

## Supplemental information

**Title:** Amplified RLR signaling activation through an interferon-stimulated gene-endoplasmic reticulum stress-mitochondrial calcium uniporter protein loop

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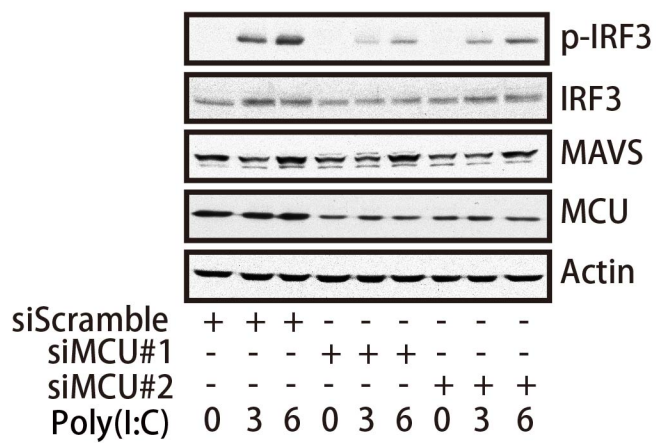
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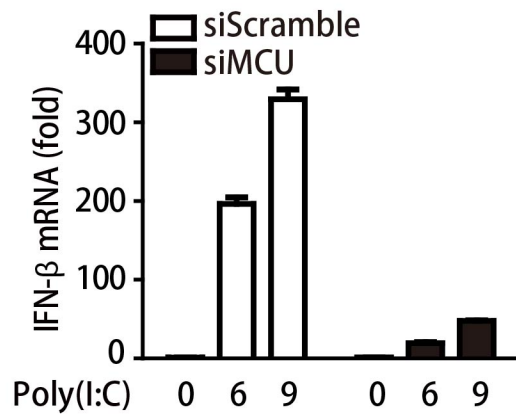
<sup>3</sup>Co-first author

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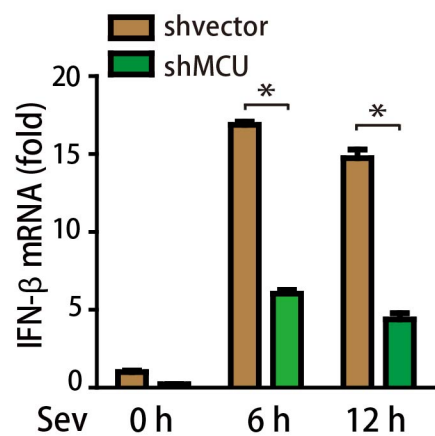
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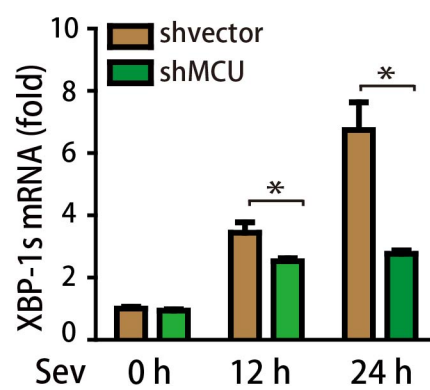
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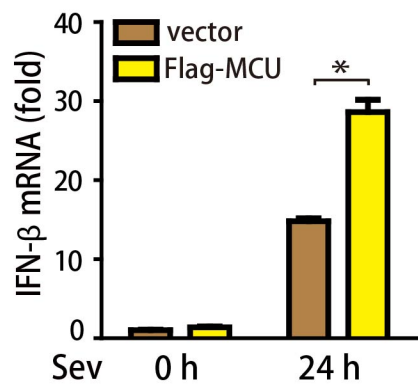
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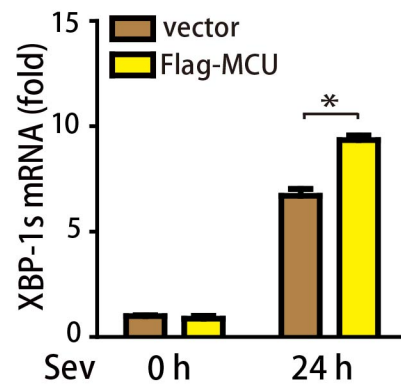
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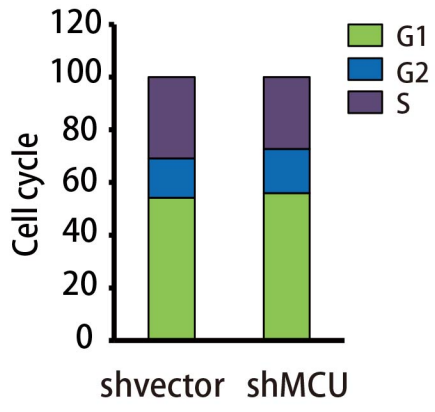
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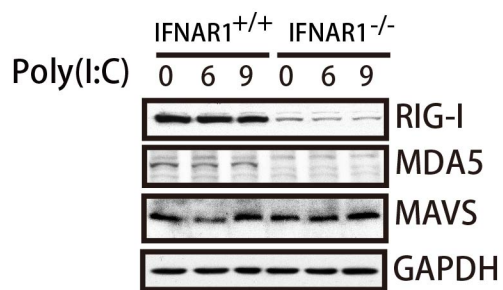
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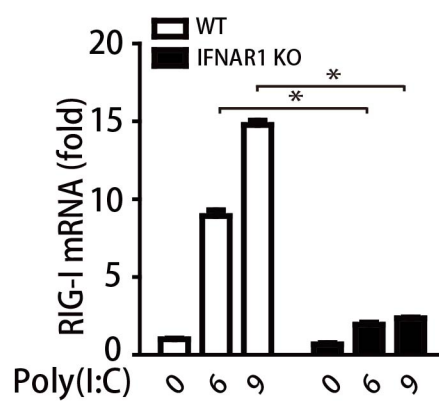
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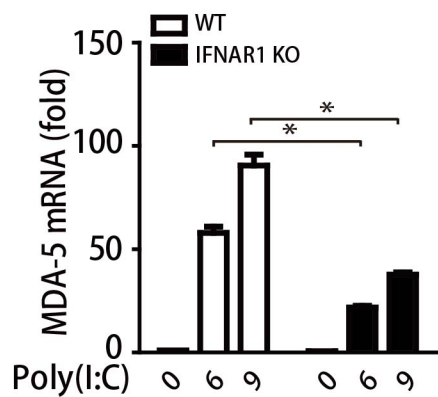
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## Figure legends

**Figure S1.** Transient knockdown of MCU decreased poly(I:C)-induced RLR signaling activation. (A) HeLa cells were transfected with MCU targeting siRNAs (siRNA#1 and siRNA#2) and then treated with 1  $\mu\text{g}/\text{mL}$  poly(I:C) for different time as indicated. Cell lysates from these cells were immunoblotted with p-IRF3, IRF-3, MAVS, MCU and Actin. (B) HeLa cells were transfected with MCU targeting siRNA#1 and then treated with 1  $\mu\text{g}/\text{mL}$  poly(I:C) for different time as indicated. IFN- $\beta$  mRNA levels from these cells were detected by qPCR.

**Figure S2.** MCU impairs SeV infection-induced RLR signaling activation and ER stress. (A, B) Stable MCU knockdown HeLa cells or control cells were treated with SeV for different time. IFN- $\beta$  mRNA levels and XBP-1s levels from these cells were detected by qPCR. (C, D) HeLa cells transfected with Flag-MCU or vector were treated with SeV for different time, and then IFN- $\beta$  mRNA levels and XBP-1s levels from these cells were detected by qPCR.

**Figure S3.** MCU knockdown did not impair cell cycle parameters. (A) Stable MCU knockdown HeLa cells or control cells were analyzed cell cycle by flow cytometry.

**Figure S4.** IFNAR1 signaling impairs the expressions of RIG-I and MDA-5. (A) WT and *IFNAR1*<sup>-/-</sup> MEF cells were treated with 1  $\mu\text{g}/\text{mL}$  poly(I:C) for different time as indicated, and then immunoblotted with RIG-I, MDA-5, MAVS and GAPDH. (B-C) mRNA levels of RIG-I and MDA-5 from these cells were detected by qPCR.