Supporting Information

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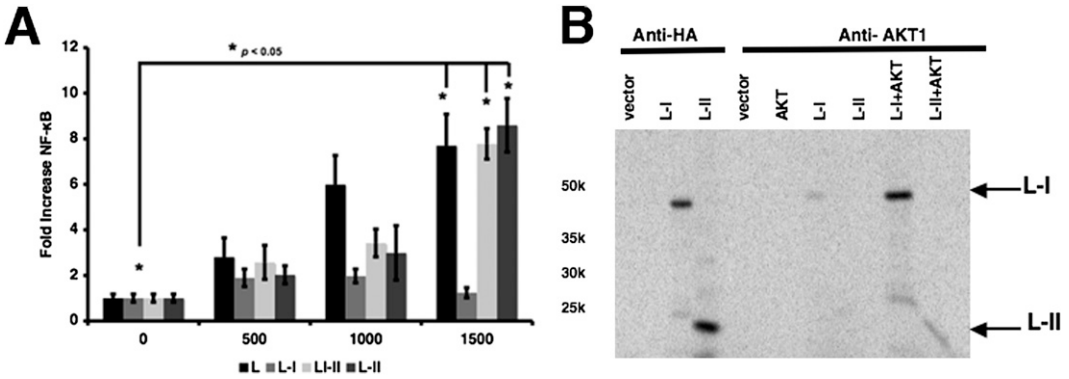

Fig. S1. Activation of NF-κB by region II of the L gene in an AKT-independent manner. (*A*) Activation of NF-κB by the L-II region of the L gene. BSR-T7 cells were transfected with a plasmid encoding a firefly luciferase gene (F-Luc) under the control of NF-κB–responsive elements and increasing amounts (0, 500, 1000, and 1500 ng) of a plasmid encoding PIV5 L, L-I, L-II, or L-I-II proteins, along with a plasmid encoding an R-Luc as an indicator of transfection efficiency. Empty vector was used to maintain a constant total of transfected DNA. Luciferase activities were measured at 1 d posttransfection. The F-Luc to R-Luc ratio serves as an indicator of reporter gene activity. These ratios were normalized to the activity of the vector alone. All transfections were carried out in replicates of four. Error bars represent SD. All *P* values were calculated using the paired *t* test and shown in the figure. (*B*) Domain I of L is important for interaction with AKT1. ^{35}S-labeled L-I and L-II were synthesized by in vitro transcription and translation. AKT1 was obtained from cells transfected with an AKT1 expression plasmid. ^{35}S-labeled L-I or L-II was mixed with cell lysate containing AKT1 and immunoprecipitated with anti-AKT1 antibody.

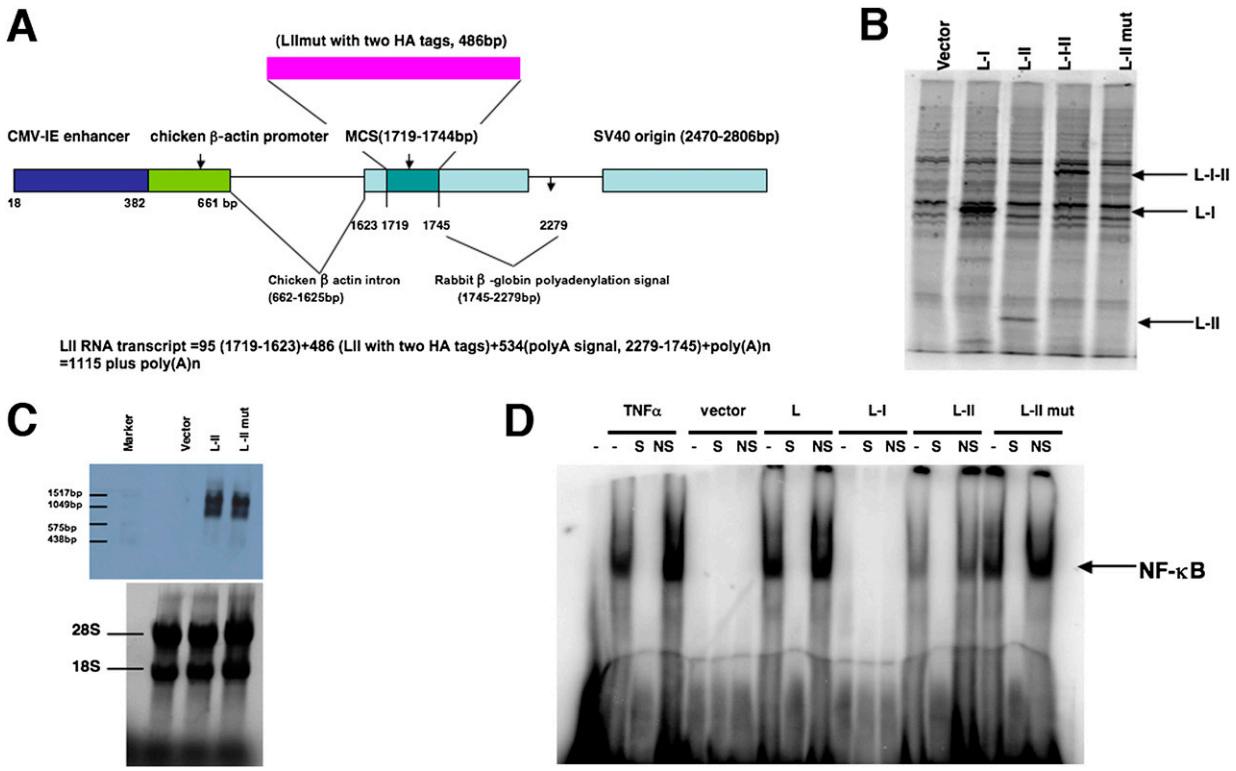

Fig. S2. L-II RNA activates NF-κB. (*A*) Schematics of the plasmid expressing L-II mut RNA. The L-II region was amplified using PCR primers that add two copies of HA tags at the C-terminal end of the L-II region, and then subcloned into the EcoRI and NheI sites of the vector pCAGGS (1). The size of the L-II RNA transcript was ~1,000 nt without poly(A). (*B*) Expression of L-II by L-II mut. The cells were transfected with vector, L-I, L-II, L-I-II, or L-II mut, and immunoprecipitation was performed to analyze the protein expression levels. (*C*) Expression of L RNA. The amounts of L-II and L-II mut RNA were compared by Northern blot analysis with anti–L-II antisense DIG-labeled RNA probe. Methylene blue staining was used to determine the total RNA levels of the samples. "Marker" indicates the DIG-labeled RNA molecular weight marker. (*D*) L-II mut activates NF-κB. A gel shift experiment was performed as described in Fig. S1*B* using appropriate competitors.

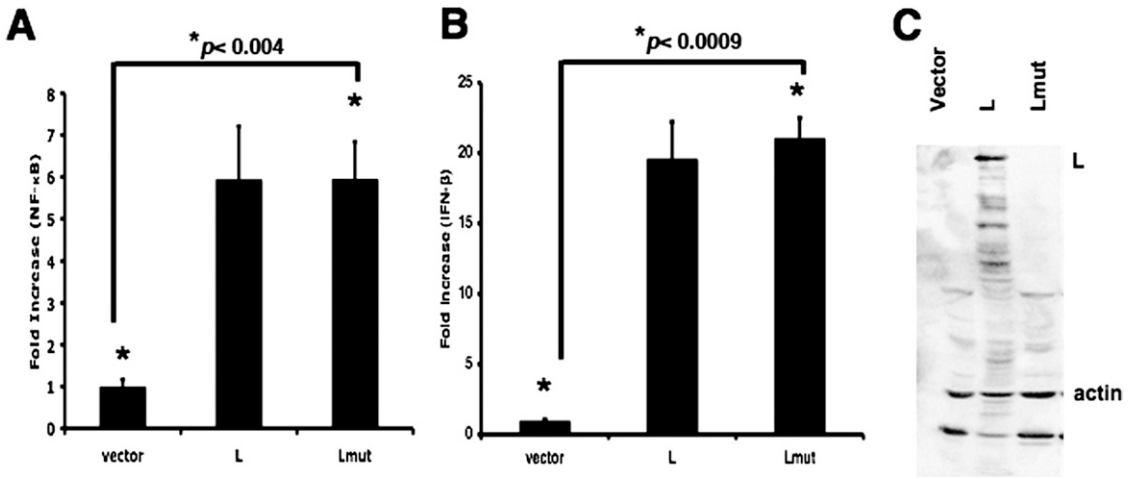

Fig. S3. Activation of NF-κB and IFN-β by L mRNA. A mutant L gene (L mut) with two in-frame stop codons located 6 nts downstream of the L start codon was generated. (*A*) Activation of NF-κB by L mut. The reporter gene assay was performed as described in Fig. 1*A*. (*B*) Activation of IFN-β promoter by L mut. A dual-luciferase assay was performed as described in Fig. 3*A*. (*C*) Expression of L mutants. The cells were transfected with vector, L, or L mut, and immunoblot analysis was performed to analyze the expression levels of the proteins.

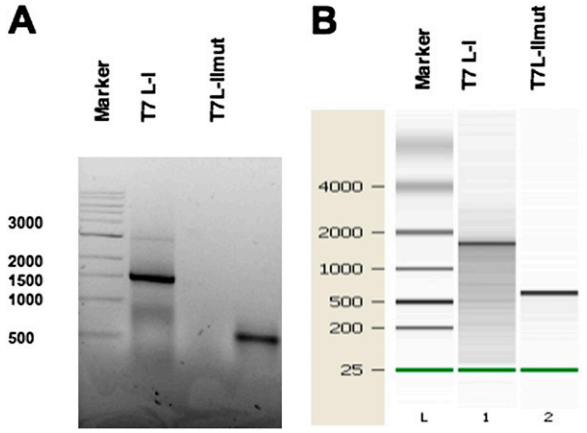

Fig. S4. Sizes of T7 RNA transcripts. Analysis of the T7 RNA transcripts in Fig. 3*H*. (*A*) Agarose gel. (*B*) Agilent Bioanalyzer. Size markers are indicated.

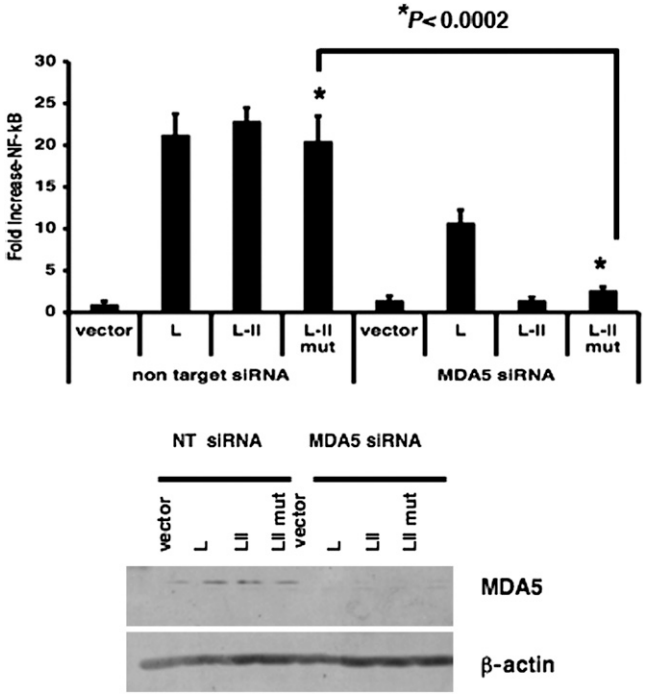

Fig. S5. Role of MDA5 in the activation of NF-κB by viral mRNA. The cells were transfected with siRNA targeting MDA5 or with control siRNA (NT siRNA). At 48 h after siRNA transfection, the cells were transfected with plasmids encoding L, L-II, or L-II mut, along with reporter luciferase genes. Luciferase activities were measured at 24 h after transfection. The amounts of MDA5 and β-actin in the lysates from the dual-luciferase assay were measured by immunoblot analysis.

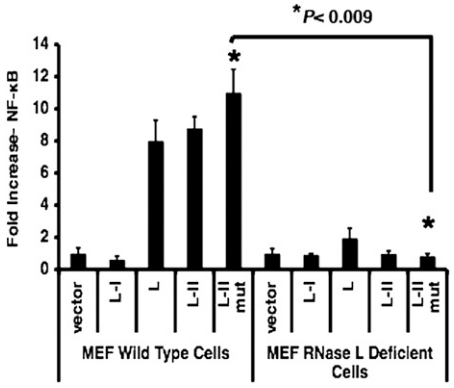

Fig. S6. Role of RNase L in activating NF-κB. A dual-luciferase assay for NF-κB activation was performed as described in Fig. 1*B* using WT or RLKO MEFs.

1. Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 108:193–199.