Supplementary Materials and Methods

Plasmids, cells, reagents and antibodies

IFN-β-pGL3 and RANTES-pGL3 luciferase reporter plasmids were kindly provided by R Lin (Montreal, Canada), pcDNA3-Myc-His-mTBK1 and pcDNA3-Myc-His-mTBK1 K38A by J Pomerantz (Maryland, USA), pRK5-Myc-Parkin by TM Dawson (Maryland, USA), pcDNA3-Flag-hIKK-*i* by T Maniatis (MA, USA), pEF-BOS-Myc-hIRF3, pEF-BOS-Myc-hIRF7, and pFLAG-CMV2-hIRF3by T Kawai (Osaka, Japan), and Flag-mIκBα by H Nakano (Tokyo, Japan). Sts-1 and Sts-2 constructs have been described previously (Hoeller et al, 2006). Full length of TBK1 or IKK-*i* were amplified by PCR and subcloned into pEGFP-C1 or pRK5-Myc respectably. All of the mutants, such as TBK1-Δ-ULD, L352A, I353A, L352AI353A, 1-383, 1-301 (wt), 1-301 (KM), IKK-*i*- Δ-ULD, L352AF353A, K38A, ULD-L352AI353A, IRF3 1-189, IRF3 1-384, IRF3 190-427, IRF3 190-384, IRF7 1-270, and IRF7 1-470 were generated by site-directed mutagenesis using PCR as described previously (Hecker et al, 2006).

HEK293T and HeLa cell lines were purchased from American Type Culture Collection and grown as the manufacture's protocol. Control and TBK1 -/- IKK-*i* -/- deficient MEF cells were cultured as described (Hemmi et al, 2004). LPS and poly(I:C) were purchased from Sigma and Amersham, respectively. α-Flag antibody (M2, Sigma), α-NUP62 antibody (BD Transduction), α-GAPDH antibody (Abcam), α-Myc antibody (9E10, Covance), α-TBK1 antibody, α-IRF3 antibody, α-p-IRF3 (Ser396) antibody (Cell signaling), α-p-IRF3 (Ser386) antibody (IBL), α-Actin antibody (Santa Cruz), and α-p-IκBα antibody (Cell Signaling) were purchased as described.

Statistic analysis

Data were analyzed by ANOVA. At least 4 experiments or samples were analyzed for the statistics.

References

Hecker CM, Rabiller M, Haglund K, Bayer P, Dikic I (2006) Specification of SUMO1- and SUMO2-interacting motifs. *J Biol Chem* **281**: 16117-16127