Supplemental Discussion:

Dkk1 and the other family members of the Wnt inhibitor family have not been extensively studied in CKD.[1] Dkk1 is developmentally important,[2] and we have shown that expression of multiple members of the Wnt inhibitor family are increased with renal injury.[3] Relevant to our model used in the studies reported here, which has insulin resistance (22weeks) progressing to type 2 diabetes (28 weeks), Dkk1 has been shown to promote hyperglycemia-induced accumulation of mesangial matrix and kidney function.[4] However, other studies have shown that overexpression of Dkk1 or delivery of Dkk1 peptide attenuated kidney disease,[5, 6] due to inhibition of noncanonical Wnt signaling in myofibroblasts. Here we show that Dkk1 levels were increased in the kidneys of sham operated animals and following electrocautery injury of the renal cortex at 12 weeks of age and contralateral nephrectomy at 14 weeks, renal expression and circulating Dkk1 levels were increased at 15 weeks during the hypertrophy of the remnant kidney. Circulating Dkk1 levels remained elevated as CKD-2 developed at 22wks, and slowly progresses towards CKD-3 at 28 wks. The neutralization of Dkk1 did not affect inulin clearance or the BUN of treated animals. Renal fibrosis was not increased (**supplemental Figure 7**), and renal klotho was unchanged (**supplemental Figure 3**)**.** Renal Wnt signaling measured by axin2 levels was increased by CKD-2, but it was not increased by Dkk1 neutralization (**supplemental Figure 6**).[7] In addition, CKD induced the expression of sclerostin and Sostdc1, two Wnt inhibitor family members not studied in our initial report of CKD stimulation of Wnt inhibitors,[3] and Dkk1 neutralization increased Sostdc1 expression suggesting that Dkk1 function in the diseased kidney is redundant. Thus, in this model of kidney disease, Dkk1 neutralization appears to be tolerated at the kidney level without untoward effects. However, our studies have been focused on the canonical Wnt pathways, and more study of the noncanonical pathways is required, especially since these pathways activated in myofibroblasts induce renal fibrosis.[5, 6]

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2. Pietilä I, Ellwanger K, Railo A, Jokela T, Barrantes IDB, Shan J, Niehrs C, Vainio SJ: Secreted Wnt antagonist Dickkopf-1 controls kidney papilla development coordinated by Wnt-7b signalling. *Developmental Biology* 353**:** 50-60, 2011.
3. Surendran K, Schiavi S, Hruska KA: Wnt-dependent- β-catenin signaling is activated after unilateral ureteral obstruction, and recombinant secreted frizzled-related protein 4 alters the progression of renal fibrosis. *J Am Soc Nephrol* 16**:** 2373-2384, 2005.
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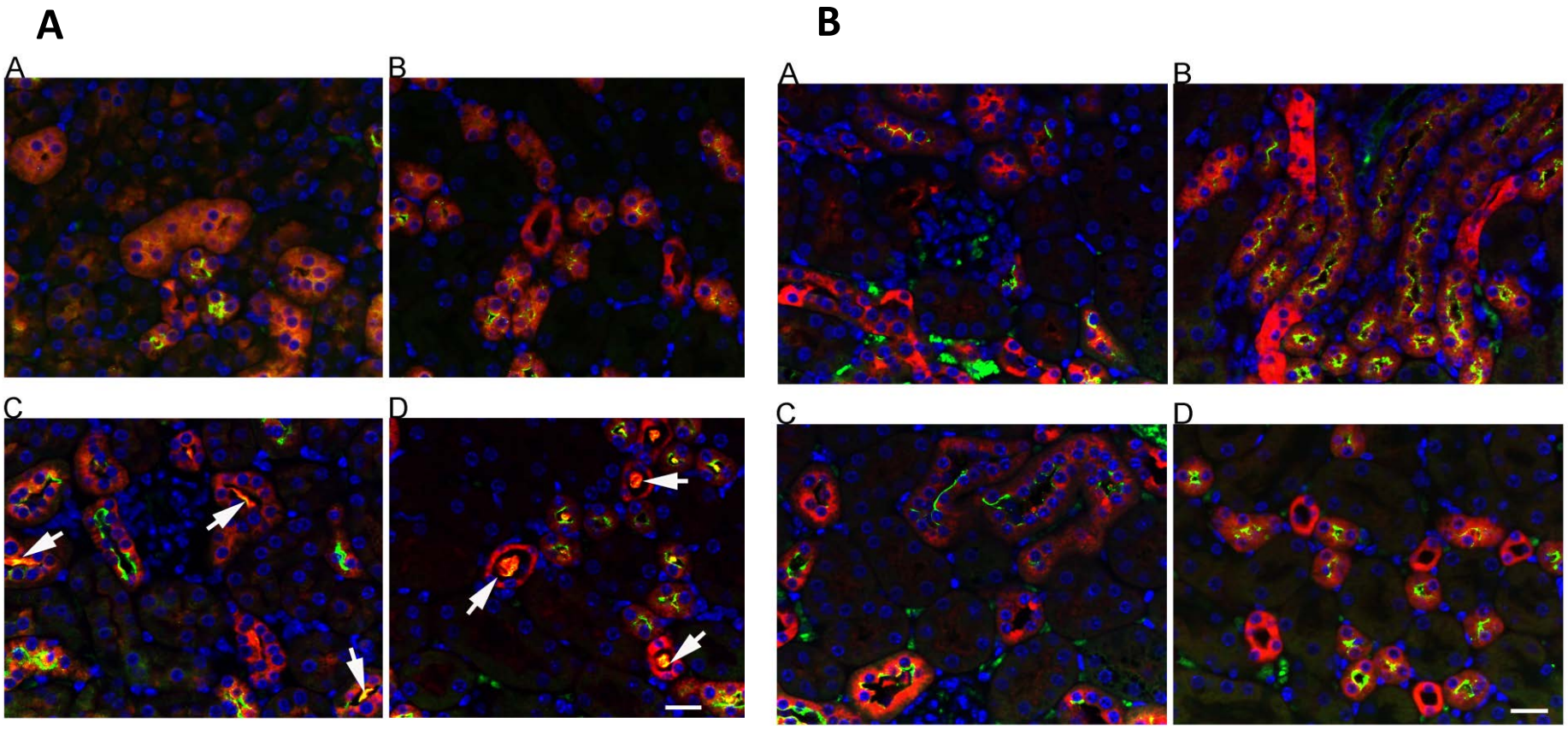
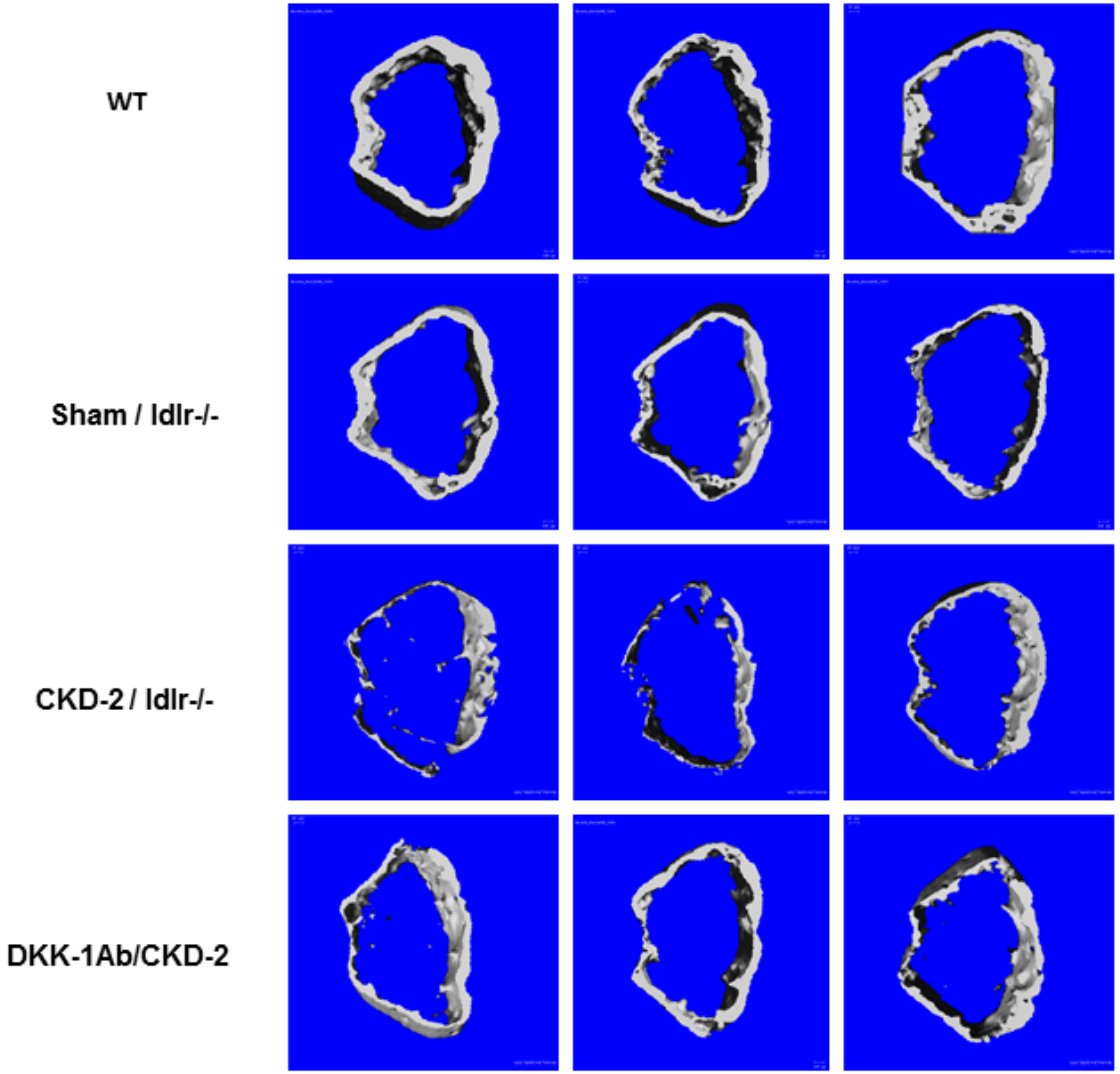
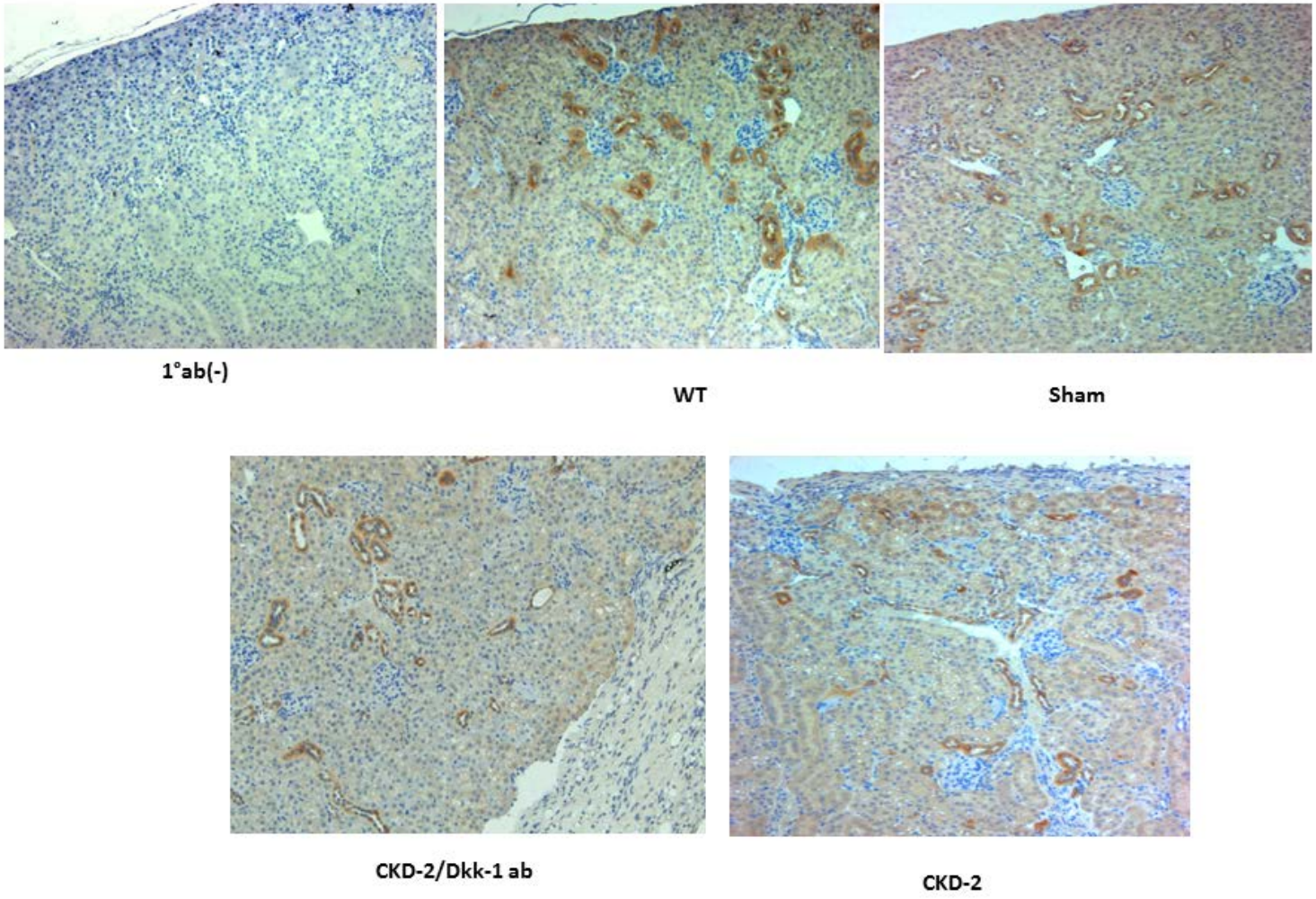


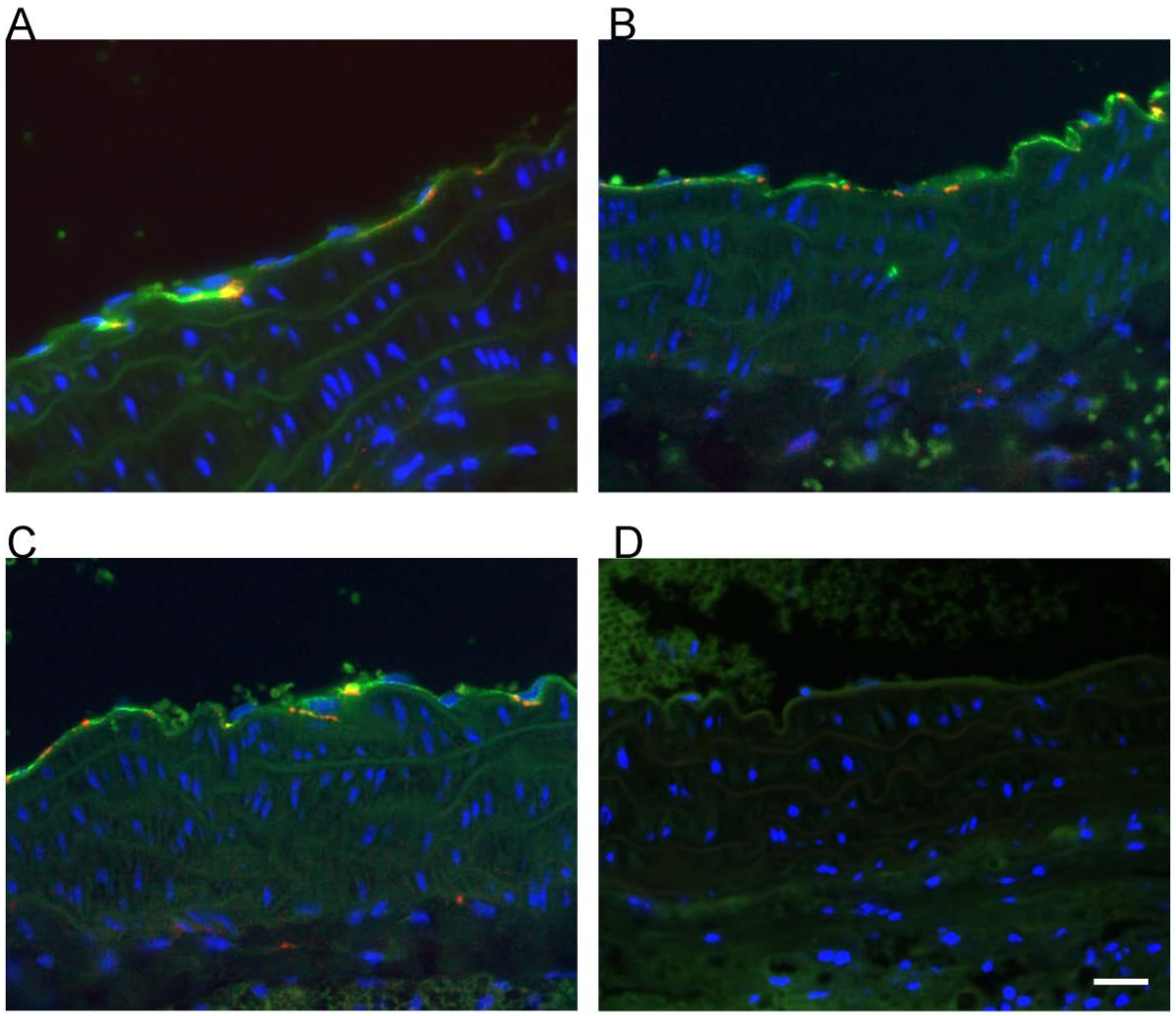
Supplemental Figure 1: Immunofluoresence microscopy of Dkk1 expression in the kidneys of the various groups of mice. Dkk-1 expression (Red) was mainly limited to the tubular epithelial cells of the thick ascending limb, distal tubule and collecting ducts as shown by co-localization with Tamm-Horsfall protein (green), aquaporin 2 (not shown) and anatomical location. Nuclei are stained with Dapi (blue). **A,** A,B, wild type; C,D, Sham operated; A,C, cortex; B,D, cortical-medullary. **B,** A,B,CKD-2; C,D,CKD-2 treated with Dkk1 mab; A,C, cortex; B,D, cortical-medullary. Dkk1 expression was increased in sham and CKD-2 mice compared to wild type. In sham mice, there was strong Dkk1 staining in tubular fluid (white arrows) but not in CKD-2 mice. The differential secretion may have contributed to the increase in plasma Dkk1 in the CKD-2 mice, which was not seen in the sham mice. Despite the significant fibrosis in the CKD-2 kidneys, there was no detectable interstitial Dkk-1 expression in the myofibroblasts.



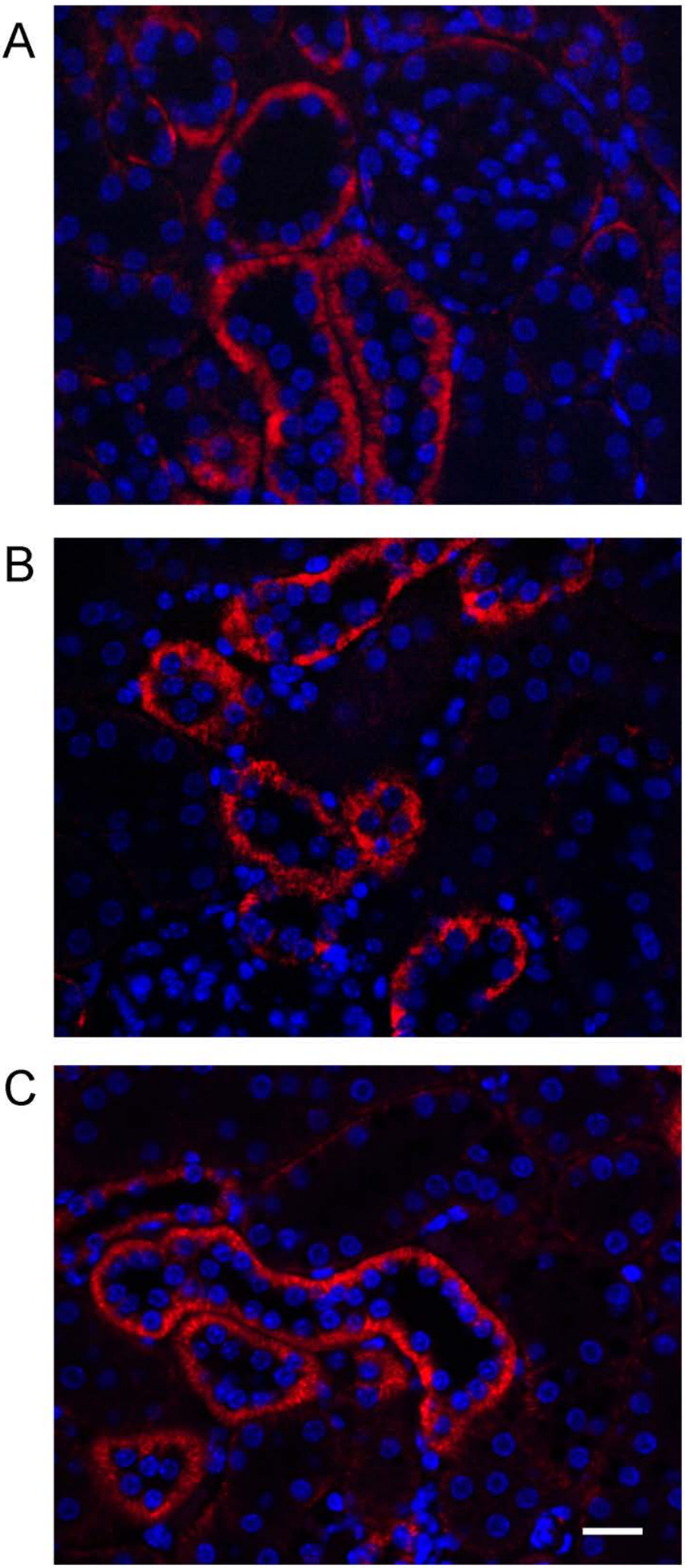
Supplemental Figure 2: Cross sectional reconstruction of microCT of mid diaphysis femoral cortices from the various groups of mice. The CKD-2 mice developed cortical thinning and decreased mineral density (porosity) which was prevented by the Dkk1 mab treatment.



Supplemental Figure 3: Immunohistochemistry of renal αklotho in the various groups of mice. Magnification 10X in each.

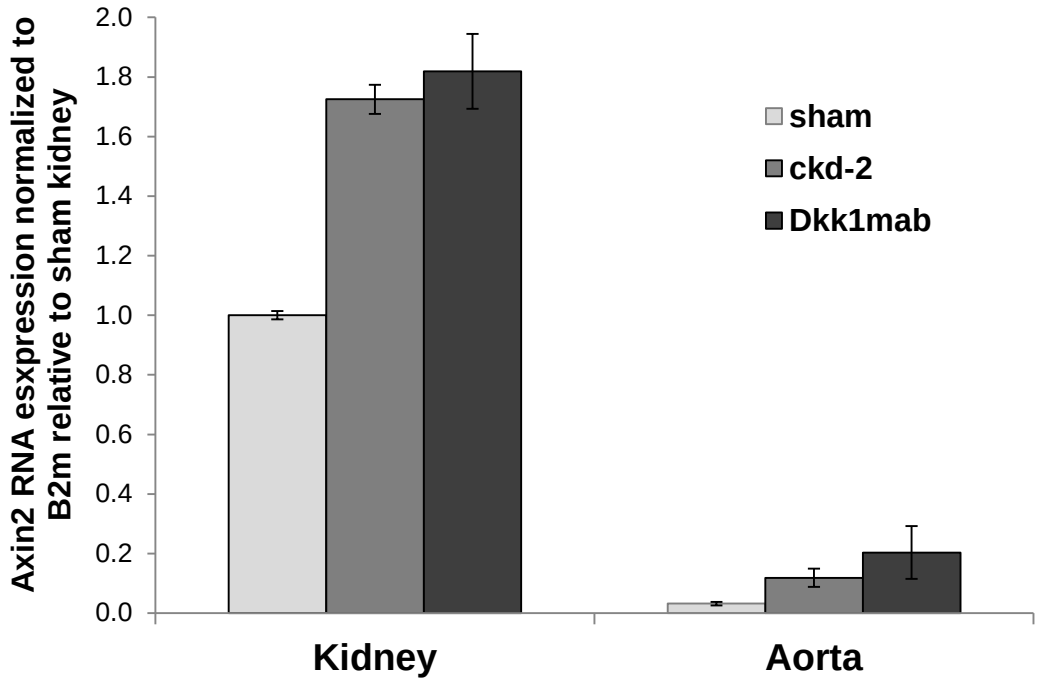


Supplemental Figure 4: Immunofluorescence microscopy of beta-catenin expression in the aortas of the various groups of mice. Red– beta catenin, – Bright green CD31, an endothelial cell marker, yellow – co-localization. **A,** Wild-type mouse aorta; **B,** aorta from a CKD-2 mouse; **C,** aorta from a CKD-2 mouse treated with the Dkk1 mab; **D,** negative control. There was no immunofluorescence for beta-catenin in the vascular smooth muscle. The elastin in the elastic lamina had background staining. There was beta-catenin expression in the endothelium and adventia aortas from CKD-2 mice.


A
B
C

Supplemental Figure 5: Immunofluorescence microscopy of beta-catenin expression in the kidneys of the various groups of mice. **A,** Cortex of kidney from a wild-type mouse, Bowman's capsule of a glomerulus, and epithelial cells of tubules positive for the Wnt inhibitors studied in this report were positive for beta-catenin **B,** Cortex of kidney from a CKD-2 mouse. **C,** Cortex of kidney from a CKD-2 mouse treated with the antibody to Dkk1.Tubular epithelial cells positive for Dkk1, sclerostin and SostDoc 1, were positive for beta-catenin, but the Dkk1 antibody did not increase beta-catenin expression. Nuclear beta-catenin was not detected.

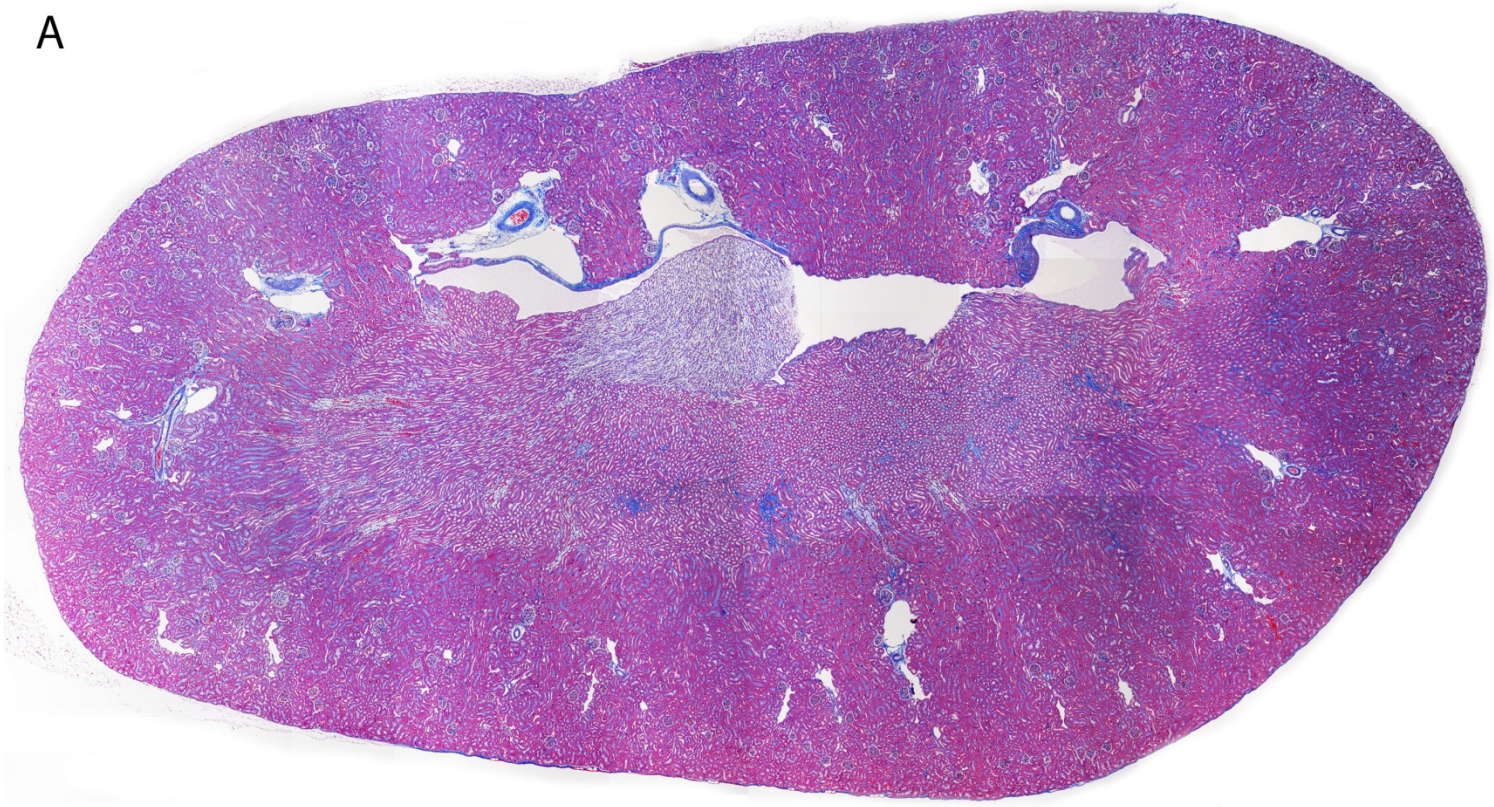
Figure 6



Supplemental Figure 6: Axin2 mRNA levels in the kidneys and aortas of the various groups of mice. RT-PCR was used to measure the message levels of Axin2, an immediate early gene transcribed following Wnt ligation of frizzled/lrp5/6 by TCF/LEF. Thus, axin2 is a biomarker of Wnt activation. Axin2 mRNA levels were much higher in the kidneys of our mouse CKD model than elsewhere in the body as expected, since disease reactivates the Wnt dependent nephrogenesis program, while elsewhere the activity of a developmental morphogen such as Wnt is low except in the skeleton. Induction of CKD-2 significantly increased renal axin2 levels, but treatment with the Dkk1 antibody had no further effect on axin2 mRNA levels. So systemic Dkk1 neutralization did not affect diseased kidney Wnt activation due to redundancy from the various Wnt inhibitors. Wnt activity in the aorta was much lower than in the kidney, in agreement with the immunofluorescent microscopy shown in **supplemental figure 4**. While induction of CKD-2 increased Wnt activity, treatment with the Dkk1 antibody doubled axin2 mRNA levels compared to CKD-2. Thus, we demonstrate that the Dkk1 antibody treatment increased aortic Wnt signaling.

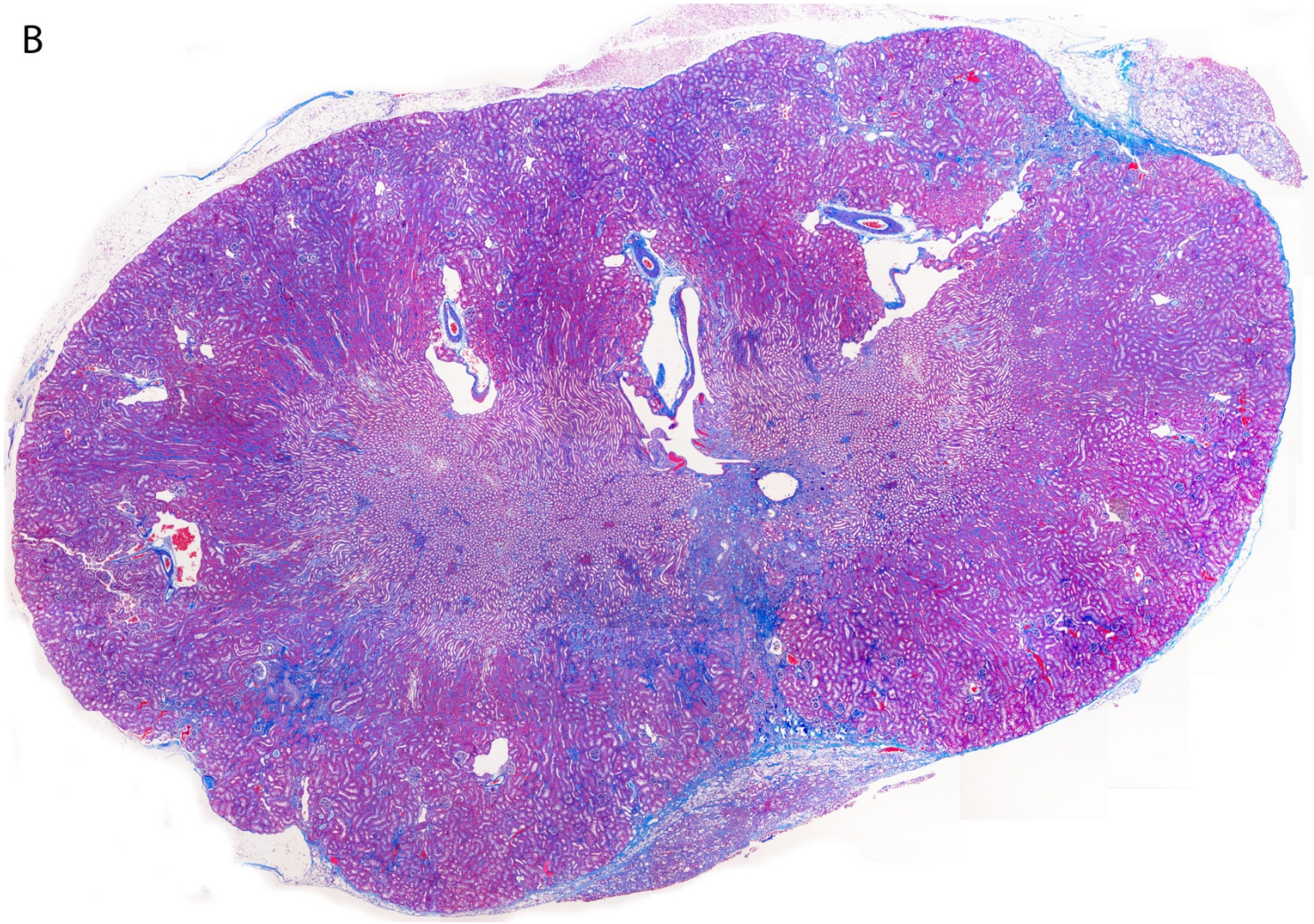
Supplemental Fig. 7A

A



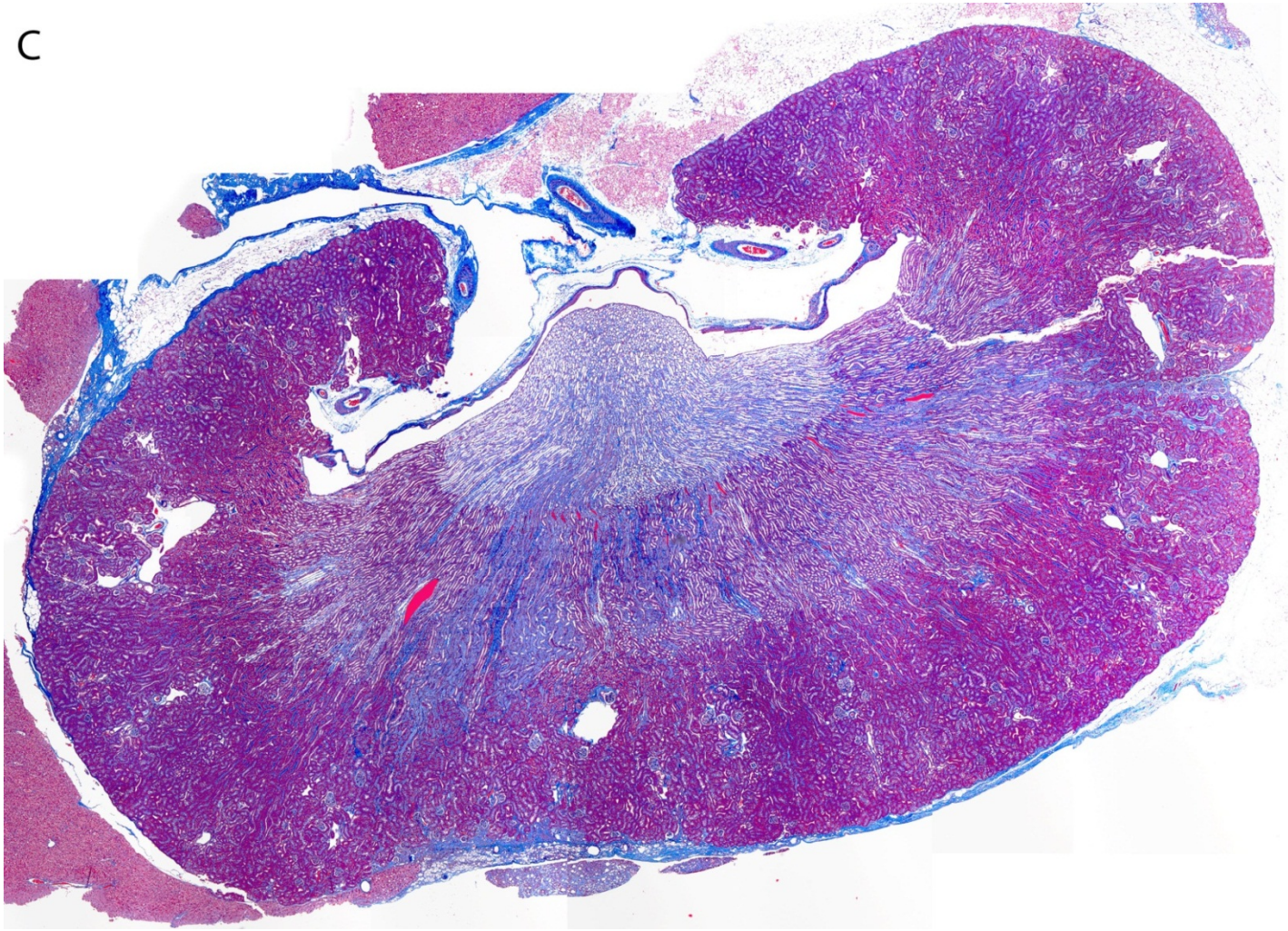
Wild type control mouse kidney

Supplemental Fig. TB



ldlr ko-CKD-2-High Fat Diet, Vehicle treatment control

Supplemental Fig. TC



ldlr ko-CKD-2-High Fat Diet, Dkk1 antibody treatment

Supplemental Figure T: A, B, C, Sagittal sections of kidneys stained with Gomori-Trichrome from wild type, CKD-2 vehicle treated and CKD-2 Dkk1 mab treated mice respectively.

Supplemental Table 1.

Laboratory data for 38 human subjects with chronic kidney disease stage 3a.

Laboratory Parameter	Value
Plasma Creatinine (mg/dl)	1.7 ± 0.4
24-hour Creatinine Clearance (ml/min/1.73m2)	46 ± 12
Plasma BUN (mg/dl)	25 ± 9
Plasma Calcium (mg/dl)	9.3 ± 0.4
Plasma Phosphorus (mg/dl)	3.4 ± 0.5
Plasma Intact Parathyroid Hormone (pg/ml)	67 ± 54

Values are displayed as mean ± SD.