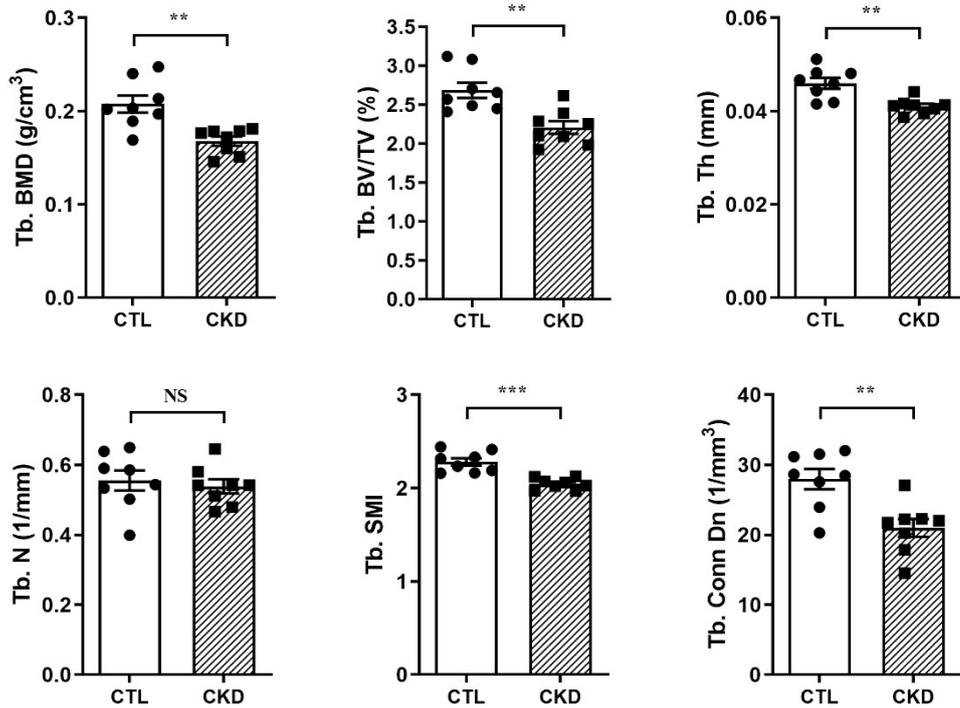
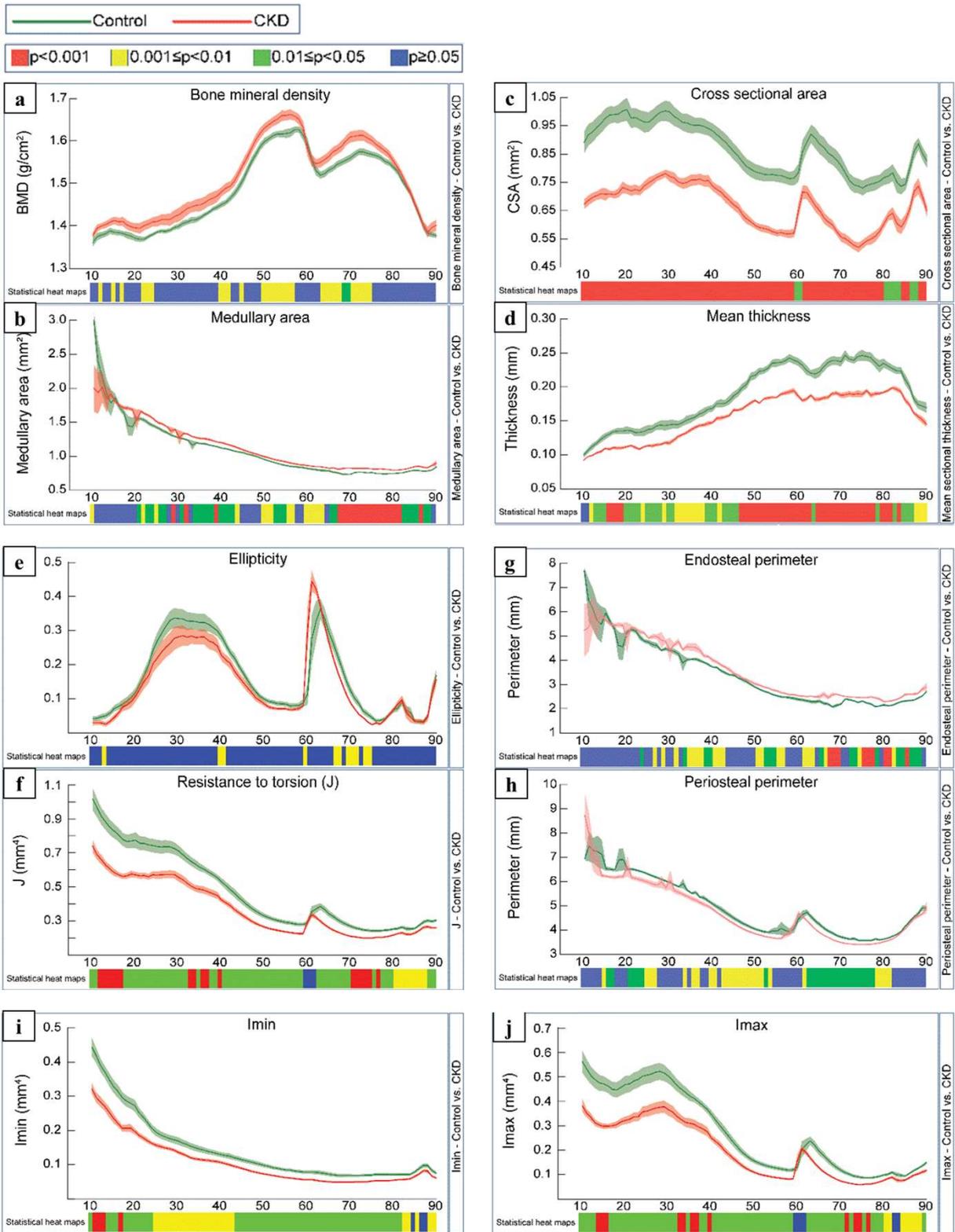


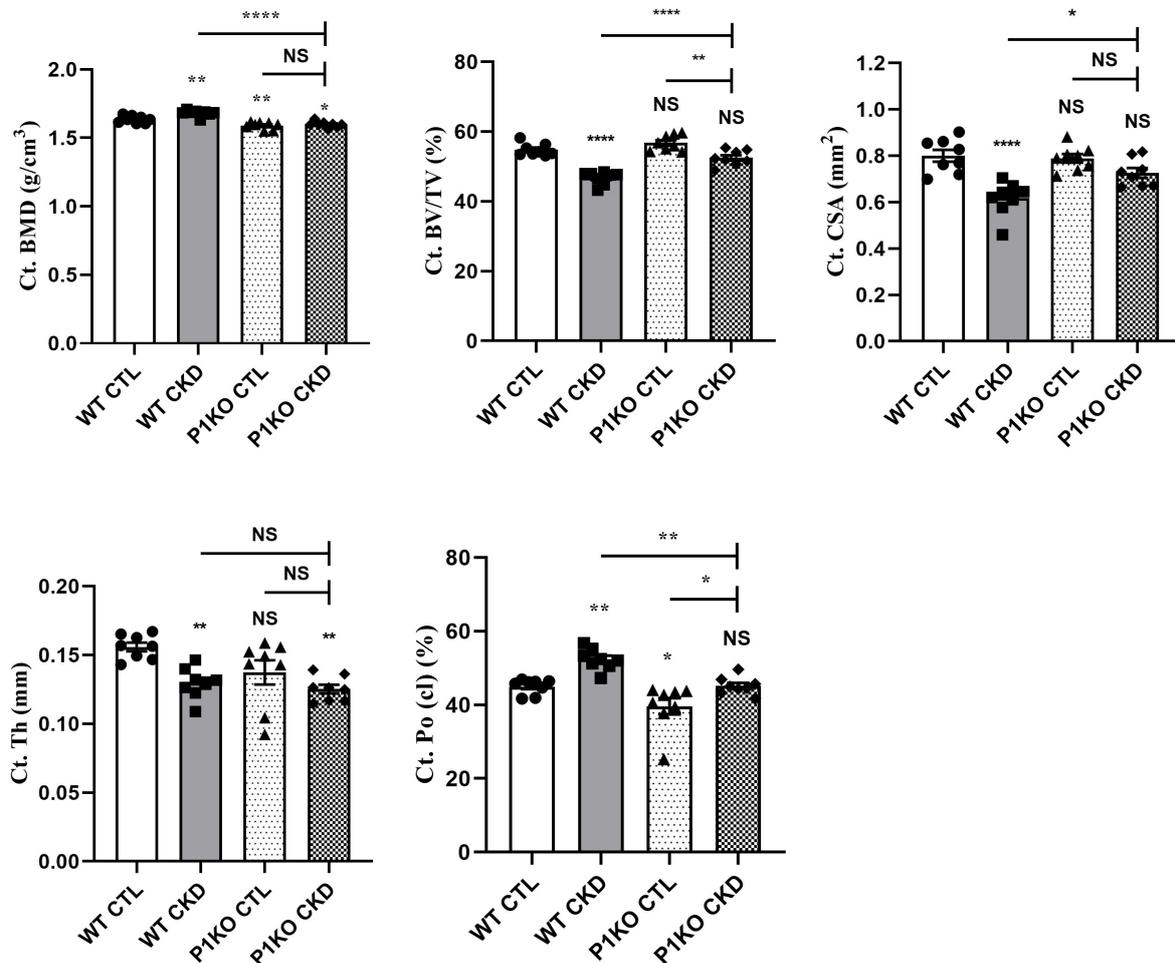
**Fig 1. Expression of osteoblast and mineralization markers in mouse femurs from CTL and CKD mice.** (A) Expression of key mineralization and osteoblast marker genes in femurs of CTL and CKD mice at end of the study (13 weeks of age). Of note, *Fgf23* and *Phospho1* expression were increased and *Alpl* expression was decreased in the femurs of the CKD-MBD mice. (B) Representative image of 2 CTL and 2 CKD-MBD femurs analyzed by western blot for PHOSPHO1 and TNAP expression (C) Quantification of PHOSPHO1 and TNAP expression indicated that PHOSPHO1 was increased and TNAP was decreased in the femur of CKD-MBD mice compared with control mice. The data are represented as the mean  $\pm$  SEM (n = 8); \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ ).



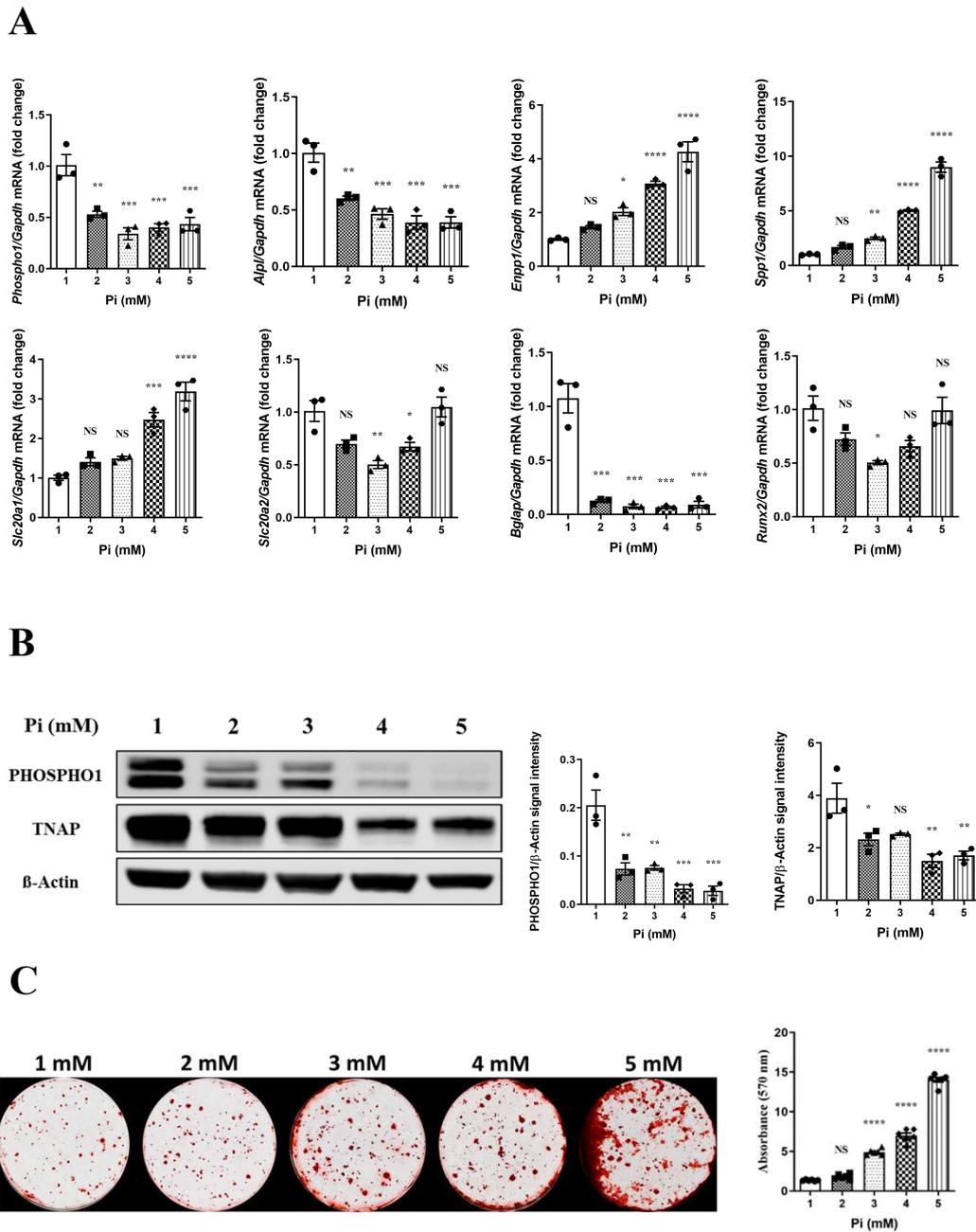
**Fig 2. Micro-CT analysis of trabecular bone of the tibia.** Micro-CT analysis of tibia from male C57BL/6 mice fed a CTL or CKD diet for 5 weeks. Tb. BMD (trabecular bone mineral density; g/cm<sup>3</sup>); Tb. BV/TV (trabecular bone volume/tissue volume; %); Tb. Th. (trabecular thickness; mm); SMI (structure model index); Tb. Conn Dn (trabecular connectivity density; mm<sup>-3</sup>) were all decreased in the CKD-MBD mice. Tb. N. (trabecular number; mm<sup>-1</sup>) was unchanged. Tibia of n = 8 (CTL mice) vs n = 8 (CKD-MBD mice) biological replicates were analysed. The data are represented as the means ± SEM. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001 versus CTL.



**Fig 3. Micro-CT analysis of whole cortical bone of the tibia.** Micro-CT analysis of tibia from male C57BL/6 mice fed a CTL or CKD diet for 5 weeks. Quantification of whole bone analyses of cortical bone between 10% and 90% of total tibial length, excluding proximal and distal metaphyseal bone, of CTL and CKD tibia at 13 weeks of age. (a) BMD (bone mineral density;  $\text{g}/\text{cm}^3$ ), (b) medullary area ( $\text{cm}^2$ ) and (g) endosteal perimeter (mm) were generally increased and (c) CSA (cross-sectional area;  $\text{mm}^2$ ), (d) mean thickness (mm), (f) resistance to torsion (J;  $\text{mm}^4$ ), (h) periosteal perimeter (mm), (i)  $I_{\text{min}}$  ( $\text{mm}^4$ ), (j)  $I_{\text{max}}$  ( $\text{mm}^4$ ) were generally decreased in the CKD-MBD bones. Tibia of  $n = 8$  (CTL mice) vs  $n = 8$  (CKD mice) biological replicates were analysed.  $p < 0.05$  was significant and  $p \leq 0.01$ – $0.05$  was noted as green,  $p \leq 0.001$ – $0.01$  as yellow and  $p \leq 0.000$ – $0.001$  as red. Not significant is noted as blue.

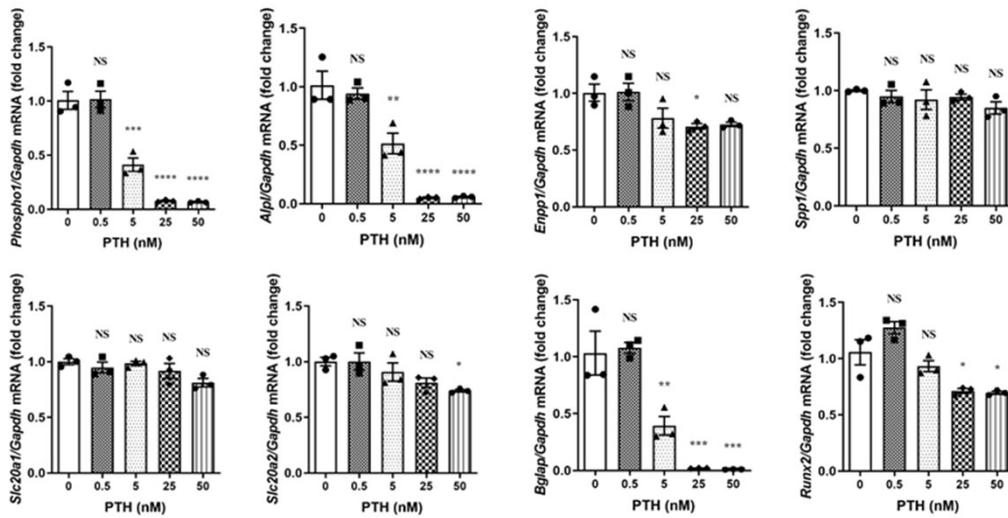


**Fig 4. Micro-CT analysis of cortical bone of wild-type (WT) and PHOSPHO1 deficient CTL and CKD mice.** Quantification of cortical bone mineral density (Ct. BMD), cortical bone volume/tissue volume (Ct. BV/TV), cortical cross-sectional area (Ct. CSA), cortical thickness (Ct. Th), and closed pore porosity (Ct Po (cl)) at 50% of the total tibial length from the top of the tibia. Of note, BMD was increased in the WT CKD-MBD tibia but not the PHOSPHO1 deficient CKD-MBD tibia when compared to their respective controls. The data are represented as the mean  $\pm$  SEM (n = 8); \*  $p < 0.05$ ; \*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  compared to WT CTL bones.

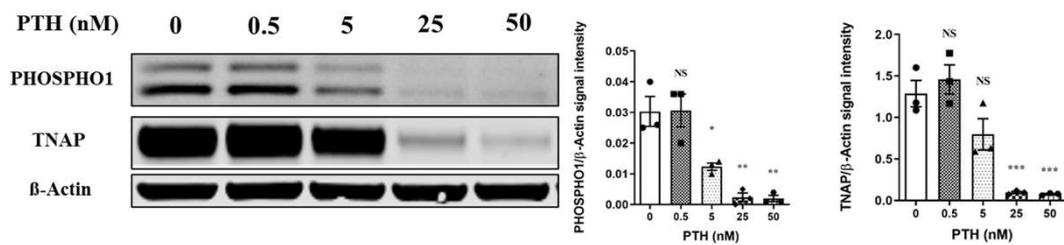


**Fig 5. Regulation of key mineralization associated genes, proteins and osteoblast extracellular matrix mineralization by Pi in primary osteoblasts.** (A) Expression analysis of *Phospho1*, *Alpl*, *Enpp1*, *Spp1*, *Slc20a1*, *Slc20a2*, *Bglap*, and *Runx2* by osteoblasts in response to Pi (1-5 mM), (B) western blotting analysis and quantification of PHOSPHO1 and TNAP expression in response to Pi and (C) representative images and quantification of alizarin red staining in response to Pi for 28 days after confluency. PHOSPHO1 and TNAP at the gene and protein level were decreased with increasing Pi concentrations whereas matrix mineralization increased with increasing Pi concentrations. The data are represented as the mean  $\pm$  SEM (n = 3); \* $p$  < 0.05; \*\*  $p$  < 0.01; \*\*\*  $p$  < 0.001; \*\*\*\*  $p$  < 0.0001 compared to 1 mM Pi cultures.

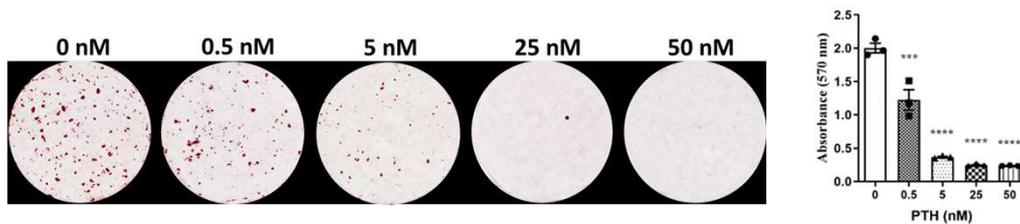
**A**



**B**

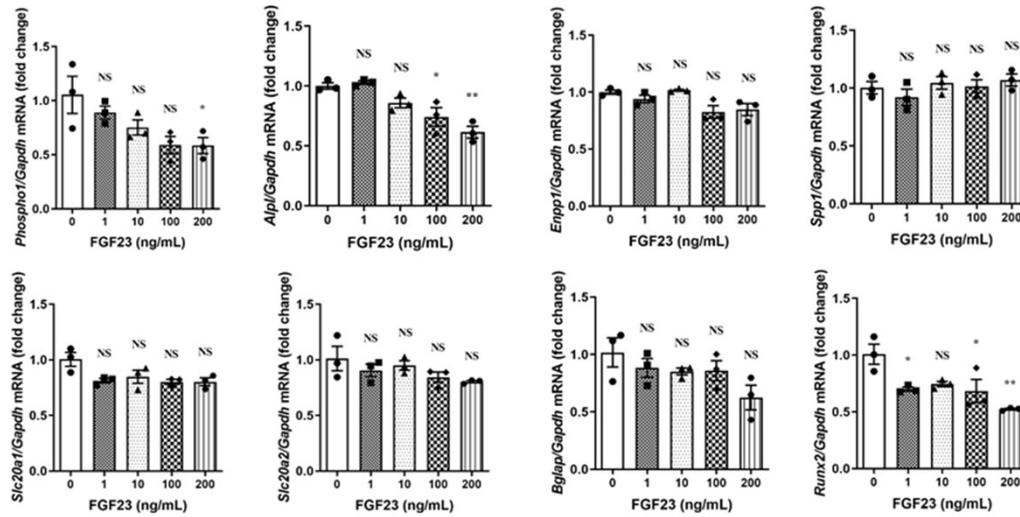


**C**

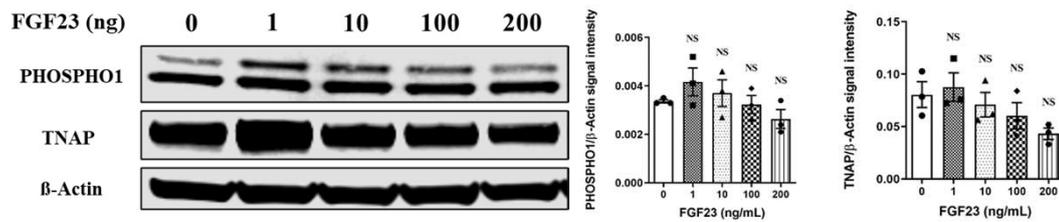


**Fig 6. Regulation of key mineralization associated genes, proteins and osteoblast extracellular matrix mineralization by PTH in primary osteoblasts.** (A) Expression analysis of *Phospho1*, *Alpl*, *Enpp1*, *Spp1*, *Slc20a1*, *Slc20a2*, *Bglap*, and *Runx2* by osteoblasts in response to PTH (0-50 nM), (B) western blotting analysis and quantification of PHOSPHO1 and TNAP expression in response to PTH and (C) representative images and quantification of alizarin red staining in response to PTH for 28 days after confluency. PHOSPHO1 and TNAP at the gene and protein level and matrix mineralization were all decreased with increasing Pi concentrations. The data are represented as the mean  $\pm$  SEM (n = 3); \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  compared to 0 nM PTH cultures.

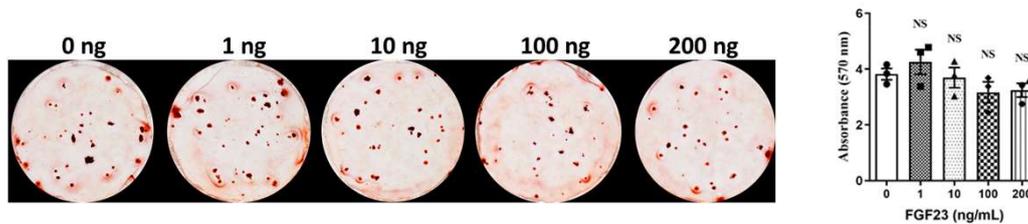
**A**



**B**



**C**



**Fig 7. Regulation of key mineralization associated genes, proteins and osteoblast extracellular matrix mineralization by FGF23 in primary osteoblasts.** (A) Expression analysis of *Phospho1*, *Alpl*, *Enpp1*, *Spp1*, *Slc20a1*, *Slc20a2*, *Bglap*, and *Runx2* by osteoblasts in response to FGF23 (0-200 ng/ml), (B) western blotting analysis and quantification of PHOSPHO1 and TNAP expression in response to FGF23 and (C) representative images and quantification of alizarin red staining in response to FGF23 for 28 days after confluency. *Phospho1* and *Alpl* gene expression were decreased at the highest FGF23 concentrations but non-significant differences were noted with PHOSPHO1 and TNAP expression and matrix mineralisation. The data are represented as the mean  $\pm$  SEM (n = 3); \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  compared to 0 nM FGF23 cultures.