Rare Variable *M. tuberculosis* Antigens induce predominant Th17 responses in human infection

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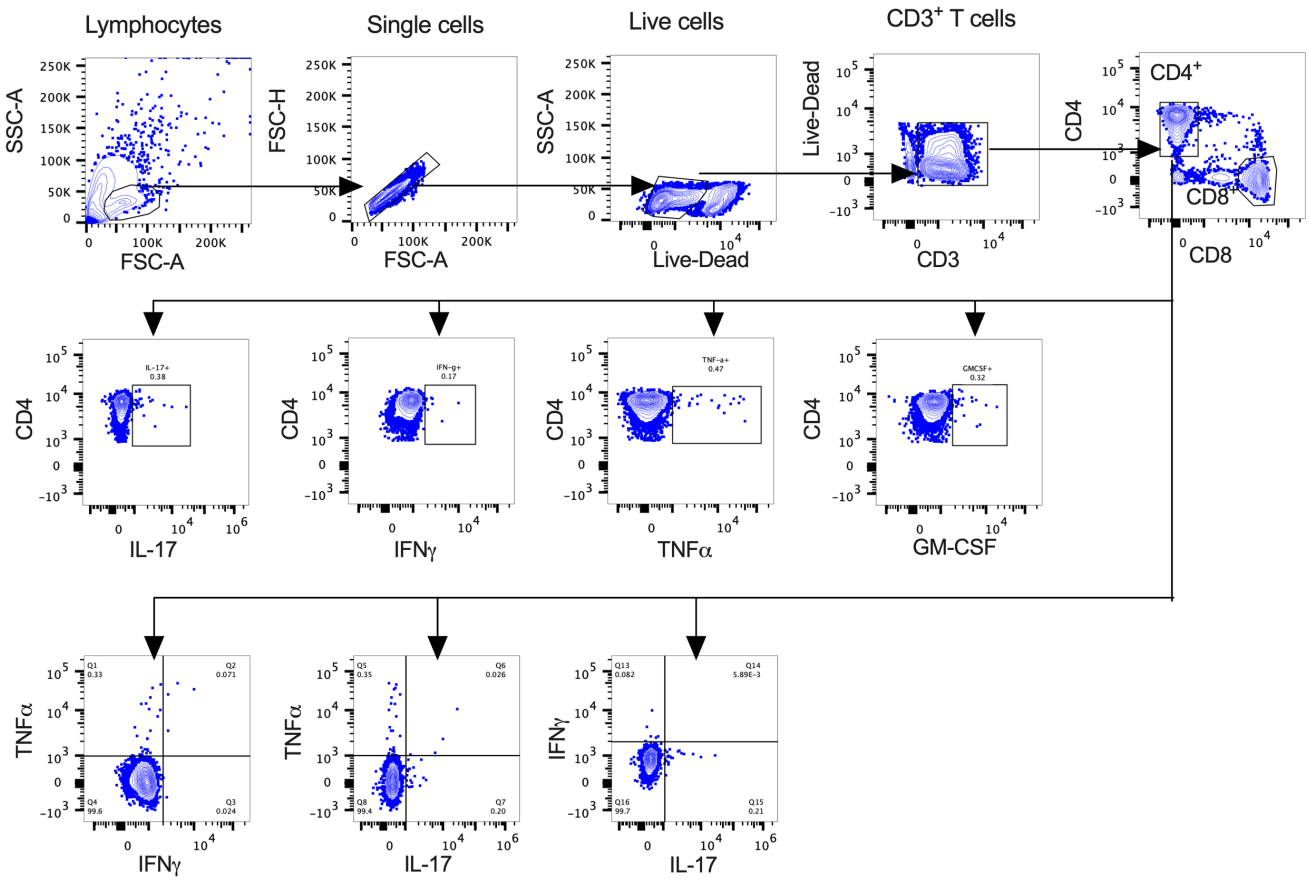
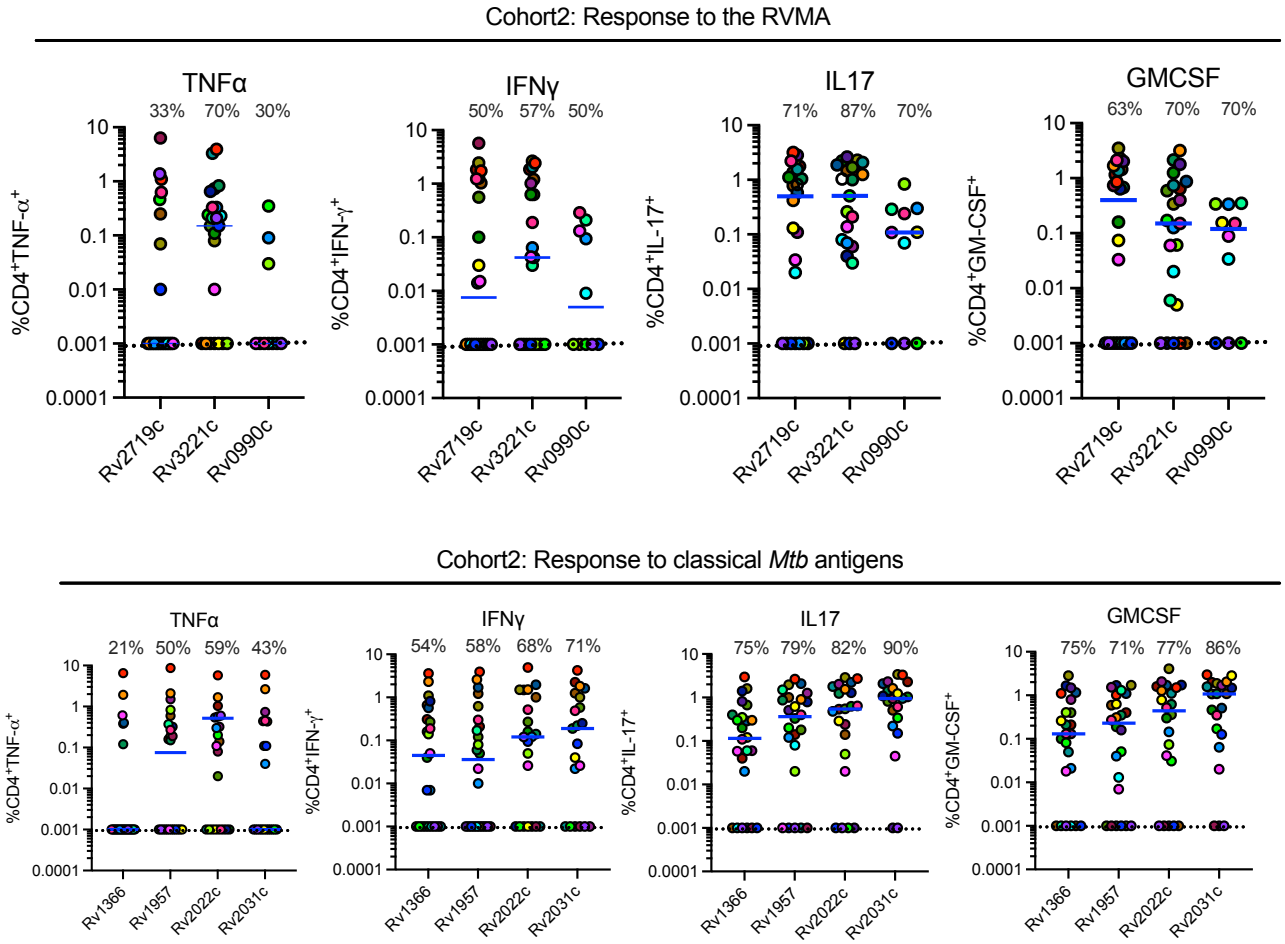
Supplementary materials

Supplementary figure 1:



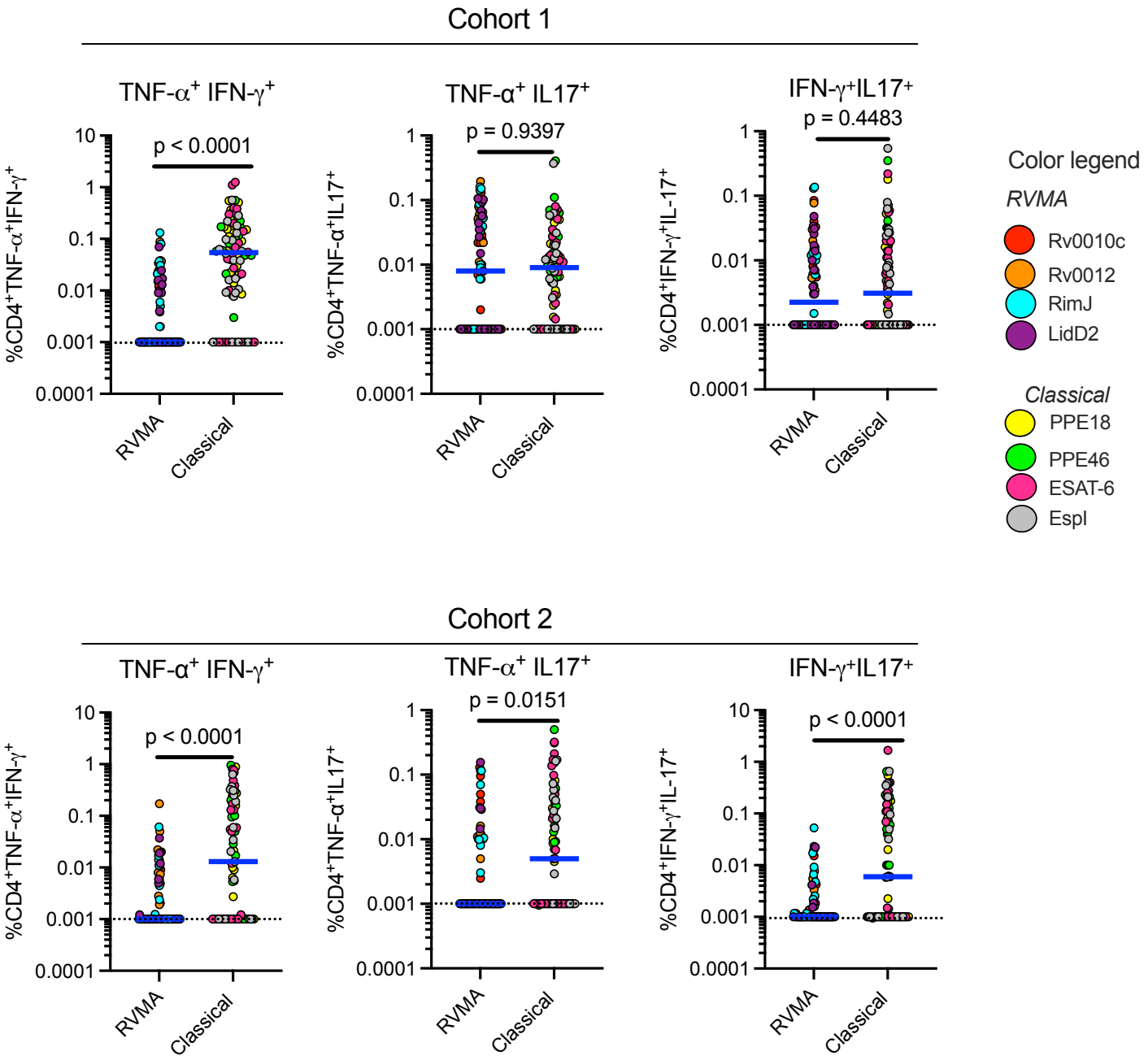
Supplementary Figure 1: Identification of *Mtb*-specific CD4$^+$ T cells. Gating strategy to detect cytokine-producing CD4$^+$ T cells after stimulation with distinct *Mtb* antigens. The shown strategy is for unstimulated PBMCs; the magnitude of *Mtb*-specific cytokine is reported after subtraction of the unstimulated background staining.

Supplementary figure 2:



Supplementary Figure 2: Distinct *Mtb* antigens elicit T cell responses with different functional properties (Cohort 2). Procedures and analyses were as described in Figure 1; the samples were obtained from participants in Cohort 2 (AHRI, Addis Ababa, Ethiopia). Results for RVMA (Rv0990c, Rv2719c, and Rv3221c) are shown in the top panel; results for LICA (Rv1366, Rv1957, Rv2022c, and Rv2031c) are shown in the bottom panel.

Supplementary figure 3:



Supplementary Figure 3: The RVMA induce significantly fewer bifunctional CD4$^+$ T cells. Cryopreserved PBMCs from participants in both cohorts were stimulated with distinct antigens (2 μg/ml) for a total of 20 hours in the presence of Golgi Stop and Golgi Plug and costimulatory antibodies anti-CD28 and anti-CD49d and dual cytokine production by CD4$^+$ T cells determined by intracellular cytokine staining. Each color code is for a distinct antigen as indicated; blue line indicates the median cytokine response. Statistics: Mann-Whitney test.

Supplemental Table 1. Demographic and clinical characteristics of study cohorts

Characteristic	**Cohort 1**	**Cohort 2**	**p***
Age, Median (Interquartile range), Y	31.5 (20, 49.5)	32.9 (26.7, 38.6)	0.6613
Sex, n (%)			
Male	15 (42%)	24 (56%)	p = 0.2611, Fisher's exact test)
Female	21 (58%)	19 (44%)	
BMI; Median (Interquartile range)	21.95 (20.18, 26.58)	21.80 (19.8, 24.7)	0.8852
HbA1c, %; Median (Interquartile range)	5.5 (5.2,5.7)	5.3 (5, 6.1)	0.7467
QFT Results, IU/mL; Median (Interquartile range)			
TB antigen minus Nil	9.03 (1.95, 10)	5.56 (2.2, 8.23)	**0.0183**
Mitogen minus Nil	5.19 (2.09, 9.46)	8.78 (7.75, 9.72)	**0.0011**
*p = Absolute p, Mann-Whitney unless otherwise specified.			

Supplemental Table 2. Cohort 2: Frequencies of CD4 T cell cytokine responses, by individual antigens

	RVMA				**IFNγ-dominant Classical**			
	Rv0010c n=17	Rv0012 n=24	RimJ n=29	LldD2 n=13	PPE18 n=21	PPE46 n=17	ESAT-6 n=21	EspI n=14
TNF	29	58	52	15	52	71	86	86
IFNγ	35	58	55	38	71	82	86	93
IL-17	59	58	62	54	67	88	86	86
GM-CSF	59	79	45	62	57	82	71	79
The values shown reflect the percent of participants whose samples yielded detectable responses, defined as >0.001% of CD4 T cells after stimulation with the indicated antigens.								

Supplemental Table 3. Cohort 2: Frequencies of responders: individual cytokines vs antigen class

	Median % (interquartile range) n = 4 antigens per category		Absolute p (Mann-Whitney)
	RVMA	**IFNγ-dominant Classical**	
TNF	40 (19, 56)	78 (57, 85)	0.0571
IFNγ	51 (38, 57)	84 (74, 90)	0.0286
IL-17	58 (54, 61)	85 (71, 87)	0.0286
GM-CSF	60 (48, 75)	74 (61, 78)	0.6857

Supplemental Table 4. Cohort 2: Magnitudes of individual cytokine responses (% of CD4 T cells) vs antigen class

	Median % (interquartile range) n = 4 antigens per category		Absolute p (Mann-Whitney)
	RVMA	**IFNγ-dominant Classical**	
TNF	0.001 (0.001, 0.12)	0.35 (0.001, 0.8)	<0.0001
IFNγ	0.001 (0.001, 0.06)	0.3(0.05, 0.72)	<0.0001
IL-17	0.04 (0.001, 0.24)	0.29 (0.09, 0.66)	<0.0001
GM-CSF	0.03 (0.001, 0.22)	0.13 (0.001, 0.32)	0.0662
Values shown for each cytokine and each antigen are median % of all CD4 T cells that express the specified cytokine. For statistical analyses, assays that yielded undetectable levels of the stated cytokine were assigned a value of 0.001%.			

Supplemental Table 5. Cohort 2: IL-17 vs IFNγ responses to individual RVMA

	Response Frequencies (% of participants with detectable cytokine$^+$ CD4 T cells)		Response Magnitudes (% of CD4 T cells that are cytokine$^+$) Median (interquartile range)		
	IL-17	**IFNγ**	**IL-17**	**IFNγ**	p*
Rv0010c	58	35	0.02 (0.001, 0.115)	0.001 (0.001, 0.047)	0.2437
Rv0012	58	58	0.07 (0.001, 0.385)	0.012 (0.001, 0.0775)	0.0054
RimJ	62	55	0.06 (0.001, 0.32)	0.01 (0.001, 0.087)	0.2157
LldD2	63	46	0.03 (0.001, 0.255)	0.001 (0.001,0.0305)	0.0391
Rv0990c	70	50	0.11 (0.001, 0.29)	0.005 (0.001, 0.15)	0.0781
Rv2719c	71	50	0.5 (0.001, 1.51)	0.008 (0.001, 1.18)	0.1269
Rv3221c	87	57	0.51 (0.06, 1.68)	0.042 (0.001, 1.01)	0.0602
*p values for the comparison of IL-17 vs IFNγ magnitudes (Wilcoxon matched pairs)					

Supplemental Table 6. Cohort 2: Magnitudes of individual cytokine responses (% of CD4 T cells) vs antigen class

	Median % (interquartile range) n = 3 RVMA and n = 4 LICA		Absolute p (Wilcoxon matched pairs)
	IL-17	**IFNγ**	
RVMA	0.26 (0.025, 1.35)	0.01 (0.001, 0.63)	0.0059
LICA	0.4 (0.05, 1.24)	0.08 (0.001, 0.58)	0.0003
Values shown for each cytokine and each antigen class are median % of all CD4 T cells that express the specified cytokine. For statistical analyses, assays that yielded undetectable levels of the stated cytokine were assigned a value of 0.001%.			

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