Lei et al. Figure S1

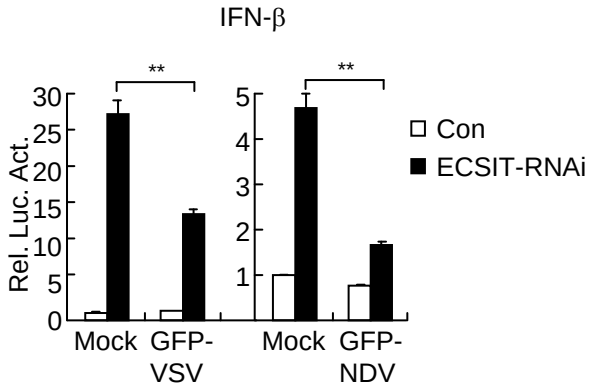

Figure S1. Effects of ECSIT-RNAi plasmid on GFP-VSV- or GFP-NDV-induced activation of the IFN-β promoter. HEK293 cells ($1x10^5$) were transfected with the ECSIT-RNAi (0.5 μg) and IFN-β reporter (0.1 μg) plasmids. Thirty-six hours after transfection, cells were left uninfected or infected with GFP-VSV for 10 hours or GFP-NDV for 24 hours before luciferase assays were performed. Graphs show mean ± SD, n = 3. **p < 0.01.

Lei et al. Figure S2

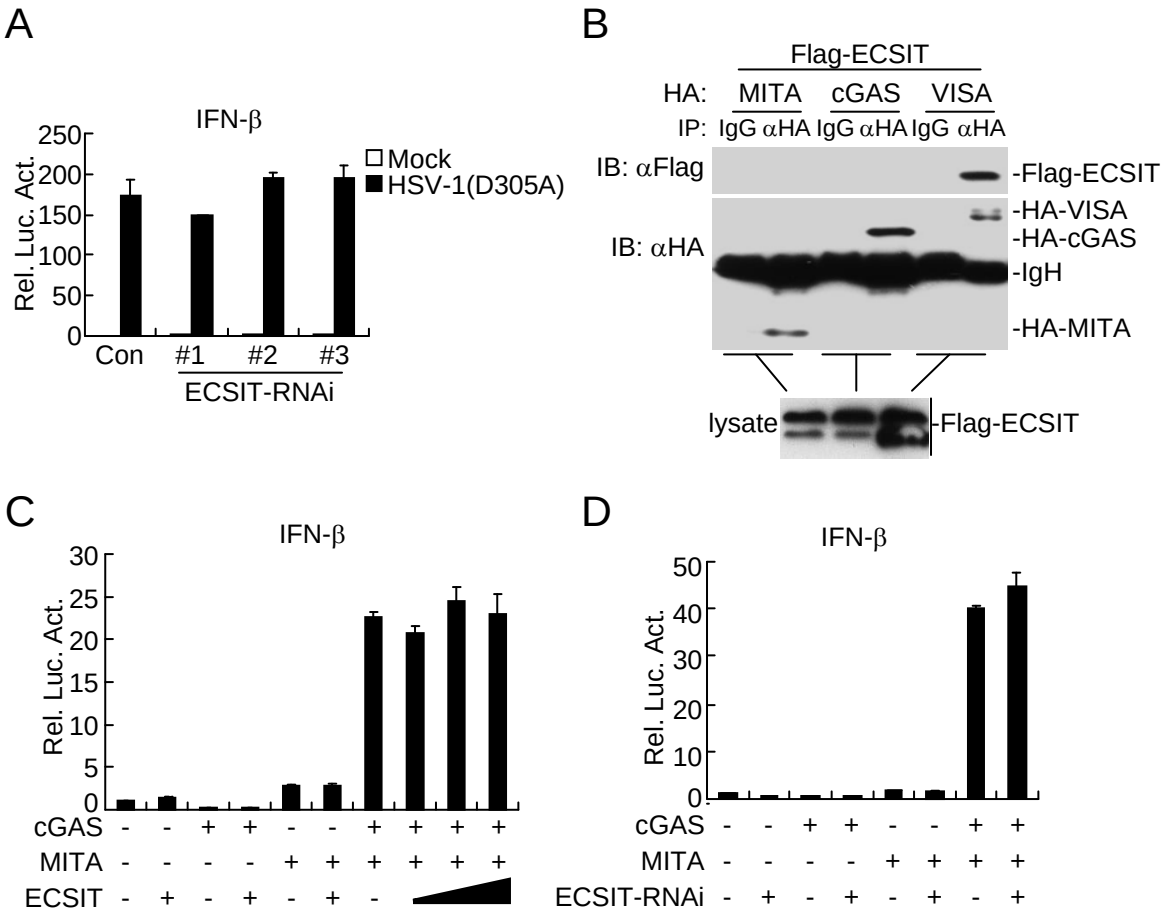

Figure S2. ECSIT is not involved in DNA virus-triggered signaling.

- Effects of ECSIT-RNAi plasmids on HSV-1(D305A)-induced activation of the IFN-β promoter. HEK293 cells ($1x10^5$) were transfected with the indicated ECSIT-RNAi (0.5 μg) and IFN-β reporter (0.1 μg) plasmids. Thirty-six hours after transfection, cells were left uninfected or infected with HSV(D305A) for 12 hours before luciferase assays were performed.
- ECSIT does not interact with cGAS and MITA. HEK293 cells ($2x10^6$) were transfected with the indicated plasmids. Twenty hours after transfection, co-immunoprecipitation was performed with anti-HA or control IgG. The immunoprecipitates were analyzed by immunoblot with anti-Flag or anti-HA (upper panel). The lysates were analyzed by immunoblots with anti-Flag (lower panels).
- Overexpression of ECSIT dose not affect cGAS and MITA-mediated activation of the IFN-β promoter. The HEK293 cells ($1x10^5$) were transfected with control or ECSIT expression plasmids and the indicated plasmids. Reporter assays were performed 24 hours after transfection.
- Knockdown of ECSIT dose not affect cGAS and MITA-mediated activation of the IFN-β promoter. The 293 cells ($1x10^5$) were transfected with control or ECSIT-RNAi plasmids (0.5 μg). Thirty-six hours later, cells were further transfected with the indicated plasmids (0.1 μg each) and IFN-β promoter reporter plasmids. Reporter assays were performed 24 hours after transfection. Graphs show mean ± SD, n = 3.

Lei et al. Figure S3

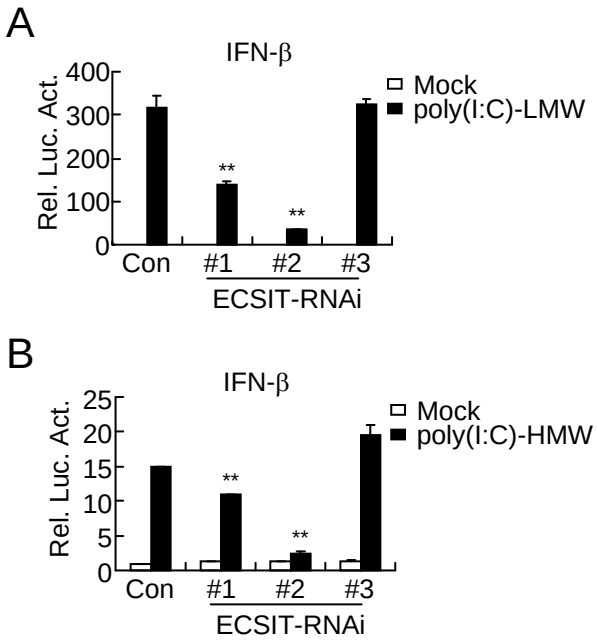

Figure S3. ECSIT plays a role in both HMW and LMW poly(I:C)-mediated signaling.

- Effects of ECSIT-RNAi plasmids on cytoplasmic poly(I:C)-LMW-induced activation of the IFN-β promoter. HEK293 cells ($1x10^5$) were transfected with ECSIT-RNAi (0.5 μg) and IFN-β reporter (0.1μg) plasmids. Thirty-six hours after transfection, cells were mock-transfected or transfected with poly(I:C)-LMW (1 μg) with Lipofectamine 2000 for 12 hours before luciferase assays were performed. LMW, low molecular weight.
- Effects of ECSIT-RNAi plasmids on cytoplasmic poly(I:C)-HMW-induced activation of the IFN-β promoter. HEK293 cells ($1x10^5$) were transfected with ECSIT-RNAi (0.5 μg) and IFN-β reporter (0.1μg) plasmids. Thirty-six hours after transfection, cells were mock-transfected or transfected with poly(I:C)-HMW (1 μg) with Lipofectamine 2000 for 12 hours before luciferase assays were performed. HMW, high molecular weight.

Graphs show mean ± SD, n = 3. **p < 0.01.