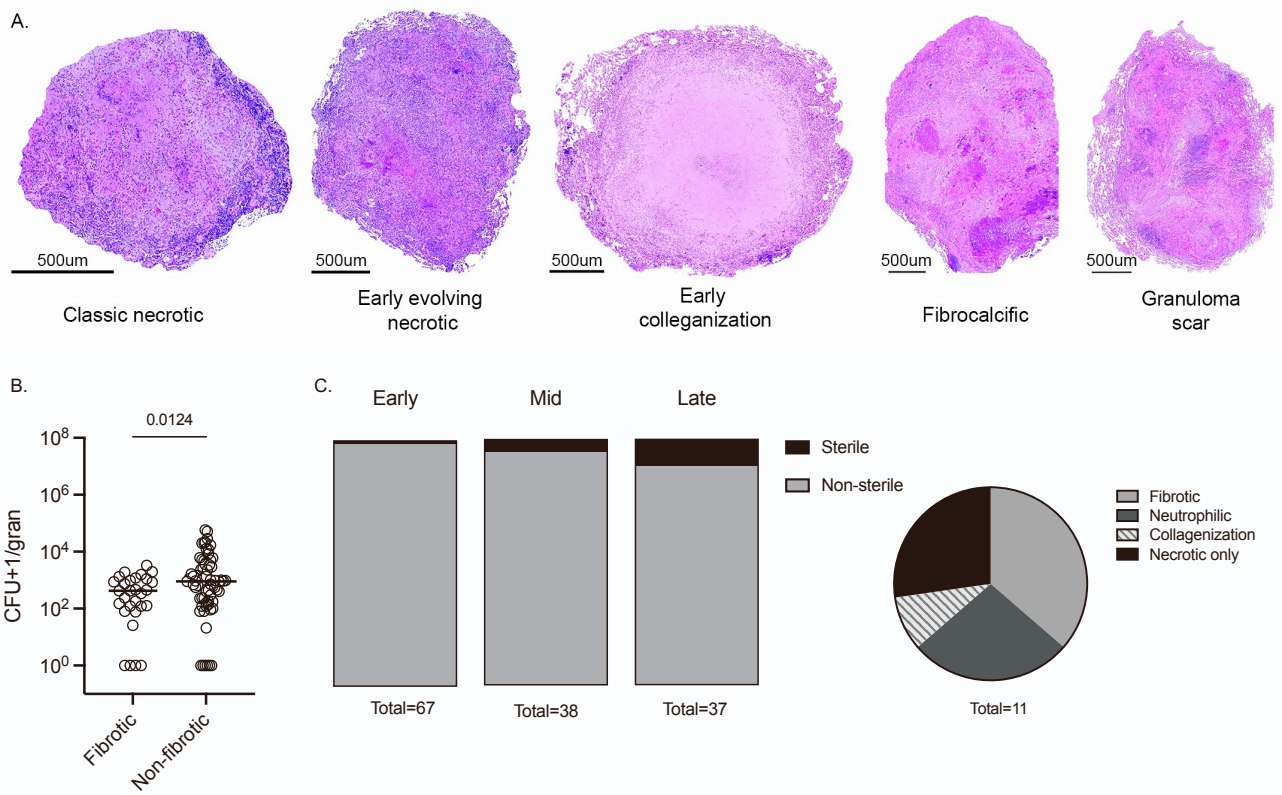


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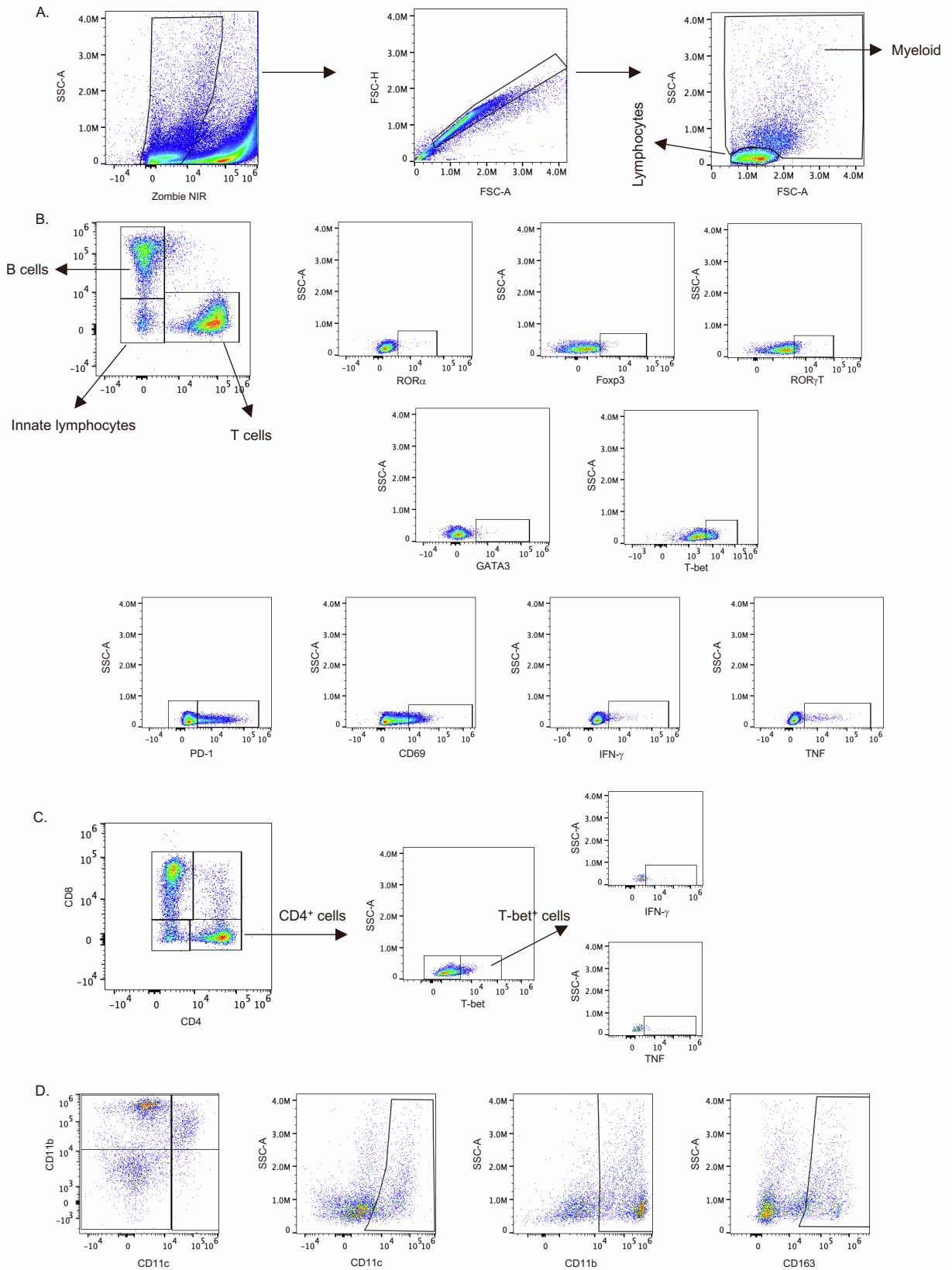
Supplemental information

**T cell transcription factor expression evolves
over time in granulomas from *Mycobacterium
tuberculosis*-infected cynomolgus macaques**

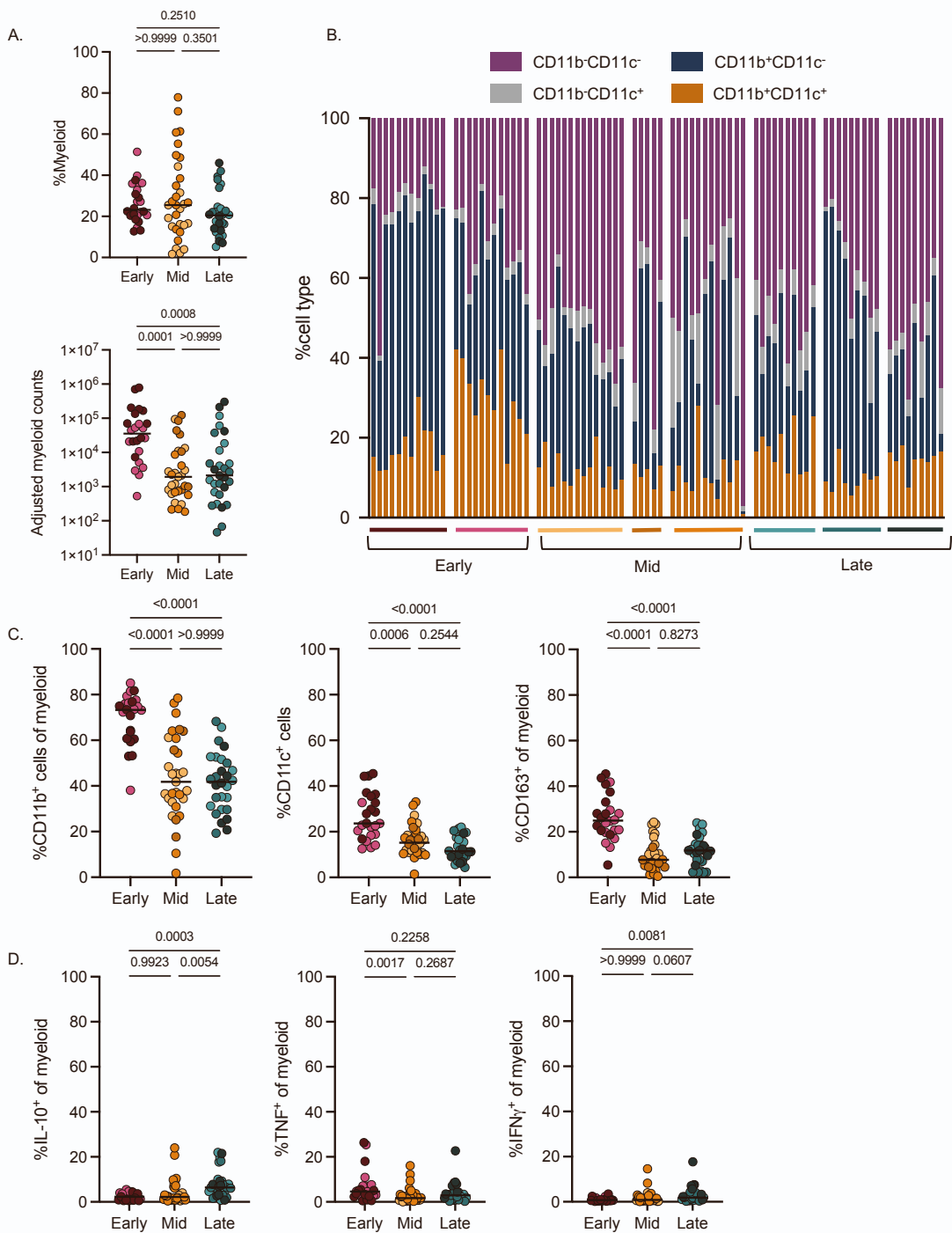
Nicole L. Grant, Pauline Maiello, Edwin Klein, Philana Ling Lin, H. Jacob Borish, Jaime Tomko, L. James Frye, Alexander G. White, Denise E. Kirschner, Joshua T. Mattila, and JoAnne L. Flynn



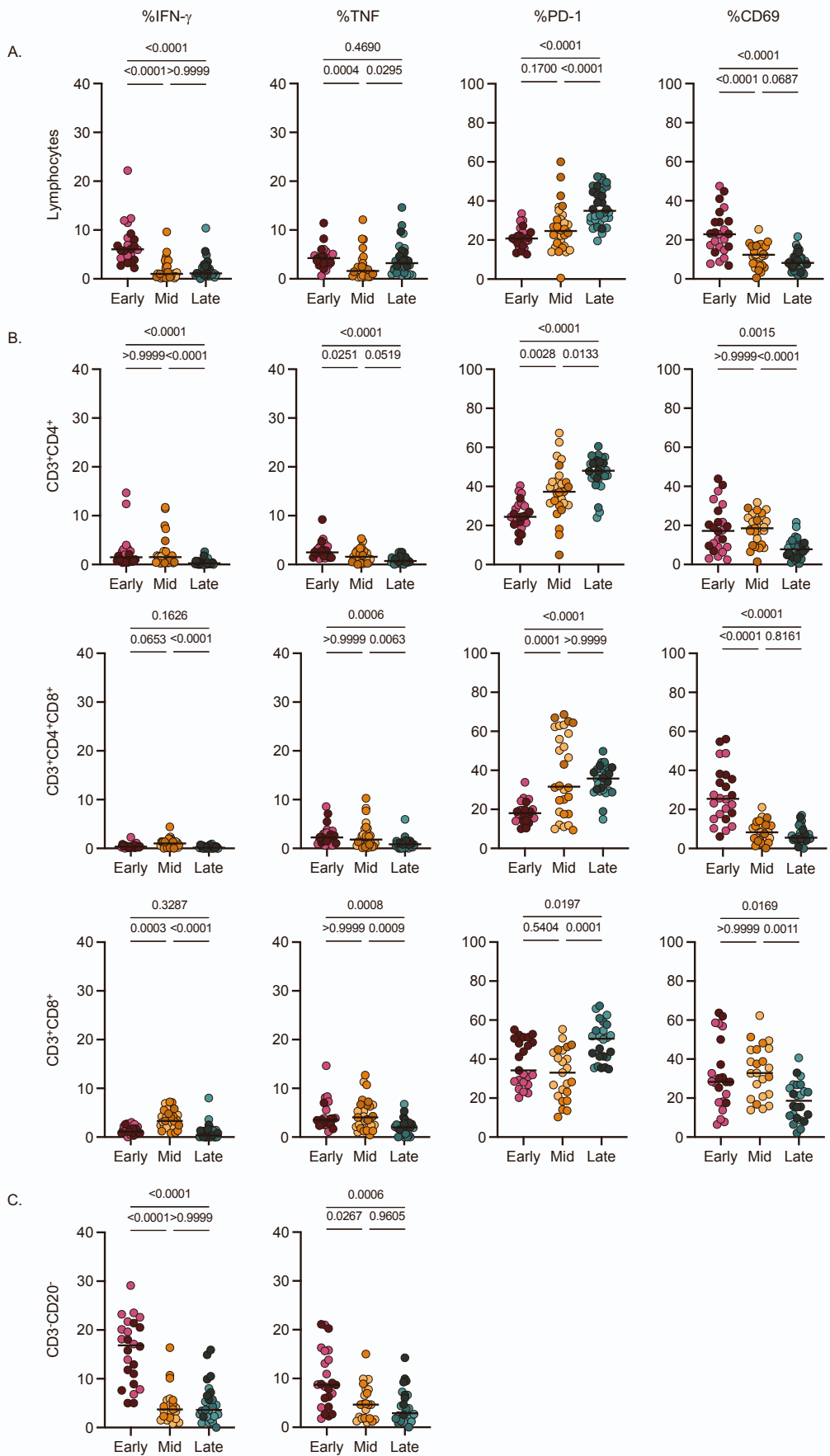
Supplementary Figure 1: Pathology and CFU in original granulomas, Related to Figure 1. (A) A range of granuloma pathologies is seen in original lesions across time points post-infection. (B) Individual granuloma CFU from fibrotic and non-fibrotic original granulomas isolated at mid and late time points. Statistics were performed using a Mann-Whitney test with p values noted on graph. (C) The proportion of sterile granulomas at each time point with total granuloma numbers assessed listed below (left). The proportion of fibrotic, neutrophilic, collagenic, or necrotic of the sterile granulomas from all time points (11 total)(right).



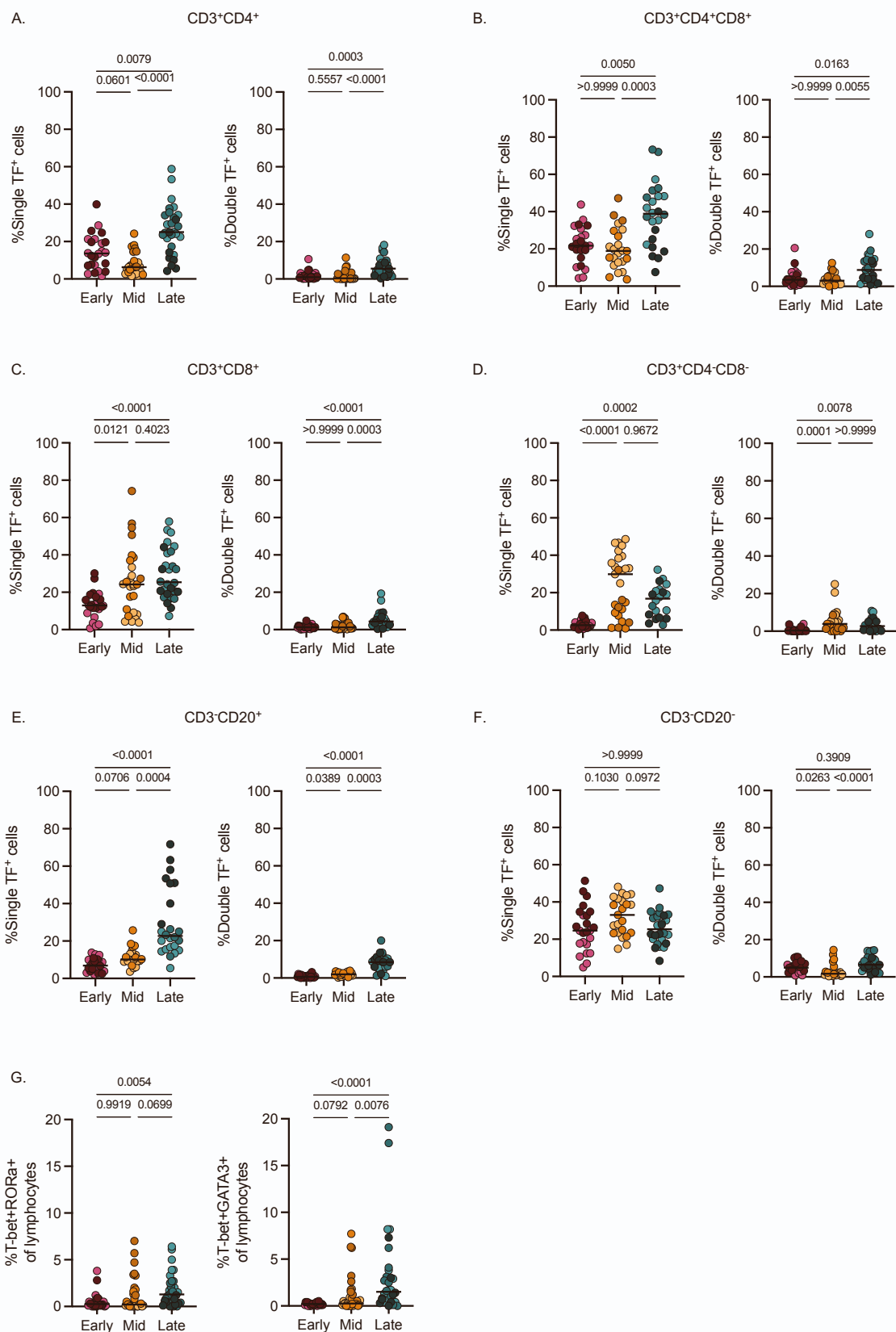
Supplementary figure 2: Flow cytometry gating strategy for lung granulomas, Related to STAR Methods. (A) Identification of live events, singlets, and myeloid and lymphoid populations. (B) From the lymphocyte parent gate, identification of B cells, innate lymphocytes (ILCs) and T cells using CD3⁺ and CD20⁺. Examples of transcription factor, activation marker, and pro-inflammatory cytokine gating. (C) From the CD3⁺ parent gate, identification of CD4⁺, CD4⁺CD8⁺, CD8⁺, and CD4⁺CD8⁻ lymphocytes. Example of gating on transcription factor⁺ cells versus transcription factor⁻ cells and then gating on pro-inflammatory cytokines. (D) From the myeloid gate, an example of CD11b versus CD11c to determine single, negative, and co-expressing populations. Examples of single identification of CD11c, CD11b, and CD163 from the myeloid parent gate.



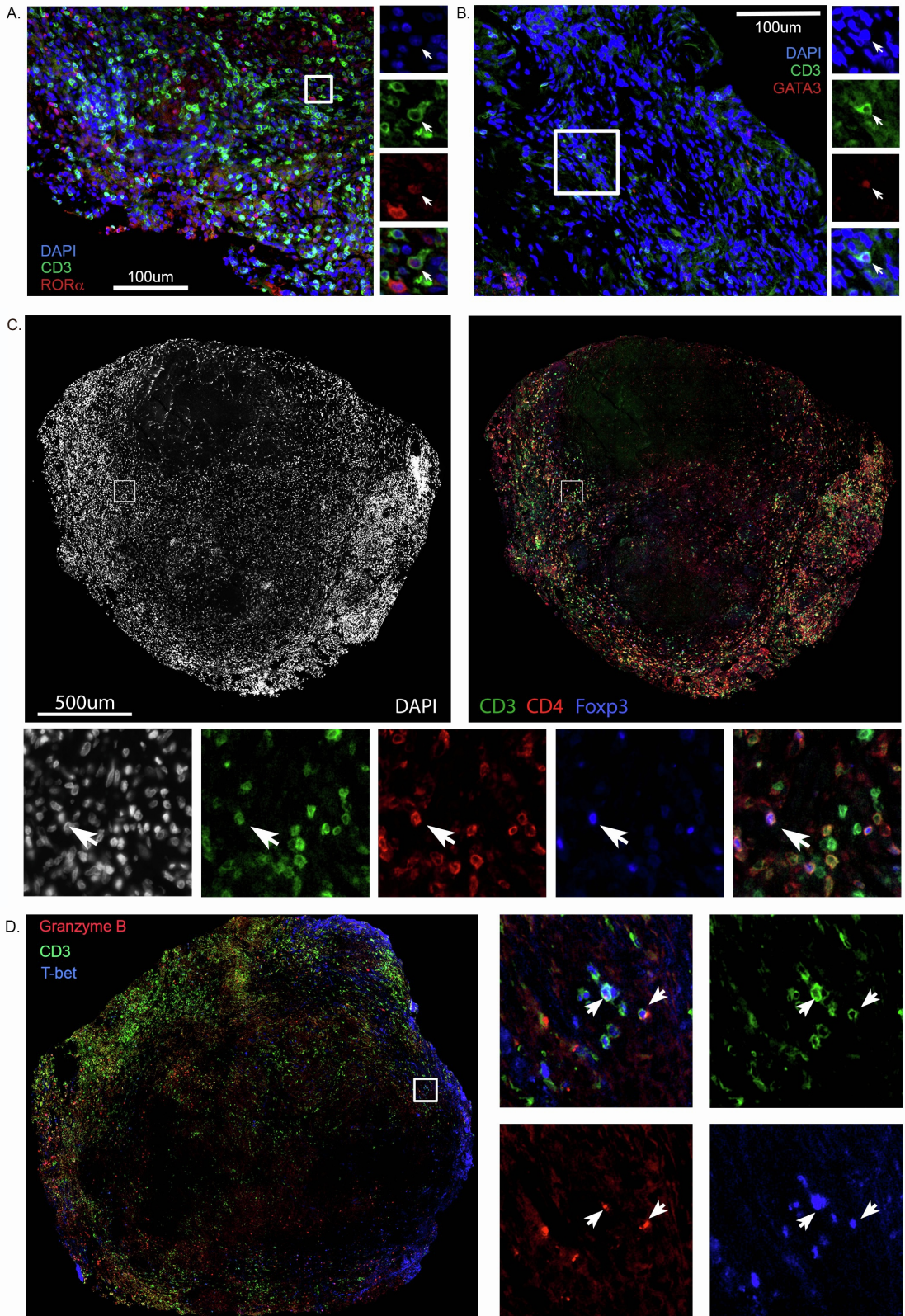
Supplementary figure 3: Mid and late granulomas have lower proportions of CD11c⁺ and CD11b⁺ myeloid cells but higher levels of IL-10, Related to Figure 2. (A) Frequency and numbers of myeloid cells in granulomas from each time point. (B) Relative frequency of the combinations of CD11c and CD11b expression in myeloid cells from each time point where each bar represents a granuloma and animals are represented by horizontal bars (Fig. 1B). (C) Frequencies of CD11b, CD11c, and CD163 expression in myeloid cells across time points. (D) Cytokine expression in myeloid cells across time points. Samples were prepared by direct ex vivo staining in the absence of in vitro stimulation. For A, C and D, individual points represent granulomas and animals are denoted by color. For A, C and D statistics, Kruskal Wallis tests were performed with Dunn's multiple comparisons-adjusted p values reported on graphs.



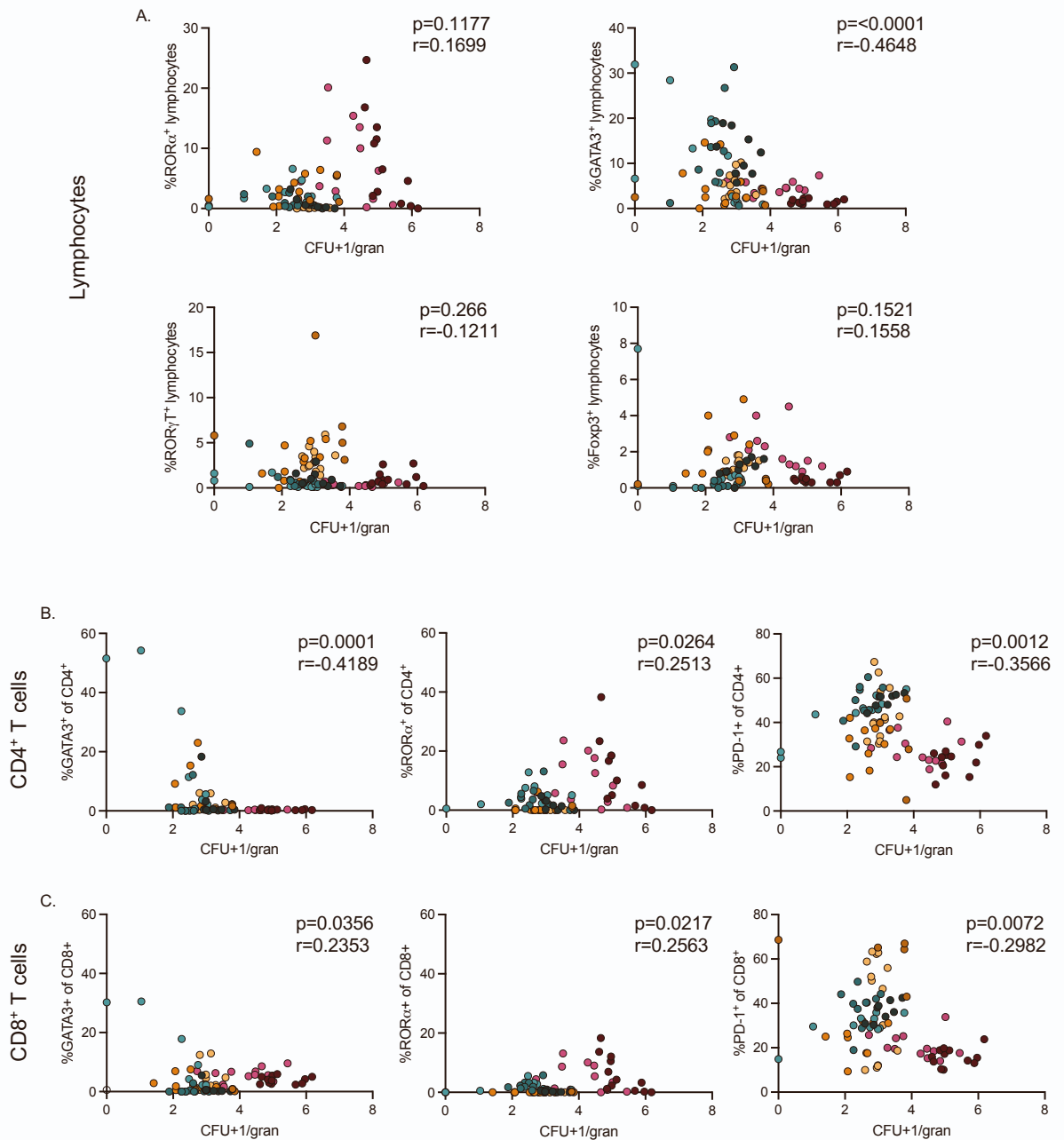
Supplementary figure 4: Lymphocytes produce low levels of pro-inflammatory cytokines at all time points despite changes in activation marker expression, Related to Figure 2. Pro-inflammatory cytokine and activation marker expression in (A) lymphocytes, (B) T cells: CD3⁺CD4⁺, CD3⁺CD4⁺CD8⁺, CD3⁺CD8⁺, and (C) CD3⁻CD20⁻ cells. Samples were prepared by direct ex vivo staining in the absence of in vitro stimulation. Kruskal Wallis tests were performed with Dunn's multiple comparisons-adjusted p values reported on graphs.



Supplementary figure 5: Transcription factor⁺ lymphocyte subpopulations in original granulomas over time, Related to Figure 3. Single or double transcription factor expression in (A) CD4⁺, (B) CD3⁺CD4⁺CD8⁺, (C) CD3⁺CD8⁺, (D) CD3⁺CD4⁻CD8⁻, (E) CD20⁺, (F) CD3⁺CD20⁻ cells. (G) Frequency of dual expression of T-bet and RORα (left) or T-bet and GATA3 (right) in all lymphocytes. Kruskal Wallis tests were performed with Dunn's multiple comparisons-adjusted p values reported on graphs.



Supplementary figure 6: Immunofluorescence staining for ROR α , GATA3, Foxp3, and Granzyme B in granulomas, Related to Figure 5. (A) Immunofluorescence for ROR α (red, arrows in inset) in an early time point granuloma can be observed (although rare) within CD3⁺ (green) cells. (B) Immunofluorescence for GATA3 (red, arrows in inset) in a late time point granuloma can be observed (although rare) within CD3⁺ (green) cells. (C) Foxp3 (blue) within CD3⁺ (green) CD4⁺ (red) cells in a mid time point granuloma. (D) Granzyme B immunofluorescence staining in a late time point granuloma is observed within CD3⁺ (green) T-bet⁺ (blue) cells as indicated by arrows.



Supplementary figure 7: Correlation analysis between CFU and frequency of lymphocyte populations, Related to Figure 7. (A) Frequency of TF expression among all lymphocytes vs log₁₀ CFU per granuloma. (B) Frequency of TF expression or PD-1 among CD4⁺ T cells vs log₁₀ CFU per granuloma. (C) Frequency of TF expression or PD-1 among CD8⁺ T cells vs log₁₀ CFU per granuloma. Each individual point represents a granuloma, colored according to animal as previously established.

Supplementary table 1: Animal and *Mtb* infection data, Related to STAR Methods.

Animal	Necropsy group	Necropsy time point	CFU @ infection	Date of infection	Date of Necropsy	# Lung granulomas	# Original granulomas	# Original granulomas for flow	Total CFU	Lung CFU	LN CFU	Necropsy score	Total Pet Hot
32719	Early	4 weeks	19	12/17/19	1/13/20	12	12	12	5.61E+06	4.40E+06	1.21E+06	21	22449.25
32819	Early	4 weeks	19	12/17/19	1/13/20	12	12	12	7.34E+05	5.96E+05	1.38E+05	20	8789.65
6219	Mid	12 weeks	10	7/17/19	10/9/19	14	17	14	1.51E+05	1.11E+05	4.05E+04	27	16627.7
6319	Mid	12 weeks	10	7/17/19	10/7/19	14	5	5	3.73E+04	2.44E+04	1.29E+04	25	4467.49
6419	Mid	12 weeks	10	7/17/19	10/9/19	19	12	12	2.98E+04	6.37E+03	2.34E+04	23	1253.85
6519	Late	20 weeks	18	8/22/19	1/6/20	19	14	12	1.23E+04	1.18E+04	5.50E+02	16	620.61
6619	Late	20 weeks	18	8/22/19	1/6/20	12	11	11	1.57E+04	4.15E+03	1.15E+04	23	2663.83
6819	Late	20 weeks	18	8/22/19	1/8/20	14	11	10	2.90E+04	1.87E+04	1.03E+04	24	6167.06

Supplementary table 2: Granulomas used for flow cytometry analysis, Related to STAR Methods.

Animal	Sample	NX Time point (wks)	NX Time	Gentle macs processed	CFU/granuloma
32819	RLL 1 (GM)	4	Early	Yes	18600
32819	RLL 2	4	Early	No	520
32819	RLL 3 (GM)	4	Early	Yes	46200
32819	RLL 7 (GM)	4	Early	Yes	3120
32819	RLL 4	4	Early	No	30000
32819	RL 5 (GM)	4	Early	Yes	5580
32819	RLL 6 (GM)	4	Early	Yes	28800
32819	RLL 8	4	Early	No	103950
32819	RLL 9 (GM)	4	Early	Yes	276000
32819	RLL 10 (GM)	4	Early	Yes	71400
32819	RLL 11	4	Early	No	3360
32819	RLL 13	4	Early	No	1880
32719	LLL gr 12	4	Early	No	930000
32719	LLL gr 2	4	Early	No	40950
32719	LLL gr 3/4	4	Early	No	45500
32719	LLL gr 6 (GM)	4	Early	Yes	762000
32719	LLL gr 7 (GM)	4	Early	Yes	1512000
32719	LLL gr 9	4	Early	No	91200
32719	LLL gr 10 (GM)	4	Early	Yes	96000
32719	LLL gr 13	4	Early	No	76800
32719	LLL gr 14 (GM)	4	Early	Yes	480000
32719	LLL gr 15 (GM)	4	Early	Yes	70200
32719	LLL gr 16 (GM)	4	Early	Yes	135600
32719	LLL gr 17 (GM)	4	Early	Yes	93000
6219	RLL cluster 6	12	Mid	No	1900
6219	RLL cluster 13	12	Mid	No	3750
6219	RLL gran 1 (GM)	12	Mid	Yes	635
6219	RLL gran 3 (GM)	12	Mid	Yes	1360
6219	RLL gran 5 (GM)	12	Mid	Yes	875
6219	RLL gran 9 (GM)	12	Mid	Yes	450
6219	RLL gran 10 (GM)	12	Mid	Yes	605
6219	RLL gran 11 (GM)	12	Mid	Yes	990
6219	RLL gran 12 (GM)	12	Mid	Yes	650
6219	RLL gran 14	12	Mid	No	975
6219	RLL gran 16	12	Mid	No	900
6219	RLL gran 17	12	Mid	No	960
6219	RLL gran 19	12	Mid	No	400
6219	RLL gran 22 (GM)	12	Mid	Yes	1355
6319	RLL cluster 5/6	12	Mid	No	975
6319	RLL gran 7 (GM)	12	Mid	No	7080
6319	RLL gran 9 (GM)	12	Mid	Yes	6000
6319	RLL gran 18 (GM)	12	Mid	Yes	0
6319	RLL gran 19 (GM)	12	Mid	Yes	6150
6419	LLL gran 1 (GM)	12	Mid	Yes	25
6419	LLL gran 2	12	Mid	No	1325
6419	LLL gran 3	12	Mid	No	700
6419	LLL gran 4 (GM)	12	Mid	Yes	335
6419	LLL gran 5 (GM)	12	Mid	Yes	120
6419	LLL gran 6A	12	Mid	No	1980
6419	LLL gran 7 (GM)	12	Mid	Yes	440
6419	LLL gran 8	12	Mid	No	50
6419	LLL gran 9 (GM)	12	Mid	Yes	115
6419	LLL gran 11 (GM)	12	Mid	Yes	470
6419	LLL gran 12	12	Mid	No	120
6419	LLL gran E	12	Mid	No	80
6519	RLL cluster 12	20	Late	No	560
6519	RLL gran H	20	Late	No	50
6519	RLL gran 1	20	Late	No	10
6519	RLL gran 3 (GM)	20	Late	Yes	870
6519	RLL gran 4 (GM)	20	Late	Yes	6030
6519	RLL gran 5 (GM)	20	Late	Yes	1105
6519	RLL gran 9 (GM)	20	Late	Yes	750
6519	RLL gran 13 (GM)	20	Late	Yes	975
6519	RLL gran 14	20	Late	No	0
6519	RLL gran 16 (GM)	20	Late	Yes	180
6519	RLL gran 17 (GM)	20	Late	Yes	300
6519	RLL gran 20	20	Late	No	0
6619	LLL cluster 2	20	Late	No	450
6619	LLL gran 1 (GM)	20	Late	Yes	240
6619	LLL gran 3	20	Late	No	850
6619	LLL gran 4 (GM)	20	Late	Yes	320
6619	LLL gran 5	20	Late	No	10
6619	LLL gran 6	20	Late	No	75
6619	LLL gran 7 old (GM)	20	Late	Yes	175
6619	LLL gran 8 (GM)	20	Late	Yes	240
6619	LLL gran 9 old (GM)	20	Late	Yes	1183
6619	LLL gran 10 (GM)	20	Late	Yes	180
6619	LLL gran 11 old (GM)	20	Late	Yes	425
6819	LLL gran 1	20	Late	No	400
6819	LLL gran 5 (GM)	20	Late	Yes	5285
6819	LLL gran 7 (GM)	20	Late	Yes	2280
6819	LLL gran 8 (GM)	20	Late	Yes	930
6819	LLL gran 9 (GM)	20	Late	Yes	1015
6819	LLL gran 10	20	Late	No	100
6819	LLL gran 12 (GM)	20	Late	Yes	720
6819	LLL gran 14 (GM)	20	Late	Yes	1650
6819	LLL gran 15 (GM)	20	Late	Yes	2940
6819	LLL gran 18	20	Late	No	260

Supplementary table 3: List of antibodies used to identify cellular populations, Related to STAR Methods

Target	Clone	Company	Catalog #	RRID	Method
CD69	TP1.55.3	Beckman Coulter	6607110	RRID:AB_1575978	Flow
ROR α	NR1F1	R&D	IC8924P-100		Flow
CD163	GHI/61	BioLegend	333614	RRID:AB_2562641	Flow
Foxp3	PCH101	Thermo Fisher	45-4776-42	RRID:AB_10854725	Flow
ROR γ T	AFKJS-9	Thermo Fisher	46-6988-80	RRID:AB_2802282	Flow
T-bet	4B10	BioLegend	644812	RRID:AB_2200540	Flow
IFN- γ	B27	BioLegend	562974	RRID:AB_2737926	Flow
CD4	L200	BD	563094	RRID:AB_2738001	Flow
CD11c	3.9	BioLegend	301628	RRID:AB_11203895	Flow
PD-1 (CD279)	EH12.1	BD	566175	RRID:AB_2739572	Flow
IL-10	JES3-9D7	eBioscience	48-7108-42	RRID:AB_10548941	Flow
TNF	Mab11	BioLegend	563418		Flow
CD3	SP34-2	BD	624072		Flow
GATA3	16E10A23	BioLegend	653806	RRID:AB_2562725	Flow
CD20	2H7	BioLegend	302318	RRID:AB_493229	Flow
CD8	RPA-T8	BD	563795	RRID:AB_2722501	Flow
CD11b	ICRF44	BD	741357	RRID:AB_2870858	Flow
ROR α	polyclonal	abcam	ab60134	RRID:AB_945289	IHC
GATA3	EPR16651	abcam	ab199428	RRID:AB_2819013	IHC
T-bet	D6N8B	Cell Signaling Technology	13232	RRID:AB_2616022	IHC
Foxp3	236A/E7	ebioscience	14-4777-82	RRID:AB_467556	IHC
CD163	10D6	Neomarkers (thermo fisher)	MS-1103-S1	RRID:AB_64140	IHC
CD11c	5D11	Novocastra- Leica	NCL-L-CD11c-563	RRID:AB_563490	IHC
CD3	CD3-12	abcam	ab11089	RRID:AB_2889189	IHC
Granzyme B	GrB-7	Dako	M7235	RRID:AB_2114697	IHC
CD8 α	D8A8Y	CST	85336S		IHC
CD3	polyclonal	Dako	A0452	RRID:AB_2335677	IHC

**Supplementary table 4: Granulomas used for IHC and immunofluorescence,
designated by figure, Related to STAR Methods**

Animal ID	Sample name	NX timing	CFU	Figure 1	Figure 5	Supplementary Figure 2	Supplementary Figure 6
17313	LLL gran 25A	Early	1.2E+04	D			
9814	RLL gran 12	Mid	4.6E+02	D	A		Foxp3
20512	RLL gran 1	Late	0.0E+00	D			
16213	RLL gran 16	Late	0.0E+00				GATA3
11114	RLL gran 5	Early	5.0E+04				ROR α
15312	Access gran 1	Late	2.4E+02				Granzyme B
6619	LLL gran 9	Late	1.2E+03			Fibrocalcific	
6619	LLL gran 7	Late	1.8E+02			Granuloma scar	
13118	RLL gran 7	Early	3.5E+04			Early evolving necrotic	
6219	RLL gran 5	Mid	8.8E+02			Classic necrotic	
9814	RLL gran 28	Mid	9.0E+02			Early colleganization	
9714	RLL gran 27	Mid	5.4E+02		B		