Supplemental Figures

Kruppel-like factor 4 contributes to high phosphate-induced phenotypic switching of vascular smooth muscle cells into osteogenic cells

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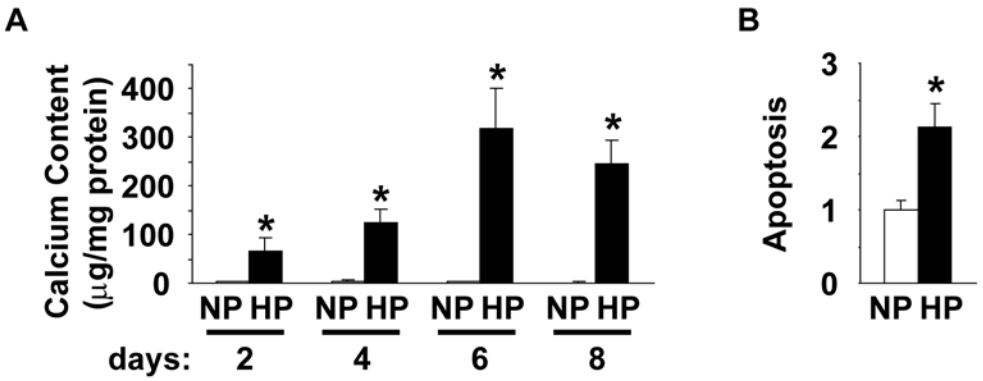
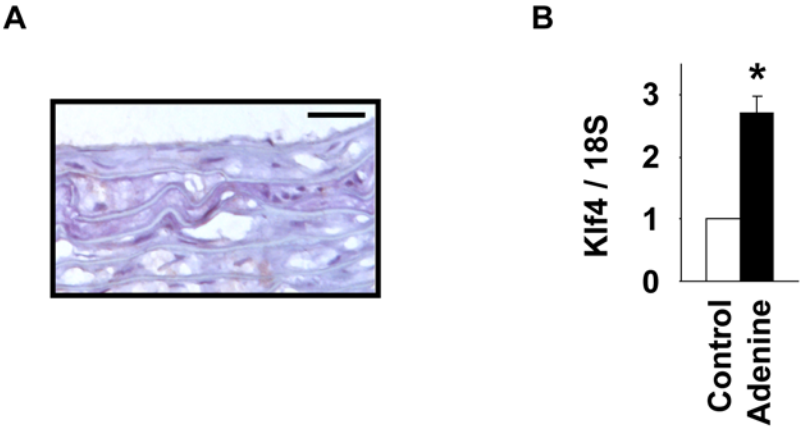
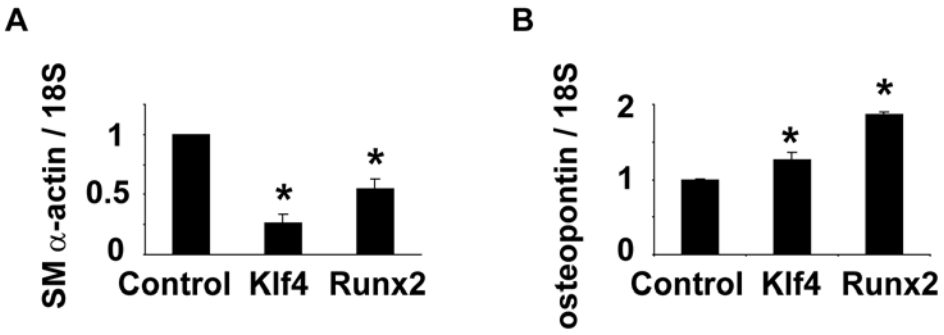


Supplemental Figure I. High phosphate concentration induced calcium deposition and apoptosis in cultured SMCs. A: Rat aortic SMCs were cultured with normal (NP) or high (HP) phosphate concentration for 2, 4, 6, and 8 days. Calcium deposition was measured and normalized by cellular protein content. B: SMCs were incubated with normal (NP) or high (HP) phosphate concentration for 24 hours. A quantitative index of apoptosis, determined by ELISA, is presented as the relative value to that with normal phosphate. Values represent the means ± SEM. *P<0.05 compared to SMCs with normal phosphate medium (n=3).



Supplemental Figure II. Klf4 was induced in the aorta of adenine-induced uremic rats. A: Negative IgG control for Figure 3H. Immunohistochemical staining was performed in the aorta of adenine-induced uremic rats using rabbit IgG, instead of anti-Klf4 antibody. Bar: 50 μm. B: *Klf4* mRNA expression in the aorta of adenine-induced uremic rats and controls was determined by real-time RT-PCR. Values represent the means ± SEM. *P<0.05 compared to controls (n=4).



Supplemental Figure III. Klf4 and Runx2 decreased *SM α-actin* expression, whereas they increased *osteopontin* expression. Rat aortic SMCs were transfected with the expression construct for Klf4 or Runx2, and expression of *SM α-actin* (A) and *osteopontin* (B) was determined by real-time RT-PCR. Values represent the means ± SEM. *P<0.05 compared to controls (n=3).