YMTHE, Volume *28*

Supplemental Information

Engineered Circular RNA Sponges Act as miRNA Inhibitors to Attenuate Pressure Overload-Induced Cardiac Hypertrophy

Annadoray Lavenniah, Tuan Danh Anh Luu, Yiqing Peter Li, Tingsen Benson Lim, Jianming Jiang, Matthew Ackers-Johnson, and Roger S.-Y. Foo

SUPPLEMENTAL DATA

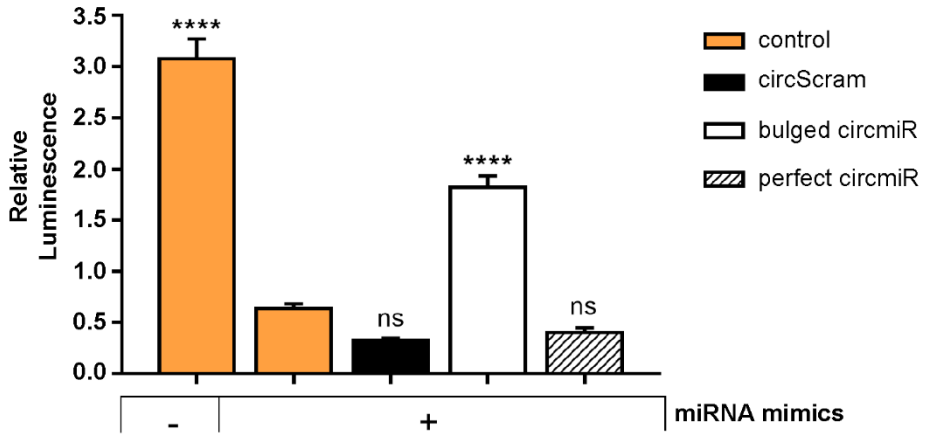

Figure S1. Efficacy of engineered circmiRs in H9C2 cardiomyocytes.

Luciferase rescue reporter assays using dual reporter constructs with miR-132 and -212 binding sites inserted into the 3'UTR of *Renilla*. H9C2 cardiomyocytes were co-transfected with the dual reporter plasmid psiCheck2, miR-132 and -212 mimics and respective circRNA expression constructs, to determine the effect of bulged circmiR or perfect circmiR versus circScram. (n=3). ****$P < 0.0001$ relative to control with mimics. One-way ANOVA with Benjamini-Hochberg adjustment.

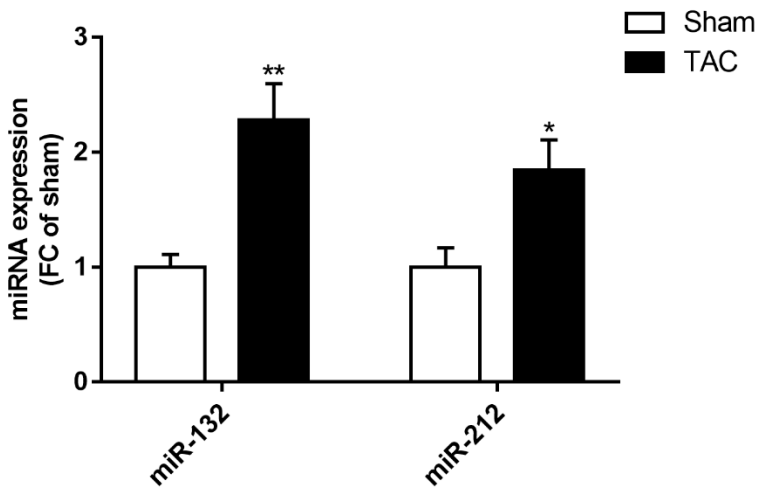

Figure S2. Cardiac miR-132 and miR-212 levels were upregulated in left ventricular pressure-overloaded mice.

Expression levels of miR-132 and miR-212 in mice ten to eleven weeks after TAC (n=7) or sham (n=6) surgery. FC: Fold change. $P < 0.05$, $^{**}P < 0.01$ relative to sham. Student's t-test.

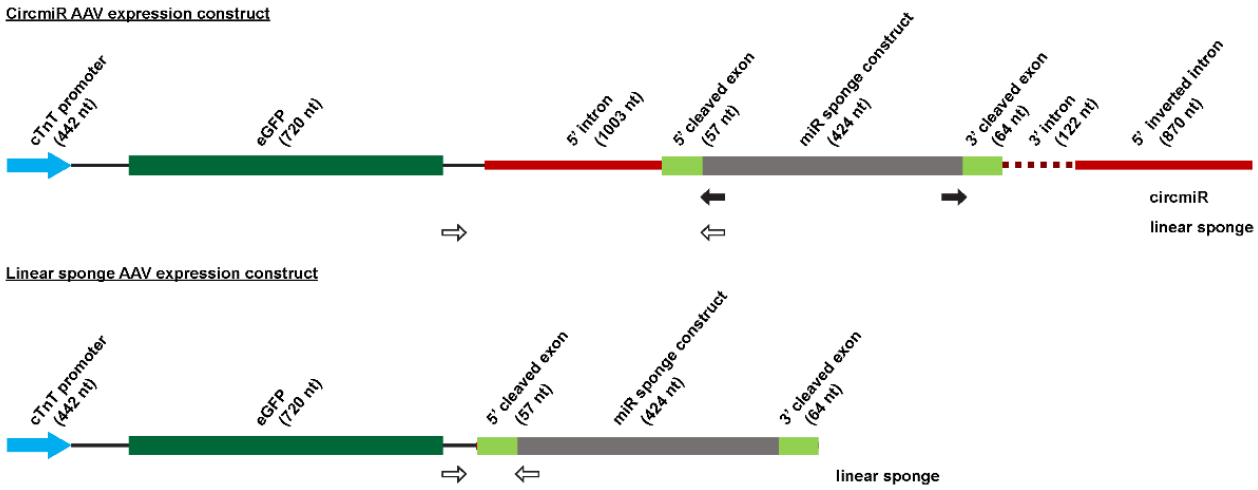

Figure S3. Primer design to distinguish between linear and circular forms of miRNA sponge.

Schematic illustration of AAV circmiR and linear sponge expression constructs, indicating positions of the circmiR-specific divergent (black arrows) and linear sponge-specific convergent (white arrows) PCR primer binding sites.

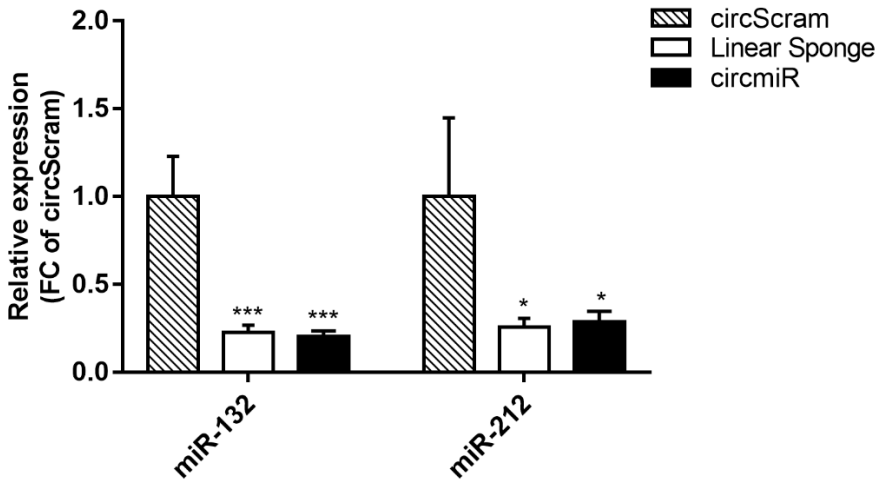

Figure S4. Administration of circmiR or linear sponge leads to reduced miR-132/212 abundance in HEK293T cells.

Expression levels of miR-132 and miR-212 in HEK293T cells 48 h after transfection FC: Fold change. (n=3); ****P < 0.0001 relative to circScram. One-way ANOVA with Benjamini-Hochberg adjustment.

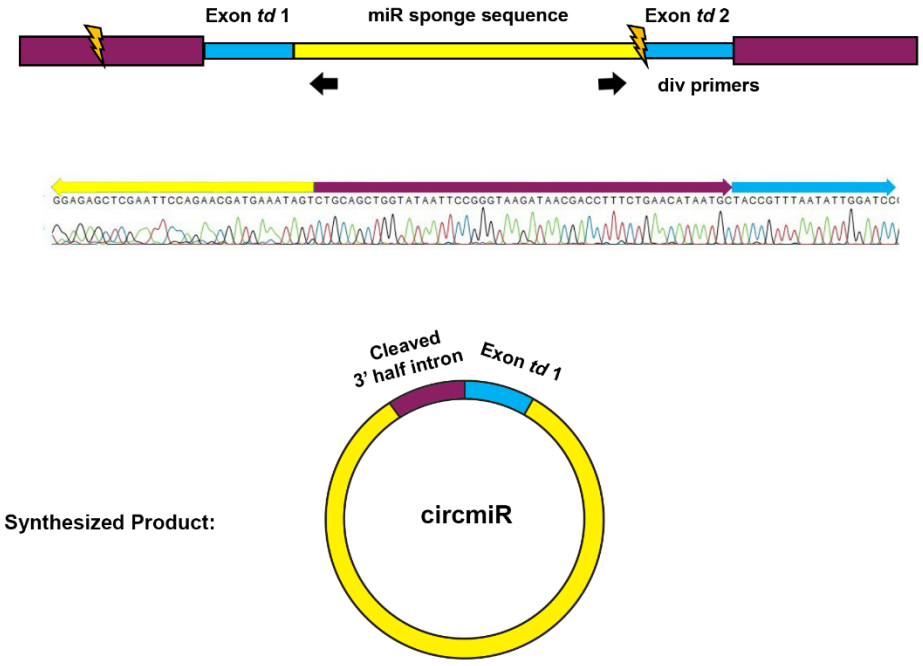

Figure S5. miRNA sponge sequence was circularised using group I permutated intron-exon splicing.

Sanger sequencing with divergent primers revealed that an auto catalytic splicing reaction took place between the 5' end of the miRNA sponge sequence and within the 3' half intron, as indicated by the orange lightning symbols.

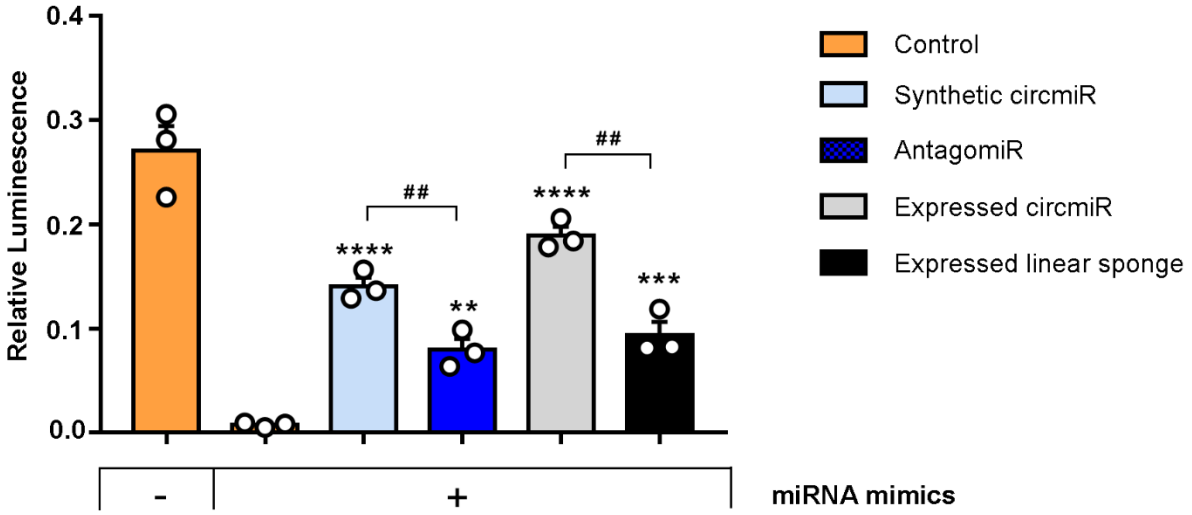

Figure S6. Engineering of synthetic circmiRs *in vitro* using group I permutated intron-exon splicing (with individual data points shown).

Luciferase reporter assays using dual reporter constructs with miR-132 and -212 binding sites inserted into the 3'UTR of Renilla. HEK293T cells were co-transfected with dual reporter plasmid psiCheck2, miR-132 and -212 mimics and respective constructs to determine the effect of antagomiRs versus circmiRs. (n=3); **P < 0.01, ***P <0.001, ****P < 0.0001 relative to control with mimics. One-way ANOVA with Benjamini-Hochberg adjustment. ##P < 0.01 as indicated. Student's t-test.

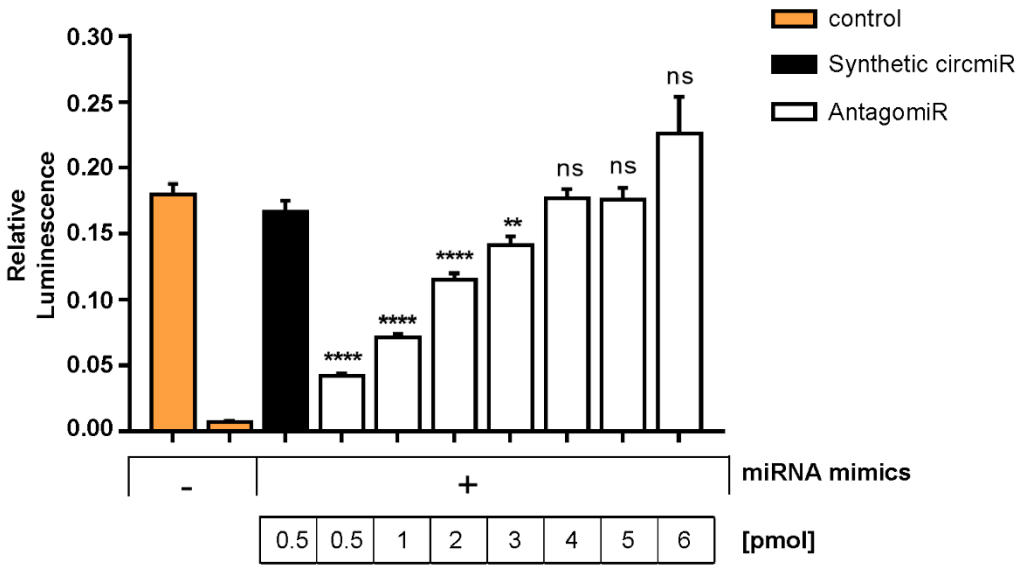

Figure S7. Dosage efficacy of synthetic circmiRs versus antagomiRs.

Luciferase reporter assays using dual reporter constructs with miR-132 and -212 binding sites inserted into the 3'UTR of *Renilla*. HEK293T cells were co-transfected with the dual reporter plasmid psiCheck2, miR-132 and -212 mimics and respective molar concentrations of inhibitor constructs to determine the effect of antagomiRs versus circmiRs. (n=3); $^{**}P < 0.01$, $^{****}P < 0.0001$ relative to synthetic circmiR. One-way ANOVA with Benjamini-Hochberg adjustment.

Supplemental Table S1.

Primer sequences used in cloning.

Cloning constructs	Forward/Reverse
5' Intron	5' -TAGAATCGCCACTCCTGCAT -3'
	5'- TTGGGTGGGAGACTTAATCG -3'
3' Intron	5'- GAGGTGGAGGGGAAGACTTT -3'
	5'- TAGAATCGCCACTCCTGCAT -3'
miR-132 perfect binding site	5'- CGACCATGGCTGTAGACTGTTA -3'
miR-132 bulged binding site	5'- CGACCATGGCTCAGACTGTTA -3'
miR-212 perfect binding site	5'-TGGCCGTGACTGGAGACTGTTA-3'
miR-212 bulged binding site	5'- TGGCCGTGACTCCGACTGTTA -3'

Supplemental Table S2.

Gene	Species	Forward/Reverse
GAPDH	Human	5'- AGCCACATCGCTCAGACACC -3'
		5'- GCCCAATACGACCAAATCC -3'
18S	Mouse	5'- TTGACGGAAGGGCACCACCAG -3'
		5'- GCACCACCACCCACGGAATCG -3'
Nppa	Mouse	5'- TCGGAGCCTACGAAGATCCA -3'
		5'- GTGGCAATGTGACCAAGCTG -3'
Nppb	Mouse	5'- GCTGCTGGAGCTGATAAGAGAA -3'
		5'- AGGTCTTCCTACAACAACTTCAGTG -3'
Myh6	Mouse	5'- CTACAAGCGCCAGGCTGAG -3'
		5'- TGGAGAGGTTATTCCTCGTCG -3'
Myh7	Mouse	5'- AGCATTCTCCTGCTGTTTCCTT -3'
		5'- TGAGCCTTGGATTCTCAAACG -3'

Primer sequences used in qPCR.