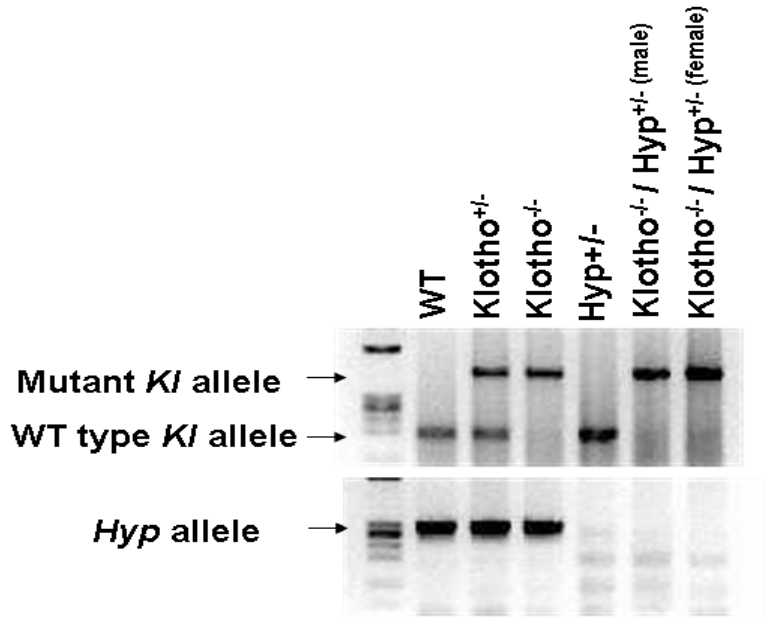
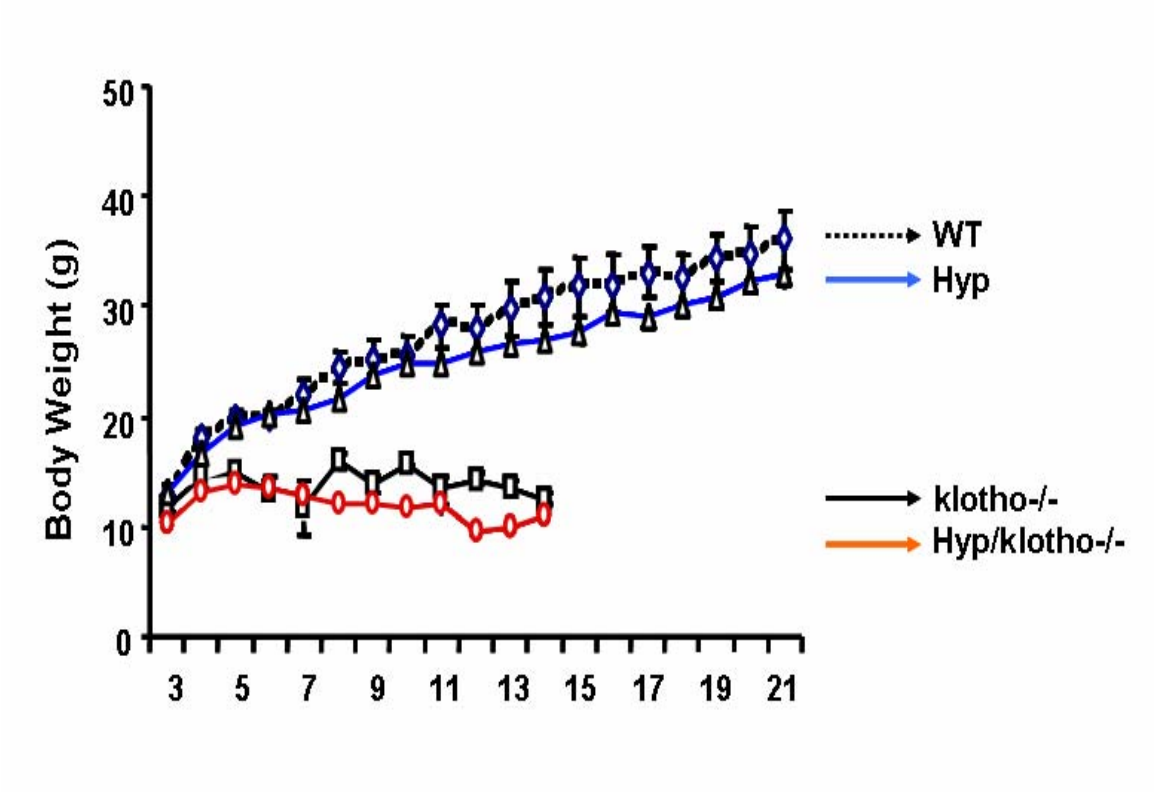
Supplementary Figure 1

PCR amplification of wild-type (WT), mutant klotho, and mutant *Hyp* alleles to identify desired genotypes of WT, *klotho*$^{-/-}$, *Hyp*/*klotho*$^{-/-}$, and *Hyp* mice.



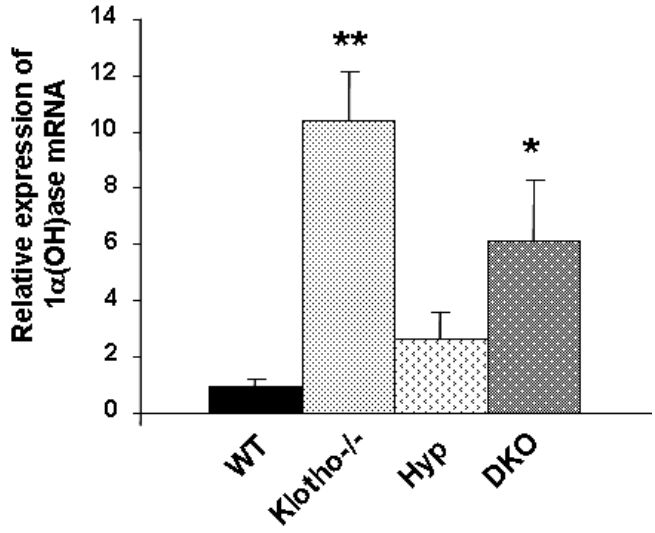
Supplementary Figure 2

Body weight patters of various genotypes. Body weight curves for wild-type (WT, n=11), *klotho*$^{-/-}$(n=22), and *Hyp*/*klotho*$^{-/-}$ (DKO, n=7) and *Hyp* (n=18) mice. The *Hyp*/*klotho*$^{-/-}$ double knockout mice are smaller than wild-type and *Hyp* mice, and similar to *klotho*$^{-/-}$ mice. Mice used to generate body weight curve were not always littermates, but of similar genetic background.



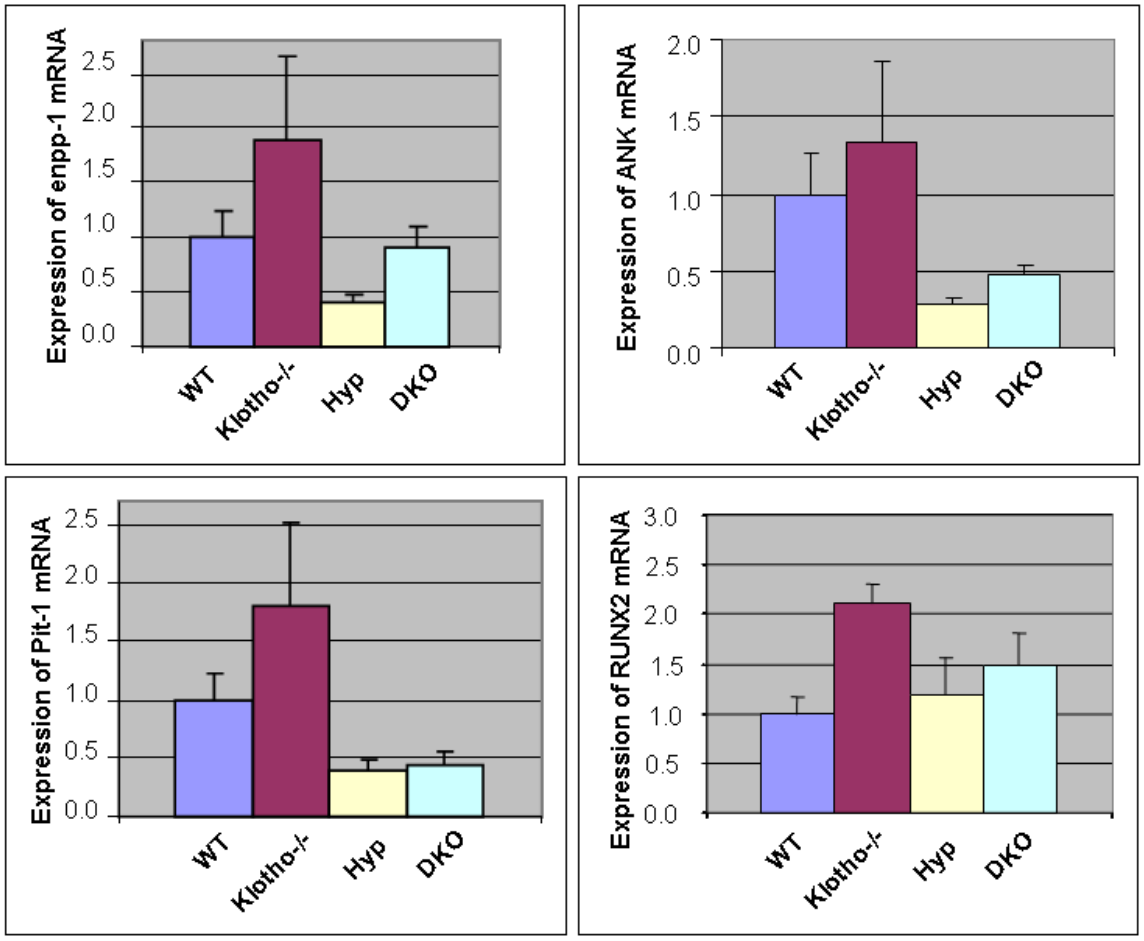
Supplementary Figure 3

Real-time PCR analysis of 1α(OH)ase. The renal expression of 1α(OH)ase was approximately 6-fold higher in *Hyp/klotho*$^{-/-}$ (DKO) mice compared to wild-type (WT) mice. Similarly, compared to WT kidneys, 1α(OH)ase expression was increased, almost 10 folds, in *klotho*$^{-/-}$ kidneys. A mild increase in the expression of 1α(OH)ase mRNA is also noted in *Hyp* mice. Data presented as 1α(OH)ase mRNA expression relative to WT, normalized with GAPDH (**p<0.01, *p<0.05, compared with WT).



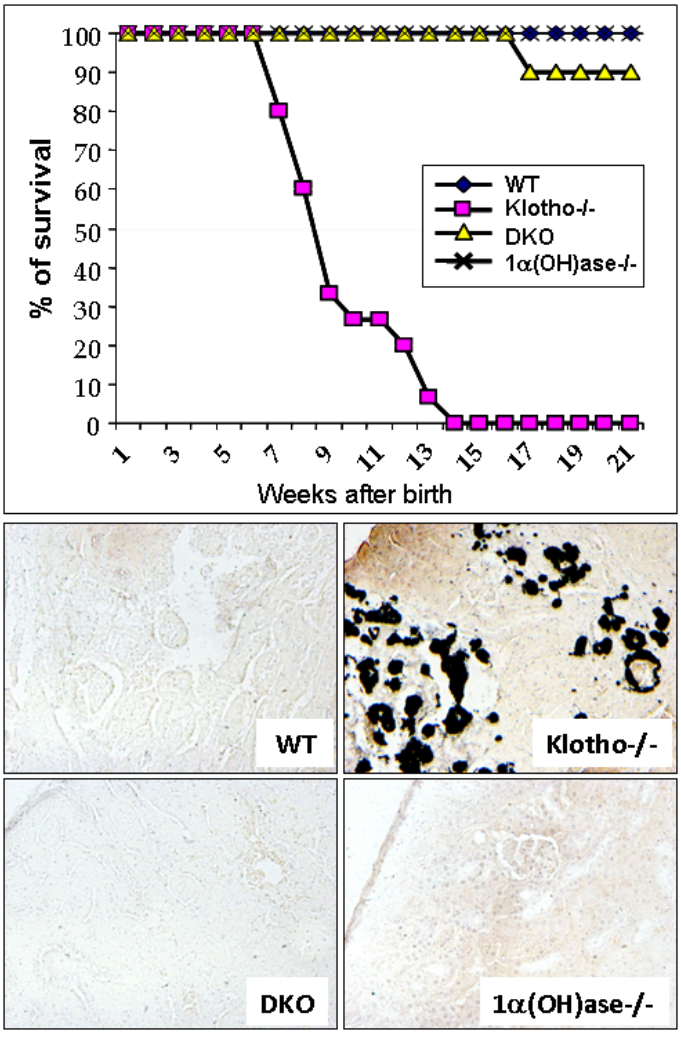
Supplementary Figure 4

Real-time PCR analysis of ennp-1, ANK, Pit-1 and RUNX2. The renal expression of ennp-1, ANK and Pit-1 was slightly reduced in *Hyp* mice, compared to the wild-type (WT) mice. RUNX2 expression was slightly higher in *klotho*$^{-/-}$ and *Hyp/klotho*$^{-/-}$ (DKO) mice, compared to the WT mice. Data presented as ennp-1, ANK, Pit-1 and RUNX2 mRNA expression relative to WT mice, normalized with GAPDH.



Supplementary Figure 5

Survival (upper panel) and soft tissue calcification (lower panel). Survival curves for wild-type (WT, n=10), *klotho*$^{-/-}$(n=15), and *klotho*$^{-/-}$/*1a(OH)ase*$^{-/-}$ double knockout (DKO, n=11) and *1a(OH)ase*$^{-/-}$ (n=18) mice **(upper panel)**. Note that the survival of *klotho*$^{-/-}$/*1a(OH)ase*$^{-/-}$ DKO mice is far better than *klotho*$^{-/-}$ mice, and very similar to the wild-type and *1a(OH)ase*$^{-/-}$ mice. Most of the *klotho*$^{-/-}$ mice died around 15 weeks of age, while most of the *klotho*$^{-/-}$/*1a(OH)ase*$^{-/-}$ DKO mice survived beyond 25 weeks of observation period. Mice used to generate survival curve were not always littermates, but of similar genetic background. Von Kossa staining of renal sections prepared from WT, *klotho*$^{-/-}$, *klotho*$^{-/-}$/*1a(OH)ase*$^{-/-}$ DKO and *1a(OH)ase*$^{-/-}$ mice **(lower panel)**. Note that the extensive calcification seen in the kidneys of *klotho*$^{-/-}$ mice is disappeared in *klotho*$^{-/-}$/*1a(OH)ase*$^{-/-}$ DKO mice. No such renal calcification is found in *1a(OH)ase*$^{-/-}$ or WT mice (magnification x20).



Supplementary Table 1

Primer sequences used in real-time PCR to examine the expression of 1α(OH)ase, ennp-1, ANK, Pit-1, RUNX2 and GAPDH.

	Forward	*Reverse*
1α(OH)ase	5'-TCAGATGTTTGCCTTTGCCC-3'	5'-TGGTTCCTCATCGCAGCTTC-3'
enpp-1	5'-GCTAATCATCAGGAGGTCAAG-3'	5'-CTGGTAGAATCCCGTCAATC-3'
ANK	5'-GATGGCACTAGAGCGAGAAG-3'	5'-TCAGAAGTTACGAGACAAGACC-3'
Pit-1	5'- ACGAGTGGGTAGAGAGTC-3'	5'- ATGGCGGATTAGAGAAAGG-3'
RUNX2	5'-CTTCACAAATCCTCCCCAAG-3'	5'- -3' GAATGCGCCCTAAATCACTG
GAPDH	5'-ACTGAGGACCAGGTTGTC-3'	5'-TGCTGTAGCCGTATTCATTG-3'