Evolutionary rewiring of host metabolism and interferon signalling by SARS-CoV-2 variants

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Summary

SARS-CoV-2 variants differ in transmissibility and immune evasion, but their effects on host-

cell metabolism and signalling remain less defined. Using integrated transcriptomic,

phosphoproteomic, and amino acid profiling in primary nasal epithelial cells, we compared

early and late host responses to pre-Omicron variants (Alpha, Beta), Delta, and Omicron

subvariants (BA.1, BA.5). Pre-Omicron strains broadly suppressed antiviral interferon-

stimulated gene expression and reprogrammed metabolism by reducing mitochondrial

oxidative phosphorylation and β-oxidation. Delta infection was associated with extensive

transcriptional and metabolic remodelling, characterised by activation of stress- and growth-

related kinases and selective retention of biosynthetic amino acids, consistent with a host

response to stress and viral modulation of interferon-associated signalling. In contrast,

Omicron infection elicited a more restrained response dominated by cytokine and survival

pathways, with limited metabolic activation and interferon suppression. Together, these

findings suggest SARS-CoV-2 has progressively evolved toward a strategy that maintains

efficient upper-airway replication while minimising epithelial stress and inflammation.

Keywords: SARS-CoV-2 variants, interferon-stimulated genes, metabolic reprogramming,

nasal epithelial cells, host-pathogen interactions

Introduction

SARS-CoV-2, the causative agent of COVID-19, has evolved into multiple variants with distinct

transmissibility, immune evasion capacity, and associated disease severity. The successive

waves of Variants of Concern (VOCs), including Alpha (PANGO lineage B.1.1.7), Beta (B.1.351),

Delta (B.1.617.2/AY sublineages), and the many Omicron sublineages (B.1.1.529/BA

sublineages, such as BA.1, BA.2, and BA.5), exhibit distinct phenotypic characteristics and

transmission advantages over preceding variants. These advantages are largely driven by

genetic changes in the viral genome, particularly in the spike protein, which enhanced

receptor binding affinity and immune escape (Carabelli *et al.*, 2023; Harvey *et al.*, 2021; Liu

and Rocklov, 2021). Additionally, VOCs exhibit varying capacities to suppress innate immune

responses, a key determinant of early viral control and disease progression (Carabelli *et al.*,

2023; Harvey *et al.*, 2021; Laine *et al.*, 2022; Reuschl *et al.*, 2024).

The nasal epithelium serves as the primary entry site for SARS-CoV-2 infection and plays a

critical role in shaping early host responses (Shi *et al.*, 2024; Sungnak *et al.*, 2020). Nasal

epithelial cells (NECs) serve as both the primary site of viral entry and replication and as an

essential frontline of antiviral immunity, producing interferons (IFNs), a family of cytokines

that coordinate antiviral defence (Desmyter *et al.*, 1968; Sposito *et al.*, 2021). Type I (e.g., IFN-

α and IFN-β) and type III (e.g., IFN-λ) interferons bind to their respective receptors on infected

and neighbouring cells, triggering the Janus kinase/signal transducer and activator of

transcription (JAK-STAT) pathway. Type II interferon (IFN-γ), primarily derived from immune

effector cells, complements these pathways by amplifying antiviral and immunomodulatory

responses once immune infiltration occurs. This leads to the induction of hundreds of

interferon-stimulated genes (ISGs), which act collectively to inhibit viral replication, degrade

viral RNA, and enhance antigen presentation (Park and Iwasaki, 2020; Schneider *et al.*, 2014).

SARS-CoV-2 has evolved multiple strategies to suppress these IFN responses, thereby evading

early immune detection and promoting viral persistence (Carabelli *et al.*, 2023; V'Kovski *et al.*,

2021; Yuen *et al.*, 2020). The Alpha and Beta strains inhibit IFN production more effectively

than earlier reference strains (Gori Savellini *et al.*, 2023; Rajah *et al.*, 2021). Delta, in contrast,

displays enhanced replication efficiency and greater host immune antagonism, leading to

higher viral loads and increased disease severity (Liu and Rocklov, 2021; Rajah *et al.*, 2021). In

comparison, several studies suggest that Omicron subvariants exhibit a reduced interaction

with the interferon response, although some evidence indicates a delayed yet stronger

induction of ISGs (Gori Savellini *et al.*, 2023; Laine *et al.*, 2022; Shi *et al.*, 2024). Collectively,

these findings suggest that each variant has evolved unique strategies to modulate IFN

signalling and evade early antiviral defenses.

Among the emerging variants, Omicron variants remain globally dominant, as they rapidly

displaced previous VOCs, through successive waves of transmission. Unlike earlier SARS-CoV-

2 variants, Omicrons show an increased preference for the upper airways and a reduced

transition to the lower respiratory tract (Shi *et al.*, 2024). Studies suggest that mutations in

the Omicron spike protein enhance viral entry into nasal epithelial cells, enabling evasion of

both constitutive and interferon-induced antiviral factors that normally limit SARS-CoV-2

entry following attachment (Gori Savellini *et al.*, 2023; Reuschl *et al.*, 2024; Shi *et al.*, 2024).

In contrast to previous variants that predominantly rely on serine transmembrane proteases

for host cell entry, Omicron strains preferentially use cysteine proteases cathepsin B (CatB)

and cathepsin L (CatL) to catalyse endosomal membrane fusion, possibly providing an

advantage in nasal epithelial infection and potentially contributing to their increased

respiratory transmissibility (Giotis *et al.*, 2022; Leach *et al.*, 2021; Shi *et al.*, 2024). These

adaptations in entry mechanisms and tissue tropism raise important questions about how

variant-specific traits impact downstream host cellular responses.

While extensive research on VOCs has focused on spike protein mutations and their role in

enhancing viral entry and immune evasion, much less is known about how these variants

differentially reprogram host cell metabolism and signalling to optimise replication and

immune escape. Viruses such as Influenza A, Dengue, HBV, HIV and others typically hijack host

metabolic networks to create an environment conducive to replication, altering energy

production, amino acid biosynthesis, and lipid metabolism to support viral genome

replication, protein synthesis, and virion assembly (Allen *et al.*, 2022; Chan *et al.*, 2009; Liu *et

al.*, 2020; Long *et al.*, 2016; Polcicova *et al.*, 2020; Ritter *et al.*, 2010; Singh *et al.*, 2020; Thaker

et al., 2019). Current understanding of SARS-CoV-2 metabolic reprogramming largely derives

from studies on early variants, which have shown that the virus targets key pathways such as

glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway (PPP) to

meet biosynthetic and redox demands (Codo *et al.*, 2020; Guarnieri *et al.*, 2024; Mullen *et al.*,

2021). A central hallmark of this reprogramming leading to the accumulation of mitochondrial

reactive oxygen species (mROS) and consequent oxidative stress is the inhibition of

mitochondrial oxidative phosphorylation (OXPHOS) (Guarnieri *et al.*, 2024). Elevated mROS

stabilises hypoxia-inducible factor 1-alpha (HIF-1α), a key regulator that redirects cellular

metabolism toward glycolysis and the PPP, enhancing the synthesis and mobilisation of

nucleotides, lipids, and amino acids, which are necessary for viral replication (Guarnieri *et al.*,

2024; Mullen *et al.*, 2021). The virus also activates the mechanistic target of rapamycin

complex 1 (mTORC1) and integrated stress response (ISR) pathways, which in-turn promote

nutrient uptake, glycolysis, and innate immune signalling, while suppressing mitochondrial

biogenesis and cytosolic protein synthesis (Guarnieri *et al.*, 2024; Li *et al.*, 2021; Mullen *et al.*,

2021; Zambalde *et al.*, 2022). In parallel, the virus disrupts cellular stress responses and

modulates key signalling pathways, such as the phosphoinositide 3-kinase/protein kinase B

(PI3K/AKT) and mitogen-activated protein kinase (MAPK) pathways, to promote cell survival

(Bojkova *et al.*, 2020; Li *et al.*, 2021; V'Kovski *et al.*, 2021). Despite these insights from early

variants, how such metabolic and signalling circuits have been refined or rewired in later

variants, particularly Delta and Omicron, remains poorly understood.

In this study, we focused on six epidemiologically and biologically representative SARS-CoV-2

lineages, which capture the principal stages of viral adaptation in replication dynamics,

immune evasion, and host metabolic engagement during the pandemic. Using an integrated

multi-omics approach in primary human nasal epithelial cells cultured at the air-liquid

interface, we compared their transcriptional, signalling, and metabolic responses at early and

late stages of infection to define lineage-specific strategies of host manipulation and their

implications for viral fitness and adaptation.

Results

Understanding how SARS-CoV-2 variants remodel host cell metabolism and signalling is

critical to deciphering their evolutionary adaptations in transmissibility and immune evasion.

To investigate these processes, primary nasal epithelial cells (NECs) from a pool of human

donors were cultured at the air-liquid interface and infected with three classes of SARS-CoV-

2 variants: early strains (IC19 (a lineage B.1 SARS-CoV-2 reference strain), the pre-Omicron:

Alpha, Beta), Delta, and Omicron subvariants (BA.1, and BA.5). NECs were derived from three

commercially available donor pools (MucilAir™, Epithelix), each comprising epithelial cells

from 10-14 independent human donors. This pooled design ensured physiological relevance

and helped minimise donor-specific variability across infection conditions. Viral replication

kinetics were measured over 72 hours. Transcriptomic analysis (RNA-seq) was conducted at

24 and 72 hpi, and phosphoproteomic and amino acid profiling were performed at 24 hpi to

characterise early post-translational and metabolic responses (Figure 1A).

Omicron and Delta variants exhibit increased replication compared to pre-Omicron strains

To compare the replication dynamics of SARS-CoV-2 variants, NECs were infected with the

variants at a multiplicity of infection (MOI) of 0.01, and viral genome copies in the culture

supernatants were quantified over time using *E* gene RT-qPCR (Figure 1B).

At 24 hpi, BA.1 exhibited the highest early replication, with significantly elevated viral genome

copies compared to the pre-Omicron reference strain IC19 ($p < 1 \times 10^{-4}$), indicating an early

replication advantage. By 48 and 72 hpi, Delta showed the highest viral loads, significantly

exceeding IC19 at both time points ($p = 1.4 \times 10^{-3}$ and 2.9×10^{-3}, respectively). BA.5 and Beta

also demonstrated increased replication relative to IC19, with significantly higher viral titres

at 48 hpi ($p = 1.65 \times 10^{-2}$ and 1.73×10^{-2}) and 72 hpi ($p = 2.61 \times 10^{-2}$ and 2.88×10^{-2}). These

findings demonstrate group-specific replication dynamics, with Delta and Omicron

subvariants exhibiting increased replication efficiency in nasal epithelial cells compared to

pre-Omicron strains.

Delta triggers the most extensive transcriptional remodelling late in infection

To characterise host transcriptional responses to SARS-CoV-2 variants, we performed RNA-

seq on NECs at 24 and 72 hpi (Figure 2A-C). Differentially expressed genes (DEGs) were

defined by a $\log_2$ fold change $\geq |1|$ and FDR ≤ 0.05 (Table S1).

At 24 hpi, pre-Omicron variants (IC19, Alpha, and Beta) elicited the most substantial early

responses, with Alpha alone triggering over 3,800 DEGs (645 up-regulated, 3,162 down-

regulated). In contrast, Delta and the Omicron subvariants (BA.1 and BA.5) triggered markedly

fewer changes, suggesting attenuated or delayed early sensing (Figure 2B).

By 72 hpi, Delta drove the most extensive transcriptional reprogramming across all variants

(1,242 up-regulated, 4,432 down-regulated genes), while BA.1 and BA.5 induced intermediate

responses. Although some overlap in down-regulated genes was observed between Delta and

Omicron, the majority of DEGs remained variant-specific (Figure 2C).

Together, these data reveal distinct temporal transcriptional patterns across SARS-CoV-2

variants, with Delta driving the broadest late-stage reprogramming and highlighting the

evolving diversity of host–virus interactions.

Reduced suppression of host interferon responses following infection with Omicron

subvariants

We next examined how SARS-CoV-2 variants modulate innate antiviral signalling by

measuring interferon-stimulated gene (ISG) expression in primary nasal epithelial cells at 24

and 72 hpi (Figure 3A–D). ISGs represent core effectors of the type I interferon pathway and

provide a transcriptional readout of antiviral activation (Park and Iwasaki, 2020) (Figure 3A).

Cells treated with recombinant interferon alpha (IFN-α) were included as a reference to

establish a benchmark for ISG induction.

At 24 hpi, ISG induction remained low across all variant-infected relative to mock samples

(Figure 3B). Alpha and Beta showed the weakest early responses, whereas Delta and the

Omicron subvariants BA.1 and BA.5 triggered modest but detectable up-regulation. By 72 hpi,

ISG expression was broadly elevated, but with clear variant-specific differences (Figure 3C).

BA.1 and BA.5 induced the largest and most diverse ISG repertoires, similar in scale to those

induced by IFN-α, while Alpha and Beta exhibited more restricted activation. In contrast, Delta

consistently induced the fewest ISGs, even as infection advanced, pointing to sustained

suppression of interferon responses.

A heatmap of the top 30 IFN-α-responsive ISGs (Figure 3D) further illustrates these

differences. BA.1 and BA.5 displayed relatively higher expression of canonical antiviral genes

by 72 hpi such as *CXCL10*, *ISG15*, *IFIT1*, and *OAS1*, key effectors of RNA sensing, translational

arrest, and immune recruitment (Schneider *et al.*, 2014; Schoggins, 2019; Schoggins *et al.*,

2011), compared with Delta. Compared with BA.1- and BA.5-infected cells, Delta-infected

cells showed fewer IFN-α–responsive genes and reduced expression levels, suggesting a

weaker activation of interferon signalling.

Progressive tuning of host metabolism from early lineages to Delta and Omicron

Previous reports have shown that early SARS-CoV-2 lineages, including IC19, Alpha, and Beta,

are associated with reduced mitochondrial activity and enhanced glycolytic flux, an

established viral strategy to redirect cellular resources toward biosynthesis (Codo *et al.*, 2020;

Guarnieri *et al.*, 2024; Mullen *et al.*, 2021). Cytosolic pathways such as glycolysis and the

pentose phosphate pathway (PPP) generate biosynthetic precursors, whereas mitochondrial

metabolism, including the tricarboxylic-acid (TCA) cycle, fatty-acid oxidation (FAO), and

oxidative phosphorylation (OXPHOS), supports ATP production, redox balance, and antiviral

signalling.

To investigate how infection with different SARS-CoV-2 variants correlates with metabolic

pathway changes in host cells, we performed gene set enrichment analysis (GSEA) of RNA-

seq data from NECs infected with the variants at 24 and 72 hpi, relative to uninfected controls

(Figure 4A-B). Normalised enrichment scores (NES) were used to quantify coordinated

transcriptional changes across curated metabolic pathways, providing a comparative measure

of pathway activation (positive NES) or suppression (negative NES) independent of gene-set

size and variance (Figure 4; full data is listed in Table S2). For interpretation, pathways with

an FDR < 0.05 were categorised by NES magnitude as follows: $|NES| < 1.0$, limited enrichment;

$1.0 \leq |NES| < 1.8$, moderate enrichment; $|NES| \geq 1.8$, strong enrichment.

At 24 hpi, metabolic responses were limited in magnitude (Figure 4A). Cells infected with most

variants showed near-neutral or mildly positive NES values in glycolysis and PPP, suggesting

modest early cytosolic engagement. FAO gene sets were consistently negative across

lineages, indicating an early block in β-oxidation. Mitochondrial carrier and import modules

were close to neutral, while OXPHOS complexes displayed small but generally positive

enrichment, apart from a minor negative signal in the Alpha/Beta Complex IV module. Thus,

early infection was associated with preserved or moderately enhanced respiratory-gene

expression rather than broad suppression.

By 72 hpi, clear lineage-specific profiles emerged (Figure 4B). Delta-infected cells exhibited

the highest and most coordinated positive NES across OXPHOS complexes I–V and several

mitochondrial modules, indicating strong respiratory activation accompanied by moderate

enrichment of glycolytic and lipid-metabolism pathways. Cells infected with the reference

strain IC19 retained moderate OXPHOS activation. Cells infected with pre-Omicron strains

displayed overall weaker enrichment and more variable mitochondrial responses. In contrast,

Omicron-infected cells showed near-neutral cytosolic NES, negative FAO and coenzyme Q

biosynthesis scores, and modest positive enrichment of mitochondrial-carrier and OXPHOS

modules, again with a negative Complex IV component. These findings suggest that Omicron

infection elicits a host response that maintains basal mitochondrial function while avoiding

extensive metabolic activation.

Grouped comparison (Figure 4B) summarises these three variant-specific trends. Cells

infected with early lineages (IC19, Alpha, Beta) show moderate OXPHOS enrichment at 24 hpi

that diminishes and becomes variable by 72 hpi. Delta-infected cells display the strongest and

most coordinated activation across respiratory and associated metabolic pathways. Omicron-

infected cells retain a selective, mitochondria-centred profile with positive overall OXPHOS

and minimal cytosolic activation.

Delta activates stress kinases, while Omicron reprograms cytokine and survival signalling

To characterise early host signalling responses, we profiled phosphorylation events at 24 hpi

in NECs infected with IC19, Delta, BA.1, and BA.5 using phospho-kinase arrays. These variants

were selected to represent the most distinct host-response patterns observed in our dataset:

IC19 as the ancestral reference, Delta as the lineage that showed the broadest transcriptional

and metabolic engagement in infected cells, and BA.1/BA.5 as Omicron subvariants that, in

our system, elicited comparatively restrained early responses but clearer cytokine-linked

signalling at later stages. Phosphorylation levels were normalised to mock controls, and

statistical significance was assessed using Welch's t-tests with Benjamini–Hochberg

correction (FDR < 0.05; Table S3).

Relative to mock, all infections were associated with phosphorylation changes in stress-,

growth-, and cytokine-associated proteins, but the magnitude and pattern differed markedly

between variants (Figure 5A–B). Compared with IC19-, Delta-infected cells displayed the

most extensive activation of cellular stress and translational pathways, with significant

increases in p53, PRAS40, and p70 kinase alongside higher phosphorylation of STAT3 and

STAT6. This profile suggests enhanced engagement of stress-responsive and translational

signalling pathways. Collectively, the pattern observed in Delta-infected cells is consistent

with a stress-associated and pro-survival phosphorylation signature dominated by the p53–

RSK–S6K and PRAS40 nodes.

By contrast, cells infected with Omicron subvariants exchibited phosphorylation patters

enriched in immune- and cytokine-linked pathways. BA.1-infected cells showed prominent

phosphorylation of p38 MAPK, PLC-γ1, RYK, and Src, alongside strong activation of JAK–STAT

components including STAT1, STAT2, and STAT5. Phosphorylation fold changes for STAT1

(11.7-fold) and STAT2 (4.0-fold) were the highest among all variants, consistent with strong

interferon- and cytokine-associated signalling. Cells infected with BA.5 showed a similar but

overall attenuated profile compared with BA.1, retaining phosphorylation of the p53, STAT3

(S727), and Src-family kinases.

Contrasting host amino acid regulation by Delta and Omicron variants

To complement the transcriptomic and phosphoproteomic analyses, intracellular amino acid

levels were quantified at 24 hpi in nasal epithelial cells infected with IC19, Delta, BA.1 or BA.5

and compared with mock-infected controls (Figure 6; Table S4). Total intracellular amino acid

abundance (Figure 6A) showed modest, non-significant reductions in IC19- and Delta-infected

cells ($q > 0.05$), despite an approximate 10% decrease in IC19 relative to mock. In contrast,

BA.1- and BA.5-infected cells exhibited significant reductions in total amino acid pools ($q <$

0.01 and $q < 0.05$, respectively). All amino acid measurements were expressed as nmol per

10^6 viable, counted cells, and viability at 24 hpi was comparable across conditions, indicating

that differences in abundance reflect infection-driven metabolic changes rather than

variation in cell number.

The variant–amino acid interaction network (Figure 6B) visualises these differences by linking

each variant to its most strongly altered amino acids. Edge width reflects the absolute $\log_2$

fold change, and colour indicates direction (red for increases, blue for decreases relative to

mock). BA.1 and BA.5 display numerous, broad negative edges, while IC19 and Delta show

fewer, thinner connections, consistent with a more limited metabolic impact.

Individual amino acid profiles (Figure 6C; Table S4) further resolved these patterns. In BA.1-

infected cells, intracellular amino acid levels showed the broadest and most significant

reductions, including all branched-chain and aromatic amino acids (leucine, phenylalanine,

tryptophan, isoleucine, valine), together with decreases in several other amino acids,

including threonine, serine, arginine and lysine. BA.5-infected cells exhibited a similar but less

pronounced profile, with significant decreases in many essential amino acids and moderate

increases in alanine and proline. Delta-infected cells displayed a mixed pattern, characterised

by significant increases in proline, alanine, glycine and glutamine, alongside reductions in

selected essential amino acids such as isoleucine and phenylalanine. IC19-infected cells

showed only small, variable changes, none of which reached significance after FDR correction.

To examine whether infection altered the internal balance of amino acids rather than total

abundance, amino acid levels were normalised to the total amino acid content within each

condition (Figure 6D; Table S4). The relative amino acid composition appeared consistent

across variants, suggesting that infection reduces overall amino acid availability without

disturbing the proportional balance within the intracellular pool.

Together, these data show that amino acid abundances in variant-infected cells differ from

those in mock-infected cells in a lineage-specific manner, with Delta-infected cells showing

comparatively higher levels of several biosynthetically relevant amino acids, whereas cells

infected with Omicron subvariants display broad amino acid depletion and limited

compensatory increases across pathways. Amino acids are central to energy production,

redox balance, protein synthesis, and immune signalling, and their intracellular abundance

provides a sensitive readout of metabolic stress and viral resource use (Thaker *et al.*, 2019).

Discussion

SARS-CoV-2 variants have evolved distinct capacities to replicate, modulate host immunity,

and influence disease severity. Although mechanisms of viral entry and antibody escape are

well characterised, far less is understood about how major lineages remodel host cellular

pathways once infection is established. To address this, we employed differentiated nasal

epithelial cells (NECs) grown at air-liquid interface, a physiologically relevant model that

recapitulates early epithelial infection in the absence of systemic immune influences. This

system enables direct examination of variant-specific host responses at the primary site of

replication, which may differ in magnitude and kinetics from those observed in bronchial or

immune-cell containing models. Through an integrated multi-omics approach, we uncovered

lineage-specific differences in the extent, timing, and coordination of transcriptional,

signalling, and metabolic responses.

Early lineages (IC19, Alpha, Beta) broadly down-regulated immune and metabolic transcripts

at 24 hpi, consistent with potent suppression of innate antiviral signalling. These results align

with previous studies showing that early SARS-CoV-2 isolates suppress mRNA synthesis and

translation through ORF6-, ORF8-, and ORF9b-mediated interference with STAT1/2 and

nuclear import (Laine *et al.*, 2022; Reuschl *et al.*, 2024; V'Kovski *et al.*, 2021; Yuen *et al.*, 2020).

By 72 hpi, infected nasal epithelial cells exhibited clear lineage-specific divergence. Cells

infected with Delta exhibited extensive transcriptional reprogramming, including activation

of stress-response, metabolic, and cytokine-related genes. In contrast, Omicron-infected cells

displayed fewer differentially expressed genes, nevertheless, showed selective enrichment of interferon-stimulated and cytokine-responsive transcripts, consistent with a delayed yet measurable antiviral response (Gori Savellini *et al.*, 2023; Reuschl *et al.*, 2024; Shi *et al.*, 2024). Consistent with previously reported effective antagonism of RIG-I/MDA5–JAK–STAT signalling (Laine *et al.*, 2022; Sievers *et al.*, 2024; Tandel *et al.*, 2022) IC19, Alpha, Beta, and Delta displayed weak ISG induction at both 24 and 72 hpi. In contrast, Omicron elicited a broader ISG response by 72 hpi, approaching that observed following exogenous IFN-α treatment, suggesting only partial suppression of interferon-driven transcription (Reuschl *et al.*, 2024; Shi *et al.*, 2024).

Phosphoproteomic profiling further distinguished variant phenotypes. Omicron infections enhanced phosphorylation within JAK–STAT and MAPK modules (STAT1/2/5, p38, PLC-γ1, Src), while Delta preferentially activated stress- and growth-associated kinases (p53, PRAS40, RSK, p70 S6K), indicating engagement of PI3K–Akt–mTORC1 and the integrated stress response (Bouhaddou *et al.*, 2020; Klann *et al.*, 2020; Li *et al.*, 2021). These patterns suggest that Omicron can replicate despite ongoing cytokine signalling, whereas Delta infection favours stress- and growth-linked pathways to sustain replication.

Our metabolic data reveal pronounced lineage-specific differences in host–virus interaction. Consistent with previous reports (Codo *et al.*, 2020; Mullen *et al.*, 2021), cells infected with pre-Omicron lineages suppressed fatty-acid oxidation and exhibited modest, heterogenous mitochondrial changes, indicating partial mitochondrial adaptation rather than broad inhibition. Conversely, Delta-infected cells displayed the strongest and most coordinated activation spanning oxidative-phosphorylation complexes I–V and several mitochondrial modules, accompanied by moderate enrichment of cytosolic pathways, including the tricarboxylic-acid cycle and lipid metabolism. These transcriptional shifts

occurred despite broadly comparable viral loads across lineages at 24-72 hpi, suggesting that

replication level alone does not account for the observed metabolic divergence. Delta-

infected NECs retained or modestly increased one-carbon intermediates such as glycine,

glutamine, and alanine, consistent with preserved anabolic and redox capacity. Targeted

metabolomic analysis further revealed that total intracellular amino acid pools declined

across infections relative to mock, but the extent varied by lineage: Delta-infected cells

exhibited the smallest reduction, BA.5-infected cells an intermediate profile, and BA.1-

infected cells the most pronounced depletion, particularly of essential and branched-chain

amino acids such as leucine and valine. These findings indicate that Delta sustains a more

biosynthetically active state, whereas Omicron subvariants replicate under more

metabolically constrained conditions, suggesting a shift towards a low-anabolism infection

strategy. When amino acid levels were normalised to total intracellular content, the overall

proportional balance of amino acids in variant-infected cells was largely preserved, with only

a small deviation detected for BA.1 compared with mock. This pattern suggests that SARS-

CoV-2 lineages primarily modulate the metabolic tone of nasal epithelial cells by altering

amino acid availability, rather than by extensively reshaping the internal composition of the

amino acid pool.

Integration of transcriptomic, phosphoproteomic, and metabolic datasets, delineates three

distinct host-virus interaction strategies. Early lineages dampened transcription, interferon

signalling, and fatty-acid oxidation while maintaining only minimal mitochondrial activity.

Delta, by contrast, exhibited coordinated activation of respiratory, cytosolic, and stress

pathways, consistent with higher metabolic demand. Omicron retained efficient replication

while preserving mitochondrial respiration and tolerating interferon-linked signalling, yet

engaging anabolic metabolism only weakly. These lineage-specific programmes likely reflect

not only viral genetic differences but also the interplay between replication dynamics and epithelial sensing thresholds in NECs. Overall, SARS-CoV-2 evolution appears to have progressively re-tuned the balance between replication, metabolism, and immune modulation. Later variants replicate effectively while imposing a smaller metabolic load and causing less epithelial stress, yet remain capable of replication in the presence of active cytokine and interferon responses. This restrained, energy-efficient infection profile mirrors clinical observations that Omicron replication is largely confined to the upper airways and associated with reduced epithelial injury and inflammation (Carabelli *et al.*, 2023; Gori Savellini *et al.*, 2023; Laine *et al.*, 2022).

The use of pooled donor–derived NECs enhances physiological relevance but may mask inter-individual variation in antiviral and metabolic responses. Future studies sampling additional time points and integrating isotope-tracing could define metabolic flux through glycolysis and the TCA cycle, while targeted perturbation of pathways such as PI3K–Akt–mTORC1, p53–RSK–S6K, and p38 MAPK in ALI cultures could establish causal links between signalling nodes and replication outcomes. Extending the approach to lower-airway or immune–epithelial co-culture models would further connect cellular rewiring to tissue-level phenotypes and transmissibility.

In summary, SARS-CoV-2 variants employ distinct reprogramming strategies in nasal epithelium. Pre-Omicron strains strongly suppress antiviral and metabolic responses; Delta drives extensive activation of stress and energy pathways; Omicron maintains mitochondrial activity while limiting broader metabolic engagement. These contrasting strategies illustrate how viral evolution has refined replication efficiency relative to metabolic demand and immune modulation, providing mechanistic insight for host-targeted therapeutic approaches.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr Efstathios Giotis (e.giotis@essex.ac.uk).

Materials availability

No new unique reagents or biological materials were generated in this study. SARS-CoV-2 variant strains were obtained from existing publicly available virus collections, as indicated in the Materials and Methods. All cell lines and virus isolates used are commercially or institutionally available.

Data and code availability

The RNA sequencing data generated during this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE271378. The phosphoproteomic and amino acid profiling datasets are available in the Supplemental information and can also be shared upon request from the Lead Contact. This study generated analysis scripts for figure production, available upon reasonable request from the Lead Contact. Scripts for data visualisation (e.g., R scripts for heatmaps, Python for statistical plots) are available from the Lead Contact upon reasonable request.

Author contributions

E.S.G. conceptualised the study, supervised the project, and contributed to methodology, investigation, formal analysis, visualisation, and funding acquisition. R.A.S., J.B., T.A., N.B., D.L. and M.Mu. performed experimental work and contributed to methodology and investigation. S.R.K. carried out bioinformatic analyses, formal analysis, data curation, and visualisation. R.A.S. also contributed to data visualisation. M.Ra. conducted amino acid

profiling and provided resources and supervision. W.S.B. and C.R. contributed to project

supervision, resources, and funding acquisition. E.S.G., R.A.S., and S.R.K. wrote the original

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Declaration of interests

The authors declare no competing interests.

Supplemental information

Supplementary Figures S1–S2.

Contains validation of RNA-seq data by qPCR across selected immune signalling and

metabolic genes. Includes:

•   **Supplementary Figure S1:** Correlation plot of RNA-seq vs qPCR fold-change values

for immune-signalling genes (*CXCL10, IFIT1, IFIT2, ISG15, OAS1, STAT1*).

•   **Supplementary Figure S2:** Correlation plot of RNA-seq vs qPCR fold-change values

for metabolism-related genes (*HK2, GMPR, ENO2, MDH1B, ATP6V1B1*).

Table S1. Excel file containing DEG lists from RNA-sequencing analysis across SARS-CoV-2

variants, related to Figures 1 and 3.

Table S2. Normalised Enrichment Scores (NES) from fast Gene Set Enrichment Analysis

(fGSEA) of oxidative phosphorylation (OXPHOS), cytosolic and mitochondrial metabolic

pathways across SARS-CoV-2 variants and time points. NES, adjusted *p*-values (FDR), and

leading-edge gene counts are reported for each pathway.

Table S3. Excel file containing phospho-kinase array raw and processed data, related to

Figure 5.

Table S4. Excel file containing raw and normalised intracellular amino acid levels (nmol/10^6

cells), related to Figure 6.

Table S5: Primer sequences and RefSeq accession numbers used for qPCR validation.

Figure Legends

Figure 1. Omicron and Delta variants exhibit enhanced replication compared to pre-

Omicron strains

(**A**) **Schematic representation of the experimental setup.** Primary human nasal epithelial cells were cultured at the air-liquid interface and infected with six SARS-CoV-2 variants: IC19 (reference strain), Alpha, Beta, Delta, Omicron BA.1, and Omicron BA.5. All infections were performed at a multiplicity of infection (MOI) of 0.01. Parallel wells treated with recombinant IFN-α served as a transcriptional benchmark. Cells were harvested at 24, 48, and 72 hpi for downstream analyses, including phosphoproteomic profiling, amino acid (AA) profiling, and RNA sequencing (RNA-seq). The timeline indicates the specific time points at which each molecular analysis was conducted.

(**B**) **Growth kinetics of SARS-CoV-2 variants.** Viral replication over a 72-hour period was quantified by RT-qPCR targeting the *E* gene. Viral RNA levels are expressed as *E* gene copies per millilitre on a log10 scale. Measurements were taken at 0, 24, 48, and 72 hpi. Variants Delta and BA.1 demonstrated significantly elevated viral loads compared to the reference strain IC19 at multiple time points. Statistical significance was evaluated using one-way ANOVA with Tukey's multiple comparisons test. Asterisks indicate significance levels: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Data are shown as mean ± standard deviation (SD) from three independent experiments.

Figure 2. Transcriptomic profiling of host responses to SARS-CoV-2 variant infection.

(B) Number of significantly up-regulated (red) and down-regulated (blue) differentially expressed genes (DEGs) at 24 and 72 hpi with indicated SARS-CoV-2 variants, based on RNA-seq analysis (log2 fold-change ≥1; FDR <0.05).

(B) Venn diagrams showing shared and unique up-regulated DEGs among variants at 24 hpi (top) and 72 hpi (bottom).

(C) Venn diagrams showing shared and unique down-regulated DEGs among variants at 24

hpi (top) and 72 hpi (bottom). Percentages represent overlap relative to the total DEGs per

variant.

Figure 3. Late ISG induction by Omicron strains versus sustained attenuation by Delta

(**A**) **Schematic of interferon signalling and ISG activation.**

(**B-C**) **Shared and unique ISG induction across variants at 24 (B) and 72 (C) hpi.** An UpSet plot

visualizes the number of significantly up-regulated ISGs (FDR < 0.05) per SARS-CoV-2 variant

and their overlaps. The bar chart at the top indicates the size of each intersection set, while

the connected dots below identify which variants contribute to each group. The colored

horizontal bars represent total ISG numbers detected per variant. The inset bar graphs show

the total number of significantly upregulated genes detected for each variant at the

corresponding time point, providing context for overall transcriptional activation.

(**D**) **Heatmaps showing log2 fold-change of top ISGs induced by IFNα treatment and during**

infection at 24 and 72 hpi. The leftmost panel shows the top ISGs up-regulated by IFNα

treatment alone, identifying baseline interferon responsiveness of the cells. The middle and

right panels show the expression of these ISGs in response to viral infection across all six SARS-

CoV-2 variants at 24 and 72 hpi, respectively. Color intensity reflects log2 fold-change, with

red indicating upregulation and blue indicating down-regulation or suppression.

Figure 4. Normalised enrichment scores (NES) depict pathway-level transcriptional

changes across SARS-CoV-2 variants and time points.

(**A**) Variant-specific NES profiles for cytosolic, mitochondrial, and oxidative phosphorylation

(OXPHOS) pathways at 24 and 72 hpi. Differential expression values (log$_2$ fold change) were

ranked and analysed with the fgsea R package using curated KEGG and literature-based

gene sets.

(**B**) Merged analysis comparing Pre-Omicron and Omicron lineages, showing composite NES

values summarizing transcriptional regulation across variants. Red indicates pathway

activation, blue indicates suppression. NES, normalised enrichment score.

Figure 5. SARS-CoV-2 variants differentially regulate host signalling pathways through

phosphorylation events.

(**A**) **Representative phospho-protein arrays** showing phosphorylation patterns in uninfected

mock controls and cells infected with IC19, Delta, Omicron BA.1, and Omicron BA.5 at 24

hpi. Key differentially phosphorylated proteins are highlighted.

(**B**) **Quantitative analysis of phosphorylation fold changes (variant-infected vs. mock)**

across all array targets grouped by signalling pathway. Dots represent mean values from at

least two independent experiments, with vertical error bars showing the standard error of

the mean (SEM). Colour coding denotes variant groups: IC19 (blue), Delta (orange), BA.1

(green), and BA.5 (red). Markers are ranked by biological pathway, with coloured

background shading corresponding to biological grouping. The vertical red dashed line

indicates the mock-infected baseline (fold change = 1).

Figure 6. SARS-CoV-2 variants induce distinct amino acid reprogramming in nasal epithelial

cells.

(**A**) **Bar plot showing total amino acid abundance** (nmol/10^6 cells) relative to mock-infected

controls at 24 hpi.

(**B**) **Variant–metabolite interaction network** showing amino acids (green nodes) connected

to each SARS-CoV-2 variant (grey nodes). Edges represent directionality of regulation

compared to mock: red edges indicate increased levels (upregulation), blue edges indicate

decreased levels (down-regulation), and black edges indicate no change.

(C) Heatmap of log$_2$-transformed amino acid levels (based on nmol/10^6 cells) for each

variant relative to mock-infected controls at 24 hpi. for each variant relative to mock-

infected controls at 24 hpi. Amino acids are annotated by metabolic pathway (left colour

bar) and biochemical role (right colour bar). Red shading indicates increased amino acid

abundance, and blue shading indicates decreased abundance.

(D) Violin plots showing the % relative amino acid composition after normalisation of each

amino acid to the total amino acid content within the same condition. This representation

highlights the internal proportional balance of amino acids rather than total abundance. The

black line indicates the median value for each distribution.

MATERIALS AND METHODS

Cells and viruses

Human nasal airway epithelial cells (NEC)s, maintained at the air–liquid interface

(MucilAir™, pooled from 14 donors, catalog no. EP02MP), were obtained in three batches

from Epithelix and cultured in MucilAir™ cell culture medium (Epithelix) at 37 °C with 5%

CO_2. SARS-CoV-2 variants were propagated and titrated as previously (Ogunjinmi *et al.*,

2024; Zhang *et al.*, 2022) in African green monkey kidney (VeroE6) cells expressing human

angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 precursor

(TMPRSS2) (VAT), kindly provided by the MRC-University of Glasgow Centre for Virus

Research, Glasgow (Rihn *et al.*, 2021). VAT cells were maintained in Dulbecco's Modified

Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1 mg/mL Geneticin

(Gibco), and 0.2 mg/mL Hygromycin B (Invitrogen). Viral stocks were generated and titrated

in VAT cells for the following SARS-CoV-2 variants: wild-type/D614G (hCoV-

19/England/IC19/2020, B.1.13, EPI_ISL_475572), Alpha (hCoV-19/England/205080610/2020,

B.1.1.7, EPI_ISL_723001), Delta (hCoV-19/England/SHEF-10E8F3B/2021, B.1.617.2,

EPI_ISL_1731019), Omicron BA.1 (hCoV-19/England/M21021166/2022, BA.1), and BA.5

(hCoV-19/England/NWLP_53/2022, BA.5).

RNA-sequencing of nasal airway epithelial cells infected with SARS-CoV-2 variants

Before infection, NEC cultures were washed with serum-free medium to remove mucus and

debris. Cells were then infected with 200 μL of virus-containing serum-free DMEM (MOI: 0.01)

and incubated at 37 °C for 1 hour. Following incubation, the inoculum was removed, and the

cells were washed twice. Time points were collected by adding 200 μL of serum-free DMEM,

incubating at 37 °C for 10 minutes, and then removing the medium for titration. Cells were

harvested at 24 and 72 hours for RNA isolation using the RNeasy Mini Plus kit (Qiagen), and

approximately 1 μg of RNA was used for sequencing library preparation.

mRNA libraries were prepared using poly-A enrichment and sequenced by Novogene. The

sequencing data (FASTQ raw files) were imported into Partek Flow (version 10.0, build

10.0.23.0531; Partek Inc.) for quality control and processing. Paired-end reads were trimmed

based on a Phred quality score threshold of >20 and aligned to the human genome (hg38)

using the STAR-2.7.8a aligner. Gene expression was quantified using the Ensembl Transcripts

release 104 v2 transcript model, and data were normalised using the counts per million (CPM)

method. Differential gene expression analysis was conducted using the DESeq2 package, with

genes considered differentially expressed if they met the criteria of FDR ≤ 0.05 and a log2 fold

change ≥ |1|. All RNA-seq data have been deposited in the Gene Expression Omnibus (GEO)

under accession number GSE271378.

Pathway enrichment and transcriptomic profiling

Normalised enrichment scores (NES) generated from the fGSEA analysis were visualised using

dot plots in R (v4.3) with the *ggplot2* (v3.4.4), *dplyr*, *tidyr*, *stringr*, and *readxl* packages. Each

dot represented a metabolic pathway under a specific condition, where the horizontal axis

denoted NES magnitude and the vertical axis listed the curated gene sets (cytosolic,

mitochondrial, and oxidative phosphorylation). Dot colour indicated the direction of

regulation (red, up-regulated; blue, down-regulated), and dot size was proportional to the

absolute NES value. Vertical reference lines at NES = 0 were included to indicate neutral

enrichment.

To aid visual interpretation, NES magnitudes were categorised as limited ($|NES| < 1.0$),

moderate ($1.0 ≤ |NES| < 1.8$), or strong ($|NES| ≥ 1.8$) enrichment. Only pathways meeting the

inclusion criteria defined in the differential expression analysis were displayed. While the dot

plots provide a comparative overview of pathway-level regulation, they represent aggregate

enrichment statistics rather than individual gene contributions. Adjusted *p*-values and false

discovery rates (FDR) computed during fGSEA were not visualised in the figure but are

reported in Supplementary Table S2. Pathway sizes (number of genes per set) and inter-gene

variance were not displayed to maintain visual clarity; therefore, pathways of different gene-

set sizes appear equally weighted. Similarly, replicate-level variability and confidence

intervals for NES values were not plotted, as the scores reflect ranked enrichment rather than

absolute expression magnitude. Relationships between overlapping pathways were not

visualised but were accounted for during pathway curation. Together, these dot plots

summarise the direction and magnitude of metabolic pathway enrichment across SARS-CoV-

2 variants and time points while maintaining a uniform visual scale for comparative

assessment.

Quantification of viral and host gene expression by RT-qPCR

Virus genome quantification was carried out using *E* gene RT-qPCR, following a previously

described method (Zhou *et al.*, 2022). Viral RNA was extracted from swab supernatant

samples using the QIAsymphony DSP Virus/Pathogen Mini Kit on the QIAsymphony

instrument (Qiagen). RT-qPCR was performed with the AgPath RT-PCR kit (Life Technologies)

on a QuantStudio 7 Flex Real-Time PCR System, using primers specific to the SARS-CoV-2 *E*

gene (Corman *et al.*, 2020). For absolute quantification of *E* gene RNA copies, a standard curve

was generated from serial dilutions of viral RNA with a known copy number. The *E* gene copies

per ml of the original virus supernatant were then determined based on this standard curve.

Validation of RNA-sequencing data by quantitative RT-PCR

To validate differential gene expression observed in RNA-seq, we performed quantitative

RT-PCR (qPCR) using independently designed primers as before (Giotis et al., 2019;

Ogunjinmi *et al.*, 2024; Zhang *et al.*, 2022). Genes were selected based on their

transcriptional regulation across SARS-CoV-2 variants and their relevance to innate immune

signalling (e.g., *CXCL10, IFIT1, IFIT2, ISG15, OAS1, STAT1*) or metabolic pathways (e.g., *HK2,*

GMPR, MDH1B, ENO2, ATP6V1B1). Total RNA was extracted from nasal epithelial cell

cultures using TRIzol™ Reagent (Fisher Scientific) according to the manufacturer's protocol.

qPCR was carried out on a CFX96 Real-Time PCR System (Bio-Rad) using PowerUp™ SYBR

Green Master Mix (Fisher Scientific). All reactions were run in triplicate to ensure

reproducibility. Primer sequences, designed based on NCBI RefSeq transcripts, are listed in

Table S5.

Phospho-kinase array and signalling analysis

Phosphorylation profiling was carried out using the Proteome Profiler Human Phospho-Kinase

Array Kit (ARY003B, R&D Systems), which detects phosphorylation at 43 kinase sites across

multiple host signalling pathways. Arrays were performed on lysates collected at 24 hpi from

the same nasal epithelial cell cultures used for transcriptomic and amino acid profiling,

ensuring matched experimental conditions across assays. Following protein extraction, total

protein was quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific), and equal

amounts of lysate were incubated with array membranes overnight at 4 °C, according to the

manufacturer's instructions. Chemiluminescent signals were developed using SuperSignal

West Femto substrate (Thermo Scientific) and imaged with the Invitrogen iBright FL1500

system. Spot intensities were quantified in ImageJ (v1.53), corrected for background, and

normalised to internal reference spots. Fold changes were calculated relative to mock-

infected controls. Each condition was assessed in at least two independent experiments. Data

visualisation was performed in Python (v3.10) using seaborn and matplotlib. Phosphorylation

sites were grouped by pathway (e.g., PI3K-Akt, MAPK, JAK-STAT) based on KEGG and

Reactome annotations (Figure 5). Dot plots represent mean fold changes ± SEM, with the

mock baseline shown as a red dashed line.

Quantitative amino acid profiling and network-based visualisation of metabolic disruption

Amino acid analysis was performed using hydrophilic interaction chromatography-tandem mass spectrometry (HILIC-MS/MS), similar as previously described (Jeffares *et al.*, 2015; Mulleder *et al.*, 2017; Rallis *et al.*, 2021). We used four biological replicates per condition, each processed in duplicate (two technical replicates), to ensure robustness and reproducibility of quantification. One million cells were harvested per condition (mock and viral infections). For extraction, cells were pelleted (3000g, 3 min), washed briefly, and resuspended in 200 µL of 80% ethanol preheated to 80 °C. The suspension was vortexed and incubated at 80 °C for 2 minutes, repeated once, then centrifuged at maximum speed for 2 minutes to clear cell debris and stored at 80 °C until further usage. For the exact amino acid quantification, we included cell free $^{15}N^{13}C$ labelled amino acids as internal standard (CNLM-6696-PK, Cambridge Isotope Laboratories). Besides, a calibration curve was generated using serial dilutions of standards. The amino acid measurements were performed by using a flowrate of 0.6 ml/min for 12 min. All proteinogenic amino acids amino expect cysteine were measured.

Final amino acid concentrations were expressed as nanomoles (nmol) per 10^6 cells to normalise for input cell number across conditions (Table S4).

To visualise SARS-CoV-2 variant-specific amino acid expression changes, a bipartite node network was constructed using Python (pandas, numpy, networkx, matplotlib). Amino acid $\log_2$ fold changes relative to mock at 24 hpi (Table S4) were used to identify the top five most dysregulated amino acids per variant (ranked by absolute $\log_2$ fold change). These variant–amino acid pairs formed the network edges. Edge thickness reflected the magnitude of the change, while colour indicated direction: red (increased abundance), blue (decreased abundance), and black (no substantial change). Amino acid nodes were sized by total

connectivity and coloured according to their dominant direction of change across variants.

The layout was manually optimised for clarity. This network allowed integrated visualisation

of shared and distinct metabolic disruptions across variants, complementing the

quantitative $\log_2$ fold-change data presented in heatmaps.

Statistical analysis

All data were analysed using GraphPad Prism software (v9) and Python (v3.10). Results are

presented as mean ± SEM. Statistical significance was defined as $p < 0.05$, with levels of

significance indicated as follows: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

To assess differences in viral replication between SARS-CoV-2 variants at each time point

relative to the reference strain IC19, a one-way ANOVA was performed at 0, 24, 48, and 72

hpi. If statistical significance ($p < 0.05$) was observed, Tukey's Honest Significant Difference

(HSD) test was conducted for post-hoc pairwise comparisons to determine specific

differences between IC19 and each variant. Adjusted p-values from Tukey's HSD test were

used to control for multiple comparisons, ensuring robust identification of significant

replication differences. Variants exhibiting p-values below the 0.05 threshold were

considered to have significant replication advantages or disadvantages relative to IC19.

Results were annotated on viral growth curves to highlight meaningful differences across the

infection timeline.

Phospho-array data were log-transformed data and analysed using Welch's t-tests, followed

by Benjamini-Hochberg correction for multiple comparisons (Table S4). Amino acid

concentrations (nmol per 10^6 cells) were measured in four biological replicates per condition,

each processed in duplicate. Technical replicates were averaged per biological replicate

before statistical analysis. For each amino acid, mean values were used to calculate the fold

change relative to mock and the corresponding $\log_2$ fold change ($\log_2 FC = \log_2[variant/mock]$).

Statistical testing was performed using Welch's two-sample t-tests (unequal variances)

applied to each amino acid for: (i) comparisons of each SARS-CoV-2 variant against mock, and

(ii) all pairwise comparisons between conditions (mock, IC19, Delta, BA.1, BA.5). Multiple

testing within each comparison was corrected using the Benjamini–Hochberg false discovery

rate (FDR) procedure (threshold $q < 0.05$). Table S4 reports, for each amino acid and

comparison, the raw p value, the FDR-adjusted q value, a Yes/No column indicating

significance at $q < 0.05$, and p-value significance symbols (* $p < 0.05$; ** $p < 0.01$; *** $p <$

0.001; **** $p < 0.0001$; ns for non-significant). Log_2FC values are provided as effect sizes.

To assess whether infection altered amino acid composition independently of total

abundance, amino acid levels were normalised to the total amino acid content within each

condition. The resulting relative proportions were compared across variants using the

Friedman test with Dunn's multiple-comparison correction (GraphPad Prism v9). Total amino

acid abundance per condition was calculated by summing per–amino acid means, with

percent change expressed relative to Mock.

Data visualisation

To illustrate shifts in amino acid levels across SARS-CoV-2 variants, heatmaps and summary

tables were used to facilitate interpretation and guide RNA-seq pathway analysis. For

phosphoproteomic analyses, heatmaps were generated using Python's matplotlib and

seaborn libraries. Heatmap of amino acid fold changes were generated using

the pheatmap package in R (v4.3), clustering both rows and columns to reveal variant-specific

metabolite patterns. Venn diagrams were created with InteractiVenn (Heberle *et al.*, 2015) to

analyse shared and unique features across different conditions.

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A.

DEGs at 24 Hours Post-Infection

Number of Genes

3000 · 2000 · 1000 · 0

IC19 Alpha Beta Delta BA.1 BA.5

■ Downregulated   ■ Upregulated

DEGs at 72 Hours Post-Infection

Number of Genes

4000 · 2000 · 0

IC19 Alpha Beta Delta BA.1 BA.5

■ Downregulated   ■ Upregulated

B.

🟢↑ 24h

🔴↑ 72h

C.

🔵↓ 24h

🔵↓ 72h

A.

B.

C.

D.

A. Total aminoacid change related to Mock (%)

B.
- Variant (grey)
- Amino Acid (green)
- Up-regulated (red edge)
- Down-regulated (blue edge)
- No change (black edge)

C. SARS-CoV-2 variants

Pathway	Role	IC19	Delta	BA.1	BA.5	
						Leucine
						Phenylalanine
						Tryptophan
						Isoleucine
						Methionine
						Valine
						Proline
						Tyrosine
						Alanine
						Threonine
						Glycine
						Glutamine
						Serine
						Asparagine
						Glutamic acid
						Histidine
						Arginine
						Lysine

log$_2$ fold-change Vs Mock

Pathway
- Energy Production
- Barrier Integrity
- Immune Response
- Redox Balance
- Structural Integrity
- Mucosal Defense
- Carbon Metabolism
- Protein Synthesis
- Detoxification
- Nitrogen Handling
- Oxidative Stress Defense
- Neurotransmission
- Histamine Regulation
- Nitric Oxide Regulation
- Ammonia Detoxification

Role
- Branched-chain
- Aromatic
- Sulfur-containing
- Non-polar
- Polar
- Acidic
- Basic

D. Amino acid pool composition (% per condition)