Supporting Information

Title: Cytosolic DNA-mediated, STING-dependent pro-inflammatory gene induction necessitates canonical NF-κB activation through TBK1

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Supporting Figure legends

Fig. S1. STING ligands-mediated signaling response in MEFs. (A) Primary MEFs (1 x 10^5 cells/well) derived from wild-type ($STING^{+/+}$) or STING-deficient mice ($STING^{-/-}$) were stimulated with 10 μg /ml of canonical 3'-5' cyclic-GMP-AMP (cGAMP) for the times indicated. The expression level of STING, IRF3 phosphorylated at Ser^{396} (p-IRF3), NFκB p65 phosphorylated at Ser^{536} (p-NF-κBp65), NF-κBp65, TBK1 phosphorylated at Ser^{172} (p-TBK1), and β-actin was determined by immunoblotting. (B) Nuclear translocation of NF-κBp65 is detected by cellular fractionation. $STING^{+/+}$ and $STING^{-/-}$ MEFs were stimulated with10 μg /ml of dsDNA90 for 3 h, and then cells were separated into cytosol and nuclear fractions. Each fraction was concentrated and subjected to immunoblotting with indicated antibodies. (C) $STING^{+/+}$ and $STING^{-/-}$ MEFs were stimulated with 10 μg /ml of dsDNA90, 5 μg /ml of Poly(dA:dT), 5 μg /ml of Poly(I:C), or 200 μM of DMXAA for 6 h, and then total RNA was extracted from these cells, and the expression level of mRNA of IFNβ, Ccl5, IL-6 and CXCL10 was determined by qRT-PCR. Data were normalized to

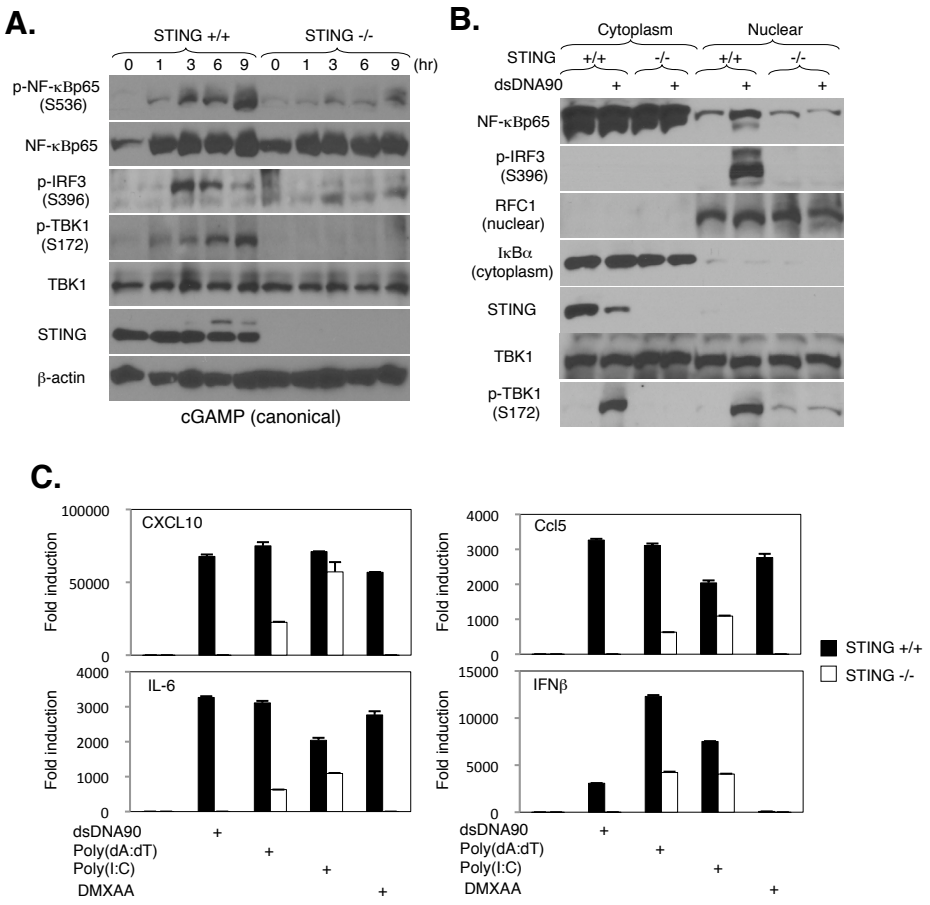
the levels of GAPDH mRNA. Error bars indicate the standard deviations (SD).

Fig. S2. HSV-1-mediated signaling response in MEFs. (A) Primary MEFs (1 x 10^5 cells/well) derived from wild-type ($STING^{+/+}$) or STING-deficient mice ($STING^{-/-}$) were stimulated with 5 μg /ml of dsDNA representing the genomes of HSV-1 or purified HSV-1 at an moi of 10 PFU/ml for the times indicated. The expression level of STING, IRF3 phosphorylated at Ser^{396} (p-IRF3), NFκB p65 phosphorylated at Ser^{536} (p-NF-κBp65), NF-κBp65, TBK1 phosphorylated at Ser^{172} (p-TBK1), TBK1, and β-actin was determined by immunoblotting.

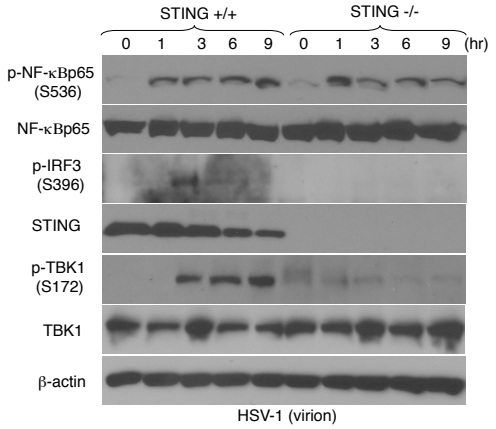
Fig. S3. TBK1 does not involve in the dsDNA-mediated MAPKs activation. (A) $TBK1^{+/+}$ and $TBK1^{-/-}$ MEFs were stimulated with 10 μg /ml of dsDNA90, or 5 μg /ml of Poly(I:C) for the times indicated. The expression level of ERK1/2 phosphorylated at Thr^{202}/Tyr^{204} (p-ERK1/2), ERK1/2, SAPK/JNK phosphorylated at Thr^{183}/Tyr^{185} (p-SAPK/JNK), JNK1/2, p38 phosphorylated at Thr^{180}/Tyr^{182} (p-p38), p38, and c-Jun phosphorylated at Ser^{63} (p-cJun) was determined by immunoblotting. (B) 293T cells were transfected with empty vector (EV) and Flag-tagged TBK1 (TBK1-FL) for 24 h, and then cell extracts were subjected to immunoblotting with indiacted antibodies.

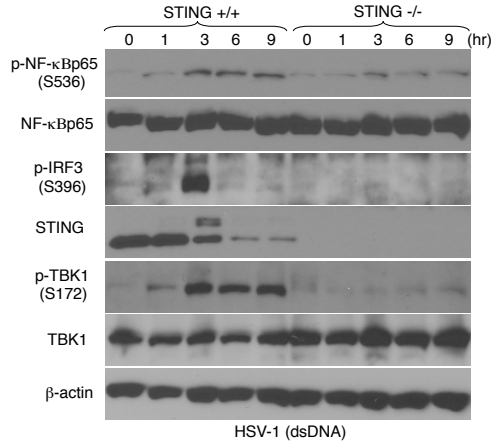
Fig. S4. IKKi/IKKε and NIK does not involve in the STING-dependent, dsDNA-mediated signaling response in MEFs. RNAi in MEFs using the non-specific (NS) and IKKi/IKKε (A) or NIK (B) were stimulated with 5 μg /ml of dsDNA90 for the times indicated. The expression level of STING, IRF3 phosphorylated at Ser^{396} (p-IRF3), NFκB p65 phosphorylated at Ser^{536} (p-NF-κBp65), NF-κBp65, TBK1 phosphorylated at Ser^{172} (p-TBK1), TBK1, NF-κBp52, NF-κBp100 phosphorylated at

Ser$^{866/870}$ (p-NF-κBp100), and β-actin was determined by immunoblotting. (C) RNAi in MEFs using the non-specific (NS) and IKKi/IKKε (upper panels) or NIK (lower panels) were stimulated with 5 μg /ml of dsDNA90 for 6 h, and then total RNA was extracted from these cells, and the expression level of mRNA of IL-6, CXCL10, and IKKi/IKKε or NIK were determined by real-time PCR, respectively. Real-time PCR data were normalized to the amount of GAPDH mRNA.

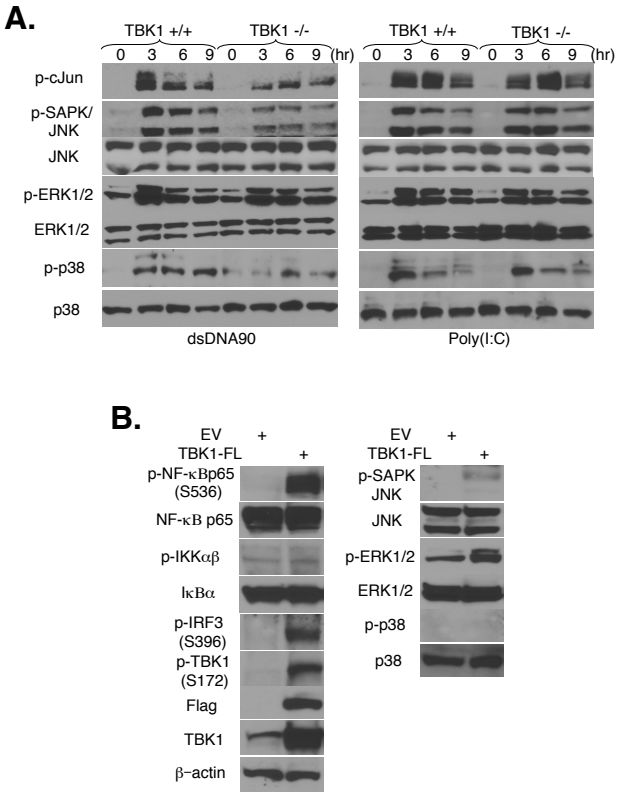

A.
STING +/+
STING -/-
0 1 3 6 9 0 1 3 6 9 (hr)
p-NF-κBp65 (S536)
NF-κBp65
p-IRF3 (S396)
p-TBK1 (S172)
TBK1
STING
β-actin
cGAMP (canonical)
B.
Cytoplasm
Nuclear
STING +/+ -/- +/+ -/-
dsDNA90 + + + +
NF-κBp65
p-IRF3 (S396)
RFC1 (nuclear)
IκBα (cytoplasm)
STING
TBK1
p-TBK1 (S172)
C.
CXCL10
Ccl5
IL-6
IFNβ
Fold induction
STING +/+
STING -/-
dsDNA90
Poly(dA:dT)
Poly(I:C)
DMXAA

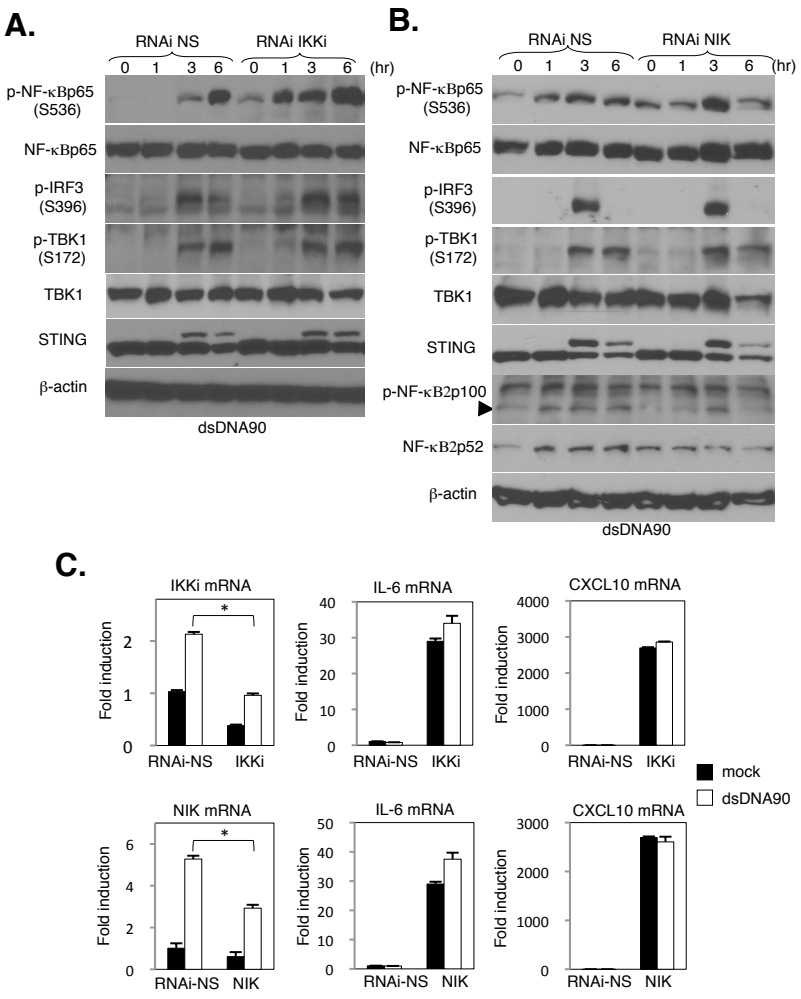
Abe et al Supporting Fig.2





Abe et al Supporting Fig.3




A.
RNAi NS
RNAi IKKi
0 1 3 6 0 1 3 6 (hr)
p-NF-κBp65 (S536)
NF-κBp65
p-IRF3 (S396)
p-TBK1 (S172)
TBK1
STING
β-actin
dsDNA90
B.
RNAi NS
RNAi NIK
0 1 3 6 0 1 3 6 (hr)
p-NF-κBp65 (S536)
NF-κBp65
p-IRF3 (S396)
p-TBK1 (S172)
TBK1
STING
p-NF-κB2p100
NF-κB2p52
β-actin
dsDNA90
C.
IKKi mRNA
IL-6 mRNA
CXCL10 mRNA
NIK mRNA
IL-6 mRNA
CXCL10 mRNA
Fold induction
RNAi-NS
IKKi
NIK
mock
dsDNA90