Supplementary Information

Nano-TiO_2 retarded fetal development by inhibiting transplacental transfer of thyroid hormones in rat

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List of Supplementary Information

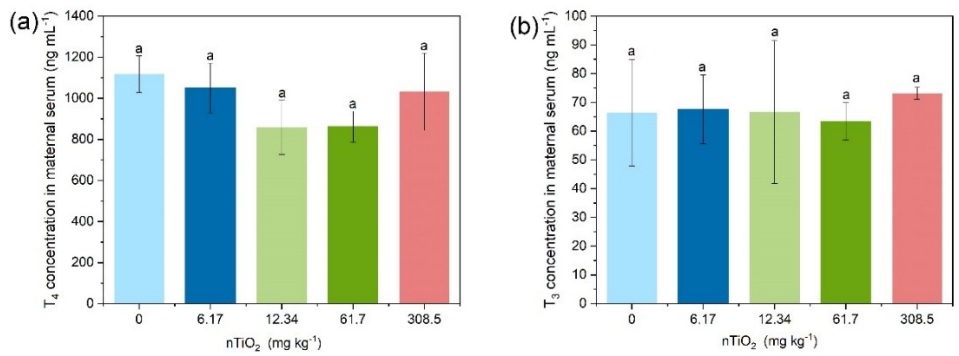

Figure S1. Concentrations of T_4 (a) and T_3 (b) in the maternal serum after exposure to $nTiO_2$ at different doses, lowercase letters indicate significant differences, n=3, $p < 0.05$.

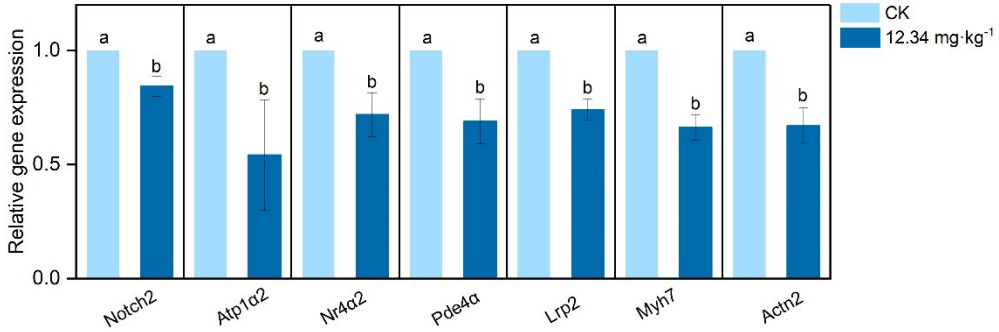

Figure S2. Expression pattern of genes encoding synthesis and signaling of THs as assayed by qPCR, lowercase letters indicate significant differences, n=3, $p < 0.05$.

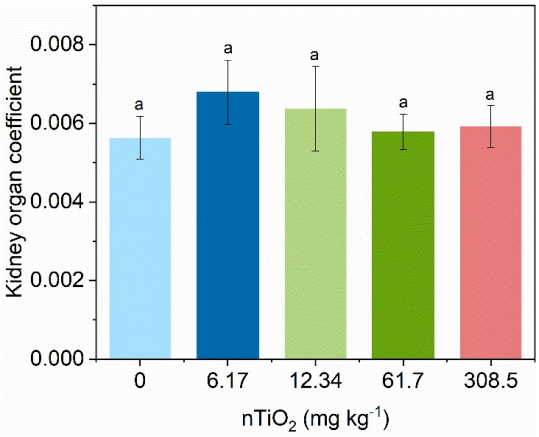

Figure S3. Coefficient of kidney on GD 18 after prenatal exposure to $nTiO_2$.

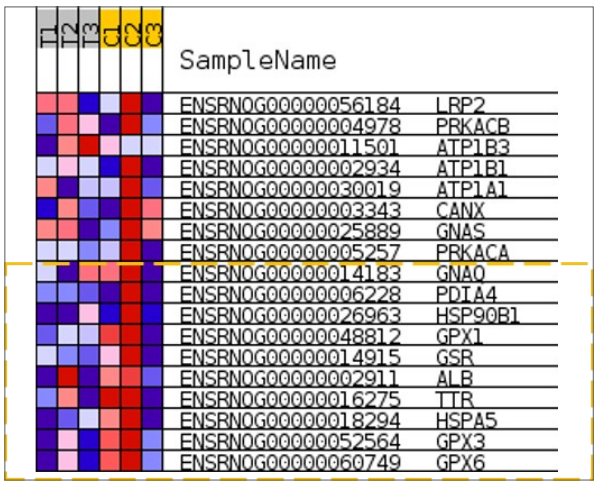

Figure S4. Abundance of altered proteins involved in the thyroid hormone synthesis pathway. Proteins in the dashed frame are the important proteins contributing to the down-regulation of this pathway. T1-3 and C1-3 stand for the three replicates of $nTiO_2$ (12.34 mg kg^{-1}) exposure and control treatment, respectively.

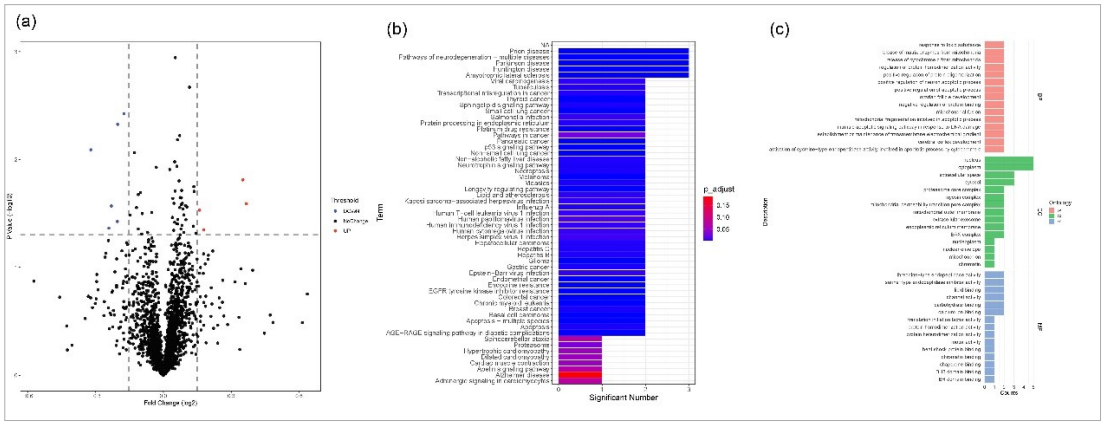

Figure S5. Volcano plot of differentially expressed proteins in placenta, and GO terms and KEGG pathways enriched in placenta upon $nTiO_2$ exposure (12.34 mg kg^{-1}).

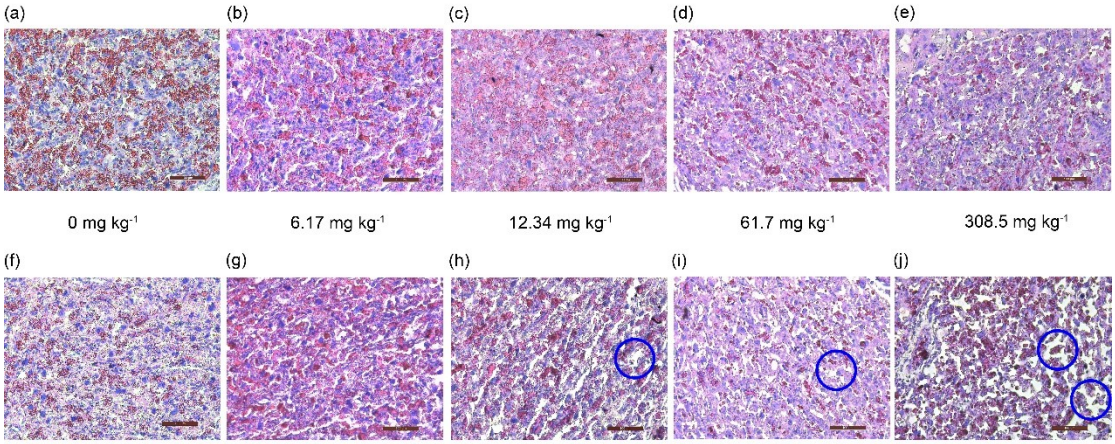

Figure S6. Pathological effects of $nTiO_2$ at different doses on the placenta. Histopathology of placental labyrinth (a−e) and trophoblast (f−j) sections after HE staining, blue circles in c−e indicate the lesions.

Text S1. ROS level in maternal liver and placenta assayed by ELISA kit.

The hepatic or placental ROS level was measured using an ELISA kit (Meimian, China). Briefly, the liver or placenta homogenates were added into a 96-well plate followed by the addition of antibody with horseradish peroxidase labeling. After 1 h incubation at 37 °C, the plate was rinsed with washing buffer for five times, and then incubated with 3,3',5,5'-tetramethylbenzidine (TMB) substrate in dark for 15 min at 37 °C. The reaction was terminated and measured by a microplate reader (Thermo Scientific, USA) at a wavelength of 450 nm.

Text S2. TMT quantitative proteomics detection of placental proteins.

Six samples of placenta from control and 12.34 mg kg^{-1} $nTiO_2$ groups (3 placentae per group) were selected for proteomics detection. Proteins were extracted in lysis buffer (8 M urea and 40 mM ammonium bicarbonate) followed by homogenization at 40 Hz

for 30 min (Scientz-48, China). The supernatant was collected by centrifuge at 12000 rpm for 15 min. Protein concentration was determined using a BCA quantification kit (CWBIO, China). After protein quantification, 200 μg protein of each sample was combined with 10 mM DTT solution and incubated at 37 °C for 60 min. Iodoacetamide (IAA, final concentration 20 mM) was then added and the mixture was incubated in dark for 45 min followed by dilution with 40 mM ammonium bicarbonate. The protein was then digested twice with trypsin (Thermo Scientific, Lithuania) for 12 h (1/50, w/w) and 4 h (1/40, w/w) at 37 °C, respectively. The digestion was terminated by TFA followed by desalting with tC18 cartridges (Sep-Pak Vac 1cc, Waters, USA). The resulting peptides from each group were labeled with tandem mass tags (TMT, Thermo Scientific, USA), TMT-126, TMT-127, TMT-128, TMT-129, TMT-130, and TMT-131, respectively, according to the manufacturer's instruction. All labeled peptides from each set were pooled together, desalted, dried, and resuspended in H_2O containing 0.1% formic acid. The sample analysis was performed with an UltiMate 3000 RSLCnano system (ThermoFisher Scientific, Germany) coupled to Q-Exactive mass spectrometer (ThermoFisher Scientific, USA). The peptides were separated by gradient elution (mobile phase A 0.1% formic acid in H_2O, mobile phase B 80% acetonitrile+0.1% formic acid, 134 min, 0.3 μL/min, gradient is listed as below) using an analytical column (50 μm × 15 cm, C18, Acclaim PepMap RSLC, 2 μm, 100A, Thermo Scientific). LC-MS/MS raw data were processed with UniProt *Rattus norvegicus* FASTA database (released on Aug 19, 2021) using Proteome Discoverer 2.2 software (Thermo Scientific). The parameters were set as follows: the mass

tolerance of precursor and fragment ions was set at 10 ppm and 0.02 Da; trypsin digestion allowed 2 missed cleavages sites; static modifications, carbamidomethylation (C, +57.021 Da), and TMT6plex (any N-terminal); dynamic modification and oxidation. The differentially expressed proteins should fulfill the following the conditions: unique peptides ≥ 2 with fold change > 1.11 (up-regulation) and < 0.9 (down-regulation) and $p < 0.05$. Differentially abundant proteins were annotated in terms of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Enrichment analysis was carried out using Wu Kong platform (https://www.omicsolution.org/wkomics/main/), and the GSEA analysis was conducted on the Omiscshare platform (https://www.omicshare.com/tools/Home/Soft/gsea).

Time	%B
0	5
5	12
100	32
125	65
126	99
134	99

Table S1. Primers used for qPCR assay.

gene name	**direction**	**primer sequence**

Lrp2	forward	CATCTTGGAAGCACAGGAGGCATAG
	reverse	CGGCATGGAGGCATCAGACATAAG
Atp1α2	forward	AGAAGCAATAAGCCACTGTCTGTATCG
	reverse	CTTCCTCATCGGCATCATCGTAGC
Nr4α2	forward	CTAGGGAGTGAGGCAGGGACAAG
	reverse	TGGCTGTTGGGATGGTTAAAGAAGG
Pde4α	forward	GTCGCCTCCTCTTCTCCATCCTAG
	reverse	CGGTGGTGGCATCTGTAATCTCTG
Notch2	forward	GTAGTGGTGACGGCTGGCATTG
	reverse	ATTCTAAGGCAGGCGATGATGTTCTC
Myh7	forward	GTGTTGGATGTTGGTTGCTGTTGG
	reverse	CCGCCTCCTCCTCTTCCTCTTC
Actn2	forward	TTGATGGTTCTGGCGATAGTTGTGAG
	reverse	CTGATTGCTGGTTCCTCTCCTTGAC
TTR	forward	TGCCAACAAGGAAGACCAAGTGAC
	reverse	ACTGAGGACAACTGCTGCTTTCG
ALB	forward	AAGCAAGGACAGCAAGGAGACATTC
	reverse	CCTAAGGTTGGCACTGGCATCG
Serpina7	forward	TCTACTGTCACTTGCCATTGCCTAAG
	reverse	AAGGAAGAAGGGTGGAGAGGATAAGAG
HNF1α	forward	GCCTGCCTCTGCCTCTTGAATG
	reverse	GGACTGGAGCCATGAATCGGTTG

HNF1β	forward	ACAGACGGATGGACAGGTGGATAG
	reverse	AGCAGGAGGAGGAAGAGAGTTAGC
HNF4α	forward	CAGGCACACTCAGAAGGCAGAAG
	reverse	CAGCACCCACATCAGGCAACTC
HNF4g	forward	TGTGATGTAGTGTGGTGTGGTTGTG
	reverse	AGGTCCAGTTGAGGGAGTGAGTG
β-actin	forward	GACGGCCAGGTCATCACTAT
	reverse	CTTCTGCATCCTGTCAGCAA