Table S1. Primers for Recombinant constructs vectors

Name	Specific sequence	Amplicon size (bp)
TGF-β1-Exon3-F	CTAGGCTAGCTATAGCAACAATTCCTGGCG	88
TGF-β1-Exon3-R	AGCTAAGCTTAGCCACTCAGGCGTATCAGTGGGGGTCA	
TGF-β1-Exon3-mutation-R	AGCTAAGCTTACGGAGTGAGGCGTATCAGTGGGGGTCA	

Note: the mutation sites were emphasized by underline.

Table S2. Over-expression of miR29b improves cardiac function at day 14 after AngII infusion

	Saline D14	AngII D14		
		AngII	Vector	miR-29b
IVS;d(mm)	0.63±0.07	1.01±0.14***	1.00±0.11***	0.83±0.16*#
LVID;d(mm)	3.83±0.52	3.33±0.52*	3.81±0.25	3.80±0.31
LVPW;d(mm)	0.81±0.08	0.85±0.33*	0.89±0.10*	0.88±0.09
IVS;s(mm)	1.06±0.07	1.40±0.15***	1.46±0.19***	1.25±0.10***#
LVID;s(mm)	2.43±0.38	2.15±0.30	2.23±0.28	2.49±0.30
LVPW;s(mm)	1.08±0.17	1.19±0.21	1.27±0.15*	1.12±0.06#
EF(%)	65.91±1.80	58.78±2.02***	58.44±2.71***	61.98±3.42*#
FS(%)	35.08±4.64	32.40±3.71	31.84±3.60	35.74±2.25#
LV Mass(mg)	100.58±11.30	126.34±16.82***	128.60±10.05***	118.07±9.07*#

IVS;d= Diastolic interventricular septal wall thickness; LVID;d= Diastolic left ventricular diameter; LVPW;d= Diastolic left ventricular posterior wall thickness; IVS;s= Systolic interventricular septal wall thickness; LVID;s= Systolic left ventricular diameter; LVPW;s= Systolic left ventricular posterior wall thickness; EF=Ejection fraction; FS=Shortening fraction.

* P<0.05; ** P<0.01; ***P<0.001 when compared with saline.

\# P<0.05; ## P<0.01; ### P<0.001 when compared with Vector control.

Table S3. The restored miR-29b improves cardiac function in established hypertensive cardiopathy

	Saline D28	AngII D14	AngII D28		
			AngII	Vector	miR-29b
IVS;d(mm)	0.62±0.05	1.02±0.15***	1.25±0.17***	1.15±0.12***	0.91±0.11***###
LVID;d(mm)	3.78±0.25	3.31±0.50*	3.22±0.39***	3.25±0.46*	3.36±0.32*
LVPW;d(mm)	0.86±0.09	0.89±0.36	0.99±0.06***	1.03±0.28	1.02±0.25
IVS;s(mm)	0.98±0.10	1.38±0.12***	1.59±0.10***	1.52±0.13***	1.31±0.19***#
LVID;s(mm)	2.40±0.40	2.19±0.40	2.05±0.31	2.07±0.27	2.30±0.16
LVPW;s(mm)	1.07±0.11	1.24±0.16*	1.44±0.14***	1.46±0.20***	1.28±0.17*
EF(%)	66.71±6.11	57.02±1.78***	42.24±3.05***	41.41±0.40***	58.76±4.90**###
FS(%)	36.51±4.71	32.09±2.97*	30.55±3.42*	31.72±1.09*	34.67±4.16
LV Mass(mg)	102.04±11.98	128.65±17.26***	133.58±10.79***	130.96±9.83**	120.39±8.82***#

Abbreviation seen Table S2.

* *P*<0.05; ** *P*<0.01; ****P*<0.001 when compared with saline control.

# *P*<0.05; ## *P*<0.01; ### *P*<0.001 when compared with empty vector control.

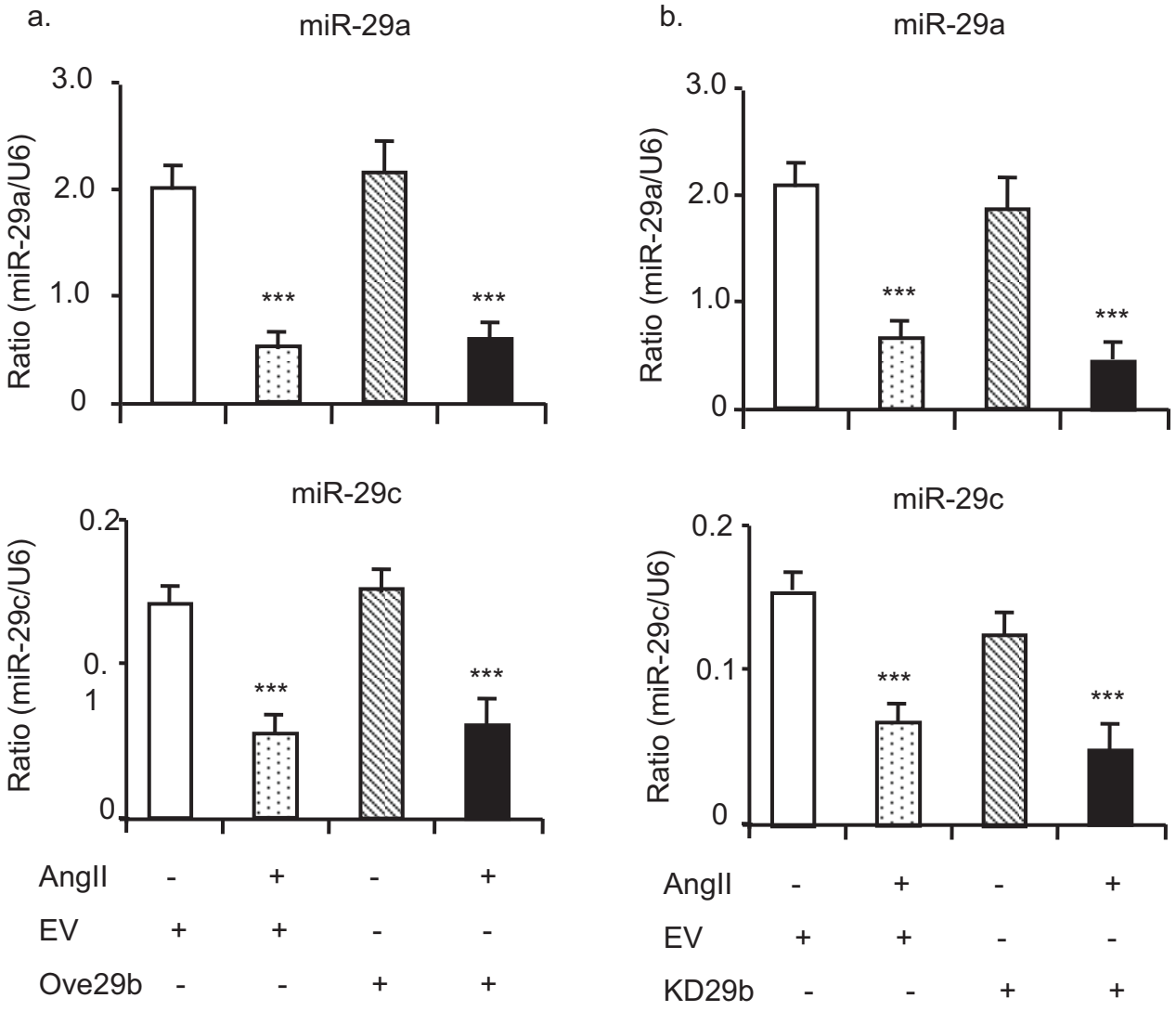
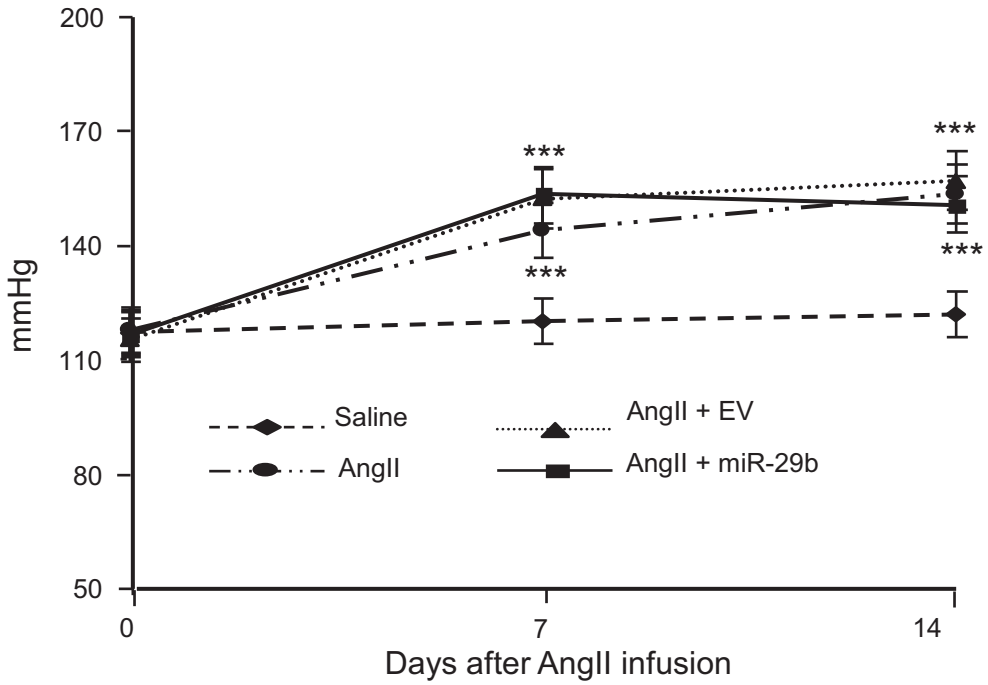


Figure S1. Effect of AngII and overexpression or knockdown of pre-miR-29b on miR-29a and miR-29c expression by cardiac fibroblasts. (CFs) (a) Effect of overexpressing pre-miR-29b on AngII (1μmol/L)-induced downregulation of miR-29a and miR-29c by CFs. (b) Effect of knocking down pre-miR-29b on AngII (1μmol/L) -induced downregulation of miR-29a and miR-29c by CFs. Results show that addition of AngII downregulates miR-29a and miR-29c in CFs, which is not altered by overexpressing (Ove29b) or knocking down (KD29b) pre-miR-29b. Each bar represents mean ± SEM for 4 independent experiments. *** $P<0.001$ when compared with the empty vector (EV) group.

a. Systolic blood pressure



b. Masson's trichrome

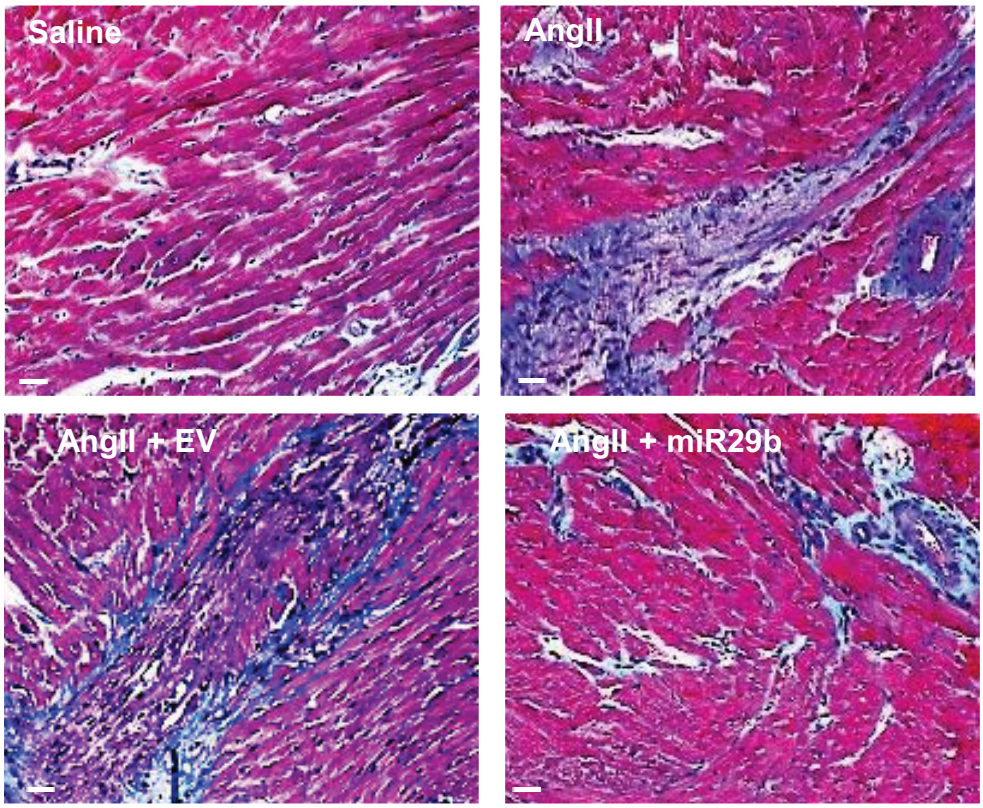


Figure S2. Over-expression of miR-29b inhibits AngII-induced cardiac fibrosis without effect on the blood pressure at day 14. (a) Systolic blood pressure. (b) Masson trichrome staining, showing green fibrotic tissues within myocardium. Data represent mean ± SEM for six mice. *** P<0.001 compared with baseline at day 0. Scale bar, 50 μM.

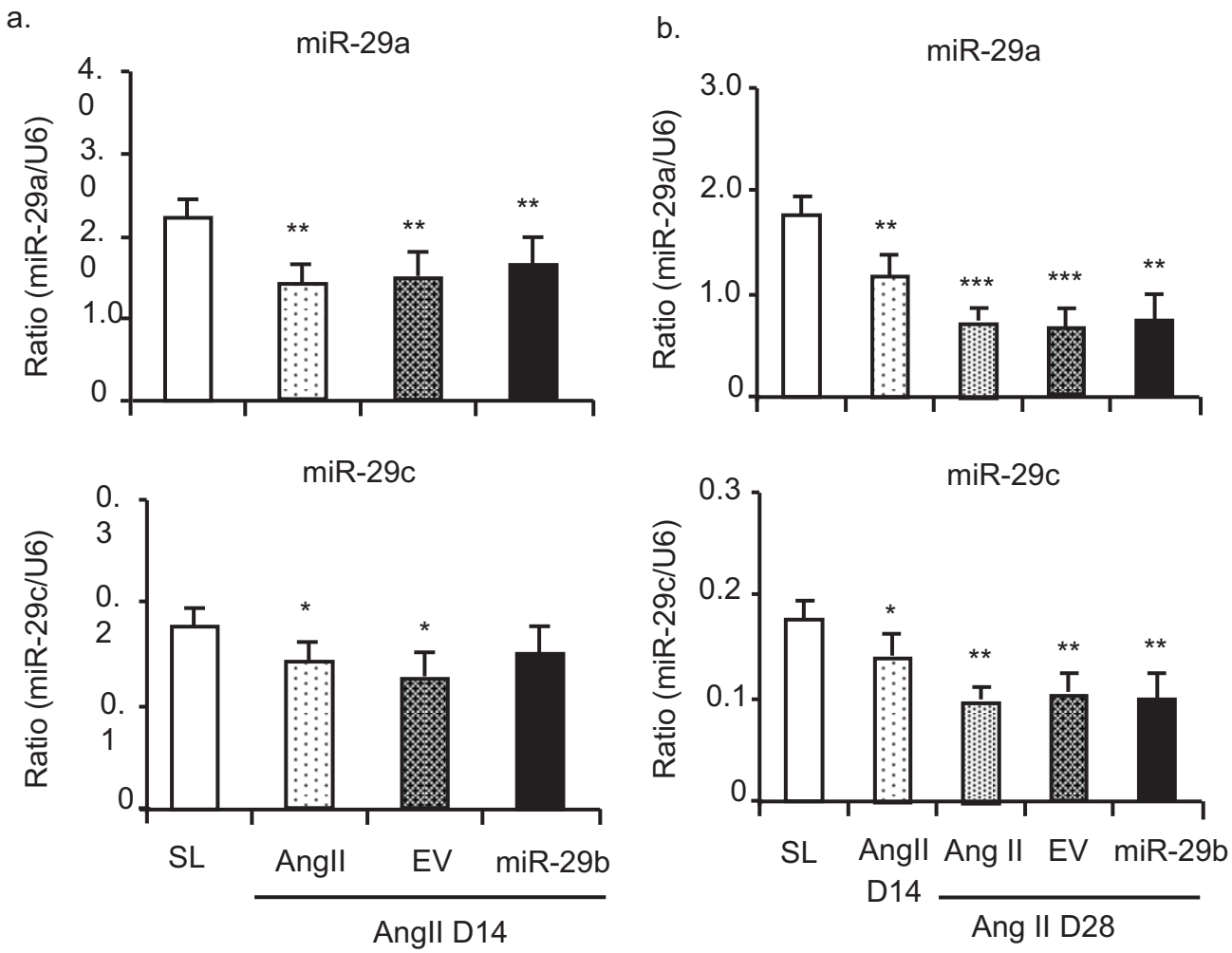


Figure S3. Effect of AngII and overexpression of pre-miR-29b on miR-29a and miR-29c expression in the hypertensive heart. (a) Effect of overexpression of pre-miR-29b on AngII-induced downregulation of miR-29a and miR-29c in a mouse model of hypertension at day 14. (b) Effect of overexpression of pre-miR-29b on AngII-induced downregulation of miR-29a and miR-29c in a mouse model of hypertension at day 28. Note that chronic AngII infusion downregulates miR-29a and miR-29c expression in the hypertensive heart, which is not altered by overexpression of pre-miR-29b. Each bar represents mean ± SEM for 6 animals. *p<0.05, **p<0.01, *** *P*<0.001 when compared with the saline-treated mice. EV, empty vector control.

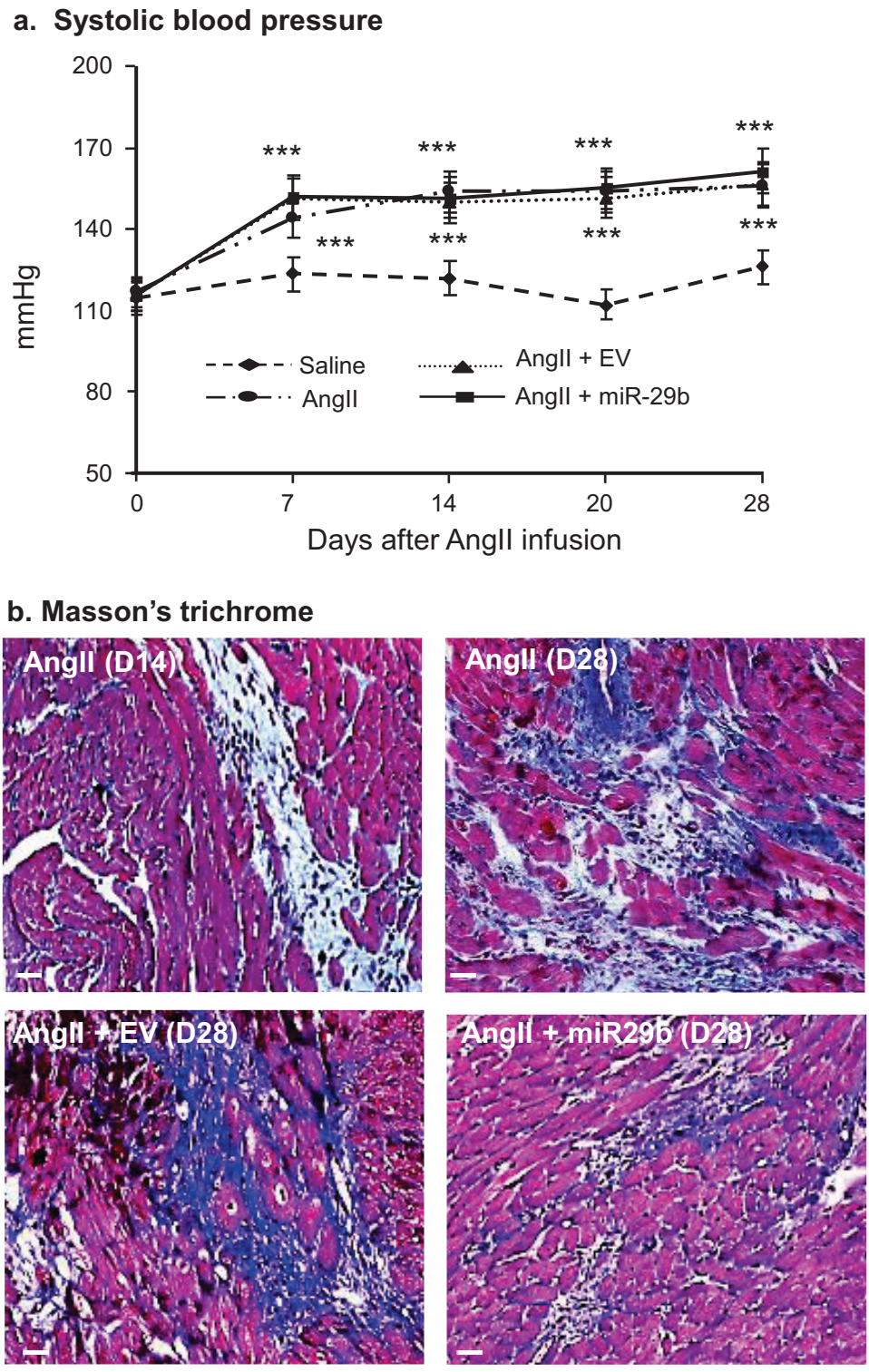


Figure S4. Therapeutic effect of miR-29b on the established hypertensive heart disease over days 14-28 after AngII infusion. (a) Systolic blood pressure. (b) Masson's trichrome staining shows that miR-29b treatment at day 14 after AngII infusion blocks progressive cardiac fibrosis. Data represent the mean ± SEM for six mice. *** P<0.001 compared with baseline at day 0. Scale bar, 50 μM.

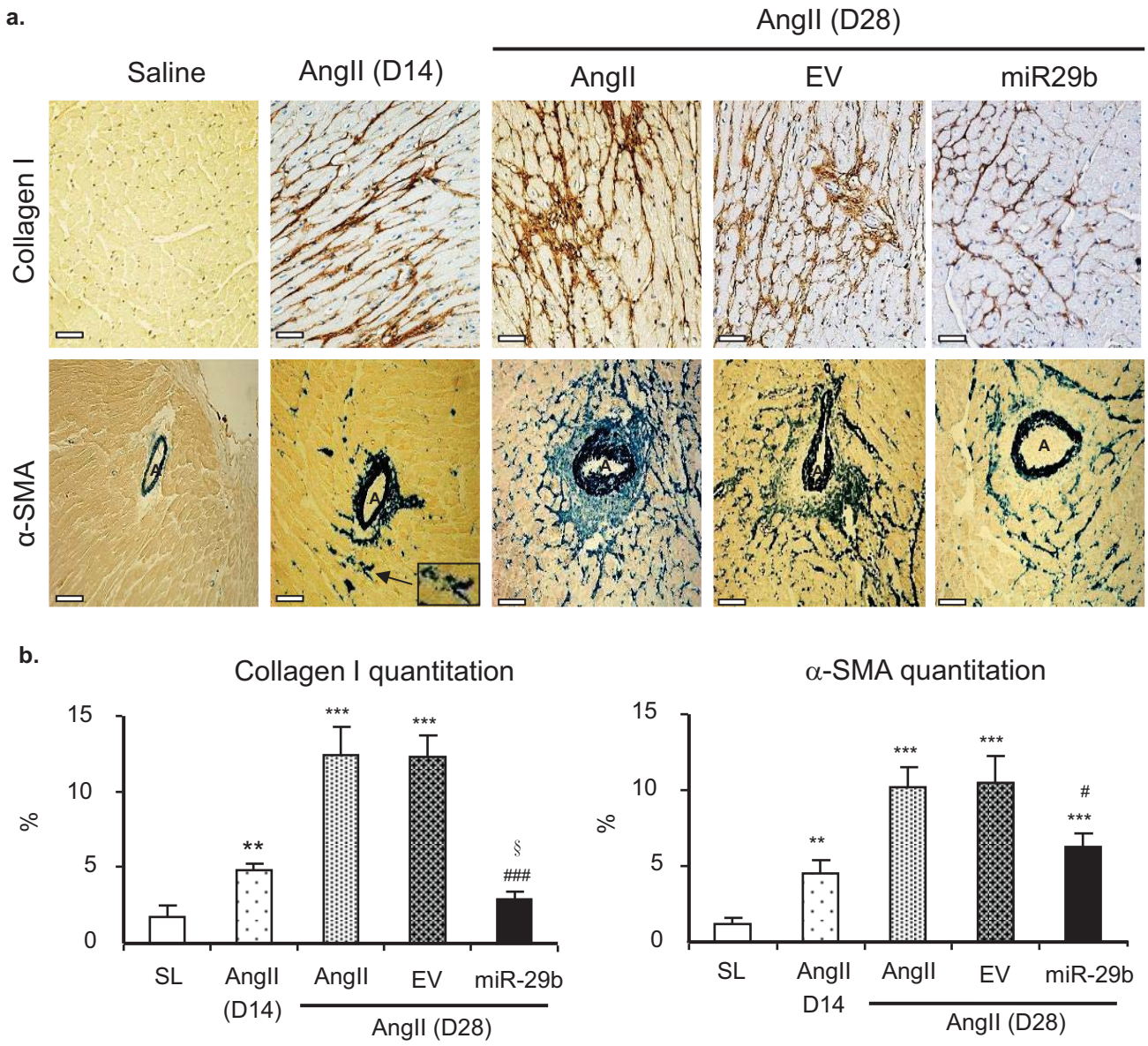
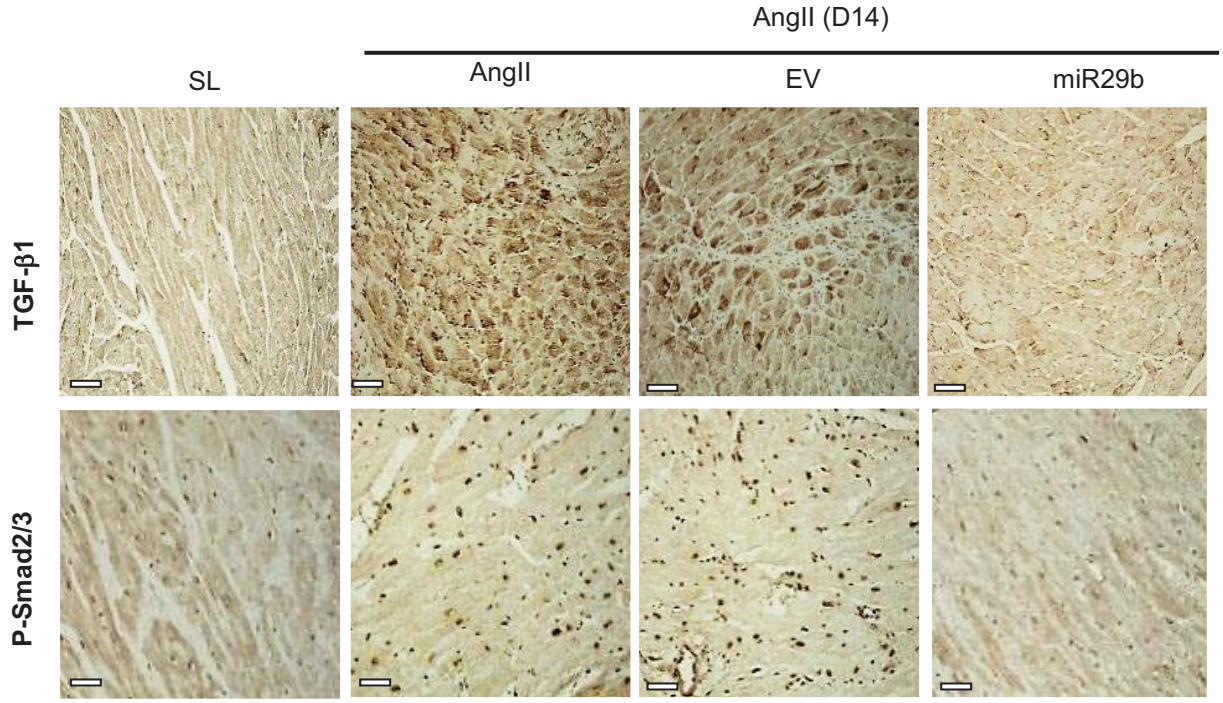


Figure S5 Therapeutic effect of miR-29b on cardiac collagen I and α-SMA⁺ myofibroblast accumulation in established hypertensive cardiac disease at day 28 after AngII infusion. (a) Immunohistochemical analysis of collagen I (Col.I) and α-SMA⁺ myofibroblast accumulation. Note that a-SMA+ myofibroblasts are illustrated in the insert. (b) Quantitative analysis of collagen I and α-SMA⁺ myofibroblast accumulation. Each bar represents mean ± SEM for six mice. ** $P<0.01$, *** $P<0.001$ compared with saline (SL); # $P<0.05$, ### $P<0.001$ compared with empty vector-treated (EV); § $P<0.05$ compared with day 14 disease animals before miR-29b treatment. A, arterioles. Scale bar, 100 μM.

a. Inhibitory effect of miR-29b on TGF-β/Smad signaling at day 14 after AngII infusion



b. Therapeutic effect of miR-29b on TGF-β/Smad signaling at day 28 after AngII infusion

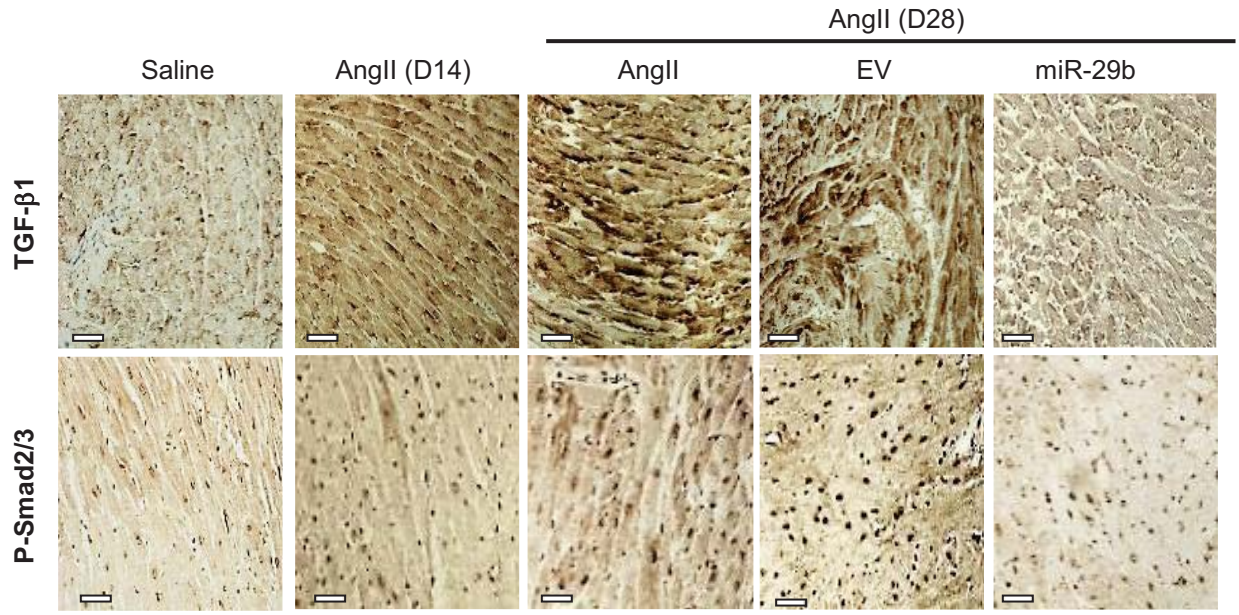


Figure S6. miR-29b treatment inhibits AngII-induced activation of TGF-β/Smad signaling in hypertensive cardiac disease. (a) Effect of miR-29b treatment over days 0-14 on TGF-β1 expression and phospho-Smad2/3 nuclear location in AngII-induced hypertensive heart. (b) Effect of miR-29b treatment over days 14-28 on TGF-ββ1 expression and phospho-Smad2/3 nuclear location in AngII-induced hypertensive heart. Data represent for groups of 6 mice. Scale bar, 50 μM.

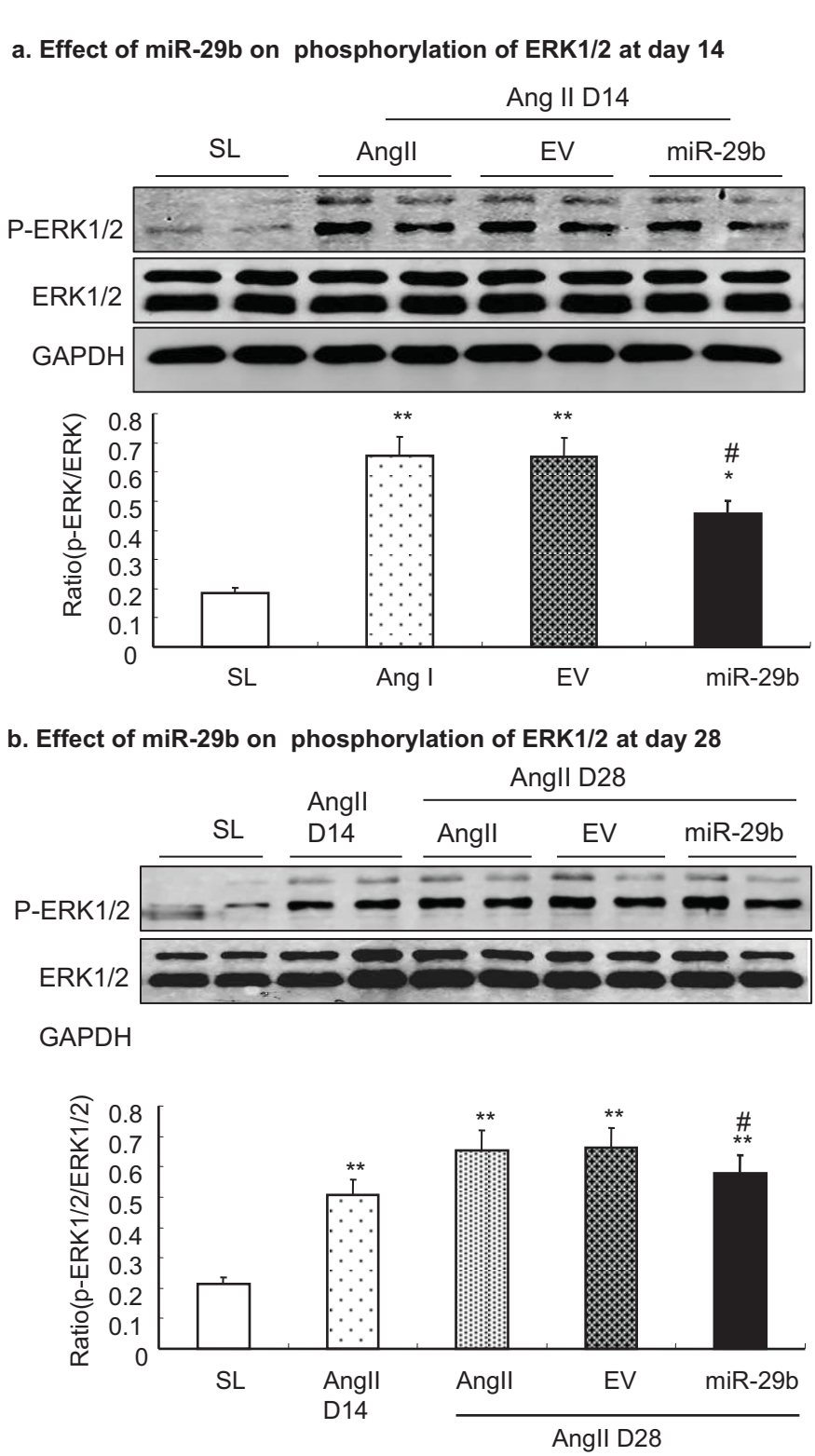


Figure S7. Effect of overexpression of miR-29b on phosphorylation of ERK1/2 in Ang II-induced hypertensive heart. (a) miR-29b treatment from day 0 to day 14 with continuous AngII infusion. (b) miR-29b treatment in the established hypertensive heart disease from day 14 to day 28 with continuous AngII infusion. Each bar represents mean ± SEM for six mice. * $P<0.05$, ** $P<0.01$ compared with saline (SL); # $P<0.05$ compared with empty vector-treated (EV).

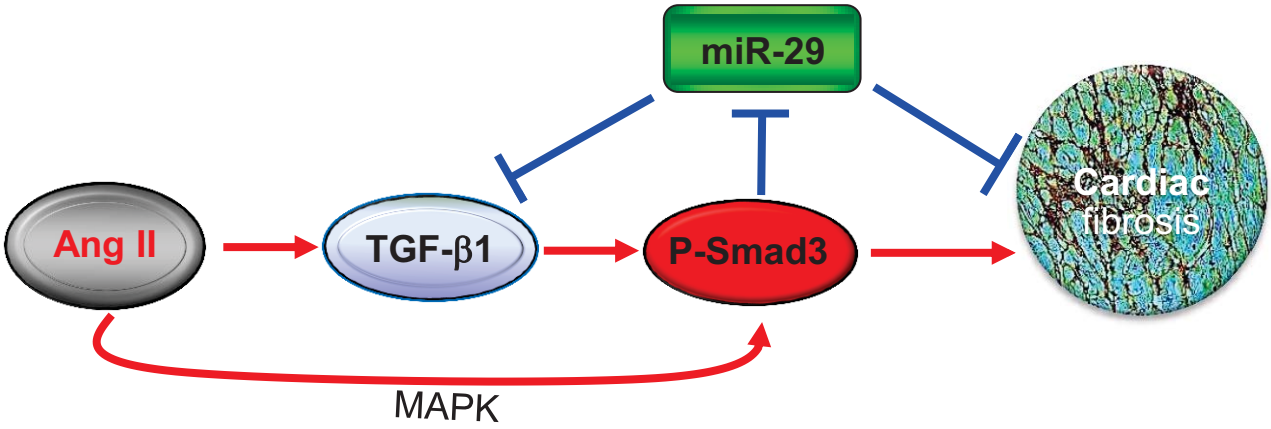


Figure S8. The AngII-Smad3-miR-29 regulatory circuit in cardiac fibrosis. AngII mediates cardiac fibrosis by activating Smad3 to down-regulate miR-29 expression via both TGF-β-dependent and independent mechanisms. In contrast, over-expression of miR-29 can inhibit AngII-induced cardiac fibrosis by directly targeting the collagen matrix synthesis and indirectly by suppressing TGF-β/Smad3 signaling.

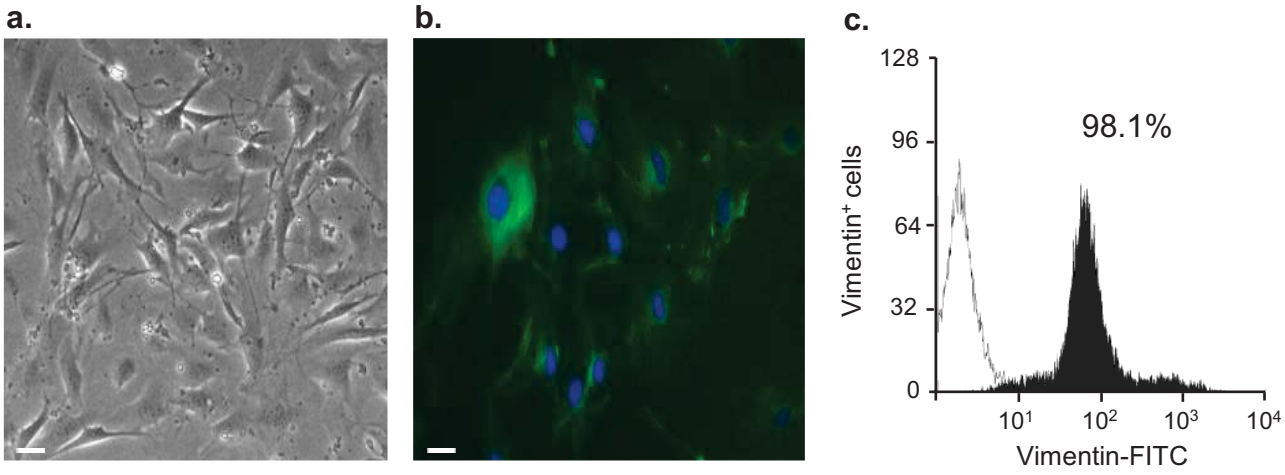


Figure S9. Characterization of primary culture of cardiac fibroblasts (CFs). (a) Morphology; (b) vimentin expression detected by immunofluorescence; (c) vimentin expression detected by flow cytometry. Data represent 4 independent experiments. Scale bar, 20 μM.