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Supplemental Information

Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1α/Glycolysis-Dependent Axis

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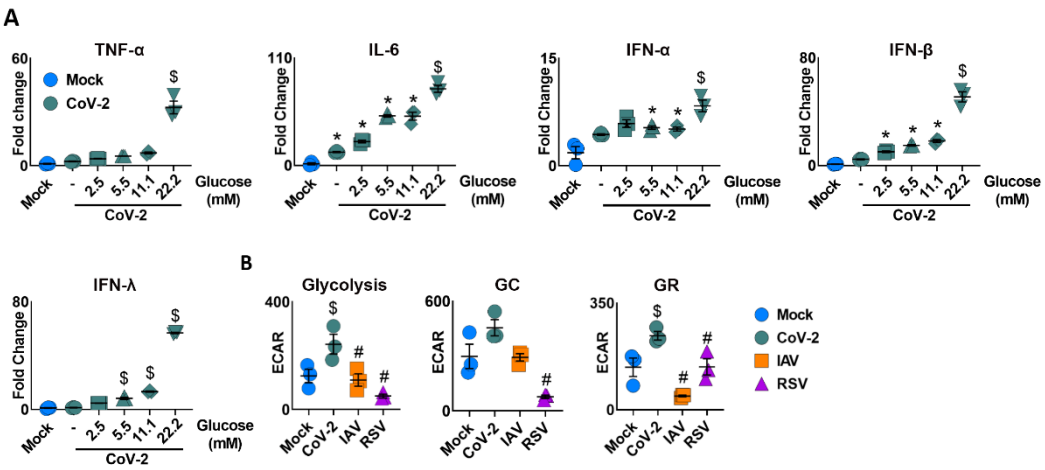

Figure S1. Related to Figure 1. SARS-CoV-2 responds to glucose concentration in extracellular environment.

(A) Human monocytes were infected with mock control or SARS-CoV-2 (CoV-2) (MOI 0.1) for 1 h under continuous agitation and incubated for 24 h in media containing different glucose concentrations (0, 2.5, 5.5, 11.1, 22.2 mM). Relative mRNA expression of cytokines (TNF-α, IL-6, IFN-α, IFN-β and IFN-λ).

(B) ECAR (extracellular acidification rate) - Analysis of Glycolysis (following Glucose injection), Glycolytic capacity (GC: following Oligomycin injection) and Glycolytic reserve (GR: glycolytic capacity - glycolysis) in monocytes infected with mock control, CoV-2, RSV or IAV.

All data represent mean ± SEM of two independent experiments performed in triplicate. *P < 0.05 compared to mock. $^{\#}$P < 0.05 compared to CoV-2. $^{\$}$P < 0.05 compared to all other groups (One-Way ANOVA and Tukey post hoc tests).

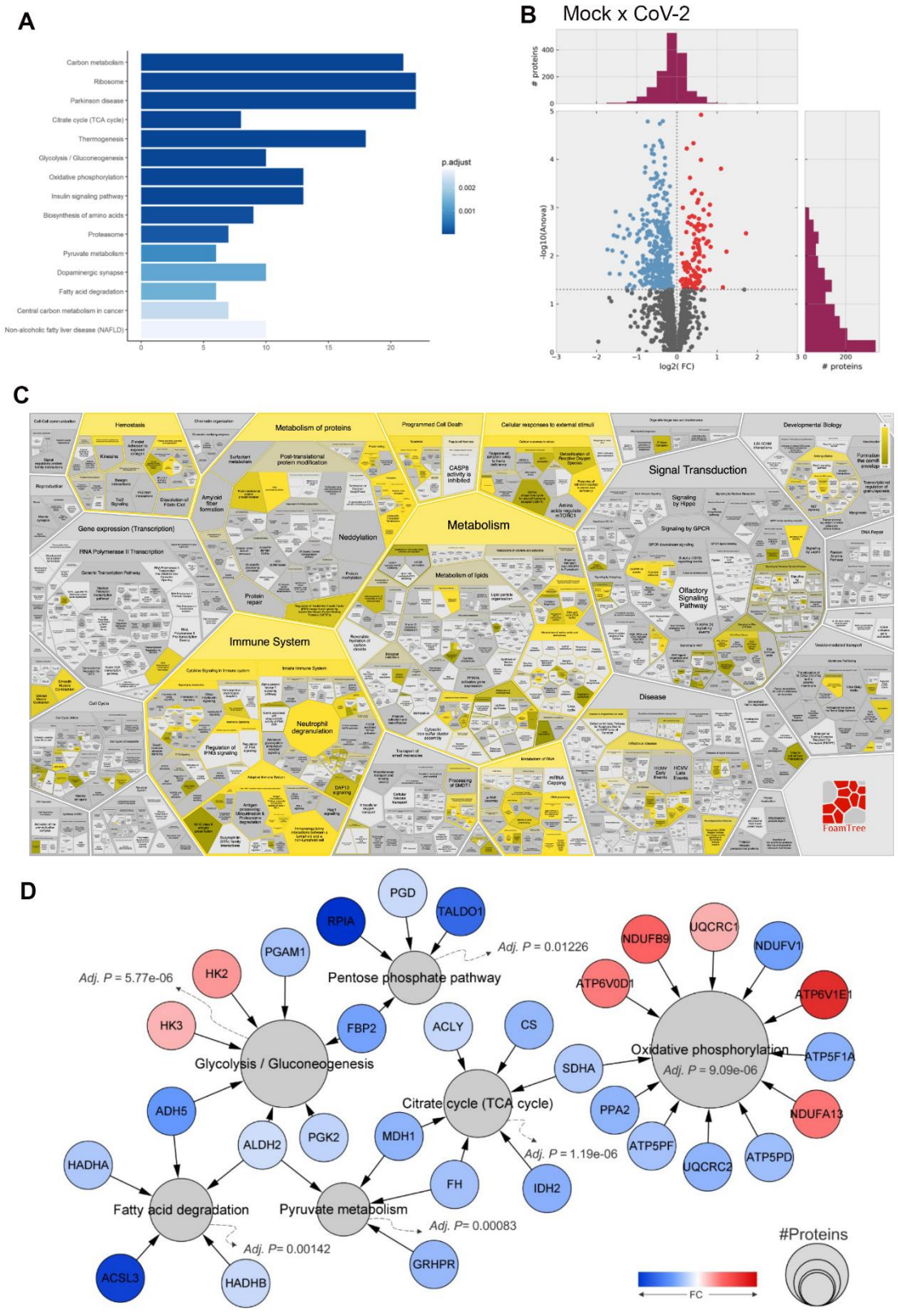

Figure S2. Related to Figure 1 and 3. Proteomic analysis of SARS-CoV-2-infected monocytes.

Human monocytes were infected with mock control or CoV-2 (MOI 0.1) for 1 h under continuous agitation and incubated for 24 h.

(A) Representation of pathways specifically associated with energy metabolism-related protein found differentially expressed.
(B) Volcano plot representing the 1509 quantified proteins, of which 510 were found significantly differentially expressed.
(C) Biological processes associated with the 510 differentially expressed proteins (Reactome).
(D) Differentially expressed proteins associated with the main energy/oxidative pathways.

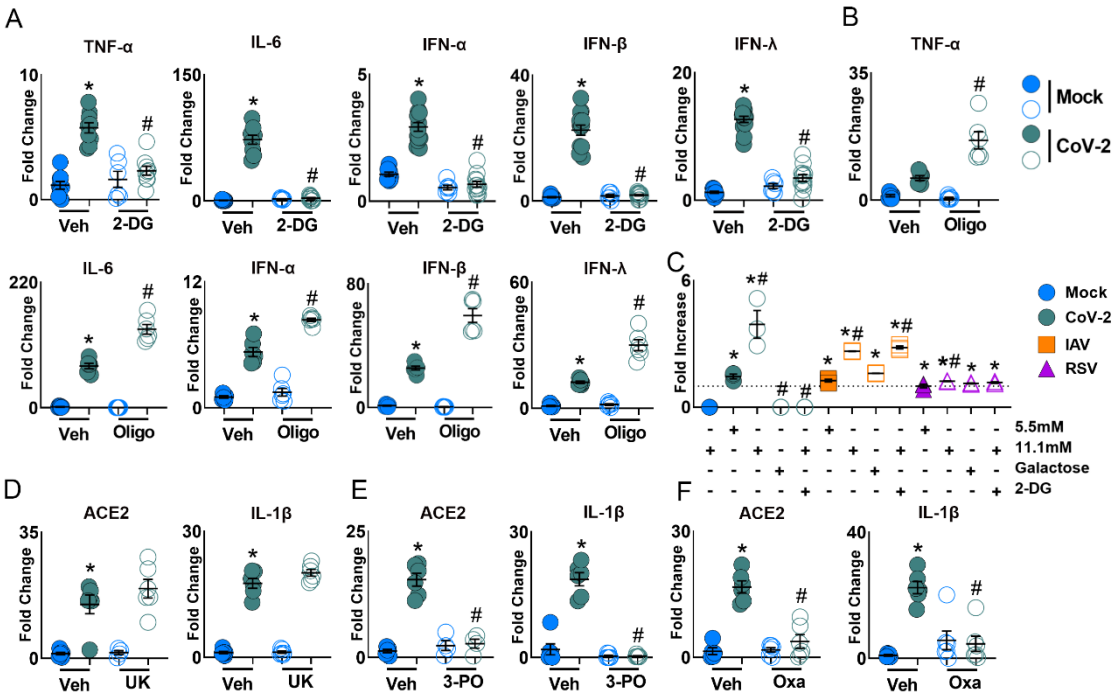

Figure S3. Related to Figure 2. Glycolysis is essential for monocyte inflammatory response to SARS-CoV-2.

Human monocytes were pre-treated with either glycolysis inhibitor 2-DG, PFKFB3 inhibitor 3-PO, LDH-A inhibitor Oxamate (OXA), mitochondrial pyruvate carrier inhibitor UK-5099 (UK) or ATP synthase inhibitor Oligomycin (Oligo) for 2 h prior to SARS-CoV-2 (CoV-2) infection (MOI 0.1) for 1 h under continuous agitation and incubation for 24 h. (A-B) Relative mRNA expression of TNF-α, IL-6, IFN-α, IFN-β and IFN-λ by qPCR in infected monocytes pre-treated with (A) 2-DG or (B) Oligo.

(C) Human monocytes were infected with mock control, CoV-2, RSV or IAV (MOI 0.1) for 1h under continuous agitation and cultured for 24 h in media containing different glucose concentrations (5.5, 11.1 mM), galactose or 2-DG and viral load was determined by qPCR.

(D-F) Relative gene expression ACE2 and IL-1β in infected monocytes pre-treated with (D) UK-5099, (E) 3-PO and (F) Oxamate.

Data are representative of at least two independent experiments performed in triplicate. Error bars represent mean ± SEM. (A-B; D-F) *P < 0.05 compared to mock. $^{\#}$P < 0.05 compared to CoV-2 (One-Way ANOVA and Tukey post hoc tests). (C) *P < 0.05 compared to mock. $^{\#}$P < 0.05 compared to its respective 5.5 mM group (One-Way ANOVA and Tukey post hoc tests).

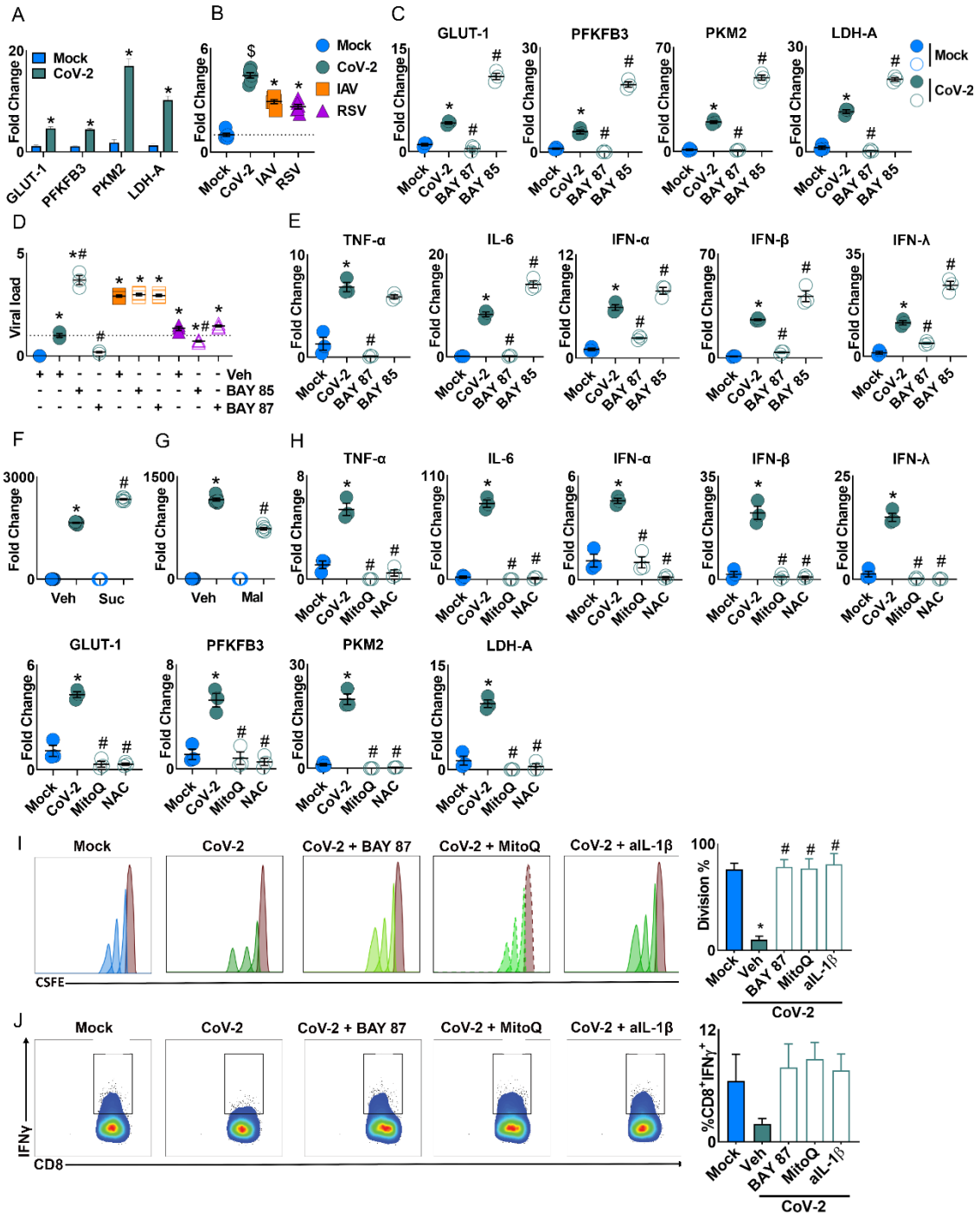

Figure S4. Related to Figure 3 and 4. mtROS/HIF-1α-axis potentiates SARS-CoV-2 induction of glycolytic enzymes and pro-inflammatory cytokines.

(A) Human monocytes were infected with mock control or SARS-CoV-2 (CoV-2) (MOI 0.1) for 1 h under continuous agitation and incubated for 24 h. Relative gene expression of target genes of HIF-1α (GLUT-1, PFKFB3, PKM2 and LDH-A).

(B) Human monocytes were infected with mock control, CoV-2, RSV or IAV (MOI 0.1) for 1 h under continuous agitation and incubated for 24 h. HIF-1α expression was assessed by flow cytometry.

(C) Human monocytes were pre-treated for 2 h with HIF-1α modulators (BAY85 and BAY87) prior to CoV-2 infection (MOI 0.1) for 1 h under continuous agitation and incubation for 24 h. Relative mRNA expression of GLUT-1, PFKFB3, PKM2 and LDH-A in monocytes pre-treated with either BAY85 or BAY87.

(D) Human monocytes were pre-treated for 2 h with HIF-1α modulators (BAY85 and BAY87) prior to CoV-2, RSV or IAV infection (MOI 0.1) for 1 h under continuous agitation and incubation for 24 h. Viral load was determined by qPCR.

(E) Human monocytes were pre-treated for 2 h with HIF-1α modulators (BAY85 and BAY87) prior to CoV-2 infection (MOI 0.1) for 1 h under continuous agitation and incubation for 24 h. Relative mRNA expression of TNF-α, IL-6, IFN-α, IFN-β and IFN-λ was determined by qPCR.

(F-G) Human monocytes were pre-treated for 2 h with cell permeable (F) Succinate (Suc) or (G) Malonate (Mal) prior to CoV-2 infection (MOI 0.1) for 1 h under continuous agitation and incubation for 24 h. Viral load was determined by qPCR.

(H) Human monocytes were pre-treated for 2 h with either MitoQ or NAC prior to SARS-CoV-2 infection (MOI 0.1) for 1 h under continuous agitation and incubation for 24 h. Relative gene expression of TNF-α, IL-6, IFN-α, IFN-β, IFN-λ, GLUT-1, PFKFB3, PKM2 and LDH-A in monocytes pre-treated with either MitoQ or NAC.

(I-J) Human lymphocytes were isolated from peripheral blood, stained with CFSE and cocultured with allogeneic PBMCs in the presence of conditioned media from mock or SARS-CoV-2 (CoV-2) monocytes pre-treated with vehicle, BAY87-2243 (BAY87) and Mitoquinol (MitoQ). Lymphocytes were also given conditioned media from CoV-2-infected monocytes with anti-IL-1β (aIL-1β). (I) Proliferation and (J) percentage of viable $CD8^{+}IFN\text{-}\gamma^{+}$ T cells were determined by flow cytometry and are shown by representative dot plots/histograms. Brown histogram represents non-proliferative cells.

All data are representative of two independent experiments performed in triplicate. Error bars represent mean ± SEM. *$P < 0.05$ compared to mock. $^{\#}P < 0.05$ compared to CoV-2 (One-Way ANOVA and Tukey post hoc test).

Table S1. Related to Figure 1 and 3. General parameters of human cohorts.

General parameters of healthy and obese/diabetic cohort				
Group	**Patient number**	**Gender**	**Age (y)**	**BMI**
Obese/Diabetic	OD1	M	52	31.1
	OD2	M	59	31.5
	OD3	F	63	36.7
	OD4	F	43	31.2
Healthy controls	HC1	M	34	26.3
	HC2	M	41	25.9
	HC3	M	58	24.7

General parameters of COVID-19 cohort					
Group	**Patient number**	**Gender**	**Age (y)**	**BMI**	**Admittance O_2 saturation (%)**
COVID-19 patients	COV1	M	50	20.2	96
	COV2	M	47	31.1	93
	COV3	M	47	24.8	91
	COV4	M	30	27.0	84
	COV5	M	57	27.1	92
Healthy controls	HC4	M	39	26.5	NA
	HC5	M	42	25.8	NA
	HC6	M	39	27.4	NA
	HC7	M	40	27.7	NA
	HC8	M	40	22.1	NA

*M, male; F, female; NA, not available.