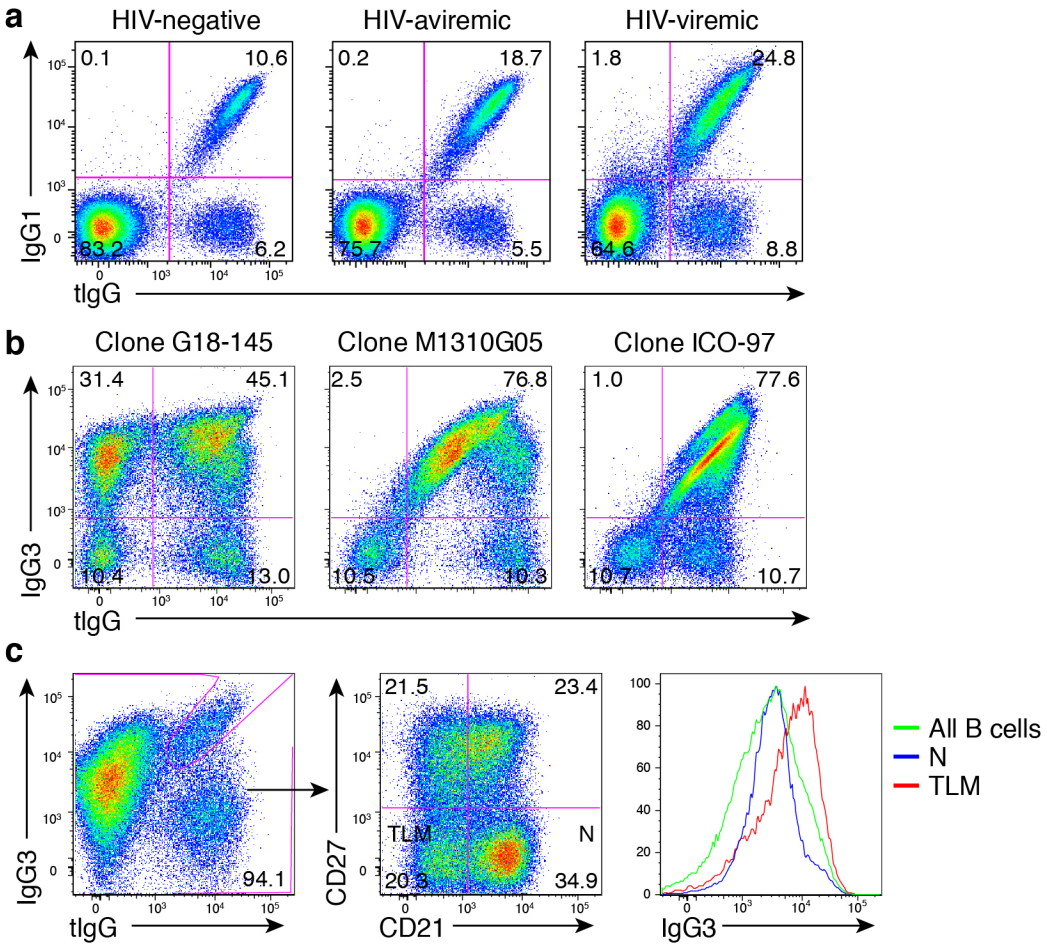
Supplementary Figure 1

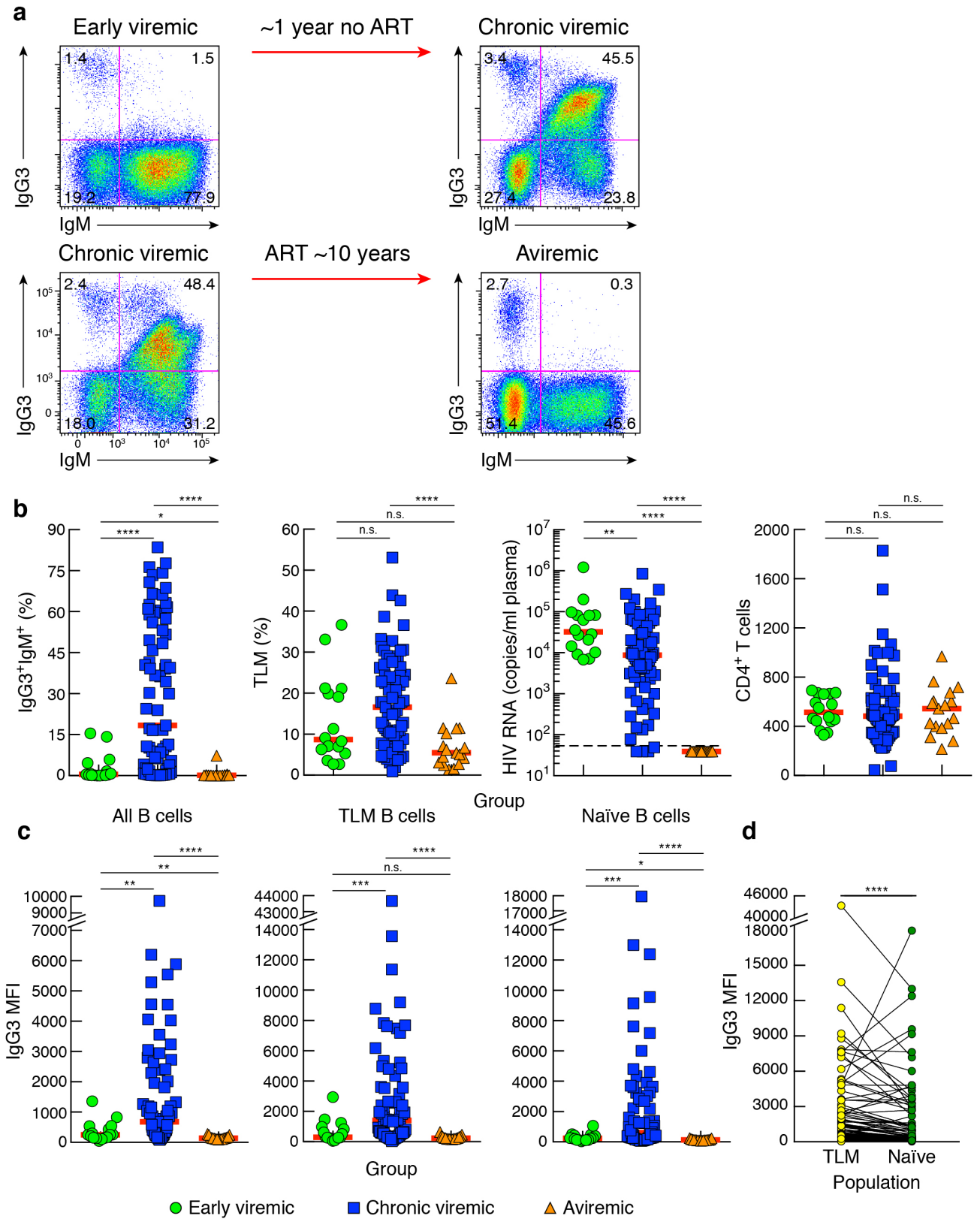


Supplementary Figure 1

Additional cell-surface analyses.

(a) Representative flow cytometry of CD20-gated B cells isolated from the peripheral blood of HIV-negative, HIV-aviremic and chronically infected HIV-viremic individuals, and stained for IgG1 and total IgG (tIgG) with pan anti-IgG mAb. (**b**) Flow cytometry of CD20-gated B cells isolated from the peripheral blood of a chronically infected HIV-viremic individual, and stained for IgG3 and tIgG with pan anti-IgG mAb clones G18-145, M1310G05 and ICO-97. (**c**) Gating strategy to evaluate mean fluorescence intensities (MFI) of B cell-bound IgG3. Staining for IgG3 and tIgG (clone G18-145) was used to identify cell-bound IgG3 by excluding tIgG/IgG3 double positive CD20-gated B cells with a clear diagonal pattern. IgG3 MFI was then determined for all B cells, as well as tissue-like memory (TLM) and naïve (N) B cells defined by expression of CD27 and CD21, as indicated in the representative plot and histograms. Data are representative of 10 (HIV-negative), 14 (HIV-aviremic) and 49 (HIV-viremic) individual experiments (**a**) or one experiment representative of eight independent experiments (**b**) or one representative of 106 individual experiments (**c**).

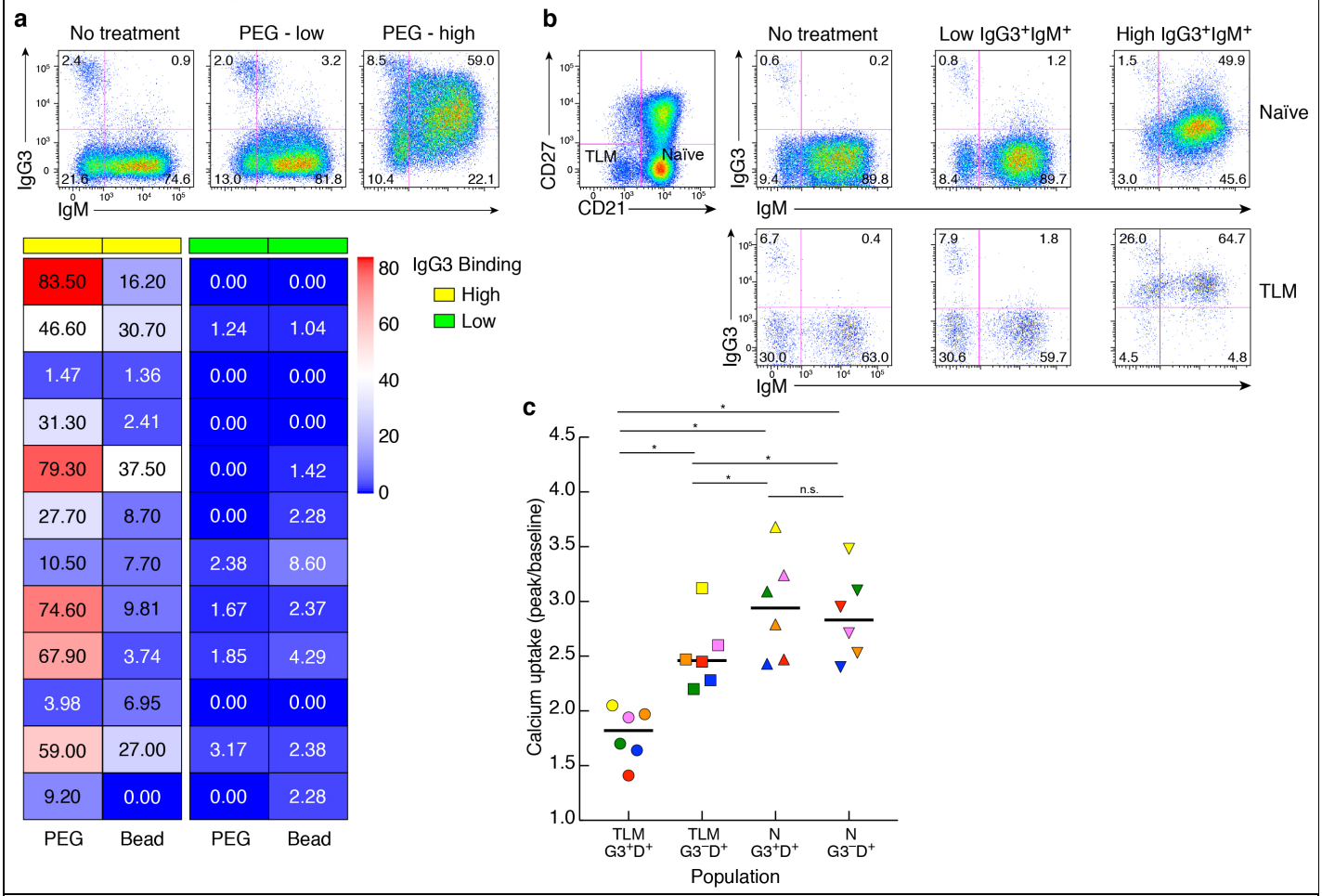
Supplementary Figure 2



Supplementary Figure 2

Factors that influence IgG3$^+$IgM$^+$ B cells.

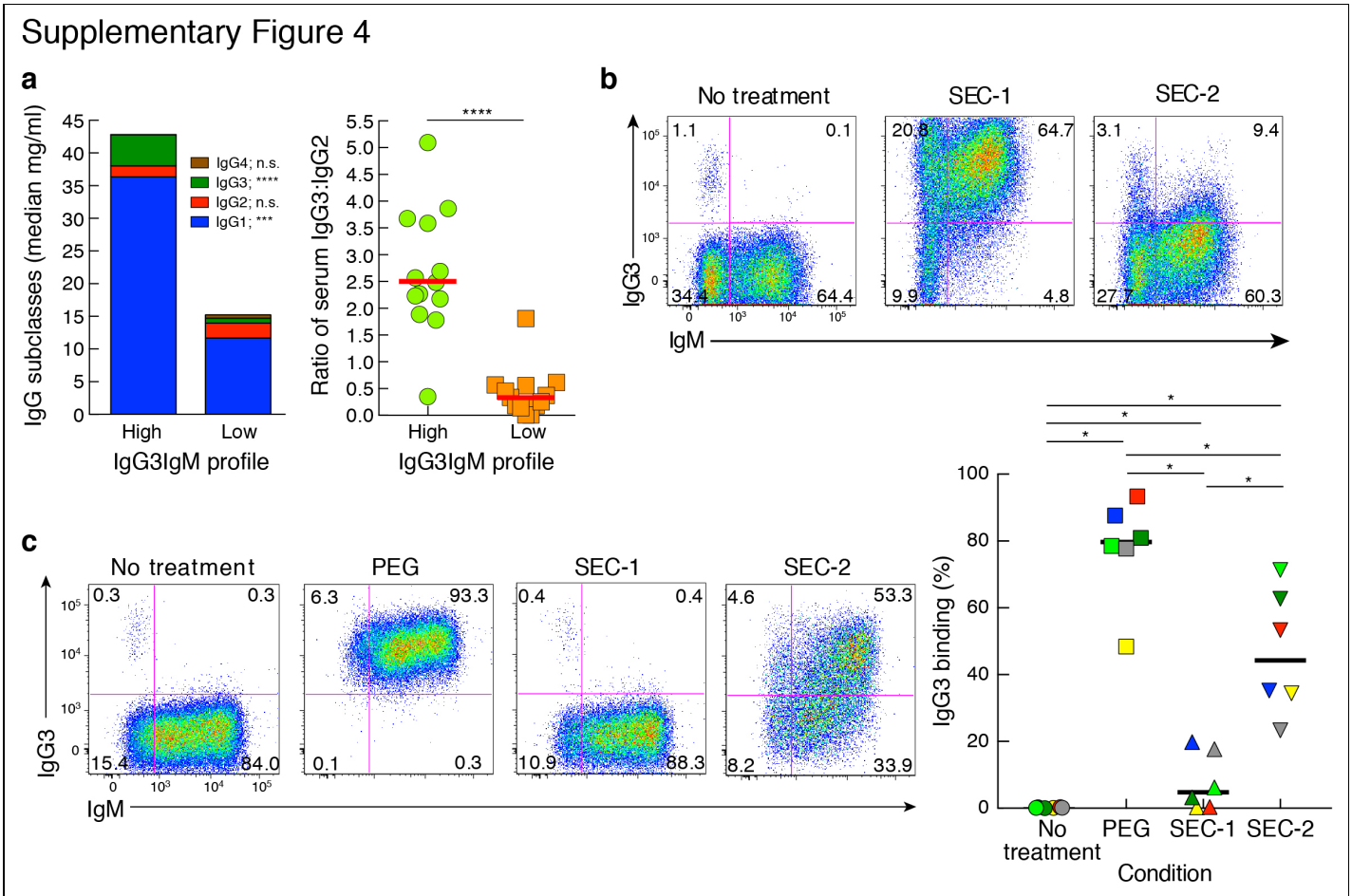
(**a**) Longitudinal flow cytometry for IgG3 and IgM of CD20-gated B cells isolated from an HIV-infected individual during early viremia and one year later in chronic stage of viremia. Similarly, B cells from a chronically infected HIV-viremic individual were stained for IgG3 and IgM before and 10 years after sustained suppression of viremia by ART. (**b**) Comparison by stage of HIV infection and status of viremia of frequencies of IgG3$^+$IgM$^+$ and TLM B cells measured by flow cytometry, as well as of HIV viral load and CD4$^+$ T-cell counts. Dotted line represents limit of detection. (**c**) Comparison by stage of HIV infection and status of viremia of mean fluorescence intensities (MFI) for IgG3 measured by flow cytometry of CD20-gated TLM and naïve B cells where IgG3-expressing B cells were excluded, as described in Methods and **Supplementary Fig. 1c**. (**d**) Comparison of IgG3 MFI on TLM versus naïve B cells of 92 HIV-viremic individuals, all stages of disease combined. Red horizontal bars represent medians; each symbol (**b**,**c**) represents an individual in early viremia (n = 16), chronic viremia (n = 73) or aviremia (n = 17); * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$ and **** $P < 0.0001$; n.s., not significant (two-tailed Mann-Whitney test after obtaining significance by Kruskal-Wallis ANOVA test on full set (**b,c**) or two-tailed Wilcoxon matched-pairs signed rank test (**d**)). Data are representative of five (early to chronic viremic) and seven (chronic viremic to aviremic) individual experiments (**a**).



Supplementary Figure 3

PEG-IgG3 isolated from serum of HIV-viremic individuals with high-intensity IgG3$^+$IgM$^+$ B cells bind to B cells of HIV-negative individuals.

(**a**) Representative flow cytometry of CD20-gated B cells of an HIV-negative individual incubated without (no treatment) or with PEG-IgG3 isolated from sera of HIV-viremic individuals with low- or high-intensity IgG3$^+$IgM$^+$ B cells. Bottom: cumulative data from paired sera of HIV-viremic individuals with high (yellow; n = 12) or low (light green; n = 12) intensity IgG3$^+$IgM$^+$ B cells. Heat map depicts % of IgM-expressing B cells from HIV-negative individuals with bound IgG3 following incubation with serum-derived PEG precipitate or bead-enriched IgG3. PEG-IgG3 isolated with serum from individuals with high-intensity IgG3$^+$IgM$^+$ B cells bound B cells of HIV-negative individuals with a median 39%, compared to 0% from those with low-intensity IgG3$^+$IgM$^+$ B cells (P = 0.005). For bead-enriched IgG3, the medians were 8.2% for high versus 1.8% for low-intensity IgG3$^+$IgM$^+$ B cells (P = 0.009). P values, two-tailed Wilcoxon matched-pairs signed rank test. (**b**) Representative flow cytometry of CD20-gated B cells of an HIV-negative individual incubated without (no treatment) or with PEG-IgG3 isolated from the serum of HIV-viremic individuals with low- or high-intensity IgG3$^+$IgM$^+$ B cells. The plots show IgG3 and IgM staining for TLM and naïve B cells, defined by the expression of CD21 and CD27. (**c**) Comparison of calcium uptake by HIV-negative IgD$^+$ (D) TLM and naïve (N) B-cell IgG3$^{+/-}$ (G3) populations treated with PEG-IgG3 isolated from serum of chronically infected HIV-viremic individuals with moderate to high-intensity IgG3$^+$IgM$^+$ B cells. Each color coded symbol represents an individual (n = 6); black horizontal bars represent medians; * P < 0.05; n.s., not significant (two-tailed Wilcoxon matched-pairs signed rank test after obtaining significance by Friedman ANOVA test on full set). Data are representative of 12 individual experiments (**b**).

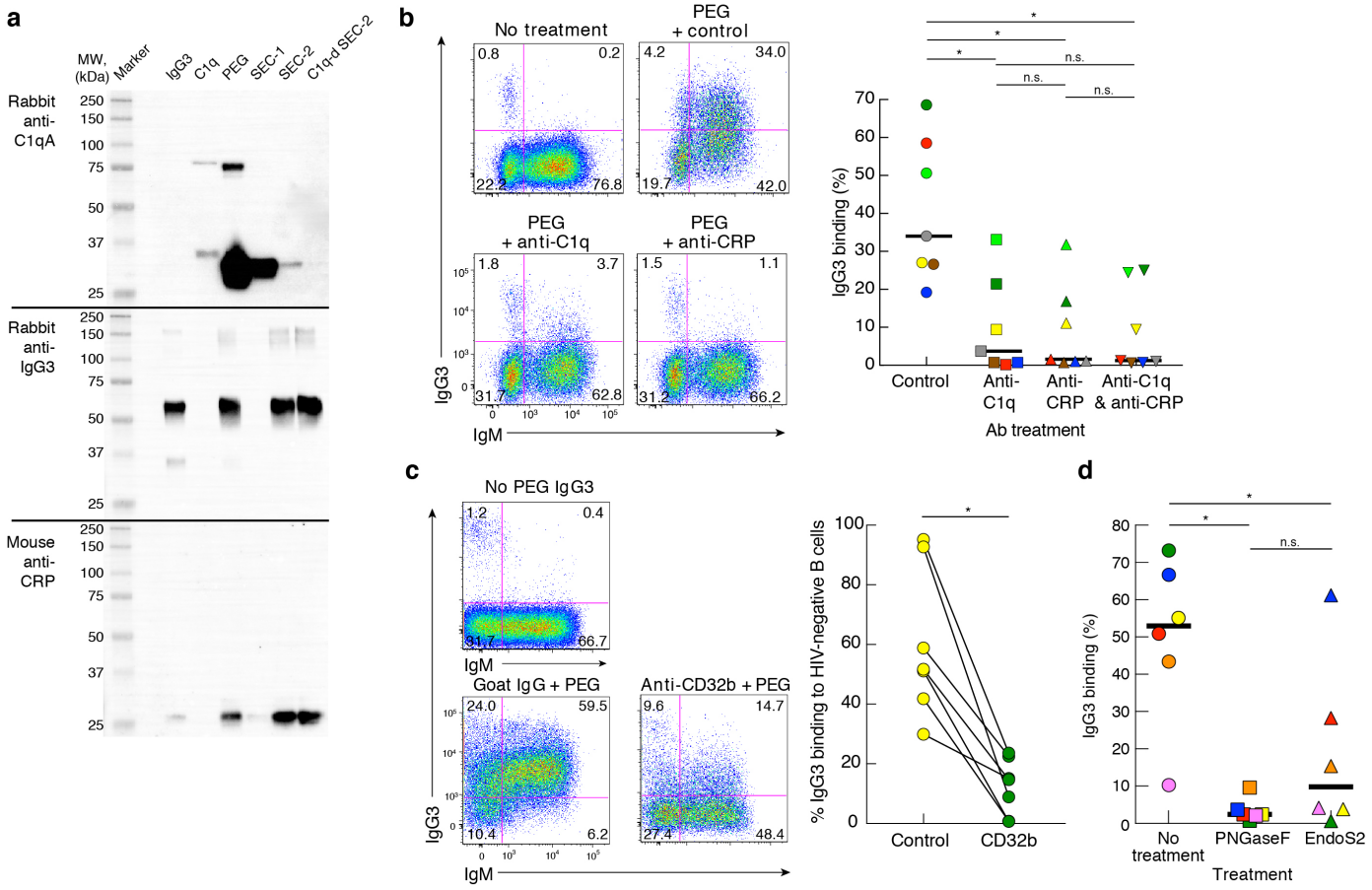


Supplementary Figure 4

IgG3 serum levels and binding to HIV-negative B cells.

(**a**) Comparison of levels and ratios of IgG subclasses in serum of HIV-viremic individuals with high (n = 12) or low (n = 12) intensity IgG3$^+$IgM$^+$ B cells (same serum used in Supplementary Fig. 3). Red horizontal bars represent medians; *** $P < 0.001$ and **** $P < 0.0001$; n.s., not significant (two-tailed Mann-Whitney test). (**b**) Representative flow plots of CD20-gated B cells of an HIV-negative individual incubated without (no treatment) or with heat-aggregated IgG3 fractionated into SEC-1 or SEC-2. (**c**) Representative flow plots of CD20-gated B cells of an HIV-negative individual incubated without (no treatment) or with PEG precipitate or with SEC-1 or SEC-2 fractions isolated from PEG-precipitated serum of an HIV-viremic individual with high-intensity IgG3$^+$IgM$^+$ B cells. Right, cumulative data from sera of HIV-viremic individuals with moderate- to high-intensity IgG3$^+$IgM$^+$ B cells. Each color coded symbol represents an individual (n = 6); black horizontal bars represent medians; * $P < 0.05$ (two-tailed Wilcoxon matched-pairs signed rank test after obtaining significance by Friedman ANOVA test on full set). Data are representative of three (**b**) or six (**c**) independent experiments.

Supplementary Figure 5



Supplementary Figure 5

Role of CD32b, C1q, CRP and glycosylation in non-aggregated IgG3 binding to B cells of HIV-negative individuals.

(**a**) Representative immunoblot analysis of C1q, IgG3 and CRP content in PEG precipitate (PEG), SEC-1, SEC-2 or SEC-2 depleted of C1q (C1q-d SEC-2) using serum of an HIV-viremic individual with moderate-intensity IgG3$^+$IgM$^+$ B cells. The gels are shown in the order the membrane was sequentially stained and stripped. Lanes marked IgG3 and C1q indicate respective protein controls. (**b**) Representative flow cytometry of CD20-gated B cells of an HIV-negative individual incubated without PEG-IgG3 (no treatment), with PEG-IgG3 from serum of an HIV-viremic individual with moderate-intensity IgG3$^+$IgM$^+$ B cells treated with control rabbit IgG or rabbit anti-C1q or rabbit anti-CRP antibody. Right, cumulative pattern from sera of chronically infected HIV-viremic individuals with moderate- to high-intensity IgG3$^+$IgM$^+$ B cells. (**c**) Representative flow cytometry of CD20-gated B cells of an HIV-negative individual stained for IgG3 and IgM. The B cells were untreated or treated with antibody to CD32b or control goat IgG prior to addition of PEG-IgG3 from an HIV-viremic individual with high-intensity IgG3$^+$IgM$^+$ B cells. Graph: cumulative effect of pre-treating B cells with anti-CD32b prior to addition of PEG-IgG3 from HIV-viremic individuals with moderate- to high-intensity IgG3$^+$IgM$^+$ B cells (n = 6). (**d**) Effect of treating PEG-IgG3 prepared from individuals with moderate- to high-intensity IgG3$^+$IgM$^+$ B cells with PNGase F or EndoS2 on binding to B cells of HIV-negative individuals. Each color coded symbol (**b,d**) represents an individual (**b**, n = 7; **d**, n = 6); black horizontal bars represent medians; * $P < 0.05$; n.s., not significant (two-tailed Wilcoxon matched-pairs signed rank test, after (**b,d**) obtaining significance by Friedman ANOVA test on full set). Data are representative of eight independent experiments (**a**).