Exploring the Role of PU.1 in Chronic Kidney Disease to Disrupt NLRP3 Inflammasome Signaling

UCONN HEALTH

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Background

Chronic kidney disease (CKD) affects over 10% of the global population and is characterized by persistent inflammation and progressive fibrosis. Macrophages contribute to CKD by promoting inflammation and tissue damage. PU.1 is a transcription factor essential for macrophage function and may regulate the NLRP3 inflammasome, a key driver of inflammatory cytokine release. Current CKD treatments focus on symptom management, but do not directly target the underlying inflammatory pathways. Investigating the PU.1-NLPR3 pathway may uncover new therapeutic strategies to better address CKD at the molecular level.

Methods

Kidney injury was modeled using tissue samples from mice subjected to unilateral ureteral obstruction (UUO) and folic acid (FA) treatment. Tissues were collected and processed for histological analysis, immunohistochemistry, and immunofluorescence to assess the PU.1 expression and localization. Western blotting was used to evaluate protein expression in kidney tissue. Gene expression analysis was conducted using PCR, and genotyping was conducted from mouse ear tissue. Additional methods included cell culture and a range of molecular biology techniques.

Results

PU.1 expression was upregulated in kidney tissue following injury in both the unilateral ureteral obstruction (UUO) and folic acid (FA) models. Immunohistochemistry and immunofluorescence revealed increased PU.1 staining in inflammatory regions, particularly in macrophage. Western blot analysis confirmed elevated PU.1 protein levels in injured samples compared to controls. Functional studies in macrophages showed that PU.1 is required for NLRP3 induction, indicating that PU.1 contributes to activation of inflammatory pathways linked to inflammasome signaling during kidney injury

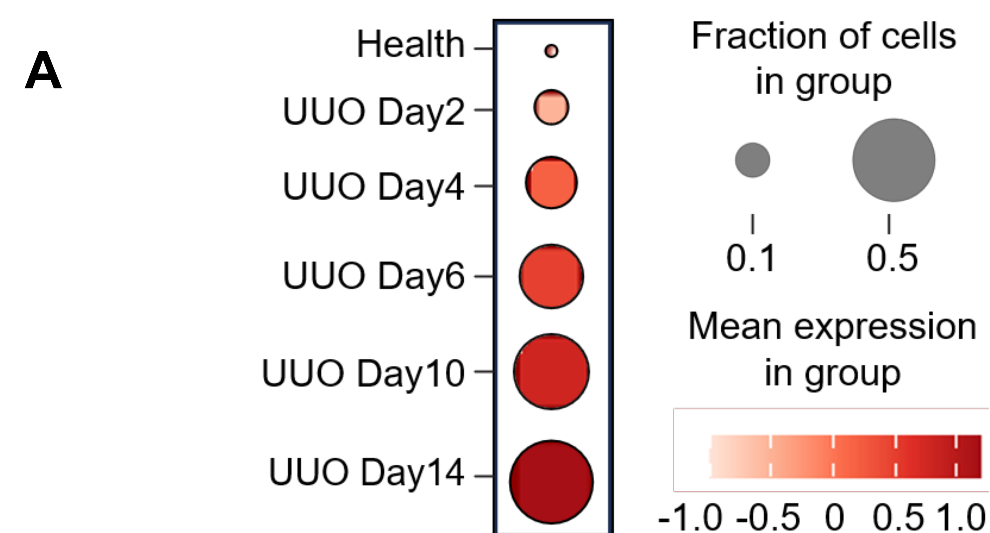

Figure 1. Single-cell analysis reveals progressive upregulation of PU.1 during UUO. Dot plot shows the mean expression and fraction of PU.1-positive cells across different time points in the UUO model of kidney injury. The color intensity reflects PU.1 expression per cell across timepoints in UUO kidneys, while the size of each dot reflects the fraction of cells expressing PU.1. PU.1 expression increases steadily from Day 2 to Day 14 post-UUO, both in expression level and the proportion of positive cells, indicating a time-dependent transcriptional activation of PU.1 in response to injury.

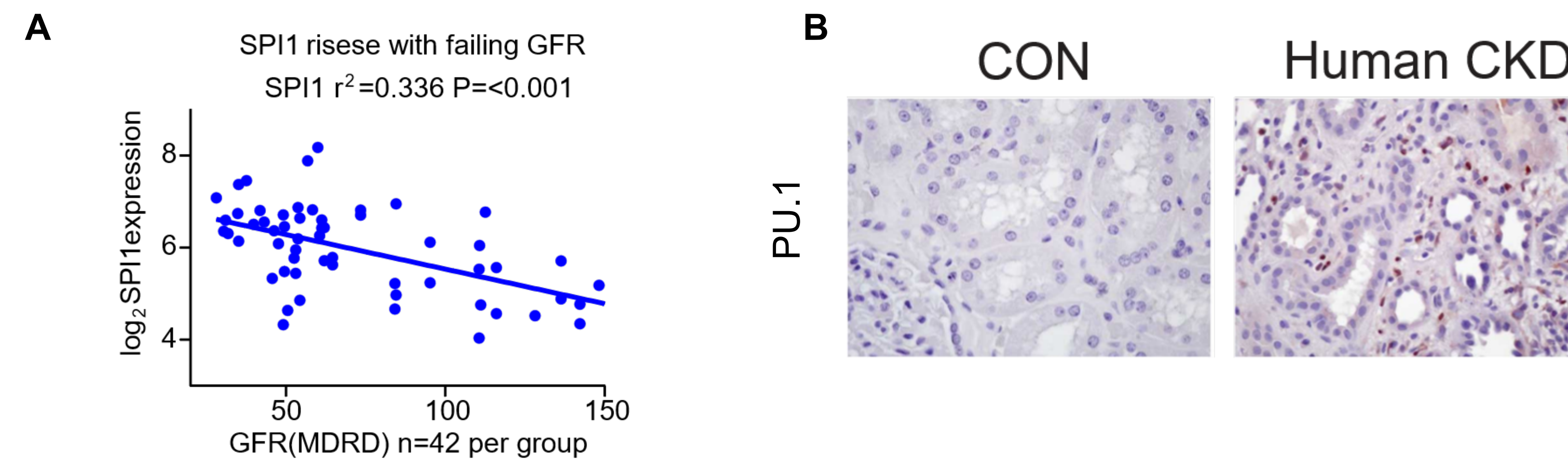

Figure 2. PU.1 expression is increased in CKD. PU.1 (SPI1) expression was analyzed in public datasets of human kidney tissue and found to inversely correlate with estimated glomerular filtration rate (GFR), indicating elevated expression in chronic kidney disease. Immunohistochemistry staining was peformed on human kidney tissue samples to evaluate PU.1 protein expression (brown). Increased PU.1 staining was observed in CKD samples compared to controls, specifically in tubulointerstitial areas.

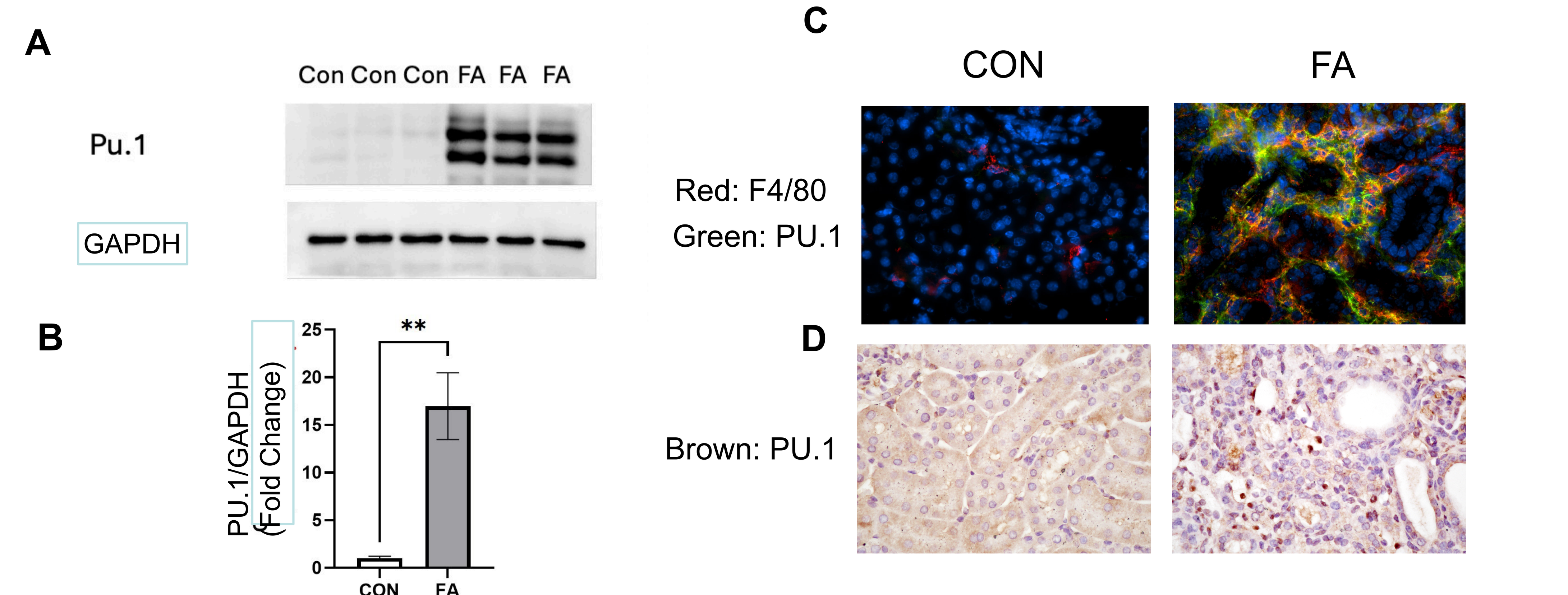

Figure 3. PU.1 expression is upregulated in folic acid (FA)- induced kidney injury. (A) Western blot analysis of kidney tissue lysates from control (Con) and FA-treated mice shows increased Pu.1 protein levels in FA-treated kidneys. GAPDH was used as a loading control. (B) Densitometric quantification of PU.1 expression (normalized to GAPDH) confirms a signficant increase in PU.1 protein levels in FA-treated kidneys compared to controls. Data are presented as mean +- SEM; p<0.01. (C) PU.1 expression in kidney sections was assessed by both immunohistochemistry (IHC) and immunofluorescence (IF). IF staining reveals PU.1 (green) nuclear localization in DAPI cells (blue) and co-localization with F4/80 fibroblasts (red) suggesting PU.1 may play a role in driving fibrotic responses.(D) IHC staining shows increased PU.1 expression in FA-treated kidneys, particularly in tubulointerstitial regions.

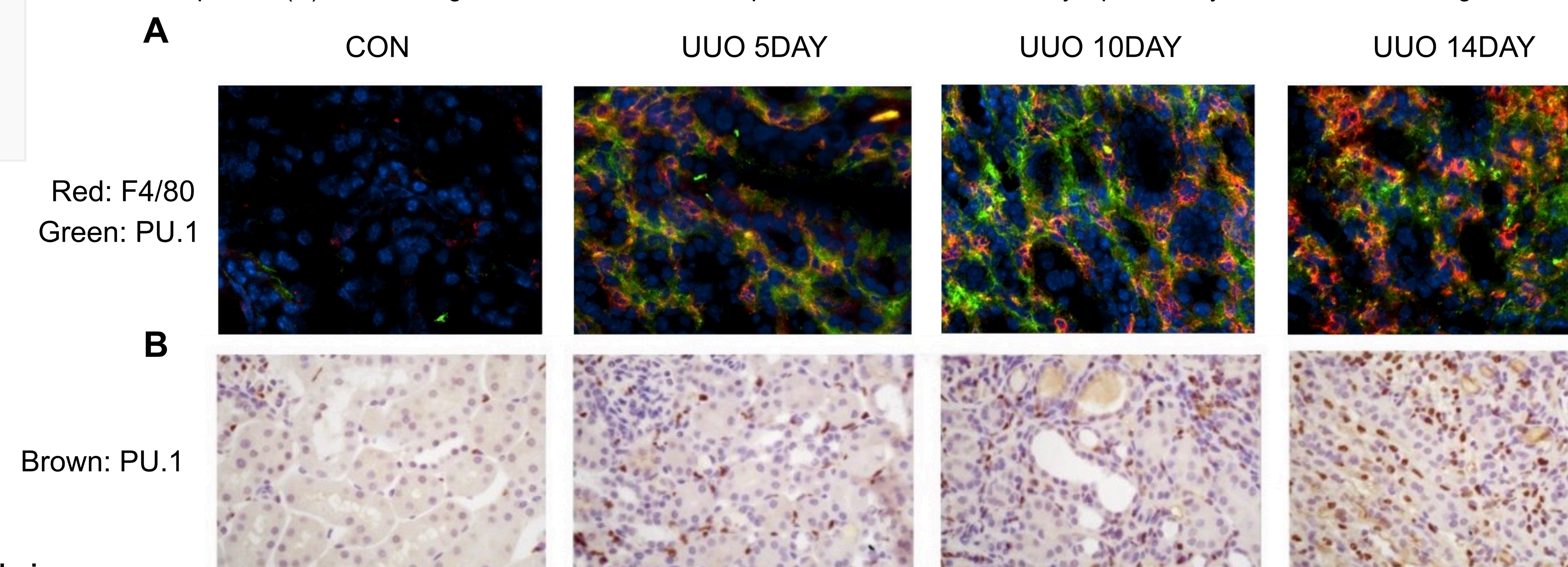

Figure 4. Time-course analysis of PU.1 expression during UUO-induced kidney injury. (A) Immunofluorescence staining of kidney sections from control and UUO mice at days 5, 10, and 14 post-obstruction. PU.1 (green) expression progressively increases with injury duration, particularly in the tubulointerstitial space. Localization of PU.1 become more prominent by day 14, suggesting PU.1 involvement in fibrotic progression. (B) Immunohistochemistry confirms increased PU.1 expression over time in UUO kidneys. Minimal PU.1 staining is observed in control kidneys, while nuclear PU.1 expression becomes more pronounced at days 10 and 14 post-UUO.

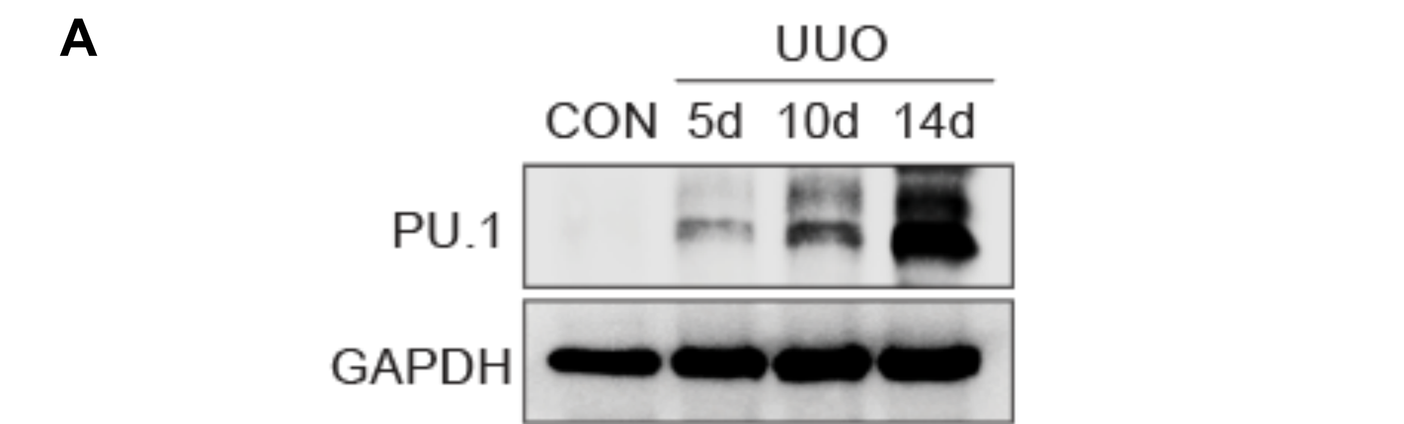

Figure 5. PU.1 protein levels increase over time in the UUO model of kidney injury. Western blot analysis was performed on kidney tissue from control and UUO mice at days 5, 10, and 14 following obstruction. A gradual increase in PU.1 expression is observed across time points, indicating enhanced PU.1 activation in response to sustained injury. GAPDH served as a loading control. These findings reinforce time-dependent PU.1 induction previously observed by immunostaining.

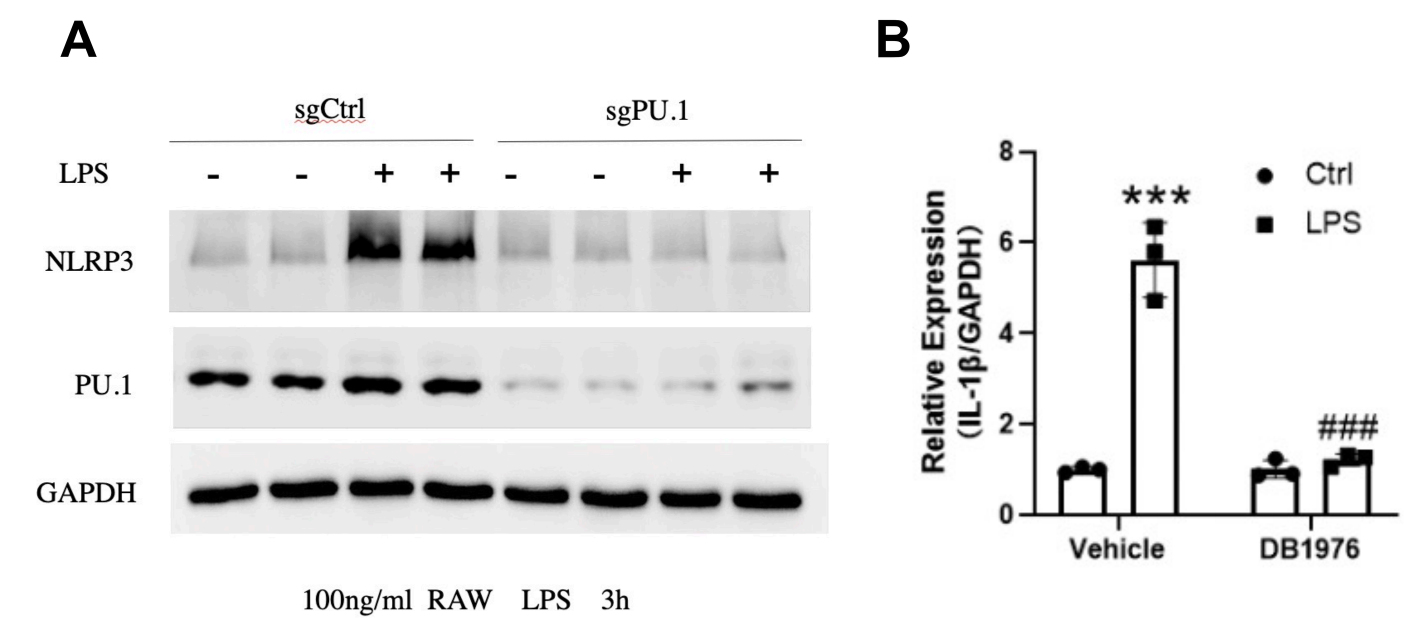

Figure 6. PU.1 regulates NLRP3 expression and inflammatory signaling in macrophages. (A) Representative Western blot of RAW macrophages treated with LPS (100 ng/mL, 3h), comparing control (sgCtrl) and PU.1 knockout (sgPU.1) conditions. PU.1 deletion reduces NLRP3 protein expression following LPS stimulation. GAPDH was used as a loading control. (B). qPCR analysis showing IL-1β mRNA levels in RAW macrophages treated with LPS, with or without the PU.1 inhibitor DB1976. LPS strongly increases IL-1β expression in untreated cells (*** p<0.001), but this increase is significantly reduced when cells are treated with DB1976 (### p<0.001 vs LPS alone).

Conclusions/Significance

Our findings demonstrate that PU.1 is upregulated in both human CKD and mouse models of kidney injury, with expression increasing over time and localizing to injured regions of the kidney. Through immunostaining, Western blotting, and transcriptomic analysis, it is shown that PU.1 is required for NLRP3 induction in macrophages, suggesting a critical role in promoting inflammatory signaling. These results identify PU.1 as a potential driver of CKD progression and support its relevance as a molecular target for therapeutic intervention. Targeting PU.1 could offer a new strategy to disrupt NLRP3-driven inflammation in chronic kindey disease.

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