## SUPPLEMENTARY FIGURES



Supplementary Figure 1. Correlation plots of the immune cell phenotypes profiled grouped in subsets. (A) Spearman correlations among CD4 T helper cell subtypes. (B) Spearman correlations among CD8 cytotoxic T cell subtypes. (C) Spearman correlations between T cells, B cells, NK cells, monocytes, and their main subtypes profiled. (D) Spearman correlations among B cell subtypes. (E) Spearman correlations among cytes, and the non-classical monocytes.



Supplementary Figure 2. Unadjusted mean percentages of CD3+ T cells, B cells, and NK cells of total live lymphocytes, unadjusted mean percentages of CD4 TEMRA cells, CD4 +CD25+cells, CD4 FoxP3 T reg cells, and Granzyme B producing CD4 cells, and unadjusted mean percentages of CD8 TEMRA cells, CD8+CD25+ cells, CD8 FoxP3 T reg cells, and Granzyme B producing CD8 cells across age groups, stratified by sex and two-group CMV status. Note: NK cells = Natural killer cells; TEMRA cells = CD45RA- re-expressing T effector memory cells; T regs cells = T regulatory cells.



Supplementary Figure 3. Strategy for T cell stimulation gating. (A) gates out dead cells. (B) gates on only lymphocytes. (C) gates on CD3+ cells. (D) gates on CD4+ and CD8+ cells within the CD3+ cells. (E–K) are C3+CD4+ (or CD8+) with isotype controls used to set negative gates for IFNg, IL4, IL17, TNFa in the CD3+ gate, and Perforin/Granzyme B, IGNg/IL7, and TNFa/IFNg in the CD4+ (or CD8+) gate, respectively. (L–R) show full data. Data for CD8+ cells was gated identically.



Supplementary Figure 4. Strategy for T, B, NK cell gating. PBMCs are gated on live cells (A), followed by lymphocytes (B). (C–H) are isotype controls. (I) is gated on live lymphocytes for CD3+, CD19+ and CD3-CD19-. (J) is gated on live lymphocytes for CD3+gdTCR+. (K) is gated on CD3-CD19- for CD56+CD16+ (NK cells). (L) is gated on CD3+ for CD38+ and HLADR+. (M) is gate on CD19+ for CD43+ and CD27+. (N) is gated on CD19+ for IgD and IgM.



Supplementary Figure 5. Regulatory T cell gating strategy. PBMCs are gated on live cells (A), then lymphocytes (B), then CD3+ cells (C) and then on either CD4+ or CD8+ cells (D). Gating on CD4+ cells, isotype controls were used to set negative gates for CD25 and FoxP3 (E), tBET and RORgT (F), CD25 and CD127 (G), FoxP3 and tBET (H) and FoxP3 and RORgT (I). CD4+ cells were then analyzed for CD25 and FoxP3 (J), tBET and RORgT (K), CD25 and CD127 (L), FoxP3 and tBET (M) and FoxP3 and RORgT (N). Similar gating was used for CD8+ isotypes controls (O–S) and test samples (T–X).



Supplementary Figure 6. Strategy for memory subset gating. PBMCs are gated on live cells (A), lymphocytes (B), CD3+ (C), CD4+ and CD8+ (D). Gating on CD4+, isotype controls were used to set negative gates for CD45RA and CD45RO (E), for CD45RA+: CD192 and CD27 (F), for CD45RO+: CD192 and CD27 (G), CD28 and CD57 (H), CD95 and CD127 (I), CD137 and CD127 (J), and CD28 and CD27 (K). CD45RA+ cells (upper left quadrant) (L) were used to gate CD197 and CD27 (M), while CD45RO+ (lower right quadrant) (L) were used to gate CD197 and CD27 (M), while CD45RO+ (lower right quadrant) (L) were used to gate CD197 and CD27 (N). Gating on CD4+ cells, were positive samples for CD28 and CD57 (O), CD95 and CD127 (P), CD137 and CD127 (Q), and CD28 and CD27 (R). A CD57 isotype control (S) and CD57+ samples gated on CD4+CD45RA+CD28+CD27+ (T) for naïve cells, CD4+CD45RO+CD28+CD27+ (U) for late differentiated cells (ED), CD4+CD45RO+CD28+CD27+ (V) for intermediate differentiated cells (ID), and CD4+CD45RA+CD28-CD27- (W) for late differentiated cells (LD). Using the CD4+, is an isotype control (X) and a CD279+ (PD1) sample (Y). CD8+ memory subsets were performed using the same gating strategy.



Supplementary Figure 7. Monocyte gating strategy. PBMCs are gated on live cells (A) followed by monocytes (B). (C) is a CD14+ sample used to set negative gates with isotypes. (D–G) are CD14+ cells with isotypes controls. (H) show gating for CD14+CD16- (Classical Monocytes), CD14+CD16+ (Intermediate Monocytes) and CD14dimCD16+ (non-Classical Monocytes). (I–L) are gated on Classical Monocytes for CD163 and HLADR (I), TREM2 and CD87 (J), CD192 and SLAN (K) and CD33 (L). (M–P) are the same as (I–L) except gated on intermediate monocytes. (Q–T) are the same as (I–L) except gated on the non-classical monocytes.