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Supplemental Information

The 3′ Untranslated Region Protects the Heart from Angiotensin II-Induced Cardiac Dysfunction via AGGF1 Expression

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Supplemental Figures

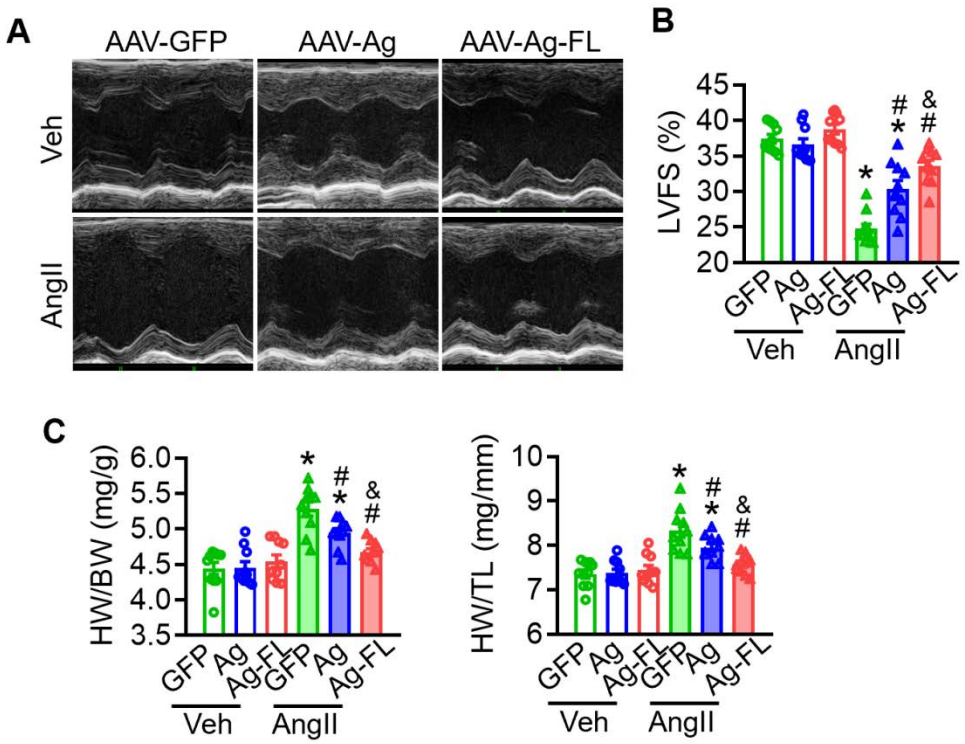

Figure S1. ***Aggf1*** **3'-UTR enhances the cardio-protective role of Aggf1 in dysfunctional heart.** 8 weeks old mice were infused with vehicle or AngII. One weeks after mini-pump implantation, the mice were injected intravenously with AAV-GFP, AAV-Ag, or AAV-Ag-FL once per week for 5 weeks (n = 10 each group). (A) Representative M-mode echocardiograms for each group. (B) LVFS levels were shown from these six group mice. (C) Ratios of heart weight to body weight (HW/BW) and heart weight to tibia length (HW/TL) were shown. * *vs* Veh, $p < 0.05$; # *vs* AAV-GFP, $p < 0.05$; & *vs* AAV-Ag, $p < 0.05$.

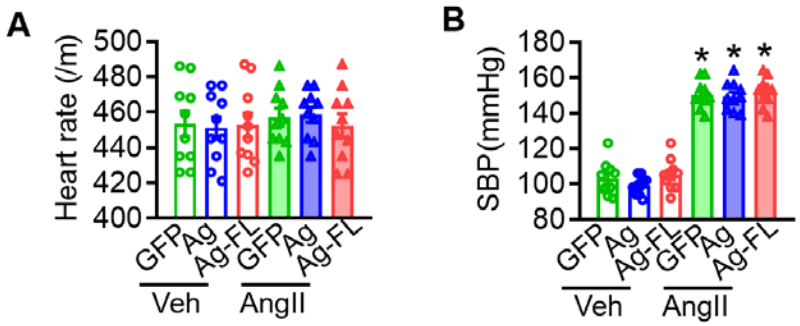

Figure S2. Neither heart rates nor blood pressures are affected after AAV-Ag or AAV-Ag-FL administration. Mice were infused with vehicle or AngII. One weeks after mini-pump implantation, the mice were injected intravenously with AAV-GFP, AAV-Ag, or AAV-Ag-FL once per week for 5 weeks. (A) Administration of either AAV-Ag or AAV-Ag-FL did not affect the heart rates in AngII-infused mice. (B) Systolic blood pressure (SBP) was measured. Statistical analysis was carried out by a Student′s t-test. n = 10, * *vs* Veh, $p < 0.05$.

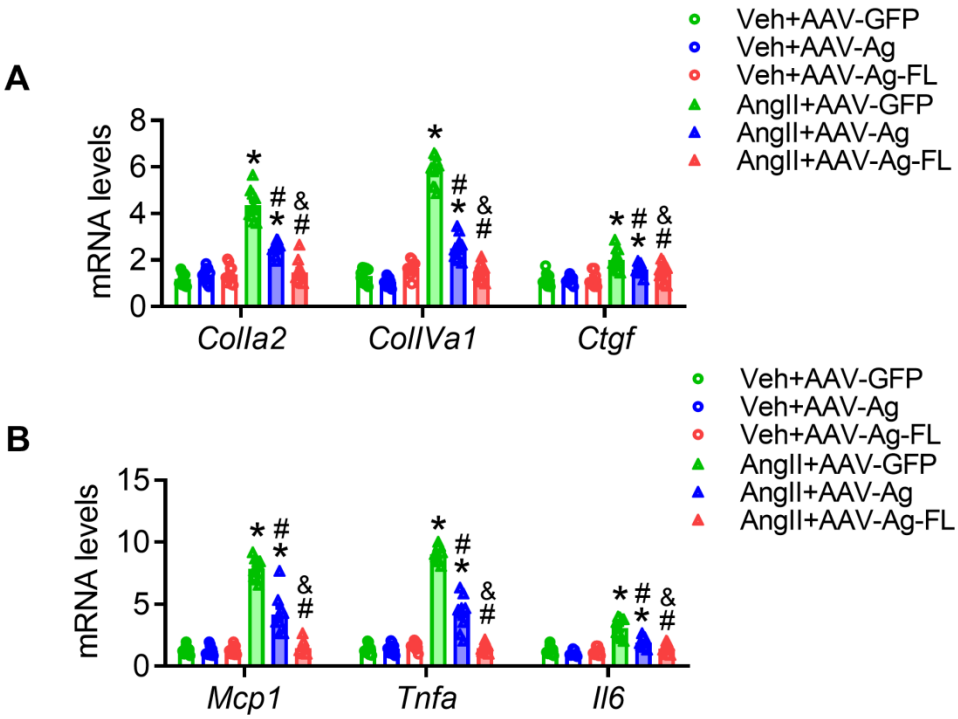

Figure S3. Inflammatory response and fibrosis are repressed by AAV-Ag and AAV-Ag-FL. Mice were infused with vehicle or AngII. One weeks after mini-pump implantation, the mice were injected intravenously with AAV-GFP, AAV-Ag, or AAV-Ag-FL once per week for 5 weeks. The heart tissues from mice were collected. Realtime-PCR analysis was performed to detect cardiac fibrotic markers, including *ColI*, *ColIV*, and *Ctgf* (A), and cardiac inflammation markers, including *Mcp1*, *Tnfα*, and *Il6* (B). * *vs* Veh, $p < 0.05$; # *vs* AAV-GFP, $p < 0.05$; & *vs* AAV-Ag, $p < 0.05$.

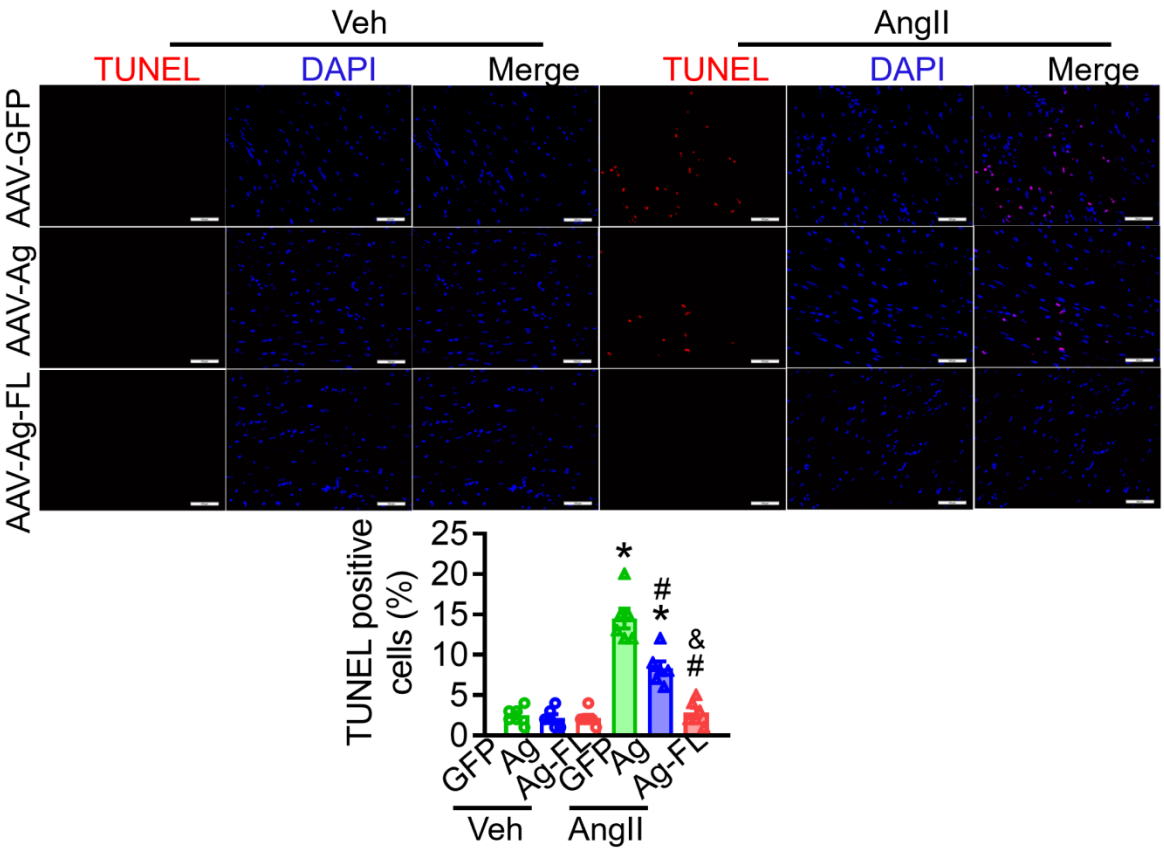

Figure S4. Apoptosis of cardiomyocytes is affected by AAV-Ag or AAV-Ag-FL. The representative images from TUNEL staining were shown in heart tissues from mice administrated with different AAVs. n = 6, * *vs* Veh, $p < 0.05$; # *vs* AAV-GFP, $p < 0.05$; & *vs* AAV-Ag, $p < 0.05$.

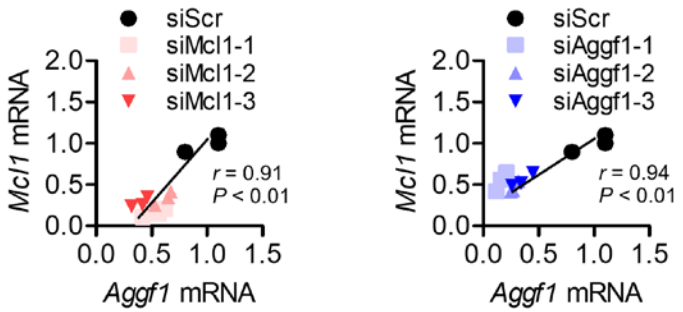

Figure S5. The reduction of *Aggf1* mRNA is associated with *Mcl1* mRNA levels. The isolated mouse neocardiomyocytes were transfected with Scramble siRNA, different siMcl1, or siAggf1 for 36 h. The association between *Aggf1* and *Mcl1* mRNA levels in *Mcl1* deficient or *Aggf1* deficient neocardiomyocytes was presented.

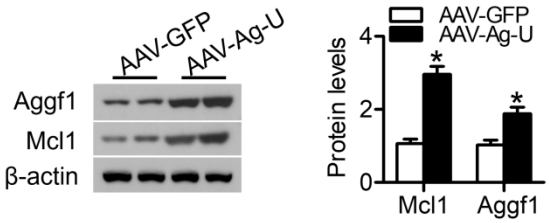

Figure S6. The expression of Aggf1 and Mcl1 is regulated by *Aggf1* 3'-UTR in neocardiomyocytes. The purified mouse neocardiomyocytes were cultured with AAV-GFP or AAV-Ag-U. Western blot assay was performed to analyze the levels of Aggf1 and Mcl1 expression. Statistical analysis was carried out by a Student's t-test. n = 6, * *vs* AAV-GFP, $p < 0.05$.

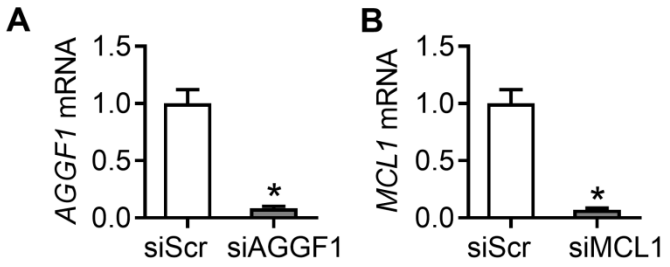

Figure S7. The mRNA levels of *AGGF1* and *MCL1* after *AGGF1* and *MCL1* knockdown in HEK293 cells. HEK293 cells were transfected with siScr, siAGGF1, or siMCL1. (A) Real-time PCR analysis for *AGGF1* mRNA level after *AGGF1* knockdown. (B) Real-time PCR analysis for *MCL1* mRNA level after *MCL1* knockdown. Statistical analysis was carried out by a Student's t-test. n = 6, * *vs* siScr, $p < 0.05$.

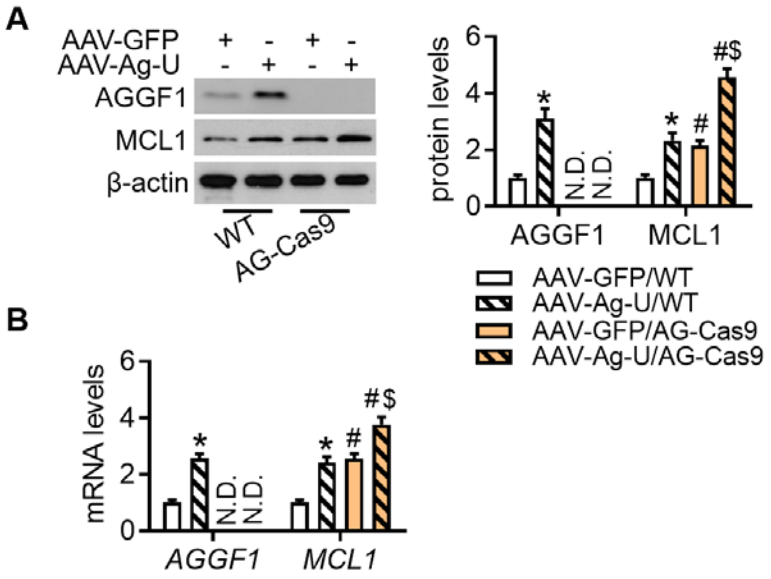

Figure S8. *AGGF1* 3'-UTR causes the increase for MCL1 expression in *AGGF1* deficient cells. *AGGF1* deficient HEK293 cells were generated with Crispr/Cas9 method. The WT and *AGGF1* deficient HEK293 cells were incubated with AAV-GFP or AAV-Ag-U. Western blot (A) and real-time PCR analyses (B) were performed to detect AGGF1 and MCL1 expression after treatment. Statistical analysis was carried out by a Student's t-test. n = 3, * *vs* AAV-GFP, $p < 0.05$; # *vs* WT, $p < 0.05$; $ *vs* AAV-GFP/AG-Cas9, $p < 0.05$.

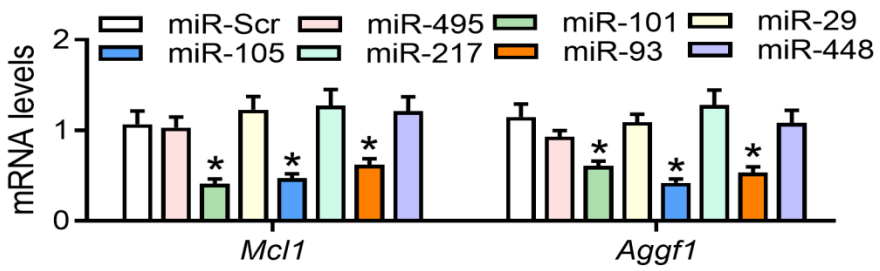

Figure S9. The mRNA levels of *Mcl1* and *Aggf1* are detected after miRNAs overexpression. The mRNA levels of *Aggf1* and *Mcl1* in mouse neocardiomyocytes after miRNAs transfection. Real-time PCR analysis was used to detect *Aggf1* and *Mcl1* mRNA levels. Statistical analysis was carried out by a Student's t-test. * *vs* miR-Scr, $p < 0.05$.

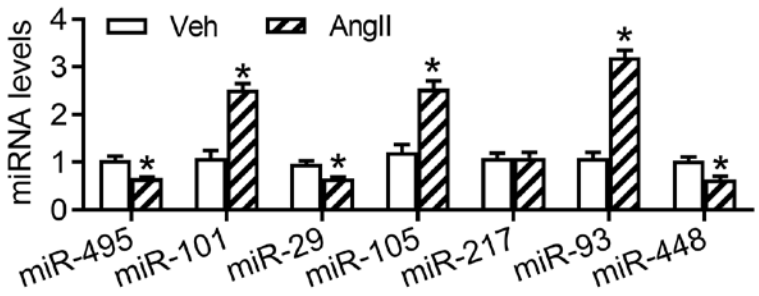

Figure S10. The levels of predictive miRNA are analyzed in hearts from AngII-infused mouse. The heart tissues were collected from mice infused with vehicle or AngII. Statistical analysis was carried out by a Student's t-test. * *vs* Veh, $p < 0.05$.

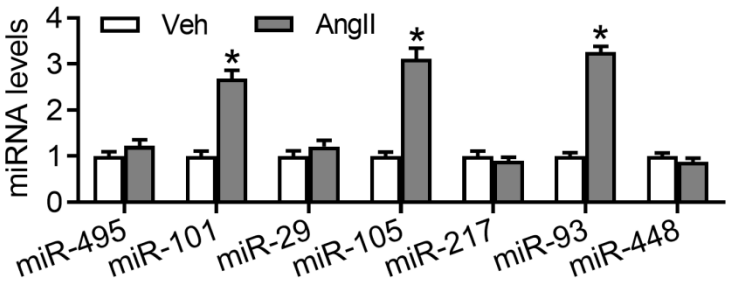

Figure S11. The levels of predictive miRNA are analyzed in AngII-treated neocardiomyocytes. Isolated mouse neocardiomyocytes were treated with AngII. The expression levels of predictive miRNAs were analyzed. Statistical analysis was carried out by a Student's t-test. n = 6, * *vs* Veh, $p < 0.05$.

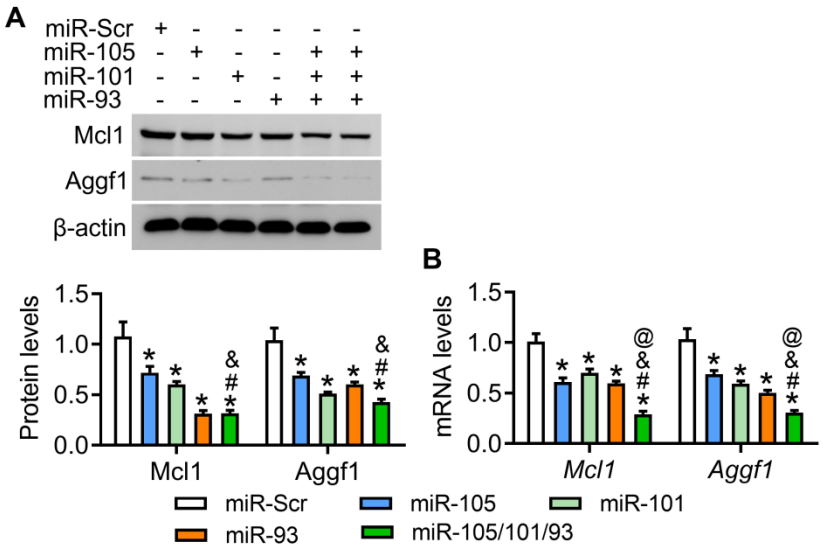

Figure S12. The expression levels of Aggf1 and Mcl1 are analyzed after miRNAs transfection in neocardiomyocytes. The purified mouse neocardiomyocytes were transfected with miR-105, miR-101, miR-93, or miR-105/101/93, respectively. Western blot (A) and real-time PCR analysis (B) were performed to detect Aggf1 and Mcl1expression. Statistical analysis was carried out by a Student's t-test. n = 6, * *vs* miScr, $p < 0.05$, # *vs* miR-105, $p < 0.05$, & *vs* miR-101, $p < 0.05$, @ *vs* miR-93, $p < 0.05$.

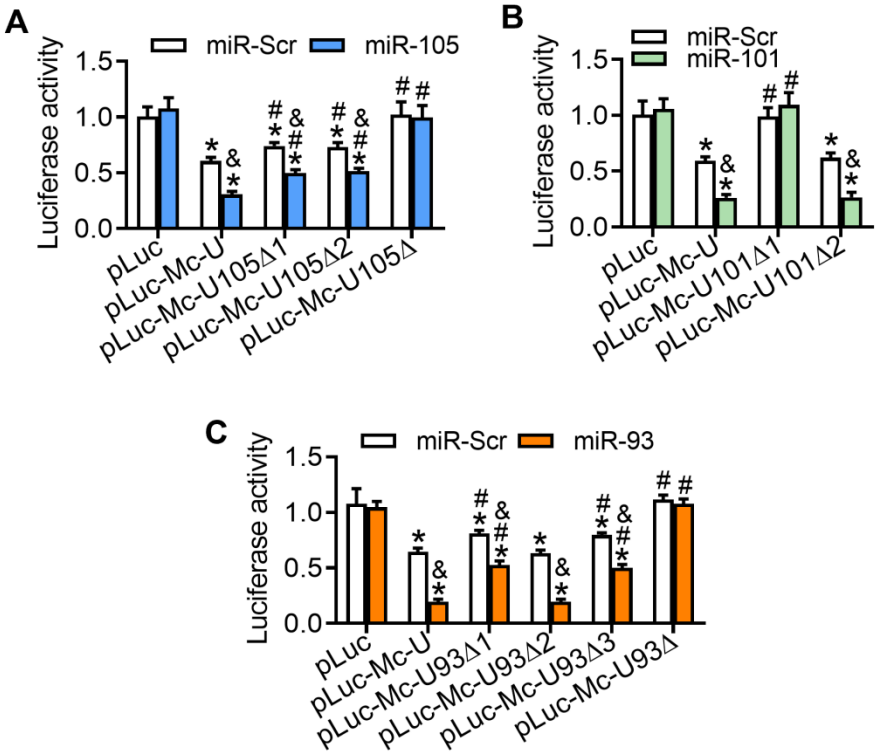

Figure S13. miRNAs binding candidates along Mcl1 3'-UTR. (A) The luciferase activities were measured in HEK293 cells transfected with different luciferase plasmids and miR-Scr or miR-105. n = 6, * *vs* pLuc, $p < 0.05$; # *vs* pLuc-Mc-U, $p < 0.05$; & *vs* miR-Scr, $p < 0.05$. (B) The luciferase activities were measured in HEK293 cells transfected with different luciferase plasmids and miR-Scr or miR-101. n = 6, * *vs* pLuc, $p < 0.05$; # *vs* pLuc-Mc-U, $p < 0.05$; & *vs* miR-Scr, $p < 0.05$. (C) The luciferase activities were measured in HEK293 cells transfected with different luciferase plasmids and miR-Scr or miR-93. Statistical analysis was carried out by a Student's t-test. n = 6, * *vs* pLuc, $p < 0.05$; # *vs* pLuc-Mc-U, $p < 0.05$; & *vs* miR-Scr, $p < 0.05$.

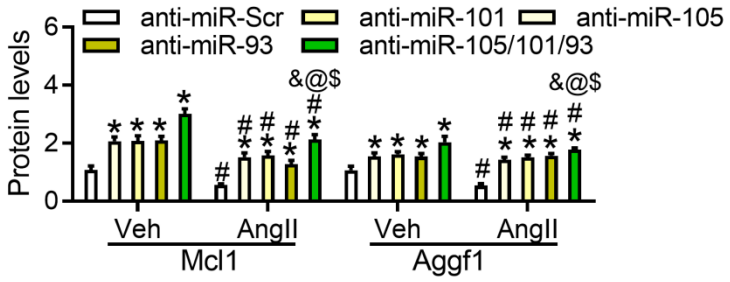

Figure S14. The protein levels of Aggf1 and Mcl1 in AngII-treated neocardiomyocytes after miRNAs transfection are quantified. Western blot analysis was performed to measure Aggf1 and Mcl1 expression after miRNA overexpression in purified mouse neocardiomyocytes. Aggf1 and Mcl1 levels were quantified by densitometric analysis of the Western blots. * *vs* anti-miR-Scr, $p < 0.05$; # *vs* Veh, $p < 0.05$; & *vs* anti-miR-105, $p < 0.05$; # *vs* anti-miR-101, $p < 0.05$; # *vs* anti-miR-93, $p < 0.05$.

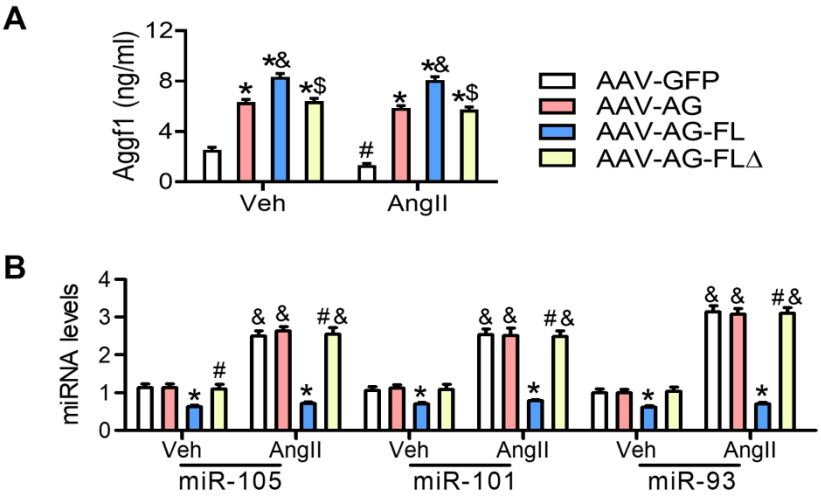

Figure S15. Gene expression in AngII-treated cardiomyocytes after *Aggf1* 3'UTR transfection. (A) The secretory Aggf1 level was measured using ELISA assay. (B) The levels of miRNAs were measured by realtime-PCR analysis. n = 6, * *vs* AAV-GFP, $p < 0.05$; # *vs* Veh, $p < 0.05$; & *vs* AAV-Ag, $p < 0.05$; $ *vs* AAV-Ag-FL, $p < 0.05$.

Supplemental Tables

Table S1. Echocardiograms for mice after AAVs administration.

	AAV-GFP / Veh	AAV-Ag / Veh	AAV-Ag-FL / Veh	AAV-GFP / AngII	AAV-Ag / AngII	AAV-Ag-FL / AngII
Number	10	10	10	10	10	10
HR (bmp)	447 ± 27	454 ± 27	457 ± 28	455 ± 32	447 ± 33	451 ± 30
IVS;d (mm)	0.74 ± 0.12	0.73 ± 0.08	0.70 ± 0.06	0.97 ± 0.11*	0.87 ± 0.16	0.78 ± 0.15#
IVS;s (mm)	1.15 ± 0.12	1.15 ± 0.12	1.09 ± 0.08	1.40 ± 0.11*	1.22 ± 0.19#	0.99 ± 0.15#&
LVID;d (mm)	3.50 ± 0.17	3.58 ± 0.21	3.46 ± 0.15	3.80 ± 0.26*	3.88 ± 0.15	3.69 ± 0.25
LVID;s (mm)	2.19 ± 0.17	2.27 ± 0.14	2.16 ± 0.14	2.86 ± 0.23*	2.72 ± 0.20	2.45 ± 0.23#&
LVPW;d (mm)	0.73 ± 0.11	0.74 ± 0.06	0.69 ± 0.07	0.97 ± 0.13*	0.78 ± 0.13#	0.71 ± 0.12#
LVPW;s (mm)	1.18 ± 0.17	1.21 ± 0.11	1.17 ± 0.15	1.44 ± 0.12*	1.18 ± 0.20#	0.98 ± 0.16#&
EF (%)	69.7 ± 3.1	68.9 ± 3.1	69.6 ± 3.2	49.7 ± 3.6*	57.6 ± 4.8#	63.2 ± 6.7#&
FS (%)	37. 5 ± 1.9	36.7 ± 2.4	38.8± 2.2	24.8 ± 2.2*	30.4 ± 3.8#	33.6 ± 2.6#&

Values are means ± SD. HR, heart rate; IVS, interventricular septum (left ventricular anterior wall); LVID, left ventricular interior diameter; LVPW, left ventricular posterior wall; EF, ejection fraction; FS, fraction shortening; d, diastole; s, systole. * *vs* AAV-GFP/ Veh, $p < 0.05$; # *vs* AAV-GFP/ AngII, $p < 0.05$; & *vs* AAV-Ag/ AngII, $p < 0.05$.

Table S2. The primers list for site-deletion of luciferase plasmids

Primers	Sense primer	Antisense primer
pLuc-AG-U-105Δ1	gaagctcctagaaagtcagtca	tttctaggagcttctacactgaag
pLuc-AG-U-105Δ2	gacacaggggacatgtggtttgtagc	tcccctgtgtcgatcaggtagcca
pLuc-AG-U-105Δ3	ctaatacttgtttacacctttag	agtattagaattgttttataaatg
pLuc-AG-U-105Δ	gaagctcctagaaagtcagtca	tttctaggagcttctacactgaag
	gacacaggggacatgtggtttgtagc	tcccctgtgtcgatcaggtagcca
pLuc-AG-U-101Δ	tggggctcgagaaatctgatgactag	gagccccattgcctgcaatggtat
pLuc-AG-U-93Δ1	ggtgacacaggagagctgccagac	cctgtgtcaccttgagcccact
pLuc-AG-U-93Δ2	ttgcaatgggactgtgctcgagaaatc	ccattgcaatggtatttatcaaaact
pLuc-AG-U-93Δ	ggtgacacaggagagctgccagac	cctgtgtcaccttgagcccact
	ttgcaatgggactgtgctcgagaaatc	ccattgcaatggtatttatcaaaact
pLuc-MC-U-105Δ1	gacaaaatggatttgtaacctac	tttgtcaccaacgttgttaattag
pLuc-MC-U-105Δ2	cctggtgtggactggttatagatttataac	acaccaggctctgcatatacactag
pLuc-MC-U-105Δ	gacaaaatggatttgtaacctac	tttgtcaccaacgttgttaattag
	cctggtgtggactggttatagatttataac	acaccaggctctgcatatacactag
pLuc-MC-U-101Δ1	ttctagcaacatagcaaaaagaaagtggc	tgctagaactggactgttaaaatcctgggcag
pLuc-MC-U-101Δ2	gcgtgttatgctcccagttccccta	taacacgccaaaaggaagtaaaggc
pLuc-MC-U-101Δ	ttctagcaacatagcaaaaagaaagtggc	tgctagaactggactgttaaaatcctgggcag
	gcgtgttatgctcccagttccccta	taacacgccaaaaggaagtaaaggc
pLuc-MC-U-93Δ1	gtcactagtcacaaagctcaataaatatc	actagtgactggcccctcttccatc
pLuc-MC-U-93Δ2	tgcacttttagccctgtctactttggc	aaagtgcacttgggactttgtcaagca
pLuc-MC-U-93Δ3	ttaggctgcagataaaaataggc	agcctaaaggtcacattctgaggc
pLuc-MC-U-93Δ	gtcactagtcacaaagctcaataaatatc	actagtgactggcccctcttccatc
	ttaggctgcagataaaaataggc	agcctaaaggtcacattctgaggc

Table S3. The list for miRNA minics used in the cell experiments

miRNAs	Species	Cat.	Company
miR-495-5p	Mouse	miR10017249-1-5	Guangzhou RiboBio
miR-101a-5p	Mouse	miR10004526-1-5	Guangzhou RiboBio
miR-29b	Mouse	miR10017063-1-5	Guangzhou RiboBio
miR-105	Mouse	miR10004856-1-5	Guangzhou RiboBio
miR-217-3p	Mouse	miR10017072-1-5	Guangzhou RiboBio
miR-93-3p	Mouse	miR10004636-1-5	Guangzhou RiboBio
miR-448-3p	Mouse	miR10001533-1-5	Guangzhou RiboBio
miR-105	Human	miR10004516-1-5	Guangzhou RiboBio
miR-101-5p	Human	miR10004513-1-5	Guangzhou RiboBio
miR-93-3p	Human	miR10004509-1-5	Guangzhou RiboBio

Table S4. The list for miRNA inhibitors used in the cell experiments

miRNAs	**Species**	**Cat.**	**Company**
miR-495-5p	Mouse	miR20017249-1-5	Guangzhou RiboBio
miR-101a-5p	Mouse	miR20004526-1-5	Guangzhou RiboBio
miR-29b	Mouse	miR20017063-1-5	Guangzhou RiboBio
miR-105	Mouse	miR20004856-1-5	Guangzhou RiboBio
miR-217-3p	Mouse	miR20017072-1-5	Guangzhou RiboBio
miR-93-3p	Mouse	miR20004636-1-5	Guangzhou RiboBio
miR-448-3p	Mouse	miR20001533-1-5	Guangzhou RiboBio

Table S5. The primers list for site-deletion AAVs

Primers	Sense primer	Antisense primer
AAV-Ag-UΔ	gaagctcctagaaagtcagtca	tttctaggagcttctacactgaag
	gacacaggggacatgtggtttgtagc	tcccctgtgtcgatcaggtagcca
	tggggctcgagaaatctgatgactag	gagccccattgcctgcaatggtat
	ggtgacacaggagagctgccagac	cctgtgtcaccttgagcccact
	ttgcaatgggactgtgctcgagaaatc	ccattgcaatggtatttatcaaaact
AAV-Mc-UΔ	gacaaaatggatttgtaacctac	tttgtcaccaacgttgttaattag
	cctggtgtggactggttatagatttataac	acaccaggctctgcatatacactag
	ttctagcaacatagcaaaaagaaagtggc	tgctagaactggactgttaaaatcctgggcag
	gcgtgttatgctcccagttccccta	taacacgccaaaaggaagtaaaggc
	gtcactagtcacaaagctcaataaatatc	actagtgactggcccctcttccatc
	ttaggctgcagataaaaataggc	agcctaaaggtcacattctgaggc
AAV-Ag-FLΔ	gaagctcctagaaagtcagtca	tttctaggagcttctacactgaag
	gacacaggggacatgtggtttgtagc	tcccctgtgtcgatcaggtagcca
	tggggctcgagaaatctgatgactag	gagccccattgcctgcaatggtat
	ggtgacacaggagagctgccagac	cctgtgtcaccttgagcccact
	ttgcaatgggactgtgctcgagaaatc	ccattgcaatggtatttatcaaaact
AAV-Mc-FLΔ	gacaaaatggatttgtaacctac	tttgtcaccaacgttgttaattag
	cctggtgtggactggttatagatttataac	acaccaggctctgcatatacactag
	ttctagcaacatagcaaaaagaaagtggc	tgctagaactggactgttaaaatcctgggcag
	gcgtgttatgctcccagttccccta	taacacgccaaaaggaagtaaaggc
	gtcactagtcacaaagctcaataaatatc	actagtgactggcccctcttccatc
	ttaggctgcagataaaaataggc	agcctaaaggtcacattctgaggc

Table S6. The primers list for miRNAs levels using realtime PCR assays

miRNAs	**Species**	**Cat.**	**Company**
miR-495-5p	Mouse	miRQ0017249-1-1	Guangzhou RiboBio
miR-101a-5p	Mouse	miRQ0004526-1-1	Guangzhou RiboBio
miR-29b	Mouse	miRQ0017063-1-1	Guangzhou RiboBio
miR-105	Mouse	miRQ0004856-1-1	Guangzhou RiboBio
miR-217-3p	Mouse	miRQ0017072-1-1	Guangzhou RiboBio
miR-93-3p	Mouse	miRQ0004636-1-1	Guangzhou RiboBio
miR-448-3p	Mouse	miRQ0001533-1-1	Guangzhou RiboBio

Table S7. The primers list for mRNAs levels using realtime PCR assays

Gene	Species	Sense primer	Antisense primer
Mcl1	Mouse	TGTAAGGACGAAACGGGACT	AAAGCCAGCAGCACATTTCT
Aggf1	Mouse	AGCACACGGAGCGACTCTA	GAGCACACTGACTCTGCTGT
Anp	Mouse	TTCTTCCTCGTCTTGGCCTTT	GACCTCATCTTCTACCGGCATCT
Bnp	Mouse	CACCGCTGGGAGGTCACT	GTGAGGCCTTGGTCCTTCAAGGTCACT
β-Mhc	Mouse	ATGTGCCGGACCTTGGAA	CCTCGGGTTAGCTGAGAGATCA
ColIa2	Mouse	GTAACTTCGTGCCTAGCAACA	CCTTTGTCAGAATACTGAGCAGC
ColIVa1	Mouse	CTGGCACAAAAGGGACGAG	ACGTGGCCGAGAATTTCACC
Ctgf	Mouse	GGGCCTCTTCTGCGATTTC	ATCCAGGCAAGTGCATTGGTA
Mcp1	Mouse	GCTCAGCCAGATGCAGTTAA	TCTTGAGCTTGGTGACAAAAACT
Tnfα	Mouse	CACGTCGTAGCAAACCACCAAGTGGA	TGGGAGTAGACAAGGTACAACCC
Il6	Mouse	TCTATACCACTTCACAAGTCGGA	GAATTGCCATTGCACAACTCTTT
Bcl-2	Mouse	CCTGGCTGTCTCTGAAGACC	CTCACTTGTGGCCCAGGTAT
Bax	Mouse	CCCGAGAGGTCTTTTTCC	GCCTTGAGCACCAGTTTG
β-actin	Mouse	CATCGTCCACCGCAAATG	CACCTTCACCGTTCCAGTT