

Expanded View Figures

Figure EV1. Anti-influenza effect of succinate is more potent in multicycle replication condition than in single cycle replication condition.

BEAS-2B were infected with influenza A/Scotland/20/ 74 (H3N2) virus (IAV) at MOI = 1 (single cycle replication) or at MOI = 10^{-3} (multicycle replication) for 4 h and treated or not with succinate (Suc) for 20 h. 2 µg/ml of TPCK treated Trypsin were added simultaneously to succinate to promote multicycle replication.

- A, B Plaque-Forming Unit assays determined the production of infectious viral particles in cell supernatants.
- C The effect of succinate on IAV transcription was assessed by RT-qPCR to quantify M1 viral mRNA. Data information: Data are represented as the mean \pm SEM of 3 independent experiments. Statistical analysis was performed using paired *t*-test (A, B) or one sample *t*-test (C), (*P < 0.05 and **P < 0.01).



Figure EV2. Nucleoprotein localization determined by flow cytometry.

BEAS-2B cells were infected with influenza A/Scotland/20/74 (H3N2) virus (IAV)) at MOI 1 for 4 h and treated or not with succinate (Suc) for 20 h. Nucleoprotein (NP) expression was assessed by flow cytometry using two different permeabilization buffers, one allowing whole cell fluorescent staining of NP, the other only the NP-associated to the cytoplasmic compartment (by subtraction, nuclear-associated NP signal was extrapolated). Data are represented as the mean \pm SEM of 4 independent experiments. Statistical analysis was performed using ANOVA with Sidak's posttest (**P < 0.01).



Figure EV3. Succinate does not alter the CRM1-dependent transport pathway or the NP oligomerization.

- A, B Human bronchial epithelial BEAS-2B cells were infected or not with the A/Scotland/20/74 (H3N2) virus (IAV) at MOI = 1 for 4 h, and subsequently treated or not with 4 mg/ml of succinate for 20 h (A) or with 10 mM of the CRM1 inhibitor leptomycin B (LMB; *middle panels* in B). The expression of CRM1 was analyzed by Western blotting (A). Expression of the viral protein NEP/NS2 was analyzed by confocal fluorescence microscopy (B). NEP/NS2 is stained in green, nuclear DNA in blue and actin cytoskeleton in red. Scale bar: 10 μM.
- C The formation of NP oligomers and monomers was assessed by Western blotting under nonreducing and reducing conditions.

Data information: Pictures are representative of three independent experiments.