Supplementary Information

Structural insights into RNA recognition by RIG-I

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Legends for Supplementary Figures and Tables

Figure S1, related to Figure 1
2Fo-Fc map at the three-ways interface between HEL1, CTD, and dsRNA.

Figure S2, related to Figure 3
Sequence alignment of the RIG-I and related helicases Sequence alignment of the closely related SF2 helicases, including RIG-I orthologs, MDA-5 and LGP-2 from *Homo sapiens*, Dicer-related-Helicase 3 (DRH-3) from *Caenorhabditis elegans*, Hef helicase from *Pyrococcus furiosus*, Rad54 from *Sulfolobus solfataricus*, Dicer from *Homo sapiens*, and eIF4A3 from *Homo sapiens*

Figure S3, related to Figure 1
SAXS analysis of RNA binding induced conformational changes of RIG-I (ΔCARDs). (**A**) Experimental SAXS data for the RIG-I (ΔCARDs) and RIG-I (ΔCARDs):dsRNA10 complex are shown as red and blue lines with error bars. The theoretical scattering curve for the RIG-I (ΔCARDs):dsRNA10 crystal structure is in black. Momentum transfer, s, is defined as $4\pi\mathrm{Sin}(\theta)/\lambda$, where 2θ is the angle between the incident and scattered radiation and λ is the wavelength of the X-ray beam. Correlation between the scattering curve for RIG-I (ΔCARDs):dsRNA10 (blue) and the theoretical curve (black) results in a $\chi 2$ value of 4.80. That $\chi 2$ is not closer to 1.0 is likely due to compaction from crystallization conditions, and because the construct contains additional N-terminal tail residues and residues from HEL2 that were not visualized or modeled in the structure. By contrast, correlation between the scattering curve for free RIG-I (ΔCARDs) protein and the theoretical curve results in a $\chi 2$ value of 20.9, which indicates that the isolated protein adopts a different conformation from the crystal structure. (**B**) The distance distribution function $P(r)$ of the RIG-I (ΔCARDs) (red) and RIG-I (ΔCARDs):dsRNA10 (blue), showing that the former species has two large centers of mass.

Figure S4, related to Figure 2 and 4
Comparison with the closely related SF2 helicases
(A) Superposition of the RIG-I (ΔCARDs) and dsRNA10 complex (red ribbon) and Archaea SF2 helicase (Hef from *Pyrococcus furiosus*, blue color), based on HEL1. **(B**) Superposition of the HEL2i. RIG-I is in red and Hef blue. (**C)** Superposition of the RIG-I (ΔCARDs) and dsRNA10 complex (red ribbon) and Eukaryotic DEAD-box helicase (eIF4A3 from *Homo sapiens*, blue color), ssRNA, ATP analog (AMPPNP-Mg^{2+}) complex, based on HEL1. (**D**) Close-up view of **(C)**, highlighting the DExx and IIa motifs.

Figure S5, related to Figure 5 and 6
IFN-β luciferase reporter assay for RIG-I signaling in Huh7.5 cells and HEK293T cells
Different amount of pUNO-hsRIG-I plasmid was transfected into either Huh7.5 cells and HEK293T cells. Huh7.5 cells showed very low unstimulated activity and high response to polyI:C stimulation at up to 3 ng of RIG-I plasmids.

Figure S6, related to Figure 7
Limited RNase digestion of RIG-I (ΔCARDs) / polyI:C complex
Low molecular weight polyI:C (Invivogen) was pre-incubated with RIG-I (ΔCARDs). The complex was then subject to RNase digestion (RNase ONE (Promega) and RNase V1 (Ambion)), 4°C overnight. The reaction was boiled and denatured with 0.1% SDS. The undigested RNA fragments were then resolved with 20% denaturing PAGE. Short oligos (dsGC10, 12, 14, 18, and 22) were loaded in the same gel as markers. Around 10-15 bases are protected by RIG-I (ΔCARDs).

Figure S7, related to Figure 7
Analytical size exclusion chromatography of RIG-I (ΔCARDs) : dsRNA oligo complexes.
Before each SEC run, RIG-I (ΔCARDs) was preincubated with 1.5 molar excess amount of dsRNA oligo, 1 mM AMPPNP (Adenosine 5′-(β,γ-imido)triphosphate, non hydrolysable ATP analog), and 1mM $MnCl_2$. Superdex^{TM} 200/10300 (GEHealthcare) is used for SEC runs. All dsRNA oligos are chemically synthesized palindromic GC repeats. See Supplementary figure 4 for denaturing PAGE profile of the oligos. Significant dimerization of RIG-I (ΔCARDs) is only observed for dsGC18 and dsGC22, see panel E and F.

Table S1
Data collection and Refinement Statistics

Table S2
Primers for mutagenesis

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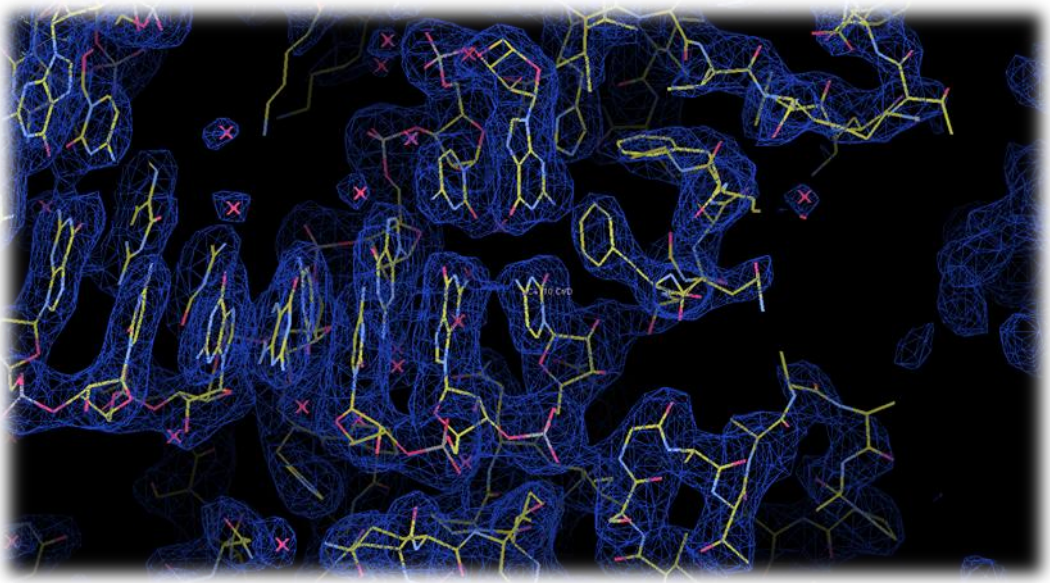
2Fo-Fc map at the three-ways interface between HEL1, CTD, and dsRNA.



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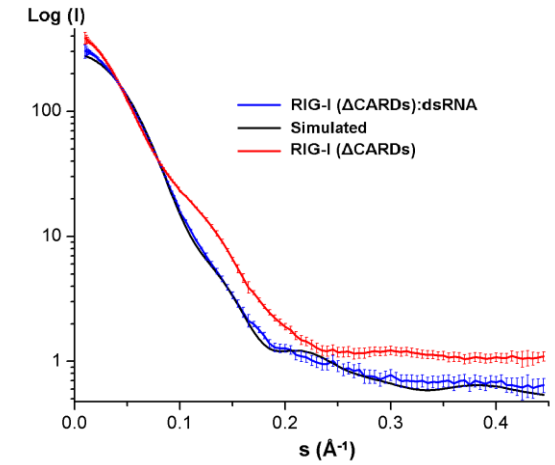
A



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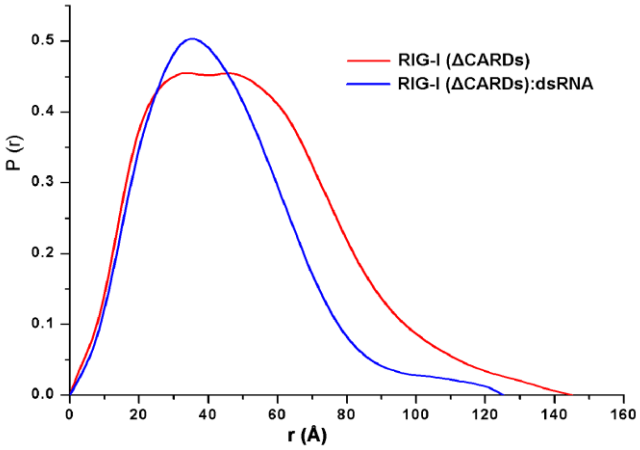

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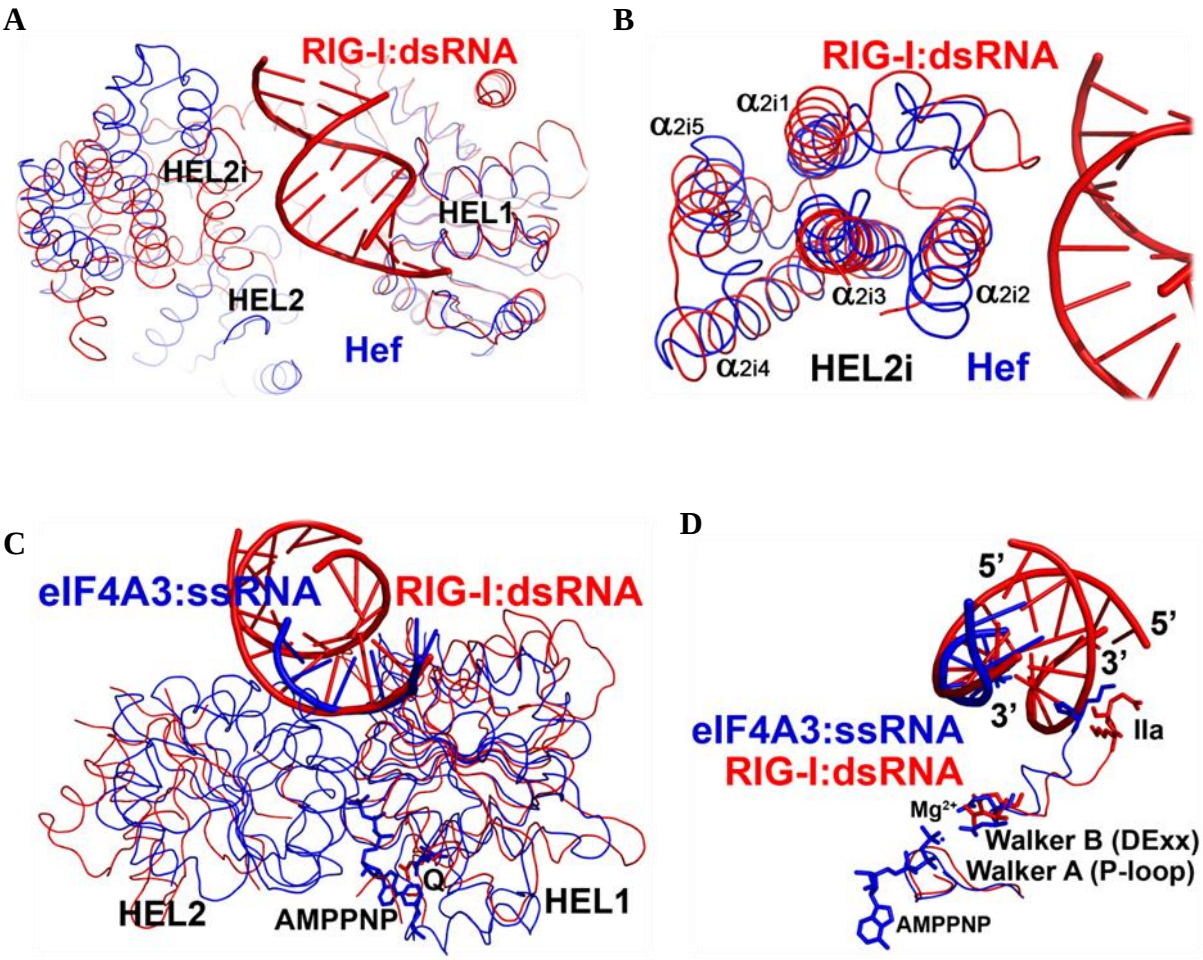

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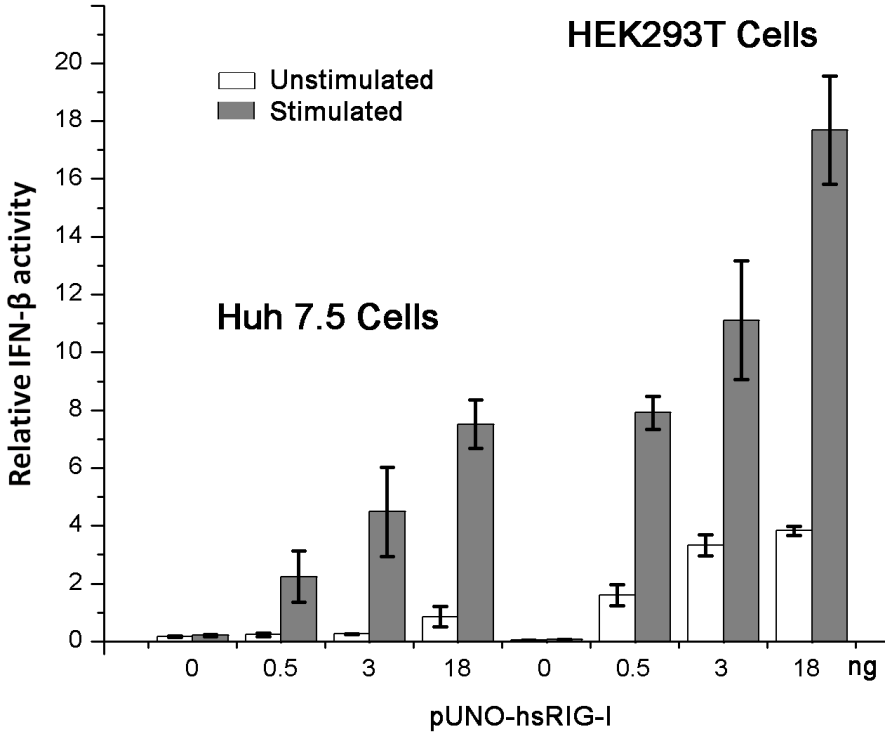

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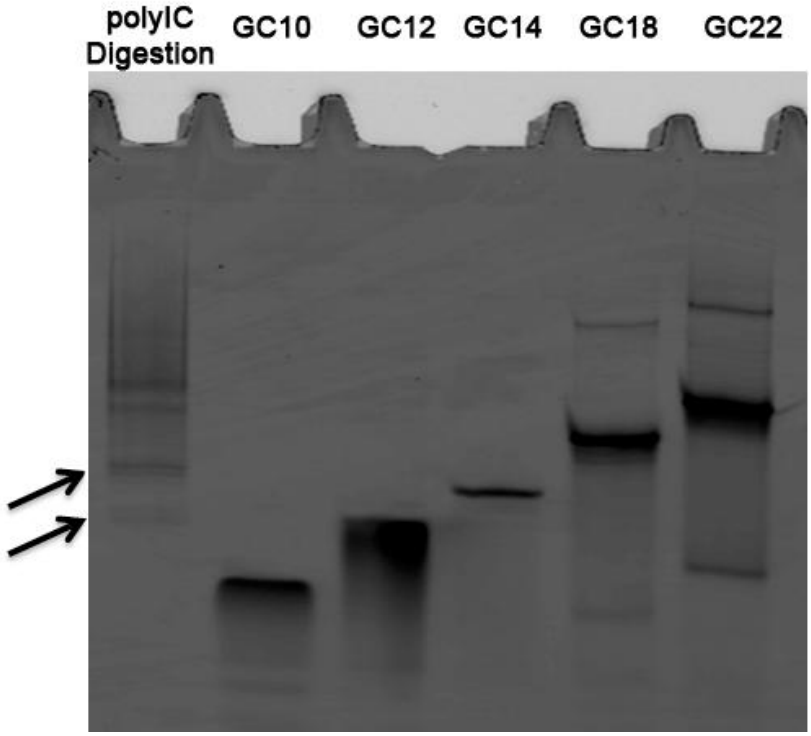

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