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Interpreting Gene Essentiality across Biological Contexts: A Causal Framework for Separating Perturbation Effects, Compensatory Responses, and Environmental Dependencies in Functional Genomics

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Abstract

Gene essentiality is often summarized as whether disruption of a gene reduces cellular or organismal fitness, yet functional-genomic evidence shows that this phenotype depends on the perturbation applied, the biological background in which it is imposed, the compensatory capacity of the system, and the environment in which fitness is measured. This article develops a causal account for interpreting context-dependent essentiality without treating screen-derived dependency scores as intrinsic, context-free properties of genes. Evidence from large-scale cancer dependency maps, cross-study CRISPR analyses, physiologic-medium screens, combinatorial perturbation studies, and in vivo functional genomics is organized around three separable components: the direct consequence of perturbing a gene, the compensatory or buffering response triggered by that perturbation, and modifiers arising from genetic background or environmental state. The framework additionally treats the observation process—perturbation modality, temporal window, assay design, and analytical model—as part of the causal interpretation problem. Gene essentiality is therefore most defensibly interpreted as a conditional relationship between a defined intervention and a fitness outcome in a specified biological and measurement context. This account provides a basis for distinguishing robust dependencies from context-limited vulnerabilities and for designing perturbation experiments that discriminate among competing causal explanations.

Keywords: Gene essentiality, Functional genomics, CRISPR screening, Genetic dependency, Paralog buffering, Gene–environment interaction

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Introduction

Genome-scale loss-of-function screening has transformed gene essentiality into quantitative maps of fitness dependencies across biological models. Large cancer dependency projects showed that disrupting the same gene can have markedly different effects across cell lines and molecular backgrounds (Tsherniak *et al.*, 2017). Yet the precision of a dependency score can conceal uncertainty about what produced the observed loss of fitness. A CRISPR-Cas9 screen measures a defined intervention under a defined assay and observation interval; it does not directly observe an abstract property called essentiality.

The distinction is not merely philosophical. Copy-number-associated cutting toxicity can generate apparent dependencies that are attenuated when the observation model explicitly accounts for copy number, demonstrating that part of a screen phenotype may arise from the measurement process rather than from loss of the targeted gene function itself (Meyers *et al.*, 2017). Perturbation modality also changes the biological question. RNA interference usually imposes partial suppression rather than a null state and can therefore expose dosage-sensitive dependencies that differ from those produced by complete gene disruption (McDonald *et al.*, 2017). Consequently, a gene may appear selectively required under one perturbation regime and broadly lethal under another without either result being intrinsically erroneous.

Large CRISPR datasets nevertheless contain reproducible biological structure. Dependency maps can prioritize vulnerabilities associated with lineage or genotype, while major screening resources show substantial agreement alongside systematic discordance (Behan *et al.*, 2019; Dempster *et al.*, 2019). Reproducibility therefore strengthens confidence that a signal is not idiosyncratic to one screen, but it does not establish invariance across environments, perturbation intensities, or biological scales.

Multi-omic analysis can associate dependencies with genomic, transcriptomic, or pathway states and improve target prioritization (Pacini *et al.*, 2024). A linked marker, however, is not automatically the mechanism creating the dependency: it may identify a modifying state, correlate with lineage, or reflect a broader network configuration. Causal interpretation must

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therefore separate predictors of dependency from mechanisms that generate it.

Translation to patient biology introduces another inferential layer. Models that connect cell-line dependencies to tumour genomic data can improve the clinical relevance of functional-genomic maps, but this step is necessarily a projection from experimentally perturbed systems to human tumours in which equivalent interventions have not usually been performed (Shi *et al.*, 2024). A rigorous account of essentiality must therefore distinguish internal evidence that a perturbation changes fitness in the screened system from external claims about whether the same dependence will persist in another biological setting.

This article develops such an account by treating essentiality as a context-dependent causal property. The central proposition is that an observed fitness defect after perturbation can reflect at least four analytically distinct components: the immediate consequence of changing the target gene, compensatory responses that reshape that consequence, genetic or environmental conditions that modify the realized dependence, and the observation process through which the phenotype is detected. The following sections first establish why gene essentiality must be indexed to biological and experimental context, then separate direct perturbation effects from compensatory responses before examining broader environmental and genetic-background interactions.

Materials and Methods

Gene Essentiality as a Context-Dependent Causal Property

Cross-study integration shows that dependency signals can be harmonized across large screening efforts, but the need for harmonization is itself informative: a gene-fitness estimate is produced within a platform, model population, and analytical pipeline (Pacini *et al.*, 2021). Operational definitions therefore matter. Classification as common essential, selective dependency, context-specific vulnerability, or nonessential can depend on sampled models, perturbation strength, scoring reference, and biological conditions.

Environmental composition provides direct evidence that this conditionality is biological rather than merely statistical. CRISPR screening in physiologic medium identified dependencies that differed from those recovered under conventional culture conditions, showing that nutrient availability can alter which gene functions are required for proliferation or survival (Rossiter *et al.*, 2021). Under this interpretation, a dependency is better expressed as a relation between a perturbation and an outcome conditional on an environment than as a permanent label attached to the gene. The same gene may participate in indispensable chemistry in one nutrient regime yet become buffered by exogenous metabolites, alternative transport routes, or pathway redundancy in another.

Lineage and disease state provide a second dimension of conditionality. Genome-scale functional-genomic analysis of multiple myeloma identified dependencies that were preferentially important in myeloma cells relative to other neoplasias (de Matos Simoes *et al.*, 2023). Chordoma-focused dependency mapping similarly showed that uncommon tumour types can exhibit vulnerabilities linked to their distinctive molecular and lineage context (Sharifnia *et al.*, 2023). These findings do not require the conclusion that lineage determines essentiality in isolation. Rather, lineage can summarize combinations of transcriptional state, chromatin configuration, metabolic demand, differentiation program, and genotype that jointly alter the consequence of gene loss.

Co-dependency patterns add useful structure but also illustrate the difference between association and causal attribution. Context-defined co-dependency analysis can identify groups of genes whose fitness effects covary and can nominate functional relationships between complexes or pathways (Chen *et al.*, 2023). However, covariation may arise because two genes respond to the same upstream state, because they participate in the same complex, or because one dependency is contingent on the other. Directionality must therefore be tested rather than inferred from correlation alone.

The conditional character of essentiality becomes especially clear when the environment is experimentally varied. CRISPR interference and activation screens across distinct nutrient settings show that transporter requirements depend on the extracellular resources available to the cell (Chidley *et al.*, 2024). Under arginine-limited conditions, for example, SLC7A5 can become required for cancer-cell growth in ways that are not reproduced under nutrient-replete conditions (Dunlap *et al.*, 2025). Such results provide a discriminating contrast: the same genetic perturbation is observed under different environmental states, allowing the environment itself to be evaluated as a modifier of the fitness effect.

A similar principle applies across biological scales. In vivo CRISPR studies can reveal perturbation consequences that differ from those observed in isolated cell culture because tumour–microenvironment interactions, host immunity, stromal support, nutrient availability, and spatial constraints become part of the causal system. MEN1 screening illustrates how a gene perturbation can acquire context-dependent effects through interaction with the tumour microenvironment (Su *et al.*, 2024). The implication is not that in vivo results are automatically more correct than in vitro results. Each experiment identifies a dependence within its own causal setting, and disagreement between settings can be mechanistically informative.

These distinctions are summarized in **Table 1**. The table treats operational definitions of essentiality as differing causal estimands rather than as interchangeable labels.

Table 1. Operational meanings of gene essentiality under distinct perturbation and biological contexts

Analytical unit	Perturbation or observation condition	What is directly measured	What may legitimately be inferred	Principal modifier of interpretation	Claim that remains unwarranted

Genome-scale knockout dependency	Near-complete loss of target function in a defined cell model	Relative fitness after gene disruption	Requirement for the targeted function under the tested conditions	Copy number, editing toxicity, growth rate, assay duration	Context-free essentiality of the gene
Partial-suppression dependency	Reduced gene activity without a complete null state	Fitness under incomplete functional depletion	Dosage-sensitive requirement under the achieved suppression	Residual protein activity, knockdown efficiency, network reserve	Equivalence to complete knockout
Lineage-selective dependency	Identical perturbation compared across molecularly distinct lineages	Differential fitness effect across model classes	Association of dependency with lineage-linked biological state	Genotype, expression program, chromatin state, metabolic demand	Universal dependency within the entire disease
Nutrient-conditional dependency	Perturbation compared across extracellular resource conditions	Change in fitness consequence as environment changes	Environment-dependent functional requirement	Metabolite availability, transport, pathway bypass	Intrinsic requirement independent of environment
Co-dependency relationship	Covariation of dependency profiles across models	Statistical association between gene-fitness patterns	Candidate shared pathway, complex membership, or conditional interaction	Common upstream state and correlated background variables	Directional causation from co-dependency alone
In vivo dependency	Genetic perturbation in an organismal or tumour-host setting	Fitness or representation under host-associated conditions	Requirement within the tested host, tissue, and temporal context	Immunity, stroma, spatial structure, nutrient supply	Direct equivalence to cell-culture or patient-wide essentiality

Together, the evidence places an essentiality observation at the intersection of perturbation, biological state, environment, and measurement. **Figure 1** represents this inferential structure without assigning any pathway universal dominance.

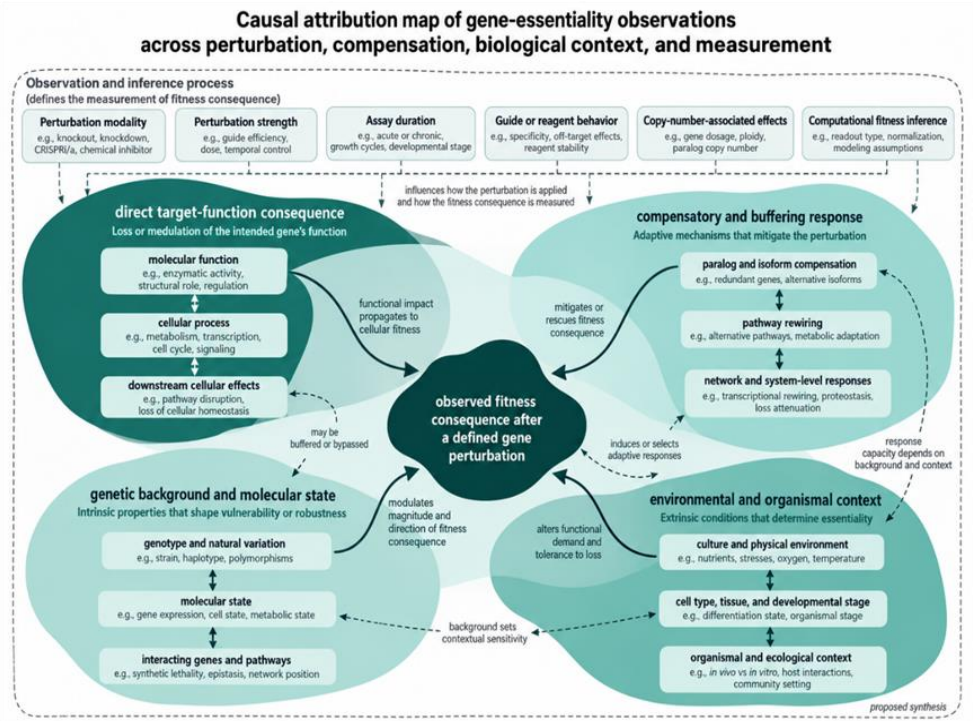


Figure 1. Causal attribution map of gene-essentiality observations across perturbation, compensation, biological context, and measurement

Direct Perturbation Effects and Compensatory Molecular Responses

A perturbation does not end when target activity is reduced or eliminated. The biological system responds. This distinction is central to causal interpretation because the measured fitness phenotype is often the result of both the immediate loss of target function and the network response that follows. Paralog buffering provides a clear example. Across cancer cell lines, the presence

and expression of a functionally related paralog can explain part of the variability in whether disruption of a gene produces a fitness defect (De Kegel & Ryan, 2019). Multiplex Cas12a screening strengthens this interpretation by showing that some dependencies hidden in monogenic knockout experiments emerge when candidate paralog pairs are disrupted together (Dede *et al.*, 2020). The absence of a phenotype after single-gene knockout can therefore represent successful compensation rather than true dispensability.

The architecture and timing of perturbation determine how well these mechanisms can be separated. Hybrid Cas9-Cas12a approaches enable combinatorial and exon-level perturbations that can reveal interactions inaccessible to conventional single-gene screens (Gonatopoulos-Pournatzis *et al.*, 2020). At the population level, CRISPR depletion is also dynamic: cells carrying strongly deleterious perturbations disappear at different rates, and explicit modeling of these trajectories can improve inference of gene-fitness effects (Dempster *et al.*, 2021). A terminal depletion score can consequently combine immediate target loss, delayed compensation, proliferation-rate differences, and selection over time.

Combinatorial screening demonstrates that paralog relationships are not uniformly redundant. Pairwise CRISPR studies identify fitness interactions in which neither single perturbation fully predicts the effect of combined loss, and DUSP4-DUSP6 provides a concrete example of a digenic dependence tied to MAPK-pathway-driven cancer states (Ito *et al.*, 2021; Thompson *et al.*, 2021). These results support a counterfactual distinction: the direct effect of losing gene A is evaluated with gene B intact, whereas the combined perturbation evaluates the system after removing a potential compensatory route. A difference between those outcomes provides stronger evidence for buffering than a single knockout phenotype alone.

Genetic background and perturbation strength further complicate this decomposition. Large-scale analyses have identified paralog interactions conditioned by expression, copy-number state, and autosomal or sex-chromosome context (Köferle *et al.*, 2022). Cross-modality comparison also shows that partial suppression can identify selective vulnerabilities when complete CRISPR knockout appears broadly lethal (Krill-Burger *et al.*, 2023). These findings imply that "the effect of gene loss" is not a single intervention unless the degree, timing, and molecular form of the perturbation are specified.

The translational appeal of paralog synthetic lethality follows from this conditionality: loss or weakening of one family member may make a second selectively important, creating a potential therapeutic window (Ryan *et al.*, 2023). Yet individual examples show why compensation should not be reduced to a binary redundant/nonredundant label. In SMARCB1-deficient cancers, dependency involving the CBP/p300 paralog pair is linked to a specific chromatin and transcriptional state (Sasaki *et al.*, 2024). Incomplete compensation between TRA2 paralogs can likewise create selective dependence when the remaining paralog cannot fully sustain the required function (Lee *et al.*, 2025). A comparable logic applies to metabolic paralogs such as CDS1/CDS2, where vulnerability emerges in defined mesenchymal-like cancer states rather than as a universal property of the pair (Arnoldus *et al.*, 2025).

The relevant causal quantity is therefore the realized effect of perturbation after the system has had an opportunity to respond. Compensation can attenuate, delay, reroute, or qualitatively alter that effect; conversely, insufficient compensation can expose dependencies that are invisible in another background. **Table 2** distinguishes these mechanisms without treating any single response pattern as the default explanation.

Table 2 distinguishes immediate perturbation consequences from compensatory responses.

Table 2. Distinguishing immediate perturbation consequences from compensatory responses in gene-dependency experiments

Perturbation situation	Immediate biological consequence under test	Compensatory process that may follow	Observation that would support compensation	Alternative explanation that must remain open	Interpretation of essentiality
Single-gene knockout with weak phenotype	Loss of targeted gene function	Paralogue or pathway substitute preserves output	Stronger defect after matched dual perturbation	Incomplete editing or low baseline requirement	Apparent nonessentiality may reflect buffering
Single-gene knockout with strong phenotype	Loss of a nonredundant or insufficiently buffered function	Limited adaptive response	Persistent phenotype despite available compensatory candidates	General growth defect or assay toxicity	Supports dependence only in the tested background
Dual-paralog perturbation	Simultaneous removal of overlapping functions	Residual network adaptation outside the paralog pair	Combined effect exceeds either single perturbation	Additive independent effects	Supports functional buffering when interaction is mechanistically validated
Partial suppression	Reduced target dosage	Residual target activity and adaptive network reserve	Selective phenotype at partial loss that differs from complete knockout	Platform-specific off-target effects	Defines a dosage-specific dependency

Time-resolved depletion	Target loss followed by population selection	Delayed transcriptional, metabolic or network adaptation	Divergence between early and late fitness effects	Growth-rate differences unrelated to compensation	Essentiality estimate is time-indexed
Background-restricted vulnerability	Perturbation imposed in a defined molecular state	Compensation constrained by genotype, expression or pathway configuration	Loss of phenotype when the modifying background changes	Correlated lineage or state variable	Dependency is conditional on the specified background

Environmental Dependencies and Genetic Background Interactions

Environmental context can alter a gene-dependency phenotype even when the perturbation itself is unchanged. In vivo CRISPR screening illustrates this most clearly because tumour cells are exposed simultaneously to host immunity, stromal signals, nutrient gradients, and treatment pressures. In lung-cancer models, perturbation screens identified tumour-cell genes including Serpinb9 and Adam2 whose effects depended on immune-therapy context (Dervovic *et al.*, 2023). Such findings place the host environment inside the causal system: a dependency may emerge because the perturbation changes how a cell interacts with immune selection rather than because it disables a universally required intracellular process.

Metabolic niches provide a complementary example. Taurine supplied by the tumour environment was shown to support glycolytic activity and leukaemogenesis in defined models (Sharma *et al.*, 2025). Here, the environment does more than modify the magnitude of a pre-existing dependency; it helps establish the metabolic state in which the dependency becomes consequential. Removing the environmental input, changing nutrient access, or altering the associated pathway can therefore change the observed fitness effect without any change in the target gene's sequence.

Immune signalling can similarly change the realized consequence of gene loss. VDAC2 loss has been linked to tumour destruction and inflammatory responses in defined cancer models (Yuan *et al.*, 2025). The relevant causal interpretation includes both tumour-intrinsic consequences and host-mediated effects. A phenotype observed in an immunocompetent setting cannot automatically be reduced to the direct intracellular function of the perturbed gene, just as a cell-culture phenotype cannot automatically predict the same response in an intact host.

Table 3 organizes these sources of variation according to the level at which they enter the dependency estimate.

Table 3. Sources of variation that can alter an observed gene-essentiality estimate

Variation source	Biological or experimental level	How the dependency estimate can change	Discriminating comparison	Interpretation boundary
Extracellular nutrient state	Cell–environment interface	Changes availability of substrates, transport requirements, and pathway bypass	Same perturbation across defined nutrient conditions	A dependency observed in one medium need not persist in another
Tumour or tissue niche	Tissue/ microenvironment	Supplies metabolites, growth signals, or spatial constraints that reshape cellular requirements	Perturbed cells tested with versus without the relevant niche condition	Niche-supported dependence is not a context-free gene property
Immune state	Tumour–host system	Alters selection, killing, inflammatory signalling, and treatment response	Matched perturbation in different immune or treatment contexts	Host-mediated fitness effects should not be assigned solely to cell-autonomous gene function
Genetic background	Gene/network/cell	Changes paralog availability, pathway reserve, oncogenic state, or synthetic-lethal relationships	Same perturbation across matched backgrounds	Background-linked dependence requires the qualifying genotype or state
Perturbation strength	Molecular intervention	Distinguishes partial loss from complete loss and may expose dosage-sensitive phenotypes	Knockdown, repression, knockout, or graded perturbation	Different interventions estimate different causal effects
Assay and analytical context	Observation process	Alters detectability through duration, population dynamics, editing toxicity, or scoring model	Replication with alternative assays or analysis	Measurement variation should not be interpreted as biological conditionality without evidence

Taken together, these examples support a multiscale view of conditional essentiality. Environmental state, genetic background, and observation design need not act independently: a genotype can alter metabolic demand, the niche can determine whether an

alternative pathway is available, and immune pressure can expose a vulnerability that is silent in vitro.

A Causal Framework for Interpreting Conditional Essentiality

A causal interpretation should begin by defining the intervention and the outcome before assigning a label to the gene. In vivo CRISPR methodology makes clear that delivery, library coverage, model choice, and readout constrain which dependencies can be observed (Santinha *et al.*, 2025). The first rule is therefore to specify the experimental system that renders the perturbation observable.

The second rule is to match perturbation semantics to the biological question. CRISPR-based functional genomics now includes knockout, repression, activation, base editing, and other variant-level interventions, each of which changes a different component of gene function (Kim *et al.*, 2024). A phenotype caused by editing one disease-associated variant does not establish that complete loss of the gene would produce the same result. Likewise, CRISPR activation interrogates consequences of increased expression and can test sufficiency-like relationships that are distinct from necessity under loss of function (Clark *et al.*, 2024).

The third rule is to identify modifiers that change the effect of the same perturbation. Acquired molecular states can create new dependencies, as illustrated by PRMT5 vulnerability in CDK4/6 inhibitor-resistant, RB-deficient breast-cancer models (Lin *et al.*, 2024). Oncogenic programmes can do the same: MYC-driven hepatocellular carcinoma shows a dependency on nuclear-to-cytoplasmic transport in the studied setting (Deutzmann *et al.*, 2024). Such results are most informative when the modifying state is retained explicitly in the claim.

The fourth rule is to treat compensation and environment as competing or interacting explanations rather than as residual noise. A weak phenotype can reflect genuine dispensability, successful paralog buffering, incomplete perturbation, or an environment that supplies a bypass. A strong phenotype can reflect direct loss of indispensable function, collapse of compensation, treatment-specific selection, or assay-associated toxicity. Discrimination requires perturbations or context changes capable of separating these possibilities.

Table 4 converts these principles into interpretation rules that can be applied without turning them into a deterministic scoring algorithm.

Table 4. Causal interpretation rules for context-dependent gene essentiality

Interpretive question	Evidence required	Competing explanation to retain	Strongest permitted conclusion
What intervention was actually imposed?	Verified perturbation modality, strength, and target engagement	Off-target effect or incomplete perturbation	Dependency under the achieved intervention
What biological state was perturbed?	Defined lineage, genotype, pathway, expression, or resistance state	Correlated state marker rather than causal modifier	State-conditioned dependency
Could compensation mask the direct effect?	Single versus combinatorial perturbation, rescue, or other mechanistic contrast	Independent additive effects or unrelated background variation	Evidence for buffering only when the contrast discriminates it
Could the environment create or remove the dependency?	Matched perturbation across defined environmental or host conditions	General stress or growth-rate differences	Environment-conditioned requirement
Does the observation process distort the phenotype?	Orthogonal assay, artifact correction, temporal analysis, or alternative scoring	Genuine biology associated with the same technical variable	Measurement-qualified dependency estimate
Can the result be generalized?	Replication across relevant models or explicit validation in the target system	Model-specific adaptation, species effect, or missing host factor	Transfer only to contexts supported by matching evidence

The framework therefore interprets essentiality claims as conditional statements linking intervention, biological state, compensatory capacity, environment, observation process, and target context of generalization. This structure does not require every experiment to manipulate all components. It requires the conclusion to remain no broader than the contrasts actually observed.

Results and Discussion

Identifiability, Perturbation Design, and Generalizability

The main challenge is identifiability: different causal processes can generate similar depletion phenotypes. Copy-number-associated Cas9 toxicity, cross-dataset discordance, and time-dependent population dynamics show that an observed fitness score can combine biological and measurement effects (Meyers *et al.*, 2017). Replication across screens helps, but agreement does not determine whether the underlying dependency is direct, compensated, or environmentally mediated (Dempster *et al.*, 2019). Temporal modeling further demonstrates that the inferred magnitude of a fitness effect depends partly on how population dynamics are represented (Dempster *et al.*, 2021).

Generalizability poses a separate problem. Projection of cell-line dependencies toward tumour cohorts can be informative, but it remains a transfer step (Shi *et al.*, 2024). In vivo studies demonstrate why the transfer cannot be assumed: host and

microenvironmental interactions can materially alter the consequence of the same gene perturbation (Su *et al.*, 2024). The appropriate question is therefore not whether one experimental system is intrinsically superior, but whether the context that generates the dependency is represented in the system to which the claim is being transferred.

Perturbation design should consequently follow the causal question. Partial suppression may be preferable when the aim is to identify dosage-sensitive vulnerabilities that complete knockout converts into pan-lethality (Krill-Burger *et al.*, 2023). In vivo screening is necessary when host-associated selection is part of the hypothesized mechanism (Santinha *et al.*, 2025). CRISPR activation is appropriate when increased expression or sufficiency is under study, but its results should not be relabeled as evidence of loss-of-function essentiality (Clark *et al.*, 2024).

A robust conditional-essentiality claim is strongest when evidence converges across distinct forms of contrast: molecular-state stratification, matched environmental manipulation, orthogonal perturbation, and validation at the biological scale relevant to the intended conclusion. Multi-omic dependency mapping can identify states associated with vulnerability (Pacini *et al.*, 2024), while in vivo immune-context studies show whether such dependencies persist when host interactions are restored (Dervovic *et al.*, 2023). Mechanistic work on context-sensitive tumour responses further shows that even an apparently strong dependency may change when the surrounding biological system changes (Yuan *et al.*, 2025). The goal is not to eliminate contextuality, but to make it explicit enough that competing causal explanations can be tested.

Conclusion

Gene essentiality is best interpreted as a conditional causal relationship rather than an intrinsic label attached permanently to a gene. The observed consequence of perturbation reflects the intervention applied, the direct function lost, the compensatory capacity of the system, the genetic and environmental state in which the perturbation occurs, and the way fitness is measured. Separating these components clarifies why dependencies can differ across cell lines, nutrient conditions, perturbation strengths, tumour states, and in vivo environments without requiring any of those observations to be dismissed as inconsistent.

This interpretation changes the scientific question from whether a gene is essential to the more informative question of when, through which mechanism, and under which perturbation and biological conditions loss of that gene becomes consequential. Claims that retain those conditions are more falsifiable, more transferable, and more useful for functional genomics than context-free classifications. The resulting framework supports mechanistic comparison across experiments while preserving the biological heterogeneity that gene-essentiality screens are designed to reveal.

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