SUPPLEMENTAL MATERIAL

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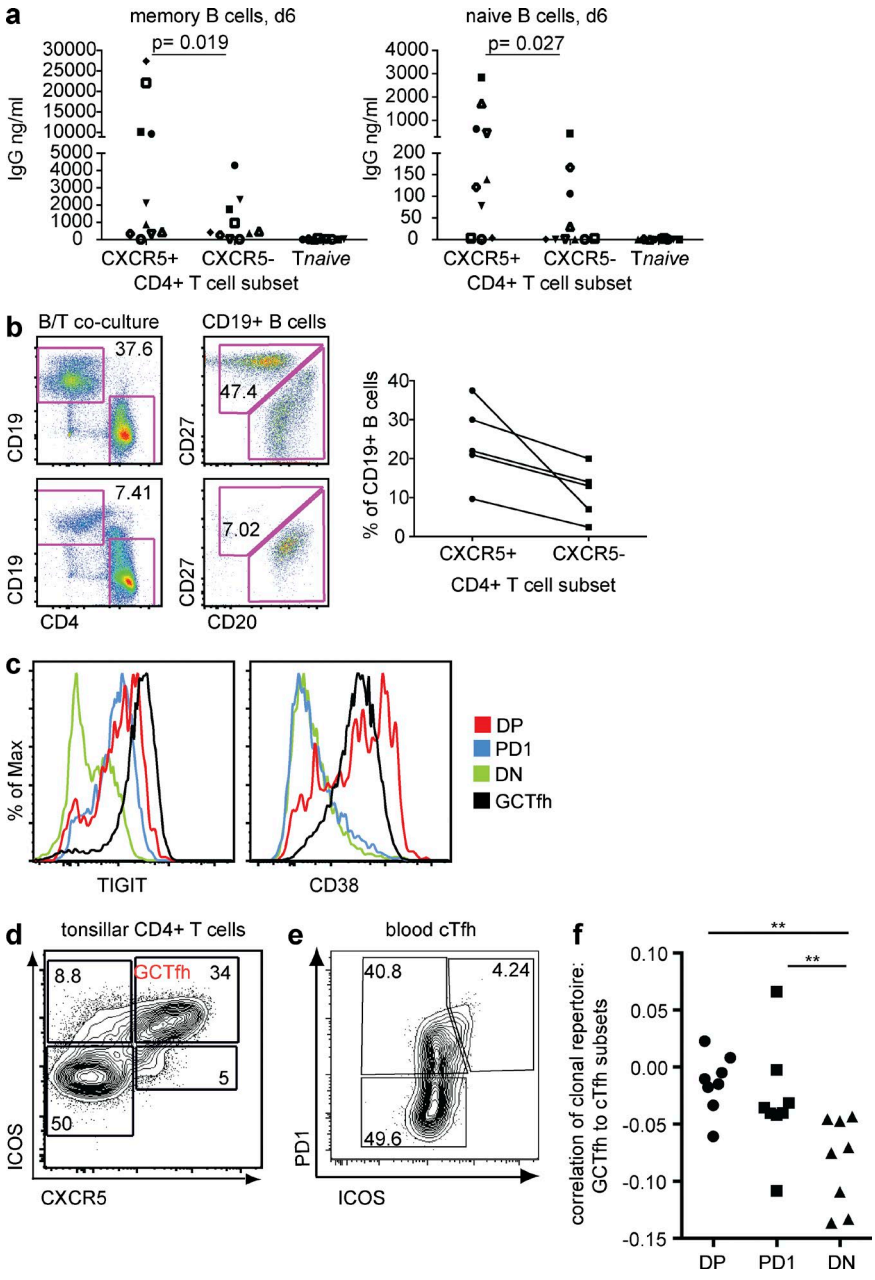

Figure S1. **Peripheral CXCR5⁺CD4⁺ T cells provide prototypical B cell helper function and phenotypically relate to GCTfh.** Purified CXCR5⁺, CXCR5⁻, and naive CD4⁺ T cell from PBMC of healthy donors (n = 10) were co-cultured with autologous memory or naive B cells and tested for their capacity to induce antibody production (a) and to support B cell differentiation and survival (b). (a) Supernatant from co-cultures (memory B cells, left; naive B cells, right) were harvested at day 6 and IgG levels were assessed by ELISA. Wilcoxon signed rank test was used to evaluate differences in antibody production among the three CD4⁺ T cell subsets. (b) Co-cultured cells were harvested and restained to differentiate naive B cells (CD19⁺CD20⁺CD27⁻), plasmablasts (CD19⁺CD20⁻CD27⁺) and CD4⁺ T cells. (left) Representative plots from one CXCR5⁺ (top) and one CXCR5⁻ (bottom) co-culture is shown. (right) Bulk CD19⁺ B cells from the same co-cultures (n = 5) were enumerated and plotted as the percentage of total cells from either CXCR5⁺ or CXCR5⁻ CD4⁺ T cell co-cultures. (c) The histograms shows expression of TIGIT and CD38 expressed by one representative donor matched pair of cTfh subsets and GCTfh, (d) Staining example for tonsillar CD4⁺T cells stratified by ICOS and CXCR5 expression is shown. (e) Staining example for the donor matched (d) circulating, blood CXCR5⁺ CD4⁺T cells further stratified by ICOS and PD1 expression is shown. (f) GCTfh (d) and cTfh subsets (e) were isolated from four donor matched tonsils/PBMC samples to high purity, followed by deep sequencing of their TCRB clonal repertoire. The correlation of TCRB profiles between GCTfh and cTfh subsets was calculated for four donors and is plotted. **, P < 0.01; or as indicated by Wilcoxon matched pair signed rank test.

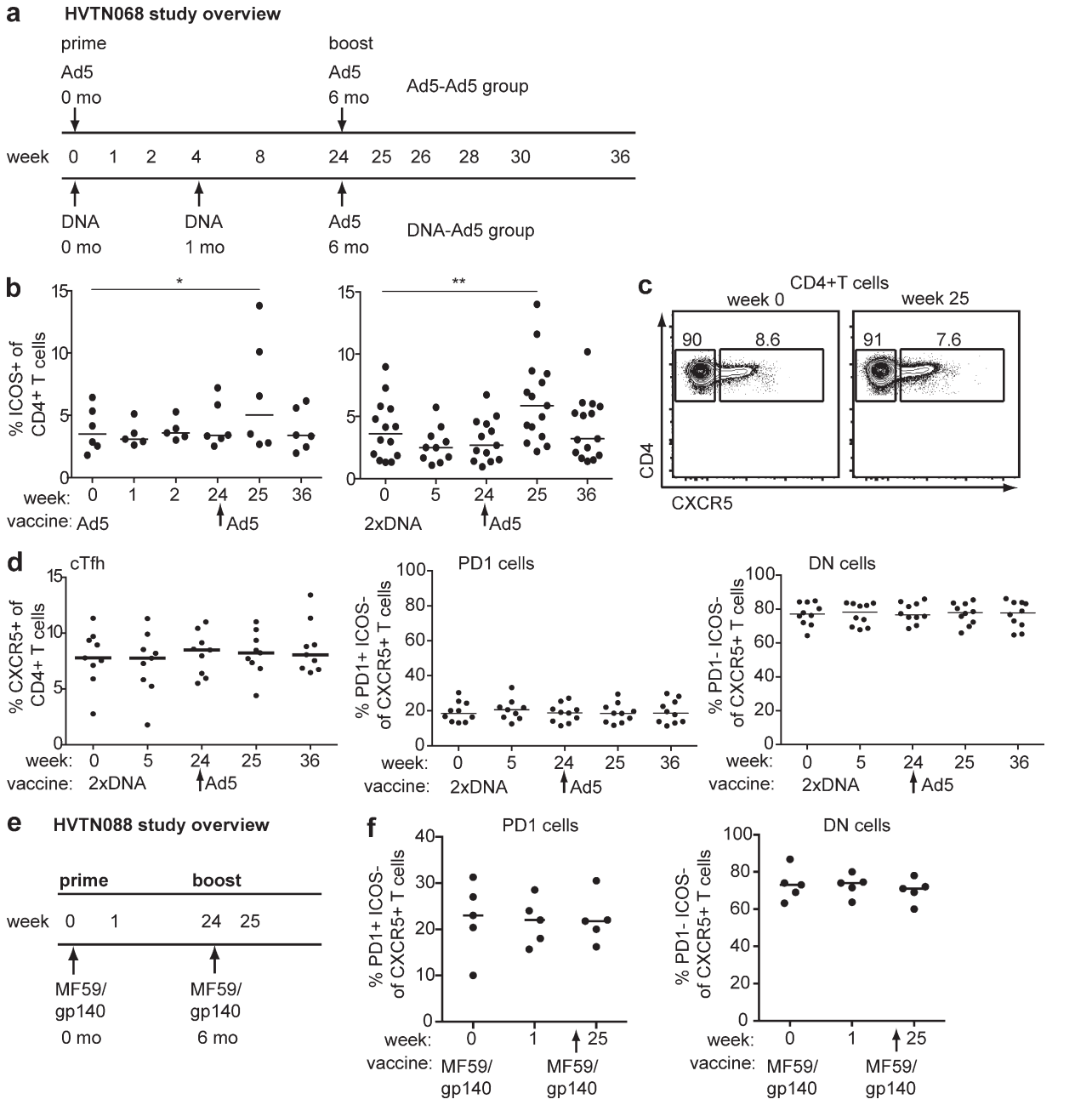

Figure S2. **Three vaccination regimens and their impact on circulating CXCR5⁺ cell subsets.** Blood specimens were collected and PBMCs were isolated and cryopreserved from study participants in HVTN068 and HVTN088 protocols evaluating experimental HIV vaccine candidates in phase 1 studies. (a) In HVTN068, healthy, Ad5-seronegative (neutralizing antibody titers <1:12) adult study participants were enrolled. Two candidate HIV-1 vaccine prime-boost regimens were compared. Group one (Ad5-Ad5 group): Ad5 prime at week zero and Ad5 boost at week 24. Group two (DNA-Ad5 group): DNA prime at week zero and four (2xDNA), followed by an Ad5 boost at week 24. (b) The percentage of total CD4⁺ T cells expressing ICOS within PBMCs collected from HVTN068 participants in the Ad5-Ad5 group (left, $n = 6$) or DNA-Ad5 group (right, $n = 15$) is plotted over time. (c) Representative staining of CXCR5 within CD4⁺ T cells in PBMCs from one DNA-Ad5 group participant at week 0 and 25. (d) Longitudinal data from DNA-Ad5 group samples matching those shown in Fig. 2 b. Bulk CD4⁺CXCR5⁺ cells, as well as PD1 and DN, are plotted over the course of vaccination. (e) In HVTN088, healthy adults were enrolled who were either primed or naive for HIV-1 Env antigens, based on previous HIV vaccine exposure. Specimens used here are from a subset of HIV-1 antigen naive participants who received a homologous prime (week 0) and boost (week 24) HIV-1 gp140 protein/MF59 adjuvant vaccine regimen. From this study, cryopreserved PBMCs were available from five donors at baseline (week 0), week 1, and week 25. (f) Longitudinal data from HVTN088 samples matching those shown in Fig. 2 e are plotted for week 0, 1, and 25 for PD1 and DN. *, $P < 0.05$; **, $P < 0.01$; by ratio-paired Student's *t* test.

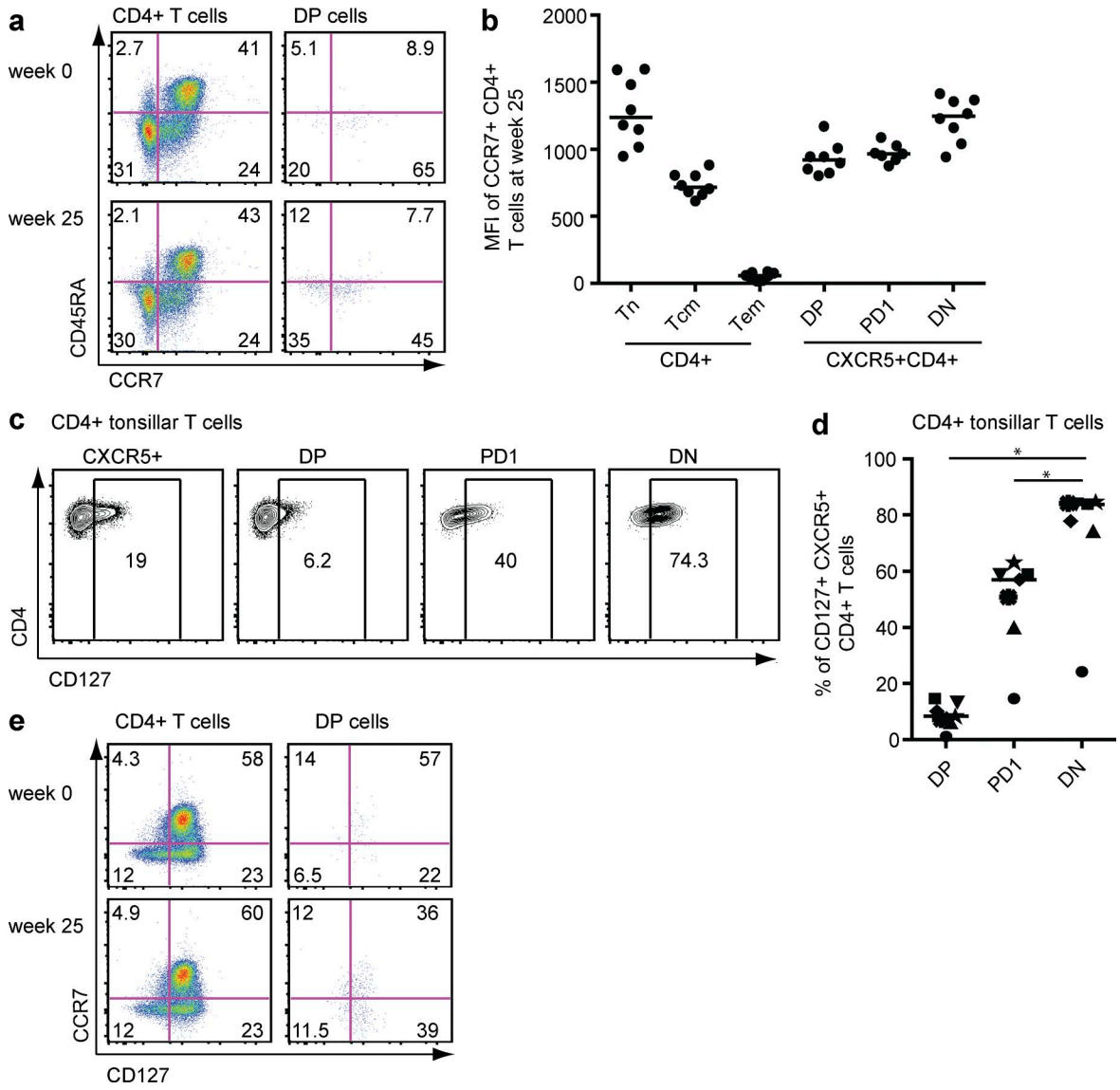

Figure S3. **Memory phenotypes of cTfh subtypes.** PBMCs collected from DNA-Ad5 recipients ($n = 8$), matching those shown in Fig. 3 at weeks 25 were surface stained with the memory T cell markers CD45RA, CCR7, and CD127 to identify naive (Tn; CD45RA$^+$CCR7$^+$), central memory (Tcm; CD45RA$^-$CCR7$^+$, effector memory (Tem; CD45RA$^-$CCR7$^-$), and effector (Teff; CD45RA$^+$CCR7$^-$) CD4$^+$ T cells (CD4$^+$CXCR5$^+$, left; CD4$^+$CXCR5$^-$, right). (a) Representative staining of subsets for CD45RA and CCR7 is shown on total CD4$^+$ and DP cells at baseline and peak response (week 0 and 25, respectively) for one DNA-Ad5 participant. (b) Comparison of the expression level of CCR7 on different CXCR5$^+$CD4$^+$ T cell subsets and bulk CD4$^+$ T cell memory populations after vaccination with DNA-Ad5. (c) CD127 expression of one representative tonsil sample stratified into bulk CXCR5$^+$CD4$^+$ T cells and the three Tfh subsets is shown. (d) The frequency of CD127$^+$CXCR5$^+$CD4$^+$ tonsillar T cells ($n = 7$) is shown for each cTfh subset. (e) One representative staining of CD127 expression on bulk CD4$^+$ T cells and DP cells at week 0 and week 25 is shown. *, $P < 0.05$ by Student's t test.

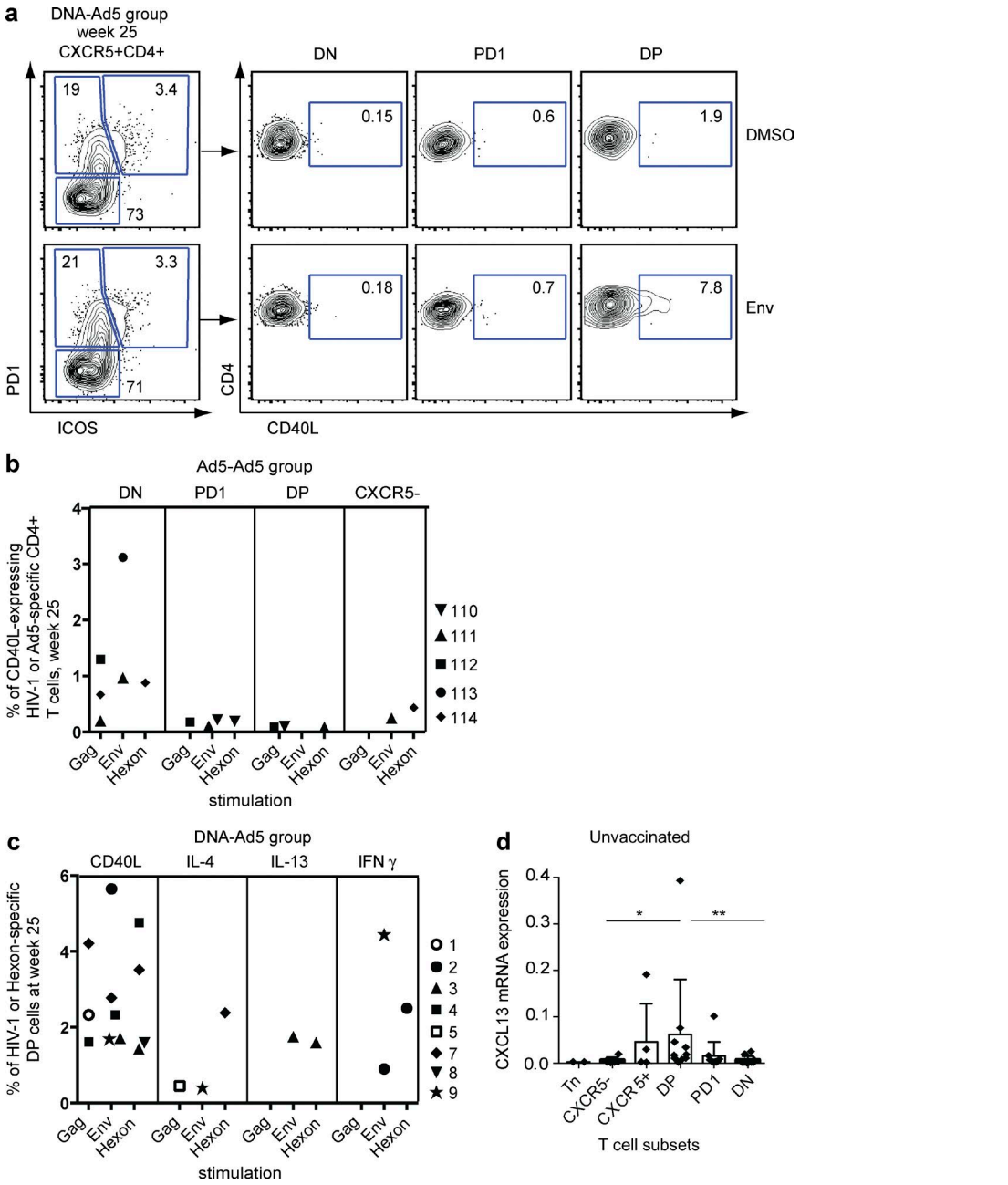

Figure S4. **Antigen-induced T cell activation of cTfh.** Total PBMC from HVTN068 participants were stimulated for five hours with the vaccine-insert matched peptide pools (Gag or Env) or Ad5 vector-associated peptide pools (Hexon). (a) Representative plots for intracellular expression of CD40L by distinct cTfh subsets from one DNA-Ad5 participant at week 25 is shown after stimulation with DMSO (top) or Env (bottom) peptide pool. (b) Percentages of CD40L-expressing $CD4^+$ T cells identified as DP, PD1, DN, or $CXCR5^-$ at week 25 are shown for the Ad5-Ad5 group; only positive responses are shown (calculated as ≥3× over background and ≥0.5% DMSO control). (c) The percentage of CD40L, IL-4, IL-13, and IFN-γ producing DP in response to stimulation with HIV-1–associated peptide pools or the Ad5 vector-associated peptide pool at week 25 are shown. Responses are only plotted when ≥3× over background and ≥0.5% DMSO control. (d) CXCL13 mRNA expression is shown in highly purified $CD3^+CD4^+CD45RO^-CXCR5^-$ (Tn), $CD3^+CD4^+CD45RO^+CXCR5^-$ ($CXCR5^-$), $CD3^+CD4^+CD45RO^+CXCR5^+$ ($CXCR5^+$), and cTfh subsets for 10 healthy unvaccinated donors. RNA was extracted for gene expression analysis by qPCR without further in vitro stimulation. mRNA levels shown are normalized to GAPDH.

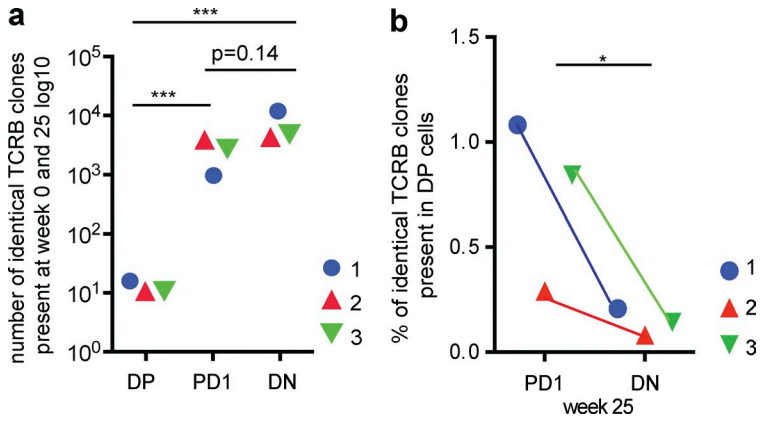

Figure S5. **The clonal repertoire relationship of cTfh.** (a) The number of TCRB clones that were present in the different cTfh subsets at both week 0 and 25 is plotted for each donor: $PD1^+ICOS^+$ (DP), $PD1^+ICOS^-$ (PD1), and $PD1^-ICOS^-$ (DN). (b) The percentage of PD1 or DN clones also present in DP at week 25 is plotted. *, $P < 0.05$; ***, $P < 0.001$.

Table S1. **Antibody information for Tfh and B cell phenotyping**

Antibody	Supplier	Clone
LIVE/DEAD Fixable Aqua Dead Cell Stain	Invitrogen	Not applicable
CD3 APC-H7	BD	SK7
CD3 BV570	BioLegend	UCHT1
CD8 BV711	BD	RPA-T8
CD4 Alexa Fluor 680	BD	RPA-T4
CD4 UV393	BD	SK3
CD45RA BV650	BioLegend	HI100
CCR7 Alexa Fluor 680	BD	150503
CXCR5 Biotin	BD	RF8B2
Streptavidin ECD	Beckman-Coulter	Not applicable
CXCR5 Alex647	BD	RF8B2
PD1 (CD279) PECy7	BioLegend	EH12.2H7
ICOS Alexa Fluor 488	BioLegend	C398.4A
CD27 BV650	BioLegend	O323
CD27 APC	BD	L128
CD127 BV421	BioLegend	A019D5
CD19 UV797	BD	SJ25C1
Slam (CD352) PE	BioLegend	NT-7
TIGIT PE	eBioscience	MBSA43
CD38 PERCPCy5.5	BD	HIT2