TUMOR NECROSIS FACTOR-LIKE WEAK INDUCER OF APOPTOSIS FAVORS PHOSPHATE-INDUCED CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS

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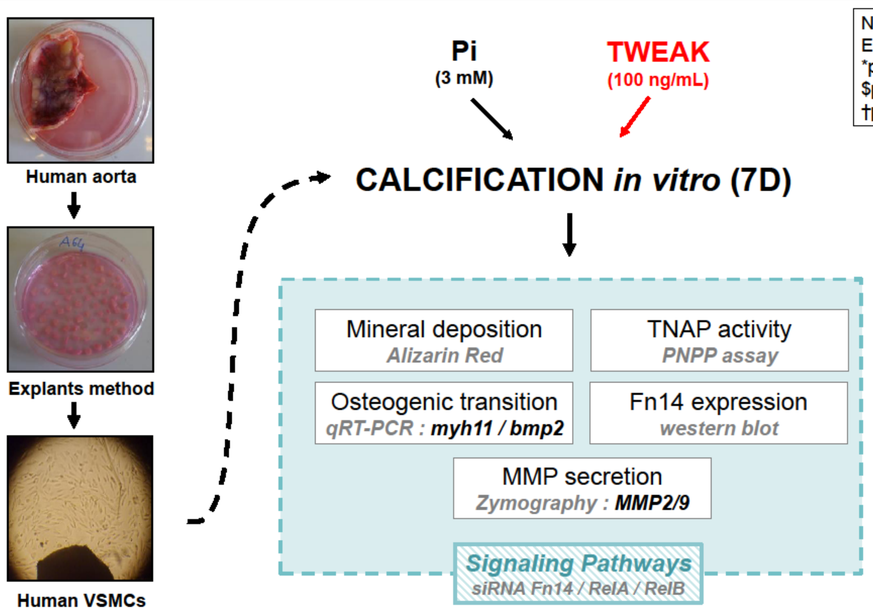
INTRODUCTION AND AIM

Medial calcification, which is a **hallmark in chronic kidney disease** (CKD), is associated with inflammatory status and is enhanced by inflammatory cytokines, such as TNF-α. **TNF-like weak inducer of apoptosis (TWEAK)**, which belongs to the TNF superfamily, recently emerged as new biomarker for the diagnosis and prognosis of cardiovascular diseases. **This study explored the involvement of TWEAK in human VSMCs (h-VSMCs) calcification *in vitro*.**

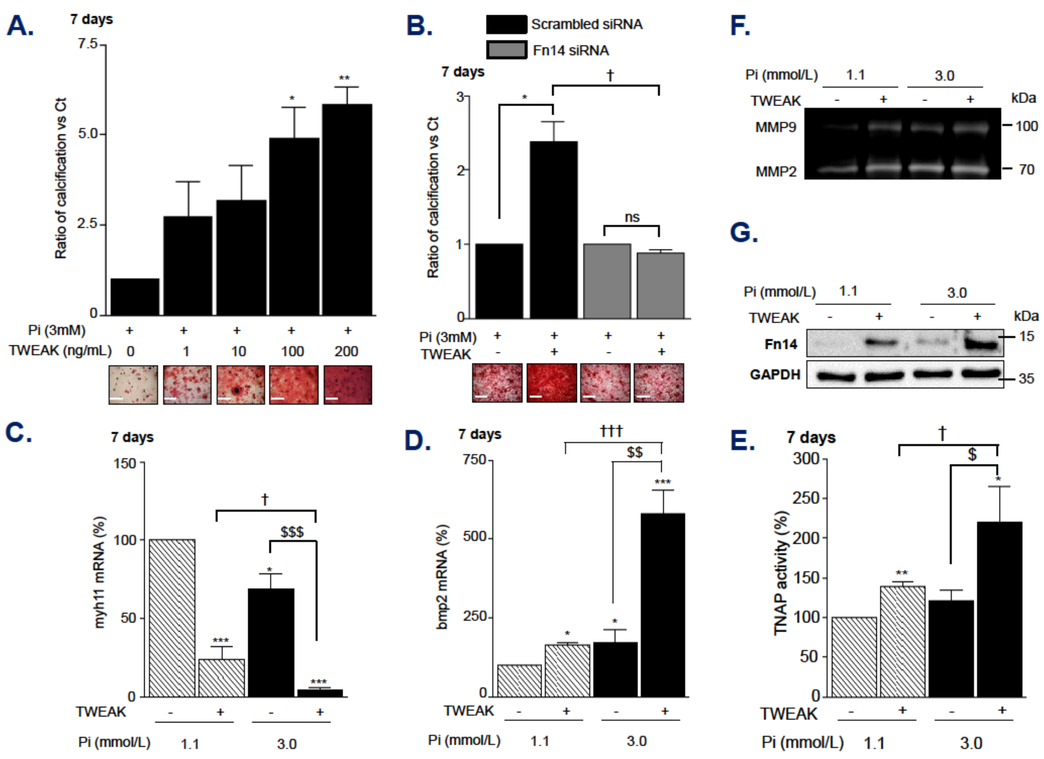
MATERIALS AND METHODS



N = 3 independent experiments
Error bars = S.E.M.
*p<0.05; **p<0.01; ***p<0.001
$p<0.05; $$p<0.01; $$$p<0.001
†p<0.05; ††p<0.01, †††p<0.001

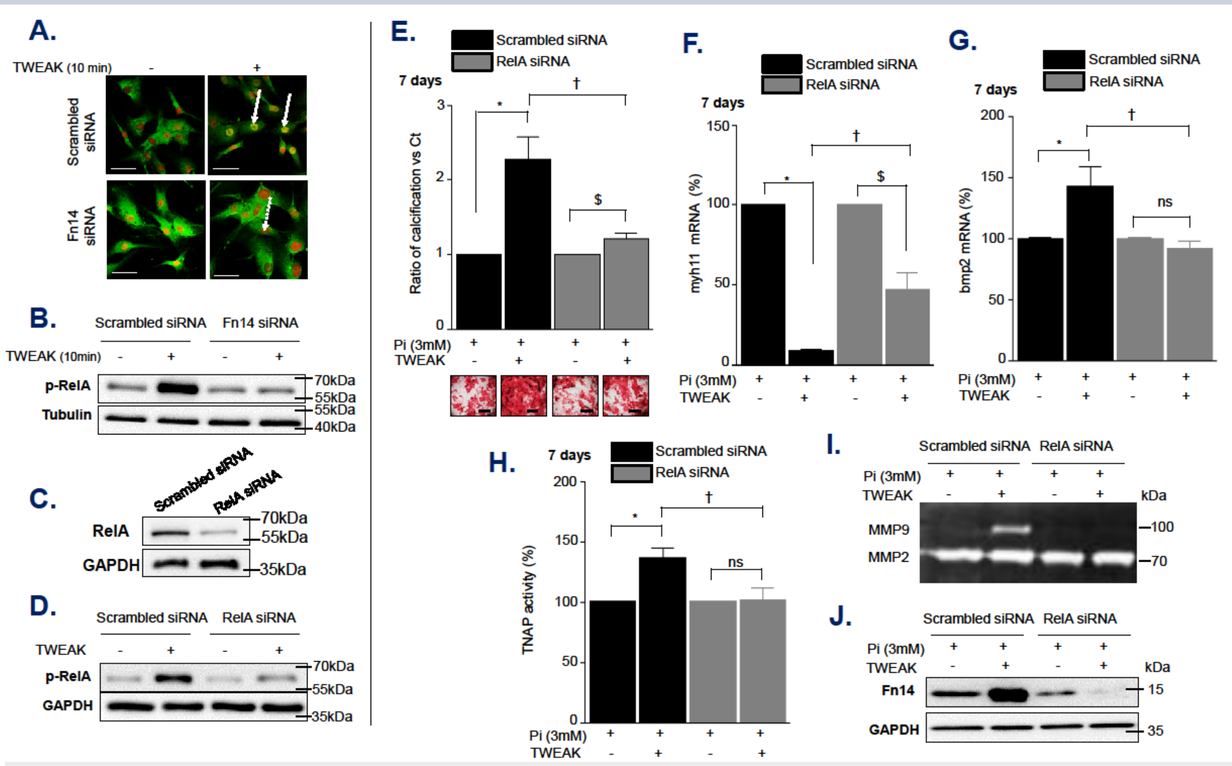
RESULTS

Figure 1. ***TWEAK promotes Pi-induced h-VSMCs calcification, and favors osteogenic transition, Fn14 expression and both TNAP and MMP9 activity***



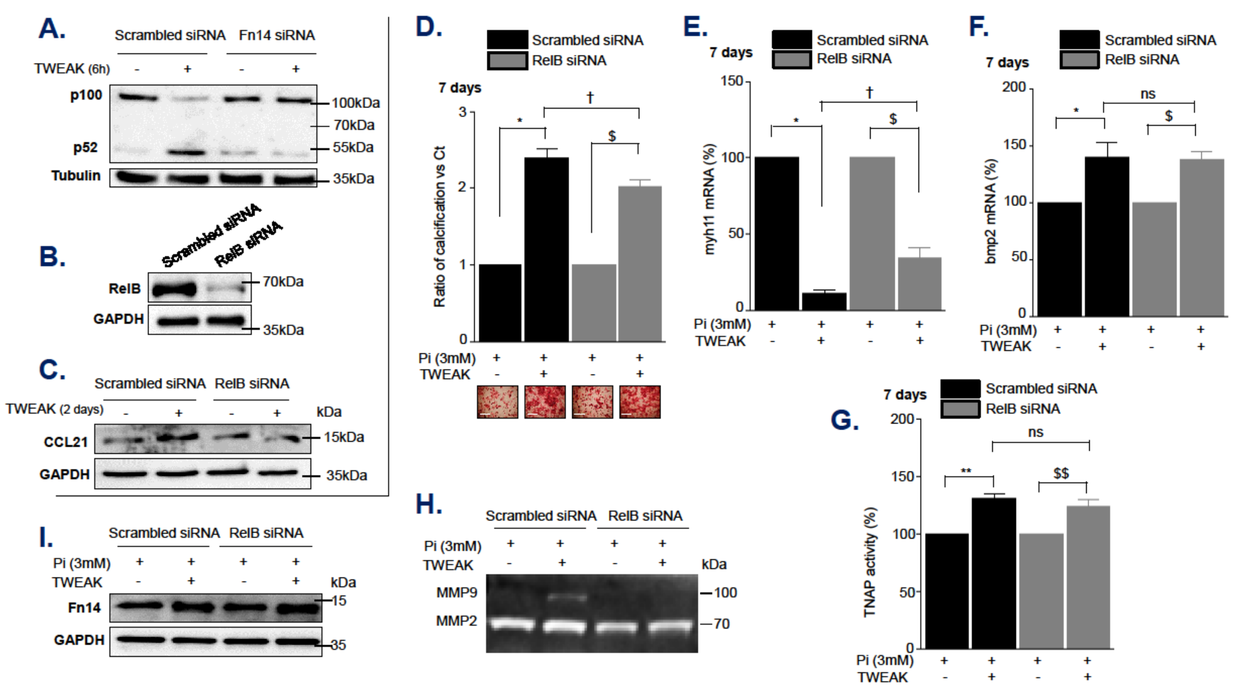
A. TWEAK promotes Pi-induced mineral deposition. *p<0.05 and **p<0.01 vs VSMCs not exposed to TWEAK. **B.** Fn14 downregulation blocks TWEAK-induced h-VSMCs calcification. Scale bars: 500µm. **C. and D.** TWEAK decreases myh11 (A.) and increases bmp2 (B.) mRNA expression. *p<0.05 and ***p<0.001 vs VSMCs cultured in 1.1 mmol/L Pi without TWEAK. **E. and F.** TWEAK promotes TNAP (C,) and MMP9 (D.) activity. *p<0.05 and **p<0.01 vs VSMCs cultured in 1.1 mmol/L Pi without TWEAK **G.** TWEAK favors Fn14 expression.

Figure 2. ***TWEAK/Fn14-induced canonical activation of NFκB pathway favors Pi-induced h-VSMCs calcification, as well as osteogenic transition, Fn14 expression and both TNAP and MMP9 activity***



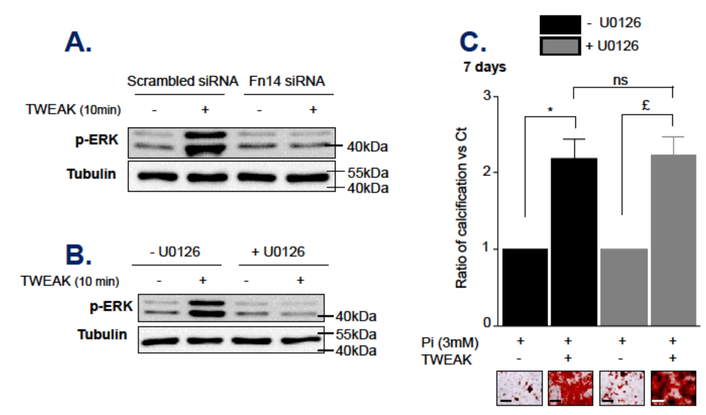
A. and B. Fn14 downregulation blocks TWEAK-induced RelA translocation (A.) and subsequent phosphorylation (B.). Images scale bars: 50µm. **C. and D.** Exposure to RelA siRNA for 2 days downregulates RelA expression by 80% (C.) and blocks TWEAK/Fn14-induced RelA phosphorylation (D.). **E.** RelA downregulation reduces TWEAK/Fn14 procalcific effects by 80%. **F., G. and H.** RelA downregulation decreases TWEAK modulation of myh11 mRNA (F.) and blocks TWEAK-induced modulation of bmp2 mRNA (G.) as well as TNAP activity (H.). **I. and J.** RelA downregulation abolishes TWEAK-induced MMP9 activity (I.) and Fn14 expression (J.).

Figure 3. ***TWEAK/Fn14-induced non-canonical activation of NFκB pathway is responsible for 20% of TWEAK procalcific properties. It favors h-VSMCs loss of contractile phenotype and increases MMP9 activity but does not modulate neither bmp2 and Fn14 expression nor TNAP activity.***



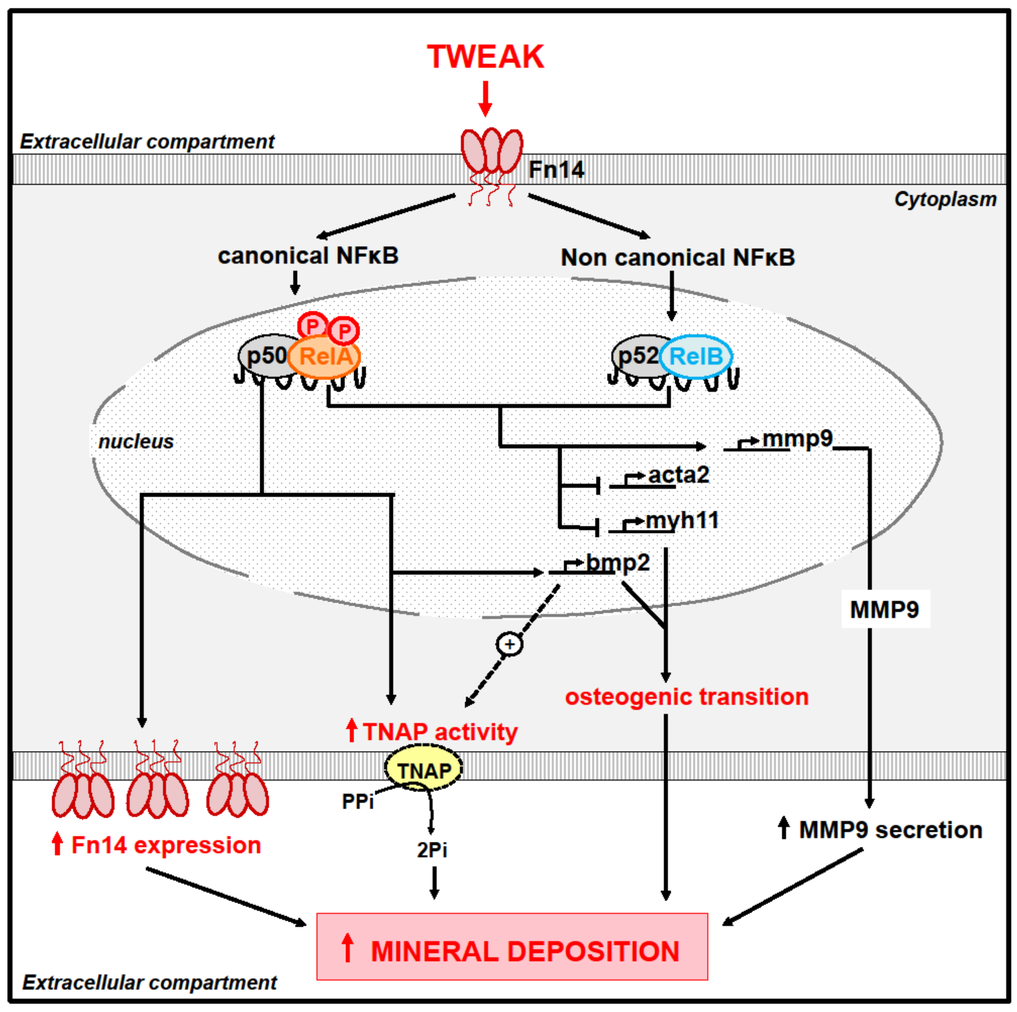
A. Fn14 downregulation blocks TWEAK-induced p100 processing into p52. **B. and C.** Exposure to RelB siRNA for 2 days downregulates RelA expression by 80% (B.) and blocks TWEAK/Fn14-induced expression of the non-canonical NFκB target gene CCL21 (C.). **D.** RelB down-regulation reduces by 20% TWEAK/Fn14-induced h-VSMCs calcification. Images scale bars: 500µm. **E., F. and G.** RelB downregulation reduces TWEAK modulation of myh11 mRNA (E.) but does not influence neither TWEAK-induced modulation of bmp2 mRNA (F.) nor TNAP activity (G.). **H. and I.** RelB downregulation abolishes TWEAK-induced MMP9 activity (H.) but does not influence Fn14 expression (I.).

Figure 4. ***TWEAK/Fn14-induced MAPK activation is not involved in TWEAK pro-calcific effects.***



A. Fn14 downregulation blocks TWEAK-induced ERK phosphorylation. **B.** U0126 blocks TWEAK/Fn14-induced ERK phosphorylation. **C.** Blockade of TWEAK/Fn14-induced ERK phosphorylation by U0126 does not influence TWEAK/Fn14 procalcific properties. Images scale bar: 500µm.

CONCLUSIONS



Conclusions. TWEAK/Fn14 strongly favors Pi-induced h-VSMCs calcification. Indeed, **80% of TWEAK/Fn14 procalcific effects are mediated through activation of canonical NFκB pathway**, which favors Pi-induced hVSMCs osteogenic transition, Fn14 expression as well as TNAP and MMP9 activity. Loss of h-VSMCs contractile markers and potentiation of MMP9 activity as a consequence of **non-canonical NFκB activation are responsible for 20% of TWEAK pro-calcific properties**. As a consequence, and given the availability of clinical-stage neutralizing anti-TWEAK strategies, **TWEAK/Fn14 axis appears as a novel therapeutic target in the prevention of CKD-related medial calcification**

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