

Supplementary Figure 1. Phenotypic analysis of CXCR5^{high}CD4^{high} T cells in LN tissues from SIV-chronic RMs. Flow cytometry contour plots showing the forward and side scatter characteristics and the expression of B cell surface markers (CD20, IgD, IgM) on CXCR5^{high} cells from a SIV-chronic LN tissue. The frequency of the gated populations is shown.

Supplementary Figure 2. RM T_{FH} cells share common characteristics with human T_{FH}. (A) Representative flow cytometry contour plots showing the expression of “human T_{FH}” surface markers (BTLA, CD127, CD69, PD-L1) on CD4 T cell populations from an SIV-chronic LN tissue. (B) Cells from a SIV-chronic LN were stimulated with superantigen (SEB) for 0, 2.5 and 5h and the expression of CD154 was assessed in individual populations. The naïve population in the absence of stimulation was used to define CD154^{high} cells. The frequency of the gated populations is shown.

Supplementary Figure 3. Localization and functional characterization of T_{FH} in RMs. (A) PD-1^{high}CD4 T cells are localized within the B cell follicles in SP from non-infected RMs. Confocal images showing the localization of CD4, CD20, PD-1 and Ki67 in SP from one SIV non-infected RM (all scale bars are set at 100 um). (B) Pooled data showing the mRNA expression of *CXCL13* in sorted CM/CD4 T cell populations from SIV-chronic LN and SP tissues. The normalized CT value for CD28^{high}CD95^{high}CCR7^{high}PD-1^{low} cells was assigned a value of 1 and

the fold change over this value for the populations tested is shown. Horizontal lines depict mean values. (C) Pooled data showing the levels of IL-5 and IL-6 in cultured supernatants analyzed for cytokines using Luminex technology. Sorted CM/CD4 T cell populations from SIV-chronic SP tissues (n=5) were stimulated with PMA/ionomycin for 14-16h. *p* values were calculated using Mann-Whitney *U* test.

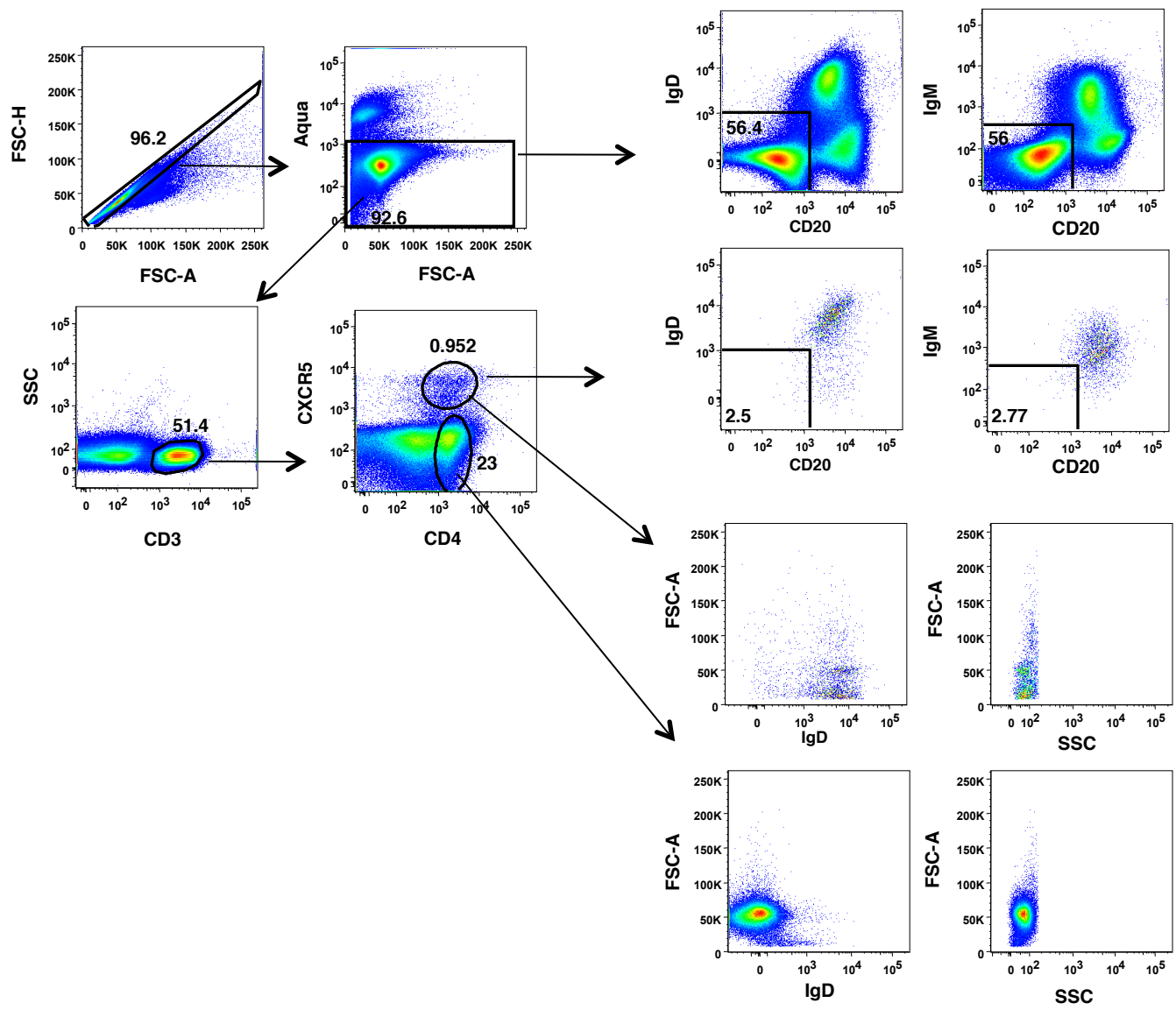
Supplementary Figure 4. (A) The gating strategy for sorting cells from the CD28^{high}CD95^{high} compartment for gene array analysis is shown. Naïve cells were used as a reference for setting the gates. The ICOS^{high}CD150^{low} population (red gate) from the CCR7^{high/low}PD-1^{dim/low} and T_{FH} compartment was analyzed. (B) The most affected network from IPA analysis (non-SIV T_{FH} compared to chronic SIV T_{FH}) for Immunological disease/Inflammatory disease/Neurological disease is shown. The red color indicates up-regulation (fold change ≥ 1.5); the green color indicates down-regulation (fold change ≤ -1.5); the grey color indicates the genes whose expression values are between -1.5 and 1.5; the white color indicates the genes that are not overlapping with the data. Shape: diamond represents enzyme; rectangle represents ligand-dependent nuclear receptor; circle represents others. (C) Canonical pathways from IPA analysis that were dominantly affected during the transition of T_{FH} from SIV-negative to SIV-chronic status are shown. The yellow line indicates a threshold *p* value of 1.5. (D) PD-1^{high}CD4 T cells are localized within the B cell follicles in LN and SP from chronically SIV infected RMs. Confocal images showing the localization of CD4, CD20, PD-1 and Ki67 in LN and SP from one chronically SIV infected RM (all scale bars are set at 100 μ m). The relative distance of CD4 T cells, expressing different amounts of PD-1, from the “center” of the B cell follicle was calculated and pooled data obtained from one SP are shown. Bars depict mean $\pm$ 95% Confidence Intervals.

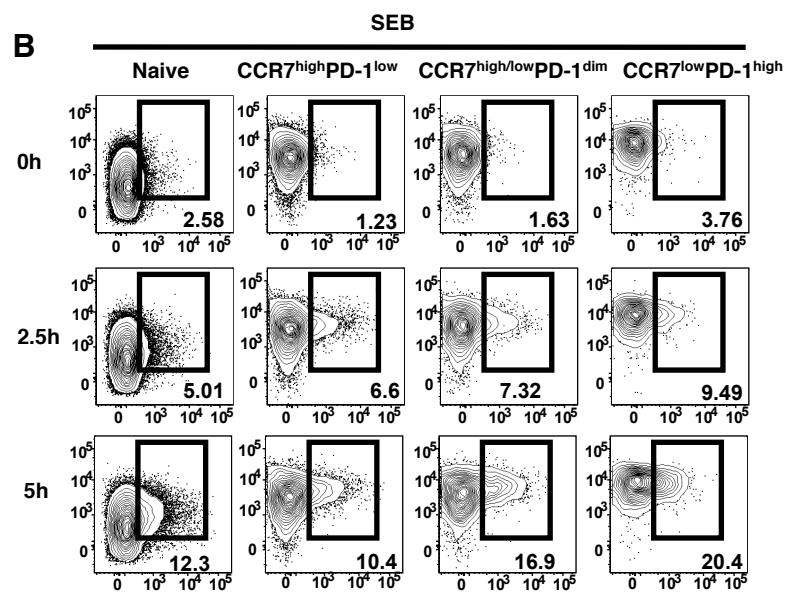
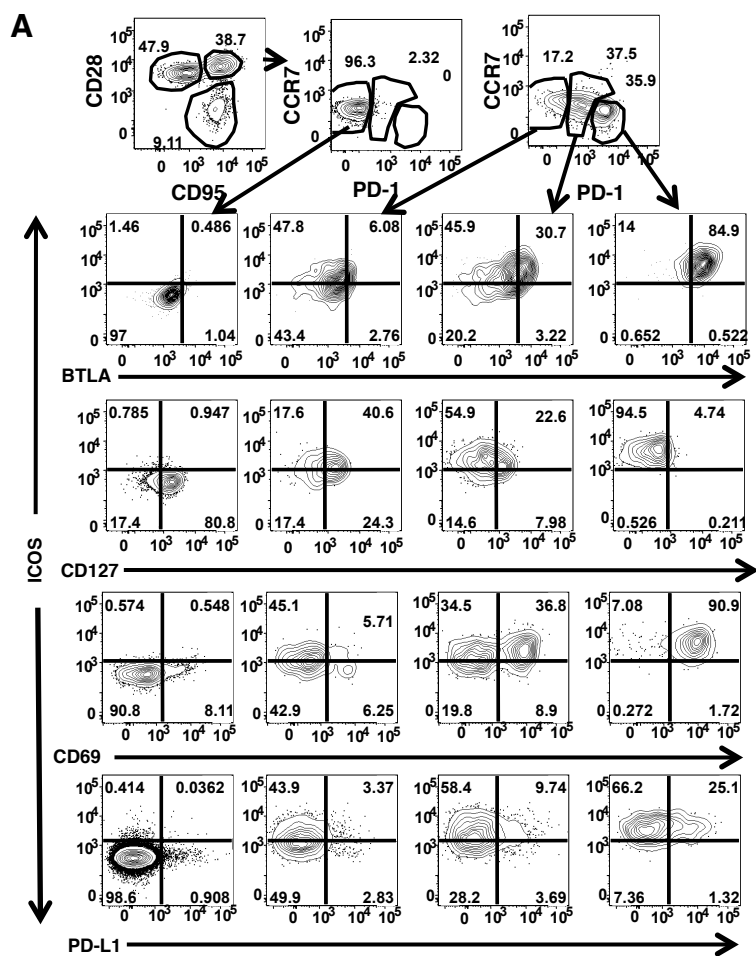
Supplementary Figure 5. (A) Pooled data showing the relative frequency of either Foxp3⁺ or Foxp3⁺CD25⁺ cells in sorted CD28^{high}CD95^{high} CD4 T cell subsets. (B) Pooled data showing the expression of PD-L1 (MFI) on IgD^{high} and IgD^{low} CD20^{high}CD3^{low} B cells in LNs from SIV- (n=10) and SVI-chronic (n=14) RMS. (C) The relative expression of the *SIPRI* mRNA in sorted cells from SIV- RMS (n=3, LN tissues) is shown. The normalized CT value for CD28^{high}CD95^{high}CCR7^{high}PD-1^{low} cells was assigned a value of 1 and the fold change over this value for the populations tested is shown. (D) The expression (MFI) of IL-6Ra on T_{FH} subsets from non-infected (n=10) (open circles), SIV-acute (n=11) (light gray circles) and SIV-chronic (n=18) (dark gray circles for low (%) T_{FH} and red circles for high (%) T_{FH}) RMS is shown. (E) Pooled data showing the expression (MFI) of IL-6R-gp130 on naïve and memory CD4 T cell populations in LN tissues from non-infected (n=6) and SIV-chronic (n=6) RMS. Horizontal lines depict mean values. *p* values were calculated using Mann-Whitney *U* test.

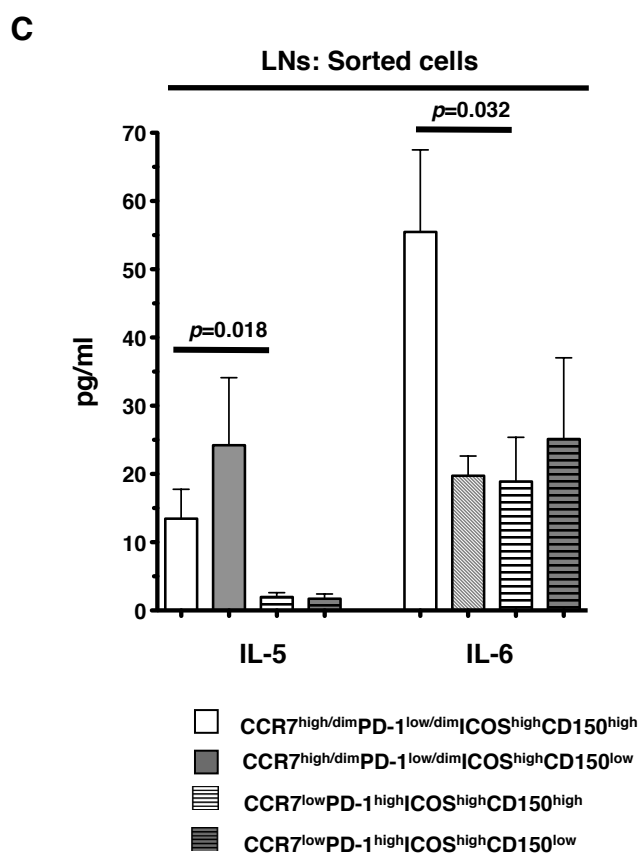
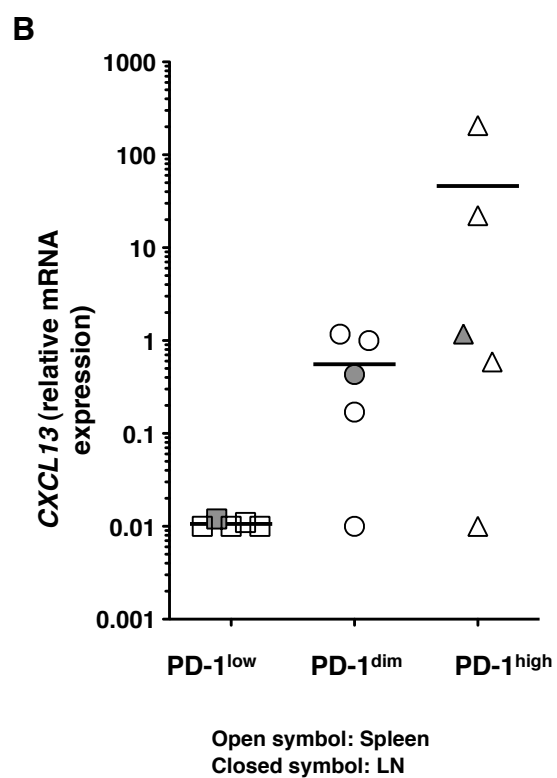
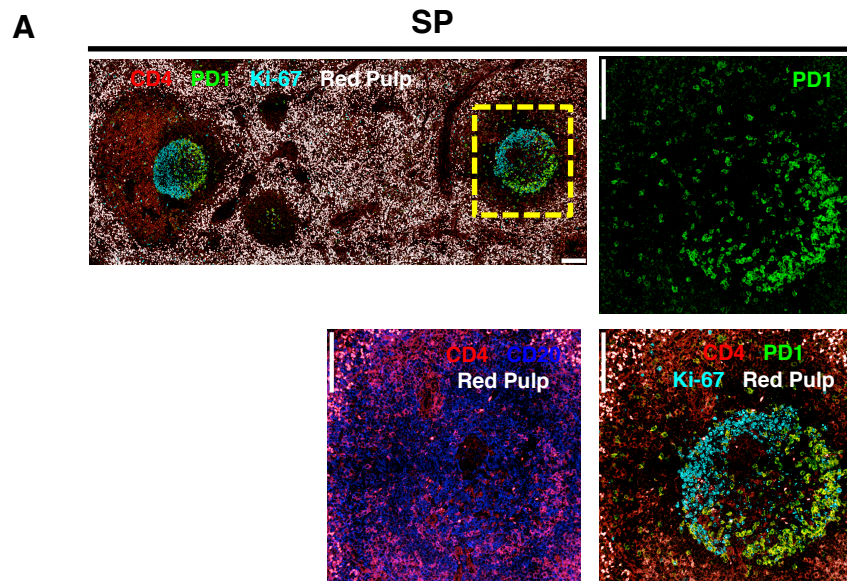
Supplementary Figure 6. (A) Flow cytometry plots showing the phosphorylated STAT-1 and STAT-3 in PD-1^{low}, PD-1^{dim}, PD-1^{high} CD4 T cells from a non-infected, a SIV-acute, a SIV-chronic with low % and a SIV-chronic with high % of TFH cells after *ex vivo* treatment with IL-6. The frequency of the gated populations is shown. (B) Pooled data showing the basal (no stimulation) levels of pSTAT-1 (*left panel*) and pSTAT-3 (*right panel*) in PD-1^{low}, PD-1^{dim} and PD-1^{high} CD4 T cells from non-infected (n=4), SIV-acute (n=4) and SIV-chronic (n=11) LN tissues. For comparison, the same scale as in figure 6E was used. Horizontal lines depict mean values. (C) Sorted cells were stimulated with anti-CD3 in the absence (solid) or presence of IL-6 (line). Representative histograms depicting the levels of Ki67 and in vitro BrdU integration in naïve and CCR7^{high/low} PD-1^{dim/low} CD4 T cells for one out of two LN tissues tested.

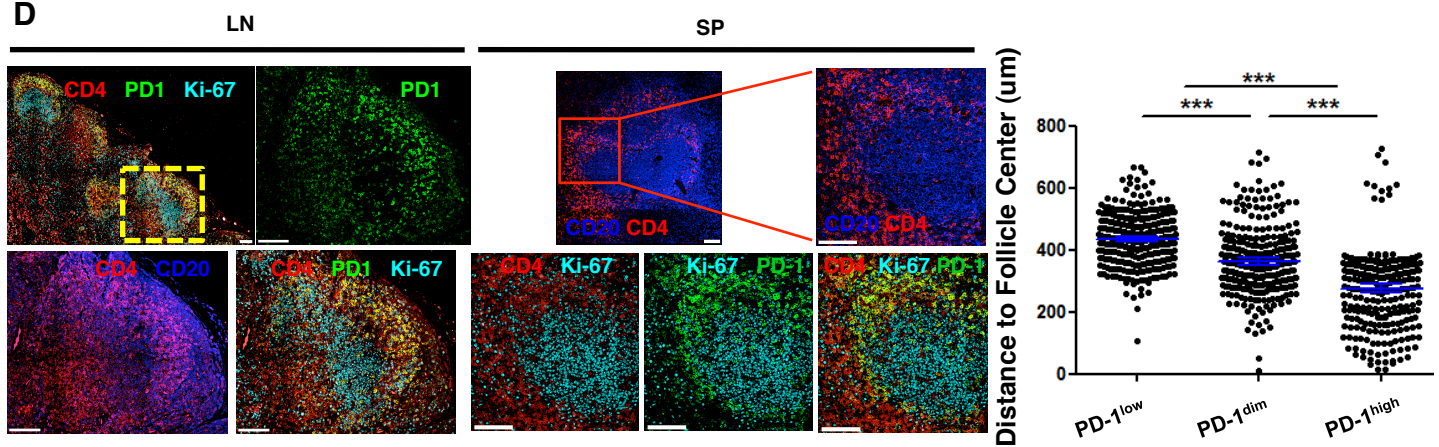
Supplementary Figure 7. (A) Flow cytometry plots showing the gating scheme for identification of CD95^{low} and CD95^{high} CD4 T cells from a LN. Cells were left untreated or treated with IL6 in the absence or presence of anti-CD3. **The frequency of the gated populations is shown.** (B) Pooled data showing the levels of ICOS (*upper panel*), CD95 (*middle panel*) and PD-1 (*lower panel*) in LN (n=5) CD4 T cell populations treated *ex vivo* with IL-6 in the presence or absence of anti-CD3.

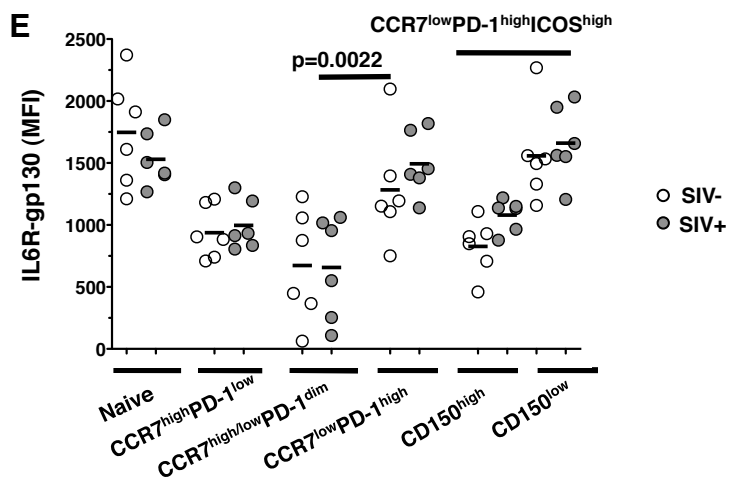
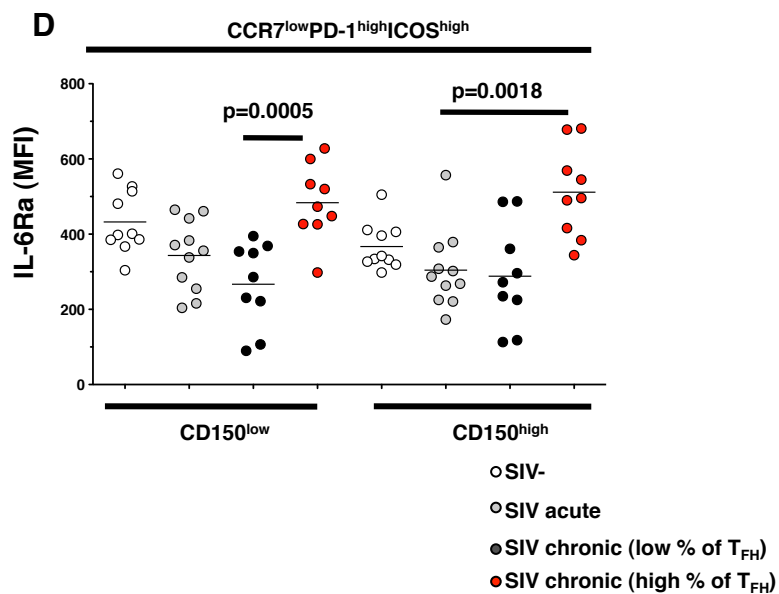
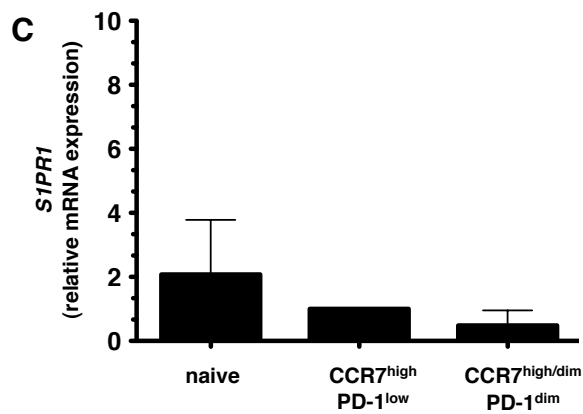
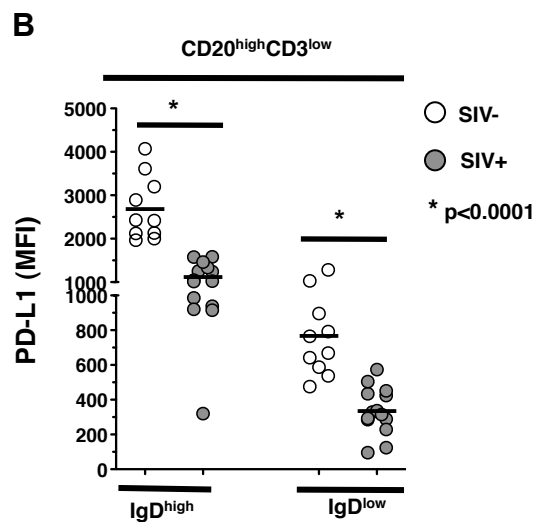
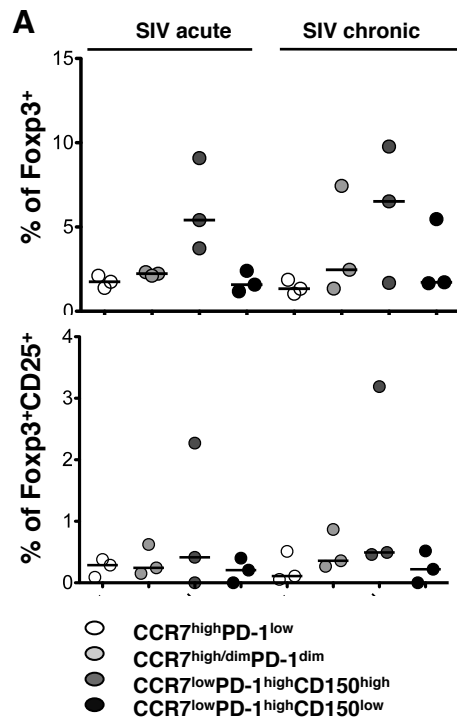
Supplementary figure 8. (A) Pooled data showing the frequency (%) of CD20^{high}CD3^{low} B cells in LNs from SIV- (n=15) and SIV-chronic RMs (n=10 for low % of T_{FH} and n=9 for high % T_{FH} LNs) (*left panel*). **The relative frequency (%) of CD20^{high}CD3^{low} CD27^{low}IgD^{high} (*middle panel*) and CD20^{high}CD3^{low} IgG^{high} (*right panel*) is also shown.** (B) Pooled data showing the frequency (%) of Ki67^{high} cells in total (*left panel*) as well as memory populations (*right panel*) of CD20^{high}CD3^{low} B cells in LNs from SIV- (n=8) and SIV-chronic RMs (n=5 for low % of T_{FH} and n=5 for high % T_{FH} LNs). (C) Pooled data showing the avidity for plasma purified IgG from SIV-chronic RMs. **Each dot represents data for a single sample from an individual animal. Animals are represented only once in each plot.** Horizontal lines depict mean values. *p* values were calculated using Mann-Whitney *U* test.



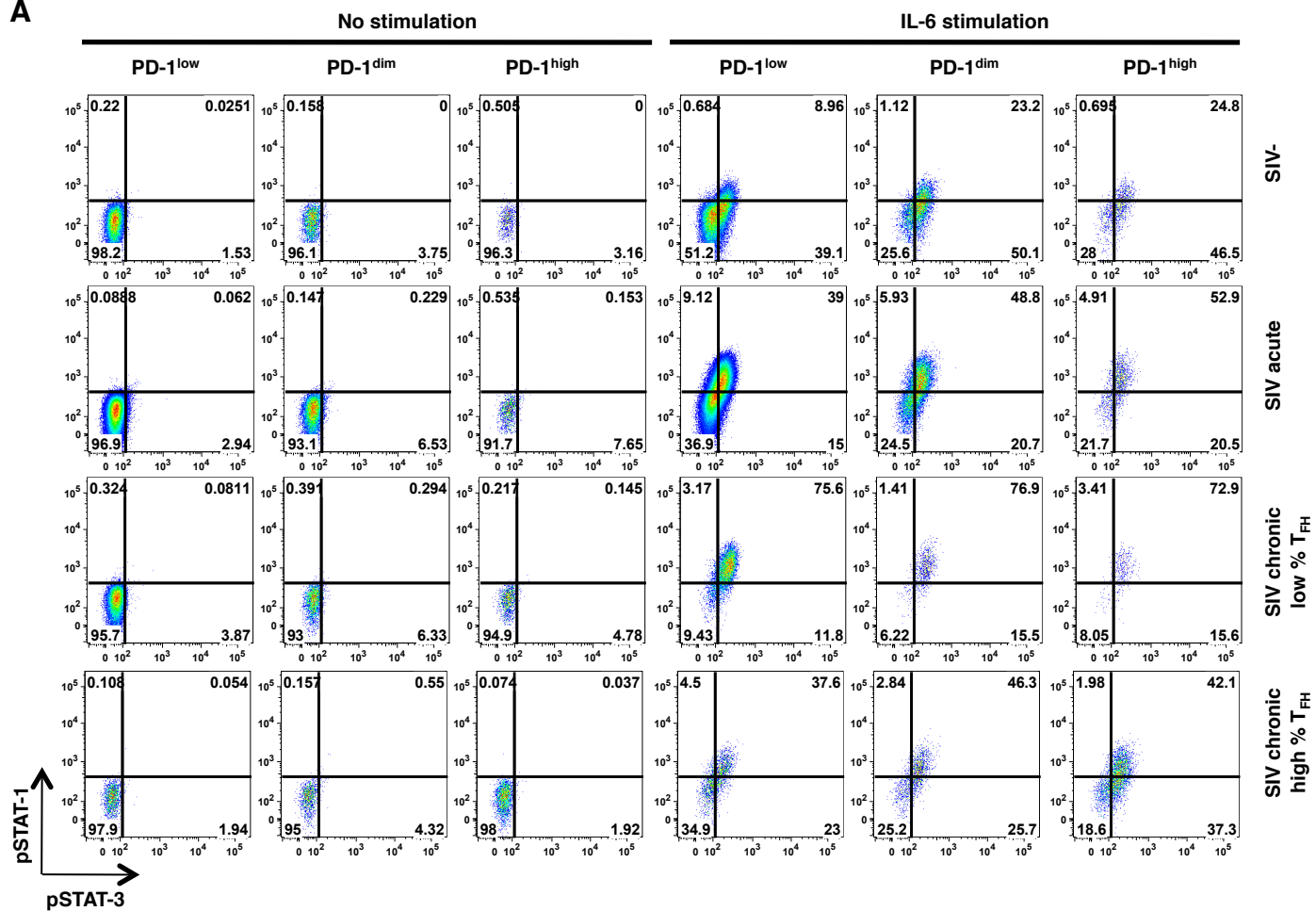




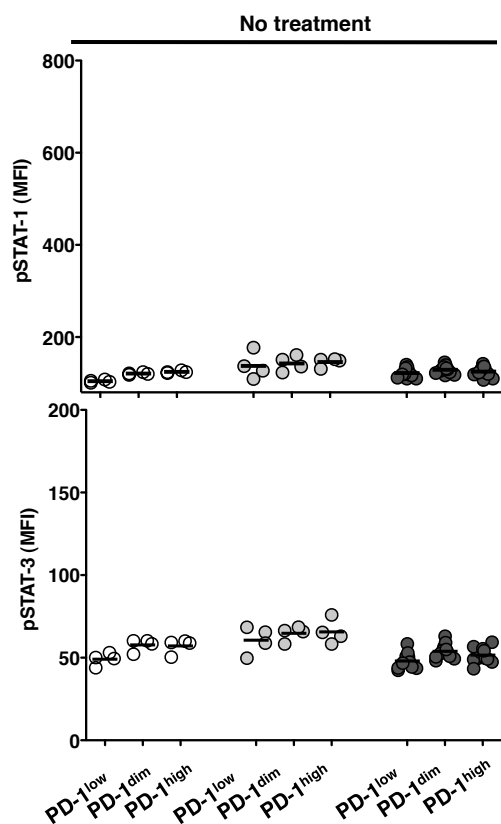




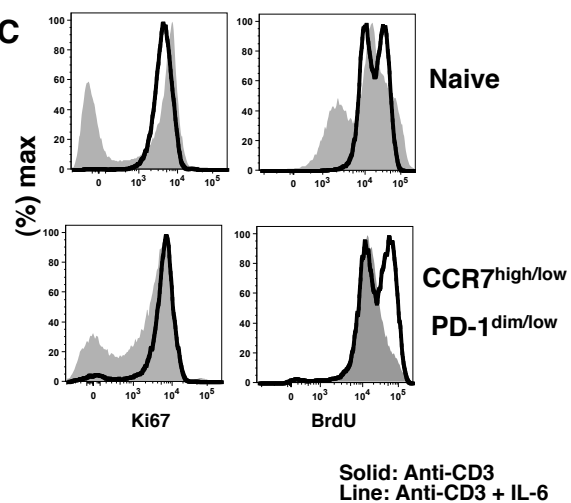
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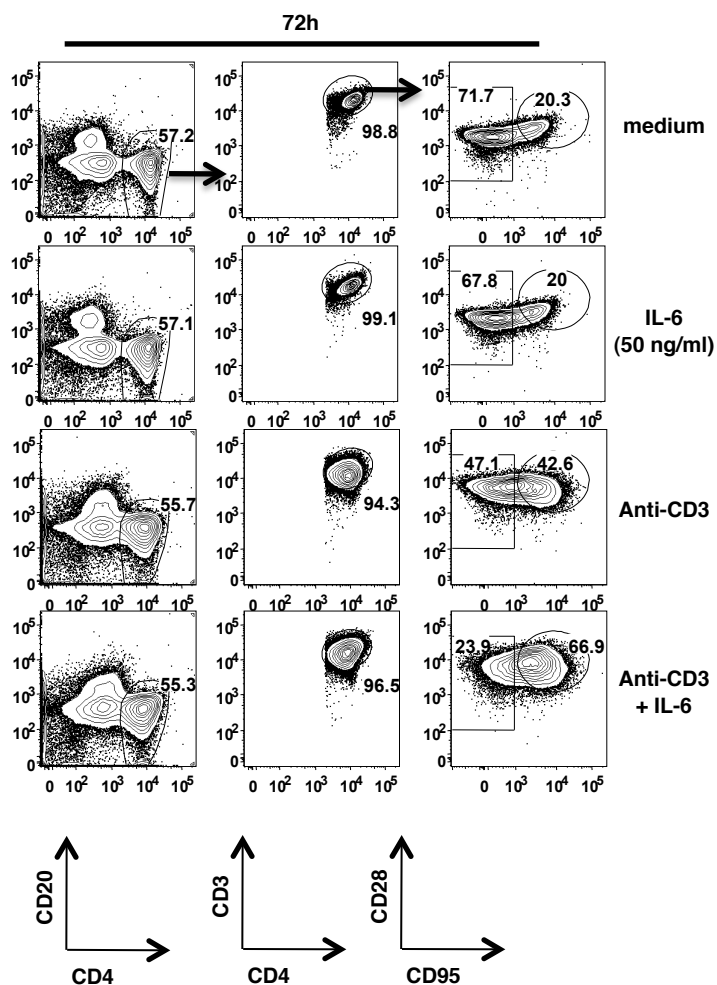
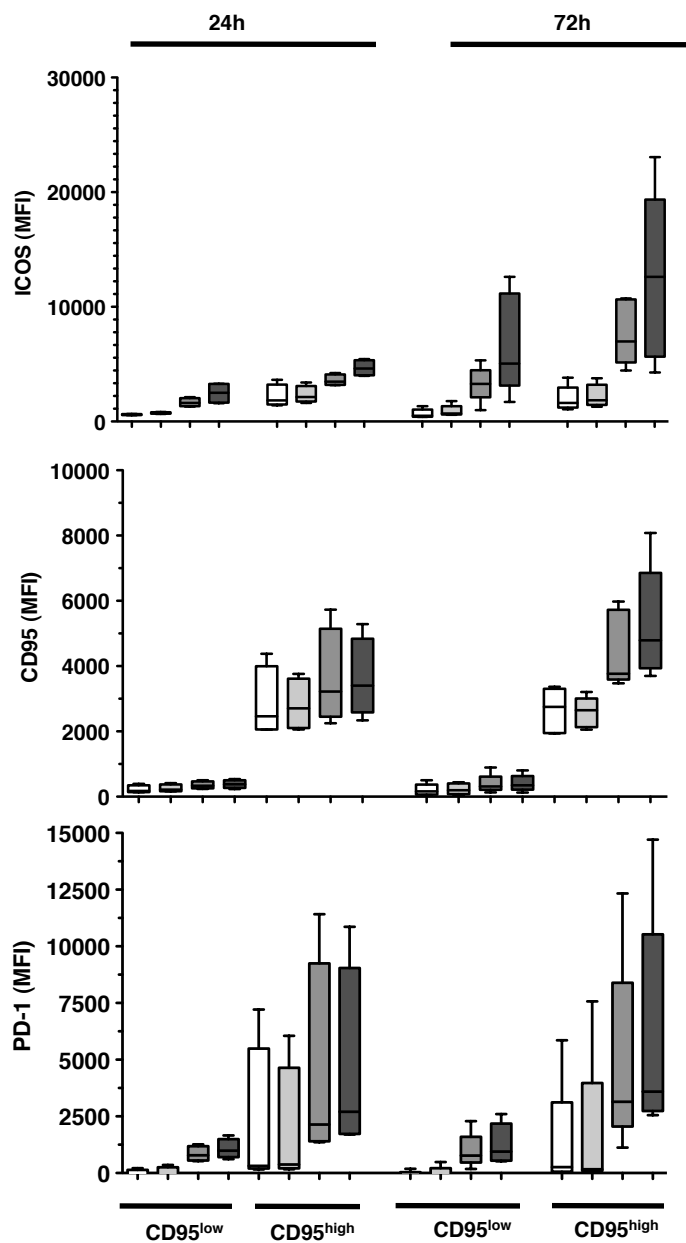


B



C



A**B**

□ medium

▒ IL-6 (50 ng/ml)

▓ Anti-CD3

■ Anti-CD3 + IL-6

