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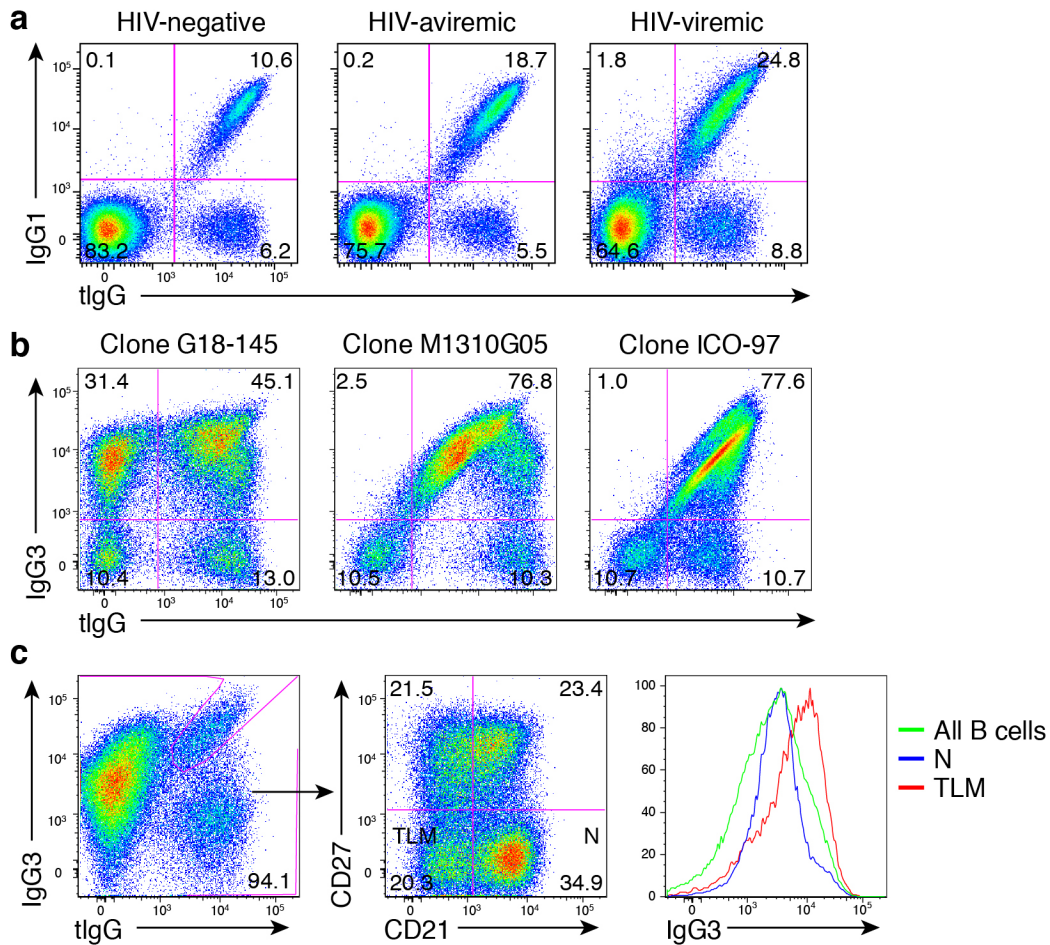
IgG3 regulates tissue-like memory B cells in HIV-infected individuals

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Supplementary Figure 1

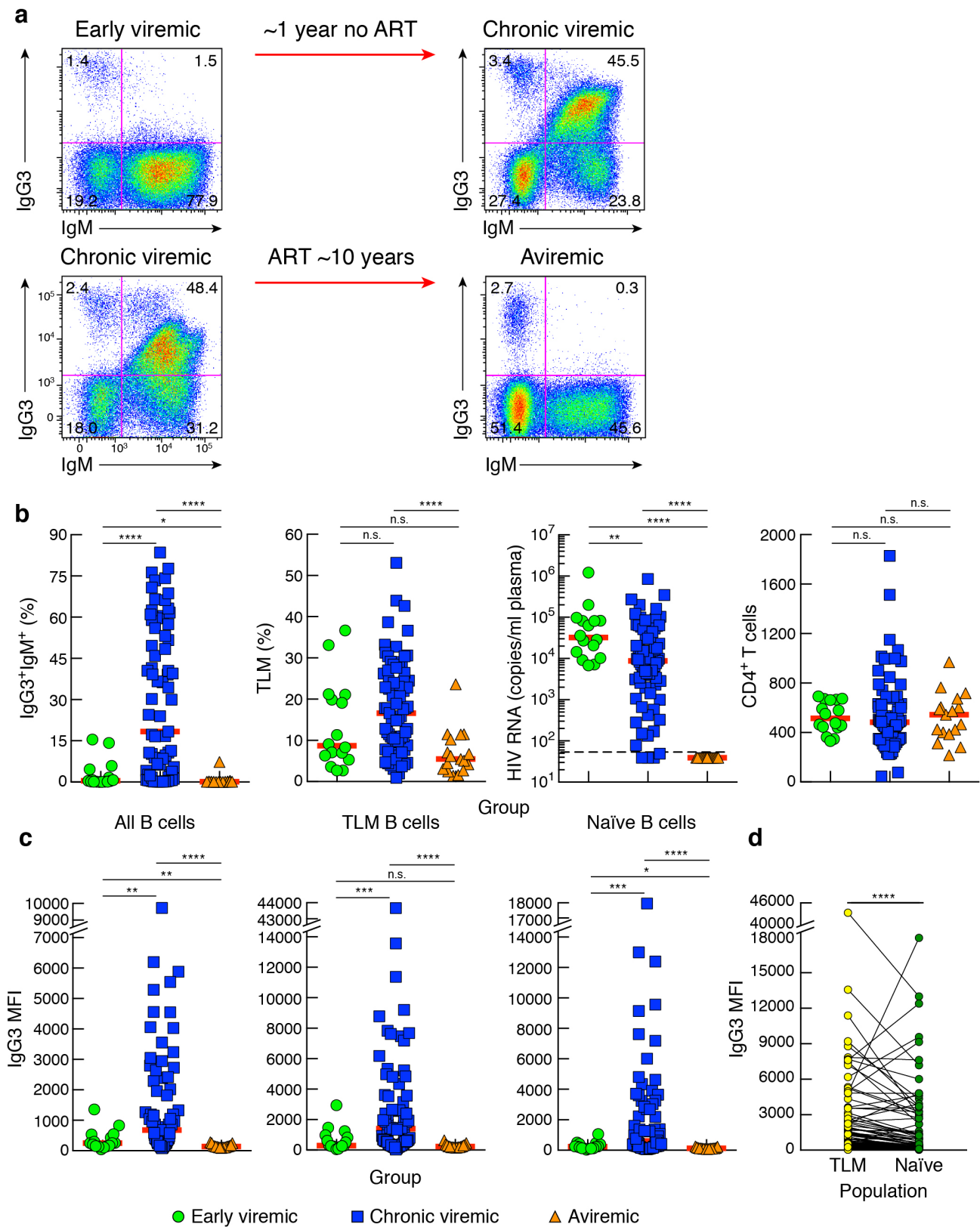


Supplementary Figure 1

Additional cell-surface analyses.

(a) 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

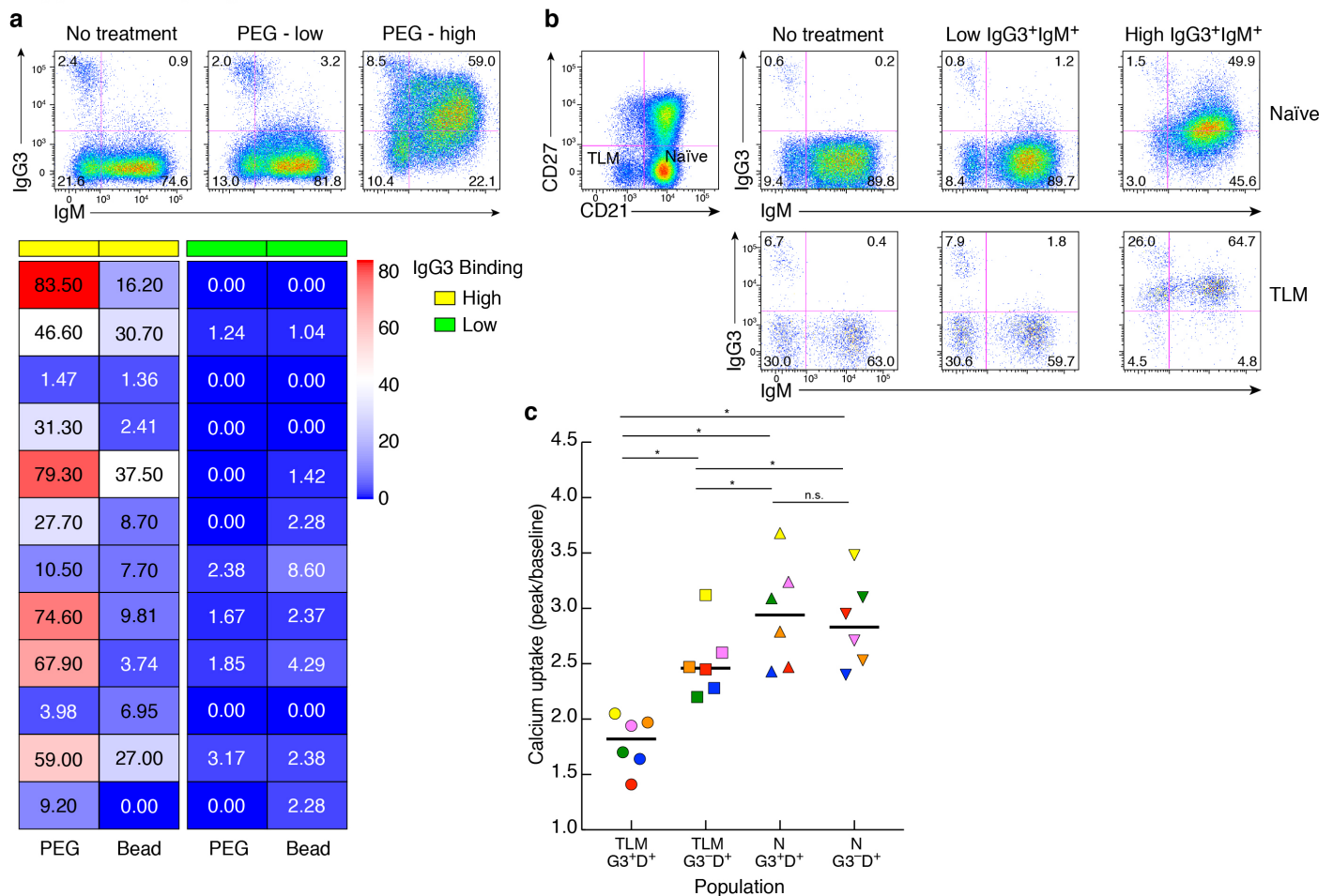


Supplementary Figure 2

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

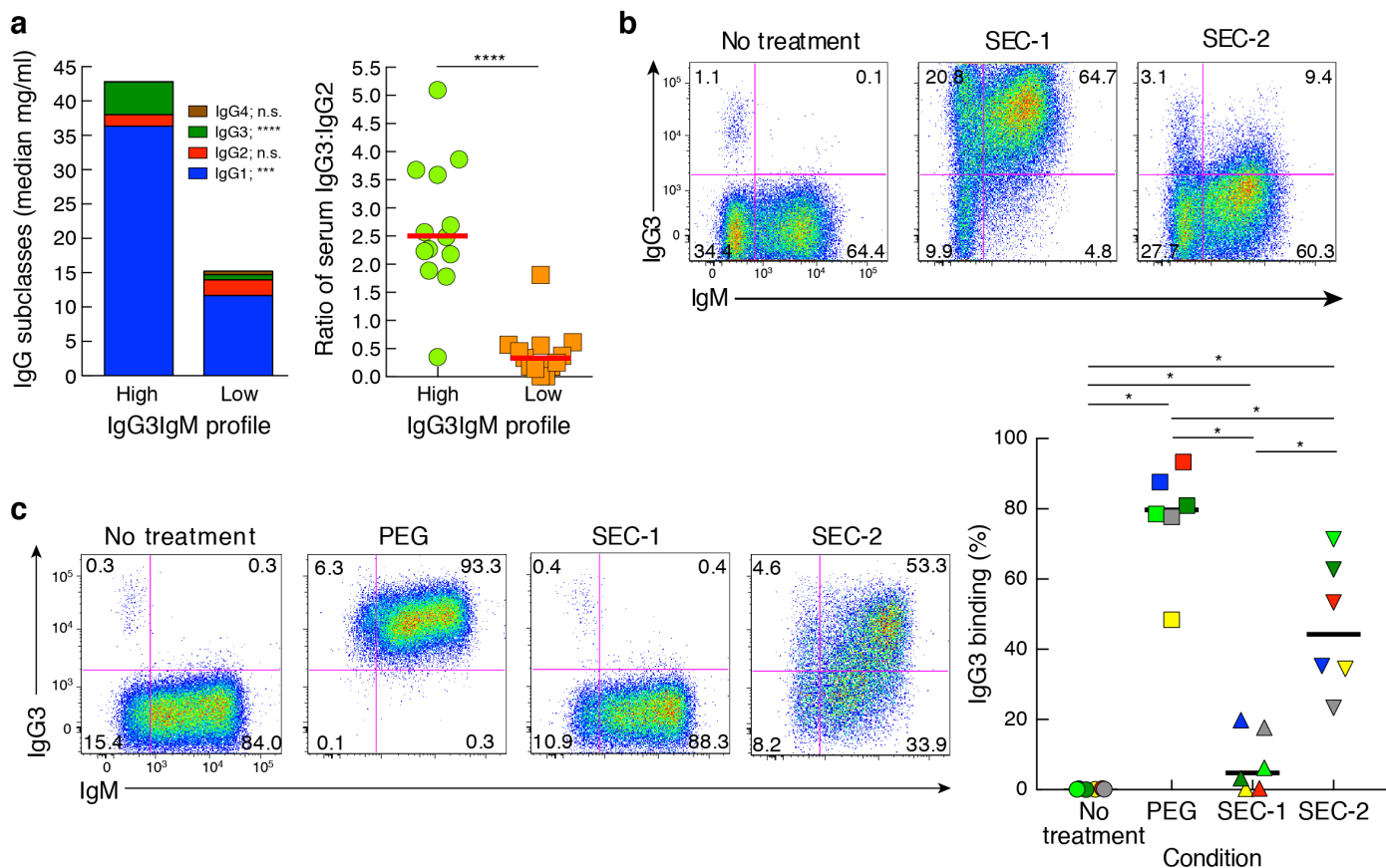


Supplementary Figure 3

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

(a) 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⁺ on 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⁺ on 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⁺ on IgM⁺ B cells bound B cells of HIV-negative individuals with a median 39%, compared to 0% from those with low-intensity IgG3⁺ on IgM⁺ B cells ($P = 0.005$). For bead-enriched IgG3, the medians were 8.2% for high versus 1.8% for low-intensity IgG3⁺ on IgM⁺ B cells ($P = 0.009$). P values, two-tailed Wilcoxon matched-pairs signed rank test. (b) 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⁺ on 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⁺ on 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

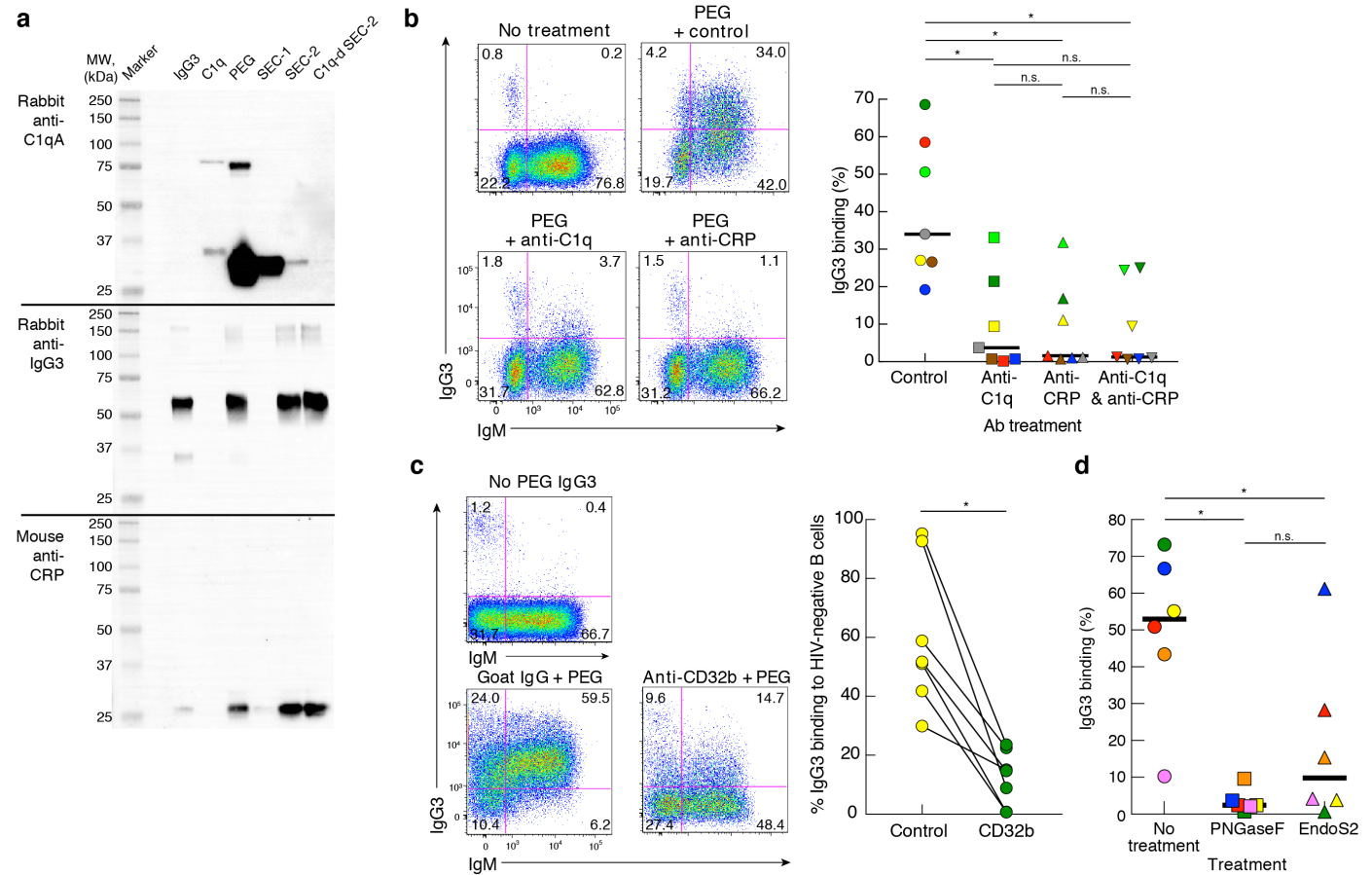


Supplementary Figure 4

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⁺ on 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) 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⁺ on IgM⁺ B cells. Right, cumulative data from sera of HIV-viremic individuals with moderate- to high-intensity IgG3⁺ on 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) 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⁺ on 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) 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⁺ on 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⁺ on IgM⁺ B cells. (c) 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⁺ on 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⁺ on IgM⁺ B cells ($n = 6$). (d) Effect of treating PEG-IgG3 prepared from individuals with moderate- to high-intensity IgG3⁺ on 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).