Supplemental material

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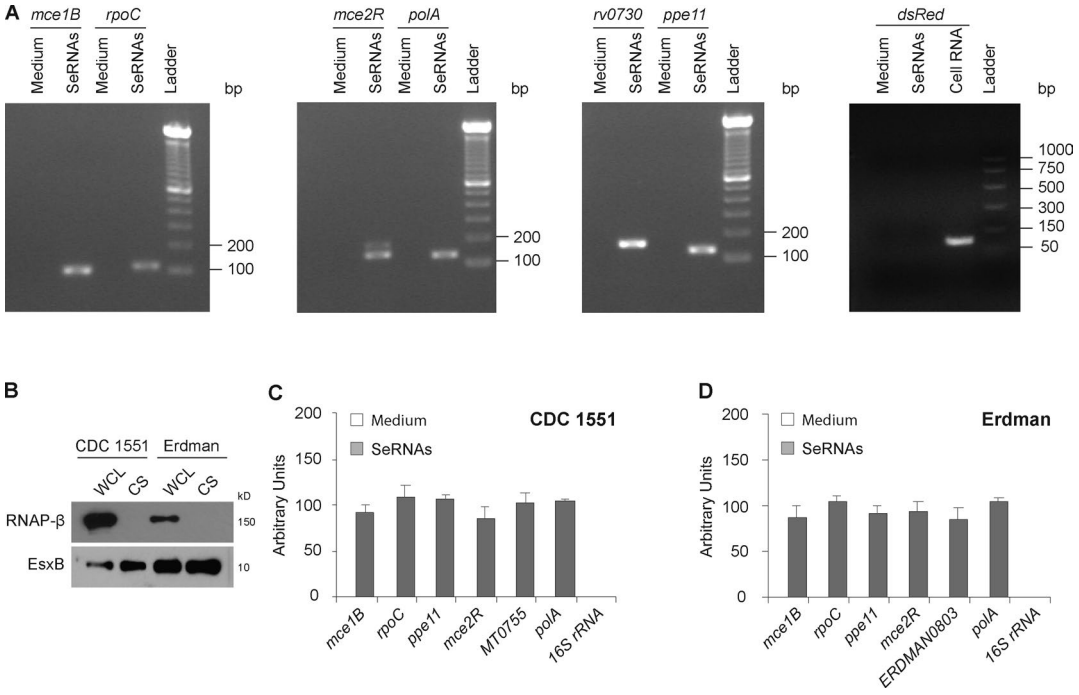

Figure S1. ***M.tb* specifically releases mycobacterial transcripts into the bacterial culture supernatant (CS).** Related to Fig. 1. **(A)** Mycobacterial RNA was isolated from the *M.tb* H37Rv expressing DsRed or the culture filtrate. The specific transcripts were amplified by RT-PCR using primers that amplify fragments of ~150 bp of the indicated genes and analyzed by gel electrophoresis. **(B)** Western blot for RNAP-β (RNA polymerase subunit β) and EsxB in WCL and culture supernatant of WT *M.tb* CDC1551 or Erdman strains. **(C and D)** qRT-PCR for *M.tb* transcripts in the supernatant of WT CDC1551 (C) and Erdman (D) strain culture (midexponential phase). Cell RNA, RNA from mycobacterial cells; medium, culture media only; seRNAs, RNA in the culture supernatant. Data are mean ± SD of triplicate qRT-PCR and are representative of two independent experiments.

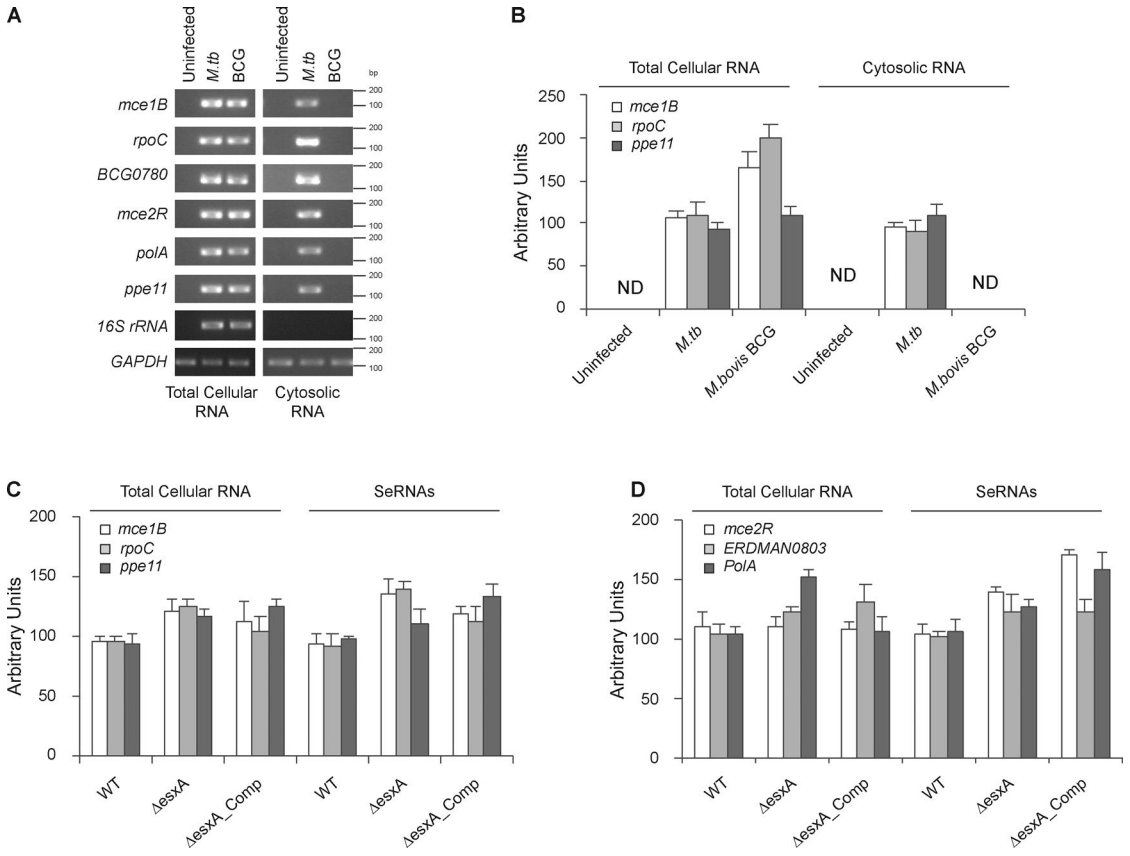

Figure S2. **The ESX-1 secretion system is important for delivery of mycobacterial mRNAs into the macrophage cytosol but not into the culture supernatant.** Related to Fig. 1. **(A)** Raw264.7 cells were left uninfected or infected for 24 h with WT *M.tb* CDC1551 or *M. bovis* BCG at an MOI of 10, and RNA was purified from infected macrophages (total cellular RNA) or the cytosol (cytosolic RNA). Mycobacterial transcripts were amplified by RT-PCR and visualized on agarose gel. **(B)** A subset of these mycobacterial transcripts was quantified by qRT-PCR. Each mycobacterial transcript was normalized to GAPDH. The abundance of each transcript was expressed relative to the *M.tb* transcript/GAPDH ratio defined for WT *M.tb*-infected cells for both total cellular and cytosolic RNA. ND, not detected. Data are mean ± SD of duplicate qRT-PCR and are representative of at least three independent experiments. **(C and D)** The *M.tb* Erdman strains were grown until midexponential phase; total cellular RNA and RNA present in the culture filtrate were purified, and the presence of the different *M.tb* transcripts was defined by qRT-PCR. For total cellular RNA, each transcript was normalized to 16S rRNA, and relative abundance was compared for each transcript to the levels observed for the WT Erdman strain. For RNA present in the culture filtrate, the relative abundance of each transcript was compared with WT Erdman, as no 16S rRNA was detected in the culture supernatant. Data are mean ± SD of duplicate qRT-PCR and are representative of at least three independent experiments.

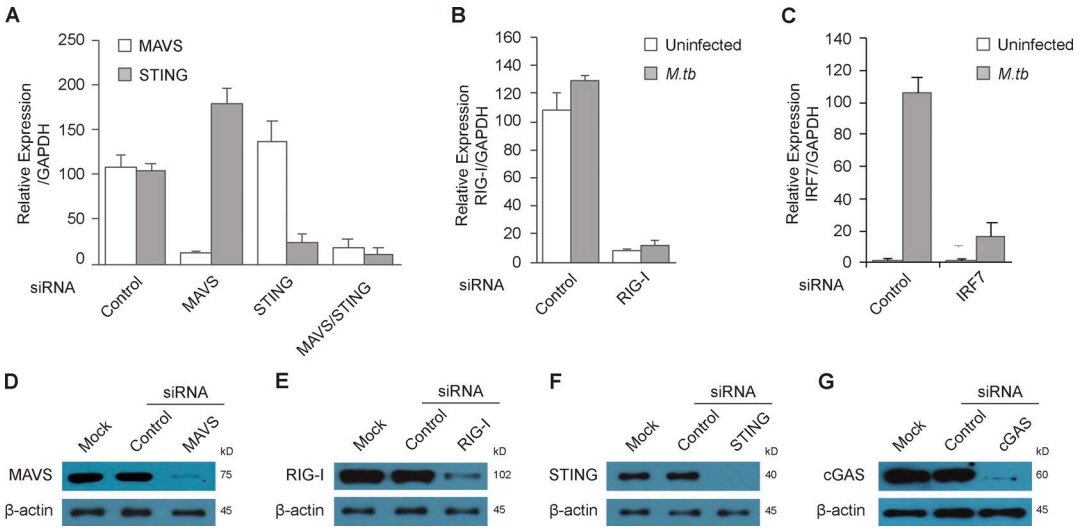

Figure S3. **MAVS, STING, cGAS, RIG-I, and IRF7 siRNA efficiency in mouse BMMs.** Related to Figs. 3, 4, and 5. **(A–C)** The level of MAVS and STING mRNA (A and B), RIG-I mRNA (B), and IRF7 mRNA (C) was determined by qRT-PCR. The mRNA levels were expressed as fold change relative to control siRNA samples in uninfected BMMs (A–C) or to IRF7 mRNA present in *M.tb*-infected BMMs. mRNA levels were normalized to GAPDH. **(D–G)** The protein abundance of MAVS (D), RIG-I (E), STING (F), and cGAS (G) was determined by Western blot. β-Actin served as a load control. Mock, untreated; Control, negative control siRNA. **(A–C)** Data are mean ± SD of triplicate qRT-PCRs and are representative of two independent experiments.

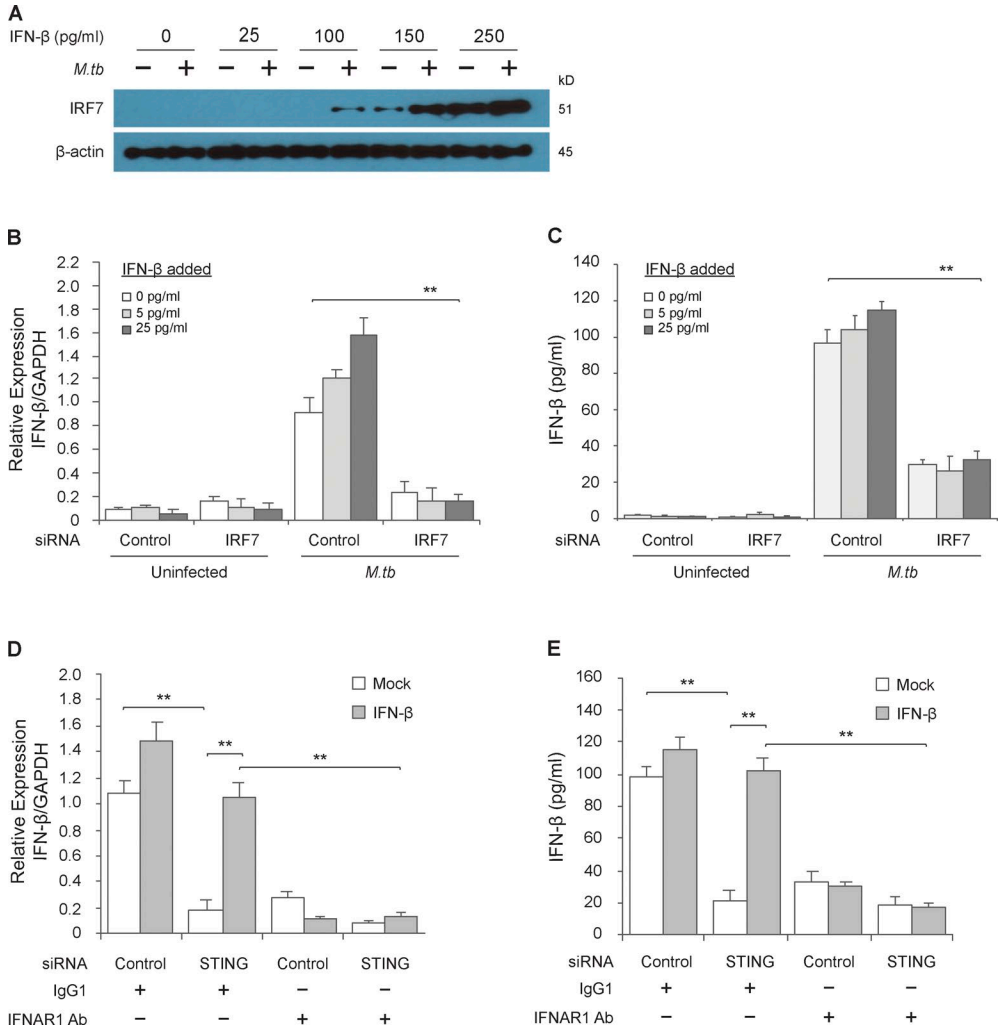

Figure S4. **Addition of exogenous IFN-β to *M.tb*-infected macrophages did not rescue IFN-β production in IRF7 knockdown cells and was blocked by pretreatment with IFNAR1 blocking antibody.** Related to Fig. 6. **(A)** Exogenous IFN-β induces IRF7 expression in *M.tb*-infected and uninfected BMMs via a dose-dependent manner. Mouse BMMs were treated with various concentration of mouse IFN-β as indicated for 4 h with or without *M.tb* (MOI = 10). **(B)** IRF7 knockdown BMMs were infected for 24 h with WT *M.tb* CDC1551 at an MOI of 10 in the absence or presence of recombinant mouse IFN-β. The expression level of IFN-β was determined by qRT-PCR and expressed as fold change relative to control siRNA-treated cells infected with *M.tb*. mRNA levels were normalized to GAPDH. **(C)** As described for B, but IFN-β protein levels in the culture supernatant were quantified by ELISA 24 h after infection. **(D)** STING knockdown BMMs were infected for 24 h with WT *M.tb* CDC1551 strain at an MOI of 10 in the absence or presence of 25 pg/ml recombinant mouse IFN-β with or without addition of the IFNAR1 blocking antibody. The expression level of IFN-β was determined by qRT-PCR. **(E)** As described for D, but IFN-β protein levels in the culture supernatant were measured by ELISA 24 h after infection. Data are mean ± SD of duplicate qRT-PCR and are representative of at least three independent experiments. ** $P < 0.005$ by two-tailed Student's *t* test.

Table S1. **Primer and siRNA sequences**

Gene name	Species	Sequence information	
		Primer sequence (RT-PCR)	
		Forward (5′->3′)	Reverse (5′->3′)
mce1B (rv0170)	*M.tb*/BCG	GAGATCGGCAAGGTCAAAGC	GCGGTCGTGGACTGATACAA
rpoC (rv0668)	*M.tb*/BCG	ATGGTGACCGGGCTGTACTA	CGCTTCGGCCGGCGAAGA
ppe11 (rv0453)	*M.tb*/BCG	CGGCACCGCAAGCAACGAG	GCGGTCCCAAGTTCCCAAGT
N-acetyltransferase (rv0730)	*M.tb*/BCG	CCAGATGAGGACCGCGACA	AACCAAGCCGCCTCCTCGT
polA (rv1629)	*M.tb*/BCG	ACGCGCCGATCCAGGGCA	CGATTTCGAACAGCAGCTCGT
Mce2R (rv0586)	*M.tb*/BCG	GGTGAGCTGGATATCTCCGT	CGATCGGATCTTCCTCAGTGT
16S rRNA	*M.tb*/BCG	CACAATGGGCGCAAGCCTGA	GCTCGCACCCTACGTATTAC
IFN beta	Mouse	TCCGAGCAGAGATCTTCAGGAA	TGCAACCACCACTCATTCTGAG
GAPDH	Mouse	TCGTCCCGTAGACAAAATGG	TTGAGGTCAATGAAGGGGTC
RIG-I	Mouse	AGAGCCAGAGTGTCAGAATCT	CTTGGCAGGCAGGGCAAGC
MAVS	Mouse	CCTCCGGGGACCTCACTCCG	TGGGGACTCTGGTGGCTGGG
STING	Mouse	GCAGCTACTGGAAGGCTGTG	GGCCAAACATCCAACTGAGG
IRF7	Mouse	AGTGGAGTTAACCTGCCACC	TCAACTGGATCCCCTGAATTG
		Primer sequence (complementary strain)	
		Forward (5′->3′)	Reverse (5′->3′)
secA2	*M.tb*	CTGCAGAATTCAGGAGTCCAGCGTGAACGTGCACGGTTGTCC	GCGTTAACTCAGCGGAACACCCCGGG
		siRNA sequence	
RIG-I	Mouse	GAAGCGUCUUCUAAUAAUU	
MAVS-1	Mouse	GAUCAAGUGACUCGAGUUU	
MAVS-2	Mouse	GGACCAAAUAGCAGUAUCA	
cGAS	Mouse	AUUUCUGCUCCUAAUGAAUU	
STING-1	Mouse	GGAUCCGAAUGUUCAAUCA	
STING-2	Mouse	CCAACAGCGUCUACGAGA	
IRF7	Mouse	SMARTpool: ON-TARGETplusIrf7siRNA	

Related to all figures.