

## Supplementary information

### **Prevention of tuberculosis in cynomolgus macaques by an attenuated *Mycobacterium tuberculosis* vaccine candidate**

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#### **This PDF file includes:**

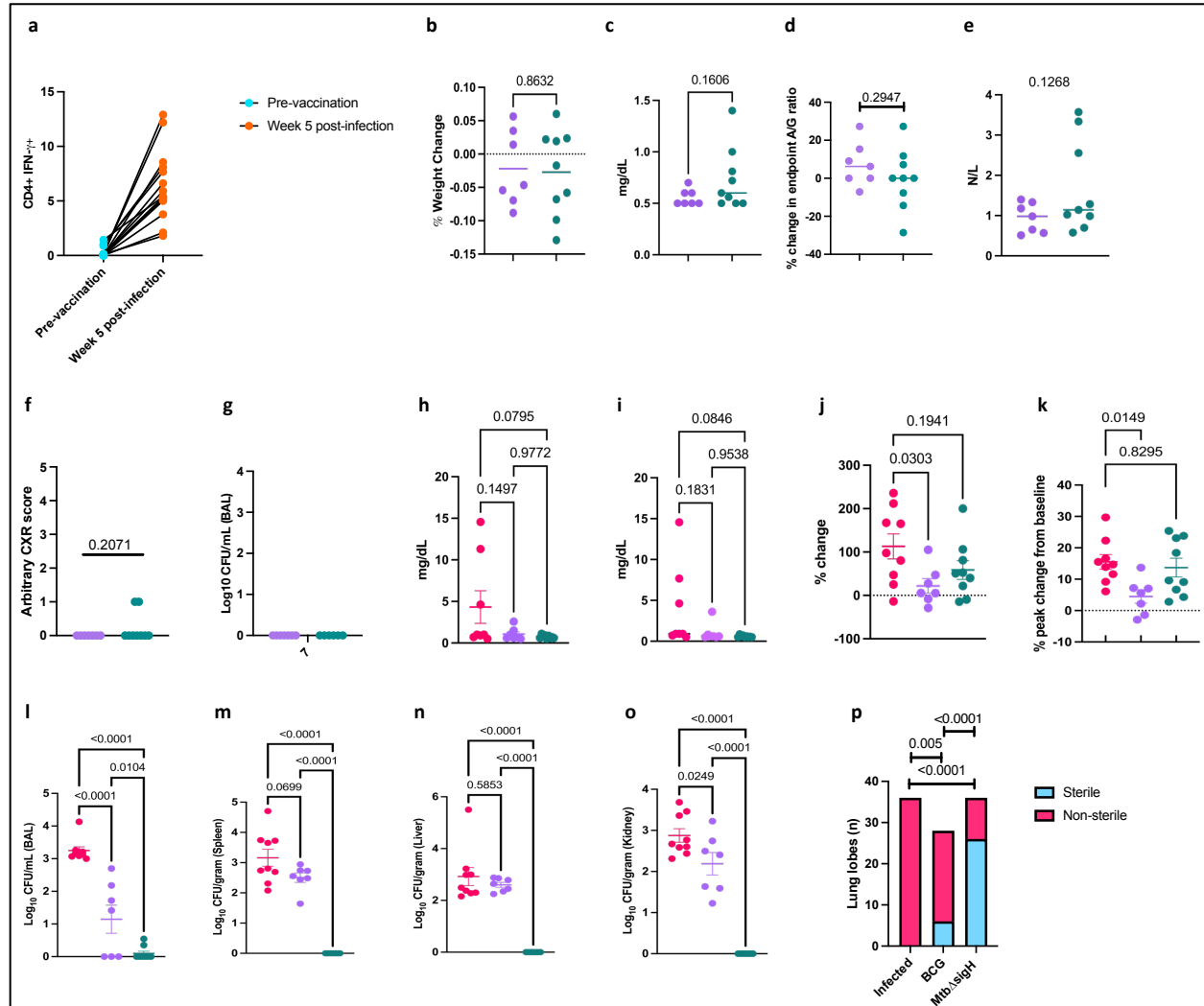
Supplementary Figures and legends:

- Figure S1
- Figure S2
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Supplementary Tables and legends:

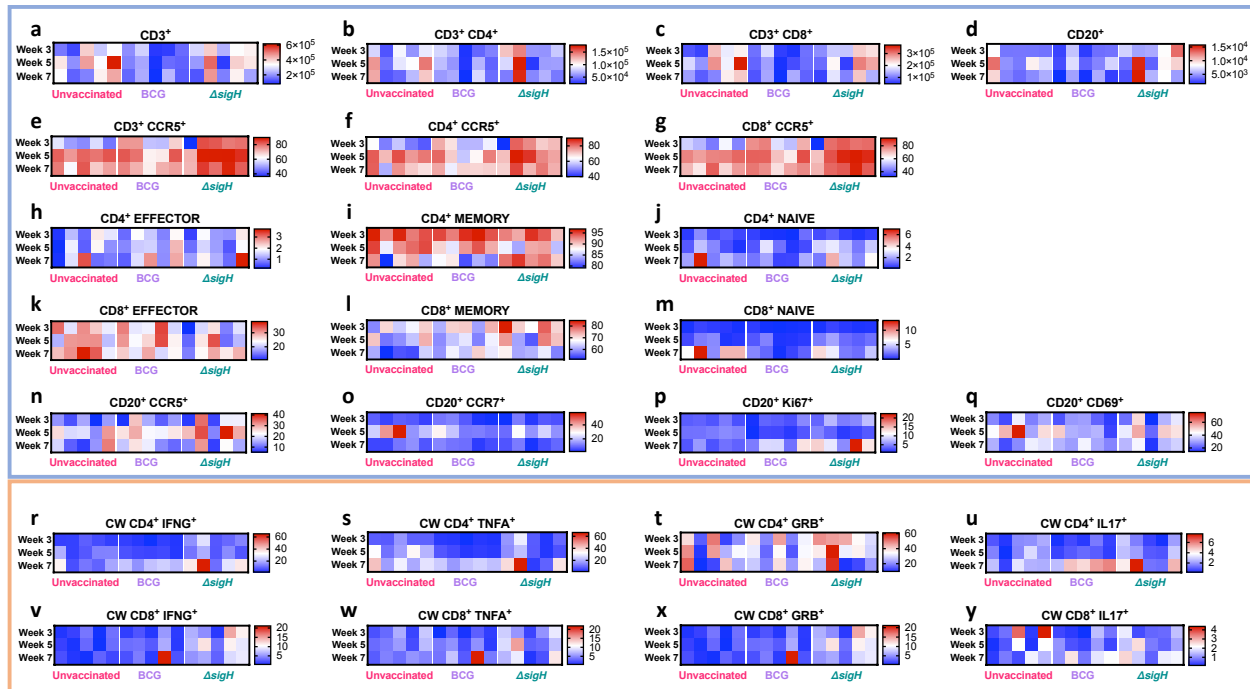
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## Supplementary figures



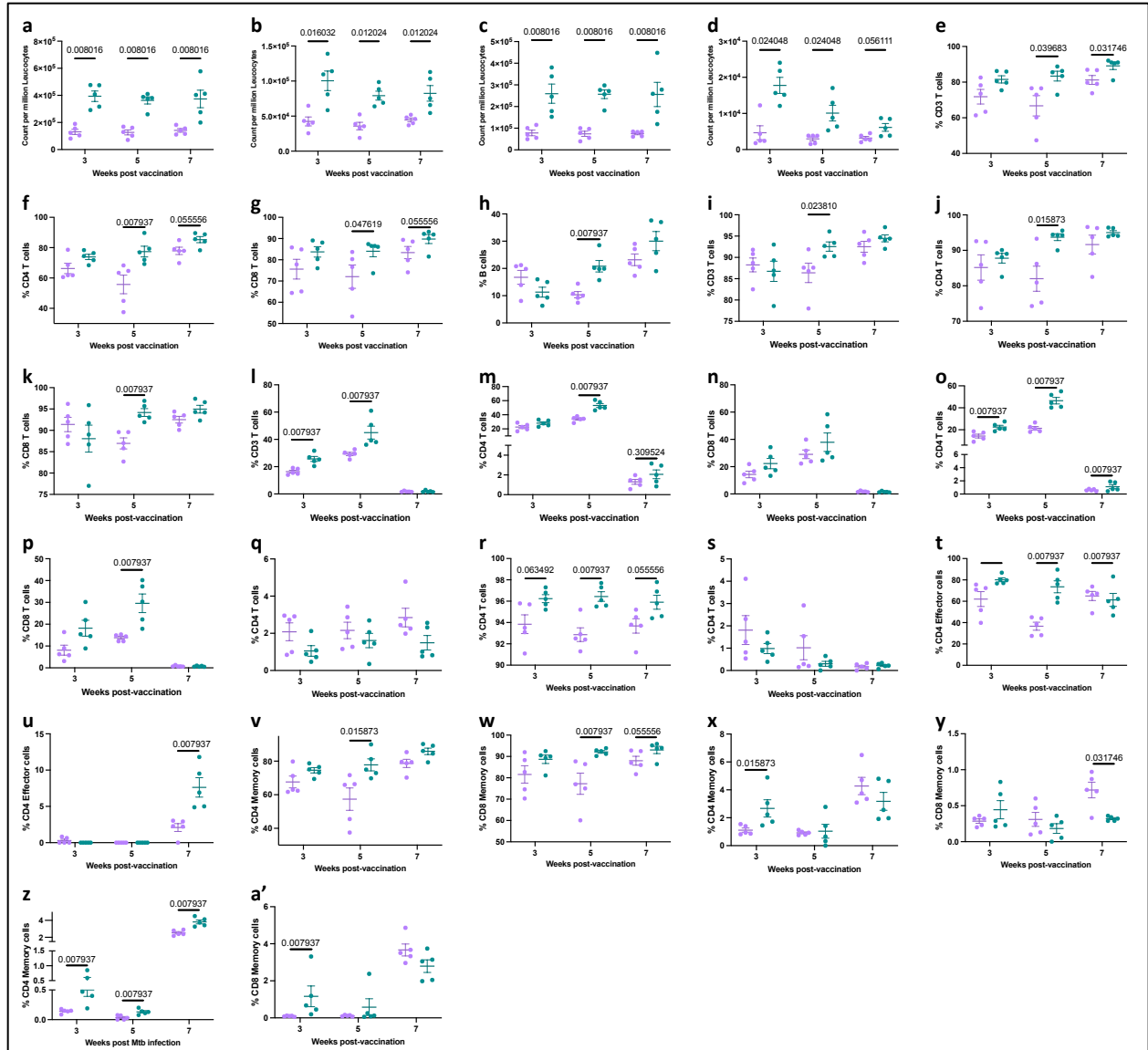
**Figure S1. Clinical, microbiological and pathological measures post-vaccination and challenge.** **a.** Antigen-specific intracellular cytokine staining pre-vaccination (turquoise) and post-challenge (orange) to measure IFNG production to confirm and validate Tuberculin Skin Test (TST) results, following stimulation with *Mtb* cell wall fraction (CW) (BEI Resources). **b.** % change in body weight at the end of the vaccination phase in BCG (lavender) and  $\Delta sigH$ -vaccinated (teal) groups relative to pre-vaccination baseline. **c.** Peak serum C-reactive protein (CRP) values (mg/dL) during the vaccination phase in BCG- and  $\Delta sigH$ -vaccinated groups. **d.** % change in serum A/G ratios at the end of the vaccination phase relative to pre-vaccination

baseline in BCG- and  $\Delta sigH$ - vaccinated groups. **e.** N/L ratio is peripheral blood at the end of the vaccination phase in BCG- and  $\Delta sigH$ - vaccinated groups. **f.** CXR scores at the end of the vaccination phase in BCG- and  $\Delta sigH$ - vaccinated groups. **g.** BAL CFUs at the end of the vaccination phase in BCG- and  $\Delta sigH$ - vaccinated groups. **h** (peak) and **i** (endpoint) serum CRP values (mg/dL) in unvaccinated (**strawberry**), BCG (**lavender**) and  $\Delta sigH$ -vaccinated (**teal**) groups during the *Mtb* challenge phase. Changes in blood neutrophil (N) percentage (**j**) and numbers (**k**) in unvaccinated, BCG- and  $\Delta sigH$ - vaccinated groups at necropsy. (**l**). BAL CFUs (at the endpoint) in unvaccinated, BCG- and  $\Delta sigH$ - vaccinated groups. CFUs in spleen (**m**), liver (**n**) and kidneys (**o**) (per gram tissue) in unvaccinated, BCG- and  $\Delta sigH$ - vaccinated groups at necropsy. (**p**) Fisher's exact test comparing sterile (**strawberry**) vs nonsterile (**turquoise**) lung lobes in the three groups of macaques. Data is presented as mean  $\pm$  SEM and P-values are derived from multiple Mann-Whitney tests with multiple hypothesis correction by false discovery rate method of Benjamini, Kreiger and Yekutielli (two stage step up) (**b-g**) except for **h-o** where one-way ANOVA with Tukey's correction was used.



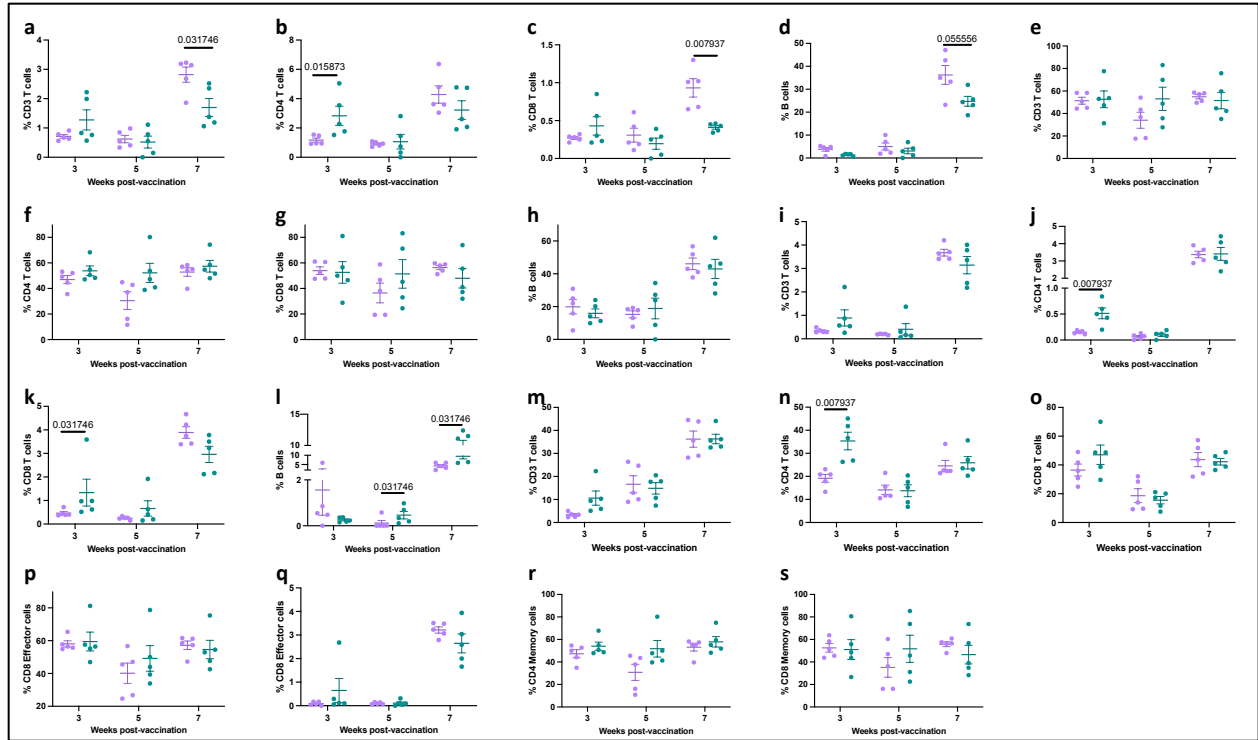
**Figure S2. Lymphocytic immune responses in BAL post-infection.** In three groups of CMs, shown are the absolute counts of CD3<sup>+</sup> (a), CD4<sup>+</sup> (b), and CD8<sup>+</sup> (c) T cells and B cells (d), in BAL at weeks 3, 5, and 7 post-infection time-point. Shown are the frequencies of CD3<sup>+</sup>CCR5<sup>+</sup> (e), CD4<sup>+</sup>CCR5<sup>+</sup> (f), and CD8<sup>+</sup>CCR5<sup>+</sup> (g) T cells in BAL, expressed as percentage of parental populations. Frequencies of CD4<sup>+</sup> T cells (CD28<sup>-</sup>CD95<sup>+</sup>) effector (h), (CD28<sup>+</sup>CD95<sup>+</sup>) memory (i) and naïve (CD28<sup>+</sup>CD95<sup>-</sup>) (j), CD8<sup>+</sup> T cells (CD28<sup>-</sup>CD95<sup>+</sup>) effector (k), (CD28<sup>+</sup>CD95<sup>+</sup>) memory (l) and naïve (CD28<sup>+</sup>CD95<sup>-</sup>) (m) in BAL at weeks 3, 5, and 7 post-infection time-point are depicted as percentage of parental population. Shown are the frequencies of CD20<sup>+</sup>CCR5<sup>+</sup> (n), CD20<sup>+</sup>CCR7<sup>+</sup> (o), CD20<sup>+</sup>KI67<sup>+</sup> (p) and CD20<sup>+</sup>CD69<sup>+</sup> (q) B cells in BAL at weeks 3, 5, and 7 post-infection time-point, expressed as percentage of B cell population. Frequencies of CD4<sup>+</sup> and CD8<sup>+</sup> T cells expressing IFNG (r, v), TNFA (s, w), GRB (t, x) and IL17 (u, y) in response to *Mtb* Cell-Wall (CW) fraction in BAL at weeks 3, 5, and 7 post-infection time-point are depicted as percentage of parental population. Each column represents an individual macaque. Results are shown for n=5 macaques per group.





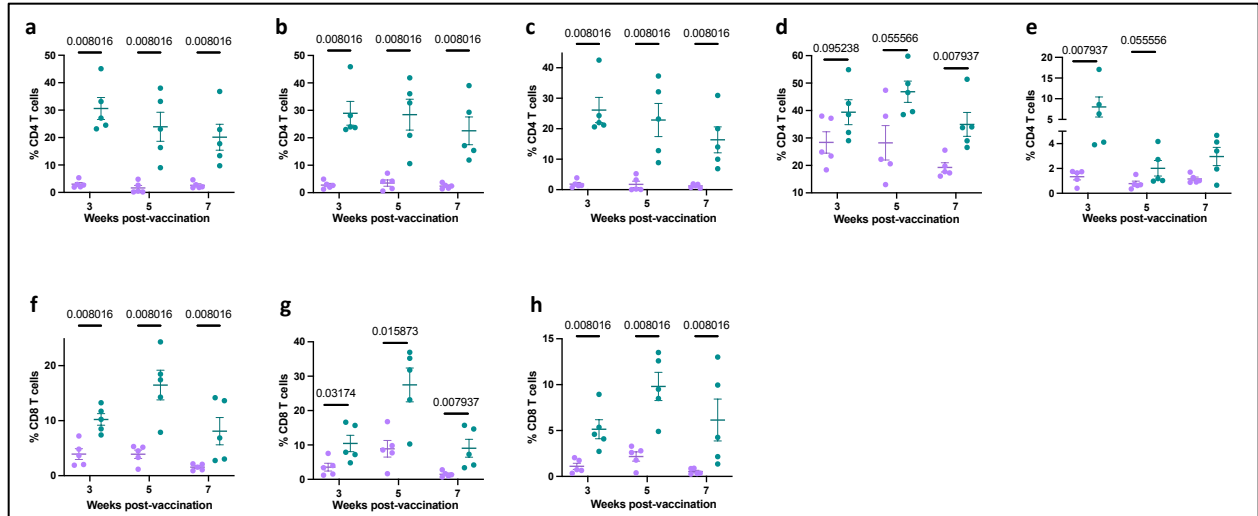
**Figure S3. T cell dynamics in the BAL post-vaccination.** Frequencies of (a) CD3<sup>+</sup>, (b) CD4<sup>+</sup>, (c) CD8<sup>+</sup>, (d) CD20<sup>+</sup>, (e) CD3<sup>+</sup>CCR5<sup>+</sup>, (f) CD4<sup>+</sup>CCR5<sup>+</sup>, (g) CD8<sup>+</sup>CCR5<sup>+</sup>, (h) CD20<sup>+</sup>CCR5<sup>+</sup>, (i) CD3<sup>+</sup>CXCR3<sup>+</sup>, (j) CD4<sup>+</sup>CXCR3<sup>+</sup>, (k) CD8<sup>+</sup>CXCR3<sup>+</sup>, (l) CD3<sup>+</sup>CCR6<sup>+</sup>, (m) CD4<sup>+</sup>CCR6<sup>+</sup>, (n) CD8<sup>+</sup>CCR6<sup>+</sup>, (o) CD4<sup>+</sup>CXCR3<sup>+</sup>CCR6<sup>+</sup>, (p) CD8<sup>+</sup>CXCR3<sup>+</sup>CCR6<sup>+</sup>, (q) CD4<sup>+</sup> Effector (CD95<sup>+</sup>CD28<sup>-</sup>), (r) CD4<sup>+</sup> memory (CD95<sup>+</sup>CD28<sup>+</sup>), (s) CD4<sup>+</sup> naïve (CD95<sup>-</sup>CD28<sup>+</sup>), (t) Effector CD4<sup>+</sup>CD69<sup>+</sup> and (u) KI67<sup>+</sup>, (v) Memory CD4<sup>+</sup>CCR5<sup>+</sup>, (w) Memory CD8<sup>+</sup>CCR5<sup>+</sup>, (x) Memory CD4<sup>+</sup>CCR7<sup>+</sup>, (y) Memory CD4<sup>+</sup>CCR5<sup>+</sup>, (z) Memory CD4<sup>+</sup>KI67<sup>+</sup>, (a') Memory CD8<sup>+</sup> KI67<sup>+</sup>,

relative to parental populations. Data is presented as mean  $\pm$  SEM and P-values were calculated by two-way ANOVA with Tukey's correction. Results are shown for n=5 macaques per group.



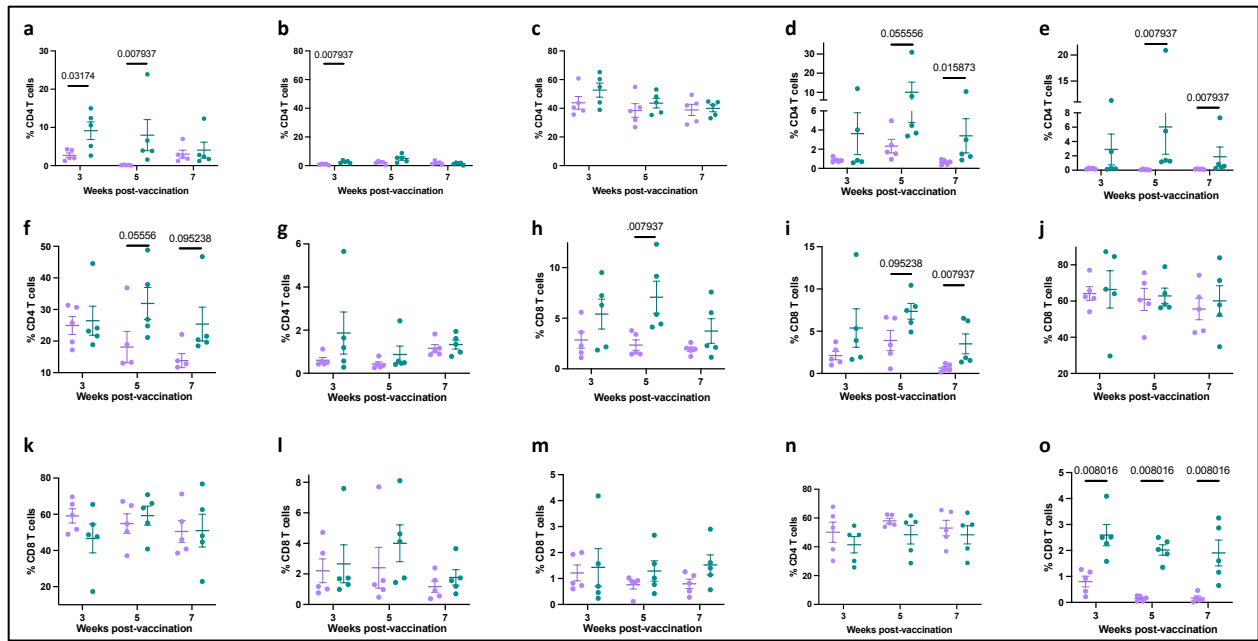
**Figure S4. T cell responses in the BAL of macaques at 3-, 5- and 7-weeks post vaccination.**

Frequencies of **a.** CD3<sup>+</sup> CCR7<sup>+</sup>. **b.** CD4<sup>+</sup> CCR7<sup>+</sup>. **c.** CD8<sup>+</sup> CCR7<sup>+</sup>. **d.** CD20<sup>+</sup> CCR7<sup>+</sup>. **e.** CD3<sup>+</sup> CD69<sup>+</sup>. **f.** CD4<sup>+</sup> CD69<sup>+</sup>. **g.** CD8<sup>+</sup> CD69<sup>+</sup>. **h.** CD20<sup>+</sup> CD69<sup>+</sup>. **i.** CD3<sup>+</sup> KI67<sup>+</sup>. **j.** CD4<sup>+</sup> KI67<sup>+</sup>. **k.** CD8<sup>+</sup> KI67<sup>+</sup>. **l.** CD20<sup>+</sup> KI67<sup>+</sup>. **m.** CD3<sup>+</sup> HLA-DR<sup>+</sup>. **n.** CD4<sup>+</sup> HLA-DR<sup>+</sup>. **o.** CD8<sup>+</sup> HLA-DR<sup>+</sup>. **p.** Effector CD8<sup>+</sup> CD69<sup>+</sup>. **q.** Effector CD8<sup>+</sup> KI67<sup>+</sup>, **r.** Memory CD4<sup>+</sup> CD69<sup>+</sup> **s.** Memory CD8<sup>+</sup> CD69<sup>+</sup>, relative to parental populations. Data is presented as mean  $\pm$  SEM and P-values are derived from multiple Mann-Whitney tests with multiple hypothesis correction by false discovery rate method of Benjamini, Kreiger and Yekutielli (two stage step up).

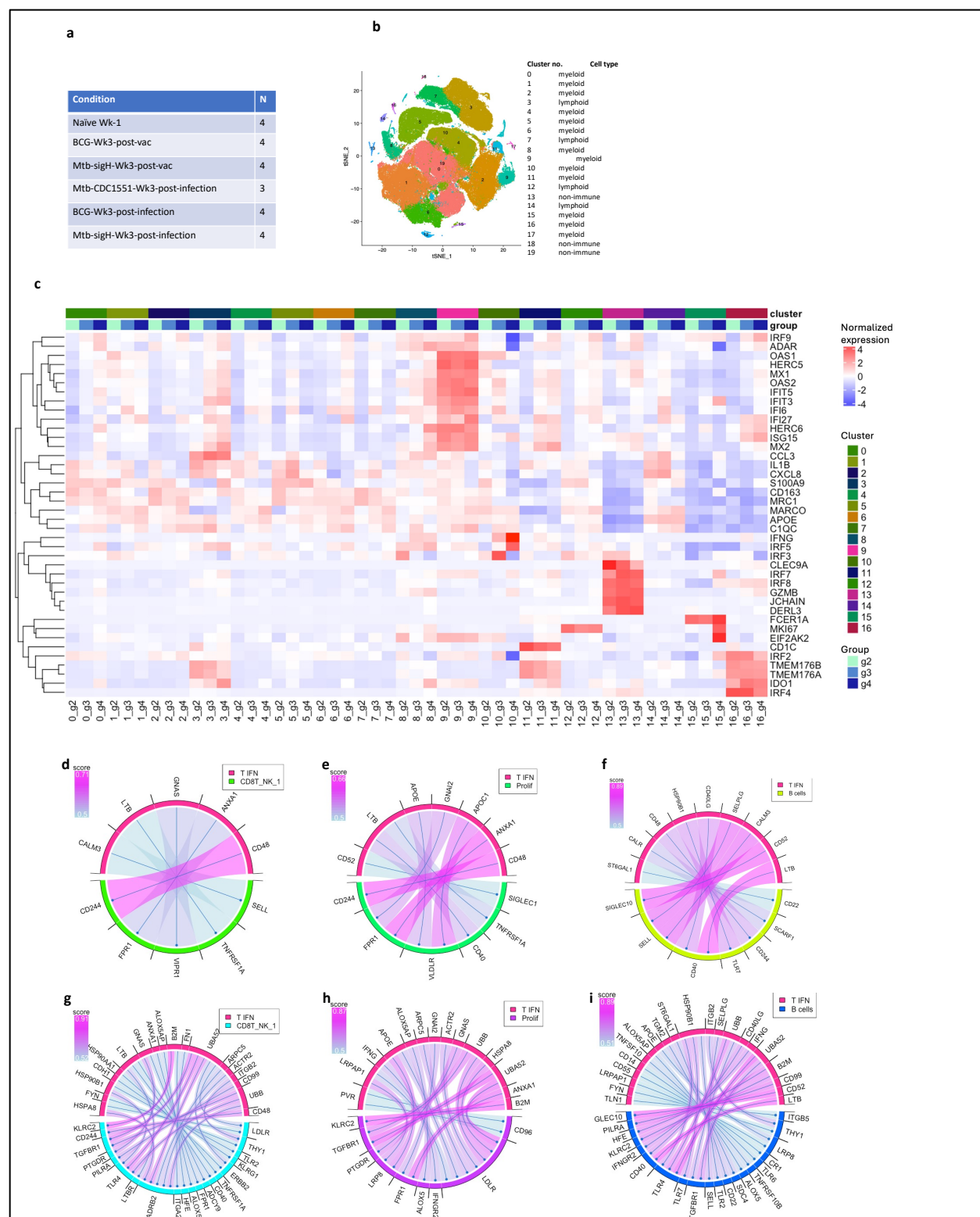


**Figure S5. *Mtb* cell wall fraction specific T cell responses in the BAL post-vaccination.**

Frequencies of **a.** CD4<sup>+</sup>IFNG<sup>+</sup>. **b.** CD3<sup>+</sup> TNFA<sup>+</sup>. **c.** CD4<sup>+</sup> IFNG<sup>+</sup>TNFA<sup>+</sup>. **d.** CD4<sup>+</sup> GZMB<sup>+</sup>. **e.** CD4<sup>+</sup> IL17<sup>+</sup>. **f.** CD8<sup>+</sup>IFNG<sup>+</sup>. **g.** CD8<sup>+</sup> TNFA<sup>+</sup>. **h.** CD8<sup>+</sup> IFNG<sup>+</sup>TNFA<sup>+</sup> in response to *Mtb* cell wall fraction (CW). P-values are derived from multiple Mann-Whitney tests with multiple hypothesis correction by false discovery rate method of Benjamini, Kreiger and Yekutielli (two stage step up). Results are shown for n=5 macaques per group.

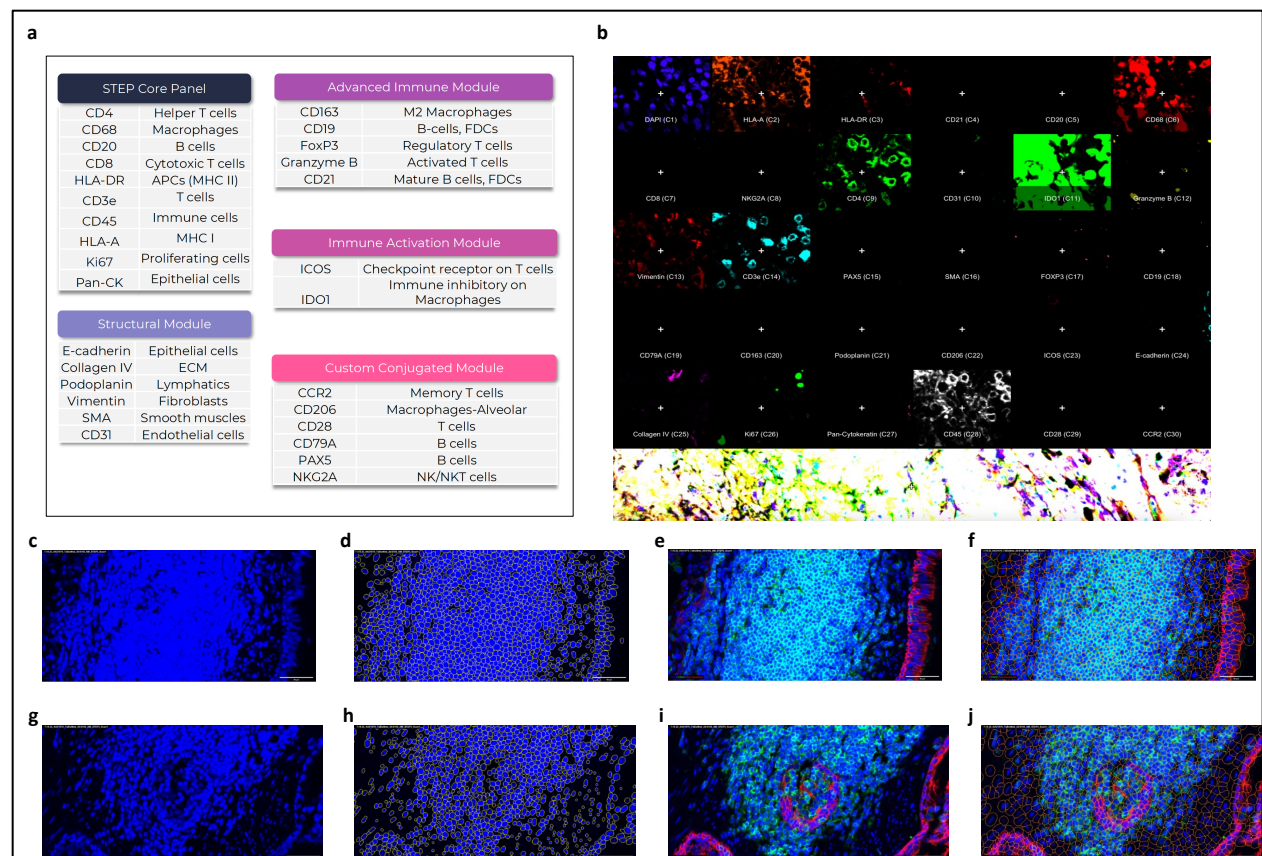


**Figure S6. *Mtb* ESAT6 and CFP10 (EC) specific T cell responses in the BAL post-vaccination.** **a.** Frequencies of CD4<sup>+</sup>IFNG<sup>+</sup> in response to EC compared to **b.** unstimulated (negative) and **c.** PMA/Ionomycin stimulated (positive control). Frequencies of **d.** CD4<sup>+</sup> TNFA<sup>+</sup>. **e.** CD4<sup>+</sup> IFNG<sup>+</sup> TNFA<sup>+</sup>. **f.** CD4<sup>+</sup> GZMB<sup>+</sup>. **g.** CD4<sup>+</sup>IL17<sup>+</sup>. **h.** CD8<sup>+</sup>IFNG<sup>+</sup>. **i.** CD8<sup>+</sup>TNFA<sup>+</sup> in response to EC. **j.** CD8<sup>+</sup>GZMB<sup>+</sup> in response to CW. **k.** CD8<sup>+</sup>GZMB<sup>+</sup> in response to EC. **l.** CD8<sup>+</sup>IL17<sup>+</sup> in response to CW. **m.** CD8<sup>+</sup>IL17<sup>+</sup> in response to EC. **n.** CD8<sup>+</sup>GZMB<sup>+</sup> unstimulated control. **o.** CD8<sup>+</sup>IFNG<sup>+</sup> TNFA<sup>+</sup> in response to EC. Data is presented as mean  $\pm$  SEM and P-values are derived from multiple Mann-Whitney tests with multiple hypothesis correction by false discovery rate method of Benjamini, Kreiger and Yekutielli (two stage step up). Results are shown for n=5 macaques per group.



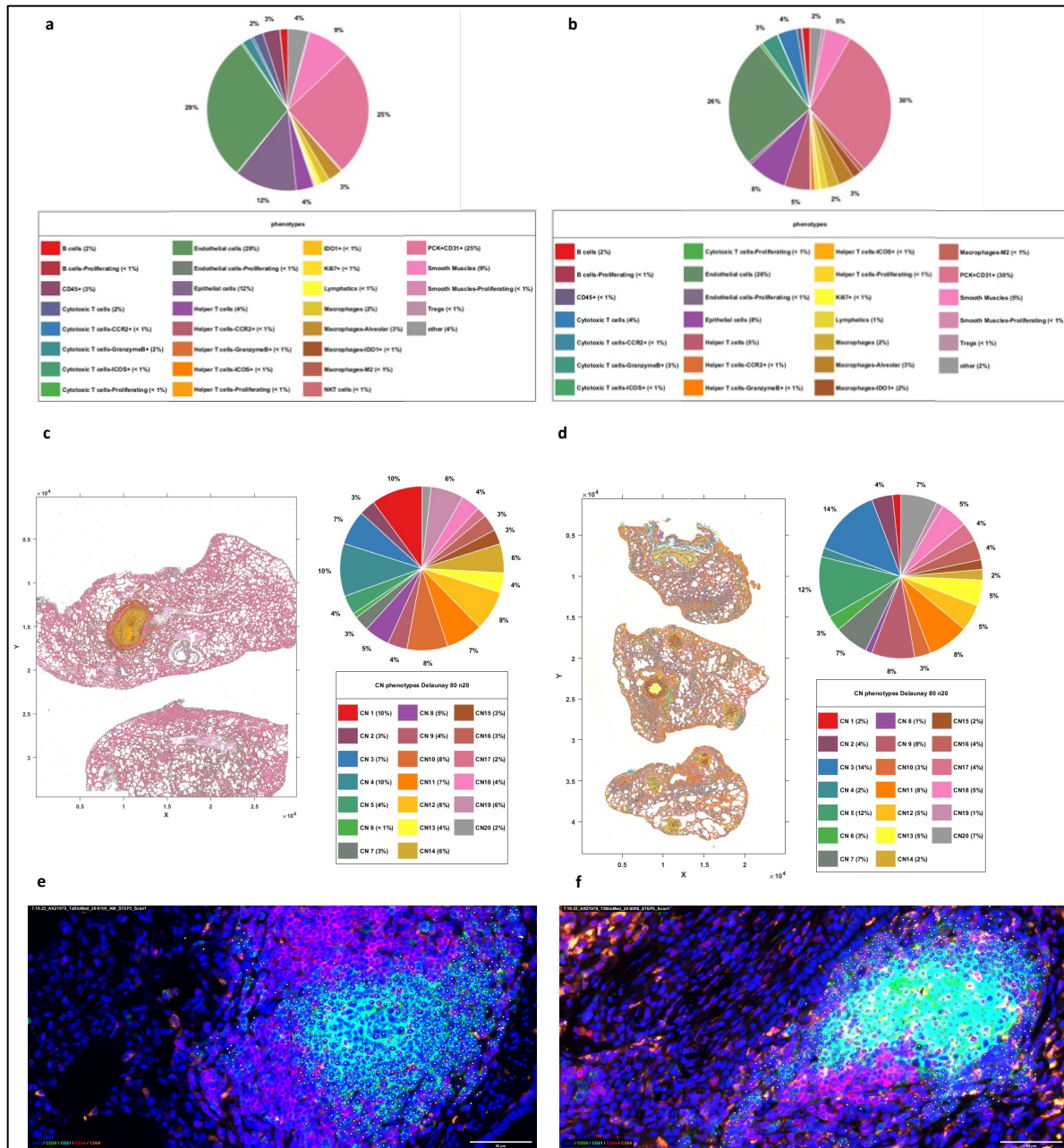
**Figure S7. scRNAseq of BAL cells.** a. scRNAseq design. b. tSNE visualization of major cell types (all conditions together). c. Expression of ISG genes detected across myeloid clusters in

the two vaccination and the unvaccinated/*Mtb* challenged groups. **d-i**. Circos plots depicting ligand-receptor interactions between genes of C9 vs C2 for BCG-vaccinated *Mtb* challenged (**d**) and  $\Delta sigH$ -vaccinated/*Mtb* challenged (**g**), C9 vs C6 for BCG-vaccinated/*Mtb* challenged (**e**) and  $\Delta sigH$ -vaccinated/*Mtb* challenged (**h**), C9 vs C7 and for BCG-vaccinated/*Mtb* challenged (**f**) and  $\Delta sigH$ -vaccinated/*Mtb* challenged (**i**).



**Figure S8. Spatial multiplexed imaging and segmentation.** **a.** CyCIF panel design. **b.** antibody staining collagen. **c-d.** Stardist nuclear segmentation on DAPI signal for the lung section obtained from an unvaccinated macaque. **e-f.** Membrane expansion mask for the lung section obtained from an unvaccinated macaque. **g-h.** Stardist nuclear segmentation on DAPI signal for the lung section obtained from a  $\Delta sigH$ -vaccinated macaque. **i-j.** Membrane expansion mask for the lung section obtained from a  $\Delta sigH$ -vaccinated macaque.





**Figure S9. Cellular subsets identified by spatial multiplexed imaging** Frequency of cellular phenotypes in the lung sections of unvaccinated (a) and  $\Delta sigH$ -vaccinated (b) macaques. Distribution of cellular neighborhoods phenotypes in the lung sections of unvaccinated (c) and  $\Delta sigH$ -vaccinated (d) macaques. Identified B cell rich (iBALT) containing cellular neighborhoods in the lung sections of unvaccinated (e) and  $\Delta sigH$ -vaccinated (f) macaques.



## Supplementary tables

Supplementary Table 1. Demographics and legends for individual animals used in the study.									
Subject	Species	Common name	Gender	Age (Years)	Weight (kg)	Group	TST Status	Origin	Study site
MF01	<i>Macaca fascicularis</i>	Crab-eating macaque	F	11 years, 69 days	6.90	$\Delta$ sigH	Positive	NIH	TNPRC
MF02	<i>Macaca fascicularis</i>	Crab-eating macaque	F	12 years, 142 days	4.10	Unvaccinated	Positive	NIH	TNPRC
MF03	<i>Macaca fascicularis</i>	Crab-eating macaque	F	13 years, 211 days	4.30	Unvaccinated	Positive	NIH	TNPRC
MF04	<i>Macaca fascicularis</i>	Crab-eating macaque	F	10 years, 80 days	6.80	BCG	Negative	NIH	TNPRC
MF05	<i>Macaca fascicularis</i>	Crab-eating macaque	F	10 years, 116 days	5.90	$\Delta$ sigH	Negative	NIH	TNPRC
MF06	<i>Macaca fascicularis</i>	Crab-eating macaque	F	10 years, 219 days	6.20	$\Delta$ sigH	Positive	NIH	TNPRC
MF07	<i>Macaca fascicularis</i>	Crab-eating macaque	F	10 years, 200 days	7.20	BCG	Positive	NIH	TNPRC
MF08	<i>Macaca fascicularis</i>	Crab-eating macaque	F	11 years, 149 days	7.10	$\Delta$ sigH	Positive	NIH	TNPRC
MF09	<i>Macaca fascicularis</i>	Crab-eating macaque	F	11 years, 105 days	4.70	Unvaccinated	Positive	NIH	TNPRC
MF10	<i>Macaca fascicularis</i>	Crab-eating macaque	F	17 years, 73 days	5.50	Unvaccinated	Positive	NIH	TNPRC
MF11	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 17 days	4.95	BCG	Positive	ENVIGO	SNPRC
MF12	<i>Macaca fascicularis</i>	Crab-eating macaque	M	5 years, 331 days	6.83	BCG	Negative	ENVIGO	SNPRC
MF13	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 20 days	6.32	BCG	Negative	ENVIGO	SNPRC
MF14	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 21 days	8.35	BCG	Negative	ENVIGO	SNPRC
MF15	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 21 days	4.66	$\Delta$ sigH	Positive	ENVIGO	SNPRC
MF16	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 35 days	5.19	Unvaccinated	Positive	ENVIGO	SNPRC
MF17	<i>Macaca fascicularis</i>	Crab-eating macaque	M	5 years, 248 days	7.84	BCG	Negative	ENVIGO	SNPRC
MF18	<i>Macaca fascicularis</i>	Crab-eating macaque	M	5 years, 354 days	4.99	$\Delta$ sigH	Positive	ENVIGO	SNPRC
MF19	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 44 days	7.02	Unvaccinated	Negative	ENVIGO	SNPRC
MF20	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 43 days	5.94	$\Delta$ sigH	Positive	ENVIGO	SNPRC
MF21	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 11 days	6.45	Unvaccinated	Positive	ENVIGO	SNPRC
MF22	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 18 days	7.01	$\Delta$ sigH	Positive	ENVIGO	SNPRC
MF23	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 44 days	5.65	$\Delta$ sigH	Positive	ENVIGO	SNPRC
MF24	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 32 days	8.63	Unvaccinated	Negative	ENVIGO	SNPRC
MF25	<i>Macaca fascicularis</i>	Crab-eating macaque	M	6 years, 20 days	5.13	Unvaccinated	Positive	ENVIGO	SNPRC

**Supplementary Table 1: Demographics for individual animals used in the study.**

Supplementary Table 2. Flow Cytometry panels														
Laser	BLUE LASER 488nm			RED LASER 640nm			VIOLET LASER 405nm				YELLOW GREEN LASER	UV LASER 355		
T cell Panel 1														
Fluorochrome	FITC	PCP-Cy5.5	APC	AL700	APC-H7	BV421	BV480	BV605			PE	PE-Cy7	BUV395	FVS440 UV
Marker	CD69	CD4	CD8	CD3	CD20	CD95	Ki67	CCR7			CCR5	CD28	CD45	LIVE/DEAD
Volume/Test (µL)	20	10	5	5	5	5	5	5			20	5	5	
T cell Panel 2														
Fluorochrome	FITC	PCP-Cy5.5	APC	AL700	APC-Cy7	BV421	BV480		BV650	BV711	PE	PE-Cy7	BUV395	FVS440 UV
Marker	CD69	CD4	CD8	CD3	HLA-DR	PD-1	CCR7		CCR5	CCR6	LAG-3	CXCR3	CD45	LIVE/DEAD
Volume/Test (µL)	20	10	5	5	2	5	5		5	5	10	5	5	
T cell Effector Panel														
Fluorochrome	eFluor520	PCP-Cy5.5	APC	AL700	APC-Cy7	BV421		BV605	BV650		PE	PE-Cy7	BUV395	BUV737
Marker	LIVE/DEAD	CD4	CD8	CD3	IFN-γ	CD95		IL-17	TNF-α		GrB	CD28	CD45	IL-2
Volume/Test (µL)		10	5	5	5	5	5	5	5		5	5	5	5

**Supplementary Table 2: Flow Cytometry panels.**

Supplementary Table 3. List of antibodies.					
Antibody	Supplier	Clone	Cat Number	Validation Statement: Reactivity	Dilution
CD69 (FITC)	BD Bioscience	FN50	555530	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
CD4 (PCP-Cy5.5)	BD Bioscience	L200	552838	Rhesus, Cynomolgus, Baboon (QC Testing) Human (Tested in Development)	As recommended by Manufacturer
CD8 (APC)	Biologend	RPA-T8	301049	Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macaque, Sooty Mangabey	As recommended by Manufacturer
CD3 (AL700)	BD Bioscience	SP34-2	557917	Rhesus, Cynomolgus, Baboon (QC Testing) Human (Tested in Development)	As recommended by Manufacturer
CD20 (APC-H7)	BD Bioscience	2H7	560853	Rhesus, Cynomolgus, Baboon (QC Testing) Human (Tested in Development)	As recommended by Manufacturer
CD95 (BV421)	BD Bioscience	DX2	562616	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
Ki67 (AF 488)	BD Bioscience	B56	558616	Human (QC Testing) Mouse (Tested in Development) Rat, Rhesus (Reported)	As recommended by Manufacturer
CCR7 (BV605)	BD Bioscience	3D12	563711	Human (QC Testing) , Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CCR5 (PE)	BD Bioscience	3A9	556042	Human (QC Testing) Rhesus, Cynomolgus (Tested in Development)	As recommended by Manufacturer
CD28 (PE-Cy7)	BD Bioscience	CD28.2	560684	Human (QC Testing), Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CD45 (BUV395)	BD Bioscience	D058-1283	564099	Rhesus, Cynomolgus, Baboon (QC Testing)	As recommended by Manufacturer
HLA-DR (APC-Cy7)	BD Bioscience	L243	335796	Human (QC Testing), Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
PD-1 (BV421)	Biologend	EH12.2H7	329920	Human, African Green, Baboon, Chimpanzee, Common Marmoset, Cynomolgus, Rhesus, Squirrel Monkey	As recommended by Manufacturer
CCR7 (BV480)	BD Bioscience	3D12	566099	Human (QC Testing), Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CCR5 (BV650)	BD Bioscience	3A9	564999	Human (QC Testing) Rhesus, Cynomolgus (Tested in Development)	As recommended by Manufacturer
CCR6 (BV711)	BD Bioscience	11A9	563923	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
LAG-3 (PE)	R&D Systems		FAB2319P	Human, Rhesus (Validated in lab, Reported)	As recommended by Manufacturer
CXCR3 (PE-Cy7)	BD Bioscience	1C6	560831	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
CD103 (FITC)	ThermoFisher	B-Ly7	11-1038-42	Human, Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CD123 (PerCP-Cy5.5)	BD Bioscience	7G3	562391	Human (QC Testing) , Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CD14 (APC)	BD Bioscience	M5E2	561383	Rhesus, Cynomolgus, Baboon (QC Testing)	As recommended by Manufacturer
CD20 (AF700)	BD Bioscience	2H7	560631	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
CD206 (BV421)	BD Bioscience	19.2	564062	Human (QC Testing) , Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CD16 (BV480)	BD Bioscience	3G8	566108	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
CD11c (BV711)	Biologend	3.9	301630	Human, African Green, Baboon, Chimpanzee, Cynomolgus, Rhesus, Squirrel Monkey	As recommended by Manufacturer
CD183 (PE)	BD Bioscience	GHI/61	556018	Human (QC Testing), Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
CD40 (PE-Cy7)	BD Bioscience	5C3	561215	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
IFN-γ (APC-Cy7)	Biologend	B27	506524	Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macaque, African Green, Sooty Mangabey	As recommended by Manufacturer
IL-17 (BV605)	Biologend	BL168	512326	Human, Rhesus (Validated in lab, Reported)	As recommended by Manufacturer
TNF-α (BV650)	Biologend	MAB11	502938	Human, Cat (Feline) 11 Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macaque, Sooty Mangabey, Swine (Pig, Porcine)	As recommended by Manufacturer
GrB (PE)	BD Bioscience	GB11	561142	Human (QC Testing), Rhesus (NHP Reagent Resource)	As recommended by Manufacturer
IL-2 (BUV737)	BD Bioscience	MQ1-17H12	612836	Human (QC Testing) Rhesus, Cynomolgus, Baboon (Tested in Development)	As recommended by Manufacturer
HLA-A	Akoya Biosciences	EP1395Y	Custom-Conjugation	Cynomolgus (QC Testing)	1:300
HLA-DR	Akoya Biosciences	EPR3692	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
CD20	Akoya Biosciences	L26	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
CD21	Akoya Biosciences	EP3093	Custom-Conjugation	Cynomolgus (QC Testing)	1:400
CD68	Akoya Biosciences	KP1	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
NG2a	Akoya Biosciences	EPR23737-127	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
CD8	Akoya Biosciences	C8/144B	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
CD4	Akoya Biosciences	EPR6855	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
CD31	Akoya Biosciences	EP3095	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
IDO1	Akoya Biosciences	V1NC3IDO	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
Vimentin	Akoya Biosciences	O91D3	Custom-Conjugation	Cynomolgus (QC Testing)	1:400
GranzymeB	Akoya Biosciences	D6E9W	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
CD3e	Akoya Biosciences	EP449E	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
SMA	Akoya Biosciences	1A4	Custom-Conjugation	Cynomolgus (QC Testing)	1:400
PAX5	Akoya Biosciences	D7H5X	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
Foxp3	Akoya Biosciences	SP97	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
CD79a	Akoya Biosciences	D1X5C	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
CD19	Akoya Biosciences	RM332	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
CD163	Akoya Biosciences	D6U1J	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
CD206	Akoya Biosciences	EPR22489-7	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
Podoplanin	Akoya Biosciences	NC-08	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
CD278 (ICOS)	Akoya Biosciences	D1K2T	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
E-cadherin	Akoya Biosciences	4A2C7	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
CollagenIV	Akoya Biosciences	EPR209660	Custom-Conjugation	Cynomolgus (QC Testing)	1:200
Pan-Cytokeratin	Akoya Biosciences	AE1/AE3	Custom-Conjugation	Cynomolgus (QC Testing)	1:300
Ki67	Akoya Biosciences	B56	Custom-Conjugation	Cynomolgus (QC Testing)	1:300
CD45	Akoya Biosciences	D9M81	Custom-Conjugation	Cynomolgus (QC Testing)	1:100
CD28	Akoya Biosciences	EPR22076	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
CCR2	Akoya Biosciences	D14H7	Custom-Conjugation	Cynomolgus (QC Testing)	1:50
LC3	Cell Signalling Technology	Polyclonal	2775s	Human, Mouse, Rat (QC Testing)	1:100

**Supplementary Table 3: List of antibodies.**

Supplementary Table 4. Sequences of oligonucleotides.			
Gene	Supplier	Forward Primer 5'→ 3'	Reverse Primer 5'→ 3'
ATG5	Sigma Aldrich	TTTGCATCACCTCTGCTTTC	TAGGCCAAAGGTTTCAGCTT
ATG7	Sigma Aldrich	CGTTGCCCACAGCATCATCTTC	CACTGAGGTTCAACATCCTTGG
b-Actin	Sigma Aldrich	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
GAPDH	Sigma Aldrich	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
GBP1	Sigma Aldrich	GTGGAACGTGTGAAAGCTGA	CAACTGGACCCTGTCGTTCT
GBP2	Sigma Aldrich	GATTTCACCCTGGAAGTGA	GGGTTCAAGCTCTTCCTCCTT

**Supplementary Table 4: Sequences of oligonucleotides.**