SUPPLEMENTAL METHODS:

Study animals, SIV infection and animal care. In the PD-1 group, nine SIV-infected RMs were treated with anti-PD-1Ab either at 10 weeks (early chronic, 5 RMs; RDb11, RFe11, RKf11, RTd11 and RWh11,) or at 90 weeks (late chronic, 4 RMs; RCe8, RDo8, RQj9 and RRk10) post SIV infection. In control group, five SIV-infected RMs were treated with Control Ab either at 10 weeks (3 RMs; RJi11, RIg11 and RPe11) or at 90 weeks (2 RMs; RFp9 and RVo6) post SIV infection. All animals except RTd11, RDb11 and RFp9 were Mamu A*01+ animals. All animals except RDb11 were Mamu B08 and B17 negative. RDb11 was positive for Mamu B17. Four doses of either PD-1 or control antibody was infused intravenously at a dose of 3mg/Kg body weight on days 0, 3, 7 and 10. PBMCs and colorectal tissue samples were collected on days 0, 14, 56 and 90 post antibody treatments for various analyses. To increase the statistical power of the control group for data presented in Figure 3C and 3D, eight additional 'no Ab control' animals were included from a parallel study. These 'no Ab control' animals were selected to match the set point viral load in the early chronic animals used for the PD-1 antibody and control antibody arms. All RMs were infected with the same stock of SIV251 intravenously except two RMs in the late chronic PD-1 antibody treated group that were infected intravenously with SIV239. Data for antibody treated animals and 'no Ab control' animals are presented wherever samples were available. Macaques were housed at the Yerkes National Primate Research Center and were cared for under the guidelines established by the Animal Welfare Act and the NIH "Guide for the Care and Use of Laboratory Animals" using protocols approved by the Emory University IACUC, Atlanta, GA, 30329, USA.

Antibodies used for therapy. The anti-PD-1 Ab (clone EH12-1540) (1) has mouse variable heavy chain domain linked to human IgG1 (mutated to reduce FcR and complement binding) and mouse variable light chain domain linked to human Kappa. The clone EH12 binds to macaque PD-1 and blocks interactions between PD-1 and its ligands *in vitro* (2). The control antibody SYNAGIS is a humanized mouse monoclonal antibody (IgG1) specific to F protein of respiratory syncytial virus (Medimmune, Gaithersberg, MD).

Transcriptional profiling of RNA. Total RNA was extracted from colorectal mucosa tissue biopsies using Qiagen RNeasy mini extraction kit according to the manufacturer's protocol. The quality and yield of isolated RNAs were assessed on an RNA 6000 Nano Lab Chip and the Agilent Bioanalyzer 2100 using the Eukaryote total RNA Nano Assay (Agilent Technologies, Palo Alto, CA). RNA samples with RNA Integrity Number (RIN) value of 8 or larger, and a 28S:18S ratio of 1.5 or higher were used.

Rhesus gene chip assays were performed at the Emory Microarray Core Facility. The RNA samples were analyzed on Rhesus macaque gene chip that consisted of 52,865 imprinted probe sets recognizing > 47,000 rhesus transcripts and >23000 genes (Affymetrix, Santa Clara, CA). Target RNA labeling, hybridization and post- hybridization processing was performed according to the Affymetrix GeneChip Expression Analysis standard protocols (3). In brief, 5µg of cRNA was reverse- transcribed using T7-Oligo (dT) primer and SuperScript II followed by second-stranded cDNA synthesis. The double-stranded cDNA was purified by use of a gene chip sample clean-up module and served as templates in the generation of biotinylated complementary RNAs (cRNAs) in the presence of T7 RNA Polymerase and a biotinylated nucleotide analog/ribonucleotide mix by in vitro transcription (IVT) reaction. The biotinylated

cRNAs were cleaned up, fragmented, and hybridized to the rhesus macaque expression arrays at 45°C for 16 h with constant rotation at 60 rpm. The gene chips were then washed and stained with Affymetrix fluidics stations 450 and scanned on Affymetrix scanner 3000. The images were processed to collect raw data with GeneChip Operating Software (GCOS) 1.4.

Analysis of Affymetrix GeneChip data. CEL and CHP files originated from Affymetrix GCOS software were analyzed in GeneSpring GX 10 software and preprocessed them with Robust Multiarray Analysis (RMA). In this analysis, the back ground correction, per gene normalization to median and probe set summarization was performed. Genespring GX 10 software was used to analyze differentially expressed genes between different groups of RMs. The differentially expressed genes were determined by comparing the gene expression intensity profiles of each gene in all individual RMs in the PD-1 group of RMs with all individual RMs in the control antibody treated group. Statistical significance analysis was performed using One way ANOVA analysis with the filters of $p \leq 0.05$, and 1.5 fold change. For analyses in the colorectum, data from 5 PD-1 antibody treated and 3 control antibody treated animals was used. For analyses in the blood, data from 3 PD-1 antibody treated and 3 control antibody treated animals was used.

The Ingenuity Pathway Analysis (IPA) was performed to determine the differentially regulated biological pathways by loading the lists of statistically significant differentially expressed genes into IPA software (Agilent technologies). The software generated statistically significant (p value of ≤ 0.05) biological pathways were reported.

Isolation of cells from blood and rectal biopsies. PBMCs were isolated from whole blood according to the standard Ficoll-hypaque separation procedures as described before (4). Lymphocytes from pinch biopsies from the rectum were obtained as described before (4). Briefly, 10–20 pinch biopsies were digested with 200 U/ml collagenase IV (Worthington, Lakewood, NJ) and DNase I (Roche, Indianapolis, IN), passed through decreasing sizes of needles (16-, 18-, and 20-gauge, five to six times with each needle), filtered through a 100μm filter and cells from filtrate were used for analysis.

LPS levels. Briefly, plasma samples were diluted 1:10 with endotoxin-free water, heated at 65°C for 10 min and vigorously mixed. 50μl of test or standard samples were equilibrated to 37°C and 50μl of limulus ameobocyte lysate (LAL) was added to the samples to allow LPS mediated activation of pro-enzyme present in LAL. After 10 min., color less PNA substrate was added to allow the color development (enzyme catalysis) and the reaction was stopped with 10% SDS (Wt/Vol) and the OD at 405nm was measured. The OD values were converted into enzyme units/ml (EU/ml) based on a standard curve.

Real-time PCR. RNA samples (0.5-1 μg) were reverse transcribed in a volume of 20μl and 0.1μl of cDNA was used for PCR analysis. Primers specific for GAPDH mRNA were used to normalize samples. In case of MX-1 gene expression, fold-changes were calculated by dividing the normalized quantity of post-infected sample with the normalized quantity of pre-infection control using the standard curve method according to the manufacturer's protocol (Applied Biosystems) and as described before (5). Tight junction gene expression analysis was performed at CFAR core facility at emory and fold changes were calculated using the relative quantitation

by ΔΔCT method (6). Primer sequences used in this study are shown in Supplemental table 3.

Cellular responses. Whole cell lysate of *Campylobacter* isolated from diagnostic blood agar plates that were used to detect infectious agents in the stools of SIV positive monkeys served as the source of coating antigen. *Salmonella typhimurium* (SL3201) lysate was prepared from over night cultures. Briefly, bacteria were collected into 10% saline solution, sonicated at 12W power for 1 min., clear soluble fraction was collected by centrifugation (10,000g for 30 min.) and the protein content was quantified (BCA protein assay kit, Pierce Protein Research). For detection of cellular responses, 2 million PBMCs were stimulated with *Campylobacter* (5μg) or *Salmonella* (0.5μg) lysates at 37°C in the presence of 5% CO2 for 8h in complete RPMI 1640 medium in a 100μl of final volume. Stimulations were performed in the presence of anti-CD28 and anti-CD49d Abs (1μg/ml; BD Pharmingen). Brefeldin A (1μg/ml) and golgi-stop (1μg/ml; BD bioscience) were added after 2 hrs of incubation. PMA (0.1μg)/ionomycin (2.5μg)-stimulated cells were used as positive controls. At the end of the stimulation, cells were washed once with FACS buffer (PBS containing 2% FBS), surface stained for 30 min at 4°C with anti-human CD3 (clone SP34-2; BD Pharmingen), anti-human CD4 (clone L200; BD Pharmingen), and anti- human CD8 (clone SK1; BD Biosciences). Cells were fixed with cytofix/cytoperm (BD Pharmingen) for 20 min at 4°C and permeabilized with 1x permwash (BD Pharmingen). Cells were then incubated for 30 min at 4°C with anti-human IFN-gamma Ab (clone B27 BD Pharmingen), washed twice with 1x permwash, once with FACS buffer, and resuspended in 1% formalin in PBS. Approximately 500,000 lymphocytes were acquired with LSRII (BD Immunocytometry Systems) and analyzed using FlowJo software (Tree Star). Lymphocytes were identified based on their scatter pattern, CD4 (CD3+, CD4+, CD8−) and CD8

(CD3+CD8+CD4−) T cells were then gated for cytokine-positive cells.

Statistics. To define differentially expressed genes in microarray analysis, one-way ANOVA statistical method was used and significance of probeset intensity changes (≥1.5 fold change) with a p value of ≤ 0.05 was determined between groups. For IPA analysis, p values (≤ 0.05) were determined by the software. Statistical analyses for comparison of decrease in LPS levels, increase in IFNg+ CD8 T cells between groups were performed using a Wilcoxon rank-sum test. Significance of differences in the level of opportunistic infections between groups was determined by a two-tailed 't' test. A Mantel–Cox log rank test was used to compare the survival curves between the PD-1 Ab and control Ab treated groups of RMs.

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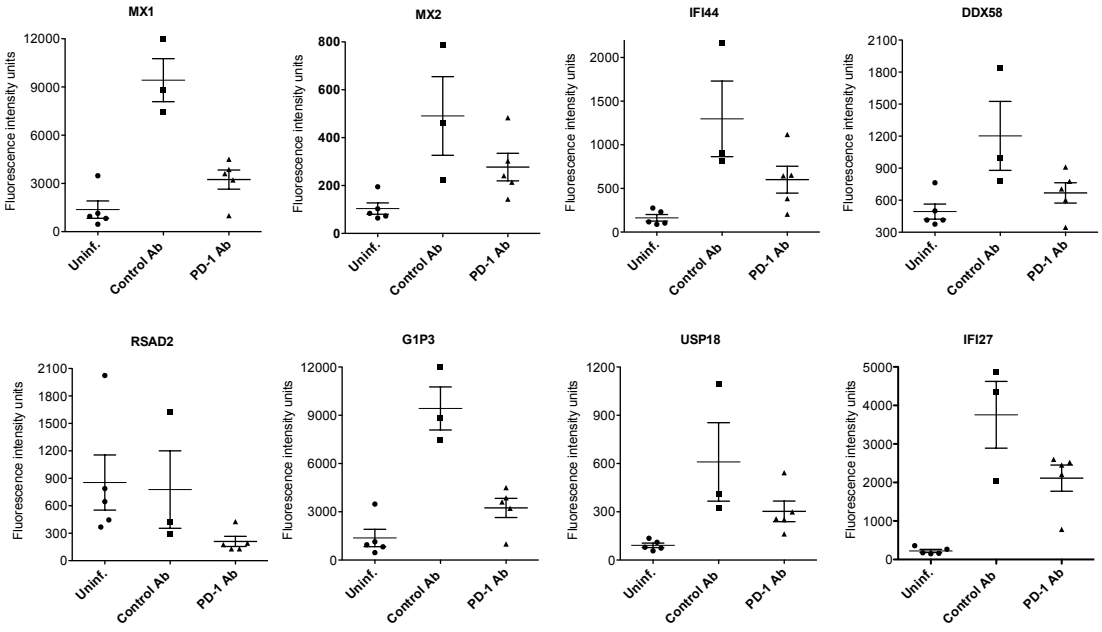
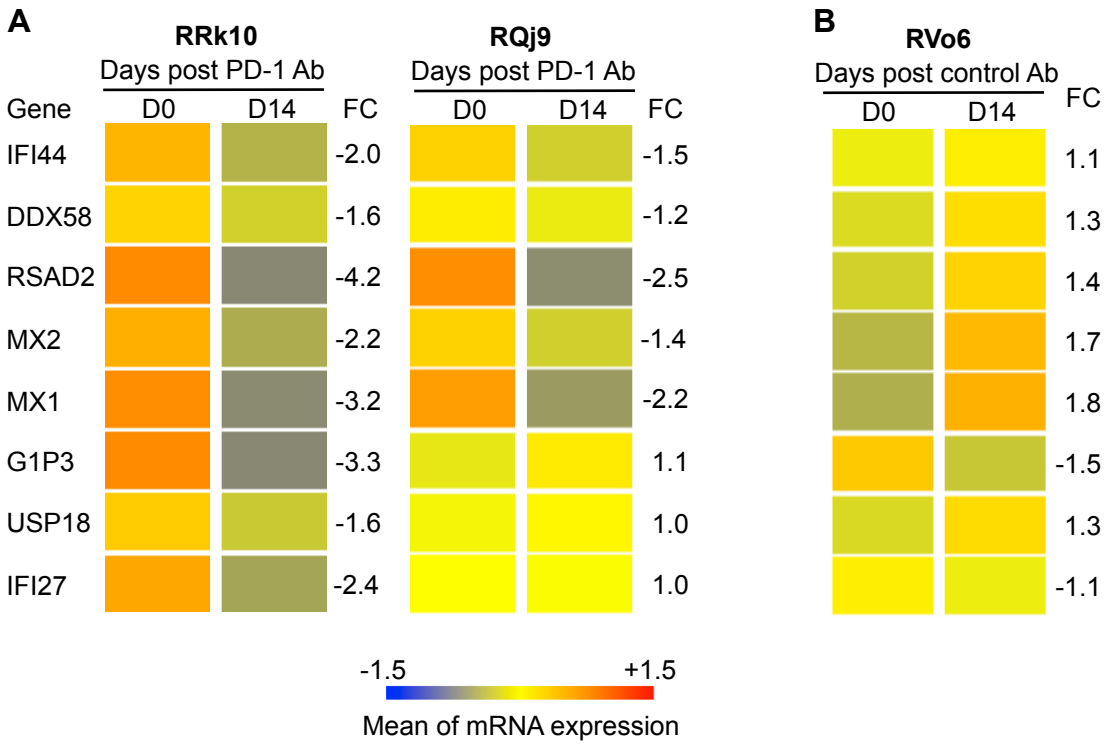
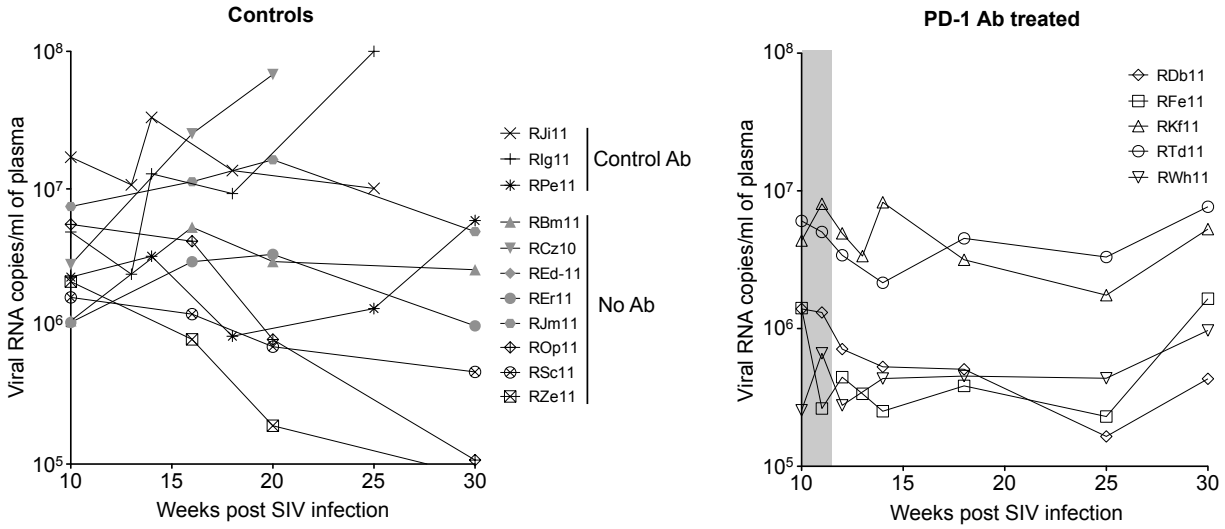
SUPPLEMENTAL FIGURES



Supplemental figure 1: Expression profiles of ISGs. Fluorescence intensity values from microarray experiments of RMs in Uninfected, Control Ab treated and PD-1 Ab treated groups are shown. The data presented for the Control Ab treated and PD-1 Ab treated groups was at day 14 following initiation of Ab treatments. Each symbol represents an individual macaque. PD-1 Ab treated group consisted data from 3 early chronic and 2 late chronic animals. Control Ab treated group consisted data from 2 early chronic and one late chronic animals.



Supplemental figure 2: Heat maps of type I IFN stimulated genes (ISGs) in gut tissue of PD-1 antibody treated (RRk10 and RQj9) or control antibody treated (RVo6) SIV-infected late chronic RMs on day 0 and day 14 post blockade.



Supplemental figure 3: Plasma viral loads of control group ('no Ab' and control Ab treated) and PD-1 Ab treated group of SIV infected early chronic RMs. The 'no Ab control' animals were selected to match the set point viral load in the early chronic animals used for the PD-1 antibody and control antibody arms.

Supplemental table 1: Incidence of opportunistic infections in control group of RMs**

S.NO	Animal ID	M1	M2	M3	M4	M5	M6	M7	M8	M9
SIV infected 'No antibody' control RMs										
1	RBm11	1							2	4
2	RCz11				3	2, 3	†			
3	REd11			1, 2 (†)						
4	REr11									1
5	RJm11		2	2		3	5	†		
6	ROp11									
7	RSc11						2, 3		6	
8	RZe11						1			
SIV infected 'Control antibody' treated RMs										
9	RIg11						†			
10	RJi11				1, 2, 5	1	†			
11	RPe11				2	2	2	2	†	

1 - *Campylobacter spp*; 2 - whipworms; 3 – *Shigella* , 4 – *Candida*, 5 – Cryptosporidium; 6 - Other bacterial infections. M1 to M9 represents months 1 to 9 post SIV infection and '†' sign indicates death of the animal.

** - Animals were tested for indicated infections due to diarrhea

Supplemental table 2: Incidence of opportunistic infections in PD-1 Ab treated group of early chronic RMs**

S.NO	Animal ID	M1	M2	M3	M4	M5	M6	M7	M8	M9
1	RDb11							3		
2	RFe11									
3	RKf11		2							
4	RTd11							2	1	
5	RWh11								1 and 2	1

1 - *Campylobacter spp*; 2 - whipworms; 3 – *Shigella*. M1 to M9 represents months 1 to 9 post SIV infection. PD-1 blockade was initiated at M2.5.

** - Animals were tested for indicated infections due to diarrhea

Supplemental table 3: Oligonucleotide primer sequences used for Real-time PCR

cDNA	FORWARD (5' to 3')	REVERSE (5' to 3')
SYBR		
GAPDH	GCACCACCAACTGCTTAGCAC	TCTTCTGGGTGGCAGTGATG
MX1	AGGAGTTGCCCTTCCCAGA	TCGTTCACAAGTTTCTTCAGTTTCA
CLDN5	TTGGCTGTTGCCTTACTTCC	GGGGAGATGTTGGAGTGAGA
JAM2	TGCTCTGAGTGGAACTGTGG	CACCTGCGATATCCAACAGA
CX45	GGAAGATGGGCTCATGAAAA	GCAAAGGCCTGTAACACCAT