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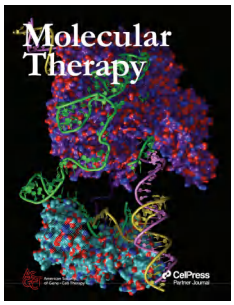

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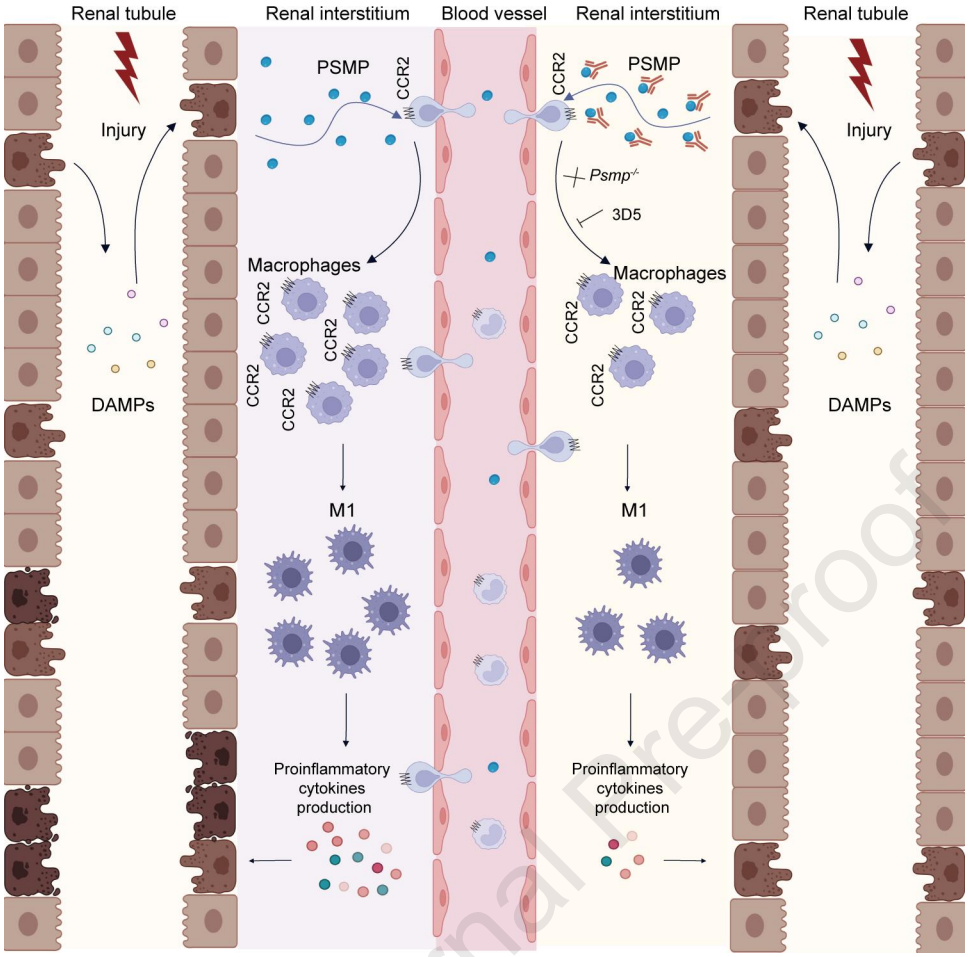

The novel potential therapeutic target PSMP/MSMP promotes acute kidney injury via CCR2

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ABSTRACT

Acute kidney injury (AKI) is a major worldwide health concern that currently lacks effective medical treatments. PSMP is a damage-induced chemotactic cytokine that acts as a ligand of CCR2 and has an unknown role in AKI. We have observed a significant increase in PSMP levels in the renal tissue, urine, and plasma of patients with AKI. PSMP deficiency improved kidney function and decreased tubular damage and inflammation in AKI mouse models induced by kidney ischemia–reperfusion injury, glycerol, and cisplatin. Single-cell RNA sequencing analysis revealed that $Ly6C^{hi}$ or $F4/80^{lo}$ infiltrated macrophages (IMs) were a major group of proinflammatory macrophages with strong CCR2 expression in AKI. We observed that PSMP deficiency decreased $CCR2^{+}Ly6C^{hi}$ or $F4/80^{lo}$ IMs and inhibited M1 polarization in the AKI mouse model. Moreover, overexpressed human PSMP in the mouse kidney, which could reverse the attenuation of kidney injury in a CCR2-dependent manner, and this effect could be achieved without CCL2 involvement. Extracellular PSMP played a crucial role, and treatment with a PSMP-neutralizing antibody significantly reduced kidney injury *in vivo*. Therefore, PSMP might be a therapeutic target for AKI, and its antibody is a promising therapeutic drug for the treatment of AKI.

INTRODUCTION

Acute kidney injury (AKI) is a clinical complication characterized by sudden renal dysfunction and is an increasing worldwide health concern.[1] AKI is associated with high morbidity, mortality, and treatment costs.[2] Furthermore, AKI also increases the development of chronic kidney disease (CKD) or end-stage renal disease and can even be life-threatening.[3,4] Unfortunately, there are no medications that can be used to stop kidney injury or promote kidney repair after injury. [5]

Inflammation is a complicated network of interactions between renal parenchymal cells and immune cells that contributes to overall renal injury and is a primary pathogenic mechanism of both AKI and CKD.[6-8] Among the immune cell populations, macrophages play a crucial role in the inflammatory response of AKI. Proinflammatory macrophages can amplify inflammation and contribute to kidney injury by releasing proinflammatory cytokines and chemokines.[9] Systemic depletion of macrophages using liposomal clodronate has been shown to mitigate early kidney injury in mice.[10-12] Targeting the recruitment and infiltration of macrophages represents a pivotal therapeutic strategy for AKI.

PC3-secreted microprotein (PSMP) or microseminoprotein (MSMP) was first found in PC3 cells and prostate cancer tissues and is expressed at low levels or not expressed in most normal tissues.[13,14] By screening candidate genes among human protein-coding genes in the human genome using peripheral blood cell chemotaxis assays, the chemotactic activity of PSMP was first discovered and identified.[14] Our previous study showed that PSMP was highly expressed in hepatocytes in human fibrotic/cirrhotic liver tissues, regardless of etiology, and exhibited low or no expression in normal human liver tissues.[15] PSMP is a damage-induced chemotactic cytokine. The damage-associated molecule pattern (DAMP) molecules IL-33 and HMGB-1 can induce mouse primary hepatocytes to produce PSMP.[15] PSMP is a ligand of CCR2 that chemoattracts peripheral blood monocytes and lymphocytes via CCR2, and the affinity of PSMP and CCR2 is comparable to that of CCL2 and CCR2.[14] The CCR2 signaling pathway has been demonstrated to play an essential role in the recruitment of monocytes to inflammatory sites.[10,16,17]

Several studies have demonstrated an association between PSMP and diseases characterized by injury, inflammation or/and fibrosis. PSMP deficiency or blockade with a PSMP-neutralizing antibody ameliorates hepatic fibrosis and DSS-induced colitis.[15,18] A study showed that PSMP

expression was considerably increased in the kidney tissue of chronic active antibody-mediated rejection (CAAMR) patients.[19] In addition, PSMP expression correlated with macrophages in CAAMR patients, and PSMP may be a histopathological diagnostic biomarker of CAAMR in kidney transplantation.[19] However, the role and function of PSMP in AKI are still unclear.

In this study, we characterized the role of PSMP in AKI patient samples and AKI mouse models. We showed that PSMP expression was considerably increased in the renal tissue, urine, and plasma of AKI patients. Then, we used PSMP-knockout mice and PSMP-neutralizing antibody to explore the physiological and pathological functions of PSMP in AKI mouse models. The results demonstrated that PSMP deficiency or blockade with a PSMP-neutralizing antibody improved kidney function and decreased tubular damage and inflammation induced by bIRI, glycerol, or cisplatin in mice. Overexpression of human PSMP (hPSMP) in the kidney demonstrated that hPSMP mediated proinflammatory and injurious effects in a CCR2-dependent manner, and these effects could be independent of CCL2 during the development of AKI. Mechanistically, our analysis through published single-cell RNA sequencing (scRNA-seq) identified $Ly6C^{hi}$ or $F4/80^{lo}$ infiltrated macrophages (IMs) as a primary proinflammatory macrophage population contributing to kidney inflammation. PSMP deficiency decreased renal $Ly6C^{hi}$ or $F4/80^{lo}$ IMs infiltration and inhibited M1 polarization in AKI. These results demonstrate that PSMP is a potential therapeutic target for AKI.

RESULTS

PSMP expression is upregulated in AKI patients and a mouse model

To investigate the relationship between PSMP and AKI, we examined PSMP expression in kidney biopsy samples obtained from AKI patients. The causes of AKI were defined as nephrotoxicity in

20 patients and ischemic injury in 7 patients. Serum creatinine levels at biopsy were 315.60±273.10 μmol/l, and the peak serum creatinine level was 664.00±446.00 μmol/l. Given the limited case numbers for these conditions, we treated all AKI patients as a single cohort for subsequent analyses. Immunohistochemical staining showed that renal PSMP expression was significantly increased in AKI patients, whereas normal control kidney sections showed almost no expression of PSMP (Figure 1A and B).

We then measured the levels of PSMP in the urine and plasma of AKI patients. The healthy control group included 8 adults (50% male and 50.58±19.04 years old). The level of PSMP in the urine of healthy controls was 2.44±1.87 ng/ml after correcting for creatinine, and the plasma level was 12.59±6.63 ng/ml. The levels of PSMP in the urine and plasma samples of AKI patients were significantly increased compared to those of healthy controls, as indicated by enzyme-linked immunosorbent assay (ELISA) (Figure 1C and D). We found a positive correlation between urine PSMP and KIM-1 (Figure 1E), suggesting an association between an increase in PSMP expression and renal tubular damage. In addition, correlation analysis showed that urine PSMP levels were positively associated with renal PSMP expression, but no significant correlation was found between plasma PSMP levels and renal PSMP expression, suggesting that urine PSMP may be excreted from kidney tissues (Figure 1F and G).

Renal PSMP expression was markedly increased in the early phase of the uIRI-induced AKI mouse model (Figure 1H and I). TUNEL staining showed that PSMP was predominantly expressed in the nonapoptotic region (Figure 1J). Furthermore, the increase in PSMP expression in the kidney was localized to lotus tetragonolobus lectin (LTL, a brush border marker)-positive cells, as shown by double immunofluorescence staining (Figure 1K). These results indicate that renal tubular epithelial cells (TECs) produced PSMP during kidney injury.

PSMP deficiency alleviates bIRI-induced AKI

We established a bIRI-induced AKI mouse model in WT and PSMP-knockout (*Psmp*$^{-/-}$) mice to explore the functions of PSMP *in vivo*. Serum creatinine and BUN were significantly decreased in *Psmp*$^{-/-}$ mice compared with WT mice, indicating improved kidney function (Figure 2A). Renal mRNA expression levels of the tubular injury marker genes *Ngal* and *Kim-1* were decreased in *Psmp*$^{-/-}$ mice (Figure 2B). PAS staining showed that *Psmp*$^{-/-}$ mice had significant reductions in intratubular cast formation, debris, and loss of the brush border, suggesting the attenuation of tubular damage (Figure 2C). Consistently, NGAL protein expression was notably decreased in *Psmp*$^{-/-}$ mice, as measured by immunohistochemical staining and western blotting (Figure 2D and E, Figure S14A). Furthermore, qPCR showed that renal mRNA expression levels of the inflammatory cytokines *Ccl2*, *Il-1β*, *Il-6*, and *Tnf-α* were reduced in *Psmp*$^{-/-}$ mice, indicating the alleviation of inflammation (Figure 2F). Renal mRNA expression of *Ccr2* was significantly decreased in *Psmp*$^{-/-}$ mice, suggesting that PSMP expression regulated the level of CCR2-positive cells (Figure 2G). These results indicate that PSMP deficiency ameliorates bIRI-induced AKI.

PSMP deficiency alleviates glycerol- and cisplatin-induced AKI

To further investigate the role of PSMP in AKI due to other causes, we used mouse models induced by glycerol and cisplatin. Following glycerol treatment, serum creatinine and BUN levels were reduced in *Psmp*$^{-/-}$ mice (Figure S1A). NGAL and KIM-1 expression analysis and PAS staining demonstrated that *Psmp*$^{-/-}$ mice had significantly reduced tubular damage (Figure S1B-E, Figure S14B and C). We observed a decrease in the mRNA expression levels of the inflammatory cytokines *Ccl2*, *Il-1β*, *Il-6*, and *Tnf-α* in *Psmp*$^{-/-}$ mice (Figure S1F). Moreover, we also observed that PSMP deficiency improved renal function, alleviated tubular damage, and decreased inflammation in cisplatin-induced AKI (Figure S2, Figure S14D). Taken together, these results

suggest that PSMP promotes the occurrence and development of AKI induced by different causes.

AAV9-hPSMP restores renal PSMP expression and promotes AKI in a CCR2-dependent manner and may be independent of CCL2

Our previous study showed that PSMP had 91.4% amino acid sequence homology between humans and mice, and both human and mouse PSMP proteins chemoattract CCR2-expressing cells *in vitro*.[14,15,18] Therefore, we investigated whether the hPSMP protein affected AKI in mice using AAV9. As expected, *Psmp*$^{-/-}$ mice that received AAV9-hPSMP had increased hPSMP expression in the kidney compared with those that received the control AAV9-null (Figure 3A, Figure S14E). Four weeks after AAV9-hPSMP injection, we established a bIRI-induced AKI mouse model. We found that hPSMP overexpression exacerbated kidney dysfunction, tubular damage, and inflammation compared to the effects observed in *Psmp*$^{-/-}$ mice injected with AAV9-null, indicating that hPSMP could aggravate AKI (Figure 3B-F, Figure S3, Figure S14E). In addition, renal mRNA expression of *Ccr2* was significantly increased after hPSMP overexpression (Figure 3G). We used PSMP- and CCR2-double-knockout (*Psmp*$^{-/-}$*Ccr2*$^{-/-}$) mice to evaluate whether CCR2 was essential for PSMP-mediated promotion AKI. The results showed that hPSMP overexpression in *Psmp*$^{-/-}$*Ccr2*$^{-/-}$ mice did not reverse the attenuation of AKI (Figure 3, Figure S3, Figure S14E).

Previous studies[20,21] and our work showed that AKI was accompanied by elevated levels of CCL2, a classic CCR2 ligand. PSMP deficiency decreased the mRNA expression of CCL2 *in vivo* (Figure 2F). We investigated the impact of PSMP on the expression of CCL2 using WT bone marrow-derived macrophages (BMDMs) *in vitro.* The results demonstrated a significant upregulation in both mRNA and protein expression levels of CCL2 following PSMP stimulation of BMDMs (Figure S4). Additionally, prior to PSMP stimulation, BMDMs were pretreated with the CCR2

antagonist RS504393, resulting in a reduction in the expression of CCL2, which indicated that PSMP regulates the expression of CCL2 through CCR2 (Figure S4). Subsequently, we used PSMP- and CCL2-double-knockout (*Psmp*$^{-/-}$*Ccl2*$^{-/-}$) mice overexpressing hPSMP to explore whether PSMP-mediated inflammation was dependent on CCL2. The results showed that hPSMP overexpression in *Psmp*$^{-/-}$*Ccl2*$^{-/-}$ mice reversed the attenuation of AKI, indicating that PSMP could promote bIRI-induced AKI independent of CCL2 (Figure S5).

Neutralization of PSMP signaling alleviates AKI

PSMP can be secreted from the cell, and markedly high expression of PSMP was detected in the plasma of AKI patients (Figure 1D). Moreover, PSMP deficiency attenuated AKI induced by different causes. Therefore, we explored whether secreted PSMP played a pivotal role in the pathogenesis of AKI. The PSMP-neutralizing antibody 3D5 can recognize and specifically neutralize PSMP *in vitro* and *in vivo*.[14,15,18] We evaluated the effects of 3D5 administered via tail vein injection before bIRI-induced AKI. As shown in Figure S6, 3D5 treatment decreased the mortality rate of mice with bIRI (ischemia time: 30 min)-induced AKI. In bIRI (ischemia time: 20 min)-induced AKI, the 3D5 treatment group exhibited significantly lower serum creatinine and BUN levels than the mIgG treatment group, indicating improved kidney function (Figure 4A). The 3D5 treatment group had decreased NGAL and KIM-1 expression and mitigated tubular damage, as evidenced by qPCR, PAS staining, immunohistochemical staining, and western blotting (Figure 4B-E, Figure S14F). The qPCR results revealed that 3D5 markedly decreased the mRNA expression levels of the inflammatory cytokines *Ccl2*, *Il-1β*, *Il-6*, and *Tnf-α* (Figure 4F). In addition, we also detected the mRNA expression level of *Ccr2*, which was decreased in the 3D5 treatment group (Figure 4G). We also investigated the protection effects of 3D5 in glycerol-induced AKI. 3D5 treatment resulted in amelioration of kidney dysfunction, attenuation of tubular

injury, and reduction of inflammation (Figure S7, Figure S14G). These results showed that blocking PSMP significantly ameliorated AKI induced by different etiologies, indicating that secreted PSMP plays a crucial role in AKI, and 3D5 could be a promising treatment for AKI.

Ly6C^{hi} or F4/80^{lo} infiltrated macrophages are proinflammatory macrophages in AKI

To characterize the function of macrophage populations in the injured kidney, we further analyzed published scRNA-seq data (GSE174324) of renal mononuclear phagocytic cell (MPC).[22] Through unsupervised clustering, these kidney MPCs were further classified into 15 macrophages based on the typical monocyte/macrophage/dendritic cell markers and the comparison with the entire gene expression based on Immunological Genome Project (ImmGen) mouse immune cell datasets. The clusters C1-C4 were defined as kidney resident macrophages (KRM), while C6-C9 were defined as Ly6C^{hi} IMs, and C12 was defined as Ly6C^{lo} IMs (Figure 5A, Figure S8, S9A, and Table S2). By utilizing the differential genes of macrophages in peripheral blood, kidney, and spleen during the steady-state period, and combining with previously published literature[22], we constructed a gene set specific to macrophages in three organs (see details in Table S3- "DEG of three organs at NC" and "Organ gene set at NC"). Then we used this dataset to analyze the macrophages in the kidneys during the acute phase of AKI, the results showed that the Ly6C^{hi} IMs in the kidney were derived from peripheral blood and almost strongly expressed CCR2 (Figure 5B, C and E). Compared to the other two clusters, Ly6C^{hi} IMs showed higher expression of inflammatory cytokine, chemokine, and chemokine receptor (Figure 5G, Figure S9B).

In addition, we also characterized the features of F4/80^{lo} and F4/80^{hi} macrophages. We employed the expression of *Adgre1* to define F4/80^{lo} and F4/80^{hi} macrophages. The clusters C1-C4 were defined as F4/80^{hi} macrophages, and the clusters C6-C9 were identified as F4/80^{lo} macrophages (Figure 5D, Figure S9A). Compared with F4/80^{hi} macrophages, F4/80^{lo} macrophages showed

higher expression of CCR2, inflammatory cytokine, chemokine, and chemokine receptor (Figure 5F, 5H, and Figure S9C).

Subsequently, we conducted an analysis of the Gene Ontology Biological Process (GOBP) of Ly6C^{hi} IMs and F4/80^{lo} macrophages high expression genes and the key regulatory transcription factors (TFs) of high expression genes (with TRRUST database) using Metascape (Figure S9D). The data revealed that the high expression genes of Ly6C^{hi} IMs and F4/80^{lo} macrophages were mainly enriched in inflammatory signaling pathways, such as "Neutrophil degradation", "Inflammatory response", "Leukocyte migration", etc. And the key regulatory transcription factors included Nfkb1, Jun, and Cebpb. Additionally, we analyzed the protein protein interaction (PPI) of Ly6C^{hi} IMs and F4/80^{lo} macrophages high expression genes in the STRING database (Figure S9E). The data showed that the high expression genes of Ly6c^{hi} IMs and F4/80^{lo} macrophages were mainly enriched in inflammatory signaling pathways, such as "Leukocyte migration", "Inflammatory response", "Cell chemotaxis", etc. These results indicate that Ly6C^{hi} or F4/80^{lo} IMs are proinflammatory macrophages in AKI.

PSMP deficiency inhibits renal proinflammatory macrophage infiltration

We next investigated renal macrophages in WT and *Psmp*$^{-/-}$ mice during bIRI- or uIRI-induced AKI by flow cytometry (Figure S10). Compared with those in WT mice, renal CD11b^{hi}Ly6C^{hi} IMs were significantly decreased in *Psmp*$^{-/-}$ mice (Figure 6A and B; Figure S11A and B). We compared CCR2^{+}Ly6C^{hi} IMs between WT and *Psmp*$^{-/-}$ mice and found a significant reduction in CCR2^{+}Ly6C^{hi} IMs in *Psmp*$^{-/-}$ mice (Figure 6C and D; Figure S11C and D). Macrophages can be identified into two subsets based on the fluorescence intensity of F4/80 and CD11b: CD11b^{hi}F4/80^{lo} IMs and CD11b^{lo}F4/80^{hi} KRM.[23,24] The data showed an increase in CD11b^{hi}F4/80^{lo} IMs and a decrease in CD11b^{lo}F4/80^{hi} KRM in bIRI- or uIRI-induced AKI (Figure

6E and F; Figure S11E). CD11b^{hi}F4/80^{lo} IMs were significantly reduced in *Psmp*$^{-/-}$ mice, while CD11b^{lo}F4/80^{hi} KRM showed no differences compared with those in WT mice (Figure 6E and F; Figure S11E).

In addition, we explored the impact of PSMP on macrophage infiltration in glycerol-induced AKI. PSMP deficiency resulted in a decrease in both the proportion and population of CD11b^{hi}Ly6C^{hi} IMs, CCR2^{+}Ly6C^{hi} IMs and CD11b^{hi}F4/80^{lo} IMs compared to WT mice (Figure S12A-C). The proportion of CD11b^{lo}F4/80^{hi} KRM was found to be increased in *Psmp*$^{-/-}$ mice compared to WT mice; however, no significant differences were observed within the population (Figure S12C). Moreover, we also observed a significant reduction in CD11b^{hi}Ly6C^{hi} IMs, CCR2^{+}Ly6C^{hi} IMs and CD11b^{hi}F4/80^{lo} IMs in *Psmp*$^{-/-}$ mice compared to WT mice following cisplatin-induced AKI (Figure S13A-C), while no differences were found in CD11b^{lo}F4/80^{hi} KRM (Figure S13C). Collectively, these findings indicate that PSMP exhibits a commonality mechanism in promoting AKI induced by different causes, primarily by facilitating the infiltration of proinflammatory macrophages.

PSMP regulates macrophage phenotypic polarization

We assessed the effect of PSMP on renal macrophage polarization *in vivo*. We used flow cytometry to detect the polarization status of kidney F4/80^{+} macrophages in mice with bIRI-induced AKI (Figure 7A). *Psmp*$^{-/-}$ mice exhibited a noticeable reduction in the proportion of M1 and increase in the proportion of M2 macrophages compared to WT mice (Figure 7 B). Moreover, we further detected the polarization status of two subtypes of macrophages: CD11b^{hi}F4/80^{lo} IMs and CD11b^{lo}F4/80^{hi} KRM (Figure 7C). PSMP deficiency significantly decreased the proportion of M1 macrophages and increased the proportion of M2 macrophages in CD11b^{hi}F4/80^{lo} IMs and CD11b^{lo}F4/80^{hi} KRM (Figure 7D and E). In addition, we measured the mRNA levels of the M1

markers *Cd86*, *Nos2*, and *Il-12*, which were also significantly decreased in *Psmp*$^{-/-}$ mice (Figure 7F).

DISCUSSION

Previously, we showed that PSMP is a damage-induced chemotactic cytokine that promotes the progression of liver fibrosis in mouse models.[15] Research has shown that PSMP expression is increased in CAAMR and is correlated with macrophages.[19] However, the function of PSMP in AKI and the mechanism remain unknown. The aim of our current study was to clarify the role of PSMP in AKI.

In this study, we examined PSMP expression in AKI patient samples. We found abundant expression of PSMP in the biopsied kidney samples of AKI patients. Multiple timepoint analysis showed a significant increase in PSMP protein expression during the early stage in the AKI mouse model, and PSMP was predominantly expressed in the TUNEL-negative area of the injured kidney. Further, through double immunofluorescence staining, we found that the increase in PSMP was localized to TECs, indicating that TECs were producing PSMP. We hypothesized that the expression of PSMP is regulated by the extracellular release of DAMPs in AKI, similar to liver injury, which requires further investigation.

To explore the effects of the increase in PSMP on AKI, we used *Psmp*$^{-/-}$ and WT mice to verify that PSMP deficiency ameliorated kidney function and alleviated tubular damage and inflammation in an AKI mouse model induced by bIRI, glycerol, and cisplatin compared with those in WT mice. More importantly, overexpressing hPSMP in *Psmp*$^{-/-}$ mice by using AAV9 vectors could reverse the attenuation of kidney injury, indicating that hPSMP could promote AKI. AKI is accompanied by increased levels of CCL2.[20,21] PSMP upregulated the expression of CCL2 in BMDMs *in vitro*. When genetic or pharmacological PSMP ablation occurred, the transcript

levels of CCL2 decreased *in vivo*. In addition, we investigated whether PSMP activity was CCL2-dependent by overexpressing hPSMP in *Psmp*$^{-/-}$*Ccl2*$^{-/-}$ mice via AAV9. The results indicated that PSMP could promote bIRI-induced AKI without CCL2 involvement.

In further clinical sample detection, we performed a retrospective association study using urine and plasma samples from AKI patients. We discovered significantly increased PSMP levels in the urine and plasma of AKI patients, and urine PSMP levels were positively correlated with urine levels of KIM-1, a tubular injury biomarker, suggesting that urine and plasma PSMP levels were related to the pathological process of AKI. Moreover, we found that urine PSMP levels in AKI patients were significantly associated with renal PSMP levels, while plasma PSMP levels showed no significant correlation with renal PSMP levels. These findings suggest that urine PSMP detection could be used to monitor kidney PSMP expression, providing a potential marker for targeted PSMP therapy and a better understanding of the role and mechanism of PSMP in kidney injury.

Previous reports have demonstrated that CCR2 deficiency ameliorates kidney injury and inflammatory infiltration in the acute phase after IRI.[17,25] Our previous study demonstrated that PSMP was a ligand of CCR2.[14] To determine whether CCR2 is required for PSMP-mediated promotion of AKI, we overexpressed hPSMP in *Psmp*$^{-/-}$ mice and *Psmp*$^{-/-}$*Ccr2*$^{-/-}$ mice and established a bIRI-induced AKI mouse model. However, the restoration of hPSMP expression in *Psmp*$^{-/-}$*Ccr2*$^{-/-}$ mice did not exacerbate kidney injury, as was observed in *Psmp*$^{-/-}$ mice, indicating that PSMP promoted AKI development in a CCR2-dependent manner.

The US Federal Drug Administration has not yet approved any drugs to treat or prevent AKI, and most clinical care has been supportive and nonspecific.[26] We created the PSMP-specific neutralizing antibody 3D5, which has significant antifibrotic effects on CCl_4-induced liver fibrosis

297 and anti-inflammatory effects on DSS-induced colitis.[15,18] Here, we observed a significant
298 reduction in the mortality rate among bIRI (ischemia time: 30 min)-induced AKI mice following
299 3D5 treatment. 3D5 significantly improved renal function, mitigated tubular damage, and
300 attenuated inflammation mice with bIRI (ischemia time: 20 min)- or glycerol-induced AKI. These
301 results indicated that PSMP, which is a secreted protein, plays a crucial role in the pathogenesis of
302 AKI and could be a therapeutic target for AKI and that its antibody is a potential treatment option
303 for AKI.

304 It is well known that proinflammatory macrophages participate in inflammation during AKI.[10]
305 scRNA-seq provides accurate information on the complete gene structure and spatiotemporal
306 expression patterns of individual cells, enabling the identification of cellular heterogeneity and
307 functional annotation. To explore the mechanism by which PSMP affects AKI, we analyzed
308 published scRNA-seq data from the AKI acute phase.[22] The analysis revealed that Ly6C^{hi} or
309 F4/80^{lo} IMs constituted major subsets of proinflammatory macrophages and that CCR2 was the
310 main receptor mediating renal injury. These findings provide support for previous studies that have
311 classified renal macrophages into distinct subsets based on the expression of Ly6C as Ly6C^{hi} and
312 Ly6C^{lo} subsets,[27] as well as based on the expression of F4/80 as F4/80^{lo} IMs and F4/80^{hi} KRM
313 subsets.[24,25] We used *Psmp*$^{-/-}$ and WT mice to identify PSMP-mediated effects on macrophages.
314 During AKI induced by different etiologies, PSMP deficiency decreased CCR2^{+}Ly6C^{hi} IMs or
315 F4/80^{lo} IMs infiltration.

316 Macrophages are a heterogeneous population, and the two main phenotypes, M1 and M2, play
317 diverse roles in mediating kidney injury and inflammation. Studies have proven that macrophages
318 can be polarized to the M1 phenotype in the early phase of kidney injury and actively participate
319 in the inflammatory response *in vitro* and in animal models.[28-31] M2 macrophages play a critical

role in resolving inflammation, promoting tissue remodeling, and facilitating recovery from AKI.[31] Our previous study showed that PSMP regulated macrophage phenotype polarization *in vivo* and *in vitro*.[18] Consistently, this study showed that PSMP deficiency inhibited M1 polarization while promoting M2 polarization. Overall, PSMP deficiency improved kidney injury by reducing proinflammatory macrophage infiltration and inhibiting M1 polarization.

In conclusion, our study demonstrated the vital role of PSMP in AKI. PSMP is expressed at low levels or not expressed in most normal tissues. We provided several lines of evidence showing that PSMP was extensively and highly expressed in AKI patients. The upregulation of PSMP, which is an upstream molecule associated with inflammatory pathogenesis, contributes to AKI in a CCR2-dependent manner, and the effect may be independent of CCL2. PSMP deficiency decreased proinflammatory macrophage infiltration and inhibited M1 polarization to alleviate AKI. A PSMP antibody reduced kidney injury *in vivo*. These findings indicate that PSMP may be a druggable target and that its antibody may be a promising therapeutic drug for AKI.

MARERIALS AND METHODS

AKI patient samples

AKI patients were from renal biopsy-AKI cross-sectional cohort during the years 2006-July 2020 in the Renal Division of Peking University First Hospital.[22] Briefly, hospitalized AKI patients with a pathological diagnosis of acute tubular injury on renal biopsy were included. The plasma and urine samples of AKI patients were collected on the day of renal biopsy. The clinical characteristics of the enrolled AKI patients were described in the previous study.[22] Normal control kidney tissues were from adjacent to renal cancer tissues (n=9). The protocol concerning the use of patient samples in this study was approved by the Biomedical Research Ethics Committee of Peking

University First Hospital (approval number: 2023[373]). All enrolled participants provided written informed consent.

Human PSMP quantification

The PSMP expression in the biopsied kidney samples of AKI patients was detected by immunohistochemical staining. Kidney samples were fixed in 10% neutral buffered formalin (Leagene) and paraffin-embedded kidney sections (4 μm). The kidney sections were deparaffinized in dimethylbenzene and hydrated in 100%I, 100%II, 95%, and 75% ethanol, and then placed in 1× phosphate-buffered saline (PBS). The prepared kidney sections used a high-pressure method to expose antigens and 3% H_2O_2 for 20 min to remove peroxidase. The sections were blocked with goat serum or 3% BSA for 40 min, then incubated with primary antibody, mouse anti-PSMP monoclonal antibody (mAb) (3D5) at 4°C overnight. Secondary antibodies (ZSGB-BIO, China) were applied and detected with a DAB kit (ZSGB-BIO, China). Subsequently, sections were briefly counterstained with hematoxylin and mounted with neutral gum. The normal control was from renal tissue adjacent to renal tumors.

Midstream samples of first voided morning urine and blood were collected from patients on the day of renal biopsy. All urine and blood samples in this study were approved by the Biomedical Research Ethics Committee of Peking University First Hospital (approval number: 2023[373]). All enrolled participants provided written informed consent. Morning urine samples from 8 healthy volunteers with informed consent were collected as healthy controls. Blood samples of 36 healthy donors with informed consent were used as healthy controls. These volunteers were from the Health Examination Center of Peking University First Hospital with normal urinalysis and blood tests and had been excluded for chronic diseases such as hypertension, cardiovascular diseases, diabetes, systemic diseases, etc. All blood and urine samples were centrifuged, and plasma and

urine supernatant were collected and stored at -80°C. The PSMP levels in the urine and plasma were measured with the human MSMP/Prostate-associated microseminoprotein ELISA Kit (EK21027, SAB) following the manufactural protocol.

Animals

Our previous study described the generation of wild-type (WT), *Psmp*$^{-/-}$, *Psmp*$^{-/-}$*Ccr2*$^{-/-}$ mice.[15] The CCL2 knockout (*Ccl2*$^{-/-}$) mice on a C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbour, USA). PSMP- and CCL2-double-knockout mice (*Psmp*$^{-/-}$*Ccl2*$^{-/-}$) were generated by crossing *Psmp*$^{-/-}$ mice with *Ccl2*$^{-/-}$ mice. Mice were housed and bred under specific-pathogen-free facility at the Peking University Health Science Center or Peking University First Hospital Animal Center. All experiments were conducted following the Guidelines for the Care and Use of Laboratory Animals and with the approval of the Ethics Committee of Peking University Health Science Center (PUIRB-LA2023285) or Peking University First Hospital Animal Center (J202134).

bIRI /unilateral IRI (uIRI) mouse models

Kidney ischemia-reperfusion injury surgery was performed as previously reported.[32] Male mice (25~27 g) were anesthetized by intraperitoneal injection of 2.5% avertin (15 ml/kg). The mouse body temperature was maintained at 37°C during the whole surgery with a temperature-controlled machine. For bIRI, both kidneys were exposed, and the renal pedicles were clamped for 30 min. In the sham group, only anesthesia and muscle incision were performed. The mice were sacrificed 24 h after surgery. For uIRI, the left back skin was cut open, and the left kidney was exposed. The renal pedicle was carefully dissected and clamped with a vascular clip for 45 min. The mice were sacrificed 0 h, 2 h, 6 h, 12 h, 24 h after surgery.

Other mouse models

For glycerol-induced AKI, male mice (28~30 g) were intramuscularly injected in each thigh caudal muscle with 50% glycerol (7.5 ml/kg body weight) (Solarbio, China) or vehicle. The mice were sacrificed 48 h after glycerol injection. For cisplatin-induced AKI, male mice (~25 g) were intraperitoneally injected with cisplatin (20 mg/kg body weight) (Sigma Aldrich, USA) or vehicle. The mice were sacrificed 72 h after cisplatin injection.

Mouse serum creatinine and blood urea nitrogen (BUN) quantification

Serum was collected from fresh mouse blood after centrifugation at 4500 rpm for 10 min. Serum creatinine and BUN were measured by assay kits following the manufactural protocols (Nanjing Jiancheng Bioengineering Institute, China).

Reverse transcription PCR and quantitative PCR (qPCR)

Total RNA was extracted and purified from frozen mouse kidney tissues using TRIzol Reagent (Life Technologies, USA). Equal RNA (2 μg) was detected using nanodrop photometric quantification (Thermo Scientific, USA), and then RNA was reverse-transcribed to cDNA using Hifair II 1st Strand cDNA Synthesis SuperMix (Yeasen Biotech, China). Real-time qPCR was performed with Hieff qPCR SYBR Green Master Mix (Yeasen Biotech, China) on an Mx3000P Real-Time PCR System (Agilent Technologies, USA), and the PCR program included 95°C for 10 min; 40 cycles of 95°C for 15 s, 60°C for 60 s, and 72°C for 30 s; 95°C for 30 s, 55°C for 30 s, and 95°C for 30 s. All gene expression levels were normalized to that of *Gapdh*. The primers used in this study are shown in Table S1.

Kidney histological analyses, immunohistochemistry, and immunofluorescence

Kidney samples were fixed in 4% neutral paraformaldehyde solution, dehydrated in 70%, 80%, 95%, 100%I, 100% II ethanol and dimethyl benzene, subsequently embedded in paraffin and cut into 4 μm sections. The sections were deparaffinized in dimethylbenzene and hydrated in 100%I, 100% II, 95%, and 75% ethanol, and then placed in 1× PBS.

For PAS staining, the prepared kidney sections were stained with PAS staining kit following the manufactural protocol (Solarbio, China). The degree of renal tubular acute injury was assessed by two renal pathologists blinded to the experimental groups. The scores were based on a 0 to 4+ scale, according to the percentage of the cortex and medullar junction region affected by the loss of renal tubular epithelial cells brush border, tubular necrosis and/or apoptosis, tubular cast, interstitial inflammation (0 = no lesion, 1+ = < 25%, 2+ = > 25 to 50%, 3+ = > 50 to 75%, 4+ = > 75 to < 100%). The sum of each score was employed to derive the ultimate tubular injury score.

For immunohistochemistry, the prepared kidney sections used a high-pressure method to expose antigens and 3% H_2O_2 for 20 min to remove peroxidase. The sections were blocked with goat serum or 3% BSA for 40 min, then incubated with primary antibody, anti-rabbit PSMP pAb or anti-goat NGAL pAb (AF1857, R&D Systems, USA) at 4°C overnight. Secondary antibodies (ZSGB-BIO, China) were applied and detected with a DAB kit (ZSGB-BIO, China). Subsequently, sections were briefly counterstained with hematoxylin and mounted with neutral gum.

For immunofluorescence, the prepared kidney sections used a high-pressure method to expose antigens, and 3% H_2O_2 to remove peroxidase. Primary antibody anti- rabbit PSMP pAb and LTL were incubated in the sections at 4°C overnight. Then, avidin-FITC was incubated in the sections at 4°C overnight. Goat anti-rabbit conjugated with Alexa Fluor 594 (ZSGB-Bio, China) was utilized as secondary antibodies for 1 h at room temperature. The sections were dyed nucleus with

DAPI (Sigma, USA) for 5 min at room temperature. Slices were mounted with Fluorescence Mounting Medium (Dako, Denmark) and imaged by BZ-X800.

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay

Kidney TUNEL staining was performed using the TUNEL Apoptosis Detection Kit (Alexa Fluor 488, Yeasen Biotech, China) following the manufactural protocol.

Western blot analysis

The samples were subjected to 15% SDS PAGE electrophoresis and transferred to PVDF membranes (Millipore Corporation, USA). The membrane was blocked with 5% BSA in TBST for 1 h at room temperature and subsequently incubated with primary antibody, anti-rabbit PSMP pAb or anti-goat NGAL pAb (AF1857, R&D Systems, USA) at 4°C overnight. Next, the membrane was incubated with IRDye 800CW donkey anti-mouse IgG, IRDye 680LT donkey anti-rabbit IgG, and IRDye 680LT donkey anti-goat IgG secondary Ab (LI-COR, USA) at room temperature for 1 h in the dark. Finally, the fluorescence intensity was detected on membranes with the LI-COR Infrared Imaging System and analyzed using Odyssey software.

Single-cell RNA sequencing data analysis

scRNA-seq data were processed as described in the published study.[22]

Flow cytometry

The kidney was cut into small fragments and incubated for 30 min at 37°C with gentle shaking in lysis solution: HBSS with 4 μg/μl collagenase type IV (Solarbio, China) and 0.5 μg/μl DNase I (Sigma-Aldrich, USA). After enzymatic digestion, cells were passed through a 70 μm nylon mesh filter and centrifuged at 2000 rpm at 4°C for 5 min to remove fragments. Then, the cell pellet was

suspended in 30% Percoll to acquire immune cells. Next, the pellet was incubated with ACK Lysing Buffer for 3 min on ice and ended with cold PBS to remove red blood cells. The prepared cells were incubated on ice with the Fc blocker (BD Biosciences, USA) for 10 min and then incubated in the dark with fluorescence-labeled antibodies for 30 min.

The antibodies used in this study included anti-mouse CD45-FITC (103108), anti-mouse CD11b-APC-Cy7 (101226), anti-mouse F4/80-PE (123109), anti-mouse F4/80-Percp-Cy5.5 (123127), anti-mouse Ly6G-PE-Cyanine7 (127616), anti-mouse Ly6C-APC (128016), anti-mouse CD206 (141705), and anti-mouse I-A/I-E (MHCII, 107613), which were purchased from BioLegend (San Diego, USA) and anti-mouse CCR2-PE (FAB5538P, R&D, USA). The cells were resuspended after unbound antibodies were removed, then cells were acquired by BD FACS Verse (BD Biosciences, USA). Data were analyzed by FlowJo software (FlowJoV10.0, TreeStar, Ashland, OR, USA).

AAV9-hPSMP construction and injection

The AAV9 delivery system that overexpresses the hPSMP gene in mouse kidneys was constructed by Vigene Bioscience (Shangdong, China). The empty associated adenovirus (AAV9-null) served as a control. Titers of the vector genome were measured by quantitative reverse-transcription PCR (qPCR) with vector-specific primers. Four-week-old male *Psmp*$^{-/-}$ mice, *Psmp*$^{-/-}$*Ccr2*$^{-/-}$ mice, and *Psmp*$^{-/-}$*Ccl2*$^{-/-}$ mice were tail vein injected with AAV9-hPSMP (1×10^{12} v.g./mice) or AAV9-null for 4 weeks before bIRI (ischemia time: 30 min)-induced AKI. The mice were sacrificed 24 h after surgery.

Mouse bone marrow-derived macrophages culture and treatment

The bone marrow cells were flushed from tibias and femurs of 8-week-old male WT mice. Red blood cells were removed by Red Blood Cell Lysis Buffer. After being washed, the cell suspension was passed through a 40 μm cell strainer and then plated in a 6-well plate at a density of 2×10^6 cells per well. The medium used for culturing the BMDMs consisted of high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin (PS), and 20 ng/mL macrophage colony-stimulating factor (M-CSF, ProteinTech, USA). The BMDMs were incubated under these conditions for 7 days and the culture medium was replaced every 3 days. To investigate the effect of PSMP on CCL2 expression, BMDMs were treatment with either fresh culture medium (as a control), or PSMP (300 ng/ml), or lipopolysaccharide (LPS, 100 ng/ml, Sigma-Aldrich, USA), or PSMP (300 ng/ml) + LPS (100 ng/ml), or pretreated with 3 μM RS 504393 (CCR2 antagonist, Sigma-Aldrich, USA) for 30 min. The cells were collected at 4 h for qPCR and the cellular supernatants were collected at 24 h for CBA.

Cytometric bead assay (CBA)

The CCL2 protein levels in cellular supernatants were quantified using a customized LEGENDplexTM mouse inflammation panel (BioLegend, USA) following the manufacturer's instructions. The data were acquired using a BD FACS Verse (BD Biosciences, USA) and analyzed with official online analysis software (https://legendplex.qognit.com). The concentration of CCL2 was determined by means of a standard curve generated during the performance of the assay.

The preparation of rabbit anti-PSMP polyclonal antibody (pAb) and mouse anti-PSMP mAb (3D5)

Rabbits and mice were immunized using the PSMP prokaryotic protein. The pAbs were purified from rabbit serum by PSMP peptide coupled to Sepharose 4B. The monoclonal hybridoma cell lines to PSMP were injected into the mouse abdominal cavity, and ascites were obtained. The mAbs were purified by protein-G.

Antibody treatments

Six-week-old male WT mice were tail vein injected with 10 mg/kg 3D5 or mIgG for 24 h before bIRI (ischemia time: 30min or 20 min)-induced AKI. The mice were sacrificed 24 h after surgery.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.2 software (GraphPad Software, USA). Sample exclusion was performed for data analyses based on identifying outliers (ROUT method) of GraphPad Prism software. Differences between the two groups were compared using Student's *t* test. Differences between multiple groups were determined using ordinary one-way ANOVA with Dunnett's multiple comparisons test or Tukey's multiple comparisons test, and two-way ANOVA with Sidak's multiple comparisons test. Pearson correlation analysis was used to assess the correlation between two variables. Comparison of Survival Curves was determined by Log-rank (Mantel-Cox) test. All data are expressed as the mean ± standard error of the mean (SEM), $P < 0.05$ was considered statistically significant.

DATA AND CODE AVAILABILITY

The scRNA-seq data used in this study have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GSE174324) and can be accessed at https://ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE174324.

KEYWORDS

Acute kidney injury; Anti-PSMP antibody; CCR2; Macrophages; inflammation

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AUTHOR CONTRIBUTIONS

Y.W., L.Y., Z.M.S., and W.J.Y. conceived the project, designed the experiments. Z.M.S. performed most of the experiments and analysis. W.J.Y performed clinical samples collection and analysis, and scRNA-seq analysis. X.K.W., Y.Q.M., Z.T.L., Q.Q.L., H.Y.H., N.L., Z.H.Z., P.L., and Y.Z. contributed to some experiments. L.J. and H.W. assessed tissue histology. Z.M.S. and W.J.Y. drafted the manuscript. Y.W. and L.Y. reviewed and edited the manuscript. All authors have read, verified, and approved this manuscript.

DECLATATION OF INTERESTS

Y.W., Z.M.S., Z.T.L., and Q.Q.L. are co-inventors on a patent covering PSMP antagonists for use in treatment of diseases.

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FIGURE LEGENDS

Figure 1. **PSMP is highly expressed in patients and mice with AKI.**

(A) Representative immunohistochemical staining of PSMP in normal and AKI patient kidneys. Scale bars, 20 μm. (B) Statistical analysis of PSMP expression levels in normal kidneys and AKI patient kidneys (NC, $n = 9$; AKI, $n = 34$). (C) Urine PSMP levels were measured by ELISA in HC ($n = 8$) and AKI patients ($n = 21$) and were corrected for creatinine. (D) Plasma PSMP levels were measured by ELISA in HC ($n = 36$) and AKI patients ($n = 36$). (E) Correlation analysis of urine KIM-1 and PSMP levels in AKI patients ($n = 16$). (F) Correlation analysis of kidney PSMP levels and the urine PSMP/creatinine in AKI patients ($n = 21$). (G) Correlation analysis of kidney PSMP and plasma PSMP levels in AKI patients ($n = 34$). (H and I) Representative immunohistochemical staining of PSMP in the kidney in uIRI-induced AKI and the statistical analysis ($n = 3$). Scale bars, 50 μm. (J) Representative immunofluorescence images of PSMP (red) and TUNEL (green) staining in uIRI-induced AKI. Scale bars, 20 μm. (K) Representative immunofluorescence images of LTL (green) and PSMP (red) in uIRI-induced AKI. Scale bars, 20 μm. The data are presented as the mean ± SEM. Statistical significance was determined by the Student's *t* test (B-D), Pearson correlation analysis (E-G), and one-way ANOVA (I). $*P<0.05$; $**P<0.01$; $****P<0.0001$. NC, normal control; HC, healthy control.

Figure 2. PSMP deficiency inhibits bIRI-induced AKI in mice.

(A) Serum creatinine and BUN were measured. (B) Renal mRNA expression levels of the tubular damage markers *Ngal* and *Kim-1* were measured by qPCR. (C) Representative histologic images of kidney sections stained with PAS, and tubular injury score. (D) NGAL protein expression in the

kidney was determined by immunohistochemical staining and statistical analysis. (E) NGAL protein expression in the kidney was determined by western blotting and statistical analysis (n = 3). (F) The expression of renal proinflammatory cytokine genes, including *Ccl2*, *Il-1β*, *Il-6*, and *Tnf-α,* was measured by qPCR. (G) *Ccr2* mRNA expression in the kidney was measured by qPCR. Scale bars, 50 μm. n = 5 for each group. The data are presented as the mean ± SEM. Statistical significance was determined by two-way ANOVA. **P<0.01; ***P<0.001; ****P<0.0001.

Figure 3. Effect of AAV9 vector-mediated hPSMP overexpression on bIRI-induced AKI in mice.

(A) The expression of hPSMP and NGAL in the kidney was measured by western blotting and statistical analysis (n = 3). (B) Serum creatinine and BUN were measured. (C) Renal mRNA expression levels of the tubular damage markers *Ngal* and *Kim-1* were measured by qPCR. (D) Representative histologic images of kidney sections stained with PAS. (E) NGAL protein expression in the kidney was determined by immunohistochemical staining. (F) The expression of renal proinflammatory cytokine genes, including *Ccl2*, *Il-1β*, *Il-6*, and *Tnf-α,* was measured by qPCR. (G) *Ccr2* mRNA expression in the kidney was measured by qPCR. Scale bars, 50 μm. n = 5 for each group. The data are presented as the mean ± SEM. Statistical significance was determined by Tukey's multiple comparisons test. *P<0.05; **P<0.01; ***P<0.001.

Figure 4. The PSMP-neutralizing antibody alleviates kidney injury in a bIRI mouse model.

(A) Serum creatinine and BUN were measured. (B) Renal mRNA expression levels of the tubular damage markers *Ngal* and *Kim-1* were measured by qPCR. (C) Representative histologic images of kidney sections stained with PAS, and tubular injury score. (D) NGAL protein expression in the kidney was determined by immunohistochemical staining and statistical analysis. (E) NGAL protein expression in the kidney was determined by western blotting and statistical analysis (n =

3). (F) Renal mRNA levels of *Ccl2*, *Il-1β*, *Il-6*, and *Tnf-α* was measured by qPCR. (G) *Ccr2* mRNA expression in the kidney was measured by qPCR. Scale bars, 50 μm. $n = 5$ for each group. The data are presented as the mean ± SEM. Statistical significance was determined by Dunnett's multiple comparisons test. *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$.

Figure 5. Characteristics of Ly6C^{hi} IMs or F4/80^{lo} macrophages in uIRI-AKI mice.

(A) UMAP plot colored by macrophages clusters showing the kidney macrophages annotation. (B) UMAP plot showing macrophage ontogeny (Ly6C^{hi} IMs, Ly6C^{lo} IMs, and KRM) in kidney macrophage.C6-C9 are Ly6C^{hi} IMs. (C) UMAP plot showing macrophage origin (kidney origin, blood origin and spleen origin) in kidney macrophage. C6-C9 are blood origin. (D) UMAP plot showing F4/80^{hi} macrophages and F4/80^{lo} macrophages classification in kidney macrophage. C6-C9 are F4/80^{lo} macrophages. (E) Dot plot showing *Ccr2* expression in kidney Ly6C^{hi} IMs, Ly6C^{lo} IMs, and KRM. (F) Dot plot showing *Ccr2* expression in kidney F4/80^{hi} macrophages and F4/80^{lo} macrophages. (G) Dot plot showing the scores of inflammatory cytokine, chemokine, and chemokine receptor in Ly6C^{hi} IMs, Ly6C^{lo} IMs, and KRM. (H) Dot plot showing the scores of inflammatory cytokine, chemokine, and chemokine receptor in F4/80^{hi} macrophages and F4/80^{lo} macrophages. UMAP, uniform manifold approximation and projection; KRM, kidney resident macrophages; IMs, infiltrated macrophages; hi, high; lo, low; Mφ, macrophage.

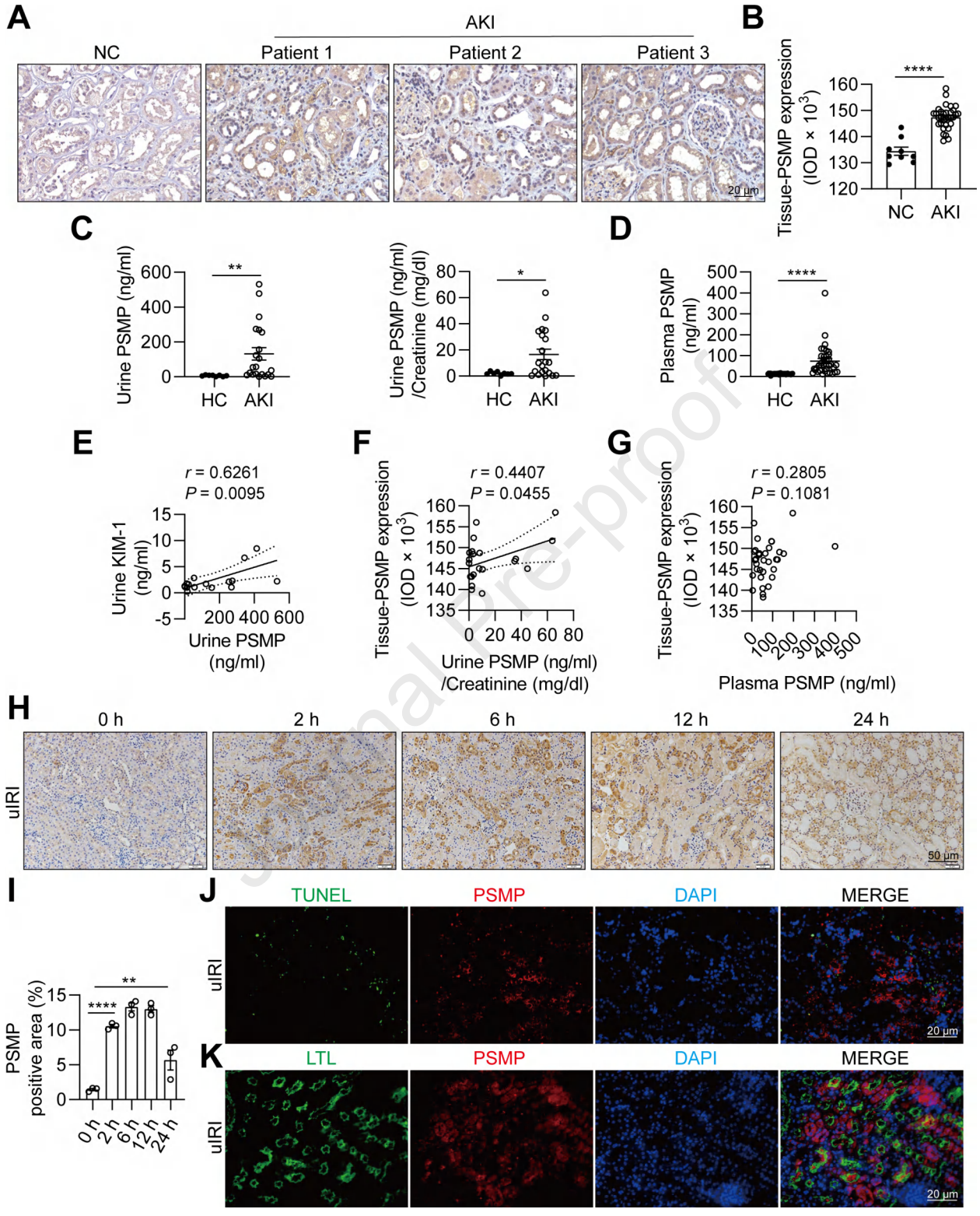
Figure 6. Effects of PSMP on macrophage infiltration in bIRI-induced AKI.

(A and B) Renal CD11b^{hi}Ly6C^{hi} IMs (CD45^{+}Ly6G^{-}) were quantified by flow cytometry. (C and D) Renal CCR2^{+}Ly6C^{hi} IMs (CD45^{+}Ly6G^{-}) were quantified by flow cytometry. (E and F) Renal CD11b^{hi}F4/80^{lo} IMs (CD45^{+}Ly6G^{-}) and CD11b^{lo}F4/80^{hi} KRM (CD45^{+}Ly6G^{-}) were quantified by flow cytometry. $n = 5$ for each group. The data are presented as the mean ± SEM. Statistical

significance was determined by two-way ANOVA. ***P<0.001; ****P<0.0001. IMs, infiltrated macrophages; KRM, kidney resident macrophages; hi, high; lo, low.

Figure 7. Effects of PSMP on macrophage polarization in AKI.

(A) Representative flow cytometry image of renal $CD11b^{+}F4/80^{+}$ macrophages. (B) M1 ($MHCII^{+}$) and M2 ($CD206^{+}$) renal $CD11b^{+}F4/80^{+}$ macrophages were quantified by flow cytometry. (C) Representative flow cytometric image of renal $CD11b^{hi}F4/80^{lo}$ IMs and $CD11b^{lo}F4/80^{hi}$ KRM. (D) M1 ($MHCII^{+}$) and M2 ($CD206^{+}$) macrophages in renal $CD11b^{hi}F4/80^{lo}$ IMs were quantified by flow cytometry. (E) M1 ($MHCII^{+}$) and M2 ($CD206^{+}$) macrophages in $CD11b^{lo}F4/80^{hi}$ KRM were quantified by flow cytometry. (F) Renal mRNA expression levels of the M1 marker genes *Cd86*, *Nos2*, and *Il-12* were measured by qPCR. $n = 5$ for each group. The data are presented as the mean ± SEM. Statistical significance was determined by Student's *t* test (B, D and E) and two-way ANOVA (F). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.


A
AKI
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Patient 3
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NC
AKI
C
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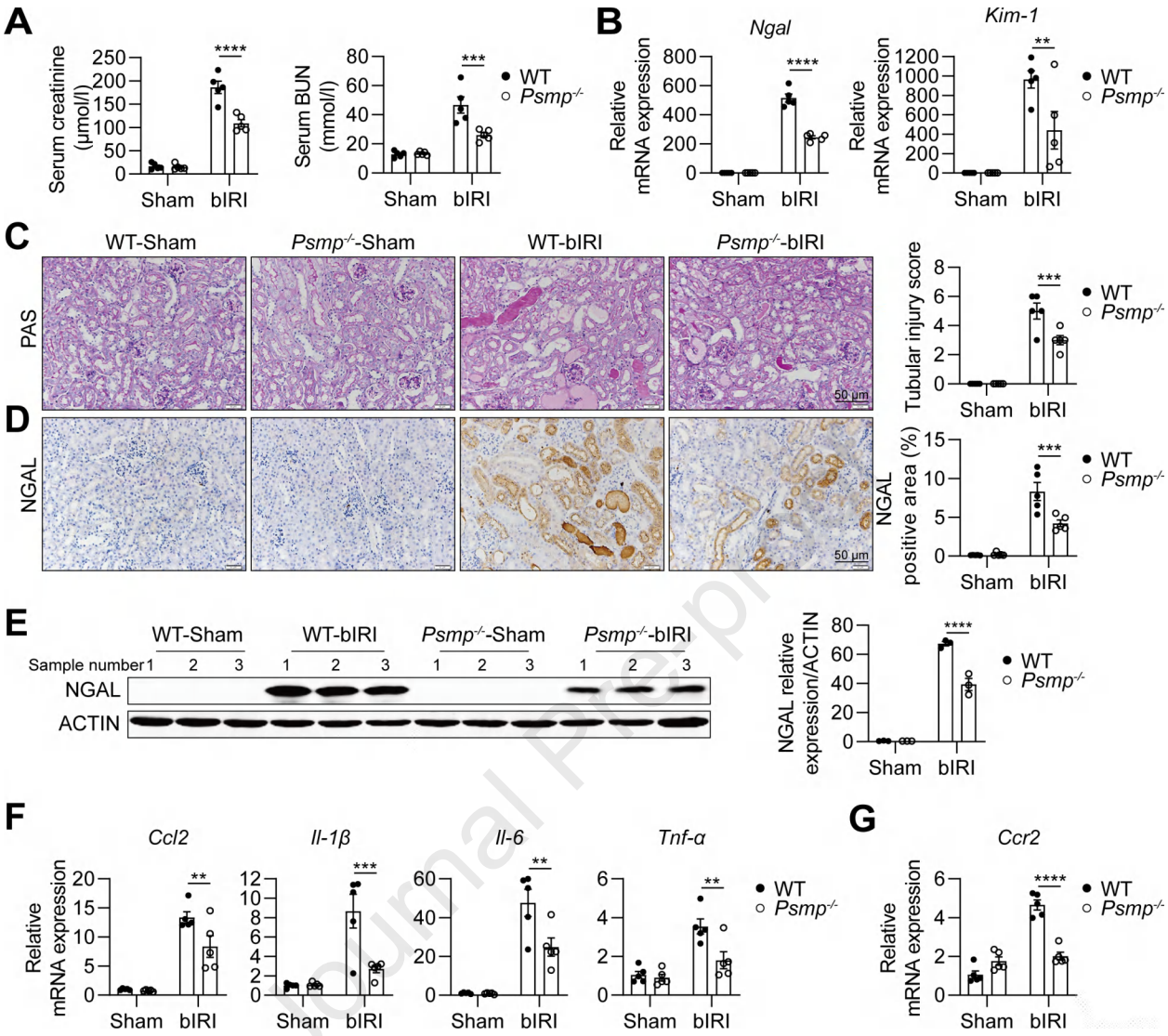
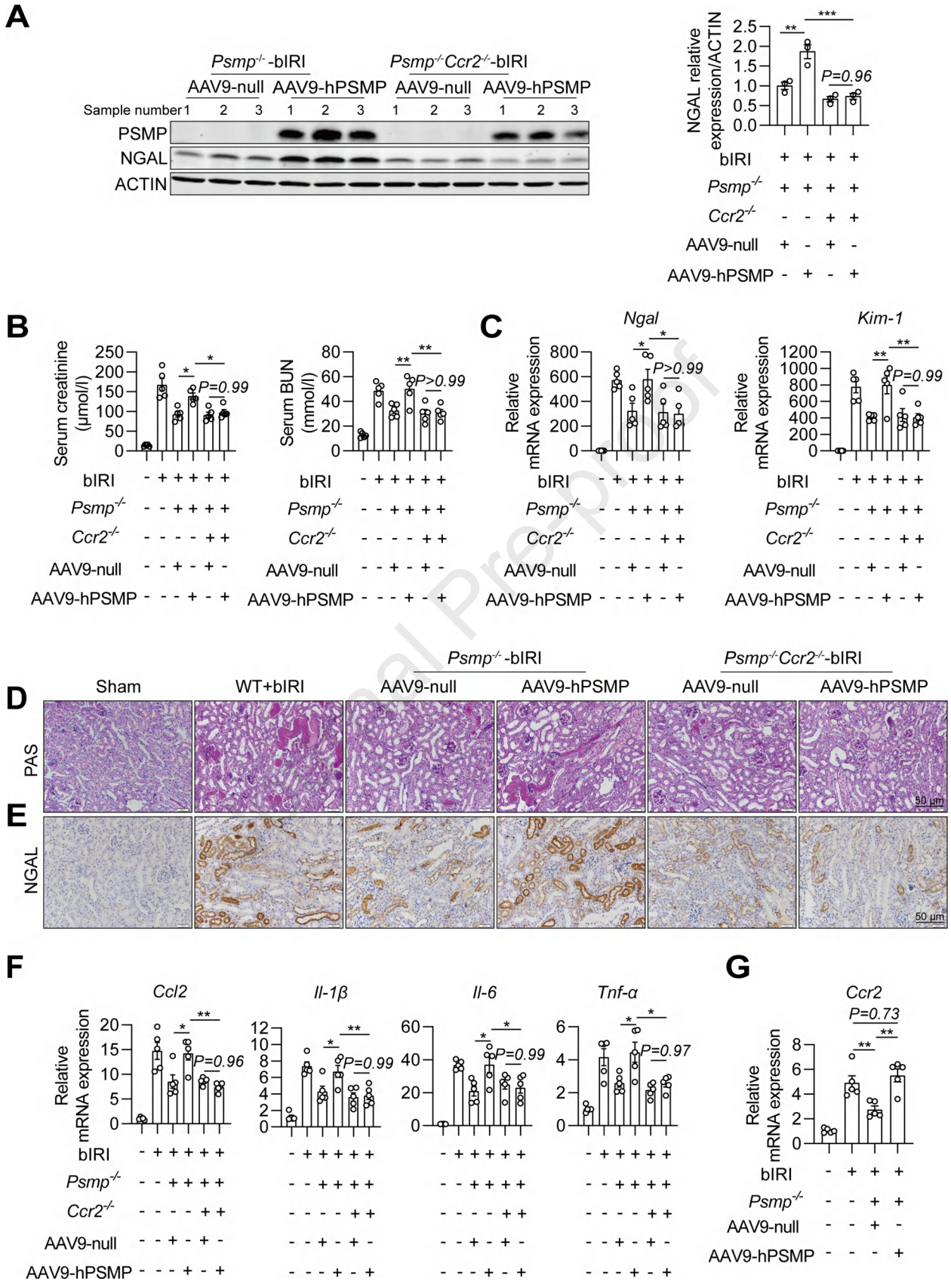
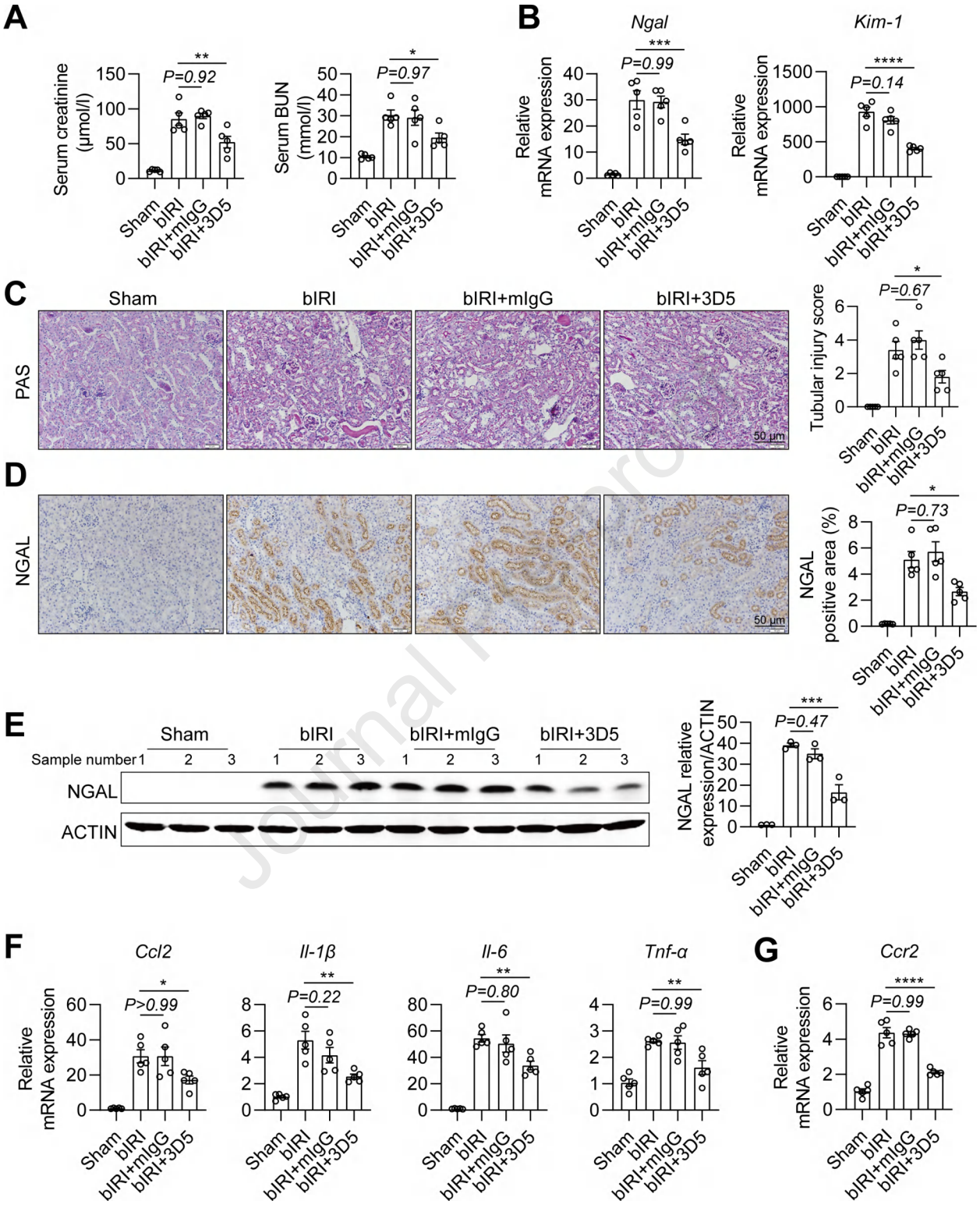
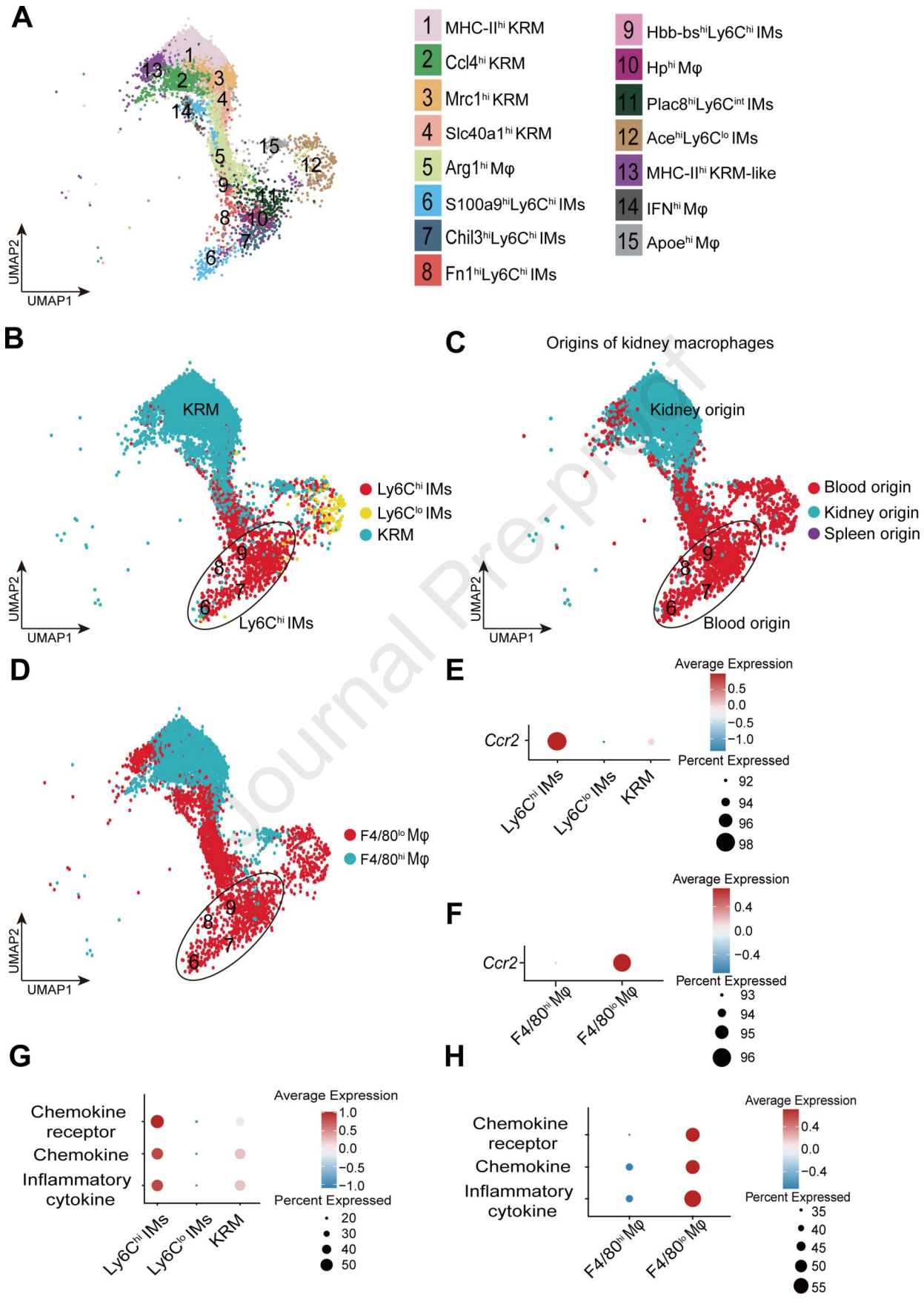
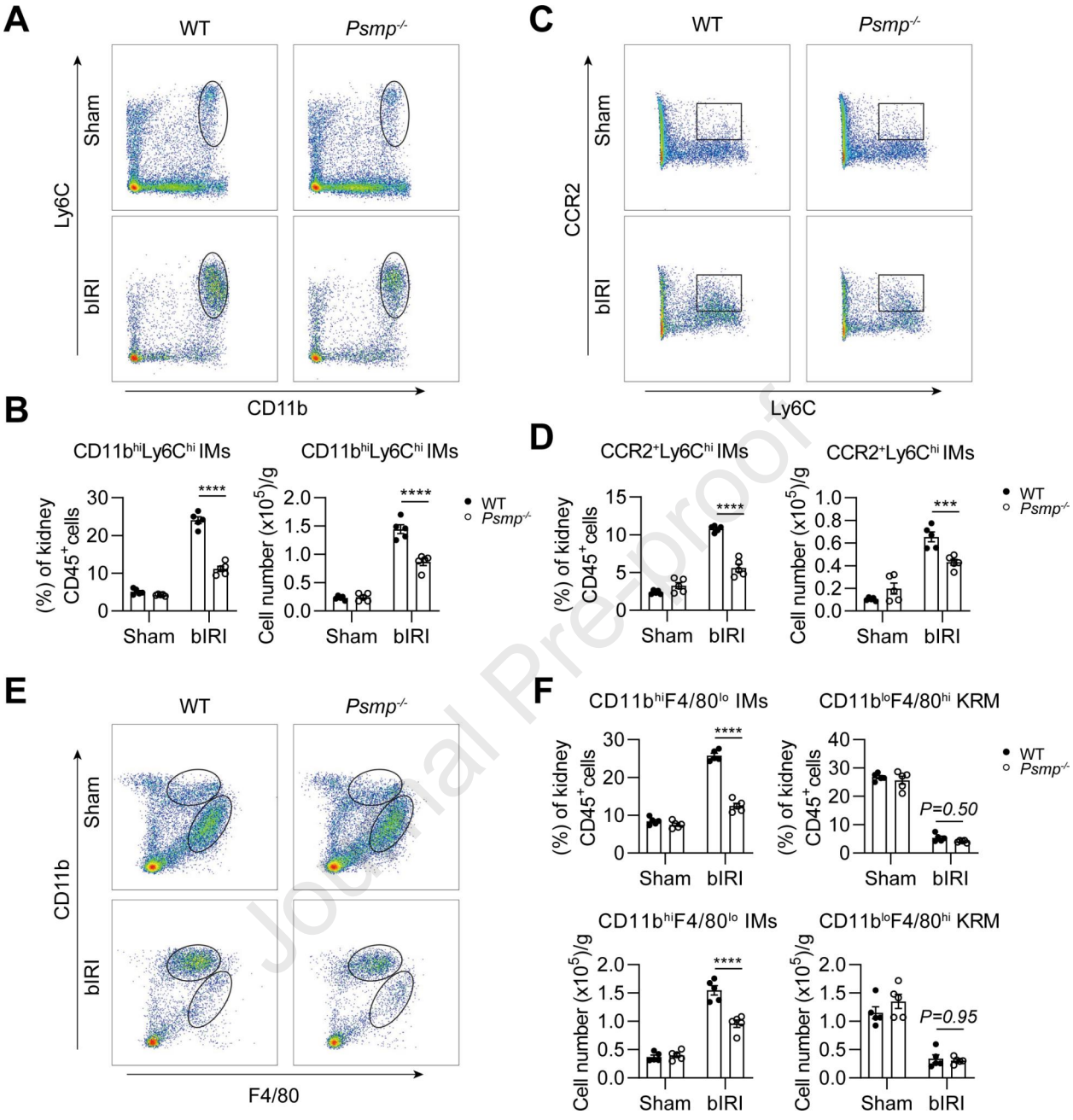
HC
AKI
Urine PSMP (ng/ml) /Creatinine (mg/dl)
*
HC
AKI
D
Plasma PSMP (ng/ml)
****
HC
AKI
E
r = 0.6261
P = 0.0095
Urine KIM-1 (ng/ml)
Urine PSMP (ng/ml)
F
r = 0.4407
P = 0.0455
Tissue-PSMP expression (IOD × 10^3)
Urine PSMP (ng/ml) /Creatinine (mg/dl)
G
r = 0.2805
P = 0.1081
Tissue-PSMP expression (IOD × 10^3)
Plasma PSMP (ng/ml)
H
0 h
2 h
6 h
12 h
24 h
uIRI
50 μm
I
PSMP positive area (%)
****
**
0 h
2 h
6 h
12 h
24 h
J
TUNEL
PSMP
DAPI
MERGE
uIRI
20 μm
K
LTL
PSMP
DAPI
MERGE
uIRI
20 μm

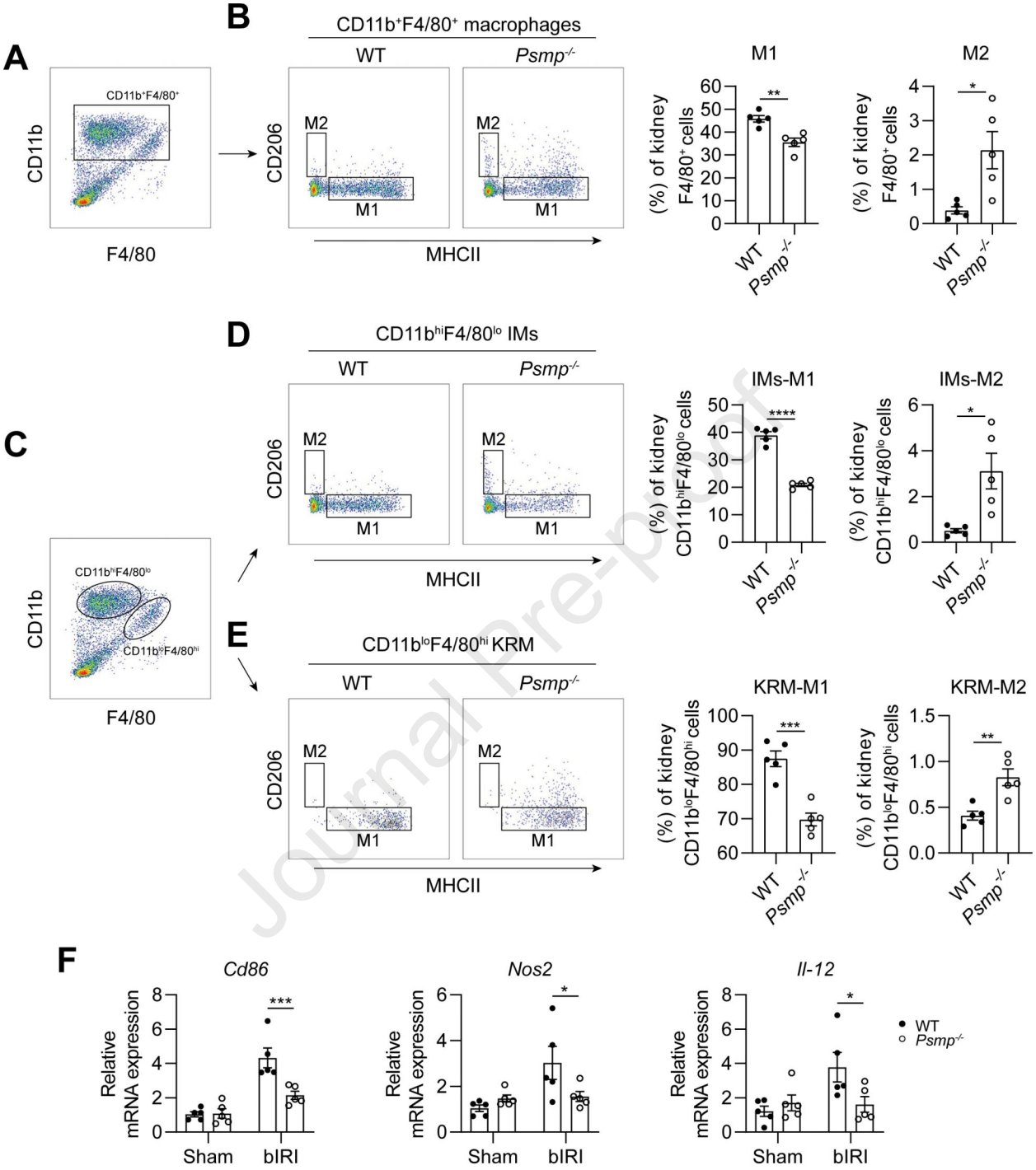



A
Psmp-/- -bIRI
Psmp-/-Ccr2-/--bIRI
AAV9-null AAV9-hPSMP AAV9-null AAV9-hPSMP
Sample number 1 2 3 1 2 3 1 2 3 1 2 3
PSMP
NGAL
ACTIN
NGAL relative expression/ACTIN
P=0.96
bIRI
Psmp-/-
Ccr2-/-
AAV9-null
AAV9-hPSMP
B
Serum creatinine (μmol/l)
P=0.99
Serum BUN (mmol/l)
P>0.99
C
Ngal
Kim-1
Relative mRNA expression
P>0.99
P=0.99
D
Sham
WT+bIRI
Psmp-/- -bIRI
Psmp-/-Ccr2-/--bIRI
AAV9-null
AAV9-hPSMP
PAS
50 μm
E
NGAL
50 μm
F
Ccl2
Il-1β
Il-6
Tnf-α
Relative mRNA expression
P=0.96
P=0.99
P=0.99
P=0.97
G
Ccr2
P=0.73
Relative mRNA expression


A
Serum creatinine (μmol/l)
P=0.92
Serum BUN (mmol/l)
P=0.97
B
Ngal
P=0.99
Kim-1
P=0.14
Relative mRNA expression
C
Sham
bIRI
bIRI+mIgG
bIRI+3D5
PAS
50 μm
Tubular injury score
P=0.67
D
NGAL
NGAL positive area (%)
P=0.73
E
Sample number
NGAL
ACTIN
NGAL relative expression/ACTIN
P=0.47
F
Ccl2
P>0.99
Il-1β
P=0.22
Il-6
P=0.80
Tnf-α
P=0.99
G
Ccr2
P=0.99







Wang, Yang and colleagues reveal that PSMP, a damage-induced chemotactic cytokine, is upregulated in patients with AKI and promotes AKI in mice through CCR2-dependent inflammation. PSMP deficiency and treatment with the PSMP-neutralizing antibody 3D5 significantly alleviate AKI in mice, suggesting that PSMP is a promising therapeutic target for AKI.