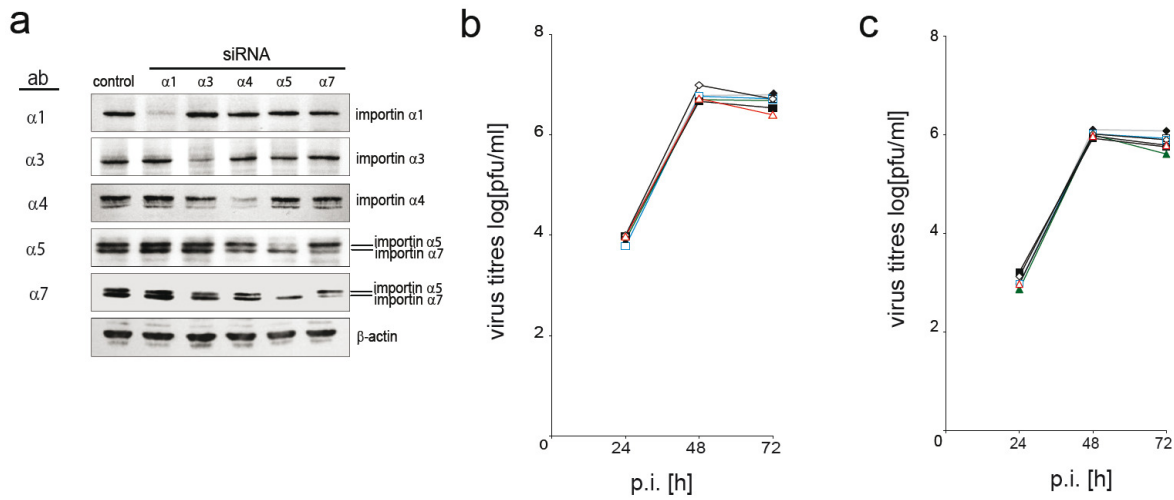


Supplementary Figure S1

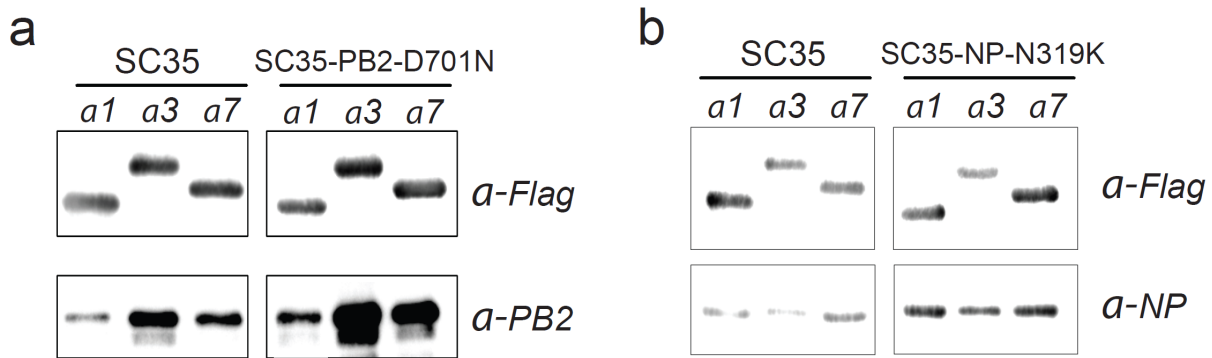
Phylogenetic analysis of human and chicken importins. Only five of six importins were studied because importin- $\alpha 6$ was shown to be testis-specific. Human and chicken importin protein sequences ($\alpha 1$, $\alpha 3$, $\alpha 4$, $\alpha 5$ and $\alpha 7$) were aligned and phylogenetic trees calculated using ClustalX and TreeView.



Supplementary Figure S2

Growth curves of SC35 and SC35M viruses in importin silenced avian cells.

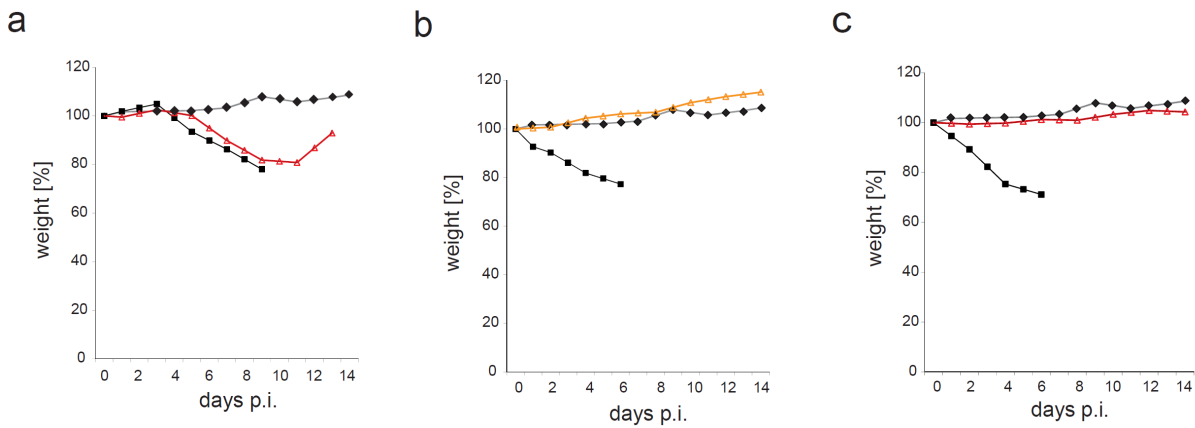
(a) Endogenous importins ($\alpha 1$ - $\alpha 7$) were silenced using siRNA in chicken fibroblasts (DF1). Importin- $\alpha 5$ antibody cross-reacts with importin- $\alpha 7$. The doublet represents importin- $\alpha 5$ (upper band) and importin- $\alpha 7$ (lower band). Importin- $\alpha 7$ antibody also cross-reacts with importin- $\alpha 5$. The doublet represents importin- $\alpha 5$ (upper band) and importin- $\alpha 7$ (lower band). (b) SC35 and (c) SC35M virus growth in importin silenced avian cells. Growth curves show controls (black, filled diamonds), $\alpha 1$ (blue, squares), $\alpha 3$ (green, filled triangles), $\alpha 4$ (black, filled squares), $\alpha 5$ (black diamonds) and $\alpha 7$ (red triangles) silenced cells.



Supplementary Figure S3

Interaction of PB2 and NP with importins.

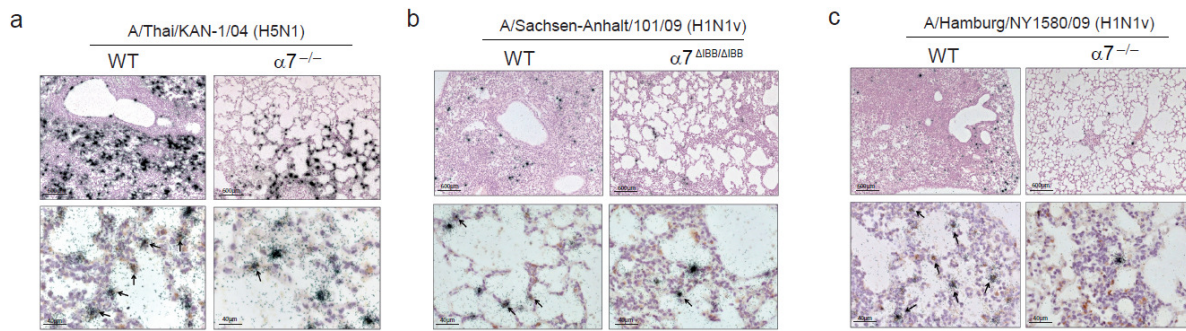
(a) 293T cells were cotransfected with SC35-PB2 or SC35-PB2-D701N and Flag-tagged importin- α 1, - α 3 and - α 7 isoforms. Immunoprecipitation was performed using the Flag-tag. (b) 293T cells were cotransfected with SC35-NP or SC35-NP-N319K and Flag-tagged importin- α 1, - α 3 and - α 7 isoforms. Immunoprecipitation was performed using the Flag-tag.



Supplementary Figure S4

Weight loss of WT and importin-knockout animals infected with H5N1 and H1N1v isolates.

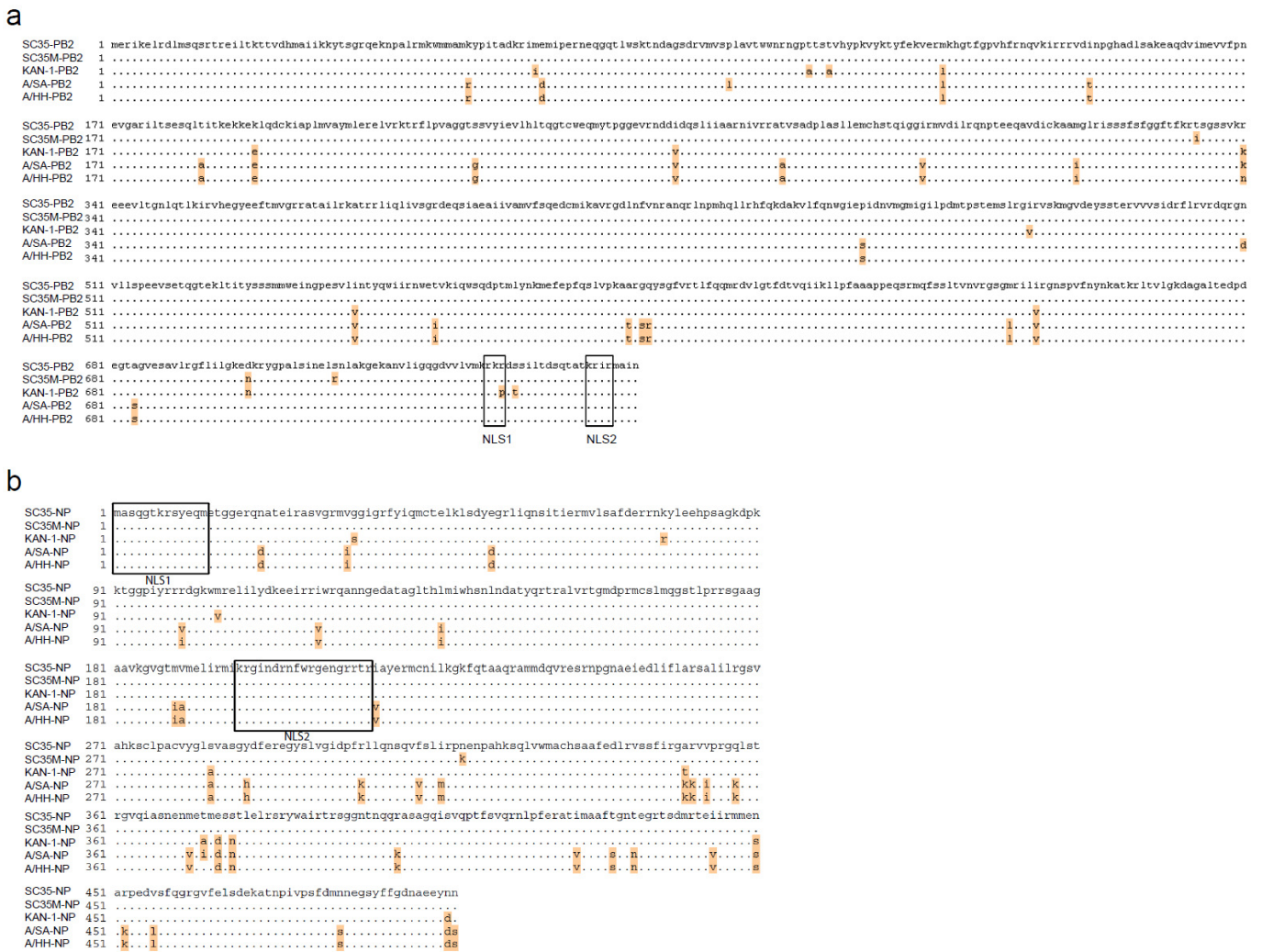
WT (black square; $n=16$), $\alpha 7^{-/-}$ (red triangle; $n=16$) or $\alpha 7^{\Delta IBB/\Delta IBB}$ (orange triangle; $n=16$) animals were infected with 50-fold LD_{50} of (a) A/Thai/KAN-1/04 (H5N1), (b) 10-fold LD_{50} of A/Sachsen-Anhalt/101/09 (H1N1v) or (c) 100-fold LD_{50} of A/Hamburg/NY1580/09 (H1N1v). Control groups received PBS (black diamond). Weight loss was monitored for 14 days.



Supplementary Figure S5

Cell tropism in the lung of H5N1 and H1N1v infected WT and importin- $\alpha 7$ -knockout mice.

In-situ hybridization and double labeling experiments demonstrate virus spread (upper panels) and virus RNA positive Mac-3+ macrophages in lungs (lower panels) of WT and $\alpha 7^{-/-}$ or $\alpha 7^{\Delta IBB/\Delta IBB}$ mice 6d p.i. with (a) A/Thai/KAN-1/04 (H5N1), (b) A/Sachsen-Anhalt/101/09 (H1N1v) or (c) A/Hamburg/NY1580/09 (H1N1v). Arrows indicate viral RNA containing macrophages. Lungs of infected WT mice are highly infiltrated and alveolar structures destroyed. In contrast, infection in the lungs of importin- $\alpha 7$ -knockout mice is localized and alveolar structures largely intact.



Supplementary Figure S6

PB2 and NP amino acid sequence alignment.

(a) PB2 and (b) NP protein sequences of SC35 and SC35M (H7N7) (GenBank accession no. DQ266097; DQ266096), A/Thai/KAN-1/04 (H5N1) (here referred as KAN-1) (GenBank accession no. AY626149; AY626145), A/Sachsen-Anhalt/101/09 (H1N1v) (here referred as A/SA) (GenBank accession no. CY045479; CY045483) and A/Hamburg/NY1580/09 (here referred as A/HH) (GenBank accession no. GU480807; HM598305) (H1N1v) were aligned using Clone Manager Professional 9 and sequence differences compared to the consensus sequence are highlighted. The PB2 bipartite NLS sequence 736-RKRX₁₂KRIR-755 as well as NP NLS sequences (1-13; NLS1) and (198-216) are highlighted.

targeted importin	siRNA sequence
human importin- α 1	GCA TGT GGC TAC TTA CGT A UU
human importin- α 3	CAA CTT ATG TCG CCA CAA A UU
human importin- α 4	ACA AGG AGG TAC CTA CAA T UU
human importin- α 5	TGG AGT TCC TCA AAC GAA A UU
human importin- α 7	AGA GCC TAG TCC TCC AAT A UU
chicken importin- α 1	GGA TAG AAG TTG TTG TGA A UU
chicken importin- α 3	GGT GAT AGA CTC CGG AAT A UU
chicken importin- α 4	TGT AAT AGA TGC TGG GCT A UU
chicken importin- α 5	GCA GAA GAC TTG TGG AAT T UU
chicken importin- α 7	GGA CAG ATT TGT TGA GTT CUU

Supplementary Table S1

siRNA sequences targeting chicken and human importin- α .