Supporting information Xu et al. Solution Structure of the *Pfu* Rpp21-Rpp29 Protein Complex

Figures

| | | | | α1 | | α2 |
|--|--|---|---|---|--|---|
| P.furiosus | | i | 0000000 10 | 20 30 | l l | 2222222222 4 0 |
| P.furiosus P.horikoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium sp. T.acidophilum M.jannaschii M.marapaludis M.vannielii A.fulgidus S.cerevisiae S.pombe H.sapiens | MV MGKKAHGGKMKPEIDE | MAKYN.E DIVKRRDWE MKKVSWE MRGKR MSRIARKQQ MIT MKKFLE MKLKKKFLE MKLKKKFLE ML NGTLLVPPP MA | KKEKKRIAK KKEKKRIAIE KREKKRVAIE PRWHLKIAEE KNLIQAIAIQ KKDVEYTARK KKLKKIAYE KKL.KKIAYE KSKKIAE KSKKIAE RTIANQDHFH MSTKSKDQHA GPVKDREAFQ | RIDILFSLAERVFP RIDTLFTLAERVAR RIDTLFTLAERVVAR IDILFTLAERVVK RIDILFFLAKSEY. RIDRLHTLARASEY. RIDKLTLARAAR RIEKLYDFAIRTGD RIDILMSLAEEEAK RIDVLMNLAEKESK ARERVFYLIKRAE RRRVFYLIKRAE RLNYLYQISAYQTR RVSYLYQASQLLFR RLNFLYQAHCVLA | YSP YSP ANP AEHP TGDD KGNW DGKA CEKK WKNID ARQKARTDAHT NVQEP QDPEN | ELAKRYVELA DLAKRYVELA DLARRYVELA HRSHRYTELA DRSERYVQLI DRAREYVRLA RRYIIEM DRAKRYVYLA DRSKNYVLLG ERSKNYVLLS ELARRYVELS PLARNYIKSM TLSRHYISTA ALARFYCYTE |
| P.furiosus | η1 2222 222 | | ² β | 3 | β4 | β5 |
| P.furiosus P.horikoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium sp. T.acidophilum M.jannaschii M.marapaludis M.vannielii A.fulgidus S.cerevisiae S.pombe H.sapiens | LLVQQKAKVKIPRKWK LEIQKKAKVKIPRKWK LEIQKKAKVKIPRKWK RNIAMKYRVRIPREWR RNISMRNRMSIPREIK RRLAERNRLTLPPAFR EHIAQRMDITLPANIK RRIAMKMRIRFPKKWK KKIAMRMRMPYPKEWK KKIAMRMRMPYPKEWK CLISKKTKTSLLPTIK KDVSQKSVMRIHPDIK RTIAKRLVLRRDPSVK | RRYCKKCHA RRYCKRCHT RRYCRKCYS NRTCCKHCYA RRFTCDDCDA RGYCKKCGT RRICKKCGS RRICKKCGS RRICKKCGS RRICKKCGS RRICKKCCS RRICKKCCS RRICKKCCS RRICKKCCS RRICKKCS S | FL VPGINARV FL IPGVNARV FL VPGFNARV FL KPGANCTV FL VPGNARY VL VPGRNARY VL VPGRNARV FL IYGRNARV FL IYGRNARV FL IYGRNARV FL IYGKNSV FL IYGKNSV LLWTPKKLEI LLVPGKSCSI LLVPGLTCTG | VRLRQKRMPH YRLRYKRMPH RLRTDRMPH YRLADGMPH YRLKDGMPH YRLKDGMPH YRLKSGMPH YRLKSGMPH YRLKSGMPH YRLKSGMPH YRLKSKRYPH YRTKAKNYPH YRTKAKNYPH YRTKAKNRP TSDGARF ROGR RCRGQRW | VVVKCLECGH VVITCLECGH VVFCLECGH VVFCCCGT VVVCCOCGT VVVCCCCGT VVVTCLECGN VVVTCLECGN VVITCLECGN VVITCLECKH VVITCLECKH VVITCLECKH VVITCLECKH VVITCLECCGT VVITCLECGF CGT VCICCCCGT | MRYPYIKEIK MRYPYLREVK MRYPYLREVK MRFPYIREKK MRYPYKKLK. ARYPYKG RFFQISR YRIPMIREKK TRIPIKTEKK TRIPIKTEKK FRIPIKKSK. RFFIGADPN KRFSDKSC QRFLNDPGHL |
| P.furiosus P.furiosus P.horikoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium sp. T.acidophilum M.jannaschii M.marapaludis M.vannielii A.fulgidus S.cerevisiae S.pombe H.sapiens | KRRKEKMEY QKRKKAT. EKRKRKKD DRRRNKIESHTTKEGT EKRRKKLEERLKAKSN NRKV. YRTYSEREGNLLNS. LWGDRPEAQLGSQADS | DEQITVGAH SQTS KPLQPLPNT | NKCGESQSDR | SEKMQTQGSSNQ | | |

Figure S1: Sequence alignment of select RPP21 homologs from Archaea and Eukarya. The alignment was generated with CLUSTALW, and illustrated using ESPRIPT2.2, in which red letters indicate a global similarity score of 0.7, and red boxed letters indicate invariant residues. Secondary structural elements represented in cartoon are observed in the NMR ensemble of *Pfu* RPP21 in complex with *Pfu* RPP29. Aligned sequences are form *Pyrococcus furiosis* (NCBI entry NP_579342), *Pyrococcus horikoshii* (NP_143456), *Pyrococcus abyssi* (NP_126253), *Methanobacterium thermoautotrophicum* (NP_276730), *Methanosarcina barkeri* (NCBI_entry YP_304815), *Halobacterium sp.* (NP_279631), *Thermoplasma acidophilum* (NP_393654), *Methanococcus jannaschii* (NP_01322736), *Archaeoglobus fulgidus* (NP_068950), *Saccharomyces cerevisiae* (NP_012280), *Schizosaccharomyces pombe* (NP_596472) and *Homo sapiens* (NP_079115).

| 1110010 | |
|---|--|
| P.furiosis P.horicoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium.sp T.acidophilum M.jannaschii M.warapaludis M.warapaludis M.vannielii A.fulgidus S.cerevisiae S.pombe H.sapiens | MDRTQTFIKDCLFTKCLEDPEKPFNENRFQDTLLLLPTDGGLTSRLQRQQRKSKLNLDNLQKVSQLESADKQLEKRDYQRINKN MKSVIYHALSQKEANDSDVQPSGAQRAEAFVRAFLKRSTPRMS |
| P.furiosis | $\begin{array}{cccc} \alpha l & \alpha 2 \\ 0.000 & 0.0000 \\ 2.0 & 3.0 \\ 1 & 1.0 & 2.0 \\ 3.0 & 3.0 \\ \end{array}$ |
| P.furiosis P.horicoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium.sp T.acidophilum M.jannaschii M.marapaludis M.vannielii A.fulgidus S.cerevisiae S.pombe H.sapiens | MWRNSEERENTSGRSQGSYQEIIGRTWIFRGAHRGRV MRRNSKERKNRATRRSQGSYQEIIGRTWIFRGAHRGRV MRRNSKERKNRATRRSQGSYQEIUGRTWIFRGAHRGRV MRRNGKERKDRTSGGSQRPYQEIVGRTWIFRGSHM.LI .MKSKVEI |
| | |
| | |
| P.furiosis | $\begin{array}{cccccccccccccccccccccccccccccccccccc$ |
| P.furiosis P.furiosis P.horicoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium.sp T.acidophilum M.jannaschii M.marapaludis M.vannielii A.fulgidus S.cerevisiae S.cprombe H.sapiens | α3 0000 β1 0 β2 0 rt 0 rt 80 rt 90 rt 100 NKKNIVWHBLIGLKVRVVNSTHPGYVGIEGYVIDETRNMLVIAGE NKVWKVPRDVCIFFFET TRRNIWHBLIGLKVRVVNSTHPAFVGIEGYVIDETRNMLVIAGE NKVWKVPRDVCIFFFEA TKRNIWHBLIGLKVRVVNSTHPAFVGIEGYVIDETRNMLVIAGE NKVWKVPRDVCIFFFEA TKRNIWHBLIGLSVRIARSVHRDIOGISGRVVDETRNMLVIVG DKVWKVPRDVCIFFFEA TPRNIFRHLIGLSVRIARSVHRDIOGISGRVVDETRNMLVIVG DKVWKVPRDVCIFFFEA TPRNIFRHLIGLEIQVIRSTNPALIGIRGRVIDETRNLIIENDG GREITVPKGIAVFHFRT PST SGVAQUPKKGATFFRLITHENDEAAAPDNGVCTAFKPA MIYDEFTGMEVSIVDSPNRSBIGTGTGLVSPETNNTLVIENDIDG GREVVIPKKGATFFRLITHENDA TPHNILRHBLIGLEIVNSTDKRLISTKGRVINETRNTLVIEKED GREVVIPKDIAVFFQL LSONILRHBLVGLNLEIVNSTDKRLISTKGRVINETRNTLVIEKEN GKEITVVVKEISIFRRIOF FSQNILRHBLVGLNLEIVNSTDKRLISTKGRVINETRNTLVIEKEN GKEITVVKKISTROF QCVELIARDWIGLMVEVKESPNHBBVGIKGEVVDETONTLKIMTE KGLKVVAKRGRTRVWY SONILRHBLVGLNLEIVNSTDKRLISTKGRVINETRNTLVIEKEN GKEITVVKKISTROF QCVELIARDWIGLMVEVKESPNHBBVGIKGEVVDETONTLKIMTE KGLKVVAKRGRTRVWY SONILRHBLVGLNLEVNSTOKKIJGIKKSLTVKYFSPNTST. GLKRVVFREAAALVLIRELYG SOSSITTSSLSKIIRTWVAENFGDVGIGKVASSLTVKYFSPNTST. GLKRVFREAAALVLIRELYG SOSSITTSSLSKIIRTWVAENFGDVGIGKVASSLTVKYFSPNTST. |
| P.furiosis P.furiosis P.horicoshii P.abyssi M.thermoautotrophicus M.barkeri Halobacterium.sp T.acidophilum M.jannaschii M.marapaludis M.vannielii A.fulgidus S.corevisiae S.pombe H.sapiens P.furiosis | $\begin{array}{cccccccccccccccccccccccccccccccccccc$ |

Figure S2: Sequence alignment of select RPP29 homologues from Archaea and Eukarya. The alignment was generated with CLUSTALW, and illustrated using ESPRIPT2.2, in which red letters indicate a global similarity score of 0.7, and red boxed letters indicate invariant residues. Secondary structural elements represented in cartoon are observed in the NMR ensemble of *Pfu* RPP29 in complex with *Pfu* RPP21. Aligned sequences are form *Pyrococcus furiosis* (NCBI entry NP_579545), *Pyrococcus horikoshii* (NP_143607), *Pyrococcus abyssi* (NP_126024), *Methanobacterium thermoautotrophicum* (10QK_A), *Methanosarcina barkeri* (YP_303669), *Halobacterium sp.* (NP_280464), *Thermoplasma acidophilum* (NP_394719), *Methanococcus jannaschii* (NP_247439), *Methanococcus marapaludis* (YP_001549311), *Methanococcus vannielii* (YP_001323236), *Archaeoglobus fulgidus* (1TSF_A), *Saccharomyces cerevisiae* (NP_009816), *Schizosaccharomyces pombe* (NP_588479) and *Homo sapiens* (NP_006618).



Figure S3. Footprinting using RNase V1 and RNase T1 to identify RPP-binding sites in *Mja* RPR. (a) Same as panel a in Figure 6 except this is a longer electrophoretic run, which was required to map protection patterns distal to the labeled termini. Mia RPR labeled at the 5'-end was incubated either without (lanes 1, 3, 5, 7 and 9) or with (lanes 2, 4, 6, 8 and 10) RNase V1 (panel a) or RNase T1 (panel b). Mia RPR was present either alone (lanes 1, 2, 7 and 8), with RPP21-RPP29 (lanes 3 and 4), with RPP30-POP5 (lanes 5 and 6) or with both binary complexes (lanes 9 and 10). Since reconstitution of the RPR with each binary RPP complex is performed in a buffer different from that used for reconstitution with both binary complexes together, two different control RNase T1/V1 digestions of the RPR are shown (lanes 1, 2 for binary RPPs and lanes 7, 8 for both binary pairs). "Alk." and "T1" represent molecular size ladders generated by subjecting end-labeled, denatured Mja RPRs to alkaline hydrolysis and partial RNase T1 digestion, respectively. The RNase T1 cleavage sites were also mapped by using primer extension assays. (b) Summary of the RPP footprinting data depicted on a secondary-structure model of Mja RPR. Circled and boxed nucleotides indicate protection to RNase T1 and RNase V1, respectively; blue and red colors indicate regions of protection by RPP30-POP5 and RPP21-RPP29, respectively. The green arrow indicates an RPR position that showed increased susceptibility to RNase T1 in the presence of either RPP30-POP5 or all four RPPs. RNase V1 cleavages around nucleotides 130-150 suggest that the secondary structure as drawn may need to be revised.



Figure S4: Two-dimensional ¹H-¹⁵N NMR spectra of *Pfu* RPP21 (a) and RPP29 (b) in complex with its (unlabeled) partner. Backbone amide assignments are indicated in red as the residue number.



Figure S5: Heteronuclear {¹H)-¹⁵N NOE data of *Pfu* RPP29 in the presence of *Pfu* RPP21 shows that the N-terminus (residues 1-16, highlighted in cyan) remains flexible, indicating this segment is not involved in binding to RPP21.



Figure S6: Interface of the *Pfu* RPP29-RPP21 complex in the ensemble. The backbones are shown in lines (RPP21 in cyan and RPP29 in cyan). The residues involving in protein-protein interactions are shown in sticks. Panels a-c are similar zoom-in regions as panel c-e in Figure 3.

| Table S1. Inter-molecular NOEs identified from chemical shifts in the ¹³ C-filtered/edited |
|--|
| NOESY spectrum recorded on [U- ¹³ C, ¹⁵ N]-RPP21 (*) and unlabeled RPP29. [†] |
| |

| RPP21* | RPP29 | RPP21* | RPP29 | RPP21* | RPP29 |
|--------|----------|--------|----------|---------|----------|
| K10HB | 121Μδ- | K10HB | 121Μδ- | K10HB | Ι 121Μδ- |
| K10Hg | L 121Μδ+ | K10Ha | L 121Μδ+ | K10Hg | L 121Μδ+ |
| K10Ha | 121Μδ- | K10Ha | 121Μδ- | K10Ha | Ι 121Μδ- |
| 113Mv2 | W45Hδ | 113Mv2 | W45Hδ | 113Mv2 | W45Hδ |
| 113Mv2 | W45HB- | S32HB- | F22HB+ | I 24Mδ+ | F30HB- |
| 113Mv2 | Η46Ηδ2 | S32HB- | E22Hv | B27Ha | 129Hß |
| 113Μδ | W45HZ3 | L35Μδ- | Ι71Μδ | V28HB | 123Μδ |
| 113Mv2 | N42Ha | L35Mδ- | Υ20Ηδ | V28Mv- | 129Mv1 |
| 113Mv2 | N42HB- | L35Μδ+ | Ι71Μδ | V28Mv- | I23Mδ |
| 113Mv2 | L121Μδ- | L35Mδ- | 123HB | V28Mv- | 129HB |
| 113Mv2 | N42Hβ+ | L35Μδ+ | I23Hβ | V28HB | I29Mδ |
| l13Μδ | W45Hc3 | L35Μδ+ | I71Mv2 | V28Mv+ | I29Hβ |
| Ι13Μδ | Η46Ηε1 | L35Mδ- | Υ20Η̈́ε | V28Μγ+ | Ι23Μδ |
| A14Ha | L121Μδ- | L35Mδ- | D72Hβ- | V28Μγ+ | F30Hζ |
| Α14Μβ | L121Μδ- | L35Mδ- | D72Hβ+ | V28Μγ- | I29Mγ2 |
| A14Mβ | L121Μδ+ | L35Μδ+ | Y20Hδ | V28Μγ- | F30Hɛ |
| A14Mβ | L121Hβ- | L35Mδ- | I23Mγ1 | V28Μγ- | Ι29Μδ |
| A14Ha | L121Μδ+ | L35Μδ+ | Y20He | V28Μγ+ | I23Mγ1 |
| A14Mβ | L121Hβ+ | L35Μδ+ | F30Hζ | V28Μγ+ | I29Mγ1 |
| E16Hγ- | Η46Ηε1 | L35Μδ+ | Ι23Μδ | V28Μγ+ | F30Hβ- |
| E16Hβ- | H46Hɛ1 | L35Μδ+ | I23Mγ1 | V28Mγ+ | I29Mδ |
| E16Hγ+ | Η46Ηδ2 | L35Μδ+ | D72Hβ- | V28Mγ- | F30Hζ |
| E16Hγ+ | Η46Ηε1 | L35Mδ- | 171Ha | V28Ha | Ι29Μδ |
| E16Hβ+ | Η46Ηε1 | L35Mδ- | l71Mγ2 | V28Ha | Ι23Μδ |
| E16Ha | Η46Ηε1 | L42Μδ+ | Ρ117Ηγ | V28Mγ+ | F30Hε |
| R17Ha | Ε47Ηβ | L42Μδ- | Ρ117Ηβ- | V28Mγ+ | l29My2 |
| I20My2 | Η34Ηβ+ | L42Μδ+ | Ι49Μδ | V28Ha | l29My2 |
| I20My2 | E47Hγ+ | L24Μδ- | A33Ha | V28Mγ- | I23Mγ1 |
| I20My2 | Ε47Ηβ | L24Μδ+ | A33Ha | Р30Нү+ | Ι29Ηβ |
| I20My2 | E47Hγ- | L24Μδ- | F30Hδ | S32Ha | E22Hy |
| I20My2 | Η34Ηδ2 | L24Μδ+ | F30Hδ | S32Hβ- | Ι23Μδ |
| I20My2 | Η34Ηβ- | L24Μδ- | F30Ha | RPP21 | RPP29 |
| L21Μδ- | Ι49Ηβ | L24Μδ+ | F30Hε | L42Μδ+ | l49Mγ2 |
| L21Μδ- | I49Mγ2 | L24Μδ- | I49Mγ2 | L42Μδ+ | Ρ117Ηβ- |
| L21Μδ- | Ι49Μδ | L24Μδ- | Α33Μβ | L42Μδ- | Ρ117Ηγ |
| L21Μδ+ | Ι49Μδ | L24Μδ- | F30Hε | L42Μδ- | Ρ117Ηβ+ |
| L21Μδ- | E47Hγ- | L24Μδ+ | G50Ha+ | L42Μδ- | Ι49Μδ |
| S23Ha | ΑЗЗΜβ | L24Μδ+ | Α33Μβ | L42Μδ- | Ρ117Ηδ- |
| S23Hβ | ΑЗЗΜβ | L24Μδ+ | Ι49Ηβ | L42Μδ+ | Ρ117Ηβ+ |
| L24Μδ+ | Ι49Μδ | L24Mδ+ | Ι49Μγ2 | L42Mδ- | E118Ha |
| L24Μδ- | G50Ha+ | L24Μδ+ | G50Ha- | L45Mδ+ | Ρ117Ηδ- |
| L24Μδ- | F30Hβ+ | L24Μδ- | G50Ha- | L45Μδ+ | L121Ha |

| RPP21* | RPP29 | RPP21* | RPP29 | | RPP21* | RPP29 |
|--------|---------|--------|---------|---|--------|---------|
| Κ10Ηβ | L121Μδ- | K10Hβ | L121Μδ- | - | K10Hβ | L121Μδ- |
| K10Ha | L121Μδ+ | K10Ha | L121Μδ+ | | K10Ha | L121Μδ+ |
| K10Ha | L121Μδ- | K10Ha | L121Μδ- | | K10Ha | L121Μδ- |
| I13Mγ2 | W45Hδ | l13Mγ2 | W45Hδ | | l13Mγ2 | W45Hδ |
| L45Μδ- | Ρ117Ηδ- | V46Hy | Ρ117Ηβ- | | Α50Μβ | L121Μδ- |
| L45Μδ- | Ρ117Ηγ | V46Hy | L121Μδ+ | | Α50Ηα | L121Μδ+ |
| L45Μδ+ | Ρ117Ηγ | V46Ha | L121Ha | | Α50Μβ | L121Hβ+ |
| V46Hy | L121Ha | V46Ha | L121Μδ+ | | | |
| V46Hy | L121Μδ- | Α50Μβ | L121Μδ+ | | | |

Table S2. Inter-molecular NOEs identified from chemical shifts in the ¹³C-filtered/edited NOESY spectrum recorded on unlabeled RPP21 and [U-¹³C,¹⁵N]-RPP29 (*).[†]

| RPP29* | RPP21 | RPP29* | RPP21 | RPP29* | RPP21 |
|--------|----------|--------|--------|--------|--------|
| S19Hβ | Ρ33Ηβ | S19Hβ | Ρ33Ηβ | S19Hβ | Ρ33Ηβ |
| S19HB | L35Mδ- | S19HB | L35Μδ- | S19Hβ | L35Mδ- |
| S19HB | L35Mδ+ | S19HB | L35Μδ+ | S19HB | L35Mδ+ |
| S19HB | L35Ha | RPP29 | RPP21 | 129My2 | L24Ha |
| S19Ha | E34Hβ1/2 | W45Hβ- | I13Mγ2 | Ι29Μδ | Υ31Ηδ |
| S19Hβ | E34Hβ1/2 | W45Hβ+ | I13Mγ2 | Ι29Μδ | V28Mγ+ |
| Y20Ha | L35Mδ- | H46Ha | Ι13Μδ | I29My2 | Y31Hδ |
| Y20Ha | L35Mδ+ | E47Ha | R17Hβ | 129My2 | R27Hδ- |
| I23My2 | Υ31Ηβ- | E47Ha | R17Ha | Ι29Μδ | V28Hβ |
| I23My2 | V28Mγ- | Ε47Ηβ | R17Ha | I29My2 | R27Hβ |
| Ι23Μδ | V28Μγ+ | l49Mγ2 | L42Μδ+ | I29My2 | R27Ha |
| I23My2 | L35Hβ- | Ι49Μδ | Υ39Ηε | Ι29Μδ | L24Ha |
| I23My2 | Υ31Ηδ | Ι49Μδ | L21Μδ- | I29My2 | V28Ha |
| I23Mγ1 | L35Μδ+ | l49Mγ2 | L24Hβ | I29My2 | V28Hβ |
| Ι23Μδ | S32Hβ+ | l49Mγ2 | L21Μδ- | 129Ha | R27Hδ+ |
| Ι23Μδ | Υ31Ηδ | Ι49Μδ | L42Hβ- | Ι29Μδ | R27Hy |
| Ι23Μδ | S32Hβ- | l49Mγ2 | L42Hβ- | I29My2 | R27Hy |
| I23Mγ2 | Υ31Ηβ+ | l49Mγ2 | L24Ha | I29Mγ1 | V28Mγ+ |
| I23Mδ | Υ31Ηβ- | l49Mγ2 | L24Hγ | Ι29Μδ | V28Ha |
| I23My2 | S32Hβ- | l49Mγ2 | L24Μδ+ | Ι29Μδ | L24Μδ- |
| I23Mδ | V28Hβ | 149Ha | L24Μδ+ | F30Ha | L24Μδ- |
| I23My2 | L35Hy | Ι49Μδ | L42Μδ+ | ΑЗЗΗα | L24Μδ- |
| I23My2 | V28Hβ | Ι49Μδ | L24Μδ+ | ΑЗЗΜβ | L24Μδ+ |
| I23My2 | S32Hβ+ | l49Mγ2 | R38Hδ+ | ΑЗЗΜβ | L24Μδ- |
| I23My2 | V28Ha | Ι49Ηβ | L21Μδ- | ΑЗЗΗα | R27Hβ |
| I23Mδ | V28Ha | l49Mγ2 | Υ39Ηε | ΑЗЗΜβ | R27Hδ+ |
| R26Hγ+ | Y31Hε | l49Mγ2 | Υ39Ηδ | ΑЗЗΜβ | R27Hδ- |
| R26Hδ- | Υ31Ηε | Ι49Μδ | R38Hδ+ | ΑЗЗΜβ | L24Ha |
| R26Hδ+ | Y31Hɛ | Ι49Μδ | Υ39Ηδ | ΑЗЗΜβ | R27Hβ |
| R26Hδ+ | Υ31Ηδ | Ι49Ηβ | L24Μδ+ | Η34Ηβ+ | I20My2 |
| R26Hγ+ | Υ31Ηδ | G50Ha+ | L24Μδ+ | H34Ha | I20Hβ |
| R26Hδ- | Y31Hβ+ | G50Ha+ | L24Μδ- | H34Ha | 120Ha |
| R26Hδ- | Υ31Ηδ | G50Ha- | L24Μδ+ | Η34Ηβ- | I20My2 |
| Ι29Ηβ | R27Hy | G50Ha- | L24Μδ- | H34Ha | I20Μδ |
| Ι29Μδ | R27Ha | l71Mγ2 | Υ39Ηδ | H34Ha | I20My2 |
| I29My2 | L24Μδ- | Ι71Μδ | L35Μδ+ | N42Ha | I13Mγ2 |
| I29Mγ1 | R27Hβ | l71Mγ2 | L35Mδ- | N42Hβ+ | I13Mγ2 |
| I29Mγ1 | Υ31Ηδ | l71Mγ2 | L35Μδ+ | Ν42Ηβ- | I13Mγ2 |
| I29Mδ | R27Hβ | Ι29Μδ | R27Hδ- | W45Hβ+ | Ι13Μδ |
| I29My2 | V28Mγ+ | 129Ha | R27Hδ- | W45Ha | I13Mγ2 |
| I29My2 | R27Hδ+ | Ι29Ηβ | R27Hβ | W45Hβ- | Ι13Μδ |
| I29My2 | Υ31Ηε | Ι29Μδ | Υ31Ηε | RPP29 | RPP21 |

| RPP29* | RPP21 | RPP29* | RPP21 | RPP29* | RPP21 |
|---------|--------|---------|--------|---------|--------|
| S19Hβ | Ρ33Ηβ | S19Hβ | Ρ33Ηβ | S19Hβ | Ρ33Ηβ |
| S19Hβ | L35Mδ- | S19Hβ | L35Mδ- | S19Hβ | L35Μδ- |
| S19Hβ | L35Μδ+ | S19Hβ | L35Μδ+ | S19Hβ | L35Μδ+ |
| Ι71Μδ | L35Μδ+ | E118Ha | L45Hβ+ | L121Μδ- | R17Ha |
| Ι71Μδ | L35Mδ- | E118Ha | L45Μδ- | L121Μδ- | Α50Ηα |
| Ι71Ηβ | L35Mδ- | L121Μδ- | l13Mγ2 | L121Μδ- | Ι13Μδ |
| l71Ha | L35Mδ+ | L121Μδ+ | Ι13Μδ | L121Μδ- | Κ10Ηβ |
| D72Ha | Υ39Ηδ | L121Μδ+ | Α50Μβ | L121Μδ+ | A50Ha |
| Ρ117Ηβ+ | L42Μδ- | L121Μδ+ | L45Mδ- | L121Μδ+ | V46Ha |
| Ρ117Ηδ+ | L42Μδ- | L121Μδ- | L45Mδ- | L121Μδ+ | R17Ha |
| Ρ117Ηγ | L42Μδ- | L121Μδ+ | Κ49Ηβ+ | | |
| Ρ117Ηα | L42Mδ- | L121Μδ+ | K10Ha | | |
| Ρ117Ηβ- | L42Mδ- | L121Μδ+ | R17Hβ | | |
| E118Ha | K49Hδ | L121Μδ- | V46Ha | | |
| E118Ha | L42Μδ+ | L121Μδ- | K10Ha | | |
| E118Ha | V46Ha | L121Μδ- | Α50Μβ | | |

Table S3. Inter-molecular NOEs identified by iterative structure-based assignment in ¹³C-separated NOESY spectra recorded on [U-¹³C,¹⁵N]-RPP21 (*) and unlabeled RPP29.[†]

| RPP21* | RPP29 | RPP21* | RPP29 | RPP21* | RPP29 |
|--------|---------|--------|---------|--------|---------|
| 113Ηα | H46Ha | L21Μδ+ | E47Hγ+ | V28Mγ- | F30Ηζ |
| I13Mγ2 | W45Hδ1 | L21Μδ+ | Ε47Ηβ | F29HN | I29Μδ |
| I13Mγ2 | N42Ha | L21Μδ+ | E47Ηγ- | P30Hγ+ | I29HB |
| l13Mγ2 | H46Ha | S23Ha | ΑЗЗΜβ | Y31HN | Ι29Μδ |
| Ι13Μδ | W45Hɛ3 | S23Hβ | ΑЗЗΜβ | S32HN | Ι23Μδ |
| Ι13Μδ | W45Hδ1 | L24Ha | ΑЗЗΜβ | S32HN | Ι29Μδ |
| Ι13Μδ | W45Ηζ3 | L24Μδ- | ΑЗЗΜβ | S32Ha | E22Hy |
| Ι13Μδ | W45Hβ- | L24Μδ- | F30Hβ+ | S32Hβ- | E22Hβ+ |
| Ι13Μδ | L121Μδ- | L24Μδ- | Η34Ηβ- | L35HN | S19Hβ |
| A14Ha | L121Hβ+ | L24Μδ- | 149Ha | L35Μδ+ | F30Ηζ |
| Α14Μβ | L121Μδ+ | L24Μδ- | G50Ha- | L35Μδ+ | 171Ha |
| E16HN | H46Hɛ1 | A50HN | L121Μδ+ | L35Μδ+ | Y20Ha |
| E16Hγ+ | N42Hβ+ | A50Ha | L121Μδ+ | L35Μδ+ | Ι23Μδ |
| E16Hγ+ | H46Hɛ1 | L24Μδ- | A33Ha | Y39HN | Ι49Μδ |
| E16Hβ+ | H46Hɛ1 | L24Μδ- | H34Ha | L42Μδ- | Ε73Ηβ |
| R17Ha | E47Hγ- | L24Μδ- | G50Ha+ | L42Μδ- | Ρ117Ηγ |
| I18HN | E47Hγ+ | L24Μδ- | F30Hδ | L42Μδ- | Ρ117Ηβ+ |
| 120HN | E47Hγ+ | L24Μδ- | F30Hε | L42Μδ- | Ρ117Ηδ- |
| 120HN | Ε47Ηβ | L24Μδ- | F30Hζ | L45Μδ- | Ρ117Ηβ+ |
| 120Ha | H34Ha | L24Μδ+ | F30Hβ- | L45Μδ- | R116Ha |
| I20My2 | Η34Ηδ2 | L24Μδ+ | G50Ha- | V46HN | L121Hβ+ |
| I20My2 | G50Ha+ | L24Μδ+ | 129Ha | V46Ha | L121Hβ+ |
| I20My2 | H34Ha | L24Μδ+ | G50Ha+ | V46Ha | L121Μδ+ |
| I20My2 | Η34Ηβ- | L24Μδ+ | F30Hε | A50HN | L121Hβ+ |
| I20My2 | Ε47Ηβ | R27HN | Ι23Μδ | Α50Μβ | L121Μδ+ |
| I20My2 | E47Hγ- | V28HN | Ι29Ηβ | | |
| I20My2 | Ι49Ηβ | V28HN | Ι29Μδ | | |
| I20My2 | ΑЗЗΜβ | V28HN | I29My2 | | |
| L21HN | E47Hγ+ | V28Ha | Ι29Μδ | | |
| L21HN | Ι49Ηβ | V28Ha | I29My2 | | |
| L21Ha | l49Mγ2 | V28Mγ- | Ι29Μδ | | |

| Table S4. Inter-molecular NOEs identifie | d by iterative structure-based assignment in ¹³ C- |
|--|---|
| separated NOESY spectra recorded on | [U- ¹³ C, ¹⁵ N]-RPP29 (*) and unlabeled RPP21. [†] |

| RPP29 | RPP21 | RPP29 | RPP21 | RPP29 | RPP21 |
|--------|--------|---------|--------|---------|--------|
| S19Hβ | S32Ha | S19Hβ | S32Ha | S19Hβ | S32Ha |
| E22Hy | S32Hβ+ | E22Hy | S32Hβ+ | E22Hy | S32Hβ+ |
| E22Hy | Y31Ha | Α33Ηα | L24Μδ- | E47Ha | L42Hβ- |
| E22Hy | S32Ha | ΑЗЗΜβ | L24Μδ- | Ι49Μδ | L42Hy |
| l23Ha | Υ31Ηβ+ | RPP29 | RPP21 | Ι49Μδ | Υ39Ηβ- |
| I23My2 | V28Hβ | l71Mγ2 | L35Hy | Ι49Μδ | Y39Ha |
| I23My2 | Υ31Ηβ+ | D72Ha | Υ39Ηδ | Ι49Μδ | Y39Ηε |
| I23My2 | S32Hβ- | D72Ha | L35Μδ- | Ι49Μδ | Υ39Ηδ |
| I23My2 | S32Hβ+ | E73HN | Υ39Ηδ | l49Mγ2 | Υ39Ηβ- |
| I23My2 | S32Ha | E73HN | L42Hy | I49Mγ2 | L21Ha |
| I23My2 | Υ31Ηδ | Ρ117Ηβ+ | L42Μδ- | l49Mγ2 | L24Ha |
| Ι23Μδ | V28Hβ | P117Ηγ | L42Hβ+ | l49Mγ2 | Y39Ha |
| Ι23Μδ | S32Hβ- | P117Ηγ | L42Μδ- | l49Mγ2 | Υ39Ηε |
| Ι23Μδ | S32Hβ+ | E118Ha | V46Ha | l49Mγ2 | Y39Hδ |
| Ι23Μδ | Y31Ha | L121Hβ- | A50Ha | G50Ha+ | L24Μδ- |
| Ι23Μδ | S32Ha | ΑЗЗΜβ | L24Μδ+ | G50Ha+ | L24Μδ+ |
| R26Hβ- | Υ31Ηβ- | ΑЗЗΜβ | R27Hβ | G50Ha- | L24Μδ+ |
| R26Hβ- | Y31He | ΑЗЗΜβ | R27Hδ- | G50Ha- | L24Μδ- |
| R26Hβ- | Υ31Ηδ | ΑЗЗΜβ | R27Hδ+ | l71Mγ2 | L35Hβ- |
| 129Ha | R27Hδ+ | ΑЗЗΜβ | 120Ha | RPP29 | RPP21 |
| Ι29Ηβ | R27Hy | ΑЗЗΜβ | S23Ha | L121Μδ+ | L13Μδ+ |
| Ι29Μδ | Υ31Ηδ | H34HN | I20My2 | L121Μδ+ | I18Hβ |
| Ι29Μδ | Y31Ha | H34Ha | Ι20Μδ | L121Μδ+ | V46Hβ |
| Ι29Μδ | R27Ha | Η34Ηβ+ | I20My2 | L121Μδ+ | V46Ha |
| Ι29Μδ | V28Ha | Η34Ηβ- | I20My2 | L121Μδ+ | A50Ha |
| Ι29Μδ | R27Hδ+ | R35HN | Ι20Ηβ | L121Μδ+ | A14Ha |
| Ι29Μδ | Υ31Ηβ+ | R35HN | Ι20Μδ | L121Μδ- | L13Μδ+ |
| Ι29Μδ | V28Hβ | G36HN | R17Ha | L121Μδ- | Α14Μβ |
| Ι29Μδ | Р30Нү+ | G36HN | E16Hβ- | L121Μδ- | A14Ha |
| l29My2 | Υ31Ηβ- | G36HN | Ι20Μδ | K122Hγ- | L45Hβ+ |
| I29My2 | Υ31Ηβ+ | G36HN | I20My2 | | |
| I29My2 | R27Ha | G36Ha+ | E16Hβ- | | |
| I29My2 | V28Ha | G36Ha- | R17Ha | | |
| I29My2 | Υ31Ηε | W45HN | I13Mγ2 | | |
| F30HN | L24Μδ- | W45Hβ- | I13Mγ2 | | |
| F30Ha | V28Μγ+ | W45Hβ- | Ι13Μδ | | |
| F30Hβ+ | L24Μδ- | W45Hβ+ | I13Mγ2 | | |
| F30Hβ+ | L24Μδ+ | W45Hβ+ | Ι13Μδ | | |
| G32Ha+ | R27Hδ+ | H46HN | Ι13Ηβ | | |
| A33HN | L24Μδ- | H46HN | I13Mγ2 | | |