

Supplemental Figure 1. Bacterial burden ($\log_{10}$ CFU/g or $\log_{10}$ CFU/mL) was determined in (A) lung, (B) lung granulomas, (C) BAL, (D) spleen at necropsy in LTBI ($n=4$), cART naive ($n=8$), cART ($n=4$), and cART+3HP ($n=6$) by homogenizing the tissues and plating on agar plates. Nonparametric Mann-Whitney test was used to compare 2 groups. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Data are presented as mean with SD.

Supplemental Figure 2. Hematoxylin & Eosin-stained lung tissue to study the cellular and granulomatous pathology in (A) LTBI ($n=3$), (B) cART naive ($n=3$), (C) cART ($n=3$), and (D) cART+3HP ($n=3$). Scale bars: 5 mm. (E) Percentage of lung involvement was calculated by a board-certified pathologist by quantification of the number of lesions per lobe in LTBI ($n=3$), cART naive ($n=3$), cART ($n=3$), and cART+3HP ($n=3$). Nonparametric Mann-Whitney test was used to compare 2 groups. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Data are presented as mean with SD.

Supplemental Figure 3. Chromogenic staining to detect CD4 and IL-21 in lung tissues from RMs infected with low dose *Mtb* infection ($n=3$). Representative image of lung section from (A,B) GP50, (C,D) HV02 and (E,F) JF47. Scale bars: 5000 μ m, 2000 μ m. CD4⁺ T cells (red) and IL-21⁺ cells (yellow) were identified, along with double-positive populations: CD4⁺IL-21⁺ cells (orange; indicated by black arrows).

Supplemental Figure 4. Chromogenic staining to detect CD4 and IL-21 in lung tissues from RMs co-infected with *Mtb*/SIV ($n=3$). Representative image of lung section from (A,B) JH07, (C,D) KG40 and (E,F) KR44. Scale bars: 5000 μ m, 2000 μ m. CD4⁺ T cells (red) and IL-21⁺ cells (yellow) were identified, along with double-positive populations: CD4⁺IL-21⁺ cells (orange; indicated by black arrows).

Supplemental Figure 5. Chromogenic staining to detect CD4 and IL-21 in lung tissues from RMs co-infected with *Mtb*/SIV and treated with cART ($n=3$). Representative image of lung section from (A,B) 33343, (C,D) 33994 and (E,F) 34741. Scale bars: 1 cm, 5000 μ m, 2000 μ m. CD4⁺ T cells (red) and IL-21⁺ cells (yellow) were identified, along with double-positive populations: CD4⁺IL-21⁺ cells (orange; indicated by black arrows).

Supplemental Figure 6. Chromogenic staining to detect CD4 and IL-21 in lung tissues from RMs co-infected with *Mtb*/SIV and treated with cART+3HP ($n=3$). Representative image of lung section from (A,B) 41876, (C,D) 41882 and (E,F) 41901. Scale bars: 5000 μ m, 2000 μ m. CD4⁺ T cells (red) and IL-21⁺ cells (yellow) were identified, along with double-positive populations: CD4⁺IL-21⁺ cells (orange; indicated by black arrows).

Supplemental Figure 7. (A) Total lung cell counts (cells per lung, μ m²) measured in uninfected (baseline) ($n=3$),

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LTBI ($n=3$), cART-naïve ($n=3$), cART ($n=3$), and cART+3HP ($n=3$) groups. Each dot represents an individual subject; horizontal lines with error bars indicate mean $\pm$ SEM. No statistically significant differences were observed between groups (ns, not significant). (B) Plasma IL-21 concentrations (pg/mL) in the same groups. Each dot represents an individual subject; horizontal lines with error bars indicate mean $\pm$ SEM. Significant differences between indicated groups are denoted by brackets and asterisks ($*P < 0.05$); ns indicates not significant. Nonparametric Mann-Whitney test was used to compare 2 groups.

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Supplemental Figure 8. Bar graphs show the percentage of IL-21⁺ CD4⁺ T cells relative to IFN- γ ⁺, IL-17⁺, and TNF α ⁺ CD4⁺ T cell subsets in LTBI ($n=3$), cART-naïve ($n=3$), cART ($n=3$), and cART+3HP ($n=3$) groups. Black bars represent IL-21⁺ CD4⁺ T cells, pink bars represent IFN- γ ⁺ CD4⁺ T cells, teal bars represent IL-17⁺ CD4⁺ T cells, and purple bars represent TNF α ⁺ CD4⁺ T cells. Individual symbols denote single subjects, and bars represent mean $\pm$ SEM. Statistical significance between indicated groups is shown by brackets with asterisks ($*P < 0.05$, $**P < 0.01$); ns indicates not significant. Nonparametric Mann-Whitney test was used to compare 2 groups.

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Supplemental Figure 9. (A-D) Representative immunohistochemistry images showing CD4⁺CD103⁺ cells in lung tissue from (A) LTBI ($n=3$), (B) cART-naïve ($n=3$), (C) cART-treated ($n=3$), and (D) cART+3HP-treated ($n=3$) animals. Red arrows indicate CD4⁺CD103⁺ T cells. (E-H) Representative images of CD4⁺CD69⁺ T cells. Representative IHC images showing CD4⁺CD9⁺ T cells in lung tissue from (E) LTBI ($n=3$), (F) cART-naïve ($n=3$), (G) cART-treated ($n=3$), and (H) cART+3HP-treated ($n=3$) animals. Red arrows indicate CD4⁺CD69⁺ T cells. Scale bars; 500 μ m, 100 μ m. (I) Quantification of total CD4⁺ T cells and CD4⁺CD103⁺ T cells per lung area (μ m²). (J) Quantification of total CD4⁺ T cells and CD4⁺CD69⁺ T cells per lung area (μ m²). Data are shown as mean $\pm$ SEM; $*P < 0.05$; ns, not significant. Nonparametric Mann-Whitney test was used to compare 2 groups.

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Supplemental Figure 10. Linear regression analysis of (A) Log10 IL-21⁺CD4⁺ T cells vs Log10 CFU/g lung burden ($n=3$) and (B) Log10 IL-21⁺CD4⁺ T cell vs % lung pathology ($n=3$). Statistical significance was assessed by calculating the p -value for the slope coefficient in a linear regression model, with significance defined as $P < 0.05$, using R version 4.2.2.

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Supplemental Figure 11. (A) Correlation between log₁₀ CD4⁺ T cell counts and lung *Mtb* burden in cART-naïve and cART-treated animals. Pearson correlation analysis showed no significant association in either group (cART-naïve: $r = -0.3906$, $P = 0.3723$; cART: $r = 0.8261$, $P = 0.1906$). (B) Correlation between log₁₀ CD4⁺ T cell counts and lung pathology scores in cART-naïve and cART-treated animals. No significant correlations were observed (cART-naïve: $r = 0.1203$, $P = 0.4616$; cART: $r = 0.9048$, $P = 0.14$). Each symbol represents an individual animal. Statistical significance was defined as $\alpha = 0.05$ (ns, not significant).

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Supplemental Figure 12. Representative chromogenic images from cART naïve RMs, (A) KG40 and (B) KR44

demonstrating the granulomatous and non-granulomatous areas of analysis. Scale bars: 5000 μ m. Stacked bar graphs showing (C) %CD4⁺IL-21⁺T cells in granulomatous and non-granulomatous areas in the lung tissue section of cART naïve RMs, JF47, KG40 and KR44. (D) %CD4⁺IL-21⁺ T cells in cells in granulomatous and non-granulomatous areas in the lung tissue section of cART naïve ($n=3$), cART ($n=3$) and cART+3HP ($n=3$) groups. Data are shown as mean $\pm$ SEM; * $P < 0.05$; ns, not significant. Nonparametric Mann-Whitney test was used to compare 2 groups.

Supplemental Figure 13. Schematic illustrating the four main steps of the Xenium platform used for spatial gene expression analysis. (A) Tissue analysis: Formalin-fixed tissue sections were mounted and prepared for spatial profiling, preserving tissue morphology and RNA integrity. (B) cDNA synthesis: In situ reverse transcription was performed, converting captured mRNA molecules into spatially barcoded complementary DNA (cDNA) while maintaining their native spatial context. (C) Gene expression detection: Targeted probes hybridized to the cDNA, enabling multiplexed detection of selected transcripts with subcellular resolution. (D) Data analysis: Spatial gene expression data were processed to generate high-resolution transcript maps, identify cell types, and quantify gene expression patterns across defined tissue regions.

Supplemental Figure 14. The figure displays gene-specific metrics such as transcript detection counts, spatial localization accuracy, and signal-to-background ratio for key genes of interest in region of interest in lung tissue sections from RMs in (A) LTBI, (B) cART-naïve, (C) cART and (D) cART+3HP treated groups. Total number of transcripts detected per gene across analyzed lung tissue sections from RMs in (E) LTBI, (F) cART-naïve, (G) cART and (H) cART+3HP treated groups using the Xenium platform. Transcript counts were derived from decoding analysis, which identifies and assigns individual RNA molecules to target genes based on probe hybridization patterns. This analysis reflects gene-specific expression abundance across all regions of interest. (I) Table displaying key decoding performance metrics across lung tissue sections analyzed using the Xenium platform. These metrics provide an overview of decoding efficiency, tissue integrity, and transcript localization quality across samples and support robust spatial gene expression analysis.

Supplemental Figure 15. (A) Pearson correlation between total IL-21⁺CD4⁺ T cell counts and IFN γ ⁺CD4⁺ T cells responding to *Mtb* cell wall (CW) antigen in cART-naïve animals. No significant association was observed ($P = 0.4321$, $R^2 = 0.6058$). (B) Pearson correlation between IL-21⁺CD4⁺ T cell counts and *Mtb* CW-specific IFN- γ ⁺CD4⁺ T cell responses in cART+3HP-treated animals. No significant association was detected ($P = 0.5237$, $R^2 = 0.4628$). Each symbol represents an individual animal. Statistical significance was defined as $P < 0.05$.

Supplemental Figure 16. Heatmap of top marker gene expression by cluster in region on interest in LTBI RM. The table provides cluster numbers, top markers and annotation.

Supplemental Figure 17. Heatmap of top marker gene expression by cluster in region on interest in cART naïve

RM. The table provides cluster numbers, top markers and annotation.

Supplemental Figure 18. Heatmap of top marker gene expression by cluster in region on interest in cART RM. The table provides cluster numbers, top markers and annotation.

Supplemental Figure 19. Heatmap of top marker gene expression by cluster in region on interest in cART+3HP RM. The table provides cluster numbers, top markers and annotation.

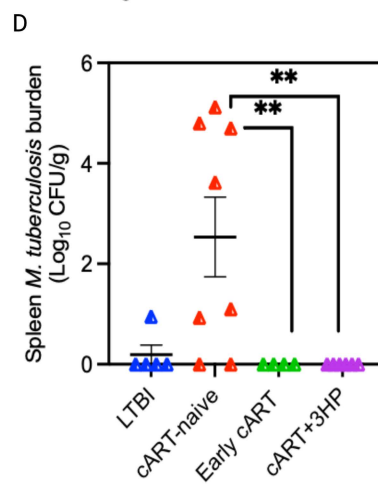
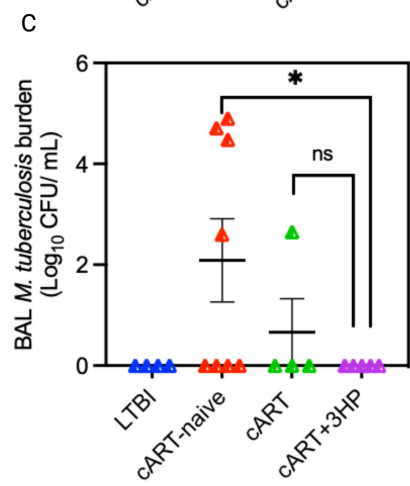
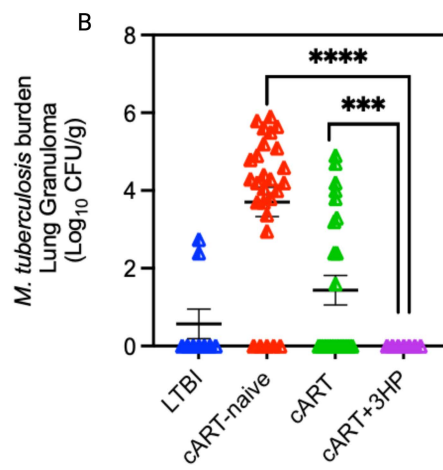
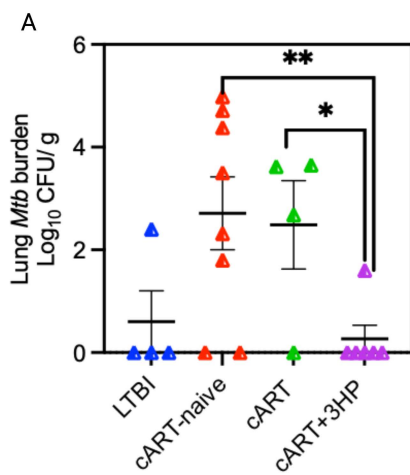
Supplemental Figure 20. Xenium morphology image QC gallery for RMs in (A) LTBI, (B) cART naïve, (C) cART and (D) cART+3HP. Image QC provides image quality based on cell segmentation. Xenium system generates morphology images, including DAPI (nuclei stain), ATP1A1/CD45/E-Cadherin for cell boundary, 18s for interior RNA and alphaSMA/Vimentin for interior protein. X axis represents the X -coordinate of a point in the image (μm) and Y axis represents the fluorescence intensity of the stained morphology images (photon equivalent - pe).

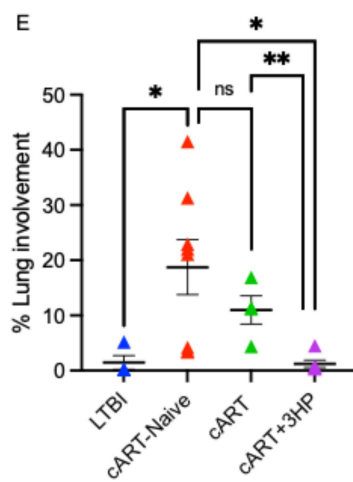
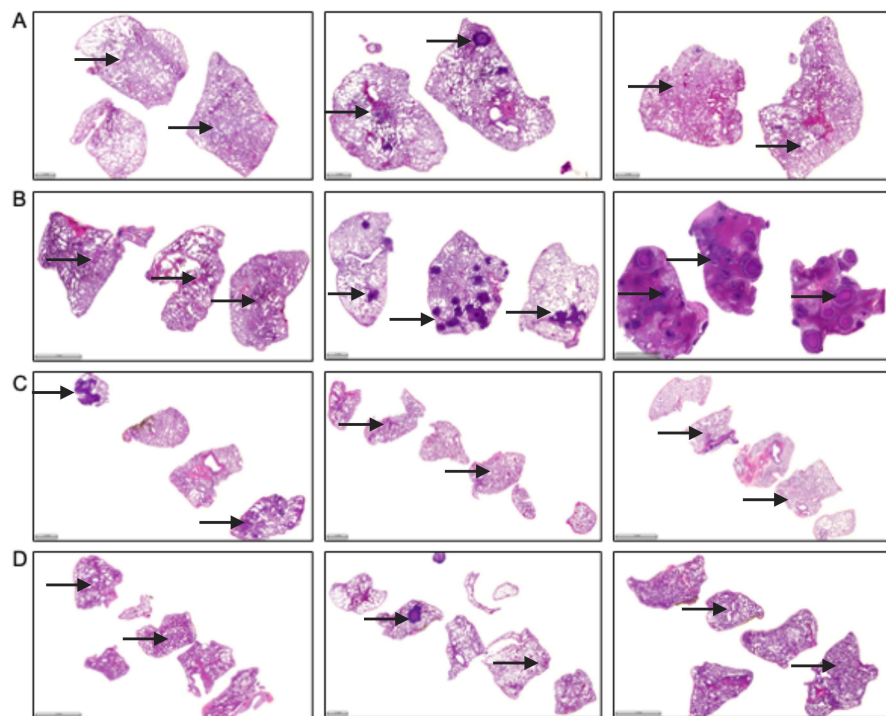
Supplemental Table 1. List of samples and experimental design information for Xenium platform. This project focuses on dissecting TB/SIV co-Infection and treatment response in macaque lung tissues. It involves a total of 4 slides, each representing an animal from one of the following groups.

Supplemental Table 2. List of reference markers used for annotation of cell clusters. After preprocessing using Seurat SCTransform workflow, to determine cell type identities, cluster-specific marker genes were extracted using the FindAllMarkers function. The resulting marker gene lists were compared against known reference markers to assign cell type annotations for each cluster.

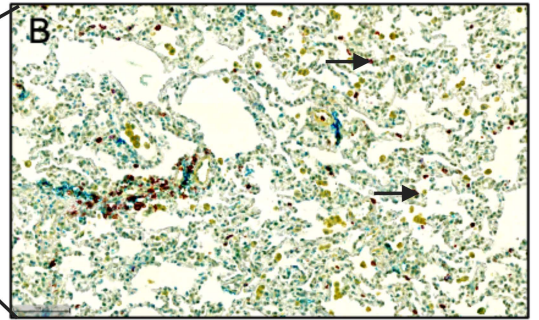
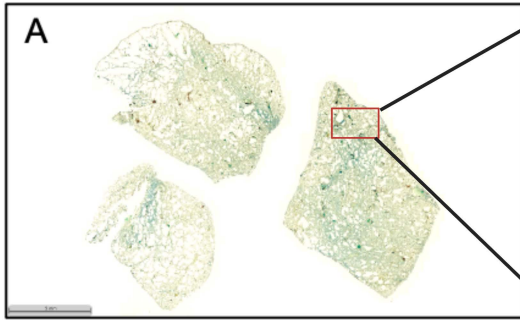
Supplemental Table 3. Demographics of the study animals including age, sex, therapy initiation, and Mamu type of study macaques.

Supplemental Table 4. Total lung tissue area (μm^2) quantified for each animal in the LTBI, cART-naive, cART-treated, and cART+3HP-treated groups. Values represent the cumulative area analyzed per animal.

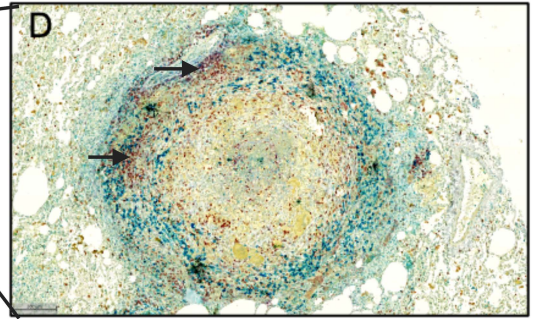
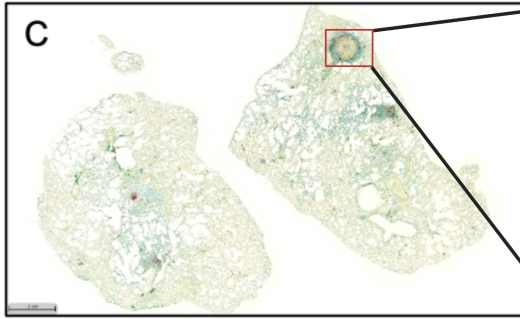




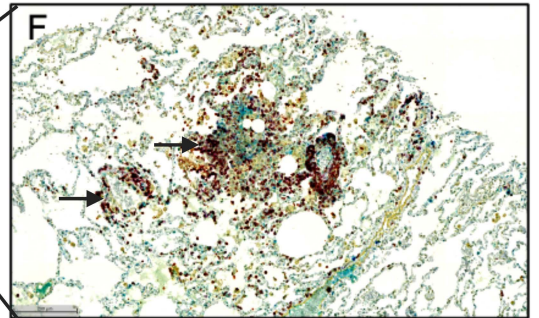
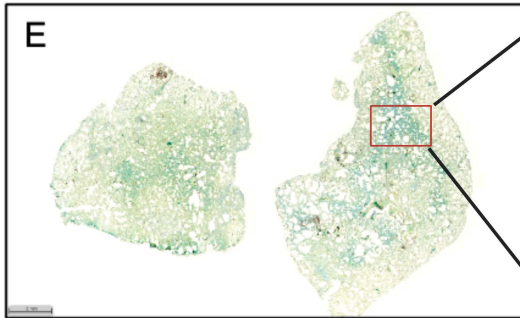
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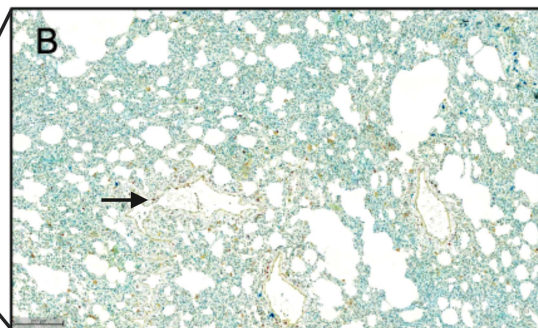
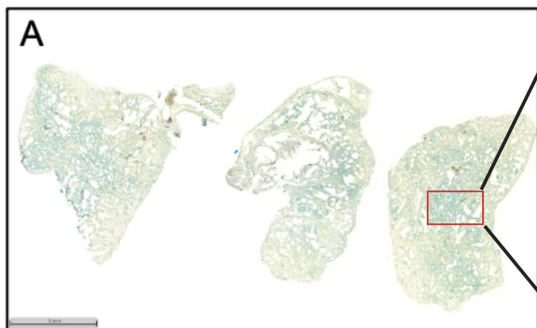
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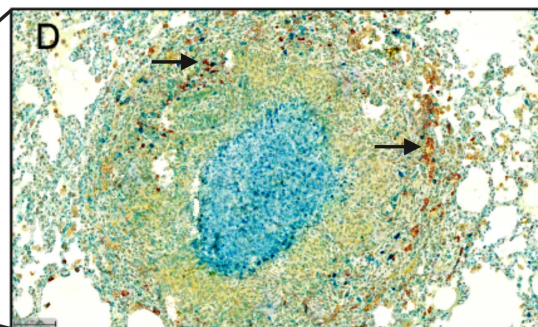
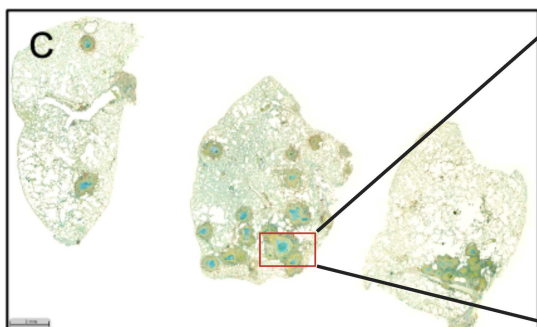
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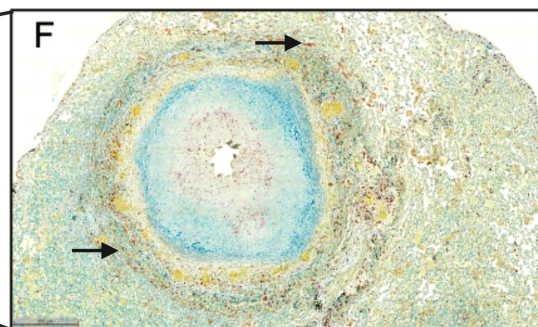
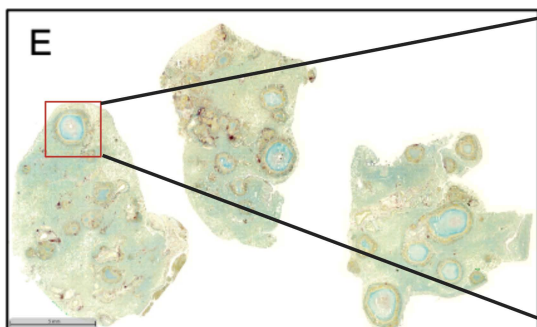
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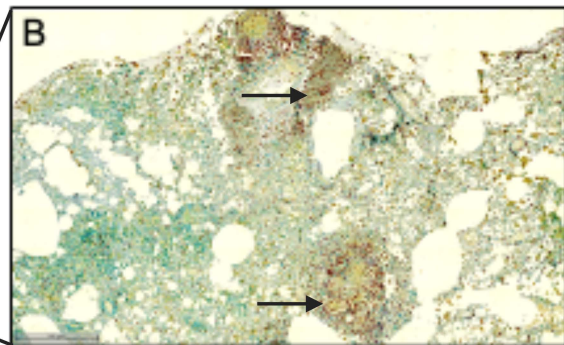
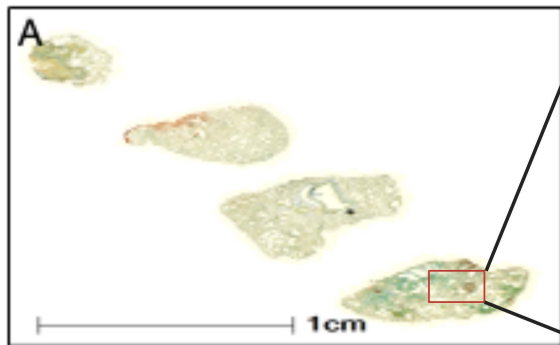
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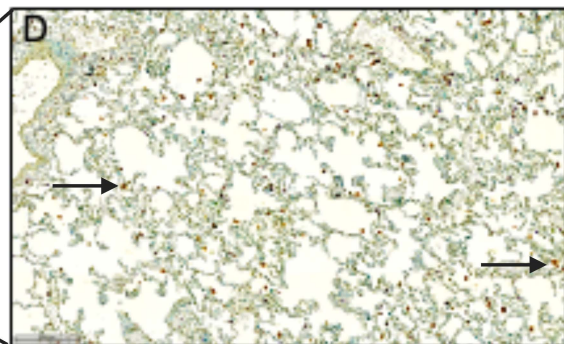
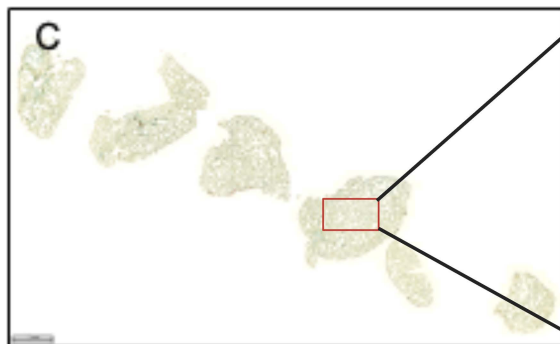
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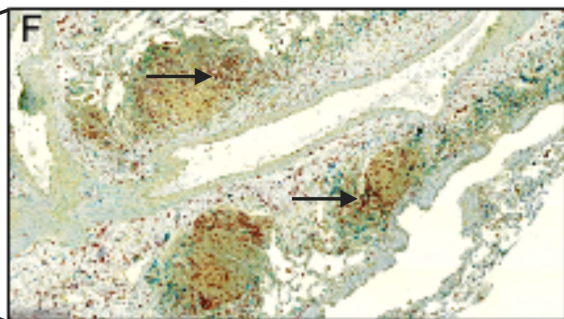
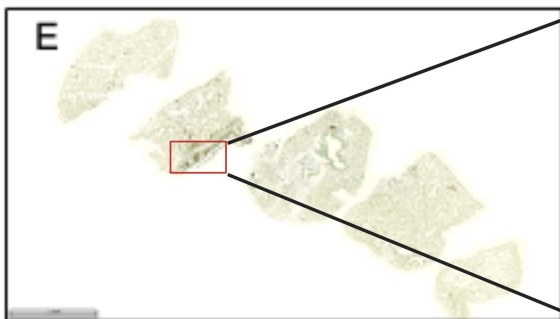
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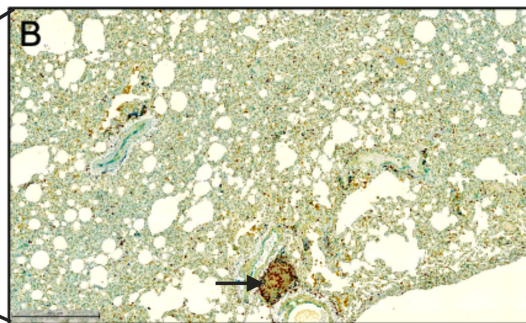
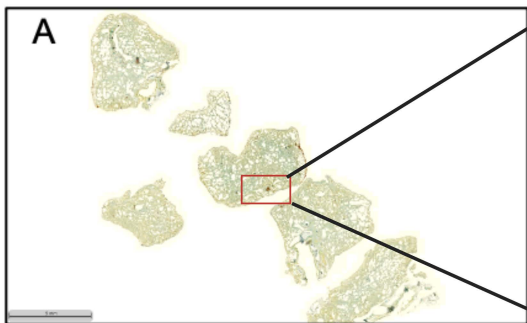
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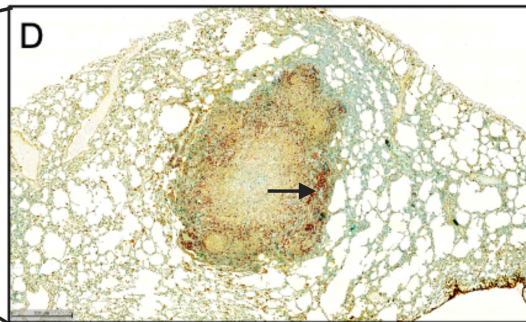
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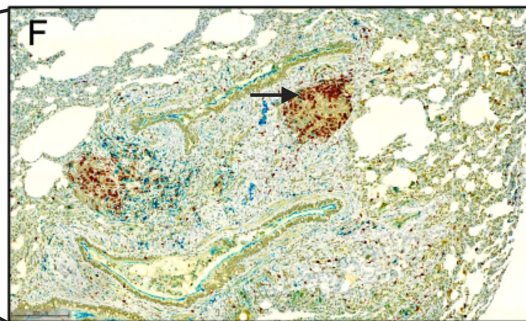
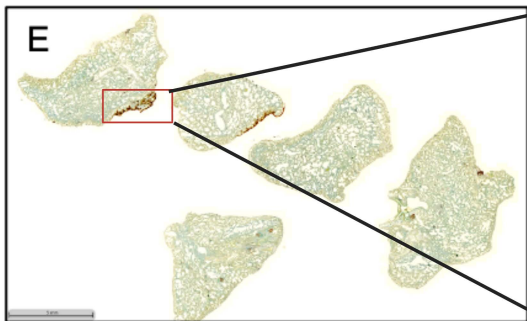
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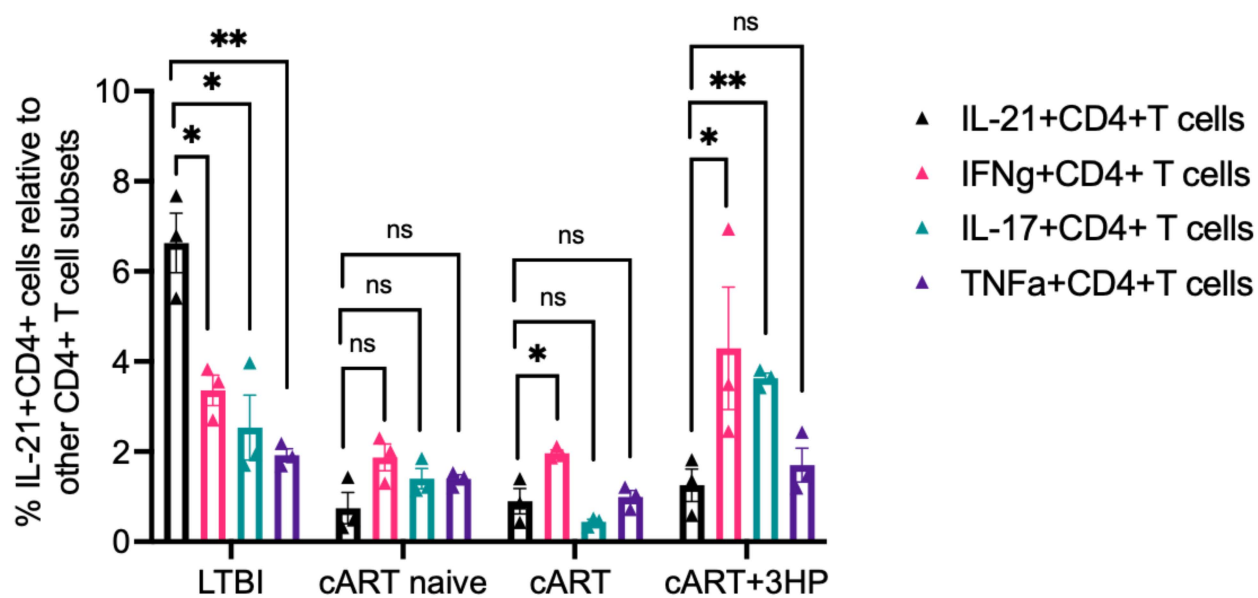


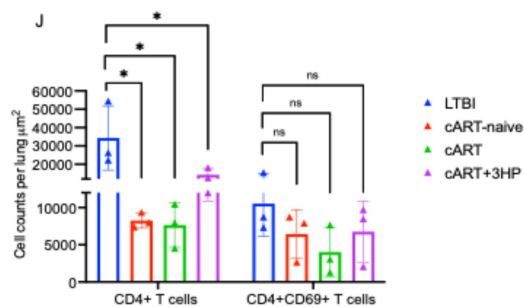
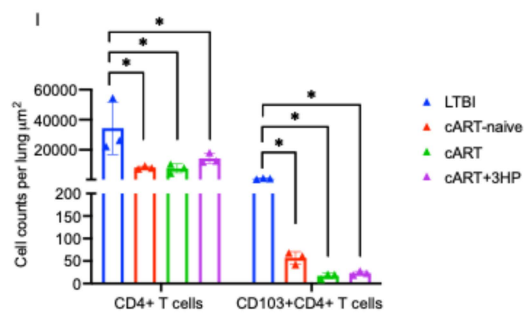
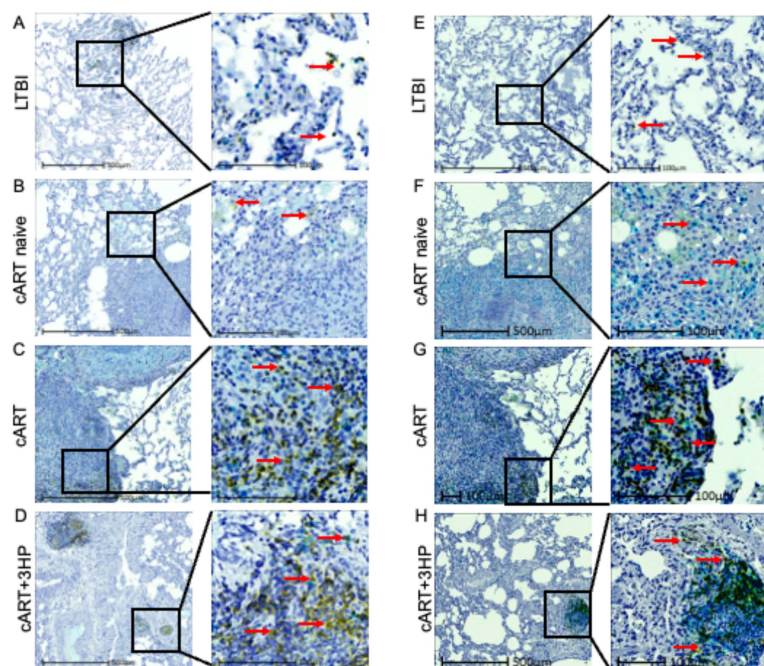
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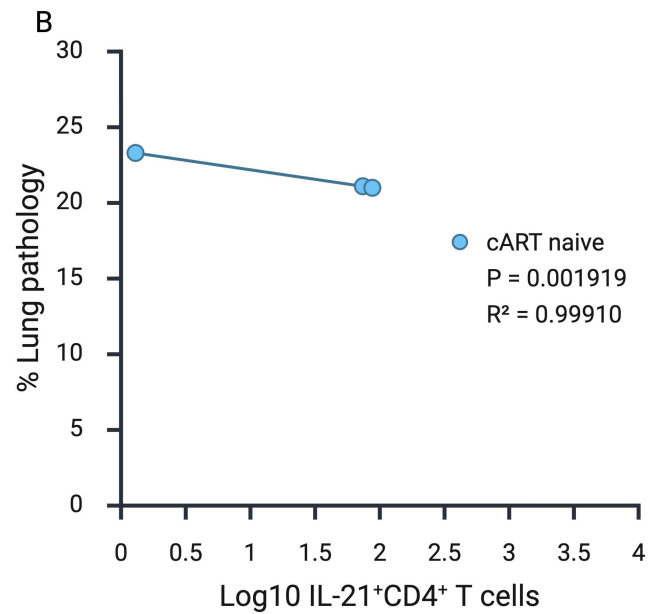
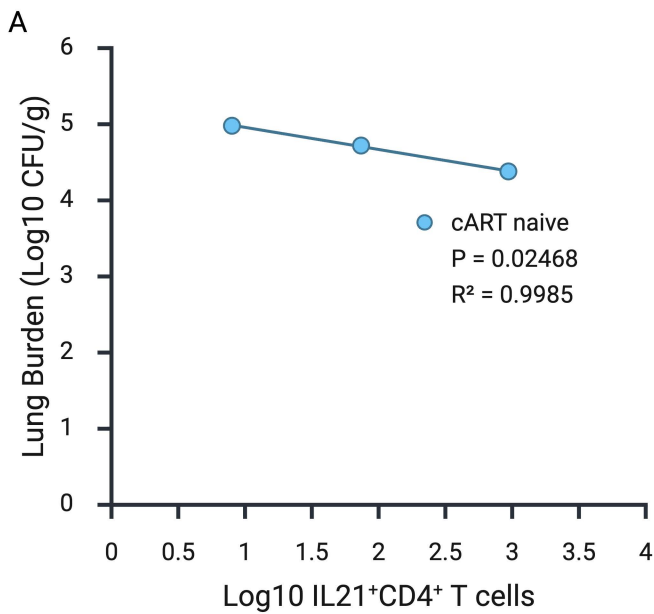


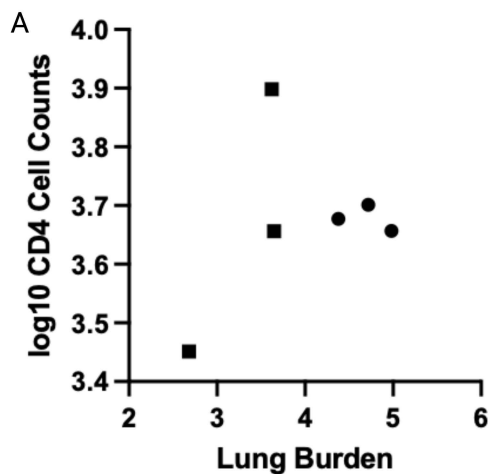
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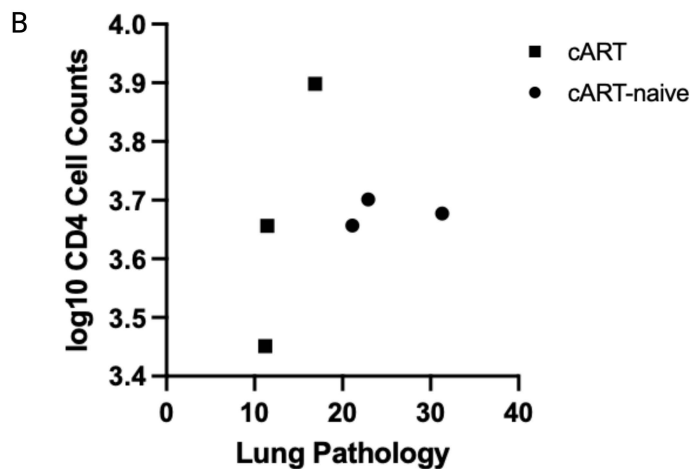




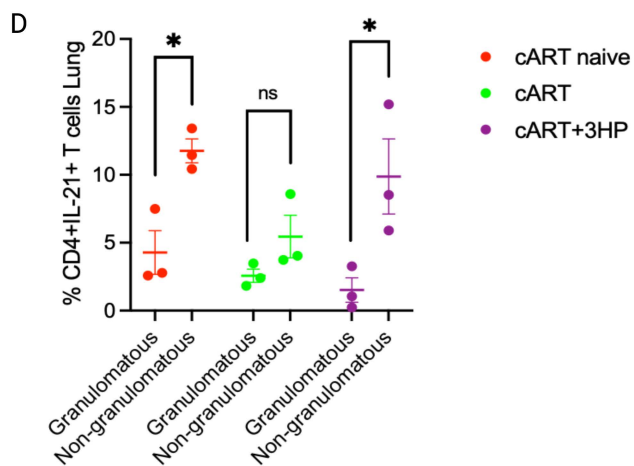
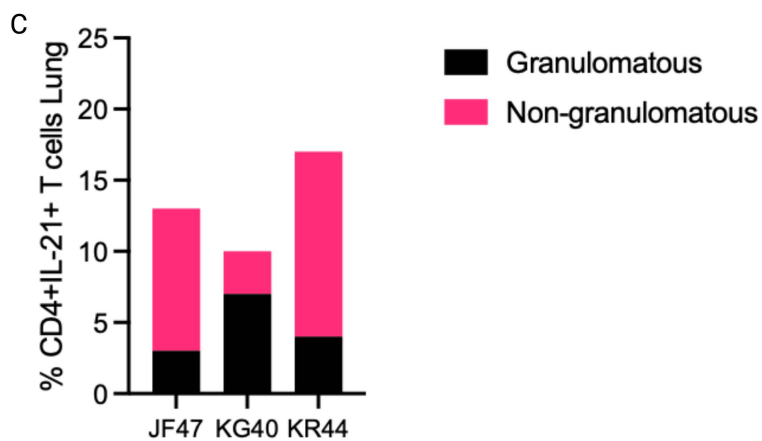
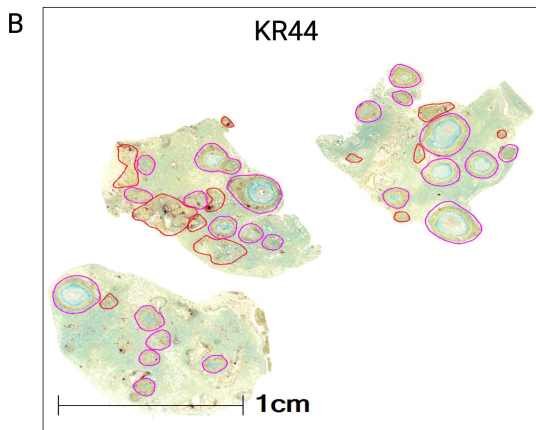
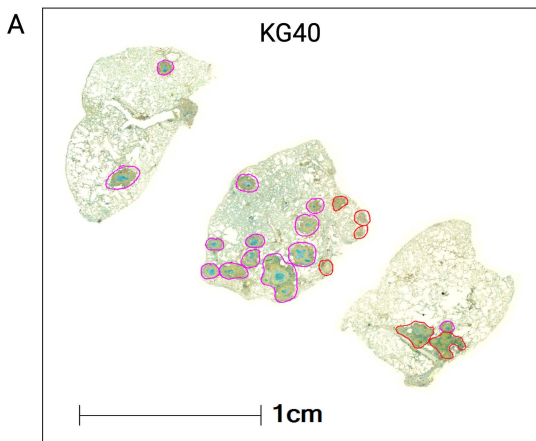




	Lung Burden vs. cART-naive	Lung Burden vs. cART
Pearson r		
r	-0.3906	0.8261
95% confidence interval		
R squared	0.1525	0.6824
P value		
P (one-tailed)	0.3723	0.1906
P value summary	ns	ns
Significant? (alpha = 0.05)	No	No

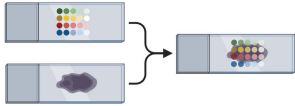


	Lung Pathology vs. cART-naive	Lung Pathology vs. cART
Pearson r		
r	0.1203	0.9048
95% confidence interval		
R squared	0.01446	0.8187
P value		
P (one-tailed)	0.4616	0.14
P value summary	ns	ns
Significant? (alpha = 0.05)	No	No

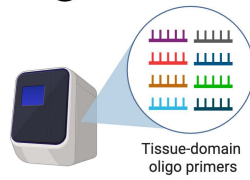


A Tissue analysis

Grid tissue section on oligo(dT) primers slide

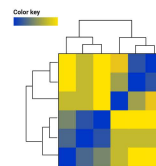


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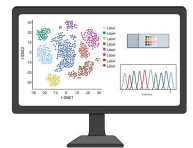
C Gene expression

Spatially-resolved genes encoded via oligo tag

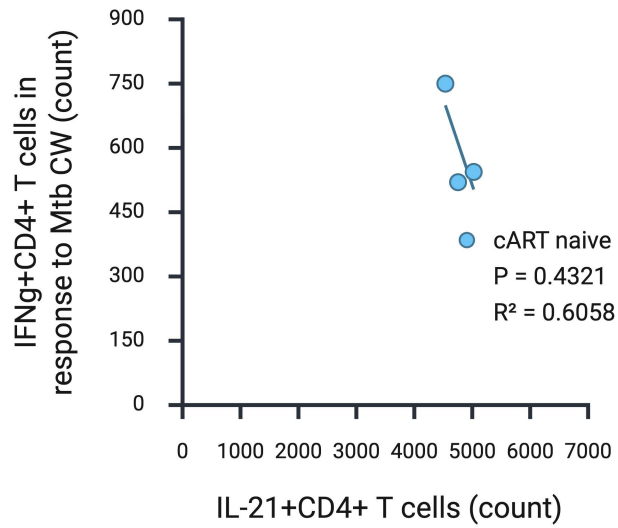


D Data analysis

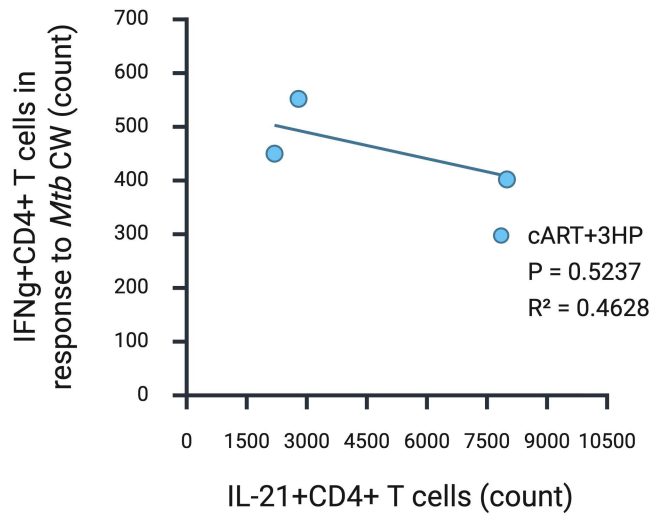
Gene clusters association to tissue domains

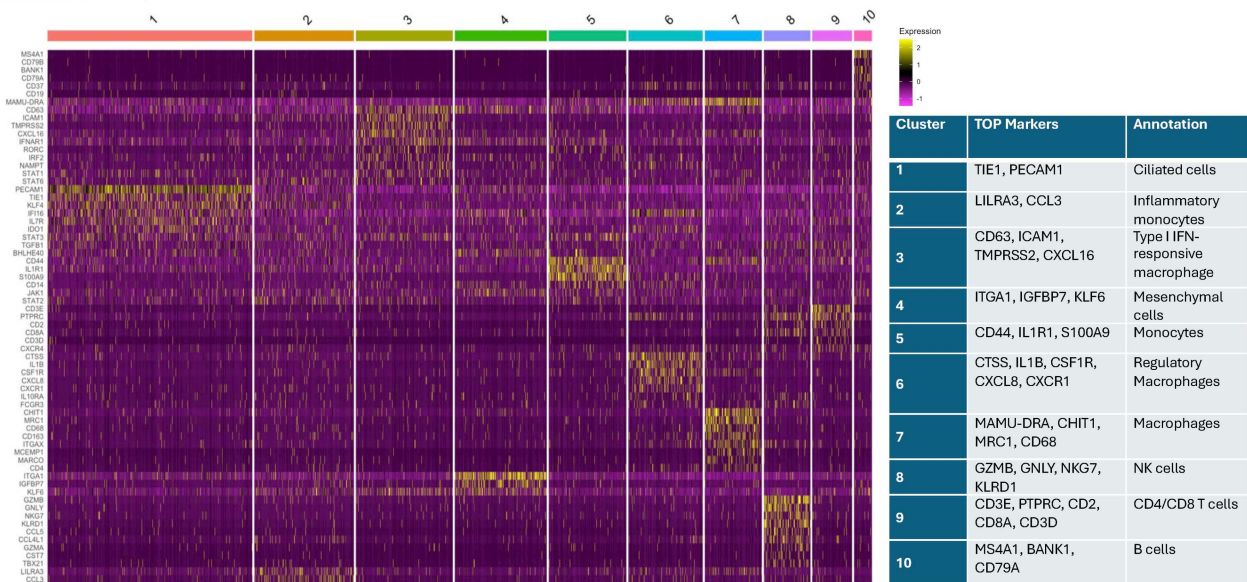


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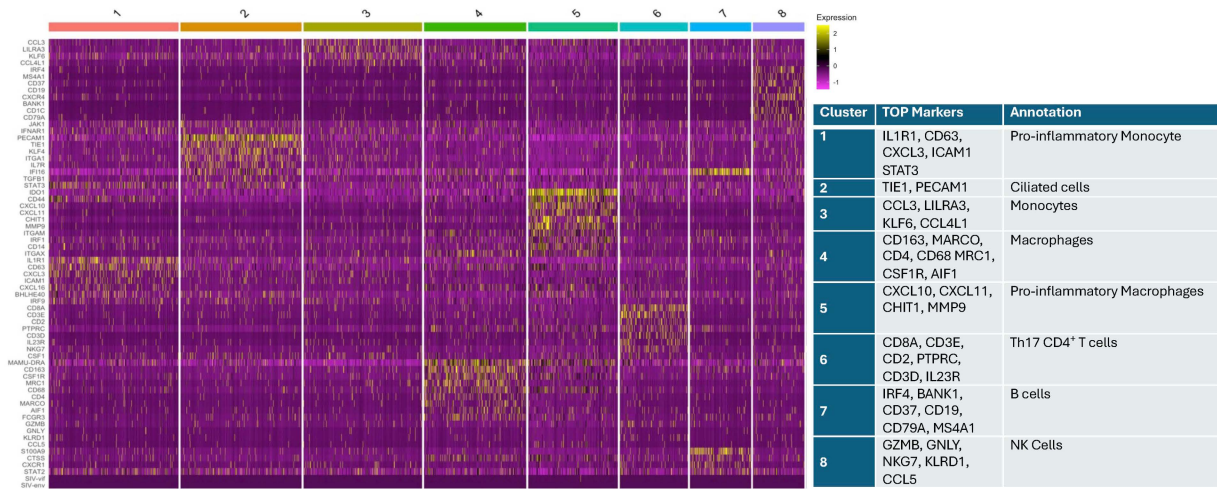


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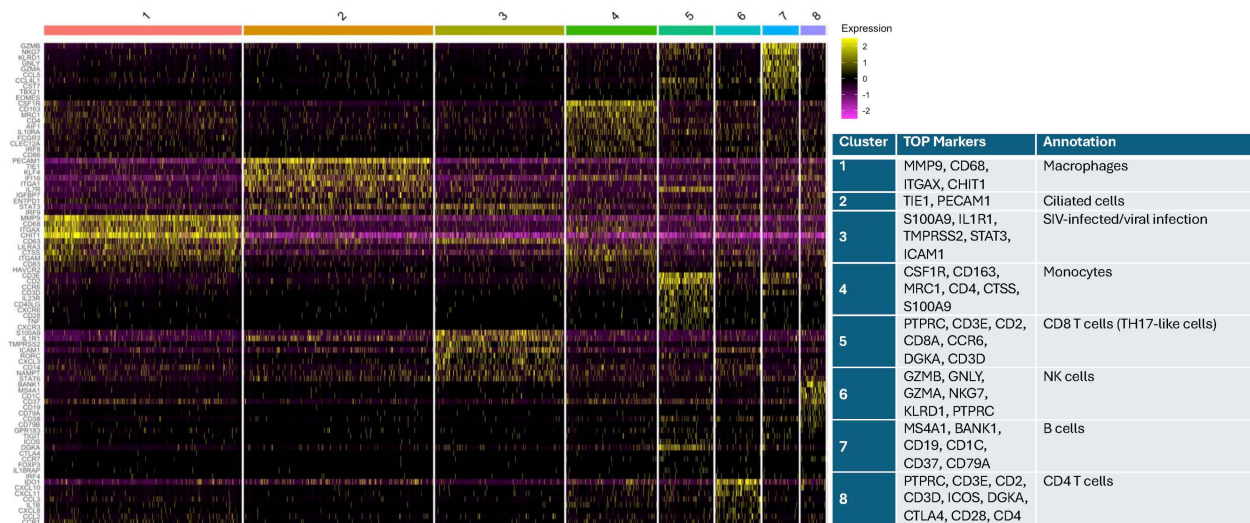




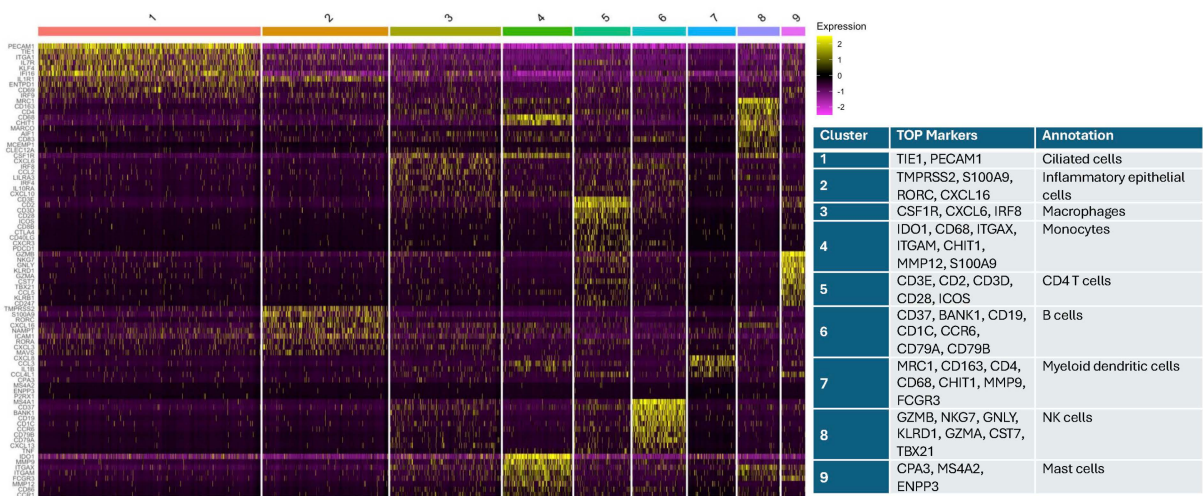
Heatmap of Top Marker Gene Expression by Cluster: LTBI



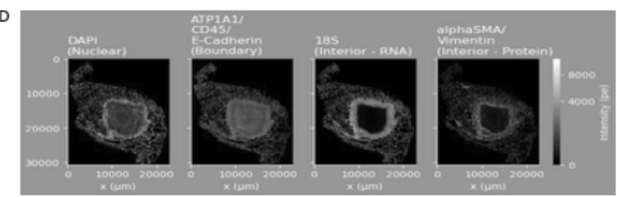
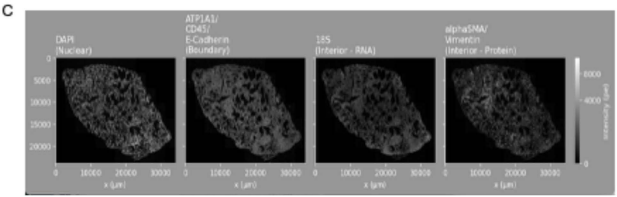
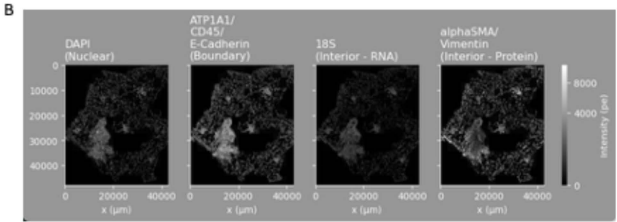
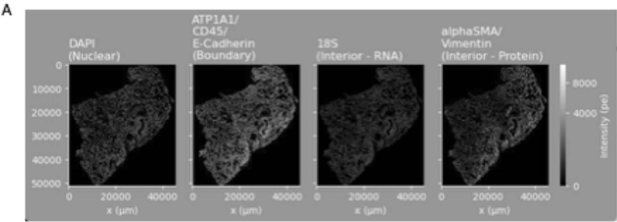
Heatmap of Top Marker Gene Expression by Cluster: cART naive



Heatmap of Top Marker Gene Expression by Cluster: cART



Heatmap of Top Marker Gene Expression by Cluster: cART+3HP



Sample ID	Group	Description
HV02	LTBI	Baseline (2 regions)
KG40	LTBI + SIV	Untreated (2 regions)
33343	LTBI + SIV + cART	cART treated (3 regions)
41882	LTBI + SIV + cART + 3HP	cART treated + TB therapy (4 regions)

Cell Type	Reference Markers
Macrophage	CD63,MRC1(CD206),CD44,PTPRC(CD45),CD163,CD83, CD14, MNDA, FCGR3(CD16)
Monocyte	MRC1(CD206),CD44,PTPRC(CD45), CD83,ITGAX, CSF3R(CD114),NAMPT, S100A9
CD4 T cells	PTPRC(CD45),CD44,CD3D,CD69,IL23R, CD3E, CD4
CD8 T cells	PTPRC(CD45),CD44,CD3D,CD8B,CD69, CD8A,CD3E
NK cells	PTPRC(CD45),CD44, NCAM1(CD56), NKG2A
B cells	PTPRC(CD45),CD44,CD79B,CD69,CD19, CD79A
Mast cells	ENPP3(CD203c),CD44,KIT(CD117),CD69, PTPRC(CD45)
Myeloid dendritic cells	ITGAM(CD11b), PTPRC(CD45), CDC1
Ciliated cells	TIE1 (CD31), PECAM1 (CD31)

Animal ID	Group	Sex	Age	Therapy initiation	MAMU Type		
					<i>A*01</i>	<i>B*08</i>	<i>B*17</i>
33343	cART treated	Female	6.30	2 wks post-SIV	Negative	Negative	Positive
33994	cART treated	Male	7.00	2 wks post-SIV	Negative	Negative	Positive
34741	cART treated	Female	5.30	2 wks post-SIV	Negative	Negative	Negative
35974	cART treated	Female	4.40	2 wks post-SIV	Negative	Negative	Positive
34561	cART+3HP treated	Female	7.90	2 wks post-SIV	Negative	Negative	Negative
41898	cART+3HP treated	Male	4.93	2 wks post-SIV	Positive	Negative	Negative
41901	cART+3HP treated	Male	4.88	2 wks post-SIV	Negative	Negative	Negative
41882	cART+3HP treated	Male	4.132	2 wks post-SIV	Negative	Negative	Negative
41876	cART+3HP treated	Male	5.102	2 wks post-SIV	Negative	Negative	Negative
41879	cART+3HP treated	Male	5.61	2 wks post-SIV	Negative	Negative	Negative
KR44	cART naïve	Male	5.50	No cART	Positive	Negative	Negative
LC88	cART naïve	Male	4.10	No cART	Negative	Negative	Negative
JH07	cART naïve	Male	7.32	No cART	Negative	Negative	Negative
JF23	cART naïve	Male	7.26	No cART	Positive	Negative	Negative
KG40	cART naïve	Male	5.51	No cART	Negative	Negative	Negative
IP88	cART naïve	Male	7.05	No cART	Positive	Negative	Negative
JI68	cART naïve	Male	6.06	No cART	Negative	Negative	Negative
JE48	cART naïve	Male	6.33	No cART	Negative	Negative	Negative
GP50	LTBI	Male	11.18	No cART	Positive	Negative	Negative
JF47	LTBI	Male	7.32	No cART	Negative	Negative	Negative
HV02	LTBI	Male	9.34	No cART	Negative	Negative	Negative
JD72	LTBI	Male	7.38	No cART	Negative	Negative	Negative

Lung	Tissue Area (μm^2)
LTBI	233556800
	239154448
	273528896
cART naïve	228770464
	166268784
	153978912
cART	170309040
	107882232
	90442016
cART+3HP	153487856
	139960048
	205737744