

Electronic Supplementary Information

Perturbation of gut microbiota plays an important role in micro/nanoplastics-induced gut barrier dysfunction

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Table S1 Physicochemical characteristics of the PS M/NPLs in different solutions. The size distribution, Zeta-potential and PDI were measured in double distilled H₂O (pH 7.0), DMEM (pH 7.6), GSF (pH 1.2) and ISF (pH 7.5) by dynamic light scattering (DLS), respectively. PDI: Polydispersity Index; GSF: Gastric simulated fluid; ISF: Intestinal simulated fluid.

| Particle type | Size by TEM (nm) | Z-average (nm) | | | | Zeta-potential (mV) | | | | PDI | | | |
|--------------------------|---------------------|------------------|-------|-------|-------|---------------------|-------|-------|-------|------------------|-------|-------|-------|
| | | H ₂ O | DMEM | GSF | ISF | H ₂ O | DMEM | GSF | ISF | H ₂ O | DMEM | GSF | ISF |
| Nano PS | 70.8±5.9 | 98.4 | 105.2 | 591.6 | 105 | -14.3 | -9.7 | 4.8 | -6.92 | 0.078 | 0.203 | 0.398 | 0.06 |
| Micro PS | 5105.5±171.8 | 6405 | 5642 | 5868 | 7380 | -17.1 | -2.02 | 6.71 | -8.38 | 0.053 | 0.007 | 0.345 | 0.08 |
| Nano PS-COOH | 70.3±10.6 | 105 | 122.1 | 122.3 | 120.8 | -23.7 | -9.24 | -3.46 | -20.2 | 0.135 | 0.141 | 0.449 | 0.25 |
| Micro PS-COOH | 5103.6±232.6 | 5275 | 5960 | 6728 | 6381 | -41.1 | -12.3 | 6.54 | -20.6 | 0.176 | 0.195 | 0.763 | 0.268 |
| Nano PS-NH ₂ | 76.5±7.8 | 187.9 | 393.5 | 225.7 | 1381 | 38.9 | -4.98 | 20.5 | -1.73 | 0.144 | 0.394 | 0.241 | 0.234 |
| Micro PS-NH ₂ | 5371.3±174.0 | 5599 | 6307 | 6178 | 5959 | 39.9 | -7.18 | 21.8 | -6.92 | 0.43 | 0.907 | 0.848 | 0.519 |

Table S2 The changed ratio of body weight (%) after exposure to various PS M/NPLs.

| Groups | Week No.1 | Week No.2 | Week No.3 | Week No.4 |
|-------------------------------------|-------------|-------------|-------------|-------------|
| Control group | | | | |
| Vehicle | -1.85±1.62 | 2.21±2.22 | 4.20±2.98 | 1.41±2.75 |
| Low dosage group (0.2 mg/kg) | | | | |
| Nano PS | -4.68±2.86* | -3.04±3.10* | -6.83±4.48* | -4.18±3.92* |
| Micro PS | -1.35±1.41 | 0.04±1.50 | 0.40±5.44 | -1.75±5.54 |
| Nano PS- COOH | -3.50±3.43 | -0.16±3.00 | 1.86±4.05 | 0.53±3.39 |
| Micro PS- COOH | -2.22±1.00 | 0.66±2.02 | 2.77±2.06 | 0.21±1.64 |
| Nano PS-NH ₂ | -2.28±2.03 | -1.35±2.66 | -0.49±4.53 | -1.62±4.02 |
| Micro PS-NH ₂ | -4.49±2.00* | -3.30±1.43* | -0.79±3.53* | -3.43±1.60* |
| High dosage group (2 mg/kg) | | | | |
| Nano PS | -4.17±2.17 | -3.76±2.97* | -5.89±7.36* | -4.82±4.12* |
| Micro PS | -2.48±2.62 | 0.87±2.43 | -0.49±3.53 | 2.21±2.55 |
| Nano PS- COOH | -4.13±1.69 | -1.01±2.48 | 0.40±3.28 | -1.75±1.22 |
| Micro PS- COOH | -2.22±1.19 | -0.43±2.46 | 0.50±2.34 | -2.38±1.91 |
| Nano PS-NH ₂ | -5.62±3.30* | -7.86±7.98* | -4.31±4.62* | -3.98±3.21* |
| Micro PS-NH ₂ | -0.67±1.61 | -2.25±6.03* | 1.50±3.82 | -1.89±5.22 |

* $P < 0.05$ vs. control group. n=6. Significant changes were marked with red color.

Table S3 The serum toxicity markers after exposure to various PS M/NPLs.

| Groups | ALT (U/L) | AST (U/L) | T-Bil ($\mu\text{mol/L}$) | CK (U/L) | ALP (U/L) | r-GT (U/L) | SCr (Serum Creatinine) ($\mu\text{mol/L}$) |
|-------------------------------------|--------------------|---------------------|--------------------------------|-----------------------|---------------------|------------------|---|
| Control group | | | | | | | |
| Vehicle | 50.35 \pm 6.39 | 158.45 \pm 27.75 | 26.94 \pm 2.69 | 669.98 \pm 263.85 | 147.25 \pm 17.67 | 6.11 \pm 1.30 | 145.65 \pm 9.11 |
| Low dosage group (0.2 mg/kg) | | | | | | | |
| Nano PS | 39.85 \pm 8.79 | 147.80 \pm 32.26 | 22.81 \pm 3.13 | 641.84 \pm 462.13 | 102.06 \pm 29.53 | 7.76 \pm 0.92 | 130.77 \pm 7.66 |
| Micro PS | 44.33 \pm 7.97 | 151.94 \pm 16.33 | 22.24 \pm 7.65 | 604.58 \pm 210.14 | 130.41 \pm 28.71 | 6.75 \pm 1.46 | 147.68 \pm 16.61 |
| Nano PS-COOH | 52.22 \pm 11.82 | 159.09 \pm 21.25 | 26.48 \pm 5.07 | 633.65 \pm 161.50 | 158.59 \pm 37.73 | 7.00 \pm 1.06 | 147.81 \pm 9.29 |
| Micro PS-COOH | 51.00 \pm 4.82 | 183.54 \pm 29.51 | 28.37 \pm 3.02 | 1389.81 \pm 359.87* | 157.61 \pm 26.94 | 7.78 \pm 1.18 | 152.48 \pm 14.81 |
| Nano PS-NH ₂ | 45.63 \pm 11.30 | 183.37 \pm 48.38 | 29.52 \pm 4.23 | 1137.44 \pm 530.77 | 137.88 \pm 15.74 | 7.44 \pm 1.01 | 173.49 \pm 8.74* |
| Micro PS-NH ₂ | 50.58 \pm 8.46 | 190.02 \pm 37.08 | 35.63 \pm 20.63 | 1503.02 \pm 644.96* | 147.18 \pm 22.62 | 9.04 \pm 2.38* | 188.73 \pm 49.37* |
| High dosage group (2 mg/kg) | | | | | | | |
| Nano PS | 45.68 \pm 11.98 | 164.95 \pm 35.90 | 23.72 \pm 2.69 | 1076.03 \pm 531.78 | 154.17 \pm 20.97 | 8.09 \pm 1.58* | 153.44 \pm 10.75 |
| Micro PS | 50.62 \pm 5.79 | 151.19 \pm 37.35 | 23.35 \pm 2.38 | 466.88 \pm 183.69 | 127.57 \pm 25.10 | 6.68 \pm 1.55 | 138.27 \pm 13.66 |
| Nano PS-COOH | 49.26 \pm 4.37 | 170.69 \pm 21.08 | 23.40 \pm 2.81 | 975.54 \pm 292.31 | 167.82 \pm 6.37 | 7.93 \pm 1.12* | 146.35 \pm 6.38 |
| Micro PS-COOH | 41.92 \pm 7.12 | 151.83 \pm 25.37 | 29.62 \pm 5.37 | 860.86 \pm 346.76 | 123.89 \pm 8.56 | 8.83 \pm 0.61* | 160.54 \pm 7.24* |
| Nano PS-NH ₂ | 43.68 \pm 6.91 | 177.29 \pm 36.00 | 28.83 \pm 3.98 | 1248.16 \pm 403.68* | 180.62 \pm 32.96* | 6.37 \pm 1.10 | 158.50 \pm 15.46 |
| Micro PS-NH ₂ | 88.26 \pm 53.86* | 235.18 \pm 34.88* | 40.86 \pm 2.26* | 1643.33 \pm 662.75* | 126.00 \pm 42.54 | 8.02 \pm 1.24* | 170.25 \pm 4.49* |

* $P < 0.05$ vs. control group. n=6. Significant changes were marked with red color.

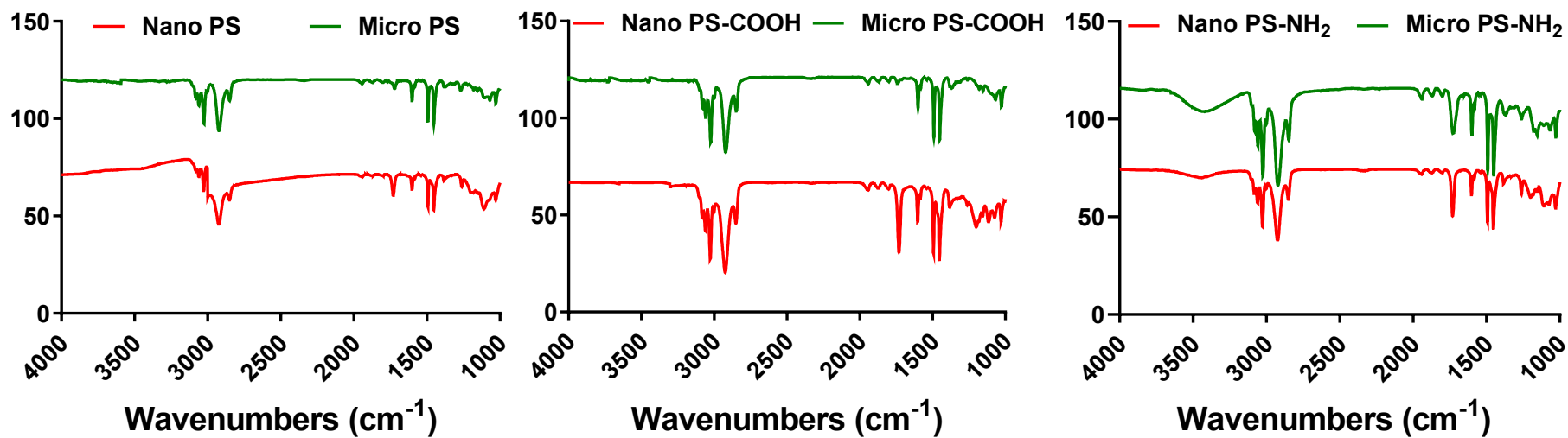


Fig. S1 Fourier transform infrared (FTIR) spectra of PS M/NPLs.

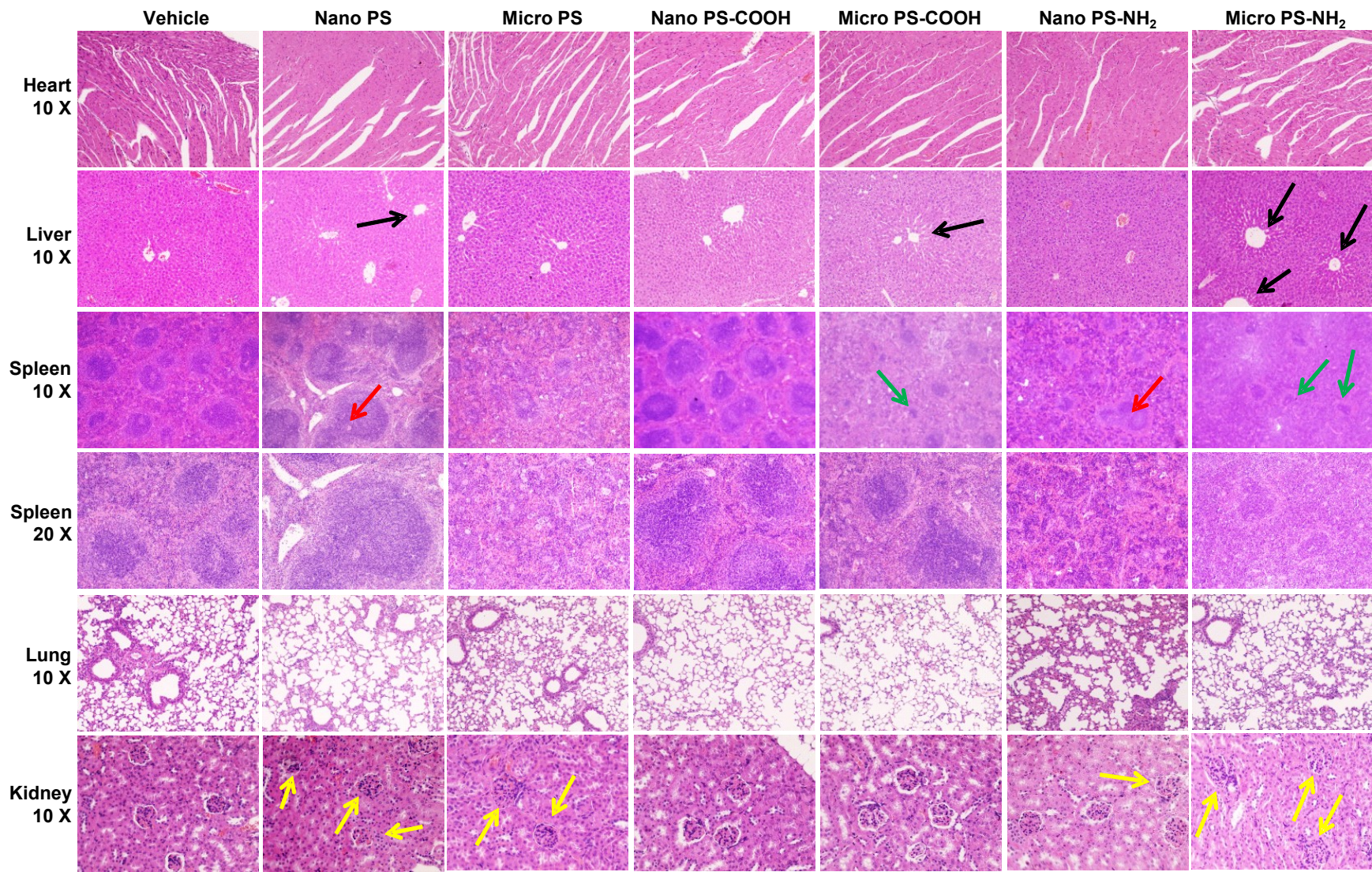


Fig. S2 Histopathological images of organ tissues of mice after oral exposure to PS M/NPLs for 28d. Black arrows show the dilatation of liver sinus and the atrophy of liver plate. The red arrows show the hyperactive germinal centers and the green arrows show the atrophy of splenic nodules. The yellow arrows show the shrinkage of renal capsular space. And the slight hydrops of renal tubular epithelial cell and infiltration of interstitial inflammatory cell are also seen. Furthermore, nano PS-NH₂ leads smaller fat cells than other exposure groups.

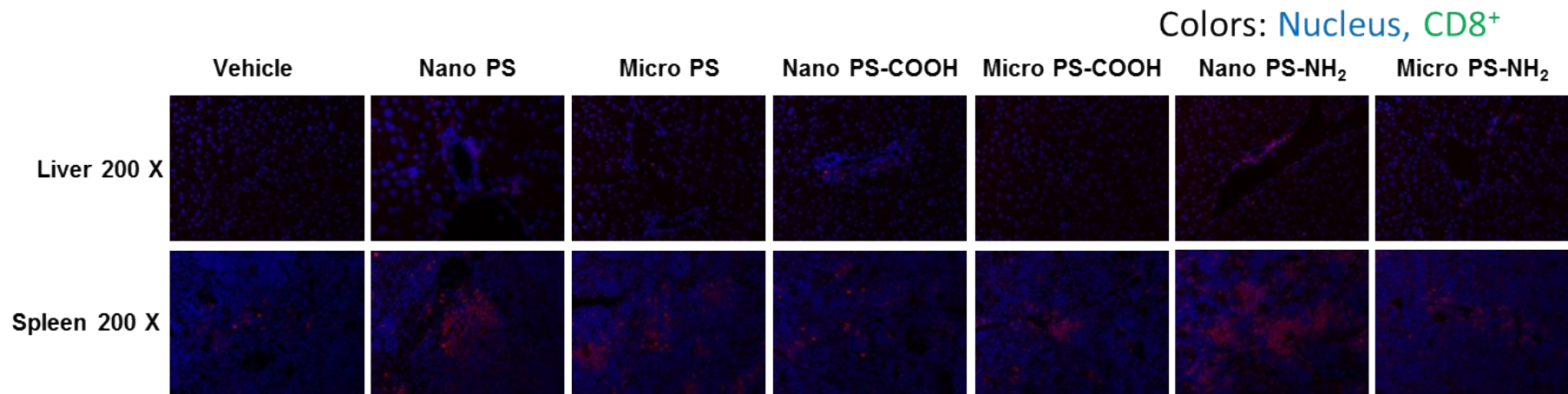


Fig. S3 Immunofluorescence test on the immunoreactivity in liver and spleen of mice after oral exposure to PS M/NPLs for 28 d. The cytotoxic T lymphocyte was labelled by CD8⁺ antibody

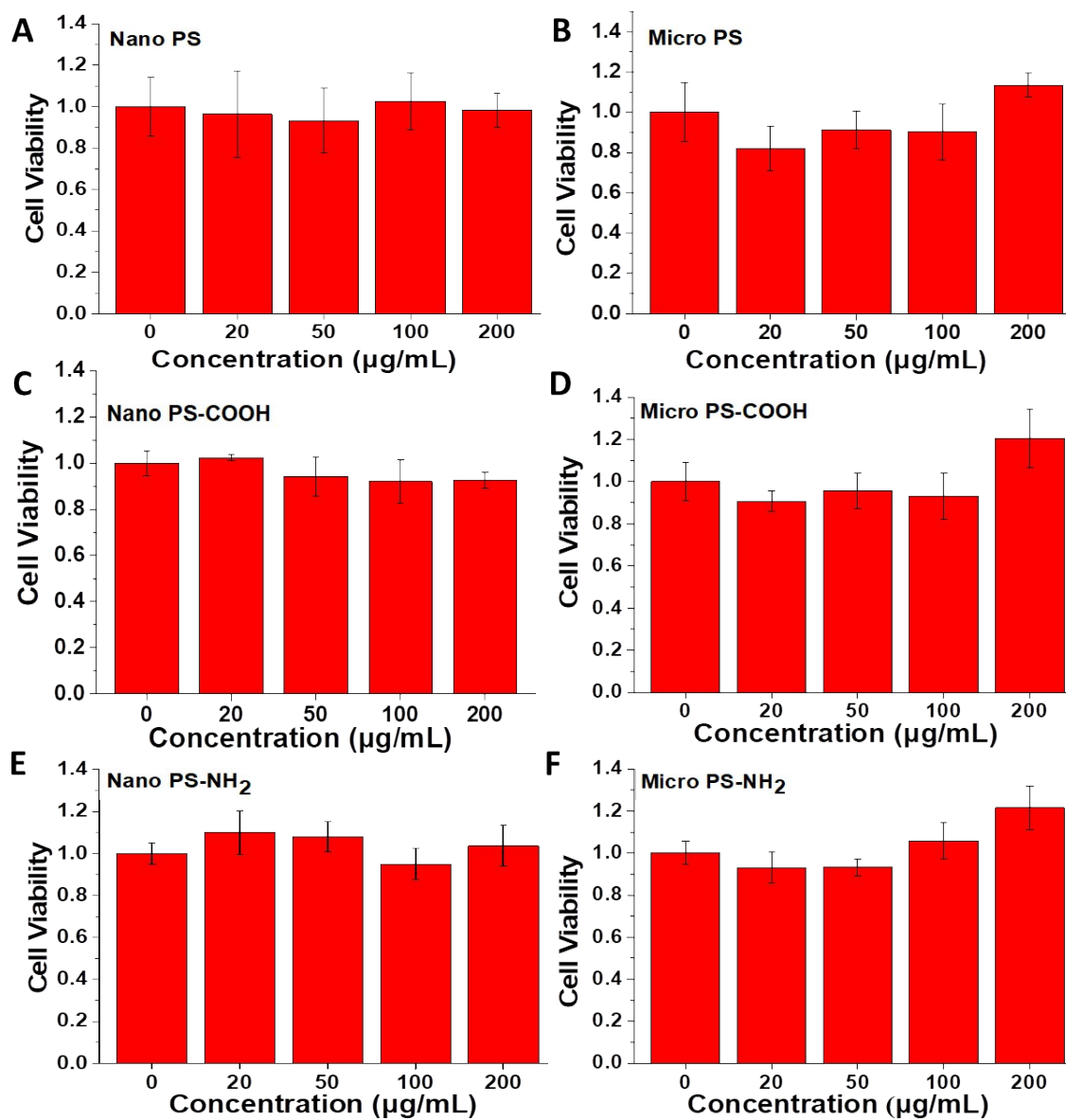


Fig. S4 The changes in cellular viability after MNPs treatment. Cell viability in Caco-2 cells determined by CCK-8 assay after exposure to different MNPs at 24 h. The data values represent the mean of at least three independent experiments normalized to untreated controls. Data expressed as mean \pm SD, n= 5.

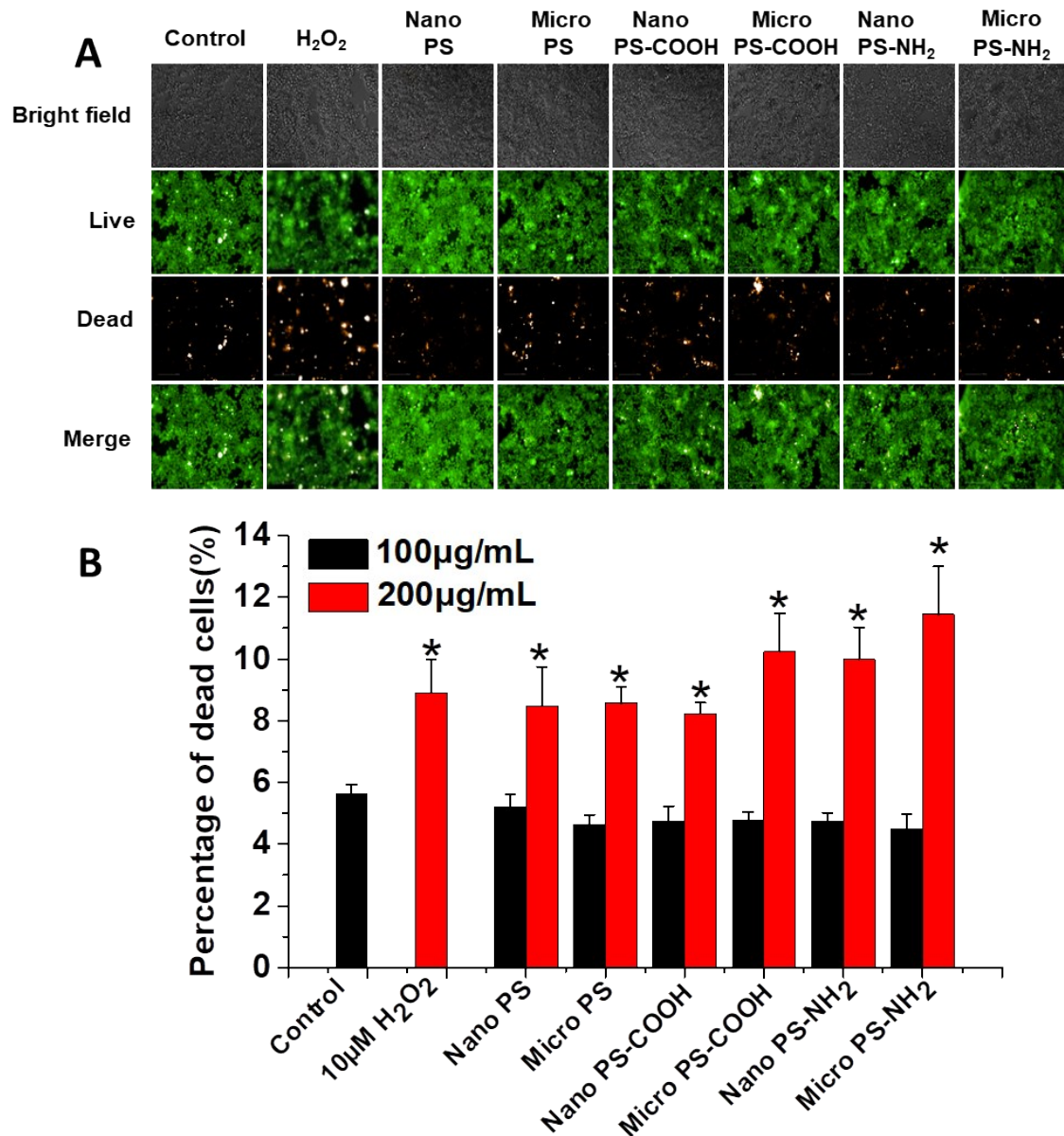


Fig. S5 High content analysis results of Caco-2 cells after incubation with MNPs for 24 h. (A) Representative images were selected for high-content screening analysis after incubating to 100 or 200 µg/mL MNPs for 24 h. These images were automatically captured by Operetta CLS (PerkinElmer, USA) with the 20×lens. (B) The results are expressed as mean ± SD of at least 3 independent experiments. * $P < 0.05$.

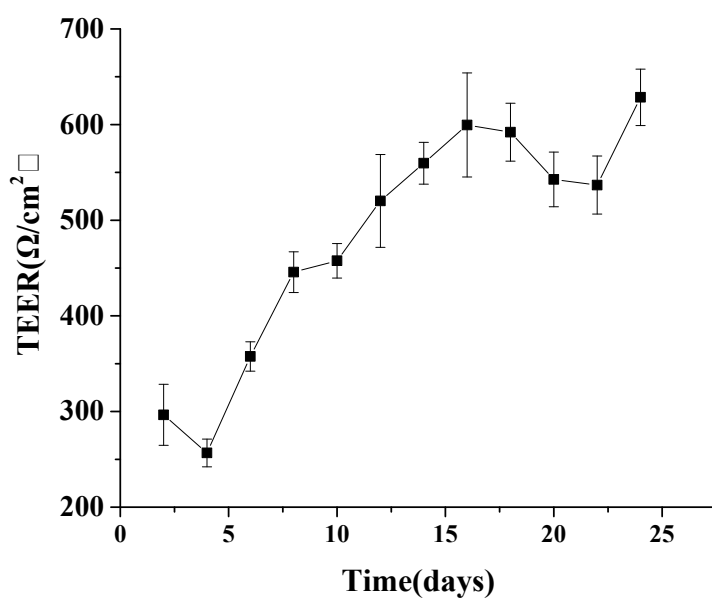


Fig. S6 The Caco-2 cells form close contact monolayer on transwell after culturing for about 3 weeks. Data expressed as mean \pm SD, n= 3. It is a representative testing result from 3 repeats.

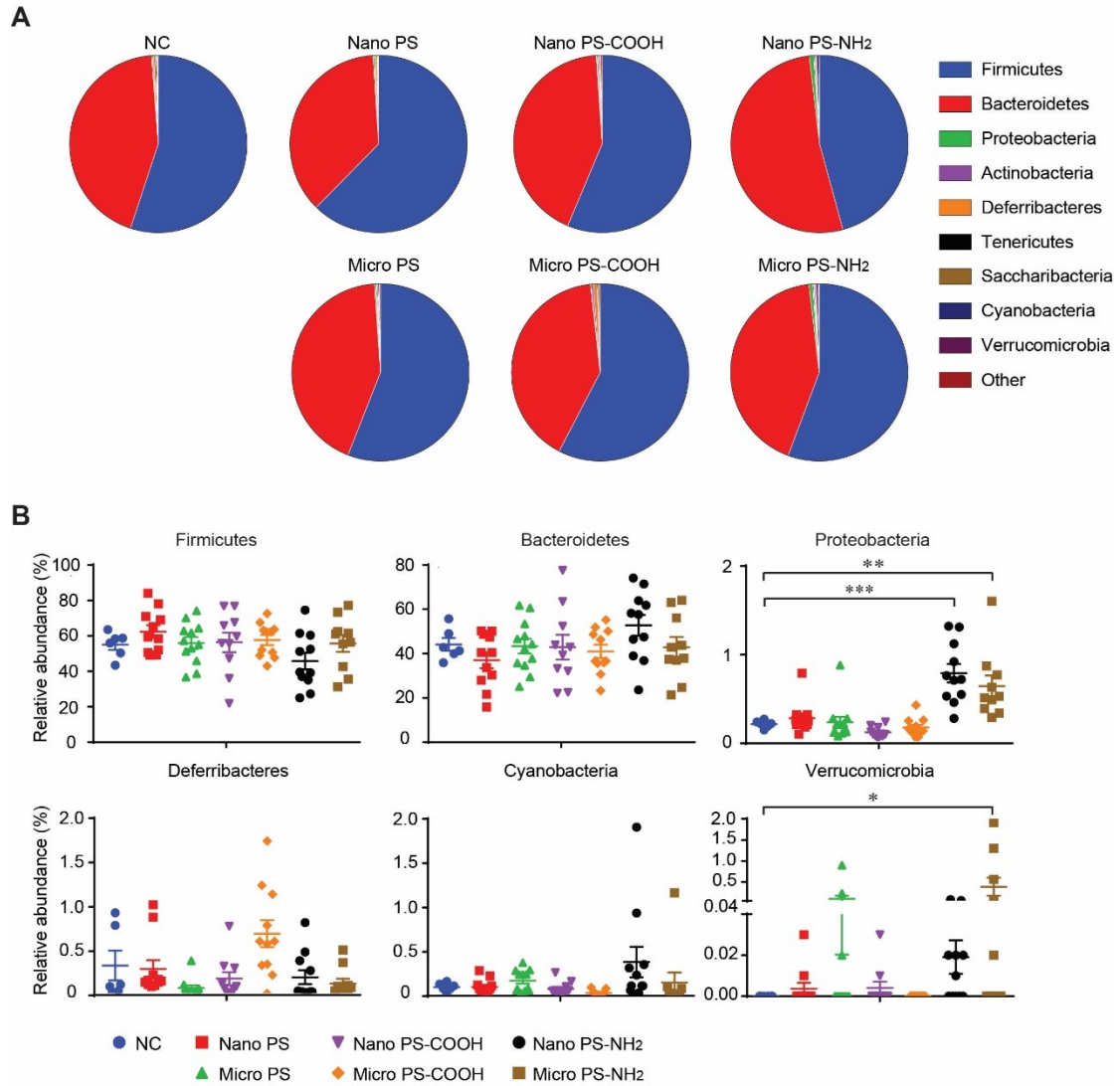


Fig. S7 The impacts of micro- and nanoplastic particles on gut microbes at the phylum level. (A) Pie charts. (B) Scalar diagrams of individual main phyla among different groups. Data are shown as means \pm SEMs. ** $P < 0.01$, *** $P < 0.001$ vs NC group.

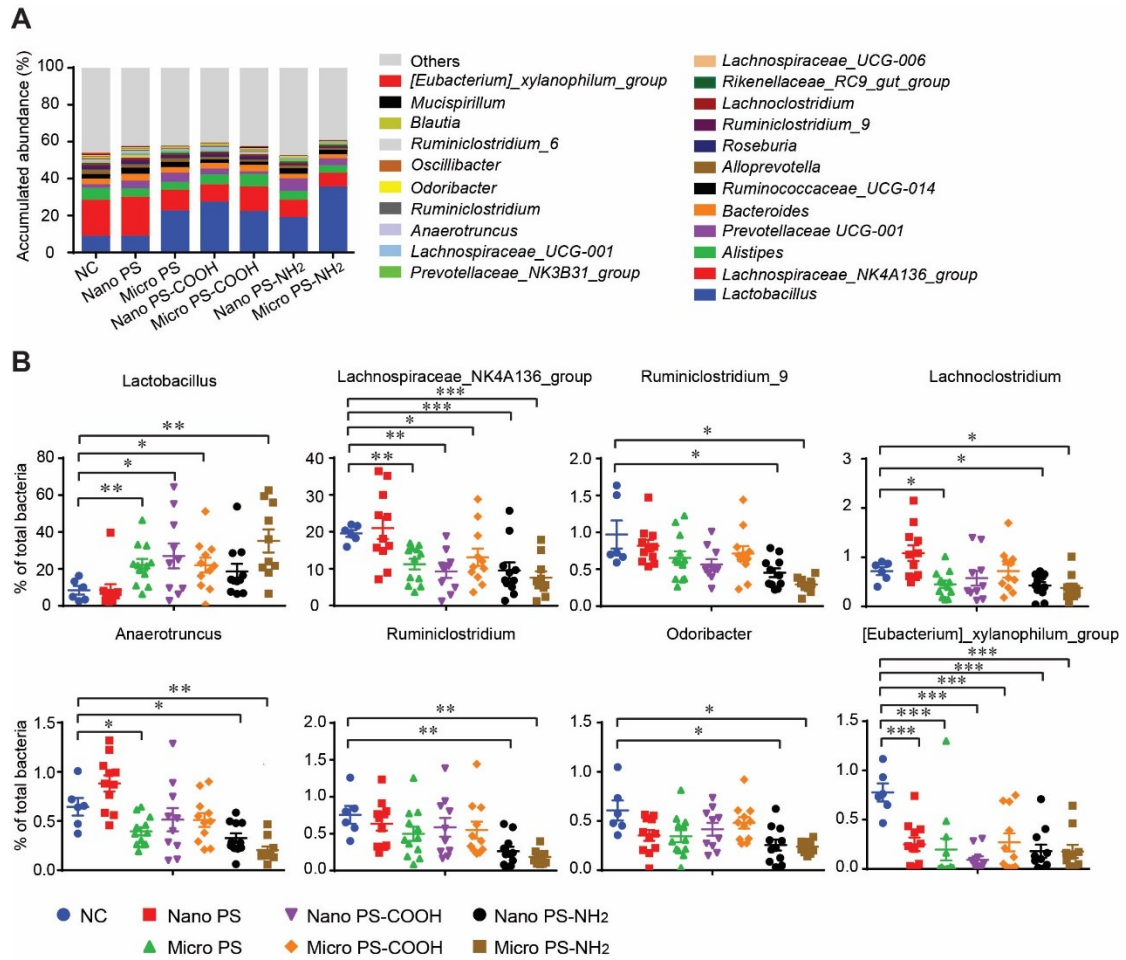


Fig. S8 The changes of the gut microbiota by micro and nanoplastic particles at the genus level. (A) The accumulative abundance of the top 23 genera among the different groups. (B) The scalar diagram of the phyla that is significantly different among the groups. Data are shown as means \pm SEMs. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs NC group.