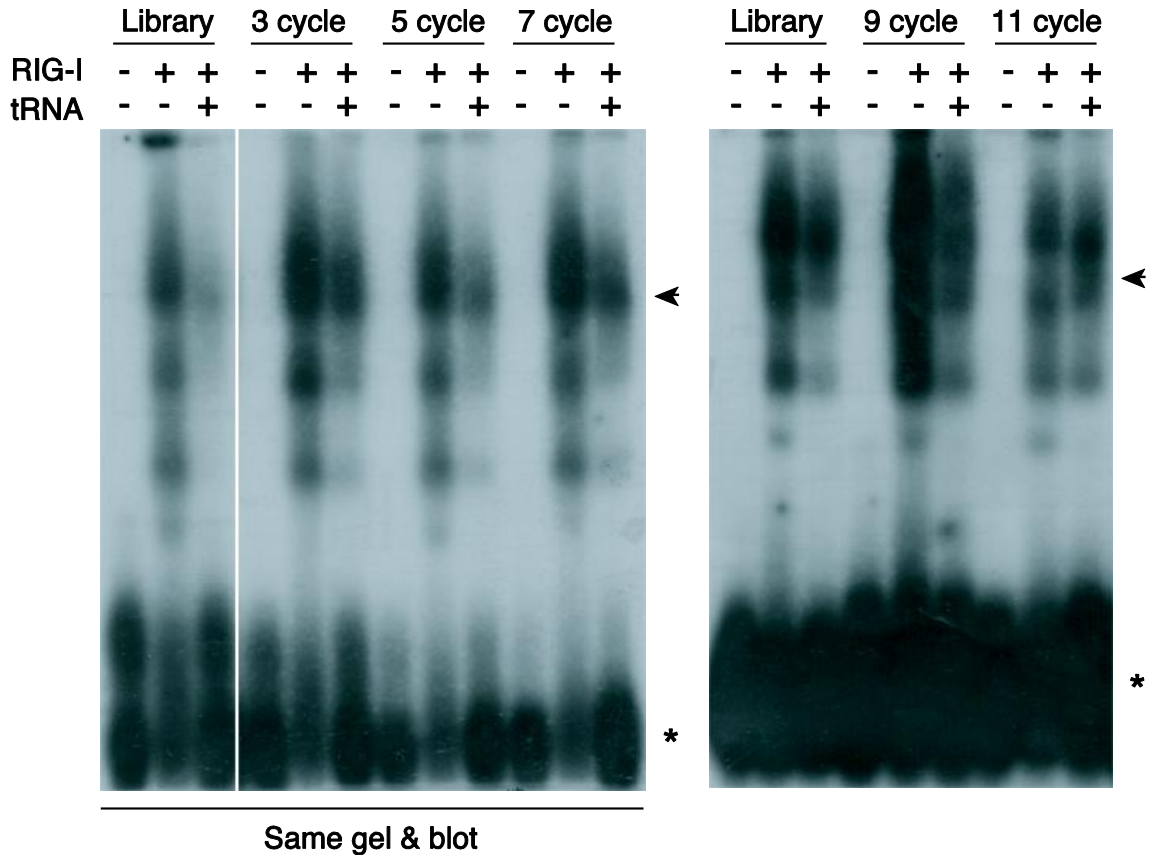
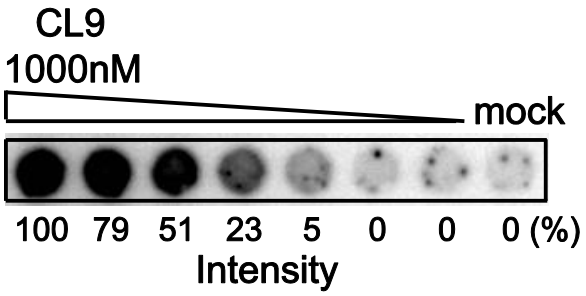
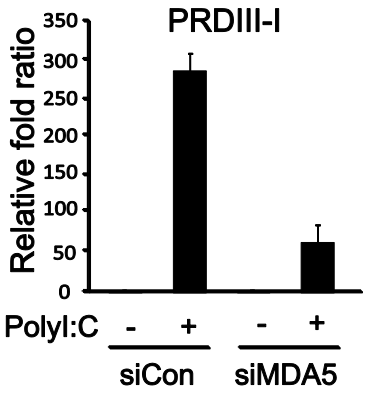
SUPPLEMENTARY INFORMATION



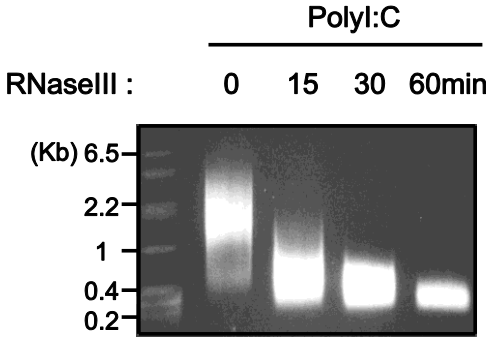
Supplementary Figure S1. RNA-EMSA using ^{32}P-labeled RNA aptamers and purified human RIG-I protein. The number of cycle indicates round of SELEX. Arrowhead indicates aptamer-RIG-I complex. Asterisk, free aptamer.



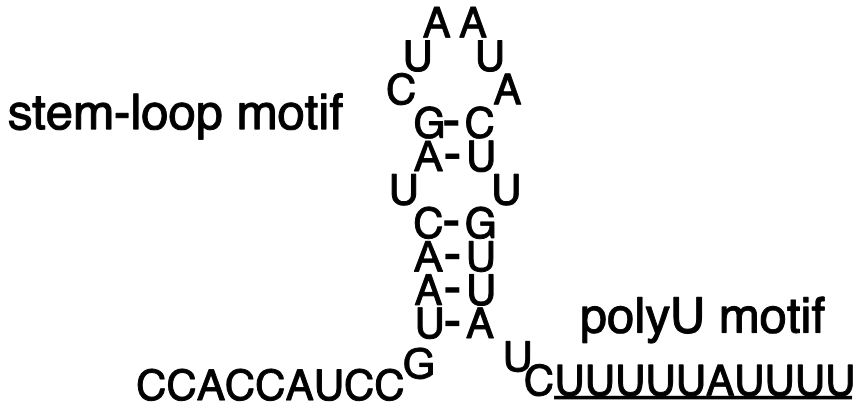
Supplementary Figure S2. The binding affinity (Kd) of AP-treated CL9 aptamer to full-length RIG-I measured by filter assay.



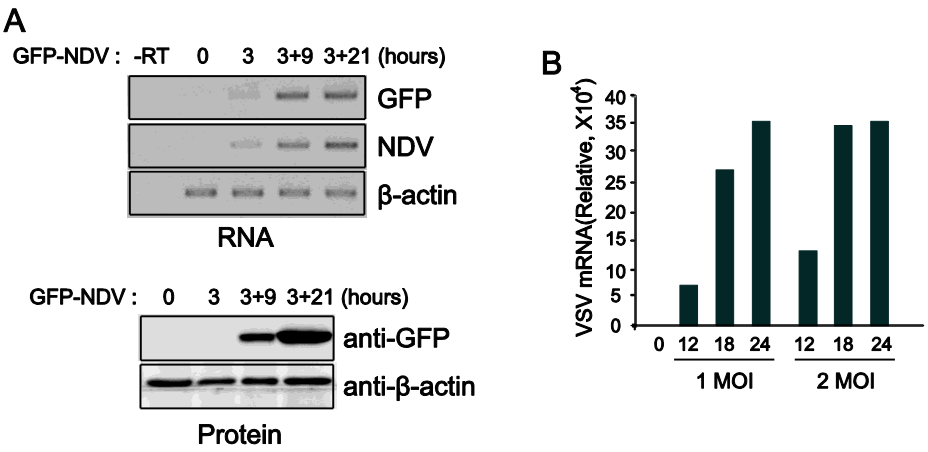
Supplementary Figure S3. HepG2 cells were transfected with siCon or siMDA5 for 36 hours, and then stimulated with PolyI:C for 6 hours.



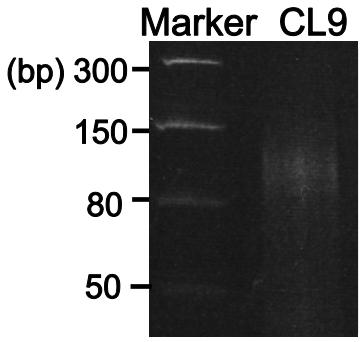
Supplementary Figure S4. To control the length of polyI:C, polyI:C was digested with RNaseIII for 1, 15, 30, and 60 minutes at 37°C and separated on a 0.8% agarose gel with EtBr staining.



Supplementary Figure S5. Predicted secondary structure of CL9. CL9 specific sequence were used for prediction of CL9 by M-fold (1).



Supplementary Figure S6. Kinetics of NDV-GFP and VSV-GFP replication. (A) HepG2 cells were infected with NDV-GFP virus for 3 hours, medium was changed, and cells were cultured for an additional 9 or 21 hours before RNA and protein analyses. **(B)** Similar to (A), except HepG2 cells were infected with VSV-GFP for indicated times.



Supplementary Figure S7. *In vitro* transcribed CL9 by T7 Polymerase was separated on a 12% Urea-PAGE with EtBr staining, and size of it was compared with ssRNA marker.

EXPERIMENTAL PROCEDURES

Binding affinity assays

We determined RNA–protein equilibrium dissociation constants (*K*d's) by the nitrocellulose-filter binding method as described (2). For all binding assays, RNAs were dephosphorylated using alkaline phosphatase(New England Biolab), and 5'-end labeled using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and γ^{32}PATP (Amersham Pharmacia Biotech, Piscataway,NJ) as described (3). Before binding assay, heat aptamer at 95℃ 3min, and then slowly ramp to 37℃ at 0.1℃ /sec in buffer (40 mM HEPES/pH 7.5, 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 0.002% tween-20) for aptamer refolding. Direct binding assays were carried out by incubating ^{32}P-labeled RNA at a concentration of less than 10 pM and protein at concentrations ranging from 1 mM to 10 fM in selection buffer at 37℃. The fraction of RNA bound was quantified with a PhosphorImager (Fuji FLA-5100 Image Analyzer, Tokyo, Japan). Raw binding data were corrected for nonspecific background binding of radiolabeled RNA to the nitrocellulose filter as described (2) and reported as the mean ± standard error of the mean (SEM) of three experiments.

In Figure 1C & Supplementary Figure S1, binding mixture of ^{32}P-labeled aptamer(1 X 10^5 CPMA), 3 μg of yeast tRNA, and100 nM of RIG-I protein was separated in 4% PAGE containing 1.33% Glycerol. To detection radioactivity of aptamer, it was exposed on X-ray film (Fujifilm).

Size control of PolyI:C

To obtain the short form of polyI:C, 1 U of RNaseIII (Takara) was incubated with 10 μg of polyI:C at 37℃ for 1 hour, and fragmented polyI:C was precipitated using phenol/ethanol precipitation (4).

REFERENCES

1. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res*, **31**, 3406-3415.
2. White, R., Rusconi, C., Scardino, E., Wolberg, A., Lawson, J., Hoffman, M. and Sullenger, B. (2001) Generation of species cross-reactive aptamers using "toggle" SELEX. *Mol Ther*, **4**, 567-573.
3. Fitzwater, T. and Polisky, B. (1996) A SELEX primer. *Methods Enzymol*, **267**, 275-301.
4. Kato, H., Takeuchi, O., Mikamo-Satoh, E., Hirai, R., Kawai, T., Matsushita, K., Hiiragi, A., Dermody, T.S., Fujita, T. and Akira, S. (2008) Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med*, **205**, 1601-1610.