Cell Reports

Supplemental Information

Acute Lung Injury Results from Innate Sensing of Viruses by an ER Stress Pathway

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Figure S1

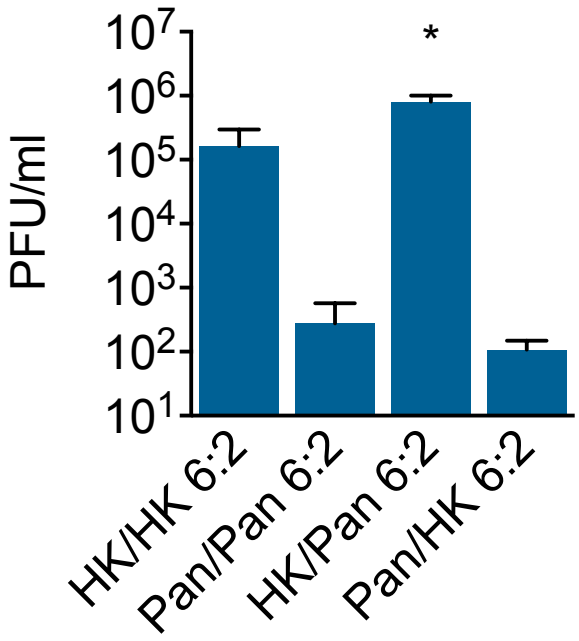

Figure S1 Pandemic surface proteins alter virus accumulation, related to Figure 1

Groups of five BALB/c mice were infected intranasally with 10^4 PFU of viruses carrying different combinations of pandemic (HK) and seasonal (Pan) HA/NA surface proteins on a 6 gene segment PR8 backbone (HK/HK 6:2, Pan/Pan 6:2, HK/Pan 6:2 and Pan/HK 6:2). Virus lung titers on d3 after infection are depicted as PFU/ml. Data are represented as mean with SD. An asterisk (*) indicates statistically significant differences ($P < 0.05$) between HK/PAN 6:2 and all other groups.

Figure S2

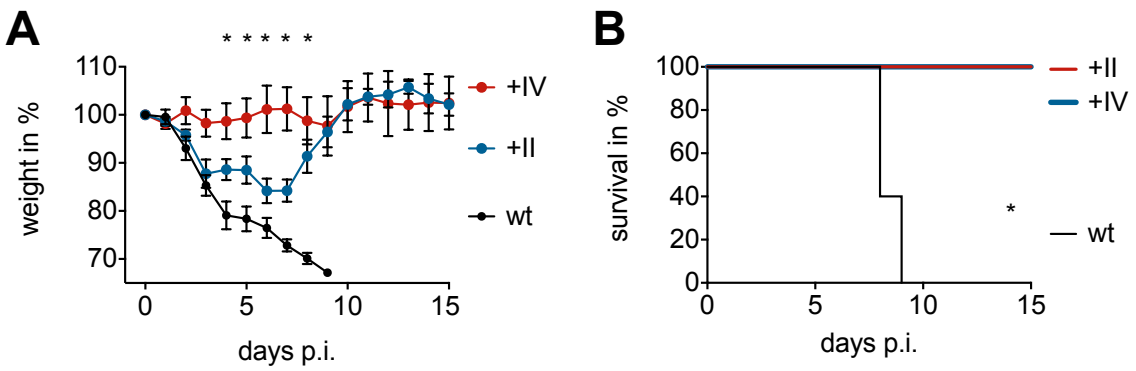

Figure S2 Pandemic HA mediated pathogenicity is mouse strain independent, related to Figure 1.

(A, B) Five C57BL/6 mice per group were infected intranasally with 10^5 PFU of isogenic viruses carrying differentially glycosylated HA globular heads (HK wt, HK +II and HK +IV). (A) Body weight change over the course of infection (15 days) is depicted as mean with SD and (B) Kaplan-Meier survival curve analysis was performed. An asterisk (*) indicates statistically significant differences ($P < 0.05$) between HK wt infected animals and all other groups.

Figure S3

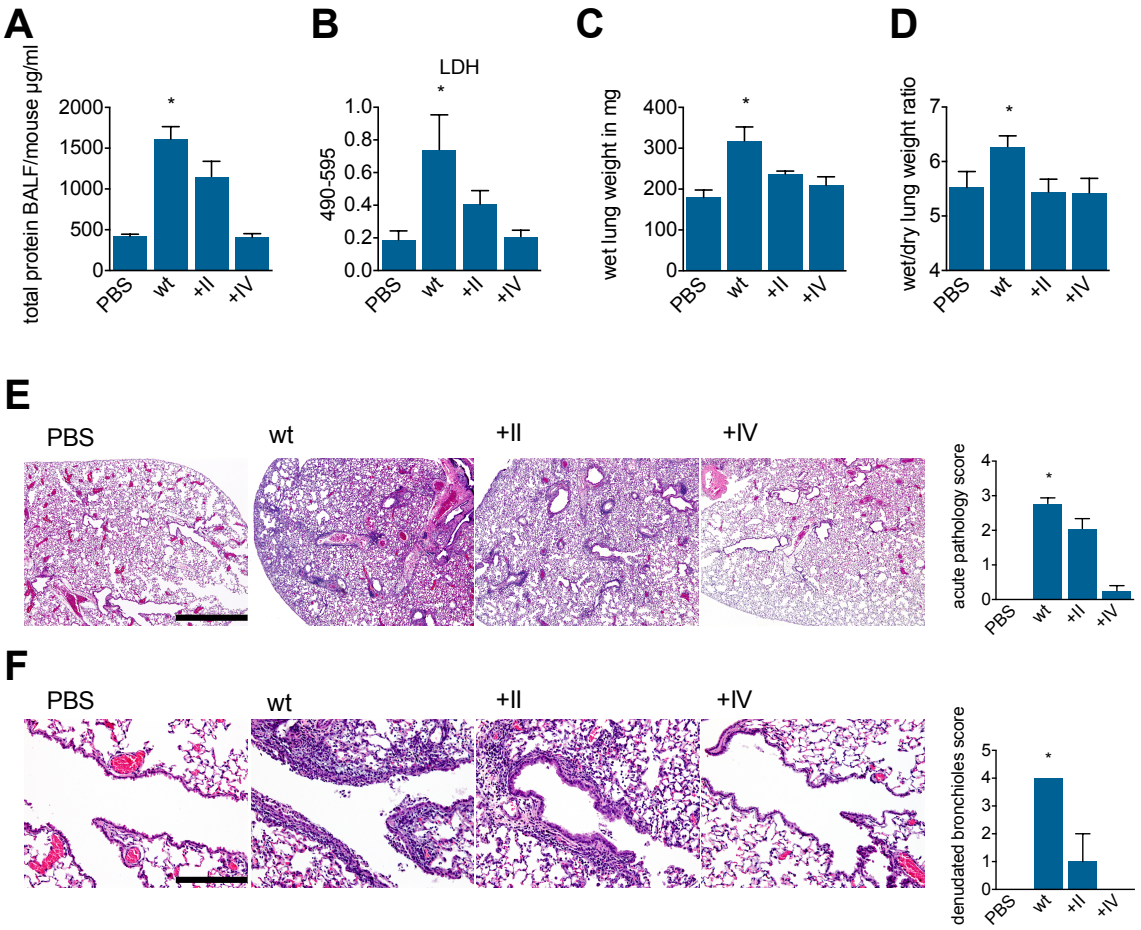

Figure S3 Severe lung pathology is associated with HA glycosylation, related to Figure 2.

(A-F) Five BALB/c mice per group were infected intranasally with $5x10^5$ PFU of isogenic viruses carrying differentially glycosylated HA globular heads (HK wt, HK +II and HK +IV) with PBS infected animals serving as control. On d5 after infection, (A, B) BALF was harvested and (A) total protein amount (µg/ml) and (B) LDH level (OD 490-595) as markers for pathogenesis are depicted. (C, D) Lungs were harvested on d7 after infection, weight, afterwards dried and weight again. (C) Wet lung weights (mg) and (D) wet/dry lung weight ratio are shown.

(E, F) Lungs were fixed, embedded, sectioned, and stained with H&E for histologic analysis on d5 after infection. Representative images for each group are shown ((E) x4 magnification (scale bar=1mM), (F) x20 magnification (scale bar=200μM)). Blind reviewed scores for (E) acute pathology and (F) denudated bronchioles are depicted. (A-D, E, F right panel) Data are represented as mean with SD. An asterisk (*) indicates statistically significant differences ($P < 0.05$) between HK wt infected animals and all other groups.

Figure S4

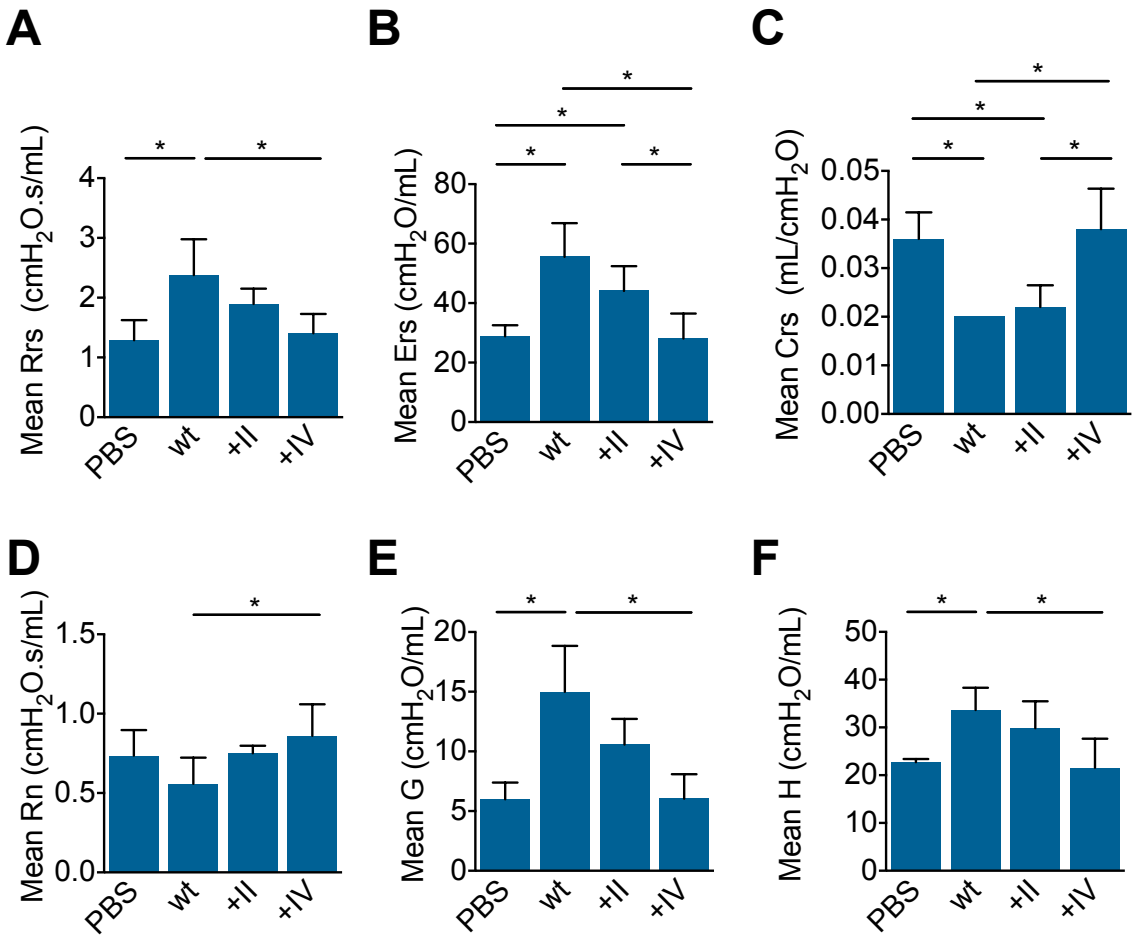

Figure S4 Alteration of lung physiology upon IAV infection depends on HA glycosylation, related to Figure 2.

Five BALB/c mice per group were infected intranasally with $5x10^5$ PFU of isogenic viruses carrying differentially glycosylated HA globular heads (HK wt, HK +II and HK +IV) with PBS infected animals serving as controls. Seven days after infection, lung physiology data were recorded. Lung physiology data were analyzed for evaluating disturbances of general lung homeostasis and lung

functionality upon infection with the different IAV to understand the physiological consequences of HA driven lung inflammation. Values for (A) respiratory system resistance (Rrs), (B) elastance (Ers), (C) compliance (Crs) and (D) Newtonian resistance (Rn), (E) tissue damping (G) and (F) tissue elastance (H) are depicted. (A-F) Data are represented as mean with SD. An asterisk (*) indicates statistically significant differences ($P < 0.05$).

Figure S5

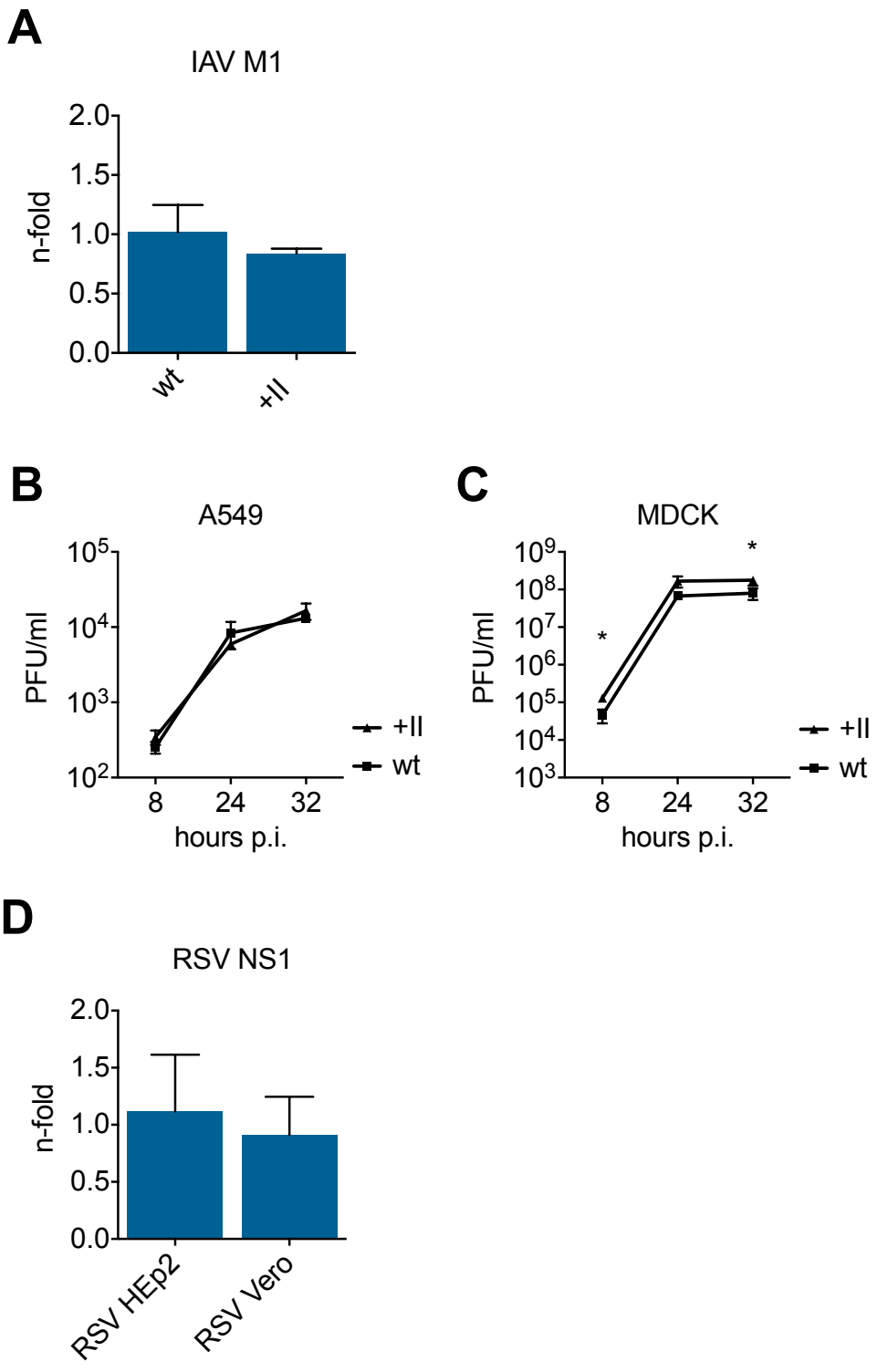
A
IAV M1
n-fold
2.0
1.5
1.0
0.5
0.0
wt
+II
B
A549
PFU/ml
10^5
10^4
10^3
10^2
8
24
32
hours p.i.
+II
wt
C
MDCK
PFU/ml
10^9
10^8
10^7
10^6
10^5
10^4
10^3
*
*
8
24
32
hours p.i.
+II
wt
D
RSV NS1
n-fold
2.0
1.5
1.0
0.5
0.0
RSV HEp2
RSV Vero

Figure S5 Differences in immune responses are not affected by alterations in viral growth, related to Figures 4 and 5.

(A, B) A549 and (C) MDCK cells were infected with isogenic IAV carrying differentially glycosylated HA globular heads (HK wt, HK +II) using (A) (MOI=5) and (B, C) (MOI=0.1). (A) Influenza M1 mRNA levels are shown as n-fold of wt infected cells 24h after infection. (B, C) 8h, 24h and 32h after infection, supernatants were collected. Virus titers were determined in standard plaque assay and are depicted in PFU/ml. (D) A549 cells were infected with two RSV A2 virus variants differing in surface protein glycosylation (RSV HEp2 and RSV Vero) (MOI=5). RSV NS1 genes levels are shown as n-fold of the RSV HEp2 infected cells 24h after infection. (A) Representative data (relate to main Fig. 4A, 4B) of three independent experiments each consisting of three biological samples are shown. (B, C) Mean values from three independent experiments and (D) representative data (relate to main Fig. 5D) of two independent experiments each consisting of four biological samples are shown. Data are represented as mean with SD. An asterisk (*) indicates statistically significant differences ($P < 0.05$) between HK wt and HK +II virus.