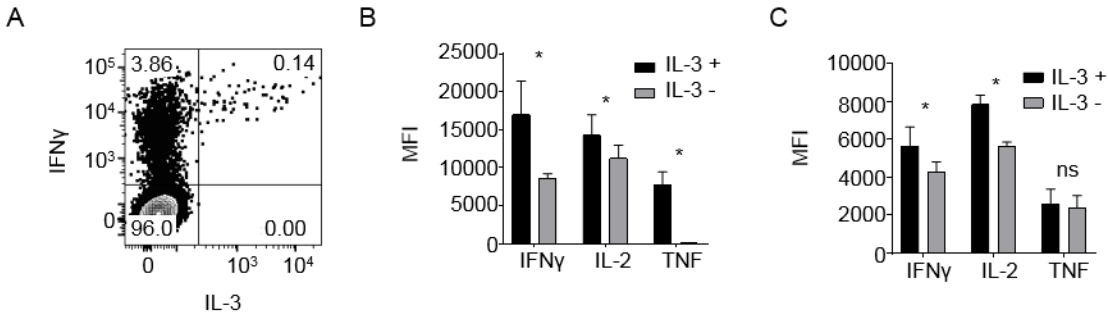


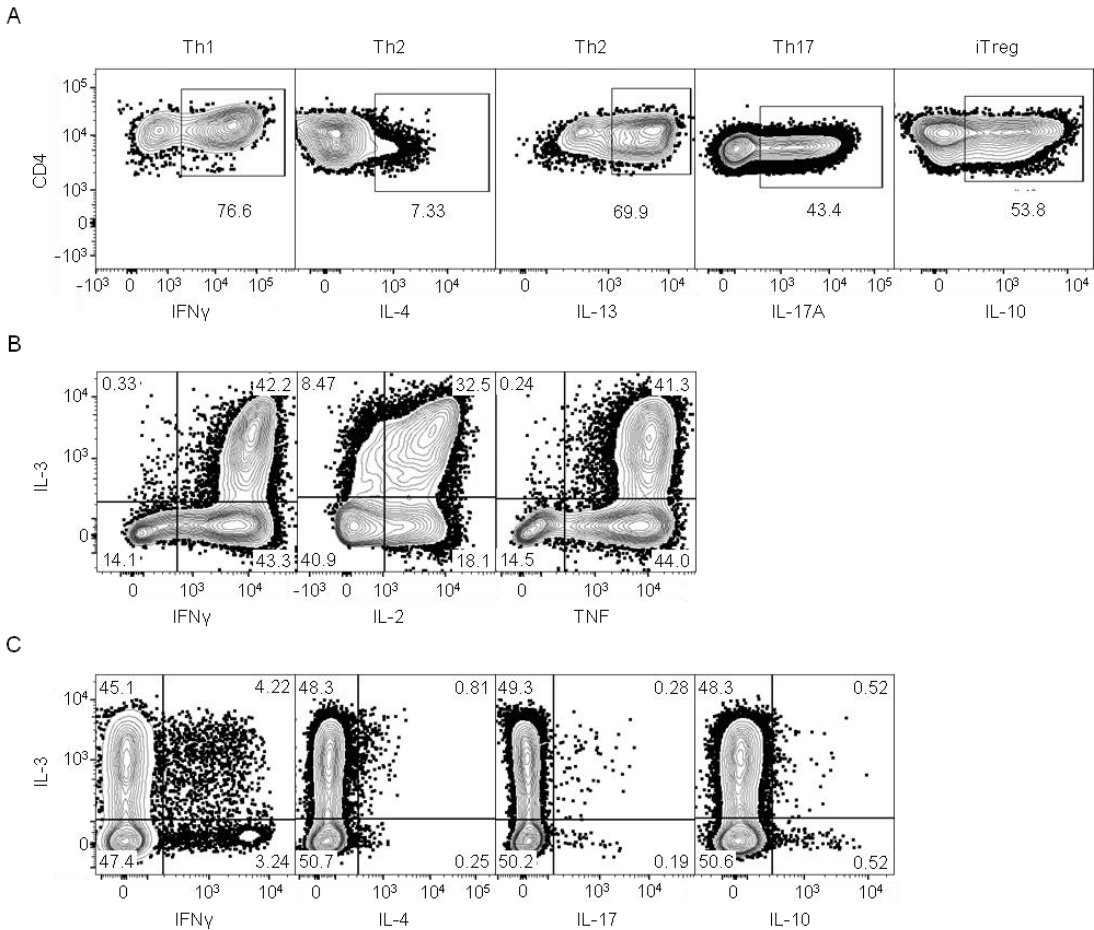
1

2 **Supplementary Figure. 1 Gating strategy.** Gating strategy used to eliminate doublets, dead
 3 cells and non-CD4⁺ T cells are shown. Each contour plot shows the cells gated in the previous
 4 plot (A-F) and the numbers indicate the percentage of parent population. Trash cocktail included
 5 antibodies to MHCII, CD8 α and B220.



1

2 **Supplementary Figure. 2 IL-3 secreting CD4⁺ T cells are multifunctional.** (A) Mice were
 3 infected with *M. tuberculosis* by the aerosol route. Four weeks later, single-cell suspensions
 4 made from mediastinal lymph nodes were re-stimulated with ESAT-6 peptide in the presence of
 5 brefeldin A and monensin. Cells were then surface stained, fixed, permeabilized, and stained for
 6 intracellular IL-3 and IFN γ . The cells shown are single cells from the lymphocyte gate that are
 7 positive for CD4 and negative for Live/Dead stain, CD8 α , B220, and MHCII. The figure
 8 represents results of two independent experiments. (B) Splenocytes from mice injected with *M.*
 9 *bovis BCG* cutaneously underwent stimulation with *M. tuberculosis* lysate and intracellular
 10 cytokine staining. Median fluorescence intensities (MFI) were calculated for each of the
 11 cytokines depicted after gating CD4⁺ T cells based on their IL-3 secretion status. The figure
 12 represents results of two independent experiments. (C) Splenocytes from mice infected with
 13 HSV-2 by cutaneous route underwent stimulation with UV-inactivated HSV-2 and intracellular
 14 cytokine staining. Median fluorescence intensities (MFI) were calculated for each of the
 15 cytokines depicted after gating CD4⁺ T cells based on their IL-3 secretion status. The figure
 16 represents results of two independent experiments. * $P < 0.05$ for IL-3⁺ versus IL-3⁻ cells
 17 (Student's t test), and ns indicates not significant.

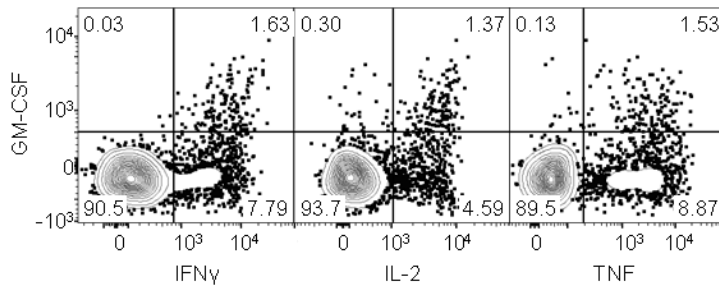


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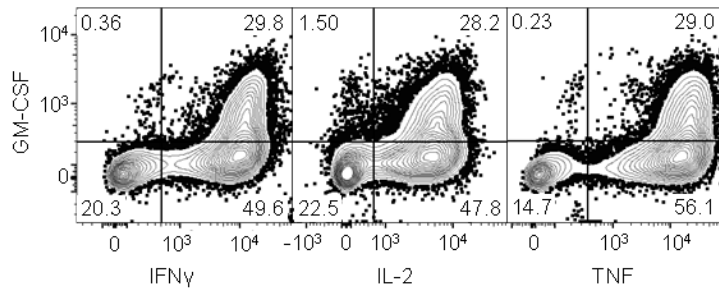
2 **Supplementary Figure. 3 Generation of IL-3 secreting CD4⁺ T cells *in vitro*.** (A) Analysis of
 3 CD4⁺ T cells from cultures prepared as in Figure 3A. The contour plots depict expression of
 4 signature cytokines by CD4⁺ T cells from cultures representing conditions that favor
 5 differentiation to the indicated functional subsets. Numbers indicate the percentage of cytokine-
 6 positive CD4⁺ T cells in each gate shown. The figure represents results of two independent
 7 experiments. (B) Splenocytes from P25 TCR transgenic mice were cultured in the presence of
 8 Antigen 85B₂₄₀₋₂₅₄ peptide, IL-12 and antibody to IL-4 to generate Th1 cells. The samples were
 9 processed and the results are displayed as in Figure 3B. The figure represents results of three
 10 independent experiments. (C) Splenocytes from P25 TCR transgenic mice were cultured in the
 11 presence of Antigen 85B₂₄₀₋₂₅₄ peptide, IL-1 α and blocking antibodies to IFN γ , IL-12 and IL-4.

- 1 The samples were then processed and the results are displayed as in Figure 4C. The figure
- 2 represents results of two independent experiments.

A



B



1

2 **Supplementary Figure. 4 Characteristics of GM-CSF secreting CD4⁺ T cells. (A)**

3 Splenocytes from mice infected with HSV-2 by cutaneous route underwent stimulation with UV-
4 inactivated HSV-2 and intracellular cytokine staining. The contour plots depict antigen-

5 experienced CD44⁺ CD4⁺ T cells that express IL-3 and cytokines that define multifunctionality.

6 The figure represents results of two independent experiments. (B) Splenocytes from OT II TCR

7 transgenic mice were cultured in the presence of ovalbumin peptide (OVA₃₂₃₋₃₃₉), IL-12 and

8 antibody to IL-4 to generate Th1 cells. The contour plots represent co-expression of GM-CSF

9 with cytokines that define multifunctionality. The figure represents results of two independent

10 experiments.