

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

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Supplementary Figures S1-S4

*E. coli*_RNase_R
*E. coli*_RNase_R
*E. coli*_RNase_II
yeast_Rrp44
mouse_Dis312
Human_Dis3
Human_Dis311
Human_Dis312

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1 MSVPAIAPRRKRLADGLSVIQKVFVRSRNGGATKIVREHYLRSDIPCLSRSCCTKCPQIVVPAQNELPKF
1 .....MLKSKTFLKKTTRAGGVMMKIVREHYLRDDIGCGAPGCAACGGAGHEGPALEPQPQ.
1 .....MLQKREKVLRLRTFQGRITLRIIVREHYLRPCVPCHSPLCPQPAACSH.....

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71 IISDSPLELSAPIGKHYVLDTNVVLQAIDLLENPNCFFDVIVPQIVLDEVR.NKSYVYVYTRRLTLCRDS
54 .....DPASSVCPQPHYLLPDTNVLLHQIDVLEDPAT.RNVIVLQIVLQEVN.NRSAPVYKRIKRDVTNNO
47 .....DGKLLSSDVTHYVIPDQVQDYLEILEFPEL.KGIIFMQTACQAVQHQRGRQRQYKLRNLLKDA

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```

140 DDHKRFIVFHNEFSEHTFVERLPNETINDRNDRAIRKTCQWYSEHLKPYD... INVVLVTNDRNLNREAA
117 ..EKHFYFTTNEHHRETYYVEQEQENANDRNDRAIRVAAKWYNEHLKMSADNQLQVIFITNDRNRKEKA
111 ..RHDCILFANEFAQCCYLPRERGESMEKQWTRSIYNAAVWYHHCQDR... MPIVMVTEDEEAIQQY

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```

206 TKEVESNIITKSL...VOYIE.....LLPNADDIRDSIPQMDSFDKDLERDTFSDFTFPEYYSTAR
1 .....MNHDPDYKLNLRSPGTPRGVSSVVGPSAVGASPG..DKSKNKSMRGKKKSIFFETYMSKED
185 IEEG...IPAFTC...EEYVK.....SLTANPELIDRLACLSEEGNE...IESGKIIFSEHLPLSK
174 GSETEG.VFVITPKNYLDNFWP.....DLKAAHELCDSILQSRRE.RENESQESHGKEYPEHLPLSK
1 .....MSHPDYRMNLRPLGTTPRGVSAVAGPHDIGASPG..DKSKNRSTRGKKKSIFFETYMSKED

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```

1 .....MSQDPFQEREA.....KYANPIPSREFILEHLTKREKPARSDELAVELHIEGE
264 VMGGLKNGVLYQGNIQISEYN.FLEGSVS...LPR...FSKPVLIQVQKLNLRNRAFNGDQVIVLLELPQSE
59 VSEGLKRGTLIQGLRINPKK.FHEAFIP.....SPDGRDIFIDGVVARNRNLNGDLVVVKLLPEEQ
237 LQQGIKSGTYLQGTFRASREN.YLEATVW.....IHGDNEENKEIILQGLKHLNRAVHEDIVAVELLPKSQ
234 LEAGIKSGRYIQGILNVNKHRAQIEAFVRLQGASSKSDSLVSDILIHGMKARNRSIHGDVVVVELLPKNE
59 VSEGLKRGTLIQGLRINPKK.FHEAFIP.....SPDGRDIFIDGVVARNRNLNGDLVVVKLLPEEH

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```

50 EQLEGLRRRLR.....AMERDQGLVFTRRQCYALPERLDLVKGTIVIGHR
1 .....MFQDNPLLAQLK..QQLHSQTPRAEGVVKATE
326 W..KAPSSIVLDSSEHFD..V.....NDNPDIEAGD.....DDNNNESSS...
121 W..KAVKPESNDKEIEATYEADIPEEGCGHHPLQQSRKQWS.GPDVIEAQFDDSDSEDRHGNTS.GLV
302 W..VAPSSVVLHDEGQN..E.....EDVE...KEE.....
304 W..KGRTVALCENDCDD..K.....
121 W..KVVKPESNDKEIEAAYESDIPEELCGHHLPQQSLKSYNDSPDVIVEAQFDGS.DSEDDGHGITQNVLV

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```

94 DGYGFLRVE...GR..KDDLYLSSEQMKTICIHGDQVLAQPLGADRKGRREARIVRVVLPKTSQIVGRYF
31 KGFGLFLEVD...AQ..K.SYFIPPPQMKKVMHGDRIIAVIHSEKERES..AEPEELVEPFLTRFVGVKQV
361 .....NTTVISDKQRRLLAKDAMIQRSS...KKIQPTAKVVYIQRRSWRQYVVGQLA
186 DGVKKLSISTPDRGKEDSSTPVMKDE..NTPIPQDTRGLSE...KSLQKSAKVVYIIEKKHSRAATGIL
325 .....ETERML...KTAVSE...KMLKPTGRVVVGIKRNWRPYCGMLS
320 .....ASGESP...SEPMPTGRVVVGIKKNWRDYVVTFF
188 DGVKKLSVVCVSEKREDDGAPVTKDE..TTCISQDTRALSE...KSLQRSKVVYIIEKKHSRAATGFL

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CSD1

Figure S1 (part 1)

*E. coli*_RNase_R

β7 → TT β8 → η2 β9 → β10

158 TEA.....GVGFVVPDDSRLSFDLIPDPQIM...GARM...GVVVVELTQRPTRRTKAVGKIV
*E. coli*_RNase_R 92 GKN.....DRLAIVDPDHPKLDK...IPCRAAR...GLNHEFKEGDWAEMRRHPLKGDERSFY...
*E. coli*_RNase_II 92 .PSSVDPQSSSTQNVFVILMDKCLP.KVRIARRA.....AELLDRRIVISIDSWPTTHKYPLGHFV
 yeast_Rrp44 409 KLLADKNSDLFKKYALFSPSDHRVP.RIYVPLKDCPQDFMTRPKDFANLFCIRIIDWKEDCNFALGQLA
 mouse_Dis312 250 .KSDIKES...RRHLFTPADKRIP.RIRIETRQA.....STLEGRRIVAIIDGWPNRSRYPNGHFV
 Human_Dis3 362 SKKEEVQSQGKNAQKILVTPWDYRIP.KIRISTQQA.....ETLQDFRVVVRIDSWESTSVYPNGHFV
 Human_Dis311 351 KLLADKNSELFRKYALFSPSDHRVP.RIYVPLKDCPQDFVARPKDYANTLFCIRIVDWKEDCNFALGQLA
 Human_Dis312 252

CSD2

*E. coli*_RNase_R

α1 α2 α3 β11

212 EVLGDNMGITGMAVDIALRTHPEYIWPQAVEQQVAGLKEEVPE...E.....AKAGRVVDL
*E. coli*_RNase_R 145AELTQYITFGDDHFVPPWVILARH...NLEKEAPD...GV.....ATEMLDEGLVREDL
*E. coli*_RNase_II 145 RDLGITFESAQAETEALLLEHDVEYRP..FSKKVLECLPAEG..HDWKAPTKLDDEPAVSKDPLLLTKRDL
 yeast_Rrp44 469 KSLGQAGEIEPETEGILTEYGVDFSD..FSSEVLECLPQSL...PWTIPP.....DEVGKRDL
 mouse_Dis312 319 RNLGDVGEKETETEVLLLEHDVPHQP..FSQAVLSFLPKMP...WSIT.....EKDMKNREDL
 Human_Dis3 418 RVLGRIQDLEGEIATILVENSISVIP..FSEAQMCEMPVNTPESPKWKVSP.....E.EEQKRDL
 Human_Dis311 412 KSLGQAGEIEPETEGILTEYGVDFSD..FSSEVLECLPQGL...PWTIPP.....E.EFSKRDL
 Human_Dis312 321

*E. coli*_RNase_R

T.T β12 → TT β13 → TT β14 → α4 β15 → TT β16

264 R.DLPLVTIDGEDARDFDDAVYCEKRRGGWRWVATADVSYYVVRPSTPLDREARNRGTSVYFSPQVIPM
*E. coli*_RNase_R 193 T.ALDFVITDSASTEDMDALFAKALPDKLQILVAIADPTAWIAEGSKLDKAAKIRAFNIVLPGFNIPM
*E. coli*_RNase_II 193 R.DKLCISIDPPGCVDDALHAHKPLNGNWEVGVHIADVTHFVKPGTALDAEGAAARGTSVYLVQKRIDM
 yeast_Rrp44 535 R.KDCIFTIIDPSTARDDALHACRRLLDGTFEVGVHIADVSYYVVRPSTPLDREARNRGTSVYLVQKRIDM
 mouse_Dis312 373 R.HLCLCSVDPPGCTDDALHCRELENGNLEVGVHIADVSHFRPAGNALDQESARRGTIVLCEKRIDM
 Human_Dis3 471 RKSHLVFSDPKGCEVDLTSVRTLNNGNLELGVHIADVTHFVAPNSYDIEARTRATTIYVLADRIDM
 Human_Dis311 469 R.KDCIFTIIDPSTARDDALSCCKPLADGNFKVGVHIADVSYYVVRPSTPLDREARNRGTSVYLVQKRIDM
 Human_Dis312 375

*E. coli*_RNase_R

α6 β17 → TT β18 → β19 α7 α8

333 IFEVLSNGLCSTNPQVDRLCMVCMTVSSKGRITG.YKFFEAVMSHARLTYTKVWHILQGDQDLREQ..
*E. coli*_RNase_R 262 IPRELSDDLCSFRANERVRPLACRMTLSADGTIEDNIEFFAATIESKAKLVYDQVSDWLENTGDWQPE...
*E. coli*_RNase_II 262 IPEMLGTDLCSFKPYVDRFAFVSEWELDDSA.NIVNVNFMKSVIRREAFSYEQALRIDDKTQ...
 yeast_Rrp44 604 VPELSSNLCSFTKCDVDRLAFSCIWEMNHNA.EILKTKFTKSVINSKASLTYAEAQLRIDSANM...
 mouse_Dis312 442 IPELSSNLCSFTKCDVDRLAFSCIWEMNHNA.EILKTKFTKSVINSKASLTYAEAQLRIDSANM...
 Human_Dis3 540 IPELSSNLCSFTKCDVDRLAFSCIWEMNHNA.EILKTKFTKSVINSKASLTYAEAQLRIDSANM...
 Human_Dis311 539 IPELSSNLCSFTKCDVDRLAFSCIWEMNHNA.EILKTKFTKSVINSKASLTYAEAQLRIDSANM...
 Human_Dis312 444 IPELSSNLCSFTKCDVDRLAFSCIWEMNHNA.EILKTKFTKSVINSKASLTYAEAQLRIDSANM...

RNB

*E. coli*_RNase_R

α9 β20 → TT β21 → α10

400YAPLVKHLEETHNLYKVLDAKREERGGISFSEEAKFIFNAERR.IERIEQQRNDIA
*E. coli*_RNase_R 330SEATAEQVRLTAQICQRRGKWRHNNHALVFKDRPDYRFILGEGKE.VLDIVAEPRRIA
*E. coli*_RNase_II 330NDELTMGMRAITLKLSSVKLQKRLAAGALNLSPEVVMDSSTDPNEVEIKKLLAT
 yeast_Rrp44 667EELPPISEPHSVVEVHQAVNLTHSIAKQLRQRFFVDGALRLDQLKLAFTLDHETGLPQGCHIEYRDS
 mouse_Dis312 569NDDITTSRLRINKLAKLKKRRIEKGALTLSSPEVRFHMDSETDHPIDLQTKELRET
 Human_Dis3 603 EFKDLDKSRQAKLEELVWVAIGKTDIARHVRARDDGCGALELEGVEVCVQLDDKKN.IHDLIPKQPLEV
 Human_Dis311 609KELPPISEPHSSVEVHQAVNLTHGIAKQLRQRFFVDGALRLDQLKLAFTLDHETGLPQGCHIEYRDS
 Human_Dis312 511

*E. coli*_RNase_R

α10 β22 → α11 TT α12

456 HKLIEECMILANISARFVEK.AKEPALFRIBDKPSTEAITSFRRSVLA.ELGLELPGGNKPEPRDYAELL
*E. coli*_RNase_R 386 NRIVEEAMIANICAAARVLRD.KLFGFIYNVHMGFDPANADALAALK.THGLHVDAAEEVLTLDGFCKLR
*E. coli*_RNase_II 386 NSLVEEMLLANISVARKIYDAPQATMLRRHAAPPSTNFEILNEMLNTRKNMSISLESSKALADSLDR
 yeast_Rrp44 724 NKLVEEMLLANISVAKKIHEEFSEHALLRKHPPAPPSPNYEILVKAAR.SRNLEIKTDTAKLSAESLDQA
 mouse_Dis312 577 NSMVEEMLLANISVAKKIHEEFSEHALLRKHPPAPPSPNYEILVKAAR.SRNLEIKTDTAKLSAESLDQA
 Human_Dis3 660 HETVAECMILANHWVAKKIWESEFPHQALLRQHPPPHQEFFSELECAK.AKGFIDTRSNKTLADSLDNA
 Human_Dis311 678 NKLVEEMLLANMAVAHKIHRAPPEQALLRRHPPPQTRMLSDLVVEFC.D.QMGLPVDVFSSAGALNKSILTQT
 Human_Dis312 579

Δ3H

*E. coli*_RNase_R

α13 β23 α14

524 ESVAD..RPDAEMLQTMLLRRSMKQAIY.....DPENRGHFGTALQSYAHFTSPIRRYDLDLTHRAIKY
*E. coli*_RNase_R 454 RELDA..QPT.GFLDSRIRRFQSFSAEI.....STEPGFHFGGLEAVATWTSPIRKYDMDINHRLLKA
*E. coli*_RNase_II 454 VDPE..PYFNTLVIRIMSTRCMMAAQYFSGAYS.Y.PDFRHYGTAVDIYTHFTSPIRRYDLDLTHRAIKY
 yeast_Rrp44 794 FGDDKYSLARKEVLTNMYSRPMQALYFCSGMQLQDQEFRHYA..NVPLYTHFTSPIRRYDLDLTHRAIKY
 mouse_Dis312 646 ESPTF..PYLNTLLRILATRCMMAAQYFCSGMQLQDQEFRHYA..NVPLYTHFTSPIRRYDLDLTHRAIKY
 Human_Dis3 729 NDPHD..PIVNRLLRSMATQAMSNALYFSTGSCAE.EEFHYHGTALDKYTHFTSPIRRYDLDLTHRAIKY
 Human_Dis311 747 FGDDKYSLARKEVLTNMYSRPMQALYFCSGMQLQDQEFRHYA..NVPLYTHFTSPIRRYDLDLTHRAIKY
 Human_Dis312 648

Figure S1 (part 2)

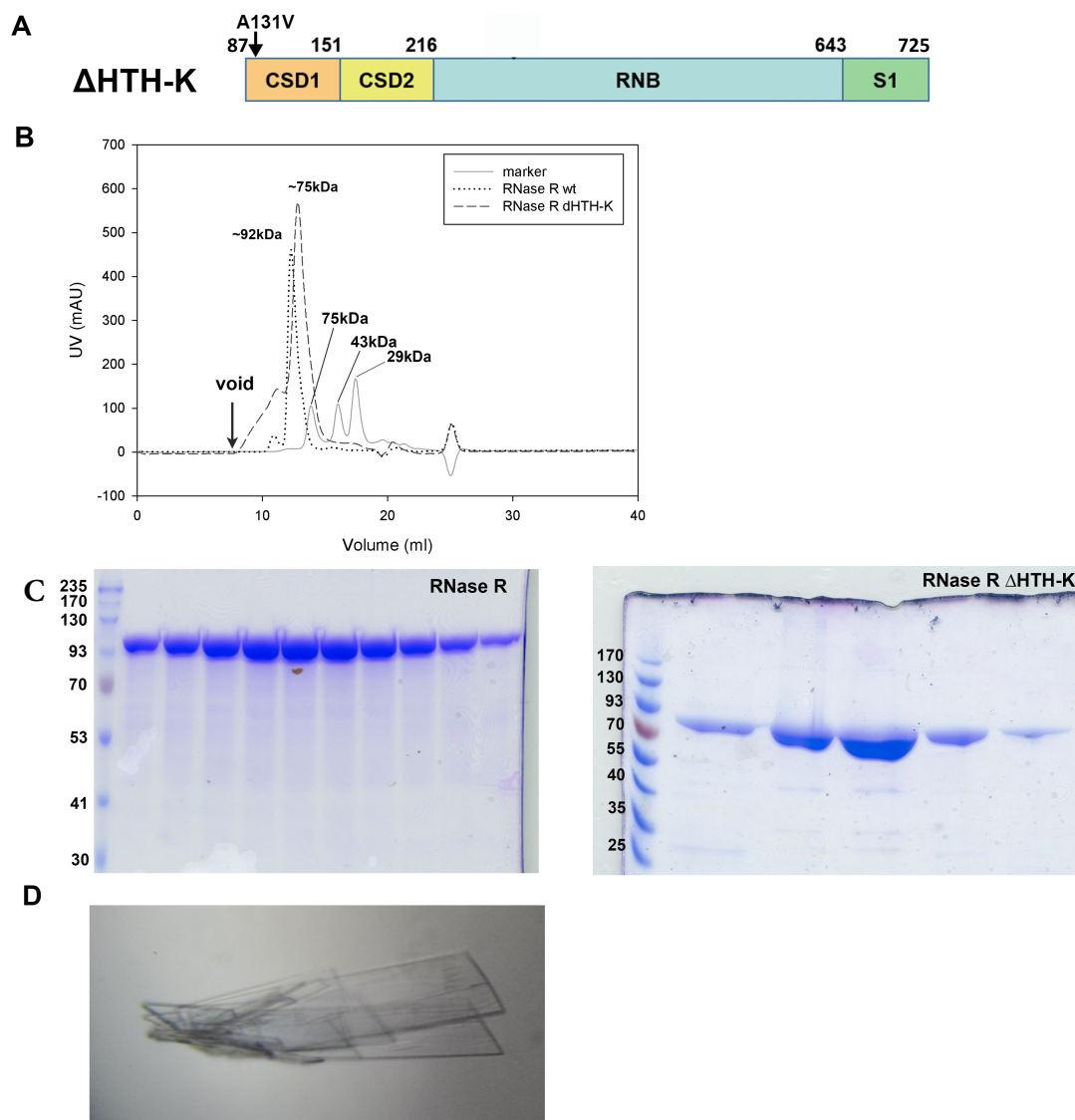


Figure S2. Purification and crystallization of RNase R Δ HTH-K. (A) Domain structure of the truncation mutant RNase R Δ HTH-K. (B) Gel filtration profiles (GE HealthCare, Superdex 200 Increase 10/300 GL) of the full-length RNase R and Δ HTH-K. (C) SDS-PAGE of the purified recombinant RNase R and Δ HTH-K.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

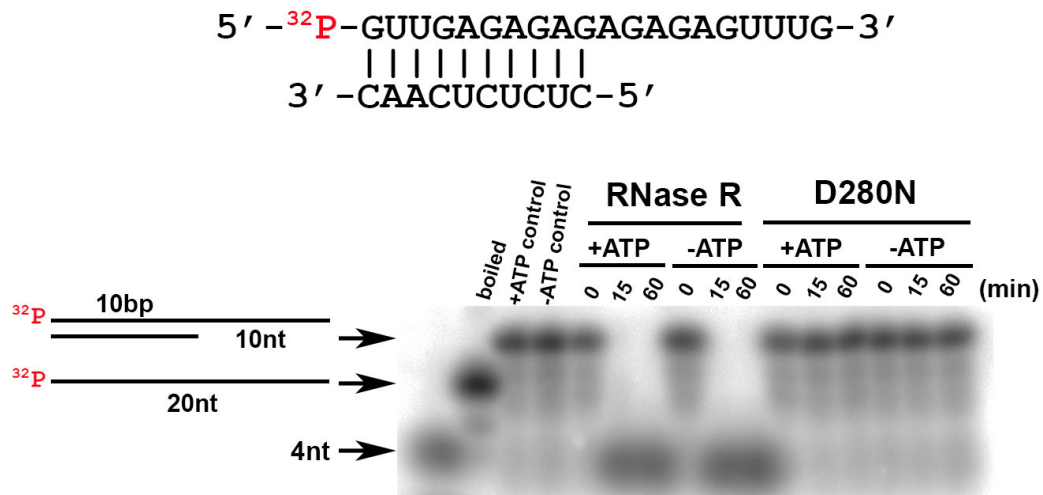


Figure S3. Duplex RNA with a 3' overhang could not be unwound by RNase R D280N mutant in the presence of ATP. A 10-bairpair double-stranded RNA with a 10-nucleotide 3' overhang (2.5 nM) was incubated with RNase R or D280N mutant (100 nM) in the presence or absence of ATP. The wild-type RNase R unwound 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 ³²P-labeled 20-nucleotide RNA with a sequence of 5'-³²P-GUUGAGAGAGAGAGAGUUUG-3' with a 10-nucleotide RNA with a sequence of 5'-CUCUCUCAAC-3' at 95°C for 5 minutes in a buffer of 20 mM HEPES (pH 7.4), 50 mM NaCl and 2 mM MgCl₂, 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 MgCl₂, 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.

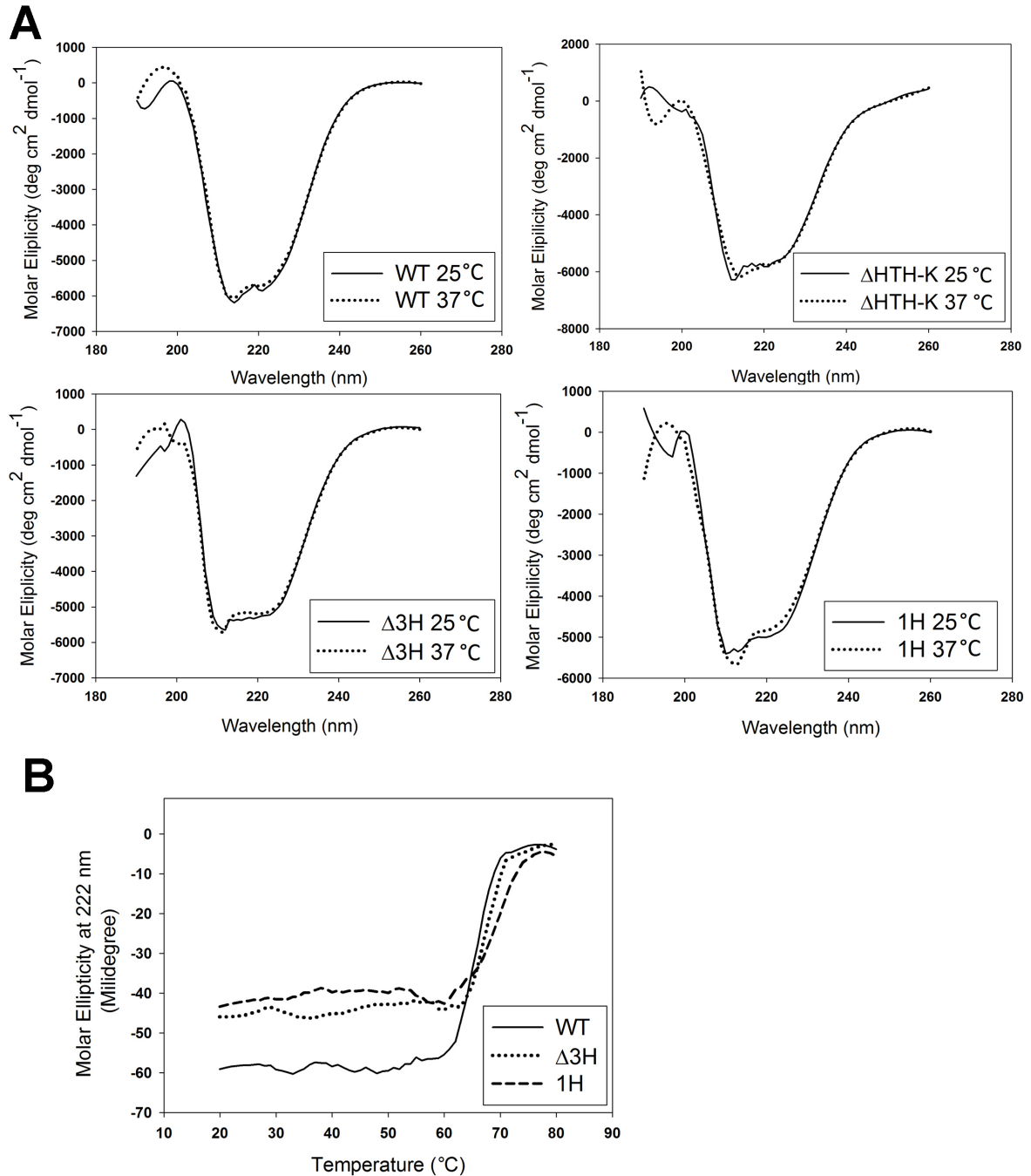


Figure S4. The CD spectra and thermal melting assays of the RNase R proteins.

(A) The CD spectra of RNase R, Δ HTH-K, Δ 3H and 1H mutants were recorded by AVIV CD400 spectrometer at 25°C and 37°C. (B) Thermal melting temperatures for RNase R, Δ 3H 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 Δ 3H, and 70.0°C for 1H mutant. The protein concentration used in the CD measurements were 10 μ M in a buffer of 50 mM Tris-HCl pH 7.5 and 500 mM NaCl. Measurements were taken three times and averaged.