SUPPLEMENTARY MATERIALS

Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging

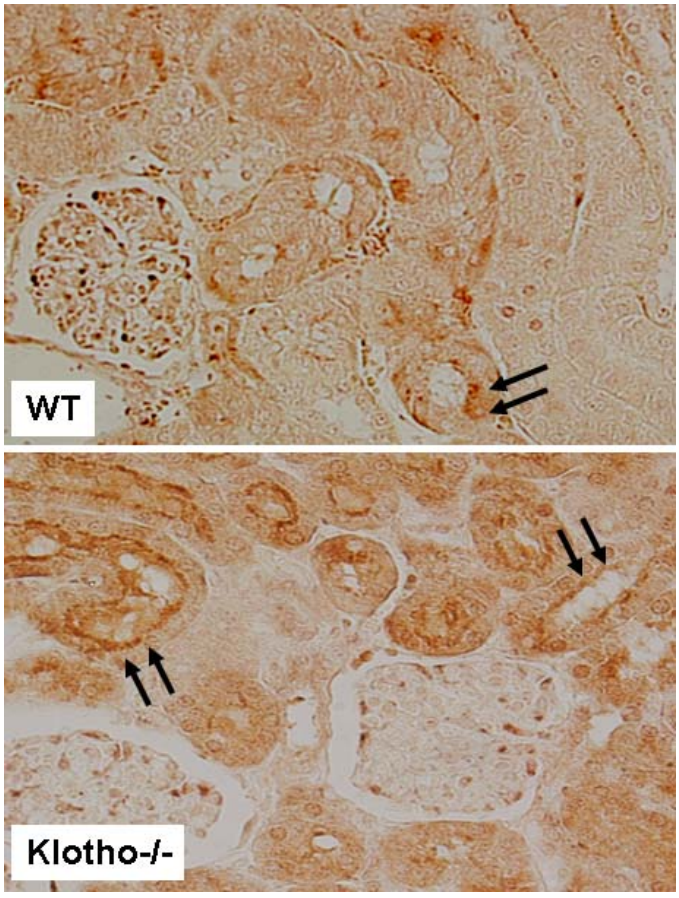
Mutsuko Ohnishi MD, PhD [1], **M. Shawkat Razzaque MD, PhD** [1, 2]

[1] Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA, USA; [2] Department of Pathology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Supplementary Figure 1

Expression of NaPi2a

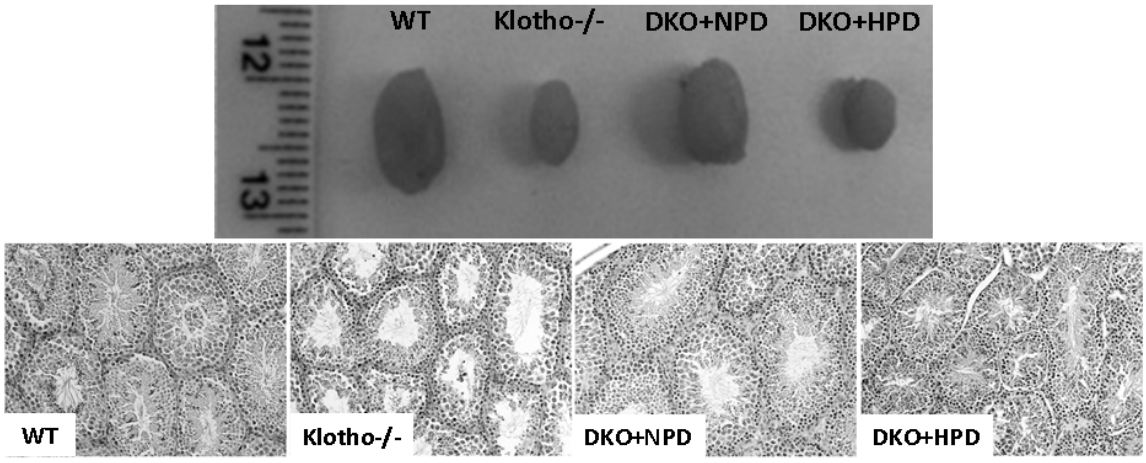
Immunostaining for NaPi2a in kidney sections prepared from wild-type (WT) and *klotho*$^{-/-}$ mice using a polyclonal antibody, as previously published (S1, S2). In contrast to WT kidney, there is markedly increased expression of NaPi2a in kidney sections from *klotho*$^{-/-}$ mice, located mostly at the luminal side of the proximal tubular epithelial cells (*arrows*) (magnification 20X).



Supplementary Figure 2

Gross and histological features of testes

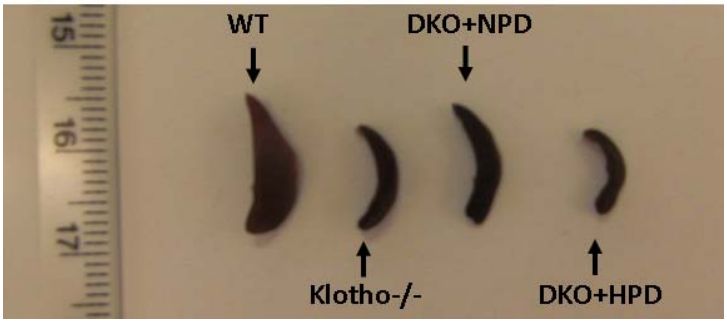
Testes obtained from wild-type (WT) mice, *klotho*$^{-/-}$ mice, *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with a normal-phosphate diet (DKO+NPD) and *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with a high-phosphate diet (DKO+HPD). Please note that, compared to DKO+NPD, testes are smaller in DKO+HPD mice and somewhat similar to hyperphosphatemic *klotho*$^{-/-}$ mice (upper panel). The animals are of similar age. The obtained testes are fixed in 10% formalin for at least 24 hours and then processed in the paraffin before sectioning and staining as detailed earlier (S3). Hematoxylin and eosin-stained sections of testes from 11-week-old WT mice, *klotho*$^{-/-}$ mice, DKO+NPD and DKO+HPD. When compared to DKO+NPD mice, there is diffuse atrophy of seminiferous tubules in testes of DKO+HPD mice with the resultant effect being infertility (magnification 20X).



Supplementary Figure 3

Gross appearance of spleen

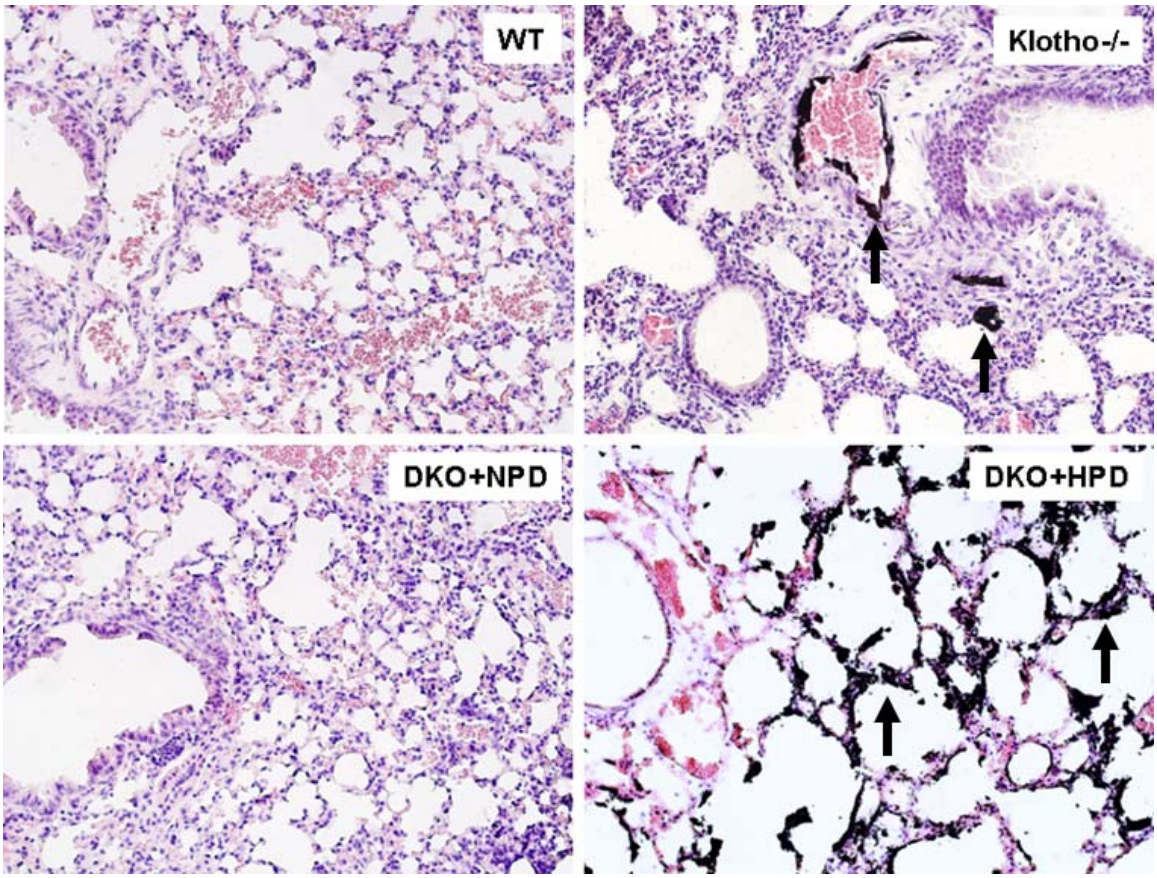
Spleens obtained from wild-type (WT) mice, *klotho*$^{-/-}$ mice, *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with a normal-phosphate diet (DKO+NPD) and *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with a high-phosphate diet (DKO+HPD). Note that, compared to DKO+NPD, the spleen is smaller in DKO+HPD mice, which is similar to hyperphosphatemic *klotho*$^{-/-}$ mice. The animals were matched for similar ages (11 week-old) and sexes.



Supplementary Figure 4

Ectopic lung calcification

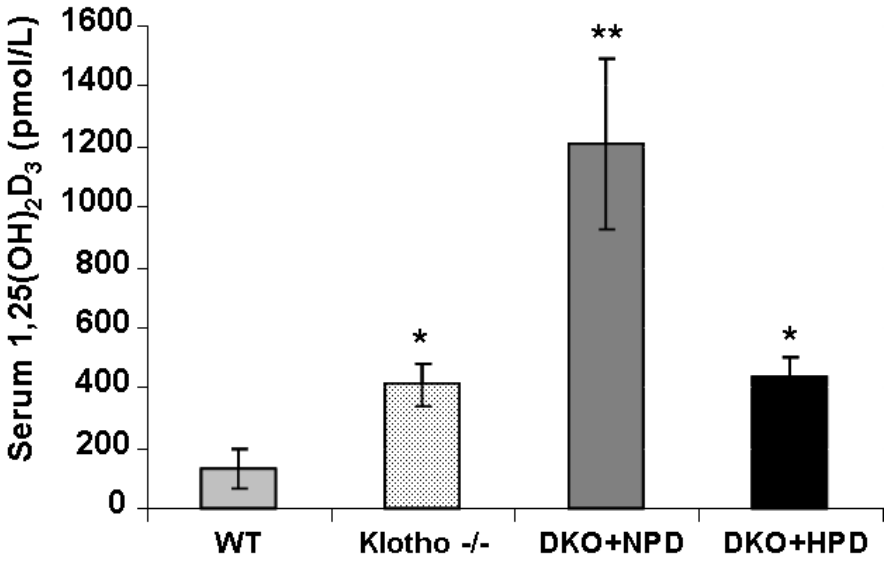
Lung sections prepared from wild-type (WT) mice, *klotho*$^{-/-}$ mice, *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with a normal-phosphate diet (DKO+NPD) and *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with a high-phosphate diet (DKO+HPD), showing pulmonary calcifications (*arrows*) in hyperphosphatemic *klotho*$^{-/-}$ mice. Inactivation of NaPi2a in *klotho*$^{-/-}$ mice eliminates this calcification from DKO+NPD mice. However, pulmonary calcification (*arrows*) reappears in DKO+HPD mice, suggesting that phosphate toxicity induces pulmonary calcification (von Kossa staining; magnification 20X).



Supplementary Figure 5

Serum 1,25 dehydroxyvitamin D levels

Serum 1,25 dehydroxyvitamin D levels in wild-type (WT), *klotho*$^{-/-}$ and *NaPi2a*$^{-/-}$/*klotho*$^{-/-}$ mice fed with either a normal-phosphate diet (DKO+NPD) or a high-phosphate diet (DKO+HPD). Serum 1,25 dehydroxyvitamin D levels are significantly higher in *klotho*$^{-/-}$ mice (n=6) when compared to WT mice (n=6), a pattern consistent with our earlier reported observations (S4, S5). Increased serum 1,25 dehydroxyvitamin D levels are also observed in DKO+NPD (n=9) and DKO+HPD (n=4) mice compared to WT controls (*: $p < 0.05$, *vs*. WT; **: $p < 0.01$, *vs*. WT).



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