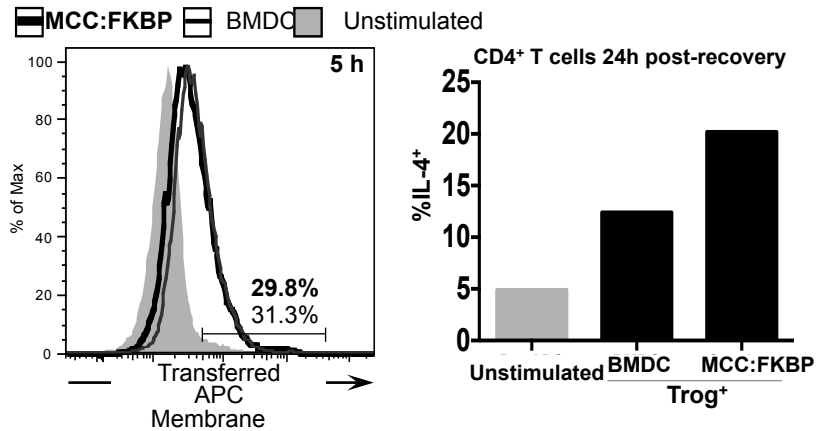
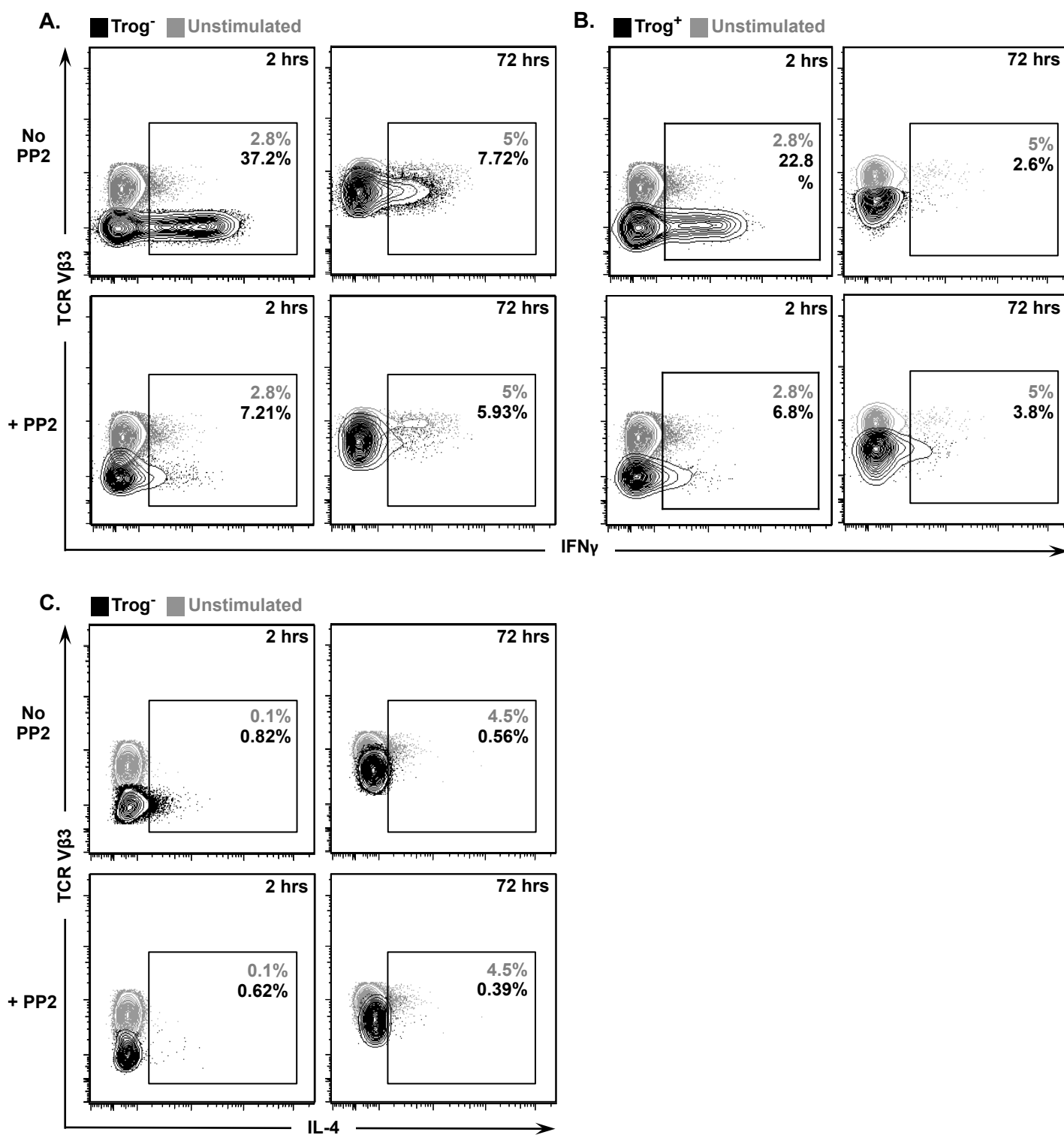


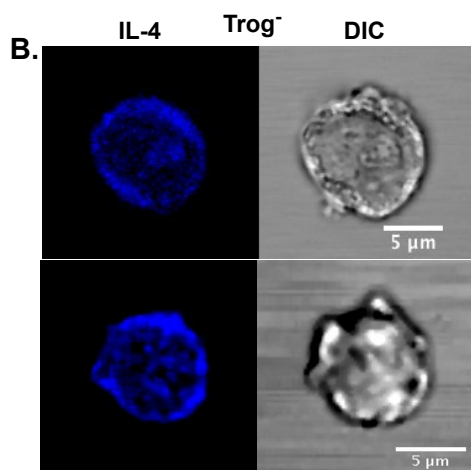
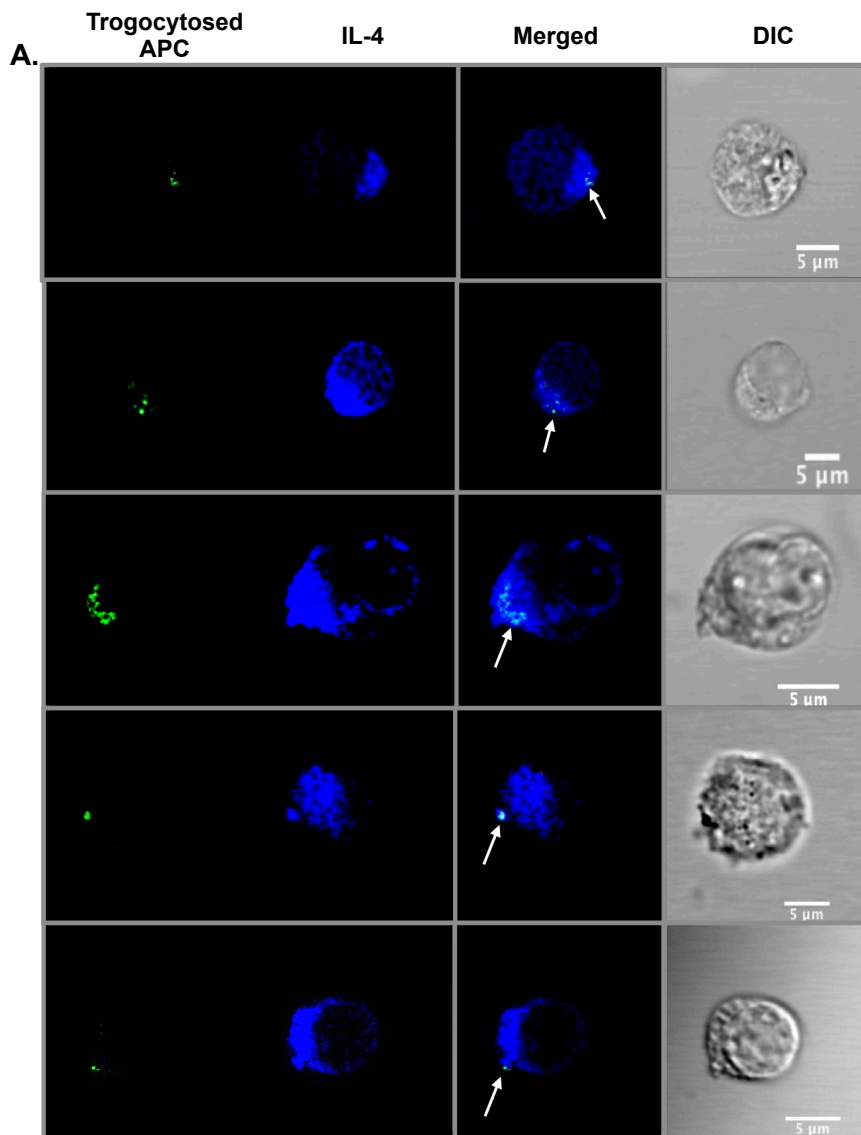
Supplemental Figure 1. *Trog*⁺ cells generated *in vitro* are identified by presence of APC membrane, *in vivo* generated *trog*⁺ cells are identified by presence of CD80/CD86 and MHCII (A) Schematic timeline of a standard *in vitro* trogocytosis assay. (B) Purity of CD4⁺ T cells following recovery from an *in vitro* trogocytosis assay. APC shown in square box and CD4⁺ cells are shown in the oval gate. Plots show recovered samples pre-magnetic separation (*left*) and post-magnetic separation of APC (*right*). (C) Identification of *in vitro* generated *trog*⁺ cells as determined by the presence of trogocytosed APC membrane. (D) Gating scheme for identification of adoptively transferred (*blue arrow*) and wild-type (*red arrow*), *in vivo* generated *trog*⁺ CD4⁺ T cells.



Supplemental Figure 2. *CD4⁺ T cells perform trogocytosis at similar frequencies from BMDC and MCC:FKBP fibroblasts, and the frequency of IL-4⁺ trog⁺ cells is similar 24 hours later.* BMDC and MCC:FKBP fibroblasts were used as APC in parallel standard *in vitro* trogocytosis-assays. **(Left)** Histograms showing frequency, and levels of trogocytosed APC membrane from BMDC (*thin grey line*) and MCC:FKBP fibroblasts (*thick black line*), with unstimulated cells (*shaded grey*) for comparison. Numbers represent frequency of trog⁺ cells from respective APC. **(Right)** Frequency of unstimulated IL-4⁺ cells (*grey*), IL-4⁺ trog⁺ cells recovered from a standard trogocytosis-assay using MCC-pulsed BMDC (*middle*) and MCC:FKBP fibroblasts (*right*), 24 hours post-recovery.



Supplemental Figure 3. *IFN γ expression in trog⁺ and trog⁻ cells is dependent on signals from APC, PP2-treated and untreated trog⁻ cells do not express IL-4.* After recovery from an *in vitro* trogocytosis assay, cells were left untreated (*top row*) or were treated for 20 minutes with 20 μ M PP2 (*bottom row*) to halt TCR signaling. Cells were analyzed for surface TCR levels and intracellular cytokine expression 2 (*left column*), or 72 hours (*right column*) after removal of PP2. (**A & B**) IFN γ and TCR V β 3 levels of (**A**) trog⁻ cells, and (**B**) trog⁺ cells. (**C**) IL-4 and TCR V β 3 levels of trog⁻ cells. Data is representative of three separate experiments.



Supplemental Figure 4. Intracellular IL-4 is polarized towards trocytosed molecules. (A) Additional representative images show trog⁺ cells 72 hours post recovery, with trocytosed APC molecules (green) and IL-4 (blue). In merged images (second from right), arrows point to trocytosed molecules. (B) IL-4⁺ trog⁻ cells 72 hours post recovery, with IL-4 shown in blue.