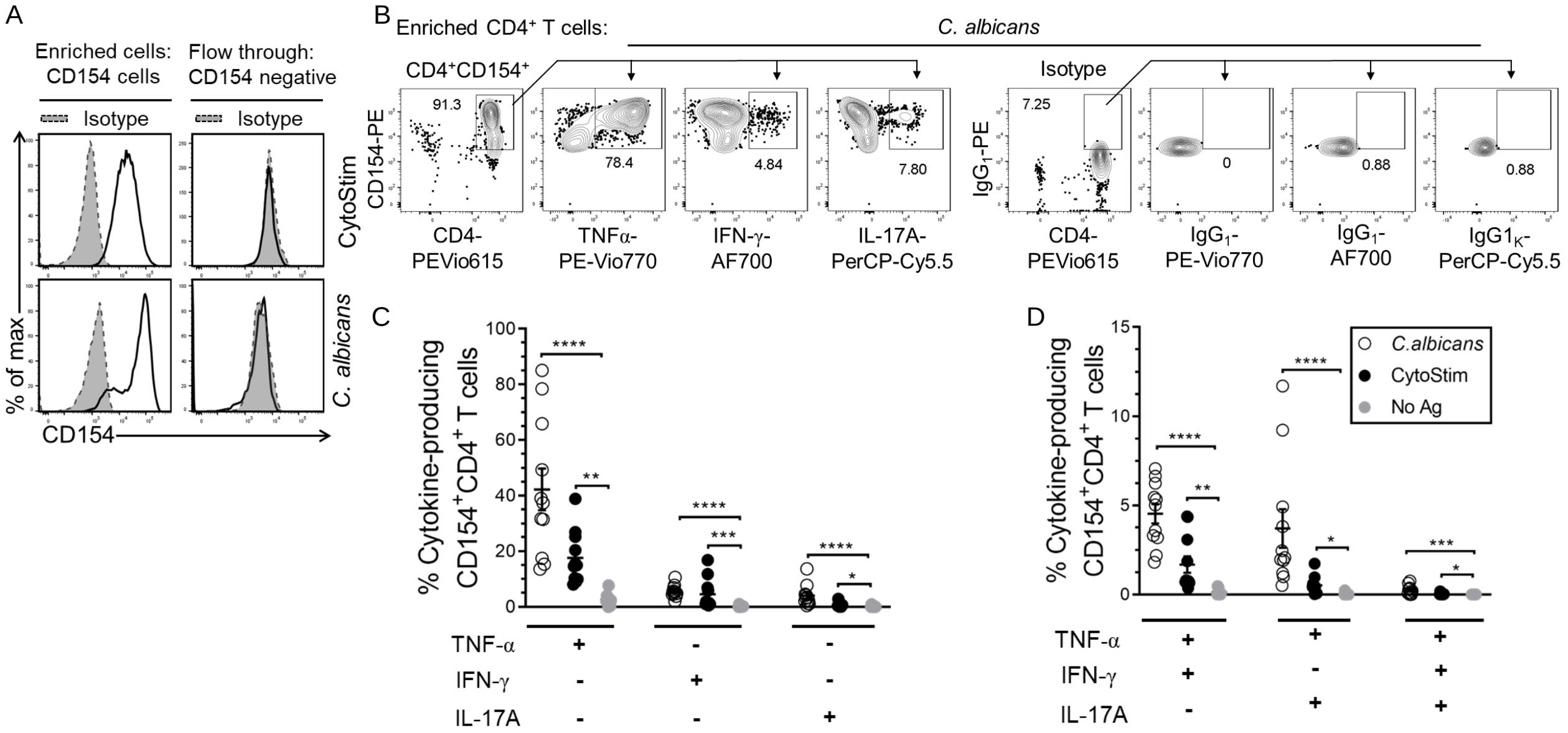

Figure S1. Detection of CD154 and cytokine expression using the CD154$^+$ enrichment assay. (A) Histogram representation of intracellular CD154 expression on enriched and flow through cells with isotype control 7h post-stimulation with CytoStim and *C. albicans*. **(B)** Contour plots representing TNF-α, IL-17A and IFN-γ producing cells among by *C. albicans*-exposed cells with (CD154 and cytokine) isotype control. **(C)** Frequency of CytoStim-stimulated or *C. albicans*-exposed CD154$^+$CD4$^+$ T cells secreting IFN-γ, IL-17A and TNF-α among Crohn's patients (n = 10). **(D)** Frequency of CytoStim-stimulated or *C. albicans*-exposed TNF-α$^+$IL-17A$^+$ and TNF-α$^+$IFN-γ$^+$ double positive CD154$^+$CD4$^+$ T cells among Crohn's patients (n = 10). Bars represent the means ± SEM of ten independent experiments; one-way ANOVA with Dunn's multiple comparison. *p<0.05, **p<0.01, ***p<0.001, ****p<0.000.

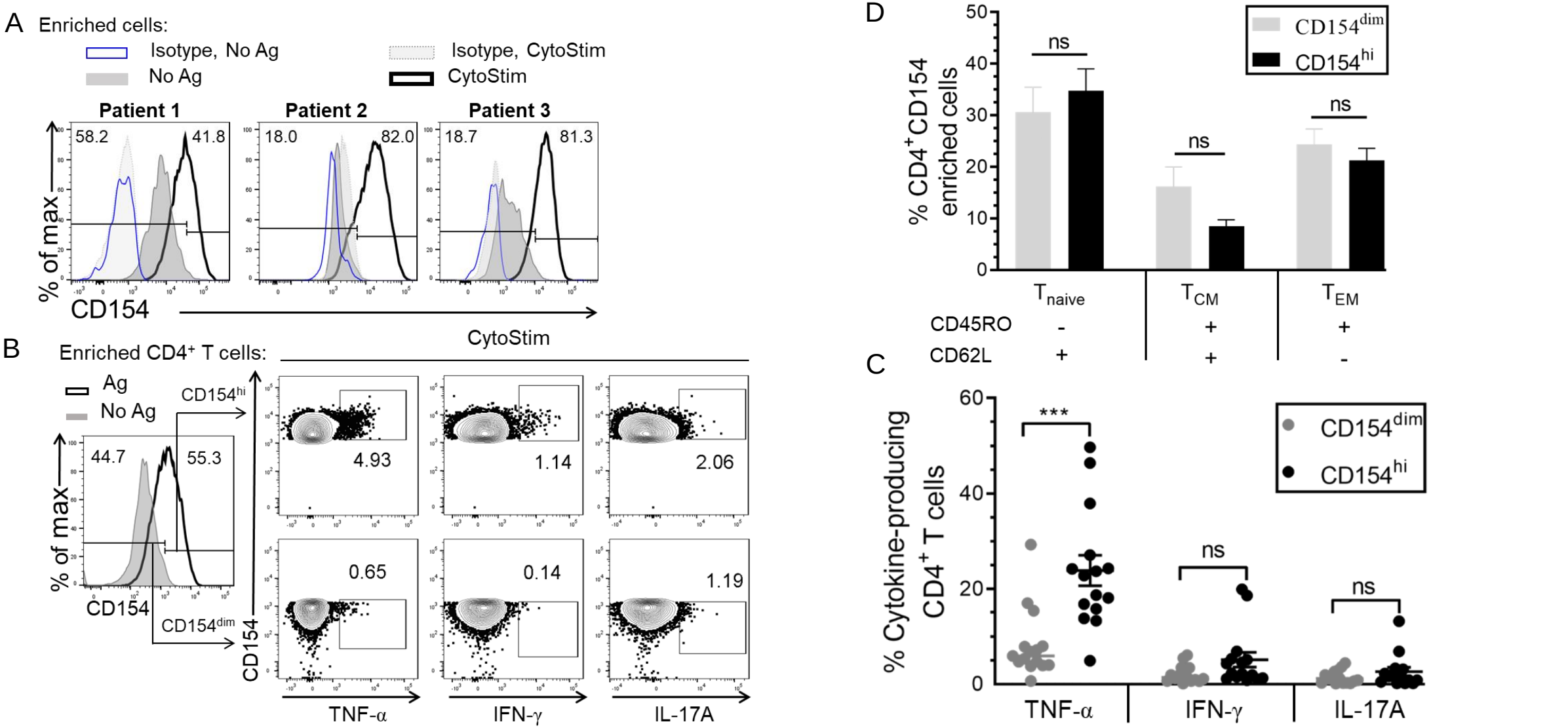

Figure S2. CD154 expression and cytokine production by CytoStim-stimulated CD4⁺ T lymphocytes. (A) CD4⁺ T lymphocyte responses to CytoStim in Crohn's patients (n = 3). Histogram overlays are shown with the CD154 isotype control for CytoStim-simulated cells (grey dotted line) and unstimulated cells (blue solid line). CD154 expression for unstimulated cells (dark grey solid line) and CytoStim-stimulated cells (black solid lines). **(B) Representative flow plots for cytokine production by CytoStim stimulated CD4⁺CD154$^{dim/hi}$ cells.** Histogram overlay plots are shown with unstimulated cells (dark grey solid lines) and CytoStim-stimulated cells (the solid line). **(C)** The frequency of TNF-α, IFN-γ, IL-17A produced by CD154$^{dim/hi}$CD4⁺ T cells following CytoStim stimulation among Crohn's patients (n = 15). Bars represent the means ± SEM of fifteen independent experiments; Mann Whitney test. ***p<0.001, ns, not statistically significant**. (D)** The percentage of CD62L and CD45RO expression by CytoStim-stimulated CD154$^{dim/hi}$CD4⁺ T cells among Crohn's patients (n = 8). Bars represent the means ± SEM of eight independent experiments; two-way ANOVA Dunnett's multiple comparison tests. ns, not statistically significant.

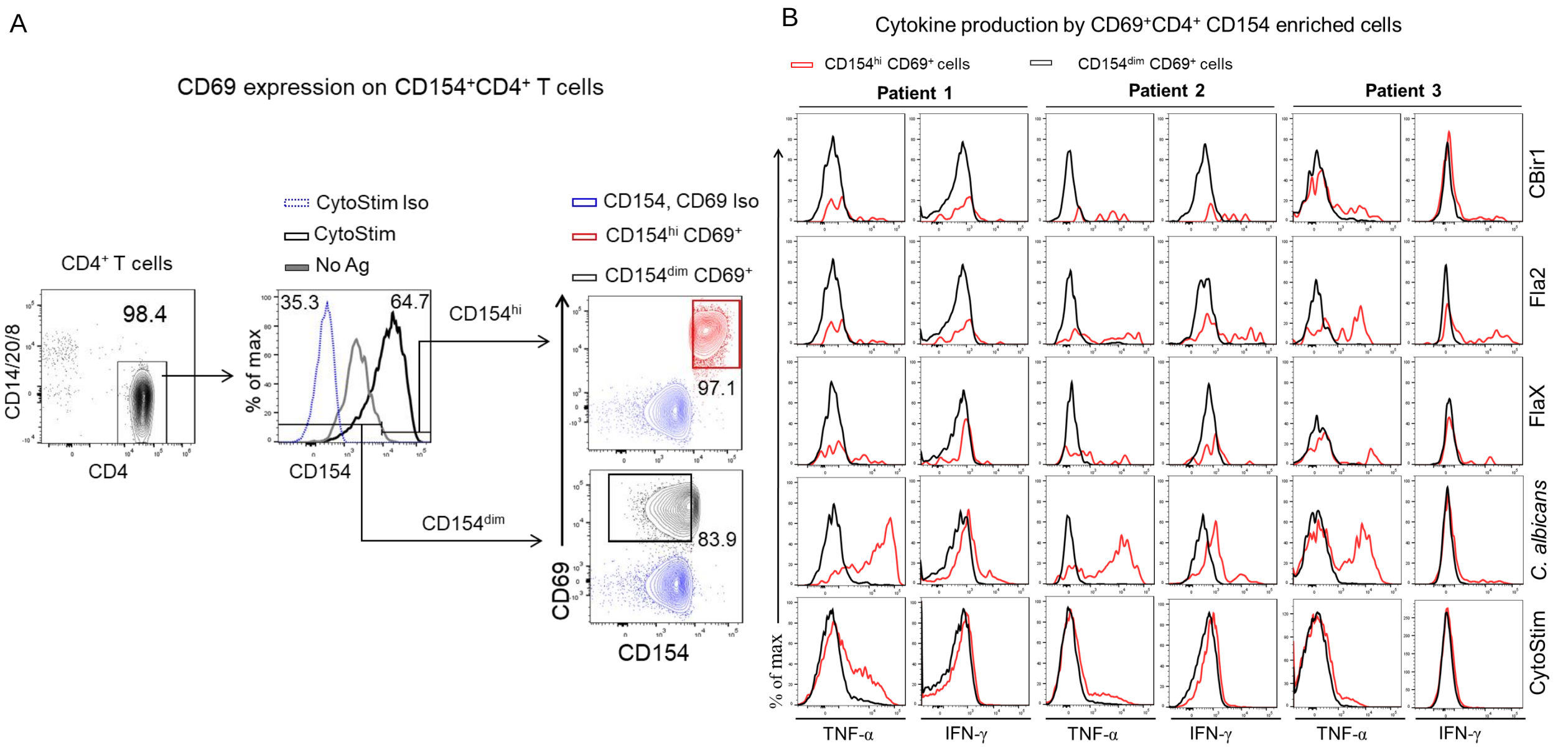

Figure S3. Co-expression of activation markers CD69 and CD154 on PBMC 7h post-stimulation. (A) Flow cytometry analysis of PBMCs from one representative active Crohn's patient. Gated on live, $CD14^-$, $CD20^-$, $CD8^-$, $CD4^+$ cells followed by histogram overlays of CytoStim-stimulated (black solid line) and antigen unexposed (grey solid line) and CD154 isotype control for CytoStim-stimulated (blue dotted line) cells. Contour plot overlays for CD154 and CD69 expression by $CD154^{hi/dim}CD4^+$ T cells with CD154 and CD69 isotype control (blue solid line). Histogram overlays of TNF-α and IFN-γ produce by CytoStim-stimulated $CD69^+CD154^{hi/dim}CD4^+$ T cells. **(B)** Histogram overlay of antigen-exposed $TNF\text{-}\alpha^+$- and $IFN\text{-}\gamma^+$-$CD69^+CD154^{hi}CD4^+$ and $CD69^+CD154^{dim}CD4^+$ T cells from 3 independent active Crohn's patients.

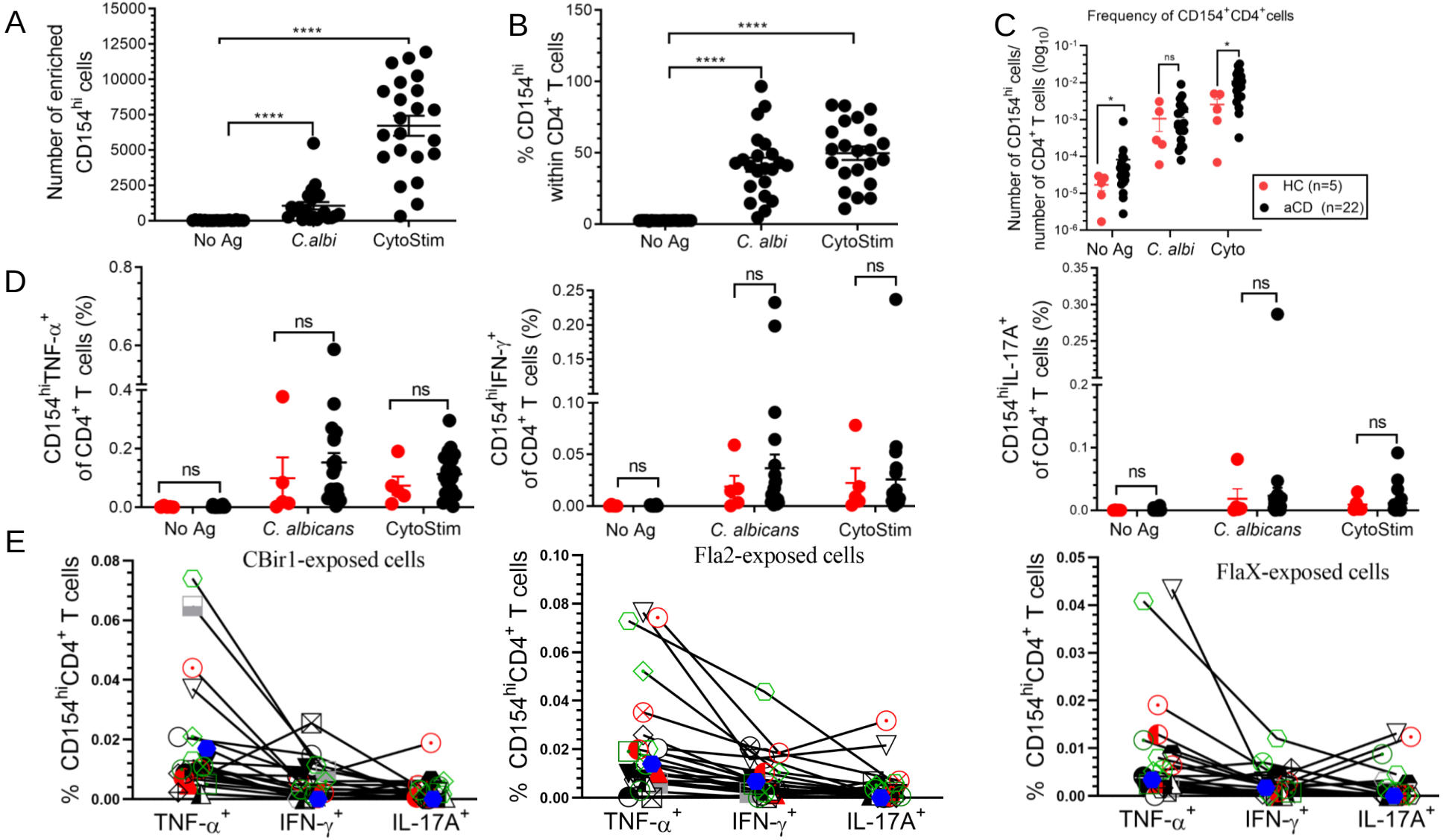

Figure S4. CD4⁺ T cell responses following antigen exposure, or polyclonal stimulation. (A) Number of *C. albicans*-, CytoStim-stimulated, or antigen unexposed enriched CD154^{hi} cells in active CD (aCD) patients (n = 22). **(B)** Percentage of CD154^{hi} cells among CD4⁺ T cells from active Crohn's (n = 22) following *C. albicans* or CytoStim stimulation. **(C)** Frequencies of *C. albicans* and CytoStim-stimulated CD154⁺CD4⁺ T cells among active Crohn's (n = 22) and healthy (n = 5) patients were calculated from the total number of CD154⁺ cells obtained after enrichment normalized to the total number of CD4⁺ cells applied on the column. **(D) Frequency of cytokine production by CD154⁺CD4⁺ T cells.** The frequency of TNF-α, IFN-γ and IL-17A producing *C. albicans* and CytoStim-stimulated T cells among active Crohn's (black dots; n = 22) and non-IBD healthy (red dots; n = 5) patients were calculated from the total number of cytokine positive cells normalized to the total number of CD154⁺ cells obtained after enrichment. Bars represent the means ± SEM of five-twenty-two independent experiments; Mann Whitney test. **p<0.01 ***p<0.001, ****p<0.0001, ns not significant. **(E)** Comparison of CD154⁺CD4⁺ T cells cytokine reactivity to different flagellin antigens within the same aCD patient. Each symbol represents an active CD patient (lines link result within same patient).