

Flow cytometry analysis of thymus cells from wild type and $PINK1^{-/-}$ mice. Left contour plots represent all cells, right contour plot has been gated solely on the double negative thymocytes to assess populations of DN1-4 T cell precursors (n = 3).



Intracellular cytokine staining of cells grown in RPMI after α -CD3/ α -CD28 stimulation. Cells were then incubated with GolgiStop, PMA and ionomycin for 5 hours before flow cytometry analysis (n = 6 +/- SD).



CD4⁺ T cells were cultured in medium containing α -CD3 (1 µg/mL) with either IL-2 (1 ng/mL) or α -CD28 (0.5 µg/mL) for 48 hours. Next, 4x10⁶ cells were plated on Cell-Tak coated plates in XF assay medium containing 25 mM glucose and 1 mM sodium pyruvate. Oligomycin (1 µM), FCCP (1.5 µM), etomoxir (200 µM) or rotenone/antimicin A (1 µM each) were added at the time points indicated by vertical bars. OCR and ECAR were measured by an XF-96 bioanalyzer. A) Graph of oxygen consumption rate (OCR) versus time as a measure of oxidative phosphorylation (n = 4 +/- SEM). B) Graph of baseline extracellular acidification rate (ECAR) to measure glycolysis (n = 3 +/- SD).



Phenotype of cells used in suppressor assay. Although the contour plots look slightly different (A), sorted populations had equivalent mean fluorescence of CD25 (B) and percentage of CD25^{hi} cells (not shown) (n=3, Mean +/- SD)



Representative histograms of the suppressor assay performed in Figure 3C (n=3).



Representative flow cytometry contour plot of Th17 polarized CD4⁺ T cells (left). Cells were cultured with α -CD3 (1 µg/mL) in medium containing TGF- β (5 ng/mL) and IL-6 (10 ng/mL) for 3 days before changing to an IL-23 (10 ng/mL) containing medium for a final 3 days. Intracellular cytokine staining was performed as described in the text. Bar graph of IL-17 expression (right) (n = 6 +/- SD).