Supplementary Data

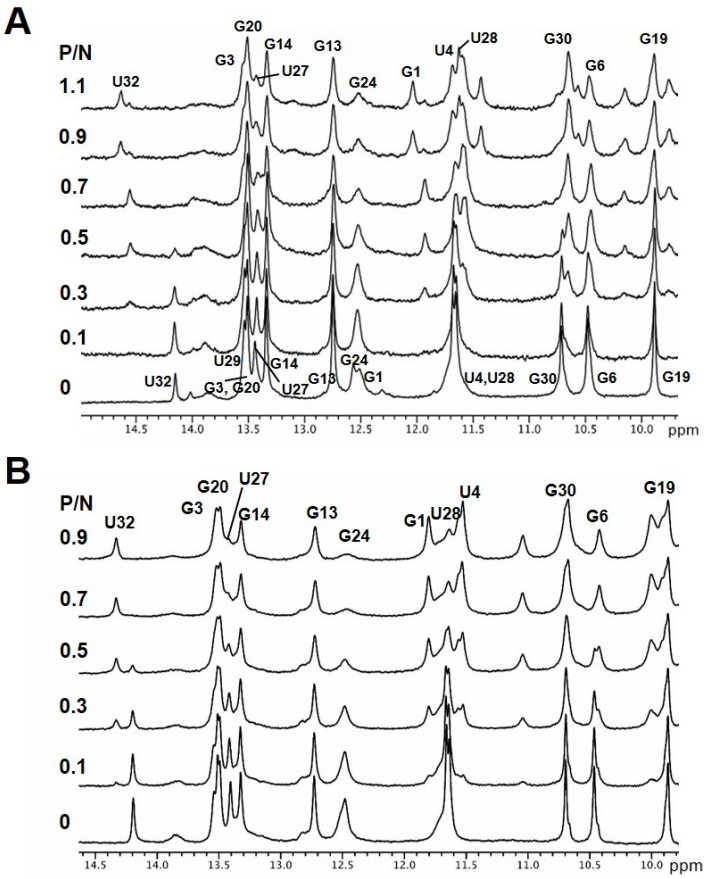

Figure S1. (**A**) Imino proton NMR spectra of 5'-PPP-iav upon titration with RIG-I CTD. P/N, protein-to-RNA molar ratios. (**B**) Imino proton NMR spectra of free and bound 5'-OH-iav RNA at various protein-to-RNA (P/N) ratios.

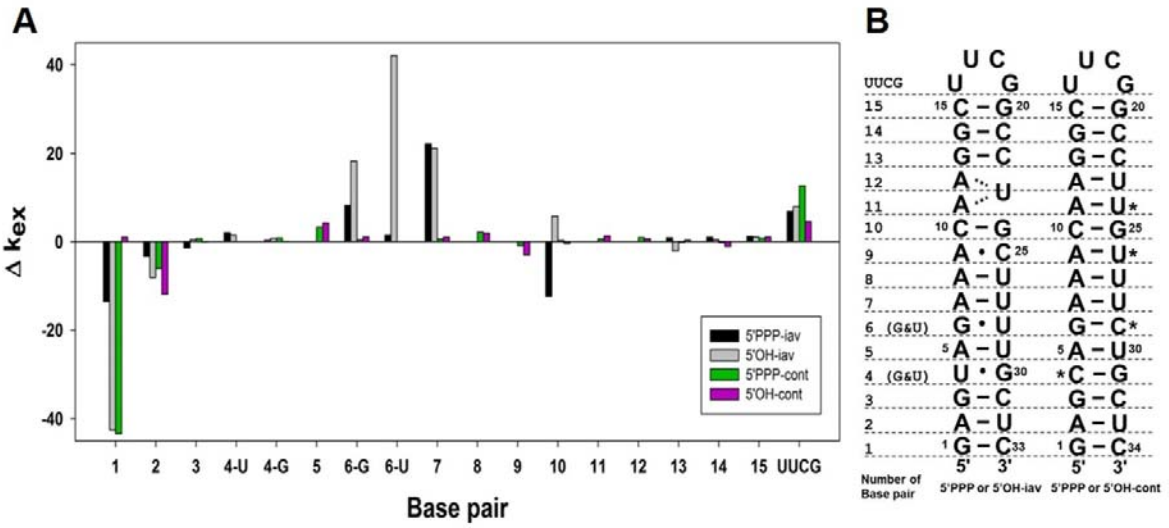

Figure S2. (**A**) Difference between k_{ex} values of free RNAs and RIG-I CTD-bound RNAs (Δk_{ex}). RNAs are 5'-PPP-iav, 5'-OH-iav, 5'-PPP-cont, and 5'-OH-cont. (**B**) Numbering of base pairs in sequences of iav RNAs and cont RNAs

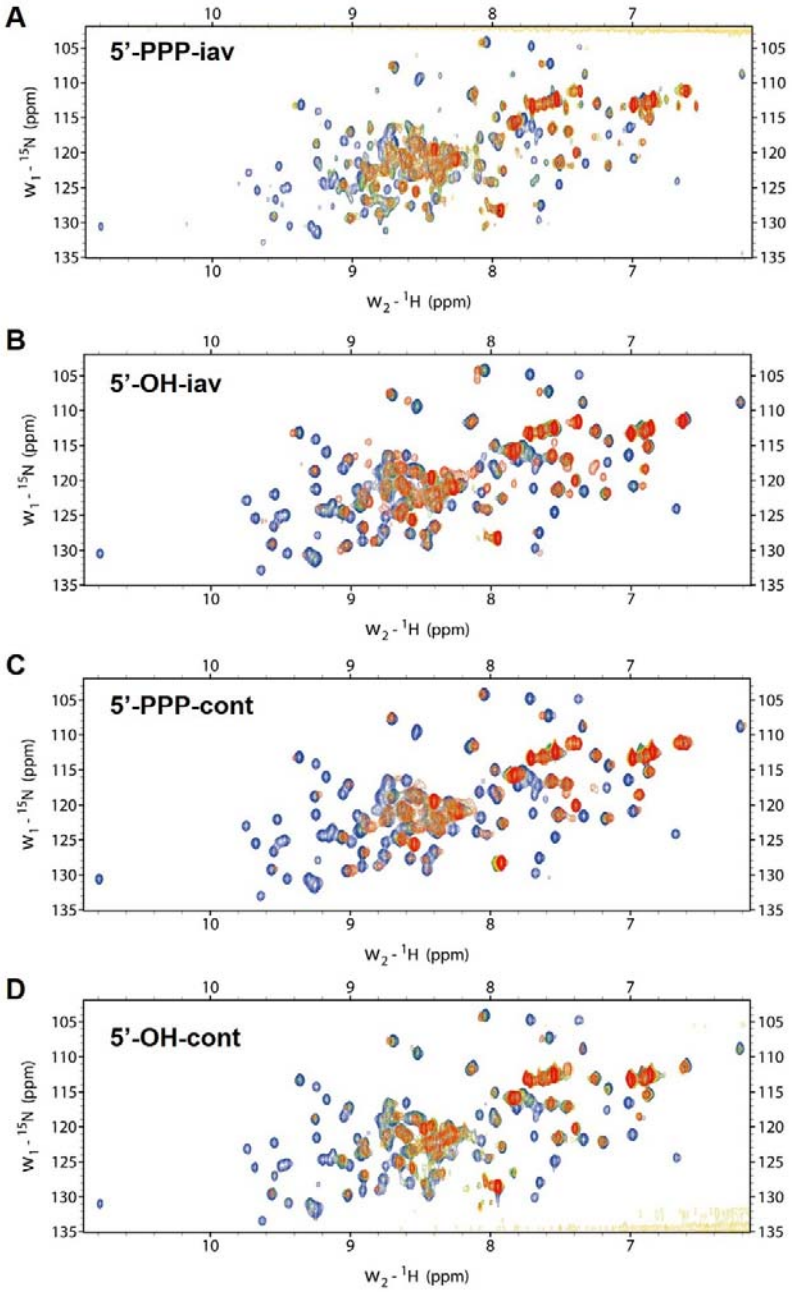

Figure S3. NMR titration of ^{15}N-labeled RIG-I CTD with iav RNAs and cont RNAs. ^{15}N-^{1}H-HSQC spectra recorded at variant RNA-to-Protein (N/P) molar ratios were overlapped (blue, N/P=0; green, N/P=0.25; yellow, N/P=0.5; red, N/P=1).

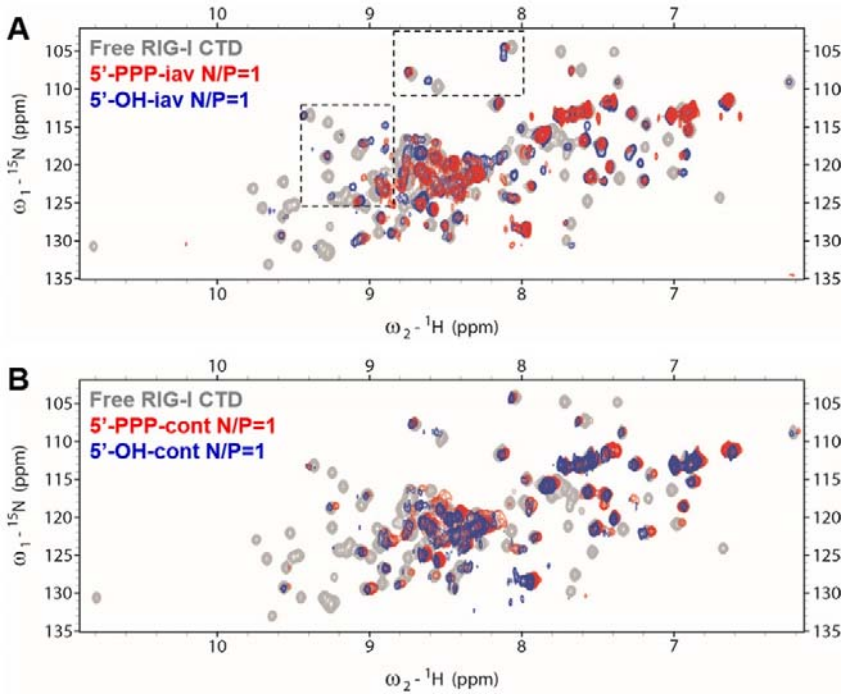

Figure S4. NMR titration of ^{15}N-labeled RIG-I CTD with iav RNAs and cont RNAs. (**A**) ^{15}N-^{1}H-HSQC spectra of free RIG-I CTD, 5'-PPP-iav/RIG-I CTD complex, and 5'-OH-iav/RIG-I CTD complex (gray, free protein; red, 1:1 complex with 5'-PPP-iav; blue, 1:1 complex with 5'-OH-iav). Amide protons in the dotted boxes revealed dramatic changes in chemical shift upon RIG-I CTD binding to 5'-OH-iav. N/P indicates RNA-to-Protein molar ratios. (**B**) ^{15}N-^{1}H-HSQC spectra of free RIG-I CTD, 5'-PPP-cont/RIG-I CTD complex, and 5'-OH-cont/RIG-I CTD complex (gray, free protein; red, 1:1 complex with 5'-PPP-cont; blue, 1:1 complex with 5'-OH-cont).

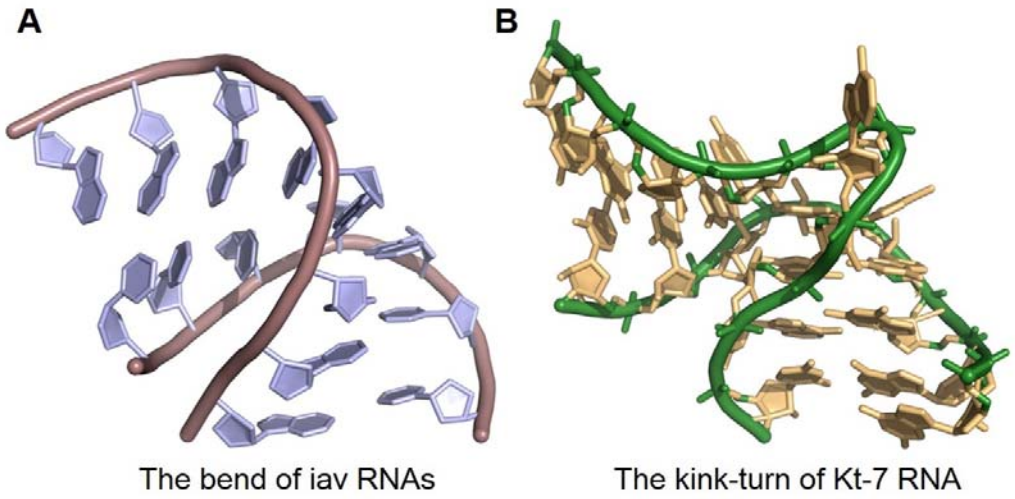

Figure S5. (A) The bend structure of iav RNAs (PDB: 1JO7). (B) The kink-turn structure of *H. marismortui* ribosomal Kt-7 RNA (PDB: 4CS1).

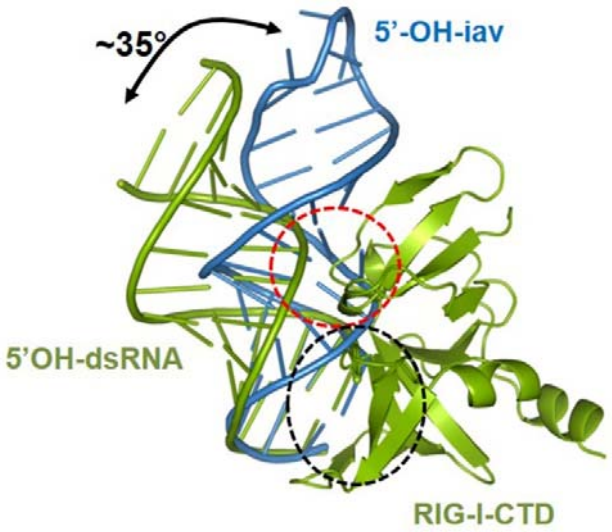

Figure S6. Influenza panhandle RNA (blue, PDB 1JO7) was aligned to the bound form of 5'-OH-14bp-GC-dsRNA (green, PDB 3OG8). Influenza RNA was aligned to the terminal 1-4 base pairs of 5'-OH-14bp-GC-dsRNAs in crystal complex. The red circle indicates alternative binding sites between 5'-OH-iav RNA and RIG-I CTD. RIG-I CTD might recognize the RNA's terminal duplex with or without 5'-PPP moiety in black circled region.

Table S1. RIG-I-dependent Induction of IFN-β by the panhandle RNAs.

Name	Ctrl siRNA	RIG-I siRNA
Poly (I:C)	12.1 ± 1.6	3.9 ± 0.3
Medium	1.0 ± 0.1	1.8 ± 0.4
5′-OH-cont	1.0 ± 0.1	1.2 ± 0.2
5′-OH-iav	7.5 ± 0.5	3.84 ± 0.02
5′-PPP-cont	9.8 ± 1.8	5.6 ± 0.1
5′-PPP-iav	9.4 ± 1.5	5.4 ± 0.2
5′-OH-Int-NS1	13.0 ± 2.1	4.6 ± 0.4
5′-OH-Bend-GC	5.5 ± 0.8	1.3 ± 0.2

Table S2. RIG-I-dependent Induction of ISG56 by the panhandle RNAs.

Name	Ctrl siRNA	RIG-I siRNA
Medium	0.10 ± 0.10	0.13 ± 0.10
5′-OH-cont	0.22 ± 0.16	0.12 ± 0.08
5′-OH-iav	0.83 ± 0.30	0.20 ± 0.14
5′-PPP-cont	0.97 ± 0.42	0.34 ± 0.25
5′-PPP-iav	1.00 ± 0.25	0.27 ± 0.12
5′-OH-Kink	0.76 ± 0.24	0.26 ± 0.20