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Supplemental information

Influenza A induces lactate formation to inhibit type I IFN in primary human airway epithelium

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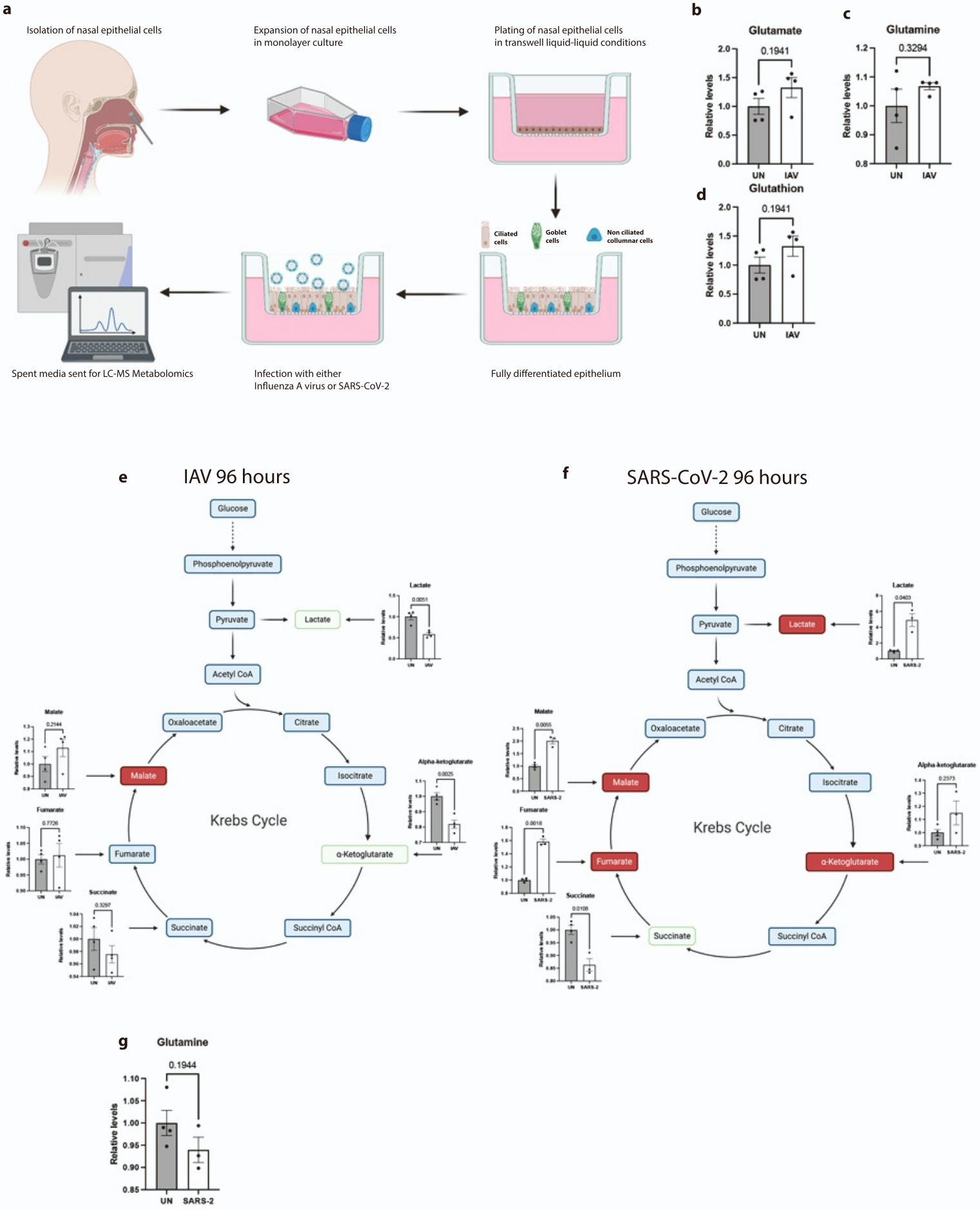

Figure S1 – HPAE-ALI cells infected with either IAV or SARS-CoV-2 yield virus specific metabolic changes (related to figure 1): (a) Graphical representation of HPAE-ALI generation and metabolomics experiment. Figure made in Biorender. (b-d) HPAE-ALI cultures either uninfected or infected with IAV for 48 hours. Measurements done on baso-lateral medium by MS-Omics. Data displayed in each figure represents one experiment with n=4. (e-f) HPAE-ALI cultures either uninfected or infected with IAV (e) or SARS-CoV-2 (f) for 96 hours. Measurements done on baso-lateral medium by MS-Omics. (g) Glutamine measurement from same experiment as (f). Data displayed in each figure represents one experiment with n=4. Error bars represent standard error of the mean (s.e.m.). Unless otherwise stated, statistical analyses by Welch's t-test.

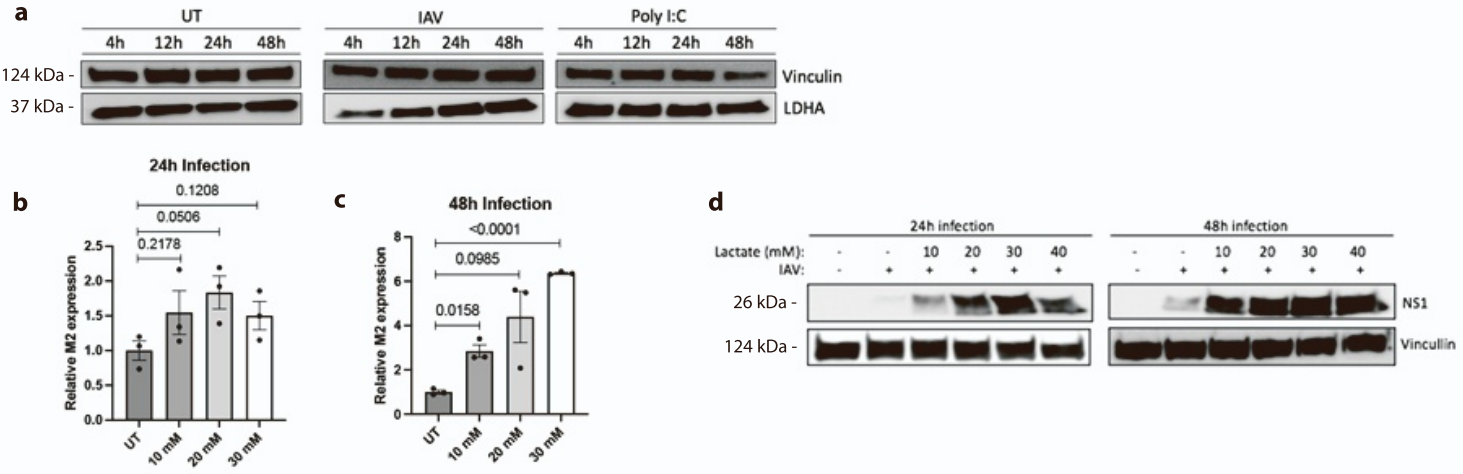

Figure S2 – IAV infection yield increased LDHA expression which produce pro-viral lactate (related to figure 2 and 3):
(a) Western blot for Vinculin and LDHA of A549 cells either left untreated (UT), infected with IAV at 60 HAU/10^6 cells or transfected with Lipofectamin (0,5 ug/ml) delivered Poly I:C (0,25 ug/ml). Data displayed represents one experiment.
(b-c) qPCR on IAV M2 RNA after 24 or 48 hours of IAV infection (60 HAU/10^6 cells) in A549 cells either untreated of with increasing concentrations of Lactate. Results repeated multiple times. n = 3. (d) Western blot on IAV NS1 protein and vinculin after 24 or 48 hours of IAV infection (60 HAU/10^6 cells) in A549 cells either untreated of with increasing concentrations of Lactate. Experiment repeated multiple times. Error bars represent standard error of the mean (s.e.m.).
Unless otherwise stated, statistical analyses by Welch's t-test.

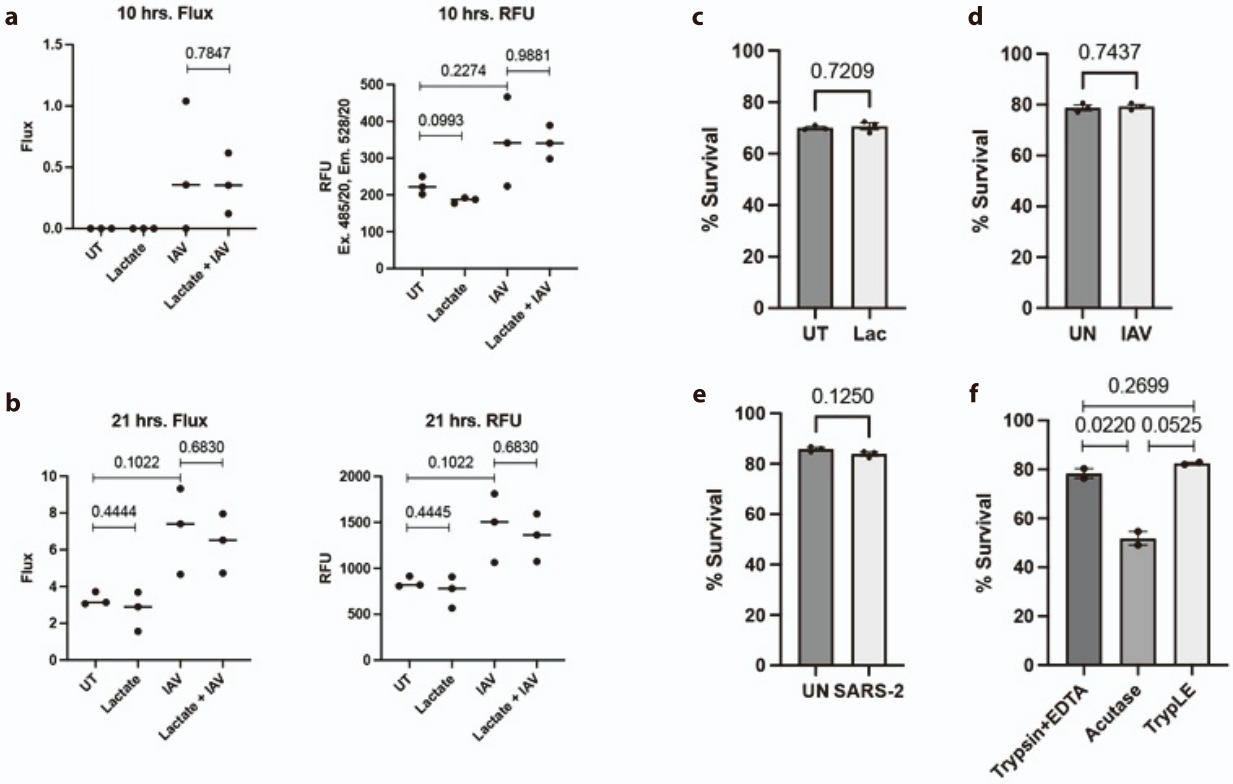

Figure S3 – Changes to morphology and survival rate of HPAE-ALI cultures (related to figure 3):
(a-b) Flux analysis using FITC-Dextran on the same HPAE-ALI cultures at 10 and 21 hrs.
Cultures were pretreated with 10 mM Lactate and infected with IAV at 60 HAU/10^6 cells.
Each dot represents the average of three technical replicates of one biological replicates.
Experiment done once with n=3. The bar represents the median value. (c) HPAE-ALI cultures pretreated with
10 mM lactate or left UT for 1 hour before harvest with Trypsin/EDTA, analyzed by flow cytometry.
Experiment done once with three biological replicates. (d) & (e) HPAE-ALI cultures infected for 1 hours with
either 300 HAU/10^6 cells for IAV or MOI 0.1 SARS-CoV-2 before harvest with Trypsin/EDTA, analyzed by flow cytometry.
Experiment done once with three biological replicates. (f) HPAE-ALI cultures harvested using either Trypsin/EDTA,
Acutase and TrypLE. Baso-lateral medium removed and harvesting agent added in both baso-lateral and apical compartments.
Cells were harvested upon release from the membranes and flow cytometry was performed to asses cell viability.
Experiment done once with two biological replicates. Each bar represents S.E.M.
Unless otherwise stated, statistical analyses by Welch's t-test.

Supp. fig. 4

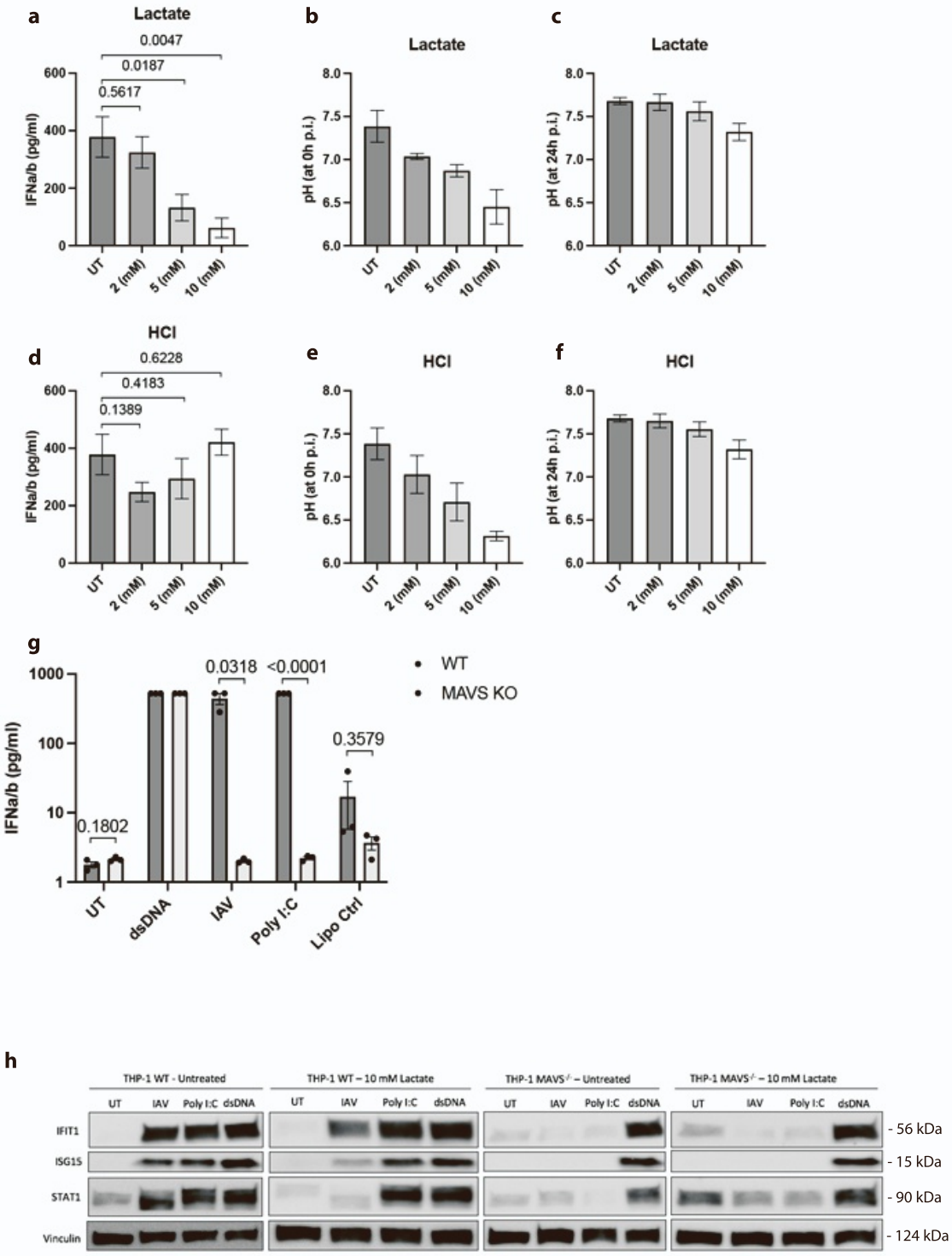

Figure S4 – Lactate negatively regulate IFN production during MAVS signaling in a pH independent way (related to figure 4): (a)HEK-blue bioassay on supernatants THP-1 cells treated with indicated concentrations of Lactate and infected with IAV at 60 HAU/10^6 cells. Experiment repeated twice with n=3 in each. (b-c) pH measurements from (a) measured at time of infection (0h) and 24 hours post infection. (d) HEK-blue bioassay on supernatants THP-1 cells treated with indicated concentrations of HCl and infected with IAV at 60 HAU/10^6 cells. Experiment repeated twice with n=3 in each. (e-f) pH measurements from (d) measured at time of infection 0h and 24 hours post infection. (g) HEK-blue bioassay on THP-1 WT or MAVSko cells either infected with IAV at 60 HAU/10^6 cells, treated with lipofectamin tranfected 0,5 ug Poly I:C or treated with lipofectamin transfected 1,75 ug dsDNA. Experiment repeated twice with n=3. (h) Western blot for IFIT1, ISG15, STAT1 and Vinculin on THP-1 WT or MAVSko cells either untreated or treated with 10 mM Lactate followed by either IAV at 60 HAU/10^6 cells, lipofectamin transfected with 0,5 ug Poly I:C or lipofectamin transfected 1,75 ug dsDNA. Error bars represent standard error of the mean (s.e.m.). Unless otherwise stated, statistical analyses by Welch's t-test.