

Supplementary Materials for
**Magnitude and kinetics of the human immune cell response associated with
severe dengue progression by single-cell proteomics**

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The PDF file includes:

Supplementary Text
Figs. S1 to S7
Legends for tables S1 to S6
References

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S6

Supplementary text:

Challenges in identifying DENV-infected cells in patient-derived PBMCs via mass cytometry

The precise immune cell targets of DENV in the human blood remain incompletely characterized. Myeloid cell types including monocytes and dendritic cells have been shown to be actively infected with DENV (98, 99). However a previous study has shown that B cells can maintain DENV infection (100) as well, and we have demonstrated that B cell populations, specifically naïve B cells, harbor the large bulk of viral RNA (10). Here, we aimed to further define DENV target cells by conjugating three antibodies targeting the DENV envelope (structural), and NS1 and NS3 (nonstructural) proteins (NS1, NS3). Titration of these anti-DENV antibodies in DENV infected Huh7 cells revealed clear bimodal signal and an inoculum dependent increase in DENV protein marker staining at 48 hours postinfection (**Figure S4B**). However, when used to detect DENV in primary patient-derived PBMCs, we detected a greatly increased noise floor, resulting in a small fraction of cells that stained positive for all three DENV proteins (**Figure S4C**). Moreover, there was no correlation between the expression level of the DENV proteins and DENV RNA level measured via RT-qPCR in patient serum (**Figure S4D**). We speculate that the DENV-specific antibodies bound viral protein debris non-specifically associated with immune cell membranes during cell staining, preventing discrimination of infected cells. Further optimization will therefore be required to detect primary DENV-infected cells in PBMCs via CyTOF.

Figure S1

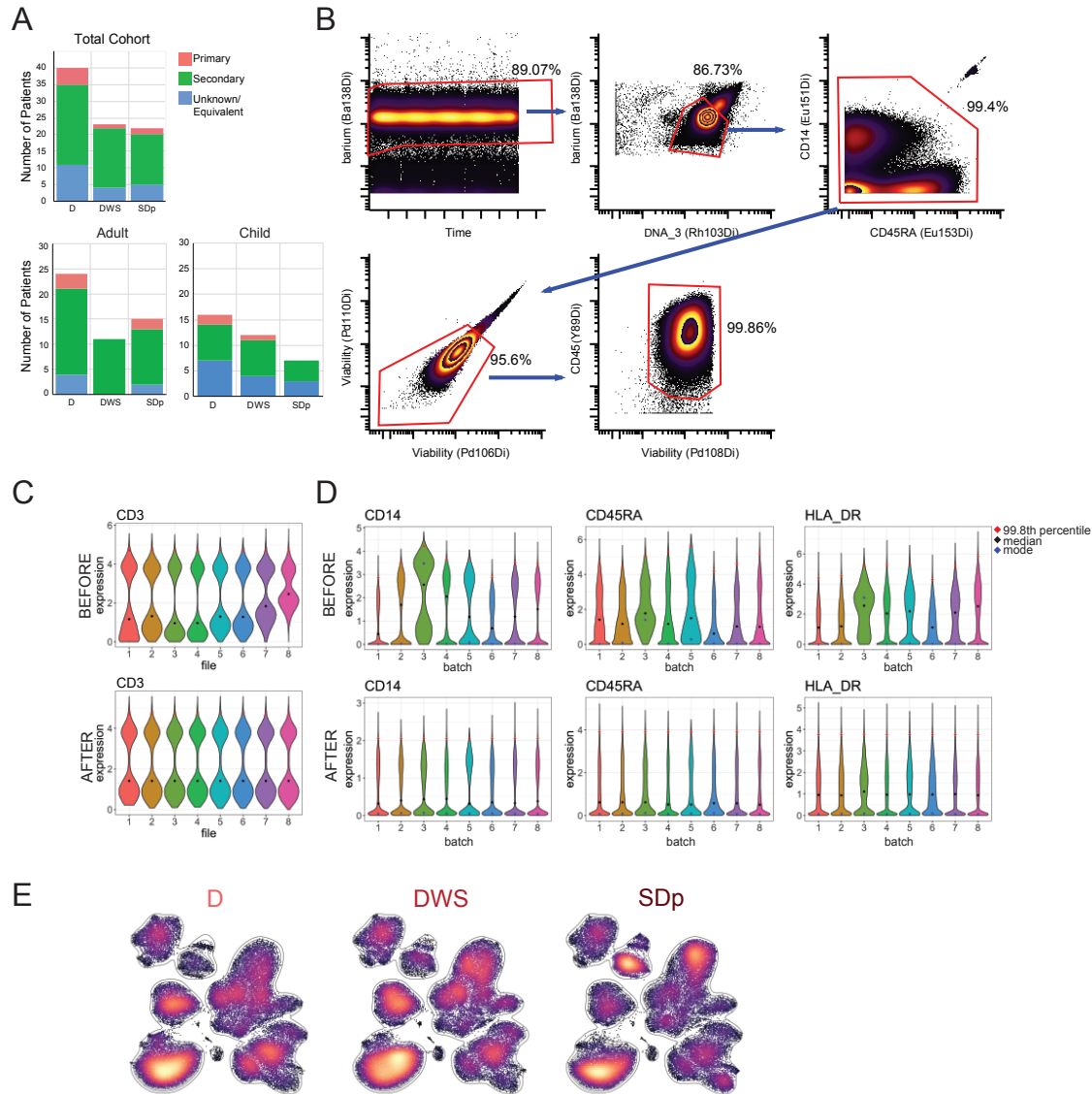


Figure S1: Patient information, gating, normalization, and unbiased segregation of cells by clinical status – related to Figure 1

- (A) Composition of cohort by dengue exposure status (colors) and clinical status (columns) in the total cohort and by age category.
- (B) Exemplative leukocyte gating strategy
- (C) Exemplative correction of CD3 within a batch. Violins represent cell distributions of barcoded files not yet deconvolved that were iteratively collected over the course of a day.
- (D) Exemplative batch correction of three protein channels. Violins represent cell distributions from a common control sample run with each batch.
- (E) UMAP plots with same coordinates as in Figure 1C, separated by disease status and colored by density.

Figure S2

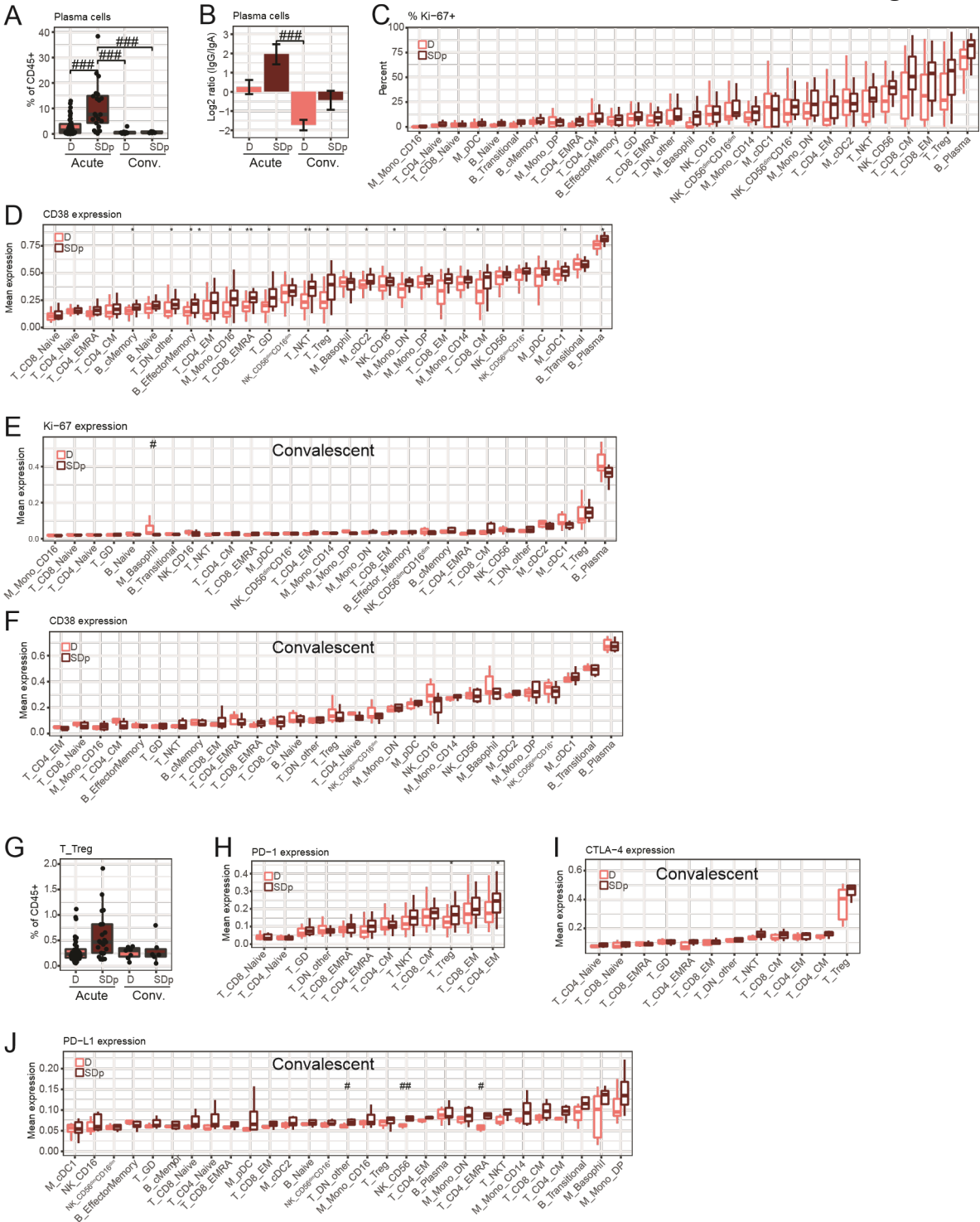


Figure S2. Additional features of immune activation and regulation in acute and convalescent SD and D samples – related to Figure 2
 (A, G) Plasma cell and Treg abundance by clinical status and time. Dots represent patients.

(B) Mean \log_2 ratio of patient IgG⁺ plasma cell abundance to IgA⁺ plasma cell abundance by clinical status and time. Error bars represent standard error of mean (SEM).

(C) % Ki-67⁺ by cell population and clinical status in acute samples.

(D, H) Mean CD38 (D) and PD-1 (H) expression by cell population and clinical status in acute samples.

(E, F, I, J) Mean Ki-67 (E), CD38 (F), CTLA-4 (I), and PD-L1 (J) expression by cell population and clinical status in convalescent samples.

In boxplots, center line signifies median, box signifies interquartile range (IQR) and whiskers signify IQR \pm 1.5*IQR. q<0.05 & |effect|>0.5; ** q<0.01 & |effect|>0.5; *** q<0.005 & |effect|>0.5; # p<0.05 & |effect|>1.0; # p<0.05 & |effect|>1.0; ## p<0.01 & |effect|>1.0; ### p<0.005 & |effect|>1.0 by Wilcoxon rank sum tests. D, dengue; SDp, SD progressors.

Figure S3

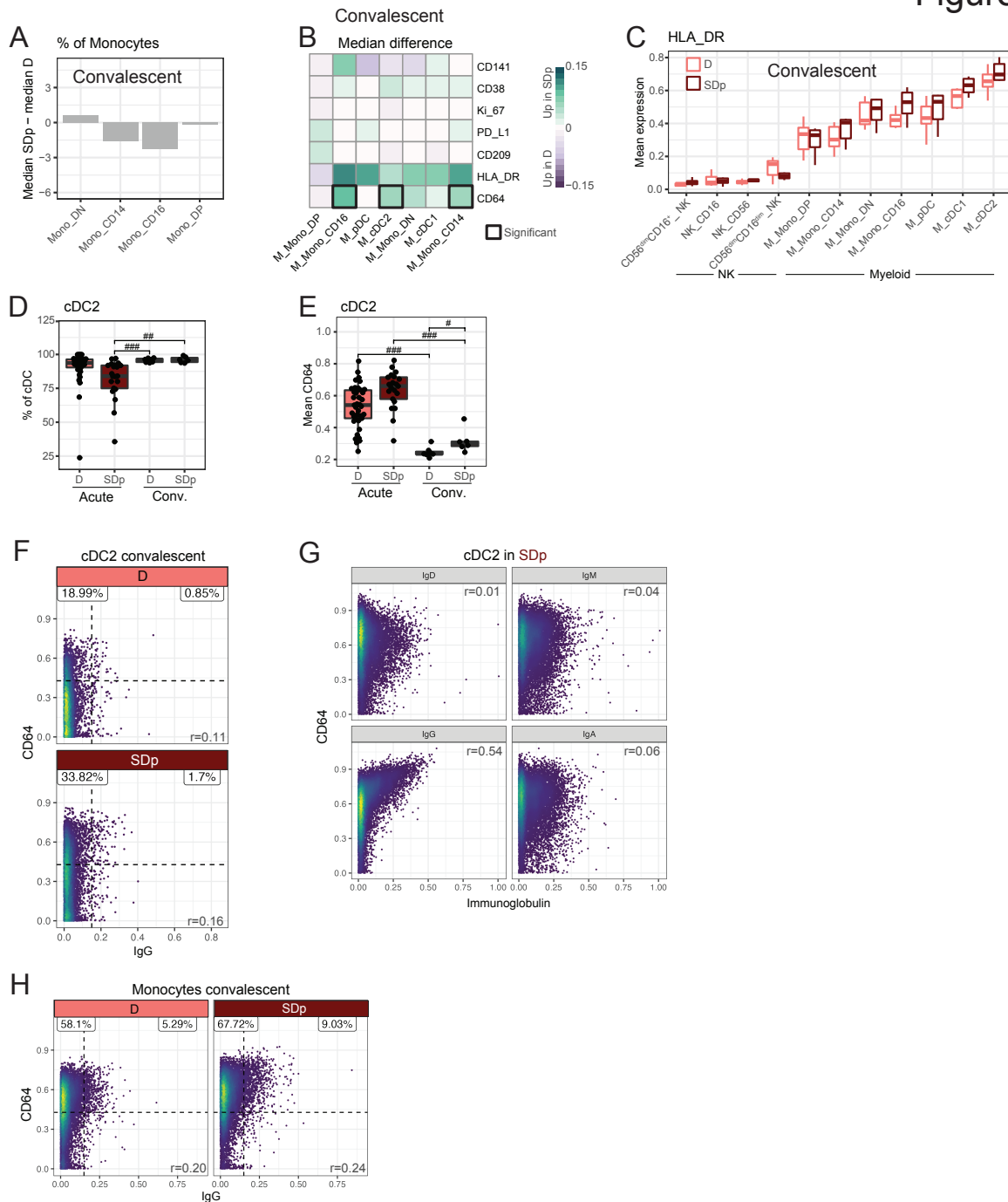


Figure S3: Additional innate immune features of acute and convalescent SDp and D samples – related to Figure 3

(A) Difference in log₂ ratio of median relative abundances of monocyte subtypes (columns) between convalescent SDp and D patients out of total monocytes. Teal bars indicate significance (q<0.05 & |effect|>0.5).

(B) Difference in cohort median of patient mean expression between convalescent SDp and D samples across molecules (rows) in myeloid cell populations (columns). Black boxes indicate significance ($p < 0.05$ & $|\text{effect}| > 0.5$).

(C) Mean HLA-DR expression in NK and myeloid cell subtypes by clinical status in convalescent samples.

(D, E) Box plots of cDC2 fraction out of total cDCs (D) and mean CD64 expression (E) by clinical status in acute and convalescent samples. Dots represent individual patients.

(F, H) Scatter plots of single cell CD64 expression versus IgG detection in convalescent samples in cDC2s (F) and monocytes (H) by clinical status. Percent proportion of cells in each quadrant, derived from an equal subsampling of cells by clinical status and patient are shown.

(G) Scatter plots of single cell CD64 expression versus immunoglobulin isotypes detection on cDC2s in acute SDp samples. Cells were derived from an equal subsampling of cDC2s by patient.

In boxplots, center line signifies median, box signifies interquartile range (IQR) and whiskers signify $\text{IQR} \pm 1.5 \times \text{IQR}$. Dots in A, C, D, F, and H represent individual patients.

* $q < 0.05$ & $|\text{effect}| > 0.5$; ** $q < 0.01$ & $|\text{effect}| > 0.5$; *** $q < 0.005$ & $|\text{effect}| > 0.5$; # $p < 0.05$ & $|\text{effect}| > 1.0$; ## $p < 0.01$ & $|\text{effect}| > 1.0$; ### $p < 0.005$ & $|\text{effect}| > 1.0$ by Wilcoxon rank sum tests.

Q-values represent FDR-corrected p-values. r in F, G, H and I was calculated by Pearson correlation. D, dengue; SDp, SD progressors; conv., convalescent.

Figure S4

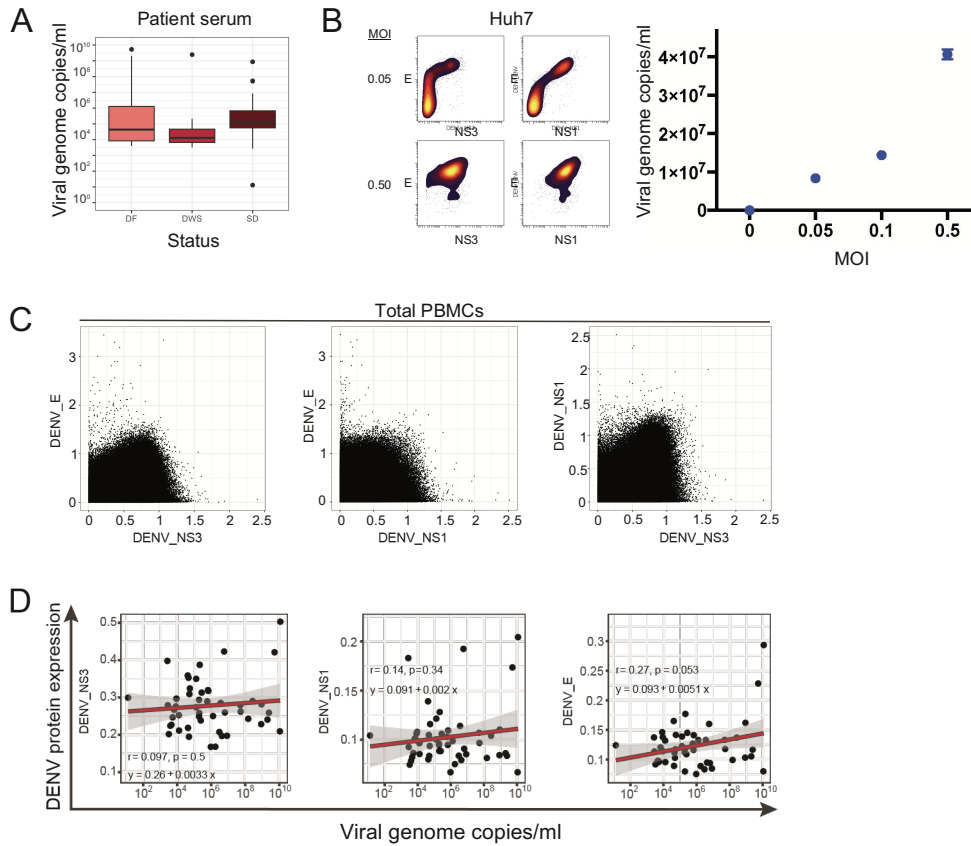


Figure S4: Quantification of DENV viral load and evaluation of DENV target cells via CyTOF

(A) Boxplot depicting level of viremia determined by qRT-PCR by dengue clinical status. In boxplots, center line signifies median, box signifies interquartile range (IQR) and whiskers signify IQR $\pm 1.5 \times$ IQR.

(B) CyTOF analysis of Huh7 cells infected with DENV-2 at MOIs of 0.05 and 0.5. Left panel: Dot plots of mean signal intensity of DENV E, NS1 and NS3 proteins. Right panel: Viral load measured via qRT-PCR for various MOIs.

(C) Contour plots of single cell DENV E, NS1 and NS3 protein expression in total PBMCs derived from the dengue Colombia cohort.

(D) Scatter plots of mean DENV E, NS1 and NS3 protein expression in PBMCs versus viral load in serum samples with detectable virus by qRT-PCR. Fitting line and 95% CI (shading) were derived by linear regression. Correlation coefficient (r) and p -values were calculated by Pearson correlation.

E, Envelope; NS1, nonstructural 1; NS3, nonstructural 3.

Figure S5

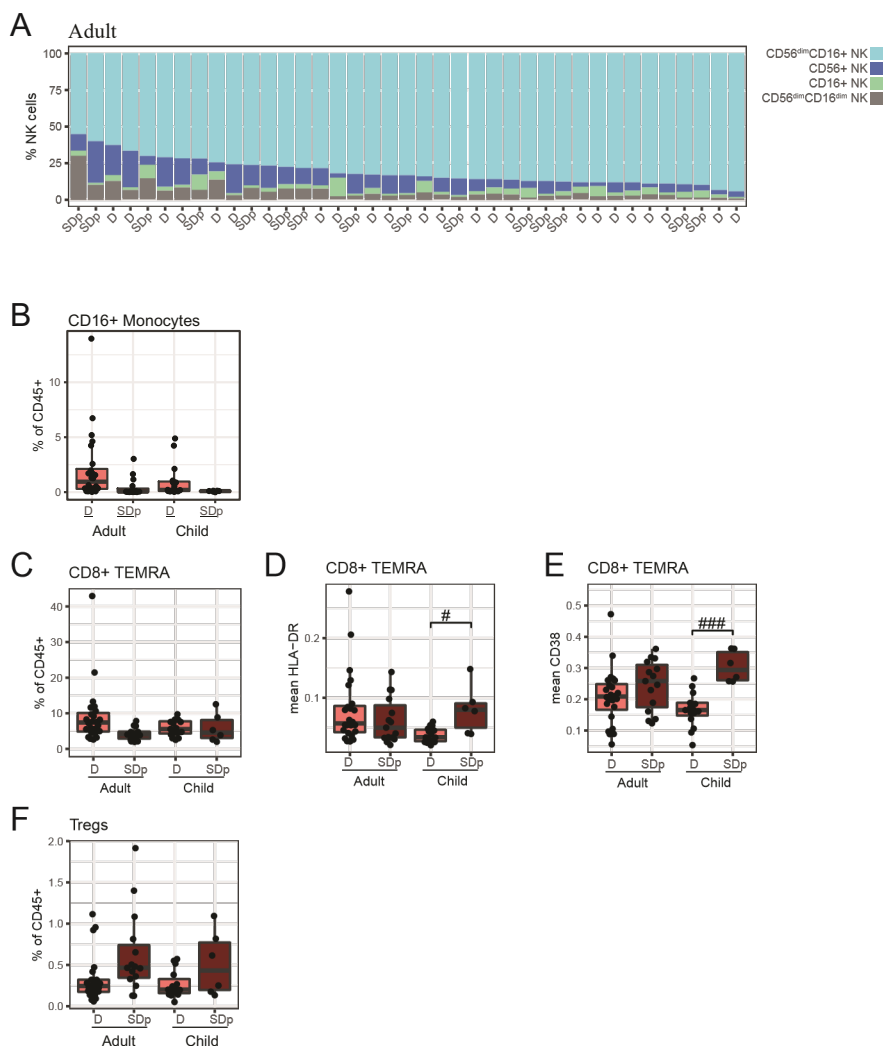


Figure S5: Additional features differentiating SDp children from SDp adults – related to Figure 4

(A) Proportion of NK cell subtypes in adults ordered by CD56^{dim}CD16⁺ NK cell abundance. Columns represent individual patients, labeled by clinical status.

(B, C, F) Box plots of CD16⁺ monocytes (B) CD8⁺ TEMRA (C), Treg (F) fractions of CD45⁺ cells by clinical status and age. Dots represent individual patients.

(D, E) Box plots of mean HLA-DR (D) and CD38 (E) expression in CD8⁺ TEMRA cells by clinical status and age. Dots represent patients.

In boxplots, center line signifies median, box signifies interquartile range (IQR) and whiskers signify IQR \pm 1.5*IQR. # $p < 0.05$ & $|\text{effect}| > 1.0$; ## $p < 0.01$ & $|\text{effect}| > 1.0$; ### $p < 0.005$ & $|\text{effect}| > 1.0$ by Wilcoxon rank sum tests. D, dengue; SDp, SD progressors.

Figure S6

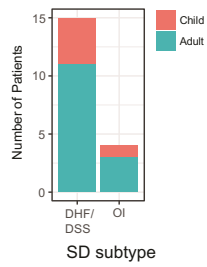


Figure S6: SDp patient distribution by SD subtypes and age – related to Figure 5
Patient composition of SD samples by age (colors) and SD subtype (columns).
DHF/DSS, Dengue hemorrhagic fever/dengue shock syndrome; OI, organ impairment.

Figure S7

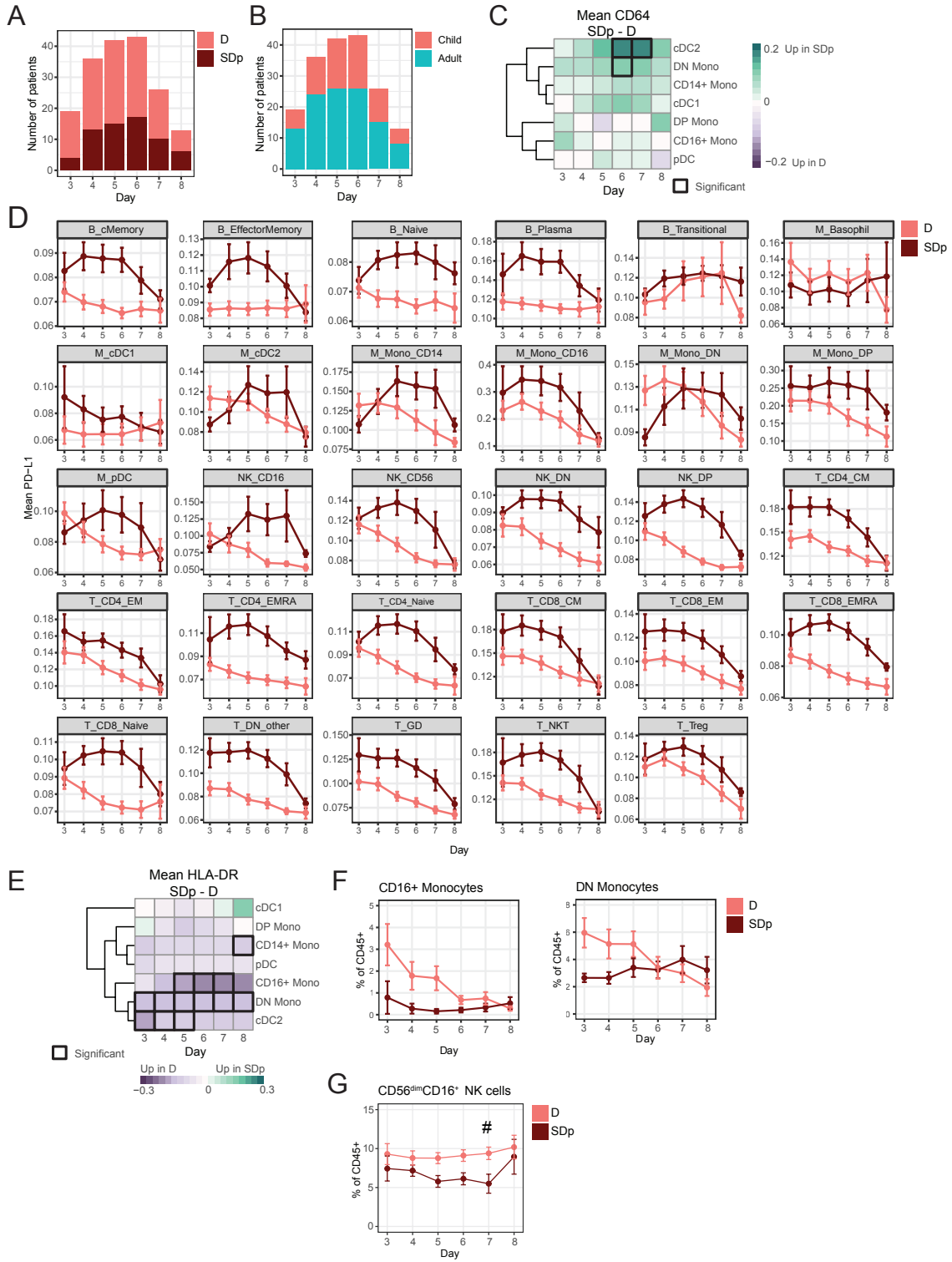


Figure S7: Additional features of acute infection by time – related to Figure 6

(A) Composition of clinical status (colors) by day.

(B) Composition of age (colors) by day.

(C, E) Difference in cohort mean of patient mean CD64 (D) and HLA-DR (E) expression between SDp and D across cell populations (rows) by day (columns). Black boxes indicate significance ($p < 0.05$ & $|\text{effect}| > 1.0$).

(D) Cohort mean of patient mean PD-L1 expression by day (columns) and clinical status (color), in the indicated cell populations. Each graph is individually scaled.

(F) Cohort mean of CD16⁺ (left) and DN (right) monocyte abundances of CD45⁺ cells by day (columns) and clinical status (color).

(G) Cohort mean of CD56^{dim}CD16⁺ NK cell abundance of CD45⁺ cells by day (columns) and clinical status (color).

Error bars represent SEM. D, dengue; SDp, SD progressor; DN, double negative.

Supplementary table captions:

Table S1: Clinical, demographic and laboratory characteristics of patients in the Colombia dengue cohort.

(S1.1) Clinical, demographic and laboratory characteristics of all the patients included in this study.

(S1.2) Clinical characteristics of severe dengue progressors included in this study.

(S1.3) Summary of patient characteristics by disease severity category.

(S1.4) Longitudinal samples analyzed.

Table S2: Effect sizes and p values of pairwise Wilcoxon rank sum tests on cell abundance and protein expression between disease categories in acute samples.

Table S3: Effect sizes and p values of pairwise Wilcoxon rank sum tests on cell abundance and protein expression between acute and convalescent samples.

Table S4: Effect sizes and p values of pairwise Wilcoxon rank sum tests on cell abundance and protein expression between adults and children.

Table S5: Effect sizes and p values of pairwise Wilcoxon rank sum tests on cell abundance and protein expression between severe dengue categories.

Table S6: Effect sizes and p values of pairwise Wilcoxon rank sum tests on cell abundance and protein expression between disease categories on days 3-8 following fever onset.

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