Supplemental Information

# Dampened STING-Dependent

Interferon Activation in Bats

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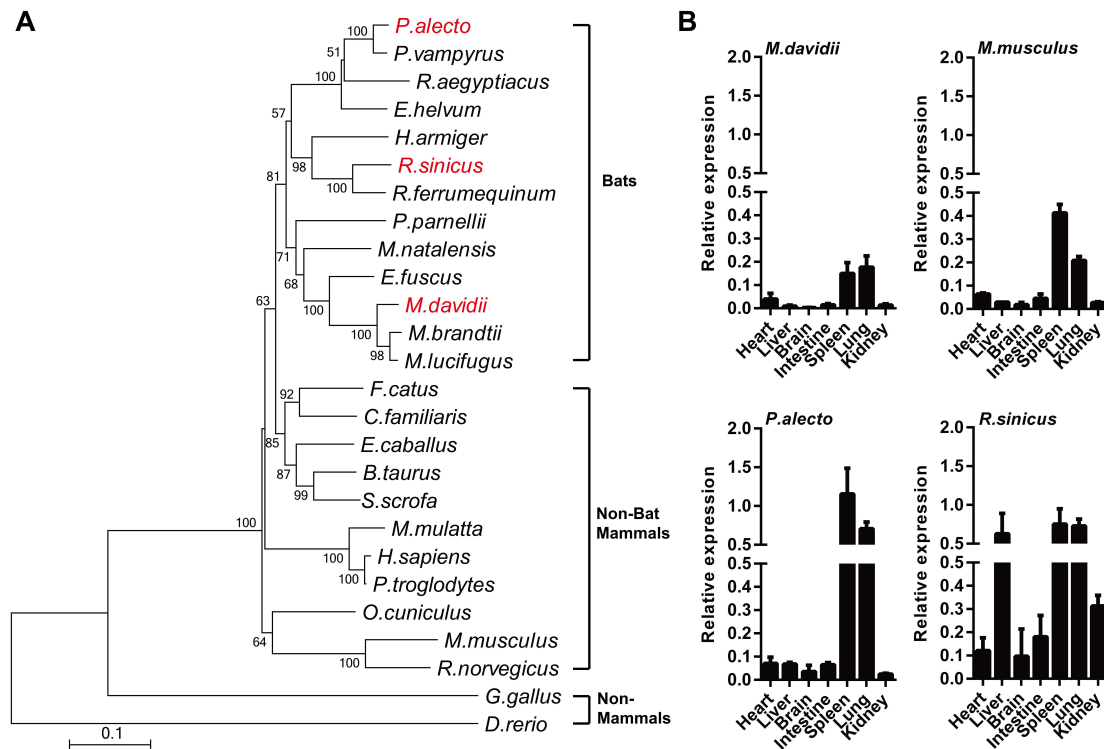
Figure S1. Phylogenetic tree of STING and Quantitation of STING mRNA in tissues of *Rhinolophus sinicus* (*Rs*), *Myotis davidii* (*Md*), *Pteropus alecto* (*Pa*) bats and mice. Related to Figure 1. (A) Phylogenetic tree of STING. The three representative bat STING genes analyzed in this study were highlighted in red. The full species name, accession numbers of STING and SRA accession numbers are listed in Table S1. Tree was constructed by MEGA (Version 4) with the neighbor-joining statistical method. Bootstrap values were calculated from 1000 replicates and values >50 are shown. (B) Tissues were collected from healthy wild-caught *Rs* (n=3), *Md* (n=3), *Pa* (n=2) and cultured BALB/c mice (n=3). The mRNA level was normalized to housekeeping gene SNRPD3 and presented as the mean ± SD.
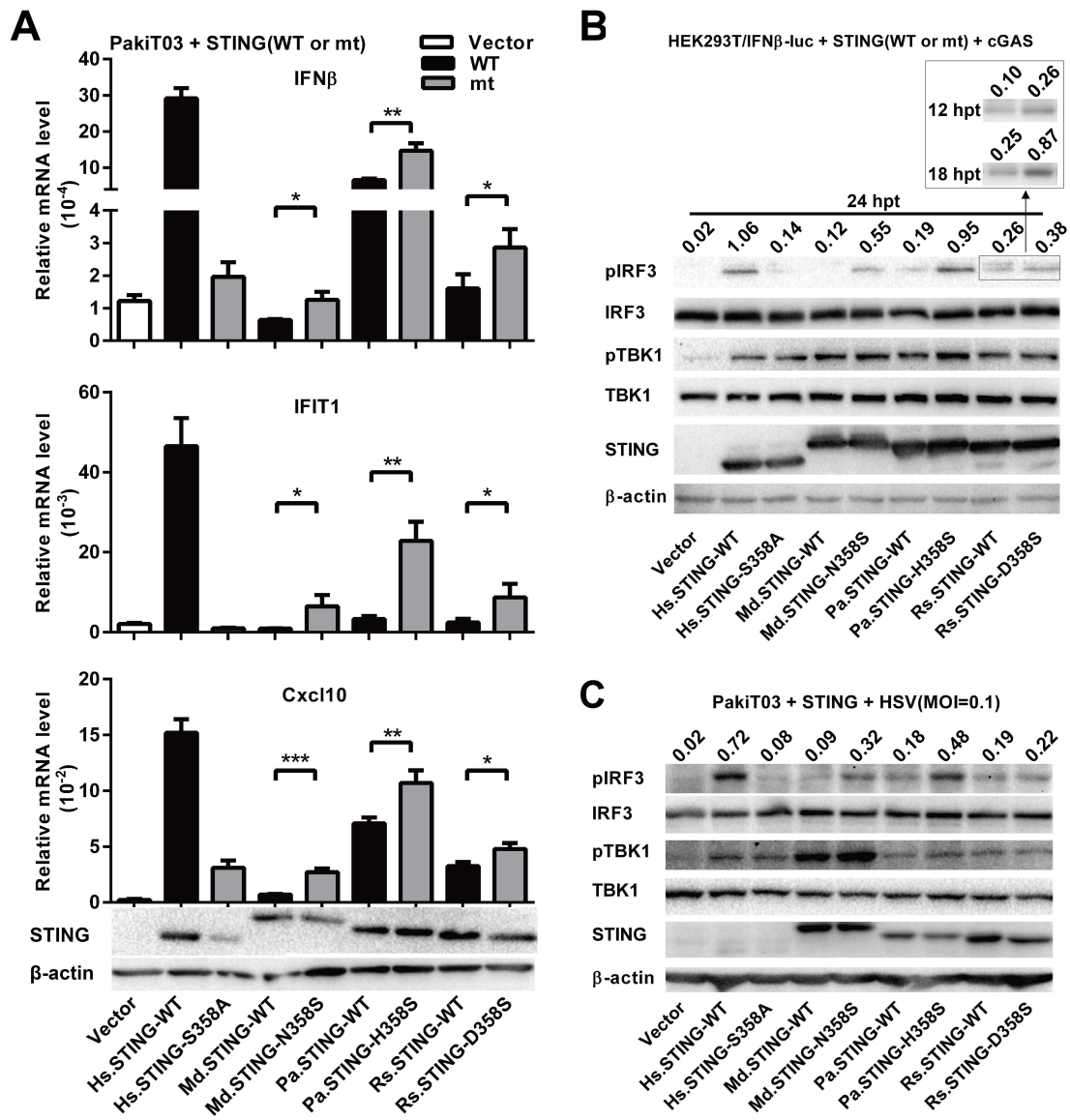
Figure S2. Effects of Serine 358 on the Dampening of IFN Activation and Phosphorylation of IRF3 and TBK1. Related to Figure 2. (A) PaKiT03 cells were transfected with indicated STING plasmids and gene expression (normalized to SNRPD3) was determined by qPCR 24 hours later. Data are presented as the mean ± SD, n=3, *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t test). (B) HEK293T cells were co-transfected with STING, cGAS, IFNβ promoter firefly luciferase and renilla luciferase plasmids. The levels of p-IRF3, IRF3, pTBK1, TBK1 and STING were determined 24 hours post transfection. For the two *R.sinicus* STING samples, the p-IRF3 levels of samples at 12, 18 hours post transfection were further indicated on the top. (C) PaKiT03 cells were transfected with indicated STING plasmids followed by

infection with HSV-luciferase at MOI=0.1 at 24 hours post transfection. At 24 hours post infection, the levels of p-IRF3, IRF3, pTBK1, TBK1 and STING were determined by Western blot. The amount of pIRF3 was counted by ImageJ software and labeled. Three independent experiments were performed. Abbreviations: WT = wild type; mt = mutant; *Hs = Homo sapiens*; *Md = Myotis davidii*; *Pa = Pteropus alecto*; *Rs = Rhinolophus sinicus*.

TableS1. Genbank accession numbers of STING sequences or SRA data ID used in this study. Related to Figure 1.

	Full name	Abbreviation	Accession numbers
Bats	*Artibeus jamaicensis*	*A.jamaicensis*	SRR539297
	Aselliscus stoliczkanus	*A.stoliczkanus*	SRR2153215
	Carollia perspicillata	*C.perspicillata*	SRR2130341-SRR2130344
	Cynopterus sphinx	*C.sphinx*	SRR2153213
	Desmodus rotundus	*D.rotundus*	SRR606902,SRR606899, SRR606908,SRR606911
	Eptesicus fuscus	*E.fuscus*	XP_008139824.1
	Eidolon helvum	*E.helvum*	MF174844
	Eonycteris spelaea	*E.spelaea*	SRR1515272
	Hipposideros armiger	*H.armiger*	XP_019517728.1
	Hipposideros pratti	*H.pratti*	SRR2153216
	Myotis brandtii	*M.brandtii*	XP_005881105.1
	Myotis davidii	*M.davidii*	XP_006772500.1
	Murina leucogaster	*M.leucogaster*	SRR2153222
	Myotis lucifugus	*M.lucifugus*	XP_006086577.1
	Megaderma lyra	*M.lyra*	SRR2153218
	Miniopterus natalensis	*M.natalensis*	XP_016059234.1
	Myotis ricketti	*M.ricketti*	SRR2153224
	Miniopterus schreibersii	*M.schreibersii*	SRR974728-SRR974741
	Pteropus alecto	*P.alecto*	XP_006923104.1
	Pteronotus parnellii	*P.parnellii*	MF174846
	Pteropus vampyrus	*P.vampyrus*	XP_011380567.1
	Rousettus aegyptiacus	*R.aegyptiacus*	XP_016021870.1
	Rhinolophus ferrumequinum	*R.ferrumequinum*	MF174845
	Rousettus leschenaultii	*R.leschenaultii*	SRR2153214
	Rhinolophus macrotis	*R.macrotis*	SRR1584445-SRR1584447
	Rhinolophus pusillus	*R.pusillus*	SRR2153217
	Rhinolophus sinicus	*R.sinicus*	XP_019595754.1
	Scotophilus kuhlii	*S.kuhlii*	SRR2153223
	Taphozous melanopogon	*T.melanopogon*	SRR2153220
	Tadarida teniotis	*T.teniotis*	SRR2153221
Non-bat animals	*Mus musculus*	*M.musculus*	NP_082537.1
	Rattus norvegicus	*R.norvegicus*	NP_001102592.1
	Homo sapiens	*H.sapiens*	NP_938023.1
	Loxodonta africana	*L.africana*	XP_003404845.1
	Pan troglodytes	*P.troglodytes*	XP_001135484.1
	Macaca mulatta	*M.mulatta*	EHH26836.1
	Bos taurus	*B.taurus*	NP_001039822.1
	Sus scrofa	*S.scrofa*	NP_001136310.1
	Felis catus	*F.catus*	XP_003980949.1
	Canis lupus familiaris	*C.familiaris*	XP_848338.2
	Equus caballus	*E.caballus*	XP_005599422.1
	Oryctolagus cuniculus	*O.cuniculus*	XP_002710295.1
	Gallus gallus	*G.gallus*	NP_001292081.1
	Danio rerio	*D.rerio*	NP_001265766.1

Table S2. Primers used in this study. Related to Figure 2 and STAR methods.

No.	Primer Name	Sequence (5' - >3')	Purpose
1	Homo/Md/Rs.STING_NotI_F	ATTTGCGGCCGC*GCCACC*ATGCCCCACTCCAGC	Clone the *Homo sapiens/Myotis davidii/Rhinolophus sinicus/Pteropus alecto* STING into pCAGGS vector (modified with an in-frame S tag at the C-terminal) with N-term Kozak sequence and no stop codon
2	Pa.STING_NotI_F	ATTTGCGGCCGC*GCCACC*ATGTCCCACTCCAGC	
3	Md/Pa.STING_NheI_R	CTAGCTAGCGAAGATATCTGTGCGGA	
4	Homo.STING_NheI_R	CTAGCTAGCAGAGAAATCCGTGCG	
5	Rs.STING_NheI_R	CTAGCTAGCGAAGACATCTGTGCGGA	
6	Md.STING_N358S-F	TCCACATTGTCCCAAGAGCCTGAGCTCCTCATC	Site-directed mutagenesis primers for *Myotis davidii* STING
7	Md.STING_N358S -R	GCTCTTGGGACAATGTGGAAGAGTCAGGCACC	
8	Homo.STING_S358A_F	TCCACGATGGCCCAAGAGCCTGAGCTCCTCATC	Site-directed mutagenesis primers for *Homo sapiens* STING
9	Homo.STING_S358A_R	CTCTTGGGCCATCGTGGAGGTACTGGGCACC	
10	Rs.STING_D358S-F	CCACGCTATCCGAAGAGCCCCAGCTCCTCA	Site-directed mutagenesis primers for *Rhinolophus sinicus* STING
11	Rs.STING_D358S-R	GGCTCTTCGGATAGCGTGGAAGGTTCCGGC	
12	Pa.STING_H358S-F	CCACGCTGTCCCAAGAGCCCGAGCTCCTCATCAG	Site-directed mutagenesis primers for *Pteropus alecto* STING
13	Pa.STING_H358S-R	GAGCTCGGGCTCTTGGGACAGCGTGGAGGAGACAGGCT	
14	Rs.STING_Age I-F	CCCACCGGTATGCCCCACTCCAGCCTACAT	Clone the *Rhinolophus sinicus* STING into pQCXIH (modified with in-frame GFP tag at the C-terminal)
15	Rs.STING_Not I_R	ATTTGCGGCCGCGAAGACATCTGTGCGGAGTGGGAG	
16	Mus.Snrpd3_QPCR_F	ATTGGTGTGCCGATTAAAGTCT	qPCR primers for mouse reference gene SNRPD3
17	Mus.Snrpd3_QPCR_R	ATACTTCCCCGGTGTTGGTCT	
18	Mus.STING_QPCR_F	TATACCTCAGTTGGATGTTTGGC	qPCR primers for mouse STING
19	Mus.STING_QPCR_R	CTGGAGTCAAGCTCTGAAGGC	
20	Mus.IRF7_QPCR_F	GAGACTGGCTATTGGGGGAG	qPCR primers for mouse IRF7
21	Mus.IRF7_QPCR_R	GACCGAAATGCTTCCAGGG	
22	Mus.IFNB_QPCR_F	AGCTCCAAGAAAGGACGAACA	qPCR primers for mouse IFNβ
23	Mus.IFNB_QPCR_R	GCCCTGTAGGTGAGGTTGAT	
24	Md.SNRPD3_QPCR_F	ACCGCGGGAAGCTCATC	qPCR primers for *Myotis davidii* reference gene SNRPD3
25	Md.SNRPD3_QPCR_R	TGTTGGACATCTGGCAGTTCA	
26	Md.STING_QPCR_F	TGTTCAAGCGAGTCTGCAGTCT	qPCR primers for *Myotis davidii* STING
27	Md.STING_QPCR_R	TCACAGCCCTCCGGTAGCT	
28	Rs.STING_QPCR-F	CCAGACACTCGCGGACATC	qPCR primers for *Rhinolophus sinicus* STING
29	Rs.STING_QPCR-R	GCAGCTTCCAGGTAGACAATGA	
30	Rs.SNRPD3_QPCR-F	TGAGACAAACACTGGTGAGGTGTA	qPCR primers for *Rhinolophus sinicus* reference gene SNRPD3
31	Rs.SNRPD3_QPCR-R	GGACATCTGGCAGTTCATGTTG	
32	Rs.IFNB1_QPCR-F	ACCTCCTGTGGCAGTTGAATG	qPCR primers for *Rhinolophus sinicus* IFNβ
33	Rs.IFNB1_QPCR-R	GCTTAAAGTCCATCCTGTCCTTGA	
34	Rs.IRF7_QPCR-F	TCCCCACACTACACCATCTAC	qPCR primers for *Rhinolophus sinicus* IRF7
35	Rs.IRF7_QPCR-R	TTCCCGTTGTACATGCTCC	
36	Pa.STING_QPCR-F	GCCGGACGCTTGAGGATAT	qPCR primers for *Pteropus alecto* STING
37	Pa.STING_QPCR-R	TCCTCTGTAGGTTCCTGGTAGACAA	
38	Pa.SNRPD3_QPCR-F	AGGTATACATCCGTGGCAGC	qPCR primers for *Pteropus alecto* SNRPD3
39	Pa.SNRPD3_QPCR-R	CCACTTGGGCCTTCAGAATA	
40	Pa.IFNB1_QPCR-F	CTCTAGCACTGGCTGGAATGAA	qPCR primers for *Pteropus alecto* IFNβ
41	Pa.IFNB1_QPCR-R	TGCCCACCGAGTGTCTCA	
42	Pa.IFIT1_QPCR-F	CCTCCACCCATCTTAGGTTTATAG	qPCR primers for *Pteropus alecto* IFIT1
43	Pa.IFIT1_QPCR-R	CATCACTGGGTACTCTCATGTC	
44	Pa.Cxcl10_QPCR-F	TGCAAGTCAATCATGTCCACAA	qPCR primers for *Pteropus alecto* Cxcl10
45	Pa.Cxcl10_QPCR-R	CAGACATCTTTTTTCCCCGTTCT	