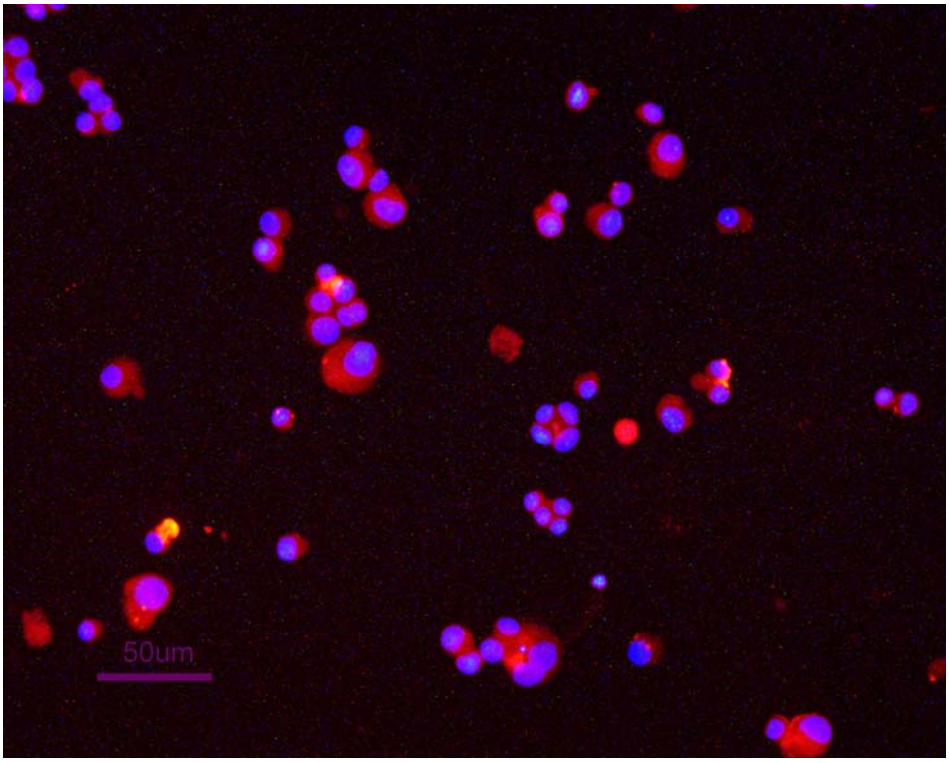
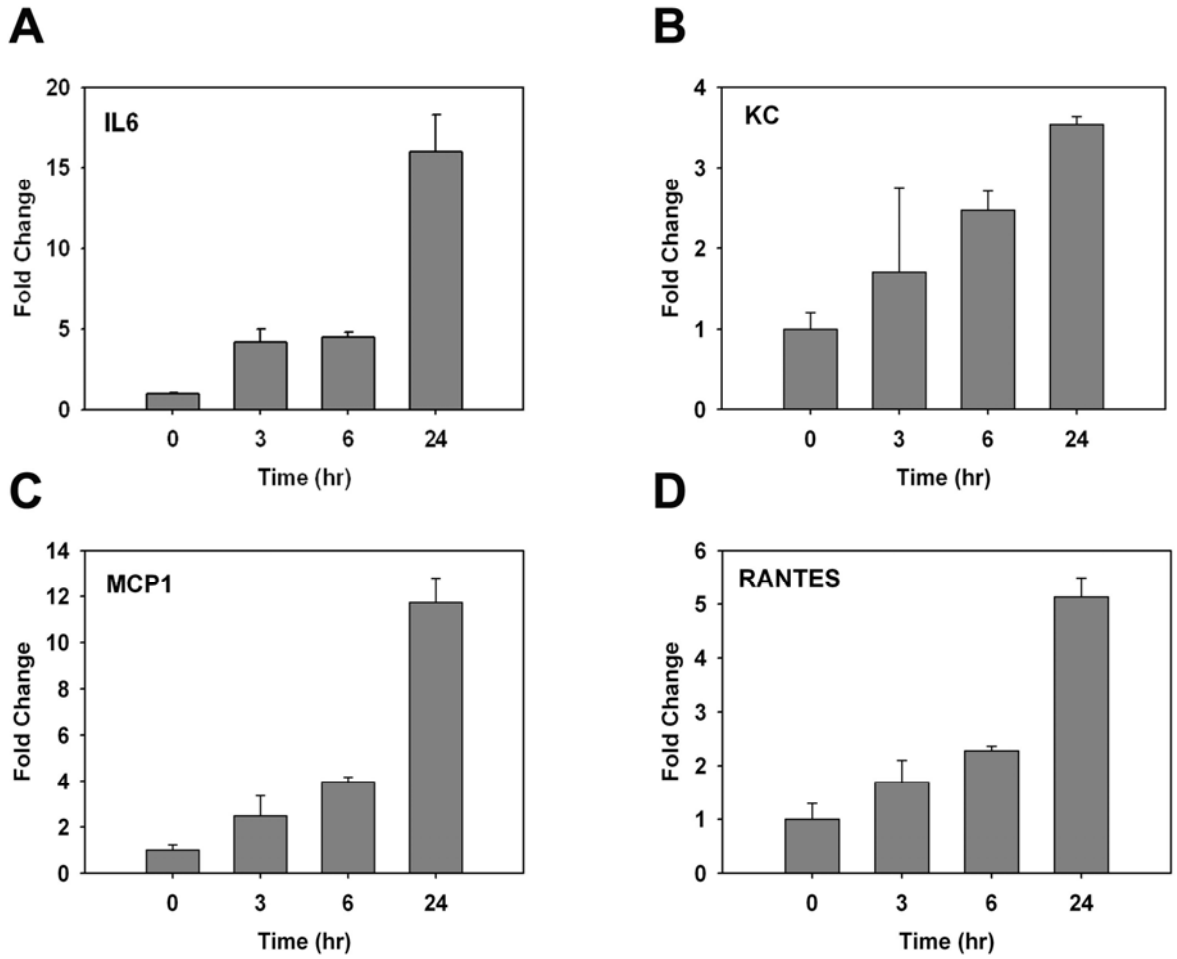
SUPPLEMENTARY DATA

Supplementary Figure 1. ***Immunofluorescence microscopy:*** To assess the purity of isolated mouse renal proximal tubular epithelial cells, 7 day-old monolayer of primary cultured tubular cells were permeabilized and used for immunostaining as described earlier (1). Following incubation with primary antibody (anti-SGLT2), cells were washed 3 times in PBS, and secondary antibody, a Alexa-488-labeled goat anti-rabbit IgG (1:100 dilution), was added for 2 hr at room temperature. Sections were again washed, and DAPI stain (1 μM in PBS; Molecular Probes) was added for 10 min for nuclear counterstaining, sections were again washed, mounted with coverslips using fluorescent mounting media (DakoCytomation, Carpinteria, CA), and viewed as described (2). Five fields were randomly photographed. Most cells stain with anti-SGLT2 antibody (red) suggesting that this preparation is enriched in proximal tubular epithelial cells, whereas blue color is nuclear DAPI stain.



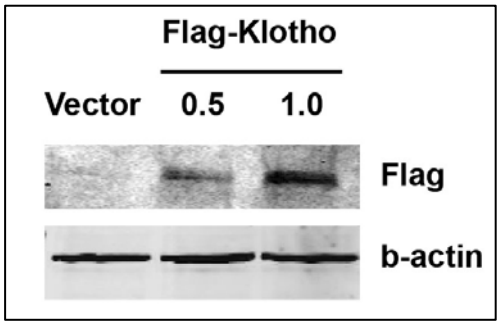
SUPPLEMENTARY DATA

Supplementary Figure 2. ***MG-HSA-induced cytokine production in culture media:*** Primary renal tubular cells were exposed to MG-HSA as described in the Method section. An aliquot of culture media was collected at various intervals and cytokine levels were measured by ELISA. Results demonstrate a time-dependent increase in cytokine secretion into the culture media.

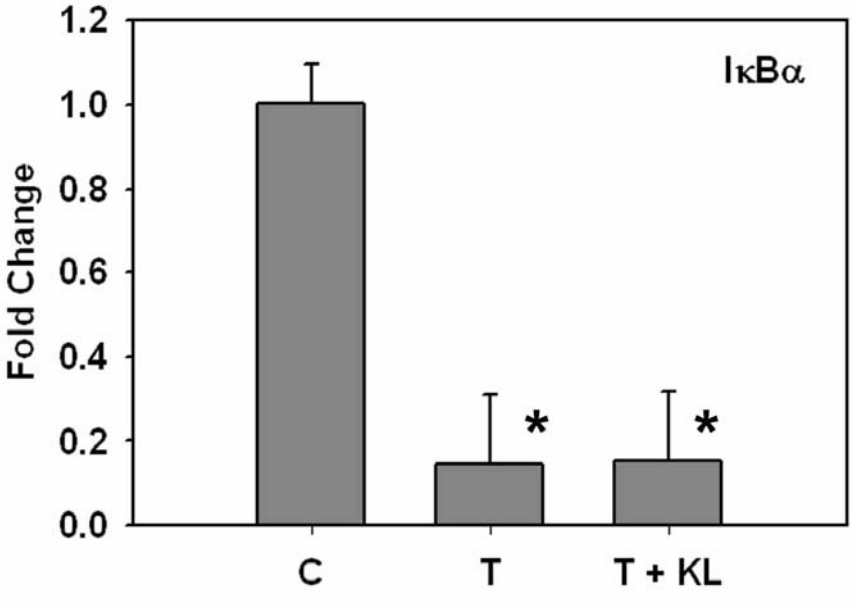


SUPPLEMENTARY DATA

Supplementary Figure 3. ***Expression of Flag-Klotho in HK-2 cells:*** HK-2 cells were transfected with either empty vector (pCDNA-3) or different concentrations of expression plasmid containing Flag-tagged Klotho using Lipofectamine 2000 reagent (Invitrogen). 48 hr later, cells were lysed in RIPA buffer and fractionated on 8 % SDS PAGE. Western blots were performed with anti-Flag antibody. - actin served as a loading control (lower panel).

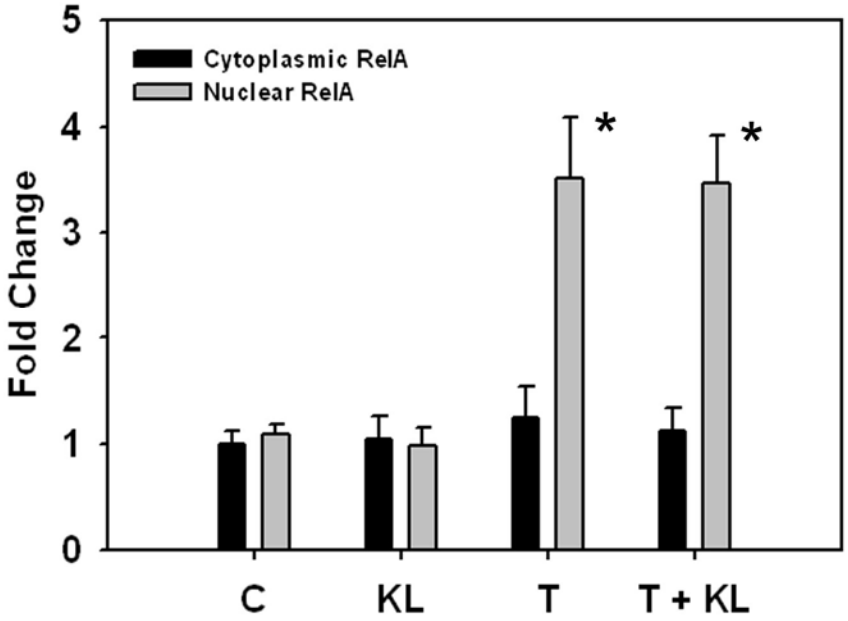


Supplementary Figure 4. ***TNFα-induced IκBα degradation is not affected by the addition of exogenous Klotho:*** As expected, TNFα significantly induced IκBα degradation; however, addition of exogenous Klotho did not interfere with this IKK-dependent IκBα degradation event. Blots from three independent experiments were quantitated and normalized to respective β-actin and expressed as fold change vs control cells. A representative blot was shown in Fig. 6A. C, Control; T, TNFα; KL, Klotho. Data represents mean ± SD of 3 independent experiments and overall significance was determined using 1 way ANOVA. Significance between groups was analyzed by performing Turkey's post-hoc test. *$p<0.05$ significantly different than control samples.



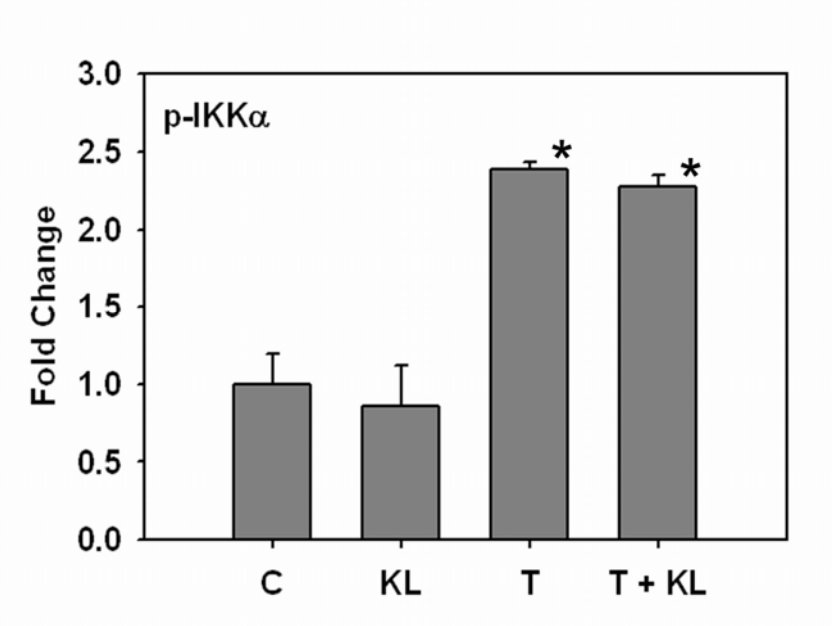
SUPPLEMENTARY DATA

Supplementary Figure 5. ***Klotho failed to inhibit TNFα-induced nuclear translocation of RelA:*** Blots from three independent experiments were quantitated and normalized to respective β-actin and expressed as fold change vs control cells. A representative blot was shown in Fig. 6B (upper panel). Results showed no change in cytoplasmic RelA concentration following TNFα treatment; however, TNFα significantly induced RelA nuclear translocation. Exogenously added Klotho failed to inhibit TNFα-induced nuclear accumulation of RelA. C, Control; T, TNFα; KL, Klotho. Data represents mean ± SD of 3 independent experiments and overall significance was determined using 1 way ANOVA. Significance between groups was analyzed by performing Turkey's post-hoc test. *p<0.01 significantly different than control samples.



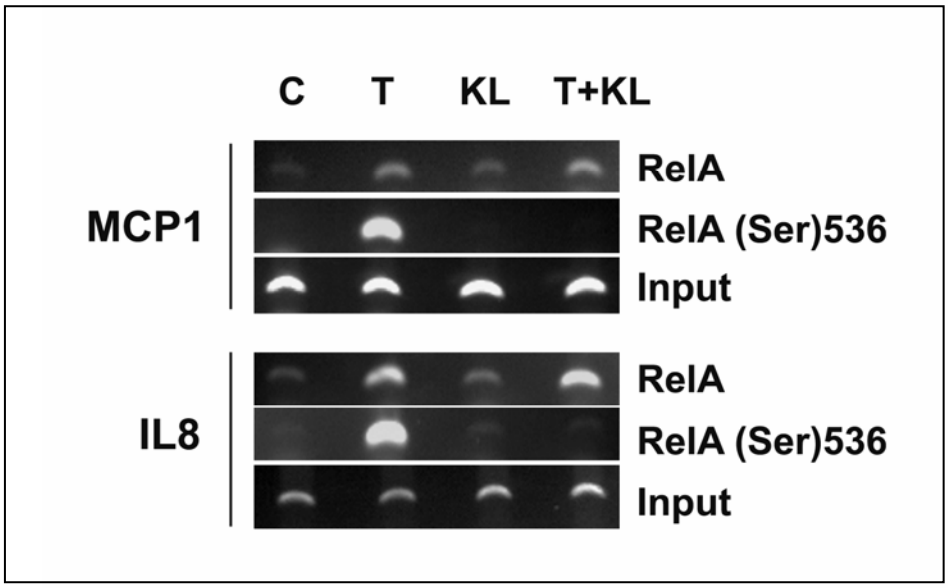
SUPPLEMENTARY DATA

Supplementary Figure 6. ***Klotho did not interfare with TNFα-induced IKKα phosphorylation:*** Blots from three independent experiments were quantitated and normalized to respective total IKKα and expressed as fold change vs control cells. A representative blot was shown in Fig. 7B. Results show TNFα significantly induced IKKα phosphorylation; however, addition of exogenous Klotho did not interfere with this phosphorylation event. C, Control; T, TNFα; KL, Klotho. Data represents mean ± SD of 3 independent experiments and overall significance determined using 1 way ANOVA. Significance between groups was analyzed by performing Turkey's post-hoc test. *p<0.01 significantly different than control samples.



SUPPLEMENTARY DATA

Supplementary Figure 7. ***Representative gel from ChIP Assay:*** Chromatin immunoprecipitated by either RelA or RelA (Ser) [536] antibodies for Fig. 8 was amplified by PCR using either IL-8 (27-cycles) or MCP-1 (31-cycles) primer and the products separated on 2 % agarose gel. For loading controls, input (chromatin before immunoprecipitation) was also amplified using same primers (14-cycles).



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