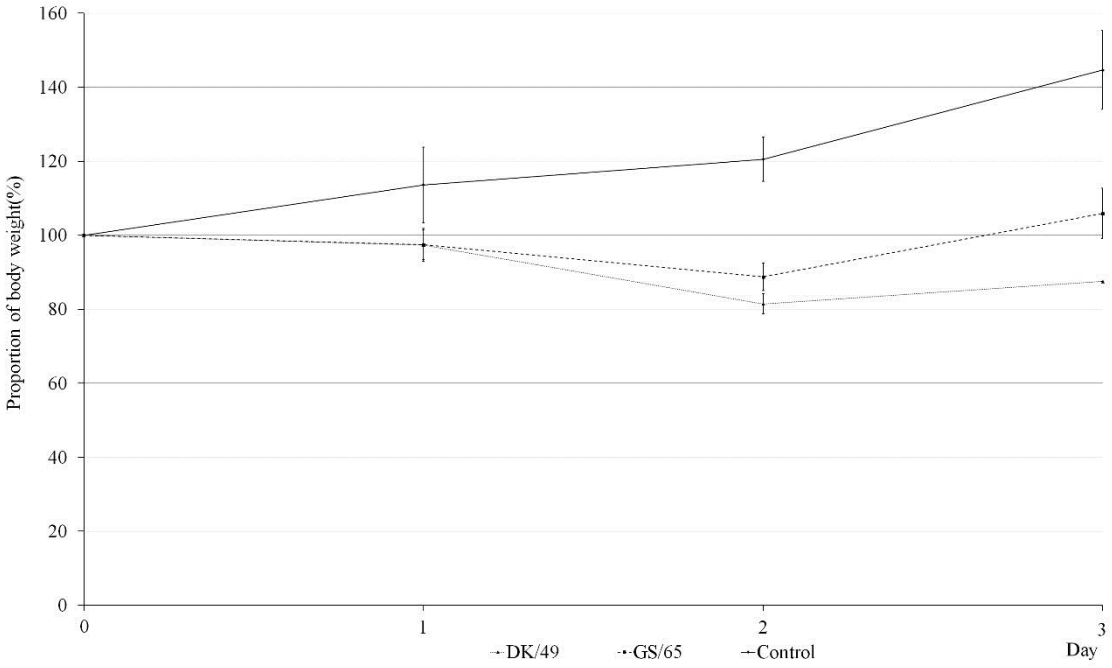
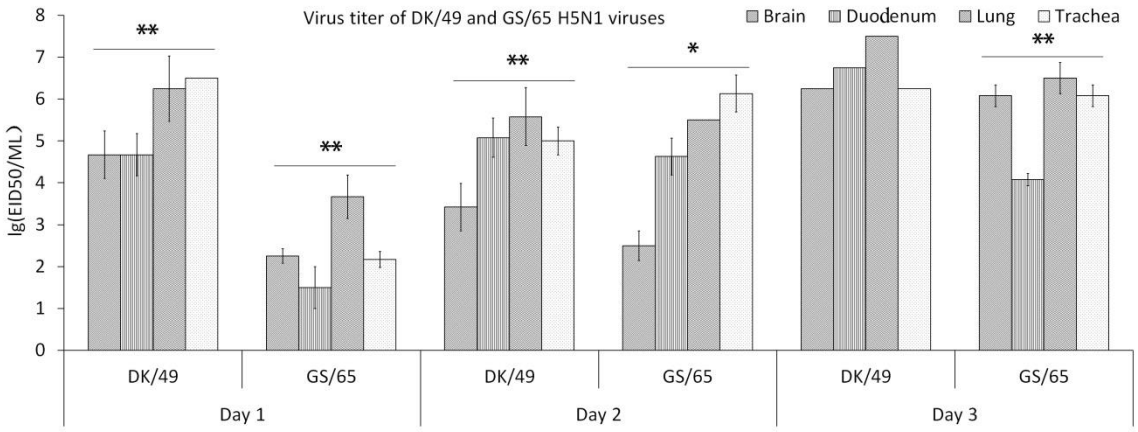
APPENDICES



Appendices A: Change in body weight of the ducks inoculated with H5N1 viruses. Data shown are the mean+-standard deviation of proportion of body weight to the corresponding one before inoculation.



Appendices B: Virus titers in ducks infected with H5N1 viruses. Data shown are the mean +- standard deviation. * $P \leq 0.05$ and ** $P \leq 0.01$ (one way ANOVA test).

Appendices C:Pathogenicity of the DK/49 and GS/65 H5N1 viruses in ducks

H5N1 viruses are recognized as highly pathogenic AIs in chickens, but they may differ in their virulence in ducks. For example, A/duck/Hubei/49/05 H5N1 virus (DK/49) is highly pathogenic, while A/goose/Hubei/65/05 H5N1 virus (GS/65) is weakly pathogenic, to the duck (Song et al. 2010). To identify genes related to immune response to H5N1 viruses, we previously inoculated intranasally 16 ducks with the DK/49 virus and 11 ducks with the GS/65 virus of 10^3 of 50% egg infections doses (EID_{50}) (Huang et al. 2013). Here we analyzed the pathogenicity of the DK/49 and GS/65 viruses in ducks. In global, the DK/49-infected ducks showed dramatic disease symptom: nine died within three days and two developed severe neurological dysfunction (such as ataxia and torticollis) on day two. In contrast, the GS/65-infected ducks showed a mild disease sign: no acute neurological symptoms or death within three days. Comparison in body weight suggested that the DK/49-infected ducks seemed to lose weight after inoculation on day one to three; while the GS/65-infected ducks lost weight after inoculation on day one and two, but regained energy and started gaining weight on day three (**Appendices A**).

We then measured virus titer of the DK/49- and GS/65-infected ducks on day one to three by calculating the EID_{50} using the Reed and Muench method (Reed et al. 1938). As shown in **Fig. 1a**, both the DK/49 and GS/65 viruses were replicated in brain, trachea, lung and duodenum of inoculated ducks. For the DK/49 virus, virus titer shed from the trachea ($10^{6.50}$ EID_{50}/g) and lung ($10^{6.25}$ EID_{50}/g) were significantly higher than those shed from the brain ($10^{4.56}$ EID_{50}/g) and duodenum ($10^{4.67}$ EID_{50}/g) on day

one ($P \leq 0.005$, one way ANOVA with turkey post poc test), virus titer shed from these four tissues ($10^{5.00\text{-}5.58}$ EID_{50}/g) were similar with the exception in brain ($10^{3.42}$ EID_{50}/g) on day two, and were increased to the similar level ($10^{6.25\text{-}7.50}$ EID_{50}/g) on day three (**Appendices B**). For the GS/65 virus, virus titer in lung ($10^{3.67}$ EID_{50}/g) was higher than those in trachea ($10^{2.17}$ EID_{50}/g), brain ($10^{1.67}$ EID_{50}/g) and duodenum ($10^{1.50}$ EID_{50}/g) on day one; virus titer in these four tissues were then significantly increased to $10^{2.50}$ to $10^{6.15}$ EID_{50}/g on day two, and continued to significantly increase in lung ($10^{6.50}$ EID_{50}/g), brain ($10^{6.08}$ EID_{50}/g), while not in trachea and duodenum on day three. Further comparison of two H5N1 viruses showed that virus titers in brain, trachea, lung and duodenum of ducks infected by the DK/49 virus were significantly higher than the corresponding ones infected by the GS/65 virus on day one. However, the difference in virus titer shed from the same tissue of ducks infected by the DK/49 virus and by the GS/65 virus was not significant on day two and three, except in trachea on day two and in duodenum on day three (**Fig. 1a**).

Immune responses in H5N1 virus infections

To understand whether the highly pathogenic H5N1 virus induced more severely dramatic and sustained expression of immune genes in their natural host than the weakly pathogenic H5N1 virus did. We firstly predicted duck genes that were homologs of 8,130 human and 2,252 mouse immune genes (comprising 7,987 unique immune genes), which were derived from updated analyses of Import, IRIS, Septic Shock Group, MAPK-NF-κβ network and immunome databases using gene families built in our previous study (Huang et al. 2013). In total, 9,162 duck genes were

clustered into 4,238 immune-related gene families, which contained at least one of the 7,987 unique immune genes. We therefore referred these 9,162 duck genes as immune genes. Detailed comparisons indicated that DK/49 infections had larger number of duck immune genes showing significantly differential expression in brain (2,231 vs 1,391) and spleen (3,099 vs 1,929) than the GS/65 infections did. Similarly, we found a large number of duck immune genes changed their gene expression significantly in brain (2,054) and spleen (2,058) tissues between the DK/49 infections when compared to the GS/65 infections.

We then investigated expression of 177 immune genes, which included in three RNA helicases, four T cell receptors, five colony stimulating factors, five interferon-induced proteins, ten toll-like receptors, and 150 cytokines annotated in our previous study (Huang et al. 2013), in the DK/49 or GS/65 infection. Transcriptomic analyses showed that, ducks infected with the DK/49 or GS/65 virus on day one to three after inoculation had a total of 98 and 114 immune genes with expression levels that were significantly changed (FDR $\leq$ 0.001 and fold change $\geq$ 2) in brains and spleens respectively (**Fig. 2**, full names are given in **Appendices F**). Collecting DEGs identified in lung (Huang et al. 2013), we found that totally 154 of 177 immune genes that were substantially changed (FDR $\leq$ 0.001 and fold change $\geq$ 2) their gene expression in these individuals. In brain, one infected with the DK/49 or GS/65 virus had 63 cytokines with expression levels that were significantly changed (FDR $\leq$ 0.001, fold change $\geq$ 2) on day one to three after inoculation when compared to control ducks. Of these cytokines, 16 growth factor genes (*BMP2, BMP3, BDNF, FGF2, FGF3, FGF10, FGF12, FGF14, FGF16, FGF19,*

GDNF, *IGF1*, *INHA*, *KITLG*, *NRG1* and *TGFB3*) had expression that was significantly decreased by 2.02- to 3.93-fold, and 11 growth factor genes (*ANGPT1*, *BMP4*, *BMP5*, *BMP7*, *BMP8*, *FGF18*, *FGF23*, *GH1*, *LEFTY*, *PGF* and *TGFB2*) had expression that was significantly increased by 2.01- to 7.12-fold, with the DK/49 or GS/65 infection. For tumor necrosis factors, interferons, interleukins or interleukin receptors and chemokines, only small part of these members (2 of 32) were substantially downregulated by 2.61- to 7.07-fold and most of them (30 of 32) were substantially upregulated by 2.02- to 6921-fold in brain of one infected with the DK/49 or GS/65 virus. In spleen, 81 cytokines' expressions were significantly changed in ones infected by the DK/49 or GS/65 virus compared to control ducks. Of these cytokines, most (28 of 41) growth factor genes (*ANGPT2*, *BDNF*, *BMP1*, *BMP2*, *BMP4*, *BMP7*, *EFNA5*, *EFNB1*, *FGF1*, *FGF7*, *FGF13*, *FGF14*, *FGF18*, *FGF23*, *FIGF*, *GDF11*, *GDNF*, *GH1*, *IGF1*, *KITLG*, *NGFB*, *NTF3*, *PDGFA*, *PDGFD*, *NRG2*, *TGFB2*, *TGFB3* and *VEGFA*) had expression that was significantly decreased by 2.05- to 858-fold, and small number (12 of 41) growth factor genes (*ANGPT1*, *BMP5*, *BMP6*, *BMP15*, *EPO*, *FGF2*, *GAS6*, *HGF*, *INHBB*, *NODAL*, *PGF* and *NRG1*) had expression that was significantly increased by 2.01- to 199-fold with the DK/49 or GS/65 infection. This is sharply contrast to the case in the other remained cytokine gene families in the DK/49- or GS/65-infected ducks, where only one tumor necrosis factor (*EDA*), one interleukin receptor (*IL7*) and one chemokine (*CXCL12*) were markedly decreased by 2.20- to 6.30-fold; while four interferons (*IFNA*, *IFNK*, *IFNG* and *IL28A*), five tumor necrosis factors (*FASLG*, *TNFSF4*, *TNFSF8*, *TNFSF10* and *TNFSF15*), twelve chemokines

(*CCL4L2*, *CCL5*, *CCL6*, *CCL19*, *CCL20*, *CCL23*, *CCL24*, *CX3CL1*, *CXCL13L1*, *CXCL13L2*, *IL8A* and *IL8B*) and fourteen interleukins or interleukin receptors (*LIF*, *IL2*, *IL6*, *IL9*, *IL10*, *IL12A*, *IL12B*, *IL15*, *IL17A*, *IL17F*, *IL18*, *IL19*, *IL22* and *IL26*) were markedly increased by 2.05- to 62486-fold.

Global transcriptomic diversity in brain, spleen and lung tissues

We examined four types of alternative splicing events including exon skipping (SE), intron retention (IR), alternative 5' splice site (A5SS) and alternative 3' splice site (A3SS), by searching against known and putative splicing junctions using the SOAPsplice software (See Materials and Methods, **Appendices H**). Alignment of the above ~2,771 million Illumina paired-end reads with the duck genome assembly (BGI_duck_1.0) suggested that totally 136,451 alternative splicing events, which comprised 64.79% expressed genes (12,657), were detected in ducks (**Appendices G**). In lung, we measured 16,866 SE, 18,925 IR, 20,223 A5SS and 30,684 A3SS, which totally comprised of 10,777 genes. These numbers were larger than their corresponding in brain (12,198 SE, 13,264 IR, 11,863 A5SS and 20,277 A3SS) and spleen (14,048 SE, 17,459 IR, 16,125 A5SS and 27,917 A3SS). Comparison in the alternative splicing variation among tissues suggested that, similar to the global variation, lung had a larger number of tissue-specific splicing (34,668 events in 8,030 genes) than the other two tissues did, where brain expressed 20,020 tissues-specific splicing events in 5,939 genes and spleen expressed 25,364 tissues-specific splicing events in 6,901 genes (**Supplementary Figure S4a**).

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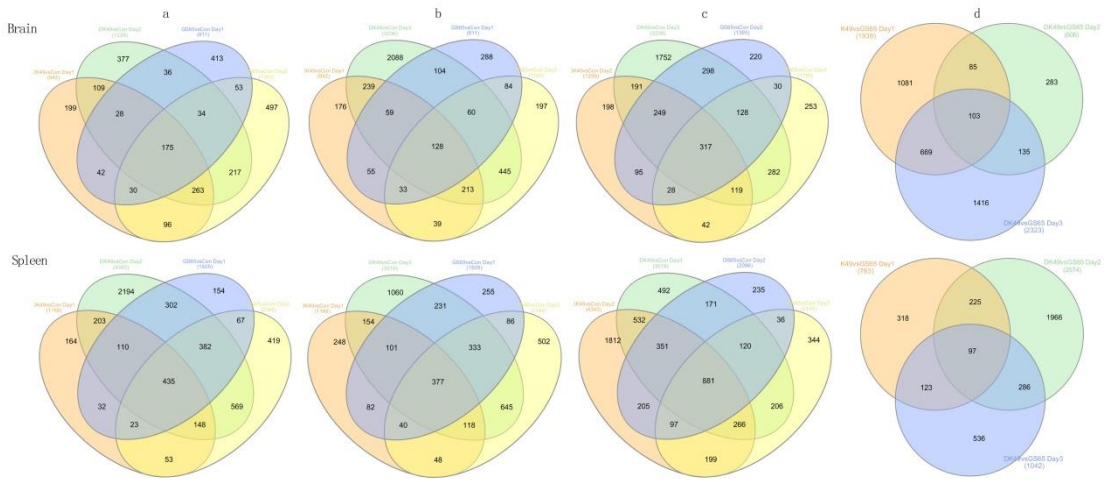
Appendices D: Primer sequences and product size of 22 genes in chicken and duck

Gene	Forward/reverse primer sequences (5′-3′)	Product size (bp)	Gene	Forward/reverse primer sequences (5′-3′)	Product size (bp)
BATF3	F: AGGAGAGAGAAGAACCGAGTTGCTG	126	LY96	F: CACGCTGCTGTTACCTGGATT	161
	R: TCCGATTTCTTTTTTCAGGGAGGTA			R: ATTGTTTCTCCTTTCAGTCCTCCA	
BCL2L14	F: GAGAGGCTCACCGATTTGTTCC	130	NCF2	F: GATTTGCTCCCCTCCAACCGC	209
	R: GGTTGTCCACAGCAGTAAGTCTGG			R: CTTCCCATTGAACATCACTGTAGCC	
BCL2L15	F: TGCGTCGTGGAAGCCTTATTTGATG	145	NCAM1	F: CTGGGAAGGGAATCAAGTGAACAT	272
	R: GCAGACGATTGGCAATAATAACCG			R: TGTCAATAGAAGGAGAGGACGGAGT	
CASP18	F: AGGGCAAAATAAAAGGCGTTGATAA	211	TDRD7	F: CATCTGGTGTGGAGACGCATACTGT	293
	R: GGAATAGAAAGCGCAGGGATAGCAT			R: CTCTGTGTTGCTTTTCACTGCTGGT	
CCR2	F: ATGCCAACAACAACGTTTGA	127	MARCO	F: GCTCCCAAGGTCCAACTGGTCAAAA	175
	R: TGTTGCCTATGAAGCCAAA			R: GCAATTCGTATGACGTTGAAGTAAC	
CD83	F: AGGCAGTGTCTTGGCACAAAATGG	224	KCNN2	F: TATCTGCTTGGAAATACTGGTGTGT	272
	R: TAATGTGATGATGCCACTCAGGTTG			R: TCATTAGAGTCTTCATAACAAAACG	
CD274	F: GGATTTTGGGCAGTCTCTCCTTCA	173	MMD2	F: TTCATCGTCTCCACCATCTTCCACA	221
	R: TCCACTCTTTGTCTCCTGTGCTTAC			R: AAGAAGAACACGTAGACGGTCCCAA	
CXCL13L2	F: CGCCACACTTCTCCTATTCCCTG	200	BCL2L15*	F: CG*ACGCGT*GCCACC ATGACAACGTTTGAGGAACAG ACGA	531
	R: CAAGGGAATGAAGGCAGTTGTGG			R: GG*GTTTAAAC*TCACTTATCGTCGTCATCCTTGT AATCGTCATCCAAGTTCTCCCATCCTCCA	

C3H8ORF80	F: AGCAGAAACTACAGGAAGCCCAGA	157	MX1*	F: CG*ACGCGT*GCCACCATGACTACTCAGCGTAACACAGACA	2214
	R: TGAATGGATACTACAAGGGACTGGT			R: GG*GTTTAAAC*CTACTTATCGTCGTCATCCTTGTAATCCAGACAGCTAAAGTCCTTCAGACAT	
DCSTAMP	F: GCAGCATTTTTTTCTCCCTGTAGTT	251	DCSTAMP*	F: CG*ACGCGT*GCCACCATGCAAGCACTTGTCTCAACAGCCCAGAATGC	1479
	R: GCAGTTCTGGCTGAGGGACGC			R: GG*GTTTAAAC*CTACTTATCGTCGTCATCCTTGTAATCCACCACATTGTCATTTACCATTGTC	
MX1	F: GCACACACCCAACTGTCAGCGA	156	DDX58*	F: G*ACGCGT*GCCACCATGACGGCGGACGAGAAGCGGAGCC	2849
	R: CCCATGTCCGAAACTCTCTGCGG			R: GG*GTTTAAAC*CTACTTATCGTCGTCATCCTTGTAATCAAATGGTGGGTACAAGTTGGACATTTCTTC	
PLAC8	F: ATCAAGAGGGACATCAATCGGAGGA	149			
	R: CGTAACTCTTTATTGGGGGCGTGAA				

*Primers for amplification of duck full-length cDNA. Sequences in italic in the "F" and "R" primers were the *MluI* and *PmeI* site respectively. Sequences underline in "R" primers are the flag sequences.



Appendices E: Venn diagram showing overlap and unique genes changing expression significantly (DEGs) response to highly (DK/49) or weakly (GS/65) pathogenic H5N1 virus infection in brain and spleen of duck. (a) DEGs on day one are compared to that on day two after infected by DK/49 or GS/65 viruses. (b) DEGs on day one are compared to that on day three after infected by DK/49 or GS/65 viruses. (c) DEGs on day two are compared to that on day three after infected by DK/49 or GS/65 viruses. (d) DEGs between DK/49 vs GS/65 on day one, two and three.

164 **Appendices F: The enrichment of significantly differential expressed ducks genes in two H5N1-viruses infections**

Tissue	Group	Name	P value	Number of molecules
Brain	DK/49 infections vs Control (DEG set 1)	Cell-to-cell signaling and interaction	1.06E-31-1.16E-06	674
		Cellular movement	5.98E-31-9.54E-07	641
		Cellular development	4.02E-30-8.94E-07	910
		Cellular growth and proliferation	4.02E-30-5.76E-07	975
		Cellular function and maintenance	1.27E-28-1.16E-06	765
	GS/65 infections vs control (DEG set 2)	Cell-to-cell signaling and interaction	8.70E-32-2.84E-06	439
		Cellular movement	3.35E-29-2.84E-06	415
		Cellular development	1.87E-24-2.69E-06	492
		Cellular growth and proliferation	1.87E-24-2.34E-06	639
		Molecular transport	2.44E-22-1.31E-06	346
	DK/49 vs GS/65 infections (DEG set 5)	Cellular movement	8.90E-24-3.19E-05	613
		Cell-to-cell signaling and interaction	6.32E-22-3.19E-05	623
		Cell signaling	2.36E-17-2.71E-05	427
		Molecular transport	2.36E-17-3.12E-05	610
		Vitamin and mineral metabolism	2.36E-17-2.48E-05	256
Spleen	DK/49 infections vs Control (DEG set 3)	Cellular movement	2.65E-24-2.84E-05	827
		Cell-to-cell signaling and interaction	3.65E-24-2.84E-05	790
		Cellular development	2.73E-13-2.64E-05	1166
		Cell signaling	3.20E-13-1.59E-06	331
		Molecular transport	3.20E-13-2.54E-05	629

GS/65 infections vs control (DEG set 4)	Cell-to-cell signaling and interaction	4.39E-15-8.71E-04	505
	Cellular movement	4.49E-11-8.42E-04	505
	Cellular growth and proliferation	3.23E-07-8.42E-04	768
	Molecular transport	8.18E-07-7.27E-04	446
	Lipid metabolism	1.14E-06-6.82E-04	288
DK/49 vs GS/65 infections (DEG set 6)	Cellular movement	2.31E-26-2.77E-05	608
	Cell-to-cell signaling and interaction	1.54E-17-2.81E-05	609
	Cellular development	1.28E-15-1.82E-05	817
	Lipid metabolism	3.03E-14-2.26E-05	410
	Molecular transport	3.03E-14-2.26E-05	484

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167 **Appendices G: Description of genes responsive to H5N1 viruses in ducks**

Gene	Full name	Gene	Full name
A2M	alpha-2-macroglobulin	*IFNE*	interferon, epsilon
ABLIM1	actin binding lim protein 1	*IFNG*	interferon, gamma
ACOT7	acyl-coa thioesterase 7	*IFNK*	interferon, kappa
ADAR	adenosine deaminase, rna-specific	*IgA*	iga secreted heavy chain constant region (alpha)
AKR1B10	aldo-keto reductase family 1, member b10 (aldose reductase)	*IGF1*	insulin-like growth factor 1
ANGPT1	angiopoietin 1	*IgM*	igm heavy chain constant region (mu)
ANGPT2	angiopoietin 2	*IL10*	interleukin 10
Anpl_DRA	duck mhc Class II, DR Alpha	*IL10RA*	interleukin 10 receptor, alpha
Anpl_UAA	duck mhc class antigen alpha chain, uaa gene	*IL12A*	interleukin 12a
Anpl_UDA	duck mhc class I antigen alpha chain, uda gene	*IL12B*	interleukin 12b
APOB	apoliporprotein b	*IL15*	interleukin 15
ARHGEF33	rho guanine nucleotide exchange factor (gef) 33	*IL17A*	interleukin 17a
ASPG	asparaginase homolog (s. cerevisiae)	*IL17D*	interleukin 17d
ATP6V0A2	atpase, h+ transporting, lysosomal v0 subunit a2	*IL17F*	interleukin 17f
AvIFIT	avian interferon-induced protein with tetratricopeptide repeats	*IL18*	interleukin 18 interon-gamma-inducing factor
AZI2	antizyme inhibitor 2	*IL19*	interleukin 19
BATF3	basic leucine zipper transcription factor, atf-like 3	*IL2*	interleukin 2

BCL2L14	bcl2-like 14 (apoptosis facilitator)	*IL22*	interleukin 22
BCL2L15	bcl2-like 15	*IL26*	interleukin 26
BDNF	brain-derived neurotrophic factor	*IL28A*	interferon, lambda 2
BMP1	bone morphogenetic protein 1	*IL6*	interleukin 6
BMP15	bone morphogenetic protein 15	*IL7*	interleukin 7
BMP2	bone morphogenetic protein 2	*IL8A*	interleukin 8 type 1
BMP3	bone morphogenetic protein 3	*IL8B*	interleukin 8 type 2
BMP4	bone morphogenetic protein 4	*IL9*	interleukin 9
BMP5	bone morphogenetic protein 5	*INHBA*	inhibin, beta a
BMP6	bone morphogenetic protein 6	*INHBB*	inhibin, beta b
BMP7	bone morphogenetic protein 7	*INHBC*	inhibin, beta c
BMP8	bone morphogenetic protein 8	*IRF1*	interferon regulatory factor 1
C15orf48	chromosome 15 open reading frame 48	*IRF7*	interferon regulatory factor 7
C1QA	complement component 1, q subcomponent, a chain	*IRF8*	interferon regulatory factor 8
C1QB	complement component 1, q subcomponent, b chain	*ISG12-2*	putative isg12-2 protein
C1R	complement component 1, r subcomponent	*KCNN2*	potassium intermediate/small conductance calcium-activated channel, subfamily n, member 2
C8orf80	chromosome 8 open reading frame 80	*KITLG*	kit ligand
CAMP	cathelicidin antimicrobial peptide	*LEFTY2*	left-right determination factor 2
CASP1	caspase 1	*LEPR*	leptin receptor
CASP18	caspase 18	*LGP2*	RIG-I-Like receptor

CCL17	chemokine (c-c motif) ligand 17
CCL19	chemokine (c-c motif) ligand 19
CCL20	chemokine (c-c motif) ligand 20
CCL21	cc chemokine ligand 21
CCL23	chemokine (c-c motif) ligand 23
CCL24	chemokine (c-c motif) ligand 24
CCL3	chemokine (c-c motif) ligand 3
CCL4L2	chemokine (c-c motif) ligand 4-like 2
CCL5	chemokine (c-c motif) ligand 5
CCL6	chemokine (c-c motif) ligand 6
CCR2	c-c chemokine receptor type 2
CCR7	c-c chemokine receptor type 7
CD274	cd274 molecule
CD3E	cd3e molecule, epsilon (cd3-tcr complex)
CD4	cd4 molecule
CD40	cd40 molecule
CD44	cd44 molecule
CD58	cd58 molecule
CD83	cd83 molecule
CD8A	cd8a molecule
CD9	cd9 molecule
LIF	leukemia inhibitory factor
LOC770718	tripartite motif-containing protein 39-like
LTB	lymphotoxin beta (tnf superfamily, member 3)
LY6E	lymphocyte antigen 6 complex, locus e
LY96	lymphocyte antigen 96
LYZ	lysozyme
MARCO	macrophage receptor with collagenous structure
Anpl_UAA	duck mhc class i antigen alpha chain, uaa gene
MITD1	mit, microtubule interacting and transport, domain containing 1
MLKL	mixed lineage kinase domain-like
MMD2	monocyte to macrophage differentiation-associated 2
MOV10	mov10, moloney leukemia virus 10, homolog (mouse)
MRPL30	mitochondrial ribosomal protein l30
MT4	metallothionein 4
MX1	mx dynamin-like gtpase 1
NGFB	nerve growth factor, beta
NLRC3	nlr family, card domain containing 3
NLRC5	nlr family, card domain containing 5
NMI	n-myc (and stat) interactor
NOD1	Nucleotide-Binding Oligomerization Domain Containing 1
NODAL	nodal homolog

CDC42	cell division cycle 42	*NOX2*	cytochrome b-245, beta polypeptide
CGN	cingulin	*NRG1*	neuregulin 1
CHDZ	chromo-helicase DNA binding protein gene	*NRG2*	neuregulin 2
CIITA	class ii, major histocompatibility complex, transactivator	*NTF3*	neurotrophin 3
CMPK2	cytidine monophosphate (ump-cmp) kinase 2, mitochondrial	*OGFR*	opioid growth factor receptor
CNKSR3	cnksr family member 3	*OTUD4*	otu domain containing 4
COL9A3	collagen, type Ⅸ, alpha 3	*PARP12*	poly (adp-ribose) polymerase family, member 12
COLEC12	collectin sub-family member 12	*PARP14A*	poly (adp-ribose) polymerase family, member 14 type 1
CSF1R	colony stimulating factor 1 receptor	*PARP14B*	poly (adp-ribose) polymerase family, member 14 type 2
CSF2RA	colony stimulating factor 2 receptor, alpha	*PARP9*	poly (adp-ribose) polymerase family, member 9
CSF2RBA	colony stimulating factor 2 receptor, beta type 1	*PDGFA*	platelet-derived growth factor alpha polypeptide
CSF2RBB	colony stimulating factor 2 receptor, beta type 2	*PDGFD*	platelet derived growth factor d
CSF3R	colony stimulating factor 3 receptor	*PFKFB3*	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
CTNNB1	catenin (cadherin-associated protein), beta 1, 88kda	*PGF*	placental growth factor
CX3CL1	c-x3-c motif chemokine 1	*PLAC8A*	placenta-specific 8, type 1
CXCL12	chemokine (c-x-c motif) ligand 12	*PLAC8B*	placenta-specific 8,type 2
CXCL13L1	chemokine (c-x-c motif) ligand 13 like 1	*PML*	promyelocytic leukemia
CXCL13L2	chemokine (c-x-c motif) ligand 13 like 2	*PPEF2*	protein phosphatase, ef-hand calcium binding domain 2
CXCL14	chemokine (c-x-c motif) ligand 14	*PRIC285*	peroxisomal proliferator-activated receptor a-interacting complex 285 kda protein

CYBB	cytochrome b-245, beta polypeptide	*PROZ*	protein z, vitamin k-dependent plasma glycoprotein
DCSTAMP	dendritic cells (dc)-specific transmembrane protein	*PTGS2*	prostaglandin-endoperoxide synthase 2
DDX60	dead (asp-glu-ala-asp) box polypeptide 60	*PXK*	px domain containing serine/threonine kinase
Anpl-DRA	major histocompatibility complex, class ii, dr alpha	*RIG-I*	Retinoic acid-inducible gene I protein
DRAM1	dna-damage regulated autophagy modulator 1	*RNF213*	ring finger protein 213
DRD3	dopamine receptor d3	*RPA1*	replication protein a1, 70kda
DUSP5	dual specificity phosphatase 5	*RPS6KA2*	ribosomal protein s6 kinase, 90kda, polypeptide 2
EDA	ectodysplasin a	*RQCD1*	rcd1 required for cell differentiation1 homolog (s. pombe)
EFNA5	ephrin-a5	*RSAD2*	radical s-adenosyl methionine domain containing 2
EFNB1	ephrin-b1	*SAMHD1*	sam domain and hd domain 1
EFNB2	ephrin-b2	*SCNN1D*	sodium channel, non voltage gated 1 deta subunit
EIF2AK2A	eukaryotic translation initiation factor 2-alpha kinase 2, type 1	*SELE*	selectin e
EIF2AK2B	eukaryotic translation initiation factor 2-alpha kinase 2, type 2	*SERPINB10*	serpin peptidase inhibitor, clade b (ovalbumin), member 10
EIF2AK2C	eukaryotic translation initiation factor 2-alpha kinase 2, type 3	*SLFN13*	schlafen family member 13
EPB41	erythrocyte membrane protein band 4.1 (elliptocytosis 1, rh-linked)	*SOCS1*	suppressor of cytokine signaling 1
EPO	erythropoietin	*SRGN*	serglycin
EPSTI1	epithelial stromal interaction 1 (breast)	*STAT1*	signal transducer and activator of transcription 1
ETV7	ets variant 7	*STAT1*	signal transducer and activator of transcription 1

FABP1	fatty acid binding protein 1, liver
FABP2	fatty acid binding protein 2, intestinal
FABP4	fatty acid binding protein 4, adipocyte
FAM196B	family with sequence similarity 196, member b
FAS	fas cell surface death receptor
FASLG	fas ligand (tnf superfamily, member 6)
FBXO18	f-box protein, helicase, 18
FGB	fibrinogen beta chain
FGF1	fibroblast growth factor 1 (acidic)
FGF10	fibroblast growth factor 10
FGF12	fibroblast growth factor 12
FGF13	fibroblast growth factor 13
FGF14	fibroblast growth factor 14
FGF16	fibroblast growth factor 16
FGF18	fibroblast growth factor 18
FGF19	fibroblast growth factor 19
FGF2	fibroblast growth factor 2
FGF23	fibroblast growth factor 23
FGF3	fibroblast growth factor 3
FGF7	fibroblast growth factor 7
FIGF	c-fos induced growth factor (vascular endothelial
STAT3	signal transducer and activator of transcription 3
TAP1	transporter 1, atp-binding cassette, sub-family b (mdr/tap)
TAP2	transporter 2, atp-binding cassette, sub-family b (mdr/tap)
TCFL5	transcription factor-like 5 (basic helix-loop-helix)
TDRD6	tudor domain containing 6
TDRD7	tudor domain containing 7
TGFB2	transforming growth factor, beta 2
TGFB3	transforming growth factor, beta 3
TLR15	toll-like receptor 15
TLR1A	toll-like receptor 1 type 1
TLR1B	toll-like receptor 1 type 2
TLR21	toll-like receptor 21
TLR2A	toll-like receptor 2 type 1
TLR2B	toll-like receptor 2 type 2
TLR3	toll-like receptor 3
TLR4	toll-like receptor 4
TLR5	toll-like receptor 5
TLR7	toll-like receptor 7
TMPRSS7	type Ⅱ transmerbrane serine protease 7
TNFSF10	tumor necrosis factor (ligand) superfamily, member 10
TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b

	growth factor d)		
FTSJD2	ftsj methyltransferase domain containing 2	*TNFSF15*	tumor necrosis factor (ligand) superfamily, member 15
GAS6	growth arrest-specific 6	*TNFSF4*	tumor necrosis factor (ligand) superfamily,member 4
GCH1	gtp cyclohydrolase 1	*TNFSF8*	tumor necrosis factor (ligand) superfamily, member 8
GDF10	growth differentiation factor 10	*TRA*	t cell receptor alpha
GDF11	growth differentiation factor 11	*TRAF3*	tnf receptor-associated factor 3
GDF2	growth differentiation factor 2	*TRANK1*	tetratricopeptide repeat and ankyrin repeat containing 1
GDNF	glial cell derived neurotrophic factor	*TRD*	t cell receptor deta
GH1	growth hormone	*TRG*	t-cell receptor gamma
GSDMA	gasdermin a	*TRIM25*	tripartite motif containing 25
HCN2	hyperpolarization activated cyclic nucleotide-gated potassium channel 2	*TRIM35*	tripartite motif containing 35
HELZ2	helicase with zinc finger 2	*TRIM39*	tripartite motif containing 39
HERC3	hect and rld domain containing e3 ubiquitin protein ligase 3	*TRIM7*	tripartite motif containing 7
HGF	hepatocyte growth factor	*TUBA8*	tubulin, alpha 8
HK2	hexokinase 2	*USP18*	ubiquitin specific peptidase 18
HSP90A	Heat shock protein 90kDa alpha	*VCAM1*	vascular cell adhesion molecule 1
HSP90AB1	Heat Shock Protein 90kDa Alpha (Cytosolic), Class B Member 1	*VEGFA*	vascular endothelial growth factor a
HSP90B1	Heat Shock Protein 90kDa Beta (Grp94), Member 1	*VIPERIN*	viperin
ICAM1	intercellular adhesion molecule 1	*XCL1*	chemokine (c motif 1/2) ligand 1

IFI35	interferon-induced protein 35	*ZC3HAV1*	zinc finger ccch-type, antiviral 1
IFIH1	interferon induced with helicase c domain 1	*ZFAND2A*	zinc finger, an1-type domain 2a
IFITM3	interferon induced transmembrane protein 3	*ZNFX1*	zinc finger, nfx1-type containing 1
IFITM5	interferon induced transmembrane protein 5	*ZP3*	zona pellucida glycoprotein
IFNA	interferon, alpha		

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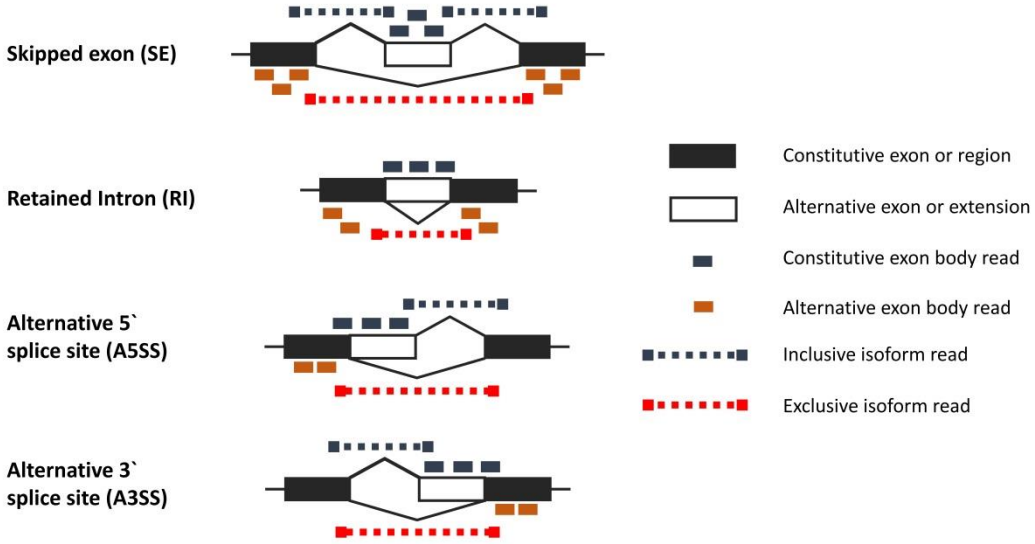

Appendices H: Four different alternative splicing events types diagrammed.

174 **Appendices I: Distribution of alternative splicing events in control and H5N1 virus-infected ducks**

Tissues	Group	Alternative 3' splicing site (A3SS)		Alternative 5' splice site (A5SS)		Intron retention (IR)		Exon skipping (SE)		Total	
		Number of events	Number of genes	Number of events	Number of genes	Number of events	Number of genes	Number of events	Number of genes	Number of events	Number of genes
Brain	Control	6108	3565	3617	2403	5690	3178	6361	2379	21776	6736
	DK/49 (day 1)	8058	4209	4631	2909	8223	4128	7085	2645	27997	7545
	DK/49 (day 2)	6975	3848	4169	2689	7127	3721	6574	2540	24845	7153
	DK/49 (day 3)	7552	4061	4658	2896	7881	4014	6701	2581	26792	7342
	GS/65 (day 1)	4883	3013	2611	1864	3599	2222	5615	2142	16708	5864
	GS/65 (day 2)	5686	3363	3237	2204	5444	3101	5778	2253	20145	6468
	GS/65 (day 3)	7062	3878	4109	2652	6813	3631	6629	2499	24613	7175
	total	20277	6811	11863	5301	13264	5729	12198	3972	57602	8808
	Tissue-specific	7816	931	4770	888	3434	971	4000	940	16420	5939
Spleen	Control	8480	4224	5347	3246	10612	4853	6581	2545	31020	7618
	DK/49 (day 1)	9762	4516	4842	3025	9876	4632	6624	2533	31104	7490
	DK/49 (day 2)	8109	3969	5215	3041	8853	4169	7084	2674	29261	7104
	DK/49 (day 3)	9590	4465	5991	3385	8638	4177	7306	2769	31525	7344
	GS/65 (day 1)	8565	4225	5258	3130	9653	4514	7226	2751	30702	7476
	GS/65 (day 2)	8835	4362	5548	3284	8708	4281	6856	2632	29947	7424
	GS/65 (day 3)	8784	4234	5170	3067	7958	3992	6970	2700	28882	7228
	total	27917	7112	16125	5948	17459	6571	14048	4459	75549	9878

	Tissue-specific	11397	585	6002	618	4253	762	3712	541	25364	6901
Lung	Control	9840	4723	6758	3788	9801	4862	7952	2967	34351	8258
	DK/49 (day 1)	9719	4459	6104	3369	9154	4396	7712	2911	32689	7735
	DK/49 (day 2)	10083	4550	7242	3731	10587	4842	8455	3104	36367	8004
	DK/49 (day 3)	10644	4886	7381	3890	10202	4926	8156	3066	36383	8169
	GS/65 (day 1)	9142	4587	6032	3511	9204	4583	7727	2873	32105	7993
	GS/65 (day 2)	10268	4703	7139	3866	9752	4767	7949	3040	35108	8093
	GS/65 (day 3)	9276	4508	6250	3516	8715	4397	7430	2765	31671	7815
	total	30684	7686	20223	6627	18925	7189	16866	5070	86698	10777
	Tissue-specific	13681	859	9545	1041	5495	1202	5948	1062	34669	8030
Total		51720	9502	31861	8404	27714	9222	25156	6465	136451	12657

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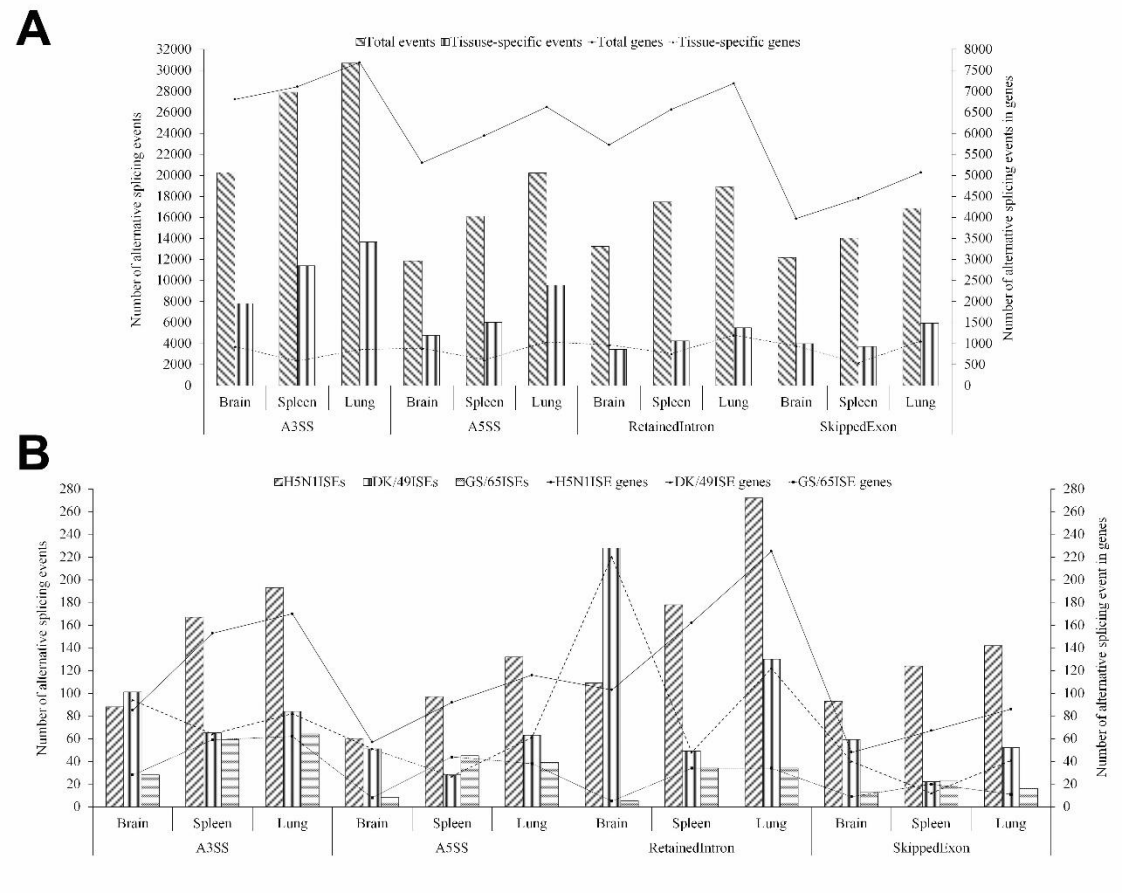
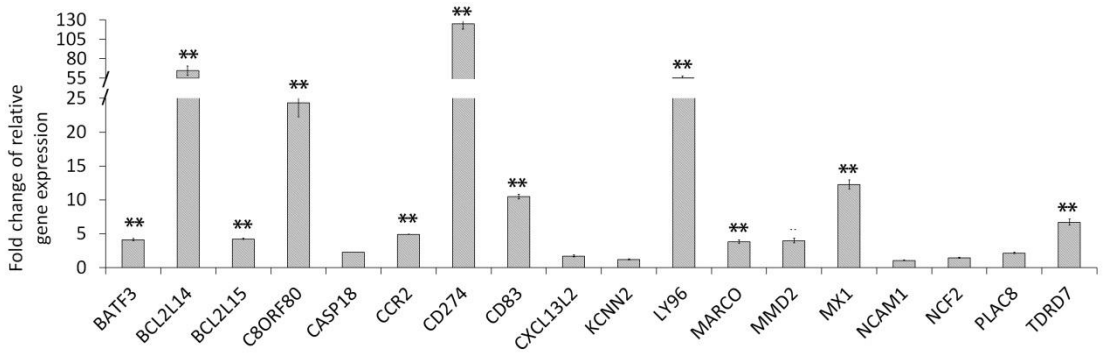
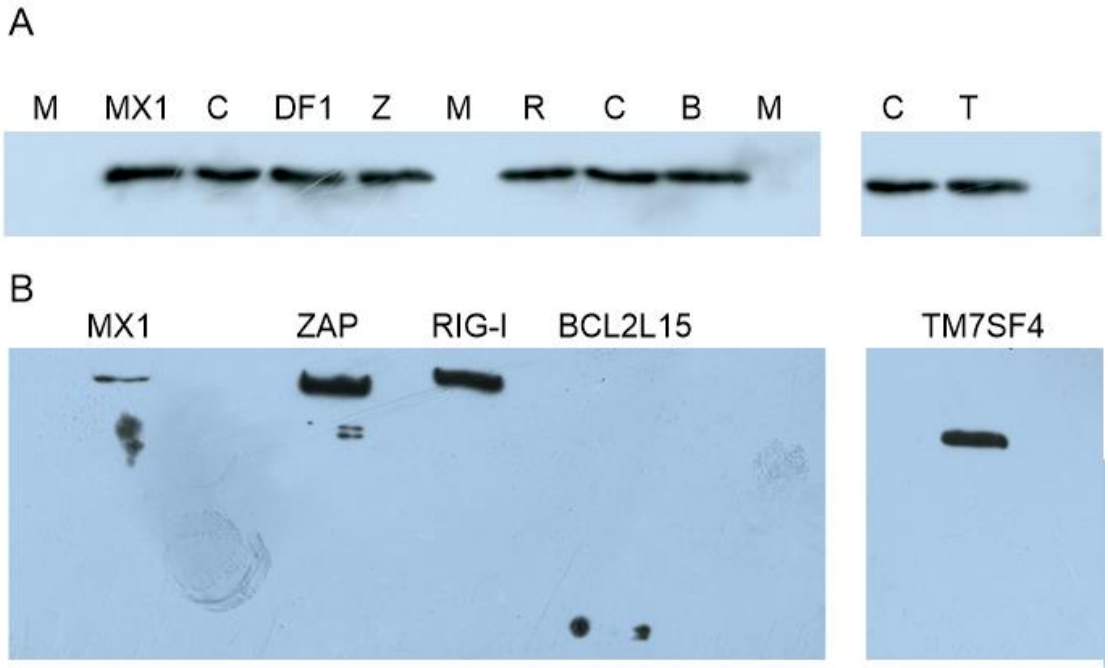


Appendices J: Identification of putative alternative splicing events responsive to H5N1 viruses in ducks. (a) Distribution of the four major types of alternative splicing events in brain, spleen and lung tissues. (b) Distribution of the four major types of putative alternative splicing events induced by avian influenza virus infection.

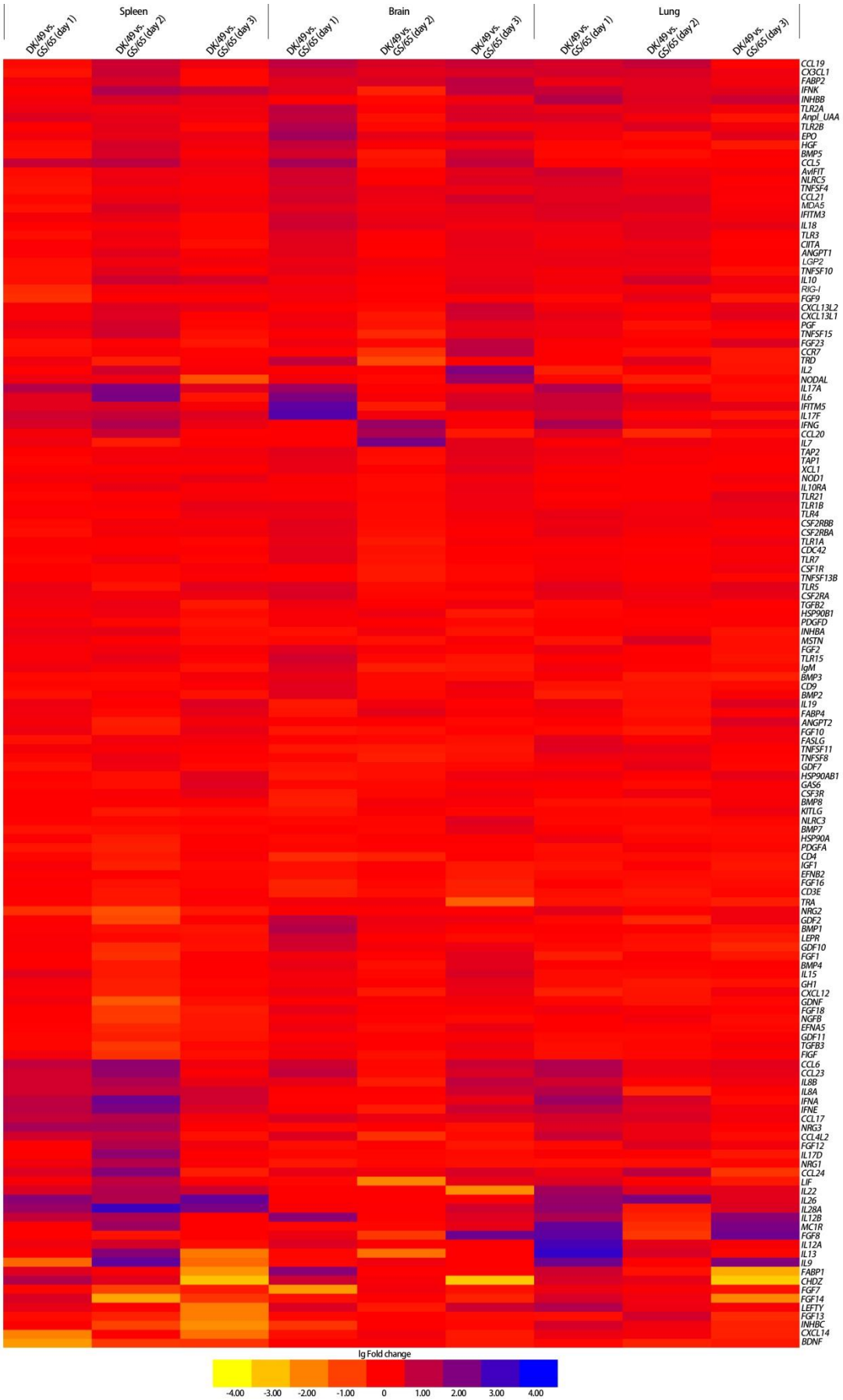


Appendices K: Quantitative RT-PCR analysis for 18 genes in DF1 cells with or without infection by DK/49 virus after 48 hours.



Appendices L: Full-length images of western blots for four duck proteins. Gene expression in DF1 cells was examined by western blotting using the anti-Flag or chicken GAPDH antibody. Samples from left to right are "molecular size marker (M)", "DF1 cells expressing duck MX1 (MX1)", "DF1 cells transfected with empty plasmid (C)", "wild DF1 cells (DF1)", "DF1 cells expressing duck ZC3HAV1 (ZAP or Z, not reported in this manuscript)", "molecular size marker (M)", "DF1 cells expressing duck RIG-I(RIG-I)", "DF1 cells transfected with empty plasmid (C)", "DF1 cells expressing duck BCL2L15(B or

BCL2L15)", "molecular size marker (M)", "DF1 cells transfected with empty plasmid (C)", "DF1 cells expressing duck DCSTAMP (also named TM7SF4, T or TM7SF4)", "molecular size marker (M)". (A) Expression of chicken GAPDH. (B) Expression of MX1, RIG-I, BCL2L15 and DCSTAMP.


Spleen
Brain
Lung
DK/49 vs. GS/65 (day 1)
DK/49 vs. GS/65 (day 2)
DK/49 vs. GS/65 (day 3)
CCL19
CX3CL1
FABP2
IFNK
INHBB
TLR2A
Anpl_UAA
TLR2B
EPO
HGF
BMP5
CCL5
AviFIT
NLRC5
TNFSF4
CCL21
MDA5
IFITM3
IL18
TLR3
CIITA
ANGPT1
LGP2
TNFSF10
IL10
RIG-I
FGF9
CXCL13L2
CXCL13L1
PGF
TNFSF15
FGF23
CCR7
TRD
IL2
NODAL
IL17A
IL6
IFITM5
IL17F
IFNG
CCL20
IL7
TAP2
TAP1
XCL1
NOD1
IL10RA
TLR21
TLR1B
TLR4
CSF2RBB
CSF2RBA
TLR1A
CDC42
TLR7
CSF1R
TNFSF13B
TLR5
CSF2RA
TGFB2
HSP90B1
PDGFD
INHBA
MSTN
FGF2
TLR15
IgM
BMP3
CD9
BMP2
IL19
FABP4
ANGPT2
FGF10
FASLG
TNFSF11
TNFSF8
GDF7
HSP90AB1
GAS6
CSF3R
BMP8
KITLG
NLRC3
BMP7
HSP90A
PDGFA
CD4
IGF1
EFNB2
FGF16
CD3E
TRA
NRG2
GDF2
BMP1
LEPR
GDF10
FGF1
BMP4
IL15
GH1
CXCL12
GDNF
FGF18
NGFB
EFNA5
GDF11
TGFB3
FIGF
CCL6
CCL23
IL8B
IL8A
IFNA
IFNE
CCL17
NRG3
CCL4L2
FGF12
IL17D
NRG1
CCL24
LIF
IL22
IL26
IL28A
IL12B
MC1R
FGF8
IL12A
IL13
IL9
FABP1
CHDZ
FGF7
FGF14
LEFTY
FGF13
INHBC
CXCL14
BDNF
lg Fold change
-4.00
-3.00
-2.00
-1.00
0
1.00
2.00
3.00
4.00

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Appendices M: Profiles of 143 immune genes in DK/49- or GS/65-infected ducks. The genes including here showed significant differences in gene expression (FDR<= 0.001, fold change >=2) in at least one experiment between the DK/49-infected ducks and GS/65-infected ducks one-three after inoculation. The heatmap was generated from hierarchical analysis of genes based on Pearson's correlation.