

Identification of an infectious bronchitis coronavirus strain exhibiting a classical genotype but altered antigenicity, pathogenicity, and innate immunity profile

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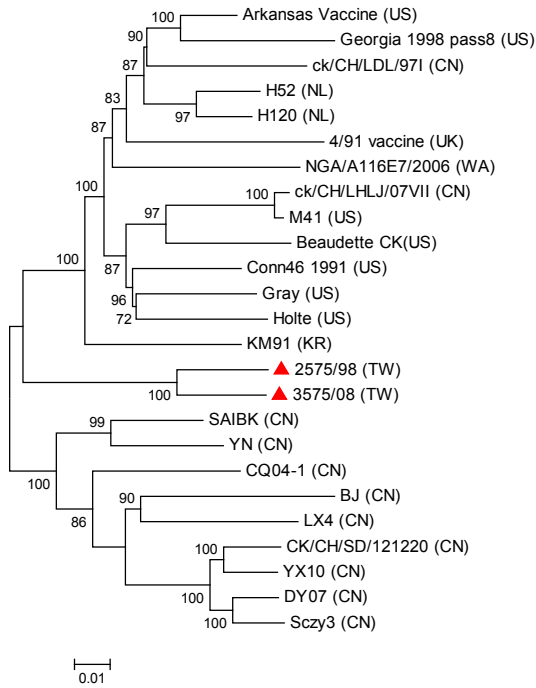
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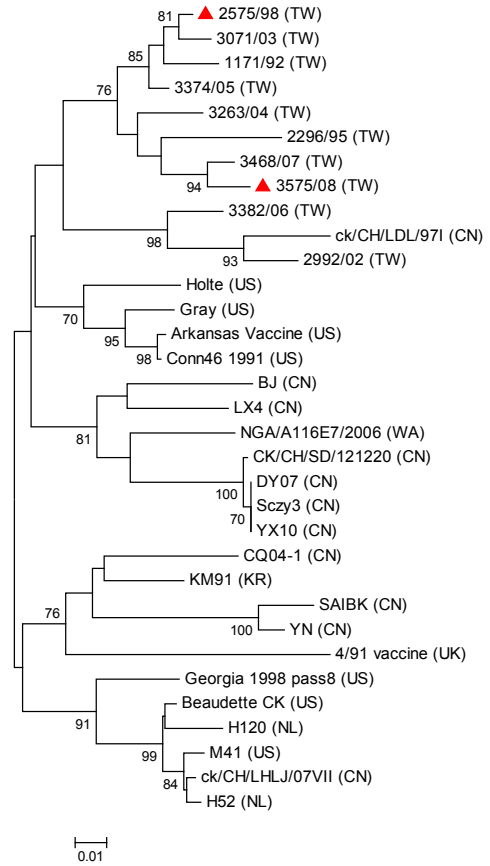
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Supplementary Figure S1. Phylogenetic analyses of full length, E (envelope), M (membrane), and N (nucleocapsid) protein genes of IBV strains. The phylogenetic trees were constructed using the MEGA version 4 by the neighbor-joining method (bootstrapping for 1,000 replicates with its value >70%). The 3575/08 and 2575/98 strains analyzed in this study are indicated with red triangles. (AU: Australia, CN: China, NL: Netherlands, TW: Taiwan, UK: United Kingdom, US: United States, WA: Western Africa)

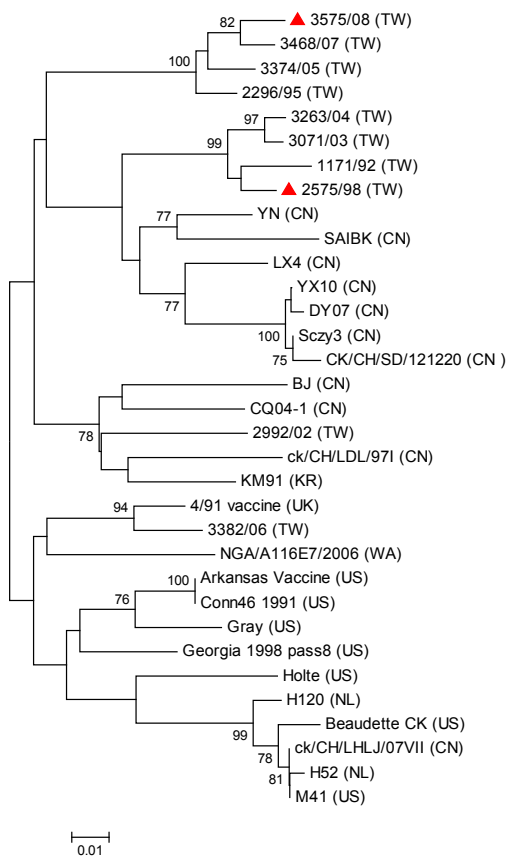
Full length



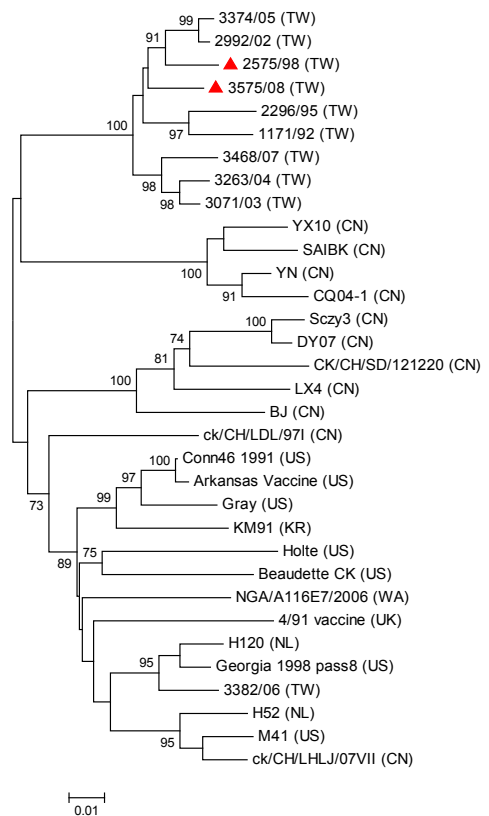
E



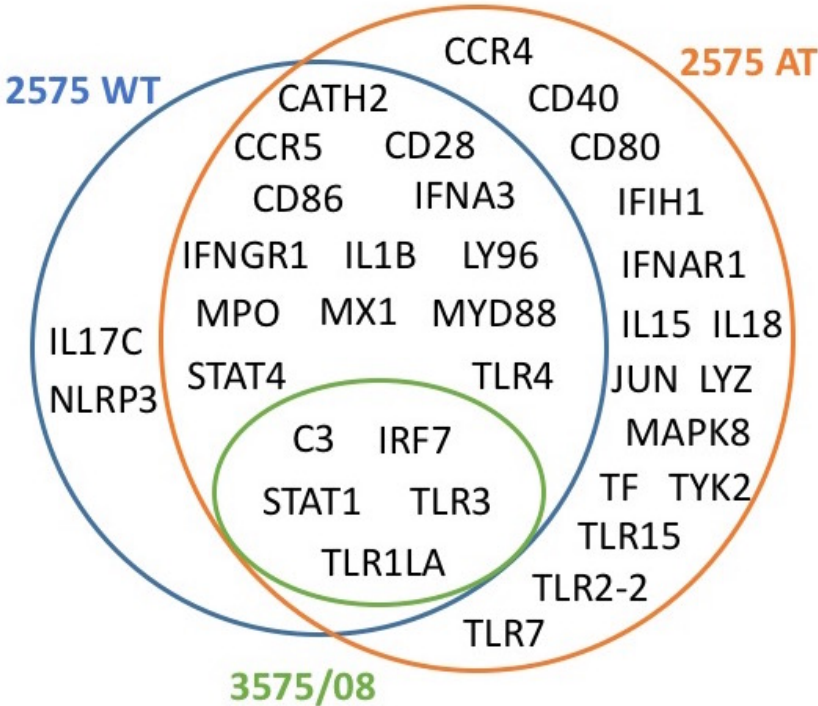
M



N



Supplementary Figure S2. Venn diagram of immune genes stimulated by IBV 3575/08, 2575 WT and 2575 AT at 24 hpi.



Supplementary Table S1. Cross neutralization test of IBVs. Homologous titers are indicated in bold.

IBV strain	Virus			
	3575/08 (TW-I)	2575/98 (TW-I)	2296/95 (TW-II)	H120 (Mass)
Serum 3575/08 (TW-I)	8192	102	34	16
2575/98 (TW-I)	128	1475	108	16
2296/95 (TW-II)	140	101	512	203
H120 (Mass)	32	32	22	531

Supplementary Table S2. Quantitative PCR Array data from infected chicken lungs. A RT² Profiler Chicken Innate & Adaptive Immune Responses PCR Array (PAGG-052ZG; SABiosciences) was performed following the manufacturer's protocol. Complete list of 84 genes that were examined in the PCR array is presented. Fold change and its 95% confidence interval (CI) were calculated based upon naive chickens for IBV 3575/08, 2575 WT or 2575 AT-infected chickens using software provided by the manufacturer at 24h and 48h post infection. Numbers in red indicate fold change greater than 3.

Gene sym.	Description	24 hpi										48 hpi			
		3575/08		2575 WT		2575 AT		3575/08		2575 WT		2575 AT			
		Fold change	95% CI	Fold change	95% CI	Fold change	95% CI	Fold change	95% CI	Fold change	95% CI	Fold change	95% CI		
C3	Complement component 3	5.00	(2.23, 7.76)	3.01	(2.23, 5.50)	8.12	(0.00001, 23.01)	8.02	(0.00001, 17.85)	0.99	(0.92, 1.05)	1.53	(0.00001, 3.43)		
CSA1	Complement component 5a receptor 1	1.08	(0.37, 1.79)	1.60	(0.07, 3.13)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.91	(0.93, 1.69)		
CAT2	Cathepsin A (cathepsin B paralog)	2.07	(0.00001, 4.80)	6.10	(2.01, 10.19)	8.12	(0.00001, 22.12)	10.46	(0.18, 20.78)	6.75	(1.72, 20.25)	2.15	(0.57, 7.73)		
CASP1	Caspase 1, apoptosis-related cysteine peptidase	0.95	(0.45, 1.45)	1.05	(0.26, 1.84)	1.83	(1.02, 2.64)	1.13	(0.11, 2.15)	0.88	(0.75, 1.01)	0.98	(0.65, 1.31)		
CASP9	Caspase 9, apoptosis-related cysteine peptidase	0.99	(0.55, 1.43)	0.89	(0.49, 1.29)	1.45	(0.84, 2.06)	0.69	(0.35, 1.03)	0.61	(0.46, 0.77)	0.87	(0.53, 1.21)		
CC14	Chemokine (C-C motif) ligand 4	1.47	(0.44, 2.50)	2.67	(1.48, 3.86)	1.92	(1.08, 2.76)	2.68	(0.00001, 5.48)	1.73	(1.4, 2.08)	1.74	(0.62, 2.86)		
CC15	Chemokine (C-C motif) ligand 5	1.07	(0.36, 1.78)	1.87	(0.67, 3.07)	1.8	(0.91, 2.69)	2.20	(0.29, 4.11)	1.62	(1.31, 1.95)	1.73	(0.77, 1.69)		
CC16	Chemokine (C-C motif) receptor 4	1.10	(0.40, 1.80)	2.24	(1.20, 3.28)	3.34	(0.00001, 6.97)	2.33	(0.37, 4.29)	1.71	(1.39, 2.06)	1.30	(0.91, 1.69)		
CCR5	Chemokine (C-C motif) receptor 5	2.74	(0.00001, 5.90)	4.77	(0.79, 8.75)	12.03	(0.00001, 37.15)	2.96	(0.00001, 7.80)	1.43	(1.16, 1.72)	1.49	(0.81, 2.50)		
CCR6	Chemokine (C-C motif) receptor 6	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
CCR8	Chemokine (C-C motif) receptor 8	0.89	(0.26, 1.52)	2.20	(1.07, 3.33)	1.92	(1.13, 2.71)	1.94	(0.24, 3.64)	1.73	(1.6, 1.85)	1.20	(0.71, 1.69)		
CD14	CD14 molecule	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
CD28	CD28 molecule	1.72	(0.00001, 3.48)	3.29	(1.19, 5.39)	14.47	(6.42, 22.52)	3.99	(0.00001, 11.75)	2.56	(1.86, 3.34)	1.77	(1.38, 5.56)		
CD4	CD4 molecule	1.71	(0.19, 3.23)	2.84	(1.43, 4.25)	2.95	(1.26, 4.64)	2.66	(0.96, 4.36)	1.73	(1.4, 2.08)	3.47	(0.70, 2.84)		
CD40	CD40 molecule, TNF receptor superfamily, member 5	1.65	(0.00001, 3.92)	2.92	(0.00001, 6.11)	4.45	(1.28, 7.62)	3.84	(0.00001, 10.30)	1.35	(1.09, 1.63)	1.78	(0.17, 3.39)		
CD40LG	CD40 ligand (TNF superfamily, member 5, hyper-IgM syndrome)	1.07	(0.28, 1.90)	2.97	(0.33, 5.61)	1.9	(0.51, 3.29)	2.37	(0.00001, 5.29)	1.48	(1.16, 1.83)	1.51	(0.32, 2.70)		
CD80	CD80 molecule	1.01	(0.46, 1.56)	2.42	(1.47, 3.37)	3.05	(0.92, 5.18)	2.47	(0.76, 4.18)	1.49	(1.21, 1.79)	1.20	(1.02, 1.38)		
CD86	CD86 molecule	1.89	(0.12, 3.66)	4.10	(0.38, 7.82)	8.25	(2.90, 13.60)	2.77	(0.00001, 5.81)	1.73	(1.4, 2.08)	1.73	(0.62, 2.84)		
CD8A	CD8a molecule	0.71	(0.20, 1.20)	2.24	(0.45, 4.03)	1.56	(0.92, 2.20)	1.54	(0.00001, 2.88)	1.13	(0.92, 1.36)	0.66	(0.54, 1.18)		
CRP	C-reactive protein, pentraxin-related	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
CSF2	Granulocyte-macrophage colony-stimulating factor	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
CXCL12	Chemokine (C-C motif) ligand 12 (stromal cell-derived factor 1)	0.87	(0.00001, 1.85)	0.56	(0.00001, 1.35)	1.43	(0.50, 2.36)	1.98	(0.00001, 4.37)	1.53	(1.45, 1.63)	0.68	(0.23, 1.13)		
CCR4	Chemokine (C-C motif) receptor 4	0.79	(0.05, 1.53)	1.14	(0.79, 2.09)	0.77	(0.00001, 1.93)	0.95	(0.50, 1.40)	0.58	(0.55, 0.61)	0.48	(0.03, 0.93)		
FAS	Fas (TNF receptor superfamily, member 6)	1.47	(0.00001, 2.97)	1.85	(0.10, 3.60)	2.35	(0.01, 4.69)	2.67	(0.00001, 6.34)	1.85	(1.15, 2.69)	0.77	(0.13, 1.41)		
FASLG	Fas ligand (TNF superfamily, member 6)	1.08	(0.37, 1.79)	2.02	(0.84, 3.20)	1.97	(1.18, 2.76)	4.17	(1.77, 6.57)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
GATA3	GATA binding protein 3	1.40	(0.09, 2.71)	2.29	(0.29, 4.29)	1.39	(0.33, 2.45)	1.04	(0.00001, 2.30)	1.01	(0.77, 1.28)	0.78	(0.03, 1.53)		
IFIH1	Interferon induced with helicase C domain 1	1.97	(0.00001, 4.34)	1.88	(0.92, 2.84)	3.35	(1.92, 4.78)	1.20	(0.25, 2.15)	1.60	(1.38, 1.83)	0.75	(0.34, 1.16)		
IFNA3	Interferon	1.20	(0.33, 2.08)	3.09	(2.04, 4.15)	27.63	(9.59, 45.67)	5.62	(1.39, 9.85)	1.36	(1.1, 1.64)	14.66	(1.99, 27.33)		
IFNA1	Interferon (alpha, beta and omega) receptor 1	1.10	(0.00001, 2.34)	2.58	(0.00001, 5.25)	3.59	(0.81, 6.37)	2.39	(0.02, 4.76)	1.90	(1.28, 2.62)	0.95	(0.00001, 1.96)		
IFNB	Interferon beta	1.21	(0.47, 1.93)	2.17	(1.09, 3.25)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
IFNG	Interferon, gamma	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
IFNGR1	Interferon gamma receptor 1	1.31	(0.00001, 3.55)	3.54	(0.00001, 7.40)	3.67	(1.96, 5.38)	3.36	(0.00001, 7.12)	1.61	(0.79, 2.71)	1.88	(0.43, 3.33)		
IL10	Interleukin 10	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
IL13	Interleukin 13	0.13	(0.00001, 0.70)	0.17	(0.00001, 0.94)	0.22	(0.00001, 1.18)	0.34	(0.00001, 1.83)	0.43	(0.2, 0.43)	0.15	(0.00001, 0.81)		
IL15	Interleukin 15	1.45	(0.00001, 2.98)	2.02	(0.44, 3.60)	5.06	(4.58, 5.54)	2.75	(0.14, 5.36)	1.29	(0.87, 3.38)	1.34	(0.98, 1.70)		
IL18	Interleukin 18 (interferon-gamma-inducing factor)	1.46	(0.44, 2.48)	2.12	(0.68, 3.56)	3.21	(2.16, 4.26)	2.20	(0.93, 3.47)	1.75	(1.66, 1.84)	1.27	(0.88, 1.66)		
IL18	Interleukin 1, beta	1.44	(0.5, 2.63)	3.83	(0.00001, 7.66)	4.96	(0.00001, 9.99)	2.79	(0.26, 3.32)	1.59	(0.39, 1.91)	1.53	(0.53, 2.51)		
IL1R1	Interleukin 1 receptor, type 1	1.54	(0.00001, 1.10)	1.18	(0.16, 2.01)	1.7	(0.00001, 3.54)	1.07	(0.00, 2.14)	0.58	(0.07, 0.59)	1.07	(0.28, 1.72)		
IL2	Interleukin 2	0.95	(0.39, 1.51)	1.59	(0.49, 2.69)	1.69	(1.13, 2.25)	2.07	(0.44, 3.70)	1.52	(1.23, 1.83)	1.15	(1.08, 1.22)		
IL4	Interleukin 4	0.99	(0.39, 1.59)	1.58	(0.34, 2.82)	1.75	(1.14, 2.36)	2.15	(0.44, 3.86)	1.58	(1.28, 1.9)	1.20	(1.06, 1.34)		
IL5	Interleukin 5	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.32	(0.94, 1.70)		
IL6	Interleukin 6 (interferon, beta 2)	1.08	(0.37, 1.79)	2.64	(0.00001, 7.59)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
IL8	Interleukin 8	0.95	(0.23, 1.67)	1.14	(0.00001, 2.71)	1.68	(0.72, 2.64)	2.06	(0.7, 3.95)	1.52	(1.23, 1.82)	1.15	(0.61, 1.69)		
IRAK1	Interleukin 1 receptor associated kinase 4	1.00	(0.10, 1.90)	1.09	(0.46, 1.72)	1.97	(1.13, 2.81)	0.67	(0.00001, 1.51)	0.66	(0.39, 1.01)	0.51	(0.00001, 1.21)		
IRF1	Interferon regulatory factor 1	2.26	(0.14, 4.38)	2.61	(0.97, 4.25)	1.86	(0.55, 3.17)	1.05	(0.00001, 2.45)	1.64	(1.3, 2.01)	0.45	(0.00001, 0.98)		
IRF6	Interferon regulatory factor 6	1.23	(0.31, 2.15)	0.71	(0.13, 2.19)	1.55	(0.52, 2.58)	1.18	(0.00001, 2.48)	1.49	(1.37, 1.6)	1.33	(0.81, 1.85)		
IRF7	Interferon regulatory factor 7	3.44	(0.00001, 8.01)	11.26	(0.40, 22.12)	4.9	(0.53, 9.27)	2.18	(0.00001, 4.63)	1.71	(1.28, 1.18)	1.40	(0.00001, 3.19)		
ITGB2	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	3.00	(1.55, 4.45)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
JAK2	Janus kinase 2 (a protein tyrosine kinase)	0.88	(0.37, 1.39)	1.59	(0.70, 2.48)	1.43	(0.66, 2.20)	1.31	(0.00001, 3.04)	0.46	(0.40, 0.49)	0.68	(0.18, 1.18)		
JUN	Jun proto-oncogene	1.16	(0.00001, 2.79)	1.77	(0.35, 3.19)	3.8	(0.00001, 7.81)	1.93	(0.27, 3.59)	0.50	(0.39, 0.63)	2.18	(0.5, 3.41)		
LITAF	Lipopolysaccharide-induced TNF factor	0.54	(0.19, 0.89)	1.08	(0.22, 1.94)	1.11	(0.50, 1.72)	0.81	(0.11, 1.51)	1.01	(0.69, 1.38)	0.26	(0.16, 0.36)		
IL17C	Interleukin-17C-like	1.08	(0.37, 1.79)	4.56	(0.00001, 18.66)	1.92	(1.08, 2.76)	2.35	(0.38, 4.32)	1.73	(1.4, 2.08)	1.31	(0.93, 1.69)		
STAT6	Signal transducer and transcription activator 6-like	1.73	(0.00001, 3.51)	2.58	(0.40, 4.76)	2.04	(0.00001, 4.12)	2.27	(0.10, 4.44)	1.34	(0.74, 2.1)	1.05	(0.00001, 2.21)		
TYK2	Similar to MGC83617 protein	1.44	(0.00001, 4.04)	2.61	(0.00001, 6.44)	3.5	(0.00001, 7.61)	1.82	(0.00001, 4.79)	0.44	(0.36, 0.53)	1.96	(0.00001, 4.34)		
TF	Lactotransferrin	1.14	(0.00001, 2.91)	1.92	(0.87, 2.97)	3.01	(0.46, 5.56)	4.67	(1.83, 7.51)	1.22	(0.78, 1.75)	1.75	(0.65, 2.85)		
LY96	Lymphocyte antigen 96	2.36	(0.00001, 5.18)	3.49	(0.00001, 7.92)	12.83	(3.23, 22.43)	2.54	(0.00001, 6.14)	2.52	(1.2, 4.31)	1.59	(0.00001, 3.20)		
LYZ	Lysozyme (renal amyloidosis)	1.82	(0.00001, 4.93)	2.43	(0.00001, 6.15)	4.14	(0.00001, 10.79)	3.03	(0.00001, 7.04)	1.99	(0.00001, 5.46)	1.44	(0.00001, 3.30)		
MAPK1	Mitogen-activated protein kinase 1	1.47	(0.50, 2.14)	1.23	(0.55, 1.91)	0.99	(0.57, 1.41)	1.25	(0.00001, 2.66)	1.16	(0.92, 1.42)	0.77	(0.53, 1.21)		
MAPK14	Mitogen-activated protein kinase 14	1.78	(0.50, 3.06)	2.11	(1.11, 3.11)	1.29	(1.59, 3.99)	2.00	(0.09, 3.91)	1.14	(0.94, 1.35)	1.06	(0.53, 1.59)		
MAPK8	Mitogen-activated protein kinase 8	2.71	(0.00001, 7.20)	2.40	(0.00001, 6.82)	8.52	(0.00001, 20.87)	3.04	(0.00001, 9.18)	0.79	(0.52, 1.11)	3.28	(0.00001, 8.05)		
MBL2	Mannose-binding lectin (protein C) 2, soluble	1.08	(0.37, 1.79)	1.30	(0.00001, 3.03)	1.92	(1.08, 2.76)	4.56	(2.41, 6.71)	1.97	(1.92, 2.1)	1.32	(0.94, 1.72)		
MPO	Myeloperoxidase	1.13	(0.42, 1.84)	3.10	(1.22, 4.98)	6.52	(3.32, 9.72)	2.35	(0.38, 4.32)	1.87	(1.34, 2.47)	1.93	(0.87, 2.39)		
MX1	Myxovirus resistance 1, interferon-inducible protein p78 (mouse)	2													

Supplementary Table S3. Primers used to amplify the complete genomic sequence of IBV 3575/08.

Location ^a	Length (bp)	Forward primer (5'→3')	Reverse primer (5'→3')
1-3009	3009	ACTGAAAATAGATATTAATATATATC	CTTGCCGGGTCTTGTATAAG
2502-4403	1902	CACCAATGTCACAACCTTGGT	CACGCCACTCAAGTATAACA
2985-5732	2748	TGGCTCTTATAACAAGACCCG	AATGTCCACGTGTACTTCCA
5011-7691	2681	GAATGAAAGTTCTTTACCTG	TGGCTGTTACAATTGGACAG
7084-9024	1941	AGTGGAAATATACAGGTGACG	CAAAGCCCATTAAGATTGTTG
8509-9961	1453	AATGCATTGCTGGCTTGTGT	CCTAAGTGGCACTGCCGT
9663-11669	2007	CACCATTTTCTACTAGTACTGC	CTACACCTGCTACGCAAGC
11572-15221	3650	CTTGACTAGGAATGGGCATAAT	ACTACACAGACGCCACAAG
14717-16297	1581	CCAAACAAGGTCTTGTAGCAG	CTTCTGGCAAGGCATTAATAGTA
16184-17725	1542	ACGATTGCACTCGTATAGTA	CATCCATTA AAAAGTGGGTAGT
17505-20183	2679	GTTAGCGGACAATTTATGCA	GAAGCATTAACTGCTGTACA
20001-21037	1037	CGTAGTAGCCAATAATGGCA	GGATTTAGGCCGTCCTGATTT
20375-22875	2501	AAACTGAACAAAAGACAGACTTAG	CCTTCCTGCATATGACCAA
21969-23915	1947	TTCTGACTTCTCGTAATGA	TGTATTGTT CAGTTACCACA
23189-25686	2498	TTTTGTGGTAGTGGAAGACA	TTGAGTTAATAGTAGGGATTT
24532-26607	2076	GTTATTGTTAACGAGTTTCCWA	GCTGCACGAGCAATAAGA
25884-27462	1579	GGATTAGATTGTGTTTACTT	AGCTACATGCCTATCTTCC

^aLocations are listed according to IBV strain 2575/98 (accession no. DQ646405).

Supplementary Table S4. IBV reference strains included in this study.

Strain	Accession no.	Country	Strain	Accession no.	Country
Vic	DQ490221 ^a	Australia	1171/92	DQ646406 ^b	Taiwan
Armidale	DQ490205 ^a	Australia	2296/95	DQ646404 ^b	Taiwan
Sczy3	JF732903	China	2575/98	DQ646405	Taiwan
YN	JF893452	China	2992/02	EU822340 ^b	Taiwan
LX4	AY338732	China	3071/03	EU822340 ^b	Taiwan
BJ	AY319651	China	3263/04	EU822338 ^b	Taiwan
YX10	JX840411	China	3374/05	EU822338 ^b	Taiwan
DY07	HM245923	China	3382/06	GQ229232 ^b	Taiwan
CK/CH/SD/121220	KJ128295	China	3468/07	EU822338 ^b	Taiwan
CQ04-1	HM245924	China	4/91 vaccine	KF377577	UK
SAIBK	DQ288927	China	Conn46 1991	FJ904719	USA
CK/CH/LHLJ/07VII	JF274479	China	Gray	GU393334	USA
GX-NN09032	JX897900	China	Holte	GU393336	USA
CK/CH/LDL/97I	JX195177	China	M41	DQ834384	USA
H52	EU817497	Netherlands	Beaudette CK	AJ311317	USA
H120	FJ888351	Netherlands	Georgia 1998 pass8	GQ504722	USA
KM91	JQ977698	South Korea	Arkansas Vaccine	GQ504721	USA
NGA/A116E7/2006	FN430415	Nigeria	--	--	--

^aSequences used for S gene comparison.

^bSequences used for S-N gene comparison.