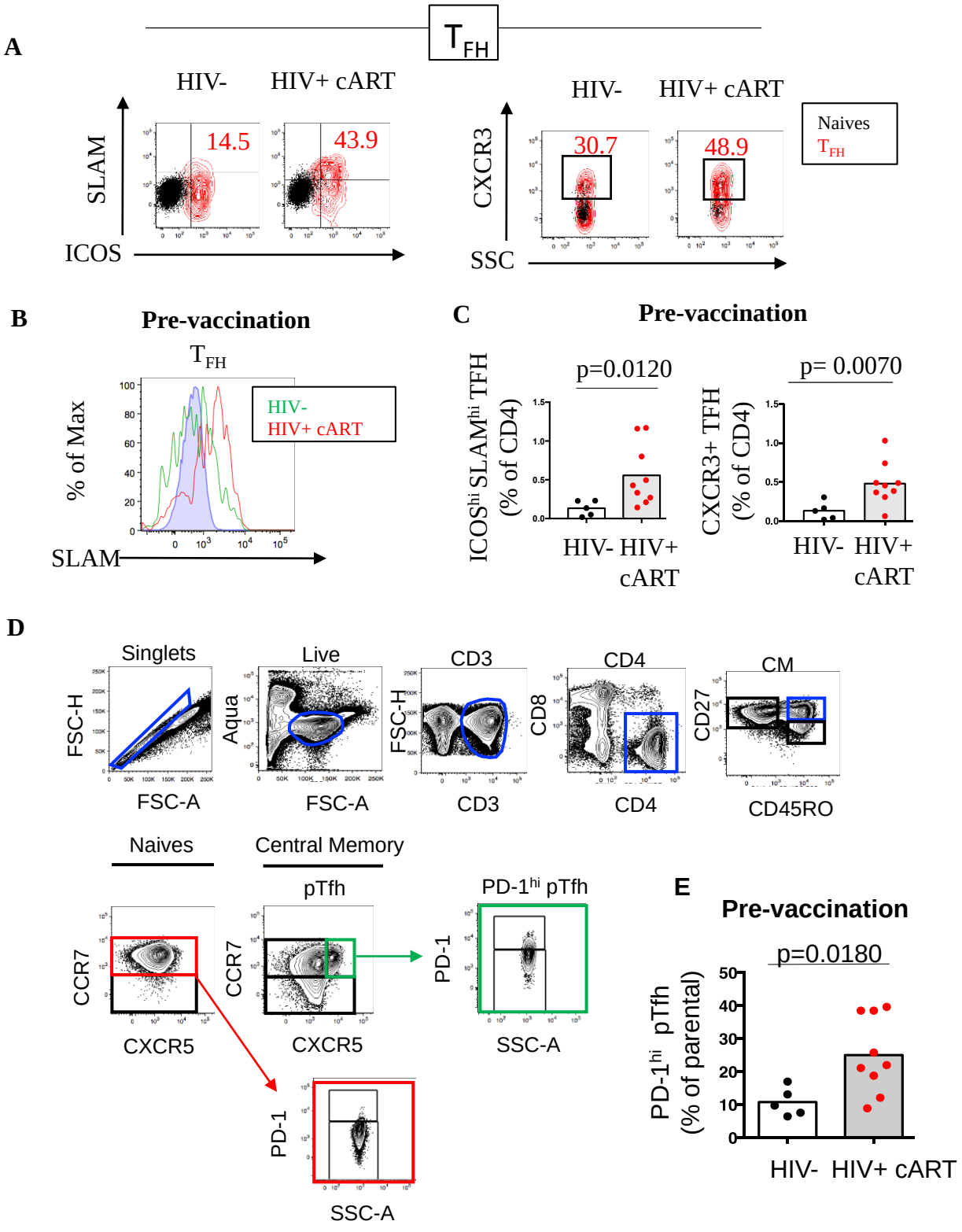
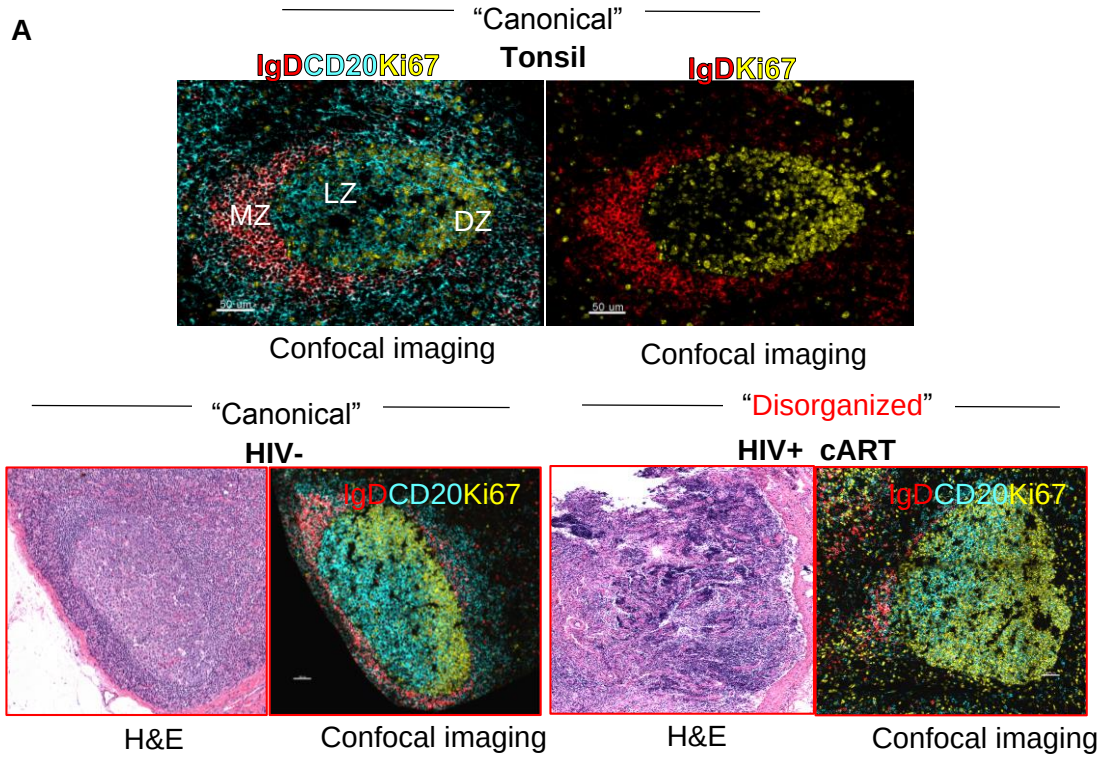
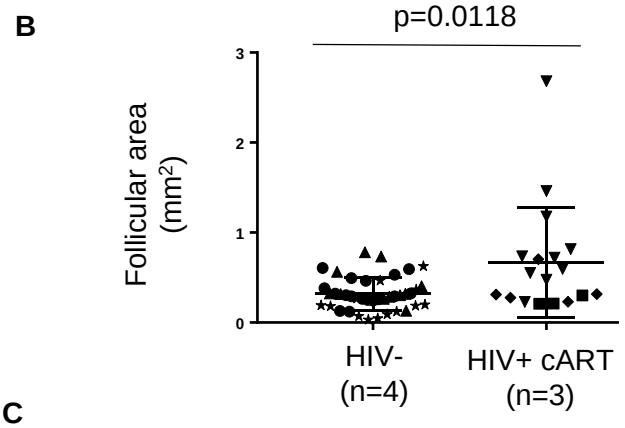
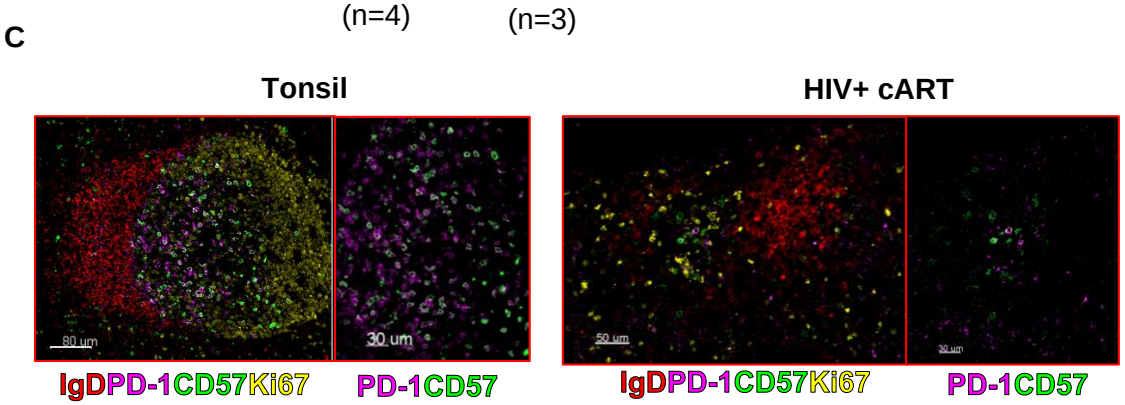
Supplemental Figure 1. (A) Representative flow cytometry plots showing the pre-vaccination distribution of CXCR3, ICOS and SLAM in LN suspensions in HC and HIV+ samples. Gates for the analysis were set based on the phenotype of the naïve population (in black in dot plots and in blue in histograms) (B) Representative histograms showing the per cell (MFI) expression of SLAM in HC T_{FH} (green), HIV+ T_{FH} (red) and HC naïve T cells (purple) (C) Cumulative plots showing expression levels of $ICOS^{hi}SLAM^{hi}$ and CXCR3+ in HC and HIV+ patients pre-vaccination (D) Gating strategy used to define circulating, peripheral blood T_{FH} (pT_{FH}) cells in flow cytometry datasets (E) Plot showing the pre-vaccination frequency of PD-1^{hi} T_{FH} cells in HC (n=5) and HIV+ (n=9) participants. Bars represent means and p values represent unpaired Mann-Whitney U-tests.



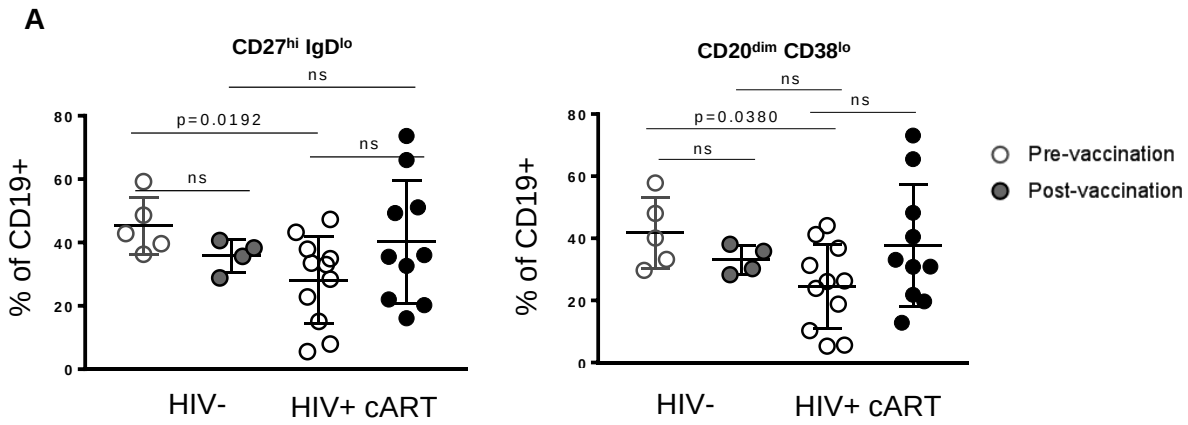
Supplemental Figure 2. (A) Representative examples of “canonical” preserved (left panel) and “disorganized” (right panel) B-cell follicles as seen in hematoxylin and eosin (H&E) staining and confocal imaging in HC and HIV+ participants respectively. For confocal imaging tonsil (upper panel) and LN (lower panel) tissue sections were stained with anti-IgD for naïve B-cell definition (red), anti-CD20 for B-cells (cyan) and Ki67 (yellow) (B) Grouped data for size of follicular areas (mm2) pre-vaccination in HC (n=4) and treated HIV+ individuals (n=3) (C) CD57+ TFH distribution in tonsillar GCs and in lymph node GCs from HC and HIV+ participants. The HIV+ cART area presented here is also shown in main figure 2A. Each symbol represents an individual follicle and follicles belonging to the same tissue share the same symbols. p value is Mann-Whitney test. All images were acquired at 40x (NA 1.3). Scale bars are 50um unless noted otherwise. MZ, mantle zone; LZ, light zone; DZ, dark zone

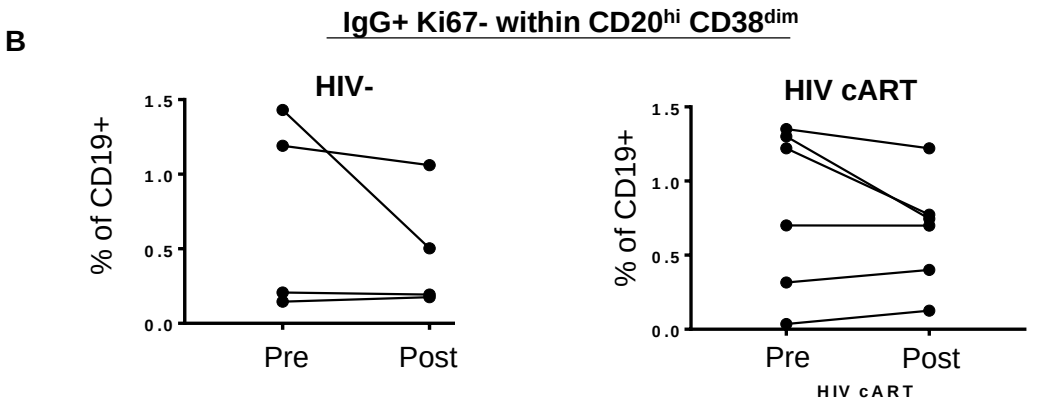


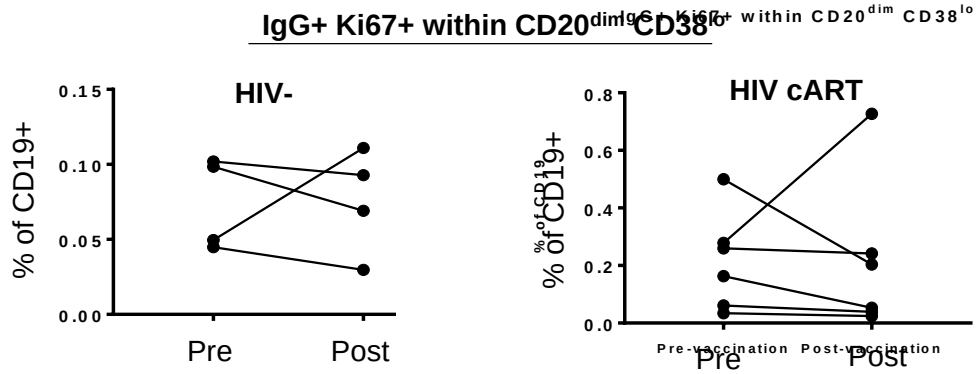


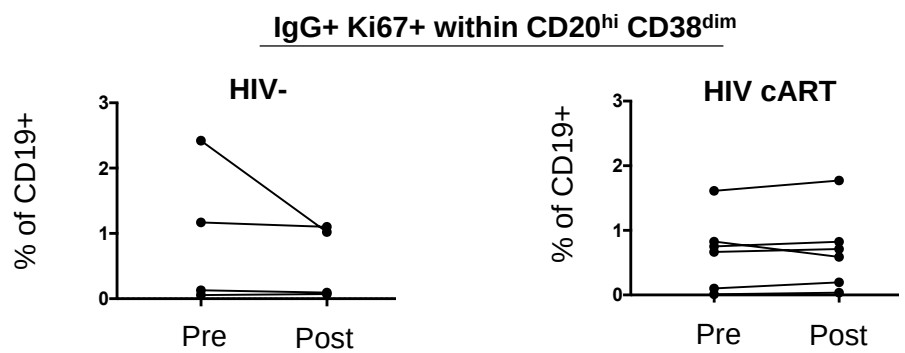


Supplemental Figure 3. (A) Cumulative frequencies of $CD20^{dim}$ $CD38^{lo}$ and $CD27^{hi}$ IgD^{lo} memory B-cells in HC and HIV+ participants pre- (open circles) and post-vaccination (closed circles) as measured using polychromatic flow cytometry. (B) Paired pre- and post-vaccination frequencies of IgG+ Ki67- and IgG+ Ki67+ populations within $CD20^{hi}CD38^{dim}$ (upper row) and $CD20^{dim}CD38^{lo}$ and $CD20^{hi}CD38^{dim}$ (middle and lower row) memory B-cells. Data are given for both HC (n=4) and HIV+ (n=6) participants. p value is a Mann-Whitney t-test.

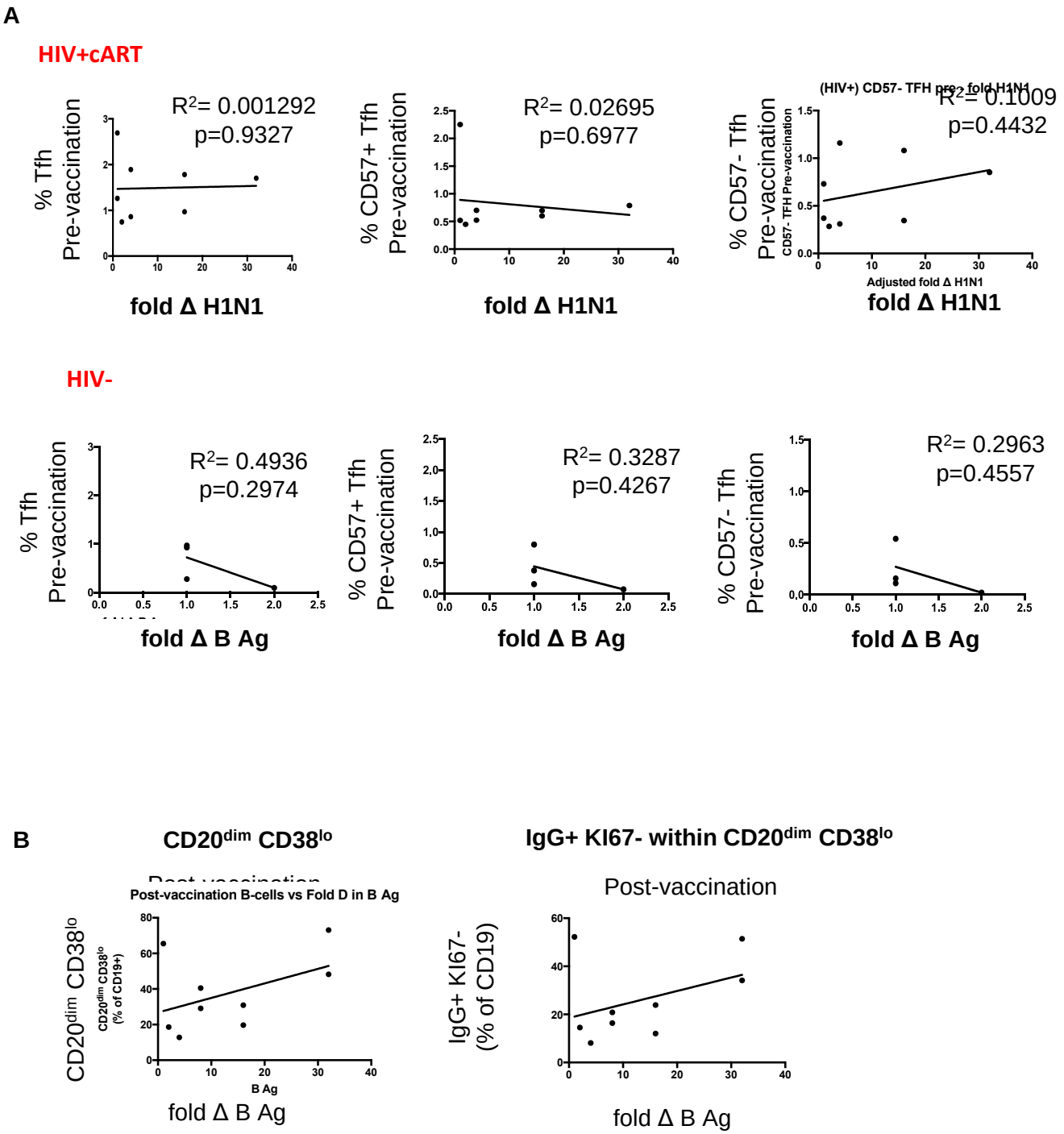




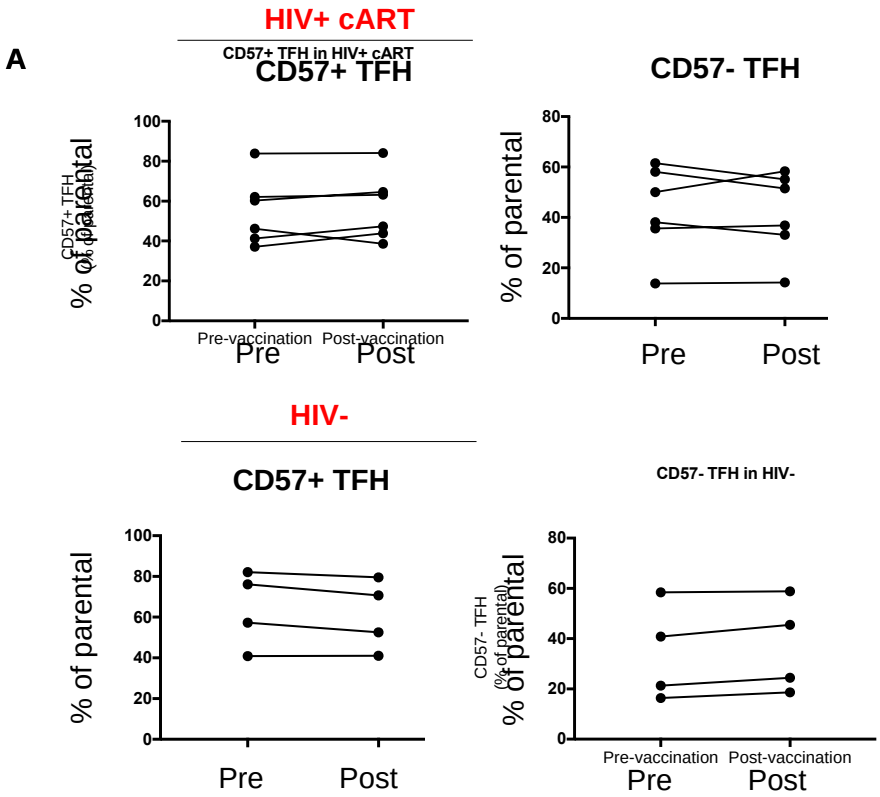


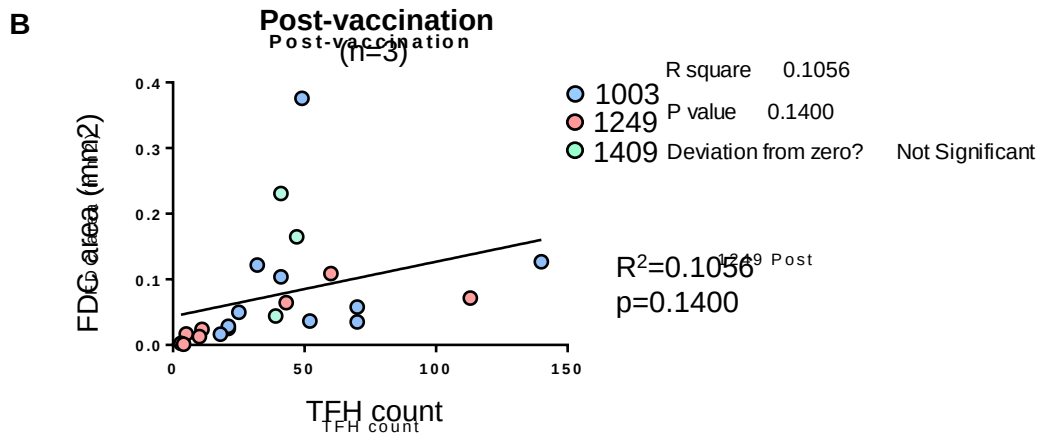


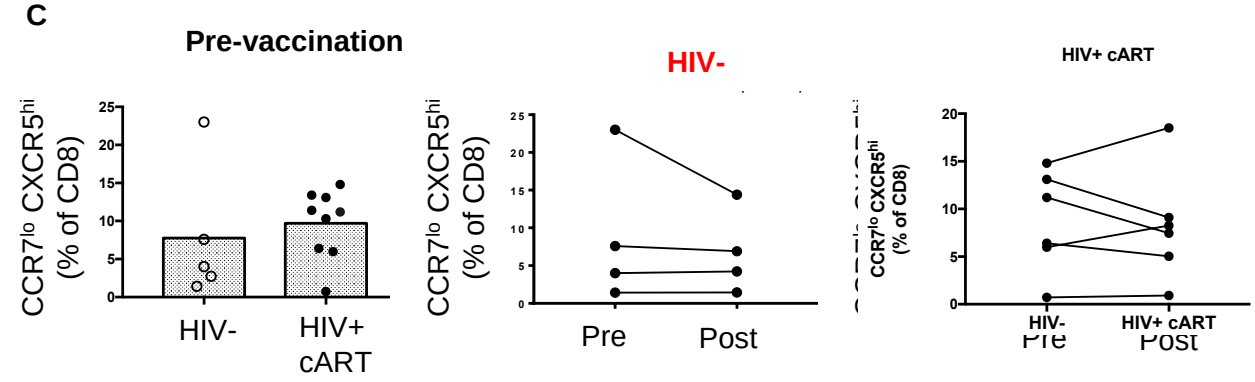
Supplemental Figure 4. (A) Percentage of T_{FH} cells, CD57+ T_{FH} and CD57- T_{FH} cells pre-vaccination as a function of the fold difference in influenza H1N1 antigen (HIV+ donors, n=8) (upper panel) or B Ag titer (HC, n=4) (lower panel) as measured by HIA (B) Percentage of $CD20^{dim}$ $CD38^{lo}$ B-cells and and IgG+ Ki67- B-cells within the $CD20^{dim}$ $CD38^{lo}$ subset as a function of the fold difference in B Ag titer as measured by HIA (n=9 for both graphs). Lines indicate correlations determined by linear regression analysis.



Supplemental Figure 5. (A) Paired frequencies of CD57+ and CD57- T_{FH} cells pre- and post-vaccination in 6 HIV+ participants (upper row) and 4 HC (lower row) expressed as a percentage of the parental population. (B) FDC area per follicle (mm2) expressed as a function of T_{FH} cell count post-vaccination in 3 HIV+ participants. T_{FH} cell count was measured using histocytometry (C) Frequencies of CXCR5+ CD8+ T-cells in LN suspensions of HC (n=5) and HIV+ (n=9) participants as measured by flow cytometry.

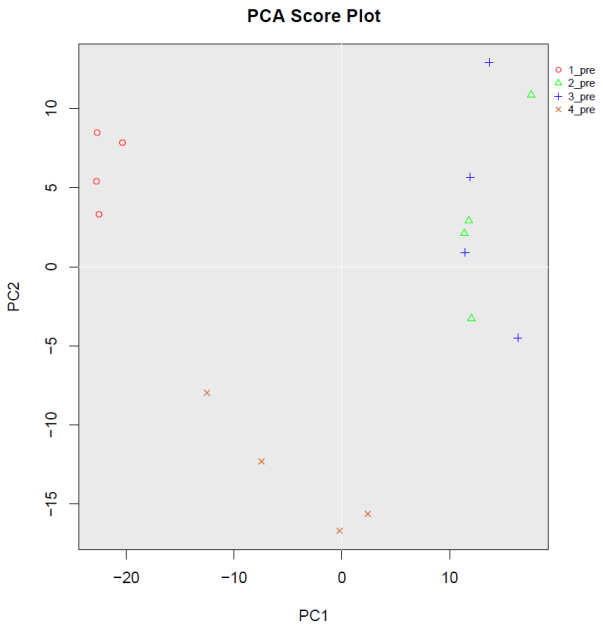






Supplemental Figure 6: Principle component analysis (PCA) score plot showing clustering of sort-purified LN CD4 T cell subsets at (A) pre-vaccination timepoint and (B) before and after vaccination in HC. Subset designations are 1: Naïve, 2: CD57+ T_{FH} cells, 3: CD57- T_{FH} cells , and 4: Non-T_{FH} Memory. Graphics were generated using SingulaR (Fluidigm).12

A HIV-
Pre-vaccination



B HIV-
Pre- and Post-vaccination

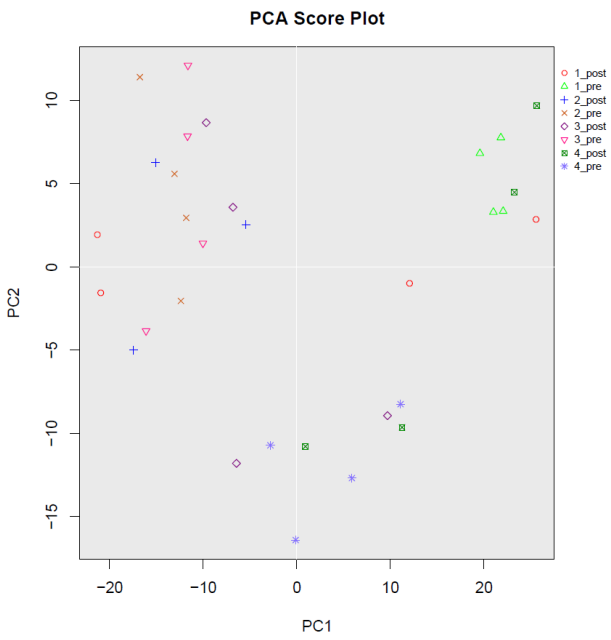


Table S1: Clinical, virological and immunological characteristics of study patients.

HIV+	CD4 (cell/µl)		CD4 IA (%)		CD8 IA (%)		H1N1 Ab response			B Ab response			Vaccine¶	Assays Pre	Assays Post
cART	T0	T1	T0	T1	T0	T1	T0	T1	FC	T0	T1	FC			
1001	855	949	3.7	3.7	12.5	12.5	1280	5120	4	160	640	4	Fluzone	F*	n/a
1002	767				7.5		1280			320			Fluzone	F*	n/a
1003		410		3.2		10.8		40			160		Fluzone	n/a	F
1219		450		1.2		5.6		320			160		Fluzone	n/a	F
1231		737		2.6		5.6		320			640		Fluzone	n/a	F
1240		945		5		8.3		160			160		Fluzone	n/a	F
1249§	893	845	6.5	6.5	10.9	10.9	160	2560	16	80	1280	16	Fluzone	F,H	H
1404	448	421	2.1	2.1	4.2	4.2	320	320	1	80	640	8	Fluzone	F,H	F
1409§	762	687	8.2	8.2	12.6	12.6	320	5120	16	80	1280	16	Fluzone	F,H	F,H
1410§	805	815	10.2	10.2	15.5	15.5	2560	2560	1	160	5120	32	Fluzone	F,H	F,H
1411	729	780	8.5	8.5	7.6	7.6	320	1280	4	320	1280	4	Fluzone	F,H	n/a
1433	668	668	3.2	3.2	5.6	5.6	160	320	2	40	320	8	Afluria	F,H	F
1421	1200	1231	2	3	5	4.2	10	320	32	10	160	16	Afluria	F,H	F
1427	253	490	15.8	15.8	39	39	40	160	4	320	1280	4	Afluria	F	F
1428	748		13.2		46.1		640		0	1280		0	Afluria	F	n/a
HC															
1250	1001	900	1.6	1.6	3.2	3.2	640	640	1	80	80	1	Fluzone	F,H	F
1407	1251		3.5		5.2		160			80			Fluzone	F,H	n/a
1451	711	827	2	2	1.5	1.5	320	640	2	160	160	1	Fluzone	F,H	F
1455	628	653	4	5.3	10.6	8.6	1280	1280	1	5120	5120	1	Afluria	F,H	F
1189	940	916	2.5	2.5	7	7	320	640	2	640	1280	2	Afluria	F	F

§ Samples used in pre- vs post-vaccination histocytometry analysis

* Partial dataset only due to limiting cell numbers

¶ Type of trivalent vaccination received. Study participants were vaccinated with either trivalent Fluzone (*Sanofi Pasteur*) consisting of A/California/07/2009, X-223A (H3N2), B/Massachusetts/02/2012 (B Yamagata lineage) or Afluria (*bioCSL*) consisting of A/California/07/2009 (H1N1); A/South Australia/55/2014 (H3N2), B/Phuket/3073/2013

F, Flow cytometry; FC, Fold Change; HC, Healthy Control; H, Histocytometry

Supplemental Table