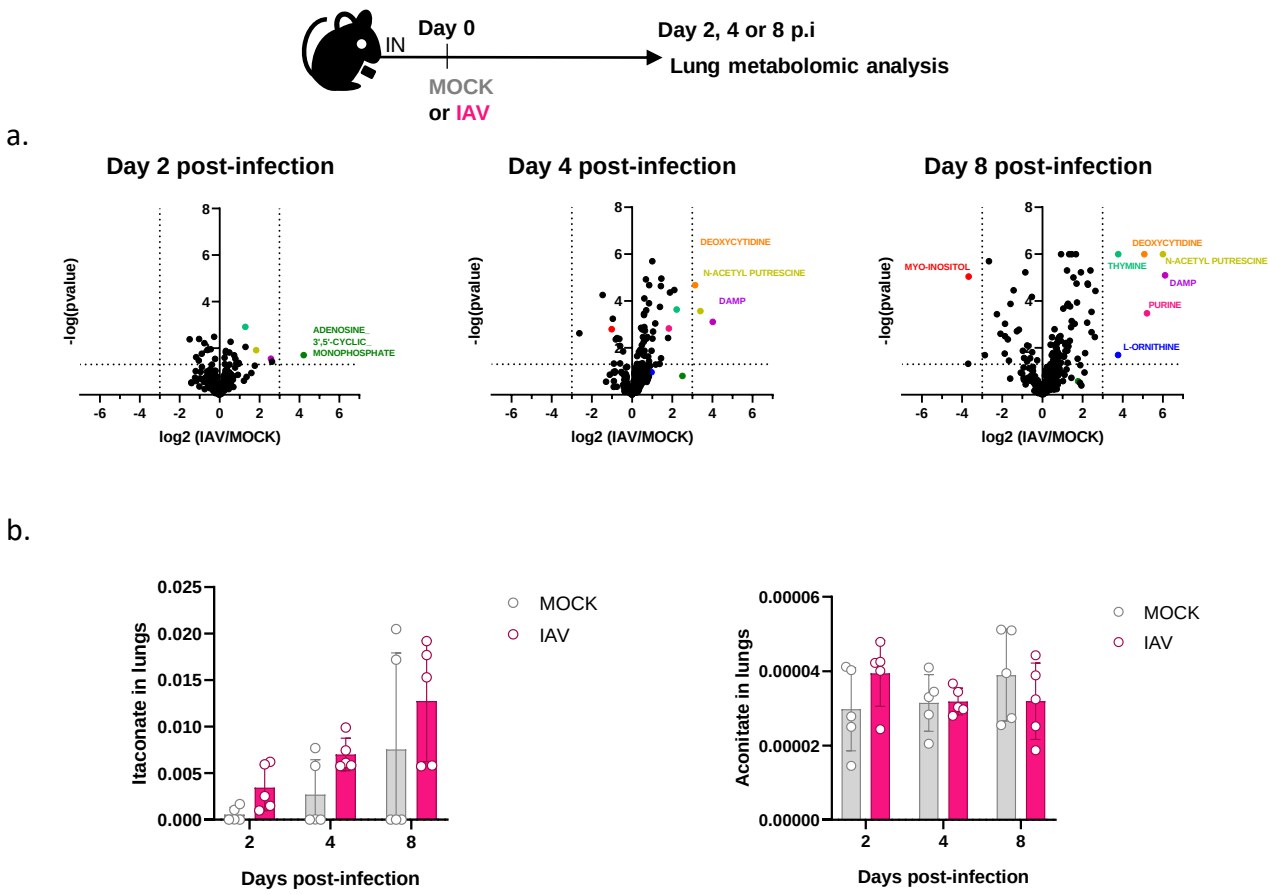
***Cis*-aconitate therapy protects against influenza mortality by dual targeting of viral polymerase and ERK/AKT/NF-κB signaling.**

Cezard *et al.*

Appendix Figures

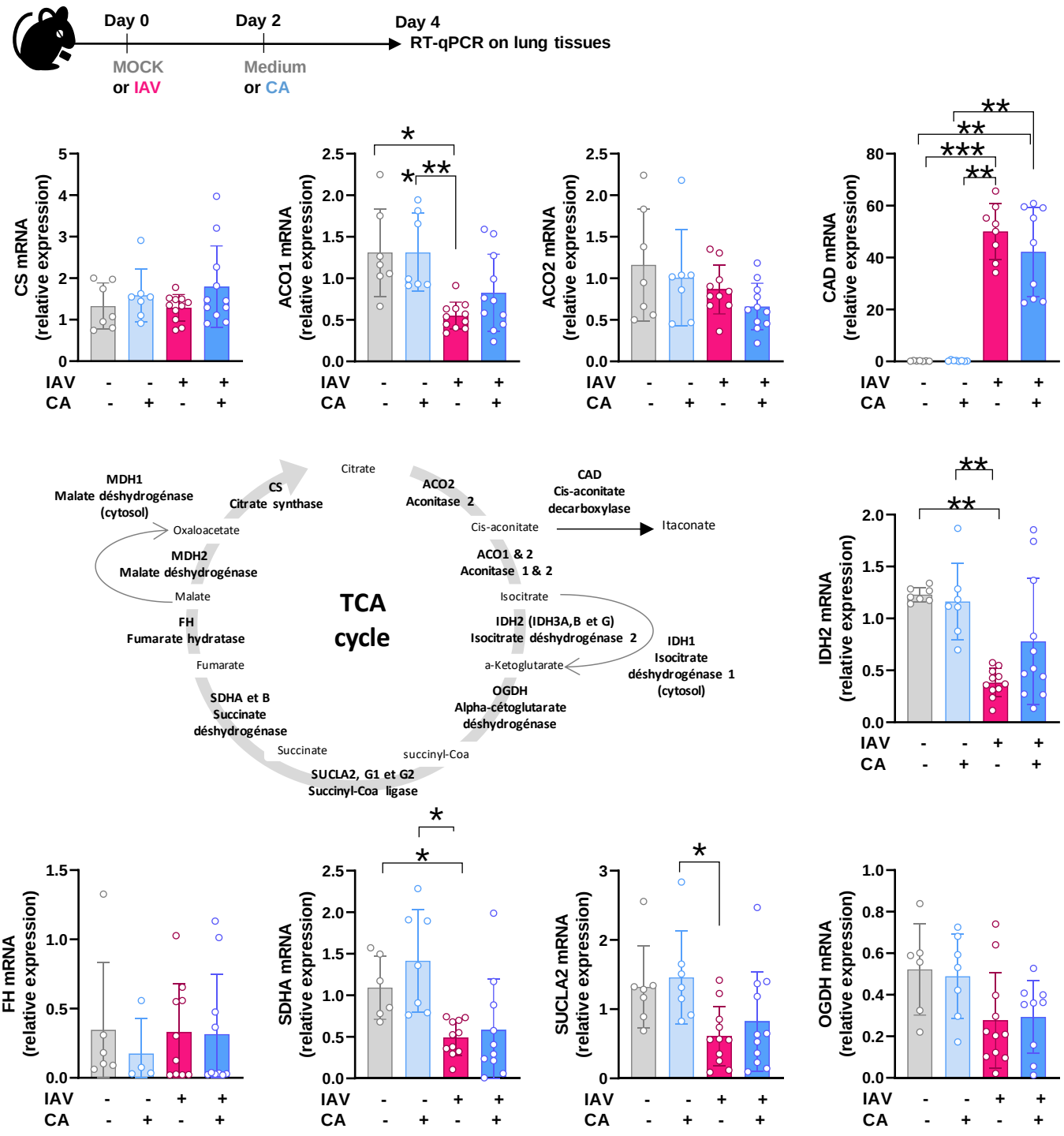
Appendix Figure S1



Appendix Figure S1. Pulmonary metabolome alterations in influenza-infected mice.

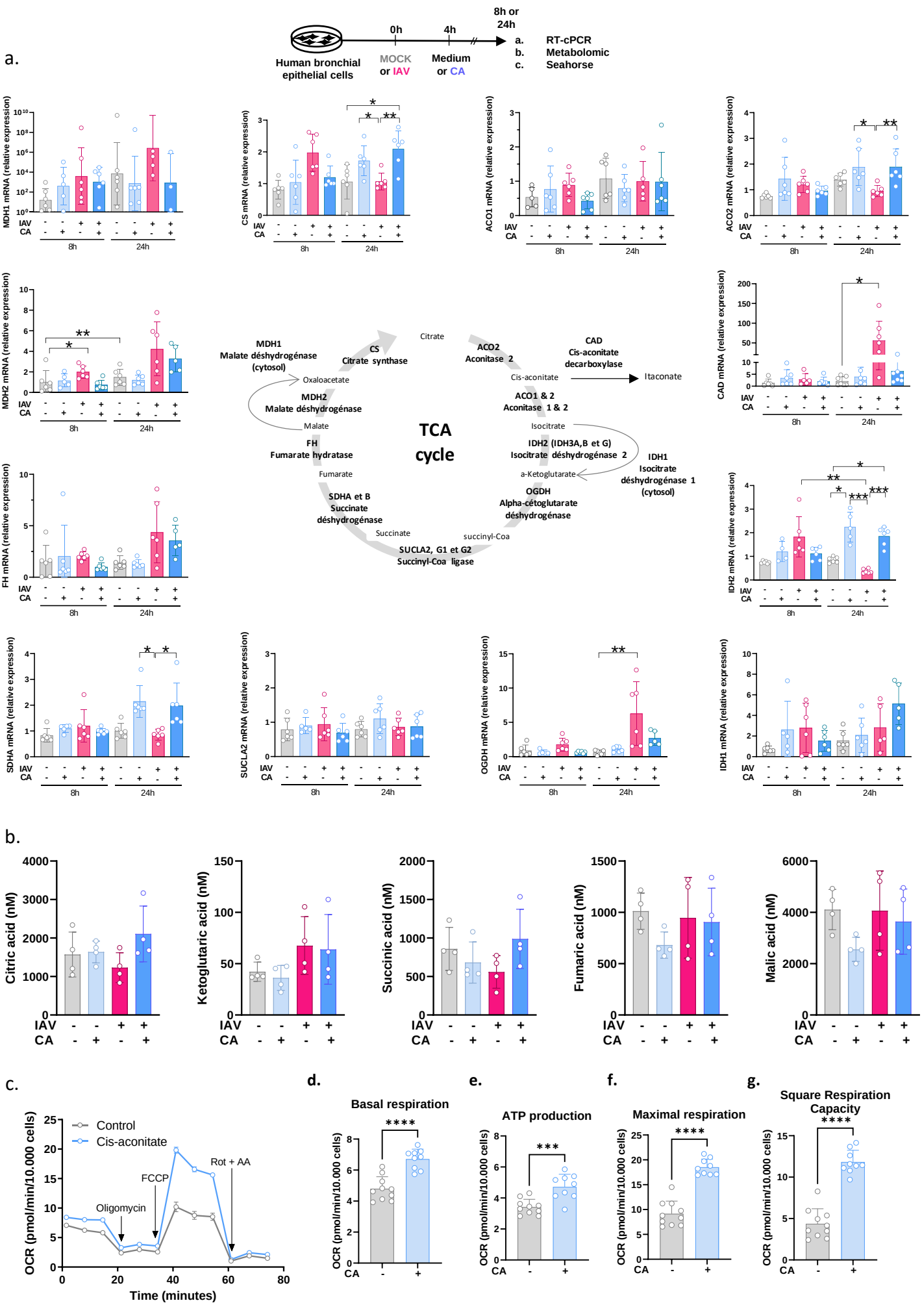
(a–e) Seven-week-old female C57Bl/6 mice were intranasally infected with 200 pfu of influenza A/Scotland/20/74 (H3N2) virus (IAV). Lungs were collected at 2, 4, and 8 days post-infection (p.i.) for metabolomic profiling by mass spectrometry. Data include results from 5 individual mice from 1 independent experiment. Principal component analysis (PCA) was used to visualize the global spectral distribution of the pulmonary metabolome (panel a). Targeted quantification of itaconate and cis-aconitate is shown in panel b. Statistical analyses were performed using multiple t-tests, with a significance threshold set at –log(p-value) ≥ 1.5 (corresponding to $p \leq 0.005$) and a log2 fold change ≥ 3 (upregulated) or ≤ –3 (downregulated)

Appendix Figure S2



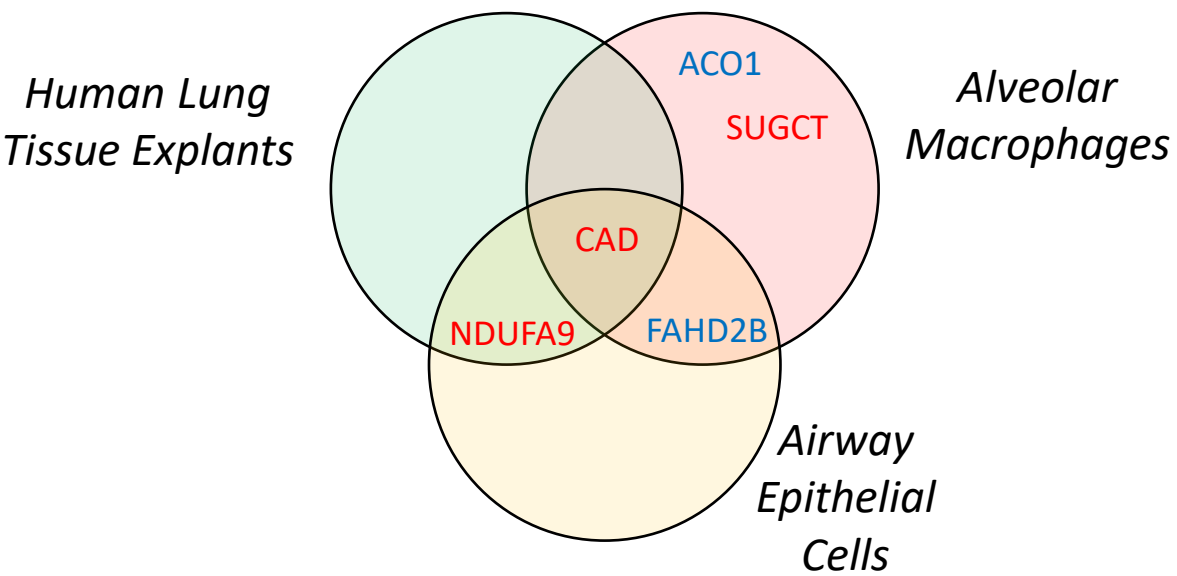
Appendix Figure S2. Influenza-induced alterations in TCA cycle enzyme expression and their modulation by cis-aconitate treatment. Seven-week-old female C57Bl/6 mice were intranasally infected with 200 pfu of influenza A/Scotland/20/74 (H3N2) virus (IAV) and treated intranasally or not with 30 mg/kg cis-aconitate (CA) at day 2 post-infection (p.i.). Lungs were collected at day 4 p.i. and the expression of key TCA cycle enzymes was quantified by RT-qPCR. Statistical analyses were performed using the Kruskal–Wallis test with Dunn's multiple comparison test. Data are presented as mean ± SEM and each dot represents one individual mouse (pooled from 3 independent experiments).

Appendix Figure S3



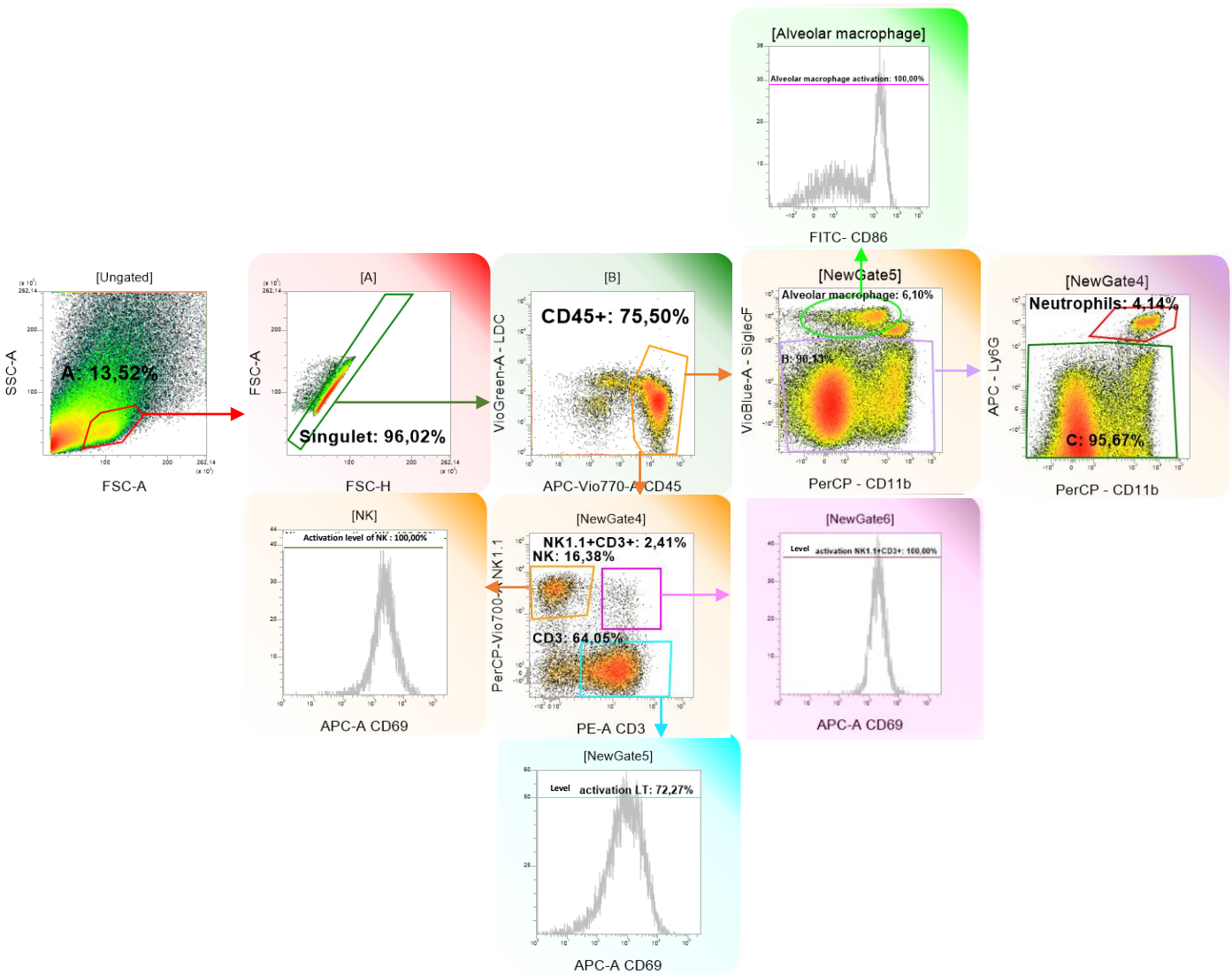
Appendix Figure S3. Influenza-induced alterations of TCA cycle enzyme expression and the effect of cis-aconitate (CA) treatment in human bronchial epithelial cells. BEAS-2B cells were infected with influenza A/Scotland/20/74 (H3N2) at a multiplicity of infection (MOI) of 1 for 4 h, then washed and treated with 3.4 mM cis-aconitate or left untreated. Expression of TCA cycle enzymes was quantified by RT-qPCR at 8 and 24 h post-infection (a), and intracellular levels of key TCA cycle metabolites were measured by mass spectrometry at 24 h post-infection (b). Mitochondrial respiration was assessed using the MitoStress test on an XFe24 analyzer (c–g), including real-time monitoring of oxygen consumption rate (OCR) in cells treated or not with cis-aconitate for 20 h (d), basal respiration prior to oligomycin injection (e), ATP-linked OCR calculated as the difference between basal OCR and OCR after oligomycin (f), and maximal respiration following FCCP injection with spare respiratory capacity calculated as the difference between maximal and basal OCR (g). Results are presented as mean ± SEM from 4 independent experiments (a–b) or individual well values (d–g). Statistical analyses were performed using the Kruskal–Wallis test with Dunn's multiple comparison test (a–b) or the Wilcoxon matched-pairs signed rank test (d–g).

Appendix Figure S4



Appendix Figure S4. Comparison of metabolic enzyme expression changes induced by influenza infection across different primary lung cell types. Expression of metabolic enzymes in alveolar macrophages, airway epithelial cells, and human lung tissue explants was analyzed based on data from Bertrams et al. [77]. Upregulated genes are shown in red, and downregulated genes in blue.

Appendix Figure S5



Appendix Figure S5. Flow cytometry gating strategy.

Surface gating used to define immune cell subsets in Fig. 5 and Supplementary figure 3.