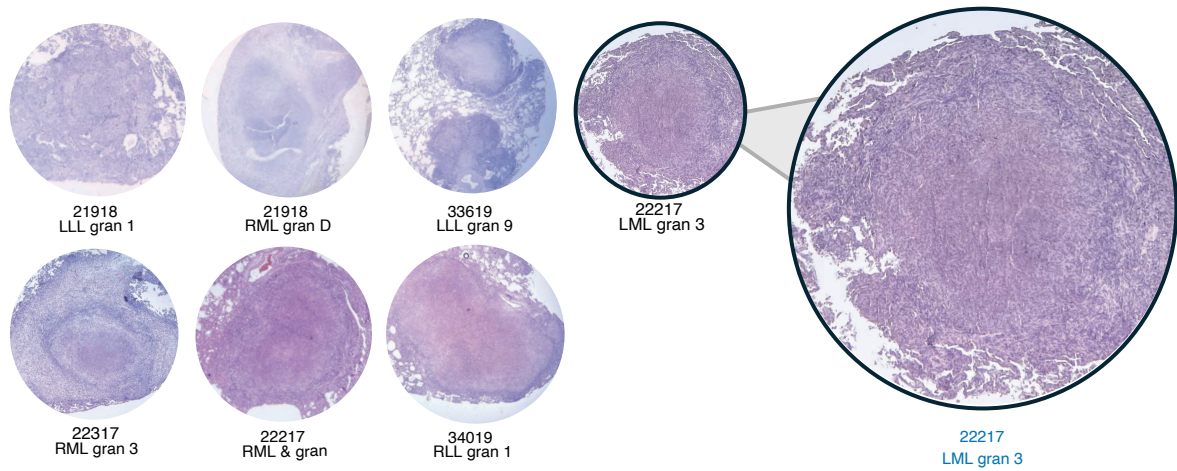
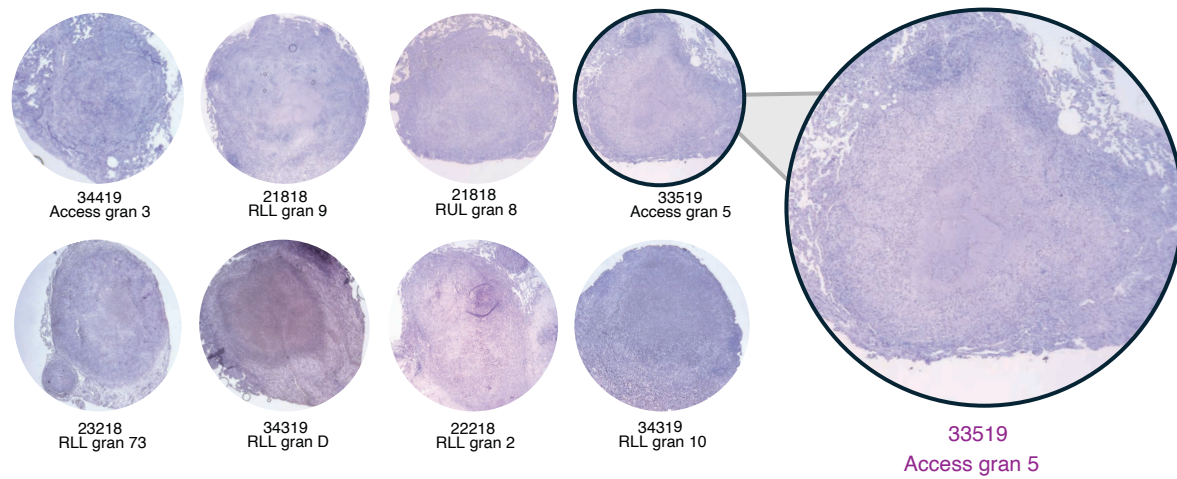


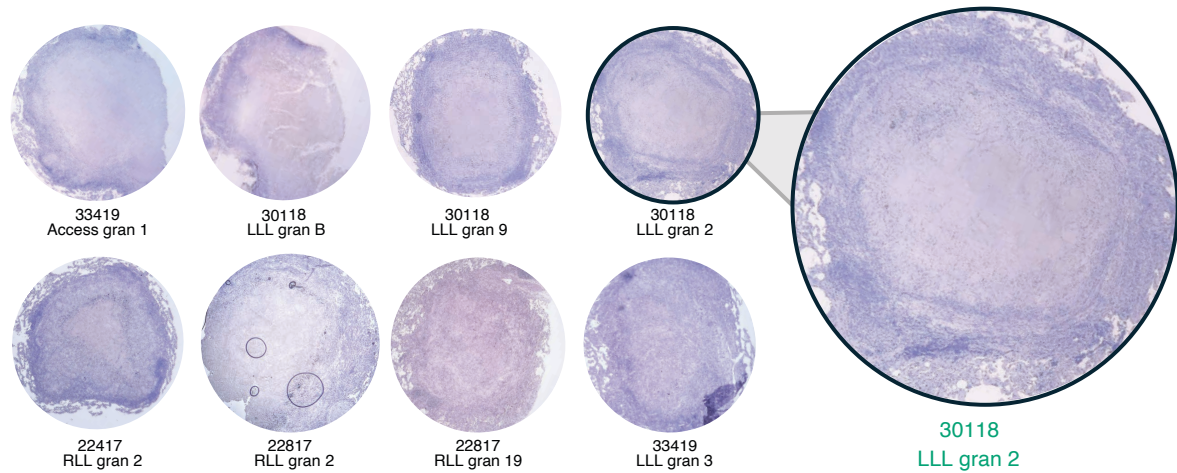
A. SIV/Mtb



B. SIV/ART/Mtb



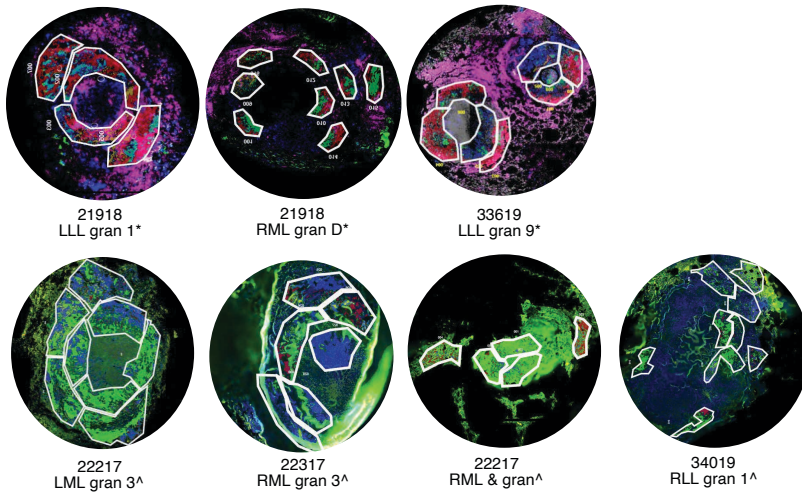
C. Mtb



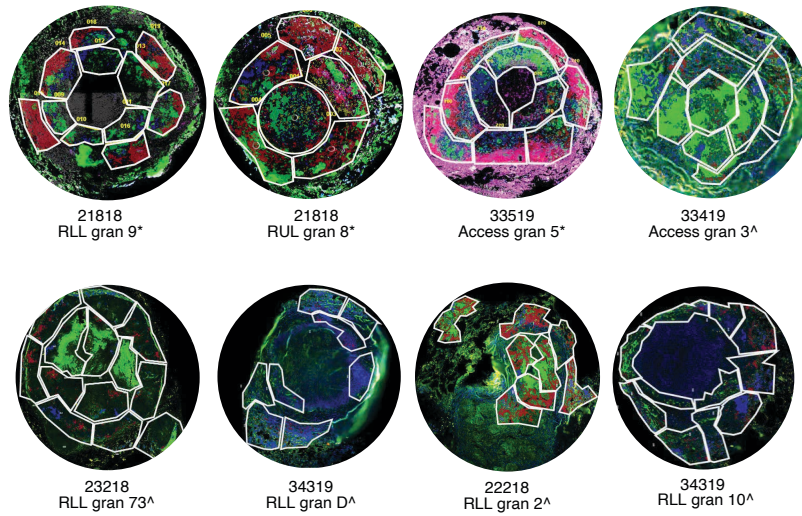
Supplemental Figure 1 Granuloma histology does not differ between animal groups.

Hematoxylin and eosin stained tissue lung granulomas (10X magnification) from SIV/Mtb (A), SIV/ART/Mtb (B), and Mtb (C) granulomas used in this study.

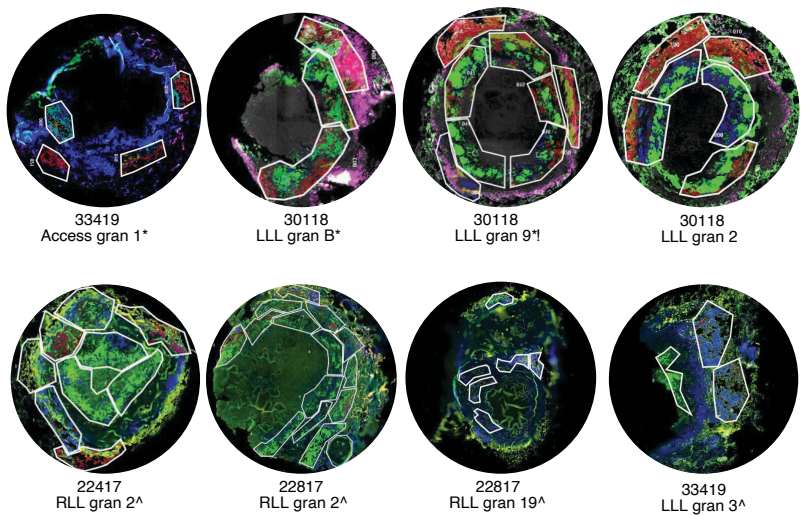
SIV/Mtb



SIV/ART/Mtb



Mtb

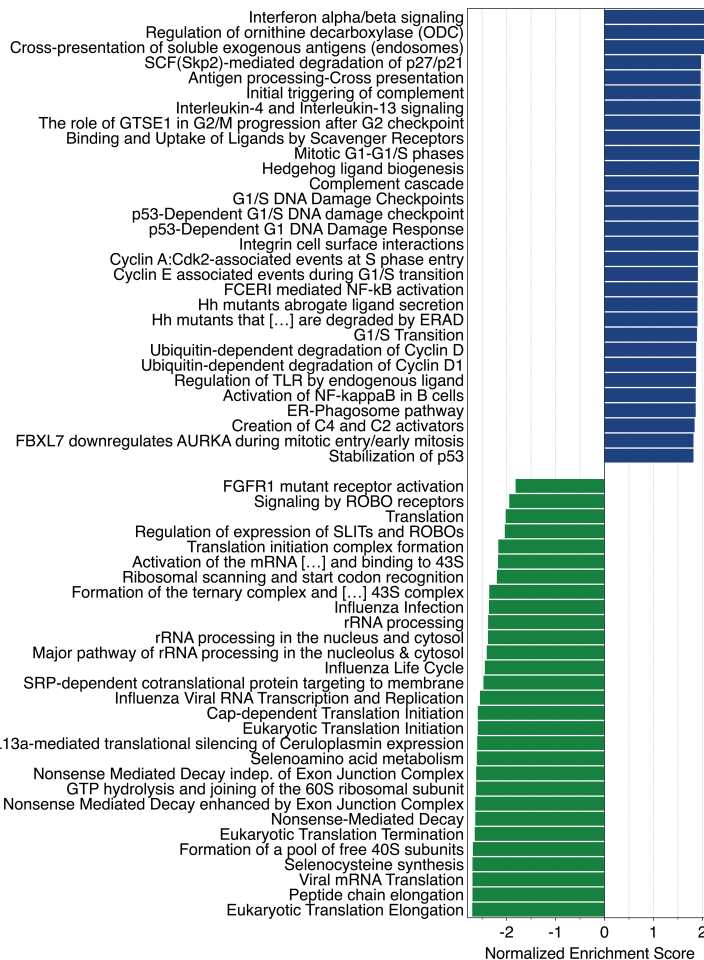


Supplemental Figure 2 GeoMX regions of interest (ROIs) from granulomas.

ROIs (inner and outer rings) from which spatial transcriptomics were assessed in SIV/Mtb (top), SIV/ART/Mtb (middle), and Mtb (bottom) granulomas. For each experimental group, the granulomas indicated with * are from batch 1, where colors indicate CD68+ [green], CD3+CD8a+ [red], CD3+CD8a- [yellow], CD3-CD8a- [blue] within ROIs, and CD68+ [blue], CD3+ [magenta], CD8a+ [green] outside ROIs. Granulomas indicated with ^ are from batch 2, where colors indicate CD68+ [green], CD3+CD8a+ [blue], CD3+CD8a- [red], and CD3-CD8a- [cyan] within ROIs, and CD68+ [green], CD3+ [yellow], and CD8a+ [magenta] outside ROIs. For details on batch correction, see Methods. All granulomas in each experimental group are shown.

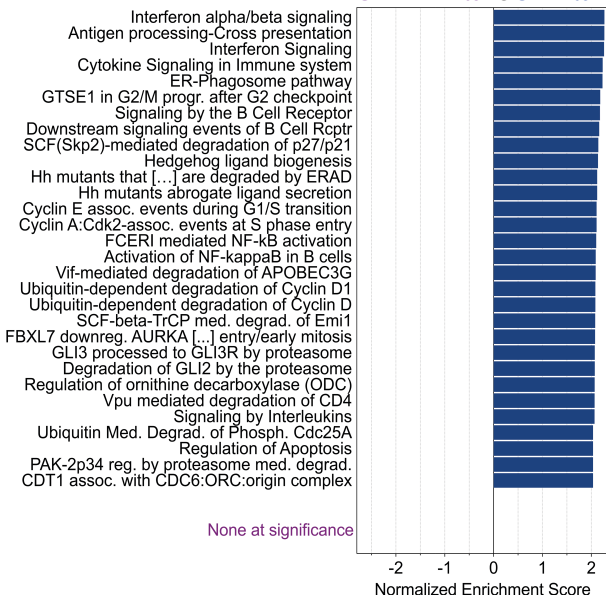
A.

Mtb vs SIV/Mtb



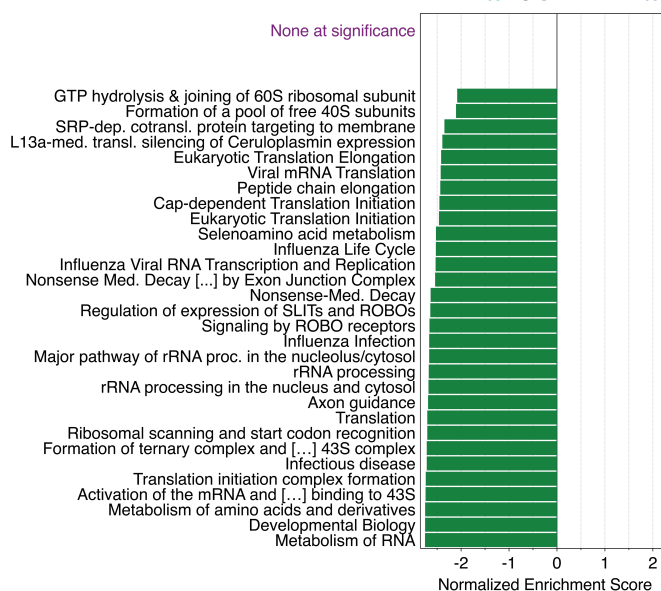
B.

SIV/ART/Mtb vs SIV/Mtb



C.

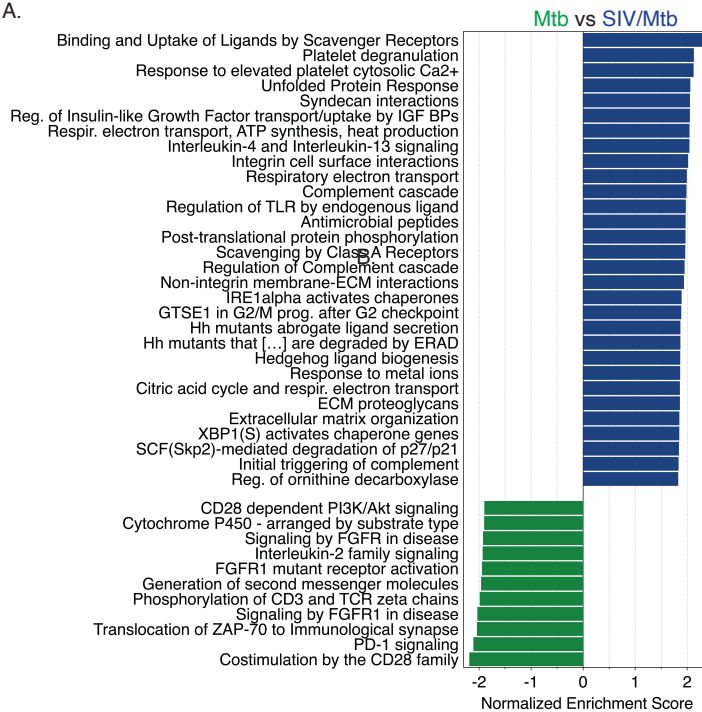
Mtb vs SIV/ART/Mtb



Supplemental Figure 3 Extended gene set enrichment analysis of myeloid cell function from total granulomas.

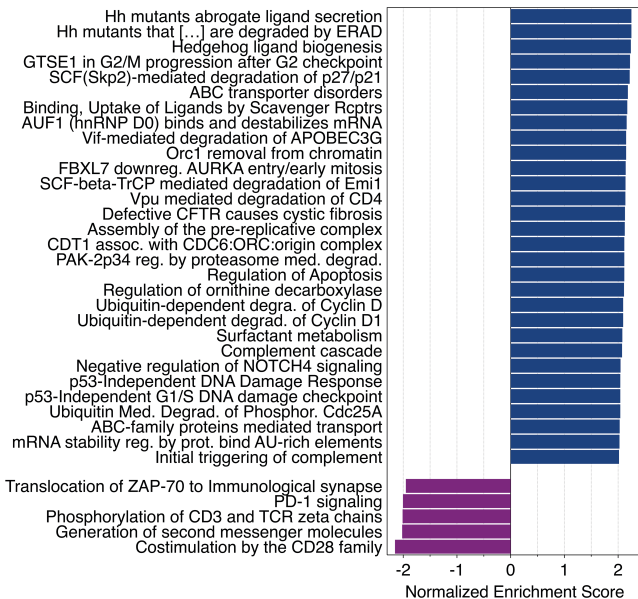
Full GSEA of all myeloid cells between (A) Mtb vs SIV/Mtb groups (B) SIV/ART/Mtb vs SIV/Mtb groups, and (C) Mtb vs SIV/ART/Mtb groups. Pathways from WebGestalt using Reactome database statistical significance set at a false discover rate < 0.05 .

A.



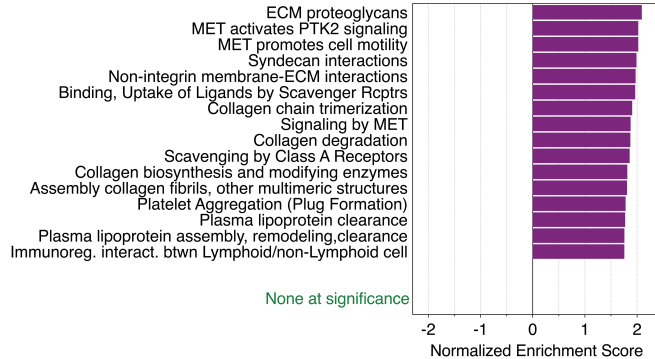
B.

SIV/ART/Mtb vs SIV/Mtb



C.

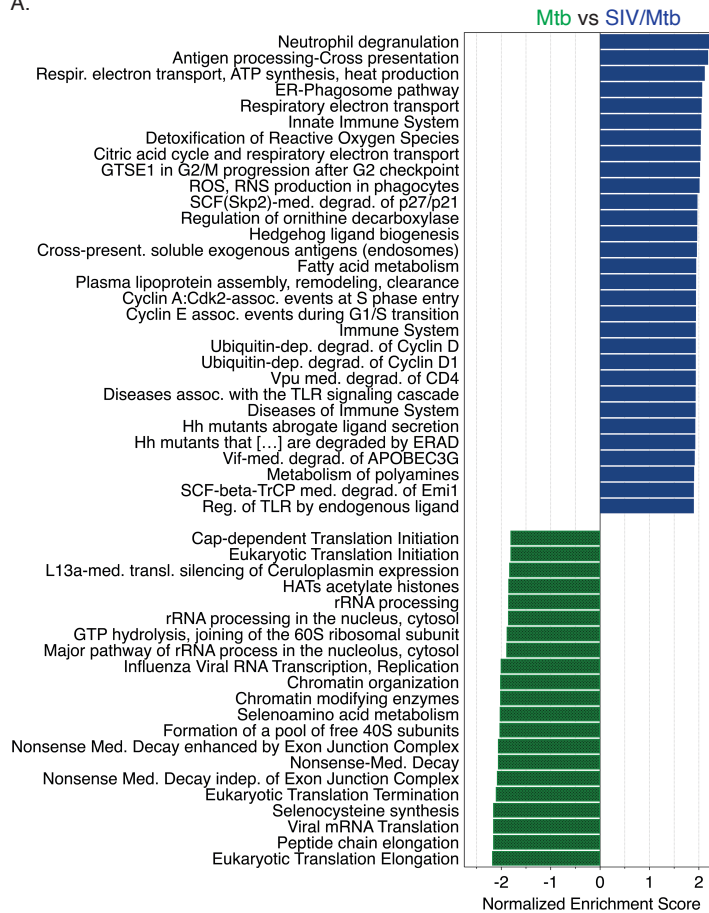
Mtb vs SIV/ART/Mtb



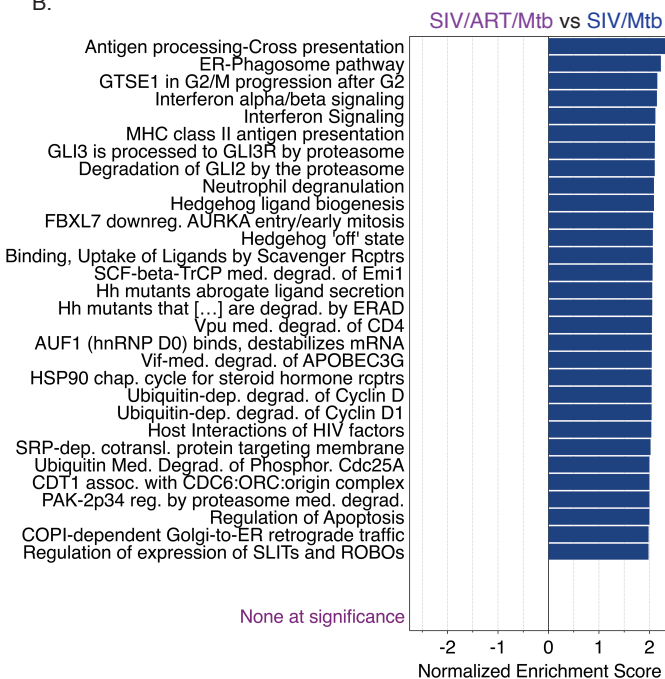
Supplemental Figure 4 Extended gene set enrichment analysis of T cell function from total granulomas.

Full GSEA of all T cells between (A) Mtb vs SIV/Mtb groups (B) SIV/ART/Mtb vs SIV/Mtb groups, and (C) Mtb vs SIV/ART/Mtb groups. Pathways from WebGestalt using Reactome database with statistical significance set at a false discover rate < 0.05 .

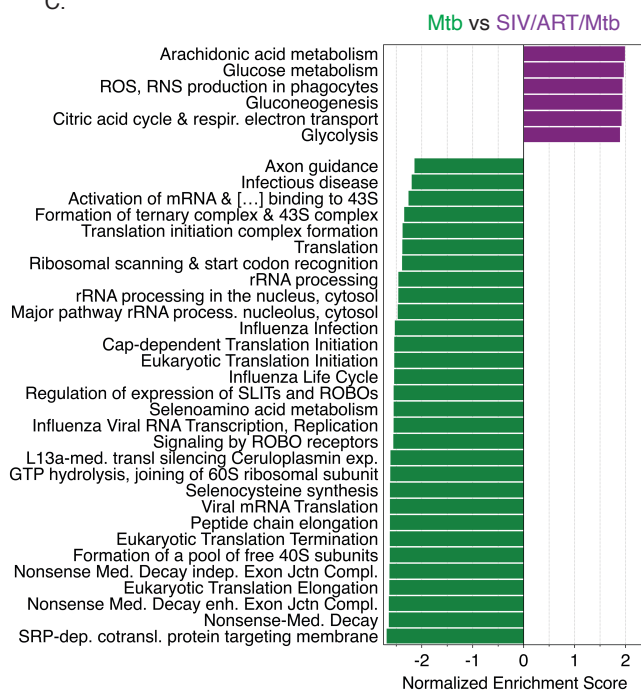
A.



B.



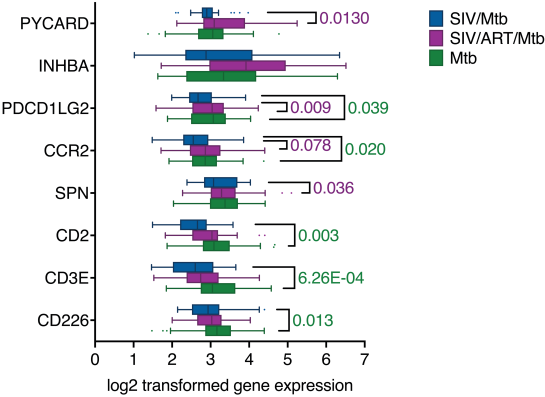
C.



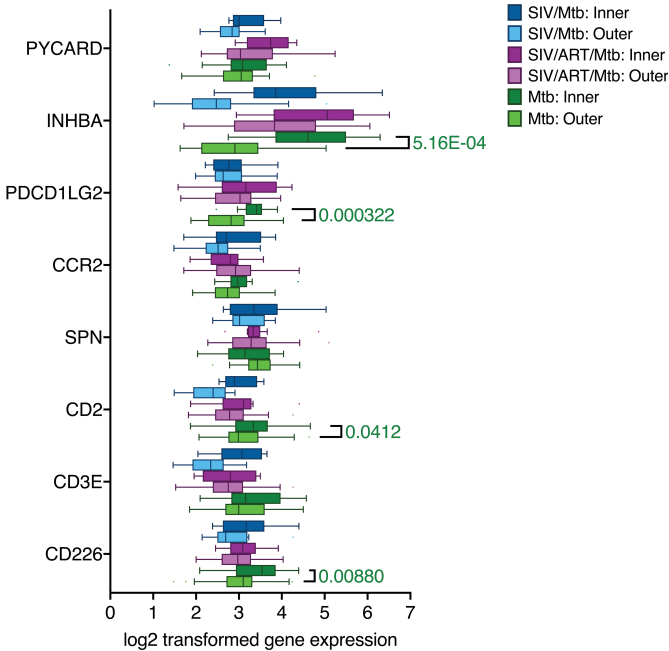
Supplemental Figure 5 Differential gene expression in myeloid cells in the outer rim of granulomas across groups.

Full GSEA of myeloid cells in the outer ring between (A) Mtb vs SIV/Mtb groups (B) SIV/ART/Mtb vs SIV/Mtb groups, and (C) Mtb vs SIV/ART/Mtb groups. Pathways from WebGestalt using Reactome database with statistical significance set at a false discover rate < 0.05.

A. Interferon- γ Production
in granulomas overall



B. Interferon- γ Production
in granulomas by region

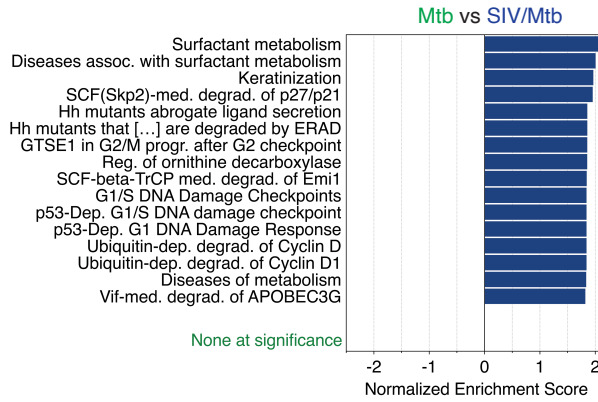


Supplemental Figure 6 Interferon- γ production in T cells from granulomas.

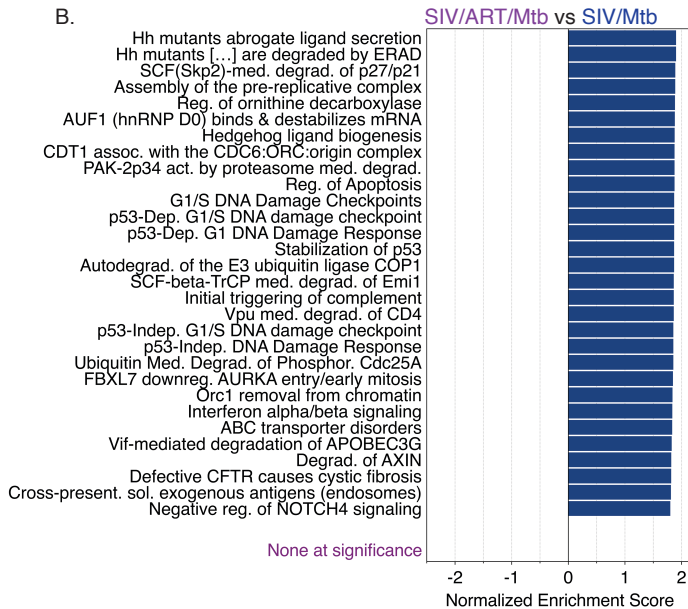
(A) Log2-transformed expression of genes in involved interferon- γ production in all T cells in granulomas. Wald test, design $\sim$ batch + treatmentgroup. Only adjusted p-values < 0.05 are indicated. Genes from the GSEA leading edge with statistical significance set at a false discover rate < 0.05 , only adjusted p-values < 0.05 listed.

(B) Log2-transformed expression of genes in interferon- γ production in inner versus outer region T cells in granulomas. Wald test, design $\sim$ batch + treatmentgroup. Only adjusted p-values < 0.05 are indicated. Genes from the GSEA leading edge with statistical significance set at a false discover rate < 0.05 , only adjusted p-values < 0.05 listed.

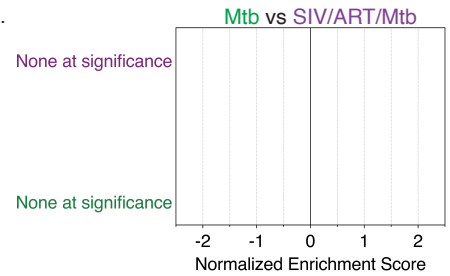
A.



B.

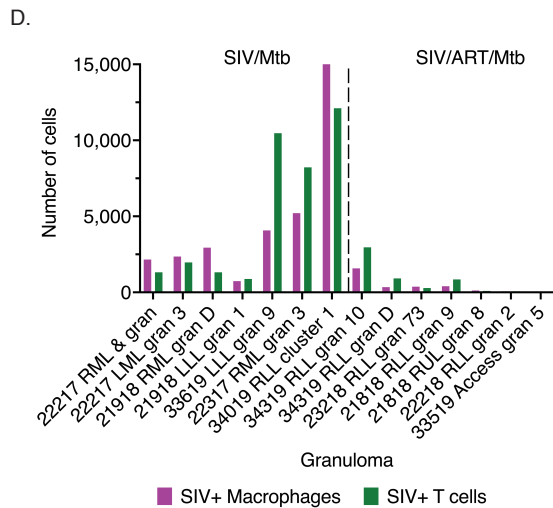
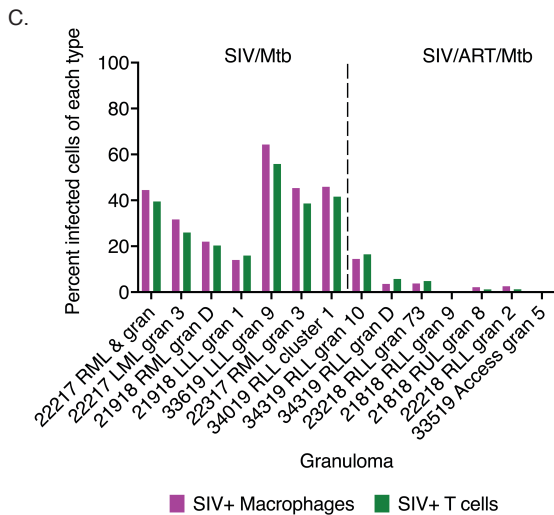
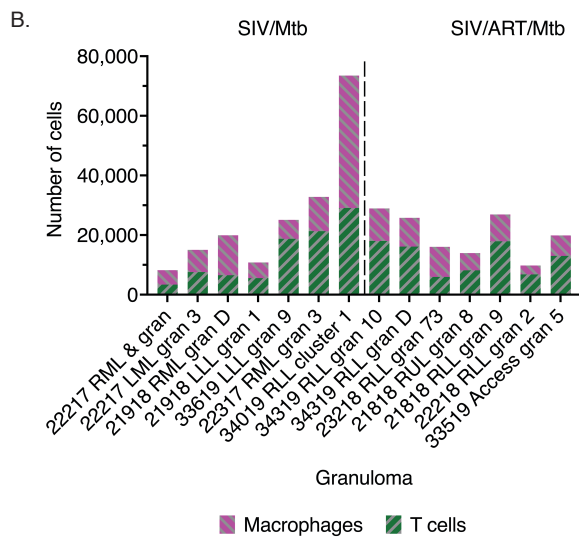
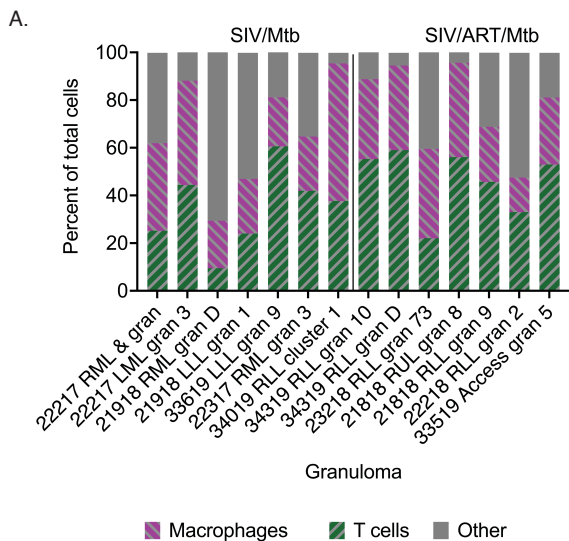


C.



Supplemental Figure 7 Differential gene expression in T cells in the inner rim of granulomas across groups.

Full GSEA of T cells in the inner ring between (A) Mtb vs SIV/Mtb groups (B) SIV/ART/Mtb vs SIV/Mtb groups, and (C) Mtb vs SIV/ART/Mtb groups. Pathways from WebGestalt using Reactome database with statistical significance set at a false discover rate < 0.05 .



Supplemental Figure 8 Expanded quantification of SIV-infected cells in granulomas.

(A) Percent of CD68+CD11c+ myeloid cells (magenta) and CD3+ T cells (green) of all total DAPI+ cells in granulomas. Grey bars are CD3-CD68-CD11c-DAPI+ cells.

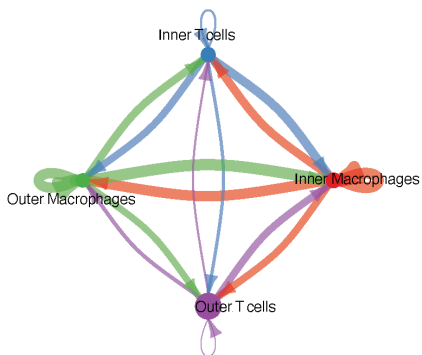
(B) Total number of CD68+CD11c+ myeloid cells (magenta) and CD3+T cells (green) in granulomas.

(C) Percent of myeloid cells (magenta) and percent of T cells (green) that had detectable SIV RNA in granulomas.

(D) Total number of SIV+ myeloid cells (magenta) and SIV+ T cells (green) in granulomas.

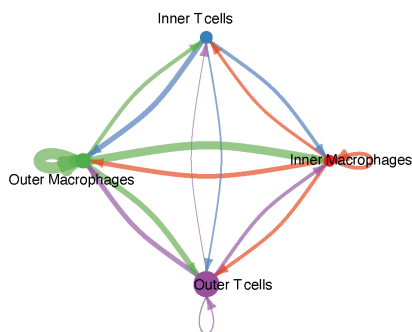
A.

Mtb



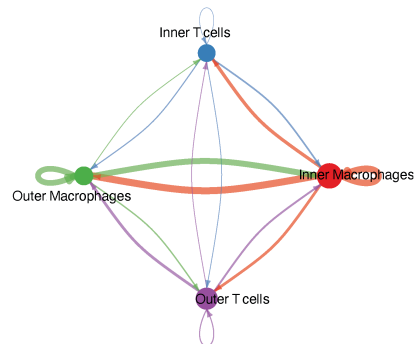
B.

SIV/ART/Mtb



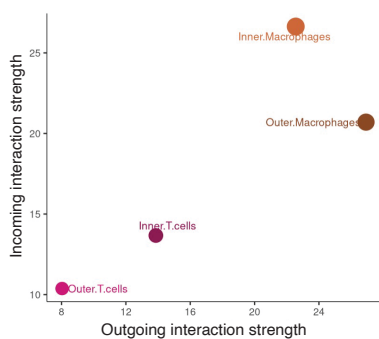
C.

SIV/Mtb

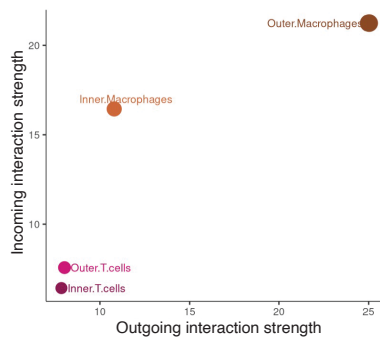


D.

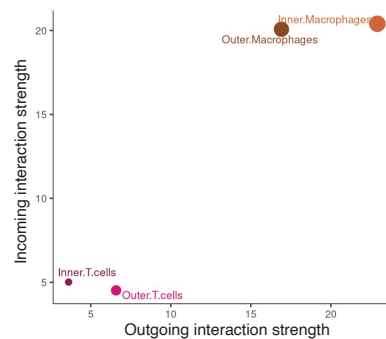
Mtb



SIV/ART/Mtb



SIV/Mtb



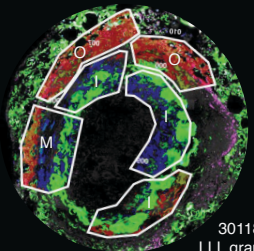
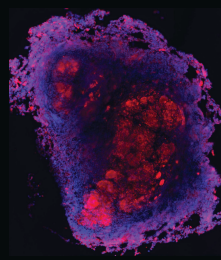
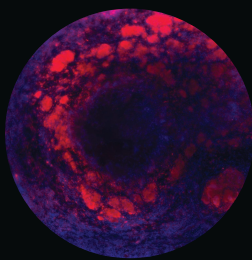
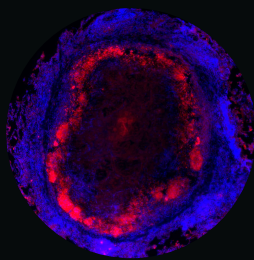
Supplemental Figure 9 Extended analysis of cell-cell communication in granulomas.

(A-C) Aggregated cell-cell communication network between inner and outer myeloid cells and T cells in (A) Mtb granulomas, (B) SIV/ART/Mtb granulomas, and (C) SIV/Mtb groups. Arrows indicate signal senders to signal receivers. Arrow thickness is proportional to the number of ligand-receptor interactions between a given pair. Node size is proportional to the number of ROIs in that cell type. Colors indicate sender group: green is outer macrophages, red is inner macrophages, purple is outer T cells, and blue is inner T cells.

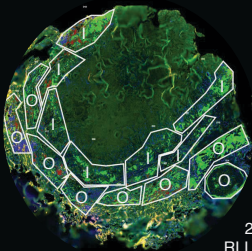
(D) Comparison of aggregated incoming and outgoing signal strength in inner and outer myeloid cells and T cells from Mtb (left), SIV/ART/Mtb (middle) and SIV/Mtb (right) groups. Dot size indicates the number of ligand-receptor pairs associated with each group.

Overexpression of a signaling genes was determined using Wilcoxon rank sum test with significance set at an adjusted p-value < 0.05 . Communication probability was determined as described in reference (1).

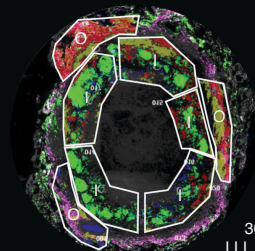
Mtb



30118
LLL gran 2*

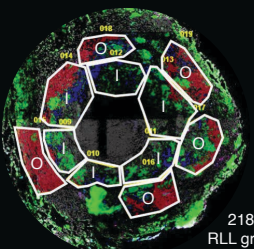
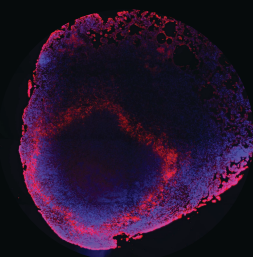
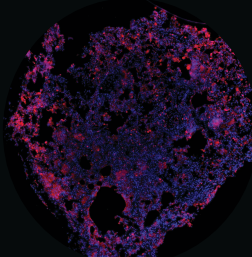
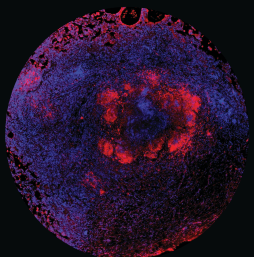


22817
RLL gran 2^

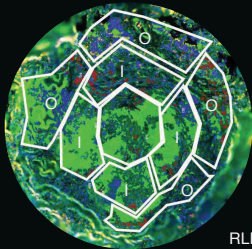


30118
LLL gran 9*

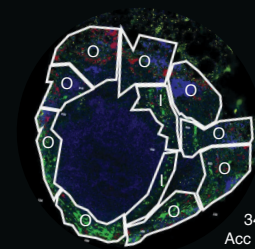
SIV/ART/Mtb



21818
RLL gran 9*

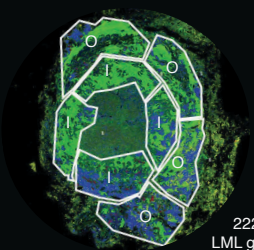
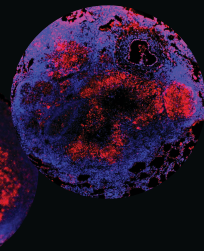
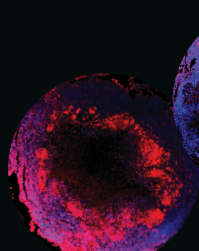
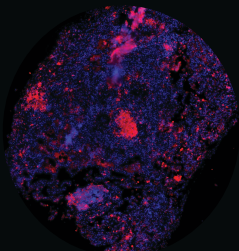
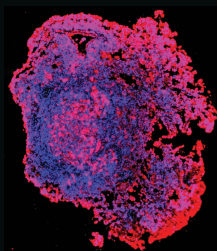


34319
RLL gran 10^

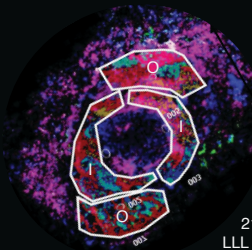


34419
Acc gran 3^

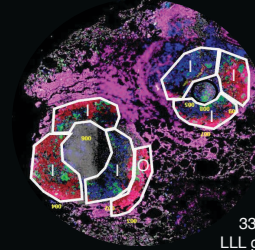
SIV/Mtb



22217
LML gran 3^



21918
LLL gran 1*



33619
LLL gran 9*

Supplemental Figure 10 Immunofluorescence (IF) validation of spatially disrupted IDO1 expression in co-infected granulomas.

For each experimental group, top rows show IF staining of IDO1 (red) and DAPI (blue). Bottom rows show GeoMX IF images of the same granuloma with the ROIs outlined in white. ROIs are (annotated as “I” (inner), “O” (outer), or “M” (mixed); only “I” and “O” ROIs were compared for spatial analysis. Granulomas indicated with * are from batch 1 (CD68+ [green], CD3+CD8a+ [red], CD3+CD8a- [yellow], CD3-CD8a- [blue] within ROIs, CD68+ [blue], CD3+ [magenta], CD8a+ [green] outside ROIs) and granulomas indicated with ^ are from batch 2 (CD68+ [green], CD3+CD8a+ [blue], CD3+CD8a- [red], and CD3-CD8a- [cyan] within ROIs, CD68+ [green], CD3+ [yellow], and CD8a+ [magenta] outside ROIs). See methods for details on batch correction. 3 granulomas per group, each granuloma is identified with a six-digit NHP ID and granuloma name indicating lung lobe of origin and ID number.