

Supplemental Information

Cellular Importin- α 3 Expression Dynamics in the Lung Regulate Antiviral Response Pathways against Influenza A Virus Infection

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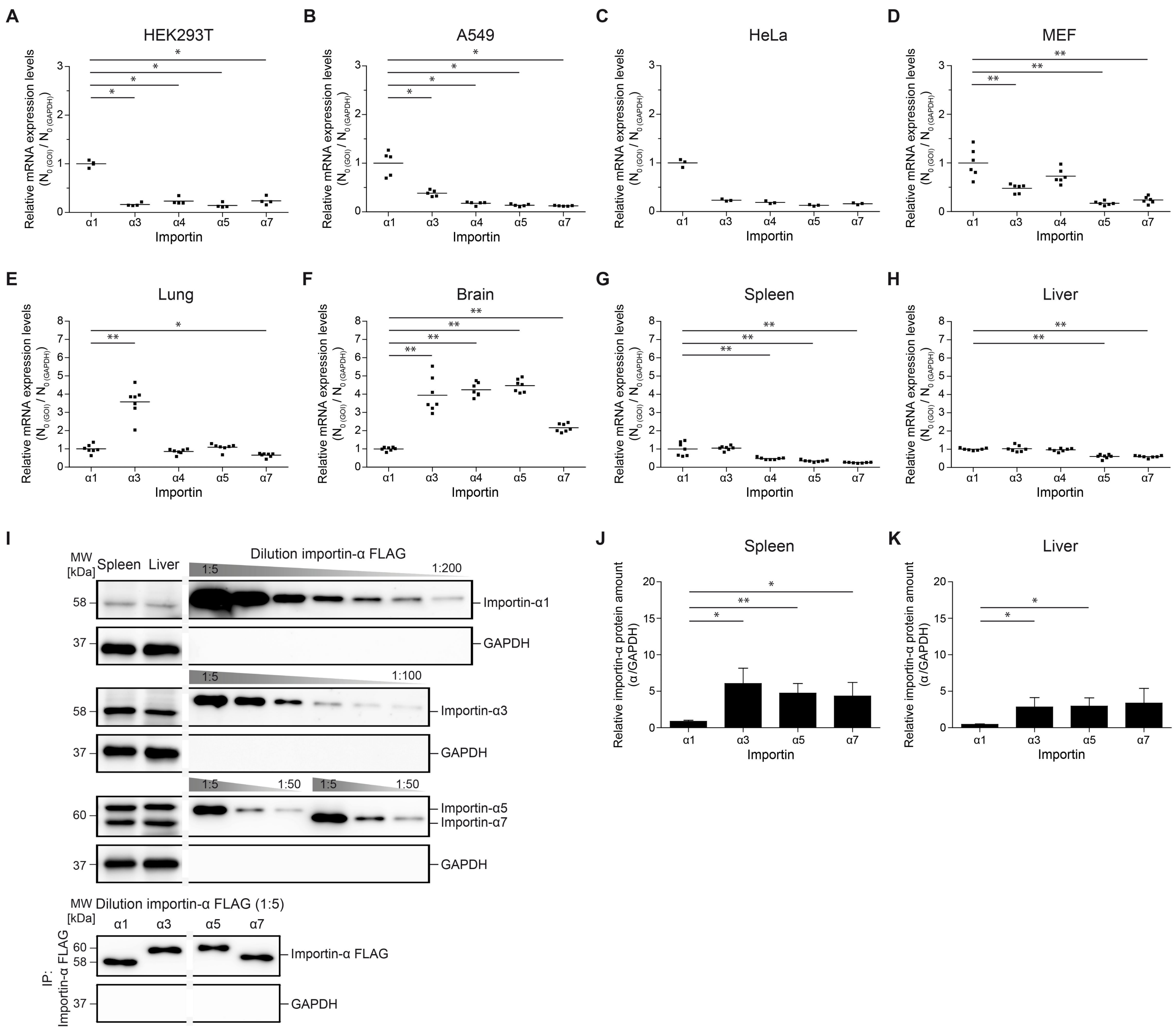


Figure S1. Importin- α mRNA and protein expression levels in immortalized mammalian cell lines and various murine organs. Related to Figure 1.

(A–D) Importin- α mRNA levels ($\alpha 1$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$) in the immortalized cell lines HEK293T (A; $n = 4$), A549 (B; $n = 5$), HeLa (C; $n = 3$), and MEFs (D; $n = 6$). (E–H) Importin- α mRNA expression levels ($\alpha 1$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$) in the murine lung (E; $n = 7$), brain (F; $n = 7$), spleen (G; $n = 7$), and liver (H; $n = 7$). RT-qPCR data were obtained using either human or mouse specific importin- α primers. Relative expression values of importin- $\alpha 1$ were set 1 after normalization against GAPDH. Each data point represents an individual sample. (I–K) Importin- α protein amounts in murine spleen and liver. Western blot analyses using importin- α isoform specific antibodies and GAPDH adjustment were performed to determine endogenous importin- α protein amounts ($\alpha 1$, $\alpha 3$, $\alpha 5$, $\alpha 7$) in murine organs (I). For relative quantification in spleen (J) and liver (K), standard curves of affinity-purified, FLAG-tagged importin- α proteins were used. Relative importin- $\alpha 1$ protein amount in murine LRT in Fig 1I) was set 1 and the values for spleen (J) and liver (K) are shown in relation to this. The anti-FLAG Western blot at the bottom validates that identical amounts of all isoforms were applied in the 1:5-dilutions of the standard curves. The gaps in (I) depict cropping of the relevant bands run on the same gel. Data shown represent means \pm SD; ($n = 3$ biological replicates; technical replicates: $n = 1-2$ per organ, $n = 1-2$ Western blot analyses). ((A–K): * $P < 0.05$, ** $P < 0.01$).

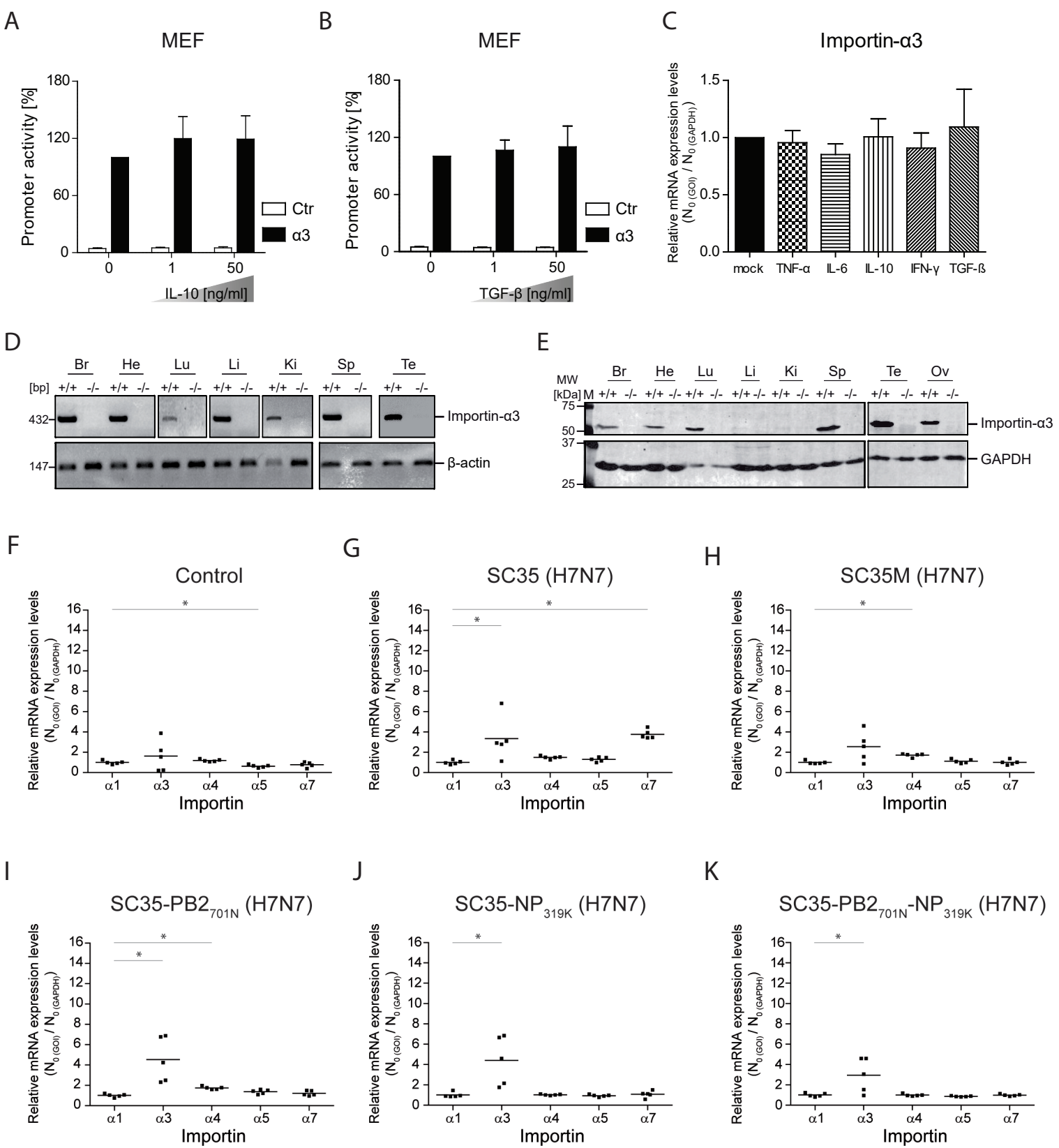


Figure S2. Importin- α 3 induction and expression *in vitro* and *in vivo*. Related to Figure 2.

(A and B) Effect of IL-10 (A) and TGF- β (B) treatment on importin- α 3 promoter activity in MEF cells upon control- (Ctr) or importin- α 3 promoter reporter construct (α 3) transfection. Relative importin- α 3 promoter activity in control-treated samples was set 100 %. (C) MEF NEMO^{-/-} cells were treated with 0.1ng TNF- α , IL-6, IL-10, IFN- γ and TGF- β and importin- α 3 mRNA levels were measured as described before. (D and E) Validation of importin- α 3 deficiency in importin- α 3^{-/-} mice. (D) Verification of importin- α 3 deletion in organs of wild type (+/+) and importin- α 3^{-/-} (-/-) mice by PCR using importin- α 3 specific, exon-spanning primers (expected fragment size: 432 bp). β -actin specific primers were used as a control (expected fragment size: 147 bp). (E) Deletion of the importin- α 3 gene was further verified by Western blot with GAPDH as loading control in brain (Br), heart (He), lung (Lu), liver (Li), kidney (Ki), spleen (Sp), testis (Te), and ovary (Ov). (F–K) Wild type (WT) mice were control-treated or infected with 6*10⁴ p.f.u. of H7N7 recombinant viruses. Importin- α mRNA expression levels (α 1, α 3, α 4, α 5, α 7) on day 1 p.i. in lungs upon control-treatment (F) or infection with SC35-PB2_{701D} (G), SC35M-PB2_{701N} (H), SC35-PB2_{701N} (I), SC35-NP_{319K} (J) or SC35-PB2_{701N}-NP_{319K} (K) viruses. Relative importin- α 1 expression values were set 1 after normalization against GAPDH. Each data point represents an individual sample ($n = 5$, * $P < 0.05$).

A

Control

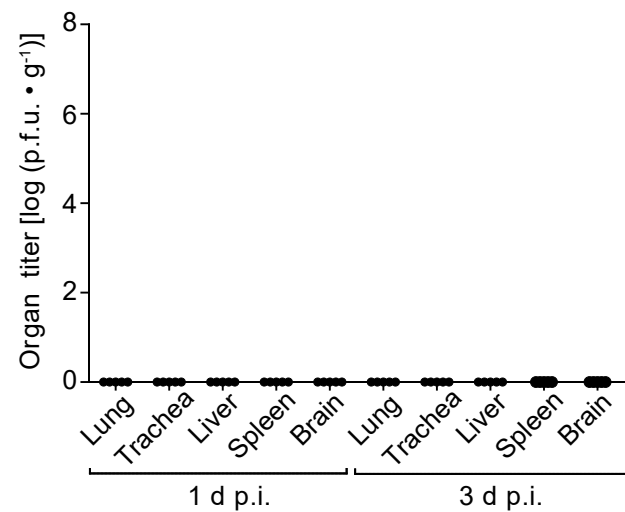
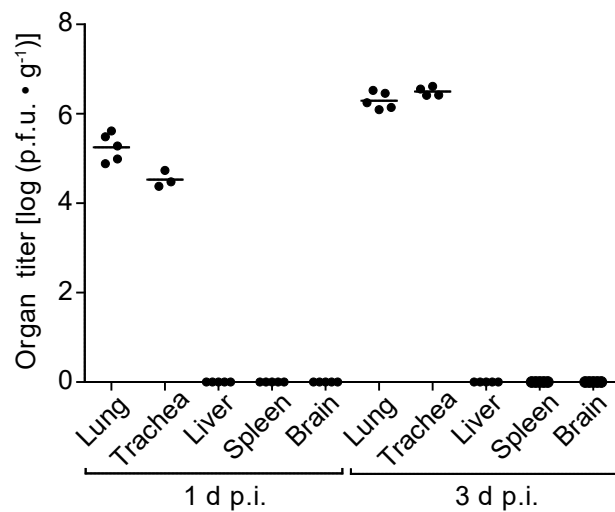
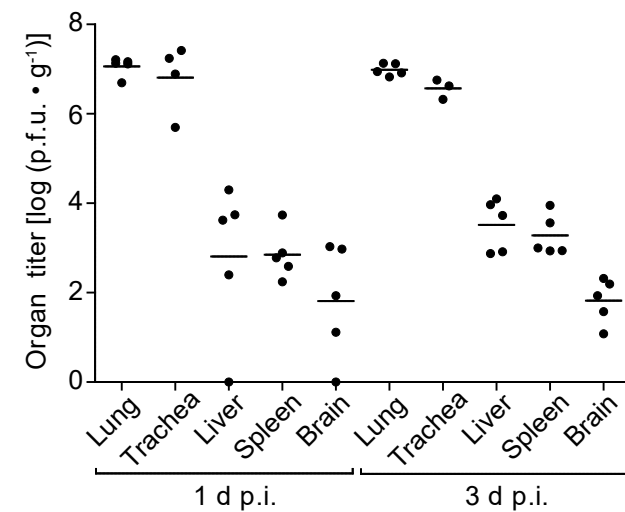
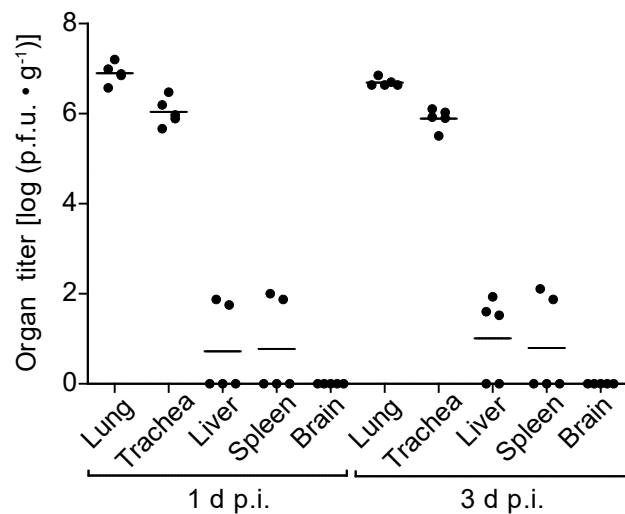
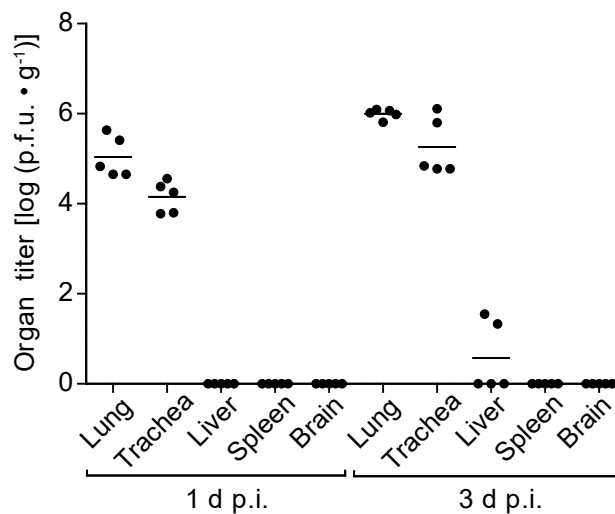
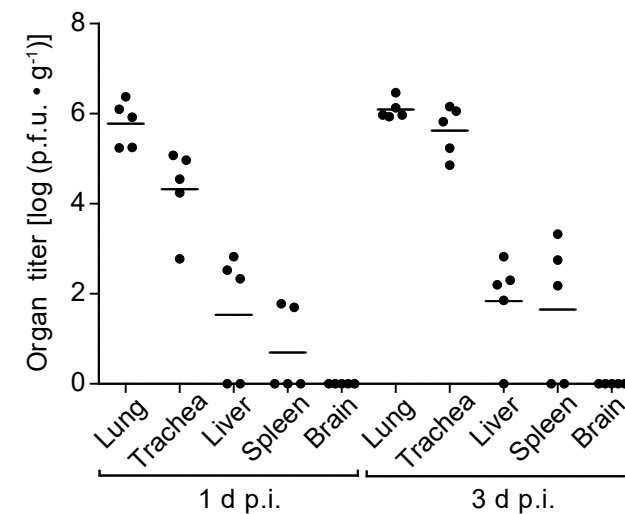
**B**SC35-PB2_{701D}**C**SC35M-PB2_{701N}**D**SC35-PB2_{701N}**E**SC35-NP_{319K}**F**SC35-PB2_{701N}-NP_{319K}

Figure S3. Avian- and human-type H7N7 HPAIV replication in mice. Related to Figure 5.

(A-F) Avian and human-type H7N7 HPAIV pathogenicity in wild type (WT) and importin- $\alpha 3^{-/-}$ ($\alpha 3^{-/-}$) mice. Virus titers given in plaque-forming units (p.f.u.) on days 1 and 3 p.i. in lung, trachea, liver, spleen, and brain of wild type mice either treated with PBS (A, Control) or infected with 6×10^4 p.f.u. of H7N7 recombinant viruses SC35-PB2_{701D} (B), SC35M-PB2_{701N} (C), SC35-PB2_{701N} (D), SC35-NP_{319K} (E) or SC35-PB2_{701N}-NP_{319K} (F). Each data point represents an individual sample; $n = 5$. (* $P < 0.05$).

Influenza

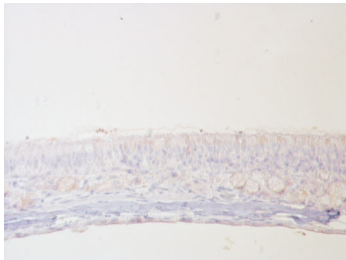
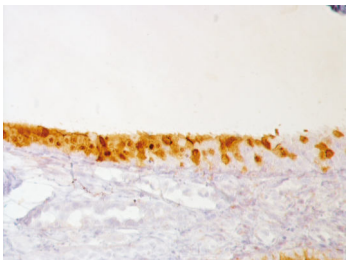
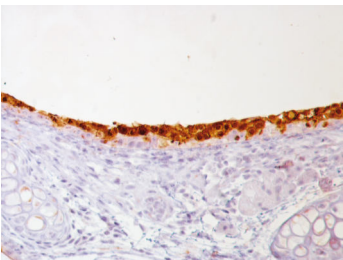
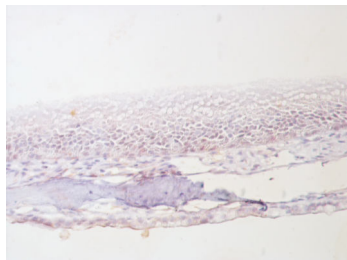
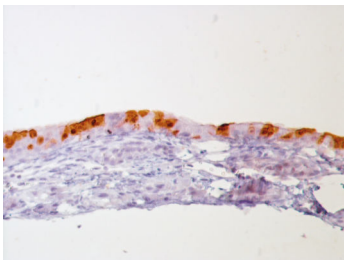
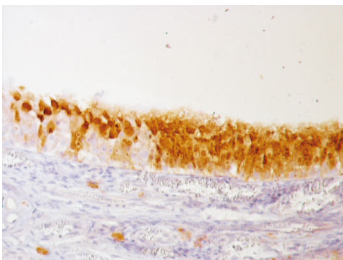
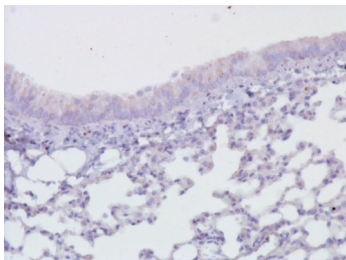
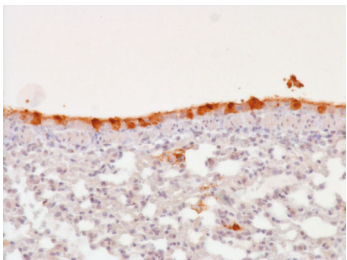
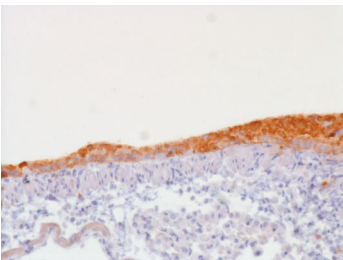
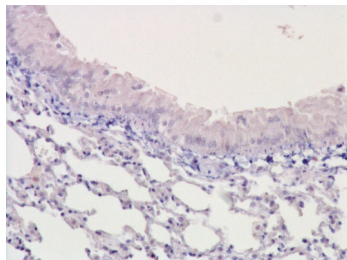
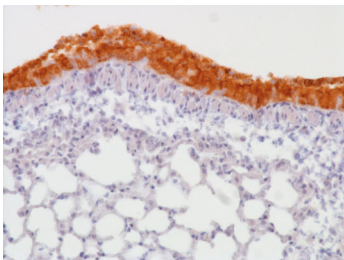
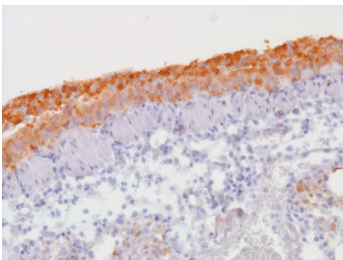
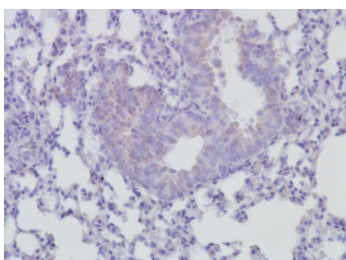
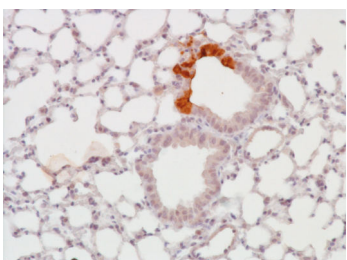
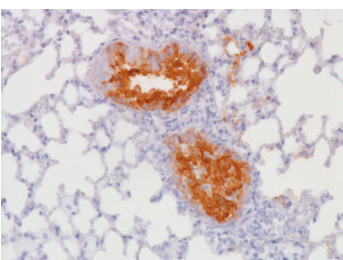
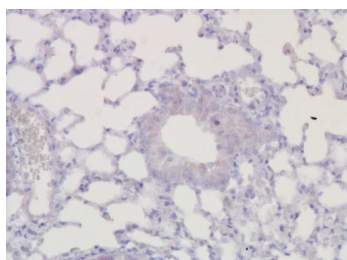
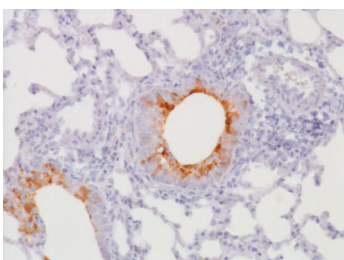
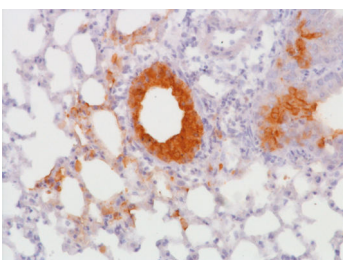
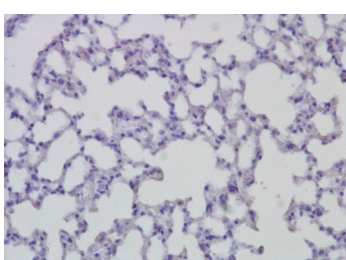
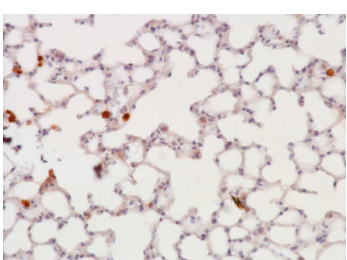
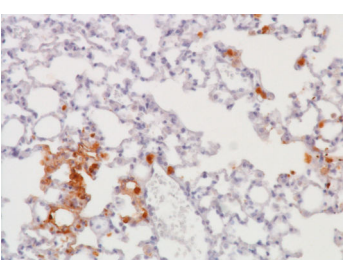
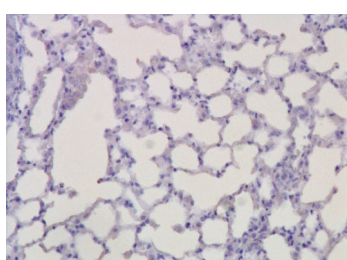
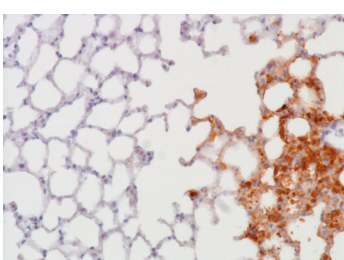
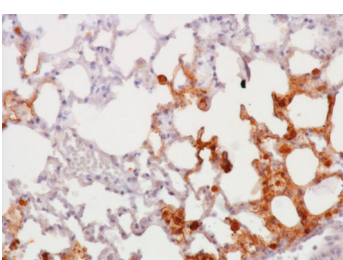
		$\alpha 3^{+/+}$			$\alpha 3^{-/-}$		
		Control	SC35-PB2 _{701D}	SC35M-PB2 _{701N}	Control	SC35-PB2 _{701D}	SC35M-PB2 _{701N}
Influenza	Nasal concha (URT)						
	Bronchus (LRT)						
	Bronchiole (LRT)						
	Alveoli (LRT)						

Figure S4. Virus replication kinetics in importin- $\alpha 3^{+/+}$ and importin- $\alpha 3^{-/-}$ mice. Related to Figure 6.

Viral antigen (NP) was stained in URT and LRT of $\alpha 3^{+/+}$ and $\alpha 3^{-/-}$ mice infected with avian-type (SC35-PB2_{701D}) or human-type HPAIV (SC35M-PB2_{701N}).

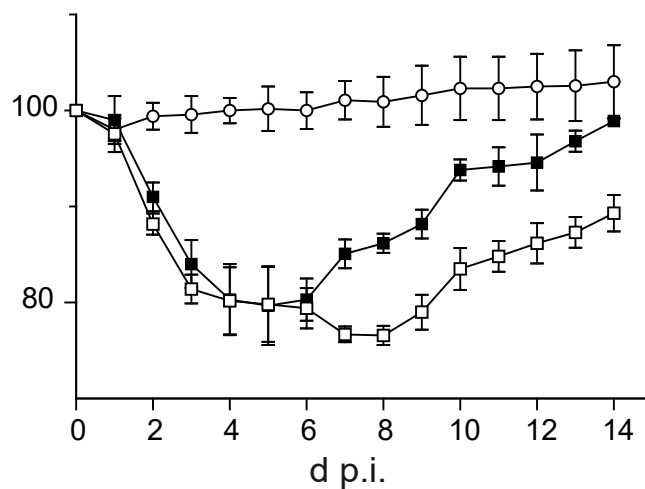
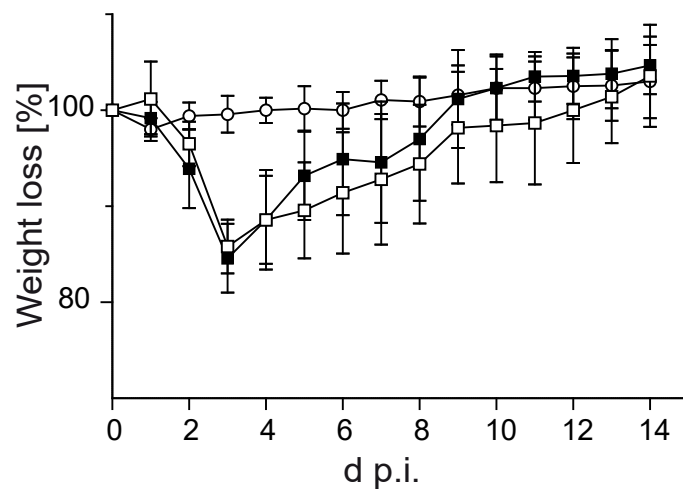
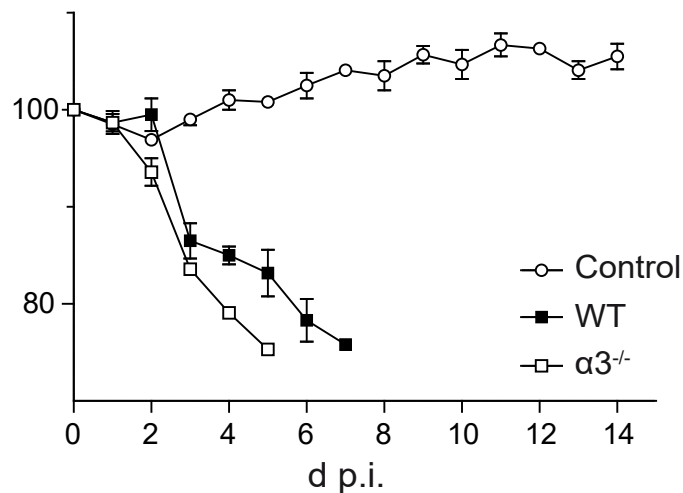
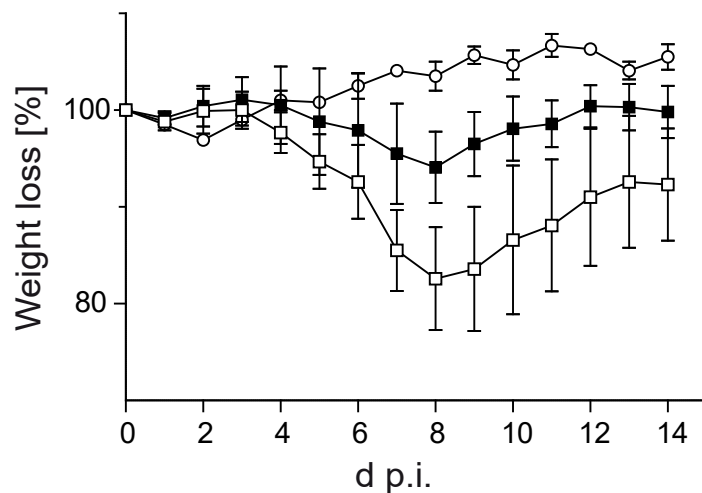
ASC35-PB2_{701D}10⁵ p.f.u.5*10⁵ p.f.u.**B**SC35M-PB2_{701N}10² p.f.u.10⁴ p.f.u.

Figure S5. Avian- and human-type H7N7 HPAIV pathogenicity in wild type and importin- $\alpha 3^{-/-}$ mice. Related to Figure 6. Differential weight loss in WT (filled square) and $\alpha 3^{-/-}$ (open square) mice upon SC35-PB2_{701D} virus infection (**A**) with 10^5 p.f.u. ($n = 6$) or $5 * 10^5$ p.f.u. ($n = 9$) or upon SC35M-PB2_{701N} virus infection (**B**) with 10^2 p.f.u. ($n = 7$) or 10^4 p.f.u. ($n = 4$). Control mice received PBS (Control, open circle). Data shown represent average values \pm SD. bp, base pairs, M, molecular weight standard, MW, molecular weight.

SUPPLEMENTARY TABLES

Table S1. Importin- α nucleotide sequence identity (in the upper triangle) and amino acid sequence identity (in the lower triangle). Related to Figure 4.

		Homo sapiens	Macaca fascicularis	Sus scrofa	Mus musculus	Gallus gallus	Anas platyrhynchos
Importin-α1	Homo sapiens	100.00	97.55	91.51	87.55	77.38	77.61
	Macaca fascicularis	99.24	100.00	90.94	86.98	77.13	76.98
	Sus scrofa	97.16	96.60	100.00	87.23	76.87	77.11
	Mus musculus	94.52	93.95	95.27	100.00	75.30	76.29
	Gallus gallus	83.52	82.95	83.52	82.20	100.00	92.75
	Anas platyrhynchos	83.93	83.36	83.93	82.99	96.40	100.00
Importin-α3	Homo sapiens	100.00	99.17	96.81	94.89	87.23	86.78
	Macaca fascicularis	100.00	100.00	96.62	94.51	87.23	86.78
	Sus scrofa	99.62	99.62	100.00	93.61	87.16	86.97
	Mus musculus	98.85	98.85	98.85	100.00	85.82	85.31
	Gallus gallus	99.04	99.04	99.04	98.27	100.00	96.04
	Anas platyrhynchos	98.27	98.27	98.46	97.50	98.85	100.00
Importin-α4	Homo sapiens	100.00	99.11	94.70	93.49	88.38	88.70

	Macaca fascicularis	99.62	100.00	95.08	93.87	88.76	89.08
	Sus scrofa	99.23	99.62	100.00	93.68	88.57	88.25
	Mus musculus	99.23	99.62	100.00	100.00	88.63	88.76
	Gallus gallus	98.08	98.46	98.85	98.85	100.00	96.31
	Anas platyrhynchos	97.89	98.27	98.66	98.66	99.43	100.00
Importin-α5	Homo sapiens	100.00	99.01	95.05	92.52	80.58	81.69
	Macaca fascicularis	100.00	100.00	95.30	92.70	80.33	81.69
	Sus scrofa	99.63	99.63	100.00	91.59	81.20	82.00
	Mus musculus	97.96	97.96	98.33	100.00	79.65	80.46
	Gallus gallus	95.91	95.91	96.28	94.80	100.00	94.37
	Anas platyrhynchos	95.91	95.91	96.10	94.42	98.70	100.00
Importin-α6	Homo sapiens	100.00	99.01	95.10	-	82.93	84.23
	Macaca fascicularis	99.63	100.00	95.34	-	82.87	84.01
	Sus scrofa	97.39	97.76	100.00	-	82.68	84.17
	Mus musculus	-	-	-	-	-	-
	Gallus gallus	94.22	94.22	93.28	-	100.00	95.53
	Anas platyrhynchos	94.22	94.06	93.28	-	99.63	100.00
Importin-α7	Homo sapiens	100.00	99.13	95.22	91.81	80.57	-

Macaca fascicularis	100.00	100.00	95.16	91.81	80.32	-
Sus scrofa	99.25	99.25	100.00	91.50	80.26	-
Mus musculus	99.25	99.25	99.25	100.00	79.64	-
Gallus gallus	94.40	94.40	94.03	94.22	100.00	-
Anas platyrhynchos	-	-	-	-	-	-

Table S2. DE Gene Cluster Characterization. Related to Figure 6.

Cluster	No. of genes	Top GO BP	BP [%]	Fold enrichment	FDR	Pathway	Pathway [%]	Fold enrichment	FDR
A1	32	GO:0009123~nucleoside monophosphate metabolic process	8.3	30.7	53.3	mmu00980:Metabolism of xenobiotics by cytochrome P450	10.5	21.7	45.0
A2	26	GO:0006032~chitin catabolic process	10.5	209.0	10.5				
A3	22	GO:0009611~response to wounding [†]	29.4	15.1	0.2	mmu04062:Chemokine signaling pathway	17.6	15.8	4.4
A4	22	GO:0006955~immune response [†]	21.4	9.6	25.8	mmu04514:Cell adhesion molecules (CAMs) [‡]	28.6	18.6	0.5
A5	6	GO:0045109~intermediate filament organization	50.0	627.1	0.005				
A6	35	GO:0006955~immune response [†]	21.7	9.6	1.3	mmu04672:Intestinal immune network for IgA production [‡]	8.7	30.4	37.5

^{†‡} Representative genes are highlighted in Table S3. BP, biological process, FDR, false discovery rate, GO, gene ontology.

Table S3. Gene Clusters. Related to Figure 6.

Cluster	No. of genes	Genes (UniProt Accession numbers)
A1	32	A9XX86, D3Z3I0, D3Z599, E9Q414, E9Q5I3, O09049, O54792, O88310, O88312, O88338, Q3TPA4, Q3UHH0, Q3UW68, Q3V1P3, Q60925, Q61456, Q8C6E1, Q8CJC6, Q8JZN1, Q8K0L2, Q8R1B0, Q920D7, Q9CXN7, Q9D0T7, Q9D6P8, Q9D6X7, Q9D733, Q9D7D2, Q9EQY5, Q9JHX2, Q9QX97, Q9WV08
A2	26	D3YV40, E0CYE1, E9PWW8, E9Q9C6, P00184, P01864, P03987, P13542, Q05421, Q0PGA1, Q149M0, Q3UN88, Q3UPY5-2, Q60604, Q61114, Q62095, Q62240, Q6RFH4-2, Q7TNJ0, Q8BNX7, Q8BZW3, Q8R400, Q91XA9, Q91Z98, Q9D7Z6, Q9QXD6
A3	22	E9PUL3, E9PWB6, F6R5P4, O35082, O35343, O55042-2, O88430 ^{†‡} , P31532 [†] , P34960, P51678 [‡] , Q497R5 [†] , Q6P205, Q8BJC0, Q8C5Y0, Q8K196, Q8VCM7 [†] , Q9D7S0, Q9JHV0, Q9JJJ3, Q9JKC0 ^{†‡} , Q9QXI6, Q9R155
A4	22	A2CG21, E9QPG8, O70165, O89093, P57774, Q3URU5 [†] , Q5SSM0 ^{†‡} , Q61003, Q61003-3, Q642U4 [‡] , Q6GTR6, Q6W9J6 ^{†‡} , Q6W9L1, Q80SX8, Q8BIF2, Q8BW88, Q8BW88-3, Q8C160, Q8C1C1, Q8QZR1, Q923L3, Q9WVS0 [†]
A5	6	P50446, P56389, P97350, Q61781, Q922U2, Q9QWL7
A6	35	A2AX52, B2RST7, D3Z1H5, E0CY86, E9Q5S9, E9Q958, G3UVT9, I6L985, J3QMN6, J3QNB7, O08850, O54689, O88890 [†] , P01592 [†] , P63239, P97347, Q14B54, Q3SXS7, Q3TP89, Q3U106, Q497L9, Q6PG97 [†] , Q8BIC8, Q8BNX1, Q8BYF0, Q91VM3-2, Q922Y2, Q925E8, Q9CPP7, Q9D8D0 ^{†‡} , Q9D8I1, Q9D8K3, Q9DCD9, Q9QWV1 ^{†‡} , Q9WVF6

^{†‡} Genes contributing to the functional enrichment described in Table S2.

Table S4. Virulence of avian-type SC35-PB2_{701D} and human-type SC35M-PB2_{701N} H7N7 HPAIV in importin- $\alpha 3^{+/+}$ and importin- $\alpha 3^{-/-}$ mice. Related to Figure 6.

Virus	Dose for infection [p.f.u.]	Survival [%]		Survival curve comparison	MLD ₅₀ [p.f.u.]	
		$\alpha 3^{+/+}$	$\alpha 3^{-/-}$		$\alpha 3^{+/+}$	$\alpha 3^{-/-}$
SC35-PB2 _{701D}	10 ⁶	22.2 (n=9)	0 (n=10)	p = 0.0220 *		
	5x10 ⁵	20 (n=10)	33.3 (n=9)	p = 0.5850	10 ^{5.8}	10 ^{5.8}
	10 ⁵	100 (n=6)	100 (n=6)	p = 1		
SC35M-PB2 _{701N}	10 ⁴	0 (n=9)	0 (n=9)	p = 0.0100 **		
	10 ³	75 (n=8)	0 (n=8)	p = 0.0446 *	10 ^{3.3}	10 ^{2.2}
	10 ²	100 (n=7)	62.5 (n=8)	p = 0.0818		

Importin- $\alpha 3^{-/-}$ ($\alpha 3^{-/-}$) mice and their wild type litter mates ($\alpha 3^{+/+}$) were infected with the indicated doses and number of animals (*n*) of either SC35-PB2_{701D} or SC35M-PB2_{701N} virus. Control mice received PBS (*n*=4). Animals were monitored for 14 days p.i.. Mouse lethal dose 50 (MLD₅₀) given in plaque-forming units (p.f.u.) was calculated as described by Reed and Muench. Statistical significance of survival curves was determined using the log-rank (Mantel-Cox) test and the data from the Kaplan-Meier survival curves (* *P*<0.05, ** *P*<0.01).

Table S5. Blocking Peptides for Importin- α Antibodies Used as Controls in Immunohistochemical Analyses. Related to STAR Methods, Experimental Model and Subject Details.

Peptide	Importin- α region and amino acid sequence		Peptide homology human / mouse	Peptide recognition	Manufacturer
Importin- α 1	aa518-529	QVQDGAPGTFNF	100 % (12/12)	importin- α 1 antibody (Abcam: ab54489; ab84440)	GenScript
Importin- α 3	aa509-521	NSSANVPTEGFQF	92 % (12/13)	importin- α 3 antibody (# NB100-93345; # NB100-81651)	Abcam (ab23144)
Importin- α 5	aa3-16	TPGKENFRLKSYKN	100 % (14/14)	importin- α 5/7 antibody ⁵⁹	GenScript
Importin- α 7-1	aa3-12	MASPGKDNRYR	100 % (10/10)	importin- α 5/7 antibody ⁵⁹	GenScript
Importin- α 7-2	aa526-536	PEAPMEGFQL	100 % (10/10)	importin- α 5/7 antibody ⁵⁹	GenScript

aa, amino acids

Table S6. Sequences of human and murine RT-qPCR primers. Related to STAR Methods, Method details.

Gene	Gene locus, GenBank-No., name and sequence (5'→3')	T _m [°C]	Amplicon [bp]
Glycerinaldehyde-3-phosphate-dehydrogenase (<i>Gapdh</i>)	human: 12p13, chromosome: 12; NC_000012.11; GenBank: NM_002046.4 F: hGAPDH_4fw GGGAAGGTGAAGGTCGGAGTCAAC	59.0	247
	R: hGAPDH_250rev TGGAGGGATCTCGCTCCTGGAAGATG	61.1	
	murine: 6 F2; 6 59.32 cM, chromosome: 6; NC_000072.6; GenBank: GU214026.1 F: mGAPDH_849fw CCACTGAAGGGCATCTTGGGCTAC	59.3	224
	R: mGAPDH_1072rev GGTGGGTGGTCCAGGGTTTCTTAC	58.9	
Hypoxanthine guanine phosphoribosyl transferase (<i>Hprt</i>)	murine: X; X 29.31 cM, chromosome: X; NC_000086 GenBank: NM_013556.2 F:mHprt_599fw GCAGTACAGCCCCAAAATGG	59.5	229
	R: mHprt_827rev TTGCAGATTCAACTTGCGCT	59.0	
Importin-α3 (<i>Kpna4</i>)	human: 3q25.33, chromosome 3; NC_000003.11; GenBank: NM_002268.4 F: hImp-a3_682fw TGTCGCCACAAAGACCCACCAC	59.5	208
	R: hImp-a3_889rev CCTGGTGGCTGAGCAGAGGAAC	59.2	
	murine: 3; 3 E2, chromosome: 3; NC_000069.6; GenBank: NM_008467.4 F: mImp-a3_987fw ACACTTCCCAGCACTCCTCACTCATC	60.0	235
	R: mImp-a3_1221rev AAGATAGGCCACTTGGTCTTTCCTTCC	58.0	
Importin-α4 (<i>Kpna3</i>)	human: 13q14.3, chromosome 13; NC_000013.10; GenBank: NM_002267.3 F: hImp-a4_699fw CCCGCCGCCTATGGAGACAGTTC	61.2	256
	R: hImp-a4_954rev CTGCTCGTCGGTGCCAGTCACTATG	61.3	
	murine: 14 D1; 14, chromosome: 14; NC_000080.6; GenBank: NM_008466.5		

	F: mImp-a4_784fw ACTGTTTGGGCGCTGTCATACTTG	58.0	217
	R: mImp-a4_1000rev GGTTTGGGAAGTGGGACAGGACATC	59.0	
Importin-α5 (<i>Kpna1</i>)	human: 3q21, chromosome 3; NC_000003.11; GenBank: NM_002264.3 F: hImp-a5_74fw GCAGGAGGAGGGAGGAAGAAGGAC	59.8	237
	R: hImp-a5_310rev GTGTTGCTGAAAGCTGTTGCTCTGGG	60.9	
	murine: 16 B4-B5; 16, chromosome: 16; NC_000082.6; GenBank: NM_008465.5 F: mImp-a5_720fw TAGAGGGAAGAGCCCACCTCCAG	58.6	239
	R: mImp-a5_958rev CAGCCCGCAAAGCAGGAGAAAC	58.9	
Importin-α7 (<i>Kpna6</i>)	human: 1p35.1, chromosome 1; NC_000001.10; GenBank: NM_012316.4 F: hImp-a7_763fw CCTGTACTGTCTCGCCTACTCTTCAGC	60.1	204
	R: hImp-a7_966rev GACGATGTTACCCACGGCTCTCAG	59.1	
	murine: 4; 4 D2.3, chromosome: 4; NC_000070.6; GenBank: NM_008468.4 F: mImp-a7_282fw TGA CTGCAGTTAGCAACCACGCAG	58.0	231
	R: mImp-a7_512rev ATGGGGACAGCTCCTGCTTCAATG	59.0	
Tumor necrosis factor α (<i>TNF-α</i>)	murine: 17 B1; 17, chromosome 17; NC_000083.6; GenBank: NM_013693.3 F: mTNFa_175fw CAGAAAGCATGATCCGCGAC	59.7	218
	R: mTNFa_392rev GGCCATAGAACTGATGAGAGGG	60.0	
Tumor necrosis factor (ligand) superfamily, member 10 (<i>Tnfsf10</i>)	murine: 3; chromosome 3; NC_000069.6; GenBank: NM_009425 F:mTnfsf10_2813_fw CCTTTGGCAGGGCTGAAAAC	59.9	203
	R: mTnfsf10_3015_rev CAGACACCATTTTCGCTTGCC	60.1	

Interferon beta 1, fibroblast (<i>Ifnb1</i>)	murine: 4; 4 41.91 cM; chromosome 4; NC_000070.6; GenBank: NM_010510.1		
	F: mIfnb1_101fw CCAGCTCCAAGAAAGGACGA	59.7	216
	R: mIfnb1_316_rev GTCTCATTCCACCCAGTGCT	59.6	
Interferon regulatory factor 2 (<i>Irf2</i>)	murine: 8, chromosome 8; NC_000074.6; GenBank: NM_008391.4		
	F: mIrf2_131fw AACAACTCACACCTTGCG	59.3	231
	R: mIrf2_361_rev AGAGCGGAGCATCCTTTTCC	60.1	
Interferon- induced GTP- binding protein (<i>Mx1</i>)	murine: 16; 16 57.46 cM; chromosome 16; NC_000082.6 GenBank: NM_010846.1		
	F: mMx1_156fw TTGACCTCATCGACACCCTG	59.4	218
	R: mMx1_373_rev ACTGGGAAGGGACCTGTTTC	59.2	
Interferon gamma (<i>Ifng</i>)	murine: 10 66.75 cM, chromosome 10; NC_000076.6; GenBank: NM_008337.4		
	F: mIfng_837fw AAGACTGTGATTGCGGGGTT	59.9	202
	R: mIfng_1038_rev ACATTCGAGTGCTGTCTGGC	60.7	
Eukaryotic translation initiation factor 2- alpha kinase 2 (<i>Eif2ak2</i>)	murine: 17; 17 49.56 cM; chromosome 17; NC_000083.6 GenBank: NM_011163.4		
	F: mEif2ak2_14fw GCCGGGAAAACGAAACAGAAG	60.3	238
	R: mEif2ak2_251_rev CCGTGCATCTGGCGGTATTT	61.1	

bp, base pairs, F, forward primer, R, reverse primer, Tm, melting temperature