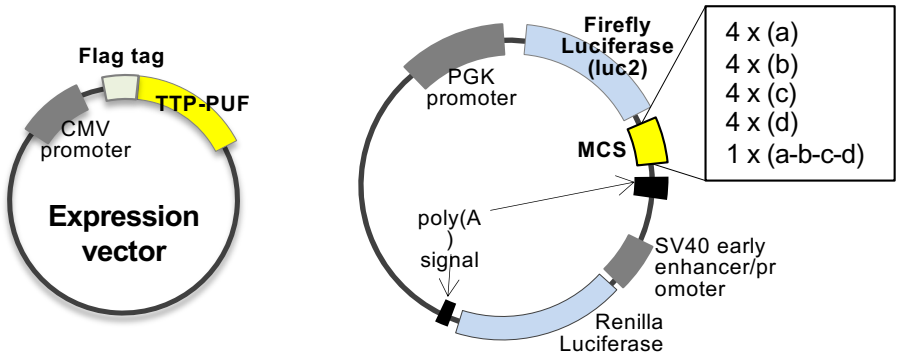
Effective RNA Regulation by Combination of Multiple Programmable RNA-Binding Proteins

Supplementary Materials

Figure S1
Figure S2
Figure S3

(a)



Reporter plasmid (pmirGLO)

(b)

4 x (a)	CTAGCCTGAGGGGAAAATTAAAACTGAGGGGTTAAGAAATACTGAGGGGTTAACAAATACTGAGGGGAATTTTAAAAG
4 x (b)	CTAGCTATCGAGAGAACAAGAACTATCGAGAGAACAAGAACTATCGAGAGAACAAGAACTATCGAGAGAACAAGAA
4 x (c)	GAATGGGGCTGACTAGAAGAAATGGGGCTGACTAGAAGAAATGGGGCTGAGTAGAAGAAATGGGGCTGAAAG
4 x (d)	CTAGCTTTCGAACCCCAGCAAACTTTCGAATAACCCGATTCCTTTCGAATTATAGCATTCCTTTCGAAG
1 x (a-b-c-d)	CTAGACTCCTGAGGGGATCCACAGCTATCGAGACACAAAGAATGGGGCTGCACCTACTACAGTGA GACACCTACTACTTTCGAACG

(c)

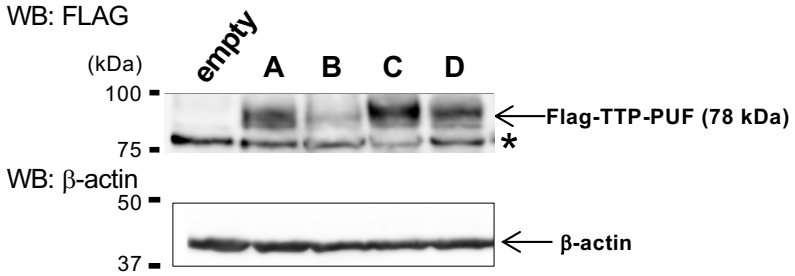


Fig S1. Plasmids used for luciferase reporter assays and expression of TTP-PUFs. (a) Plasmids used for luciferase reporter assays. (b) The DNA fragments corresponding to each PUF binding sequences (shown in color) were inserted into MCS of reporter plasmids. After transfection, the firefly luciferase gene is transcribed and PUF binding sequences should be at 3'UTR of the firefly luciferase mRNA. (c) Expression of each TTP-PUFs was confirmed by western blotting assay. From left to right: empty vector, TTP-PUF A, TTP-PUF B, TTP-PUF C and TTP-PUF D. * indicates nonspecific bands.

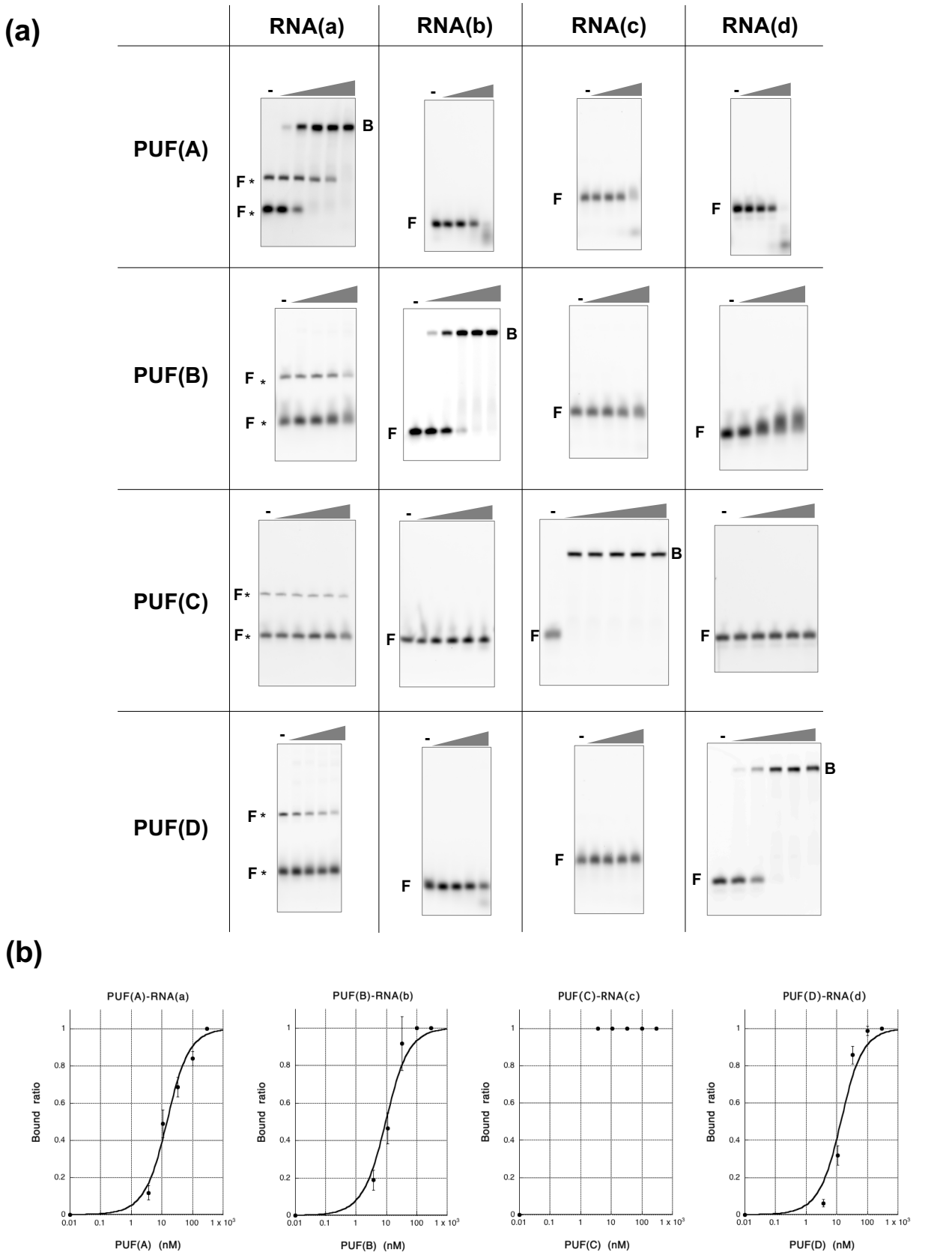


Fig S2. Representative results of EMSA. (a) Three-fold serially diluted concentrations of PUF(A)~(D) (0-300 nM) were incubated with RNA(a)~(d) (10 nM) at 25˚C for 30 min and the mixtures were gel-electrophoresed. "F" and "B" indicate protein-free RNA and protein-bound RNA, respectively. RNA(a) showed two protein-free bands (shown by *). (b) The fractions of PUF-bound RNA were plotted against the PUF concentrations and fit to the 1:1 binding equation curve; Fraction bound = $\{([P]+[R]+K_D)- (([P]+[R]+K_D)^2-4[P][R])^{0.5}\}/2[R]$, where [P]=protein concentrations and [R]=RNA concentrations (10 nM). The calculated K_D values were shown in Table 1.

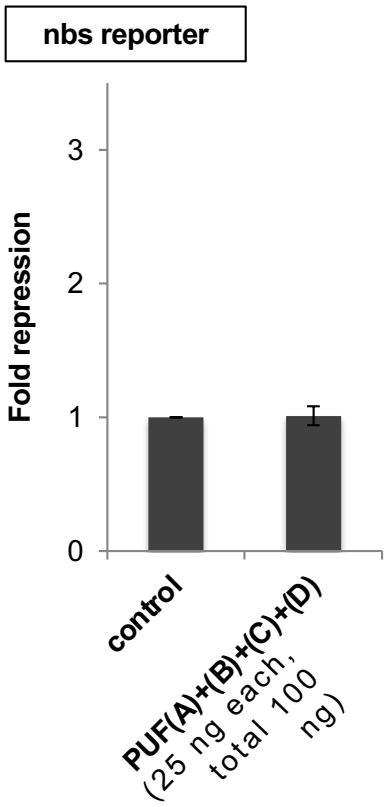


Fig S3. Effect of the mixture of TTP-PUFs on the nbs reporter. The mixture of TTP-PUF(A)~(D) did not repress the luciferase activity of non-binding-site vector.