Supplementary Information (SI) for Environmental Science: Nano.

Integrated transcriptomic and metabolomic analysis to decipher the regulatory mechanisms of polystyrene nanoplastic-induced metabolic disorders in hepatocytes

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Text S1

1. Materials and methods

In vivo fluorescence imaging

The six-week-old C57BL/6 male mice were employed to determine the targeted effector organs after single gavage with red-fluorescent labelled PSNPs. Mice without PSNPs exposure served as control. We appreciate the reviewer's inquiry regarding the dosage rationale. The 150 mg/kg·bw PSNPs dose for gastric gavage was determined based on epidemiological data on human oral microplastic intake (0.1-5 g/week)[1]. Using body surface area normalization for interspecies dose aconversion[2], this mouse-equivalent dose translates to a human equivalent dose of 12.195 mg/kg/day for a standard 60 kg adult. This corresponds to a weekly intake of 5.0 g, which aligns precisely with the estimated human exposure range. After a single gavage of 150 mg/kg·bw of particles, changes in fluorescence intensity were observed at intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h. After 12 h and 24 h of exposure to red fluorescent labeled PSNPs, mice from each group were sacrificed. Tissues were collected and rinsed with saline. Subsequently, the tissues were meticulously arranged on a black background. The fluorescence signal of the whole body and each organ (heart, lung, liver, spleen, kidney, intestine) were visualized at an excitation wavelength/emission wavelength of 640/680 nm using a small animal in vivo analysis system (PerkinElmer, USA). All animal care and experimentation were conducted according to National Guidelines for Animal Care and Use, and approved by the Animal Experimental Ethics Committee of Capital Medical University (Ethical number, AEEI-2024-013).

2. Results

PSNP accumulated in mouse liver and entered the hepatocytes

To observe the distribution of PSNPs in vivo, the fluorescence imaging in mice after oral exposure was performed. By gavage of 150 mg/kg·bw red fluorescence-labelled PSNPs into mice, we observed continuous changes in fluorescence signal intensity over 24 h (Figure S1a). The fluorescence signals tended to disappear after 24 h after a single oral gavage. We then collected multiple organs (heart, lungs, liver, spleen, kidneys and intestine) from mice after 12 and 24 hours of gavage. PSNPs were

clearly distributed in the liver (Figure S1b and 1c). Additionally, immunofluorescence of liver paraffin sections showed that PSNPs were internalized into hepatocytes (Figure S1d). Therefore, this study was conducted in the human normal hepatocytes to investigate the regulatory mechanisms and effects of PSNP exposure on hepatic metabolism.

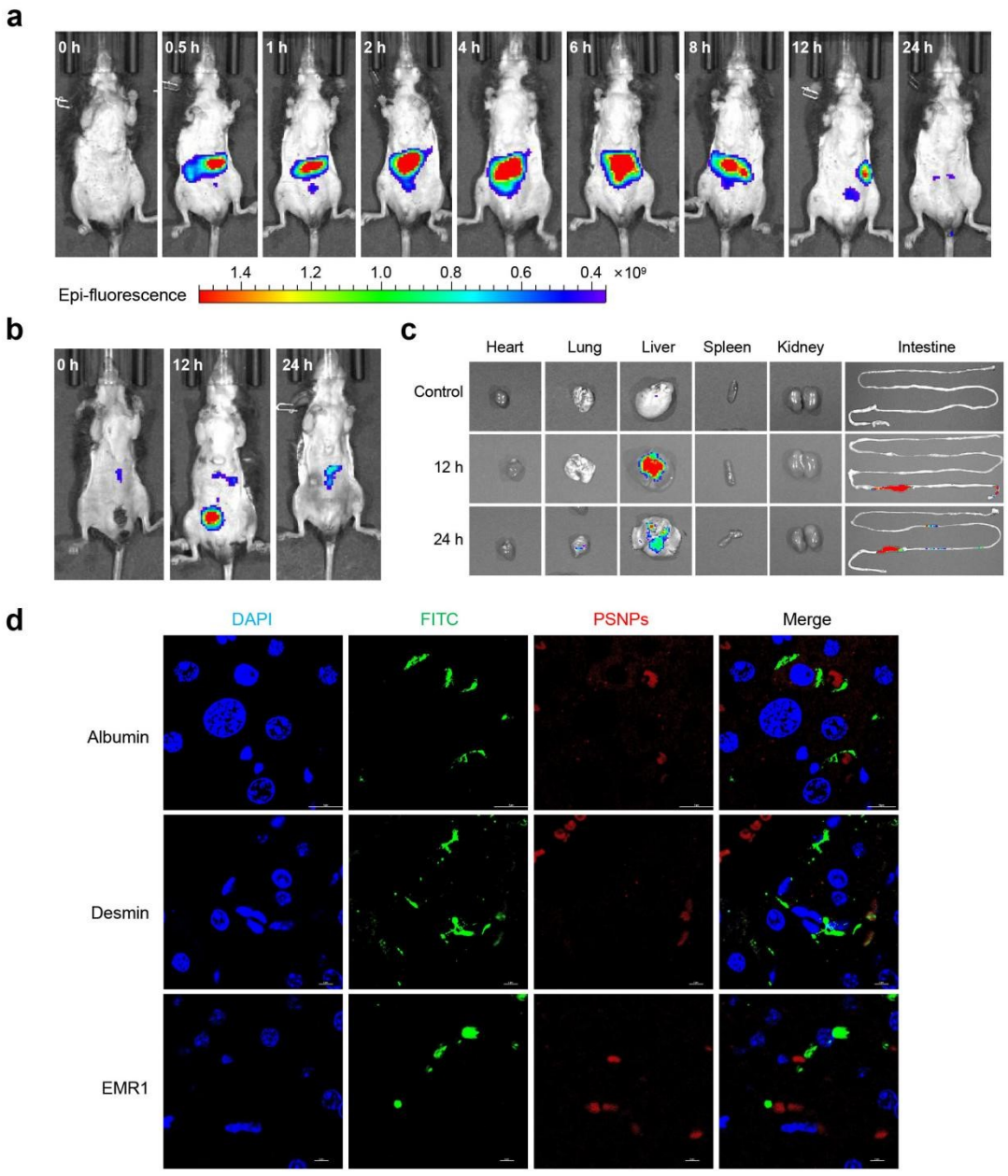


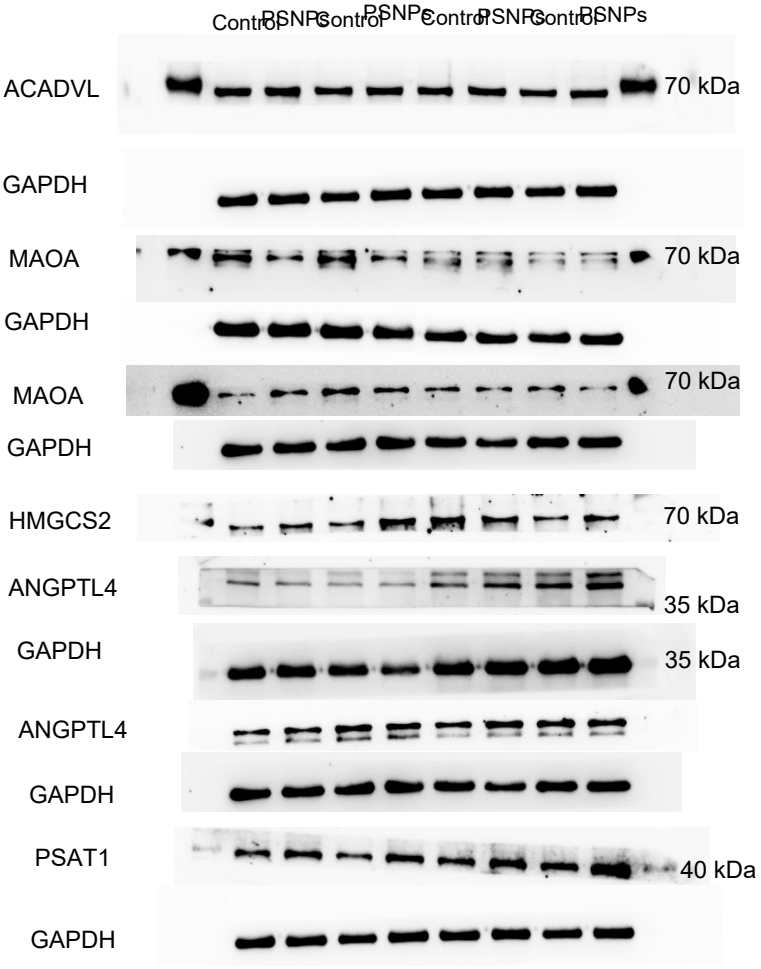
Figure S1 PSNPs accumulated in mouse liver after a singal oral gavage. (a) Representative fluorescence images of whole-body distribution of red-fluorescent labelled PSNPs in living mice. After a single gavage of 150 mg/kg·bw of particles, changes in fluorescence intensity were observed at intervals of 0, 0.5, 1, 2, 4, 6, 8, 12,

and 24 h by a small animal in vivo imaging analysis system. (b-c) Mice at each sample time (0, 12, 24 h) were sacrificed to collect tissues. Ex vivo images of fluorescence intensities for each organ (heart, lung, liver, spleen, kidney, intestine) indicated the accmulation of PSNPs in the liver of mice. (d) Immunofluorescence of liver tissue sections from red-fluorescent labelled PSNPs-administered mice (150mg/kg·bw, 24h). Red fluorescence representing PSNPs appeared in hepatocytes, indicating the main cell type in which they were internalised . DAPI nuclear; FITC: albumin: hepatocytes, desmin: hepatic stellate cells, EMR: macrophage; Red: red-fluorescent labelled PSNPs. Scale bar = 5 μm.

Table S1 The list of primer sequences

Gene	Forward (5′-3′)	Reverse (3′-5′)	Amplicon length (bp)
ANGPTL4	GGCTCAGTGGACTTCAACCG	CCGTGATGCTATGCACCTTCT	103
ACSBG1	ACACTGTGCATCGGATGTTCT	AGGAGATGTGTTCCCACTTGT	96
CPT1A	TCCAGTTGGCTTATCGTGGTG	TCCAGAGTCCGATTGATTTTTGC	98
ACADVL	ACAGATCAGGTGTTCCCATACC	CTTGGCGGGATCGTTCACTT	114
HMGCS2	GACTCCAGTGAAGCGCATTCT	CTGGGAAGTAGACCTCCAGG	179
PSAT1	TGCCGCACTCAGTGTTGTTAG	GCAATTCCCGCACAAGATTCT	141
PSPH	GAGGACGCGGTGTCAGAAAT	GGTTGCTCTGCTATGAGTCTCT	131
PHGDH	CTGCGGAAAGTGCTCATCAGT	TGGCAGAGCGAACAATAAGGC	154
MAOA	TTCAGGACTATCTGCTGCCAA	GGTCCCACATAAGCTCCACC	147
COMT	TACTGCGAGCAGAAGGAGTG	CCAGCGAAATCCACCATCC	227

Western blot raw data



References

1 K. Senathirajah, S. Attwood, G. Bhagwat, M. Carbery, S. Wilson and T. Palanisami, *J Hazard Mater*, 2021, **404**, 124004.

2 A. B. Nair and S. Jacob, *Journal of Basic and Clinical Pharmacy*, 2016, **7**, 27.