

Supplementary information, Figure S1

Figure S1 N-terminal binding of TRAF3 to MAVS is not required for IFN activation. (A) Schematic representation of the MAVS wt and MAVS-Nterm AAA. The highly conserved sequence in region 143-PVQET-147 of MAVS, as well as the mutations sites of MAVS-Nterm AAA is shown. The CARD, PRO and TM domains are also shown. (B) HEK293 cells were transfected with either: empty vector, FLAG-TRAF3, MYC-MAVS wt or MYC-MAVS-Nterm AAA alone or in co-transfection with FLAG-TRAF3. Co-immunoprecipitation was performed using an anti-MYC antibody followed by immunoblot with an anti-FLAG to reveal interaction with FLAG-TRAF3 (top panel).Immunoprecipitated MAVS was revealed by immunoblot with anti-MYC antibody (second panel). Input for TRAF3 is shown (bottom panel). (C) qPCR analysis of total RNA isolated from HeLa cells transfected either with empty vector, MAVS wt or MAVS-Nterm AAA. Relative fold expression levels of IFNB1, IFNA2, IFIT1 (ISG56), IL6 and IP-10 versus ACTIN mRNA are shown. Data is representative of at least two experiments run in duplicate. (D) HeLa cells were transfected with IFNα4-Luc (IRF7), IFNβ- Luc, ISG56-Luc, or NF-kB reporter plasmid, and expression plasmids encoding either: empty, MAVS wt or MAVS-Nterm AAA as indicated. Luciferase activity was analyzed at 24 hours post-transfection and fold activation was determined compared to empty vector; values represent the average \pm S.D. Results are representative of at least three experiments run in triplicate.