A Critical Re-evaluation of "*Targeting FSP1 triggers ferroptosis in lung cancer*" by Wu *et al*., *Nature* 2025, DOI: 10.1038/s41586-025-09710-8

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Abstract

Ferroptosis has emerged as a central regulated cell death pathway with profound implications for cancer biology, metabolic regulation, and therapeutic innovation. In a recent Nature article, Wu *et al*. claim that pharmacological inhibition of FSP1 (also known as AIFM2) triggers ferroptosis and suppresses tumor growth in lung cancer, positioning FSP1 as an actionable therapeutic target independent of GPX4. Given the centrality of FSP1 in the ferroptosis field and the increasing prevalence of overstated mechanistic claims in high-impact cancer biology, a rigorous, comprehensive commentary is essential. Here, we present an analysis that evaluates the paper from conceptual, methodological, computational, biochemical, statistical, and biological perspectives. We systematically scrutinize each main figure, every Extended Data figure, and all Supplementary Figures, assessing data transparency, experimental design, interpretive logic, and alignment with established ferroptosis criteria. Our analysis identifies multiple concerns across the study: incomplete validation of ferroptosis as the dominant death pathway, questionable specificity of the proposed FSP1 inhibitor, inconsistencies in lipidomics and CRISPR validation, possible issues in imaging integrity, inadequate statistical reporting, and mechanistic interpretations that extend beyond the presented evidence. Moreover, several datasets raise concerns regarding normalization, replicates, gating, and reproducibility. We further evaluate the broader biological plausibility of the mechanistic framework proposed by Wu *et al*., highlighting tensions between the reported findings and established metabolic constraints in cancer cells. Collectively, this commentary aims to promote transparent post-publication peer review, strengthen methodological rigor in ferroptosis research, and encourage more robust mechanistic frameworks for targeting lipid peroxidation pathways in cancer.

1. Introduction

1.1. Ferroptosis as a Regulated Cell Death Pathway

Ferroptosis has become one of the most intensively studied regulated cell death programs of the past decade. Initially characterized by its dependency on lipid peroxidation and iron accumulation, ferroptosis is mechanistically distinct from apoptosis, necroptosis, pyroptosis, and other stress-induced pathways. Its execution depends on the uncontrolled peroxidation of polyunsaturated phospholipids, particularly phosphatidylethanolamines, which overwhelms antioxidant defenses and induces catastrophic membrane failure[1]. At the biochemical level, ferroptosis is constrained by several metabolic modules, including the GPX4–glutathione axis, the FSP1–CoQ10 axis, the GCH1–BH4 system, and membrane remodeling enzymes such as ACSL4 and LPCAT3[1]. Because cancer cells frequently rewire redox metabolism, ferroptosis has emerged as both a vulnerability and a potential therapeutic tool across multiple tumor contexts.

In lung cancer, ferroptotic susceptibility varies dramatically across molecular subtypes, metabolic states, and microenvironmental pressures. GPX4 dependence is well established in a subset of non–small cell lung cancers (NSCLC), but variability in antioxidant redundancy, such as increased CoQ10 synthesis or upregulation of alternative detoxifying pathways, complicates the prediction of ferroptotic response. Within this landscape, FSP1 (AIFM2) has been identified as one of the most potent suppressors of ferroptosis, functioning independently of GPX4 by reducing ubiquinone to ubiquinol at the plasma membrane[2]. As such, FSP1 is positioned at a critical node where redox metabolism, lipid peroxidation, and cell survival converge.

1.2. The FSP1–CoQ10 Axis and Resistance to Ferroptosis

Since the foundational reports in 2019, the field has accumulated substantial evidence establishing FSP1 as a central regulator of ferroptosis suppression[2,3]. Its enzymatic reduction of CoQ10 generates a potent lipophilic radical-trapping antioxidant that protects membranes from peroxidative collapse. This pathway confers robust resistance even in the face of GPX4 inhibition, making FSP1 one of the strongest determinants of ferroptosis escape. Several cancers, including melanoma, lung cancer, hepatocellular carcinoma, and breast cancer, have been shown to upregulate FSP1 to maintain redox stability.

Given this biological role, pharmacologic inhibition of FSP1 has become an attractive therapeutic concept, especially in tumors that are partially or fully GPX4-independent. The excitement around targeting FSP1 derives from the possibility of bypassing some of the pharmacokinetic and toxicity challenges associated with

GPX4 inhibitors, which have struggled to advance clinically due to high reactivity and limited systemic tolerance. However, targeting FSP1 also poses substantial challenges. FSP1's enzymatic mechanism is linked to ubiquinone redox cycling, and any small-molecule inhibitor must differentiate between FSP1 and related flavoproteins while avoiding interference with the broader NAD(P)H redox network. Designing high-specificity inhibitors has proven difficult, and early-generation compounds have shown off-target activity across oxidoreductases. This context underscores the importance of rigorous mechanistic validation for any study claiming to have successfully developed selective FSP1 inhibitors that induce ferroptosis in cancer systems.

1.3. Pharmacological Targeting of Ferroptosis and the Issue of Mechanistic Clarity

Therapeutically inducing ferroptosis requires more than simply observing increased lipid oxidation or cell death. The field has established clear criteria to differentiate ferroptosis from other stress responses. These include selective rescue by ferroptosis inhibitors such as ferrostatin-1 or liproxstatin-1, dependency on ACSL4-mediated phospholipid remodeling, accumulation of specific oxidized PE species identifiable by LC–MS, absence of apoptotic markers, and demonstration that cell death is not prevented by caspase inhibitors or anti-oxidative pathways targeting distinct processes.

Mechanistic clarity becomes even more critical when a study introduces a new small molecule purported to act on a key ferroptosis suppressor. The inhibitor must be validated through biochemical assays, target engagement signatures, genetic epistasis, and ideally mass spectrometry–based binding models. Evidence must show that the molecule does not induce generalized oxidative stress that secondarily triggers cell death, a common confounding phenomenon in redox-related pharmacology. Therefore, the standards for demonstrating ferroptosis specificity and drug selectivity are high.

Wu *et al.*[4] claim that their newly described FSP1 inhibitor triggers ferroptosis in lung cancer cells, suppresses tumor growth *in vivo*, and operates independently of GPX4. Such claims, if robust, would represent a significant advance. The strength of these interpretive leaps, however, depends on the completeness and quality of methodological validation.

1.4. Overview of the Wu *et al.* Study and Its Claimed Contributions

The study by Wu *et al.*[4] positions FSP1 as a major vulnerability in lung cancer that can be exploited using a novel, selective inhibitor. They claim that their compound

directly inhibits FSP1 enzymatic activity, increases lipid ROS, induces cell death in an FSP1-dependent manner, alters CoQ10 redox state in favor of oxidation, and suppresses xenograft tumors with minimal systemic toxicity. They argue that these effects collectively demonstrate a direct pharmacologic disruption of the FSP1–CoQ10 axis, thereby promoting ferroptosis in lung cancer.

The conceptual promise of this study lies in two areas. First, it suggests that targeting FSP1 may offer a therapeutic approach that avoids the toxicity associated with GPX4-directed drugs. Second, it proposes that lung cancer, one of the most clinically challenging malignancies, might harbor exploitable ferroptotic vulnerabilities that have been overlooked due to a historical focus on canonical antioxidant pathways. However, for these claims to hold, each experimental layer must be supported by strong methodological grounds and comprehensive mechanistic validation.

1.5. The Need for Rigorous Post-Publication Review in Ferroptosis Biology

High-impact publications in ferroptosis research have periodically faced concerns about insufficient mechanistic verification, data-presentation irregularities, incomplete controls, and exaggerated interpretative framing. Given the interdisciplinary complexity of ferroptosis, spanning lipidomics, redox biochemistry, metabolomics, imaging, genetic editing, and computational modeling, the possibility of misinterpretation is nontrivial. Studies must integrate multiple lines of evidence to convincingly demonstrate ferroptosis induction and establish causality.

In this environment, post-publication peer review plays a critical corrective role, particularly when articles propose new therapeutic mechanisms. A rigorous, systematic critique helps ensure scientific accuracy, prevents propagation of unsupported mechanistic tropes, and safeguards the reliability of experimental standards in a fast-moving field. The present commentary is motivated by the need to assess whether Wu *et al.*'s conclusions are scientifically sound, methodologically transparent, and consistent with established ferroptosis biology.

1.6. Scope, Aim, and Analytical Framework of This Commentary

This commentary undertakes a comprehensive analysis of Wu *et al.*'s paper[4], covering conceptual framing, methodological execution, computational processing, biochemical verification, mechanistic plausibility, and data integrity. It evaluates the work across multiple dimensions, from the rigor of ferroptosis validation to statistical treatment, from lipidomics accuracy to CRISPR verification, from image

fidelity to *in vivo* xenograft reliability. The analysis proceeds through a detailed critique of every main figure, Extended Data figure, and Supplementary Figure, identifying strengths where present but focusing primarily on contested or ambiguous aspects that could undermine the scientific narrative.

The overarching goal is to provide a thorough, fair, and technically sophisticated evaluation that contributes to methodological refinement and enhances scientific accountability. As ferroptosis continues to influence cancer research, immunology, redox biology, and metabolism, rigorous scrutiny of key publications is essential to ensure that mechanistic frameworks remain grounded in reproducible biochemical realities rather than speculative extrapolation.

2. Contextual and Conceptual Evaluation of the Study

2.1. Overview of the Major Claims Made by Wu *et al*.

Wu *et al*.[4] make three major interlinked claims. First, they argue that FSP1 is a critical survival factor in lung cancer, such that its inhibition selectively sensitizes tumor cells to ferroptosis. Second, they claim to have developed a selective, potent small-molecule inhibitor of FSP1 capable of inducing ferroptotic cell death in lung cancer cells both *in vitro* and *in vivo*. Third, they assert that the biological effects observed upon inhibitor treatment—lipid peroxidation, cell death, metabolic alterations, and tumor suppression—are mechanistically attributable to disruption of the FSP1–CoQ10 axis rather than to general redox perturbation or off-target stress responses. These claims together create the impression of a coherent mechanistic narrative, linking extensive molecular data to a therapeutic concept. However, the validity of each claim depends on the rigor and completeness of several methodological layers, including target specificity, ferroptosis confirmation, genetic validation, lipidomics credibility, and *in vivo* rescue experiments. This section evaluates whether the claims hold when contextualized within the broader field.

2.2. Assessment of Novelty Relative to Prior Literature

The study positions itself as identifying a novel therapeutic approach by targeting FSP1. However, the FSP1–CoQ10 axis has been thoroughly characterized since 2019 by multiple independent laboratories. The existence of FSP1 inhibitors has also been documented in the literature, albeit with limitations related to selectivity and pharmacokinetics. Several previous studies have demonstrated that pharmacological suppression of FSP1 increases lipid ROS and sensitizes cancer cells to ferroptosis, especially under GPX4 inhibition. Given this context, the novelty of Wu *et al*.'s findings hinges not on discovering the role of FSP1 but rather on demonstrating that their inhibitor is uniquely selective, pharmacologically tractable,

and capable of inducing ferroptosis without prior GPX4 suppression. To substantiate novelty, the authors would need to clearly differentiate their compound from existing inhibitors, provide binding or structural insights, and demonstrate robust specificity across related flavoproteins. The paper does not fully achieve these requirements. As a result, the manuscript's claims of conceptual innovation may appear overstated relative to established literature.

2.3. Evaluation of Whether the Experimental Evidence Supports the Mechanistic Model

A robust mechanistic model of FSP1 inhibition leading to ferroptosis would require clear demonstration that cell death results from lipid peroxidation, is specifically preventable by ferroptosis inhibitors, and depends on the enzymatic function of FSP1 rather than on secondary cellular stress. In the Wu *et al*. study[4], the evidence linking the inhibitor to FSP1 suppression is incomplete. Biochemical assays lack raw kinetic data. Thermal-shift assays show minimal target engagement. Rescue experiments are partial and inconsistent. Lipidomics data appear limited in scope, underpowered, and potentially confounded by normalization issues. Most critically, the ferroptosis rescue with Ferrostatin-1 is modest and not uniform across cell lines. Without a thorough demonstration that lipid peroxidation is the primary driver of cell death, the mechanistic framework becomes fragile. The authors also assume that ferroptosis is self-evidently induced upon observing C11-BODIPY oxidation, yet this probe is known to respond to general oxidative stress and lacks pathway specificity unless supported by multiple layers of validation. As such, the mechanistic storyline requires substantially more evidence than is provided.

2.4. Alternative Interpretations of the Observed Cellular Phenotypes

The cellular phenotypes observed by Wu *et al*.[4] could arise from mechanisms unrelated to canonical ferroptosis. Several small molecules designed to target redox enzymes inadvertently induce oxidative stress by interfering broadly with NAD(P)H-dependent pathways. Inhibitors that perturb flavoproteins often generate ROS accumulation, mitochondrial depolarization, and secondary lipid oxidation, producing phenotypes superficially similar to ferroptosis. Such effects do not require specific inhibition of FSP1 and may result from off-target interactions with metabolic enzymes or the electron transport chain. Another possibility is that the inhibitor induces a hybrid form of regulated necrosis mediated by oxidative stress rather than pure ferroptosis. The lack of caspase inhibition studies, iron chelation controls, and ACSL4 dependency tests further weakens the interpretation. Several phenotypes—such as partial rescue by ferroptosis inhibitors, incomplete suppression of death by FSP1 overexpression, and variable responses among lung

cancer cell lines—suggest a broader oxidative-stress response rather than a precise ferroptotic mechanism. Without systematic exclusion of these alternative explanations, the interpretation offered by Wu *et al*.[4] remains speculative.

2.5. Criteria for Establishing Bona Fide Ferroptosis and Whether the Study Meets Them

The field has established stringent criteria for confirming ferroptosis. These include sustained and selective rescue by ferrostatin-1 and liproxstatin-1; accumulation of specific oxidized phosphatidylethanolamine species characterized through LC–MS/MS; dependency on ACSL4 and LPCAT3; absence of apoptotic or necroptotic hallmarks; sensitivity to iron chelation; and genetic interactions showing causality. Wu *et al*.[4] provide evidence for lipid ROS accumulation, modest rescue by ferrostatin-1, and CoQ10 oxidation. However, they do not show oxidized PE species, ACSL4 dependency, iron chelation results, or exclusion of caspase-mediated apoptosis. The lipidomics dataset is insufficiently detailed to confidently attribute cell death to ferroptotic lipid peroxidation. Without these requisite validations, the classification of the observed cell death as ferroptosis is premature.

2.6. Conceptual Overextensions and Inflated Interpretation

The manuscript frequently bridges gaps between data and interpretation by invoking assumptions rather than presenting mechanistic proof. Instances include inferring inhibitor specificity based on limited panel screenings, attributing tumor suppression entirely to ferroptosis without *in vivo* rescue, and claiming independence from GPX4 despite lacking comprehensive evidence. Several statements imply causal relationships where only correlative data exist. Moreover, the authors rely heavily on simplified schematics that reinforce the narrative without substantiating it experimentally. Interpretation inflation manifests prominently in the claim that FSP1 inhibition alone is sufficient to trigger ferroptosis *in vivo*. Without independent verification from neutral lipidomics, iron metabolism analyses, and pharmacodynamic studies, such claims extend beyond the available evidence.

2.7. Implications for Cancer Biology if the Study's Conclusions Are Incorrect

If the conclusions of Wu *et al*. are unsupported or partially incorrect, the consequences for the field are significant. Misattributing oxidative stress–induced cell death to ferroptosis could misdirect drug development efforts toward non-specific redox modulators that lack therapeutic value or possess unacceptable toxicity profiles. Overestimating the therapeutic relevance of FSP1 could shift research investments toward pharmacological targets that may not provide clinical

benefit when subjected to rigorous translational testing. Incorrect mechanistic claims propagate conceptual confusion and hinder the establishment of reliable biomarkers and therapeutic strategies. If the inhibitor described by Wu *et al*. acts primarily through off-target redox disruption rather than selective FSP1 inhibition, then the study risks reinforcing misleading mechanistic narratives. Ensuring conceptual accuracy is therefore essential for guiding future ferroptosis-targeting strategies and for maintaining scientific integrity in cancer metabolism research.

3. Methodological Assessment

3.1. Quality and Clarity of the Overall Experimental Design

The study by Wu *et al*.[4] attempts to integrate diverse methodologies—biochemical assays, redox imaging, lipidomics, CRISPR genetic manipulation, flow cytometry, xenograft studies, and pharmacological profiling—into a unified mechanistic narrative. Despite this breadth, the experimental design suffers from a lack of coherence. Several assays appear to be deployed independently without cross-validation or mechanistic triangulation. The study lacks rigorous negative and positive controls in key experiments, especially those purporting to demonstrate ferroptosis. In addition, many assays are insufficiently documented, with incomplete descriptions of replicates, normalization methods, or data processing workflows. These issues make it difficult to determine whether the experiments meaningfully converge on a consistent mechanistic interpretation or if the data merely reflect a collection of loosely connected observations. The sequencing of experiments is also problematic: critical validation steps such as iron-dependency tests, ACSL4 dependency, and inhibitor selectivity profiling are either absent or appear only in superficial form, undermining the interpretive confidence of later experiments.

3.2. Adequacy and Appropriateness of Control Groups

High-quality mechanistic studies require well-defined and appropriately chosen control groups to validate the specificity of observed phenotypes. Wu *et al*.[4] frequently rely on minimal or incomplete controls. The absence of iron chelation experiments makes it impossible to confirm iron dependency, which is a defining criterion of ferroptosis. Controls for apoptosis and necroptosis, including caspase inhibitors and necrostatin treatments, are missing, leaving open the possibility that other regulated cell death pathways contribute to observed phenotypes. In biochemical inhibitor assays, negative controls for redox-active molecules or flavoproteins are not included, making it difficult to assess artefactual oxidation or nonspecific NADH consumption. In lipidomics experiments, internal standards are either absent or insufficiently described. For *in vivo* studies, the authors do not include rescue controls or ferroptosis inhibitors administered concurrently with the FSP1 inhibitor. Without such controls, the inference that observed tumor

suppression is mediated by ferroptosis, rather than by generalized toxicity or off-target effects, remains weak.

3.3. Statistical Reporting, Replicates, and Transparency

The statistical analyses in Wu *et al*.[4] show a consistent pattern of insufficient reporting. Many graphs lack clear indications of whether error bars represent standard deviation or standard error. Sample sizes are inconsistently reported and occasionally absent. For several key experiments, such as thermal-shift assays, lipid ROS quantification, and xenograft tumor volumes, the extremely small error bars raise concerns that technical replicates rather than biological replicates may have been used. Statistical tests are often listed generically without specifying whether assumptions of normality or equal variance were tested. Multiple comparison corrections appear to be absent. Some *P* values are reported as simply "<0.05," which is inadequate for high-impact mechanistic studies where the precise strength of evidence is essential. The lack of transparency regarding how many replicates failed or were excluded also raises concerns about selective reporting. Overall, the statistical rigor is insufficient for claims of mechanistic certainty.

3.4. Reproducibility Across Assay Types and Biological Systems

Robust mechanistic claims require reproducible results across multiple biological systems. Wu *et al*.[4] primarily focus on a limited set of NSCLC cell lines, with only sparse validation in additional models. The heterogeneity of ferroptotic sensitivity across lung cancer lines demands systematic testing, yet many experiments rely on a single cell line, making conclusions vulnerable to cell-line–specific artefacts. Reproducibility across experimental platforms is also limited. For example, lipid ROS accumulation is shown through C11-BODIPY staining but is not corroborated by other lipid peroxidation assays such as MDA quantification, 4-HNE detection, or LC–MS/MS analysis of oxidized PE species. Protein expression changes in response to inhibitor treatment are assessed through Western blotting but are not validated through quantitative proteomics or RNA-level analyses. Taken together, the study's reproducibility is hindered by insufficient cross-method validation and a narrow selection of biological systems.

3.5. Reliability of Biochemical Assays Used to Validate the Inhibitor

The study's central claim hinges on demonstrating that the proposed compound directly inhibits FSP1 enzymatic activity. However, the biochemical assays used are limited in diagnostic power. The NADH oxidation assay used to quantify FSP1 activity is inherently prone to artefacts because many small molecules can alter

fluorescence or modulate NADH redox cycling independently of the enzyme. The study does not provide raw kinetic traces, which limits the ability to assess curve fitting or detect anomalies such as signal drift or background interference. Additionally, the authors do not compare the inhibitor's effects on related flavoproteins, leaving open the possibility of off-target inhibition. Without enzymatic specificity validation, the biochemical foundation of the study remains unstable.

3.6. Transparency and Integrity of Imaging Data

Imaging-based assays, including C11-BODIPY staining, immunohistochemistry, mitochondrial tracking, and electron microscopy, are central to the study's mechanistic argument but exhibit several shortcomings. Many images appear over-contrasted or sharpened, potentially obscuring subtle features. Electron microscopy images show suspiciously uniform mitochondrial swelling patterns and repeated morphologies that raise concerns about duplication. The study fails to provide raw TIFF files or metadata for imaging conditions, hindering independent evaluation. Moreover, quantification of imaging data is poorly documented, with no description of how thresholds were determined, how cells were segmented, or whether analyses were performed in blinded fashion. The inconsistent scale bars and magnifications across panels further diminish confidence in the imaging integrity.

3.7. Validity of *In Vivo* Models and Experimental Conditions

The xenograft experiments in Wu *et al*. form the basis of the study's therapeutic claims, yet the design of these experiments is methodologically limited. The number of animals per group appears low, raising concerns about statistical power. Randomization and blinding procedures are not described. The administration schedule, dosing, and pharmacokinetic context of the inhibitor are insufficiently documented. The reduction in tumor growth could result from systemic toxicity or immune modulation rather than ferroptosis induction, especially since no *in vivo* rescue experiments were performed. Histological analyses of tumor tissues suffer from overexposure and lack of quantification. Toxicity assessments rely primarily on gross histology rather than on quantitative serum chemistry or organ-specific biomarkers, limiting their interpretive value.

3.8. Lack of Formal Discrimination Between Death Pathways

One of the central methodological weaknesses is the failure to rigorously differentiate ferroptosis from other death pathways. The study lacks caspase inhibition assays, MLKL phosphorylation analyses, or autophagy-related markers that could exclude apoptosis, necroptosis, or autophagy-dependent cell death. The absence of iron chelation studies is particularly problematic, as ferroptosis is

uniquely iron-dependent. Lipid ROS accumulation alone is insufficient to claim ferroptosis, since oxidative stress from mitochondrial disruption or ROS-generating compounds can produce similar signatures. Without pathway-specific discriminative experiments, the study cannot convincingly attribute cell death to ferroptosis.

3.9. Off-Target Concerns for the Proposed FSP1 Inhibitor

The inhibitor's specificity is central to the study but inadequately evaluated. The authors test only a limited panel of enzymes and do not examine potential interactions with mitochondrial oxidoreductases, cytochrome P450 enzymes, or redox-sensitive metabolic proteins. Lack of chemoproteomic profiling leaves open the possibility that the compound binds multiple proteins. Off-target redox perturbation is a pervasive issue with electrophilic or redox-active small molecules, and such effects can easily masquerade as ferroptosis. The absence of structure–activity relationships further limits confidence in the proposed mechanism.

3.10. Absence of Multi-omics Validation Across Modalities

Mechanistic studies of ferroptosis increasingly rely on integrated multi-omics approaches, including proteomics, metabolomics, transcriptomics, and lipidomics. Wu *et al*.[4] perform lipidomics but do so in a limited, narrowly targeted fashion without MS/MS validation of key species. Transcriptomic and proteomic analyses are absent, preventing systematic evaluation of antioxidant pathways, iron metabolism, or compensatory responses. Without multi-omics triangulation, the study lacks the robustness necessary for a mechanistic claim of this magnitude.

4. Figures: Detailed Figure-by-Figure Critique

4.1. Figure 1: FSP1 Expression, Dependency, and Clinical Relevance

Figure 1 attempts to establish FSP1 as broadly upregulated and functionally essential in lung cancer, yet the data presented suffer from several methodological and interpretive weaknesses. The RNA-seq datasets are not accompanied by a clear description of normalization procedures, making it uncertain whether the comparisons across tumor and normal tissues are valid. Without clarifying whether TPM, FPKM, or DESeq2-normalized counts were used, or whether purity estimation was applied to adjust for stromal infiltration differences, the expression changes shown may reflect technical or compositional biases rather than genuine tumor biology. FSP1 expression varies widely across NSCLC subtypes, yet the authors aggregate adenocarcinoma, squamous carcinoma, and small-cell lung cancer without stratification. This aggregation obscures biological heterogeneity and inflates perceived significance. The CRISPR dependency analysis is similarly limited.

The DepMap-derived signatures appear smoothed, with an unusual clustering pattern that raises questions about data preprocessing. The absence of comparison to GPX4 dependency across the same cell lines is a notable omission, as it prevents contextualizing the relative importance of FSP1. Immunohistochemistry is undermined by over-contrasting, inconsistent staining intensities, and insufficient quantification. Claims of prognostic relevance are unsupported because survival curves show minimal separation and lack multivariate analysis. Altogether, **Figure 1** provides insufficient evidence to conclude that FSP1 is clinically relevant, functionally essential, or universally overexpressed in lung cancer.

4.2. Figure 2: Discovery and Characterization of the Proposed FSP1 Inhibitor

Figure 2 is pivotal to the paper's mechanistic narrative, yet it presents the weakest empirical foundation. The structure of the inhibitor is shown without explanation of its design rationale or comparison to existing FSP1 inhibitors such as iFSP1. The biochemical assays use a NADH-based readout that is highly susceptible to artefacts, particularly when evaluating redox-active compounds. The IC_{50} values reported show implausibly small variance, suggesting that technical replicates or smoothed data may have been used. No kinetic data or raw fluorescence traces are provided, limiting the ability to evaluate enzyme behavior. Target engagement assessed by thermal-shift assays yields only minimal ΔT_m shifts that are indistinguishable from noise. Without chemoproteomic, binding, or structural validation, the evidence for direct interaction between the compound and FSP1 remains weak. Cellular assays show dose-response curves that deviate from expected pharmacologic behavior and may indicate broad oxidative stress rather than specific target inhibition. Rescue experiments using FSP1 overexpression produce only partial reversal of cytotoxicity, which is inconsistent with a selective inhibitor. Overall, **Figure 2** fails to establish specificity, potency, or mechanistic fidelity of the compound, undermining the central premise of the study.

4.3. Figure 3: Evidence for Ferroptosis Induction

Figure 3 presents data intended to demonstrate that inhibitor treatment induces ferroptosis, yet the evidence is indirect and incomplete. The C11-BODIPY flow cytometry plots lack gating documentation, compensation, or unstained controls, making fluorescence shifts difficult to interpret. The BODIPY probe is sensitive to general ROS and cannot serve as a standalone indicator of ferroptotic lipid peroxidation. Rescue by ferrostatin-1, the strongest evidence offered, is partial and inconsistent across cell lines, which contradicts the typical near-complete rescue seen in bona fide ferroptosis. Western blot analyses of GPX4 and other regulators present inconsistent band intensities, unclear normalization, and potential splicing

artifacts. CRISPR knockout validation is limited to protein-level assessments without sequencing confirmation of indels. Overexpression rescue experiments involve supraphysiological levels of FSP1 that may introduce artefacts rather than reflect mechanistic specificity. The combined data in **Figure 3** do not convincingly demonstrate that ferroptosis is the dominant mode of cell death induced by the inhibitor.

4.4. Figure 4: Lipidomics and CoQ10 Redox State

Figure 4 is intended to provide biochemical depth by linking FSP1 inhibition to CoQ10 oxidation and lipid peroxidation patterns. However, the lipidomics workflow is insufficiently detailed, and the data presented lack the breadth and specificity required to substantiate ferroptosis. The measurement of CoQ10 and CoQ10H2 is highly sensitive to extraction and handling conditions; the absence of internal standards or antioxidant stabilizers raises concerns about artefactual oxidation during sample processing. The lipid species shown represent only a narrow subset of ferroptosis-associated phospholipids, and the study does not identify oxidized phosphatidylethanolamines, which are central biochemical hallmarks of ferroptosis. The chromatograms shown have unusually uniform baselines and lack MS/MS fragmentation data, preventing confident molecular identification. Lipid droplet imaging appears to have undergone contrast enhancement, and the quantification lacks methodological clarity regarding segmentation or thresholding. Without rigorous lipidomics pipelines and validation, **Figure 4** does not provide mechanistic support for FSP1-dependent lipid peroxidation.

4.5. Figure 5: Xenograft Studies and Claims of Therapeutic Efficacy

Figure 5 forms the translational core of the paper, yet it suffers from major design and interpretive weaknesses. Tumor growth curves display unrealistically small variation, suggesting possible data smoothing or insufficient biological replication. There is no description of randomization, blinding, or power calculations. Survival curves lack appropriate statistical reporting and censoring information. Histological staining of tumour tissues is inconsistently exposed, limiting interpretability. The authors rely on TUNEL staining to infer ferroptosis, despite TUNEL marking DNA fragmentation associated with apoptosis or other stress responses. Pharmacokinetic data show anomalies in concentration–time curves that are incompatible with typical small-molecule distribution and metabolism. The toxicity assessment is superficial and lacks serum chemistry or organ-specific biomarkers. Perhaps most critically, the study does not include *in vivo* rescue experiments using ferroptosis inhibitors, making it impossible to determine whether tumor reduction is mediated

by ferroptosis. Without mechanistic confirmation, **Figure 5** cannot support claims that the inhibitor produces ferroptosis-driven tumor suppression.

5. Extended Data Figures: Exhaustive Critique

5.1. Extended Data Figure 1: Expanded Expression Analyses

Extended Data Figure 1 presents additional expression datasets intended to reinforce the conclusion that FSP1 is upregulated in lung cancer. However, the figure repeats the methodological issues identified in **Figure 1**. The authors rely on multiple publicly available datasets but provide no description of batch-correction methods or alignment procedures. The heatmaps displayed lack color-scale legends, making the magnitude of change opaque. Several datasets appear to contain duplicated patient identifiers, suggesting possible data mislabeling or transcription errors. The PCA analyses show unusually tight clustering patterns, which may indicate aggressive batch correction, overfitting, or selective sample inclusion. In the absence of raw data availability or computational reproducibility, the extended expression analyses add volume but not validity to the central claim.

5.2. Extended Data Figure 2: Specificity Screening

Extended Data Figure 2 attempts to demonstrate the inhibitor's specificity across a limited panel of enzymes. The presentation is unconvincing. Many IC50 values appear identical across different enzymes, which is statistically implausible for independent biochemical assays. Error margins are uniformly minimal, suggesting technical replicates or copied placeholders rather than genuine independent experiments. The figure includes only a narrow selection of enzymes and omits key off-target candidates such as cytochrome P450 isoforms, mitochondrial oxidoreductases, flavoproteins, and electron transport chain components. Given the structural similarity of the inhibitor to known redox-active molecules, a more comprehensive screen would be essential. Without such breadth, **Extended Data Figure 2** does not substantiate the claim of specificity.

5.3. Extended Data Figure 3: Ferroptosis Markers

Extended Data Figure 3 presents additional ferroptosis-associated markers, including ACSL4 and LPCAT3 levels, MDA quantification, and intracellular iron measurements. The Western blots show over-saturated bands with unclear background levels, making it difficult to assess relative expression. The MDA assay is presented as a single bar graph with no documentation of calibration curves or normalization procedures. MDA quantification is sensitive to technical variability, and without raw chromatographic data or standard curves, its reliability is questionable. The iron measurements rely on FerroOrange fluorescence, which is

nonspecific and easily influenced by changes in cellular redox state. The figure thus fails to provide credible extensions of ferroptosis validation.

5.4. Extended Data Figure 4: ROS and Mitochondrial Assessments

Extended Data Figure 4 presents ROS imaging and mitochondrial morphology analyses. The ROS imaging relies on DCFDA and MitoSOX, which detect general ROS rather than lipid-specific peroxides associated with ferroptosis. The figure does not include proper controls for probe oxidation, nor is the imaging presented with identical exposure times. Some images appear to share visual patterns across panels, raising concerns about duplication or template reuse. Mitochondrial morphology is shown through low-resolution images that appear post-processed. The uniformity of mitochondrial swelling across replicates is biologically implausible, suggesting either selective image presentation or manipulation. Overall, the figure does not provide trustworthy ROS or mitochondrial evidence.

5.5. Extended Data Figure 5: Electron Microscopy Evidence

This figure aims to demonstrate ultrastructural changes associated with ferroptosis, particularly condensed mitochondrial membranes and cristae disruption. The electron micrographs, however, are problematic. Several images appear duplicated with contrast changes, a hallmark of digital reuse. The resolution is insufficient to identify hallmark ferroptotic features such as decreased mitochondrial volume or membrane rupture patterns. Scale bars differ in size relative to objects in inconsistent ways across panels, undermining quantitative interpretation. Without raw TIFF files and metadata, the ultrastructural claims cannot be independently verified.

5.6. Extended Data Figure 6: CRISPR Validation Panels

Extended Data Figure 6 attempts to validate FSP1 knockout cell lines using sequencing and Western blotting. The Sanger sequencing traces are blurry and lack annotation of guide RNA target sites, making interpretation impossible. The Western blot lanes show abrupt edges that appear digitally altered, suggesting potential splicing of bands. The knockout verification is incomplete because off-target edits, indels, and clonal variation are not assessed. Without definitive evidence of knockout efficiency and clonal integrity, the genetic experiments underlying several mechanistic claims are compromised.

5.7. Extended Data Figure 7: Lipidomics Validation

The lipidomics validation figure suffers from the same issues as **Figure 4**. The chromatograms show identical baselines across distinct lipid species, which is unusual for LC–MS data. Quantification is provided to three decimal places, an

unrealistic precision for this type of analysis. The absence of MS/MS fragmentation spectra prevents confident structural identification of oxidized lipid species. Normalization is described vaguely, with no reference to internal standards. Without rigorous lipidomics protocols, the lipid peroxidation claims remain unsubstantiated.

5.8. Extended Data Figure 8: Animal Toxicity Panels

Extended Data Figure 8 provides organ histology and weight measurements intended to demonstrate the inhibitor's safety. The histology images are low resolution and appear to be reused or obtained from stock sources due to the striking similarity between panels. The absence of serum biomarkers, organ weights, or toxicokinetic parameters undermines any claims about safety. The sample sizes are unclear, and the quantification of histological abnormalities is missing. Without comprehensive toxicology, **Extended Data Figure 8** cannot support assertions of tolerability.

6. Supplementary Figures: Full Evaluation

6.1. Supplementary Figure S1: Flow Cytometry Gating

Supplementary Figure S1 is intended to provide methodological transparency for the flow cytometry experiments shown in the main figures. However, the figure does not address the major concerns raised by the C11-BODIPY analyses. The gating strategy lacks definition and appears to have been drawn post hoc to fit the observed fluorescence shifts. The forward scatter and side scatter plots show improbable uniformity across replicates, raising concerns that data may have undergone smoothing or filtering. There is no evidence of compensation controls, which are critical for lipid ROS probes that often spill into adjacent channels. Additionally, the fluorescence intensity profiles display noise-free patterns inconsistent with genuine cytometry acquisition. Because flow cytometry is central to the claim of lipid peroxidation, the lack of rigorous gating documentation makes the entire dataset less credible. This figure fails to establish that the observed changes reflect biological variation rather than gating artefacts or data-processing choices.

6.2. Supplementary Figure S2: qPCR and Gene Expression

Supplementary Figure S2 presents qPCR data for genes involved in ferroptosis, oxidative stress, and metabolism. However, the figure lacks primer efficiency validation, standard curves, and melt curve analyses, all of which are essential for ensuring specificity and linearity. The authors use GAPDH as the sole reference gene despite its known variability under oxidative stress and metabolic perturbation. A stable housekeeping gene panel or geNorm validation is necessary for reliable

normalization. The qPCR data show remarkably small error bars, which may indicate technical replicates rather than biological replicates. The lack of consistency between these expression patterns and the protein-level observations presented elsewhere in the paper further raises questions about reproducibility. Overall, **Supplementary Figure S2** provides minimal reassurance regarding transcriptional changes and does not meaningfully reinforce the mechanistic claims.

6.3. Supplementary Figure S3: Cell Line Expansion Studies

Supplementary Figure S3 attempts to generalize the findings by demonstrating inhibitor sensitivity across additional lung cancer cell lines. However, the number of lines included is limited, and the selection appears arbitrary. The figure does not include normal lung epithelial cells, which would be necessary to support claims of tumor selectivity. The sensitivity patterns shown are inconsistent with known ferroptotic profiles of these cell lines, and several cell viability curves exhibit irregularities characteristic of data smoothing. The lack of accompanying genetic characterization makes it difficult to interpret heterogeneity among cell lines. Without broader representation and rigorous validation, the figure does not support the assertion that FSP1 inhibition broadly induces ferroptosis in lung cancer.

6.4. Supplementary Figure S4: Organ Histology

Supplementary Figure S4 presents additional organ histology to support claims about the inhibitor's safety. The images are low resolution and lack annotations indicating magnification or staining conditions. Many panels appear nearly identical across treatment groups, suggesting image reuse or insufficient sampling. There is no quantification of tissue integrity, inflammatory infiltration, or structural abnormalities. Histology presented in this manner does not provide meaningful toxicological insight. Comprehensive safety assessment requires serum chemistries, organ weights, and blinded histopathological scoring, none of which are provided.

6.5. Supplementary Tables: Missing Metadata and Incomplete Documentation

Accompanying tables in the supplementary materials exhibit numerous deficiencies. Several datasets do not contain sample identifiers, making it impossible to track which experiments correspond to which biological replicates. Metadata describing experimental conditions, machine settings, or normalization strategies are either incomplete or absent. For lipidomics and proteomics-derived tables, the absence of raw peaks, retention times, and MS/MS spectra precludes independent verification. Some tables contain contradictory values or formatting inconsistencies indicative of manual editing or dataset merging errors. Without detailed reporting standards or

accessible raw data, the supplementary tables do not provide transparency or reproducibility.

6.6. Supplementary Methods: Inadequate Experimental Detail

Although not presented as a figure, the **Supplementary Methods** section is crucial for interpreting supplementary data figures. Unfortunately, the descriptions provided are cursory and omit several essential details. For flow cytometry, there is no mention of instrument configuration, threshold settings, or controls for probe auto-oxidation. For lipidomics, there is no outline of extraction solvents, gradient conditions, ionization modes, collision energies, or calibration strategies. CRISPR editing protocols do not describe clonal selection, screening procedures, or sequencing depth. Without these methodological details, the supplementary materials cannot serve as a foundation for replication or independent scrutiny.

6.7. Integration of Supplementary Data with Main Findings

The supplementary materials are intended to reinforce and validate claims made in the main text. Instead, they highlight methodological weaknesses and inconsistencies. Many supplementary datasets do not align coherently with the main figures. For example, lipid ROS measurements in **Supplementary Figure S1** differ from those shown in **Figure 3**, suggesting batch effects or selective data inclusion. qPCR expression changes do not correspond to Western blot trends. Histology in **Supplementary Figure S4** contradicts claims of minimal toxicity. These inconsistencies undermine the narrative presented in the main text.

6.8. Overall Assessment of Supplementary Data

The supplementary figures and tables do not provide the methodological depth or transparency required for a high-impact mechanistic claim. Instead of clarifying or validating key experiments, the supplementary materials expose gaps in experimental rigor and raise concerns about reproducibility and data handling. The lack of raw data, missing metadata, inconsistent presentation, and questionable image integrity collectively weaken the scientific foundation of the paper. Rather than strengthening the conclusions of Wu *et al*., the supplementary figures significantly magnify the uncertainties surrounding the study's experimental and mechanistic claims.

7. Synthesis of Technical, Methodological, and Conceptual Problems

7.1. Mechanistic Ambiguity Between Ferroptosis and Oxidative Stress

A central weakness of the study lies in its inability to distinguish ferroptosis from broader oxidative stress phenotypes. The authors present increased lipid ROS and partial rescue by Ferrostatin-1 as definitive evidence for ferroptosis, yet these signals are non-specific and can arise from numerous perturbations of NAD(P)H homeostasis. Several redox-active inhibitors generate ROS accumulation through mitochondrial disruption or flavoprotein inhibition without engaging ferroptotic pathways. Because Wu *et al*. do not demonstrate ACSL4 dependency[4], iron-dependency reversal, or oxidized PE signature formation, the mechanistic boundary between ferroptosis and general oxidative cytotoxicity remains unresolved. This ambiguity affects the interpretation of virtually all subsequent figures and calls into question whether the cell death observed reflects the ferroptotic program or simply non-specific redox collapse.

7.2. Inadequacy of Mechanistic Validation

Mechanistic validation is incomplete at multiple levels. The study lacks biochemical demonstration that the compound directly binds FSP1, lacks structural or chemoproteomic assays to map interaction sites, and does not provide comparative inhibition profiles against related oxidoreductases. Without such validation, the mechanistic premise remains speculative. The genetic rescue experiments are limited by incomplete knockout verification and supraphysiological overexpression in rescue experiments. Furthermore, the authors do not interrogate whether the compound's cytotoxicity persists in FSP1-null cells, a crucial experiment for establishing dependency. Collectively, these omissions prevent the establishment of a causal mechanistic chain linking inhibitor treatment to FSP1 suppression and ferroptosis activation.

7.3. *In Vivo* Ferroptosis Evidence Weakness

The xenograft experiments represent a critical translational claim, yet they lack ferroptosis-specific *in vivo* validation. There is no demonstration that tumor reduction is reversed by ferroptosis inhibitors, no measurement of oxidized lipid species in tumor tissues, and no evidence of iron-dependent peroxidation pathways being activated *in vivo*. The reliance on TUNEL staining, which detects general DNA fragmentation rather than ferroptosis-specific features, further weakens the authors' interpretation. Without ferroptosis-specific biomarkers in tumor samples, the assertion that FSP1 inhibition induces ferroptosis *in vivo* is unsupported. The

possibility remains that tumor suppression arises from broad cytotoxicity, impaired proliferation, or altered tumor microenvironment rather than ferroptosis.

7.4. Off-Target Effects of the Inhibitor

One of the most concerning issues is the lack of robust off-target profiling. Redox-active compounds frequently inhibit multiple NAD(P)H-dependent enzymes or interfere with mitochondrial electron transport. Without a comprehensive chemoproteomic assessment or enzyme panel screening, off-target effects cannot be ruled out. The compound's structure suggests potential interactions with flavins and quinones, raising suspicions about interference with mitochondrial energetics or the broader ubiquinone cycle. Such off-target effects could easily explain the observed lipid peroxidation patterns and cytotoxicity. The authors' limited screening does not provide sufficient confidence that FSP1 inhibition is the primary driver of the observed phenotypes. This limitation undermines the mechanistic interpretation of nearly every experiment.

7.5. Data Integrity and Reproducibility Issues

Across main **Figures**, **Extended Data figures**, and **Supplementary Figures**, concerns arise regarding image integrity, raw data transparency, replicate consistency, and methodological documentation. Several imaging panels appear digitally altered or duplicated with different contrast settings. Western blot lanes exhibit abrupt transitions suggestive of splicing. Flow cytometry plots lack raw gating, compensation controls, or unstained baselines. Lipidomics tables show identical baseline values and implausible precision. Xenograft tumor curves display unusually low variability suggestive of smoothing or insufficient biological replication. These issues collectively erode confidence in data reliability. Reproducibility concerns are exacerbated by missing metadata, undocumented normalization procedures, and a lack of deposited raw datasets.

7.6. Overinterpretation Relative to Data Strength

Wu *et al*. repeatedly draw conclusions that exceed the inferential strength of their data. They claim to demonstrate selective FSP1 inhibition, yet provide no binding or specificity data. They assert ferroptosis as the mechanism of cell death without fulfilling established biochemical or genetic criteria. They infer therapeutic relevance despite minimal evidence of *in vivo* ferroptosis. They describe the inhibitor as safe despite lacking comprehensive toxicological evaluation. They present a coherent mechanistic model without systematically excluding alternative explanations. This pattern of interpretive inflation is characteristic of several high-impact publications in rapidly evolving fields, where conceptual enthusiasm may

outpace mechanistic rigor. The study repeatedly treats correlative observations as mechanistic proof, which introduces conceptual fragility into the overall narrative.

7.7. Disconnect between Biochemical, Genetic, and Cellular Phenotypes

The biochemical inhibition assays do not convincingly align with genetic data or cellular ferroptosis phenotypes. For example, the partial rescue by FSP1 overexpression suggests either incomplete inhibition of FSP1 or involvement of additional pathways. Similarly, CRISPR knockout cells do not exhibit the predicted sensitivity shift, raising questions about whether FSP1 is truly essential in the tested lines. The lack of correspondence between lipidomics data, ROS measurements, and viability assays suggests that the observed cellular responses may involve parallel pathways rather than a unified mechanistic program. This disconnect undermines the coherence of the mechanistic model.

7.8. Absence of Systems-Level Consideration

The study does not account for broader metabolic constraints that shape ferroptosis susceptibility. The FSP1–CoQ10 axis functions within a larger network of NAD(P)H-dependent redox pathways, membrane-remodeling enzymes, and antioxidant systems. Perturbing FSP1 alone may be insufficient to induce ferroptosis unless accompanied by parallel disruptions in glutathione, BH4, ACSL4-mediated PUFA activation, or cellular iron homeostasis. By ignoring these interconnected pathways, the authors present a simplified view that fails to integrate systems-level redox biology. This omission makes the proposed mechanistic framework biologically incomplete and reduces its translational relevance.

7.9. Implications for Mechanistic and Therapeutic Interpretation

The cumulative methodological and conceptual problems identified in this section raise serious concerns about the validity of the study's central conclusions. If the inhibitor's effects derive from off-target redox disruption rather than FSP1 inhibition, then the therapeutic implications are fundamentally altered. If observed phenotypes reflect oxidative stress rather than ferroptosis, then the mechanistic claims collapse. If xenograft tumor reduction results from nonspecific toxicity, then the translational significance evaporates. The study's narrative depends on a mechanistically coherent chain of evidence, which is not convincingly established. As a result, the paper risks contributing to conceptual confusion and misleading drug development priorities in the ferroptosis field.

8. Statistical and Computational Concerns and Biological Plausibility

8.1. Statistical Power, Replicate Handling, and Significance Inflation

A recurring issue throughout Wu *et al*.'s study is the lack of clarity regarding sample sizes, the type of replicates used, and the statistical validity of reported findings. Many figures display extremely narrow error bars inconsistent with expected biological variability, suggesting the reliance on technical replicates rather than true biological replicates. Statistical power is not discussed, and no power analyses are provided for critical experiments such as xenograft tumor measurements or lipidomics quantification. Several *P* values are reported as a generic "<0.05," which obscures the strength of evidence and prevents assessment of robustness or sensitivity. The absence of multiple comparison corrections further contributes to significance inflation, especially in datasets involving numerous parallel assays such as expression profiling. Collectively, these omissions call into question the reliability of the statistical framework and the degree to which the data genuinely support the study's mechanistic claims.

8.2. Questionable Assumptions about Data Distribution

The statistical analyses implicitly assume that key datasets follow normal distributions, yet no tests for normality or variance homogeneity are reported. Cell viability assays, ROS measurements, and lipidomics data frequently exhibit skewed or multimodal distributions, which require nonparametric treatment. Without documentation of distributional properties or validation of model assumptions, the reported means and error bars may not accurately represent the underlying data. In addition, the authors use parametric tests for datasets where sample sizes appear to be below the thresholds required for reliable estimation of distribution parameters. This lack of statistical rigor amplifies uncertainty about the robustness of observed differences.

8.3. Model Fitting, Curve Behavior, and Lack of Cross-Validation

The inhibitor dose–response curves and enzymatic inhibition curves often show shapes and slopes that do not resemble standard pharmacologic behavior. Several curves display abrupt inflections or plateau at inconsistent levels. The absence of Hill coefficient reporting limits interpretability regarding cooperative or non-cooperative interaction patterns. In enzyme assays, the fitted IC_{50} values exhibit implausibly tight confidence intervals, inconsistent with expected variability in biochemical systems. No cross-validation of computational fits is shown, nor do the

authors provide goodness-of-fit metrics. This lack of transparency undermines confidence not only in the inhibitor's potency but also in the computational validity of key biochemical conclusions.

8.4. Batch Effects and Normalization Inconsistencies

The computational handling of RNA-seq, lipidomics, and ROS datasets reveals several inconsistencies. RNA-seq heatmaps lack description of batch-correction procedures or normalization pipelines. When multiple public datasets are combined, batch effects are inevitable, yet the PCA plots present artificially clean clustering that raises suspicion of overcorrection or selective sample removal. In lipidomics data, normalization appears to be performed against total lipid content without internal standards, which is insufficient for compensating for extraction variability or ionization efficiency. Flow cytometry data fail to show compensation or normalization to unstained controls, making fluorescence intensity values unreliable. These normalization inconsistencies distort interpretation and diminish replicability.

8.5. Potential Computational Bias in RNA-seq, Lipidomics, and Flow Cytometry

The computational workflows for RNA-seq and lipidomics are insufficiently described, preventing assessment of analytic bias. The RNA-seq differential expression analyses lack information on read alignment, quantification algorithms, filtering criteria, or differential expression thresholds. The absence of raw FASTQ files or alignment statistics prevents independent evaluation. In lipidomics, the limited number of identified features and the perfect peak alignment across replicates are inconsistent with standard LC–MS variability, suggesting either aggressive filtering or algorithmic smoothing. Flow cytometry plots show noise-free histograms, which is unusual for biological samples and suggests heavy post-processing. The lack of algorithmic transparency raises the possibility that results may reflect computational artefacts rather than biology.

8.6. Biological Plausibility of the Proposed FSP1 Inhibitor Mechanism

The mechanism proposed by Wu *et al*.[4] posits that their small molecule selectively inhibits FSP1, leading to CoQ10 oxidation and ferroptosis. While conceptually attractive, the biological plausibility of this mechanism remains unconvincing given the data. FSP1 functions as part of an extensive NAD(P)H-dependent redox network; inhibitors targeting its active site must avoid interference with related oxidoreductases, including NQO1, POR, and mitochondrial complex I. The structural features of the inhibitor depicted in the study resemble known redox-active

scaffolds rather than highly specific enzyme inhibitors. Without structural studies, binding assays, or differential metabolite profiling, it is difficult to accept that the observed phenotypes arise solely from FSP1 inhibition rather than broad disruption of NAD(P)H homeostasis. The biological context surrounding FSP1 function thus makes the selective mechanism proposed by the authors implausible without substantially stronger evidence.

8.7. Compatibility of Findings with Established Ferroptosis Biochemistry

The canonical ferroptosis pathway requires coordinated involvement of ACSL4, LPCAT3, and iron-dependent peroxidation of PUFA-containing phospholipids. The study does not demonstrate changes in ACSL4 dependency or oxidized PE species, which are hallmarks of ferroptosis. The authors' observation of lipid ROS and CoQ10 oxidation could reflect any number of oxidative insults unrelated to ferroptosis. Additionally, the partial rescue by Ferrostatin-1 contradicts the expectation of near-complete protection in bona fide ferroptosis induction. The incomplete alignment of observed phenomena with established biochemistry suggests that the authors' interpretation overlooks essential components of ferroptotic execution.

8.8. Alternative Pathways Better Explaining the Data

The phenotypes described by Wu *et al*. are equally explained by mitochondrial dysfunction, inhibition of unrelated flavoproteins, or induction of oxidative stress. Several redox-active compounds induce C11-BODIPY oxidation, TUNEL positivity, and lipid peroxidation through mechanisms unrelated to ferroptosis. The absence of iron chelation rescue data further raises questions about whether iron-dependent lipid peroxidation is truly necessary for the observed cell death. Under this alternative interpretation, the inhibitor functions as a general oxidizing agent, and FSP1 suppression is merely correlative rather than causal.

8.9. Systems-Level Coherence with Lung Cancer Metabolism

Lung cancer cells maintain intricate redox buffering systems involving glutathione, thioredoxin, NADPH-generating pathways, and mitochondrial metabolism. The study's proposal that FSP1 inhibition alone is sufficient to destabilize this system neglects the redundancy and plasticity of cancer redox networks. Systems-level analyses, such as metabolomics or isotope tracing, would be required to support such a claim. Without these, the proposed mechanism is not well integrated into the known metabolic logic of NSCLC. The biological system's inherent robustness casts further doubt on the plausibility of the inhibitor acting selectively and exclusively through the FSP1–CoQ10 axis.

9. Alternative Interpretations and Mechanistic Possibilities

9.1. Off-Target Cytotoxicity

A central alternative explanation for the findings in Wu *et al.*[4] is that the proposed inhibitor induces off-target cytotoxicity unrelated to FSP1. Many redox-active compounds influence multiple NAD(P)H-dependent enzymes, mitochondrial flavoproteins, or ubiquinone-interacting proteins. Such interference can create widespread ROS accumulation and lipid oxidation without requiring FSP1 inhibition. Because Wu *et al.*[4] do not provide chemoproteomic profiling, binding studies, or comprehensive off-target screening, it remains plausible that the observed cellular effects derive from broad disruption of redox homeostasis. This interpretation also explains the partial rescue by FSP1 overexpression, inconsistent with a specific pharmacological interaction. Without definitive evidence that the inhibitor engages FSP1 selectively, off-target cytotoxicity remains a more parsimonious explanation.

9.2. Mixed Forms of Regulated Cell Death

The phenotypes reported by Wu *et al.*[4] may reflect a combination of regulated cell death pathways rather than pure ferroptosis. Many hallmarks described in the study, such as TUNEL positivity, mitochondrial swelling, and fluctuating ROS levels, can arise from apoptosis, necroptosis, or parthanatos. The absence of caspase inhibition assays, necroptosis pathway analyses, or PARP activity measurements leaves these possibilities unexplored. In some contexts, oxidative stress induced by redox-active compounds triggers hybrid forms of cell death involving mitochondrial permeability transition, which produces lipid peroxidation but is not ferroptotic in origin. The partial protection by Ferrostatin-1 observed in the study aligns with a mixed phenotype rather than pathway-selective ferroptosis. This mixed-death model better accommodates the incomplete rescue effects and variable sensitivity across cell lines.

9.3. Metabolic Crisis Rather Than Ferroptosis

Another plausible alternative mechanism is that inhibitor treatment causes metabolic collapse by disrupting mitochondrial electron transport or NADH recycling. The mitochondrial membrane potential and ROS levels shown in Wu *et al.* exhibit patterns consistent with mitochondrial depolarization rather than ferroptosis. Inhibition of flavoproteins involved in oxidative phosphorylation can trigger broad lipid oxidation due to impaired ATP generation and increased electron leak. Such stress does not rely on ACSL4-driven PUFA peroxidation and thus cannot be accurately categorized as ferroptotic. The absence of detailed mitochondrial

assays, such as oxygen consumption rate measurements, hinders the authors' ability to distinguish ferroptosis from metabolic crisis. This alternative mechanism explains the general oxidative stress signals without invoking ferroptosis-specific biochemical pathways.

9.4. Non-Ferroptotic Lipid Peroxidation

The accumulation of lipid ROS observed in the study may arise from non-ferroptotic lipid peroxidation pathways. Autoxidation of unsaturated lipids occurs readily under elevated ROS conditions, and C11-BODIPY oxidation can be driven by general oxidative stress rather than the enzymatic processes characteristic of ferroptosis. The lipidomics data do not provide evidence of oxidized phosphatidylethanolamine species, which are the primary substrates of ferroptosis-associated lipid peroxidation. In the absence of these specific oxidized lipids, lipid peroxidation cannot be attributed to ferroptosis. Non-ferroptotic oxidation of membrane lipids is a common outcome of redox imbalance and aligns with the study's incomplete rescue by Ferrostatin-1. This alternative pathway also aligns with the inhibitor's likely interference with general redox metabolism, which would promote lipid oxidation irrespective of ACSL4 or iron-dependent mechanisms.

9.5. Stress-Adaptive Responses and Cell-State Transitions

Lung cancer cells exhibit dynamic cell-state transitions in response to oxidative stress, including shifts toward quiescence, mesenchymal-like states, or inflammatory phenotypes. Some of the transcriptional and metabolic changes reported by Wu *et al*.[4] may reflect stress-adaptive reprogramming rather than cell death pathways. Cells undergoing oxidative stress frequently upregulate antioxidant responses, modify membrane lipid composition, and undergo partial mitochondrial remodeling. Because the study lacks longitudinal tracking of cell-state dynamics or single-cell analyses, it is unclear whether observed phenotypes represent terminal death pathways or transient adaptive responses. This alternative interpretation fits with incomplete cell death rescue and the modest effects observed in some assays.

9.6. Heterogeneity among Lung Cancer Cell Lines

Lung cancer cell lines exhibit substantial heterogeneity in ferroptotic sensitivity, redox capacity, and lipid metabolism. The limited number of cell lines used in Wu *et al*. does not allow the authors to ascribe mechanistic uniformity across NSCLC. In some cell lines, FSP1 is dispensable due to redundant antioxidant systems. In others, GPX4 remains the dominant ferroptosis suppressor. The inconsistent responses across the few lines tested suggest that the inhibitor may act differently depending on a cell's metabolic wiring rather than through a uniform mechanistic pathway. A more heterogeneous dataset could reveal that the inhibitor's effects correlate better

with intrinsic redox vulnerability or mitochondrial dysfunction rather than FSP1 expression or activity. Without systematic profiling, the authors' interpretation remains vulnerable to cell-line bias.

9.7. Tension between Proposed Mechanism and *In Vivo* Biology

In vivo environments impose constraints that differ markedly from those observed *in vitro*. The tumor microenvironment contains variable oxygen tension, diverse metabolic niches, immune components, and extracellular matrix interactions. Ferroptosis induction *in vivo* typically requires strong lipid peroxidation triggers or significant disruption of antioxidant networks. The relatively mild biochemical changes observed in Wu *et al*.'s *in vitro* experiments do not appear robust enough to sustain ferroptotic pressure in a complex *in vivo* environment. The modest tumor regression observed in xenografts may reflect systemic toxicity, altered angiogenesis, or microenvironmental changes rather than ferroptosis-driven tumor suppression. Without ferroptosis biomarkers in tumor tissues or *in vivo* rescue data, the plausibility of *in vivo* ferroptosis induction remains low.

9.8. Interference with CoQ10 and NAD(P)H Pathways as an Underlying Mechanism

CoQ10 and NAD(P)H are central hubs of redox metabolism. Any compound that disrupts their recycling or function produces widespread metabolic consequences independent of FSP1. The data presented by Wu *et al*. show changes in CoQ10 redox state that could arise from perturbation of mitochondrial complex I, NQO1, or other NADH-dependent enzymes. This broad-interference model explains both the lipid peroxidation signals and the partial rescue by ferroptosis inhibitors. It also aligns with the incomplete FSP1 dependency observed in some experiments. Without direct evidence that the inhibitor specifically binds FSP1 and alters its enzymatic function, the pathway most consistent with the data remains generalized redox disruption.

9.9. Summary of Alternative Mechanistic Frameworks

Collectively, the alternative interpretations presented here offer more coherent and biologically plausible explanations for the observed phenotypes in Wu *et al*. than the selective-FSP1-inhibition model proposed by the authors. Off-target redox disruption, mixed cell death modalities, metabolic collapse, non-ferroptotic lipid oxidation, and stress-adaptive transitions all align more closely with the data and do not require assumptions unsupported by the experimental evidence. Unless these alternatives are systematically excluded through rigorous mechanistic experiments, the central claims of the study remain speculative and insufficiently validated.

10. Implications for Ferroptosis Biology and Cancer Research

10.1. Risks of Misinterpreting Regulated Cell Death Pathways

The claims made by Wu *et al*.[4] carry significant implications for the ferroptosis field, particularly because ferroptosis has rapidly evolved into a highly influential paradigm in cancer research. Misinterpreting oxidative stress–mediated death as ferroptosis risks conflating mechanistically distinct processes. Such conflation undermines the precision necessary to delineate lipid peroxidation pathways, iron dependency, and membrane remodeling dynamics that define ferroptosis. If researchers adopt inhibitors or biomarkers that are insufficiently validated, they may propagate conceptual errors that distort the literature. These risks are magnified in therapeutic development, where mechanistic accuracy is essential for predicting efficacy, toxicity, and resistance mechanisms. The study's ambiguities therefore present broader challenges for maintaining conceptual clarity within regulated cell death biology.

10.2. Impact on Therapeutic Development Pipelines

Therapeutically inducing ferroptosis has generated considerable enthusiasm because ferroptotic vulnerability appears in multiple malignancies, including lung cancer. However, the translational relevance of ferroptosis is contingent on the development of selective, reliable modulators that avoid general oxidative toxicity. If the inhibitor described by Wu *et al*. acts primarily through nonspecific redox disruption rather than selective FSP1 inhibition, then efforts to advance it into preclinical development would be misguided. Misinterpreting off-target ROS induction as ferroptosis creates a false impression of mechanistic novelty and distorts prioritization within drug development pipelines. A compound that disrupts broad NAD(P)H metabolism may show initial cytotoxicity but is unlikely to be tolerable systemically. The absence of rigorous toxicology and mechanistic specificity in the study highlights the need for caution before integrating such compounds into translational programs.

10.3. Importance of Standardized Ferroptosis Criteria

One of the central implications of this paper is the need for standardized criteria for ferroptosis assessment. The field has matured to the point where reliance on lipid ROS staining and partial rescue by Ferrostatin-1 is no longer sufficient. A rigorous ferroptosis diagnosis requires demonstration of iron dependency, oxidized phosphatidylethanolamine species, ACSL4–LPCAT3 axis contribution, and pathway-specific genetic interactions. The widespread variability in ferroptosis sensitivity across cell lines and tissues further underscores the necessity of broad validation.

Wu *et al*. fail to meet these criteria, reminding the field that standardized frameworks are essential to prevent ambiguity and ensure reproducibility. Establishing such criteria would help elevate methodological standards and protect against oversimplified or premature mechanistic claims.

10.4. Translational Implications for NSCLC Treatment

The potential therapeutic implications of targeting FSP1 in NSCLC are substantial. If FSP1 could indeed be selectively inhibited to bypass GPX4 dependency and induce ferroptosis, it would open new avenues for treating tumors resistant to conventional therapies. However, the incomplete mechanistic foundation presented in the study weakens its relevance to real-world translational challenges. NSCLC exhibits substantial metabolic heterogeneity, variable antioxidant buffering capacity, and diverse tumor microenvironment interactions. These factors complicate the predictability of ferroptotic responses. The modest tumor regression observed in the study may reflect generic cytotoxicity rather than a ferroptosis-driven therapeutic effect. Without robust *in vivo* evidence of ferroptosis, claims of translational promise remain speculative and risk misleading clinical research agendas. More comprehensive validation using patient-derived xenografts, ferroptosis-specific *in vivo* markers, and combination studies with existing therapies would be necessary before the therapeutic concept could be credibly advanced.

10.5. Broader Consequences for Research Integrity

Beyond mechanistic concerns, the study raises issues relevant to research integrity. The questionable image quality, inconsistent statistical analyses, incomplete reporting, and absence of raw data deposition exemplify broader challenges in preclinical science. Such deficiencies can propagate irreproducible findings, erode trust in high-impact publications, and burden the scientific community with incorrect assumptions. As ferroptosis continues to attract substantial attention, ensuring rigorous data presentation and analytical transparency becomes critical. The issues present in Wu *et al*.'s work highlight the need for journals, reviewers, and investigators to adopt stricter standards for data integrity, including raw data availability, standardized imaging pipelines, and computational reproducibility frameworks. Failure to uphold these standards risks undermining the field's long-term credibility.

10.6. Lessons for Future Mechanistic Oncology Studies

Wu *et al*.'s paper[4] illustrates several lessons applicable to mechanistic oncology more broadly. First, mechanistic claims require multidimensional validation that integrates biochemical, genetic, metabolic, and computational evidence. Second, pathway-specific rescue experiments are essential for establishing causality,

particularly in complex cell death pathways. Third, broad off-target testing is necessary to avoid confounding effects when introducing new small molecules. Fourth, *in vivo* studies require rigorous design, including randomization, blinding, toxicology assessment, and mechanistic validation. Fifth, the integration of multi-omics datasets can provide more robust mechanistic insights and help prevent oversimplified interpretations. Future studies targeting ferroptosis or similar pathways should adopt a systems-level approach that accounts for metabolic heterogeneity, microenvironmental context, and compensatory regulatory circuits.

10.7. Implications for the Future of Ferroptosis Research

The rapid expansion of ferroptosis research has brought both conceptual innovation and methodological inconsistency. The issues highlighted in Wu *et al.*'s work underscore the importance of establishing rigorous standards for ferroptosis induction, detection, and interpretation. The field would benefit from consensus guidelines detailing required assays, validation steps, and controls. Additionally, integrating ferroptosis research with metabolomics, structural biology, computational modeling, and *in vivo* imaging could enhance mechanistic depth. Wu *et al.*'s study demonstrates that prematurely attributing broad oxidative phenotypes to ferroptosis risks diluting the conceptual clarity of the field. Ensuring that ferroptosis remains a precisely defined and mechanistically grounded form of regulated cell death is essential for its future translation into clinical applications.

10.8. Summary of Translational and Conceptual Implications

Overall, the implications of Wu *et al.*'s work extend beyond the specific dataset to reflect broader challenges in ferroptosis research and mechanistic oncology. The study illustrates how incomplete validation, statistical uncertainty, and conceptual oversimplification can distort mechanistic narratives. The paper reminds the field of the critical importance of rigorous methodology, multidimensional validation, and transparency. Without these components, promising therapeutic strategies risk being built on unstable foundations. Strengthening these aspects will be essential for guiding the next generation of ferroptosis-targeted interventions and ensuring their scientific and translational relevance.

11. Recommendations for Clarification, Correction, and Future Research

11.1. Required Experimental Corrections and Data Transparency

The most immediate need for improving the scientific reliability of Wu *et al.*'s study is the release of raw data. This includes original Western blot TIFF files, unprocessed flow cytometry FCS files, raw LC–MS chromatograms, and complete

xenograft tumor measurements from individual mice. Without these datasets, multiple concerns raised about data integrity and reproducibility cannot be resolved. Corrections should also include proper annotation of sequencing traces for CRISPR clones, full documentation of imaging acquisition parameters, and transparent presentation of controls. Given that several figure panels suggest possible duplication or digital alteration, providing primary data is essential for independent validation. A revised version of the study would benefit greatly from a public repository containing all raw and processed data accompanied by detailed metadata.

11.2. Missing Rescue Controls and Pathway-Specific Validation

To establish ferroptosis convincingly, the authors must include missing rescue controls in both *in vitro* and *in vivo* contexts. These include liproxstatin-1, Trolox, iron chelators such as deferoxamine, and CoQ10 supplementation. Demonstration that tumor inhibition can be reversed by ferroptosis inhibitors *in vivo* is essential for supporting the central claim. The absence of such evidence leaves open the possibility that tumor suppression results from off-target toxicity rather than ferroptosis. Additionally, the study needs ACSL4 dependency assays, LPCAT3 knockdown or inhibition studies, and lipidomics demonstrating accumulation of oxidized phosphatidylethanolamine species. Without these, ferroptosis remains an unverified assumption rather than a demonstrated mechanism.

11.3. Improved Biochemical and Imaging Validation

The biochemical assays require more rigorous validation. Enzyme inhibition studies should include raw kinetic traces, background-corrected rates, and comparative analyses across related oxidoreductases. Structural or computational docking studies would help support claims of specificity. Chemoproteomic pull-down assays could identify binding partners and clarify whether the inhibitor directly engages FSP1. Imaging assays must be performed with standardized exposure settings, blinded analysis, and quantification pipelines described in detail. Electron microscopy should be repeated at higher resolution and include multiple independent fields to validate the ultrastructural claims. Without these improvements, the mechanistic inferences drawn from biochemical and imaging data remain weak.

11.4. Need for Multi-Omics Integration and Systems-Level Approaches

A comprehensive mechanistic study on ferroptosis requires integration of multi-omics datasets. Transcriptomics could clarify whether ferroptosis-related genes or antioxidant pathways are activated. Proteomics could reveal changes in redox

enzymes, membrane proteins, or ferroptosis regulators. Untargeted metabolomics could identify alterations in glutathione metabolism, TCA cycle intermediates, or NADPH flux. A broader lipidomics pipeline is required to assess diversity and specificity of lipid peroxidation products. Integrating these datasets through network analyses would provide a systems-level perspective on how the inhibitor influences cellular metabolism. Such an approach would clarify whether the observed phenotypes arise from selective perturbation of the FSP1–CoQ10 axis or from widespread redox disruption.

11.5. Transparency in Data Deposition and Computational Pipelines

To strengthen the study's reproducibility, the authors should deposit all computational pipelines. This includes code for RNA-seq alignment, differential expression analysis, batch correction, lipidomics preprocessing, and flow cytometry gating. Sharing scripts and parameters is essential for evaluating whether noise filtering, smoothing, or arbitrary thresholds influenced the results. Additionally, public deposition of FASTQ files, mass spectrometry raw files, and cytometry data would enable independent researchers to replicate analyses. Journals increasingly require such transparency for high-impact mechanistic studies. Without it, the computational integrity of Wu *et al.*'s findings remains in question.

11.6. Guidelines for Future Inhibitor Development Studies

The issues identified in Wu *et al.*'s inhibitor characterization illustrate broader challenges in developing selective ferroptosis modulators. Future studies should adopt rigorous medicinal chemistry strategies including structure–activity relationships to confirm specificity, assessment of redox reactivity to avoid confounding effects, and evaluation across large panels of oxidoreductases and flavoproteins. Binding studies using crystallography, cryo-EM, or NMR are essential to determine whether compounds interact with the target's active site. Early incorporation of chemoproteomic profiling can identify off-target proteins and refine inhibitor selectivity. Pharmacokinetic and toxicity studies must include serum chemistry, organ histopathology, bioavailability measurements, and detailed dose–response analyses. These practices would reduce reliance on incomplete biochemical assays and prevent premature mechanistic interpretation.

11.7. Strengthening *In Vivo* Mechanistic Evidence

Future studies focusing on ferroptosis *in vivo* should incorporate direct biochemical markers. Detection of oxidized phospholipids in tumors using LC–MS/MS is essential. Iron accumulation should be verified through chemical probes or synchrotron-based imaging. Ferroptosis inhibitors should be tested in combination

with candidate drugs to validate pathway specificity. Genetic models, such as FSP1 knockout xenografts or tumor models expressing catalytically inactive FSP1 mutants, would greatly strengthen claims of pathway dependence. Without such *in vivo* mechanistic evidence, attributing tumor inhibition to ferroptosis remains speculative.

11.8. Addressing Statistical and Computational Weaknesses

The study would benefit from comprehensive statistical recalibration. This includes reporting exact P values, confidence intervals, and descriptions of statistical tests used. Validation of data distribution, variance homogeneity, and appropriate test selection is necessary for robust inference. Batch correction procedures should be explicitly documented. Biological replicates must be clearly differentiated from technical replicates, and sample sizes should be justified through power calculations. For computational analyses, transparency in algorithms and parameters is essential. Addressing these weaknesses would significantly enhance the reliability of the study.

11.9. Reframing the Mechanistic Narrative with Appropriate Caution

The authors' mechanistic narrative should be reframed to reflect the uncertainties identified. Rather than presenting selective FSP1 inhibition as firmly established, the narrative should acknowledge the possibility of off-target effects, oxidative stress, or mixed cell death mechanisms. Claims about therapeutic potential should be tempered until *in vivo* ferroptosis is demonstrated. A more cautious and evidence-based narrative would strengthen the scientific contribution and provide a more realistic foundation for future investigation.

12. Conclusion

12.1. Summary of Major Critiques

This comprehensive commentary has identified substantial methodological, statistical, conceptual, and interpretive weaknesses in the study by Wu *et al*.[4] The central claim that selective inhibition of FSP1 induces ferroptosis in lung cancer is not adequately supported by the experimental evidence presented. Across biochemical assays, imaging analyses, genetic studies, lipidomics, and *in vivo* experiments, the data exhibit inconsistencies, insufficient controls, and interpretative inflation. Many of the core mechanistic inferences rely on indirect or incomplete evidence rather than pathway-specific validation. The lack of raw data, limited off-target profiling, and insufficient ferroptosis-specific rescue experiments collectively weaken confidence in the central narrative. While the study introduces

an interesting hypothesis regarding FSP1 modulation, the evidence falls short of establishing this mechanism convincingly.

12.2. Importance of Rigorous Methodology

The challenges identified in this study underscore the broader importance of methodological rigor in contemporary ferroptosis research. As the field expands rapidly, the complexity of redox biology and lipid peroxidation demands multidimensional validation. High-quality mechanistic studies require integration of genetic, biochemical, pharmacological, and multi-omics evidence, along with careful statistical treatment and transparent documentation. The absence of standardized criteria for ferroptosis induction risks allowing speculative or incomplete interpretations to enter the literature. Wu *et al.*'s work illustrates how methodological shortcuts can undermine otherwise promising hypotheses and complicate downstream research. Rigorous methodology is essential not only for scientific accuracy but also for guiding translational efforts.

12.3. Preventing Mechanistic Inflation

Mechanistic inflation—where minimal or non-specific evidence is overinterpreted to support sweeping mechanistic claims—is a recurring challenge in fast-moving fields. The misclassification of oxidative stress–induced phenotypes as ferroptosis represents a common form of such inflation. Without careful discrimination between ferroptosis and other oxidative cell death modalities, the conceptual boundaries of the field become blurred. Wu *et al.*'s reliance on non-specific measurements such as C11-BODIPY oxidation, incomplete rescue experiments, and underpowered lipidomics exemplifies this issue. Preventing mechanistic inflation requires adherence to established criteria, rigorous exclusion of alternative pathways, and a commitment to cautious interpretation, especially when proposing clinically actionable mechanisms.

12.4. Ensuring Reproducibility and Scientific Integrity

Scientific integrity depends on transparent reporting, availability of raw data, rigorous statistical analyses, and reproducible computational workflows. Several concerns raised in this commentary—such as possible image manipulation, lack of raw flow cytometry files, inconsistent replicate reporting, and absence of deposited sequencing or lipidomics data—reflect systemic weaknesses that compromise reproducibility. In high-impact mechanistic studies, journals must enforce stringent data transparency requirements, and authors must adopt open-science practices to ensure that results can be independently validated. Ensuring reproducibility is essential not only for confirming the validity of individual studies but also for maintaining trust in the broader research landscape.

12.5. Final Perspective on FSP1-Targeted Ferroptosis in Cancer

Despite the shortcomings of Wu *et al*.'s study, the idea of targeting the FSP1–CoQ10 axis remains scientifically intriguing. FSP1 has emerged as a potent ferroptosis suppressor, and its regulation of lipid peroxidation offers a promising avenue for therapeutic exploitation. However, the evidence presented in this paper does not yet establish FSP1 inhibition as a robust or selective mechanism for inducing ferroptosis in lung cancer. The mixed cell death phenotypes observed, the incomplete ferroptosis validation, and the high likelihood of off-target redox disruption suggest that further investigation is required. Future studies should build upon more rigorous biochemical characterization, comprehensive off-target profiling, and *in vivo* ferroptosis-specific validation. Only with such rigor can the FSP1 pathway be credibly positioned as a therapeutic target.

Reference

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