REFEREE PDF EXPORT

Full Manuscript Report

SIMULATED EDITORIAL RISK ESTIMATE

Major Revision Risk

This is a simulation for pre-submission manuscript preparation. It is not an official editorial decision, does not impersonate a journal or editor, and should not be treated as a publication forecast.

JOURNAL

Journal of Cell Biology

SIMULATED OUTCOME SIGNALS

Desk Reject **10-20%** Reject After Review **20-30%** Major Revision **35-45%** Minor Revision **15-25%**

Directional outcome simulation based on high-tier journal stringency, published/selectivity priors where available, and this manuscript's quality scores. A stronger manuscript shifts simulated outcomes toward review and revision rather than rejection.

Directional simulation only. This is not an official journal review, editorial decision, or publication forecast. Referee Bio is independent and is not affiliated with or endorsed by any journal, publisher, editor, or society named in this report.

Overview

How to interpret this report

Use this as a pre-submission risk map. The strongest signal is not the exact percentage; it is the pattern of objections, ranked fixes, methods gaps, and journal-fit pressure points that repeat across the report.

What Referee cannot know

Referee cannot know the actual editor, reviewer panel, confidential journal priorities, competing papers under review, informal reviewer biases, or the final decision. Treat the output as preparation, not prophecy.

Review Scores & Criteria

Statistical Methods Audit

PASS

Paper type: In vitro cell biology study combining arrayed CRISPR screening with biochemical validation and patient-derived cell lines

✓ **Multiple comparisons correction**

Holm and Holm-Sidak corrections applied explicitly at single time points and for planned comparisons; Benjamini-Hochberg FDR applied to genome-wide screen enrichment; two planned Bliss comparisons corrected with Holm.

✓ **Blinding / randomization**

TEM scoring performed 'on randomized image identifiers by an analyst blinded to genotype and treatment'; SPR injections performed 'in randomized order'; third validation campaign used 'independently seeded cells' and 'separately prepared OA treatment stocks'.

✓ **Exact statistical tests named**

All comparisons explicitly named: two-way repeated-measures ANOVA, Kruskal-Wallis with Dunn correction, one-way ANOVA with Dunnett, robust Z-scores with MAD scaling, four-parameter logistic curve fitting, mixed-effects models, Mann-Whitney sensitivity analysis.

✓ **Replicate definitions (biological vs technical)**

Comprehensive Table defining biological replicates per experiment type: 'Single cells nested within biological replicate' for imaging, 'one sgRNA-pool well per gene per campaign' for screening, 'independent protein preparations' for biochemistry; explicitly states 'prevent inflation of n from single-cell counts'.

✓ **Effect sizes reported**

All major comparisons report 95% confidence intervals with point estimates: e.g., '24 h OA-BafA1 difference = 23.4 puncta/cell, 95% CI 20.8 to 26.0'; 'MIRA1 KO response at 2 mM versus vehicle mean difference = +12.5, 95% CI +10.1 to +14.9'; log KD differences reported as '1.9, CI 1.5 to 2.3'.

✓ **Normality / distribution checks**

Statement provided: 'Parametric tests were applied to replicate-level summaries or log-transformed affinity estimates when assumptions were adequate; otherwise nonparametric tests were used,' demonstrating awareness of distribution assumptions for small samples (n=3–4 biological replicates).

✓ **Sample size / power calculation**

Prospective sample-size justification stated: 'Sample sizes were selected prospectively on practical and precision grounds rather than post hoc power calculations, which is now stated explicitly. For imaging experiments, n = 3-4 biological replicates was chosen to estimate effect sizes with narrow confidence intervals given the large cell-level sampling per replicate.'

✓ **Exclusion criteria**

Well-level and cell-level exclusion criteria defined: 'Wells were excluded if nuclear count, reporter expression, or segmentation quality failed predefined thresholds'; MIRA1 guides validated 'without violating nuclear-count or reporter-intensity gates'; baseline TEM scoring criteria ('swollen when cross-sectional area exceeded the 90th percentile', 'cristae disrupted when more than half...fragmented') clearly specified.

Critical gaps: None identified: the manuscript explicitly addresses all major applicable statistical reporting standards for this study type. · Minor: confidence interval reporting for replicate-level correlations in genome-wide screening (Pearson r 0.93 with CI 0.92–0.94 is provided, but individual-guide robustness by bootstrap is mentioned only in Figure S1 legend, not quantified in main text).

This dummy manuscript exemplifies comprehensive statistical transparency and is a model for moderate-high rigor in cell biology screens. No revisions needed; recommend as a reference standard for reviewer guidance on reporting biological replicates, multiple-comparison correction, and effect-size confidence intervals in arrayed screening combined with cell-based validation.

Structural Word Count Benchmarks

Based on 15 papers in this journal in the Referee corpus

Journal of Cell Biology guidelines: Main text ≤9,000 words · ≤8 figures — ≤9,000 w total; figure panels ≤8

Your manuscript: **10,365 words** total (limit: 9,000)

Section	Your count	Typical range (p25–p75)	Median		Status
Introduction	488	562.5–930.5	707		✓ Typical
Results	3,896	3,865.5–6,680.5	5,383		✓ Typical
Discussion	969	1,074–1,427	1,140		✓ Typical

Reviewer Understanding

Reviewers converge on two principal concerns for Journal of Cell Biology: first, the genome-wide screen rests on only two independent campaigns, which is below the reproducibility standard this journal expects for a receptor-discovery claim built on a single new gene; second, MIRA1 is never benchmarked head-to-head against established membrane-resident receptors NIX, BNIP3, or FUNDC1 under identical depolarization conditions, leaving the 'selective receptor' designation insufficiently supported relative to JCB's mechanistic bar. The neuronal validation, biochemical binding orthogonality, and statistical transparency are genuine strengths that place this manuscript within revision range.

Major Concerns

- **Screen reproducibility: only two genome-wide campaigns support the primary receptor assignment**

  The genome-wide CRISPR screen was performed in n=2 independent campaigns (Figure 2 legend: 'Biological n = 2 independent genome-wide screening campaigns'). Although a third validation campaign was added for MIRA1 specifically (Figure S1), the full ranked hit list — including MIRA1's rank-1 position — derives from only two campaigns. Journal of Cell Biology expects genome-wide perturbation screens that underpin mechanistic receptor claims to demonstrate hit-list stability across at least three independent experimental runs, not post-hoc single-gene validation. The current design cannot exclude that the MIRA1 rank-1 assignment reflects campaign-specific variance rather than a stable signal, because no competing strong depletion hit was re-screened at guide level to calibrate false-positive rate at the top of the distribution.

- **No head-to-head comparison with established OMM receptors NIX, BNIP3, or FUNDC1 under matched conditions**

  The manuscript claims MIRA1 is 'a selective mitophagy receptor for damaged-mitochondria clearance' and positions it within the receptor-amplification network alongside OPTN and CALCOCO2. However, NIX, BNIP3, and FUNDC1 — the three most characterized outer-mitochondrial-membrane ATG8-binding receptors — are never depleted in the same mito-FLARE assay under the oligomycin/antimycin A conditions used throughout the study. Without matched mito-FLARE flux data for NIX-KO, BNIP3-KO, and FUNDC1-KO cells, the claim that MIRA1 is a strong determinant specifically of depolarization-induced mitophagy cannot be placed in quantitative context. JCB reviewers will ask: is the MIRA1 effect size larger, smaller, or comparable to these established receptors? The absence of this comparison leaves the receptor designation qualitatively asserted rather than experimentally supported.

Minor Concerns

- **Figure numbering disorder: Figure 7 appears after Figure 8 in the results narrative**
  The results section describes Figure 8 (neuronal validation) before Figure 7 (patient-cell TOMM20 data), creating a numbering inconsistency that will draw reviewer and production attention. All figure numbers should follow the order of first citation in the text.
- **Coiled-coil domain functional data (DeltaCC, CCmut) lacks a main-text figure**
  The coiled-coil deletion and CCmut experiments described in the results section are mentioned without a dedicated figure number. The SEC-MALS oligomerization data and co-IP results supporting the receptor-assembly conclusion are important for the mechanistic model but have no figure anchor, which means they cannot be evaluated for quantification quality or statistical reporting by reviewers.
- **Table 2 statistical checklist is useful but partially redundant with figure legends**
  Table 2 reproduces statistical details already present in the figure legends. While the redundancy aids readers, the table format creates a maintenance burden: any legend revision must also update Table 2. JCB does not typically require a statistical checklist table; the information should remain in the legends and Table 2 should be removed or condensed into a supplementary methods note.
- **Proximity-labeling miniTurbo experiment is described in methods but has no results section text or figure**
  The Methods section describes a low-expression MIRA1-miniTurbo proximity-labeling experiment with biotinylated protein capture and mass-spectrometry or immunoblot quantification, but neither the Results nor any figure legend reports the outcome of this experiment. If performed, the data should be reported; if not performed, the methods description should be removed to avoid implying data exist that are not shown.
- **Carbonate extraction and protease-protection topology data lack quantification reporting**
  The results text states that carbonate extraction 'suggested membrane association' and protease-protection 'supported a topology' with cytosol-exposed domains, but no figure is cited, no quantification is provided, and no statistical test is reported. JCB's imaging and biochemistry rigor standards require that topology claims be supported by quantified blot data with reported n and effect sizes.

Methods Critique

The Methods section meets JCB's reporting standard better than most submissions at this stage: biological n, analysis units, sgRNA validation depth, passage-matching rules, and SPR chip-regeneration criteria are all explicitly stated. The primary reporting gap is the absence of normalization details for LC3B-II/I ratio quantification — the text states bands were 'quantified in Fiji' and the ratio was 'normalized to loading control' but does not specify whether the loading control was total protein (Ponceau or fluorescent total-protein stain) or a single housekeeping band, which matters for LC3B-II detection in depolarized cells where housekeeping protein abundance may shift. A secondary gap is that the mito-FLARE reporter integration site and copy number are not specified, which is relevant to whether reporter expression stability across clones reflects genuine flux or position-effect silencing.

Novelty & Significance

The manuscript's novelty architecture is well-suited to JCB's moderate-high novelty threshold: a genome-wide arrayed screen using a flux-competent reporter (rather than a static colocalization readout) that identifies a previously unannotated OMM protein with direct ATG8-binding activity and a separable oligomerization function is a legitimate conceptual advance over the current receptor landscape. The primary novelty risk is that the fictional designation of MIRA1 as 'previously unannotated' cannot be independently evaluated, but within the simulation framework the combination of screen-to-biochemistry-to-cell-biology-to-neuronal-model workflow represents the kind of mechanistic completeness JCB expects for a receptor-discovery paper. The mito-FLARE reporter itself represents a secondary methodological advance that is sufficiently differentiated from existing tandem-tag reporters (mito-QC, mt-Keima) to be considered an independent contribution, though this differentiation should be stated more explicitly in the introduction.

Statistics Note

The most consequential statistical gap is the use of a flat Kruskal-Wallis test on individual cells in Figure 4 without explicit modeling of the culture-level nesting structure, which risks pseudoreplication given n=4 cultures with 170+ cells each; the mixed-effects approach used correctly in Figure 8 should be applied consistently to Figure 4.

Suggested Experiments

- Perform mito-FLARE flux measurement in NIX-KO, BNIP3-KO, and FUNDC1-KO U2OS cells under the same oligomycin/antimycin A conditions used throughout the study, and present the resulting effect sizes alongside MIRA1-KO in a single comparative panel — this single experiment directly defends the 'selective receptor' designation and answers the benchmarking question that Reviewer 2 will make a revision requirement.
- Add a third independent genome-wide screening campaign and report MIRA1's rank and robust Z-score from each of the three campaigns individually, promoting Figure S1 per-guide data to the main text as a sub-panel of Figure 2 — this converts the n=2 campaign limitation from a potential desk-rejection flag into a strength.
- Report the coiled-coil deletion and CCmut experiments in a dedicated main-text figure with quantified co-IP band intensities, SEC-MALS elution profiles with mass calibration, and mito-FLARE rescue data shown side-by-side with WT and W214A controls — this closes the only remaining mechanistic gap in the domain-function analysis.

Strengths

- The mito-FLARE reporter design measures completed lysosomal delivery rather than an autophagosome-stage intermediate, providing a biologically interpretable single-cell flux metric that is directly coupled to the screen readout — this design decision strengthens every downstream experiment.
- Per-figure statistical reporting is exceptionally thorough: biological n, analysis units, named tests, multiple-comparison corrections, and effect sizes with 95% confidence intervals are provided for every main figure, which is above JCB's typical submission standard.
- The three-orthogonal-format biochemical binding validation (GST pull-down, SPR, ITC-style) with matched negative controls (W214A, GST alone, scrambled peptide) and explicit fragment-boundary analysis converts the LIR-dependent receptor model from a genetic inference into a direct biochemical claim.
- The Bliss sensitivity analysis around the ATG7-defined baseline is a sophisticated and pre-emptive response to the most obvious epistasis-methodology critique, and the conclusion is appropriately narrowed to 'partially shared receptor-amplification module' rather than overclaiming full redundancy.
- The neuronal validation framework — four independent differentiations, mixed-effects model with batch as random intercept, chronic rotenone alongside acute OA, and MAP2-positivity gating — is proportionate to the parkinsonism framing and represents a genuine elevation above U2OS-only receptor papers.

Journal Fit

Journal names are used only for submission-planning context. Referee Bio is independent and is not affiliated with, endorsed by, sponsored by, or operated by any listed journal, publisher, editor, or society.

REACH **Nature Cell Biology** 76%

Field-leading mechanistic cell biology; high bar for causality.

BEST FIT **Journal of Cell Biology** 83%

Rigorous cell biology with strong image/data integrity culture and mechanistic expectations.

FALLBACK **Molecular Biology of the Cell** 86%

ASCB community journal emphasizing solid mechanistic cell biology, reproducibility, and fair review.

Other Good Options

PLOS ONE 88%

Multidisciplinary research across science and medicine assessed primarily for technical soundness rather than perceived novelty or impact.

Scientific Reports 88%

Broad multidisciplinary research across natural, clinical, biological, physical, and applied sciences, assessed primarily for technical validity rather than perceived impact.

Molecular Ecology 86%

Uses molecular tools (genomics, population genetics, phylogenomics) to answer evolutionary and ecological questions. Population structure, local adaptation, speciation, and conserv

Proceedings of the Royal Society B 86%

Broad biological sciences — evolution, ecology, behavior, physiology, and comparative biology. Values clear biological questions, rigorous methods, and broad relevance. Open to div

Journal of Ecology 86%

Evolution 86%

Plant ecology, vegetation science, and plant-animal interactions. Requires clear ecological hypotheses, field validation, and statistical rigor. Mechanistic understanding of plant

Theoretical and empirical evolutionary biology covering microevolution, macroevolution, speciation, adaptation, and population genetics. Quantitative genetics and phenotypic evolut

BMC Biology 86%

Broad biology open-access journal; favors solid general-interest biology with transparent methods and accessible framing.

Current Biology 85%

Interesting biological advance; scope can be focused if broadly accessible.

Full Ranked List

#	Journal	Fit %	Editorial Risk	Note
1	PLOS ONE	88%	low	Multidisciplinary research across science and medicine assessed primarily for technical soundness rather than perceived novelty or impact.
2	Scientific Reports	88%	low	Broad multidisciplinary research across natural, clinical, biological, physical, and applied sciences, assessed primarily for technical vali
3	Molecular Biology of the Cell	86%	low	ASCB community journal emphasizing solid mechanistic cell biology, reproducibility, and fair review.
4	Molecular Ecology	86%	low	Uses molecular tools (genomics, population genetics, phylogenomics) to answer evolutionary and ecological questions. Population structure, l
5	Proceedings of the Royal Society B	86%	low	Broad biological sciences — evolution, ecology, behavior, physiology, and comparative biology. Values clear biological questions, rigorous m
6	Journal of Ecology	86%	low	Plant ecology, vegetation science, and plant-animal interactions. Requires clear ecological hypotheses, field validation, and statistical ri
7	Evolution	86%	low	Theoretical and empirical evolutionary biology covering microevolution, macroevolution, speciation, adaptation, and population genetics. Qua
8	BMC Biology	86%	low	Broad biology open-access journal; favors solid general-interest biology

#	Journal	Fit %	Editorial Risk	Note
				with transparent methods and accessible framing.
9	Current Biology	85%	low	Interesting biological advance; scope can be focused if broadly accessible.
10	PLOS Biology	85%	low	Broad biological sciences emphasizing conceptual advances, openness, rigor, and interest across biological disciplines.
11	Nature Communications	85%	low	Technically complete, significant but not necessarily field-defining.
12	Ecology Letters	84%	low	High-impact ecology requiring conceptual novelty beyond description. Strong preference for work with broad implications for ecological theor
13	Global Change Biology	84%	low	Mechanistic understanding of biodiversity and ecosystem responses to global change (climate, land use, invasions, pollutants). Requires clea
14	American Naturalist	84%	low	Theoretical and conceptual advances in evolutionary ecology. Highly selective for papers with broad conceptual novelty. Mathematical/theoret
15	PNAS	84%	low	Broad significance, less punitive than N/C/S but still selective.
16	Science Advances	84%	low	High-quality broad science with strong technical support.
17	Journal of Cell Biology	83%	low	Rigorous cell biology with strong image/data integrity culture and

#	Journal	Fit %	Editorial Risk	Note
				mechanistic expectations.
18	eLife	83%	low	Constructive, transparent, rigor-heavy review culture.
19	Nature Cell Biology	76%	low	Field-leading mechanistic cell biology; high bar for causality.

Section Analysis

FIX THESE FIRST

Everything else is noise until you clear this bar at Journal of Cell Biology.

1 Screen reproducibility insufficient for a rank-1 receptor assignment: n=2 genome-wide campaigns

Figure 2's legend explicitly states 'Biological n = 2 independent genome-wide screening campaigns.' MIRA1's entire mechanistic story derives from its rank-1 position in this screen, yet no third campaign data exists for the full ranked list. Figure S1 adds per-guide and third-campaign validation for MIRA1 specifically, but this is a post-hoc single-gene check, not a demonstration that the screen's hit architecture is reproducible. JCB associate editors reviewing receptor-discovery manuscripts built on genome-wide screens routinely require three independent campaigns before sending to review, because two-campaign rank-1 assignments carry a non-trivial false-positive rate that downstream mechanistic investment cannot retroactively correct. If an associate editor applies this criterion at desk, the manuscript does not survive triage in its current form.

2 No benchmarking against established OMM-resident ATG8 receptors NIX, BNIP3, or FUNDC1 under matched conditions

The manuscript claims MIRA1 is 'a selective receptor for damaged-mitochondria clearance' and positions it as a strong determinant of depolarization-induced mitophagy. Yet NIX, BNIP3, and FUNDC1 — the three outer-mitochondrial-membrane proteins that directly bind ATG8-family proteins through LIR motifs, the exact functional category to which MIRA1 is being assigned — are never depleted in the mito-FLARE assay under the oligomycin/antimycin A conditions used throughout the paper. The epistasis section benchmarks MIRA1 against OPTN and CALCOCO2, which are cytosolic ubiquitin-binding adaptors, not LIR-containing OMM receptors. A JCB associate editor evaluating a receptor-discovery paper will ask: compared to the existing members of the same functional class, how strong is the MIRA1 effect? The absence of this comparison means the 'selective receptor' designation is supported only relative to a non-parallel receptor class, which is insufficient to clear JCB's mechanistic novelty threshold at desk triage.

Introduction 72%

Does the gap statement justify this journal's scope?

> **Top risk:** Gap statement does not differentiate mito-FLARE from existing tandem-tag reporters by name

The introduction correctly frames the gap as the need for a flux-competent, genome-scale arrayed screen that can separate true mitophagy loss from confounding phenotypes, and it positions this against the known limitations of pooled CRISPR and static-readout approaches. For JCB's specific readership, the gap statement would be stronger if it named mito-QC and mt-Keima directly and explained why a tandem-tag OMM reporter addresses limitations those tools have in arrayed perturbation formats. The transition from 'receptor logic is incomplete' to 'we built a screen' is logically sound but does not explain why an unbiased screen is more likely to identify new receptors than candidate-based biochemistry — a sentence justifying the discovery strategy would sharpen the gap for JCB's mechanistically oriented readers.

Methods 81%

Does it match the reporting standard this journal enforces?

> **Top risk:** LC3B-II/I normalization method unspecified; reporter integration site and copy number absent

The Methods section is above average for JCB in specificity: sgRNA validation depth, passage-matching rules, SPR chip-regeneration criteria, and Bliss formula are all explicitly stated. The primary compliance gap is LC3B-II/I normalization: the text states 'normalized to loading control' without specifying whether total protein or a housekeeping band was used, which is consequential for an immunoblot readout in metabolically stressed cells. A secondary gap is that the mito-FLARE reporter's lentiviral integration site and copy number are unspecified, relevant to clone-to-clone expression stability claims.

Results

76%

Are figures ordered to build the argument correctly?

> **Top risk:** Figure 7/8 numbering inversion and missing figure for coiled-coil domain data disrupt argument flow

The results section builds a coherent causal argument: reporter validation, screen, domain prediction, biochemical binding, knockout/rescue, coiled-coil function, neuronal validation, epistasis, pharmacological bypass, and network placement. The primary flow problem is that Figure 7 and Figure 8 are cited out of numerical order in the text (Figure 8 neuronal validation is presented before Figure 7 patient-cell TOMM20 data), which disrupts the logical progression from neuronal flux to mitochondrial retention phenotype. The coiled-coil domain results lack a figure anchor entirely, creating a results paragraph that reviewers cannot evaluate quantitatively.

Discussion

78%

Is there overclaiming that would trigger editorial pushback?

> **Top risk:** Mechanistic model of site-selective ATG8 recruitment is not directly tested and extends beyond the epistasis and biochemical evidence

The Discussion is notably self-aware about the fictional context and explicitly limits disease claims: 'The fictional human genetics section should be read as a demonstration of manuscript structure rather than evidence' and deferiprone is framed as 'a mechanistic competence test rather than a therapeutic hypothesis.' For a real submission, the highest overclaiming risk is the sentence 'MIRA1 stabilizes ATG8-family recruitment at selected outer-membrane sites to increase the probability of productive isolation-membrane growth' — this mechanistic model of site selection is not directly tested by any experiment in the manuscript and would be flagged by a JCB associate editor as speculation exceeding the epistasis and biochemical data. The phrase 'selected outer-membrane sites' implies spatial patterning that would require super-resolution or CLEM evidence to support.

Fix First

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Rank	What to fix first	Acceptance impact	Effort
1	Add matched mito-FLARE flux data for NIX-KO, BNIP3-KO, and FUNDC1-KO cells under identical OA conditions and present as a comparative panel alongside MIRA1-KO	+high	moderate
2	Perform a third full genome-wide screening campaign, report per-campaign MIRA1 ranks individually, and promote Figure S1 per-guide data to a main-text panel within Figure 2	+high	high
3	Replace the Kruskal-Wallis analysis in Figure 4 with a mixed-effects model matching the approach used in Figure 8, explicitly modeling culture as a random intercept	+moderate	low
4	Assign a figure number to the coiled-coil deletion and CCmut experiments and include quantified co-IP and SEC-MALS data with stated n and effect sizes in the main text	+moderate	low
5	Revise Figure 3 to include at least one representative SPR sensorgram in the main-text panel alongside the KD bar summary, and move Figure S3 sensorgrams to a main-text supplementary panel explicitly cross-referenced in the Figure 3 legend	+moderate	low
6	Specify in the Discussion that the 'selected outer-membrane sites' mechanistic model is a hypothesis requiring super-resolution or CLEM validation, and replace the site-selection language with a more conservative statement about receptor-density amplification supported by the coiled-coil oligomerization data	+low	low

- [ ] **R1**: Add matched mito-FLARE flux data for NIX-KO, BNIP3-KO, and FUNDC1-KO cells under identical OA conditions and present as a comparative panel alongside MIRA1-KO

- [ ] **R2**: Perform a third full genome-wide screening campaign, report per-campaign MIRA1 ranks individually, and promote Figure S1 per-guide data to a main-text panel within Figure 2

- [ ] **R3**: Replace the Kruskal-Wallis analysis in Figure 4 with a mixed-effects model matching the approach used in Figure 8, explicitly modeling culture as a random intercept

- [ ] **R4**: Assign a figure number to the coiled-coil deletion and CCmut experiments and include quantified co-IP and SEC-MALS data with stated n and effect sizes in the main text

- [ ] **R5**: Revise Figure 3 to include at least one representative SPR sensorgram in the main-text panel alongside the KD bar summary, and move Figure S3 sensorgrams to a main-text supplementary panel explicitly cross-referenced in the Figure 3 legend

- [ ] **R6**: Specify in the Discussion that the 'selected outer-membrane sites' mechanistic model is a hypothesis requiring super-resolution or CLEM validation, and replace the site-selection language with a more conservative statement about receptor-density amplification supported by the coiled-coil oligomerization data

Upgrade Path

Upgrade Path: Journal of Cell Biology → Nature Cell Biology

The manuscript demonstrates strong technical execution and statistical rigor but lacks the mechanistic completeness and multi-system validation that Nature Cell Biology requires; novelty is solid but the scope is confined to a single experimental system without orthogonal in vivo or physiologically relevant validation.

These are the 3 specific changes most likely to close the gap between your submission journal and the reach journal. Ranked by likely impact on desk-triage risk.

1 **Add orthogonal validation in a physiologically relevant system** HIGH IMPACT

High effort

Perform the core mechanism validation in a second model system—either primary cells, organoid culture, or in vivo tissue (depending on your mechanism). If your main findings are in cultured cells, add a parallel experiment in acute tissue explant, whole-

organism imaging, or primary cell isolation. Include matched quantification and statistical analysis in this second system to demonstrate mechanism generalizability beyond your primary model.

Your next step: Identify which physiological context your mechanism is most relevant to (tissue type, developmental stage, disease state), then design a validation experiment using that system with the same readouts as your cultured cell work—run it in parallel with your current revisions.

Why Nature Cell Biology requires this: Nature Cell Biology reviewers weight studyScale 15% (vs 4% at JCB) and reject single-system findings as a rejection pattern; orthogonal systems with consistent mechanistic output demonstrate conceptual maturity and field leadership rather than optimization of one platform.

2

Map complete temporal and causal hierarchy of the mechanism

HIGH IMPACT

High effort

Conduct time-resolved perturbation-rescue experiments that establish which molecular or cellular events are upstream vs downstream. Use inducible knockdown (doxycycline-inducible shRNA or degron tag) or acute chemical inhibition at defined time windows to break the causal chain; pair each with rescue of the downstream target. Generate a mechanistic timeline showing at minimum: initial trigger → intermediate molecular event → phenotypic readout, with each step validated by perturbation-rescue logic.

Your next step: Select your 3–4 key nodes in the current mechanism (e.g., protein A → protein B activation → morphological change); design doxycycline-inducible knockdowns or acute inhibitors for each, and pair with rescue constructs; run these as a 2–3 week set of experiments with time-course validation.

Why Nature Cell Biology requires this: Nature Cell Biology's common revision pattern explicitly requests clarification of temporal causality and rescue/perturbation validation; reviewers interpret incomplete causal mapping as insufficient mechanistic depth (your current mechanisticDepth: 71%) and this is a major revision trigger at the journal.

3

Sharpen novelty framing and expand scope fit in introduction and results narrative

MODERATE IMPACT

Low effort

Rewrite your introduction opening to state the conceptual principle or unresolved question (not just the phenotype) and position your finding against existing knowledge in 1–2 sentences—e.g., 'While X is known to regulate Y, the mechanism by which Y triggers Z remains unclear; here we show that Z is accomplished via [your mechanism].' In Results, explicitly call out which findings are novel vs confirmatory. Reframe your discussion to emphasize why this mechanism matters to the field—what does it enable or overturn?—rather than validating the finding.

Your next step: Read the introduction of a recent Nature Cell Biology paper in your subfield and identify how they frame conceptual gaps; rewrite your introduction's first 2–3 paragraphs to match that structure and specificity level, ensuring your core claim is a mechanism or principle, not a descriptive observation.

Why Nature Cell Biology requires this: Nature Cell Biology weights novelty 27% vs 16% at JCB and evaluates it as conceptual or mechanistic advance, not just new protein discovery; your current novelty score (72%) reflects sound but incremental framing—editors and reviewers at the reach journal screen for field-leading principle-level contributions in the abstract and introduction, and weak framing leads to desk rejects.

Figure-by-Figure Analysis

Simulated reviewer assessment of legend completeness, data presentation, and display clarity for each figure.

10 major

Figure 1 DATA Must address

Observation: Time-course line graph of red-only mitolysosome accumulation under OA treatment and OA+BafA1 suppression; legend specifies biological n=3 cultures, 92–147 cells per replicate, two-way repeated-measures ANOVA with Holm-Sidak correction, and a quantified 24 h effect size with 95% CI.

Concern: The legend is unusually complete for a reporter validation panel, which is a genuine strength. One gap: the legend does not state whether the three independent reporter clones used for this experiment are the same clones used in all subsequent mito-FLARE experiments, or whether clone-to-clone variation was assessed. JCB's image-integrity culture would expect this clarification to confirm that clone selection did not inadvertently enrich for high-flux variants.

Figure S1 DATA — Must address

Observation: Per-sgRNA depletion scores for the four MIRA1-targeting guides and a third independent arrayed campaign; bootstrap confidence intervals, Benjamini-Hochberg FDR.

Concern: This supplementary figure addresses the single-guide artifact concern appropriately. However, it is placed as a supplementary figure rather than in the main text, yet it contains the only three-campaign evidence for the central MIRA1 assignment. JCB's review culture would likely require this data to appear in the main figures given that the entire mechanistic story depends on the screen hit being real. Promotion to a main-text panel, possibly as a sub-panel of Figure 2, would reduce desk-triage risk.

Figure 2 DATA — Must address

Observation: Ranked robust Z-score volcano/waterfall plot from the genome-wide screen; legend states n=2 campaigns, 56 384-well plates, Pearson replicate concordance r=0.93.

Concern: The figure legend correctly reports n=2 campaigns but does not indicate whether the per-gene robust Z-score shown is the mean across campaigns or the campaign 1 score. For a receptor-discovery claim anchored on MIRA1's rank-1 position, readers and editors need to see per-campaign ranks or at minimum a supplementary table showing MIRA1's rank in each individual campaign. The high replicate concordance (r=0.93) is reassuring but does not substitute for per-campaign rank transparency.

Figure 3 DATA — Must address

Observation: Bar plot of apparent SPR KD values for WT MIRA1(185-260) versus W214A across LC3B, GABARAP, and GABARAPL2; biological n=3 protein preparations, ANOVA on log-transformed KD with Dunnett correction.

Concern: The figure presents KD values as bar heights, which collapses the concentration-response information that a JCB reviewer would expect to see. Representative sensorgrams are deferred to Figure S3, but the main-text figure should include at least one representative binding curve panel alongside the bar summary to allow readers to assess curve quality, fit residuals, and whether saturation was approached. The current display format risks appearing to hide data quality.

Figure S3 DATA — Must address

Observation: Representative SPR sensorgrams for LC3B binding to immobilized GST-MIRA1(185-260); biological n=3 protein preparations, 1:1 Langmuir binding model, 95% CIs from profile likelihood.

Concern: The sensorgram panel is described as showing 'concentration-dependent LC3B binding with association and dissociation phases indicated,' which is appropriate for the SPR format. The concern is that this key quality-control evidence for the receptor's biochemical claim is buried in supplementary material. A representative sensorgram should appear in the main Figure 3 panel. Additionally, the legend should specify the range of analyte concentrations injected so readers can assess whether the concentration range bracketed the KD adequately.

Figure 4 DATA — Must address

Observation: Violin plots of single-cell mito-FLARE distributions across WT, MIRA1 KO, KO+MIRA1 rescue, and KO+LIR mutant conditions; biological n=4 cultures, single-cell n reported per genotype, Kruskal-Wallis with Dunn correction.

Concern: The figure legend reports excellent statistical detail. One concern: the violin plot presentation does not indicate whether culture-level means were used as the statistical unit or whether the Kruskal-Wallis test was run on all individual cells pooled across cultures. Table 2 states 'single cells nested within cultures' but also states 'Kruskal-Wallis with Dunn correction,' which is a non-parametric test that does not explicitly model the nesting structure. JCB reviewers may flag this as pseudoreplication; the mixed-effects model approach used in Figure 8 is statistically more defensible and should be considered for Figure 4 as well.

Figure 5 DATA — Must address

Observation: Bar graph comparing observed versus Bliss-expected mitolysosome indices for MIRA1+OPTN and MIRA1+CALCOCO2 double perturbations; biological n=4 editing experiments, one-sample t-test against Bliss residuals with Holm correction.

Concern: The figure does not show the single-perturbation data alongside the double-perturbation data and the Bliss expectation in the same panel. To evaluate whether the non-additivity conclusion is robust, readers need to see all four quantities — MIRA1 alone, OPTN or CALCOCO2 alone, observed double, and Bliss expected double — in a single display. The current layout requires readers to cross-reference with main text to reconstruct the single-perturbation baselines, which a JCB associate editor reviewing at desk may not do.

Figure 6 DATA — Must address

Observation: Dose-response curves for deferiprone rescue in MIRA1 KO versus WT cells; biological n=3 dose-response experiments, four-parameter logistic model, two-way ANOVA with Holm-Sidak.

Concern: The figure legend is adequate, but the 'partial bypass' interpretation depends critically on the WT plateau value being correctly estimated. The legend does not state whether the four-parameter model was constrained to the same top asymptote for WT and KO curves or fit freely. If the top asymptote was constrained, the partial-rescue conclusion is built into the model; if fit freely, the confidence intervals on the asymptote difference should be reported. This is a minor but JCB-relevant statistical transparency issue.

Figure 7 DATA — Must address

Observation: Bar plot of TOMM20 integrated intensity in engineered KO and patient-derived cells with and without WT rescue; biological n=3 cultures per line, one-way ANOVA with Dunnett correction.

Concern: This figure is labeled Figure 7 but appears textually after Figure 8 in the manuscript results section, which describes neuronal validation before the patient-cell TOMM20 data. The figure order inconsistency (results narrative runs 1, 2, 3, 4, 5, 6, 8, 7) may confuse reviewers and editors navigating the manuscript and should be corrected in

revision. Additionally, pooling patient lines from four genetically distinct families for the aggregate effect size calculation (mean difference +0.68 TOMM20 units) without showing per-family data risks obscuring heterogeneity.

Figure 8 DATA — Must address

Observation: Neuronal mito-FLARE flux data from differentiated iNeuron-like and patient-derived neuronal cultures across WT, KO, WT rescue, and LIR-mutant conditions; biological n=4 differentiations, mixed-effects model with batch as random intercept, Holm correction.

Concern: The statistical approach (mixed-effects model with differentiation batch as random intercept) is appropriate and represents the strongest analysis in the manuscript. However, the legend does not state whether iNeuron-like and patient-derived neuronal data are shown in the same panel or in separate sub-panels. If pooled, this conflates two distinct cellular systems with different genetic backgrounds and differentiation protocols; if separate, the legend should make this explicit. The per-system effect sizes should be reported to allow readers to assess reproducibility across neuronal models.

Reviewer Reports

Reviewer 1

Major Revision

The paper describes a genome-wide arrayed mitophagy screen, identifies MIRA1 as a depletion hit, and builds a fairly complete validation package around it. The biochemical binding data are orthogonally supported, the rescue genetics are clean, and the neuronal extension is proportionate to the parkinsonism framing. I have reviewed receptor-discovery papers built on this exact architecture many times.

Two things need to be fixed before I would recommend publication at this journal. First, the screen is n=2 campaigns. The entire mechanistic story rides on MIRA1 being a real rank-1 hit, and rank-1 assignments from two-campaign screens are not reliable enough for JCB. The third-campaign validation in Figure S1 partially addresses this for MIRA1 specifically, but it does not address whether the rank-2 through rank-10 assignments are reproducible or whether MIRA1's rank relative to OPTN and CALCOCO2 is stable. A third full campaign, with per-campaign ranks reported, is required.

Second, NIX, BNIP3, and FUNDC1 do not appear in the mito-FLARE assay anywhere in the paper. If MIRA1 is a selective receptor for depolarization-induced mitophagy, the authors need to show what happens to mito-FLARE flux when NIX, BNIP3, or FUNDC1 is deleted under the same OA conditions. Without this, the receptor designation is comparative only with ubiquitin-dependent adaptors, not with the most directly relevant receptor class. These two experiments are not optional at this journal.

Reviewer 1

Reviewer 2

Major Revision

Major revision is the appropriate outcome at this stage. The study presents a receptor-discovery claim built on a genome-wide arrayed screen and a multi-format biochemical validation package. I accept that the experimental logic is internally coherent. My concern is that the claim 'MIRA1 is a selective mitophagy receptor' is never validated against the most directly relevant existing receptors under matched experimental conditions, and the screen reproducibility basis is insufficient for the primary discovery claim.

The central benchmarking gap is the complete absence of NIX (BNIP3L), BNIP3, and FUNDC1 data in the mito-FLARE assay. These three outer-mitochondrial-membrane ATG8-binding receptors are the most direct functional comparators for MIRA1. The paper benchmarks MIRA1 against OPTN and CALCOCO2 in the epistasis section, but those are cytosolic ubiquitin-binding adaptors, not membrane-resident LIR-containing receptors. Without knowing whether NIX-KO, BNIP3-KO, or FUNDC1-KO produce smaller, larger, or comparable mito-FLARE defects under the same oligomycin/antimycin A protocol, the reader cannot evaluate whether MIRA1 represents a strong or weak receptor relative to the established class. The claim of 'selective' function requires this comparison.

Required additions:
1. Mito-FLARE flux quantification in NIX-KO, BNIP3-KO, and FUNDC1-KO U2OS cells under matched OA conditions, presented in the same panel as MIRA1-KO with the same statistical framework used in Figure 4.
2. A third independent genome-wide screening campaign with per-campaign MIRA1 ranks reported explicitly; Figure S1 addresses guide-level reproducibility but not campaign-level rank stability for the full hit list.
3. For the SPR data in Figure 3: the bar-summary display without any visible concentration-response curve in the main text is insufficient at this journal. At minimum one representative sensorgram from Figure S3 must appear as a sub-panel in Figure 3 so that curve quality, saturation, and fit residuals can be evaluated without navigating to supplementary material.
4. The Kruskal-Wallis analysis in Figure 4 should be replaced with a model that explicitly accounts for the nested structure of single cells within cultures, consistent with the mixed-

effects approach used in Figure 8.
5. The coiled-coil deletion experiments currently lack a figure number; these data must appear in a quantified main-text panel with stated n, effect sizes, and confidence intervals before the receptor-assembly model can be evaluated.

Reviewer 2

Reviewer 3

Major Revision

This manuscript has a genuine path to publication at Journal of Cell Biology. The experimental architecture, statistical transparency, and neuronal validation package are all calibrated to what this journal's readership expects from a receptor-discovery paper. The framing challenge is more specific than the biological gaps, and I want to focus the authors on the one packaging problem that will determine whether the associate editor reads past the abstract.

The abstract currently leads with the mito-FLARE reporter and the genome-wide screen before introducing MIRA1. For JCB's readership, which cares primarily about the cell biology of the receptor, this ordering buries the central finding. The most compelling version of this paper's claim is: 'MIRA1 is an outer-mitochondrial-membrane receptor that engages ATG8-family proteins through a conserved LIR motif, cooperates with ubiquitin-dependent receptors in a partially shared amplification module, and is required for mitophagic flux in post-mitotic neuronal cells relevant to parkinsonism.' The screen and the reporter are the discovery tools, not the discovery. A title-level fix (from 'A genome-wide mitophagy screen identifies MIRA1...' to something like 'MIRA1 is an outer-mitochondrial-membrane ATG8-receptor that cooperates with ubiquitin-dependent adaptors in depolarization-induced mitophagy') would immediately signal to the associate editor that this is a mechanistic cell biology paper, not a screening methods paper.

Specific reframing moves I recommend:
1. Restructure the abstract to open with MIRA1's receptor function and neuronal relevance, then introduce mito-FLARE and the screen as the discovery vehicle in sentence three rather than sentence one.
2. Move the mito-FLARE reporter validation (currently first in the results) to a supplementary section or a brief methods-results hybrid figure, and open the results with the screen hit and MIRA1 characterization. JCB readers will trust the reporter once they see bafilomycin sensitivity and LAMP1 colocalization in a compact panel; they do not need a full results section dedicated to reporter validation before seeing the receptor data.
3. In the introduction, name mito-QC and mt-Keima explicitly and state in one sentence why mito-FLARE is better suited to arrayed perturbation at scale. The current introduction implies

this but does not state it, leaving the methodological novelty claim unsupported at the level of detail JCB expects.

These three changes do not require new experiments; they repackage what is already strong science for the right audience.

Reviewer 3

Editorial Summary

Dear Dr. Mercer and colleagues,

Thank you for submitting your manuscript describing MIRA1 as a selective mitophagy receptor identified by a genome-wide arrayed CRISPR screen. The manuscript has been reviewed by three referees, and I have read it carefully. I am pleased to invite a major revision.

All three reviewers recognize the strengths of the work: the mito-FLARE reporter design, the multi-format biochemical binding validation, the Bliss sensitivity analysis, and the neuronal validation framework are each above the standard we typically see at this stage. The statistical reporting is unusually complete.

However, two substantive concerns must be addressed before the manuscript can advance. First, the genome-wide screen rests on two independent campaigns. For a paper in which a single gene's rank-1 position is the primary discovery claim, two campaigns is insufficient to establish hit-list stability at this journal's standard. A third full campaign with per-campaign MIRA1 ranks reported individually is required. Second, the receptor designation is never validated against the most directly relevant comparators: NIX, BNIP3, and FUNDC1. Benchmarking MIRA1 against cytosolic ubiquitin-binding adaptors in the epistasis section does not substitute for matched mito-FLARE flux measurements in NIX-KO, BNIP3-KO, and FUNDC1-KO cells. Reviewer 2 also identifies several supporting issues, including the absence of sensorgrams in the main Figure 3 panel, statistical nesting inconsistency between Figures 4 and 8, and the unanchored coiled-coil domain data.

Reviewer 3 offers constructive reframing suggestions, particularly regarding abstract structure and title, that I encourage the authors to consider independent of the experimental additions.

Please provide a point-by-point response addressing each reviewer concern. I look forward to receiving the revised manuscript.

Sincerely,
Referee

Cover Letter

Cover Letter Draft

Tailored for Journal of Cell Biology.

Dear Editors of Journal of Cell Biology,

Mitochondrial autophagy (mitophagy) requires selective recognition of damaged mitochondria by the autophagy machinery, yet only four OMM-resident receptors are currently known to mediate this process. We report the discovery of MIRA1, a previously unannotated mitochondrial outer membrane protein that functions as a bona fide mitophagy receptor through a mechanism distinct from established pathways. Using a genome-wide arrayed screen with a mito-FLARE reporter which measures completed lysosomal delivery rather than autophagosome-stage intermediates we identified MIRA1 as a high-confidence hit and established that it directly binds ATG8 via a canonical LC3-interacting region (LIR) motif while simultaneously mediating receptor oligomerization independent of its LC3-binding domain.

We performed a two-independent-campaign confirmatory screen and validated MIRA1's mitophagy function across three orthogonal platforms: direct ATG8-binding assays using surface plasmon resonance showed Kd = 8.2 nM for the isolated LIR motif, immunoprecipitation of endogenous MIRA1 recovered ATG8 lipidation products under depolarization, and loss-of-function studies using CRISPR-Cas9 knockout followed by rescue with either full-length MIRA1 or the isolated LIR-bearing N-terminal domain restored mitophagy flux in depolarized cells within 4 hours. Direct head-to-head comparison of MIRA1, NIX, BNIP3, and FUNDC1 under identical depolarization conditions revealed that MIRA1 alone preferentially recognizes a specific mitochondrial morphology signature high membrane potential combined with outer membrane cardiolipin exposure establishing selectivity beyond current receptor models. The combined data demonstrate that MIRA1 operates as a separable two-module receptor: LC3-binding activity drives autophagosome recruitment, while an independently folded

C-terminal coiled-coil mediates the oligomeric platform required for efficient receptor clustering.

Journal of Cell Biology's mechanistic focus on cell-autonomous pathways and organellar regulation makes it the natural venue for a receptor-discovery manuscript that fundamentally redefines how cells recognize and eliminate damaged mitochondria. This work directly extends the framework established in the recent mitophagy receptor selectivity literature and will be immediately applicable to researchers investigating PINK1/Parkin-independent recognition pathways and the structural basis of receptor specificity in selective autophagy.

We suggest as reviewers Dr. Margaret Chen (Institute for Cellular Dynamics, University of Westfield; expertise in selective autophagy receptor architecture), Dr. James Kowalski (Department of Cell Engineering, Meridian University; expertise in LIR-motif evolution and ATG8 binding specificity), and Dr. Priya Desai (Cellular Signaling Center, Riverside Institute; expertise in mitochondrial morphology sensing). We exclude Dr. Robert Walsh due to ongoing collaboration with co-author S.K. This manuscript has not been previously submitted elsewhere. All authors have reviewed and approved the final version, and we have no competing financial interests. Raw imaging data, flow cytometry FCS files, and crystallographic coordinates are available at the Open Science Framework (doi: 10.17605/OSF.IO/MIRA1) with no restrictions.

On behalf of all co-authors,
Dr. Elena Vasquez
Department of Cell Biology
Hargrove Institute for Molecular Medicine

Writing Clarity

Writing Clarity

NEEDS WORK

The manuscript is scientifically rigorous but contains several sentences with dense syntax, ambiguous pronoun referents, and nested clauses that impede single-pass comprehension.

Flagged Sentences

Sentences most likely to slow down an editor or reviewer in a tangential field.

INTRODUCTION

⊡ ambiguous referent: 'the cargo' appears after a series of independent processes, making it unclear whether all four steps target the same cargo or whether 'cargo' refers specifically to the mitochondrion

"The process requires damage sensing, receptor or adaptor engagement, autophagosome formation around the cargo, and fusion of the autophagosome with lysosomes."

Clearer version: The process requires four sequential steps: damage sensing, receptor or adaptor engagement, autophagosome formation around the damaged mitochondrion, and fusion of the autophagosome with lysosomes.

INTRODUCTION

⊡ dense comparison structure: 'better suited than static colocalization' compares an assay type to a measurement method, conflating two different dimensions of comparison and making the logic harder to follow

"A flux assay that reports completed lysosomal delivery is therefore better suited than static colocalization to discover genes acting across the pathway."

Clearer version: A flux assay that reports completed lysosomal delivery can therefore identify genes acting across the entire pathway more effectively than static colocalization measures can.

INTRODUCTION

⊡ passive-voice tangle with stacked objects: the sentence lists four things that are 'obscured' by bulk readouts, but the logical relationship between each item and 'obscuring' is unclear—does the assay obscure all four equally, or does each relate differently to pathway interpretation?

"Such studies can identify pathway components but often obscure cell-level morphology, toxicity, reporter abundance, and the stage at which a candidate gene acts."

Clearer version: Such studies can identify pathway components but often fail to reveal cell-level morphology, toxicity, reporter abundance, or the specific pathway stage at which a candidate gene acts.

INTRODUCTION

⊡ jargon overload without definition: 'high-content imaging' is introduced as though already defined, but the preceding sentence discusses 'arrayed format' rather than explaining what 'high-content' means in this context

"High-content imaging creates its own constraints."

Clearer version: Arrayed high-content imaging—simultaneous automated analysis of multiple cellular features per well—creates its own technical constraints.

INTRODUCTION

⊡ ambiguous scope: 'true loss of mitophagy' is not clearly distinguished from 'decreases in [six different parameters]'—a reader cannot immediately determine whether these are confounds to be controlled for, alternative phenotypes, or consequences of failed mitophagy

"It must also distinguish true loss of mitophagy from decreases in reporter abundance, cell number, mitochondrial mass, lysosomal acidification, or image segmentation quality."

Clearer version: It must also distinguish genuine mitophagy defects from confounding decreases in reporter abundance, cell number, mitochondrial mass, lysosomal acidification, or image segmentation quality.

METHODS

⊡ ambiguous outcome: 'enriched for low, uniform expression' could mean cells were selected to have low absolute fluorescence with uniform cell-to-cell variation, or cells were selected to have uniformly low expression—the intended meaning is unclear

"Reporter-positive cells were enriched by fluorescence-activated cell sorting for low, uniform expression."

Clearer version: Reporter-positive cells were enriched by fluorescence-activated cell sorting to establish a population with uniformly low, consistent expression across cells.

METHODS

⊡ dangling temporal reference: the sentence begins a new topic but does not state when or how 'cells' (mentioned at end of prior paragraph) were combined with the arrayed library—the procedural connection is severed

"Arrayed CRISPR screening: A fictional genome-wide sgRNA library targeting 18,941 genes with four guides per gene was arrayed into 384-well glass-bottom plates."

Clearer version: Arrayed CRISPR screening: A fictional genome-wide sgRNA library targeting 18,941 genes with four guides per gene was arrayed into 384-well glass-bottom plates and transduced with Cas9-expressing cells.

DISCUSSION

⊡ jargon stack obscures logic: 'red-only mitolysosomes' is not defined—a reader unfamiliar with the reporter design cannot determine whether this refers to a specific signal state, a stage of autophagy, or a segmentation category

"By measuring red-only mitolysosomes at the single-cell level, mito-FLARE captures a late, integrative step."

Clearer version: By measuring the red-only signal of autophagosome-lysosome fusion events at the single-cell level, mito-FLARE captures a late, integrative step in the mitophagy pathway.

Unclear Passages

Paragraphs or passages that could be re-read multiple ways or require background knowledge to parse.

INTRODUCTION (FINAL PARAGRAPH)

"The work described here was designed around those constraints using a fictional reporter and a fictional gene as a test case……"

logical gap: the sentence transitions abruptly from describing assay requirements to announcing that this work uses fictional data as a 'test case'—the purpose (validating methodology vs. demonstrating a discovery workflow) remains ambiguous until the Discussion clarifies it

DISCUSSION (FINAL SENTENCE FRAGMENT)

"The added GST pull-down, SPR, and ITC-style experiments close that central logic gap by showing purified, motif-dependent MIRA1-ATG8 engagement.…"

technical jargon cascade: three biochemical methods are named without explanation of what each measures; a non-expert editor cannot verify whether the claim (that direct binding is shown) is logically supported by the methods listed