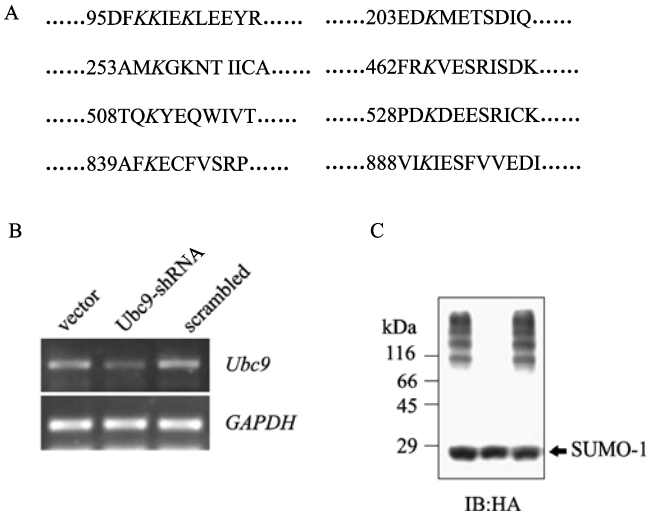
Supplements:

Supplemental Figure 1. Ubc9 knockdown in HeLa cells by RNAi. (A) The putative sumoylation sites (italics) in RIG-I were predicted by SUMOplot Analysis Program. (B–C) 2×10^6 HeLa cells were pre-infected with 2MOI lentivirus vector, lentivirus containing Ubc9-shRNA or Ubc9-shRNA scrambled. (B) Forty-eight hours post-infection, cells were collected and performed to detect Ubc9 mRNA level after different treatment by RT-PCR. *hGAPDH* as loading control. (C) Twelve hours post lentiviral infection, cells were transfected with pCMV-HA-SUMO-1. Thirty-six hours post-transfection, cells were lysed in 1×SDS loading buffer and boiled for 5 minutes, then the cell lysate was subjected to separation by SDS-PAGE and Immunobloted with anti-HA antibody.



Supplemental Figure 2. K172R mutation has no effect on RIG-I SUMOylation and SUMO-1 attachment site in RIG-I might be non-consensus. HEK293T cells were co-transfected with pCMV-HA-SUMO-1, pcDEF-Myc-Ubc9, pEF-Flag-RIG-I or its K/R mutation mediated by calcium phosphate method. Forty-eight hours post-transfection, cells were lysed in IP buffer. The cell lysates were immunoprecipiated with anti-Flag antibody and immunobloted with anti-Flag or anti-HA antibody.