

Supplementary data from the original manuscript by Christensen et al entitled:

Renoprotective Effects of Metformin are Independent of Organic Cation Transporters 1 & 2 and AMP-activated Protein Kinase in the Kidney.

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	SHAM	SHAMmet	UUO	UUOmet
Metformin (ng/mL)	nd	2200 ± 36	nd	3289 ± 47
Creatinine (μmol/L)	8.17 ± 0.28	8.17 ± 0.37	11.83 ± 0.68*	11.67 ± 0.77*
Urea (mmol/L)	8.52 ± 0.55	7.15 ± 0.34	10.83 ± 0.49*	9.67 ± 0.43*

Supplementary table 1: Effect of metformin on renal function in response to 3 days unilateral ureteral obstruction. Plasma metformin, creatinine and urea levels in sham and UUO mice treated with and without metformin.

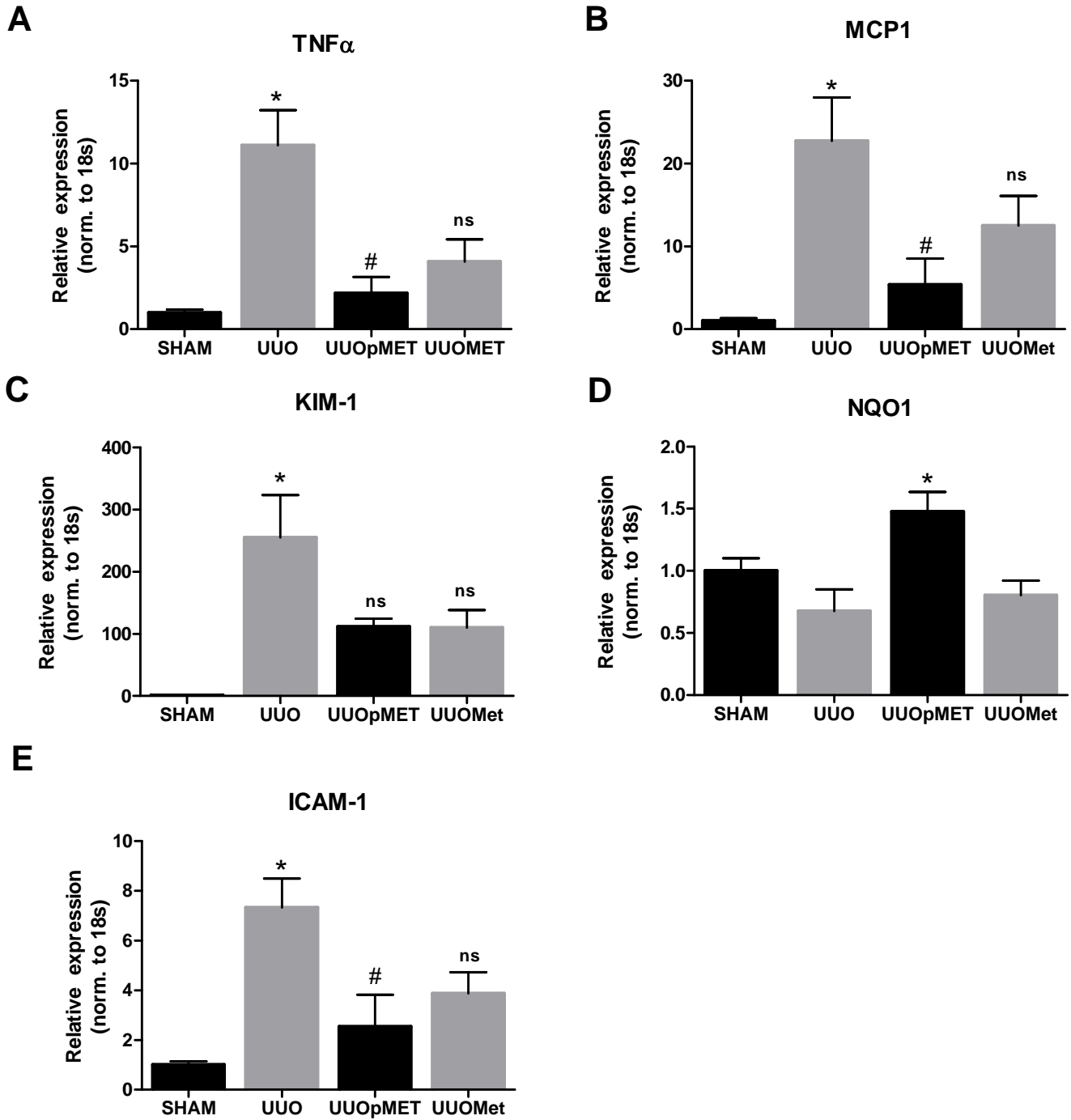
	WT	WTmet	OCT1/2KO	OCT1/2KOmet
Metformin (ng/mL)	nd	2892 ± 335	nd	4614 ± 783
Creatinine (μmol/L)	8.6 ± 0.67	8.5 ± 0.39	9.5 ± 0.31*	12.4 ± 0.61*#
Urea (mmol/L)	12.08 ± 0.67 ns	12.27 ± 0.27	9.00 ± 0.53*	9.16 ± 0.63*

Supplementary table 2: Effect of metformin on renal function in response to 3 days unilateral ureteral obstruction in OCT1/2 KO mice. Plasma metformin, creatinine and urea levels in sham and UOU OCT1/2 KO mice treated with and without metformin.

Supplementary table 3: QPCR primers sequences.

Target	Forward primer	Reverse primer
TNFα	5'- AGGCTGCCCCGACTACGT-3'	5'-GACTTTCTCCTGGTATGAGATAGCAAA-3'
IL-6	5'- GATGCTACCAAAGTGGATATAATC-3'	5'-GGTCCTTAGCCACTCCTTCTGTG-3'
IL-1β	5'-CAGGCAGGCAGTATCACTCA-3'	5'-TGTCCTCATCCTGGAAGGTC-3'
F4/80	5'-TGCTAGTGGAGGCAGTGATG-3'	5'-CTGTATTCAACCAGCAGCGA-3'
Itgax	5'-ACTGACCTGGTCTGATTGG-3'	5'-CAGCACCTCTGTTCTCCTCC-3'
Mac2	5'-GCTTATCCTGGCTCAACTG C-3'	5'-TTCAGTGTGGCCCATGATTGT-3'
KIM1	5'-CGGTACAACCTAAAGGGGCA-3'	5'-GACGTGTGGGAATCTCTGGT-3'
Nqo1	5'-CAGATCCTGGAAGGATGGAA-3'	5'-TCTGGTTGTCAGCTGGAATG-3'
Vcam1	5'-GTGGTGCTGTGACAATGACC-3'	5'-ACGTCAGAACAACCGAATCC-3'
Icam1	5'-TCCAATTCACACTGAATGCC-3'	5'-GTCTGCTGAGACCCCTCTTG-3'
MCP1	5'-CAAGAAGGAATGGGTCCAGA-3'	5'-GTGCTGAAGACCTTAGGGCA-3'
OCT3	5'-AATATCCTGTTTCGGCGTTG-3'	5'-TCACGATCACGAAGCAAGTC-3'
Pmat	5'-GTCTTCTCGCTGCTAATGGG-3'	5'-ATGGTGTTCCTGCCAGTTC-3'
Mate1	5'-CTGCTCTTCAGACAGGACCC-3'	5'-TGACAAGGTTAGCTGCGATG-3'
Mate2	5'-TCTTCTGCAGCGTTCCTAT-3'	5'-CTCCACTCCTGGAAGCACTC-3'
C-Myc	5'-CAACGTCTTGAACGTCAGA-3'	5'-TCGTCTGCTTGAATGGACAG-3'

Supplementary figure 1



Supplementary figure 1: The effect of metformin in mice subjected to 3 days unilateral ureteral obstruction (UUO).

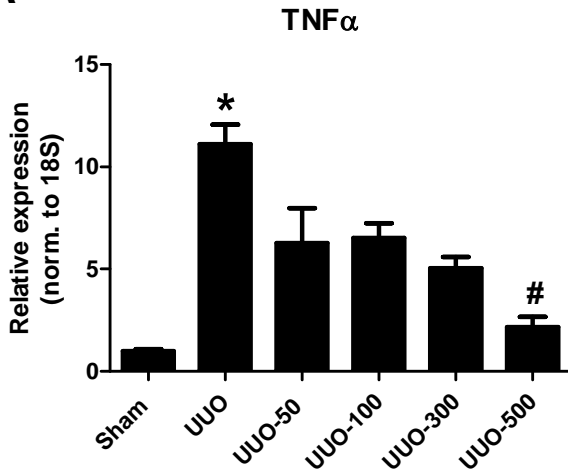
UUO mice were treated with metformin (500 mg/kg/day) after UUO damage. The kidneys were processed for qPCR analysis (n = 4-5 mice in each group) to evaluate the different doses on the expression of inflammatory, injury and oxidative stress markers. Regulation of the inflammatory and injury markers (A) TNF α , (B) MCP1, (C) KIM1, (D) NQO1 and (F) ICAM1 in mice subjected to 3dUUO and metformin treatment normalized to ribosomal 18S RNA. Each bar represents the mean \pm SEM. *P<0.05 compared to the sham group. # P<0.05 compared to the UUO group.

Data demonstrated that metformin administered at a dose of 500 mg/kg/day after induction of UUO had some renoprotective effects in mice subjected to 3dUUO.

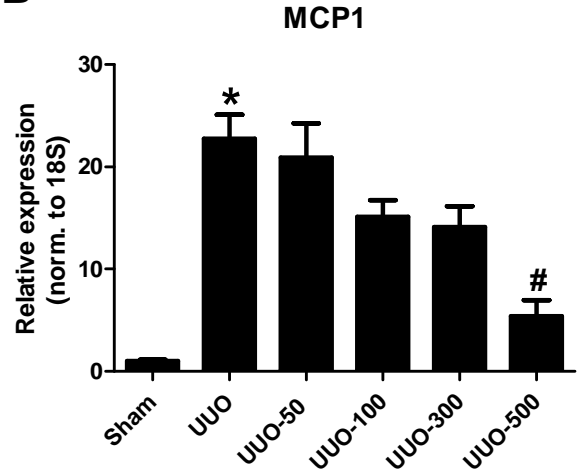
UUOpMET: UUO mice were pretreated with metformin. UUOMet: UUO mice were treated with metformin after UUO damage.

Supplementary figure 2

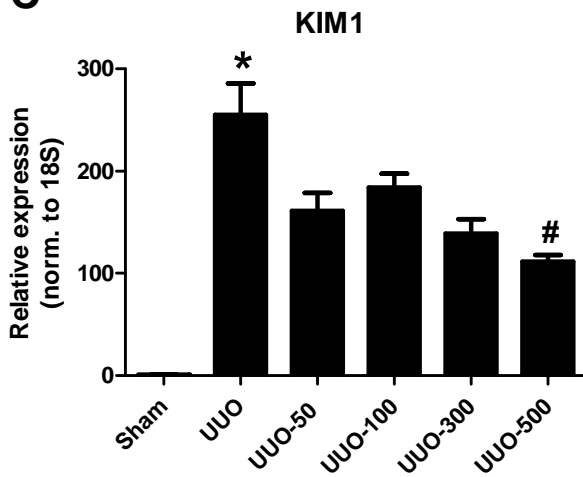
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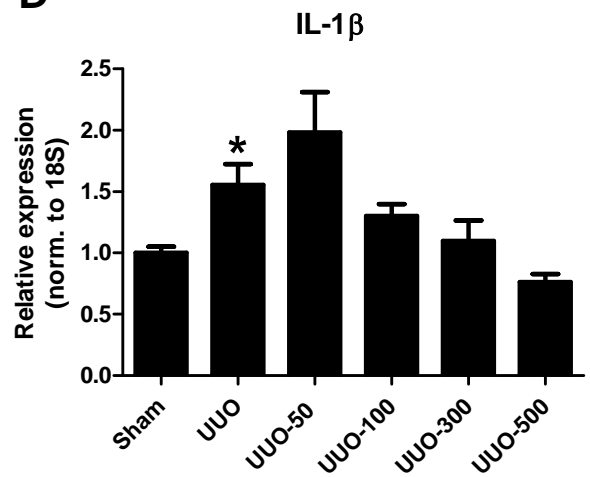
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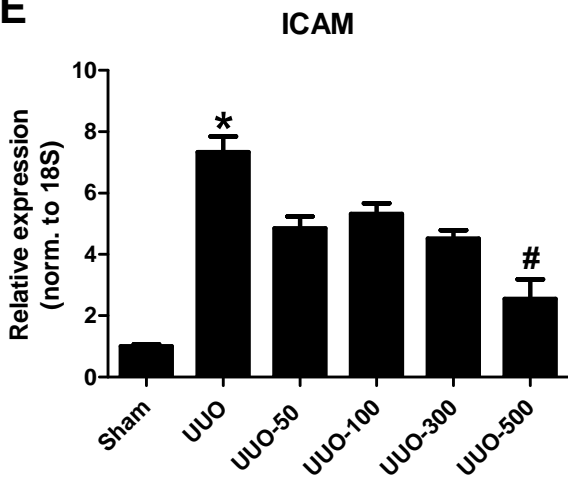
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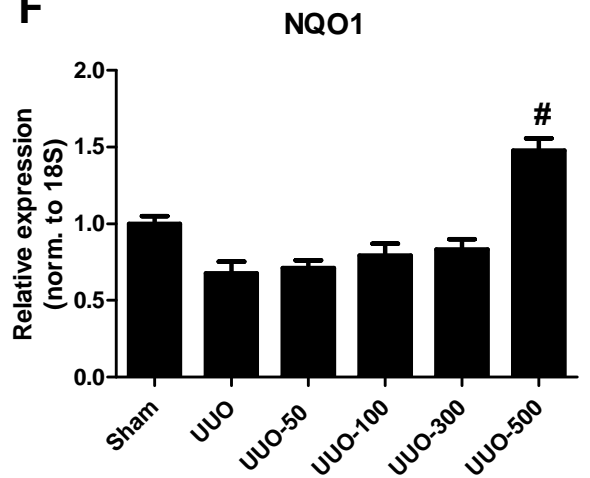
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E



F

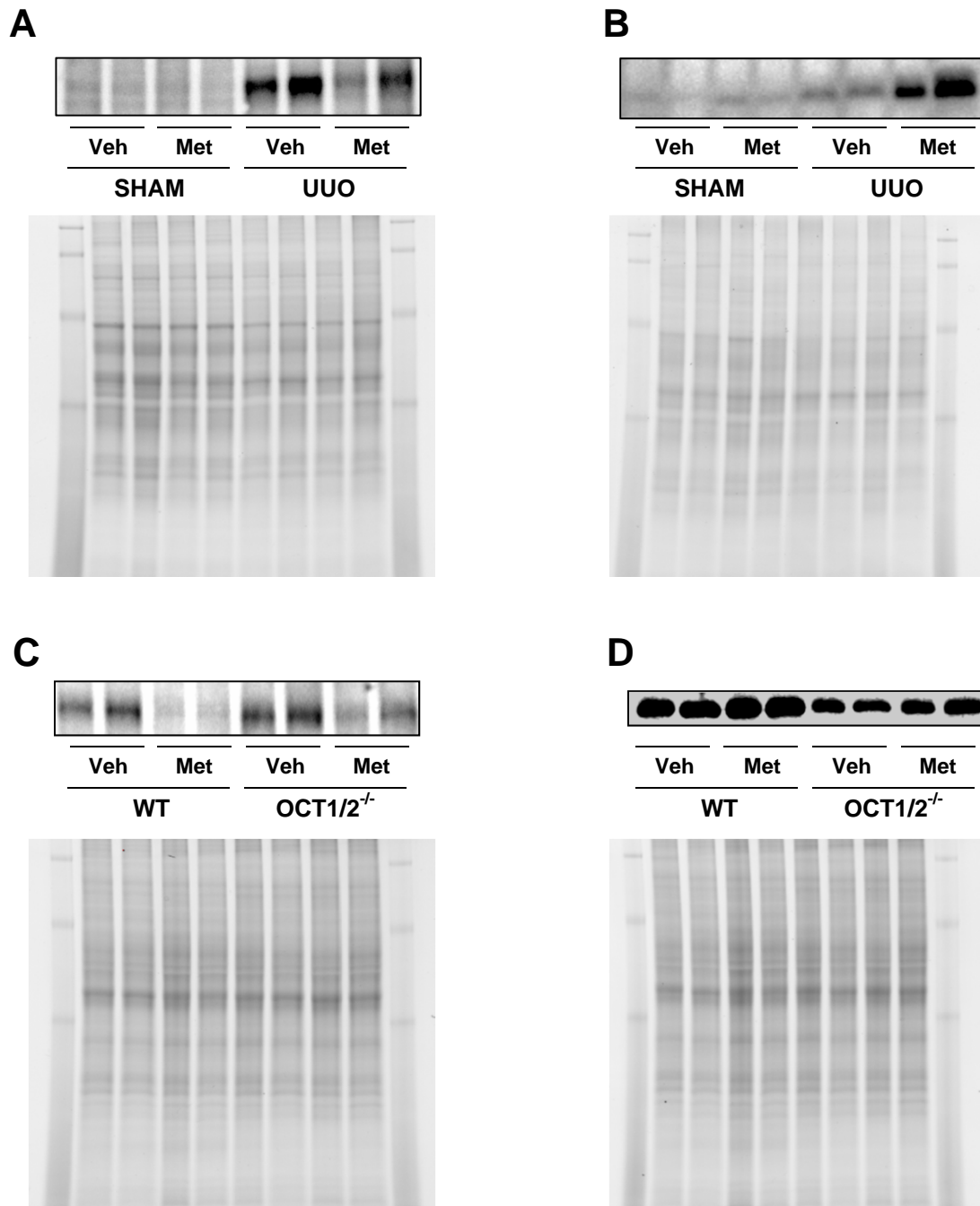


Supplementary figure 2: Dose-response experiment in mice subjected to 3 days unilateral ureteral obstruction (UUO) with different doses of metformin.

UUO mice were administered 4 different doses of metformin (50, 100, 300 and 500 mg/kg/day). The kidneys were processed for qPCR analysis (n = 4-5 mice in each group) to evaluate the different doses on the expression of inflammatory, injury and oxidative stress markers. Regulation of the inflammatory and injury markers (A) TNF α , (B) MCP1, (C) KIM1, (D) IL-1 β , (E) ICAM and the oxidative stress marker (F) NQO1 in mice subjected to 3dUUO and metformin treatment normalized to ribosomal 18S RNA. Each bar represents the mean \pm SEM. *P<0.05 compared to the sham group. # P<0.05 compared to the UUO group.

Data demonstrated that metformin administered at a dose of 500 mg/kg/day reduced significantly renal inflammation, renal injury and oxidative stress in mice subjected to 3dUUO whereas the lower doses had no significant effect.

Supplementary figure 3



Supplementary figure 3 – Total protein gels for western blots in figure 3, 4 and 5. Western blots with corresponding total proteins. A: KIM-1 from figure 3, B: HO-1 from figure 4, C: KIM-1 from figure 5 and D: HO-1 from figure 5.