

Supplemental Figure 1

- A. Gating strategy to phenotype total T cells derived from a cervical cytobrush and PBMC sample from a representative HSV-2 infected participant. Gating focused on live CD3+ cells in the lymphocyte forward/side scatter regions that were CD4+ and CD4-negative, the latter of which was considered CD8+ for quantitation. The expression of CCR7/CD45RA and CD69/CD103 are displayed for the CD3+CD4+ and CD3+CD4- (CD8+) populations.
- **B.** Measuring cervical cytobrush-derived HSV-2 reactive CD4+ T cell responses from 2 HSVneg participants. Cells isolated from cervical cytobrushes obtained from 2 HSVneg participants were cultured with UV-mock (bottom graphs) or UV-HSV-2 (top graphs) and the resultant CD4+ T cells were analyzed by ICS and flow cytometry for the expression of IFN-γ. Net % CD4+/IFNγ+ (UV-HSV-2 minus UV-mock) was 0.0% for Participant 18 and 0.01% for Participant 19. In both cases, the proportion of CD4+/IFN-γ+ cells stimulated with UV-HSV-2 was not statistically significantly different than those stimulated with UV-mock (Fisher's Exact Test P>0.05).