Investigating the nature of fluorescent vesicles released by mineralizing osteoblast reporter line MC3T3

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Biomineralization of bone is an essential developmental process, providing the inorganic components of bone. Bones are made up of three types of cells, osteoblasts, osteoclasts and osteocytes. Osteoblasts are crucial for production of a mineralized extracellular matrix (EMC). Following secretion of ECM by osteoblasts, they line the bone and secrete Matrix Vesicles (MV's). These are small membrane-bound vesicles (50-300nm) that concentrate calcium and phosphate leading to the production of crystalline calcium phosphate, hydroxyapatite. This project aimed to investigate the nature of fluorescent vesicles released by mineralising matrix vesicle (MV) reporter osteoblast (PHaNTAM) cells (a reporter cell line created prior to the start of the project) through understanding the characterisation and trafficking of the vesicles.

Vesicle isolation

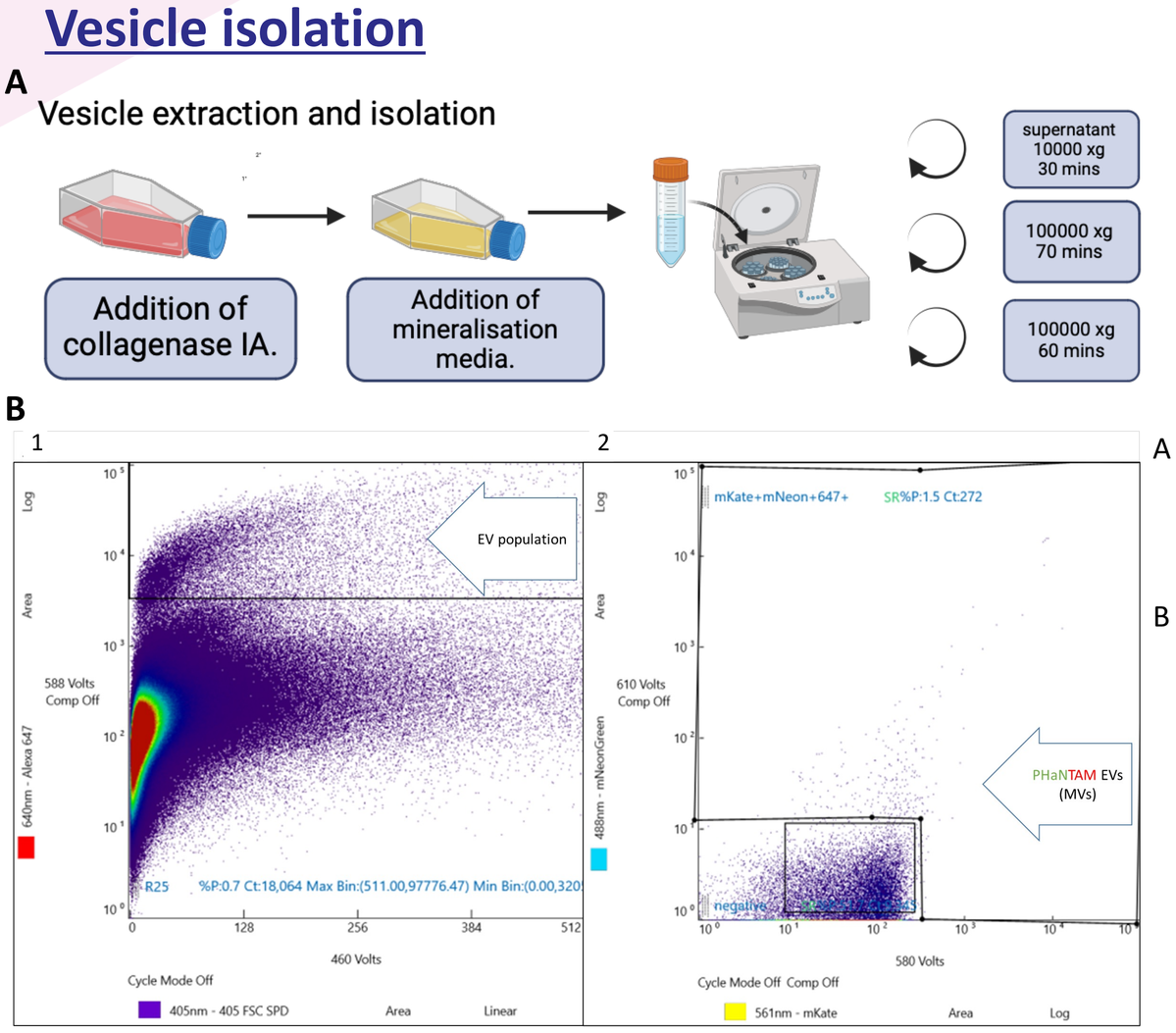

Figure 2: (A) Vesicle isolation workflow of mineralising PHaNTAM cells. (B)(1) Small Particle Detection using a Bigfoot Spectral cell sorter showed EVs produced by mineralising PHaNTAM cells after 6 days in osteogenic media, stained by lipid membrane dye CellMask647.Distinct population of EVs above background is marked by arrow. (2) Within total observed EVs, a 488nm (PHOSPHO1-MnG) and 561nm (TNAP-mKate2) positive population of varying fluorescent intensity is marked by arrow (MVs).

Results: The small particle detection through cell sorting showed Extracellular vesicles (EVs) are produced by mineralising PHaNTAM cells after 6 days in osteogenic media.

Western blot

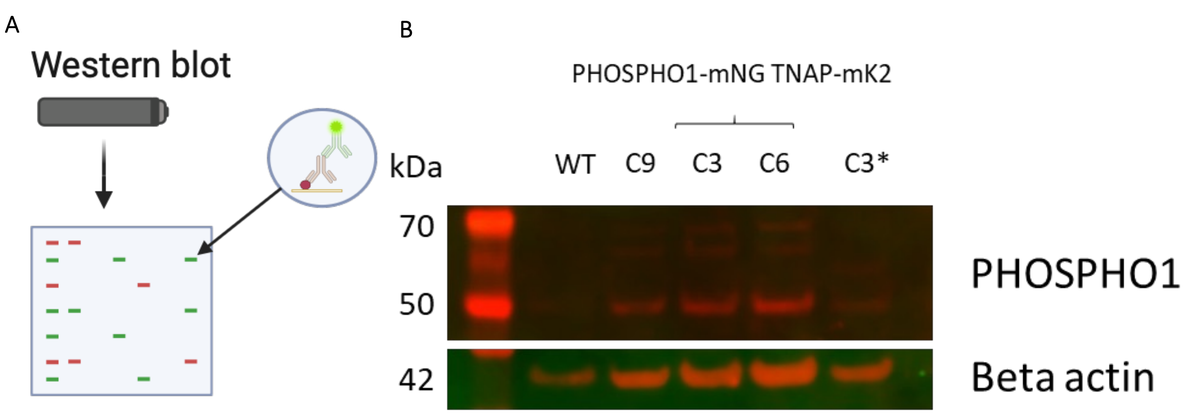

Figure 3 : (A) western blot methodology red and green colours show banding presence and the use of a loading ladder. (B) Western blot showing the presence of PHOSPHO1-mNG TNAP-mK2 at 50 kDa, and beta actin at 42kDa (loading control) imaged using Licor oddesy Fc camera system.

Results: Western blot analysis showed the presence of bands of PHOSPHO1 at 50 kDa in across samples and not in the WT control. Confirming the expression of PHOSPHO1 in this cell line.

Golgi trafficking

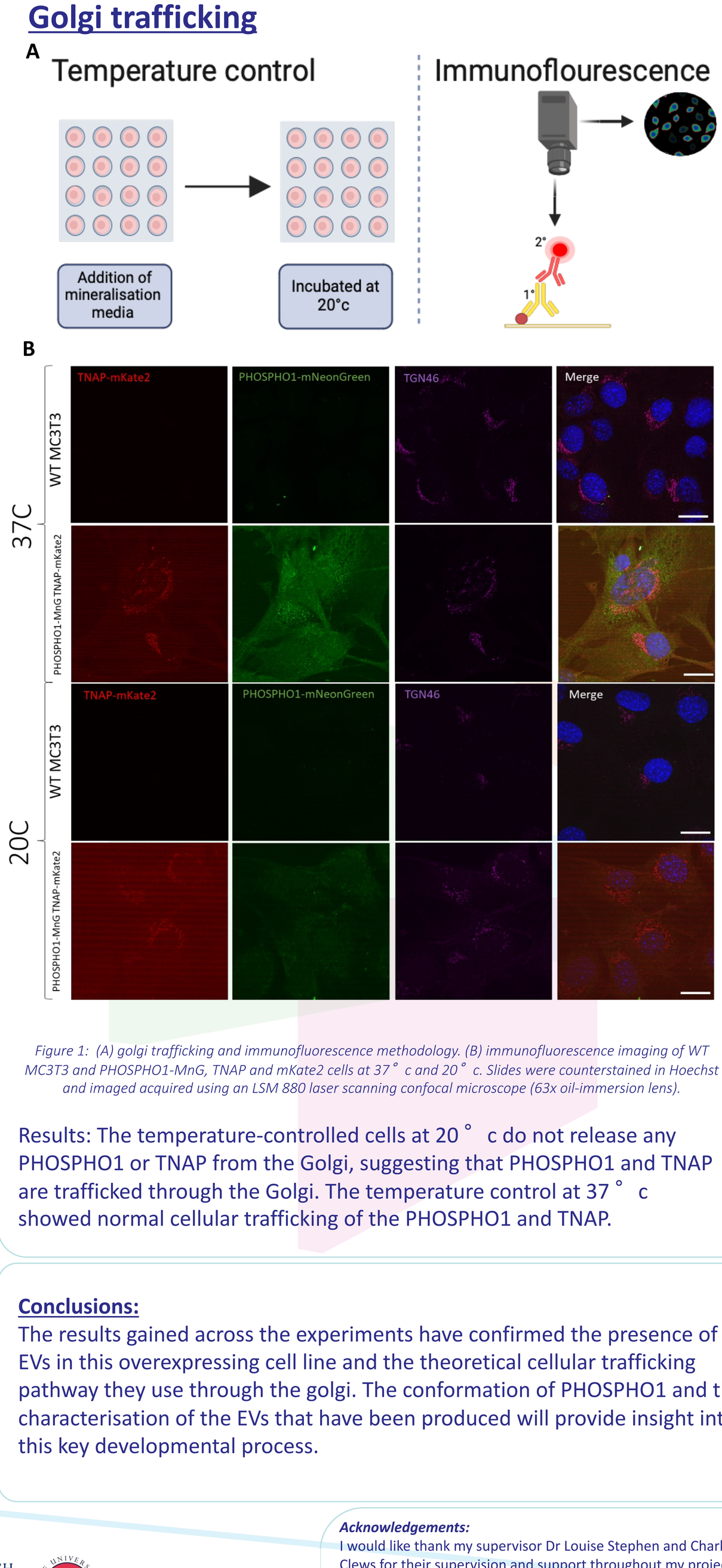

Figure 1: (A) golgi trafficking and immunofluorescence methodology. (B) immunofluorescence imaging of WT MC3T3 and PHOSPHO1-MnG, TNAP and mKate2 cells at 37° c and 20° c. Slides were counterstained in Hoechst and imaged acquired using an LSM 880 laser scanning confocal microscope (63x oil-immersion lens).

Results: The temperature-controlled cells at 20° c do not release any PHOSPHO1 or TNAP from the Golgi, suggesting that PHOSPHO1 and TNAP are trafficked through the Golgi. The temperature control at 37° c showed normal cellular trafficking of the PHOSPHO1 and TNAP.

Conclusions:

The results gained across the experiments have confirmed the presence of EVs in this overexpressing cell line and the theoretical cellular trafficking pathway they use through the golgi. The conformation of PHOSPHO1 and the characterisation of the EVs that have been produced will provide insight into this key developmental process.

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