

Screening for H3N2 influenza A and influenza B virus activating proteases in murine airways by transcriptome profiling and protease inhibition studies

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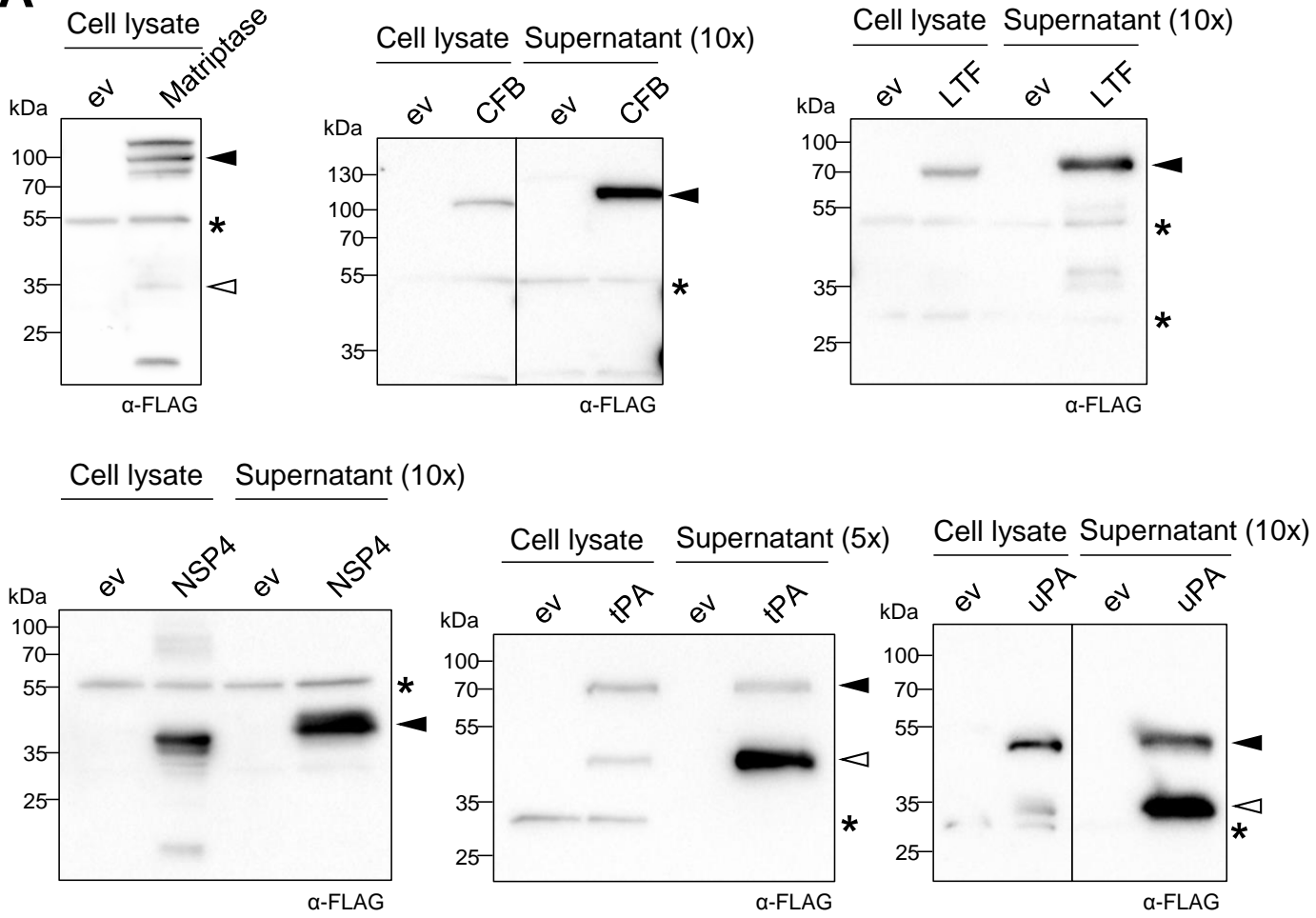
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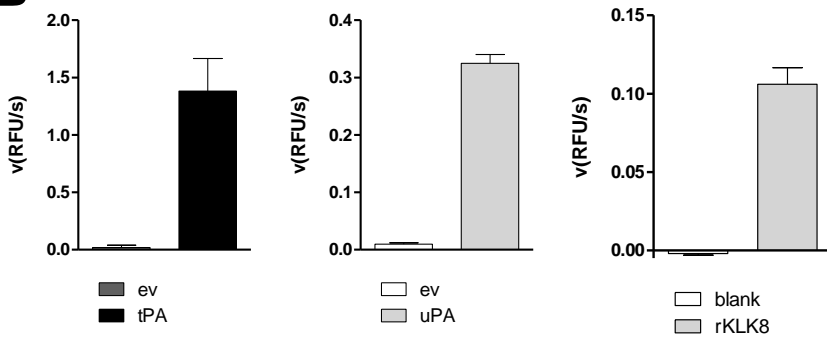
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Figure S1

A



B



C

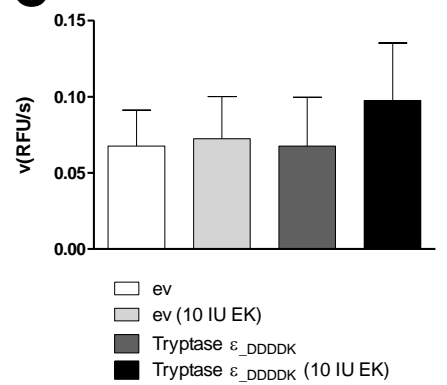


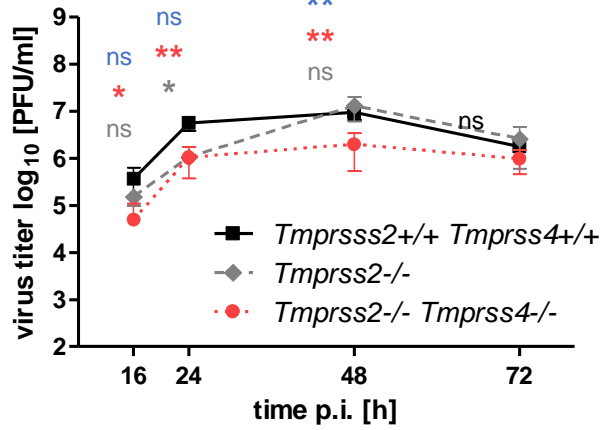
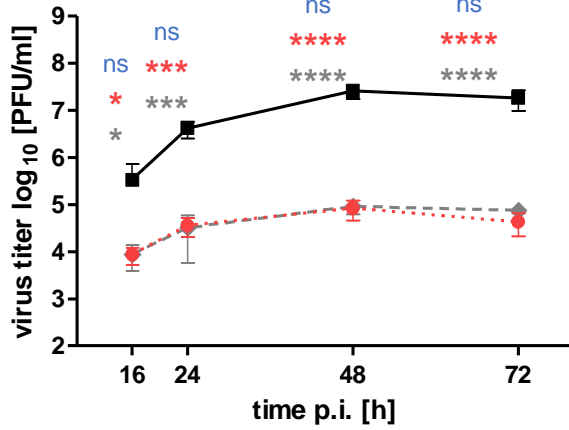
Figure S1: (A) Expression of murine matriptase, CFB, LTF, NSP4, tPA and uPA in HEK293 cells. Cells were transfected with protease-encoding plasmids or empty vector (ev) as control and incubated for 48 h. Cell supernatants were cleared from cell debris and concentrated 5x (tPA) or 10x (uPA, LTF, NSP4, CFB) by ultrafiltration. Cell lysates and concentrated supernatants were subjected to SDS-PAGE under reducing conditions and Western blot analysis with FLAG-specific antibodies that recognize the C-terminal DDK tag. Zymogens and mature forms are indicated by filled and open arrowheads, respectively. Non-specific bands are indicated by an asterisk. (B) Enzymatic activity of tPA and uPA in HEK293 supernatants. HEK293 were transfected with protease-encoding plasmids or empty vector (ev) and incubated for 48 h. Cell supernatants were diluted with buffer (1:4 for tPA and 1:5 for uPA) or recombinant rKLK8 (1:4) and incubated with 50 μ M of fluorogenic peptide substrate Mes-dArg-Gly-Arg-AMC (tPA, uPA) or Boc-Val-Pro-Arg-AMC (rKLK8) to measure protease activity over a period of 30 min at room temperature with 15 detection cycles. Steady-state rates in relative fluorescence units (RFU) per sec ($v(\text{RFU/s})$) were calculated from the linear rate equation of enzymatic substrate turnover. Supernatants of cells transfected with empty vector were subtracted as background of unspecific substrate turnover and remaining protease activity (in %) in presence of inhibitor was standardized to untreated controls. Data shown are mean values \pm SD of \geq three independent experiments. (C) Enzymatic activity of tryptase ϵ DDDDK mutant in HEK293 supernatants. Cells were transfected with a tryptase ϵ DDDDK mutant-encoding plasmid or empty vector (ev). At 24 h post transfection cell supernatants were treated with or without 10 UI of enterokinase (EK) for 24 h, harvested and cleared from cell debris, concentrated (5x) by ultrafiltration and subjected to SDS-PAGE and Western blot analysis. Protease activity was assayed as described above. Data shown are mean values \pm SD of \geq three independent experiments.

Figure S2

A

Anhui/H7/PR8

Aichi/H3N2/PR8



B

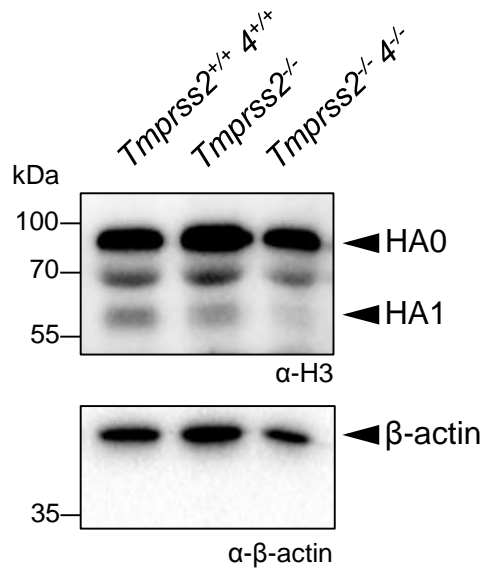
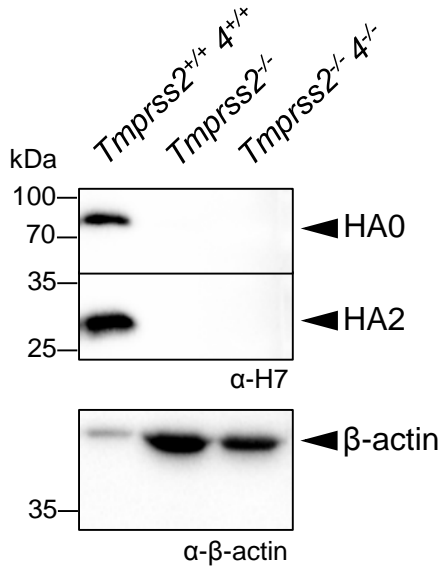


Figure S2: (A) Proteolytic activation and multicycle replication of Anhui/H7/PR8 and Aichi/H3N2/PR8 in AECII of *Tmprss2*^{-/-} and *Tmprss2*^{-/-}*Tmprss4*^{-/-} or wild-type mice. Primary AECII of *Tmprss2*^{-/-} or *Tmprss2*^{-/-}*Tmprss4*^{-/-} or wild-type mice were infected at a MOI of 0.01 and incubated for 72 h. Virus titers were determined by plaque assay at indicated time points. Data are mean values \pm SD (n = 3-4). Log-transformed data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test according to the number of parameters and groups being compared. Statistical significance is color-coded (gray: wild-type vs. *Tmprss2*^{-/-}, red: wild-type vs. *Tmprss2*^{-/-}*Tmprss4*^{-/-}, blue: *Tmprss2*^{-/-} vs. *Tmprss2*^{-/-}*Tmprss4*^{-/-}). No significance between all groups is indicated by ns in black letters. (B) At 72 h post infection cell lysates were analysed by SDS-PAGE under reducing conditions and Western blot using HA-specific antibodies against HA. β -actin served as loading control.

Table S-1: Distribution of trypsin-like serine protease mRNAs in FPKM

Gene name	Trachea					Bronchie					Lung				
	1	2	3	mean	SD	1	2	3	mean	SD	1	2	3	mean	SD
C2	1.61	2.46	2.02	2.03	0.42	3.99	5.93	8.96	6.29	2.51	6.32	5.93	5.95	6.06	0.22
Cfb	16.21	35.95	43.92	32.03	14.27	20.87	22.96	18.96	20.93	2.00	15.03	14.96	13.95	14.65	0.60
Corin	1.10	4.28	1.15	2.18	1.82	11.46	15.98	22.47	16.64	5.53	0.40	0.24	0.27	0.30	0.09
F10	0.07	0.09	0.13	0.09	0.03	0.26	1.17	0.61	0.68	0.46	4.15	6.03	4.64	4.94	0.98
F2	0.55	0.80	0.98	0.78	0.22	1.21	1.35	2.21	1.59	0.54	0.12	0.19	0.19	0.17	0.04
Gzma	0.27	1.10	0.93	0.77	0.44	0.86	1.13	3.40	1.80	1.40	18.05	19.09	12.34	16.49	3.63
Hgfac	0.34	0.59	0.83	0.59	0.24	0.38	0.49	0.65	0.51	0.14	1.43	1.44	1.38	1.42	0.03
Hpn	9.89	6.92	9.22	8.67	1.56	4.80	6.71	3.36	4.96	1.68	4.32	4.88	4.99	4.73	0.36
Klk1	0.69	0.92	0.69	0.77	0.13	1.70	1.00	0.91	1.20	0.44	1.84	3.88	2.61	2.78	1.03
Klk11	3.69	6.78	10.05	6.84	3.18	7.74	6.53	3.83	6.03	2.00	1.32	1.20	1.13	1.21	0.10
Klk14	0.16	0.15	0.15	0.15	0.01	0.28	0.16	0.11	0.18	0.09	0.98	1.98	1.19	1.38	0.53
Klk1b21	1.14	0.29	0.73	0.72	0.42	0.62	0.42	0.35	0.46	0.14	0.25	0.27	0.47	0.33	0.12
Klk1b22	1.86	0.07	0.15	0.70	1.01	0.00	0.04	0.03	0.02	0.02	0.03	0.03	0.00	0.02	0.02
Klk1b9	1.37	0.02	0.09	0.50	0.76	0.03	0.00	0.00	0.01	0.02	0.06	0.10	0.00	0.05	0.05
Klk8	2.18	2.10	2.00	2.09	0.09	2.21	2.45	2.44	2.37	0.13	6.53	5.94	7.25	6.58	0.66
Ltf	3090.69	2451.06	6548.47	4030.08	2204.32	987.25	632.69	505.18	708.37	249.79	9.29	8.04	10.21	9.18	1.09
Plat	8.51	21.78	19.36	16.55	7.06	15.98	20.09	18.36	18.14	2.07	17.80	22.65	20.01	20.15	2.43
Plau	2.72	3.17	2.57	2.82	0.31	2.04	2.91	2.98	2.64	0.52	6.02	5.08	6.00	5.70	0.54
Plg	0.01	0.01	0.00	0.00	0.00	0.00	0.00	1.26	0.42	0.73	0.02	0.02	0.04	0.03	0.01
Proc	0.43	0.76	0.76	0.65	0.19	2.13	1.18	1.24	1.52	0.53	0.50	0.46	0.59	0.52	0.07
Prss12	0.93	1.71	1.71	1.45	0.45	1.45	1.59	1.70	1.58	0.13	5.96	5.89	4.93	5.59	0.58
Prss22	2.06	3.46	5.10	3.54	1.52	3.39	2.82	3.92	3.38	0.55	0.37	0.69	0.63	0.56	0.17
Prss32	4.12	2.67	4.20	3.66	0.86	0.86	0.35	0.29	0.50	0.31	0.00	0.03	0.01	0.01	0.01
Prss36	2.79	1.99	2.57	2.45	0.41	2.13	1.86	2.25	2.08	0.20	1.83	1.48	1.69	1.66	0.18
Prss8	78.60	26.26	48.32	51.06	26.27	20.96	23.86	12.84	19.22	5.71	40.35	41.24	42.19	41.26	0.92
St14	36.12	27.08	37.90	33.70	5.80	25.75	24.76	16.89	22.46	4.86	16.16	16.46	17.53	16.72	0.72
Tmprss11a	1.35	2.90	2.00	2.08	0.78	0.17	0.06	0.01	0.08	0.08	0.00	0.00	0.00	0.00	0.00
Tmprss11g	0.21	0.47	0.27	0.31	0.14	0.09	0.13	0.29	0.17	0.11	0.00	0.00	0.01	0.00	0.00
Tmprss13	4.35	6.53	7.47	6.12	1.60	4.14	3.21	2.76	3.37	0.70	1.26	1.01	0.99	1.09	0.15
Tmprss2	21.95	27.66	23.44	24.35	2.96	22.32	20.59	15.45	19.45	3.57	26.51	25.69	26.66	26.29	0.52
Tmprss3	0.36	0.08	0.51	0.32	0.22	0.04	0.06	0.03	0.04	0.01	0.02	0.03	0.00	0.02	0.02
Tmprss4	3.76	6.06	6.26	5.36	1.39	7.28	7.71	4.24	6.41	1.89	10.38	9.92	10.15	10.15	0.23
Tmprss5	0.43	0.46	0.24	0.38	0.12	0.38	0.52	0.47	0.46	0.07	0.31	0.31	0.37	0.33	0.04
Tmprss6	0.25	0.30	0.51	0.35	0.14	0.36	0.33	0.41	0.37	0.04	0.05	0.07	0.10	0.07	0.03

Table S-2: Distribution of trypsin-like serine protease mRNAs in FPKM

Gene name	MLE-15					AECII				
	1	2	3	mean	SD	1	2	3	mean	SD
C2	0.98	1.01	0.91	0.97	0.06	1.63	1.55	1.03	1.40	0.33
Cfb	1.04	0.86	0.79	0.90	0.12	5.53	5.88	5.85	5.75	0.19
Hgfac	0.01	0.03	0.10	0.05	0.05	1.60	1.16	1.54	1.43	0.24
Hpn	1.11	1.05	0.92	1.03	0.10	8.82	9.34	7.59	8.58	0.90
Klk11	0.22	0.29	0.53	0.35	0.17	0.04	0.01	0.00	0.02	0.02
Klk14	1.35	1.15	0.55	1.02	0.42	0.00	0.00	0.02	0.01	0.01
Klk8	0.07	0.02	0.02	0.03	0.03	0.62	0.58	0.91	0.71	0.18
Ltf	0.05	0.07	0.05	0.05	0.01	56.37	49.62	75.33	60.44	13.33
Plat	13.97	14.26	19.19	15.80	2.93	12.00	12.84	13.63	12.82	0.82
Plau	12.05	12.34	15.45	13.28	1.89	68.25	50.79	54.54	57.86	9.19
Proc	0.62	0.57	0.36	0.52	0.14	0.72	1.19	0.84	0.92	0.24
Prss12	9.55	9.60	8.28	9.15	0.75	0.20	0.26	0.28	0.25	0.04
Prss22	3.93	3.44	5.98	4.45	1.34	132.54	115.70	94.43	114.23	19.10
Prss27	0.03	0.05	0.04	0.04	0.01	0.47	0.45	0.35	0.42	0.07
Prss32	0.13	0.26	0.49	0.29	0.18	0.42	0.53	0.60	0.52	0.09
Prss36	1.84	1.71	1.57	1.71	0.14	0.52	0.36	0.51	0.46	0.09
Prss57	0.14	0.20	0.27	0.20	0.07	14.80	11.57	8.61	11.66	3.10
Prss8	20.09	21.95	19.65	20.56	1.22	9.64	12.52	13.04	11.73	1.83
St14	14.15	13.97	12.14	13.42	1.11	117.63	111.79	101.71	110.37	8.05
Tmprss13	0.05	0.05	0.01	0.04	0.03	1.58	1.60	1.25	1.48	0.20
Tmprss2	0.03	0.03	0.05	0.04	0.01	11.93	14.37	15.94	14.08	2.02
Tmprss4	0.55	0.50	0.30	0.45	0.13	1.35	1.43	1.89	1.55	0.29

Table S-3: Differential protease gene expression between AECII compared with MLE-15 cells

Gene name	AECII vs MLE-15 cells	
	log2FC	p-value
C2	0.76	1.0E-03
Cfb	2.90	1.1E-84
Hgfac	5.29	1.1E-23
Hpn	3.28	1.2E-102
Klk11	4.32	8.1E-06
Klk14	-7.30	1.5E-08
Klk8	4.60	2.8E-10
Ltf	10.36	1.2E-160
Plat	-0.07	6.4E-01
Plau	2.35	4.4E-41
Proc	1.04	2.9E-03
Prss12	-4.98	2.0E-134
Prss22	4.91	1.3E-94
Prss27	3.56	4.2E-07
Prss32	1.06	4.1E-02
Prss36	-1.65	9.5E-14
Prss57	6.09	3.6E-85
Prss8	-0.58	5.6E-05
St14	3.27	4.5E-170
Tmprss13	5.46	5.9E-38
Tmprss2	8.84	6.9E-105
Tmprss4	2.00	8.4E-12