

Figure S1. Validation of ATF3 knockdown, Klotho overexpression and ATF3 overexpression in mouse kidney tissues. Kidney tissue lysates were subjected to western blotting using antibodies against ATF3 and Klotho. β -actin served as the loading control. Representative blots from three independent experiments are shown. Densitometric analysis of ATF3 and Klotho protein levels normalized to β -actin is presented as mean $\pm$ SD (n=3 per group). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***P<0.001 vs. Sham group; #P<0.05, ##P<0.01, ###P<0.001 vs. UUO group; &P<0.05, &&P<0.001 vs. UUO +oe-Klotho group. ATF3, activates transcription factor 3; UUO, unilateral ureteric obstruction; oe, overexpression.

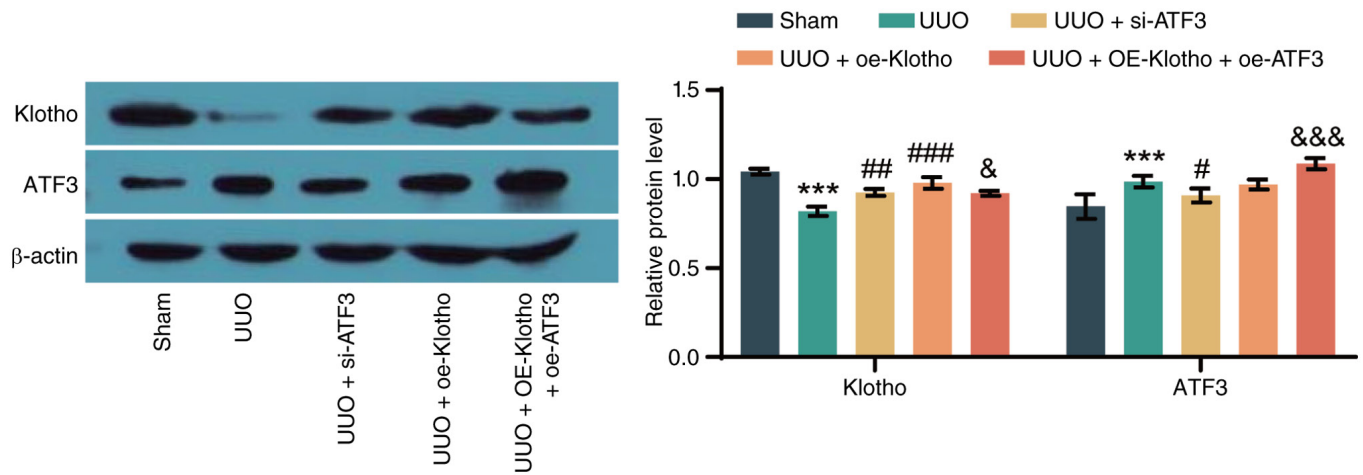


Figure S2. Validation of Klotho and ATF3 overexpression efficiency in HK2 cells. (A) Western blot analysis confirmed that the expression of the Klotho overexpression plasmid (oe-Klotho) group was significantly higher when compared with that of the empty vector (oe-NC) group and the normal control (NC) group. (B) Western blot analysis confirmed that the expression of the ATF3 overexpression plasmid (oe-ATF3) group was significantly higher when compared with that of the empty vector (oe-NC) group and the normal control (NC) group. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. *** $P < 0.001$ vs. the NC/oe-NC group. ATF3, activates transcription factor 3; oe, overexpression; nc, negative control.

