000 nM EHDPP	160.0	148.0	145.6
	Background	137.4	134.6	137.1
Gray value of β-actin (Intden/Area; AU)		1#	2#	3#
	DMSO	225.1	227.0	214.3
	100 nM EHDPP	224.6	226.8	206.3
	1,000 nM EHDPP	219.7	228.1	205.7
	10,000 nM EHDPP	222.6	224.5	212.4
	Background	158.7	153.7	151.6
Average intensity of CD71 (2 d; AU)	Control (DMSO)	138.6 ± 5.5		
	EHDPP (10, 000 nM)	141.4 ± 20.8		

TOM, trophoblast organoid medium; AU, arbitrary units; PD, pyruvate dehydrogenase complex; CD71, transferrin receptor

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

Experiments	Dose	Numeric data
Relative expression of IGF1 (RNA level; RU)	DMSO	1.00 ± 0.20
	100 nM EHDPP	0.98 ± 0.12
	1,000 nM EHDPP	0.86 ± 0.19
	10,000 nM EHDPP	0.91 ± 0.22
Relative expression of IGF2 (RNA level; RU)	DMSO	1.00 ± 0.33
	100 nM EHDPP	1.84 ± 0.40
	1,000 nM EHDPP	2.11 ± 0.24
	10,000 nM EHDPP	1.47 ± 0.21
Average Intensity of Ki67 (2 d; AU)	Control (DMSO)	204.6 ± 12.3
	EHDPP (10, 000 nM)	69.6 ± 32.7
Average Intensity of TP63 (2 d; AU)	Control (DMSO)	113.7 ± 12.5
	EHDPP (10, 000 nM)	44.3 ± 15.9
Average Intensity of CD71 (2 d; AU)	Control (DMSO)	126.1 ± 5.0
	EHDPP (10, 000 nM)	122.3 ± 15.1
Average Intensity of Ki67 (10 d; AU)	Control (DMSO)	273.5 ± 10.9
	EHDPP (10, 000 nM)	60.1 ± 30.1
Average Intensity of TP63 (10 d; AU)	Control (DMSO)	142.6 ± 7.13
	EHDPP (10, 000 nM)	81.3 ± 21.4
Average Intensity of CD71 (10 d; AU)	Control (DMSO)	132.3 ± 11.9
	EHDPP (10, 000 nM)	62.2 ± 17.2
Average Intensity of HLA- G (10 d; AU)	Control (DMSO)	62.2 ± 17.2
	EHDPP (10, 000 nM)	41.6 ± 10.7

IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; TP63, tumor protein 63; AU, arbitrary units; RU, relative unit; CD71, transferrin receptor; HLA-G, human leucocyte antigen protein-G

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

Experiments	Dose	Numeric data		
Average Intensity of TPBPA (mean ± SDs; AU)	Control	39.9 ± 6.0		
	10 mg/kg EHDPP	35.2 ± 2.8		
Average Intensity of TFAP2C (mean ± SDs; AU)	Control	48.2 ± 8.2		
	10 mg/kg EHDPP	42.4 ± 3.4		
Average Intensity of HAND1 (mean ± SDs; AU)	Control	31.0 ± 4.3		
	10 mg/kg EHDPP	21.7 ± 6.8		
Placenta p-IGF1R (Gray value; Intden/Area; AU)		1#	2#	3#
	Control	159.2	139.9	168.8
	0.4 mg/kg EHDPP	148.4	130.5	160.8
	2 mg/kg EHDPP	138.2	130.1	160.5
	10 mg/kg EHDPP	145.8	132.9	159.6
	Background	108.6	120.4	147.9
Placenta IGF1R (Gray value; Intden/Area; AU)		1#	2#	3#
	Control	184.6	137.7	183.8
	0.4 mg/kg EHDPP	179.8	138.7	205.9
	2 mg/kg EHDPP	207.9	173.6	179.3
	10 mg/kg EHDPP	184.4	158.2	193.5
	Background	97.6	104.2	161.9
Placenta PD (Gray value; Intden/Area; AU)		1#	2#	3#
	Control	198.9	184.6	212.6
	0.4 mg/kg EHDPP	188.6	173.9	177.7
	2 mg/kg EHDPP	175.0	154.4	192.5
	10 mg/kg EHDPP	169.1	153.0	189.0
	Background	138.0	126.8	129.1
Placenta β-actin (Gray value, with PD; Intden/Area; AU)		1#	2#	3#
	Control	200.0	224.5	228.9
	0.4 mg/kg EHDPP	188.6	223.0	225.9
	2 mg/kg EHDPP	192.1	218.7	221.3
	10 mg/kg EHDPP	191.5	224.6	223.1
	Background	127.5	113.3	160.9
Placenta cytochrome C (Gray value; Intden/Area; AU)		1#	2#	3#
	Control	204.7	145.6	178.1
	0.4 mg/kg EHDPP	193.9	139.9	176.3
	2 mg/kg EHDPP	171.8	144.2	175.2
	10 mg/kg EHDPP	169.0	135.9	174.9
	Background	141.8	125.8	163.8
Placenta β-actin (Gray value, with cytochrome C; Intden/Area; AU)		1#	2#	3#
	Control	197.6	225.6	226.9
	0.4 mg/kg EHDPP	196.8	223.0	225.1
	2 mg/kg EHDPP	195.3	226.7	223.0
	10 mg/kg EHDPP	191.0	228.0	222.2
	Background	136.0	152.8	164.1

TPBPA, trophoblast-specific protein α; AU, arbitrary units; TFAP2C, transcription factor AP-2γ; HAND1, crest derivatives-expressed protein 1; p-IGF1R, phosphorylated-IGF1R; IGF1R, insulin-like growth factor 1 receptor; PD, pyruvate dehydrogenase complex

Sequence of HA-GFP

gacattgatt attgactagt tattaatagt aatcaattac ggggtcatta gttcatagcc catatatgga gttccgcgtt acataactta
cggtaaatgg cccgcctggc tgaccgccca acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg
ccaataggga ctttccattg acgtcaatgg gtggagtatt tacggtaaac tgcccacttg gcagtacatc aagtgtatca
tatgccaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgcct ggcattatgc ccagtacatg accttatggg
actttcctac ttggcagtac atctacgtat tagtcatcgc tattaccatg gtgatgcggt tttggcagta catcaatggg
cgtggatagc ggtttgactc acggggattt ccaagtctcc accccattga cgtcaatggg agtttgtttt ggcaccaaaa
tcaacgggac tttccaaaat gtcgtaacaa ctccgcccca ttgacgcaaa tgggcggtag gcgtgtacgg tgggaggtct
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acccaagctg gctagcatgt acccatacga cgtaccagat tacgctagca agggagaaga actctttact ggtgttgtcc
caattctggt tgagctggat ggtgatgtga atggccacaa attctctgtg tctggtgaag gtgaaggaga tgcaacttat
ggaaagctga ctctgaagtt catttgtaca acaggaaagc tgccagtgcc ttggccaact ctggtgacca ccctgactta
tggtgttcaa tgtttcagca ggtaccctga ccacatgaag cagcatgact tctttaaatc tgcaatgcca gaaggttatg
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