Common orthopaedic trauma may explain 31,000-year-old remains

https://doi.org/10.1038/s41586-023-05756-8

Received: 24 September 2022

Accepted: 25 January 2023

Published online: 15 March 2023

Open access

Check for updates

Nicholas J. Murphy[1], Joshua S. Davis[2,3], Seth M. Tarrant[1] & Zsolt J. Balogh[1]✉

ARISING FROM T. R. Maloney et al. *Nature* https://doi.org/10.1038/s41586-022-05160-8 (2022)

The fascinating discovery of skeletal remains in Borneo of an individual (TB1) with absent left distal tibia, fibula and foot from 31,000 years ago[1] has been proposed as evidence of a contemporaneous sophisticated amputation procedure. Maloney et al.[1] infer from the bony abnormalities that surgical amputation is the only possible explanation and, furthermore, that the limb shows no evidence of infection. We dispute the conclusion that these skeletal remains provide evidence of a transosseous surgical amputation and that the limb shows no signs of infection. We propose that the skeletal findings have more plausible alternative explanations, such as the natural history of an injury pattern commonly encountered in blunt orthopaedic trauma, an open distal tibia/fibula fracture with growth-plate involvement.

From the perspective of orthopaedic trauma surgeons practicing in this field, the exclusion of blunt trauma as a potential mechanism of injury is a rather reductionist approach to a differential diagnostic puzzle with several missing pieces from thousands of years ago. Maloney et al.[1] dismissed blunt trauma with the assertion that it "typically causes comminuted and crushing fractures". To support this statement, they cited an isolated case report of an axis fracture[2] (the second cervical vertebra in the neck). We disagree with the statement and suggest the supporting citation is inadequate. Oblique fracture patterns such as TB1's are frequently observed from blunt trauma in clinical practice[3].

Physeal (growth plate) fractures of the distal tibia and fibula are common injuries in adolescents[4]. The most common subtype[5] involves fracture through the distal tibial physis and into the metaphysis (Salter–Harris type II[6]), and typically occurs concomitantly with a distal fibula fracture[7]. It is not uncommon that the medial apex of the fracture pierces through the skin[8,9], while the foot displaces laterally, as seen in Fig. 1. The mean age of people presenting with this Salter–Harris type II injury to the distal tibia and fibula is 12–13 years[7], which corresponds to the predicted age of injury for the individual of whom the skeletal remains were discovered in Borneo, given he or she is estimated to have died aged 19–20 (a predicted 6–9 years after suffering the injury)[1]. The typical mechanism of these injuries is forced inversion or eversion while the foot is fixed in position on the ground. In Borneo 31,000 years ago, this may have eventuated from any slip or misstep whilst running, or a jump from low height. Today, open ankle fractures are managed with intravenous antibiotics, tetanus prophylaxis and expeditious debridement with open reduction and internal fixation in the operating theatre.

In Borneo 31,000 years ago, the natural history of an open physeal ankle fracture without modern surgical care could quite plausibly have produced the findings encountered in TB1's skeletal remains. In some cases, the inoculation of bacteria into exposed bone could result in acute infection progressing to overwhelming sepsis and death. In other cases, a chronic osteomyelitis can develop, often with the formation of a life-long draining sinus[10]. Survival with chronic osteomyelitis was described on many occasions in the pre-antibiotic era[11]. In the 1830s, Nathan Smith, a professor of surgery at Yale University, suggested that most people with osteomyelitis he observed survived with the condition, writing "a very great majority of patients survive the attack, albeit with long confinement, protracted suffering and great emaciation."[12]. The "long confinement, protracted suffering" was most probably TB1's fate during his young adulthood. It is highly implausible that either surgical amputation or an open fracture, in the absence of antiseptics, anaesthetics or antibiotics, could occur without a subsequent established infection. Furthermore, the bony changes shown in figure 3 of Maloney et al.[1] are typical of chronic osteomyelitis—the cortical thickening of the distal tibia and fibula are consistent with involucrum, and the small bony defect of the distal tibia could represent an area of sequestrum. The evidence of bone lysis and necrosis at the distal tibial and fibula, which Maloney et al.[1] refer to in the caption of figure 3b, could quite plausibly occur secondary to infection[13]. The suggested mechanism explains other characteristics of the skeletal findings—the missing malleolar parts of the distal tibia and fibula are consistent with common adolescent fracture patterns, and the small size of the left tibia and fibula relative to the right is highly suggestive of physeal arrest, which is a common complication of displaced physeal fractures[7,14].

With a distal tibia and fibula fracture already present and necrosis of the bone and surrounding soft tissues occurring due to infection, terminalization of the limb—that is, cutting through the remaining soft tissues—is a more plausible scenario. This is a substantially different proposition from the primary transosseous surgical amputation described in Maloney et al.[1], which states that the bone must have been cut with a sharp instrument. It is impossible to know whether loss of the foot occurred around the time of injury, or weeks to months later. If an arterial injury accompanied the initial bony injury, and the limb suffered distal ischaemia as a result, a dry gangrenous process may even have autoamputated the limb without any assistance.

We cannot exclude the possibility of rarer causes to explain these skeletal remains—for example, congenital transverse deficiency of the lower limb, a rare congenital anomaly that can manifest as a hypoplastic limb with absent foot[15]. If TB1 were born with this condition, weightbearing on the footless lower limb without a durable heel pad could have caused ulceration and the chronic infective changes that we observe in the bony architecture of TB1's distal tibia and fibula.

[1]Department of Traumatology, John Hunter Hospital & University of Newcastle, Newcastle, New South Wales, Australia. [2]School of Medicine and Public Health, University of Newcastle, Newcastle, New South Wales, Australia. [3]Department of Infectious Diseases, John Hunter Hospital, Newcastle, New South Wales, Australia. ✉e-mail: Zsolt.balogh@health.nsw.gov.au

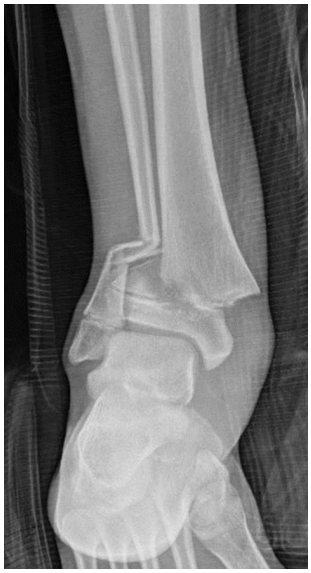

Fig. 1 | Plain radiograph of a distal tibia and fibula fracture. A plain radiograph of a distal tibia and fibula fracture involving the physis (growth plate) in a 14-year-old individual.

Overall, we find that the conclusions drawn by Maloney et al.[1] are unconvincing. Performing primary supra-articular transosseous surgical amputation through the thick cortices of the tibia without specialized metallic tools (at least chisel and saw) would be very difficult and is highly improbable. If the people in Borneo were performing lower-limb amputation using 'sharp instruments', it would have been easier to perform transarticular amputation through the soft tissues of the ankle joint, where it is not necessary to transect thick cortical bone. This is not the pattern observed in these skeletal remains.

We suggest that interdisciplinary input from expert orthopaedic trauma surgeons and bone and joint infection experts would be of value in archaeological studies such as this to aid in formulating plausible explanations of injury mechanism and infectious processes, as palaeopathology is unlikely to cover the breadth of specialized understanding required. Unfortunately, our concerns are not limited to the explanation of the missing part of the skeleton. Figure 3a of Maloney et al.[1] contains a photographed reconstruction of TB1's bony anatomy, with the distal portion of the right tibia placed back-to-front such that the medial malleolus is incorrectly articulating with the distal fibula. As an image that is likely to be frequently reproduced, and that has already had considerable media attention, this requires correction. Although we cannot support the conclusions of Maloney et al.[1], we nevertheless consider the findings described to be of great interest. Even if TB1 did not undergo a transosseous surgical amputation, the findings demonstrate evidence of an individual who, 31,000 years ago, must have had enormous kin support to survive for several years after a severe open injury, which of itself seems a notable detail about our ancestors.

Reporting summary

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

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/s41586-023-05756-8.

Data availability

All relevant data are included in the Article.

1. Maloney, T. R. et al. Surgical amputation of a limb 31,000 years ago in Borneo. *Nature* **609**, 547–551 (2022).
2. Aydin, K. & Cokluk, C. A fracture of unilateral pars interarticularis of the axis: a case report. *Turk. Neurosurg.* **17**, 155–157 (2007).
3. Domzalski, M. E., Lipton, G. E., Lee, D. & Guille, J. T. Fractures of the distal tibial metaphysis in children: patterns of injury and results of treatment. *J. Pediatr. Orthop.* **26**, 171–176 (2006).
4. Podeszwa, D. A. & Mubarak, S. J. Physeal fractures of the distal tibia and fibula (Salter-Harris type I, II, III, and IV fractures). *J. Pediatr. Orthop.* **32**, S62–S68 (2012).
5. Spiegel, P. G., Cooperman, D. R. & Laros, G. S. Epiphyseal fractures of the distal ends of the tibia and fibula. A retrospective study of two hundred and thirty-seven cases in children. *J. Bone Joint Surg. Am.* **60**, 1046–1050 (1978).
6. Salter, R. B. & Harris, W. R. Injuries involving the epiphyseal plate. *J. Bone. Joint Surg.* **45**, 587–622 (1963).
7. Russo, F., Moor, M. A., Mubarak, S. J. & Pennock, A. T. Salter-Harris II fractures of the distal tibia: does surgical management reduce the risk of premature physeal closure? *J. Pediatr. Orthop.* **33**, 524–529 (2013).
8. Nandra, R. S., Wu, F., Gaffey, A. & Bache, C. E. The management of open tibial fractures in children. *Bone Joint J.* **99-B**, 544–553 (2017).
9. Simske, N. M., Audet, M. A., Kim, C.-Y. & Vallier, H. A. Open ankle fractures are associated with complications and reoperations. *OTA Int.* **2**, e042 (2019).
10. Panteli, M. & Giannoudis, P. V. Chronic osteomyelitis: what the surgeon needs to know. *EFORT Open Rev.* **1**, 128–135 (2016).
11. Klenerman, L. A history of osteomyelitis from the *Journal of Bone and Joint Surgery*: 1948 to 2006. *J. Bone Joint Surg. Br.* **89**, 667–670 (2007).
12. Smith, N. *Medical and Surgical Memoirs* 97 (WA Francis, 1831).
13. Ciampolini, J. & Harding, K. G. Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often?. *Postgrad. Med. J.* **76**, 479–483 (2000).
14. Herman, M. J. & Dean MacEwen, G. Physeal fractures of the distal tibia and fibula. *Curr. Orthop.* **17**, 56–62 (2003).
15. Gold, N. B., Westgate, M.-N. & Holmes, L. B. Anatomic and etiological classification of congenital limb deficiencies. *Am. J. Med. Genet. A* **155**, 1225–1235 (2011).

Author contributions Z.J.B. initiated this Comment. N.J.M. prepared the first draft manuscript. All of the authors revised and approved the final manuscript.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-023-05756-8.

Correspondence and requests for materials should be addressed to Zsolt J. Balogh.

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

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

nature portfolio

Corresponding author(s): Nicholas Murphy, Zsolt Balogh

Last updated by author(s): Dec 8, 2022

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	Not applicable
Data analysis	Not applicable

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 Authors confirm that all relevant data are included in the article.

Human research participants

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

Reporting on sex and gender	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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	Not applicable
Data exclusions	Not applicable
Replication	Not applicable
Randomization	Not applicable
Blinding	Not applicable

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

Palaeontology and Archaeology

Specimen provenance	Not applicable. This is a letter to the editor concerning a publication regarding paleopathology. No new data was collected for our letter.
Specimen deposition	Not applicable

Dating methods	Not applicable

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

Ethics oversight	No ethical approval was required as this was a letter to the editor concerning a previous publication. No data was collected or used that is not publicly available for this letter.

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

Reply to: Common orthopaedic trauma may explain 31,000-year-old remains

https://doi.org/10.1038/s41586-023-05757-7

Published online: 15 March 2023

Open access

Check for updates

Melandri Vlok[1,13]✉, Tim Maloney[2,3,4,13]✉, India Ella Dilkes-Hall[5,13], Adhi Agus Oktaviana[6,7,13], Pindi Setiawan[8,14], Andika Arief Drajat Priyatno[9,13], Marlon Ririmasse[7], I. Made Geria[7], Muslimin A. R. Effendy[9], Budy Istiawan[9], Falentinus Triwijaya Atmoko[9], Shinatria Adhityatama[6], Ian Moffat[10], Renaud Joannes-Boyau[11,12], Adam Brumm[3] & Maxime Aubert[2,3,11,13]

REPLY TO Murphy et al. *Nature* https://doi.org/10.1038/s41586-023-05756-8 (2023)

We appreciate the accompanying technical Comment by Murphy et al.[1]—a group of practicing orthopaedic surgeons—on our original paper[2]. However, we strongly disagree with their conclusion that a reductionist approach was taken in the diagnosis of surgical amputation in a 31,000-year-old individual (TB1) from Borneo. We note that a complete systematic differential diagnosis was indeed completed (Extended Data Table 1); this process involved careful consideration of the most common and banal conditions first, such as accidental fracture, before considering the possibility of more rare and unusual circumstances. Through this iterative process, fracture was first eliminated as a possibility, followed by natural causes of amputation.

Surgical amputation was the remaining scenario left that completely described the characteristics that we observed in the bone. As is standard for palaeopathological analysis, a detailed description of the pathology was undertaken, including recording of the location and aspect of affected bone, the type of bone affected, the mechanism of injury, the degree of healing, complications to healing, force and fracture type. This detailed analysis means that certain aspects of trauma were excluded from the differential diagnosis due to the specific location of the injury. It was at this stage that physeal fractures, which Murphy et al.[1] correctly recognize as common fractures in early adolescence, were disregarded from the differential diagnosis as the affected portion of the bone was at the site of the mid to distal lower third diaphysis and not near to the distal metaphyseal region (Fig. 1). We surmise that Murphy et al.[1] may have mistaken the thin cortices of TB1's tibia and fibula for that at the diaphyseal-to-metaphyseal transition in the bone that is naturally thin. However, with TB1, the cortices in these bones (and indeed also the left femur) are thin due to extreme atrophy that probably occurred over a number of years. We acknowledge that two-dimensional photographs and radiographs can misrepresent to readers injuries that, in reality, occur in three dimensions. Thus, we provide publicly available three-dimensional computed tomography files of the amputation.

Moreover, the age of 6 to 9 years after surgery is a minimum age based on the minimal timing required for the completion of bone remodelling in the major long bones and, given the size of the lower limb bones, it is probable that the injury occurred in childhood. As Murphy et al.[1] are aware, physeal stasis can have diverse traumatic origins as well as stasis of longitudinal growth in general[3,4]. Experimental animal studies demonstrate the importance for muscular activity to initiate longitudinal growth of bones through biomechanical strain. Thus, the small size of the left limb bones can be readily attributed to the existing evidence for bone atrophy related to muscle wastage[5].

We are uncertain what Murphy et al.[1] are referring to in their second paragraph when relating the cervical fracture to the force applied to the amputation site. Although it is possible that the cervical vertebral fracture occurred in the same event that led to the need for amputation of the lower limb, the limitations of bone response prevent us from investigating this possibility any further. Owing to the lack of empirical evidence, we refrain from speculating on the motivation or underlying cause that led to the decision to amputate. It is of course possible that the trauma described by Murphy et al.[1] was the ultimate mechanism of injury that led to the child's limb being surgically amputated at the location of the distal diaphysis. We clarify that we are not saying oblique fractures of the long bone shafts do not occur from blunt force trauma but are atypical in cases from an accident (excluding modern situations including transport), particularly one where the fibula and tibia were both fractured.

Murphy et al.[1] point out in detail the requirements for their proposed scenario to have occurred but do not see the improbability of such a condition in the context of the Pleistocene tropics of Borneo. They do suggest soft tissue-only surgery as an alternative that would have involved antisepsis and debridement, which is arguably a far more sophisticated (and therefore less parsimonious) form of care that would have required a complex understanding of the anatomical basis for infection to specifically remove the infected tissue (rather than performing an entire amputation). If the fracture was not reduced through fixation, as is the case in modern Western surgical practices, a dead foot would have probably been an extreme impediment for the rugged mountainous terrain, and far more painful than a stump. Moreover, the fractured foot would have been susceptible to repeated infection as it was carried throughout the environment.

Murphy et al.[1] incorrectly describe the remodelled bone as osteomyelitis. To support their argument, they report an anecdote in a review on the history of osteomyelitis that is from a single memoir of an American surgeon published in 1831, whose patients received

[1]Sydney Southeast Asian Centre, University of Sydney, Sydney, New South Wales, Australia. [2]Griffith Centre for Social and Cultural Research, Griffith University, Gold Coast, Queensland, Australia. [3]Australian Research Centre for Human Evolution, Griffith University, Nathan, Queensland, Australia. [4]Research into Deer Genetics and Environment, RIDGE Group Inc, Ascot, Western Australia, Australia. [5]Archaeology, School of Social Sciences, University of Western Australia, Crawley, Western Australia, Australia. [6]School of Humanities, Languages and Social Science, Griffith University, Gold Coast, Queensland, Australia. [7]BRIN, OR Arkeologi, Bahasa dan Sastra, Pusat Riset Arkeometri, Jakarta, Indonesia. [8]Faculty of Art and Design, Bandung Institute of Technology, Bandung, Indonesia. [9]Balai Pelestarian Cagar Budaya Kalimantan Timur, Samarinda, Indonesia. [10]Archaeology, College of Humanities, Arts and Social Sciences, Flinders University, Bedford Park, South Australia, Australia. [11]Geoarchaeology and Archaeometry Research Group (GARG), Southern Cross University, Lismore, New South Wales, Australia. [12]Palaeo-Research Institute, University of Johannesburg, Johannesburg, South Africa. [13]These authors contributed equally: Melandri Vlok, Tim Maloney, India Ella Dilkes-Hall, Adhi Agus Oktaviana, Andika Arief Drajat Priyatno, Maxime Aubert. [14]Deceased: Pindi Setiawan. ✉e-mail: Melandri.vlok@sydney.edu.au; t.maloney@griffith.edu.au

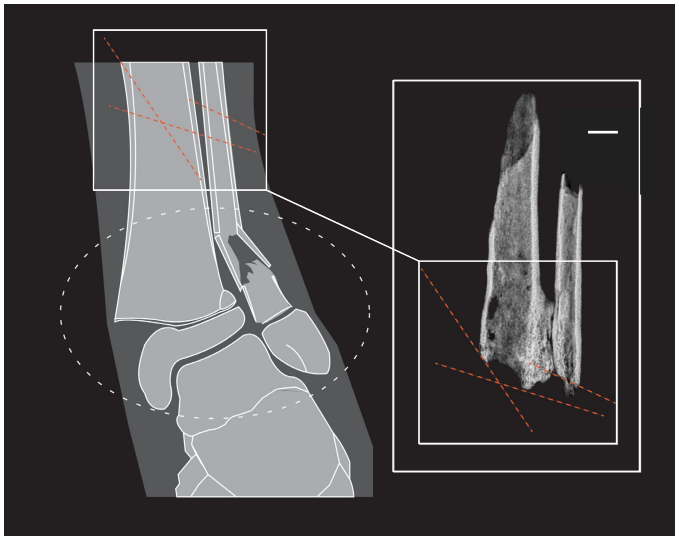

Fig. 1 | A Salter–Harris type II fracture of left tibia and fibula similar to the one presented by Murphy et al. compared with TB1's amputation. The amputation site is more proximal (white box) to the region of Salter–Harris physeal fractures (white dashed oval). At minimum, three different angles of force are present in TB1's amputation (red dashed lines) as opposed to one angle of force in Salter–Harris type II. Medial physis fracture of the tibia is absent in TB1 as is necessary for Salter–Harris type II classification with fibular involvement (see figure 1 of Murphy et al.[1]). Scale bar, 10.0 mm.

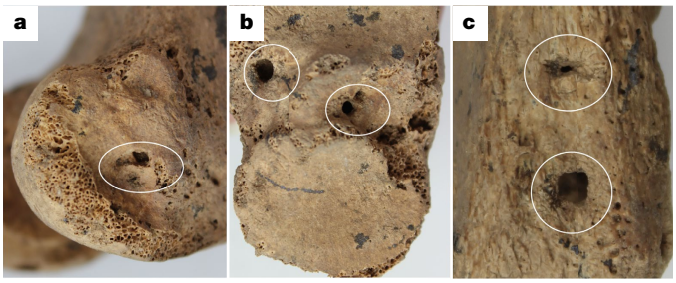

Fig. 2 | Cloacae of the right femur and tibia compared to carnivore puncture holes of the left tibia. a–**c**, Comparison of cloacae of the right femur (**a**) and tibia (**b**) with the carnivore puncture holes of the left tibia (**c**). The left tibia holes are clearly caused by punctures in dry bone resulting in square jagged margins to the cavities. By contrast, the margins of the holes in the right tibia and femur are rounded due to the constant remodelling process in the development of the cloacae. The femoral cloaca (**a**) also presents with a clear lytic channel consistent with infection.

treatment in hospital[6]. Osteomyelitis in the tropics is more aggressive owing to the greater diversity of the pathogens that cause osteomyelitis, the suitability of *Staphylococcus aureus*—the most common cause of osteomyelitis—to the humidity in the tropical belt and, potentially, the reduced amount of clothes worn in tropical environments increasing the infection risk of exposed wounds[7,8]. Although the mortality rate of untreated sepsis is not documented in the tropics, antibiotic-era in-hospital mortality rates in post-amputation contexts are reported to be as high as 10% and, in the Vietnam War, sepsis was attributed to 12% of deaths in surgical patients, the third leading cause of mortality in that conflict[9–11]. Osteomyelitis, both pyogenic and non-pyogenic, is readily observed in archaeological bone. In pyogenic forms, death of bone leading to sequestrum is readily observable surrounded by a shell of bone known as involucrum. Cloacae—pus draining holes—form to drain the pus from the medullary canal. Although there are circular holes in the bone, these are clearly a result of carnivore puncture and beetle scavenging marks, which are very common causes of post-mortem skeletal damage observed in Southeast Asian archaeological human skeletons (Fig. 2).

The radiographs of TB1's amputated limb (Fig. 1) show a lack of bone radiolucency associated with the development of sequestra, and the localization of radiodense bone only intermediate to the tibia and fibula is consistent with myositis ossification, and not with osteomyelitis, which will result in subperiosteal inflammation and subsequent new bone development on a more diffuse scale around the infected site. The complete lack of subperiosteal change to the tibia and fibula away from the ossified region, as well as the initiation of the subperiosteal new bone, from both the tibia and the fibula, to meet intermediately, is consequently not consistent with osteomyelitis. Moreover, chronic osteomyelitis is expected to be associated with some level of continued subperiosteal activity observed as woven bone and, in this case, the bone is entirely lamellar. Evidence of osteomyelitis in the right limb is available for comparison as well as dry bone examples from prehistoric Southeast Asia associated with and without fracture[12,13].

We do concede the error that the medial malleolus of the right tibia is not placed in anatomical position in figure 3a of our original paper[2]. However, the aim of this figure is to represent the general completeness of the skeleton, and the relationship of the size of the left and right limbs, which we believe the figure succeeds in presenting. Given the medial malleolus is barely discernible, we believe the matter of anatomical correctness to be negligible.

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/s41586-023-05757-7.

Reporting summary

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

Data availability

CT data are available at Figshare (https://figshare.com/projects/CT_Data_Tebo_TB1_Borneo_Kalimantan/150765).

1. Murphy, J., Davis, J., Tarrant, S. & Balogh, Z. Common orthopaedic trauma may explain 31,000-year-old remains. *Nature* https://doi.org/10.1038/s41586-023-05756-8 (2023).
2. Maloney, T. R. et al. Surgical amputation of a limb 31,000 years ago in Borneo. *Nature* **609**, 547–551 (2022).
3. Westberry, D. E., Davids, J. R. & Pugh, L. I. The Boyd amputation in children: indications and outcomes. *J. Pediatr. Orthop.* **34**, 86–91 (2014).
4. Krajbich, I. J. Lower-limb deficiencies and amputations in children. *JAAOS* **6**, 358–367 (1998).
5. Hall, B. K. & Herring, S. Paralysis and growth of the musculoskeletal system in the embryonic chick. *J. Morphol.* **206**, 45–56 (1990).
6. Smith, N. *Medical and Surgical Memoirs* (WA Francis, 1831).
7. Schaumburg, F., Alabi, A., Peters, G. & Becker, K. New epidemiology of *Staphylococcus aureus* infection in Africa. *Clin. Microbiol. Infect.* **20**, 589–596 (2014).
8. Munshi, B., MacFater, W., Hill, A. G. & McCaig, E. H. Paediatric osteomyelitis in Fiji. *World J. Surg.* **42**, 4118–4122 (2018).
9. Bickler, S. W. & Sanno-Duanda, B. Bone setter's gangrene. *J. Pediatr. Surg.* **35**, 1431–1433 (2000).
10. Shittu, A. et al. Tropical pyomyositis: an update. *Trop. Med. Int. Health* **25**, 660–665 (2020).
11. Blyth, D. M., Yun, H. C., Tribble, D. R. & Murray, C. K. Lessons of war: combat-related injury infections during the Vietnam War and Operation Iraqi and Enduring Freedom. *J. Trauma Acute Care Surg.* **79**, S227–S235 (2015).
12. Scott, R. M. et al. Domestication and large animal interactions: skeletal trauma in northern Vietnam during the hunter-gatherer Da But period. *PLoS ONE* **14**, e0218777 (2019).
13. Vlok, M., Paz, V., Crozier, R. & Oxenham, M. A new application of the bioarchaeology of care approach: a case study from the metal period, the Philippines. *Int. J. Osteoarchaeol.* **27**, 662–671 (2017).

14. Rouhani, A., Elmi, A., Aghdam, H. A., Panahi, F. & Ghafari, Y. D. The role of fibular fixation in the treatment of tibia diaphysis distal third fractures. *Orthop. Traumatol. Surg. Res.* **98**, 868–872 (2012).
15. Bonnevialle, P. et al. Distal leg fractures: how critical is the fibular fracture and its fixation? *Orthop. Traumatol. Surg. Res.* **96**, 667–673 (2010).
16. Donnally, C. et al. Orthopedic injuries associated with jet-skis (personal watercrafts): a review of 127 inpatients. *Orthop. Traumatol. Surg. Res.* **104**, 267–271 (2018).
17. Pennoyer, G. P. Traumatic amputation of the thigh, complicated by both tetanus and gas gangrene with recovery. *JAMA* **95**, 342–343 (1930).
18. Wen, A. P. Y. et al. Successful ankle replantation in two cases with different presentations. *Arch. Plast. Surg.* **47**, 182–186 (2020).
19. Pannier, S. Congenital pseudarthrosis of the tibia. *Orthop. Traumatol. Surg. Res.* **97**, 750–761 (2011).
20. Abbas, Z. G. & Archibald, L. K. Epidemiology of the diabetic foot in Africa. *Med. Sci. Monit.* **11**, RA262–RA270 (2005).
21. Eisenberg, K. A. & Vuillermin, C. B. Management of congenital pseudoarthrosis of the tibia and fibula. *Curr. Rev. Musculoskelet. Med.* **12**, 356–368 (2019).
22. Anderson, E., Peluso, S., Lettice, L. A. & Hill, R. E. Human limb abnormalities caused by disruption of hedgehog signaling. *Trends Genet.* **28**, 364–373 (2012).
23. Tadmor, O., Kreisbe, G., Achiront, R., Porat, S. & Yagel, S. Limb amputation in amniotic band syndrome: serial ultrasonographic and Doppler observations. *Ultrasound Obstet. Gynecol.* **10**, 312–315 (1997).
24. Baker, C. J. & Rudolph, A. J. Congenital ring constrictions and intrauterine amputations. *Am. J. Dis. Child.* **121**, 393–400 (1971).
25. Fisher, R. & Cremin, B. Limb defects in the amniotic band syndrome. *Pediatr. Radiol.* **5**, 24–29 (1976).
26. Barber, C. G. Immediate and eventual features of healing in amputated bones. *Ann. Surg.* **90**, 985–992 (1929).
27. Burns, K. R. *Forensic Anthropology Training Manual* (Routledge, 2015).

Acknowledgements We thank H. Rice for his collaboration on producing the CT scan data.

Author contributions M.V., T.M., I.E.D.-H. and A.A.D.P. conceived and wrote the paper. T.M., I.E.D.-H. and A.A.D.P carried out the excavation of the site and burial. M.A. and A.B. conceived the study and contributed to the paper. Site access, project coordination and field logistics were facilitated by P.S., M.R., A.A.O., F.T.A., I.M.G., M.A.R.E., B.I. and S.A. M.V. conducted the osteological analyses. I.M. conducted the geophysical survey. R.J.-B. conducted the US–ESR dating analyses and the Bayesian modelling. All of the authors contributed to editing the paper.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-023-05757-7.

Correspondence and requests for materials should be addressed to Melandri Vlok or Tim Maloney.

Extended Data Table 1 | Differential Diagnosis of Tebo 1

Diagnosis	Description of pathology	Relative expected frequency	Reasons for exclusion	Probability	Ref.
Fracture with non-union and/or traumatic amputation at distal diaphysis	External forces causing trauma to the left tibia and fibula.	Frequent	Fractures at the location of both distal tibia and fibula are associated with comminution or crushing fractures from: - Animal attack - Rock fall - Fall from height Multiple sites of force; however, the directions of force are not consistent with a comminution or crushing fracture (i.e., margin is clean). Traumatic amputations very rarely lead to fibular amputation except where a metal blade has been involved or in a high impact accident such as in a motor vehicle collision. Pattern of remodeled bone is not consistent with any evidence for osteomyelitis (e.g., lack of clear development of involucrum, cloacae or sequestrum). Such a fracture and removal of limb would likely be associated with a length of time where pathogens could enter the bloodstream. The probability of survival is minimal.	Implausible	14-18
Amputation-Post traumatic pseudoarthrosis or gangrene	Ischemia or gangrene leads to the loss of the foot and distal leg.	Rare	The lack of evidence for infection or severe ischemia rules out gangrene, a common condition in traditional bone setting contexts in the tropics. There are reports of up to 10% mortality when surgical and medical intervention (in hospital mortality) occurs in tropical environments. Examples of gangrenous lower legs in tropic contexts in diabetics indicate a mortality rate of more than 50%. Bone pattern not consistent with atrophic (tapered), hypertrophic (splayed) forms.	Ruled out	9-11,19,20
Amputation-congenital pseudoarthrosis	Congenitally impaired local vascularization	Rare	Congenital pseudoarthrosis of both tibia and fibula is exceedingly rare, and in most cases does not involve a true non-union of the bone; instead, is associated with bowing not observed in TB1. Requires operative treatment.	Ruled out	19,21
Abnormal Congenital limb development	Portion of the distal limb fails to develop embryonically	Rare	Extremely rare. Occurs spontaneously as well as in several genetic conditions. Bone end develops as a closed limb. No evidence for inclining margins as observed in TB1.	Ruled out	22
Amputation- in utero (amniotic band syndrome)	Tissue originating from the fetal membrane ties, constricts and eventually removes the distal limb.	Rare	Results in very clean margins with a single constrictive force on bones rather than multiple directions of force as observed in TB1. Commonly associated with other malformations such as syndactyly, club foot, cleft lip and palate, and anencephaly. Can result in both open and closed soft tissue around the amputation. Usually multiple amputations.	Ruled out	23-25
Amputation-surgical	Surgical removal of the lower limb	Unknown	See original paper[2].	Plausible	26,27

Refs. [14–27]

Paper ID: 2022-09-15483A

nature portfolio

Corresponding author(s): Tim Maloney

Double-blind peer review submissions: write DBPR and your manuscript number here instead of author names.

Last updated by author(s): 2022-11-22

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	
X	☐	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
X	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
X	☐	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.*
X	☐	A description of all covariates tested
X	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
X	☐	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)
X	☐	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.*
X	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X	☐	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: *Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.* N/A

Data analysis: *Provide a description of all commercial, open source and custom code used to analyse the data in this study, specifying the version used OR state that no software was used.* N/A

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

Provide the data availability statement here. CT data is available on figshare: https://figshare.com/projects/CT_Data_Tebo_TB1_Borneo_Kalimantan/150765

Human research participants

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

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	*Describe how sample size was determined, detailing any statistical methods 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.*
Data exclusions	*Describe any data exclusions. 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.*
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	*Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.*
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

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	*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.*

Data collection	*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.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*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.*
Non-participation	*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.*
Randomization	*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.*

Ecological, evolutionary & environmental sciences study design

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

Study description	*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.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi 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.*
Sampling strategy	*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.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*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*
Data exclusions	*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.*
Reproducibility	*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.*
Randomization	*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.*
Blinding	*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.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

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
[x]	[ ] Antibodies
[x]	[ ] Eukaryotic cell lines
[ ]	[x] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] 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: East Kalimantan, Borneo- radiocarbon dates 31kya

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: Currently at Griffith University, Gold Coast, Australia

Indicate where the specimens have been deposited to permit 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: Research permits, approval for access to skeletal remains, photography and radiography were granted by National Research and Innovation Agency, Indonesia.

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	*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.*
Reporting on sex	*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.*
Field-collected samples	*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.*
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.

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	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

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	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

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

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	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*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.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*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.*

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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*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.*
Cell population abundance	*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.*
Gating strategy	*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.*

☐ 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	*Indicate task or resting state; event-related or block design.*

Design specifications

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.

Behavioral performance measures

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).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

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

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.