Supplemental Data

Wnt4/β–catenin signaling in medullary kidney myofibroblasts

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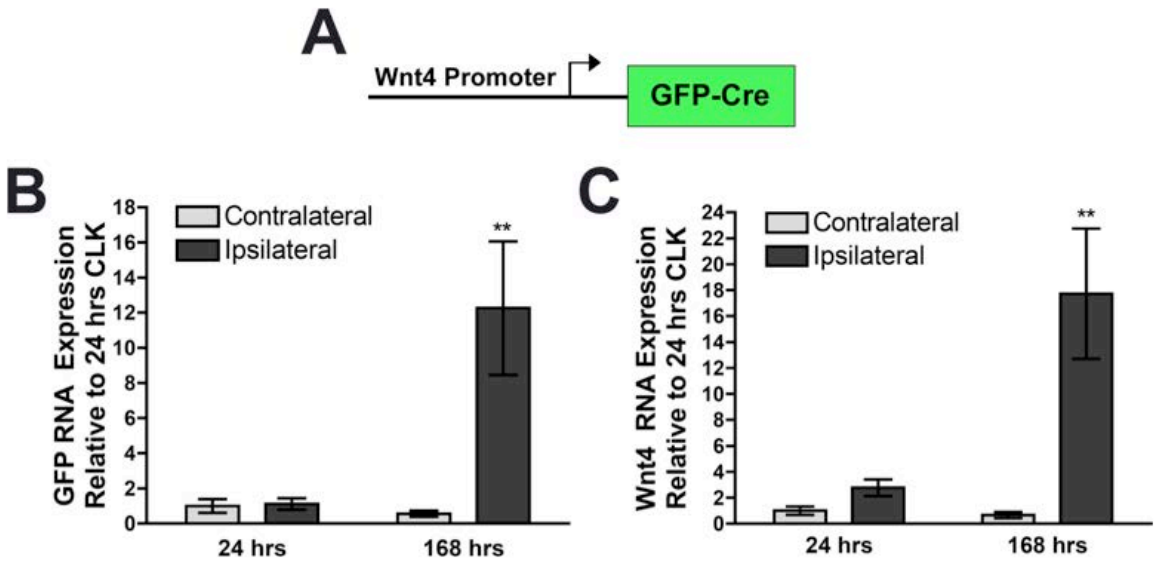

Figure S1. GFP transcript increases similarly to Wnt4 transcript in *Wnt4*$^{GC/+}$ reporter mice. (A) *Wnt4*$^{GC/+}$ have a GFP-Cre recombinase fusion protein knocked-in downstream of the promoter region on one allele of the Wnt4 locus. (B,C) GFP and Wnt4 (respectively) transcripts were significantly increased in injured kidney 7 days after U-IRI compared to contralateral kidney. Data analyzed by two-way ANOVA in B *and* C, $^{**}p < 0.01$ (Bonferroni's post-test). B and C, analyzed by t-test, $n = 3$ per group.

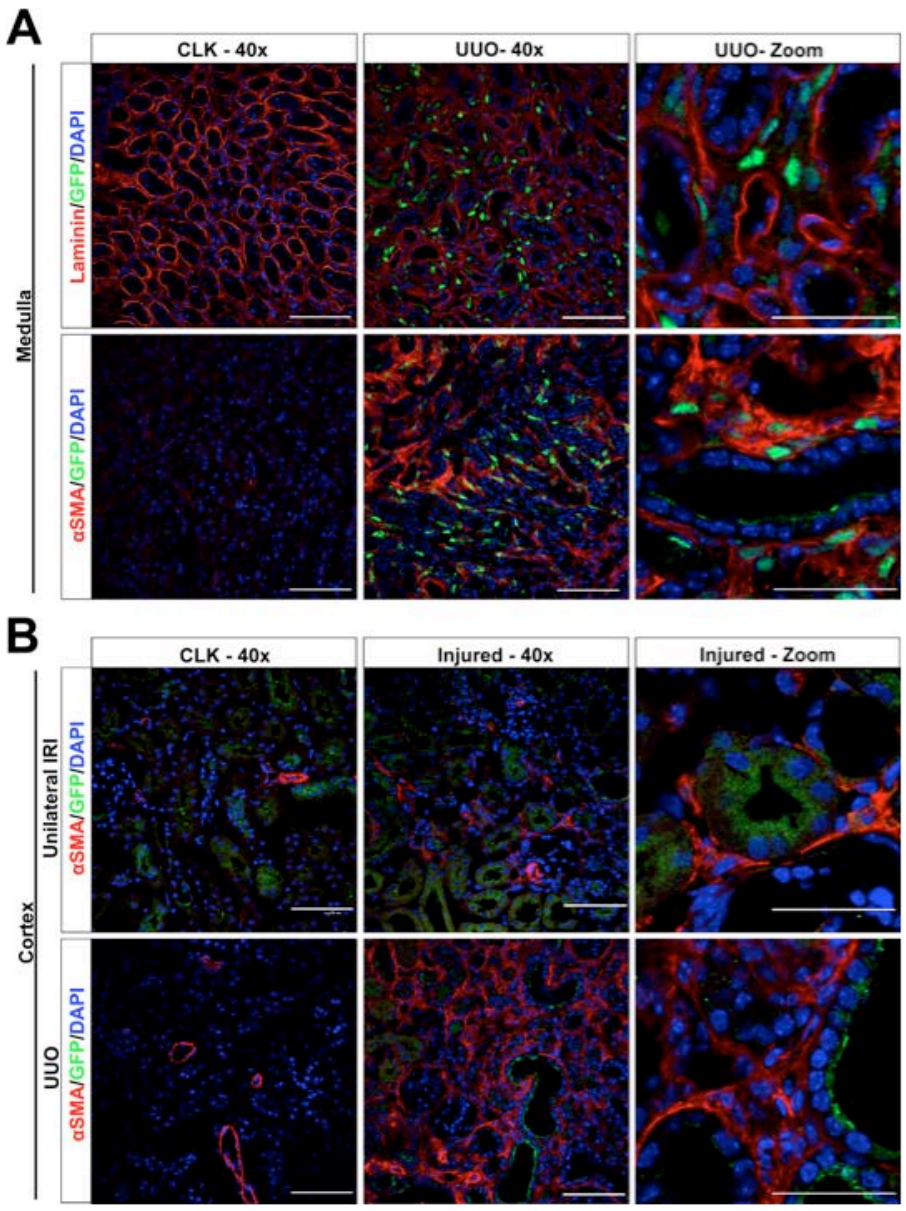

Figure S2. Wnt4 is increased in medullary interstitial myofibroblasts following unilateral ureteral obstruction and not in cortex during fibrosis. (A) 7 days Following UUO, CLK and injured kidney sections were stained with anti-GFP antibody in combination with anti-laminin and anti-αSMA. GFP+ cells are located outside of tubules in αSMA+ medullary myofibroblasts. (B) immunofluorescent staining of cortex in CLK and UUO and U-IRI kidney 7 days after injury. Co-staining with anti-GFP and anti-αSMA antibodies indicate that while there are αSMA+ myofibroblasts in the cortex, they are negative for GFP. Scale bars = 50μM.

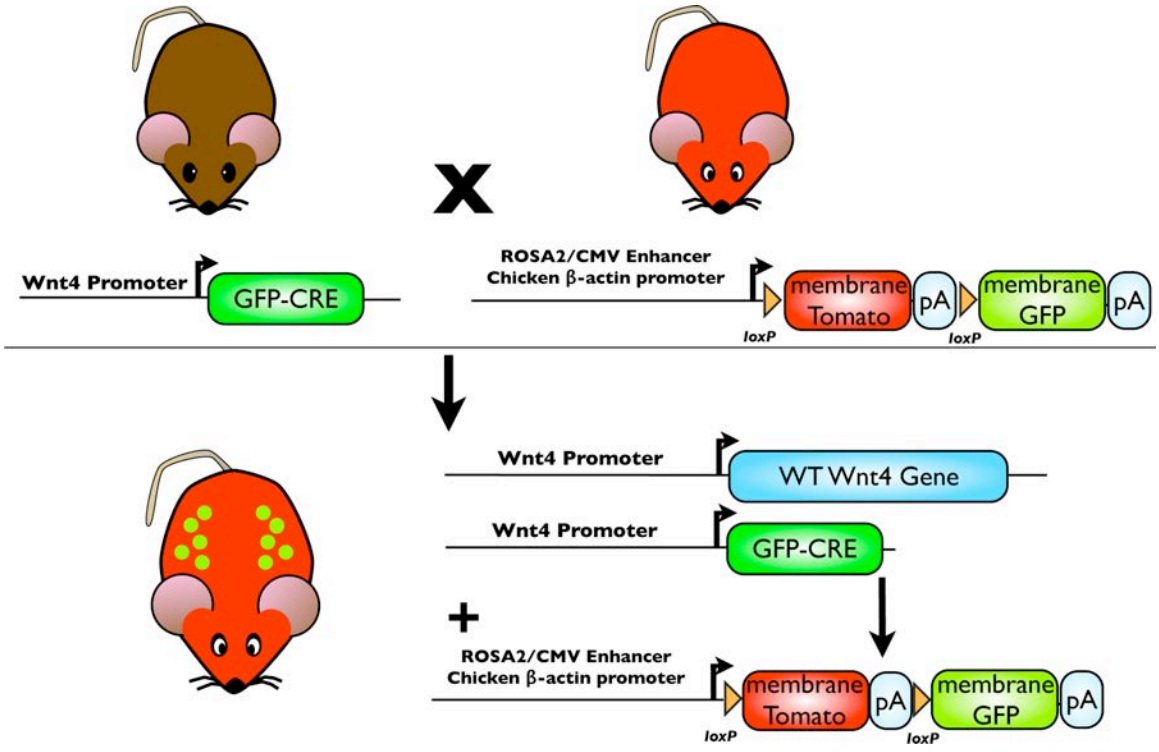

Figure S3. Breeding strategy to generate *Wnt4*$^{GC/+}$*;R26*$^{mTmG/+}$ mice**.** *Wnt4*$^{GC/+}$ were crossed with *R26*$^{mTmG/mTmG}$ to yield bigenic *Wnt4*$^{GC/+}$*;R26*$^{mTmG/+}$ mice. The Wnt4 locus will have one WT allele and one allele with a GFP-Cre recombinase fusion protein knocked in downstream of the Wnt4 promoter. In cells that have expressed Wnt4 throughout the lifetime of the mouse, the GC fusion protein will excise the floxed membrane-tomato gene and those cells were then permanently express membrane-GFP.

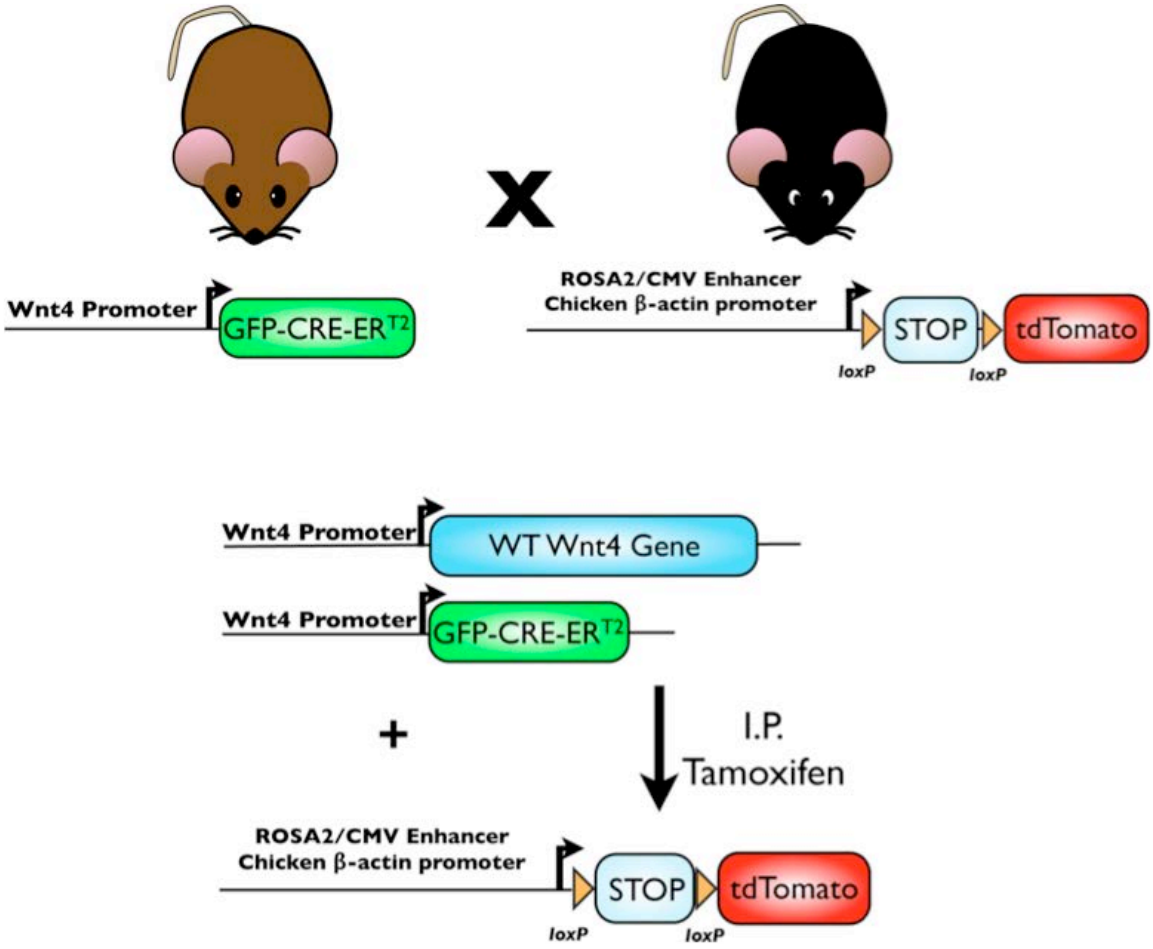

Figure S4. Breeding strategy to generate *Wnt4*$^{GCE/+}$;*R26*$^{tdTomato/+}$ mouse. *Wnt4*$^{GCE/+}$ were crossed with *R26*$^{tdTomato/tdTomato}$ to yield bigenic *Wnt4*$^{GCE/+}$;*R26*$^{tdTomato/+}$ mice. The Wnt4 locus will have one WT allele and one allele with a GFP-Cre recombinase-ER^{T2} fusion protein knocked in downstream of the Wnt4 promoter. Following injection of tamoxifen, the fusion protein will translocate to the nucleus and excise a floxed *STOP* codon upstream of the *tdTomato* gene and all cells expressing Wnt4 at the time of tamoxifen injection will be permanently labeled with tdTomato.

10x
40x
80x
Choroid Plexus
Tomato/PDGFRß/DAPI
Ureteral Stroma/Epithelia
Tomato/CK18/DAPI
180x
Hepatic Stellate Cells
Tomato/CK18/DAPI
Tomato/PDGFRß/DAPI

Figure S5. *Wnt4*$^{GCE/+}$;*R26*$^{tdTomato/+}$ reporter mice faithfully reproduce Wnt4 expression reported in hepatic stellate cells of the liver and cells in the ureter as well as identfies new cells in the choroid plexus. Brain sections from *Wnt4*$^{GCE/+}$;*R26*$^{tdTomato/+}$ mice were stained with PDGFRβ to identify pericytes lining capillary loops in the choroid plexus of the third ventricle. tdTomato is expressed in these PDGFRβ+ cells in this part of the brain. In the bladder, Cytokeratin18 demarcates the epithelial cell boundary and tdTomato+ cells are seen within the boundary and presumably in stromal cells outside of the epithelial cell layers. In the liver staining with CK18 shows that tdTomato+ reside in interstitial space and appear to wrap around capillaries to interact with endothelial cells with dendritic-like projections. Staining liver sections with PDGFRβ shows that tdTomato+ cells colocalize with this marker. Error bars in 10x, 40x, and 80x are 100μM, 50μM, and 10μM, respectively. Error bars in 180x images are 10μM.

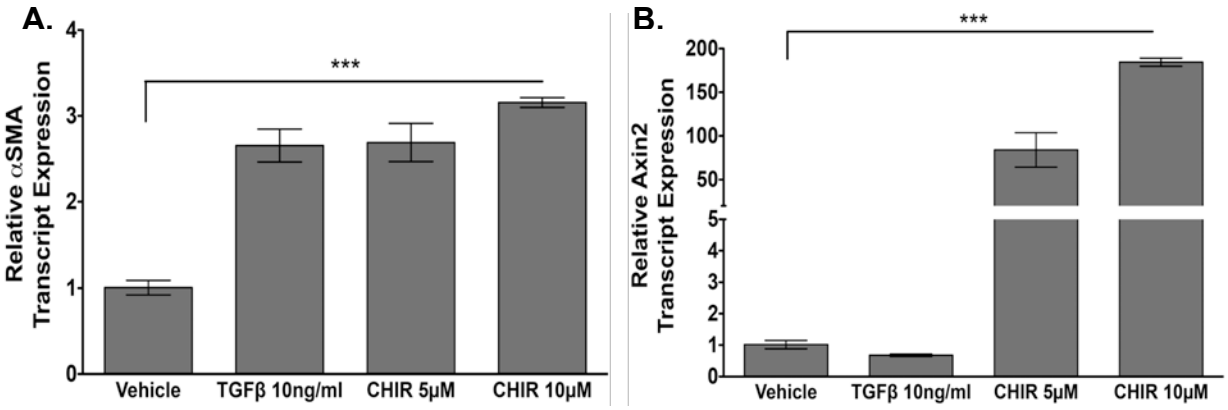

Figure S6. Treatment of 10T1/2 cells with TGFβ and CHIR99021 increases αSMA expression. (A), TGFβ treatment and CHIR treatment at 5 and 10μM significantly, and comparably, increased αSMA levels. (B), CHIR also significantly increased the canonical β-catenin signaling pathway readout, Axin-2, as expected. Data analyzed by one-way ANOVA n = 3 per condition, ***$p < .0001$.

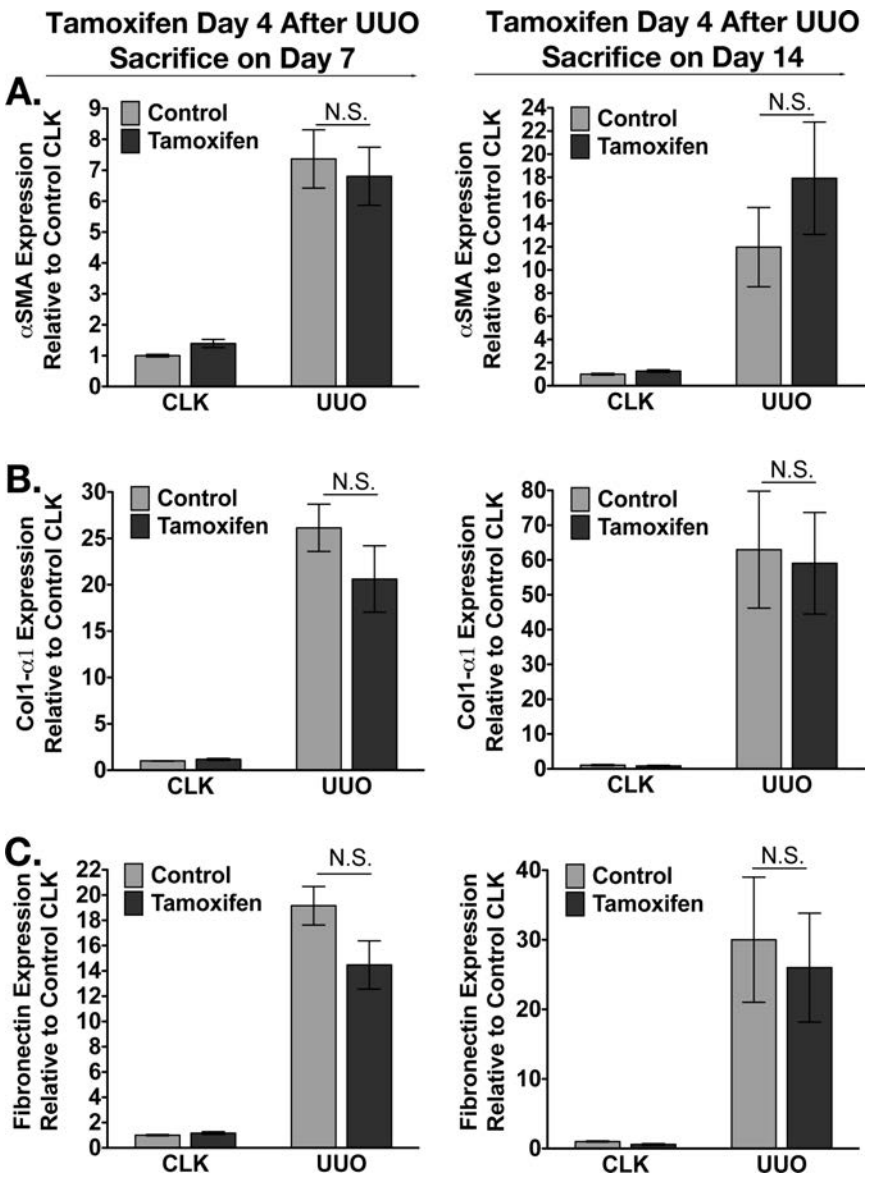

Figure S7. Tamoxifen treatment schedule does not effect fibrosis. Tamoxifen treatment schedule for this experiment replicates treatment schedule used for subsequent experiments. Tamoxifen treatment did not effect levels of αSMA (A), Col1-α1 (B), or fibronectin (C) on days 7 or 14 after UUO when 3mg of tamoxifen was administered I.P. on day 4 after UUO surgery. Data analyzed by two-way ANOVA n = 4 per group (control day 7, tamoxifen day 7, control day 14 and tamoxifen 15). NS = not significant.

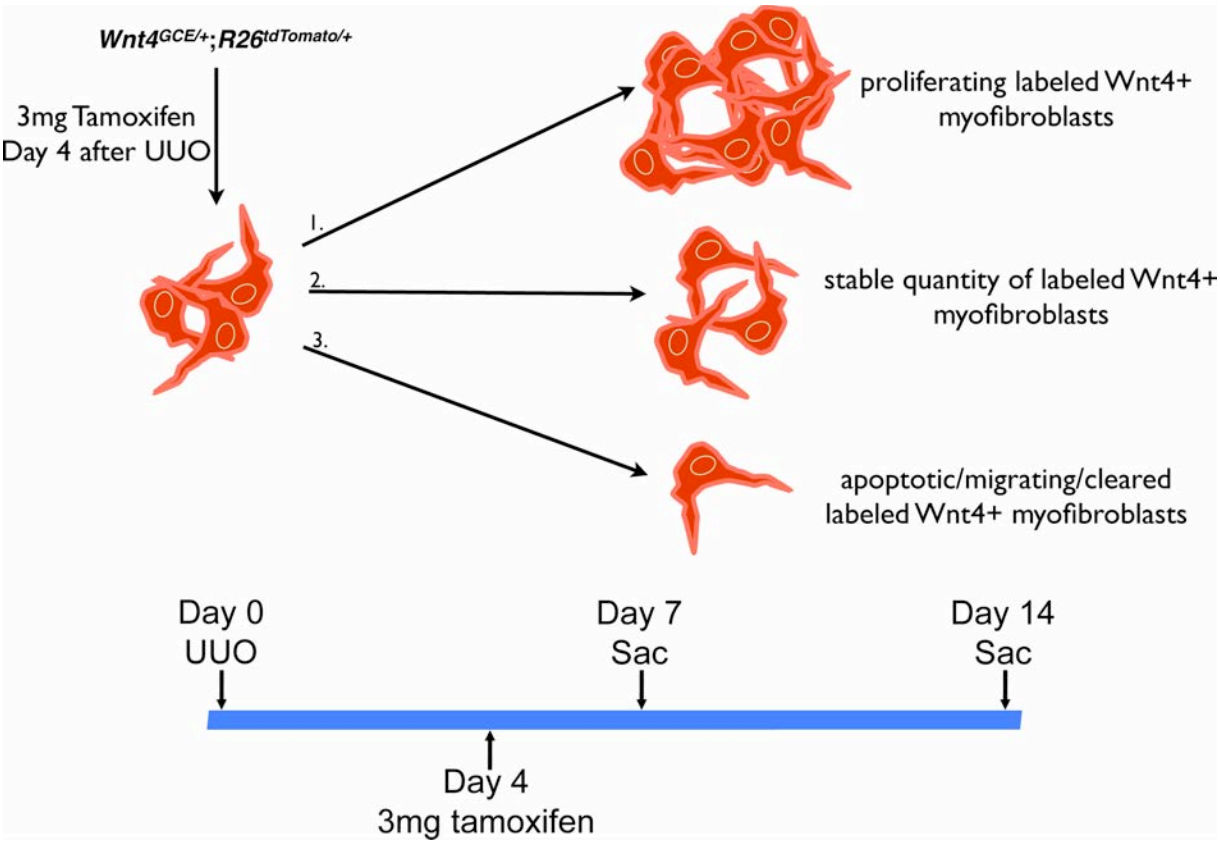

Figure S8. Schematic of possible outcomes for tdTomato+ myofibroblasts labeled at Day 4 after UUO and quantified on Day 7 and Day 14 after UUO. *Wnt4*$^{GCE/+}$;*R26*$^{tdTomato/+}$ mice are treated with one 3mg pulse of tamoxifen i.p. on day 4 after UUO and recombination is allowed to take place for 72 hours. One group of mice will be sacrificed at day 7 and a baseline number of permanently labeled tdTomato+ cells will be recorded. A second group of mice that were injected with tamoxifen on day 4 will be sacrificed on day 14 and tdTomato+ cells are quantified. We hypothesize three scenarios: 1. On day 14 there will be a greater number of tdTomato+ as a result of the labeled cells dividing over the chase period. 2. There will be a similar amount of tdTomato+ cells. 3. There may be less tdTomato+ cells perhaps as a result of apoptosis, cell migration out of the kidney, or clearance by phagocytic cells in the kidney.

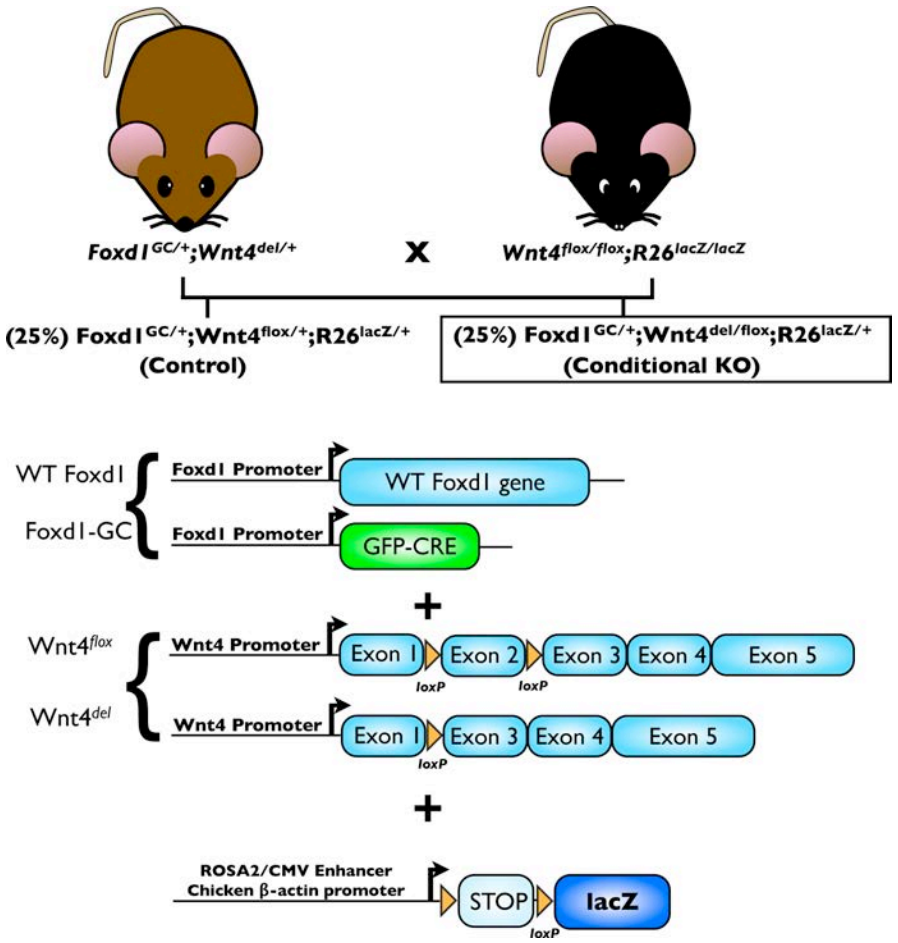

Figure S9. Breeding strategy to generate the trigenic control *Foxd1*$^{GC/+;}$*Wnt4*$^{flox/+}$*;R26*$^{lacZ/+}$ mice and the trigenic cell specific Wnt4 knockout mouse *Foxd1*$^{GC/+}$*;Wnt4*$^{del/flox}$*;R26*$^{lacZ/+}$. *Foxd1*$^{GC/+}$*;Wnt4*$^{del/+}$ mice were crossed with *Wnt4*$^{flox/flox}$*;R26*$^{lacZ/lacZ}$ mice resulting in 25% of the litter being the control genotype, *Foxd1*$^{GC/+;}$*Wnt4*$^{flox/+}$*;R26*$^{lacZ/+}$ and 25% being the conditional knockout genotype, *Foxd1*$^{GC/+}$*;Wnt4*$^{del/flox}$*;R26*$^{lacZ/+}$. The control mice have the one wildtype *Foxd1* allele and the GFP-Cre recombinase fusion protein inserted into the other *Foxd1* allele. At the *Wnt4* locus exon 2 of one allele is floxed while the other is wildtype. They are also heterozygous for expression of a floxed *STOP lacZ* at the *ROSA* locus. The conditional knockout mice are different at the *Wnt4* locus where one allele is deleted and the other maintains the floxed exon 2. The *Foxd1* promoter will drive expression of the GFP-Cre recombinase in the progenitor cells of pericytes, mesangial cells, and vascular smooth muscle cells, with the expectation that Wnt4 expression will be knocked out/down in these cells in the adult mouse. The lacZ protein will only be expressed in the descendants of Foxd1+ cells.

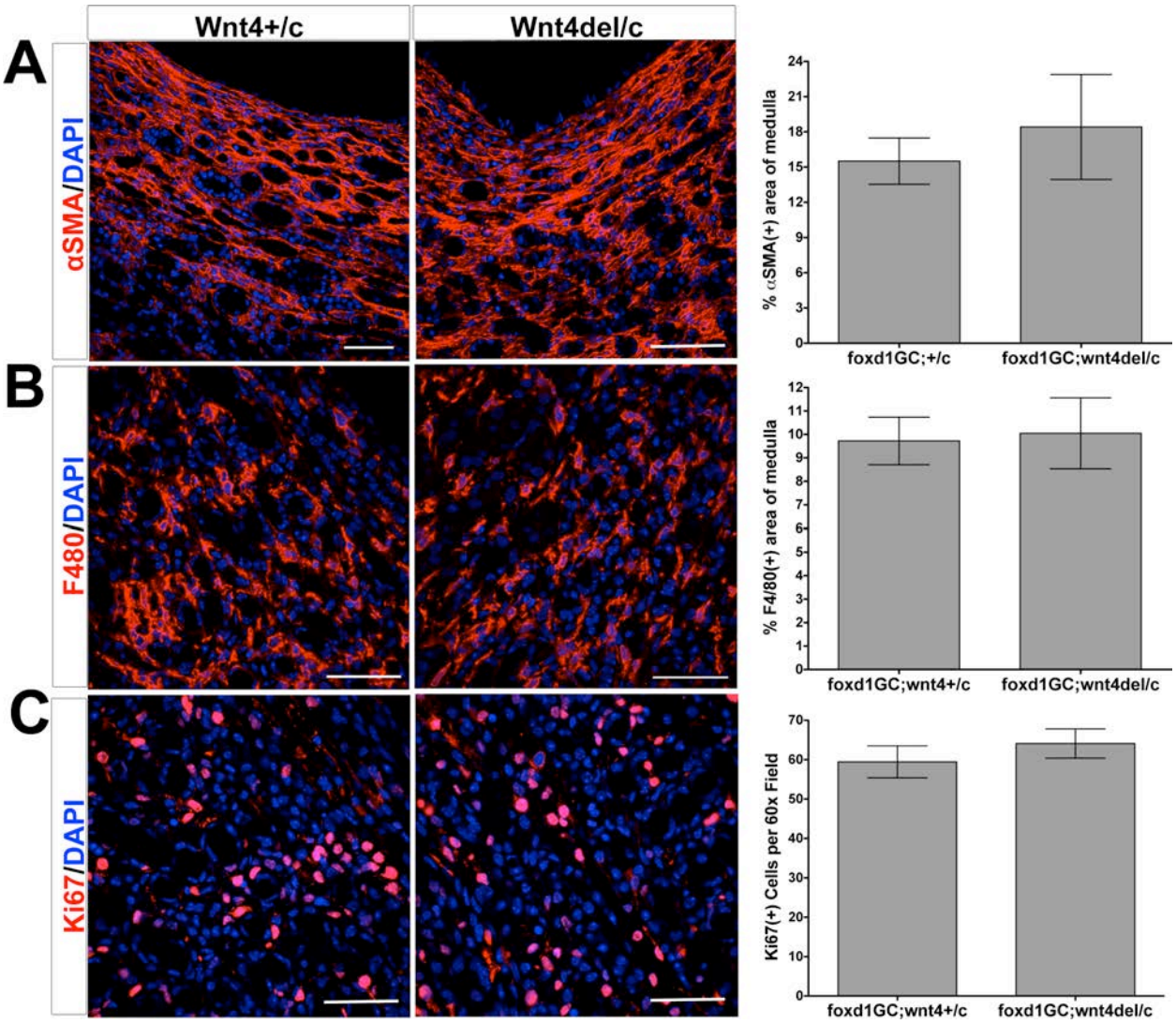

Figure S10. **Quantification of histological samples of medulla from *Wnt4*$^{flox/+}$ and *Wnt4*$^{del/flox}$ mice after UUO.** (A) Histological sections of kidney from control and knockout mice were stained with anti-αSMA antibodies and the percentage of αSMA+ area of kidney medulla was calculated for both genotypes. (B) Sections were stained with anti-F480 antibody and F480+ area of medulla was quantified in both genotypes. (C) Sections were stained with anti-Ki67 antibody and the average number of Ki67+ cells per high power field was obtained. Scale bars = 50μM.

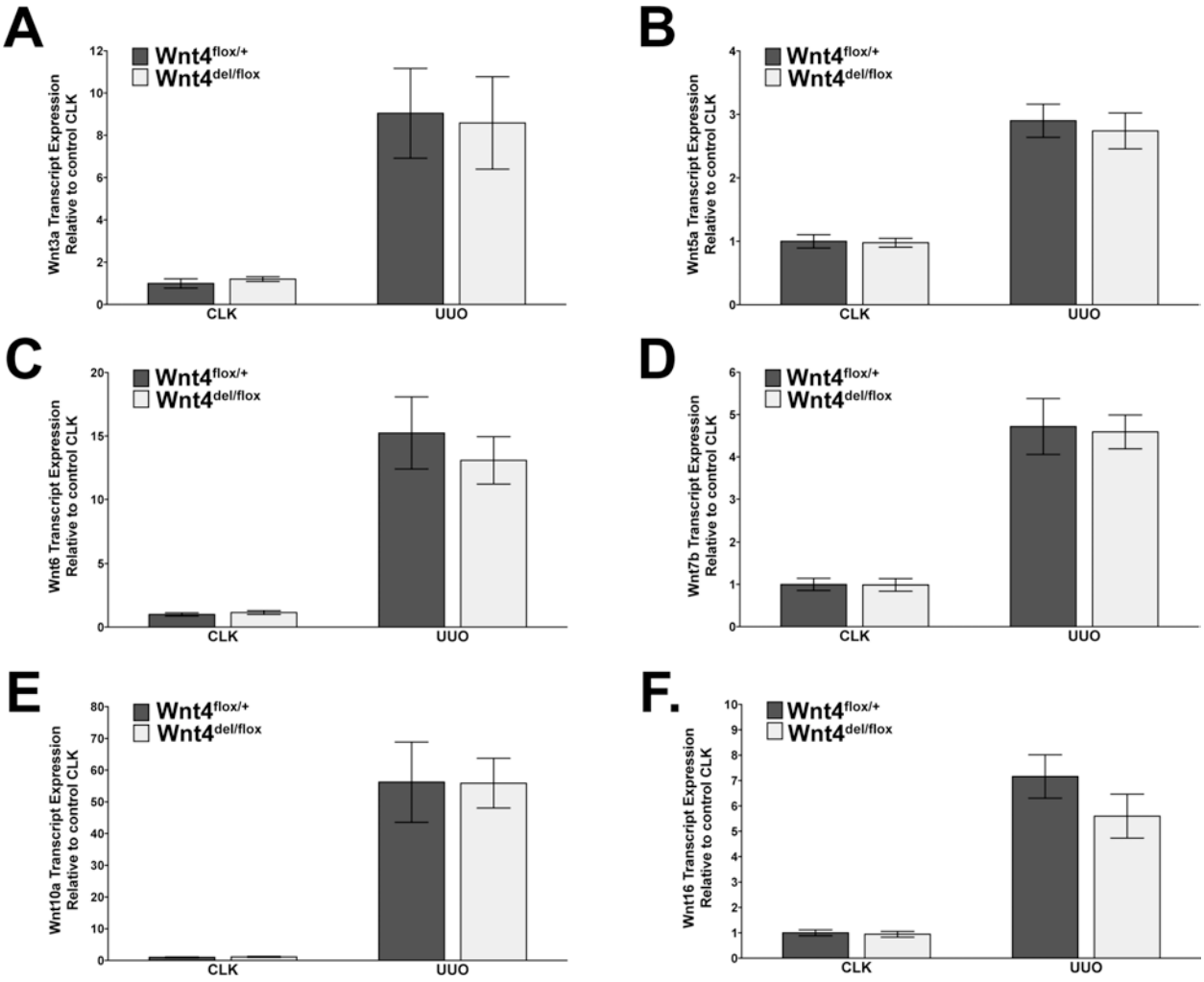

Figure S11. Various Wnt ligands are increased in UUO kidney compared to CLK kidney and are increased to similar levels in *Wnt4*$^{flox/+}$ and *Wnt4*$^{del/flox}$ mice. (A-F) Transcripts of Wnt ligands Wnt3a, Wnt5a, Wnt6, Wnt7b, Wnt10a, and Wnt16 (respectively) were analyzed by qPCR. Data in *A-F* analyzed by two-way ANOVA comparing between genotypes in CLK and UUO conditions.

Gene	Primer Sequences
Wnt4	For: 5'-GGTGGTGACACAAGGGACCCG-3' Rev: 5'-GCACTGTCCGGTCACAGCCA
Axin2	For: 5'-TGACTCTCCTTCCAGATCCCA-3' Rev:5'-TGCCCACACTAGGCTGACA-3'
Lef1	For: 5'-CACACATCCCGTCAGATGTC-3' Rev: 5'-TGATGGGATAAACAGGCTGA-3'
Wnt3a	For: 5'-TCACTGCGAAAGCTACTCCA-3' Rev: 5'-CACCACCGTCAGCAACAG-3'
Wnt5	For: 5'-CCGGGCTTAATATTCCAATG-3' Rev: 5'-ACGCTTCGCTTGAATTCCT-3'
Wnt6	For: 5'-CCTGCAGATGCTGGTAGGAT-3' Rev: 5'-ACTGCTGCTGCTCTTGT-3'
Wnt7b	For: 5'-CCAGGCCAGGAATCTTGTT-3' Rev: 5'-ACGTGTTTCTCTGCTTTGGC-3'
Wnt10a	For: 5'-GCACTCTCTCGAAAACCTCG-3' Rev: 5'-GAGTGCCAGCATCAGTTCC-3'
Wnt16	For: 5'-TTTTCCAGCAGGTTTTCACA-3' Rev: 5'-TCTACACAACAACGAAGCGG-3'
Col-1α1	For: 5'-TGACTGGAAGAGCGGAGAGT-3' Rev: 5'- GTTCGGGCTGATGTACCAGT-3'
αSMA	For: 5'-CTGACAGAGGCACCACTGAA-3' Rev: 5'-CATCTCCAGAGTCCAGCACA-3'

Figure S12. Primers used in study.