Figure S1

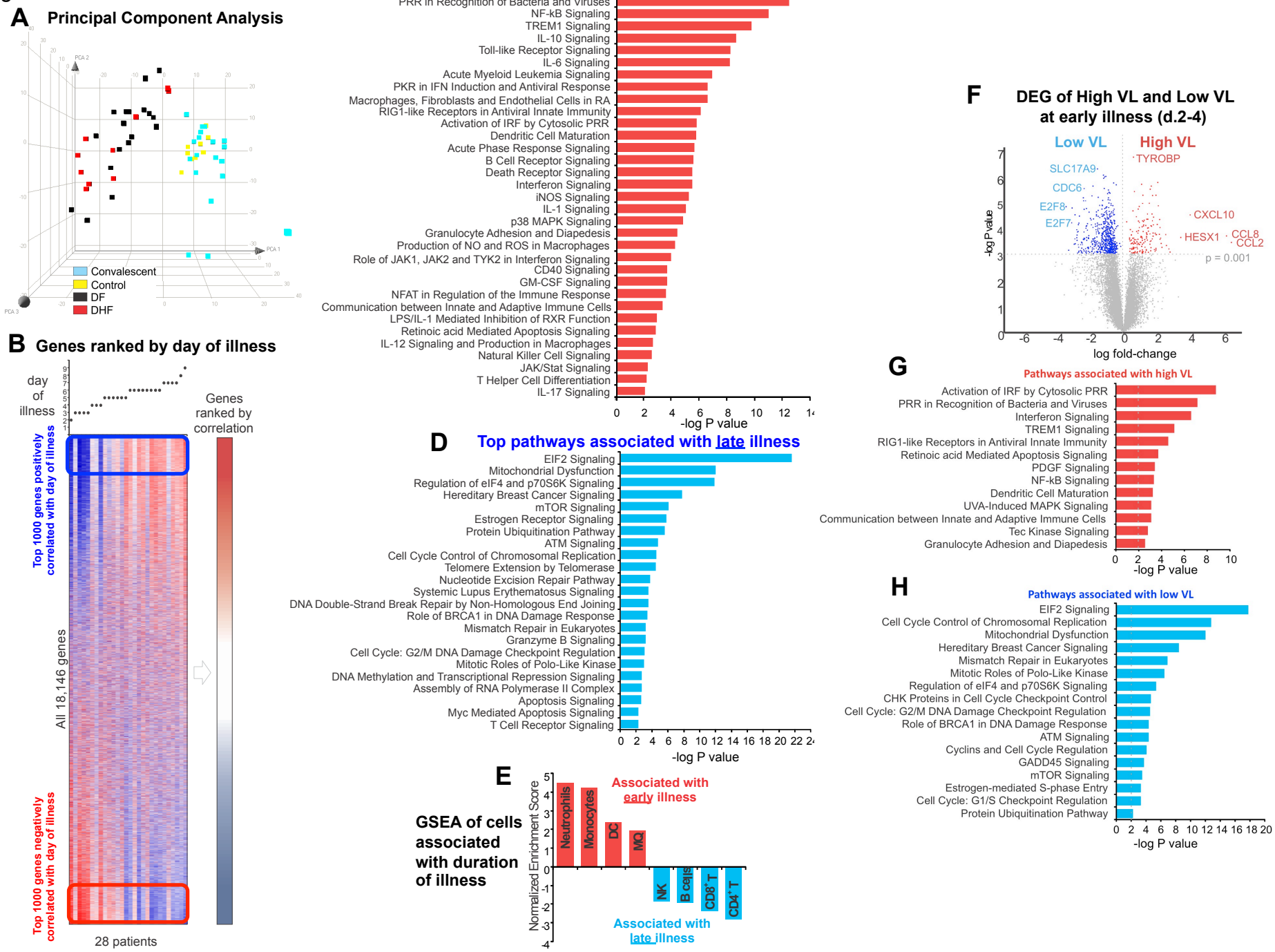
A
Principal Component Analysis
PCA 1
PCA 2
PCA 3
Convalescent
Control
DF
DHF
B
Genes ranked by day of illness
day of illness
Genes ranked by correlation
Top 1000 genes positively correlated with day of illness
All 18,146 genes
Top 1000 genes negatively correlated with day of illness
28 patients
C
Top pathways associated with early illness
PRR in Recognition of Bacteria and Viruses
NF-kB Signaling
TREM1 Signaling
IL-10 Signaling
Toll-like Receptor Signaling
IL-6 Signaling
Acute Myeloid Leukemia Signaling
PKR in IFN Induction and Antiviral Response
Macrophages, Fibroblasts and Endothelial Cells in RA
RIG1-like Receptors in Antiviral Innate Immunity
Activation of IRF by Cytosolic PRR
Dendritic Cell Maturation
Acute Phase Response Signaling
B Cell Receptor Signaling
Death Receptor Signaling
Interferon Signaling
iNOS Signaling
IL-1 Signaling
p38 MAPK Signaling
Granulocyte Adhesion and Diapedesis
Production of NO and ROS in Macrophages
Role of JAK1, JAK2 and TYK2 in Interferon Signaling
CD40 Signaling
GM-CSF Signaling
NFAT in Regulation of the Immune Response
Communication between Innate and Adaptive Immune Cells
LPS/IL-1 Mediated Inhibition of RXR Function
Retinoic acid Mediated Apoptosis Signaling
IL-12 Signaling and Production in Macrophages
Natural Killer Cell Signaling
JAK/Stat Signaling
T Helper Cell Differentiation
IL-17 Signaling
-log P value
D
Top pathways associated with late illness
EIF2 Signaling
Mitochondrial Dysfunction
Regulation of eIF4 and p70S6K Signaling
Hereditary Breast Cancer Signaling
mTOR Signaling
Estrogen Receptor Signaling
Protein Ubiquitination Pathway
ATM Signaling
Cell Cycle Control of Chromosomal Replication
Telomere Extension by Telomerase
Nucleotide Excision Repair Pathway
Systemic Lupus Erythematosus Signaling
DNA Double-Strand Break Repair by Non-Homologous End Joining
Role of BRCA1 in DNA Damage Response
Mismatch Repair in Eukaryotes
Granzyme B Signaling
Cell Cycle: G2/M DNA Damage Checkpoint Regulation
Mitotic Roles of Polo-Like Kinase
DNA Methylation and Transcriptional Repression Signaling
Assembly of RNA Polymerase II Complex
Apoptosis Signaling
Myc Mediated Apoptosis Signaling
T Cell Receptor Signaling
-log P value
E
GSEA of cells associated with duration of illness
Normalized Enrichment Score
Associated with early illness
Associated with late illness
Neutrophils
Monocytes
DC
MΦ
NK
B cells
CD8+ T
CD4+ T
F
DEG of High VL and Low VL at early illness (d.2-4)
Low VL
High VL
SLC17A9
CDC6
E2F8
E2F7
TYROBP
CXCL10
HESX1
CCL8
CCL2
p = 0.001
-log P value
log fold-change
G
Pathways associated with high VL
Activation of IRF by Cytosolic PRR
PRR in Recognition of Bacteria and Viruses
Interferon Signaling
TREM1 Signaling
RIG1-like Receptors in Antiviral Innate Immunity
Retinoic acid Mediated Apoptosis Signaling
PDGF Signaling
NF-kB Signaling
Dendritic Cell Maturation
UVA-Induced MAPK Signaling
Communication between Innate and Adaptive Immune Cells
Tec Kinase Signaling
Granulocyte Adhesion and Diapedesis
-log P value
H
Pathways associated with low VL
EIF2 Signaling
Cell Cycle Control of Chromosomal Replication
Mitochondrial Dysfunction
Hereditary Breast Cancer Signaling
Mismatch Repair in Eukaryotes
Mitotic Roles of Polo-Like Kinase
Regulation of eIF4 and p70S6K Signaling
CHK Proteins in Cell Cycle Checkpoint Control
Cell Cycle: G2/M DNA Damage Checkpoint Regulation
Role of BRCA1 in DNA Damage Response
ATM Signaling
Cyclins and Cell Cycle Regulation
GADD45 Signaling
mTOR Signaling
Estrogen-mediated S-phase Entry
Cell Cycle: G1/S Checkpoint Regulation
Protein Ubiquitination Pathway
-log P value

Figure S1, Related to Figures 1 and 2. Transcriptomic analysis identifies molecular signatures that correlate with duration of symptomatic disease. (A) Unsupervised Principal Component Analysis (PCA) was used to assess clustering of samples (transcriptional profiles) according to their expression data. Expression data from 7,258 genes (top 40% genes with highest variance across all samples) were used for this analysis. (B) Heat map of probe sets (rows) and subjects (columns) ranked by the day of illness reported at specimen collection (top), colors in map indicate relative gene expression. Right margin demonstrates probe sets with negative (blue) or positive correlation (red) to day of illness. $P < 0.05$ (Pearson). Brackets visualize an approximate range of top 1000 genes positively (red) or negatively (blue) correlated to day of illness used for analysis in C-D. (C and D) Top Ingenuity pathways that negatively (C, early illness) or positively (D, late illness) correlate with day of illness. Dotted line represents a cut-off value (–log p=2). (E) GSEA analysis of the whole blood transcriptome in blood of the acute dengue patients (DF and DHF) using cell-specific data sets. Normalized enrichment score (NES) indicates correlation of distinct cell-specific gene sets to day of illness. MQ, macrophages; DC, dendritic cells; NK, natural killer. (F-H) Analysis of DEGs in dengue patients with high VL (n=5) and low VL (n=3) enrolled in the study at the reported days 2-4 of symptomatic illness. (F) Volcano plot showing genes differentially expressed between dengue patients with high viral load (red) and low viral load (blue) at early days of illness (days 2-4). The - log10 P-value of a student's t-test and the log2 fold-change between high VL and low VL patients are represented in the y-axis and x-axis, respectively. (G, H) Ingenuity Pathway analysis of the top pathways that positively (G) or negatively (H) correlated to VL in patients enrolled at early (d. 2-4) days of illness.

Figure S2

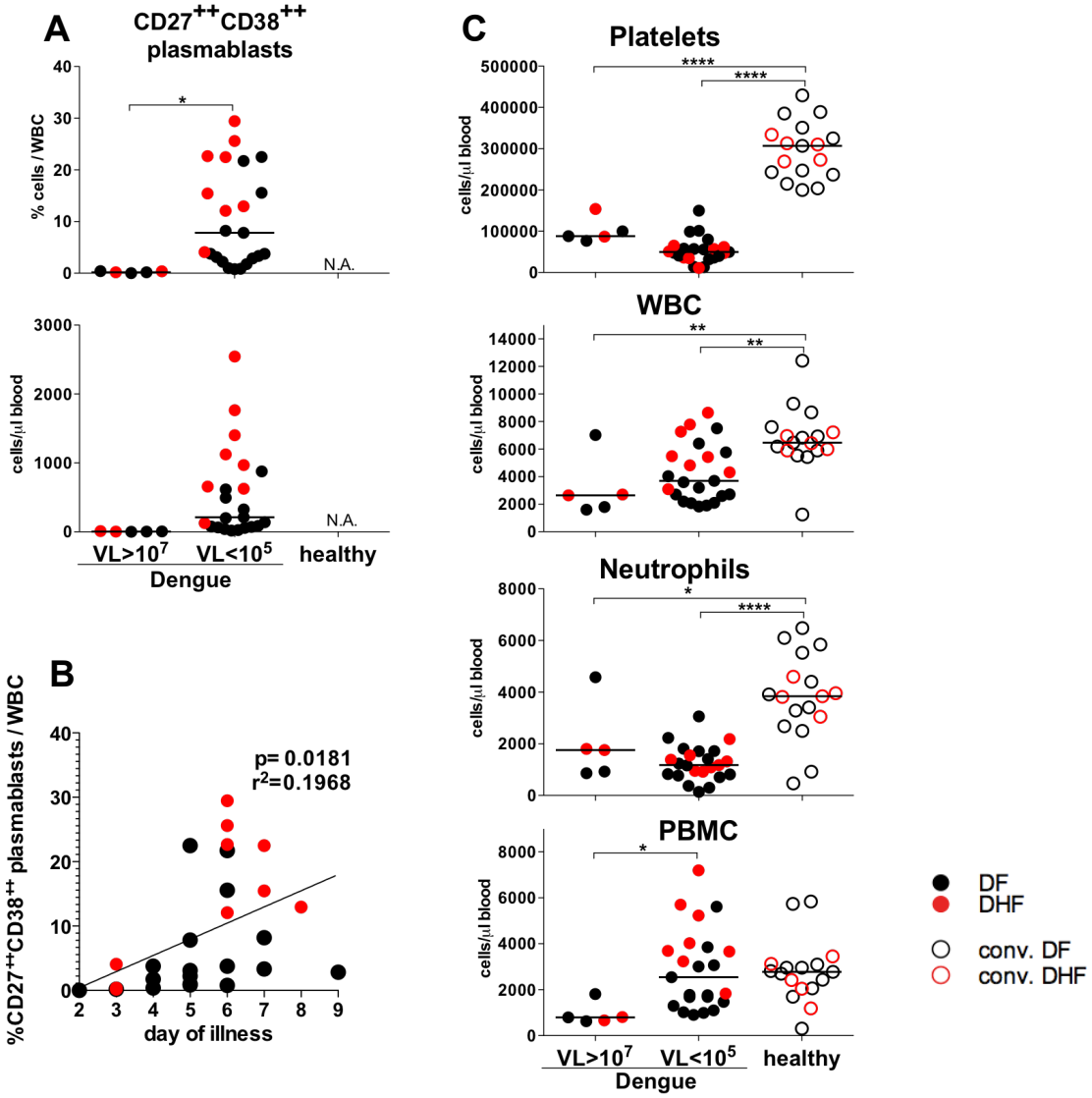
A
CD27++CD38++ plasmablasts
% cells / WBC
cells/µl blood
N.A.
VL>10^7
VL<10^5
healthy
Dengue
B
%CD27++CD38++ plasmablasts / WBC
p= 0.0181
r2=0.1968
day of illness
C
Platelets
WBC
Neutrophils
PBMC
DF
DHF
conv. DF
conv. DHF

Figure S2, Related to Figures 2 and 3. Blood cell populations in dengue patients. (A) Proportion in WBC and absolute counts of $CD20^{-}CD19^{dim}CD27^{++}CD38^{++}$ plasmablast B cells as measured by flow cytometry in fresh blood specimens in dengue patients. $CD27^{++}CD38^{++}$ plasmablast B cells were not assessed in healthy individuals. N.A., not available. (B) Correlation analysis of $CD27^{++}CD38^{++}$ plasmablast B cells with a duration of acute dengue disease at the specimen collection represented as day of illness. P-value was calculated for correlation efficiency and was considered significant at p=0.05. Spearman's rank correlation coefficient values are indicated (r^2). Diagonal line shows linear regression. (C) Absolute counts of platelets, white blood cells (WBC), neutrophils and peripheral blood mononuclear cells (PBMC: lymphocytes and monocytes) were measured by CBC analysis in whole blood specimens. (A and C) represent comparison analysis between high VL ($VL>10^7$, n=5), low VL ($VL<10^5$, n=23) and healthy individuals (convalescent, n=19). Symbols represent: acute dengue (DF black dots, DHF red dots) and healthy convalescent (DF black circles, DHF red circles). ANOVA with Bonferroni, SEM, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S3

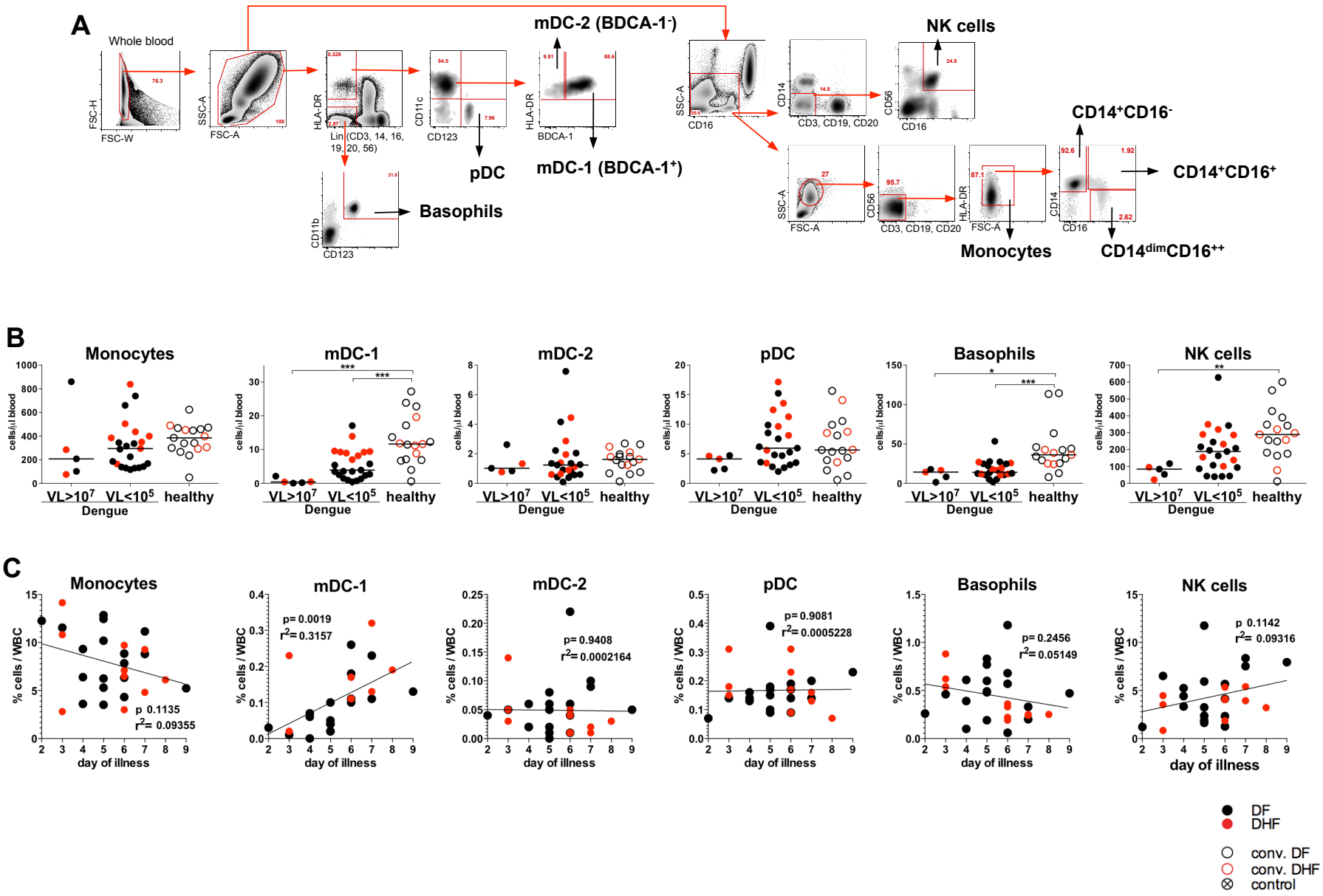
A
Whole blood
FSC-H
FSC-W
SSC-A
FSC-A
HLA-DR
Lin (CD3, 14, 16, 19, 20, 56)
CD11c
CD123
pDC
mDC-2 (BDCA-1-)
BDCA-1
mDC-1 (BDCA-1+)
CD11b
Basophils
SSC-A
CD16
CD14
CD3, CD19, CD20
NK cells
CD56
CD14+CD16-
CD14+CD16+
CD14dimCD16++
Monocytes
B
Monocytes
mDC-1
mDC-2
pDC
Basophils
NK cells
cells/µl blood
VL>10^7
VL<10^5
healthy
Dengue
C
% cells / WBC
day of illness
p 0.1135
r2= 0.09355
p= 0.0019
r2= 0.3157
p= 0.9408
r2= 0.0002164
p= 0.9081
r2= 0.0005228
p= 0.2456
r2= 0.05149
p 0.1142
r2= 0.09316
DF
DHF
conv. DF
conv. DHF
control

Figure S3, Related to Figure 3. Innate cells subsets in blood of dengue patients. (A) Cell-gating strategy for flow cytometry analysis of whole blood specimens. All cells were identified within the singlets gate (FSC-H/FSC-W). Myeloid DCs (mDC) were gated within the Lineage (Lin.: CD3, CD14, CD16, CD19, CD20, CD56)^{neg}, HLA-DR^{+} CD11c^{+} cells as BDCA-1^{+} mDC-1 and BDCA-1^{-} mDC-2. Plasmacytoid DCs (pDC) were identified as Lin^{neg} HLA-DR^{+} CD123^{+} cells, and basophils as Lin^{neg} HLA-DR^{-} CD123^{+}CD11b^{+} cells. Monocytes were identified within the SSC-A^{hi} FSC-A^{hi} cells as the CD3^{-}CD19^{-}CD20^{-}CD56^{-} HLA-DR^{+} population and gated for the CD14^{+}CD16^{-}, CD14^{+}CD16^{+}, and CD14^{dim}CD16^{++} subsets. NK cells were identified as the CD3^{-}CD19^{-}CD20^{-}CD14^{-} as a CD56^{+}CD16^{+} double positive population. (B) Absolute counts and (C) % of WBC of monocytes, mDC-1, mDC-2, pDC, NK cells and basophils in whole blood specimens. In (B) numbers of specific cell subsets were compared between groups of dengue patients with high VL (VL>10^{7}, n=5), low VL (VL<10^{5}, n=23), and healthy (convalescent, n=19, controls n=9). (C) Correlation analysis of proportions of distinct cell subsets with duration of acute dengue at the time of specimen collection (day of illness). Symbols represent: acute dengue (DF black dots, DHF red dots), convalescent individuals (DF black circles, DHF red circles) and controls (crossed circles). ANOVA with Bonferroni, SEM, *p<0.05, **p<0.01, ***p<0.001.

Figure S4

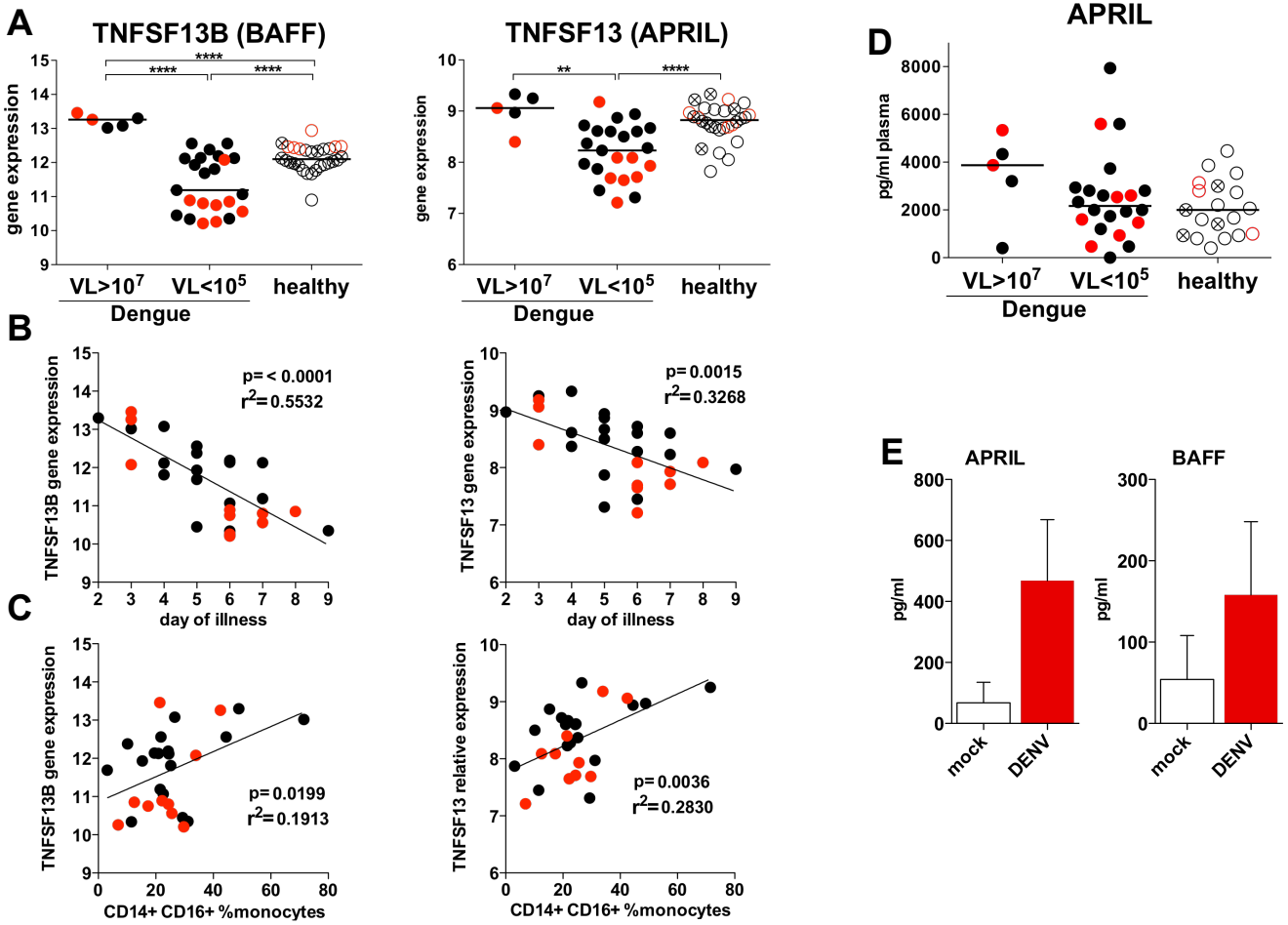
A
TNFSF13B (BAFF)
gene expression
VL>10^7
VL<10^5
healthy
Dengue
TNFSF13 (APRIL)
B
TNFSF13B gene expression
p= < 0.0001
r^2=0.5532
day of illness
TNFSF13 gene expression
p= 0.0015
r^2=0.3268
C
p= 0.0199
r^2=0.1913
CD14+ CD16+ %monocytes
TNFSF13 relative expression
p= 0.0036
r^2=0.2830
D
APRIL
pg/ml plasma
E
APRIL
BAFF
pg/ml
mock
DENV

Figure S4, Related to Figure 5. BAFF and APRIL in blood of dengue patients and infected monocytes. (A-C) Expression of BAFF (TNFSF13B, left) and APRIL (TNFSF13, right) in dengue patients was measured by gene array in RNA isolated from whole blood. In (A) relative genes expression were compared between groups of dengue patients with high VL ($VL>10^7$, n=5), low VL ($VL<10^5$, n=23), and healthy (convalescent, n=19 and controls n=9). Correlation analysis of gene expression with (B) duration of acute dengue disease at the specimen collection represented as day of illness and (C) proportion of the CD14+CD16+ monocytes in blood. P-values were calculated for correlation efficiency and were considered significant at p=0.05. Spearman's rank correlation coefficient values are indicated (r^2). Diagonal lines represent linear regression. (D) APRIL protein was measured by ELISA in plasma of dengue patients with high VL ($VL>10^7$, n=5), low VL ($VL<10^5$, n=22), and healthy (convalescent, n=15 and controls n=4). (E) BAFF and APRIL were measured in supernatants of monocytes cultured in medium or with DENV-2 (MOI=1) for 48 hr, representative data from a single experiment are shown. For (A) and (D), ANOVA with Bonferroni, SEM; for (E) T-test, +/- SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Symbols represent: acute dengue (DF black dots, DHF red dots), convalescent individuals (DF black circles, DHF red circles) and controls (crossed circles).

Figure S5

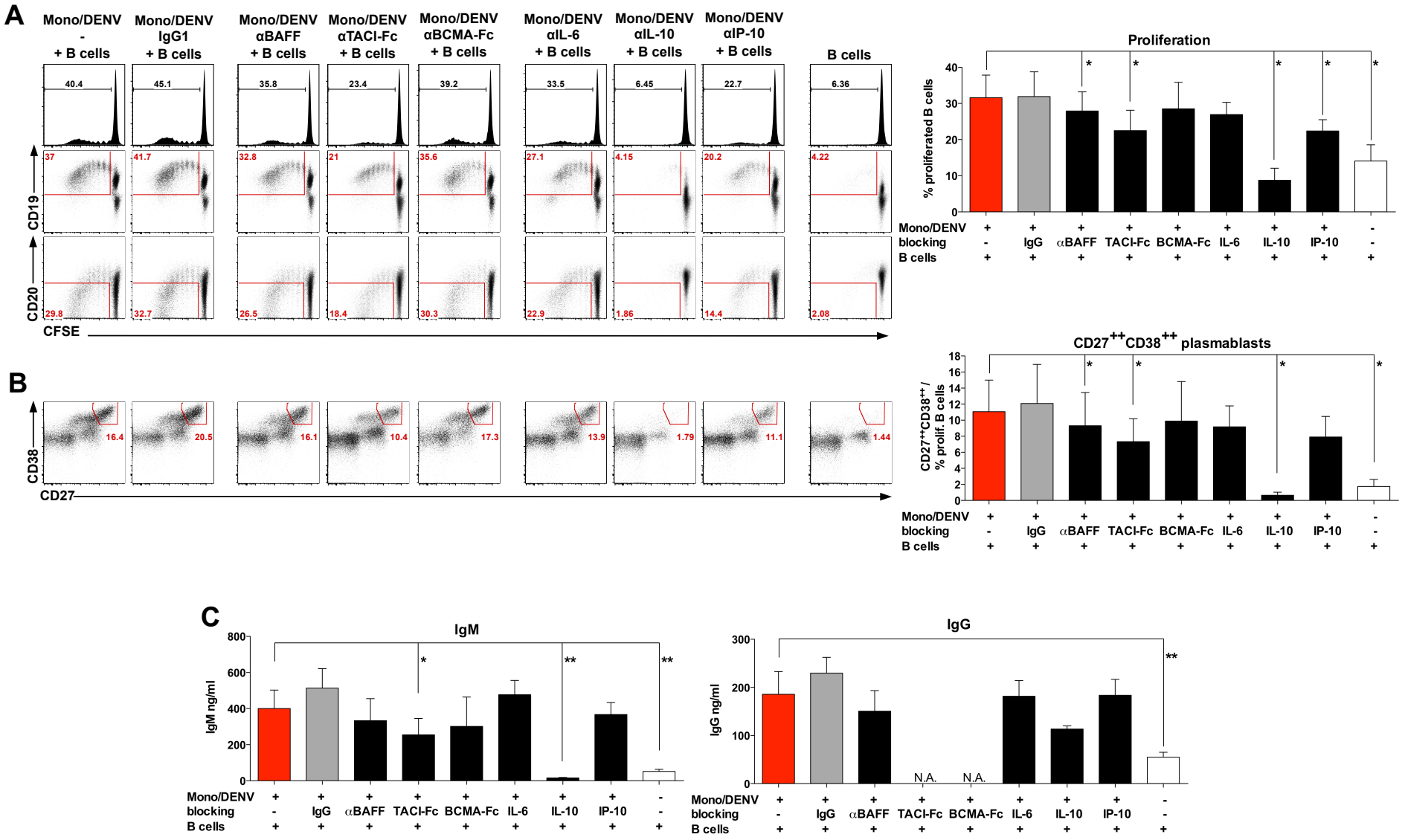
A
Mono/DENV - + B cells
Mono/DENV IgG1 + B cells
Mono/DENV αBAFF + B cells
Mono/DENV αTACI-Fc + B cells
Mono/DENV αBCMA-Fc + B cells
Mono/DENV αIL-6 + B cells
Mono/DENV αIL-10 + B cells
Mono/DENV αIP-10 + B cells
B cells
40.4
45.1
35.8
23.4
39.2
33.5
6.45
22.7
6.36
37
41.7
32.8
21
35.6
27.1
4.15
20.2
4.22
29.8
32.7
26.5
18.4
30.3
22.9
1.86
14.4
2.08
CD19
CD20
CFSE
Proliferation
% proliferated B cells
Mono/DENV
blocking
B cells
IgG
αBAFF
TACI-Fc
BCMA-Fc
IL-6
IL-10
IP-10
B
16.4
20.5
16.1
10.4
17.3
13.9
1.79
11.1
1.44
CD38
CD27
CD27++CD38++ plasmablasts
CD27++CD38++ / % prolif. B cells
C
IgM
IgM ng/ml
IgG
IgG ng/ml
N.A.
N.A.

Figure S5, Related to Figure 5. APRIL and IL-10 contributes to $CD27^{++}CD38^{++}$ plasmablast differentiation and IgM production by DENV-infected monocytes in vitro. Monocytes were infected with DENV-2, MOI=1 for 48 hr and subsequently co-cultured with allogenic, CFSE-labeled $CD19^{+}$ B cells for 6 d in the presence of 50 nM CpG and 20 U/ml IL-2 and with one of the following antibodies: control IgG1, α-BAFF, TACI-Fc or BCMA-Fc, α-IL-6, α-IL-10 and α-IP-10. (A) Histograms (top) and dot blots show B cell proliferation measured by CFSE dilution. Expression of CD19 (middle) or CD20 (bottom) in cultured B cells after 6d, numbers in gates indicate % of proliferated cells. Bar-graph indicates proportion of proliferated B cells measured by CSFE dilution, mean of 4 individual experiments. (B) Plasmablast differentiation was measured by surface expression of CD38 and CD27 within total B cells after 6 d culture. Bar-graphs indicate frequency of $CD27^{++}CD38^{++}$ plasmablasts within total B cells, mean of 4 individual experiments. (C) Total IgM and IgG were assayed in cultures by ELISA on day 6. (A-C) Mean data are shown from 4 independent tests with 8 different blood donors. T-test, SEM, *$p<0.05$, **$p<0.01$, ***$p<0.001$. N.A. not available.

Figure S6

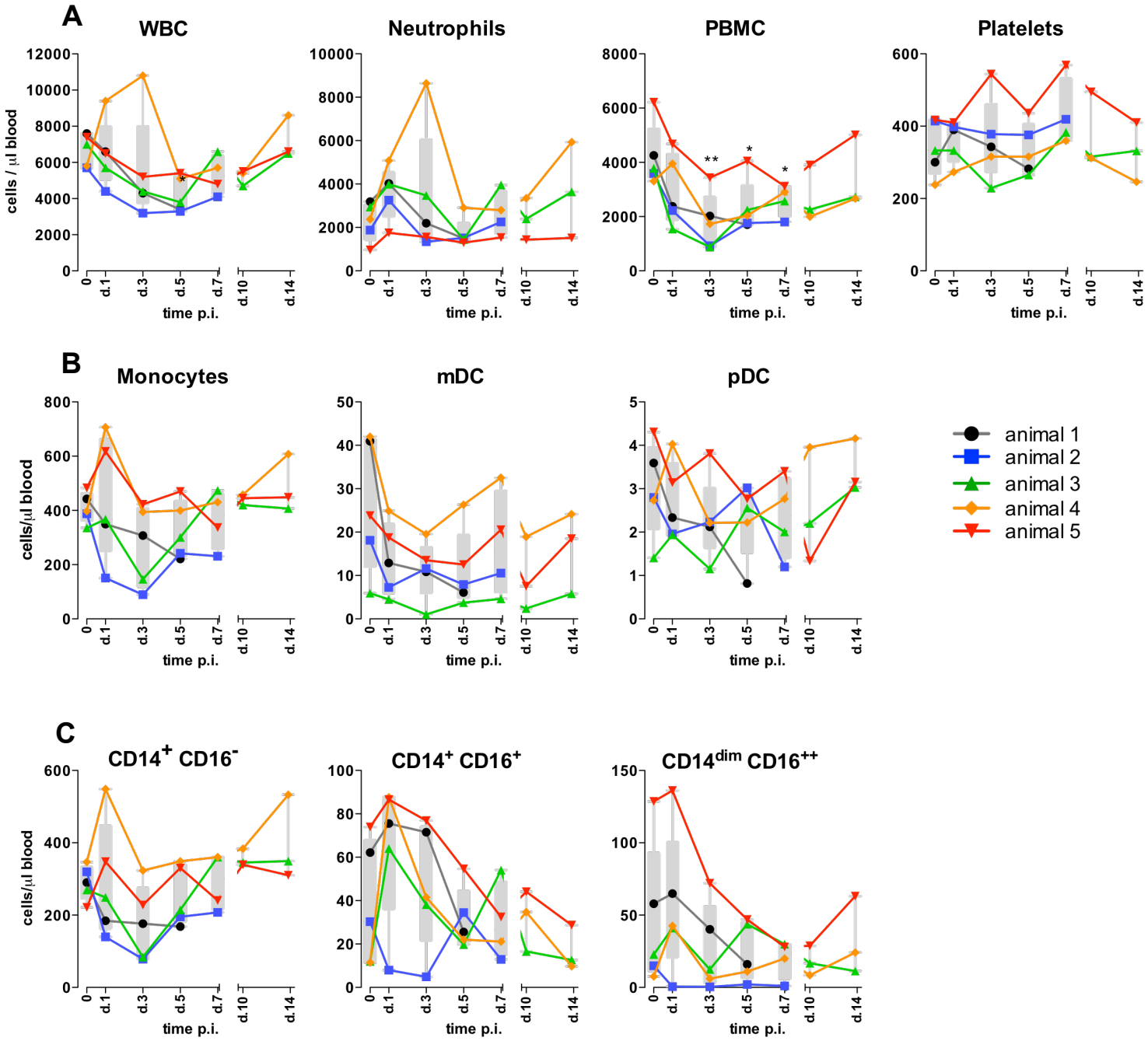
A
WBC
Neutrophils
PBMC
Platelets
cells / µl blood
time p.i.
B
Monocytes
mDC
pDC
cells/µl blood
C
CD14+ CD16-
CD14+ CD16+
CD14dim CD16++
animal 1
animal 2
animal 3
animal 4
animal 5

Figure S6, Related to Figure 6. Absolute cell counts after DENV infection in rhesus macaques. (A) Absolute counts of white blood cells (WBC), neutrophils, peripheral blood mononuclear cells (PBMC, lymphocytes and monocytes) and platelets in whole blood specimens. (B and C) Cell populations in NHP blood were assessed by flow cytometry. Total monocytes were identified within the SSC-A^{hi} FSC-A^{hi} PBMCs as the $CD3^{neg}CD8^{neg}CD20^{neg}$ HLA-DR^{+} population. Myeloid DCs (mDC) were identified as the (Lin.: CD3, CD8, CD20, CD14, $CD16)^{neg}$, HLA-DR^{+} $CD11c^{+}$ BDCA-1^{+} cells, and plasmacytoid DCs (pDC) as Lin^{neg} HLA-DR^{+} $CD123^{+}$ cells. (B) Kinetics of absolute counts of monocytes, mDC and pDC. (C) Kinetics of absolute counts of the $CD14^{+}CD16^{-}$, $CD14^{+}CD16^{+}$ and $CD14^{dim}CD16^{++}$ cell populations. (A-C) Color coded symbols represent individual animals in the cohort. Statistical analysis shows significant change relevant to the baseline, mean ± SEM, t-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$. Gray box bars show values between the first and the 99th percentile, whiskers indicate min and max.

Figure S7

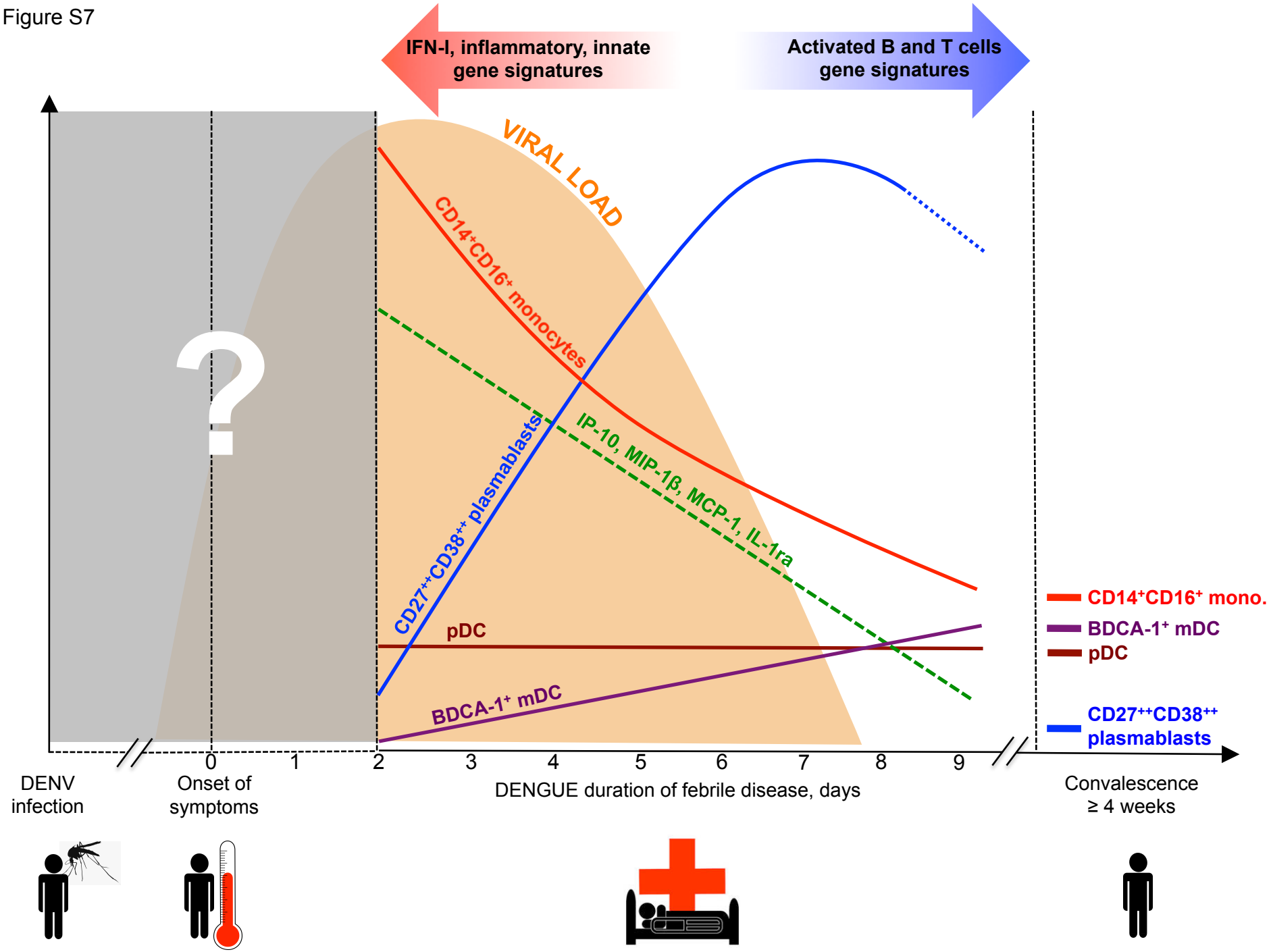
IFN-I, inflammatory, innate gene signatures
Activated B and T cells gene signatures
VIRAL LOAD
CD14+CD16+ monocytes
IP-10, MIP-1β, MCP-1, IL-1ra
CD27++CD38++ plasmablasts
pDC
BDCA-1+ mDC
?
0
1
2
3
4
5
6
7
8
9
DENV infection
Onset of symptoms
DENGUE duration of febrile disease, days
Convalescence ≥ 4 weeks
CD14+CD16+ mono.
BDCA-1+ mDC
pDC
CD27++CD38++ plasmablasts

Figure S7. Summary Figure. Systems biological analysis of the innate immune responses to dengue infection in humans. Early symptomatic dengue (days 2-4 of febrile disease) correlates with high levels of dengue virus (DENV) and expression of genes encoding the pro-inflammatory mediators and type I IFN related proteins in blood. In the late phase of acute illness (days 5-8 of febrile disease), transcriptome analysis reveals genes associated with activated T and B cells. Flow cytometric analysis reveals an expansion in the numbers of $CD14^{+}CD16^{+}$ monocytes and depletion of BDCA-1^{+} mDC, that inversely correlates with the increase in the $CD27^{++}CD38^{++}$ plasmablasts B cells. The early symptomatic dengue is associated with the increase in the plasma pro-inflammatory cytokines.

SUPPLEMENTAL TABLES

Table S1, Related to Figure 1. Clinical characteristics of the study cohort including acute dengue patients (days 2-9 of acute illness), convalescent patients (≥ 4weeks after release) and uninfected controls.

Dengue patients	**Acute (day 2-9 of reported febrile illness)**	**Convalescent (≥ 4 weeks after release)**	**Controls**
Total, n	28	19	9
Age group, years, n (%):			
Infants, 0-2	2 (8)	1 (5)	0 (0)
Children, 3-15	5 (18)	4 (21)	0 (0)
Adult, 16-50	21 (75)	14 (74)	9 (100)
Gender, n (%):			
male	20 (72)	12 (63)	2 (22)
female	8 (28)	7 (37)	7 (78)
Clinical severity, n (%):			
DF	18 (64)	13 (68)	-
DHF-I	7 (25)	5 (26)	-
DHF-II	3 (11)	1 (5)	-
DHF-III	0 (0)	0 (0)	-
DHF-IV (DSS)	0 (0)	0 (0)	-
DENV serotype, n (%):			
DENV-1	12 (43)	-	-
DENV-2	8 (29)	-	-
DENV-3	7 (25)	-	-
DENV-4	0 (0)	-	-
undefined	1 (3)	-	-
Illness day, average	5.3	-	-
patients admitted (n) / reported day of illness (d)	n=1 / d2 n=4 / d3 n=3 / d4 n=6 / d5 n=8 / d6 n=4 / d7 n=1 / d8 n=1 / d9		

Table S2, Related to Figures 1 and 3. Complete Blood Count (CBC) in dengue patients (DF and DHF) during acute disease (days 2-9 of febrile illness) and convalescence (≥ 4weeks after release).

CBC	Acute	Convalescent	p value
Platelets cells x10^3/μl (range)	62.3 (11 – 154)	295 (204 – 429)	<0.0001
WBC cells/μl (range)	4104 (1600 – 8640)	6765 (1250 – 12420)	0.0003
Neutrophils cells/μl (range)	1489 (378 – 5230)	3572 (895 – 6942)	0.0001
Lymphocytes cells/μl (range)	1376 (253 – 3635)	2125 (237 – 4781)	0.03
HCT % (range)	41.68 (33.2 – 49.4)	40.61 (29.2 - 49.3)	0.45
Hemoglobin gm/dL (range)	14.28 (11.3 - 16.3)	13.54 (9.9 - 16.3)	0.53

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Experimental cohort and blood collection. A total of 37 patients were enrolled in the study. Six of the individuals were excluded from the analysis with an unidentified, non-dengue disease, 2 were excluded from the analysis because of the incomplete clinical data collection and 1 due to the defective gene array chip hybridization. Routine laboratory measurements (CBC, urine and blood chemistry) and clinical manifestations of dengue infection were recorded and used for absolute cell count analysis. A final diagnosis and severity classification were done at the conclusion of the trial with a full review of all the clinical and laboratory data using the WHO guidelines (WHO, 1997). Within the cohort of 28 individuals with clinically and laboratory confirmed dengue, 18 were diagnosed with DF and 10 with uncomplicated DHF (DHF-I n=7, DHF-II n=3), though none of the enrolled patients was diagnosed with the DSS. Dengue patients tested positive for DENV serotype 1 (DENV-1, n=12), DENV-2 (n=8) and DENV-3 (n=7) and one remained with an undefined serotype, however none of the subjects was classified with DENV-4, a serotype distribution typical for the endemic dengue region of the study (Klungthong et al., 2004). The experimental group included 21 adults (age 15-50) and 7 adolescent individuals with a majority of male patients (n=20; Table S1). The control cohort (n=9) consisted of local, healthy, young adults (males n=2 and female n=7) without any symptoms of febrile disease. All the control samples were collected at the same time frame as the dengue patients during the season of 2009 in Bangkok, Thailand. All dengue patients in the analyzed cohort had substantial leucopenia with significantly reduced absolute counts of white blood cells (1.65-fold) when compared with convalescent samples (Table S1 and Figure S2C). Consistent with typical dengue symptoms (Peeling et al., 2010), patients in our cohort presented severe thrombocytopenia with a mean absolute count of platelets at 62,321 cells/ml of blood (4.7-fold decrease to convalescent, Table S2 and Figure S2C). Also, as measured by the ratio of serum IgM and IgG, only 2 of patients out of 28 were classified as primary infection, with a vast majority (n=26) of secondary dengue cases (data not shown). Blood was collected on CPT tubes (Vacutainer® with Sodium Citrate; BD). Whole blood specimens were stained with monoclonal Abs for flow cytometry analysis or preserved on RNALater buffer (Ambion) and stored in -80^{o}C. Plasma samples were isolated from CPT tubes according to the manufacturer's protocol and stored in -80^{o}C.

Microarrays and analysis. All RNA samples were checked for purity using a ND-1000 spectrophotometer (NanoDrop Technologies) and for integrity by electrophoresis on a 2100 BioAnalyzer (Agilent Technologies). Two-round in vitro transcription amplification and labeling was performed starting with 50 ng intact, total RNA per sample, following the Affymetrix protocol. Samples were hybridized on Human U133 Plus 2.0 Arrays (using GeneTitan platform, Affymetrix, or individual cartridges).

Unsupervised clustering analysis. Analysis was performed using the 7,258 genes with highest variance within the whole blood genome expression. Samples from 18 DF, 10 DHF, 19 convalescent patients and 9 controls were RMA normalized and probe sets that map to same gene symbol were collapsed by choosing the probe set with the highest mean expression value across all the samples. Variance of each gene of all 56 samples was calculated and only the top 40% of them were used in the analyses.

Pathway analyses. Analyses were performed using Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005) and Ingenuity Pathway Analysis (Ingenuity Systems) software. A meta-analysis was performed using data from the GSE22886 study (Abbas et al., 2005) and monocytes specific meta-analysis was performed using the data from Cros et al.,2010 (Cros et

al., 2010). Genes were first pre-ranked based on their correlation values between expression and VL or days of illness. Then, gene set enrichment analysis (GSEA) was used to determine if gene signatures of different cell types (gene sets) were enriched among the positively (high VL) or negatively (low VL) correlated genes or if correlated with the duration of symptomatic dengue. We compared the transcriptional profile between any two cell populations (at least 2-fold difference in mean expression and p-value < 0.05). For the cell population signature analysis, genes were classified as highly expressed in a given subset if they were up-regulated in this subset compared to at least 6 other subsets (out of 7 possible comparisons).

FACS staining and antibodies. PBMCs and plasma from NHP were isolated using CPT tubes (EDTA, Vacutainer; BD). Plasma samples were cryopreserved for further analysis. The NHP LNs were cleaned to remove excessive tissue and injected with 1 ml of the solution of collagenase type 4 (Worthington, Lakewood, NJ), chopped, and digested for 1 hour in 37°C. Digested LNs were further disrupted and cells were washed in PBS/1mM EDTA and filtered twice through a cell strainer (70μm) to remove undigested tissue. Freshly isolated PBMC or LN cells were resuspended in PBS/1mM EDTA/5% FBS and incubated with an appropriate Ab cocktail, washed and fixed, and analyzed on a LSRII flow cytometer (BD).

Antibodies used for staining of human blood: BD: Lineage cocktail 1 (lin 1): CD3, CD14, CD16, CD19, CD20, CD56 (Clones: NCAM16.2, MφP9, L27, SJ25C1, 3G8, SK7), HLA-DR (L-243), CD11b (ICRF44), CD11c (S-HCL-3), CD123 (7G3), CD14 (MQp9 and M5E2), CD16 (3G8), CD56 (NCAM16.2), CD3 (SK7), CD19 (SJ25C1), CD20 (L27), CD38 (HB7), CD27 (M-T271). Miltenyi: BDCA-1/CD1c (AD5-8E7), Biolegend: BDCA-1/CD1c (L161), CD11c (3.9), CD16 (3G8), CD56 (HCD56).

Antibodies used for in vitro assays: Biolegend: CD14 (M5E2), CD20 (2H7), CD11c (3.9), CD115 (9-4D2-1E4), CD169 (7-239), CD163 (GHI-61), CD1a (SK9), CD16 (3G8), BD: CD206 (19.2), CCR5 (2D7/CCR5), CD19 (SJ25C1), CD38 (HB7), CD27 (M-T271). RnD: CCR2 (FAB5538A).

In rhesus macaques total monocytes were identified within the SSC-A^{hi} FSC-A^{hi} PBMCs as the $CD3^{neg}CD8^{neg}CD20^{neg}$ HLA-DR^{+} population. Myeloid DCs (mDC) were identified as the (Lin.: CD3, CD8, CD20, CD14, CD16)^{neg}, HLA-DR^{+} $CD11c^{+}$ BDCA-1^{+} cells, and plasmacytoid DCs (pDC) as Lin^{neg} HLA-DR^{+} $CD123^{+}$ cells. Antibodies used for staining of rhesus macaques PBMC or LN cells: BD Pharmingen: CD3 APC-Cy7 (SP34-2), CD8 APC-Cy7 (SK-1), CD20 APC-Cy7 (L27), HLA-DR PE-TxRed (L243), CD123 PerCp-Cy5.5 (7G3), CD11c APC (S-HCL-3), Biolegend: CD14 PacBlue and ALexa-700 (M5E2), CD16 PacBlue and ALexa-700 (3G8), CD11c PE-Cy7 (3.9); Miltenyi: BDCA-1 APC (AD5-8E7); Caltag: HLA-DR PE-TxRed (TU36).

Viral Load and NS-1 ELISA and plasma IgM. VLs and plasma IgM were measured according to a method described previously (Onlamoon et al., 2010). Briefly, RNA was extracted from 140 μL of plasma using the QIAmp Viral RNA Mini kit (QIAGEN). The appropriate DENV serotype probes defined at the clinical screening for human samples and DENV-2 probes for rhesus samples and their flanking primers were prepared and custom synthesized by Operon. The viral RNA copy number was determined using a real-time one-step quantitative reverse-transcribed polymerase chain reaction (RT-PCR) assay using the TaqMan RT kit (Perkin Elmer Applied Biosystem) and Bio-Rad iCycler system using a standard control for virus quantitation by the quantitative RT-PCR assay. The limit of detection is approximately 100 copies of RNA

equivalent viral genome per milliliter plasma. DENV-specific IgM antibodies in rhesus plasma were determined by antibody capture enzyme-linked immunosorbent assay (ELISA). The increase of antibody titers was expressed as a percentage of the optical density (OD) values obtained on samples from the same monkey on day 0 (before infection).

Measurement of the levels of NS1 in serum of dengue patients was performed as previously described (Clark et al., 2012). Briefly, collected patient sera were stored in freezer until assay. Standard ELISA was set up to quantify the level of NS1 antigen in the collected serum samples by using purified NS1 antigen (CTK Biotech. Inc, San Diego, CA) to derive a standard curve. Serum samples and various concentrations of NS-1 were incubated with coating buffer on ELISA plates (Nunc Maxisorp) overnight at 4C. After 2 washes with PBS, samples were blocked with 5% milk in PBS-Tween 20 for 30 minutes at RT. Polyclonal rabbit anti NS-1 antibody (2ug/ml, CTK Biotech. Inc, San Diego, CA) in 5% milk was incubated for 1 hour at 37C. Plates were washed and incubated with HRP conjugated donkey anti rabbit IgG (1:2500) in 5% milk for 1 hour at 37C. Tetramethylbenzidine OptEIA substrate (BD) was prepared and 50 ul was dispensed into individuals wells of the microtiter plates and incubated for 5 minutes. The samples were neutralized with 25ul 4N H_2SO_4 and read at OD 490. Values obtained using the NS-1 standard were plotted and the plot utilized to calculate the amount in the experimental sample.

In vitro Assays.

Monocyte phenotyping and cytokines secretion. $CD14^+$ monocytes were incubated in complete RMPI media (Corning) with 10% FBS (HyClone), 100u-100ug/ml Pen-Strep (HyClone) in 96-well plates and $1x10^5$ cells/well were infected with DENV-2 (clone 16681) at MOI=1 or MOI=0.1 or incubated with LPS (TLR4-L) 100ng/ml or R-848 (TLR7/8-L) 1ug/ml in duplicates. After 48hr supernatants were collected and stored at -20^{o}C for cytokine analysis. Cells were washed in PBS stained with the live/dead marker Alexa 430 (Invitrogen) and appropriate Ab cocktail. Cells were washed in PBS/5% FBS, fixed and analyzed on a LSRII flow cytometer (BD).

B cell proliferation assay. Monocyte-derived DCs (MDDCs) were differentiated from monocytes by a standard method by culturing with GM-CSF (1000U/10^6 cells, Miltenyi) and IL-4 (500U/10^6, Peprotech) for 6 days. Then, $1x10^5$ $CD14^+$ monocytes or MDDC were infected with the DENV-2 at MOI=1. After 48hr cells were washed twice with complete cell culture medium to remove free virus. Total, allogenic $CD19^+$ B cells were stained with CFSE (Invitrogen) and co-cultured in 96-well plates with the MDDC or $CD14^+$ monocytes at the ratio of 5:1 in the presence of 20U/ml of IL-2 (Peprotech) and 50nM CpG type B 2006 (TriLink Biotech) in duplicates. In some cultures the following antibodies were added at the concentration of 10μg/ml for blocking experiments: R&D: anti-BAFF (148725), TACI-Fc (174-TC), BCMA-Fc (193-BC), anti-IL-6 (6708), anti-IP-10 (33036), BD: anti-IL10 (554497). After 6 days of culture supernatants were collected and stored at -20^{o}C for total IgG, IgA and IgM analysis. Cells were washed in PBS stained with the live/dead marker Alexa 430 (Invitrogen) and appropriate Ab cocktail. Cells were washed in PBS/5% FBS, fixed and analyzed for CFSE dilution and B cell phenotype on a LSRII flow cytometer (BD).

ELISAs. Total IgG, IgA and IgM secreted in vitro for 6 days by B cells stimulated with DENV-infected monocytes or MDDC were analyzed in duplicates by a standard plate ELISA using anti-human IgG, IgA and IgM antibodies (Bethyl Laboratories). The final concentration of each Ab in the supernatant was calculated using a standard serum provided by the manufacturer

(Bethyl Laboratories). APRIL and BAFF concentrations were assessed by ELISA (eBioscience) in monocytes ($1x10^5$ cells/well) infected with DENV-2 at MOI 1 for 48hr.

Electron Microscopy. All sections prepared for electron microscopy were washed in PBS before being post-fixed in osmium tetroxide (1% solution in PBS) for 20 min. They were then washed five times (5 min each) in PBS, and dehydrated in a graded series of ethanol and propylene oxide. Uranyl acetate (1%) was added to the 70% ethanol to improve contrast in the electron microscope. The sections were then embedded in resin (Durcupan, ACM; Fluka, Buchs, Switzerland) on microscope slides, and put in the oven for 48 h at 60 °C. After examination with the light microscope, areas of interest in the GP were cut out from the slides, glued onto resin blocks, cut into ultrathin 60-nm-thick sections with a Leica (Nussloch, Germany) UCT ultramicrotome, and collected on pioloform-coated single-slot copper grids. They were then stained with lead citrate (Reynolds, 1963), and examined with a Zeiss EM-10C electron microscope.

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