

SUPPLEMENTAL MATERIAL

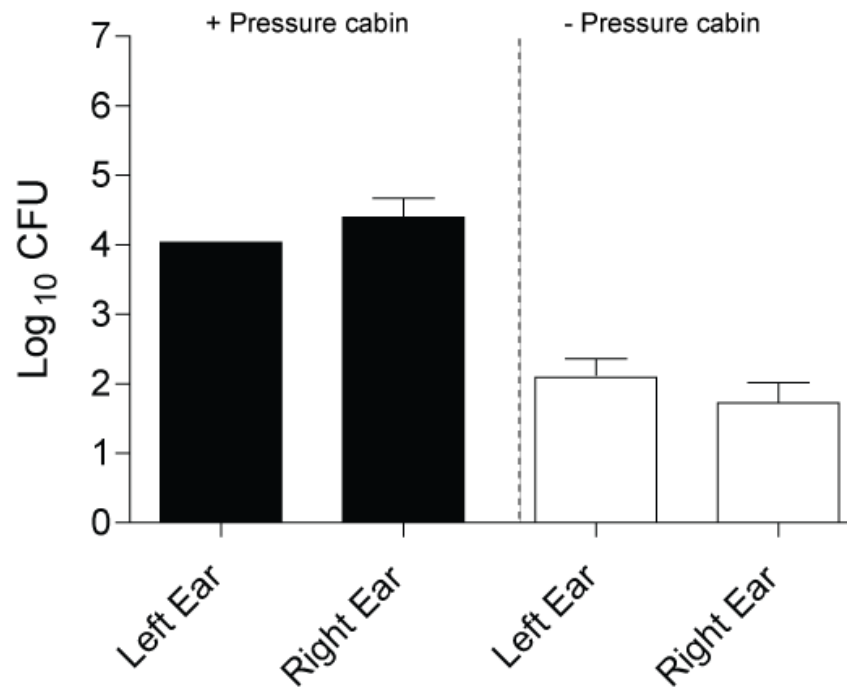


Fig. S1. The pressure cabin delivers an intranasal inoculum to the middle ear. Fourteen day old mice were administered 10^6 CFU of *S. pneumoniae* intranasally and then exposed to the pressure cabin as described in the materials and methods. Mice were then euthanized and the middle ears were collected for enumeration of bacterial load (“+ pressure cabin”). Alternatively, mice were administered *S. pneumoniae* intranasally in the absence of the pressure cabin and then euthanized for enumeration of bacterial load in the middle ear (“- pressure cabin”). Data shown represents the mean \pm SEM.

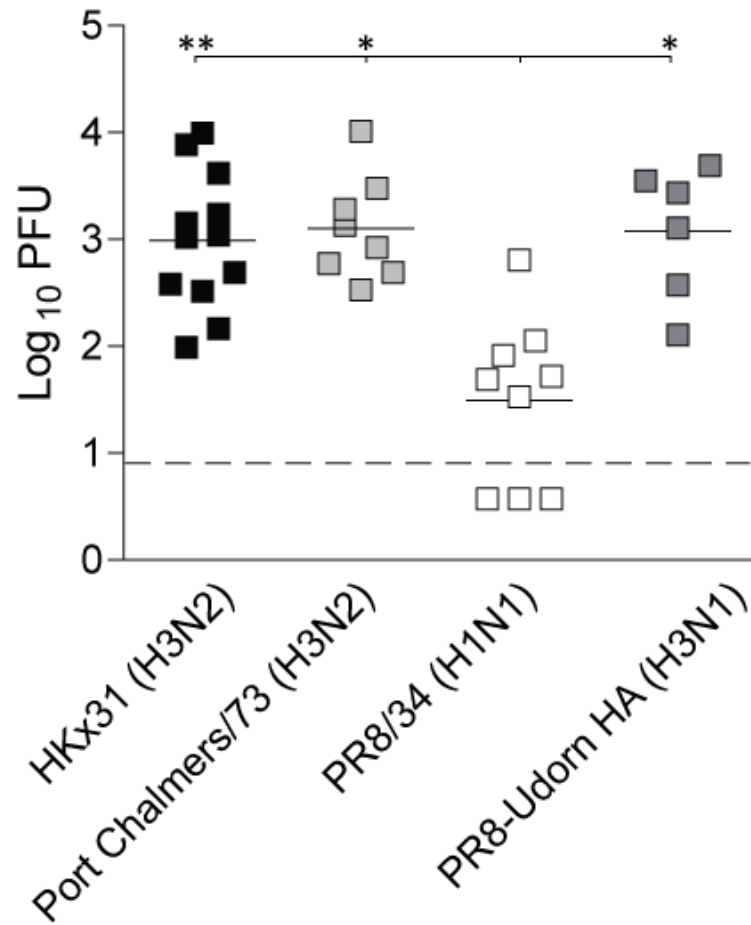


Fig. S2. Titers of different IAV strains in the middle ears of mice six days post-intranasal IAV infection. Viral titers are represented as the average of titers derived from the left and right ear of each mouse. Statistical significance was determined using a Mann-Whitney *U*-Test and Bonferroni post-test and was analyzed relative to PR8/34. Statistical significance is denoted by two asterisks ($p < 0.01$) or one asterisk ($p < 0.05$). Data are pooled from a minimum of two independent experiment and the dashed line indicates the detection limit of the assay.

Table S1: Genes upregulated in the middle ears of Udorn/72 infected mice relative to Udorn-PR8 HA infected mice

Sequence ID	Gene ID	Gene name	P value Udorn/Udorn- PR8 HA	Q value Udorn/Udorn- PR8 HA	Fold change Udorn/Udorn- PR8 HA
AK002830	54123	Irf7	0.000208	0.00948967	4.02
BC018331	68774	Ms4a6d	4.71E-09	2.57E-05	4.04
BC044865	20558	Slfn4	0.0000575	0.004373765	4.08
AK076430	17857	Mx1	0.000108	0.006303825	4.10
AK159883	54123	Irf7	0.000104	0.006194856	4.14
AK165065	16145	Igtp	0.0000016	0.000728961	4.15
BC116911	50501	Prok2	0.0000158	0.002214919	4.15
BC057889	12475	Cd14	0.0000401	0.003517138	4.17
BC132518	20756	Sprr2b	0.00215	0.03614913	4.17
BC132100	331535	Serpina7	0.000000319	0.000327007	4.17
BC050835	15958	Ifit2	0.0000359	0.003354665	4.20
BC096369	20531	Slc34a2	0.00000307	0.001126244	4.28
AK152337	16145	Igtp	0.00000308	0.001126244	4.30
BC132557	16145	Igtp	0.00000257	0.000980283	4.30
DQ021879	17885	Myh8	0.00133	0.02733603	4.31
BC146444	408196	Gm5416	0.00000838	0.001673554	4.33
NM_010846	17857	Mx1	0.000073	0.005127701	4.34
XM_485980	434219	Gm5598	0.00000108	0.000580779	4.35
U44731	55932	Gbp3	0.0000193	0.002492529	4.37
BC019195	55932	Gbp3	0.0000165	0.002255222	4.40
BC016101	64177	Trpv6	8.26E-08	0.000175702	4.40
BC007143	100702	Mpa2l	0.0000084	0.001673554	4.41
BC003768	15957	Ifit1	0.0000738	0.005150806	4.59

AK155452	56619	Clec4e	0.0000888	0.005740566	4.67
BC156163	20762	Sprr2h	0.0000301	0.003010297	4.68
BC052900	19225	Ptgs2	0.000238	0.01021881	4.69
BC066841	60533	Cd274	0.00000529	0.001363384	4.73
E01225	N/A	N/A	0.0000455	0.003807518	4.86
AK088858	21822	Tgtp	0.00000345	0.001154808	5.33
BC057684	71898	Apol9b	0.00000341	0.001154808	5.34
BC114209	626578	Gbp10	0.0000105	0.001841893	5.36
BC057969	100702	Mpa2l	0.00000467	0.001276593	5.37
XM_905096	631323	Gm12250	0.00000467	0.001276593	5.38
BC020489	223672	Apol9a	0.00000105	0.000574057	5.61
BC112402	99899	Ifi44	0.0000272	0.002845707	5.64
BC002084	12992	Csn1s2b	0.000178	0.00862229	5.67
NM_001005858	667370	I830012O16Rik	0.0000111	0.001886611	5.80
BC002085	14939	Gzmb	0.0000045	0.001272539	5.81
BC061126	20306	Ccl7	3.86E-08	0.000126621	5.81
BC034256	21822	Tgtp	0.0000033	0.001154808	6.03
BC058555	229898	Gbp5	4.88E-08	0.000140507	6.05
BC023105	667597	BC023105	0.00000079	0.000498013	6.06
BC117014	330122	Cxcl3	0.000013	0.001974269	6.16
BC003804	15959	Ifit3	0.00000388	0.001210006	6.17
AK150926	17474	Clec4d	0.0000448	0.003807215	6.22
NM_001101605	667373	Gm14446	0.000029	0.002954329	6.24
AK172047	60440	Iigp1	0.000000456	0.000364401	6.35
AK170692	17474	Clec4d	0.0000513	0.004064749	6.36
XM_001471581	667597	BC023105	0.000000404	0.00034875	6.55
AK155919	17474	Clec4d	0.0000137	0.002061488	6.61
BC003727	16175	Il1a	0.000091	0.005807577	6.65
BC004649	60440	Iigp1	0.00000115	0.000608447	6.73

BC003343	17329	Cxcl9	5.14E-08	0.000140507	6.85
AJ007971	60440	Iigp1	0.000000148	0.000242744	6.87
BC011336	14469	Gbp2	6.63E-08	0.000158627	7.04
BC020033	58203	Zbp1	0.00000051	0.000364401	7.26
BC117057	21926	Tnf	0.0000255	0.002778124	7.45
AK155874	20310	Cxcl2	0.0000766	0.005213128	7.74
BC032882	14469	Gbp2	9.5E-09	3.46E-05	7.98
BC024392	20311	Cxcl5	0.00000207	0.000859528	8.10
AK152620	667370	I830012O16Rik	0.00000168	0.000744722	8.24
AK149459	331535	Serpina7	0.000000818	0.000498013	8.88
BC042532	16181	Il1rn	7.75E-10	6.36E-06	9.87
AK030414	229898	Gbp5	0.000000384	0.00034875	10.17
AK171037	16181	Il1rn	5.73E-09	2.57E-05	10.47
BC003218	56619	Clec4e	0.000000485	0.000364401	10.65
BC111443	20302	Ccl3	8.57E-08	0.000175702	10.92
BC120715	80885	Niacr1	0.00000149	0.00070836	11.83
BC040425	226695	Ifi205	1.9E-09	1.25E-05	12.26
BC119257	20303	Ccl4	0.000000175	0.000243501	14.72
XM_127883	16365	Irg1	0.00000135	0.000670975	14.73
BC011437	16176	Il1b	0.000000244	0.000287311	16.87
AK224980	16176	Il1b	0.000000169	0.000243501	17.68
BC030067	15945	Cxcl10	2.77E-10	3.03E-06	17.93
BC119511	20310	Cxcl2	0.000000108	0.000202287	23.86
BC055885	20210	Saa3	0.00000851	0.001673554	24.41
BC049931	15930	Ido1	1.35E-11	4.43E-07	27.33
AK089891	16365	Irg1	3.52E-11	5.77E-07	158.98

Table S2: Genes downregulated in the middle ears of Udorn/72 infected mice relative to Udorn-PR8 HA infected mice

Sequence ID	Gene ID	Gene name	P value Udorn/Udorn- PR8 HA	Q value Udorn/Udorn- PR8 HA	Fold change Udorn/Udorn- PR8 HA
BC065108	56410	Cbln3	0.000518	0.01594003	0.198395118
BC094611	18012	Neurod1	0.000637	0.01805735	0.203279257
BC072635	268934	Grm4	0.000387	0.01349081	0.239306405
BC058965	18013	Neurod2	0.0000275	0.002845707	0.241170958
BC018241	18012	Neurod1	0.00359	0.0491091	0.242510915
XM_001476613	100041343	Gm3282	0.000000179	0.000243501	0.243253872

Table S3: Statistical analysis (Kruskal-Wallis test accompanied by Dunn's multiple comparisons test) of middle ear inflammation induced by different IAV strains

IAV strains	Significant?
PUHA (H3N1) vs. PR8- HKx31 HA (H3N1)	No ^a
PUHA (H3N1) vs. Udorn-PR8 NA (H3N1)	No
PUHA (H3N1) vs. PR8 (H1N1)	Yes ^b
PUHA (H3N1) vs. HKx31 (H3N2)	No
PUHA (H3N1) vs. Udorn (H3N2)	No
PUHA (H3N1) vs. Udorn-PR8 HA (H1N2)	Yes
PUHA (H3N1) vs. PR8 HKx31 NA (H1N2)	Yes
PUHA (H3N1) vs. PR8-Auck HA (H1N1)	Yes
PUHA (H3N1) vs. PR8-Udorn NA (H1N2)	Yes
PUHA (H3N1) vs. Port Chalmers/73 (H3N2)	No
PR8- HKx31 HA (H3N1) vs. Udorn-PR8 NA (H3N1)	No
PR8- HKx31 HA (H3N1) vs. PR8 (H1N1)	Yes
PR8- HKx31 HA (H3N1) vs. HKx31 (H3N2)	No
PR8- HKx31 HA (H3N1) vs. Udorn (H3N2)	No
PR8- HKx31 HA (H3N1) vs. Udorn-PR8 HA (H1N2)	Yes
PR8- HKx31 HA (H3N1) vs. PR8 HKx31 NA (H1N2)	Yes
PR8- HKx31 HA (H3N1) vs. PR8-Auck HA (H1N1)	Yes
PR8- HKx31 HA (H3N1) vs. PR8-Udorn NA (H1N2)	Yes
PR8- HKx31 HA (H3N1) vs. Port Chalmers/73 (H3N2)	No
Udorn-PR8 NA (H3N1) vs. PR8 (H1N1)	Yes
Udorn-PR8 NA (H3N1) vs. HKx31 (H3N2)	No
Udorn-PR8 NA (H3N1) vs. Udorn (H3N2)	No
Udorn-PR8 NA (H3N1) vs. Udorn-PR8 HA (H1N2)	Yes
Udorn-PR8 NA (H3N1) vs. PR8 HKx31 NA (H1N2)	No
Udorn-PR8 NA (H3N1) vs. PR8-Auck HA (H1N1)	No
Udorn-PR8 NA (H3N1) vs. PR8-Udorn NA (H1N2)	Yes
Udorn-PR8 NA (H3N1) vs. Port Chalmers/73 (H3N2)	No
PR8 (H1N1) vs. HKx31 (H3N2)	Yes
PR8 (H1N1) vs. Udorn (H3N2)	Yes
PR8 (H1N1) vs. Udorn-PR8 HA (H1N2)	No
PR8 (H1N1) vs. PR8 HKx31 NA (H1N2)	No
PR8 (H1N1) vs. PR8-Auck HA (H1N1)	No
PR8 (H1N1) vs. PR8-Udorn NA (H1N2)	No
PR8 (H1N1) vs. Port Chalmers/73 (H3N2)	No
HKx31 (H3N2) vs. Udorn (H3N2)	No
HKx31 (H3N2) vs. Udorn-PR8 HA (H1N2)	Yes
HKx31 (H3N2) vs. PR8 HKx31 NA (H1N2)	Yes
HKx31 (H3N2) vs. PR8-Auck HA (H1N1)	No
HKx31 (H3N2) vs. PR8-Udorn NA (H1N2)	Yes
HKx31 (H3N2) vs. Port Chalmers/73 (H3N2)	No
Udorn (H3N2) vs. Udorn-PR8 HA (H1N2)	Yes
Udorn (H3N2) vs. PR8 HKx31 NA (H1N2)	Yes
Udorn (H3N2) vs. PR8-Auck HA (H1N1)	Yes
Udorn (H3N2) vs. PR8-Udorn NA (H1N2)	Yes

Udorn (H3N2) vs. Port Chalmers/73 (H3N2)	No
Udorn-PR8 HA (H1N2) vs. PR8 HKx31 NA (H1N2)	No
Udorn-PR8 HA (H1N2) vs. PR8-Auck HA (H1N1)	No
Udorn-PR8 HA (H1N2) vs. PR8-Udorn NA (H1N2)	No
Udorn-PR8 HA (H1N2) vs. Port Chalmers/73 (H3N2)	No
PR8 HKx31 NA (H1N2) vs. PR8-Auck HA (H1N1)	No
PR8 HKx31 NA (H1N2) vs. PR8-Udorn NA (H1N2)	No
PR8 HKx31 NA (H1N2) vs. Port Chalmers/73 (H3N2)	No
PR8-Auck HA (H1N1) vs. PR8-Udorn NA (H1N2)	No
PR8-Auck HA (H1N1) vs. Port Chalmers/73 (H3N2)	No
PR8-Udorn NA (H1N2) vs. Port Chalmers/73 (H3N2)	Yes

^a $p \geq 0.05$

^b $p < 0.05$