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CTRITICAL REVIEW

Organoid Models through the Lens of Metabolomics: A Systematic Review of Experimental Applications and Analytical Approaches

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Organoid models have transformed experimental biology by enabling three-dimensional systems that recapitulate tissue architecture, cellular heterogeneity, and metabolic activity patterns more faithfully than conventional in vitro cultures. In parallel, metabolomics has emerged as a systems-level approach to interrogate the biochemical processes integrating genetic programs, environmental cues, and phenotypic outcomes. Despite this promise, the application of metabolomics to organoids remains analytically fragile, challenged by low sample biomass, complex extracellular matrices, heterogeneous culture conditions, and substantial variability in experimental and computational workflows. This systematic review critically examines metabolomics and lipidomics applications across intestinal, hepatic, renal, cerebral, vascular, and tumor-derived organoids, spanning development, disease modeling, toxicology, and drug response. We synthesize how metabolic profiling provides functional insights often inaccessible to transcriptomic or morphological analyses alone. Particular emphasis is placed on analytical design and quality control, highlighting how matrix-aware strategies, normalization choices, and QC-driven preprocessing critically shape metabolite recovery, reproducibility, and biological interpretability. By comparing targeted and untargeted approaches, mass spectrometry– and NMR-based platforms, and extracellular matrix mitigation strategies, we identify recurring sources of analytical variability and interpretative bias. We further propose a minimal, context-aware QC framework tailored to the specific constraints of organoid-based metabolomics. Collectively, this work provides a critical analytical reference to strengthen reproducibility, comparability, and translational robustness in 3D organoid metabolomics.

Introduction

The emergence of human organoids has profoundly reshaped experimental models of human biology by enabling three-dimensional architectures that recapitulate key cellular, genetic, and organizational features of native tissues. Through intrinsic self-organization and tissue-specific lineage commitment, organoids occupy a conceptual and experimental space between reductionist two-dimensional cultures and human in vivo systems, which remain constrained by ethical and practical limitations. Their rapid adoption across developmental biology, oncology, toxicology, and regenerative medicine reflects a broader shift toward experimental models that prioritize human relevance over experimental convenience [1]. Organoids are self-organizing three-dimensional cellular constructs derived from primary tissues or pluripotent stem cells that reproduce essential structural and functional characteristics of native organs. Unlike conventional two-dimensional cultures, organoids develop spatial architecture, apico–basal polarity, multicellular lineage diversification, and microenvironmental gradients within an extracellular matrix scaffold. This structural and cellular complexity enhances their physiological relevance for modeling tissue development, disease progression, and therapeutic responses. At the same time, the very features that confer biological fidelity—matrix embedding, heterogeneous cell populations, variable differentiation states, and intrinsically low biomass—also introduce analytical constraints that are largely absent in monolayer cultures. As a result, organoids represent experimentally sophisticated yet analytically demanding substrates in which measurement decisions can critically influence biological interpretation.

Despite their morphological and cellular sophistication, early organoid studies have relied predominantly on transcriptomic, genetic, and phenotypic readouts to infer biological function. While these approaches provide valuable structural and regulatory insight, they offer only indirect access to the biochemical state of the system. Cellular metabolism represents the most immediate functional layer of biology, integrating genetic programs, environmental cues, and multicellular interactions into measurable molecular outputs. Accordingly, metabolomics and lipidomics provide a uniquely sensitive

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framework to interrogate organoid physiology, enabling the detection of dynamic pathway activity, metabolic plasticity, and functional maturation that are not readily captured by other omics layers [2].

In recent years, the integration of organoid models with mass spectrometry– and NMR-based metabolomics has enabled direct measurement of metabolic states in human-relevant three-dimensional systems. These approaches have been applied across diverse biological contexts, including cancer metabolism, tissue development, toxicological responses, and neurobiology, demonstrating that organoids can reproduce hallmark metabolic features of human tissues and diseases [3]. Metabolic profiling has further revealed pathway-level vulnerabilities, tumor–stroma metabolic crosstalk, and developmental bottlenecks intrinsic to in vitro differentiation [4]. At the same time, the application of metabolomics and lipidomics to organoids has highlighted fundamental analytical and conceptual challenges. These include limited biomass, interference from extracellular matrices, statistical fragility associated with small sample sizes, and a lack of standardized workflows for sample preparation, data acquisition, and analysis [2]. Such sources of variability complicate cross-study comparison and hinder the translational interpretation of metabolic phenotypes.

Notably, the expansion of organoid metabolomics has been driven as much by technological innovation as by biological discovery. Advances in chromatographic miniaturization, high-resolution mass spectrometry, spatially resolved mass spectrometry imaging, and low-input analytical workflows now permit metabolic and lipidomic profiling from hundreds—or even individual—organoids [5,6]. These developments have shifted the field away from pooled, population-level measurements toward higher-resolution and spatially informed analyses, while simultaneously increasing methodological heterogeneity [7].

Given this rapidly evolving landscape, a critical synthesis of organoid-based metabolomics is timely to integrate biological insight with methodological rigor and translational realism. In particular, distinguishing metabolic features that genuinely reflect the structural, functional, and genetic characteristics of human organs from those driven by model-specific or analytical constraints is essential for the field's future development.

Beyond their experimental relevance, organoid-based metabolomics also holds increasing translational potential. By enabling metabolic characterization in human-derived, physiologically structured systems, organoids provide an intermediate platform between reductionist in vitro models and clinical samples, with potential applications in therapeutic stratification, functional biomarker discovery, and preclinical model refinement.

In this review, we provide a structured and critical overview of metabolomics applications in human organoid research. We examine key methodological considerations spanning sample preparation, analytical platforms, and data processing, highlight recurrent sources of variability, and discuss emerging best practices. We then synthesize biological insights across major application domains, outlining how metabolic profiling in organoids is advancing our understanding of disease mechanisms, developmental trajectories, and tissue-specific functions. By integrating methodological trends with biological interpretation, this work aims to provide a critical and analytically grounded overview of the current state of the art.

Materials and Methods

A systematic literature search was conducted on PubMed in November 2025 to identify original research articles applying metabolomics and/or lipidomics approaches to organoid-based experimental models. The search was performed using the query "metabolomics AND organoids", without temporal restrictions, yielding a total of 347 records. The search strategy was intentionally broad in order to capture the diversity of biological models, analytical platforms, and experimental objectives characterizing metabolomics and lipidomics investigations in organoids. PubMed was selected as the primary biomedical database to ensure consistency and reproducibility of search results within a clinically oriented context.

The study selection process was performed independently by two authors, with any discrepancies resolved through discussion until consensus was reached. All retrieved records were screened according to a two-stage selection process consistent with PRISMA 2020 guidelines [8]. Studies were included if they met all of the following criteria: (i) applied metabolomics and/or lipidomics as a primary analytical approach on organoid three-dimensional models, including human-derived organoids (patient-derived, iPSC-derived, or primary cell-derived) as well as non-human organoids;

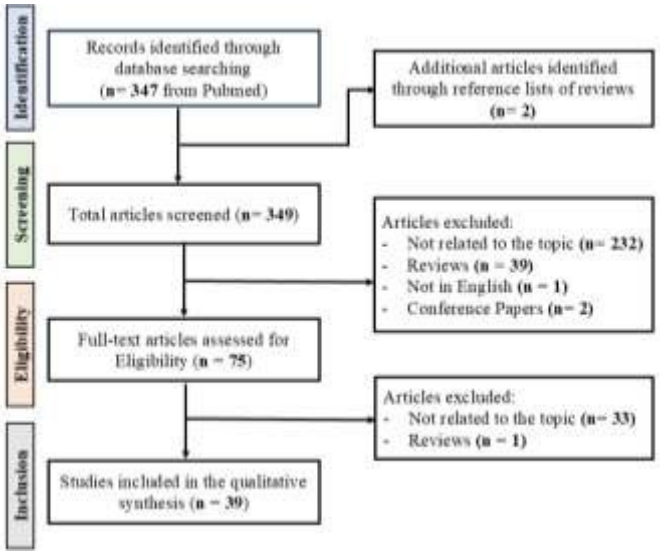

Figure 1. PRISMA-style flow diagram of the literature search and study selection process. The diagram depicts the identification, screening, eligibility, and inclusion phases of the systematic literature search conducted for this review. Records were identified through PubMed and complementary reference list screening. Following removal of non-relevant records and exclusion based on predefined criteria, 39 studies were included in the qualitative synthesis.

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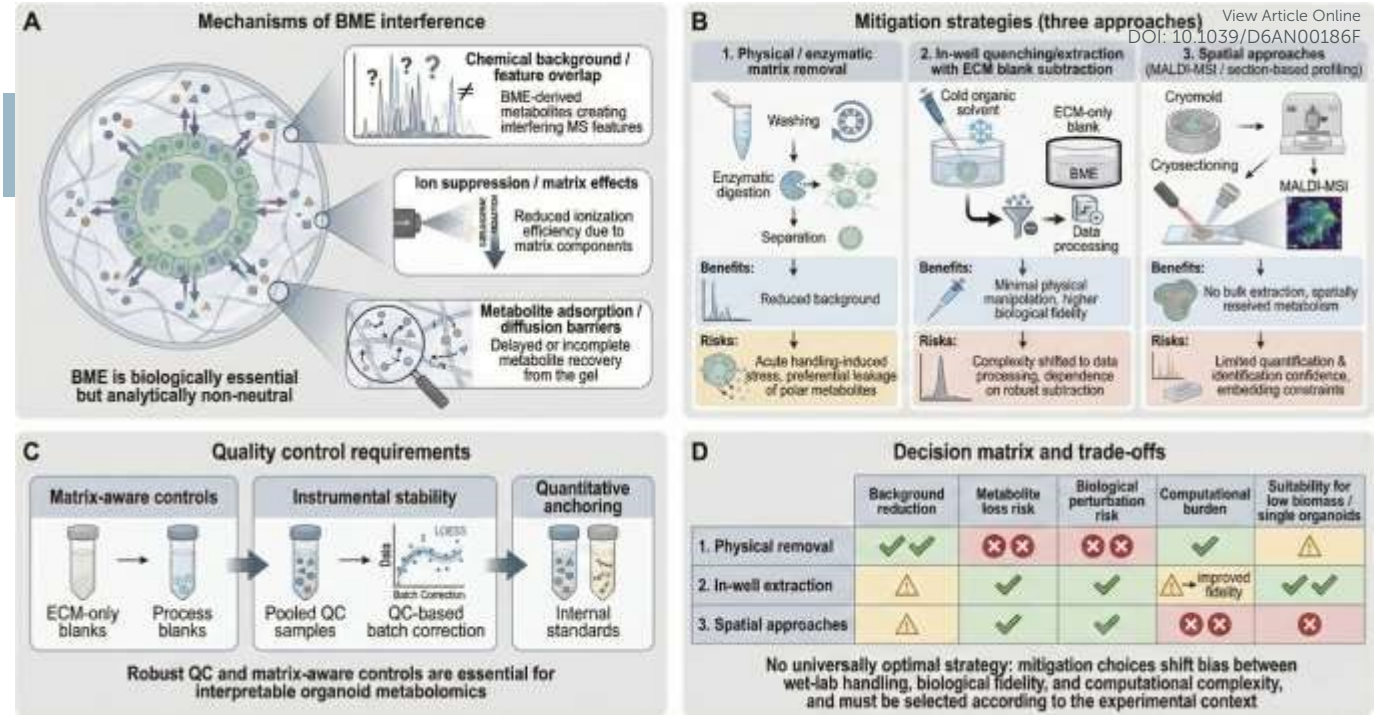

Figure 2. Extracellular matrix (basement membrane extract, BME) interference and mitigation strategies in organoid-based metabolomics. Human organoids are typically cultured within basement membrane extracts (BME), such as Matrigel, which are biologically indispensable for three-dimensional growth and differentiation but analytically non-neutral for metabolomics and lipidomics. Panel A illustrates the principal mechanisms through which BME interferes with metabolomic measurements, including chemical background and feature overlap from matrix-derived metabolites, ion suppression effects during electrospray ionization, and metabolite adsorption or diffusion barriers that delay or prevent quantitative recovery from the gel. Panel B summarizes three major mitigation strategies adopted in the literature. Physical or enzymatic matrix removal reduces analytical background but may induce acute handling-related stress and preferential leakage of polar metabolites. In-well quenching and extraction combined with ECM-only blank subtraction minimize physical manipulation and better preserve biological fidelity, at the cost of increased dependence on matrix-aware data processing. Spatially resolved approaches, such as MALDI–MSI, bypass bulk extraction altogether and retain spatial information but are constrained by limited quantification and metabolite identification confidence. Panel C highlights the quality control (QC) requirements necessary to support these strategies, emphasizing the role of matrix-aware controls (ECM-only and process blanks), instrumental stability monitoring through pooled QC samples and batch correction, and quantitative anchoring via internal standards. Panel D integrates these elements into a comparative decision matrix, illustrating that no universally optimal strategy exists; rather, mitigation choices shift analytical bias between wet-lab handling, biological perturbation, and computational complexity. Collectively, the figure underscores that managing BME-related interference requires explicit trade-off awareness rather than protocol-level optimization alone.

(ii) provided original experimental data (i.e., non-review articles); (iii) reported sufficient methodological detail regarding sample handling and analytical workflows. Furthermore, studies were excluded if they were not written in English and did not have full text available. As a result, a total of 39 articles were included in the qualitative synthesis.

The flow diagram of the study selection is outlined in Figure 1. The reviewed literature reveals not only biological diversity but methodological asymmetry, which forms the basis of the analytical critique developed below.

Results and Discussion

The body of literature identified through the systematic search reflects a rapidly expanding yet methodologically diverse field applying metabolomics and lipidomics to human organoid models. Notably, despite the absence of temporal restrictions in the search strategy, all studies meeting the inclusion criteria were published within the last five years, underscoring both the recent emergence of this research area and its ongoing phase of methodological consolidation.

This temporal clustering reflects the recent convergence of organoid technology and metabolomics, underscoring the novelty of the field rather than limitations in the search strategy.

This heterogeneity in biological context and analytical design, synthesized in Table 1, provides the basis for the critical evaluation of methodological practices and biological insights presented in the following sections. Table 1 provides a conceptual aggregation of the reviewed literature by organoids, biological context, and analytical strategy- while a study-by-study inventory is provided in supplementary table S1- and is intended to provide insights in the major application domains discussed below.

Sample Preparation

Sample preparation represents the primary determinant of data quality and biological interpretability in metabolomics and lipidomics studies conducted on human 3D organoids. In contrast to two-dimensional cultures or bulk tissues, organoids combine three-dimensional architecture, marked cellular heterogeneity, and pronounced variability in size and biomass, rendering the direct transfer of conventional metabolomics workflows inherently problematic [9–11]. As a result, current practices reflect a fragmented methodological landscape,

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largely driven by case-specific adaptations rather than shared best-practice frameworks.

As schematically summarized in Figure 2, the analytical challenges associated with organoid sample preparation—particularly those arising from the use of basement membrane extracts—do not admit a single optimal solution, but instead require explicit trade-offs between background reduction, biological perturbation, and computational complexity.

Extracellular Matrix Interference and the Low Biomass Challenge

A defining methodological challenge in organoid metabolomics arises from the near-universal reliance on basement membrane extracts (BME), most commonly Matrigel, to sustain three-dimensional growth and differentiation. The principal mechanisms through which BME interferes with metabolomic measurements are illustrated in Figure 2A, including matrix-derived background signals, ion suppression effects, and metabolite retention within the gel. While biologically indispensable, these matrices are chemically complex, metabolically active, and poorly defined, introducing substantial background signals and ion suppression effects in mass spectrometry–based analyses [10,12]. As a result, the majority of studies attempt to physically remove the extracellular matrix prior to metabolite extraction through repeated cold PBS washes, low-speed centrifugation, or enzymatic dissociation using dispase or Cell Recovery Solution (CRS) [13–15]. While effective in reducing matrix-derived background, these procedures inherently increase the risk of metabolite leakage and stress-induced metabolic alterations, particularly in fragile or highly differentiated organoids [16]. These handling-related trade-offs are explicitly represented in Figure 2B, which contrasts physical or enzymatic matrix removal with alternative, matrix-aware extraction strategies.

An alternative and conceptually innovative strategy was introduced by Neef et al. [10], who proposed an in-well sampling approach in which cold extraction solvents are added directly to organoids embedded in the ECM, thereby minimizing physical manipulation. To compensate for matrix-derived background signals, the authors implemented a rigorous bioinformatic filtering strategy based on ECM-only blank samples, enabling the removal of over 70% of uninformative features and allowing reliable metabolomic profiling from fewer than 500 cells per injection [10]. This approach exemplifies the class of in-well extraction and ECM-blank–driven mitigation strategies summarized in Figure 2B, in which analytical background is addressed primarily through computational filtering rather than physical matrix removal.

Beyond matrix interference, the intrinsically low biomass of organoid cultures represents a pervasive bottleneck for metabolomics and lipidomics. Many studies report insufficient

Table 1. Aggregated overview of organoids, biological contexts, and analytical strategies represented in the reviewed literature. Studies included in this review are grouped by organoids models, species of origin, primary biological context, and omics strategy. For each category, all relevant studies are explicitly cited to ensure bibliographic completeness, while detailed study-specific information is provided in Supplementary Table S1.

Organoid model	Species / Origin	Primary Biological Context	Omics Strategy	Included Studies
Intestine (Colon & Small Intestine)	Human (iPSC, ESC, PDO), Mouse	CRC, Toxicity (5-FU, Microplastics), Nutrition (Formula vs. Breast milk), Host-microbe interaction	Metabolomics (LC-MS, NMR), Transcriptomics (RNA-seq), Lipidomics (MALDI-MSI), Multi-omics	10,11,14,16–23
Liver & Biliary	Human (iPSC, HepG2, PDO), Rat (DLS)	Hepatotoxicity (DBP), Glucose/Lipid Metabolism, Bile Duct Engineering	Lipidomics, Metabolomics, Transcriptomics	9,24–26
Kidney	Human (Fetal, iPSC, PDO), HEK293T	Nephrogenesis, Pediatric Cancer (Wilms, MRT), Adhesion mechanisms	Spatial Metabolomics (MALDI-MSI), Metabolomics (LC-MS, NMR), scRNA-seq	27–30
Brain & Nervous System	Human (iPSC, Brain Organoids)	Neurodegeneration (Alzheimer's), Toxicology (Graphene Oxide), Neuropsychiatry	Lipidomics, Metabolomics, Proteomics, Transcriptomics	6,13,31,32
Pancreas	Human (PDO), Cell lines	PDAC (Cancer metabolism), KRAS/p53 mutations, Drug response	Metabolomics (Targeted/Untargeted), Flux Analysis (13C), Proteomics	12,33,34
Vascular / Heart	Human (iPSC)	Microvascular integrity, Diabetic retinopathy, Biomanufacturing	Proteomics (Secretome), Metabolomics (Seahorse)	35,36
Lung / Airway	Mouse (Tracheal basal cells)	Epithelial differentiation, Drug toxicity (Anti-TB drugs)	Metabolomics, Transcriptomics	37,38
Sensory Organs	Human (iPSC), Mouse	Eye (Cornea/Aniridia), Ear (Cochlear development)	Metabolomics (GC-MS), Transcriptomics	39,40
Adipose	Mouse (SVF)	Immunometabolism, Inflammation	Lipidomics	41
Endocrine	Human (Parathyroid PDO)	Hyperparathyroidism	Metabolomics (Untargeted)	15
Tumor (General)	Human (Slices), Mouse	Cancer metabolism, Method development	Metabolomics, Proteomics	42,43
Methodological	Human (Plasma, DBS, PDO)	Analytical method optimization (Microbore columns)	Metabolomics (UHPLC-HRMS)	44

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signal intensity when analyzing individual organoids, particularly during early differentiation stages or when working with patient-derived material characterized by slow growth rates [29,40]. The most widespread mitigation strategy is therefore the pooling of multiple organoids or entire culture wells, with some investigations aggregating material from up to 40 wells per biological replicate [29,40].

While pooling effectively increases analyte abundance, it fundamentally alters the analytical question being addressed, shifting the focus from organoid-level phenotypes to population-averaged metabolic states.

This approach obscures inter-organoid heterogeneity, limits the detection of rare metabolic phenotypes, and complicates the interpretation of patient-specific responses, therefore limiting potential precision medicine applications [12,34]. As highlighted in Figure 2D, such strategies effectively redistribute analytical bias by trading organoid-level resolution for increased signal intensity. Despite these drawbacks, pooling remains prevalent due to the lack of widely accessible high-sensitivity analytical solutions.

Recent methodological advances, however, are beginning to redefine these constraints. The adoption of microbore liquid chromatography systems with 1.0 mm internal diameter columns has been shown to substantially enhance sensitivity by increasing analyte concentration at the electrospray source. Using this configuration, Gadara et al. [13] demonstrated the quantification of more than 350 lipid species from a single cerebral organoid, achieving approximately fourfold sensitivity gains relative to conventional setups. Similarly, La Gioia et al. [44] observed a significant increase in MS1 feature detection and metabolome coverage in colorectal cancer organoids containing fewer than 10^4 cells. These studies collectively signal a methodological transition from pooled analyses toward single-organoid–resolved metabolomics, with profound implications for the study of intra-model heterogeneity. Despite these gains, however, analytical miniaturization introduces its own limitations. Microbore systems are often optimized for specific metabolite classes, such as polar metabolites or phospholipids, potentially biasing coverage toward selected chemical spaces [44]. Moreover, reduced chromatographic robustness and increased susceptibility to clogging may limit long-term reproducibility, particularly in large-scale or multi-center studies.

Complementary to chromatographic miniaturization, spatially resolved techniques such as mass spectrometry imaging (MSI) offer an alternative solution by eliminating bulk extraction altogether. High-resolution MALDI-MSI approaches have been successfully applied to organoid sections embedded in fish gelatin or cellulose-based matrices, enabling lipid mapping at cellular or subcellular resolution while preserving tissue architecture [6,16]. However, these approaches require highly specialized embedding protocols and remain analytically and computationally demanding.

Quenching Strategies and Metabolic Snapshot Fidelity

The preservation of an authentic metabolic snapshot requires the rapid and irreversible cessation of enzymatic activity at the time of sampling. Across the reviewed literature, snap-freezing or flash-freezing in liquid nitrogen remains the most commonly employed quenching strategy [12,15,27]. This approach is favored for its simplicity and effectiveness across a broad range of metabolites.

For spatially resolved applications, alternative quenching strategies are required to preserve tissue morphology. In these contexts, organoids are frequently quenched using ethanol and dry ice prior to embedding and cryosectioning, enabling high-resolution mass spectrometry imaging while maintaining structural integrity [6,16]. Although effective for lipid mapping, these protocols introduce additional constraints on metabolite coverage and require careful optimization of embedding media to prevent analyte delocalization.

Extraction protocols across studies converge toward solvent systems that maximize metabolite coverage while precipitating proteins and minimizing enzymatic reactivation. Chilled methanol/acetonitrile/water mixtures (typically 2:2:1 or 5:3:2, v/v/v) are most commonly employed for polar metabolomics, reflecting their compatibility with low-input samples and broad chemical coverage [22,27]. For lipidomics, MTBE-based or Matyash protocols are increasingly favored over classical chloroform-based extractions due to improved phase separation, reduced protein contamination, and enhanced reproducibility [9,15].

Analytical Platforms

The analytical platforms employed in metabolomics and lipidomics critically determine both the depth of molecular coverage and the biological inferences that can be drawn from

Table 2. Overview of analytical platform categories in organoid-based metabolomics and lipidomics. Representative studies, typical instrumental configurations, metabolome or lipidome coverage, quantification strategies, and key strengths and limitations are summarized for each major analytical approach, highlighting the methodological trade-offs inherent to metabolomic analyses of low-input, three-dimensional organoids.

Platform category	Typical application	Main strength	Key limitation
Untargeted LC–MS	Global metabolic profiling	Broad coverage, discovery-driven	Annotation uncertainty, relative quantification
Targeted LC–MS	Pathway-focused studies	Quantitative robustness	Limited metabolome coverage
Lipidomics (LC/MS)	Membrane remodeling, development	High sensitivity for lipids	Limited structural resolution
Microbore LC–MS	Low biomass / single organoids	Increased sensitivity	Reduced robustness
GC–MS	Central carbon metabolism	High ID confidence	Limited lipid coverage
MSI	Spatial metabolism	Spatial resolution	Limited quantification
NMR	Non-destructive profiling	Reproducibility	Low sensitivity
SIRM / multi-platform	Mechanistic flux analysis	Functional insight	High complexity

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these systems. Table 2 provides a structured overview of the main analytical strategies used for organoid-based studies. Liquid chromatography–mass spectrometry (LC–MS) platforms dominate organoid metabolomics and lipidomics studies, with high-resolution Orbitrap and Q-TOF instruments representing the most frequently adopted configurations [6,27,44]. These platforms offer the mass accuracy (<5 ppm) and dynamic range required to detect low-abundance metabolites in samples often comprising fewer than 10^4 cells, a prerequisite for meaningful organoid-level profiling [13].

Untargeted LC–MS approaches are widely used to explore global metabolic perturbations across diverse biological contexts, including cancer metabolism, developmental transitions, and toxicological responses [15,17,24]. Reported coverage in these studies ranges from fewer than 100 annotated metabolites to several thousand detected features, depending on chromatographic mode, ionization polarity, and data processing thresholds [10,21].

However, it is important to note that high feature counts do not necessarily translate into confident metabolite identification, as many studies rely on putative annotations without validation against authentic standards [17,26].

Targeted LC–MS workflows, by contrast, prioritize quantitative robustness and biological interpretability over breadth. Examples include targeted lipidomics of hepatocyte-derived organoids using HILIC-MS/MS [9], absolute quantification of central carbon metabolites using serially diluted standards [14], and MRM-based lipid profiling in scalable bioreactor systems [35]. While these approaches inherently limit discovery potential, they provide higher confidence in pathway-level conclusions and are particularly well suited for translational and comparative studies.

Gas chromatography–mass spectrometry (GC–MS) remains an important complementary platform in organoid metabolomics, particularly for the analysis of central carbon metabolism, amino acids, and organic acids [12,38,39]. GC–MS offers superior chromatographic resolution and well-established spectral libraries, facilitating confident metabolite identification. However, its reliance on chemical derivatization, longer run times, and relatively lower sensitivity compared to LC–MS limit its applicability to low-input samples and large metabolite panels. In several studies, GC–MS analyses are restricted to a limited number of significantly altered metabolites, often focusing on tricarboxylic acid cycle intermediates and amino acids [12,38].

While these targeted insights are biologically informative, they provide only a partial view of the metabolic landscape and are insufficient to capture lipid remodeling or pathway-level reprogramming beyond primary metabolism.

Lipidomics: Coverage Versus Structural Resolution

Lipidomics represents a particularly prominent application of metabolomics technologies in organoid research, especially in studies of neural development, hepatobiliary function, and adipose tissue biology [6,13,41]. Both LC–MS/MS–based targeted lipidomics and infusion-based shotgun approaches are widely employed, enabling the detection of hundreds of lipid species across multiple subclasses.

However, a recurring limitation across lipidomics studies is the reliance on sum composition annotations, in which lipid species are identified based on total carbon number and degree of unsaturation without resolving fatty acyl chain position or stereochemistry [13,34]. While sufficient for comparative analyses, this level of resolution constrains mechanistic interpretation, particularly when investigating membrane dynamics, signaling lipids, or enzyme-specific remodeling processes. Only a minority of studies employ advanced MS/MS strategies or reference standards to achieve deeper structural elucidation [9,41].

Spatially Resolved and Non-Destructive Platforms

Mass spectrometry imaging has emerged as a powerful tool for spatial metabolomics and lipidomics in organoids, enabling the visualization of molecular distributions within intact three-dimensional structures [6,16,29]. MALDI-MSI approaches allow the correlation of lipid and metabolite patterns with histological features, such as neurogenic rosettes or differentiated tubular regions, providing insights inaccessible to bulk extraction methods.

Nevertheless, MSI workflows introduce trade-offs in terms of metabolite coverage, quantification accuracy, and structural validation. Formalin fixation, embedding media, and matrix application can affect lipid recovery and delocalization, while quantitative interpretation often relies on relative intensity measures rather than absolute concentrations [16,29].

High-resolution magic angle spinning (HR-MAS) NMR spectroscopy represents a complementary, non-destructive alternative for intact organoid profiling [19,28]. While offering unparalleled reproducibility and the ability to recover samples for downstream analyses, NMR-based approaches are inherently limited in sensitivity and metabolite coverage, restricting their applicability to relatively abundant metabolites.

Quantification Strategies and Interpretative Constraints

Across platforms, the vast majority of organoid metabolomics and lipidomics studies rely on relative quantification strategies, including peak-sum normalization, TIC normalization, or z-score transformations [12,17,34]. Absolute quantification, achieved through external calibration or isotopically labelled internal standards, is comparatively rare but is increasingly recognized as essential for cross-study comparability and translational relevance [14,41,42].

The predominance of relative quantification complicates the integration of datasets generated using different platforms, extraction protocols, or normalization schemes. This limitation is particularly relevant for studies aiming to compare metabolic states across organoid biobanks, patient cohorts, or experimental laboratories.

Data Processing, Statistical Modeling, and Sources of Analytical Fragility

Across the reviewed studies, substantial heterogeneity emerges in preprocessing pipelines, normalization strategies,

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multivariate analyses, and validation practices, reflecting the inherent tension between high-dimensional molecular data and

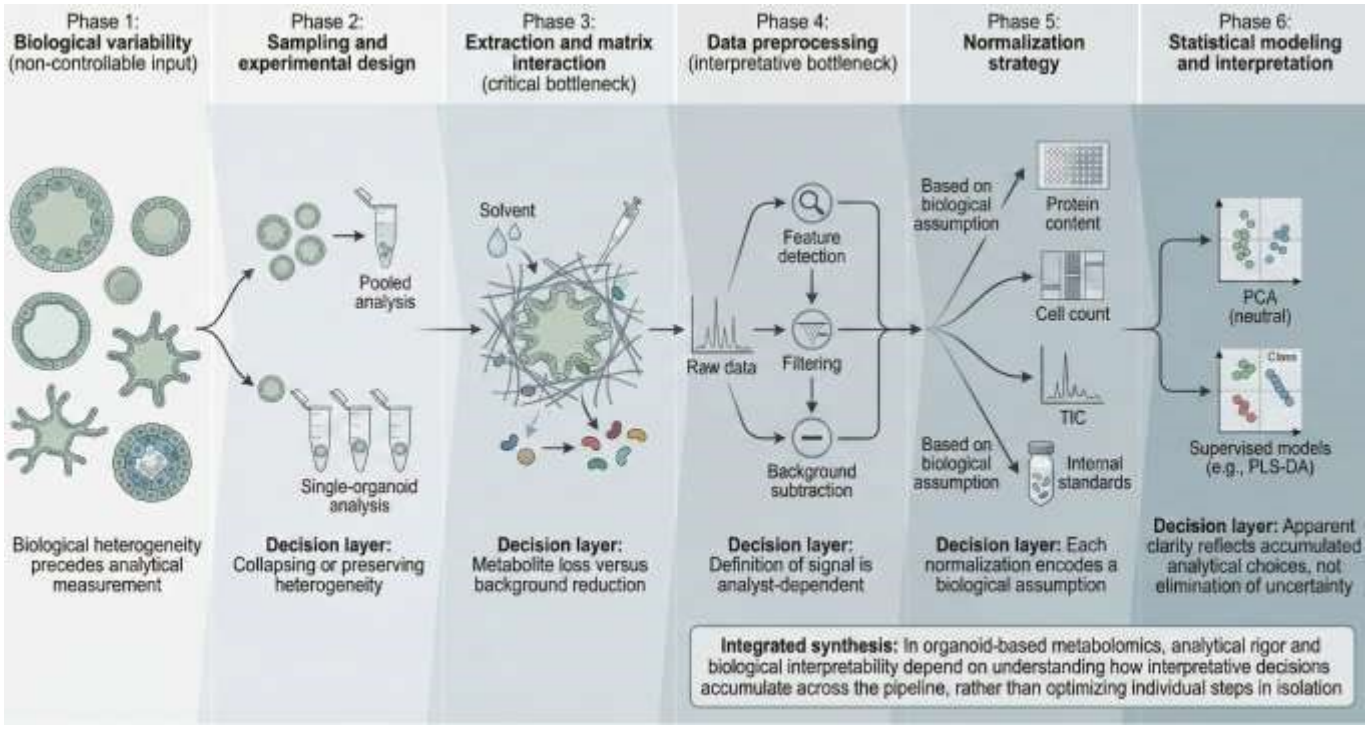

Figure 3. Propagation of analytical uncertainty through layered decision-making in organoid metabolomics. Metabolomic analysis of human organoids is characterized by low-input material, intrinsic biological heterogeneity, and extensive reliance on analytical and computational decisions. This figure conceptualizes the organoid metabolomics pipeline as a sequence of layered interpretative decisions, through which analytical uncertainty accumulates not as a single source of error but as the result of successive constraints imposed on the data. Early phases reflect non-controllable biological variability and sampling design choices, where pooling and small-N experimental designs may collapse or amplify heterogeneity before measurement. Extraction and matrix interaction represent a critical bottleneck, introducing an explicit trade-off between metabolite loss and background reduction. Data preprocessing constitutes a further interpretative bottleneck, as feature detection, filtering, and background subtraction define what is retained as signal. Normalization strategies impose additional biological assumptions, yielding alternative quantitative representations of the same underlying data. In the final phase, statistical modeling and interpretation compress these layered decisions into apparently clear results, particularly when supervised models are applied without adequate validation. The integrated synthesis highlights that analytical rigor and biological interpretability in organoid-based metabolomics depend on understanding how such decisions stratify and accumulate across the pipeline, rather than on optimizing individual steps in isolation. This framework provides a conceptual basis for the need for robust QC practices and transparent reporting standards in the field.

the limited number of biological replicates that typically characterize organoid experiments. As conceptualized in Figure 3, these sources of analytical fragility do not act independently but accumulate through successive layers of interpretative decisions across the organoid metabolomics pipeline. Most studies rely on established vendor software or open-source pipelines for peak detection, alignment, and deconvolution, including Sciex OS, Agilent Profinder, Compound Discoverer, MS-DIAL, and custom workflows [12,17,39,44]. However, filtering criteria such as minimum detection frequency, intensity thresholds, or relative standard deviation (RSD) cutoffs are often applied inconsistently and are rarely justified in relation to sample size or analytical variance. As a consequence, feature sets retained for downstream analysis can vary dramatically even among studies addressing similar biological questions.

More rigorous preprocessing strategies are observed in studies that explicitly incorporate quality control–driven filtering and background correction.

The systematic removal of features with high RSD values in pooled QC samples (typically >20–30%) improves analytical reproducibility and reduces noise propagation into multivariate models [15,22]. In parallel, matrix-aware preprocessing approaches that subtract extracellular matrix–derived background signals using ECM-only blanks have proven essential for 3D organoids embedded in Matrigel or BME, particularly under low-biomass conditions [10], demonstrating that preprocessing decisions are not neutral technical steps but active determinants of biological signal recovery. Within the layered decision framework illustrated in Figure 3, preprocessing represents a critical interpretative bottleneck, as filtering and background subtraction directly define what is retained as signal.

Normalization as an Unresolved Source of Variability

Given the pronounced variability in organoid size, cellular composition, and growth kinetics across patient lines and differentiation stages, the choice of normalization strategy can profoundly influence downstream statistical interpretation. Accordingly, normalization constitutes one of the most consequential decision layers in the framework depicted in Figure 3, as each strategy implicitly encodes assumptions regarding biomass equivalence and biological comparability.

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The most frequently adopted approach involves normalization to total protein content, typically measured via bicinchoninic acid (BCA) assays, which provides a pragmatic surrogate for biomass [9,15].

Alternative strategies include normalization to cell counts, total ion current (TIC), summed peak intensities or data-driven transformations such as z-score scaling and median fold change, each of which rests on distinct assumptions regarding sample comparability and analytical stability [9,11,12,17]. In isotope tracing and stable isotope–resolved metabolomics studies, the use of isotopically labeled internal standards is increasingly recognized as essential to control for extraction efficiency, instrumental drift, and batch effects, particularly in longitudinal or biobank-scale investigations [15,42]. Despite these advances, no consensus has yet emerged, and normalization strategies are often selected post hoc, limiting cross-study comparability. This lack of consensus further amplifies the stratification of analytical decisions outlined in Figure 2, reinforcing the need for explicit reporting of normalization assumptions.

Multivariate Modeling and Overfitting Risk

Unsupervised multivariate analyses, including PCA, hierarchical clustering, and dimensionality reduction methods such as UMAP, are widely used for exploratory purposes and generally pose a low risk of statistical overinterpretation when applied transparently [29,34]. In contrast, supervised methods—most notably PLS-DA and OPLS-DA—are frequently employed under conditions of limited sample size, often with fewer than five biological replicates per group [12,17,39].

Several studies report extremely high model performance metrics (R^2 and Q^2 values >0.9) without adequate external validation or clearly documented permutation testing, raising concerns regarding model overfitting and inflated effect sizes [17,39]. The routine use of VIP-based feature selection and S-plots in small-N datasets further amplifies this risk, as these tools can inadvertently reinforce noise-driven class separation. These issues are particularly problematic in organoid studies, where biological variability between lines is often substantial and may be misinterpreted as condition-specific metabolic reprogramming. As illustrated in Figure 3, such apparently robust outcomes may reflect the compression of multiple upstream analytical decisions into simplified statistical summaries, rather than genuine biological separation.

Overall, validation strategies across the reviewed literature range from minimal internal checks to more robust permutation-based and QC-driven approaches. While some studies rely solely on nominal statistical thresholds or confidence intervals, others implement explicit permutation testing to assess model stability and guard against overfitting [14,22]. Batch effects and technical drift are often addressed inconsistently; however, the application of QC-based LOESS correction, RUVSeq, or dataset integration frameworks such as Seurat represents a clear advancement toward reproducible multi-batch analyses [10,14,29].

Notably, a subset of studies adopts knowledge-driven validation strategies, integrating metabolomics with transcriptional footprinting or mechanistic network inference to constrain interpretation within established regulatory frameworks [11,36]. These approaches reduce reliance on purely statistical separation and enhance biological plausibility, albeit at the cost of increased analytical complexity. Together, these observations support the view presented in Figure 3, in which analytical rigor and biological interpretability depend on understanding how interpretative decisions accumulate across the pipeline, rather than on optimizing individual steps in isolation.

Biological Insights Gained from Organoid-Based Metabolomics and Lipidomics

Beyond methodological considerations, metabolomics and lipidomics have provided a functional dimension to organoids that extends their utility well beyond morphological or transcriptional characterization. Across the reviewed studies, metabolic profiling has enabled the identification of pathway-level reprogramming, developmental bottlenecks, and context-specific vulnerabilities, often recapitulating key aspects of human metabolism while simultaneously exposing intrinsic limitations of in vitro three-dimensional models.

Metabolic Reprogramming and Therapeutic Vulnerabilities in Cancer Organoids

Cancer organoids constitute the most extensively investigated application of metabolomics and lipidomics, particularly in relation to metabolic plasticity, drug resistance, and pathway dependency. Early untargeted analyses of pancreatic ductal adenocarcinoma (PDAC) patient-derived organoids revealed that early recurrent disease is characterized by a pronounced anaplerotic phenotype, with elevated fumarate, malate, and glutamate sustaining tricarboxylic acid cycle activity [12]. Subsequent studies demonstrated that PDAC organoids segregate into distinct metabolic subtypes, including a glucose-dependent “glucomet” phenotype driven by the GLUT1/ALDOB/G6PD axis, associated with enhanced pentose phosphate pathway flux, increased nucleotide synthesis, and resistance to 5-fluorouracil [11].

Comparable principles emerge in colorectal cancer organoids, where genetic perturbations intersect with metabolic rewiring. EGFR deletion in KRAS-mutant organoids induces a shift toward anaplerotic glutaminolysis, coupled to stemness-associated signaling programs [14], while metastatic colorectal liver organoids exhibit MYC-driven glycolysis and histone lactylation, directly linking metabolic flux to epigenetic regulation of metastatic gene expression [45]. Beyond carbohydrate metabolism, organoid-based metabolomics has consistently identified de novo nucleotide biosynthesis as a critical vulnerability, exemplified by malignant rhabdoid tumor organoids displaying marked sensitivity to antifolate and DHODH-targeting strategies [27].

Comparable principles are increasingly supported by evidence from breast cancer organoid models. Patient-derived breast cancer organoids have been shown to faithfully preserve tumor heterogeneity, including genomic alterations and subtype-specific features, while maintaining a high degree of concordance with parental tumors. Importantly, these systems enable functional interrogation of therapy response, with

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several studies demonstrating that organoid-based drug screening can recapitulate patient-specific sensitivity and resistance patterns, thereby supporting their application in precision oncology. Moreover, the integration of advanced models such as patient-derived xenograft–derived organoids, co-culture systems, and organoid-on-chip platforms further enhances their physiological relevance by incorporating microenvironmental interactions and dynamic signaling processes, ultimately improving their predictive power for therapeutic response [46].

Collectively, these findings indicate that cancer organoids do not merely recapitulate established metabolic hallmarks of malignancy, but expose the context-dependent plasticity of tumor metabolism under controlled microenvironmental constraints, thereby enabling functional interrogation of therapeutically actionable metabolic states.

Metabolic changes during organoids' differentiation

A second major class of insights concerns metabolic maturation, defined here as the progressive acquisition of tissue-specific oxidative metabolism and functional biochemical programs during organoid differentiation. Multiple studies converge on the observation that differentiation is accompanied by a transition from glycolysis toward oxidative metabolism, mirroring in vivo developmental trajectories. In kidney organoids, incomplete activation of fatty acid oxidation reflects reduced expression of CPT1a, the rate-limiting enzyme of mitochondrial β-oxidation. This metabolic immaturity can be partially rescued through targeted metabolite supplementation [29]. Similarly, serine-dependent regulation of S-adenosylmethionine availability and DNA methylation emerges as a key metabolic determinant of early nephrogenesis [30].

In cochlear organoids, insufficient levels of α-ketoglutarate and NAD^+ constrain mitochondrial respiration and hair cell differentiation, identifying cofactor availability as a limiting factor in functional maturation [40]. Importantly, these limitations are not universal: parathyroid patient-derived organoids retain high metabolic fidelity to matched tissues and preserve hormone secretion capacity, underscoring that metabolic immaturity is context-dependent rather than intrinsic to organoid technology [15].

Together, these observations indicate that metabolic state is not a passive consequence of differentiation in organoids, but an active determinant of lineage specification and functional maturation. Rather than representing a fixed limitation of in vitro three-dimensional models, metabolic immaturity emerges as a tunable variable—one that can be experimentally modulated to accelerate, constrain, or redirect differentiation trajectories.

Several studies further demonstrate that targeted metabolic modulation can actively drive organoid differentiation and functional maturation. In cochlear organoids, supplementation with α-ketoglutarate promotes hair cell differentiation through coordinated metabolic and epigenetic reprogramming, identifying metabolite availability as a limiting factor in sensory regeneration [40]. In renal organoids, dynamic shifts in glycolytic and oxidative metabolism accompany nephron specification, highlighting metabolic plasticity as a determinant of stem cell fate decisions [30]. Similarly, corneal organoids modeling aniridia-associated keratopathy exhibit disease-specific disruptions in amino acid and energy metabolism downstream of PAX6 mutations, providing mechanistic insights inaccessible through transcriptomics alone [39].

Environmental Toxicology and Cellular Stress Responses

Organoid-based metabolomics has emerged as a particularly sensitive framework for detecting early metabolic perturbations induced by environmental and pharmacological stressors. Across multiple organoid models, metabolic alterations consistently precede overt structural or viability changes, underscoring the capacity of metabolomics to capture functional disruption at the pathway level.

In hepatic organoids, exposure to aged microplastics induced perturbations in homocysteine metabolism and mitochondrial electron transport, generating a state of reductive stress at environmentally relevant concentrations [26]. Complementary findings in polypropylene microplastic–exposed liver organoids further demonstrated disruption of central carbon metabolism and lipid remodeling, reinforcing mitochondrial dysfunction as a recurrent hepatic vulnerability [47]. Together, these studies indicate that organoid metabolomics can resolve subtle redox imbalances that remain undetectable by conventional cytotoxicity assays.

In intestinal organoids, nanoplastic exposure impaired AKT/mTOR signaling and altered lipid and nucleotide metabolism prior to activation of apoptotic pathways [23]. Similarly, graphene oxide treatment in cerebral organoids resulted in selective ceramide accumulation through ER stress–linked mechanisms [31], identifying membrane lipid remodeling as a sensitive axis of environmental neurotoxicity. These findings highlight lipid metabolism as a cross-organ vulnerability node in response to particulate stressors.

Pulmonary organoid models exposed to low-dose radiation exhibited early alterations in glycerophospholipid composition and calcium signaling that preceded ferroptotic outcomes [32]. Such observations extend the concept of metabolomics as an early-warning system to radiation-induced injury, illustrating how pathway-level changes anticipate irreversible phenotypic consequences.

Collectively, these studies demonstrate that organoid metabolomics enables mechanistically resolved characterization of toxicant-induced stress responses across tissue contexts. However, the interpretation of early metabolic signatures remains dependent on rigorous normalization strategies, matrix-aware controls, and appropriate statistical validation, reinforcing the central role of analytical robustness in toxicological applications.

Neurodevelopmental and Rare Disease Mechanisms

In neuroscience and rare disease models, lipidomics has been particularly informative in linking genotype to functional phenotype. High-sensitivity lipidomics revealed that cerebral organoids carrying the APOE4/4 genotype—homozygous for the ε4 allele of apolipoprotein E, the strongest genetic risk

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factor for late-onset Alzheimer's disease—exhibit impaired incorporation of polyunsaturated fatty acids into membrane phospholipids, providing a mechanistic framework for lipid dysregulation in Alzheimer's disease models [13]. Spatial lipidomics further demonstrated selective enrichment of sphingomyelin and ceramide species within neurogenic niches, implicating membrane lipid composition in progenitor cell behavior [6].

Similarly, in corneal organoids modeling aniridia-associated keratopathy, metabolomics uncovered profound disruptions in branched-chain amino acid metabolism downstream of PAX6 mutations, revealing metabolic constraints not readily apparent from transcriptomic analyses alone [39].

Importantly, several studies demonstrate that the integration of metabolomics with complementary omics layers, including transcriptomics, proteomics, and stable isotope–resolved approaches, enables a more comprehensive interpretation of organoid biology. By linking gene expression programs to functional biochemical outputs, multi-omics strategies facilitate the identification of regulatory–metabolic coupling, pathway activity, and context-dependent cellular states that are not accessible through single-layer analyses alone.

However, the application of multi-omics in organoid systems also introduces substantial analytical and interpretative challenges. The combination of high-dimensional datasets with inherently low sample biomass and limited replicate numbers increases the risk of overfitting, data sparsity, and integration bias. Moreover, differences in temporal resolution, normalization strategies, and platform-specific sensitivity complicate cross-omics alignment and quantitative interpretation. As a result, while multi-omics approaches hold significant promise, their effective implementation in organoid models requires carefully designed experimental frameworks and robust data integration methodologies.

Nutrient Handling and Tissue-Specific Physiology

Beyond disease modeling, organoid metabolomics has provided quantitative insight into tissue-specific physiological functions and nutrient handling. In small intestinal organoids, integrated transcriptomic–metabolomic analyses demonstrate that human breast milk induces broader metabolic maturation programs than infant formula, promoting lipid absorption, epithelial differentiation, immune-related pathways, and steroid biosynthesis [20–22]. In adipose spheroids, lipidomic profiling revealed dynamic remodeling of triacylglycerol and phospholipid pools in response to inflammatory cues, highlighting the relevance of three-dimensional immune-competent adipose models for studying metabolic disease [41]. In hepatic systems, adult donor–derived liver organoids exhibit the closest lipidomic resemblance to primary human hepatocytes among commonly used in vitro models and have been employed to evaluate nutraceutical interventions, with metabolomics revealing hepatoprotective effects mediated through antioxidant and lipid metabolic pathways [9,24].

Collectively, these findings indicate that organoid metabolomics is not limited to pathological modeling but can authentically capture tissue-specific metabolic physiology, enabling quantitative assessment of functional maturation, nutrient processing, and context-dependent metabolic adaptation in human-relevant 3D models.

Taken together, these findings indicate that organoid-based metabolomics extends beyond descriptive characterization, enabling functional interrogation of metabolic states with potential relevance for therapeutic targeting, patient stratification, and the identification of context-specific vulnerabilities in human disease models.

Quality Control Strategies in Organoid-Based Metabolomics and Lipidomics

Across the studies included in this review, data reliability in organoid-based metabolomics, lipidomics, and multi-omics workflows emerges as critically dependent on the implementation of multi-layered quality control (QC) strategies spanning the wet-lab, instrumental, and data analysis phases. Within this framework, quality control strategies represent the primary means by which uncertainty propagation—highlighted in Figures 2 and 3—can be monitored, constrained, and made explicit across organoid metabolomics workflows. Given the intrinsic low biomass of organoid samples, the pervasive presence of extracellular matrix–derived background, and the frequent use of small-N experimental designs, QC procedures are not ancillary technical steps but central determinants of analytical robustness and biological interpretability.

Beyond conventional static cultures, metabolomics has been applied to monitor large-scale and engineered organoid models. In bioreactor-based vascular organoid production, metabolic and proteomic profiling of secreted extracellular vesicles has been used as a quality attribute to assess functional consistency during upscaling [35]. Additionally, NMR-based metabolomics has been employed in interdisciplinary contexts to link cellular metabolic states with biomaterial properties, demonstrating the versatility of metabolomic readouts beyond classical biological perturbations [28].

Wet-Lab and Instrumental Quality Control

At the experimental level, the primary objectives of QC are to ensure instrumental stability, minimize carryover, and control for technical variability unrelated to biological effects. Sample randomization across injection sequences is routinely adopted to mitigate the impact of signal drift and time-dependent fluctuations on downstream statistical comparisons [12,26]. In parallel, the systematic inclusion of blank injections—typically consisting of extraction solvent alone—serves to monitor carryover effects and maintain chromatographic cleanliness. In high-sensitivity workflows, blanks are frequently interspersed at regular intervals, as exemplified by Gadara et al. [13], who introduced solvent blanks every four matrix-containing injections to prevent signal accumulation.

A defining QC requirement unique to 3D organoids is the use of matrix-aware blanks. Several studies explicitly analyze extracellular matrix (ECM)-only samples, such as Matrigel or basement membrane extract (BME), to disentangle organoid-derived metabolic features from matrix-associated background

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signals [10]. This strategy is particularly critical under low-input conditions, where ECM-derived metabolites can dominate the detected feature space and confound biological interpretation if not properly controlled.

The use of internal standards (IS) represents a near-universal QC practice across platforms, enabling normalization for extraction efficiency and instrumental response. In lipidomics workflows, class-specific internal standards are commonly employed to ensure quantitative consistency across lipid subclasses [9]. Untargeted metabolomics studies often combine internal standard normalization with peak-sum or total signal scaling [12], while NMR-based approaches rely on reference compounds such as TSP or alanine for chemical shift calibration and relative quantification [19]. Together, these strategies provide an essential anchor for technical reproducibility across heterogeneous analytical platforms.

QC-Driven Data Processing and Signal Stabilization

Post-acquisition QC strategies are predominantly centered on the identification and removal of analytically unstable features. Filtering based on coefficient of variation (CV) or relative standard deviation (RSD) in pooled QC samples represents the most widely adopted criterion for assessing reproducibility. Thresholds vary across studies, reflecting different tolerances for analytical noise: features with RSD values exceeding 30% are commonly excluded [13,26], while more stringent cutoffs of 25% or even 20% are applied in workflows prioritizing high quantitative fidelity [10,15]. Although heterogeneous, these practices converge on the recognition that QC-based filtering is indispensable for preventing noise propagation into multivariate models.

Signal drift correction constitutes an additional layer of QC-driven data stabilization. Several studies implement locally weighted regression approaches, such as LOESS smoothing, applied to pooled QC injections to correct for time-dependent intensity fluctuations within and between analytical batches [10,17]. This approach is particularly relevant in extended acquisition sequences and multi-batch designs, where uncorrected drift may masquerade as biologically meaningful variation.

Handling of missing values further reflects the tension between data completeness and analytical rigor. Common strategies include the removal of features exceeding predefined missingness thresholds (e.g., >50%) followed by imputation using low-intensity replacement or data-driven methods such as k-nearest neighbors [26,36]. While necessary for downstream statistical modeling, these steps introduce additional assumptions that reinforce the interpretative nature of data preprocessing in organoid metabolomics.

Normalization, Isotopic Standards, and Model-Specific Constraints

Normalization strategies represent a critical interface between QC and biological interpretation. Across the reviewed literature, normalization to total protein content or cell count is preferentially adopted for organoid samples, reflecting their limited availability and heterogeneous size distributions [9,15]. In contrast, total ion current (TIC) normalization is predominantly used in mass spectrometry imaging (MSI) studies, where spatially resolved signal intensities rather than absolute concentrations constitute the primary analytical output [7].

The use of isotopically labeled standards further enhances QC rigor, particularly in workflows aiming for quantitative or semi-quantitative interpretation. Stable isotope–labeled compounds are frequently introduced as internal standards to monitor extraction efficiency and ionization stability [15,44]. Importantly, a conceptual distinction must be maintained between isotopes used for QC purposes and those employed in stable isotope–resolved metabolomics (SIRM), where labeled substrates serve as biological tracers rather than technical controls [27,42]. Failure to differentiate these roles may obscure the analytical intent of isotopic labeling strategies.

Toward Matrix-Aware and Context-Specific QC Frameworks

A recurring theme across organoid studies is the inadequacy of QC frameworks directly inherited from tissue or biofluid metabolomics. The presence of ECM-derived background, coupled with low biomass and pronounced inter-organoid heterogeneity, necessitates context-specific QC adaptations. Neef et al. [10] exemplify this need by implementing fold-change–based filtering against ECM-only blanks, in addition to conventional statistical criteria, to systematically eliminate non-biological features. Such approaches highlight the importance of explicitly modeling matrix contributions rather than treating them as generic background noise.

Collectively, the reviewed literature converges toward a multi-tiered QC paradigm encompassing pre-analytical randomization and pooling, analytical control through internal standards and matrix-aware blanks, and post-analytical stabilization via QC-driven filtering and drift correction. As emphasized by the conceptual frameworks presented in Figures 2 and 3, robust quality control in organoid-based metabolomics is not achieved through isolated technical optimizations but through the transparent integration of QC practices across the entire analytical pipeline. Establishing harmonized, matrix-aware QC guidelines will therefore be essential to improve reproducibility, enable cross-study comparability, and support the translational potential of organoid metabolomics. Together, these practices illustrate that quality control in organoid-based metabolomics cannot be treated as an auxiliary technical step, but must be integrated into experimental design, data processing, and reporting standards to enable meaningful cross-study comparison.

Toward Standardization: A Minimal QC Framework for Organoid-Based Metabolomics

Based on the converging practices observed across the reviewed studies and informed by ongoing community-driven initiatives for metabolomics quality assurance (e.g., mQACC [48]), a set of minimal, context-aware QC principles can be delineated for organoid-based metabolomics and lipidomics. Rather than prescribing rigid protocols, these recommendations aim to define transparent decision points that enhance reproducibility while preserving experimental flexibility.

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(1) Mandatory matrix-aware controls. For organoids embedded in basement membrane extracts, the inclusion of ECM-only blanks should be considered essential rather than optional. These controls enable explicit modeling and subtraction of matrix-derived background features, which represent a dominant source of analytical interference under low-biomass conditions. Reporting of ECM-associated feature filtering criteria (e.g., fold-change thresholds relative to blanks) should be standardized to improve cross-study interpretability.

(2) Systematic use of pooled QC samples. Pooled QC samples should be incorporated at three levels: (i) initial system conditioning, (ii) periodic monitoring of instrumental stability throughout the analytical sequence, and (iii) post-acquisition assessment of feature reproducibility. Injection frequency and acceptance thresholds (e.g., CV or RSD cutoffs) should be explicitly reported, recognizing that different analytical platforms may require distinct tolerance levels.

(3) Explicit separation of biological tracers and technical standards. Isotopically labeled compounds used for stable isotope–resolved metabolomics (SIRM) should be clearly distinguished from isotopic internal standards employed for QC and quantification. This distinction is critical to avoid conflation between biological flux interpretation and technical performance assessment, particularly in multi-platform and tracer-based studies.

(4) Context-appropriate normalization strategies. Normalization choices should be reported together with their underlying biological assumptions. For organoid samples, normalization to total protein content or cell number is generally more appropriate than volume- or weight-based approaches used in tissue or biofluid metabolomics. When alternative strategies such as TIC normalization are adopted (e.g., in MSI workflows), their implications for quantitative interpretation should be explicitly acknowledged.

(5) QC-driven preprocessing transparency. Feature filtering, missing value handling, and drift correction should be anchored to QC-derived metrics rather than arbitrary thresholds. Reporting of preprocessing parameters—including CV cutoffs, drift correction algorithms (e.g., LOESS), and imputation strategies—should be sufficiently detailed to allow independent reproduction of analytical pipelines.

(6) Validation proportional to experimental complexity. Given the frequent use of small-N designs in organoid studies, supervised modeling approaches should be accompanied by explicit validation procedures, such as permutation testing or QC-informed model diagnostics. Apparent model performance should be interpreted in light of cumulative analytical decisions rather than as direct evidence of biological separation.

Taken together, these principles outline a minimal QC framework tailored to the specific constraints of organoid-based metabolomics. Importantly, they reinforce the central message of this review: analytical rigor and biological interpretability in organoid models do not emerge from isolated technical optimizations, but from the transparent integration of matrix-aware controls, QC-driven data processing, and assumption-aware statistical interpretation across the entire analytical pipeline.

Future Directions and Unresolved Challenges in Organoid Metabolomics

Despite rapid advances over the past decade, organoid-based metabolomics remains an emerging field characterized by substantial methodological, conceptual, and translational challenges that must be addressed to fully realize its potential.

One of the most critical unresolved issues is the lack of standardized experimental and analytical workflows. Significant variability in organoid culture conditions, extracellular matrix composition, and analytical procedures contributes to limited reproducibility and cross-study comparability. The absence of harmonized protocols and quality control frameworks further amplifies analytical heterogeneity, hindering the identification of robust and transferable metabolic signatures [49,50].

A second major limitation concerns the integration of metabolomics with other layers of biological information. Although multi-omics approaches are increasingly recognized as essential for capturing system-level complexity, their implementation in organoid systems remains fragmented. The lack of standardized pipelines for integrating metabolomic, transcriptomic, and proteomic data constrains mechanistic interpretation and limits the reconstruction of coherent biological models [51,52].

From a biological standpoint, current organoid models only partially recapitulate the complexity of in vivo tissue environments. In particular, the limited inclusion of stromal, immune, and vascular components restricts the study of metabolic crosstalk and niche-specific reprogramming. Emerging platforms, including co-culture systems and organoid-on-chip technologies, aim to address these limitations, although their adoption is still constrained by technical complexity and lack of standardization [53,54].

Looking forward, several key directions are expected to shape the evolution of the field. The development of chemically defined and matrix-independent culture systems will be essential to reduce variability and improve reproducibility. Advances in single-organoid and spatial multi-omics technologies are likely to enable the resolution of intra-organoid heterogeneity and metabolic gradients. In parallel, artificial intelligence and machine learning approaches are expected to play a central role in the integration and interpretation of complex multi-omics datasets [55].

Importantly, the clinical and regulatory translation of organoid-based metabolomics will require the establishment of validated, reproducible, and scalable analytical pipelines. This includes the definition of minimal reporting standards, benchmarking datasets, and cross-laboratory validation studies. Greater alignment between academia, industry, and regulatory agencies will be essential to enable the integration of organoid-based platforms into precision medicine and drug development pipelines [56].

Collectively, addressing these challenges will be critical to transform organoid metabolomics from a promising experimental approach into a robust, standardized, and clinically relevant platform.

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Conclusions

Metabolomics and lipidomics have emerged as complementary biochemical layer that substantially enhance the interpretability of human organoid models. The reviewed literature demonstrates that metabolic profiling can recapitulate key metabolic features of human tissues while uncovering pathway-level reprogramming, developmental bottlenecks, and context-specific vulnerabilities that are often inaccessible to transcriptomic or morphological analyses alone. The strong temporal clustering of the available literature further supports the interpretation of organoid-based metabolomics as an emerging field still undergoing methodological maturation. At the same time, these insights remain highly sensitive to experimental design, analytical platform choice, and data processing strategies, underscoring the methodological fragility of the field. The lack of standardized workflows for sample preparation, normalization, and statistical validation currently limits cross-study comparability and translational robustness. In this context, multi-omics integration represents a promising yet still evolving strategy to bridge molecular regulation and functional metabolic phenotypes, although its application in organoid systems remains constrained by analytical complexity, data integration challenges, and limited standardization. Moving forward, the integration of harmonized analytical frameworks with biologically informed validation strategies will be essential to fully exploit the potential of metabolomics and lipidomics in organoid-based research.

Looking ahead, several technological and conceptual developments are expected to reshape organoid-based metabolomics. Advances in analytical miniaturization and microbore chromatography are progressively enabling metabolomic profiling at the level of single organoids, opening new avenues for resolving intra-model heterogeneity and patient-specific metabolic phenotypes. In parallel, spatially resolved approaches, including high-resolution mass spectrometry imaging, are expanding the ability to map metabolic organization within three-dimensional structures, linking biochemical gradients to tissue architecture.

The integration of metabolomics with stable isotope–resolved approaches (SIRM) and fluxomics is also emerging as a critical direction, enabling dynamic interrogation of metabolic pathway activity beyond static abundance measurements. These strategies provide a more mechanistic understanding of metabolic reprogramming and may help bridge the gap between descriptive and functional metabolomics in organoid systems.

At the computational level, the increasing adoption of machine learning and data integration frameworks offers opportunities to extract biologically meaningful patterns from high-dimensional datasets, although these approaches remain constrained by small sample sizes and limited standardization.

Despite these advances, the field still faces significant challenges, including the need for improved sensitivity, robust matrix-aware workflows, and harmonized reporting standards. Addressing these limitations will be essential to fully realize the potential of organoid-based metabolomics as a scalable and translationally relevant platform.

When applied rigorously, these approaches position organoids as experimentally advantageous systems at the interface between mechanistic biology and translational medicine, with emerging potential to support functionally informed preclinical modeling and metabolic stratification strategies.

Author contributions

J.T. and M.L. conceived the review, coordinated the study, and led the writing and critical revision of the manuscript. M.L. contributed extensively to literature screening, data extraction, and manuscript drafting. A.I. contributed to the selection of the literature, data curation, and the writing of specific sections of the manuscript. A.S. contributed to the critical evaluation of the content and to editorial revision. All authors reviewed and approved the final version of the manuscript.

Conflicts of interest

J.T., A.I. and M.L. are affiliated with Theoreo S.r.l., a university spin-off company operating in the field of metabolomics. The remaining authors declare that there are no conflicts of interest.

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References

*1 J. Bustos, J. Alvarado Gonzalez, D. De Abreu, H. Liebisch-Rey, A. Silva, D. Ortiz, L. Ramírez, J. Ortega and L. Celis Regalado, J Biomed Res Environ Sci, 2021, **2**, 272–279.*

2 S. E. ; S. Murphy J., The Analyst, DOI:10.1039/d2an00599a.

*3 A. L. Caipa Garcia, J. E. Kucab, H. Al-Serori, R. S. S. Beck, M. Bellamri, R. J. Turesky, J. D. Groopman, H. E. Francies, M. J. Garnett, M. Huch, J. Drost, M. Zilbauer, V. M. Arlt and D. H. Phillips, Chem. Res. Toxicol., 2024, **37**, 234–247.*

*4 N. Glibetic, S. Bowman, T. Skaggs and M. Weichhaus, IJMS, 2024, **25**, 10503.*

View Article Online
DOI: 10.1039/D6AN00186F

5 S.-A. Iakab, F. Keller, S. Schmidt, J. Cordes, Q. Zhou, J. L. Cairns, F. Fischer, R. Schneider, I. Wolf, R. Rudolf and C. Hopf, Cancer Biology, 2022, preprint, DOI: 10.1101/2022.12.05.519157.

6 G. Cappuccio, S. M. Khalil, S. Osenberg, F. Li and M. Maletic-Savatic, Front. Mol. Biosci., 2023, **10**, 1181965.

7 D. Hadavi, I. Tosheva, T. P. Siegel, E. Cuypers and M. Honing, Front. Bioeng. Biotechnol., 2023, **11**, 1197760.

8 P. Tugwell and D. Tovey, Journal of Clinical Epidemiology, 2021, **134**, A5–A6.

9 F. Bonanini, M. Singh, H. Yang, D. Kurek, A. C. Harms, A. Mardinoglu and T. Hankemeier, Experimental Cell Research, 2024, **437**, 114008.

10 S. K. Neef, N. Janssen, S. Winter, S. K. Wallisch, U. Hofmann, M. H. Dahlke, M. Schwab, T. E. Mürdter and M. Haag, Metabolites, 2020, **10**, 494.

11 D. Rodrigues, T. De Souza, L. Coyle, M. Di Piazza, B. Herpers, S. Ferreira, M. Zhang, J. Vappiani, D. C. Sévin, A. Gabor, A. Lynch, S.-W. Chung, J. Saez-Rodriguez, D. G. J. Jennen, J. C. S. Kleinjans and T. M. De Kok, Arch Toxicol, 2021, **95**, 2691–2718.

12 L. M. Braun, S. Lagies, R. F. U. Klar, S. Hussung, R. M. Fritsch, B. Kammerer and U. A. Wittel, Cancers, 2020, **12**, 1440.

13 D. Gadara, V. Berka and Z. Spacil, Anal. Chem., 2024, **96**, 59–66.

14 D. Krauß, V. Moreno-Viedma, E. Adachi-Fernandez, C. De Sá Fernandes, J.-W. Genger, O. Fari, B. Blauensteiner, D. Kirchhofer, N. Bradaric, V. Gushchina, G. Fotakis, T. Mohr, I. Abramovich, I. Mor, M. Holcmann, A. Bergthaler, A. Haschemi, Z. Trajanoski, J. Winkler, E. Gottlieb and M. Sibilia, EMBO Mol Med, 2025, **17**, 1355–1392.

15 K. R. Sekhar, S. G. Codreanu, O. C. Williams, J. C. Rathmell, W. K. Rathmell, J. A. McLean, S. D. Sherrod and N. Baregamian, Front. Endocrinol., 2023, **14**, 1223312.

16 A. A. M. Duivenvoorden, B. S. R. Claes, L. Van Der Vloet, T. Lubbers, K. Glunde, S. W. M. Olde Damink, R. M. A. Heeren and K. Lenaerts, Anal. Chem., 2023, **95**, 18443–18450.

17 L. Chen, Z. Dai, C. Ge, D. Huang, X. Zhou, K. Pan, W. Xu, J. Fu and J. L. Du, Journal of Chromatography B, 2022, **1203**, 123260.

18 L. Chen, Z. Dai, Y. Zhang, H. Sheng, B. Hu, J. Du, J. Chang, W. Xu and Y. Hu, Bioscience Reports, 2025, **45**, 1–12.

19 W. J. M. Van Der Kemp, M. T. Grinde, J. O. Malvik, H. W. M. Van Laarhoven, J. J. Prompers, D. W. J. Klomp, B. Burgering, T. F. Bathen and S. A. Moestue, NMR in Biomedicine, 2023, **36**, e4882.

20 X. Wang, Y. Zhong, C. Zheng, C. Huang, H. Yao, Z. Guo, Y. Wu, Z. Wang, Z. Wu, R. Ge, W. Cheng, Y. Yan, S. Jiang, J. Sun, J. Li, Q. Xie, X. Li and H. Wang, Food Research International, 2024, **195**, 114999.

21 X. Wang, W. Zhang, C. Zheng, C. Huang, H. Yao, Z. Wang, K. Huang, Y. Yan, S. Jiang, J. Sun, Q. Xie, Y. Zhang, X. Li and H. Wang, Molecular Nutrition Food Res, 2025, **69**, e70068.

22 X. Wang, S. Yang, C. Zheng, C. Huang, H. Yao, Z. Guo, Y. Wu, Z. Wang, Z. Wu, R. Ge, W. Cheng, Y. Yan, S. Jiang, J. Sun, X. Li, Q. Xie and H. Wang, Nutrients, 2024, **16**, 2951.

23 L. Xuan, J. Luo, C. Qu, P. Guo, W. Yi, J. Yang, Y. Yan, H. Guan, P. Zhou and R. Huang, Science of The Total Environment, 2024, **913**, 169606.

24 Q. Feng, H. Chen, M. Ren, Y. Qiao, J. Zou, X. Liang, L. Yu, Y. Wu, S. Chen, Y. Sun, C. Bao, X. Yang, P. Ma and S. Lu, Food Research International, 2025, **219**, 116976.

25 J. Chen, S. Ma, H. Yang, X. Liang, H. Yao, B. Guo, D. Chen, J. Jiang, D. Shi, J. Xin, K. Ren, X. Zhou, Y. Li, L. Geng and J. Li, Bioactive Materials, 2023, **26**, 452–464.

26 W. Cheng, Y. You, H. Chen, Y. Zhou, Y. Feng and Y. Wang, Environmental Research, 2025, **280**, 121820.

27 M. M. G. Kes, F. Morales-Rodriguez, E. A. Zaal, T. De Souza, N. Proost, M. Van De Ven, M. M. Van Den Heuvel-Eibrink, J. W. A. Jansen, C. R. Berkers and J. Drost, Cell Reports Medicine, 2025, **6**, 101878.

28 O. V. Ochima, O. Alonge, J. P. Pollak, B. Wang and D. Kuila, Front. Bioeng. Biotechnol., 2025, **13**, 1652675.

29 G. Wang, B. Heijs, S. Kostidis, R. G. J. Rietjens, M. Koning, L. Yuan, G. L. Tiemeier, A. Mahfouz, S. J. Dumas, M. Giera, J. Kers, S. M. Chuva De Sousa Lopes, C. W. Van Den Berg, B. M. Van Den Berg and T. J. Rabelink, Cell Stem Cell, 2022, **29**, 1580-1593.e7.

30 Q. Wang, Y. Xiong, S. Zhang, Y. Sui, C. Yu, P. Liu, H. Li, W. Guo, Y. Gao, A. Przepiorski, A. J. Davidson, M. Guo and X. Zhang, Front. Genet., 2021, **12**, 632810.

31 X. Liu, C. Yang, P. Chen, L. Zhang and Y. Cao, Science of The Total Environment, 2022, **849**, 157815.

32 M. Notaras, A. Lodhi, E. Barrio-Alonso, C. Foord, T. Rodrick, D. Jones, H. Fang, D. Greening and D. Colak, Mol Psychiatry, 2021, **26**, 7760–7783.

33 T. Toyoda, N. Miura, S. Kato, T. Masuda, R. Ohashi, A. Matsushita, F. Matsuda, S. Ohtsuki, A. Katakura and K. Honda, Cancer Science, 2024, **115**, 3622–3635.

34 Y. Li, S. Tang, X. Shi, J. Lv, X. Wu, Y. Zhang, H. Wang, J. He, Y. Zhu, Y. Ju, Y. Zhang, S. Guo, W. Yang, H. Yin, L. Chen, D. Gao and G. Jin, Cell Reports Medicine, 2023, **4**, 101162.

35 J. Ene, C. Liu, F. Syed, L. Sun, D. Berry, P. Durairaj, Z. L. Liu, C. Zeng, S. Jung and Y. Li, Stem Cell Res Ther, 2025, **16**, 207.

36 S. G. Romeo, I. Secco, E. Schneider, C. M. Reumiller, C. X. C. Santos, A. Zoccarato, V. Musale, A. Pooni, X. Yin, K. Theofilatos, S. C. Trevelin, L. Zeng, G. E. Mann, V. Pathak, K. Harkin, A. W. Stitt, R. J. Medina, A. Margariti, M. Mayr, A. M. Shah, M. Giacca and A. Zampetaki, Nat Commun, 2023, **14**, 5552.

37 J. E. Druso, R. Aboushousha, M. MacPherson, C. Erickson, V. Mori, E. Hickman, A. R. D'Amico, D. Seward, J. A. Reisz, J. E. Rager, Y. Janssen-Heininger and E. Corteselli, BMC Mol and Cell Biol, 2025, **26**, 31.

38 X. Li, Y. Liu, G. Xu, S. Wang, Z. Hou, H. Shao, J. Wu, W. Yang and H. Chen, Toxicology and Applied Pharmacology, 2025, **505**, 117546.

39 A. C. Koc, V. Sari, G. Kocak, T. Recber, E. Nemutlu, D. Aberdam and S. Güven, BMC Ophthalmol, 2025, **25**, 14.

40 Q. Liu, L. Zhang, Z. Chen, Y. He, Y. Huang, C. Qiu, C. Zhu, D. Zhou, Z. Gan, X. Gao and G. Wan, Advanced Science, 2024, **11**, 2308032.

41 J. Taylor, J. Sellin, L. Kuerschner, L. Krähl, Y. Majlesain, I. Förster, C. Thiele, H. Weighardt and E. Weber, Sci Rep, 2020, **10**, 21104.

42 T. W.-M. Fan, J. Yan, C. F. L. Goncalves, J. M. M. Islam, P. Lin, M. M. Y. Kaddah, R. M. Higashi, A. N. Lane, X. Wang and C. Zhu, Journal of Biological Chemistry, 2025, **301**, 108495.

View Article Online
DOI: 10.1039/D6AN00186F

*43 K. Eckenstein, B. Cengiz, M. E. K. Chang, J. M. Cartier, M. R. Flory and G. V. Thomas, Metabolites, 2025, **15**, 393.*
*44 D. La Gioia, E. Salviati, M. G. Basilicata, C. Felici, O. A. Botrugno, G. Tonon, E. Sommella and P. Campiglia, Anal Bioanal Chem, 2025, **417**, 2837–2847.*
*45 J. Zhou, W. Dai, R. Wang, W. Xu, Z. Xiang, Y. Wang, T. Zhang, Y. Zhao, L. Wang and A. Mao, Acta Pharmacol Sin, 2025, **46**, 1404–1418.*
*46 D. Leung, J. Kaur, G. Richardson and T. Jardé, Critical Reviews in Oncology/Hematology, 2025, **214**, 104914.*
*47 W. Cheng, H. Chen, Y. Zhou, Y. You, D. Lei, Y. Li, Y. Feng and Y. Wang, Environment International, 2024, **191**, 108949.*
*48 J. A. Kirwan, H. Gika, R. D. Beger, D. Bearden, W. B. Dunn, R. Goodacre, G. Theodoridis, M. Witting, L.-R. Yu and I. D. Wilson, Metabolomics, 2022, **18**, 70.*
*49 C. Zhou, Y. Wu, Z. Wang, Y. Liu, J. Yu, W. Wang, S. Chen, W. Wu, J. Wang, G. Qian and A. He, Cancer Medicine, 2023, **12**, 14375–14386.*
*50 R. Yang, Y. Qi, X. Zhang, H. Gao and Y. Yu, Chinese Medical Journal, 2024, **137**, 3050–3060.*
*51 D. Singh, A. Thakur, Rakesh and A. Kumar, Drug Development Research, 2025, **86**, e70121.*
*52 Y. Gu, W. Zhang, X. Wu, Y. Zhang, K. Xu and J. Su, Clinical & Translational Med, 2023, **13**, e1499.*
*53 M. Hofer and M. P. Lutolf, Nat Rev Mater, 2021, **6**, 402–420.*
*54 X. Jiang, L. Oyang, Q. Peng, Q. Liu, X. Xu, N. Wu, S. Tan, W. Yang, Y. Han, J. Lin, L. Xia, M. Peng, Y. Tang, X. Luo, M. Su, Y. Shi, Y. Zhou and Q. Liao, Front. Cell Dev. Biol., 2023, **11**, 1232528.*
55 Maramraju S; Kowalczewski A; Kaza A; Liu X; Singaraju J; Albert MV; Ma Z; Yang H, Bioengineering & Translational Medicine, DOI:10.1002/btm2.10641.
*56 S. Kim, T. Lee, M. Ryu, Y. Park and S. Moon, J of Applied Toxicology, 2026, **46**, 1124–1146.*

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Jacopo Troisi, PhD

on behalf of all authors