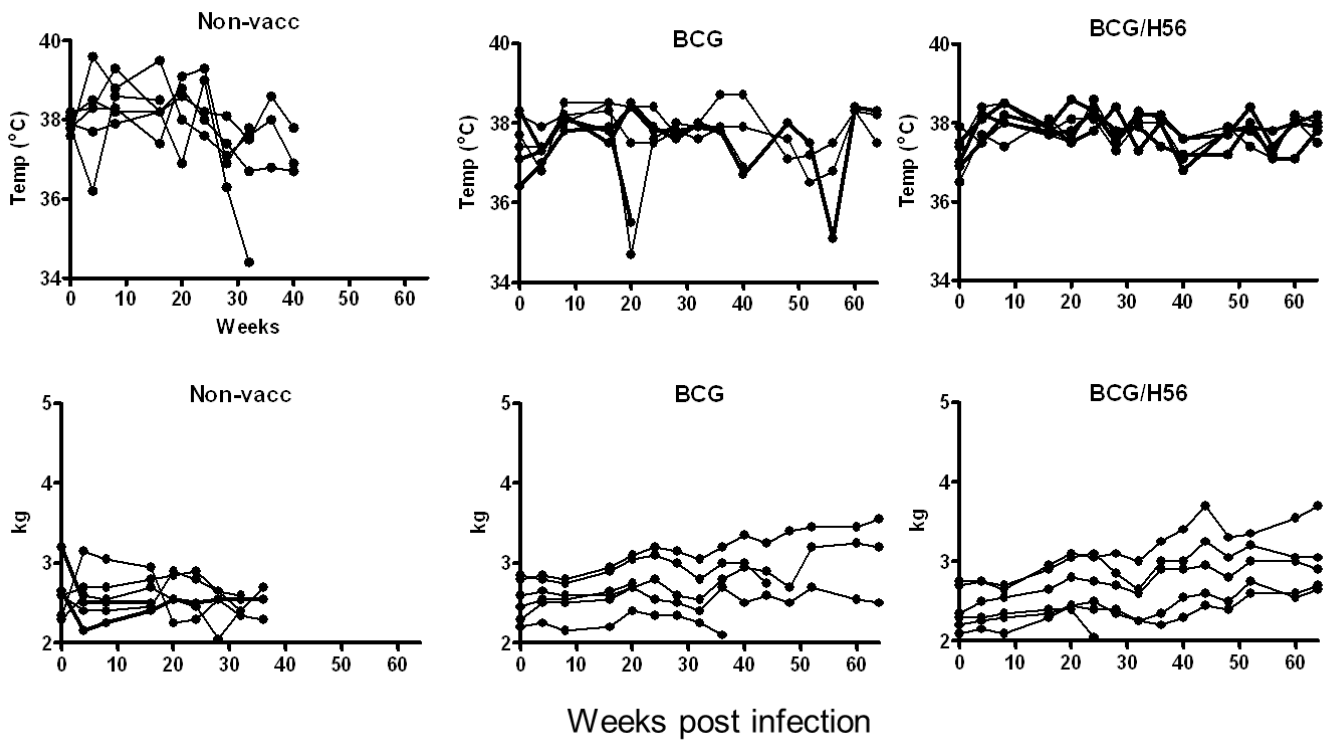


1 **Supplemental figure 1. Temperature and weight curves in monkeys after high dose *M.***
2 ***tuberculosis* infection**

3 Non-vaccinated, BCG vaccinated and BCG vaccinated/H56 boosted monkeys were infected with
4 *M.tuberculosis* (500 CFU) and evaluated from 0-64 weeks (every 4 weeks) post challenge. Weight
5 and temperature of the individual animals were determined at the indicated time points.

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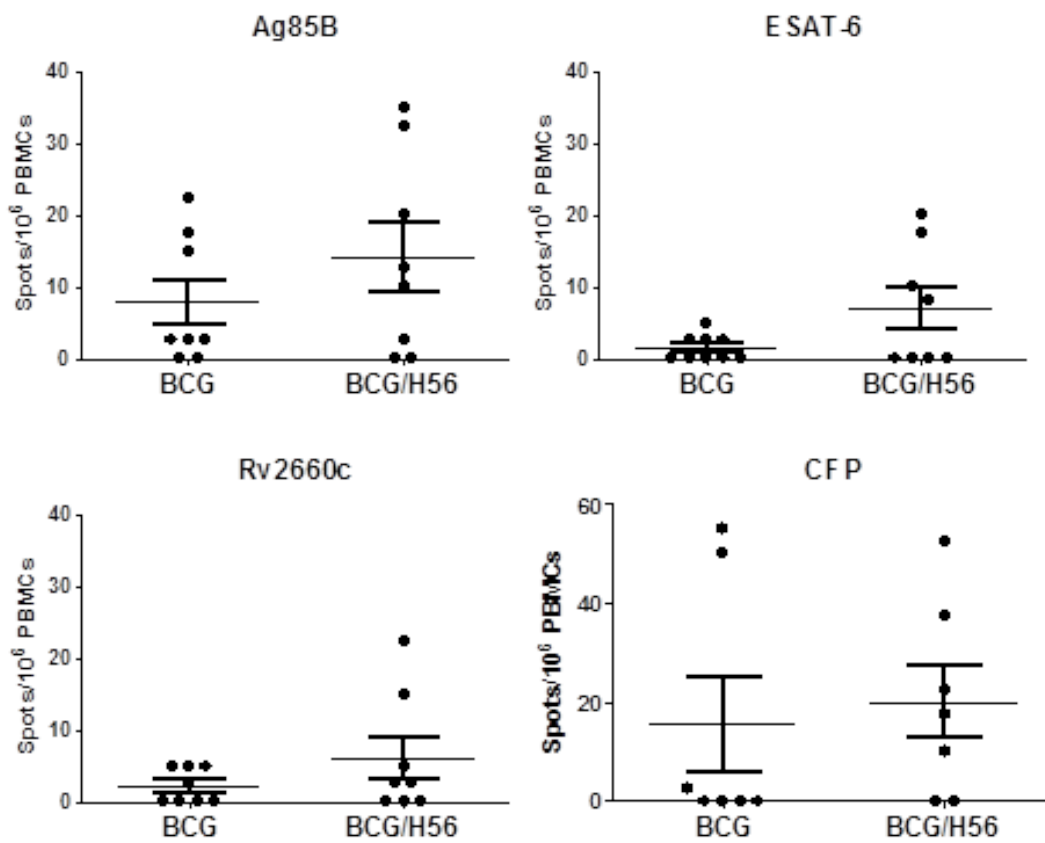
13

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15 **Supplemental figure 2. IL-2 ELISPOT after vaccination**

16 IL-2 ELISPOT was performed on 200,000 PBMCs/well from BCG and BCG vaccinated/H56
17 boosted monkeys, stimulated with Ag85B, ESAT-6, Rv2660c, media (negative control, not shown)
18 or CFP (culture filtrate proteins) for 48 hours as described in materials and methods.

19



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21

21 **Supplemental figure 3. Primer and probe sequences.**

22 flanking forward (ff) and flanking reverse (fr) for pre-amplification, forward (f) and reverse, (r)
23 primers and TaqMan probe (p) for real-time PCR.

24

25

Rv2660c:

(ff) gcagcaacaggccaggctag,
(fr) acaaaatctgggctgcgtgaac,
(f) gcggcaggcgcacatct,
(r) gaactgactcggtgcaacca,
(p) tgcccacgccgaca.

Rv3875:

(ff) ggaagcagtcacctgaccaagctc,
(fr) gccttcggtcgaagccattg,
(f) accaggggtgtccagcaaaaatg,
(r) accggcttcgctgatcgt,
(p) agctgaacaacgcgctg.

Rv1886c:

(ff) ggtactaccagtcgggactgtcgatag,
(fr) cctgttgccggacaaccattg,
(f) gcgggcagtcagcttctac,
(r) ggtcaggaaggttcccactg,
(p) ctgcggaaggctgg.

Rv2225:

(ff) gcgcacatgcggtcaagct,
(fr) gcgtcggcgcgatggttggc,
(f) ccgagcaaatgcctgtct,
(r) gccgccaagggttg,
(p) tgatggcacacatcg.

Rv3051c:

(ff) catatcaagtcggtgccctcaatc,
(fr) tgcccgcaacgcgtgatac,
(f) gatcgggctaggacagatgaac,
(r) ggtgaagtcgatgccttcgt,
(p) ccgggaacgcatct.

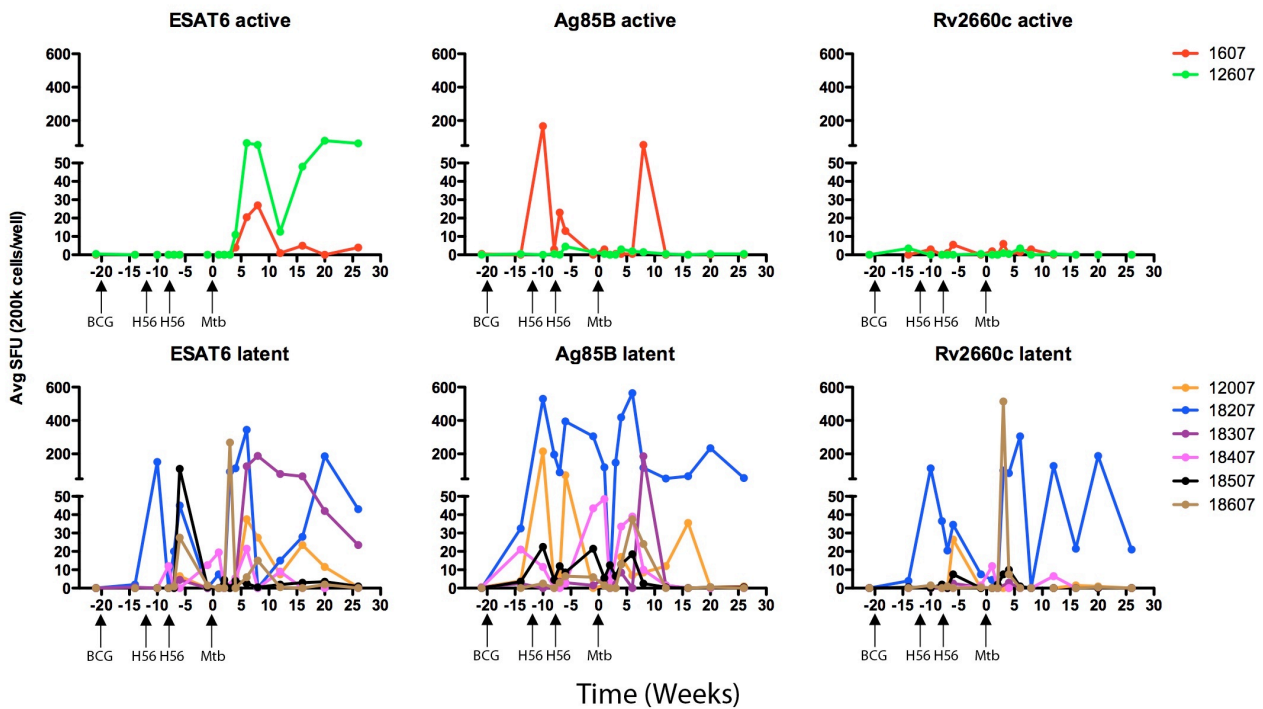
Rv3127:

(ff) caaagacgttgcatgcttgatg,
(fr) ggcgtctgatccagcgtatc,
(f) cgactggtgtagcgtcaca,
(r) ggtgaagtccaccattcgagtt,
(p) cgggacgatccgtac

25 **Supplemental figure 4. Antigen specific IFN- γ ELISPOT responses in individual monkeys.**

26 Frequency of IFN- γ producing T cells specific for vaccine antigens is elevated prior to challenge
27 and early (<4 weeks) post-challenge in the BCG/H56-vaccinated animals that would become latent
28 (N=6), but low or absent in the BCG/H56-vaccinated animals (N=2) that developed active TB. The
29 top 3 panels show responses (IFN- γ ELISPOT) from the animals that developed active disease;
30 three antigens are shown (ESAT-6, Ag85B, Rv2660c) and each line represents an individual
31 monkey. The bottom 3 panels show the responses from the monkeys who developed latent
32 infection; each line represents an individual monkey.

33



34

35 **Supplemental table 1. Clinical parameters for individual animals after high dose challenge**

36 Non-vaccinated, BCG vaccinated and BCG vaccinated/H56 boosted monkeys were infected with
 37 *M.tuberculosis* (500 CFU) and evaluated from 0 to 64 weeks (every 4 weeks) post challenge.
 38 Weight and temperature of the animals were determined at the indicated time points. Erythrocyte
 39 sedimentation rates were determined on whole blood. X-ray: the animals were scored according the
 40 following definitions: (-): negative, SN: scattered nodules, BP(u): unilateral bronchopneumonia,
 41 BP(b): bilateral bronchopneumonia, BP/Con: Bronchopneumonia with consolidation of lung ,
 42 BP/At: Bronchopneumonia with Atelectasis.

Weeks	Non Vacc						BCG						BCG/H56					
	3008 L	5566 E*	8197 A	2861 I	7156 C	6651 D	7705 B	6784 C*	7947 B	197 BJ	4418 J	5985 C	8262 A	S 4708 EC	5923 D	S 2934 N	7531 C	1290 GB
0	2.35	2.6	2.6	2.3	2.65	3.2	2.85	2.3	2.45	2.2	2.6	2.8	2.7	2.1	2.75	2.2	2.3	2.35
4	3.15	2.4	2.7	2.6	2.15	2.5	2.8	2.5	2.55	2.25	2.65	2.85	2.75	2.15	2.75	2.25	2.3	2.5
8	3.05	2.4	2.7	2.55	2.25	2.5	2.75	2.5	2.55	2.15	2.6	2.8	2.7	2.1	2.65	2.3	2.35	2.55
16	2.95	2.45	2.8	2.7	2.4	2.5	2.9	2.55	2.65	2.2	2.6	2.95	2.9	2.3	2.95	2.35	2.4	2.65
20	2.25	2.3	2.85	2.55	2.55		3.05	2.7	2.75	2.4	2.7	3.1	3.05	2.45	3.1	2.45	2.4	2.8
24	2.3	2.8	2.9	2.45	2.5		3.1	2.55	2.35	2.8	3.2	3.1	3.1	2.5	3.05	2.4	2.05	2.75
28	2.55	2.65	2.65	2.05	2.55		3	2.5	2.35	2.6	3.15	2.85	2.35	3.1	2.4			2.7
32	2.35	2.4	2.6	2.4	2.55		2.8	2.4	2.25	2.55	3.05	2.65	2.25	3	2.25			2.6
36	2.3			2.7	2.55		3	2.7	2.1	2.8	3.2	3	2.2	3.25	2.35			2.9
40							3	2.5		2.95	3.35	3	2.3	3.4	2.55			2.9
44							2.75	2.6		2.9	3.25	3.25	3.25	2.45	3.7	2.6		2.95
48							2.5			2.7	3.4	3.05	2.4	3.3	2.5			2.8
52								2.7		3.2	3.45	3.2	2.6	3.35	2.75			3
56								2.55		3.25	3.45	3.05	2.6	3.55	2.55			3
60								2.5		3.2	3.55	3.05	2.7	3.7	2.65			2.9
64																		
0	37.8	38.1	38.2	37.6	37.9	37.8	38.2	38.3	36.4	37.1	37.7	37.4	37.4	37.5	36.9	37	36.5	37.9
4	38.3	38.5	38.3	39.6	37.7	36.2	37.9	37.3	37	37.3	36.8	37.4	38.4	38.1	37.5	38.2	37.7	37.5
8	39.3	38.2	38.3	38.8	37.9	38.6	38.2	38.1	37.8	38.2	38.5	38	38.5	38.5	38.2	38	37.4	38
16	38.2	38.2	37.4	39.5	38.2	38.5	38.3	37.8	37.9	37.5	38.5	38.5	37.7	37.9	37.9	37.7	38.1	37.8
20	38.8	36.9	39.1	38	38.6		37.5	38.4	35.5	38.5	38.4	34.7	37.5	38.6	37.5	37.8	37.6	38.1
24	38	39	39.3	37.6	38.2		37.5	37.8		37.9	38.4	37.6	38.6	38.3	37.8	38.3	38.4	38.1
28	37.4	36.3	36.9	37.1	38.1		37.8	37.8		37.6	37.7	38	37.6	37.5	38.4	37.8		37.3
32	36.7	34.4	37.8	37.6	37.5		37.6	37.9		38	38	37.9	38.3	38.2	37.3	37.9		38
36	36.8			38	38.6		37.9	37.8		37.8	38.7	37.9	38.1	38.2	38	37.4		38
40							36.9	36.7			38.7	37.9	37.6	37.6	36.8	37.2		37.1
44								38			37.1	37.6	37.9	37.7	37.8	37.2		37.8
48								37.5			37.2	36.5	37.4	38.4	37.9	38		37.8
52								35.1			37.5	36.8	37.1	37.4	37.8	37.1		37.2
56								38.4			38.3	38.3	38.2	38	38	37.1		38.1
60								38.3			37.5	38.2	38	38.2	37.9	37.8		37.5
64																		
0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
4	0	0	2	0	0	0	1	0	0	0	0	2	0	0	0	0	1	1
8	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
16	10	0	3	0	0	4	0	0	3	0	2	0	1	0	0	0	5	0
20	0	3	0	0	2		1	0	3	1	0	0	0	0	0	0	13	0
24	0	4	2	0	7		0	0	0	0	0	1	0	0	0	0	5	0
28	0	21	9	0	6		0	0	5	0	0	0	0	0	1	0		0
32	0	9	24	0	2		0	0	7	0	0	0	0	0	0	0		1
36	20		11	2	2		1	0	10	0	0	0	0	0	1	1		0
40							4	1		3	1	0	0	2	0	0		2
44								1		3	1	0	0	0	0	0		1
48							0			0	0	0	0	0	0	0		1
52							0			0	0	0	0	0	0	1		0
56							0			0	0	0	0	0	0	0		0
60							0			0	0	0	0	0	1	0		0
64							0			0	0	0	0	1	0	0		0
0	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4	BP(u)	BP(u)	(-)	(-)	(-)	(-)	(-)	SN	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
8	(-)	BP(b)	(-)	(-)	(-)	(-)	(-)	SN	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
16	(-)	BP(b)	BP(At)	(-)	BP(b)	BP(u)	(-)	SN	BP(u)	BP(b)	(-)	(-)	(-)	(-)	(-)	(-)	BP(b)	(-)
20	(-)	BP(b)	BP	(-)	BP(b)		(-)	SN	BP(b)	BP(b)	BP(u)	(-)	SN	(-)	(-)	(-)	BP(b)	(-)
24	SN	BP(b)	BP/At	SN	BP(b)		(-)	SN	BP(b)	BP(b)	BP(u)	(-)	(-)	(-)	(-)	(-)	BP/Con	(-)
28	SN	BP(b)	BP/At	BP(u)	BP(b)		(-)	SN	BP(b)	BP(b)	BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
32	(-)	BP(b)	BP/At	BP(u)	BP(b)		(-)	SN	BP(b)	BP(b)	BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
36	(-)		BP/At	BP(u)	BP(b)		(-)	SN	BP/Con	BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
40							(-)	SN	BP/Con	BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
44							(-)	SN		BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
48								BP(u)		BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
52								(-)		BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
56								(-)		BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
60								(-)		BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
64								(-)		BP(u)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

59 **Supplemental table 2. Pathology scores at necropsy after high dose challenge**

60 Gross pathology in lungs and other organs. Pathology scores (maximum score is 10) given as
 61 individual organ scores of lungs and other organs in the vaccine and control groups after infection
 62 with *M. tuberculosis*. “Additional organs” include Pericardium, Stomach and bone.

Animal number	SCORE	RT. LUNG	LF. LUNG	SPLEEN	KIDNEY	LIVER	PERICARDIUM	STOMACH	BONE	THORACIC LYPMH N.
Non-vacc.										
6651 D	22	7	7	7	0	0	0	0	0	1
3008 L	19	3	0	3	0	7	0	0	6	0
5566 E	24	7	6	3	0	4	0	0	4	0
8197 A	30	10	7	4	0	5	0	0	4	0
2861 I	08	2	2	0	2	0	0	0	0	2
7156 C	16	4	4	3	3	2	0	0	0	0
BCG										
7705 B	02	0	2	0	0	0	0	0	0	0
6784 C	00	0	0	0	0	0	0	0	0	0
7947 B	27	10	9	5	3	0	0	0	0	0
197 BJ	35	10	7	0	5	7	2	0	4	0
4418 J	09	7	0	2	0	0	0	0	0	0
5985 C	00	0	0	0	0	0	0	0	0	0
BCH/H56										
8262 A	00	0	0	0	0	0	0	0	0	0
S4708 EC	00	0	0	0	0	0	0	0	0	0
5923 D	00	0	0	0	0	0	0	0	0	0
S2934 N	05	0	5	0	0	0	0	0	0	0
7531 C	34	10	10	4	0	3	0	0	4	3
1290 GE	00	0	0	0	0	0	0	0	0	0