NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods, Experimental design, Lines [91-129]; Supplementary Fig. 1-2. Experimental. Methods, Sections 2.2–2.3 (individual mouse as the experimental unit).
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	The primary experiment included 15 mice per group (60 mice in total); exact n values for downstream analyses are provided in the corresponding figure legends. The FMT experiment included 15 mice per group (60 mice in total). Methods, Lines [91-128]. No formal a priori sample size calculation was performed; group sizes were based on our previous study using the same model and prior experimental experience.
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	mice that were 8 weeks of age at the start of acclimentation (purchased at 7 weeks of age, followed by a one week acclimatization period) free of overt disease were included. No formal exclusion criteria for data points were predefined. Outlier testing was performed during data Results, Sections 3.1–3.5 and corresponding figure legends; exclusion status is not explicitly stated. The exact n for each analysis is reported in the corresponding figure legends.
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods, Lines [93-94]. Methods, Lines [98-128]. Potential confounders were minimised by maintaining otherwise identical environmental and experimental conditions across groups, except for the prespecified housing intervention.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Methods, Lines [135 137]. Personnel conducting group allocation and interventions were aware of group assignments, whereas behavioural outcome assessment and data analysis were performed by investigators blinded to group allocation.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Methods, Sections 2.4–2.12, Lines [131-270]. Outcomes included SPT sucrose preference, TST immobility time, OFT vertical exploratory activity, SNpc The primary outcome measure was sucrose preference percentage in the sucrose preference test (SPT). All other outcomes were secondary. This primary outcome was not used for sample size calculation. No formal a priori sample size calculation was performed.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods, Section 2.13, Lines [263 – 270]. Statistical analyses were performed using GraphPad Prism 8.0. Group comparisons were conducted using one- or two-way ANOVA followed by Tukey – Kramer post hoc tests. and microbiota – behaviour associat Normality of continuous variables was assessed using the Shapiro – Wilk test.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods, Sections 2.1–2.2. Mice were 7 weeks old at arrival; after 1 week of acclimatization, interventions began at 8 weeks of age. Methods, Section 2.1, Lines [79-89]. Mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and maintained under specific pathogen-free
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Methods, Sections 2.2 – 2.12, Lines [91-261]. Group-specific housing, saline or rotenone administration, antibiotic pretreatment, FMT, behavioural testing, tissue collection. and downstream laboratory Methods, Sections 2.2–2.4, Lines [91-157]. Rotenone and social-isolation interventions lasted four consecutive weeks; FMT recipients received two weeks of antibiotic Methods, Sections 2.1 and 2.4, Lines [91-157]. Animals were maintained at the Experimental Animal Center of Xuzhou Medical University under specific Methods, Sections 2.2–2.4, Lines [91-157]. Rotenone was used to establish a behaviourally validated PD model, individual housing was used to induce social isolation.
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Results, Sections 3.1 3.5, Lines 274 460; Figures 1 5 and corresponding legends. Results are presented as mean – SEM, with the exact n for each experimental group reported in the figure legends. e size estimation relied on preliminary experimental findings and published relevant literature. Statistical inference was performed using hypothesis testing approaches including ANOVA followed by post-hoc comparisons and Spearman ' s correlation.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract, Lines 5 – 34. The abstract summa
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction, Lines 46 – 76. The scientifi c background, knowledge gap, and rational e concerning PD-associated depression, so cial isolation, and the microbiota – gut – brain axis are described. lation, enabling investigation of SI-aggr avated depression-like and gut – brain-axi s abnormalities under PD-like conditions. The relevance to human PD and the limita tions of these animal models are discusse d.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction, Lines 42-76. The study inves
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Methods, Section 2.1, Lines 80-89. All animal procedur es were approved by the Lab oratory Animal Welfare and Ethics Committee of Xuzhou Medical University (Approva l No. 202309T002).
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods, Sections 2.1 2.2, Lines 79-105. Mice
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Methods, Sections 2.1 2.3, Lines 79-129. All procedures were conducted under institutional ethical approval, and mice were euthanised with sodium pentobarbital at the end of the experiments. No additional No animals died unexpectedly du ring the experiment, and no anim als or data points were excluded f rom analyses. Humane endpoints, monitored signs and monitoring frequency are not reported. The primary experimental mice were euthanised with sodium pentobarbital (100 mg/kg, i.p.) after behavioural testing; FMT recipients
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion, Lines 463-554. The findings are interpreted in relation to the study objectives and relevant literature, supporting a model in which social isolation aggravates behavioural, neurochemical, intestinal, and Discussion, Lines 568-584. Limitations include unresolved mechanisms of rotenone-associated microbiota alterations, the limited functional resolution of 16S rRNA sequencing, unidentified causal taxa and
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion, Lines 555-567; Methods, Lines 91-128. The findings may have relevance to human PD because social isolation is a potentially modifiable psychosocial factor and the microbiota gut brain axis may represent a possible intervention target. However, generalisation should be cautious because the study used male C57BL/6J mice, a
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	The manuscript does not state whether a study protocol (including the research question, key design features and analysis plan) was prepared before the study or whether/where it was registered.
Data access	20	Provide a statement describing if and where study data are available.	Availability of Data and Materials, Section S7.
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Conflicts of Interest, Section S11. NA