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Supplemental Information

Developing Human Skin Contains Lymphocytes Demonstrating a Memory Signature

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Supplemental Figures

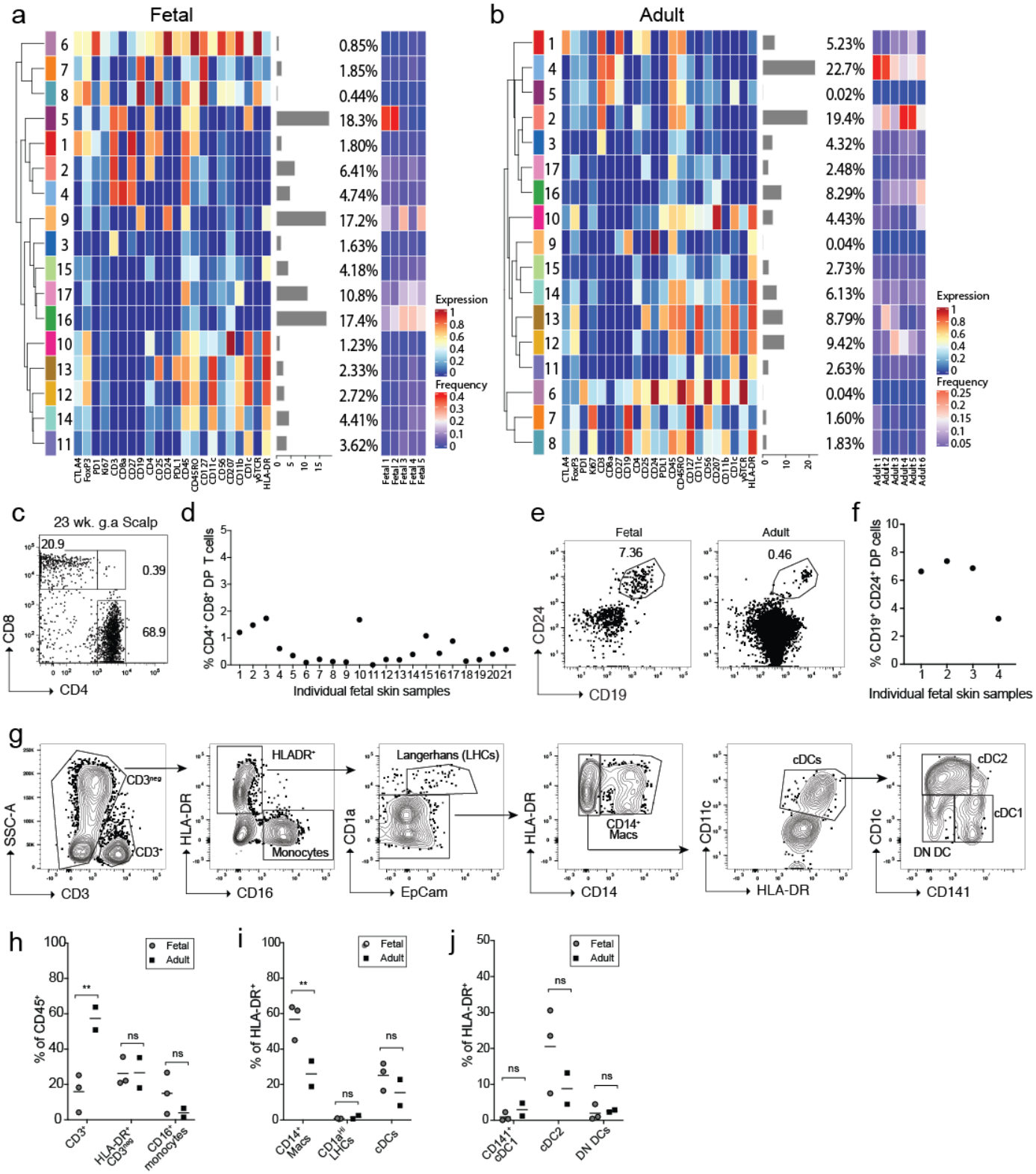

Figure S1: Relative frequency of major immune cells subsets in fetal vs. adult human skin. (Related to Figure 1) 23 week g.a. fetal and adult skin torso samples were analyzed in parallel for 22 markers using mass cytometry. Heatmap for (a) fetal and (b) adult skin demonstrating relative expression of 22 markers by cluster as well as cluster frequency on a group and individual sample basis. In confirmatory studies cells were isolated from 20-23 week g.a. fetal skin (scalp and/or torso), as well as adult skin where noted, and analyzed by flow cytometry. (c) Representative flow plots of CD4 and CD8 expression by live CD3+ lymphocytes in fetal skin. (d)

Percentage of double-positive CD4+CD8+ lymphocytes across 21 fetal skin samples. (e) Representative flow plots of CD19+CD24+ double positive cells in fetal vs. adult skin (gated on live CD45+ cells). (f) Percentage of CD19+CD24+ double positive cells among live CD45+ cells across 4 fetal skin samples. (g) Flow cytometry gating strategy for skin myeloid subsets (fetal skin example shown). (h-j) Relative amounts of various major subsets in fetal vs. adult torso skin, shown as (h) % of live CD45+ cells for CD3+ T cells, HLA-DR+ APCs and CD16+ monocytes and (i-j) and as % of HLA-DR+ for APC types including macrophages, Langerhans cells (LHC) and classical DC (cDC) subsets.

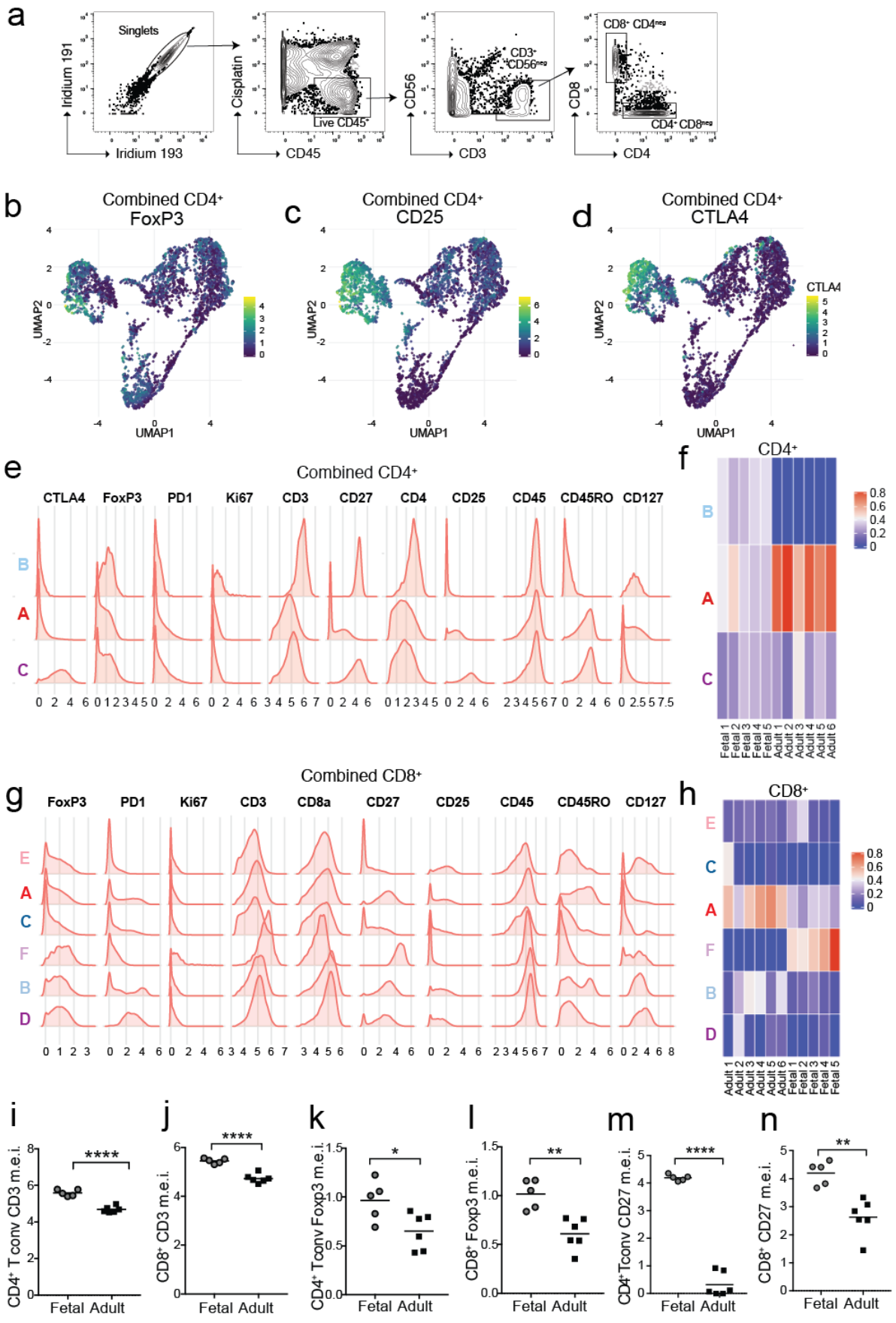

Figure S2: Age-based expression of key markers on skin αβ T cell subsets. (Related to Figure 2) (a) Gating strategy used to identify CD4+ and CD8+ single-positive cells by CyTOF. (b-d) UMAP plots of single positive CD4+ cells from combined fetal and adult

skin, colored by intensity of (b) Foxp3, (c) CD25, and (d) CTLA4 expression. (e) Histograms depicting relative expression of key markers on CD4+ clusters. (f) Heatmap of relative CD4+ cluster frequency in individual fetal and adult samples. (g) Histograms depicting relative expression of key markers on CD8+ clusters. (h) Heatmap of relative CD8+ cluster frequency in individual fetal and adult samples. (i-n) Median expression of CD3 (i-j), Foxp3 (k-l) and CD27 (m-n) on skin CD4+ Tconv and CD8+ T cells by age. Each point represents data from an individual donor.

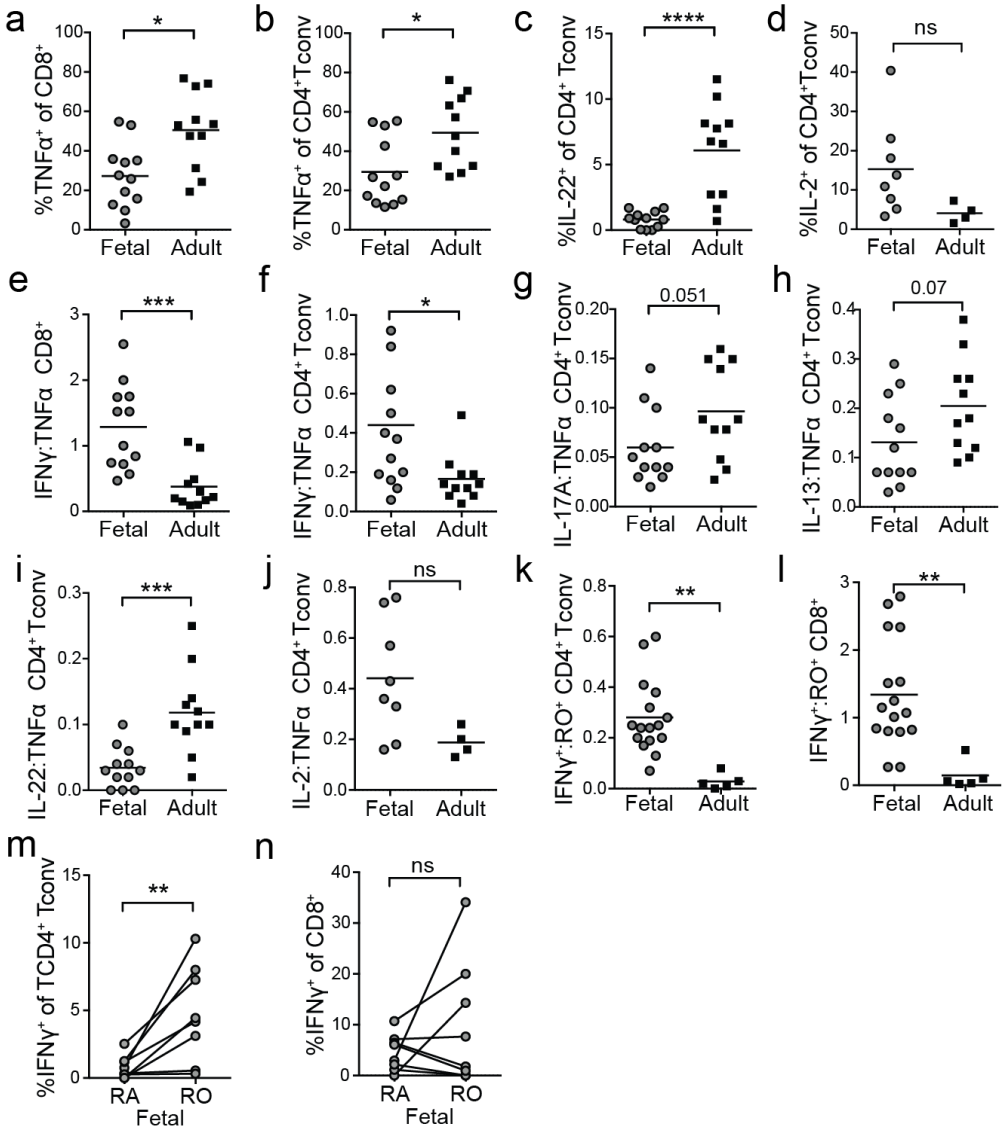

Figure S3: Fetal skin T cells have heightened capacity for IFNγ production. (Related to Figure 3) Cells were isolated from 23 week g.a. fetal skin (scalp and/or torso) as well as adult (torso) skin and analyzed by flow cytometry following PMA/ionomycin re-stimulation. Percentage TNFα-producing (a) CD8+ T cells and (b) CD4+ Tconv in skin by age. Percentage (c) IL-22 and (d) IL-2 producing CD4+ Tconv by age. (e-j) Cytokine expression in fetal vs. adult skin was normalized on a per sample basis to the percentage of TNFα+ cells. Normalized IFNγ production by (e) CD8+ T cells and (f) CD4+ Tconv in fetal vs. adult skin. Normalized (g) IL-17A, (h) IL-13, (i) IL-22 and (j) IL-2 by CD4+ Tconv. IFNγ production normalized to the percentage of CD45RO+ cells for (k) CD4+ Tconv and (l) CD8+ T cells. IFNγ production by CD45RO+ vs. CD45RA+ cells among fetal (m) CD4+ Tconv and (n) CD8+ T cells. Each point in represents data from an individual tissue sample; for some fetal samples data from scalp and torso skin from the same fetal donor are included as separate points.

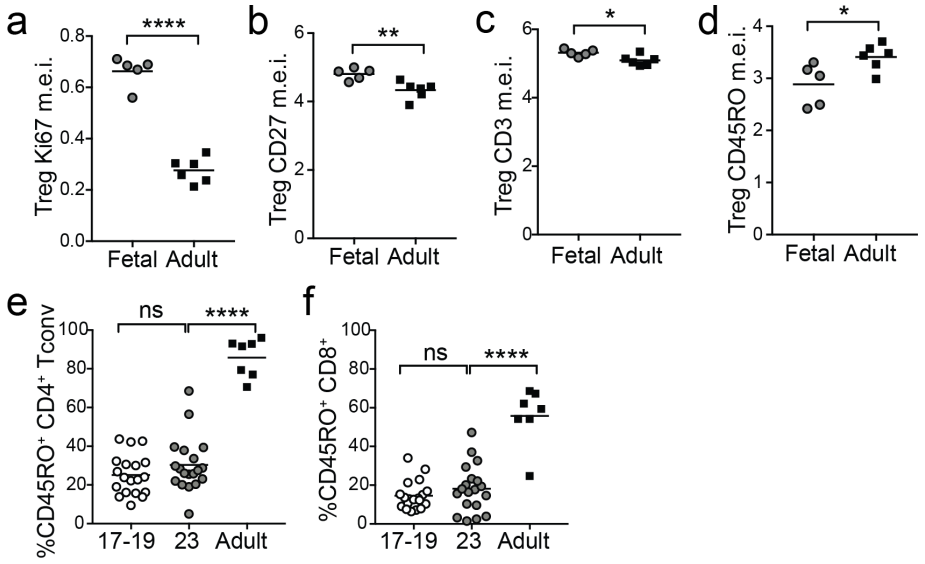

Figure S4: Expression of key markers by fetal vs. adult skin Tregs and of CD45RO by skin CD4+ Tconv and CD8+ T cells. (Related to Figure 4) (a-c) 23 week g.a. fetal torso skin along with healthy adult skin torso samples were analyzed in parallel for 22 markers using mass cytometry. Median expression of (a) Ki-67, (b) CD27, (c) CD3 and (d) CD45RO on skin Tregs by age. Each point represents data from an individual donor. (e-f) Cells were isolated from 17 to 23 week g.a. fetal skin (scalp and/or torso) as well as adult (torso) skin and analyzed by flow cytometry. Percentage of CD45RO+ (e) CD4+ Tconv cells and (f) CD8+ T cells by age. Points represent data from an individual tissue sample; for some fetal samples data from scalp and torso skin from the same fetal donor are included as separate points.

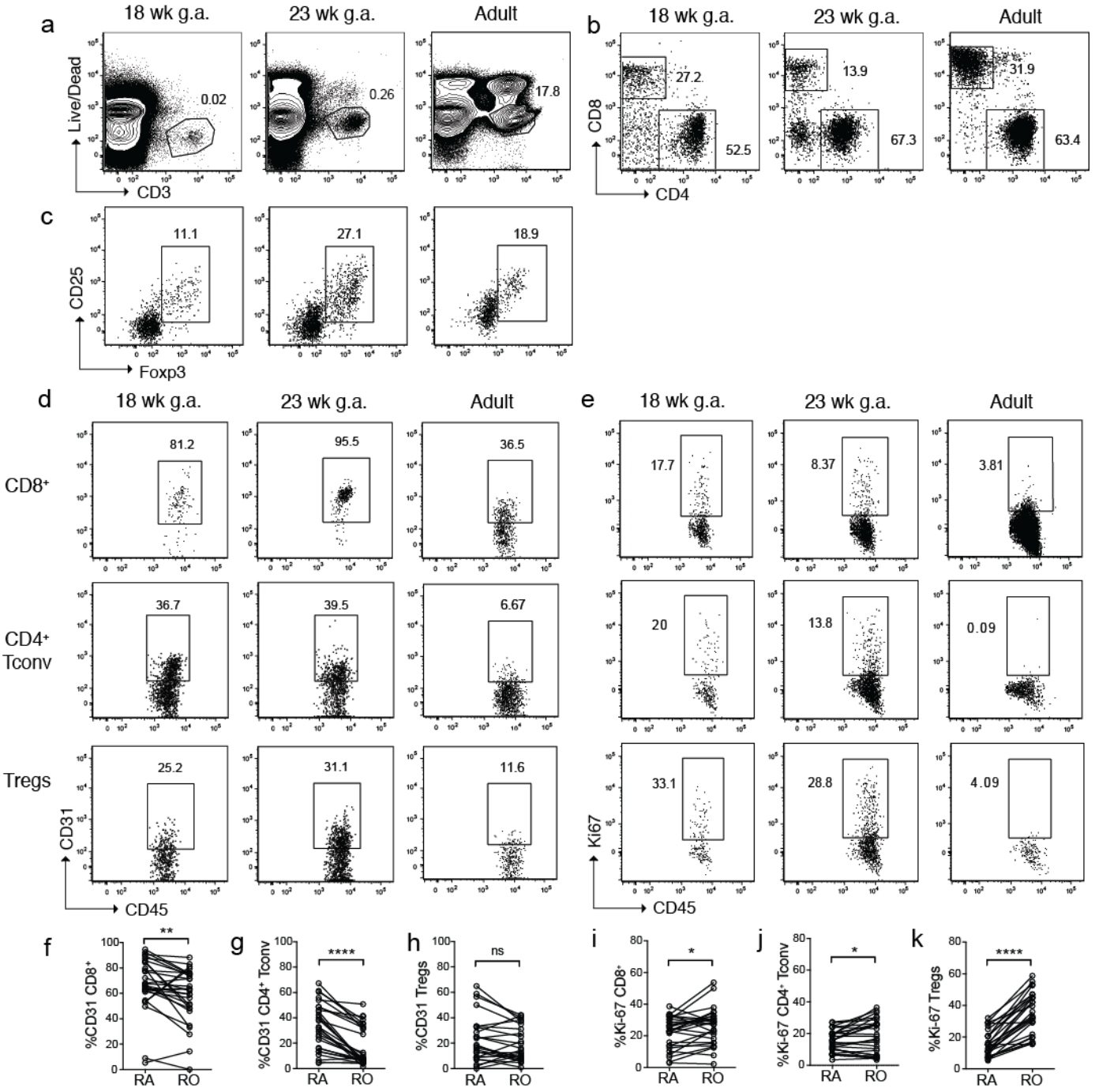

Figure S5: Abundance of αβ T cell subsets and their expression of CD31 and Ki-67 vary by age in human skin. (Related to Figure 5) Cells were isolated from 17 to 23 week g.a. fetal skin (scalp and/or torso) as well as adult (torso) skin and analyzed by flow cytometry. Representative flow cytometry plots by age demonstrating (a) live CD3$^+$ T cells (pre-gated on singlets), (b) CD4$^+$ and CD8$^+$ expression by T cells (pre-gated on live CD3$^+$) and (c) Tregs (pre-gated CD3$^+$CD8^{lo}CD4$^+$). Representative flow plots by age showing (d) CD31 and (e) Ki-67 expression by skin αβ T cell subsets. Percentage of CD31$^+$ cells among CD45RA$^+$ vs. CD45RO$^+$ (f) CD8$^+$ T cells, (g) CD4$^+$ Tconv cells, and (h) Tregs in fetal skin. Percentage of Ki-67$^+$ cells among CD45RA$^+$ vs. CD45RO$^+$ (i) CD8$^+$ T cells, (j) CD4$^+$ Tconv cells, and (k) Tregs in fetal skin. (f-k) Paired data points represent CD45RA$^+$ vs CD45RO$^+$ subsets from the same tissue sample.