

Supplementary Information

Supplementary Materials and Methods

Constructs and cloning

The sequence encoding full-length *Mus musculus* RIG-I was amplified from total mouse cDNA (courtesy of Dr. Stefan Bauersachs) and cloned into pET28 (Novagen). Δ RD RIG-I was subcloned in a modified pET21a (Novagen) to express 6xHis-MBP-TEV-RIG-I (1-797). The mutants (T348E, E374Q, K380E, double mutant Q508AQ512A, R547E, and R731A,) were generated by site directed mutagenesis with PfuUltra (Stratagene).

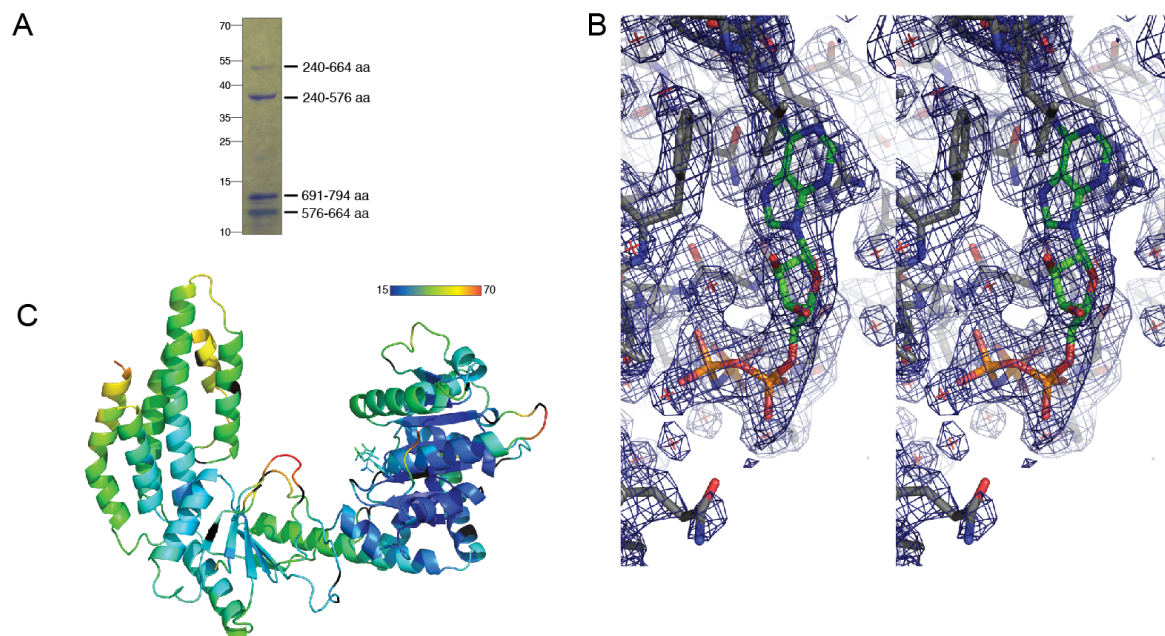
Expression and protein purification

All constructs were expressed in *E.coli* Rosetta (DE3) or B834 (DE3) strains. Native protein was grown in LB media and selenomethionine labeled protein in modified M9 media. Bacteria were grown until an OD₆₀₀ of 0.6 to 0.8 and induced at 18°C for 16 to 18 hrs with 0.1mM IPTG. Proteins were purified by Ni-NTA agarose resin and when applicable incubated with TEV protease (ratio 1:50) at 4°C for 20 to 22 hrs to remove the 6xHis-MBP-tag. The proteins were further purified by ion exchange followed by size exclusion chromatography using a Superdex 200 column (GE Healthcare) equilibrated in 20mM TRIS pH 7.5, 150mM NaCl and 1mM DTT. Purified RIG-I Δ RD was concentrated to 27.5 mg/ml for crystallization. All purified proteins were frozen in liquid N₂ and stored at -80°C.

Crystallization

Selenomethionine labeled RIG-I^{SF2} was crystallized using an in-drop proteolysis approach. To purified RIG-I (27.5 mg/ml) 1:10 (v/v) of 50 mM of ATP derivative adenosine 5'-(β,γ -imido)triphosphate (AMP-PNP) and 1:500 (w/w) 1mg/ml subtilisin were added and the mix was crystallized by sitting drop vapor diffusion method in crystallization solution [100mM BIS-TRIS pH 6.6 and 22% (w/v) PEG3350]. The crystals appeared in 1 day at 20°C and were soaked in cryoprotectant solution (15% ethane-1,2-diol in crystallization solution) and flash frozen. SDS-PAGE analysis of the crystals (Suppl. Fig. 1A) shows that the protease cleaved between the N-terminal CARDs and SF2 as well as removing two internal loops of SF2.

Supplementary Figures

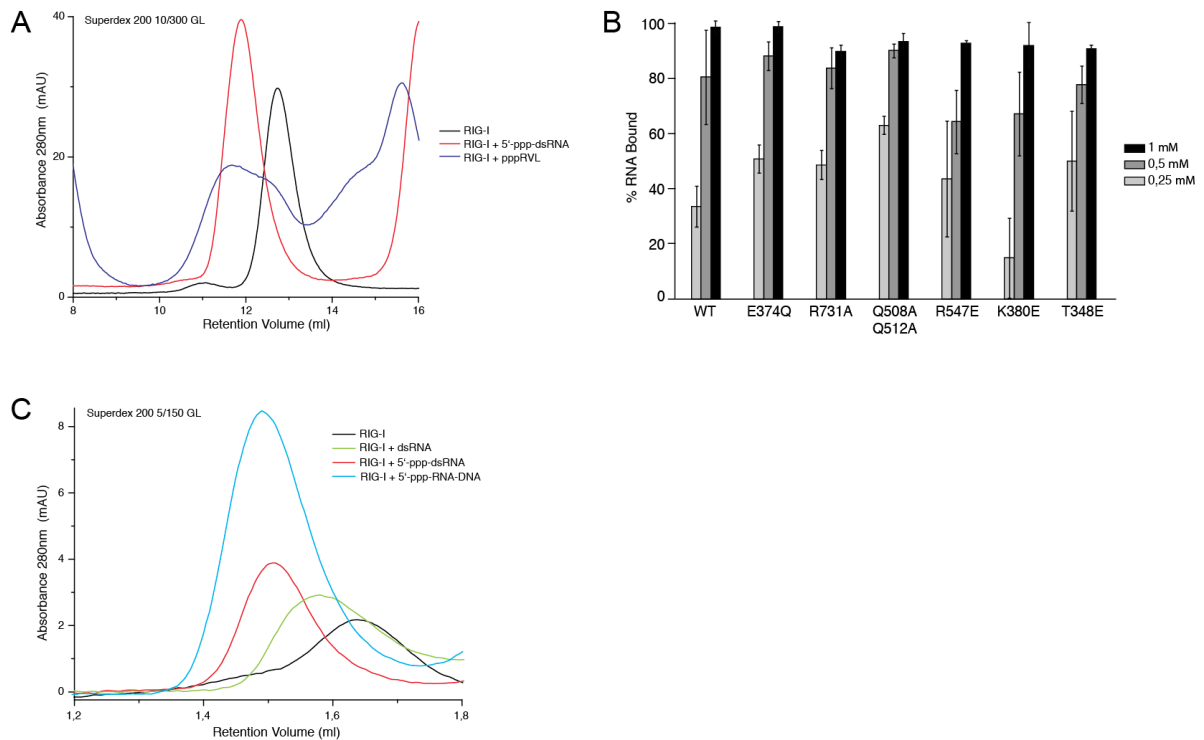


Supplementary Figure 1: Structure of RIG-I SF2 Domain

A) Crystals of RIG-I SF2 domain were collected and washed three times with reservoir solution and loaded on SDS-PAGE. The bands were assigned the indicated regions based on the ordered parts in crystal structure.

B) 2Fo-Fc electron density overlaid with the final model around the AMP-PNP molecule. The $2F_o - F_c$ map is contoured at 1 σ .

C) View of the RIG-I SF2 domain structure in the orientation of Fig 1A, colored according to the C α B-factors. Black residues are glycines.

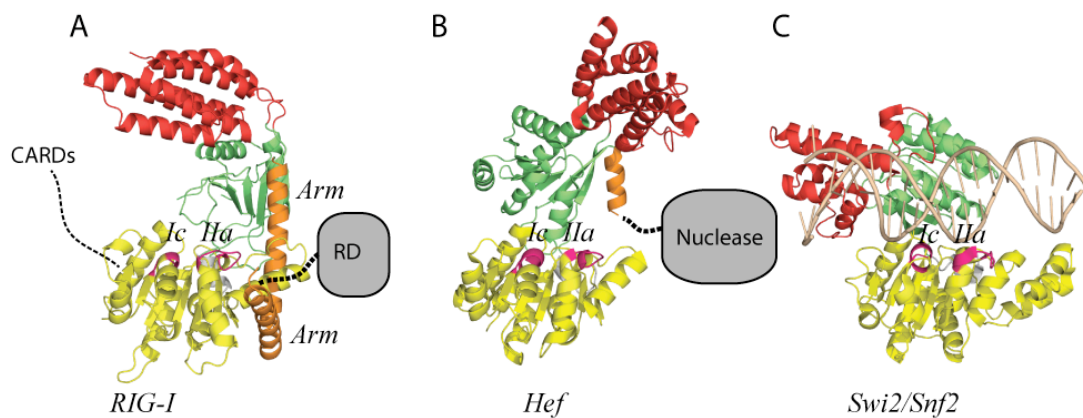


Supplementary Figure 2: RNA dependent activation of RIG-I

A) Size exclusion chromatography analysis of RIG-I (black) preincubated with either chemically synthesized 5'-ppp-dsRNA (25-mer) (red) or *in vitro* transcribed 5'-ppp containing rabies virus leader RNA 58-mer (pppRVL) (blue). Upon 5'-ppp-dsRNA binding RIG-I dimerizes as in the case of pppRVL (Cui et al, 2008).

B) Electrophoretic mobility shift analysis of RIG-I mutants bound to 25-mer dsRNA. Plotted bars: mean \pm sd (n=3)

C) Size exclusion chromatography analysis of RIG-I (black) preincubated with 5'-ppp-dsRNA (25-mer) (red) or dsRNA (25mer) (green) or 5'-ppp-RNA-DNA hybrid (cyan). Hybrid ppp-nucleic acid dimerizes RIG-I like ppp-dsRNA



Supplementary Figure 3: Comparison to Hef and Swi/Snf2 dsDNA binding proteins

Comparison of crystal structures of the SF2 domains of RIG-I (A, this work), Hef (B, (Nishino et al, 2005)) and Swi/Snf2 in complex with dsDNA (C, (Durr et al, 2005)) RIG-I and Hef have a related domain structure, although the orientation of domains 1 (yellow) and 2 (green) are more canonical in Hef, while oriented in a rather non-canonical orientation in RIG-I by the RLR specific helical arm. RD and CARDs are attached to SF2 by linkers, while Hef contains a linked nuclease domain. Swi/Snf2 binds dsDNA at motifs Ic and Ila, which we identify by mutational analysis in RIG-I. The overall similarity of domains 1a in all three structures suggests a related mode of double-stranded nucleic acid binding.

Supplementary references

Cui S, Eisenacher K, Kirchhofer A, Brzozka K, Lammens A, Lammens K, Fujita T, Conzelmann KK, Krug A, Hopfner KP (2008) The C-terminal regulatory domain is the RNA 5'-triphosphate sensor of RIG-I. *Mol Cell* **29**(2): 169-179

Durr H, Korner C, Muller M, Hickmann V, Hopfner KP (2005) X-ray structures of the *Sulfolobus solfataricus* SWI2/SNF2 ATPase core and its complex with DNA. *Cell* **121**(3): 363-373

Nishino T, Komori K, Tsuchiya D, Ishino Y, Morikawa K (2005) Crystal structure and functional implications of *Pyrococcus furiosus* hef helicase domain involved in branched DNA processing. *Structure* **13**(1): 143-153