SUPPLEMENTARY INFORMATION

Title: Nucleic acid sensing activates the innate cytosolic surveillance pathway and promotes parasite survival in visceral leishmaniasis

Authors: Sushmita Das[1]*, Ashish Kumar[2], Abhishek Mandal[3], Kumar Abhishek[3], Sudha Verma[3], Ajay Kumar[3], Pradeep Das[3].

Authors affiliations:

[1] Department of Microbiology, All-India Institute of Medical Sciences (AIIMS), Patna, India.

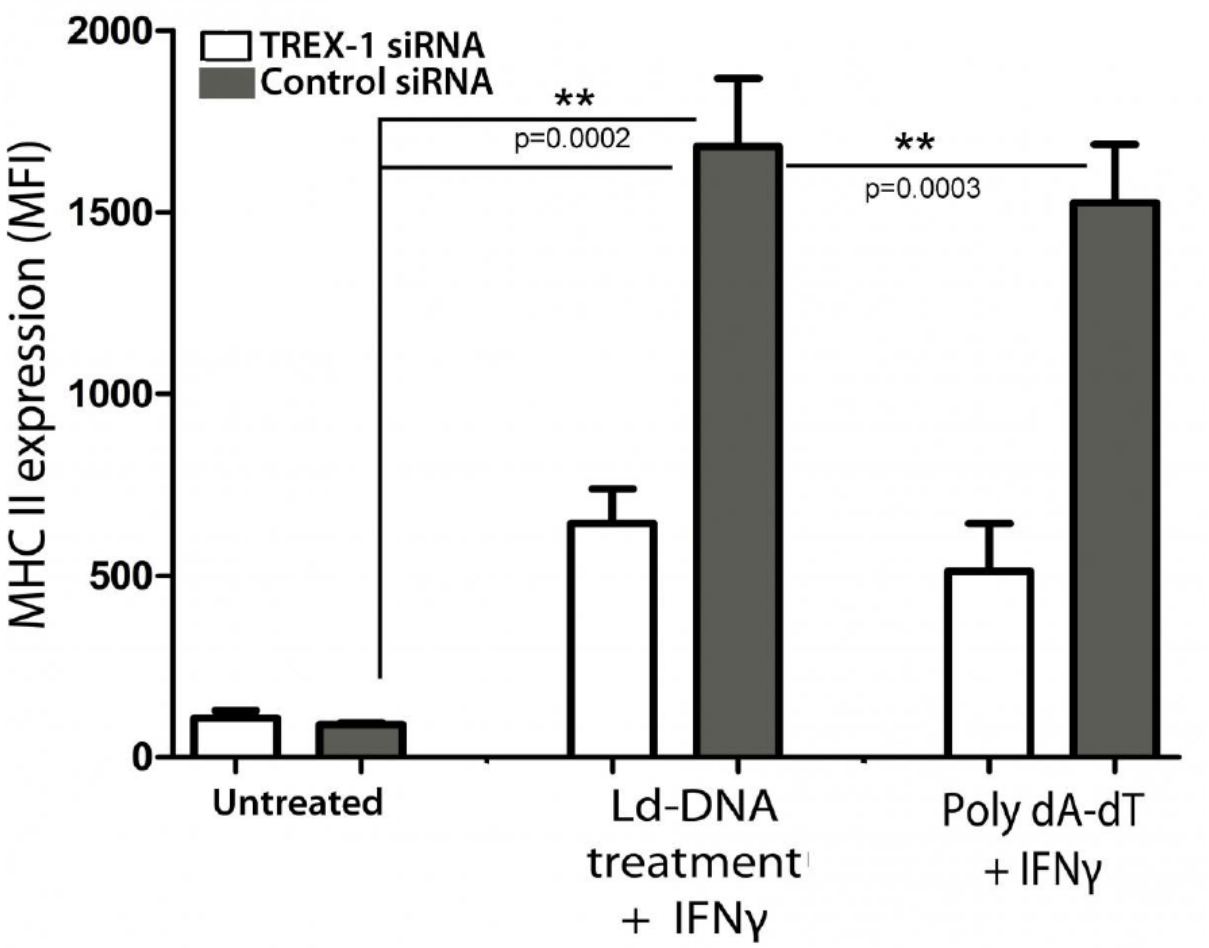
[2] Department of Biochemistry, ICMR-Rajendra Memorial Research Institute of Medical Sciences, Patna, India.

[3]Department of Molecular biology, ICMR-Rajendra Memorial Research Institute of Medical Sciences, Patna, India.

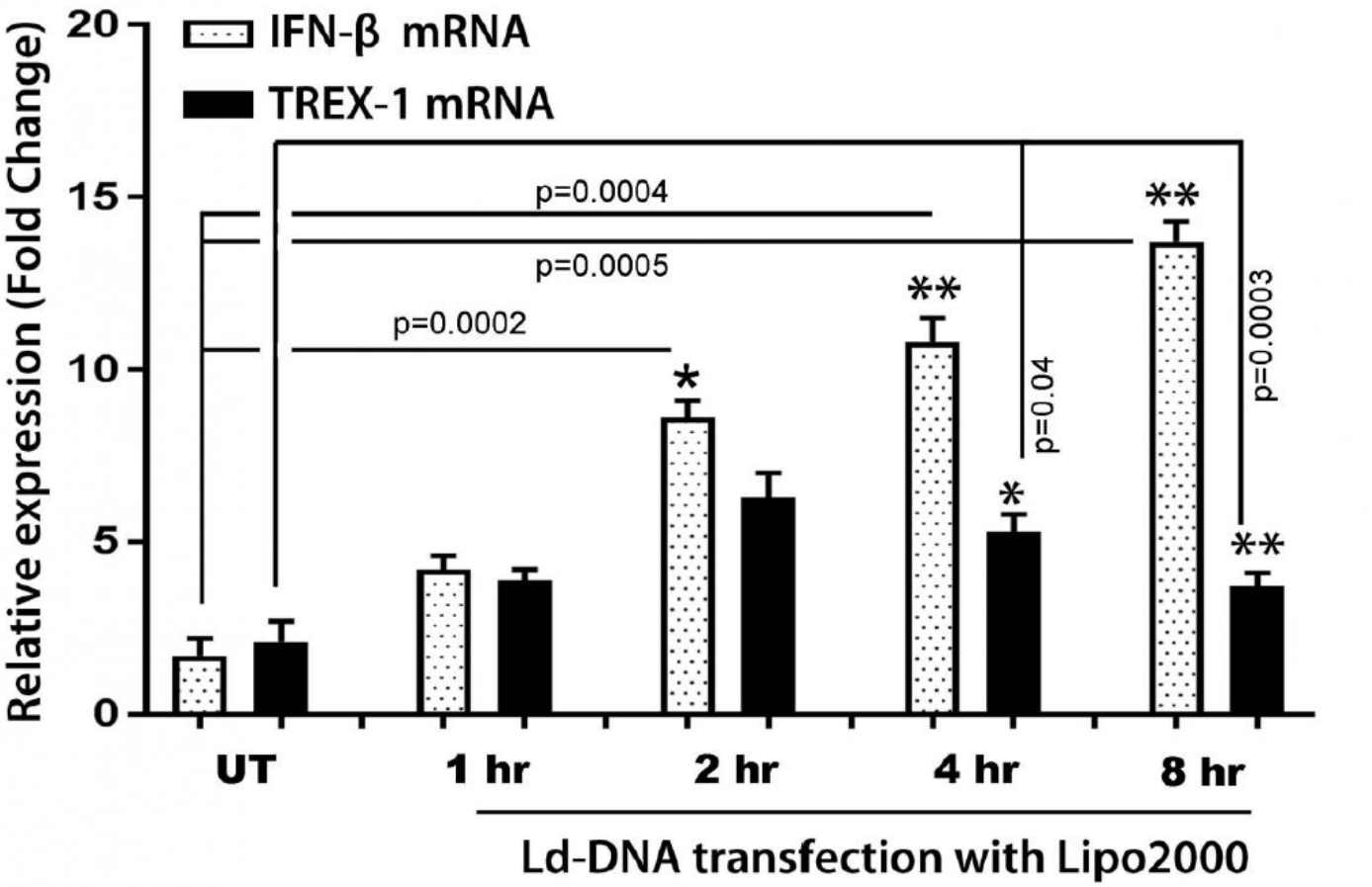
Running title: Cytosolic DNA sensing in Leishmaniasis

Correspondence to:

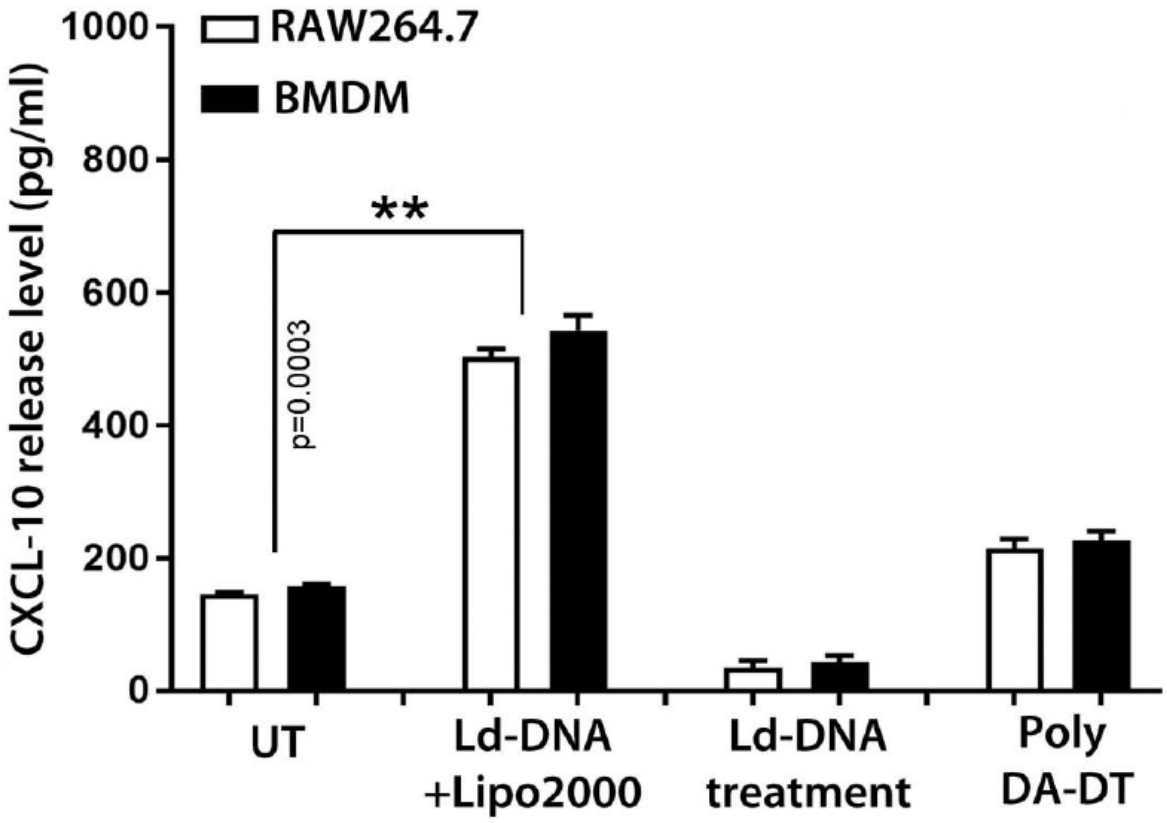
*Dr. Sushmita Das, Associate Professor, Department of Microbiology, All-India Institute of Medical Sciences (A.I.I.M.S.), Patna, India; Phone: 91-612-2631565; Facsimile: 91-612-2634379; E-mail: sushmita.de2008@gmail.com



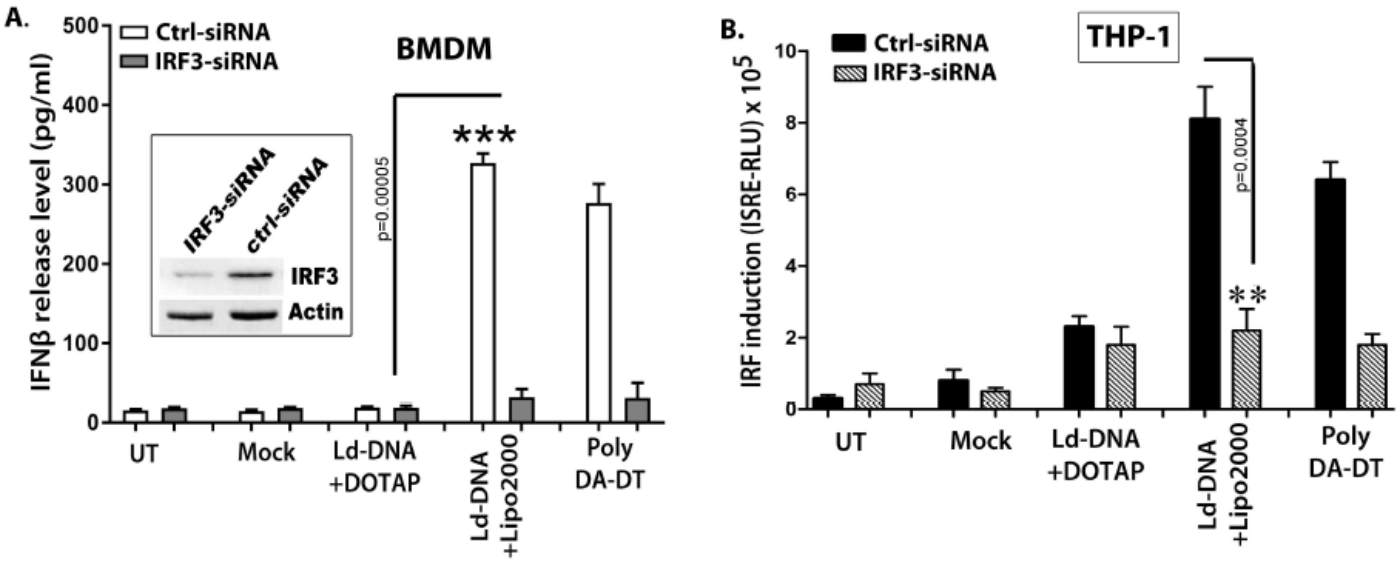
Supplementary figure 1: Human THP-1 cells were pre-transfected with targeted TREX-1 siRNA or non-targeted (NT) control siRNA (E). In both sets, cells were either mock transfected or Ld-DNA transfected [with DOTAP or Lipofectamine 2000] or transfected with dsDNA control Poly(dA-dT); 2 hrs prior to the addition of fresh media containing no (0=UT) or 100U/ml IFN-γ treatment for 30 mins. Mean intensity of three independent experiments are presented.



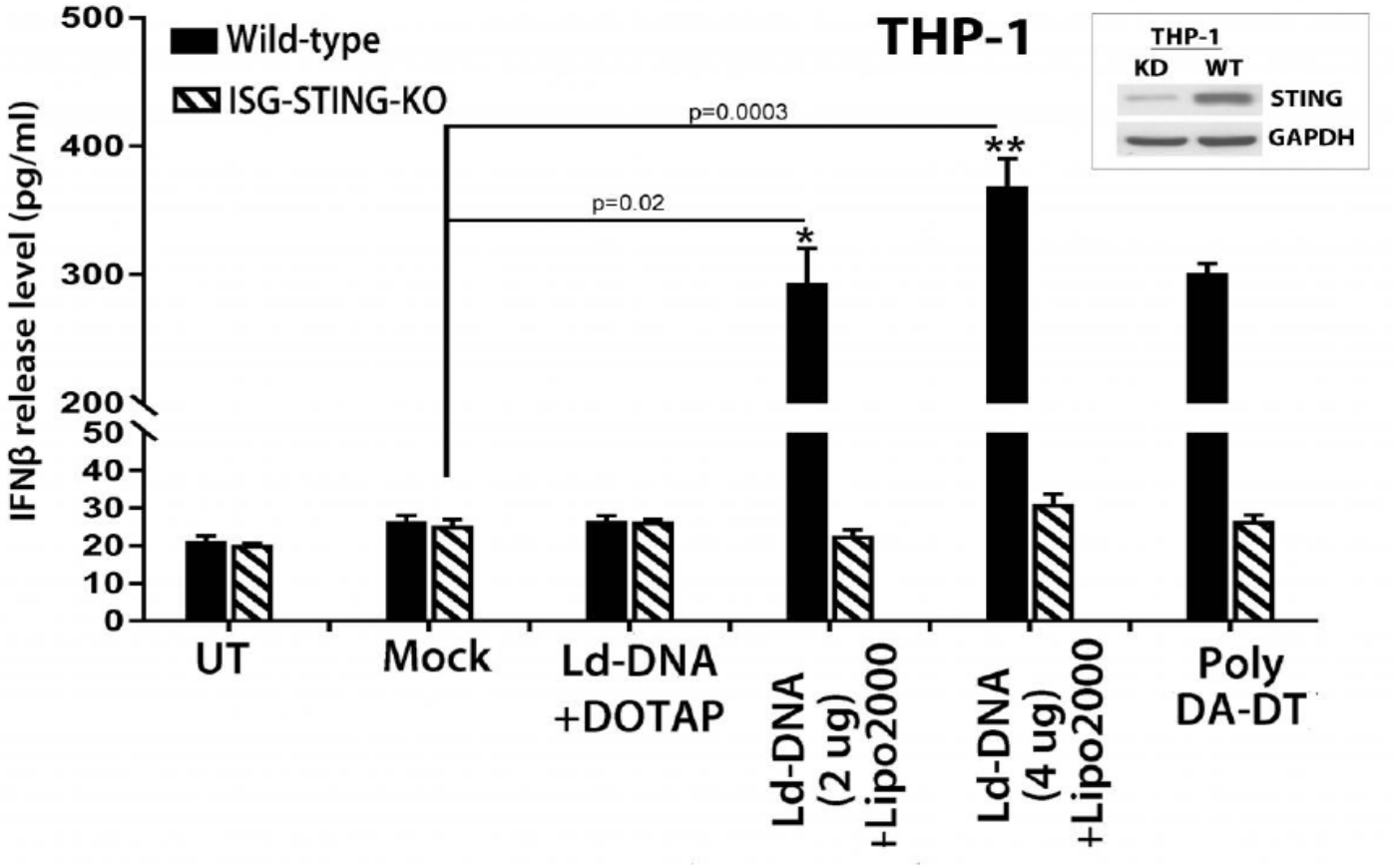
Supplementary figure 2: Mouse RAW264.7 cells were pre-transfected with Ld-DNA [2.5 ug/ml with DOTAP or Lipofectamine 2000] for different indicated time points or left untreated. Total RNA were extracted and relative transcript levels of IFNβ was analyzed by qRT-PCR. Mean of relative expression (fold change) from three sets of experiments were determined and normalized to GAPDH expression.



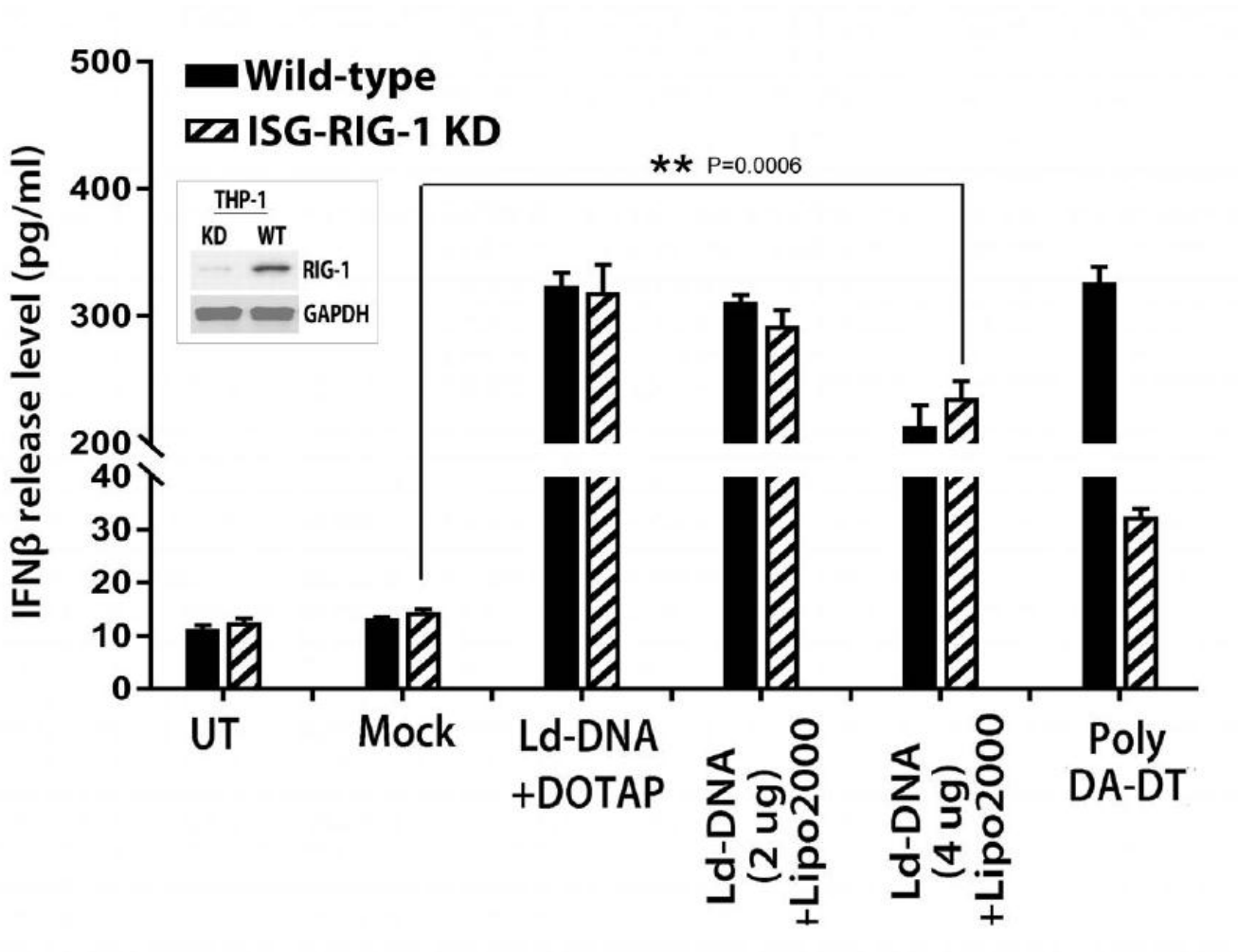
Supplementary figure 3: In two parallel sets of experiment, either mouse RAW264.7 or BALB/c mouse BMDM cells were pre-transfected with either mock transfected (UT) or Ld-DNA transfected [2.5 ug/ml with DOTAP or Lipofectamine 2000] or treated with 2.5 ug/ml Ld-DNA or transfected with double-stranded (ds) DNA Poly(dA-dT) as positive control for indicated time periods [4 hrs]. Production of CXCL-10 was estimated at the release level in the culture supernatant by ELISA.



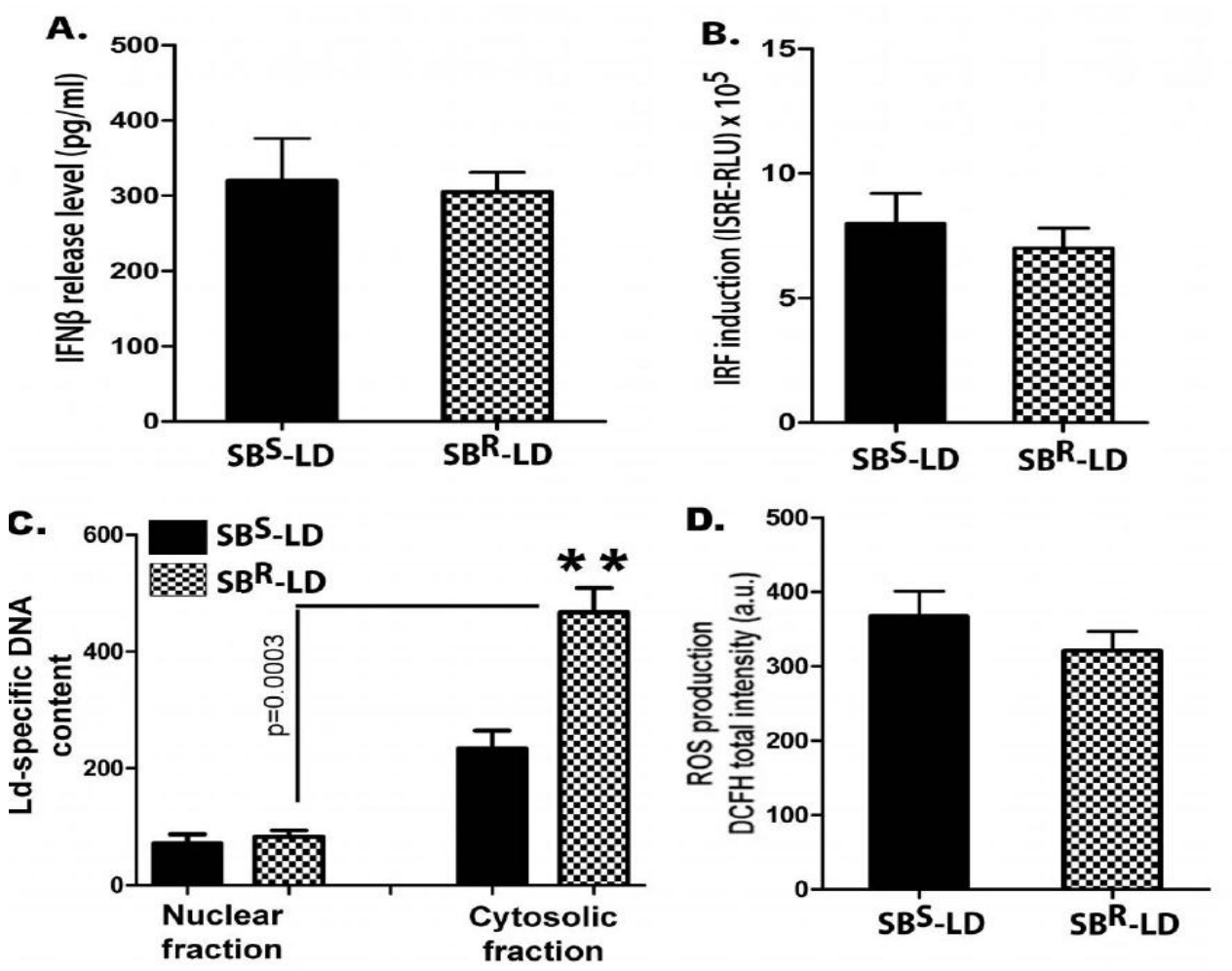
Supplementary figure 4: (A) BALB/c mouse BMDM pre-transfected with targeted IRF-3 siRNA or non-targeted (NT) control siRNA. In both sets, cells were either mock transfected or Ld-DNA transfected [with DOTAP or Lipofectamine 2000] or transfected with dsDNA control Poly(dA-dT). Production of IFN-β was estimated at the release level in the culture supernatant by ELISA. (B) THP-1 Dual™ cells (Invivogen) were activated by PMA and transfected with control siRNA or IRF3-siRNA. In both sets, cells were either mock transfected or Ld-DNA transfected [with DOTAP or Lipofectamine 2000] or transfected with dsDNA control Poly(dA-dT). Twenty-four hours after transfection, the culture supernatants were used for estimation of IRF pathway activation by luciferase reporter assay (Quanti-Luc). Mean values of three independent experiments are presented.



Supplementary figure 5: THP-1 Dual^{TM} cells (Invivogen) derived STING ablated [STING KO] cells and control cells [wild type] were either mock transfected or with Ld-DNA [2.5 ug/ml with DOTAP or Lipofectamine 2000]. The production of IFN-β was estimated at the release level in the culture supernatant by ELISA. Mean values of three independent experiments are presented.

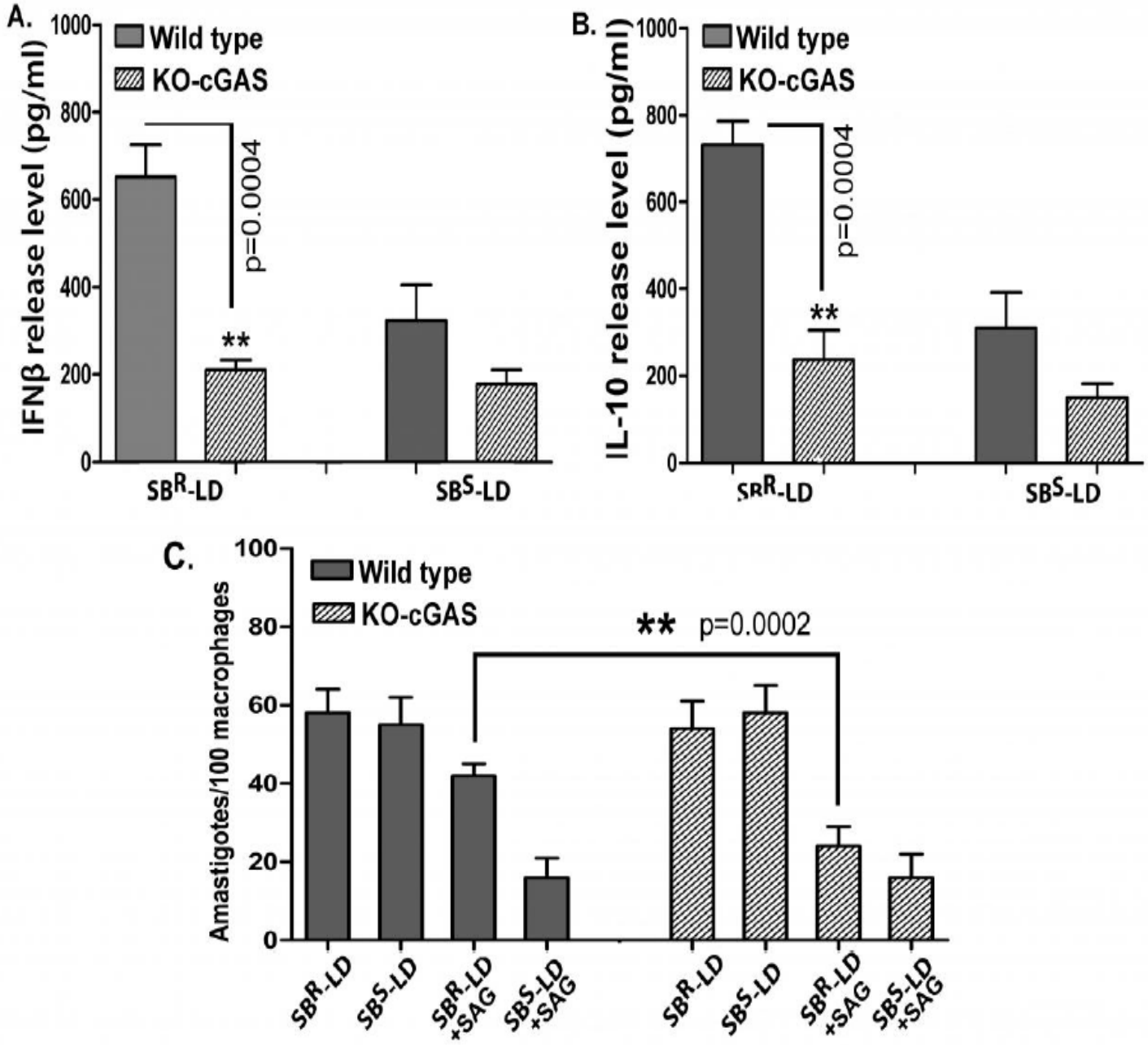


Supplementary figure 6: THP-1 Dual™ cells (Invivogen) were pre-transfected with targeted RIG-1 siRNA or non-targeted (NT) control siRNA as per suppliers' instructions. These cells were later mock transfected or Ld-DNA transfected [2.5 ug/ml with DOTAP or Lipofectamine 2000] or left untreated or transfected with dsDNA control Poly(dA-dT). The production of IFN-β was estimated at the release level in the culture supernatant by ELISA. Mean values of three independent experiments are presented. Inset: Western blot analysis of RIG-1 and GAPDH (loading control) of RIG-1 knockdown (KD) and control (WT) THP-1 cells shows 50% reduction of RIG-1 protein levels in the KD cells in immunoblot with specific antibodies and subsequent densitometric quantification. Mean values of three independent experiments are presented.



Supplementary figure 7: (A-B) Mouse RAW264.7 cells were transfected with equal amounts of with either antimony resistant (SB^R-LD) or antimony sensitive (SB^S-LD) *L.donovani* parasite DNA [2.5 ug/ml with Lipofectamine 2000]. Production of IFN-β was estimated at the release level in the culture supernatant by ELISA (A), IRF pathway activation was measured by luciferase reporter assay (B) and ROS generation was measured by loading with H2DCFDA (DCFH) for 30 min at 37°C to monitor its oxidation by flow cytometry (D). Mean values of three independent experiments are presented. (C) Mouse RAW264.7 cells were either infected with antimony resistant (SB^R-LD) or antimony sensitive (SB^S-LD) *L. donovani* parasites (parasite: macrophage= 10: 1). Uninfected parasites were washed off. The cytosolic and nuclear fractions were prepared after 8 hrs post transfection of the cells. The

DNA in the respective fractions were then subjected to qRT-PCR of *L.donovani* specific kDNA sequence and were normalized to the inputs.



Supplementary figure 8: (A-B) THP1-Dual™ KO-cGAS cells were generated from THP1-Dual™ cells by stable knockout of the cGAS gene and cultured as per suppliers' instructions. Cells (wild-type or KO-cGAS) were either infected with antimony resistant (SB^R-LD) or antimony sensitive (SB^S-LD) *L.donovani* parasites (parasite : macrophage= 10: 1). Production of IFN-β (A) and IL-10 (B) was estimated at the release level in the culture supernatant by ELISA. Mean values of three

independent experiments are presented. (C) In another set of infection experiment, THP1-Dual™ KO-cGAS cells were generated from THP1-Dual™ cells by stable knockout of the cGAS gene and cultured as per suppliers' instructions. Cells (wild-type or KO-cGAS) were either infected with antimony resistant (SB^R-LD) or antimony sensitive (SB^S-LD) *L.donovani* parasites (parasite : macrophage= 10: 1) and later treated/untreated with SAG (60 ug/ml). Number of intracellular amastigotes per 100 macrophages on infection was microscopically evaluated on stained coverslip preparations from each experiment.

Primers used in this study:

Sr No.	Name	Sequence
1	m cGAS-F	5'-TAGCGGTCTCAACTCAAG-3'
2	m cGAS-R	5'-TGGTGTCTGTTCATAGCA-3'
3	m TREX-1-F	5'-CAATAGCCACTCTGTATG-3'
4	m TREX-1-R	5'-TGACCGCTATGACTTTCC-3'.
5	m IFN-B F	5'-GCACTGGGTGGAATGAGACTATTG-3'
6	m IFN-B R	5'-TTCTGAGGCATCAACTGACAGGTC-3'
7	Leish k-DNA F	5'-TCTGTGG CCCATTTGTTGTA-3'
8	Leish k-DNA R	5'-CATTTTTCGGTTTTCGGAGA-3'
9	m GAPDH F	5'-AGATTGTTGCCATCAACGAC-3'
10	m GAPDH R	5'-ATGACAAGCTTCCCATTCTC-3'