Expanded View Figures

Figure EV1. Contemporary IAV strains lacking PB1-F2 do not display an *in vivo* phenotype in mice or murine cells.

A THP-1-derived macrophages were infected with the wild-type (Wt) and ΔF2 H5N1 (VN) virus at MOI 5 for 24 h. Levels of IL-1β were measured by ELISA. The mean ± standard deviation of five independent experiments is shown. Statistical analysis was performed by one-way ANOVA, and *P*-values are indicated.

B THP-1-derived macrophages were infected with the wild-type (Wt) and ΔF2 H5N1 (VN) virus at MOI 10 for 12 h. Levels of IL-1β were measured by ELISA. The mean ± standard deviation of three independent experiments is shown. Statistical analysis was performed by one-way ANOVA, and *P*-values are indicated.

C Lung viral loads from 5 individual mice on day 3 post-infection were assessed by plaque assay on MDCK cells. Median titers are indicated Statistical analysis was performed by Student's *t*-test as compared to mock samples (*$P < 0.05$, **$P < 0.01$).

D Groups of $n = 5$ mice were mock treated or infected with 40 pfu of the VN Wt or VN ΔF2 strains for 3 days. LDH assay was performed on BAL samples in technical duplicates ($^{\#}P < 0.05$ compared with wt **$P < 0.01$ compared with mock).

E ELISA for IL-1β in BALF of mock ($n = 3$), VN Wt ($n = 5$) or VN ΔF2 ($n = 5$) infected mice.

F–O Groups of mice were mock treated ($n = 2$) or infected with 40 pfu of the VN Wt ($n = 5$) or VN ΔF2 ($n = 4$–5, of note for F–H the staining for macrophages for the red sample failed due to technical problems and is not indicated) for 7 days. Flow cytometry data for indicated cell populations. Statistical analysis was performed by one-way ANOVA, and *P*-values are indicated. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

P Groups of $n = 3$–5 mice were infected with 40 pfu of the VN Wt or VN ΔF2 strains for 8–11 days. Lung viral loads were assessed by plaque assay on MDCK cells.

Q *Streptococcus pneumoniae* colonies in serial dilutions of lung homogenate are shown in trypcase soy agar +5% sheep blood plates. Representative results are shown in technical triplicates

R *Streptococcus pneumoniae* bacteria in the lungs (cfu/organ) 24 h post-infection from mock treated, VN Wt or VN ΔF2 pre-infected animals (mock $n = 4$, virus infected $n = 5$). Median values ± SD are indicated. Statistical analysis was performed by one-way ANOVA *P*-values toward mock samples are indicated. (**$P < 0.01$, ***$P < 0.001$).

S ELISA for IL-1β secretion in BMDMs ($n = 3$) mock treated of infected with VNWt and ΔF2 strains. Statistical analysis was performed by one-way ANOVA, and *P*-values are indicated. Median values ± SD are indicated. *P*-values toward mock samples are indicated. (****$P < 0.0001$).

T Immunoblot of cell lysates from (S). For all panels, the median of the indicated independent experiments is shown.

Source data are available online for this figure.

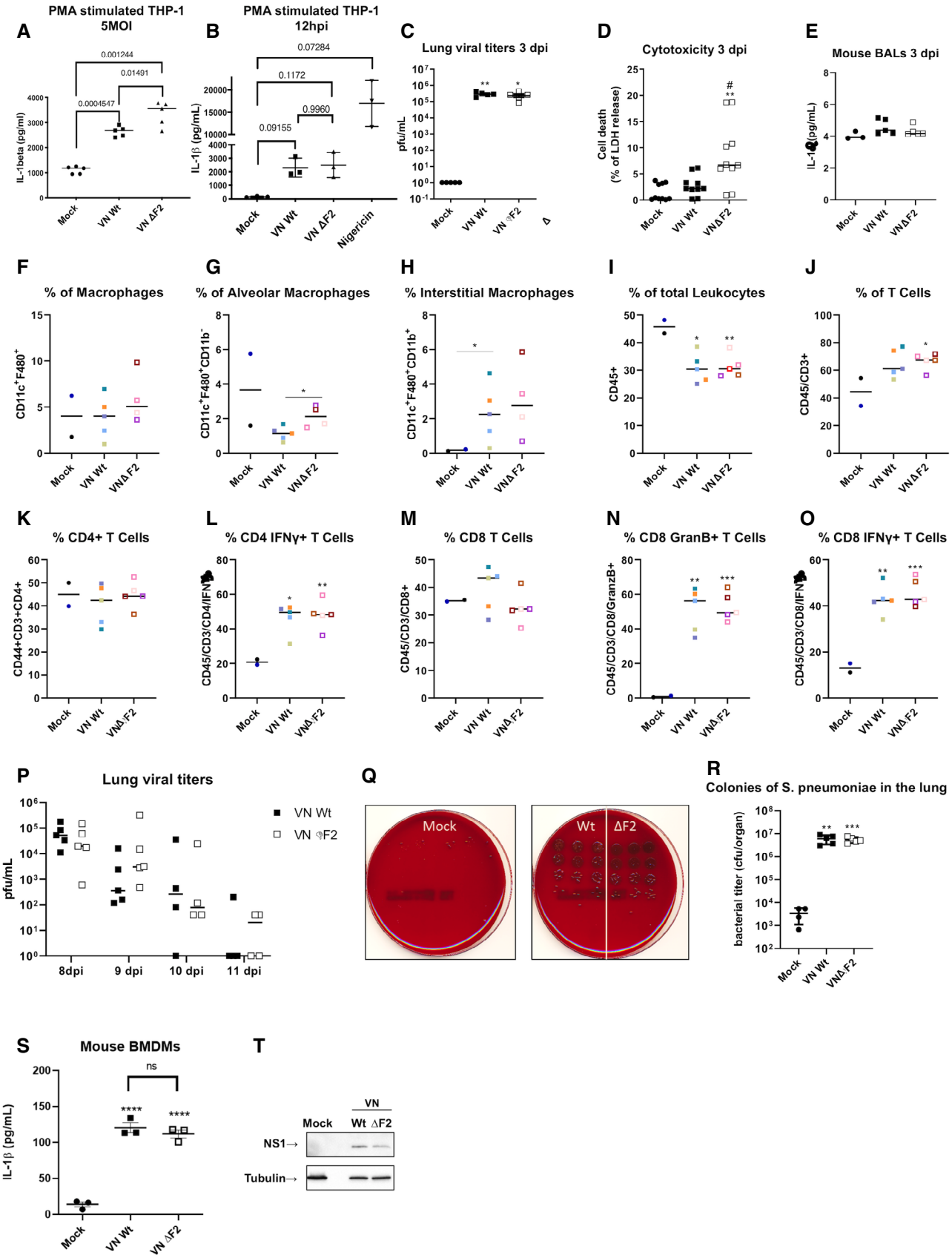

Figure EV1.

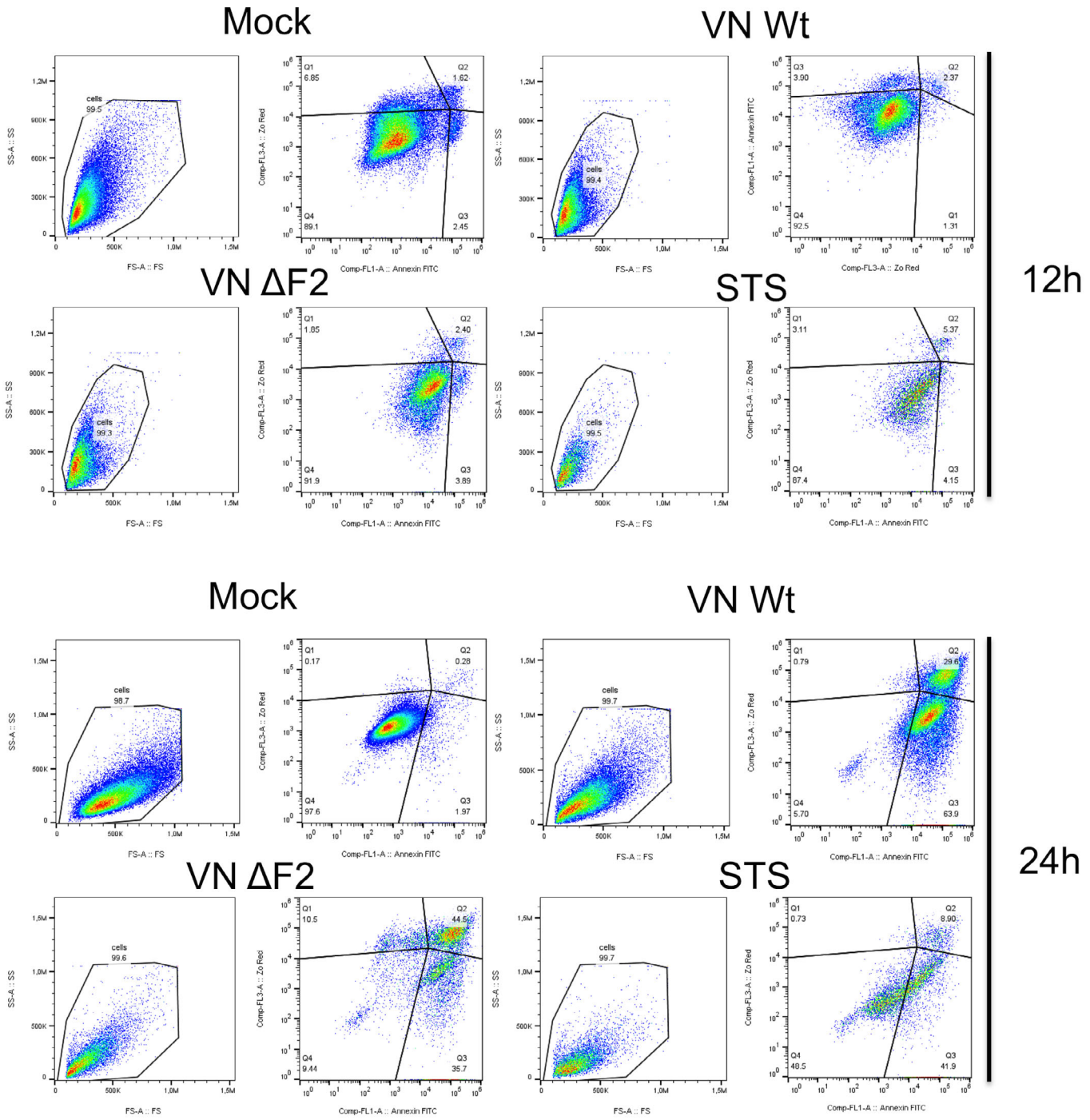

Figure EV2. Representative FACS plots for Annexin V and Zombie-Red staining.

Upper panels show representative panels for Fig 2A/B, lower panels show representative panels for Fig 2C/D.

Figure EV3. Induction of NF-κB-dependent genes upon H5N1 infection.

A–X THP-1 cells were mock treated or infected with the VN Wt and ΔF2 strains for 4 h (A–H), 8 h (I–O), 18 h (P), and 24 h (Q–X) at MOI MOI 6 (I–O)-10 (A–H and P–X). Relative expression levels normalized to the average of mock were determined by RT–qPCR for indicated mRNAs normalized to 18S rRNA and calibrated to mock. The mean ± standard deviation of 2–6 independent experiments with at least two technical replicates each is shown (each dot represents an independent biological sample). Statistical analysis was performed by paired two-tailed Student's *t*-test, and *P*-values are indicated.

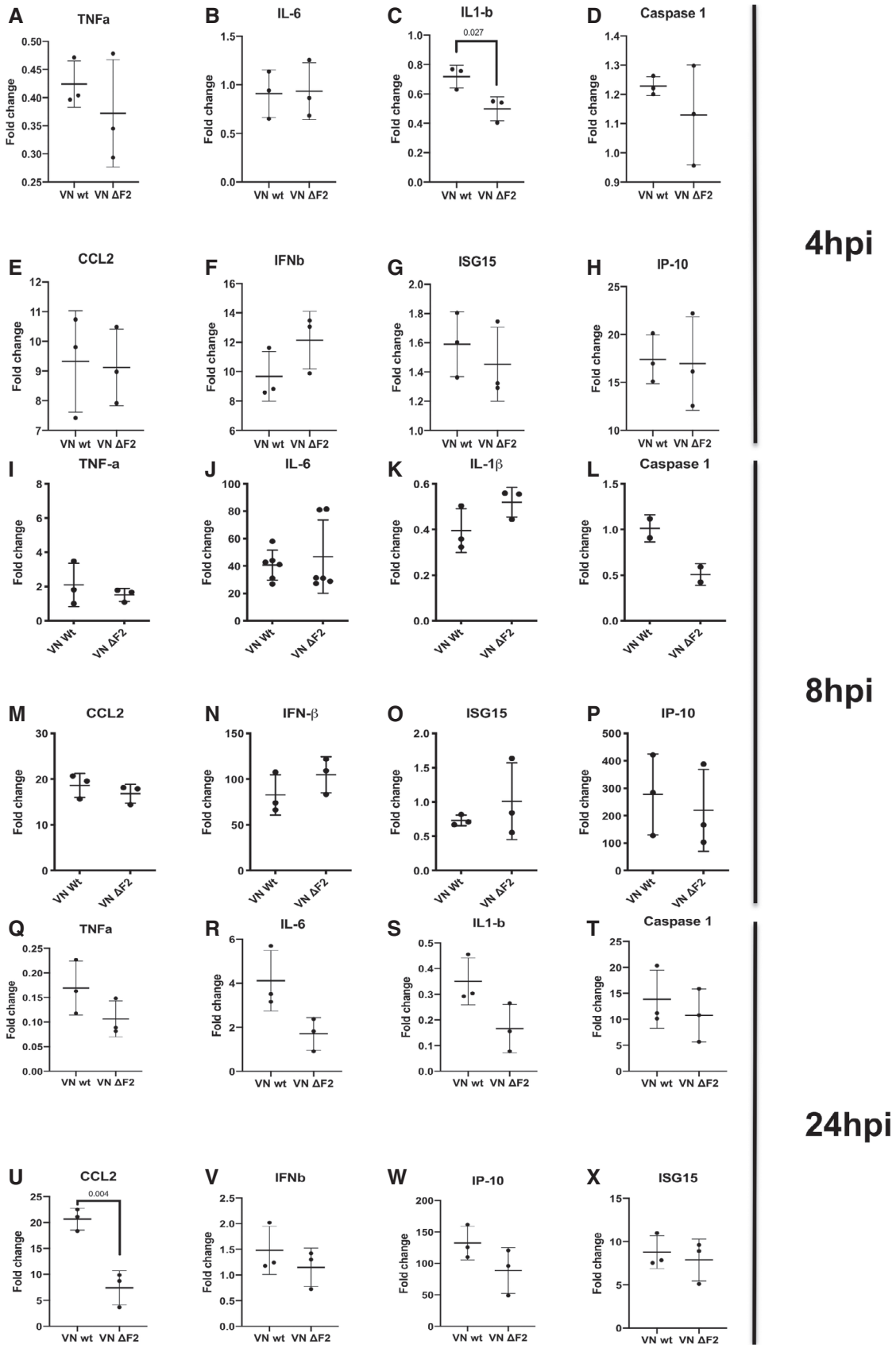
A TNFa
B IL-6
C IL1-b
0.027
D Caspase 1
E CCL2
F IFNb
G ISG15
H IP-10
4hpi
I TNF-a
J IL-6
K IL-1β
L Caspase 1
M CCL2
N IFN-β
O ISG15
P IP-10
8hpi
Q TNFa
R IL-6
S IL1-b
T Caspase 1
U CCL2
0.004
V IFNb
W IP-10
X ISG15
24hpi
Fold change
VN wt
VN ΔF2
VN Wt

Figure EV3.

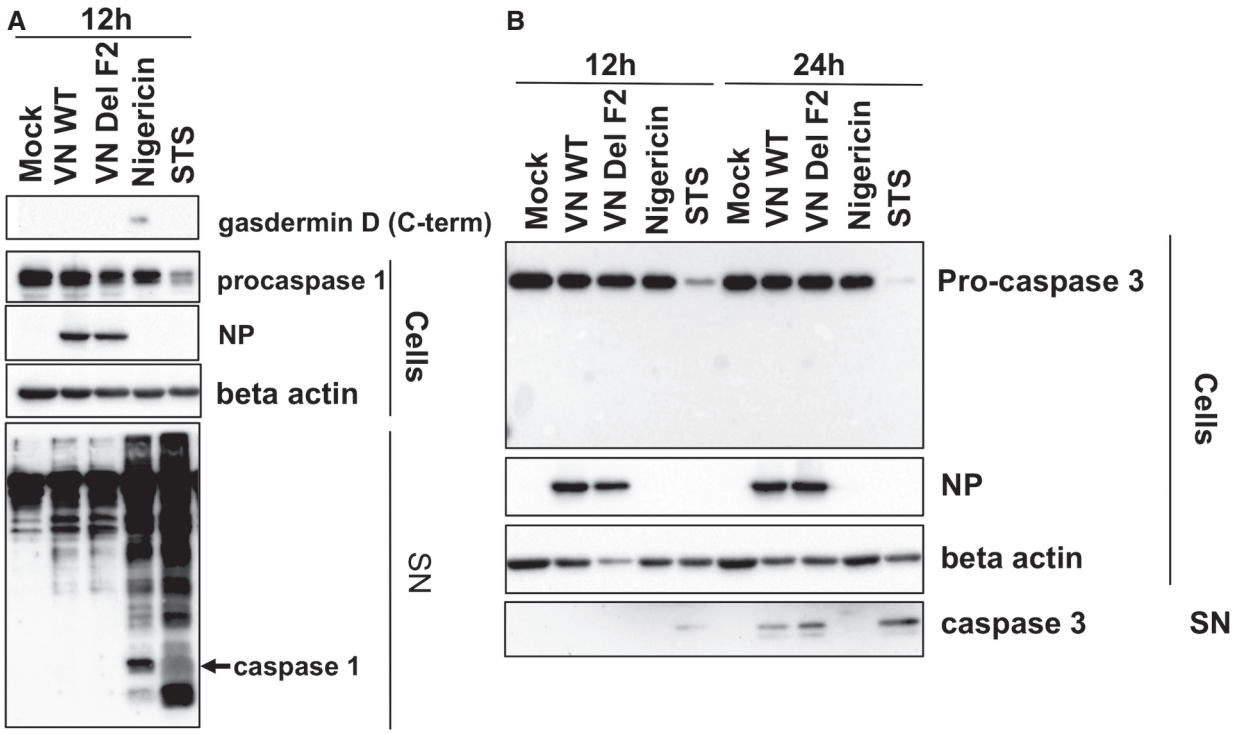

Figure EV4. IAV dependent activation of the NLRP3 inflammasome and caspase 3 after 12 and 24 h.

A Supernatants of THP-1 cells infected with VN Wt and ΔF2 virus at a MOI 10 for 12 h or treated with 6.7 μM of nigericin for 45 min or treated with 1 μM of staurosporine for 12 h or 24 h were analyzed by immunoblotting against gasdermin D, caspase 1, NP, and beta actin ($n = 3$).

B Supernatants and cell lysates of THP-1 cells infected with VN Wt and ΔF2 virus at a MOI 10 for 12 h or treated with 6.7 μM of nigericin for 45 min or treated with 1 μM of staurosporin for 12 h were analyzed by immunoblotting against caspase 1, NP, and beta actin ($n = 3$).

Source data are available online for this figure.

