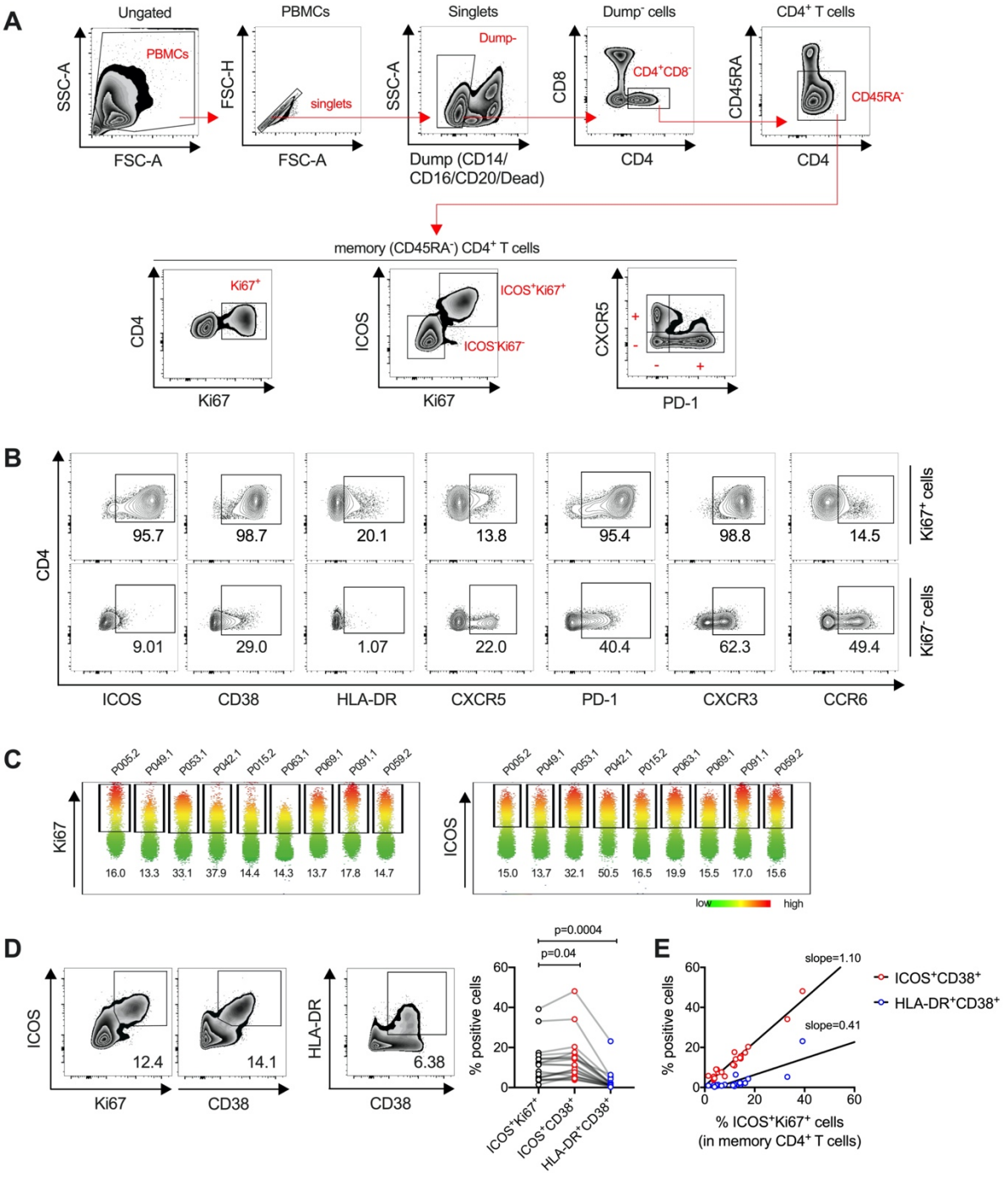

Fig. S1. Phenotypic analyses of activated CD4⁺ T cells in blood of acute dengue patients, Related to Fig. 1

(A) Gating strategy for the multi-parametric flow cytometry analysis of memory CD4⁺ T cells: PBMCs side-scatter gate, singlets, Dump negative cells, CD4⁺ T cells, memory (CD45RA negative) cells.

(B) Representative FACS plots showing the gating schemes for the surface expression of markers in Ki67 positive and negative T cell subsets as shown in Fig. 1D.

(C)FACS plots display the expression and frequency of Ki67 and ICOS in equally concatenated memory $CD4^+$ T cells from 9 DENV samples used in tSNE analysis.

(D) Representative plots (left) showing co-expression of ICOS and Ki67 or ICOS and CD38, and HLA-DR and CD38. Paired dotplot (right) shows the frequency of above mentioned double positive cells in samples from acute dengue patients (n=21).

(E) *xy*-plot shows the correlation between frequency of ICOS/CD38 and ICOS/Ki67 double-positive cells (red dots), and the correlation between frequency of HLA-DR/CD38 and ICOS/Ki67 double-positive cells (blue dots).

Statistics: (D), Friedman test and corrected using Dunn's multiple comparisons test, (E) simple linear regression.

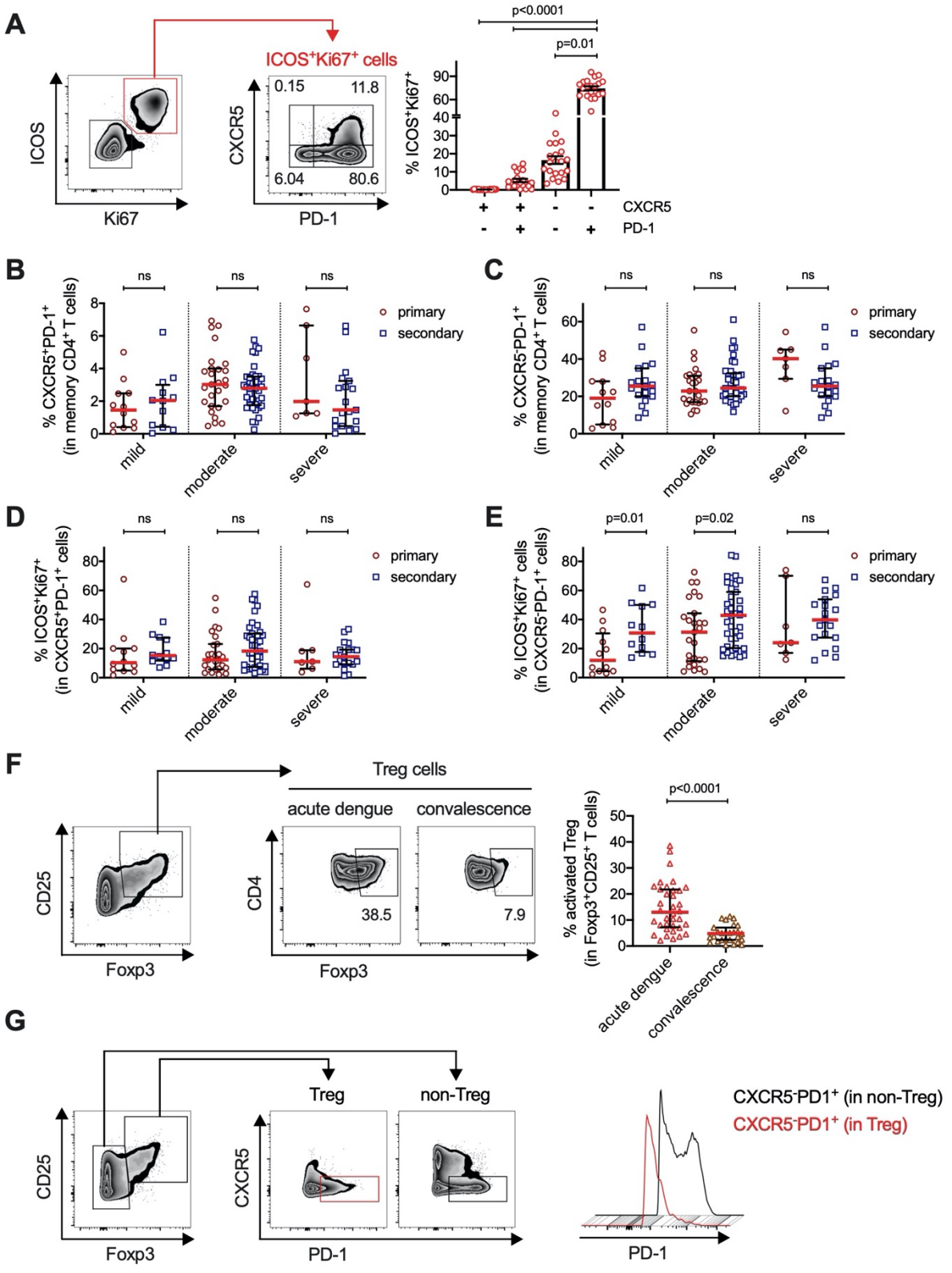

Fig. S2. Activated CXCR5⁻PD-1⁺ CD4⁺ T cells accumulate in dengue patients with exacerbating severity, Related to Fig. 2

(A) Representative FACS plots (left) and scatter barplot (right) showing the staining of CXCR5 and PD-1 on activated ($ICOS^{+}Ki67^{+}$) memory $CD4^{+}$ T cells and quantify the frequency of the resulting four subsets by the expression of CXCR5 and PD-1. Data are shown as mean ± SEM.

(B-E) Scatter dotplots quantifying the frequency of (B) $CXCR5^{+}PD\text{-}1^{+}$ and (C) $CXCR5^{-}PD\text{-}1^{+}$ subsets in memory $CD4^{+}$ T cells, and frequency of activated cells within (D) $CXCR5^{+}PD\text{-}1^{+}$ and (E) $CXCR5^{-}PD\text{-}1^{+}$ subsets in primary (n=46) and secondary (n=70) acute DENV samples with increasing degree of severity.

(F) Example FACS plot of intranuclear FOXP3 staining and dotplot quantifying the frequency of activated ($FOXP3^{hi}$) cells in Treg subset of acute (n=36) and convalescent (n=27) DENV samples.

(G) Left panel: Representative plots show the gating for regulatory T cells (Treg) ($CD25^{+}FOXP3^{+}$) and non-Tregs ($FOXP3^{-}$) in memory $CD4^{+}$ T cells and gating of $CXCR5^{-}$ $PD\text{-}1^{+}$ cells within Treg and non-Treg populations. Right panel: Overlayed histogram depicts the comparative expression of PD-1 in two populations.

Statistics: (A), Friedman test and corrected using Dunn's multiple comparisons test. (B-E), multiple t tests and corrected using Holm-Sidak method, (F), Mann-Whitney test. ns, non-significant. Data represented as median ± IQR.

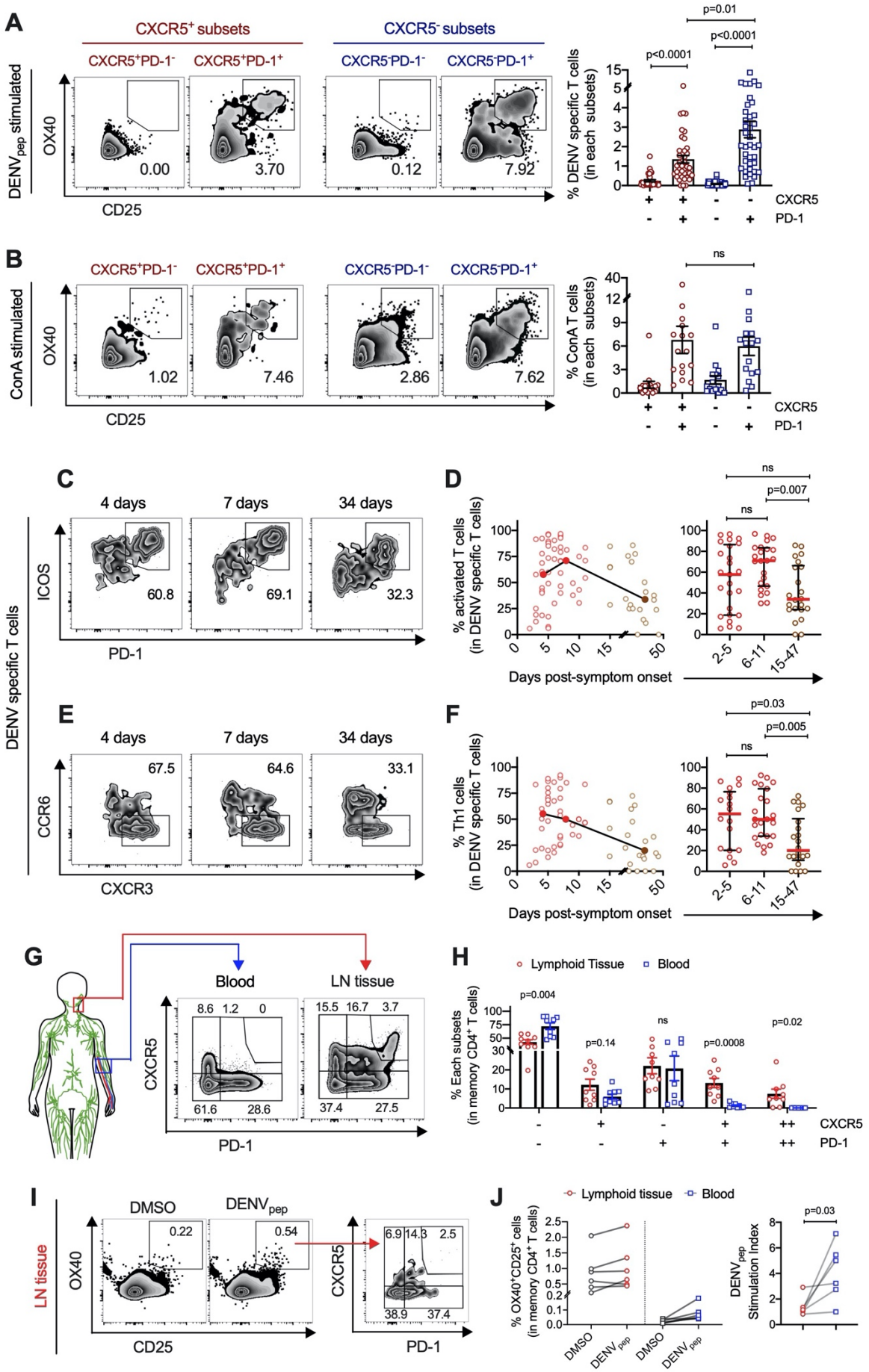
A
CXCR5+ subsets
CXCR5- subsets
CXCR5+PD-1-
CXCR5+PD-1+
CXCR5-PD-1-
CXCR5-PD-1+
DENVpep stimulated
OX40
CD25
0.00
3.70
0.12
7.92
% DENV specific T cells (in each subsets)
p=0.01
p<0.0001
p<0.0001
CXCR5
PD-1
B
ConA stimulated
1.02
7.46
2.86
7.62
% ConA T cells (in each subsets)
ns
C
4 days
7 days
34 days
DENV specific T cells
ICOS
PD-1
60.8
69.1
32.3
D
% activated T cells (in DENV specific T cells)
ns
p=0.007
Days post-symptom onset
2-5
6-11
15-47
E
67.5
64.6
33.1
CCR6
CXCR3
F
% Th1 cells (in DENV specific T cells)
p=0.03
p=0.005
G
Blood
LN tissue
8.6 1.2 0
61.6 28.6
15.5 16.7 3.7
37.4 27.5
CXCR5
PD-1
H
Lymphoid Tissue
Blood
% Each subsets (in memory CD4+ T cells)
p=0.004
p=0.14
ns
p=0.0008
p=0.02
I
LN tissue
DMSO
DENVpep
0.22
0.54
6.9 14.3 2.5
38.9 37.4
J
Lymphoid tissue
Blood
% OX40+CD25+ cells (in memory CD4+ T cells)
DENVpep Stimulation Index
p=0.03

Fig. S3. CXCR5^{-}PD-1^{+} cells are present in dengue virus specific memory CD4^{+} T cells pool and circulate persistently in blood of dengue recovered individuals, related to Fig. 3

(A-B) Representative FACS plots and scatter barplot quantifying the frequency of antigen-specific (OX40^{+}CD25^{+}) cells in the four subsets defined by expression of CXCR5 and PD-1 in memory CD4^{+} T cells of acute dengue PBMCs after (A) stimulation with DENV peptides megapool (DENV$_{pep}$) (n=39) or (B) treatment with concanavalin A (ConA) (n=16) for 18-20 h. Data are shown as mean ± SEM.

(C) Representative FACS plots depicting the frequency of activated (ICOS^{+}PD-1^{+}) cells in DENV-specific memory CD4^{+} T cells at various time points post-symptom onset.

(D) Scatter dotplots showing the frequency of activated (ICOS^{+}PD-1^{+}) cells in DENV-specific memory CD4^{+} T cells in continuous and grouped time-points (days post-symptom onset) from cross-sectional DENV samples (n=70).

(E) Representative FACS plots depicting the frequency of Th1 cells in DENV-specific memory CD4^{+} T cells on various time-points as shown in Fig. S3C.

(F) Scatter dotplots showing the frequency of Th1 cells in DENV-specific memory CD4^{+} T cells in continuous and grouped time-points from cross-sectional DENV samples (n=63).

(G-H) Shown is the (G) representative of CXCR5 and PD-1 staining and (H) frequency of indicated subsets in paired LN tissue and blood samples from a virus exposed individual.

(I) Example FACS plots showing the frequency of OX40^{+}CD25^{+} T cells in DMSO and DENV$_{pep}$ stimulation conditions. OX40^{+}CD25^{+} cells are further gated to show the various subsets by the expression of CXCR5 and PD-1.

(J) Frequency of OX40^{+}CD25^{+} T cells in memory CD4^{+} T cells of paired LN tissue and blood samples from virus exposed individuals (n=6) stimulated with DMSO and DENV$_{pep}$.

Statistics: (A-B), Friedman test followed by Dunn's multiple comparisons test, (D, F), Kruskal-Wallis test and corrected using Dunn's multiple comparisons test, (H) multiple t tests and corrected using Holm-Sidak method, (J) Mann-Whitney test. ns, non-significant. Data represented as median ± IQR.

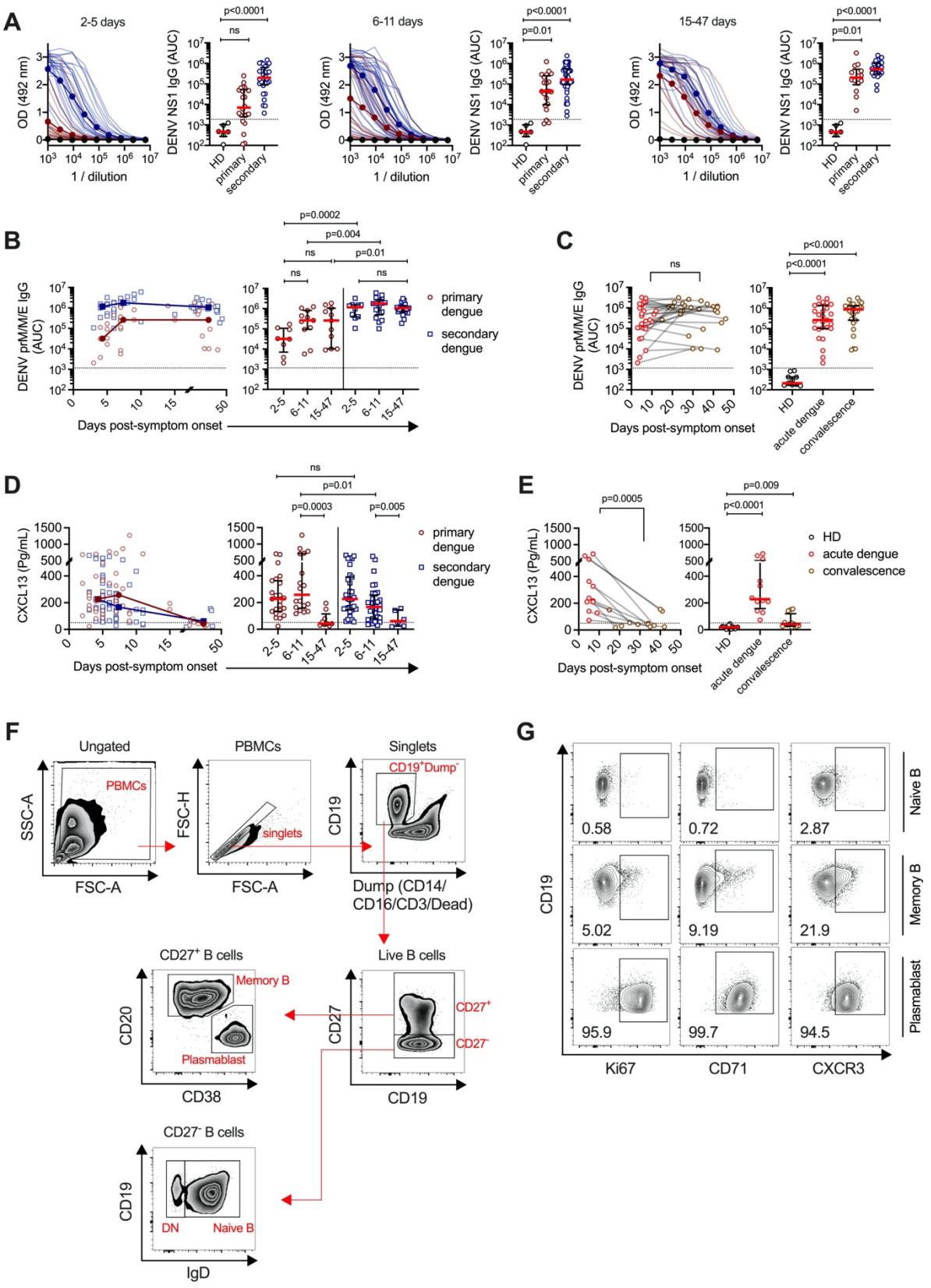

Fig. S4. Activated CXCR5⁻PD-1⁺ CD4⁺ T cells positively associate with antibody responses and plasmablast in dengue, related to Fig. 4

(A) ELISA OD curves of anti-NS1 IgG in serially diluted plasma samples at indicated intervals post-symptom onset. Respective scatter plots show quantity of anti-NS1 IgG measured as AUC in DENV seronegative HD, primary dengue and secondary dengue patient samples.

(B) Quantity of anti-prM/M/E IgG antibodies (AUC) in continuous and grouped time-points (days post-symptom onset) from cross-sectional cohorts of primary (n=30) and secondary (n=46) dengue samples.

(C) Longitudinal analysis of anti-prM/M/E IgG (AUC) in paired acute and convalescent dengue samples (n=27), in comparison with background AUC in DENV seronegative HD samples.

(D) Quantitation of CXCL13 cytokine in the plasma of cross-sectional dengue samples in continuous and grouped time-points (days post-symptom onset) from primary (n=50) and secondary (n=69) dengue.

(E) Longitudinal measurement of CXCL13 in paired acute and convalescent DENV samples (n=12), in comparison with HD samples.

(F) Gating strategy for the flow cytometry analysis of B cells: PBMCs side-scatter gate, singlets, live $CD19^{+}$ B cells, $CD27^{+}$ and $CD27^{-}$ B cells, naive and DN B cells, memory B cells and plasmablast.

(G) FACS plots showing the gating schemes for the expression of markers Ki67, CD71 and CXCR3 on respective B cell population, as shown in Fig. 4F.

Statistics: (A-E), Kruskal-Wallis test followed by Dunn's multiple comparisons test, (C, E), paired analysis by Wilcoxon matched-pairs signed rank test. ns, non-significant. Dotted line in A-E represent the limit of positivity. Data are shown as median ± interquartile range (IQR).

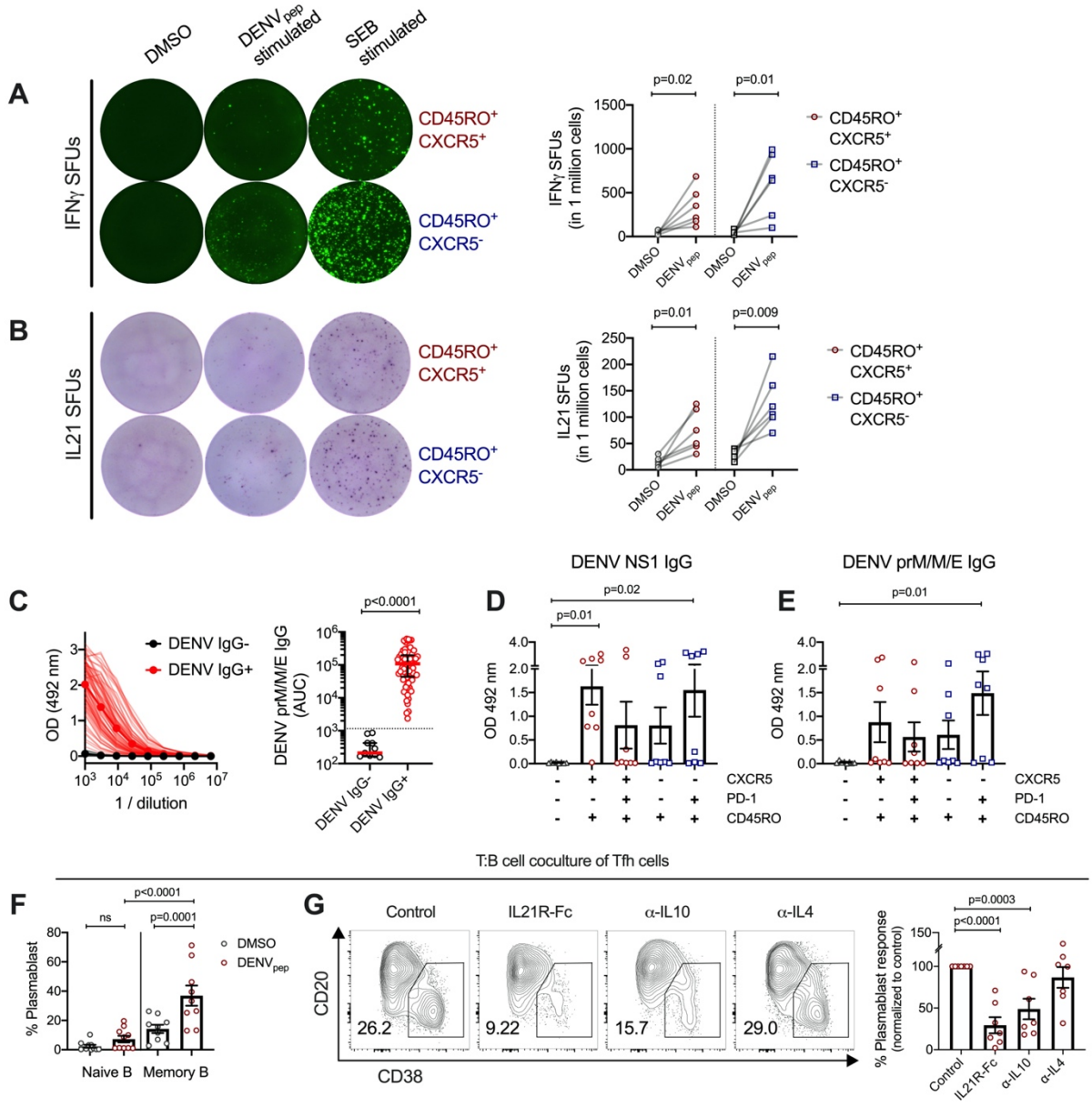

Fig. S5. DENV specific CXCR5^{-}PD-1^{+} CD4^{+} T cells are functionally potent and capable of driving plasmablast and antibody responses, related to Fig. 5

(A-B) Analyses of IFNγ and IL21 secreting cells in FACS sorted CD45RO^{+}CXCR5^{+} and CD45RO^{+}CXCR5^{-} memory subsets from acute dengue samples.

(A) Representative FluoroSpot images of IFNγ spot forming cells (SFUs) in CXCR5^{+} and CXCR5^{-} memory subsets stimulated for overnight in presence of antigen presenting cells (CD20^{+}/CD14^{+}/CD16^{+} cells) with DMSO, DENV$_{pep}$, Staphylococcal enterotoxin B (SEB). Paired dotplot show the number of IFNγ SFUs calculated over one million of cells.

(B) Example ELISpot images of IL21 SFUs in $CXCR5^+$ and $CXCR5^-$ memory subsets stimulated as in panel A. Paired dotplot depict the number of IL21 SFUs per million of cells.

(C) ELISA OD curves of anti-prM/M/E DENV IgG antibodies in serially diluted plasma from dengue seronegative (DENV IgG^-) (n=11) and seropositive (DENV IgG^+) (n=77) individuals. Serostatus was confirmed using commercial DENV IgG screening kits. Scatter plot shows the quantity of anti-prM/M/E antibodies (AUC) in the two groups. Data are represented as median ± IQR.

(D-E) Barplots showing the levels of (D) anti-NS1 and (E) anti-prM/M/E DENV IgG secreted in T:B co-cultures in presence of $DENV_{pep}$ as shown in Fig. 5G, 5H.

(F) Barplots showing the plasmablast frequency in co-cultures of Tfh ($CXCR5^+$) cell subset and naive ($CD27^-$) or memory ($CD27^+$) $CD20^+$ B cells.

(G) Representative plots showing the plasmablast frequency in T:B cocultures of Tfh ($CXCR5^+$) cells and $CD20^+$ B cells in presence of $DENV_{pep}$ with or without blocking conditions with IL21R-Fc, anti-IL10 and anti-IL4. Data in scatter barplot are background subtracted from DMSO and normalized to control (without blocking) condition.

Statistics: (A-B), paired t-test, (C), Mann-Whitney test, (D-E), 1-way ANOVA with Tukey's multiple comparisons test, (F), 1-way ANOVA with Bonferroni's multiple comparisons test, (G), 1-way ANOVA with Dunnett's multiple comparisons test. ns, non-significant. Data represented as mean ± SEM.

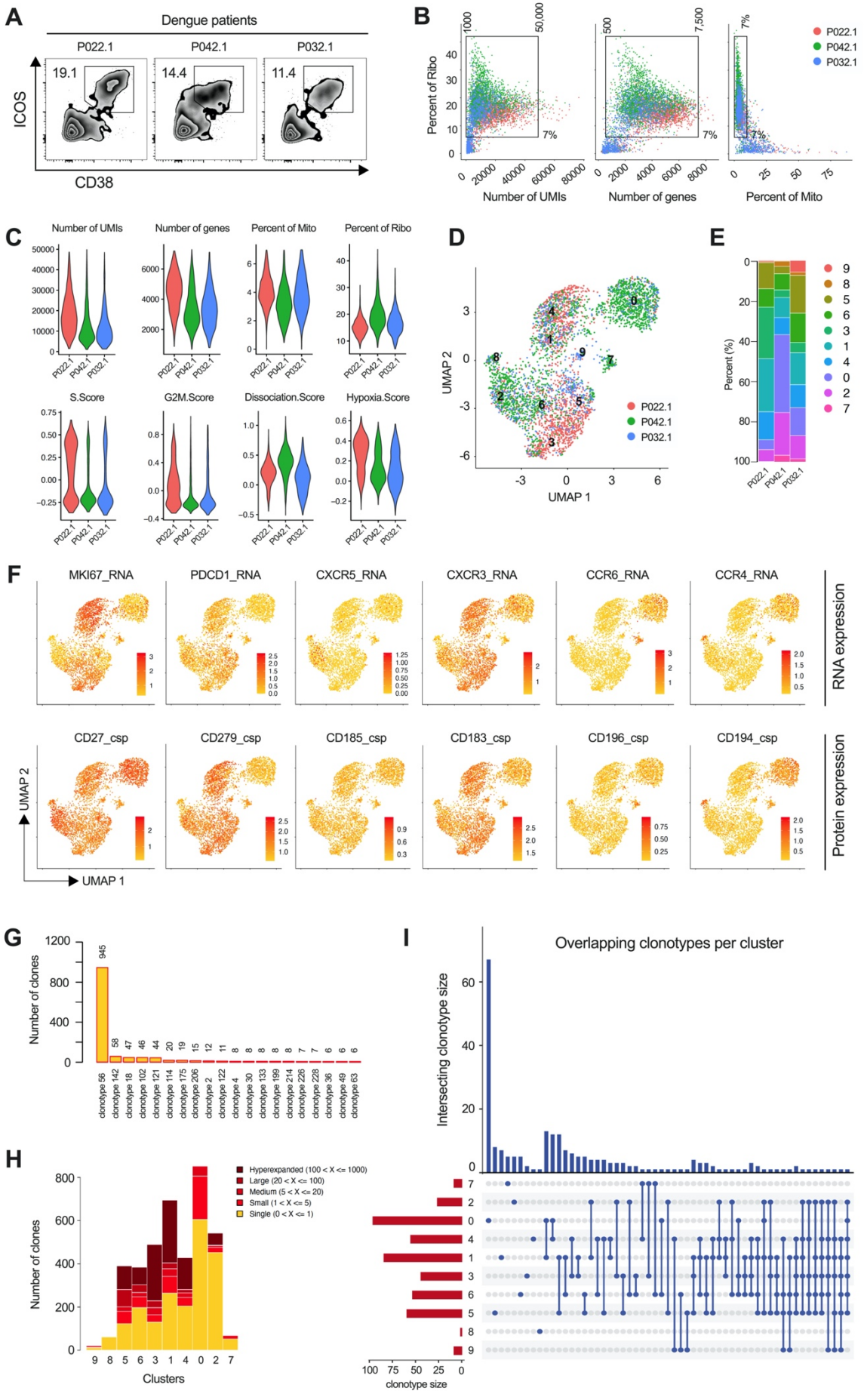
A
Dengue patients
P022.1
P042.1
P032.1
19.1
14.4
11.4
ICOS
CD38
B
Percent of Ribo
Number of UMIs
Number of genes
Percent of Mito
7%
P022.1
P042.1
P032.1
C
Number of UMIs
Number of genes
Percent of Mito
Percent of Ribo
S.Score
G2M.Score
Dissociation.Score
Hypoxia.Score
D
UMAP 2
UMAP 1
E
Percent (%)
F
MKI67_RNA
PDCD1_RNA
CXCR5_RNA
CXCR3_RNA
CCR6_RNA
CCR4_RNA
RNA expression
CD27_csp
CD279_csp
CD185_csp
CD183_csp
CD196_csp
CD194_csp
Protein expression
G
Number of clones
clonotype 56
clonotype 142
clonotype 18
clonotype 102
clonotype 121
clonotype 114
clonotype 175
clonotype 206
clonotype 2
clonotype 122
clonotype 4
clonotype 30
clonotype 133
clonotype 199
clonotype 214
clonotype 226
clonotype 228
clonotype 36
clonotype 49
clonotype 63
H
Hyperexpanded (100 < X <= 1000)
Large (20 < X <= 100)
Medium (5 < X <= 20)
Small (1 < X <= 5)
Single (0 < X <= 1)
Number of clones
Clusters
I
Overlapping clonotypes per cluster
Intersecting clonotype size
clonotype size

Fig. S6. Single cell RNAseq and TCRseq analyses of activated $CD4^+$ T cells of acute dengue patients reveal heterogeneity in $CXCR5^-PD\text{-}1^+$ population. related to Fig. 6

(A) FACS plots show the expression and frequency of $ICOS^+CD38^+$ $CD4^+$ T cells in the samples from acute dengue patients used for single cell RNAseq analyses.

(B) Scatter plots showing the parameters used for filtering out dead/low quality and likely doublets. Cells undergone analyses were containing at least 1000 transcripts (UMIs) and 500 genes, and a maximum of 50,000 UMIs and 7,500 genes with <7% mitochondrial genes and >7% ribosomal genes. Colour represent the cells from three dengue patients.

(C) Violin plots showing individually each donors with levels of potential variables: number of UMIs, genes, percent of mitochondrial genes, ribosomal genes, scores for S and G2M cell cycle phases, scores for dissociation (associated with sample processing) and hypoxia.

(D) UMAP showing the uniform distribution of filtered cells after data integration of three DENV patients samples using seurat CCA-based integration.

(E) Barplot shows the relative proportion of all ten clusters (0-9) in each patient's sample.

(F) UMAP plots showing the expression of RNA transcripts (*top*) and surface proteins (*bottom*) of selected genes.

(G) Bar plot showing the number of cell clones in top 20 expanded clonotypes.

(H) Bar plot depicting the number of cell clones belonging to different clonal sizes in each cluster.

(I) Plot shows the level of overlapping clonotypes between cells from different clusters. Blue bars (*top*) represent the size of intersecting clonotypes in indicated clusters, whereas horizontalred bar indicates the total expanded (clonal size > 1) clonotypes per cluster. Bottom panel shows the intersections among clusters as depicted by vertical blue lines connecting the clonotypes marked in the row against each cluster.

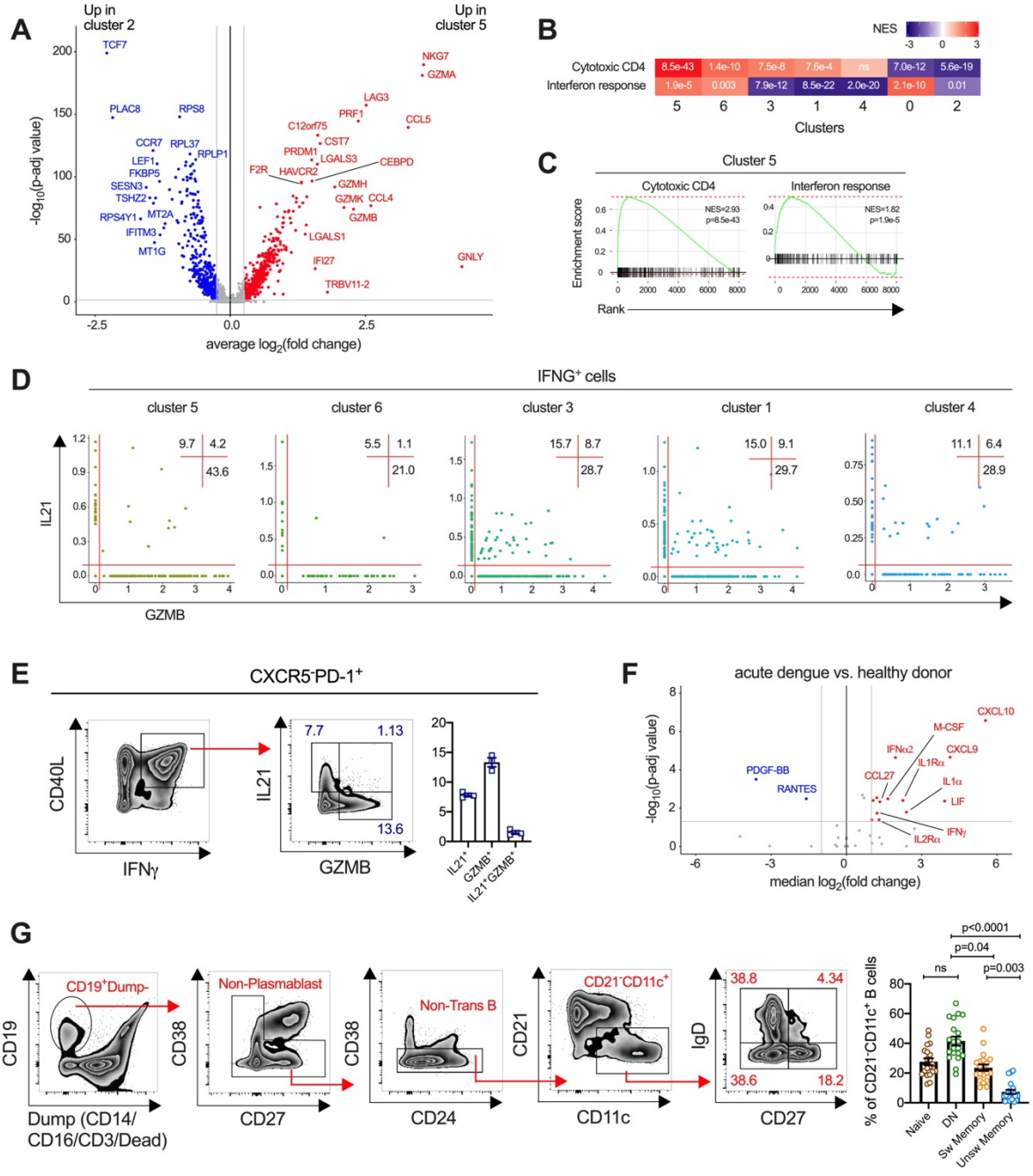

Fig. S7. Distinct features and diverse clonotypes of *helper* subset in CXCR5^{-}PD-1^{+} T cells and their strong association with extrafollicular B cell response, related to Fig. 7

(A) Volcano plot shows the differentially expressed genes (DEGs) between the cluster 5 and cluster 2. Significant DEGs were calculated by seuratFindMarkersfunction with adjusted p-value < 0.05, $\log_2$|fold change| > 0.25, and 10% as minimum percentage of cells expressing DEGs.

(B) Gene set enrichment analysis (GSEA) for signature genes of cytotoxic CD4 and interferon response in a given cluster compared to cells in remaining of clusters. Heatmap showing the normalized enrichment scores (NES) and adjusted p-values are indicated within each cluster.

(C) Enrichment plots showing signature genes of cytotoxic CD4 and interferon response in cluster 5.

(D) xy-plots showing the expression and percentage of *IFNG* positive cells expressing *GZMB* alone (*GZMB*$^+$), *IL21* alone (*IL21*$^+$) and both (*GZMB*$^+$*IL21*$^+$) in the indicated clusters.

(E) FACS plots showing the intracellular co-staining of GZMB and IL21 in CD40L$^+$IFNγ$^+$ cells of CXCR5$^-$PD-1$^+$ T-cell subset activated with SEB.

(F) Volcano plot depicting the differentially regulated cytokines in plasma sample of acute dengue patients versus HD donors. Significantly regulated cytokines were calculated with adjusted p-value < 0.05, $\log_2$|fold change| > 1.

(G) Representative FACS plots depicting the phenotype of naive (IgD$^+$CD27$^-$) , double negative (IgD$^-$CD27$^-$), switched (IgD$^-$CD27$^+$) and unswitched (IgD$^+$CD27$^+$) memory B cells gated in CD21$^-$CD11c$^+$ cells after excluding plasmablast and transitional B cells. Scatter barplot shows the frequency of each of the mentioned subsets in CD21$^-$CD11c$^+$ cells of acute dengue samples (n=20). Data are shown as mean ± SEM.

Statistics: (F), multiple t tests and corrected using Holm-Sidak method, (G), 1-way ANOVA followed by Dunn's multiple comparisons test. ns, non-significant.