Supplementary Materials

Supplementary Materials and Methods

Study Design

The study was conducted in the UK at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), University of Oxford, Oxford, and the NIHR WTCRF in Southampton. Healthy, malaria-naïve males and non-pregnant females aged 18-50 were invited to participate in the study. Allocation to study groups occurred at screening based on sequential recruitment of groups and volunteer preference. For safety reasons the first volunteer who received a new vaccine dose was vaccinated alone and there was at least a 48 hour gap before subsequent volunteers were vaccinated. A further two volunteers could be vaccinated 48 hours after the first, and then at least another 48 hours gap had to elapse before the rest of the volunteers receiving that dose of vaccine could be vaccinated. Safety stopping and holding rules were used in this study to ensure participant safety, particularly given that this was a first-in-human dose escalation study, as detailed below.

Group Holding Rules

The study would have been put on hold if any of the following criteria were reached:

Solicited local adverse events:

- If more than 25% of doses of a vaccine were followed by Grade 3 solicited local adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 48 h.

Solicited systemic adverse events:

- If more than 25% of doses of a vaccine were followed by Grade 3 solicited systemic adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 48 h.

Unsolicited adverse events:

- If more than 25% of volunteers developed a Grade 3 unsolicited adverse event (including the same laboratory adverse event) that was considered possibly, probably or definitely related to vaccination and persisted at Grade 3 for > 48 h. For the ChAd63 RH5 vaccination, Groups

2A and 2B were considered as one group as they were enrolled simultaneously and received the same vaccine dose.

A serious adverse event considered possibly, probably or definitely related to vaccination, a death or a life-threatening reaction occurred.

Individual Stopping Rules (applied to all vaccinated individuals)

In addition to the above stated group holding rules, stopping rules for individual volunteers applied (i.e. indications to withdraw individuals from further vaccinations). Volunteers would have been withdrawn from further vaccinations if any of the events listed below occurred and were considered possibly, probably or definitely related to vaccination.

Local reactions:

- Injection site ulceration, abscess or necrosis.

Laboratory AEs:

- If the volunteer developed a Grade 3 laboratory adverse event considered possibly, probably or definitely related within 7 days after vaccination which persisted continuously at Grade 3 for > 72 h.

Systemic solicited adverse events:

- If the volunteer developed a Grade 3 systemic solicited adverse event considered possibly, probably or definitely related within 2 days after vaccination (day of vaccination and one subsequent day) which persisted continuously at Grade 3 for > 72 h.

Unsolicited adverse events:

- If the volunteer had a Grade 3 adverse event, which persisted continuously at Grade 3 for > 72 h.
- If the volunteer had a serious adverse event.

If the volunteer had an acute allergic reaction or anaphylactic shock following the administration of the vaccine investigational product.

Safety reviews were carried out by the Local Safety Monitor (LSM) prior to each dose escalation, and no concerns were raised with any of the vaccine doses.

Participants in Groups 2B and 2C received the ChAd63 RH5 and MVA RH5 at days 0 and 56 (nominal study days are used throughout; a window period of ± 7 days was permitted in the protocol).

Monitoring

The LSM provided safety oversight, and Good Clinical Practice (GCP) compliance was independently monitored by the University of Oxford Clinical Trials and Research Governance (CTRG) Office.

Inclusion and Exclusion Criteria

A medical history and physical examination were conducted at the screening visit, as well as baseline blood tests including a full blood count; urea and electrolytes; liver function tests; and hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) serology. Dipstick urinalysis for all volunteers and pregnancy testing for all female volunteers were conducted at screening. Pregnancy testing was also carried out prior to each vaccination. A full list of inclusion and exclusion criteria is shown below:

Inclusion Criteria

The volunteer had to satisfy all of the following criteria to be eligible for the study:

- Healthy adult aged 18 to 50 years;
- Able and willing (in the Investigator's opinion) to comply with all study requirements;
- Willing to allow the discussion of their medical history with their GP;
- For females only, willingness to practice continuous effective contraception during the study and a negative pregnancy test on the days of screening and vaccination;
- Agreement to refrain from blood donation during the course of the study;
- Provide written informed consent.

Exclusion Criteria

The volunteer could not enter the study if any of the following applied:

- Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment or during the study period;
- Prior receipt of an investigational malaria vaccine or any other investigational vaccine likely to impact on interpretation of the trial data;
- Administration of immunoglobulins and/or any blood products within the three months preceding vaccination;
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within 6 months preceding vaccination (inhaled and topical steroids were allowed);
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine, e.g. egg products;
- Any history of anaphylaxis in relation to vaccination;
- Pregnancy, lactation or willingness/intention to become pregnant during the study;
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ);
- History of serious psychiatric condition likely to affect participation in the study;
- Any other serious chronic illness requiring hospital specialist supervision;
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week;
- Suspected or known injecting drug abuse in the 5 years preceding enrolment;
- Seropositive for hepatitis B surface antigen (HBsAg);
- Seropositive for hepatitis C virus (antibodies to HCV);
- History of clinical malaria (any species);
- Travel to a malaria endemic region during the study period or within the previous six months;

- Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis;
- Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data;
- Inability of the study team to contact the volunteer's GP to confirm medical history and safety to participate.

Safety Analysis

Following each vaccination, volunteers completed an electronic diary card for 28 days with any adverse event data. Participants were asked to record both solicited and unsolicited AEs. Data on solicited AEs were collected for 7 days after each vaccination, whereas details of any unsolicited AEs were collected for the duration of each diary. Solicited AEs were those expected following an intramuscular vaccination and included local AEs (pain, erythema, warmth, swelling and itching) and systemic AEs (headache, malaise, myalgia, arthralgia, feverishness, nausea, fatigue, and measured fever). If these AEs occurred outside of the first seven days they were defined as unsolicited. Any solicited AEs occurring during the diary card period were defined as being at least possibly related to vaccination.

Volunteers graded all AEs as mild, moderate or severe:

- **GRADE 0:** None.
- **GRADE 1:** Transient or mild discomfort (< 48 h); no medical intervention/therapy required.
- **GRADE 2:** Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required.
- **GRADE 3:** Marked limitation in activity, some assistance usually required; medical intervention/therapy required; hospitalization possible.

Adverse event data also included the results of the hematology (full blood count) and biochemistry (liver function tests, urea and electrolytes) carried out at all visits during the diary card period, except those occurring 2 days post-vaccination.

For each unsolicited AE, an assessment of the relationship of the AE to the study intervention(s) was undertaken. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to vaccination were considered. The likely causality of all unsolicited AEs was assessed as per the criteria below:

- **No Relationship:** No temporal relationship to study product ***and*** alternate aetiology (clinical state, environmental or other interventions); ***and*** does not follow known pattern of response to study product.
- **Unlikely:** Unlikely temporal relationship to study product ***and*** alternate aetiology likely (clinical state, environmental or other interventions) ***and*** does not follow known typical or plausible pattern of response to study product.
- **Possible:** Reasonable temporal relationship to study product; ***or*** event not readily produced by clinical state, environmental or other interventions; ***or*** similar pattern of response to that seen with other vaccines.
- **Probable:** Reasonable temporal relationship to study product; ***and*** event not readily produced by clinical state, environment, or other interventions ***or*** known pattern of response seen with other vaccines.
- **Definite:** Reasonable temporal relationship to study product; ***and*** event not readily produced by clinical state, environment, or other interventions; ***and*** known pattern of response seen with other vaccines.

All unsolicited AEs that were assessed as being possibly, probably or definitely related to either ChAd63 RH5 or MVA RH5 are shown in Table S1.

ChAd63 and MVA RH5 Vaccines

The RH5 sequence was codon optimized for human expression and is cloned in frame at the N-terminus to the human tissue plasminogen activator (tPA) leader sequence (aa 1–32, GenBank Accession #K03021). Two potential sites of N-linked glycosylation within the RH5 sequence were mutated asparagine to glutamine (N38Q and N214Q) (1). This transgene cassette is inserted at the E1 site of the E1/E3-deleted ChAd63 vector, under the control of the CMV immediate-early promoter. In this case, the CMV promoter is the 1.9 kbp promoter including the regulatory element, enhancer and intron A (2). The promoter was also engineered to include tandem tetracycline operators in proximity to the TATA box by ligation of a fragment containing these into the SacI restriction site (1). The entire E4 locus of the ChAd63 vector is also replaced with the *E4Orf6* gene from human adenovirus serotype 5. In the case of MVA, the transgene cassette is inserted at the thymidine kinase (TK) locus with expression driven by the vaccinia P7.5 early/late promoter without any additional marker (3).

For cGMP manufacture of ChAd63 RH5, the overall manufacturing process was divided into the following steps: i) production of the ChAd63 RH5 Drug Substance, and ii) Vaccine formulation and filling. Briefly, 15 L of the packaging cell line Procell92.S were infected with the pre-GMP seed stock virus at MOI=100 in Wave Bioreactors and dedicated to the generation of the purified Drug Substance.

Two days post-infection, the infected cells were lysed by adding a detergent based lysis buffer and a Benzonase treatment was performed directly into the Wave Bioreactors by incubating cells for 2 h at 37 °C. After the lysis, the bulk harvest was clarified by depth filtration, using D0HC filters in order to remove large aggregates and cellular debris. The clarified lysate was concentrated 10-fold by Tangential Flow Filtration (TFF), using 300 KDa PES ultrafiltration cassettes and subjected to a second Benzonase treatment prior to being aliquoted and stored at -80 °C. The day after, the Drug Substance was thawed at 37 °C in a water bath, pooled and diafiltered against 10 volumes of buffer. Adenovirus purification was performed by Anion Exchange Chromatography using Source 15Q resin, a strong anion exchanger able to interact with the highly negative adenovirus surface charge. The

virus is bound by the resin with high affinity and is separated from the protein and nucleic acid contaminants by a NaCl gradient. The anion exchange purified virus was then formulated by TFF, using 300 KDa PES ultrafiltration cassettes and exchanging 10 volumes of formulation buffer A438 at constant sample volume, in order to ensure a complete buffer exchange. The formulated virus was then filtered using a TPP 0.22 μm PES vacuum filter, sampled, dispensed into aliquots and frozen at -80 °C for subsequent storage. The formulated Drug Substance was thawed at room temperature, pooled, diluted at the desired concentration and filtered through a Pall PVDF Mini Kleenpak 0.22 μm filter into a sterile bag. The final bulk product was dispensed semi-automatically using a filling machine set to deliver 650 μL into borosilicate type 1 glass 2 mL vials. The ChAd63 RH5 vaccine is a liquid formulation contained in vials with 0.5 mL extractable volume.

Each vaccine lot underwent comprehensive quality control analysis to ensure that the purity, identity, and integrity of the virus met pre-defined specifications. Testing and specifications for the ChAd63 RH5 Drug Substance and Drug Product were done at Advent in accordance with Ph. Eur. 5.14 monograph Gene transfer medicinal products for human use. §Adenovectors for human use (01/2011) and included testing for transgene sequencing, for absence of extraneous agents (as per Ph. Eur. 2.6.16), residual impurities (DNA, HCP, benzonase, BSA), absence of replication-competent adenovirus (RCA), tests for pH, osmolality, sterility, extractable volume, and endotoxins. Biological activity (potency) was quantified using: i) anti-hexon immuno-staining that quantitatively measures infectious virus titer; ii) quantitation of total vector particle concentration by qPCR; iii) a calculated determination of total *versus* infectious particle ratio; and iv) determination of expression of the RH5 transgene by Western blot.

MVA RH5 was manufactured under cGMP conditions by IDT Biologika GmbH, Germany, as previously described (4).

Final certification of both the ChAd63 and MVA RH5 viral vaccines and associated labelling took place at the Clinical Biomanufacturing Facility (CBF) in Oxford. Vaccine lots were stored at the clinical site at -80 °C and the temperature was monitored.

The stability studies of ChAd63 RH5 vaccine were performed at the manufacturing facility Advent in Pomezia, Italy for up to 48 months and at the recommended storage condition below -65 °C. Potency related tests (Western blot, vector particle concentration, infectious virus titer, and infectious virus titer ratio) were performed at each time-point, since these parameters are considered to be main indicators of the stability of the vaccine. pH was also performed at each time-point, while the sterility test was performed annually. The stability studies were conducted concurrent with the clinical trial and confirmed ChAd63 RH5 vaccine stability throughout this period of time. MVA RH5 stability was confirmed by annual re-titrations on chick embryo fibroblast (CEF) cells and immuno-potency was monitored by performing annual *in vivo* immunogenicity assays in mice.

Study Design and Approvals

Vaccination of volunteers was carried out at the CCVTM in Oxford and NIHR WTCRF in Southampton. The four volunteers in Group 1 were vaccinated with 5 x 10^9 vp of ChAd63 RH5. Following a safety review, twelve volunteers (Groups 2A and 2B) were vaccinated with 5 x 10^{10} vp and eight of these (Group 2B) went on to receive MVA RH5 1 x 10^8 pfu eight weeks later. A further eight volunteers were enrolled into the final group (Group 2C), and a safety review was carried out prior to the full dose MVA RH5 vaccinations (2 x 10^8 pfu) given eight weeks after ChAd63 RH5 5 x 10^{10} vp.

Vaccination visits occurred on days 0 (all groups) and 56 (Groups 2B and 2C). Volunteers also attended follow-up visits on days 2, 7, 14, 28, 56 and 84 in Group 1, on days 2, 7, 10 14, 28, 56, 63, 84 and 140 in Group 2A and on days 2, 7, 10, 14, 28, 58, 63, 84 and 140 in Groups 2B and 2C, with a final follow-up phone call for groups 1 and 2A on day 180 and on day 240 for Groups 2B and 2C. Nominal study days are reported throughout.

All vaccinations were given intramuscularly (IM) into the deltoid muscle, preferentially into the non-dominant arm. Blood tests for exploratory immunology were carried out at baseline (day 0) and at all physical visits after vaccination except days 2 and 58.

Participants

All volunteers signed written consent forms, and consent was confirmed before each vaccination. Allocation to study groups (Figure 1) occurred at screening based on sequential recruitment of groups and volunteer preference.

Ghanaian Plasma Samples

Samples were provided from a study conducted in Asutsuare, a town within the Shai-Osudoku district, Greater Accra Region and approved by the Noguchi Memorial Institute for Medical Research IRB (study number NMIMR-IRB: CPN: 010/12-13). Written informed consent was obtained from study participants. Malaria transmission rate in the area has being reported to be stable all year round with two seasonal peaks; major season April-July and minor season September-November (5). The dominant circulating malaria species within the study area is *P. falciparum* with a few cases of *P. ovale* and *P. malariae* reported. 79 healthy adults from Asutsuare who were exposed to malaria but malaria negative by rapid diagnostic test kit were recruited at the end of the April-July malaria season in August 2014. The cohort was 38:41 female:male; age range 16-69 years; haemoglobin level range 8.3-18.2 g/dL. Venous blood samples were collected in EDTA vacutainers from each participant. Plasma was separated by centrifugation and stored at -20 °C until use.

Kenyan Serum Samples

Sera were collected during adult cross-sectional surveys between 2006 and 2008 from the villages surrounding the Chonyi area in Kilifi, Kenya which experiences moderate malaria transmission with an EIR of 10-100 infective bites/person/year (6). These adults are considered to have substantial naturally acquired immunity as evidenced by the decline in clinical episodes of malaria with age (7). Scientific and ethical approvals for the Kenyan serum samples were granted by the Kenya National Scientific and Research Ethics Committees respectively, SSC No. 1131.

Peripheral Blood Mononuclear Cell (PBMC) and Serum Preparation

Blood samples were collected into lithium heparin-treated vacutainer blood collection systems (Becton Dickinson, UK). PBMC were isolated and used within 6 hours in fresh assays as previously

described (8). Excess cells were frozen in foetal calf serum (FCS) containing 10% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen. Plasma samples were stored at -80 °C. For serum preparation, untreated blood samples were stored at room temperature (RT) and then the clotted blood was centrifuged for 5 min (1000 *xg*). Serum was stored at -80 °C.

Peptides

Peptides for *ex-vivo* IFN-γ ELISPOT were purchased from NEO Scientific (Cambridge, MA, USA). The peptides, 20 aa in length and overlapping by 10 aa, covered the entire RH5 insert present in the viral vectored vaccines (Table S2). Peptides were reconstituted in 100% DMSO at 50-200 mg/mL and combined into various pools for the ELISPOT assay. Overlapping peptides for ELISA have been reported previously (9). Briefly, biotinylated 20mer peptides spanning the RH5 vaccine insert (running from residue E26–Q526), each offset by 8 aa from the previous peptide (i.e. overlapping by 12 aa), were synthesized by Mimotopes, Australia. The K33-K51 peptides for ELISA were purchased from NEO Scientific (Cambridge, MA, USA) (Table S3). Each peptide stock was reconstituted to 50 mg/mL in DMSO.

Recombinant RH5 Proteins

Recombinant RH5 proteins for ELISA and B cell assays were produced from stably transfected *Drosophila melanogaster* Schneider 2 (S2) cell lines. Cell line generation and growth conditions have been previously described in detail (10, 11). In brief, the RH5_FL protein encodes the full-length ectodomain of the PfRH5 antigen (aa E26-Q526) based on the sequence of the 3D7 clone *P. falciparum* parasite, and all four putative N-linked glycosylation sequons (N-X-S/T) were mutated Thr to Ala – as performed for a previous PfRH5 protein vaccine produced in mammalian HEK293 cells and tested in rabbits (10, 12) and *Aotus* monkeys (13).

Protein disorder was predicted using PONDR (http://www.pondr.com/).

The RH5ΔNL construct has been described previously (14), initiating at residue K140 and running through to Q526, but lacking the internal disordered loop N248-M296. Both constructs also contained

an N-terminal 18 aa BiP insect signal peptide (MKLCILLAVVAFVGLSLG) and a C-terminal four amino acid (EPEA) C-tag for purification (11).

Clarified supernatant from a 4-day batch culture of S2 cells was concentrated 15-20 fold and buffer exchanged using a TFF system fitted with Pellicon 3 Ultracel 10 kDa membrane (Merck Millipore, UK). Purification was performed on an AKTA Pure 25 system (GE Healthcare, UK), consisting of an affinity step with CaptureSelect™ C-tag column (Thermo Fisher Scientific, UK) and a polishing size exclusion chromatography (SEC) using Superdex 200 16/60 PG (GE Healthcare, UK) in 20 mM Tris-HCl, 150 mM NaCl, pH 7.4 (TBS). Purified protein was quantified by Nanodrop (Thermo Fisher Scientific, UK) and stored at -80 °C until further use.

Mono-biotinylated RH5Nt protein (residues F25-K140 with a C-terminal rat CD4 domains 3+4 (d3+4) tag) for ELISA was produced by transient transfection of HEK293-6E cells as previously described (15).

The construct used for RH5_FL CFCA was a gift from Gavin Wright (Addgene plasmid # 47780) (16), including, in brief, an exogenous signal peptide of mouse origin, the full-length RH5 ectodomain with the four potential sites of N-linked glycsolylaion mutated threonine to alanine followed by C-terminal tags rat CD4 d3+4 and enzymatic biotinylation sequence. The construct used for RH5ΔNL CFCA included a murine Ig leader sequence (mouse variable κ light chain 7-33 (17)) followed by i) RH5ΔNL (*P. falciparum* 3D7 clone sequence, with potential sites of N-linked glycosylation mutated threonine to alanine (10, 16), residues K140-N506 with the N248-M296 internal disordered loop removed); ii) rat CD4 d3+4; iii) an AviTag™ (GLNDIFEAQKIEWHE) to allow for mono-biotinylation; and iv) C-terminal C-tag (EPEA) (11). Both constructs were co-transfected with plasmid encoding *E. coli* biotin ligase (BirA) using the transient EXPI293 system (Thermo Fisher Scientific, UK). Culture supernatants were harvested after 4 days, concentrated and buffer exchanged in PBS using a TFF system fitted with Pellicon 3 Ultracel 10 kDa membrane (Merck Millipore, UK). Samples were stored at -20 °C until further use.

Ex-vivo IFN-γ ELISPOT

Fresh PBMC were used in all assays using a previously described protocol (8), except that 50 µL/well RH5 peptide pools (Table S2) (final concentration each peptide 5 µg/mL) were added to triplicate test wells, 50 µL/well R10 and DMSO control were added to negative unstimulated wells, and 50 µL/well Staphylococcal enterotoxin B (SEB) (final concentration 0.02 µg/mL) plus phytohemagglutinin (PHA) (final concentration 10 µg/mL) was added to positive control wells. Spots were counted using an ELISPOT counter (Autoimmun Diagnostika (AID), Germany). Results are expressed as IFN-γ spot-forming units (SFU) per million PBMC. Background responses in unstimulated control wells were almost always less than 20 spots, and were subtracted from those measured in peptide-stimulated wells.

Total IgG and Peptide ELISAs

ELISAs were performed using the standardized methodology as previously described (4, 8), except that plates were coated with recombinant RH5_FL and RH5ΔNL protein produced from the *Drosophila* S2 cells at a concentration of 2 µg/mL in PBS. The reference serum used to generate the standard curve was prepared from a pool of day 84 serum samples from high-responding volunteers in this trial. The reciprocal of the dilution giving an optical density at 405nm (OD_{405}) of 1.0 in the standardized assay was used to assign an ELISA unit value of the standard. The standard curve and Gen5 ELISA software v2.07 (BioTek, UK) was used to convert the OD_{405} of individual test samples into arbitrary units (AU). These responses in AU are reported in µg/mL following generation of a conversion factor by calibration-free concentration analysis (CFCA).

For the ELISA against denatured antigen, RH5_FL protein was heated for 10 min at 80 °C using a PCR machine and coated on 96 well Nunc-Immuno Maxisorp plates at a concentration of 2 µg/mL in DPBS and left at 4 °C over-night. Plates were then washed with PBS containing 0.05% Tween 20 (PBS/T) x6 and blocked for 1 h with Casein block solution (Pierce). After another wash step, d84 test sera diluted to a level calculated to give an OD_{405}=1.0 in casein block solution were added to the plates for 2 h. Day 0 test sera diluted 1:100 and anti-RH5 chimeric human IgG1 monoclonal

antibodies 4BA7 and 2AC7 (9) (Douglas AD *et al.*, in preparation) were included as controls. Plates were washed again and alkaline phosphatase-conjugated goat anti-human IgG (γ-chain) (Sigma) diluted 1:1000 in casein block solution was added, before development with *p*-nitrophenylphosphate substrate (Sigma) diluted in diethanolamine buffer (Thermo Fisher Scientific). OD_{405} was read using a microplate reader (Biotek) and Gen5 v2.07 software.

For the RH5Nt ELISA, biotinylated protein was coated on streptavidin coated plates (Pierce) at a concentration of 2 µg/mL in DPBS and left at 4 °C over-night. Plates were then washed with PBS/T x6 and blocked for 1 h with Casein block solution (Pierce). After another wash step, d0 and d84 test sera diluted 1:100 in casein block solution were added to the plates for 2 h. Plates were washed again and alkaline phosphatase-conjugated goat anti-human IgG (γ-chain) (Sigma) diluted 1:1000 in casein block solution was added, before development with *p*-nitrophenylphosphate substrate (Sigma) diluted in diethanolamine buffer (Thermo Fisher Scientific). OD_{405} was read using an Infinite F50 microplate reader (Tecan) and Magellan v7.0 software.

For peptide ELISAs, streptavidin-coated plates (Pierce) were coated with individual peptides at a concentration of 10 µg/mL in PBS and left at 4 °C over-night. Plates were then washed with PBS/T x6 and blocked for 1 h with Casein block solution (Pierce). After another wash step, test sera diluted 1:100 in casein block solution were added to the plates for 2 h. Plates were washed again and alkaline phosphatase-conjugated goat anti-human IgG (γ-chain) (Sigma) diluted 1:1000 in casein block solution was added, before development with *p*-nitrophenylphosphate substrate (Sigma) diluted in diethanolamine buffer (Thermo Fisher Scientific). OD_{405} was read using an Infinite F50 microplate reader (Tecan) and Magellan v7.0 software.

Calibration-Free Concentration Analysis (CFCA)

Anti-RH5_FL total IgG ELISA AU were converted to antigen-specific IgG concentration in µg/mL as follows. CFCA was performed with a method similar to that previously described (18-20), using a Biacore X100 instrument, a Biotin CAP chip, and X100 control and evaluation software (GE Lifesciences, UK) (Figure S4). Mono-biotinylated RH5_FL supernatant was produced for use in the

CFCA by transient transfection of suspension EXPI293 cells as described above. Supernatant was clarified, and dialyzed against PBS using Snakeskin (Life Technologies) at 4 °C. CFCA was subsequently performed using serum samples from subjects in Group 2C and volunteers from another on-going clinical trial of a RH5-based vaccine (Minassian AM *et al.*, Clinicaltrials.gov NCT02927145 – to be reported elsewhere) with a range of RH5_FL-specific IgG antibody responses as assessed by ELISA. Each individual's serum was diluted 1:100 in running buffer (18). Mass-transport limited binding conditions were obtained by capturing a minimum of 3000 response units (RU) of RH5_FL antigen on the active flow cell. The chip was regenerated with the manufacturer's supplied regeneration and CAP reagents (diluted 1:2 in HBS EP+) and fresh antigen (neat supernatant) prior to each application of antibody. Antigen-specific antibody binding was measured by double reference subtraction, firstly of binding to a flow cell coated only with the biotin capture reagent, and secondly of the binding of a day 0 serum sample (from the same volunteer) from that of the test sample (Figure S4A). Initial rates of antigen-specific binding at 5 µL/min and 100 µL/min were measured and compared to permit measurement of concentration and the level of mass-transport limitation. Parameters (initial binding rate >0.3 RU/s at 5 µL/min flow rate and >0.13 for quality control (QC) ratio) recommended by the manufacturer were used for quality control of data (Figure S4B). Initial binding rates were in the range 0.39 – 2.93 RU/s at 5 µL/min flow, and calculated QC ratios were all >0.3 (reflecting adequate mass transport limitation for concentration estimation). The binding model used a molecular weight of 150 kDa for IgG. The viscosity of the running buffer at 20 °C was 1.0562 mPa*s (measured using a densitometer equipped with a viscometer module (20)) giving a measured diffusion coefficient of IgG at 20 °C in a solution with the viscosity of water, pH 7.4, of 4.8×10^{-11} m^2/s. The diffusion coefficient of IgG under the test conditions (25 °C) was therefore calculated to be 5.494×10^{-11} m^2/s. The CFCA-measured RH5_FL-specific antibody concentrations for each individual were analysed by linear regression with the corresponding total IgG ELISA AU data, with the slope of the line used to derive an AU-to-µg/mL conversion factor (Figure S4C). A similar analysis was undertaken as above for RH5ΔNL using serum samples from subjects in Groups 2B and 2C (Figure S4D).

Memory B cell and ASC ELISPOT

B cell ELISPOT assays were performed as described in detail elsewhere (21). In brief, to measure mBC responses, frozen PBMC were thawed before culturing with a polyclonal B cell stimulation mix containing *Staphylococcus aureus* Cowan strain Pansorbin cell 'SAC' (Calbiochem), the human TLR agonist CpG ODN-2006 (Invivogen) and pokeweed mitogen 'PWM' (Sigma) for 6 days, allowing mBC to differentiate into ASC. On day five of the experiment, ELISPOT plates were coated with recombinant RH5_FL protein produced from the *Drosophila* S2 cells to measure the antigen-specific response and polyvalent goat-anti human IgG (Caltag) to measure the total IgG response. PBS coated wells were used as a negative control. On day six, cultured cells were transferred to the ELISPOT plate and incubated for 18-20 h before developing with an anti-human IgG (γ-chain) antibody conjugated to alkaline phosphatase (Calbiochem) followed by a substrate buffer. Plates were counted using an AID ELISPOT plate reader. *Ex-vivo* ASC ELISPOT assays were performed exactly as above but using fresh PBMC directly prepared and added to the ELISPOT plate with no preceding 6 day culture.

Serum IgG Concentration

Total serum IgG concentrations were determined using a Bio-Monolith Protein G column on an Agilent 1260 HPLC system (Agilent, Cheshire, UK). Separation was performed at 1 mL/min using PBS and 0.2 M Glycine pH 2.0 as mobile phases with detection at UV 280 nm. A calibration curve was produced using purified human IgG.

Assay of Growth Inhibition Activity (GIA)

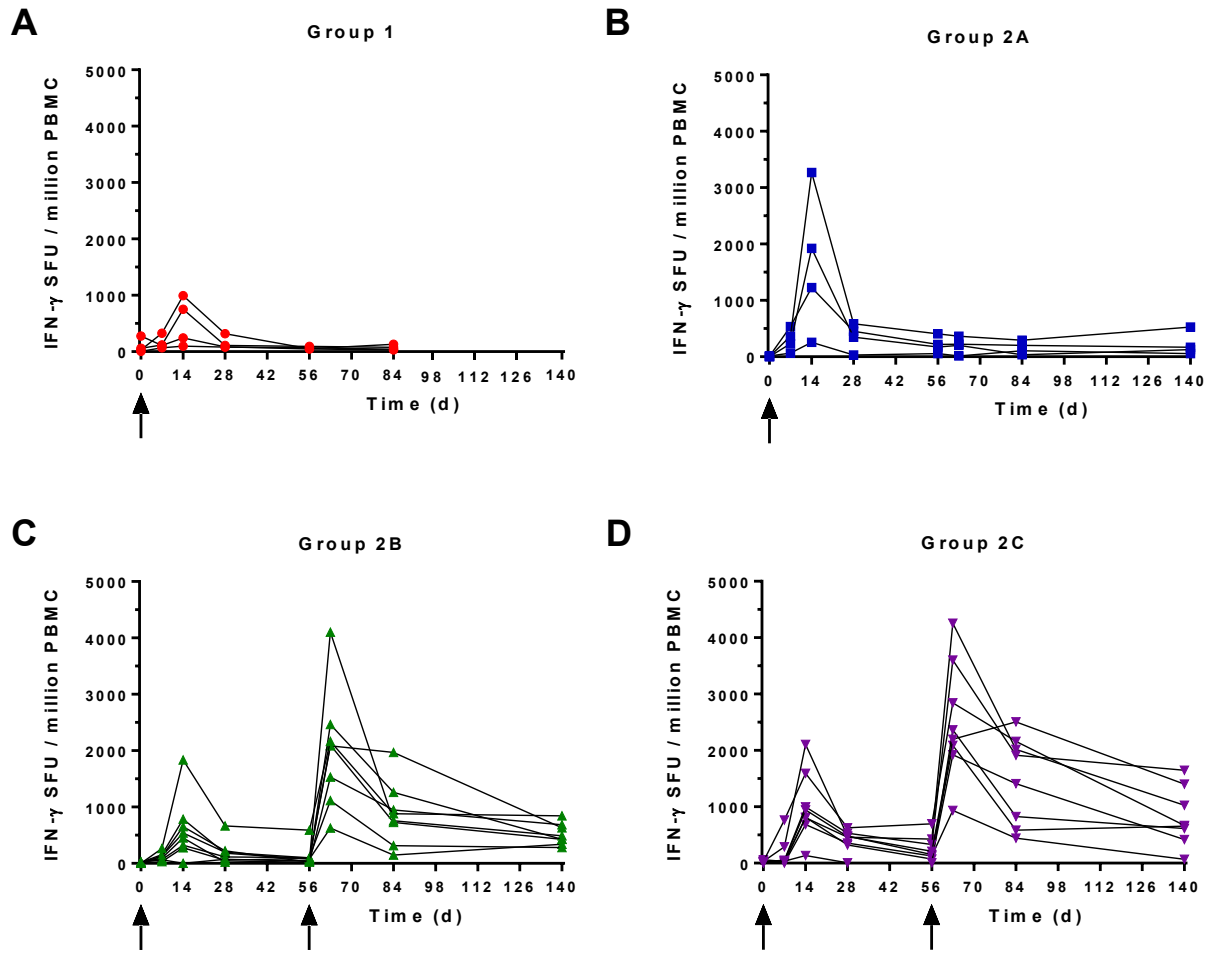
Protein G purified IgG samples were incubated with RBC infected with synchronized *P. falciparum* parasites in a final volume of 40 μL for 40 h at 37 °C, and the final parasitemia in each well was quantified by biochemical determination of parasite lactate dehydrogenase. Six laboratory-adapted parasite lines (3D7, 7G8, Dd2, FVO, GB4 and MCamp) plus two short-term culture-adapted parasite isolates from Cambodian patients with malaria (Cp845, Cp806) (18) and one from an Australian resident who contracted malaria in Ghana (HMP002) (22) were utilized for the regular assay of GIA.

The blood collected for the master cell bank (HMP002) was leukodepleted prior to the addition of the cryopreservation agent Glycerolyte 57 in 1:2 ratio as previously described (23). To determine the effect of complement, additional assays of GIA were performed with a slight modification using 3D7 clone parasites. In the assay, 25 % v/v of non-heat-inactivated or heat-inactivated US normal serum was used for the 40-hour assay, instead of 10 % v/v of non-heat inactivated serum in a regular GIA assay. The complement activity of non-heat-inactivated serum (and no activity in heat-inactivated serum) was confirmed by EZ Complement Cells-CH50 Test (Diamedix, Miami Lakes, FL, USA). All human sera and RBC were purchased from Interstate Blood Bank, USA.

Avidity-based Extracellular protein Interaction Screen (AVEXIS)

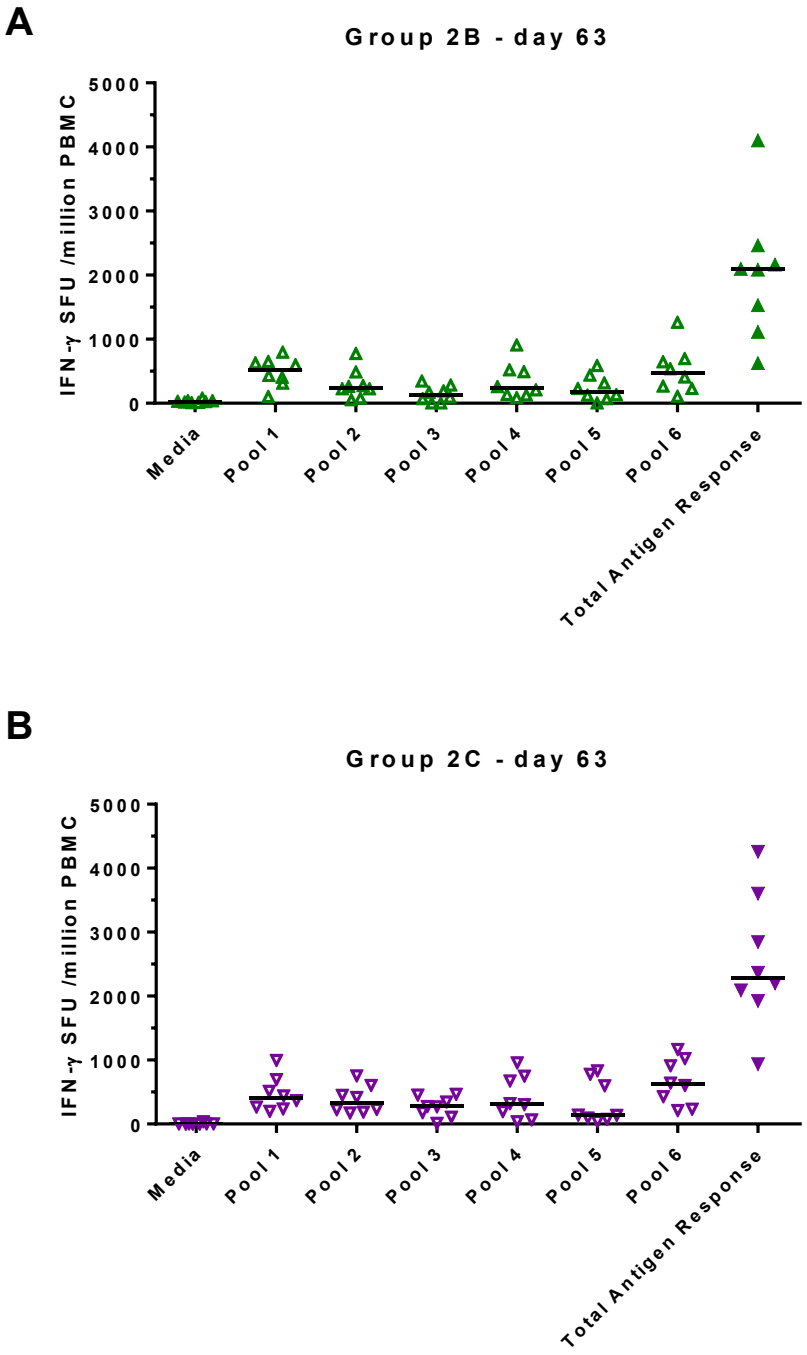
Monomeric biotinylated bait proteins and highly avid pentameric β-lactamase-tagged prey protein activities were prepared and their expression levels normalized as described (24) prior to their use in interaction inhibition screening. Briefly, biotinylated RH5 bait was immobilized in streptavidin-coated 96-well microtiter plates, washed with PBS/T (PBS with 0.1 % Tween-20), incubated with test sera diluted in PBS with 2 % BSA, washed four times with PBS/T, incubated with prey proteins and washed three times with PBS/T. Any captured preys were quantified by adding the colorimetric β-lactamase substrate nitrocefin and measuring the absorbance of the hydrolysis products at 485 nm. The protein constructs were all full-length ectodomains with threonine to alanine mutations to remove sites of potential N-linked glycosylation (except for basigin) as previously reported (15, 25): RH5 amino acids F25-Q526; CyRPA amino acids D29–E362; P113 amino acids Y23-K942; and basigin isoform 2/BSG-S (25) amino acids M1-A23 followed by G140-L322.

Supplementary Figures



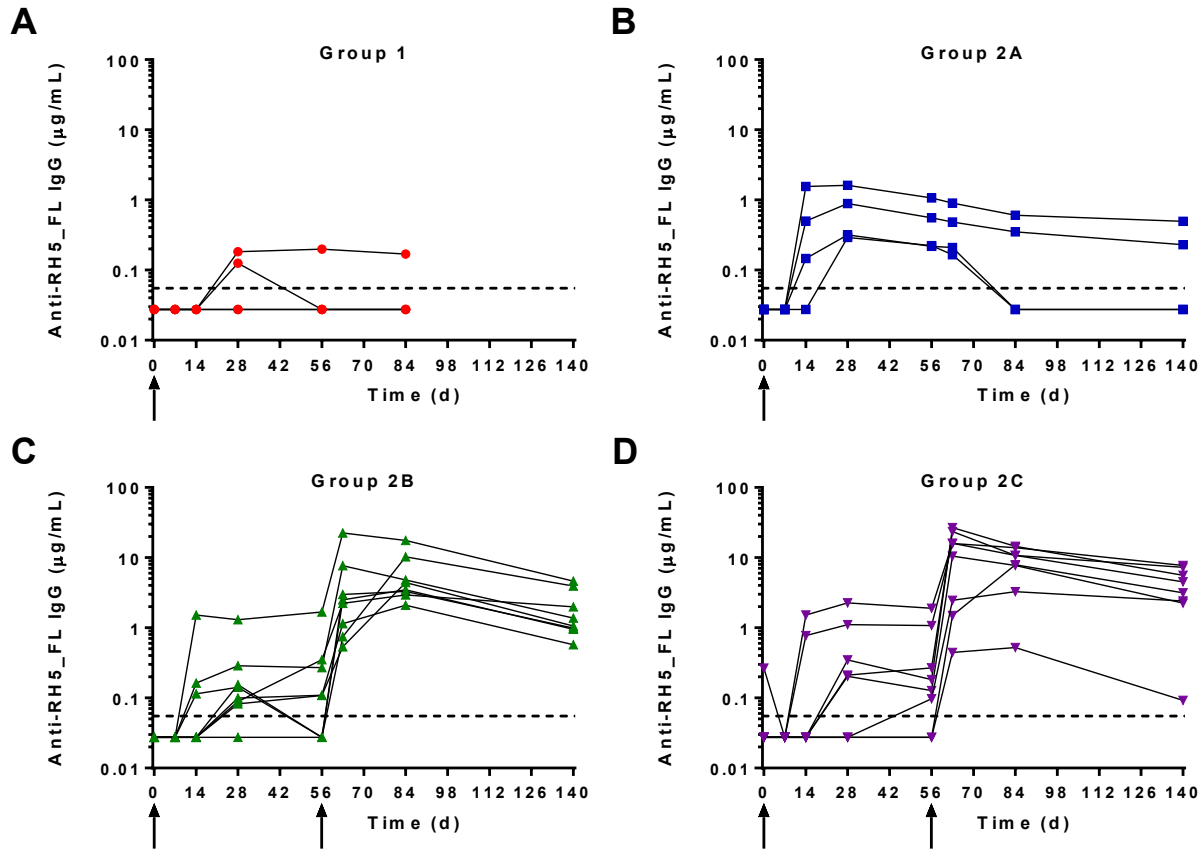
Supplementary Figure 1. Individual *ex-vivo* IFN-γ ELISPOT data.

ELISPOT responses to the RH5 insert (summed response across all the individual peptide pools) are shown over time following immunization in **(A)** Group 1 (n=4); **(B)** Group 2A (n=4); **(C)** Group 2B (n=8); and **(D)** Group 2C (n=8). Individual responses are shown for each volunteer.



Supplementary Figure 2. *Ex-vivo* IFN-γ ELISPOT data according to peptide pool.

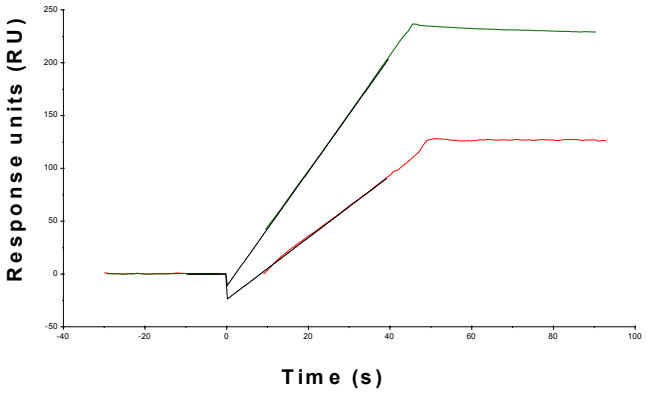
ELISPOT responses to the RH5 insert at day 63 for Groups 2B and 2C are shown according to the six peptide pools used in the assay (Table S2). Individual and median responses are shown for each pool, the media control and the total summed response.



Supplementary Figure 3. Individual ELISA data.

Serum total IgG ELISA responses to recombinant RH5_FL are shown over time following immunization in **(A)** Group 1 (n=4); **(B)** Group 2A (n=4); **(C)** Group 2B (n=8); and **(D)** Group 2C (n=8). The horizontal dotted line indicates the limit of detection of the assay. Individual responses are shown for each volunteer. One volunteer (in Group 2C) showed a weak response above background at baseline (day 0).

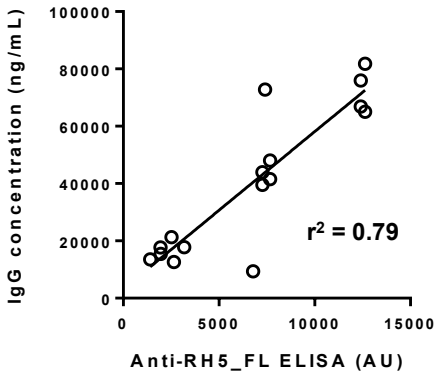
A



B

Sample	Calculated Concetration (µg/mL)	Fitted initial binding rate (RU/s)		QC ratio fit	ELISA (AU)
		5 µL/min flow	100 µL/min flow		
1	17.8	0.59	0.97	0.38	3185
2	13.6	0.39	0.58	0.30	1410
3	12.7	0.55	1.12	0.62	2643
4	15.4	0.66	1.34	0.61	1959
5	17.7	0.63	1.10	0.43	1950
6	21.3	0.84	1.59	0.52	2525
7	39.5	1.62	3.16	0.56	7266
8	75.9	2.93	5.43	0.50	12411
9	81.8	2.92	5.10	0.43	12626
10	41.5	1.73	3.45	0.58	7672
11	72.8	2.40	3.96	0.38	7400
12	9.4	0.50	1.35	1.00	6787
13	43.9	0.46	1.26	1.00	7266
14	66.9	0.70	1.84	0.96	12411
15	65.0	0.69	1.86	1.00	12626
16	48.0	0.51	1.38	1.00	7672

C

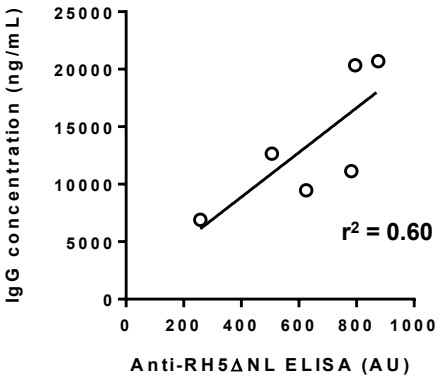


RH5_FL conversion factor (slope)

5.5 (95% CI: 3.9 – 7.1) ng/mL

per 1 AU (ELISA unit)

D



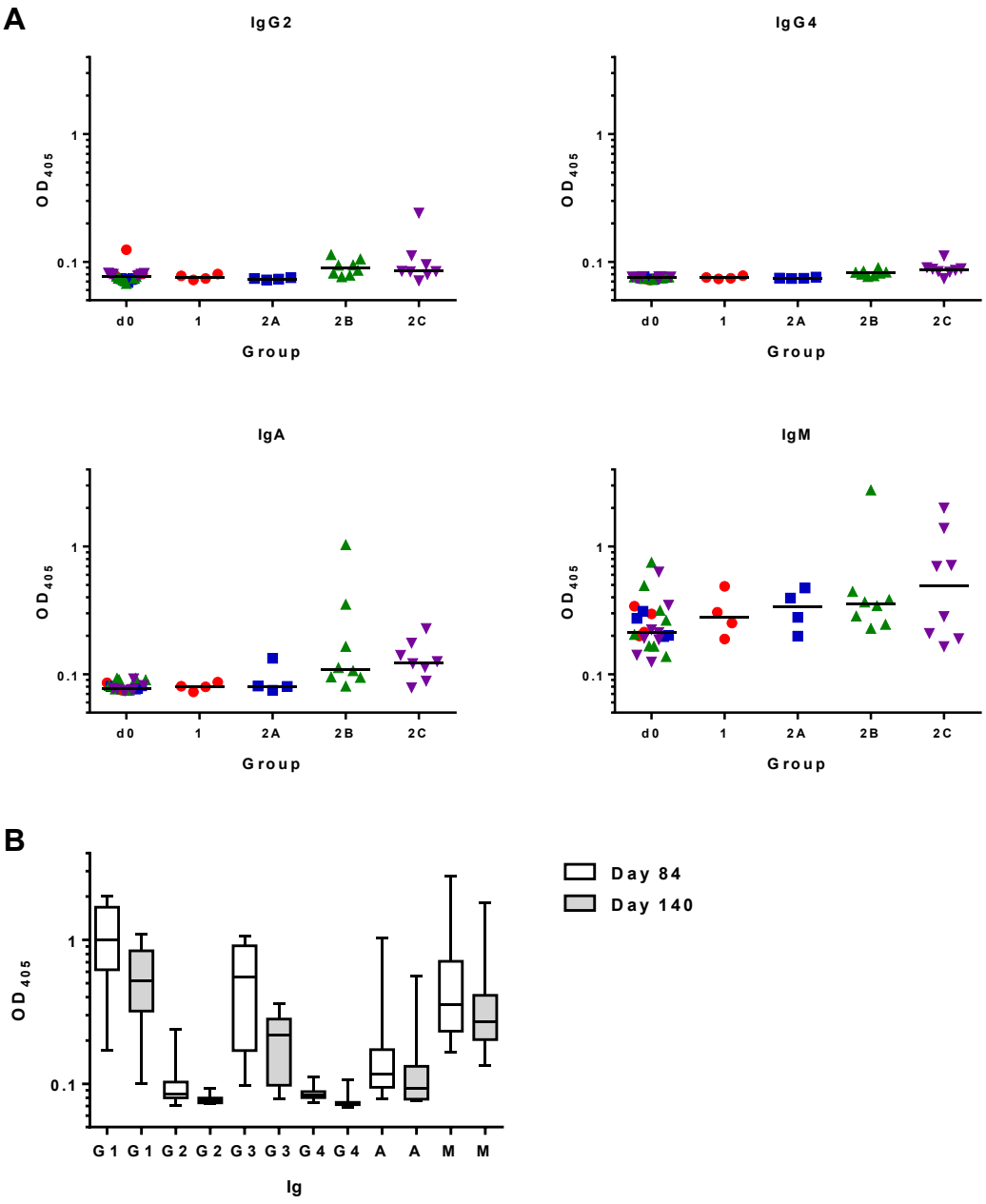
RH5ΔNL conversion factor (slope)

19.4 (95% CI: -2.5 – 41.2) ng/mL

per 1 AU (ELISA unit)

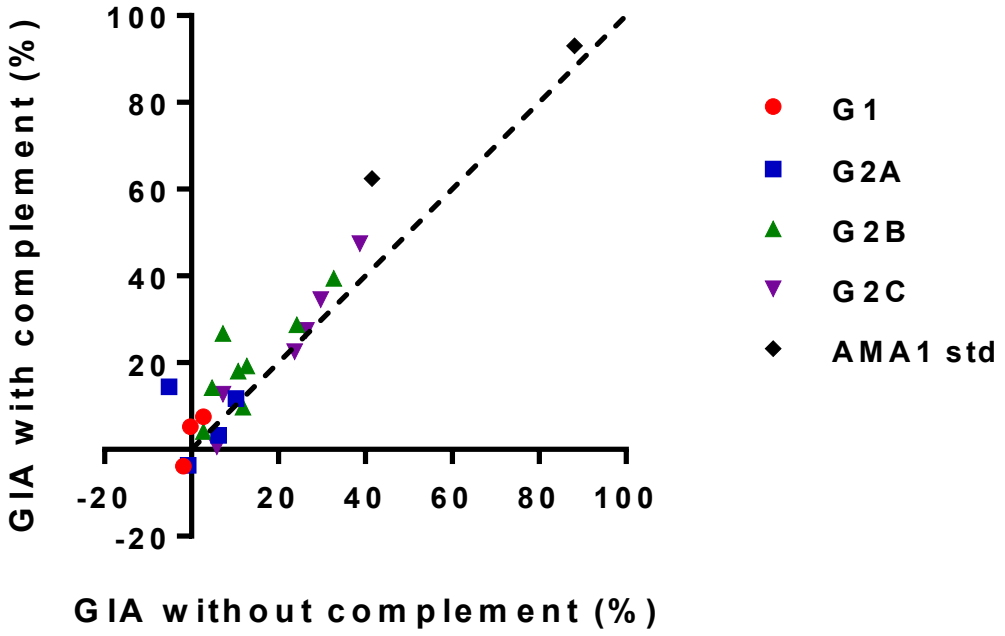
Supplementary Figure 4. Calibration-free concentration analysis (CFCA).

CFCA was performed using a Biacore X100 instrument in order to measure absolute (μg/mL) concentrations of RH5_FL-specific antibody in serum samples from vaccinated subjects. (**A**) Binding at low sample flow rate (5 μL/min) is indicated in red while binding at high flow (100 μL/min) is indicated in green. Example data are shown for one test sample with the graph showing final double-subtracted RH5_FL-specific binding at the two flow rates. Sample binding to the non-RH5_FL coated reference flow cell (Fc1) was subtracted from the RH5_FL coated active flow cell (Fc2), followed by subtraction of volunteer-matched pre-immune d0 serum binding (also calculated as Fc2-Fc1). Colored lines show the test sample data, and the black lines the fitted data generated by X100 evaluation software. The slopes of these solid lines were used to calculate antigen-specific antibody concentration in the test sample. (**B**) Tabulates CFCA and relevant ELISA results for the 16 samples. (**C**) Correlation of ELISA AU for each sample and the RH5_FL-specific IgG concentration measured by CFCA. Linear regression r^2 value is shown; with the slope used to define the conversion factor between ELISA AU and antigen-specific IgG concentration in ng/mL. (**D**) A similar CFCA analysis was performed for RH5ΔNL, with final analysis shown as in panel C.



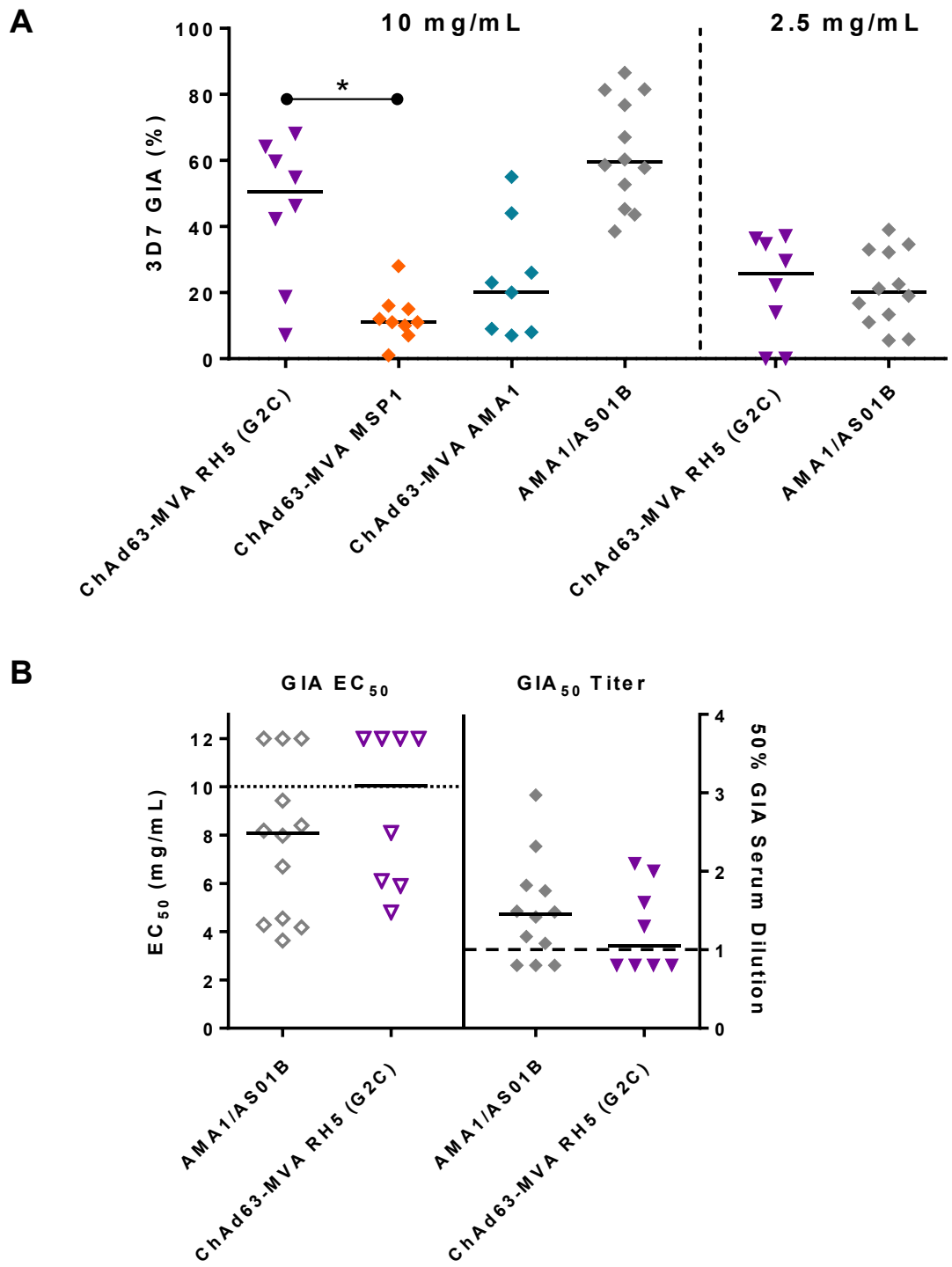
Supplementary Figure 5. Isotype ELISA data.

(**A**) Isotype profiles of serum antibody responses against RH5_FL were assessed by ELISA. Responses are shown at baseline (d0) and for all groups at day 84. Individual and median responses are shown for IgG2, IgG4, IgA and IgM. IgG1 and IgG3 are shown in Figure 4D. (**B**) Profiles are shown for Groups 2B and 2C combined (n=16) at the day 84 versus day 140 time-points. Box and whisker plots show median, interquartile range and range.



Supplementary Figure 6. Comparison of GIA with and without addition of complement.

In vitro GIA of purified IgG from d84 serum samples was assessed at 10 mg/mL with 25 % v/v of non-heat-inactivated or heat-inactivated US normal serum against 3D7 clone *P. falciparum* parasites at the GIA Reference Center. Individual results are shown according to group. The assay control is also shown – a rabbit anti-AMA1-C2 standard IgG tested at 0.25 and 1.5 mg/mL.



Supplementary Figure 7. Comparison of functional GIA induced by RH5-, AMA1- and MSP1-based vaccines in healthy UK adults.

In vitro GIA of purified IgG was assessed at 10 or 2.5 mg/mL against 3D7 clone *P. falciparum* parasites at the NIH GIA Reference Center. Individual and median results are shown. The ChAd63-

MVA viral vaccines encoding AMA1 or MSP1, and the AMA1 recombinant protein (called FMP2.1) formulated in AS01B adjuvant have been tested previously in healthy UK adults at trials in Oxford and the results reported elsewhere (26, 27). Data are included here for comparison purposes. * $P <$ 0.05, according to statistical analysis as reported in the Results. (**B**) Individual GIA assay EC_{50} of each purified IgG in mg/mL is shown (left). Samples for which the GIA was <50% at 10 mg/mL were plotted as 12 mg/mL. To relate these GIA assay results (using a normalized concentration of purified IgG) back to the original sera, the concentration of IgG in each original serum sample was also measured by HPLC. This enabled calculation of the GIA_{50} serum titer (right), defined previously as the dilution factor of each serum sample required to reach the concentration of purified IgG that gives 50% GIA (13). Individual data and median results are shown for the ChAd63-MVA RH5 Group 2C versus the previously reported AMA1 FMP2.1/AS01B vaccine (26). Samples for which the GIA_{50} serum titer could not be calculated (because they did not achieve ≥50% GIA using 10 mg/mL purified IgG) are plotted arbitrarily at 0.8.

Supplementary Tables

Unsolicited AEs following ChAd63 RH5				
Group	**Subject ID**	**Time-point(s)**	**Symptom**	**Severity**
1	MVT-0571005	1	Abdominal cramping	3
2A	MVT-0571003	0	Numbness around the lips	1
2A	MVT-0571003	9, 11	Headache	2
2A	MVT-0571003	9	Fatigue	1
2A	MVT-0571009	9	Painful injection site	1
2A	MVT-0571010	1 to 2	Sore throat	1
2B	MVT-0571015	10	Fatigue	2
2C	MVT-0571014	7 to 8	Temp 37.2 °C; feeling unwell	2
2C	MVT-0571014	8	Weakness	2
2C	MVT-0571014	8	Fatigue	2
2C	MVT-0571014	11	Headache	1
2C	MVT-0572211	2	Felt light headed and dizzy after going for a run	1
2C	MVT-0572205	1	Palpitations	2
2C	MVT-0572205	1	Insomnia	2
2C	MVT-0572210	7 to 8	Sore throat	1
2C	MVT-0572210	9 to 12	Mild sinus / nasal congestion	1
Unsolicited AEs following MVA RH5				
Group	**Subject ID**	**Time-point(s)**	**Symptom**	**Severity**
2B	MVT-0571012	1 to 11	Coryzal symptoms, cough and congestion	1
2B	MVT-0571018	0 to 1	Abdominal pain	1
2B	MVT-0571020	4 to 5	Coryzal symptoms	1
2B	MVT-0572204	10	Headache	1
2C	MVT-0571014	8	Vomited x 1	2
2C	MVT-0572211	0 to 3	Disturbed sleep	1
2C	MVT-0572205	1	Tinnitus	1
2C	MVT-0571017	0 to 1	Small swelling / hematoma at vaccination site	1

Supplementary Table 1. Unsolicited AEs considered possibly, probably or definitely related to vaccination.

Maximum reported severity shown. Time-point(s) = days post-vaccination.

Name	Sequence	Peptide Pool	
RH5-1	ENAIKKTKNQENQLTLLPIK	**P1**	N-terminal
RH5-2	ENQLTLLPIKSTEEEKDDIK	**P1**	
RH5-3	STEEEKDDIKNGKDIKKEID	**P1**	
RH5-4	NGKDIKKEIDNDKENIKTNN	**P1**	
RH5-5	NDKENIKTNNAKDHSTYIKS	**P1**	
RH5-6	AKDHSTYIKSYLNTNVNDGL	**P1**	
RH5-7	YLNTNVNDGLKYLFIPSHNS	**P1**	
RH5-8	KYLFIPSHNSFIKKYSVFNQ	**P1**	
RH5-9	FIKKYSVFNQINDGMLLNEK	**P1**	
RH5-10	INDGMLLNEKNDVKNNEDYK	**P1**	
RH5-11	NDVKNNEDYKNVDYKNVNFL	**P1**	
RH5-12	NVDYKNVNFLQYHFKELSNY	**P1**	
RH5-13	QYHFKELSNYNIANSIDILQ	**P1**	
RH5-14	NIANSIDILQEKEGHLDFVI	**P2**	Start of 45 kDa fragment
RH5-15	EKEGHLDFVIIPHYTFLDYY	**P2**	
RH5-16	IPHYTFLDYYKHLSYNSIYH	**P2**	
RH5-17	KHLSYNSIYHKSSTYGKCIA	**P2**	
RH5-18	KSSTYGKCIAVDAFIKKIQE	**P2**	
RH5-19	VDAFIKKIQETYDKVKSKCN	**P2**	
RH5-20	TYDKVKSKCNDIKNDLIATI	**P2**	
RH5-21	DIKNDLIATIKKLEHPYDIN	**P2**	
RH5-22	KKLEHPYDINNKNDDSYRYD	**P3**	Disordered Loop
RH5-23	NKNDDSYRYDISEEIDDKSE	**P3**	
RH5-24	ISEEIDDKSEETDDETEEVE	**P3**	
RH5-25	ETDDETEEVEDSIQDTDSNH	**P3**	
RH5-26	DSIQDTDSNHTPSNKKKNDL	**P3**	
RH5-27	TPSNKKKNDLMNRTFKKMMD	**P3**	
RH5-28	MNRTFKKMMDEYNTKKKKLI	**P4**	Structured 1
RH5-29	EYNTKKKKLIKCIKNHENDF	**P4**	
RH5-30	KCIKNHENDFNKICMDMKNY	**P4**	

RH5-31	NKICMDMKNYGTNLFEQLSC	**P4**	
RH5-32	GTNLFEQLSCYNNNFCNTNG	**P4**	
RH5-33	YNNNFCNTNGIRYHYDEYIH	**P4**	
RH5-34	IRYHYDEYIHKLILSVKSKN	**P4**	
RH5-35	KLILSVKSKNLNKDLSDMTN	**P4**	
RH5-36	LNKDLSDMTNILQQSELLLT	**P5**	
RH5-37	ILQQSELLLTNLNKKMGSYI	**P5**	
RH5-38	NLNKKMGSYIYIDTIKFIHK	**P5**	
RH5-39	YIDTIKFIHKEMKHIFNRIE	**P5**	
RH5-40	EMKHIFNRIEYHTKIINDKT	**P5**	
RH5-41	YHTKIINDKTKIIQDKIKLN	**P5**	
RH5-42	KIIQDKIKLNIWRTFQKDEL	**P5**	Structured 2
RH5-43	IWRTFQKDELLKRILDMSNE	**P5**	
RH5-44	LKRILDMSNEYSLFITSDHL	**P6**	
RH5-45	YSLFITSDHLRQMLYNTFYS	**P6**	
RH5-46	RQMLYNTFYSKEKHLNNIFH	**P6**	
RH5-47	KEKHLNNIFHHLIYVLQMKF	**P6**	Structured 3
RH5-48	HLIYVLQMKFNDVPIKMEYF	**P6**	
RH5-49	NDVPIKMEYFQTYKKNKPLT	**P6**	
RH5-50	QTYKKNKPLTQ	**P6**	

Supplementary Table 2. RH5 overlapping peptides for ELISPOT assays.

20mer peptides overlapping by 10 aa were generated for the whole of the RH5 vaccine 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 pools used in the ELISPOT assay indicated. These pools corresponded to regions of interest identified from RH5 structural and biochemical analysis (14, 15). RH5ΔNL protein lacking the N-terminal region of RH5 (14) begins at residue K140 (highlighted in yellow in pool 1), and also lacks the internal disordered loop N248-M296 (highlighted in yellow in pool 3).

#	N-term	Sequence	C-term
1	Amine-	ENAIKKTKNQEN**Q**LTLLPIKGSG	Lys(Biotin)-Amide
2	Biotin-	SGSGNQEN**Q**LTLLPIKSTEEEKDD	-Amide
3	Biotin-	SGSGLPIKSTEEEKDDIKNGKDIK	-Amide
4	Biotin-	SGSGEKDDIKNGKDIKKEIDNDKE	-Amide
5	Biotin-	SGSGKDIKKEIDNDKENIKTNNAK	-Amide
6	Biotin-	SGSGNDKENIKTNNAKDHSTYIKS	-Amide
7	Biotin-	SGSGNNAKDHSTYIKSYLNTNVND	-Amide
8	Biotin-	SGSGYIKSYLNTNVNDGLKYLFIP	-Amide
9	Biotin-	SGSGNVNDGLKYLFIPSHNSFIKK	-Amide
10	Biotin-	SGSGLFIPSHNSFIKKYSVFNQIN	-Amide
11	Biotin-	SGSGFIKKYSVFNQINDGMLLNEK	-Amide
12	Biotin-	SGSGNQINDGMLLNEKNDVKNNED	-Amide
13	Biotin-	SGSGLNEKNDVKNNEDYKNVDY**K**N	-Amide
14	Biotin-	SGSGNNEDYKNVDY**K**NVNFLQYHF	-Amide
15	Biotin-	SGSGDY**K**NVNFLQYHFKELSNYNI	-Amide
16	Biotin-	SGSGQYHFKELSNYNIANSIDILQ	-Amide
17	Biotin-	SGSGNYNIANSIDILQEKEGHLDF	-Amide
18	Biotin-	SGSGDILQEKEGHLDFVIIPHYTF	-Amide
19	Biotin-	SGSGHLDFVIIPHYTFLDYYKHLS	-Amide
20	Biotin-	SGSGHYTFLDYYKHLSYNSIYHKS	-Amide
21	Biotin-	SGSGKHLSYNSIYHKSSTYGKCIA	-Amide
22	Biotin-	SGSGYHKSSTYGKCIAVDAFIKKI	-Amide
23	Biotin-	SGSGKCIAVDAFIKKI**Q**ETYDKVK	-Amide
24	Biotin-	SGSGIKKI**Q**ETYDKVKSKCNDIKN	-Amide
25	Biotin-	SGSGDKVKSKCNDIKNDLIATIKK	-Amide
26	Biotin-	SGSGDIKNDLIATIKKLEHPYDIN	-Amide
27	Biotin-	SGSGTIKKLEHPYDINNK**N**DDSYR	-Amide
28	Biotin-	SGSGYDINNK**N**DDSYRYDISEEID	-Amide
29	Biotin-	SGSGDSYRYDISEEIDDKSEETDD	-Amide
30	Biotin-	SGSGEEIDDKSEETDDETEEVEDS	-Amide
31	Biotin-	SGSGETDDETEEVEDSIQDTDSNH	-Amide
32	Biotin-	SGSGVEDSIQDTDSNHTPSNKKKN	-Amide
33	Biotin-	SGSGDSNHTPSNKKKNDL**M**NRTFK	-Amide
34	Biotin-	SGSGKKKNDL**M**NRTFKKMMDEYNT	-Amide
35	Biotin-	SGSGRTFKKMMDEYNTKKKKLIKC	-Amide
36	Biotin-	SGSGEYNTKKKKLIKCIKNHENDF	-Amide
37	Biotin-	SGSGLIKCIKNHENDFNKICMDMK	-Amide
38	Biotin-	SGSGENDFNKICMDMKNYGTNLFE	-Amide
39	Biotin-	SGSGMDMKNYGTNLFEQLSCYNNN	-Amide
40	Biotin-	SGSGNLFEQLSCYNNNFCNTNGIR	-Amide
41	Biotin-	SGSGYNNNFCNTNGIRYHYDEYIH	-Amide
42	Biotin-	SGSGNGIRYHYDEYIHKLILSVKS	-Amide
43	Biotin-	SGSGEYIHKLILSVKSKNLNKDLS	-Amide
44	Biotin-	SGSGSVKSKNLNKDLSDMTNILQQ	-Amide
45	Biotin-	SGSGKDLSDMTNILQQSELLLTNL	-Amide
46	Biotin-	SGSGILQQSELLLTNLNKKMGSYI	-Amide
47	Biotin-	SGSGLTNLNKKMGSYIYIDTIKFI	-Amide

48	Biotin-	SGSGGSYIYIDTIKFIHKEMKHIF	-Amide
49	Biotin-	SGSGIKFIHKEMKHIFNRIEYHTK	-Amide
50	Biotin-	SGSGKHIFNRIEYHTKIINDKTKI	-Amide
51	Biotin-	SGSGYHTKIINDKTKIIQDKIKLN	-Amide
52	Biotin-	SGSGKTKIIQDKIKLNIWRTFQKD	-Amide
53	Biotin-	SGSGIKLNIWRTFQKDELLKRILD	-Amide
54	Biotin-	SGSGFQKDELLKRILDMSNEYSLF	-Amide
55	Biotin-	SGSGRILDMSNEYSLFITSDHLRQ	-Amide
56	Biotin-	SGSGYSLFITSDHLRQMLYNTFYS	-Amide
57	Biotin-	SGSGHLRQMLYNTFYSKEKHLNNI	-Amide
58	Biotin-	SGSGTFYSKEKHLNNIFHHLIYVL	-Amide
59	Biotin-	SGSGLNNIFHHLIYVLQMKFNDVP	-Amide
60	Biotin-	SGSGIYVLQMKFNDVPIKMEYFQT	-Amide
61	Biotin-	SGSGNDVPIKMEYFQTYKKNKPLT	-Amide
62	Biotin-	SGSGDVPIKMEYFQTYKKNKPLTQ	-Acid
K33-K51-Biotin	Amine-	KNQENNLTLLPIKSTEEEKSGSGK	-Biotin
Biotin-K33-K51	Biotin-	SGSGKNQENNLTLLPIKSTEEEK	-Acid

Supplementary Table 3. Biotinylated RH5 overlapping peptides for ELISA assays.

20mer peptides overlapping by 12 aa were generated for the whole of the RH5 vaccine insert present in the ChAd63 and MVA vaccines. Underlined aa sequences were included as linkers between the biotin and 20mer. Chemistries at the two termini are also indicated. The N38Q and N214Q amino acid substitutions in the vaccine construct are highlighted in bold and yellow. Residues relevant to the RH5ΔNL (14) protein are highlighted in pink: K140 (first residue), and also N248-M296 (internal disordered loop). Two further peptides were used corresponding to the P113-binding region mapped within RH5 to residues K33-K51 (15). These peptides were based on native RH5 sequence, without the N38Q amino acid substitution in the vaccine construct to remove a site of potential N-linked glycosylation (green highlight), and were tested with both N- and C-terminal biotinylation.

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