Supplementary information

Elucidating the Underlying Toxic Mechanisms of Nanoplastics on Zebrafish Hematological and Circulatory Systems

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Table S1. The temporal parameters of zebrafish exposure to PS-NPs, including the initiation and duration of exposure as well as the time point of sample collection.

Initial exposure time point	exposure duration	Collect zebrafish time point
0 hpf	12 hours	12 hpf
12 hpf	14 hours	26 hpf
12 hpf	18 hours	30 hpf
24 hpf	24 hours	48 hpf
24 hpf	35 hours	59 hpf
24 hpf	48 hours	72 hpf

Table S2. Primers used in this study

Genes	Accession No.	Primer sequences (5'-3' F/R)	Size (bp)	Usage
nppa	NM_198800.3	ACAGAGACCGAGAGGAAG	166 bp	Heart failure
		AGGGTGCTGGAAGACCCTAT		
nppb	NM_001327776.1	CATGGGTGTTTTAAAGTTTCTCC	101 bp	Heart failure
		CTTCAATATTTGCCGCCTTTAC		
ho1	NM_001127516.1	GGACTTGGAGCACTTCTTCGG	216 bp	Oxidative stress
		ATGCCCTTGTTTCCAGTCAGC		
keap1	NM_182864.2	TGTGATCTGGTTCTGCATGTC	121 bp	Oxidative stress
		ACTCCTTGAAGTTGCTGGTG		
nrf2	NM_182889.1	CACCCAACATGAATCAACTG	137 bp	Oxidative stress
		ATTTCCGCCATCTGATGTAAT		
apex1	NM_213421.2	AATAAAGTGTTGGGTGTACGTG	251 bp	BER pathway
		CAGGAGGTGATCTTCATATTGG		
creb1	NM_200909.1	AGGAGCGTGGAGAACCATAAA	151 bp	BER pathway
		GGCAGAGCCATCAGCGAC		
crem	XM_677932.9	GGAACAACACCATCAGATCC	226 bp	BER pathway
		CCTGAGTGATTGCAATGTACTG		
p62	NM_001312913.1	CCTGGGTTTCCGTTCACT	564 bp	Mitophagy
		TACTTTGGTCCGCTTTCC		
pink1	NM_001008628.1	GGGAGTGGATCATCTGTGC	519 bp	Mitophagy
		TCTGCCATCTGGACCTTGT		
parkin	NM_001423707.1	GCTGCGGGTCATCTTTGCT	440 bp	Mitophagy
		CTCCGTGAATCCGGTTTGG		
β-actin	NM_131031.2	CTCCCCTTGTTCACAATAACCTACTA	185 bp	Internal reference
		ATACACAGC		
		TTCTGTCCCATGCCAACCATCACTC		

Table S3. Mortality of zebrafish treated with different concentrations of G-PS-NPs on 48, 56, and 72 hpf

Concentration （PS-NPs）	0 μg/mL	10 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
48hpf	0	0	0	0	0	0
56hpf	0	0	0	0	0	0
72hpf	0	0	0	2.06%	6.18%	31.25%

Table S4. Comparative summary of previously published studies on hematological and circulatory systems toxicities

NPs type	Species	Size（nm）	Concentration range	Exposure duration	Main toxicological findings	Reference
PS-NPs	mice	40 nm	16, 40, 100 μg/day	1/4/12 weeks	Cardiotoxicity mitochondria damage	1
PS-NPs	mice	100 nm	10, 50, 100 μg/mL	30/180 days	vascular toxicity tiRNA-Glu-CTC/Cacna1f axis	2
PS-NPs	mice	100 nm	5, 10 mg/kg	3 months	Atherosclerosis Oxidative stress	3
PS-NPs	mice	50 nm	25 mg/kg BW	28 days	Blood-brain barrier Inhibiting Ferroptosis	4
PS-NPs	mice	100 nm	0.1, 0.5, 2.5 mg/day	6 weeks	cardiotoxicity upregulated HIPK2 activated the P53 and TGF-beta	5
PS-NPs	zebrafish	80 nm	1, 10, 100 μg/mL	7 days	Cardiotoxicity disrupting the Notch and Wnt signaling pathways	6
PS-NPs	mice	80 nm	0, 30, 60, and 120 μg d	42 days	hematopoietic damage cell oxeiptosis and senescence	7
PS-NPs	zebrafish	20 nm	2, 5, 8 mg/L	22/46/70h	vascular malformation interfering with the VEGFA/VEGFR pathway	8
PS-NPs	mice	100 nm	100 μg/mL	30/180 days	vascular toxicity regulation of the PIWI-interacting RNA expression	9
PS-NPs	mice	50 nm	2.5, 25, 250 mg kg^{-1}	19 weeks	atherosclerosis upregulating MARCO	10

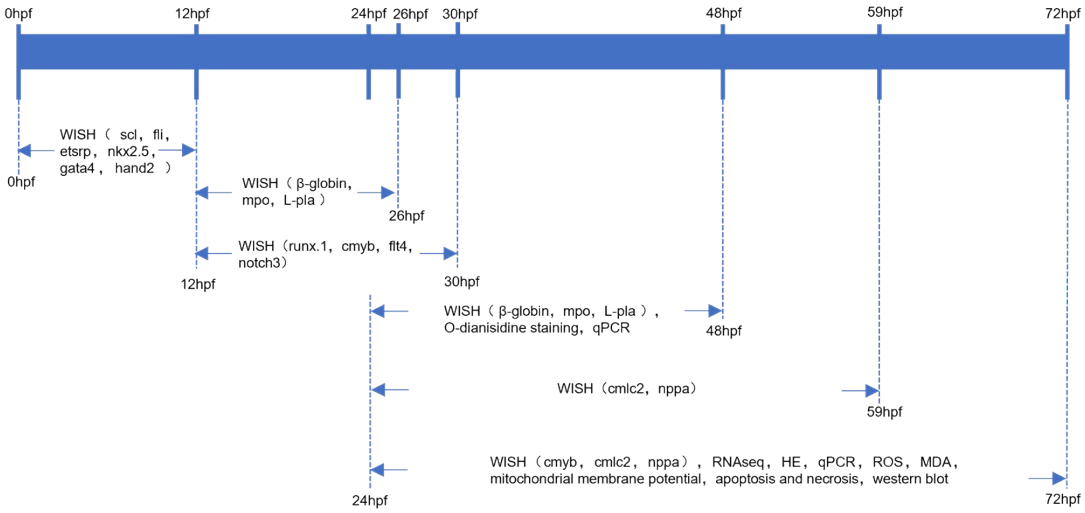

Figure S1. The flow chart depicting the experimental design for acute exposure during embryonic stages.

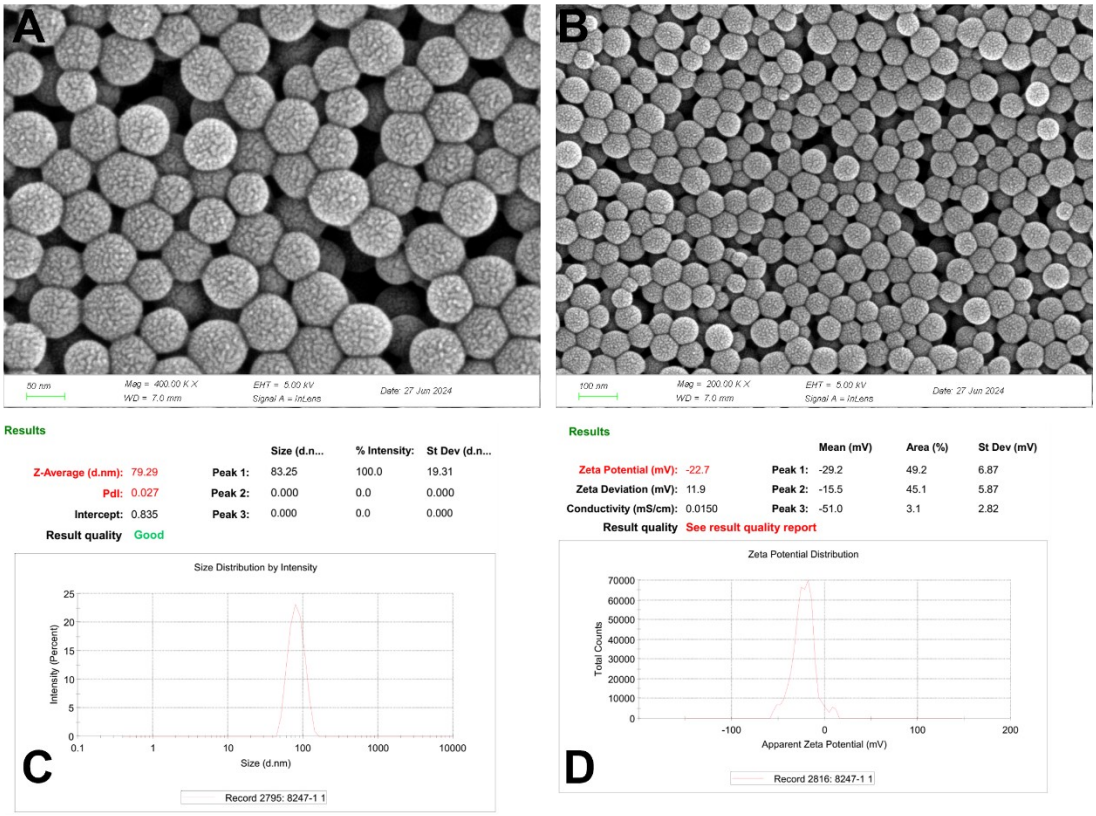

Figure S2. The characteristics of PS-NPs. (A-B) Morphology of PS-NPs (80 nm) was obtained by the SEM; (C) The hydrodynamic diameters of PS-NPs; (D) The zeta potential of PS-NPs.

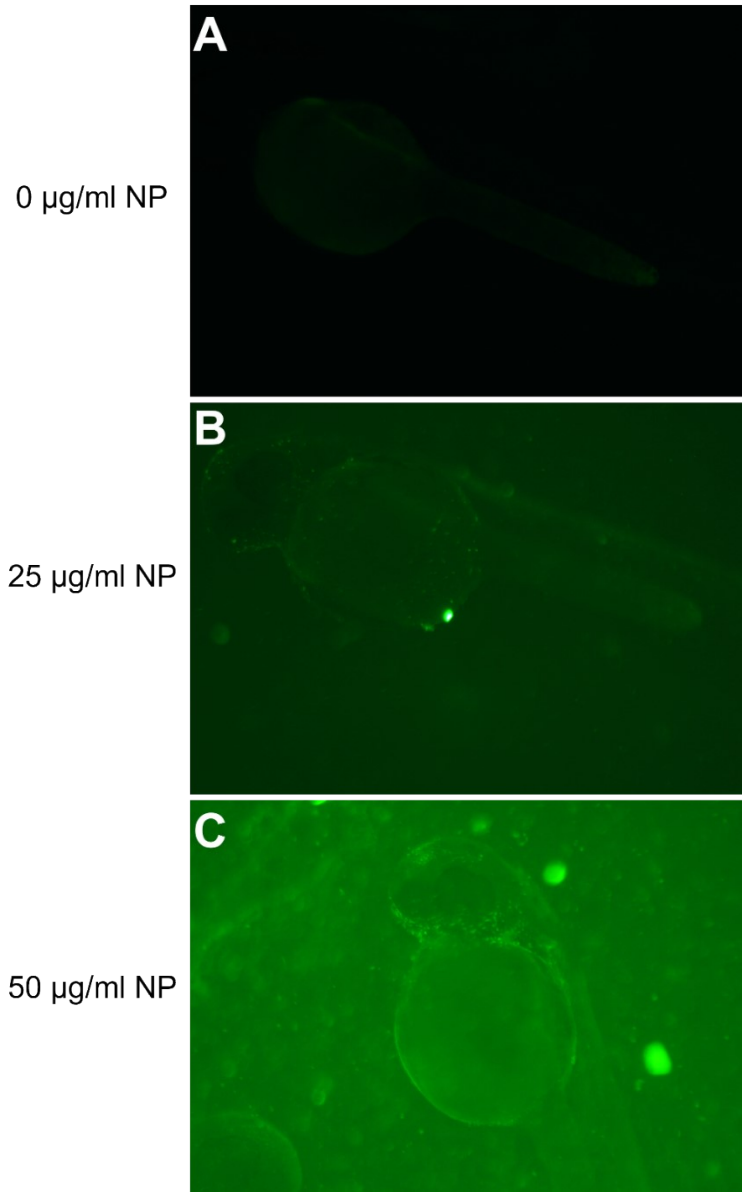

Figure S3. The distribution of G-PS-NPs with green fluorescence (25 μg/mL and 50 μg/mL) in zebrafish at 72 hpf. (A) 0 μg/mL G-PS-NPs. (B) 25 μg/mL G-PS-NPs. (C) 50 μg/mL G-PS-NPs. Images are taken at the same exposure time.

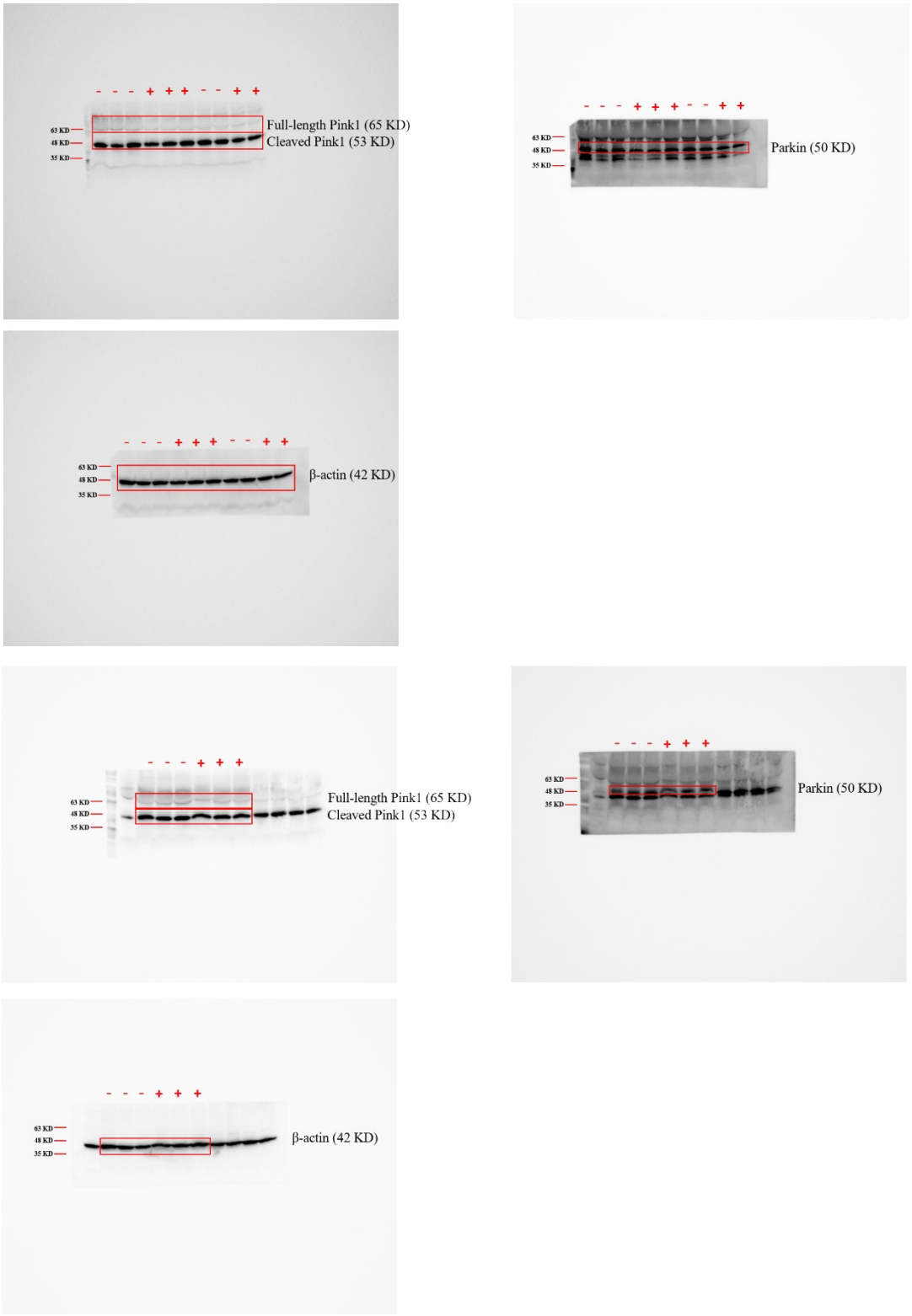

Figure S4. The raw data of western blot. The red box represents the target band. "-" represents PS-NPs untreated zebrafish, and "+" represents PS-NPs treated zebrafish.

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