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Supplemental information

Manganese enhances DNA- or RNA-mediated innate immune response by inducing phosphorylation of TANK-binding kinase 1

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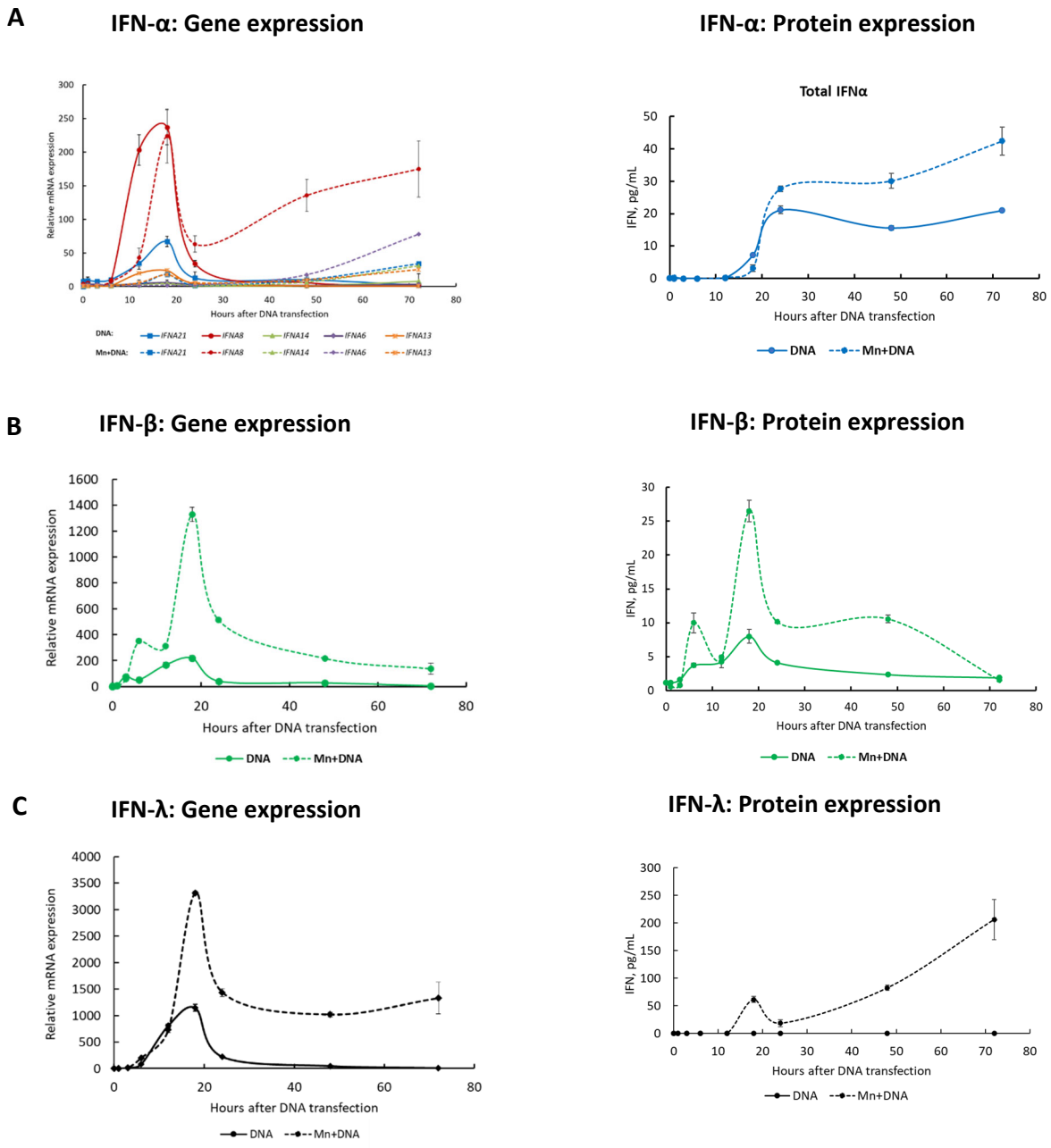


Figure S1. The time course of Mn-enhanced DNA-mediated innate immune response, related to Fig. 1. Human primary macrophages were seeded in a six-well plate and treated with or without Mn^{2+} at a concentration of 25 μM. The cells were then transfected with DNA, and the cell lysate or cell supernatants were harvested at the indicated time points and subjected to real-time RT-PCR (upper panels) for gene expression levels of **(A)** *IFNA21, IFNA8, IFNA14, IFNA6, IFNA13*; **(B)** *IFNB*; and **(C)** *IFNL1* or to ELISA (lower panels) for protein expression detection of **(A)** IFN-α, **(B)** IFN-β, and **(C)** IFN-λ. One representative experiment of at least three independent experiments is shown. And each was done as triplicate. All the data are shown as mean ± SD of three replicates (n=3).

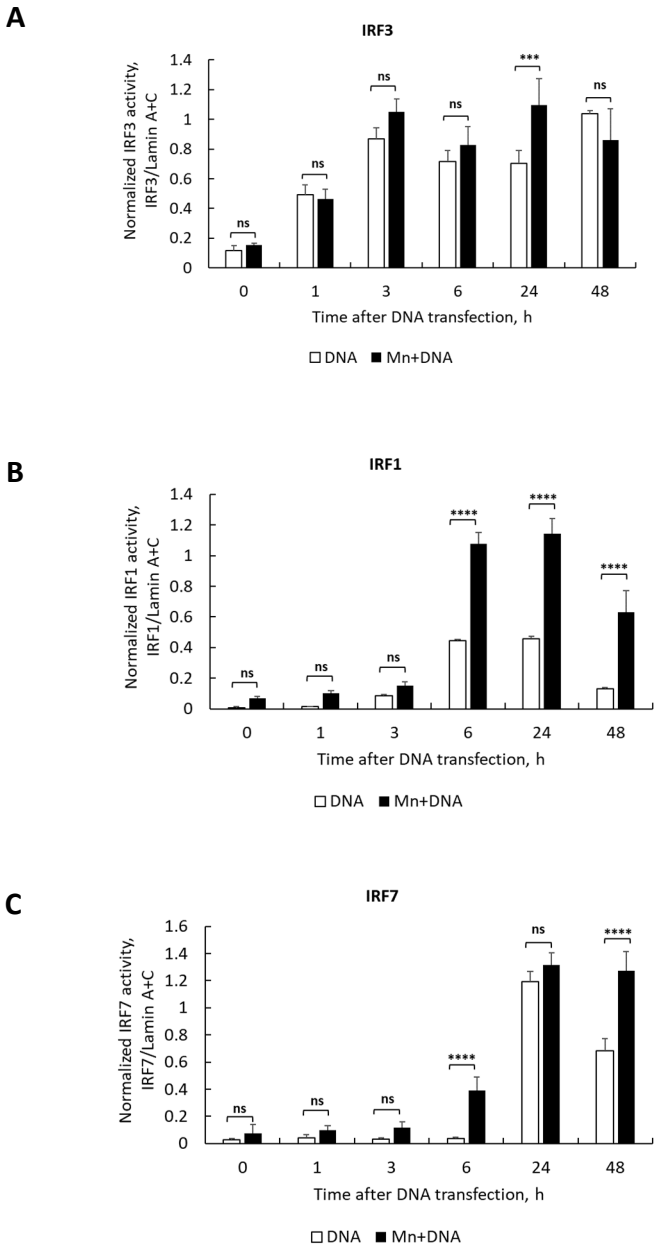


Figure S2. Mn enhances DNA-mediated IRF3, IRF1 and IRF7 activity to induce innate immune response, related to Fig. 3B. The data in Fig. 3B was further analyzed by Image J. The intensity of the band for **(A)** IRF3, **(B)** IRF1, **(C)** IRF7 was normalized by the intensity of Lamin A+C. The densitometry analysis was performed for three independent times, and the data are shown as mean ± SD (n=3). One-way ANOVA was performed to determine if Mn significantly enhances DNA-mediated IRF3, IRF1 and IRF7 activity, ****P<0.0001, ***P<0.001, ns (not significant) P > 0.05 (One-way ANOVA) were indicated in the figures.

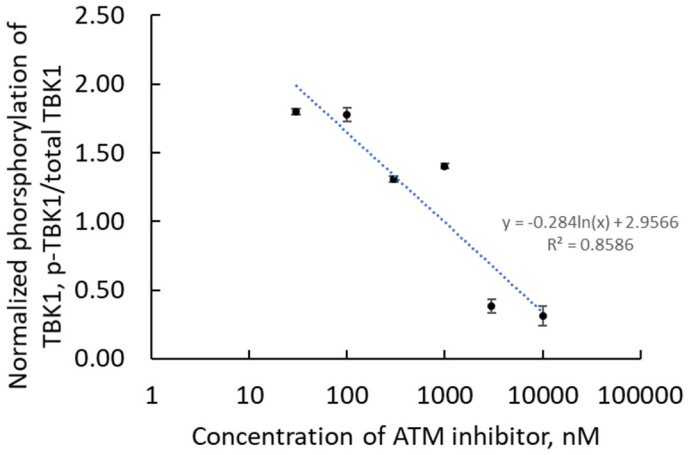


Figure S3. ATM inhibitor KU60019 does-dependently inhibited phosphorylation of TBK1 in macrophages, related to Fig. 4D. The data in Fig. 4D was further analyzed by Image J. The densitometry analysis was performed for three independent times, and the data are shown as mean±SD (n=3). The intensity of the band for phosphorylated TBK1 (p-TBK1) was normalized by the intensity of total TBK1, and the value for p-TBK1/TBK1 vs. the concentration of ATM inhibitor was plot in the figure, a trend line (blue, curve fitting)) is indicated in the figure with equation and R-square value.

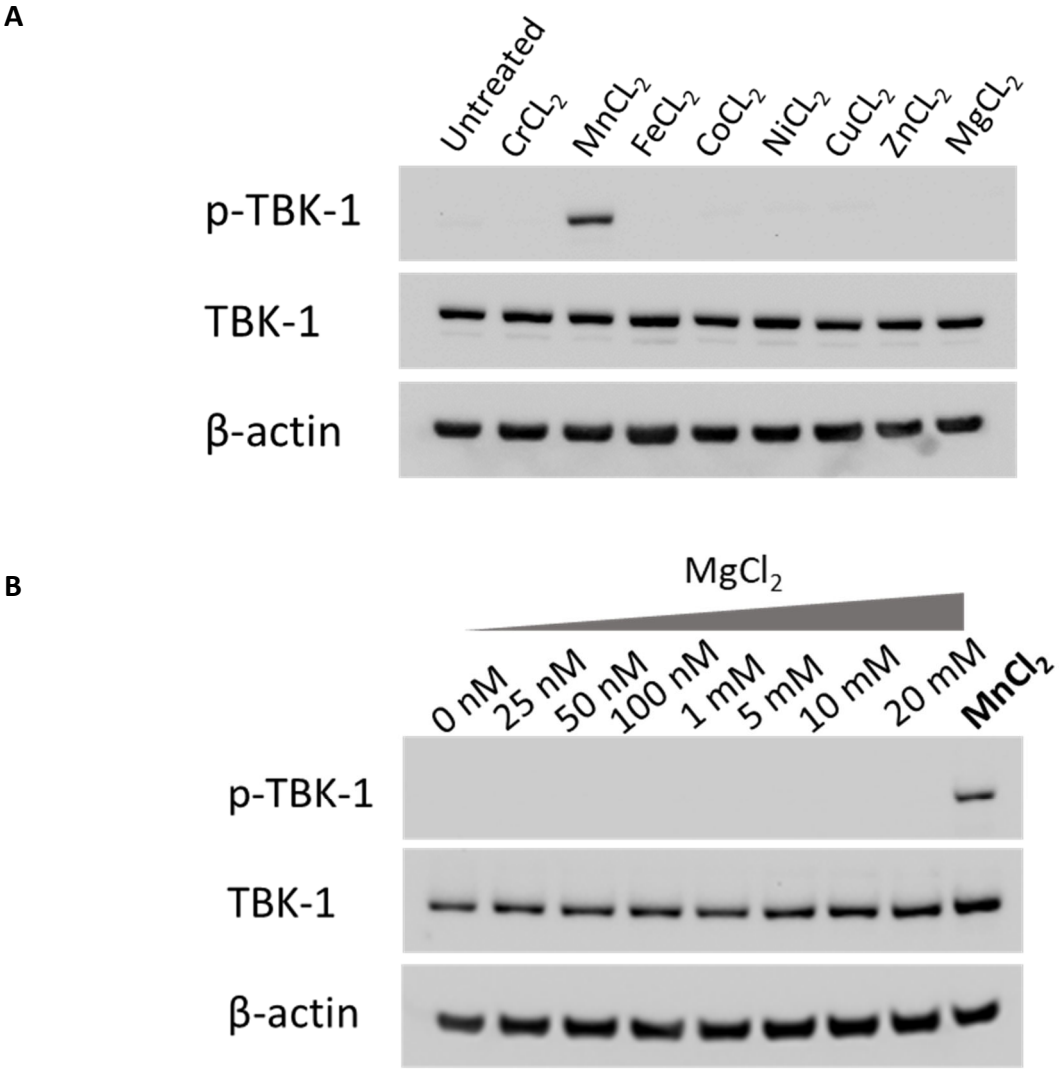


Figure S4. Among all the metal ions tested in the study, only Mn induces the phosphorylation of TBK1, related to Fig. 4. (A) Human primary macrophages were seeded in a six-well plate and treated with various metal solutions at a concentration of 25 μM. The whole-cell lysate was collected at 48 hours after treatments and was followed by Western blotting to check p-TBK1 and total TBK1. β-actin was also included as the loading control. One representative experiment of at least three independent experiments is shown. **(B)** Macrophages were treated with $MgCl_2$ at different concentrations, and the whole-cell lysate was collected at 48 hours after treatments. The cell lysate was subjected to Western blotting probed by p-TBK1, TBK1, and β-actin antibodies. One representative experiment of at least three independent experiments is shown.

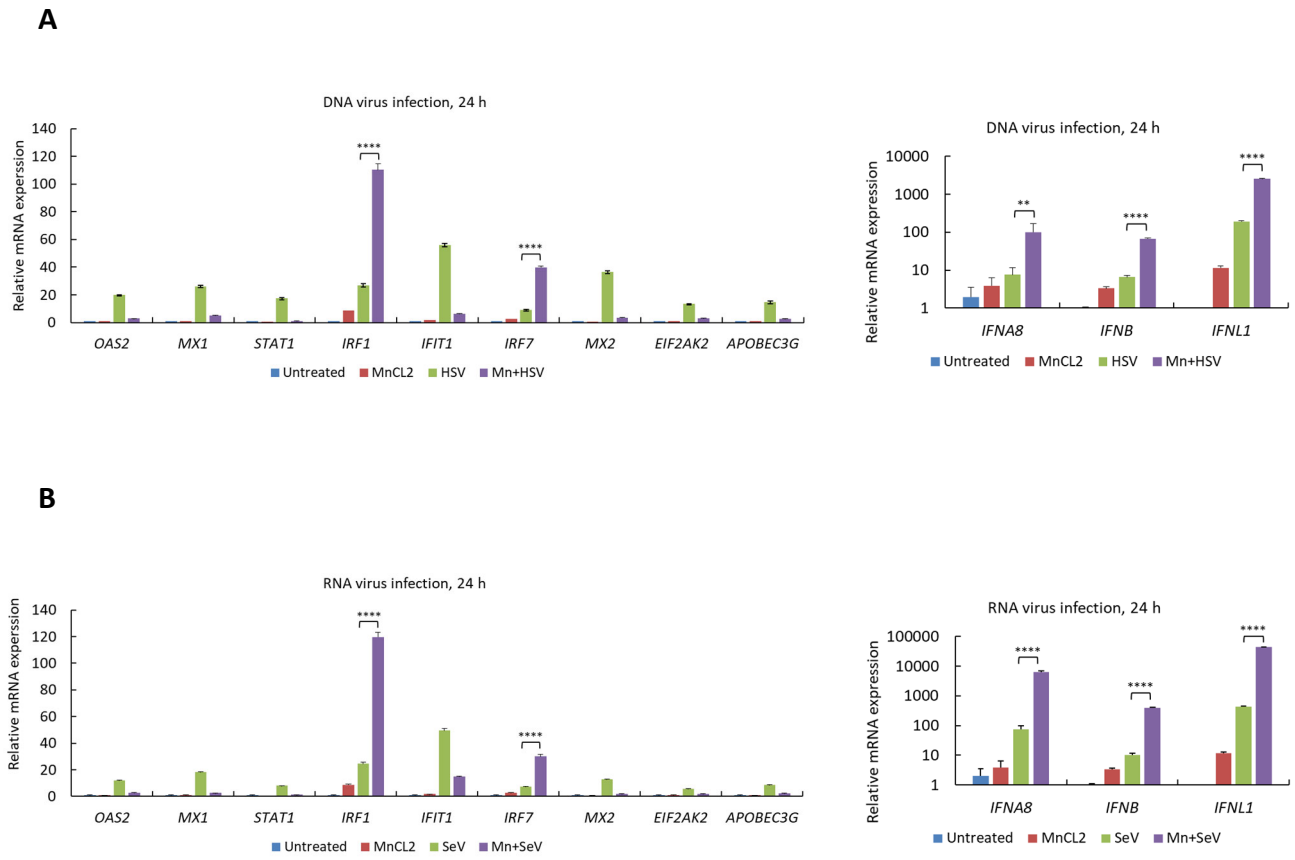
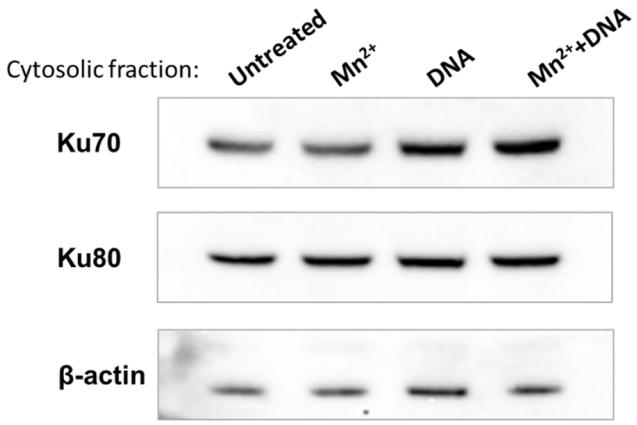


Figure S5. Mn-enhanced virus-mediated IFN induction induces early induction of some ISGs, related to Fig. 5 & Fig. 6. Human primary macrophages were seeded in a six-well plate and treated with or without Mn^{2+} at a concentration of 25 μM. The cells were then infected by **(A)** HSV-1 virus or **(B)** SeV virus at MOI of 1 and 40, respectively, and the total RNA was extracted at 24 h after virus infection and subjected to real-time RT-PCR for gene expression levels of *OAS2, MX1, STAT1, IRF1, IFIT1, IRF7, MX2, EIFAK2, IFNA8, IFNB and IFNL1*; One representative experiment of at least three independent experiments is shown. All the data are shown as mean ± SD of three replicates (n=3). ****P<0.0001, **P<0.01 (One-way ANOVA) were indicated in the figures.

A



B

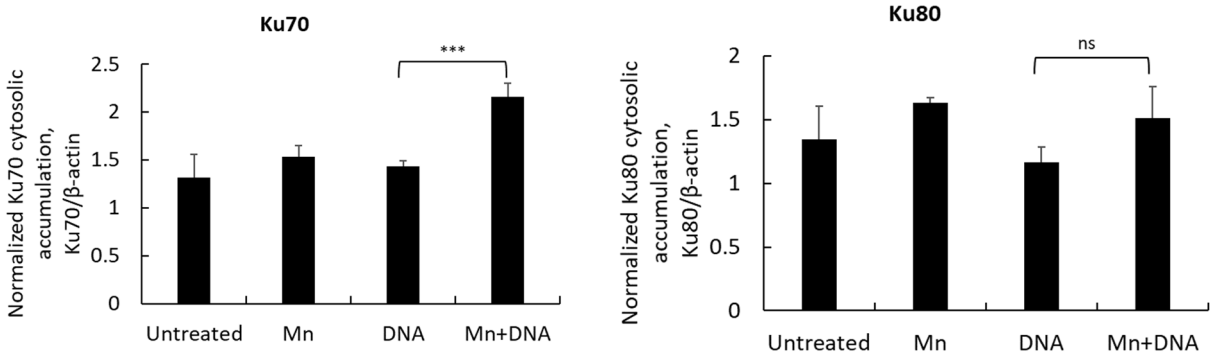


Figure S6. Mn slightly promoted the cytoplasmic translocation of Ku70, related to discussion. (A) HEK cells were treated with Mn^{2+} at 25 μM, then further transfected with DNA after 24 hours of Mn treatment. The cytosolic fraction was prepared and subjected to Western blotting for anti-Ku70, anti-Ku80 detection. The β-actin antibody was included as the loading control for the experiments. One representative experiment of at least three independent experiments is shown. **(B)** The intensity of band Ku70 or Ku80 was normalized by that of β-actin. The densitometry analysis was performed for three independent times, and the data are shown as mean ± SD (n=3). ***P<0.001, ns (not significant) $P > 0.05$ (One-way ANOVA) were indicated in the figures.