

Hyperglycemia-associated immunometabolic rewiring worsens mitochondrial dysfunction and inflammatory imbalance during *Burkholderia cepacia* infection

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35 **Abstract**

36 **Background:** Diabetes mellitus significantly raises the risk of serious bacterial infections. In this
37 study, we investigated how a hyperglycemic environment worsens immune and metabolic
38 dysfunction. We focused on how it drives inflammation during *Burkholderia cepacia* infection in
39 a K562 cell model.

40 **Methods:** K562 cells were grown under normal (5.5 mM) or high (15 mM) glucose conditions
41 and then infected with *B. cepacia*. We assessed global metabolic changes using untargeted gas
42 chromatography-mass spectrometry (GC-MS), while targeted metabolomics measured
43 tricarboxylic acid (TCA) cycle intermediates. We evaluated mitochondrial health, including
44 membrane potential and oxidative stress, with multiparametric flow cytometry (JC-1, H2DCFDA,
45 and MitoSOX). We measured inflammatory markers, antioxidant defenses, and changes in
46 signaling pathways through qRT-PCR, immunoblotting, and ELISA.

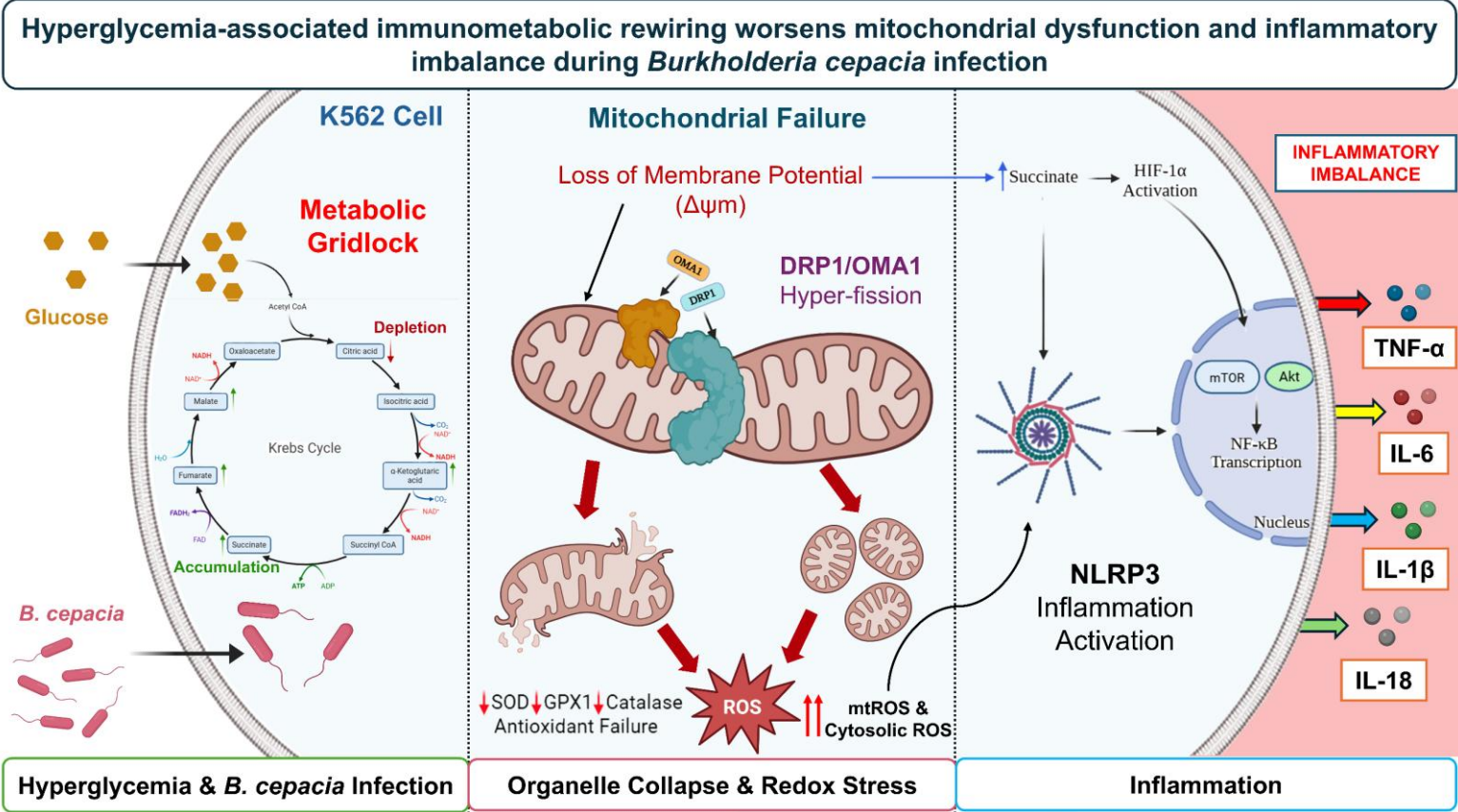
47 **Results:** Untargeted metabolomics showed that infection shifts metabolism away from central
48 carbon metabolism toward lipid use. This process is severely affected by high blood sugar levels.
49 Targeted analysis found that metabolic stress from hyperglycemia disrupts the flow of the TCA
50 cycle, leading to a significant drop in citrate levels and a harmful buildup of succinate. This
51 metabolic traffic jam causes serious mitochondrial damage, indicated by rapid membrane
52 depolarization and irregular fission-fusion dynamics, with increased DRP1 and decreased PGC-
53 1 α . As a result, infected cells in a high glucose environment show a sharp rise in mitochondrial
54 superoxide and cytosolic reactive oxygen species. Furthermore, hyperglycemia reduces host
55 antioxidant defenses, including superoxide dismutase and glutathione synthetase, leading to
56 severe lipid damage. This unchecked oxidative damage significantly activates the NLRP3
57 inflammasome and NF- κ B pathways through alterations in the mTOR-Akt signaling axis,
58 resulting in a marked release of IL-1 β , IL-18, IL-6, and TNF- α .

Conclusion: Hyperglycemia traps the host in a difficult energy crisis. It worsens infection-induced disruption of the TCA cycle, leads to severe mitochondrial fragmentation, and depletes antioxidant defenses. This combined oxidative stress overactivates the NLRP3 inflammasome, highlighting the importance of mitochondrial health and metabolic flexibility as key treatment targets for individuals with metabolic issues who face bacterial infections.

Keywords

Immunometabolism, *Burkholderia cepacia*, Hyperglycemia, Mitochondrial dysfunction, Oxidative stress, NLRP3 inflammasome, Metabolomics

Graphical Abstract



70 **Introduction**

71 The link between metabolic diseases and an increased risk of infection is a major issue in clinical
72 immunology[1]. Diabetes mellitus, characterized by long-lasting high blood sugar levels, is a
73 significant global health problem. It significantly weakens the immune system, making individuals
74 more vulnerable to serious, stubborn bacterial infections[2]. Among opportunistic pathogens, the
75 *Burkholderia cepacia* complex (Bcc), a group of tough, drug-resistant Gram-negative bacteria,
76 poses a serious risk to individuals with weakened immune systems and metabolic issues[3].
77 Although it is well known that systemic metabolic problems lead to worse infection outcomes, the
78 exact ways in which high blood sugar affects a host cell's ability to deal with a pathogen such as
79 *B. cepacia* are still not fully clear. Recent advances in immunometabolism have shown that
80 effective immune responses depend on important shifts in cellular energy processes[4]. When host
81 cells encounter a bacterial pathogen, they often undergo rapid metabolic changes, shifting from
82 oxidative phosphorylation to aerobic glycolysis, resembling the Warburg effect[5]. This metabolic
83 change enables rapid ATP production and the buildup of key metabolic intermediates such as
84 succinate[6]. These intermediates serve as secondary messengers that promote the production of
85 pro-inflammatory cytokines and reactive oxygen species (ROS)[7]. Mitochondria act as the main
86 center for this immunometabolic response[8]. In addition to their usual role as the powerhouse of
87 the cell, mitochondria play a key role in regulating innate immunity[9]. They achieve this by
88 fundamentally changing the tricarboxylic acid (TCA) cycle. Instead of generating ATP, the cycle
89 is intentionally disrupted to gather specific metabolites that serve as inflammatory signaling
90 molecules[10]. For instance, accumulated succinate stabilizes hypoxia-inducible factor 1-alpha
91 (HIF-1 α)[11]. This stabilizes HIF-1 α , which drives the production of pro-inflammatory cytokines,
92 such as IL-1 β . Exported citrate is used for lipid and prostaglandin synthesis[12]. In addition to this
93 metabolic shift, mitochondria play a role in the immune response by changing their structure. They
94 shift towards fragmented fission to manage cellular stress responses[13]. Moreover, they actively

95 produce mitochondrial reactive oxygen species (mtROS) to support bactericidal activity and
96 inflammasome activation[14]. During a controlled immune response, a short burst of mtROS is
97 crucial for killing bacteria and activating the NLRP3. This activation drives the maturation of
98 interleukin (IL-1 β)-1 β and interleukin (IL-18)[15]. However, this process requires tight control
99 by the cell's antioxidant defense systems[16]. We believe that hyperglycemia disrupts this delicate
100 homeostasis, placing the mitochondria in a state of metabolic gridlock that the cell cannot resolve
101 during active infection. When cells are exposed to consistently high glucose levels, their baseline
102 metabolic load increases[17]. This increases basal ROS production and forces reliance on
103 metabolic pathways other than carbohydrate metabolism[18].

104 *Burkholderia cepacia* is an adaptable, multidrug-resistant, opportunistic pathogen. These
105 pathogens can cause serious and often deadly systemic and respiratory infections in individuals
106 with weakened immune systems, especially those with chronic metabolic disorders, such as
107 diabetes mellitus[19]. Understanding the cellular processes that cause this vulnerability to infection
108 is important for developing new therapies that target the host. This study directly supports the
109 United Nations Sustainable Development Goal 3 (SDG 3), which seeks to fight infectious diseases
110 and lower early deaths from conditions such as diabetes[20]. Intracellular pathogens often alter
111 host lipid droplets and fatty acid oxidation pathways to gain essential nutrients and escape immune
112 detection[21]. However, the mechanism by which *B. cepacia* conducts lipid hijacking inside a host
113 cell with altered metabolism due to pre-existing hyperglycemia remains largely unknown.
114 Additionally, intracellular bacteria actively compete with the host for central carbon metabolites
115 and energy sources[22]. When *B. cepacia* infects hyperglycemic stressed cells, the struggle for
116 resources quickly drains the host's remaining energy reserves[23]. This leads to a significant crisis
117 in cellular energy levels. We hypothesized that pre-existing hyperglycemia places host
118 mitochondria in a state of metabolic gridlock during active infection. As the TCA cycle breaks
119 down and antioxidant defense enzymes, such as superoxide dismutase and glutathione peroxidase,

are depleted, this uncontrolled oxidative damage leads to severe mitochondrial depolarization and a harmful cytokine storm. To investigate this hypothesis, we used K562 cells. This model is reliable and has been validated for studying metabolic and inflammatory responses in human myeloid lineages[24]. To investigate this, we aimed to map immunometabolic changes during *B. cepacia* infection under both normoglycemic and hyperglycemic conditions. By combining untargeted metabolomics, targeted TCA cycle analysis, multiparametric flow cytometry, and cytokine profiling, this study aimed to provide a clear understanding of how high glucose levels worsen intracellular oxidative stress, disrupt mitochondrial function, and activate the inflammasome pathway.

Methods

Study Design

An *in vitro* infection cell line model was used to evaluate the effects of glucose availability on host cell immunometabolic remodeling, mitochondrial dysfunction, and inflammatory responses during *Burkholderia cepacia* infection. Four experimental conditions were analyzed: normoglycemic control (NG), hyperglycemic control (HG), infection under normoglycemia (ING), and infection under hyperglycemia (IHG). NG was defined as 5.5 mM (~99 mg/dL) glucose and HG as 15 mM (~270 mg/dL) glucose to establish physiological conditions similar to those in people with diabetes.

Cell line and culture conditions

K562 cells (ATCC CCL-243) were maintained at 37 °C in a Heracell™ VIOS 160i CO₂ Incubator (Thermo Fisher Scientific) with 5% CO₂. Cells were cultured in RPMI-1640 no-glucose base medium with L-glutamine (#cat no.- AT150 (Himedia) supplemented with 10% Fetal Bovine Serum (South American origin, #cat no.- RM9955, Himedia) and 1X Antibiotic-Antimycotic (100X stock, #cat no.- 15240062, Gibco™). D-(+)-Glucose (#cat no Osaka, Ltd., Pure Chemical

Industries, Japan) G7021 (Sigma-Aldrich) was added to the RPMI-1640 no-glucose base medium to generate NG (5.5 mM) and HG (15 mM) media for all downstream experiments.

Bacterial strain and growth kinetics

Burkholderia cepacia ATCC 25416 was used in all experiments. Bacterial cultures were streaked and grown in a 37 °C incubator (Span Automation) on *Burkholderia cepacia*-specific agar (BCSA; #cat no.- MU2089, Himedia), containing casitose (10 g/L), lactose (10 g/L), sucrose (10 g/L), sodium chloride (5 g/L), yeast extract (1.5 g/L), phenol red (0.08 g/L), crystal violet (0.002 g/L), and agar (14 g/L), supplemented with BCSA selective supplement (#cat no. FD361 (Himedia) containing polymyxin B (60000 IU/mL), gentamycin (100 µg/mL), and vancomycin (250 µg/mL)). Then, a single colony was picked and transferred to LB liquid medium (#cat no.- G1245, Himedia) in a 37 °C incubator (Span Automation) until the A₆₀₀ reached 0.6-0.8. To guide infection timing and inoculum selection, bacterial growth kinetics were assessed by monitoring the culture turbidity (optical density at 600 nm) over time. Growth curves were generated under the indicated media conditions (LB broth, NG-RPMI-1640 medium, and HG-RPMI-1640 medium) with OD₆₀₀ values of 0.01, 0.02, and 0.05. OD₆₀₀ was measured using a Varioskan™ LUX Multimode Microplate Reader (Thermo Fisher Scientific) to measure bacterial growth in these cultures.

Cell Viability Assays

Trypan Blue Dye-exclusion Assay

The Trypan Blue dye exclusion assay was used to assess infection-associated cytotoxicity over time under normal glucose (NG) and high glucose (HG) conditions. Cells were seeded at 1×10⁵/mL in tissue-culture-treated, flat-bottom 96-well plates (#P21024, Abdos). The absolute cell count per mL was measured over 0–48 h post-infection. At each time point, the cells were mixed with 0.4% Trypan Blue solution in DPBS (#cat no.- TCL046 (Himedia) at a 1:10 ratio, and

168 viable (dye-excluded) versus non-viable (dye-positive) cells were enumerated using a Neubauer
169 chamber to calculate the percentage viability relative to the appropriate uninfected control.

170 **MTT Assay**

171 Cell viability/metabolic activity was quantified using the Cell Proliferation Kit I (MTT) (#cat no.-
172 11465007001, Roche). For each condition, 100 μ L of 2×10^5 /mL cells were seeded and processed
173 as per the manufacturer's instructions, including MTT labelling and solubilization. Absorbance
174 was measured at 570 nm with reference/background measurement at 630 nm using a Varioskan™
175 LUX Multimode Microplate Reader (Thermo Fisher Scientific), and viability was calculated
176 relative to each matched, uninfected control.

177 **Global metabolomic profiling by GC–MS**

178 Global metabolomic profiling was performed using gas chromatography–mass spectrometry
179 (GC–MS) on a GCMS-QP2020 NX system (Shimadzu Corporation, Kyoto, Japan) equipped with
180 an SH-I-5Sil MS capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness). For each
181 experimental condition, 16×10^6 cells were harvested to ensure normalization by cell number
182 prior to metabolite extraction. Cell lysates were extracted using LC-MS-grade methanol
183 containing heptadecanoic acid (0.5 mg/mL in ethyl acetate) as an internal standard, followed by
184 oximation with hydroxylamine hydrochloride and trimethylsilyl derivatization using N,O-
185 bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS).
186 The derivatized extracts were transferred to GC vials for further analyses.

187 GC–MS analysis was performed in splitless mode with a 1 μ L injection volume. Helium was used
188 as the carrier gas at a constant column flow rate of 1.69 mL/min. The oven temperature was
189 initially held 75 °C for 4 min, ramped 280 °C 4 °C/min, and maintained at the final
190 temperature for 1.56 min. The injector, ion source, and transfer line temperatures were maintained
191 at 250, 230, and 290 °C, respectively. Mass spectra were acquired in electron ionization (EI)

192 mode over an m/z range of 40–600 with a solvent delay of 3 min. Metabolites were identified by
193 spectral matching against the NIST 2011 mass spectral library.

194 Metabolite peak areas were used for downstream statistical analyses after log₂ transformation.
195 Unsupervised principal component analysis (PCA) was performed to visualize intrinsic metabolic
196 variations and identify clustering patterns among the samples. Supervised partial least squares
197 discriminant analysis (PLS-DA) was employed to maximize group separation and identify
198 metabolites that contribute to differences between glucose conditions and infection status.
199 Variable importance in projection (VIP) scores were calculated, and metabolites with VIP scores
200 >1.0 were considered major contributors to the discrimination between groups. All statistical
201 analyses were performed using R (v4.3.0) with the mixOmics package for multivariate analysis
202 and ggplot2 for visualization.

203 The functional interpretation of significantly altered metabolites was performed using
204 MetaboAnalyst (v6.0), with pathway mapping based on the Kyoto Encyclopedia of Genes and
205 Genomes (KEGG) database. Pathway enrichment analysis was conducted using the
206 hypergeometric test, and pathway topology analysis employed relative betweenness centrality.
207 Pathways with an adjusted P -value <0.05 were considered significantly enriched.

208 **Targeted metabolic measurements**

209 Intracellular lactate and pyruvate levels were quantified, and the lactate-to-pyruvate ratio (L/P
210 ratio) was calculated. Tricarboxylic acid (TCA) cycle intermediates were quantified to assess
211 infection-associated perturbations in mitochondrial metabolism, including citrate depletion and
212 succinate accumulation patterns observed under infected conditions. Targeted measures were
213 compared across the four groups.

214 **RNA extraction and Quantitative real-time PCR (qRT-PCR)**

215 Cells were washed twice with cold Dulbecco's phosphate-buffered saline without phenol red,
216 calcium, and magnesium (#cat no.- TL1006, Himedia) and harvested using an appropriate volume

217 of RNAiso Plus (#cat no.- 9109, Takara Bio Inc.). Chloroform was then added to 1/5 of the
218 RNAiso Plus volume, and the mixture was centrifuged at 13000 rpm for 10 min at 4 °C using a
219 Sorvall ST-8R (Thermo Fisher Scientific). Post-centrifugation, the aqueous layer was transferred
220 to a separate RNase-free tube, and isopropanol was added to 1/2 the volume of RNAiso Plus
221 reagent. The mixture was centrifuged at 13000 rpm for 10 min at 4 °C. After removing the
222 supernatant, 500 µL of 70% ethanol was added, and the mixture was centrifuged at 7000 rpm for
223 10 min at 4 °C. The supernatant was discarded and air-dried, followed by dissolving the pellet in
224 non-DEPC-treated nuclease-free water (#cat no.- AM9938, Invitrogen™). The concentration of
225 isolated RNA samples was checked using a NanoDrop One Microvolume UV-Vis
226 Spectrophotometer, and purity was confirmed by measuring A_{260/280} and A_{260/230} ratios, where an
227 A_{260/280} value of ~2 and an A_{260/230} ratio between 2.0 and 2.2 were considered for cDNA synthesis.
228 ABScript II cDNA First-Strand Synthesis Kit (#cat no.- RK20400 (Abclonal) was used for cDNA
229 synthesis according to the manufacturer's protocol. Primers for qRT-PCR were designed using
230 Primer-BLAST and Primer3 software, with sequences provided in **Supplementary Table S1**.
231 Genious 2X SYBR Green Fast qPCR Mix (#cat no. RK21204 (Abclonal) was used to perform
232 qRT-PCR experiments to measure relative gene expression levels, normalized to glyceraldehyde
233 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene on a Bio-Rad CFX Opus 96
234 instrument and acquired using Bio-Rad CFX Maestro software. The thermal profile was as
235 follows: 95 °C for 2 min, followed by 40 cycles at 95 °C for 10 s, 58 °C for 20 s, and 72 °C for
236 30 s, followed by a concluding melt curve analysis (65 °C to 95 °C with increments at 0.5 °C for
237 5 s at each point). Relative gene expression was analyzed using the $\Delta\Delta C_t$ method.

238 **Mitochondrial fluorescence imaging**

239 Mitochondrial membrane potential and mass were assessed by fluorescence microscopy using
240 MitoTracker Red FM and 10-N-Nonyl Acridine Orange (NAO), respectively, with Hoechst 33342
241 used as a nuclear counterstain. Following the respective experimental treatments, the cells were

242 incubated with 75 nM MitoTracker Red FM, 50 nM NAO, and 3 µg/mL Hoechst 33342 under
243 optimized staining conditions. Excess dye was removed by washing with phosphate-buffered
244 saline (PBS), and the stained cells were imaged immediately using an upright fluorescence
245 microscope. Fluorescence images of representative fields were analyzed using ImageJ software
246 (National Institutes of Health, Bethesda, MD, USA).

247 **Flow Cytometry Analysis**

248 Flow cytometry was performed to assess mitochondrial mass, mitochondrial membrane potential,
249 and cytoplasmic and mitochondrial reactive oxygen species (ROS) levels post-infection. Cells
250 were washed twice with DPBS and stained with LIVE/DEAD™ Fixable Near-IR (1:1000,
251 #L10119, Invitrogen) for 30 min at room temperature in the dark and then washed twice with
252 PBS+2% FBS. Subsequently, each group was stained with cell-permeant 2',7'-
253 dichlorodihydrofluorescein diacetate (H₂DCFDA) (10 µM, #D399, Invitrogen) for cytosolic ROS
254 measurement, MitoSOX™ Mitochondrial Superoxide (2.5 µM, #M36005, Invitrogen) for
255 mitochondrial ROS, and JC-1 Dye (2 µM, #T3168, Invitrogen) to measure mitochondrial
256 membrane potential, followed by incubation for 20-30 min at 37 °C in the dark. Cells were then
257 washed twice with PBS, acquired on a BD FACSCanto™ II Flow Cytometry System using
258 FACSDiva software version 8.0 (BD Biosciences), yielding a minimum of 100000 total events
259 for each sample, and analyzed using FlowJo™ v10 software. All flow cytometry experiments
260 were performed in independent biological replicates (n = 3 per group). Appropriate unstained cells
261 and positive controls were used to set the gates for each assay. The positive controls were as
262 follows: heat-killed cells for LIVE/DEAD™ Fixable Near-IR, hydrogen peroxide for H₂DCFDA,
263 Antimycin A for MitoSOX, and FCCP for JC-1 dye. Flow cytometry analysis was performed
264 using a uniform sequential gating strategy for all samples. Events were first inspected on a time
265 vs. FSC-A plot to exclude periods of flow instability, followed by the selection of the cell
266 population on FSC-A vs. SSC-A to remove debris. Singlets were then identified using FSC-A

267 versus FSC-H, and viable cells were defined as the LIVE/DEAD near IR negative population,
268 with the threshold set by employing a heat-killed positive control, which shows live cells as dim
269 and dead cells as a bright population owing to amine reactive dye uptake (**Supplementary Figure**
270 **S2**). Within the viable population, functional readouts were quantified as the geometric mean
271 fluorescence intensity (gMFI) and/or the frequency of stain-positive cells using gates set from the
272 unstained and single-stained controls. For JC-1, mitochondrial depolarization was defined as the
273 $J_{\text{aggregate}}$ (red) to J_{monomer} (green) ratio using an FCCP-treated positive control gate.

274 **Protein extraction and immunoblotting**

275 Post-infection, cells were washed with $1 \times$ DPBS and lysed with the appropriate volume of
276 radioimmunoprecipitation assay (RIPA) buffer (#Cat no.- TCL161 (Himedia) along with Pierce
277 protease and phosphatase inhibitor (#Cat no.- A32959 (Thermo Fisher Scientific) and sonicated,
278 followed by centrifuging at 10000 rpm for 15 min at 4 °C. After discarding the pellet, the total
279 protein content in the supernatant was quantified using the Pierce™ BCA Protein Assay kit (#cat
280 no.- 23225 (Thermo Fisher Scientific) according to the manufacturer's protocol. Total protein (30
281 µg) was normalized throughout all groups, and $4 \times$ Bolt™ LDS Sample Buffer (#cat no.- B0008,
282 Invitrogen) and 10X Bolt™ Sample Reducing Agent (#cat no.- B0009, Invitrogen) were added,
283 followed by resolution on a 12% Bis-Tris gel and a 16% Tricine gel (for low-molecular-weight
284 proteins). Resolved proteins were transferred to Immobilon® -P PVDF Membranes (#cat no.-
285 IPVH08100, Millipore) and probed with primary antibodies against mitochondrial proteins such
286 as OPA1 (1:1000, #80471, Cell Signaling Technology), OMA1 (1:1000, #95473, Cell Signaling
287 Technology), DRP1 (1:1000, #8570, Cell Signaling Technology), Mitofusin-1 (1:1000, #14739,
288 Cell Signaling Technology), AFG3L2 (1:1000, #A26340, Abclonal), PGC1α (1:1000, #A12348,
289 Abclonal), inflammatory signaling pathway markers such as mTOR (1:1000, #2983, Cell
290 Signaling Technology), phospho-mTOR (Ser2448) (1:1000, #5536, Cell Signaling Technology),
291 Akt (1:1000, #9272, Cell Signaling Technology), phospho-Akt (Ser473) (1:2000, #4060, Cell

292 Signaling Technology), NLRP3 (1:2000, #A28144, Abclonal), Caspase-3 (1:1000, #14220, Cell
293 Signaling Technology), and β -actin (1:10000, #AC026, Abclonal). Membranes were blocked with
294 5% non-fat milk for 2 h, washed with phosphate-buffered saline containing 0.1% Tween-20, and
295 incubated with primary antibodies overnight at 4 °C. After incubation, the membranes were
296 incubated with HRP-conjugated Goat Anti-Rabbit IgG(H+L) (1:10000, #4030-05, Southern
297 Biotech) for 1.5 h, and bands were visualized using Clarity Western ECL Substrate (#1705061,
298 Bio-Rad). Densitometric analysis was performed using ImageJ software and normalized to β -actin
299 as a control. Original uncropped chemiluminescent images for all immunoblots are provided in
300 the Supplementary Material (**Supplementary Figures S3**).

301 **Enzyme-Linked Immunosorbent Assay (ELISA)**

302 Extracellular pro-inflammatory cytokines and intracellular oxidative stress markers were
303 measured using specific human sandwich ELISA kits. To assess cytokine release, we collected
304 K562 cell culture supernatants 3 h after infection. The collected samples were centrifuged at 300
305 $\times$ g for 5 min at 4 °C to eliminate non-adherent cells and debris. We quantified the levels of
306 interleukin-6 (IL-6) (Cat. #EH0201, FineTest), tumor necrosis factor-alpha (TNF- α) (Cat.
307 #EH0302, FineTest), interleukin-1 beta (IL-1 β) (Cat. #EH0185, FineTest), and interleukin-18 (IL-
308 18) (Cat. #EH0011, FineTest). To assess intracellular antioxidant capacity and lipid peroxidation,
309 we harvested, washed twice with cold DPBS, and lysed infected and uninfected cells. We then
310 analyzed the intracellular lysates to determine the concentration of total superoxide dismutase
311 (SOD) (Cat. #EH4706-1, FineTest), glutathione peroxidase 1 (GPX1) (Cat. #EH0826, FineTest),
312 glutathione synthetase (GSS) (Cat. #EH4071, FineTest), and catalase (Cat. #EH0643, FineTest),
313 and malondialdehyde (MDA) (Cat. #EH4174, FineTest). All ELISAs were conducted according
314 to the manufacturer's recommended protocols. Optical densities were measured using a
315 Varioskan™ LUX Multimode Microplate Reader (Thermo Fisher Scientific). We calculated the

absolute concentrations for all targets by interpolating from standard curves generated using the recombinant protein standards provided in each kit.

Statistical Analysis

All experiments were performed in at least three biological replicates, with data reported as the mean $\pm$ standard error of the mean (SEM), unless stated otherwise. Subsequent statistical analyses were performed using GraphPad Prism v.10. Group differences were analyzed using one-way or two-way ANOVA, followed by Tukey's multiple comparison test to determine the significance between the groups. The significance threshold was set at $\alpha = 0.05$, corresponding to a 95% confidence interval (CI). Significance is denoted as ns (non-significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

Results

Burkholderia cepacia growth kinetics and dose-dependent cytotoxicity

Determining the basic parameters of host-pathogen interactions under different metabolic conditions is a prerequisite for assessing subsequent cellular responses. The baseline growth of *Burkholderia cepacia* in cell-free environments was assessed by monitoring the optical density at 600 nm (OD₆₀₀). Growth curves were initiated with starting inocula of 0.01, 0.02, and 0.05 OD₆₀₀. These correspond to the infectious burden used in the subsequent host-cell assays. The experiments were conducted using Luria-Bertani (LB), normoglycemic (NG), and hyperglycemic (HG) complete RPMI-1640 media. The correlation coefficients obtained for the exponential growth model were high in all cases, reflecting strong bacterial adaptation. For starting inocula with OD₆₀₀ values of 0.01, 0.02, and 0.05, the generation times (G) in LB medium were 68.2 min ($R^2 = 0.9998$), 84.4 min ($R^2 = 0.9797$), and 76 min ($R^2 = 0.9904$), respectively. In NG medium, the generation times (G) were 82.1 min ($R^2 = 0.0008$), 94.9 min ($R^2 = 0.9688$), and 137 min ($R^2 = 0.9949$) for the corresponding MOIs. Likewise, in the HG condition, the generation times (G) for growth were 79.9 min ($R^2 = 0.9643$), 160.6 min ($R^2 = 0.9909$), and 137 min ($R^2 = 0.9921$) (Figure 1). Our host mammalian cell-free growth studies showed that *B. cepacia* replicates at

342 similar rates in normal and high-glucose environments. This means that the pathogen does not
343 gain an advantage from high glucose levels. Thus, the severe cytotoxicity observed during K562
344 infections under high-glucose conditions is likely due to the weakened metabolic resilience of the
345 host cells rather than an increased number of bacteria (**Figure 2**). Subsequent analysis of K562
346 cells with different multiplicities of infection (MOI of 1:5, 1:10, and 1:25) showed that *B. cepacia*
347 significantly affected the viability of host cells in a time- and dose-dependent manner from 0th to
348 48th hour. The cytotoxic effect was more pronounced with a higher inoculum of MOI 1:25 than
349 with MOI 1:10, which was consistently severe under both normoglycemic and hyperglycemic
350 conditions. This trend was strongly supported by both MTT metabolic activity assays (**Figure 2**)
351 and trypan blue exclusion assays (**Supplementary Figure S1**).

352 **Global Metabolic Segregation and Reprogramming**

353 A comprehensive assessment of the cellular metabolome is necessary to uncover the biochemical
354 basis of this extreme cytotoxicity. Principal Component Analysis (PCA) of the untargeted GC-MS
355 dataset revealed significant metabolic differences between *B. cepacia-infected* and uninfected K562
356 cells. This separation was largely driven by Principal Component 1 (PC1), which accounted for the
357 largest share of metabolic variance (29.4%). PC1 effectively separated the experimental groups
358 based on infection status. Principal Component 2 (PC2), the second axis capturing 18.1% of the
359 variance, further distinguished the profiles based on other experimental factors, such as different
360 glucose microenvironments (**Figure 3A**). The Variable Importance in Projection (VIP) values
361 revealed key amino acids (L-valine and glycine) and lipids (linoleyl acetate, hexadecanoic acid,
362 and ethyl carbamate) that contributed to this difference (**Figure 3B**). Class-level metabolite
363 profiling revealed a significant shift from central carbon metabolism to lipid and amino acid
364 metabolism. In the uninfected controls, fatty acids, identified as fatty acyls, accounted for 28% of
365 the metabolome under normal glucose (NG) conditions and 25% under high glucose (HG)
366 conditions. Amino acids, which fall under carboxylic acids and their derivatives, remained steady

367 at 9.4% under both conditions. After *B. cepacia* infection, the most important metabolic change
368 occurred in the amino acid pool. In normoglycemic cells, the relative abundance of amino acids
369 increased sharply from 9.4% to 22.2%, indicating a strong shift towards protein-driven metabolism.
370 However, this response was much weaker in the hyperglycemic environment, where amino acid
371 levels increased by only 14.6%. In contrast, infection led to an overall increase in fatty acids, but
372 HG cells showed a disproportionate lipid buildup, rising to 40% compared to 31.2% in NG cells.
373 This change was accompanied by a decrease in the levels of carbohydrate-related metabolites
374 **(Figure 4)**. Overall, these patterns indicate that the hyperglycemic state drives host cells to rely
375 excessively on lipids while attenuating the healthy, amino acid-rich metabolic response that
376 typically occurs during infection. Pathway enrichment analysis predicted that this shift would
377 radically affect mitochondrial and peroxisomal metabolism, focusing on enzymes involved in the
378 intracellular transport of fatty acids and β -oxidation **(Figure 5)**. To identify the specific functional
379 bottlenecks causing lipid accumulation, we conducted a targeted Metabolite Set Enrichment
380 Analysis (MSEA). This analysis helped us map the key metabolites to their corresponding enzymes
381 and transport systems within the cells. The results indicated severe dysfunctions, mainly in
382 mitochondria and peroxisomes **(Figure 6)**. In both the normoglycemic (ING) and hyperglycemic
383 (IHG) groups, the most notable functional sets included fatty acid transport, fatty-acid-CoA ligase
384 activity, and β -oxidation of long-chain fatty acids. Importantly, a hyperglycemic environment
385 exacerbated this issue. In the IHG group, enzymes involved in the carnitine shuttle, specifically
386 carnitine O-acetyltransferase and the mitochondrial carnitine-acetyl carnitine carrier, showed
387 significantly higher enrichment ratios and strong statistical relevance ($-\log_{10}(p) > 3.0$) than those in
388 the ING group. The carnitine shuttle is essential for transporting long-chain fatty acids into the
389 mitochondria for β -oxidation. Therefore, the predicted failure of these specific transport enzymes
390 explains the large 40% intracellular buildup of unoxidized lipids observed in hyperglycemic K562
391 cells **(Figure 5)**.

392 The systemic metabolic shift and possible organelle dysfunction subsequently prompted a focused
393 analysis of the central bioenergetic pathways. Principal Component Analysis (PCA) of the selected
394 TCA cycle intermediates confirmed that infection was the primary driver of metabolic variation.
395 Specifically, Principal Component 1 (PC1), the main axis that accounts for the largest variance in
396 the dataset, explained 55.1% of the total variance and effectively separated the groups by infection
397 status. Principal Component 2 (PC2), the orthogonal axis capturing the second largest variance,
398 explained 31.5% of the separation, further separating the cells based on secondary metabolic
399 changes, such as the glucose microenvironment (**Figure 7**). Infection mainly caused this metabolic
400 difference. However, this effect was further enhanced in a hyperglycemic environment. In terms of
401 magnitude, infection with *B. cepacia* resulted in severe mitochondrial gridlock, characterized by a
402 substantial reduction in intracellular citrate ($p < 0.001$) and a sudden accumulation of alpha-
403 ketoglutarate, fumarate, and malate. This was accompanied by an exponential increase in succinate
404 accumulation ($p < 0.0001$) (**Figure 8**). This drastic reduction in forward TCA cycle flux indicates
405 a severe metabolic bottleneck. Although these targeted intermediate profiles do not directly
406 measure electron transfer capabilities, this gridlock strongly correlates with the pathway enrichment
407 for redox stress that we previously observed in untargeted metabolomic analyses (**Figure 5**). This
408 suggests a significant disruption in the cellular redox balance. The infected cells showed a drastic
409 reduction in intracellular pyruvate concentration ($p < 0.0001$) and a substantial increase in the
410 lactate-to-pyruvate ratio ($p < 0.01$ in NG and $p < 0.0001$ in HG groups) (**Figure 9**). This elevated
411 L/P ratio indicates a problem with oxidative phosphorylation and suggests the presence of
412 pseudohypoxic stress[25]. However, to confirm this change in the intracellular redox state, targeted
413 LC-MS/MS profiling of the NAD⁺/NADH couple is required in the future.

414 **Hyperglycemia worsens infection-related mitochondrial depolarization and remodeling**

415 Fluorescence microscopy using Hoechst 33342, nonyl acridine orange (NAO), and MitoTracker
416 Red showed that *B. cepacia* infection caused significant mitochondrial depolarization without

417 completely losing mitochondrial mass. NAO staining remained partially intact, whereas
418 MitoTracker Red intensity decreased sharply (**Figure 10**). This was validated by quantifying the
419 mitochondrial membrane potential ($\Delta\psi_m$) using JC-1 flow cytometry (**Supplementary Figure**
420 **S2**). Uninfected NG cells exhibited healthy polarization, with 56.0% JC-1 monomers and 43.5%
421 aggregates. After infection (ING), the population changed significantly to 93.1% monomers and
422 only 6.34% aggregates. Hyperglycemia worsened this drop, as the hyperglycemic group with
423 infection had 95.1% monomers and only 4.21% aggregates. This was reflected by a significant
424 decrease in the red/green median fluorescent intensity (MFI) ratio ($p < 0.0001$) (**Figure 11**). This
425 significant loss of membrane potential is closely associated with major changes in mitochondrial
426 dynamics, quality control, and production. Immunoblotting showed that the fragmentation
427 mediator DRP1 and the stress-activated inner-membrane protease OMA1 were upregulated by
428 more than three times in the IHG group compared to the NG controls ($p < 0.001$) (**Figure 12**;
429 **uncropped blots provided in Supplementary Figure S3**). In contrast, the host cell's ability to
430 fuse mitochondria was significantly impaired, as indicated by a significant decrease in the outer-
431 membrane fusion protein MFN1 ($p < 0.0001$). Additionally, the expression of the crucial matrix
432 quality-control protease AFG3L2 was significantly reduced under hyperglycemic infection,
433 suggesting a severe collapse of mitochondrial protein balance. Importantly, this excessive
434 structural fragmentation occurred alongside a complete halt in mitochondrial renewal. The
435 expression of PGC-1 α , the main coactivator of mitochondrial biogenesis and oxidative
436 metabolism, was significantly reduced, showing approximately a 50% decrease in the IHG group
437 compared to the controls ($p < 0.01$) (**Figure 12**). Overall, these findings indicate that the
438 hyperglycemic infection environment traps host cells in a state of irreversible mitochondrial
439 failure, marked by unchecked DRP1-mediated fragmentation, loss of fusion and quality-control
440 mechanisms, and a complete halt in PGC-1 α -driven mitochondrial biogenesis.

441 **Depletion of Antioxidant Defenses and Combined Oxidative Damage**

Flow cytometric co-staining with MitoSOX™ Red (targeting mitochondrial superoxide) and H₂DCFDA (targeting cytosolic ROS) showed that the loss of metabolic function caused significant oxidative stress (**gating strategy shown in Supplementary Figure S2**). Uninfected cells maintained normal ROS levels, whereas infected cells exhibited a marked increase in double-positive populations (MitoSOX⁺ / DCFDA⁺), indicating elevated levels of both mitochondrial superoxide and cytosolic ROS (**Figure 13A-C**). ELISA quantification showed that hyperglycemia reduced the host antioxidant capacity during infection. While total Superoxide Dismutase (SOD), Glutathione Peroxidase 1 (GPX1), and catalase levels increased in the infected normoglycemic group as a compensatory defense, their concentrations were significantly lower in the infected hyperglycemic group ($p < 0.0001$). This antioxidant failure directly causes lipid peroxidation, which is measured by malondialdehyde (MDA) concentration. MDA levels increased from approximately 2,000 ng/mL in uninfected normoglycemic controls to approximately 10,000 ng/mL in infected normoglycemic cells, peaking at nearly 14,000 ng/mL in the infected hyperglycemic group ($p < 0.0001$) (**Figure 13D-H**).

Hyper-activation of the inflammasome and systemic cytokine storm

First, the transcriptional profiles of key inflammatory mediators were evaluated to determine whether localized mitochondrial damage and oxidative stress led to a systemic immune response. Quantitative RT-PCR revealed a significant increase in the activity of the NF- κ B signaling pathway and NLRP3 inflammasome after *B. cepacia* infection. While cells infected with normal sugar levels responded strongly, high sugar levels significantly worsened the response. The mRNA levels of NF- κ B1 and NLRP3 were much higher in the infected hyperglycemic (IHG) group than in the normoglycemic infected (ING) group ($p < 0.0001$) (**Figure 14A, B**). As a result, the downstream targets of NF- κ B, such as TNF- α and IL-6, showed dramatic increases of 60-fold and 45-fold, respectively, in the IHG group (Figure 14C, D). Similarly, the levels of

466 inflammasome-dependent precursors IL-1 β and IL-18 were greatly elevated under high-sugar
467 conditions ($p < 0.0001$) (**Figure 14E, F**).

468 This strong transcriptional priming led to a significant cytokine storm at the protein level in the
469 present study. The levels of key NF- κ B drivers increased in the extracellular space. For instance,
470 IL-6 levels increased from approximately 500 pg/mL to 1,300 pg/mL in ING cells, while they
471 increased to over 3,000 pg/mL in IHG cells ($p < 0.0001$) (**Figure 15A**). TNF- α levels also showed
472 a similar increase during hyperglycemic infection ($p < 0.0001$) (**Figure 15B**). Moreover, oxidative
473 damage to mitochondria leads to robust assembly and activation of the NLRP3 inflammasome.
474 The release of mature inflammasome-dependent cytokines IL-1 β and IL-18 peaked in the IHG
475 group ($p < 0.0001$) (**Figure 15C-D**). Immunoblot densitometry further confirmed this activation,
476 showing a significant increase in NLRP3 protein levels ($p < 0.01$) and active caspase-3 cleavage
477 ($p < 0.001$) (**Figure 16A-C; uncropped blots are provided in Supplementary Figure S3**).

478 1. Discussion

479 The association between metabolic diseases and the body's capacity to combat infections reveals
480 a notable deficiency in the innate immune response[26]. Diabetes mellitus impairs the immune
481 system[27]. However, the exact intracellular processes underlying this effect remain unclear. This
482 study offers a new understanding of how a high-sugar environment worsens immunometabolic
483 failure and inflammatory imbalance caused by *Burkholderia cepacia* infection. This bacterium is
484 a recognized risk factor for patients with diabetes[28]. The existing literature shows that cells
485 cultured under normal glucose levels can mount a temporary defense against early metabolic
486 stress[29]. Our data demonstrate, for the first time, that the pre-existing energy strain from high
487 glucose levels traps the host in severe mitochondrial failure, ongoing oxidative stress, and a
488 chaotic inflammatory response during *B. cepacia* infection.

489 Before investigating downstream intracellular signaling pathways, it is important to separate
490 active host responses from the passive effects of bacterial overgrowth or late-stage cell death[30].

491 In our model, growth curve analyses showed that *B. cepacia* replicates at similar rates under
492 normoglycemic and hyperglycemic conditions (**Figure 1**). At the primary 3-hour post-infection
493 timepoint, host cell viability remained largely intact (**Figure 2 and Supplementary Figure S1**).
494 This supports the idea that the significant mitochondrial depolarization, TCA cycle blockade, and
495 cytokine storms identified in this study are genuine immunometabolic effects of host-pathogen
496 interactions, not merely signs of cell death. Intracellular pathogens can rapidly alter host cell
497 processes to create a niche for replication; however, the drop in host cell viability we observed at
498 24 and 48 hours highlights the harmful trajectory this manipulation takes once early antioxidant
499 and metabolic defenses are exhausted[31].

500 Initiating this serious trajectory, our untargeted metabolomic profiling revealed significant
501 changes in the host central carbon metabolism during early stage infection (**Figure 3**). This change
502 involved a clear shift from carbohydrate to lipid and amino acid utilization (**Figure 4**). Research
503 has shown that intracellular pathogens, such as *Burkholderia* species, manipulate host lipid
504 droplets and fatty acid oxidation (FAO) pathways to obtain the necessary nutrients[32]. Under
505 normal glucose levels, immune cells can adapt their metabolism to manage these changes[33].
506 However, we demonstrated that high glucose levels significantly limited the ability to adapt.
507 Pathway enrichment analysis revealed notable increases in fatty acid transport, β -oxidation of
508 long-chain fatty acids, and amino acid metabolism (**Figures 5 and 6, respectively**). Rather than
509 indicating successful lipid oxidation, the large accumulation of fatty acids in the infected
510 hyperglycemic (IHG) group suggests that high glucose levels provide an excess of metabolic
511 substrates that infected cells struggle to metabolize. Such severe imbalances in substrates are
512 known to cause lipotoxicity[34]. Therefore, our findings indicate that this imbalance leads to
513 metabolic stagnation in the IHG group.

514 Mechanistically, this global bioenergetic standstill is due to a significant physical blockage in the
515 mitochondrial tricarboxylic acid (TCA) cycle. This cycle typically acts as the central control point

516 of mammalian cellular metabolism[35]. Our focused analysis showed a drastic decrease in
517 intracellular citrate levels and a rapid and massive increase in succinate levels in the infected
518 groups (**Figure 8**). Existing research highlights that exporting and using citrate is vital for
519 maintaining metabolic balance, and its depletion indicates a serious metabolic slowdown[36,37].
520 While an increase in succinate is a known sign of immunometabolic changes and
521 inflammation[38,39], our findings indicate that under hyperglycemic conditions, this switch to
522 alternative substrates ultimately fails to restore ATP production and worsens lipid accumulation
523 within cells. Because forward flow in the TCA cycle is impaired, downstream intermediates are
524 produced via alternative anaplerotic pathways[40–42]. Our observations of succinate
525 accumulation showed both increased anaplerotic carbon input and reduced mitochondrial use. In
526 addition to being a simple metabolic intermediate, research shows that succinate acts as a potent
527 immunometabolic signaling molecule. It stabilizes HIF-1 α and boosts IL-1 β production[43,44].
528 Similarly, we found that the infected cells had reduced pyruvate oxidation and a significantly
529 higher lactate-to-pyruvate ratio (L/P) (**Figure 9**). While a high L/P ratio usually indicates severe
530 pseudohypoxic stress[45], reflecting the known Warburg-like shift in activated immune cells[46],
531 our data show that hyperglycemic cells cannot effectively resolve this shift. Instead, the ongoing
532 disruption of TCA cycle flow, constant citrate depletion, and increasing succinate accumulation
533 suggest that hyperglycemia pushes infected host cells beyond adaptive metabolic changes into a
534 state of extreme bioenergetic fatigue. To fully understand these complex interactions, future
535 isotopic tracing studies using ¹³C-labeled nutrients will be crucial for accurately tracking the
536 interrupted fluxes in the TCA cycle during active infections.

537 This biochemical gridlock in the mitochondrial matrix leads to severe structural and electrical
538 failure throughout the organelle network. In this study, fluorescence microscopy and flow
539 cytometry-based JC-1 assay revealed that *B. cepacia* infection induced widespread mitochondrial
540 depolarization prior to the complete loss of mitochondrial mass (**Figures 10-11 and**

541 **Supplementary Figure S2).** We found that hyperglycemia worsens functional failure by
542 disrupting the three interconnected components of mitochondrial homeostasis: biogenesis,
543 dynamics, and quality control (**Figure 12**). First, our study identified complete suppression of
544 PGC-1 α -driven mitochondrial biogenesis during *B. cepacia* infection (**Figure 12G and 12I**). This
545 collapse is closely linked to the altered redox state observed earlier (**Figure 8**). The significantly
546 increased L/P ratio (**Figure 9**) may serve as a clear indicator of severe NAD⁺ depletion inside the
547 cells. As the metabolic sensor SIRT1 requires NAD⁺ to deacetylate and activate PGC-1 α , the
548 NAD⁺ shortage caused by the infection may essentially shut down the entire SIRT1-PGC-1 α
549 signaling pathway[47]. Consequently, the sharp drop in PGC-1 α during hyperglycemic infection
550 leaves the host cell unable to produce new, healthy organelles to remove stored lipids or restore
551 normal flow through the TCA cycle. Second, this failure to renew the network puts the existing
552 mitochondria under extreme changes in their structural dynamics. We observed a marked increase
553 in the fission mediator DRP1 and the stress-activated inner-membrane protease OMA1, along
554 with a simultaneous breakdown of the opposing fusion machinery, MFN1 (**Figure 12A-F and**
555 **Supplementary Figure S3**). While DRP1-induced fragmentation is known to cause energy
556 failure in the literature[48], our data show that this excessive fission traps the remaining
557 mitochondria of the host cell in a permanently fragmented and depolarized state. Finally, this
558 structural failure is exacerbated by a complete breakdown of mitochondrial protein quality
559 control, as evidenced by the significant loss of the key matrix protease AFG3L2 (**Figure 12G-H**
560 **and Supplementary Figure S3**). Without effective quality control to manage or remove these
561 damaged fragments, the organelle network approaches a state of critical functional collapse.
562 The physical fragmentation of the mitochondrial network and the loss of membrane potential
563 severely disrupt the electron transport chain. This leads to significant electron leakage[49]. We
564 confirmed this by observing a large increase in both mitochondrial superoxide (quantified using
565 MitoSOX™ Red) and cytosolic reactive oxygen species (quantified using H₂DCFDA) (**Figure**

566 **13A-C and Supplementary Figure S2).** Previous studies have shown that a temporary burst of
567 ROS is necessary for effective bactericidal activity[50]. However, our data indicate that the high-
568 glucose environment completely undermined the host's ability to manage this oxidative burst. The
569 hyperglycemic-infected group experienced a significant drop in key antioxidant enzymes,
570 including SOD, catalase, GPX1, and GSS (**Figure 13D-G**). The simultaneous loss of glutathione
571 synthetase (GSS) explains this decline, as the cells can no longer produce the glutathione needed
572 to maintain peroxidase activity[51]. While uncontrolled lipid peroxidation is known to produce
573 malondialdehyde (MDA) as a final marker of membrane damage[52], our tests confirmed a
574 significant, harmful accumulation of MDA in these affected cells (**Figure 13H**).

575 Ultimately, uncontrolled oxidative fallout directly triggers severe inflammatory response. Our
576 RT-qPCR and ELISA tests showed significant transcriptional priming of the NF- κ B signaling
577 pathway and NLRP3 inflammasome (**Figure 14**). This leads to a cytokine storm (**Figure 15**).

578 While high levels of mitochondrial ROS and the release of oxidized mitochondrial DNA are well-
579 known triggers for NLRP3 inflammasome assembly in existing research[53–55], our findings
580 expand on this understanding by showing the hyperphosphorylation of Akt and mTOR under high
581 sugar conditions (**Figure 16D-G and Supplementary Figure S3**). Research has shown that
582 overactive Akt/mTOR signaling reduces the activation thresholds of inflammatory pathways,
583 driving premature hyperactivation[56,57]. Therefore, our data indicate that a high-glucose
584 environment prepares the body for a heightened inflammatory response during *B. cepacia*
585 infection[58]. This ultimately resulted in the robust assembly of caspase-3 (**Figure 16A-C and**
586 **Supplementary Figure S3**) and substantial secretion of IL-1 β and IL-18.

587 By mapping this pathway from excess nutrients to cytokine release, our targeted and untargeted
588 metabolomic data provide novel direct biochemical evidence of ongoing immunometabolic
589 failure. The decrease in citrate levels and the accumulation of succinate indicate a disrupted
590 mitochondrial network. Along with a significant increase in lipid accumulation within cells, our

findings show that hyperglycemia not only increases reliance on lipid sources but also hinders their effective breakdown. This, in turn, drives the overactivation of the NLRP3 inflammasome and triggers a harmful cytokine storm.

These findings emphasize the importance of mitochondrial health in maintaining a balanced immune response. Targeting mitochondrial reactive oxygen species, restoring mitochondrial function, and improving metabolic flexibility are potential treatment strategies for this condition. Future experiments with mitochondria-targeted antioxidants, such as MitoQ, to specifically reduce mitochondrial reactive oxygen species[59], or with NAD⁺ precursors, such as nicotinamide riboside, to reactivate the SIRT1-PGC-1 α production pathway[60], will be important. These experiments will help determine whether metabolic issues can be separated from excessive inflammasome activation. Finally, it is crucial to test these *in vitro* changes in a living system. This shift is particularly urgent because of the growing threat of the *Burkholderia cepacia* complex, a serious hospital-acquired infection[3], with a clinical severity similar to that of *Burkholderia pseudomallei* (melioidosis)[61]. As we have previously pointed out, there are gaps in the understanding of host-pathogen interactions during *Burkholderia* sp. infections[62], and closing the divide between molecular evasion strategies and systemic metabolic failure is vital. Therefore, future research should use established diabetic mouse models, such as *db/db* mice (*lepr^{db}*), to test the targeted inhibitors of mitochondrial dynamics, such as Mdivi-1. Researchers must determine whether halting excessive hyperfission can restore systemic immune balance and ultimately reduce infection-related mortality in metabolically weakened hosts.

2. Conclusion

In conclusion, this study shows that hyperglycemia significantly weakens the natural immune response against *Burkholderia cepacia* by causing profound immunometabolic reprogramming. The basal metabolic stress imposed by high glucose synergizes with the intracellular bacterial burden to disrupt tricarboxylic acid (TCA) cycle flux, cause severe mitochondrial fission, and

616 rapidly deplete the body's antioxidant supply. Integrating untargeted and targeted metabolomic
617 analyses, our findings demonstrate that infection promotes metabolic rewiring, characterized by
618 the enrichment of lipid and amino acid metabolic pathways, intracellular citrate depletion, and
619 succinate accumulation, indicating impaired mitochondrial carbon metabolism and altered
620 substrate utilization in hyperglycemic conditions. This structural and functional failure leads to
621 an uncontrolled buildup of mitochondrial reactive oxygen species (mtROS) and succinate,
622 triggering excessive activation of the NLRP3 inflammasome and harmful inflammatory response.
623 Our findings highlight the key role of mitochondrial balance in determining the outcome of
624 infection. They suggest that improving the body's metabolic flexibility through targeted
625 scavenging of mtROS or drug-based mitochondrial restoration could be an effective way to reduce
626 severe bacterial infections in individuals with diabetes.

627 **List of abbreviations**

628 AFG3L2, AFG3 Like Matrix AAA Peptidase Subunit 2; Akt, Protein Kinase B; ANOVA,
629 Analysis of Variance; BCA, Bicinchoninic Acid; Bcc, *Burkholderia cepacia* complex; BCSA,
630 *Burkholderia cepacia*-specific agar; BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; cDNA,
631 Complementary DNA; CI, Confidence Interval; DPBS, Dulbecco's Phosphate-Buffered Saline;
632 DRP1, Dynamin-related protein 1; ECL, Enhanced Chemiluminescence; EI, Electron Ionization;
633 ELISA, Enzyme-Linked Immunosorbent Assay; FAO, Fatty Acid Oxidation; FBS, Fetal Bovine
634 Serum; FCCP, Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; FSC, Forward Scatter
635 (FSC-A: Area, FSC-H: Height); GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GC-MS,
636 Gas Chromatography-Mass Spectrometry; gMFI / MFI, Geometric Mean Fluorescence Intensity
637 / Median Fluorescent Intensity; GPX1, Glutathione Peroxidase 1; GSS, Glutathione Synthetase;
638 H2DCFDA, 2',7'-dichlorodihydrofluorescein diacetate; HG, Hyperglycemic Control / High
639 Glucose; HIF-1 α , Hypoxia-inducible factor 1-alpha; HRP, Horseradish Peroxidase; IHG,
640 Infection under Hyperglycemia; IL-1 β , Interleukin-1 beta; IL-6, Interleukin-6; IL-18, Interleukin-
641 18; ING, Infection under Normoglycemia; KEGG, Kyoto Encyclopedia of Genes and Genomes;
642 L/P ratio, Lactate-to-Pyruvate ratio; LB, Luria-Bertani; MDA, Malondialdehyde; MFN1,
643 Mitofusin-1; MOI, Multiplicity of Infection; MSEA, Metabolite Set Enrichment Analysis; mTOR,
644 Mammalian Target of Rapamycin; mtROS, Mitochondrial Reactive Oxygen Species; NAD⁺ /
645 NADH, Nicotinamide Adenine Dinucleotide; NAO, 10-N-Nonyl Acridine Orange; NF- κ B,
646 Nuclear Factor kappa B; NG, Normoglycemic Control / Normal Glucose; NIST, National Institute
647 of Standards and Technology; NLRP3, NLR Family Pyrin Domain Containing 3; OD600, Optical
648 Density at 600 nm; OMA1, Overlapping with the m-AAA protease 1; OPA1, Optic Atrophy 1;
649 PBS, Phosphate-Buffered Saline; PCA, Principal Component Analysis; PGC-1 α , Peroxisome
650 proliferator-activated receptor gamma coactivator 1-alpha; PLS-DA, Partial Least Squares
651 Discriminant Analysis; qRT-PCR, Quantitative Real-Time Polymerase Chain Reaction; RIPA,
652 Radioimmunoprecipitation Assay; ROS, Reactive Oxygen Species; SD, Standard Deviation;
653 SEM, Standard Error of the Mean; SIRT1, Sirtuin 1; SOD, Superoxide Dismutase; SSC, Side

654 Scatter (SSC-A: Area); TCA, Tricarboxylic Acid; TMCS, Trimethylchlorosilane; TNF- α , Tumor
655 Necrosis Factor-alpha; VIP, Variable Importance in Projection; $\Delta\Psi m$, Mitochondrial Membrane
656 Potential

657 **Declarations**

658 **Ethics approval**

659 The experimental protocols and study design were reviewed and approved by the Institutional
660 Ethics Committee (IEC) of Kasturba Medical College (KMC), Manipal Academy of Higher
661 Education (MAHE), Manipal, India (Approval No: IEC1:162/2022). As this study exclusively
662 utilized an *in vitro* K562 cell line model and involved no human participants or live animal
663 models, norms regarding human research (such as the Declaration of Helsinki) and guidelines for
664 animal experiments are not applicable.

665 **Consent to participate**

666 Not applicable.

667 **Consent for publication**

668 Not Applicable since our *in vitro* study is based on a cell line model and includes no identifying
669 images, personal details, or clinical data from participants.

670 **Availability of data and material**

671 Original uncropped chemiluminescent images for all immunoblots are provided in the
672 Supplementary Material. The datasets generated and analyzed during the current study are
673 available from the corresponding author on reasonable request.

674 **Competing interests**

675 The authors declare that the research was conducted without any commercial or financial
676 relationships that could be construed as potential conflicts of interest.

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681 **Authors' contributions**

682 SM and AM conceived the study, performed methodology, software, validation, formal analysis,
683 data curation, visualization, and wrote the original draft. MB and YSPM performed the
684 methodology and formal analysis. SGB and PC conducted methodology. SLKL performed formal
685 analysis and investigation. CM provided resources and supervised the study. SGD provided
686 resources, supervised the study, and acquired funding. RGD conceptualized and supervised the
687 study, administered the project, acquired funding, and contributed to methodology, validation,
688 formal analysis, investigation, resources, data curation, and writing the original draft. All authors
689 reviewed and edited the manuscript, and all authors read and approved the final manuscript.

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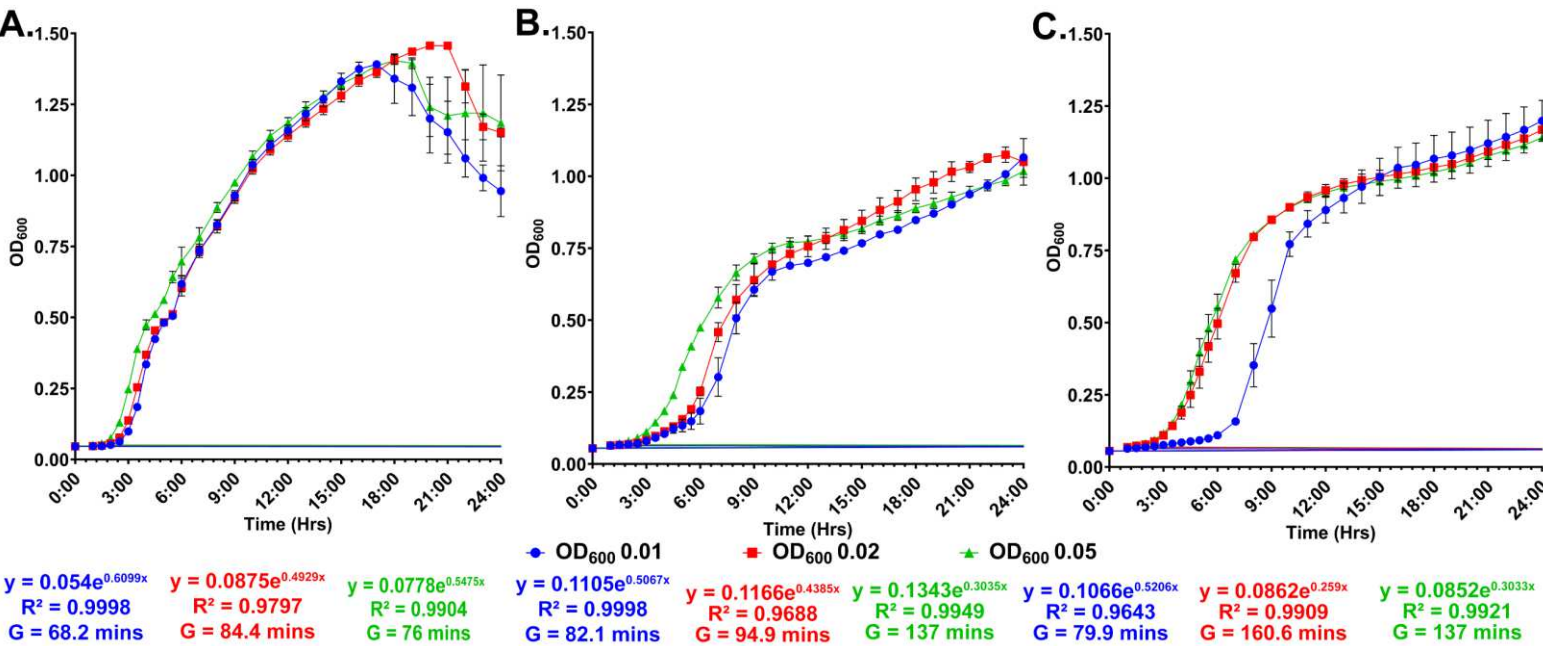
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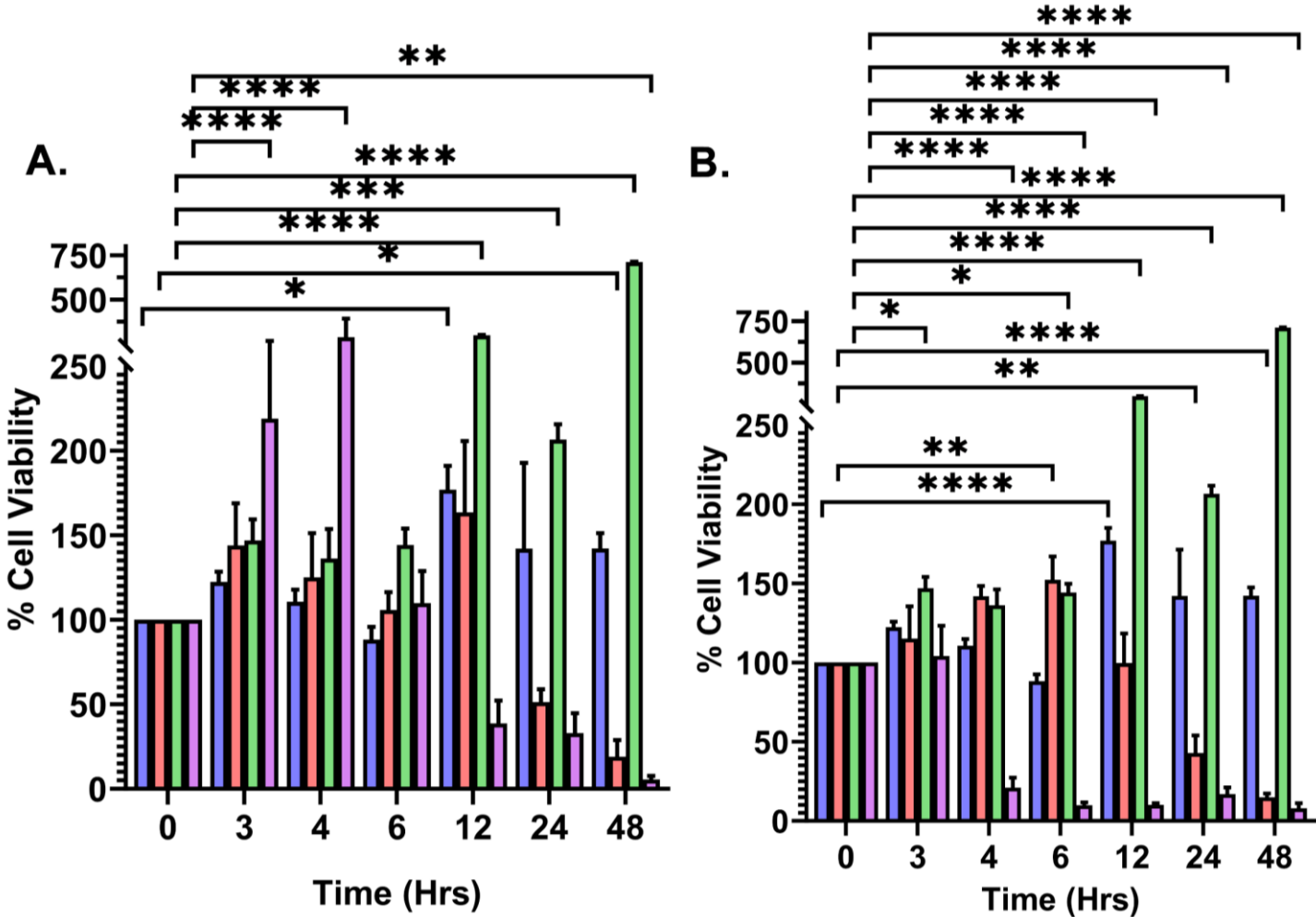
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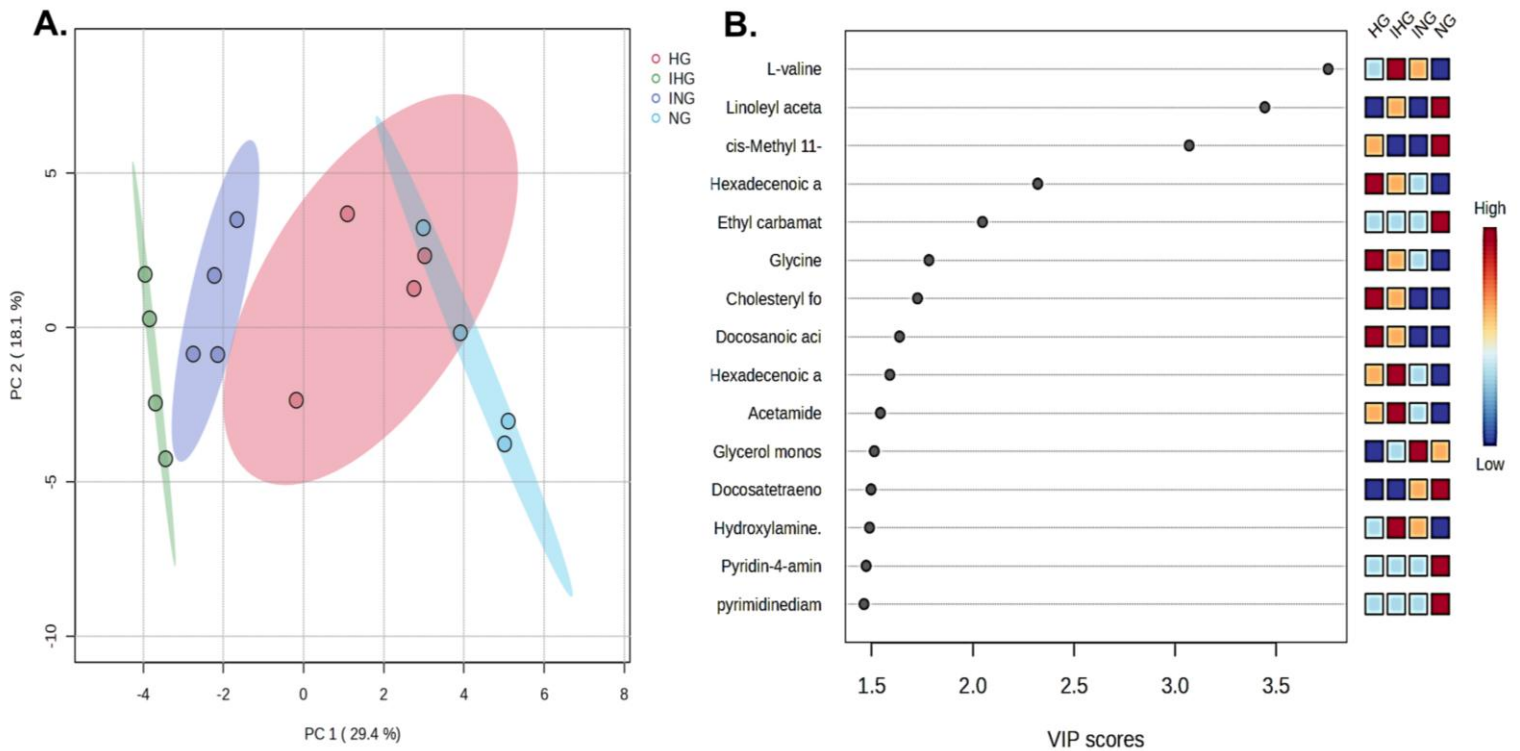
854 **Figures**
 855 **Figure 1. Growth kinetics of *B. cepacia* in LB or complete RPMI-1640 medium under**
 856 **normoglycemic and hyperglycemic conditions.**



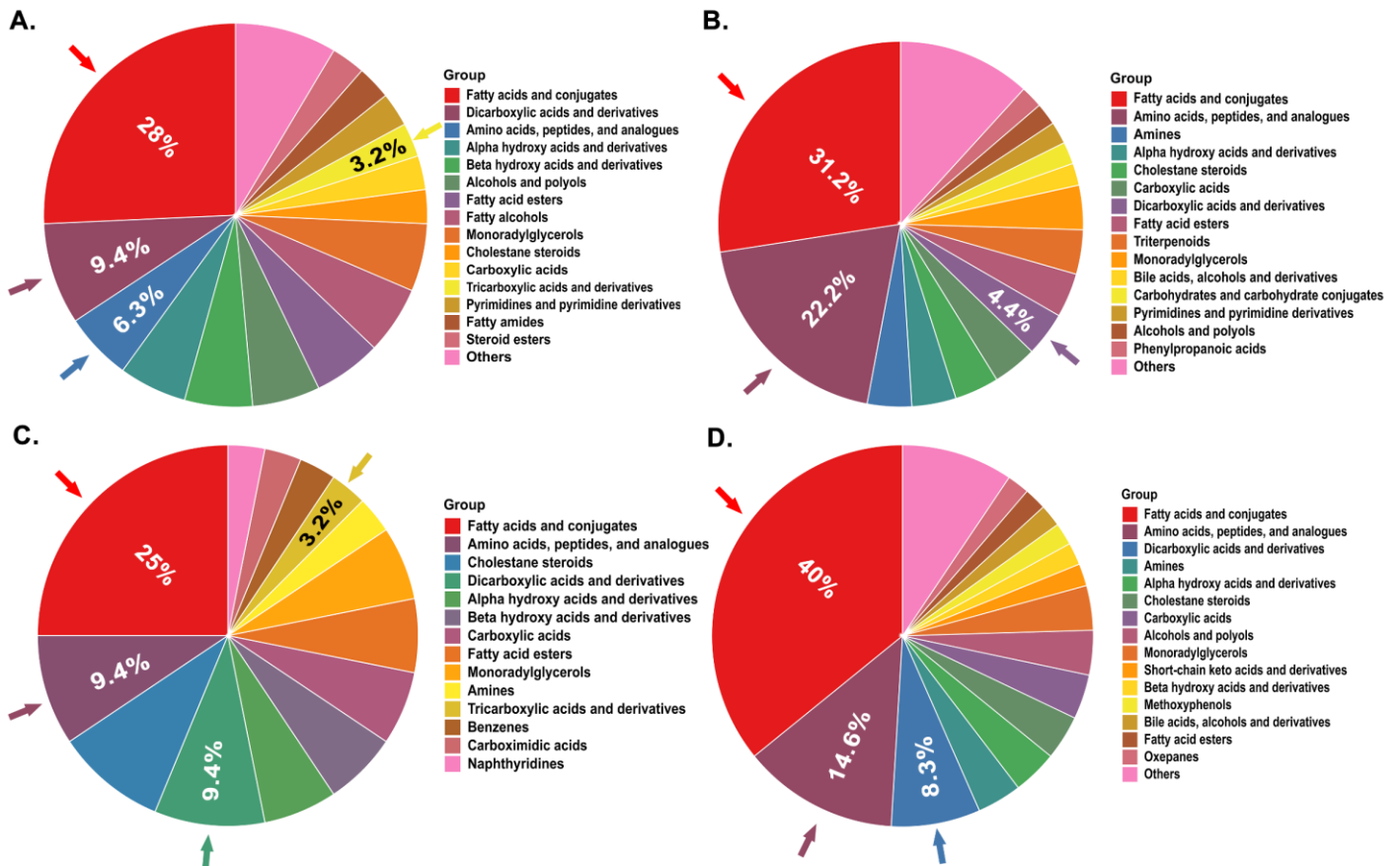
857 **Figure 2. *Burkholderia cepacia* infection reduced K562 cell viability in a time-dependent**
 858 **manner, with greater cytotoxicity observed at higher inoculum levels.**



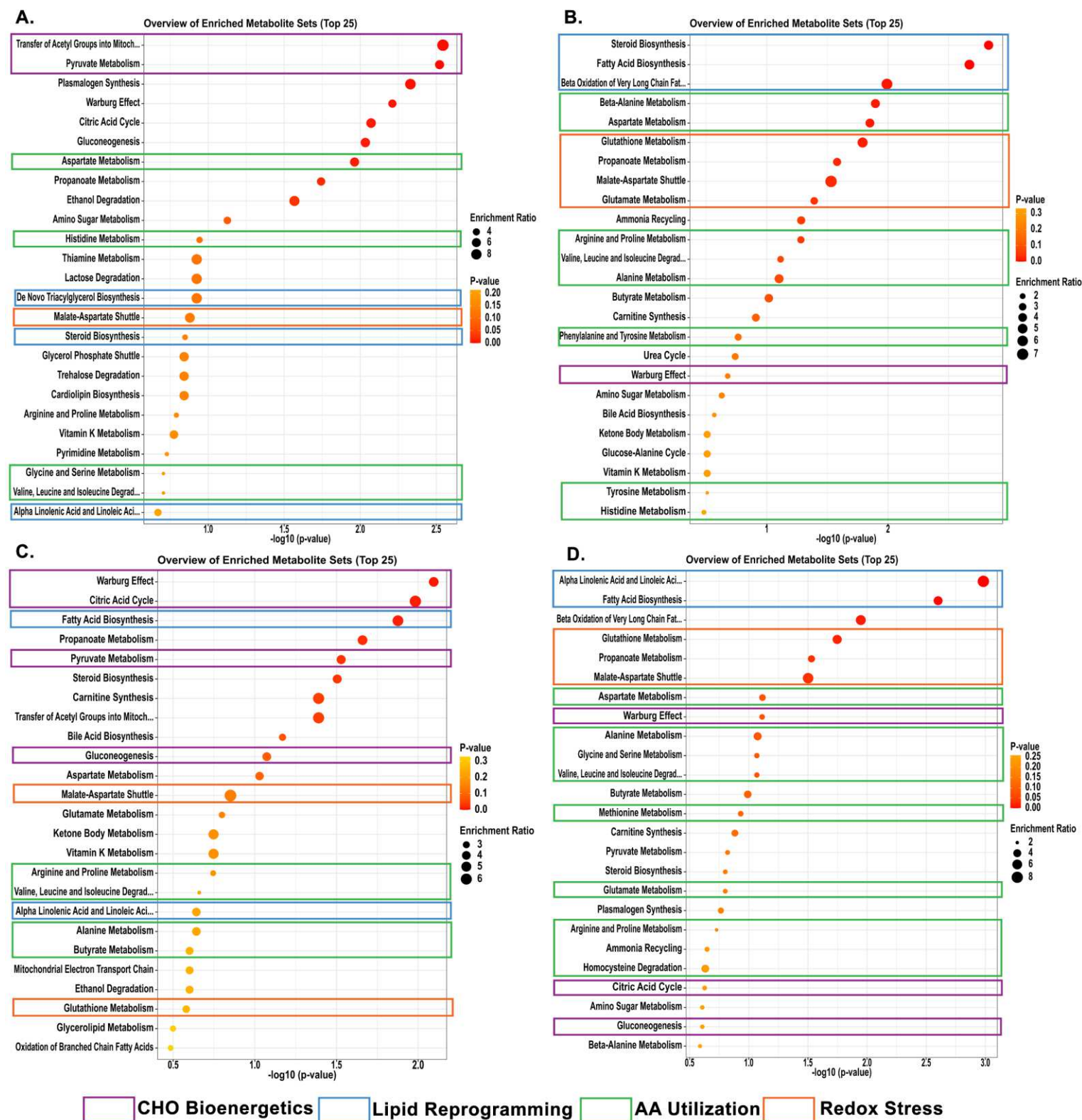
859 **Figure 3. Multivariate statistical analysis identifying discriminant metabolic profiles of host-**
860 **pathogen interactions under varying glycemic conditions**



861 **Figure 4. Infection and glycemic status drive distinct shifts in metabolite superclass**
862 **compositions.**

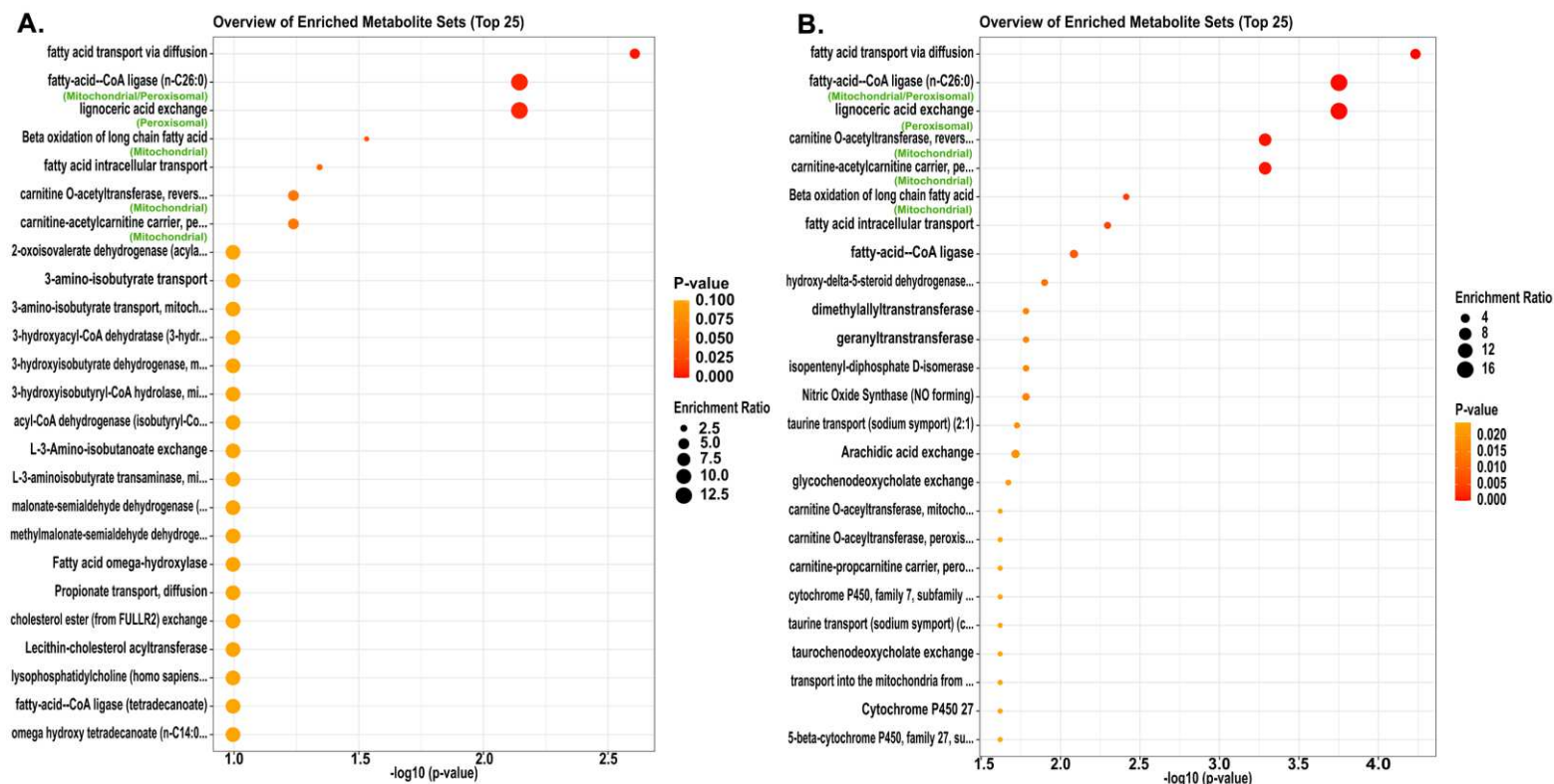


863 **Figure 5. Infection-induced metabolic pathway reprogramming under normoglycemic and**
864 **hyperglycemic conditions**

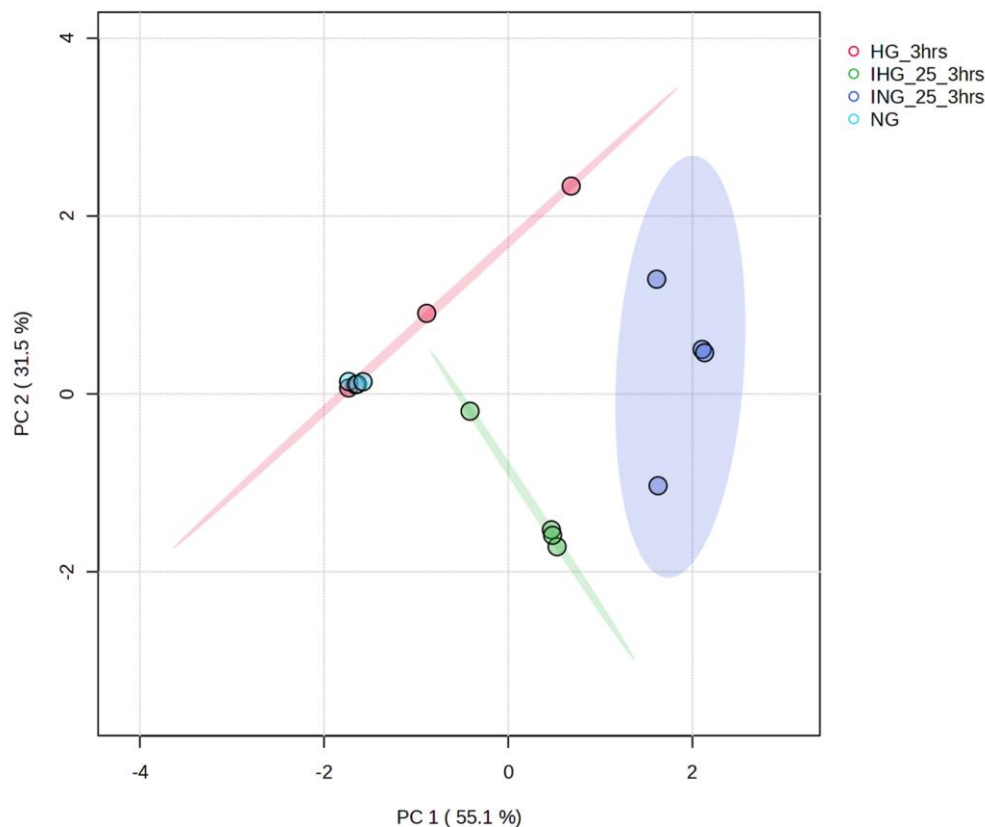


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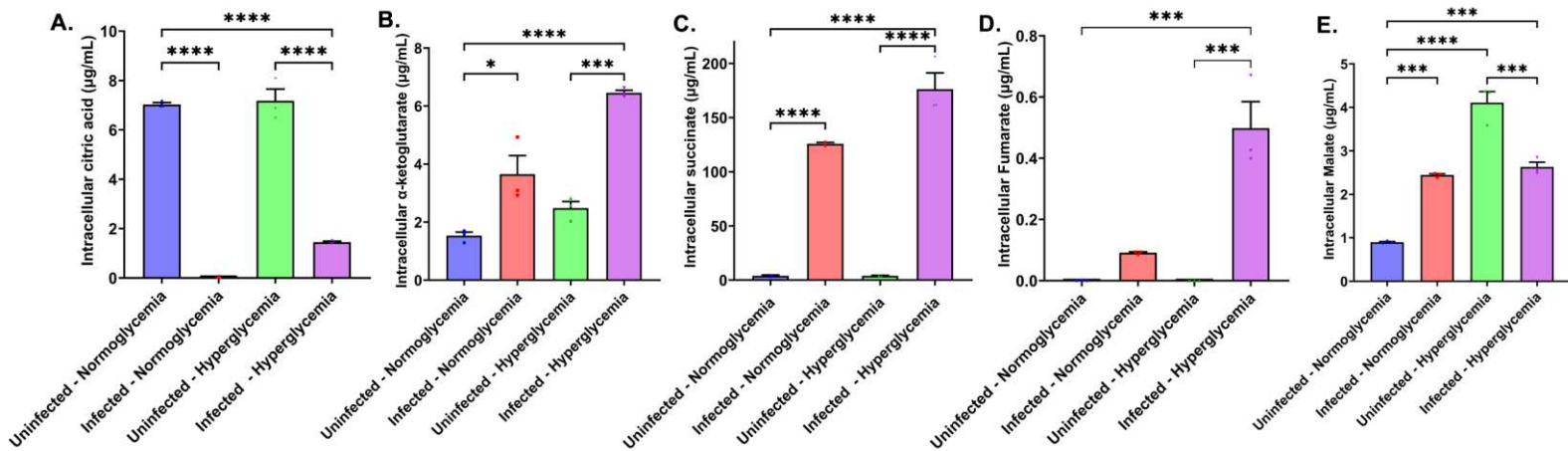
869 **Figure 6. Metabolite set enrichment analysis identified specific enzymes and transport**
870 **dysfunctions in mitochondrial and peroxisomal lipid metabolism.**



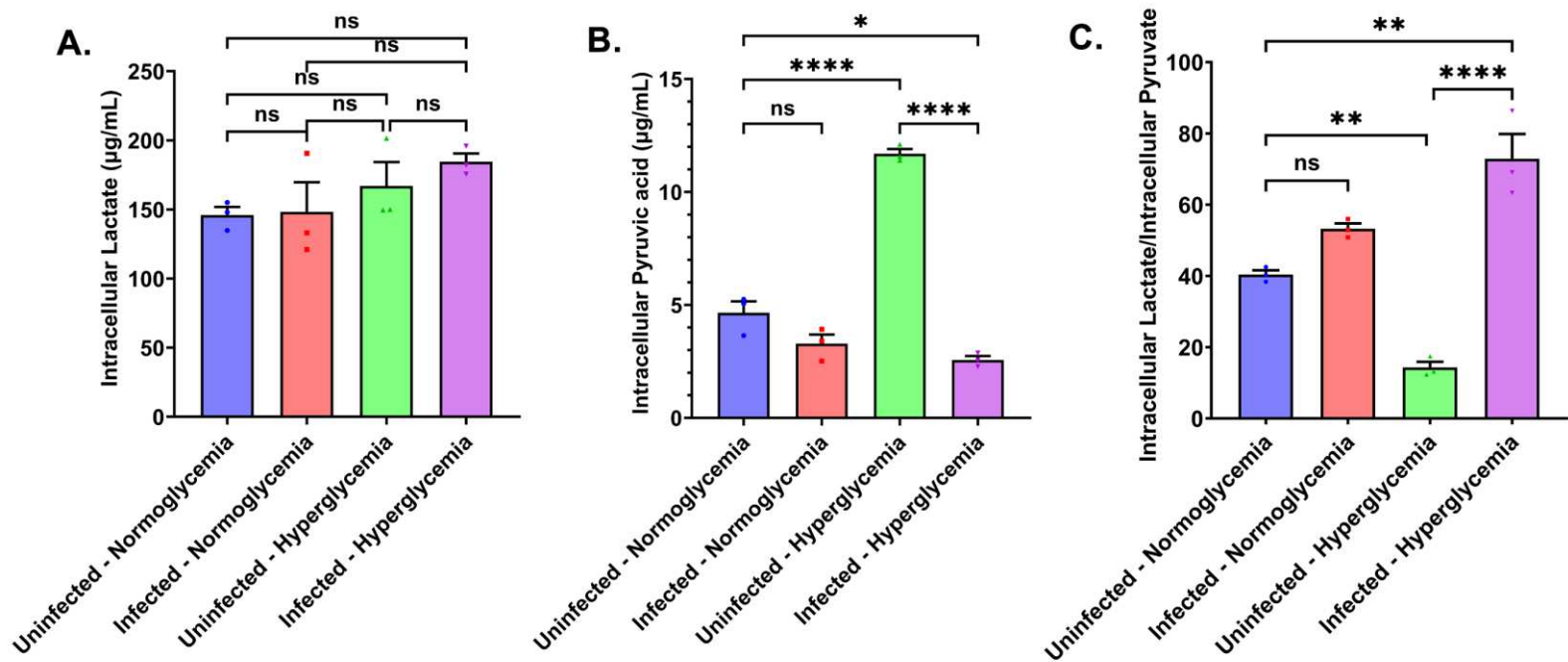
871 **Figure 7. Principal component analysis (PCA) revealed distinct targeted metabolic**
872 **reprogramming driven by *Burkholderia cepacia* infection and hyperglycemic stress**



873 **Figure 8. Targeted metabolomic profiling demonstrates severe disruption of tricarboxylic**
874 **acid (TCA) cycle flux during *Burkholderia cepacia* infection.**

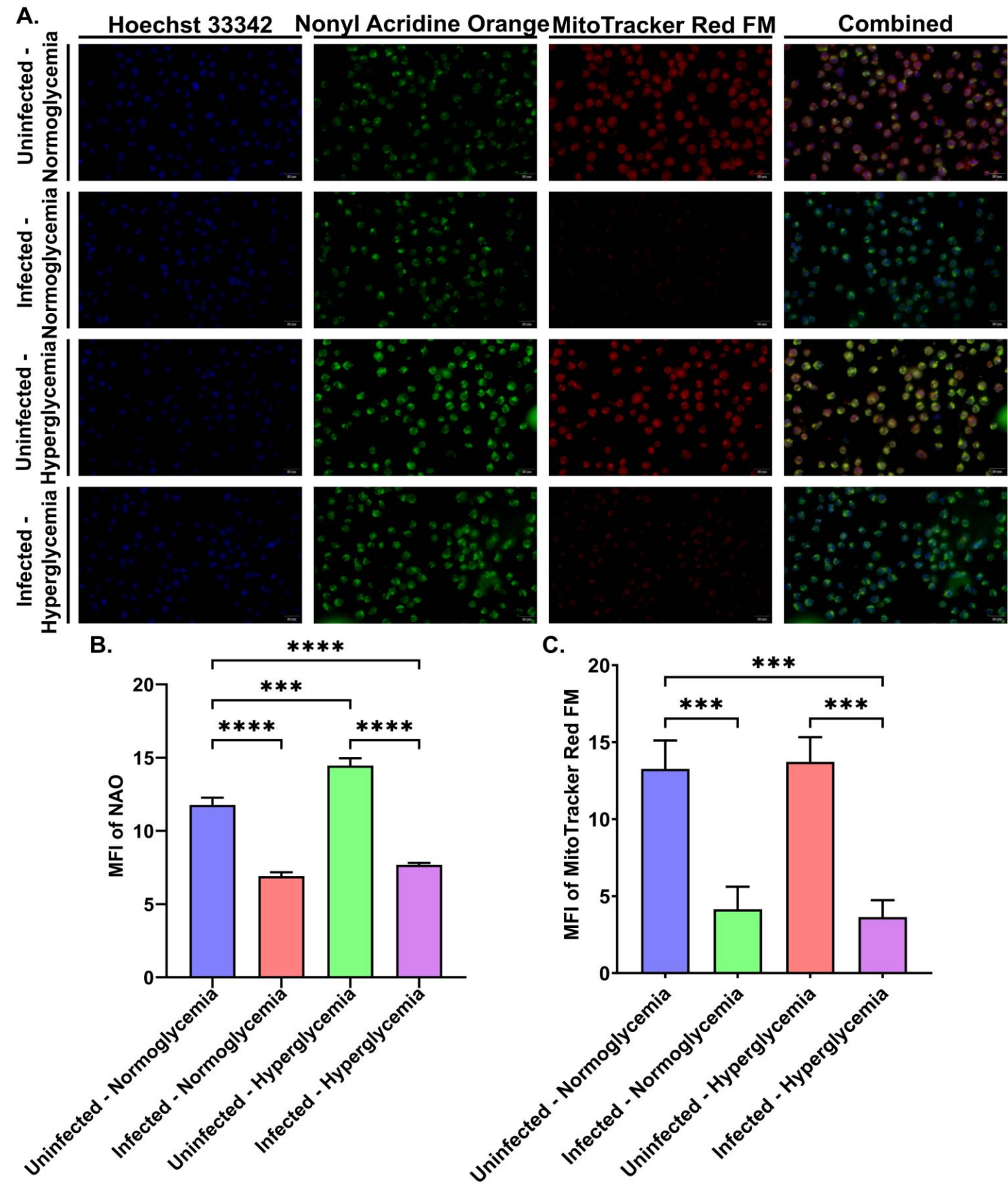


875 **Figure 9. *Burkholderia cepacia* infection alters pyruvate metabolism and elevates the**
876 **intracellular L/P ratio in hyperglycemic conditions.**

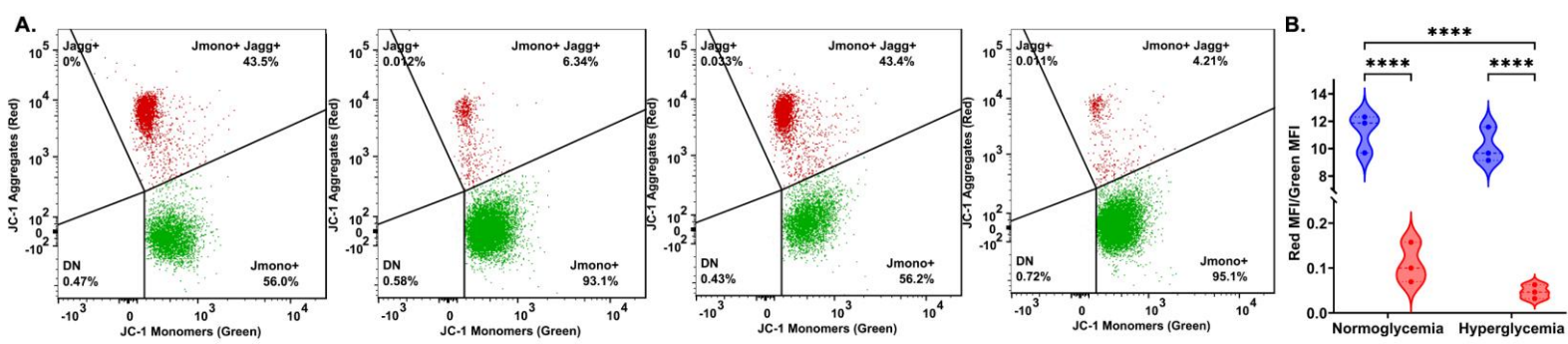


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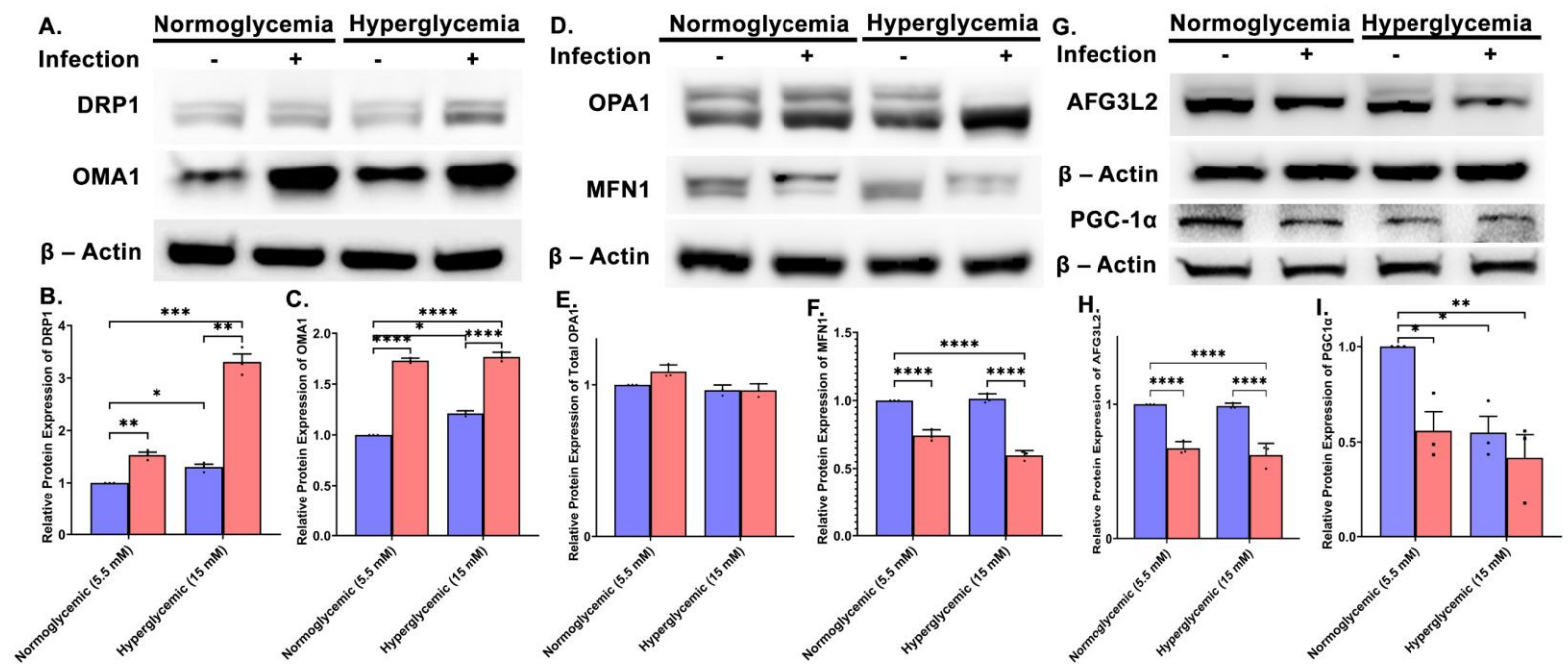
879 **Figure 10. *Burkholderia cepacia* Infection Causes Mitochondrial Depolarization and Partial**
880 **Loss of Mitochondrial Mass.**



881 **Figure 11. Hyperglycemia worsens infection-related loss of mitochondrial membrane**
882 **potential in K562 cells**

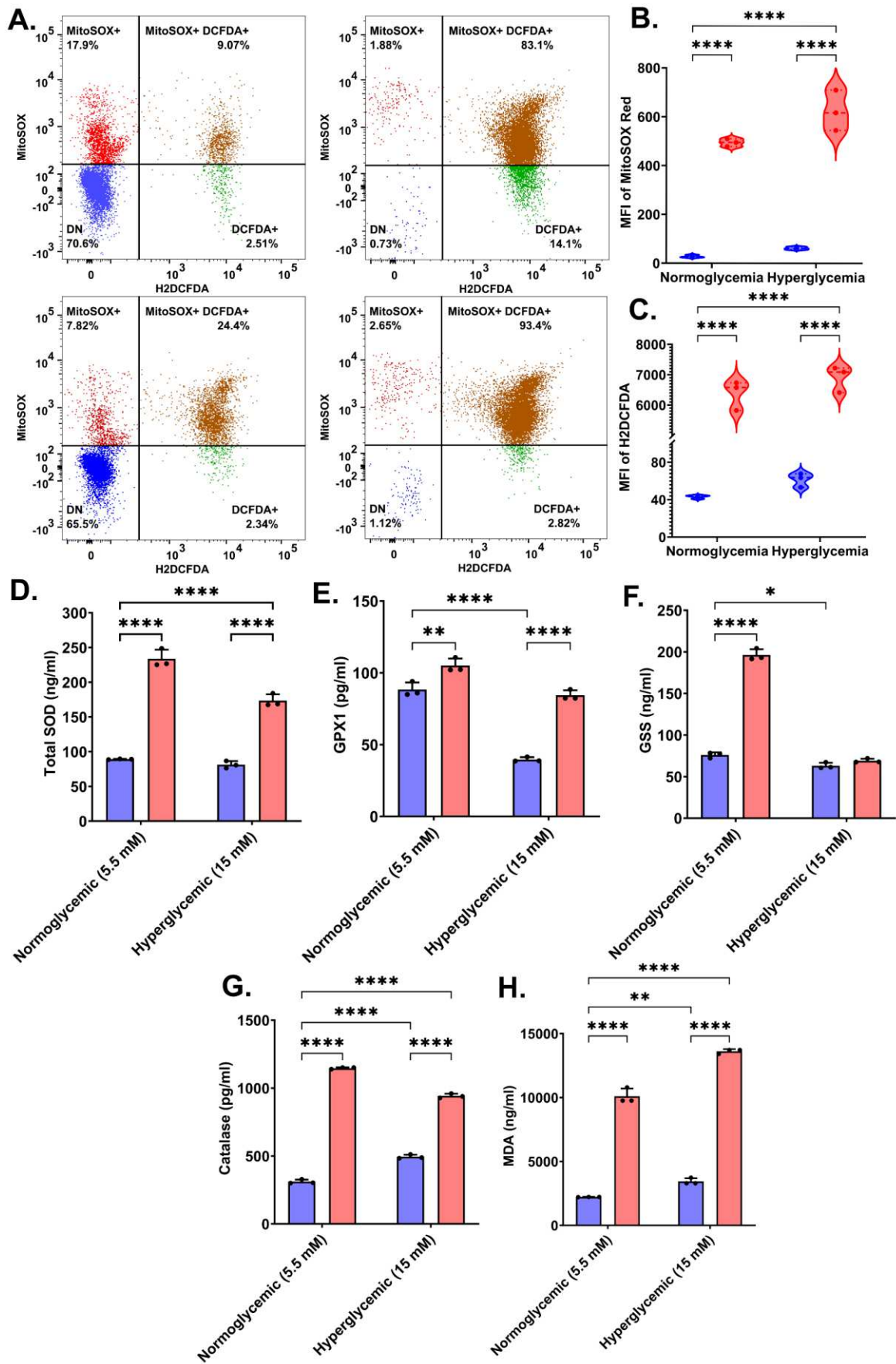


883 **Figure 12. Hyperglycemia and *Burkholderia cepacia* infection synergistically drive profound**
884 **alterations in host mitochondrial dynamics, quality control, and biogenesis.**

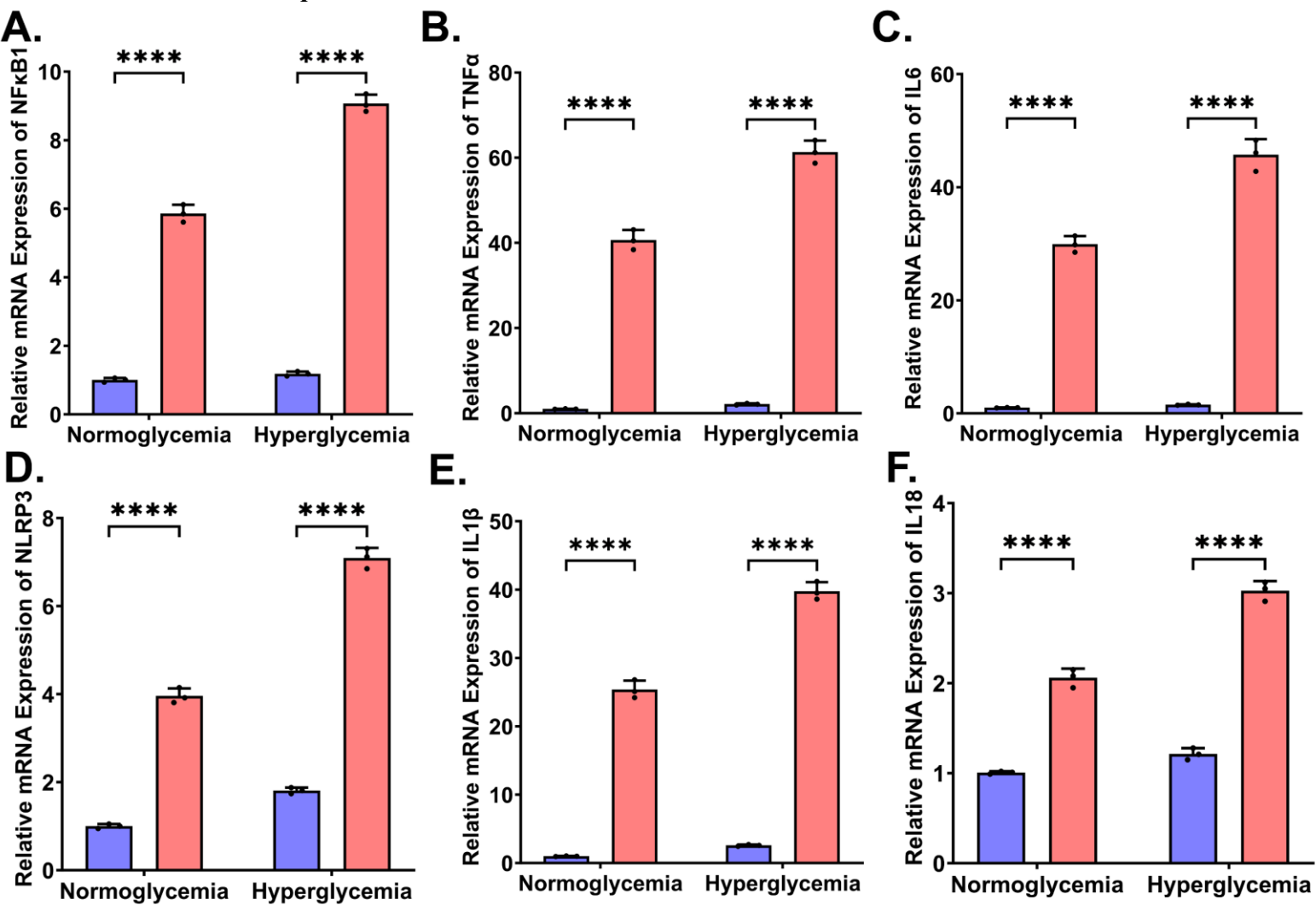


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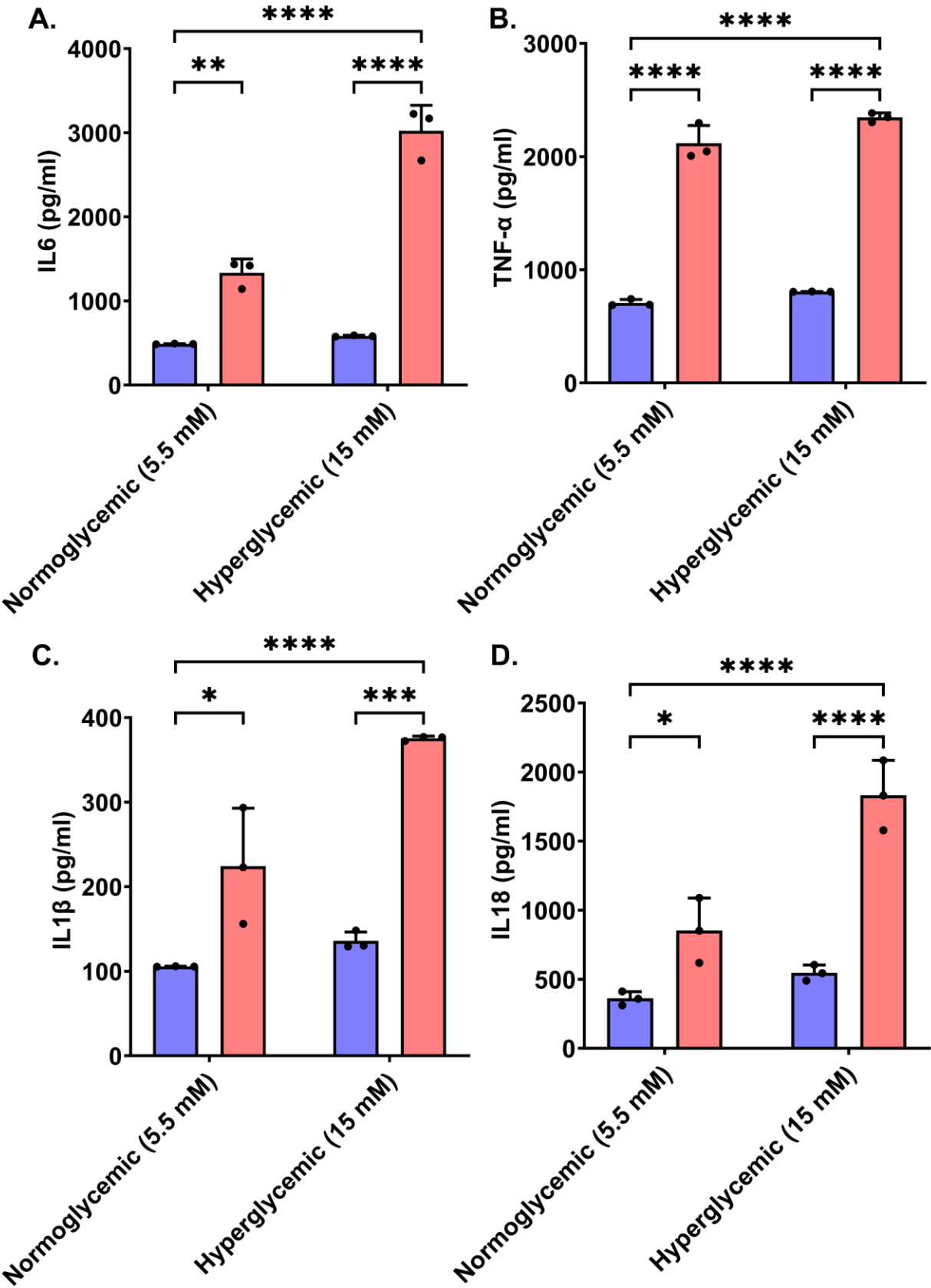
887 **Figure 13. Hyperglycemia worsens infection-related oxidative stress and depletes**
888 **antioxidant defense**



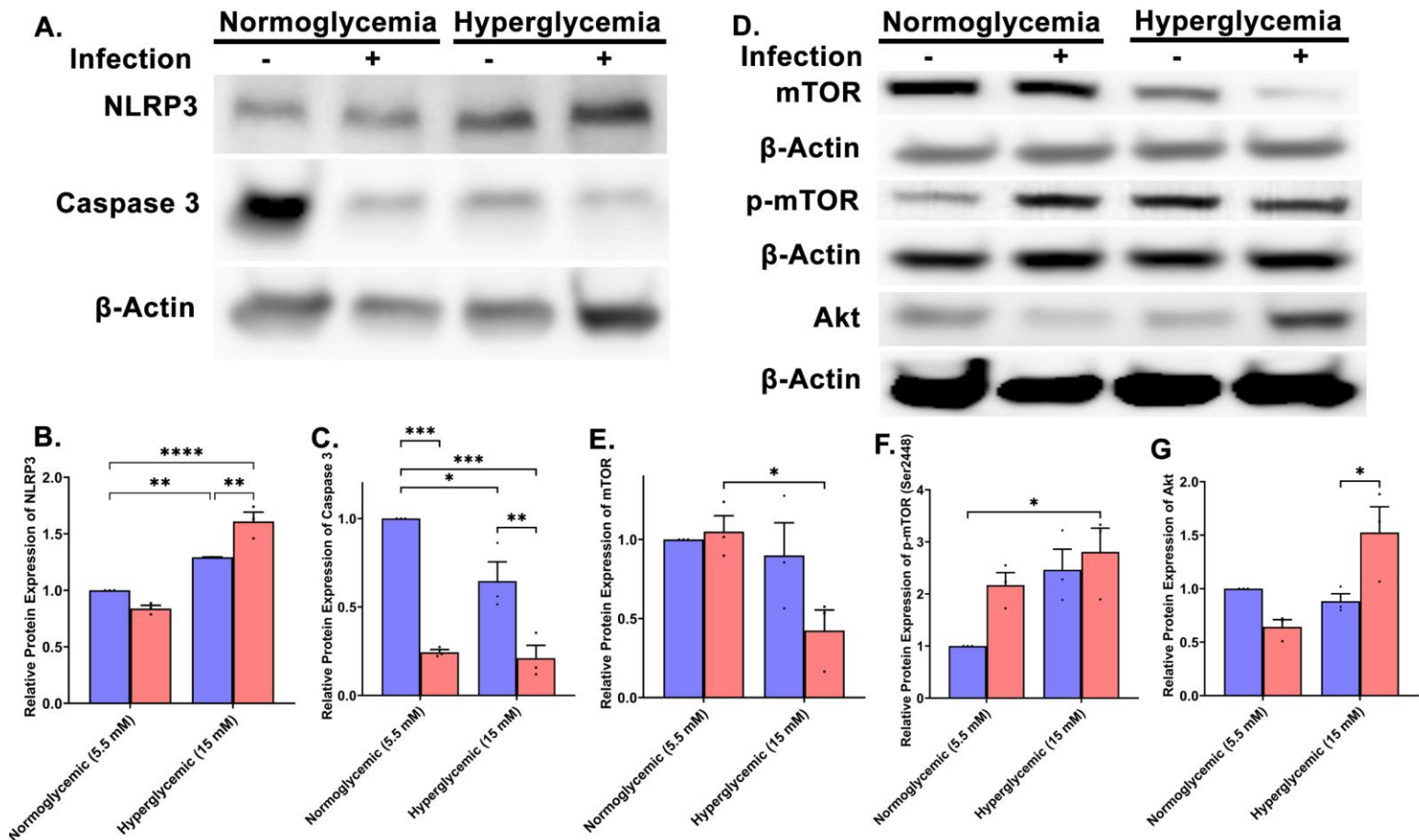
889 Figure 14. Hyperglycemia primes the host transcriptional inflammatory response to
890 *Burkholderia cepacia* infection



894 Figure 15. Hyperglycemia increases proinflammatory cytokine and inflammasome
895 responses during infections.



898 **Figure 16. Hyperglycemia rewires inflammasome- and mTOR–Akt-associated signaling**
899 **during *Burkholderia cepacia* infection.**



900

901 Figure Legends

902 **Figure 1. Growth kinetics of *B. cepacia* in LB or complete RPMI-1640 medium under**
903 **normoglycemic and hyperglycemic conditions.** Bacterial growth was monitored by measuring
904 the optical density at 600 nm (OD₆₀₀) over time for cultures initiated at starting OD₆₀₀ values
905 of 0.01, 0.02, and 0.05, which corresponded to the infectious equivalents used in subsequent
906 cellular assays. These inocula are represented by blue circles (0.01 OD₆₀₀), red squares (0.02
907 OD₆₀₀), and green triangles (0.05 OD₆₀₀) in (A) LB, (B) normoglycemic (NG), and (C)
908 hyperglycemic (HG) media. The data points represent the mean values, and the error bars indicate
909 the standard deviation of the replicate measurements. Solid curves represent exponential growth
910 model fits (fit equations and R² values are displayed within each panel), and the corresponding
911 generation times (G) are reported below each panel.

912
913 **Figure 2. *Burkholderia cepacia* infection reduced K562 cell viability in a time-dependent**
914 **manner, with greater cytotoxicity observed at higher inoculum levels.** (A) shows MOI 1:10
915 across normoglycemic and hyperglycemic conditions over 0–48 h, and (B) shows the
916 corresponding kinetics at MOI 1:25, with bars indicating mean ± SEM (n=3) and lines overlaying
917 time course- trends.

918
919 **Figure 3. Multivariate statistical analysis identifying discriminant metabolic profiles of host-**
920 **pathogen interactions under varying glycemic conditions (A)** Principal Component Analysis
921 (PCA) score plot providing an unsupervised overview of global metabolic variance and natural
922 clustering among the four experimental groups: Normoglycemic Control (NG), Infection in
923 Normoglycemic Condition (ING), Hyperglycemic Control (HG), and Infection in Hyperglycemic
924 Condition (IHG). The shaded regions indicate 95% confidence ellipses. **(B)** Variable Importance
925 in Projection (VIP) score plot. The plot ranks the top 15 discriminant metabolites driving the
926 metabolic separation between experimental conditions. Metabolites with a VIP score > 1.0 are
927 considered highly influential, and the adjacent colored boxes indicate the relative concentration
928 level (ranging from dark blue for the lowest to dark red for the highest) of each metabolite across
929 the respective groups.

930
931 **Figure 4. Infection and glycemic status drive distinct shifts in metabolite superclass**
932 **compositions.** Comparative pie charts illustrate the relative abundance (%) of major metabolite
933 superclasses across the four experimental conditions: **(A)** normoglycemic control (NG), **(B)**
934 infection in normoglycemic condition (ING), **(C)** hyperglycemic control (HG), and **(D)** infection
935 in hyperglycemic condition (IHG). Each colored sector represents a distinct chemical class
936 identified during global profiling: carboxylic acids and derivatives, fatty acyls, hydroxyl acids and
937 derivatives, keto acids and derivatives, and pyridines and derivatives. Data labels within each
938 sector denote the specific percentage contribution of each chemical class to the total discriminant
939 metabolome of the respective experimental group.

940
941 **Figure 5. Infection-induced metabolic pathway reprogramming under normoglycemic and**
942 **hyperglycemic conditions.** Comparative pathway enrichment analysis of the untargeted
943 metabolomic profiles of K562 cells. The bubble plots illustrate the significantly enriched KEGG
944 metabolic pathways across four experimental cohorts: **(A)** normoglycemic control, **(B)** infection

in normoglycemic condition (ING), (C) hyperglycemic control, and (D) infection in hyperglycemic condition (IHG). In each panel, the y-axis denotes statistical significance as $-\log(p)$, while the x-axis represents the Pathway Impact score derived from the relative-betweenness centrality topology analysis. Bubble color gradients (from yellow to red) indicate the raw p-value, with darker shades of red indicating greater statistical significance. The bubble size is proportional to the Pathway Impact score. The results demonstrated a distinct metabolic shift from central carbon metabolism to lipid and amino acid-associated pathways post-infection, a trend that was markedly amplified under hyperglycemic conditions. Significance was evaluated using the hypergeometric test ($p < 0.05$).

Figure 6. Metabolite set enrichment analysis identified specific enzymes and transport dysfunctions in mitochondrial and peroxisomal lipid metabolism. Bubble plots illustrating the top 25 significantly enriched enzyme and transport functional sets predicted from the intracellular metabolomic profiles of K562 cells following *B. cepacia* infection. (A) Infection under normoglycemic conditions (ING) and (B) infection under hyperglycemic conditions (IHG). The y-axis defines the specific enzymatic or transport pathways, with green text annotations highlighting the critical functions localized to the mitochondria and the peroxisomes. The x-axis denotes statistical significance as $-\log_{10}(p\text{-value})$. Bubble color gradients (ranging from yellow/orange to red) indicate the raw p-value, with darker red shades indicating higher statistical significance. The bubble size is strictly proportional to the calculated Enrichment Ratio.

Figure 7. Principal component analysis (PCA) revealed distinct targeted metabolic reprogramming driven by *Burkholderia cepacia* infection and hyperglycemic stress. Unsupervised PCA score plot derived from untargeted GC-MS metabolomic profiling of K562 cells. The primary axis, Principal Component 1 (PC1), captured 55.1% of the total metabolic variance and primarily segregated the cohorts based on infection status. Principal Component 2 (PC2) accounted for 31.5% of the variance, further delineating the metabolic divergence driven by the glucose microenvironment. The experimental groups included normoglycemic uninfected controls (NG, light blue circles), hyperglycemic uninfected controls (HG_3hrs, pink circles), normoglycemic cells infected with *B. cepacia* at an MOI of 1:25 for 3 h (ING_25_3hrs, dark blue circles), and infected hyperglycemic cells (IHG_25_3hrs, green circles). The shaded regions and linear fits indicate the clustering and separation trajectories of the biological replicates.

Figure 8. Targeted metabolomic profiling demonstrates severe disruption of tricarboxylic acid (TCA) cycle flux during *Burkholderia cepacia* infection. Intracellular concentrations of key TCA cycle intermediates were quantified in K562 cells cultured under normoglycemic (5.5 mM glucose) or hyperglycemic (15 mM glucose) conditions, either left uninfected or infected with *B. cepacia* (MOI 1:25) for 3 h. Bar graphs represent the absolute concentrations ($\mu\text{g/mL}$) of (A) citric acid, (B) α -ketoglutarate, (C) succinate, (D) fumaric acid, and (E) malic acid. The profiles illustrate a marked depletion of forward citrate flux coupled with the pathological accumulation of downstream intermediates (succinate, α -ketoglutarate, fumarate, and malate) post-infection, a metabolic bottleneck that was significantly exacerbated by the hyperglycemic microenvironment. Data are presented as mean $\pm$ SD of independent biological replicates ($n = 3$), with individual data points. Statistical significance was assessed using one-way ANOVA

followed by Tukey's multiple comparisons test (* $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant).

Figure 9. *Burkholderia cepacia* infection alters pyruvate metabolism and elevates the intracellular L/P ratio in hyperglycemic conditions. Targeted quantitative analysis of central carbon metabolites in K562 cells. Bar graphs illustrate (A) intracellular lactate levels ($\mu\text{g/mL}$), (B) intracellular pyruvate levels ($\mu\text{g/mL}$), and (C) calculated intracellular lactate/pyruvate (L/P) ratio across four experimental cohorts: uninfected normoglycemia (blue), infected normoglycemia (red), uninfected hyperglycemia (green), and infected hyperglycemia (purple). While basal lactate production remained largely unchanged, infections significantly impaired the oxidation of pyruvate. The resulting elevated L/P ratio, which was most severe in the hyperglycemic-infected cohort, indicates an altered ratio and a profound metabolic shift toward anaerobic respiration and pseudohypoxic stress. Bars represent the mean $\pm$ SEM of independent biological replicates for each group. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant, $p > 0.05$).

Figure 10. *Burkholderia cepacia* Infection Causes Mitochondrial Depolarization and Partial Loss of Mitochondrial Mass. Representative fluorescence micrographs of K562 cells stained with Hoechst 33342 (nuclei), nonyl acridine orange (NAO; mitochondrial mass), and MitoTracker Red (mitochondrial membrane potential). Both normoglycemic- and hyperglycemic-infected cells show a marked reduction in MitoTracker Red intensity with partially preserved NAO staining, indicating mitochondrial depolarization without significant loss of mitochondrial mass. Scale bar 20 μm . One-way ANOVA followed by Tukey's post hoc test. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant ($p > 0.05$).

Figure 11. Hyperglycemia worsens the infection-related loss of mitochondrial membrane potential in K562 cells (A). Flow cytometry dot plots of K562 cells stained with JC-1. Cells were maintained at normal or high glucose levels, then left uninfected (control) or infected with *B. cepacia* (MOI 1:25, 3 h). The Y-axis (Comp-PE-A) shows the JC-1 aggregates found in healthy, polarized mitochondria, whereas the X-axis (Comp-FITC-A) shows the JC-1 monomers that indicate depolarized mitochondria. The plots, from left to right, are: Uninfected normoglycemia, infected normoglycemia, uninfected hyperglycemia, and infected hyperglycemia. (B) Analysis of $\Delta\psi\text{m}$ is presented as the ratio of red (PE) to green (FITC) median fluorescent intensity (MFI). Violin plots display the data distribution, with the central dashed line indicating the median and the dotted lines indicating the interquartile ranges. Individual data points represent separate biological replicates ($n=3$). We evaluated statistical significance using two-way ANOVA followed by Tukey's multiple comparisons test. **** $p < 0.0001$.

Figure 12. Hyperglycemia and *Burkholderia cepacia* infection synergistically drive profound alterations in host mitochondrial dynamics, quality control, and biogenesis. K562 cells cultured under normoglycemic (5.5 mM) or hyperglycemic (15 mM) conditions were evaluated as uninfected (–) or following *B. cepacia* infection at an MOI of 1:25 for 3 h (+). Representative immunoblots and corresponding densitometric quantification of the mitochondrial fission mediators DRP1 and OMA1, demonstrating their massive upregulation during infection. Representative immunoblots and quantification of the fusion-associated proteins OPA1 and MFN1 revealed a severe depletion of MFN1-driven fusion capacity after infection. Representative immunoblots and quantitative analyses of the essential matrix protease AFG3L2 and biogenesis

coactivator PGC-1 α indicated a collapse in protein quality control and organelle renewal. All protein expression levels were normalized to the internal loading control, β -actin. Data are presented as mean $\pm$ SD of independent biological replicates (n = 3), with individual data points. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test (*p < 0.05, p < 0.01, ***p < 0.001, ****p < 0.0001).

Figure 13. Hyperglycemia worsens infection-related oxidative stress and depletes antioxidant defenses (A) Representative flow cytometry dot plots of K562 cells co-stained with MitoSOXTM Red and H2DCFDA to evaluate mitochondrial superoxide (Y-axis, Comp-PE-A) and general cytosolic reactive oxygen species (ROS) (X-axis, Comp-FITC-A). Cells were grown under normal (5.5 mM) or high-glucose (15 mM) conditions. They were assessed as uninfected (control) or as having been infected with *B. cepacia* (MOI 1:25, 3 h). Quantitative analysis of (B) mitochondrial superoxide and (C) general cytosolic ROS is shown as the median fluorescence intensity (MFI) in violin plots. The dashed lines represent the median, and the dotted lines represent the interquartile range. (D–G) ELISA-based measurements of intracellular antioxidant enzyme levels: (D) total superoxide dismutase (SOD), (E) glutathione peroxidase 1 (GPX1), (F) glutathione synthetase (GSS), and (G) catalase. (H) Measurement of malondialdehyde (MDA) concentrations, which serve as an indicator of cellular lipid peroxidation. In the bar graphs (D–H), the data are shown as the mean $\pm$ SD. Each data point across all panels represents an independent biological replicate (n = 3). Statistical significance was assessed using two-way ANOVA followed by Tukey's multiple comparisons test (*p < 0.05, **p < 0.01, ****p < 0.0001).

Figure 14. Hyperglycemia primes the host transcriptional inflammatory response to *Burkholderia cepacia* infection. Quantitative RT-PCR analysis of key inflammatory genes in K562 cells cultured under normoglycemic (5.5 mM) or hyperglycemic (15 mM) conditions and left uninfected (blue bars) or infected with *B. cepacia* (MOI 1:25) for 3 h (red bars). Relative mRNA expression levels of (A) NF- κ B1, (B) NLRP3, (C) TNF- α , (D) IL-6, (E) IL-1 β , and (F) IL-18 were normalized to GAPDH internal controls and expressed relative to normoglycemic uninfected conditions. Data are presented as mean $\pm$ SD of independent biological replicates (n = 3). Statistical significance was assessed using a two-way ANOVA followed by Tukey's multiple comparisons test (****p < 0.0001)

Figure 15. Hyperglycemia increases proinflammatory cytokine and inflammasome responses during infections. Levels of pro-inflammatory cytokines released from K562 cells grown under normoglycemic (5.5 mM) or hyperglycemic (15 mM) conditions. Cells were either left uninfected as controls (blue bars) or infected with *B. cepacia* (red bars) at an MOI of 1:25 for 3 h. The panels show the levels of key NF- κ B pathway drivers: (A) interleukin-6 (IL-6) and (B) tumor necrosis factor-alpha (TNF- α), along with NLRP3 inflammasome-dependent cytokines: (C) interleukin-1 beta (IL-1 β) and (D) interleukin-18 (IL-18). Data are shown as mean $\pm$ SD. Each data point represents an independent biological replicate (n = 3). Statistical significance was assessed using a two-way ANOVA followed by Tukey's multiple comparisons test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Figure 16. Hyperglycemia rewires inflammasome- and mTOR–Akt-associated signaling during *Burkholderia cepacia* infection. (A) Representative immunoblots of NLRP3 and caspase-3 in cells cultured under normoglycemic (5.5 mM glucose) or hyperglycemic (15 mM glucose)

1083 conditions and left uninfected (–) or infected with *Burkholderia* (MOI 1:25) for 3 h (+); β -actin
1084 served as a loading control. Densitometric quantification of NLRP3 **(B)** and caspase-3 **(C)**
1085 normalized to β -actin and expressed relative to the normoglycemic, uninfected condition. **(D)**
1086 Representative immunoblots of mTOR, phospho-mTOR (Ser2448), and Akt under the same
1087 conditions, with β -actin as the loading control for each blot. Densitometric quantification of total
1088 mTOR **(E)**, phospho-mTOR (Ser2448) **(F)**, and Akt **(G)**, normalized to β -actin and expressed
1089 relative to normoglycemic uninfected conditions. Bars represent mean $\pm$ SEM (n = 3 independent
1090 experiments), and statistical significance is indicated by asterisks as determined by two-way
1091 ANOVA with Tukey's multiple comparisons test (*P < 0.05, **P < 0.01, ***P < 0.001, ****P <
1092 0.0001)

Supplementary Files

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