Supplementary Figures and Tables

Supplementary Tables.

Supplementary Table S1: Cluster markers for pTfh clusters in healthy donors *attached as excel*

Supplementary Table S2: DEGs between Tfh1_cyto and Tfh1_CCR7 clusters *attached as excel*

Supplementary Table S3: Phenotyping panel

Target	Fluorochrome	Clone	Manufacturer	Catalog #	Dilution
LAG-3	BUV661	3DS223H	Invitrogen	376-2239-42	1 in 25
CD49b	FITC	eBioY418	Invitrogen	11-0498-42	1 in 50
CD25	BUV395	2A3	BD Biosciences	564034	1 in 100
CXCR5	BUV615	MU5UBEE	Invitrogen	366-9185-42	1 in 50
CXCR3	BV421	1C6/CXCR3	BD Biosciences	562558	1 in 25
CCR6	BV605	11A9	BD Biosciences	562724	1 in 50
OX40	BV650	ACT35	BD Biosciences	563658	1 in 50
CCR7	BV711	150503	BD Biosciences	566602	1 in 50
CD3	BV786	UCHT1	BD Biosciences	565491	1 in 500
CD4	Spark blue 574	SK3	Biolegend	389714	1 in 100
CD69	PerCP-eFluor 710	FN50	ThermoFisher	46-0699-42	1 in 100
TIM-3	PE-CF594	7D3	BD Biosciences	565560	1 in 50
PD-1	PE-Fire 640	EH12.2H7	Biolegend	329968	1 in 50
TIGIT	PE-Fire 810	A151513G	Biolegend	372745	1 in 100
CD45RA	redFluor 710	HI100	Cytek Biosciences	80-0458-T100	1 in 400
ICOS	APC-H7	DX29	BD Biosciences	567142	1 in 100
CD38	APC-Fire 810	HIT2	Biolegend	303550	1 in 200
Viability	LD-Blue		ThermoFisher	L23105	1 in 5000
Intracellular:					
CTLA-4	BUV737	14D3	ThermoFisher	367-1529-42	1 in 50
Ki-67	BUV805	B56	BD Biosciences	569636	1 in 50
Granzyme B	BV510	GB11	BD Biosciences	563388	1 in 800
Granzyme K	RB780	G3H69	BD Biosciences	569225	1 in 50
NKG7	PE	E6S2A	Cell Signalling Technology	84835	1 in 2000
c-Maf	PECy7	sym0F1	ThermoFisher	25-9855-82	1 in 400
FOXP3	RB613	259D/C7	BD Biosciences	571087	1 in 100
SAP	eFluor660	XLP-1D12	ThermoFisher	50-9787-42	1 in 100

Supplementary Table S4: PMA/Io panel

Target	Fluorochrome	Clone	Manufacturer	Catalog #	Dilution
CD8	BUV496	RPA-T8	BD Biosciences	612942	1 in 100
CD45RA	BUV563	HI100	BD Biosciences	612926	1 in 400
CD3	BUV805	SK7	BD Biosciences	612893	1 in 50
CD14/CD19	BV510	M5E2 / SJ25C1	Biolegend	301842/ 363019	1 in 100
CCR4	BV605	L291H4	Biolegend	359418	1 in 100
CCR6	BV650	11A9	BD Biosciences	563922	1 in 50
CXCR5	BV711	J252D4	Biolegend	356934	1 in 50
CD4	BV785	OKT4	Biolegend	317442	1 in 50
CXCR3	PE-CF594	1C6/CXCR3	BD Biosciences	562451	1 in 50
PD1	PE-Cy7	EH12.1	BD Biosciences	561272	1 in 50
Vd2	APC-Fire	B6	Biolegend	331420	1 in 50
Viability	LD-blue		Thermofisher	L23105	1 in 5000
Intracellular:					
IFNy	BUV395	B27	BD Biosciences	563563	1 in 50
IL-10	BV421	JES3-9D7	Biolegend	501422	1 in 10
TNF	BV750	MAb11	BD Biosciences	566359	1 in 100
IL-17a	FITC	BL168	Biolegend	512304	1 in 25
IL-21	PE	3A3-N2.1	BD Biosciences	560463	1 in 10
IL-4	APC	MP4-25D2	Biolegend	500812	1 in 10

Supplementary Table S5: Genes used in input for scType prediction *attached as excel*

Supplementary Table S6: Cluster markers for predicted annotated cell clusters in CHMI data set *attached as excel*

Supplementary Table S7: DEGs in each Tfh subset during malaria infection identified by edgeR pseudobulk *attached as excel*

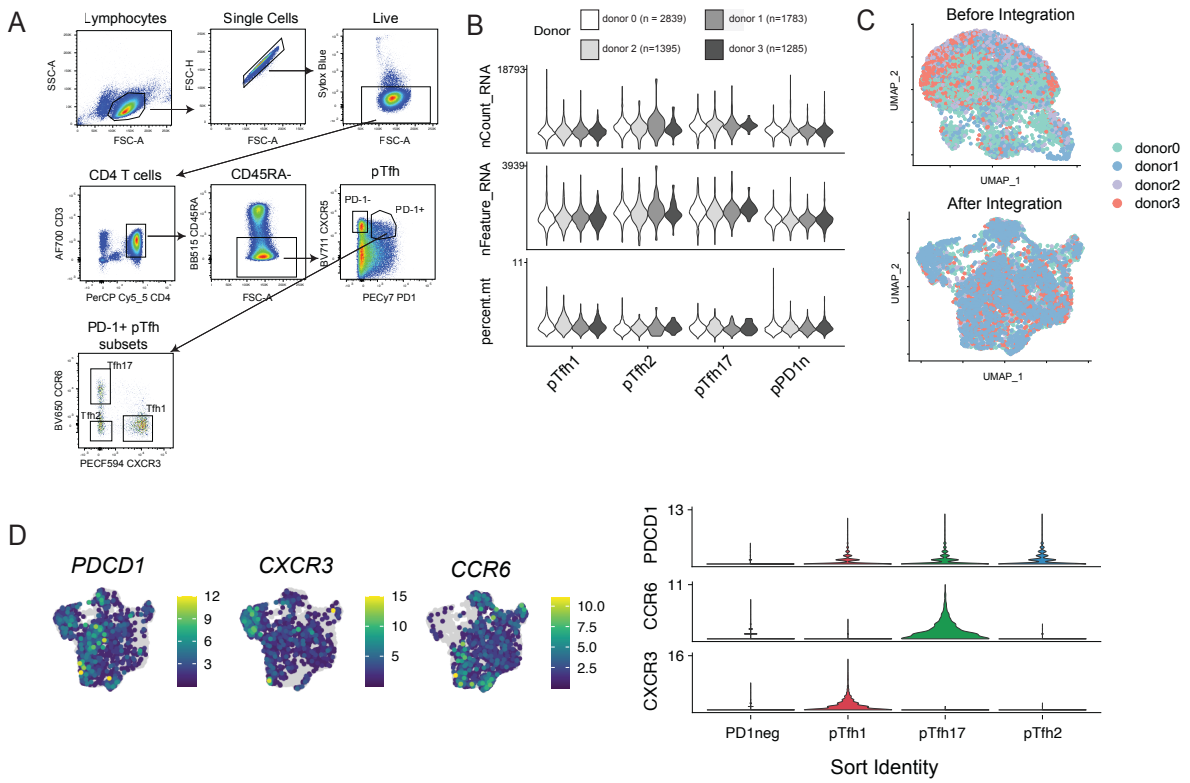
Supplementary Table S8: Antibody panel for sort purification of Tfh cells for scRNAseq of healthy samples

Target	Fluorochrome	Clone	Manufacturer	Catalog #	Dilution
CD45RA	BB515	HI100	BD Biosciences	564552	1 in 400
CD4	PerCP-Cy5.5	OKT4	Biolegend	317428	1 in 50
CXCR3	PE-CF594	1C6/CXCR3	BD Biosciences	562451	1 in 50
PD1	PE-Cy7	EH12.1	BD Biosciences	561272	1 in 50
CD3	AF700	OKT3	Biolegend	317340	1 in 50
	Sytox Blue		Invitrogen	S34857	1 in 1000
CCR6	BV650	11A9	BD Biosciences	563922	1 in 50
CXCR5	BV711	J252D4	Biolegend	356933	1 in 50

Supplementary Table S9: Antibody panel for sort purification of Tfh cells for scRNAseq of CHMI samples

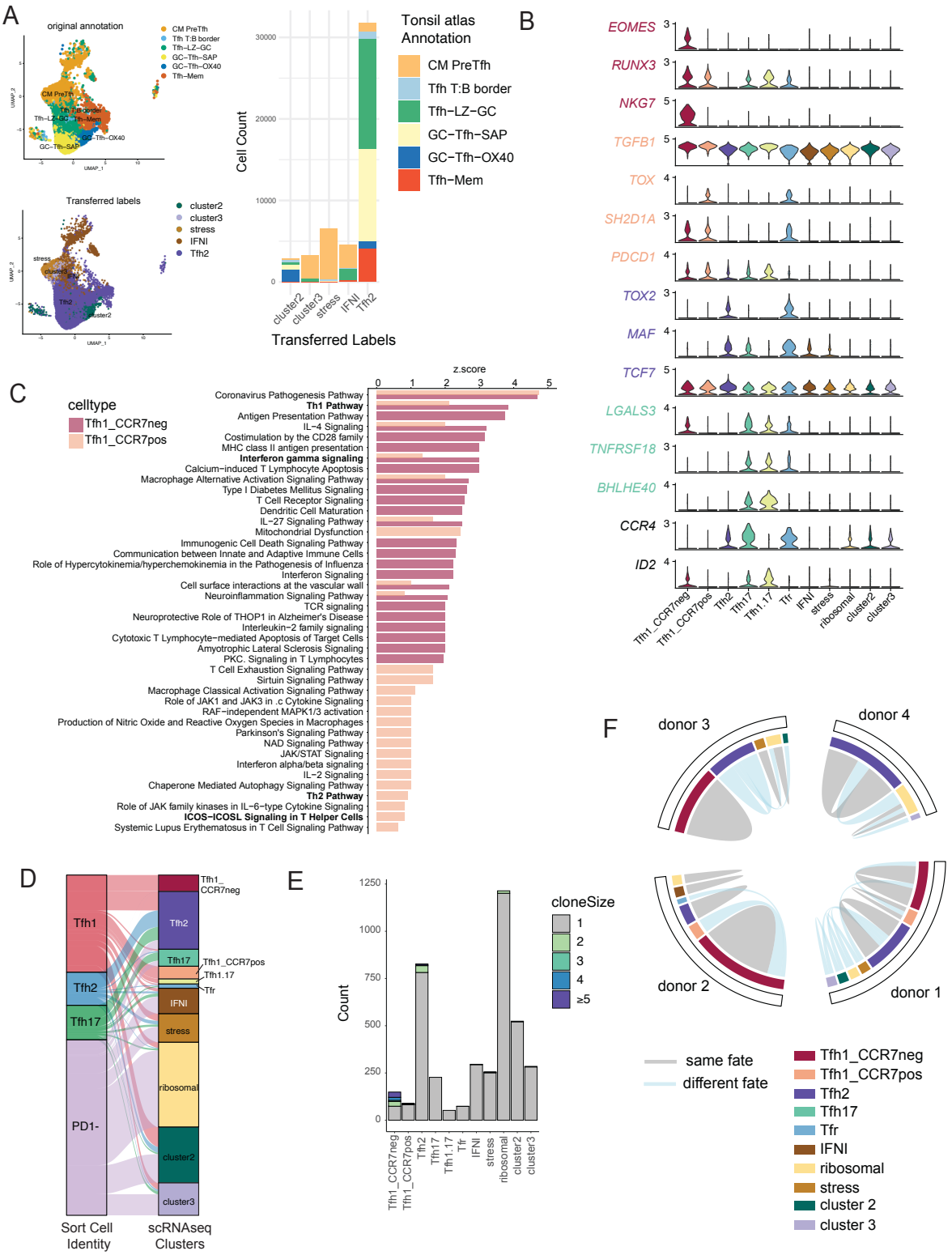
Target	Fluorochrome	Clone	Manufacturer	Catalog #	Dilution
Stain 1:					
TCR gD	FITC	B1	Biolegend	331208	3 in 25
CXCR5	BV711	J252D4	Biolegend	356933	2 in 25
Stain 2:					
CD4	PerCpCy5.5	OKT4	Biolegend	317428	1 in 50
CD19	PE	HIB19	BD Biosciences	555413	1 in 100
PD1	PE-Cy7	EH12.1	BD Biosciences	563922	1 in 50
Vd2	APC	B6	Biolegend	331418	1 in 50
CD3	AF700	OKT3	Biolegend	317340	1 in 50
	Sytox Blue		Invitrogen	S34857	1 in 1000
CD56	BV510	HCD56	Biolegend	318340	1 in 100
CD45RA	BV570	HI100	Biolegend	304132	1 in 400
HLADR	BV785	L243	Biolegend	307642	1 in 200

Supplementary Figures



Supplementary Figure 1. Single cell RNA sequencing of pTfh cells from healthy donors.

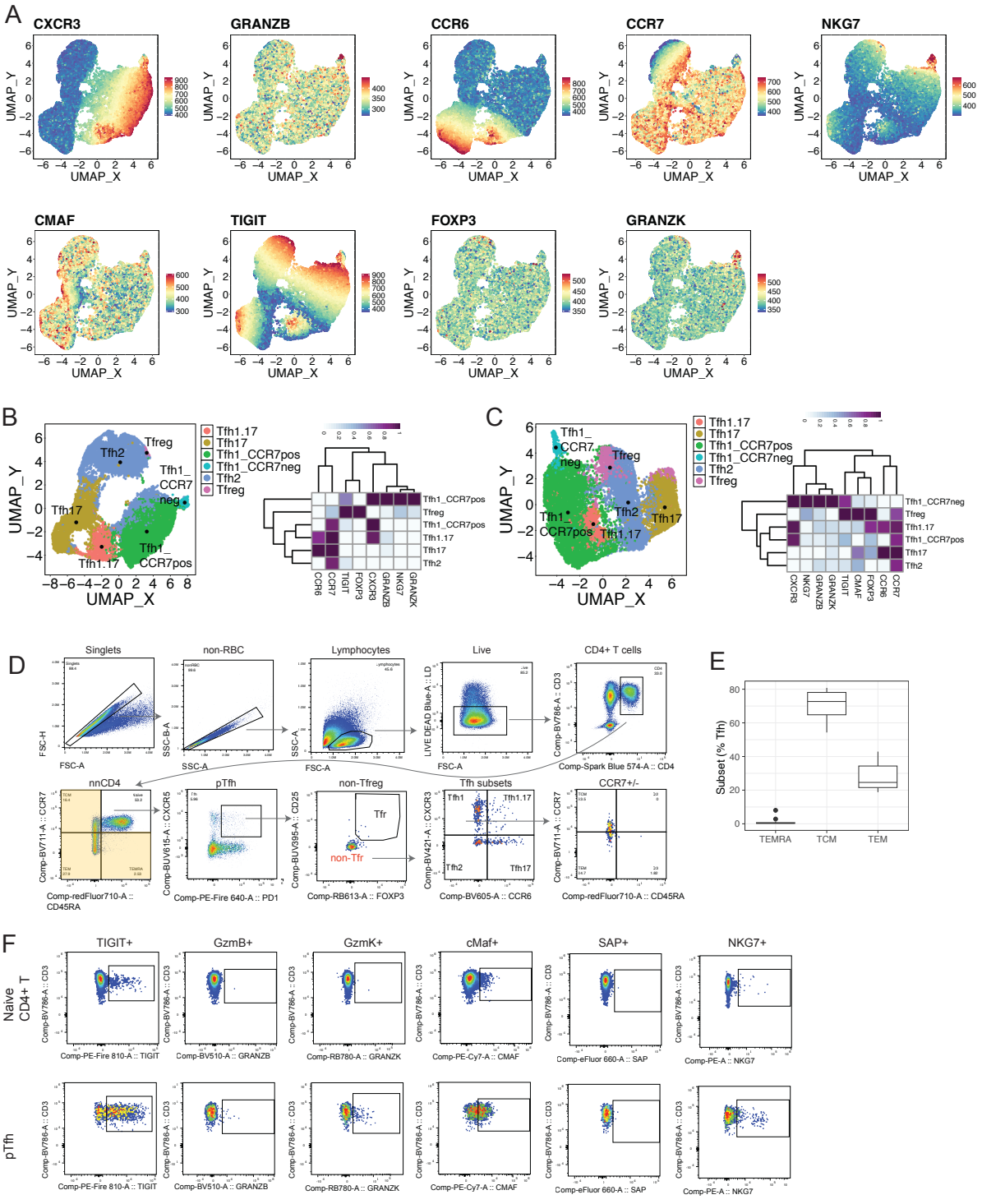
A) Gating strategy for sorting pTfh cell populations scRNAseq. Non-naïve CD4 T cells were identified as CD3+/CD4+, CD45RA- cells. Tfh1 (CXCR3+CCR6-), Tfh2 (CXCR3-CCR6-), Tfh17(CXCR3-CCR6+) were sorted from CXCR5+PD1+ cells, and a resting Tfh cell population from CXCR5+PD1- cells. ***B)*** *QC of cells from each donor and subset, nCount_RNA, nFeature_RNA and percent mitochondrial content is shown.* ***C)*** *UMAP of data before and after integration for donor.* ***D)*** *Gene expression of key phenotyping markers PD1 (encoded by PDCD1), CXCR3 and CCR6 used to sort Tfh subsets phenotypically. UMAP and Vlnplot in each sorted population shown.*



Supplementary Figure 2. Cluster identification in single cell RNA sequencing of pTfh cells from health donors

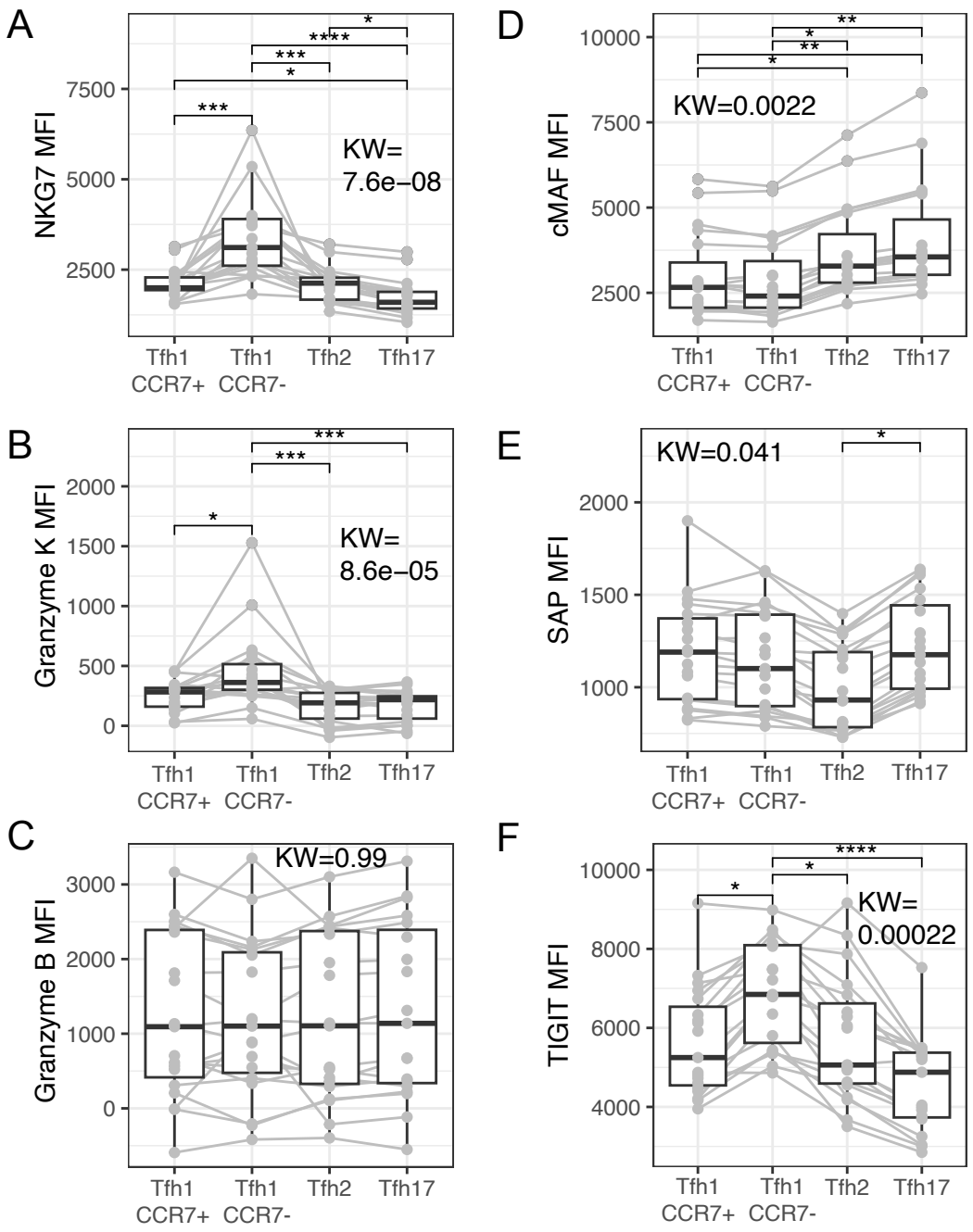
A) UMAP and bar chart showing the number of cells in each predicted cell cluster grouped by their original cell annotation from the human tonsil atlas. ***B)*** *Expression of genes with various roles in Tfh and CD4 T cell development across different Tfh cell clusters.* ***C)****. Ingenuity Pathway Analysis identified top 25 pathways from cluster marker genes (relative to all other Tfh clusters) for Tfh1-CCR7^{neg} and Tfh1-CCR7^{pos} subsets. Number of genes identified in enriched pathway indicated for*

each subset. Pathways of interest in bold. ***D)*** *Relationship between cell sorted identity and transitional cell cluster identify.* ***E)*** *Frequency of individual clones that occurred with clonal size of 1, 2, 3, 4, >5 in each subset.* ***F)*** *Circos plot of clonal overlap between subsets. Each cell of clones (≥2) is represented in a node in the ring, with a cell of each clone indicated by the inner coloured ring and donor by the outer labelled ring. Clones that exist in a single Tfh subset are joined by grey lines, while clones that are shared across Tfh subsets are joined by light blue lines. No clones were shared across donors.*



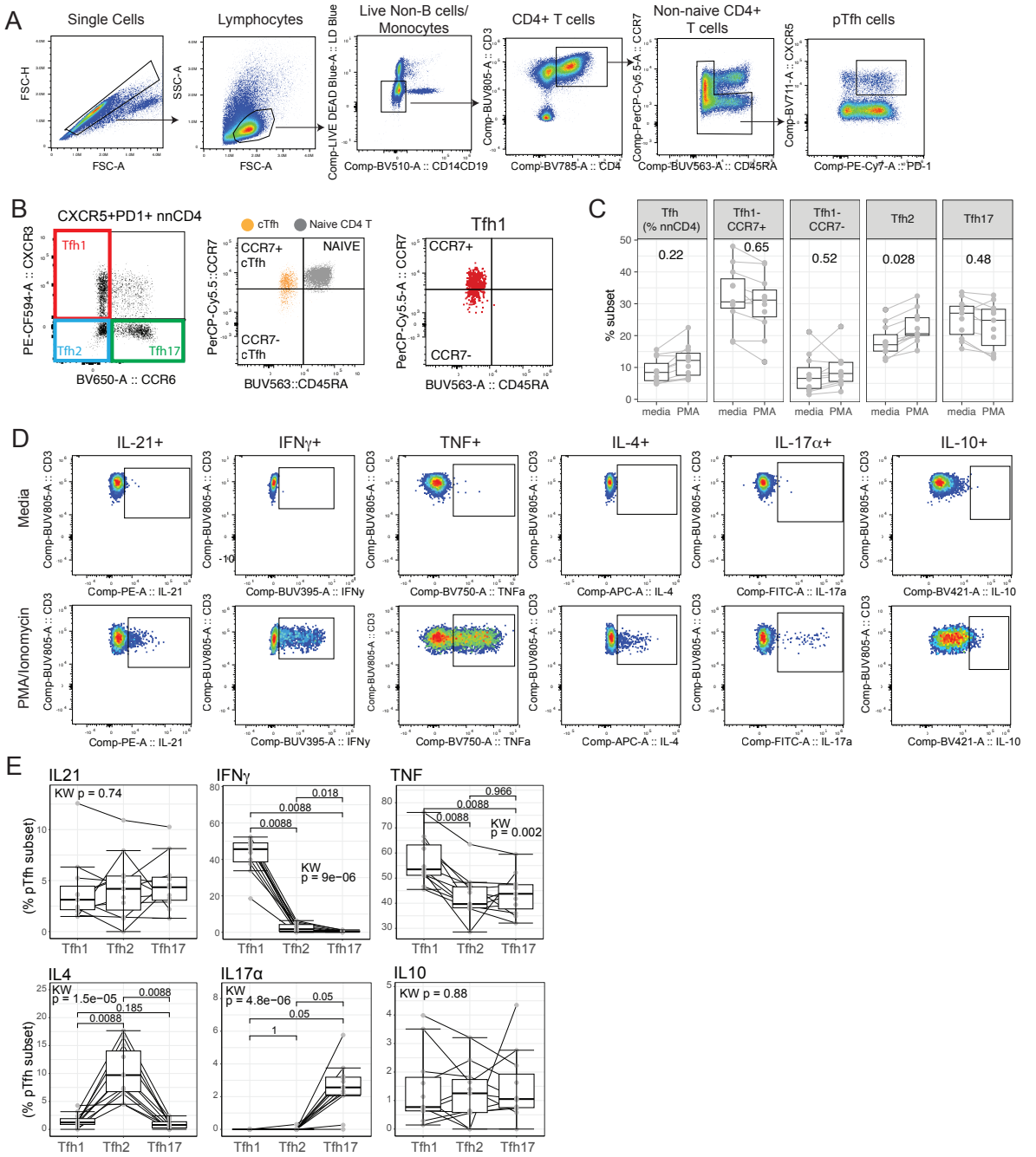
Supplementary Figure 3: Phenotypic diversity within pTfh subsets based on CCR7 expression.

**A)** Expression of each marker used in unbiased analysis (see Figure 2). **B/C)** UMAP of unbias analysis of a single experiment (n=6 for B and n=6 for C). **D)** Gating strategy for identify pTfh cells. pTfh cells were identified as CXCR5+PD1+ non-naïve CD4 T cells. Within pTfh cells, Tfr cells were identified as FoxP3+/CD127^{lo} cells, pTfh subsets identified based on CXCR3 and CCR6, and CCR7 expression analysed. **E)** Proportion of central memory (TCM, CCR7+CD45RA-), effector memory (TEM, CCR7+CD45RA-) and effector memory re-expressing CD45RA (TEMRA, CCR7-/CD45RA+) cells within Tfh cell population. **F)** Marker expression in naïve CD4 T cells and pTfh cells.



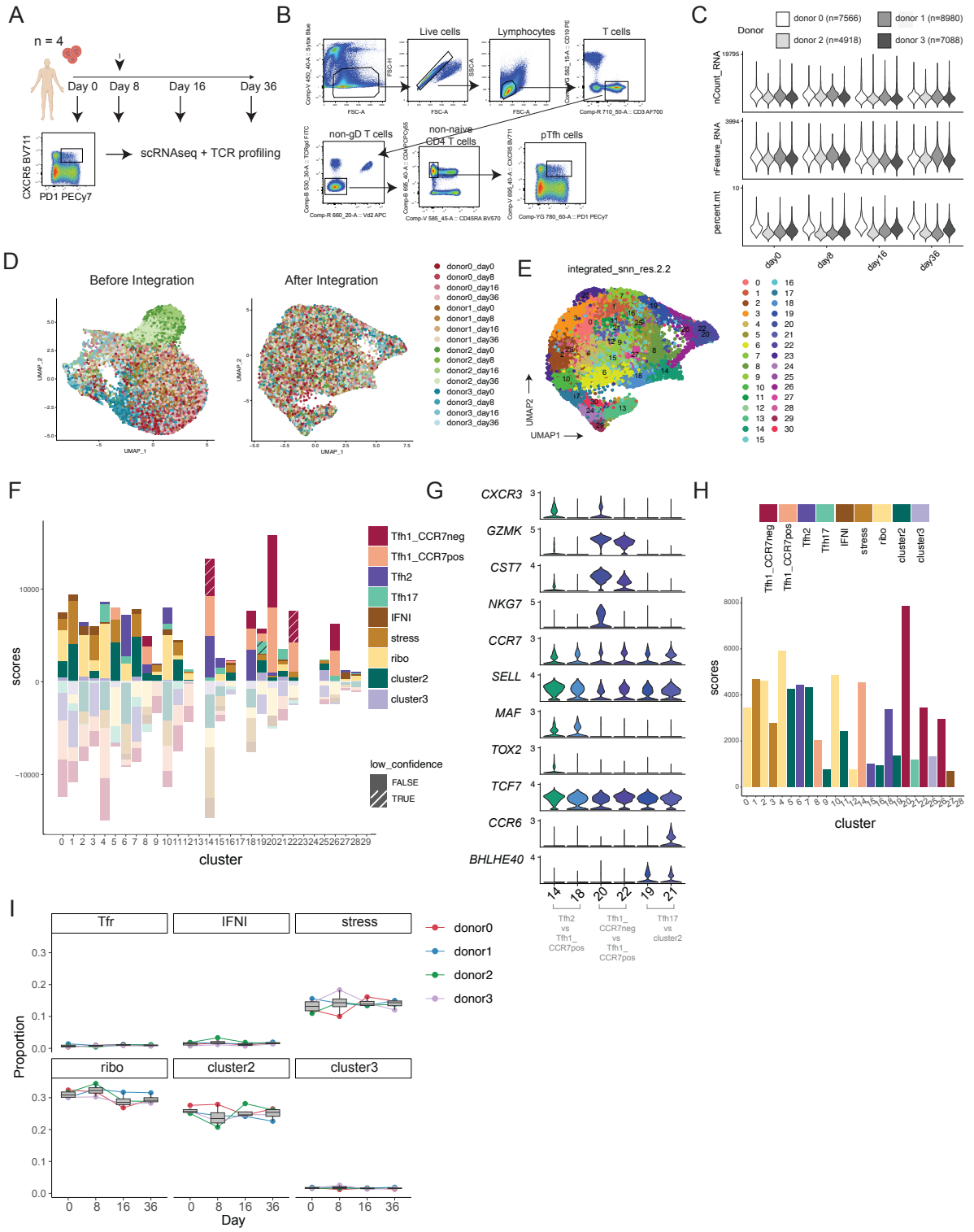
Supplementary Figure 4: Marker expression as MFI on Tfh subsets identified by flow cytometry.

*__A)__ NKG7, __B)__ granzyme K, __C)__ granzyme B, __D)__ cMAF, __E)__ SAP and __F)__ TIGIT MFI on each Tfh subset/phenotypic state in healthy individuals (n=19). Data are marker expression as MFI for each Tfh subset. Kruskal-Wallis test is used for the global comparison. P is post-hoc dunns test with FDR correction for multiple comparisons. Only significant differences (p<0.05) are shown. * <0.05-<0.01, ** <0.01-<0.001, ***<0.001.*



Supplementary Figure 5: Gating strategy for pTfh cells and cytokine expression following PMA/Io stimulation.

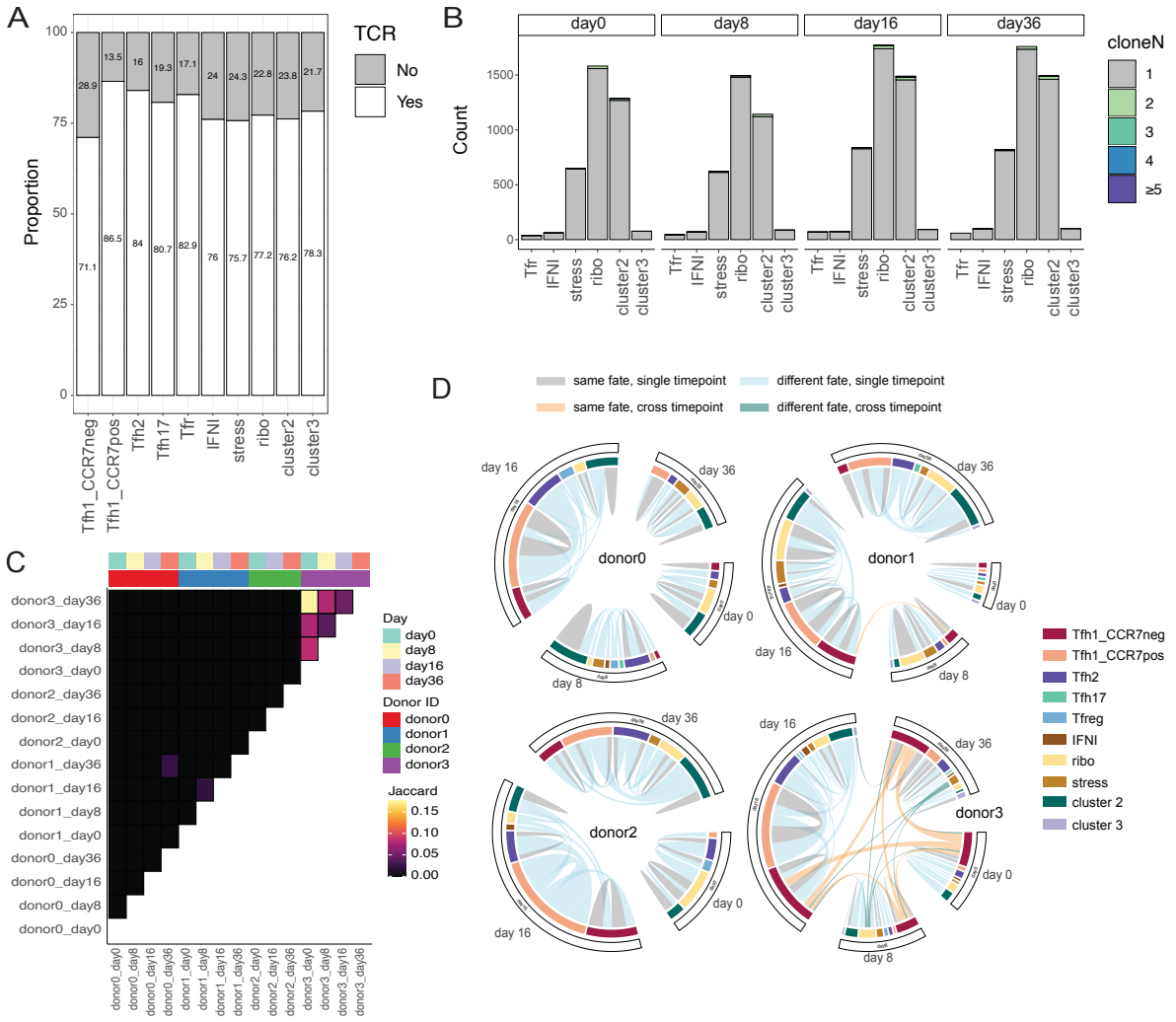
PBMCs were stimulated with PMA/Ionomycin to assess cytokine production. ***A)*** *pTfh cells were identified as CXCR5+PD1+ CD4 T cells.* ***B)*** *A gradient of CCR7 could be detected within pTfh1 cell susbsets, and pTfh1 clusters can be divided into CCR7^{pos} and CCR7^{neg} cells.* ***C)*** *Subset distribution before and after stimulation. Tfh is % of non-naïve CD4, while Tfh subsets are % of total Tfh. P is the paired Wilcoxon signed-rank test.* ***D)*** *Representative data of cytokine expression in pTfh cells in unstimulated (media) or PMA/Ionomycin stimulated cells.* ***E)*** *pTfh cells subsets were identified by CXCR3 and CCR6 expression as pTfh1 (CXCR3+CCR6+), pTfh2 (CXCR3-CCR6-) and pTfh17 (CXCR3-CCR6+) cells (grouping CCR7^{pos} and CCR7^{neg} Tfh1 cells). P is the paired Wilcoxon signed-rank test between groups, adjusted for multiple comparisons with Holm's FDR, and Kruskal Wallis group comparison test.*



Supplementary Fig 6. scRNAseq analysis of Tfh cells in CHMI

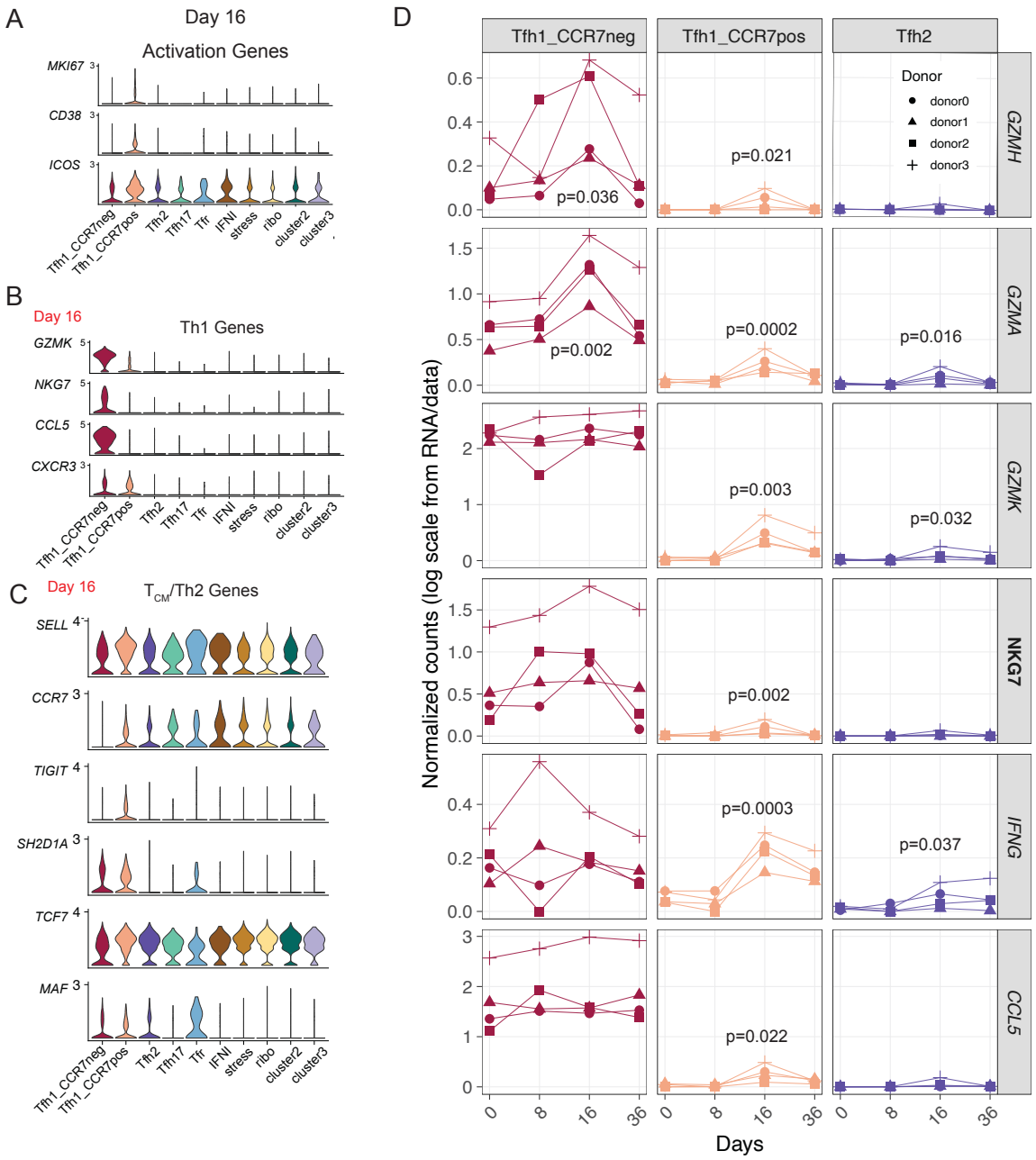
A) *scRNAseq experimental set up for Tfh cells during controlled human malaria infection (CHMI). Tfh cells (CXCR5+PD1+) identified within non-naïve CD4 T cells were sorted from four individuals at day 0, 8, 16 and end-of-study during CHMI. Individuals were treated at day 8 (indicated by dotted arrow).* ***B)*** *Gating strategy for Tfh cell sorting for scRNAseq.* ***C)*** *QC of data from each donor across time points nCount_RNA, nFeature_RNA and percent mitochondrial content is shown.* ***D)*** *Data before and after integration based on donor and CHMI day.* ***E)*** *Over clustering of data prior to scType label*

transferer. ***F)*** *scType was used to label transfer cell signatures (top 20 up and down regulated genes) for each cell subset identified in healthy 'map' data onto over-clustered CHMI data. Each cell cluster was scored for each reference signature (left), and the majority score (right) used to collapse clusters into cell subsets.* ***G)*** *Average expression of Tfh cell cluster markers in each subset for manual adjustment of "low-confidence" predicted cluster identities.* ***H)*** *Final predicted label for each cell cluster.* ***I****) Proportion of other Tfh cell subsets identified over time.*



Supplementary Figure 7: TCR analysis of Tfh cells in CHMI

__A)__ Proportion of cells with TCR captured across each subset. __B)__ Clonal size of other Tfh cell subsets identified over time. __C)__ Clonal overlap between donor and day. __D)__ Circos plot of clonal overlap between subsets for each donor. Each cell of clones (≥2) is represented in a node in the ring, with a cell of each clone indicated by the inner coloured ring and donor by the outer labelled ring. Clones that exist in a single Tfh subset are joined by grey lines, clones that exist in a single Tfh subset across more than one timepoint are in peach, clones that are shared across different Tfh subsets at a single timepoint are joined by light blue lines, and clones that are shared across different Tfh subsets and different timepoints are in green. No clones were shared across donors.



111

112 ***Supplementary Figure 8: Tfh cell activation profiles at day 16 following CHMI***

113 *DEGs were identified in each subsets comparing day 0 to subsequent time points during infection.* ***A)***
114 *Expression of activation genes for all clusters at Day 16.* ***B-C)*** *Despite upregulation of genes*
115 *associated with inflammation, expression profiles were still different between Tfh1_cyto and*
116 *Tfh1_CCR7 subsets. Tfh cell subsets maintained expression profiles of identifying cluster markers.*
117 ***D)*** *Expression of selected inflammatory and cytotoxic genes in each individual in each Tfh subset at*
118 *day 0, 8, 16 and 36 of CHMI. P is adjusted from pseudobulk analysis at day 16 compared to Day 0.*

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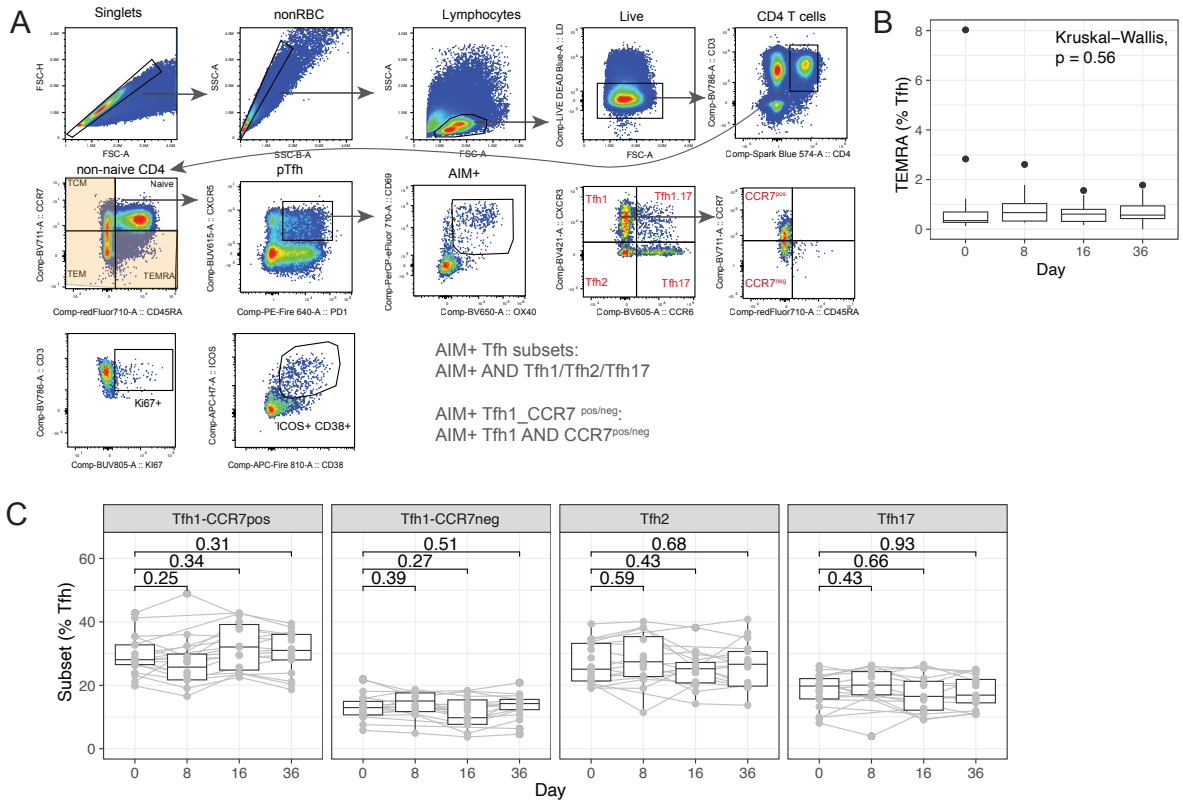
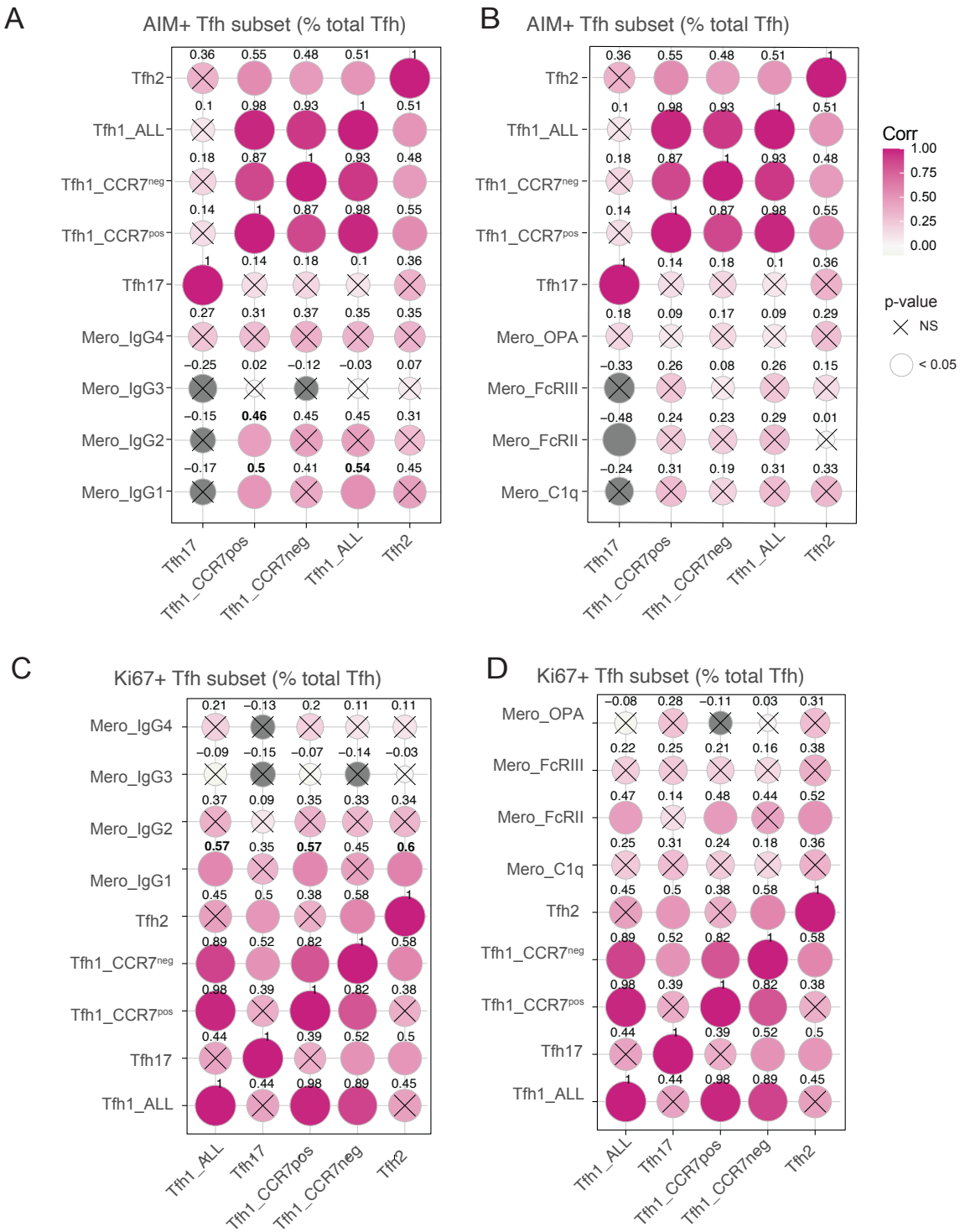


Supplementary Figure 9: Validating Tfh cell profiles in CHMI

A) Gating strategy for identification of malaria specific pTfh cells in individuals undergoing CHMI using Activation Induced Marker assay. ***B)*** *Proportion effector memory re-expressing CD45RA (TEMRA, CCR7-/CD45RA+) within total Tfh population across CHMI. Kruskal Wallis indicated.* ***C)*** *Frequencies of Tfh subsets within the total Tfh compartment across CHMI. P is Wilcoxon rank-sum test.*



Supplementary Figure 10: Correlations between Tfh cells and antibody development in CHMI.

*Correlation matrix between malaria-specific Tfh subsets **(A/B)** or Ki67 expression of Tfh subsets **(C/D)** at day 36 with either IgG subclasses to merozoite **(A/C)** or functional capacity to fix complement (C1q), bind dimeric FcγRIIa or FcγRIIIa (surrogates for IgG antibody capacity to crosslink cellular receptors), and promote opsonic phagocytosis (OPA) **(B/C)**. Spearman's rho and p-value are indicated.*