Estimating the replicability of Brazilian biomedical science

The Brazilian Reproducibility Initiative[1]

Warning note:

In following up with the project by discussing replications individually with participant labs, we have detected a few errors in the data that are included in this preprint. The ones found up to now change some replication rates slightly (i.e. from 15-45% to 19-45% in the primary analysis) but do not make a meaningful difference in the article's conclusions.

This, however, has raised concerns that our original data checking process (which was performed asynchronously) was likely suboptimal. We have thus decided to hold off revision of the preprint and submission to a journal until we have discussed each experiment individually with the labs that replicated it, in order to check the original data and clear up any doubts.

This process should be concluded in December 2025, when a revised preprint should be expected. In the meantime, keep in mind that the results in the final version are bound to present minor changes when compared to those in this version.

[1] The full author list and correspondence information for the consortium are provided at the end of the manuscript.

Abstract

Concerns over the replicability and reproducibility of published research have grown in many research fields, but empirical data to inform policies are still scarce. Biomedical research in Brazil expanded rapidly over the last three decades, with no systematic assessment of the replicability of its findings. With this in mind, we set up the Brazilian Reproducibility Initiative, a multicenter replication of published experiments from Brazilian science using three common experimental methods: the MTT assay, the reverse transcription polymerase chain reaction (RT-PCR) and the elevated plus maze (EPM). A total of 56 laboratories performed 143 replications of 56 experiments; of these, 97 replications of 47 experiments were considered valid by an independent committee. Replication rates for these experiments varied between 15 and 45% according to five predefined criteria. In median terms, relative effects (expressed as ratios between group means) were 60% larger in original experiments than in replications, while coefficients of variation were 60% smaller. Effect size decrease was smaller for MTT, cell line experiments and original results with less variability, while t values for replications were positively correlated with researcher predictions about replicability. Deviations from preregistered protocols were very common in replications, most frequently due to reasons inherent to the experimental model or related to infrastructure and logistics. Our results highlight factors that limit the replicability of results published by researchers in Brazil and suggest ways by which this scenario can be improved.

Introduction

Concerns over the replicability and reproducibility of published results have increased in many areas of science over the past decade. Basic biomedical science has not been immune to these concerns: reports from the pharmaceutical industry (Begley & Ellis, 2012; Prinz et al., 2011) and systematic assessments of replicability in specific fields, such as cancer biology (Errington, Mathur, et al., 2021), spinal cord injury (Steward et al., 2012) and amyotrophic lateral sclerosis (Scott et al., 2008), have reported success rates ranging from 0 to 40%. Nevertheless, these projects have focused on selected subsets of papers in specific areas of science, and the dimension of the problem in the biomedical literature at large remains unknown.

Although concerns over reproducibility are global, many of the proposed solutions to address it are local in scope, as institutions and funders are instrumental in setting up policies and incentives that can foster reproducible research (Munafò et al., 2017). Actionable data on the replicability and reproducibility of research produced within specific institutions, regions or countries, however, is extremely scarce. With this in mind, we set up the Brazilian Reproducibility Initiative, a multicenter assessment of the replicability of experiments published by Brazilian institutions using common laboratory methods (Amaral et al., 2019; Neves et al., 2020).

This assessment of a representative sample of experiments published by national researchers aims to provide systematic data to raise awareness of the issue and inform scientific policy within the country. In addition to generating data on replicability, the project's first-person, naturalistic approach can identify challenges faced by the laboratories conducting replications, which are drawn from institutions similar to those that produced the original results. Consequently, our findings can offer insights into problems and potential solutions that are relevant to the country's reality, as well as guide future replication efforts in lab biology.

Methods

Lab recruitment

We began by systematically reviewing a random sample of life sciences articles from Brazil to identify common experimental models and methods (see https://osf.io/f2a6y). Based on these findings, we chose 10 experimental methods, using rodents or cell lines as models, to select studies for replication (see https://osf.io/qhyae). We then opened a public call for Brazilian labs that could replicate experiments using these methods and models, advertised by email, social media and lectures

in conferences and institutions, to which 73 labs initially responded. Based on the expertise of respondents and a feasibility analysis by the coordinating team, we selected 3 outcome assessment methods for replication: the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) reduction assay, the reverse transcription polymerase chain reaction (RT-PCR) and the elevated plus maze test of anxiety (EPM; see https://osf.io/qxdjt for details). Three further calls for labs were opened later to fill in specific gaps, leading to another 24 responses.

Experiment selection

We searched the Web of Science for publications from 1998 to 2017 with affiliations in Brazil, using R code to identify the methods listed above (see https://osf.io/57f8s for details and https://osf.io/4sy2g for code). We then manually screened for articles that met the following criteria: (a) had at least 50% of authors (including the corresponding one) affiliated with a Brazilian institution; (b) had at least one quantitative result using one of the selected methods and models that (c) was statistically significant, (d) was mentioned in the title or abstract and (e) used only commercially available materials (for details, see https://osf.io/u5zdq). A data extraction step collected information on the biological models, experimental procedures and results for the first comparison in the article that presented a significant difference between two groups. Manuals used for extraction were based on existing guidelines for methods reporting (Bustin et al., 2009; Kilkenny et al., 2010; NPQIP Collaborative group, 2019), and are available at https://osf.io/udjr7, https://osf.io/tr6xa, https://osf.io/7uhb6 and https://osf.io/rkvtm. After this step, we excluded experiments that did not report standard deviation, standard error of mean or confidence interval, or had an estimated cost greater than BRL 5,000 (around USD 1,300 at the time) per replication.

We then shared summaries of the eligible experiments' methods with collaborating laboratories, according to their expertise, to confirm their capability to replicate them. Our replication sample comprised the first 20 experiments for each method that could be assigned to three independent labs. If labs withdrew from the project before completing their replications, those experiments were reassigned to other labs within our network or to new labs recruited by additional calls. Further information about (a) the selected experiments, (b) the original article, journal, authors and respective institutions, and (c) the teams performing replications were collected as outlined in https://osf.io/enjxy.

Protocol development

For each experiment, the coordinating team transcribed the methodological information available in the original article into a protocol, with missing information included as questions. Each lab assigned to replicate an experiment received this protocol, without information about the original publication or results (for details, see https://osf.io/gsvy2). They were instructed to fill in protocol gaps and elaborate on procedures when necessary, keeping as close to the original methods as possible (see

instructions at https://osf.io/29vh7). Adaptations were allowed in cases of necessity (e.g. different equipment or unavailable reagents), or when the replicating lab considered the original protocol inadequate to measure the desired outcome, either due to methodological errors (e.g. incorrect primer sequences) or to significant risk of bias (e.g. lack of a vehicle group). Guidelines used to decide about adaptations are available at https://osf.io/e7zs9.

Protocols then received at least two external revisions, usually by another lab in the network that was not performing the same experiment and by a member of the coordinating team, both of whom had access to the original publication (see https://osf.io/g6ph5**)**. Instructions to reviewers are available at https://osf.io/k97r4. For some protocols, a second coordinating team member replaced the independent lab as a reviewer. Reviewer comments were incorporated into the protocol as additional questions for revision and completion by the replicating labs. This process was iterated until the lab and the coordinating team deemed the protocol complete. Experiments involving animals were submitted to local institutional animal ethics review boards at this stage, and protocols were adjusted if required. After approval, the final version was reviewed once more by an independent member of the coordinating team, who raised additional questions if necessary, and then preregistered at the OSF (see https://osf.io/vzam6/ for the complete version history of registered protocols). A flowchart of the protocol development process is presented in **Figure S1**.

Sample size calculations were performed to achieve 95% statistical power to detect the original standardized mean difference in each individual replication (see details at https://osf.io/wxzr7). Code and data for the calculations are available at https://osf.io/vs5rp and https://osf.io/atkd7, respectively. Authors of the original articles were contacted for missing information and raw data (see contact protocol at https://osf.io/3dn4x), but these were intended for future analyses and were not used in developing the replication protocols.

Researcher predictions

Before the start of experiments, an open call was issued to experimental researchers willing to make predictions regarding the success of individual replications. Seventy participants selected one of the three methods and received a survey containing a summary of the 20 experiments associated with that method, including the original result and associated statistics. They were asked to predict (a) the probability of replication success, (b) the expected effect size in the replication, and (c) how technically challenging the replication would be (see details at https://osf.io/tm76h and full survey at https://osf.io/29mq5). Fifty-seven of these researchers also participated in prediction markets, in which they received USD 50 in vouchers to bet on the replication success of individual experiments. An analysis plan for this part of the project, which will be explored in future publications, was

preregistered at https://osf.io/pjhgd, and anonymized survey data is deposited at https://doi.org/10.7910/DVN/2RLSMG.

Experimental procedures

For each experiment, a custom data collection spreadsheet was built by the coordinating team based on the protocol. This was meant to document not only the experimental results but also the execution of each protocol step to make the experiment auditable. Labs received these spreadsheets along with a detailed manual on how to execute and document the replications (https://osf.io/ackyd) and on how to deal with protocol adaptations when necessary (https://osf.io/e7zs9).

Experimental materials were acquired by the coordinating team and delivered to the lab performing the replication unless they were already available there. Labs were expected to only use materials within their expiration date; however, due to the frequent postponing of experiments because of COVID-19 restrictions or difficulties with suppliers, the use of reagents with expired validity was allowed if their activity could be clearly demonstrated – either by the method demonstrably working as expected (e.g. PCR kits, molecular weight scales) or by a separate test of biological activity with a prespecified expected result (e.g. a test of cell growth with a particular culture medium). These cases were documented in data collection forms, compiled by the coordinating team and evaluated by the validation committee (see below).

Labs then performed replications and sent the data collection sheets to the coordinating team when experiments were completed. If any difficulties arose, labs were free to contact the coordinating team for orientations. If results suggested a failure to implement the method adequately (Neves & Amaral, 2020), labs were allowed to repeat experiments (see **Figure S1**), and general guidelines used to deal with these situations were later compiled in https://osf.io/m35s6.

Post-experiment debriefing

After an experiment was finished, data collection spreadsheets were sent to the coordinating team, who reviewed them and created a document with questions concerning (a) experimental steps deviating from the preregistered protocol and (b) unclear or missing information. These were sent to the replicating lab, which was asked to confirm the observations on protocol deviations, answer the coordinating team's questions and rate (on a scale of 1 to 5) how much the executed protocol deviated from the preregistered one (data available at https://osf.io/g9jsp, https://osf.io/64whp and https://osf.io/dfp3h).

After undergoing this step and answering further questions by the coordinating team if needed, labs received access to the original article from which the experiment was drawn. They were asked to fill in a form answering whether they felt the original result was successfully replicated, with a

justification for their answer. They also rated how relevant the differences between the original protocol and the replication were (on a scale of 1 to 5). Data is available at https://osf.io/dqwr4, and more details on each debriefing step are presented at https://osf.io/xgth2.

Based on the responses to the first debriefing form, a final notes document on each experiment was compiled by the coordinating team, containing (a) a list of deviations from the original protocol, (b) a list of additional observations on the experiment, (c) a list of reagents used after the expiration date (if any) and (d) clarifications about the data collection spreadsheet.

Validation of experiments

To adjudicate whether the performed experiments constituted valid replications and should be included in the main analysis, a validation committee was formed for each method, including members of the participating labs using that method and the coordinating team. Each experiment was evaluated by three members of this committee, who received the registered protocol, the original article, the final notes document, and (in some cases) the data collection spreadsheets and/or additional material, but with no access to the replication results. Each member of the validation committee received instructions (https://osf.io/rnemd) on how to evaluate these documents and was asked to answer how much the executed protocol deviated from the original experiment (on a scale of 1 to 5) and whether they considered the experiment a valid replication of the original one, providing justifications for their answers.

If any of the three evaluators or the replicating lab considered the replication invalid or attributed a deviation rating greater than 3, or if the sum of the 4 evaluation scores was greater than 10, the experiment was discussed by the validation committee to decide whether the replication should be included in the primary analysis. The committee could also suggest the exclusion of individual experimental units, or the use of an experimental unit different from the one suggested by the lab. Committee members did not participate in the discussion of their own replications. After all experiments were discussed, decisions were reviewed for consistency, and inconsistent decisions were rediscussed to align criteria. **Figure S2** provides an overview of the debriefing and validation process; for more details, see https://osf.io/e3fjg. Decisions are available at https://osf.io/9ta45.

Lab self-assessment and project evaluation

After the conclusion of the validation process, replicating labs received the final notes documents, validation committee scores and validation decisions. They were then asked to fill in a final debriefing form (see https://osf.io/xgth2) where they should answer about their agreement with these scores and decisions. They were also asked to elaborate on reasons for protocol deviations,

whether those could have been prevented, and what they would change in the replication protocol if they were to start over. Data for this step is available at https://osf.io/dp54y.

In addition to these lab responses, individual lab members were asked to optionally fill in an anonymous project evaluation form, in which they could evaluate the project and their participation, and elaborate on challenges, difficulties, and learning opportunities. The 121 responses to this form were complemented by 18 semi-structured interviews with project participants. Responses to open questions about protocol deviations and project difficulties were categorized by the coordinating team following a taxonomy available at https://osf.io/5gjb7. After this, the coordinating team built its own list of difficulties faced by the project. For more on the project evaluation process, see https://osf.io/nfr6y.

Data cleaning and checking

Primary data from the experiments recorded in data collection spreadsheets (optical density measurements for MTT and conventional PCR, Ct values for quantitative PCR (RT-qPCR), and behavioral outcomes for EPM) were manually compiled by the coordinating team into a single spreadsheet for each method, along with experiment-level metadata (available at https://osf.io/pwfze). When inconsistencies in calculations or discrepancies between subsections of the data collection spreadsheets were detected, they were cleared up by direct contact with the replicating lab. After this, results were summarized by code for each experiment into spreadsheets and scatter plots and shared with the replicating labs, who were asked to check for discrepancies with their calculations. If issues were found, the lab and coordinating team reviewed them via email until the sources of errors were identified and corrected (see https://osf.io/58vsx for details).

Data analysis

The protocol for analyzing replication rates and predictors was initially registered at https://osf.io/9rnuj, before any experimental results had been reviewed by the coordinating team. It was updated at a time point when experimental results were available, but no analyses had been performed, to account for steps added after the experimental procedures (such as the validation process). The final dataset used for the analyses included in the manuscript is deposited at https://doi.org/10.7910/DVN/ZJRDIV (Brazilian Reproducibility Initiative, 2025) . Analysis code in R was developed based on the protocol and is available both in the data repository and at https://github.com/BrazilianReproducibilityInitiative/bri-analysis. A list of deviations, additions and specifications added to the protocol after the analysis had started is available at https://osf.io/9hj7t.

In summary, results from the available replications of each experiment were aggregated by meta-analysis using the R package metafor (Viechtbauer, 2010), with the log-transformed ratio of

means as the effect measure for MTT and EPM. For RT-qPCR experiments, we used the mean difference in ΔCt values (which is already in log scale), while for conventional PCR we used the log-transformed relative band density between the gene of interest and the reference gene. The results of these meta-analyses were used for comparison with the original result (also transformed into the natural logarithm of the ratio of means). Pairing between units in the experimental and control groups was implemented for calculating ratios when this was done in the original study, as described in the methods or inferred from the lack of error bars in the control group (see https://osf.io/wxzr7).

Replication rates for the sample were calculated on the basis of 5 dichotomous criteria: (a) whether the original estimate was within the 95% prediction interval of a random-effects meta-analysis of the replication, (b) whether the estimate of a random-effects meta-analysis of the replications was within the 95% confidence interval of the original results, (c) whether the point estimate of a fixed-effects meta-analysis of the replication was statistically significant at $p < 0.05$ and had the same sign as the original result, (d) whether at least half of individual replications were statistically significant in the same direction as the original result and (e) whether at least half of labs considered the original result successfully replicated in their subjective assessment. Prediction and confidence intervals were based on t distributions with degrees of freedom based on the number of experimental units, combined between groups within replications and between replications within meta-analyses using the Welch-Satterthwaite equation (Welch, 1947). Sensitivity analyses use z distributions or the Knapp-Hartung approach to calculate degrees of freedom (Knapp & Hartung, 2003). Agreement between replication criteria was calculated by Cohen's kappa for pairs and Fleiss' kappa for the aggregate of the 5 criteria.

These rates were initially calculated for the primary analysis set, which included only the replications endorsed by the validation committee. Rates were also calculated for other sets of replications, namely (a) all replications, (b) all replications considered valid by the lab, (c) only experiments with at least 2 valid replications, (d) only experiments with at least 3 valid replications, (e) only replications achieving 80% post-hoc power on aggregate to detect the original relative difference in means, considering the standard deviation, sample size and correlation between pairs found in the replication (see https://osf.io/tbkvz for experiments within each analysis set and results of post-hoc power calculations). The primary analysis used the experimental unit as defined by the validation committee, while sets (a) and (b) above considered the lab's definition. A multiverse analysis using all possible replication criteria and sets of experiments is presented as a specification curve (Simonsohn et al., 2020).

The correlation between effect sizes from the replication and original experiments was evaluated using Pearson's *r*, with sensitivity analyses removing a prominent outlier or using Spearman's ρ. Coefficients of variation from the original study were compared to the mean coefficient

of variation of its replications using Wilcoxon's signed rank test. The mean absolute difference between the effect sizes of the original study and its replications (both expressed as log ratios of means) was compared to that between individual replications of the same experiment, also using Wilcoxon's signed rank test. Predictors of replication success at the level of original experiments and at the level of replications were evaluated using Spearman's correlation coefficient. A complete list of tested predictors is available in the analysis protocol and list of deviations.

Results

Lab recruitment and selection of experiments

Throughout the project, 97 labs across Brazil applied to replicate experiments in response to 4 public calls. Of these, 75 joined the Initiative at some point and 56 contributed data. The geographic distribution of these labs is displayed in **Figure S3** and **Table S1**, with information on team members available in **Table S2**.

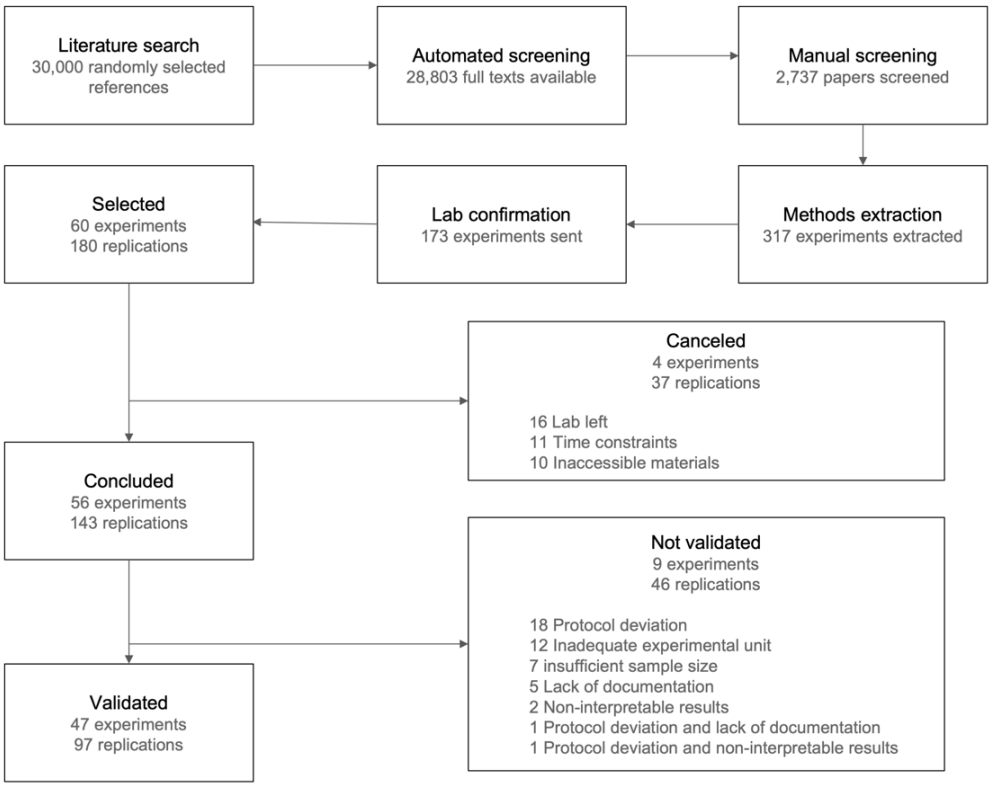

Figure 1. Flowchart describing the selection and execution of replication experiments. Top row shows the number of articles that underwent automated and manual screening. The methods of selected experiments were extracted, and their summaries were sent to labs to determine the final replication sample. Numbers of concluded and validated experiments (i.e. those with at least one replication) and replications are shown in the bottom rows, with reasons for lack of completion or invalidation shown on the right.

The selection of experiments for replication and their execution are summarized in **Figure 1**. A total of 317 experiments using the 3 selected methods (MTT, PCR and EPM) were identified by full-text screening and had protocol data extracted. 173 protocol summaries (https://osf.io/7kagj) were shared with participating labs until we could assign 20 experiments with each method to at least 3 labs. Information on selected experiments and their articles of origin can be found at https://osf.io/cyjsz and is summarized in **Table S3**, while replication protocols developed by labs are available at https://osf.io/vzam6/.

Concluded experiments

Out of the 180 planned replications, 143 were carried out by labs, covering a total of 56 experiments, of which 34 had three replications, 19 had two replications and 3 had only one (**Table S4**). 137 of these had enough data (i.e. more than one valid data point in each group) to be analyzed. Canceled replications were due to inaccessible materials and to labs leaving the project or not finishing replications by the project's deadline.

Experiments submitted by labs then underwent validation by our designated committee. Out of 143 replications, 46 were excluded in this step due to protocol deviations (18), using an inadequate experimental unit (12), insufficient sample size (7), insufficient documentation (5), non-interpretable results (2) or a combination of these reasons (2) (see **Figure 1**). Results of the validation process are available at https://osf.io/9ta45/ and a description of reasons for invalidation can be found in **Table S5**. Validation decisions for different interpretations of the experimental unit in cell culture experiments are detailed in **Table S6**. Labs agreed with the committee's decisions on all validated experiments and on 76% of invalidated ones.

This left us with 97 valid independent replications and 3 replications with insufficient sample size and n > 1 / group (which were included in meta-analyses but not analyzed independently), covering 47 different experiments in our primary analysis. Only 12 experiments remained with three valid replications, while 26 had two and 9 had only one. A list of materials concerning each replication is compiled at https://osf.io/6av7k/wiki/Individual_Replications/, while aggregated data for each analysis set is available at https://osf.io/6av7k/wiki/Aggregated_Replications/. For the primary analysis, unit-level data are available as tables at https://osf.io/uzm97/files/, and forest plots for meta-analyses are available at https://osf.io/sx9gv.

Replication rates

Replication rates for the 47 experiments included in the primary analysis are shown in **Table 1**. In 18 out of 40 experiments with multiple replications (45%), the original estimate was within the 95% prediction interval of the replication meta-analysis, while 12 out of 47 aggregate replication

estimates (26%) and 25 out of 97 individual replications (26%) were within the original experiment's 95% confidence interval. Nine (19%) aggregate estimates and 13 (13%) individual replications showed a statistically significant effect in the same direction as the original, with half or more of the available replications significant in 7 experiments (15%). Thirty-one replications (32%) were considered successful by the replicating lab, with at least half of them making this judgment in 19 experiments (40%). That said, many labs used loose criteria for success, such as an effect in the same direction or of comparable size, regardless of statistical analyses (see **Table S7**). Ten (21%) experiments were replicated by at least half of the applicable criteria, and agreement between different criteria is shown in **Figure S4**, with Fleiss' kappa coefficients of 0.36 for the 5 experiment-level criteria and 0.35 for the 3 replication-level criteria.

By experiment	**All**	**MTT**	**PCR**	**EPM**
Original in replication's 95% PI	**18/40 (45%)**	9/16 (56%)	8/13 (62%)	1/11 (9%)
Replication in original 95% CI	**12/47 (26%)**	4/18 (22%)	5/15 (33%)	3/14 (21%)
Same-sign significance ($p<0.05$)	**9/47 (19%)**	4/18 (22%)	3/15 (20%)	2/14 (14%)
≥50% replications significant	**7/47 (15%)**	2/18 (11%)	3/15 (20%)	2/14 (14%)
≥50% subjectively replicated	**19/47 (40%)**	7/18 (39%)	8/15 (53%)	4/14 (29%)
By replication	**All**	**MTT**	**PCR**	**EPM**
Replication in original 95% CI	**25/97 (26%)**	9/39 (23%)	6/29 (21%)	10/29 (34%)
Same-sign significance ($p<0.05$)	**13/97 (13%)**	6/39 (15%)	5/29 (17%)	2/29 (7%)
Subjectively replicated	**31/97 (32%)**	13/39 (33%)	12/29 (41%)	6/29 (21%)

Table 1 - Replication rates in the primary analysis. Replication rates for the primary analysis using multiple criteria. Effect size comparisons are based on random-effects meta-analyses, while same-sign significance is based on fixed-effects meta-analysis estimates. The 95% prediction interval criterion only uses experiments with more than one replication. All statistical tests use t distributions based on the number of experimental units. PI, prediction interval; CI, confidence interval; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay; PCR, reverse transcription polymerase chain reaction; EPM, elevated plus maze. For more information on replication criteria, see https://osf.io/9rnuj.

A major caveat for the interpretation of these replication rates is the low statistical power of some replications. Although we calculated sample sizes for each individual replication to have 95% power to detect the original standardized difference (with the primary analysis including only those achieving at least 80% power for this purpose), coefficients of variation were on average much higher among replications, particularly for *in vitro* experiments (**Figure S5**). This led statistical power to detect the original relative difference to be lower than planned for most experiments (**Figure S6**),

biasing replication rates based on statistical significance downwards and those based on prediction intervals upwards (as also occurs when there is low agreement among replication results).

Considering only experiments with at least 80% statistical power for the aggregate of replications in an *a posteriori* power analysis using the original relative difference and the variability achieved in replications (n=27) leads to an increase in replication rates based on statistical significance and a decrease in those based on prediction intervals, particularly for MTT and PCR experiments. This leads to convergence of replication rates, with 31% of original estimates in the replications' 95% prediction interval, 26% of replication estimates in the original 95% confidence interval and 30% of experiments statistically significant in the same direction as the original (**Table S8**).

By experiment	**Primary**	**Lab's choice**	**All Exps**	**≥ 2 copies**	**3 copies**	**≥80% power**
Original in replication's 95% PI	**18/40 (45%)**	23/43 (53%)	27/53 (51%)	18/39 (46%)	7/12 (58%)	8/26 (31%)
Replication in original 95% CI	**12/47 (26%)**	15/56 (27%)	14/56 (25%)	11/39 (28%)	5/12 (42%)	7/27 (26%)
Same-sign significance (p<0.05)	**9/47 (19%)**	11/56 (20%)	11/56 (20%)	9/39 (23%)	4/12 (33%)	8/27 (30%)
≥50% replications significant	**7/47 (15%)**	8/56 (14%)	7/56 (12%)	7/39 (18%)	1/12 (8%)	5/27 (19%)
≥50% subjectively replicated	**19/47 (40%)**	20/56 (36%)	20/56 (36%)	15/39 (38%)	5/12 (42%)	11/27 (41%)
By replication	**Primary**	**Lab's choice**	**All Exps**	**≥ 2 copies**	**3 copies**	**≥80% power**
Replication in original 95% CI	**25/97 (26%)**	32/116 (28%)	36/137 (26%)	24/89 (27%)	14/36 (39%)	19/62 (31%)
Same-sign significance (p<0.05)	**13/97 (13%)**	17/116 (15%)	18/137 (13%)	13/89 (15%)	5/36 (14%)	10/62 (16%)
Subjectively replicated	**31/97 (32%)**	35/116 (30%)	41/137 (30%)	27/89 (30%)	14/36 (39%)	20/62 (32%)

Table 2. Replication rates for different analysis sets. Replication rates for the primary and secondary analyses. Effect size comparisons are based on random-effects meta-analysis, while same-sign significance is based on a fixed meta-analysis estimate. The prediction interval criterion only uses experiments with more than one replication. Subsets for secondary analyses include all experiments judged valid by the replicating lab (Lab's Choice), all concluded experiments (All Exps) – both of which use the experimental unit as defined by the lab –, only experiments with at least 2 and 3 replications, and only experiments with ≥ 80% *a posteriori* power. All statistical tests use t distributions based on the number of experimental units. PI, prediction interval; CI, confidence interval. For more information on replication criteria, see https://osf.io/9rnuj.

Table 2 displays replication rates for these and other sets of experiments, including all experiments irrespectively of the validation results (n=56), all experiments considered valid by labs (n=56), and only experiments with at least two (n=39) or three (n=12) replications (broken down by method in **Tables S9-S12**). Disregarding the validation process increases sample size but has little impact on replication rates, whereas analyzing only experiments with multiple replications leads to higher rates by some criteria, but with a much lower sample size. Replacing t by z distributions in meta-analyses (**Tables S13-S14)** decreases replication rates based on confidence intervals, which

become narrower, while slightly increasing statistical significance rates. Basing degrees of freedom for meta-analyses on the Knapp-Hartung approach (**Tables S15-S16**), on the other hand, increases replication rates based on prediction intervals and lowers those based on meta-analytical significance, as estimates incorporate more uncertainty. Analyzing relative expression in a linear scale for PCR experiments also leads to lower rates of statistical significance and wider prediction intervals, as it increases variability within experiments (**Table S17**). A multiverse analysis for all combinations of experimental sets, replication criteria and analysis options is presented as a specification curve in **Figure 2**, which shows a median replication rate of 26% (interquartile range = 17-36%).

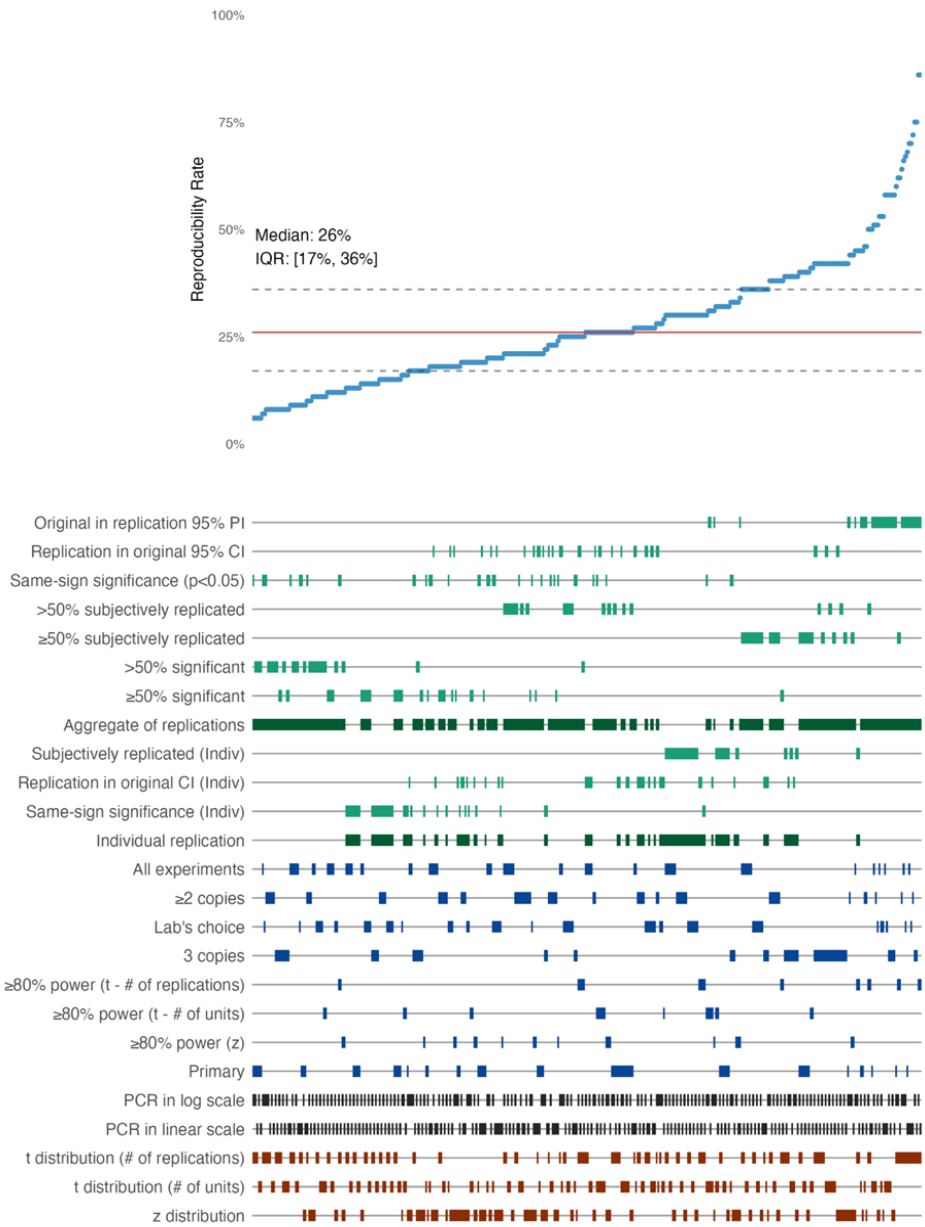

Figure 2. Specification curve analysis of reproducibility rates. Curve shows reproducibility rates arising from the combination of 10 replication criteria (green; 5 criteria for the aggregate of experiments, with 2 different ways to resolve ties for those involving voting, and 3 criteria for individual replications), 8 sets of experiments with different criteria for inclusion (blue), 2 ways of handling PCR data (black), and 3 different distributions for statistical analysis (red). Replication rates range from 6 to 86%, with a median of 26% and an interquartile range (IQR) of 17 to 36%.

Effect size and variability comparison

A comparison between the effect sizes from each original experiment and those of replications in the primary analysis is shown in **Figure 3A**, while the correlation between the aggregate effect size of replications and the original one is shown in **Figure 3B**. The high linear correlation ($r = 0.76$, $p = 4.1 \times 10^{-10}$) is due to one prominent outlier with very large effect sizes in both the original and replication; removal of this experiment leaves a weak correlation with $r = 0.2$ ($p = 0.19$; **Figure 3C**). Using a non-parametric approach for the whole sample also leads to a lower correlation coefficient (Spearman's $\rho = 0.34$, $p = 0.02$).

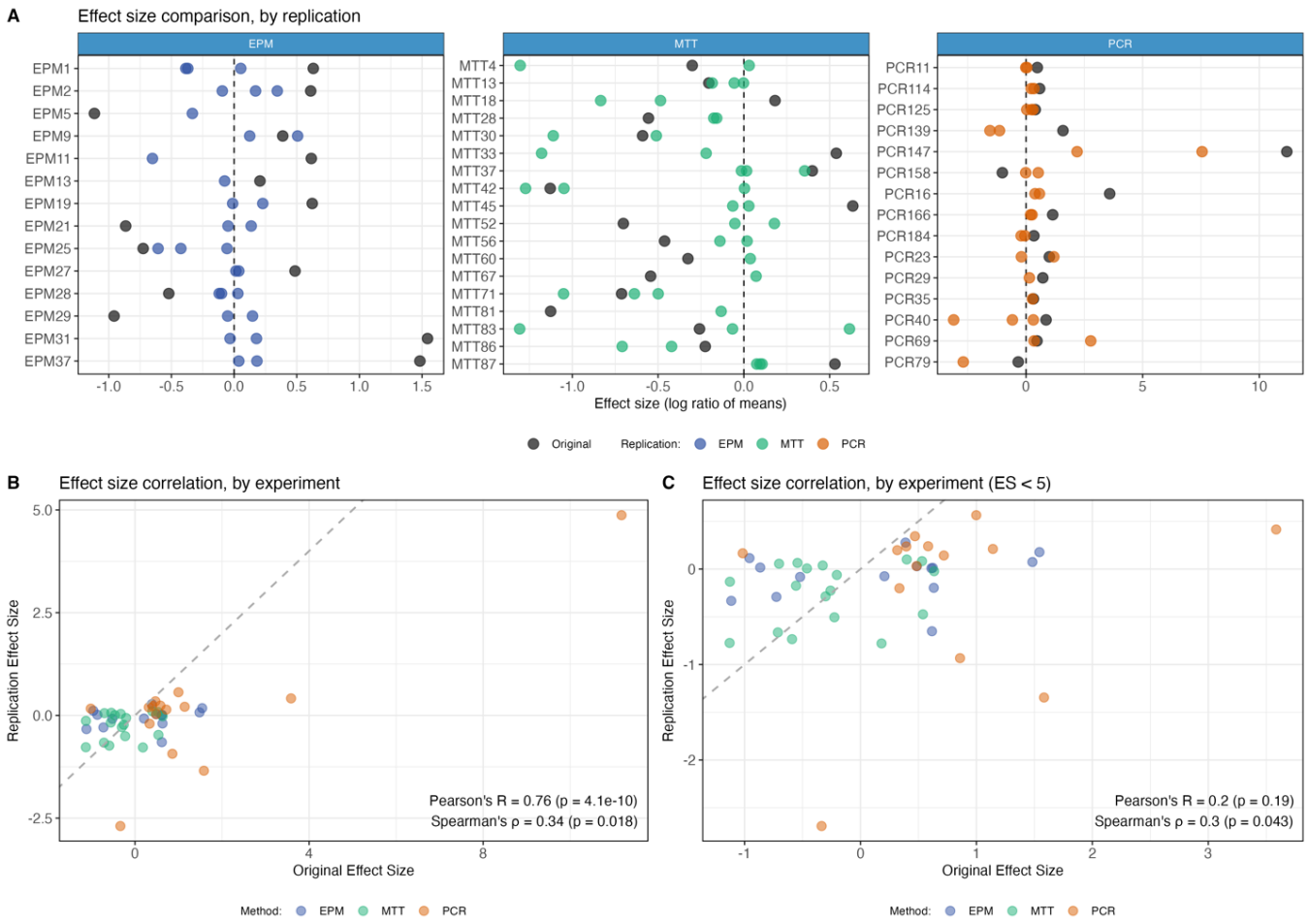

Figure 3. Effect size comparisons and correlations. (A) Effect size comparison between original experiments (dark) and individual replications (light) for EPM (blue), MTT (green) and PCR (orange) experiments. X axes represent effect sizes as the natural logarithm of the ratio between the means of the experimental and control groups in primary behavioral outcomes (EPM), optical density (MTT) or relative gene expression (PCR), with 0 indicating no difference between groups. **(B)** Correlation between the effect sizes of original experiments (x axis) and the aggregate result of their respective replications (y axis), again expressed as log ratios of means ($r = 0.76$, $p = 4.1 \times 10^{-10}$; $\rho = 0.34$, $p = 0.02$). Colors are the same as in A. **(C)** The same analysis excluding the prominent outlier in PCR147, which yields a much weaker linear correlation ($r = 0.2$, $p = 0.19$; $\rho = 0.3$, $p = 0.04$). All results use the primary analysis set and t distributions based on the numbers of experimental units.

Forty-four out of 47 effect sizes (94%) were larger in the original than in the replication aggregate, with a median ratio between the original and replication relative effect sizes (both expressed as ratios of means) of 1.6 (1.45 for MTT, 1.71 for PCR and 2.01 for EPM) (**Table 3**). While this may reflect exaggeration of original effect sizes or publication bias (Ioannidis, 2008), some degree of inflation is expected even in its absence, as our selection process filtered for original experiments with significant differences. Coefficients of variation were lower in the original than in the average of replications in 39 out of 47 experiments (83%), with a median ratio of 0.4 (Wilcoxon's signed rank test, $p = 1.1x10^{-8}$). This difference was more marked in MTT and PCR experiments (ratios of 0.23 and 0.24, $p = 3.2x10^{-4}$ and $1.2x10^{-4}$, respectively) than in EPM ones (ratio of 0.75, p = 0.06). Sign errors (i.e. significant effects in the opposite direction of the original) were infrequent, occurring in 2 out of 47 experiments (4%) and accounting for 18% of significant replication results.

By experiment	**All** (n=47)	**MTT** (n=18)	**PCR** (n=15)	**EPM** (n=14)
Original effect size (ratio of means)	**1.8** (1.2 - $7x10^5$)	1.71 (1.2 – 3.1)	2.05 (1.4 - $7x10^5$)	1.87 (1.2 - 4.7)
Replication effect size (ratio of means)	**1.1** (0.3 - 130.6)	1.1 (0.5 - 2.2)	1.23 (0.3 - 130.6)	1.02 (0.5 - 1.4)
Effect size ratio (original/replication)	**1.6** (0.1 - 548.3)	1.45 (0.8 - 2.8)	1.71 (0.1 - 548.3)	2.01 (1.1 - 4.1)
Coefficient of variation (original)	**0.21** (0.01 - 1)	0.08 (0.03 - 0.2)	0.22 (0.01 - 0.5)	0.47 (0.1 - 1)
Coefficient of variation (replication)	**0.6** (0.1 - 2.8)	0.33 (0.1 - 1.1)	0.66 (0.4 - 2.8)	0.67 (0.2 - 1.4)
Coefficient of variation ratio (original/replication)	**0.4** (0.02 - 2.5)	0.23 (0.05 - 2.4)	0.24 (0.02 – 1.4)	0.75 (0.3 - 2.5)
Mean effect size difference (original vs. replications)	**1.87** (1.1 - 550.7)	1.56 (1.1 - 3.4)	2.5 (1.2 - 550.7)	1.67 (1.2 - 4.4)
Mean effect size difference (between replications)	**1.27** (1 - 212.4)	1.27 (1 - 3.8)	1.5 (1.1 - 212.4)	1.23 (1 - 1.5)
Ratio of mean ES differences (orig-rep/rep-rep)	**1.34** (0.3 - 18.5)	1.16 (0.5 - 1.9)	1.36 (0.3 - 18.5)	1.55 (0.8 - 3.5)
Sign error (% of total)	**2/47** (4%)	1/18 (6%)	1/15 (7%)	0/14 (0%)
Opposite sign (total)	**16/47** (34%)	7/18 (39%)	4/15 (27%)	5/14 (36%)

Table 3. Comparisons between results of replications and original studies in the primary analysis. Continuous variables are shown as median (range), while categorical ones are shown as proportion (percentage). Original effect sizes represent the ratio between the higher and lower mean of the two groups in the original study and are thus always above 1. Replication results respect the same order as the original: thus, effects above 1 are in the same direction, and those below 1 are in the opposite direction. Ratios between original and replication effect sizes are thus ratios between ratios and are calculated by exponentiating differences between log ratios. Coefficients of variation are calculated as the pooled mean of both groups divided by the pooled standard deviation; for PCR experiments, this is done for relative expression values in linear scale. Mean effect size differences are obtained from absolute differences between effect sizes in log ratio of means, in order to measure discrepancies between the original experiment and its replications or between individual replications. These are also exponentiated and thus represent the mean ratio between the higher and lower value. Sign errors refer to effects in the opposite direction of the original with $p < 0.05$, while opposite sign (total) includes all differences in the opposite direction (irrespective of significance). All results use the primary analysis set and t distributions based on the number of experimental units. ES, effect size; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay; PCR, reverse transcription polymerase chain reaction; EPM, elevated plus maze.

Our multicenter design also allowed us to compare the variation between individual replications to that between replications and original studies. Relative differences (expressed as ratios between the higher and lower mean) between original effects and individual replications were larger than those between replications of the same experiment, with a median ratio of 1.34 (Wilcoxon signed-rank test between pairs, $p = 0.006$). Differences were larger and more consistent among EPM studies (median ratio = 1.55, $p = 0.007$) than among PCR (ratio = 1.36, $p = 0.22$) or MTT experiments (ratio = 1.16, $p = 0.35$) (**Table 3**). This suggests that part of the irreplicability observed in our study is due to factors specific to published experiments that were not present in replications; nevertheless, a sizable amount of variation was still observed among replications, as can be seen in **Figure 3A**.

Predictors of replication success

We analyzed whether multiple factors at the level of the original experiment and replication were correlated with replication success, as measured by the strength of evidence for an effect in the same direction (the t value for the replication or aggregate of replications) and by effect size exaggeration (the difference between the effect sizes of the original experiment and replication, both expressed as log ratios of means). A summary of these correlations in the primary analysis is shown as a heat map in **Figure 4**, while individual scatter plots are available at https://osf.io/fdpbe.

At the original experiment level (**Figure 4A)**, replication success as measured by t value was weakly correlated with researcher predictions about the probability of replication success ($\rho = 0.31$, $p = 0.04$) (see **Figure S7** for survey results). Predictions correlated more strongly with dichotomous same-sign significance ($\rho = 0.43$, $p = 0.003$), which was the outcome participants were directly asked to predict. Meanwhile, less effect size exaggeration was correlated with lower coefficients of variation in the original experiment ($\rho = -0.44$, $p = 0.002$) – an expected result when original results are filtered for significance –, MTT experiments ($\rho = 0.31$, $p = 0.03$), and cell line experiments ($\rho = 0.27$, $p = 0.06$), although these variables are heavily correlated among themselves, making confounding likely (see **Figure S8** for correlations among predictors). Less exaggeration was also correlated with better

reporting of the experiment's methods (ρ = 0.25, p = 0.09) and, surprisingly, with lower use of bias control measures in EPM experiments (ρ = -0.6, p = 0.02), although the latter analysis had a very low sample size (n=14). Most of these correlations are weak and, due to the multiplicity of tested predictors, could plausibly have arisen by chance.

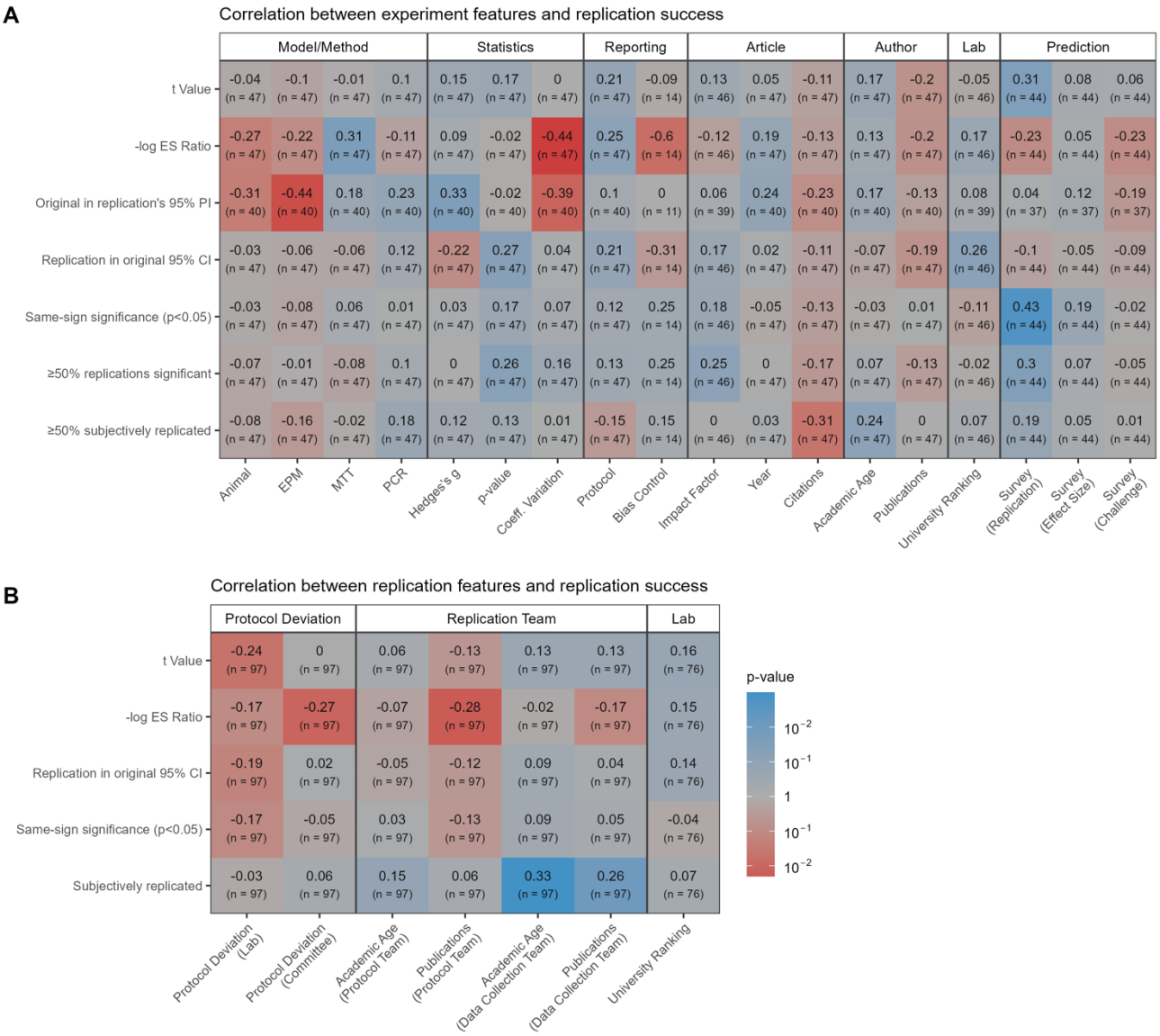

Figure 4. Predictors of replication success. (A) Predictors of replication success at the level of original experiments (x axis) in the primary analysis. Categories include experimental model and method (animal vs. cell, EPM/MTT/PCR vs. others), statistics (standardized effect size, p value and coefficient of variation), reporting (protocol reporting score, bias control measures), article features (journal impact factor, year and citations in the first 2 years), author features (years since first publication and number of publications at the time of original article publication), lab features (university ranking) and researcher predictions (about replication probability, expected effect size and difficulty). For details on each predictor, see https://osf.io/9rnuj. Y axis represents different continuous (aggregate replication t value, effect size differences in log scale) and dichotomous outcomes (same as in Table 1); effect size differences are expressed as replication minus original so that blue indicates correlations with less exaggeration/higher replicability and red with more exaggeration/lower replicability. Numbers show univariate correlations as Spearman's ρ, while color intensity represents p values. **(B)** Predictors at the level of replications, including degree of protocol deviation (judged by the lab and validation committee), features of the replication team (mean number of years since first publication and number of publications by the protocol and data collection teams) and of the lab (university ranking). Other conventions are the same as in A, but outcomes and sample size refer to individual replications. Scatter plots for individual correlations are available at https://osf.io/fdpbe.

At the replication level (**Figure 4B**), t values were negatively correlated with the degree of change between the original article and replication, as assessed by the replicating lab ($\rho = -0.24$, $p = 0.02$); however, as this score was attributed after seeing the results, unsuccessful replications could have led researchers to attribute greater value to protocol changes. Smaller ratios between original and replication effect sizes were also negatively correlated with this score ($\rho = -0.17$, $p = 0.1$), as well as with that attributed by the validation committee, which was blind to the replication results ($\rho = -0.27$, $p = 0.008$). Curiously, replicating teams with larger numbers of published articles found greater decreases in effect size between the original study and replication ($\rho = -0.28$, $p = 0.005$ for protocol-developing teams, $\rho = -0.17$, $p = 0.09$ for data collection teams). More experienced data collection teams were also more likely to consider the replication successful in their subjective assessments ($\rho = 0.33$, $p = 0.001$ for years since first publication and $\rho = 0.26$, $p = 0.009$ for number of publications). Once more, the multiplicity of tested predictors and the non-independence between replications with overlapping teams make these correlations tentative at best.

Self-assessment by labs and coordinating team

Protocol deviations occurred frequently in our sample and were registered by the coordinating team in 91% of replications (76% for MTT, 98% for PCR and 100% for EPM). On a scale of 1 to 5 (with 1 being no deviation and 5 being a deviation that invalidates the experiment as a direct replication), replications in the overall sample had a mean (± SD) rating of 1.9 ± 1 when evaluated by the lab and 2.4 ± 1 when rated by the validation committee. Among experiments included in the primary analysis, these ratings were 1.7 ± 0.8 and 2.1 ± 0.8, respectively. When analyzing all experiments (see **Figure S7**), deviation scores by the committee were higher for EPM experiments ($\rho = 0.45$, $p = 3.4 \times 10^{-8}$), older experiments ($\rho = -0.35$ vs. year of publication, $p = 2.2 \times 10^{-5}$) and those judged more technically challenging in the prediction survey ($\rho = 0.33$, $p = 9.5 \times 10^{-5}$).

Reasons for protocol deviations provided by labs are presented in **Figure 5**, with illustrative examples presented in **Table S18**. Most deviations were due to issues intrinsic to the experiment, such as the experimental model behaving differently than expected. Reasons related to the infrastructure of the lab and animal facility were also frequent, as were logistical problems involving suppliers or regulatory requirements. A smaller fraction of deviations was due to deliberate choices or errors by the lab performing the replication. An assessment of general difficulties by participating researchers (**Figure S9** and **Table S19**) placed COVID-19 restrictions, delayed delivery of reagents, and difficulties with the experimental model as the top challenges faced by labs.

The coordinating team also assessed the obstacles faced by the project, with top-ranked difficulties shown in **Table S20** and a complete list available at https://osf.io/q76vj. Lack of common terminologies for describing aspects of experiment design, problems with reagent delivery, poor

methodological descriptions in the original articles, and experimental models not working as expected were considered the most relevant difficulties. These were grouped into five categories encompassing the key challenges faced by the project (**Table 4**). These challenges, along with associated opportunities for improvement, will be discussed more extensively in future publications.

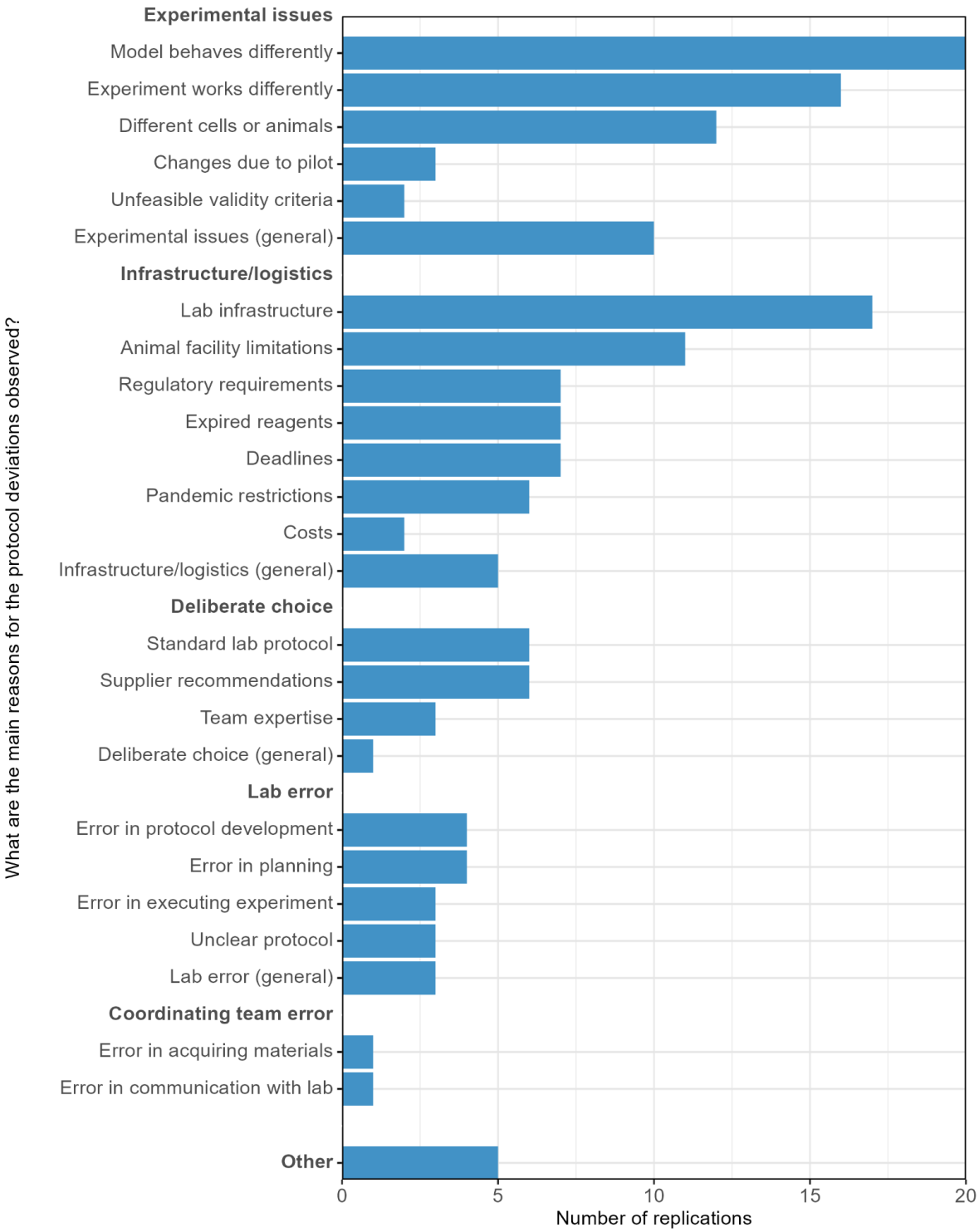

Figure 5. Reasons for protocol deviations. Reasons were provided as open answers by the lab and categorized by the coordinating team as described at https://osf.io/5gjb7. They are divided into general categories (bold) and subcategories. Examples for each category are shown in **Table S18**.

Category	Examples
Lack of standardized terminologies for experimental design and reporting	Unstandardized descriptions of methods and results in original articles. Discrepancies in terminology used to describe issues such as experimental units and controls by replicating labs.
Tension between replicating protocol exactly and getting methods to work	Lack of consensus between essential and adaptable steps in protocols. Experimental models behaving differently across labs. Difficulties in preregistration in the absence of pilot experiments. Difficulties in establishing criteria for methodological validity.
Multicenter project management	Communication difficulties between the coordinating team and participating labs. Lack of formal training in project management by the coordinating team. Participant labs not used to external supervision or standardization of procedures. Heterogeneous data management by labs.
Logistics and infrastructure	Difficulties in obtaining licenses with regulatory agencies. Inefficiency of national suppliers in delivering reagents. Limitations in animal facilities and general laboratory infrastructure. High prevalence of junior scientists in participating teams.
General difficulties for large-scale projects	Difficulties in anticipating loss of labs and experiments during the project. Difficulties in data management and statistical analysis of complex data. Difficulties in maintaining documentation practices throughout the project.

Table 4. Challenges in performing a large-scale multicenter replication of experiments from Brazilian biomedical science. Main categories of challenges faced by the project, based on the coordinating team's assessment of an initial list of difficulties (see **Table S20** and https://osf.io/q76vj).

Discussion

In this large multicenter replication of published biomedical experiments from Brazil, replication rates in our primary analysis varied from 15 to 45%, depending on the criteria used to define replicability. These numbers are in a similar range as previous estimates from the biomedical literature (Begley & Ellis, 2012; Errington, Mathur, et al., 2021; Prinz et al., 2011; Scott et al., 2008; Steward et al., 2012). Among these, our study is unique in trying to assess a representative sample of articles by randomly selecting experiments from a wide range of publications based on methods, rather than filtering by research area, importance or citation counts. It is also the first attempt to evaluate replicability within a specific country, in order to provide data that can be of more direct use for local funders, institutions and researchers. That said, the absence of similar studies elsewhere precludes comparisons with other regions.

When compared to originally positive experiments in the Reproducibility Project: Cancer Biology (RP:CB) (Errington, Mathur, et al., 2021) – perhaps the closest counterpart to our study – replication rates in our primary analysis were lower when measured by statistical significance in the same direction (19% *vs*. 39%) or inclusion of the replication effect size in the original 95% confidence interval (26% *vs*. 43%). Correlations between effect sizes were also lower in our study (Spearman's $\rho = 0.34$ *vs*. 0.47). Multiple factors could account for these differences, including sampling strategy (general literature *vs*. highly cited articles), experimental selection (first significant experiment *vs.* most relevant ones), replication facilities (academic labs *vs*. commercial services), protocol development (with *vs*. without the participation of original authors), subfield (general *vs*. cancer biology) and region of origin (Brazil *vs*. worldwide).

Our study is also unique in employing a multicenter replication strategy, allowing us to assess the extent to which interlaboratory variability may account for discrepancies between the original study and its replications. Differences in effect sizes between the original study and replication were larger than those among replications, suggesting that irreplicability is partly due to factors that are intrinsic to published articles, such as publication bias, selective reporting, low statistical power and incentives for positive results (Smaldino & McElreath, 2016). That said, differences between replications were also large in many cases. As replicating labs were blind to the original result and had no obvious incentives towards particular results, heterogeneity between replications is more likely due to differences in experimental conditions or in how experiments were conducted. These may include protocol adaptations, different levels of adherence to the original protocol, or undetected errors by replicating labs. The finding of significant interlaboratory variability is also consistent with previous attempts to replicate identical protocols across multiple sites for animal (Crabbe et al., 1999) and cell line studies (Elliott et al., 2017; Niepel et al., 2019).

Within the biomedical literature, our study is also unique in assessing predictors of replicability. Although the power of this analysis was limited by the small number of successful replications, MTT and cell-line experiments exhibited smaller decreases in effect sizes between the original and replication than EPM and animal experiments. Statistical significance rates also rose more expressively for MTT and PCR experiments than for EPM ones when only replications with high *a posteriori* power were considered, suggesting that reasons for irreplicability differ between methods. In *in vitro* experiments, non-significant results were partly explained by coefficients of variation being higher in replications than in original studies, leading to low statistical power. In animal experiments, power was usually adequate, and non-significant replication effects were closer to zero, suggesting that original results are more likely to represent false-positive or ungeneralizable results.

Although researcher predictions overestimated replicability in our sample, they had some success in forecasting the evidence strength of replications as measured by their t values; however, prediction accuracy was lower than that reported by previous replication projects in psychology and the social sciences (Gordon et al., 2021). This finding might be expected, given that the replicability of findings in biomedical science is probably less intuitive to the average researcher than that of psychology studies, for which prediction accuracy is above chance even among laypeople (Hoogeveen et al., 2020). In contrast, greater use of measures to control risk of bias in animal experiments, such as randomization and blinding (Landis et al., 2012), was correlated with greater effect size decreases in replications. Although this is likely to be a chance finding, as both sample size for the analysis and plausibility are low, it suggests that researcher predictions depend on tacit knowledge that does not rely on these traditionally used proxies for methodological rigor. Researcher justifications for predictions and analyses of whether prediction markets can improve this accuracy will be explored in further work.

Our analyses have multiple caveats, most of which are due to difficulties faced by participating labs performing replications. Twenty-one percent of planned replications ended up not being performed, either because materials could not be acquired (5%) or because the labs to which they had been assigned left the project or could not meet its deadlines (15%). This is a much smaller fraction than the 74% unfinished replications in RP:CB (Errington, Denis, et al., 2021), likely due to our selection of simpler experiments to replicate. On the other hand, our validation process had a large impact on our sample, excluding around 30% of replications from the primary analysis. Interestingly, this filtering process did not have a large impact on replication rates, as included and excluded replications had similar rates of success. This may be explained by the fact that, while some issues raised during the validation process would be expected to lower replicability (e.g. deviations from the original protocol), others could artificially raise it (e.g. insufficient biological variation among experimental units or biases in randomization).

Statistical power was further limited by the fact that coefficients of variation were around 2.5 times larger in replications than in original studies, with a 4-fold median difference for MTT and PCR. This may either indicate that replicating labs had more difficulty in controlling technical error or that variability was underestimated in the originally published results. Underestimation can plausibly occur due to elimination of outliers (Gress et al., 2018; Holman et al., 2016), incorrect labeling of error bars (Cumming et al., 2007; Vaux, 2004), or insufficient biological variation among experimental units in the original study (Lazic et al., 2018; Vaux et al., 2012). Filtering the sample by achieved power suggests that higher variability in replications biased replication rates based on statistical significance

downwards, at least for *in vitro* studies; nevertheless, even when considering only experiments with high power, replication rates remained between 19 and 41%.

For experiments with high agreement among replications, it is reasonable to assume that the reasons for irreplicability are more likely to lie in the published results. Nevertheless, as we replicated comparisons between individual groups, lack of replication success does not apply to the whole article, or even to the whole experiment from which the comparison was drawn. Due to our choice of selecting the first comparison with a significant difference in an article, it was not infrequent to select an initial experiment or a low treatment dose, meaning that an unsuccessful replication does not necessarily call the main conclusions of a study into question. More importantly still, low agreement among replications and/or a lower number of replications than originally planned limited our ability to judge many of the original experiments.

Our results should thus be thought of as naturalistic estimates of real-life academic labs trying to replicate individual experiments from the published literature. That said, due to our crowdsourced, country-based design, the population of labs that performed replications was largely similar to the one that produced the original results. Thus, whether the original estimate or the replication one is a more accurate description of the phenomenon under study does not change the conclusion that Brazilian researchers have difficulties replicating findings from other research groups within the same community.

In fact, the observation that most labs faced difficulties in following preregistered protocols and controlling experimental variability provided an opportunity to glimpse at possible causes for replication failures. While all we can say about the original results is how closely they were replicated by our labs – which provides little information about the reasons for failed replications – our data collection process allowed an extensive audit of how closely replication protocols were followed by participant labs. This was complemented by a self-assessment process that allowed us to investigate relevant difficulties and opportunities for improvement.

Deviations from preregistered protocols were present in most replications. They occurred most commonly due to issues that were intrinsic to the experiment – such as difficulties with growing cells in the conditions reported in the original publication, or with performing experimental procedures as stated in the protocol. In many cases, labs reported that these issues could not have been anticipated without direct experience with the experimental model. Similar limitations were observed in the RP:CB, in which two thirds of preregistered protocols required modifications after experimental work had begun (Errington, Denis, et al., 2021).

This should be considered when interpreting replication rates, as difficulties in adequately implementing a method or experimental model are likely to decrease the probability of replication

success. Thus, "one-shot" replication projects such as our own, in which extensive efforts are made to define the protocol in advance, are different from "best-shot" attempts at replication which allow for tinkering with the protocol over the course of multiple replication attempts – which are arguably closer to how experiments are typically replicated by academic labs (Guttinger, 2019). While the former are likely to underestimate replicability due to the difficulty in defining methods upfront, the latter can overestimate it due to the active effort in obtaining results that are close to the original.

The challenge of prespecifying methods also poses limits to the applicability of protocol preregistration in laboratory biology – or in any other area in which data collection depends on complex methods. Although we believe that preregistration of experimental methods is worthwhile, it may be more useful when performed after pilot experiments have been carried out, in order to reduce the need for deviations. Preregistering analysis methods poses fewer difficulties when data is relatively simple, as in our experiments; nevertheless, more data-intensive methods can involve steps of data filtering or complex analytical decisions (e.g. Carp, 2012) that may be equally hard to specify in advance.

Our attempts to establish predefined inclusion/exclusion criteria to assess the validity of individual samples or experiments (Neves & Amaral, 2020) were also less successful than we had expected. Labs had difficulties in establishing these criteria in advance, frequently setting very high standards for markers, such as RNA purity, that turned out to be difficult to achieve and led them to break their own criteria. An independent validation process performed after the experiment turned out to be more feasible for assessing methodological validity and may be more compatible with the reality of academic labs. Importantly, this process was blind to the replication results, in order to prevent replication outcomes from contaminating judgments about methodological adequacy.

Other reasons for protocol deviations included infrastructural problems, such as difficulties with the delivery of materials, cells or animals on time, limitations in access to equipment, and lack of personnel to perform replications. The COVID-19 pandemic proved to be a particularly complex challenge, as many labs and institutions were closed for extended periods, and even after normal activity was resumed, changes in replicating teams and lab priorities meant that many experiments ended up performed in non-ideal conditions. Unsurprisingly, this was the most frequently reported difficulty by participants in our project evaluation survey.

All that said, there remains a fraction of protocol deviations that had no particular reason, in which labs simply made a different methodological choice from the preregistered one. This suggests that the idea of preregistration as a commitment to a particular methodology is still poorly understood by biomedical researchers. Most of them seem to consider protocols as general guidelines, assuming – perhaps correctly – that many steps will change after the experiment begins, and sometimes preferring vague descriptions over precise specifications to allow flexibility in the process. In some

cases, this also led to a lack of clarity when developing protocols, leading to misunderstandings when experiments were performed by a different researcher.

These communication issues were particularly evident when determining the appropriate experimental unit in cell culture experiments. Although this was stated in advance by the coordinating team in the protocols as different passages of a cultured cell line, or as primary cultures from independent pools of animals, many labs opted for other ways to define biological replicates. Not all adaptations were accepted, leading many replications to be excluded from our primary analysis. Moreover, merely understanding what experimental unit was used by a particular lab could be challenging, due to the lack of standardized terminology to define this among researchers (Lazic et al., 2018; Vaux et al., 2012). To a lesser extent, this also applied to other aspects of experimental design, such as positive and negative controls, blanks and randomization, which were described using distinct terminology by individual labs. This suggests that the adoption of consensus taxonomies that systematically and unambiguously describe these issues could potentially improve reproducibility.

A final lesson learned from this project is that organizing large-scale initiatives in laboratory biology is hampered by the lack of experience of academic labs with working in coordinated fashion. While collaboration in biomedical research is frequent, the culture of repeating the same experiment across labs and standardizing procedures is confined to specific research fields (Brancato et al., 2024) or projects (Lyden et al., 2022). As a striking example of this limitation, a recent systematic review of the biomedical literature found only sixteen examples of multicenter animal studies (Hunniford et al., 2023). Consequently, managing large-scale projects is a skill for which researchers receive little training; moreover, being externally managed or supervised is also an unusual experience for academic labs, which is sometimes met with resistance (Maysami et al., 2016). If large-scale, crowdsourced research is to flourish in lab biology, cultural changes are important to capacitate the academic workforce for such collaborative endeavors (Amaral & Neves, 2021; Coles et al., 2022; Uhlmann et al., 2019). Possible solutions in this direction will be elaborated in a future article dedicated to these issues.

As a final reflection, our project leaves contributions beyond the results reported here. Our quantitative data describing replications are fully shared and open to scrutiny. Similarly, the majority of our textual data – including protocols, final notes, justifications for researcher predictions and other documents – remain to be analyzed in more depth. The collaborative nature of the project has constituted an important learning experience for the many researchers involved, including many early-career scholars, and the visibility we received (e.g. de Oliveira Andrade, 2019) has cast a spotlight on replicability and reproducibility in academic debates across the country. Finally, we leave spinoffs such as the Brazilian Reproducibility Initiative in Preclinical Systematic Review and Meta-Analysis

(BRISA) (http://en.reprodutibilidade.bio.br/brisa), an ongoing collaboration to train biomedical researchers in systematic review methods, and the Brazilian Reproducibility Network (http://en.reprodutibilidade.org), a multidisciplinary organization to promote reproducible research practices across the country. We expect this legacy to help place reproducibility at the forefront of Brazil's national research agenda.

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