Metabolomics assessment of vitamin D impact in Pam_3CSK_4 stimulation.

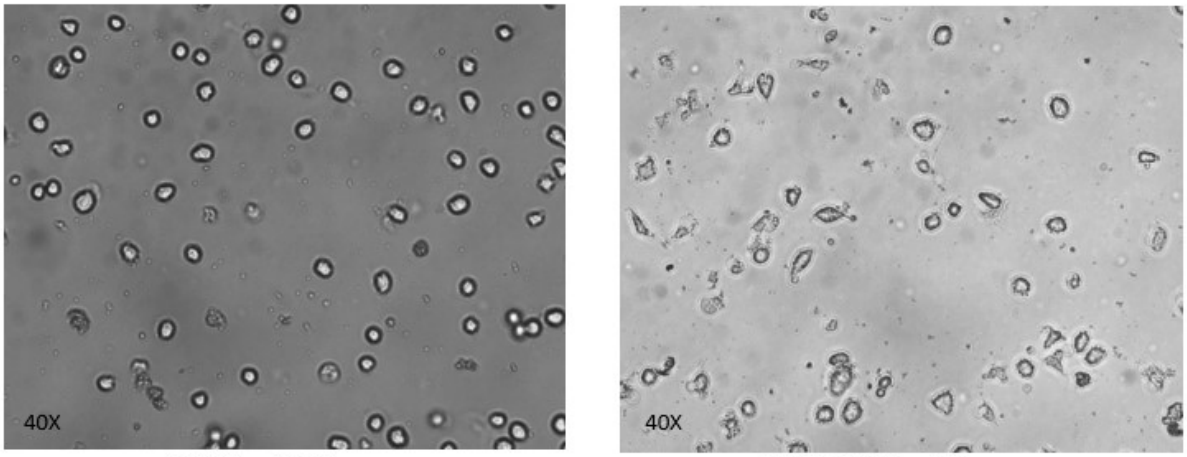
Baloyi NN, Tugizimana F and Sitole LJ*.

*Department of Biochemistry, University of Johannesburg, PO Box 524, Auckland Park, Johannesburg 2006, South Africa. lsitole@uj.ac.za

**Address correspondence to this author at the Biochemistry Department, University of Johannesburg, Johannesburg 2006, South Africa. Tel: +27115592984; E-mail: lsitole@uj.ac.za*

Supplementary data.

1. Micrographs of U937 cells supplemented with or without phorbol myristate acetate (PMA) for 24-hrs.



A. Without PMA. B. With PMA for 24 hrs.

Figure S1: Cellular morphology assessment under the microscope: Micrographs of U937 cells supplemented without (A) or with PMA (B) for 24-hrs treatment. The micrographs show the differences in the morphology of cells between the two conditions. In the absence of PMA, cells are round in shape and have smooth edges. On the contrary, PMA supplemented cells have uneven, irregular shape with rough edges. Cells were viewed under 40X magnification on a Zeiss Axiovert 25 Phase Inverted light microscope before the micrographs were acquired using AxioVision 3.1.

2. Flow cytometry analysis of U937 monocyte-macrophage CD14 count.

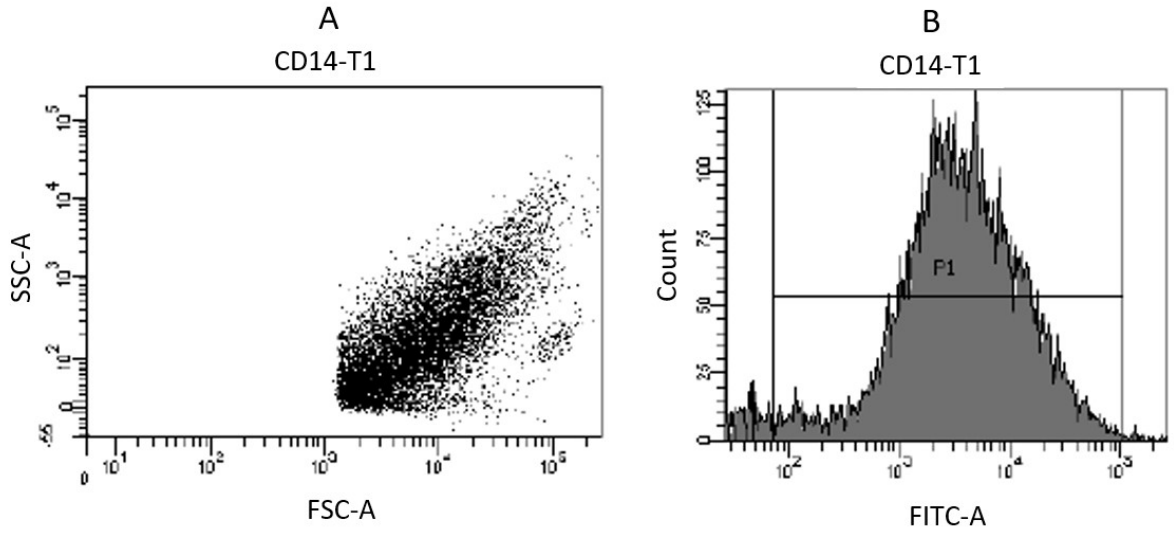


Figure S2: Flow cytometry analysis of monocyte-macrophage CD14 count. (**A**) A **dot plot** provides the forward scatter (FSC-A) which is the measure of the size of cells and the side scatter (SSC-A), the granularity or density of the cell. (**B**) A **histogram** of CD14 labelled monocyte-macrophage cell populations. Gate P1 is the CD14 expressing cells labelled with antibody fluorochrome, FITC-A.

3. CD14 count of U937 cells supplemented with or without phorbol myristate acetate (PMA).

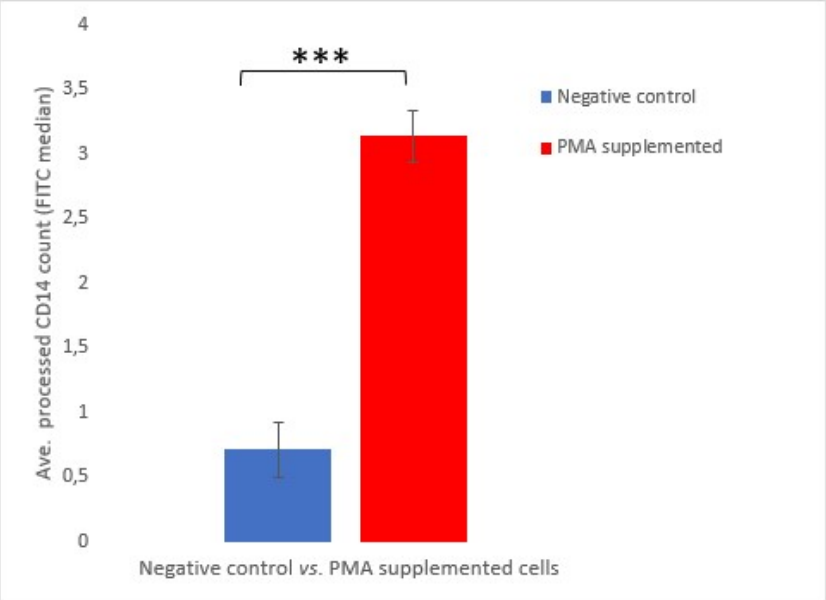


Figure S3: Flow cytometry CD14 count. CD14 count of U937 cells supplemented with 100 ng/mL PMA *vs*. negative control cells incubated for 24-hrs. Each error bar represents the standard deviation. The two groups show statistically significant differences in CD414 counts, with $p<0.001$ ***.

4. PLS-DA modelling and variable selection – Control *vs*. Pam_3CSK_4.

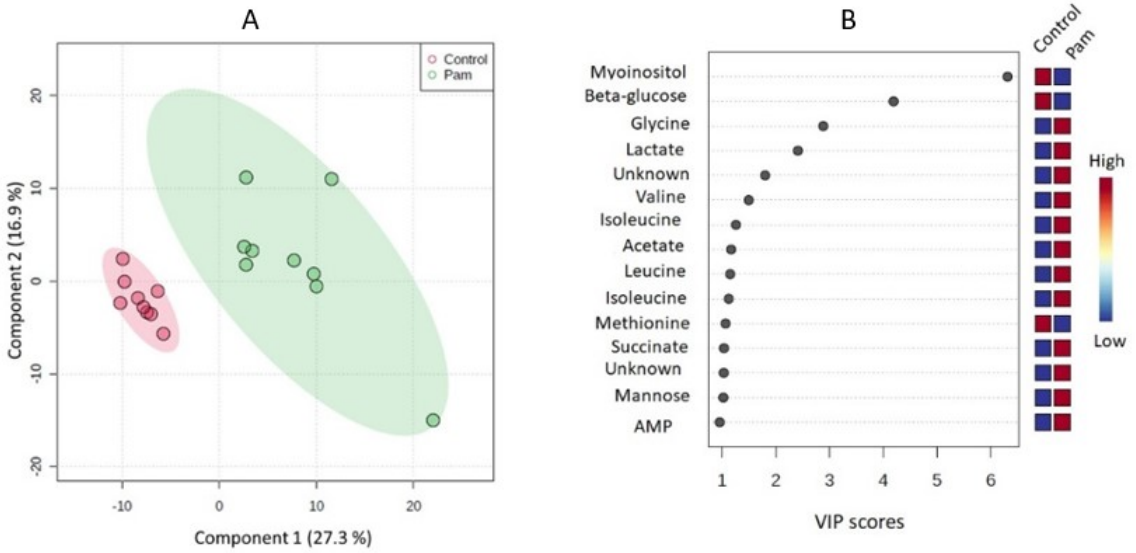


Figure S4: PLS-DA modelling and variable selection. (**A**) The PLS-DA scores models separating control *vs*. Pam_3CSK_4. (**B**) The variables (metabolites) responsible for the separation observed in PLS-DA scores model were identified using VIP scores.

5. PLS-DA modelling and variable selection – Control *vs*.$1,25(OH)_2D_3$.

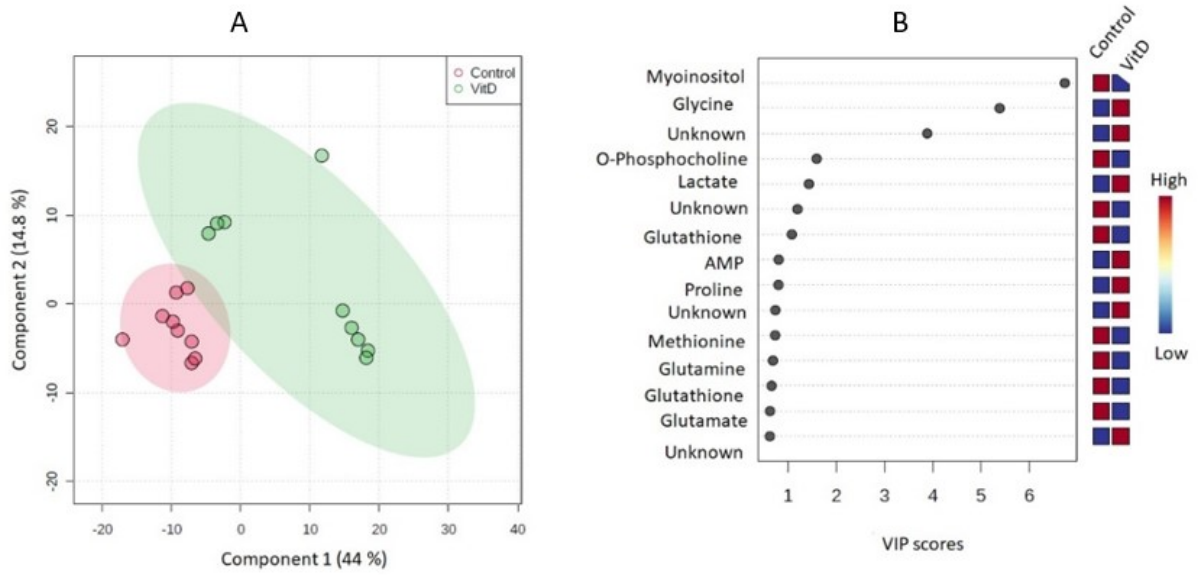


Figure S5: PLS-DA modelling and variable selection. (**A**) The PLS-DA scores models separating control *vs*. $1,25(OH)_2D_3$. (**B**) The variables (metabolites) responsible for the separation observed in PLS-DA scores model were identified using VIP scores.