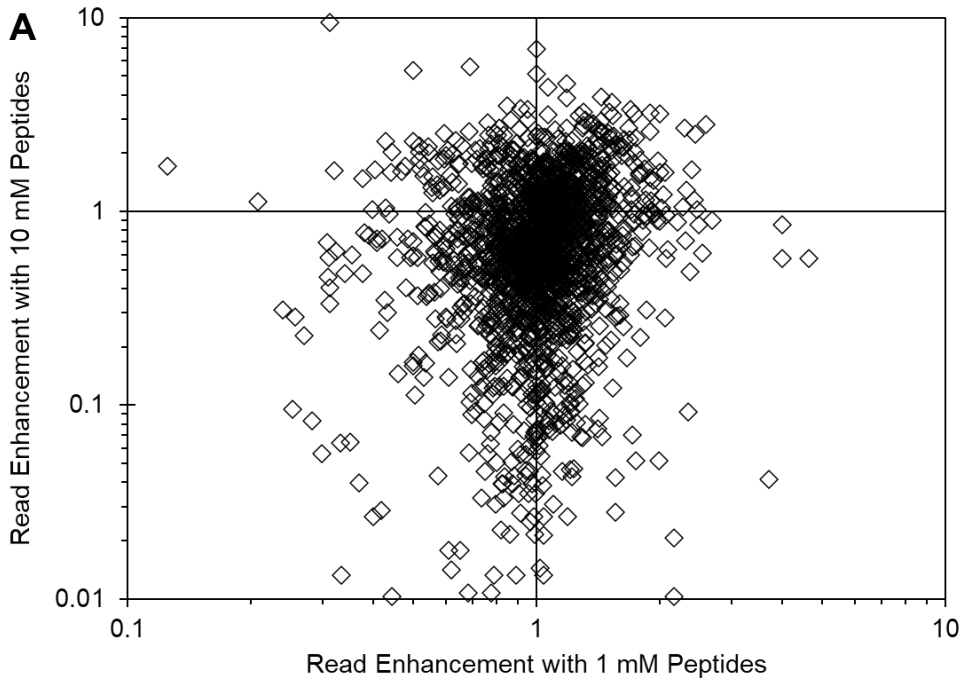


Figure S2



B

1 mM Peptide	10 mM Peptide	Number	Percentage (%)
>1	>1	358	18.9
<1	>1	193	10.2
>1	<1	639	33.8
<1	<1	648	34.3
=1	>1	14	0.7
=1	<1	28	1.5
>1	=1	7	0.4
<1	=1	3	0.2

Figure S2 Effect of peptides on RNA sequence enrichment. (A) Correlation plot between two different peptide concentrations (1 mM and 10 mM) for the enrichment of RNA sequences in the presence of individual peptides. Each diamond represents a single RNA sequence (the peak sequence of a cluster) and its enrichment with one peptide at two different peptide concentrations. The majority of symbols is near the graph's origin, suggesting no strong influence of peptides or their concentration on RNA enrichment under selection conditions. **(B)** The table below the graph displays the number and percentage of sequence / peptide pairs in each quadrant of the plot in (A). Pairs that lie exactly on one of the axes (i.e. no peptide effect) are listed separately in the table. Most sequences showed lower enrichment at 10 mM peptide compared to 1 mM peptide. Since the enrichment of sequences is relative between sequences, it is not possible to state whether the absolute activity of RNAs would decrease at higher peptide concentration. However, the data suggest that on average, 10 mM peptide concentrations cause a larger difference in activity between RNA sequences than 1 mM peptide concentrations.

Figure S3

C0 **GAGACCGCAAGAGAC**GAAAAAAGCACCAGCATTTGGTTGCCATATACTATATCCACATGATATATGTGGTGTGTAGGGAAGACTCGTGTAGGCCG
AAAAGTATCAATATCGCTCCAATGTTTTCCGTAAGGAAAACCTCGGAGCTGACTACTCCTTCTAGCCGAC **CATGGTTCAGACTACAAC**

C1 **GAGACCGCAAGAGAC**GAGATGAACATATAAACGTTATAGATAGAGCCTTATCCGATATATGAGGATTTGTGAGTGATGCCGTTTCGAGCACAATTT
TTGTATATATATACACGACAAGATGCACGATAATTGTGGAGATGCGTCACCCTGTAATACAGGAACATAT **CATGGTTCAGACTACAAC**

C2 **GAGACCGCAAGAGAC**TCTGCCGCTGTCTGTGGCGAGTGGATTAGGGAAGCCAAAACCCACTTGAGTTTCGTATGAATTATACGGGACCAAATA
AAATATCAGGAGTATTGGAAATGTCAACATCGACAAAGACTGCTTTTATGAGAGGCACCTTCAATTGCT **CATGGTTCAGACTACAAC**

C5 **GAGACCGCAAGAGAC**GGAAGTCAACTGATGGGGGATGGGCTGAACGACTTGAATGCCCTGTTCATCTTGGAGCCTTGAAAACTGATAAGC
CCGTGTAACATCTAAAGTAGACAAGGCTTAGTATTGGAAGGGTTCCTTATGGTGCACCTAAATGTTGGTTGA **CATGGTTCAGACTACAAC**

C8 **GAGACCGCAAGAGAC**ACTAGATGGAAACACACACCAAAGAAGGGTCTTCAGTGAACACTACCGAGCCTCCAACCGGACGCTGACGTATACGTT
CGTGGAGAGTGGATTCCATCTGGAAACCTAGTCGGTACCACAATAACTGCATAACGTAAGGAATAAGTTTA **CATGGTTCAGACTACAAC**

C9 **GAGACCGCAAGAGAC**CAAGGACACATGACAAGGATTTTCGTTAAGTGAACCGTTCGAAATGATAATTGCGGAGATGTTGTTTCACGATTTTCTG
CGAAGTGGGAAGGGCTCTCTACGCAAGAGTCGATACTCTTAAAGAGGGCCAAATTGATTGTAGGAGAACGGT **CATGGTTCAGACTACAAC**

C10 **GAGACCGCAAGAGAC**TAAGGCTTTGGTGTGGCGGTATACTAACCTACGTTCCCTTACCAATATCTTGTCTGTAGTTATTGCCCAAACCTAAT
AGAAAATTAAGGACAGATGCAACCACCTAATGTTAGGAGGTCATCGGAAAAGCTAAGTGTGTCTGACTTTG **CATGGTTCAGACTACAAC**

C11 **GAGACCGCAAGAGAC**AAAGGGGATTTGCCGTTCCCGAATTGTCAACCAACGATTTGCGTAAACCGGAAAAGAATAGCCGAAATATTGA
CGAATCTGTCGCGACAACCTGCAAAATCCCGCTTTCAGTATATGAATCGGTGTGTTGAAATAG **CATGGTTCAGACTACAAC**

C12 **GAGACCGCAAGAGAC**GTGAATGAGATTTCTTGGGGCAAACCCACCACACTGGTGAAGAGGAGGTGCATTTGTTCCCAACGATGCTCAAAAT
TATTTTTGGAAAGAATAGCGATCGATTCTGTGGCCTGATTTTGAACATAAAGTTTGCCTTGGTATAGATC **CATGGTTCAGACTACAAC**

C36 **GAGACCGCAAGAGAC**GTATGAACPTTGGATCTACGTAAAGTCAATAGGCACGATAAAAACGTTGCGGAGAAGCGGTGTGCCCTTCTTACCTTTG
TTTTTTGGATTAGGAAAATACACAATTAAGAAAATGAGAGTGAAGTACGCATCTAGATGATGTAGAATAATC **CATGGTTCAGACTACAAC**

C37 **GAGACCGCAAGAGAC**TCTCAGAAAGGGGATCATGTTCCCGATGTTGAATCCATCAACTGATAAGCTGTACCGGAAAAGGAAGACTCCGGTT
GAAACGAAGATCGGAATTAGCATTTTCAGTAGATACCTGCAATGAAAGGTTTTGGTCTATAAAGTGAAGGAG **CATGGTTCAGACTACAAC**

C48 **GAGACCGCAAGAGAC**CAGTAATACATACATGGATCGCAGGGAATTTTACCTGATAGGTTTCAGTAAAAGCGCTTGGATAGGTTTTCTGATTAG
AACAAATCAATCGTCCGTCCTAAACGAAAACCCGAGTGGGAGAATCCACCCGAGTAGATAAAATAAGTAGTA **CATGGTTCAGACTACAAC**

C54 **GAGACCGCAAGAGAC**GAAGAGTTAAGTTGAATTGCTTAAACCTGAGCGACACAGGCACTGAAAACCTTCGATCAAGAGATTTCTGAATCTAACC
CATTCCGAGATCCATAGGACTCAGTTTCTAGATAGTGAACGTTGATTCCTAGGCGAAGTACCAGACTAACT **CATGGTTCAGACTACAAC**

C64 **GAGACCGCAAGAGAC**TATGTAGCCGTTTAGGTGAAGAACTGCCAACCATACCGGTCACAATTCACATTTGCAAACCGGCTAAGGTAATAGGT
AAGAAATGCGCGGAGAACTAATACTCACTAAGGGAGTGTGGTCTTGAACACACGAAACCACAGCTA **CATGGTTCAGACTACAAC**

C67 **GAGACCGCAAGAGAC**TAAAGTATAACAAACATCGCGAGTGTATTTGCTCGTGTGTTTTAAGAGGGTTAAATGACGAGTCAAGGACGCCAGAA
TCAATTCGAAAAGAAGGTAATTACAAATTTATGTTATTTTTATTCGTGATATCATGGAATGATCGGTTTGATT **CATGGTTCAGACTACAAC**

C74 **GAGACCGCAAGAGAC**ACAGATGGGACTAGAACAAAGGCTGACGACGTTCTCGGACAGAGGCAAGTGCAAAATCCGTGGGCATGCAATAACT
ATCATAACAAAGGAACGAGTTGGCCATAGCTACCGACAATTAATGGCTGCAACGTTGCAACCCCATG **CATGGTTCAGACTACAAC**

C78 **GAGACCGCAAGAGAC**AAATTAATTTCTCTTAAACACGTTTGGAGAGTGTCAACAATAATGGATGTAGTCTGGATGTGCTAGAGGAACA
CCGCGGCGCATCCCAAACGAGCTGGAATTTGAGAGAAAAGTACGGTGTGTTGGGCTCGGTCGTCTACA **CATGGTTCAGACTACAAC**

C80 **GAGACCGCAAGAGAC**GAAATAACTTTATTAACCGATTTTAGGATGATCTGTTGTGTAGGGAAGACTGTGTTGACTCGAGTTGTGATTTCT
GCTGTGCTGTCCCTAGCTAATGCTATAAGGCAAAACAGGCTGCAAGCTTAACACTATATCGATCGAGACAA **CATGGTTCAGACTACAAC**

C84 **GAGACCGCAAGAGAC**GTAGTGAACCGTGGGTTATGATGGAATAAGAGCCATCTTCTGACTAGTTTCGACCCCTTATTCCGAAATGGGTCAA
TAGGGGCGCAATGCTTCCGGGACATGGATGAAGCGAACGTTGCTTCATGCAACCCCTGCTGAATGTTCCGTAG **CATGGTTCAGACTACAAC**

C147 **GAGACCGCAAGAGAC**CCGAGAAGAAGTTTGAATGCTTCCTTGTGTACATGGTGACGAGCGAGAATTTGACGACGCATTATACATGGAAGGG
AGTACACGGATCTACAGTTAAAAGTGTCTGTGGAACACGATTTGACGGAAAAAGGTGAAGGACTCTAACCGT **CATGGTTCAGACTACAAC**

C175 **GAGACCGCAAGAGAC**TGGAAGTTGTGGTGGCAATCCGAAAACATAATGGATCGAGTCACTGCCCTATTATAAGCAACCGGCGAGGGGAAA
TAGTAGTACTGAAGGAGCGAATGAGAAGAATCAGGGTAGTAAAGGTAAGATTCACACTGCCCTGAAACTCGT **CATGGTTCAGACTACAAC**

C181 **GAGACCGCAAGAGAC**GGAACCTGATGGGCTTTGCAAGAGCTTCGTTTATGCGTAGCACAATATGACAATTGAAGCATATAGTGTGGGCGCA
ACTAAATGGGCTTGTGCCGTATGACAGTAAAGGACGCGATCCGTTGAAACTACGTACACTGTGTACGAAGGCC **CATGGTTCAGACTACAAC**

C204 **GAGACCGCAAGAGAC**AATACAATAAAGAAAGGAGCGTAGTCTGTGGCAAAACAGCTGTGAATGAAGAGCAGTGCAGAACAGAGATATGTG
CTAGTTGCTCCTATTGGACCAATCTTGGGCTGTGCAGAGACATAGTCGACATATGGAACATTTCCATAA **CATGGTTCAGACTACAAC**

C221 **GAGACCGCAAGAGAC**AACGTAACCGTGCATTTGCGAGTGGCAATGTGTTTGTAGTAAAAATGTTCCAACATAAGATATCCGAGGAATTAATG
GCACTCTGAAAATTTAGATGCCAGGCGAGCGGAAGTTGATGAAGTCAACTAATGGTAGAGATAACACTTA **CATGGTTCAGACTACAAC**

C235 **GAGACCGCAAGAGAC**GATGAACCTAAGATGGTGCATTTATTCAGTCAAGTAAAAAAGAGTGCACCTTATGTTGAAAAAAGTGGT
AGGGGTCCCGGACGCTTAATTGCCATCGAAGGCACTACCTGAAAATAAACTGACATAGCCAGAGTGGATAATG **CATGGTTCAGACTACAAC**

C238 **GAGACCGCAAGAGAC**TATGTATTGGGAAGTACGAGTGCAGTGAAGGCTGTGCGGCTCTATAAGCTGGACTACAAAATCTAGTCC
CTTGAACCTGTCTCTTGTCAACAAGGATTTGACAGGAGATGAAGAGCGGAGAAACCAAGACTGACCTA **CATGGTTCAGACTACAAC**

C308 **GAGACCGCAAGAGAC**TGGAAGTTAGCGGTGGTACTTTATAAGGGGATGGTTAACTAGCATAAACTGTAGAGTGAATTTCTTAAACAGGTGCG
CTACTCTTTCTCACTTATAAAGCTAGACTCGAAGACGGGCGAGTTGAACCAATGTTACCTCAAAATGGAGTT **CATGGTTCAGACTACAAC**

C337 **GAGACCGCAAGAGAC**ACTGGATTCGTGTTAAGCTAAAACAGACATCTGCCCTGGCTGGGACTGGGAAGTTGTCCCAAAGACTATTTTTTG
GGTCTTCCGGGATCAGGCTTAAACCTTTAGATATTAGTAAATGCACCAAACCGGCACTTACTAAAA **CATGGTTCAGACTACAAC**

C368 **GAGACCGCAAGAGAC**GAAGGAGTGTACACGTGGGAGAACTTTCGGGTGTTTCGACTGGAACGTTTGTATTGTAAGACTGCCGATCAATTTT
TATAAGAGCGCTAGTTCCGATACTCGAATCGTCTCCCTTTGTGAAAGCTGGTATGAACGACGCTAGGCACCTC **CATGGTTCAGACTACAAC**

C408 **GAGACCGCAAGAGAC**AAAATTAATTTTCAAGACCATGCAGTTAACATACAAGATATAAATTAGCTAAAATAACTCTTGATATACCTCGACG
CTAGCACCCGAGAACAAGTAGGAGTGTGGCCTCGAAGTAGCGGAAGAGCTAACAAAGTATTGCAAACGTAATA **CATGGTTCAGACTACAAC**

Figure S3. Ribozyme sequences that were tested biochemically. Each sequence is the peak of a sequence cluster that was identified, and found promising for peptide benefit by HTS analysis. For each sequence, the 5'-constant regions (blue) and 3'-constant regions (red) are indicated, with the black sequence stemming from the N150 randomized region.

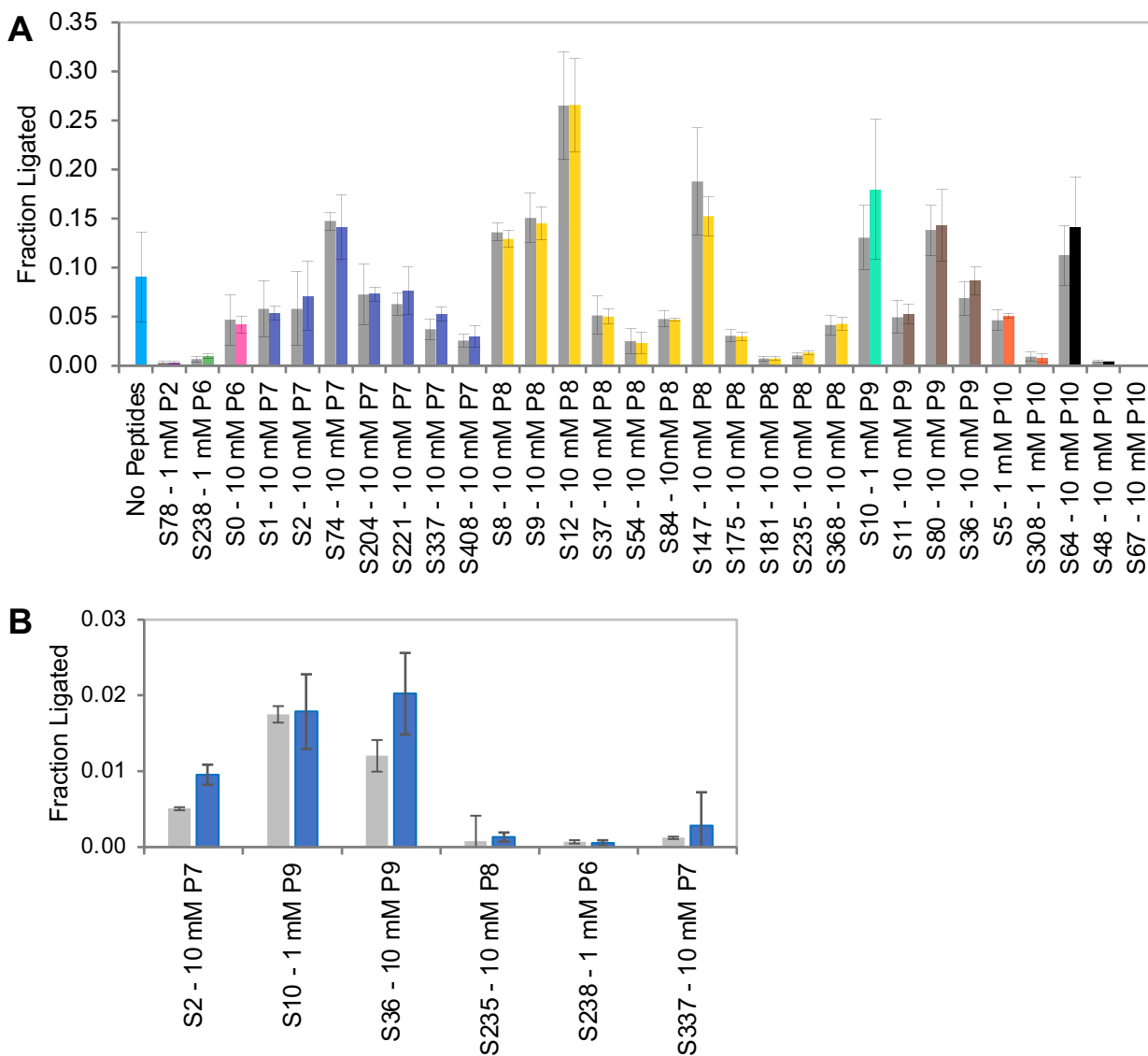
Figure S4

Figure S4. Biochemical testing of peptide effects in ribozymes based on predictions from HTS analysis. (A) Activity assay of 30 ribozymes in the absence, and presence of the one peptide that was predicted from HTS data (figure 2B) to increase ribozyme activity. The incubation with cTnp was done under selection conditions (50 mM Tris/HCl pH 8.3, 100 mM MgCl₂, 50 mM Na₃cTnp). Grey columns indicate the activity without peptide, colored columns indicate the activity with peptide. The identity of the peptide, and its concentration in the assay are given in the column labels. Error bars are standard deviations from triplicate experiments. (B) Activity assay of six promising ribozymes in the absence, and presence of the same peptide as in (A) but under suboptimal reaction conditions, i.e. at a decreased reaction pH. The incubation with cTnp was done under selection conditions (50 mM HEPES/NaOH pH 7.0, 100 mM MgCl₂, 50 mM Na₃cTnp). Grey columns indicate the activity without peptide, colored columns indicate the activity with peptide. The identity of the peptide, and its concentration in the assay are given in the column labels. Error bars are standard deviations from triplicate experiments.

Figure S5

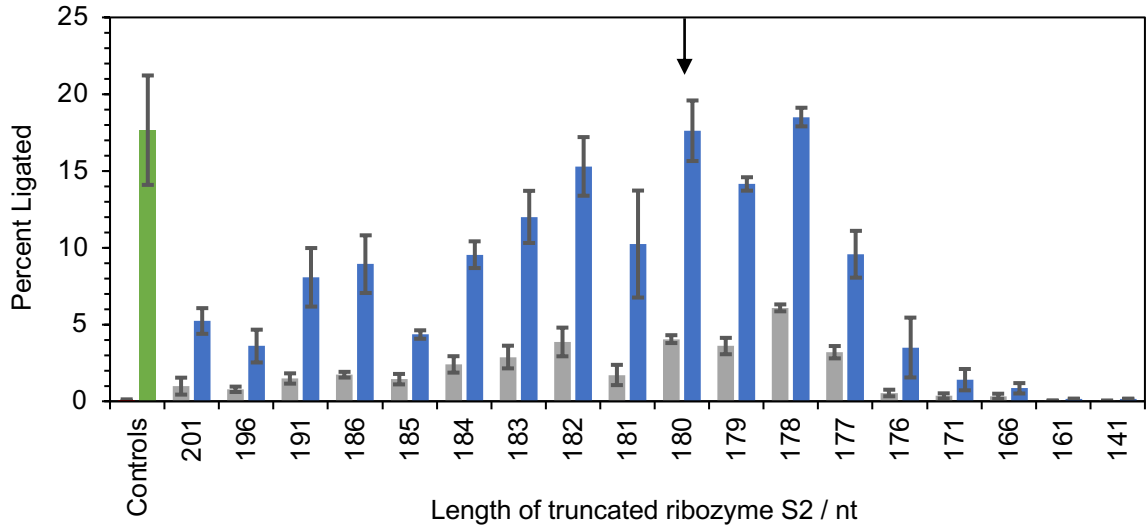


Figure S5: Truncation analysis of ribozyme S2. (A) Ribozyme activity shown as function of its length, using truncations at its 3'-terminus. Blue columns indicate the activity with 10 mM peptide P4; gray columns indicate ribozyme activity without peptide. The chosen ribozyme S2T18 (180 nt length) is indicated by an arrow. Error bars are standard deviations from triplicate experiments.

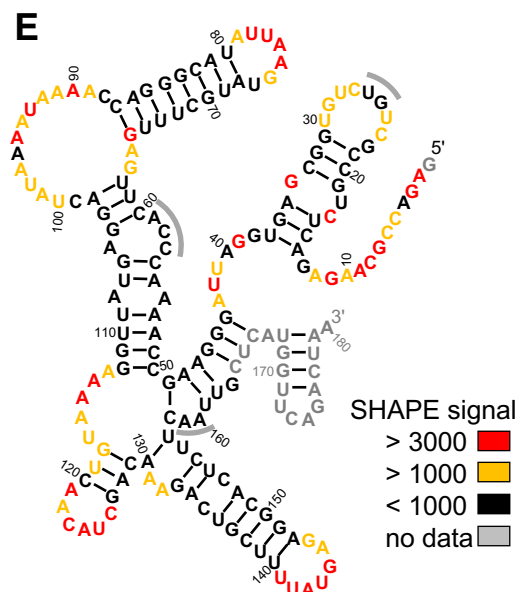
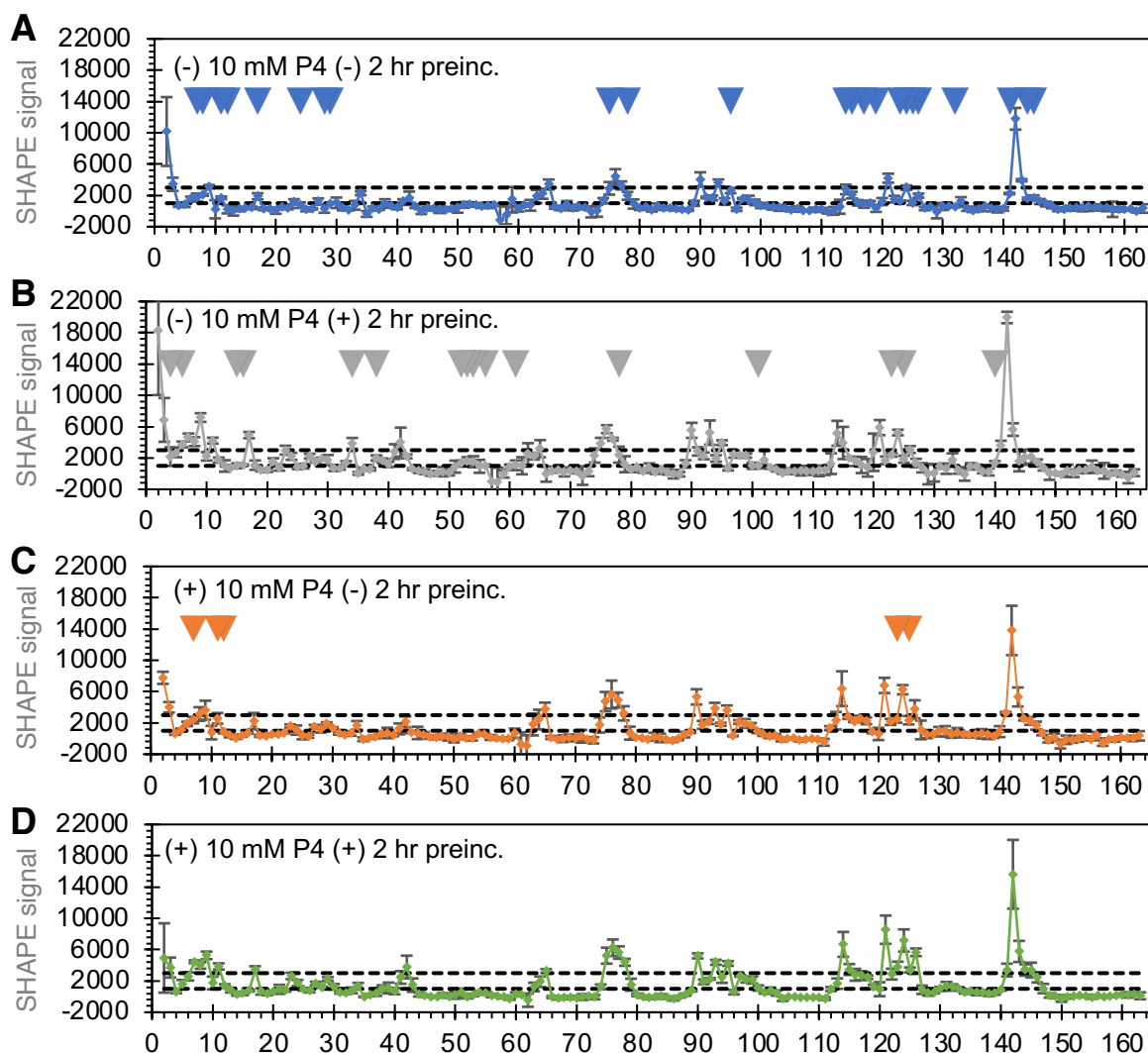
Figure S6

Figure S6. Secondary structure probing of ribozyme S2, using SHAPE. **(A)** SHAPE profile of ribozyme S2 in the absence of peptide, in the presence of 100 mM MgCl₂, 50 mM Na₃Ctmp, 50 mM MES/NaOH pH 6.0, 3.3 mM NaOH, 1% DMSO (to dissolve 1M7, and pre-incubated for 2 minutes at 22°C. **(B)** As in (A) but pre-incubated for 2 hours. **(C)** As in (A) but in the presence of 10 mM peptide P4. Note that this peptide preparation increased the pH by about 1. **(D)** As in (A) but with 10 mM peptide P4, and pre-incubated for 2 hours. Error bars are standard deviations from triplicate experiments. Arrowheads indicate positions where error bars do not overlap with those from the data set (D). **(E)** Secondary structure model based on the constraints of a 2-hour pre-incubation with 10 mM peptide 4 (B) because we speculated that this data set was likely most consistent with the catalytically active structure.