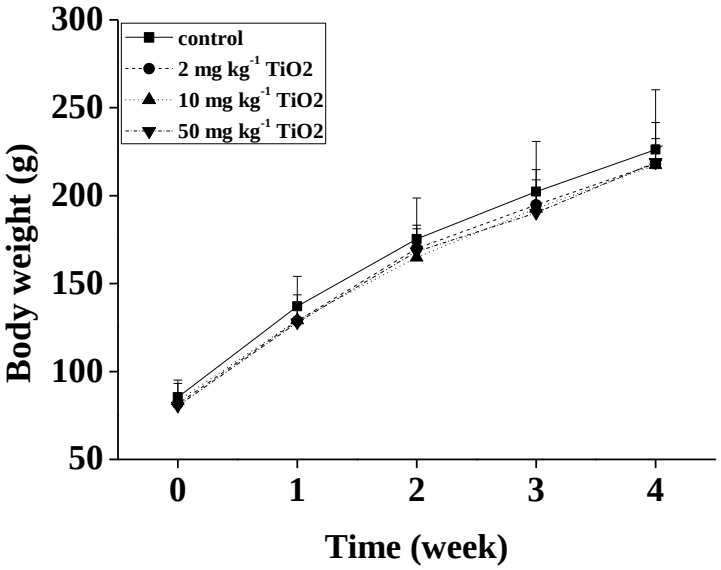

Figure S1. Body weight of the rats after oral exposure to TiO_2 nanoparticles for 30 days (mean ± SD, n = 6).

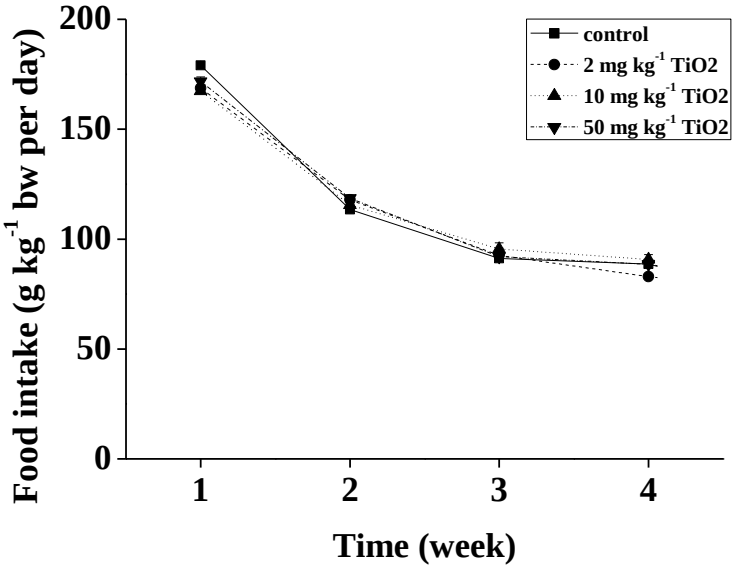

Figure S2. Food intake of the rats after oral exposure to TiO_2 nanoparticles for 30 days (mean ± SD, n = 6).

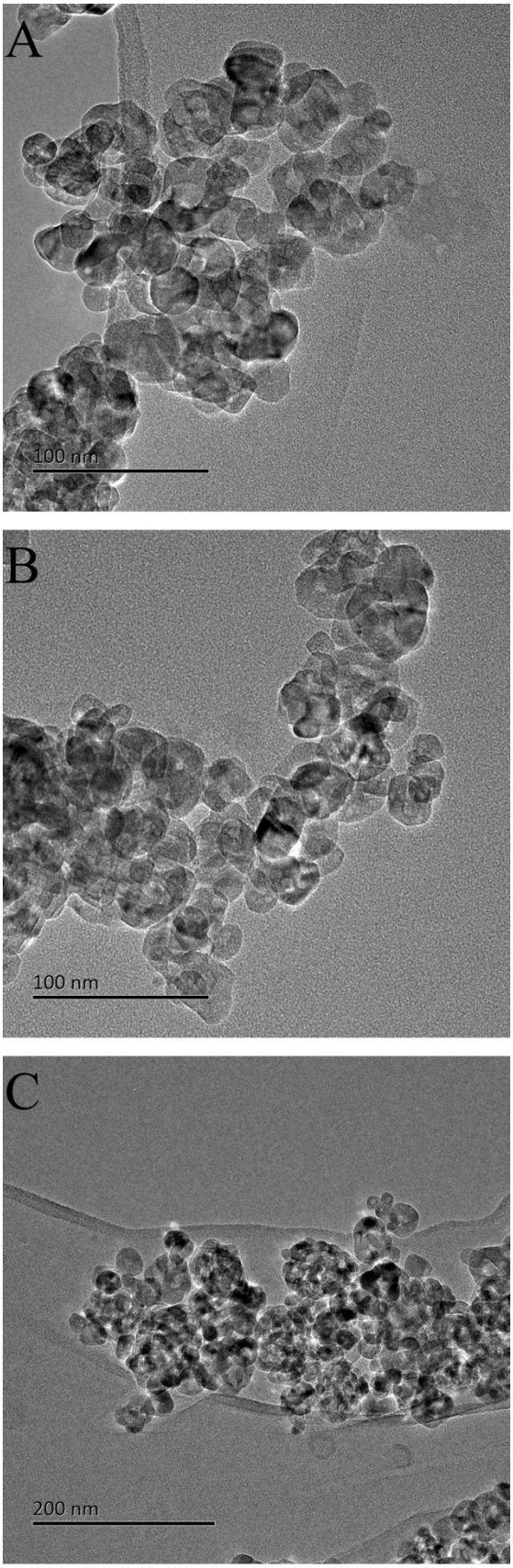

Figure S3. The representative transmission electron microscope (TEM) image of TiO_2 NPs in different solutions. TiO_2 NPs were disperse in H_2O (A), AGJ (B) and AIJ (C). AGJ: artificial gastric juice; AIJ: artificial intestinal juice.

Table S1. Key parameters for metabolite identification by Compound Discoverer software.

Parameters	QE HF
Alignment// RT tolerance （min）	0.2
Select spectra// min peak count	1
Select spectra// S/N threshold	3
Detect Compounds// S/N threshold	1.5
Alignment// model	Adaptive curve
Min Peak Intensity	500 000
Match Ion Activation Energy	True
Ion Activation Energy Tolerance	50
Fragment Data Selection// Preferred Ions	[M+H]+1; [M-H]-1
Identity Search	HighChem HighRes
Similarity Search	Confidence Forward
Gap filling// S/N thresholda	1.5
Gap filling// Mass Tolerance	5ppm

Table S2. Differential expressed metabolites in feces of rats exposed to TiO_2 NPs orally for 30 days, analyzing by OPLS-DA method.

Super class	Metabolite name	Molecular formula	Retention time, RT (min)	molecular mass	Maximum peak area	HMDB ID	KEGG ID	Change fold (log_2N)
Organic acids and derivatives	N-Acetylhistamine	C7H11N3O	1.79	153.08959	257839487.3	HMDB0013253	C05135	9.2921
Organoheterocyclic compounds	Caprolactam	C6H11NO	0.997	113.08392	50306020.06	-	-	9.0679
Lipids and lipid-like molecules	Glycerophosphocholine	C8H20NO6P	8.508	257.10189	10058327.42	HMDB0000086	C00670	8.1662
Organoheterocyclic compounds	Pyridoxine	C8H11NO3	1.512	169.07333	25766033.85	HMDB0000239	C00314	3.7117
Organoheterocyclic compounds	2,3,5,6-Tetramethylpyrazine	C8H12N2	0.902	136.09945	55027963.83	-	-	2.8741
Organoheterocyclic compounds	Tryptamine	C10H12N2	2.128	160.09946	6070015.952	HMDB0000303	C00398	2.6282
Organoheterocyclic compounds	Cytosine	C4H5N3O	2.784	111.04319	113810771.3	HMDB0000630	C00380	1.9498
Nucleosides, nucleotides, and analogues	Adenosine	C10H13N5O4	2.252	267.09567	11566671.36	HMDB0000050	C00212	1.7002
Organic acids and derivatives	D-Pipecolinic acid	C6H11NO2	5.892	129.07861	3718138.761	HMDB0005960	-	0.66524
Organoheterocyclic compounds	Urocanic acid	C6H6N2O2	5.489	138.04255	9627965.588	HMDB0000301	C00785	-0.46766
Organic oxygen compounds	N-Acetyl-D-galactosamine	C8H15NO6	4.442	221.08922	46771695.16	HMDB0000212	C01074	-0.75542
Organic acids and derivatives	N6-Acetyl-L-lysine	C8H16N2O3	7.226	188.11551	4840058.308	HMDB0000206	C02727	-1.1743
Organic acids and derivatives	L-Phenylalanine	C9H11NO2	4.196	148.05186	100250926.9	HMDB0000159	C00079	-1.1882
Organic acids and derivatives	N-Acetylornithine	C7H14N2O3	8.579	174.10004	6231015.797	HMDB0003357	C00437	-1.6379
Organic acids and derivatives	Gamma-Aminobutyric acid GABA	C4H9NO2	8.074	103.0635	46514035.24	HMDB0000112	C00334	-1.7989
Organic acids and derivatives	Glycyl-L-leucine	C8H16N2O3	5.23	188.11544	2674661.209	HMDB0028929	-	-2.1839

Table S2 (continued). Differential expressed metabolites in feces of rats exposed to TiO_2 NPs orally for 30 days, analyzing by OPLS-DA method.

Super class	Metabolite name	Molecular formula	Retention time, RT (min)	molecular mass	Maximum peak area	HMDB ID	KEGG ID	Change fold (log_2N)
Organic acids and derivatives	Diaminopimelic acid	C7H14N2O4	12.199	190.09488	1341797.772	HMDB0001370	C00666	-2.5981
Organoheterocyclic compounds	4-Picoline	C6H7N	3.2	93.05815	1953343.396	-	-	-2.6693
Organic acids and derivatives	L-Valine	C5H11NO2	5.878	117.07894	5542425.427	HMDB0000883	C00183	-3.0192
Organoheterocyclic compounds	Imidazolelactic acid	C6H8N2O3	6.745	156.05309	682091.3611	HMDB0002320	C05132	-3.7642
Organic acids and derivatives	L-Methionine	C5H11NO2S	5.024	149.05053	39229713.7	HMDB0000696	C00073	-3.8696
Organoheterocyclic compounds	Thiamine	C12H16N4OS	5.493	264.10346	31544046.6	HMDB0000235	C00378	-4.7906
Organic acids and derivatives	L-Ornithine	C5H12N2O2	12.971	132.08962	83614610.21	HMDB0000214	C00077	-8.5952
Organic acids and derivatives	L-Histidine	C6H9N3O2	8.914	155.0691	29915498.98	HMDB0000177	C00135	-9.5719
Organoheterocyclic compounds	4-Methyl-5-thiazoleethanol	C6H9NOS	5.48	143.04008	7452113.814	HMDB0032985	C04294	-9.6926

Table S3. Key parameters for GC-MS/MS analysis.

	Parameters
Injection volume	2 μL
Front Inlet Mode	2:1
Carrier Gas	Helium
Column	DB-FFAP (30m x 0.25mm x 0.25um)
Column Flow	0.85 mL/min
Oven Temperature Ramp	90 ℃ hold on 1min, raised to 130 ℃ at a rate of 15 ℃/min ,raised to 230 ℃ at a rate of 20 ℃/min, hold on 4min, after running for 5 min
Front Injection Temperature	230 ℃
Transfer Line Temperature	230 ℃
Ion Source Temperature	230 ℃
Quad Temperature	150 ℃
Electron Energy	70 eV
Scan mode	MRM
Solvent Delay	3.3 min