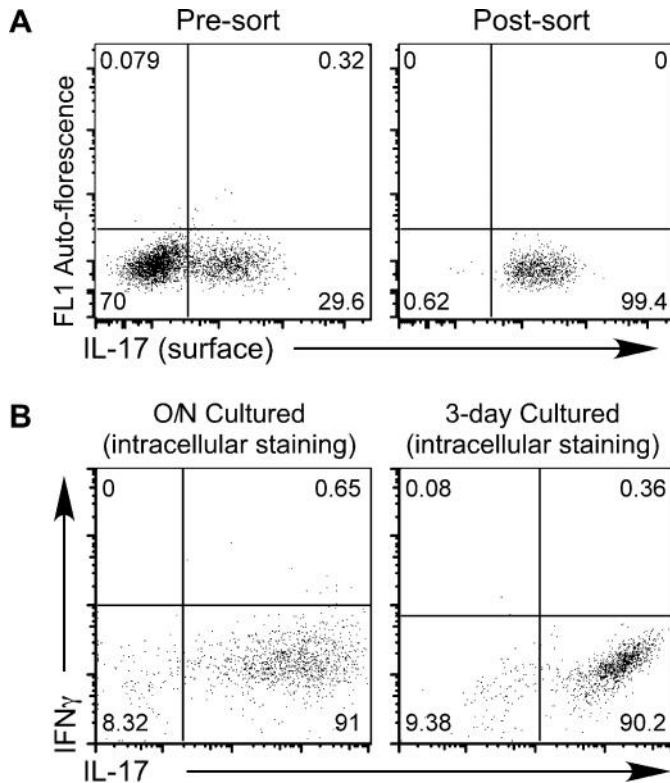


Islet antigen-specific Th17 cells can induce TNF α -dependent autoimmune diabetes

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Supplemental Figure 1. Purification of Th17 cells by surface IL-17 staining and FACS sorting.

(A) CD4⁺ T cells were activated under Th17 differentiation conditions. After 5 days of differentiation culture, cells were harvested and cultured in complete RPMI medium without further stimulation for 2 days (resting). Cells were restimulated with PMA and Ionomycin without Brefeldin A for 3 hours and stained for surface CD4 and IL-17 (left panel). CD4⁺IL-17^{surface+} cells were sorted by FACS (right panel). (B) Sorted CD4⁺IL-17^{surface+} cells were cultured in complete RPMI medium without any stimulation. At different time points, cells were analyzed for the expression of IL-17 and IFN γ by intracellular staining and FACS, after restimulation with PMA, Ionomycin, and Brefeldin A (or GolgiPlugTM). Shown are representative IL-17 and IFN γ staining of over-night (O/N, left panel) and 3-day (right panel) cultured cells.