nature portfolio

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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	No open source or custom code was used in the collection of these data. AD Instruments LabChart v7.3.8 was used to collected MAP data. Applied Photophysics Pro-Data SX software was used to collect stopped flow data. Agilent UV-VIs Chemstation software was used to collect UV-Visible data. NO chemiluminescence measurements were collected using EcoPhysics software paired with eDAQChart for data export or Zysense NOAnalysis for the Nitric Oxide Analyzer (NOA 280i). EPR data were collected using Bruker Xepr software on a LINUX workstation. Flow cytometry data were collected using BD CellQuest.
Data analysis	No open source or custom code was used in the analysis of these data. AD Instruments LabChart v7.3.8 was used to start analysis on MAP data. Open source ImageJ was used to process the image of the red cell membranes. eDAQChart software was used for processing some NO chemiluminescence measurements. Otherwise Microsoft Excel v16.69 and GraphPad Prism v9.5 was used for all analyses and figure generation.

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

The datasets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Both male and female volunteers were recruited and information was not gathered with respect to gender.
Population characteristics	Healthy volunteers were recruited between the ages of 17 and 60 but no limitation were made on recruiting older adults.
Recruitment	Volunteers were recruited by word of mouth. Study staff asked around for volunteers.
Ethics oversight	Atrium Health Wake Forest Baptist, Wake Forest Medical School, Internal Review Board (BG01-168).

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	As the magnitude of the effect was unknown, we determined sample sizes empirically. We estimated a priori a sample size of at least 4 treated mice and control mice each. We observed significant differences between the groups (p = 0.00075 for 75 nM dose vs normal saline) and thus the sample size was considered adequate.
Data exclusions	No data were excluded from the study
Replication	Within the number of animals tested per group per condition, the result was replicated.
Randomization	Mice were randomly allocated into groups; usually one control and one treatment group was given per day
Blinding	Investigators were not blinded to experiments. Blinding was not possible due to color of treatment solution (red) vs controls (colorless). Also treatment resulted in precipitous changes in MAP.

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

Methods

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

Antibodies

Antibodies used	FITC labeled PAC-1 (BD Biosciences 340507) and Per-CP labeled CD61 antibodies (BD Biosciences 340506, Clone: RUU-PL7F12)
Validation	All experiments were conducted with baseline and ADP activated platelets which demonstrate expected behavior of the antibodies.

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	Male C57BL/6 mice (from the Jackson Laboratory), aged 12 to 14 weeks
Wild animals	Wild animals were not used in this study.
Reporting on sex	Male mice were used in this study since fluctuating hormone levels in females affect blood pressure.
Field-collected samples	No field collected samples were obtained or used in these experiments.
Ethics oversight	All animal studies were performed using protocols approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh and in accordance with National Institutes of Health guidelines (Protocol # 21110109)

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

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	Fresh human blood was drawn into sodium citrate tubes with the first tube being discarded and platelet rich plasma (PRP) collected after sedimentation of the red blood cells. Platelet activation was measured as described previously using platelet agonist ADP (Wajih, N. et al. Potential therapeutic action of nitrite in sickle cell disease. Redox Biology 12, 1026–1039 (2017).)
Instrument	BD FACSCalibur Analyzer
Software	BD CellQuest
Cell population abundance	Cell population was set for 10,000 events for FITC fluorescence to quantify platelet bound PAC1. Regrading purity, theses cells are from platelet rich plasma from freshly drawn blood that we doubly label platelets with antibodies.
Gating strategy	Based on side and forward scattering compared to red blood cells and then also for activated platelets. Platelets are labelled along the x axis and activation on the y axis. Activation is considered the upper right quadrant divided by the sum of the two right quadrants.

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