

Malaria vaccine protection against intradermal or venous parasites: a randomized phase 2b human challenge trial

Received: 20 January 2025

Accepted: 31 October 2025

Published online: 6 January 2026

 Check for updates

A list of authors and their affiliations appears at the end of the paper

Two licensed vaccines block *Plasmodium falciparum* malaria sporozoites through anticircumsporozoite protein antibodies. In animal models, intradermal (ID) sporozoites are more readily blocked than intravenous sporozoites. We hypothesized that this complicates human studies, where infectious mosquito bites deliver a mixture of ID and venous sporozoites. Here, to test whether vaccine efficacy varies by route of inoculation, we undertook a phase 2b open, randomized controlled trial, recruiting healthy volunteers in Kenya for randomization to the circumsporozoite protein-based R21/Matrix-M vaccine ($n = 38$), thrombospondin-related adhesive protein fused to a multi-epitope string (ME-TRAP)-based vaccines ($n = 24$) or to control ($n = 18$). We enrolled 37 of these volunteers to controlled human malaria infection (CHMI) using ID or direct venous injection (DVI) of sporozoites, with PCR monitoring of parasitemia. Systemic and local postvaccination adverse events and systemic CHMI-related events were detected in 4.8%, 12.9% and 72.9% of volunteers, respectively, most commonly fever, headache and fatigue. No serious or severe adverse events were seen. Seven of 8 (88%) control volunteers and 11 of 12 (92%) ME-TRAP vaccinated volunteers, but none of the 12 R21 vaccinated volunteers receiving ID challenge met the prespecified treatment criteria for the primary endpoint. However, five of five R21 vaccinated volunteers receiving DVI sporozoites met the primary endpoint ($P < 0.0005$ by log rank across all groups). Secondary efficacy outcomes were similar; that is, R21 vaccinated volunteers receiving ID challenge did not meet any secondary endpoints, whereas volunteers for other groups met secondary endpoints between days 7 and 21 ($P < 0.0005$, $P = 0.0001$, $P = 0.0007$ and $P < 0.0005$ by log rank for time to parasitemia >20 , >500 , $>1,000$ and $>10,000$, respectively). R21/Matrix-M was highly protective against CHMI using ID. inoculation of sporozoites, but not against DVI sporozoites. CHMI is used in clinical development to select efficacious vaccines and to define correlates of efficacy. Correlates of efficacy for antibodies to sporozoites should also be assessed by separate DVI and ID challenges. The study was registered with ClinicalTrials.gov (NCT03947190) and PACTR (PACTR202108505632810).

Plasmodium falciparum (Pf) malaria remains a pressing public health problem. The Pf life cycle includes (1) sporozoites, injected by infected mosquitoes during feeding and then invade hepatocytes and (2) merozoites, which develop in hepatocytes and then initiate blood stage infection through repeated red blood cell invasion. Two malaria vaccines have recently been licensed (that is, RTS,S/AS01 (ref. 1) and R21/Matrix-M²), both of which induce immunity to the major antigen on the sporozoite surface, that is, the circumsporozoite protein (CSP). The passive transfer of anti-CSP antibodies is also partially protective³.

RTS,S/AS01 and R21/Matrix-M are both recommended by the World Health Organization (WHO) for use in young children and are predicted to have major health benefits on morbidity and mortality when given as a three-dose primary course between 5 and 9 months of age with a booster at 2 years⁴. Over the first year of follow-up, efficacy is reported at 78% for R21/Matrix-M² and 56% for RTS,S/AS01 (ref. 5). Efficacy appears to wane over time as antibodies fall^{6,7} but can be restored with booster doses for both vaccines^{1,8}. Reductions in mortality were seen in a 4-year implementation trial of RTS,S/AS01 (ref. 9).

It is unclear why antibody-based protection remains partial despite high levels of anti-CSP antibodies induced by vaccines or high concentrations of circulating monoclonal antibodies. Empirically, protection appears to be 'leaky', with given antibody levels being inconsistently protective¹⁰.

Controlled human malaria infection (CHMI) involves exposing healthy volunteers to challenge with malaria parasites, using either laboratory-reared infectious mosquitoes or cryopreserved parasites¹¹. The challenge controls the dose, timing and genotype of the exposure, in contrast to the heterogeneity of exposures seen in field studies¹². CHMI studies were undertaken in the initial clinical development of RTS,S/AS01E and R21/Matrix-M using infectious mosquito bites, where efficacy was estimated at 50%¹³ and 64–82%¹⁴, respectively.

Infectious mosquito bites mostly deliver Pf sporozoites into dermal layers and sporadically into capillaries¹⁵. Direct venous injection (DVI) of sporozoites infects human hosts with sevenfold fewer sporozoites than intradermal (ID) injection^{16,17}, with many sporozoites failing to reach capillaries¹⁸. Furthermore, in animal models, dermal sporozoites are more readily blocked by anti-CSP antibodies compared to intravenous sporozoites^{19,20}. We therefore hypothesized that vaccine protection might vary according the route of inoculation (that is, ID versus DVI).

To our knowledge, there are no CHMI studies that compare anti-CSP antibodies against different routes of inoculation of sporozoites in human volunteers. We undertook a CHMI efficacy study in volunteers vaccinated with R21/Matrix-M in Kilifi on the Kenyan Coast, comparing DVI versus ID challenge.

An alternative vaccination strategy to the induction of anti-CSP antibodies is the induction of T cells using viral vectors²¹. Previous studies of T cell-inducing vaccinations with a recombinant chimpanzee adenovirus (ChAd63) then modified vaccinia Ankara (MVA), both encoding the Pf antigens full-length pre-erythrocytic antigen thrombospondin-related adhesive protein (TRAP) fused to a multi-epitope (ME) string (ME-TRAP), have shown efficacy against CHMI among UK adults²², no efficacy in West African children²³, but efficacy in Kenyan adults²⁴. We therefore included an additional group to retest the efficacy of ChAd63/MVA ME-TRAP in Kenyan adults to test whether this was a chance finding. T cell immunity is directed at liver stages, and there was therefore no biological reason to expect the route of sporozoite inoculation to influence efficacy, hence we restricted ME-TRAP vaccinated volunteers to ID challenge.

CHMI studies in for clinical vaccine development are typically done using infectious mosquito bites. Arguably, this reflects the challenge experienced in field studies more closely than the artificial injection of cryopreserved sporozoites and predicts outcomes in subsequent field studies²⁵. However, where sensitivity to the anti-CSP antibodies induced by vaccination may vary for ID and for intravenous sporozoites, then

the mixture of inoculation routes in infectious mosquito bites introduces noise and precludes a definitive measurement of correlates of efficacy. To test whether vaccine efficacy varies by route of sporozoite inoculation for R21/Matrix-M, we randomized R21 vaccinated volunteers to CHMI using ID or DVI of sporozoites.

Results

Participant demographics

One hundred thirty-five volunteers were screened for eligibility and 80 were randomized and vaccinated between 20 July 2022 and 27 December 2022. All completed scheduled vaccinations and the first cohort (that is, $n = 40$) were eligible for CHMI undertaken from 27 November 2022 to 17 December 2022. Three did not proceed to CHMI owing to SARS-CoV-2 positive results ($n = 2$) and increase in liver enzymes (alanine aminotransferase (ALT), $n = 1$), so that 37 completed CHMI (Fig. 1). One volunteer was qPCR positive for malaria parasites before vaccination, and all were qPCR negative for malaria parasites before CHMI. There was a predominance of young male volunteers (28/40, 70%) with a mean age of 28.3 years (Table 1). Volunteers were from the Mijikenda ethnic group. The male predominance among volunteers appears to relate to established gender roles²⁶.

The prespecified primary endpoint was time to meeting treatment criteria (that is, reaching the parasitaemia threshold of 500 parasites μl^{-1} or any parasitaemia plus important clinical symptoms). Prespecified secondary endpoints were (1) immunology (specifically antibody responses to CSP measured by enzyme-linked immunosorbent assay (ELISA) and T cell responses to ME-TRAP peptides measured by ELISPOT) and (2) efficacy (time to parasitemia >20 , >500 , $>1,000$ and $>10,000$, respectively). All these prespecified outcomes are reported here and are not being published elsewhere.

Primary outcomes

CHMI was conducted 28 days after the last vaccination. All 8 unvaccinated control volunteers and all 12 volunteers vaccinated with ME-TRAP became PCR positive for malaria parasites following ID challenge with cryopreserved sporozoites produced by Sanaria (PfSPZ), with observed typical parasite growth curves on qPCR. Seven of eight control volunteers (88%) and 11 of 12 ME-TRAP volunteers (92%) met the criteria for the primary endpoint and received malaria treatment during CHMI, and one control and one ME-TRAP volunteer remained just below the parasitaemia threshold and so did not meet the primary endpoint (Fig. 2a,c and Table 2).

In contrast, none of the 12 volunteers vaccinated with R21 met the primary endpoint following challenge with ID PfSPZ. Three out of the nine volunteers were briefly positive by PCR for malaria parasites but with no evidence of parasite growth. Hence they were protected and did not meet the primary endpoint (Fig. 2b and Table 1).

Historically unvaccinated control volunteers who had previously undergone CHMI with PfSPZ challenge by DVI showed similar growth rates to PfSPZ challenge ID volunteers, with 31 out of 34 (91%) volunteers meeting the primary endpoint, 2 out of 34 being PCR negative (6%) and 1 out of 34 being PCR positive but not meeting the primary endpoint (3%) (Fig. 2d). However, when the five volunteers vaccinated with R21 underwent CHMI with PfSPZ challenge by DVI sporozoites, all five showed typical growth curves of parasites and met the primary endpoint (Fig. 2e).

Hence, none of the volunteers vaccinated by R21 who received the ID PfSPZ challenge met a primary endpoint, whereas volunteers for all other groups met primary endpoints between days 12 and 21 ($P < 0.0005$ by log-rank survival across all groups; Fig. 3). Parasites were genotyped for AMA1, confirming the challenge parasite strain (NF54) in all cases.

Secondary outcomes. Immunogenicity outcomes were the secondary outcomes. Following the vaccination course with R21, anti-NANP antibodies rose from baseline levels of just under 10 ELISA units (EU) to above 1,000 EU in the R21-vaccinated volunteers, but remained below

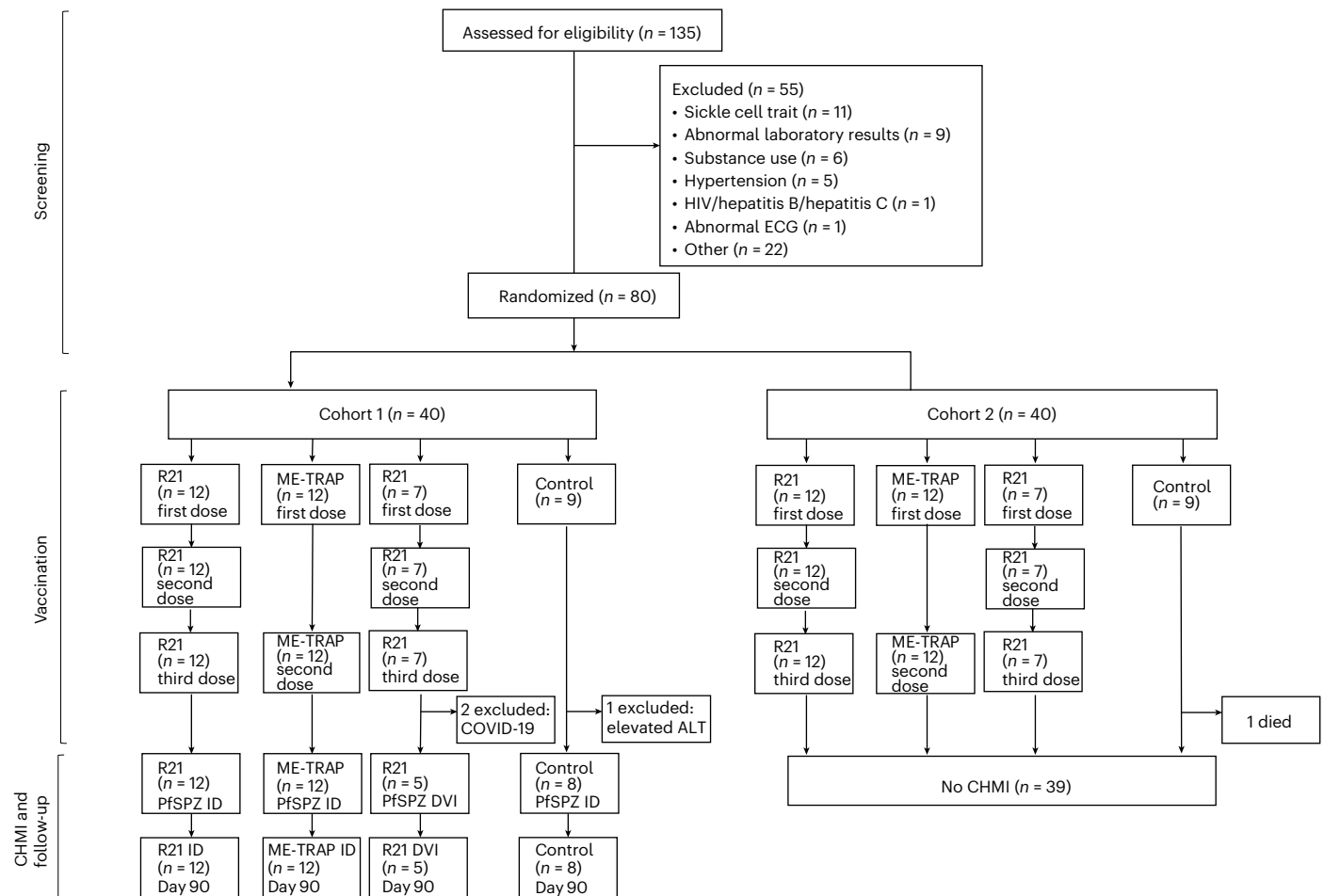


Fig. 1 | CONSORT diagram showing volunteer flow. Out of 135 volunteers screened, 80 volunteers who were eligible and met the enrollment criteria were randomized into one of four groups across two enrollment cohorts 4 weeks apart. Of the 55 excluded volunteers, those with abnormal laboratory results included low hemoglobin levels (<10 g dl⁻¹ for females (*n* = 6)), thrombocytopenia (*n* = 2) and elevated levels of ALTs (*n* = 1). Other exclusions included being eligible but did not turn up for screening results (*n* = 4), not being on any method of contraception (*n* = 3), number required attained (*n* = 3), did not meet location of

residence criteria (*n* = 2) and did not attend enrollment visit (*n* = 2). Other reasons, all with a frequency of one (*n* = 8) were written informed consent not signed, unstable blood pressure values, positive pregnancy test, abdominal hernia, neurofibromatosis, allergy to ibuprofen, failed test of understanding and history of schizophrenia. Seven volunteers had more than one screening failure and these were elevated laboratory values (*n* = 4), abdominal mass (*n* = 1), abnormal electrocardiogram (ECG) (*n* = 1) and substance use (*n* = 1). First dose, second dose and third dose indicate the respective vaccine doses.

Table 1 | Baseline characteristics based on vaccine enrollment and CHMI group

	Challenged				Not challenged				Historical control DVI (<i>n</i> = 34)
	R21 ID (<i>n</i> = 12)	R21 DVI (<i>n</i> = 7)	ME-TRAP ID (<i>n</i> = 12)	Control ID (<i>n</i> = 9)	R21 ID (<i>n</i> = 12)	R21 DVI (<i>n</i> = 7)	ME-TRAP ID (<i>n</i> = 12)	Control ID (<i>n</i> = 9)	
Age in years, mean (s.d.)	28.2 (6.1)	26.1 (4.1)	28.8 (5.1)	30.8 (8.1)	27.4 (6.8)	25.4 (6.0)	28.1 (7.1)	26.7 (3.5)	28.0 (8.0)
Male, % (<i>n</i> / <i>N</i>)	83.3% (10/12)	71.4% (5/7)	66.7% (8/12)	55.6% (5/9)	83.3% (10/12)	71.4% (5/7)	58.3% (7/12)	77.8% (7/9)	79% (27/34)
Female, % (<i>n</i> / <i>N</i>)	16.7% (2/12)	28.6% (2/7)	33.3% (4/12)	44.4% (4/9)	16.7% (2/12)	28.6% (2/7)	41.7% (5/12)	22.2% (2/9)	21% (7/34)
BMI, kg m ⁻² , mean (s.d.)	21.69 (1.7)	19.72 (1.8)	23.88 (4.8)	20.55 (1.9)	21.74 (2.4)	20.71 (1.8)	23.29 (3.9)	19.79 (1.5)	31.0 (3.5)

Data are presented either as mean (s.d.), percentage and (*n*/*N*) or median and (min–max). BMI, body mass index; *n*, number of healthy volunteers enrolled to each group.

10 among the control group (Extended Data Fig. 1). Peak responses were geometric means of 2,152 and 1,113 and minimum to maximum ranges of 750–5,500 versus 610–3,300 for ID versus DVI challenged groups, respectively (*P* = 0.14).

Following vaccination with viral vectors encoding ME-TRAP, spot-forming units (s.f.u.) per million peripheral blood mononuclear cells (PBMCs) increased from geometric mean of 104 s.f.u. (95% CI 70–153) at baseline to a peak of 735 (95% CI 364–1,485 s.f.u., *P* = 0.01 for both comparisons with baseline) and 335 s.f.u. (95% CI 162–691 s.f.u.) on

the day of challenge, compared with 127 s.f.u. (95% CI 74–409 s.f.u.) among the control group; *P* = 0.064, *P* = 0.001 and *P* = 0.084 for comparisons between all controls and ME-TRAP vaccinated volunteers at days 0, 63 and 84, respectively (Extended Data Fig. 2).

Volunteers had evidence of past exposure to malaria, as evidenced by prevaccination IgG antibodies against schizont extract (Extended Data Fig. 3). Historical controls had higher titers than the newly recruited cohort (geometric means of 1,244,000 EU (95% CI 693,000–2,235,000 EU) versus 394,000 EU (95% CI 258,000–602,000 EU),

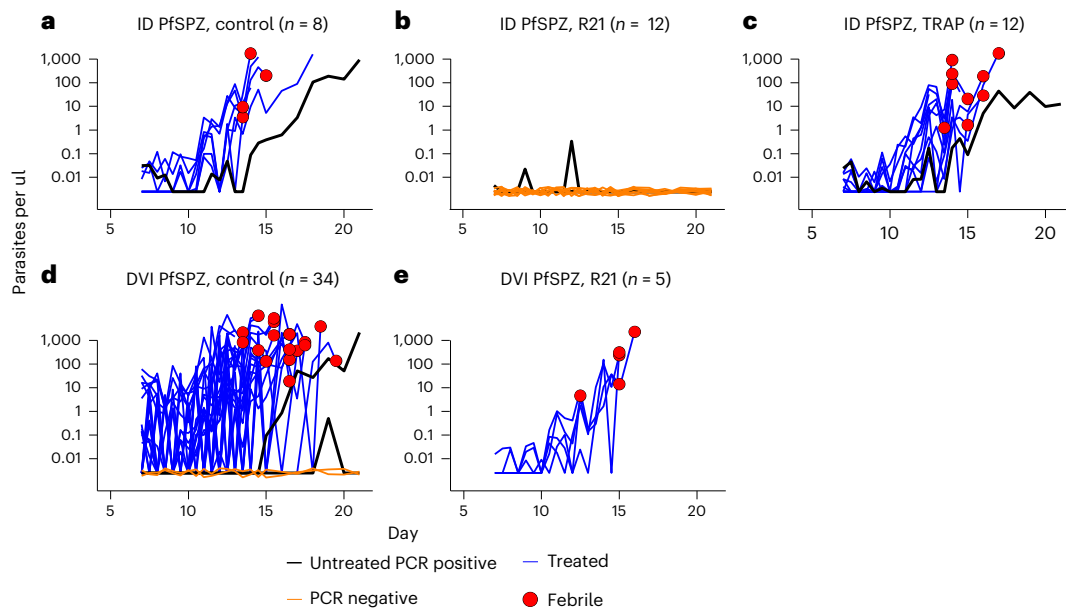


Fig. 2 | Falciparum qPCR outcome following CHMI. **a–e**, The qPCR results (y axis, log transformed) by time after inoculation (x axis) showing ID challenge with PfSPZ, control group ($n = 8$) (**a**); ID challenge with PfSPZ, vaccinated with R21/Matrix-M ($n = 12$) (**b**); ID challenge with PfSPZ, vaccinated with viral vectors encoding ME-TRAP ($n = 12$) (**c**); DVI challenge with PfSPZ, control group ($n = 34$) (**d**); and DVI challenge with PfSPZ, vaccinated with R21/Matrix-M ($n = 5$) (**e**).

Parasitemia was determined by asexual 18S ribosomal RNA gene qPCR done in Kilifi. The blue lines represent individuals who reached the required malaria diagnosis criteria, green lines represent individuals who did not meet the criteria for diagnosis but were qPCR positive, orange lines represent individuals who were qPCR negative throughout monitoring and red dots denote individuals who were febrile based on the criteria for diagnosis.

respectively, $P = 0.0009$), and the DVI and ID challenged groups had similar schizont extract antibodies (geometric means of 284,000 EU (95% CI 158,000–511,000 EU) versus 434,000 EU (95% CI 252,000–745,000 EU), respectively, $P = 0.4$).

Secondary efficacy outcomes were consistent with the primary outcome, that is, none of the volunteers vaccinated by R21 who received the ID PfSPZ challenge met the secondary endpoints, whereas volunteers for all other groups met secondary endpoints between days 7 and 21, none of the 12 volunteers in the R21 ID group met any of the secondary endpoints, whereas 75/78 (96.1%), 60/78 (76.9%), 56/78 (71.8%) and 39/78 (50%) met secondary endpoints ($P < 0.0005$, $P = 0.0001$, $P = 0.0007$ and $P < 0.0005$ by log-rank survival across all groups for time to parasitemia >20 , >500 , $>1,000$ and $>10,000$, respectively; Extended Data Fig. 4).

Safety. Following vaccination, self-limiting local solicited adverse events and general adverse events such as headache or fatigue were detected in a minority of volunteers (Table 3). No event was reported as severe and four events were reported as moderate, with a median and maximum duration of 4.5 and 5 days, respectively. General adverse events were similar in the R21 and ME-TRAP groups after the first vaccination. Specifically, headache occurred in 0/24, 1/14 (7.1%) and 5/24 (21%); fatigue in 2/24 (8.3%), 0/14 and 3/24 (12%); and malaise in 2/24 (8.3%), 0/14 and 2/24 (8.3%) of volunteers allocated to the R21 ID, R21 DVI and ME-TRAP ID groups, respectively. Fever was a more common general adverse event at the final vaccination for ME-TRAP with 5/24 (20.8%) and 8/24 (33.3%) experiencing headache, but only 1/38 volunteers vaccinated with R21 experienced fever (2.6%) and 2/38 experienced headache (5.2%). Local adverse events were rare and limited to pain at the injection site in 1/24 (4.2%) and 2/24 (8.3%) in the R21 ID and ME-TRAP ID groups, respectively.

During CHMI, there were no immediate or early adverse events, but after day 7, fever, headache and fatigue were common in 22/37 (59.5%), 17/37 (45.9%) and 12/37 (32.0%) of volunteers, respectively (Extended Data Table 1). Six events were reported to be moderate and none were reported as severe. All events resolved on follow-up.

Abnormal blood tests, primarily transient elevations in liver enzymes and/or low white blood counts were identified in several volunteers but were not clinically significant and resolved on follow-up (Extended Data Table 2). There were clinically nonsignificant minor elevations in liver enzymes among the R21 ID group, and during CHMI there were clinically nonsignificant, transient abnormalities of liver enzymes and platelet counts (Extended Data Table 3). The safety findings were consistent with those reported previously in the same population²⁷.

Sensitivity analysis. We saw one-off PCR positive signals on a single time point among 3 of the 12 volunteers (25%) vaccinated with R21 who received PfSPZ Challenge by ID. If we include these volunteers as meeting an exploratory endpoint for purposes of a sensitivity analysis, protection is nevertheless substantial by ID (9 out of 12, 75%), and statistically significantly different from DVI (0 out of 5 protected, $P = 0.009$ by Fisher's two-sided test).

Post hoc analysis. The geometric mean parasite densities by PCR were similar over time following PfSPZ challenge for the control group by DVI, the control group by ID challenge, ME-TRAP vaccines by ID challenge and R21 vaccines by DVI (Extended Data Fig. 5). Hence, parasite inoculum and growth rates appear similar among these four groups.

Discussion

Our results extend previous observations of protective efficacy by R21 against CHMI delivered by infective mosquito bites in UK adults¹⁴ and observations of protective efficacy by R21 against natural challenge in the field in West and East African children². Instead of using infectious mosquito bites, we injected the PfSPZ challenge by needle and syringe to compare the protective efficacy of DVI versus ID inoculation of sporozoites.

R21 provided high levels of protective efficacy against PfSPZ challenge by ID, but we found no evidence of efficacy against PfSPZ challenge by DVI (that is, 0 out of 12 volunteers challenged by ID met the primary endpoint of requiring treatment versus 5 out of 5 challenged

Table 2 | Malaria diagnosis outcome by vaccination and challenge group

qPCR outcome	Control ID n=8	R21 ID n=12	ME-TRAP ID n=12	Historical control DVI (n=34)	R21 DVI n=5
PCR negative, % (n/N)	0% (0/8)	75% (9/12)	0% (0/12)	5.9% (2/34)	0% (0/5)
Untreated PCR positive, % (n/N)	12.5% (1/8)	25% (3/12)	8.3% (1/12)	2.9% (1/34)	0% (0/5)
Treated, febrile, % (n/N)	50.0% (4/8)	0% (0/12)	75% (9/12)	55.9% (19/34)	100% (5/5)
Treated, nonfebrile, % (n/N)	37.5% (3/8)	0% (0/12)	17% (2/12)	35.3% (12/34)	0% (0/12)

Data are presented as the percentage and n/N. Treated, nonfebrile refers to volunteers who met parasitemia thresholds for treatment without developing objective fever; treated, febrile refers to volunteers who met lower parasitemia thresholds and were treated owing to the presence of objective fever and/or other clinical signs.

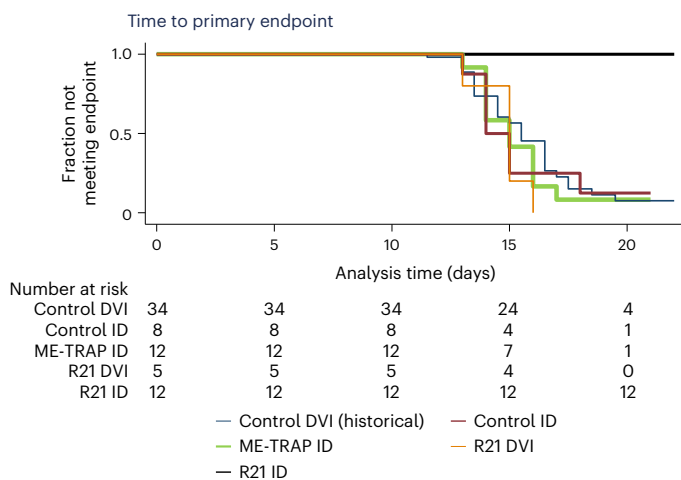


Fig. 3 | Time to primary endpoint survival analysis. Kaplan–Meier curve of the fraction of volunteers that did not meet the primary efficacy endpoint criteria for treatment (that is, 500 parasites per μ l, or any parasite density plus clinically significant symptoms) following CHMI. $P < 0.0005$ by log-rank testing across all groups.

by DVI, $P < 0.001$ by Fisher’s two-sided test). Hence, despite the small sample size and the inclusion of three low-density PCR signals in a sensitivity analysis, the variation in protective efficacy of R21 by challenge route was strongly statistically significant.

Was this difference in protective efficacy by challenge route the result of differing PfSPZ doses? PfSPZ challenge is more infectious when given by DVI. To ensure comparable infectivity, we selected PfSPZ challenge doses that reliably infected unvaccinated volunteers in previous studies (that is, a sevenfold reduction for DVI at 3,200 PfSPZ challenge to 22,500 cryopreserved PfSPZ challenge ID)^{16,17}. Among the unvaccinated volunteers in our study, neither infection rates (that is, 8/8 for ID challenge versus 32/34 (94%) for DVI challenge) nor growth curves (Extended Data Fig. 5) showed variation by challenge route.

We conclude that the different doses resulted in a similar effective inoculum reaching the liver in unvaccinated volunteers, and therefore that the differences seen among R21 vaccinated volunteers imply a qualitatively different interaction between anti-CSP antibodies and PfSPZ challenge that is dependent on the route of challenge. Furthermore, our data are consistent with animal models. Prior studies in mice have shown that at a given level, antisporezoite antibodies are protective against dermal inoculation but not against intravenous inoculation, and furthermore that more potent monoclonal antibodies are required to inhibit intravenous sporozoites compared to ID sporozoites^{19,20}.

Taking together that (1) infectious mosquito bites deliver a variable proportion of Pf sporozoites into capillaries or dermal layers¹⁵, (2) that anti-CSP antibodies correlate noisily with protection against sporozoite infection¹⁰, with protection appearing leaky²⁸, and (3) the data presented here that R21 vaccination was ineffective against PfSPZ challenge by DVI but effective against PfSPZ challenge by ID injection, we conclude that

sporozoites causing infection in the face of high-titer anti-CSP antibodies induced by RTS,S or by R21 result from the occasional injection of capillary sporozoites by mosquito bites, producing noise in the correlation between antibody titers and the endpoint, which explains the leaky protection. The infrequent injection of sporozoites into capillaries (that is, one in five mosquito feeds¹⁵) is consistent with high levels of efficacy achieved with anti-PfCSP antibody induction despite this lack of efficacy against PfSPZ challenge by DVI: that is, if most mosquito bites deliver only dermal sporozoites, then anti-PfCSP antibodies are likely to be protective most of the time, whereas every fifth bite delivering sporozoites directly into a capillary would lead to breakthrough infection.

Correlates of protection have been defined for many other vaccines²⁹ and have been used to guide policy decisions on vaccination schedules and doses for vaccines against yellow fever virus³⁰, pneumococcus³¹, meningococcus A³² and hepatitis B³³. Hence, defining a correlate of protection for a vaccine may translate to substantial public health benefits. While there is little doubt that anti-PfCSP antibodies are mechanistic correlates of protection, the strength of the association has varied by trial, and understanding the sources of noise in field trials will support public health decisions on future vaccine schedules.

More potent or higher-titer anti-CSP antibodies may be protective against DVI. In mouse models, high-potency monoclonal antibodies are able to inhibit sporozoites in blood whereas lower-potency monoclonals can inhibit sporozoites in skin or blood³⁴, and this is further supported by findings in Saimiri monkeys challenged with vivax sporozoites³⁵. Furthermore, vaccines acting through mechanisms apart from anti-CSP antibodies may not exhibit route-dependent protection³⁶.

ME-TRAP was not protective against CHMI in our study and T cell induction, determined by ELISpot, was lower than that previously seen in European and Kenyan volunteers among whom protective efficacy was observed (that is, 735 s.f.u. at the peak in our study versus 2,068 and 1,451 s.f.u., respectively)^{22,24}. Immunogenicity to this regimen may be suppressed by prior malaria exposure³⁷ and may explain variable efficacy. In any case, the finding is not relevant to testing our hypothesis regarding antisporezoite antibodies, since the mechanism of action for ME-TRAP vaccines is cellular immunity against liver parasite stages³⁸.

Our study has some limitations. We relied on historical controls for PfSPZ challenge by DVI and prior malaria exposure appears to have been higher in this group (Extended Data Fig. 4). However, the outcomes for DVI versus ID PfSPZ challenge among R21 vaccinees were based on a contemporaneous and randomized comparison, as was the comparison of R21 vaccinees versus control vaccines for PfSPZ challenge by ID, and prior malaria exposure did not differ between these groups (Extended Data Fig. 4).

Challenge by ID and DVI of cryopreserved sporozoites may not precisely duplicate infectious mosquito bites, and the latter is probably more representative of natural challenge in the field. For instance, with R21, ID and DVI PfSPZ challenge would under or overestimate protection in the field, respectively, whereas mosquito bite challenge more accurately predicted subsequent efficacy in the field¹⁴. On the other hand, our data suggest advantages in studying ID and/or DVI PfSPZ challenge in making comparisons between products inducing anti-CSP antibodies.

Table 3 | Local and general adverse events postvaccination

Local adverse events	Day 0			
	R21 ID, n=24	R21 DVI, n=14	ME-TRAP ID, N=24	Total, N=62
Pain at injection site	1 (4.2%)	0	2(8.3%)	3(4.8%)
Induration	0	0	0	0
Erythema	0	0	0	0
General adverse events				
Arthralgia	0	0	1(4.2%)	1(4.2)
Chills	0	0	1(4.2%)	1(4.2)
Fatigue	2 (8.3%)	0	3(12%)	5(8.1%)
Fever	0	1 (7.1%)	1(4.2%)	2(3.2)
Headache	0	1 (7.1%)	5(21%)	6(9.7%)
Malaise	2 (8.3%)	0	2(8.3%)	4(6.5%)
Myalgia	0	0	1(4.2%)	1(4.2%)
Day 28				
Local adverse events	R21 ID n=24	R21 DVI n=14		Total N=38
Pain at injection site	0	0		0
Induration	0	0		0
Erythema	0	0		0
General adverse events				
Arthralgia	0	0		0
Chills	1 (4.2%)	1 (7.1%)		2 (5.3%)
Fatigue	0	1 (7.1%)		1 (2.6%)
Fever	1 (4.2%)	1 (7.1%)		2 (5.3%)
Headache	0	1 (7.1%)		1 (2.6%)
Malaise	0	0		0
Myalgia	1 (4.2%)	2 (14%)		3 (7.9%)
Day 56				
Local adverse events	R21 ID n=24	R21 DVI n=14	ME-TRAP ID N=24	Total N=62
Pain at injection site	0	0	4 (16.7%)	4 (6.5%)
Induration	0	0	1 (4.2%)	1 (1.6%)
Erythema	0	0	0	0
General adverse events				
Arthralgia	0	0	1 (4.2%)	1 (1.6%)
Chills	0	0	1 (4.2%)	1 (1.6%)
Fatigue	0	0	1 (4.2%)	1 (1.6%)
Fever	1 (4.2%)	0	5 (20.8%)	6 (9.7)
Headache	0	2 (14%)	8 (33.3%)	10 (16.1%)
Malaise	0	0	3 (12.5%)	3 (4.8%)
Myalgia	1 (4.2)	0	3 (12.5%)	4 (6.5%)

A further limitation is the sample size. Five R21 vaccinated volunteers were challenged by DVI and 12 R21 vaccinated volunteers were challenged by ID injection. In mitigation of the sample size, the outcomes completely segregated according to challenge route and were found to be significant by the primary analysis (that is, log rank for time to outcome, $P < 0.0005$).

We recruited volunteers from a specific area of Kilifi County (that is, Ngerenya), which has been at low malaria transmission since the early 2000s³⁹. Previous work has shown that a high proportion of

CHMI participants from this location meet the primary endpoint and that baseline antibody levels to schizont extract are relatively low²⁷. We therefore recruited from this location for the present study and included data from previous participants resident in Ngerenya as a historical control population.

CHMI with infectious mosquito bites will probably remain an important step in clinical development of vaccines and is physiologically closer to challenge in the field. However, mosquito bites deliver a combination of dermal and venous sporozoites, and the concern raised by animal models is that dermal sporozoites are more readily blocked than venous sporozoites. Our study extends this finding to humans. Therefore, determining correlates of protection for anti-CSP antibodies will require either (1) large sample sizes to account for the noise introduced by mixed inoculation routes through mosquito bites or (2) a model where dermal and venous sporozoite inoculations can be studied separately. Our data suggest that the latter approach is tractable and could be used to derive antibody thresholds for protection to facilitate immunobridging studies for new products that also induce anti-CSP antibodies. Furthermore, superiority trials for new anti-CSP antibody inducing products will be underpowered in classic CHMI studies, but CHMI studies based on DVIPFSPZ can be used to specifically test for higher potency antibodies that could lead to higher levels of protection in the field against infectious mosquito bites.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-025-04107-6>.

References












- RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* **386**, 31–45 (2015).
- Dattoo, M. S. et al. Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, phase 3 trial. *Lancet* **403**, 533–544 (2024).
- Kayentao, K. et al. Subcutaneous administration of a monoclonal antibody to prevent malaria. *N. Engl. J. Med.* **390**, 1549–1559 (2024).
- Penny, M. A. et al. Public health impact and cost-effectiveness of the RTS,S/AS01 malaria vaccine: a systematic comparison of predictions from four mathematical models. *Lancet* **387**, 367–375 (2016).
- RTS,S Clinical Trials Partnership et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N. Engl. J. Med.* **365**, 1863–1875 (2011).
- Olotu, A. et al. Seven-year efficacy of RTS,S/AS01 malaria vaccine among young African children. *N. Engl. J. Med.* **374**, 2519–2529 (2016).
- Campo, J. J. et al. Duration of vaccine efficacy against malaria: 5th year of follow-up in children vaccinated with RTS,S/AS02 in Mozambique. *Vaccine* **32**, 2209–2216 (2014).
- Dattoo, M. S. et al. Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial. *Lancet Infect. Dis.* **22**, 1728–1736 (2022).
- World Health Organization. *Statistical Report, Malaria Vaccine Pilot Evaluation (MVPE), Analysis of Data to Month 46* (WHO, 2024); [https://www.who.int/publications/m/item/statistical-report-malaria-vaccine-pilot-evaluation-\(mvpe\)-analysis-of-data-to-month-46](https://www.who.int/publications/m/item/statistical-report-malaria-vaccine-pilot-evaluation-(mvpe)-analysis-of-data-to-month-46)


10. Olotu, A. I., Fegan, G. & Bejon, P. Further analysis of correlates of protection from a phase 2a trial of the falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults. *J. Infect. Dis.* **201**, 970–971 (2010).
11. Kibwana, E., Kapulu, M. & Bejon, P. Controlled human malaria infection studies in Africa—past, present, and future. *Curr. Top. Microbiol. Immunol.* **445**, 337–365 (2024).
12. Bejon, P. et al. Analysis of immunity to febrile malaria in children that distinguishes immunity from lack of exposure. *Infect. Immun.* **77**, 1917–1923 (2009).
13. Kester, K. E. et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. *J. Infect. Dis.* **200**, 337–346 (2009).
14. Venkatraman, N. et al. R21 in Matrix-M adjuvant in UK malaria-naive adult men and non-pregnant women aged 18–45 years: an open-label, partially blinded, phase 1–2a controlled human malaria infection study. *Lancet Microbe* **0**, 100867 (2024).
15. Ponnudurai, T., Lensen, A. H. W., Gemert, G. J. A., Bolmer, M. G. & Meuwissen, J. H. T. Feeding behaviour and sporozoite ejection by infected *Anopheles stephensi*. *Trans. R. Soc. Trop. Med. Hyg.* **85**, 175–180 (1991).
16. Shekalaghe, S. et al. Controlled human malaria infection of Tanzanians by intradermal injection of aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites. *Am. J. Trop. Med. Hyg.* **91**, 471–480 (2014).
17. Mordmüller, B. et al. Direct venous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection: a dose-finding trial in two centres. *Malar. J.* **14**, 117 (2015).
18. Hopp, C. S. et al. Longitudinal analysis of *Plasmodium* sporozoite motility in the dermis reveals component of blood vessel recognition. *Elife* **4**, e07789 (2015).
19. Flores-Garcia, Y. et al. Antibody-mediated protection against *Plasmodium* sporozoites begins at the dermal inoculation site. *mBio* **9**, 02194–18 (2018).
20. Aliprandini, E. et al. Cytotoxic anti-circumsporozoite antibodies target malaria sporozoites in the host skin. *Nat. Microbiol.* **3**, 1224–1233 (2018).
21. Kimani, D. et al. Translating the immunogenicity of prime-boost immunization with ChAd63 and MVA ME-TRAP from malaria naive to malaria-endemic populations. *Mol. Ther.* **22**, 1992–2003 (2014).
22. Hodgson, S. H. et al. Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naive individuals. *J. Infect. Dis.* **211**, 1076–1086 (2015).
23. Tiono, A. B. et al. First field efficacy trial of the ChAd63 MVA ME-TRAP vectored malaria vaccine candidate in 5–17 months old infants and children. *PLoS ONE* **13**, e0208328 (2018).
24. Ogwang, C. et al. Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against *Plasmodium falciparum* infection in Kenyan adults. *Sci Transl Med* **7**, 286re5 (2015).
25. Ogwang, R., Adan, M., Bejon, P. & Kapulu, M. C. Controlled human malaria infection studies: insights into recent advances and key immunological and ethical implementation lessons. *Front. Immunol.* **16**, 1672945 (2025).
26. Njue, M. et al. Ethical considerations in controlled human malaria infection studies in low resource settings: experiences and perceptions of study participants in a malaria challenge study in Kenya. *Wellcome Open Res.* **3**, 39 (2018).
27. Kapulu, M. C. et al. Safety and PCR monitoring in 161 semi-immune Kenyan adults following controlled human malaria infection. *JCI Insight* **6**, e146443 (2021).
28. Olotu, A. et al. Four-year efficacy of RTS,S/AS01E and its interaction with malaria exposure. *N. Engl. J. Med.* **368**, 1111–1120 (2013).
29. Plotkin, S. A. Correlates of protection induced by v_vaccination. *Clin. Vaccine Immunol.* **17**, 1055 (2010).
30. Juan-Giner, A. et al. Immunogenicity and safety of fractional doses of 17D-213 yellow fever vaccine in children (YEFE): a randomised, double-blind, non-inferiority substudy of a phase 4 trial. *Lancet Infect. Dis.* **23**, 965–973 (2023).
31. Gallagher, K. E. et al. Fractional doses of pneumococcal conjugate vaccine—a noninferiority Trial. *N. Engl. J. Med.* **391**, 2003–2013 (2024).
32. Sow, S. O. et al. Immunogenicity and safety of a meningococcal A conjugate vaccine in Africans. *N. Engl. J. Med.* **364**, 2293–2304 (2011).
33. Zanetti, A. R. et al. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* **366**, 1379–1384 (2005).
34. Aguirre-Botero, M. C. et al. Cytotoxicity of human antibodies targeting the circumsporozoite protein is amplified by 3D substrate and correlates with protection. *Cell Rep.* **42**, 112681 (2023).
35. Charoenvit, Y. et al. Inability of malaria vaccine to induce antibodies to a protective epitope within its sequence. *Science* **251**, 668–671 (1991).
36. Roozen, G. V. T. et al. Single immunization with genetically attenuated PfΔmei2 (GA2) parasites by mosquito bite in controlled human malaria infection: a placebo-controlled randomized trial. *Nat. Med.* <https://doi.org/10.1038/s41591-024-03347-2> (2025).
37. Bejon, P. et al. The induction and persistence of T cell IFN- γ responses after vaccination or natural exposure is suppressed by *Plasmodium falciparum*. *J. Immunol.* **179**, 4193–4201 (2007).
38. Kimani, D. et al. Translating the immunogenicity of prime-boost immunization with ChAd63 and MVA METRAP from malaria naive to malaria-endemic populations. *Mol. Ther.* **22**, 1992–2003 (2014).
39. O’Meara, W. P. et al. Relationship between exposure, clinical malaria, and age in an area of changing transmission intensity. *Am. J. Trop. Med. Hyg.* **79**, 185 (2008).

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

Melissa C. Kapulu ^{1,2} , **Francesca Orenge**¹, **Domtila Kimani**¹, **Elizabeth Kibwana**¹, **Hillary Kibet**¹, **Mary Mutahi**¹, **Mehreen S. Dattoo** ³, **Duncan Bellamy**³, **Janet Musembi**¹, **Omar Ngoto**¹, **Hamisi Rashid**¹, **Stellamaris Akinyi**¹, **Mwaganyuma H. Mwatasa**¹, **Lydia Nyamako**¹, **Kelvias Keter**¹, **Rose Gatheru**¹, **Agnes Mutiso**¹, **Jennifer Musyoki**¹, **Jedidah Mwacharo**¹, **Yonas Abebe**⁴, **Eric R. James**⁴, **Peter F. Billingsley**⁴, **Caroline Ngetsa**¹, **Moses Mosobo**¹, **Johnstone Makale**¹, **Brian Tawa**¹, **Kevin Wamae**¹, **Lynette I. Ochola-Oyier** ¹, **Alison Lawrie**³, **Fernando Ramos-Lopez** ³, **Rachel Roberts**³, **Thomas L. Richie** ⁴, **B. Kim Lee Sim** ⁴, **Stephen L. Hoffman** ⁴, **Katie J. Ewer**³, **Adrian V. S. Hill** ³, **Mainga Hamaluba** ^{1,2} & **Philip Bejon** ^{1,2,5}

¹Centre for Geographic Medicine Research (Coast), Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya. ²Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University Oxford, Oxford, UK. ³Jenner Institute, University of Oxford, Oxford, UK. ⁴Sanaria Inc., Rockville, MD, USA. ⁵Department of Modernising Medical Microbiology, Nuffield Department of Medicine, University Oxford, Oxford, UK.  e-mail: mkapulu@kemri-wellcome.org

Methods

We undertook a phase IIb open-label, unblinded, randomized single-center study. Randomization and vaccination was undertaken between 20 July 2022 and 27 December 2022, and the first cohort were eligible for CHMI undertaken between 27 November 2022 and 17 December 2022 at the KEMRI Wellcome Trust Research Programme in Kilifi Kenya. One hundred thirty-five volunteers were screened for eligibility and 80 were randomized and vaccinated. The first cohort ($n = 40$) were eligible for CHMI. Three did not proceed to CHMI owing to SARS-CoV-2 positive results ($n = 2$) and an increase in liver enzymes (ALT, $n = 1$), so that 37 completed CHMI (Fig. 1). This cohort included four groups (R21/Matrix-M ID, R21/Matrix-M DVI, ChAd63/MVA ME-TRAP ID and control ID). R21 was adjuvanted with Matrix-M (R21/Matrix-M) in a 0-, 1-, 2-month schedule (Supplementary Methods). ChAd63/MVA ME-TRAP was given as two sequential doses in a 0- and 2-month schedule. For CHMI, we used *P. falciparum* NF54 sporozoites (Sanaria PfSPZ challenge (NF54)) using two alternative inoculation routes: injecting either 22,500 PfSPZ challenge ID or 3,200 PfSPZ challenge DVI.

The study was open label as (1) placebo for the different doses and routes would have been impractical and (2) outcomes were based on objective PCR data and the laboratory team were blind to allocations.

Ethics

We complied with all relevant ethical requirements. Before commencing activity, approvals were obtained from a National IRB in Kenya and the relevant Oxford IRB (ERU (KEMRI/SERU/CGMR-C/158/3844) and OxTREC (OxTREC 32-19)) and from the medicines regulatory authority in Kenya (Pharmacy and Poisons Board (ECCT/19/11/01)). The study was registered with ClinicalTrials.gov (NCT03947190) on 07 May and PACTR (PACTR202108505632810) on 24 August 2021 to comply with in-country regulatory requirements. The use of Sanaria PfSPZ challenge (NF54) was done in accordance with an investigational new drugs application with the US Food and Drug Administration. The protocol underwent minor amendments to include methods for COVID testing and then for completion of study procedures after the first cohort underwent CHMI when a manufacturing hold was placed on PfSPZ, which prevented a second cohort CHMI.

Study volunteers and eligibility

Following written informed consent, we recruited healthy adult men and women aged between 18 and 45 years from Kilifi North on the Kenyan Coast, where there is currently very low malaria transmission but with previous low to moderate malaria transmission. Recruitment was conducted by fieldworkers who were resident in the study areas, combined with community events to explain the study. Prospective participants were self-referring. Gender was self-reported, and given the sample size and low frequency of women volunteers, gender subgroup analyses were not conducted.

Inclusion criteria were

- (1) Healthy adults aged 18–45 years
- (2) Able and willing (in the Investigator's opinion) to comply with all study requirements
- (3) Nonpregnant, nonlactating adult female or adult male
- (4) Agreement to refrain from blood donation during the study
- (5) Use of effective method of contraception for the duration of study for female participants.
- (6) Written informed consent
- (7) Plan to remain resident in the study area for 1 year following first dose of vaccination

Exclusion criteria were

- (1) Clinically significant congenital abnormalities as judged by the study clinicians

- (2) Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections; or >14 days immunosuppressant medication within the past 6 months
- (3) Sickle cell disease
- (4) Anaphylaxis in relation to vaccination
- (5) Clinically significant laboratory abnormality as judged by the study clinician
- (6) Blood transfusion within one month of enrollment
- (7) Hemoglobin less than 11.3 g dl⁻¹ for men and less than 10 g dl⁻¹ for in women, where judged to be clinically significant in the opinion of the investigator
- (8) Administration of immunoglobulins and/or any blood products within the last 3 months
- (9) Participation in another research study involving receipt of an investigational product in the 30 days preceding enrollment or planned use during the study period
- (10) Seropositive for hepatitis B surface antigen or hepatitis C (HCV IgG)
- (11) Use of systemic antibiotics with known antimalarial activity within 30 days of challenge
- (12) Women only; pregnancy, or an intention to become pregnant a day before challenge
- (13) Any significant disease, disorder or situation (including confirmed COVID-19 PCR positivity) which, in the opinion of the Investigator, may either put the participants at risk, or may influence the result of the trial
- (14) Confirmed parasite positive by PCR a day or 3 days before challenge

Compensation was informed by an existing guideline and approved by IRBs, and included out of pocket costs and transport, costs of contraception where applicable, an additional KES 2000 per overnight stay, applicable ATM or mobile money charges and KRA taxation.

Enrollment and randomization

Volunteers were considered enrolled at the time of randomization, and vaccination was done on the same day. Enrollment took place within 90 days of screening. The volunteers were randomly assigned with randomization via a computer-generated sequence by an independent statistician. A randomization list in the form of password protected spreadsheet was generated using STATA and the data manager setup randomization in REDCap. Study clinicians only clicked to randomize after confirmation of eligibility criteria and immediately before vaccination for each volunteer, after which REDCap would reveal the volunteers' randomization arm that could not be edited. The volunteers were randomized to either one of four groups.

Vaccines and vaccination

R21 is a pre-erythrocytic protein-in-adjuvant malaria vaccine candidate. It is adjuvanted with Matrix-M based on the CSP produced by using recombinant Hepatitis B surface antigen particles expressing the central NANP repeat and the C terminus. Volunteers received three vaccinations with R21 at 4-week intervals. R21 was thawed to room temperature then mixed with Matrix-M before administration (10 µg mixed with Matrix-M 50 µg) and administered intramuscularly.

The viral vectors used were ChAd63 ME-TRAP and MVA ME-TRAP. Volunteers received a single intramuscular dose of each vaccine which was given, sequentially, 8 weeks apart. Both vaccines were thawed to room temperature before administration.

CHMI

CHMI was undertaken 4 weeks after the final vaccination. Volunteers were tested for malaria blood stage infection by qPCR and tested for SARS-CoV-2 infection by RT-PCR on naso-pharyngeal swab. Volunteers positive for either were excluded from proceeding to CHMI.

The CHMI agent (that is, Sanaria PfSPZ challenge (NF54)) in cryovials was thawed by partial submersion of each vial for 30 s in a 37 ± 1 °C water bath. Trained study staff prepared, diluted and dispensed PfSPZ challenge to clinical staff using the diluents PBS and 25% human serum albumin within 30 min of thawing²⁷. Challenge was administered in a volume of 0.5 ml using a needle and syringe by DVI at the standard dose of 3,200 PfSPZ challenge. The ID injections were administered in two separate syringes in both left and right arms with each syringe containing 11,250 PfSPZ challenge in 0.05 ml (that is, 22,500 in total). The injection sites were covered with a sterile dressing, removed no earlier than 1 h after inoculation.

The study endpoint criteria following CHMI for treatment and/or malaria diagnosis were as previously defined^{27,40}, that is reaching parasitemia threshold of 500 parasites μl^{-1} or any parasitemia plus important clinical symptoms. Volunteers who reached day 22 were treated without meeting the endpoint. Secondary efficacy outcomes were defined as time to parasitemia >20 , >500 , $>1,000$ and $>10,000$, respectively.

Safety assessments

Solicited adverse events were recorded for 7 days after each vaccination, volunteers were assessed within 1 h of vaccination on the days of vaccination. Volunteers were provided with diary cards, rulers and thermometers and trained on the measurement and recording of cutaneous reactions or swelling and auxiliary temperature. Clinicians telephoned vaccinees daily for 7 days to remind them to record solicited events and to record and assess unsolicited adverse events. Further in-person examination was organized if necessary. Any unsolicited adverse events occurring between 7 and 30 days of each vaccination were recorded based on recall at 4 weeks. Serious adverse events were collected throughout the study. Causality was assessed by clinicians.

Immunology

Plasma samples were separated from whole blood for serology. We conducted ELISAs for levels of anti-NANP antibody and anti-whole schizont antibody (3D7 strain)⁴¹.

PBMCs were separated by density gradient centrifugation from heparinized whole blood. Ex vivo ELISpot assays were performed over 18 h of incubation using Multiscreen IP ELISpot plates (Millipore) and Mabtech IFN γ SA-ALP antibody kits (Mabtech). Peptide pools were tested in duplicate with 250,000 PBMCs per well. TRAP peptides were 20 amino acids in length, overlapping by 10 amino acids (ProImmune). Responses were averaged across duplicates, the background from negative control wells was subtracted and summed across TRAP pools plus ME. Plates were counted using an AID automated ELISpot counter (AID Diagnostika GmbH), using identical settings for all plates. The lower limit of detection for the assay was 20 s.f.u.

qPCR for parasite detection

Venous blood was collected twice daily from days 8 to 15 after PfSPZ challenge inoculation and then daily from days 16 to 22 for the detection of the 18S ribosomal RNA *P. falciparum* gene using qPCR in triplicates in a TaqMan assay using primers and probes synthesized by Eurofins Genomics: 18S Pf forward-5'-GTAATTGGAATGATAGGAATTACAAGGT 3'; 18S Pf Reverse-5'-TCACTACGAACGTTTAACTGCAAC 3'; 18S Pf MGB-5'-FAM-AACAATTGGAGGGCAAG-NFQ-MGB 3' (ref. 42). No-template water (no-template control) was used as a negative control and cultured parasites of known quantity were used as a positive control, with

sample parasite quantification undertaken against DNA extracted from known cultured parasite standards using eight serial dilutions. Standard curves were checked against the WHO external quantified quality control sample.

Parasite genotyping

Parasite genotyping targeted the *ama1* (PF3D7_1133400) gene using amplicon deep sequencing⁴³. Briefly, *ama1* amplicons spanning nucleotides 441–946 were generated from each sample, in duplicate. Deep sequencing of the sequencing library was performed on the SpotON FlowCell R10.4.1 (Oxford Nanopore Technologies, FLOW-MIN114-1) at the KEMRI-Wellcome Trust Laboratories. Sequence data analysis was performed in SeekDeep version 3.0.110, and *ama1* microhaplotypes with fewer than 250 reads or those detected at less than 5% minor allele frequency were discarded unless the microhaplotype was independently detected in additional samples at $>5\%$ frequency.

Statistical analysis

The intent-to-treat (ITT) cohort included volunteers receiving one or more vaccines. The according-to-protocol cohort included all evaluable volunteers meeting eligibility criteria and complied with study procedures. Safety is reported ITT, immunogenicity is reported according-to-protocol and efficacy in CHMI is reported ITT for the cohort undergoing CHMI. Safety data were tabulated descriptively and immunogenicity was described by geometric means with 95% confidence intervals. CHMI data were reported by the description of quantitative PCR over time, then by frequency of outcome categories according to vaccine group. Using a comparison of proportions and assuming $P = 0.05$ and 100% infection rates in the control group, we predicted 90% power to detect 40% efficacy with $n = 20$ groups and 80% power to detect 60% efficacy with $n = 10$.

Inclusion and ethics statement

The research was conducted and led in Kenya. Twenty-five of the 38 authors were resident in Kenya at the time of the study, and 23 authors are Kenyan citizens. The Kenyan authors played full roles in study design, implementation and data ownership. Malaria is a substantial public health problem in Kenya and a key research priority of KEMRI-Wellcome, the institution leading the research. Research roles and responsibilities were specified in the protocol, which was reviewed by IRBs in Kenya and the UK. A Kenyan author was registered for a PhD based on work from the study. CHMI studies have been previously undertaken in both the UK and Kenya and there are no severe restrictions or prohibitions. Animal welfare and environmental protection was not relevant, and biocontainment of falciparum malaria was undertaken as specified in the protocol reviewed by IRBs and with laboratory safety officer oversight. CHMI studies involve minimal risk to participants, with careful clinical supervision to reduce this risk as specified above. Relevant citations for previous CHMI and malaria studies in Kenya are included.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data are available through the online repository for KEMRI-Wellcome Trust Research Programme: Harvard Dataverse at <https://doi.org/10.7910/DVN/TNHS14> (ref. 44). Individual-level data, excluding personal identifiers, will be made available in accordance with an application to the Data Governance Committee, which meets monthly (dgc@kemri-wellcome.org). There are no timing restrictions on the availability of data.

References

40. Kapulu, M. C. et al. Controlled human malaria infection (CHMI) outcomes in Kenyan adults is associated with prior history of malaria exposure and anti-schizont antibody response. *BMC Infect. Dis.* **22**, 86 (2022).
41. Sang, S. et al. Safety and immunogenicity of varied doses of R21/Matrix-MTM vaccine at three years follow-up: a phase 1b age de-escalation, dose-escalation trial in adults, children, and infants in Kilifi-Kenya. *Wellcome Open Res.* **8**, 450 (2023).
42. Kibwana, E. et al. Quantification of *Plasmodium falciparum*: validation of quantitative polymerase chain reaction assays for detection of parasites in controlled human malaria infection studies. *Front. Malar.* **3**, 1497613 (2025).
43. Wamae, K. et al. Targeted amplicon deep sequencing of *ama1* and *mdr1* to track within-host *P. falciparum* diversity throughout treatment in a clinical drug trial. *Wellcome Open Res.* **7**, 95 (2024).
44. Kapulu, M. C et al. Replication data for: The malaria vaccine R21 is protective against intradermal but not intravenous *Plasmodium falciparum* sporozoites in controlled human malaria infection in Kenyan adults *Biosciences Dataverse* <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/TNHS14> (2025).

Acknowledgements

This work was supported by a Wellcome (203077) to P.B. as the lead applicant. This publication was produced by the Multi-Stage Malaria Vaccine Consortium (MMVC), which is part of the EDCTP2 program supported by the European Union (RIA2016V-1649-MMVC), to A.V.S.H. as the lead applicant. The views and opinions of authors expressed herein do not necessarily state or reflect those of EDCTP. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The trial sponsor was University of Oxford, who took responsibility for sponsorship roles as specified by ICH-GCP. We are grateful to all the study volunteers and to the study teams (clinical, nursing, pharmacy, laboratory and fieldworkers) that have supported the study, including the collaborating teams at Sanaria and the Data Safety Monitoring Board and Local Safety Monitor. We are grateful to the teams at Novavax, Serum Institute India and Clinical Biomanufacturing Facility at Oxford University for the vaccines. All study team members were notified of the manuscript and reviewed

the manuscript. This manuscript is published with permission/approval from KEMRI Director.

Author contributions

Conceptualization by M.C.K., P.B., A.V.S.H. and S.L.H. Formal analysis by M.C.K., H.K., M.M. and P.B. Funding acquisition by K.J.E., A.V.S.H., M.H. and P.B. Investigation by F.O., D.K., E.K., M.S.D., D.B., J. Musembi, O.N., H.R., S.A., M.H.M., L.N., K.K., R.G., A.M., J. Musyoki, J. Mwacharo, C.N., M.M., J. Makale, B.T., K.W. and L.I.O.-O. Project administration by A.L., F.R.-L. and R.R. Resources by Y.A., E.R.J., P.F.B., T.L.R., B.K.L.S. and S.L.H. Supervision by M.C.K., K.J.E., A.V.S.H. and P.B. Writing—original draft by M.C.K. and P.B. Writing—review and editing by all co-authors.

Competing interests

A.V.S.H. and K.J.E. are named as co-inventors on patent applications related to R21/MM. K.J.E. was an employee of the University of Oxford at the time of the work and is now an employee of GSK and owns restricted shares in GSK. A.V.S.H. is a co-inventor of the R21/MM vaccine and chief investigator of the published phase III trial, and may derive some royalties from vaccine sales in keeping with the University of Oxford's royalty sharing arrangements. Y.A., E.R.J., P.F.B., T.L.R., B.K.L.S. and S.L.H. are salaried, full-time employees of Sanaria, the manufacturer of Sanaria PfSPZ Challenge. The other authors declare no competing interests.

Additional information

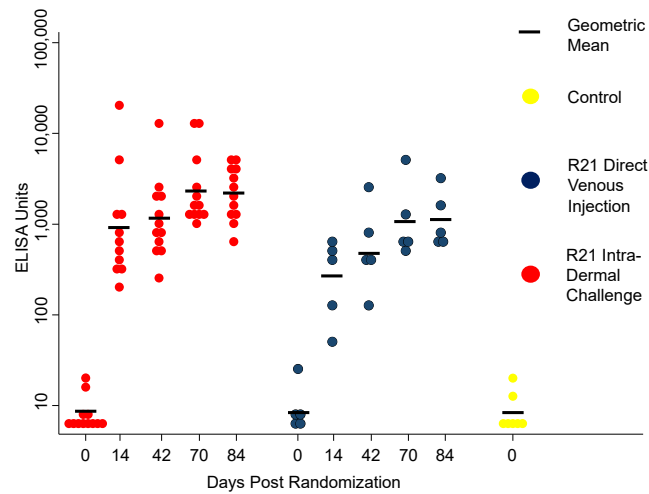
Extended data is available for this paper at <https://doi.org/10.1038/s41591-025-04107-6>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-025-04107-6>.

Correspondence and requests for materials should be addressed to Melissa C. Kapulu.

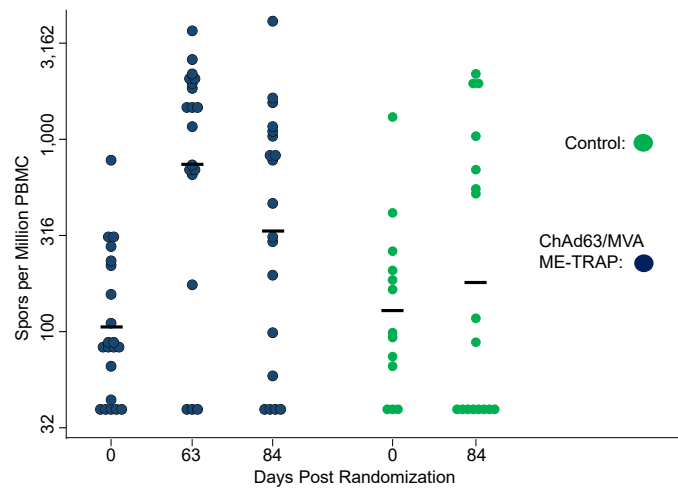
Peer review information *Nature Medicine* thanks Stefan Kappe and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Lia Parkin, in collaboration with the *Nature Medicine* team.

Reprints and permissions information is available at www.nature.com/reprints.



Extended Data Fig. 1 | NANP vaccine-induced antibody responses over time. NANP IgG ELISA units are shown over time with individual scatter dot plots per vaccination group. Peak responses were geometric means of 2,152 and 1,113 and

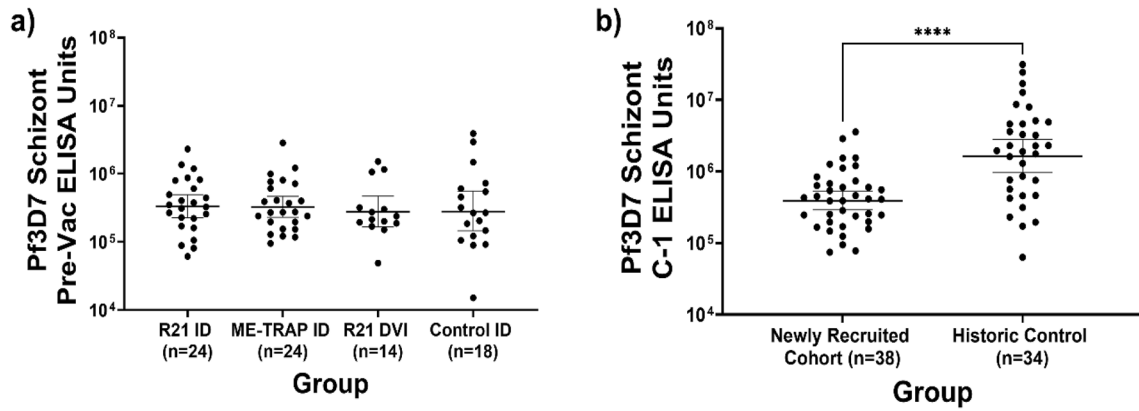
minimum to maximum ranges of 750-5,500 vs 610-3,300 for ID vs DVI challenged groups, respectively ($p = 0.14$ by Kruskal-Wallis testing). Analysis is restricted to those undergoing CHMI, that is $n = 12$ for R21 ID, $n = 5$ for R21 DVI, $n = 8$ for control.



Extended Data Fig. 2 | ME-TRAP vaccine-induced T cell responses over time.

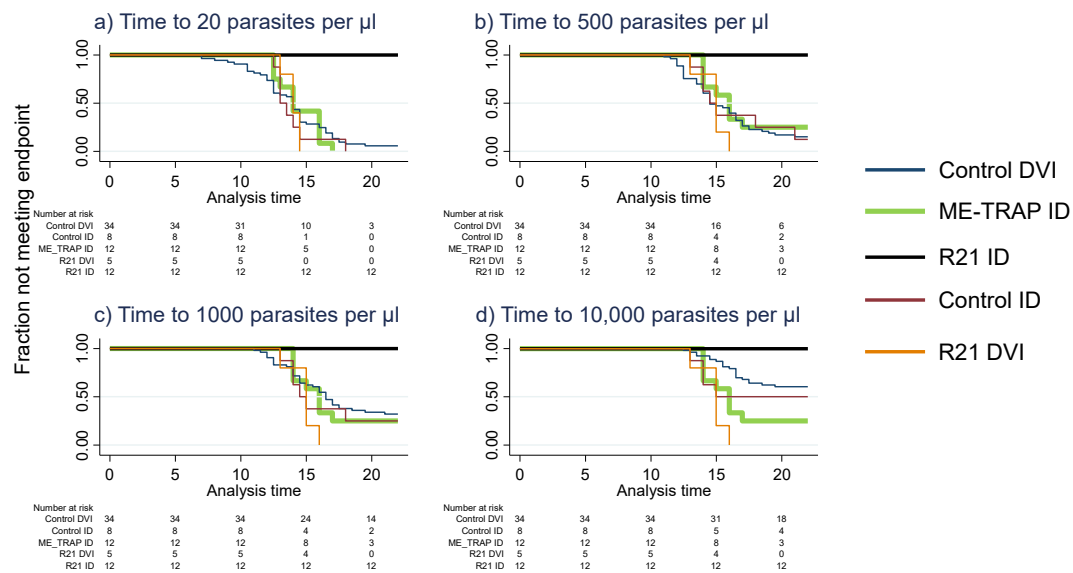
ME-TRAP IFN γ positive spots by ELISpot per million PBMC are shown over time with individual scatter dot plots per group and time point. Spot forming units per million PBMC (sfu) increased from geometric mean of 104 sfu (95%CI 70-153) at baseline to a peak of 735 (95%CI 364-1485, $p = 0.01$ for both comparisons with baseline) and 335 sfu (95%CI 162-691) on the day of challenge, compared with 127

sfu (95%CI 174 sfu (95%CI 74-409) among the control group, $p = 0.064$, $p = 0.001$, $p = 0.084$ for comparisons between all controls and ME-TRAP vaccinated volunteers at days 0, 63 and 84, respectively. The sample size varied according to timepoint according to the availability and viability of cells, hence for ChAd63/MVA ME-TRAP $n = 21, 19$ and 19 at days 0, 63 and 84, respectively, and for Control $n = 13$ and 17 for days 0 and 84, respectively.



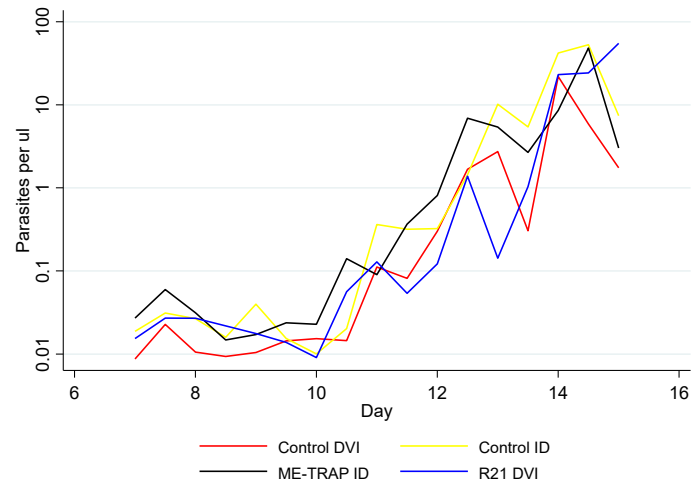
Extended Data Fig. 3 | Past exposure antibody data. Schizont IgG ELISA units are shown measured at (a) pre-vaccination (Pre-Vac) and (b) day before challenge (C-1) against Pf3D7 schizont extract giving the geometric mean and error bars showing 95% confidence intervals. Historic controls had higher titres than the newly recruited cohort (geometric means of 1,244,000 EU (95% CI 693,000-

2,235,000) vs 394,000 EU (95% CI 258,000-602,000), respectively, $p = 0.0009$), and the DVI and ID challenged groups had similar schizont extract antibodies (geometric means of 284,000 EU (95% CI 158,000-511,000) vs 434,000 EU (95% CI 252,000-745,000) respectively, $p = 0.4$). These tests were not pre-specified.



Extended Data Fig. 4 | Time to: a) 20 parasites per μl ; b) 500 parasites per μl ; c) 1000 parasites per μl ; d) 10,000 parasites per μl . None of the volunteers vaccinated by R21 and challenged by ID PfSPZ Challenge met the secondary endpoints, whereas volunteers for all other groups met secondary endpoints between days 7 and 21. Hence, none of 12 volunteers in the R21 ID group met any

of the secondary endpoints, whereas 75/78 (96.1%), 60/78 (76.9%), 56/78 (71.8%) and 39/78 (50%) met secondary endpoints at $p < 0.0005$, $p = 0.0001$, $p = 0.0007$, $p < 0.0005$ by log rank survival across all groups for time to parasitemia >20 , >500 , >1000 , $>10,000$, respectively.



Extended Data Fig. 5 | Parasite growth curves following ID and DVI inoculation. Geometric means of log PCR parasite densities following CHMI by group and day for: R21 DVI (blue line); Control ID (green line); ID ChAd63/MVA

ME-TRAP (black line); and Historic Control DVI (red line). $n = 8$ for control DVI, $n = 12$ for ME-TRAP ID, $n = 8$ for control ID and $n = 34$ for historic control DVI. No data are shown for R21 ID as volunteers were not PCR positive.

Extended Data Table 1 | General adverse events between day 8 of CHMI to day of treatment, and day of treatment to exit of study

Day 8 of CHMI to Day of Treatment					
Systemic solicited	R21 ID n=12	R21 DVI n=5	ME-TRAP ID n=12	Control ID n=8	Total N=37
Fever	1 (8.3)	5 (100)	11 (91.7)	5 (62.5)	22 (59.5)
Headache	2 (16.7)	4 (80)	8 (66.7)	3 (37.5)	17 (45.9)
Fatigue	1 (8.3)	2 (40)	5 (41.7)	4 (50)	12 (32.0)
Low back pain	0	2 (40)	5 (41.7)	3 (37.5)	10 (27.0)
Myalgia	1 (8.3)	1 (20)	3 (25)	2 (25)	7 (18.9)
Arthralgia	0	1 (20)	2 (16.7)	3 (37.5)	6 (16.2)
Chills	0	2 (40)	2 (16.7)	2 (25)	6 (16.2)
Anorexia	0	2 (40)	2 (16.7)	2 (25)	5 (13.5)
Nausea	1 (8.3)	0	0	2 (25)	3 (8.1)
Sweating	0	0	1 (8.3)	2 (25)	3 (8.1)
Rigors	0	0	0	0	0
Vomiting	0	0	0	0	0
Day of Treatment to Exit					
Systemic solicited	R21 ID n=12	R21 DVI n=5	ME-TRAP ID N=12	Control ID n=8	Total N=37
Fever	0	3 (60)	2 (16.7)	2 (25)	7 (18.9)
Headache	1 (8.3)	3 (60)	3 (25)	4 (50)	11 (29.7)
Fatigue	2 (16.7)	1 (20)	2 (16.7)	4 (50)	9 (24.3)
Myalgia	0	1 (20)	4 (33.3)	3 (37.5)	8 (21.6)
Low back pain	0	0	2 (16.7)	2 (25)	4 (10.8)
Arthralgia	0	1 (20)	2 (16.7)	3 (37.5)	6 (16.2)
Anorexia	0	1 (20)	1 (8.3)	2 (25)	4 (10.8)
Nausea	0	0	1 (8.3)	3 (37.5)	4 (10.8)
Chills	0	1 (20)	1 (8.3)	1 (12.5)	3 (8.1)
Sweating	0	0	0	2 (25)	2 (5.4)
Rigors	0	0	0	0	0
Vomiting	0	0	0	0	0

Data are presented as n (%). Percentages indicate volunteers with adverse events over the total number of volunteers per vaccination group. All adverse events are reported based on maximum severity over the follow-up period. The median duration of events is 1 day.

Extended Data Table 2 | Volunteers with out-of-range safety blood results during vaccination (n=62)

Parameter	Gender	Limit	Treatment Group	Timepoint			
				D28	D56	C-1	
Chemistry							
Creatinine	Male	>115	R21 ID	0	0	0	
			R21 DVI	1	1	0	
			ME-TRAP ID	0	1	0	
			Control ID	0	0	1	
	Female	>91	R21 ID	0	0	0	
			R21 DVI	1	0	0	
			ME-TRAP ID	0	1	1	
			Control ID	0	0	0	
	AST	Male	>47	R21 ID	4	3	5
				R21 DVI	0	2	1
				ME-TRAP ID	0	0	4
				Control ID	0	0	1
Female		>42	R21 ID	0	0	1	
			R21 DVI	0	0	1	
			ME-TRAP ID	0	1	3	
			Control ID	0	0	1	
ALT	Male	>35	R21 ID	6	4	10	
			R21 DVI	1	3	2	
			ME-TRAP ID	0	2	8	
			Control ID	0	0	5	
	Female	>35	R21 ID	1	0	3	
			R21 DVI	0	0	1	
			ME-TRAP ID	0	1	3	
			Control ID	0	0	1	
Haematology							
Hb	Male	<11.5	R21 ID	0	0	0	
			R21 DVI	0	0	0	
			ME-TRAP ID	0	0	0	
			Control ID	0	0	0	
	Female	<9.5	R21 ID	0	0	0	
			R21 DVI	0	0	0	
			ME-TRAP ID	0	0	1	
			Control ID	0	0	0	
	WBC (Total)	Male	<3.3 OR >8.4	R21 ID	6	6	1
				R21 DVI	3	3	0
				ME-TRAP ID	0	1	1
				Control ID	0	0	1
Female		<3.3 OR >8.2	R21 ID	0	0	0	
			R21 DVI	0	0	0	
			ME-TRAP ID	0	0	2	
			Control ID	0	0	0	
Platelets	Male	<124 OR >358	R21 ID	0	0	1	
			R21 DVI	1	1	0	
			ME-TRAP ID	0	0	1	
			Control ID	0	0	0	
	Female	<127 OR >397	R21 ID	0	1	1	
			R21 DVI	0	0	0	
			ME-TRAP ID	0	0	0	
			Control ID	0	0	1	

ALT denotes alanine transaminase; AST aspartate aminotransferase; D Day after vaccination; Hb, Haemoglobin; WBC white blood count. None of the episodes were judged to be clinically significant or caused symptoms. All abnormalities resolved on follow up.

Extended Data Table 3 | Volunteers with out-of-range safety blood results during CHMI (n=37)

Parameter	Gender	Limit	Timepoint					
			C+6	C+8	C+10	DoT	C+36	C+91
Chemistry								
Creatinine	Male	>115	1	0	0	4	0	0
	Female	>91	0	0	0	2	0	0
AST	Male	>47	2	2	3	7	0	0
	Female	>42	2	2	2	2	0	0
ALT	Male	>35	11	11	11	14	0	0
	Female	>35	4	4	4	3	0	0
Haematology								
Hb	Male	<11.5	0	0	0	0	0	0
	Female	<9.5	0	0	0	0	0	0
WBC (Total)	Male	<3.3 OR >8.4	0	0	0	2	3	2
	Female	<3.2 OR >8.2	0	2	0	0	0	2
Platelets	Male	<124 OR >358	0	1	0	4	3	0
	Female	<127 OR >397	0	1	0	0	2	0

ALT denotes alanine transaminase; AST aspartate aminotransferase; C+ day after challenge; Hb, Haemoglobin; WBC white blood count. None of the episodes were judged to be clinically significant or caused symptoms. All abnormalities resolved on follow up.

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

NA

Data analysis

NA

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data are available through the online repository for KEMRI-Wellcome Trust Research Programme: Harvard Dataverse: <https://doi.org/10.7910/DVN/TNHS14>

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	There was a predominance of young male volunteers (28/40, 70%) with a mean age of 28.3 years (Table 1). Volunteers were from the Mijikenda ethnic group. The male predominance among volunteers appears to relate to established gender roles. Gender was self-reported, and given the sample size and low frequency of women volunteers, gender sub-group analyses were not conducted (i.e. 2-4 women per group). Further analysis would risk inadvertent breach of privacy due to cardinality with these group sizes.
Reporting on race, ethnicity, or other socially relevant groupings	There was a predominance of young male volunteers (28/40, 70%) with a mean age of 28.3 years. Volunteers were from the Mijikenda ethnic group.
Population characteristics	Table 1
Recruitment	We recruited healthy adult men and women aged between 18-45 years from Kilifi North on the Kenyan Coast, where there is currently very low malaria transmission but with previous low to moderate malaria transmission. Recruitment was conducted by fieldworkers who were resident in the study areas, combined with community events to explain the study. Prospective participants were self-referring.
Ethics oversight	Prior to commencing activity, approvals were obtained from a National IRB in Kenya and the relevant Oxford IRB (i.e. SERU (KEMRI/SERU/CGMR-C/158/3844) and OxtREC (OxtREC 32-19)) and from the medicines regulatory authority in Kenya (i.e. Pharmacy and Poisons Board (ECCT/19/11/01)).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Using a comparison of proportions and assuming $p=0.05$ and 100% infection rates in the control group, we predicted 90% power to detect 40% efficacy with $n=20$ groups, and 80% power to detect 60% efficacy with $n=10$. In the first cohort, $n=40$ was divided as $n=8, 12, 12$ and 5 for Control ID, ME-TRAP ID, R21 ID and R21 IV, respectively and $n=34$ for IV controls from historical comparisons.
Data exclusions	None
Replication	Not applicable
Randomization	The volunteers were randomly assigned with randomization via a computer-generated sequence by an independent statistician. A randomization list in the form of password protected spreadsheet was generated using STATA and the data manager set up randomization in REDCap. Study clinicians only clicked to randomize after confirmation of eligibility criteria and immediately prior to vaccination for each volunteer, after which REDCap would reveal the volunteers' randomization arm that could not be edited. The volunteers were randomized to either one of four groups.
Blinding	The study was undertaken open label as a) placebo for the different doses and routes would have been impractical; b) outcomes were based on objective PCR data and the laboratory team were blind to allocations.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy	<i>Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.</i>
Data collection	<i>Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.</i>
Timing	<i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Non-participation	<i>State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.</i>
Randomization	<i>If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.</i>

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.</i>
Research sample	<i>Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i>, all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i>
Sampling strategy	<i>Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.</i>
Data collection	<i>Describe the data collection procedure, including who recorded the data and how.</i>
Timing and spatial scale	<i>Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Reproducibility	<i>Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.</i>
Randomization	<i>Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.</i>
Blinding	<i>Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.</i>

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions	Kilifi community, described
Location	Kilifi, Kenya
Access & import/export	NA
Disturbance	NA

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Clinical data
- Dual use research of concern
- Plants

Methods

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

Antibodies used

Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.

Validation

Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.

Authentication

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

Mycoplasma contamination

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.

Wild animals	<i>Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Reporting on sex	<i>Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT03947190
Study protocol	Attached to submission
Data collection	qPCR and clinical observations, inpatient facility, described in methods
Outcomes	<p>Primary Outcomes: CHMI was conducted 28 days after the last vaccination. All eight unvaccinated control volunteers, and all 12 volunteers vaccinated with ME-TRAP, became PCR positive for malaria parasites following ID challenge with cryopreserved sporozoites produced by Sanaria (PfSPZ), with observed typical parasite growth curves on quantitative PCR. Seven of 8 control volunteers (88%) and 11 of 12 ME-TRAP volunteers (92%) met criteria for the primary endpoint and received malaria treatment during CHMI, and one control and one ME-TRAP volunteer remained just below the parasitaemia threshold and so did not meet the primary endpoint (Fig. 2, Panels A and C, Table 2).</p> <p>In contrast, none of the 12 volunteers vaccinated with R21 met the primary endpoint following challenge with ID PfSPZ. Three out of the nine volunteers were briefly positive by PCR for malaria parasites but with no evidence of parasite growth. Hence they were protected and did not meet the primary endpoint (Fig 2, Panel B, Table 1).</p> <p>Historical unvaccinated control volunteers who had previously undergone CHMI with PfSPZ Challenge by DVI showed similar growth rates to PfSPZ Challenge ID, with 31 of 34 (91%) volunteers meeting the primary endpoint, 2 of 34 being PCR negative (6%) and 1 of 34 being PCR positive but not meeting the primary endpoint (3%) (Fig. 2, Panel D).</p> <p>However, when the 5 volunteers vaccinated with R21 underwent CHMI with PfSPZ Challenge by DVI sporozoites, all 5 showed typical growth curves of parasites, and met the primary endpoint (Fig 2, Panel E).</p> <p>Hence, none of the volunteers vaccinated by R21 who received ID PfSPZ Challenge met a primary endpoint, whereas volunteers for all other groups met primary endpoints between days 12 and 21 ($p < 0.0005$ by logrank survival across all groups, Fig 3). Parasites were genotyped for AMA1, confirming the challenge parasite strain (NF54) in all cases.</p> <p>Secondary Outcomes: Immunogenicity outcomes were secondary outcomes. Following the vaccination course with R21, anti-NANP antibodies rose from baseline levels of just under 10 ELISA Units (EU) to above 1000 EU in the R21-vaccinated volunteers but remained below 10 among the control group (Extended Data Fig 1). Peak responses were geometric means of 2,152 and 1,113 and minimum to maximum ranges of 750-5,500 vs 610-3,300 for ID vs DVI challenged groups, respectively ($p = 0.14$).</p> <p>Following vaccination with viral vectors encoding ME-TRAP, spot forming units per million PBMC (sfu) increased from geometric mean of 104 sfu (95%CI 70-153) at baseline to a peak of 735 (95%CI 364-1485, $p = 0.01$ for both comparisons with baseline) and 335 sfu (95%CI 162-691) on the day of challenge, compared with 127 sfu (95%CI 174 sfu (95%CI 74-409) among the control group, $p = 0.064$, $p = 0.001$, $p = 0.084$ for comparisons between all controls and ME-TRAP vaccinated volunteers at days 0, 63 and 84, respectively (Extended Data Fig 2).</p> <p>Volunteers had evidence of past exposure to malaria as evidenced by pre-vaccination IgG antibodies against schizont extract (Extended Data Fig 3). Historic controls had higher titres than the newly recruited cohort (geometric means of 1,244,000 EU (95% CI 693,000-2,235,000) vs 394,000 EU (95% CI 258,000-602,000), respectively, $p = 0.0009$), and the DVI and ID challenged groups had similar schizont extract antibodies (geometric means of 284,000 EU (95% CI 158,000-511,000) vs 434,000 EU (95% CI 252,000-745,000) respectively, $p = 0.4$).</p> <p>Secondary efficacy outcomes were consistent with the primary outcome: i.e. none of the volunteers vaccinated by R21 who received ID PfSPZ Challenge met the secondary endpoints, whereas volunteers for all other groups met secondary endpoints between days 7 and 21 (none of 12 volunteers in the R21 ID group met any of the secondary endpoints, whereas 75/78 (96.1%), 60/78 (76.9%), 56/78 (71.8%) and 39/78 (50%) met secondary endpoints at $p < 0.0005$, $p = 0.0001$, $p = 0.0007$, $p < 0.0005$ by log rank survival across all groups for time to parasitemia > 20, > 500, > 1000, $> 10,000$, respectively, Extended Data Fig 4).</p>

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | No | Yes | |
|-------------------------------------|--------------------------|----------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Public health |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | National security |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Crops and/or livestock |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Ecosystems |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Any other significant area |

Experiments of concern

Does the work involve any of these experiments of concern:

- | No | Yes | |
|-------------------------------------|--------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Demonstrate how to render a vaccine ineffective |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Increase transmissibility of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Alter the host range of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enable evasion of diagnostic/detection modalities |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enable the weaponization of a biological agent or toxin |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Any other potentially harmful combination of experiments and agents |

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

ChIP-seq

Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session
(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates	<i>Describe the experimental replicates, specifying number, type and replicate agreement.</i>
Sequencing depth	<i>Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.</i>
Antibodies	<i>Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Peak calling parameters	<i>Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.</i>
Data quality	<i>Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.</i>
Software	<i>Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.</i>

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	<i>Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.</i>
Instrument	<i>Identify the instrument used for data collection, specifying make and model number.</i>
Software	<i>Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.</i>
Cell population abundance	<i>Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.</i>
Gating strategy	<i>Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.</i>

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	<i>Indicate task or resting state; event-related or block design.</i>
Design specifications	<i>Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.</i>
Behavioral performance measures	<i>State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).</i>

Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI Used Not used

Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: Whole brain ROI-based Both

Statistic type for inference

(See [Eklund et al. 2016](#))

Correction

Models & analysis

n/a | Involved in the study

Functional and/or effective connectivity

Graph analysis

Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis