Supplemental Table S1.

										MAMU type		
Animal ID	Group	Sex	Age at enrollment	Mtb CDC1551 dose (CFU)	SIVmac239	ART therapy days	Survival since Mtb challenge	TST Status(pre-Mtb challenge)	TST status (3 weeks post challenge)	***A*01***	***B*08***	***B*17***
KV48	ART	Male	4.82	10	300	35	127	NNN	PPP	+	-	-
LA04	ART	Male	4.22	10	300	44	136	NNN	PPP	-	-	-
LA46	ART	Male	4.18	10	300	63	155	NNN	PPP	-	-	-
LE24	ART	Male	4.03	10	300	14	106	NNN	PPP	-	-	-
KR44	ART-naïve	Male	5.5	10	300	0	121	NNN	PPP	+	-	-
LC88	ART-naïve	Male	4.1	10	300	0	155	NNN	PPP	-	-	-
JH07	ART-naïve	Male	7.32	10	300	0	152	NNN	PPP	-	-	-
JF23	ART-naïve	Male	7.26	10	300	0	152	NNN	PPP	+	-	-
KG40	ART-naïve	Male	5.51	10	300	0	124	NNN	PPP	-	-	-
IP88	ART-naïve	Male	7.05	10	300	0	131	NNN	PPP	+	-	-
JI68	ART-naïve	Male	6.06	10	300	0	164	NNN	PPP	-	-	-
JE48	ART-naïve	Male	6.33	10	300	0	120	NNN	PPP	-	-	-
GP50	LTBI	Male	11.18	10	--	0	166	NNN	PPP	+	-	-
JF47	LTBI	Male	7.32	10	--	0	174	NNN	PPP	-	-	-
HV02	LTBI	Male	9.34	10	--	0	166	NNN	PPP	-	-	-
JD72	LTBI	Male	7.38	10	--	0	174	NNN	PPP	-	-	-

Supplemental Table S2.

Table: Summary of semi-quantitative RNAscope ISH scores

		ISH score		
Group	Animal ID	Lung	Lymph node	Spleen
ART-naïve	LC88	1	4	4
ART-naïve	KR44	1	2	0
ART	KV48	0	2	2
ART	LA46	0	2	2
ART	LE24	0	3	3

The tissue sections were analyzed per field of 40X objective and score was assigned as '0' - if no positive staining or less than 1 dot per 10 cells in one microscopic field; '1'- for one to three dots per cell in one microscopic field; '2'- for four to nine dots per cell or very few dot clusters; '3'- for 10-15 dots per cell and/ or less than 10% dots are in a cluster; and '4' for more than 15 dots per cell and/ or more than 10% dots are in a cluster in one field. The score represented in the table is an average of the three fields studied under 40X objective.

Figure S1.

Clinical correlates of TB reactivation in ART-treated NHPs with *Mtb/SIV* co-infection:

(A) Percent weight loss (kg) as compared to baseline. (B) Percent change in temperature (°F) compared to baseline. Data represented as (mean ± SEM)

Figure S1.

A **B**

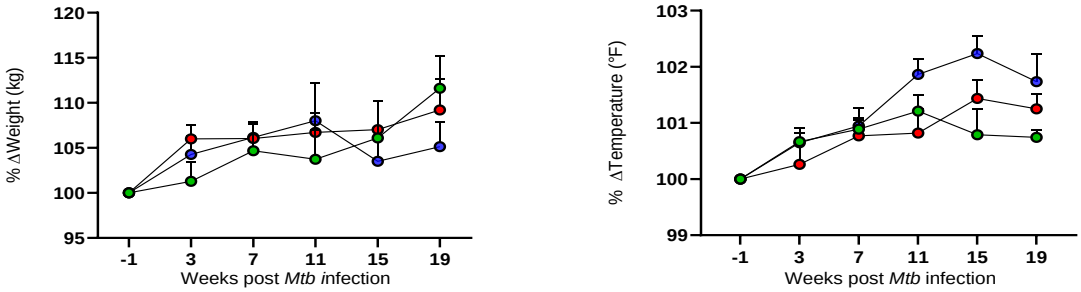
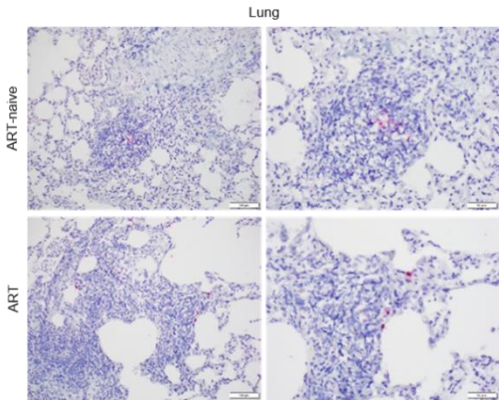


Figure S2.

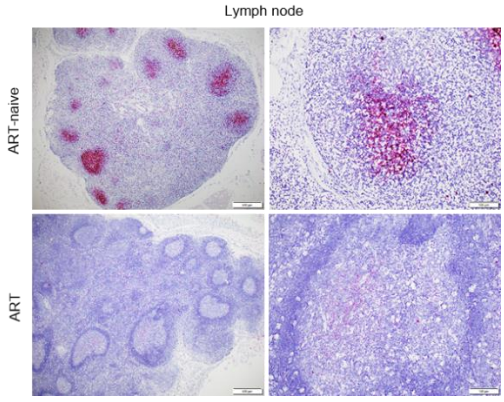
RNAscope ISH - to study SIV viral load in various tissues.

Serial sections from formalin-fixed paraffin-embedded tissue blocks (FFPE) of macaque lung, lymph node, and spleen were used for *In Situ Hybridization* (ISH) staining procedure RNAscope using SIVmac239 specific probe. Fig S2A shows representative low magnification (Total 200X; eyepiece 10X, Objective 20X) and high magnification (Total magnification 400X; eyepiece 10X, objective 40X) images of the lung, while Fig S2B show lymph node with images low magnification (Total 40X; eyepiece 10X, Objective 4X) and high magnification (Total magnification 200X; eyepiece 10X, objective 20X), and S2C show spleen images with low magnification (Total 20X; eyepiece 10X, Objective 2X) and high magnification (Total magnification 200X; eyepiece 10X, objective 20X) from ART-naïve (n=3) and ART (n=3) animals.

Figure S2.



B



C

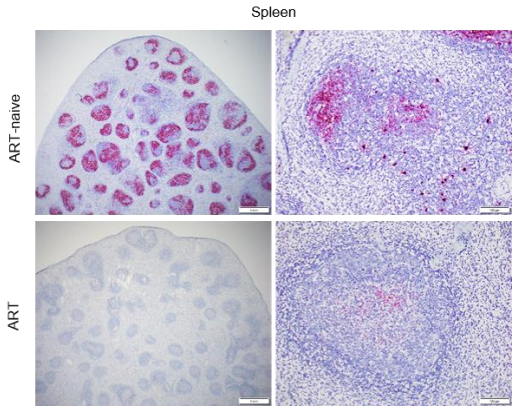


Figure S3.

Multi parameter flowcytometry to identify markers of chronic immune activation HLADR+ CD4+ T cells, CD69+ CD4+ T cells , PD1+ CD4+ T cells and CXCR3+CCR6+ CD4+ T cells in BAL and whole blood samples The three groups studied are *Mtb* infection only i.e. LTBI (n=4, shown in green), *Mtb*/SIV co-infection i.e. ART-naïve (n=8, shown in red) and *Mtb*/SIV co-infection with ART treatment i.e. ART (n=4, shown in blue); (A-F) data represent as (mean ± SEM); One-way ANOVA with Tukey's mulitiple corrections test; CD4+ T cell represented as percent of CD3+ T cells; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Figure S3.

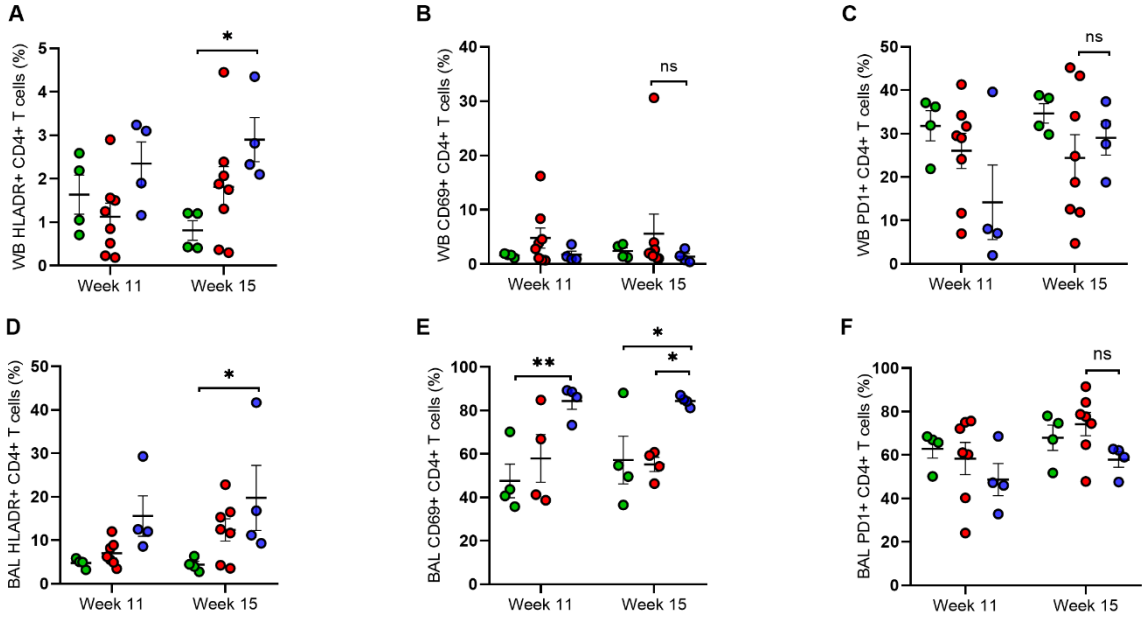


Figure S4.

High parameter flow cytometry to study markers of Th1 and Th17 responses, (S4A) CCR6+ CD4+ T cells, (S4B) CXCR3+ CD4+ T cells, and (S4C) CXCR3+ CCR6+ CD4+ T cells respectively in LTBI (n=4, green), ART-naïve (n=8, red) and, ART (n=4, blue) study group respectively. S4A-C data represented as (mean ± SEM), one-way ANOVA with Tukey's multiple comparison test (S4A-C). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. CD4+ T cell represented as percent of CD3+ T cells.

Figure 4.

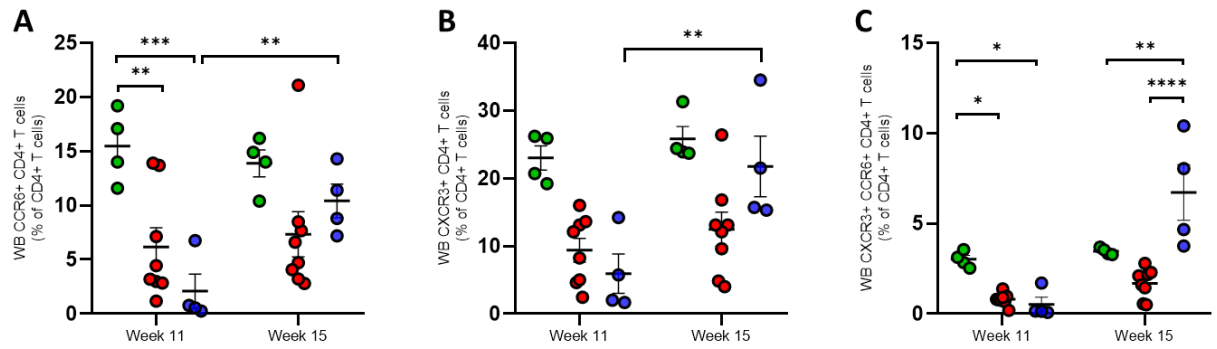


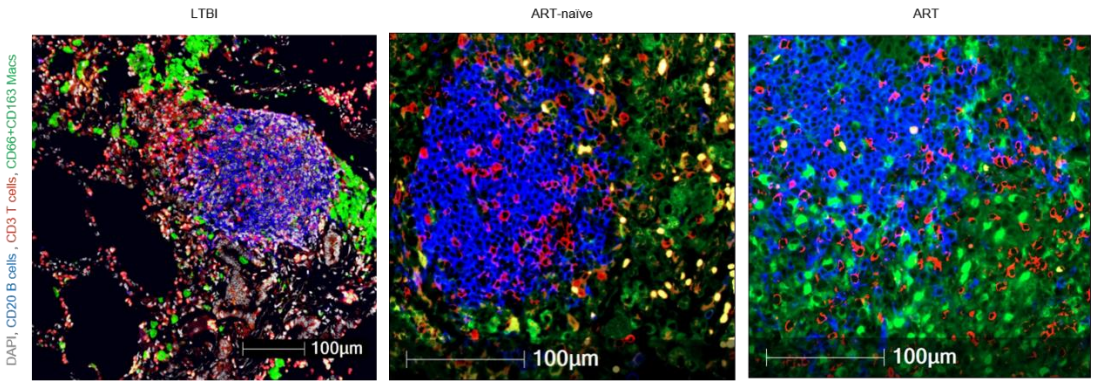
Figure S5.

Cellular characterization of iBALT in the *Mtb*/SIV co-infection model with ART therapy.

Immunohistochemistry staining and confocal imaging of formalin-fixed, paraffin-embedded (FFPE) lung sections from *Mtb/SIV* infected macaques with/without ART. Quantitative analysis was performed using HALO™ image analysis software to characterize the cellular composition of iBALT in the lung sections. (Fig S5A) show cell composition of iBALT with DAPI (grey), CD20+ B cells (blue), CD3+ T cells (red), and CD68+CD163+ macrophages (green) in the lung of LTBI, ART-naïve and ART groups. The iBALT regions in each lung section were manually identified (area including an airway, blood vessel, CD20+ B cell aggregates, and other immune cell aggregates) to perform quantitative analysis using HALO. The three groups studied are *Mtb* infection only i.e. LTBI (n=3), *Mtb*/SIV co-infection i.e. ART-naïve (n=3) and *Mtb*/SIV co-infection with ART treatment i.e. ART (n=3). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; (B) one way ANOVA with Tukey's multiple comparison test; (B) data is presented as mean ± SEM.

Figure S5

A



B

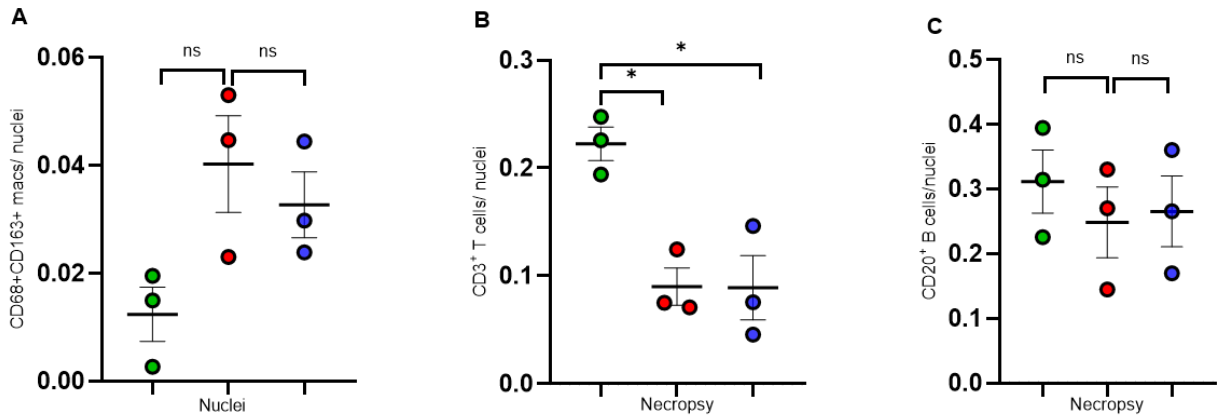


Figure S6.

Cellular characterization of lung interstitium in Mtb/SIV co-infection model with ART therapy.

Immunohistochemistry staining and confocal imaging of formalin-fixed, paraffin-embedded (FFPE) lung sections from *Mtb/SIV* infected macaques with/without ART. Quantitative analysis was performed using HALO™ image analysis software (Akoya Bioscience, Marlborough, MA, USA) to quantify CD68+CD163+ macrophages in the interstitium of the lung. (Fig S6) show DAPI (blue), CD4+ T cells (red), and CD68+CD163+ macrophages (green) in the lung of LTBI, ART-naïve and ART groups. The lung interstitium (lung parenchyma – alveolar spaces/glass) reported, (mean ± SEM), 472.17 ± 239.01 counts/mm^2, 171.13 ± 54.32 counts/mm^2 of CD68+CD163+ macrophages, with no significant difference, p = 0·2654, between ART-naïve (n=4) group and ART (n=4) group respectively. High count of macrophages in this section can be due to the infiltration of macrophages and neutrophils in the surrounding tissue as seen in TB reactivation. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; two-tailed Student's t-test.

Figure S6.