

Multivariate profiling of African green monkey and rhesus macaque T lymphocytes

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Figure S1. Gating strategy for the identification of T cell subsets. After exclusion of dead cells, T cells ($CD3^+$) were classified into $CD4^+CD8^-$, $CD4^-CD8^+$, $CD4^+CD8^+$ (double positive) and $CD4^-CD8^-$ (double negative). Each of these four categories was further divided into naïve ($CD28^{lo}CD95^-$), central memory (CM) ($CD28^{hi}CD95^+$) and effector memory (EM) ($CD28^-CD95^+$). The same analysis was applied to all African green monkey ($n = 8$) and rhesus macaque ($n = 19$) samples.

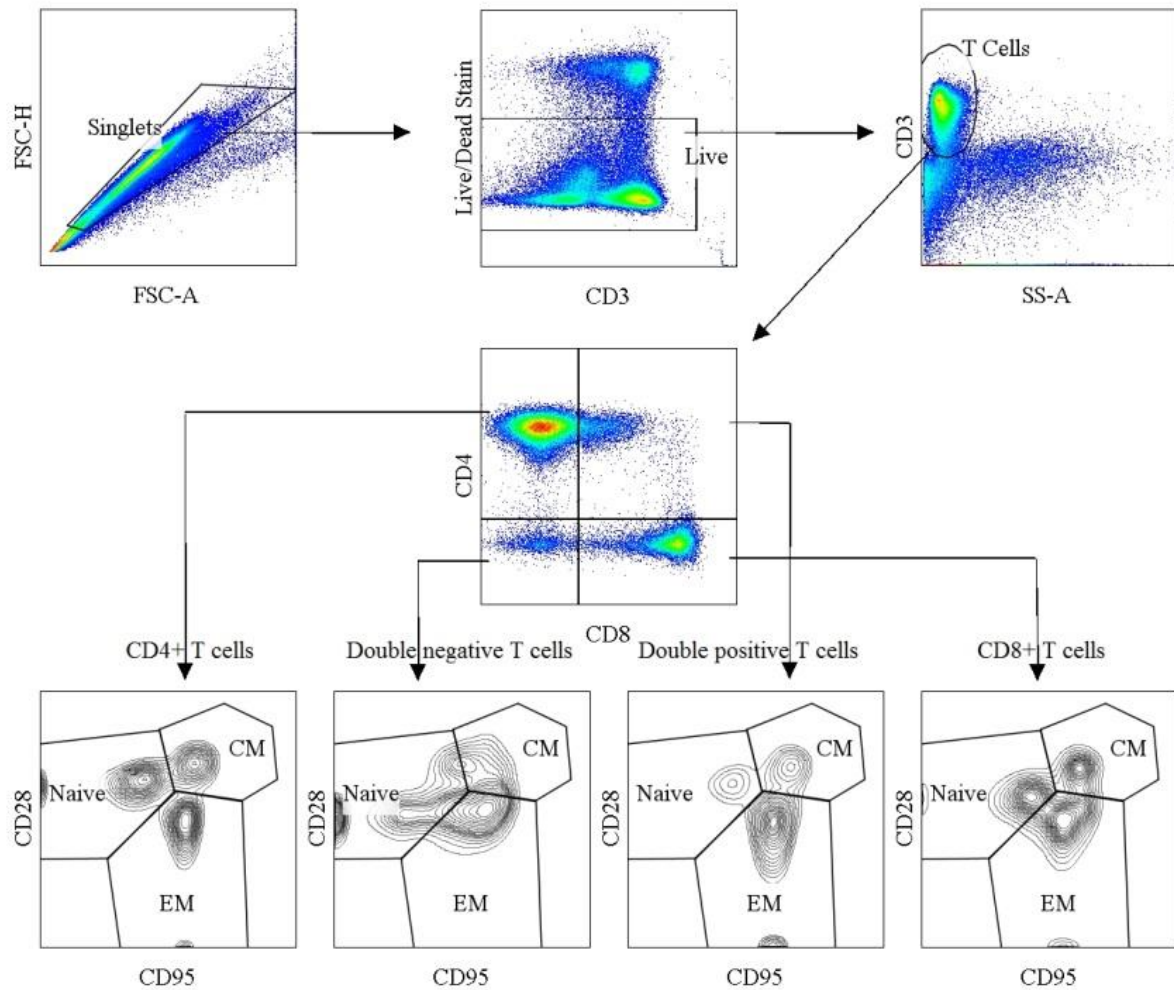


Figure S2. Hierarchical clustering and multidimensional scaling analysis of African green monkey (green; n = 8) and rhesus macaque (red; n = 19) T-cell subpopulations. Analysis based on percent (A, B) and absolute count (C, D) data are shown. Hierarchical clustering (A, C) and multidimensional scaling (B, D) were based on similarity calculated using Canberra distances (see Methods for description of similarity calculation).

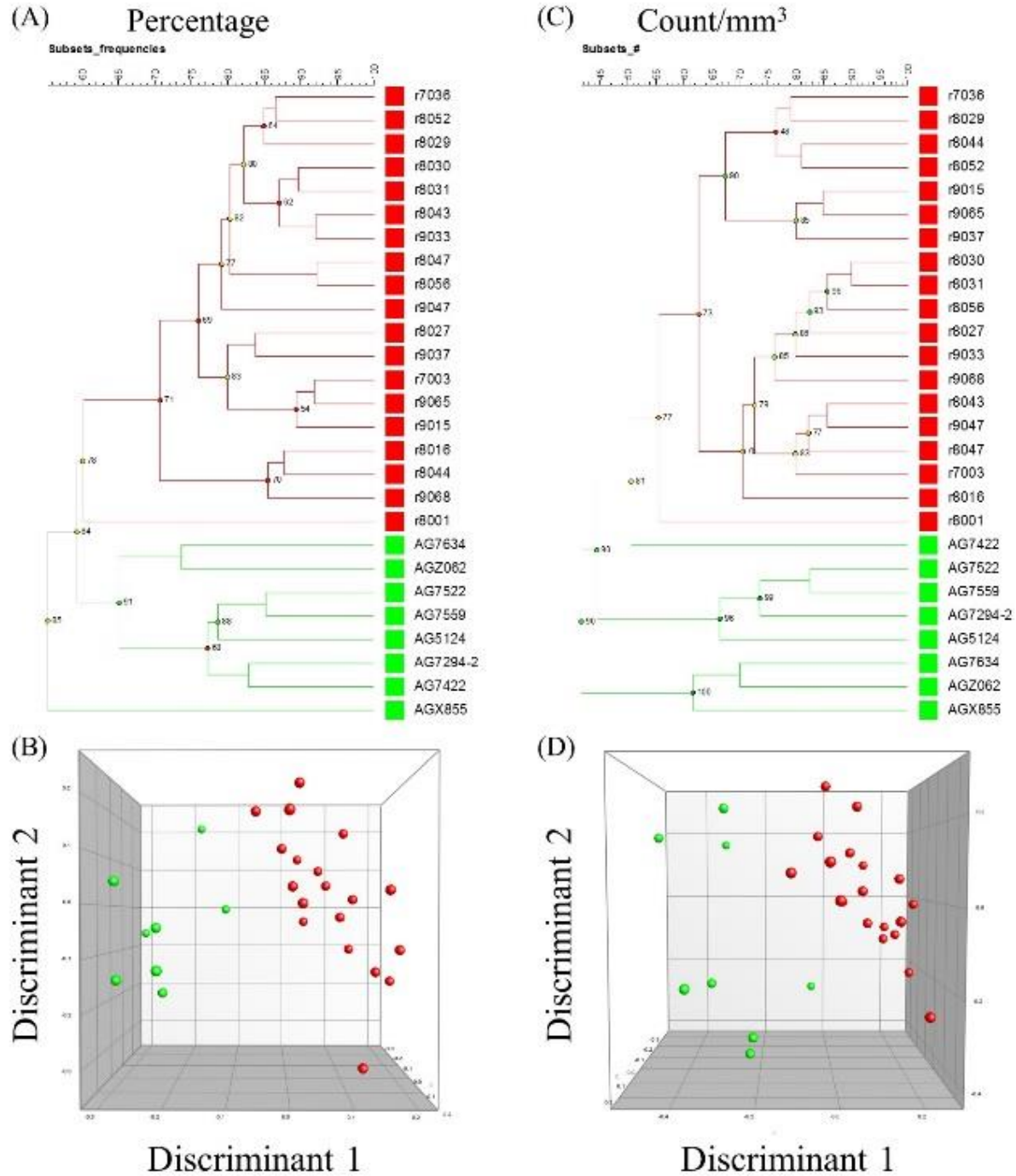


Figure S3. Representative dot plots showing surface-marker gating strategies. Shown in this figure are CD25, CD28 and CD95 gating in CD4⁺ and CD8⁺ T cells of African green monkeys (n = 8) and rhesus macaques (n = 19).

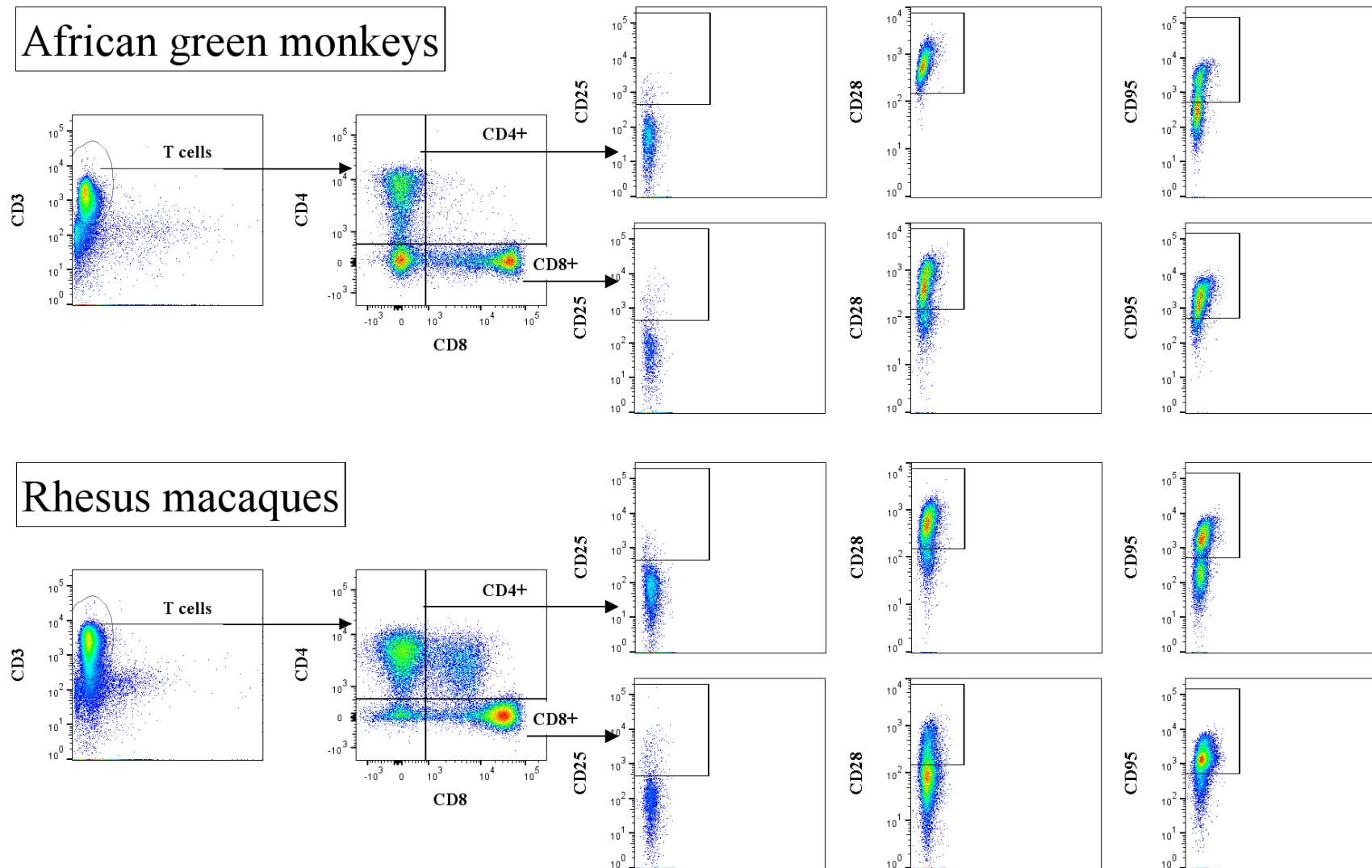
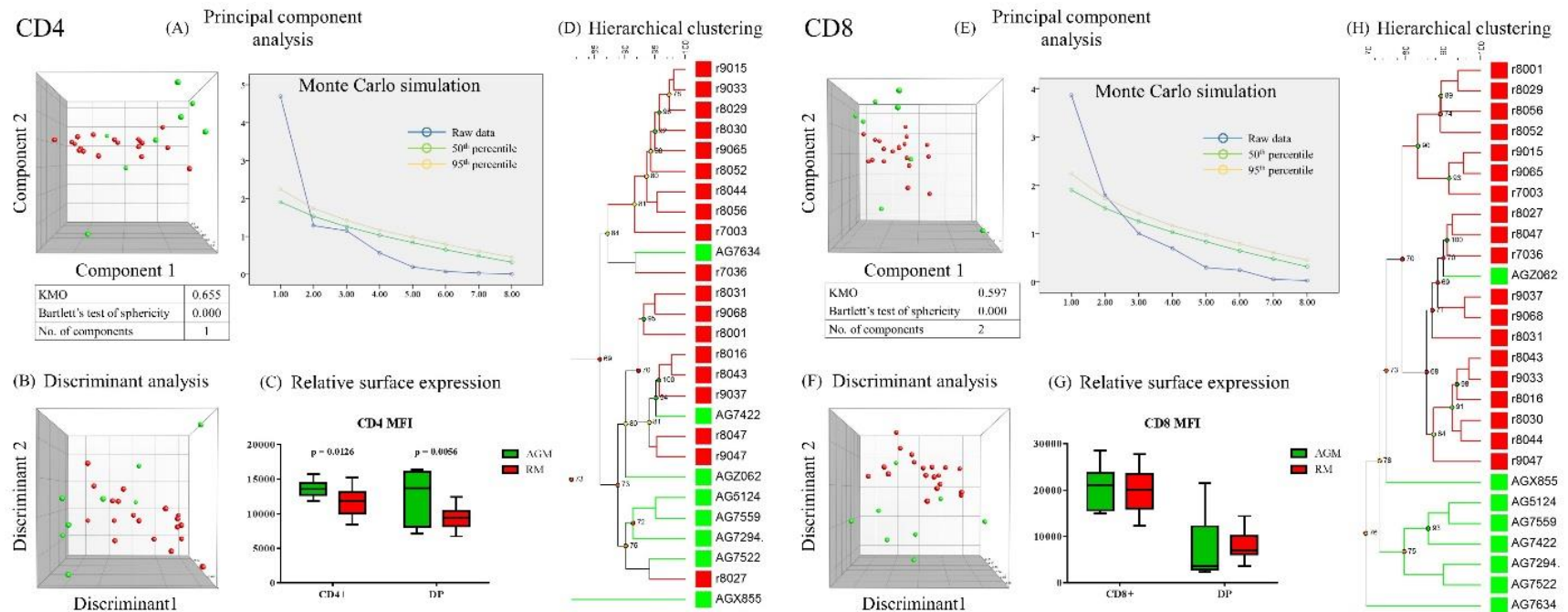
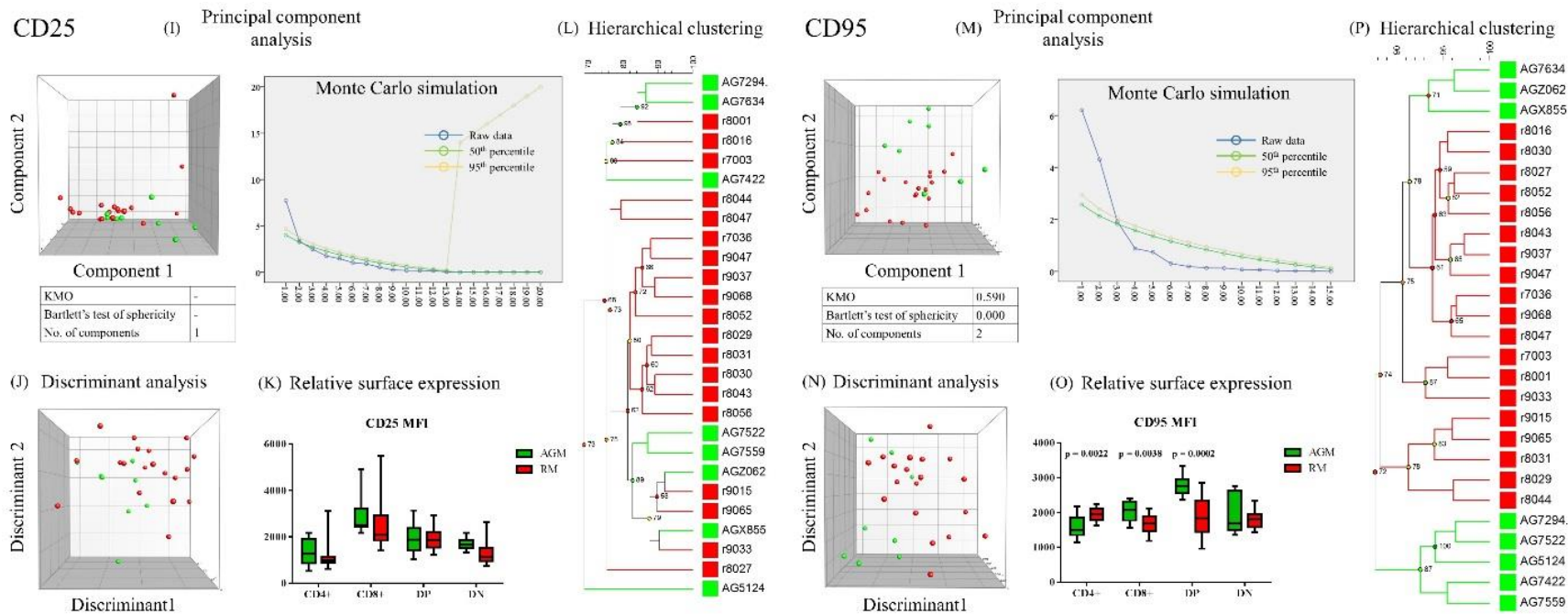


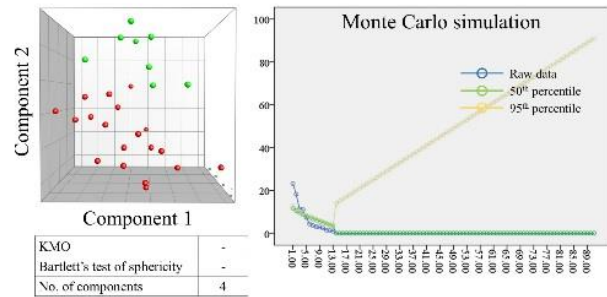
Figure S4. Segregation of African green monkeys (AGM; green; n = 8) and rhesus macaques (RM; red; n = 19) based on T-cell mean surface expression of CD3, CD4 (A-D), CD8 (E-H), CD25 (I-L), CD28 and CD95 (M-P). Data were analysed using principal component analysis (A, E, I, M, Q), multidimensional scaling (B, F, J, N, R) and hierarchical clustering (D, H, L, P, S) on individual surface proteins (A-P) or a combined profile of all six surface molecules (Q-S). To determine component significance in principal component analysis, scree plots showing raw eigenvalues and eigenvalues modelled at 50th and 95th percentile (blue, green and yellow, respectively) were used. Components were considered significant when their raw eigenvalues were higher than the corresponding 95th percentile modelled values. Kaiser-Meyer-Olkin measure of sampling adequacy (KMO), Bartlett's test of sphericity *p* values and the number of significant components are indicated below each plot. Surface expression levels of CD4, CD8, CD25 and CD95 as determined by mean fluorescence intensity of conjugated antibody stained cells are shown (C, G, K, O). Statistical significance of differences between African green monkeys and rhesus macaques in surface-molecules' expression was determined using multiple t-tests with Holm-Sidak correction for multiple comparisons. *P* values below 0.05 are indicated.



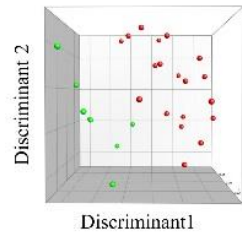


Composite

(Q) Principal component analysis



(R) Discriminant analysis



(S) Hierarchical clustering

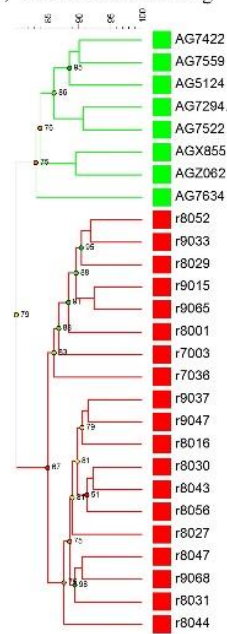


Figure S5. Representative dot plots showing gating strategies used to identify cytokine-secreting total T cells and CD4⁺ T cells of African green monkeys (n = 8) and rhesus macaques (n = 19). Dot plots: x axes show side scatter, y axes show brightness of cytokine staining in arbitrary units. Histograms: x axes show brightness of cytokine staining in arbitrary units, y axes show cell counts normalized to mode (%). Overlaid data of phorbol 12-myristate 13-acetate and ionomycin (PMA + I)-stimulated and unstimulated cells are shown in each histogram. IFNg: gamma interferon; IL-2: interleukin 2; TNFa: tumour necrosis factor alpha.

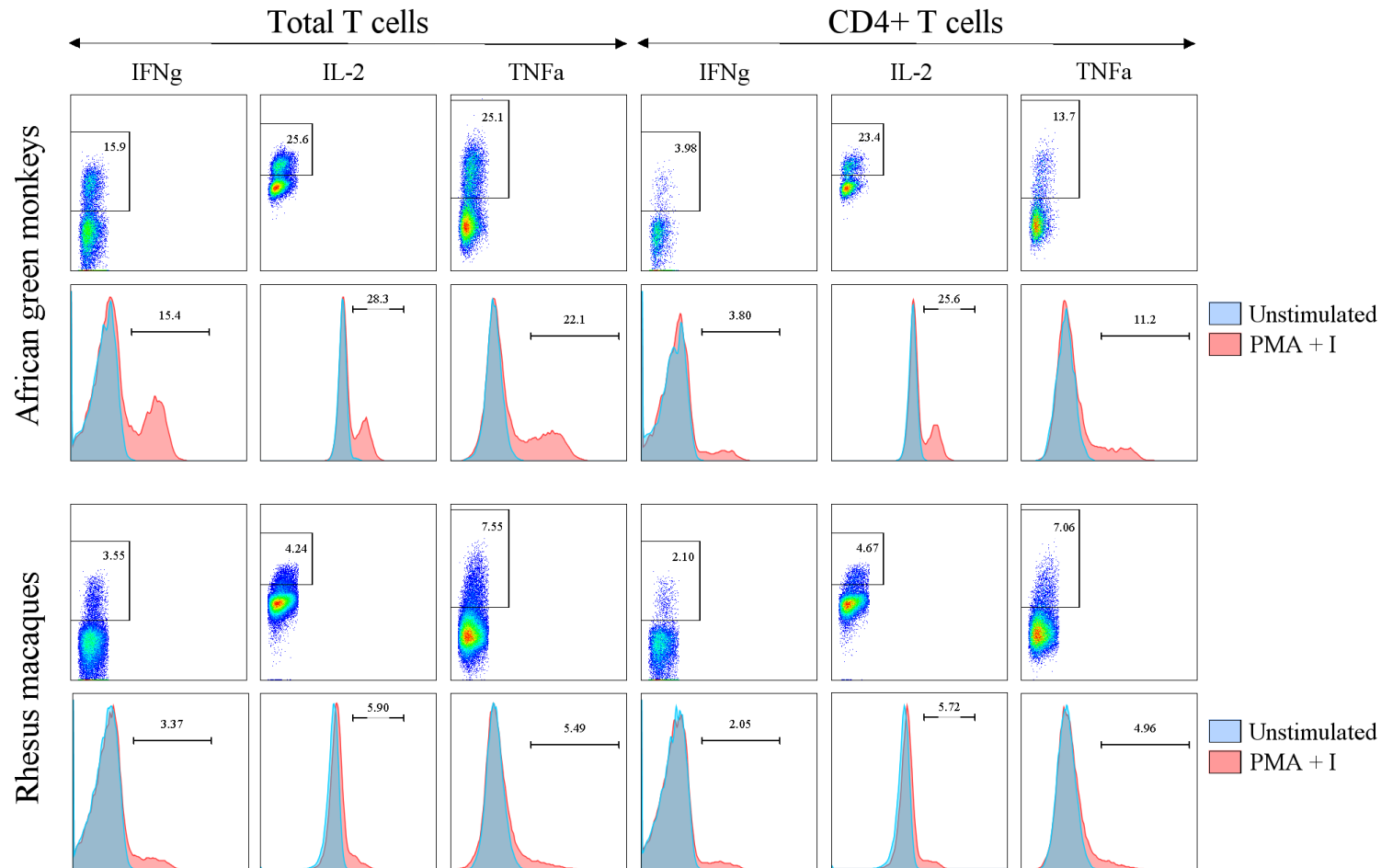


Figure S6. Cytokine-secretion patterns of the central memory, effector memory and naïve compartments of total T cells and CD4⁺ T cells of African green monkeys (n = 8) and rhesus macaques (n = 19). Pie slices 1 through 7 represent IFN⁺IL2⁺TNF⁺, IFN⁻IL2⁺TNF⁺, IFN⁺IL2⁻TNF⁺, IFN⁺IL2⁺TNF⁻, IFN⁻IL2⁻TNF⁺, IFN⁻IL2⁺TNF⁻ and IFN⁺IL2⁻TNF⁻ cell populations, respectively.

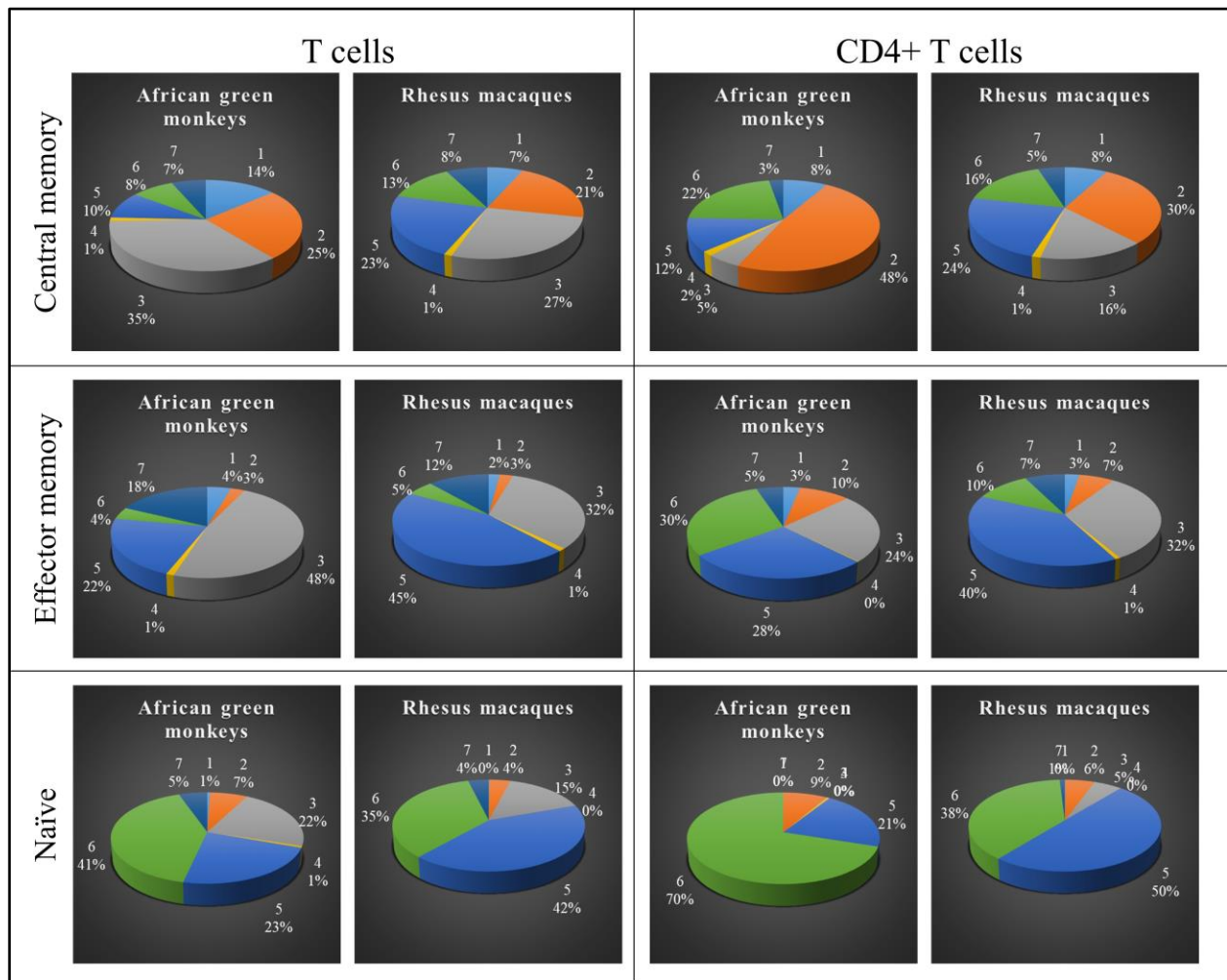


Figure S7. Representative dot plots showing gating on CD4⁺ central memory T cells of African green monkeys (n = 8) and rhesus macaques (n = 19). Cytokine secretion is shown in overlaid histograms from phorbol 12-myristate 13-acetate and ionomycin (PMA + I)-stimulated and unstimulated cells. IFN γ : gamma interferon; IL-2: interleukin 2; TNF α : tumour necrosis factor alpha.

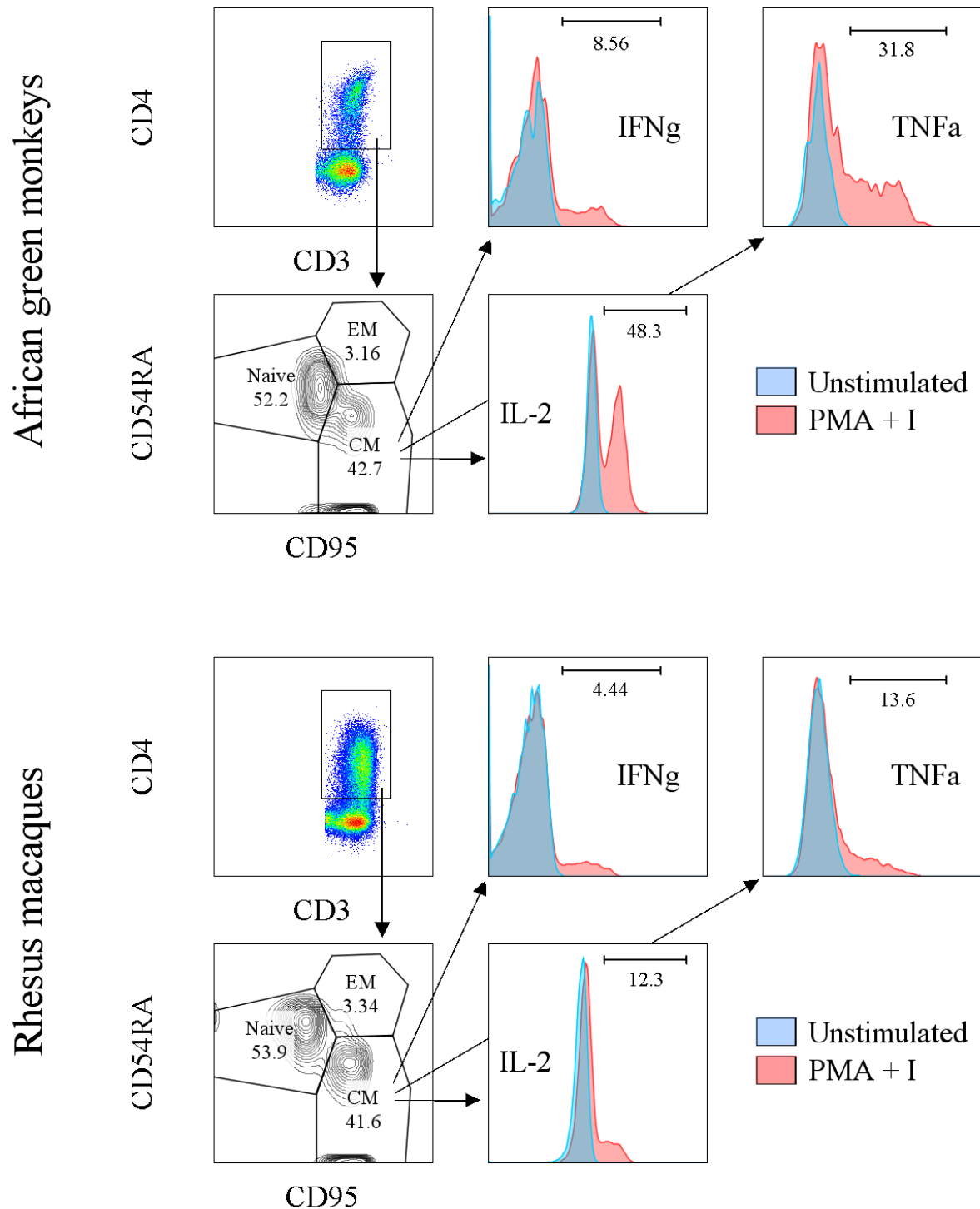


Figure S8. Cytokine-secretion patterns of central memory CD4⁺ T cells of African green monkeys (n = 8) and rhesus macaques (n = 19) after mitogenic stimulation. IFN: gamma interferon; IL2: interleukin 2; TNF: tumour necrosis factor alpha.

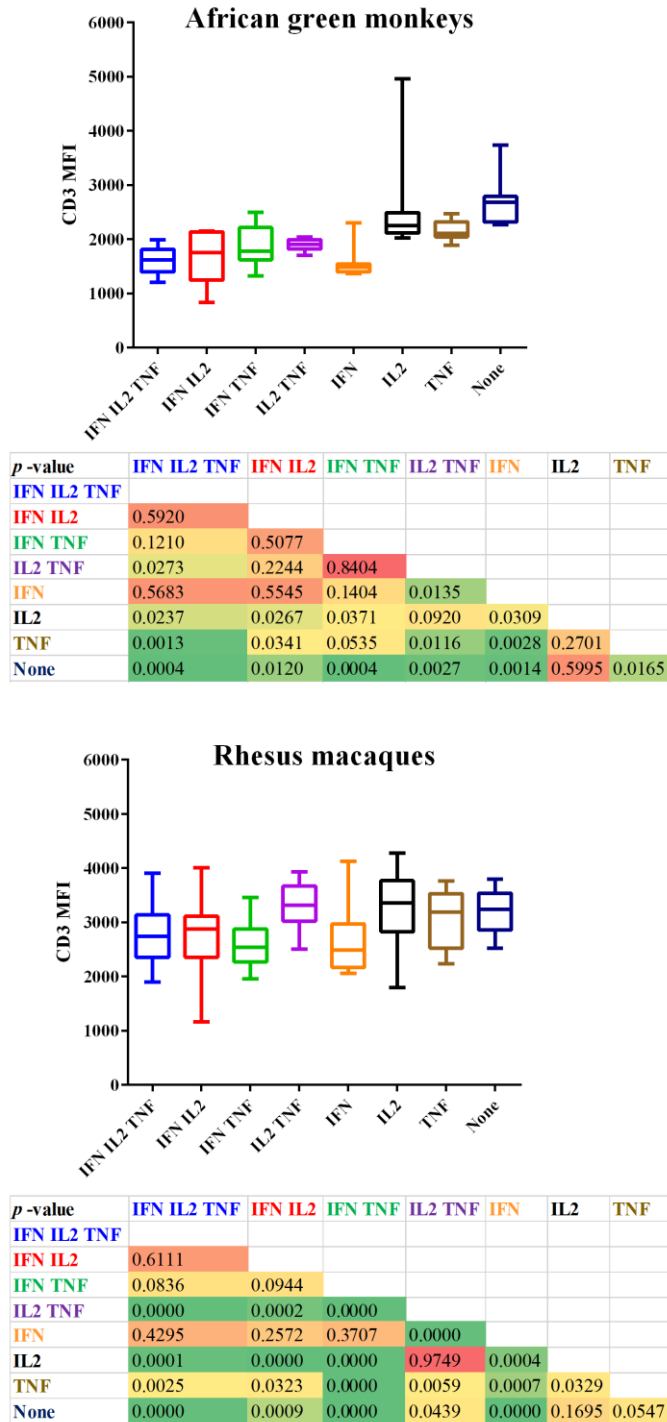


Figure S9. Representative dot plots showing gating strategies used to delineate cytokine-secreting total T cells and CD4⁺ T cells of cynomolgus macaques. Shown data are from an animal that has been infected with wild-type SIVmac239. The same gating method was applied to all cynomolgus macaques samples, including those infected with wild-type (n = 4) or Δ nef (n = 4) viruses. PMA + I: 12-myristate 13-acetate and ionomycin; IFN γ : gamma interferon; IL-2: interleukin 2; TNF α : tumour necrosis factor alpha.

