



Research Article

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Chronic nanoplastic exposure drives immune dysfunction through hematopoietic reprogramming and adaptive immune impairment

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Abstract: Nanoplastics (NPs) have emerged as ubiquitous environmental contaminants, yet their long-term immunological consequences in vertebrates remain mechanistically uncharacterized. Using adult zebrafish exposed to environmentally relevant and high concentrations of polystyrene NPs (PS-NPs), we used single-cell RNA sequencing, flow cytometry, and functional immune assays to comprehensively dissect NP-induced immune dysregulation. Single-cell transcriptomic analysis revealed that while overall kidney immune cell frequencies remained stable, chronic NP exposure profoundly reprogrammed hematopoietic stem and progenitor cell differentiation trajectories toward myeloid-biased differentiation, indicating impaired lymphoid regeneration capacity. Concurrently, NPs induced global activation of infection-associated and programmed cell death pathways across immune populations despite the absence of microbial challenge, establishing a state of sterile inflammation in which NP particles function as danger-associated molecular patterns. At the cellular level, NP exposure selectively depleted regulatory T cells while expanding NK cell populations, coupled with marked suppression of pro-inflammatory cytokines and Treg-associated immunoregulatory genes, creating a dysbalanced inflammatory state. Mechanistically, antigen-presenting cells (APCs) from NP-exposed fish indicated severely impaired antigen uptake and suppressed co-stimulatory molecule expression. Consequently, T cells exhibited transcriptional exhaustion characterized by baseline downregulation of effector cytokines and complete ablation of antigen-responsive gene upregulation, resulting in severely diminished T cell proliferation following antigen stimulation in a dose-dependent manner. These multi-scale mechanistic findings establish that chronic NP exposure causes an organized immune dysfunction distinct from cytotoxic immune depletion. This study provides single-cell resolution evidence of NP-induced immunotoxicity and highlights the urgent need for environmental and health risk assessments of chronic NP contamination.

Key words: Nanoplastics; Immunotoxicity; Zebrafish; Single-cell RNA sequencing; Adaptive immunity suppression

1 Introduction

Plastic pollution has emerged as a pervasive environmental threat, with microplastics (MPs; <5 mm) and nanoplastics (NPs; <1 µm) now ubiquitously detected in aquatic ecosystems worldwide (Al-Thawadi, 2020; Peng et al., 2020). These particles originate from the degradation of macroplastics, direct industrial emissions, and the fragmentation of consumer products (Amobonye et al., 2021; Arif et al., 2024). Beyond their direct

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physical presence, these particles pose multifaceted hazards at both organism and ecosystem scales (Boyle and Örmeci, 2020; Choudhury et al., 2023; Pencik et al., 2023; Lu et al., 2024a). First, they accumulate harmful compounds, including heavy metals and persistent organic pollutants on their surfaces, serving as vectors for bioaccumulation of toxic chemicals (Yin et al., 2021; Dong et al., 2023). Second, emerging evidence shows that plastic particles directly disrupt endocrine function by interfering with signaling pathways in key regulatory organs (pituitary, thyroid, adrenal, and reproductive tissues), compromising hormonal homeostasis (Saha et al., 2024). Increasing attention has been directed toward the direct impacts of plastic particles on aquatic organisms. Studies have shown that the uptake and accumulation of these particles in fish can result in a range of physical and toxicological effects, including inflammation, oxidative stress, reproductive and developmental impairments, which ultimately alter feeding behavior, growth rates, and overall health (Hu and Palić, 2020; Yin, et al., 2021; Marcelino et al., 2022; Zhang et al., 2022; Bhat et al., 2024; Sun et al., 2024; Verma et al., 2024; Yi et al., 2024).

Recent studies have underscored the immunotoxic impacts of plastic pollution, revealing significant alterations in immune cell populations, cytokine production, and overall immune function across multiple fish species (Hirt and Body-Malapel, 2020; Bai et al., 2022). For instance, exposure to MPs in gilthead seabream has been shown to induce inflammatory responses and compromise phagocytic activity in macrophages, which are critical components of the innate immune system (Espinosa et al., 2018; Del Piano et al., 2023). Additionally, the interactions between NPs/MPs and immune cells can disrupt cytokine signaling pathways, thereby affecting the efficacy of the adaptive immune response, which is essential for pathogen-specific immunity and immunological memory (Lieschke and Trede, 2009; Rauta et al., 2012; González-Fernández and Cuesta, 2022; Beans, 2023; Banaee et al., 2024). Given the pivotal role of the adaptive immune response in combating infections and maintaining homeostasis, any disruption caused by plastic pollutants warrants comprehensive investigation (Hirt and Body-Malapel, 2020; Bai, et al., 2022; González-Fernández and Cuesta, 2022). Teleost fish, including zebrafish, have adaptive immune mechanisms conserved across vertebrates, wherein T and B lymphocytes coordinate humoral and cellular immune responses (Page et al., 2013; Wan et al., 2017). The zebrafish model offers substantial advantages for immunotoxicological research: genetic tractability enabling precise molecular manipulation; transparent larvae permitting real-time visualization of immune processes; and most critically, conservation of immune pathways, hematopoietic organization, and organ physiology with mammalian systems (Lieschke and Currie, 2007; Renshaw and Trede, 2012). These features make zebrafish an ideal sentinel organism for evaluating how environmental contaminants compromise immune function in vertebrates (Jiang et al., 2025). However, despite growing recognition of NP accumulation in aquatic environments and emerging evidence that plastic pollution impairs immune function, the cellular and molecular mechanisms through which chronic NP exposure dysregulates immune homeostasis remain largely unresolved.

To bridge these knowledge gaps, we present a comprehensive, multi-level analysis of NP-induced immunotoxicity by integrating histopathology with bulk transcriptomic profiling, and single-cell sequencing after prolonged PS-NP exposure. Our findings reveal that chronic exposure leads to preferential accumulation of NPs in the kidney, inducing a cascade of interrelated immune dysfunctions, such as altered hematopoiesis, impaired antigen-presenting cell activity, T cell exhaustion, and loss of regulatory T cells, alongside persistent activation of effector immune subsets, resulting in an ineffective inflammatory response. This work offers a high-resolution, single-cell view of NP-driven immune disruption, deepening insights into the mechanisms by which environmental NPs undermine immune defenses in vertebrates and highlighting the potential need for strengthened regulatory attention to this emerging immunotoxic threat.

2 Results

2.1 Nanoplastic exposure suppresses immune responses in zebrafish

To investigate whether NP exposure impairs immune function, adult zebrafish were exposed to environmentally relevant (0.1 and 1 mg/L) and high (10 mg/L) concentrations of NPs for 60 d, alongside untreated controls, with all other rearing conditions standardized across groups (Jiang et al., 2020; Cheng et al., 2022). We assessed immune competence through two complementary approaches: bacterial pathogen challenge and humoral immune stimulation. Following NP pre-exposure, zebrafish were challenged with *A. hydrophila* or immunized with KLH, a soluble model antigen (**Fig. 1a**). In the bacterial challenge experiment, zebrafish pre-exposed to 1 mg/L and 10 mg/L NP concentrations exhibited significantly reduced survival rates compared to controls (**Fig. 1b**). Correspondingly, spleen and kidney bacterial loads, markers of immune containment in primary lymphoid tissues, were markedly elevated in the NP-exposed groups, indicating compromised anti-microbial defenses (**Fig. 1c**). In the humoral immune response assay, KLH stimulation triggered characteristic spleen enlargement in control zebrafish, reflecting typical immune activation. By contrast, NP-exposed zebrafish showed attenuated spleen volume expansion in a dose-dependent manner (**Fig. 1d**). Gene expression analysis of head kidney leukocytes revealed that baseline pro-inflammatory cytokine expression (*il1b*, *il6*, *tnfa*) increased progressively with NP concentration, whereas the immunoregulatory gene *il10* showed biphasic kinetics, modest upregulation at lower concentrations (0.1, 1 mg/L) but significant suppression at 10 mg/L. Critically, KLH-induced cytokine upregulation was substantially blunted in all NP-exposed groups compared to controls (**Fig. 1e**). These results collectively suggested that sub-chronic NP exposure causes dose-dependent immune dysfunction, characterized by impaired pathogen clearance, attenuated antigen-specific responses, and dysregulated inflammatory gene expression in zebrafish.

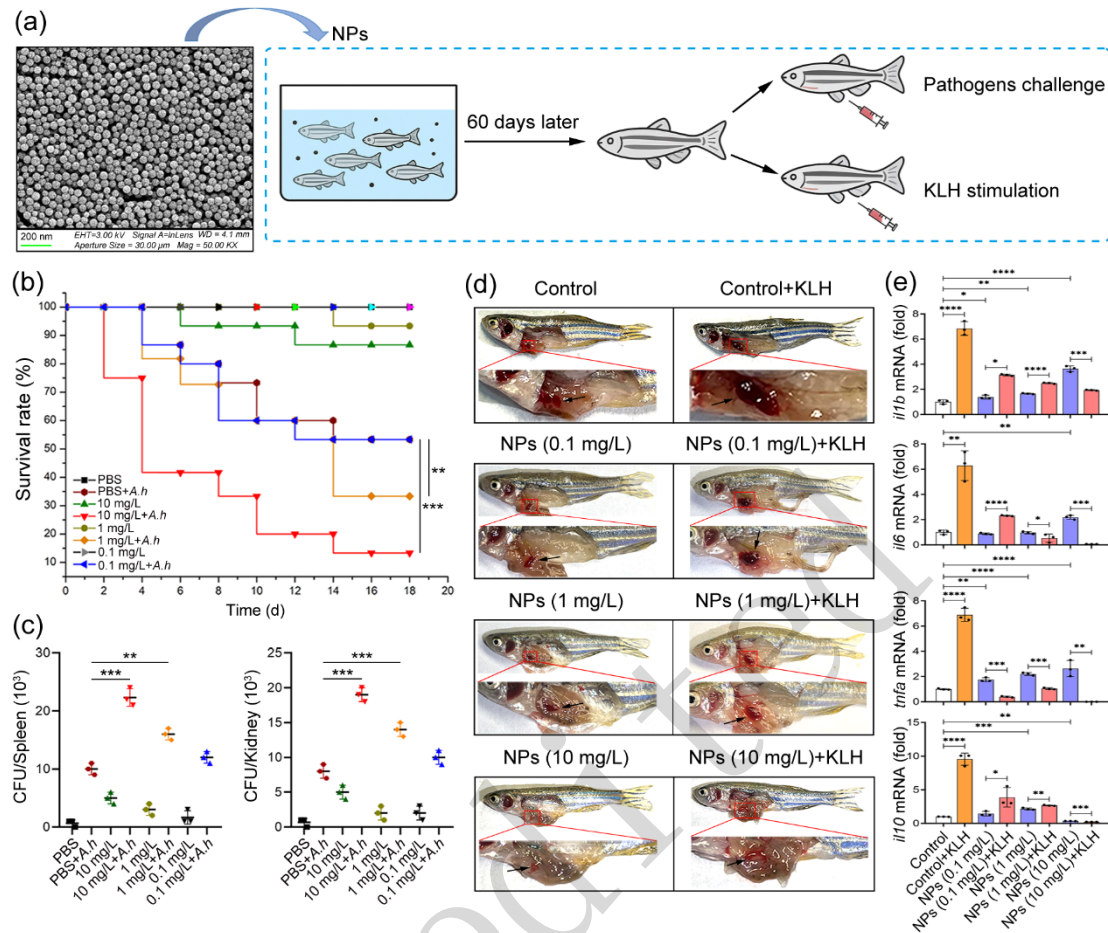


Fig. 1 NP exposure inhibits immune responses in zebrafish. (a) Scanning electron microscopy images of NPs and an experimental schematic illustrating the overall workflow of the study. (b) Survival rate of zebrafish after *A. hydrophila* infection and pre-exposure to low (0.1 mg/L), medium (1 mg/L), or high (10 mg/L) concentrations of NPs ($n = 15$). (c) Quantification of bacterial load in the spleen and kidney, the primary immune organs of zebrafish exposed to NPs ($n = 15$). (d) Illustrates the morphological changes in the spleen of control group zebrafish and zebrafish exposed to NPs before and after stimulation with the KLH antigen. (e) The inhibition of inflammation and induction of immune dysregulation in zebrafish by different concentrations of NPs. RT-qPCR analysis was conducted to assess the expression levels of inflammatory cytokines (*il1b*, *il6* and *tnfa*) and the immune regulatory cytokine *il10* in zebrafish from different treatment groups before and after antigen stimulation. Error bars represent SD. All data were obtained from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

2.2 Sub-chronic NP exposure induces accumulation, structural damage, and cell death pathways in zebrafish kidneys

Following the 60-day NP exposure period (10 mg/L), we collected zebrafish kidney and spleen tissues for ultrastructural and histological analysis. Transmission electron microscopy (TEM) revealed substantial accumulation of NP particles within kidney tissue, localized to both intercellular spaces and intracellular compartments (**Fig. 2a**). In control kidneys, cuboidal epithelial cells displayed characteristic ultrastructure: abundant mitochondria with well-defined cristae, extensive rough endoplasmic reticulum, and basally positioned round nuclei. In contrast, NP-exposed kidneys indicated marked ultrastructural deterioration, including dra-

matically reduced mitochondrial density, ER fragmentation, and evidence of cytoplasmic disorganization. Correspondingly, H&E histological analysis revealed extensive architectural distortion in NP-exposed kidneys, characterized by cellular disarray and loss of tubular organization (**Fig. 2b**). Notably, no NP accumulation was detected in the spleen, indicating preferential accumulation in the kidney under the present experimental conditions (**Fig. S1**).

To characterize the molecular mechanisms underlying NP-induced kidney damage, we performed RNA-sequencing on kidney tissue. A total of 1,595 significantly differentially expressed genes (DEGs) were identified, with 761 (47.7%) upregulated and 834 (52.3%) downregulated (**Figs. 2c-2f**). Heatmap and GO enrichment analyses revealed prominent upregulation of the “Immune response” pathway (**Figs. 2g and 2h**). To further validate these findings, selected immune-related factors were examined at the protein level. Western blot and immunofluorescence analyses showed that exposure to NPs significantly increased the expression of inflammatory molecules, including IL-1 β , IL-6, and TNF- α , in zebrafish kidneys (**Figs. 2i and 2j**). GSEA, KEGG, and heatmap analyses further identified enrichment of cell death-related pathways, including “Apoptosis,” “Necroptosis,” and “Ferroptosis,” accompanied by disruption of structural processes such as “Cell projection organization,” consistent with histological evidence of epithelial disorganization (**Figs. 2k-2n**). In addition, KEGG pathway analysis revealed upregulation of “Endocytosis” and “Calcium signaling” pathways, suggesting activation of cellular stress responses and calcium-dependent apoptotic cascades (**Fig. 2l**). Notably, GSEA highlighted significant downregulation of “Development”-related pathways in kidneys exposed to NPs compared with controls. This finding is consistent with the structural loosening observed in tissue sections, as well as the pronounced activation of pathogen-recognition pathways in the absence of microbial challenge (**Figs. S3a and 3b**). Protein-protein interaction (PPI) network analysis identified necroptosis and apoptosis-related genes as central hub regulators coordinating the cell death response (**Fig. S4b**). Collectively, these transcriptomic changes paint a picture of kidney tissue under stress: heightened cell death signaling, aberrant immune activation, disrupted cytoskeletal dynamics, and metabolic dysfunction.

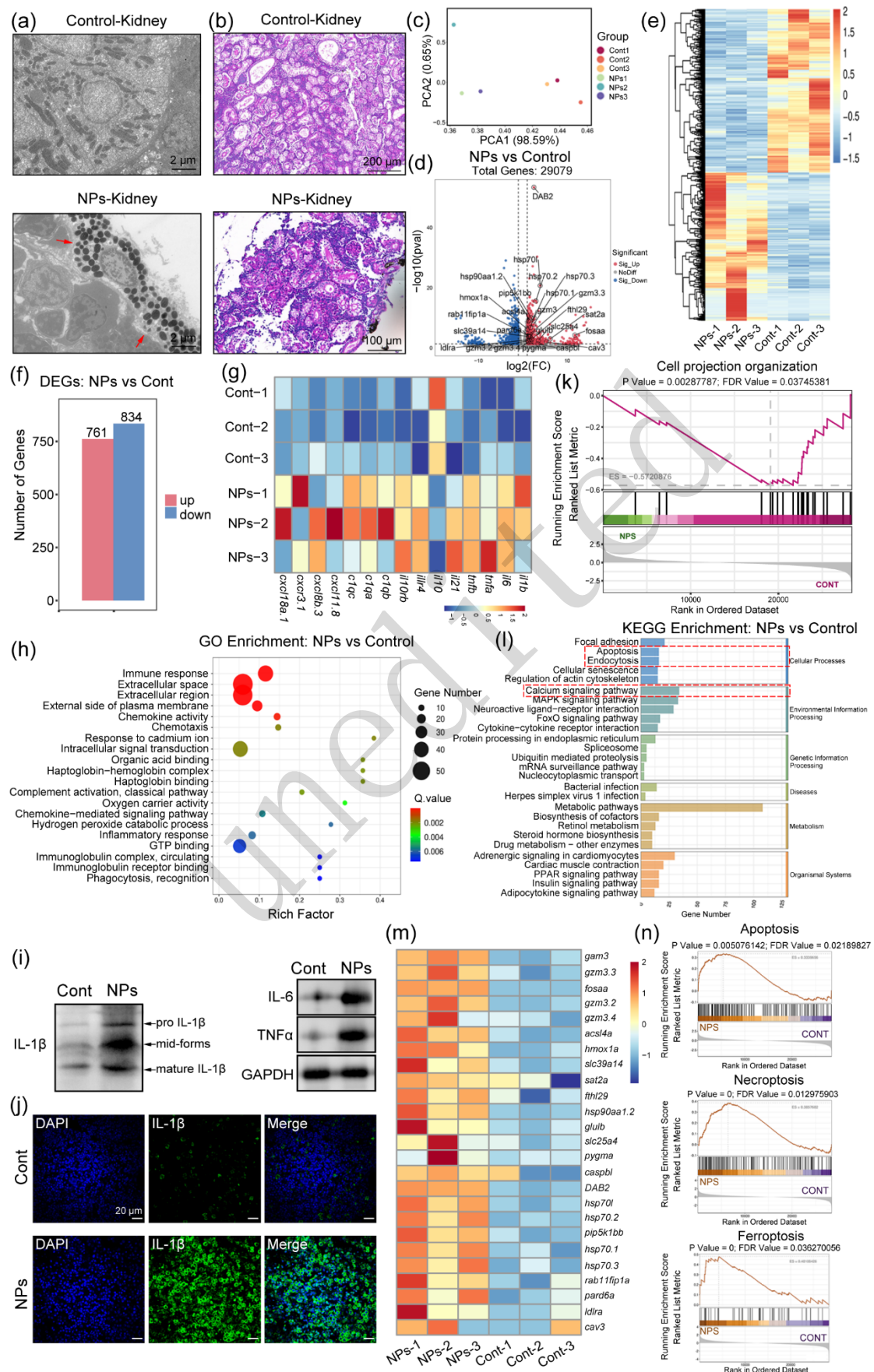


Fig 2. Chronic NP exposure (10 mg/L) induces particle accumulation, structural damage, and activation of cell death pathways in the zebrafish kidney. (a) TEM detection of zebrafish kidney in the control and the NP treatment groups. The red arrows indicate NP particles in the kidney of zebrafish. (b) H&E staining of zebrafish kidney in the control and NP treatment groups. (c) Principal component analysis (PCA) analysis of RNA-seq data to visualize sample-to-sample variation. The PCA plot shows clear clustering of control (Cont) versus NP treatment samples, with three biological replicates per group. (d) Volcano plot depicting significantly upregulated genes related to cell death in the kidney of the NP treatment group compared to the control group. (e) Hierarchical clustering analysis showing differential gene expression between the NP-treated group and the control group. (f) Differentially expressed gene numbers in the NP treatment group relative to the control group. (g) Heatmap showing the differential expression of inflammation-related genes in the NP treatment group compared to the control group. (h) GO enrichment analysis of differentially expressed genes (DEGs) after NP (10 mg/L concentration) exposure. The color and size of the points were scaled with respect to Q-values and the number of related DEGs. (i) Western blot analysis of IL-1 β , IL-6 and TNF α expression in the kidney before and after NP exposure. (j) Immunofluorescence analysis of IL-1 β expression in the kidney before and after NP exposure. (k) GSEA plot showing the downregulation of the “Cell projection organization” pathway in the NP treatment group. (l) Bar plot showing KEGG enrichment analysis of the kidney in the NP treatment group relative to the control group. (m) Heatmap illustrating the differential expression of cell death-related genes in the kidney of the NP treatment group and control group. (n) GSEA enrichment analysis of cell death-related pathways in the control group compared to the NP treatment group. GSEA plots illustrating the upregulation of cell death-related pathways including “Apoptosis”, “Necroptosis” and “Ferroptosis” in the NP treatment group.

2.3 NP exposure triggers persistent infection-like immune activation without altering immune cell proportions in zebrafish kidney

To investigate whether sub-chronic NP exposure alters kidney immune cell composition, we performed scRNA-seq on kidney tissue from control and NP-treated zebrafish (10 mg/L, 60 days). Libraries were generated from pooled cells (six fish per group) using the DNBelab C4 platform. Quality metrics were comparable between groups: control samples yielded 14,812 mean reads and 1,048 median genes per cell, while NP-exposed samples generated 16,002 mean reads and 1,000 median genes per cell. Following stringent quality control, 14,531 cells (control) and 14,054 cells (NP-exposed) were retained for analysis, spanning 23,754 and 23,916 detected genes, respectively (**Table S2 and Fig. S5**). Unsupervised clustering using Seurat identified 12 distinct cell populations with characteristic transcriptomic signatures (**Fig. 3a**). Clear boundaries among clusters were evident in the heatmap (**Fig. 3b**). Cell identity was assigned based on canonical lineage markers, and dot plot analysis confirmed that the PCA-based cell separation approach effectively captured cellular heterogeneity (**Figs. 3c, 3d, S6 and Table S2**).

Notably, NP exposure did not significantly alter the frequency or proportional composition of major immune cell populations relative to controls (**Figs. 3e and 3f**). However, comparative pathway analysis revealed striking transcriptomic dysregulation across immune cell clusters. KEGG enrichment analysis identified five dominant pathways significantly enriched in NP-exposed kidney immune cells: Salmonella infection, Herpes simplex virus 1 infection, Protein processing in endoplasmic reticulum, Phagosome, and Metabolic pathways (**Figs. 3g, 3h and S7c-7g**). This enrichment pattern, typically associated with active pathogenic challenge indicates that sub-chronic NP exposure establishes a persistent infection-like immune state despite the absence of actual microbial infection. Analysis of cell death pathways revealed widespread activation of apoptosis, necroptosis, and ferroptosis programs across immune populations. Notably, neutrophils (clusters 0, 1) exhibited

pronounced enrichment in death-receptor and programmed cell death pathways, suggesting these sentinel cells are particularly vulnerable to NP-induced stress (Fig. 3i). This sustained cell death signaling, coupled with continuous antigen presentation and immune activation, creates a pathological inflammatory microenvironment.

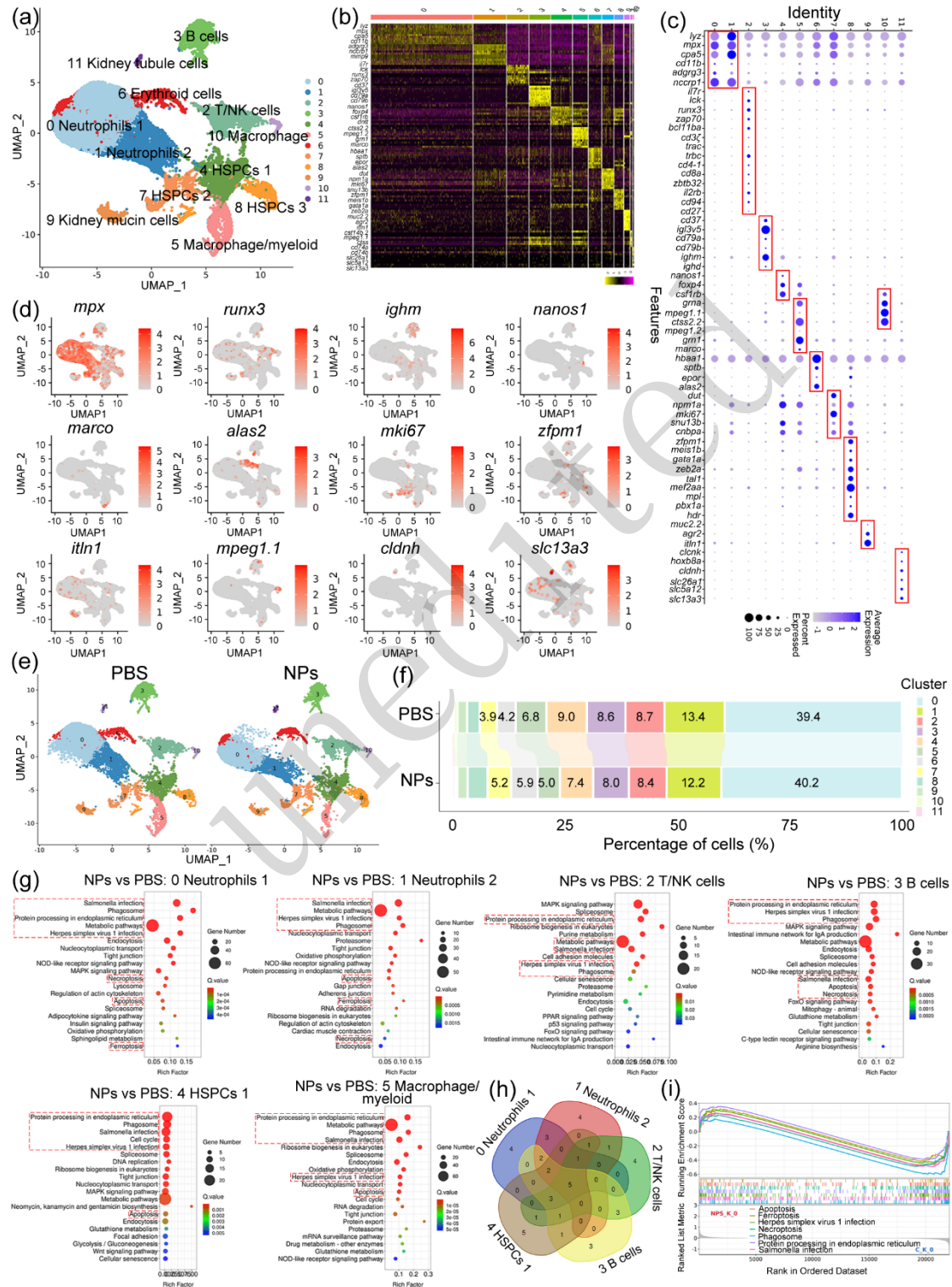


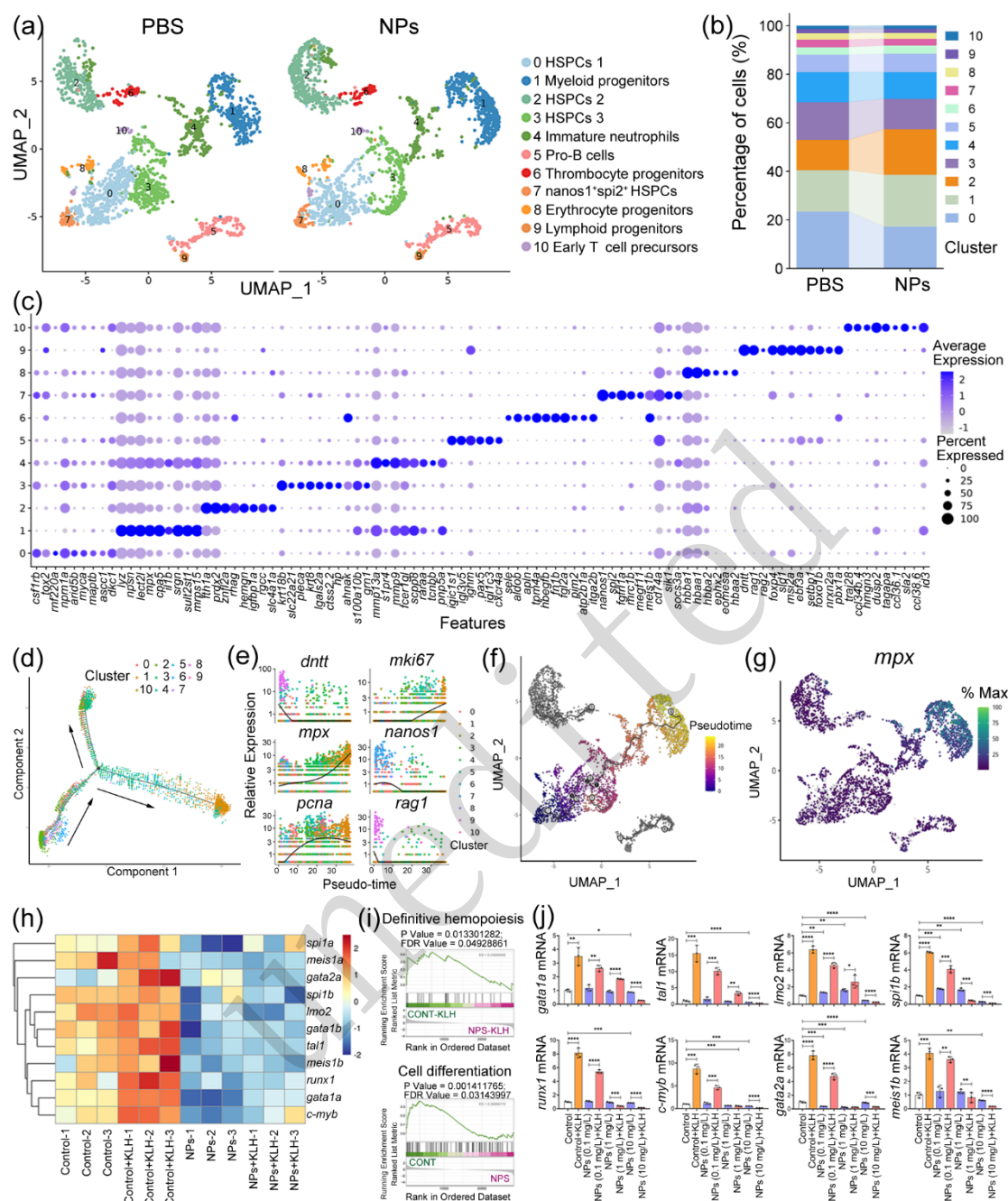
Fig 3. scRNA-seq characterization of immune cell types reveals that NP exposure triggers persistent

infection-like immune activation in the zebrafish kidney. (a) UMAP visualization of zebrafish immune cells and kidney cells. (b) Heatmap displaying marker genes specific to each cluster. Columns depict distinct cell subtypes, while rows correspond to genes. Expression levels are color-coded, with intense yellow indicating high expression and deep purple representing low expression. (c) Dot plots illustrating marker gene expression levels and the respective percentages of cluster-expressing cells. (d) UMAP results depicting molecular marker distribution for immune and kidney cell types. Each dot signifies a cell, with dark dots indicating elevated expression of the marker gene in a given cell. (e) UMAP visualization showing 12 immune and kidney cell clusters identified in both the control (PBS) and NP-exposed groups. (f) Histogram illustrating the differences in the proportions of immune and kidney cell clusters between the control (PBS) and NP-exposed groups. (g) KEGG pathway enrichment analyses of Neutrophils 1 (cluster 0), Neutrophils 2 (cluster 1), T/NK cells (cluster 2), B cells (cluster 3), HSPCs (cluster 4), and Macrophage/Myeloid cells (cluster 5) comparing the NP-exposed group with the control group (PBS). (h) Venn diagram illustrating the overlap among the top 20 enriched pathways across the five major immune cell types. (i) GSEA enrichment analysis of Neutrophils 1 (cluster 0), showing significantly upregulated signaling pathways following NP exposure.

2.4 NP exposure traps HSPCs in a dysfunctional state with significantly reduced responsiveness to immune stimulation

Building on evidence that sub-chronic NP exposure induces sustained immune activation without altering overall immune cell frequencies, we hypothesized that NPs may reprogram the differentiation fate of hematopoietic stem and progenitor cells (HSPCs), which are responsible for continuous immune cell regeneration. To test this, we performed subclustering analysis on the three primary HSPC populations identified in the initial analysis. Detailed subclustering revealed 11 distinct HSPC subpopulations (H0-H10) with characteristic transcriptomic signatures (**Figs. 4a, 4c, S8 and Table S3**). Pseudotime trajectory analysis identified a primary differentiation axis with three branch points, with early multipotent populations (H0, H2, H3, H6, H7, H8, H9) positioned proximally and lineage-restricted progenitors (H1, H4, H5) at distal locations, indicating distinct stages of hematopoietic commitment (**Figs. 4d-4g and S9**). Proportional analysis revealed that NP exposure induced a selective rebalancing of HSPC differentiation fates (**Fig. 4b**). While frequencies of early multipotent populations (H5-H10) remained largely stable, myeloid progenitors (H1) increased, indicating a preferential commitment to myelopoiesis. Paradoxically, immature neutrophils (H4) were reduced despite elevated myeloid progenitor frequencies, suggesting either accelerated terminal differentiation or selective loss of immature neutrophils under sub-chronic inflammatory stress. These population dynamics indicated that NP exposure biases HSPC differentiation toward the myeloid lineage while constraining downstream neutrophil maturation.

To validate this phenotype across the transcriptomic landscape, we reanalyzed bulk kidney RNA-seq data and identified significant downregulation of canonical hematopoietic regulatory genes (**Fig. 4h**). GSEA revealed substantial suppression of “Definitive hematopoiesis” and “Cell differentiation” pathways in NP-exposed kidneys, indicating global impairment of normal hematopoietic maturation (**Figs. 4i and S10**). To further assess effect of this dysregulated differentiation during immune stimulation, we treated control and NP-exposed zebrafish with KLH and quantified hematopoietic transcription factor expression. In controls, KLH stimulation robustly upregulated key regulators of hematopoietic fate specification (*gata1a*, *tall*, *lmo2*, *spilb*, *runx1*, *c-myb*, *gata2a*, *meis1b*; all $p < 0.05$). Critically, this hematopoietic transcriptional response was severely blunted in NP-exposed kidneys, with inhibition intensifying in a dose-dependent manner (**Figs. 4j and S2**). This persistent suppression of hematopoietic transcriptional programs indicated that chronic NP accumulation traps HSPCs in a dysfunctional state with significantly reduced responsiveness to normal immune stimulation cues, thereby preventing adaptive regeneration of the immune system.



genes in leukocytes in the kidney of zebrafish across different treatment groups through RNA-seq analysis. (i) GSEA plot showing the downregulation of “Definitive hematopoiesis” and “Cell differentiation” in the NP treatment group. (j) RT-qPCR analysis of hematopoiesis-related gene expression across different treatment groups. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

2.5 NP exposure disrupts T/NK cell homeostasis and promotes inflammatory responses in zebrafish kidney

Given the evidence that NP exposure dysregulates hematopoiesis while inducing immune exhaustion, we checked whether specific T/NK cell lineages were selectively compromised. Subclustering analysis of the T/NK cell population identified 10 functionally distinct subsets (T0-T9) with characteristic transcriptomic profiles (**Figs. 5a, 5c, S11 and Table S4**). Analysis of subset composition before and after NP exposure revealed a striking remodeling of the T/NK cell landscape (**Fig. 5b**). Cytotoxic T cells (T0) and regulatory T cells (Treg, T3), populations essential for effective viral immunity and immune tolerance, respectively, were significantly reduced. Conversely, all three NK cell subsets (T1, T4, T9) were proportionally increased, indicating a bias toward innate lymphocyte expansion and a simultaneous loss of adaptive immune control mechanisms (**Fig. 5b**). This compositional shift correlated with profound transcriptomic dysregulation. Pro-inflammatory cytokine genes (*il1b*, *il6*, *tnfa*) were markedly upregulated across multiple clusters, while Treg-associated immunoregulatory genes (*foxp3a*, *il10*, *ctla4*) were substantially downregulated, and paradoxically, Th2-associated genes (*il4*, *gata3*) were upregulated, reflecting a complex, dysbalanced inflammatory phenotype (**Figs. 5d and 5e**). Pathway analysis focusing on the cytotoxic T cell compartment (T0) revealed significant enrichment in stress-response pathways, including “Necroptosis”, “Salmonella infection”, and “Herpes simplex virus 1 infection”, indicating that cytotoxic T cells experience heightened programmed cell death signaling and infection-mimetic stress despite the absence of actual pathogenic challenge (**Fig. 5f**). In contrast, regulatory T cells were associated with unexpected activation of innate immune pathways, particularly the NOD-like receptor signaling cascade, a pattern typically associated with pathogenic sensing rather than immune homeostasis (**Fig. 5g**) (Wen et al., 2013). This aberrant Treg activation suggests that NP particles may be recognized as danger-associated molecular patterns (DAMPs), triggering innate immune surveillance in cells normally dedicated to immune tolerance. Collectively, these single-cell findings reconcile earlier observations of impaired pathogen control and blunted antigen-specific responses.

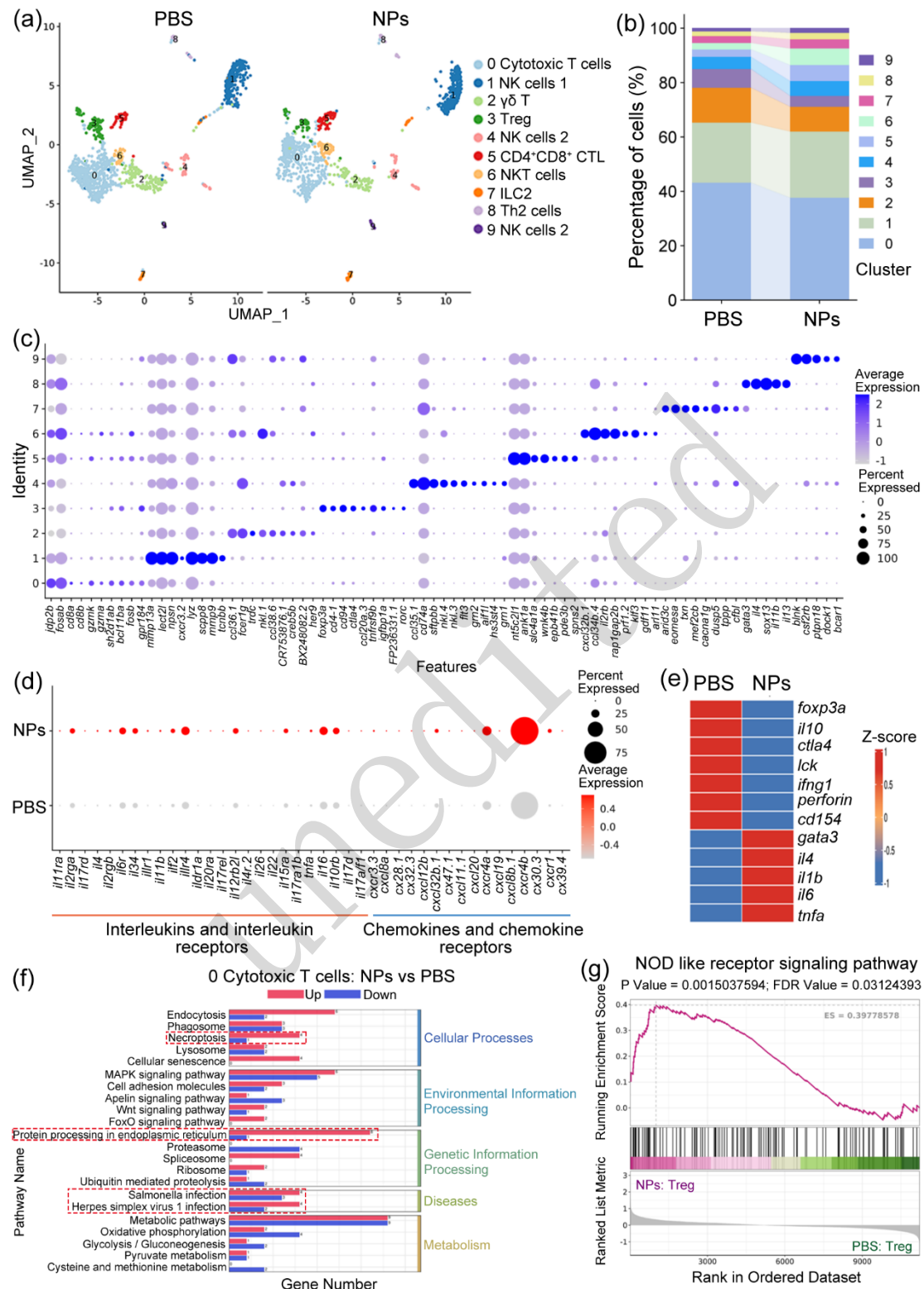


Fig 5. NP exposure disrupts T/NK cell homeostasis and promotes inflammatory responses in zebrafish kidney. (a) UMAP visualization showing 10 distinct T/NK cell subclusters identified in both the control (PBS) and NP-exposed groups. (b) Bar plot illustrating the proportional differences in T/NK cell subsets between the control and NP-exposed groups. (c) Dot plot showing the expression patterns of representa-

tive marker genes across T/NK cell subtypes. (d) Dot plot showing the upregulation of inflammation-related cytokine genes in the control (PBS) and NPs-exposed groups. (e) Heatmap depicting differential expression of inflammation- and T cell activation-related genes between the two groups. (f) KEGG pathway enrichment analysis of cluster 0 (cytotoxic T cells) following NP exposure relative to the PBS control. (g) GSEA enrichment analysis of cluster 3 (Treg cells) following NP exposure relative to the PBS control.

2.6 Sub-chronic NP exposure suppresses T cell proliferation and blunts KLH-induced adaptive immune responses

To directly measure whether NP accumulation compromises T cell effector functions, we quantified T cell proliferation and activation markers in control and NP-exposed fish using gene expression and flow cytometry approaches (**Fig. 6a**). Leukocyte populations from each group were analyzed for expression of canonical T cell activation genes: *lck* (T cell lineage marker), *t-bet* (Th1 transcription factor), *il2* (T cell growth factor), *ifng* (interferon-gamma; Th1 cytokine), *cd154* (CD40 ligand; T cell costimulatory molecule), and *perforin* (cytotoxic effector molecule). In control zebrafish, KLH antigen stimulation robustly upregulated all six markers, reflecting coordinated T cell activation (**Fig. 6b**). However, NP-exposed fish exhibited dose-dependent suppression of this transcriptional response. Remarkably, even in the absence of KLH challenge, the high-concentration NP treatment group (10 mg/L) showed significant baseline downregulation of *t-bet*, *ifng*, *cd154*, and *perforin* compared to controls, indicating constitutive impairment of effector T cell programs. Critically, this impaired baseline state prevented further upregulation upon KLH stimulation, suggesting that NP-exposed T cells occupy a functionally exhausted state refractory to antigen-driven activation (**Fig. 6b**). Flow cytometric analysis confirmed the suppressed transcriptomic phenotype at the protein level. In control fish, KLH stimulation increased the CD4⁺CD154⁺ T cell population from 3.94% ± 0.20% (baseline) to 7.31% ± 0.45% (post-KLH), representing an 85.8% expansion (**Fig. 6c**). Similarly, CD8⁺CD154⁺ T cells expanded from 7.53% ± 0.31% to 13.00% ± 0.40%, a 72.8% increase (**Fig. 6d**). In contrast, zebrafish pre-exposed to medium (1 mg/L) and high (10 mg/L) NP concentrations showed abolished or severely diminished KLH-induced T cell proliferation responses, with no statistically significant differences in CD4⁺CD154⁺ or CD8⁺CD154⁺ frequencies before and after antigen stimulation. Low-dose NP treatment (0.1 mg/L) partially preserved the proliferative response, though it was still substantially reduced relative to controls (**Figs. 6c and 6d**). Collectively, these findings suggested that chronic NP exposure severely impairs the adaptive immunity of zebrafish, leading to significant inhibition of T cell proliferation and activation.

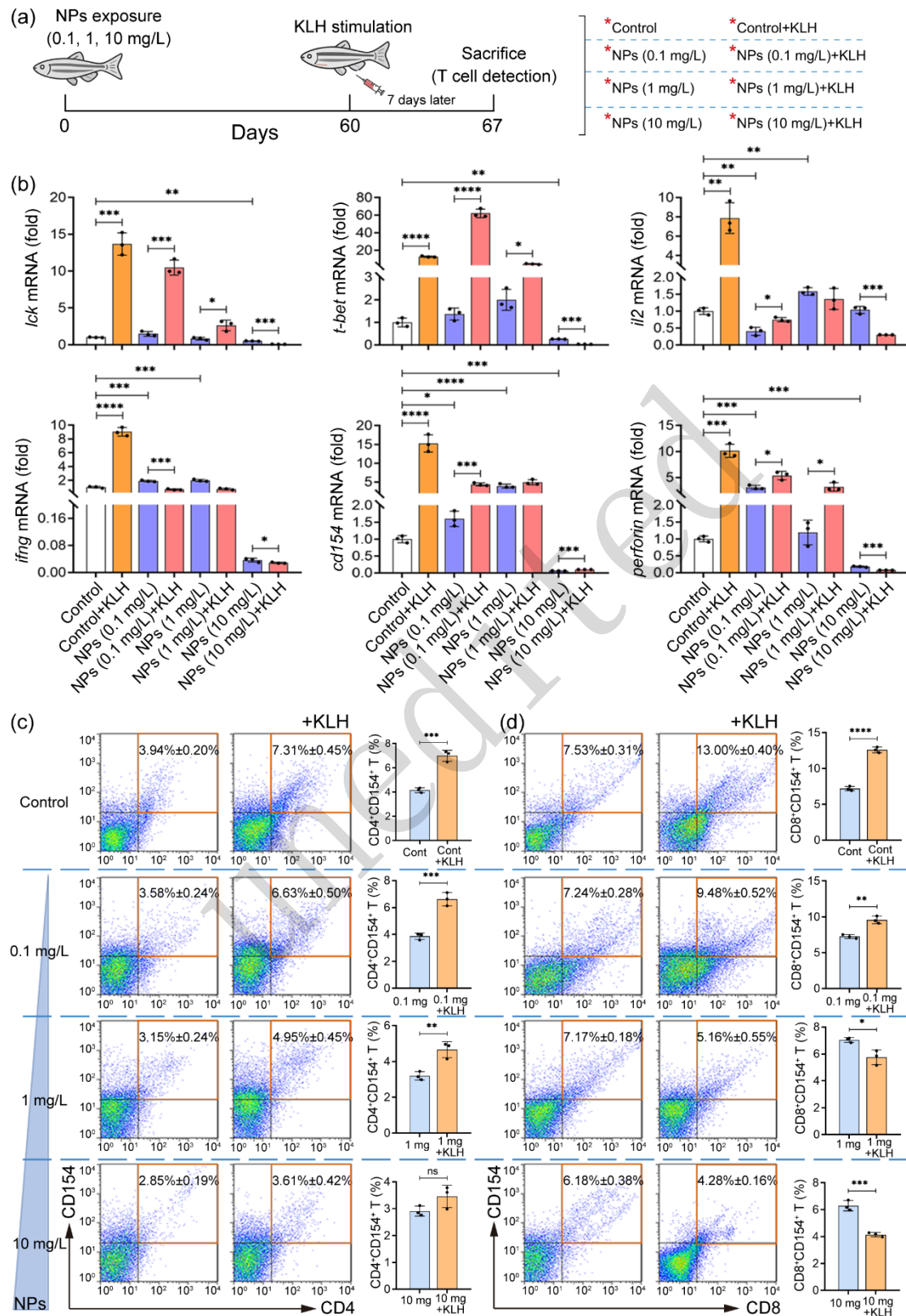


Fig 6. NPs inhibit T cell proliferation and activation in zebrafish. (a) Schematic illustrating the experimental assessment of impaired T cell immune responses to antigen stimulation in zebrafish following NP

exposure. (b) RT-qPCR analysis of T cell activation-related gene expression across different treatment groups. (c) Flow cytometry assessment of CD4⁺CD154⁺ T cell percentages before and after KLH antigen stimulation in the control group and zebrafish exposed to low, medium, or high concentrations of NPs. (d) Flow cytometry assessment of CD8⁺CD154⁺ T cell percentages before and after KLH antigen stimulation in the control group and zebrafish exposed to low, medium, or high concentrations of NPs. Data represent three independent experiments. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

2.7 NP exposure impaired antigen-presenting cell (APC)-mediated antigen presentation in zebrafish

Next, we evaluated APC function *in vivo* and *in vitro* using FITC-labeled KLH as a model antigen and measuring both antigen uptake and co-stimulatory molecule expression. FCM analysis revealed that control zebrafish mounted a robust antigen presentation response, with the frequency of KLH-FITC⁺ APCs increasing from a baseline of $0.33\% \pm 0.03\%$ to $6.73\% \pm 0.14\%$ following intraperitoneal KLH-FITC administration (Figs. 7a and 7b). In contrast, zebrafish exposed to high concentrations of NPs exhibited markedly impaired antigen capture, with KLH-FITC⁺ APC frequencies increasing only from $0.25\% \pm 0.01\%$ to $0.43\% \pm 0.04\%$ (Figs. 7a and 7b). Consistently, immunofluorescence analysis demonstrated substantial antigen uptake in APCs from the control group, whereas APCs from the high-concentration NP-exposed group showed severely attenuated antigen capture (Fig. 7c). This dramatic reduction in antigen-uptake capacity indicates that NP-induced APC dysfunction occurs at the fundamental level of pathogen/antigen recognition. To assess whether this functional deficit extended to APC activation markers, we quantified expression of the co-stimulatory molecules *cd80*, *cd83*, and *cd40*, which are essential for T cell priming. Control zebrafish exhibited robust KLH-induced upregulation of all three molecules (Fig. 7d), providing the necessary costimulatory signals for T cell activation. By contrast, NP-exposed zebrafish showed marked suppression of KLH-stimulated expression of these co-stimulatory molecules across all NP concentrations tested, indicating that even APCs capable of contacting antigen fail to differentiate into fully activated, T cell-priming cells (Fig. 7d). The parallel suppression of antigen uptake and co-stimulatory activation suggested that NP exposure compromises multiple checkpoints in APC function.

To validate these findings in a controlled *ex vivo* setting, we isolated leukocytes from control and NP-exposed fish and exposed them to KLH-FITC in culture. Both KLH-FITC uptake and expression of MHC-II (a marker of APC maturation and antigen presentation-competence) were assessed by flow cytometry. Control leukocytes indicated efficient antigen capture and MHC-II upregulation, with KLH-FITC⁺ cells reaching $18.80\% \pm 0.56\%$ and KLH-FITC⁺MHC-II⁺ cells comprising $14.30\% \pm 0.21\%$ of the population (Figs. 7e and 7f). Leukocytes from NP-exposed fish showed dramatic dose-dependent declines in both parameters: at 0.1 mg/L NP exposure, KLH-FITC⁺ cells decreased to $12.20\% \pm 0.21\%$ and KLH-FITC⁺MHC-II⁺ cells to $12.10\% \pm 0.14\%$; at 1 mg/L, these fell to $10.04\% \pm 0.13\%$ and $9.27\% \pm 0.26\%$, respectively; and at 10 mg/L, only $7.65\% \pm 0.15\%$ and $7.08\% \pm 0.17\%$ remained (Figs. 7e and 7f). This concentration-dependent suppression indicated that chronic NP exposure directly impairs the intrinsic capacity of APC populations to recognize, capture, and present antigen.

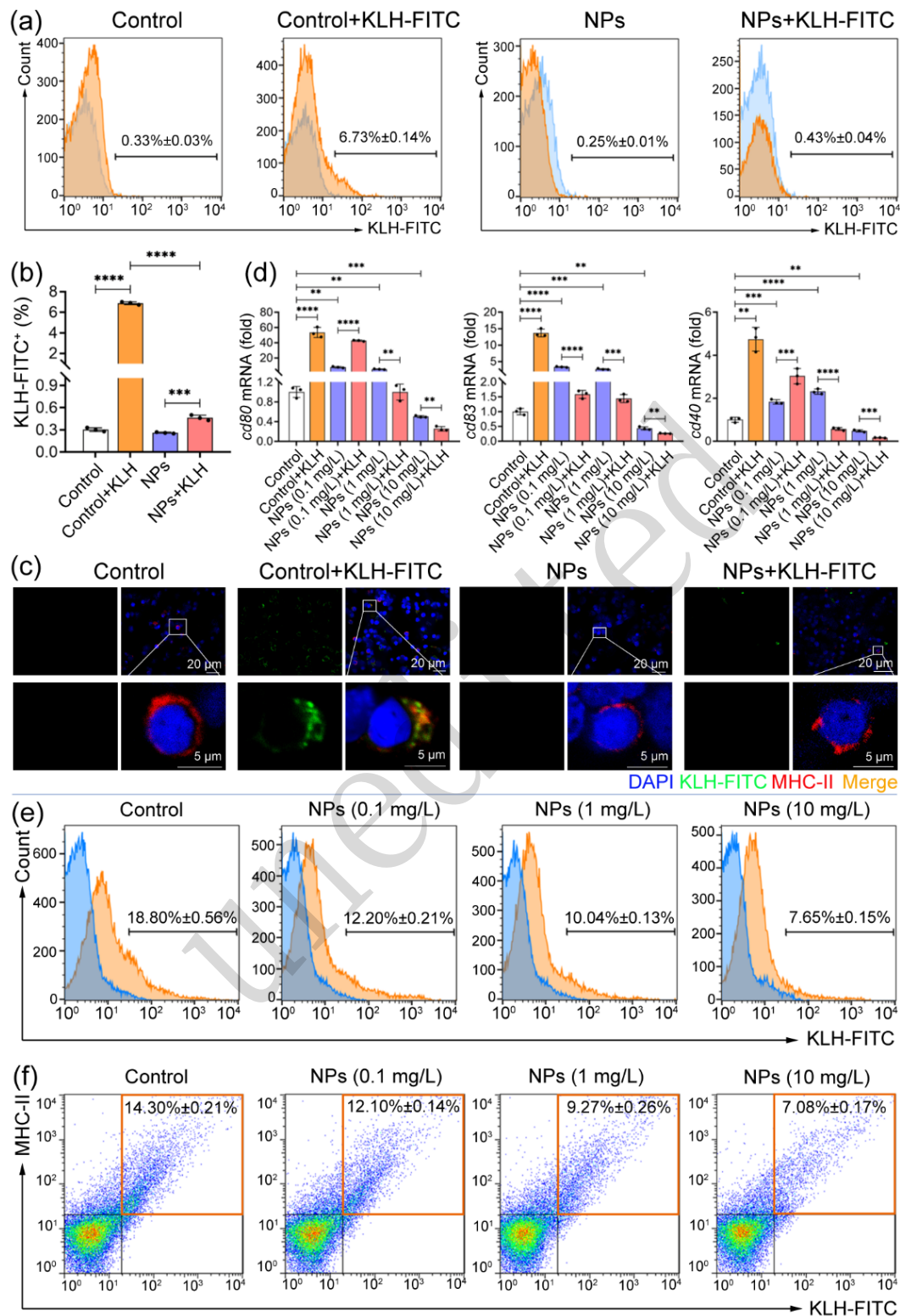


Fig 7. In vivo and in vitro analysis of the inhibitory effects of NPs on antigen presentation by zebrafish APCs. (a) In vivo FCM assessment of phagocytosis of soluble antigen KLH-FITC in zebrafish across different treatment groups after KLH antigen stimulation, characterized by the presence of KLH-FITC⁺ cells. (b) Bar chart showing the proportion of KLH-FITC⁺ cells across different treatment groups. (c)

Immunofluorescence analysis of antigen uptake by APCs. Blue, green, and red signals indicate DAPI-stained nuclei, KLH-FITC, and MHC-II, respectively. Scale bars: 20 μm and 5 μm . **(d) RT-qPCR analysis of the expression of co-stimulatory molecules related to APC activation in zebrafish across different treatment groups before and after KLH antigen stimulation.** **(e) *In vitro* FCM analysis of the phagocytic activity of zebrafish APCs from different treatment groups in response to the soluble antigen KLH-FITC.** **(f) *In vitro* FCM analysis of the proportion of KLH-FITC⁺MHC-II⁺ cells following the phagocytosis of the soluble antigen KLH-FITC by zebrafish APCs in different treatment groups.** Data represent three independent experiments, error bars represent SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3 Discussion

Nanoplastics have emerged as pervasive environmental contaminants with multifaceted effects on aquatic ecosystems and the organisms that inhabit them (Wang and Wang, 2014; Trevisan et al., 2022; Arif, et al., 2024). Our findings extend previous work by demonstrating that chronic NP exposure in adult zebrafish causes selective accumulation within the kidney, an organ central to both excretion and hematopoiesis. This tissue specificity is particularly consequential, as disruption of renal immune homeostasis can propagate systemic immune dysfunction. Consistent with earlier reports of NP-induced oxidative stress and histopathological damage in larvae (Yang et al., 2024), our single-cell transcriptomic analysis revealed that long-term NP exposure reprograms immune and hematopoietic gene networks at unprecedented resolution, demonstrating that adult immunity is also profoundly and durably altered by these particles.

The kidney and spleen constitute the primary lymphoid and hematopoietic compartments in teleosts, serving as the anatomical foundation of zebrafish adaptive immunity (Lewis et al., 2019; Hu et al., 2023; Hu et al., 2024). Our multi-scale analysis identifies three hierarchically organized mechanistic layers through which chronic NP exposure suppresses adaptive immune responses in a concentration-dependent manner. First, impaired hematopoietic regeneration: single-cell analysis of HSPCs revealed that NP exposure induces myeloid-biased hematopoiesis, a hallmark of stress-induced emergency hematopoietic reprogramming. Critically, this bias simultaneously suppresses lymphoid commitment pathways, indicated by downregulation of canonical hematopoietic transcription factors (*gata1a*, *tal1*, *lmo2*, and *spilb*) following KLH stimulation. This transcriptional reprogramming means that chronic NP exposure corrupts the developmental pathways responsible for regenerating the adaptive immune system itself, fundamentally narrowing long-term capacity for immune recovery and establishing a self-perpetuating cycle of immunodeficiency. Second, disrupted antigen presentation: NP-exposed antigen-presenting cells (APCs) present marked deficiencies at multiple functional checkpoints. Quantitative analysis revealed that NP exposure reduces KLH antigen uptake to 6.39% of control levels and dramatically suppresses the expression of co-stimulatory molecules (*cd80*, *cd83*, and *cd40*) essential for T cell priming (Sharpe and Freeman, 2002; Gong et al., 2009). This APC dysfunction represents a critical checkpoint failure wherein insufficient or absent co-stimulatory signals block naive T cell activation entirely, regardless of T cell intrinsic capacity (Lever et al., 2014). By establishing a bottleneck at the initiation of adaptive responses, impaired APC function weakens downstream immunity independent of direct T cell damage. However, the relationship between these observations remains mainly correlative. It is unclear whether the observed T-cell abnormalities arise mostly because of impaired antigen presentation or reflect direct effects of NPs on T cells. Future studies incorporating co-culture or separation-based experimental designs will be required to directly resolve this mechanism. Third, T cell transcriptional exhaustion coupled with loss of immune tolerance. T cells from NP-exposed fish exhibit a characteristic exhaustion phenotype: baseline downregulation of effector cytokines (*ifng1*, *lck*, *cd154*), reduced granzymes (*perforin*), and ablation of antigen-responsive gene upregulation. This manifests functionally as profound suppression of both CD4⁺CD154⁺ and CD8⁺CD154⁺ T cell expansion

following antigen stimulation. Concurrently, single-cell analysis revealed a selective reduction in regulatory T cells (Tregs) accompanied by a compensatory expansion of NK cell populations, indicating a disruption of immune tolerance mechanisms. The paradoxical combination, concurrent pro-inflammatory cytokine upregulation (*il1b*, *il6*, *tnfa*) with Treg suppression creates a dysbalanced inflammatory state: excessive chronic stimulation sufficient to damage tissue but insufficient to drive effective pathogen clearance. Previous studies have shown that such cytokine overproduction forms a self-amplifying loop, where NF- κ B activation induces cytokine release, which in turn further stimulates NF- κ B signaling, ultimately leading to chronic inflammation and tissue injury (Liu et al., 2018; Wang et al., 2018). Importantly, this inflammatory activation is not protective but maladaptive: persistent cytokine dysregulation is a hallmark of immunotoxicity and is frequently associated with concurrent immunosuppression (Wang et al., 2020). This apparent paradox, hyperinflammation alongside impaired immune function reflects a breakdown of immune homeostasis rather than effective immune activation.

Together, these three layers establish a unified pathological phenotype: NP-induced immunotoxicity operates through simultaneous failures in adaptive immunity regeneration, initiation, and termination in a dose-dependent manner. This reprogramming is particularly insidious because it preserves immune cell architecture while rendering immune responses non-functional, a state of “immune exhaustion” that mimics, but mechanistically differs from, immunosuppression from traditional cytotoxic agents (Blackburn et al., 2009). Furthermore, the absence of direct measurements of internal NP accumulation in kidney or hematopoietic cells limits our ability to establish a definitive link between external exposure and internal dose. Therefore, future studies should incorporate quantitative assessments of NP uptake and tissue distribution (e.g., via fluorescence labeling or spectroscopic approaches) to strengthen mechanistic understanding. Note that, for scRNA-seq, each library was generated from pooled kidneys of six fish per group to reduce inter-individual variability and ensure sufficient cell yield for single-cell profiling. However, the scRNA-seq dataset represents a single pooled biological sample per experimental group and therefore lacks independent biological replicates. To address this limitation, key single-cell findings were validated using independent experimental approaches (e.g., histological, cellular and molecular assays). Accordingly, we emphasize that the scRNA-seq data serve mainly as a high-resolution discovery dataset.

Transcriptomic pathway enrichment revealed pronounced activation of infection-associated pathways, such as “Salmonella infection” and “Herpes simplex virus 1 infection”, across multiple immune cell clusters despite the absence of microbial challenge. This paradoxical activation of pathogen-recognition programs in the absence of actual infection defines a state of sterile inflammation, in which NPs may act as DAMPs or pathogen-associated molecular pattern (PAMP) mimics (Fleshner, 2013; Zindel and Kubes, 2020). The concurrent activation of apoptosis, necroptosis, and ferroptosis gene programs further indicates that NPs impose acute cellular stress, consistent with observations in MP/NP toxicity studies (Li et al., 2023; Lu et al., 2024b). These cell death programs are closely interconnected and often co-regulated by oxidative stress and mitochondrial dysfunction. While apoptosis contributes to immune cell depletion, excessive or dysregulated cell death can also release endogenous danger signals, which act as DAMPs to further stimulate innate immune responses (Liu, et al., 2018). This combination, simultaneous activation of infection-mimetic pathways and cell death programs creates a self-reinforcing inflammatory loop: particle-induced cell damage activates pattern recognition receptors (PRRs), such as Toll-like and NOD-like receptors, and downstream NF- κ B/MAPK signaling, promoting sustained cytokine production that amplifies immune stimulation while paradoxically impairing adaptive responsiveness (Takeuchi and Akira, 2010; Carroll et al., 2024). This sterile, pseudo-infectious state represents a pathological immune phenotype distinct from either acute infection or normal homeostasis, establishing a chronic inflammatory ground state that persists as long as particles remain (Gong et al., 2020). However, future studies should incorporate direct validation of PRR activation and downstream signaling pathways to substantiate this hypothesis.

The immunotoxic effects of NPs in fish have been increasingly documented across multiple species and developmental stages, and our findings align with and substantially extend this growing body of literature. Saputra et al. showed that polystyrene nanoplastic (PS-NP) exposure in early zebrafish development induced immune toxicity characterized by dysregulated expression of immune-related genes (IL-1 β , IL-6, NF- κ B, TNF- α , TLRs), indicating inflammation and innate immune activation triggered by oxidative damage (Saputra et al., 2025). Similarly, Ahmadifar et al. reported that dietary PS-NP exposure in adult zebrafish significantly upregulated pro-inflammatory cytokines (IL-1, IFN- γ , TNF- α) by 202%, 154% and 307%, respectively, accompanied by elevated oxidative stress markers (Ahmadifar et al., 2024). These observations of NP-induced pro-inflammatory cytokine elevation are consistent with our single-cell transcriptomic findings, which revealed concurrent upregulation of IL-1 β , IL-6 and TNF- α across multiple immune cell clusters. However, our study provides an unprecedented mechanistic resolution by revealing that this pro-inflammatory state occurs paradoxically alongside severe suppression of adaptive immune responses, a finding not captured by bulk transcriptomic or cytokine-level analyses in previous studies. Beyond inflammatory signatures, our results also corroborate and extend prior work on NP-mediated hematopoietic disruption. Gou et al. reported that PS-NP exposure in zebrafish caused aberrant specification of early mesodermal hemangioblasts and cardiac progenitor cells, impairing both primitive and definitive hematopoiesis (Gou et al., 2024). More recently, a single-cell study by a separate group demonstrated that PS-NPs disrupted erythrocyte differentiation, evidenced by decreased mature erythrocytes and increased immature erythrocytes (Kwon et al., 2025). While these studies focused on embryonic erythropoiesis, our single-cell analysis of adult HSPCs revealed that chronic NP exposure induces myeloid-biased hematopoiesis while suppressing lymphoid commitment pathways, demonstrating that NP-mediated hematopoietic reprogramming persists into adulthood and compromises adaptive immune regeneration. Furthermore, our observed selective accumulation of NPs in the kidney aligns with multiple reports documenting NP bioaccumulation in zebrafish kidney tissue across independent studies, validating the kidney as a primary target organ for NP immunotoxicity (Bashirova et al., 2023).

These findings suggest potential ecological relevance. As a representative vertebrate model for aquatic species, zebrafish provide insights into how chronic NP exposure may influence immune function and hematopoiesis. The observed immune suppression and hematopoietic alterations could be associated with increased susceptibility to opportunistic pathogens and reduced resilience to environmental stressors. In natural systems, such effects may contribute to population-level consequences, including altered survival and reproductive performance, although these outcomes would depend on complex ecological contexts. Beyond aquatic organisms, the conservation of hematopoietic and immune processes across vertebrates indicates potential, but not definitive, relevance to human health. Previous studies suggest that NP exposure may influence inflammatory pathways and immune regulation in mammals, potentially affecting the balance between innate and adaptive immunity (Collins et al., 2021; Yuan et al., 2022). However, direct extrapolation to human disease remains limited and requires further investigation. In addition, NPs may act as carriers for co-contaminants such as heavy metals or organic pollutants, which could further modulate immune responses under environmentally relevant conditions. Overall, these findings highlight NPs as potential immunomodulatory stressors and underscore the need for further mechanistic and cross-species studies to better evaluate their ecological and health implications.

Collectively, our study provides an integrated, multi-level characterization of NP-induced immunotoxicity combining organismal physiology, bulk transcriptomics, and single-cell genomics. Rather than depleting immune cell populations, chronic NP exposure appears to reprogram transcriptional networks governing hematopoiesis, antigen presentation, and T cell differentiation, suggesting a state of severe immune dysregulation characterized by pseudo-infectious sterile inflammatory responses and marked adaptive immune suppression (**Fig. 8**). This mechanistic understanding underscores the urgent need for mitigation strategies targeting NP source reduction and bioaccumulation prevention, coupled with long-term surveillance studies evaluating

ecological and human health consequences of chronic environmental NP exposure.

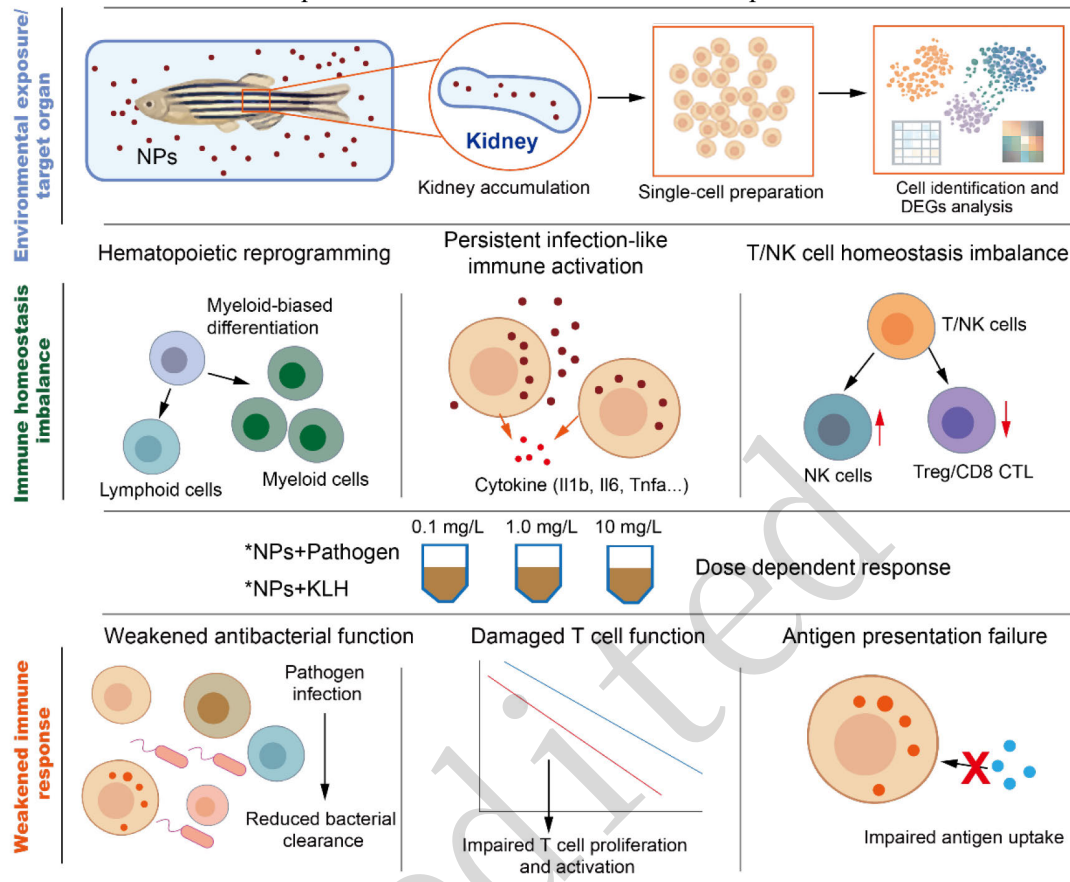


Fig. 8. Chronic exposure to polystyrene NPs (0.1-10 mg/L) causes selective kidney accumulation in adult zebrafish, reprogramming hematopoietic stem and progenitor cells toward myeloid-biased, dysfunctional states. This hematopoietic disruption, combined with impaired antigen uptake and co-stimulatory signaling in antigen-presenting cells, leads to T cell exhaustion, loss of regulatory T cells, and expansion of NK cells. As a result, adaptive immune responses to bacterial infection and antigenic stimulation are severely blunted, establishing sterile, infection-like inflammation and increased pathogen susceptibility with potential population-level consequences.

4 Conclusions

In this study, we provided a comprehensive multi-level characterization of the immunotoxic effects of chronic NPs exposure in adult zebrafish. By integrating organismal phenotyping, bulk transcriptomics, single-cell RNA sequencing, and functional immune assays, we demonstrate that prolonged exposure to polystyrene NPs induces profound immune dysfunction without markedly altering the overall composition of immune cell populations. Instead, NPs reprogram key transcriptional and cellular networks governing immune homeostasis.

Mechanistically, chronic NPs exposure preferentially accumulates in the kidney and triggers persistent sterile inflammation accompanied by activation of infection-associated and programmed cell death pathways. At the hematopoietic level, NPs drive myeloid-biased differentiation of hematopoietic stem and progenitor cells while suppressing lymphoid regenerative capacity, thereby impairing immune system renewal. Concurrently, antigen-presenting cells exhibit severely reduced antigen uptake and diminished expression of co-stimulatory

molecules, resulting in defective antigen presentation. These upstream defects are accompanied by profound adaptive immune impairment characterized by regulatory T-cell depletion, expansion of NK-cell populations, transcriptional exhaustion of T cells, and a marked loss of antigen-induced T-cell activation and proliferation. Importantly, our findings reveal that NPs-induced immunotoxicity is not simply a consequence of immune cell loss, but rather reflects a coordinated breakdown of immune regulation involving disrupted hematopoiesis, impaired antigen presentation, chronic inflammatory activation, and adaptive immune suppression. This dysfunctional immune state compromises host defense against pathogenic challenge and reduces responsiveness to antigenic stimulation.

Materials and methods

The detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

Data will be made available from the corresponding author upon request.

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Author contributions

Chongbin HU: Writing-original draft, Software, Methodology, Investigation, Formal analysis, Data curation. Qiong ZHAO: Writing-review & editing, Investigation, Methodology. Yang JIANG: Investigation, Methodology. Zhen SU: Visualization. Jing ZHENG: Methodology. Jingjun ZHOU: Methodology. Qiufen HE: Methodology. Dongdong FAN: Resources. Lixin XIANG: Resources. Hangjun ZHANG: Resources. Wei WANG, Writing-review & editing, Conceptualization. Jianzhong SHAO: Writing-review & editing, Supervision, Project administration. Ye CHEN: Writing-review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Compliance with ethics guidelines

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The study protocol was approved by the Institutional Review Board of Zhejiang University and all animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee at Zhejiang University (ZJU20241120).

Declaration on the use of generative AI tools

The authors declare that no generative artificial intelligence (AI) tools or AI-assisted technologies were used in any part of the writing, drafting, editing, or revision process of this manuscript. All content, including text, data analysis, discussions, and conclusions, is the original work of the authors. The authors take full responsibility for the authenticity, accuracy, and academic integrity of the entire manuscript.

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Supplementary information

Figs. S1-S11; Tables S1-S6; Materials and methods