SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Fluorescence in situ hybridization (FISH)

The ViewRNA Tissue Kit (Thermo Fisher #19931 & #19932) was employed according to manufacturer's instructions. The following ViewRNA probes were used for detection: miR-129-5p (VM1-10479-VCP), mouse Tnnt2 (VB6-3202146), mouse Pecam1 (VB6-12921), mouse Acta2 (VB6-12923). Tissue slides were mounted with Advantage Mounting Medium (Innovex, #NB300) and imaged on a Nikon confocal microscope.

Conditioned medium experiments

H9C2 cardiac myoblasts were transfected with negative control or miR-129-5p mimics (miRVana, ThermoFisher #4464058, #4464066, 40nM) using Lipofectamine RNAiMAX (ThermoFisher) in Optimem medium (ThermoFisher) for 6h, after which medium was replaced with DMEM+1%FCS+ 1%antibiotic/antimycotic. After 24h, conditioned medium from H9C2 cells was transferred to mouse primary cardiac fibroblasts in a 1:1 ratio with fresh DMEM-F12+0.5% FCS. After 24h incubation, total RNA was isolated from cardiac fibroblasts using Trizol (Thermo Fisher). For mRNA RT-qPCR, reverse transcription was performed using the High Capacity cDNA Reverse Transcription kit (Thermo, #4368814) and qPCR was performed using Power Up SYBR Green Master Mix (Thermo, # A25779) on a BioRad CFX Connect PCR detection system. Primers for *postn* and *runx2* are listed in Supplemental Table I.

SUPPLEMENTAL TABLE

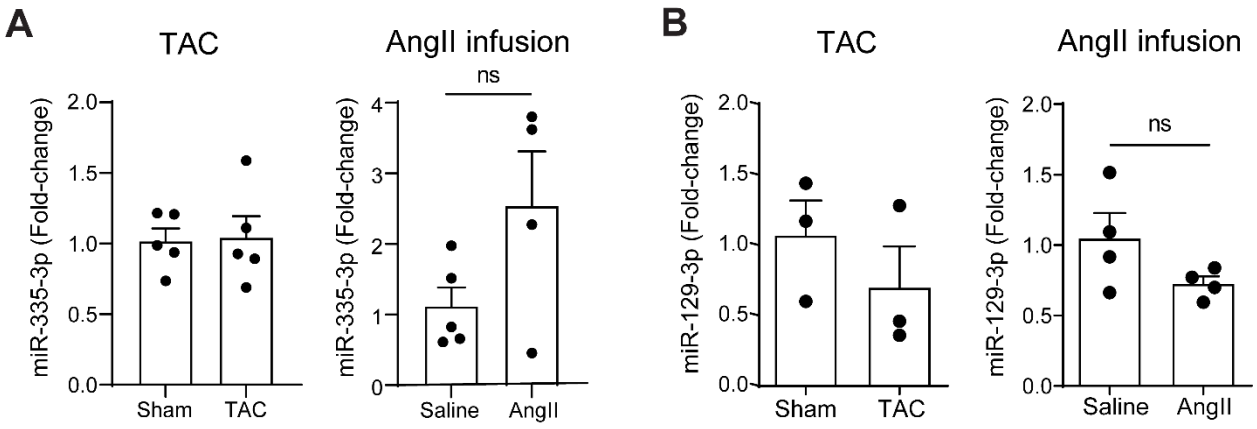
Mouse primer	Forward	Reverse
Alp3	aacccagacacaagcattcc	gcctttgaggtttttggtca
Aspn	aggacacgttcaagggaatg	aggccttttggaattgaggt
Postn	agtgctctgaggccatcact	aggtcggtgaaagtggtttg
Rplp0	ctctcgctttctggagggtg	acgcgcttgtacccattgat
Runx2	gccgggaatgatgagaacta	ggaccgtccactgtcacttt
Sox9	agctcaccagaccctgagaa	tcccagcaatcgttaccttc

Supplemental Table I. Mouse primer sequences. Alp3: alkaline phosphatase, aspn: asporin, postn: periostin, rplp0: ribosomal protein lateral stalk subunit P0, runx2: runt-related transcription factor 2, sox9: SRY-box transcription factor 9.

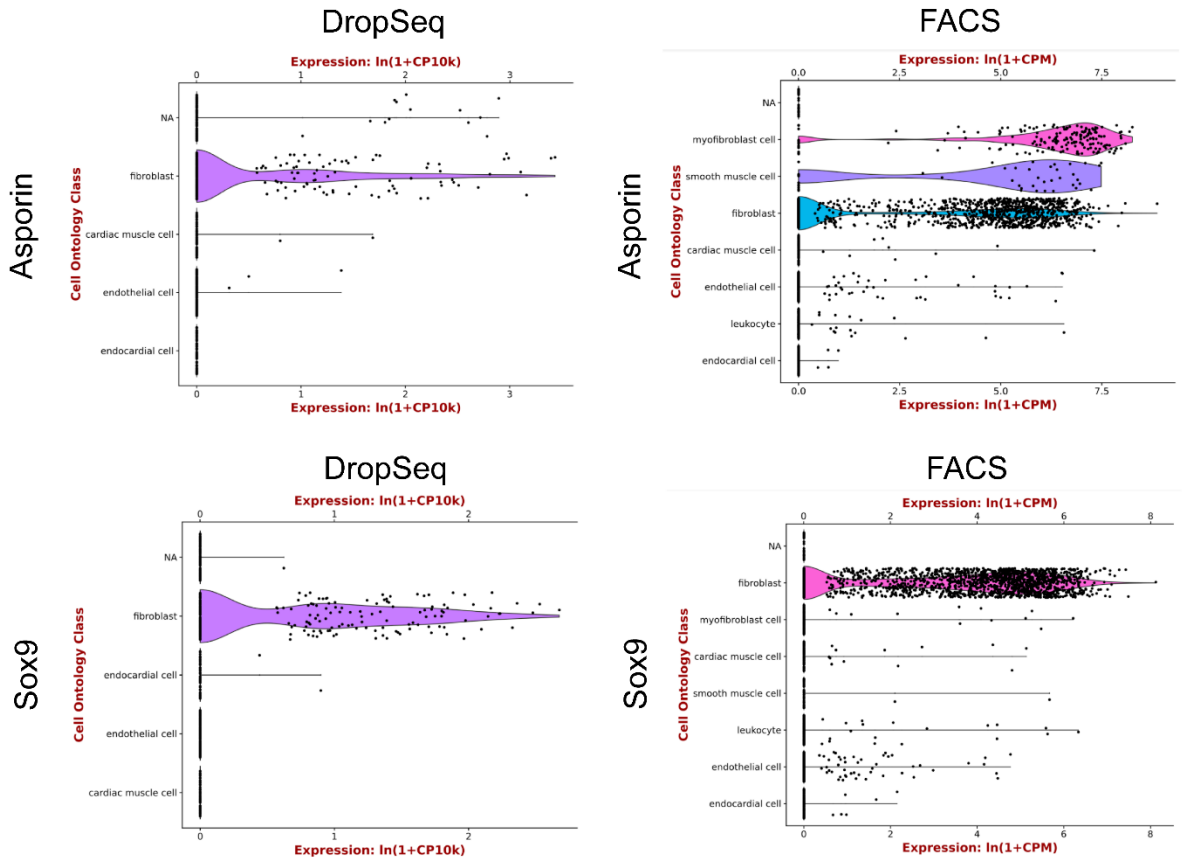
	Control 1	Control 2	Control 3	Control 4
Age	63	91	67	30
Sex	Male	Female	Female	Female
Diagnosis	Head & neck cancer	Urinary tract infection, DM	Neurodegenerative disease	Chorioamnionitis, HELLP syndrome
LVEF %	60-65%	N/A	N/A	N/A
Diastolic dysfunction	N/A	N/A	N/A	N/A
E/A ratio	N/A	N/A	N/A	N/A
Pathology/autopsy: Fibrosis	N/A	N/A	N/A	N/A
Pathology/autopsy: Cardiomegaly	Mild	N/A	N/A	N/A
Pathology/autopsy: Cardiomyocyte hypertrophy	N/A	N/A	N/A	N/A
	HF 1	**HF 2**	**HF 3**	**HF 4**
Age	55	67	62	78
Sex	Male	Male	Female	Female
Diagnosis	CHF, HTN, ESRD, DM, CAD	CHF, HTN, ESRD, CAD	CHF, DM, CAD	CHF, HTN, DM
LVEF %	46	79	N/A	75
Diastolic dysfunction	Grade III	Grade II	N/A	Grade I
E/A ratio	2.1	1.4	N/A	1.8
Pathology/autopsy: Fibrosis	Posterior and anterior LV	Patchy interstitial	Subendocardial, patchy interstitial and perivascular in LV and IVS	Interstitial and perivascular
Pathology/autopsy: Cardiomegaly	Yes	N/A	Yes	Yes
Pathology/autopsy: Cardiomyocyte hypertrophy	Yes	Yes	N/A	Yes

Supplemental Table II. Patient characteristics. CAD: coronary artery disease; CHF: congestive heart failure; DM: diabetes mellitus; ESRD: end-stage renal disease, HTN: hypertension, LVEF: left ventricular ejection fraction

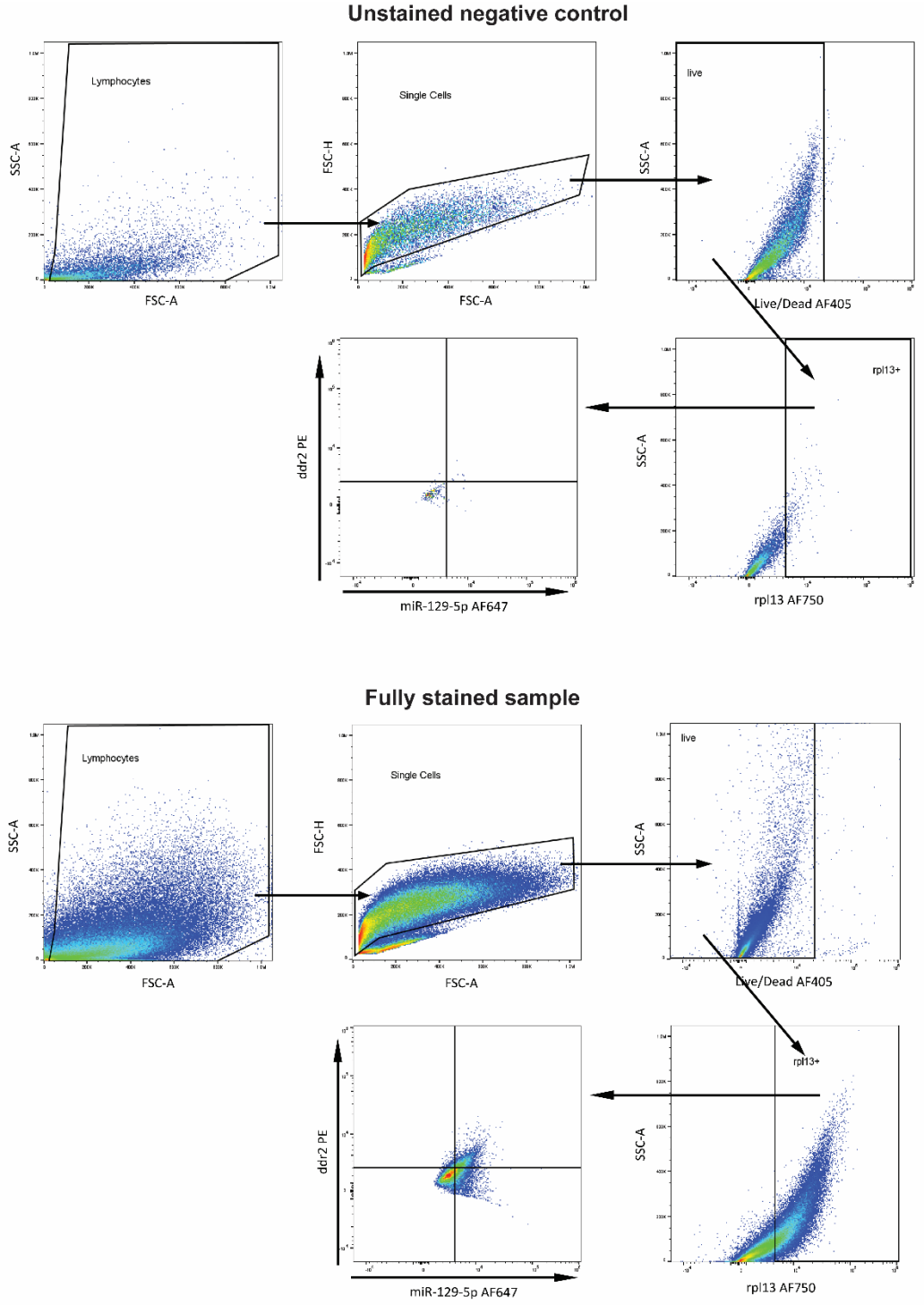
SUPPLEMENTAL DATA



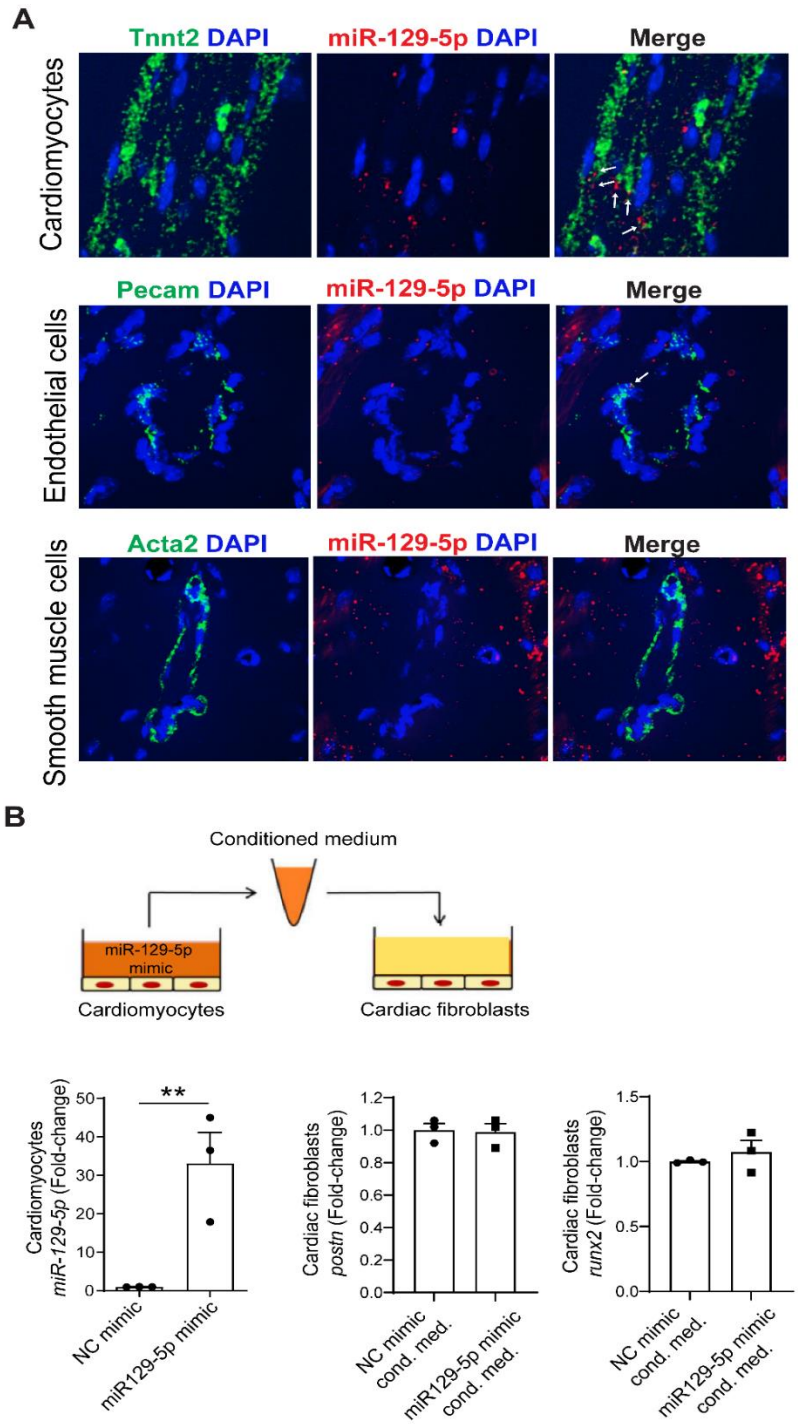
Supplemental Figure I. Validation of bioinformatic analysis. A. miR-335-3p expression in mouse LV measured by qPCR. **B.** miR-129-3p expression in mouse LV measured by qPCR. Data presented as mean+SEM, student's t-test. AngII: Angiotensin II, FACS: flow-assisted cell sorting, Ns: non-significant, TAC: transverse aortic constriction.



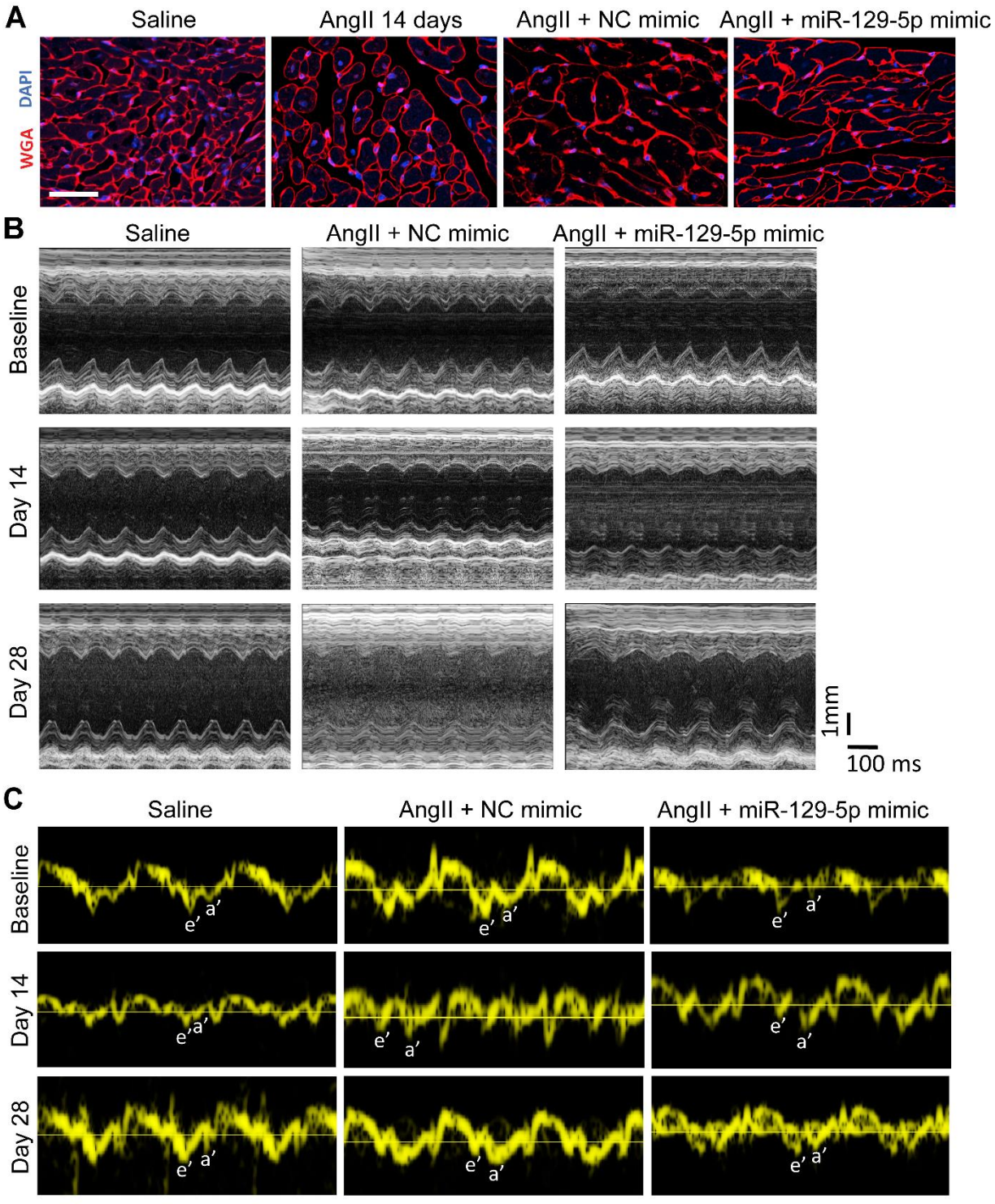
Supplemental Figure II. Expression of Asporin and Sox9 in different cell types of the healthy mouse heart. Measured by DropSeq and FACS in the Tabula Muris publically-available dataset.



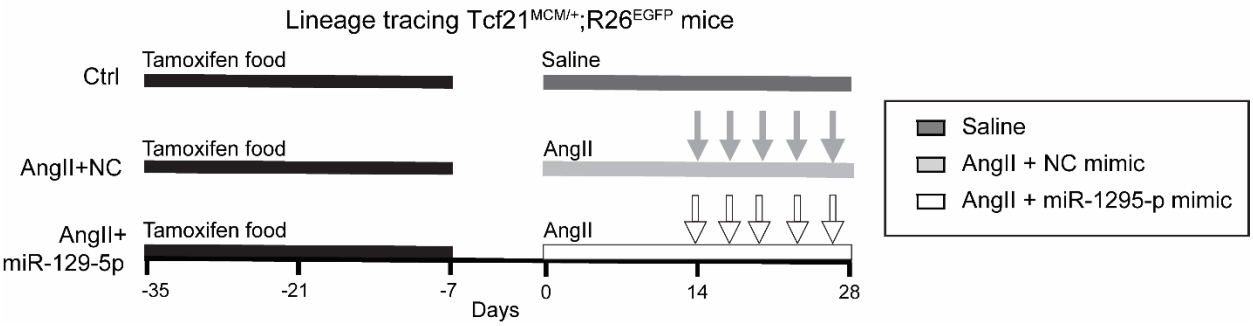
Supplemental Figure III. Gating strategy for PrimeFlow experiments in mouse cardiac single cell suspension with examples of a negative control unstained sample and a fully stained sample.



Supplemental Figure IV. miR-129-5p expression in cardiac cell types. A. miR-129-5p expression in cardiomyocytes, endothelial cells, and smooth muscle cells in the healthy mouse hearts as assessed by fluorescence *in situ* hybridization. Tnnt2: cardiac muscle troponin t, Pecam1: platelet and endothelial cell adhesion molecule 1, Acta2: actin alpha 2 smooth muscle **B.** Expression of myofibroblast gene *postn* and osteogenic fibroblast gene *runx2* in cardiac fibroblasts after incubation with conditioned medium from cardiomyocytes differentially expressing miR-129-5p. **>p=0.01, Student's t-tes**t**



Supplemental Figure V. Cardiac parameters in mice. A. Representative images of cardiomyocyte hypertrophy in mouse hearts assessed by wheat germ agglutinin staining. **B.** Representative M-mode traces. **C.** Representative tissue Doppler traces. **D.** Il-6 expression in hearts of AngII-infused mice treated with negative control or miR-129-5p mimic as assessed by qPCR. *>p=0.05, **>p=0.01, ANOVA with Holm Bonferonni correction. AngII: Angiotensin II. NC: negative contro



Supplemental Figure VI. Experimental setup for tracing of CF of the transcription factor 21 (Tcf21) lineage in mice ($Tcf21^{MCM/+};R26^{EGFP}$ mice). $Tcf21^{MCM/+};R26^{EGFP}$. Mice were fed a Tamoxifen diet 4 weeks prior to AngII pump implantation to activate eGFP expression in Tcf21-lineage CF. After 14 days of AngII infusion, mice received either miR-129-5p mimics or negative control mimics intravenously every 3 days for the remainder of the experiment.