Supporting information

RNA Aptamer-Mediated Gene Activation Systems for Inducible Transgene Expression in Animal Cells

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Table S1. Sequences of trigger RNAs

Trigger RNA	Sequence (5' → 3')
MPx1	GGUGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCG
MPx2	GGUCCACCAGAGUAGAGCAUCAGCCUACUCCGGACAAGCAGCAGAGGAUAUGGCCUCGCUGCGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCG
MPx4	GGUUCUGCCAUGAGGAAUGACCAUCAGGCAUUCCGAUCCGAGGAGCAGACGAUAUGGCGUCGCUCCGGCUGCAGAAGUGACGAUCACGCACUUCGGAGUGACCAGCAGAGCAUAUGGGCUCGCUGGGCCACCAGAGUAGAGCAUCAGCCUACUCCGGACAAGCAGCAGAGGAUAUGGCCUCGCUGCGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCG
MPx1S	GGUGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCGUCACACAAACUACUCUUU
MPx2S	GGUCCACCAGAGUAGAGCAUCAGCCUACUCCGGACAAGCAGCAGAGGAUAUGGCCUCGCUGCGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCGUCACACAAACUACUCAAAUGUCCGAAAGGUGGA
MPx4S-α	GGUUCUGCCAUGAGGAAUGACCAUCAGGCAUUCCGAUCCGAGGAGCAGACGAUAUGGCGUCGCUCCGGCUGCAGAAGUGACGAUCACGCACUUCGGAGUGACCAGCAGAGCAUAUGGGCUCGCUGGGCCACCAGAGUAGAGCAUCAGCCUACUCCGGACAAGCAGCAGAGGAUAUGGCCUCGCUGCGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCGUCACACAAACUACUCAAAUGUCCGAAAGGUGGCAAACACUCCAAAGCAGCCAAACGGAUCAAACAUGGCAGCGGUGCUU
MPx4S-β	GGUUCUGCCAUGAGGAAUGACCAUCAGGCAUUCCGAUCCGAGAAGUGACGAUCACGCACUUCGGCUGCAGGAGCAGACGAUAUGGCGUCGCUCCGGAGUGACCAGCAGAGCAUAUGGGCUCGCUGGGCCACCAGAGUAGAGCAUCAGCCUACUCCGGACAAGCGAGGAGCAUCAGCCCUCGCGAGUAGAGCAGCAGAGGAUAUGGCCUCGCUGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCGUCACACAAACUACUCAAAUGUCCGAAAGGUGGCAAACACUCCAAAGCAGCCAAACGGAUCAAACAUGGCAGCGGUGCUU
MPx4S-γ	GGUUCUGCCAUGAGGAAUGACCAUCAGGCAUUCCGAUCCGAGAAGUGACGAUCACGCACUUCGGCUGCAGAGUAGAGCAUCAGCCUACUCGGAGUGAGCGAGGAGCAUCAGCCCUCGCGCCACCAGGAGCAGACGAUAUGGCGUCGCUCCCGGACAACCAGCAGAGCAUAUGGGCUCGCUGGGAGUAGAGCAGCAGAGGAUAUGGCCUCGCUGCGUGUGACGAGCAGACCAUAUGGGGUCGCUCGUCACACAAACUACUCAAAUGUCCGAAAGGUGGCAAACACUCCAAAGCAGCCAAACGGAUCAAACAUGGCAGCGGUGCUUU
MCx4S	GGUUCUGCCAUGAGGAAUGACCAUCAGGCAUUCCGAUCCGACUGAAUGCCUGCGAGCAUCGGCUGCAGAAGUGACGAUCACGCACUUCGGAGUGACUGAAUGCCUGCGAGCAUCGCCACCAGAGUAGAGCAUCAGCCUACUCCGGACAACUGAAUGCCUGCGAGCAUCGAGUAGAGCGAGGAGCAUCAGCCCUCGCGUGUGACUGAAUGCCUGCGAGCAUCUCACACAAACUACUCAAAUGUCCGAAAGGUGGCAAACACUCCAAAGCAGCCAAACGGAUCAAACAUGGCAGCGGUGCUUU

BPx4S	GGUUCUGCCAUGAGGGCCCUGAAGAAGGGCCCGAUCCGAGGAGCAGACGAU AUGGCGUCGCUCCGGCUGCACGGCCCUGAAGAAGGGCCGGGAGUGACCAGC AGAGCAUAUGGGCUCGCUGGGCCACCACCGCCCUGAAGAAGGGCGGCGGAC AAGCAGCAGAGGAUAUGGCCUCGCUGCGAGUAGAGGGCCCUGAAGAAGGGC CCGUGUGACGAGCAGACCAUAUGGGGUCGCUCGUCACACAAACUACUCAAA UGUCCGAAAGGUGGCAAACACUCCAAAGCAGCCAAACGGAUCAAACAUGGC AGCGGUGCUUU

Table S2. Oligonucleotide sequences for real-time PCR

		Sequence (5' → 3')
GFP	FW primer	CGAGGACAGCGTGATCTTC
	RV primer	CCACGGTGGCGTTGCT
	Taqman probe	CCGACAAGATCATCC
RFP	FW primer	TCAAGGAGGCCGACAAAGAG
	RV primer	GTACTTGGCCACAGCCATCTC
	Taqman probe	CCTACGTCGAGCAGCA

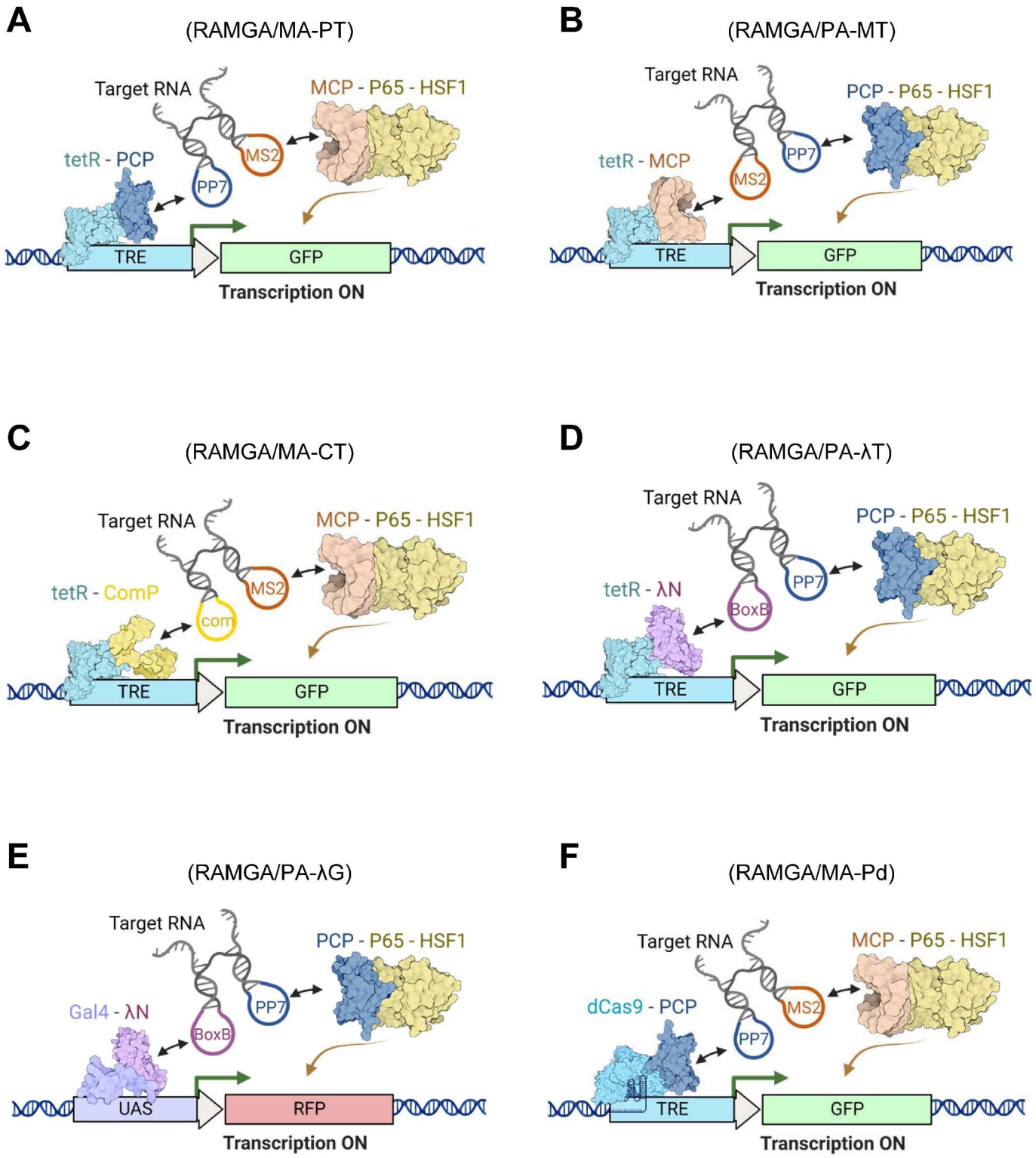

Figure S1. Schematic illustrations of transgene expression based on the RNA Aptamer-Mediated Gene Activation (RAMGA) system. (A) The RAMGA/MA-PT system consists of tetR-PCP and MCP-P65-HSF1, which can recognize and respond to RNA containing MS2-PP7 aptamers. (B) The RAMGA/PA-MT system consists of MCP-tetR and PCP-P65-HSF1, which can recognize and respond to RNA containing MS2-PP7 aptamers. (C) The RAMGA/MA-CT system consists of MCP-P65-HSF1 and COM-tetR, which can recognize and respond to RNA containing MS2-com aptamers. (D) The RAMGA/PA-λT system consists of PCP-P65-HSF1 and λN-tetR, which can recognize and respond to RNA containing PP7-BoxB aptamers. (E) The RAMGA/PA-λG system consists of PCP-P65-HSF1 and λN-Gal4, which can recognize and respond to RNA containing PP7-BoxB aptamers. (F) The RAMGA/MA-Pd system consists of MCP-P65-HSF1 and PCP-dCas9, which can recognize and respond to RNA containing MS2-PP7 aptamers.

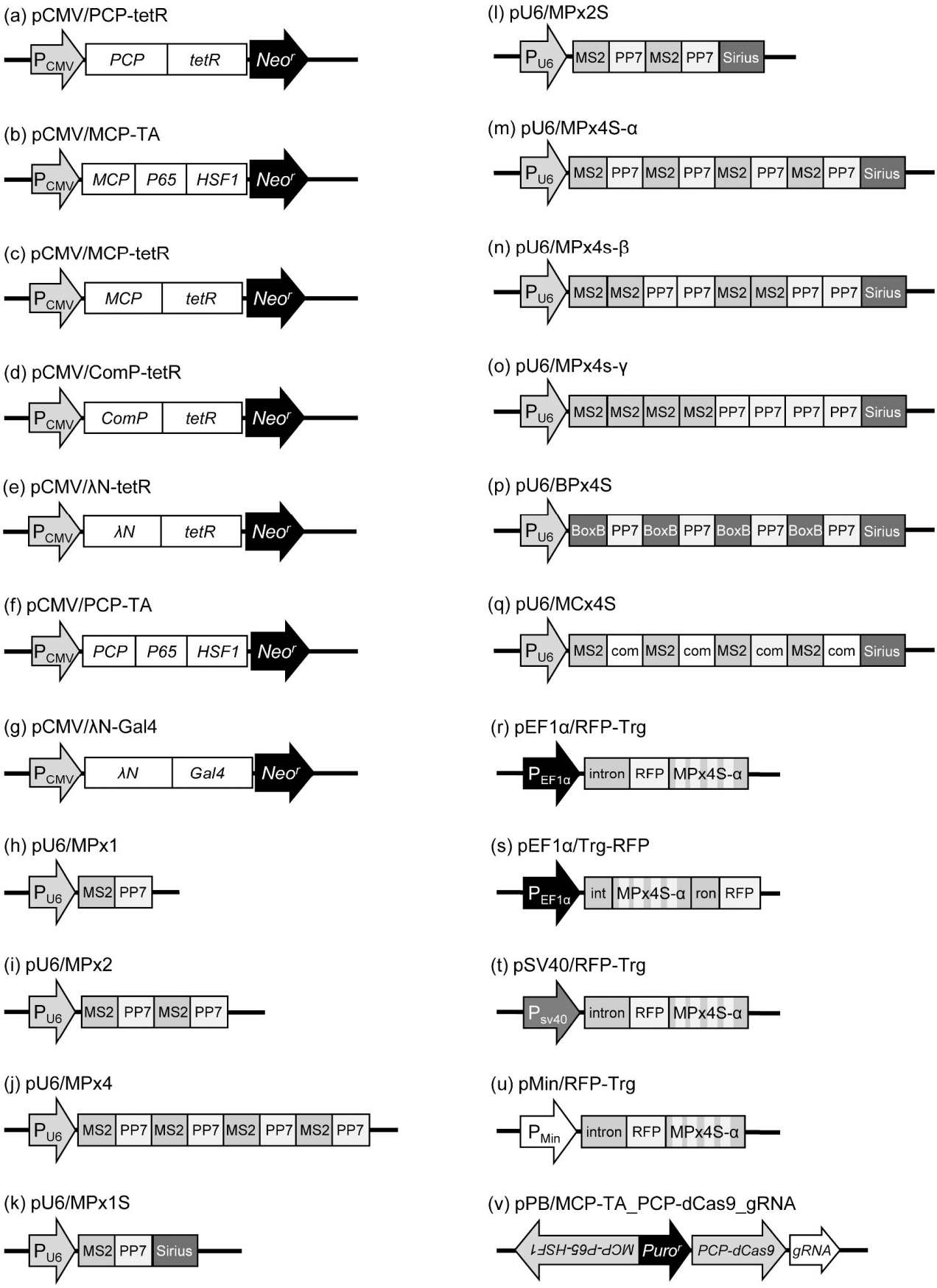

Figure S2. Schematic diagrams of plasmid structures. (a–g) Fusion protein expression vectors. (h–q) Trigger RNA expression vectors with a U6 promoter. (r–u) mRNA expression vectors with promoters EF1α, SV40, and minimal promoter. (v) All-in-one vector for expressing MCP-P65-HSF1, PCP-dCas9, and gRNA.

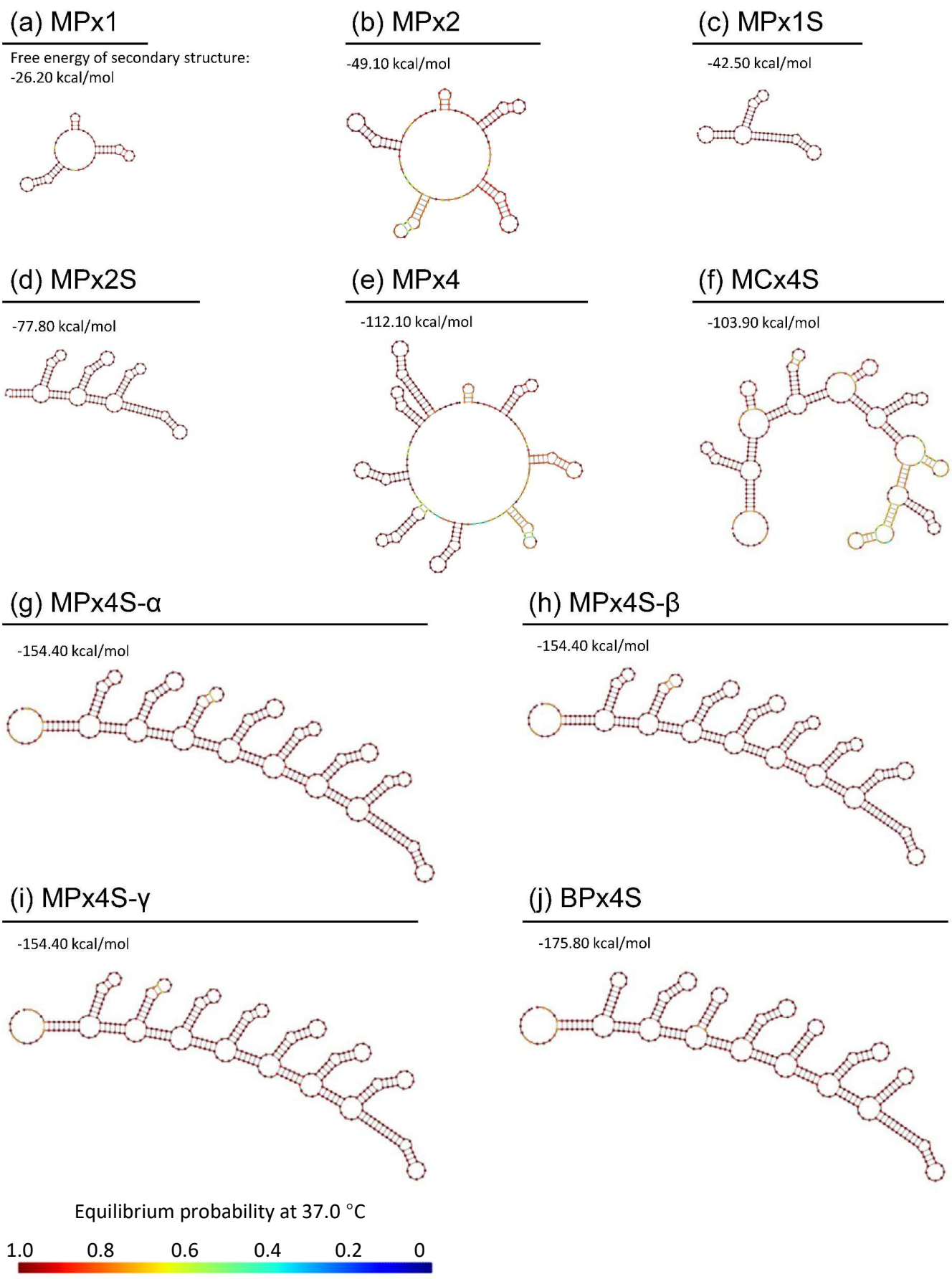

Figure S3. Predicted secondary structures of trigger RNAs along with their free energy calculations. The predictions were performed using NUPACK software.

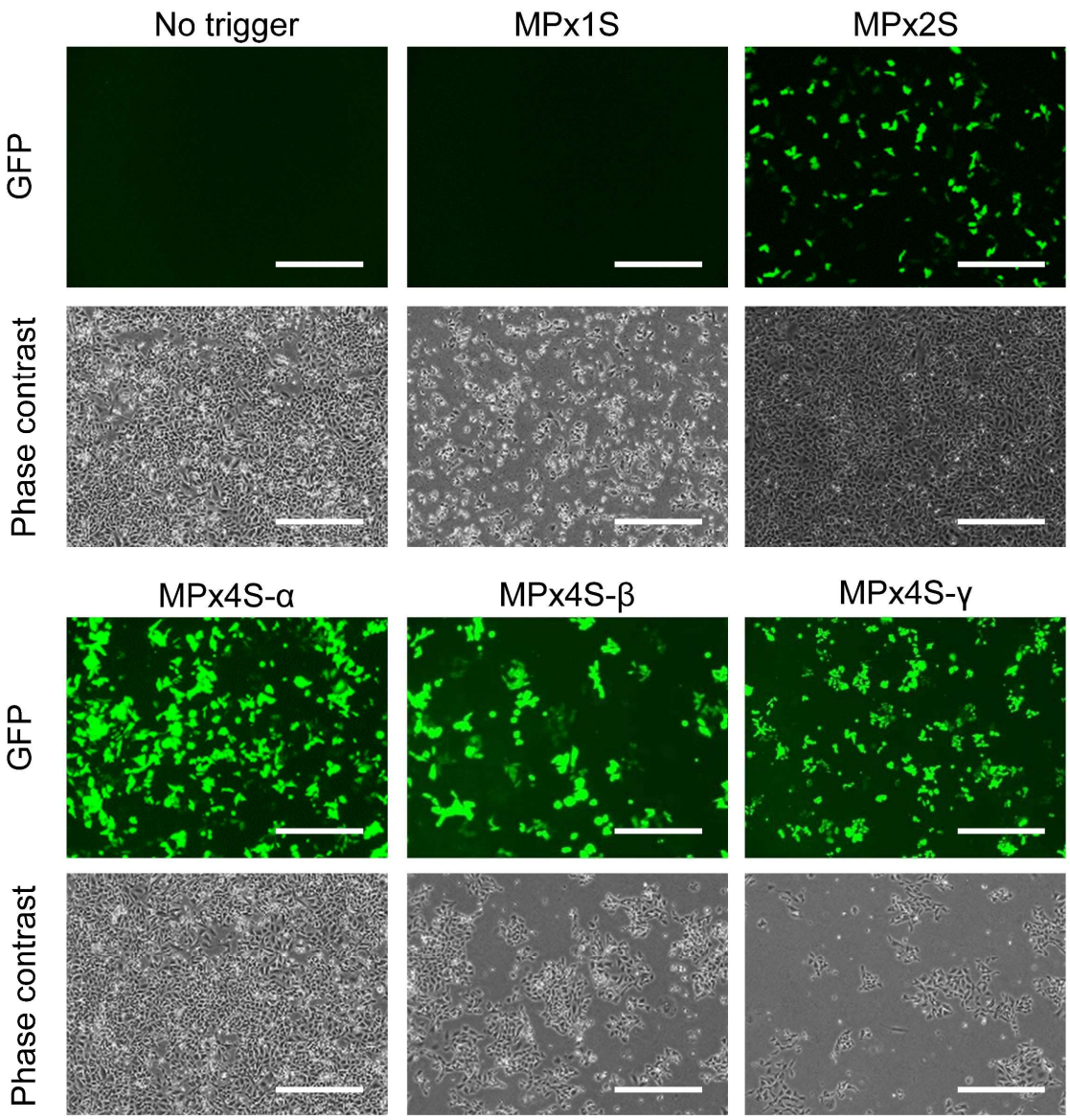

Figure S4. Phase-contrast and fluorescence images of CHO cells transfected with the RAMGA/MA-PT system, either with or without the presence of MS2-PP7 trigger RNA variants, which include MPx1S, MPx2S, MPx4S-α, MPx4S-β, and MPx4S-γ. GFP, Green Fluorescent Protein. Scale bars = 500 μm.

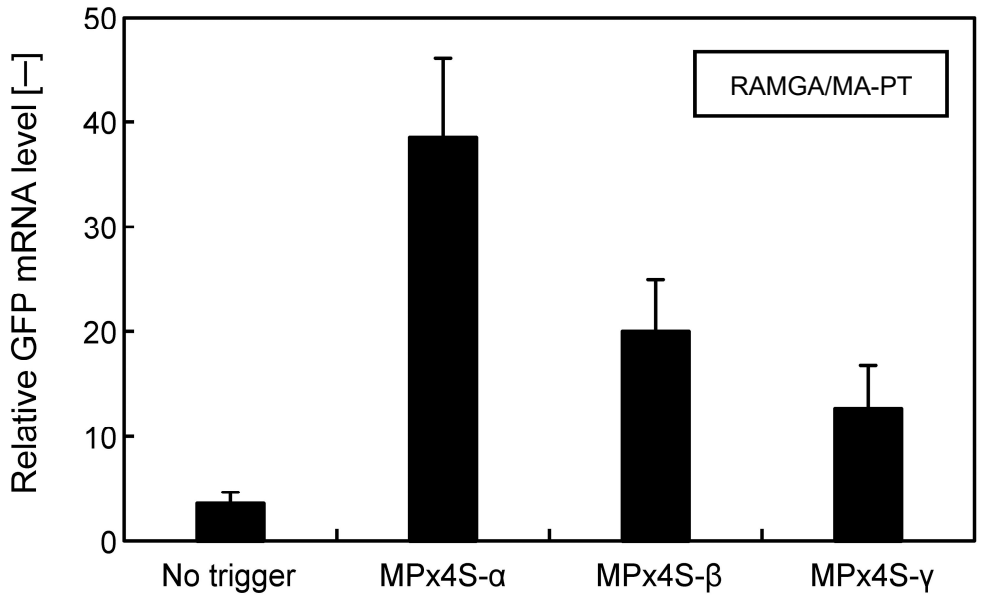

Figure S5. Relative GFP mRNA levels in CHO cells transfected with the RAMGA/MA-PT system in the presence or absence of MS2-PP7 trigger RNA variants containing MPx4S-α, MPx4S-β, and MPx4S-γ. Data represent the mean ± SD.

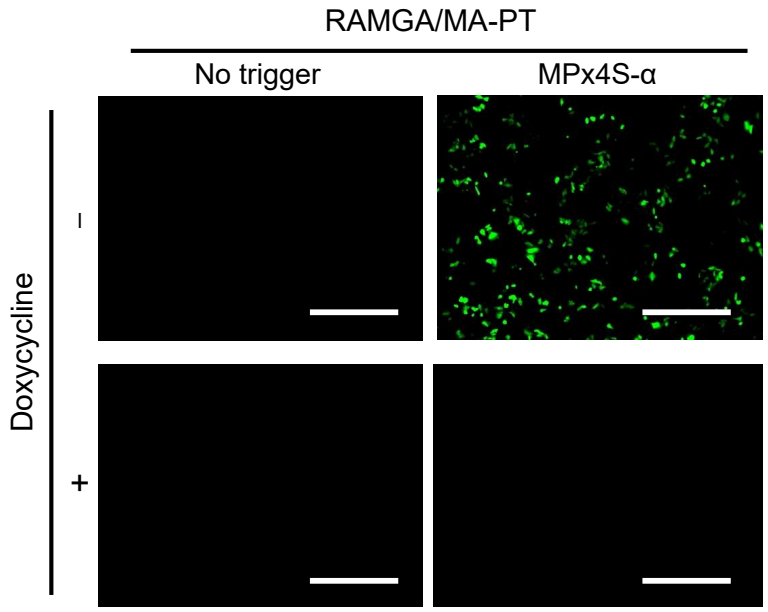

Figure S6. Phase-contrast and fluorescence images of CHO cells for post-transfection using RAMGA/MA-PT system with or without MPx4S-α as a trigger, in the absence (–) or presence (+) of doxycycline. Scale bars = 500 μm.

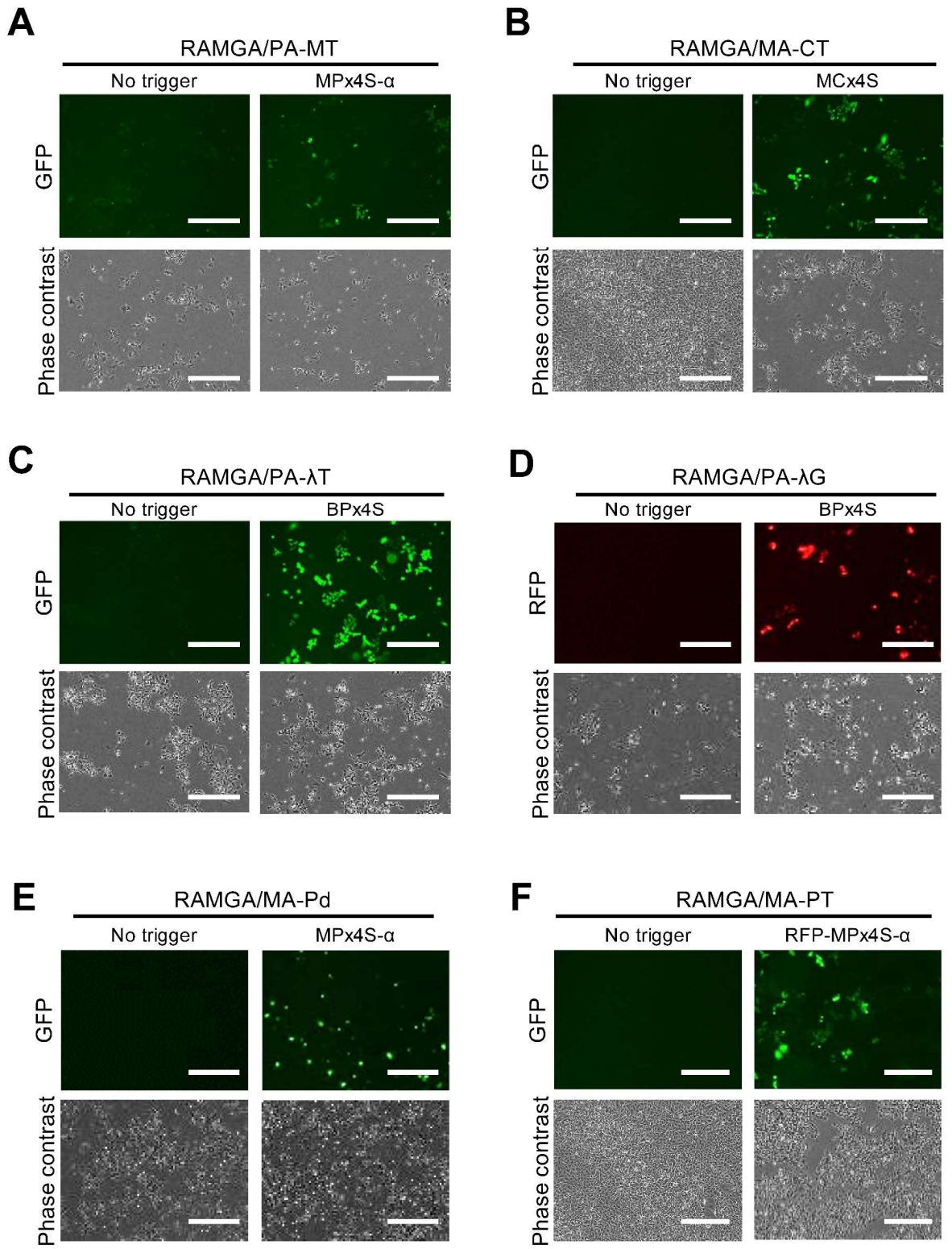

Figure S7. Phase-contrast and fluorescence images of CHO cells for post-transfection using various RAMGA systems. (A) Response of the RAMGA/PA-MT system to MPx4S-*α*. (B) Response of the RAMGA/MA-CT system to MCx4S. (C) Response of the RAMGA/PA-*λ*T system to BPx4S. (D) Response of the RAMGA/PA-*λ*G system to BPx4S. (E) Response of the RAMGA/MA-Pd system to MPx4S-*α*. (F) Response of the RAMGA/MA-PT system to RFP mRNA containing MPx4S-*α*. Scale bars = 500 μm.