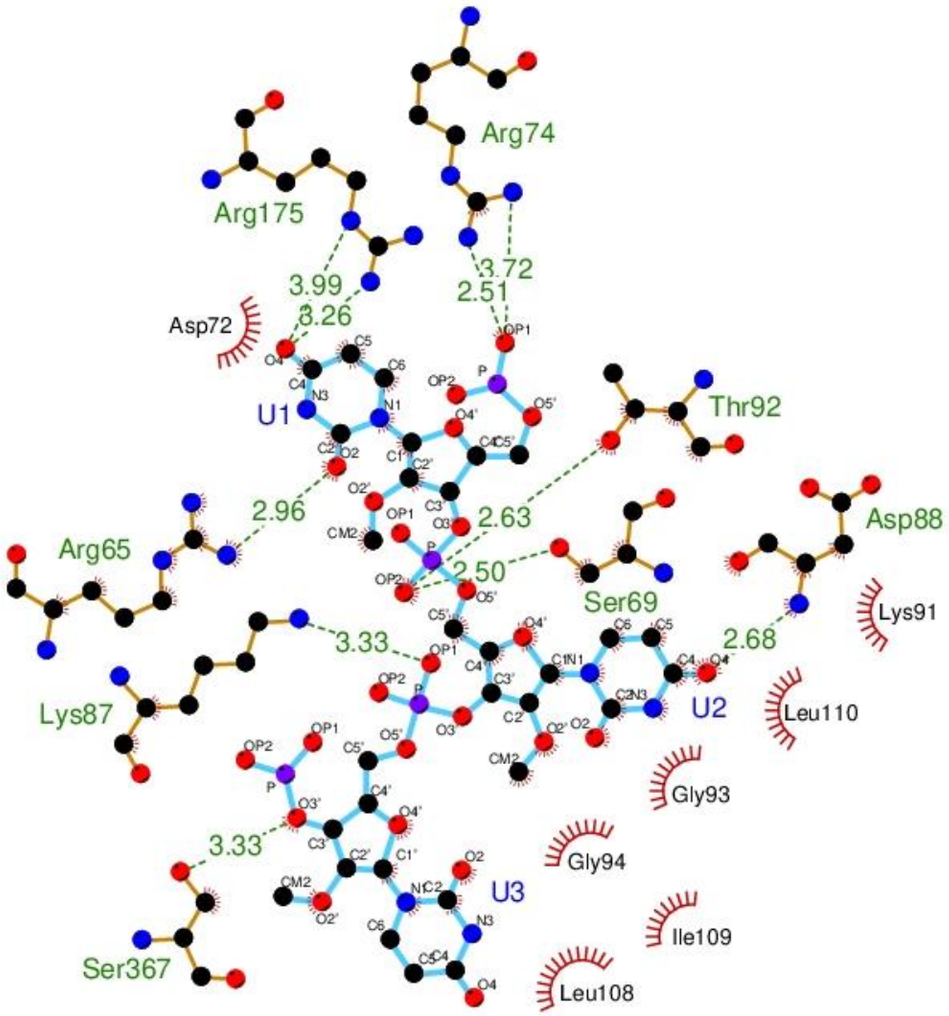
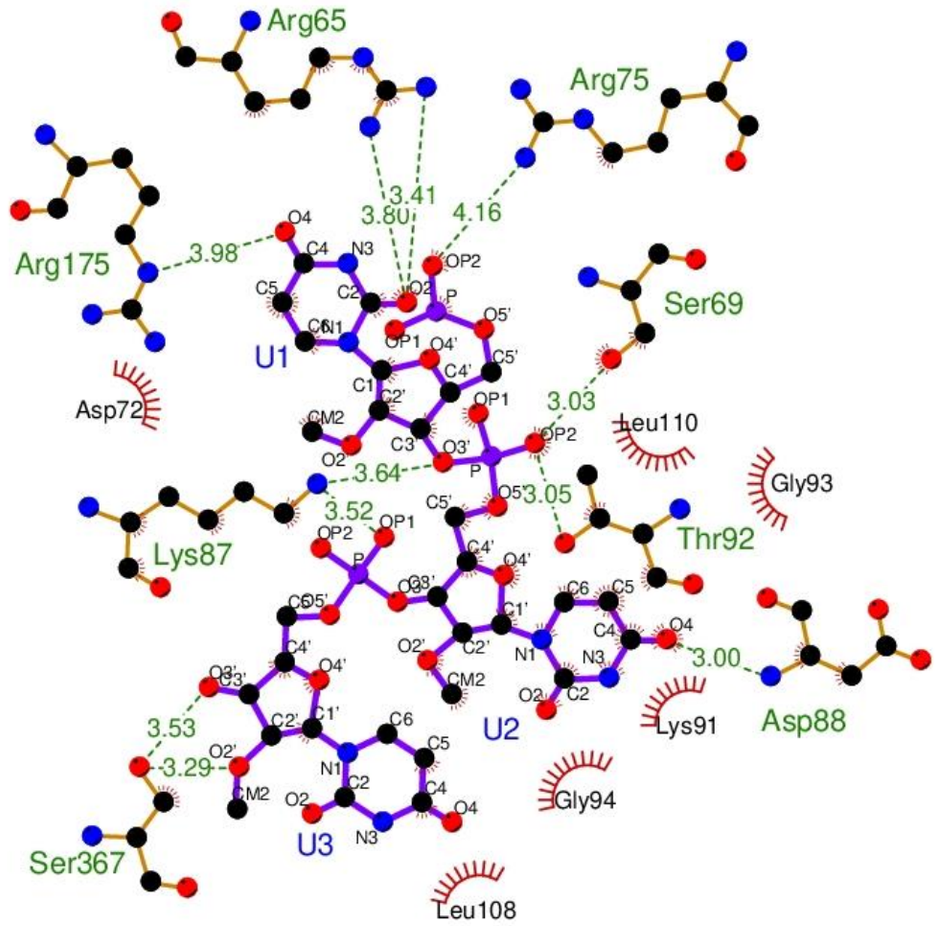
Supplementary Figures and Table

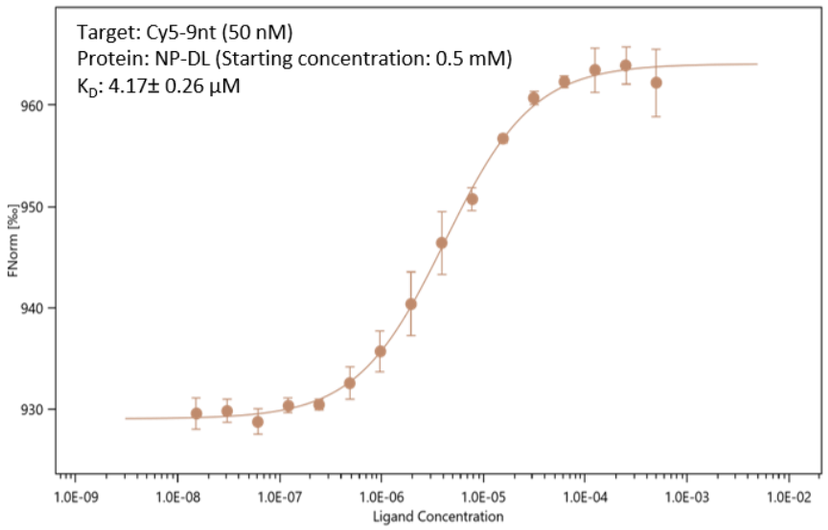


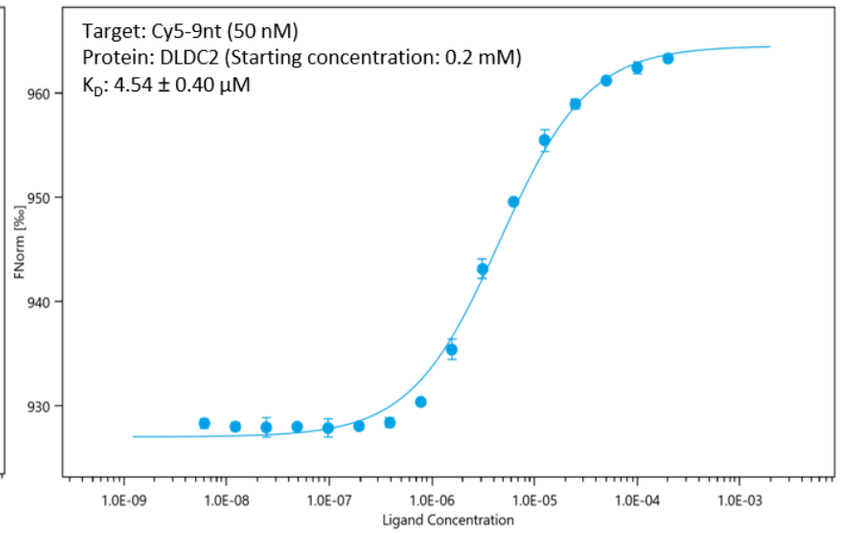
NP-DL / RNA complex

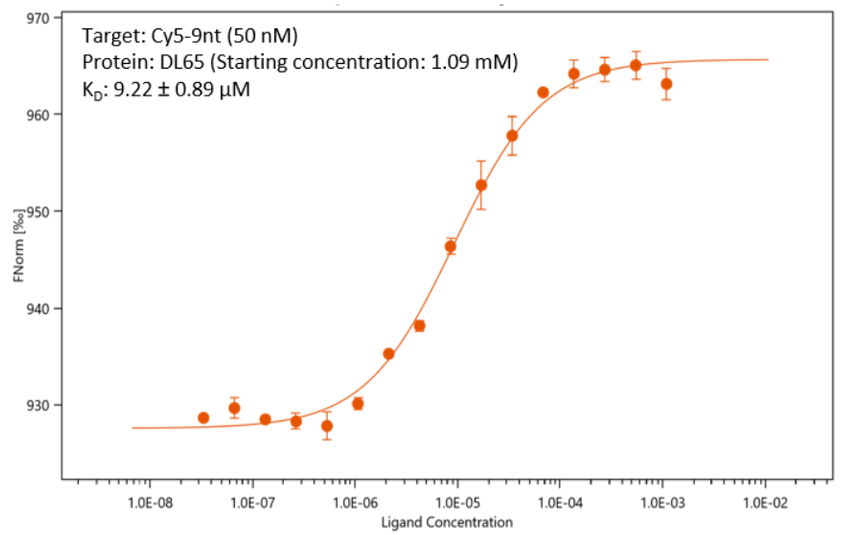


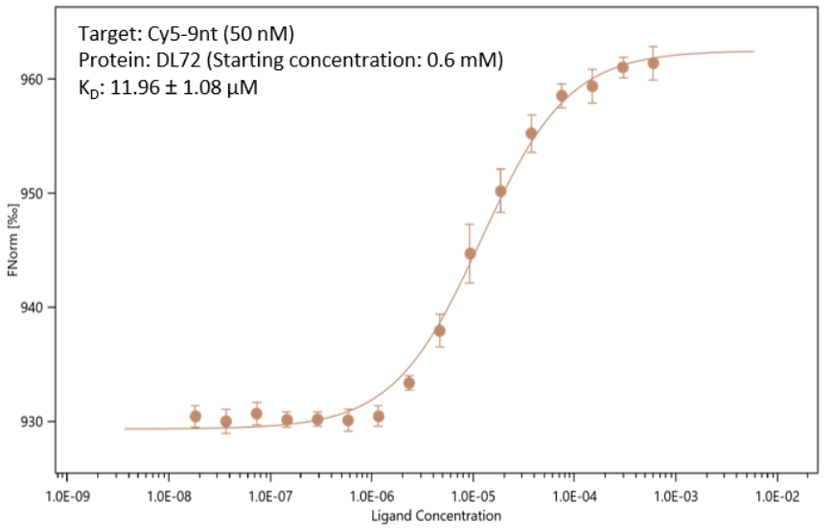
DLDC2 / RNA complex

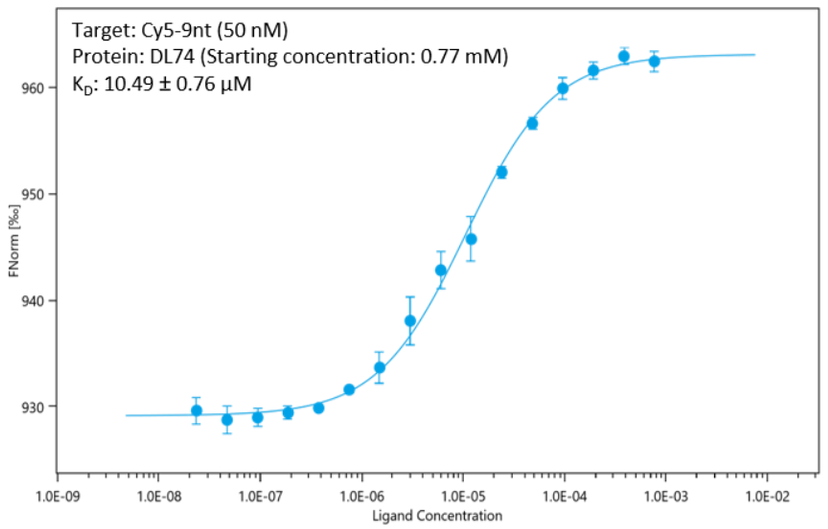
Supplementary figure 1: Ligplot+ analyses for RNA nucleotides on NP-DL (left) and DLDC2 (right). Possible polar and hydrophobic contacts between RNA nucleotides and the protein core are visualized in two-dimension. All distances are shown in Å.

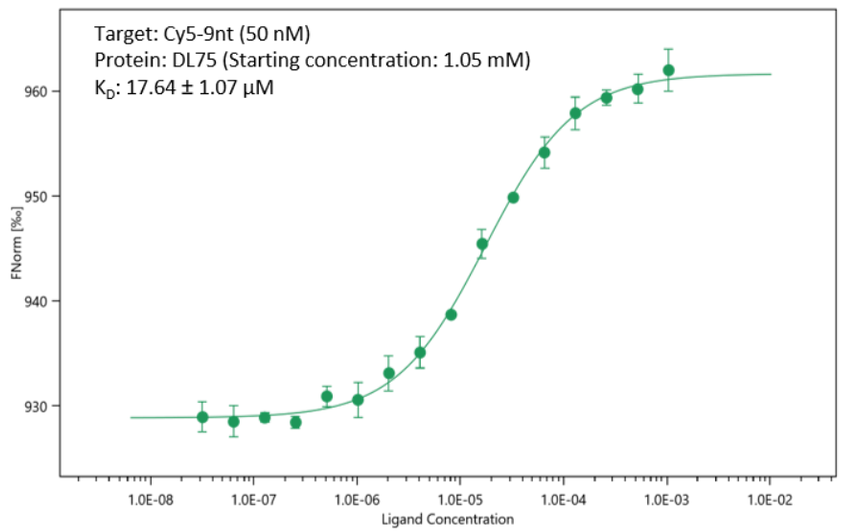


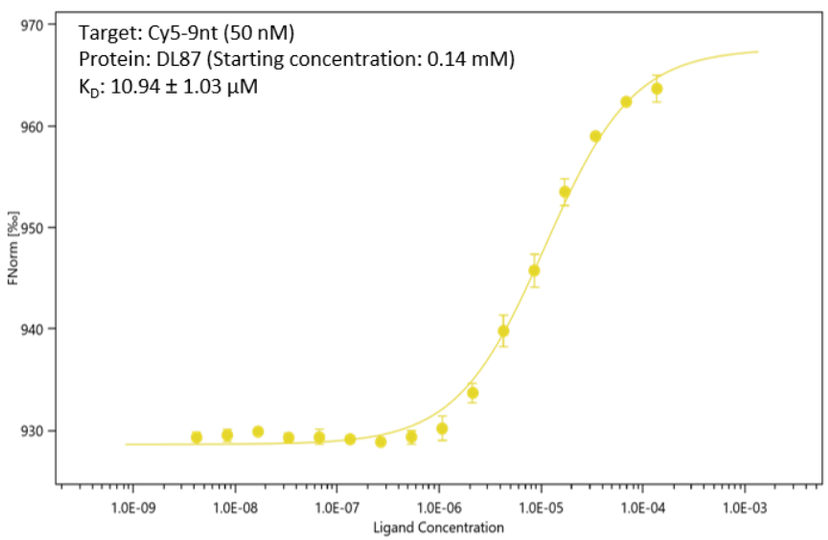


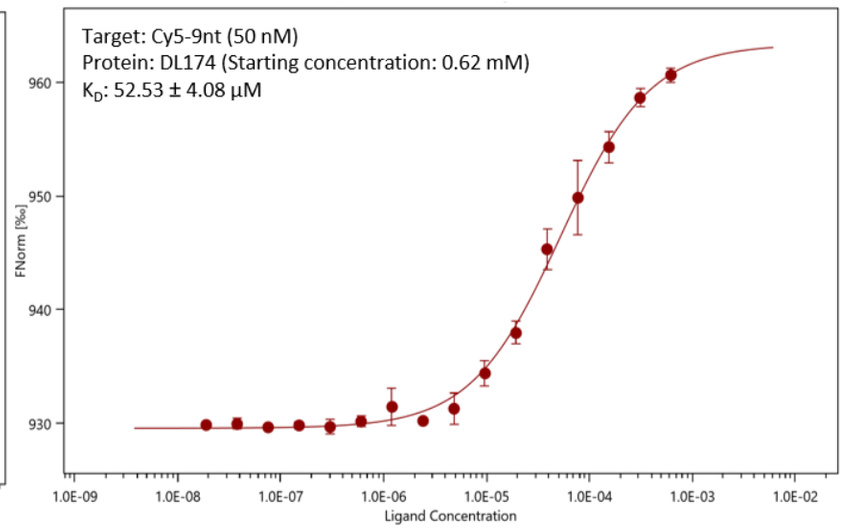




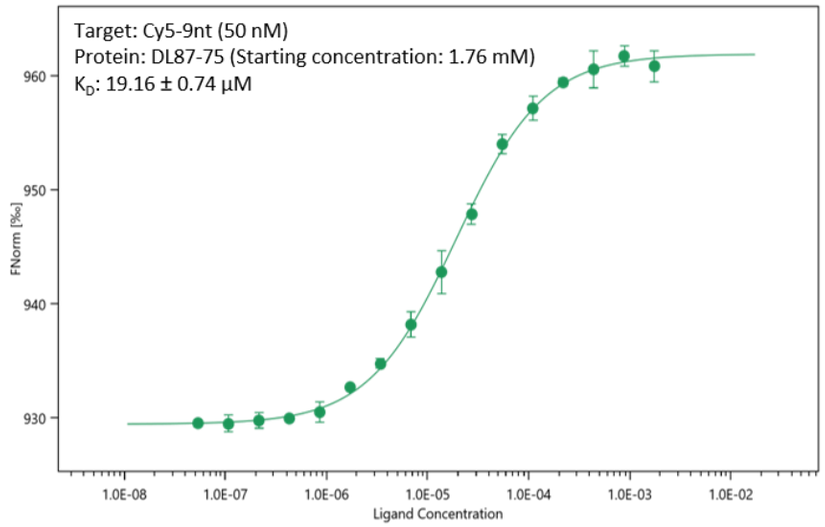


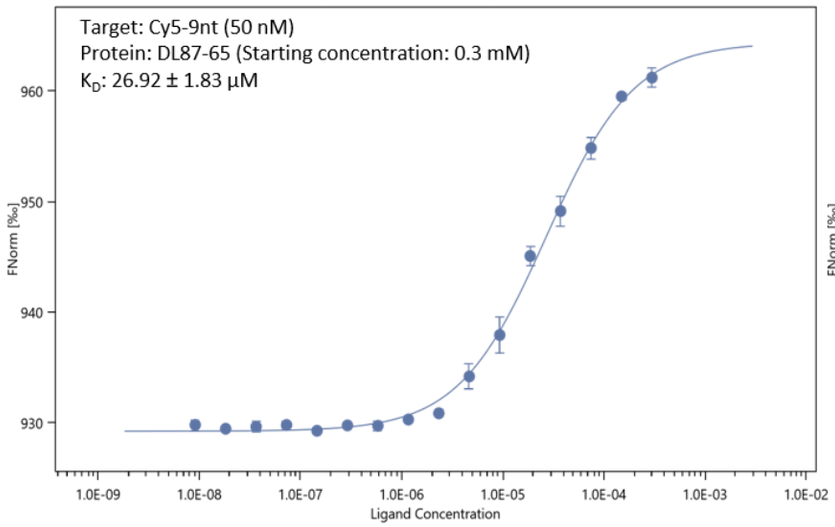


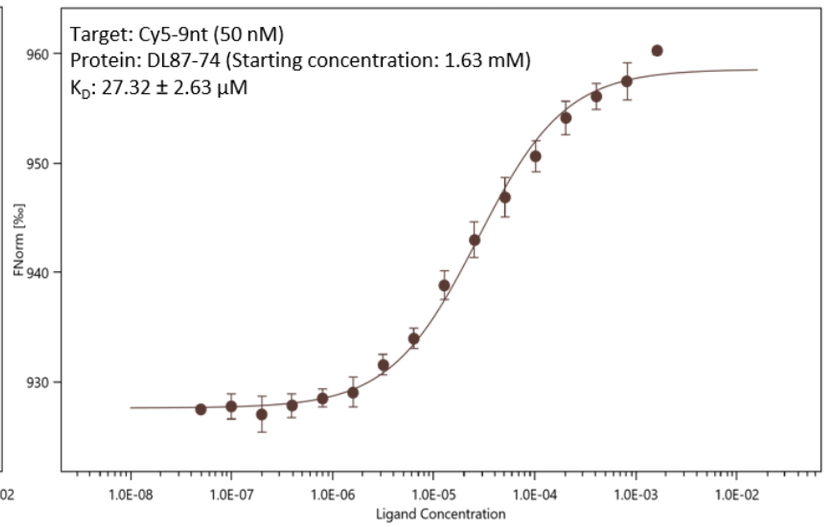


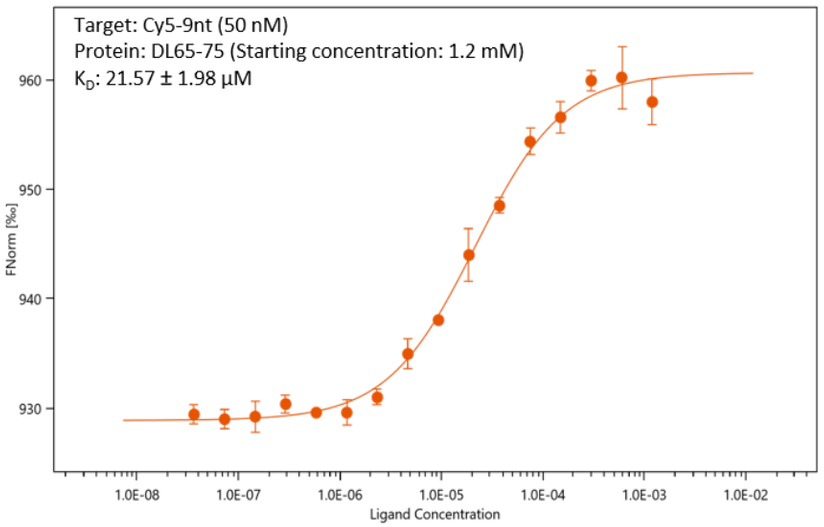


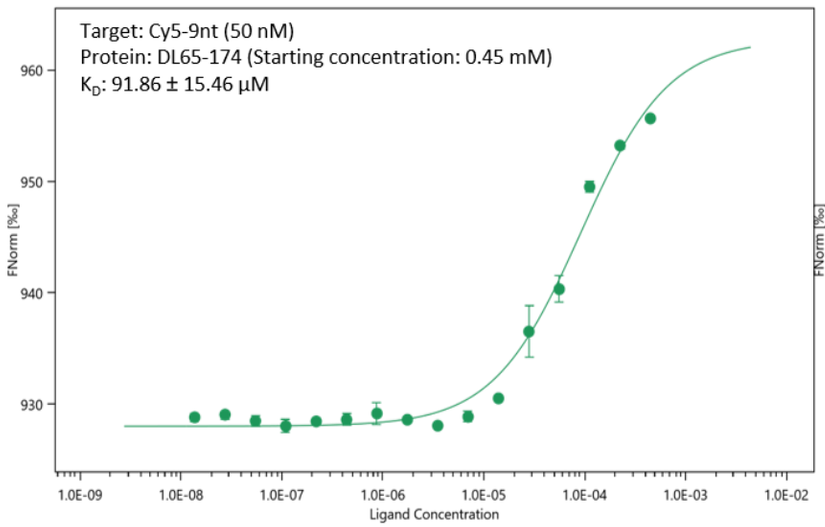
Supplementary figure 2: MST analysis of single-point mutants showed that RNA-binding was attenuated. MST curves are expressed as F_{norm} (0/00) versus concentration. Data represent mean values from at least three independent measurements. Error bars represent standard deviation.

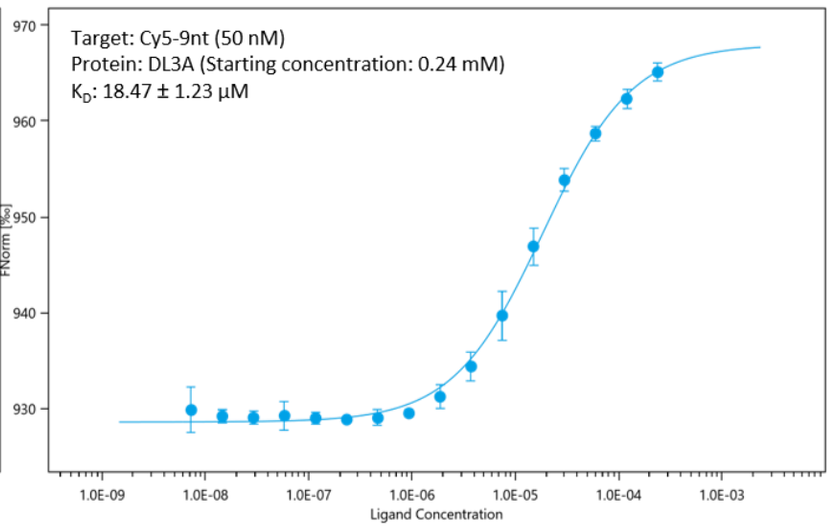




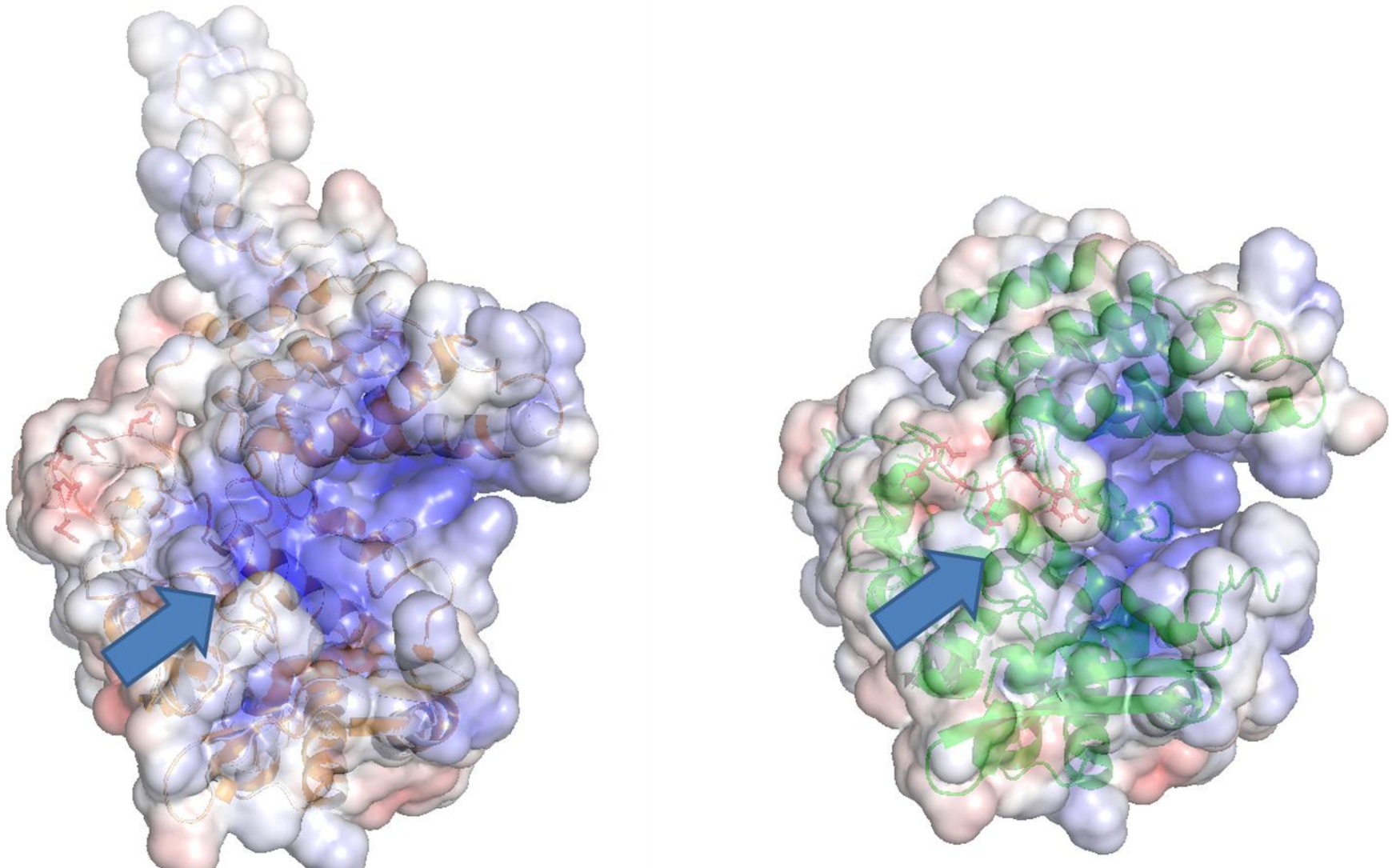








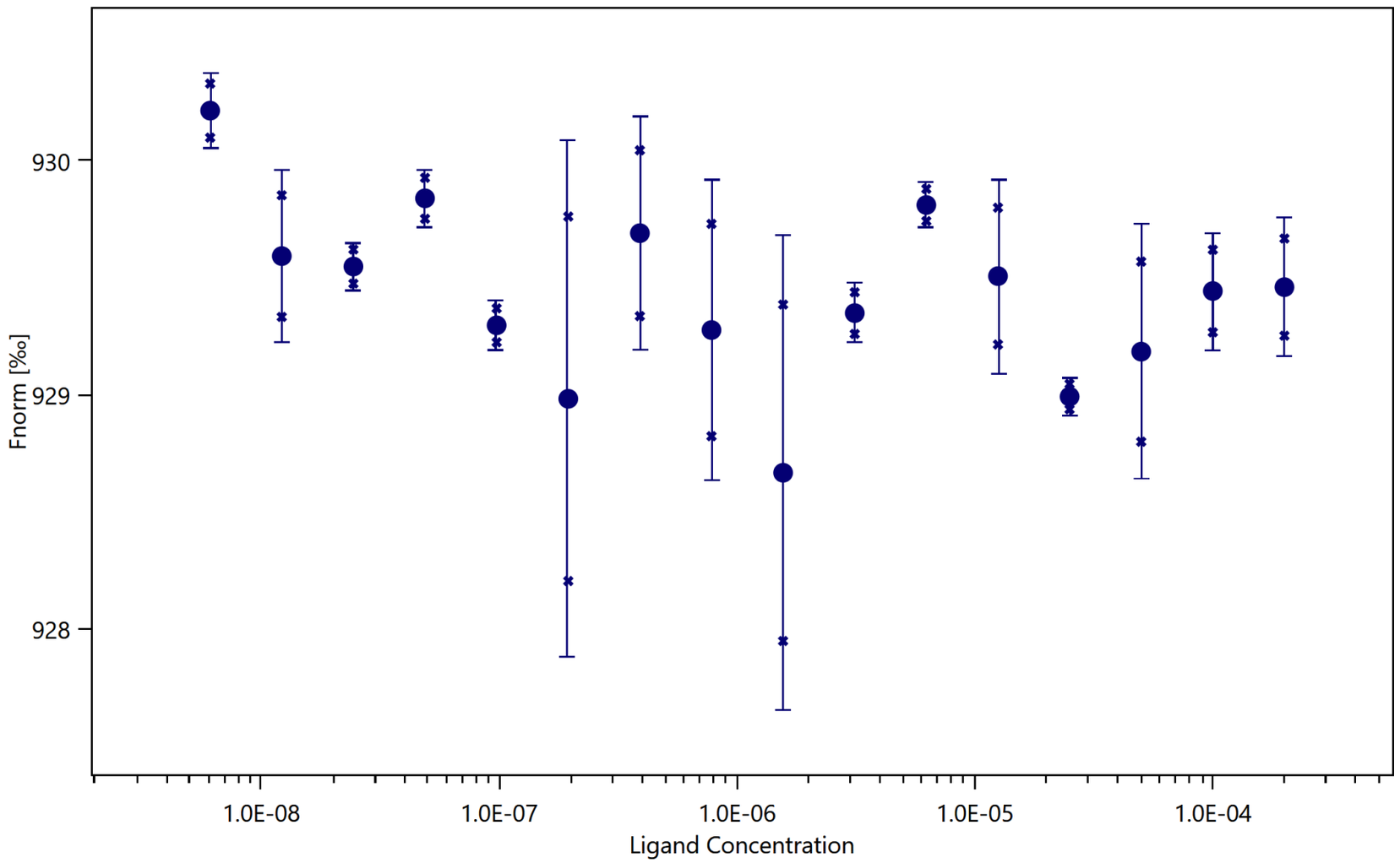
Supplementary figure 3: MST analysis showed that RNA-binding was attenuated in all double-point mutants tested. MST curves are expressed as F_{norm} (0/00) versus concentration. Data represent mean values from at least three independent measurements. Error bars represent standard deviation.



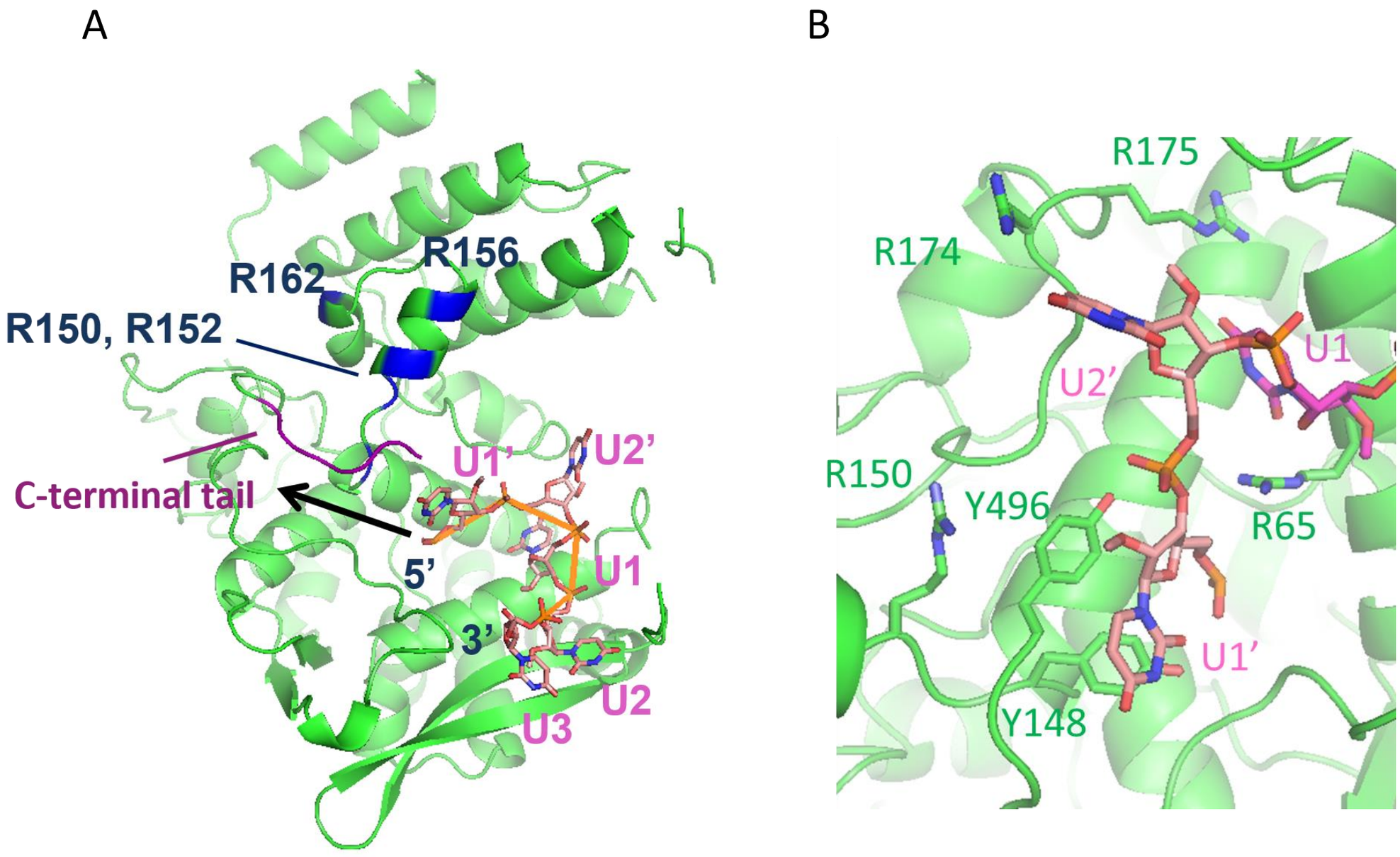
Surface potential of apo-NP (2Q06)

Surface potential of NP-DL

Supplementary figure 4: Surface potential for wild-type NP (left, PDB 2Q06) and NP-DL (right) is shown. Blue arrows indicate the position of G2 groove. In NP-DL the groove is covered by the C-terminal tail rendering it less basic than wild-type NP where the C-terminal tail is displaced.



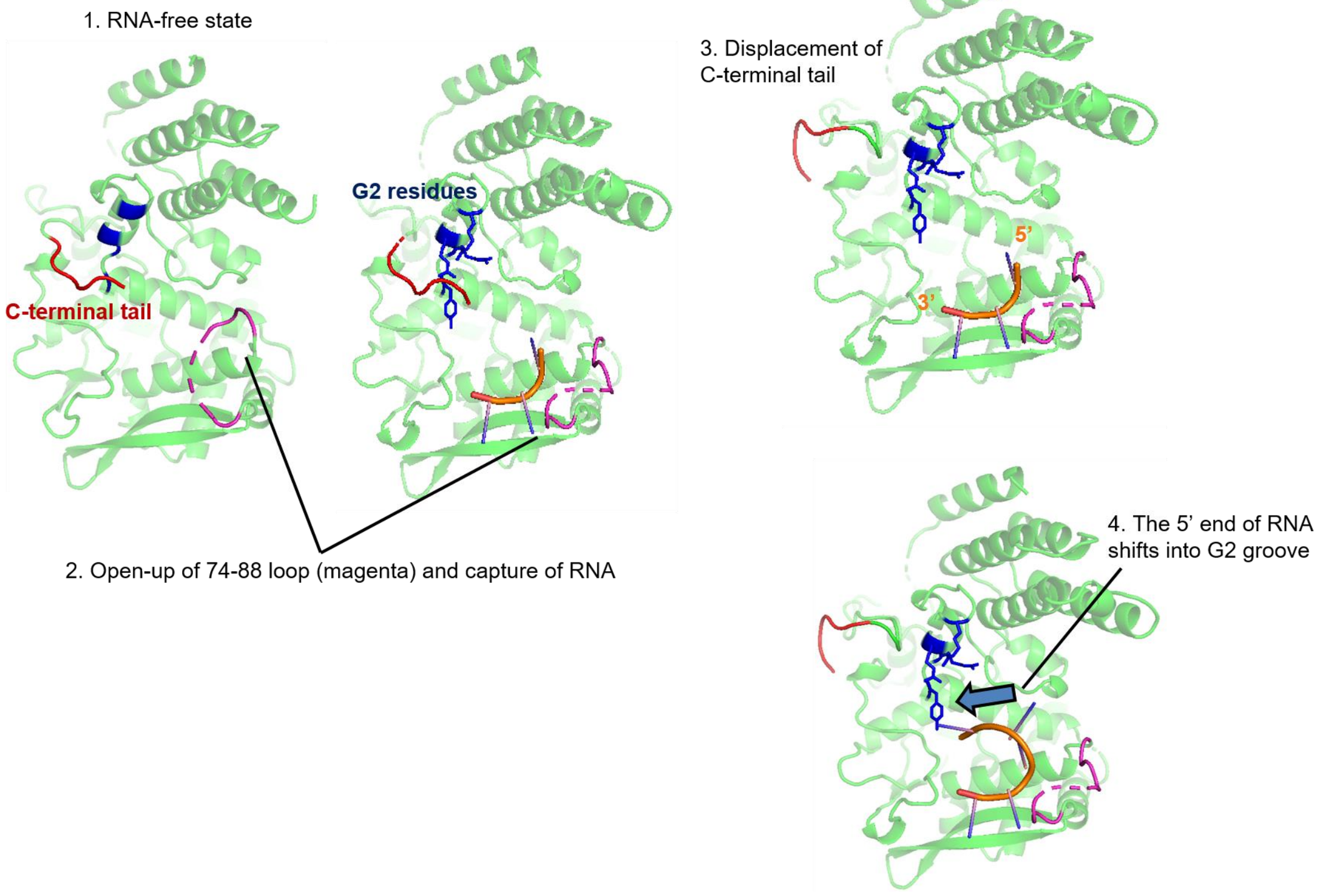
Supplementary figure 5: 50 nM of Cy5 tagged 9-mer RNA was titrated against a serial dilution of tail peptide (starting from 0.2 mM) to confirm the absence of interaction between the two species by MST. Experiment was performed twice.



Supplementary figure 6: Modelling of two extra nucleotides (U1', U2') at the 5' end based on DLDC2-RNA complex. In (A) G2 residues R150, R152, R156, R162 are labelled blue and the NP C-terminal tail is labelled violet. The two extra nucleotides at the 5' end tend to extend towards the G2 groove and exhibit good protein-RNA interactions. In (B) important NP residues in vicinity of U1' and U2' are displayed, demonstrating possible protein-RNA interactions, such as R65 to hold the pan-ultimate phosphate and the potential π-π stacking between the ultimate U base with Y148.

Supplementary Table 1: Intermolecular distances between DLDC2 and the two additional nucleotides at 5' end

Side chain interactions with RNA		
Interacting atoms on DLDC2 protein (residue / atom label)	Interacting atoms on the additional two nucleotides (nucleotide / atom label)	Distance in Å
R65/NH1	U1'/O5'	3.7
R65/NH2	U1'/O3'	3.9
Y148/OH	U1'/OP1	2.9
Y148/OH	U1'/O2	3.2
Y496/OH	U1'/O2'	3.9
Shortest distances between aromatic rings		
Y148/CE1	U1'/N3	4.2
Y496/CD1	U1'/C6	4.6



Supplementary figure 7: A three step mechanism of RNA binding by NP is proposed. The RNA is first packaged by the opening of G1 groove (steps 1 to 2), owing the 74-88 loop flexibility. Secondly C-terminal tail (red) displaces to free G2 groove (step 3). Finally, the 5’ end of the RNA extends into G2 groove defined by positive and aromatic residues around (Y148, R150, R152, R156 in blue color).