

**Cell Reports**

**Supplemental Information**

**Genome-Derived Cytosolic DNA Mediates  
Type I Interferon-Dependent Rejection  
of B Cell Lymphoma Cells**

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## **Supplemental Figures**

### **Figure S1. Accumulation of Cytosolic DNA in Human Cancer Cells in Response to DNA Damage, Related to Figure 1**

(A-B) The human colon colorectal carcinoma cell line HCT116 (A) and the cervix adenocarcinoma cell line HeLa (B) were treated with DMSO (Upper rows) or 4 µM aphidicolin (Lower rows) for 16 h and stained with ssDNA- (Left panel) or dsDNA-specific (Right panel) antibodies (Red). Cells were co-stained for the mitochondrial marker COX IV (Green) in the presence of DAPI (Blue). Z-stack images were acquired by confocal microscopy and analyzed using Imaris to generate iso-surface plots. Scale bars denote 10 µm. Data are representative of at least three independent stainings. (C) BC2 cells treated with DMSO or 10 µM Ara-C for 16 h were digested with 10 U/ml ssDNA-specific S1 nuclease for 1 h before staining for ssDNA (Red) and COX IV (Green) in the presence of DAPI (Blue). Scale bars denote 20 µm. (D) BC2 cells treated with DMSO or 10 µM Ara-C for 16 h were treated with 10 µg/ml DNase I for 1 h before staining for cytosolic dsDNA (Red) and COX IV (Green) in the presence of DAPI (Blue). Data are representative of at least three independent stainings.

### **Figure S2. Presence of Cytosolic DNA in Yac-1 Cells and MRC5 Cells, Related to Figures 1 and 3**

(A-B) The mouse lymphoma cell line Yac-1 (A) and the human lung fibroblast cell line MRC5 (B) were stained for cytosolic DNA using ssDNA- (Left panel) or dsDNA-specific (Right panel) antibodies (Red). The cells were co-stained

for the mitochondrial marker COX IV (Green) in the presence of DAPI (Blue). (C) Confocal image of A549 cells stained with 3  $\mu$ l/ml of PicoGreen for 1 h and 0.25  $\mu$ g/mL of CellMask for 5 min. Z-stack images were acquired by confocal microscopy and analyzed using Imaris to generate iso-surface plots. (D) Z-stack image of PicoGreen staining (Green) of BC2 cells treated with 10  $\mu$ M Ara-C for 16 h. Cells were co-stained with MitoTracker (Red). White box indicates nuclear DNA that may give rise to cytosolic DNA. Scale bars denote 10  $\mu$ m. Data are representative of at least three independent stainings.

**Figure S3. Extracted Cytosolic DNA does not contain genomic DNA, Related to Figure 4**

(A-B) Two cytosolic DNA fractions from Ara-C-treated BC2 cells used for cloning of cytosolic DNA were amplified for the presence of a mouse *Gapdh* intron (A) and an interleukin-2 exon. (B). 0.5  $\mu$ g of a 1kb DNA ladder (SM0314, Thermo Scientific) (A) or a 100 bp Plus DNA ladder (SM0322, Thermo Scientific) (B) was loaded in the first lane. Non-template PCR reactions served as negative controls ( $H_2O$ ).

**Figure S4. Effects of *Rnaseh1* on cytosolic DNA, Related to Figure 6**

(A) Immunoblot analysis of RNASEH1 protein levels in BC2 cells transduced with a retroviral vector encoding *Gfp* (Ctrl) or *Rnaseh1-IRES-Gfp*. (B) Immunoblot analysis of BC2 transduced with a retroviral vector encoding *Gfp* (Ctrl) or *Rnaseh1-IRES-Gfp* probed with antibodies specific for phospho-H2AX-Ser139 ( $\gamma$ H2AX-P), phospho-p53-Ser15 (p53-P), p53 and GAPDH after treatment with DMSO or 10  $\mu$ M Ara-C for 16 h. (C) EpM1 cells were

transduced with a lentiviral GIPZ non-silencing control shRNA (Ctrl) or *Rnaseh1* shRNA construct. GFP<sup>+</sup> EpM1 cells were analyzed for the presence of cytosolic ssDNA or dsDNA (Red) using specific antibodies in presence of DAPI. (D-E) Quantification of the average intensity of ssDNA (D) or dsDNA (E) stainings in control (White bar) and *Rnaseh1* shRNA transduced (Grey bar) EpM1 cells shown in (C). (F) The relative transcript levels of *Rnaseh1* in *Rnaseh1* shRNA transduced EpM1 cells (Grey bar) were normalized to control shRNA-transduced EpM1 cells (White bar). Error bars indicate  $\pm$ SD. NS= not significant.

**Figure S5. Knockdown of *RNASEH1* and *TREX1*, Related to Figure 6**

(A) A549 (Upper panels) and HeLa (Lower panels) cells were transfected with 24  $\mu$ g *RNASEH1*-specific or control siRNAs. 48 h after transfection, cells were stained for dsDNA (Red) and RNASEH1 (Green) in the presence of DAPI (blue). Quantification of the dsDNA stainings is shown on the right. Scale bars denote 10  $\mu$ m. \* $p<0.05$ ; \*\*\* $p<0.001$ . (B) Immunoblot analysis of TREX1 protein levels in BC2 cells transduced with a retroviral vector encoding *Gfp* (Ctrl) or *Rnaseh1*-IRES-*Gfp*. Data are representative of two independent experiments. (C) HeLa cells transduced with a retroviral vector encoding *Gfp* (CTRL) or *TREX1*-IRES-*Gfp* were treated with DMSO (Upper row) or 4  $\mu$ M aphidicolin (Lower row) for 16 h. The presence of cytosolic ssDNA was analyzed by using ssDNA-specific antibodies (Red) in the presence of DAPI (Blue). (D) Quantification of the average intensity of cytosolic ssDNA stainings in HeLa cells transduced and treated as described in (C). (E) HeLa cells transduced with control shRNA (CTRL) or *TREX1*-specific shRNA were

treated with DMSO (Upper row) or 4 µM aphidicolin (Lower row) for 16 h. The presence of cytosolic ssDNA was analyzed by using ssDNA-specific antibodies (Red) in the presence of DAPI (Blue). (F) Quantification of the average intensity of cytosolic ssDNA stainings in HeLa cells transduced and treated as described in (E). Only cytoplasmic regions were analyzed and calculations were based on at least 100 cells and 4 different areas of the slide. Error bars denote  $\pm$ SD. \*\* $p$ <0.01; \*\*\* $p$ <0.001. Scale bars denote 20 µm.

**Figure S6. Cytosolic DNA Clones Induce Expression of IFN-β, Related to Figure 7**

(A) IFN-β level in the supernatant of murine C57BL/6 fibroblasts 18 h after transfection with 2.5 µg of the indicated cytosolic ssDNA and dsDNA clones derived from Ara-C-treated BC2 cells. IFN-β levels in the supernatant were measured by ELISA 18 h post transfection. Supernatant of mock-transfected murine fibroblasts served as control. Error bars indicate  $\pm$ SD. (B) 50 base pairs of cytosolic DNA sequences present in dsDNA2 and dsDNA3 clones (See supplemental experimental procedures) with the potential to form triple-stranded DNA were annealed with reverse complement DNA or RNA oligonucleotides of the same sequence. 2.5 µg of annealed DNA:DNA and RNA:DNA complexes were transfected into murine C57BL/6 fibroblasts. IFN-β levels in the supernatant were measured by ELISA 18 h post transfection. Supernatant of mock-transfected murine fibroblasts served as control. (C) Immunoblot analysis of RNASEH1 protein levels in EµM1 cells transduced with a retroviral vector encoding *Gfp* (Ctrl) or *Rnaseh1-IRES-Gfp*. (D) HeLa cells transduced with a retroviral vector encoding *Gfp* (CTRL) or *TREX1*-

IRES-*Gfp* were treated with DMSO (Upper row) or 4 µM aphidicolin (Lower row) for 16 h. The relative transcript levels of *IFNA4* (White) and *IFNB* (Grey) in control shRNA and *TREX1* shRNA-transduced HeLa cells after 16 hrs of DMSO or aphidicolin treatment were normalized to control shRNA transduced HeLa cells treated with DMSO. (E) HeLa cells transduced with control shRNA (*CTRL*) or *TREX1*-specific shRNA were treated with DMSO (Upper row) or 4 µM aphidicolin (Lower row) for 16 h. The relative transcript levels of *IFNA4* (White) and *IFNB* (Grey) in control shRNA and *TREX1* shRNA-transduced cells after 16 h of DMSO or aphidicolin treatment were normalized to control shRNA-transduced HeLa cells treated with DMSO. Error bars denote ±SD. \* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ . (F)  $1 \times 10^6$  PKH26-labeled *Rnaseh1*-IRES-*Gfp*-transduced EµM1 cells (Grey column) were mixed with  $1 \times 10^6$  control IRES-*Gfp*-transduced EµM1 cells (White column). 5 µg/ml of blocking INFAR1 antibody or isotype was added to cell cultures. The next day, total cell numbers were counted and the ratio of PKH26<sup>+</sup> to PKH26<sup>-</sup> EµM1 cells was determined by flow cytometry. Cell numbers were normalized to the initial cell numbers. Error bars indicate ±SD. (G) Cells described in (A) were also analyzed for Annexin V expression and propidium iodide (PI) incorporation by flow cytometry. PI or Annexin-V positive cells were considered apoptotic. Data are representative results from two independent transductions. Error bars indicate ±SD. (H) NK cell depletion in the blood (Upper panel), spleen (Middle panel) and bone marrow (Bottom panel) was examined in C57BL/6 mice 24 h after intraperitoneal administration of isotype control antibodies or anti-NK1.1 antibodies (500 µg). Tissue samples were stained for CD3 and DX5

expression and analyzed by flow cytometry. Boxes indicate the electronic gating strategy used to identify NK cells and T cells.

**Figure S7. Schematic diagram of generation and function of cytosolic DNA in tumor cells, Related to Figures 1-7**

Formation of R-loops in the genome of tumor cells lead to the presence of DNA in the cytosol of B-cell lymphomas. Recognition of cytosolic DNA by innate sensor pathways induce the expression of type I interferon and potentially other immunomodulatory molecules, which contribute to the rejection of B-cell lymphomas.

**Movie S1. Presence of cytosolic DNA in Ara-C-treated BC2 cells, Related to Figure 1**

Z-stack image of PicoGreen staining (Green) of BC2 cells treated with 10  $\mu$ M Ara-C for 16 h. Cells were stained with 3  $\mu$ l/ml PicoGreen and 100 nM MitoTracker (Red) for 1 h. Z-stack images were acquired by confocal microscopy and analyzed using Imaris to generate iso-surface plots.

**Movie S2. Cytosolic dsDNA transiently co-localizes with lysosomes, Related to Figure 2**

Live cell imaging of A549 cells stained with 3  $\mu$ l/ml PicoGreen (Green) for 1 h and 50 nM Lysotracker (Red) for 30 min before taking pictures every 9 sec.

**Movie S3. Cytosolic dsDNA is partially associated with mitochondria, Related to Figure 3**

Live cell imaging of A549 cells stained with 3  $\mu$ l/ml PicoGreen (Green) for 1 h and 100 nM MitoTracker (Red) for 30 min before taking pictures every 9 sec.

**Movie S4. Cytosolic ssDNA is partially associated with mitochondria Related to Figure 3**

Live cell imaging of A549 cells stained with 3  $\mu$ l/ml OliGreen staining (Green), 100 nM MitoTracker (Red) and 1  $\mu$ g/ml Hoechst (Blue) for 1 h before taking pictures every 9 sec.

**Movies S5. Aphidicolin induces the increased presence of cytosolic dsDNA in HeLa cells, Related to Figure 3**

Live cell imaging of HeLa cells treated with 4  $\mu$ M aphidicolin for 16 h. Cells were stained with 3  $\mu$ l/ml PicoGreen (Green) for 1 h and 100 nM MitoTracker (Red) for 30 min before taking pictures every 9 sec.

**Movies S6. Cytosolic dsDNA is partially associated with mitochondria in DMSO-treated HeLa cells, Related to Figure 3**

Live cell imaging of DMSO treated HeLa cells stained with 3  $\mu$ l/ml PicoGreen (Green) for 1 h and 100 nM MitoTracker (Red) for 30 min before taking pictures every 9 sec.

**Movie S7. Cytosolic dsDNA in HeLa cells after recovery from aphidicolin treatment, Related to Figure 3**

Live cell imaging of HeLa cells treated with 4  $\mu$ M aphidicolin for 16 h followed by another 16 h culture in fresh medium. Cells were stained with 3  $\mu$ l/ml

PicoGreen (Green) for 1 h and 100 nM MitoTracker (Red) for 30 min before taking pictures every 9 sec.

## Supplemental Experimental Procedures

### Primers and PCR

The following primers were used: *Hprt*-5': tgggaggccatcacattgt; *Hprt*-3': gctttccagttcaactaatgaca; *Ifna4*-5': agtgaccagcatctacaagacc; *Ifna4*-3': gaggcaggtcacatcctagag; *Ifnb*-5': aatttctccagcactgggtg; *Ifnb*-3': tctcccacgtcaatcttcc; *GAPDH*-5': gccagttagctcccggtca, *GAPDH*-3': atcaccatctccaggagcga; *TREX1*-5': ctaggaccaagccaagacca, *TREX1*-3': aagatccttgttacccctgc; *IFNA4*-5': agaggccgaagttcaaggta, *IFNA4*-3': tgtgggtctgaggcagatca; *IFNB*-5': aaactcatgagcagtctgca, *IFNB*-3': aggagatctcagttcggagg.

Total RNA was isolated using the RNeasy kit (Qiagen). PCR assays were performed using an ABI GeneAmp system 2700. 2 µg total RNA were reverse transcribed using a M-MLV reverse transcriptase according to the manufacturer's instructions (Promega). Mouse *Rnaseh1* and human *TREX1* were amplified from reverse-transcribed cDNA using Phusion high-fidelity DNA polymerase (Thermo Fisher Scientific) with the following primers: *Rnaseh1*-5': 5'-atgcgcggctgctgccgtgtccgcacagt-3'; *Rnaseh1*-3': 5'-tcagtccctcagactgctcgctccgtg-3'; *TREX1*-5': 5'- atggccctggagctcgacaca-3'; *TREX1*-3': 5'- ctactccccaggtgtggccaggata-3'. PCR thermocycling parameters were 98°C for 30 sec, 30 cycles of 98°C for 10 sec, 58°C for 15 sec and 72°C for 1 min with final extension at 72°C for 10 min. Amplified PCR product was sequenced (1st Base) and cloned into retroviral MSCV 2.2

construct containing IRES-GFP (MSCV-GFP). The MCV-GFP vector was used as negative control. Transfection and transduction were performed as described in (Diefenbach et al., 2003).

### **Generation and maintenance of primary murine fibroblasts**

Ears and tails of C57BL/6 mice were harvested and cut into smaller pieces. For tissue dissociation and cell isolation, the pieces were incubated for 90 min with shaking at 37°C in a cocktail of RPMI 1640 medium (Invitrogen, Singapore) supplemented with 5 % FCS (Invitrogen, Singapore), 2.5 mg/ml of collagenase D (Roche, Singapore) and 450 U/ml of pronase (Merck Millipore, Germany). The cells were then centrifuged at 1700 rpm for 7 min at 4°C and cultured in RPMI 1640 medium (Invitrogen, Singapore) supplemented with 5% heat inactivated FCS (Invitrogen, Singapore), 150 µM β-mercaptoethanol (Sigma, Singapore), 1.4 µM of L-glutamine (Sigma, Singapore), 20 U/ml of penicillin-streptomycin (Invitrogen, Singapore) and 20 mM of HEPES (Hyclone, USA). The isolated primary fibroblasts were grown in culture for 12 - 24 days before being used for experiments.

### **Cloning of cytosolic DNA**

Briefly, live cells were harvested after Ficoll-paque (GE healthcare, Singapore) isolation and washed with PBS. Cytoplasmic fraction were extracted in extraction buffer containing 50 mM HEPES, 150 mM NaCl, 10% glycerol, 1 mM DTT, 2 mM pH 8.0 EDTA and 2.5% digitonin. Cleared extracts were treated with 1 mg/ml proteinase K (Promega) for 1 h at 55°C, extracted with phenol:chloroform, treated with RNase A (1 mg/ml) for 1.5 h at 37°C,

extracted sequentially with phenol:chloroform and chloroform. DNA was precipitated by adding 1/10 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of cold 100% ethanol followed by incubation at -80°C for 30 min and centrifugation for 15 min at 12 x 10<sup>3</sup> rpm in a bench-top centrifuge. The pellet was washed with 70% ethanol, air-dried and resuspended in water. Purified cytosolic DNA was amplified for an intron of mouse *Gapdh* gene and C57BL/6 genotyping internal control primers to exclude the possibility of genomic DNA contamination. Primers used are as follows: *Gapdh-5'*: AATCTTGAGTGAGTTGTCA; *Gapdh-3'*: CGTATTGGCGCCTGGTCA; oIMR7338-5': CTAGGCCACAGAATTGAAAGATCT; oIMR7339-3': GTAGGTGGAAATTCTAGCATCATCC.

For dsDNA cloning, purified cytosolic DNA was treated with DNA polymerase I, Large (Klenow) fragment (1 U/μg DNA; NEB) supplemented with 33 μM of each dNTP to blunt DNA. Blunted DNA fragments were precipitated by ethanol as described above. 3'-A-overhangs were added to DNA fragments by using standard *Taq* polymerase (NEB). dsDNA fragments were cloned into pCR4-TOPO vector according to the manufacturer's instructions (Invitrogen). Cytosolic ssDNA was cloned as described in ref. (Stetson et al., 2008).

### **Analysis of Cloned Cytosolic DNA**

Sequences were mapped to the mouse genome using the BLASTN search function of Ensembl (<http://www.ensembl.org>) deselecting the RepeatMasker option. Sequences were analyzed for the presence of endogenous retroelements using RepeatMasker (<http://www.repeatmasker.org>). The triplex

search tool (<http://helix.fi.muni.cz/triplex/www>) and the non-B DNA database (<https://nonb-abcc.ncifcrf.gov/apps/nBMST/default>) was used for the analysis of non-B DNA motifs.

### **Fluorescence in situ hybridization**

Briefly, cytospins were prepared and cells were fixed in 4% paraformaldehyde for 10 min at room temperature. After fixation, cells were washed twice in PBS and permeabilized in 0.2% Triton X-100 for 10 min at room temperature. Cells were washed twice in PBS before treatment with 0.1 mg/ml DNase I-free RNase A (Sigma) for 90 min at 37°C followed by washing twice in PBS. Biotin-14-dCTP (Invitrogen) was incorporated into DNA fragments by using standard *Taq* polymerase (New England Biolabs (NEB), USA) supplemented with 200 µM of each dATP, dGTP, dTTP, 40 µM dCTP and 160 µM biotin-14-dCTP.

2.5 ng/µl biotinylated cloned DNA probes #1 (5'-  
cttataagagttgcatggatgttcacagccatagaaatcctaacaaggactgacaaaata  
agattcctgccaaggaagtggcattcagagtacacacgcatacctcccagagactcctgtgcattctgg  
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tttagacacacctggagtggagctgctctgcatgttctggactggctaaggagttggcccaaggctgct  
aggc-3') or biotinylated *gfp* DNA negative control probe (5'-  
atggtagcaagggcgaggagctgttaccgggggtggccatcctggcgagctggacggcgacgta  
aacggccacaagttcagcgttccggcgagggcgagggcgatgccacctacggcaagctgaccctgaa  
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ctgaggcaagaccccaacgagaagcgcgatcacatggcctgctggagttcgtgaccggccggatc  
actctcggcatggacgagctgtacaagtaatag-3') were dissolved in hybridization buffer  
(0.2% BSA, 2 x sodium citrate buffer, 10% dextran sulfate, 50% formamide  
with 0.5 µg/µl competitor salmon sperm DNA (Invitrogen) and denatured by

heating at 65°C for 10 min and then briefly transferred to ice. Meanwhile slides were denatured in a solution of pre-warmed 70% formamide/2 x SSC in 79°C water bath for 2.5 min and probes were spotted immediately on the denatured cell pellet. Slides were overlaid and sealed with one piece of parafilm (Sigma), then left to hybridize overnight at 37°C in a dark and humidified hybridization oven (Labnet, Singapore). Next day slides were rinsed in descending concentrations of SSC at 37°C. After washing the slides were equilibrated in 4 x SSC for 1-2 min. Slides were incubated with streptavidin-HRP conjugate (Invitrogen) and signals were amplified by using TSA Plus Cyanine 3 System as instructed by the manufacturer (Perkin Elmer, Singapore). Finally, samples were rinsed in PBS, counterstained with 0.5 µg/ml DAPI for 10 min, washed once in PBS and mounted in fluorescence mounting medium (Dako). Images were taken on a Zeiss Axio Imager Z1 fluorescent microscope equipped with AxioVision 4.8 software (Carl Zeiss MicroImaging) using a 100 x oil immersion objective.

### **Western blotting**

Whole cell extracts were prepared and electrophoresed in 12% SDS-PAGE gels, and blotted onto nitrocellulose membranes. Antibodies specific for phospho-H2AX-Ser139 ( $\gamma$ H2AX-P), phospho-p53-Ser15 (p53-P-Ser15), p53,  $\beta$ -tubulin (216) (Cell Signaling Technology, USA), Trex1 (611987, BD Biosciences), RNase H1 (C-18) (sc-30319, Santa Cruz) and GAPDH (Sigma) and horseradish peroxidase-coupled second stage reagents (Santa Cruz) were used to develop the blots (Thermo Fisher Scientific). Blots were exposed on X-ray film (Thermo Fisher Scientific).

## ELISA

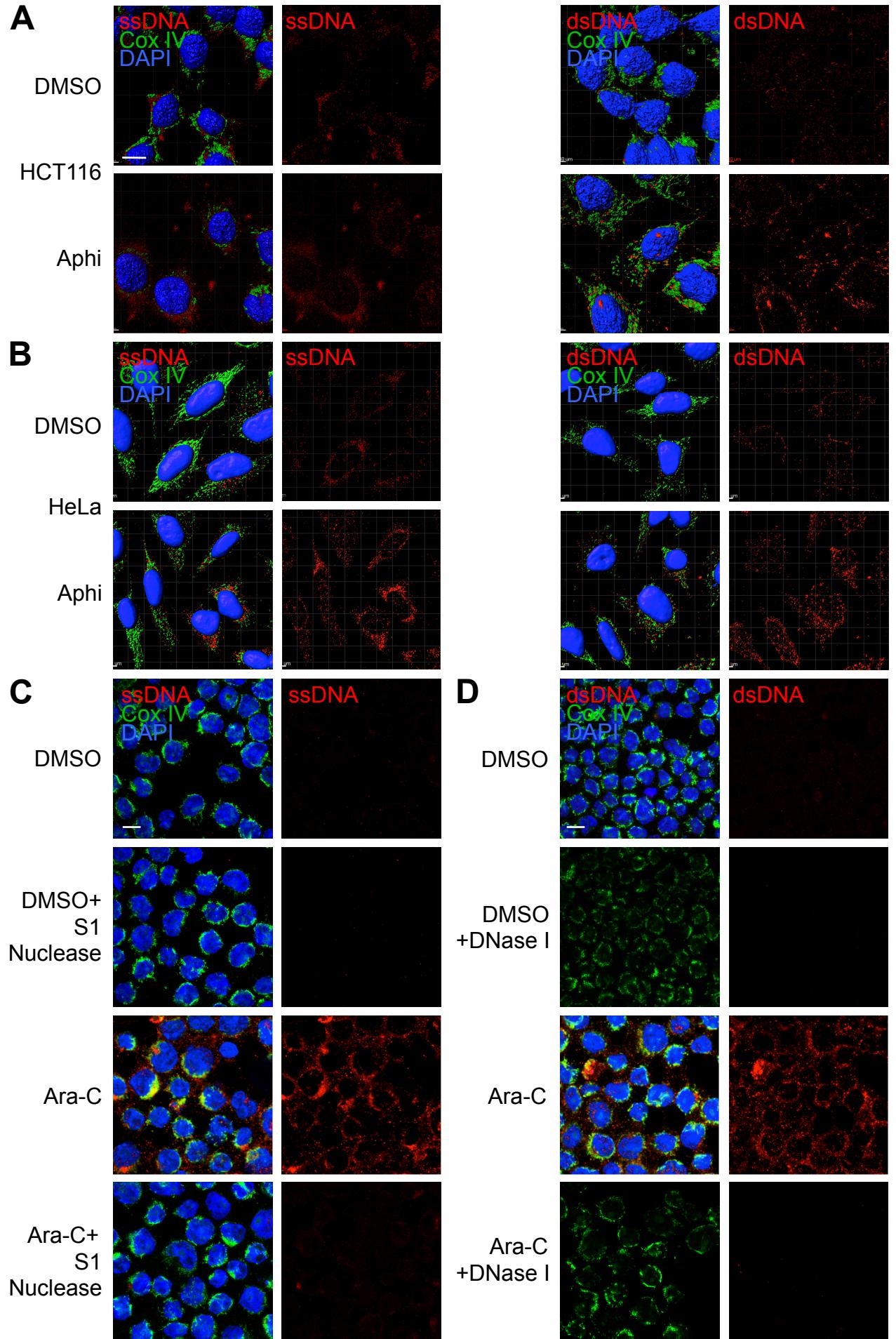
1 x 10<sup>6</sup> IRES-*Gfp*- and *Rnaseh1*-IRES-*Gfp*-transduced EμM1 cells were seeded in a 24-well plate, supernatant were harvested 24 h later. Primary murine fibroblasts were transfected with 2.5 μg of ssDNA, cytosolic clones dsDNA1, dsDNA2 and dsDNA5 derived from Ara-C-treated BC2 cells using Lipofectamine 2000 according to the manufacturer's instructions (Life Technologies, Singapore). ssDNA: TACAGATCTACTAGTGATCTATGACT GATCTGTACATGATCTACA; dsDNA1: ATACCAGCCATAGAAGAAACAC TAGTCCACAACACAGGGTTGCGCCAATCAGGCCACTCCCACCCACTCT TTAAAATGTGAGAATGCAGAGGACATCTAGGAAGAAAGTCCTTGATTCC ATCGATCAATGATGGTCCACACCGCGTAGAATTATCGTCATCACTGCTG TACGTAACGCCCTCAAGCTTTCGTCAAATCCTCAGACCAGTTCCGTAGA AGAAACAAAGACAATTCTTAGATAGATTAGCTGCATGTGTCCAATTTCA TATTGGACACTAGTAATAACATAAGGCTCCTGATTCTTATCAC; dsDNA2: A TATCCTTGTCTCTAATAGTTAATTGGTACTAATATTACATGGCACAAGA AATCTTGTGAACATATATTCTATCATTCCATAAGCCCCTTCCCTTAAAT TCTTATATAGTCTAATAGTAATATTATTAAATATTATTGGCATATATTGG TTTATGCTAAATGAAAAATTATTTGTGTTATATATTGTTCCCTTAAGG GTGTTCCATTGGAAACACTGAGCAGCTAGGAAAAGCTGTATCTTCA GAAGAACCCACTGGCAT; dsDNA3: GGTCCAGCTTGTGTTGATACCGA AACTACAGCTGGACCATTGTCTTTAGGGTGGAGATTAGCGCTGTTCT GAGGAGGACCCATAGCTACTGGTAAGCTCACAGAAGGAAAGAAGTGTGT AGCTGGAGCAACTGGGAATACAGTGTTCAGAGCCCTCCTCCTCCCC GGTTCCCAATGATAAAAGAAAGCAAAC TGCA GCCGGCTGC

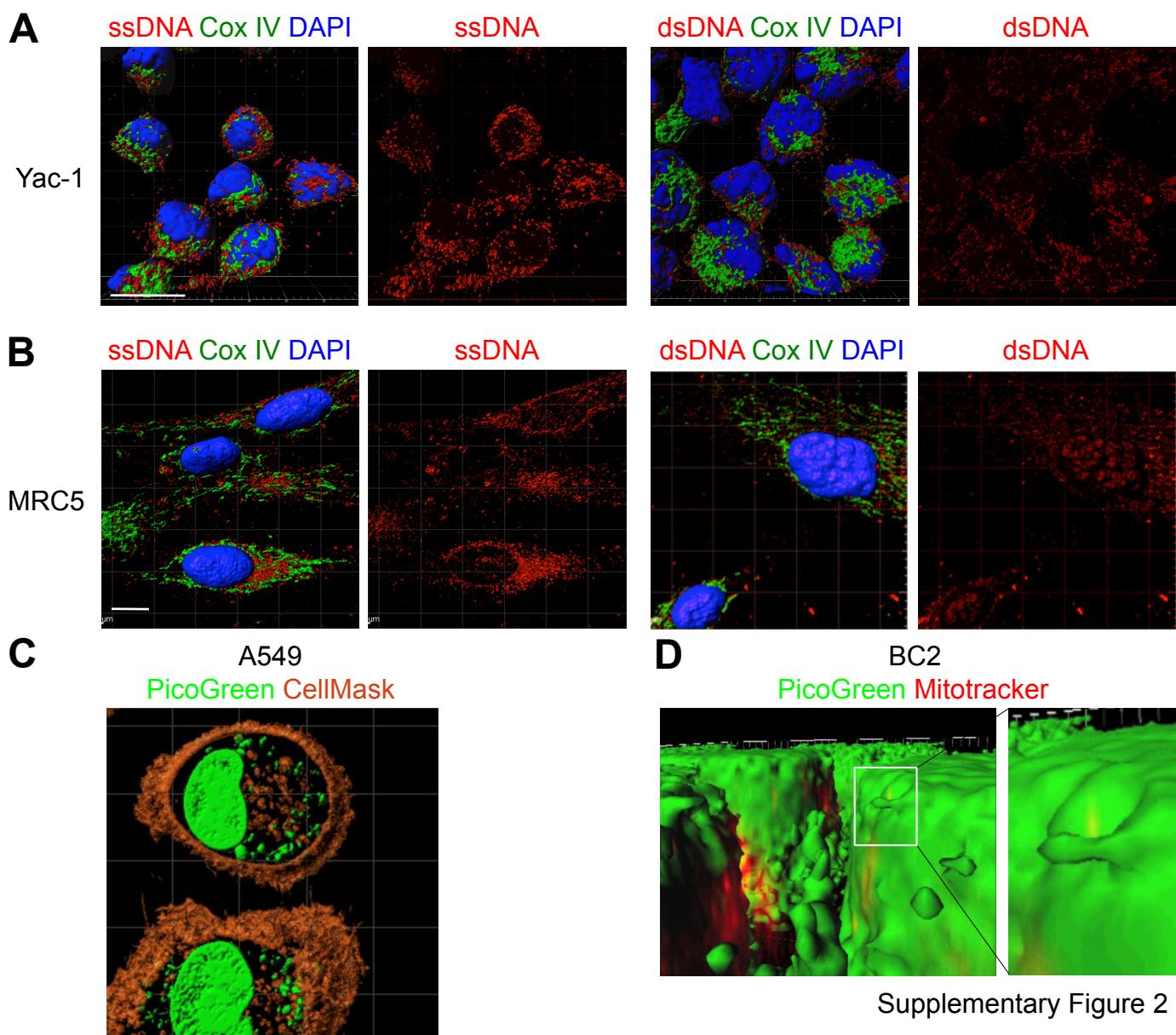
TAGGAATACAGCCTGGAGAGCTGCGATGCTCAGAAGCCCTCAGTCCTGT  
CCAAGATACTAAAGAAATTCTATCGCTGATGTTGCCTGGAGTTT  
ACGAGCCT; dsDNA5: GTCCCCAATAAGAATTTCCTCATGAGAGTTG  
CATTGGTCATGGTGTCTCTAACAGCAATGAAGCCCTAAGTAAGATAGAT  
TTTTTGTTACCAGGGACTTGGTATTCTGTGATAGGCCTGACCATGCTT  
GTTTCTGTTGGAGGAATGTGGAATTGGGGAC. Fibroblasts were also  
transfected with 2.5 µg of dsDNA and RNA:DNA oligonucleotides highlighted  
in green in dsDNA2 and dsDNA3. DNA oligonucleotides shown in green and  
the corresponding reverse complement DNA and RNA oligonucleotides were  
ordered from 1<sup>st</sup> Base (Singapore). Supernatants were harvested 18 h later  
and IFN-β concentrations in the supernatant were measured according to the  
manufacturer's instructions (PBL Interferon source).

### **siRNA experiments**

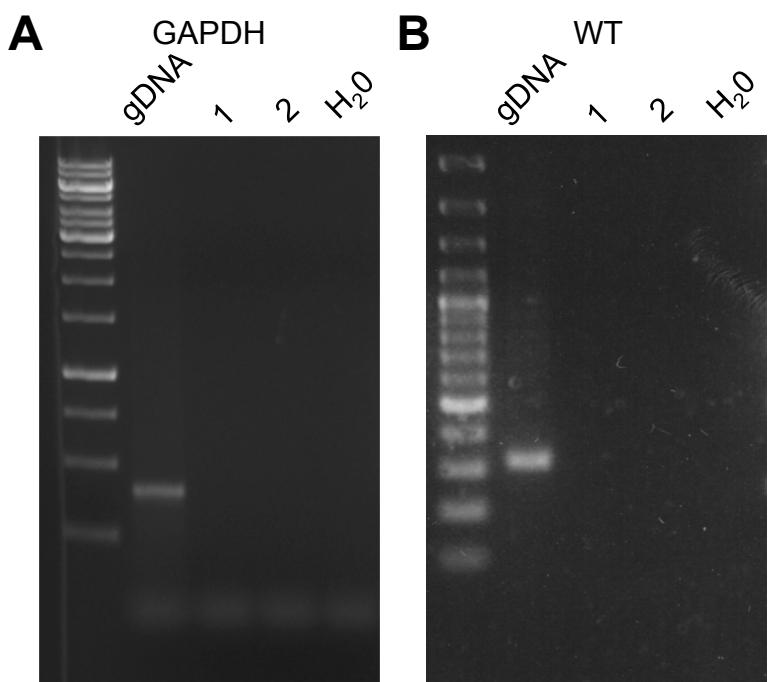
A549 and HeLa cells were transfected with 24 µg RNASEH1-specific siRNA  
or control siRNA using Tranfection (BioRad) according to the manufacturer's  
instructions. The following siRNA constructs were used RNASEH1#1:  
GACCGGUUUCCUGCUGCCAGAUUA[dT][dT]; RNASEH1#2: CCGGAAGU  
UUCAGAAGGGCAUGAAA[dT][dT]; and the negative control SIC001 (Sigma).  
48 hours after tranfections cells were stained for dsDNA and RNASEH1 as  
described in ref. (Lam et al., 2014). A RNASEH1-specific antibody (C-18,  
Santa Cruz) and the anti-goat IgG-Alexa488 antibody (Jackson  
ImmunoResearch) were used for the RNASEH1 stainings.

Supplementary Figure 1

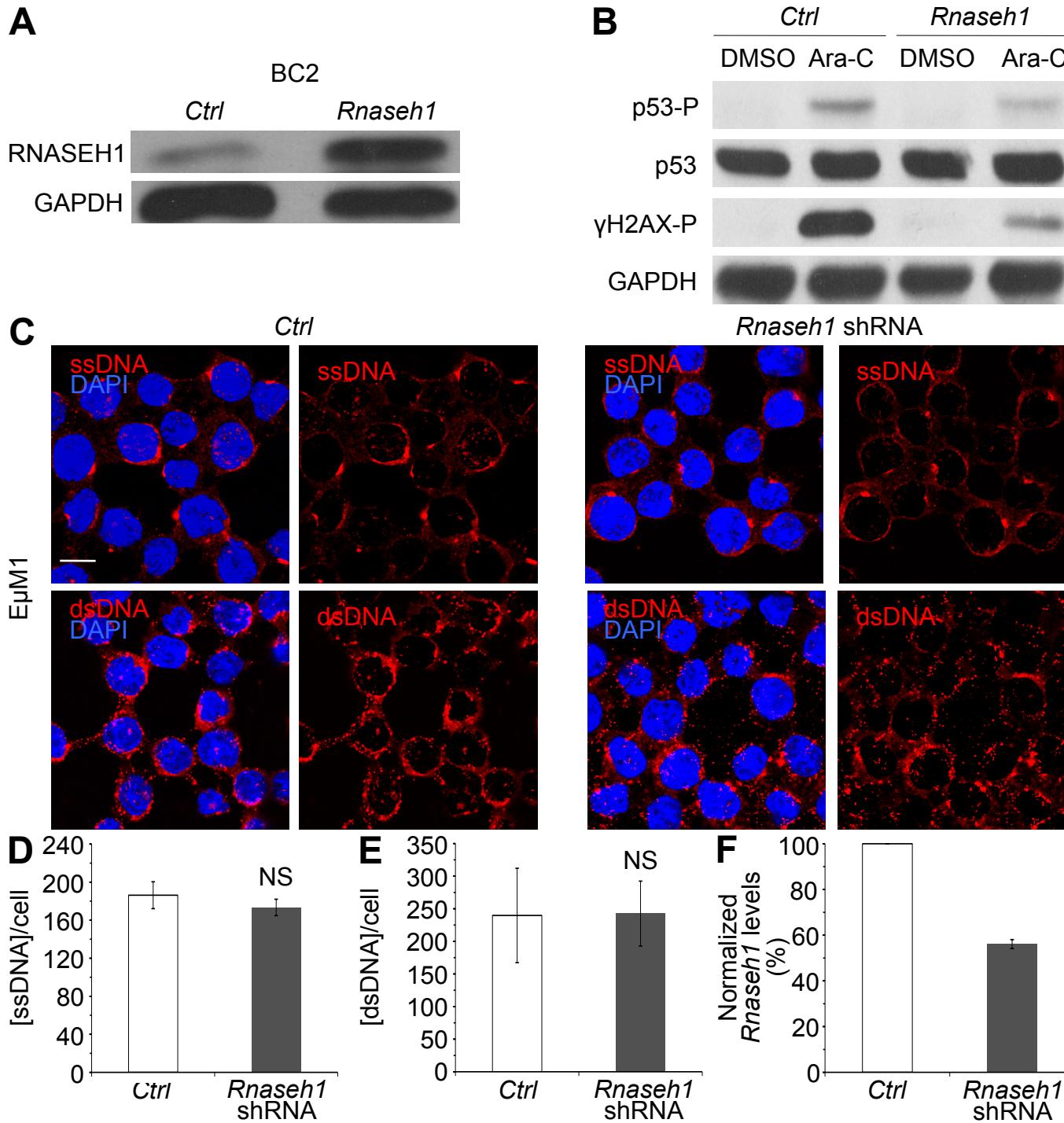




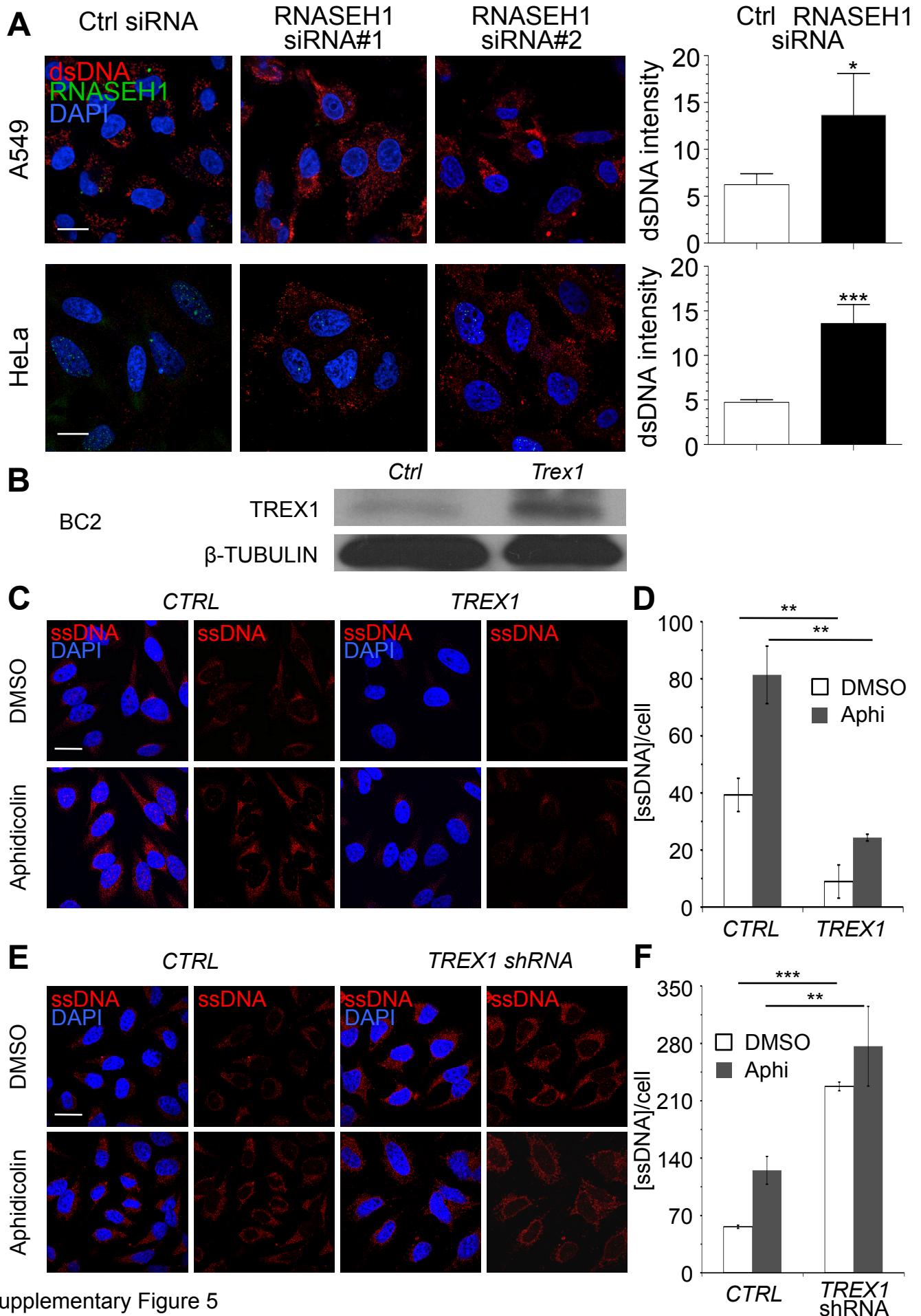
Supplementary Figure 2



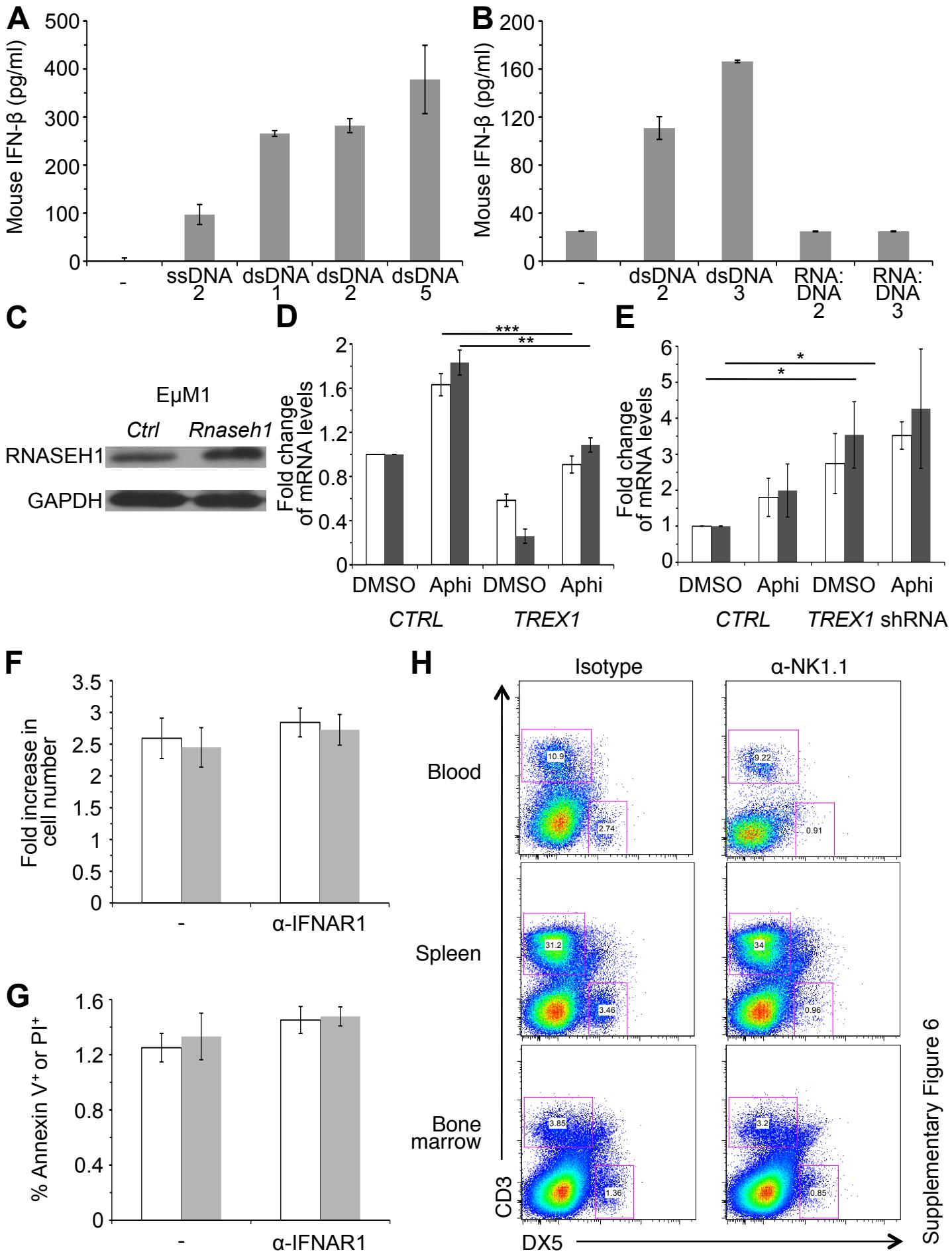
Supplementary Figure 3

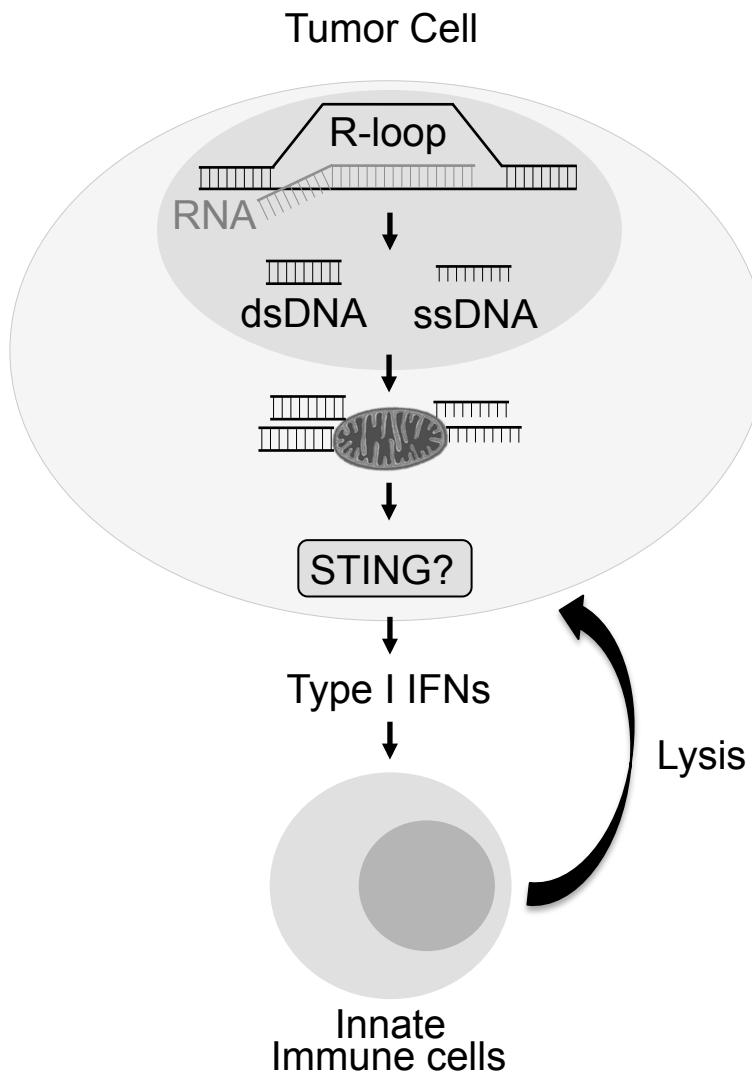


Supplementary Figure 4



Supplementary Figure 5





Supplementary Figure 7