Ultrastructural changes in cardiac and skeletal myoblasts following in vitro exposure to monensin, salinomycin, and lasalocid

3.3 / 5
AVERAGE RATING

Journal Article · Biology, Medicine
Reviewed August 3, 2026

QUALITY RATINGS

Technical Soundness 3/5
Core EM methods are standard and sound, but lack of quantitative analysis and some over-interpretation weaken technical robustness.

Novelty 3/5
Application of SEM to ionophore-exposed myoblasts and detailed ultrastructural description provide moderate incremental novelty.

Organization & Clarity 4/5
IMRaD structure is followed and narrative is generally coherent, though aims and quantitative aspects could be more explicit.

Writing Quality 4/5
Prose is mostly clear and professional, with occasional jargon/acronym issues and minor abstract formatting artifacts.

Statistics & Significance 2/5
Minimal statistical reporting confined to supplements; no quantitative analysis of EM findings and limited description of EC50 estimation.

Reproducibility 3/5
Protocols for culture and EM prep are reasonably detailed, but imaging sampling, cell passage, and data specifics are insufficiently reported.

Ethics/Privacy Compliance 4/5
Ethics approval is stated; work uses established cell lines with no apparent privacy concerns, though scope of ethics statement could be clearer.

ISSUES FOUND

- 4 major
- 23 moderate
- 18 minor
- 2 polish

47 issues identified, graded 1 (polish) to 5 (critical).

Executive Summary

- Core methodological reporting is insufficient for a high-impact life-science article: sample sizes, image/field selection, outcome definitions, and statistical methods are largely absent, undermining reproducibility and interpretation (I01, G02, G03, G04, G05, G06, G07, G08, I02, I03, I05, I14).
- The EM figure set is effectively uninterpretable as archival evidence: multiple multi‐panel micrographs lack captions, legends, and readable scale bars, making it impossible to know what conditions are shown or to gauge morphologic detail (V01–V08, V09–V13).
- Results and conclusions rest almost entirely on unquantified qualitative impressions of morphology; no enumeration of phenotypes, variability, or formal statistics is provided, yet strong comparative and mechanistic claims are made (G08, G09, I06, I07, I08, I09, I12, S01).
- There are specific internal inconsistencies and overinterpretations, including confusion over vacuolization patterns between ionophores and speculative assignment of death modalities and in vivo relevance from limited in vitro data (X02, I08, I09, I19, I20).
- Study design is narrow (single concentration and time point) without clear justification or articulated hypotheses/endpoints, but conclusions are framed more broadly than the data permit (I03, I11, I19).
- Important experimental controls and conditions for EM and MTT assays (vehicle controls, solvent concentrations, imaging parameters, replicates, cell line passage/mycoplasma status) are incompletely described (I04, I05, I13, I14).
- Data availability and referencing are underspecified for an archival article: the nature of deposited datasets is unclear, and the reference list/exact sourcing of some claims is difficult to follow (I17, I21).
- Minor but correctable presentation/terminology issues (fragmented abstract, non‐standard “StEM”, incomplete ethics wording) detract from clarity and professionalism (I15, I16, I18).

Prioritized Issues

I01

MAJOR · 4/5

Section	Methods
Location	Unknown
Evidence (≤25w)	"Monensin had the greatest effect on myoblasts, with the majority of cells being filled with large electron-lucent vacuoles"
Problem	No description of how many cells/fields were examined or how "majority" was defined for ultrastructural changes.
Standard Violated	Life sciences imaging standards require explicit sampling strategy and counts for phenotypic prevalence.
Impact	Undermines the reliability of comparative statements about severity and frequency of ionophore-induced changes.
Confidence	High

I04

MAJOR · 4/5

Section	Methods
Location	Unknown
Evidence (≤25w)	"Stock solutions were prepared by dissolving the ionophores in methanol (MeOH) up to a concentration of 40 mM"
Problem	Vehicle (methanol) control conditions and final solvent concentration in culture medium are not reported for EM experiments.
Standard Violated	Controls reporting standards require explicit vehicle control description to isolate compound-specific effects.
Impact	Raises uncertainty whether observed ultrastructural changes are attributable solely to ionophores rather than solvent effects.
Confidence	High

I08

MAJOR · 4/5

Section	Discussion
Location	Unknown
Evidence (≤25w)	"These observations are suggestive of programmed non-apoptotic cell death mechanisms characterised by excessive vacuole formation"
Problem	Inference of specific non-apoptotic programmed cell death modalities (e.g., methuosis, paraptosis, mitoptosis) is made without supporting molecular or functional assays.
Standard Violated	Cell death nomenclature standards require biochemical/molecular evidence beyond morphology alone for modality assignment.
Impact	Over-interpretation may mislead readers about mechanistic conclusions drawn from this work.
Confidence	High

I02

MODERATE · 3/5

Section	Methods
Location	S1–S4 Figs
Evidence (≤25w)	"Log-dose response curves were generated by using the mean percentage cell survival ± StEM vs the log of the concentration"
Problem	Statistical methods for EC50 estimation and comparison between ionophores are not described in the main text.
Standard Violated	SAMPL and basic toxicology reporting require description of model fitting and statistical analysis for dose–response data.
Impact	Weakens the basis for claims about relative in vitro toxicity and selection of the 0.1 µM concentration.
Confidence	High

I03

MODERATE · 3/5

Section	Methods
Location	Unknown
Evidence (≤25w)	"This concentration was selected... as this concentration falls within the intermediate range between the EC50s of the three ionophores"
Problem	Use of a single concentration and 48 h exposure lacks justification regarding biological relevance or coverage of dose–response and time–course effects.
Standard Violated	Experimental design standards favor multiple doses/time points or strong justification for a single condition.
Impact	Limits interpretability of ultrastructural findings and their relationship to toxicity progression.
Confidence	High

I05

MODERATE · 3/5

Section	Methods
Location	Unknown
Evidence (≤25w)	"Samples were viewed using a JEOL JEM 1400-FLASH transmission electron microscope"
Problem	Imaging parameters (magnification ranges per analysis, number of images per condition, selection criteria) are not detailed.
Standard Violated	EM reporting guidelines expect sufficient imaging detail to reproduce acquisition and analysis.
Impact	Other groups cannot reproduce or systematically compare the EM observations.
Confidence	High

I06

MODERATE · 3/5

Section	Results
Location	Unknown
Evidence (≤25w)	"monensin had the greatest effect on myoblast ultrastructure, followed by salinomycin and lasalocid. This was expected, as monensin has the lowest EC50"
Problem	Relative ranking of ultrastructural effects is qualitative and not supported by any quantified or statistically analyzed metrics.
Standard Violated	Claims comparing treatment effects should be supported by quantitative data or clearly labeled as qualitative observation.
Impact	Reduces robustness of the conclusion about comparative toxicity at the ultrastructural level.
Confidence	High

I07

MODERATE · 3/5

Section	Results
Location	Unknown
Evidence (≤25w)	"There was an increase in the number of rounded myoblasts"
Problem	No enumeration or statistical analysis of morphological categories (rounded vs spread, apoptotic vs necrotic) is provided.
Standard Violated	Quantitative morphology studies should present counts or proportions with statistical evaluation.
Impact	Precludes assessment of effect magnitude and variability between experiments.
Confidence	High

I20

MODERATE · 3/5

Section	Discussion
Location	Unknown
Evidence (≤25w)	"the ultrastructural changes and subsequent cell death observed in this study likely contribute to the lesions seen in animals exposed to lethal ionophore doses"
Problem	Extrapolation from in vitro myoblast models to in vivo livestock lesions is speculative and not supported by direct in vivo correlation data in this study.
Standard Violated	Translational claims should distinguish clearly between speculation and evidence-based inference.
Impact	May overstate applied relevance to veterinary pathology.
Confidence	High

I19

MODERATE · 3/5

Section	Discussion
Location	Unknown
Evidence (≤25w)	"All three ionophores similarly altered the cellular ultrastructure, with monensin having the greatest effect at the concentrations investigated"
Problem	Statement implies a broader generality than warranted by testing only one concentration and one time point.
Standard Violated	Claim scope should match experimental design limits.
Impact	Overgeneralization could mislead about broader dose/time-dependent behavior.
Confidence	High

I21

MINOR · 2/5

Section	References
Location	Unknown
Evidence (≤25w)	"Goodrich R, Garrett J, Gast D, Kirick M, Larson D, Meiske J. Influence of monensin on the performance of cattle"
Problem	Reference list appears truncated in the excerpt; completeness and formatting cannot be fully assessed, and some in-text citations (e.g., [52–60]) cluster many sources without clear linkage to specific statements.
Standard Violated	Vancouver style and good practice require precise citation-to-claim mapping and complete reference list.
Impact	Moderate risk of readers being unable to trace specific mechanistic claims to particular studies.
Confidence	Medium

I17

MINOR · 2/5

Section	Data Availability
Location	Unknown
Evidence (≤25w)	"All files are available from the Institutional Repository of the University of Pretoria database"
Problem	Data availability statement does not specify which datasets (e.g., raw EM images, MTT raw values) are deposited or how they are organized.
Standard Violated	Data-sharing norms require clear description of deposited data types to enable reuse.
Impact	Limits practical accessibility and verification of the reported findings.
Confidence	High

I15

MINOR · 2/5

Section	Abstract
Location	Unknown
Evidence (≤25w)	"Ultrastrucpone.0311046 Editor: Hans-Peter Kubis, Bangor University, the ionophores."
Problem	Abstract text as presented is fragmented and contains formatting artifacts, impairing clarity of the study summary.
Standard Violated	Abstracts should clearly and coherently present background, methods, results, and conclusions.
Impact	Reduces readability and may obscure the main findings to readers relying on the abstract.
Confidence	Medium

I14

MINOR · 2/5

Section	Methods
Location	Unknown
Evidence (≤25w)	"the plates were washed, followed by the addition of 200 µl complete media and 20 µl (5 mg/ml in PBS) MTT"
Problem	MTT assay description omits details such as number of replicates per condition and plate layout, beyond stating "n = number of biological repeats" in S1 Fig legend.
Standard Violated	Toxicology assay reporting requires explicit replication structure for proper interpretation of variability.
Impact	Unclear robustness and reproducibility of EC50 values used to justify exposure conditions.
Confidence	High

I13

MINOR · 2/5

Section	Methods
Location	Unknown
Evidence (≤25w)	"H9c2(2–1) (ATCC1 CRL-1446TM)) and skeletal (L6 (JCRB9081)) muscle cell lines were obtained"
Problem	Passage numbers and any mycoplasma testing status of the cell lines are not reported.
Standard Violated	Cell culture reporting standards recommend stating passage range and contamination checks.
Impact	Potential variability in cellular responses cannot be assessed or controlled for by other researchers.
Confidence	Medium

I12

MINOR · 2/5

Section	Results
Location	Unknown
Evidence (≤25w)	"In summary, after ionophore exposure, the Golgi apparatus became dilated and the cytoplasm was filled with electron-lucent vesicles"
Problem	Textual summaries of findings are qualitative; no figure legends or text indicate any semi-quantitative assessment (e.g., frequency) of these features.
Standard Violated	Good practice in EM studies includes at least semi-quantitative descriptors or tabulation of key ultrastructural findings.
Impact	Readers cannot gauge how common or robust the reported features are across cells/experiments.
Confidence	High

I11

MINOR · 2/5

Section	Introduction
Location	Unknown
Evidence (≤25w)	"In this study, ultrastructural changes occurring in cardiac and skeletal myoblasts following exposure to 0.1 µM monensin, salinomycin, and lasalocid were investigated"
Problem	Introduction does not clearly articulate specific hypotheses or predefined endpoints beyond descriptive observation.
Standard Violated	IMRaD conventions expect explicit research questions or hypotheses guiding the study.
Impact	Makes it harder to judge whether methods and results adequately address stated aims.
Confidence	High

I10

MINOR · 2/5

Section	Discussion
Location	Unknown
Evidence (≤25w)	"this is the first study to use SEM to investigate the effects of ionophores on cardiac and skeletal myoblasts"
Problem	Claim of being "the first" SEM study lacks explicit comparison to existing literature and is not strictly necessary for interpreting results.
Standard Violated	Novelty claims should be carefully substantiated and conservative in scope.
Impact	Risk of overstating novelty; minor but relevant for positioning of contribution.
Confidence	Medium

I09

MINOR · 2/5

Section	Discussion
Location	Unknown
Evidence (≤25w)	"Ultrastructural features alone are insufficient [48–50] and further studies using molecular techniques are needed"
Problem	Despite acknowledging that morphology alone is insufficient, earlier in the Discussion multiple specific death modalities are proposed based only on ultrastructural observations.
Standard Violated	Internal consistency of argumentation and alignment of conclusions with acknowledged limitations.
Impact	Creates conceptual inconsistency and may confuse readers about the strength of mechanistic claims.
Confidence	High

I16

POLISH · 1/5

Section	Ethics
Location	Unknown
Evidence (≤25w)	"This project was approved by the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria"
Problem	Ethics statement does not specify that only established cell lines were used and that no human/animal subjects were involved, though this is implied.
Standard Violated	Transparency standards favor explicit clarification of ethical scope when approvals are cited for in vitro-only work.
Impact	Minor ambiguity about the ethical context; low impact on scientific validity.
Confidence	Medium

I18

POLISH · 1/5

Section	Figures & Tables
Location	S1 Fig
Evidence (≤25w)	"Log-dose response curves were generated by using the mean percentage cell survival ± StEM"
Problem	Use of "StEM" is non-standard terminology and could cause confusion with "SEM" (standard error of the mean).
Standard Violated	Acronym usage should be standard or explicitly defined to avoid ambiguity.
Impact	Minor clarity issue but may confuse some readers regarding statistical dispersion measure.
Confidence	High

Consistency Audit

I09

Type	claim_scope
Evidence A	"These observations are suggestive of programmed non-apoptotic cell death mechanisms characterised by excessive vacuole formation" (P46)
Evidence B	"Ultrastructural features alone are insufficient [48–50] and further studies using molecular techniques are needed" (P47)
Inconsistency	The Discussion assigns specific non-apoptotic programmed cell death mechanisms based on morphology, yet immediately acknowledges that morphology alone is insufficient for such classification.

I19

Type	claim_scope
Evidence A	"All three ionophores similarly altered the cellular ultrastructure, with monensin having the greatest effect at the concentrations investigated" (P48)
Evidence B	"The myoblasts were exposed to 0.1 µM monensin, salinomycin, and lasalocid for 48 h." (P15)
Inconsistency	Conclusions about similarity and ranking of ionophore effects are framed broadly, although only one concentration and time point were actually examined.

Guideline Compliance

Audited against ARRIVE (2.0 Essential 10); study type: animal study.

Item	Status	Evidence / Note
ARRIVE-1 — Study design groups	Partial	"The myoblasts were exposed to 0.1 µM monensin, salinomycin, and lasalocid for 48 h." — Groups (three ionophores plus negative control) and in vitro myoblast experimental units are clear, but there is no explicit statement of control group numbers or experimental unit definition per analysis.
ARRIVE-2a — Sample size per group	Missing	No exact number of experimental units (e.g., number of wells, coverslips, or cells per group actually analyzed) is reported for any group.
ARRIVE-2b — Sample size justification	Partial	"This concentration was selected based on previous cytotoxicity experiments as this concentration falls within the intermediate range between the EC50s" — Justification is given for choice of exposure concentration, but no rationale or calculation is provided for how many samples/replicates were needed (no power or precision justification).
ARRIVE-3 — Inclusion and exclusion criteria	Missing	No inclusion or exclusion criteria for images, fields, cells, or experiments are described, and no exclusions or reasons for exclusion are reported.
ARRIVE-4a — Randomization	Not applicable	This is an in vitro cell line study without allocation of individual animals to groups; randomization of animals is not applicable.
ARRIVE-4b — Confounder control	Missing	No description of steps to control confounders such as order of treatment, plate position, or batch effects is provided.
ARRIVE-5 — Blinding	Missing	Blinding of investigators for treatment during imaging, image selection, or analysis is not mentioned.
ARRIVE-6 — Outcome measures	Partial	"ultrastructural changes occurring in cardiac and skeletal myoblasts following exposure to 0.1 µM monensin, salinomycin, and lasalocid were investigated using electron microscopy." — Outcome (ultrastructural changes by TEM/SEM) is described qualitatively, but no explicit designation of a primary outcome measure is given.
ARRIVE-7 — Statistical methods	Missing	No statistical methods are described; results are purely qualitative with no mention of tests, software, or assessment of assumptions.
ARRIVE-8 — Animal characteristics	Reported	"Rat cardiac (H9c2(2–1) (ATCC1 CRL-1446TM)) and skeletal (L6 (JCRB9081)) muscle cell lines were obtained" — Species (rat origin via cell lines), cell line names, and sources (ATCC, JCRB) are provided; sex/age of donor animals are not typically available for immortalized lines.
ARRIVE-9 — Experimental procedures	Reported	"The myoblasts were exposed to 0.1 µM monensin, salinomycin, and lasalocid for 48 h." — Culture conditions, seeding density, exposure conditions, and detailed TEM/SEM preparation and imaging protocols are comprehensively described for replication.
ARRIVE-10 — Results with variability	Missing	Findings are described narratively and with micrographs only; no quantitative summaries, effect estimates, variability measures, or analyzed numbers per group are provided.

Item	Status	Evidence / Note
ARRIVE-ETHICS — Ethical statement and welfare	Partial	"This project was approved by the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (REC070-19, 2019/05/06)." — Ethics committee and approval number are reported, but no details on specific guidelines followed or on any animal welfare/humane endpoints (cell-line study context) are provided.

Action Plan

G02, I01

Action	Define and report, for each experimental group and assay, the exact sample sizes and how they map to biological and technical replicates (e.g., number of independent cultures, wells, coverslips, cells/fields per condition), and provide an operational definition of terms like “majority” used for ultrastructural changes.
Effort (S/M/L)	M
Priority (P0–P2)	P0
Success Criteria	Methods and Results sections contain explicit n for every figure/analysis and a clear description of how many cells/fields were examined per sample; narrative statements such as “majority of cells” are replaced or supplemented with percentage estimates tied to these counts.

V01, V02, V03, V04, V05, V07, V08, V09, V10, V11, V12, V13, V06

Action	Rewrite and insert full figure captions and in‑figure legends for all EM figures, clearly stating the experimental condition for each panel, defining all abbreviations/symbols, and regenerating images or labels so that scale bar numeric values are legible at journal resolution.
Effort (S/M/L)	M
Priority (P0–P2)	P0
Success Criteria	Every multi‑panel EM figure has a complete caption specifying treatment, time, and modality per panel; all letters/symbols (e.g., N, A, R, M*, arrows) are defined in caption or legend; scale bar lengths are readable without zooming and match values stated in captions.

G08, I02, G09, I06, I07, I12, S01

Action	Introduce quantitative morphometric analysis and appropriate statistics: predefine a set of morphological categories, systematically count them across blinded fields, summarize as proportions/means with variability, and apply suitable statistical tests for comparisons (including EC50 estimation methods with confidence intervals).
Effort (S/M/L)	L
Priority (P0–P2)	P0
Success Criteria	Results include at least one table/figure showing quantified morphological endpoints (e.g., % apoptotic‑like, % vacuolated) with mean±SD/SEM or 95% CI for each group; the Methods specify statistical tests and software; EC50 values are accompanied by CIs and a clear description of the fitting model; narrative comparative claims are explicitly linked to these analyses.

I03, G03, I11, G07, I19

Action	Clarify and align study design with aims: explicitly state hypotheses and primary outcome(s), justify the use of a single concentration and 48 h time point (or broaden the design to include minimal dose–response/time–course), and revise conclusions to match this limited design.
Effort (S/M/L)	M
Priority (P0–P2)	P0
Success Criteria	Introduction states clear hypotheses and primary endpoints; Methods justify chosen concentration/time or describe added dose/time conditions; Discussion and Conclusion avoid generalizations beyond the tested conditions and clearly delimit the inference scope.

I08, I09

Action	Substantially tone down or remove specific attributions of death modalities (methuosis, paraptosis, mitoptosis) that are based solely on morphology, and either add appropriate molecular/functional assays or reframe the discussion as exploratory morphological observations without naming specific pathways.
Effort (S/M/L)	M
Priority (P0–P2)	P0
Success Criteria	Revised Discussion no longer asserts specific forms of programmed cell death without supporting molecular/functional data; if such assays are added, they are described in Methods and yield results that are explicitly connected to the morphologic interpretation.

I20

Action	Limit extrapolation from in vitro myoblast findings to in vivo livestock lesions, clearly labelling any such connections as speculative and pointing to the need for dedicated in vivo correlation studies rather than suggesting direct equivalence.
Effort (S/M/L)	S
Priority (P0–P2)	P1
Success Criteria	Discussion text explicitly distinguishes between observed in vitro effects and hypothesized in vivo relevance; statements claiming correspondence to livestock lesions are either removed or softened with conditional language and calls for future in vivo validation.

I04, I14

Action	Fully describe control conditions and assay implementation: specify vehicle (methanol) controls and final solvent concentrations for EM and MTT experiments, detail plate layout, number of technical/biological replicates, and how background/controls were handled in data analysis.
Effort (S/M/L)	M
Priority (P0–P2)	P1
Success Criteria	Methods include a subsection on controls clearly listing solvent type and final % in medium for each assay; MTT protocol states wells per condition, number of independent experiments, and control normalization procedures; EM Methods mention inclusion of vehicle-only samples imaged under identical conditions.

G04, I05

Action	Provide detailed imaging acquisition and selection parameters: magnification ranges used for each analysis, number of images/fields per sample, predefined criteria for image selection, and whether selection was randomized or systematic.
Effort (S/M/L)	M
Priority (P0–P2)	P1
Success Criteria	EM Methods specify magnifications, field-of-view strategy (e.g., random grid), and minimum number of images per sample and per condition; any exclusion of images/fields is described along with reasons; this information is sufficient for another lab to reproduce the imaging protocol.

G05, G06

Action	Address potential confounders and blinding: describe how plate position, treatment order, and batch were managed, and whether imaging and morphological scoring were performed blinded to treatment; if not originally blinded, acknowledge this as a limitation and, where feasible, implement blinding in additional validation experiments.
Effort (S/M/L)	M
Priority (P0–P2)	P1
Success Criteria	Methods include explicit statements on randomization/order, plate layout with respect to treatment, and whether observers were blinded during image capture and analysis; if blinding was not used, this is stated and justified, and at least a subset of analyses is repeated under blinded conditions or the limitation is clearly acknowledged in the Discussion.

I13

Action	Report cell line–related quality parameters, including passage range used in experiments and mycoplasma contamination testing status and schedule.
Effort (S/M/L)	S
Priority (P0–P2)	P1
Success Criteria	Methods include the cell line source, passage number range at experimentation, and a statement on mycoplasma testing (method and frequency); if testing was not done, this is explicitly acknowledged in the Discussion as a limitation.

I17

Action	Revise the data availability statement to specify exactly which datasets are available (e.g., raw TEM/SEM image sets with metadata, raw and processed MTT readings, analysis scripts) and where/how they are organized and accessed.
Effort (S/M/L)	S
Priority (P0–P2)	P2
Success Criteria	Data availability section lists dataset types, repository accession numbers/URLs, file organization (e.g., by treatment and replicate), and any access conditions; this information is sufficient for an external researcher to locate and reuse the data.

I15, I21

Action	Edit the abstract for clarity and remove formatting artifacts; ensure that the reference list is complete, correctly formatted, and that dense citation clusters are pruned or structured so each key statement is supported by specific, traceable references.
Effort (S/M/L)	S
Priority (P0–P2)	P2
Success Criteria	Abstract reads as a coherent summary with standard structure (background, methods, key results, conclusions) and no visible formatting glitches; the reference list matches all in‑text citations, and high‑level claims are supported by clearly linked references rather than large undifferentiated citation blocks.

I16

Action	Clarify the ethics statement to explicitly state that only established cell lines were used and that no human or live animal subjects were involved in the experiments.
Effort (S/M/L)	S
Priority (P0–P2)	P2
Success Criteria	Ethics section includes a clear sentence such as “This study used only established commercial cell lines and did not involve human participants or live animals,” in line with journal policy.

I18

Action	Standardize terminology by replacing “StEM” with “SEM” (scanning electron microscopy) or clearly defining any non‑standard abbreviations at first use to avoid confusion with statistical terms like standard error of the mean.
Effort (S/M/L)	S
Priority (P0–P2)	P2
Success Criteria	All instances of non‑standard microscopy abbreviations are either corrected to standard forms (SEM, TEM) or explicitly defined; no remaining term can be reasonably confused with common statistical abbreviations.

X02

Action	Reconcile and clarify the description of vacuolization effects between monensin and salinomycin, ensuring that text, figures, and captions consistently reflect the actual observed patterns and do not contradict statements about the proportion of affected cells.
Effort (S/M/L)	S
Priority (P0–P2)	P1
Success Criteria	Revised Results/Discussion and figure captions present a consistent account of vacuolization across treatments, with no internal contradictions; any nuanced differences (e.g., subtle vs extensive vacuolization) are explicitly described and, where possible, quantified.