Influenza A virus-induced host caspase and viral PA-X antagonise the antiviral host factor, histone deacetylase 4

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Items: Supplementary Figures

Figure S1

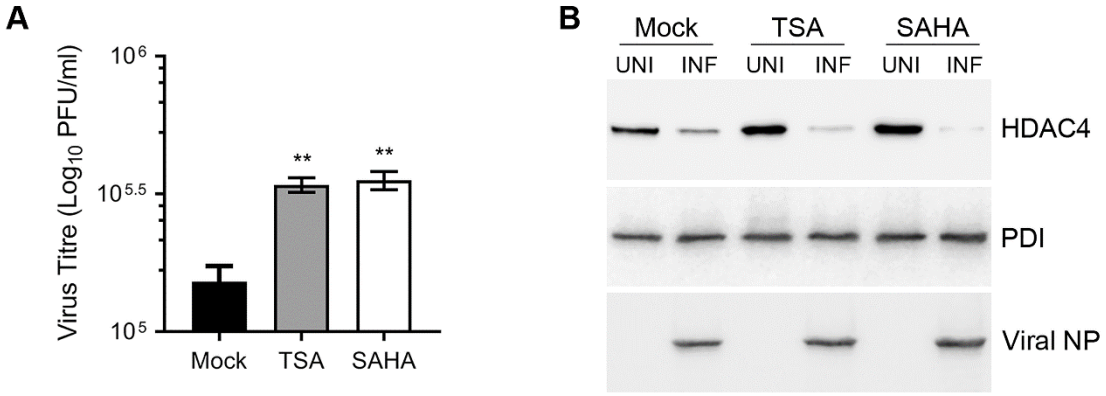

Fig. S1. The treatment with HDAC inhibitors enhances IAV infection. A549 cells were infected with PR8 at a MOI of 1.0 and, after removing the virus inoculum treated with 1 µM of TSA or SAHA. After 24 h of infection, the culture media and the cells were harvested separately. (A) The culture medium was titrated by microplaque assay to determine the titres of released viral progeny. Error bar represents the means ± standard errors of the means of three biological replicates. The asterisks represent P values (0.002 for TSA and 0.001 for SAHA) calculated by ANOVA, and indicate significant differences in means. (B) The total lysates of uninfected (UNI) and infected (INF) cells were prepared, and the HDAC4, PDI and viral NP polypeptides were detected by WB. Note: The HDAC4 blot was reused to probe for PDI.

Figure S2

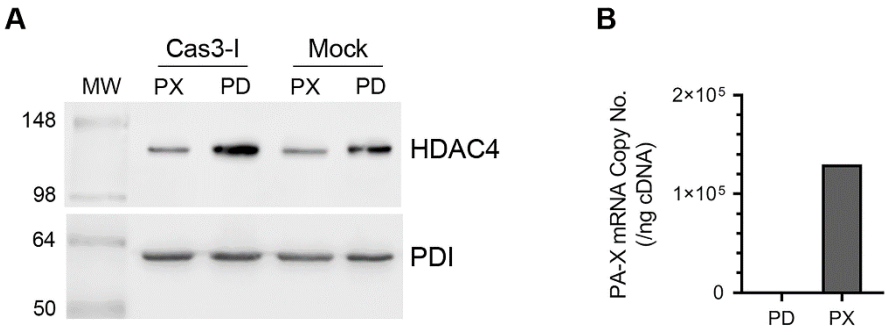

Fig. S2. Treatment of PA-X overexpressing cells with caspase 3 inhibitor. A549 cells were transfected with either empty plasmid pcDNA3 (PD) or PA-X plasmid (PX) for 48 h with (Cas3-I) or without (Mock) caspase 3 inhibitor (40 µM), the latter in duplicate. (A) Cells were harvested and the HDAC4 and PDI polypeptides were detected in total cell lysates by WB. Note: The HDAC4 blot was reused to probe for PDI. (B) The second set of mock-treated cells was processed to determine the PA-X mRNA copy number by absolute qPCR using the PA-X plasmid calibrator as described in Fig. 5F.

Figure S3

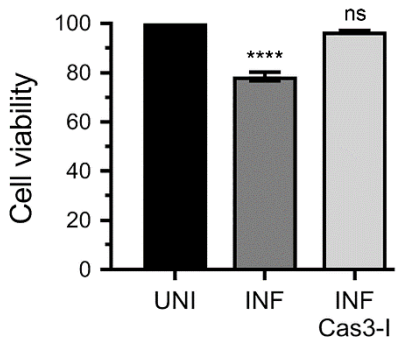

Fig. S3. Viability of IAV infected cells. A549 cells were infected with PR8 at a MOI of 1.0 and, after removing the virus inoculum cells were treated with or without caspase 3 inhibitor (40 µM). After 24 h, the viability of cells was determined by MTT assay. Then, the viability of respective uninfected cells (UNI) was considered 100% to compare the viability of infected cells treated with (INF-Cas3-I) or without (INF) caspase 3 inhibitor. The asterisks represent P value (<0.0001) calculated by ANOVA, and indicate significant differences in means; ns, non-significant.