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## Supplemental Information

# miR-1468-3p Promotes Aging-Related Cardiac Fibrosis

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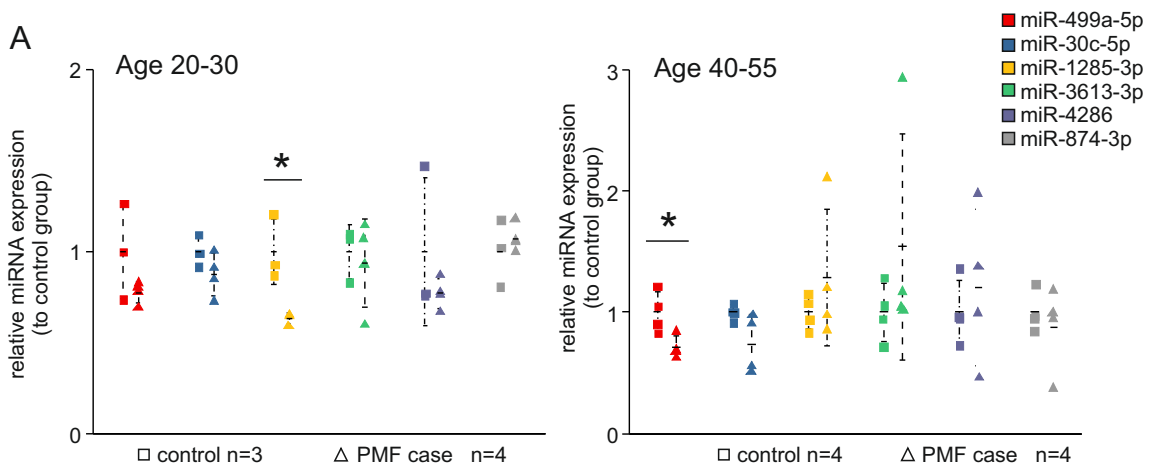


Figure S1. Analysis for microRNA expression in left ventricular samples of healthy control subjects (control) and victims of sudden cardiac death (SCD) with primary myocardial fibrosis (PMF case). The data are shown as relative to respective age control.

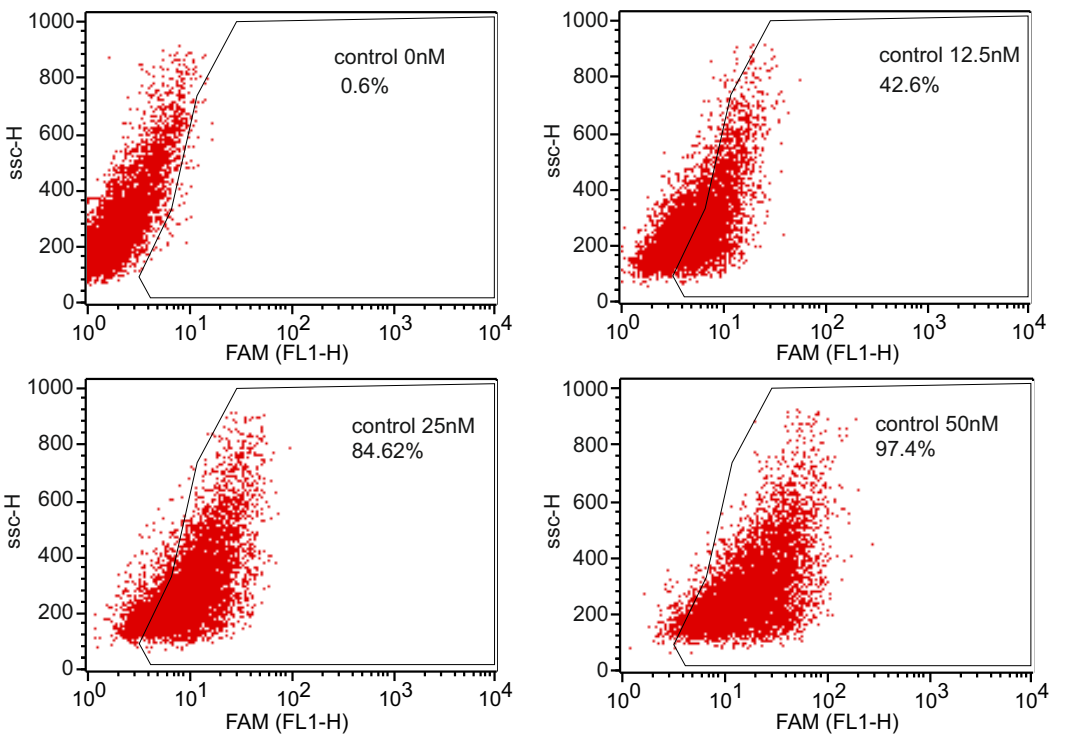


Figure S2. Human cardiac fibroblasts (hCFs) were transfected with different concentrations of fluorescence-labeled synthetic control sequence. Shown is fluorescence-activated cell sorting of hCFs at 24h after transfection.

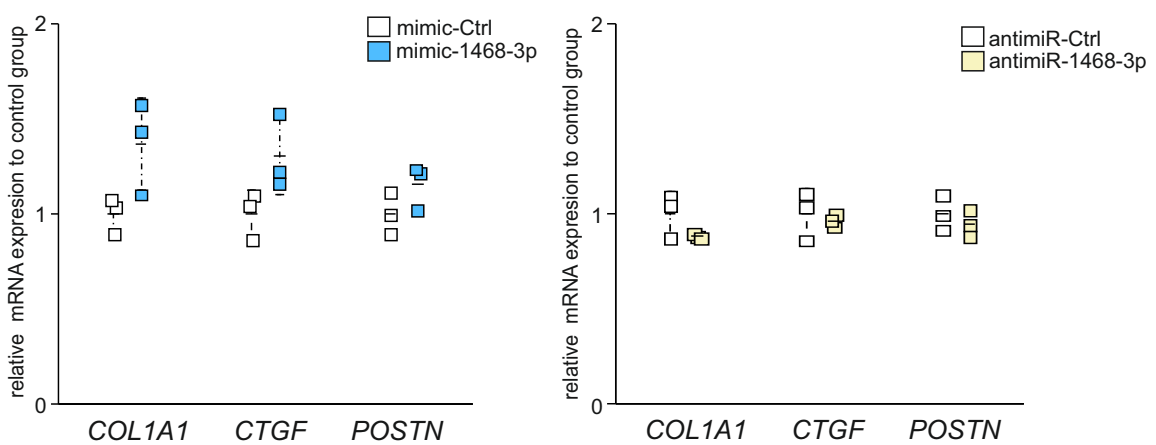


Figure S3. Left panel: Cultured human cardiac fibroblasts (hCFs) were treated with 20 nM of mimic-1468-3p or control sequence (mimic-Ctrl). Right panel: hCFs were treated with 50 nM of antimiR-1468-3p or control sequence (antimiR-Ctrl). Shown is qPCR analysis for collagen 1a1 (Col1a1), connective tissue growth factor (CTGF) and periostin (Postn).

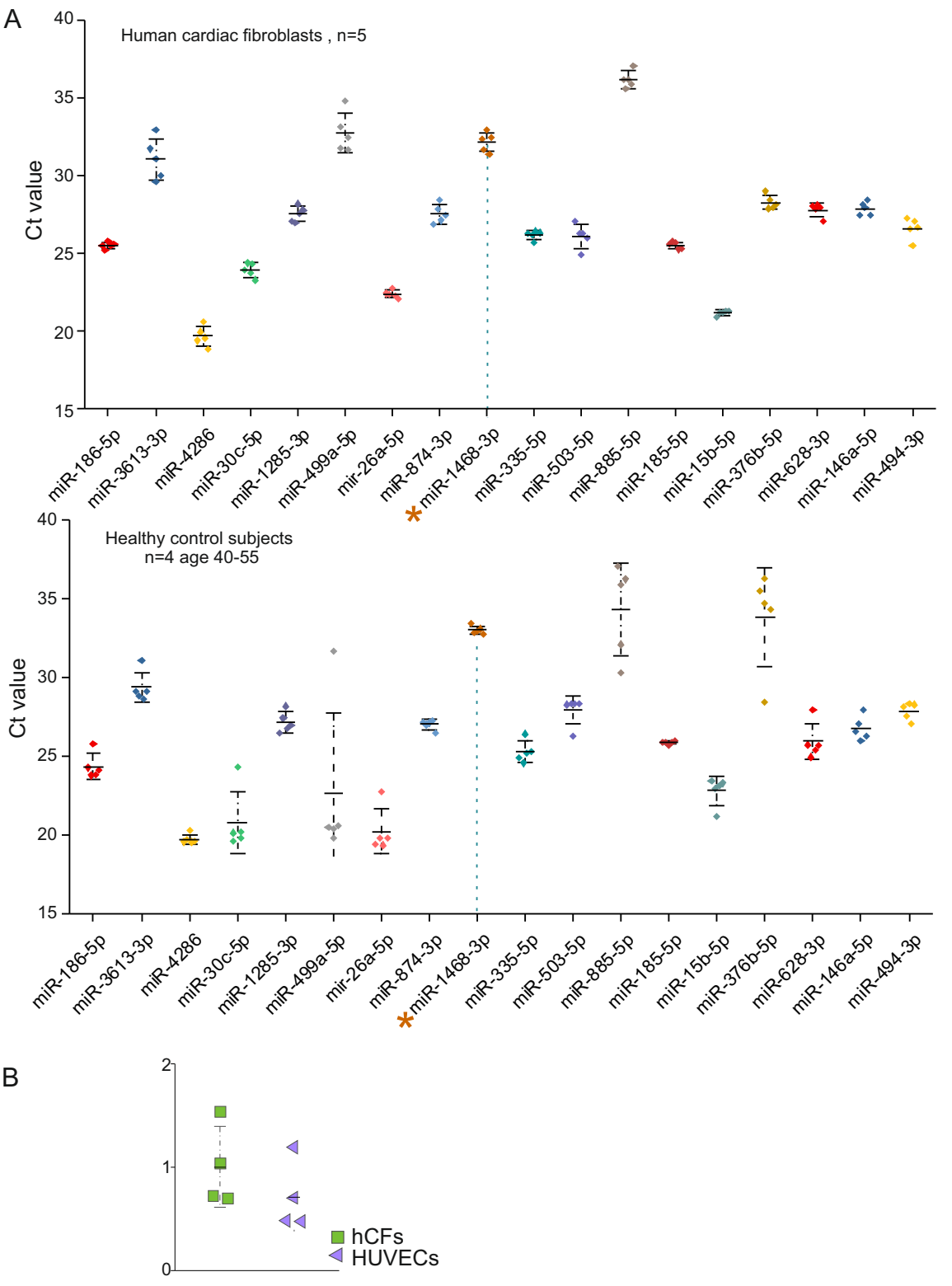


Figure S4. (A) Analysis for microRNA expression levels in cultured human cardiac fibroblasts (upper panel) and in left ventricular samples of healthy control subjects (lower panel). Shown are qPCR threshold levels (midpoint of the linear phase of qPCR amplification curve) for individual microRNAs. (B) Shown is qPCR analysis for hsa-miR-1468-3p expression in human cardiac fibroblasts and human umbilical vein endothelial cells.***p<0.001 vs mimic-Ctrl. N.S., no significance.

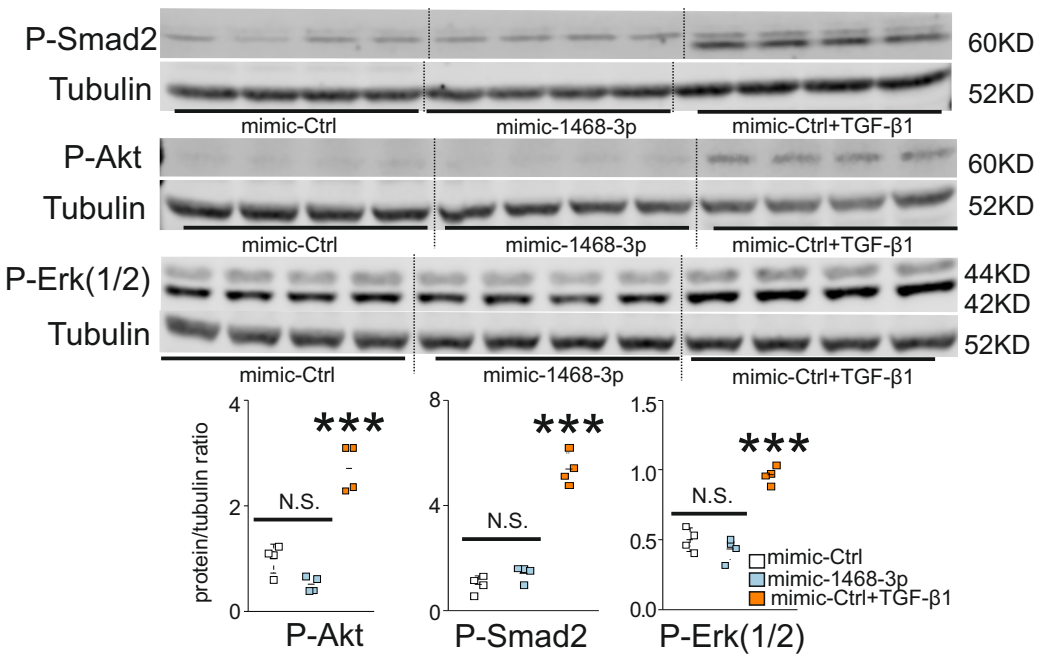


Figure S5. Human cardiac fibroblasts were treated with 20 nM of mimic-1468-3p or control sequence (mimic-Ctrl) for 24h and treated with TGF-β1 (5 ng/ml) for 20h where indicated. Shown is Western blot and densitometry analysis (lower pannel) for phosphorylated Smad2, phosphorylated Akt and phosphorylated extracellular signal-regulated kinase (ERK1/2). Tubulin was used as a loading control. Data are expressed as mean±SD. One-way ANOVA followed by Tukey´s post hoc test was used. ***p<0.001 vs mimic-Ctrl. N.S., no significance.

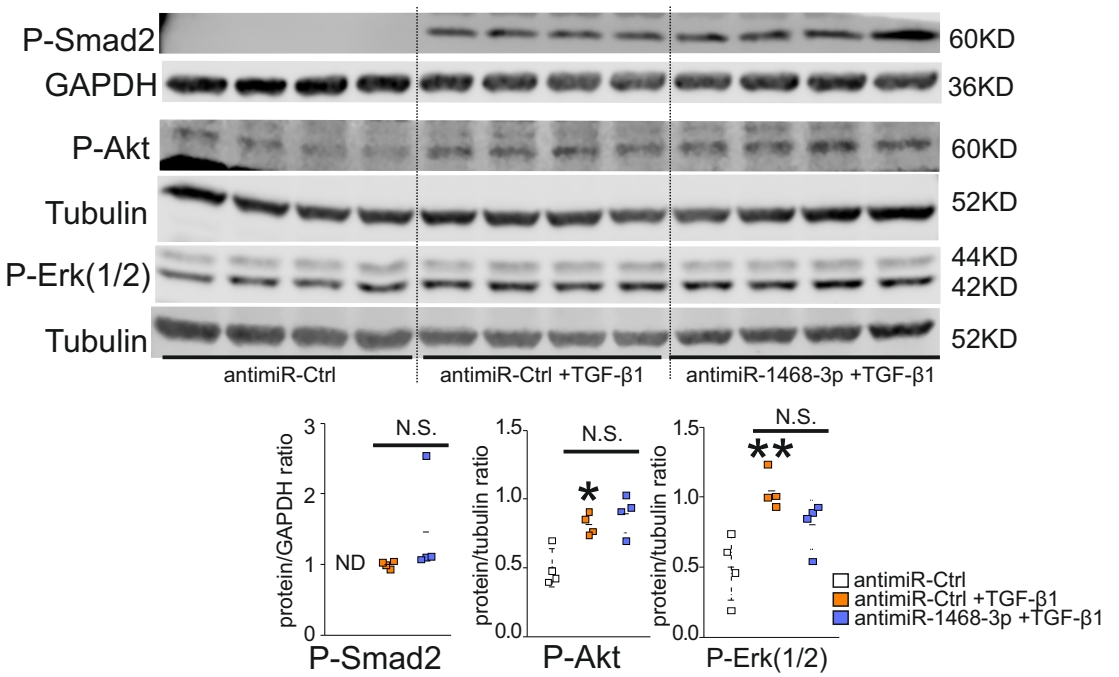


Figure S6. Human cardiac fibroblasts were treated with 50 nM of antimiR-1468-3p or control sequence (antimiR-Ctrl) for 24h and treated with TGF-β1 (5 ng/ml) for 20h where indicated. Shown is Western blot analysis and densitometry analysis (lower panel) for phosphorylated Smad2, phosphorylated Akt and phosphorylated extracellular signal-regulated kinase (ERK1/2). Tubulin was used as a loading control. Data are expressed as mean±SD. One-way ANOVA followed by Tukey´s post hoc test was used. *p<0.05, **p<0.01, vs antimiR-Ctrl. N.S., no significance.

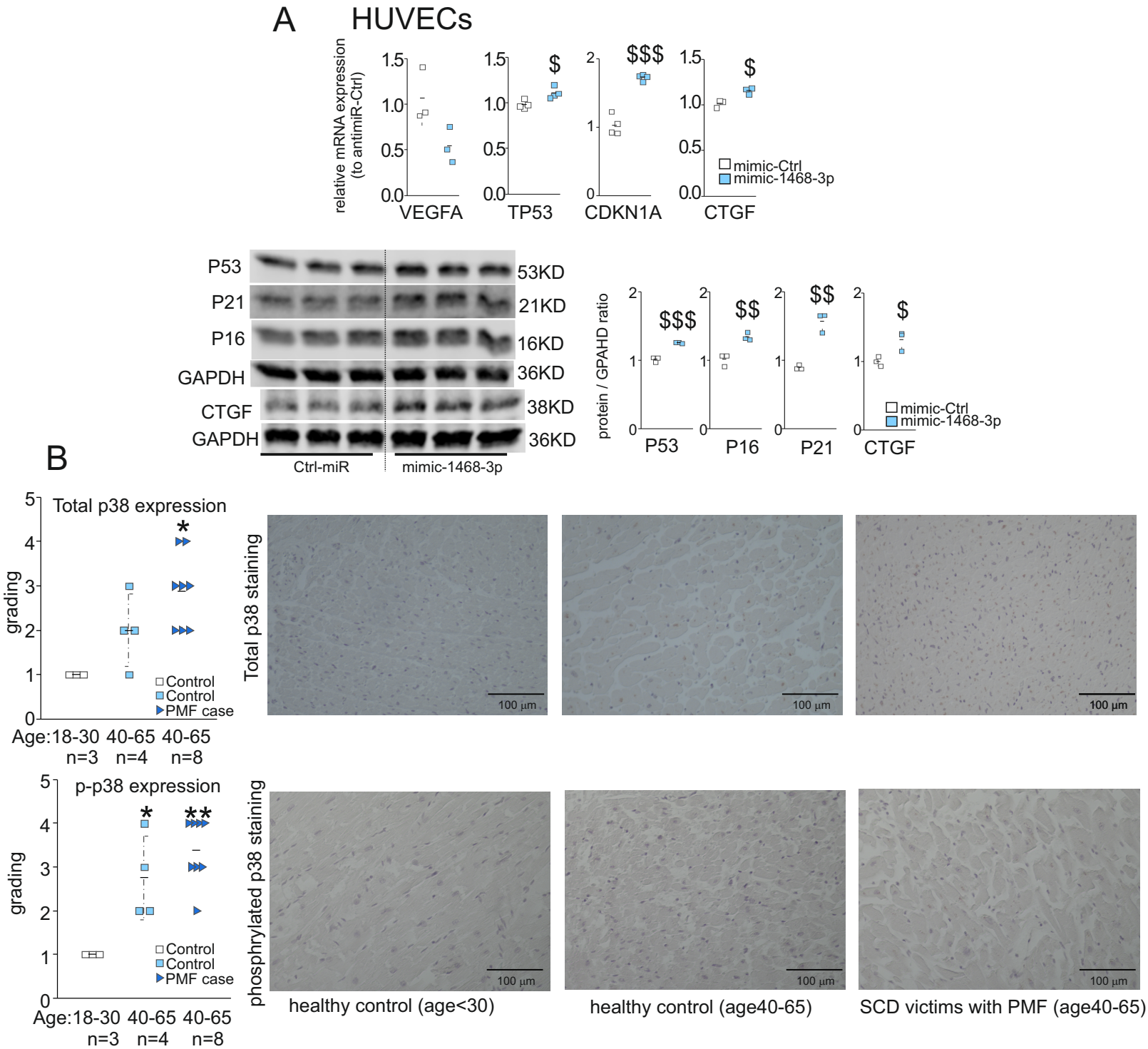


Figure S7 (A) Cultured human umbilical vein endothelial cells (HUVECs) were treated with 20 nM of mimic-1468-3p or control sequence (mimic-Ctrl). Upper panel: shown is qPCR analysis for Vascular endothelial growth factor A (VEGFA), Tp53, CDKN1A and connective tissue growth factor (CTGF). Lower panel: shown is Western blot analysis (left panel) and densitometry analysis (right panel) for p53, p21, p16 and CTGF. GAPDH was used as a loading control. (B) Analysis for total p38 and phosphorylated p38 expression in healthy control hearts at the age of 18-30yrs or 40-65 yrs, and in hearts of sudden cardiac death (SCD) victims with primary myocardial fibrosis (PMF) at the age of 40-65 yrs. Blind-grading (left panel) and representative staining of total p38 and phosphrylated p38 in each indicated group. Data are expressed as mean±SD. $p<0.05, $$p<0.01, $$$p<0.001 vs mimic-Ctrl. *p<0.05, **p<0.01 vs healthy control hearts (age:18-30yrs)

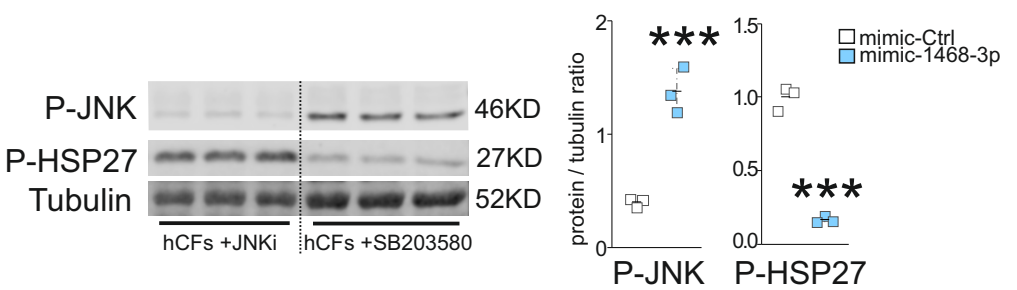


Figure S8. Human cardiac fibroblasts were treated with 2 μM JNK inhibitor I (JNKi) or 5 μM SB203580 (p38 inhibitor) for 24h. Shown are Western blot analysis and densitometry analysis (right panel) for phosphorylated c-Jun N-terminal kinase (p-JNK) and phosphorylated heat shock protein 27 (p-HSP27). Tubulin was used as a loading control. Data are expressed as mean±SD. ***p<0.001 vs mimic-Ctrl.

Table S1. RNA sequencing analysis of differential expressed (DE) genes after antimiR-1468-3p administration in TGF-β1 treated cells. Human cardiac fibroblasts (hCFs) were treated with antagomir of miR-1468-3p (antimiR-1468-3p) or control sequence (antimir-Ctrl) for 24h and treated with TGF-β1 (5 ng/ml) for 20h. At the end of the experiment, RNA was collected for RNA sequencing analysis. The DE transcripts were filtered by fold change (FC) >1.5 increase (labeled red) or decrease (labeled blue) in relative expression ratio and FDR-adjusted p-value (FDR p) <0.05 between compared groups.

Table S2. Treatment of human cardiac fibroblasts with antimiR-1468-3p modulates expression of genes related to cell cycle. Human cardiac fibroblasts (hCFs) were treated with antagomir of miR-1468-3p (antimiR-1468-3p) or control sequence (antimiR-Ctrl), and after 24h treated TGF-β1 (5 ng/ml) for 20h. At the end of the experiment, RNA samples were collected for RNA sequencing. Shown are Gene Ontology (GO) terms identified by the analysis of the 353 genes differently expressed in antimiR-1468-3p treated hCFs.

Table S3. Analysis for miR-1468-3p regulation of fibrotic and fibrosis-related signaling genes.
Human cardiac fibroblasts (hCFs) were treated with antagomir of miR-1468-3p (antimiR-1468-3p) or control sequence (antimiR-Ctrl) for 24h and treated with TGF-β1 (5 ng/ml) for 24h where indicated. At the end of the experiment, RNA was collected for RNA sequencing analysis. Shown are normalized expression levels of TGF-β/SMAD, p38/MAPK, phosphorylation and fibrosis related genes that were investigated. Student's t tests with equal variances were performed between antimiR-Ctrl +TGF-β1 vs antimiR-1468-3p+TGF-β1 treated cells, and genes that were significantly regulated by antimiR-1468-3p were labelled in red.