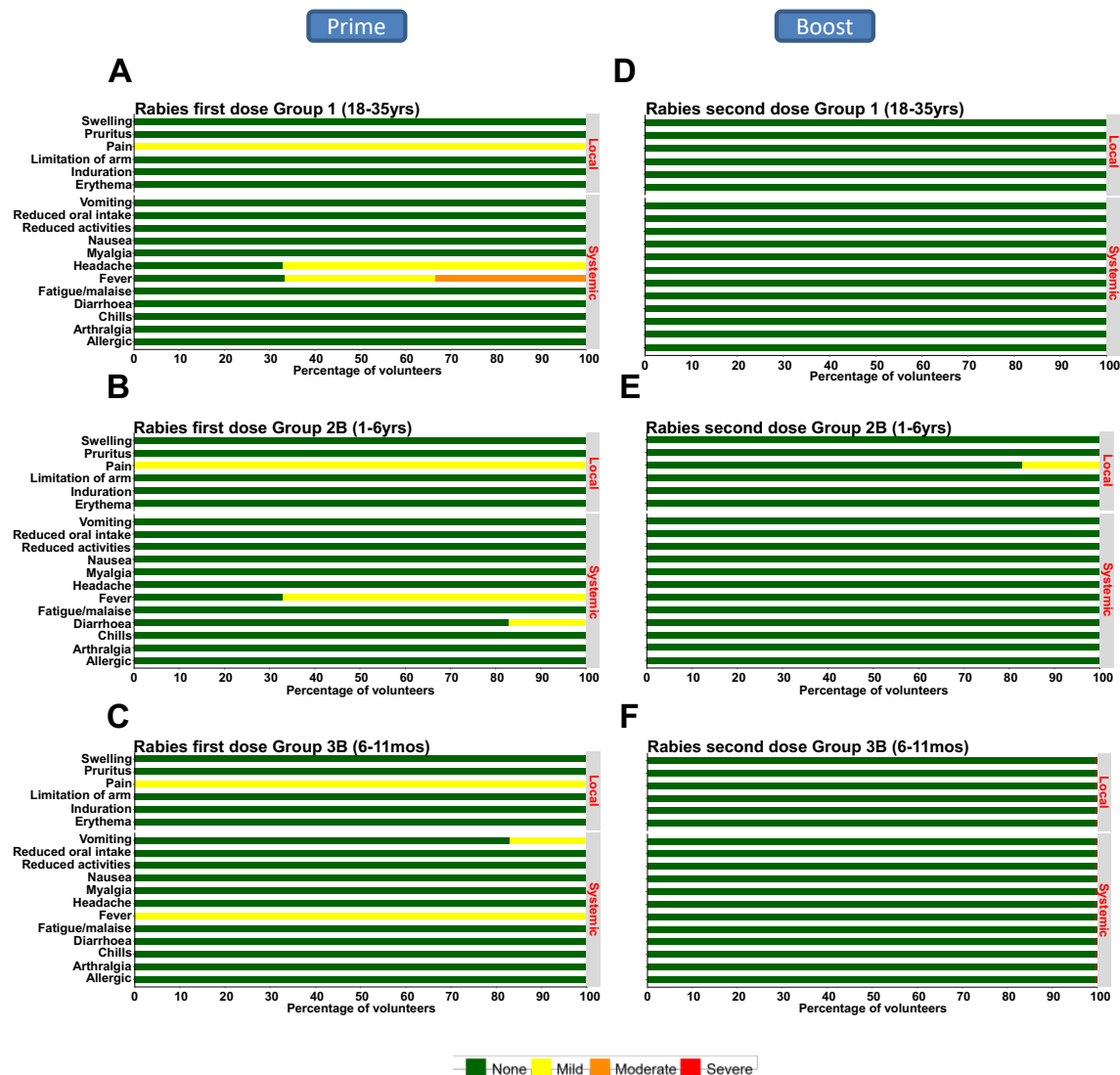
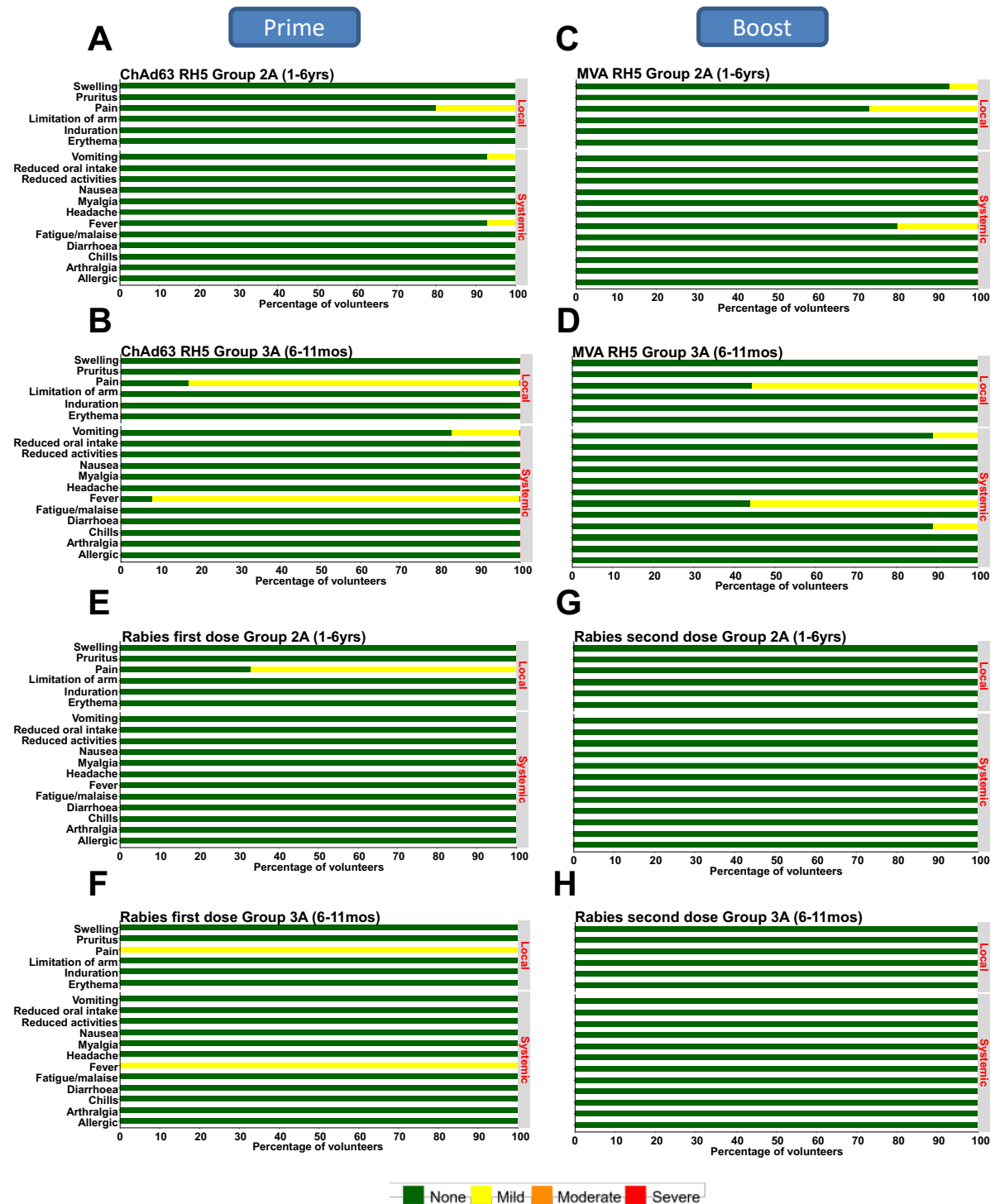


Supplementary Figures



Supplementary Figure 1. Solicited AEs following vaccination with rabies vaccine control in Groups 1, 2B and 3B; related to Figure 2.

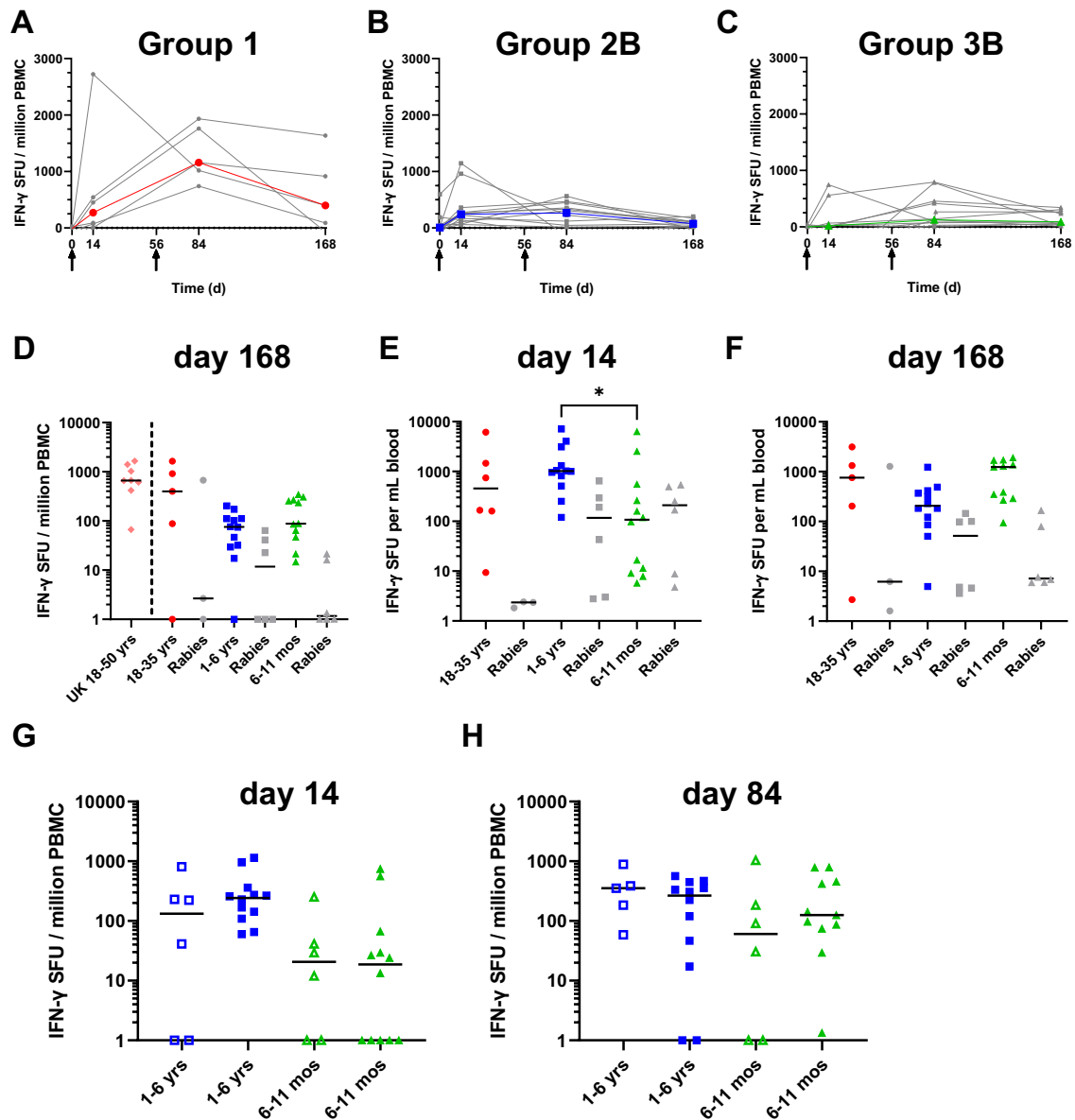
The solicited local and systemic AEs recorded for 7 days following prime and boost with rabies vaccine control are shown at the maximum severity reported by all volunteers within the VAC070 trial Groups 1, 2B and 3B. Volunteers aged (A) 18-35 years (n=3); (B) 1-6 years (n=6); and (C) 6-11 months (n=6) received rabies vaccine control on day 0, and the same volunteers aged (D) 18-35 years (n=3); (E) 1-6 years (n=6); and (F) 6-11 months (n=6) received a second dose of the same control on day 56.



Supplementary Figure 2. Solicited AEs following vaccination with a lower “lead in” dose of ChAd63 RH5 and MVA RH5 or rabies vaccine control in Groups 2A and 3A; related to Figure 2.

The solicited local and systemic AEs recorded for 7 days following the lower “lead in” dose of ChAd63 RH5 prime and MVA RH5 boost, or rabies vaccine control, are shown at the maximum

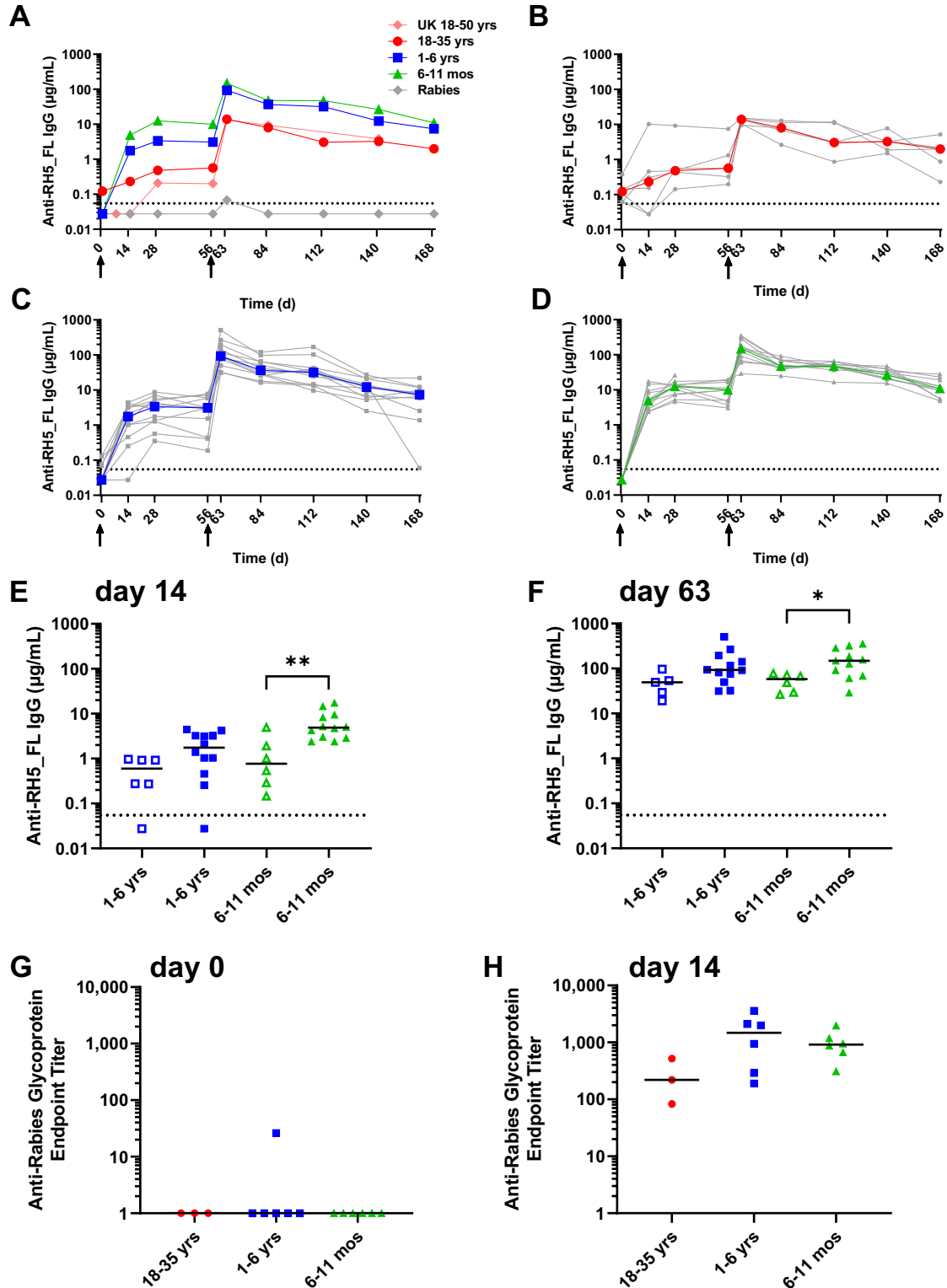
severity reported by all volunteers within the VAC070 trial Groups 2A and 3A. Volunteers aged **(A)** 1-6 years (n=6); and **(B)** 6-11 months (n=6) received the lower “lead in” priming dose of 1×10^{10} vp ChAd63 RH5 on day 0. The same volunteers aged **(C)** 1-6 years (n=5); and **(D)** 6-11 months (n=6) received the lower “lead in” boosting dose of 1×10^8 pfu MVA RH5 on day 56. Volunteers aged **(E)** 1-6 years (n=3); and **(F)** 6-11 months (n=3) received rabies vaccine control on day 0, and the same volunteers aged **(G)** 1-6 years (n=3); and **(H)** 6-11 months (n=3) received a second dose of the same control on day 56.



Supplementary Figure 3. *Ex-vivo* IFN- γ ELISPOT data; related to Figure 3.

Median (in color) and individual (in grey) *ex-vivo* IFN- γ ELISPOT responses to RH5 per million PBMC are shown over time (days 0, 14, 84 and 168) for all groups receiving the full dose of ChAd63-MVA RH5 vaccines. Responses are shown for (A) Group 1 adults 18-35 years, N=5 or 6; (B) Group 2B children 1-6 yrs, N=12; and (C) Group 3B infants 6-11 months, N= 11 or 12. (D) Median and individual *ex-vivo* IFN- γ ELISPOT responses to RH5 per million PBMC are shown for all groups receiving the full dose of ChAd63-MVA RH5 vaccines and rabies vaccine control at day 168, sixteen

weeks post-MVA RH5 boost. Group 1 adults 18-35 years, N=5; Group 2B children 1-6 years, N=12; Group 3B infants 6-11 months, N=11; rabies vaccine controls shown in grey to the right of each age group with same symbol, N=3, 6 and 6, respectively. Historical data testing the identical dose and regimen of the ChAd63-MVA RH5 vaccine in healthy UK adults 18-50 years are shown for comparison only, N=8 (note in the UK trial these data were taken at day 140, the closest comparator time-point) ¹. **(E)** Median and individual *ex-vivo* IFN- γ ELISPOT responses to RH5 per mL peripheral blood are shown for all groups receiving the full dose of ChAd63-MVA RH5 vaccines and rabies vaccine control at day 14, and **(F)** day 168. Groups as per **Figure 3A** and panel (D), respectively. Analysis using Kruskal-Wallis test with Dunn's multiple comparison test across Groups 1, 2B and 3B only; * $P < 0.05$. **(G)** Median and individual *ex-vivo* IFN- γ ELISPOT responses to RH5 per million PBMC are also shown at day 14, two weeks post-ChAd63 RH5 prime, and **(H)** day 84, four weeks post-MVA RH5 boost in Group 2A children 1-6 years, N=5 or 6, and Group 3A infants 6-11 months, N=6, who received the lower "lead in" doses of ChAd63-MVA RH5 (open symbols) alongside those who received the full dose of vaccine in Groups 2B and 3B (closed symbols; data as per **Figure 3A,B**).

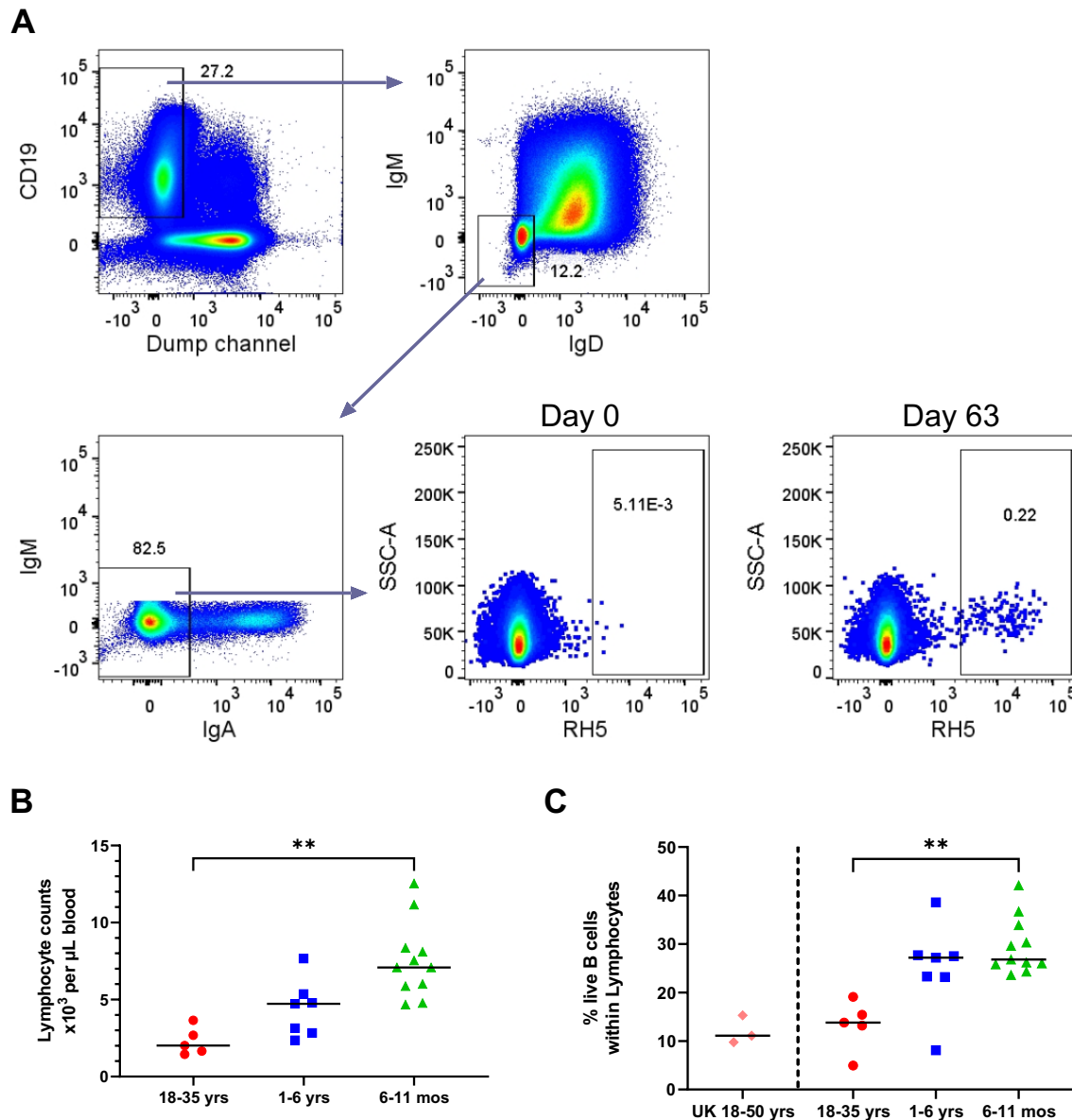


Supplementary Figure 4. Serum antibody ELISA data; related to Figure 4.

(A) Median anti-RH5_FL serum total IgG responses as measured by ELISA are shown over time for all groups receiving the full dose of ChAd63-MVA RH5 vaccines: Group 1 adults 18-35 years (red),

N=5 or 6; Group 2B children 1-6 yrs (blue), N=12; and Group 3B infants 6-11 months (green), N= 11 or 12; as well as rabies vaccine control (grey), all groups and ages pooled, N=15. Historical data testing the identical dose and regimen of the ChAd63-MVA RH5 vaccine in healthy UK adults 18-50 years (pink) are shown for comparison only: N=20 (up to day 56) and N=8 (onwards from day 63) ¹.

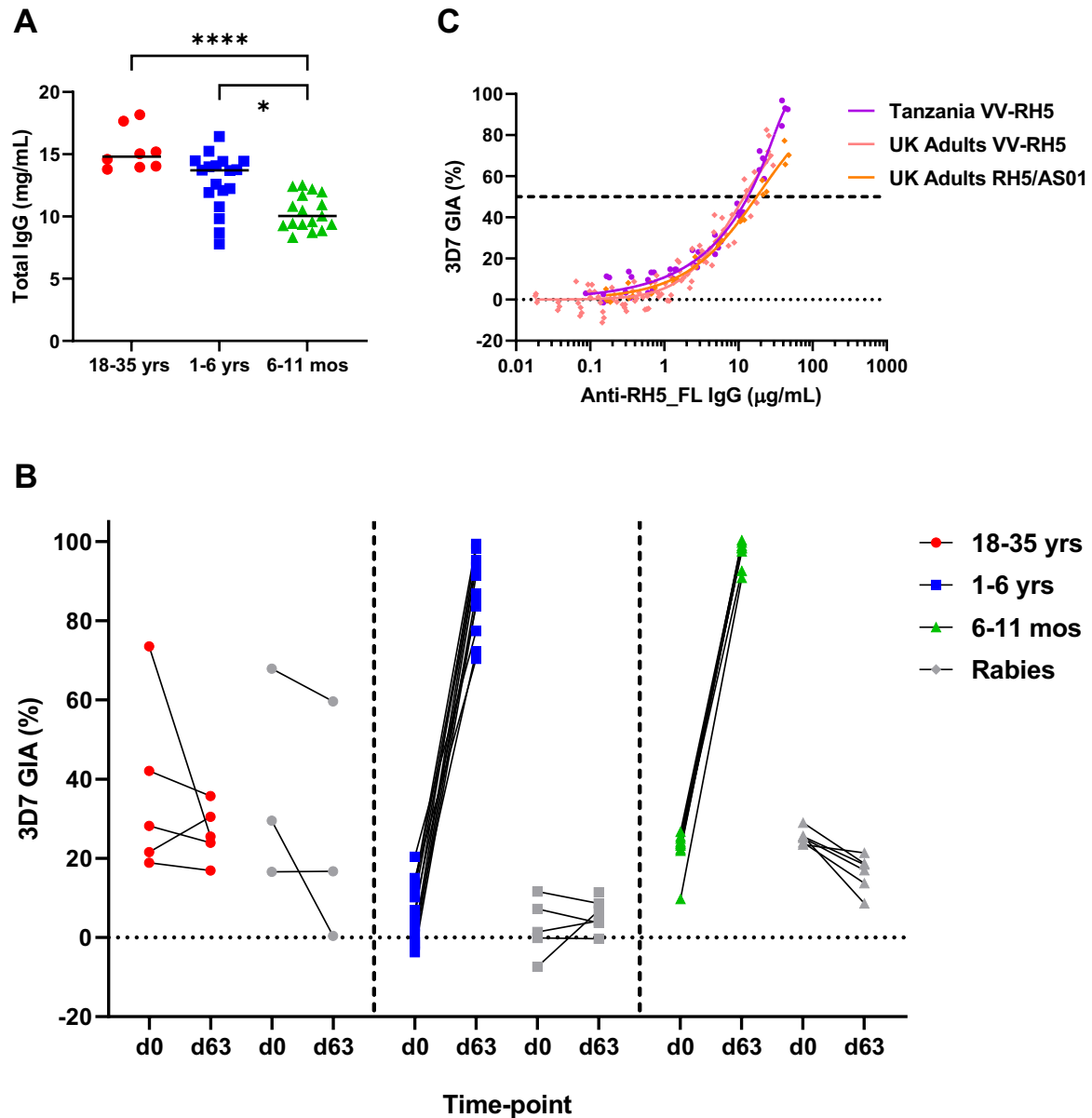
(B) Median (in color) and individual (in grey) anti-RH5_FL serum total IgG responses as measured by ELISA are also shown over time for Group 1 adults 18-35 years, N=5 or 6, receiving the full dose of ChAd63-MVA RH5 vaccines, with the same also shown for **(C)** Group 2B children 1-6 yrs, N=12; and **(D)** Group 3B infants 6-11 months, N= 11 or 12. Arrows indicate vaccination time-points, and the horizontal dotted line indicates the limit of detection of the assay. Median and individual anti-RH5_FL serum total IgG responses as measured by ELISA are also shown at **(E)** day 14, two weeks post-ChAd63 RH5 prime, and **(F)** day 63, one week post-MVA RH5 boost in Group 2A children 1-6 years, N=5 or 6, and Group 3A infants 6-11 months, N=6, who received the lower “lead in” doses of ChAd63-MVA RH5 (open symbols) alongside those who received the full dose of vaccine in Groups 2B and 3B (closed symbols; data as per **Figure 4A,B**). Analysis using Kruskal-Wallis test with Dunn’s multiple comparison test comparing only within Group 2 (2A vs 2B) and within Group 3 (3A vs 3B); * $P < 0.05$, ** $P < 0.01$. **(G)** Median and individual anti-rabies glycoprotein serum total IgG responses as measured by ELISA in day 0 samples (pre-vaccination) and **(H)** day 14 samples (after one vaccination) are shown for individuals receiving rabies vaccine control: Group 1 adults 18-35 years, N=3; Group 2B children 1-6 years, N=6; Group 3B infants 6-11 months, N=6.



Supplementary Figure 5. Analyses of RH5-specific B cells; related to Figure 5.

(A) Gating strategy for identification of RH5-specific cells within the live CD19⁺ IgD⁻ IgM⁻ IgA⁻ B cell population. Representative RH5 probe staining is shown for matched Day 0 and Day 63 samples from a single vaccinee. Dump channel includes: anti-human CD3, anti-human CD14, anti-human CD56, and a cell viability stain. See Methods for further details. (B) Lymphocyte counts per microliter of blood for samples analyzed by flow cytometry. Group 1 adults 18-35 years, N=5; Group 2B children 1-6 years, N=7; Group 3B infants 6-11 months, N=11. (C) Percentage of live CD19⁺ B cells within lymphocytes as assessed by flow cytometry. Same individuals as shown in (B). Data from

baseline samples for three healthy UK adult volunteers 18-50 years of age enrolled in a previous trial of the ChAd63-MVA RH5 vaccine ¹ are shown for comparison only. Individual and median results are shown in each panel. Analyses used Kruskal-Wallis test with Dunn's multiple comparison test across the three vaccinated groups; ** $P < 0.01$.



Supplementary Figure 6. Serum IgG concentration and GIA assay data; related to Figure 6.

(A) Total serum IgG concentrations in mg/mL at the day 63 time-point for the vaccinated volunteers in the VAC070 trial as measured by HPLC. Median and individual results are shown for all volunteers (RH5 vaccine and rabies vaccine control): Group 1 adults 18-35 years (red), N=8; Group 2B children 1-6 yrs (blue), N=18; and Group 3B infants 6-11 months (green), N= 17. (B) *In vitro* growth inhibition activity (GIA) of purified IgG was assessed against 3D7 clone *P. falciparum* parasites. Total IgG purified from serum at i) baseline (day 0) pre-vaccination, and ii) day 63, one week post-

MVA RH5 boost vaccination, was initially tested at each sample's physiological total serum IgG concentration as measured in (A). Individual linked data shown for Group 1 adults 18-35 years, N=5 vaccinees and N=3 control; Group 2B children 1-6 years, N=12 vaccinees and N=5 or 6 control (one baseline sample was lost during analysis); and Group 3B infants 6-11 months, N=11 vaccinees and N=6 control. (C) Samples from three different RH5 vaccine clinical trials were compared for anti-RH5 IgG "functional quality" in an independent head-to-head experiment in the same GIA assay. Samples included those from i) the Tanzanian VAC070 trial (only children and infants) of the viral-vectored ChAd63-MVA RH5 (VV-RH5) vaccine reported here; ii) the VAC057 trial of the VV-RH5 vaccine tested previously in healthy UK adults ¹; and iii) the VAC063 trial of the RH5.1/AS01_B protein-in-adjuvant vaccine tested previously in healthy UK adults ². Purified total IgG samples were i) titrated in the GIA assay and ii) tested by ELISA to quantify anti-RH5_FL IgG per mg total IgG. The relationship between the measured GIA and concentration of anti-RH5_FL IgG used in the assay are plotted. Non-linear regression curves are shown for all samples combined from VAC070 (purple line, $r^2 = 0.97$, N=45); VAC057 (pink line, $r^2 = 0.92$, N=90); and VAC063 (orange line, $r^2 = 0.98$, N=27). Dashed line indicates the EC₅₀ (concentration of anti-RH5_FL polyclonal IgG that gives 50% GIA).

Supplementary Tables

Age Group	Demographic	ChAd63 RH5 / MVA RH5	Rabies	Total
6-11 mos	N =	18	9	27
	Age (mos)	9.6 (5.9-11.9)	9.1 (8.0-10.2)	9.4 (5.9-11.9)
	Male	10 (55.6%)	4 (44.4%)	14 (51.9%)
	Female	8 (44.4%)	5 (55.6%)	13 (48.1%)
	Weight (kg)	8.6 (7.0-10.5)	8.6 (7.0-10.0)	8.6 (7.0-10.5)
	Height (cm)	71.1 (59.0-81.1)	69.6 (66.0-72.2)	70.6 (59.0-81.1)
	BMI (kg/m²)	17.2 (13.7-20.8)	17.6 (14.5-20.4)	17.3 (13.7-20.8)
	MUAC (cm)	14.6 (12.0-16.6)	14.4 (13.4-16.0)	14.5 (12-16.6)
1-6 yrs	N =	18	9	27
	Age (yrs)	3.4 (1.2-5.6)	4.0 (2.0-5.6)	3.6 (1.2-5.6)
	Male	8 (44.4%)	3 (33.3%)	11 (40.7%)
	Female	10 (55.6%)	6 (66.7%)	16 (59.3%)
	Weight (kg)	13.0 (9.0-17.0)	13.7 (10.0-17.6)	13.3 (9.0-17.6)
	Height (cm)	94.6 (75.9-107.0)	98.8 (83.6-111.0)	96.0 (75.9-111.0)
	BMI (kg/m²)	14.5 (12.5-16.5)	14.0 (12.7-15.9)	14.4 (12.5-16.5)
	MUAC (cm)	15.4 (13.5-17.2)	15.2 (14.0-16.7)	15.3 (13.5-17.2)
18-35 yrs	N =	6	3	9
	Age (yrs)	24.8 (19.0-30.2)	27.7 (24.6-30.7)	25.8 (19.0-30.7)
	Male	5 (83.3%)	2 (66.7%)	7 (77.8%)
	Female	1 (16.7%)	1 (33.3%)	2 (22.2%)
	Weight (kg)	56.7 (52.0-60.0)	59.0 (58.0-60.0)	57.4 (52.0-60.0)
	Height (cm)	164.9 (152.0-173.5)	166.3 (159.0-176.0)	165.4 (152.0-176.0)
	BMI (kg/m²)	21.0 (18.0-24.2)	21.4 (19.4-23.3)	21.1 (18.0-24.2)

Supplementary Table 1. Demographics of VAC070 trial participants; related to Figure 1.

Baseline characteristics are presented as a frequency (%) or mean value with range. BMI = body mass index; MUAC = mid-upper arm circumference.

Age Group	MedDRA system organ class	MedDRA preferred term	ChAd63-MVA RH5	Rabies	Total
6-11 mos	Gastrointestinal disorders	Diarrhoea	0	3	3
	Respiratory, thoracic and mediastinal disorders	Viral upper respiratory tract infection	7	4	11
	Ear and labyrinth disorders	Otitis media	1	0	1
1-6 yrs	General disorders and administrative site conditions	Pyrexia	1	0	1
	Respiratory, thoracic and mediastinal disorders	Viral upper respiratory tract infection	1	2	3
	Skin and subcutaneous tissue disorders	Folliculitis	0	1	1
	Infections and infestations	Cutaneous larva migrans	0	1	1
18-35 yrs	Respiratory, thoracic and mediastinal disorders	Viral upper respiratory tract infection	1	0	1
		Tonsillitis	0	1	1
	Nervous system disorders	Headache	1	0	1

Supplementary Table 2. Unsolicited AEs following vaccination with ChAd63-MVARH5, or rabies control vaccine; related to Figure 2.

Unsolicited AEs were collected for 28 days after each dose of vaccine and are presented as frequency. None of the reported unsolicited AEs were assessed as possibly, probably or definitely related to vaccination.

Lab Parameters	ChAd63-MVA RH5				Rabies				Total			
	Number of abnormalities	Grade			Number of abnormalities	Grade			Number of abnormalities	Grade		
		1	2	3		1	2	3		1	2	3
Infants 6-11 mos												
Increased WBC	8	1	3	4	1	0	1	0	9	1	4	4
Decreased WBC	1	1	0	0	0	0	0	0	1	1	0	0
Increased Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Haemoglobin	7	6	1	0	2	2	0	0	9	8	1	0
Decreased Platelets	1	0	1	0	0	0	0	0	1	0	1	0
Increased Neutrophil	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Neutrophil	0	0	0	0	0	0	0	0	0	0	0	0
Increased Eosinophil	0	0	0	0	0	0	0	0	0	0	0	0
Increased Creatinine	1	1	0	0	0	0	0	0	0	0	0	0
Increased ALT	3	3	0	0	1	1	0	0	4	4	0	0
Children 1-6 yrs												
Increased WBC	1	1	0	0	1	1	0	0	2	2	0	0
Decreased WBC	1	1	0	0	1	1	0	0	2	2	0	0
Increased Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Lymphocytes	2	2	0	0	1	1	0	0	3	3	0	0
Decreased Haemoglobin	1	0	1	0	1	1	0	0	2	1	1	0
Decreased Platelets	0	0	0	0	0	0	0	0	0	0	0	0
Increased Neutrophil	0	0	0	0	1	1	0	0	1	1	0	0
Decreased Neutrophil	0	0	0	0	0	0	0	0	0	0	0	0
Increased Eosinophil	1	1	0	0	1	1	0	0	2	2	0	0
Increased Creatinine	0	0	0	0	0	0	0	0	0	0	0	0
Increased ALT	2	2	0	0	0	0	0	0	2	2	0	0
Adults 18-35 yrs												
Increased WBC	0	0	0	0	1	1	0	0	1	1	0	0
Decreased WBC	2	2	0	0	0	0	0	0	2	2	0	0
Increased Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Lymphocytes	1	1	0	0	1	1	0	0	2	2	0	0
Decreased Haemoglobin	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Platelets	0	0	0	0	1	1	0	0	1	1	0	0
Increased Neutrophil	0	0	0	0	0	0	0	0	0	0	0	0
Decreased Neutrophil	0	0	0	0	0	0	0	0	0	0	0	0
Increased Eosinophil	0	0	0	0	0	0	0	0	0	0	0	0
Increased Creatinine	0	0	0	0	1	1	0	0	1	1	0	0
Increased ALT	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table 3. Laboratory AEs related to vaccination with ChAd63-MVA RH5 or rabies vaccine control; related to Figure 2.

Table shows laboratory AEs recorded within 28 days of each vaccine administration and considered possibly, probably or definitely related to vaccination. Total numbers vaccinated with ChAd63-MVA RH5: Group 1 adults 18-35 years, N=6; Group 2 children 1-6 years, N=18; Group 3 infants 6-11 months, N=18. Total numbers vaccinated with rabies control: Group 1 adults 18-35 years, N=3; Group 2 children 1-6 years, N=9; Group 3 infants 6-11 months, N=9.

WBC = white blood cells (leukocytes); ALT = alanine aminotransferase.

Name	Sequence	Pool	
RH5 1	ENAIKTKNQENLALLPIK	P1	Disordered N-terminus
RH5 2	ENNALLPIKSTEEKDDIK	P1	
RH5 3	STEEKDDIKNGDKIKEID	P1	
RH5 4	NGDKIKEIDNDKENIKTNN	P1	
RH5 5	NDKENIKTNNAKDHSTYIKS	P1	
RH5 6	AKDHSTYIKSYLNTNVNDGL	P1	
RH5 7	YLNTNVNDGLKYLFIPIHNS	P1	
RH5 8	KYLFIPIHNSFIKKYSVFNQ	P1	
RH5 9	FIKKYSVFNQINDGMLLNEK	P1	
RH5 10	INDGMLLNEKNDVKNNDYK	P1	
RH5 11	NDVKNNDYKNDYKNNVFL	P1	
RH5 12	NVDYKNNVFLQYHFKELSNY	P1	
RH5 13	QYHFKELSNYNIANSIDILQ	P1	
RH5 14	NIANSIDILQKEGHLDFVI	P2	Start of 45 kDa Fragment
RH5 15	EKEGHLDFVIIPHYTFLDY	P2	
RH5 16	IPHYTFLDYKHLSYNSIYH	P2	
RH5 17	KHLSYNSIYHKSSTYKCIA	P2	
RH5 18	KSSTYKCIADVAFIKKINE	P2	
RH5 19	VDAFIKKINEAYDKVSKCN	P2	
RH5 20	AYDKVSKCNDIKNDLIATI	P2	
RH5 21	DIKNDLIATIKKLEHPYDIN	P2	
RH5 22	KKLEHPYDINNKNDDSYRYD	P3	Disordered Loop
RH5 23	NKNDDSYRYDISEIDDKSE	P3	
RH5 24	ISEIDDKSEETDDETEEVE	P3	
RH5 25	ETDDETEEVEDSIQDTSNH	P3	
RH5 26	DSIQDTSNHAPSNNKKNDL	P3	
RH5 27	APSNNKKNDLMNRAFKMMMD	P3	
RH5 28	MNRAFKMMMEYNTKKKKLI	P4	Ordered Region 1
RH5 29	EYNTKKKKLIKCIKNHENDF	P4	
RH5 30	KCIKNHENDFNKICMDMKNY	P4	
RH5 31	NKICMDMKNYGTNLFQQLSC	P4	
RH5 32	GTNLFQQLSCYNNNFNTNG	P4	
RH5 33	YNNNFNTNGIRYHYDEYIH	P4	
RH5 34	IRYHYDEYIHKLILSVKSKN	P4	
RH5 35	KLILSVKSKNLNKDLSMTN	P4	
RH5 36	LNKDLSMTNLQQSELLT	P5	Ordered Region 2
RH5 37	ILQQSELLTNLNKKMGSIYI	P5	
RH5 38	NLNKKMGSIYIYDTIKFIHK	P5	
RH5 39	YIDTIKFIHKEMKHIFNRIE	P5	
RH5 40	EMKHIFNRIEYHTKIINDKT	P5	
RH5 41	YHTKIINDKTIIQDKIKLN	P5	
RH5 42	KIIQDKIKLNIWRTFQKDEL	P5	
RH5 43	IWRTFQKDELLKRILDMANE	P5	
RH5 44	LKRILDMANEYSLFITSDHL	P6	Ordered Region 3
RH5 45	YSLFITSDHLRQMLYNTFY	P6	
RH5 46	RQMLYNTFYSEKHLNNIFH	P6	
RH5 47	KEKHLNNIFHLYVLQMKF	P6	
RH5 48	HLIYVLQMKFNDVPIKMEYF	P6	
RH5 49	NDVPIKMEYFQTYKKNKPLT	P6	
RH5 50	QTYKKNKPLTQ	P6	Disordered C-terminus

Supplementary Table 4. RH5 overlapping peptides for ELISPOT assays; related to

Figure 3.

20mer peptides overlapping by 10 aa were used to measure T cell responses by *ex-vivo* IFN- γ

ELISPOT assay against the whole of the RH5 insert present in the ChAd63 and MVA vaccines

(except for the final peptide RH5-50 which is an 11mer overlapping by 10aa). Peptide sequences are

shown, and peptide pools (P) 1-6 used in the ELISPOT assay indicated. These pools corresponded to

regions of interest identified from RH5 structural and biochemical analysis ^{3,4}. Summed total responses across all the RH5 peptide pools in the ELISPOT assay are reported. Two potential or predicted sites of N-linked glycosylation (at sequons N-X-S/T) were mutated Asn (N) to Gln (Q) in the viral vaccine insert (at positions N38 and N214; green letters) ¹; whereas, in a protein version of this vaccine, called RH5.1, all four possible sequons (starting at N38, N214, N284 and N297) were mutated Thr (T) to Ala (A) (green, blue and red letters) ⁵. The peptides used in this study corresponded to the RH5.1 protein vaccine sequence, and thus had small mismatches to the RH5 vaccine insert used in the ChAd63 and MVA viral vaccines. This could have potentially led to small under-estimations of the vaccine-induced T cell response if these mutations occurred in critical residues of T cell epitopes.

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