Supplementary Information

Structural insights into RNA unwinding and degradation by RNase R

Lee-Ya Chu, Tung-Ju Hsieh, Bagher Golzarroshan, Yi-Ping Chen,
Sashank Agrawal and Hanna S. Yuan

This document includes:
Supplementary Figures S1-S4

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E.coli_RNase_R

E.coli_RNase_R          ......................................................................
E.coli_RNase_II         ......................................................................
yeast_Rrp44         1   MSVPAIAPRRKRLADGLSVTQKVFVRSRNGGATKIVREHYLRSDIPCLSRSCTKCPQIVVPDAQNELPKF
mouse_Dis3l2            ......................................................................
Human_Dis3          1   ................MLKSKTFLKKTRAGGVMKIVREHYLRDDIGCGAPGCAACGGAHEGPALEPQPQ.
Human_Dis3l1        1   .............MLQKREKVLLLRTFQGRTLRIVREHYLRPCVPCHSPLCPQPAACSH...........
Human_Dis3l2            ......................................................................


E.coli_RNase_R

E.coli_RNase_R          ......................................................................
E.coli_RNase_II         ......................................................................
yeast_Rrp44        71   ILSDSPLELSAPIGKHYVVLDTNVVLQAIDLLENPNCFFDVIVPQIVLDEVR.NKSYPVYTRLRTLCRDS
mouse_Dis3l2            ......................................................................
Human_Dis3         54   ....DPASSVCPQPHYLLPDTNVLLHQIDVLEDPAI.RNVIVLQTVLQEVR.NRSAPVYKRIRDVTNNQ
Human_Dis3l1       47   ....DGKLLSSDVTHYVIPDWKVVQDYLEILEFPEL.KGIIFMQTACQAVQHQRGRRQYNKLRNLLKDA
Human_Dis3l2            ......................................................................


E.coli_RNase_R

E.coli_RNase_R          ......................................................................
E.coli_RNase_II         ......................................................................
yeast_Rrp44       140   DDHKRFIVFHNEFSEHTFVERLPNETINDRNDRAIRKTCQWYSEHLKPYD....INVVLVTNDRLNREAA
mouse_Dis3l2            ......................................................................
Human_Dis3        117   ..EKHFYTFTNEHHRETYVEQEQGENANDRNDRAIRVAAKWYNEHLKKMSADNQLQVIFITNDRRNKEKA
Human_Dis3l1      111   ..RHDCILFANEFQQCCYLPRERGESMEKWQTRSIYNAAVWYYHHCQDR.....MPIVMVTEDEEAIQQY
Human_Dis3l2            ......................................................................


E.coli_RNase_R

E.coli_RNase_R          ......................................................................
E.coli_RNase_II         ......................................................................
yeast_Rrp44       206   TKEVESNIITKSL....VQYIE........LLPNADDIRDSIPQMDSFDKDLERDTFSDFTFPEYYSTAR
mouse_Dis3l2        1   ..........MNHPDYKLNLRSPGTPRGVSSVVGPSAVGASPG..DKKSKNKSMRGKKKSIFETYMSKED
Human_Dis3        185   IEEG...IPAFTC....EEYVK........SLTANPELIDRLACLSEEGNE....IESGKIIFSEHLPLSK
Human_Dis3l1      174   GSETEG.VFVITFKNYLDNFWP........DLKAAHELCDSILQSRRE.RENESQESHGKEYPEHLPLEV
Human_Dis3l2        1   ..........MSHPDYRMNLRPLGTPRGVSAVAGPHDIGASPG..DKKSKNRSTRGKKKSIFETYMSKED


E.coli_RNase_R

E.coli_RNase_R      1   .........MSQDPFQEREAE............KYANPIPSREFILEHLTKREKPASRDELAVELHIEGE
E.coli_RNase_II         ......................................................................
yeast_Rrp44       264   VMGGLKNGVLYQGNIQISEYN.FLEGSVS....LPR...FSKPVLIVGQKNLNRAFNGDQVIVELLPQSE
mouse_Dis3l2       59   VSEGLKRGTLIQGVLRINPKK.FHEAFIP.......SPDGDRDIFIDGVVARNRALNGDLVVVKLLPEDQ
Human_Dis3        237   LQQGIKSGTYLQGTFRASREN.YLEATVW....IHGDNEENKEIILQGLKHLNRAVHEDIVAVELLPKSQ
Human_Dis3l1      234   LEAGIKSGRYIQGILNVNKHRAQIEAFVRLQGASSKDSDLVSDILIHGMKARNRSIHGDVVVVELLPKNE
Human_Dis3l2       59   VSEGLKRGTLIQGVLRINPKK.FHEAFIP.......SPDGDRDIFIDGVVARNRALNGDLVVVKLLPEEH


                                                                                      β1
E.coli_RNase_R                                                                       --->  T

E.coli_RNase_R     50   EQLEGLRRRLR........................AMERDGQLVFTRRQCYALPERLDLVKGTVIGHR
E.coli_RNase_II     1   ..........................MFQDNPLLAQLK..QQLHSQTPRAEGVVKATE
yeast_Rrp44       326   W..KAPSSIVLDSEHFD..V.....................NDNPDIEAGD.......DDDNNESSS....
mouse_Dis3l2      121   W..KAVKPESNDKEIEATYEADIPEEGCGHHPLQQSRKGWS.GPDVIIEAQFDDS.DSEDRHGNTS.GLV
Human_Dis3        302   W..VAPSSVVLHDEGQN..E.....................EDVE...KEE...................
Human_Dis3l1      304   W..KGRTVALCENDCDD..K..................................................
Human_Dis3l2      121   W..KVVKPESNDKETEAAYESDIPEELCGHHLPQQSLKSYNDSPDVIVEAQFDGS.DSEDGHGITQNVLV


                     β2                β3      η1        β4              β5          β6
E.coli_RNase_R      T  --->               -->     ℓℓℓ   TT  ---->          ------->    -----

E.coli_RNase_R     94   DGYGFLRVE....GR..KDDLYLSSEQMKTCIHGDQVLAQPLGADRKGRREARIVRVLVPKTSQIVGRYF
E.coli_RNase_II    31   KGFGFLEVD....AQ..K.SYFIPPPQMKKVMHGDRIIAVIHSEKERES..AEPEELVEPFLTRFVGKVQ
yeast_Rrp44       361   ..................NTTVISDKQRRLLAKDAMIAQRS....KKIQPTAKVVYIQRRSWRQYVGQLA
mouse_Dis3l2      186   DGVKKLSISTPDRGKEDSSTPVMKDE..NTPIPQDTRGLSE....KSLQKSAKVVYILEKKHSRAATGIL
Human_Dis3        325   .........................ETERML....KTAVSE....KMLKPTGRVVGIIKRNWRPYCGMLS
Human_Dis3l1      320   ...............................ASGESP....SEPMPTGRVVGILQKNWRDYVVTFP
Human_Dis3l2      188   DGVKKLSVCVSEKGREDGDAPVTKDE..TTCISQDTRALSE....KSLQRSAKVVYILEKKHSRAATGFL

                                    CSD1
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Figure S1 (part 1)

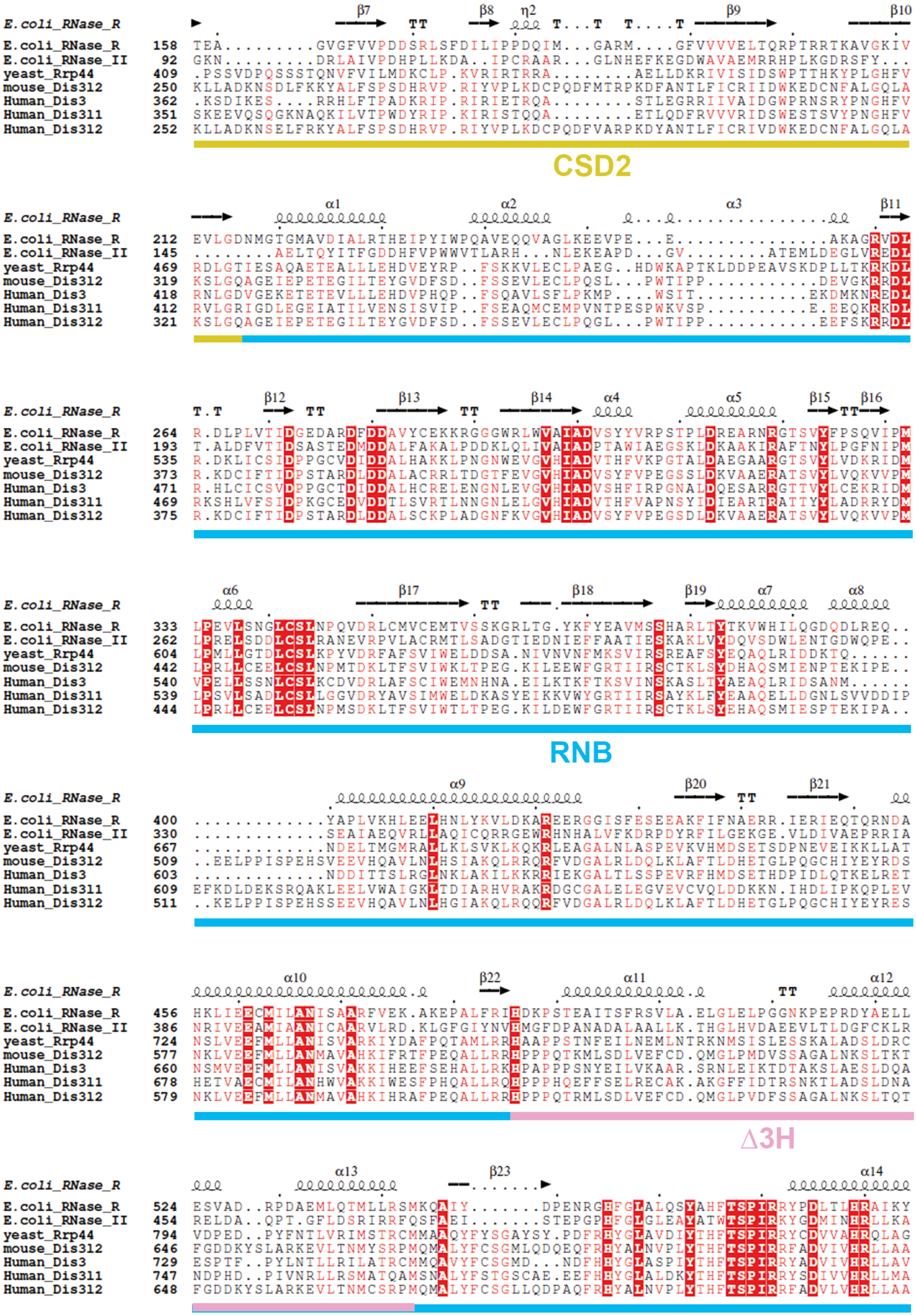

Figure S1 (part 2)

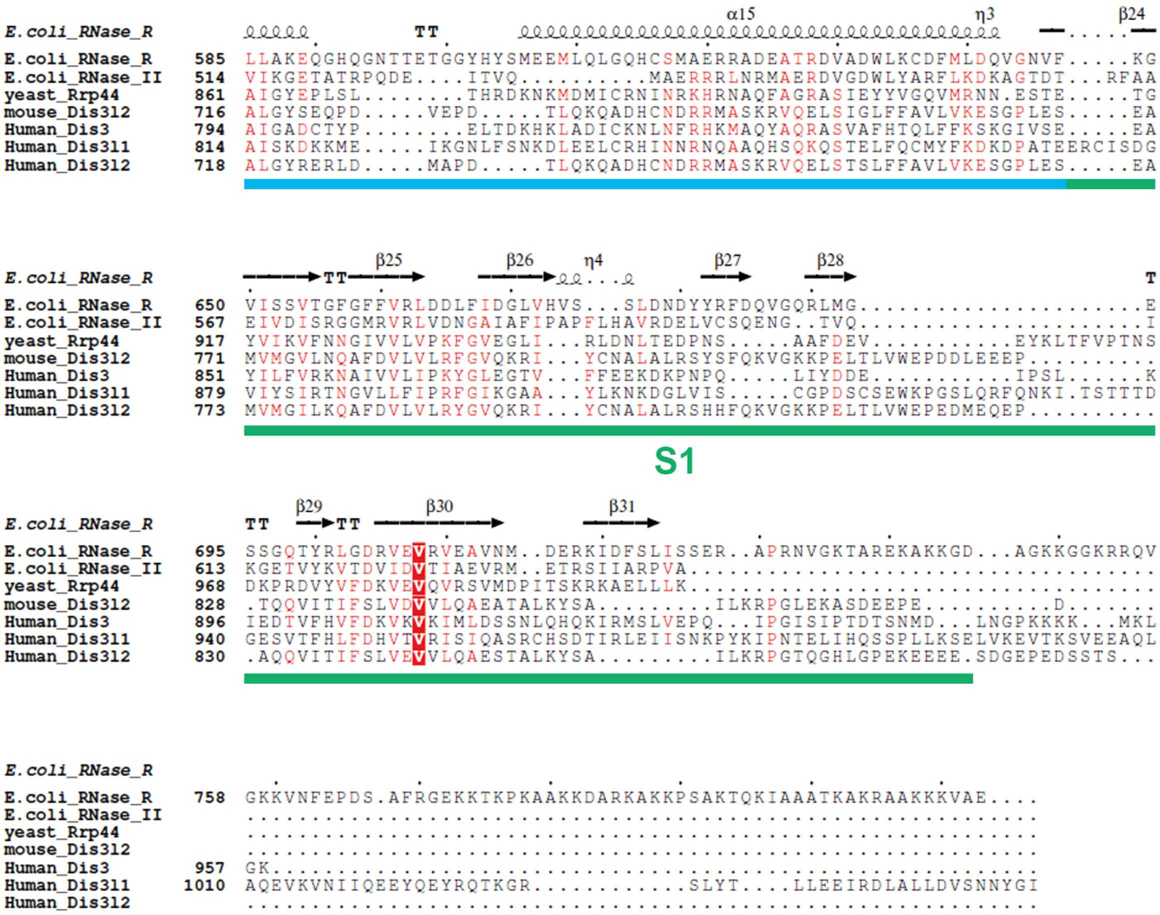

Figure S1. Multiple sequence alignment of the conserved domains in RNase II family proteins; RNase R, RNase II, Rrp44, Dis3l2, Dis3l1 and Dis3l2. The first row displays the secondary structures derived from the crystal structure of RNase R (PDB entry: 5XGU) determined in this study; α-helices are presented as wires and β-strands are presented as arrows. The last row indicates each domain region of RNase R; CSD1 in orange, CSD2 in yellow, RNB in blue, S1 in green and the tri-helix wedge region in pink. Residues in red letters are similar residues and residues shaded in red are conserved among the seven proteins in the alignment.

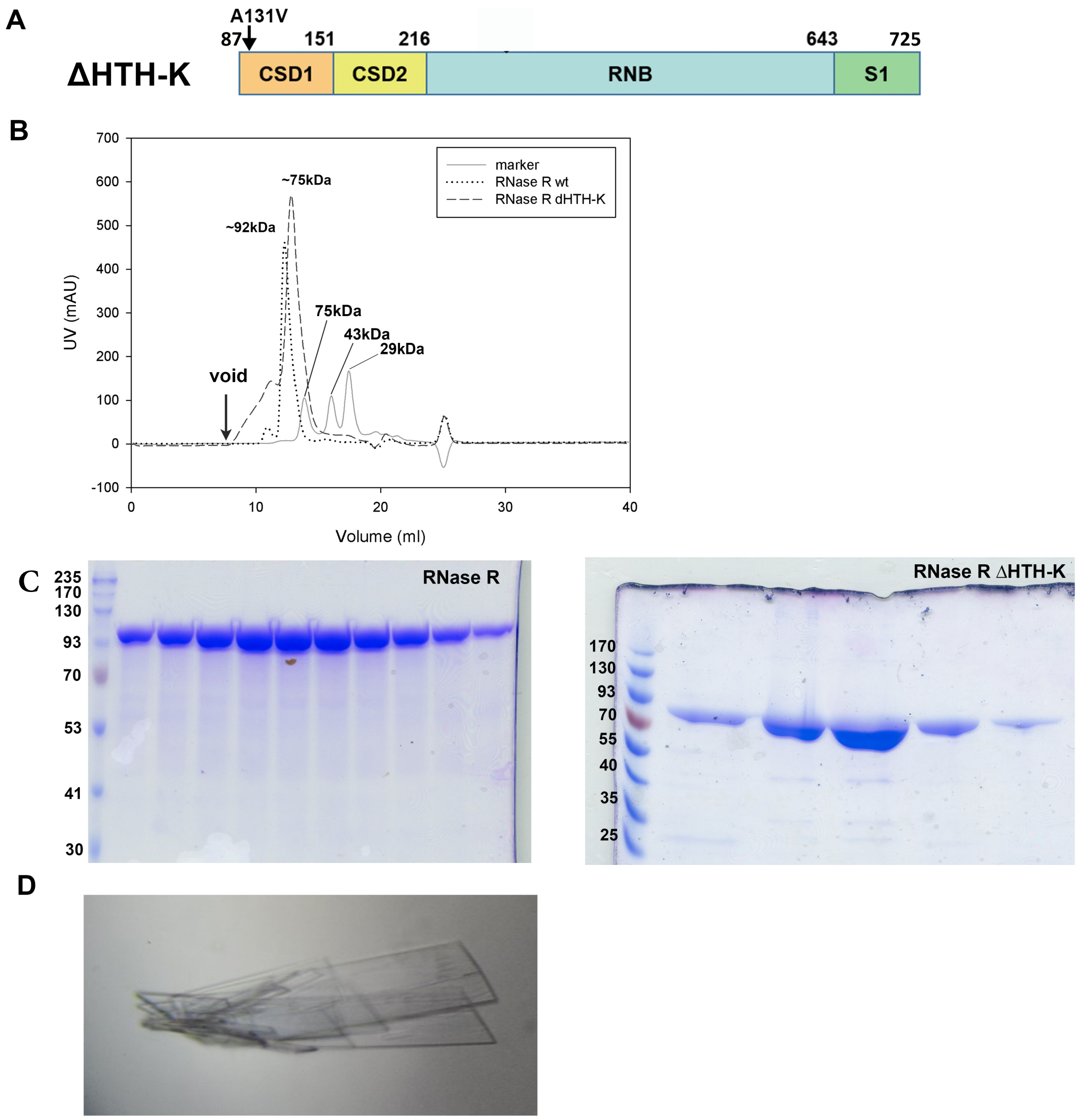

Figure S2. Purification and crystallization of RNase R ΔHTH-K. (A) Domain structure of the truncation mutant RNase R ΔHTH-K. (B) Gel filtration profiles (GE HealthCare, Superdex 200 Increase 10/300 GL) of the full-length RNase R and ΔHTH-K. (C) SDS-PAGE of the purified recombinant RNase R and ΔHTH-K. (D) Crystals of RNase R ΔHTH-K.

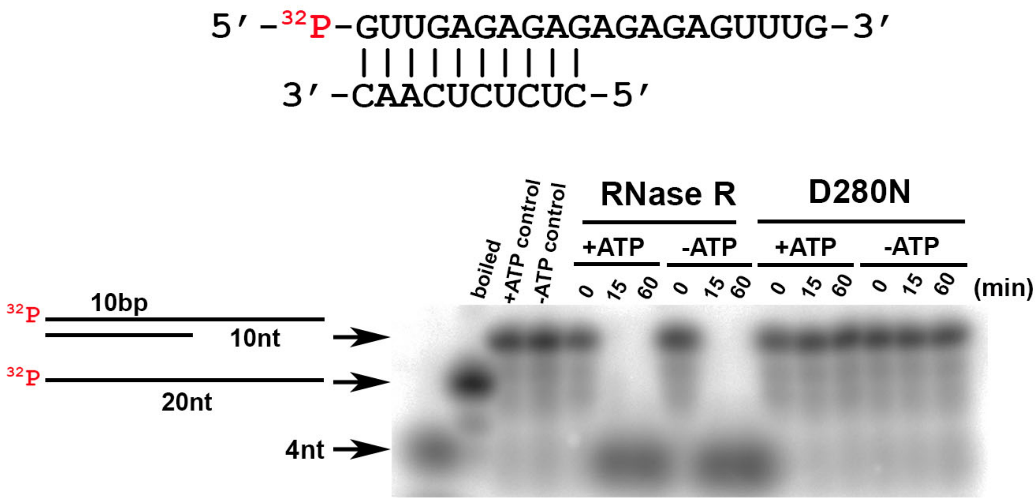

Figure S3. Duplex RNA with a 3′ overhang could not be unwound by RNase R D280N mutant in the presence of ATP. A 10-bairpair double-stranded RNA with a 10-nucleotide 3′ overhang (2.5 nM) was incubated with RNase R or D280N mutant (100 nM) in the presence or absence of ATP. The wild-type RNase R unwound and degraded dsRNA, however, RNase R D280N mutant could not unwind the duplex RNA in the presence of ATP. The RNA substrate was annealed by mixing a 5'-end ^{32}P-labeled 20-nucleotide RNA with a sequence of 5'-^{32}P-GUUGAGAGAGAGAGAGUUUG-3' with a 10-nucleotide RNA with a sequence of 5'-CUCUCUCAAC-3' at 95^{o}C for 5 minutes in a buffer of 20 mM HEPES (pH 7.4), 50 mM NaCl and 2 mM $MgCl_2$, followed by gradual cooling to room temperature over 2 hours. The duplex RNAs were unwound by boiling the sample (see the marker lanes). Full-length RNase R or D280N mutant (100 nM) was incubated with the duplex RNA (2.5 nM) in a buffer containing 20 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM DTT and 0.25 mM $MgCl_2$, with or without 5 mM ATP at 37˚C for 0-60 minutes. Reactions were stopped by adding 2 mg/ml Protease K and 25 mM EDTA at different time points as indicated in the figure. After the reaction, 6X DNA loading dye (30% (v/v) glycerol, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF) was added and the samples were loaded on a 20 % TBE gel for gel-electrophoresis.

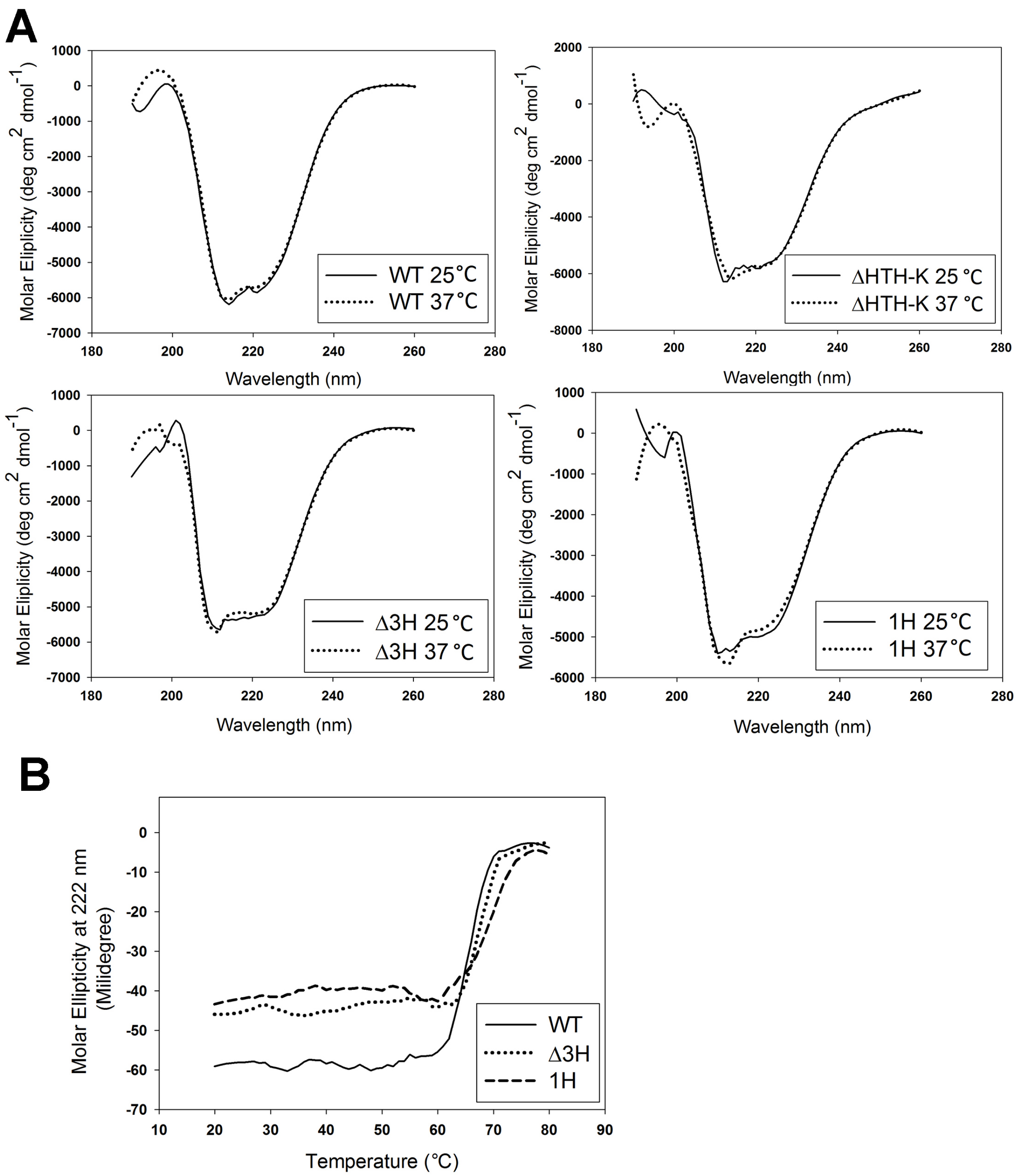

Figure S4. The CD spectra and thermal melting assays of the RNase R proteins. (A) The CD spectra of RNase R, ΔHTH-K, Δ3H and 1H mutants were recorded by AVIV CD400 spectrometer at 25˚C and 37˚C. (B) Thermal melting temperatures for RNase R, Δ3H and 1H were determined by measuring the ellipticity at 222 nm as a function of temperature from 20 to 80˚C. The thermal melting points were 65.7˚C for RNase R, 68.0˚C for Δ3H, and 70.0˚C for 1H mutant. The protein concentration used in the CD measurements were 10 μM in a buffer of 50 mM Tris-HCl pH 7.5 and 500 mM NaCl. Measurements were taken three times and averaged.