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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒ An indication of 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 statistics 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
☐	☒ Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Crystallographic data collected at Advanced Photon Source (APS) at Argonne National Laboratory at beamline 24-ID-C and processed onsite through the RAPD software pipeline. Flow cytometry data was collected using FACSDiva software (BD Biosciences).
Data analysis	Crystallographic data collected at Advanced Photon Source (APS) at Argonne National Laboratory at beamline 24-ID-C and processed onsite through the RAPD software pipeline. Bromine SAD phasing was calculated using the SHELX suite. A partial model of the bromine-soaked protein was built by the PHENIX suite. This model was then used in arp/wARP as a starting model for automated building against the native data to 1.4Å resolution. Several cycles of automated building with arp/wARP, refinement with REFMAC, and manual inspection and model building led to a final model with and R/Rfree of 13.0%/16.9%. LC-MS/MS analysis of glycopeptide raw files, converted to mgf format by MSConvert (proteowizard.sourceforge.net), were searched against the VSG3 protein sequence using the Mascot software (v.2.4.0, Matrix Science Inc., Boston, MA). LC-MS analysis of intact and de-N-glycosylated VSG3 scans were collected over m/z 335-3200 and spectra were deconvoluted using the Maximum Entropy software. Flow cytometry data was analyzed using FlowJo software. Data plotting and statistical analyses were performed using Prism (Graphpad) and R.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Coordinates and structure factors of VSG3 have been uploaded to the RCSB PDB (www.rcsb.org) with PDB ID: 6ELC.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	We did not have an a priori prespecified effect size from which to preemptively determine an appropriate sample size for our mouse infection experiments (Fig. 4 and Supplementary Figs. 8 and 9) but the sample sizes were large enough to detect the effect observed and to verify reproducibility. Also see statement in Methods, page 19.
Data exclusions	No data were excluded.
Replication	Non-immunized mouse infection findings were reproduced for a total of n = 15 mice/experimental group (Figure 4a and b and Supplementary Fig. 8a-d) as well as n = 6 mice/experimental group for a different mouse strain (Extended Data Fig8e-f). Pre-immunized mouse infection findings were reproduced for n = 12 mice / experimental group (Fig. 4c and Supplementary Fig. 9a). Antibody binding assays were reproduced with 3 independent mouse-derived antiserum samples for each experimental group. All results were found to be reproducible.
Randomization	Mice were assumed to be sufficiently randomized by Jackson Laboratory and no additional randomization was performed.
Blinding	Experimenters were not blinded to mouse group allocation, but measurements (survival assessment, parasitemia counts, FACS MFI) were not subjective.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	We have used our own monoclonals for screening VSG3 and VSG3 mutant knock inclones (reported in PMID: 22952449 and mad e freely available to the community since then, through the monoclonal antibody facility of Rockefeller University).
Validation	These antibodies were initially validated in PMID: 22952449, and have been used in multiple subsequent studies (e.g. PMCID: PMC5635023).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Trypanosoma brucei Lister 427 strain; George Cross Laboratory, Rockefeller University. See also Methods, page 18.
Authentication	RNAseq.
Mycoplasma contamination	Tested and found negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C57BL6J and CD-1 mice were used. All mice were wild type males or females, aged 6-9 weeks at experiment start (Jackson Laboratory) [see methods, p. 18-20]. All experiments described were approved by the Rockefeller University Institutional Animal Care and Use Committee under prot ocol #16894 and comply with all NIH mandated ethical regulations regarding the use of vertebrate animals. (Statement also included in methods).
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.

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	See section "FACS analyses to determine IgM binding" in Methods, page 20
Instrument	BD-FACSCalibur and BD-LSRII
Software	FACSDiva software was used for sample collection, FlowJo software was used for analysis.
Cell population abundance	n/a. Samples are not mixed cell types
Gating strategy	Samples are gated via forward and side scatter to eliminate debris and cell aggregates, and dead cells are excluded via elimination of cells which stain positive in the propidum iodide channel (see methods, page 20).

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