Supplementary Information

Inadequate T follicular cell help impairs B cell immunity during HIV infection

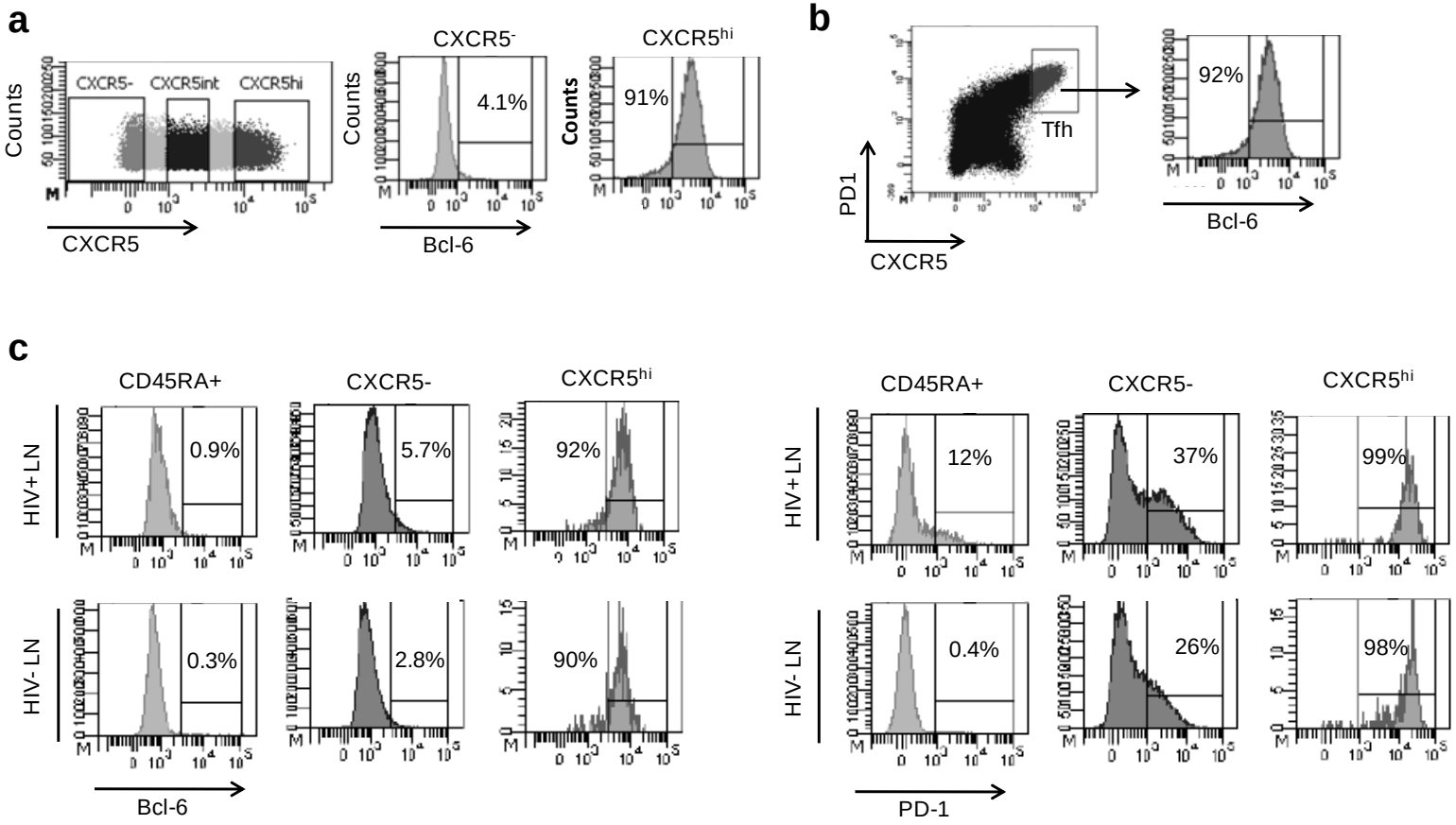
Rafael A. Cubas[1], Joseph C. Mudd[2], Anne-Laure Savoye[3], Matthieu Perreau[3], Julien van Grevenynghe[1], Talibah Metcalf[1], Elizabeth Connick[4], Amie Meditz[4], Gordon J. Freeman[5], Guillermo Abesada-Terk Jr.[6], Jeffrey M. Jacobson[7], Ari D. Brooks[8], Shane Crotty[9,10], Jacob D. Estes[11], Giuseppe Pantaleo[3], Michael M. Lederman[2], Elias K. Haddad[1]

[1]Vaccine and Gene Therapy Institute of Florida, Port St. Lucie, Florida, USA.
[2]Division of Infectious Diseases, Center for AIDS Research, Case Western Reserve University/University Hospitals, Case Medical Center, Cleveland, Ohio, USA.
[3]Divisions of Immunology and Allergy, Swiss Vaccine Research Institute, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland.
[4]Division of Infectious Diseases, University of Colorado Denver, Colorado, USA.
[5]Department of Medical Oncology, Dana-Faber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA.
[6]Robert and Carol Weissman Cancer Center, Martin Memorial Health Systems, Stuart, Florida, USA.
[7]Division of Infectious Diseases and HIV Medicine, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA.
[8]Department of Surgery, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA.
[9]Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology (LIAI), La Jolla, California, USA.
[10]Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID)
[11]The AIDS and Cancer Virus Program, SAIC-Frederick, National Cancer Institute, NIH, Frederick, Maryland, USA.

Supplementary Table 1 Sample data from uninfected and HIV-infected subjects

HIV-		HIV+			
ID	**Age**	**ID**	**Age**	**CD4 Count (cells/µl)**	**Viral Load (copies/ml)**
LN048	71	LNB-4	39	606	4862
LN055	57	LNB-6	43	386	6050
LN056	43	LNB-7	47	429	5791
LN061	46	LNB-10	29	389	17960
LN062	46	LNB-11	32	650	6640
LN063	56	P4133	24	762	2745
LN065	41	P3925	39	465	52855
LN066	26	AVIB1024	45	709	46318
LN068	68	AVIB1014	43	788	416096
LN071	54	CNA2108	76	1074	216700
LN073	74	P3194	54	866	4733
Average	**53**	**Average**	**43**	**648**	**70977**

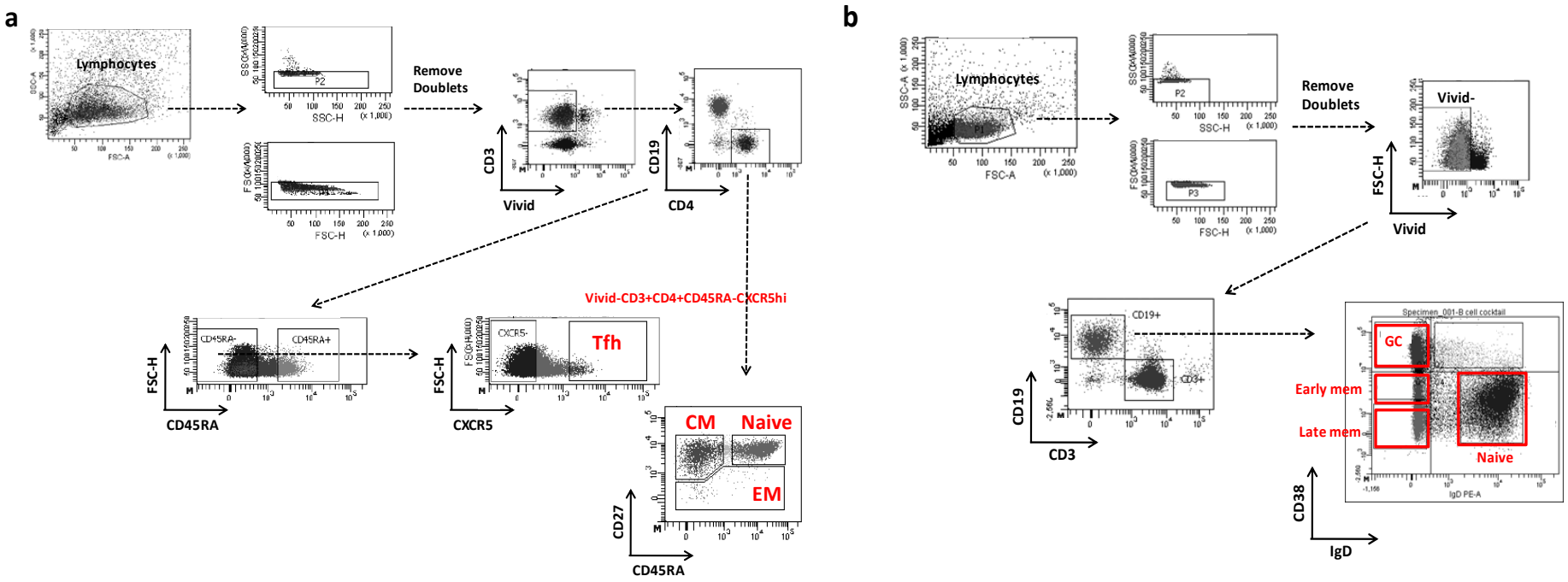
Supplementary Fig. 1 Tfh cells are enriched in the $CD4^{+}CXCR5^{hi}$ T cell population



(**a**) The dot plot depicts the distribution of CXCR5 and Bcl-6 expression on $Vivid^{-}CD3^{+}CD4^{+}CD45RA^{-}$ cells from LNMCs. (**b**) Expression of PD-1 in the $CXCR5^{hi}$ subset. (**c**) Enrichment for $Bcl\text{-}6^{+}$ (first group of panels) and $PD\text{-}1^{+}$ (second group of panels) cells in the $CXCR5^{hi}$ T cell subset from HIV-infected and uninfected LNs. Non-Tfh cells ($CD45RA^{+}$ and $CD45RA^{-}CXCR5^{-}$) are included for comparison of Bcl-6 and PD-1 staining. Representative plots are shown.

Cubas, et al.

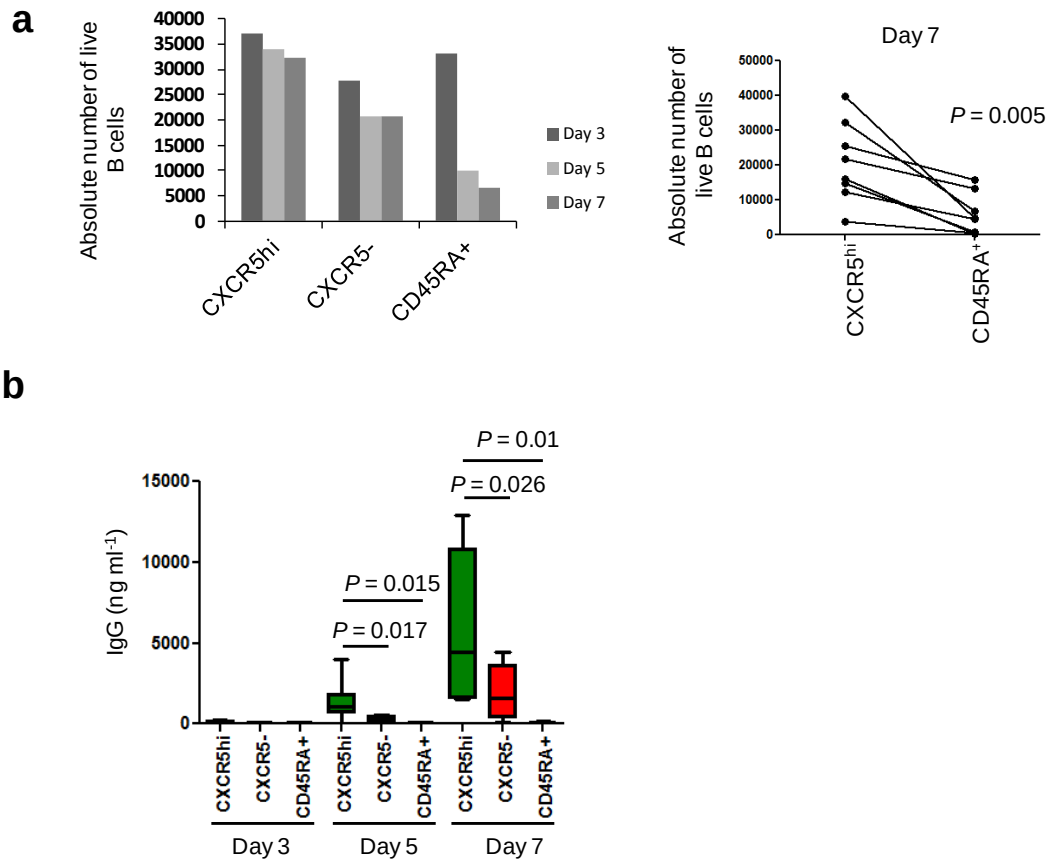
Supplementary Fig. 2 Gating strategies for T cell and B cell subsets from LNMCs



Cells were gated after removing doublets and dead cells. (**a**) T cell subsets were defined as: naïve ($CD3^+CD4^+CD45RA^+CD27^+$), central memory ($CD3^+CD4^+CD45RA^-CD27^+$), effector cells ($CD3^+CD4^+CD45RA^-CD27^-$) and Tfh cells ($CD3^+CD4^+CD45RA^-CXCR5^{hi}$). (**b**) B cell subsets were defined as: naïve ($CD3^-CD19^+CD38^-IgD^+$), GC ($CD3^-CD19^+CD38^{++}IgD^-$), early memory ($CD3^-CD19^+CD38^+IgD^-$) and late memory ($CD3^-CD19^+CD38^-IgD^-$).

Cubas, et al.

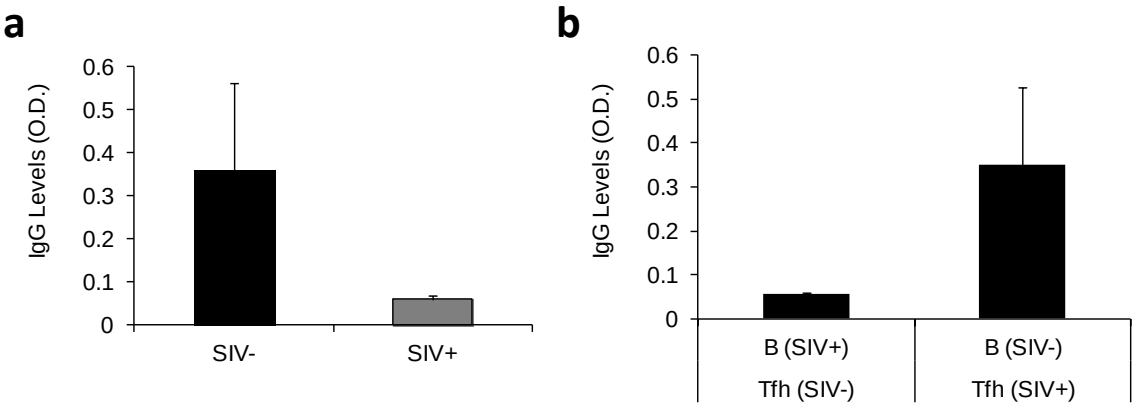
Supplementary Fig. 3 Coculture of Tfh cells (CXCR5^{hi}) with GC-enriched B cells from uninfected TMNCs leads to increased B cell help and antibody production



GC enriched B cells were cocultured with sorted autologous T cells (1 x 10^5) at a 1:1 ratio in the presence of 100 ng ml^{-1} of SEB. B cells were cocultured with either Tfh (CXCR5^{hi}) or non-Tfh cells (CXCR5$^-$ and CD45RA$^+$) for 3, 5 and 7 d. (**a**) Coculture of B cells with Tfh cells leads to a pronounced increase in the absolute number of live B cells at day 7 when compared to non-Tfh cells (CD4$^+$CD45RA$^+$) (n=8). The bar graph depicts a representative result for the absolute number of live B cells at different time points during the coculture. (**b**) Total IgG in the supernatant after coculture for 3, 5 and 7 days. (n=8).

Cubas, et al.

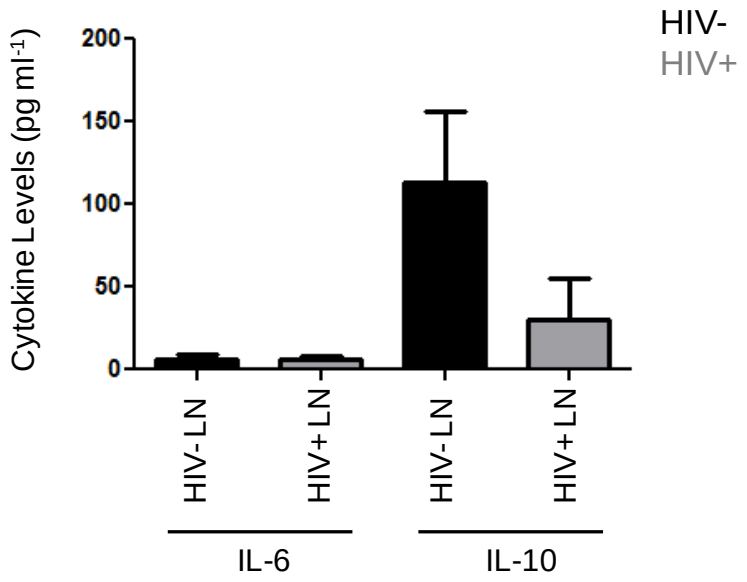
Supplementary Fig. 4 Cocultures of Tfh cells and GC-enriched B cells from SIV-infected macaques show reduced levels of immunoglobulin production



Tfh cells ($CD3^+CD4^+CD45RA^-PD\text{-}1^{hi}$) and activated B cells ($CD20^+CD21^-$) from age-matched LNs of SIV-infected or uninfected macaques were sorted and placed in coculture at equal numbers in the presence of SEB. Supernatants were harvested after 7 d to measure total immunoglobulin production. (**a**) Total levels of IgG in cocultures from uninfected and SIV-infected macaques (n = 2). (**b**) Tfh cells and B cells from the same macaques before (SIV^-) and after infection with SIV (SIV^+) were mismatched and placed in coculture at equal numbers in the presence of SEB. After 7 d the supernatants where collected to measure the total levels of IgG (n = 2).

Cubas, et al.

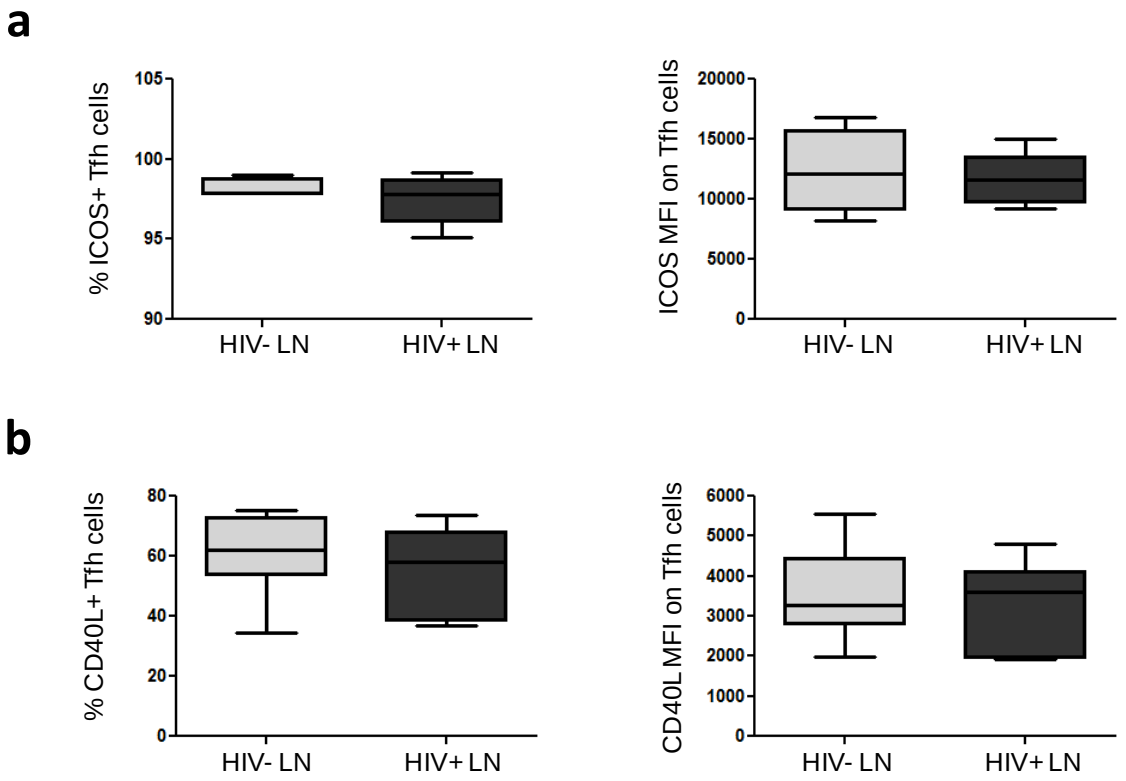
Supplementary Fig. 5 Cocultures of Tfh cells and GC-enriched B cells from HIV-infected LNs show lower levels of IL-10



LNMCs from HIV$^-$ and HIV$^+$ subjects were sorted into Tfh and GC-enriched B cells. Cells were cocultured in the presence of SEB for 7 days and the supernatants were collected to analyse the levels of IL-6 and IL-10 by Cytometric Bead Assay (CBA) (n=4).

Cubas, et al.

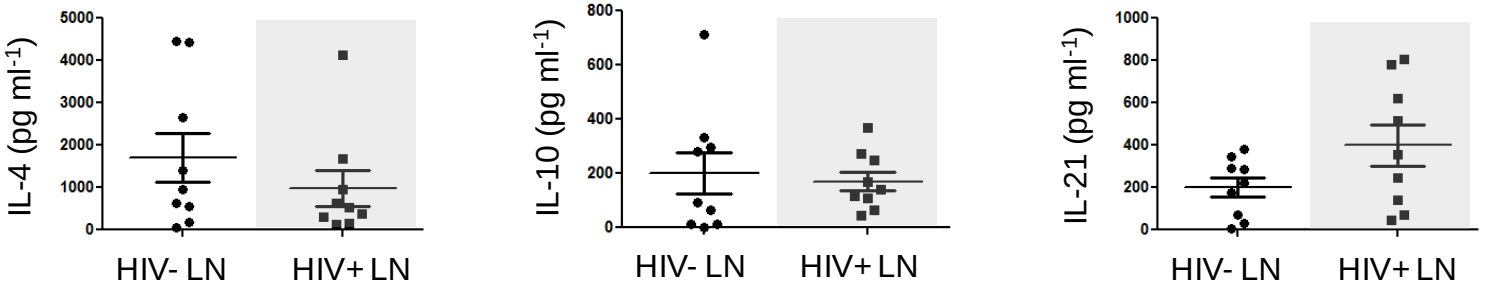
Supplementary Fig. 6 Expression levels of ICOS and CD40L in Tfh cells from LNs of HIV-infected and uninfected individuals



Tfh cells were defined as $CD3^{+}CD4^{+}CD45RA^{-}CXCR5^{hi}$ cells. (**a**) Frequency of $ICOS^{+}$ Tfh cells and expression level for ICOS (MFI) on Tfh cells. (**b**) Frequency of $CD40L^{+}$ Tfh cells and expression level for CD40L (MFI) on Tfh cells ($n \geq 4$ for both HIV^{-} and HIV^{+} LN samples).

Cubas, et al.

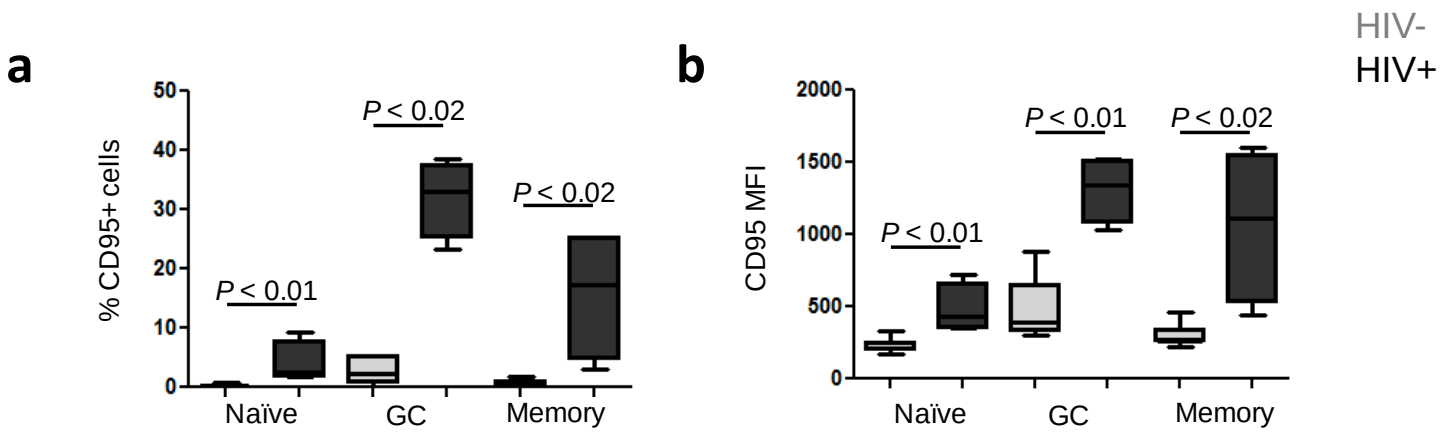
Supplementary Fig. 7 Production of cytokines from Tfh cells as assessed by Luminex from uninfected and HIV-infected subjects



Tfh cells were sorted from LNMCs of HIV uninfected (n=9) and infected subjects (n=9). Sorted cells were then stimulated with phorbol myristate acetate (PMA 100 ng mL^{-1}) and ionomycin (1 µg mL^{-1}) in complete RPMI for 18 h at 37 °C. Supernatants were then collected and analyzed for the presence of cytokines (IL-4, IL-10 and IL-21). Plots depict the amount of cytokines produced (pg ml^{-1}) from Tfh cells after stimulation.

Cubas, et al.

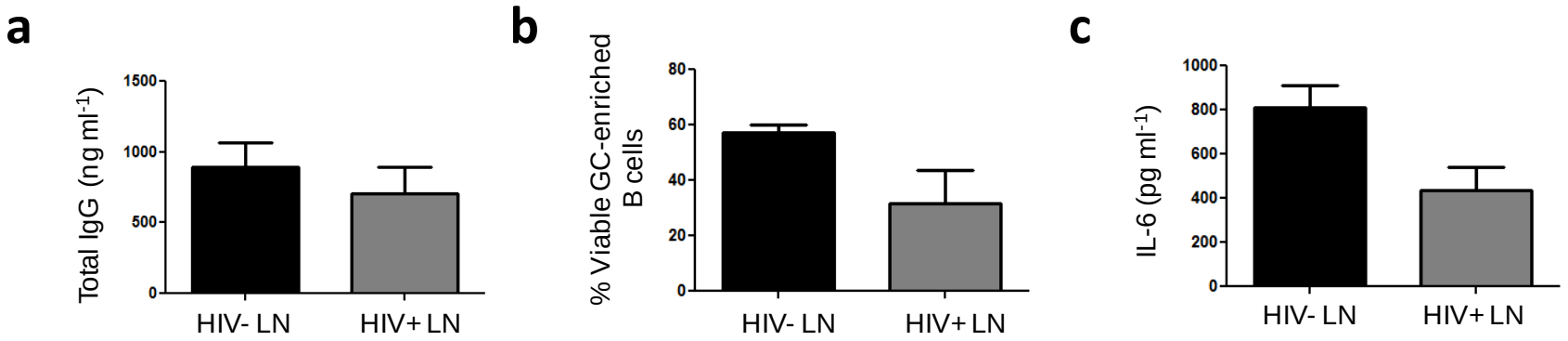
Supplementary Fig. 8 Expression levels of CD95 on B cell subsets from LNs of HIV-infected and uninfected individuals



B cells subsets were defined as naïve ($CD3^-CD19^+CD38^-IgD^+$), GC ($CD3^-CD19^+CD38^{++}IgD^-$) and memory ($CD3^-CD19^+CD38^{+/-}IgD^-$). (**a**) Frequency and (**b**) level of CD95 expression on different B cell subsets as measured by flow cytometry analysis ($n \geq 4$ for both HIV^- and HIV^+ LN samples).

Cubas, et al.

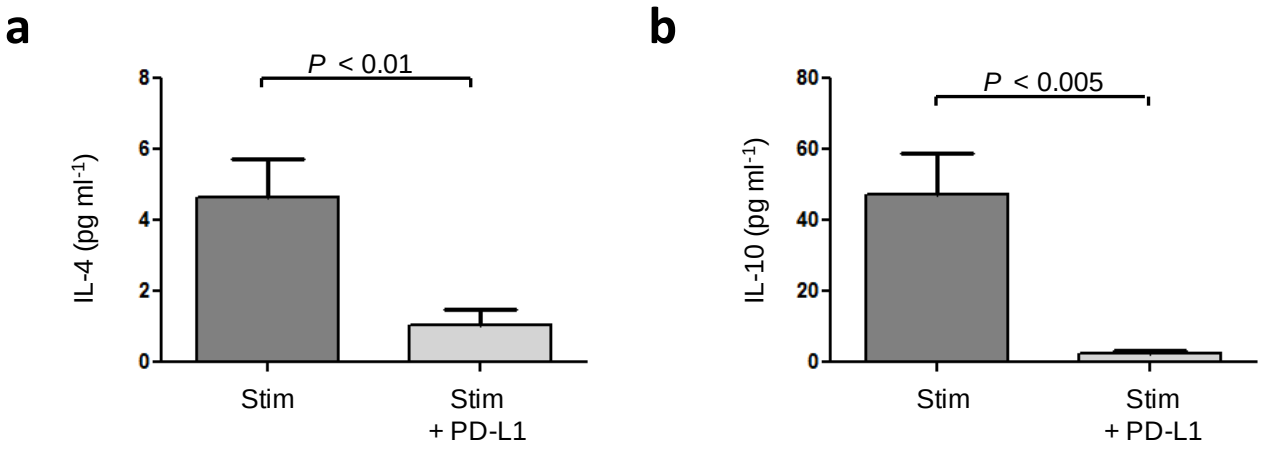
Supplementary Fig. 9 Effect of polyclonal B cell activation on IgG production, viability and cytokine output



GC-enriched B cells from HIV-infected and uninfected LNs were sorted and placed in culture (1 x 10^5 cells) with 2.5 µg ml^{-1} of CpG-B (ODN-2006) (InvivoGen) in complete media. After 4 d the supernatants were collected and the cells harvested for further analysis. (**a**) Total levels of IgG in the culture supernatant as measured by ELISA. (**b**) Frequency of viable GC-enriched B cells (defined as $Vivid^-Annexin\text{-}V^-$) after 4 d in culture under polyclonal stimulation. (**c**) Total levels of IL-6 in the culture supernatants following polyclonal B cell stimulation as measured by CBA (pg ml^{-1}) ($n \geq 3$).

Cubas, et al.

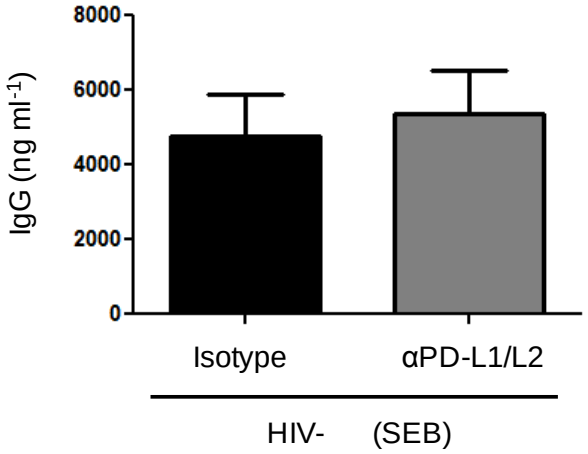
Supplementary Fig. 10 Effect of PD-1 triggering on cytokine production from Tfh cells



Tonsil mononuclear cells (TMNCs) from uninfected individuals were stained and sorted to highly purify Tfh cells. A total of 2 x 10^5 cells were cultured for 3 d in the presence or absence of anti-CD3, anti-CD28 and isotype coated beads (Stim) or anti-CD3, anti-CD28 and PD-L1 coated beads (Stim + PD-L1). Supernatants were collected to analyze the levels of different cytokines by CBA. (**a**) Total levels of IL-4 and (**b**) IL-10 (pg ml^{-1}) (n=8).

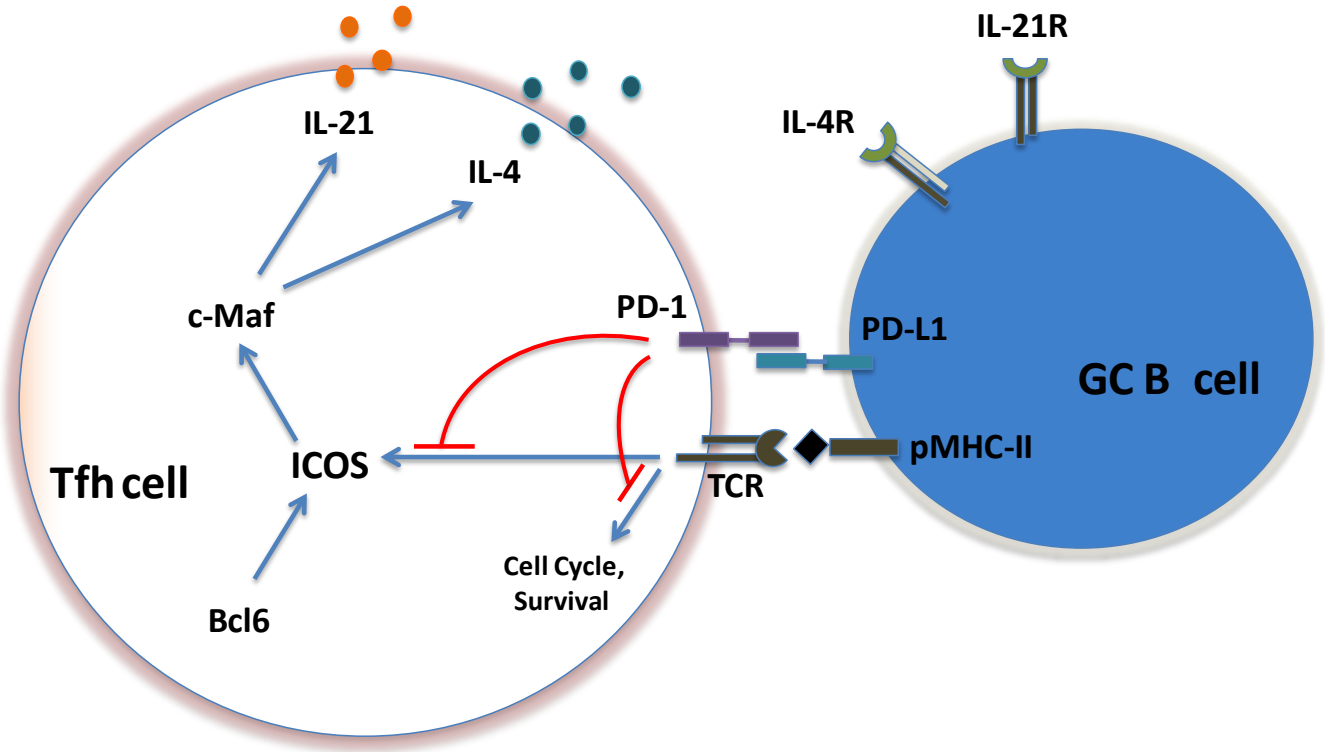
Cubas, et al.

Supplementary Fig. 11 Effect of PD-L1/L2 blocking antibodies on cocultures from Tfh and GC-enriched B cells from uninfected individuals



TMNCs from HIV$^-$ subjects were sorted into Tfh and GC-enriched B cells. B cells were treated with anti-PD-L1/L2 or isotype control antibodies (20 μg ml^{-1}) for 20 min at 37 °C before addition of Tfh cells. Cells were then cocultured in the presence of SEB for 7 d. Supernatants were collected to measure the total level of IgG by ELISA (n=3).

Supplementary Fig. 12 Proposed model for the induction of Tfh functional impairment during HIV infection



Interaction of Tfh cells with GC B cells in LNs from HIV^{+} subjects can trigger PD-1 on Tfh cells. Engagement of PD-1 on Tfh cells results in the inhibition of cell proliferation, activation, survival and ICOS expression. This reduction in the levels of ICOS can in turn affect downstream transcription factors like c-Maf leading to reduced levels of IL-4 and IL-21 cytokine secretion which are critical in preventing GC B cell apoptosis and inducing B cell survival, proliferation and differentiation into antibody secreting plasma cells. PD-1 triggering on Tfh cells can therefore affect GC B cell responses and immunoglobulin production.