Supplementary information

Influenza A viruses alter the stability and antiviral contribution of host E3-ubiquitin ligase Mdm2 during the time-course of infection

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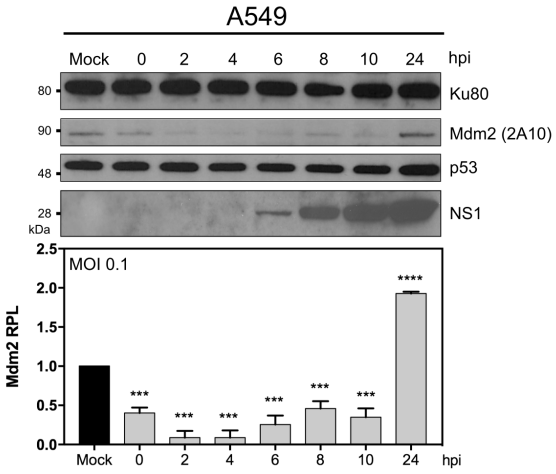
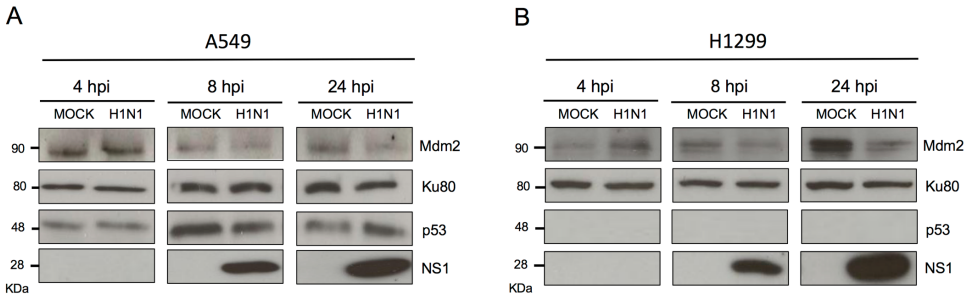
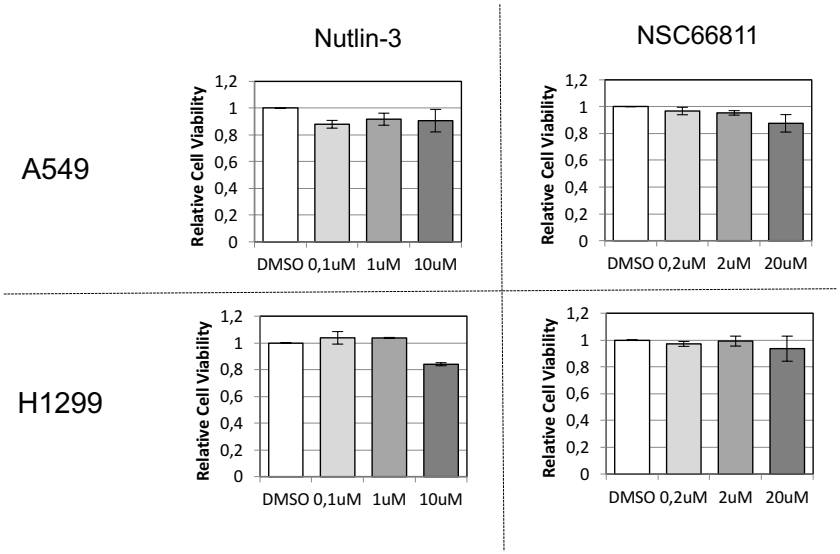


Supplementary Figure 1. Mdm2 expression is strongly impacted by IAV infection, mostly at post-transcriptional levels. A. Human lung A549 cells were mock-infected or infected by influenza A/Moscow/10/99 (H3N2) at a MOI of 0.1 and cell lysates were analyzed by western blot for the expression of Mdm2 (2A10 antibody), p53 and IAV NS1 at different time-points post infection. Ku80 was used as a loading control. Mdm2 relative protein levels (Mdm2 RPL) were measured by densitometry and calculated from data from three independent experiments. *** and **** for *P*-value < 0.001 and 0.0001, respectively.



Supplementary Figure 2. Mdm2 expression is strongly impacted by IAV infection. Human lung A549 cells (A) or H1299 cells (B) were mock-infected or infected by influenza A/PuertoRico/8/1934 (H1N1) at a MOI of 4 or 0.1 and cell supernatants and lysates were harvested at 4, 8 or 24hpi, respectively. Endogenous Mdm2 and p53, IAV NS1 expression were measured at protein level by western blot. Ku80 was used as a loading control.



Supplementary Figure 3. Cell viability after Nutlin-3 or NSC66811 treatment. Human lung A549 cells or H1299 cells were treated with DMSO or NTlin-3/NSC66811 at different concentrations. Cell viability was measured at 72 h post-treatment using a colorimetric cell proliferation assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS), Promega). Results were obtained from three independent experiments and were expressed as relative values compared to DMSO control treatment.