# Role of PHOSPHO1 in chondrocyte matrix vesicle mineralization: an AFM study

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## BACKGROUND

- Matrix vesicles (MVs) are a special class of extracellular vesicles (EVs) that initiate mineralization in cartilage and other vertebrate tissues by accumulating $Ca^{2+}$ and inorganic phosphate ($P_i$) and forming crystalline mineral deposits.[1]
- During the first stages of mineralization, mineral deposits of $Ca^{2+}$ and $P_i$ within the MV lumen are not crystalline and form the so called nucleation core (NC).[2]
- We have recently shown that MV calcification is regulated by PHOSPHO1. The genetic ablation of *Phospho1* impairs the formation of mineral deposits within the MV lumen, suggesting that intra-vesicular production of $P_i$ is necessary for the correct $Ca^{2+}/P_i$ stoichiometry for NC formation.[3]

## OBJECTIVE

- Investigate the role of PHOSPHO1 on MV biogenesis and volume growth.
- Monitor the mineralization status of MVs with differing mineralization potential, *i.e.* mineralization-competent (WT) and mineralization-compromised (*Phospho1*$^{-/-}$) MVs.

## METHODS

- Tapping-mode AFM (TM-AFM) topography imaging under light tapping has been used to characterize the volume and number of WT and *Phospho1*$^{-/-}$ MVs.
- TM-AFM phase imaging under inelastic cantilevered tip-sample interactions has been used to map elastic property variation in WT and *Phospho1*$^{-/-}$ MVs and enable compositional mapping.

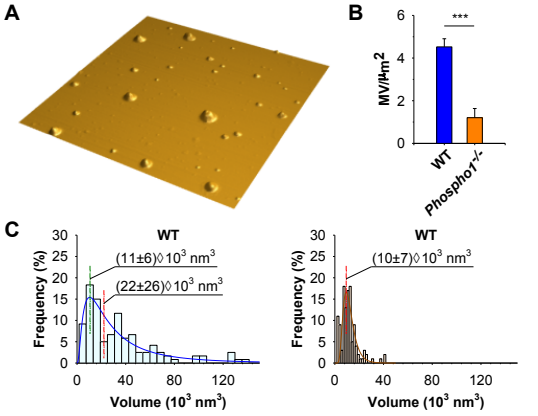


**Figure 1. Morphology, number and volume distribution of WT and *Phospho1*$^{-/-}$ MVs. A.** TM-AFM 3D topography images of WT MVs. Scan size 3μm × 3μm. **B.** Number of WT and *Phospho1*$^{-/-}$ MVs in a scan size with an area of 1μm$^2$. Results are expressed as mean ± SEM. Statistical differences between samples were calculated by non-parametric Mann–Whitney U analysis. *** $p < 0.001$. **C.** Volume distribution for WT and *Phospho1*$^{-/-}$ MVs.

## RESULTS

- WT and *Phospho1*$^{-/-}$ MVs appeared as globular flattened features (Figure 1A). The number of WT MVs was statistically greater than the number of *Phospho1*$^{-/-}$ MVs (Figure 1B). WT MVs had a left-skewed volume distribution with mode and mean value of $11\times10^3$ nm$^3$ and $22\times10^3$ nm$^3$, respectively, whereas the distribution of volumes for *Phospho1*$^{-/-}$ MVs had a narrow distribution with both mode and mean value of $10\times10^3$ nm$^3$ Figure 1C).
- WT MVs showed changes in surface morphology and phase with vesicle volume (Figure 2). Phase distribution showed the presence of a portion of MV lumen stiffer than other portions. We posit that this portion is the NC.
- The portion of WT MV lumen surrounding the NC was composed by two parts: one was less stiff than other vesicle portions, the other had values of stiffness lower than the NC and formed clusters around the NC within bigger vesicles.
- *Phospho1*$^{-/-}$ MVs did not show any appreciable changes in surface morphology and phase with changes in vesicle volume.

## CONCLUSIONS

- AFM topography and phase imaging enabled us to track the changes in the lumen of WT and *Phospho1*$^{-/-}$ MVs and validate the role of PHOSPHO1 in regulating the intra-vesicular level of $P_i$ necessary to trigger NC formation.
- Future studies will aim at relating compositional variation in MV lumina to vesicle nanoscale elastic modulus. These studies will use a combination of Raman spectroscopy and AFM nano-indentation on volume-fractionated MVs.

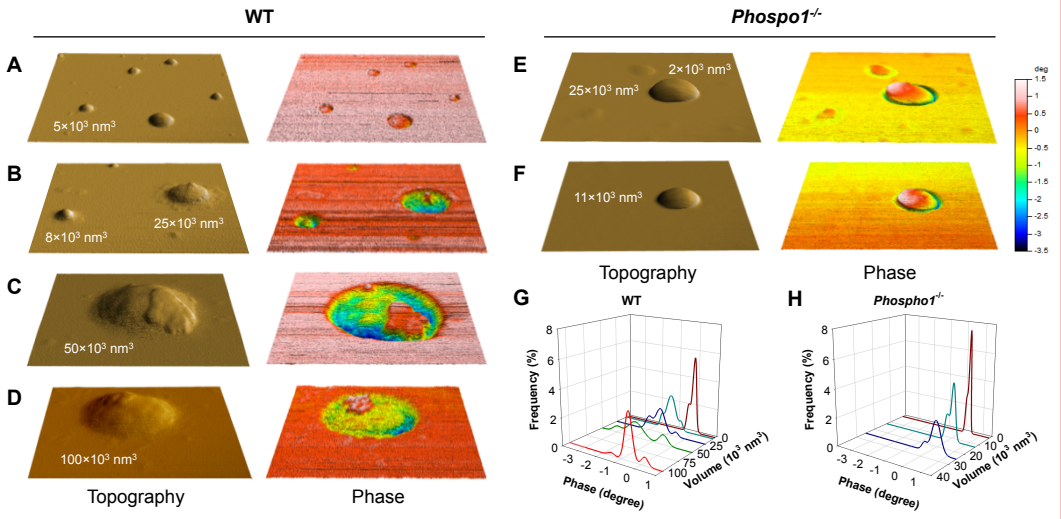


**Figure 2. Phase changes with vesicle volume for WT and *Phospho1*$^{-/-}$ MVs. A – F.** 3D topography and phase images of WT (A. to D.) and *Phospho1*$^{-/-}$ (E. and F.) MVs with different volumes. Scan size 1μm × 1μm (A), 600nm × 600nm (B), 350nm × 350nm (C- F). Phase scale bar on the right is common for all the depicted phase images. **G** and **H.** 3D graphs of the distributions of phase values for WT (G) and *Phospho1*$^{-/-}$ (H) MVs represented in the AFM images on the left. These graphs show the changes in lumen viscoelasticity of WT and *Phospho1*$^{-/-}$ MVs with vesicle volume. The distributions of phase values across MV surface were calculated by considering the average phase value of mica substrate equal to zero.

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**Disclosure.** All authors report no conflicts of interest

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