Supplementary Information

Directed evolution of a synthetic RNA-protein module to create a new translational switch

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1. Materials and Methods

Protein preparation

L7Ae and its modified L7KK were expressed and purified as previously reported [1]. Briefly, the pET 28-b+ vector (Novagen) was selected for the cloning and expression of the recombinant protein L7Ae from *A. fulgidus*. The plasmid (pET 28-b+ - L7Ae) was transformed into *E. coli*. BL21(DE3) (pLysS) cells. Protein expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the culture was incubated overnight at 30°C. The cells were harvested by centrifugation at 6,000 r.p.m. for 20 min at 4°C and resuspended in sonication buffer (50 mM phosphate buffer [pH 8.0] and 300 mM NaCl) at 4°C. The suspension was sonicated, and the lysate was incubated at 80°C for 15 min to denature the endogenous proteins, which were removed by centrifugation at 6,000 r.p.m. for 20 min at 4°C. The supernatant contained the recombinant hexahistidine-tagged L7Ae protein. L7Ae was purified from the supernatant using Ni-NTA agarose according to the manufacturer's directions (QIAGEN). The purity of the protein was confirmed by sodium dodecyl sulfate (SDS)-PAGE. The eluted protein was concentrated using a YM-3 Microcon device (Millipore), and dialyzed against a buffer containing 20 mM HEPES-KOH (pH 7.5), 150 mM KCl, 1.5 mM $MgCl_2$ and 5% glycerol. The concentration of the purified L7Ae protein was determined using the

Bradford protein assay (Bio-Rad). The purified L7Ae protein was stored in storage buffer (20 mM HEPES-KOH (pH 7.4), 150 mM KCl and 1.5 mM $MgCl_2$ containing 40% glycerol) at –20°C.

RNA preparation for EMSA and SPR assays

The DNA templates and primers used in this study were purchased from Hokkaido System Science or GeneDesign. The single-stranded templates annealed with the T7 annealing primer were transcribed using T7 RNA polymerase for approximately 4 h at 37°C. The DNA templates were then degraded using TURBO DNase (MEGAshortscript™, Ambion) for 15 min at 37°C. The resulting RNA products were purified by denaturing PAGE and precipitated using ethanol. RNA sequences used in this study are shown in page S9-S12.

Construction of the RNA library

The DNA templates for the RNA pool were constructed by PCR using three synthetic oligonucleotides: N30-a (5'-CCGGGGATCCTCTAGAGTCGGG-N30-CCCCA TTGGTATATCTCCTTCCTATAGTGAGTCGTATTAGC-3'), N30-b (5'-GCTAATAC GACTCACTATAGGAAGGAGATATACCA-3'), where the underlined sequence is the

T7 promoter sequence) and N30-c (5'-CCGGGGATCCTCTAGAGTC-3'). The PCR amplifications were performed using KOD-PLUS-DNA polymerase (TOYOBO). After purification, the PCR products were transcribed in the presence of [^{32}P-α] GTP (PerkinElmer) for the 1st-9th round of selection to monitor the rate of RNA collection in the *in vitro* selection. The 10-14 rounds of RNA selection did not include [^{32}P-α] GTP labeling. After DNA degradation, the resulting RNA products were purified as described above.

In vitro selection of RNA aptamers

In vitro selection was performed at room temperature in a selection buffer containing 20 mM HEPES-KOH (pH 7.5), 150 mM KCl, 1.5 mM $MgCl_2$, 2 mM DTT, 3% glycerol, and 100 μg tRNA. The total volume of the reaction mixture and concentrations of the RNA pool and target protein (L7KK) are listed in Supplementary Table S1. The pooled RNA was dissolved in buffer, incubated for 5 min at 80°C and cooled to 25°C using a thermal cycler. Ni-NTA magnetic agarose beads (QIAGEN) were used for the *in vitro* selection. The beads were washed twice with buffer (without tRNA) and mixed with L7KK dissolved in a wash buffer containing 20 mM HEPES-KOH (pH 7.5), 150 mM KCl, 1.5 mM $MgCl_2$, 2 mM DTT, 3% glycerol. The L7KK solution and beads were

incubated for 30 min on ice, mixed with RNA solution and then incubated for 30 min at room temperature. The unbound RNA was removed, and the beads were washed five times with the wash buffer. The beads were then mixed with an elution buffer containing 20 mM HEPES-KOH (pH 7.5), 150 mM KCl, 1.5 mM $MgCl_2$, 2 mM DTT, 3% glycerol, 100 mM EDTA, and 1 M imidazole, and incubated for 5 min at room temperature to elute the bound RNA. The supernatant was collected, mixed with SDS solution (0.1% final concentration), and incubated for 10 min at 37°C. Phenol extraction, diethyl ether extraction and ethanol precipitation were then performed. The selected candidates were reverse transcribed using ReverTra Ace (TOYOBO) with primer N30-c and amplified by PCR with primers N30-b and N30-c. In the 7th round, before the selection of the RNA candidates bound to L7KK, any RNA that bound nonspecifically to the beads was removed by mixing the beads with the wash buffer and collecting the supernatant. To characterize the selected aptamers, DNA templates isolated after the 14th round of selection were cloned into the pGEM vector (Promega), and 42 clones were sequenced.

During rounds 1-9 of selection, the RNA pool was ^{32}P-labeled to monitor the proportion of RNA that bound to L7KK, which was saturated by the sixth round (**Supplementary Table S1**). After the sixth round, we performed negative selection to

exclude the bead-binding RNAs. We conducted five additional rounds of selection with non-radiolabeled RNA to simplify the experimental procedure.

Electrophoretic mobility shift assays (EMSAs)

The EMSA assays were performed in 20 µl of RNP-binding buffer [20 mM HEPES-KOH (pH 7.5), 150 mM KCl, 1.5 mM $MgCl_2$, 2 mM DTT, and 3% glycerol]. The RNAs dissolved in the buffer were denatured by heating at 80°C for 3 min; the mixture was then cooled to 4°C. After various concentrations of recombinant L7KK were added to the solution containing 40 nM of RNA, the solutions were incubated on ice for 30 min. The RNA-protein complex was separated from the free RNA by electrophoresis on 12% native polyacrylamide gels at 4°C for approximately 2 hours. After electrophoresis, the gels were stained with SYBR Green II (Molecular Probes) and observed with an FLA-3000 or FLA-7000 phosphoimager (Fujifilm).

Surface plasmon resonance (SPR) assay

The SPR analysis was performed in binding buffer containing 20 mM HEPES-KOH (pH 7.4), 150 mM KCl, 1.5 mM $MgCl_2$, 2 mM DTT, 5% glycerol, 0.01% Tween-20, 125 µg/ml tRNA, and 62.5 µg/ml BSA using a BIAcore 3000 instrument (GE Healthcare).

The proteins were immobilized on the CM5 sensor chip *via* amine coupling according to the manufacturer's instructions. The RNA solutions were prepared in the same buffer, denatured at 96°C for 5 min, refolded at room temperature, and serially diluted. A 40-µl aliquot of each RNA solution was injected onto the sensor chip at a flow rate of 20 µl/min. The surface of the sensor chip was regenerated with 0.1 N NaOH for 5 s and equilibrated with buffer prior to the next RNA injection.

Plasmid construction for cellular assays

The C/D box-EGFP- and H23-EGFP-expressing plasmids were constructed using whole-plasmid PCR of pEGFP-N1 (Clontech) with the forward primers 5'-GGGCGTGATGCGAAAGCTGACCCTGTGAGCAAGGGCGAGGAGCTG-3' or 5'-GGGGATGACGCGAAAGCTGACCCTGTGAGCAAGGGCGAGGAGCTG-3', respectively, and the reverse primer 5'-CATGGTGGCGACCGGTGGATC-3', as previously reported [1]. The BFP open reading frame was amplified by PCR using a plasmid derived from pTagBFP-C (Evrogen) and the primers 5'-GGCCCGGGA TCCACCGGTCGCCACCATGGGGGATGACGCGAAAGCTGACCCTAGCGAGCT GATTAAGGAGAACATGC-3' and 5'-TTATGATCTAGAGTCGCGGCCGCTT TAGTGCCCCAGTTTGCTAGGGAG-3'. The H23-BFP-expressing plasmid was

constructed by replacing the EGFP gene between the *Bam*HI and *Not*I sites in pEGFP-N1 with the PCR product. The L7Ae- or L7KK-expressing plasmid in mammalian cells was constructed by incorporating the OneStrep-L7Ae (L7KK)-myc-His-IRES-DsRed Ex coding sequence between the *Hin*dIII and *Not*I sites in pcDNA5TO, as previously reported [2].

Translational regulation assay in HeLa cells

HeLa cells (0.5×10^5 per well) established in 24-well plates were cultured at 37°C in DMEM/F-12 medium (Invitrogen) supplemented with 10% (v/v) FBS and an antibiotic/antimycotic solution (A5955, Sigma Aldrich). The incubator atmosphere was enriched with 5% CO_2. After 1 day, 70-80% confluent cells were co-transfected with plasmids using 1 μl of Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The media were changed 4 h after transfection. The HeLa cells were collected by trypsinization and resuspension in 200 μl DMEM/F12 medium at 1 day after transfection. A total of 10,000 cells per sample were analyzed by fluorescence-activated cell sorting using a FACSAria instrument (BD Biosciences). The dead cells were excluded by gating out events with low FSC and SSC signals.

RNA sequences used in this study

H23

5’-GGGGAUGACGCGAAAGCUGACCC-3’

shH23

5’-GGCAUCAAGGUGAACUUCAGGGGGAUGACGCGAAAGCUGACCCCUGA

AGUUCACCUUGAUGCCGA-3’

sh C/D box

5’-GGCAUCAAGGUGAACUUCAGCUGACCCGAAAGGGCGUGAUGCUGAAG

UUCACCUUGAUGCCAG-3’

D12

5’-GGAAGGAGAUAUACCAAUCGGGCACGGUCCAAGAUAUCGAUGAAUUC

CAGUCCUCGACUCUAGAGGAUCCCCGG-3’

G2

5’-GGAAGGAGAUAUACCAACGGGCCACCGGAAAAAGUGUAUGU*CGAU*GA

GUUCCCCGGACUCUAGAGGAUCCCCGG-3’

G6

5’-GGAAGGAGAUAUACCAUGGGGAACCGAUGAAGGAGCUUACCGGUGCC

CUUCCCCGACUCUAGAGGAUCCCCGG-3’

G10

5’-GGAA*GGAG*AUAUACCAAUGCGUGCAAUACCAUGGGUGUGU*CGAU*GAU

CUCCGCCGGACUCUAGAGGAUCCCCGG-3’

H1

5’-GGAAGGAGAUAUACCAACGGGGAAGACUCCCGGCAUAUCGAUGAACU

CCAAGCCCGACUCUAGAGGAUCCCCGG-3’

H2

5’-GGAAGGAGAUAUACCAACGGGGUCGCGCUGACCACACACAAGUGGGA

UGACGCACGACUCUAGAGGAUCCCCGG-3’

H5

5’-GGAAGGAGAUAUACCACGGGCAGCGAUGACCUGAUAAAAACUCAGUG

AGCUCCCGACUCUAGAGGAUCCCCGG-3’

H8

5’-GGAAGGAGAUAUACCAAUGAGGCAAGAUACGCGGAGGUAGUCUCCCC

AACCUCUCGACUCUAGAGGAUCCCCGG-3’

H10

5’-GGAAGGAGAUAUACCAAUGGAGCAGGGCGGGAUGAUACAUUCGUGU

GGACCGCCCGACUCUAGAGGAUCCCCGG-3’

Kt-15

5’-GGCAUCAAGGUGAACUUCACUGAACCCGAAAGGGGAUGUGGUGAAGU

UCACCUUGAUGCCAG-3’

Kt-7

5’-GGCAUCAAGGUGAACUUCAGCGGAGCCGAAAGGCGAAGAAGCUGAAG

UUCACCUUGAUGCCAG-3’

lysC lysine riboswitch

5’-GGCAUCAAGGUGAACUUCAGUGGAGACGAAAGUCUGUGAAACUGAA

GUUCACCUUGAUGCCAG-3’

SAM Kt (*B.subtilis*)

5’-GGCAUCAAGGUGAACUUCAGCAGAGGCGAAAGCCGACGAAGCUGAAG

UUCACCUUGAUGCCAG

archaeal H/ACA

5’-GGCAUCAAGGUGAACUUCAGCUGAUUGGUGAGGCUGAAGUUCACCUU

GAUGCCAG-3’

sR8 C’/D’ box

5’-GGCAUCAAGGUGAACUUCAGCUGAGUCUGUGAUGCUGAAGUUCACCU

UGAUGCCAG-3’

H23-L2-G

5’-GGCAUCAAGGUGAACUUCAGGGGGGUGACGCGAAAGCUGACCCCUGA

AGUUCACCUUGAUGCCGA-3’

H23-L3-C

5’-GGCAUCAAGGUGAACUUCAGGGGGACGACGCGAAAGCUGACCCCUGA

AGUUCACCUUGAUGCCGA-3’

H23-1b-C

5’-GGCAUCAAGGUGAACUUCAGGGGGAUCACGCGAAAGCUGACCCCUGA

AGUUCACCUUGAUGCCGA-3’

SAM Kt L3-U

5’-GGCAUCAAGGUGAACUUCAGCAGAGGCGAAAGCCGAUGAAGCUGAAGU

UCACCUUGAUGCCAG-3’

Kt-15-2b-A

5’-GGCAUCAAGGUGAACUUCACUGAACCCGAAAGGGGAUGAGGUGAAGU

UCACCUUGAUGCCAG-3’

2. Supplementary Figures

Supplementary Figure S1

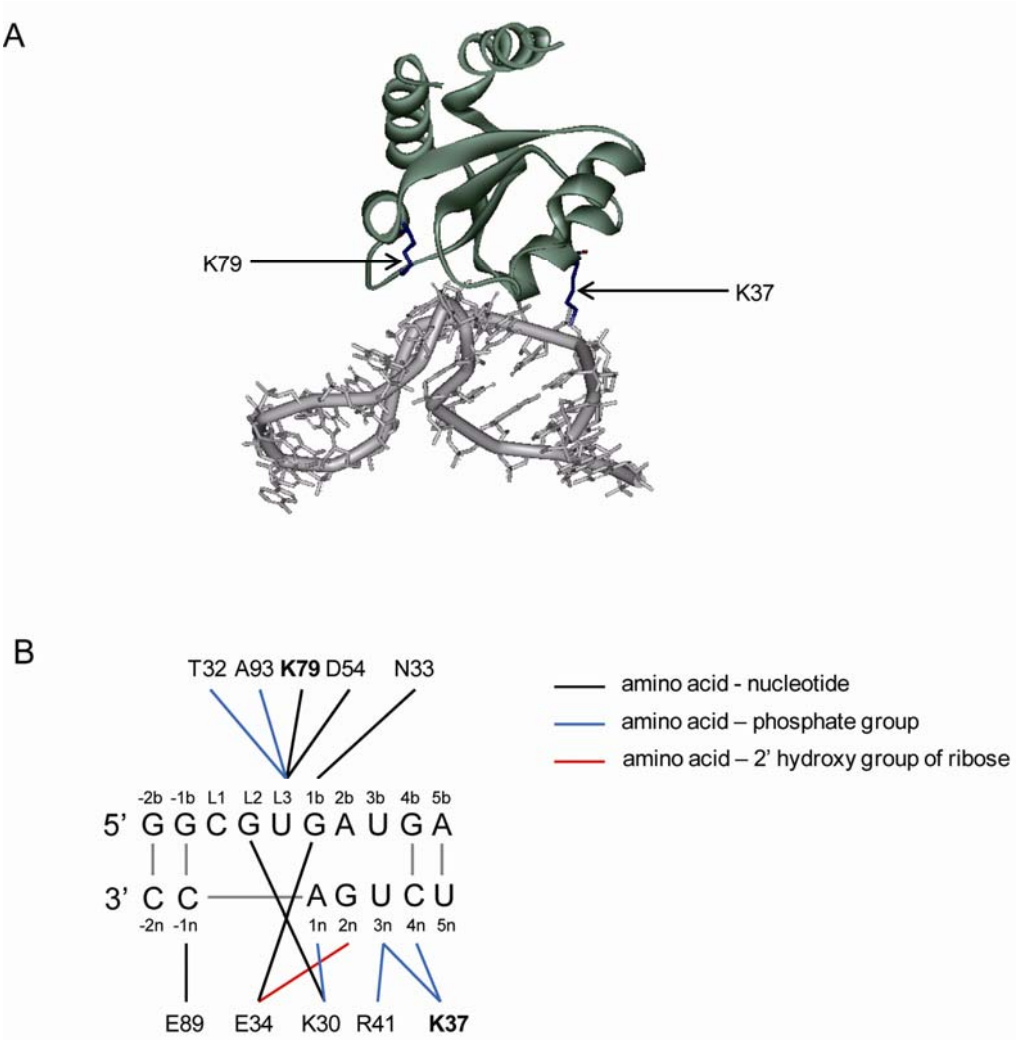

Figure S1. The interaction between L7Ae and the C/D box.

(A) The locations of K37 and K79, which are estimated to interact with the C/D box, are shown on the protein crystal structure (PDB ID 1RLG). To construct L7KK, these two amino acids (K37 and K79) were mutated to alanine. **(B)** The hydrogen bonds between L7Ae and the C/D box were predicted by Discovery Studio (Accelrys).

Supplementary Figure S2

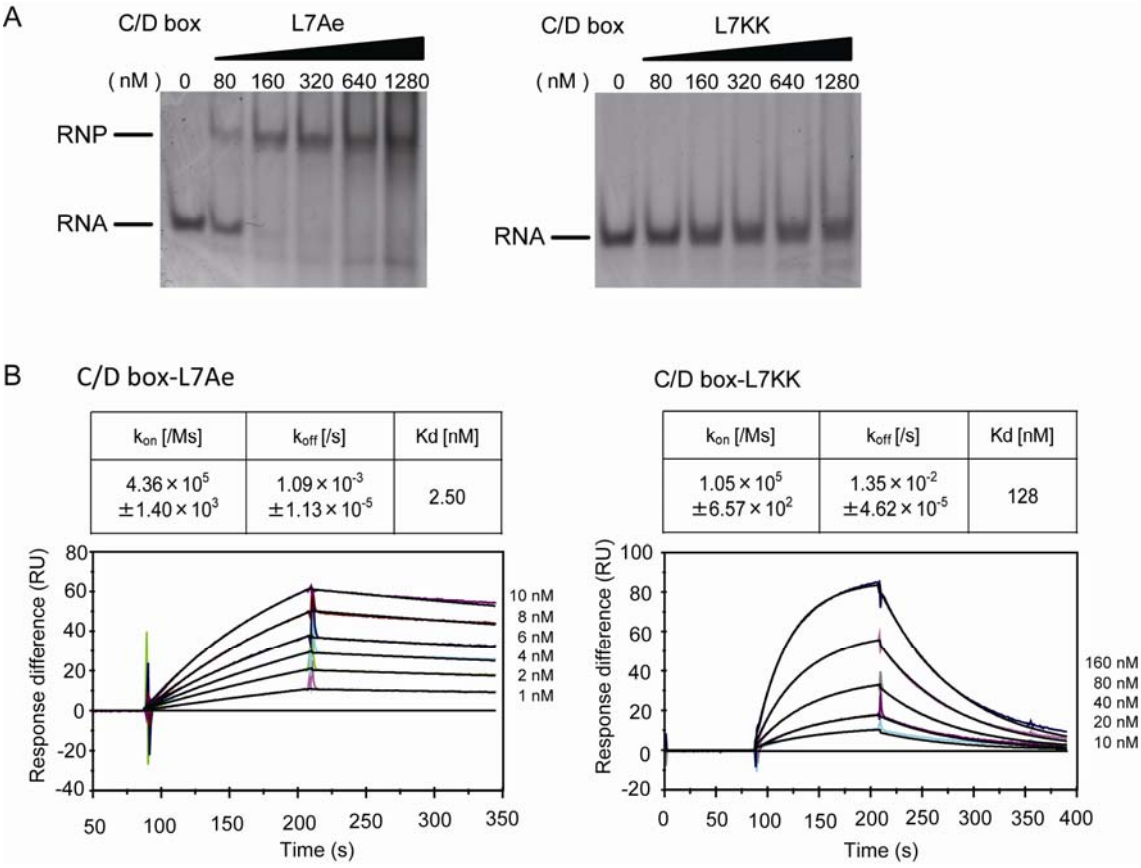

Figure S2. Binding assays of L7Ae / L7KK and the C/D box.

(A) EMSA with a 40 nM short hairpin RNA containing the C/D box (C/D box-shRNA) and various concentrations (0, 80, 160, 320, 640, and 1280 nM) of L7Ae and L7KK. The RNA was stained with SYBR Green. **(B)** Surface plasmon resonance (SPR) measurements. L7Ae or L7KK was immobilized on the CM5 sensor chip, and various concentrations of C/D box-shRNAs were tested (L7Ae - 0, 1, 2, 4, 6, 8, and 10 nM; L7KK - 0, 10, 20, 40, 80, and 160 nM). The measurement curve for 0 nM was taken as the baseline. The black curves are fitted curves calculated using BIAevaluation software.

Supplementary Figure S3

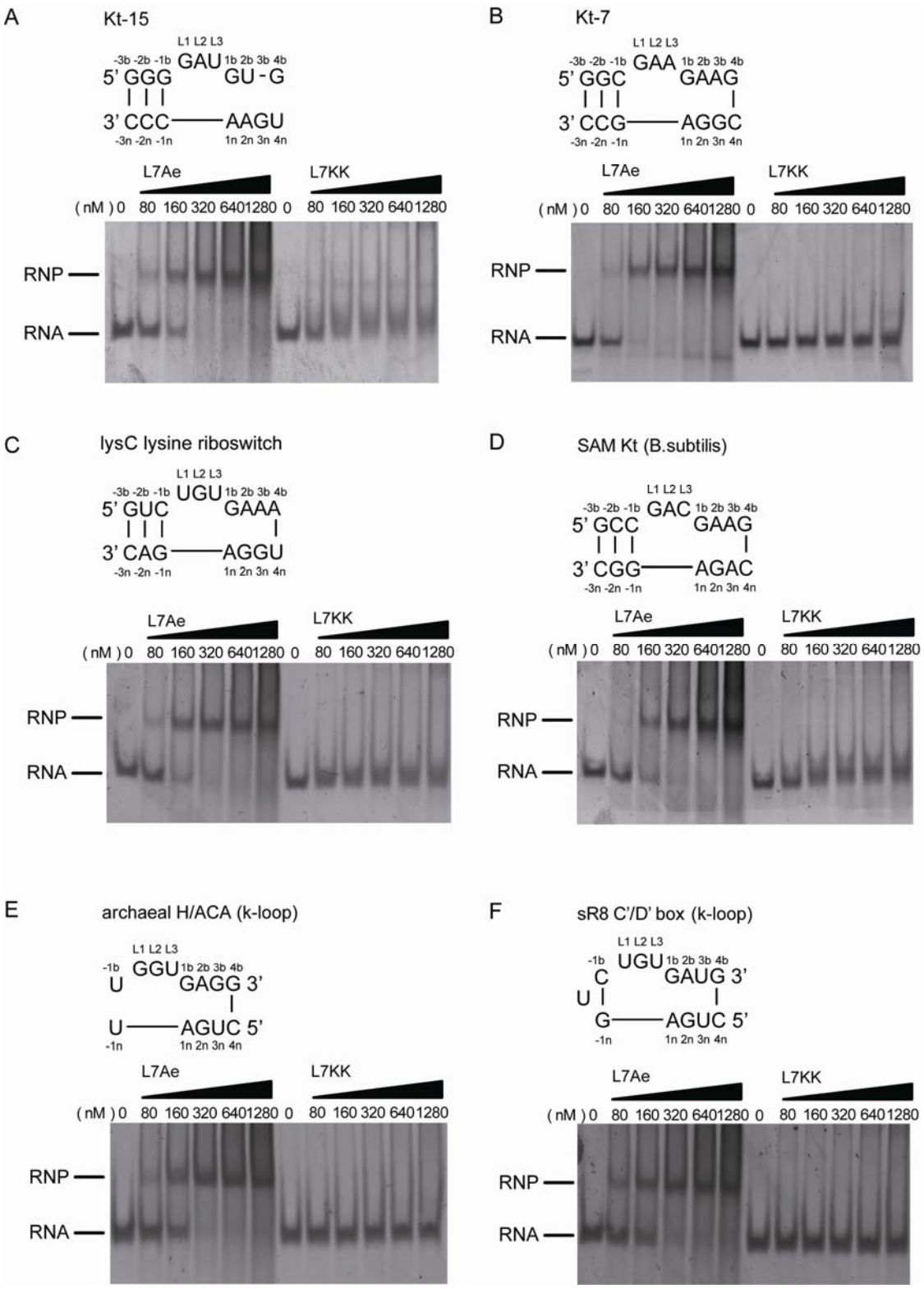

Figure S3. EMSA between natural kink-turn or K-loop RNA and L7Ae or L7KK. The sR8 C'/D' box and archaeal H/ACA are K-loops, and the other K-turn RNAs were tested for interactions with L7Ae and L7KK. A total of 40 nM of K-turn (K-loop)-containing shRNAs and various concentrations (0, 80, 160, 320, 640, and 1280 nM) of L7Ae or L7KK protein were used for the analyses. The RNA was stained with SYBR Green. The sequences and secondary structures of each K-turn and K-loop are shown at the top of each gel.

Supplementary Figure S4

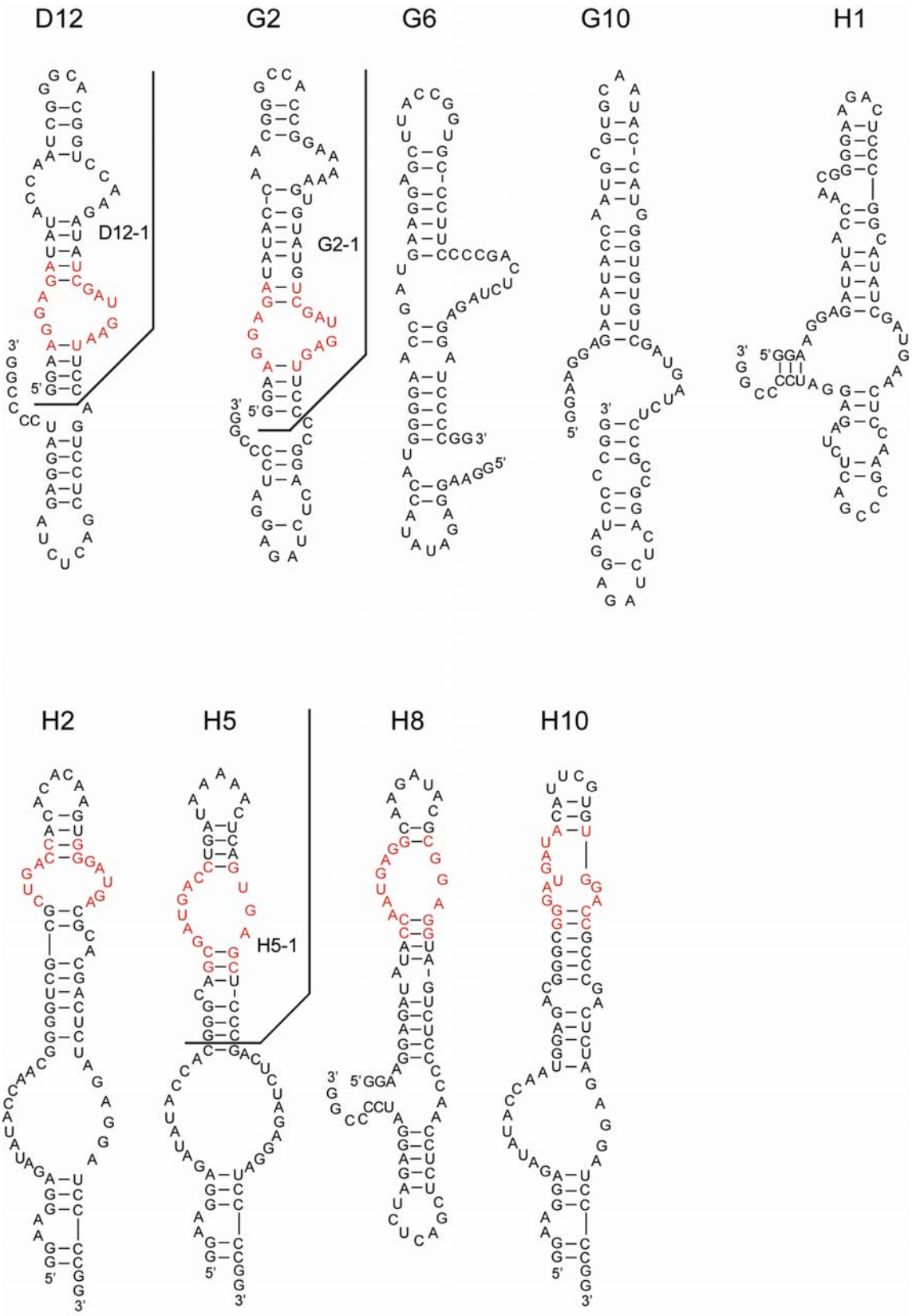

Figure S4. The secondary structures of the nine representative aptamers (D12, G2, G6, G10, H1, H2, H5, H8, and H10) predicted by RMDetect.

D12, G2, H2, H5, H8 and H10 were predicted to have K-turn structures. The predicted K-turn sequences are shown in red. The D12-1, G2-1 and H5-1 variants were derived from the regions of the D12, G2, and H5 aptamers, respectively, that are indicated by black lines.

Supplementary Figure S5

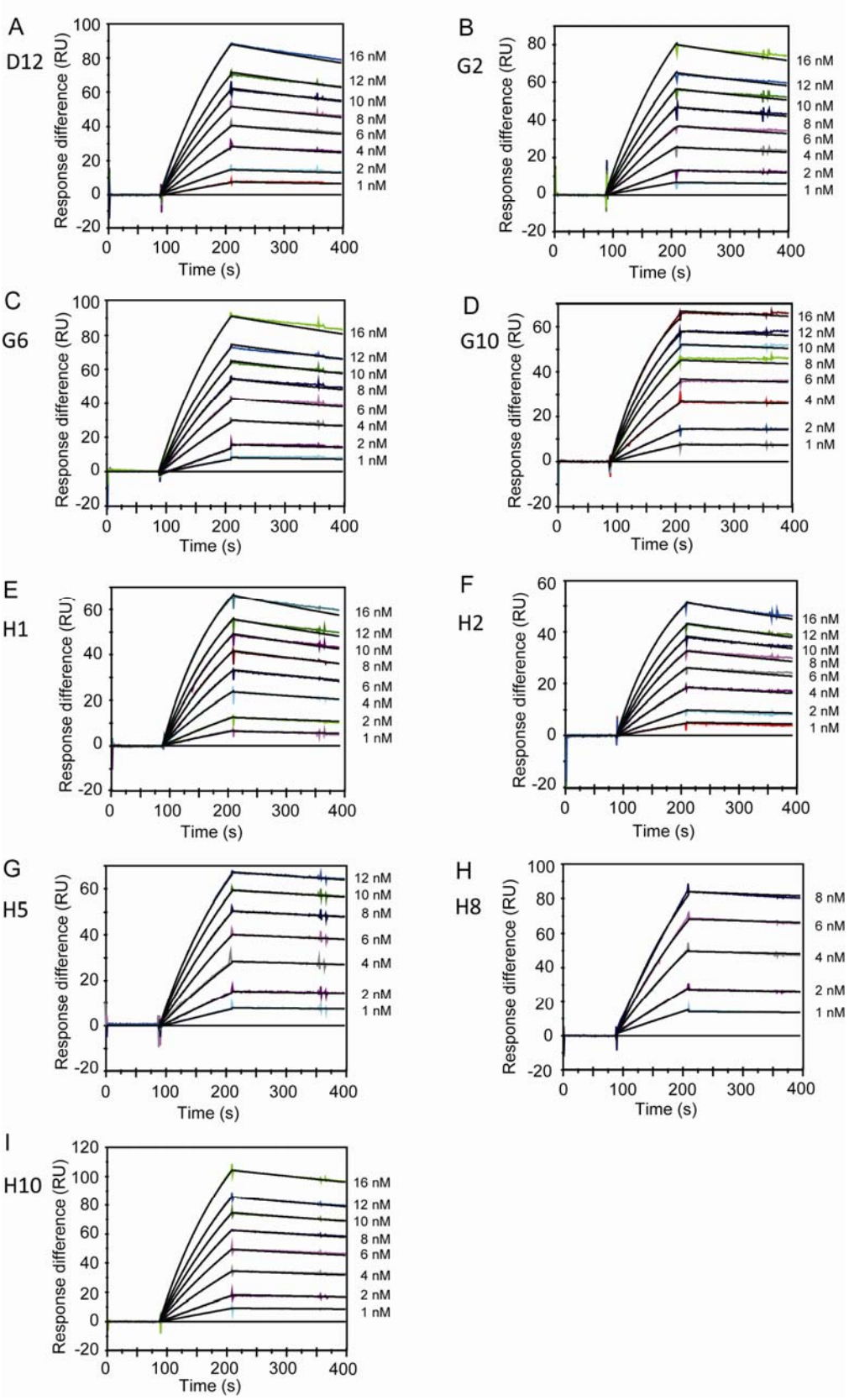

Figure S5. SPR sensorgrams of the nine representative aptamers (D12, G2, G6, G10, H1, H2, H5, H8, and H10) binding to L7KK immobilized on the CM5 sensor chip. RNAs at the indicated concentrations were injected onto the chip at 90 s. The measurement curve for 0 nM was taken as the baseline. The black curves are fitted curves calculated using BIAevaluation software.

Supplementary Figure S6

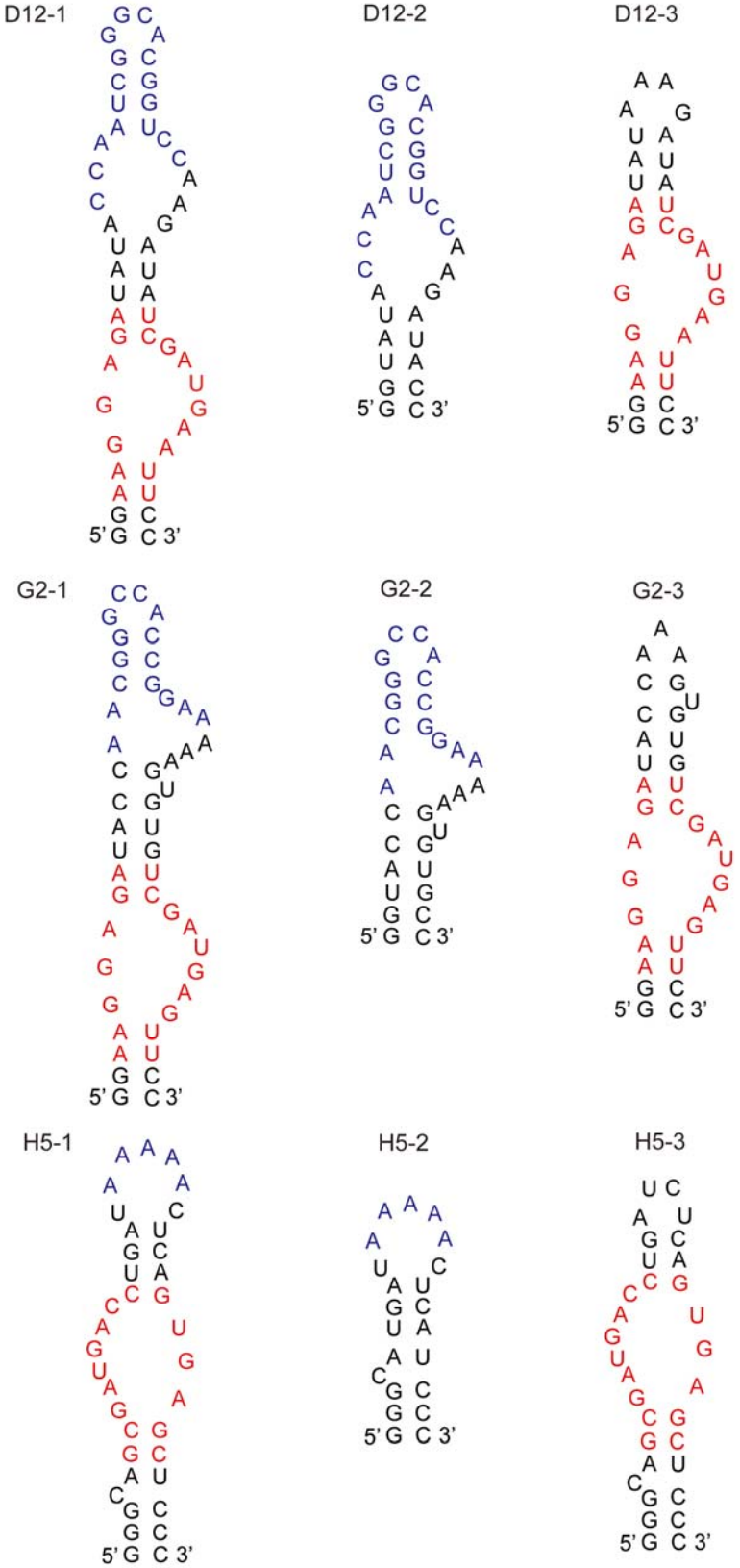

Figure S6. Secondary structures of the three aptamer (D12, G2, and H5) derivatives. The predicted K-turn sequences are shown in red, and the terminal loop sequences are shown in blue. The D12-2, G2-2, and H5-2 aptamers did not contain the K-turn motifs. We decided to choose H5-3 for further studies because the m-fold web server for RNA folding [3] predicted that the secondary structure of H5-3 is more stable (ΔG =-8.60 kcal/mol) compared with the rest (D12-3; ΔG =-5.10 kcal/mol, G2-3; ΔG =-5.50 kcal/mol).

Supplementary Figure S7

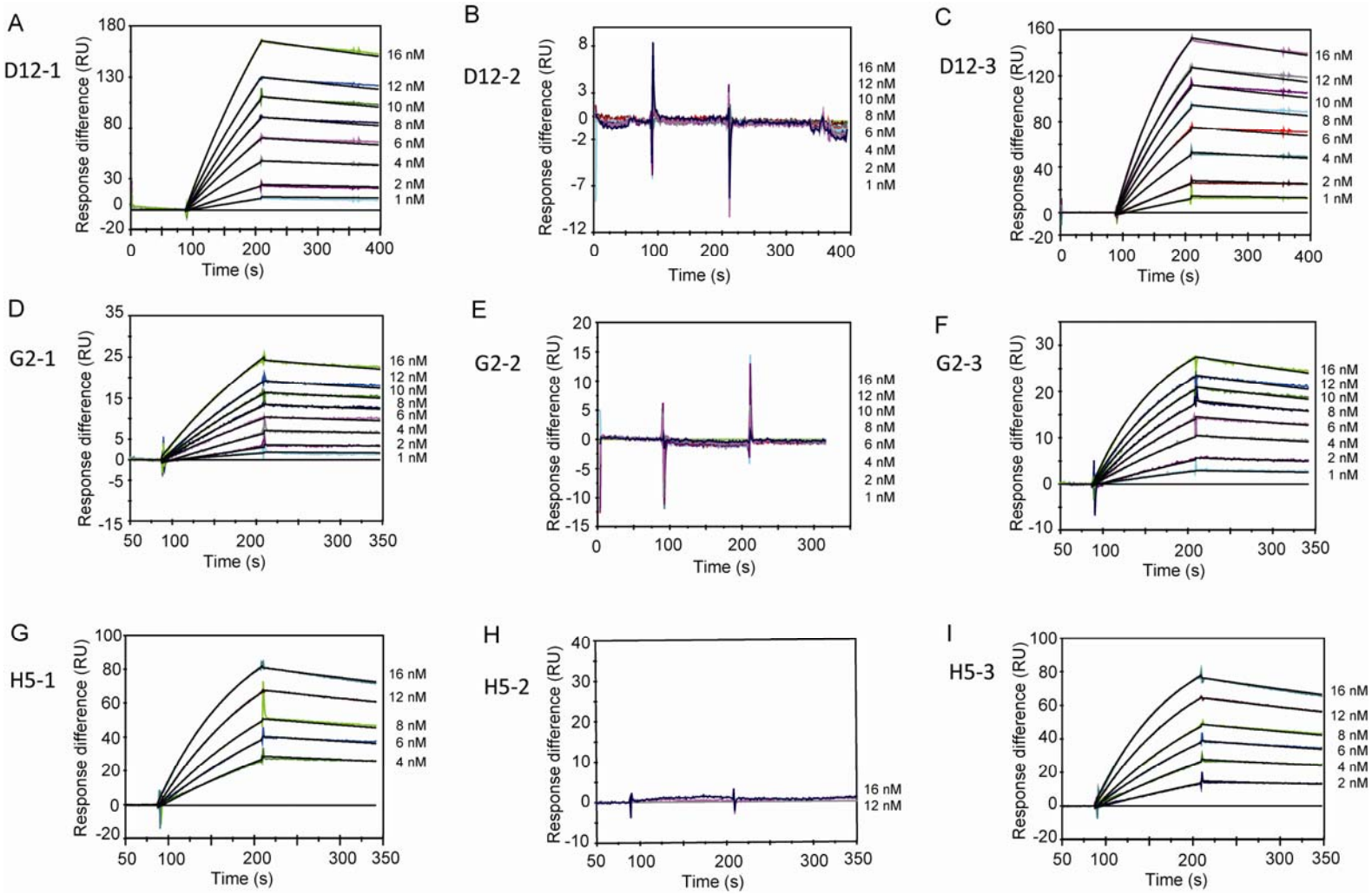

Figure S7. SPR sensorgrams of the aptamer derivatives (D12-1-3, G2-1-3, and H5-1-3) binding to L7KK immobilized on the CM5 sensor chip.

The indicated concentrations of RNAs were injected onto the chip at 90 s. The measurement curve for 0 nM was taken as the baseline. The black curves are fitted curves calculated using BIAevaluation software.

Supplementary Figure S8

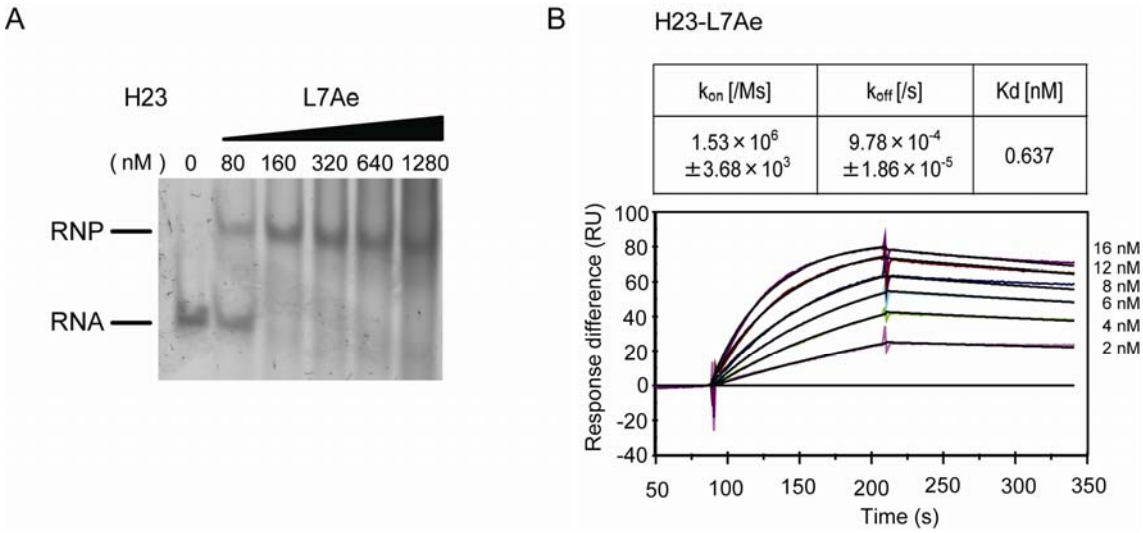

Figure S8. Analyses of L7Ae binding to the H23 aptamer. (A) EMSA with 40 nM of H23-shRNA and various concentrations (0, 80, 160, 320, 640, and 1280 nM) of L7Ae. The RNA was stained with SYBR Green. **(B)** Surface plasmon resonance (SPR) measurements. L7Ae was immobilized on the CM5 sensor chip, and various concentrations (0, 2, 4, 6, 8, 12, and 16 nM) of the H23 aptamer were used for the kinetic analyses. The measurement curve for 0 nM was taken as the baseline. The black curves are fitted curves calculated using BIAevaluation software.

Supplementary Figure S9

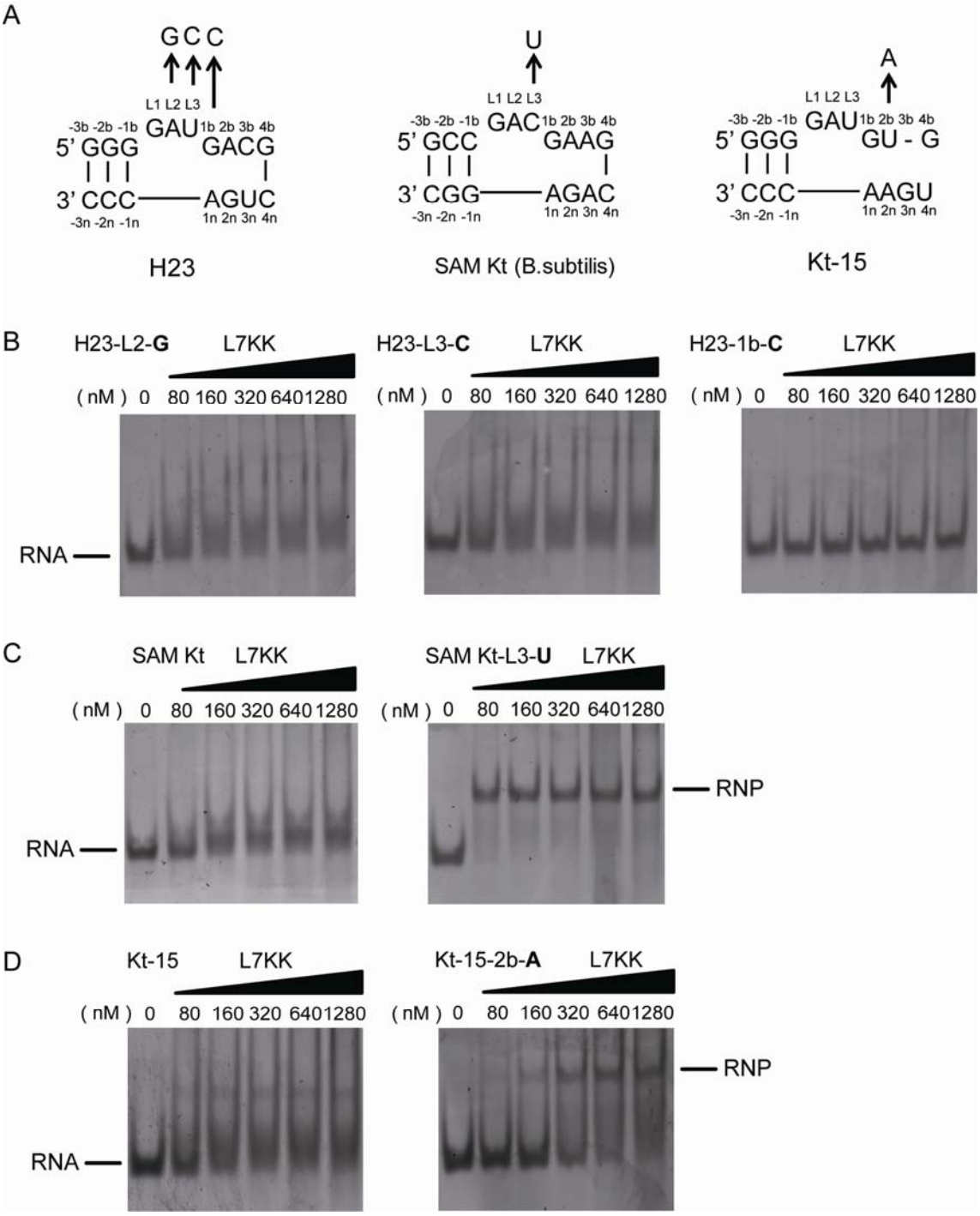

Figure S9. Mutational analysis of H23 and two K-turn sequences. (**A**) Three mutated H23 molecules, H23-L2-G, H23-L3-C and H23-1b-C, were constructed by disrupting a portion of the consensus sequence of the H23 aptamer (left). Two mutated K-turn RNA constructs. SAM Kt-L3-U (middle) and Kt-15-2bA (right), contained a portion of the consensus L2-AUGA-2b motif found in the K-turn$_{AUGA}$ sequence. (**B**) EMSA with 40 nM H23 variants and various concentrations (0, 80, 160, 320, 640, and 1280 nM) of L7KK protein. (**C, D**) EMSA with 40 nM SAM Kt (-L3-U) (**C**) or Kt-15 (-2bA) (**D**) and various concentrations (0, 80, 160, 320, 640, and 1280 nM) of L7KK protein. RNA was stained with SYBR Green. The combinations of RNA and protein are indicated above the image of the gel. The modified H23 and K-turn motifs were incorporated into the loop region of the short hairpin RNAs (shRNAs), and the generated RNAs were used in all EMSAs.

Supplementary Figure S10

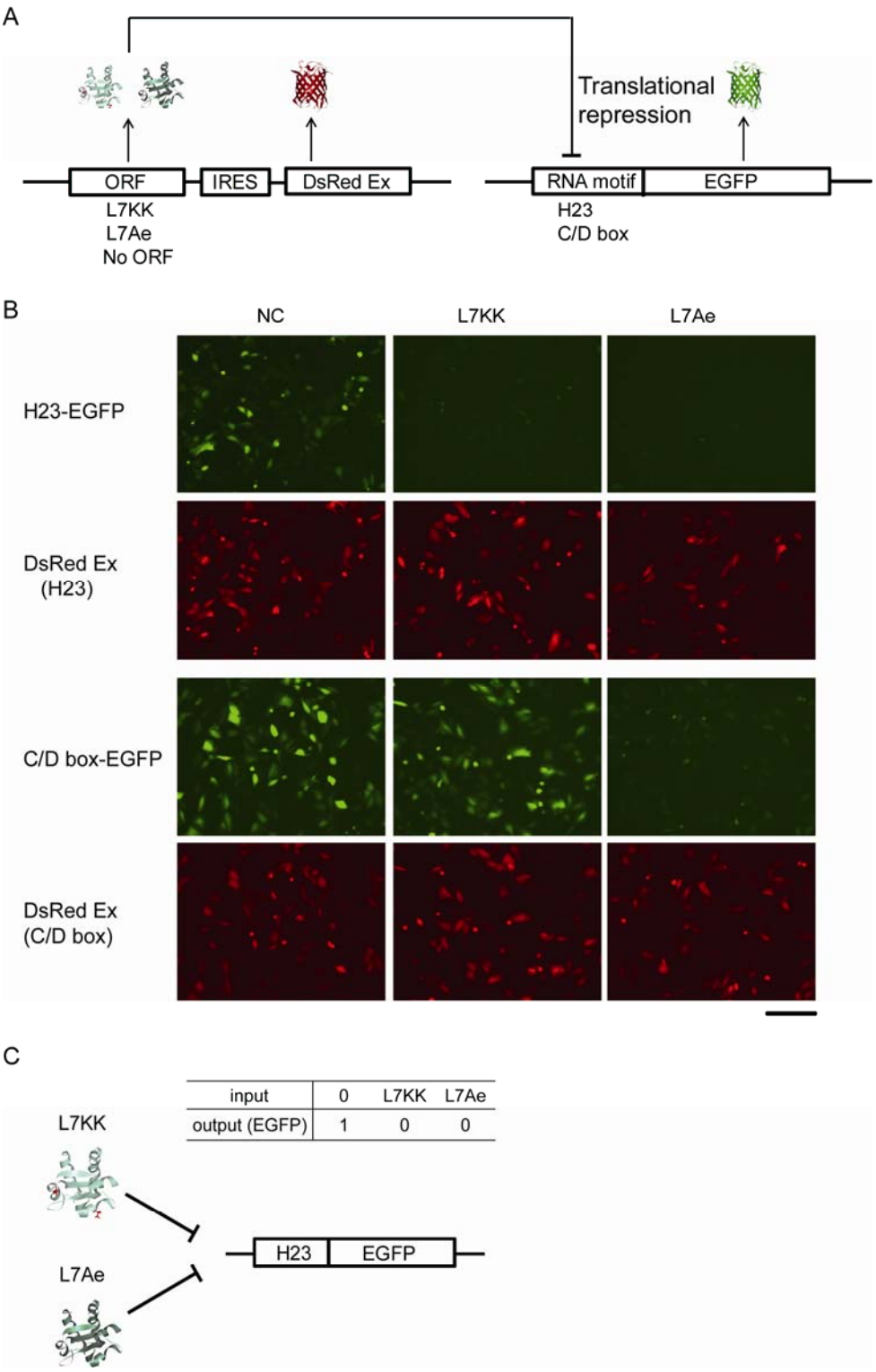

Figure S10. Translational repression in HeLa cells.

(**A**) A schematic representation of the translational repression of H23- or C/D box containing EGFP mRNAs by L7KK or L7Ae in HeLa cells. (**B**) Fluorescence microscopic images of cells. A total of 0.6 μg plasmid, H23 (C/D box)-EGFP mRNA-expressing plasmid (0.1 μg) and pNC (0.5 μg), pL7KK (0.2 μg) + pNC (0.3 μg) or pL7Ae (0.2 μg) + pNC (0.3 μg) were cotransfected in HeLa cells. The cells were observed by fluorescence microscopy at 24 hours after the transfection. A scale bar represents 200 μm. (**C**) Schematic representation of an NOR gate implemented by L7KK and L7Ae.

Supplementary Figure S11

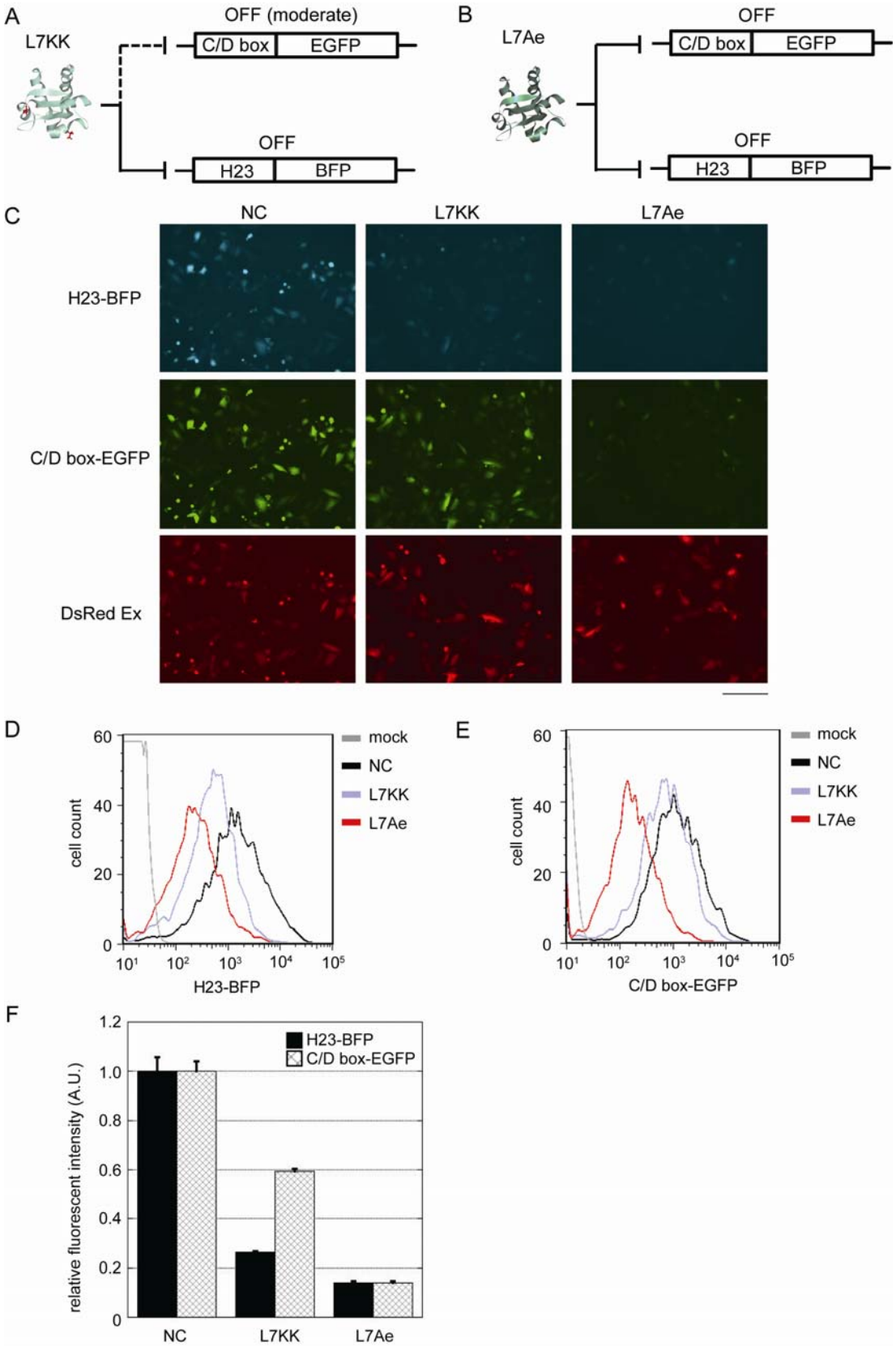

Figure S11. **Simultaneous translational regulation in HeLa cells.** (**A,B**) Schematic representation of the translational switches that enable the fine-tuning of the expression from the two mRNAs in a single tube. L7KK moderately and strongly represses the translation of the C/D box-EGFP mRNA and H23-BFP mRNA, respectively (left). L7Ae strongly represses the translation of both mRNAs (right). (**C**) Fluorescence microscopic images of cells. HeLa cells were cotransfected with three plasmids, pL7KK or pL7Ae (0.6 μg), pH23-BFP (0.2 μg) and pC/D box-EGFP (0.2 μg). The cells were observed by fluorescence microscopy at 24 hours after transfection. A scale bar represents 200 μm. (**D,E**) Histogram of EGFP and BFP fluorescence, as analyzed by flow cytometry. The DsRed-positive cells were gated and analyzed at 24 hours after transfection. (**F**) The mean relative EGFP and BFP fluorescence values of DsRed-positive cells were measured by flow cytometry.

3. Supplementary Tables

Supplementary Table 1

cycle	RNA pool (pmol)	L7KK (pmol)	total volume (μl)	% captured
1	500	50	200	0.62
2	300	30	200	1.87
3	200	20	200	5.56
4	200	20	200	5.6
5	200	20	200	7.52
6	200	20	200	6.91
7	168	20	200	11.0
8	100	10	100	8.9
9	100	10	100	14.6
10	200	40	200	-
11	200	40	200	-
12	200	20	200	-
13	200	20	200	-
14	200	20	200	-

Table S1. The conditions for the in vitro selection experiment.

A ^{32}P-labeled RNA pool was used during first nine rounds to monitor the ratio of captured to non-captured RNA, and a non-RI-labeled RNA pool was used in the tenth through fourteen rounds of selection. Before the seventh round, negative selection was conducted to eliminate bead-binding RNAs. We did not monitor the binding rate of the RNAs during the last five rounds of selection.

Supplementary Table 2

D12	AUCGGG |CACGGUCCAAGAUAUC**GAUGA**AUUCCAGUC| CUC
E1	AUGCGG |CGUAUC**AAUGA**CUUCCCUACUCCUCUGGGU| CCU
E2	AUGAUG |GCGCAUCGCCGGAGGGACCCUUUCCAUCCU| CUC
E3	CGGGCA |GCUGAGGACCCAACCCC**GAUGA**CGCUCG| CCC
E4	AUGCGA |UCGGAAGC**AAUGA**CUAAUGUGUAAGUUCU| CCU
E5	UGGGGG |UGAGCCUCCUGGC**GAUGA**CCCCCCGCUUC| CUC
E6, F3	AUGGGG |GAGGGCC**GAUGA**AUGCAAGACAGCAGGAGG| CCU
E8	AUAGGG |CUGGCAUGACGACGUAAG**GAUGA**CUCACGG| CCA
E9	AAGGGC |CGCGG**GAUGA**UGGCUAAACAUGCCGGAUC| GCG
E10	ACGGGG |AUGCC**AAUGA**CAGAAGUCACUCCUUGCCAU| CUC
E11	AGGGAG |CGAACCAAGGAUGGCGAGGCAUGCUUUCU| CCA
E12	AUGAGG |CAGCGAGCAUACCAUUACCGGUUGC| AAU
F2	AUGAGG |CUGUAUC**GAUGA**AUUCUCCGUGCCUCUAGG| CCU
F5	AAGGGG |UCGGCCGCCGGACGCAG**GAUGA**UGGCGU| CCC
F6	ACGGGG |CGACGCAAG**GAUGA**CCCCCGCCUCUAGGCA| CCU
F7	AAGGGG |**GAUGA**CAGGCCCAAGCUCUAAGGUUGUGGC| CUC
F8	AUGAGG |CAGGA**GAUGA**UGUCAAUACAACGACUGAU| CCU
F9	AACGGC |GACCC**GAUGA**CGGAGCACUACGCUUUCAG| CCA
F10	AUGGGG |AGCGAACAGC**GAUGA**UCCAAAGACCUUCCC| CUC
F11	AUGACG |UGACUCCCCGAGGUAACUCGCCUCCCUAUC| CUC
F12,G11	AUGCGG |CAACCCCGUUCGUAAUUAUC**GAUGA**UUUCCC| CGG
G2	ACGGGC |CACCGGAAAAAGUGUAUGUC**GAUGA**GUUCC| CCG
G3,G12	AUGGAG |CUGGCCGACGUGGUACUUCCUAG**GAUGA**CG| CCA
G4	UGGGGG |UACGGGGCGAGCAGAUUUGCGAUGUCCGU| CUC
G5	AUAGGG |CAUC**AAUGA**CGUGGAAAAUUUACACUGAC| CUC
G6	UGGGGA |ACC**GAUGA**AGGAGCUUACCGGUGCCCUUC| CCC
G7	AUCGGG |CGAGCAACAGC**GAUGA**ACCCGCCCUCCUUCC| CCG
G8	AUGGGG |AGCGAAGAGCGAUGAUCCAAAGACCUUCCC| CUC
G9	ACGGGG |ACCC**AAUGA**CAUGAGCAAAAUCCCUACCUU| CCC
G10	AUGCGU |GCAAUACCAUGGGUGUGUC**GAUGA**UCUCCG| CCG
H1	ACGGGG |AAGACUCCCGGCAUAUC**GAUGA**ACUCCAAG| CCC
H2,H7	ACGGGG |UCGCGCUGACCACACACAAGUGG**GAUGA**CG| CAC
H3	AUGAGG |CGGACCAACUCACGCGGAGGUGACCCUCCC| CCG
H4	AUGGAG |ACGGAAG**GAUGA**ACCUUGGAUAUCUUCUCC| CCC
H5	CGGGCA |GC**GAUGA**CCUGAUAAAAACUCAGUGAGCU| CCC
H6	AUGAGG |CACCAGACCGAGUUAUAUUUUCCGCCGGCA| CUC
H8	AUGAGG |CAAGAUACGCGGAGGUAGUCUCCCCAACCU| CUC
H10	AUGGAG |CAGGGCGG**GAUGA**UACAUUCGUGUGGACCG| CCC

Table S2. Selected RNA sequences.

The bold and underlined letters indicate the conserved G/A-AUGA sequences of the selected aptamers. The region of random sequences are flanked with a " | " symbol. The entire sequence of each aptamer is 5'-GGAAGGAGAUAUACCA-(shown sequence)-GACUCUAGAGGAUCCCCGG-3'.

Supplementary Table 3

RNA sample ID	k_{on} [/Ms]	k_{off} [/s]	Kd [nM]
D12	$4.15\times10^{5}\pm1.99\times10^{3}$	$6.42\times10^{-4}\pm1.50\times10^{-5}$	1.55
G2	$3.71\times10^{5}\pm9.43\times10^{2}$	$5.86\times10^{-4}\pm7.24\times10^{-6}$	1.58
G6	$3.81\times10^{5}\pm1.38\times10^{3}$	$7.01\times10^{-4}\pm1.06\times10^{-5}$	1.84
G10	$7.58\times10^{5}\pm2.10\times10^{3}$	$1.83\times10^{-4}\pm1.40\times10^{-5}$	0.241
H1	$5.53\times10^{5}\pm1.57\times10^{3}$	$8.00\times10^{-4}\pm1.12\times10^{-5}$	1.45
H2	$5.55\times10^{5}\pm2.06\times10^{3}$	$7.19\times10^{-4}\pm1.47\times10^{-5}$	1.30
H5	$5.43\times10^{5}\pm1.28\times10^{3}$	$2.81\times10^{-4}\pm6.92\times10^{-6}$	0.518
H8	$7.58\times10^{5}\pm4.35\times10^{3}$	$1.57\times10^{-4}\pm1.66\times10^{-5}$	0.207
H10	$4.22\times10^{5}\pm7.80\times10^{2}$	$4.34\times10^{-4}\pm5.90\times10^{-6}$	1.03

Table S3. Surface plasmon resonance measurements of the binding constants between L7KK and the nine representative aptamers D12, G2, G6, G10, H1, H2, H5, H8, and H10. The parameters were calculated using BIAevaluation software and were based on the curves shown in **Supplementary Figure S5**.

Supplementary Table 4

RNA sample ID	k_{on} [/Ms]	k_{off} [/s]	Kd [nM]
D12-1	$2.07 \times 10^5 \pm 1.45 \times 10^3$	$5.15 \times 10^{-4} \pm 1.18 \times 10^{-5}$	2.49
D12-3	$5.00 \times 10^5 \pm 2.11 \times 10^3$	$5.61 \times 10^{-4} \pm 1.55 \times 10^{-5}$	1.12
G2-1	$2.40 \times 10^5 \pm 2.45 \times 10^3$	$6.77 \times 10^{-4} \pm 1.97 \times 10^{-5}$	2.82
G2-3	$6.78 \times 10^5 \pm 2.28 \times 10^3$	$1.03 \times 10^{-3} \pm 1.55 \times 10^{-5}$	1.51
H5-1	$5.46 \times 10^5 \pm 2.19 \times 10^3$	$8.31 \times 10^{-4} \pm 1.62 \times 10^{-5}$	1.52
H5-3	$5.95 \times 10^5 \pm 1.67 \times 10^3$	$1.01 \times 10^{-3} \pm 1.27 \times 10^{-5}$	1.69

Table S4. Surface plasmon resonance measurements of the binding constants between L7KK and the aptamer derivatives D12-1/3, G2-1/3, and H5-1/3. The parameters were calculated using BIAevaluation software and were based on the curves shown in **Supplementary Figure S7**. Parameter sets were not determined for D12-2, G2-2 and H5-2 because these sequences did not interact with L7KK.

4. References

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