Supporting information

Engineering light-up aptamers for the detection of RNA hairpins through kissing interaction.

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Table S1: Sequences of the parent MG aptamer, pre-miR let7b, K4 and malaswitch (Msw) variants.

Table S2: Sequences of pre-miR 206, K206 and MDMD malaswitch variants.

Figure S1: Predicted secondary structures (Mfold) of full-length pre-miRNAs.

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Figure S3: Specificity of Msw6.1 and MDMD1 malaswitches.

Figure S4: Test of sensing properties of chemically modified MDMD1.2

Candidates	Sequences (5' to 3')
MG aptamer:	GGAUCCCGACUGGC GAGA GCCAGGUAACGAAUGGAUCC
pre-miR let7b	CGGGGUGAGGUAGUAGGUUGUGUGGUUUCAGGGCA GUGAUGU UGCCCCUCGGAAGAUAACUAUACAACCUACUGCCUUCCCUG
K4	GCA GUGAUGU UGC
Msw1	GGAUCCCGA CU ACAUCAC AG GUAACGAAUGGAUCC
Msw2	GGAUCCCGA CU CAUCAC AG GUAACGAAUGGAUCC
Msw3	GGAUCCCGA CU ACAUCA AG GUAACGAAUGGAUCC
Msw4	GGAUCCCGA CU UCAUCA AG GUAACGAAUGGAUCC
Msw5	GGAUCCCGA CU ACAUCU AG GUAACGAAUGGAUCC
Msw6	GGAUCCCGA CU ACAUCAG AG GUAACGAAUGGAUCC
Msw1.1	GGAUCCCGA CU GACAUCAC AG GUAACGAAUGGAUCC
Msw1.2	GGAUCCCGA CU ACAUCAC CG GUAACGAAUGGAUCC
Msw1.3	GGAUCCCGA UU ACAUCAC AA GUAACGAAUGGAUCC
Msw1.4	GGAUCCCGA AU ACAUCAC AG GUAACGAAUGGAUCC
Msw1.5	GGAUCCCGA CG ACAUCAC AG GUAACGAAUGGAUCC
Msw4.1	GGAUCCCGA CU GCAUCAU AG GUAACGAAUGGAUCC
Msw4.2	GGAUCCCGA CC CAUCAU GG GUAACGAAUGGAUCC
Msw5.1	GGAUCCCGA CU GACAUCU AG GUAACGAAUGGAUCC
Msw6.1	GGAUCCCGA CU GACAUCAG AG GUAACGAAUGGAUCC
Msw6.2	GGAUCCCGA CG ACAUCAG AG GUAACGAAUGGAUCC

Table S1: Sequences of the parent MG aptamer, pre-miR let7b, K4 and malaswitch (Msw) variants. Predicted apical loops are underlined.

Candidates	Sequences (5' to 3')
Pre-miR 206	CCAGGCCACAUGCUUCUUUAUAUCCUCAUAG AUAUCUCAGCA CUAUGGAAUGUAAGGAAGUGUGUGGUUUUGG
K206	CAUAG AUAUCUCAGCA CUAUG
MDMD1	GGAUCCCGA CU AGCUGAGAUAG AG GUAACG AAUGGAUCC
MDMD2	GGAUCCCGA CU AGCUGAGAUGA AG GUAACG AAUGGAUCC
MDMD3	GGAUCCCGA CU ACGUGAGAUGA AG GUAACG AAUGGAUCC
MDMD4	GGAUCCCGA CU ACGAGAGAUAUG AG GUAACG AAUGGAUCC
MDMD5	GGAUCCCGA CU AGCUGAGAAUA AG GUAACG AAUGGAUCC
MDMD6	GGAUCCCGA CU AGCUGAGAAUAG AG GUAACG AAUGGAUCC
MDMD7	GGAUCCCGA CU AGCUGAGUAUA AG GUAACG AAUGGAUCC
MDMD8	GGAUCCCGA CU ACGUGAGAAUA AG GUAACG AAUGGAUCC
MDMD1.1	**GGAUCCCGA CU AGCUGAGAUAG AG GUAACG AAUGGAUCC**
MDMD1.2	**GGAU**CCCGA CU A**GCUGAGAUA**G AG GUAACG AAUGG**AUCC**

Table S2: Sequences of pre-miR 206, K206 and MDMD malaswitch variants. Predicted apical loops are underlined. For MDMD 1.1 and 1.2 bold letters indicate 2'-O-methyl nucleotides.

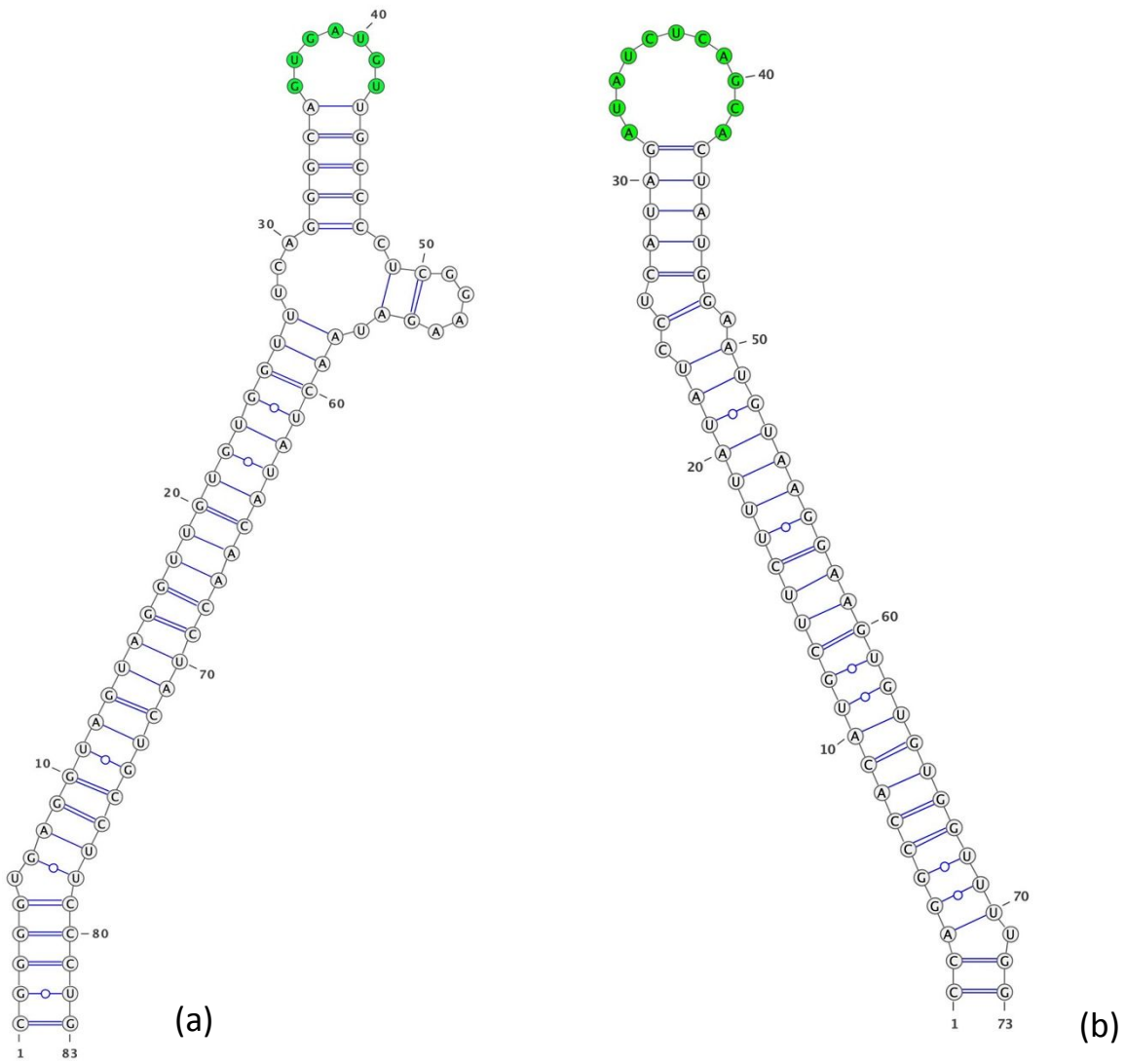

Figure S1: Predicted secondary structures (Mfold) of full-length pre-miRNAs (a) pre-miR-let7b (b) pre-miR206. Nucleotides of the apical loops are indicated in green.

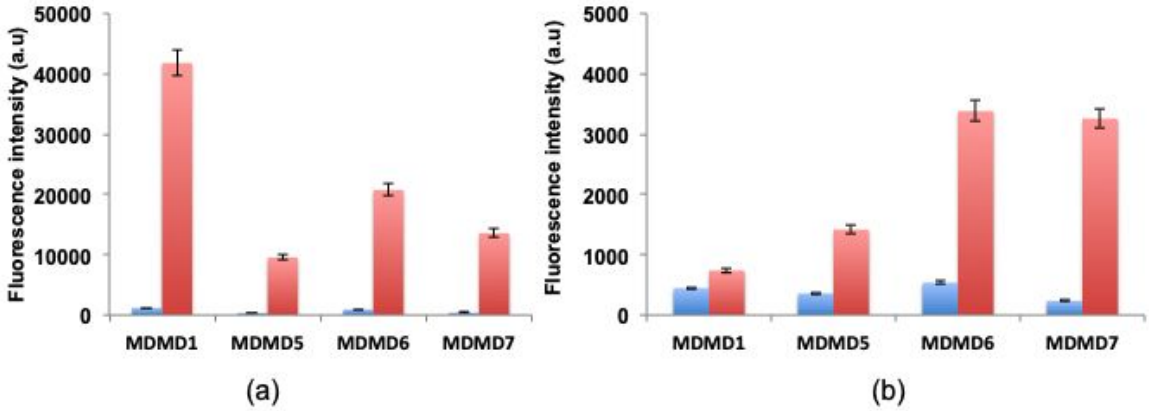

Figure S2: Effect of magnesium on the fluorescence of MDMD-MG-K206 complexes. (a) 10mM magnesium, (b) 50mM magnesium. MDMD, MG and K206 concentrations were adjusted to 0.1 μM, 2 μM and 2 μM, respectively. Blue and red bars correspond to measurements in SE buffer without and with aptakiss K206, respectively. (λ_{Exc} = 610 nm, λ_{Em} = 650 nm). Measurements were performed in triplicate and standard error was calculated accordingly.

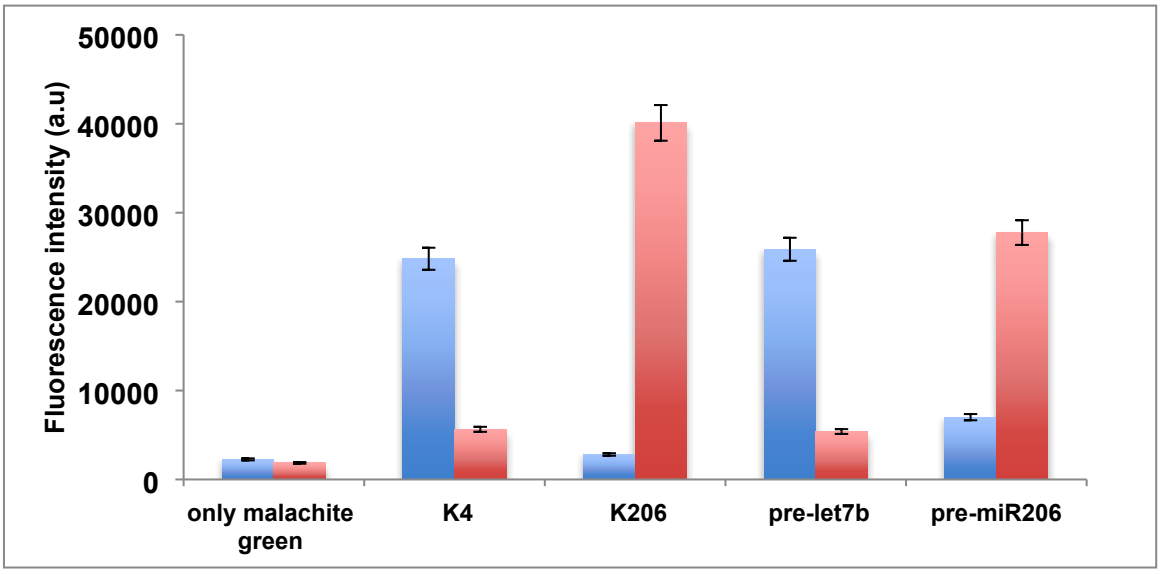

Figure S3: Specificity of Msw6.1 and MDMD1 malaswitches. 0.1 μM of both malaswitches were treated with 2 μM of hairpin loops and miRNA precursors. Blue and red bars correspond to Msw6.1 and MDMD1 malaswitches, respectively. All the measurements were performed in triplicate and standard error was calculated accordingly.

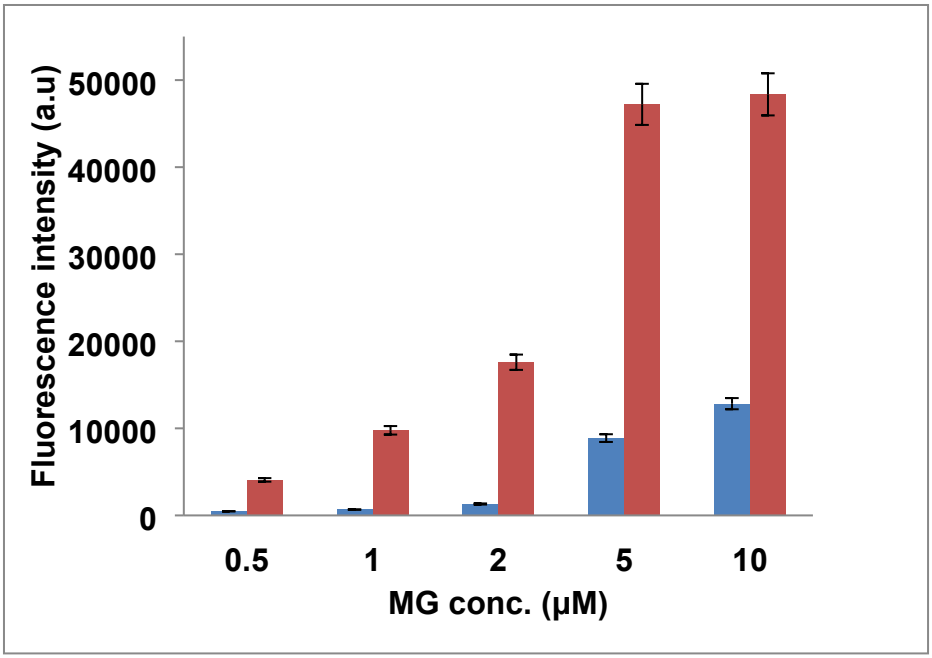

Figure S4: Test of sensing properties of chemically modified MDMD1.2 (see Table S2 for modifications). Fluorimetric titration of MDMD1.2 – pre-miR206 complexes by malachite green (MG) in SE buffer. MDMD1.2 and pre-miR206 were adjusted at 0.1and 2 μM, respectively. Measurements were performed in triplicate. (λ_{Exc} = 610 nm, λ_{Em} = 650 nm) Blue and red bars correspond to the signal obtained in absence and presence of pre-miR206, respectively.