

Supplementary materials

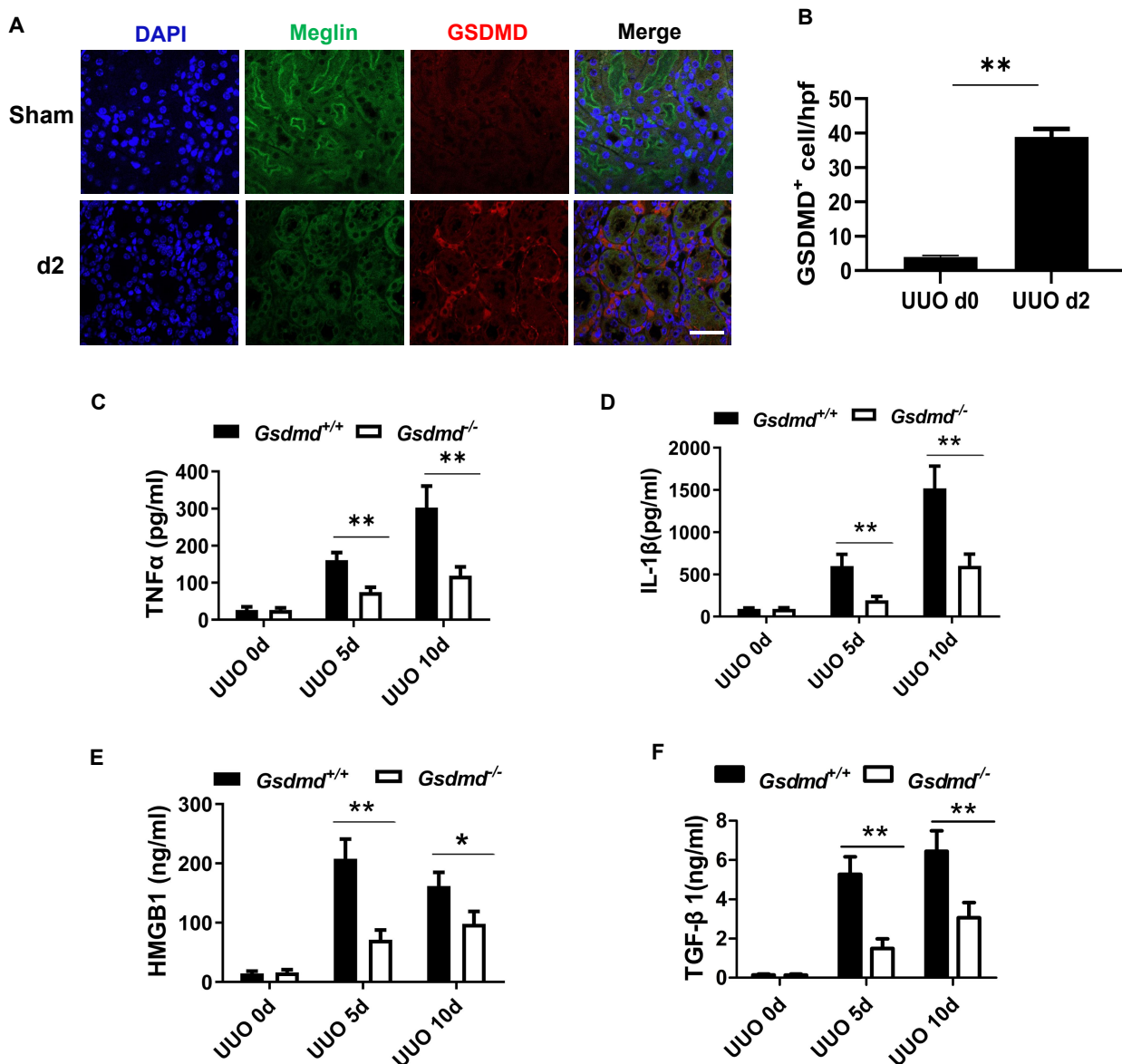
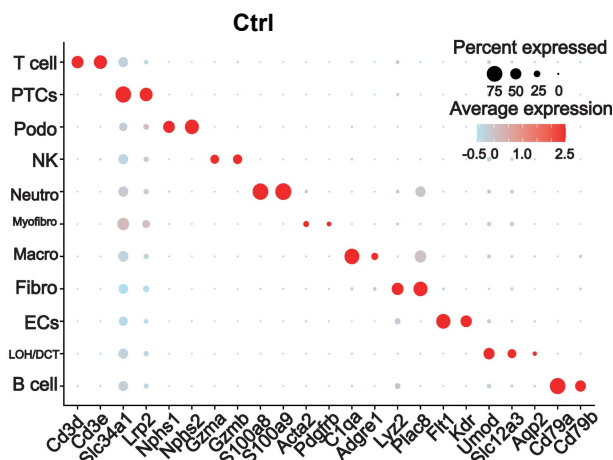


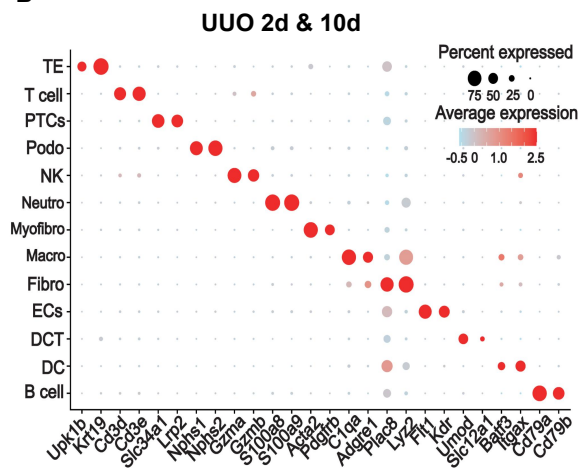
Figure S1. UUO induced GSDMD activation in the kidney and *Gsdmd* deletion inhibited inflammatory cytokines production after UUO. (A) Representative images of immunofluorescence staining of kidney sections from wild-type mice on day 0 and day 2 after UUO, showing the expression of megalin (green) and GSDMD (red). DAPI (blue) was used for nuclear staining. (B) Quantification of GSDMD-expressed cells by immunofluorescence. n=6. (C-F) TNF-α, IL-1β, HMGB1 and TGF-β production in kidney on day 0, 5 and 10 after UUO were detected by ELISA. n=5. **P* < 0.05; ***P* < 0.01.

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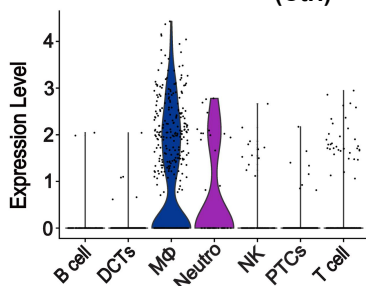
A



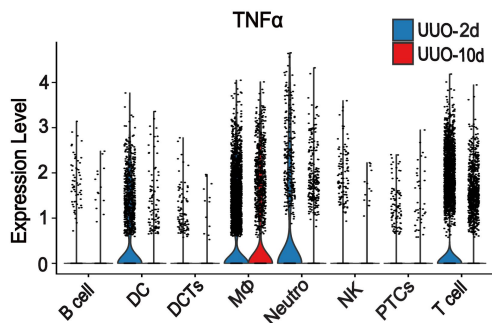
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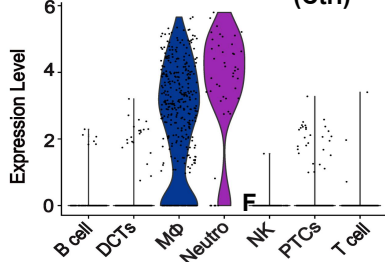
C **TNF α (Ctrl)**



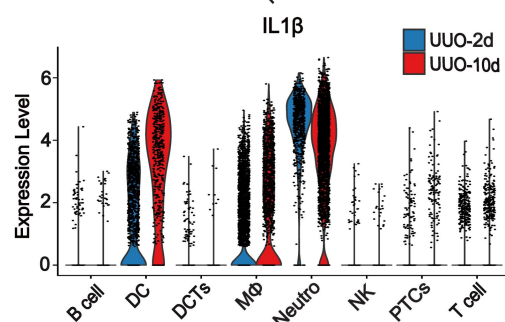
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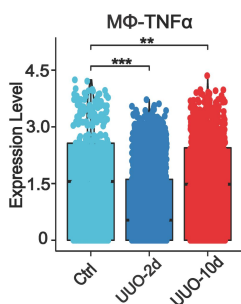
E **IL1 β (Ctrl)**



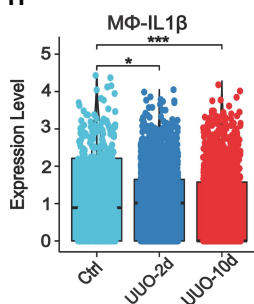
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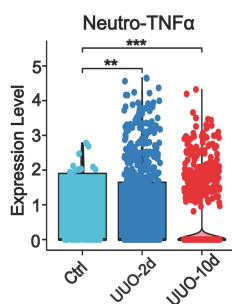
G



H



I



J

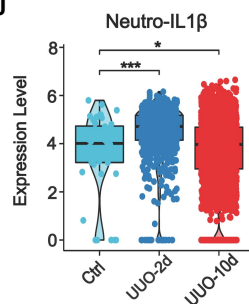


Figure S2. Single-cell sequencing showed the elevated gene expression of TNF- α and IL-1 β in macrophages and neutrophils. (A, B) Dot plot of cluster-enriched gene expressions. Cell clusters of control kidney (left) and kidneys undergoing UUO (right) were identified by kidney cell-specific marker expression. (C-F) Violin plots of log-transformed gene expression of TNF- α and IL-1 β in each cell population (B cell, dendritic cell, distal tubular cell, macrophage, neutrophil, natural killer cell, proximal tubular cell and T cell) of control kidney and kidneys undergoing UUO. (G-J) Comparison of gene expression of TNF- α and IL-1 β in macrophages and neutrophils in the kidneys on day 2 and 10 after UUO. * $P < 0.05$; ** $P < 0.01$; * $P < 0.001$.**

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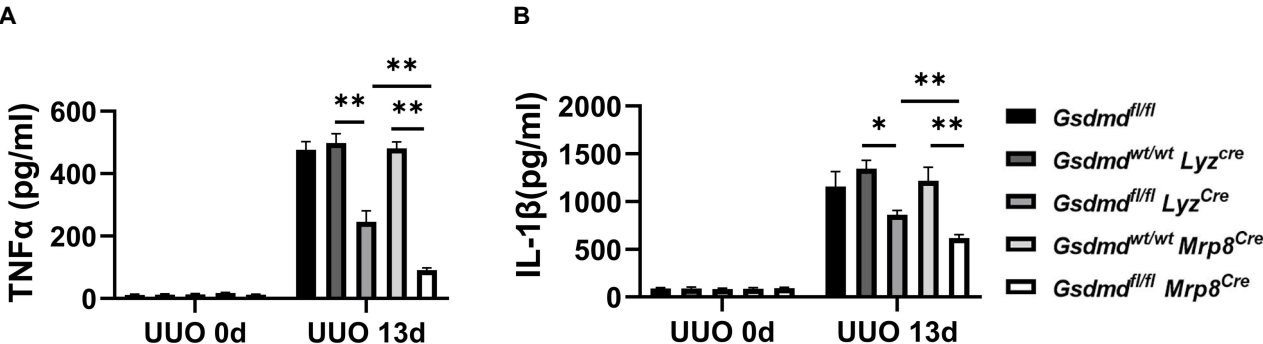


Figure S3. Specific deletion of *Gsdmd* in neutrophils inhibited TNF- α and IL-1 β production in kidney after UUO. (A, B) TNF- α and IL-1 β production in kidney on day 13 after UUO were detected by ELISA. n=5. * $P < 0.05$; ** $P < 0.01$.

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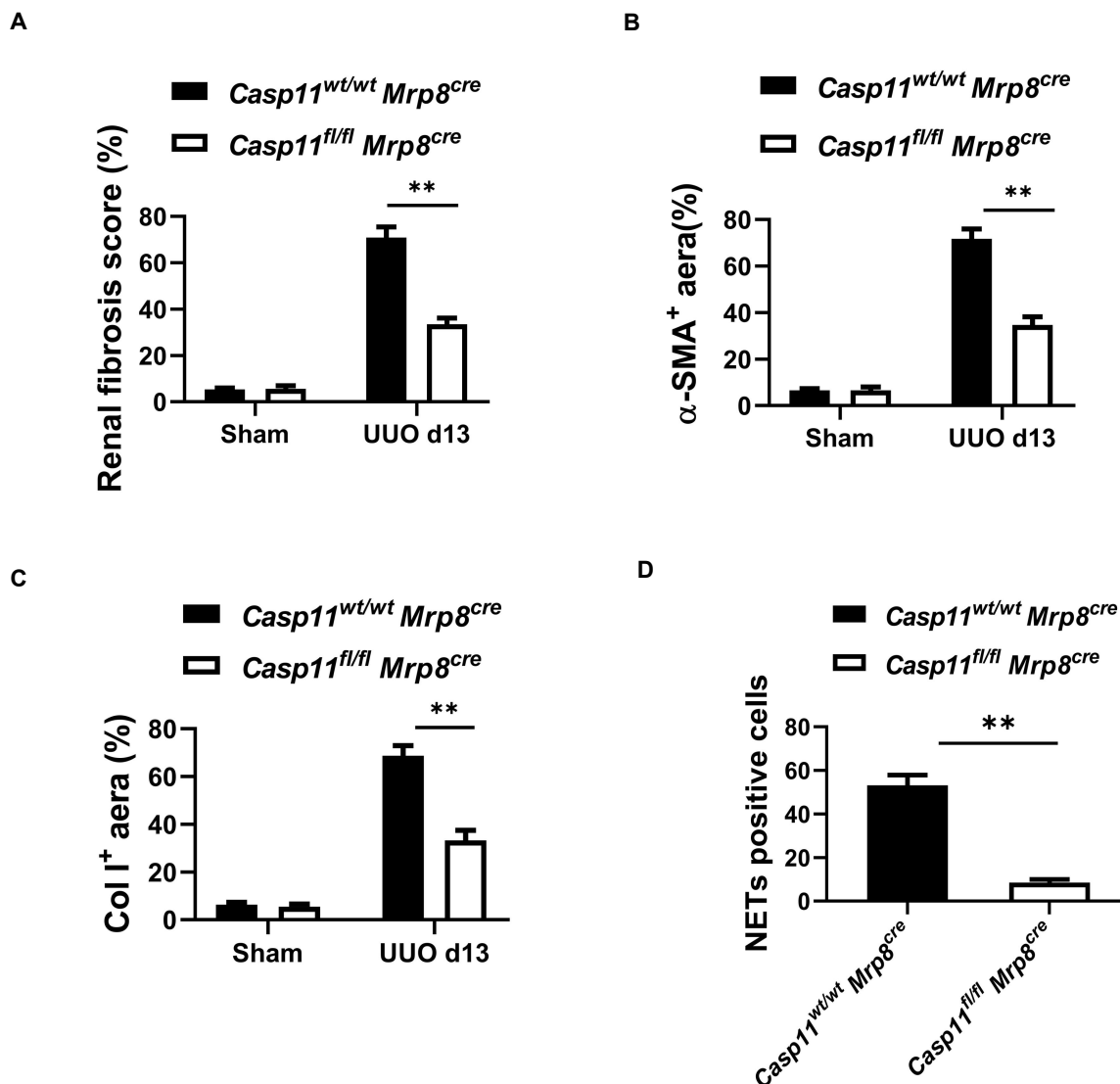


Figure S4. Quantification of renal fibrosis scores, α-SMA and Col I expression and NETs formation after UUO in the kidney of wildtype versus *Casp11^{fl/fl} Mrp8^{cre}* mice. (A) Quantification of renal fibrosis scores evaluated by Masson trichrome staining. Kidneys were isolated from *Casp11^{wt/wt} Mrp8^{cre}* mice and *Casp11^{fl/fl} Mrp8^{cre}* mice on day 13 after UUO. (B, C) Quantification of α-SMA (B) and Col I (C) expression by immunofluorescence. (D) Quantification of cells undergoing NETs. n=6. ***P* < 0.01.

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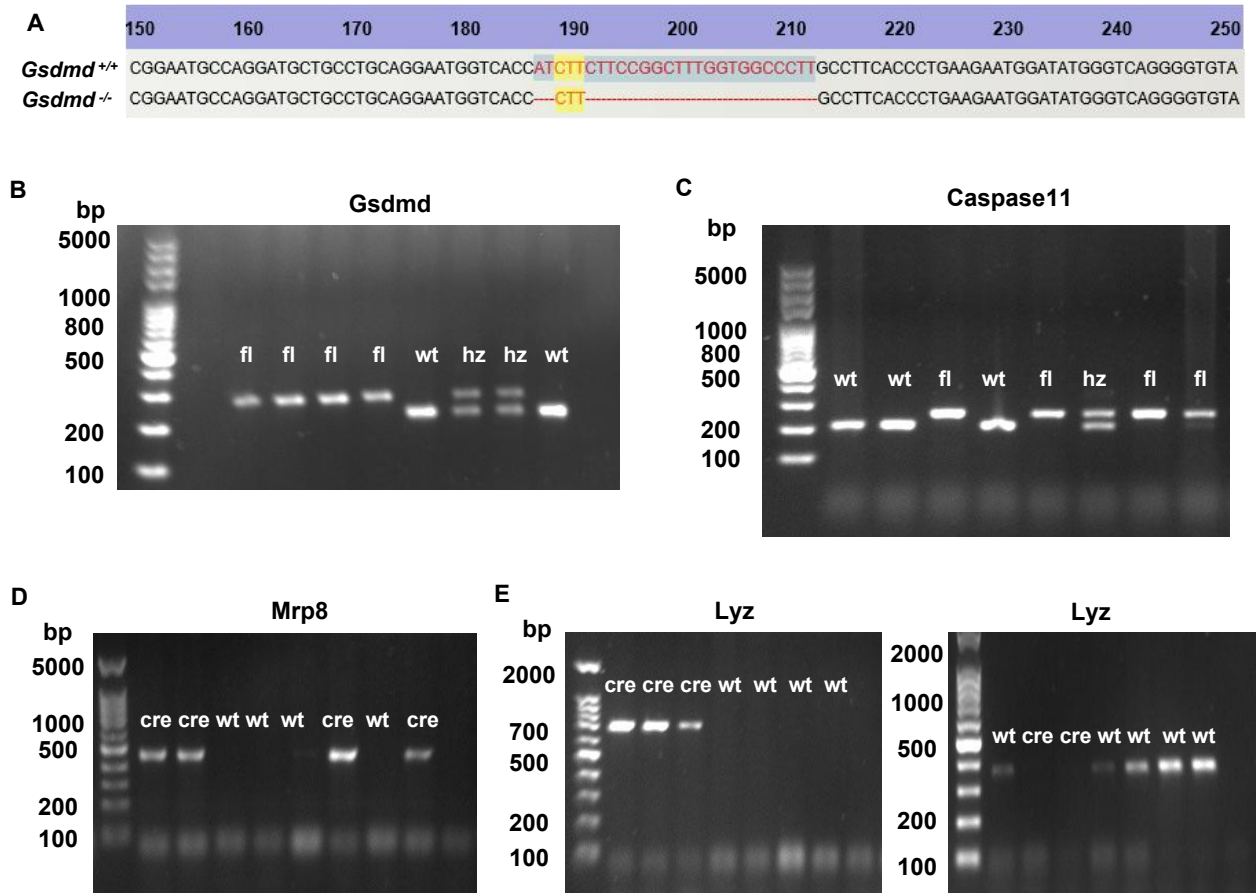


Figure S5. Identification of gene-editing mice. (A) Gene sequences of the targeted region of *Gsdmd*^{-/-} mice. (B) Representative DNA gel of *Gsdmd*^{fl/fl}, *Gsdmd*^{wt/wt} mice and heterozygotes. One DNA band is observed for *Gsdmd*^{fl/fl} or *Gsdmd*^{wt/wt} mice, the higher one for *Gsdmd*^{fl/fl} mice and the lower one for *Gsdmd*^{wt/wt} mice. Two DNA bands are observed for heterozygotes (HZ). (C) Representative DNA gel of *Casp11*^{fl/fl}, *Casp11*^{wt/wt} mice and heterozygotes. The higher band indicated for *Casp11*^{fl/fl} mice and the lower one indicated for *Casp11*^{wt/wt} mice. Two DNA bands are observed for heterozygotes. (D) Representative DNA gel of *Mrp8*-cre mice. (E) Representative DNA gel of *Lyz*-cre mice and wildtype pups. One DNA band at about 700 bp is observed for *Lyz*-cre mice, while the one at about 350 bp is observed for wildtype pups. If both bands are shown, indicating for heterozygotes.