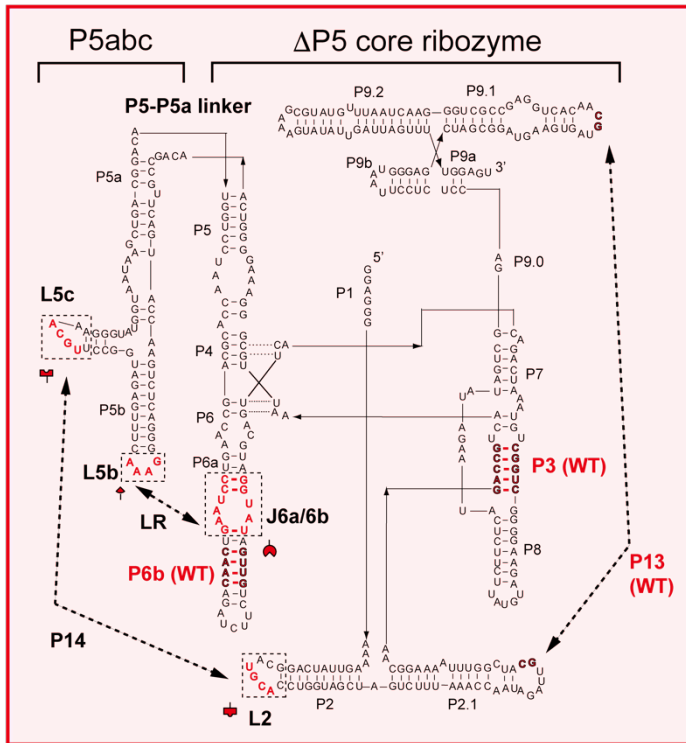
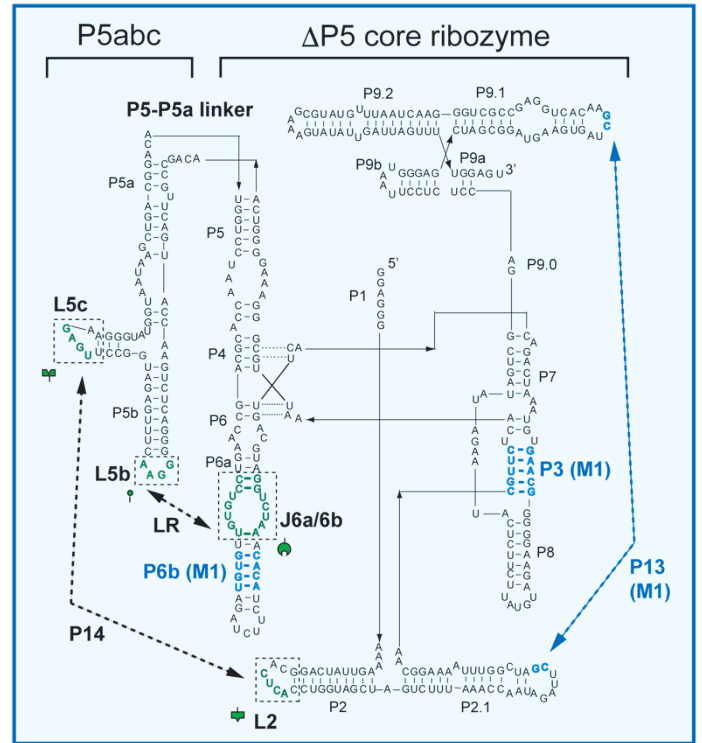


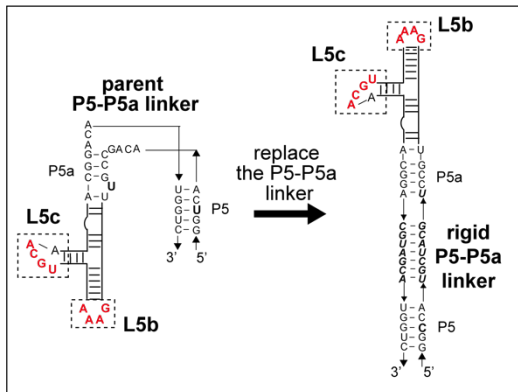
A Wild-type ribozyme



B M1 variant ribozyme



C



D

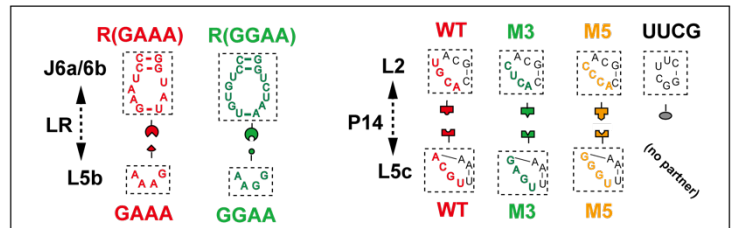


Figure S1

Sequences and secondary structures of the *Tetrahymena* group I ribozymes and their structural elements used for modular engineering in this study.

(A, B) Secondary structures of the wild-type *Tetrahymena* ribozyme (A) and its variant (M1 type) ribozyme (B). Lines with two arrowheads indicate tertiary interactions. Nucleotides shown in red in A and green in B participate directly in tertiary interactions between the P5abc module and Δ P5 module. Nucleotides shown in bold are structural elements (P3, P6b, and P13) that differ between the wild-type and M1 type.

(C) Nucleotide sequences of the parent P5-P5a linker region and a rigid variant used in RzM.

(D) Nucleotide sequences of RNA motifs involved in J6a/6b-L5b and L2-L5b tertiary interactions. Corresponding icons used for indication of RzM:RzM interfaces are also shown.

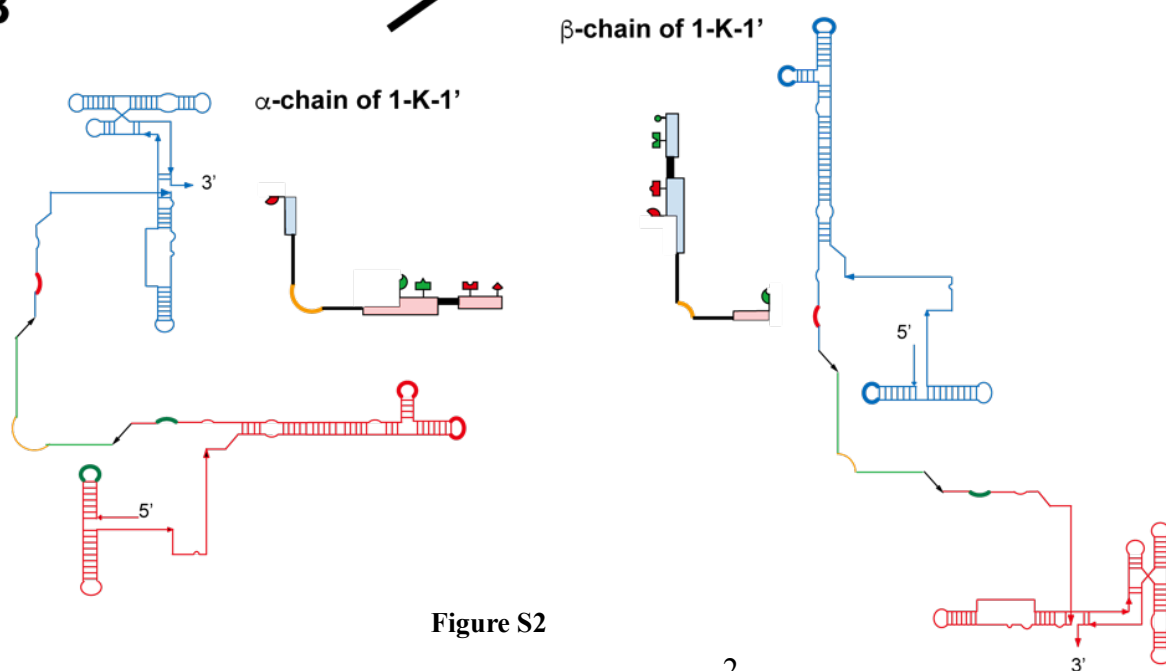
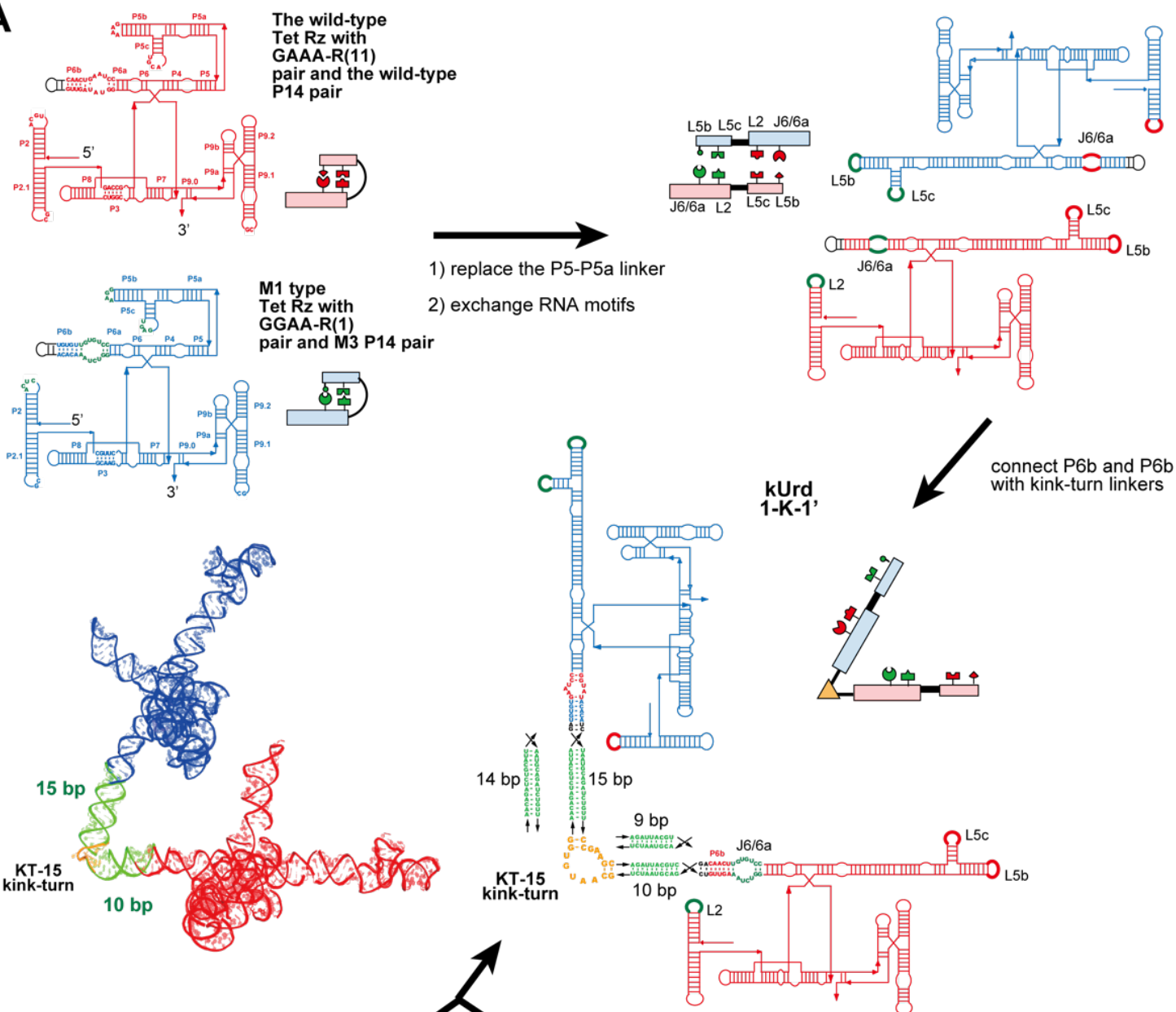


Figure S2

Scheme of rational redesign of unimolecular *Tetrahymena* ribozymes to generate kUrd **1-K-1'**.

(A) Stepwise redesign of wild-type and M1 type ribozymes to construct kUrd **1-K-1'**.

(B) Formation of kUrd **1-K-1'** through assembly of its α - and β -chains.

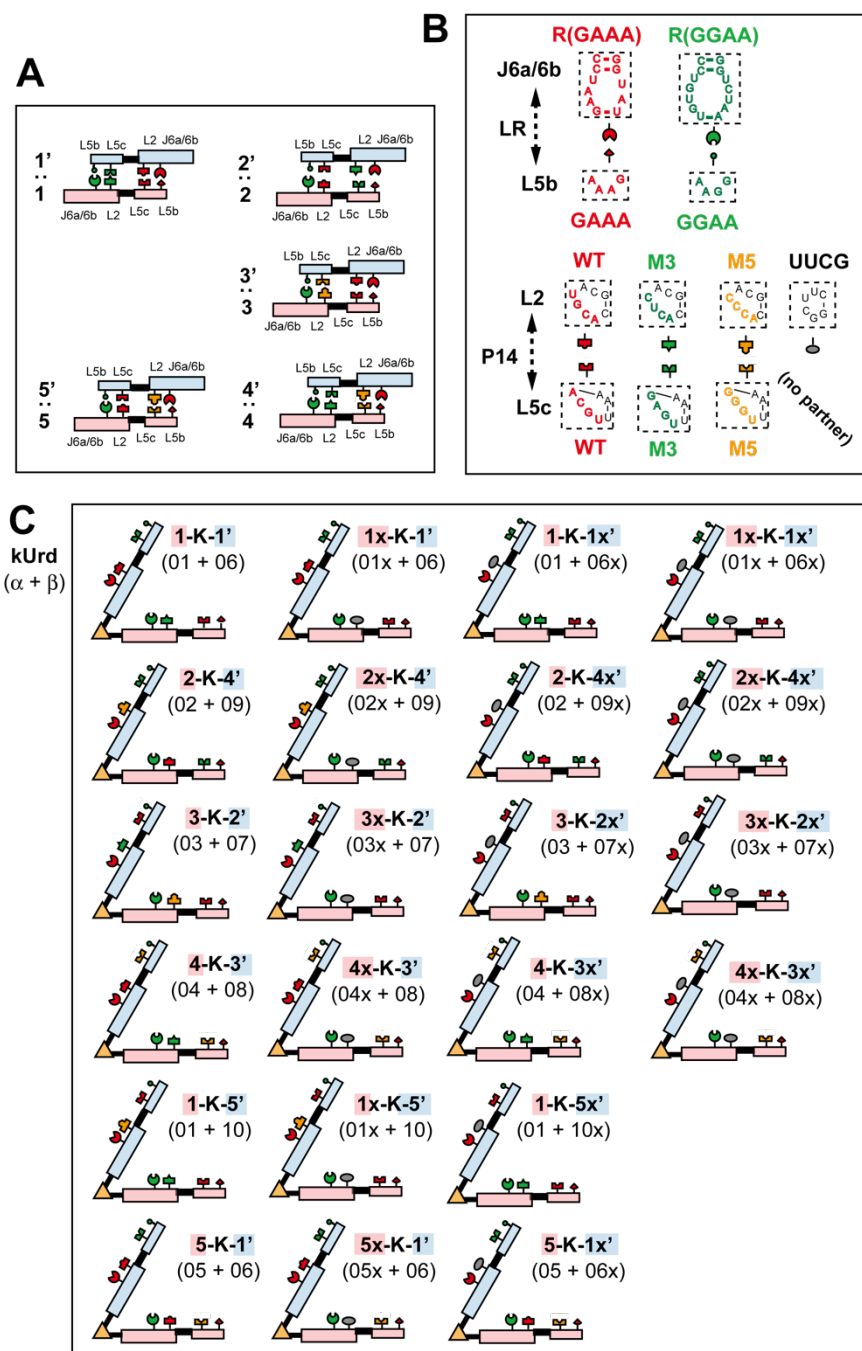


Figure S3

Kink-turn unit ribozyme dimers (kUrds) employed in this study.

(A) Five distinct pairs of RzM:RzM interfaces used in this study.

(B) Nucleotide sequences of RNA motifs involved in J6a/6b-L5b and L2-L5b tertiary interactions. Corresponding icons used for indication of RzM:RzM interfaces are also shown.

(C) Structural organization of kUrds employed in this study. Each kUrd is formed by the assembly of two RNA-chains (α - and β -chains), identity of which is shown in parentheses by the number used in Table S1.

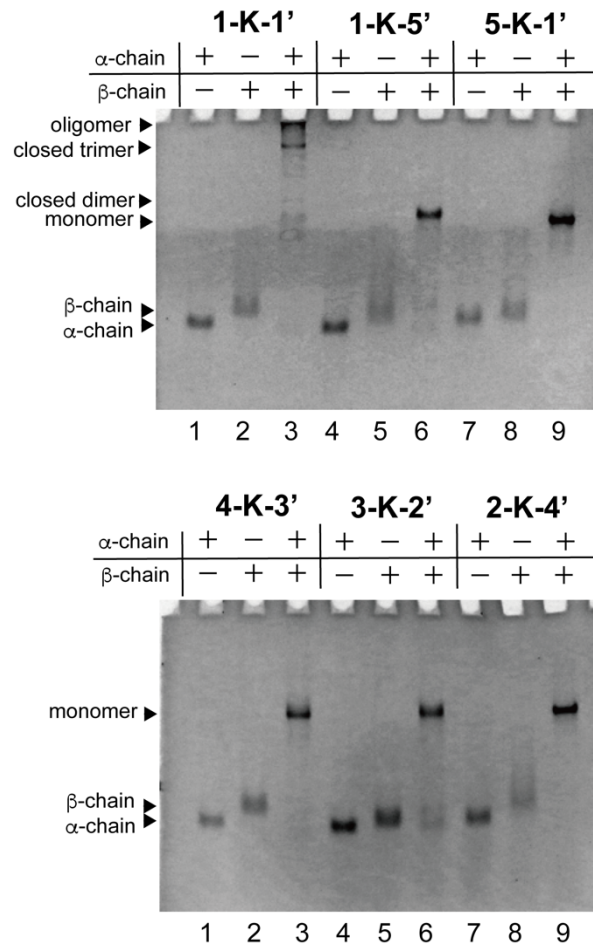


Figure S4

Formation of kUrds through the assembly of their α -chain and β -chain RNAs.

EMSA of α -chain RNA, β -chain RNA and their equimolar mixture of each kUrds used for homopolymerization (top), diblock copolymerization (top), and triblock copolymerization (bottom). EMSA were performed with 0.125 μ M each RNA chain in the presence of 15 mM Mg^{2+} . RNAs were visualized by ethidium bromide staining.

A 10 mM Mg²⁺ [0.125 μM RNA chain each]

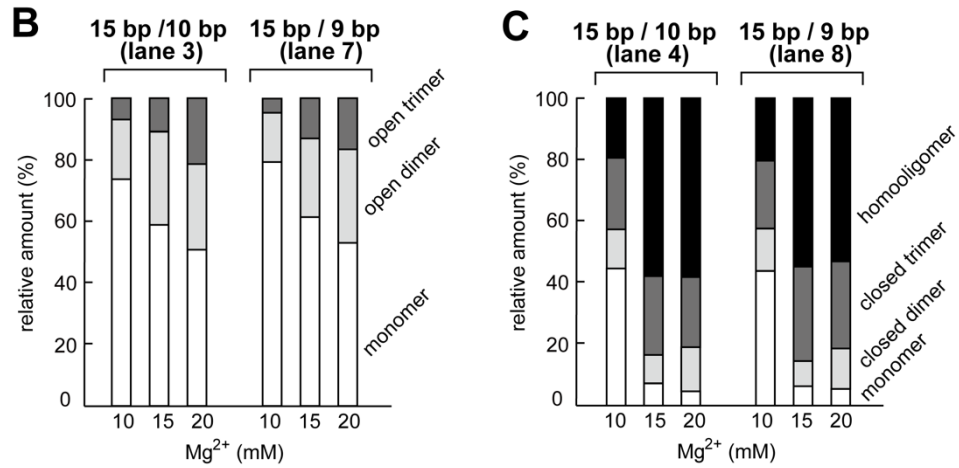
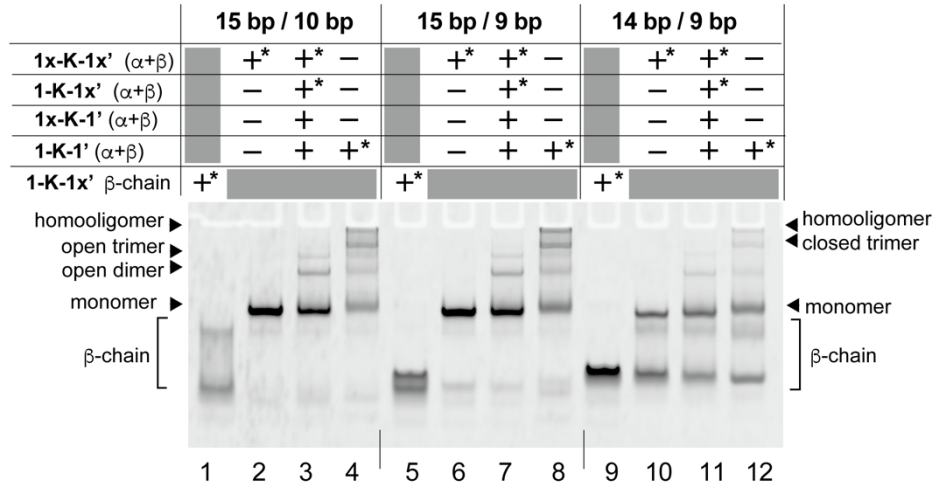


Figure S5

Oligomerization of **1-K-1'** and its mutants.

(A) EMSA of **1-K-1'** homopolymers formed in the presence of 10 mM Mg²⁺. Asterisks indicate RNA chains labeled with BODIPY fluorophore.

(B) Relative amounts of monomers and open oligomers of kUrds in the presence of different concentration of Mg²⁺. Relative amounts were calculated from lanes 3 and 7 of Figures S5A, 2B, and 2C.

(C) Relative amounts of monomeric and closed oligomers of kUrds in the presence of different concentration of Mg²⁺. Relative amounts were calculated from lanes 4 and 8 of Figures S5A, 2B, and 2C.

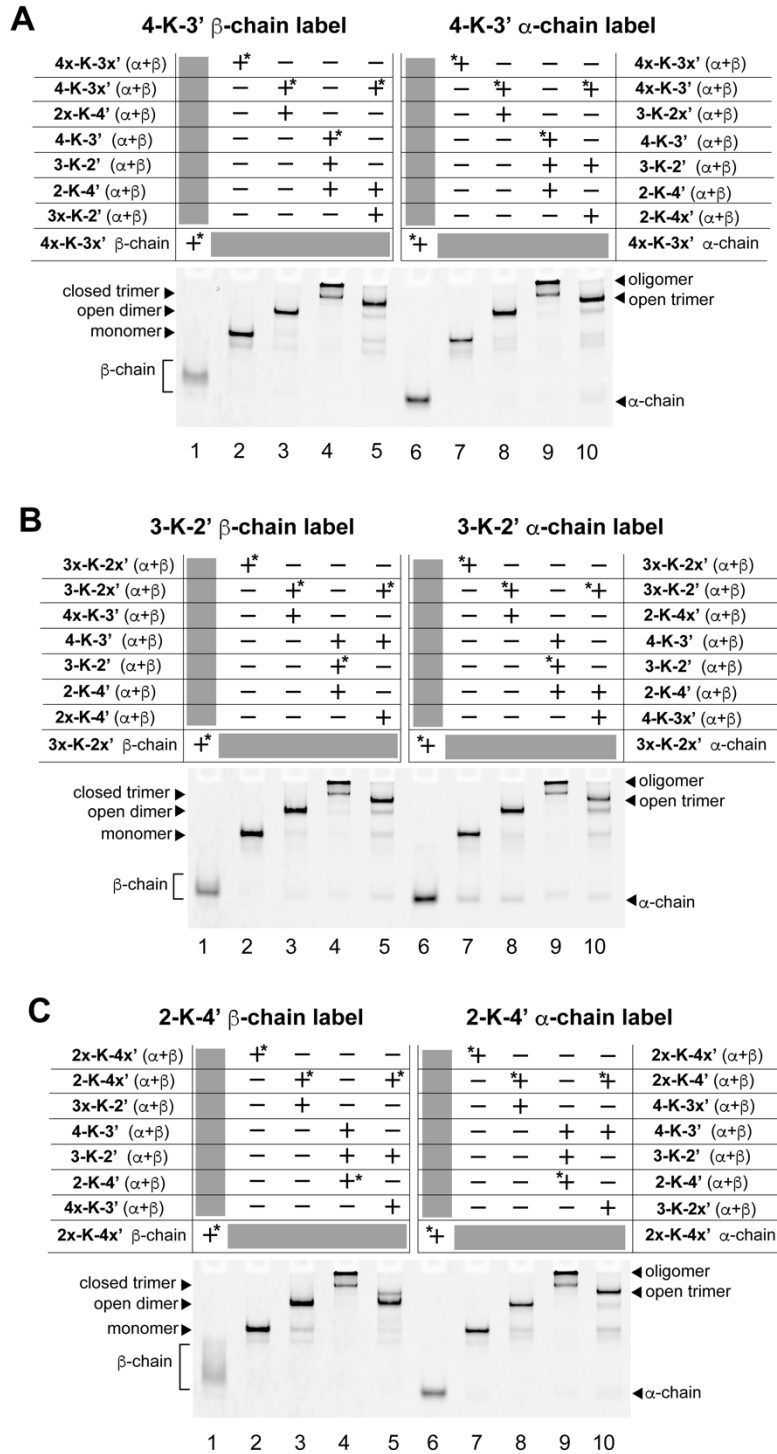


Figure S6

EMSA of triblock kUrd copolymers formed in the presence of 20 mM Mg^{2+} .

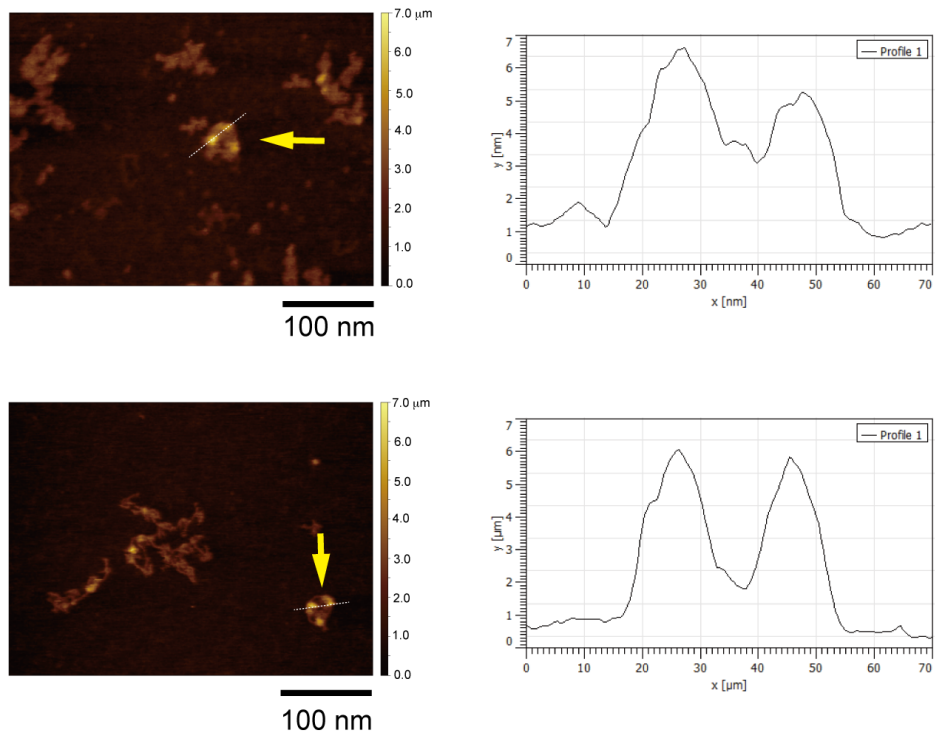
Asterisks indicate RNA chains labeled with BODIPY fluorophore.

(A) EMSA of triblock kUrd copolymers labeled with β -chain (left) or α -chain (right) of 4-K-3'.

(B) EMSA of triblock kUrd copolymers labeled with β -chain (left) or α -chain (right) of 3-K-2'.

(C) EMSA of triblock kUrd copolymers labeled with β -chain (left) or α -chain (right) of 2-K-4'.

A kUrd closed heterotrimer



B kUrd closed heterodimer

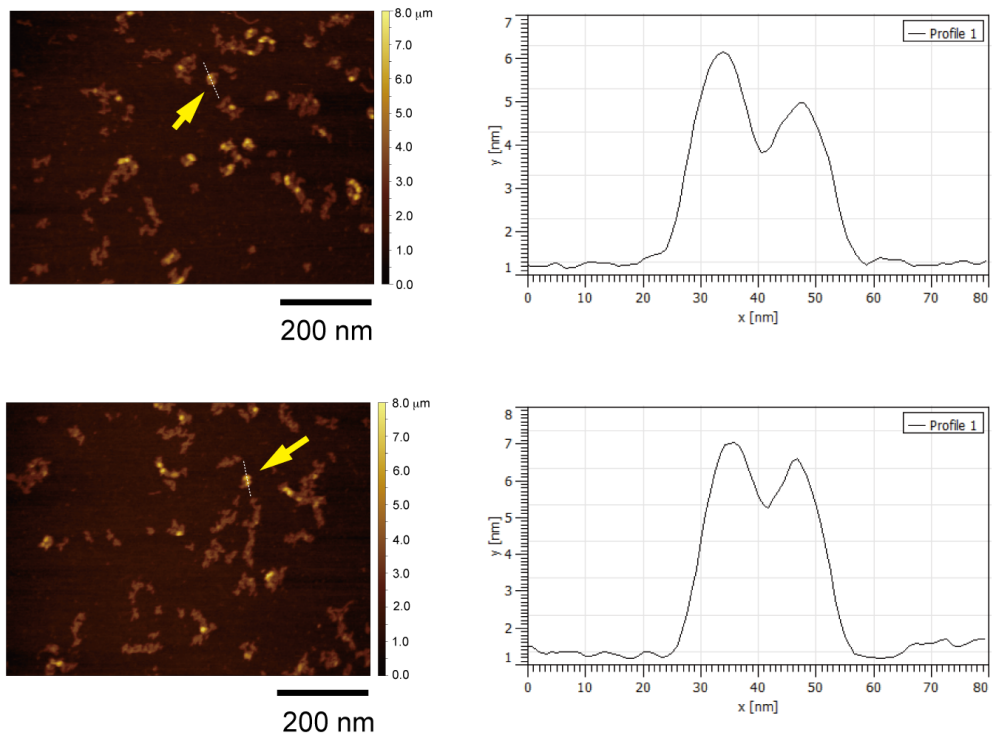


Figure S7

AFM images of the closed heterotrimer and heterodimer and their cross sections.

(A) AFM images of the closed heterotrimer (indicated by an yellow arrow) with 17.5 mM Mg^{2+} and their cross sections.

(B) AFM images of the closed heterodimer (indicated by an yellow arrow) with 17.5 mM Mg^{2+} and their cross sections.

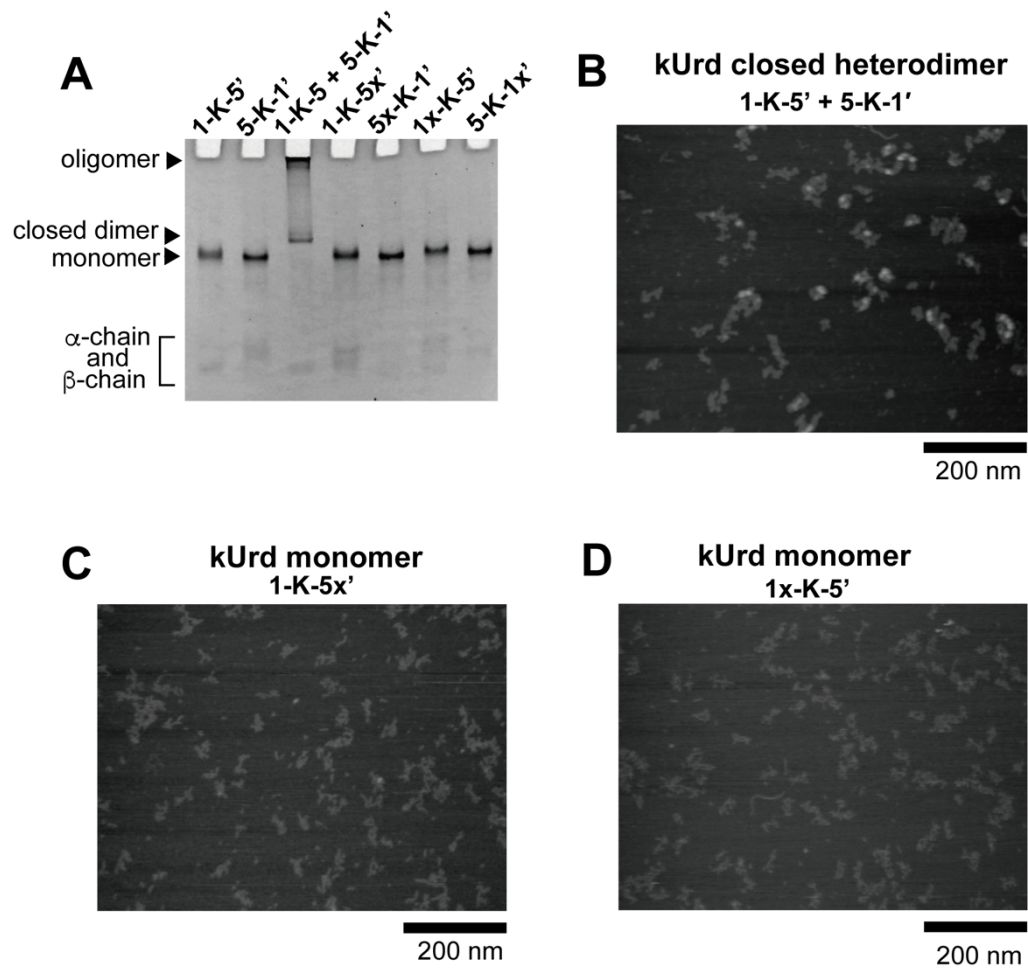


Figure S8

AFM images of the closed heterodimer and its mutant monomers

(A) EMSA of kUrds in the presence of 17.5 mM Mg^{2+} . RNAs were visualized by ethidium bromide staining.

(B) AFM imaging of a mixture of equal amounts of 1-K-5' and 5-K-1' in the presence of 17.5 mM Mg^{2+} .

(C) AFM imaging of 1-K-5x' in the presence of 17.5 mM Mg^{2+} .

(D) AFM imaging of 1x-K-5' in the presence of 17.5 mM Mg^{2+} .

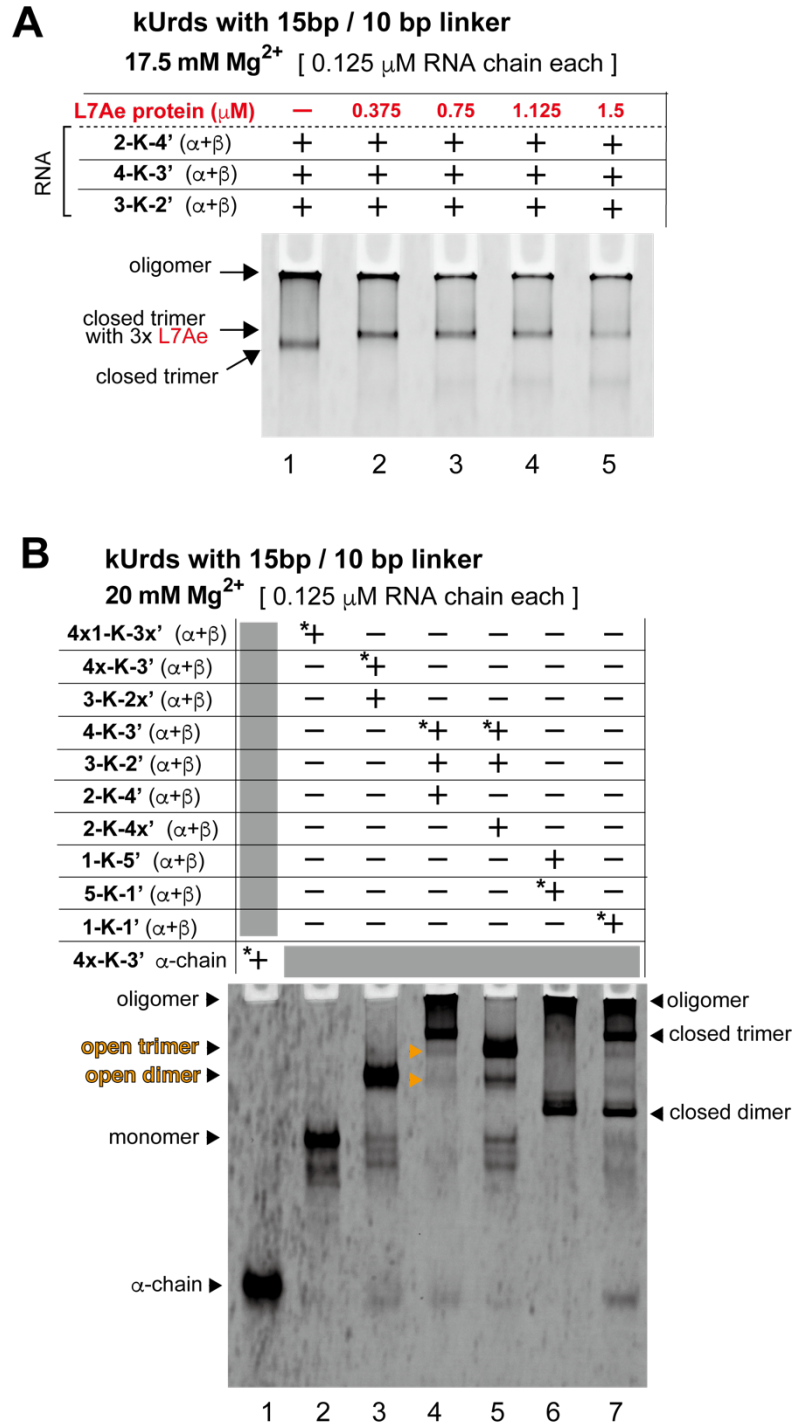


Figure S9

Complex formation of L7Ae proteins with KT-15 kink-turn motifs in a closed kUrd heterotrimer.

(A) EMSA of a closed kUrd heterotrimer in the absence and presence of L7Ae protein.

(B) EMSA of a closed kUrd heterotrimer in the absence and presence of L7Ae protein and L7Ae-EGFP fusion protein. Orange arrows indicate bands corresponding to open forms of kUrd dimer and trimer.

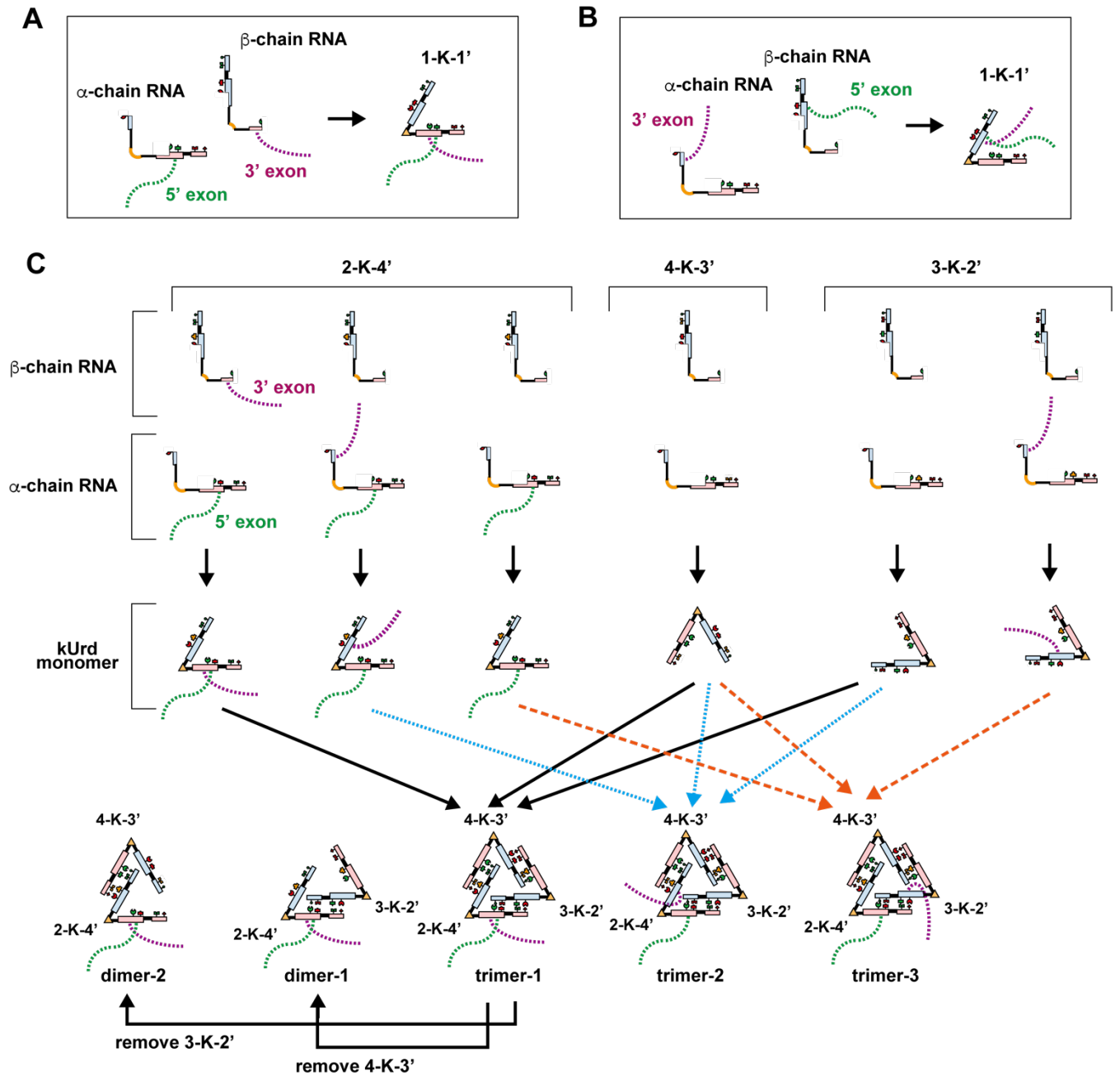


Figure S10

Closed kUrd trimers and their partial dimers used for analysis of the *trans*-splicing reaction.

- (A) Formation of **1-K-1'** possessing the 5' exon and 3' exon in its α -chain and β -chain, respectively, to provide the wild-type Δ P5 ribozyme unit.
- (B) Formation of **1-K-1'** possessing the 5' exon and 3' exon in its β -chain and α -chain, respectively, to provide the M1 type Δ P5 ribozyme unit.
- (C) Formation of kUrd monomers used in analysis of *trans*-splicing reaction promoted by ribozyme unit 2 in triblock kUrd copolymer.

Table S1

Nucleotide sequences of α - and β -chain RNAs employed for EMSA, AFM imaging, and activity assays

NNNNN :	P1 substrate recognition element, type-a: 5'GGAGGG3', type-b: 5'GUGGCU3'
<u>thick underline</u> :	sequence for KT-15 motif
<u>wavy underline</u> :	sequence for the two linker duplexes connecting KT-15 motif and two RzM units
<u>dashed underline</u> :	sequence for M1 ribozyme unit
<i>bold italic</i> :	nucleotides removed in modulation of the length of the two linker duplexes
AGCUAGCU :	nucleotides forming RNA motifs that specify RzM:RzM interfaces

01 α -chain for 1-K-1', 1-K-1x', 1-K-5 and 1-K-5x', which provides type-a P1 to 1

NNNNNAAAAGUUAUCAGGCA**CUCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUG
CAUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGCA**AAGGGUAUGGUAAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAACAG**GAC**
GUAACUG**GCAAUGUGGAACAGAU**CUCAU**ACACAUAUGG**AUGCAGUUCACAG
ACUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAUGUCGGACCUCUC
CUUAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGGAGCCGCUGGGAAACUAAUU
UGUAUGCGAAAGUAUAUUGAUUUAGUUUUGGAGU

01x α -chain of 1x-K-1' and 1x-K-1x', which provides type-a P1 to 1x

GGAGGGAAAAGUUAUCAGCU**UCCG**GUAGCUAGUCUUUAAACCAAUAGAUUGCA
UCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGCA**AAGGGUAUGGUAAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAACAG**GACGU**
AAUCUG**GCAAUGUGGAACAGAU**CUCAU**ACACAUAUGG**AUGCAGUUCACAGACU
AAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAUGUCGGACCUCUCCU
AAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGGAGCCGCUGGGAAACUAAUUUGU
AUGCGAAAGUAUAUUGAUUUAGUUUUGGAGU

02 α -chain of 2-K-4' and 2-K-4x', which provides P1 to 2

NNNNNAAAAGUUAUCAGGCA**UGCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUG
CAUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGAG**AAGGGUAUGGUAAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAACAG**GAC**
GUAACUG**GCAAUGUGGAACAGAU**CUCAU**ACACAUAUGG**AUGCAGUUCACAGAC
ACUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAUGUCGGACCUCUC
CUUAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGGAGCCGCUGGGAAACUAAUU
UGUAUGCGAAAGUAUAUUGAUUUAGUUUUGGAGU

02x α -chain of 2x-K-4' and 2x-K-4x', which provides type-a P1 to 2x

GGAGGGAAAAGUUAUCAGCU**UCCG**GUAGCUAGUCUUUAAACCAAUAGAUUGCA
UCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGAG**AAGGGUAUGGUAAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAACAG**GACGU**
AAUCUG**GCAAUGUGGAACAGAU**CUCAU**ACACAUAUGG**AUGCAGUUCACAGAC
UAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAUGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGGAGCCGCUGGGAAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUUAGUUUUGGAGU

03 α -chain of 3-K-2' and 3-K-2x', which provides P1 to 3

NNNNNAAAAGUUAUCAGGCA**CCCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUG
CAUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG

UCAGUACCAAGUCUCAGGGGAAA CUUUGAGAUGGCCU UGCA AAGGGUAUGGUAUAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU CCUGUGU UCAACAGGAC
GUAUCUGCAAUGUGGAACAGAUUCUGCAUUACUACACA UAUGG AUGCAGUUCACAG
ACUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUC
CUUAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUU
UGUAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

03x α -chain of 3x-K-2' and 3x-K2x', which provides type-a P1 to 3x

GGAGGG AAAAGUUAUCAGCUUCGGCUGGUAGUCUUAACCAAUAGAUUGCA
UCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGGGAAA CUUUGAGAUGGCCU UGCA AAGGGUAUGGUAUAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU CCUGUGU UCAACAGGACGU
AAUCUGCAAUGUGGAACAGAUUCUGCAUUACUACACA UAUGG AUGCAGUUCACAGAC
UAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

04 α -chain of 4-K-3' and 4-K3x', which provides P1 to 4

NNNNNN AAAAGUUAUCAGGCA CUCACUGGUAGCUAGUCUUAACCAAUAGAUUG
CAUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGGGAAA CUUUGAGAUGGCCU UGGG AAGGGUAUGGUAUAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU CCUGUGU UCAACAGGAC
GUAUCUGCAAUGUGGAACAGAUUCUGCAUUACUACACA UAUGG AUGCAGUUCACAGAC
ACUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUC
CUUAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUUG
UGUAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

04x α -chain of 4x-K-3' and 4x-K-3x', which provides type-a P1 to 4x

GGAGGG AAAAGUUAUCAGCUUCGGCUGGUAGCUAGUCUUAACCAAUAGAUUGCA
UCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGGGAAA CUUUGAGAUGGCCU UGGG AAGGGUAUGGUAUAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU CCUGUGU UCAACAGGACGU
AAUCUGCAAUGUGGAACAGAUUCUGCAUUACUACACA UAUGG AUGCAGUUCACAGAC
UAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

05 α -chain of 5-K-1', which provides type-a P1 to 5

GGAGGG AAAAGUUAUCAGGCA UGCA CCUGGUAGCUAGUCUUAACCAAUAGAUUG
CAUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGGGAAA CUUUGAGAUGGCCU UGGG AAGGGUAUGGUAUAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU CCUGUGU UCAACAGGAC
GUAUCUGCAAUGUGGAACAGAUUCUGCAUUACUACACA UAUGG AUGCAGUUCACAGAC
ACUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUC
CUUAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUUG
UGUAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

05x α -chain of 5x-K-1', which provides type-a P1 to 5

GGAGGG AAAAGUUAUCAGCUUCGGCUGGUAGCUAGUCUUAACCAAUAGAUUGCA
UCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGGGAAA CUUUGAGAUGGCCU UGGG AAGGGUAUGGUAUAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU CCUGUGU UCAACAGGACGU
AAUCUGCAAUGUGGAACAGAUUCUGCAUUACUACACA UAUGG AUGCAGUUCACAGAC
UAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUUG

UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

06 β -chain of 1-K-1', 1x-K-1', 5-K-1' and 5x-K-1', which provides type-a P1 to 1'

GGAGGGAAAAGUUAUCAGGCAUGCAACUGGUAGCUAGUCUUUAAACCAAUAGAUUC
GAUCGGUUUAAAAGGCAACGUUCUCAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGGGGAAACUUUGAGAUGGCCUUGAGAAGGGUAUGGUAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGUCCUAAGUGUGUAGUAAU
GCAGAUUCUGUUCGGAAGCAGAUUACGUCCUGUUGAUAUCUGGAUGCAGUUCACAGAC
UAAAUGUCGGUCCGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

06x β -chain of 1-K-1x' and 1x-K-1x', which provides type-a P1 to 1x'

GGAGGGAAAAGUUAUCAGCUUCGGCUGGUAGCUAGUCUUUAAACCAAUAGAUUCGA
UCGGUUUAAAAGGCAACGUUCUCAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGGGGAAACUUUGAGAUGGCCUUGAGAAGGGUAUGGUAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGUCCUAAGUGUGUAGUAAUGC
AGAUCUGUUCGGAAGCAGAUUACGUCCUGUUGAUAUCUGGAUGCAGUUCACAGACU
AAAUGUCGGUCCGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
AAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUGU
AUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

07 β -chain of 3-K-2' and 3x-K-2' which provides P1 to 2'

NNNNNNAAAAGUUAUCAGGCAUCACACUGGUAGCUAGUCUUUAAACCAAUAGAUUC
GAUCGGUUUAAAAGGCAACGUUCUCAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGGGGAAACUUUGAGAUGGCCUUGCAAGGGUAUGGUAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGUCCUAAGUGUGUAGUAAU
GCAGAUUCUGUUCGGAAGCAGAUUACGUCCUGUUGAUAUCUGGAUGCAGUUCACAGAC
UAAAUGUCGGUCCGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

07x β -chain of 3-K-2'x and 3x-K-2x' which provides type-a P1 to 2x'

GGAGGGAAAAGUUAUCAGCUUCGGCUGGUAGCUAGUCUUUAAACCAAUAGAUUCGA
UCGGUUUAAAAGGCAACGUUCUCAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
AGUACCAAGUCUCAGGGGGAAACUUUGAGAUGGCCUUGCAAGGGUAUGGUAUAAG
CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGUCCUAAGUGUGUAGUAAUGC
AGAUCUGUUCGGAAGCAGAUUACGUCCUGUUGAUAUCUGGAUGCAGUUCACAGACU
AAAUGUCGGUCCGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
AAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUGU
AUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

08 β -chain of 4-K-3' and 4x-K-3', which provides P1 to 3'

NNNNNNAAAAGUUAUCAGGCAUGCAACUGGUAGCUAGUCUUUAAACCAAUAGAUUC
GAUCGGUUUAAAAGGCAACGUUCUCAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGGGGAAACUUUGAGAUGGCCUUGGGAAGGGUAUGGUAUA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGUCCUAAGUGUGUAGUAAU
GCAGAUUCUGUUCGGAAGCAGAUUACGUCCUGUUGAUAUCUGGAUGCAGUUCACAGAC
UAAAUGUCGGUCCGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

08x β -chain of 4-K-3x' and 4x-K-3x', which provides type-a P1 to 3x'

GGAGGGAAAAGUUAUCAGCUUCGGCUGGUAGCUAGUCUUUAAACCAAUAGAUUCGA

UCGGUUUAAAAGGCAACGUUCUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
 AGUACCAAGUCUCAGGG **GGAA** CUUUGAGAUGGCCU **UGGG** AAGGGUAUGGUAUAAG
 CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU **CCUAAG** UGUGUAGUAAUGC
 AGAUCUGU **UCCGAAGCAGAUUACGUCCUGUUGA** **AAUCUGG** AUGCAGUUCACAGACU
 AAAUGUCGGUCGGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
 AAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUGU
 AUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

09 β-chain for 2-K-4' and 2x-K-4', which provides P1 to 4'

NNNNNN AAAAGUUAUCAGGCA **CCCA** CCUGGUAGCUAGUCUUUAAACCAAUAGAUUC
 GAUCGGUUUAAAAGGCAACGUUCUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
 UCAGUACCAAGUCUCAGGG **GGAA** CUUUGAGAUGGCCU **UGAG** AAGGGUAUGGUAUA
 AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU **CCUAAGU** UGUGUAGUAAU
 GCAGAUUCUGU **UCCGAAGCAGAUUACGUCCUGUUGA** **AAUCUGG** AUGCAGUUCACAGAC
 UAAUUGUCGGUCGGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
 UAAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUG
 UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

09x β-chain for 2-K-4x' and 2x-K-4x', which provides type-a P1 to 4x'

GGAGGG AAAAGUUAUCAGCU **UCCG** CUGGUAGCUAGUCUUUAAACCAAUAGAUUCGA
 UCGGUUUAAAAGGCAACGUUCUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
 AGUACCAAGUCUCAGGG **GGAA** CUUUGAGAUGGCCU **UGAG** AAGGGUAUGGUAUAAG
 CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU **CCUAAGU** UGUGUAGUAAUGC
 AGAUCUGU **UCCGAAGCAGAUUACGUCCUGUUGA** **AAUCUGG** AUGCAGUUCACAGACU
 AAAUGUCGGUCGGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
 AAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUGU
 AUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

10 β-chain of 1-K-5', which provides type-a P1 to 5'

GGAGGG AAAAGUUAUCAGGCA **CCCA** CCUGGUAGCUAGUCUUUAAACCAAUAGAUUC
 GAUCGGUUUAAAAGGCAACGUUCUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
 UCAGUACCAAGUCUCAGGG **GGAA** CUUUGAGAUGGCCU **UGCA** AAGGGUAUGGUAUA
 AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU **CCUAAGU** UGUGUAGUAAU
 GCAGAUUCUGU **UCCGAAGCAGAUUACGUCCUGUUGA** **AAUCUGG** AUGCAGUUCACAGAC
 UAAUUGUCGGUCGGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
 UAAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUG
 UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

10x β-chain of 1-K-5x', which provides type-a P1 to 5'

GGAGGG AAAAGUUAUCAGCU **UCCG** CUGGUAGCUAGUCUUUAAACCAAUAGAUUCGA
 UCGGUUUAAAAGGCAACGUUCUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGUC
 AGUACCAAGUCUCAGGG **GGAA** CUUUGAGAUGGCCU **UGCA** AAGGGUAUGGUAUAAG
 CUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU **CCUAAGU** UGUGUAGUAAUGC
 AGAUCUGU **UCCGAAGCAGAUUACGUCCUGUUGA** **AAUCUGG** AUGCAGUUCACAGACU
 AAAUGUCGGUCGGGGAAGAUGUAUUCUUCUCAUAAGAUAUAGUCGGACCUCUCCU
 AAUGGGAGCUAGCGGAUGAAGUGAUGCAACACUGGAGCCGCUGGGAACUAAUUUGU
 AUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

Table S2

Nucleotide sequences of α - and β -chain RNAs employed for *trans*-splicing reaction to yield Spinach aptamer

black letters:	nucleotides belonging to kUrd
red letters:	nucleotides belonging to Spinach aptamer
GAGUGU GCACUC :	5' splice-site recognition elements forming P1 duplex
<u>thick underline</u> :	sequence for KT-15 motif

α -chain of 2-K-4' to provide 5'-exon to 2

GGGACGCGACUGAAUGAAAUGGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAG
UA**GAGUGU**AAAUAGCAUAUUGGAGUUA
GCACUCAAAAGUUAUCAGGCA**UGCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUGC
AUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGU
CAGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGAG**AAGGGUAUGGUAAUAA
GCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAAACAGGACG
UAAUCUG**GCAAUGUGGA**ACAGAUUCUGCAUUACUACACA**UAUGG**AUGCAGUUCACAGA
CUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUCC
UUAUUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUU
GUAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU

α -chain of 2-K-4' to provide 5'-exon to 2 and 3'-exon to 4'

GGGACGCGACUGAAUGAAAUGGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAG
UA**GAGUGU**AAAUAGCAUAUUGGAGUUA
GCACUCAAAAGUUAUCAGGCA**UGCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUGC
AUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCGU
CAGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGAG**AAGGGUAUGGUAAUAA
GCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAAACAGGACG
UAAUCUG**GCAAUGUGGA**ACAGAUUCUGCAUUACUACACA**UAUGG**AUGCAGUUCACAGA
CUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUCC
UUAUUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUU
GUAUGCGAAAGUAUAUUGAUUAGUUUUGGAGUACUCG**GAGCUCCGUAACUAGUCG**
GUC

α -chain of 3-K-2' to provide 3'-exon to 2'

GGAGGGAAAAGUUAUCAGGCA**CCCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUG
CAUCGGUUUAAAAGGCAAGACCGUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGG**GAAA**CUUUGAGAUGGCCU**UGCA**AAGGGUAUGGUAAUAA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUGUGU**UCAAACAGGAC
GUAUUCUG**GCAAUGUGGA**ACAGAUUCUGCAUUACUACACA**UAUGG**AUGCAGUUCACAG
ACUAAAUGUGAACGGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUC
CUAAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUU
UGUAUGCGAAAGUAUAUUGAUUAGUUUUGGAGU
ACUCG**GAGCUCCGUAACUAGUCGCGUC**

β -chain of 2-K-4' to provide 3'-exon to 2

GGAGGGAAAAGUUAUCAGGCA**CCCA**CCUGGUAGCUAGUCUUUAAACCAAUAGAUUC
GAUCGGUUUAAAAGGCAACGUUCUCAAAUUGCGGGAAAGGGGUCAUGCUACGUCCG
UCAGUACCAAGUCUCAGGG**GGAA**CUUUGAGAUGGCCU**UGAG**AAGGGUAUGGUAAUAA
AGCUGACGGACGUAGCAUGGUCCUAACCACGCAGCCAAGU**CCUAAGU**GUGUAGUAAU
GCAGAUUCUGU**UCCGAAGC**AGAUUACGUCCUGUUGA**AAUCUGG**AUGCAGUUCACAGAC
UAAAUGUCGGUCCGGGGAAGAUGUAUUCUUCUCAUAAGAUUAAGUCGGACCUCUCCU
UAAUGGGAGCUAGCGGAUGAAGUGAUCGAACACUGGAGCCGCUGGGAACUAAUUUG
UAUGCGAAAGUAUAUUGAUUAGUUUUGGAGUACUCG**GAGCUCCGUAACUAGUCGCG**
UC