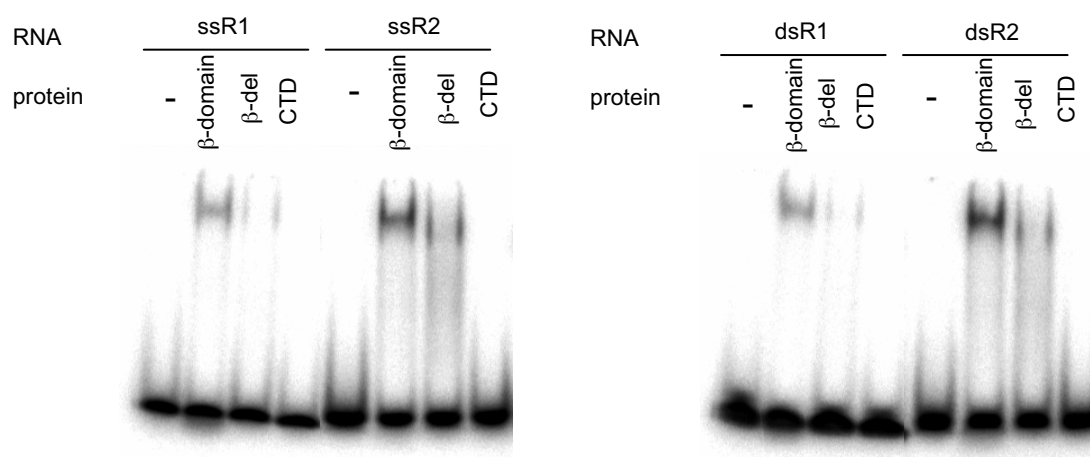


# Supporting Information

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**Fig. S1.** EMSA demonstrates that the  $\beta$ -finger domain ( $\beta$ -domain) binds ss and dsRNA weakly and without sequence specificity.  $\beta$ -finger deletion ( $\beta$ -del) reduces binding, and the C-terminal domain (CTD) of Prp8 does not obviously bind RNA. ssR1 is a 13-nt RNA with arbitrary sequence (GCUUUACGGUGCU). ssR2 is a 13-nt RNA (GAGAUUUUUUCG) from U6 that forms Helix II in the first-step complex. For dsR1 and dsR2, the perfect complement was annealed to ssR1 and ssR2 before addition of protein. Protein concentrations for the  $\beta$ -finger domain,  $\beta$ -del, and CTD are 32, 33, and 33  $\mu$ M, respectively.

**Table S1. Data collection and refinement statistics**

## Data collection

Space group	$P2_12_12_1$ , $a = 78.17$ , $b = 84.81$ , $c = 94.01$ Å, $\alpha = \beta = \gamma = 90^\circ$ , two molecules per a.u.
Resolution, Å	2.05
Wavelength, Å	0.9795
Redundancy*	12.4 (5.6)
Completeness, %	94.5 (68.8)
$R_{\text{merge}}^\dagger$	0.101 (0.322)
$\  \sigma(I) \ $	33.8 (2.8)
Refinement statistics ( $ F  > 0$ )	
Resolution, Å	2.05
$R_{\text{working}}$ (number of reflections)	0.214 (61,442)
$R_{\text{free}}^\ddagger$ (number of reflections)	0.236 (6,712)
Number of protein atoms	4,122
Number of nonprotein atoms	247 water molecules
rmsd from ideal bond length, Å	0.007
rmsd from ideal bond angle, °	1.239
Ramachandran statistics (for non-Pro/Gly residues)	
Most favored	438 residues
Additionally allowed	30 residues
Generously allowed	6 residues
Disallowed	0 residues

\*Numbers in parentheses represent values for the highest-resolution bin.

$^\dagger R_{\text{merge}} = \sum |I_{\text{obs}} - I_{\text{avg}}| / \sum I_{\text{avg}}$ .

$^\ddagger R_{\text{free}}$  was calculated with 10% of reflections.