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The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells

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In the version of this supplementary file originally posted online, the sensor DDX41 was suggested to recognize Z-form DNA in addition to conventional B-form DNA. Although GC-rich DNA has the potential to adopt a left-handed Z-DNA conformation under conditions of high salt or ethanol, whether the GC-rich oligonucleotides used the original study actually adopted the Z-DNA conformation remains uncertain. Therefore, the file has been corrected throughout to reflect whether poly(dA:dT) or poly(dG:dC) was used to stimulate cells. The error has been corrected in this file as of 17 November 2011.

Supplementary Information

DDX41 senses intracellular DNA mediated by STING in Dendritic Cells

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Content

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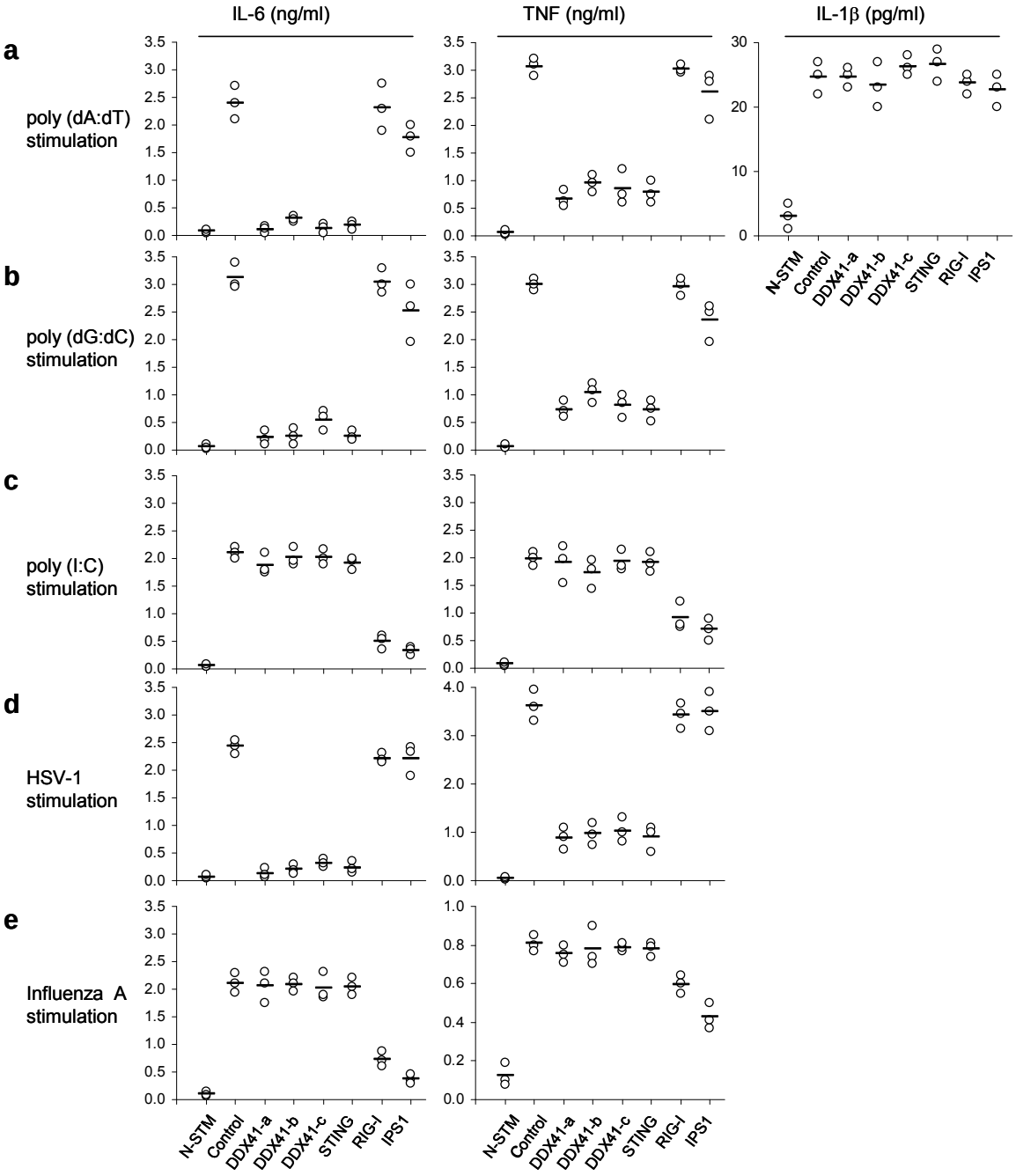
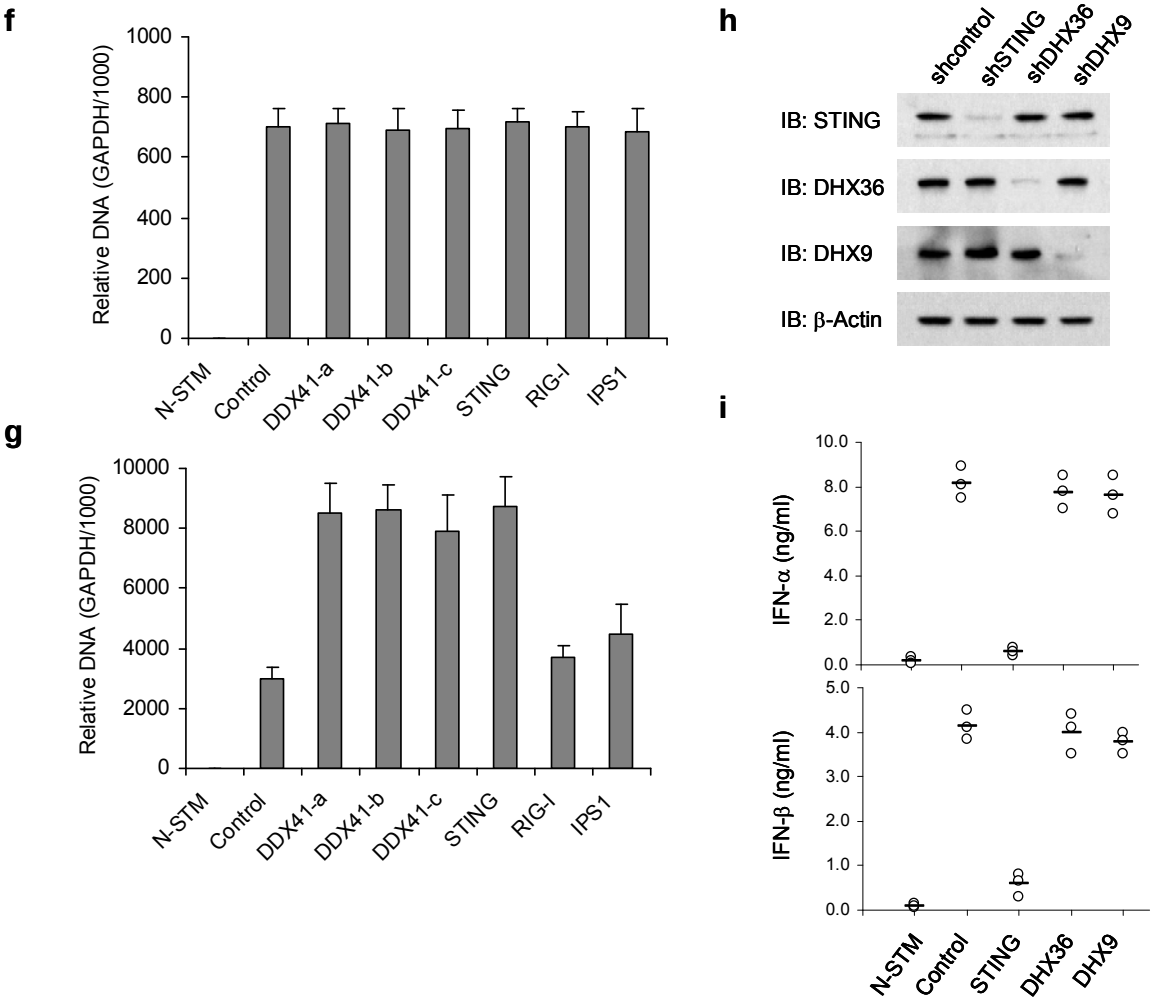
Fold-induction	Control Wildtype	DDX41 knockdown	STING knockdown
Cxlc10	512	1	0
Ccl5	96	1	1
Ifih1	176	2	1
Ish15	37	0	0
Ifit1	319	1	2

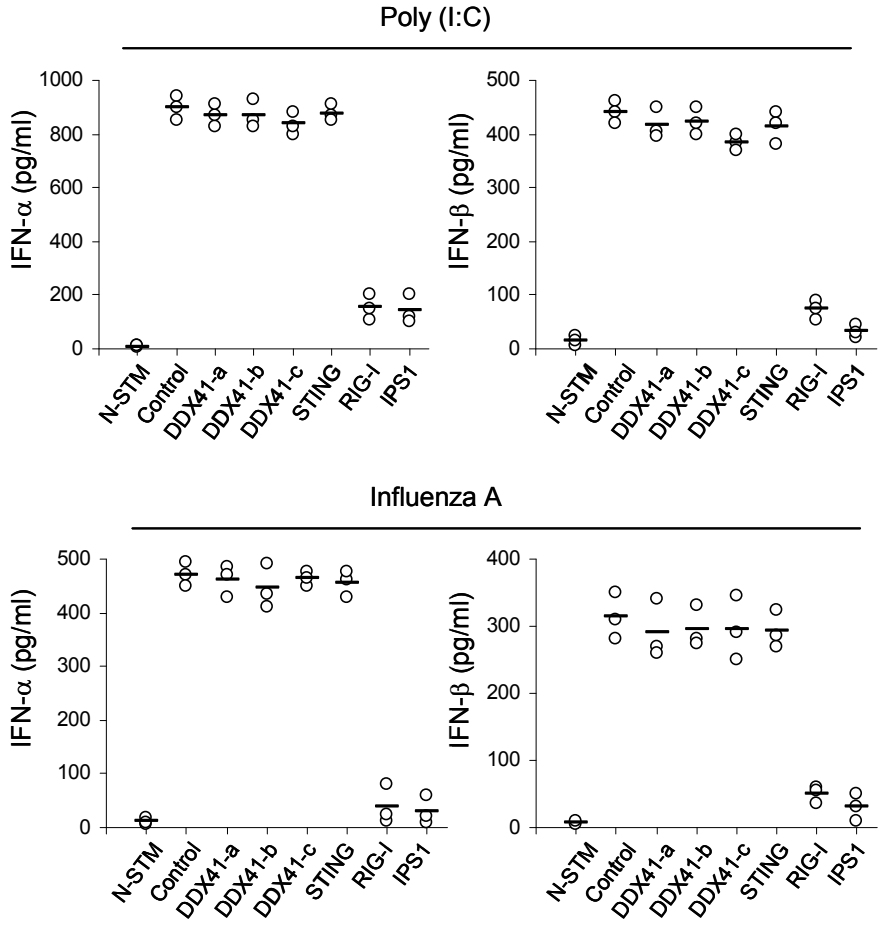
Supplementary Table 1. Gene expression profiles of wild type, DDX41- and STING-knockdown BMDCs stimulated for 8 h with poly (dA:dT). The right 3 columns indicate the fold increase in the poly (dA:dT) treated cells versus the mock-treated cells. Data is representative of three independent experiments.



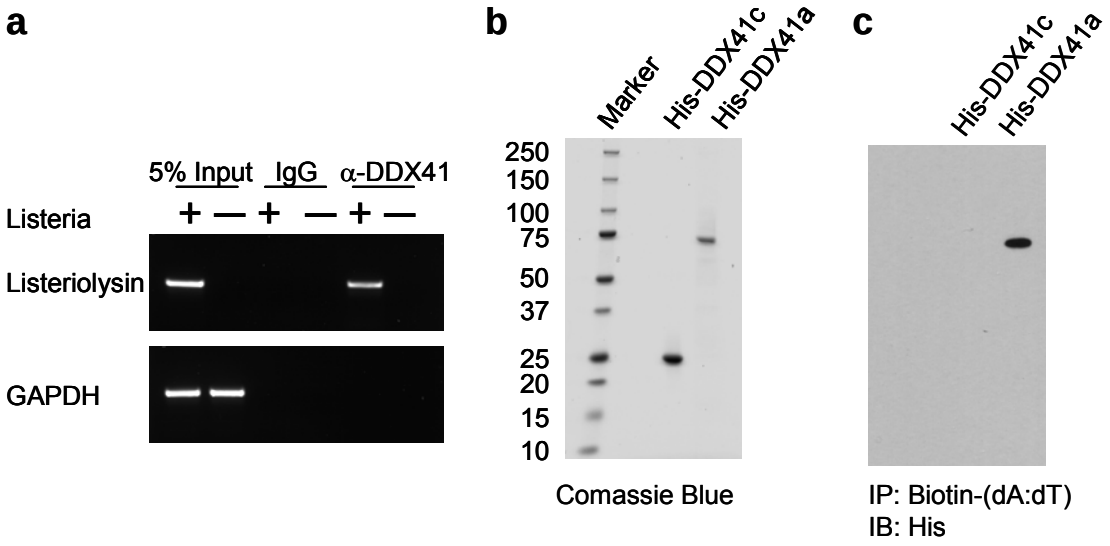


Supplementary Figure 1. DDX41- or STING- knockdown causes a reduction in IL-6 and TNF production in response to DNA and not RNA. ELISA of IL-6, TNF or IL-1β production from D2SC cells with the indicated shRNAs after 16 h stimulation with (**a**) 1 μg/ml of poly (dA:dT); (**b**) 1 μg/ml of poly (dG:dC); (**c**) 2.5 μg/ml of poly (I:C) (LMW, 0.2-1 kb); (**d**) HSV-1 at MOI = 10; or (**e**) influenza A virus at MOI = 10. DNA and poly (I:C) were delivered by Lipofectamine 2000. N-STM, D2SC cells without stimulation. (**f**) shRNA knockdown D2SC cells were challenged with equivalent titers of HSV-1. QPCR

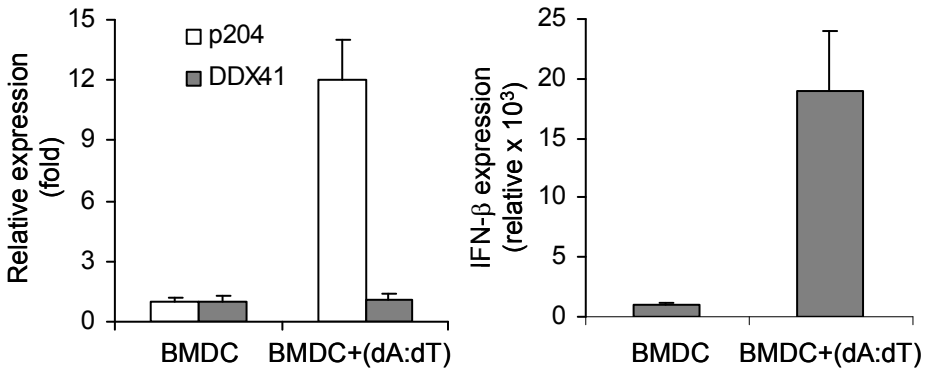
of HSV-1 DNA copies from D2SC cells incubated with HSV-1 virus at MOI of 10 for 1 h, followed by DNA extraction. (**g**) QPCR of HSV-1 DNA copies from D2SC cells with shRNA knockdown of indicated protein. D2SC cells were incubated with HSV-1 at MOI of 10 for 1 h. Cells were collected, washed with PBS and cultured for an additional 20 h followed by DNA extraction. (**h**) Immunoblot (IB) showing the knockdown efficiency of shRNA targeting the indicated genes in D2SC cells. Non-targeting shRNA served as a control (first left lane). β-actin blots are shown as loading controls (bottom). (**i**) ELISA of IFN-α/β production by D2SC cells with the indicated shRNA after 16 h stimulation with 1 μg/ml of poly (dA:dT). Individual circle represents the value from each independent experiment (performed in triplicate). Error bars represent the average value from at least three independent experiments (**a**, **b**, **c**, **d**, **e**, and **i**). Data is representative of three independent experiments (mean and s.d. in **f** and **g**).



Supplementary Figure 2. DDX41- or STING-knockdown in BMDCs have normal cytokine responses to poly (I:C) and Flu A virus. ELISA of INF-α/β production from bone marrow-derived GM-CSF DCs stimulated for 16 h with (**a**) 2.5 μg/ml of poly (I:C) or (**b**) Flu A at MOI of 10. Individual circle represents the value from each independent experiment (performed in triplicate). Error bars represent the average value from at least three independent experiments.



Supplementary Figure 3. DDX41 binds DNA. (**a**) Visualization of amplified DNA from ChIP assay on 1.5% argarose gel. ChIP assay was performed in BMDCs with (+) or without (-) Listeria infection. 5% of lysate was used for the input control. The Listeriolysin DNA and GAPDH DNA (control) was amplified by PCR. (**b**) Visualization of full size recombinant DDX41 (DDX41a) and the truncated version of DDX41 lacking the DNA binding domain (DDX41c) expressed and purified from *E. coli* BL21 (DE3). (**c**) Immunoblot using anti-His after immunoprecipitation assays in which purified His-DDX41a or His-DDX41c were incubated with biotinylated poly (dA:dT), followed by addition of NA-beads. Data is representative of three independent experiments.



Supplementary Figure 4. Mouse DDX41 is not an IFN-inducible gene. (**a**) Relative mRNA abundance of DDX41 and p204 in unstimulated (BMDC) or poly (dA:dT) stimulated BMDCs (BMDC+(dA:dT)); (**b**) Relative IFN-β mRNA abundance in unstimulated (BMDC) or poly (dA:dT) stimulated BMDCs (BMDC+(dA:dT)). Data is representative of three independent experiments.

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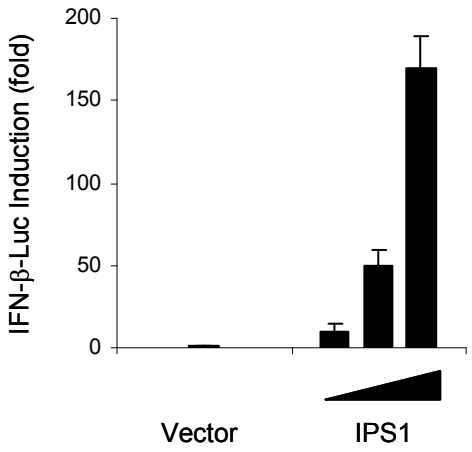
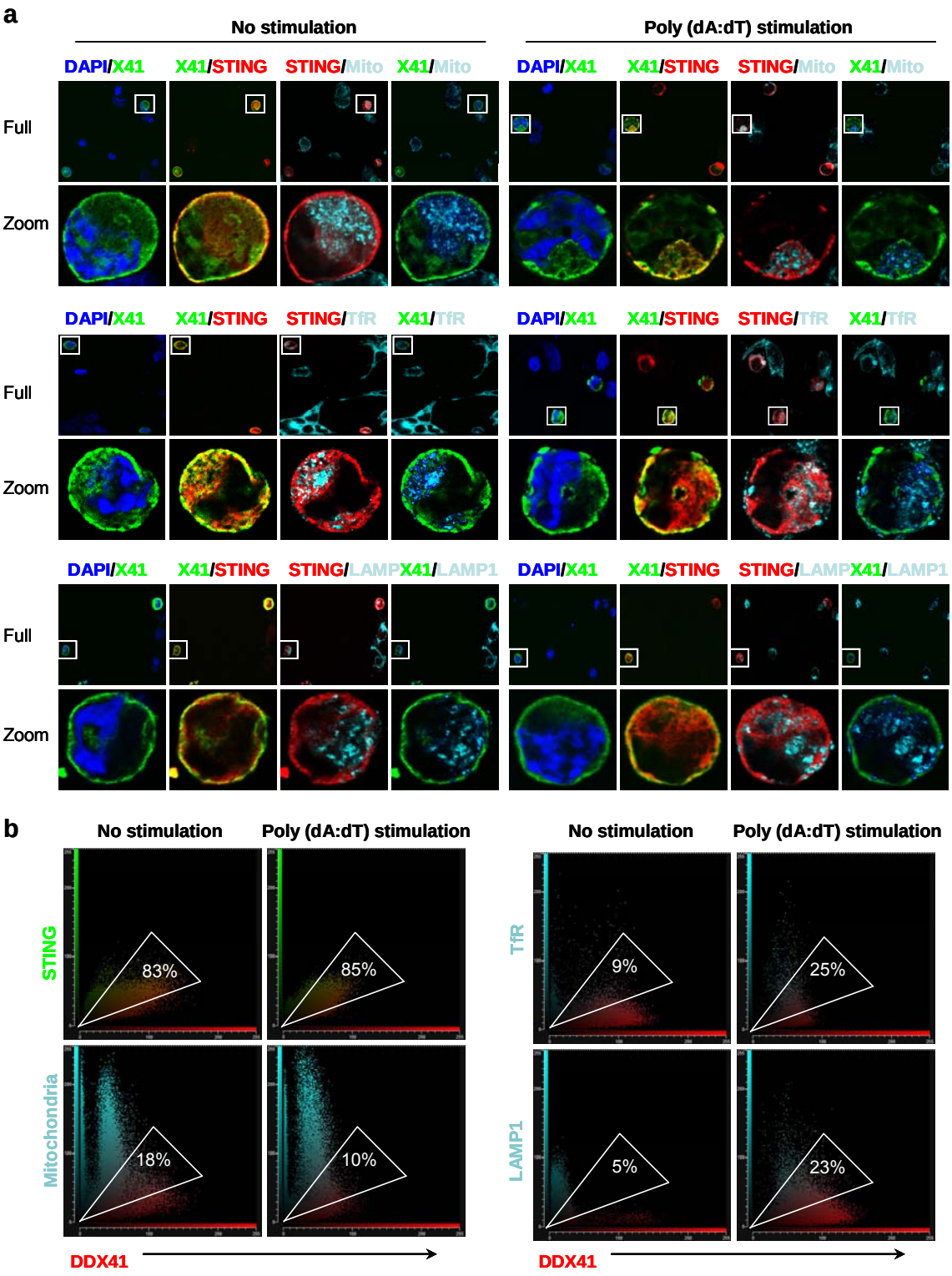


Supplementary Figure 5. Fold activation of IFN-β promoter in HEK293T cells.

HEK293T cells transfected with IFN-β luciferase reporter (100 ng) plus increasing concentrations (20, 100 or 200 ng) of IPS1 expression vector. The Renilla-luciferase reporter gene (2 ng) was transfected simultaneously for the internal control. Data is representative of three independent experiments.



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Supplementary Figure 6. DDX41 translocates to endosomes following poly (dA:dT) activation. (**a**) Confocal image of HEK293T cells co-transfected with HA-STING or Myc-DDX41 expression plasmids. Anti-HA or anti-Myc stained cells without stimulation (left), or stimulated with poly (dA:dT) for 4 h (right). DAPI served as the nuclei marker. MitoTracker was used to probe the mitochondrion. TfR and LAMP1 served as the endosome markers. (**b**) The quantification on the co-expression of DDX41 and STING in different subcellular localization. The images of zoomed single cells in supplemental **Fig. 6a** were quantified using Leica Confocal Software. Data is representative of three independent experiments.

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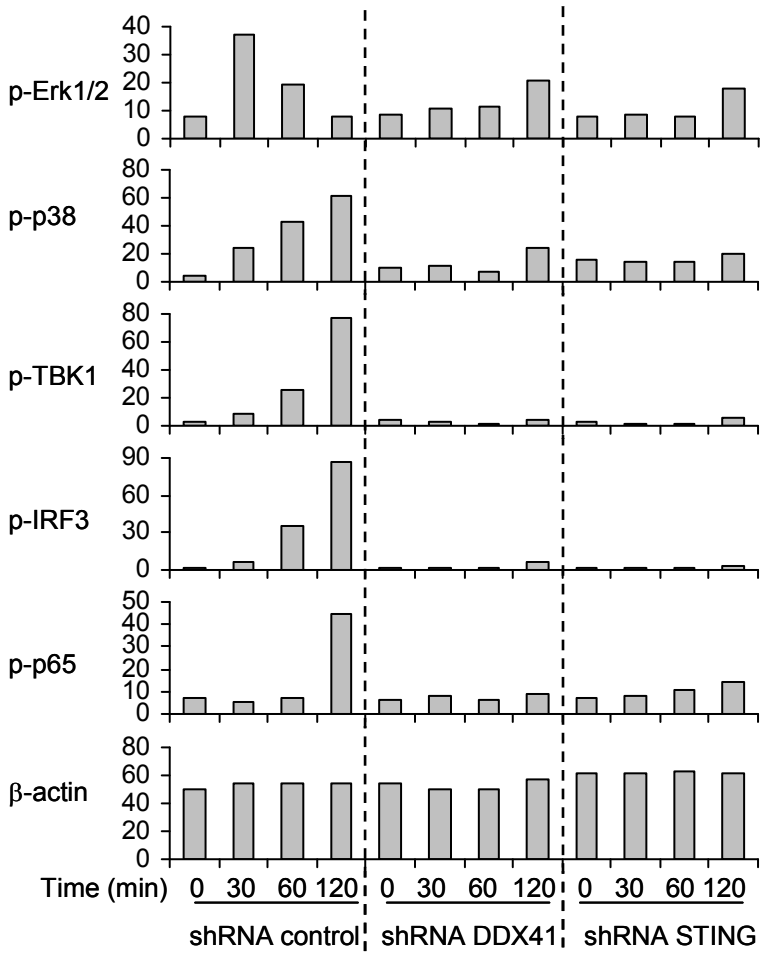

Supplementary Figure 7. The protein amounts shown in the blots of Figure 7b. The protein amounts were quantified by scanning the bands.

SUPPLEMENTARY METHODS

Expression and purification of full-length and truncated DDX41 proteins. The full length and the C-terminus of mouse DDX41 were cloned into the pET-30-(a) expression vector (Novagen) with a His fusion at the N-terminus. Plasmids were transformed into *Escherichia coli* BL21(DE3), and histidine tagged versions of each protein were expressed and then purified on Ni-NTA-agarose according to the manufacturer's instructions (Qiagen).

Chromatin Immunoprecipitation. GM-CSF bone marrow derived mDCs collected on day 7 of culture. Listeria was added to the cells at CFU of 20. Cells washed with PBS after 2 h infection. ChIP assay was performed using EZ-ChIP kit (Millipore). 2×10^6 cells was used for each Immunoprecipitation. Anti-DDX-41 (Santa Cruz, sc-166225, C-3) was used for immunoprecipitation. Mouse IgG was served as the isotype control. The Listeria Genus Primer set (Bioo Scientific Corporation, Cat# 375201) was used to amplify Listeria DNA. These primers are targeting the phosphoribosyl pyrophosphate synthetase (prs) gene (Listeriolysin, Accession # NC_003212) of Listeria. The following primers were used to detect GAPDH (control): 5′-AACTTTGGCATTGTGGAAGG-3′ and 5′-ACACATTGGGGGTAGGAACA-3′. The final PCR products were visualized on 1.5% argarose gel.

Primers for mRNA assay or QPCR. To assay IFN-inducible gene expression, the mRNA abundance of expressed genes was detected with real-time RT-PCR using the

following primers: CXCL10: 5′-GGATGGCTGTCCTAGCTCTG-3′ and 5′-ATAACCCCTTGGGAAGATGG-3′; CCL5: 5′-CCCTCACCATCATCCTCACT-3′ and 5′-CCTTCGAGTGACAAACACGA-3′; MDA5: 5′-GTGCCAATCTTGATGCCTTT-3′ and 5′-GGCCCACTGCTCATAATGTT-3′. ISG15: 5′-AAGCAGCCAGAAGCAGACTC-3′ and 5′-TAAGACCGTCCTGGAGCACT-3′; Ifit1: 5′-AGGCTGGAGTGTGCTGAGAT-3′ and 5′-TCTGGATTTAACCGGACAGC-3′; GAPDH: 5′-AACTTTGGCATTGTGGAAGG-3′ and 5′-ACACATTGGGGGTAGGAACA-3′; IFN-β: 5′-CCCTATGGAGATGACGGAGA-3′ and 5′-TCCCACGTCAATCTTTCCTC-3′.

shRNA knockdown efficiency in BMDCs was detected with real-time RT-PCR using the following primers: DDX41: 5′-CCAGCACCTCAAAGAGAAGG-3′ and 5′-CCTCAGACATGCTCAGGA-3′; STING: 5′-AAATAACTGCCGCCTCATTG-3′ and 5′-TGGGAGAGGCTGATCCATAC-3′; RIG-I: 5′-AGAGCCAGCGGAGATAACAA-3′ and 5′-CCTTGATCATGTTCGCCTT-3′; IPS1: 5′-AGAGCAACTCCTCCAGACCA-3′ and 5′-AACGGTTGGAGACACAGGTC-3′; p204: 5′-ATTCTGGATTGGGCAAACTG-3′ and 5′-CTCTTCCTGGGTTGCAGAAG-3′; GAPDH: 5′-AACTTTGGCATTGTGGAAGG-3′ and 5′-ACACATTGGGGGTAGGAACA-3′.

shRNA knockdown efficiency in human THP1 was detected with real-time RT-PCR using the following primers: DDX41: 5′-AGGCCAAGATGGTGTACCTG-3′ and 5′-AGGCAACGTCTGTGGCTACT-3′; STING: 5′-ACTGTGGGGTGCCTGATAAC-3′ and 5′-TGGCAAACAAAGTCTGCAAG-3′; IFI16: 5′-ATGACCCCAAGAGCATGAAG-3′ and 5′-ATGAACGGTCCTGGAAAATG-3′;

GAPDH: 5′-GAGTCAACGGATTTGGTCGT-3′ and 5′-TTGATTTTGGAGGGATCTCG-3′.

For QPCR assay, the following primers were used:

GAPDH: 5′-AACTTTGGCATTGTGGAAGG-3′ and 5′-ACACATTGGGGGTAGGAACA-3′.

HSV-1 DNA: 5′-TGGGACACATGCCTTCTTGG-3′ and 5′-ACCCTTAGTCAGACTCTGTTACTTACCC-3′

RNA-mediated interference. The following shRNA lentiviral vectors targeting mouse genes were purchased from Open Biosystems: DDX41, catalog number RMM4534-NM_134059, clone TRCN 0000104010, TRCN 0000104011 and TRCN 0000104013; STING, catalog number RMM1766-96890503, clone V2MM_103341; RIG-I, catalog number RMM4534-NM_172689, clone TRCN0000103886; IPS1, catalog number RMM4534-NM_144888, clone TRCN0000124772. The siRNA target 3'-UTR of DDX41 was purchased from Dharmacon. The following shRNA lentiviral vectors targeting human genes were purchased from Open Biosystems: DDX41, catalog number RMM4534-NM_134059, clone TRCN 0000104013 and TRCN 0000104014; IFI16, catalog number RHS4533-NM_005531, clone TRCN0000019079 and TRCN0000019083; STING, catalog number RHS4533-NM_198282, clone TRCN0000163296.