

Inhibition of O-GlcNAc transferase alters the differentiation and maturation process of human monocyte derived dendritic cells

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Methods

Viability assay

Viability of moDCs was measured by PI staining (ThermoFischer Scientific). Briefly, 2×10^5 treated or untreated moDCs were stained by 2.0 $\mu\text{g/mL}$ of PI for 5 min. Measurements were performed on Attune NxT flow cytometer (ThermoFisher Scientific).

Results

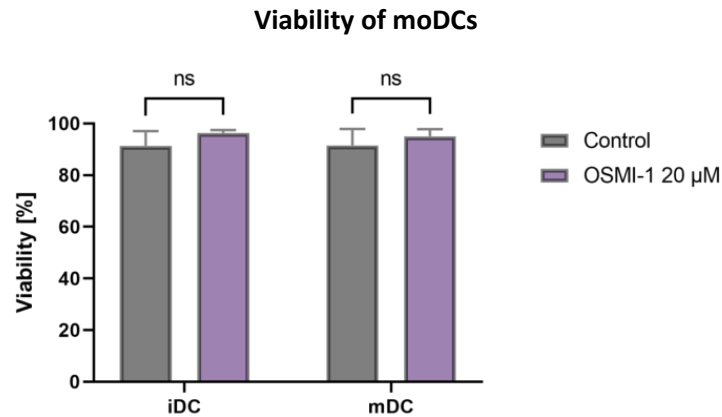


Figure S1. *Viability of moDCs.*

Viability of untreated or OSIM-1 treated immature and mature moDCs was assessed by PI. Results are presented as percentage of viable cells (PI negative) \pm SD (4 different donors). Statistical significance between individual pairs was calculated using Student's t-test; p-value < 0.05 was considered statistically significant. iDCs, immature moDCs; mDCs, mature moDCs.

OGT inhibitor OSMI-1 affects the MEK/ERK and mTOR/AKT signalling axis in moDCs

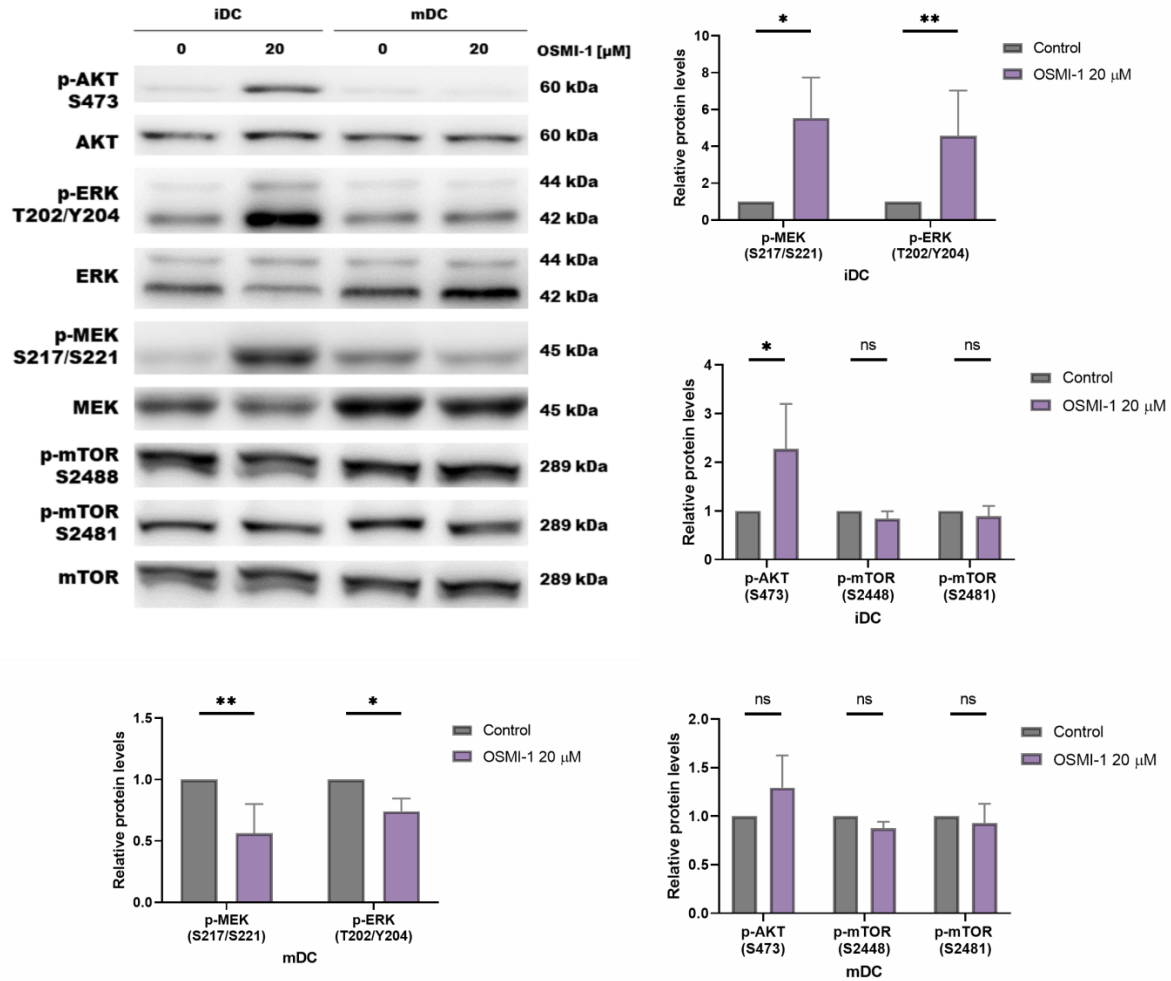


Figure S2. Characterisation of mTOR/AKT and MEK/ERK signalling pathways in the presence of OSMI-1.

Representative immunoblots of phosphorylated ERK, MEK, AKT and mTOR, and their total expression in untreated or OSMI-1 treated immature (day 6) and mature moDCs (day 8). The results in charts are presented as a ratio of phosphorylated/total protein levels normalized to untreated moDCs \pm SD (4-6 different donors). Statistical significance between individual pairs was calculated using Student's t-test; p-value < 0.05 was considered statistically significant. iDCs, immature moDCs; mDCs, mature moDCs.

Phenotype of tolDCs and mature OSMI-1-moDCs obtained from nontreated immature moDCs

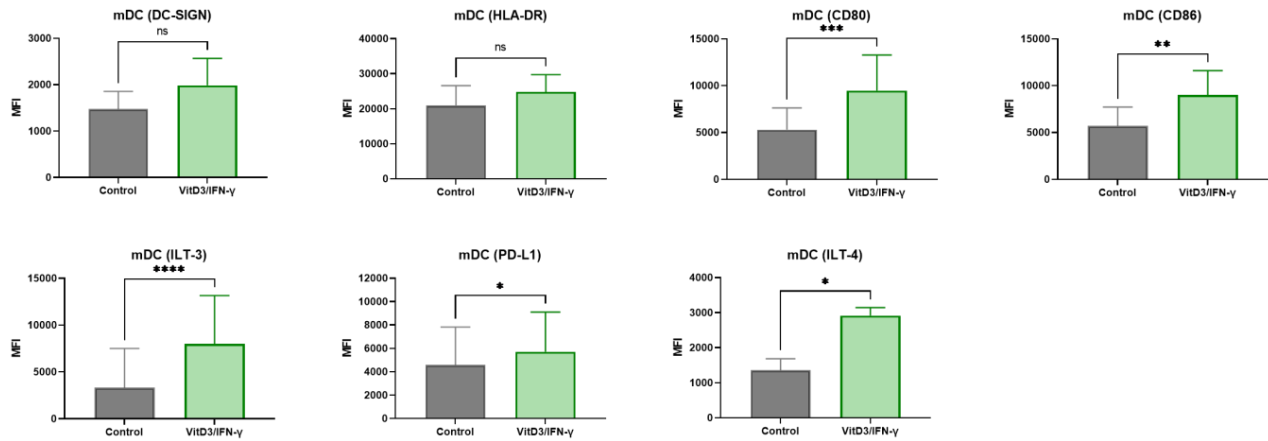


Figure S3. Characterisation of specific DC surface markers in the presence of Vitamin D₃ together with IFN- γ (tolDCs).

Shown are mean MFI values \pm SD of n=6-8 independent experiments. tolDC were prepared from immature moDCs following 2 days incubation with the cytokine cocktail and the presence of Vitamin D₃ together with IFN- γ . Statistical significance between individual pairs was calculated using Student's t-test; p-value < 0.05 was considered statistically significant. iDCs, immature moDCs; mDCs, mature moDCs.

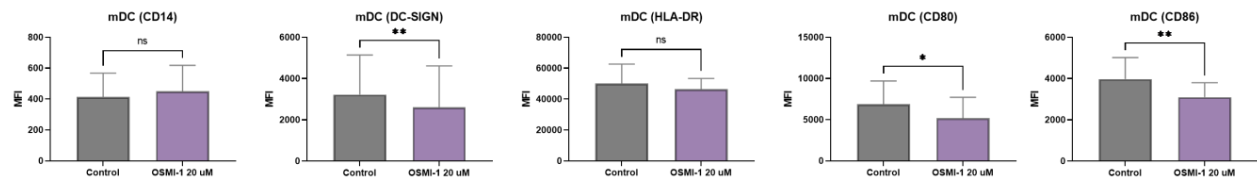


Figure S4. Characterisation of specific DC surface markers in the presence of OSMI-1 during maturation.

Shown are mean MFI values \pm SD of n=6-8 independent experiments. Mature moDCs were prepared from control immature moDCs following 2 days incubation with the cytokine cocktail. The immature moDCs (control) were either untreated or treated with 20 μ M of OSMI-1 for additional 2 days. Statistical significance between individual pairs was calculated using Student's t-test; p-value < 0.05 was considered statistically significant. iDCs, immature moDCs; mDCs, mature moDCs.