



A kidney-targeted MOF nanozyme combats calcium oxalate crystal-induced kidney injury

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ABSTRACT

Calcium oxalate crystal-induced kidney injury (COC-KI) is characterized by excessive oxidative stress and inflammation. Nanozymes can partially mitigate COC-KI through superoxide dismutase (SOD)- and catalase (CAT)-like activities, but their therapeutic efficacy is often compromised by the coexisting peroxidase (POD)- and oxidase (OXD)-like activities, which may paradoxically generate reactive oxygen species (ROS). Additionally, the lack of targeted delivery remains another challenge. To overcome these limitations together, metal-organic framework (MOF)-based nanozyme PCN222-Mn (PM), which exhibits prominent SOD- and CAT-like activities with negligible POD/OXD-like activities, was chosen for the treatment of COC-KI. To achieve precise renal targeting, we functionalized PM with hyaluronic acid (HA), yielding PCN222-Mn@HA (PMH). Both *in vitro* and *in vivo* studies demonstrated that PMH effectively accumulated in injured renal tubular epithelial cells (RTECs) and provided superior protection against COC-KI. Mechanistic investigation *via* RNA sequencing revealed that PMH exerted its renoprotective effects primarily by suppressing NF- κ B signaling, which in turn downregulated the expression of NLRP3 and MCP-1, thereby inhibiting inflammasome activation and macrophage recruitment. Collectively, this work proposes a novel and effective targeted nanozyme strategy for the treatment of COC-KI.

1. Introduction

Kidney stones, clinically referred to as nephrolithiasis, represent a prevalent urological disorder worldwide. In recent years, alterations in diet, climate, and lifestyle have contributed to a rising incidence of this disease [1]. In addition to its high prevalence, nephrolithiasis is also characterized by a high recurrence rate, with up to 50% of patients experiencing a relapse within five years and 75% within two decades [2]. Among various types of kidney stones, calcium oxalate (CaOx) stones represent the most prevalent subtype [1]. The formation of CaOx kidney stones is highly complex, involving urinary supersaturation with

oxalate and calcium ions, as well as an imbalance between urinary crystal inhibitors and promoters, ultimately resulting in intratubular crystal nucleation and precipitation. Intratubular crystal deposition can induce renal tubular epithelial damage and upregulate adhesion molecules, including CD44 and osteopontin (OPN) [3]. The enhanced expression of these molecules facilitates crystal-cell adhesion and retention, further promoting crystal accumulation and aggravating renal tubular injury, ultimately contributing to calcium oxalate crystal-induced kidney injury (COC-KI) [4]. Furthermore, accumulating evidence associates COC-KI with an increased risk of systemic conditions, including hypertension, obesity, and diabetes [5,6]. Although minimally

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invasive techniques are able to remove existing stones, they do not address the underlying crystal-induced cellular injury or prevent kidney stones recurrence [7]. Potassium citrate, a common preventive agent, can partially inhibit stone formation and alleviate COC-KI; however, its prolonged use is often limited by gastrointestinal intolerance, which reduces patient adherence and compromises therapeutic efficacy [8,9]. Therefore, there is an urgent public health need for developing effective anti-lithiasis medications with favorable safety profiles.

Recently, nanozymes have emerged as a promising strategy for the treatment of cardiovascular and cerebrovascular diseases, metabolic disorders, infectious diseases, wound healing, and renal injuries [10–12]. Research has also demonstrated that CeO₂-based nanozymes can alleviate COC-KI and stone formation by scavenging excess renal reactive oxygen species (ROS) [13]. Similarly, MOF-818 nanozymes have been reported to mitigate COC-KI by suppressing oxidative stress and inflammatory responses [14]. Although these nanozymes have shown promising advances, a significant limitation exists in that some nanozymes usually possess multiple enzymatic activities such as superoxide dismutase (SOD)-, catalase (CAT)-, peroxidase (POD)- and oxidase (OXD)-like activities. When these complex catalytic reactions coexist, their pathways and outcomes could antagonize or interfere with each other, potentially leading to adverse therapeutic effects [15]. In previous studies, we found that metal-organic framework PCN222-Mn (PM), as a type of MOF nanozyme, exhibited comprehensive and potent antioxidant activities (prominent SOD- and CAT-like activities) while demonstrating minimal pro-oxidant activities (negligible POD- and OXD-like activities) [16–18]. In this study, we selected PM for COC-KI therapy based on its negligible pro-oxidant activities. To further enhance its therapeutic potential, we engineered PM to confer active

targeting capability by functionalizing it with hyaluronic acid (PCN222-Mn@HA, referred to as PMH), which could bind to CD44 receptors overexpressed on injured renal tubular epithelial cells (RTECs) [19–21].

In this study, we systematically evaluated the therapeutic efficacy of the PMH nanozyme against COC-KI using an oxalate-induced injury model. Our findings demonstrated that PMH was efficiently internalized by injured RTECs via HA-mediated targeting, and exhibited excellent intracellular ROS-scavenging ability. Both *in vitro* and *in vivo* studies confirmed that PMH provided comprehensive renal protection by alleviating oxidative stress, suppressing tubular cells apoptosis, inhibiting inflammatory responses and reducing crystal deposition. Furthermore, RNA sequencing analysis and corresponding *in vivo* studies revealed that PMH ameliorated COC-KI by inhibiting NF- κ B activation, thereby suppressing NLRP3 inflammasome activation and macrophage recruitment (Fig. 1). Overall, our study introduced a promising nanozyme-based therapeutic strategy for the treatment of COC-KI and provided mechanistic insights into PMH-mediated treatment.

2. Results and discussion

2.1. Synthesis and characterization of PM and PMH

PCN222-Mn (PM) was synthesized as previously reported [16,22–24]. Typically, the ligand TCPP-Mn was mixed with Zr cluster. The mixture was then heated at 90 °C for 5 h to obtain the raw products (Fig. 2A). The raw products were repeatedly washed with DMF to get PM for later use. The morphology of the as-synthesized PM was first examined by transmission electron microscopy (TEM) imaging (Fig. 2B), which revealed uniform nanoparticles with an average size of 98.3 ±

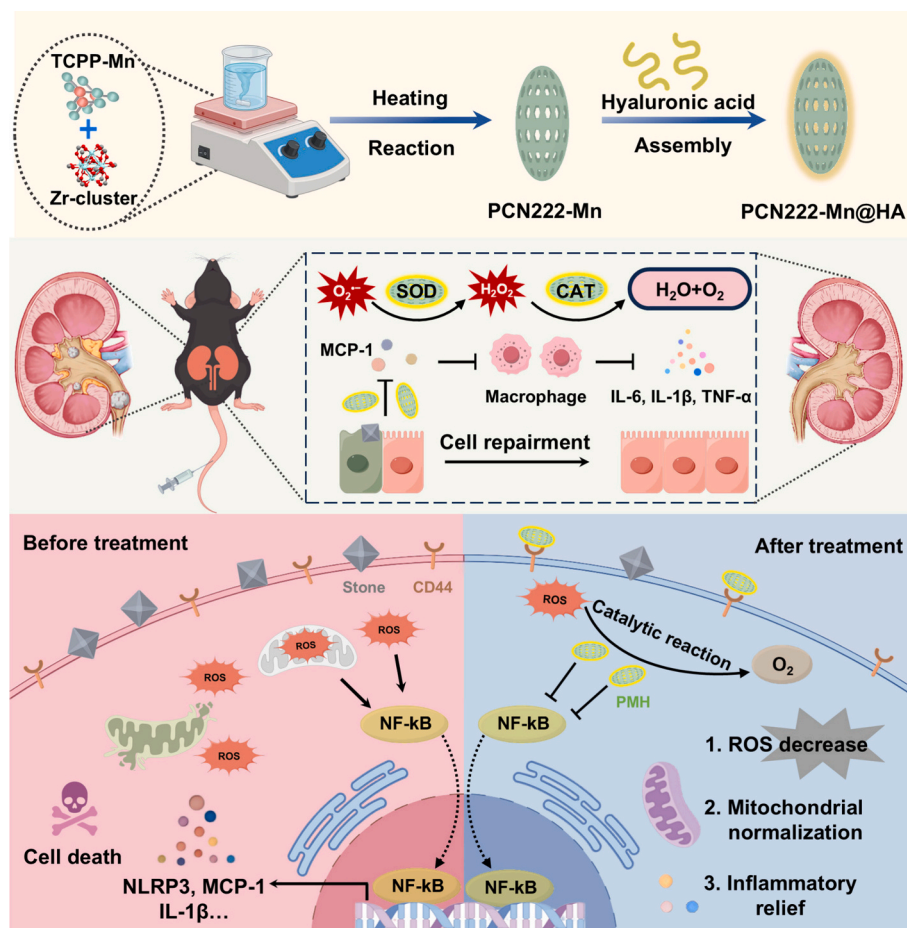


Fig. 1. Schematic illustration of the kidney-targeted nanozyme with cascade ROS-scavenging activity for treating COC-KI.

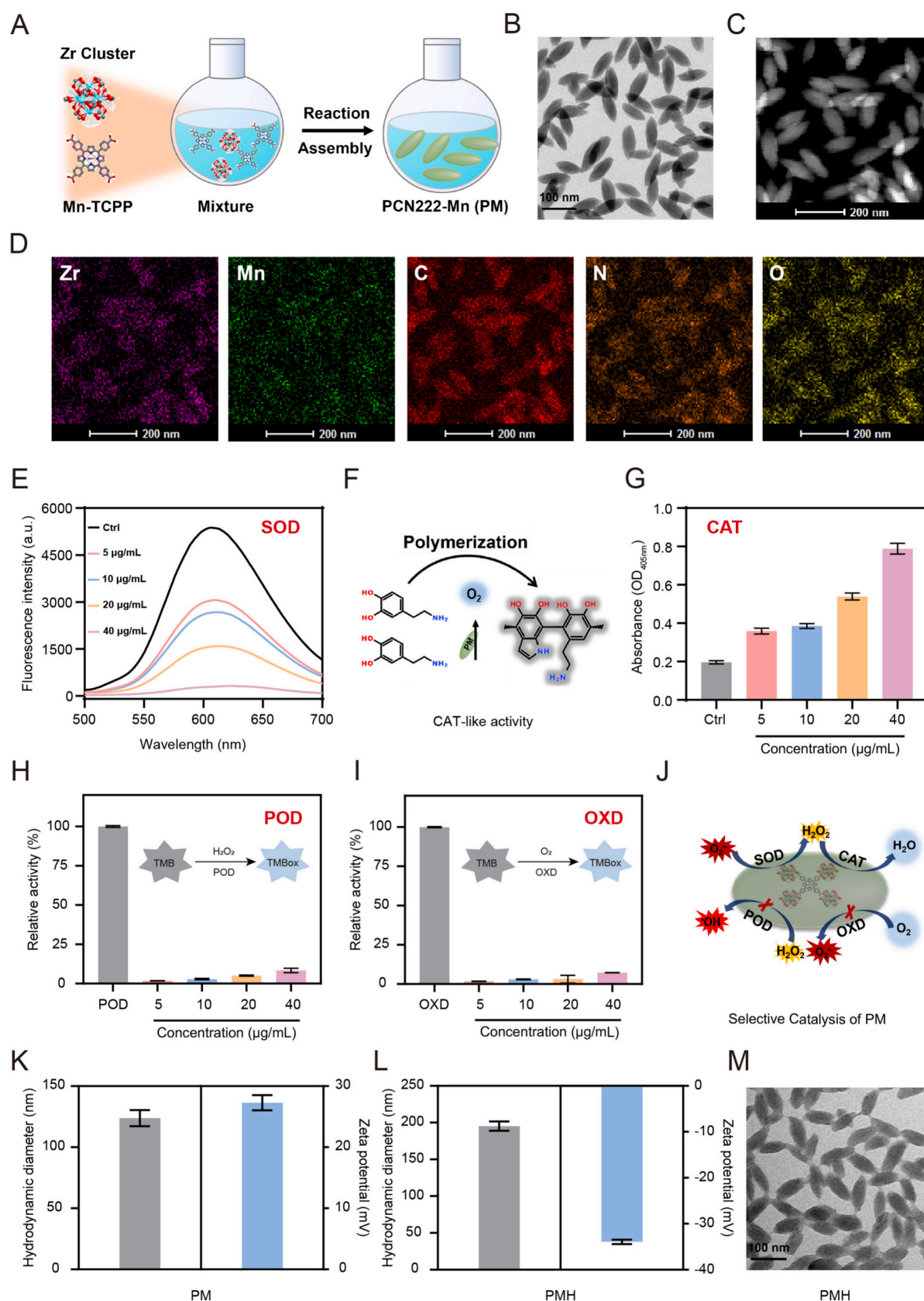


Fig. 2. Synthesis and characterization of PM and PMH. (A) Schematic illustration of the synthetic route for PM. (B) TEM image showing uniform morphology of PM nanoparticles. Scale bar: 100 nm. (C, D) HAADF-STEM image of PM and corresponding elemental mapping. Scale bar: 200 nm. (E) SOD-like activity of PM. (F) Schematic diagram of the dopamine-based CAT-like activity detection method. (G) CAT-like activity of PM. (H, I) POD- and OXD-like activities of PM. (J) Schematic of the PM-mediated ROS scavenging cascade without secondary radical production. (K, L) Zeta potential and hydrodynamic diameter of PM and PMH. (M) TEM image of PMH. Scale bar: 100 nm.

2.3 nm. Dynamic light scattering (DLS) analysis further revealed that PM exhibited low polydispersity index (PDI) values ($PDI = 0.155$), indicating its excellent colloidal dispersity and uniform size distribution (Fig. S1A). We also performed the high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) analysis (Fig. 2C–D) and the results showed that all elements were uniformly distributed. To identify the crystalline structure of PM, X-ray diffraction (XRD) analysis was performed. The pattern of PM was similar to the simulated one, further confirming the successful synthesis of PM (Fig. S2).

Next, we evaluated the enzyme-like activities of the synthesized PM, including SOD-, CAT-, POD- and OXD-like activities. The SOD-like activity was evaluated using hydroethidine (HE). PM demonstrated potent superoxide anion ($O_2^{\bullet-}$) scavenging ability in a dose-dependent manner (Fig. 2E). To further validate the SOD-like catalytic activity of PM, electron paramagnetic resonance (EPR) spectroscopy was employed to directly detect superoxide radicals using the spin-trapping method with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trap. The attenuated characteristic signal of superoxide radicals confirmed the efficient SOD-like activity of PM (Fig. S3). Since hydrogen peroxide (H_2O_2) is a product of the SOD-like reaction, the decomposition of H_2O_2 was assessed to evaluate the CAT-like activity of PM via a dopamine-based assay (Fig. 2F) [25]. In this assay, H_2O_2 is decomposed into H_2O and O_2 in the presence of substances with CAT-like activity. The generated O_2 subsequently oxidizes dopamine to dopamine quinone, which further polymerizes into polydopamine with a characteristic absorbance at 405 nm. Therefore, a higher absorbance intensity indicates stronger CAT-like activity. PM showed a pronounced CAT-like activity (Fig. 2G), indicating its ability to form a cascade reaction for both superoxide anion and H_2O_2 scavenging. Furthermore, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were also used to evaluate PM's antioxidant ability. The consistent results demonstrated that PM has great antioxidant ability in a dose-dependent manner (Fig. S4). A robust ROS scavenging ability is essential for nanozyme-based antioxidative therapy, however, some nanozymes often exhibit multiple enzyme-like activities, such as POD- and OXD-like activities, which may counteract their antioxidant effects. Herein, the POD- and OXD-like activities of PM were investigated. As shown in Fig. 2H and I, PM exhibited no detectable POD- or OXD-like activities. Collectively, these results demonstrated that PM can form a cascade reaction for ROS elimination without generating secondary radicals (Fig. 2J).

To enhance renal targeting and thereby maximize the antioxidant efficacy of PM, we functionalized PM with HA to construct a kidney-targeting nanozyme PCN222-Mn@HA (denoted as PMH). PMH was obtained by incubating PM and HA at a mass ratio of 1:10. As shown in Fig. 2K, PM exhibited a strong positive charge as determined by DLS analysis, which could thereby facilitate the adsorption of the negatively charged HA to construct PMH. Compared to PM, PMH exhibited an increased hydrodynamic diameter ($PDI = 0.198$) (Fig. 2K and S1B) and a pronounced shift in zeta potential from positive to negative (Fig. 2L). In addition, TEM imaging (Fig. 2M) revealed that PMH displayed a well-dispersed and uniform morphology. Together, these characterizations confirmed the successful construction of PMH.

2.2. CD44-mediated cellular uptake and kidney targeting

Given that CD44, a receptor for HA, has been reported to be upregulated under hyperoxaluric conditions [26], we first assessed its expression by Western blot. As expected, CD44 was significantly upregulated both in oxalate-injured HK-2 cells and the kidneys of glyoxylate (Gly)-induced mice (Fig. 3A–B and S5). Immunohistochemical (IHC) staining further confirmed the enhanced expression of CD44 in injured RTECs (Fig. S6). The upregulation of CD44 in injured RTECs provides a favorable microenvironment for HA-mediated recognition and preferential accumulation of HA-modified nanozymes in injured renal tissues.

Next, we evaluated the targeting capability of nanozymes using Cy5.5-labeled PMH, with PM as the control. Both PM and PMH exhibited time-dependent cellular accumulation (Fig. 3C and E), while PMH demonstrated significantly higher uptake than PM at all time points, which was attributed to HA-mediated targeting [27,28]. To further verify the role of HA-CD44 binding in promoting cellular targeting, we performed a competitive inhibition assay using free HA molecules [29]. Preincubation of HK-2 cells with free HA significantly reduced the cellular uptake of PMH in a concentration-dependent manner (Fig. 3D and S7), further confirming that the enhanced targeting of PMH toward injured HK-2 cells was mediated by HA-CD44 interactions.

Next, we investigated the kidney targeting ability of PMH *in vivo*. A COC-KI mouse model was established via intraperitoneal injection of Gly, and then Cy5.5-labeled PM and PMH were intravenously administered for *in vivo* imaging analysis. As shown in Fig. 3F, PMH and PM predominantly accumulated in the liver and kidneys. The accumulated fluorescence intensity of PMH showed a time-dependent profile, peaking at 12 h and remaining at a high level until 36 h, with nearly complete clearance by 96 h (Fig. S8). No significant difference was observed in hepatic accumulation between PM and PMH, while PMH exhibited significantly higher renal accumulation than PM. Consistent with the *in vitro* HA blocking assay, the renal accumulation of PMH was markedly reduced when CD44 was pre-blocked with free HA. To further evaluate the biodistribution of PMH in major organs in more detail, mice were sacrificed 12 h post-injection, and organs including heart, liver, spleen, lung, and kidneys were collected for *ex vivo* fluorescence imaging. Both PM and PMH predominantly localized in the liver and kidneys, whereas PMH demonstrated significantly higher renal retention than PM. Consistently, HA blockade substantially decreased the renal enrichment of PMH in the mouse model (Fig. 3G–H). In order to identify the specific target cells of PMH in the kidney, we co-stained RTECs with their specific marker, aquaporin-1 (AQP1) [30]. A distinct co-localization of PMH with RTECs was observed, indicating efficient uptake of PMH by RTECs (Fig. 3I). Taken together, these results demonstrated that PMH possesses precise renal targeting ability, laying a solid foundation for its targeted antioxidant effects in the kidney.

2.3. The biocompatibility and cytoprotective effects of PMH

To evaluate the biocompatibility of PMH *in vitro*, we first examined its cytotoxicity in HK-2 cells. After 24 h co-incubation with PMH, cell viability was determined by the CCK-8 assay. As shown in Fig. 4A, no significant cytotoxicity was observed even at PMH concentrations up to 160 $\mu\text{g/mL}$. Given that nonspecific interactions between nanomaterials and erythrocytes may induce hemolysis, which is a major concern for clinical translation [29], we further evaluated the hemocompatibility of PMH. A hemolysis test was performed by incubating PMH with fresh mouse erythrocytes. After centrifugation, the supernatant was visually inspected and spectrophotometrically analyzed to quantify hemoglobin release. PMH demonstrated negligible hemolysis even at a high concentration of 100 $\mu\text{g/mL}$ (Fig. S9). To further evaluate the biocompatibility of PMH *in vivo*, we assessed the structure and function of major organs in mice. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in serum were measured to evaluate hepatic function. No significant alterations in ALT or AST levels were observed either in the PM- or PMH-treated groups (Fig. S10). Furthermore, major organs including the heart, liver, spleen, and lung were collected for histopathological examination via hematoxylin and eosin (H&E) staining. No obvious pathological damage was observed following PM and PMH treatment (Fig. S11). Collectively, these findings demonstrated that both PM and PMH exhibit favorable biosafety and biocompatibility profiles *in vitro* and *in vivo*.

Previous studies have established that urinary oxalate supersaturation activates NADPH oxidase, thereby leading to ROS overload and cellular oxidative stress [31]. Concurrently, the interaction between supersaturated oxalate and calcium ions in urine promotes CaOx crystal

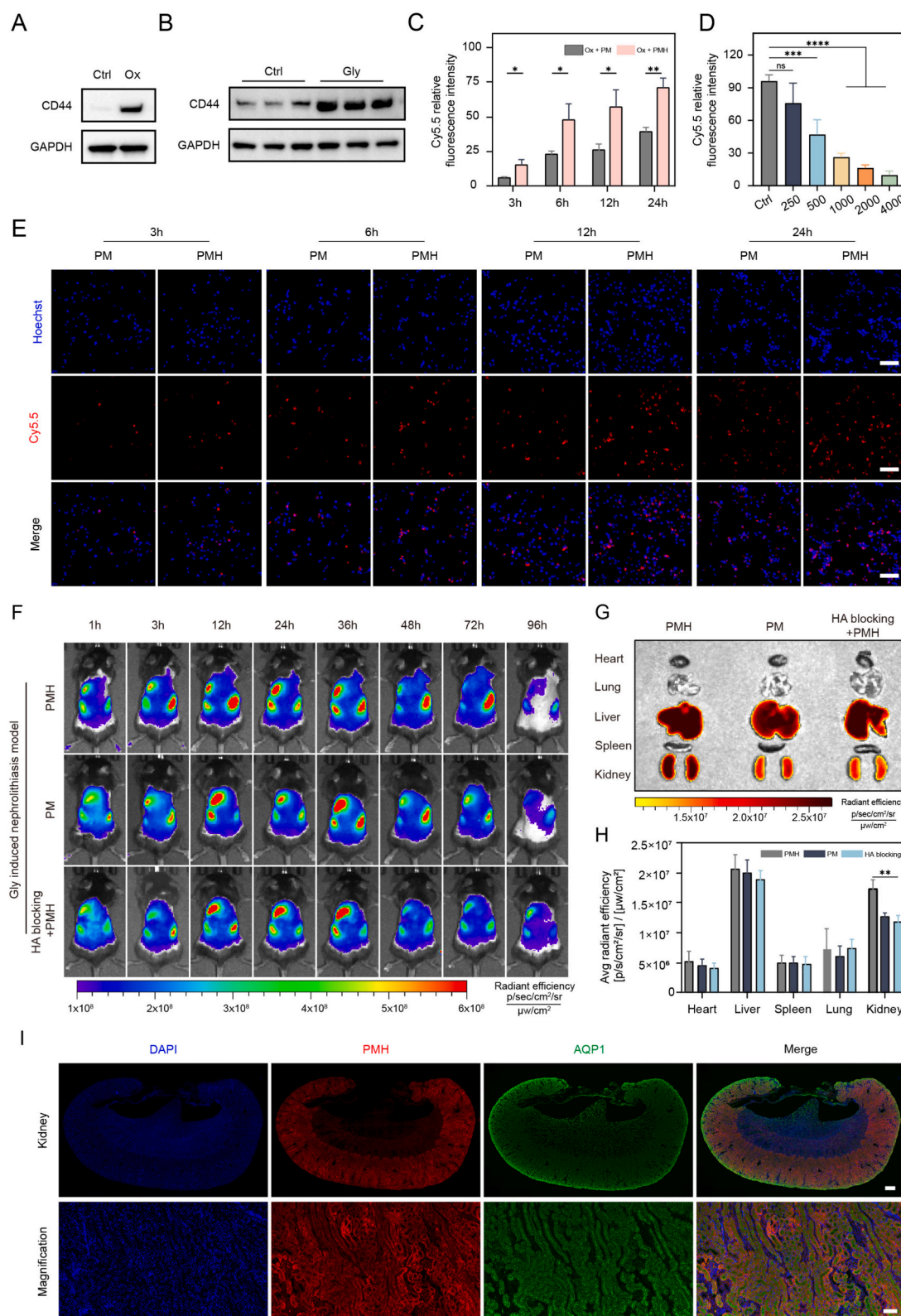


Fig. 3. Cellular uptake and renal targeting ability of PMH. (A, B) Western blot analysis of CD44 in HK-2 cells and mouse renal tissues. (C) Semi-quantitative analysis of PM and PMH internalization in oxalate-injured HK-2 cells. (D) Semi-quantitative analysis of PMH internalization at 24 h in oxalate-injured HK-2 cells pretreated with different concentrations of free HA (μg/mL). (E) Fluorescence imaging of Cy5.5-labeled PM and PMH uptake in oxalate-injured HK-2 cells. Scale bar: 50 μm. (F) Representative *in vivo* fluorescence images of Cy5.5-labeled PM and PMH at multiple time points post-intravenous injection. (G, H) Representative *ex vivo* fluorescence images and corresponding semi-quantitative analysis of major organs harvested 12 h post-intravenous injection of PM and PMH. (I) Immunofluorescence co-localization of PMH (red) with proximal tubule marker AQP1 (green) in renal sections. Scale bar: 500 μm; magnification scale bar: 100 μm. Data were presented as mean ± SD, $n = 3$.

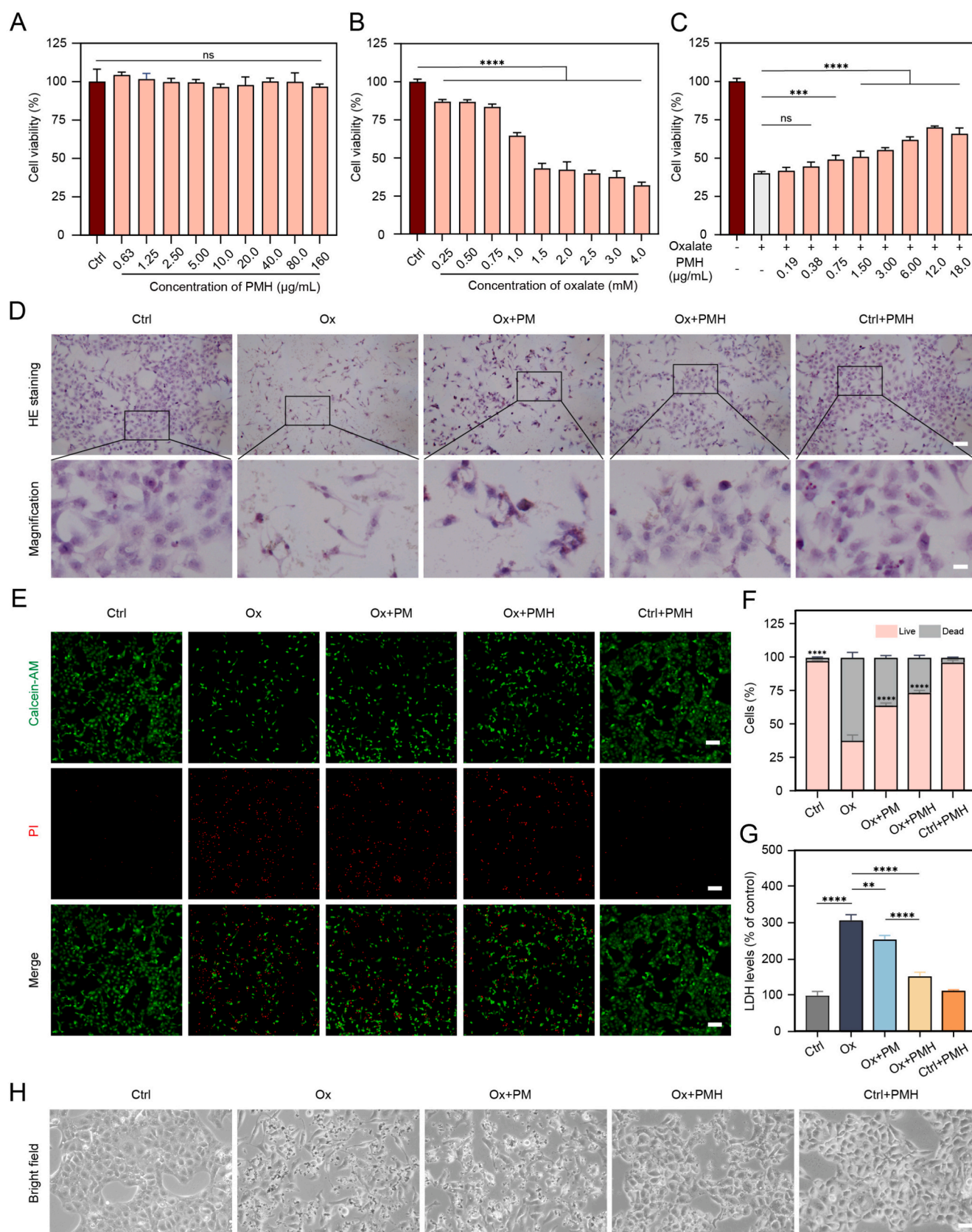


Fig. 4. Evaluation of the cytoprotective effect of PMH against oxalate-induced cytotoxicity in HK-2 cells. (A) Cell viability after 24 h treatment with PMH at different concentrations ($n = 4$). (B) Viability of HK-2 cells treated with different concentrations of oxalate for 24 h ($n = 4$). (C) PMH restored the viability of oxalate-injured HK-2 cells ($n = 4$). (D) Assessment of morphological alterations in HK-2 cells by H&E staining after various interventions ($n = 3$). 100 $\times$, Scale bar: 100 μm ; 400 $\times$, scale bar: 25 μm . (E, F) Calcein-AM/PI staining and corresponding semi-quantification ($n = 3$). Scale bar: 100 μm . (G) Quantification of LDH release from HK-2 cells under different treatments ($n = 3$). (H) Representative images of HK-2 cell-crystal adhesion. Scale bar: 50 μm . Data were presented as mean $\pm$ SD.

formation, which adheres to RTECs and exacerbates cellular injury. To simulate the high oxalate microenvironment, we established an oxalate (Ox)-induced injury model in HK-2 cells. The effect of oxalate on cell viability was evaluated to determine an appropriate concentration for injury induction. As shown in Fig. 4B, 1.5 mM oxalate elicited a moderate cytotoxic effect, consistent with previous reports [32], and this concentration was selected for subsequent experiments. Next, we investigated the protective effect of PMH *in vitro*. HK-2 cells were co-treated with oxalate and different concentrations of PMH (0.19–18 $\mu\text{g/mL}$) for 24 h, followed by a CCK-8 assay. The results showed that oxalate reduced cell viability by approximately 50%, whereas co-treatment with PMH significantly improved cell viability in a dose-dependent manner. Notably, treatment with 12 $\mu\text{g/mL}$ PMH increased cell viability to approximately 75% (Fig. 4C), thus, it was chosen for subsequent cellular experiments.

Under physiological conditions, HK-2 cells exhibited a robust, densely packed morphology. In contrast, exposure to oxalate induced significant morphological alterations, characterized by cell shrinkage and elongation of cellular processes [33]. PM treatment partially ameliorated oxalate-induced cellular alterations, while PMH administration exhibited a substantial restoration of cell density and normal architecture, demonstrating its superior therapeutic efficacy compared to PM (Fig. 4D). To further evaluate the cytoprotective effect of PMH, Calcein-AM/PI staining was performed. Both PM and PMH treatments markedly enhanced green fluorescence (representing live cells) and attenuated red fluorescence (representing dead cells), with PMH showing a better protective effect (Fig. 4E–F). Moreover, lactate dehydrogenase (LDH), a well-established marker of membrane disruption during cell death, was detected. Treatment with oxalate resulted in an approximately threefold increase in LDH release relative to the control group, while PMH administration significantly suppressed LDH release compared to PM (Fig. 4G). To investigate whether PMH-mediated protection against oxalate-induced cellular injury could further reduce crystal adhesion on RTECs, the formation and attachment of crystals on the HK-2 cell surface were examined. As shown in Fig. 4H, extensive crystal deposition was observed on the surface of HK-2 cells after 24 h of oxalate incubation. In contrast, treatment with both PM and PMH markedly reduced crystal adhesion, with PMH exhibiting a more pronounced inhibitory effect on crystal deposition. Collectively, these results demonstrated that PMH exhibits favorable biocompatibility and potent cytoprotective effects against oxalate-induced cellular damage.

2.4. PMH attenuates oxalate-induced oxidative stress injury *in vitro*

Oxidative stress is a pivotal driver of COC-KI. Supersaturated urinary oxalate promotes excessive ROS generation within RTECs, leading to oxidative damage and activation of apoptotic pathways [34]. Hence, scavenging intracellular ROS represents a rational strategy to mitigate oxidative damage and reestablish redox homeostasis. To assess the antioxidant capacity of PMH *in vitro*, intracellular ROS levels were monitored using the DCFH-DA fluorescent probe. HK-2 cells subjected to oxalate exhibited strong green fluorescence, indicating substantial ROS accumulation. Both PM and PMH treatments significantly attenuated green fluorescence intensity, with PMH at 12 $\mu\text{g/mL}$ demonstrating the most effective ROS clearance (Fig. 5A–B). ROS scavenging ability was also evaluated by flow cytometry, which yielded consistent results and further confirmed the superior ROS-scavenging ability of PMH (Fig. 5C). To further investigate the intracellular free radical scavenging ability of PMH, the levels of specific ROS, including hydroxyl radical ($\bullet\text{OH}$), H_2O_2 , and $\text{O}_2^{\bullet-}$, were subsequently examined. The results showed that oxalate stimulation markedly increased the intracellular levels of $\bullet\text{OH}$, H_2O_2 , and $\text{O}_2^{\bullet-}$ in HK-2 cells, whereas PMH treatment significantly reduced the levels of these reactive species. Notably, PMH exhibited superior ROS-scavenging efficacy compared with PM (Fig. S12A–B and S12E–F). Meanwhile, oxalate injury induced a significant increase in malondialdehyde (MDA) content in HK-2 cells compared to the control group.

After treatment with PM and PMH, MDA levels were significantly reduced, with PMH showing the greater reduction (Fig. 5D).

Mitochondria, a central regulator of cellular energy metabolism, are highly vulnerable to oxidative stress induced by oxalate [35]. Excessive ROS could disrupt the electron transport chain, leading to mitochondrial dysfunction and further promoting ROS generation, thereby forming a self-perpetuating cycle that exacerbates cellular injury [36]. To evaluate the mito-protective effects of PMH, mitochondrial superoxide production and membrane potential were examined. Both PM and PMH suppressed mitochondrial ROS production, as measured using the Mito-SOX Red probe, while PMH exhibited a stronger inhibitory effect than PM (Fig. 5E and G). Mitochondrial membrane depolarization, as an early hallmark of apoptosis, was assessed with JC-1 staining. Consistently, both PM and PMH markedly alleviated mitochondrial membrane depolarization compared to the oxalate injury group, with PMH showing a more pronounced effect than PM (Fig. 5F and H). Overall, these findings demonstrated that both nanozymes could protect HK-2 cells from oxalate-induced injury by scavenging ROS and preserving mitochondrial function, with PMH exhibiting superior efficacy.

2.5. PMH ameliorates Gly-induced crystal deposition and kidney injury *in mice*

Administration of Gly in mice is a well-established method for inducing COC-KI [37]. To explore the therapeutic potential of PMH *in vivo*, a COC-KI mouse model was established (Fig. 6A), followed by treatment with PM or PMH (intravenous injection of 5 mg/kg PM or PMH on days 8, 10, and 12). The H&E staining results showed no crystal deposition in either the control group or the group treated only with PMH. Extensive CaOx crystal deposition was observed in the Gly group under polarized light microscopy, whereas both PM- and PMH-treated groups showed a significant reduction in renal crystal deposition, with the PMH-treated group exhibiting the most pronounced effect (Fig. 6B–D). This phenomenon was further confirmed by Von Kossa staining (Fig. 6E and G). These results together verified that PMH could effectively reduce renal CaOx crystal deposition.

Gly-induced renal CaOx crystal deposition can cause severe tubular injury, characterized by tubular dilation, loss of brush borders, and cell detachment [38]. To determine whether PMH could prevent these effects, Periodic Acid-Schiff (PAS) staining was performed to assess tubular injury. Both PM and PMH treatments lessened renal tubular damage, as shown by reduced tubular dilation and restored brush border structure, with PMH providing a greater protective effect (Fig. 6F and I). Kidney injury molecule-1 (KIM-1), as a sensitive biomarker of proximal tubular damage, was also evaluated by immunofluorescence staining. PM and PMH treatments effectively downregulated KIM-1 expression, with PMH exhibiting a more pronounced reduction (Fig. 6H and J). Furthermore, serum creatinine (Scr) and blood urea nitrogen (BUN) levels, as key indicators of renal function, were evaluated. Compared to the control group, both Scr and BUN levels were significantly elevated in the Gly group. In contrast, PM and PMH treatments restored renal function, with PMH showing superior renal protective effect (Fig. 6K–L). Meanwhile, treatment with PMH alone did not result in any significant structural or functional harm to the kidney.

Taken together, the above findings indicated that both PM and PMH treatments can alleviate Gly-induced CaOx deposition and renal injury in mice. Notably, PMH exhibited superior therapeutic efficacy compared to the non-targeted PM. Moreover, PMH administration alone did not induce renal crystal deposition or tissue damage, further confirming its favorable biosafety and biocompatibility.

2.6. PMH alleviates CaOx-induced oxidative stress and apoptosis in the kidneys

Given that CaOx crystal deposition triggers severe oxidative stress in the kidneys, we further evaluated the oxidative status in the COC-KI

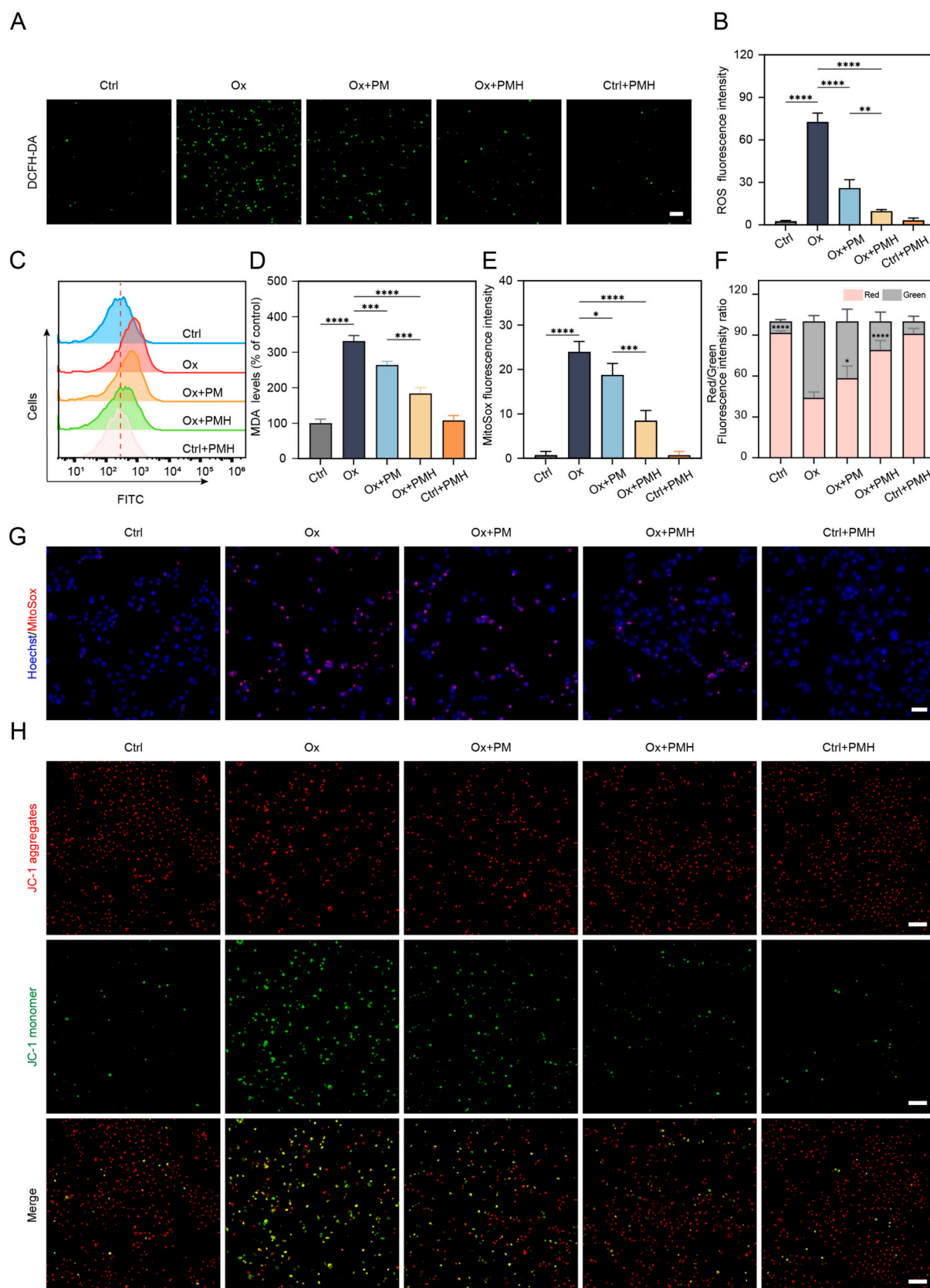


Fig. 5. PMH alleviates oxalate-induced oxidative injury and mitochondrial dysfunction in HK-2 cells. (A, B) Representative fluorescence images of intracellular ROS in HK-2 cells under different treatments and the corresponding semi-quantitative analysis. Scale bar: 100 μ m. (C) Flow cytometry analysis of intracellular ROS levels in HK-2 cells. (D) Levels of MDA in oxalate-injured HK-2 cells after treatment with PM or PMH. (E, G) Mitochondrial superoxide levels assessed by MitoSOX Red staining and corresponding semi-quantitative analysis. Scale bar: 50 μ m. (F, H) Mitochondrial membrane potential evaluated by JC-1 staining and semi-quantification of the red/green fluorescence ratio. Scale bar: 50 μ m. All data were presented as mean $\pm$ SD, $n = 3$.

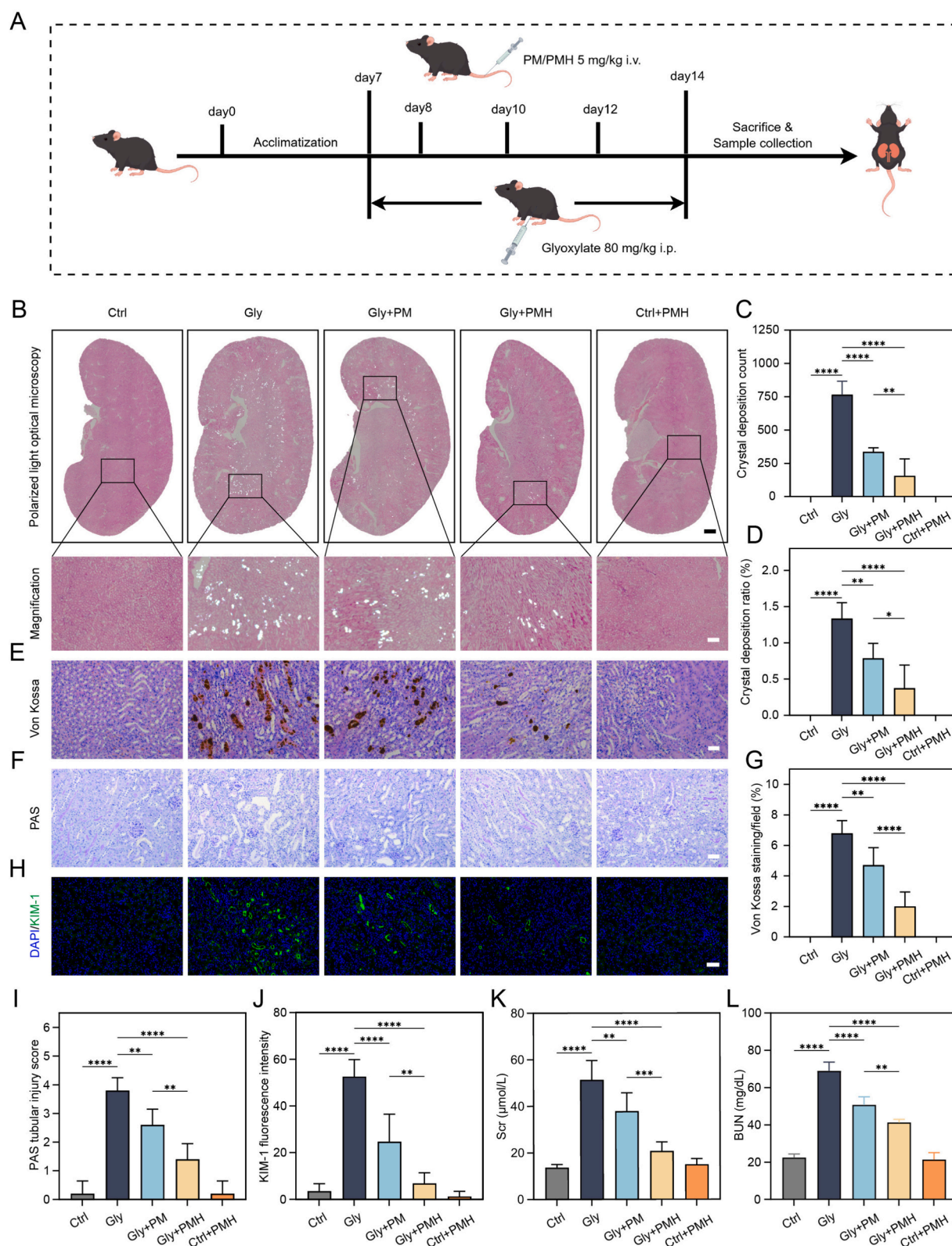


Fig. 6. PMH inhibits Gly-induced CaOx crystal deposition and kidney damage. (A) Schematic diagram of the treatment schedule in the Gly-induced COC-KI mouse model. The illustration was created with figdraw.com. (B) Detection of renal CaOx crystals by polarized light optical microscopy in different groups. Scale bar: 500 μ m; magnification scale bar: 125 μ m. (C, D) Statistical results of crystal deposition by number and total area (deposition ratio, %). (E, G) Von Kossa staining for CaOx deposition and corresponding semi-quantitative analysis of the positive area in renal tissues. Scale bar: 50 μ m. (F, I) PAS staining of kidney sections and corresponding quantification of the tubular injury score. Scale bar: 50 μ m. (H, J) KIM-1 immunofluorescence staining with semi-quantitative analysis of the fluorescence intensity. Scale bar: 50 μ m. (K, L) Assessment of renal function by measuring Scr and BUN levels. All data were presented as mean $\pm$ SD, $n = 5$.

mouse model. Kidney sections from each group were stained with dihydroethidium (DHE) to evaluate the effect of PMH on renal superoxide radical accumulation. Relative to the control group, the Gly group displayed notably stronger DHE-derived red fluorescence, suggesting that CaOx deposition triggered excessive ROS generation in renal tissue. Treatment with both PM and PMH effectively diminished this red fluorescence signal, with PMH showing a more potent inhibitory effect (Fig. 7A–B). Furthermore, the levels of specific ROS, including $\bullet\text{OH}$ and H_2O_2 , were examined in renal tissues. Compared with the control group, the Gly group exhibited significantly elevated levels of $\bullet\text{OH}$ and H_2O_2 , whereas PMH treatment markedly reduced the accumulation of these ROS (Fig. S12C–D). Notably, PMH demonstrated superior ROS-scavenging efficacy in renal tissues compared with PM. Renal oxidative stress was further assessed by measuring renal MDA levels, as well as total SOD and CAT activities. Relative to the control group, mice in the Gly group displayed markedly higher MDA levels accompanied by reduced SOD and CAT activities. Conversely, PM treatment partially normalized these changes, whereas PMH administration more effectively restored renal antioxidant capacity (Fig. 7C–E). NOX2, a superoxide-generating enzyme that promotes ROS production, was also significantly upregulated in the Gly group. In contrast, PM and PMH could effectively restore renal redox balance by suppressing NOX2 expression while upregulating SOD2 and CAT expression, with PMH showing superior efficacy (Fig. 7F–I). As sustained oxidative stress is well known to trigger apoptosis in RTECs [33], we further assessed the anti-apoptotic effect of PMH *in vivo* by TUNEL staining. Few apoptotic cells appeared in either the control or PMH-only groups. In contrast, the Gly-treated group exhibited an obvious increase in apoptotic cells. Both PM and PMH treatments reduced apoptotic cell death, with PMH exerting a more pronounced effect than PM (Fig. 7J–K).

Overall, these consistent results highlighted the strong antioxidant and anti-apoptotic properties of PMH *in vivo*, emphasizing its therapeutic potential for COC-KI.

2.7. Mechanism analysis of PMH in suppressing COC-KI by RNA sequencing

To elucidate the therapeutic mechanisms of PMH against COC-KI, kidney tissues were collected for RNA sequencing analysis. Principal component analysis (PCA) and Pearson correlation analysis demonstrated strong intra-group consistency (Fig. 8A and S13). The Gly group exhibited a distinct transcriptomic profile relative to the control group, while PMH treatment shifted the gene expression pattern toward the normal state. Differentially expressed genes (DEGs) were identified using thresholds of $|\log_2\text{FC}| > 1$ and adjusted p -value < 0.05 . A total of 2980 DEGs were detected between the control and Gly groups, and 584 DEGs between the PMH-treated and Gly groups. In particular, the expression levels of NLRP3 and MCP-1 were significantly upregulated in the Gly group and markedly downregulated following PMH treatment (Fig. 8B–C).

To further explore the therapeutic mechanism of PMH, we intersected the genes upregulated in the Gly group with those downregulated after PMH treatment (Fig. S14). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of these overlapping genes revealed significant enrichment in pathways related to inflammation, chemotaxis, and immune regulation (Fig. 8D–E), suggesting that PMH likely protected kidneys through suppression of inflammatory responses and modulation of immune processes.

Previous work has demonstrated that ROS overload in RTECs triggered by oxalate or CaOx deposition activates the NF- κB signaling cascade, which in turn upregulates key genes involved in inflammasome activation and chemotaxis [39,40]. As a key mediator of renal inflammation, the NLRP3 inflammasome assembles upon agonist stimulation. This process triggers Caspase-1 activation and the release of the pro-inflammatory cytokine IL-1 β [41]. Given the central role of NF- κB in orchestrating oxalate-induced inflammatory and immune responses

[42], we assessed whether PMH influenced this pathway and its downstream effects. Transcriptomic analysis revealed that PMH treatment significantly downregulated a core set of NF- κB pathway-related genes (Fig. 8F). A similar expression pattern was observed for key inflammasome-related genes, including NLRP3, ASC, and Caspase-1 (Fig. S15). Gene set enrichment analysis (GSEA) further demonstrated that pathways related to ROS signaling, inflammatory response, NF- κB signaling, and apoptosis were significantly activated in the Gly group and markedly attenuated following PMH treatment (Fig. 8G–I and S16).

In summary, the transcriptomic findings suggest that PMH may mitigate renal inflammation and tubular injury by scavenging excess renal ROS and modulating ROS-induced NF- κB activation, thereby reducing the transcription of key inflammasome- and chemokine-related genes.

2.8. PMH alleviates renal inflammation by suppressing inflammasome activation and macrophage infiltration *in vivo*

To further validate the anti-inflammatory effects of PMH suggested by RNA sequencing, IHC analysis of key proteins within the NF- κB /NLRP3 axis was performed *in vivo*. The IHC staining results showed that CaOx crystal deposition promoted NF- κB nuclear translocation in RTECs, together with elevated expression of inflammasome components (NLRP3, ASC, Caspase-1) and increased IL-1 β release. Both PM and PMH treatments suppressed NF- κB nuclear translocation and downregulated the expression of inflammasome-related proteins, thereby attenuating renal inflammation (Fig. 9A–F).

Previous studies have proved that damage to RTECs can trigger the secretion of MCP-1, which promotes macrophage infiltration and the subsequent release of pro-inflammatory mediators (e.g., IL-1 β , TNF- α , IL-6) [43,44]. To validate the downregulation of MCP-1 by PMH treatment, as indicated by RNA sequencing, we assessed MCP-1 expression levels through IHC staining and RT-qPCR assay. Compared to the control group, the expression level of MCP-1 was substantially elevated in the Gly group but significantly reduced by both nanozyme treatments, with PMH exerting a stronger suppressive effect (Fig. 9J–K and S17). The downregulation of MCP-1 led to a consequential reduction in macrophage recruitment, as evidenced by F4/80 immunofluorescence showing extensive infiltration in the Gly group that was attenuated by PM and more potently suppressed by PMH (Fig. 9L–M). Consistent with the reduced macrophage infiltration, the upregulated expression of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) was significantly diminished following PMH treatment (Fig. 9G–I).

Collectively, these results indicated that PMH could alleviate CaOx-induced renal inflammatory injury by modulating ROS-mediated NF- κB activation, thereby suppressing NLRP3 inflammasome activation and reducing MCP-1-driven macrophage recruitment.

3. Conclusion

In conclusion, this study developed a kidney-targeted nanozyme PMH for the effective treatment of COC-KI by ingeniously integrating the selective antioxidative capacity of PM (possessing SOD- and CAT-like activities while lacking pro-oxidant POD/OXD-like activities) with the targeting function of HA toward CD44. *In vitro* experiments confirmed enhanced cellular uptake and potent ROS-scavenging capacity of PMH in oxalate-injured HK-2 cells, accompanied by restored mitochondrial function and reduced cell death. *In vivo* assessments in a Gly-induced COC-KI mouse model showed that PMH preferentially accumulates in injured RTECs, thereby significantly reducing crystal deposition, alleviating tubular damage, and restoring renal function. Mechanistic investigations through transcriptomic analysis and *in vivo* validation indicated that PMH exerts its protective effects through modulation of ROS-mediated NF- κB signaling, thereby inhibiting NLRP3 inflammasome activation and macrophage infiltration. The targeted design of PMH confers superior therapeutic efficacy compared to the

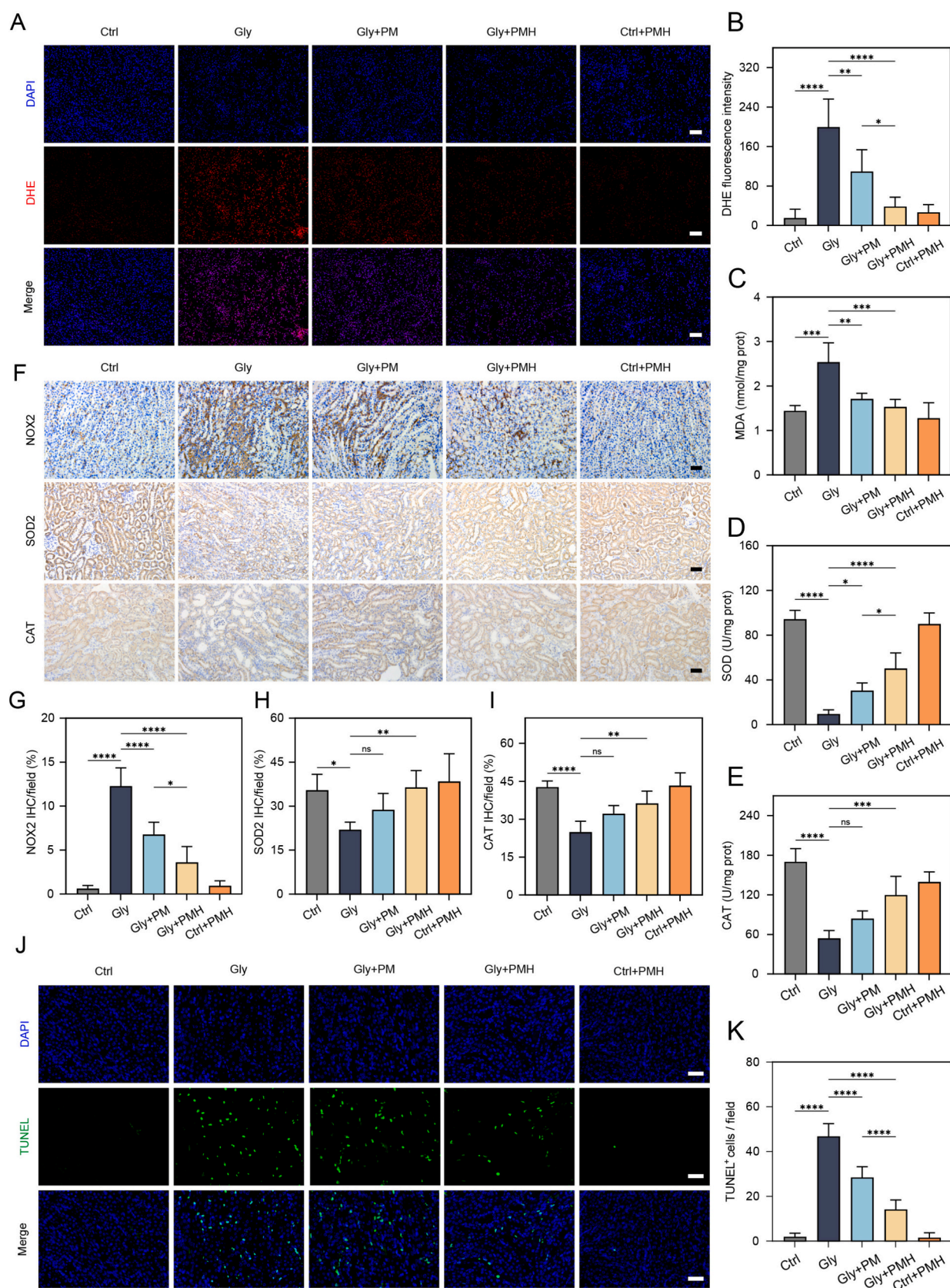


Fig. 7. PMH mitigates renal oxidative stress and apoptosis *in vivo*. (A, B) Representative fluorescence images of renal superoxide anion accumulation detected by DHE staining and corresponding semi-quantitative analysis ($n = 5$). Scale bar: 50 μ m. (C-E) MDA, SOD, and CAT levels in different groups ($n = 4$). (F) IHC staining for the expression of key redox regulators (NOX2, SOD2, and CAT) in kidney tissue from different groups ($n = 5$). Scale bar: 50 μ m. (G-I) Semi-quantitative analysis of the immunohistochemical staining shown in (F). (J, K) TUNEL staining for apoptotic cells in kidney sections and semi-quantification of TUNEL-positive cells per field ($n = 5$). Scale bar: 50 μ m. Data were presented as mean $\pm$ SD.

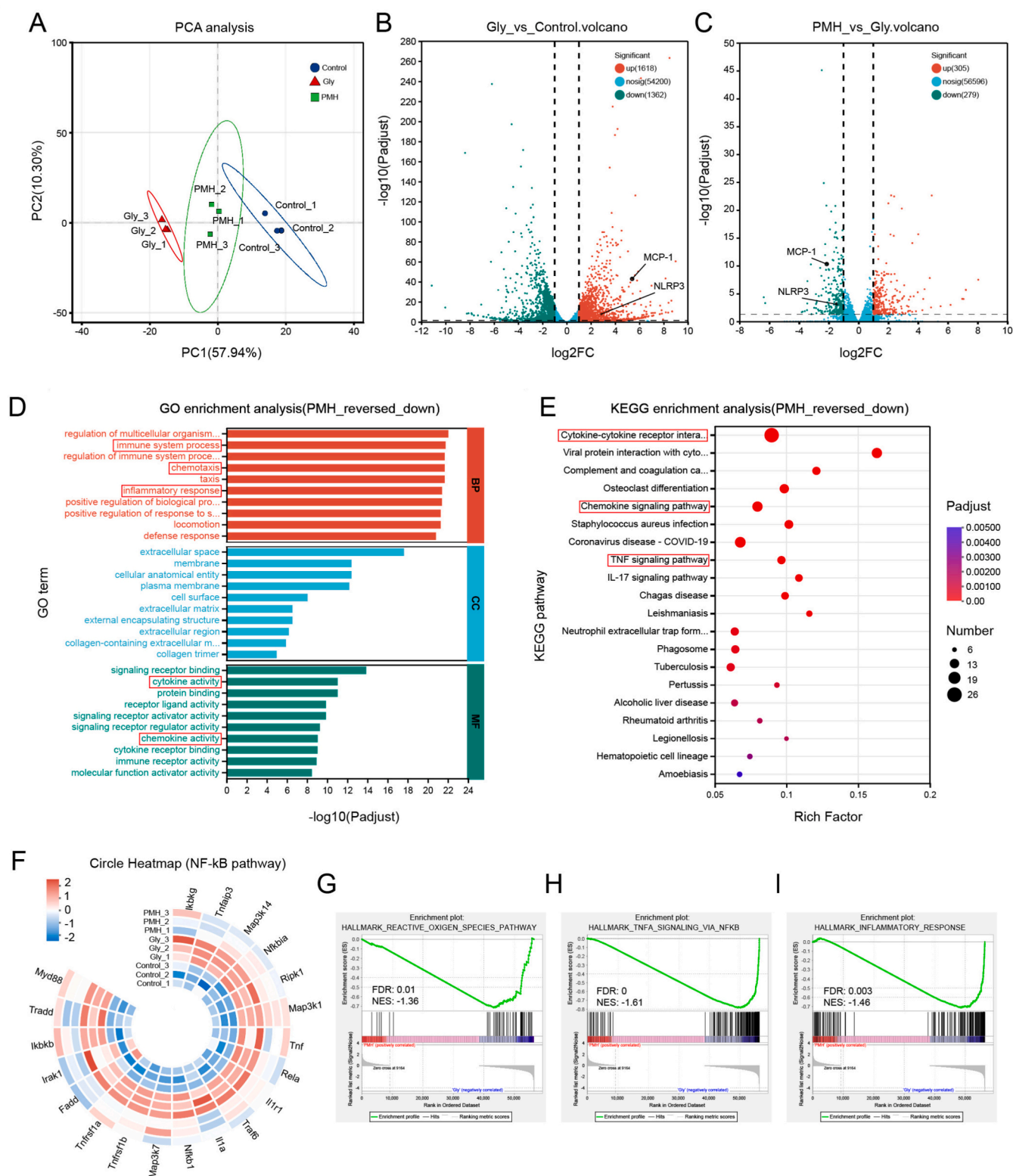


Fig. 8. RNA sequencing reveals the mechanism of PMH in alleviating CaOx crystal-induced kidney injury. (A) PCA plot of renal transcriptomes from different groups. (B) Volcano plot of DEGs between the Gly and control groups. (C) Volcano plot of DEGs between the Gly and PMH-treated groups. (D, E) GO and KEGG enrichment analyses of the overlapping DEGs reversed by PMH treatment. (F) Heatmap showing expression patterns of NF-κB signaling pathway-related genes in Control, Gly, and PMH-treated groups. (G-I) GSEA demonstrating PMH-mediated suppression of (G) reactive oxygen species, (H) NF-κB signaling, and (I) inflammatory response pathways.

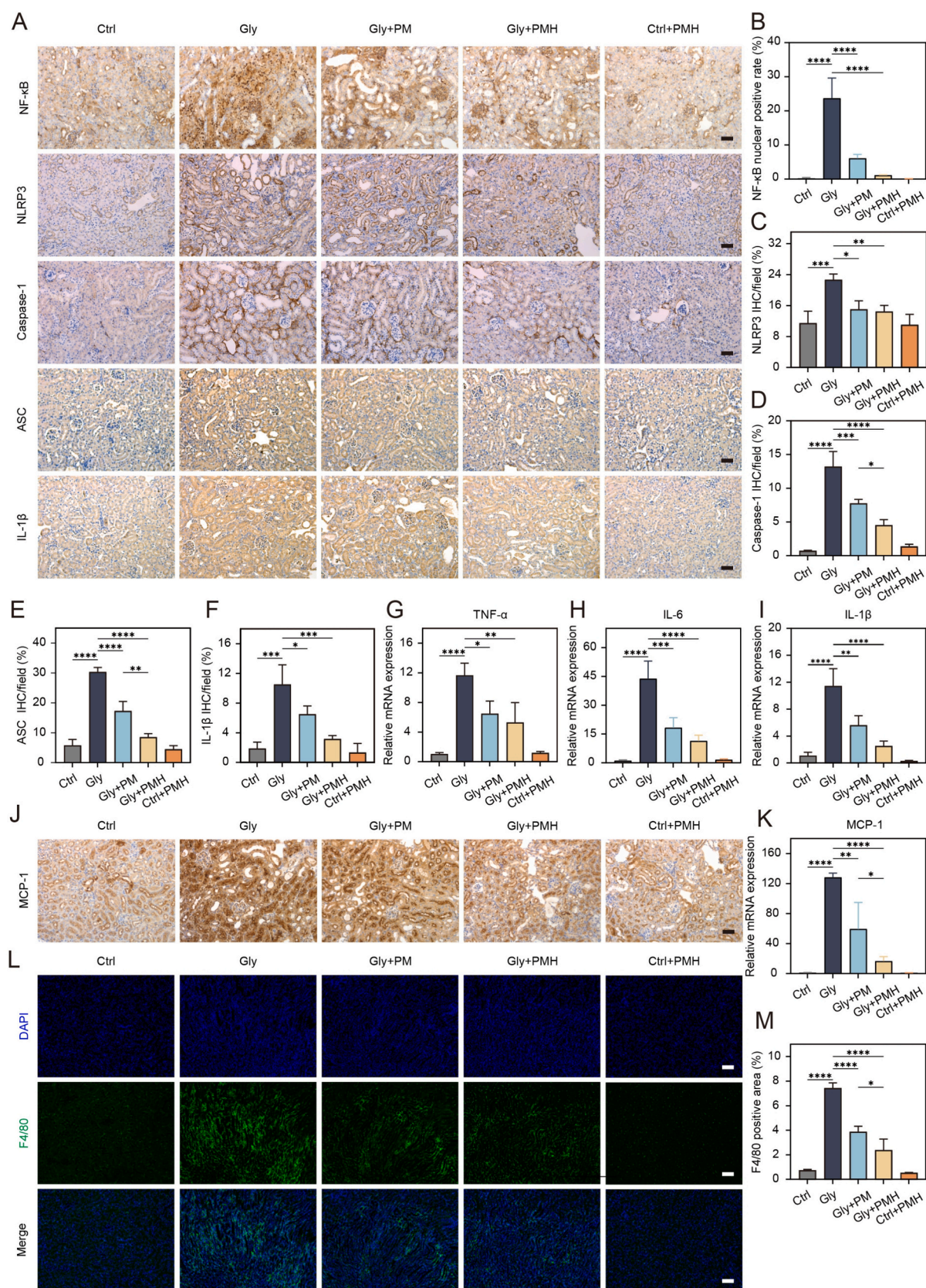


Fig. 9. PMH attenuates renal inflammation by suppressing inflammasome activation and macrophage infiltration. (A) IHC staining of renal sections showing expression of NF- κ B, NLRP3, Caspase-1, ASC, and IL-1 β . Scale bar: 50 μ m. (B) Percentage of NF- κ B positive nuclei. (C–F) Semi-quantitative analysis of the positive staining area for NLRP3, Caspase-1, ASC, and IL-1 β . (G–I) Relative mRNA expression of pro-inflammatory cytokines (TNF- α , IL-6, and IL-1 β) in renal tissues determined by RT-qPCR. (J, K) IHC staining and RT-qPCR analysis of MCP-1 in kidney tissues. Scale bar: 50 μ m. (L, M) F4/80 immunofluorescence staining with semi-quantitative analysis of the positive area. Scale bar: 100 μ m. All data were presented as mean $\pm$ SD, $n = 3$.

non-targeted PM nanozyme, while maintaining an excellent biosafety profile. Collectively, this study establishes PMH as a promising targeted therapeutic candidate for the clinical management of COC-KI.

4. Experimental section

4.1. Materials

Human proximal tubular epithelial cells (HK-2, CL-0109) were purchased from Procell (Wuhan, China). Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F-12, G4612), phosphate-buffered saline (PBS, G4202) and penicillin/streptomycin (G4003) were purchased from Servicebio (Wuhan, China). Fetal bovine serum (FBS, SA211.02) was purchased from Cellmax (Beijing, China). Sodium oxalate (S818088) was purchased from Macklin Biochemical (Shanghai, China). Glyoxylate (Gly, G10601) was purchased from Sigma-Aldrich (MO, USA). The following kits were purchased from Beyotime Biotechnology (Shanghai, China): Cell Counting Kit-8 (CCK-8, C0037), Hematoxylin and Eosin (H&E) Staining Kit (C0105M), Live/Dead cell double staining kit (C2015S), LDH assay kit (C0018S), MDA assay kit (S0131S), SOD assay kit (S0101S), JC-1 mitochondrial membrane potential assay kit (C2003S), Reactive Oxygen Species Assay Kit for Superoxide Anion with DHE (S0064S), and Mitochondrial superoxide assay kit (S0061S). 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA, HY-D0940) was purchased from MedChemExpress (MCE, USA). Anti-CD44 (85163-5-RR), Anti-HAVCR1 (30948-1-AP), Anti-CAT (21260-1-AP) were sourced from Proteintech (Wuhan, China); Anti-AQP1 (ab168387), Anti-F4/80 (ab300421) and Anti-NF- κ B (ab32536) were sourced from Abcam (Cambridge, MA, USA); Anti-SOD2 (ET1701-54), Anti-NOX2 (HA723224) were sourced from Huabio (Hangzhou, China); Anti-NLRP3 (DF7438), Anti-Caspase-1 (AF5418), Anti-ASC (DF6304), Anti-IL-1 β (AF5103), and Anti-MCP-1 (DF7577) were sourced from Affinity (Cincinnati, OH, USA).

4.2. Synthesis of PM and PMH

Typically, the synthesis of PM was performed as previously reported. TCPP-Mn (10 mg), $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ (30 mg), and benzoic acid (250 mg) were mixed in DMF (20 mL), then the mixture was reacted at 90 °C for 5 h to obtain the raw product PM. The pure PM was gained by washing the raw products with DMF several times and then stored for later use. The PMH was assembled by adding HA into PM solution. Briefly, HA (10 mg/mL, 2 mL) was added in PM solution (0.5 mg/mL, 4 mL), then the two were mixed together with ultrasound for complete assembly. The mixture was washed with water three times and then centrifuged to obtain pure PMH.

4.3. Analysis of enzyme-like activities

HE-mediated SOD-like activity analysis: Typically, xanthine (0.6 mM), xanthine oxidase (0.05 U/mL), and PM (5, 10, 20, and 40 $\mu\text{g/mL}$) were mixed in Tris-buffer (0.1 M, pH 7.4) at 37 °C for 20 min. Then, HE (0.5 mg/mL) was added to the mixture and reacted for 15 min. Finally, the fluorescence spectra were recorded for assessing the SOD-like activity (EX: 470 nm, EM: 610 nm).

DPPH-mediated radical scavenging ability analysis: Different concentrations of PM (5, 10, 20, and 40 $\mu\text{g/mL}$) were mixed with DPPH solution (0.25 mM). Then the mixture was reacted for 30 min. After reaction completion, the DPPH radical scavenging ability of PM was calculated by detecting the absorption spectra at 517 nm.

ABTS-mediated radical scavenging ability analysis: Firstly, ABTS solution was mixed with potassium persulfate to produce $\text{ABTS}^{+\bullet}$ radicals. Then different concentrations of PM (5, 10, 20, and 40 $\mu\text{g/mL}$) were added into the solution containing $\text{ABTS}^{+\bullet}$ radicals, respectively. The $\text{ABTS}^{+\bullet}$ scavenging ability of PM was recorded by the optical density value at 734 nm.

Dopamine (DA)-enabled assay for CAT-like activity analysis: PM at different concentrations (5, 10, 20, and 40 $\mu\text{g/mL}$) was mixed with DA (2 mg/mL) and 5 μL H_2O_2 (0.1 mM) in Tris-buffer (10 mM, pH 8.5). Then the solution was quickly transferred into an anaerobic device and incubated at 37 °C for 30 min. Finally, the CAT-like activity was assessed by recording the absorbance at 405 nm.

TMB-enabled assay for POD and OXD-like activities analysis: TMB was chosen as the substrate for both POD and OXD-like activities analysis. For POD-like activity, TMB (final concentration: 0.5 mM) was mixed with PM (5, 10, 20, and 40 $\mu\text{g/mL}$) in 0.2 M acetate buffer (pH 4.5), then H_2O_2 (final concentration: 0.5 mM) was supplemented. Commercial peroxidase was used as the positive control at a working concentration of 0.05 U/mL. The mixture was incubated at 37 °C for 20 min, and the POD-like activity was determined by measuring the absorbance at 652 nm. For OXD-like activity detection, the procedure was similar to POD-like activity analysis, but without adding H_2O_2 . Briefly, TMB was incubated with PM at various concentrations (5, 10, 20, and 40 $\mu\text{g/mL}$) in 0.2 M acetate buffer (pH 4.5) for 20 min, and the absorbance at 652 nm was subsequently measured. Commercial oxidase served as the positive control for analysis, with a working concentration of 0.05 U/mL.

4.4. Biosafety analysis of synthesized nanozymes

For the cytotoxicity assessment of PMH *in vitro*, HK-2 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24 h. Then the cells were treated with PMH at concentrations ranging from 0 to 160 $\mu\text{g/mL}$ for an additional 24 h. Finally, the CCK-8 assay was used to assess cell viability.

A hemolysis assay was performed to evaluate the blood compatibility of PMH. Fresh blood obtained from C57BL/6 J mice was centrifuged at 3000 rpm for 10 min at 4 °C. After removing the plasma and buffy coat, the collected erythrocytes were washed three times with PBS and resuspended to obtain a 2% (v/v) suspension. Aliquots of the erythrocyte suspension were incubated with various concentrations of PMH (1.25 to 100 $\mu\text{g/mL}$). PBS and double-distilled water (ddH_2O) served as the negative and positive controls, respectively. Following incubation at 37 °C for 1 h, the samples were centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 540 nm using a microplate reader. Then the hemolysis percentage was calculated as follows:

$$\frac{A_{\text{Sample}} - A_{\text{Negative}}}{A_{\text{Positive}} - A_{\text{Negative}}} \times 100\%$$

where A_{Sample} , A_{Negative} , and A_{Positive} represent the absorbance values of the PMH group, PBS group, and ddH_2O group, respectively.

For *in vivo* biosafety assessment, major organs including heart, liver, spleen, and lung were collected from each group. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned for H&E staining. Additionally, serum levels of ALT and AST were measured to evaluate the potential impact of PMH on hepatic function.

4.5. Cell culture and cell viability assay

HK-2 cells were cultured in DMEM/F-12 medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C with 5% CO_2 . For subsequent experiments, cells were divided into the following five groups: a) control group (Ctrl): cultured in serum-free medium; b) oxalate injury group (Ox): treated with 1.5 mM oxalate; c) PM treatment group (Ox + PM): treated with 1.5 mM oxalate and 12 $\mu\text{g/mL}$ PM; d) PMH treatment group (Ox + PMH): treated with 1.5 mM oxalate and 12 $\mu\text{g/mL}$ PMH; e) PMH control group (Ctrl + PMH): treated with 12 $\mu\text{g/mL}$ PMH alone in serum-free medium. All cells were incubated for 24 h.

For assessment of oxalate-induced cytotoxicity, HK-2 cells were seeded in a 96-well plate and cultured for 24 h. Cells were then treated with oxalate at concentrations ranging from 0.5 to 4.0 mM for an

additional 24 h. After treatment, 10 μ L CCK-8 reagent was added to each well and the plate was incubated at 37 °C for 2 h. The absorbance was measured at 450 nm using a microplate reader.

To evaluate the protective effects of PMH against cellular damage caused by oxalate, HK-2 cells were seeded in 96-well plates and categorized into three groups: a) control group: maintained in serum-free culture medium; b) oxalate-injured group: treated with 1.5 mM oxalate; c) PMH treatment group: co-incubated with 1.5 mM oxalate and PMH at concentrations ranging from 0.19 to 18 μ g/mL. After 24 h of incubation, cell viability was measured with the CCK-8 assay according to the manufacturer's instructions.

4.6. Western blot analysis

Following previously described protocols [45], collected renal tissues and cells were lysed using protein lysis buffer. After centrifugation, the supernatants were collected, and protein concentrations were determined. Equal amounts of protein samples were separated by SDS-PAGE, transferred onto membranes, blocked, and incubated with specific primary and secondary antibodies. Protein bands were visualized using an enhanced chemiluminescence (ECL) system, and the relative expression levels were quantified using ImageJ software.

4.7. Cell uptake study

HK-2 cells were seeded in 12-well plates at a density of 1×10^5 cells per well and cultured for 24 h. After attachment, oxalate-treated HK-2 cells were incubated with Cy5.5-labeled PM or PMH for 3, 6, 12, and 24 h, respectively. Following Hoechst staining and PBS washing, the fluorescence images of cells were acquired using a fluorescence microscope.

A HA competitive inhibition assay was conducted to verify CD44-mediated enhanced cellular uptake of PMH. Oxalate-injured HK-2 cells were pretreated with HA solutions of varying concentrations (250, 500, 1000, 2000, and 4000 μ g/mL) for 0.5 h prior to PMH incubation. Following 24 h of co-culture, cellular internalization of PMH was quantified by fluorescence analysis.

4.8. Influence on cell morphology by synthesized nanozymes

HK-2 cells were seeded in 6-well plates at a density of 2×10^5 cells per well. After 24 h of culture, cells were subjected to the respective treatments for an additional 24 h. Subsequently, cells were fixed with 4% paraformaldehyde and processed for H&E staining following the manufacturer's protocol. Cellular morphology was examined using a light microscope to evaluate structural alterations.

4.9. Calcein-AM/PI staining

Cells were plated in a 12-well plate and then subjected to different treatments for 24 h. Following intervention, live and dead cells were stained using the Calcein-AM/PI cell double staining kit, with images captured by fluorescence microscope.

4.10. Crystal-cell adhesion assay

After cell attachment, oxalate was added and incubated with HK-2 cells for 24 h. Following incubation, the culture medium was removed, and the cells were washed three times with Hanks' Balanced Salt Solution (HBSS) to eliminate non-adherent crystals. During each washing step, the plates were placed on an orbital shaker at 120 rpm for 3 min. Subsequently, the adhesion of crystals on the cell surface was observed using a light microscope.

4.11. Intracellular ROS detection

Briefly, HK-2 cells were loaded with DCFH-DA fluorescent probe (1:1000 dilution in serum-free medium) at 37 °C for 20 min. Following three washes with PBS to remove extra probe, intracellular ROS generation was visualized using a fluorescence microscope. Relative ROS levels were further quantified by flow cytometric analysis.

4.12. Detection of specific ROS in cells and renal tissues

To further characterize the changes in individual ROS species, the levels of $\bullet$ OH and H_2O_2 in cells and renal tissues were determined using commercial assay kits, including the Hydroxyl Radical Assay Kit (KTB1094, Abbkine) and Hydrogen Peroxide Assay Kit (KTB1041, Abbkine), according to the manufacturer's instructions.

The levels of $O_2^{\bullet-}$ in HK-2 cells and renal tissues were evaluated using dihydroethidium (DHE) fluorescence staining. Briefly, cells and frozen kidney sections were incubated with DHE staining solution according to the manufacturer's protocol. After staining, fluorescence images of cells and renal sections were captured using a fluorescence microscope.

4.13. Mitochondrial membrane potential and mitochondrial superoxide measurement

Mitochondrial membrane potential ($\Delta\psi_m$) and mitochondrial superoxide generation were assessed using JC-1 and MitoSOX Red fluorescent probes, respectively. Following experimental treatments, cells were incubated with JC-1 or MitoSOX Red working solutions at 37 °C for 30 min. After thorough washing with PBS, fluorescence signals were examined under an inverted fluorescence microscope.

4.14. COC-KI model construction

All experimental procedures were approved by the Animal Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine (Ethical code: SAHZU 2025-220). Male C57BL/6 J mice (6–8 weeks, 22–24 g) were housed under specific pathogen-free (SPF) conditions with *ad libitum* access to food and water. Following one week of acclimatization, the mice were randomly assigned to five groups ($n = 5$ per group): a) Control group (Ctrl), receiving daily intraperitoneal injections of normal saline; b) Glyoxylate model group (Gly), treated with 80 mg/kg Gly via intraperitoneal injection for 7 consecutive days; c) PM treated group (Gly + PM): administered 5 mg/kg PM via tail vein injection on days 8, 10, and 12 during Gly induction; d) PMH treated group (Gly + PMH): administered 5 mg/kg PMH via tail vein injection on days 8, 10, and 12 during Gly induction; and e) PMH control group (Ctrl + PMH): receiving 5 mg/kg PMH via tail vein injection on days 8, 10, and 12 without Gly induction. All mice were euthanized on day 14 for collection of renal tissues and blood samples. Renal tissues were fixed in 4% paraformaldehyde for subsequent histological analysis.

4.15. Renal targeting assessment

To investigate the renal targeting capability *in vivo*, Cy5.5-conjugated PM and PMH were prepared. After successful establishment of the COC-KI model, mice received intravenous administration of 5 mg/kg Cy5.5-labeled nanozymes to monitor their *in vivo* bio-distribution. For CD44 blockade assay, the HA blocking group was pretreated with free HA (5 mg/kg) via intravenous injection 0.5 h prior to PMH administration. *In vivo* imaging was performed at multiple time points (1, 3, 12, 24, 36, 48, 72, and 96 h post-injection) under anesthesia

using a small animal live imaging system (Revvity, IVIS Spectrum 2, USA).

To investigate the accumulation of nanozymes in key organs and their cellular localization, mice were euthanized 12 h after injection, and the heart, liver, spleen, lung, and kidneys were then collected for *ex vivo* imaging. Proximal tubules were identified by AQP1 immunostaining, and co-localization of PMH with AQP1-positive tubules was analyzed to verify specific targeting to RTECs.

4.16. Detection of renal crystal deposition

Renal tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Tissue sections were stained with H&E and examined under a polarized light microscope to identify birefringent crystals. To further evaluate CaOx deposition, Von Kossa staining was also employed on the kidney sections. The percentage area and number of crystal deposits in each section were quantified using ImageJ software.

4.17. Tubular injury evaluation

Tubular injury was evaluated by PAS staining. Pathological features included tubular dilation, cast formation, and epithelial cell detachment. Tubular injury was semi-quantitatively scored based on the percentage of affected tubules: 0 (none), 1 (<25%), 2 (25–50%), 3 (51–75%), and 4 (>75%).

4.18. Immunofluorescence and immunohistochemistry staining

Kidney sections were deparaffinized, rehydrated, and subjected to antigen retrieval. After blocking, sections were incubated with primary antibodies overnight at 4 °C, followed by incubation with appropriate secondary antibodies. For immunohistochemistry staining, sections were incubated with HRP-conjugated secondary antibodies and visualized using a DAB substrate. For immunofluorescence staining, fluorophore-conjugated secondary antibodies were employed, followed by DAPI staining. Stained sections were examined by bright-field microscopy for immunohistochemistry and fluorescence microscopy for immunofluorescence.

4.19. Biochemical assay

LDH release was measured using a commercial assay kit. Key oxidative stress-related parameters, including MDA levels and SOD, CAT activities were evaluated according to the manufacturer's instructions. Serum levels of Scr, BUN, ALT, and AST were quantified using an automated chemistry analyzer (Chemray 240, Rayto, China) to evaluate renal and hepatic function.

4.20. TUNEL staining

Apoptosis was evaluated using a TUNEL apoptosis detection kit according to the manufacturer's protocol. Briefly, deparaffinized sections underwent antigen retrieval and permeabilization, followed by incubation with TdT enzyme and labeling solution for 1 h at 37 °C in a humidified dark chamber. Nuclei were counterstained with DAPI. All sections were imaged under a fluorescence microscope.

4.21. RT-qPCR assay

The Ultrapure RNA Kit (CoWin Biosciences, CW0581M) was used to extract total RNA. According to the manufacturer's instructions, 1 µg of RNA from each sample was converted into cDNA using RT SuperMix for

qPCR (Servicebio, G3337). Quantitative PCR was conducted on a Roche LightCycler 480 system using the SYBR Green qPCR Master Mix (Servicebio, G3326). The primer sequences employed in this study were provided in Supplementary Table S1.

4.22. RNA sequencing

Kidney samples were collected from control, Gly-induced nephrolithiasis, and PMH-treated groups ($n = 3$ per group). After rinsing with ice-cold RNase-free PBS, tissues were snap-frozen and stored at -80°C . Total RNA was extracted and submitted to Majorbio Bio-Pharm Biotechnology Co., Ltd. (Shanghai, China) for transcriptome sequencing using the Illumina platform (San Diego, CA). Subsequent bioinformatic analysis was performed using the Majorbio Cloud platform (www.majorbio.com).

4.23. Statistical analysis

Data were analyzed using Origin 2021, GraphPad Prism 10, and ImageJ. Results were shown as mean $\pm$ standard deviation (mean $\pm$ SD). Statistical differences between groups were evaluated using Student's *t*-test for two-group comparisons and one-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test for comparisons among multiple groups. Statistical significance was defined as $P < 0.05$, and * indicated $P < 0.05$, ** indicated $P < 0.01$, *** indicated $P < 0.001$, **** indicated $P < 0.0001$, with ns indicating no significant difference.

CRediT authorship contribution statement

Xuhong He: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Min Zhou:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Zhengxin Liang:** Supervision, Methodology. **Shiyi Jin:** Supervision, Investigation. **Kunze Yu:** Data curation. **Longxiyu Meng:** Validation, Methodology. **Zhicong Tan:** Supervision, Software. **Jinming Xu:** Resources, Project administration. **Jiaju Xu:** Resources, Project administration, Methodology. **Chongjun Xiang:** Data curation. **Jingchao Wei:** Validation, Supervision. **Wenjun Gao:** Supervision, Methodology. **Jiaming Wen:** Supervision, Resources. **Hui Wei:** Writing – review & editing, Supervision, Resources. **Bohan Wang:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Ethics declarations

All animal experiments were performed in accordance with the guidelines approved by the Second Affiliated Hospital of Zhejiang University School of Medicine (Ethical code: SAHZU 2025–220).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2026.181083>.

Data availability

Data will be made available on request.

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