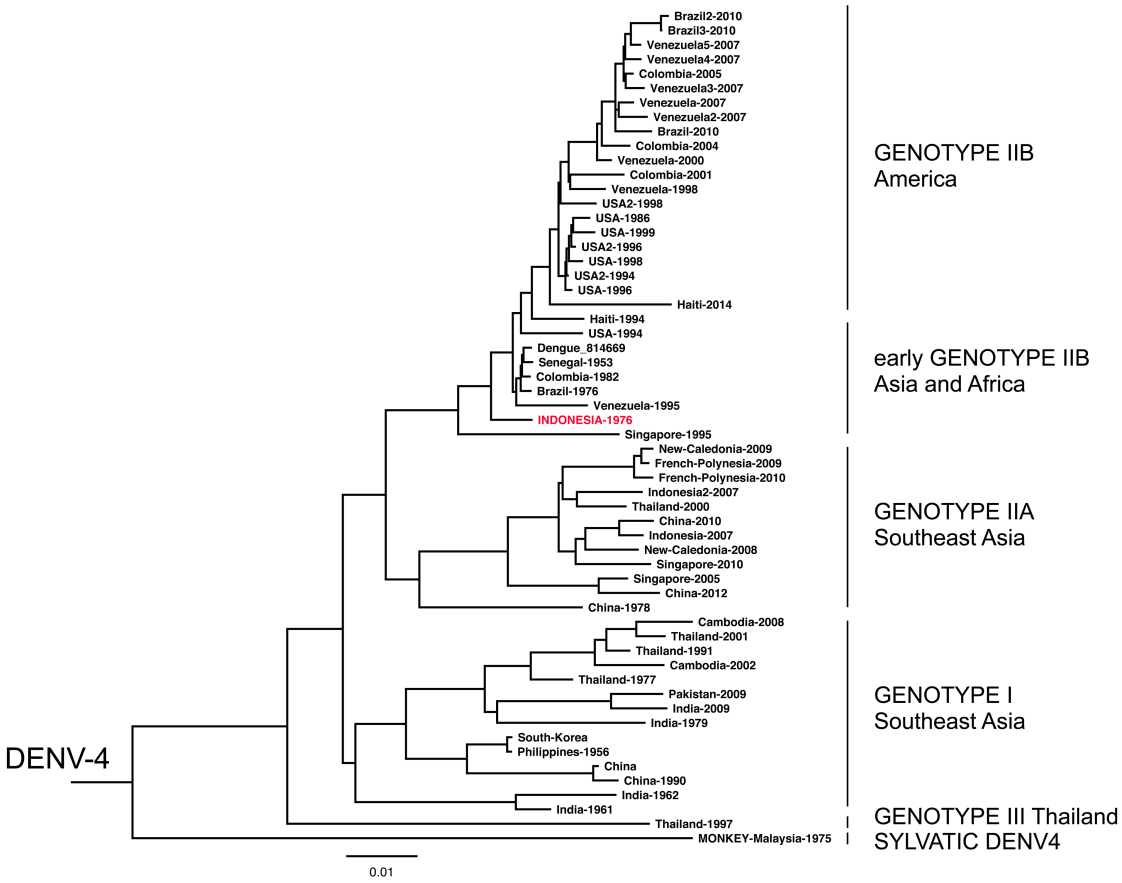
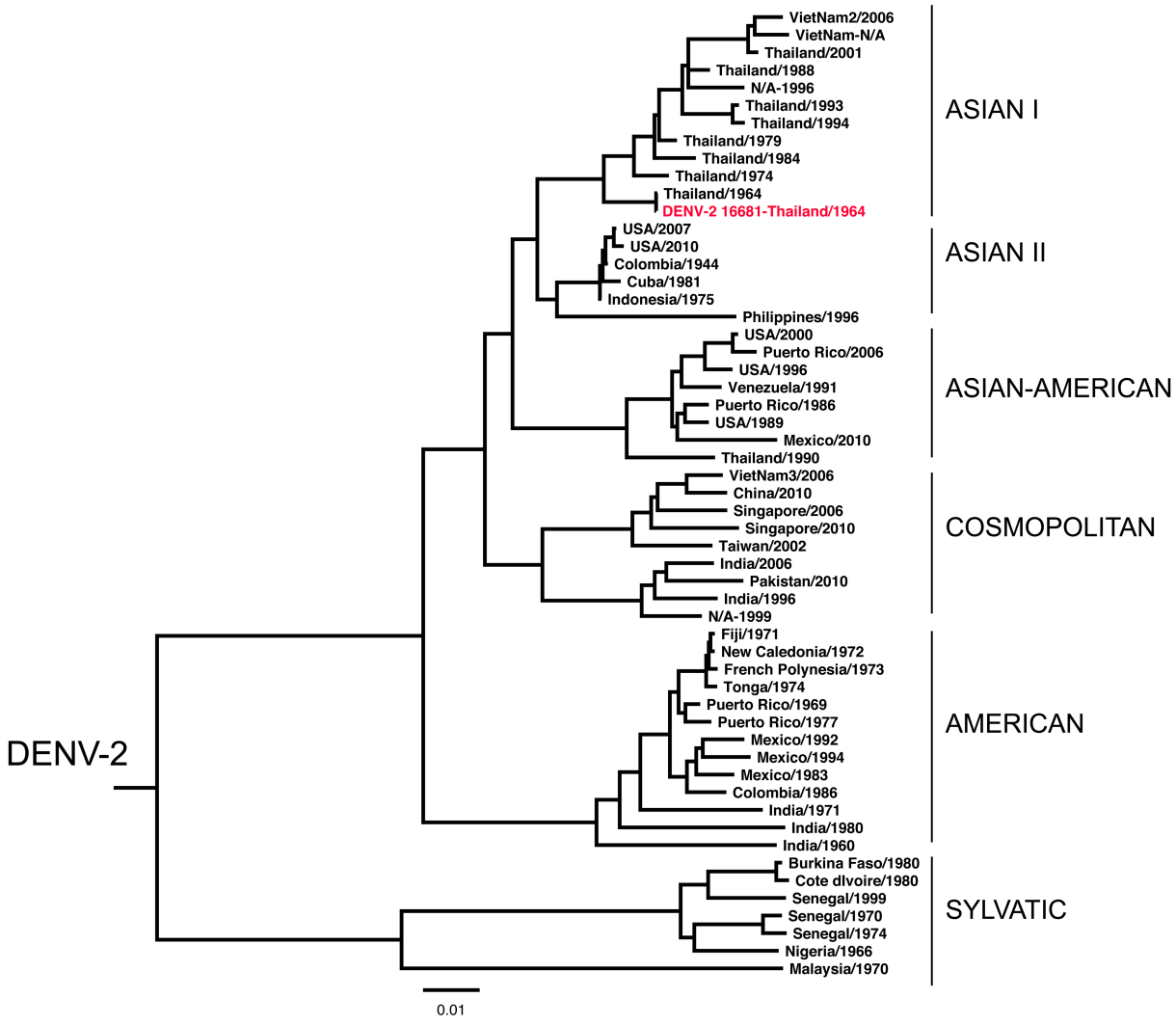
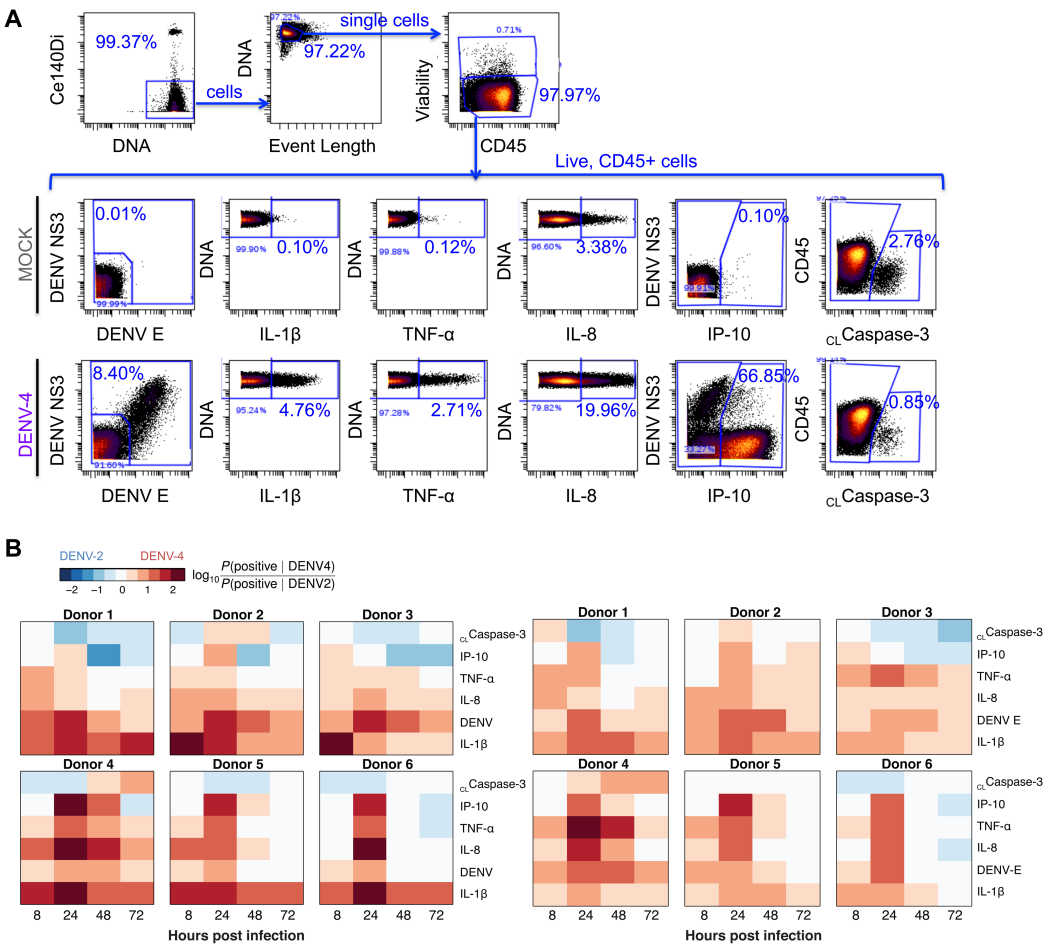


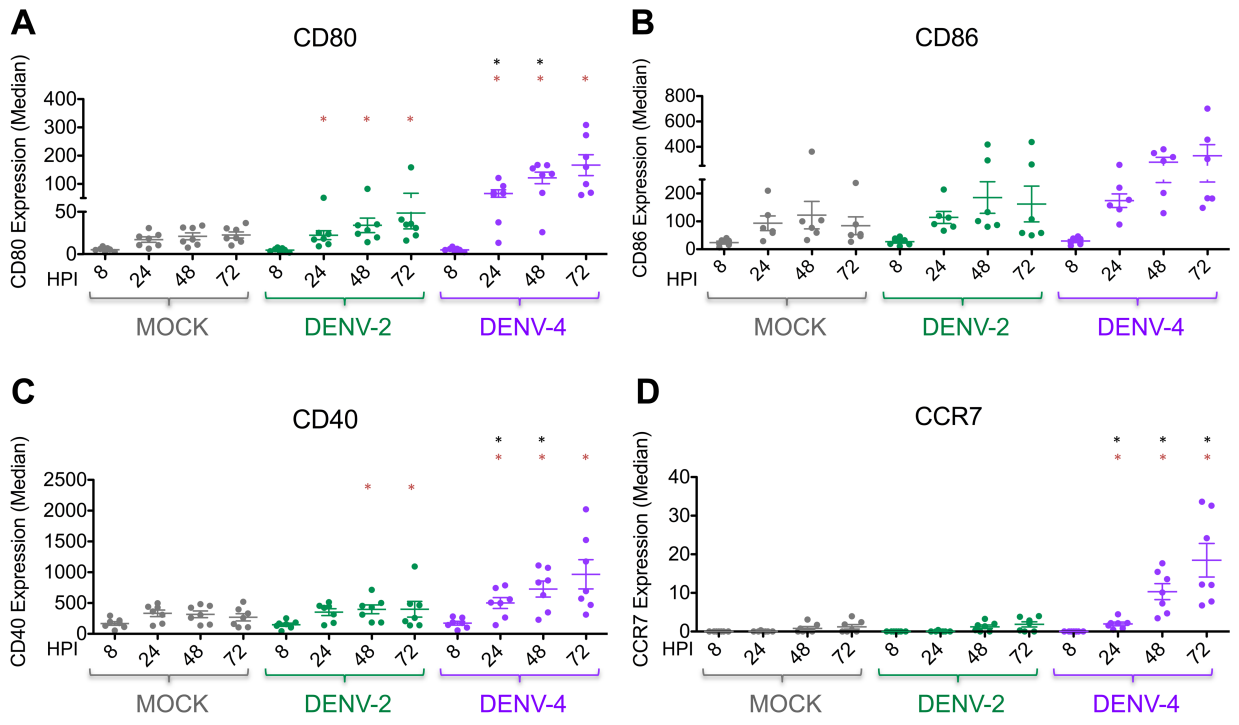
Supplemental Figure 1. Phylogenetic tree of representative genomes of DENV-4. A selection of complete genomes of dengue virus (DENV)-4 was downloaded from the National Institute of Allergy and Infectious Diseases (NIAID) Virus Pathogen Database and Analysis Resource (ViPR) through the web site http://www.viprbrc.org/. The selection was manually trimmed to contain 57 isolates representative of the complete genetic diversity and the sequence of Indonesia 1976 (strain 1036; GeneBank accession number KX812530) was added to the list (highlighted in red). A consensus neighbor-joining phylogenetic tree from 1000 replicates was obtained using the program Geneious (www.geneious.com). The tree was visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and rooted at midpoint for clarity. The scale bar represents genetic distance as nucleotides substitutions per site.



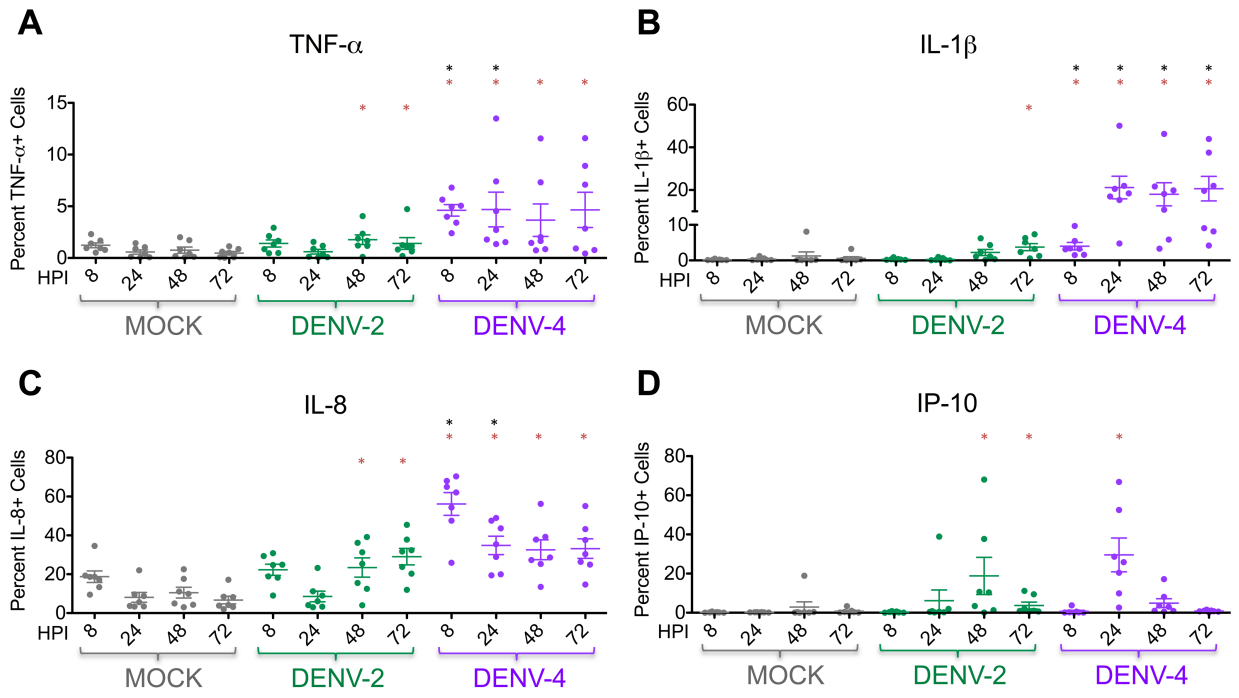
Supplemental Figure 2. Phylogenetic tree of representative genomes of DENV-2. A neighbor-joining phylogenetic tree of 55 dengue virus (DENV)-2 complete genomes was obtained as described for DENV-4 (Supplemental Figure 1). The isolate used in this study is highlighted in red. The scale bar represents genetic distance as nucleotides substitutions per site.



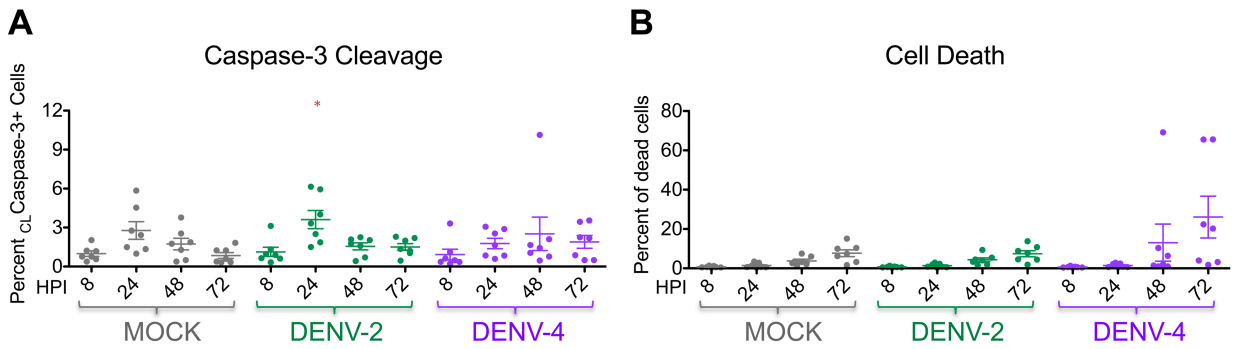
Supplemental Figure 3. CyTOF manual and autogating analyses. (A) Representative manual gating scheme for mass cytometry by time-of-flight (CyTOF) analysis. One representative donor out of seven is shown. Cells were first identified based on DNA content, and residual cell-bead doublets were excluded based on Ce140. Single cells were determined by DNA content and event length. Live, CD45+ cells were determined by surface CD45 staining and exclusion of Rh103+ dead cells. Live, CD45+ cells were then gated for intracellular markers (DENV envelope (E) protein, DENV non-structural 3 (NS3) protein, IL-1β, TNF-α, IL-8, IP-10, and cleaved caspase-3) based on generally negative staining in mock-infected cells at 24 hours post infection (hpi). IP-10 gating took into account some cross-talk with the DENV NS3 channel due to known isotopic impurity. **(B)** Manual gating versus autogating for CyTOF analysis across multiple DC donors. Heat maps comparing marker expression during DENV-2 versus DENV-4 infection of six (out of seven) independent DC donors are shown. Red indicates greater marker expression during DENV-4 infection, blue indicates greater marker expression during DENV-2 infection, and white indicates no difference in marker expression between DENV-2 and DENV-4 infection. These calculations were made using the event counts from CyTOF data as determined by either **(left panel)** manual gating of cells positive for each marker, or by **(right panel)** auto-gating of cells positive for each marker based on the 99th percentile threshold, which was set for mock treatment at 8 hpi. The comparative effect of DENV-4 versus DENV-2 infection on each marker was calculated within each donor as the $\log_{10}$ of the relative risk for positive marker frequency between the two infection conditions.



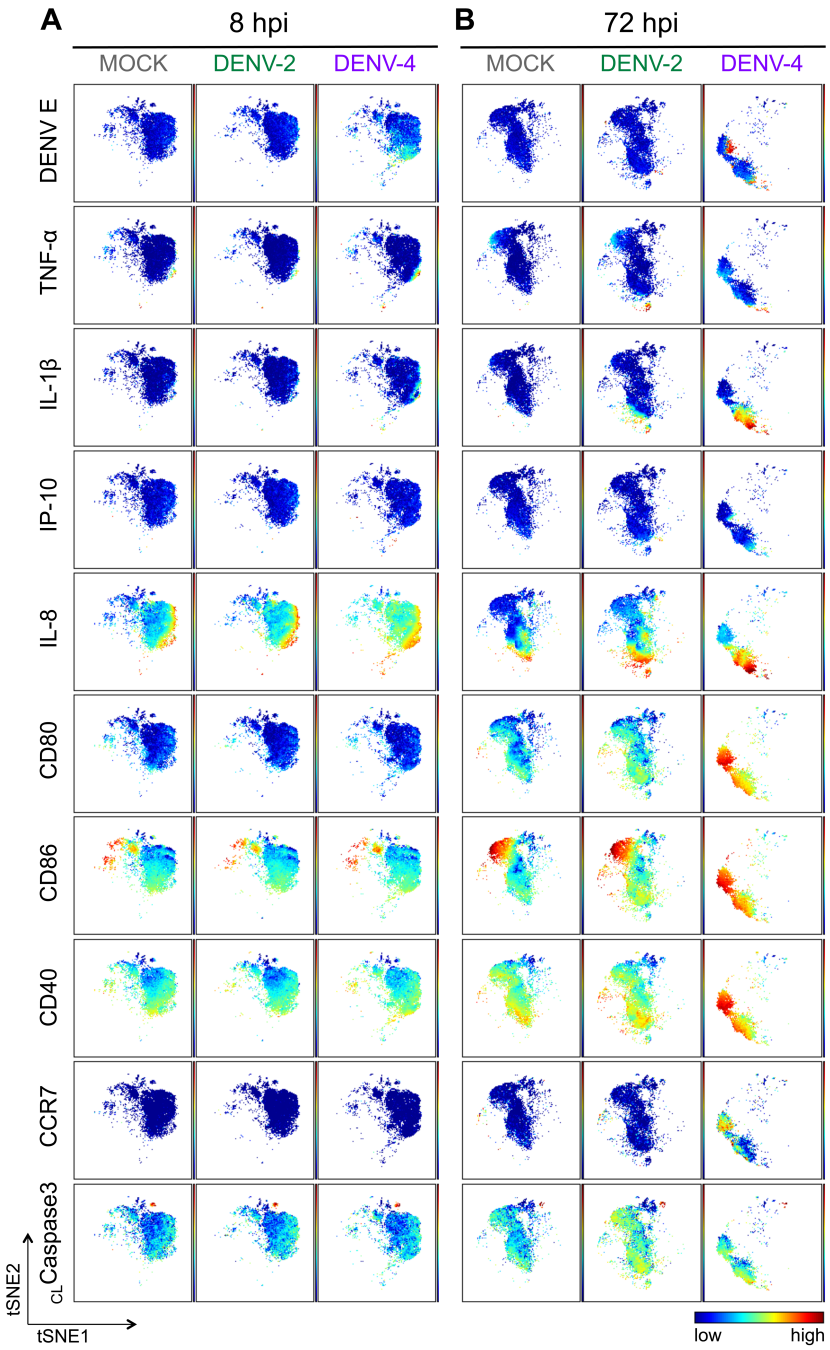
Supplemental Figure 4. DENV-4 infection leads to higher upregulation of DC activation markers compared to DENV-2 infection. Median expression of the DC surface markers **(A)** CD80, **(B)** CD86, **(C)** CD40 and **(D)** CCR7 by mass cytometry by time-of-flight (CyTOF) analysis of mock or dengue virus (DENV)-2- or DENV-4- infected DCs. Cells were gated on live single CD45+ cells, and data from seven DC donors are shown. Mean ± SEM are shown. Black asterisks represent statistical significance by the paired, two-tailed Wilcoxon signed rank test, comparing DENV-4 to DENV-2 at each timepoint. Red asterisks represent statistical significance for either DENV-2 or DENV-4 compared to mock-infected cells at each timepoint. The Benjamini-Hochberg procedure was performed within each time point to adjust the significance level for multiple comparisons (* $p \leq 0.05$).



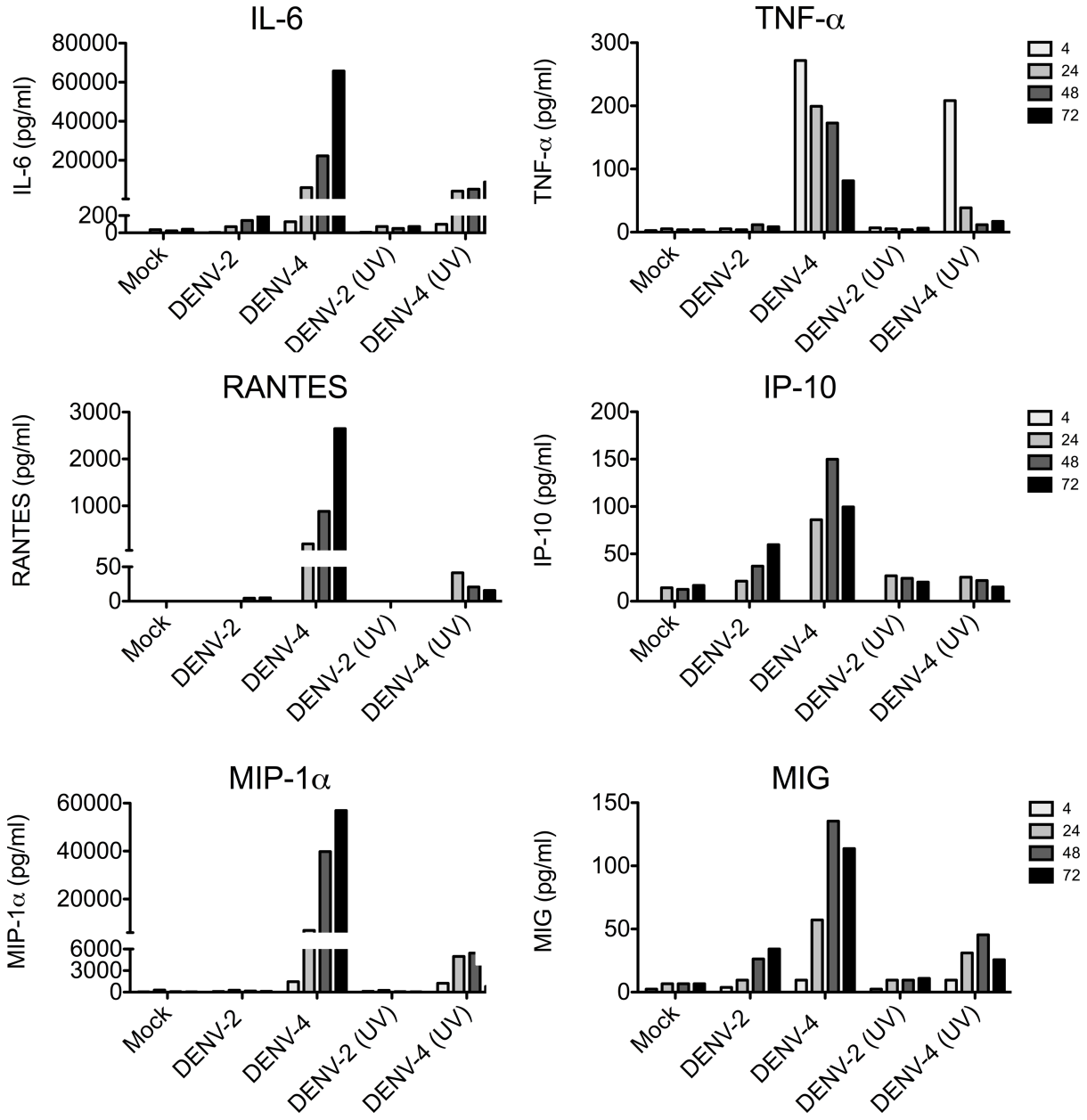
Supplemental Figure 5. DENV-4 infection upregulates intracellular cytokine production compared to DENV-2 infection. The percentage of DCs positive for intracellular cytokine production of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-8 and **(D)** IP-10 by mass cytometry by time-of-flight (CyTOF) analysis of mock, dengue virus (DENV)-2-, or DENV-4-infected DCs. Cells were gated on live single CD45+ cells, and data from seven DC donors are shown. Positive cells were determined by setting a negative threshold on mock-infected cells at 8 hours post infection (hpi) (Supplemental Figure 3A). Mean ± SEM are shown. Black asterisks represent statistical significance by the paired, two-tailed Wilcoxon signed rank test, comparing DENV-4 to DENV-2 at each timepoint. Red asterisks represent statistical significance for either DENV-2 or DENV-4 compared to mock-infected cells at each timepoint. The Benjamini-Hochberg procedure was performed within each time point to adjust the significance level for multiple comparisons (* $p \leq 0.05$).



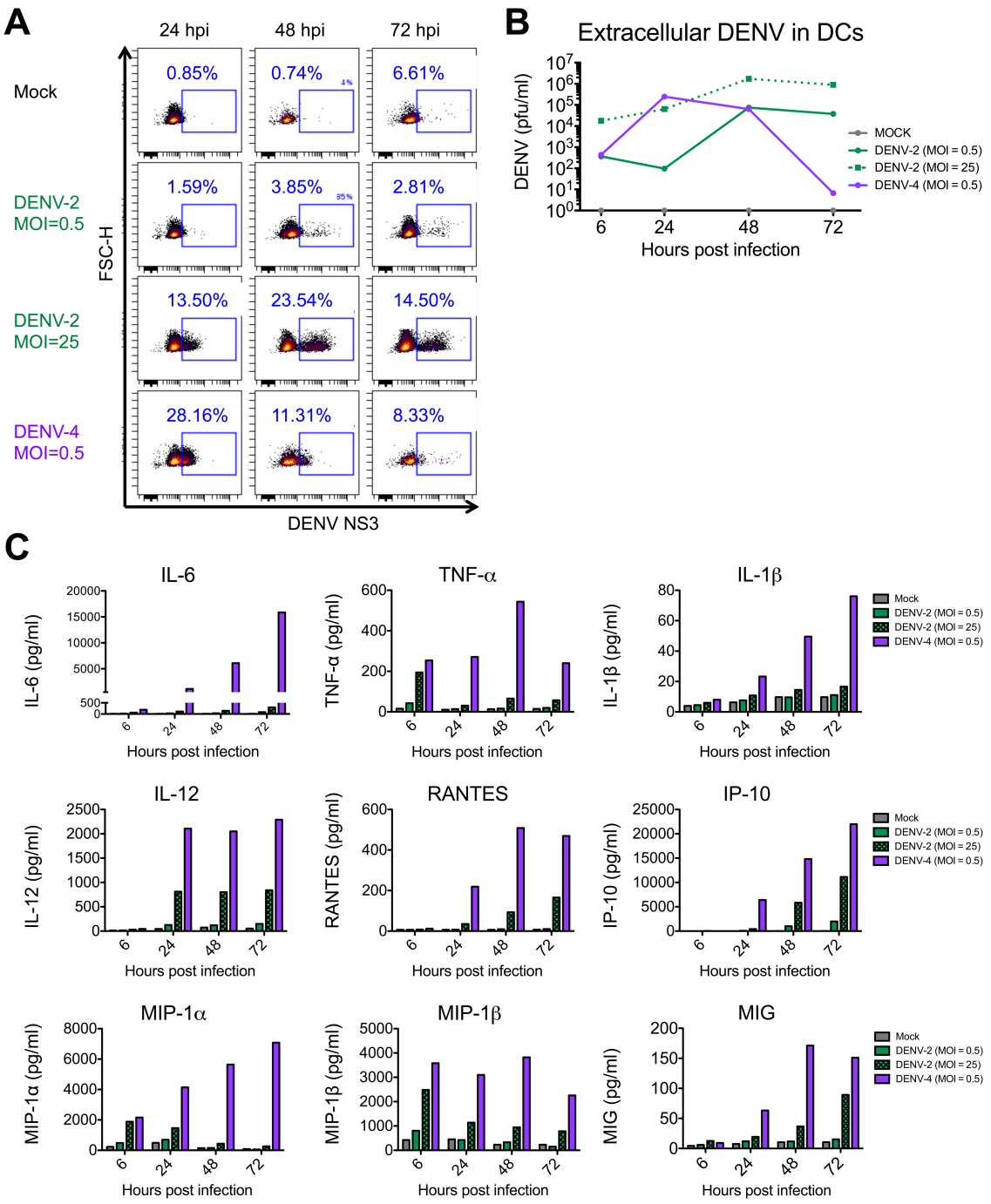
Supplemental Figure 6. Lack of statistical difference in caspase-3 cleavage or cell death during DENV-2 versus DENV-4 infection of DCs. (**A**) The percentage of DCs positive for caspase-3 cleavage by mass cytometry by time-of-flight (CyTOF) analysis of mock, dengue virus (DENV)-2-, or DENV-4-infected. Cells were gated on live single CD45+ cells. Positive cells were determined by setting a negative threshold on mock-infected cells at 8 hours post infection (hpi) (Supplemental Figure 3A). (**B**) The percentage of cell death for the aforementioned DC cultures based on CyTOF viability staining gating (Supplemental Figure 3A). Data from seven DC donors are shown. Mean ± SEM are shown. The red asterisk represents statistical significance for either DENV-2 or DENV-4 compared to mock-infected cells at each timepoint. The Benjamini-Hochberg procedure was performed within each time point to adjust the significance level for multiple comparisons (* $p \leq 0.05$).



Supplemental Figure 7. The phenotypic landscape is different during DENV-2 versus DENV-4 infection in DCs. Live single CD45+ cells from representative donor #2 (Supplemental Figure 3B) out of seven DC donors were used to create viSNE plots, which were generated using all 18 phenotypic markers used for mass cytometry by time-of-flight (CyTOF) staining except markers for dengue virus (DENV). Each point in the plot represents a single cell, and the axes show arbitrary units based on the t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm. The relative position of a cell on the plot represents its general phenotype in multidimensional space. For (**A**) 8 hpi and (**B**) 72 hpi, cells are colored according to staining intensity of the indicated marker, enabling the comparison of expression patterns across markers for different infection conditions and timepoints post infection.



Supplemental Figure 8. The continuous upregulation of cytokine and chemokine secretion is dependent on active DENV replication. DCs were either mock-infected or infected with dengue virus (DENV)-2 or DENV-4 (MOI = 0.5) or with an equivalent number of UV-inactivated DENV-2 or DENV-4 particles. Multiplex ELISA was performed on DC culture supernatants at the indicated timepoints (hours post infection) to measure cytokine and chemokine secretion. One representative donor out of three is shown.



Supplemental Figure 9. Cytokine production is dependent on both DENV strain and MOI. DCs were either mock-infected or infected with dengue virus (DENV)-2 (MOI = 0.5 or MOI = 25) or DENV-4 (MOI = 0.5). **(A)** The percent of live DENV-infected DCs was measured by flow cytometry using an anti-DENV non-structural 3 (NS3) antibody. **(B)** Replication kinetics were determined by plaque assay of infectious DENV particles released into DC culture supernatants. DENV-2 is represented in green, while DENV-4 is in purple. Solid lines indicate MOI = 0.5 while the dotted line indicates DENV-2 at MOI = 25. **(C)** Multiplex ELISA was performed on DC culture supernatants to measure cytokine and chemokine secretion. Data from one donor is shown.

Supplemental Table 1. Mass cytometry by time-of-flight (CyTOF) phenotyping panel.

Isotope	Target	Clone
Surface		
89Y	CD45	HI30
148 Nd	CD16	3G8
153 Eu	CD1c	L161
154 Sm	CD86	IT2.2
159 Tb	CD11c	Bu15
160 Gd	CD14	M5E2
162 Dy	CD80	2D10.4
164 Dy	CD40	5C3
167 Er	CCR7	G043H7
172 Yb	CD209	9E9A8
174 Yb	HLA-DR	L243
209Bi	CD11b	ICRF44
Intracellular		
142 Nd	$_{CL}$Caspase-3	D3E9
147 Sm	IL-1β	H1b-98
152 Sm	TNF-α	MAb11
156 Gd	IL-6	MQ2-13A5
165 Ho	DENV E	D1-4G2-4-15
173 Yb	IL-8	E8N1
175 Lu	DENV NS3	E1D8
176 Yb	IP-10	J034D6
Cell ID		
103 Rh	Viability	
191/193 Ir	DNA	
102-110 Pd	Barcoding	