

**Table S1** Primer sets used for PCR in this study

Primer	Sequence of oligonucleotides (5' → 3')
RIG-I-F	ATGACGGCCGAGGAGCGGCGAAA
RIG-I-R	TCATTCGGGTATTTCTGGAGAACCAAAGGGTATCTTC
RIG-I-CARD-F	ATGACGGCCGAGGAGC
RIG-I-CARD-R	TTTGTCCACAATCCACAGTTGACTGA
RIG-I-△CARD-F	ATGGATGTTGAACTGAAAGTTCTTGAGGAT
RIG-I-△CARD-R	TCATTCGGGTATTTCTGGAGAACCAA
CIV PB2-F	ATGGAGAGAATAAAAGAATTAAGAG
CIV PB2-R	TCACATTGATGGCCATCCGAATTCTTTTGGT
CIV PB1-F	ATGGATGTCAATCCGACTTTACTTT
CIV PB1-R	TCACTTTTGGCGTCTGAGCTCT
CIV PA-F	ATGGAAGACTTTGTGCGACAAT
CIV PA-R	TCACTTTCAGTGCATGTGTG
CIV HA-F	ATGAAAACTGTTATTGCTTTAAGCTAC
CIV HA-R	TCACAATGCAAATGTTGCACC
CIV-NP-F	ATGGCGTCTCAAGGCACCAA
CIV-NP-R	TCACATTGTCATACTCTTCTACATTGTCTCCGAA
CIV-NA-F	ATGAACCCAAATCAGAAGATAATAGCAATAGGCTC
CIV-NA-R	TCACTATAGGCATGAAGTTGATATTCGCCCCAT
CIV-M1-F	ATGAGTCTTCTAACCGAGGTC

CIV-M1-R	TCACTTAAATCGCTGCAT
CIV-M2-F	ATGAGTCTTCTAACCGAGGTCGAA
CIV-M2--R	TTACTCCAGCTCTATGTTGACAAA
CIV-NS1-F	ATGGATTCCAACACTGTGTCAAGC
CIV-NS1-R	TCACAATTTCTGACTCAATTGTTCTCGC
Flag-homologous arm-F	GACGATGACAAGCTTGCGGCCGCG
Flag-homologous arm-R	CAGGGATGCCACCCGGGATCC
Myc-homologous arm-F	TGGCCATGGAGGCCCGAATTCAA
Myc-homologous arm-R	CCTCGAGAGATCTCGGTCGACAA
VC/VN-homologous arm-F	ACCGGACTCAGATCTGAATTCC
VC/VN-homologous arm-F	CTCCACCCTCGAGCGGGATCCC

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**Table S2** Primer pairs used for quantitative RT-PCR analysis

<b>Primer</b>	<b>Sequence of oligonucleotides (5' → 3')</b>	<b>Gene accession NO.</b>
RIG-I-qF	GTTGCTGATGAAGGCATCGACATTG	XM_005626701
RIG-I-qR	CACTTGCTACCTCTTGCTCTTCCTC	
IRF-3-qF	CCAAGGATGAAGATGGAGACCTGTTC	XM_005616307
IRF-3-qR	GCGTGGTGAGTGTCGGCTTC	
NF-κB-qF	AACACTGGAAGCACGAATGACAGAG	NM_001003344
NF-κB-qR	CATGAGCCGCACCACACTGAG	
IFN-β-qF	CCAGTTCCAGAAGGAGGACA	NM_001135787
IFN-β-qR	TGTCCCAGGTGAAGTTTTC	
Mx1-qF	GATTCGGACGAGGAGAAGAAGAAGAAG	NM_001003134
Mx1-qR	GATGTGGCTGGAGATGCGGTTG	
OAS-qF	ATCCCCTAACCCCTCTACACA	NM_001048131
OAS-qR	ATGCAAAATGCTCCACACAGG	
STAT-1-qF	AGTGGAAGCGGAGACAGCAGAG	XM_843260
STAT-1-qR	CAGCGACTACGGTGAACCAAGTTC	
GAPDH-qF	AATTCCACGGCACAGTCAAGGC	NM_001003142
GAPDH-qR	ACAACATACTCAGCACCAGCATCAC	

**Table S3** Sequences of siRNA used in RNA interference

Primer	Sequence of oligonucleotides (5' → 3')
psiRIG-I-1	GGCTGAGAAGAACAATAAA
psiRIG-I-2	TGAGTAAGTTACACCCTAA

**Table S4** Antibody used in this study

<b>Antibody</b>	<b>Company</b>	<b>Host species</b>	<b>Catalog number</b>
RIG-I	Millipore	Mouse	MABF297
Flag tag	GeneTex	Mouse	GTX82562
Myc tag	GenteTex	Rabbit	GTX115046
IAV M1	GeneTex	Rabbit	GTX125928
IAV M2	GenteTex	Rabbit	GTX125951
IRF-3	CST	Rabbit	#29047
Phospho-IRF-3 (Ser396)	CST	Rabbit	#11904
Alexa Fluor® 488	Abcam	Goat	AB150113
Alexa Fluor® 594	Abcam	Goat	AB150080

A

[ Canis ] 1 VTALEBRRNIAAFRDYVIKILDPAYISLYAPWFKDDEVOYI CAEKNKNGPNEAASLFLKCLLEI QEEGWRFGLDALAHAGYSGLYEAI ESWDFQ  
[ Felis ] 1 VTALEBRRNIAAFRDYVIKILDPAYISLYAPWFKDDEVOYI CAEKNKNGPNEAASLFLKCLLEI QEEGWRFGLDALAHAGYSGLYEAI ESWDFQ  
[ Homo ] 1 VTALEBRRNIAAFRDYVIKILDPAYISLYAPWFKDDEVOYI CAEKNKNGPNEAASLFLKCLLEI QEEGWRFGLDALAHAGYSGLYEAI ESWDFQ  
[ Nus ] 1 VTALEBRRNIAAFRDYVIKILDPAYISLYAPWFKDDEVOYI CAEKNKNGPNEAASLFLKCLLEI QEEGWRFGLDALAHAGYSGLYEAI ESWDFQ

[ Canis ] 96 KMSELEFYRLLLKRLQPEFKTTVNPNDILPKISELLISOEEELI QICSNKGLVAGAERNVVECLLRSDKENWPKTKLALDEEESKFSQWIVDR  
[ Felis ] 96 TIIRLEEFYRLLLKRLQPEFKTTVNPNDILPKISELLISOEEELI QICSNKGLVAGAERNVVECLLRSDKENWPKTKLALDEEESKFSQWIVDR  
[ Homo ] 96 KIIRLEEFYRLLLKRLQPEFKTTVNPNDILPKISELLISOEEELI QICSNKGLVAGAERNVVECLLRSDKENWPKTKLALDEEESKFSQWIVDR  
[ Nus ] 96 KIIRLEEFYRLLLKRLQPEFKTTVNPNDILPKISELLISOEEELI QICSNKGLVAGAERNVVECLLRSDKENWPKTKLALDEEESKFSQWIVDR

[ Canis ] 191 --DVELKVLDEEELITSEVOI FYKIEPECONLSQDPCTPS VAST--CTIPKPRNYQLELALPAKEGKNTIICAPTGGCKTFVALI CEHHLKK  
[ Felis ] 191 GAKNI DVKVLDEEEMTSIDIQI FYKIEPECONLSQDPCTPS VAST--CTIPKPRNYQLELALPAKEGKNTIICAPTGGCKTFVALI CEHHLKK  
[ Homo ] 191 GI KDVEDELEDE--KMTSDIQI FYKIEPECONLSQDPCTPS VAST--CTIPKPRNYQLELALPAKEGKNTIICAPTGGCKTFVALI CEHHLKK  
[ Nus ] 191 GF KAESKADLEDEGAASTIQI QI FYKIEPECONLSQDPCTPS VAST--CTIPKPRNYQLELALPAKEGKNTIICAPTGGCKTFVALI CEHHLKK

[ Canis ] 280 FPGQGGKGVFFANQIPVYEQCKSVFSRYFERLGKVGAGISGATSENISVEQI MENNDI ILLTPQI LVNNEKNGTI PSLSVFTLM FDECHNTSK  
[ Felis ] 284 FPGQGGKGVFFANQIPVYEQCKSVFSRYFERLGKVGAGISGATSENISVEQI MENNDI ILLTPQI LVNNEKNGTI PSLSVFTLM FDECHNTSK  
[ Homo ] 285 FPGQGGKGVFFANQIPVYEQCKSVFSRYFERLGKVGAGISGATSENISVEQI MENNDI ILLTPQI LVNNEKNGTI PSLSVFTLM FDECHNTSK  
[ Nus ] 286 FPGQGGKGVFFANQIPVYEQCKSVFSRYFERLGKVGAGISGATSENISVEQI MENNDI ILLTPQI LVNNEKNGTI PSLSVFTLM FDECHNTSK

[ Canis ] 375 HIPYNNMNNYLDQKLGSSDPLPQVGLTASVGGDANKTMAVEYICRLCASLTSVIATVKDNLLELEI VYKPKQKFRKVESRTTDRKCI  
[ Felis ] 379 HIPYNNMNNYLDQKLGSSDPLPQVGLTASVGGDANKTMAVEYICRLCASLTSVIATVKDNLLELEI VYKPKQKFRKVESRTTDRKCI  
[ Homo ] 380 QIPYNNMNNYLDQKLGSSDPLPQVGLTASVGGDANKTMAVEYICRLCASLTSVIATVKDNLLELEI VYKPKQKFRKVESRTTDRKCI  
[ Nus ] 381 HIPYNNMNNYLDQKLGSSDPLPQVGLTASVGGDANKTMAVEYICRLCASLTSVIATVKDNLLELEI VYKPKQKFRKVESRTTDRKCI

[ Canis ] 470 ISQLMRETESLAKNI FDELGTITLENVSQIQNRNFGTKQYEQWVSYOKACNVFQLPNRDEESRI CKALFLYTSHLRKYNDALI ISEBARMDAL  
[ Felis ] 474 ISQLMRETESLAKNI FDELGTITLENVSQIQNRNFGTKQYEQWVSYOKACNVFQLPNRDEESRI CKALFLYTSHLRKYNDALI ISEBARMDAL  
[ Homo ] 475 ISQLMRETESLAKNI CKDL----ENLSQIQNRNFGTKQYEQWVSYOKACNVFQLPNRDEESRI CKALFLYTSHLRKYNDALI ISEBARMDAL  
[ Nus ] 476 ISQLMRETESLAKNI FDELGTITLENVSQIQNRNFGTKQYEQWVSYOKACNVFQLPNRDEESRI CKALFLYTSHLRKYNDALI ISEBARMDAL

[ Canis ] 565 DYLKDFTTIVRAAGFDEI EQDLIRRFEEKLOKLESVSNPSSNENPKLDLCTI LOEEYHLNPESTRITLIFVKTRALVDALRKM EISELSFLKPG  
[ Felis ] 569 DYLKDFTTIVRAAGFDEI EQDLIRRFEEKLOKLESVSNPSSNENPKLDLCTI LOEEYHLNPESTRITLIFVKTRALVDALRKM EISELSFLKPG  
[ Homo ] 565 DYLKDFTTIVRAAGFDEI EQDLIRRFEEKLOKLESVSNPSSNENPKLDLCTI LOEEYHLNPESTRITLIFVKTRALVDALRKM EISELSFLKPG  
[ Nus ] 566 DYLKDFTTIVRAAGFDEI EQDLIRRFEEKLOKLESVSNPSSNENPKLDLCTI LOEEYHLNPESTRITLIFVKTRALVDALRKM EISELSFLKPG

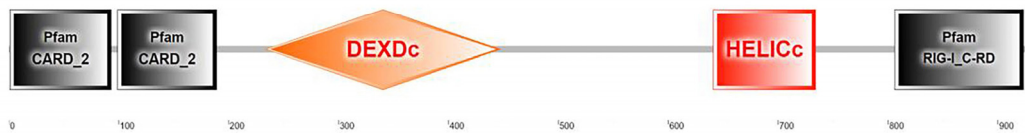
[ Canis ] 660 IITGRGRTNNTGNTLPAGKQIDAFRTDGLKKI LIATSVADEGI DIAECNLVILYEEYGVNVI RMOTRGRGRARCSKCFLLTS SAVI EKEKAS  
[ Felis ] 664 IITGRGRTNNTGNTLPAGKQIDAFRTDGLKKI LIATSVADEGI DIAECNLVILYEEYGVNVI RMOTRGRGRARCSKCFLLTS SAVI EKEKAS  
[ Homo ] 660 IITGRGRTNNTGNTLPAGKQIDAFRTDGLKKI LIATSVADEGI DIAECNLVILYEEYGVNVI RMOTRGRGRARCSKCFLLTS SAVI EKEKAS  
[ Nus ] 661 IITGRGRTNNTGNTLPAGKQIDAFRTDGLKKI LIATSVADEGI DIAECNLVILYEEYGVNVI RMOTRGRGRARCSKCFLLTS SAVI EKEKAS

[ Canis ] 755 KKEKLNLYREKLNDSISLGLMNNFAVKEKVLQIQI GERNIRDSQERVELVPDKNKKLLGRCKRAFACYTADI RVVETSCHYTVVGDAIKERFV  
[ Felis ] 759 KKEKLNLYREKLNDSISLGLMNNFAVKEKVLQIQI GERNIRDSQERVELVPDKNKKLLGRCKRAFACYTADI RVVETSCHYTVVGDAIKERFV  
[ Homo ] 755 KKEKLNLYREKLNDSISLGLMNNFAVKEKVLQIQI GERNIRDSQERVELVPDKNKKLLGRCKRAFACYTADI RVVETSCHYTVVGDAIKERFV  
[ Nus ] 756 KKEKLNLYREKLNDSISLGLMNNFAVKEKVLQIQI GERNIRDSQERVELVPDKNKKLLGRCKRAFACYTADI RVVETSCHYTVVGDAIKERFV

[ Canis ] 850 SRLHPKPKPSFGHTEKAKI FCARRNGQIDWGI EAVRYKTFEI PVIKI ESEFVVEDIATGATLYS KWKDFEERIQDPAEKSV  
[ Felis ] 854 SRLHPKPKPSFGHTEKAKI FCARRNGQIDWGI EAVRYKTFEI PVIKI ESEFVVEDIATGATLYS KWKDFEERIQDPAEKSV  
[ Homo ] 850 SRLHPKPKPSFGHTEKAKI FCARRNGQIDWGI EAVRYKTFEI PVIKI ESEFVVEDIATGATLYS KWKDFEERIQDPAEKSV  
[ Nus ] 851 SRLHPKPKPSFGHTEKAKI FCARRNGQIDWGI EAVRYKTFEI PVIKI ESEFVVEDIATGATLYS KWKDFEERIQDPAEKSV

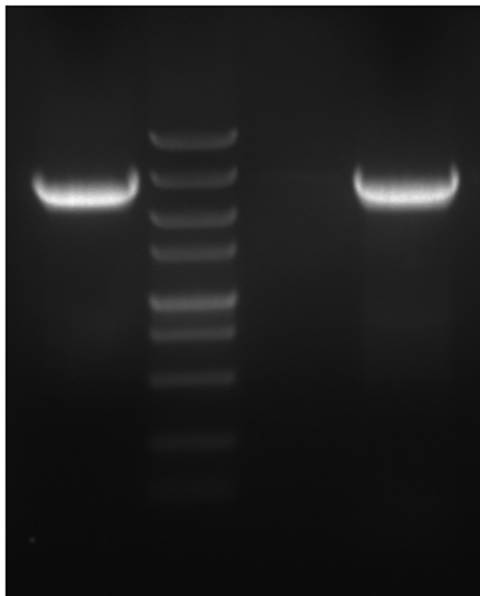
———— CARD  
—— DEXD box  
----- Helicase  
\*\*\*\*\* C-terminal Regulatory

B

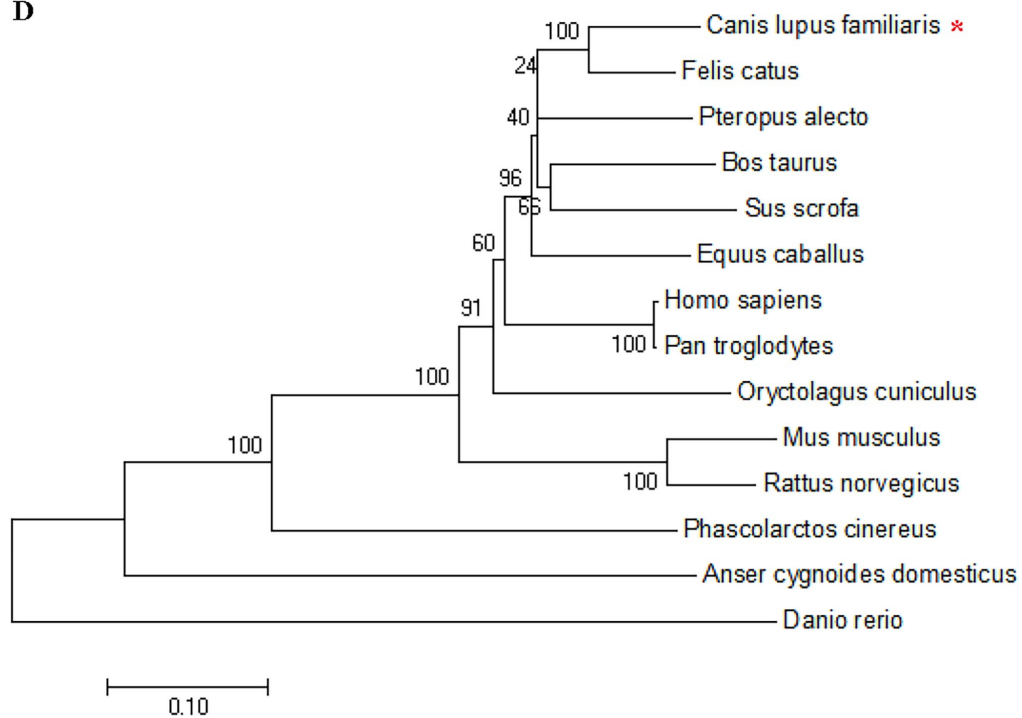


C

Tissue DL5000 MDCK Cell



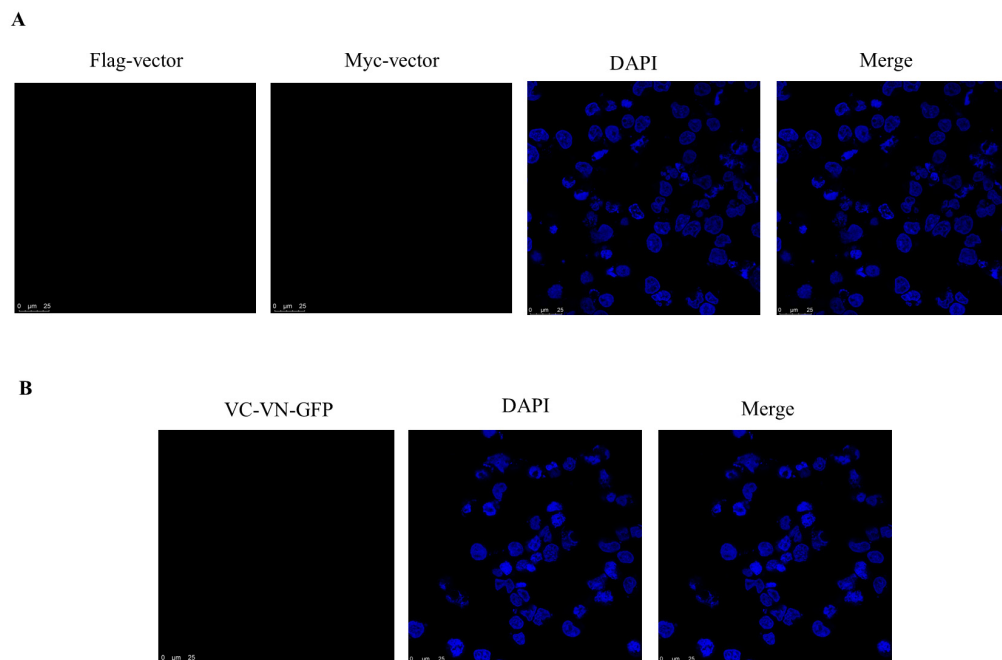
D



**Figure S1 Bioinformatic analysis of canine RIG-I gene.** (A) Amino acid multiple alignments of canine, feline, human, and mouse. Black considers as consensus. Alignment was performed using Clustal X program and edited with Boxshade. (B) The schematic representation of canine RIG-I. (C) The agarose electrophoresis of

canine RIG-I (Left track: canine RIG-I cloned from MDCK. Right track: canine RIG-I cloned from beagle dog). (D) A phylogenetic tree based on the relationship of RIG-I between canine and other species. The scale bar is 0.1. GenBank accession nos.:

*Canis lupus familiaris*: MG835367; *Felis catus*: XM\_006939199.4; *Pteropus Alecto*: NM\_001290158.1; *Bos taurus*: NM\_001034527.2; *Sus scrofa*: NM\_213804.2; *Equus caballus*: XM\_005605049.3; *Homo sapiens*: NM\_014314.3; *Pan troglodytes*: XM\_001156662.4; *Oryctolagus cuniculus*: XM\_002708040.3; *Mus musculus*: NM\_172689.3; *Rattus norvegicus*: NM\_001106645.1; *Phascolarctos cinereus*: XM\_020976230.1; *Anser cygnoides domesticus*: NM\_001311190.1; *Danio rerio*: NM\_001306095.1.



**Figure S2 Negative control of IFA and BiFC assays.** (A) HEK 293T cells were transfected with Flag tag vector and Myc tag vector together. The Flag tag and Myc tag primary antibody were used for IFA. (B) HEK 293T cells were transfected with



vector Venus VN-173 and vector Venus VN-173 together. After 24 h transfection, the sample was observed by confocal scanning microscopy.