**Supplementary Information for “OpenCyto: An Open Source Infrastructure for Scalable, Robust, Reproducible, and Automated, End-to-End Flow Cytometry Data Analysis”**

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**Supporting Text S1**

*Model Description*

In order to identify cell subsets that exhibit antigen-specific changes upon vaccination, we fit a linear mixed effects model to the background subtracted proportions of cytokine positive cells within each population and gating method. Without loss of generality, for a fixed cell subset and stimulation condition, we let

$y\_{ijk}^{s} , y\_{ijk}^{u}$ be the proportions of cytokine positive cells in the antigen stimulated ($s$) and non-stimulated ($u$) sample from subject$ i$, visit $j$, and vaccine regimen $k$, and $y\_{ijk}^{∆}=y\_{ijk}^{s}-y\_{ijk}^{u}$ be the background subtracted proportion of cytokine positive cells. We model:

$y\_{ijk}^{∆}=μ+ γ\_{i}+a\_{j}+b\_{k}+c\_{jk}+ϵ\_{ijk}$,

where $μ$ is the intercept for the cell subset,$ γ\_{i}$ is a subject-level random effect with $γ\_{i}∼N(0,σ\_{I})$ , $a\_{j}$ is the effect of visit $j$, $b\_{k}$ is the effect of vaccine regimen $k$, and $c\_{jk}$ is the interaction between visit $j$ and vaccine regimen $k$, and $ϵ\_{ijk}∼N(0,σ\_{ϵ})$. We want to test whether $c\_{jk}>0$ (i.e., whether there is an increase in the proportion of cytokine positive cells at the post-vaccine time-point compared to the pre-vaccine time-point) for each vaccine regimen. We fit the model with the R’s *lmer* function from the *lme4* package, and perform linear hypothesis tests using the *glht* and *contrasts* packages.