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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   This study used deidentified human PBMCs as starting material, however it was not designed as a clinical study per se, so there were no projections or power calculations a priori, as this is not relevant to our approach. The goal of our study was to isolate and characterize potently neutralizing monoclonal antibodies with novel properties. We studied PBMC samples from de-identified survivor PBMCs unitl we identified potent neutralizing monoclonal antibodies. Our sample size of 13 individuals with history of laboratory-confirmed WNV infection was sufficient for isolating clones of interest.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analyses

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Findings presented were based on at least two independent experiments, as stated in the figure legends.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   In Figure 4, 10 mice were allocated into each treatment group across two independent experiments

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Blinding was not used during data collection and/or analysis as the clinical outcomes (survival and death) were not amenable to subjective bias of interpretation.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	GraphPad Prism v6.0g

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Plasmids used throughout the study to create reporter virus particles are available upon request. Please contact Ted Pierson; piersontc@mail.nih.gov

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Human antibodies used were generated as part of our study using previously described methods (Smith SA, et al. J Virol 2012. doi: 10.1128/JVI.06335-11. Murine antibodies used have been described extensively elsewhere (Oliphant T, et al. Nat Med 2005. doi:10.1038/nm1240; Oliphant T, et al. J Virol 2006. doi:10.1128/JVI.01732-06). Commercial antibodies are listed by company name and catalog number.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Cell lines were obtained from ATCC
b. Describe the method of cell line authentication used.	Raji-DCSIGNR cells lines used thought out for neutralization studies were stained for DC-SIGNR expression (detailed provided in the manuscript). DC-SIGNR expression level does not significantly impact neutralization sensitivity, as detailed in PMID: 23312596.
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were not tested for mycoplasma prior to these experiments. Cell banks from which these cells were grown were are mycoplasma-free as evaluated using the Universal Mycoplasma Detection Kit (ATCC).
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Animals were purchased from Jackson laboratories and housed in at pathogen-free facility at Washington University until experimentation. Age and sex information for these animals are provided in the revised manuscript.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This work describes a discovery based experimental approach to identify individual monoclonal antibodies for structure function studies. Although the starting materials were human PBMCs, we did not seek to analyze or attribute any clinical or demographic features to the molecular studies here. The samples were de-identified prior to lab study. Therefore, we did not collect metadata about covariates other than history of WNV infection, as these features were not relevant to our studies.