阻断免疫抑制信号在艾滋病防治中的作用

Blockade of inhibitory pathway for controlling HIV infection

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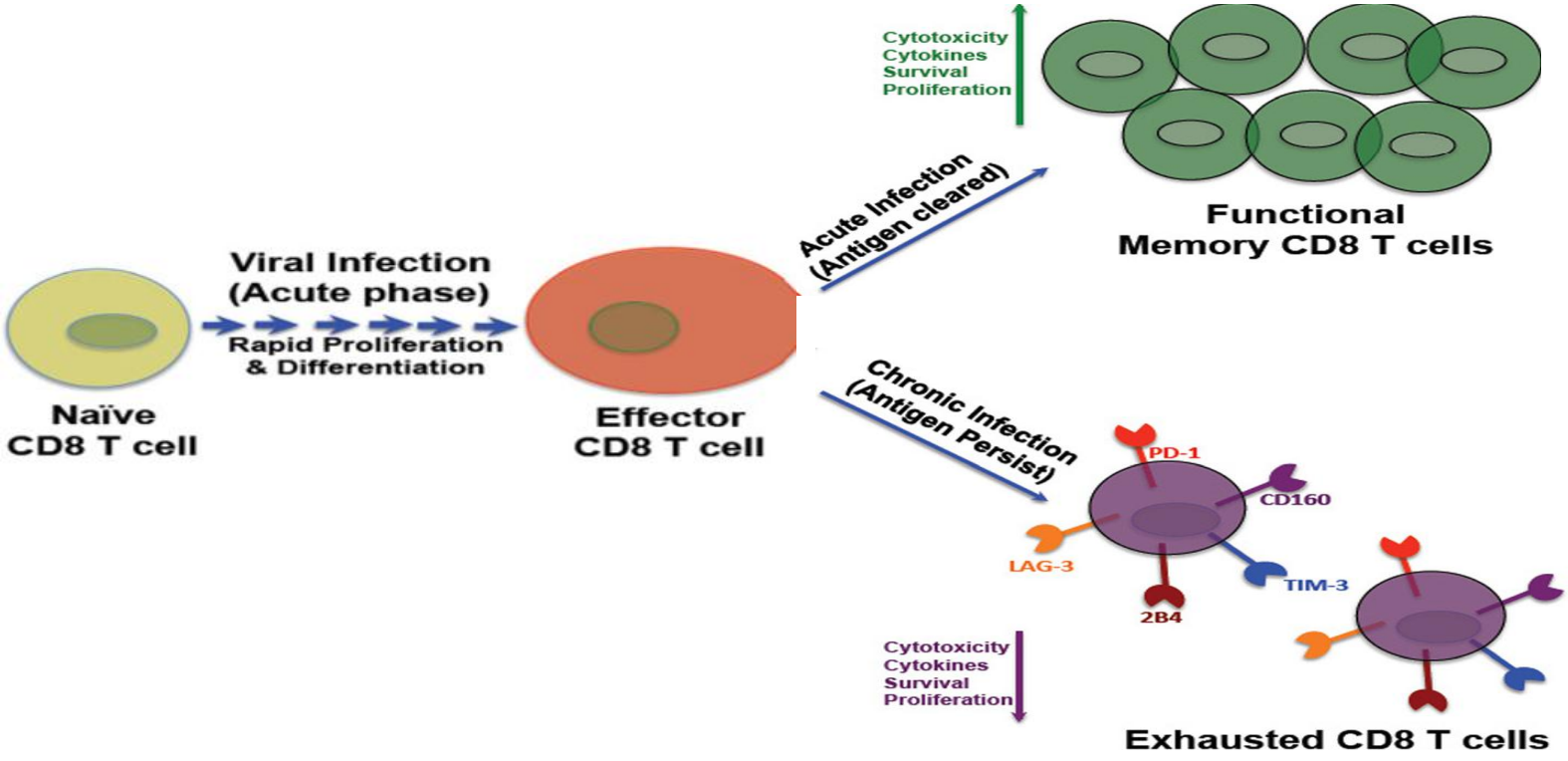
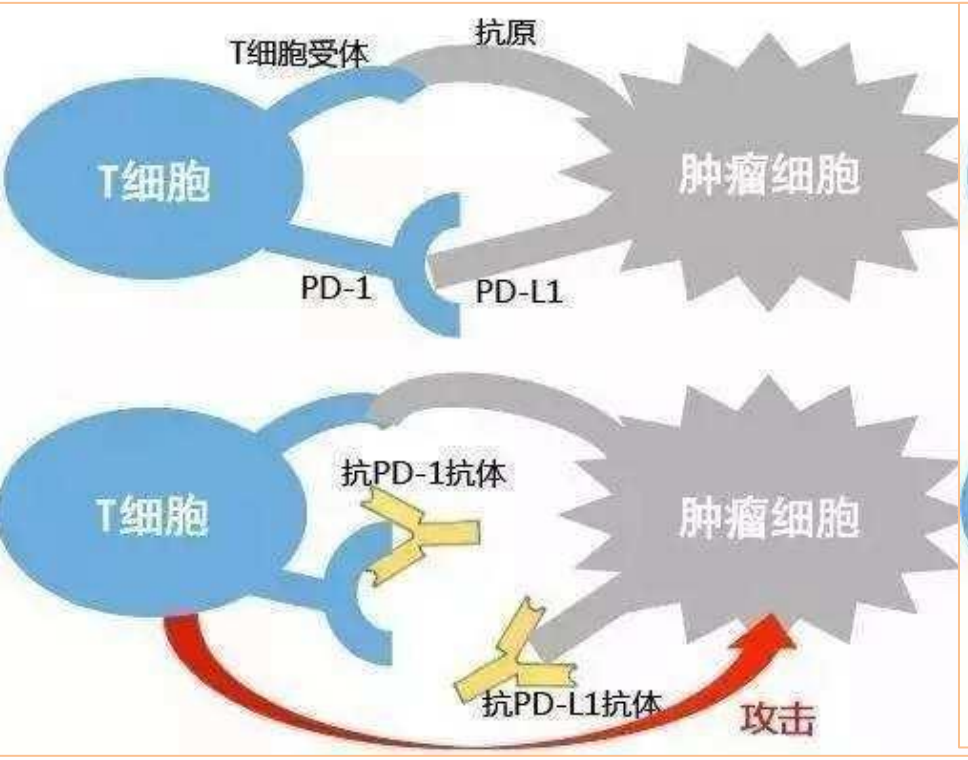
CTLs eliminate viral-infected cells, but persistent chronic infection leads to T cell exhaustion due to elevated immune-inhibitory signals

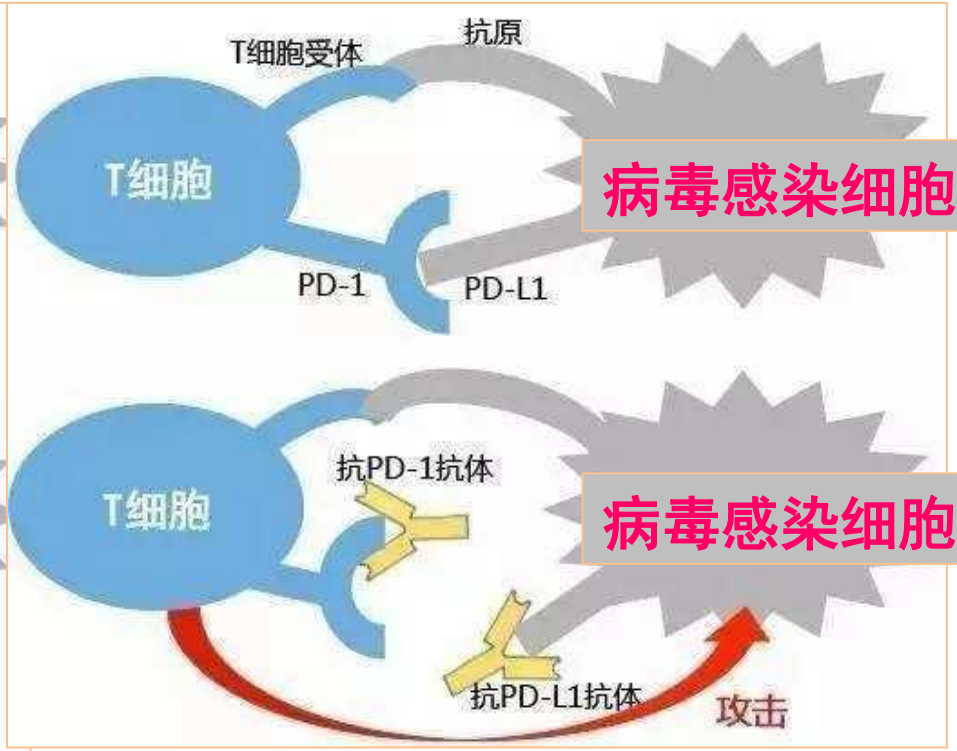


Roles of immune checkpoint molecules during HIV infection

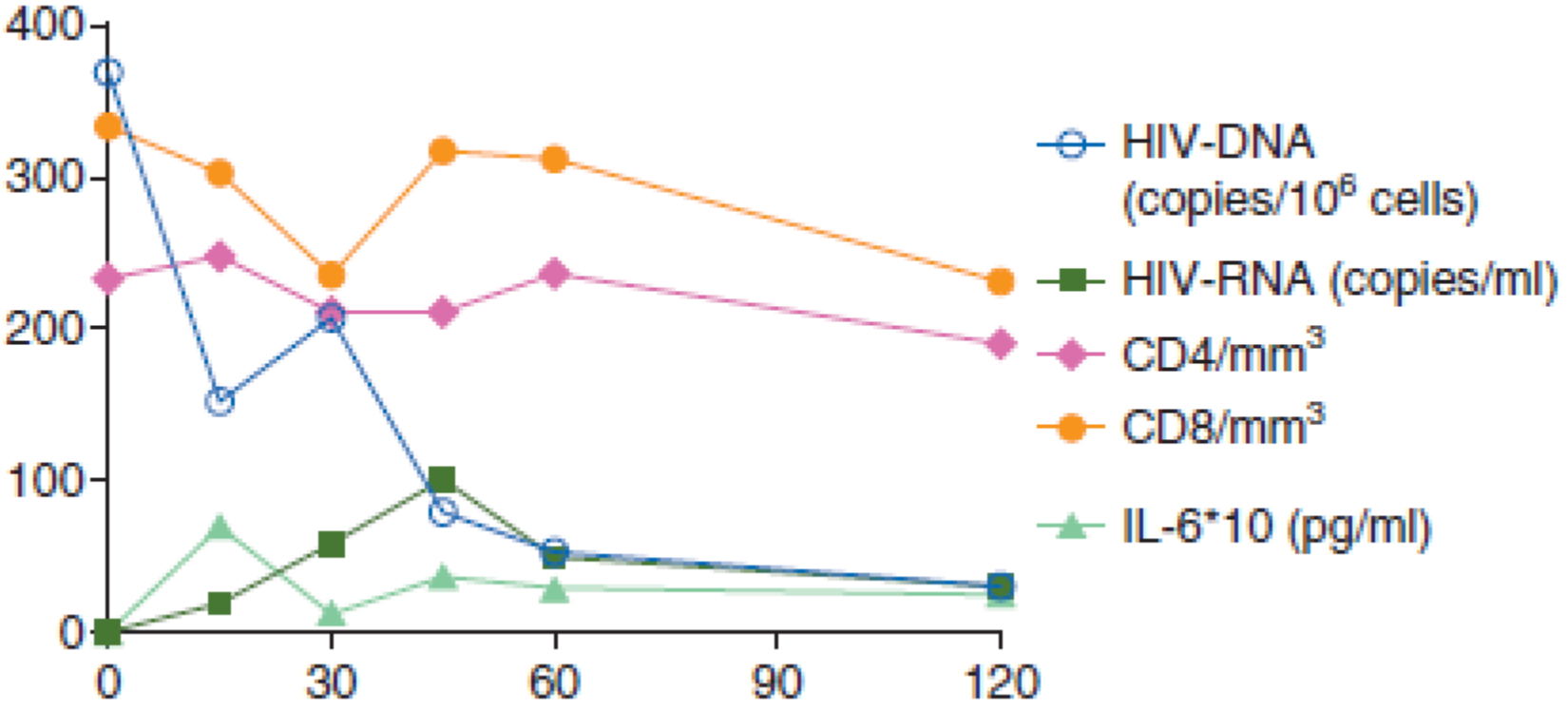
Molecule	Expression	Function	Role in HIV Infection	Effects of Blocking Antibodies
Cytotoxic T-lymphocyte-associated protein 4 (**CTLA4**)	T cells	Regulates early stage of T cell activation	Upregulation on HIV-specific CD4+ T cells and often co-expressed with PD-1	Blocking CTLA4 pathway In vitro increased proliferation of HIV-specific CD4+ T cells
Programmed cell death protein 1 (**PD-1**)	T cells B cells Natural killer (NK) cells Monocytes Dendritic cells (DCs)	Limits the activity of immune cells in the periphery e.g., inhibits cytokine production, proliferation, survival of activated T cells	Elevated numbers of cells expressing PD-1 and upregulated expression on HIV-specific CD8+ T cells elevated during chronic infection with loss of CD28 and perforin expression Upregulation of PD-1 on HIV-specific CD4+ T cells Upregulation of PD-1 and PD-L1 expression on B cells Upregulated expression of PD-1 and ligand PD-L1 on monocytes, macrophages, dendritic cells and neutrophils	In vivo blockade with anti-PD-L1 antibody increased proliferation capacity and cytokine production in HIV-specific CD8+ and CD4+ T cells. The first study of PD-L1 blocking in vivo: enhancement of HIV-1 specific responses by increasing percentage of gag-specific CD8+ T cells producing IFN in 2/6 individuals. In vitro blockade of PD-1 pathway increases responses, survival, and proliferation of memory B cells in HIV infection In vivo blockade in SIV increased the amount of antibodies produced against SIV antigens
Lymphocyte-activation gene 3 (**LAG3**)	T cells NK cells	Negatively regulates T cell signaling and controls memory T cell pool size	Elevated numbers of LAG3-expressing CD4+ and CD8+ T cells with increased density of LAG3 on these cells	Blocking LAG3 ex vivo enhances proliferation and effector function of HIV-specific T cells
T cell immunoglobulin and mucin-domain-containing-3 (**TIM3**)	Cells of innate and adaptive immune system	Regulating innate and adaptive immunity with both stimulating and inhibitory mechanisms	Elevated TIM3 expression on CD4+ and CD8+ T cells; Upregulation on HIV-specific CD8+ T cells in progressive HIV infection with a lack of proliferation markers and cytokine production in response to HIV-specific antigens ex vivo.	Blocking TIM3 signaling ex vivo restored CD8+ T cell functionality and enhanced their expansion

Blockade of inhibitory signals restore immune functions against cancer and infectious diseases





Increase of HIV RNA and decrease of HIV reservoir in a patient with anti-PD1 therapy for a lung cancer patient with HIV infection



Guihot A. et al. Ann Oncol. 2018

Summary table for immune checkpoint inhibitors in regulating the latent HIV/SIV reservoirs

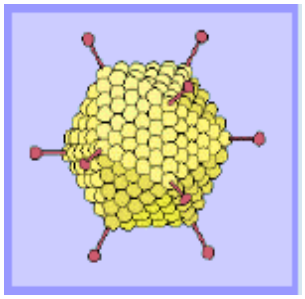
Model type /trial type	target	Findings	
		Virological	Immunological
SIV-infected rhesus macaques	PD-1 (clone EH12-1540)	Significant reduction in plasma viremia. Macaques during late chronic stage of infection had viral RNA copies drop below pre-treatment levels and delayed disease progression.	Rapid expansion of SIV-specific CD8+ T cells.
SIV-infected rhesus macaques	CTLA-4 (MDX-010)	Significant increase in plasma viremia.	Increased levels of T cell activation
Case report of HIV/HCV co-infected patient with malignancy (melanoma)	CTLA-4 (ipilimumab) then PD-1 (pembromlizumab)	Viral loads did not increase following administration of treatment.	No immune-related adverse events experienced.
HIV-infected participants with malignancy (NSCLC)	PD-1 (nivolumab)	Viral loads remained undetectable.	CD4+ T cell counts remained stable.
HIV-infected participants with malignancy	PD-1 (nivolumab or pembromlizumab)	No consistent changes in CD4+ T cell-associated HIV RNA or DNA or plasma viremia.	PD-1 binding decreased following initiation of therapy. No consistent changes in frequency of total or activated CD4+ or CD8+ T cells.
Case report of HIV-infected patient with malignancy (NSCLC)	PD-1 (nivolumab)	Transient increase in plasma HIV copies. Overall decrease in cell-associated HIV DNA.	Total CD4+ and CD8+ counts remained stable. Decrease in PD-1+ T cells.
Case report of HIV-infected patient with malignancy (NSCLC)	PD-1 (nivolumab)	Transient increase in cell-associated HIV DNA levels.	Transient increase in IL-6 levels. Transient increase in CD4+ and CD8+ T cell counts. Decrease of PD-1 expression by T cells.
Case report of HIV-infected patient with malignancy (melanoma)	CTLA-4 (ipilimumab)	Cyclical decrease in plasma HIV RNA levels following each dose of antibody, with an overall decline from 60 to 5 copies/ml. Cell-associated unspliced HIV RNA from CD4+ T cells increased ~ 20 fold.	Increase in total CD4+ T cell numbers.
Case report of HIV-infected patient with malignancy (melanoma)	PD-1 (nivolumab)	Cell-associated unspliced HIV RNA increased ~25 fold. Ratio of cell-associated unspliced HIV RNA:HIV DNA significantly increased.	No changes reported.
Otherwise healthy HIV-infected participants	PD-L1 (BMS-936559)	No consistent changes in cell-associated RNA or DNA.	Significant increase in HIV-specific CD8+ T cells in 2 of 6 treated participants.

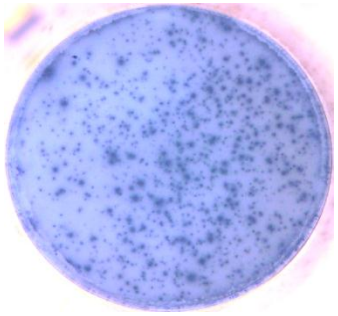
Scientific question

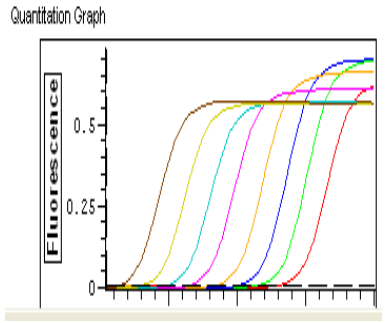
- Recently, some case reports suggested that anti-PD1 therapy for cancer patients with HIV infection might affect the level of cell-associated HIV RNA and DNA. Several ex vitro studies also showed that cells expressing CTLA4, PD-1, LAG-3 and the activation marker HLA-DR are enriched to harbor HIV provirus. **However, it is not fully clarified the exact interactions between PD-1 signal modulation and latent reservoirs.**

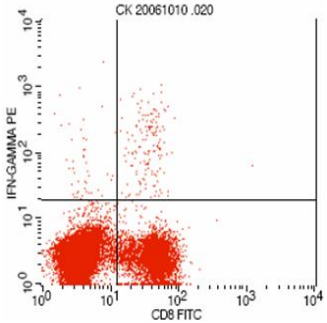
- In this study, we therefore investigated **how PD-1 blockade affect the immunogenicity and protection of prophylactic vaccines,** and **whether PD-1 signal modulation is correlated with viral rebound in chronic SIV-infected macaques**, and further explore the underlying mechanisms of preferential formation and maintenance of latent reservoirs in PD-1+CD4+T cells, indicating the **feasibility to develop the potentially preventive and therapeutic strategies against HIV infection by targeting PD-1-expressing CD4+T and CD8+T cells.**

Establishment of animal model of SIV-infected rhesus monkeys

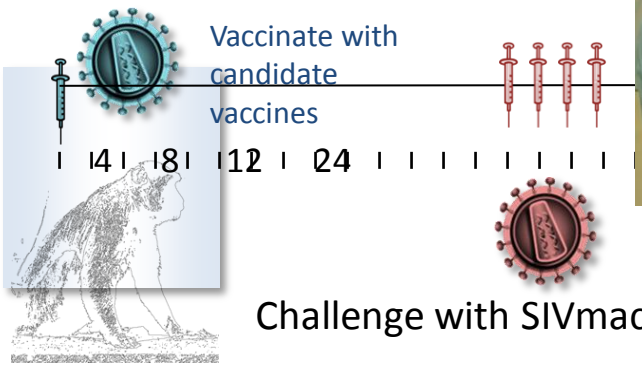








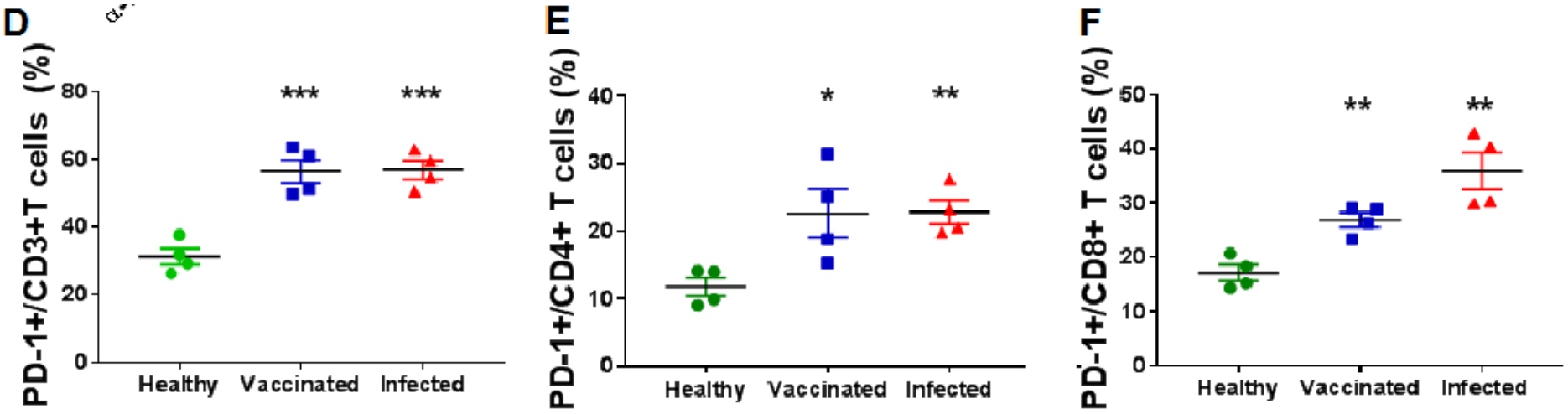
- 建立和完善了中国猕猴/SIV感染模型用于评价抗艾滋病药物和疫苗的保护效果。



Challenge with SIVmac239 (IV or intrarectal)

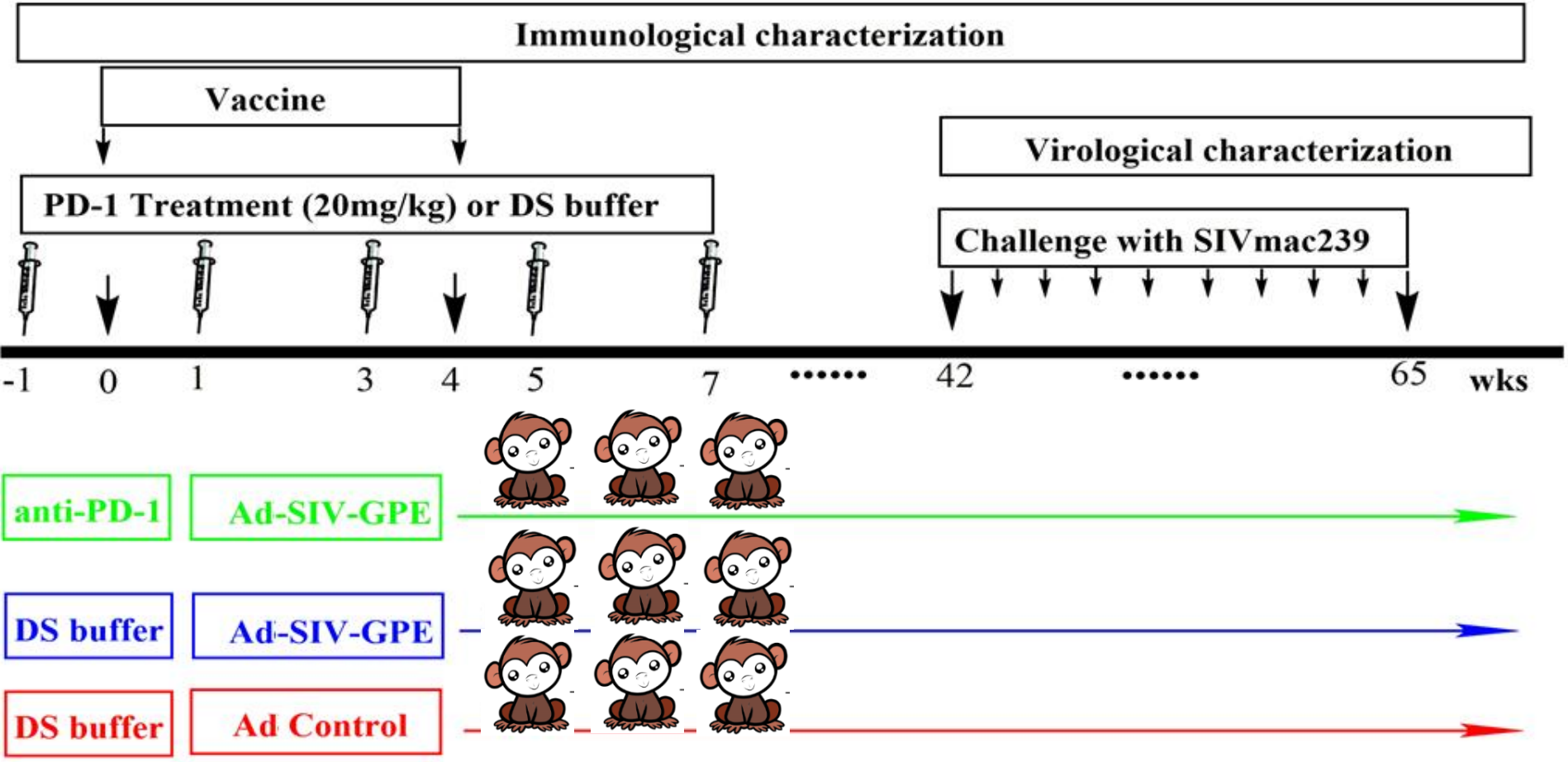
Sun et al. J Infect. 2010； Sun et al. J Virol., 2012; Sun et al. J Virol., 2013; Wang et al. Adv Mat, 2016; Pan et al. Front Immuo., 2018; Yang et al. Adv Sci., 2019

PD-1 pathway is involved in response to SIV infection as well as vaccination

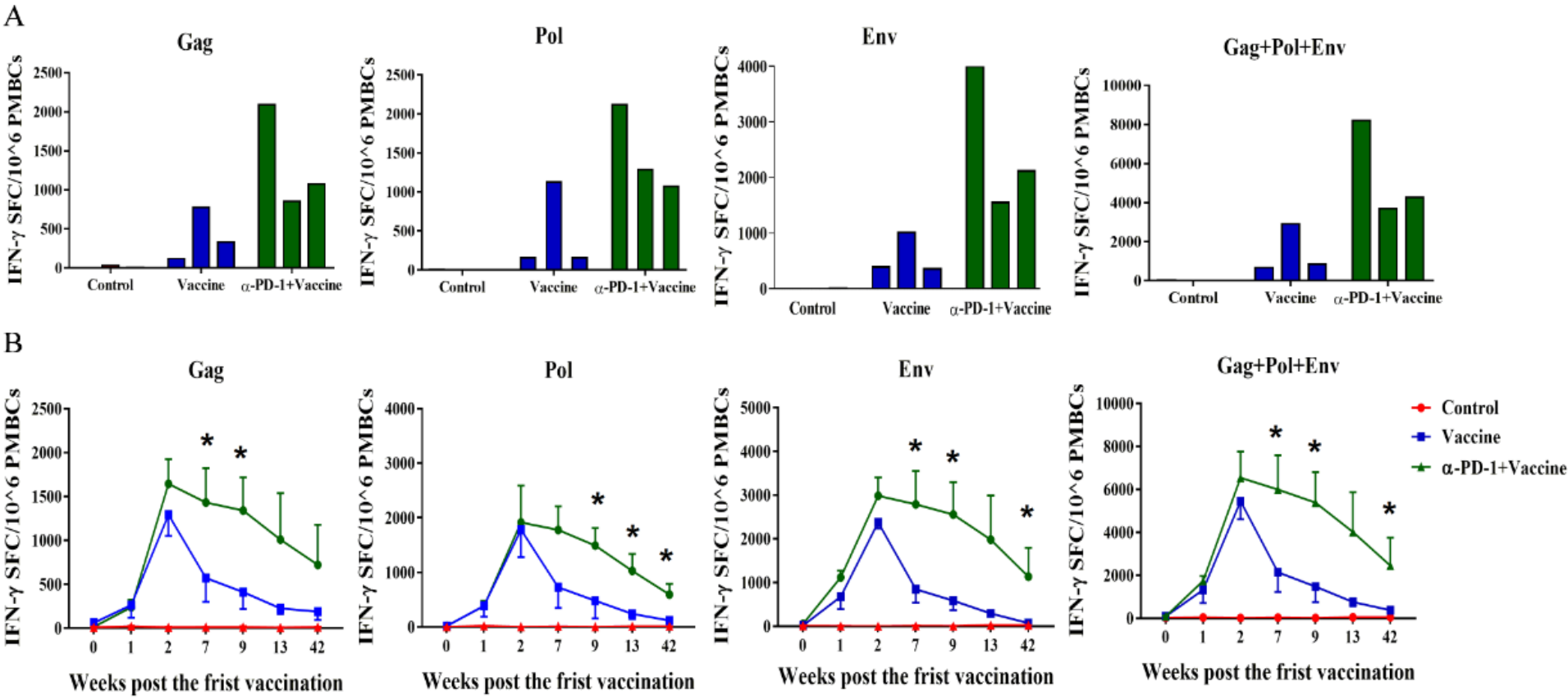


Pan et al. Front Immunol., 2018

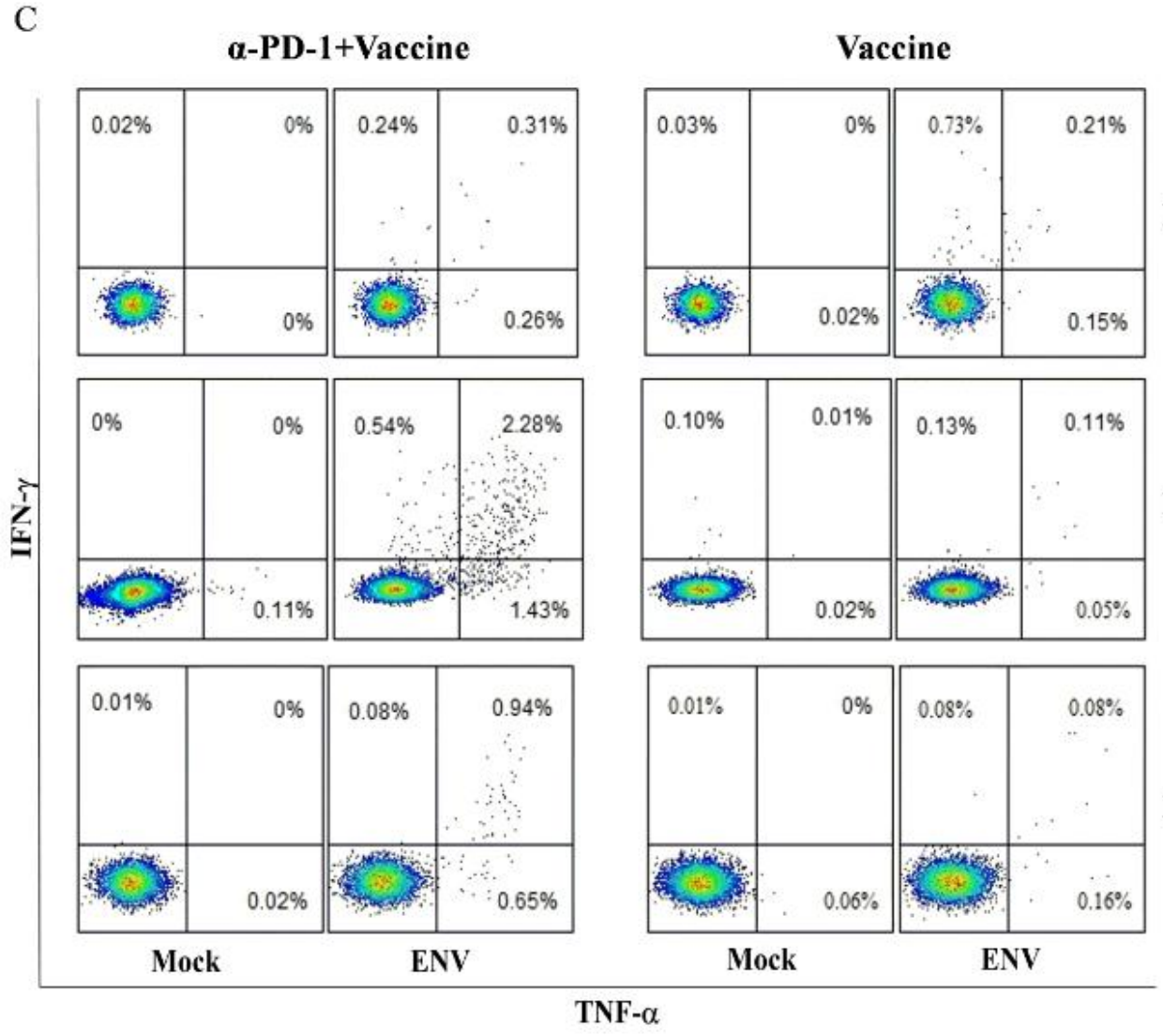
Experimental design for preventive vaccination

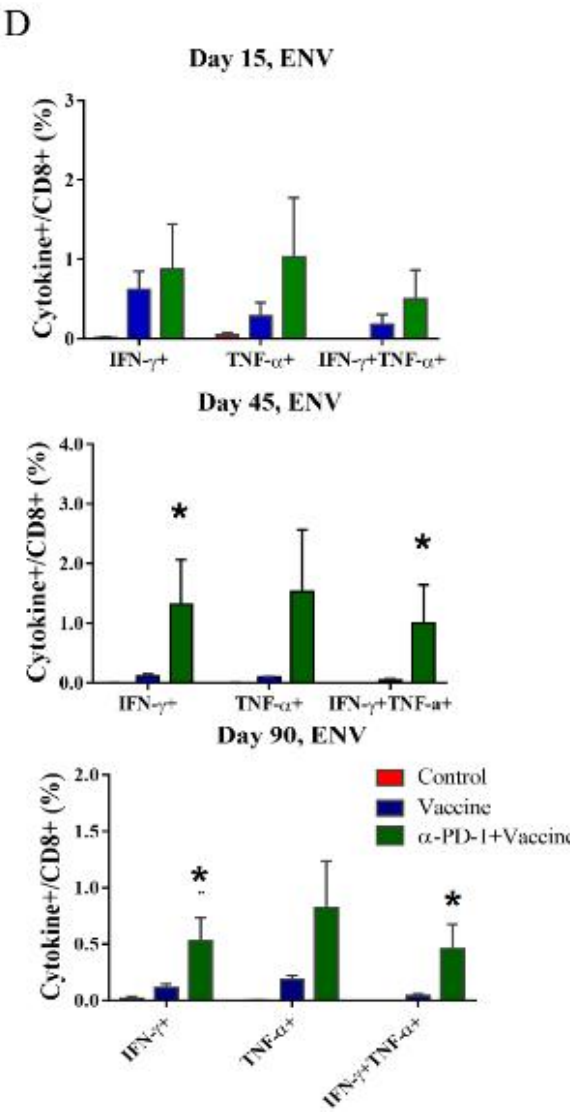


Robust and Sustained SIV-specific T responses by PD-1 blockade in monkeys



Elevated SIV-specific T responses with poly-functionality by PD-1 blockade in monkeys





Improved Immune Protection Against SIV Infection by PD-1 Blockade in Monkeys

"+" represents SIVmac239 $TCID_{50}$:

+:1000;

++:5000;

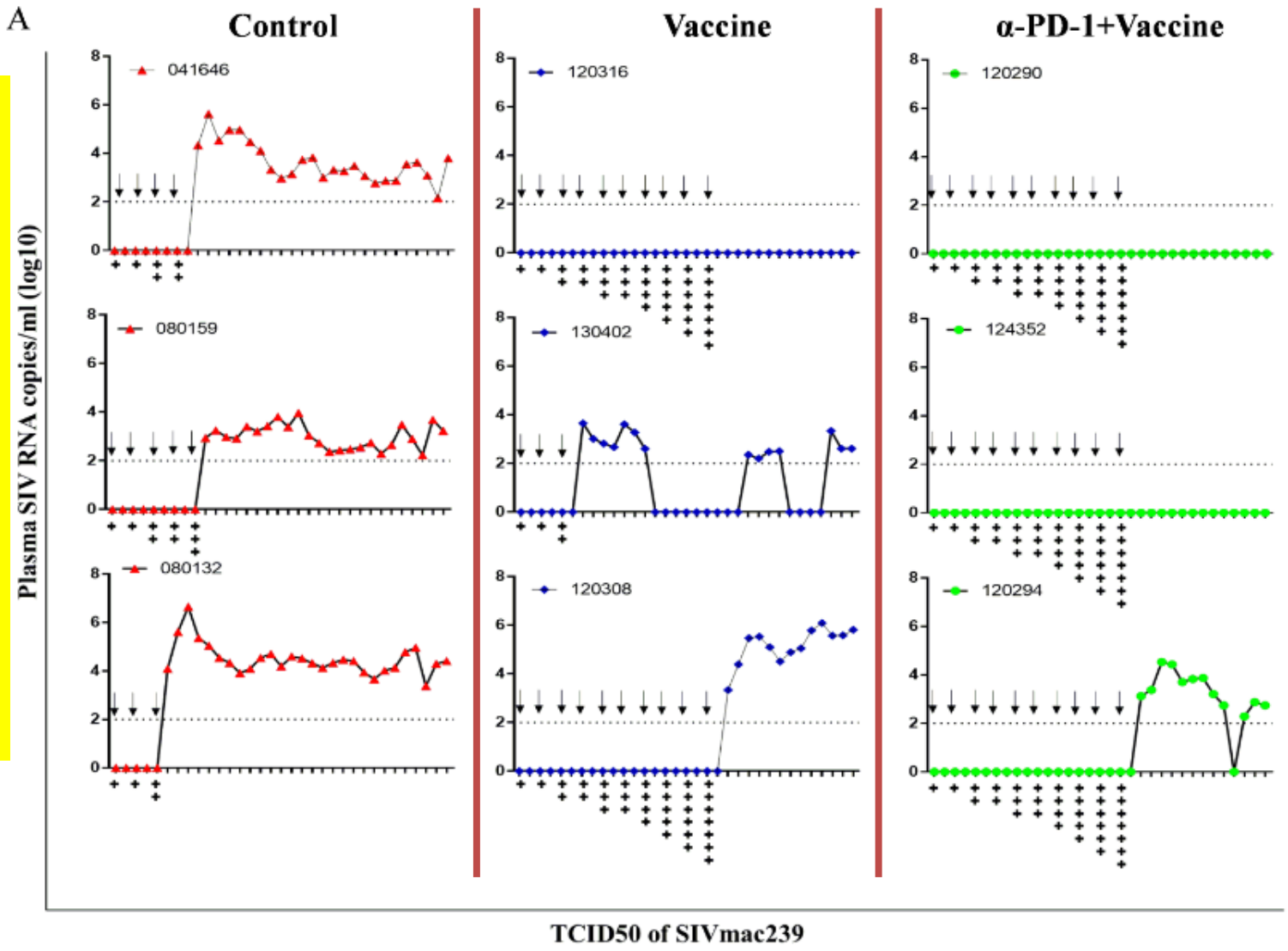
+++:10000;

++++:15000;

+++++:25000;

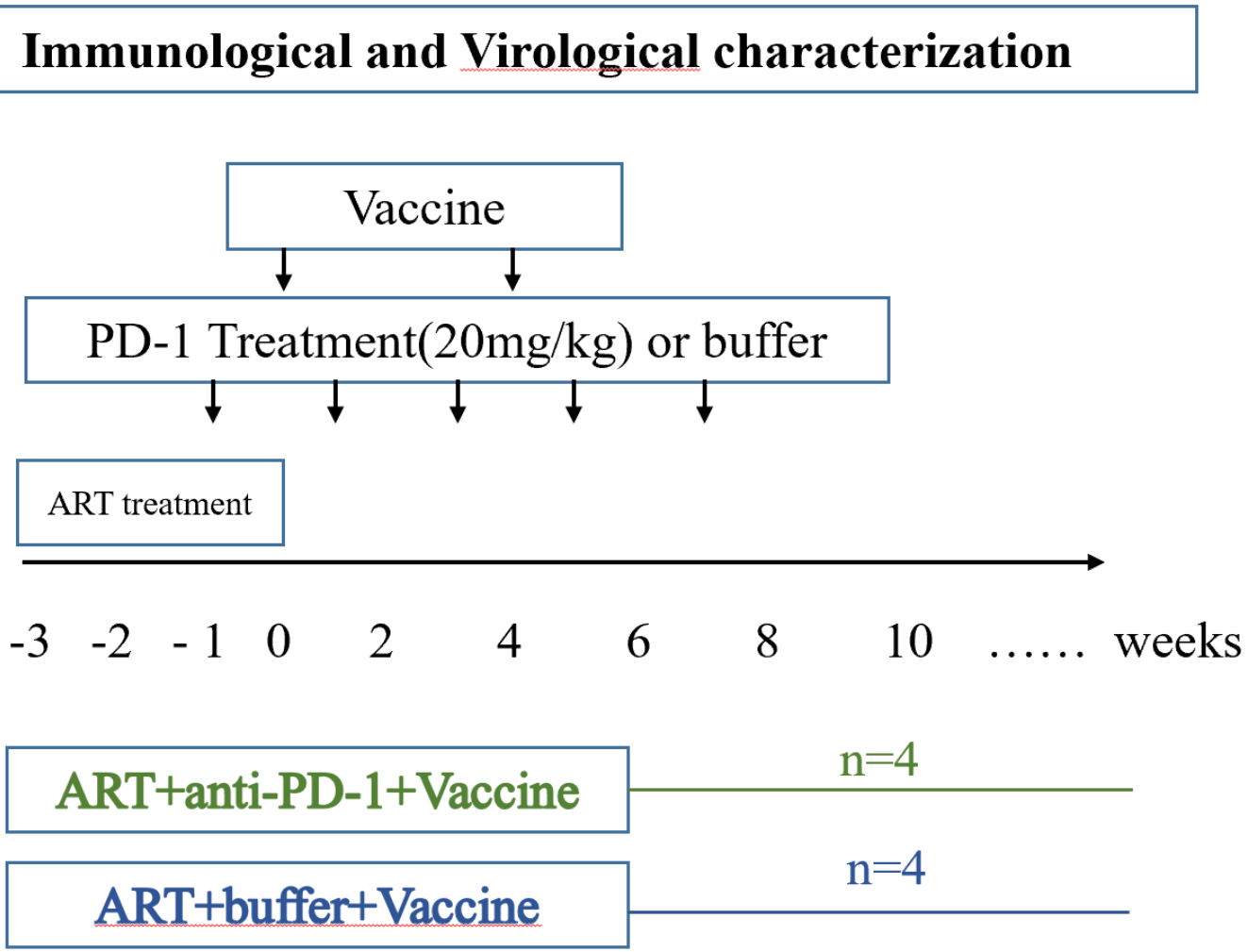
++++++:50000;

+++++++:100000.



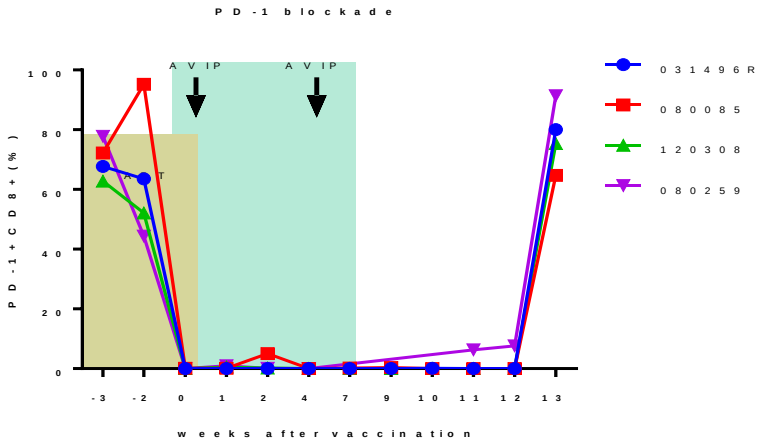
Pan et al. Front Immuno., 2018

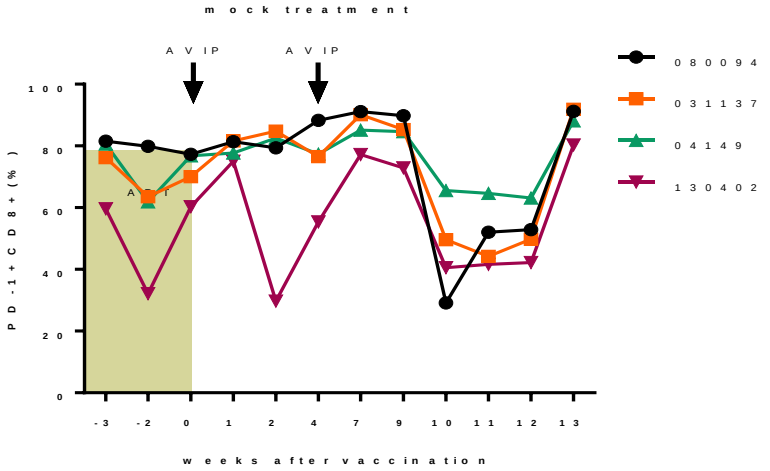
Experimental design for therapeutic vaccination in SIV-infected macaques

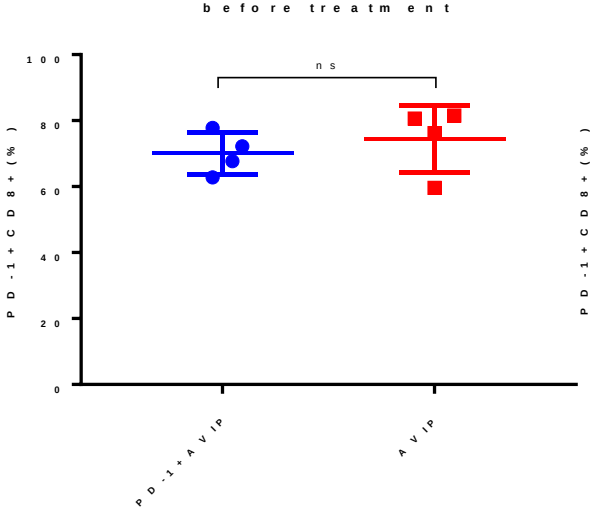


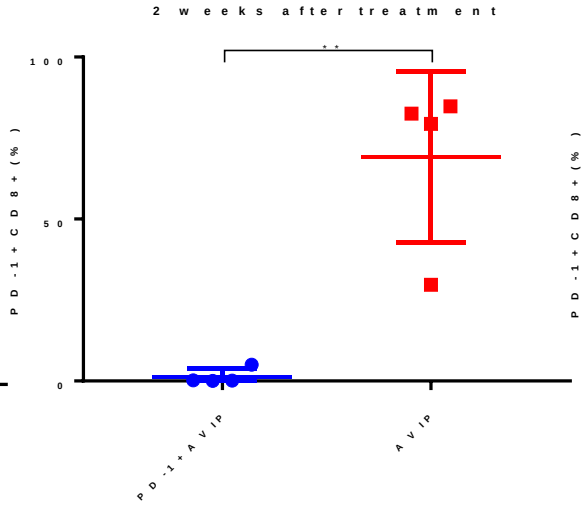
Data not published

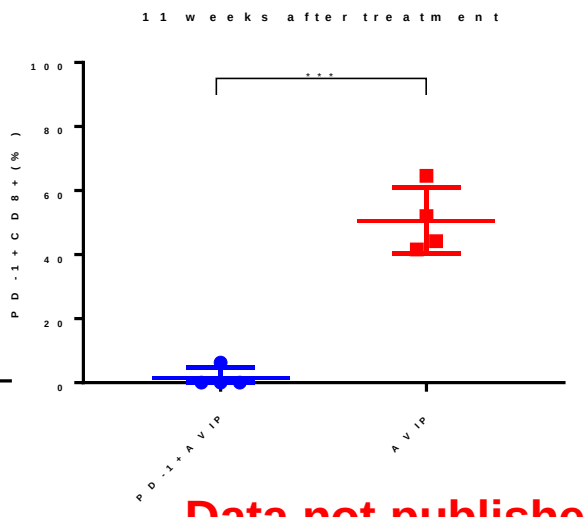
GB226 antibody effectively blocked PD-1 signal on T lymphocyte surface in SIV-infected macaques











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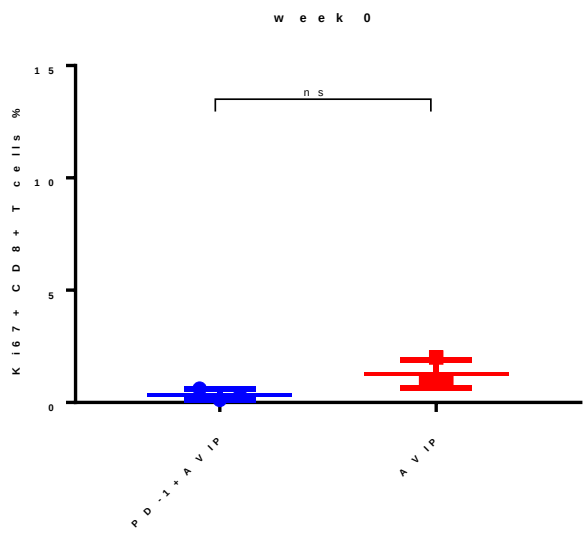
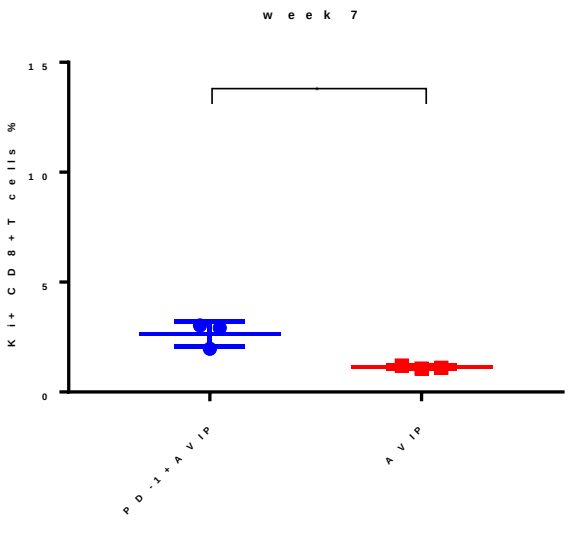
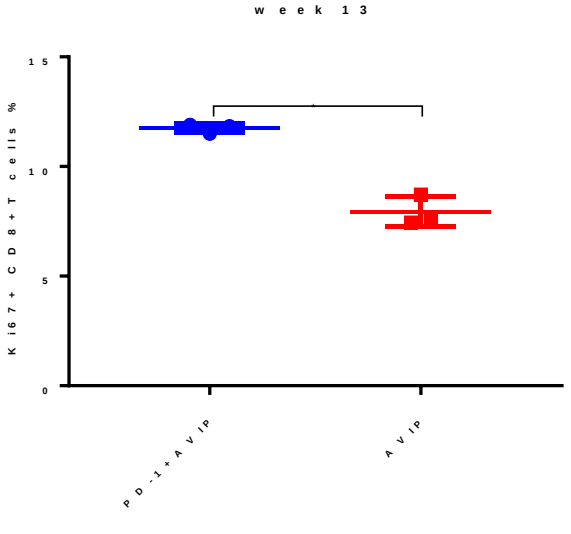
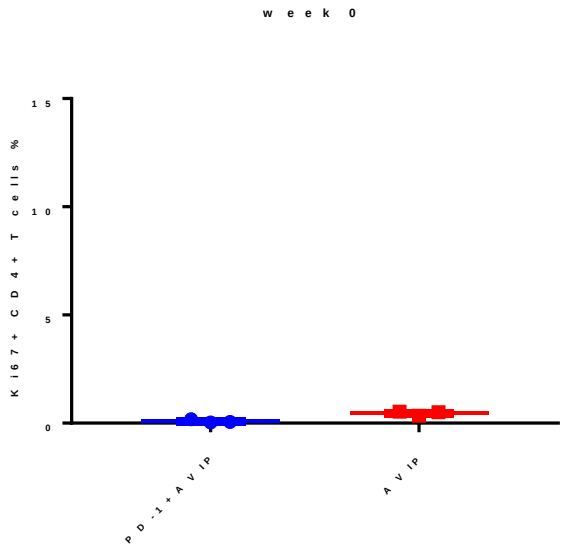
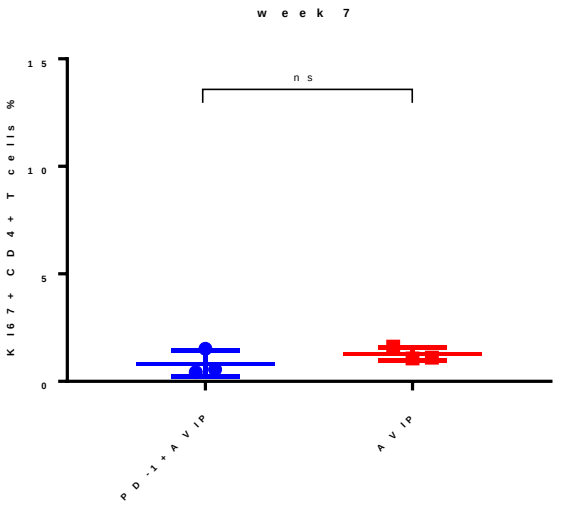
Improved non-specific proliferation of T lymphocytes by PD-1 blockade in SIV-infected monkeys

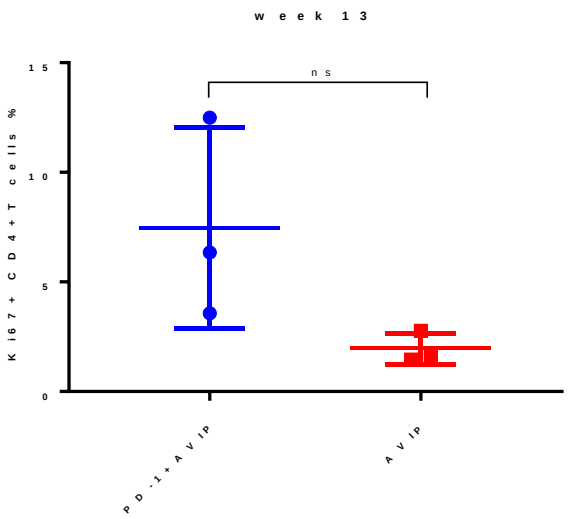




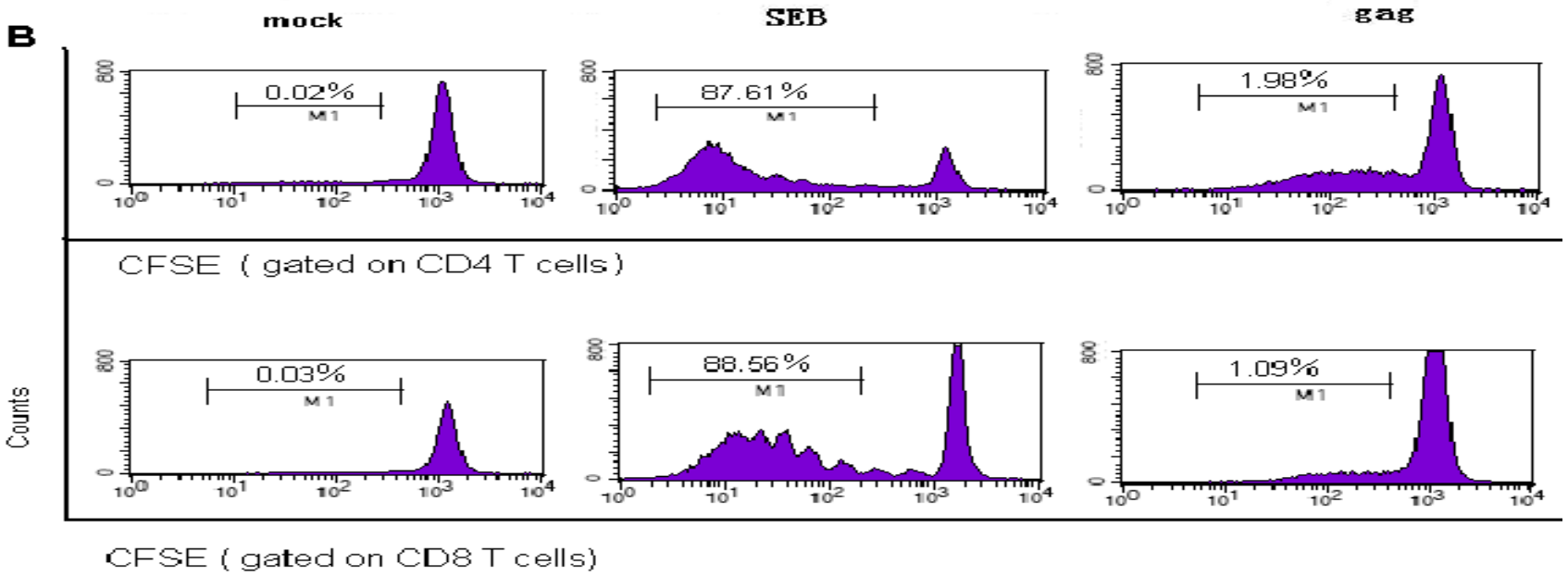


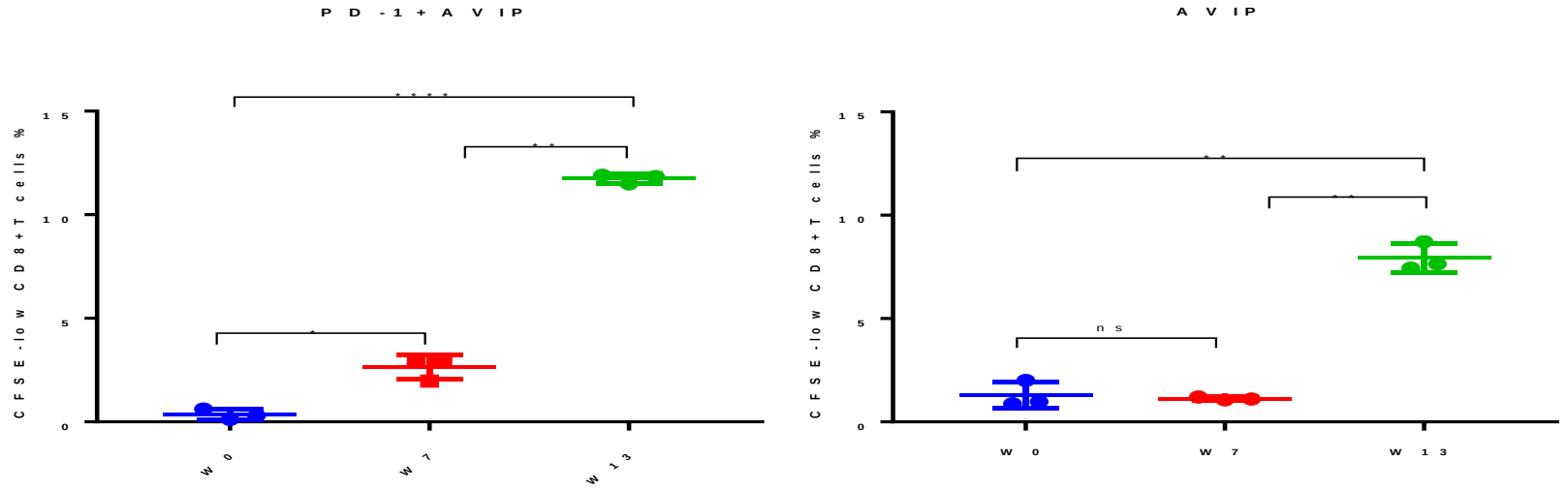




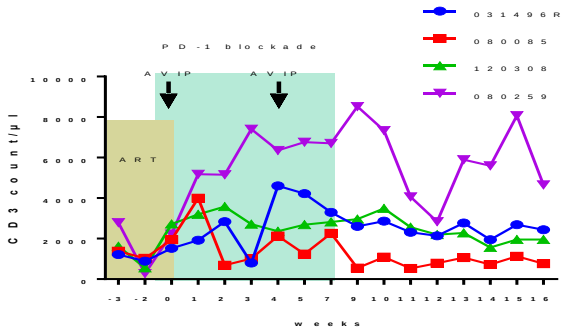


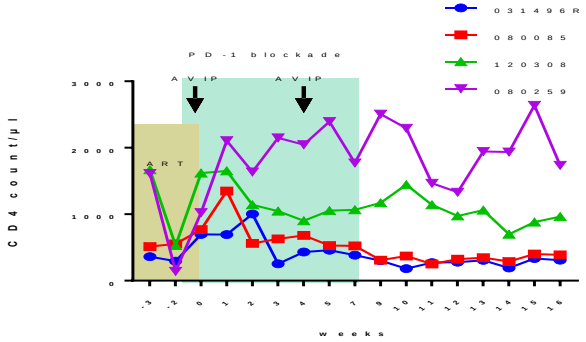
Improved SIV-specific proliferation of T lymphocytes by PD-1 blockage in SIV-infected monkeys

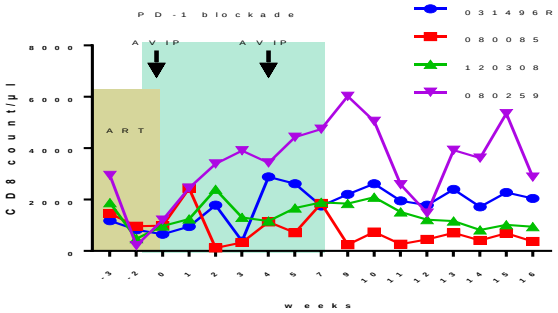


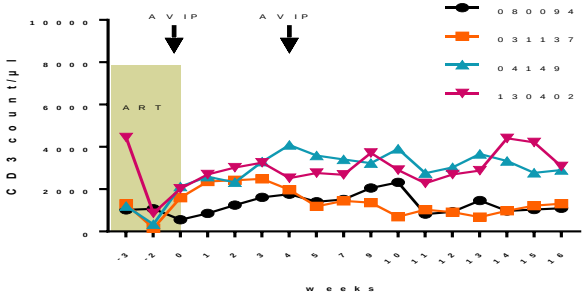


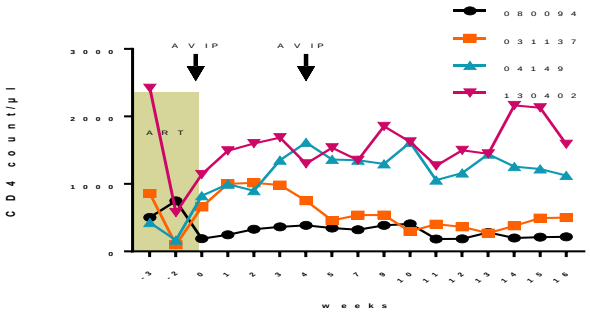
PD-1 blockade promote the quantity of CTLs in ART-treated SIV-infected macaques

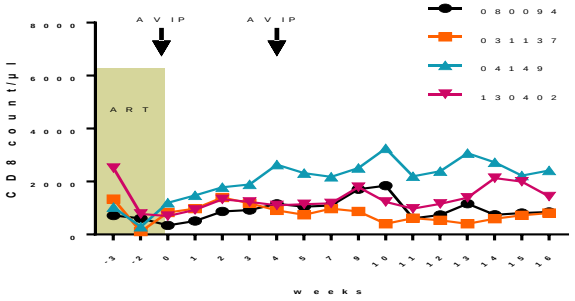


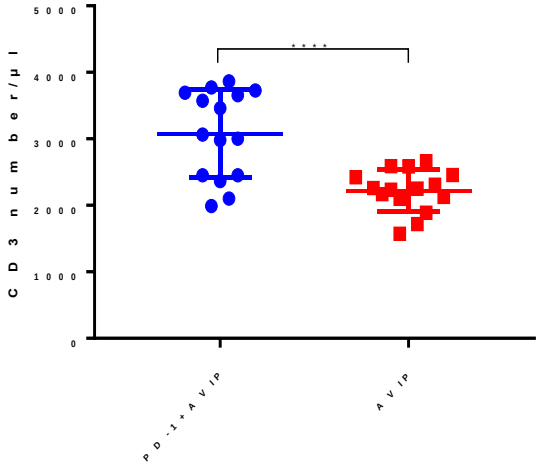


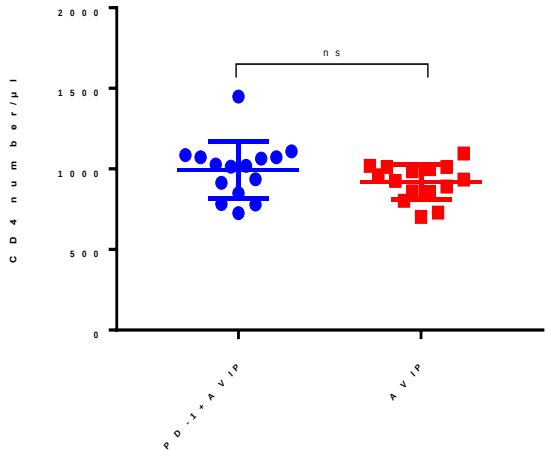


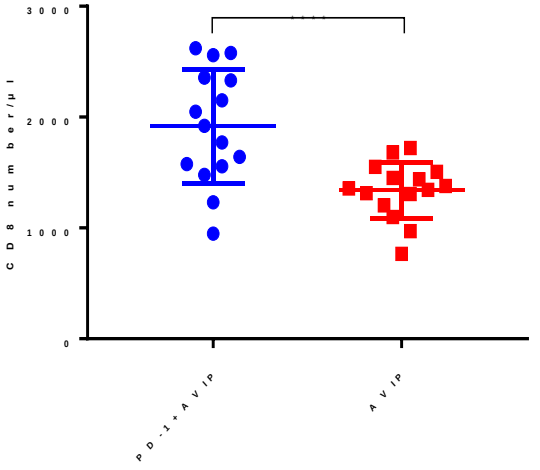




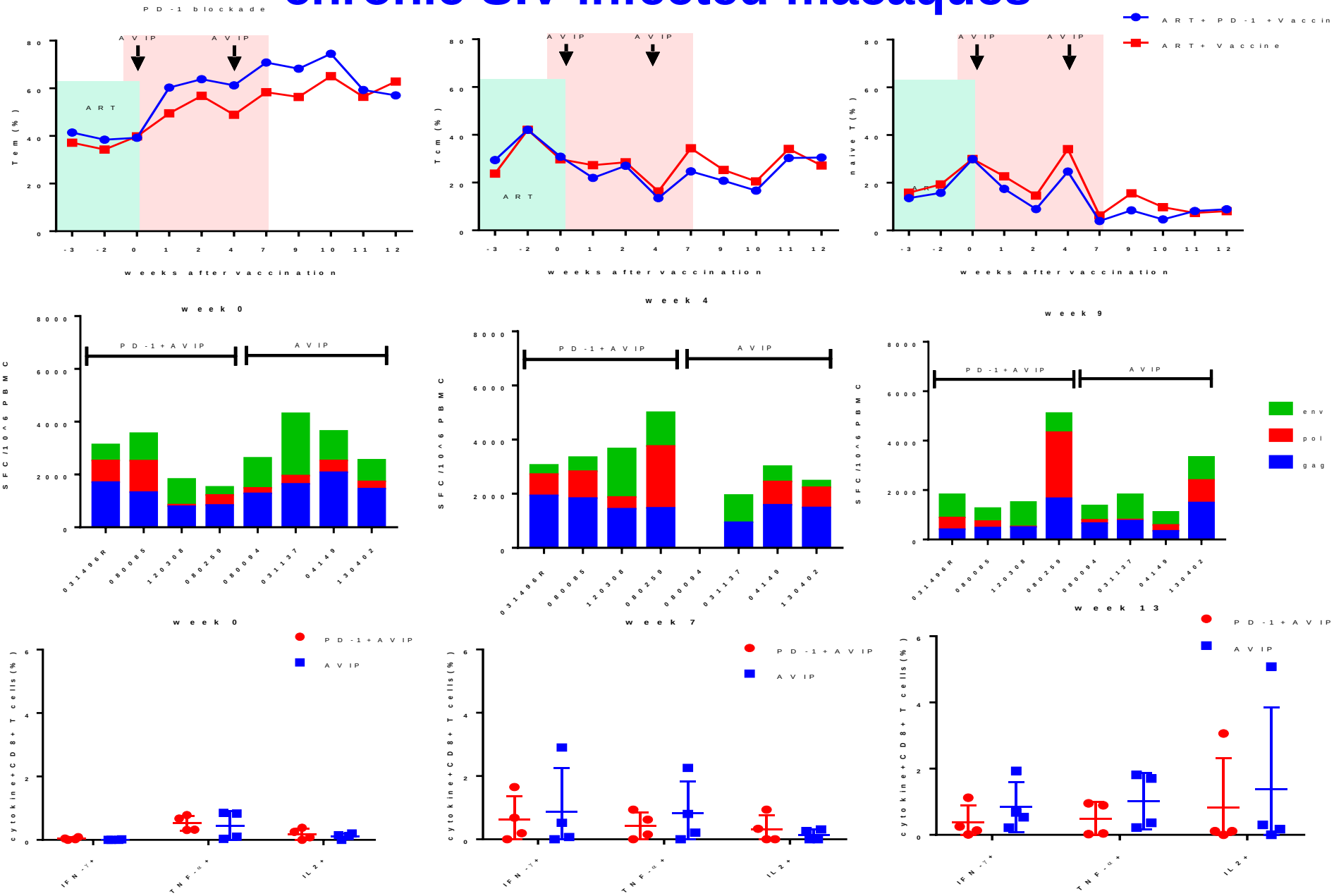




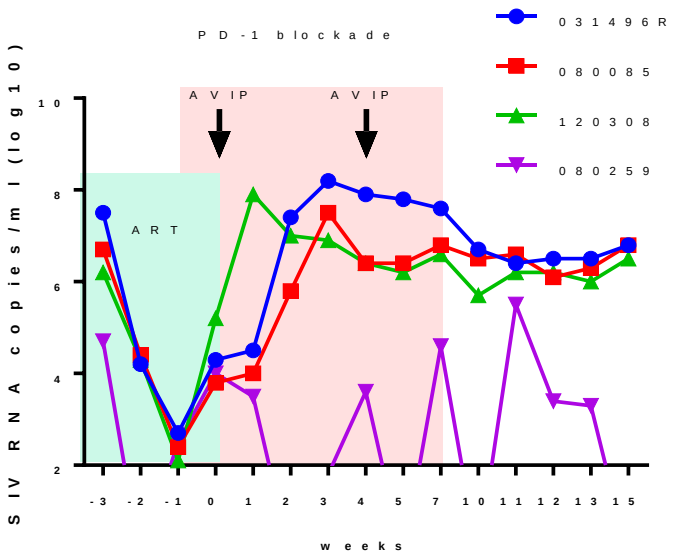


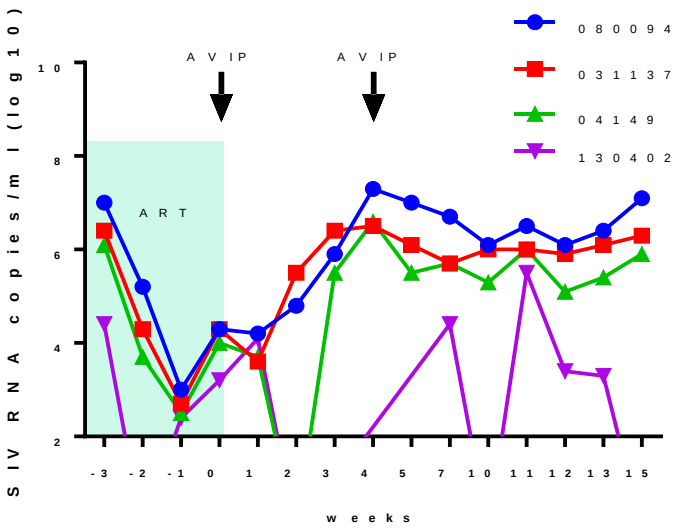


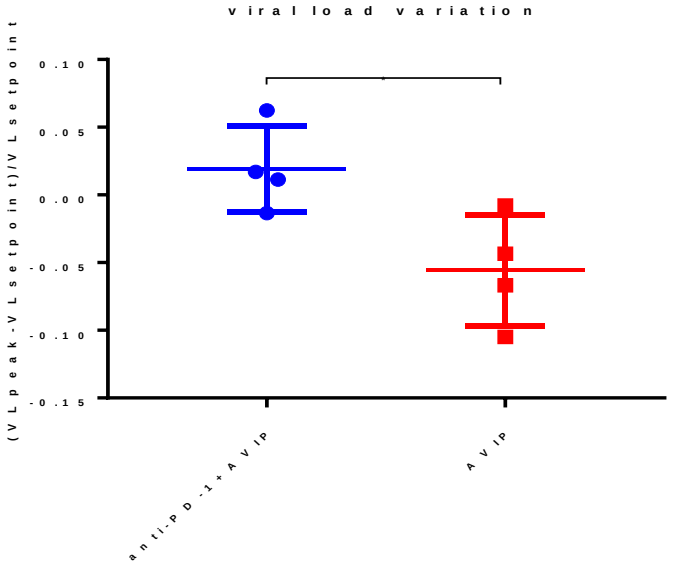
PD-1 blockade restored in part immune functions in chronic SIV-infected macaques

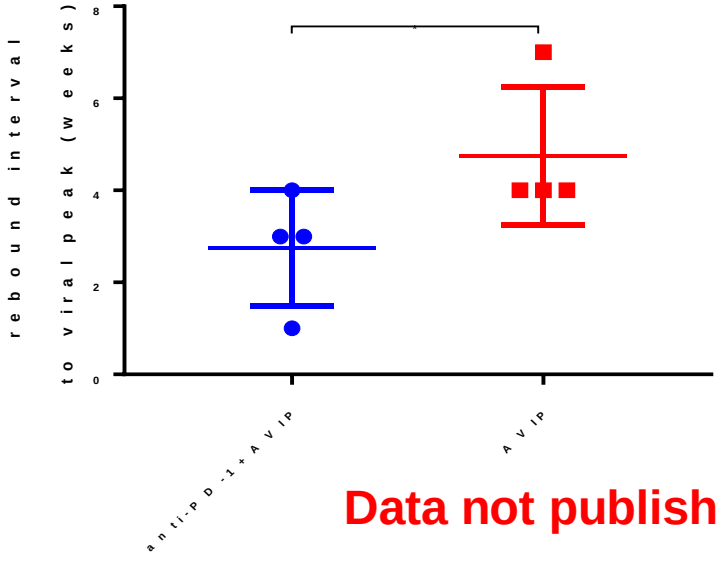


After ART termination, PD-1 blockade improve viral rebound in SIV-infected macaques



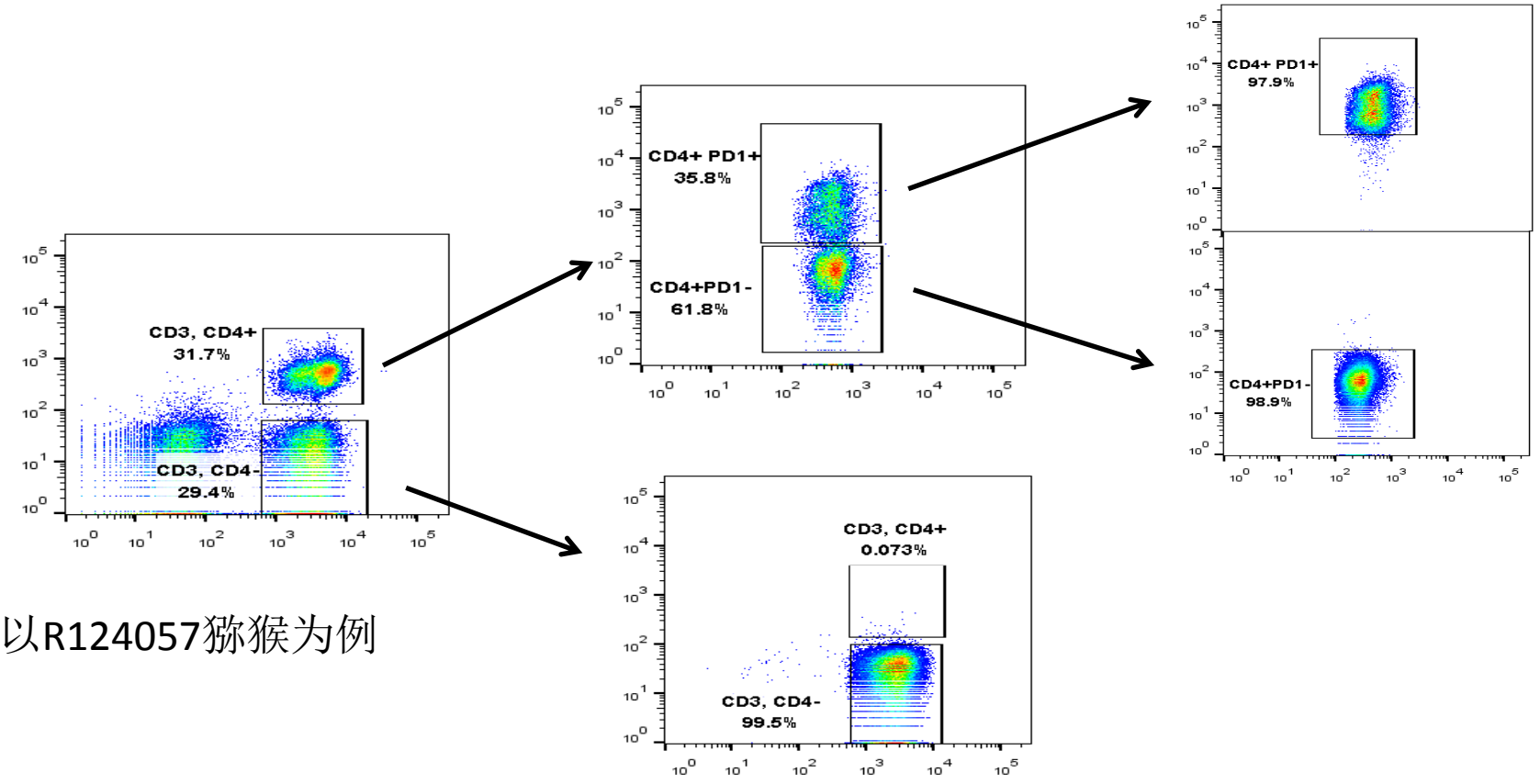






Data not published

Sorting PD1+CD4+ and PD1-CD4+ T cell population from SIV-infected macaques

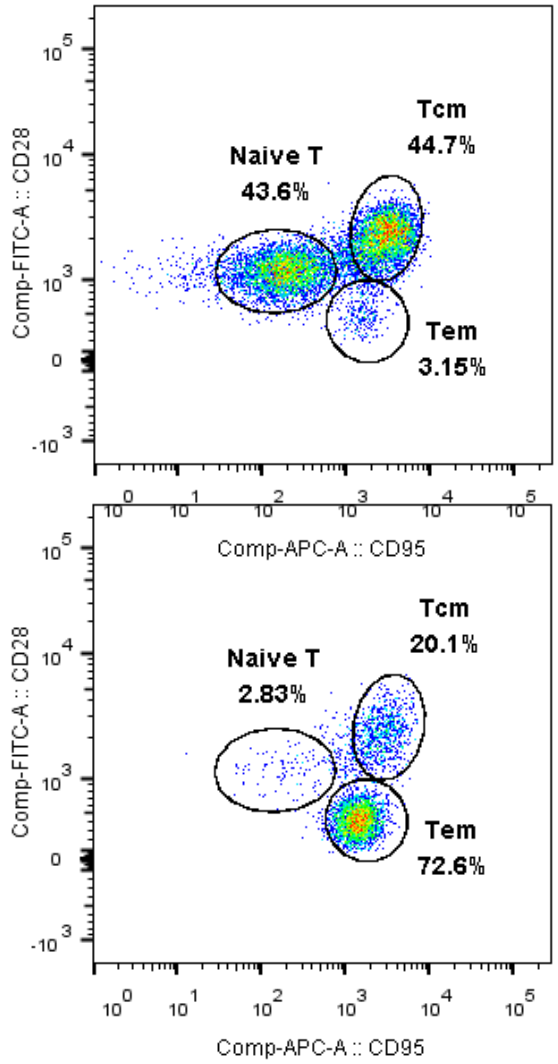


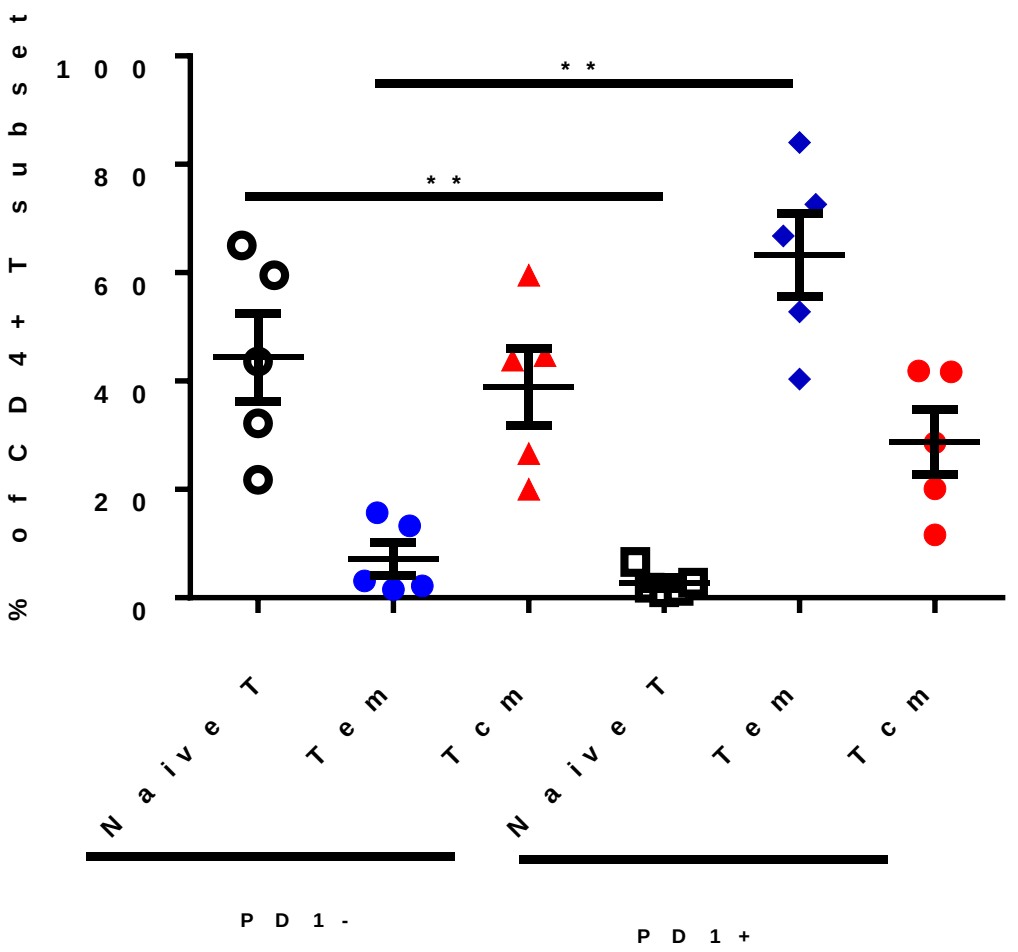
以R124057猕猴为例

Compared to PD1-CD4+T cells，phenotype of PD1+ CD4+T cells represents a greater fraction of effector memory subsets，and a lower fraction of naïve cell subsets

PD1-

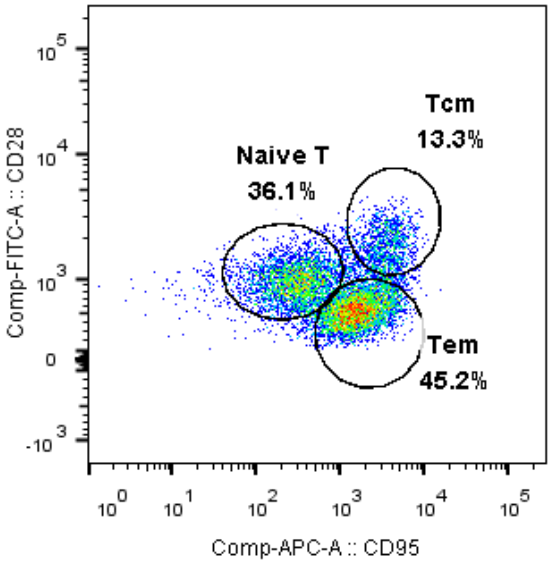
PD1+



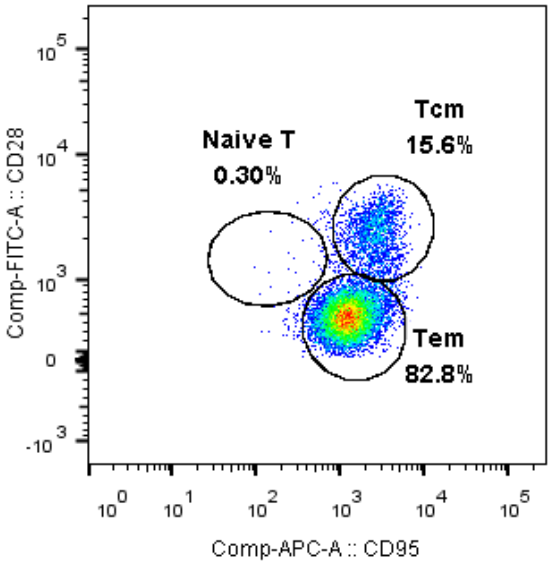


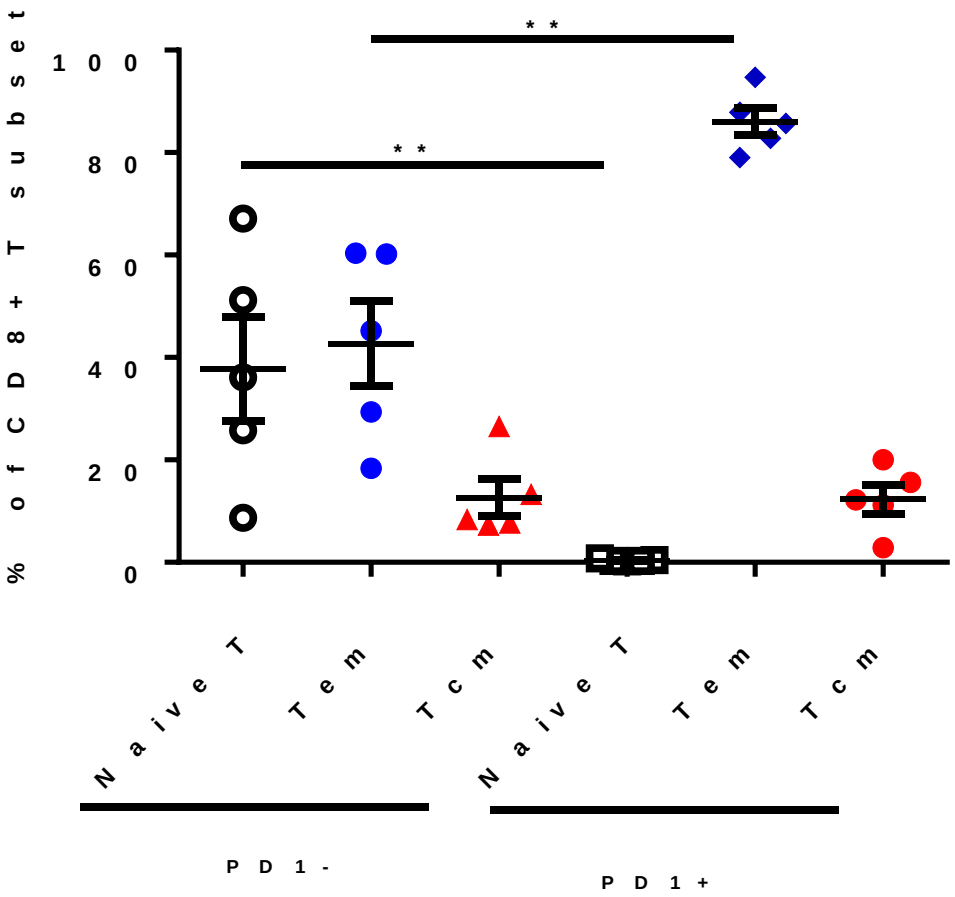
Compared to PD1-CD8+T cells，phenotype of PD1+ CD8+T cells represents a greater fraction of effector memory T subsets，and a lower fraction of naïve T cells

PD1-

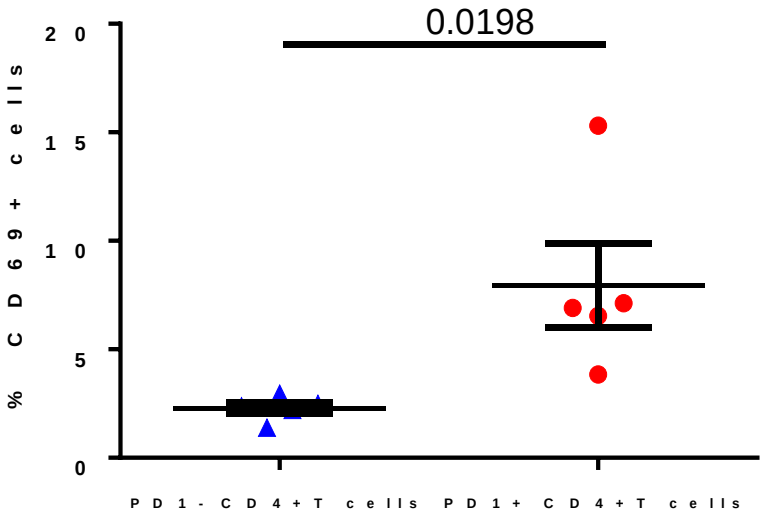


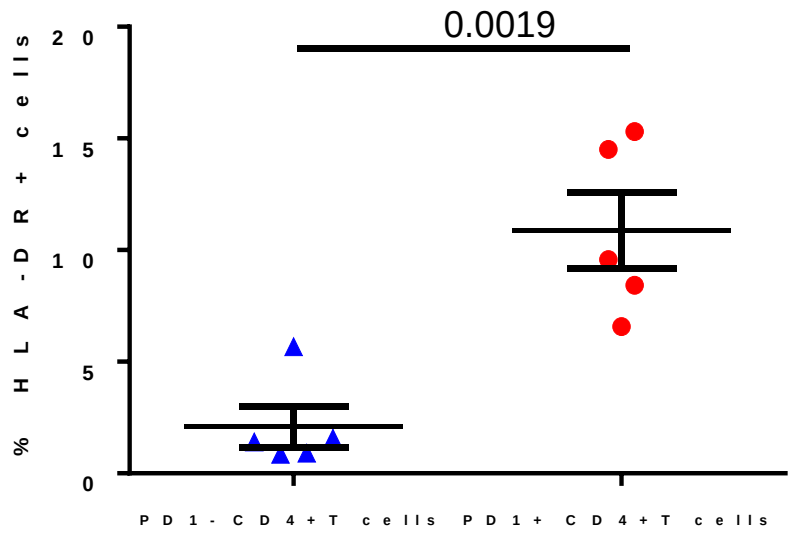
PD1+

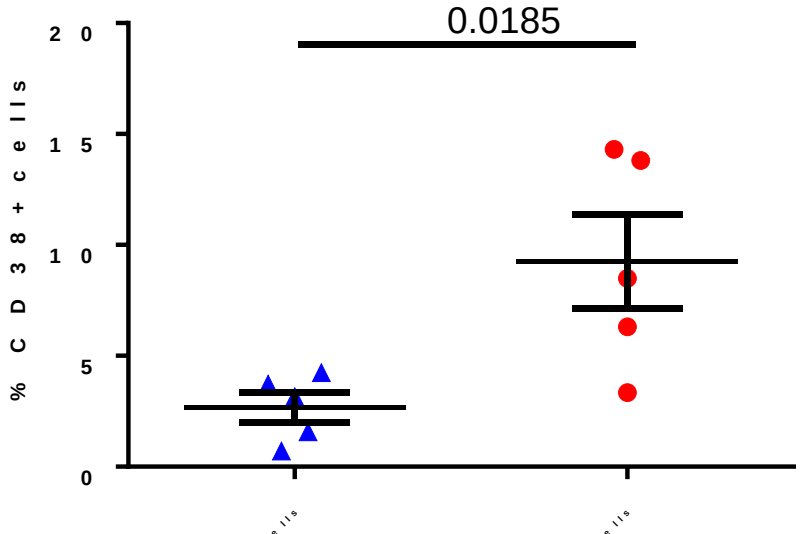


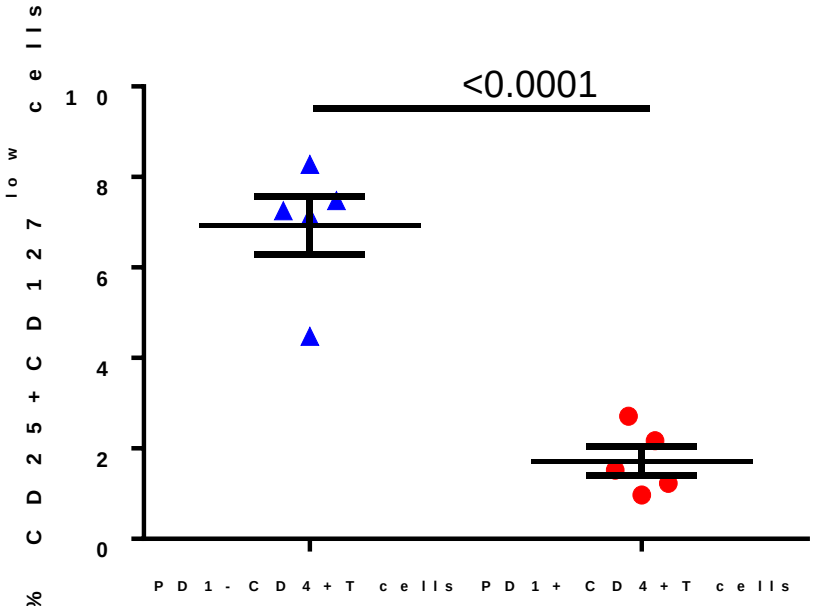


PD1+ CD4+ T cells mainly exhibit the status of activation and exhaustion

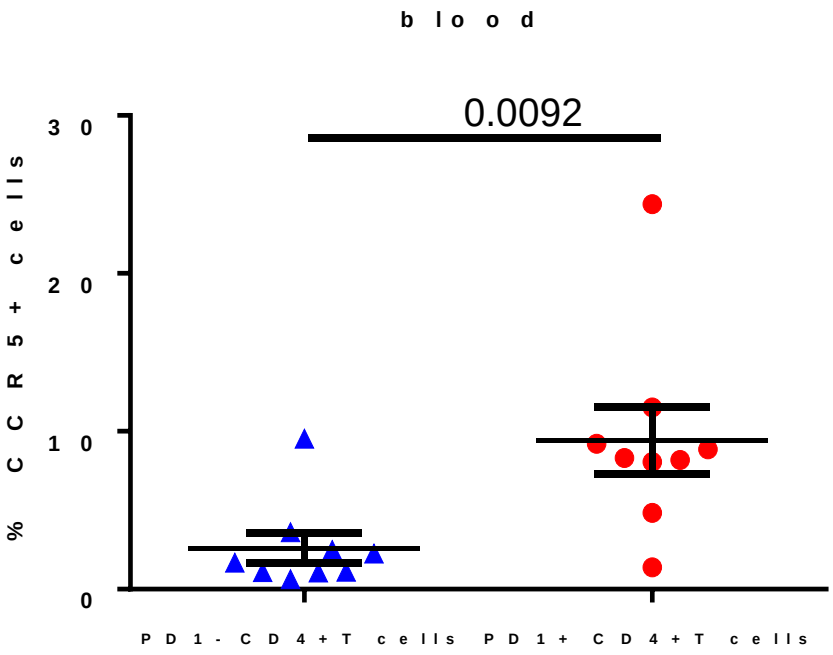


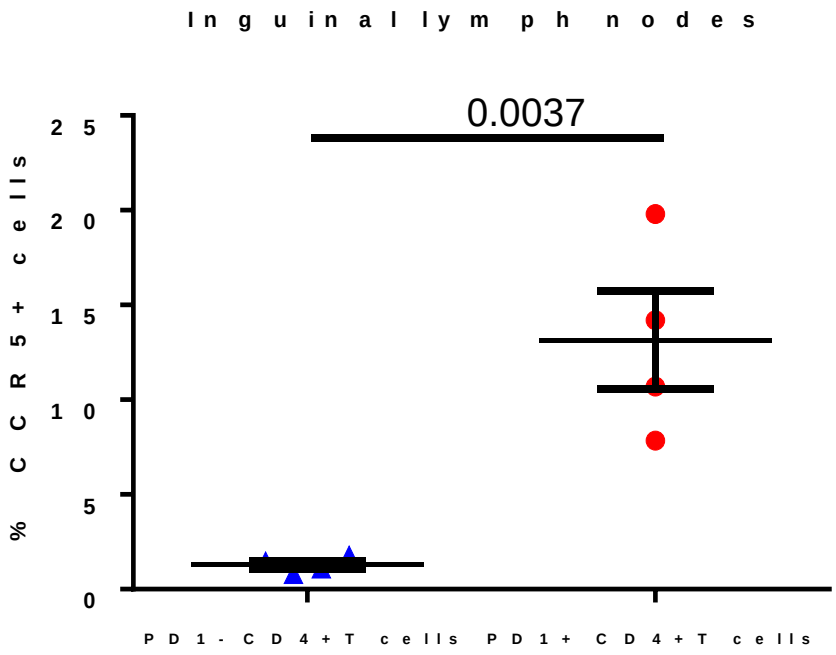






PD1+ CD4+ T cells in peripheral blood and lymph nodes exhibit a higher level of CCR5, indicating which are more susceptible to HIV/SIV entry

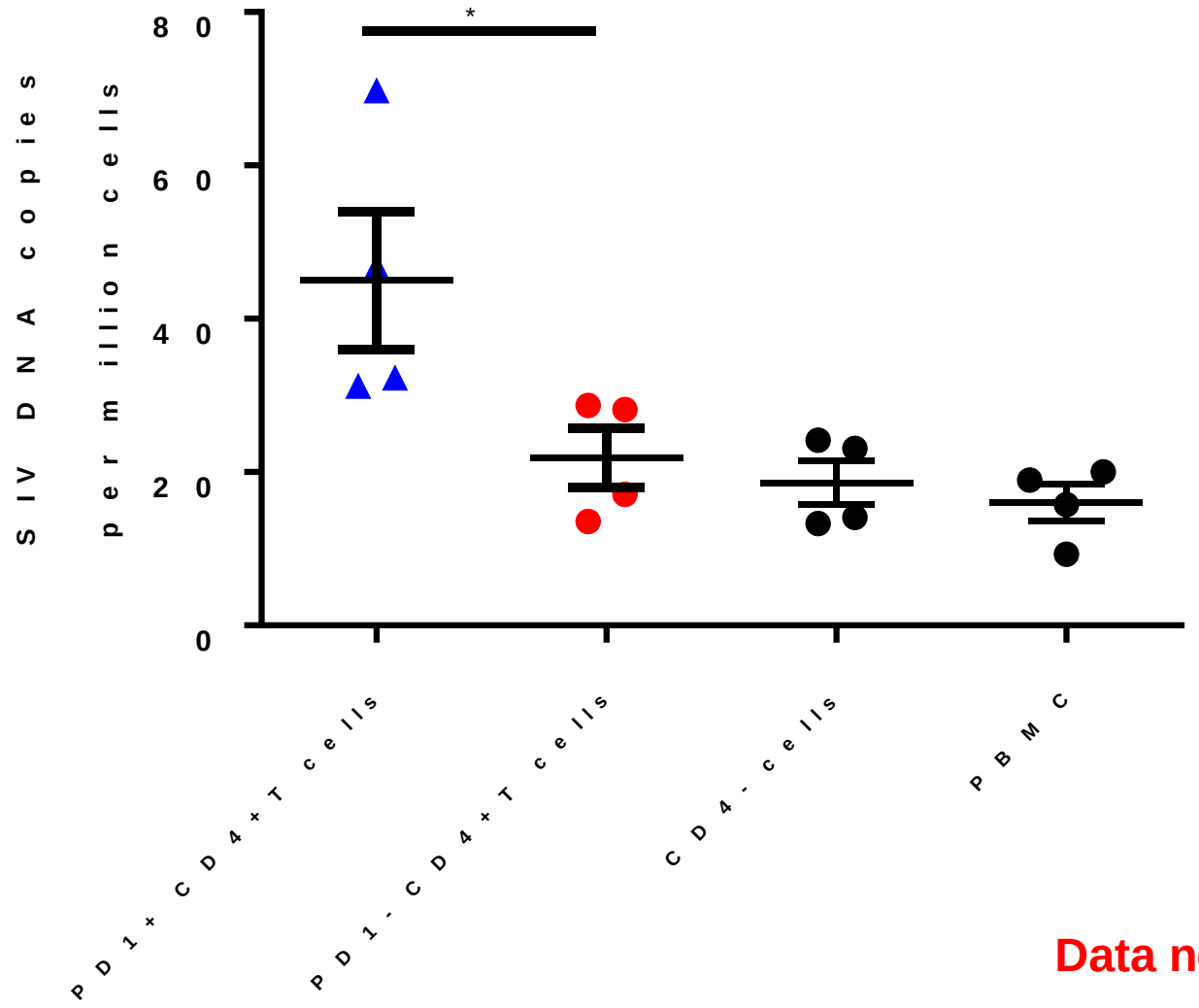




There is no significant difference for the expression of CD4 and CXCR4 in PD- and PD+ subsets.

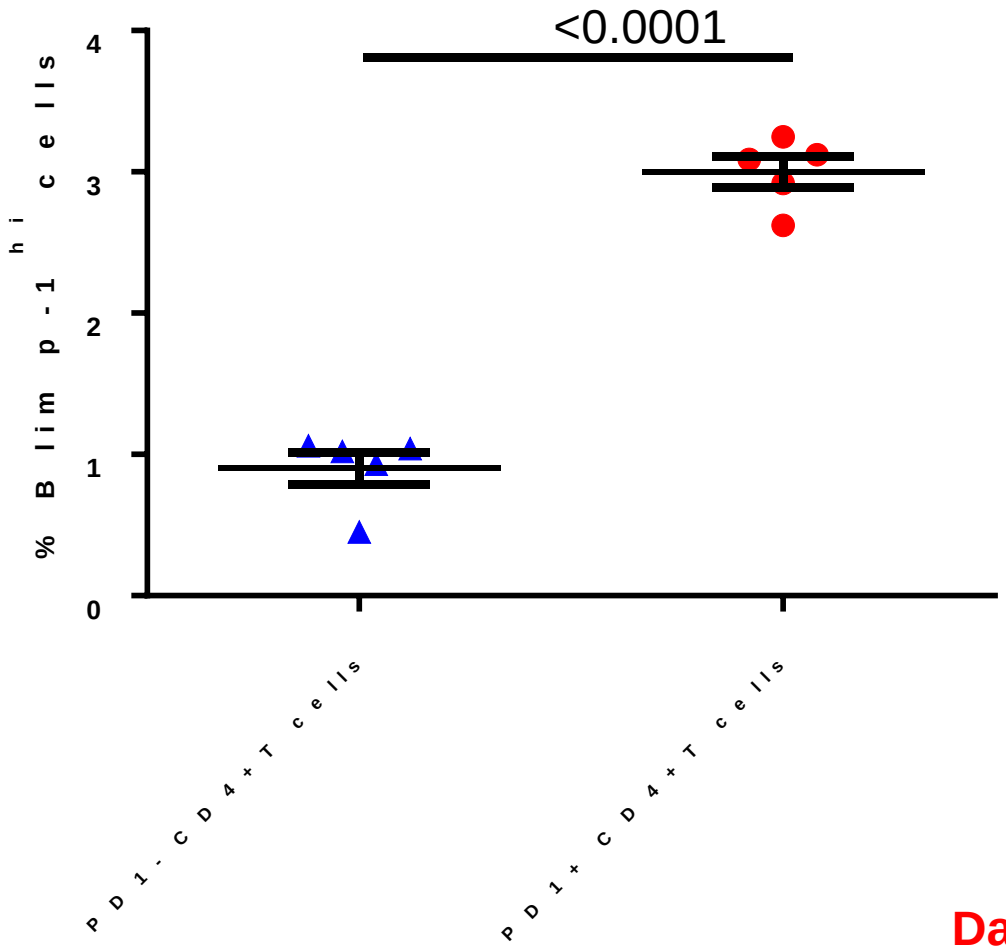
Data not published

SIV provirus are preferentially enriched in PD-1+ CD4+ T cells than PD-CD4+T cells



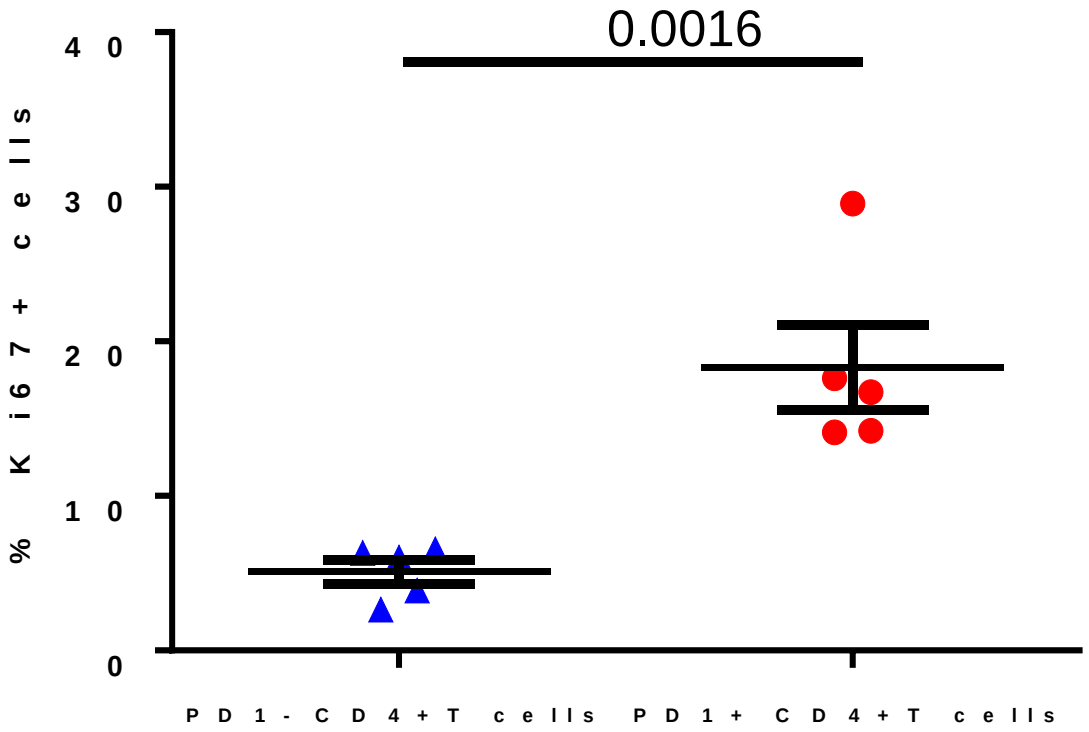
Data not published

PD1+ CD4+T cells exhibit a higher level of Blimp-1 transcriptional repressor, therefore inhibit the transcription of IFNa Promotor and latent HIV promotor



Data not published

PD1+ CD4+ T cells exhibit more potent proliferation with a higher level of Ki67, which might facilitate the turnover and maintenance of HIV/SIV provirus



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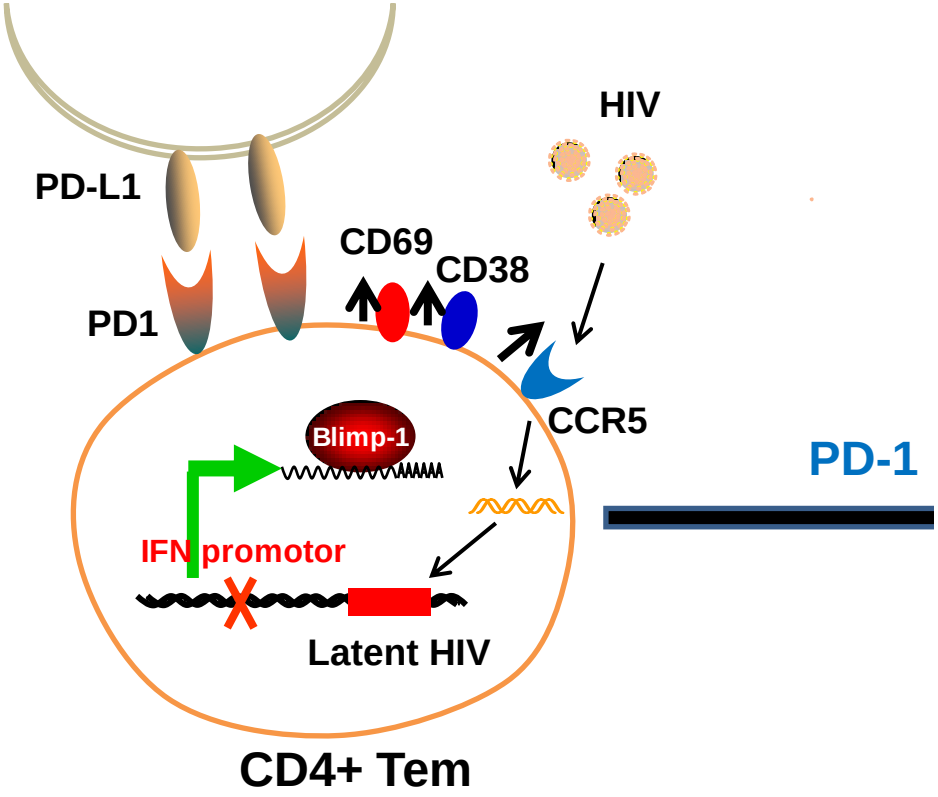
Pattern to illustrate the controlling HIV/SIV infection by modulating immune inhibitory pathway



Susceptibility of HIV entry;
Inhibition of HIV transcription;
Proliferation of latent reservoir.

Formation, turnover and maintenance of latent reservoirs

Summary and perspective

- Immuno-inhibitory pathways are involved in the pathogenesis of infectious diseases, as well as in response to vaccination.
- Blockade of inhibitory signal can enhance the functions of vaccinated T cells, and our previous findings demonstrated that preventive vaccination combined with PD-1 blockade generated distinct immune response profiles and conferred better control of pathogenic SIV infection in monkeys.
- Due to susceptibility of viral entry (by CCR5), inhibition of SIV transcription (by Blimp-1) and potent ability of proliferation, the latent SIV provirus are preferentially enriched in PD-1+ CD4+ T cells.
- Our findings showed that PD-1 blockade might enhance latent SIV transcription in chronic SIV-infected monkeys, allowing for rational design for therapeutic vaccination with ART to eliminate these reactivated reservoirs.

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Let's image a world without AIDS!

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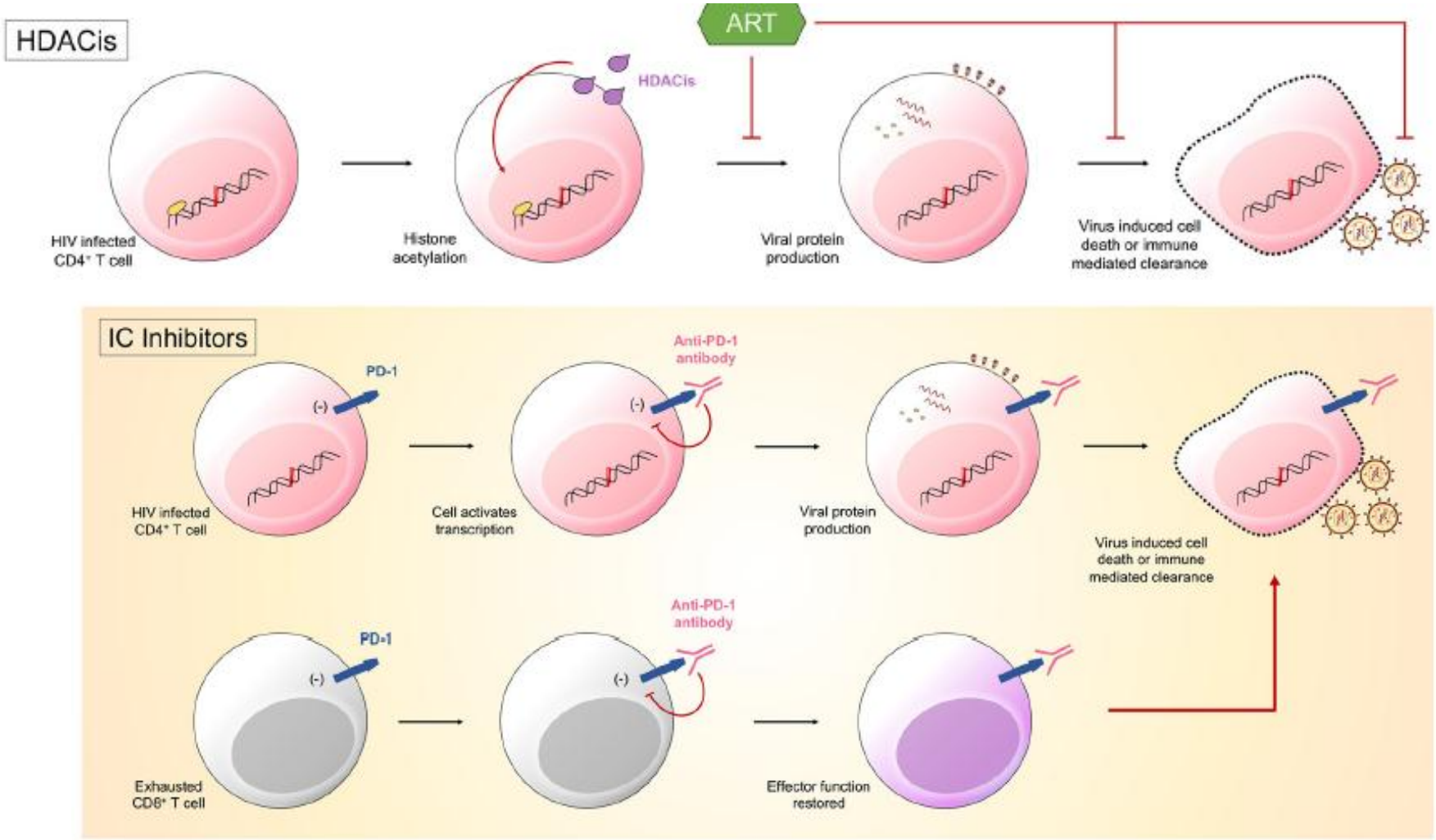
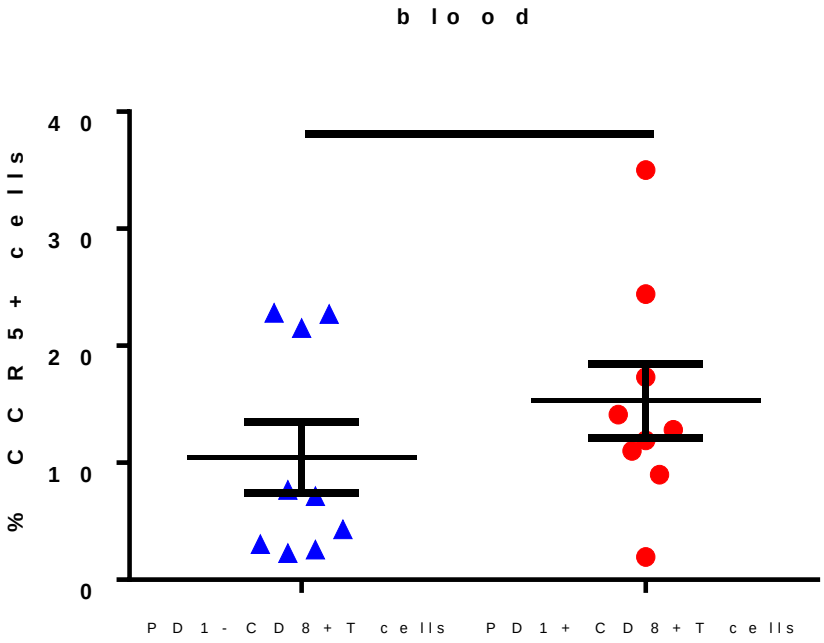
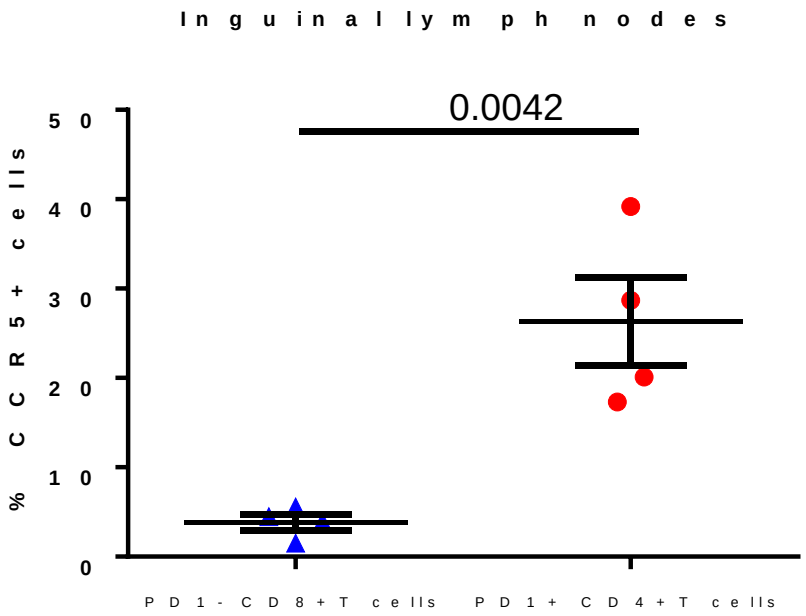


Latency reversing agents (LRA) are one half of the “shock and kill” approach. They function to induce HIV transcription. Histone deacetylase inhibitors (HDACis) promote histone acetylation and induce transcription of viral products. Immune checkpoint (IC) inhibitors could also function as LRA. Anti-PD-1 antibodies
bind PD-1 on both HIV infected CD4+ T cells and HIV-specific CD8+ T cells. The binding of PD-1 by the antibody will disinhibit signals sent into the cells by the
immune checkpoint molecule. Cells will become transcriptionally active, with CD4+ T cells producing viral products that ultimately result in the death of the cell, and
CD8+ T cells having effector function restored, allowing them to target infected CD4+ T cells.

PD1+ CD8+ T cells in lymph nodes exhibit a higher level of CCR5 expression





Data not published