“Rigor and reproducibility in data reporting”

General Issues of Reliability and Reproducibility

- Science advances through the publication of novel results, followed by efforts to reproduce them. Such replication of experimental findings distinguishes science from other forms of intellectual inquiry.

- Many factors can affect the outcomes of experiments. Variability of reagents, methods, and resources is difficult to avoid—particularly in biology—and can have an effect on experimental outcomes.

- Recent well-publicized allegations of the inability to reproduce published biomedical research have raised questions within the research community and among public stakeholders.

(adapted from FASEB policy)

General Issues of Reliability and Reproducibility

- **Replicability:** the ability to duplicate (i.e., repeat) a prior result using the same source materials and methodologies. This term should only be used when referring to repeating the results of a specific experiment rather than an entire study.

- **Reproducibility:** the ability to achieve similar or nearly identical results using comparable materials and methodologies. This term may be used when specific findings from a study are obtained by an independent group of researchers.

- **Transparency:** the reporting of experimental materials and methods in a manner that provides enough information for others to independently assess and/or reproduce experimental findings.

(adapted from FASEB policy)

Why Improve Transparency?

- Irreproducibility of pre-clinical studies
- Negative impact on political and public will to fund research……

Reason for irreproducibility

- Poor methodological descriptions
- Reagents not described adequately and/or validated – antibodies, siRNAs, small molecules
- Inappropriate data interpretation

http://www.nih.gov/research-training/rigor-reproducibility

Efforts Toward a Solution.....

Improve transparency of JBC papers by:

1. **Improving description of methods, including reagents**
2. **Clearly defining reproducibility (technical and biological replicates)**

1. Biological Materials

Instructions to Authors:

Descriptions of biological materials should include enough information to uniquely identify materials, such as:

- **Repository accession numbers when available**
- **Antibodies - source, dilutions, and validation criteria**
- **Cell lines - source, authentication, derivation, and contamination (such as mycoplasma) status**
- **Animals - source, species, strain, sex, age, husbandry**
- **Transgenic animals – genetic background**

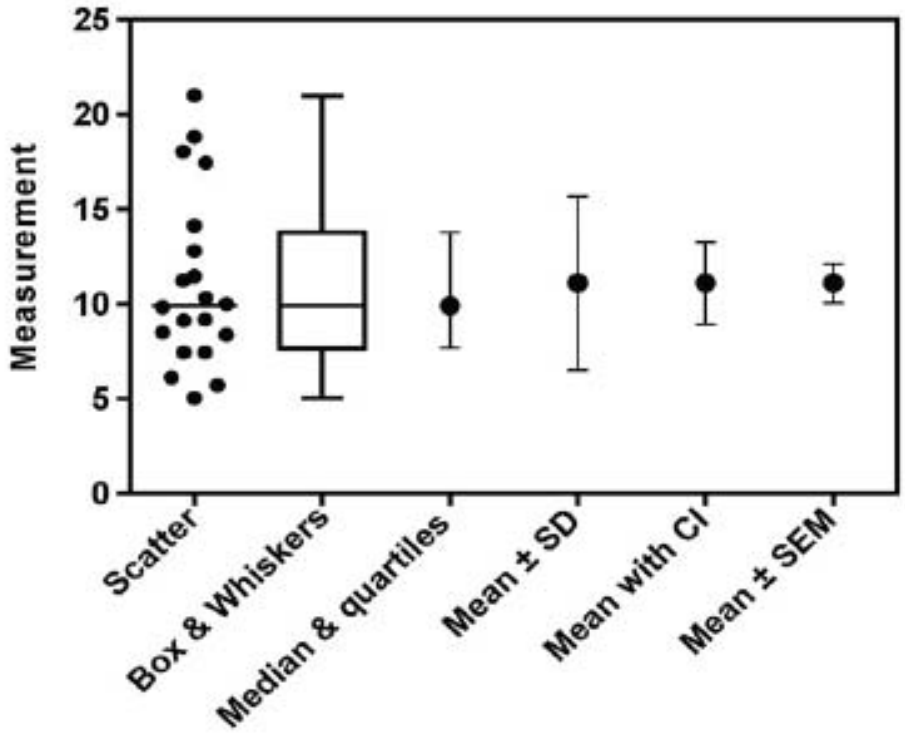
2. Experimental uncertainty, statistics

JBC Instructions to Authors:

- Authors must include information on uncertainty and reproducibility of data in figure legends.
- Authors must state numbers of independent samples (biological replicates) and replicate samples (technical replicates) analyzed and report how many times each experiment was repeated.
- Variation/precision should be reported by standard deviation (SD) (preferable), confidence intervals (CI) or standard error of the mean (SEM).

2. Experimental uncertainty, statistics

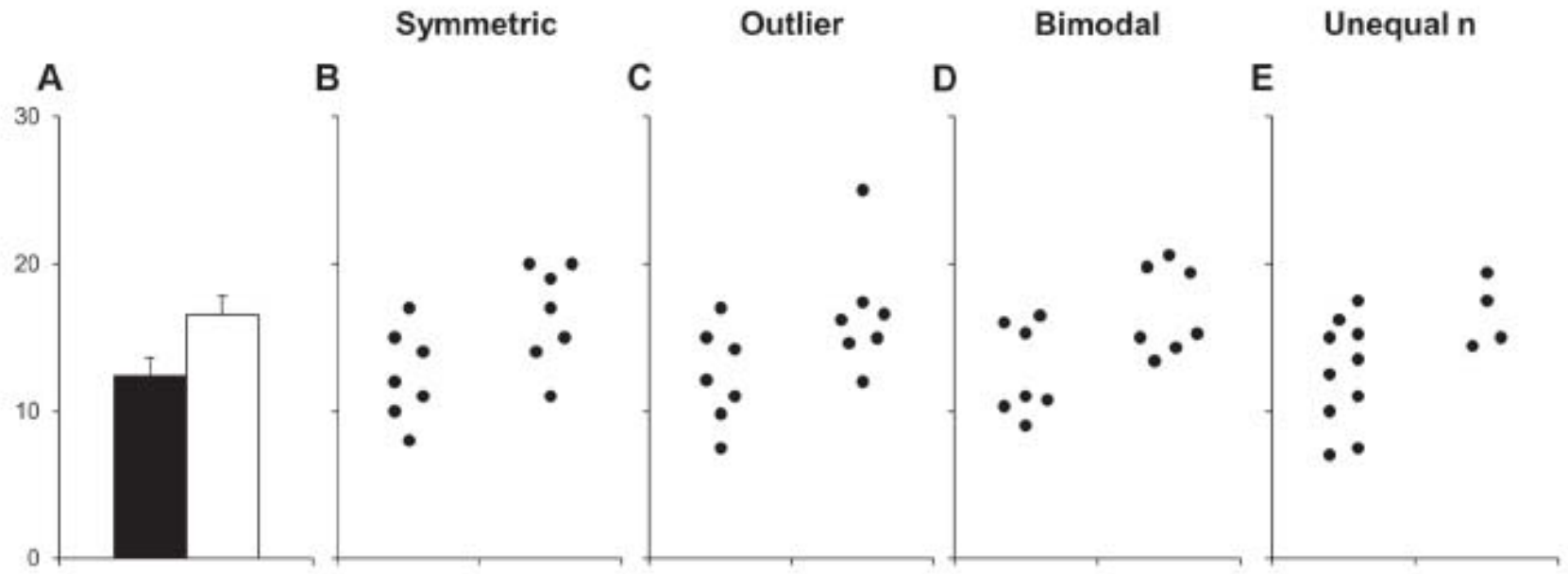
Same data analyzed and plotted differently:



Motulsky, 2014. J. Pharmacol. Exp. Ther. 351:200

3. Graphical presentation of data

Mean ± SEM bar graphs can be misleading



Weissgerber et al., 2015. PLOS Biology 13: e1002128

3. Graphical presentation of data

Instructions to authors:

- **Scatter plots strongly recommended for small data sets (can overlay a mean ± SEM on top of the individual data points); box and whisker plots for large data sets.**

2. Experimental uncertainty, statistics

Instructions to Authors:

Additional issues for animal studies

- State whether or not animals and/or samples analyzed were randomized, and indicate how.
- State whether studies were blinded or not. If so, indicate the method of blinding.
- State whether any data were excluded. If so, indicate the reason and/or criteria for exclusion.

4. Western blots

Instructions to authors:

- Define species of origin and source of all antibodies used, including catalog/lot numbers.
- Describe how novel antibodies were generated, including preparation/purification of epitope/antigen.
- Describe data supporting antibody specificity, including post-translational modifications or neoepitopes.
- If possible, demonstrate loss of immunoreactivity after genetic or other molecular modification to the antigen.

4. Western blots

Instructions (semi-quantitative analyses):

- Explain how data were obtained, whether signal intensity was linear with antigen loading, and how protein loading was normalized. Some detection methods (e.g., ECL) have a very limited linear range.
- Strongly prefer normalizing signal intensity to total protein loading (assessed by staining membranes for total protein). "House-keeping" proteins should not be used for normalization without evidence that manipulations do not affect expression.
- Phospho-(or other PTM-) specific antibody signals should be normalized to total levels of target protein.

Image Issues

- EMBO Press and Rockefeller University Press routinely screen accepted manuscripts for image issues
- ~20-25% of manuscripts have issues with the images, ~1% amount to serious manipulation and acceptance is revoked
- Questions regarding image validity can lead to delays in publication, corrections to published articles, requests to withdraw manuscripts, and/or retractions of articles

Guidelines for Best Practices in Image Processing

- Images should be treated as data
- Images should be minimally processed
- If an image must be processed, the original unmanipulated image must be retained
- Acquisition settings on instruments can compromise image data from the beginning- over-saturating or over-contrasting an image during acquisition results in loss of data that cannot be recovered

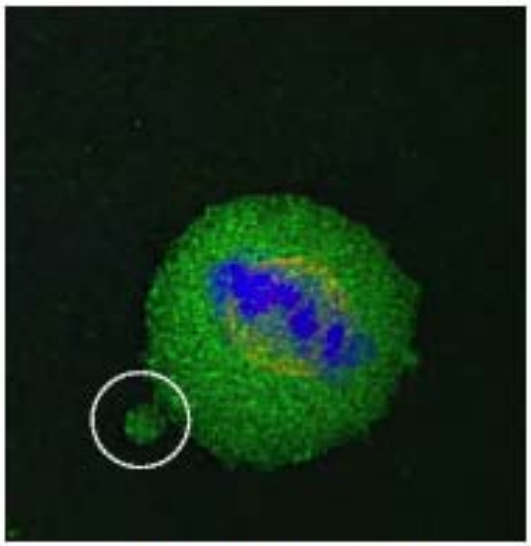
Types of Manipulation

Image manipulation falls into three different categories, with different criteria for what constitutes as inappropriate

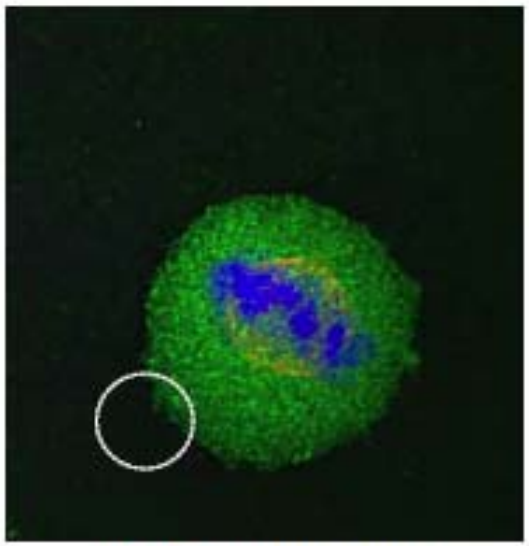
- Editing
- Grouping
- Adjustment

Inappropriate Editing

- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced



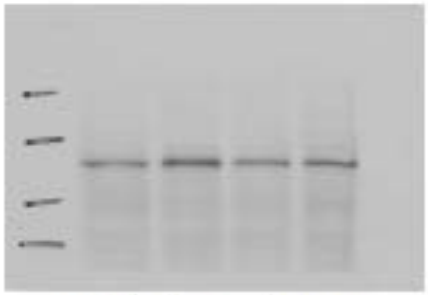
Original data



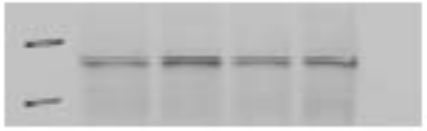
NOT acceptable
(feature removed)

Cropping

- Immunoblots should be cropped in a way that retains information about antigen size and antibody specificity
- Cropped images should retain sufficient area around the bands of interest, ideally including the positions of at least one molecular weight marker above and below the band



Original data

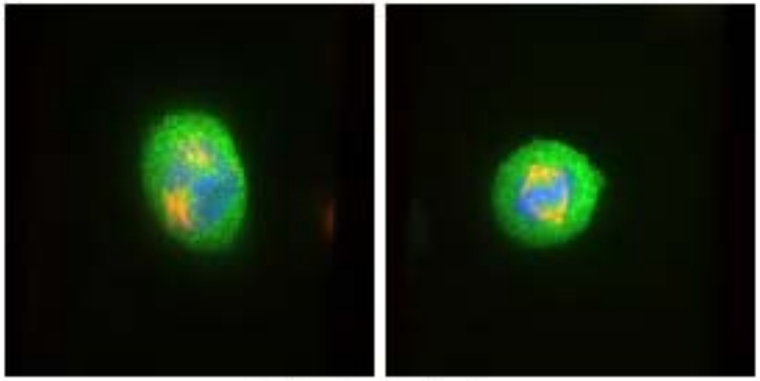


Acceptable

NOT acceptable

Improper Grouping

- Groupings of images from different exposures must be made explicit by the arrangement of the figure and in the text of the figure legend



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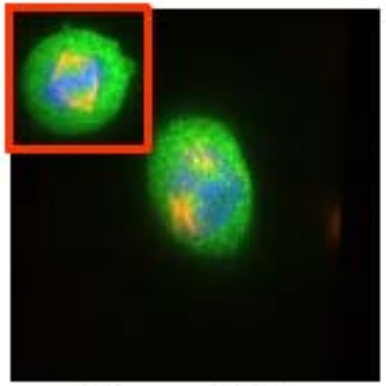
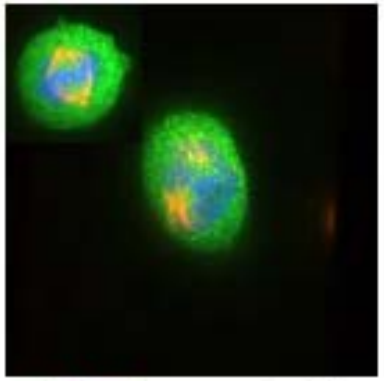


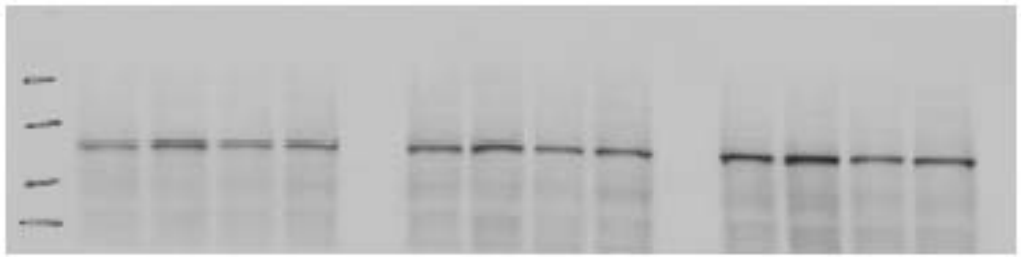
Acceptable
(with designation)



NOT acceptable

Splicing

- Splicing should be used ONLY if it is essential to remove lanes from the original blot
- Splice positions must be clearly indicated and explained in the figure legend



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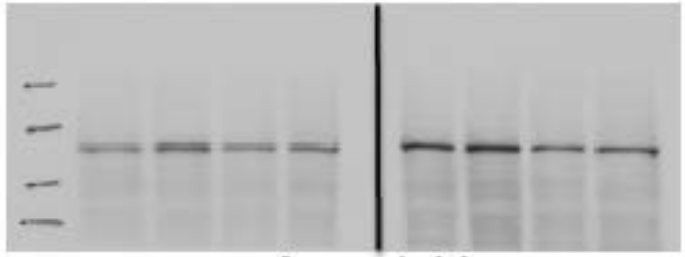
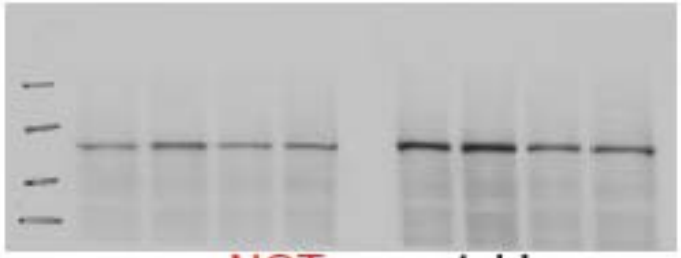


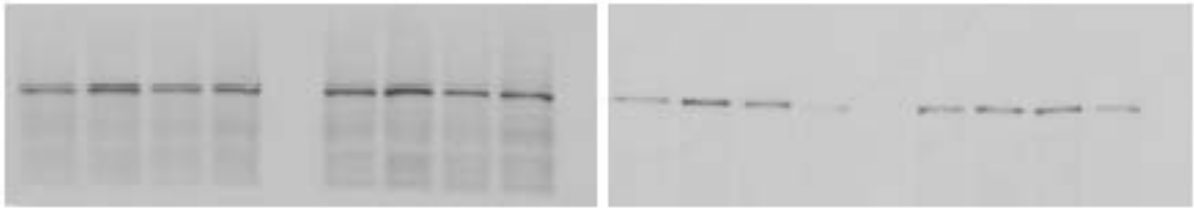
Acceptable



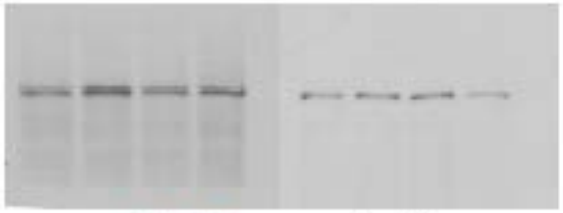
NOT acceptable

Splicing

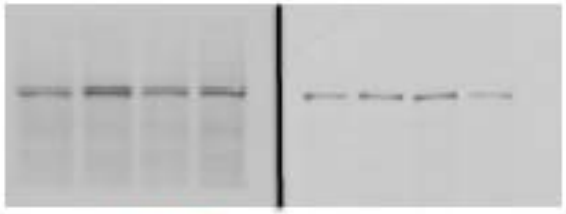
- Splicing should NEVER be used between different blots



Original data



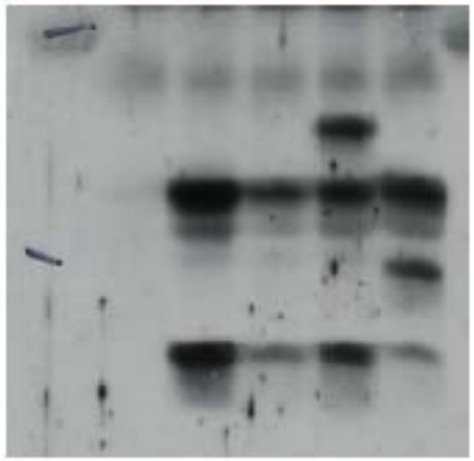
NOT acceptable



NOT acceptable

Inappropriate Adjustment

- Adjustments of brightness, contrast, or color balance are acceptable if they are applied to every pixel in the image and as long as they do not obscure, eliminate, or misrepresent any information present in the original, including the background.



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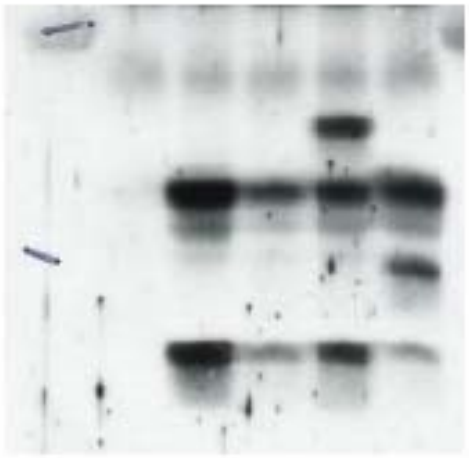
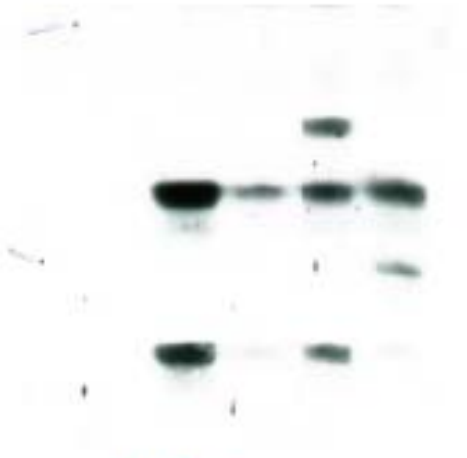


Acceptable

Increasing contrast



NOT acceptable
(too much contrast)

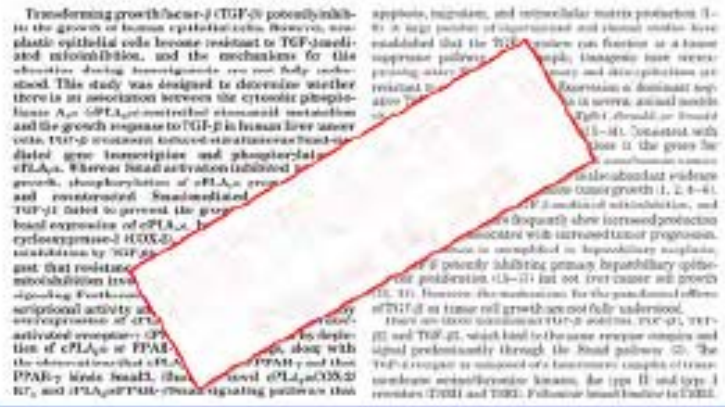
Other Issues

- Re-use of images that represent the same experimental conditions in more than one panel or figure is highly discouraged and must be disclosed and well-justified

- These examples are not limited to immunoblot and microscopy images- they are applied equally to any graphics/data shown in a paper

Consequences

- Any allegation of inappropriate image manipulation will be investigated
- Manuscripts containing violations may be rejected without scientific review
- Articles containing violations may be retracted after publication
- Institutional officials may be notified of articles with manipulated images
- http://www.jbc.org/site/misc/edpolicy.xhtml#ethics



jbc the journal of biological chemistry

ASBMB American Society for Biochemistry and Molecular Biology

Public Perception

Retraction Watch

Tracking retractions as a

The Retraction Watch Leaderboard

with 18 comments

Who has the most retractions? Here's our unofficial list (see notes on methodology), which we'll update as more information comes to light:

1. Yoshitaka Fujii (total retractions: 183) Sources: Final report of investigating committee, our reporting
2. Joachim Boldt (94) Sources: Editors in chief statement, additional coverage
3. Peter Chen (60) Source: SAGE
4. Diederik Stapel (58) Source: Our cataloging
5. Adrian Maxim (48) Source: IEEE database
6. Hua Zhong (41) Source: Journal
7. Shigeaki Kato (38) Source: Our cataloging
8. Hendrik Schön (36) Sources: PubMed and Thomson Scientific
9. Hyung-In Moon (35) Source: Our cataloging
10. James Hunton (33.5, counting partial retraction as half) Source: Our cataloging

www.retractionwatch.com

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Search Publications

www.pubpeer.com

jbc the journal of biological chemistry

ASBMB American Society for Biochemistry and Molecular Biology

JBC Policies

- Policy on image manipulation:

http://www.jbc.org/site/misc/ifora.xhtml#manipulation

- Policy on preparing figures with immunoblots:

http://www.jbc.org/site/misc/ifora.xhtml#blots

- Policy on microscopic imaging data:

http://www.jbc.org/site/misc/ifora.xhtml#micro

Resources

ORI: ori.hhs.gov

Online Learning Tool for Research Integrity and Image Processing: ori.hhs.gov/education/products/RIandImages/default.html

JCB editorial: "What's in a picture? The temptation of image manipulation" J. Cell Biol. 2004 Jul 5;166(1):11-5.

jbc

AVOIDING ETHICAL VIOLATIONS:

a primer for Journal of Biological Chemistry authors

	Issue	How to avoid
Plagiarism	Using another person's text, figures, graphs, or data without attribution	Be sure to cite work from other sources.
Self-plagiarism	Re-using cata from your own previously published paper	Ensure that all data are new and original for each paper, including control experiments.
Falsification of data	Manipulating data so that the published figure does not exactly match the original	Ensure that no specific feature is enhanced, obscured, moved, removed, or introduced. Groupings of images must be disclosed. Images must be uniformly adjusted and must not obscure, eliminate, or misrepresent any information present in the original image. Keep all original data.

Fabrication of data	Making up data to improve the experimental results	Make sure that the results are accurately represented.
Conflicts of interest	Disclosing any actual or perceived conflict that could affect scientific jucgment	Make certain that any affiliations, financial relationships, personal relationships, or funding sources that could be perceived as influencing an author's objectivity are disclosed.
Animal and preclinical research studies	Reporting research using animals in a transparent manner	Ensure that all research has been reviewed and approved by an Institutional Animal Care and Use Committee. Consult the ARRIVE guidelines for reporting animal research.

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Authorship issues	Adding, deleting, or changing the order of the authors	Agree on authorship before writing the manuscript. Authorship is based on substantial contributions.
Duplicate submission	Submitting a manuscript or substantial parts of a manuscript to more than one journal	Withdraw your paper or wait until it's declined before submitting to another journal.

CONSEQUENCES: Violations of these policies may result in delay of publication, retraction of the article, or notification of your institution (http://www.jbc.org/site/misc/edpolicy.xhtml)