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Supporting Information

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Supporting Information

for

Histone H3 N-Terminal Peptide Directly Binds to Its Own mRNA: A Possible Mode of Feedback Inhibition to Control Translation

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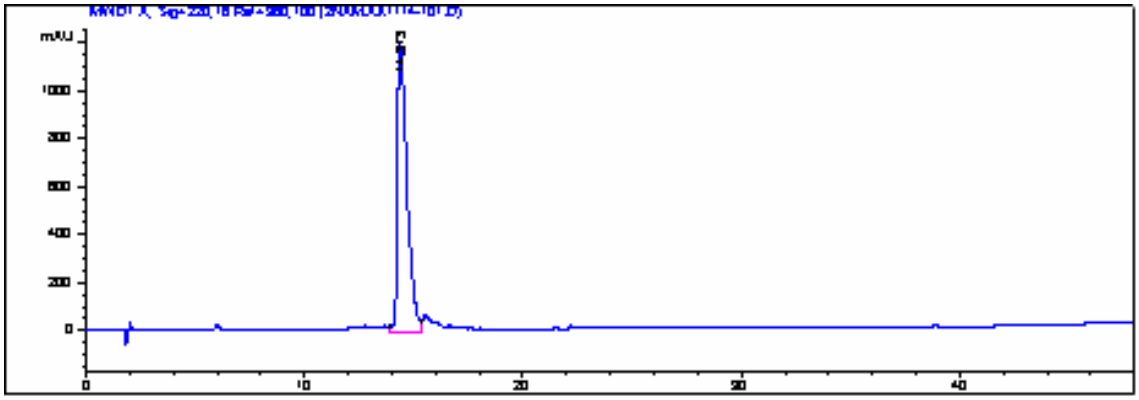
Experimental details of the followings:

I. Preparation of Peptides

Solid phase synthesis. Histone peptides were synthesized using a protocol for the solid-phase synthesis. Biotin was labeled on the C-terminal of the peptide, so that the peptide could be attached to a solid support to facilitate selection and evaluation. Briefly, after 4-Fmoc-hydrazinobenzoyl AM NovaGel^{TM} resins (50 mg, 30 μmol) had been deprotected with 20% piperidine in dimethylformamide (DMF), they were washed with DMF and MC (methylene chloride, 10 x 1 mL). The resulting solution was mixed with a solution of Fmoc-protected first amino acid (6 equiv), from C-terminal, benzotriazole-1-yl-oxy-trispyrridino-phosphonium hexafluoro-phosphate (PyBOP, 6 equiv), and *N,N*-diisopropyl-ethylamine (DIPEA, 12 equiv) in DMF (1 mL). The suspension was stirred at room temperature for 3 h to complete the reaction, which was monitored by 2,4,6-trinitrobenzenesulfonic acid (TNBS) test or Kaiser test. Resins were filtered and washed with DMF (5 x 1 mL) and MC (5 x 1 mL). Coupling reactions were repeated until the last amino acid was added. Resins were then treated with a cleavage cocktail (5 mL, trifluoroacetic acid (TFA)/triisopropylsilane/water = 95:2.5:2.5) for 2 h at room temperature. Resins were then removed by filtration, and washed with TFA (2 x 1 mL). The combined filtrate was concentrated by a stream of nitrogen. To the resulting substance, a 50 mL mixture of diethyl ether and *n*-hexane (50:50 *v/v*) was added to precipitate the peptide. The resulting suspension was centrifuged at 1500 rpm for 10 min at room temperature. After carefully decanting the supernatant, a pellet was washed with a 50 mL mixture of diethyl ether and *n*-hexane (50:50 *v/v*) twice and was dissolved in DMSO (0.2 mL).

Purification of peptides. Histone peptides were purified using HPLC (C18, 5 μm, 4.6 mm x 15 cm for the stationary phase; Buffer A, H_2O containing 0.1% TFA, buffer B, CH_3CN containing 0.1% TFA for the mobile phase). Peptides were eluted at 18~25% of buffer B, and was lyophilized, affording white powder (about 20~30% yield, >95% purity). Mass spectra of peptides were measured using Voyager^{TM} MALDI-TOF mass spectrometer (Applied Biosystems). MS ($M+H^+$) for H3 18-mer peptide: 1941.2 (calcd.), 1941.4 (found). MS ($M+H^+$) for biotinylated H3 18-mer peptide (Figure S1): 2167.2 (calcd.), 2167.2 (found).



II. Procedures for SELEX

A DNA library containing random sequences of 50 nucleotides[1] was synthesized by Integrated DNA Technology (USA). The random region was flanked by defined sequences containing the T7 promoter and restriction enzyme sites for in vitro transcription and cloning [5'-CGGAATTCGTAATACGACTCACTATAGGGACGCGTGGTACC(N50)AAGCTTCCGCGGGGATCCAA-3']. The DNA library was amplified by general PCR condition [95 ℃ 5 min, (95 ℃, 30 s; 5 5 ℃, 30 s; 72 ℃, 30 s) 10 cycles, 72 ℃, 8 min 30 s] using 5'-primer (5'-CGGAATTCGTAA TACGACTCACTATAGGGACGCGTGGTACC-3') and 3'-primer (5'-TTGGATCCCCGCGGAAGCTT-3'). The RNA library was prepared by in vitro transcription using T7 polymerase [40 mM Tris (pH 7.9 at 25 ℃), 6 m M $MgCl_2$, 10 mM NaCl, 2 mM spermidine]. Prepared RNA library containing 1 x 10^{14} different molecules was used for first cycle. In each cycle, in order to remove bead binders, pre-clearing step was carried out. Then, RNA molecules was incubated with 10 μM of biotinylated H3 18-mer peptide in 200 μL of binding buffer [30 mM Tris (pH 7.5 at 25^{o}C), 150 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT] with 40 units of RNase inhibitor for 30 min at room temperature. Streptavidin-agarose (30 μL) was added to the sample and then the sample was subjected to shaking for 30 min at room temperature. Peptide-RNA complexes were pelleted and washed three times with binding buffer (500 μL). And then, RNA was eluted by incubating with 200 μL of 1 M NaCl, 10 mM NaOH for 5 min at room temperature. The resulting mixture was subjected to phenol/chloroform extraction for eluting RNA aptamers from peptides bound to beads. The eluted RNAs were purified by micro-spin G-25 column (Amersham) and ethanol precipitation. It was reverse transcribed by M-MuLV reverse transcriptase (Roche) using 3' primer (500 μM) and each of dNTPs (1 mM) and amplified

by 20 cycles of PCR. The amplified DNA was then transcribed into RNA followed by next selection cycle as described above.

III. Cloning and sequencing of the selected RNA

After 6 cycles of selection, the selected and amplified DNA was inserted into pUC19 vector using EcoRI, BamHI restriction site and T4 DNA ligase. The recombinant DNA was transformed into *E. coli*. Plasmid DNA was prepared from individual clones and sequenced.

IV. Pull-down assay for the selected clones of RNA

Pull-down assay was carried out with same procedure as described above (selection step in SELEX cycle). Briefly, each 100 nM of 5'-^{32}P end labeled[2] C1 and C2 RNA was mixed with in the presence or absence of 10 μM of H3 18-mer peptide in binding buffer for 30 min at room temperature. Streptavidin-agarose was added to each solution and incubated for 30 min at room temperature with gentle shaking. The resulting suspensions were centrifuged and the supernatant containing unbound RNA was discarded. The pelleted beads were washed three times with binding buffer. Bound RNA was eluted with 200 μL of 1 M NaCl, 10 mM NaOH for 5 min at room temperature, and fractionated and analyzed by 6% polyacrylamide/7 M urea gel electrophoresis and autoradiography (Figure S2).

V. Pull-down assay using cellular total RNAs

Preparation of cellular total RNAs from HL60 cells. Cellular total RNAs from HL60 cells were purified by using Trizol reagent (Invitrogen) employing the manufacturer's protocol. About 1 mg of RNA was obtained from more than 5 x 10^7 cells.

***Pull-down assay*.** The pull-down assay was carried out as described above. Each 50 μg of cellular total RNA along with the presence or absence of 10, 100 μM of H3 18-mer peptide was stirred in binding buffer for 30 min at room temperature. Streptavidin-sepharose (50 μL, High performance, GE healthcare) was added to each solution and incubated for 30 min at room temperature with gentle shaking. The resulting suspensions were centrifuged and the supernatant (150 μL) containing un-

bound RNA was saved for control PCR reactions. The pelleted beads were washed five times with binding buffer. Bound RNA was eluted with 0.5 M ammonium acetate, 1 mM EDTA, 0.1% (*w/v*) SDS for 1 h at 37 ℃, and the resulting mixture was subjected to phenol/chloroform extraction. The eluted RNAs were purified by micro spin-column (Nucleogen) and ethanol precipitation. It was reverse transcribed (10% fraction of RNAs was used for reaction of supernatant) by M-MuLV reverse transcriptase (Roche) using 3'-primer [500 μM, 5'-TGGGTGGCTCTGAAAAGAGCCTTT-3' for H3 detection, GAPDH 3'-primer (5'-TCCTTGGAGGCCATGTGGGCCAT-3') for GAPDH detection] and each of dNTPs (1 mM) and then amplified by 30 cycles of PCR using each primer [primers for H3 detection (H3c 213-340 5'-prmer; 5'-CCGTAATACGACTCACTATAGGAGCTGCTGATCCGCAAGCTGCCCTTC-3', H3c 213-340 3'-primer; 5'-CCAGGTAGGCCTCGCTGGCCTCCT-3'), primers for GAPDH detection (GAPDH 5'-prmer; 5'-TGATGACATCAAGAAGGTGGTGAAG-3'), GAPDH 3'-primer]. The PCR for H3c 213-340 sequences and GAPDH amplified 127 b.p. and 240 b.p. DNA products, respectively. PCR products were identified by 2% agarose gel electrophoresis.

VI. Measurement of binding affinity between peptide and RNA using BIAcore and a pull-down assay

Preparation of H3c internal hairpin RNA. H3c hairpin RNA was prepared by in vitro transcription from synthesized double-stranded DNA template containing T7 promoter (sense strand, 5'-CCGTAATACGACTCACTATAGGGCGCAGGACTTTAAGACGGACCTGCGCCC-3'). Mutant H3 hairpin RNAs were prepared by DNA templates containing substituted nucleotides.

Pull-down assay. ^{32}P-end labeled H3 hairpin RNA probe (~100 pmol) was mixed with cold RNAs to be used in the described concentration for further study. For each binding reaction, each solution of biotinylated H3 18-mer peptide and 100 nM of labeled H3 hairpin RNA (pre-annealed) with 1 μM $tRNA^{mix}$ was incubated for 30 min in the indicated concentration. Streptavidin sepharose high performance beads (GE Healthcare) were washed with 0.1% BSA containing binding buffer (30 mM Tris (pH 7.5 at 25^{o}C), 150 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT) once and binding buffer three times beforehand to reduce nonspecific binding to resins. Ten microliters of ~70% sepharose bead slurry were aliquoted to each tube of various concentrations of chemicals. Each reaction mixture was incubated for 30 min at room temperature. Un-

bound RNA was washed away with 500 μL of binding buffer twice. One hundred fifty microlitters of elution buffer (0.5 M ammonium acetate, 1 mM EDTA, 0.1% SDS) were added to each tube and incubated for 1 h at 37 ℃. Eluted RNA was purified by precipitation (ethanol : 3 M NaOAc : 10 mg/mL glycogen = 300 μL : 15 μL : 2 μL). To the resulting precipitates, 10 μL of loading buffer (95% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol FF, 5 mM EDTA and 0.025% SDS at pH 8.0) were added. Each sample (10 μL) was loaded on the 15% polyacrylamide gel with 7 M urea and run at 230 V in 1 x TBE running buffer for 20 min. The gel was exposed to a phosphorimager screen and individual bands were quantified on a FLA-3000 and analyzed with Multi Gauge Ver. 3.0 software (Fuji Photo). The results are shown in Figure S3.

BIAcore assay. Assay was carried out on a BIAcore 3000 instrument at room temperature. Streptavidin solution (5 mg/mL) was diluted to 1/200-1/400 in 10 mM sodium acetate (pH 5.0). The flow rate was adjusted as 5 μL/min. The carboxymethyl dextran matrix of CM5 sensor chip was activated by injection of 35 μL of coupling solution [200 mM *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide, 50 mM *N*-hydroxysuccinimide]. The diluted streptavidin solution was then injected into the activated flow cell. Unreacted NHS esters were deactivated by injection of ethanolamine (35 μL of 1 M at pH 8.5), affording surfaces of flow cell 1 to 4 that gave a final change in response units (RU) of streptavidin was 4000. Flow cell 1 was used as a reference and biotinylated H3 18-mer peptide was injected into the streptavidin coated flow cell 2. A final change in RU of H3 peptide was 600. For the binding assay, flow rate was adjusted as 30 μL/min. H3 hairpin RNA, RRE or pre-miR 24 RNA and H3 hairpin RNA mutants (mt1~6) at various concentrations (250, 500 nM, 1, 2, 4, and 8 μM in the presence of 20 μM of $tRNA^{mix}$) were injected by using the serial automated method which is comprised of sample injection (60 μL), dissociation (240 s), and regeneration (10 μL of 20 mM NaCl, 0.2 mM NaOH). The signals from a reference flow cell (streptavidin coated flow cell 1 surface) were subtracted from raw data (Figure S4). Binding affinity of H3 hairpin RNA to H3 18-mer peptide was calculated by using BIAevaluation software (Table S1, 1:1 Langmuir binding model).

VII. Hydroxyl radical RNA footprinting of truncated H3c mRNA

Preparation of truncated H3c mRNA (sequences 213-340). The DNA template for truncated H3c mRNA preparation was obtained from pCMV-SPORT6-H3c[3] construct by PCR using designed primers [5'-primer (5'-CCGTAATACGACTCACTA-TAGGAGCTGCTGATCCGCAAGCTGCCCTTC-3'), 3'-primer (5'-CCAGGTAGGCCT-CGCTGGCCTCCT-3')]. The truncated H3c mRNA was prepared by in vitro transcription and PAGE purification.

Hydroxyl radical RNA footprinting. A solution of 20 nM 5'-^{32}P end labeled truncated H3c mRNA (sequences 213-340) in binding buffer was heated at 65^{o}C for 5 min and cooled to room temperature. 5, 20, 40 and 80 μM of H3 18-mer peptide was added to the prepared RNA solution (final 20 μL) and mixed with freshly prepared hydroxyl radical mixture 4 μL [1 μL of 50 mM $Fe(NH_4)_2(SO4)_26H_2O$, 100 mM EDTA, 2.5% (*v/v*) H_2O_2 and 250 mM ascorbate] and then incubated for 5 min at 4^{o}C. Fragmented RNA was obtained by ethanol precipitation. The resulting RNA was loaded on 10% polyacrylamide/7 M urea gel. The gel was dried and radioactivity of the individual bands was monitored (Figure S5) by using a phosphorimager (FLA-3000) and was analyzed by Multi Gauge Ver. 3.0 software (Fuji Photo).

VIII. In vitro translation assay

In vitro translation assay was carried out with a modified manufacturer's protocol (Promega, rabbit reticulocyte lysate system, nuclease treated). Briefly, template H3c DNA containing T7 polymerase promoter was obtained from pCMV-SPORT6-H3c construct by PCR using designed primers containing kozak sequences (bold sequences) for enhanced translation efficiency [5'-primer (5'-ATATAATACGACTCA-CTATAGG**GATT**ATGGCCCGTACTAAGCAGACTG-3'), 3'-primer (5'-TCTAGAGCG-GCCGCCCTTTT-3')]. H3c mRNA was prepared by in vitro transcription reaction by using T7 polymerase. H3c mRNA was heated for 3 min at 65 ℃ and then cooled on ice. 1 μL of template H3c mRNA (1 μg/μL) was mixed with 0.5 μL of 1 mM amino acid mix without Met, 0.5 μL of RNase inhibitor (40 unit/μL), 1 μL of Redivue L-[^{35}S]-methionine (GE healthcare, 15 mCi/mL), 17.5 μL of rabbit reticulocyte lysate (Promega) and various concentrations (final 1, 5, 10, 20 and 30 μM) of H3 18-mer peptide (or full-length H3 protein[4]). Then, final 25 μL of each reaction mixture was incubated for 1.5 h at 30^{o}C. To complete the reaction, 1 μL of RNase A (1 mg/mL) was added and

incubated for 5 min at room temperature. 5 μL of aliquot was added to 20 μL of SDS loading dye. Samples were heated for 20 min at 65^{o}C and then cooled on ice. After centrifugation, 20 μL of sample was loaded on 15% SDS polyacrylamide gel. The gel was dried and radioactivity of the individual bands was monitored by a phosphorimager (FLA-3000) and was analyzed by Multi Gauge Ver. 3.0 software (Fuji Photo). Newly in vitro translated 17 KDa H3 proteins were labeled with ^{35}S-methionine and band was confirmed by comparison with protein standard marker (Figure S6). 1 μg of luciferase mRNA was used as a control.

IX. Reporter gene assay

Preparation of reporter gene constructs containing histone H3 hairpin or RRE RNA hairpin. The H3 hairpin or RRE RNA hairpin was inserted into HindIII site on 5'-UTR of pGL3-control vector (Promega). For direct ligation, DNA templates containing the HindIII site were synthesized [Sense strand of the H3 hairpin insert (5'-AGCTTGCGCAGGACTTTAAGACGGACCTGCGC A-3'); Antisense strand of H3 hairpin insert (5'-AGCTTGCGCAGGTCCGTCTTAAAGTCCTGCGC A-3'); Sense strand of RRE insert (5'-AGCTTGGTGGGCGCAGCTTCGGCTGACGGTACACCA-3'); Antisense strand of RRE insert (5'-AGCTTGGTGTACCGTCAGCCGAAGCTGC-GCCCACC A-3')]. Mutant H3 hairpin mt2 and mt3 inserts were also synthesized by same manner. The H3 or RRE hairpin DNA was inserted into pGL3-control vector using HindIII restriction site and T4 DNA ligase. The recombinant DNA was transformed into *E. coli*. Plasmid DNA was prepared from individual clones and sequenced. Cloned reporter DNAs were prepared for transfection in cells.

Cell culture, transfection and luciferase assay. Human 293A embryonic kidney cells were grown in DMEM containing 10% FBS. In a 6-well plate, the cells (1.5 x 10^{5} cell in 2 mL) were incubated at 37^{o}C for 24 h until the cells reached around 70% confluent. Each of pGL3-control plasmid variants (250 ng) were co-transfected with of pRL-CMV (100 pg) with manufacturer's protocol. After 16 h incubation, cells were subjected to luciferase assay. Luciferase activity was measured using dual-luciferase reporter assay system and Glomax^{TM} 20/20 luminometer (Promega). Firefly luciferase activity was normalized by using renilla luciferase activity. In each sample, total RNAs were purified using Trizol reagent and then the same amount of RNA was subjected to RT-PCR using luciferase-specific primers [Primers for 656-835 se-

quences of firefly luciferase mRNA (5'-prmer; 5'-CTCGCATGCCAGAGATCCTA-3', 3'-primer; 5'-TCCTGAAGGCTCCTCAGAAAC-3', primers for 337-603 sequences of renilla luciferase mRNA (5'-primer; 5'-AAGATCATTTTTGTCGGCCA-3', 3'-primer; 5'-TGCTGCAAATTCTTCTGGTTC-3')].

X. Sequences of *Homo sapiens* histone H3c cDNA inserted in pCMV-SPORT6 vector

Sequences of *H. sapiens* H3c cDNA inserted in pCMV-SPORT6 vector are shown [A red region, coding region; A blue region, H3 hairpin region; Underlined sequences, palindromic termination element (stem-loop); NCBI accession number NM 021059].

1 CGCCGCTGCGCTGGTAAGCCTGTGTTTTGGTTCGCTATGGCCCGTACTAAGCAGACTGCT
61 CGCAAGTCGACCGGCGGCAAGGCCCCGAGGAAGCAGCTGGCCACCAAGGCGGCCCGCAAG
121 AGCGCGCCGGCCACGGGCGGGGTGAAGAAGCCGCACCGCTACCGGCCCGGCACCGTAGCC
181 CTGCGGGAGATCCGGCGCTACCAGAAGTCCACGGAGCTGCTGATCCGCAAGCTGCCCTTC
241 CAGCGGCTGGTACGCGAGATC**GCGCAGGACTTTAAGACGGACCTGCGC**TTCCAGAGCTCG
301 GCCGTGATGGCGCTGCAGGAGGCCAGCGAGGCCTACCTGGTGGGGCTGTTCGAAGACACG
361 AACCTGTGCGCCATCCACGCCAAGCGCGTGACCATTATGCCCAAGGACATCCAGCTGGCC
421 CGCCGCATCCGTGGAGAGCGGGCTTAAGAAGTGGCGGTTCGGCCGGAGGTTCCATCGTAT
481 CCAAAAGGCTCTTTTCAGAGCCACCCACATCAGCACTTGGAAGAAGCTGTACCGCTTGCC
541 CTCCGTGCTCCTCCGGCATTAGAGCGGGGAAGGCACTTCCGCTTAGGCTCCCAACAAAAC
601 GGGGTCGGGTCCCCCTGCTGCGGTGCCGTCTTGAGCTTTGCCGACTTCGGCCAATTGGTC
661 TATCCGCACTGGCCGGGCTGCGGACGCTTTCCTGGGCGCCGGCTCGCGGGCTTTGGCGGT
721 CCGGCTCCCCCCGCGCGTGACCGGGGCCCTGCGTGGCGGGGGGGCCGGGGCAGGAGAGAC
781 GGGGCGTCGGGCACGAGGCCAGGGGTCACTAGGGGGACGCTCAGGGCTCGCGGTCCTGAG
841 CGCGGCTGGCCTTTGCGGAAGTGGAGCGCCACACGCCCGTGCTTCGCGCCCAAACCGGCC
901 TCACCCGGCGCGGCCATCCGGCTAGGGAGGGAAATGACCACCTCGGCCTCGCCGGGGTTC
961 TGCGGACAAACGTGGGGAGAACTGCGCTGGGGCTGCCGTTCAGTCCCCGCGCCACCTCCA
1021 CTAGCACAGTGGTAACCCCCATATCCTGCCCCTAAGCCAAAATTCCCGGGGGCACGGGGT
1081 GTGGGCGGAATTTTGTGAATCTGGGCTTTATCGAGCCGTTCCCTGTCCATGTCCACAGAA
1141 GGAGATTCACTATTCCAGTGAAGGCACTCGGTCCTCTTAGAAGCTGCATGGTATGTCTGG
1201 ATTTTCTGTCCAGACTCAGTAGTTCCTTTACGTTGTTTTCGTCGGACATGTGGGCAGACG
1261 CTGGTGCCCGAGTTCAGATCCTTGGGAAGACAGTAGATCAGACAGGAAGAACGTGGGCCT
1321 CCGTTGGGCAAATGAAGCTCTTCGCCTTAGGAAATCGTGACAGGAGGCCACAGAGGTGAT
1381 CTCTACAGTCTCTGGGAGCTGGGAAGTCCAAGGCCTCTTAGTGGGAAAGTGAGGCAGGGT
1441 CTTGCAGAGCCCACTCTACACACGGCCAGATACAAATGGCTGTAGAGGTCTTAGATAACA

1501 CCTTCTCGGTCATTTATTTGCACCTCAGAAGAAGCCAGGGCTTCGGTTTTCTGTTTGTTT

1561 CATCACAGTTGACAGGTTAAAAGCATTCACTGCAGCGATCTATGAGAATTAAGAGGGGAG

1621 AAGGGGAAATGGAATAAAACTTCGTTTTGTAAAGCTAAAAAAAAAAAAAAAA

Table S1. Calculated values of binding affinities of the H3 hairpin RNAs using BIA-core 3000 instrument. Binding affinities of H3 hairpin RNAs to H3 18-mer peptide were calculated by using BIAevaluation software (1:1 Langmuir binding model). N.C. means not calculable.

	K_a (1/Ms)	K_d (1/s)	K_D (M)
H3 hairpin	1.3×10^{4}	1.5×10^{-2}	1.2×10^{-6}
H3 hairpin mt1	3.6×10^{4}	5.2×10^{-2}	1.4×10^{-6}
H3 hairpin mt2	N.C.	N.C.	N.C.
H3 hairpin mt3	N.C.	N.C.	$\geq 1 \times 10^{-5}$
H3 hairpin mt4	1.7×10^{4}	3.9×10^{-2}	2.4×10^{-6}
H3 hairpin mt5	N.C.	N.C.	N.C.
H3 hairpin mt6	3.8×10^{4}	8.3×10^{-2}	2.2×10^{-6}

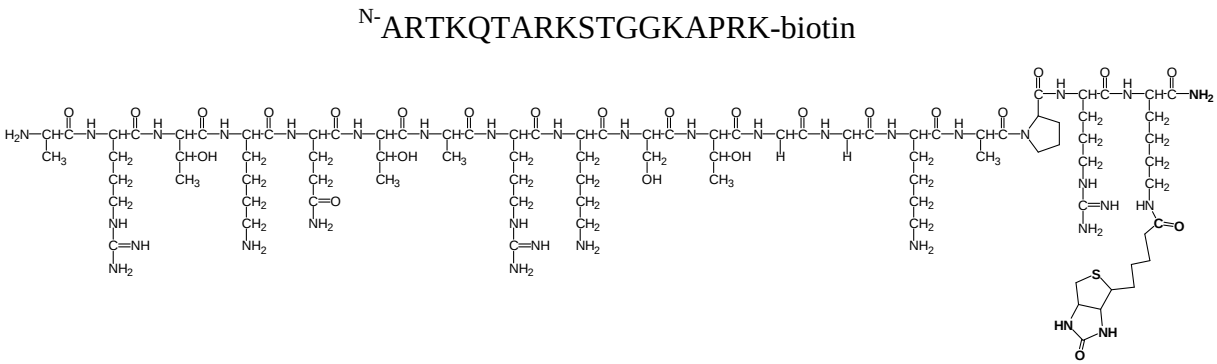


Figure S1. Structure of H3 peptide used as bait.

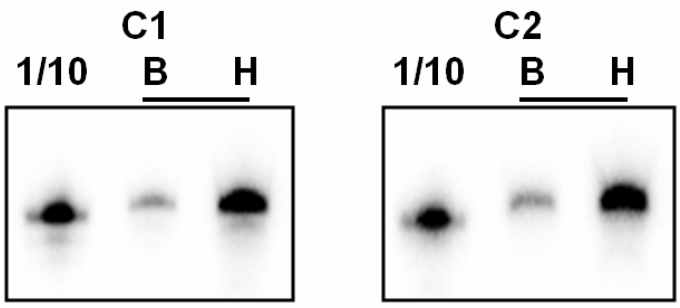
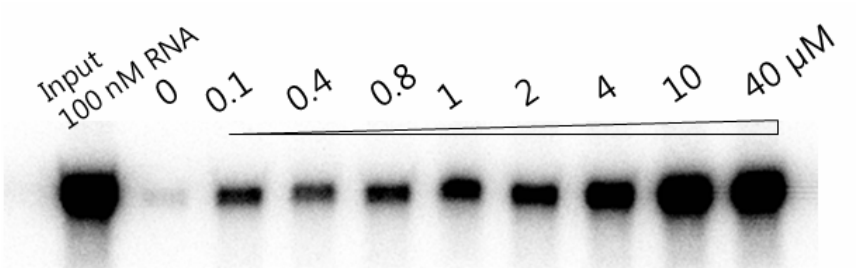


Figure S2. Specific binding of C1 and C2 RNA aptamer to histone H3 18-mer peptide. 100 nM of ^{32}P-labeled each RNA aptamer was used for a pull-down assay (1/10, 10% input; B, Bead alone; H, Biotinylated histone H3 18-mer peptide 10 μM).

A)



K_d = 8.2 ± 1.4 μM

B)

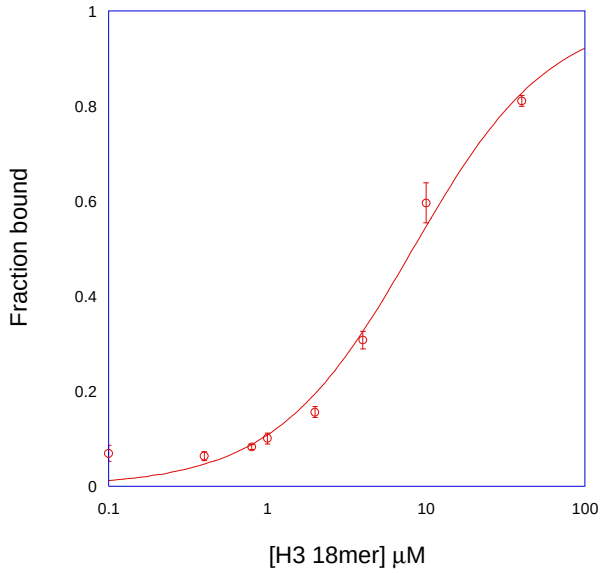
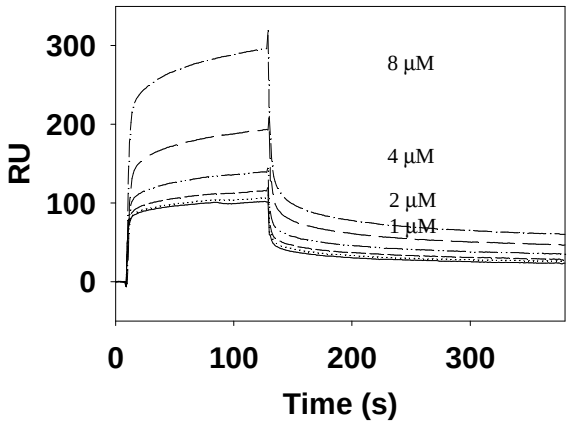
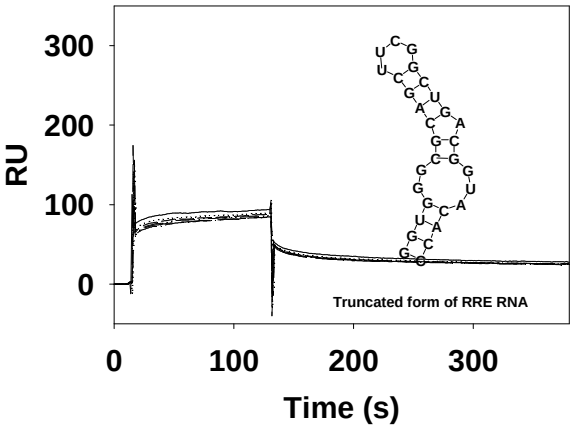


Figure S3. Binding affinity of H3 hairpin RNA to H3 18-mer peptide using pull-down method. **A)** Representative autoradiogram for pull-down assay. Indicated increasing amounts of peptides were mixed with radioactively labeled RNA (100 nM) and tRNA^{mix} (1 μM). The value indicates average and one standard deviation of individual three experiments. **B)** Fitting curve for binding affinity.

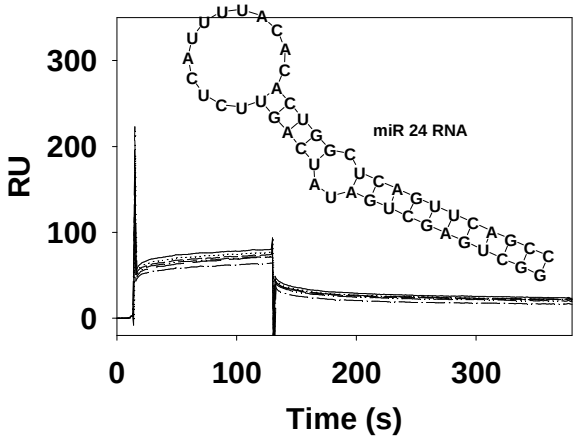
A)


8 μM
4 μM
2 μM
1 μM
RU
Time (s)

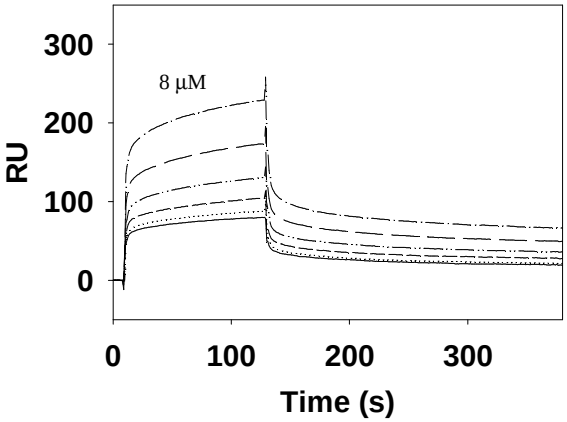
B)


Truncated form of RRE RNA
RU
Time (s)

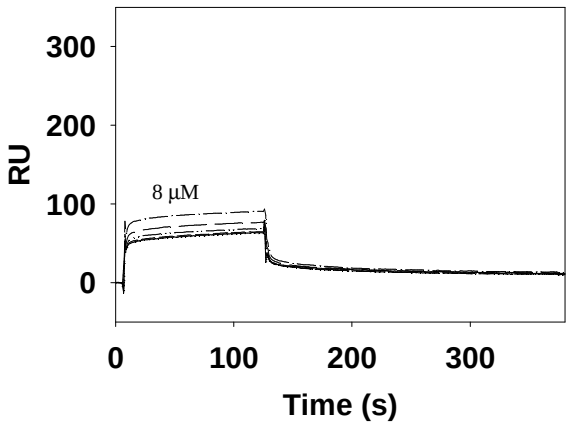
C)


miR 24 RNA
RU
Time (s)

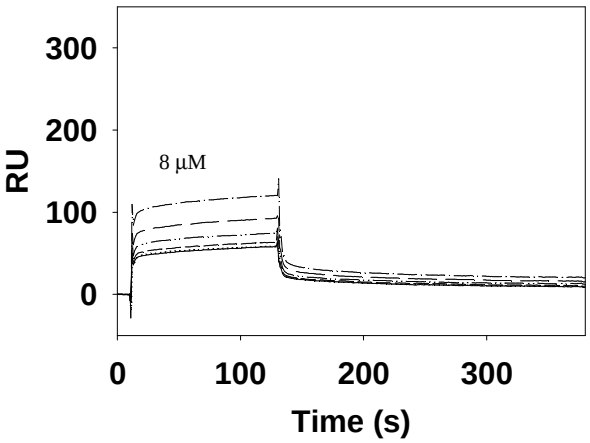
D)


8 μM
RU
Time (s)

E)


8 μM
RU
Time (s)

F)


8 μM
RU
Time (s)

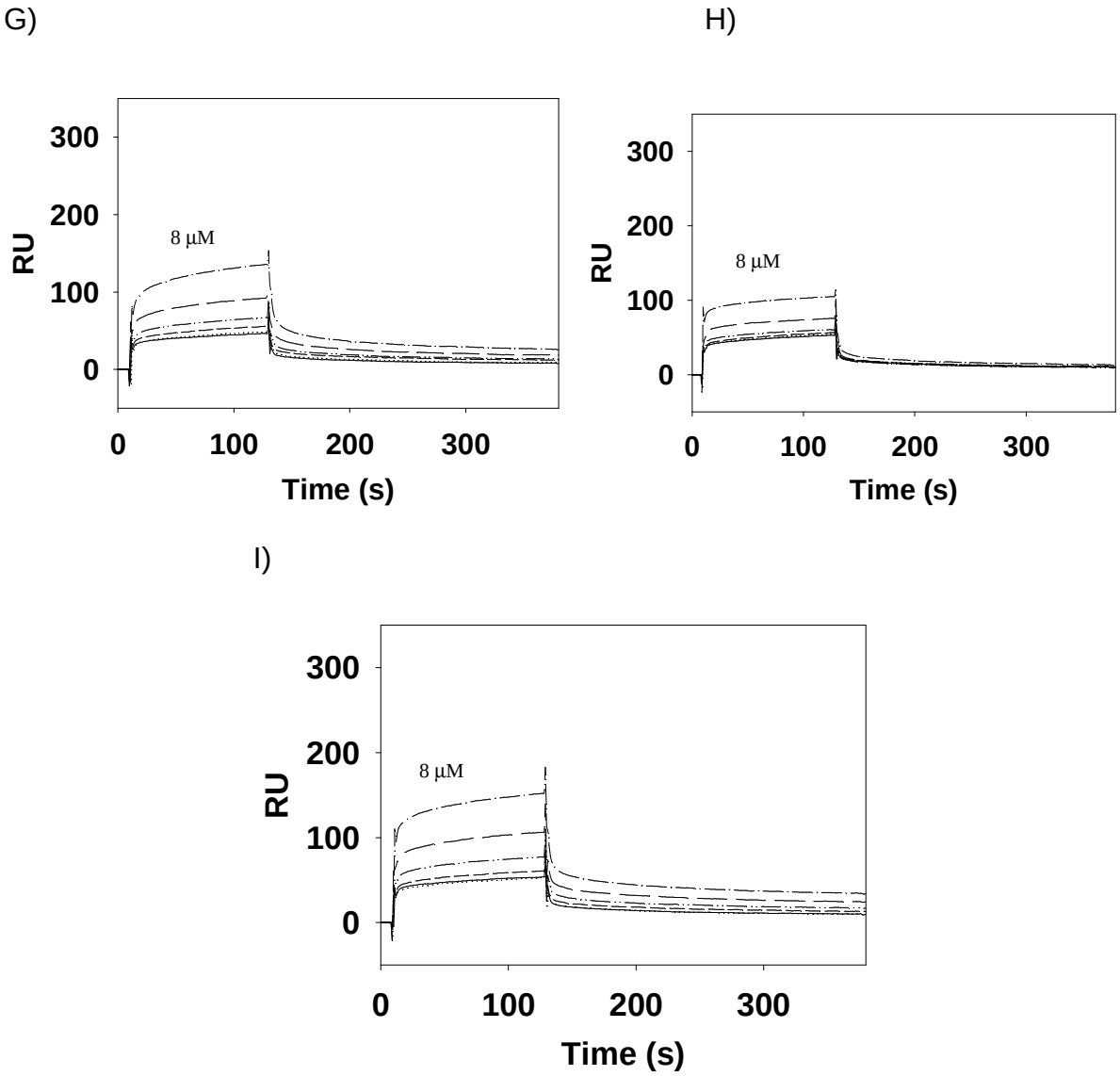


Figure S4. Binding specificity of H3 hairpin RNA to H3 18-mer peptide using BIAcore. A) Sensorgrams of H3 hairpin RNA binding to H3 18-mer peptide. As a control, B) RRE and C) pre-miR-24 RNA were injected. D)~I) Sensorgrams of mutant (mt) H3 hairpins mt1-6. Various concentrations of hairpin RNAs were injected (250, 500 nM, 1, 2, 4, and 8 μM in the presence of 20 μM of $tRNA^{mix}$). RU, response unit.

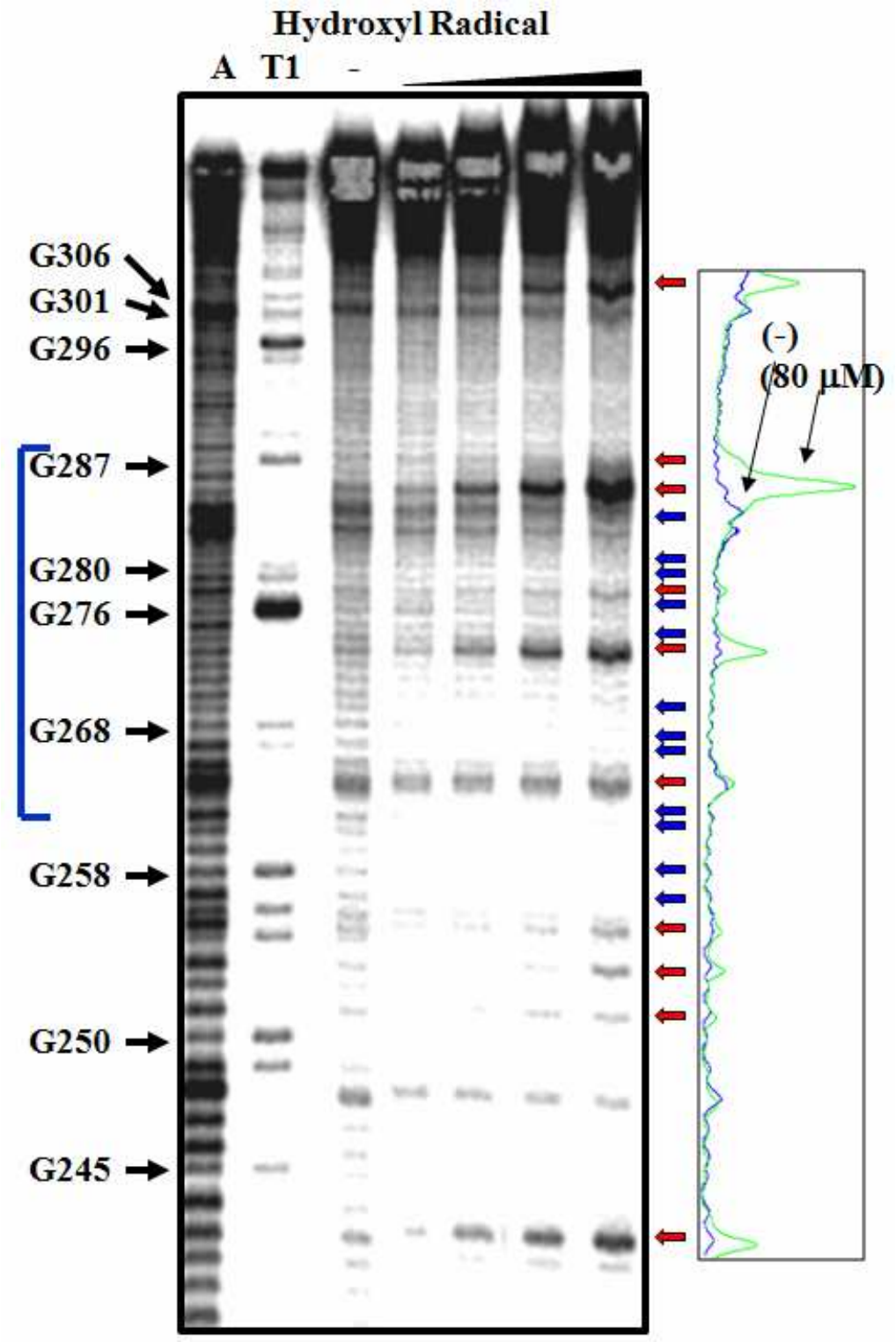


Figure S5. Autoradiogram of the hydroxyl radical cleavage of truncated (sequences 213-340) H3c mRNA (A, alkaline hydrolysis ladder; T1, RNase T1 marker; −, no peptide; 5, 20, 40 and 80 μM of H3 18-mer peptide; A red arrow means enhancement of cleavage; A blue arrow means protection from cleavage; A blue line, hairpin region).

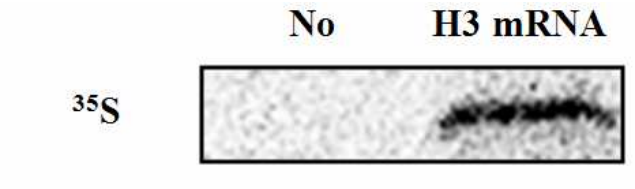


Figure S6. In vitro translated histone H3 protein labeled with S^{35} (No, no RNA template; H3c mRNA, 1 µg of H3c mRNA containing kozak sequence as a template).

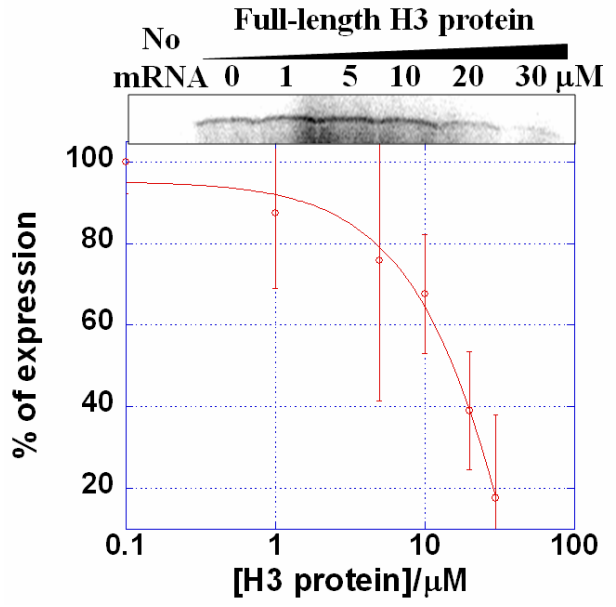


Figure S7. Inhibition of in vitro translation of the H3 protein caused by addition of the full-length H3 protein. Indicated concentrations (1-30 µM) of H3 protein and 1 µg of H3c mRNA were used for each reaction.

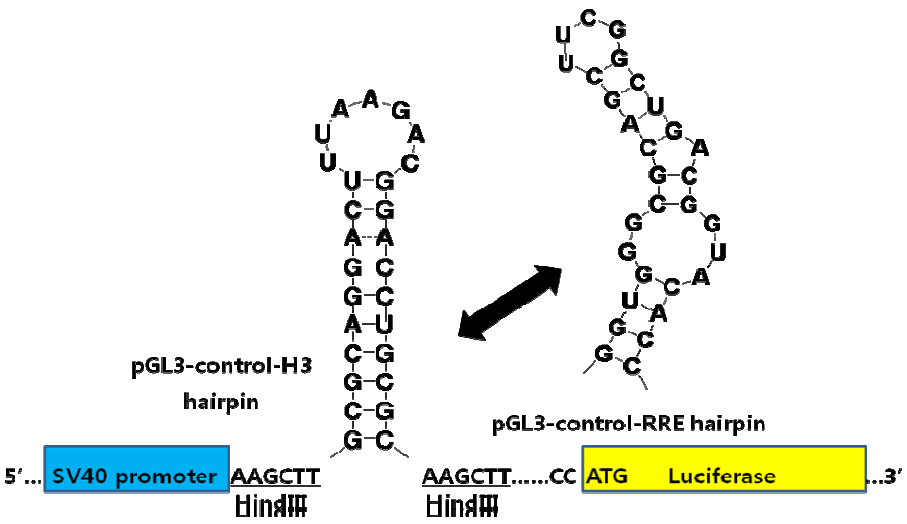


Figure S8. Schematic representation of pGL3-control-H3 hairpin and -RRE RNA hairpin constructs. H3 or mt2, 3, RRE hairpin sequences were inserted in downstream of SV40 promoter on 5'-UTR.

References and Notes

1 M. Y. Kim, S. Jeong, *Mol. Cells* **2003**, *16*, 413-417.

2 K. H.Lee, S. Jeong, E. G. Yang, Y. –K. Park, J. Yu, *Bioorg. Med. Chem.* **2007**, *15*, 7545-7552.

3 The clone was gifted from 21C Human Gene Bank, Genome Research Center, KRIBB, Korea.

4 Recombinant full-length histone H3 protein was purchased from Upstate.