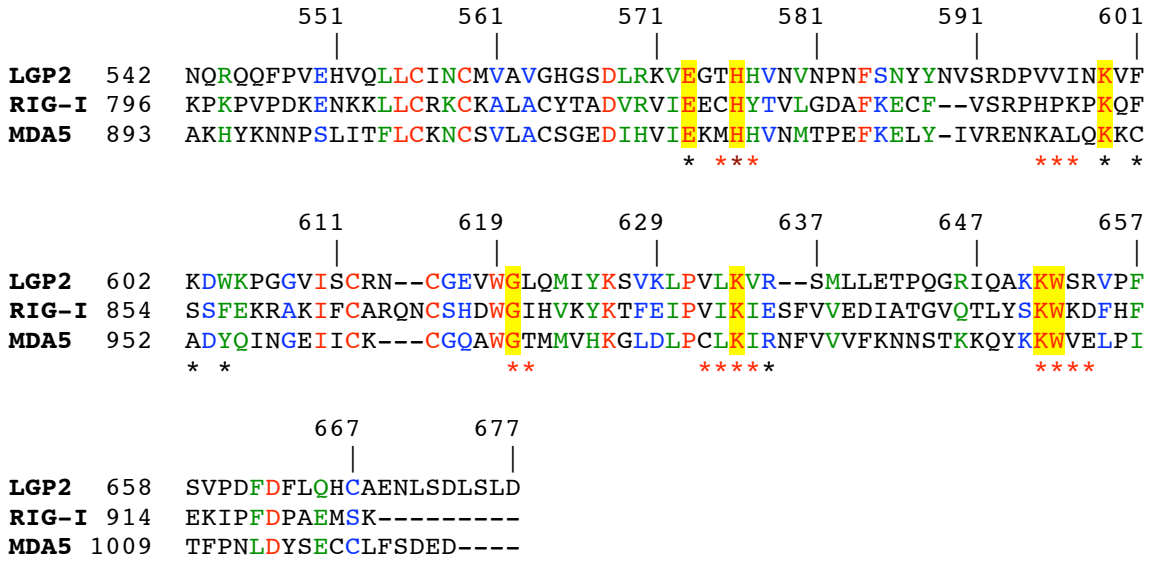
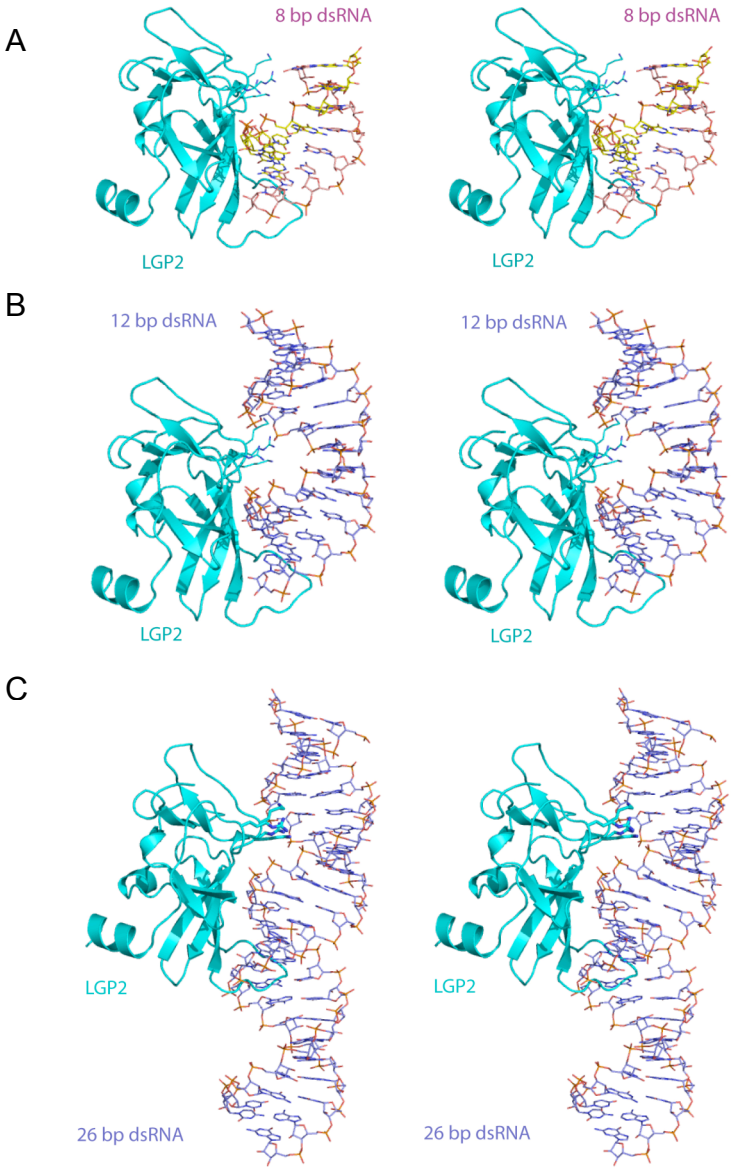
Supplementary Material for "The RIG-I like Receptor LGP2 recognizes the Termini of Double-stranded RNA"

Xiaojun Li, C. T. Ranjith-Kumar, Monica T. Brooks, S. Dharmaiah, Andrew B. Herr, C. Cheng Kao, Pingwei Li *

* To whom correspondence should be addressed: Tel: 979-845-1469; Email: pingwei@tamu.edu



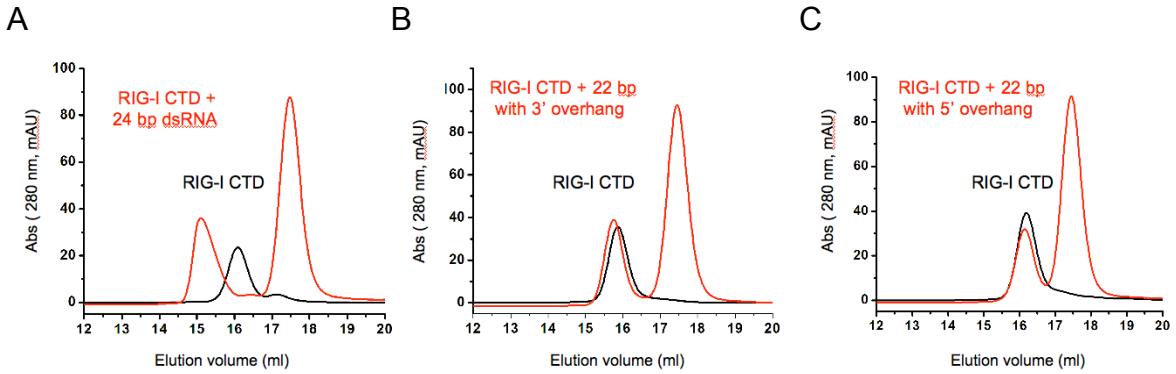
Supplementary Figure 1 Sequence alignment of human LGP2, RIG-I, and MDA5 C-terminal domains. Residues of LGP2 involved in binding with the 5' end of the dsRNA are labeled with red asterisks; residues involved in binding with the 3' end of the dsRNA are labeled with black asterisks. His576, which is involved in binding of both strands, is labeled with brown asterisk. Residues conserved in all three proteins are in red. Conserved residues involved in dsRNA binding highlighted in yellow. Conservatively replaced residues are in green and blue.



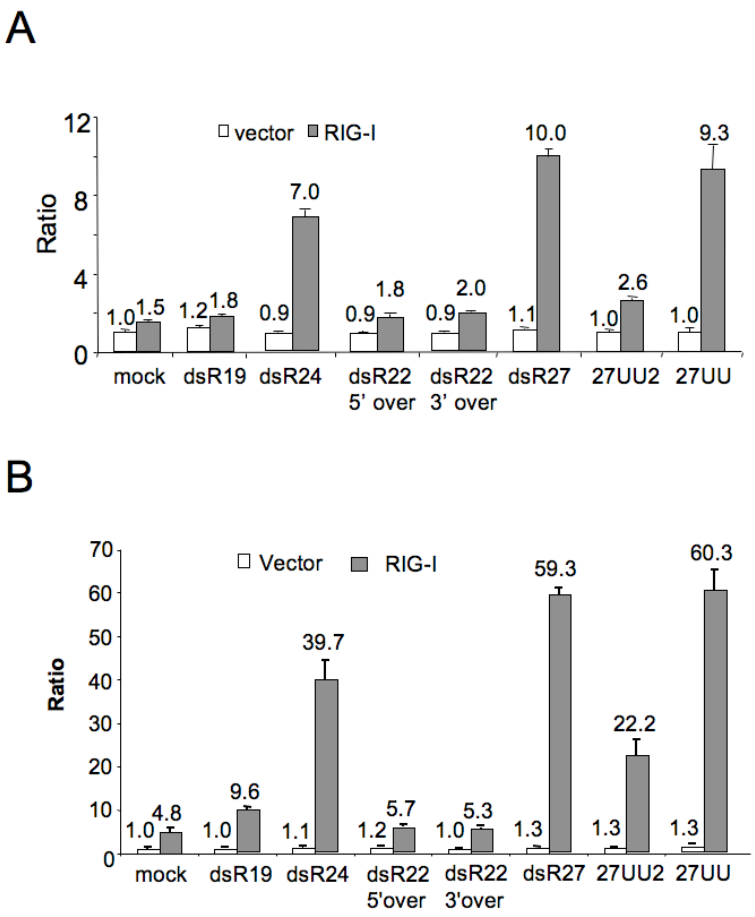
Supplementary Figure 2 Molecular models of LGP2 CTD bound to a 12 bp and a 26 bp dsRNA. A, Stereo view of the structure of LGP2 CTD bound to the 8 bp dsRNA. Residues Arg636, Lys650, Lys651, and Arg654 are shown as stick models. B, Stereo view of the molecular model of LGP2 CTD bound to a 12 bp dsRNA. C, Stereo view of the molecular model of LGP2 CTD bound to the middle of a 26 bp dsRNA.

Supplementary Table 1 Sequences of dsRNA used in binding studies, analytical ultracentrifugation, and cell-based assays.

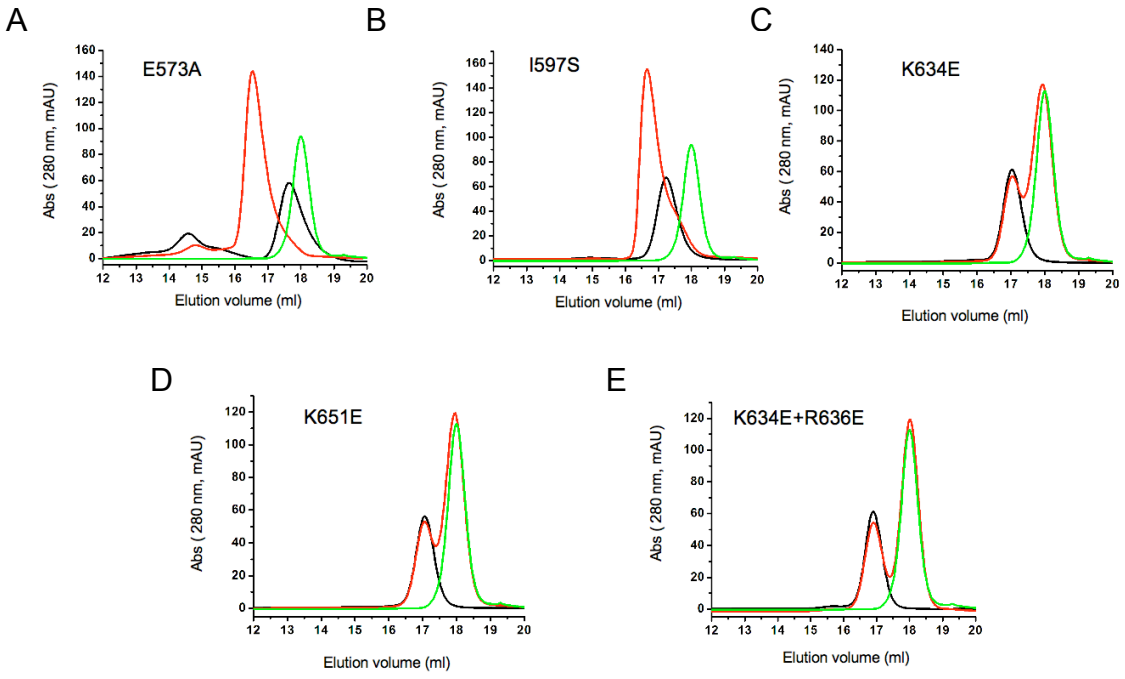
8 bp dsRNA	5' GCGCGCGC 3' CGCGCGCG
24 nucleotides small hairpin RNA (shRNA)	AAA 5' CCGCAUUG A 3' GGCGUAAC G GUU
24 bp dsRNA (dsR24) 24 bp 5' ppp dsRNA	5' GCGCGCAUGCGCGCGCAUGCGCGC 3' CGCGCGUACGCGCGCGUACGCGCG
22 bp dsRNA with 5' overhangs	5' AAGCGCGCUGCGCGCAGCGCGC 3' CGCGCGACGCGCGUCGCGCGAA
22 bp dsRNA with 3' overhangs	5' GCGCGCUGCGCGCAGCGCGCAA 3' AACGCGCGACGCGCGUCGCGCG
19 bp dsRNA (dsR19)	5' UGGUUUACAUGUUCCAAUA 3' ACCAAAUGUACAAGGUUAU
27 bp dsRNA (dsR27)	5' AAGCUGACCCUGAAGUUCAUCUGCACC 3' UUCGACUGGGACUUCAAGUAGACGUGG
27 bp dsRNA with 3' overhangs (27UU2)	5' AAGCUGACCCUGAAGUUCAUCUGCACCUU 3' UUUUCGACUGGGACUUCAAGUAGACGUGG
27 bp dsRNA with only one 3' overhang (27UU)	5' AAGCUGACCCUGAAGUUCAUCUGCACCUU 3' UUCGACUGGGACUUCAAGUAGACGUGG



Supplementary Figure 3 Binding studies of RIG-I CTD with different dsRNAs by gel filtration chromatography. A, RIG-I CTD binding with a 24 bp dsRNA containing two blunt ends. B, RIG-I CTD binding with a 22 bp dsRNA containing two overhanging nucleotides at the 3' ends. C, RIG-I CTD binding with a 22 bp dsRNA with two overhanging nucleotides at the 5' ends.



Supplementary Figure 4 Double-stranded RNAs with blunt end can activate RIG-I signaling. Different forms of dsRNA were transfected into HEK 293T cells with RIG-I-expressing vector (grey bars) or an empty vector control (white bars) and luciferase driven from an NF-κB (A) or IFN-β (B) promoter. All signals shown are the ratios of the NF-κB or IFN-β luciferase versus *Renilla* luciferase driven from a constitutive herpesvirus thymidine kinase promoter. dsRNA with blunt ends (dsR24, dsR27, and 27UU) stimulated the activation of RIG-I more efficiently than dsRNA with either 5' or 3' overhangs (dsR22 5' over, dsR22 3' over, and 27UU2).



Supplementary Figure 5 Analysis of LGP2 CTD mutant proteins binding to dsRNA using gel filtration chromatography. Each panel contains the elution profile of the 8 bp dsRNA (green chromatogram), LGP2 CTD mutant protein (black chromatogram), and a 2:1 mixture of the protein and the RNA (red chromatogram). A, Analysis of LGP2 CTD mutant E573A. B, Analysis of mutant I597S. C, Analysis of mutant K634E. D, Analysis of mutant K651E. E, Analysis of a double mutant K634E/R636E.