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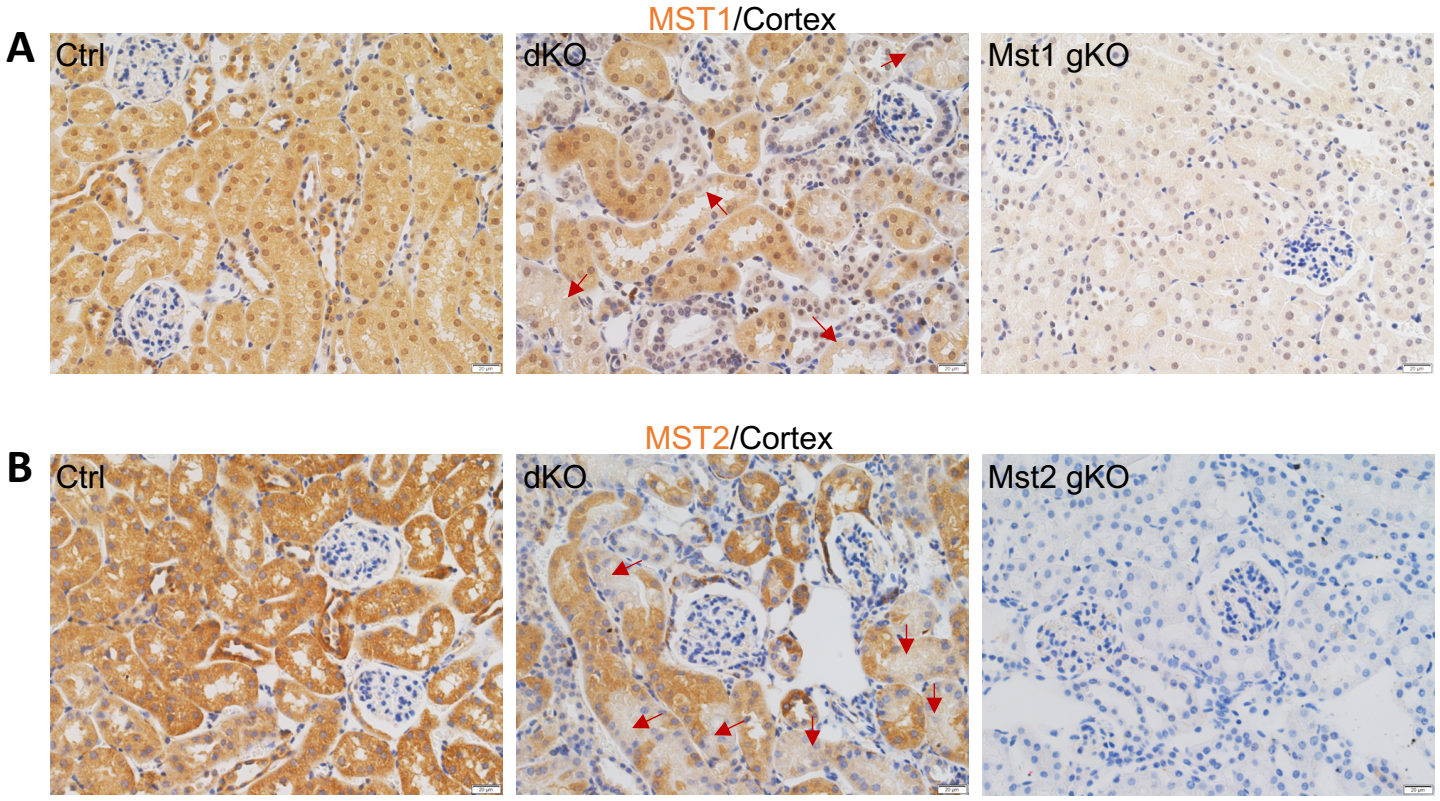
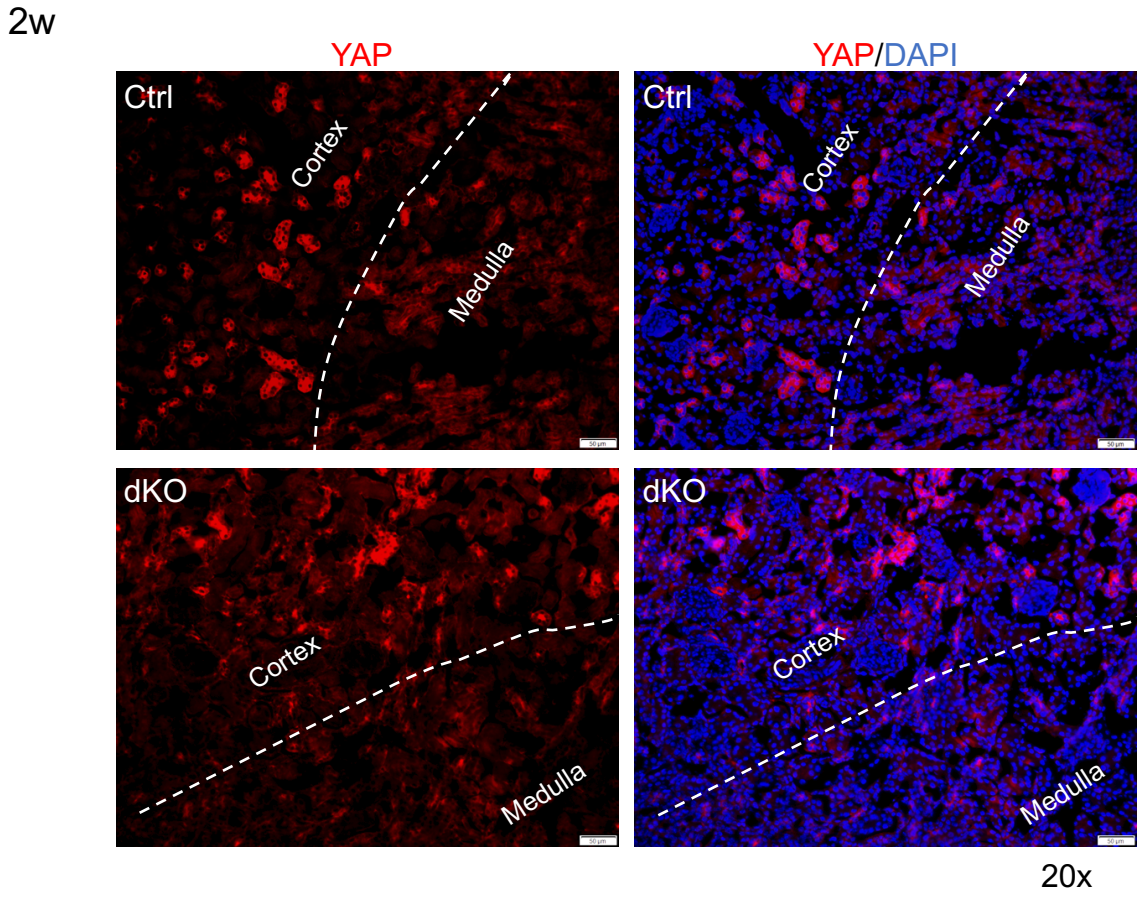
Supplemental Figure 1



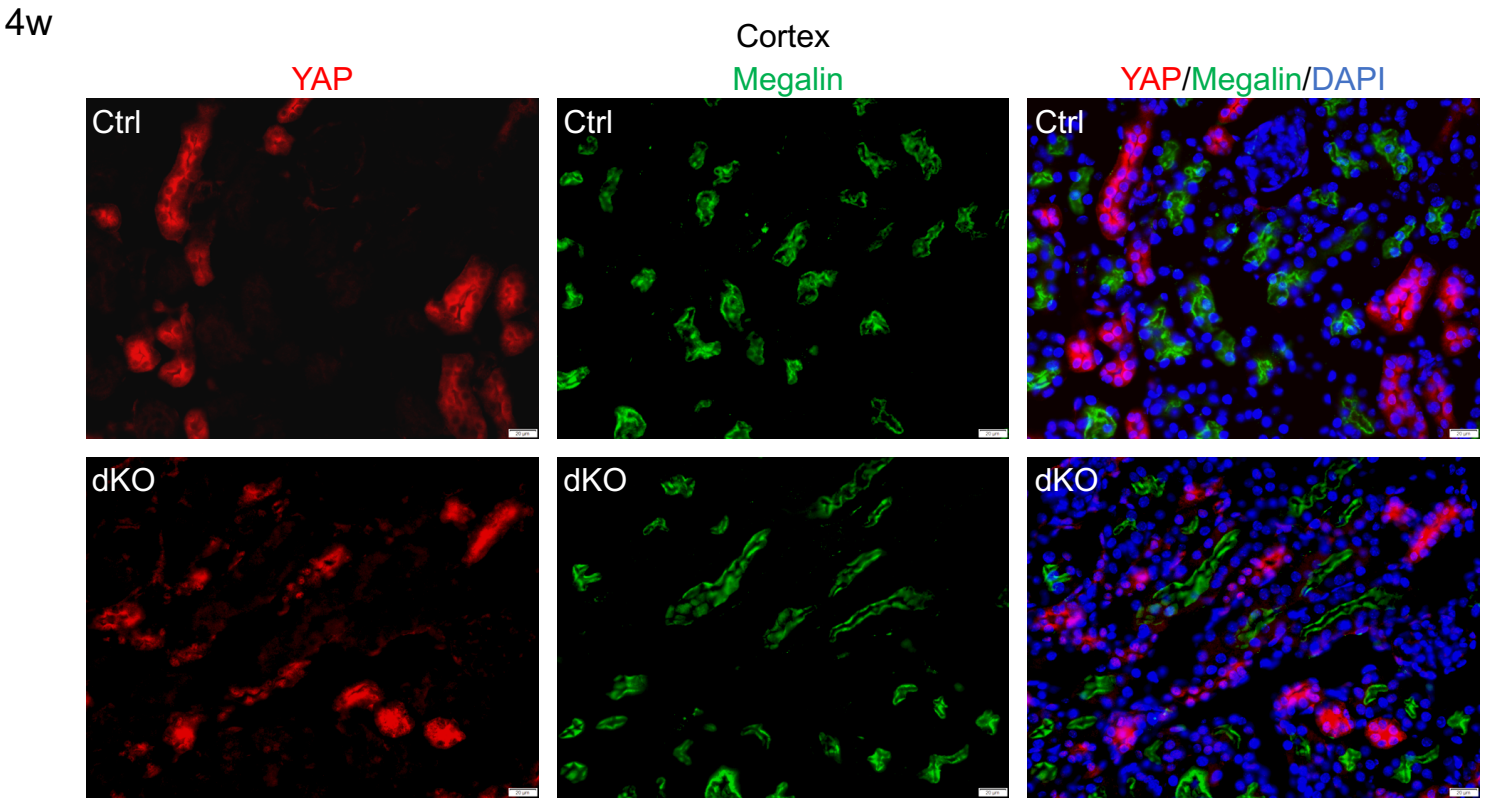
Supplemental Figure 1. Immunohistochemistry for MST1 and MST2 in control and Mst1/2 dKO kidneys. Paraffin kidney sections from 8-week old mice were used for immunohistochemistry for MST1 (A) and MST2 (B) staining (brown). Mst1 global KO (gKO) and Mst2 gKO kidneys were used as respective negative controls. Cortical regions are shown. Red arrows point to clusters of proximal cells, which do not express MST1 (A) or MST2 (B).

Supplemental Figure 2



Supplemental Figure 2. YAP localization in control and Mst1/2 dKO kidneys at 2 weeks of age. Frozen kidney sections from Ctrl and Mst1/2 dKO mice at 2 weeks of age were used for immunofluorescence for YAP (red). DAPI was used to stain nuclei. Cortex and medulla were labelled.

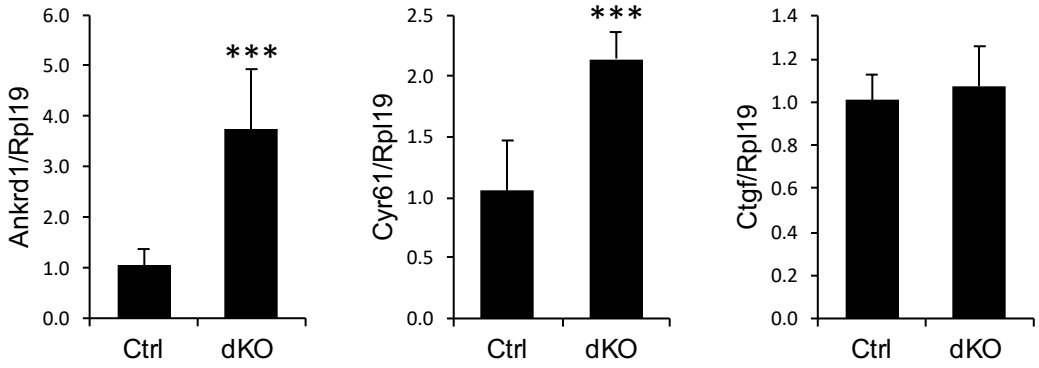
Supplemental Figure 3



Supplemental Figure 3. YAP localization in control and Mst1/2 dKO kidneys at 4 weeks of age. Frozen kidney sections from Ctrl and Mst1/2 dKO mice at 4 weeks of age were used for immunofluorescence for YAP (red) and megalin (a marker for the proximal tubule) (green). DAPI was used to stain nuclei. The cortical regions are shown.

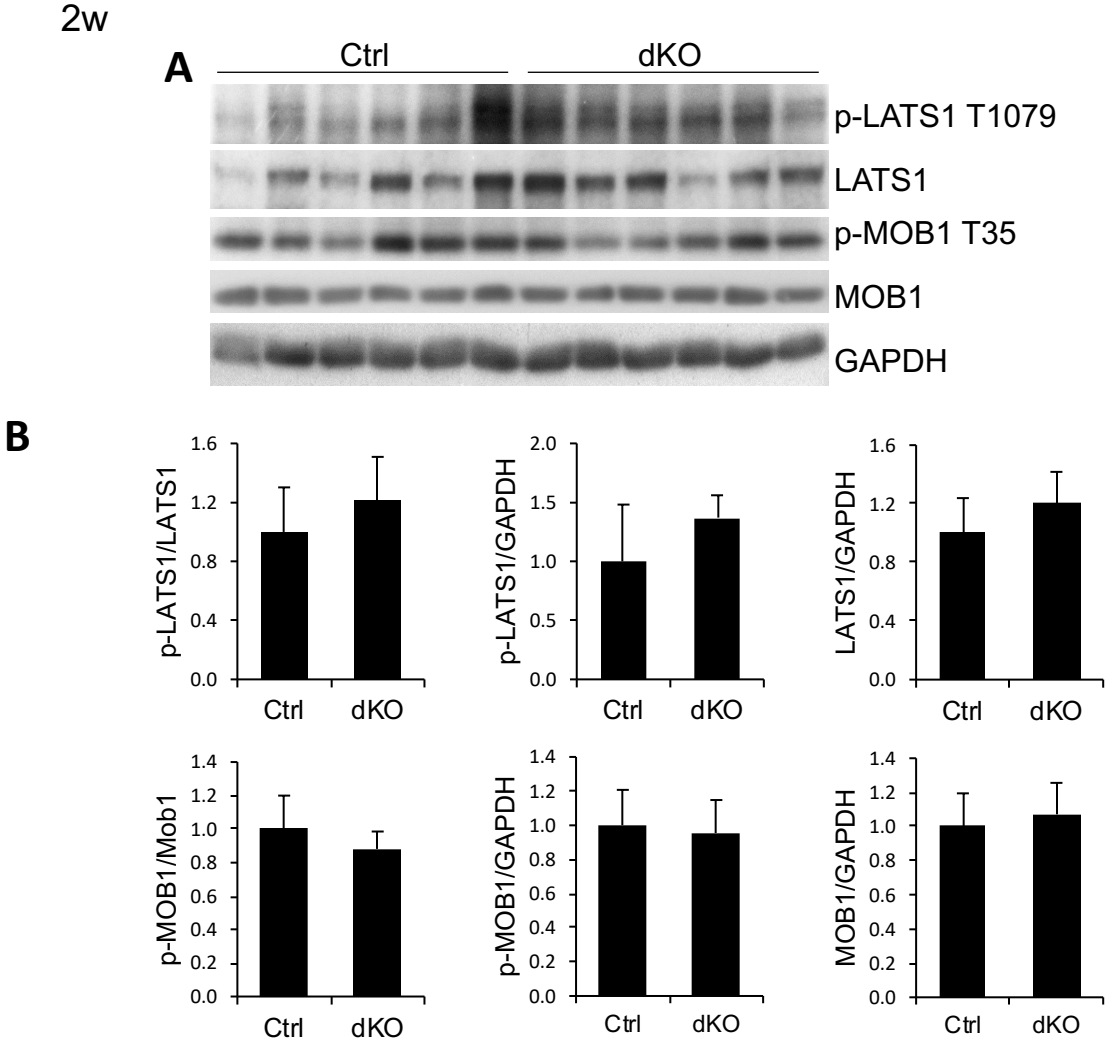
Supplemental Figure 4

Female
4w



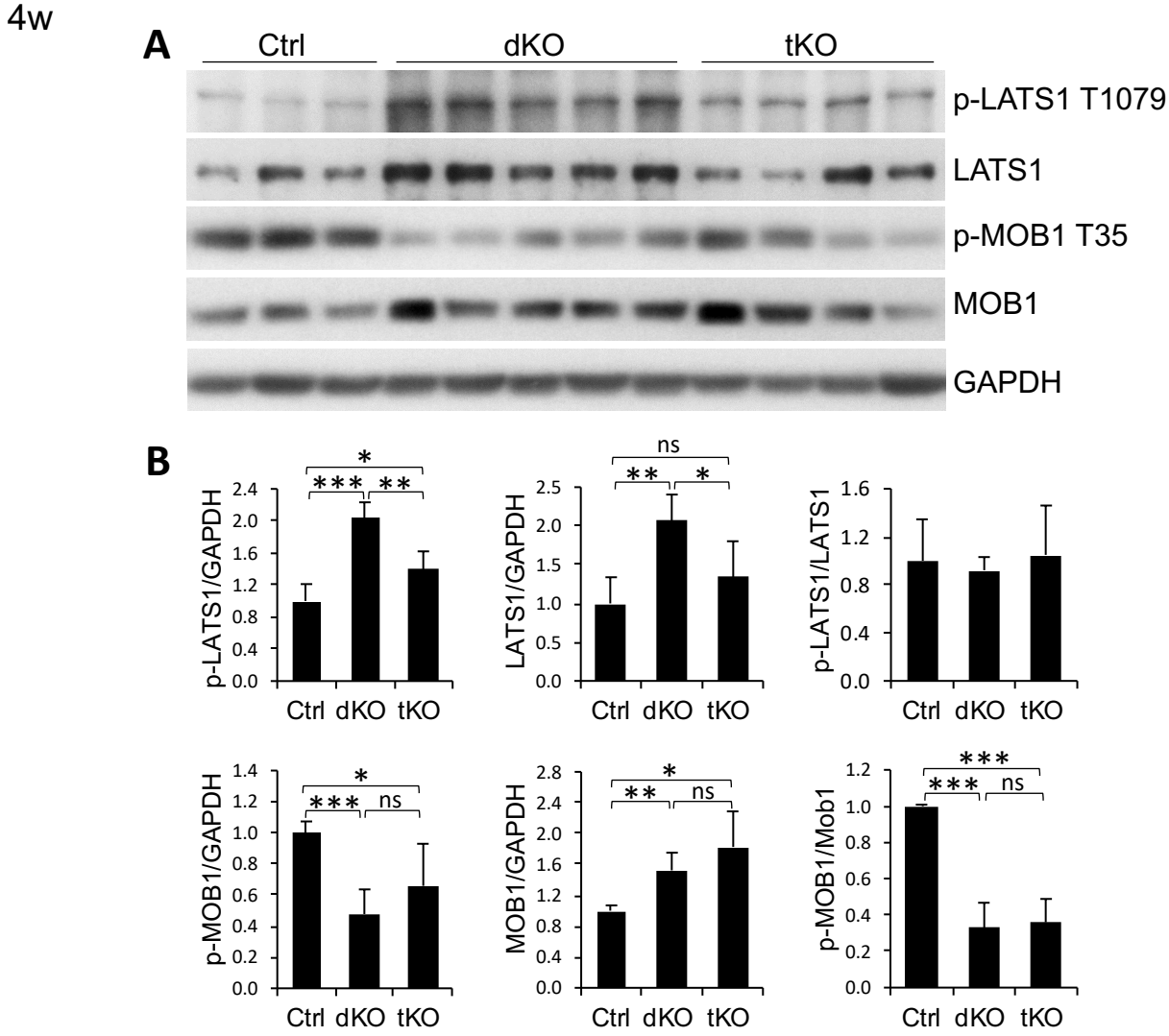
Supplemental Figure 4. Expression of the YAP target genes Ankrd1, Cyr61 and Ctgf in female control and Mst1/2 dKO kidneys at 4 weeks of age. Kidneys collected from female mice were analyzed for Ankrd1, Cyr61 and Ctgf mRNA levels by real-time PCR. *** $P < 0.001$. n = 5.

Supplemental Figure 5



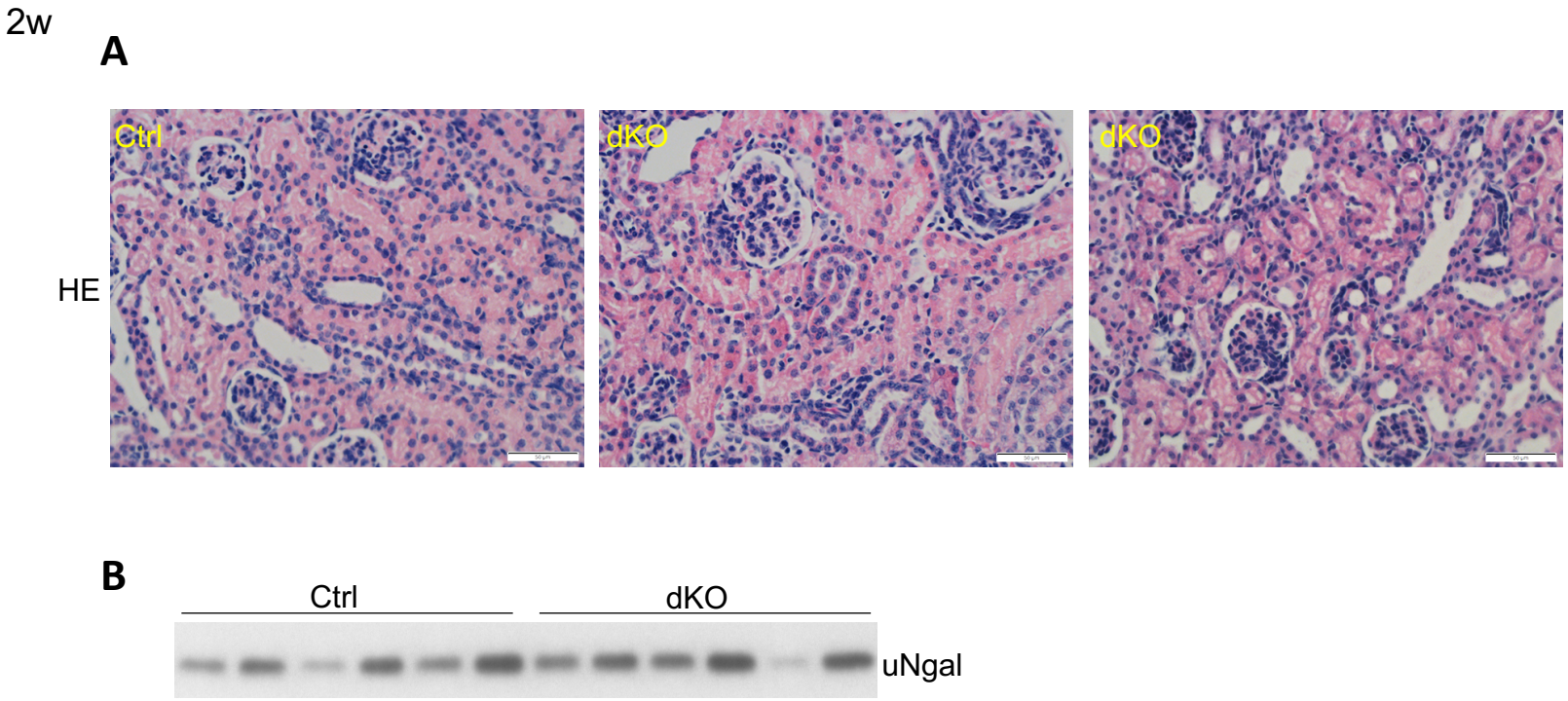
Supplemental Figure 5. Expression of phosphorylated and total LATS1 and MOB1 in the kidneys of control and Mst1/2 dKO mice at 2 weeks of age. (A) Kidney lysates from Ctrl and Mst1/2 dKO mice at 2 weeks of age (male) were used for Western blotting for phospho-LATS1, LATS1, phospho-MOB1 and MOB1. (B) Quantitative analysis was performed.

Supplemental Figure 6



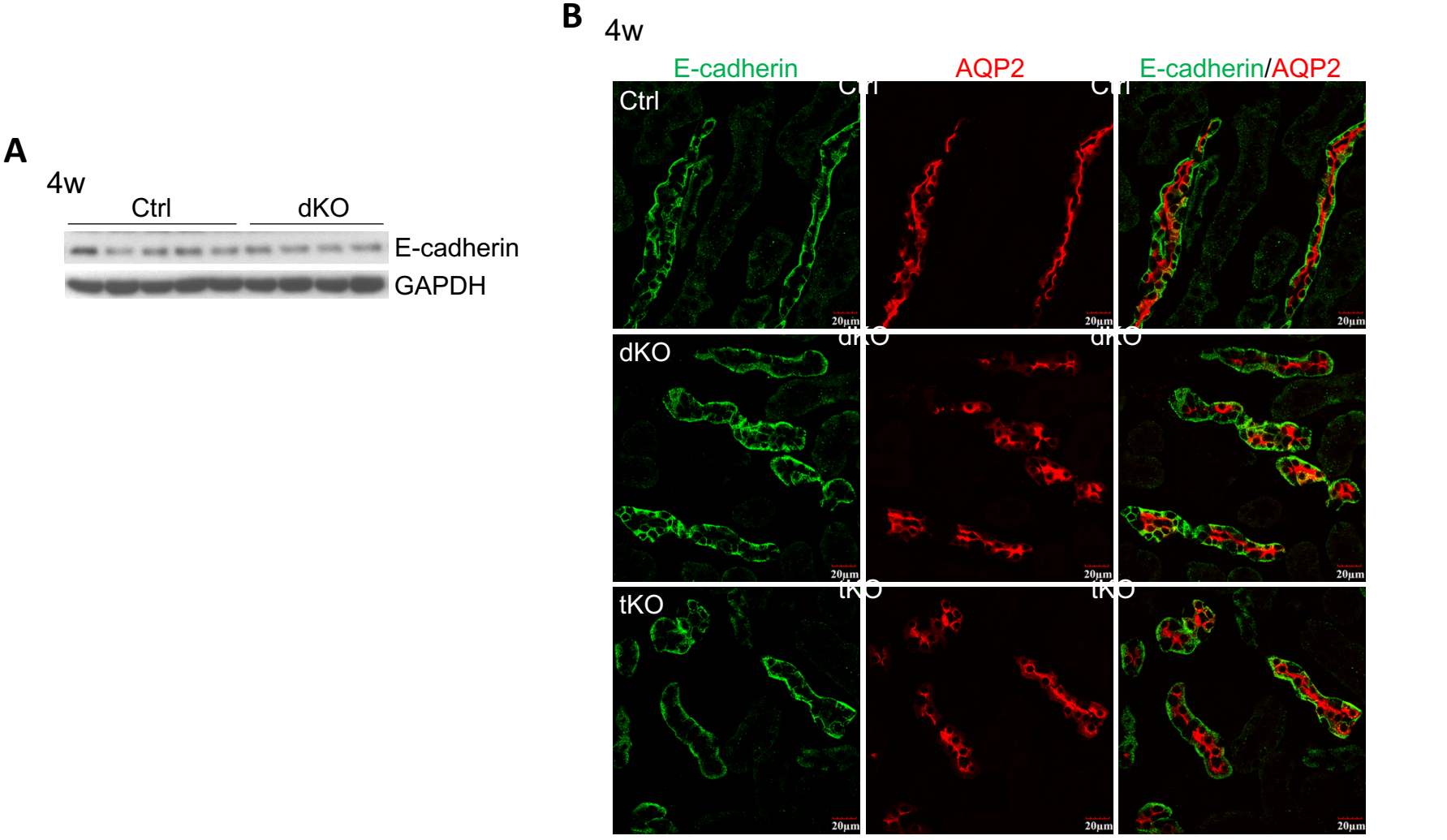
Supplemental Figure 6. Expression of phosphorylated and total LATS1 and MOB1 in the kidneys of control, Mst1/2 dKO and Mst1/Mst2/Yap tKO mice at 4 weeks of age. (A) Kidney lysates from Ctrl, Mst1/2 dKO and Mst1/Mst2/Yap tKO mice at 4 weeks of age (male) were used for Western blotting for phospho-LATS1, LATS1, phospho-MOB1 and MOB1. (B) Quantitative analysis was performed. **P* < 0.05; ***P* < 0.01; *** *P* < 0.001; ns, no significance.

Supplemental Figure 7



Supplemental Figure 7. Kidney structure and urinary Ngal levels in control and Mst1/2 dKO mice at 2 weeks of age. Kidney and urine samples were from Ctrl and Mst1/2 dKO mice at 2 weeks of age. Kidneys were used for H.E. staining (A), and urine samples were used for Western blotting for Ngal (uNgal) (B).

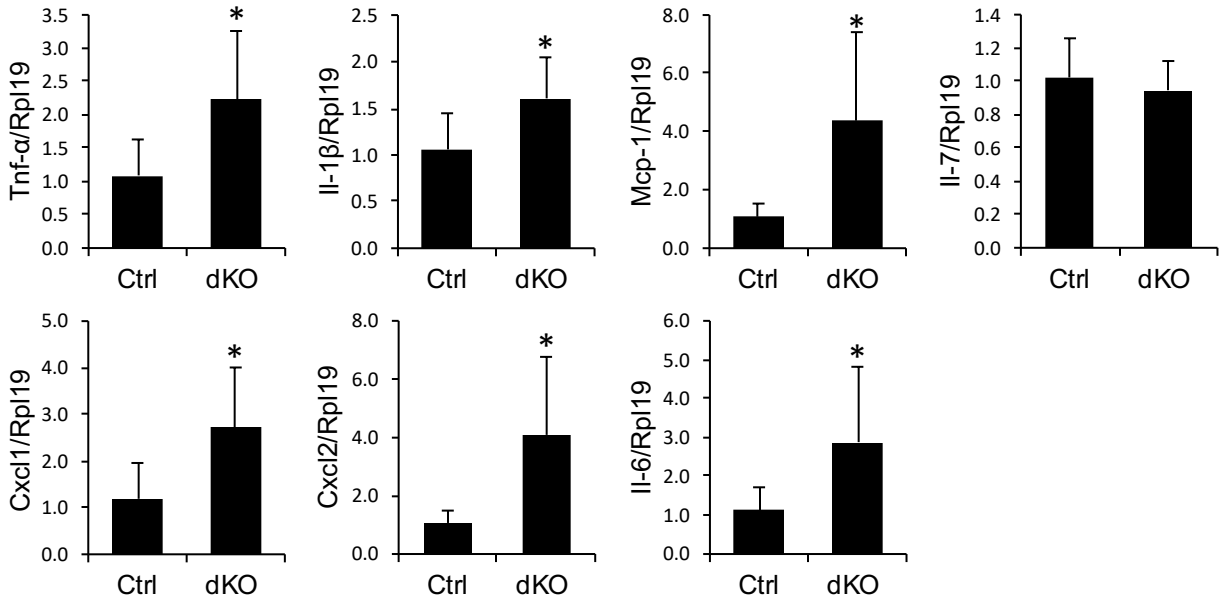
Supplemental Figure 8



Supplemental Figure 8. E-cadherin localization in control, Mst1/2 dKO and Mst1/Mst2/Yap tKO kidneys. (A) Western blotting for E-cadherin expression in Ctrl and Mst1/2 dKO mice at 4 weeks of age. GAPDH was used as the loading control. (B) Localization of E-cadherin in control, Mst1/2 dKO and Mst1/Mst2/Yap tKO kidneys. Frozen kidney sections from Ctrl, Mst1/2 dKO and Mst1/Mst2/Yap tKO mice were used for immunofluorescence for E-cadherin. Co-staining with AQP2, a marker for the principal cells of collecting ducts, is shown.

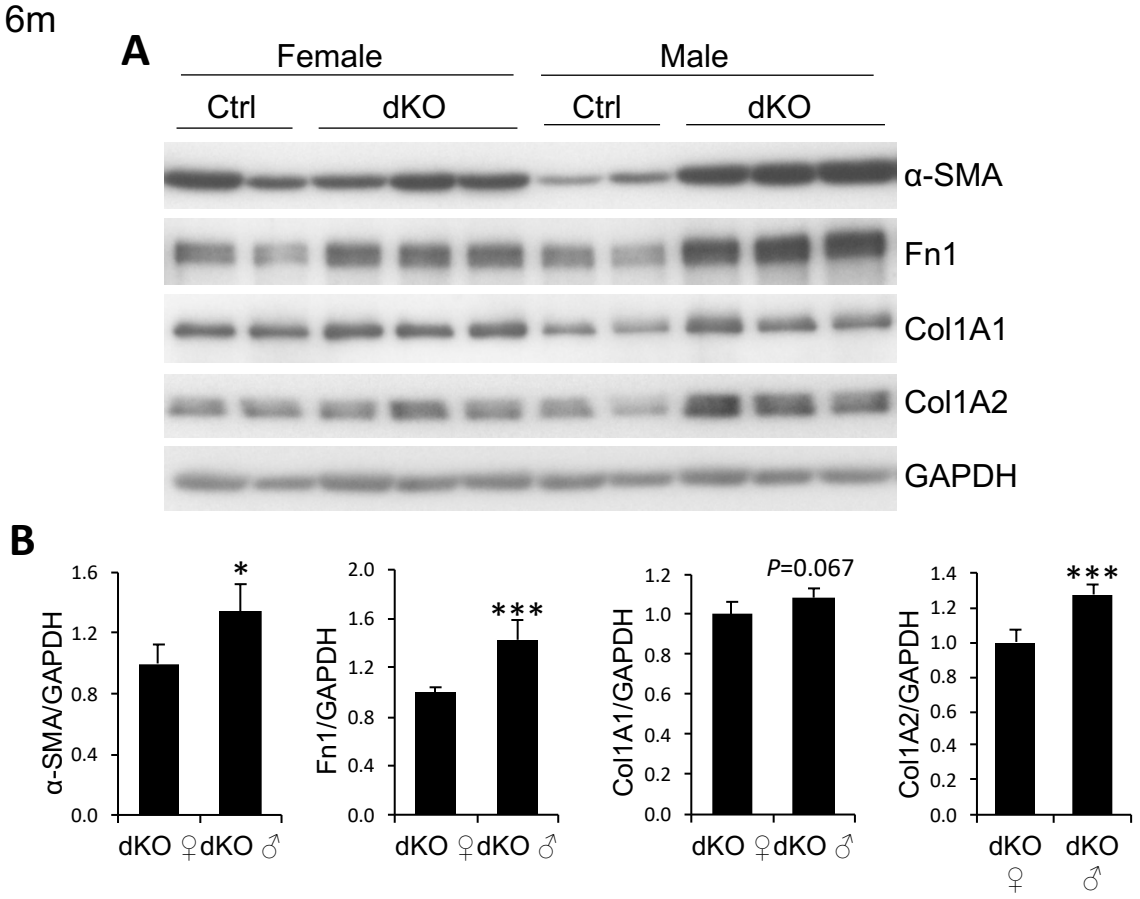
Supplemental Figure 9

Female
4w



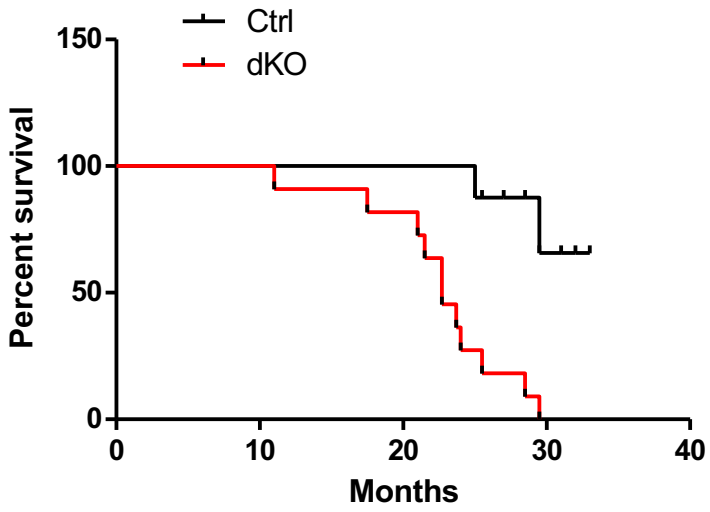
Supplemental Figure 9. Inflammation in Mst1/2 dKO kidneys in female mice. Expression of inflammatory factors in Mst1/2 dKO kidneys. Kidneys collected from female control and Mst1/2 dKO mice at 4 weeks of age were analyzed for Tnf-α, Il-1β, Mcp-1, Il-7, Cxcl1, Cxcl2 and Il-6 mRNA levels by real-time PCR. Rpl19 was used as internal control for real-time PCR. *$P < 0.05$. n = 5.

Supplemental Figure 10



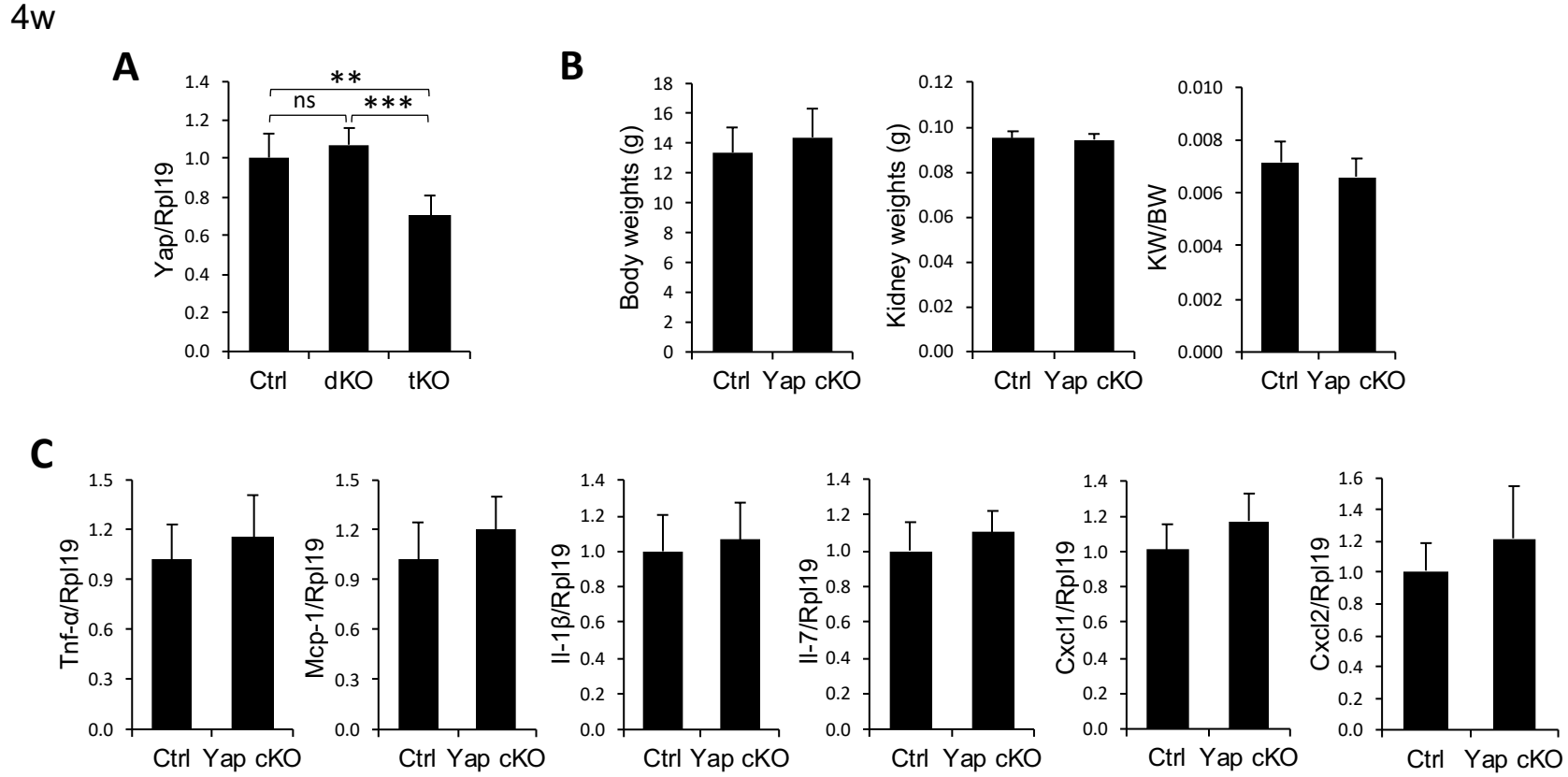
Supplemental Figure 10. Comparison in fibrotic responses in the kidney between male and female Mst1/2 dKO mice. (A) Expression of α-SMA, fibronectin 1 (Fn1) and type I collagen α (Col1A1 and Col1A2) in the kidneys of male and female control and Mst1/2 dKO mice at 6 months of age. GAPDH was used as loading control. (B) Quantitative analysis of α-SMA, Fn1, Col1A1 and Col1A2 protein levels was performed. **P* < 0.05; ****P* < 0.001.

Supplemental Figure 11



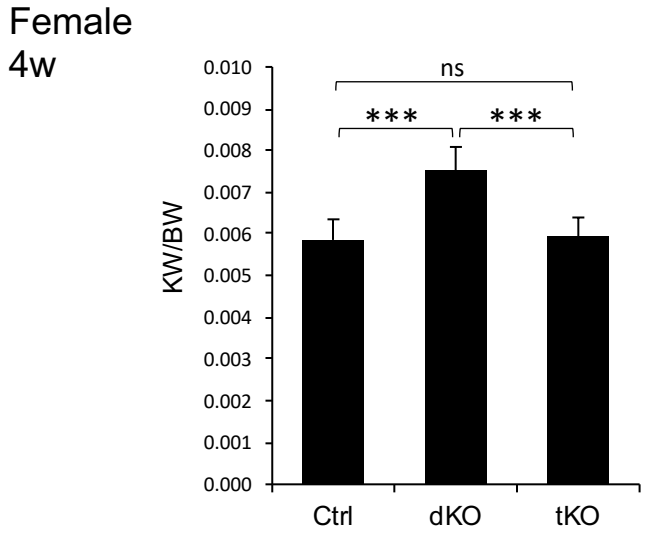
Supplemental Figure 11. Survival curves comparing control and Mst1/2 dKO mice. 8 Ctrl and 11 Mst1/2 dKO mice were used. *P* = 0.0004, Long-rank (Mantel-Cox) test.

Supplemental Figure 12



Supplemental Figure 12. Yap expression in Mst1/Mst2/Yap tKO mice and kidney sizes and inflammatory factors in tubule-specific Yap conditional knockout (cKO) mice at 4 weeks of age. (A) Yap mRNA levels in Ctrl, Mst1/2 dKO and Mst1/Mst2/Yap tKO kidneys. Kidney samples collected from male control, Mst1/2 dKO and Mst1/Mst2/Yap tKO mice at 4 weeks were used for real-time PCR analysis for Yap. (B) Body weight and kidney weight in Ctrl and Yap cKO mice. Body weights, kidney weights and ratios of kidney weights over body weights of male control and Yap cKO mice at 4 weeks of age are presented. (C) Expression of inflammatory factors in Yap cKO kidneys. Kidneys collected from male control and Yap cKO mice at 4 weeks were analyzed for Tnf-α, Mcp-1, Il-1β, Il-7, Cxcl1 and Cxcl2 by real-time PCR. n = 5 for panels A-C. Rpl19 was used as the internal control. $^{**}P < 0.01$; $^{***}P < 0.001$; ns, no significance.

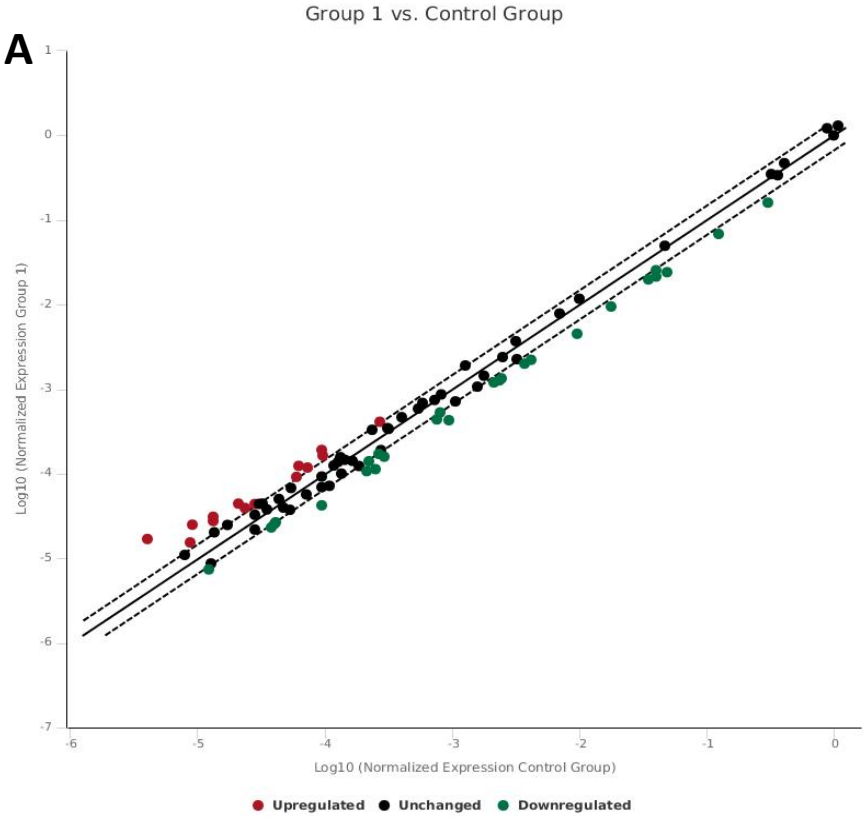
Supplemental Figure 13



Supplemental Figure 13. Ratios of kidney weight over body weight in female control, Mst1/2 dKO and Mst1/Mst2/Yap tKO mice. Female mice at 4 weeks of age were used to calculate the ratios of kidney weight over body weight. ***$P < 0.001$. n = 6/5/4 (Ctrl/dKO/tKO).

Supplemental Figure 14

A



B

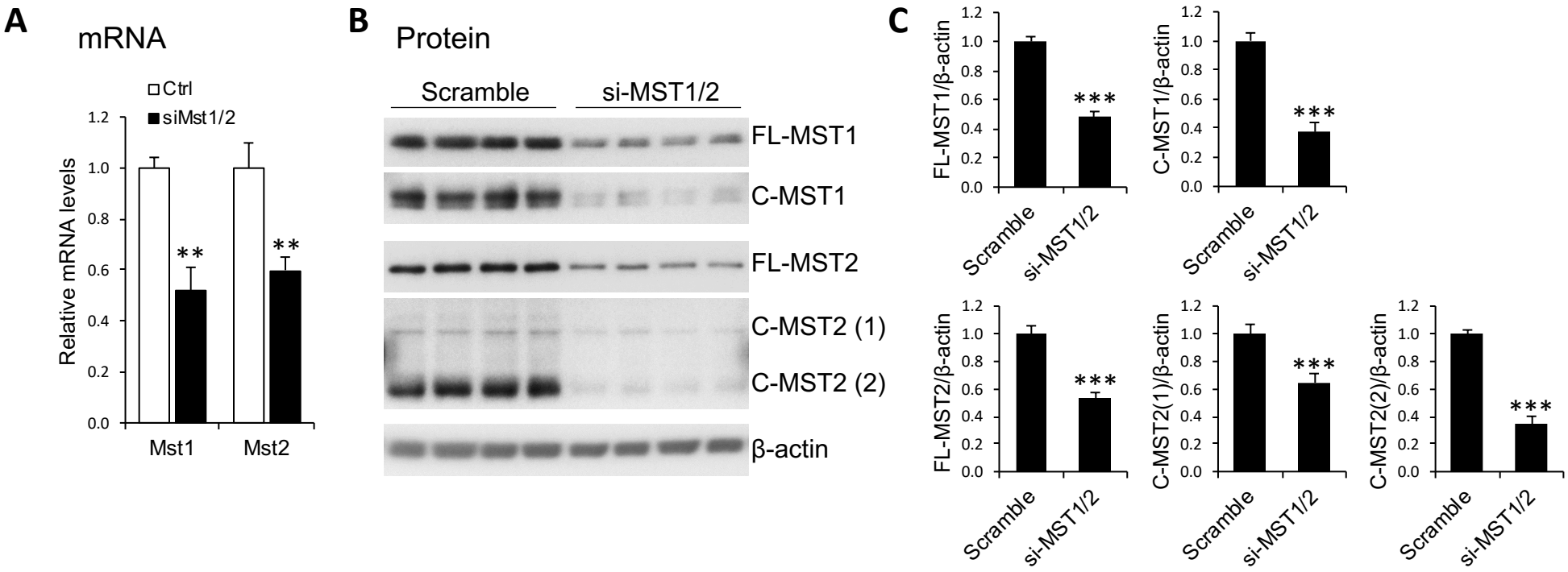
Genes Over-Expressed in Group 1 vs. Control Group		
Gene Symbol	Fold Regulation	Comments
Il3	4.19	B
Il24	2.75	B
Il22	2.35	B
Lta	2.13	A
Il6	2.11	B
Lif	2.04	
Ccl20	1.98	
Il21	1.76	B
Tnf	1.71	
Nodal	1.68	A
Il1rn	1.64	
Il9	1.56	A
Il4	1.53	
Ltb	1.52	

C

Genes Under-Expressed in Group 1 vs. Control Group		
Gene Symbol	Fold Regulation	Comments
Cxcl9	-2.24	
Cxcl11	-2.21	
Ccl5	-2.17	
Il15	-2.1	
Bmp4	-2	
Il12a	-1.98	
Gpi1	-1.89	
Bmp7	-1.88	
Bmp6	-1.87	
Ccl19	-1.86	
Bmp2	-1.85	
Ccl17	-1.84	
Ctf1	-1.83	
Il16	-1.81	
Cxcl12	-1.79	
Cntf	-1.78	
Cxcl16	-1.74	
Ccl11	-1.71	
Cd70	-1.64	B
Il17a	-1.64	B
Il11	-1.57	
Cx3cl1	-1.56	
Cd40lg	-1.55	B
Il10	-1.54	B
Ccl4	-1.52	
Ccl2	-1.51	

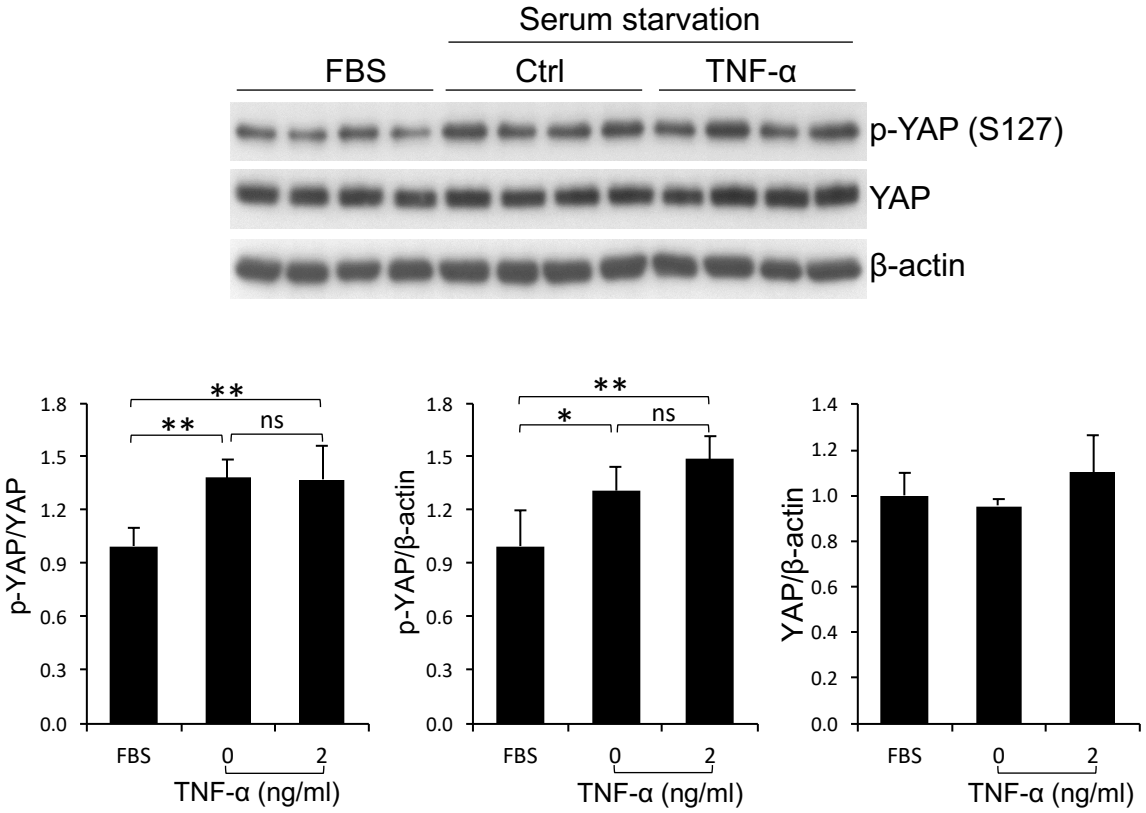
Supplemental Figure 14. PCR array analysis for inflammatory cytokines and chemokines in the kidneys of Mst1/2 dKO mice at 2 weeks of age. (A) The scatter plot compares the normalized expression of every gene on the array between Mst1/2 dKO (Group 1) and control kidneys. The central line indicates unchanged gene expression. The dotted lines indicate the selected fold regulation threshold (1.5). (B) Genes over-expressed in Mst1/2 dKO kidneys vs control kidneys. (C) Genes under-expressed in Mst1/2 dKO kidneys vs control kidneys. A, this gene's average threshold cycle is relatively high (> 30) in either the control or Mst1/2 kidneys, and is reasonably low in the other sample (< 30). B, this gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and Mst1/2 kidneys.

Supplemental Figure 15



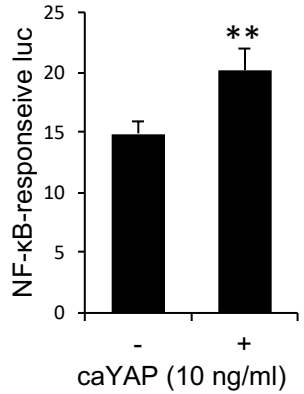
Supplemental Figure 15. Effects of Mst1 and Mst2 siRNAs on Mst1 and Mst2 expression in IMCD3 cells. Cells were transfected with control (Ctrl/Scramble) or Mst1/2 siRNAs (siMst1/2, 120 nM each). 24 h after transfection, cells were serum starved for 16 h before cells were harvested for real-time PCR analysis for Mst1 and Mst2 mRNA levels (A) or for Western blotting for MST1 and MST2 proteins (B). Quantitative analysis of full-length (FL-) and cleaved (C-) MST1 and MST2 protein levels was performed by densitometry (C). n = 3 for panel A. $^{**}P < 0.01$; $^{**}P < 0.001$.

Supplemental Figure 16



Supplemental Figure 16. Effects of a low dose of TNF-α on YAP activity in IMCD3 cells. Cells at 75-80% confluency were serum starved for 16 h before the cells were replaced with complete medium or treated with or without TNF-α at 2 ng/ml in FBS free DMEM containing 0.1% BSA, for 2 h. Cells were collected for Western blotting for phospho-YAP and total YAP. The band densities were quantified. *$P < 0.05$; **$P < 0.01$; ns, no significance.

Supplemental Figure 17



Supplemental Figure 17. Effects of caYAP on NF-κB responsive luciferase activity. IMCD3 cells were transiently transfected with an NF-κB responsive luciferase reporter, in combination with pTK-RL, with or without co-transfection with caYAP plasmid. 24 h after transfection, cells were serum starved for 16 h before the cells were analyzed for luciferase and renilla activities. n = 4. $^{**}P < 0.01$.