SFigure 1.
Plasma SIV RNA viral loads (copies/mL) in individual RMs in PD-1 Ab or saline control groups at (A) time of infection through 24 weeks of infection and (B) at day -10 of phase I (start of study). Limit of detection is 60 copies/mL.
Figure 2. (A) Frequency of CD4+ T cells in the blood, CD4+ T cells in the rectum, and GagCM9+ CD8+ T cells in the blood at 24 weeks of infection for RMs in PD-1 Ab or saline control groups. (B) Frequency of Tcm CD4+ T cell in the rectum, and SIV Gag and Env specific IFNγ and TNFα producing CD4+ and CD8+ T cells in the blood at day -10 of phase I (start of study) for RMs in PD-1 Ab or saline control groups.
Ki-67 expression on Cell Trace Violet-labeled PBMCs from RMs chronically infected with SIVmac251 for 30 or more weeks that were stimulated with SIV Gag peptides and cultured in the presence or absence of primatized anti-PD-1 antibody (10 µg/ml) for 5 days (n = 8). (B) Plasma concentrations of primatized PD-1 Ab (EH12 IgG4) in PD-1 blockade treated RMs as determined by ELISA. (C) OD450 values measuring levels of anti-EH12 responses generated in plasma of PD-1 Ab treated RMs. (D) Frequency of Ki-67 expressing CD4+ and CD8+ TCM and TEM cells in the blood. (E) Frequency of Ki-67+ CD4+ and CD8+ T cells in the rectum. (F) Frequency of SIV Gag and Env specific IFN-γ and TNF-α producing CD8+ T cells for individual RMs. (G) Frequency of GagCM9+ CD8+ T cells in the blood (saline, n = 6; PD-1 Ab treated, n = 5). Data are shown as mean ± SEM. *, p < 0.05; **, p < 0.01; ***, p < 0.001. Two-tailed paired Student’s t test (A, F) or two-way ANOVA (D, E) were used. N = 10 per group unless otherwise noted. Shaded grey area depicts anti-retroviral treatment.
Figure 4

(A) GSEA plots comparing day 10 to day 0 of Phase I for PD-1 Ab and saline treated groups. Leading edge genes from gene sets are shown as black outlined dots. (B) Heat map of log2-fold change of gene expression for leading edge genes of select gene sets comparing day 10 to day 0 during Phase I. (C) Heat map of log2-fold change of gene expression from day 10 of treatment over day 0 during Phase I of selected genes. Numbers indicate nominal p-values for change in gene expression. Exact p values are shown.

Saline, n = 5; PD-1 Ab treated, n = 10.
Figure 5.
(A) Plasma concentrations of primatized PD-1 Ab (EH12 IgG4) in PD-1 blockade treated RMs as determined by ELISA during Phase II. (B) OD450 values measuring levels of anti-EH12 responses generated in plasma of PD-1 Ab treated RMs. (C) Ki-67 expression of T<sub>CM</sub> and T<sub>CM</sub> CD4<sup>+</sup> T cells at day 0, 7, and 14 after first PD-1 Ab infusion under ART. (D) Ki-67 expression on CD4<sup>+</sup>, CD8<sup>+</sup>, and GagCM9 Tet<sup>+</sup> CD8<sup>+</sup> T cells from PBMCs after second and third PD-1 Ab infusion (right: saline, n = 2; αPD-1 Single Treated (ST), n = 3; αPD-1 Double Treated (DT), n = 5). Data are shown as mean ± SEM. (E) Frequency of SIV Gag and Env-specific IFNγ and TNFα producing CD4<sup>+</sup> (left) and CD8<sup>+</sup> (right) T cells for individual RMs after first PD-1 Ab infusion. (F) Frequency of granzyme B+ CD8<sup>+</sup> T cells (saline, n = 5), CXCR5<sup>+</sup> GagCM9+ CD8<sup>+</sup> T cells (saline, n = 3; ST, n = 3; DT, n = 5), and CXCR5+ CD4<sup>+</sup> T cells (saline, n = 5) in peripheral blood at two weeks after last PD-1 Ab infusion. Plasma SIV RNA viral loads (copies/mL) during phase II. Limit of detection is 60 copies/mL. (G) Number of transient increases in plasma viremia per animal. Exact p values are shown. Two-way ANOVA (C, D) or two-tailed paired Student’s t test (E) were used. Saline, n = 4; αPD-1 ST, n = 5; αPD-1 DT, n = 10 unless otherwise noted. Shaded grey area depicts anti-retroviral treatment.
Biochemical parameters detected in the blood of PD-1 Ab treated animals during Phase II. Orange symbols are αPD-1 double treated and blue symbols are αPD-1 single treated RMs. Shaded grey area depicts normal ranges. *, p < 0.05. Two-tailed paired Student’s t-test were used to compare week 4 or week 8 of phase II to week 0.
Complete blood count of PD-1 Ab treated animals during Phase II. Orange symbols are αPD-1 double treated and blue symbols are αPD-1 single treated RMs. Shaded grey area depicts normal ranges. *, p < 0.05. Two-tailed paired Student’s t-test were used to compare week 4 or week 8 of phase II to week 0.
SFigure 8.
Heat map of log₂ fold change of gene expression for leading edge genes of select gene sets comparing day 7 to day 0 during first PD-1 Ab infusion of Phase II. PD-1 Ab double and single treated combined, n = 9.
**Figure 9.**

(A) Frequency of Ki-67+ (left), Ki-67+ CXCR5+ (middle), and perforin+ (right) CD8+ T cells from the blood after ART interruption (ARTi).

(B) Frequency of SIV Gag and Env-specific IFNγ and TNFα producing CD8+ T cells in the blood after ARTi. Data are shown as geometric means.

(C) Frequency of Ki-67+ CXCR5+ CD4+ T cells as percentage of CD3+ T cells in the blood after ARTi.

(D) Frequency of TCM CD4+ T cells as percentage of CD3+ T cells in the blood after ARTi.

(E) Boolean analysis of expression of CXCR5, granzyme B, perforin, and Ki-67 on TCM CD8+ T cells 3 weeks after ARTi. Data and bars are shown as means unless otherwise indicated. * p < 0.05; **, p < 0.01; ***, p < 0.001. Two-tailed unpaired Student's t-test with Welch's correction (C left), two-way ANOVA (A-D), or two-tailed unpaired Student's t-test (E) were used. Saline, n = 4; αPD-1 Single Treated, n = 5; αPD-1 Double Treated, n = 10.