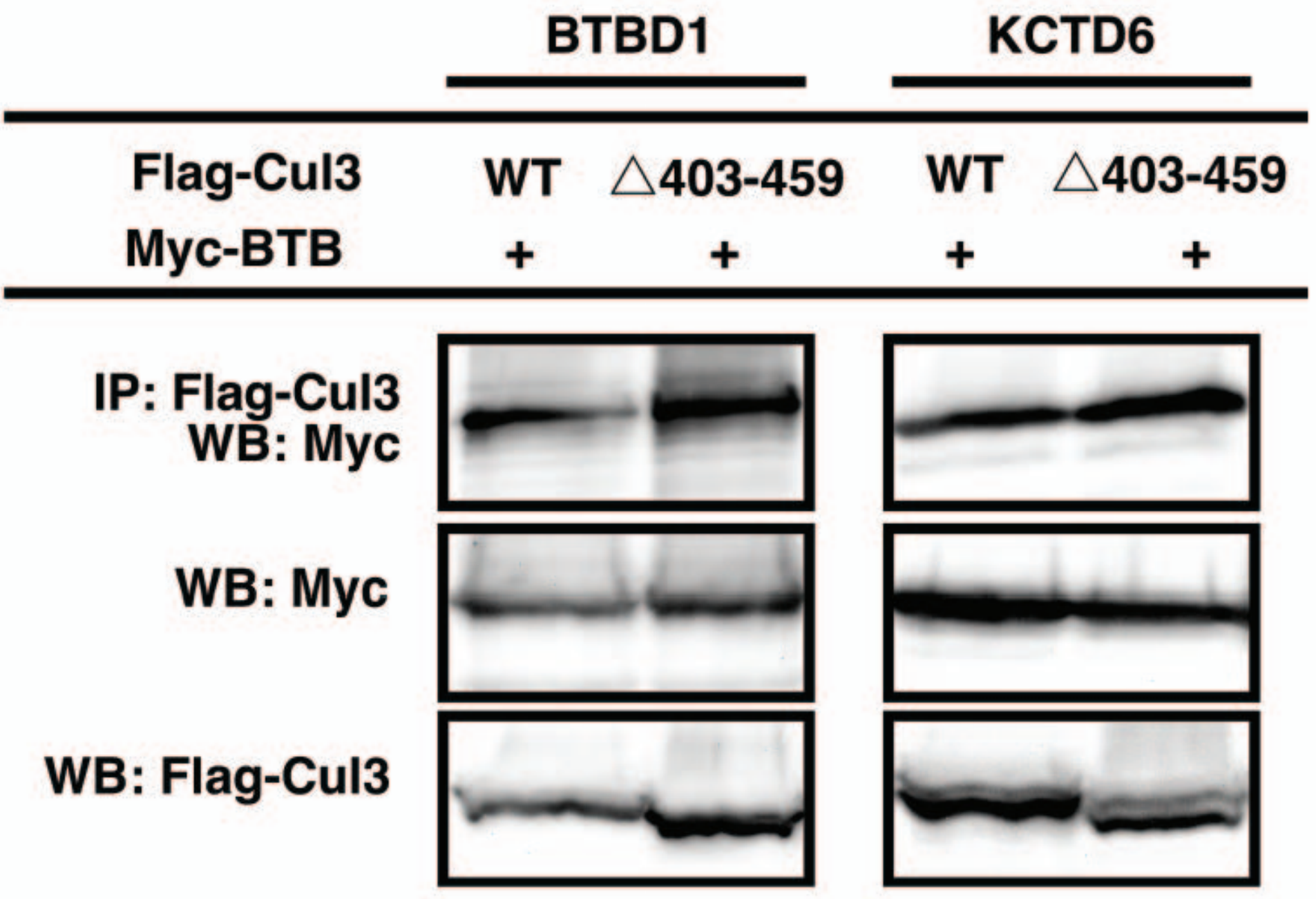
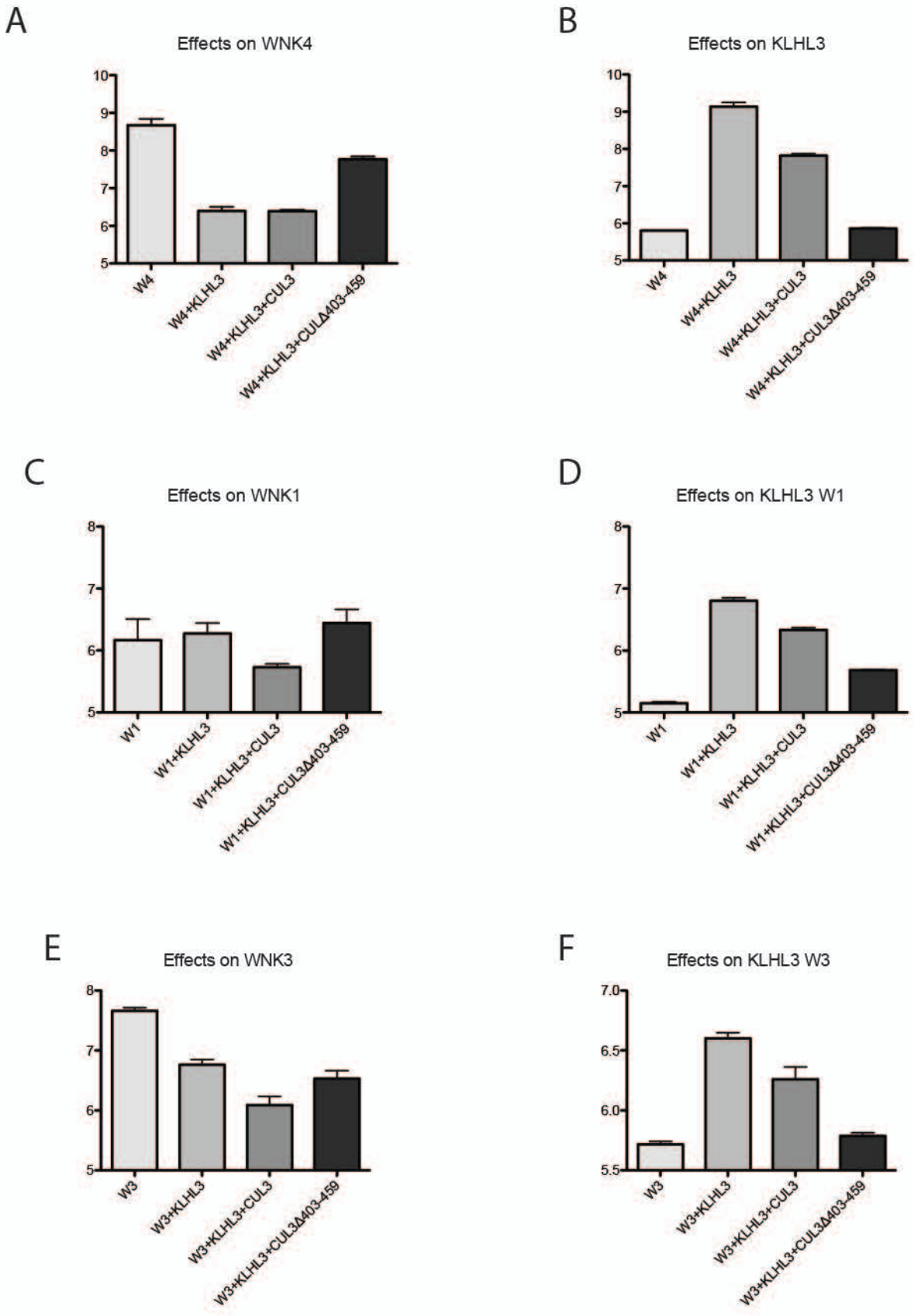
Supp Figure 1



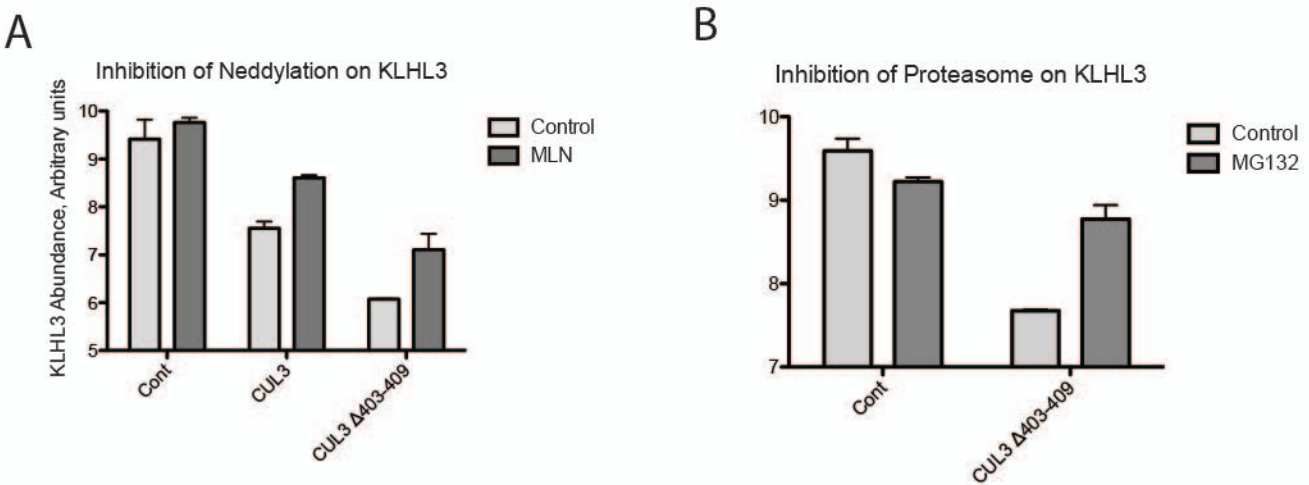
Supplementary Figure 1: Enhanced binding of CUL3 Δ403-459 to BTBD1 and KCTD6, two canonical BTB proteins. IP and western blot are shown.

Supp Figure 2



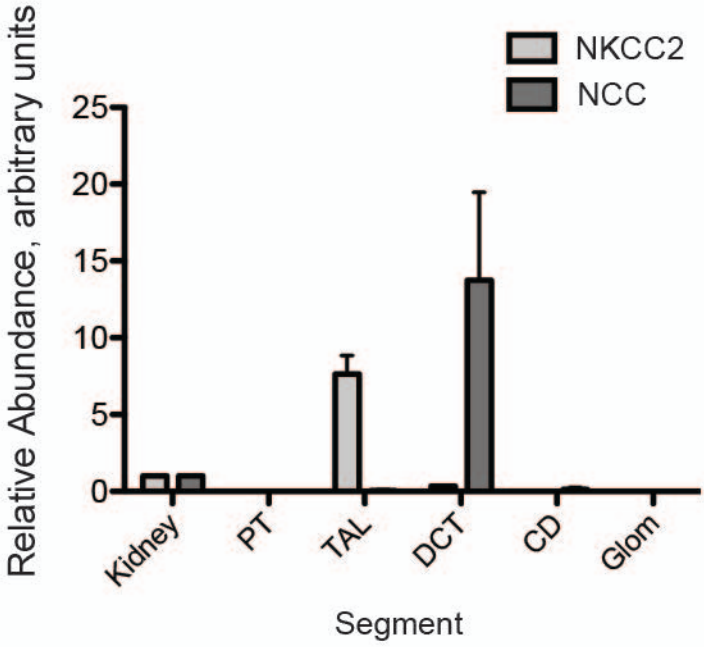
Supplementary Figure 2: Quantification CUL3 Δ403-459 effects on WNK and KLHL3 abundance. Panels show quantification of blots from Figure 2. Panels A, C, and E show effects of CUL3 and CUL3 Δ403-459 on WNK4, WNK1, and WNK3 abundance, as assessed by densitometry. The effects on WNK4 and WNK3 were highly significant by ANOVA. In both cases, the difference between CUL3 Δ403-459 and CUL3 on WNK kinase abundance was significant, based on Tukey's post hoc analysis. Panels B, D, and F show effects of CUL3 Δ403-459 and CUL3 on KLHL3 abundance. The differences were all highly significant by AVOVA and post hoc analysis. Note the consistent patterns of effects.

Supp Figure 3

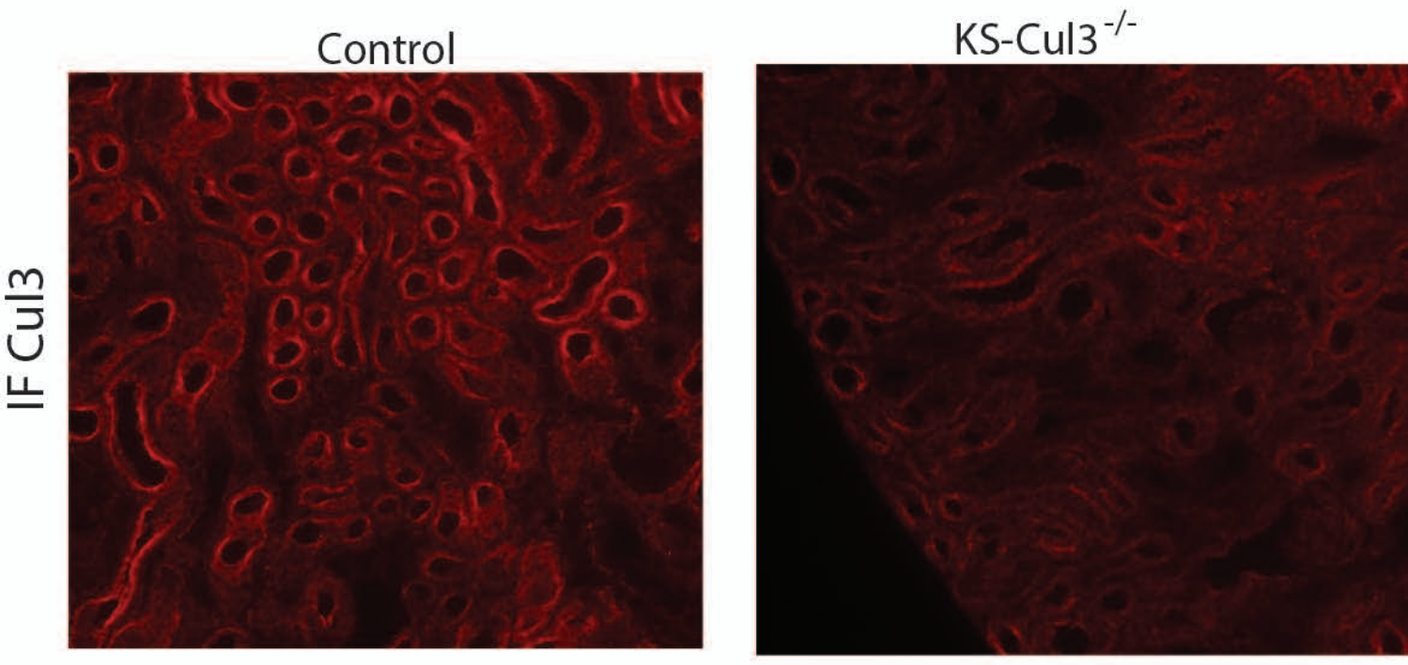


Supplementary Figure 3: quantification of effects of inhibition of neddylation and proteasomal activity on WNK kinase abundance. Panel A shows effects of inhibiting neddylation on KLHL3 abundance. Note that inhibiting neddylation increases the abundance of KLHL3 in the presence of CUL3 Δ403-459. Panel B shows effects of inhibiting the proteasome on WNK4 abundance. Two-way analysis of variance showed significant effects of both proteasomal inhibition and CUL3.

Supp Figure 4

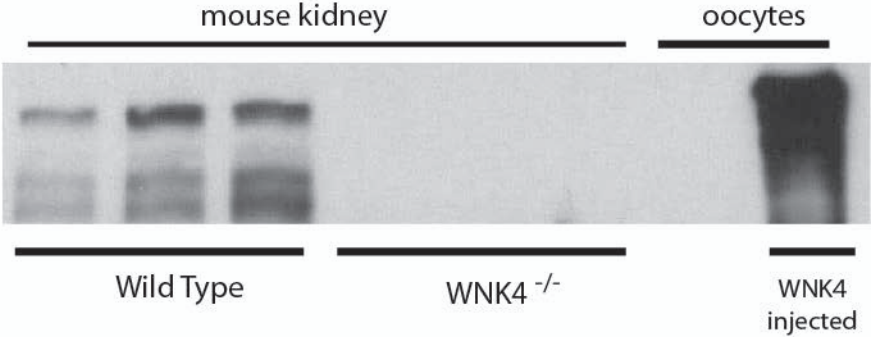


Supplementary Figure 4: Validation of nephron dissection. Quantitative PCR was used to analyze dissected segments for NKCC2 and NCC, as markers for thick ascending limb and distal convoluted tubule respectively.



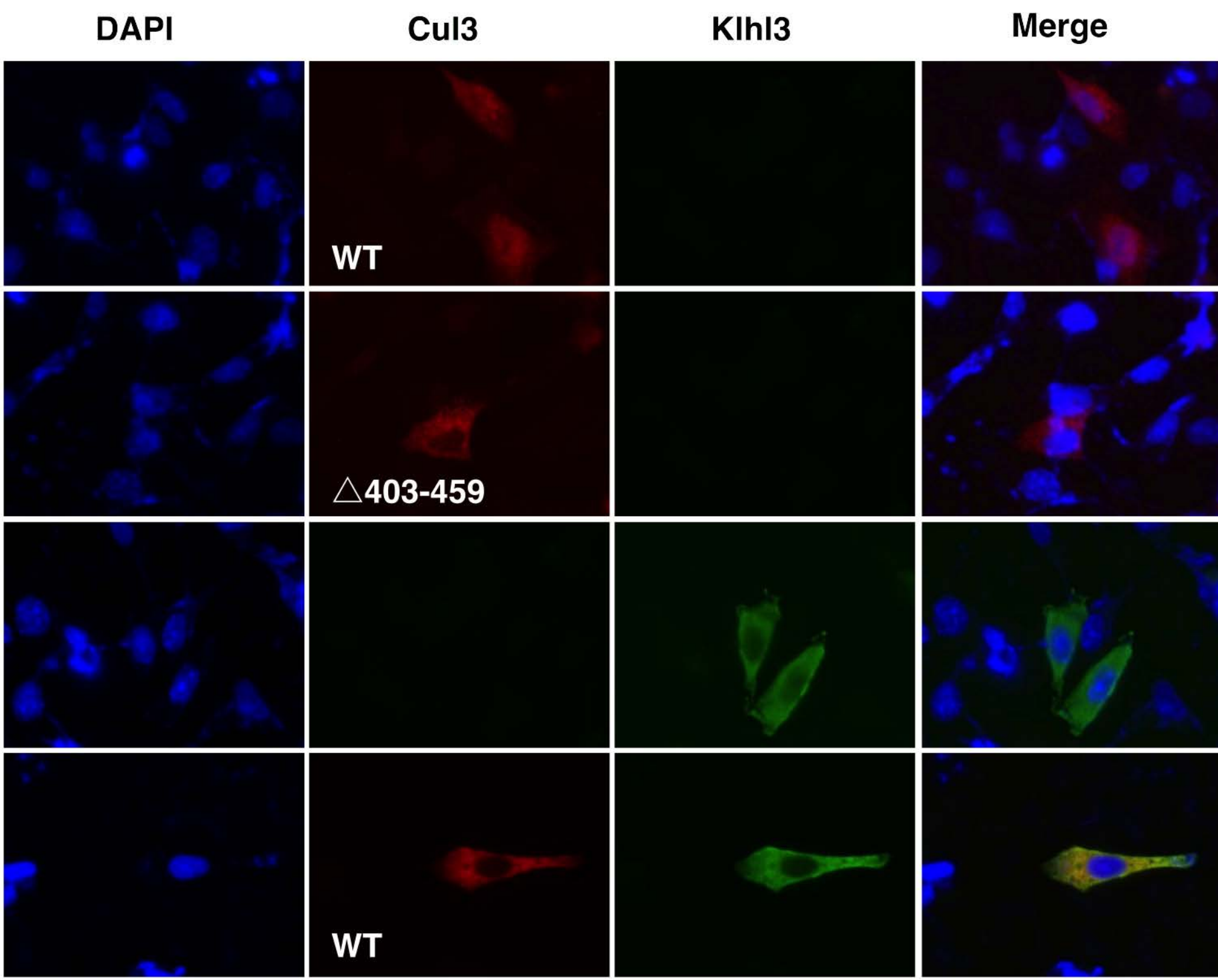
Supplementary Figure 5: Validation of CUL3 antibody. Immunofluorescence of kidney cortex from control and KS-Cul3-/- mice.

Supp Figure 6



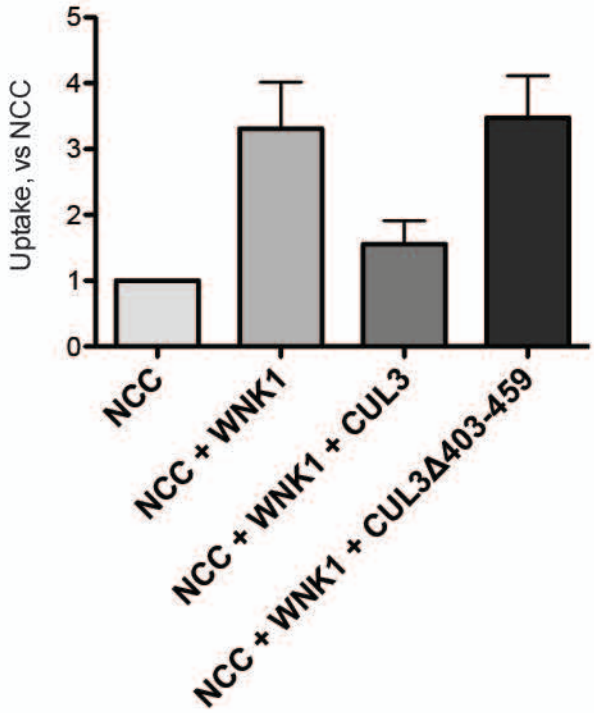
Supplemental Figure 6: western blots of kidney from wild type and WNK4 knockout mice (as reported by Gamba in PNAS), and from oocytes, injected with WNK4 mRNA.

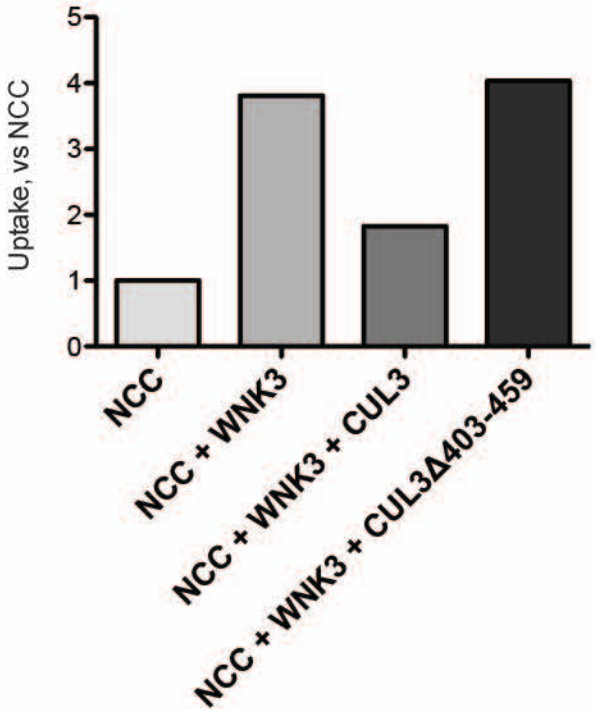
Supp Figure 7



Supplementary Figure 7: Immune fluorescence of mouse DCT cells transfected and analyzed as in Figure 1. Although wild type CUL3 is not entirely nuclear in these cells, cotransfection with KLHL3 drives it away from the nucleus. CUL3 Δ403-459 is non-nuclear at baseline.

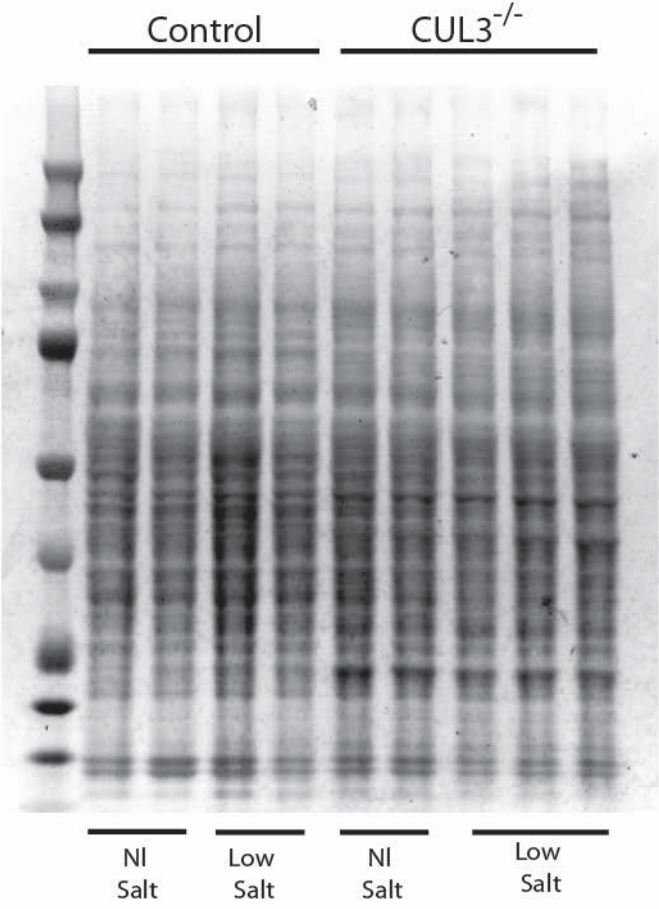
Supp Figure 8





Supplementary Figure 8: Sodium uptake into oocytes injected with either WNK1 or WNK3, showing that results are similar. These results were pooled for Figure 2.

Supp Figure 9



Supplementary Figure 9: staining with Ponceau, as a loading control for figure 8D