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Supplemental Information

***miR-27a-3p* Targets ATF3 to Reduce Calcium**

Deposition in Vascular Smooth Muscle Cells

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Figure S1

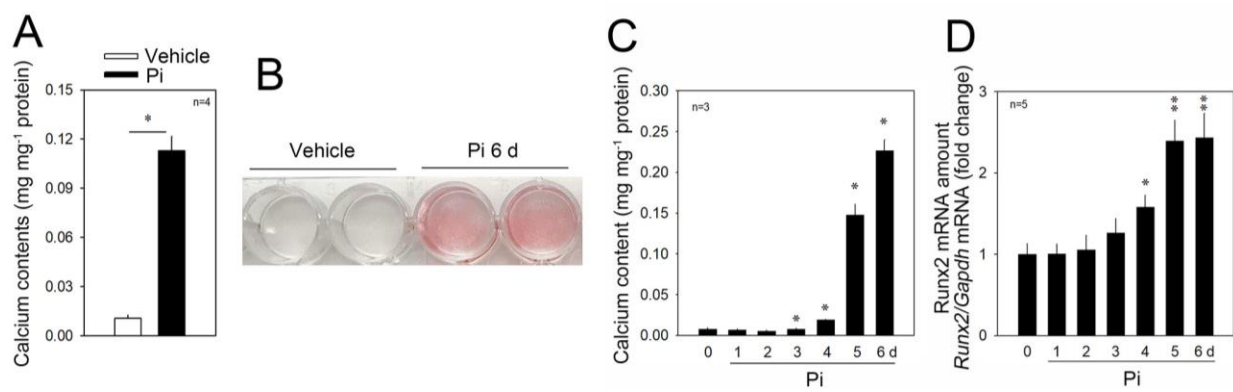


Figure S1. Inorganic phosphate (Pi) induce vascular calcification in vitro condition. (A) Treatment with inorganic phosphate (Pi) successfully induced calcium deposition in rat vascular smooth muscle cells (RVSMCs). (B) Treatment with Pi increased in a time-dependent fashion in A10 cells. (C) Alizarin red S staining shows that Pi for 6 days increased the calcium deposition. Pi: inorganic phosphate. Error bars indicate SD. * p<0.05, ** p<0.01.

Figure S2

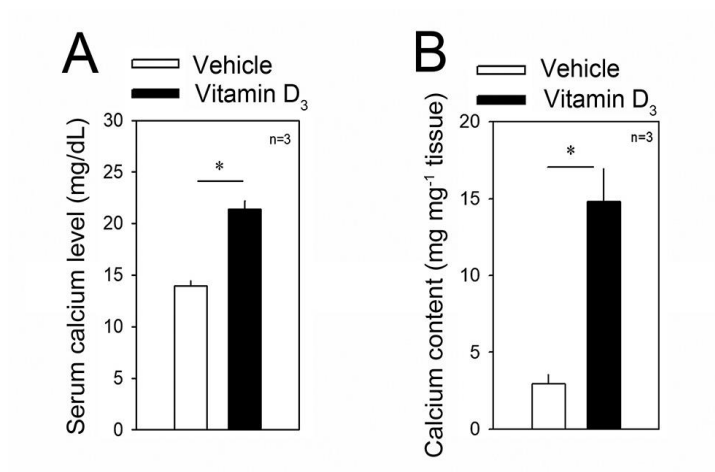


Figure S2. Vitamin D₃ induces vascular calcification in mouse aorta. Vitamin D₃ was administered daily for the first 3 consecutive days and then maintained for the following 6 days. (A) The serum calcium level was increased by vitamin D₃. (B) Calcium deposition was increased in the vitamin D₃-administered mouse aorta. Error bars indicate SD. * p<0.05, ** p<0.01.

Figure S4

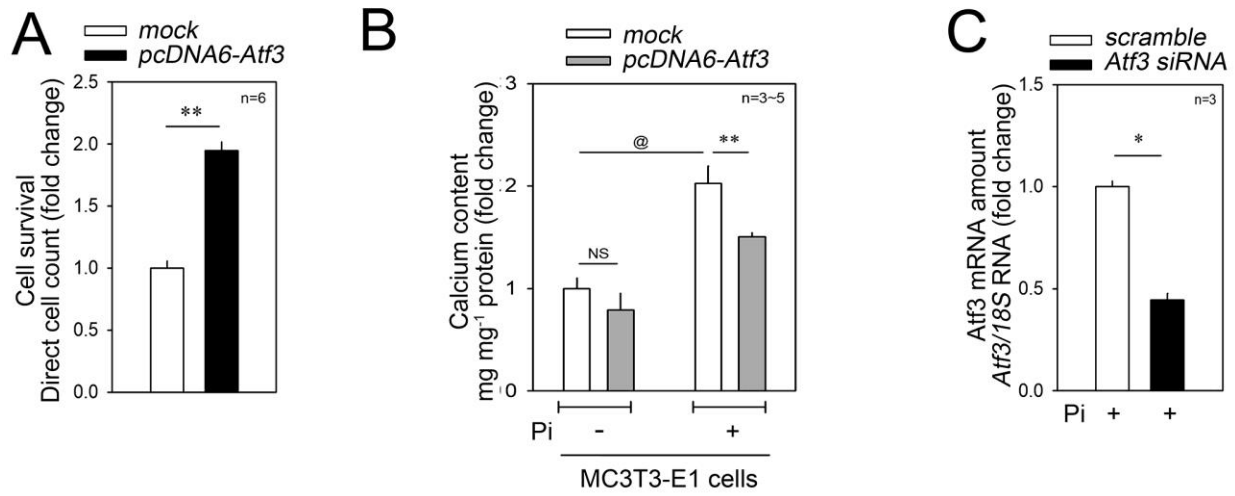


Figure S4. Effect of Atf3. (A) Transfection of *pcDNA6-Atf3-myc* increased the cell survival. (B) Atf3 blocked the Pi-induced increase in calcium deposition in MC3T3-E1 osteogenic precursor cells. (C) *Atf3 siRNA* reduced the mRNA level of Atf3 in A10 cells. Error bars indicate SD. NS: not significant, * p<0.05, ** and @@ p<0.01.

Figure S5

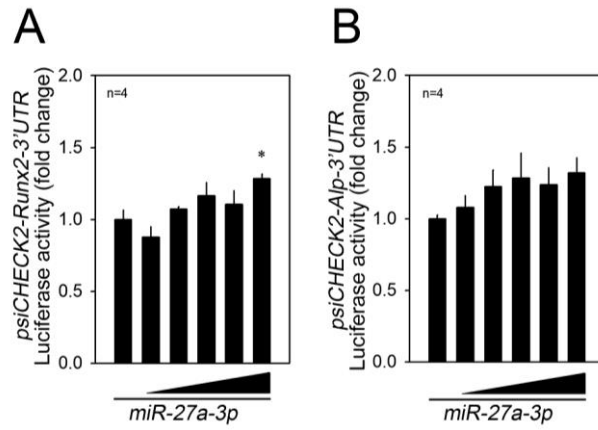


Figure S5. Effect of *miR-27a-3p* mimic transfection on luciferase activities of 3'UTRs of both Runx2 and Alp. (A) *miR-27a-3p* mimic failed to reduce *psiCHECK2-Runx2-3'UTR* luciferase activity. (B) *miR-27a-3p* mimic did not inhibit *psiCHECK2-Alp-3'UTR* luciferase activity. Error bars indicate SD. * $p < 0.05$.