Supplementary materials:

Echocardiography

Transthoracic echocardiography was performed at 2 and 4 weeks after second hand smoke exposure (SHS) using a Hewlett-Packard Sonos 5500 ultrasound machine with a 15-MHz linear-array transducer, as described previously. Animals were anesthetized with a half dose of the ketamine-xylazine-atropine mixture used. M-mode images were recorded and analyzed offline. The following parameters were measured and calculated: left ventricular interior diastolic dimention(LVIDd). left ventricular interior systolic dimention(LVIDs). Interventricular septum at systolic and diastolic (LVIDs, LVIDd), LV posterior wall thickness at systole and diastole (LVPWs, LVPWd), fractional shortening (FS%) and ejection fractional (EF%).

Western blotting analysis

Heart tissue was homogenized with a Polytron mixer in PBS buffer. Homogenates were then centrifuged at 12,000 rpm for 30 min at 4 ℃. SDS-PAGE was carried out with polyacrylamide gels. Forty micrograms of supernatant samples was electrophoresed at 100 V for 1.5 hr. Electrophoresed proteins was transferred to PVDF papers using a Hoefer Scientific Instruments Transphor unit at 150 mA for 2 hr. Subsequently, membranes were blocked with 5% non-fat milk for 1 hr at room temperature. Monoclonal antibodies were diluted 1:200 in antibody binding buffer (TBS) and afterwards incubated overnight (4℃) with the the following antibodies (1:500): MMP 2, MMP 9, TIMP-1, TIMP-2, TIMP-3, TIMP-4, α-tubulin (Cell Signaling, Leiden, The Netherlands). Incubations were performed at room

temperature for 3.5 hr. Washed the immunoblots three times in 5 mL blotting buffer for 10 min and then immersed in the Horseradish peroxidase-conjugated anti-mouse (1:1000) or anti-rabbit (1:500) secondary antibodies (Santa Cruz Lab, CA). The membranes were then washed in blotting buffer for 10 min three times. Color development was presented in chemiluminescent detection of proteins by ECL chemiluminescence.

Statistical analysis

Quantitation was carried out by scanning and analyzing the intensity of the hybridization signals using FUJIFILM Imagine program for western blot analysis. Statistical analysis of the data was performed using SigmaStat software. Results were expressed as mean ± SEM. Statistical analysis was performed using the analysis of variance. When assessing multiple groups, two-way ANOVA was utilized with turkeys post hoc test, unpaired, two-sided student's t test was used when indicated.

Supplementary Legend:

Figure S1

SHS exposure causes left ventricular hypertrophy in young SHS exposure, old control and old SHS exposure rats.

(A). Heart weight (g). (B). Representative M-model echocardiography short axis view from young control (left up-panel), young SHS-exposed (right up-panel), old control (left down-panel), old-exposed (right down-panel) rat hearts. (C). Quantification analysis of M-model echocardiography results from young control, young SHS-exposed, old control, old-exposed rat hearts. Data are presented as means ± SEM. $^{*}p<0.05$ compared with young control. $^{\#}p<0.05$ compared with aging control. (D). left ventricular fractional shortening (LVFS); left ventricular ejection fractional (LVEF). Data are presented as means ± SEM. $^{*}p<0.05$ compared with young control. $^{\#}p<0.05$ compared with aging control.

Figure S2

Reduced MMPs and increased TIMPs in SHS-exposed aging heart.

(A). Western blot analyses of protein levels of MMP 2 and MMP 9 in young control, young SHS-exposed, old control, old-exposed rat hearts. α-tubulin expression is the protein loading control. Data are presented as mean ± SEM, $^{*}p<0.05$, $^{**}p<0.01$ compared with young control, $^{\#}p<0.05$ compared with aging control. (B). Western blot analyses of protein levels of TIMP-1, TIMP-2, TIMP-3 and TIMP-4 in young control, young SHS-exposed, old control, old-exposed rat hearts. α-tubulin expression is the protein loading control. Data are presented as mean ± SEM, $^{*}p<0.05$, $^{**}p<0.01$ compared with young control, $^{\#}p<0.05$ compared with aging control.

Figure S1

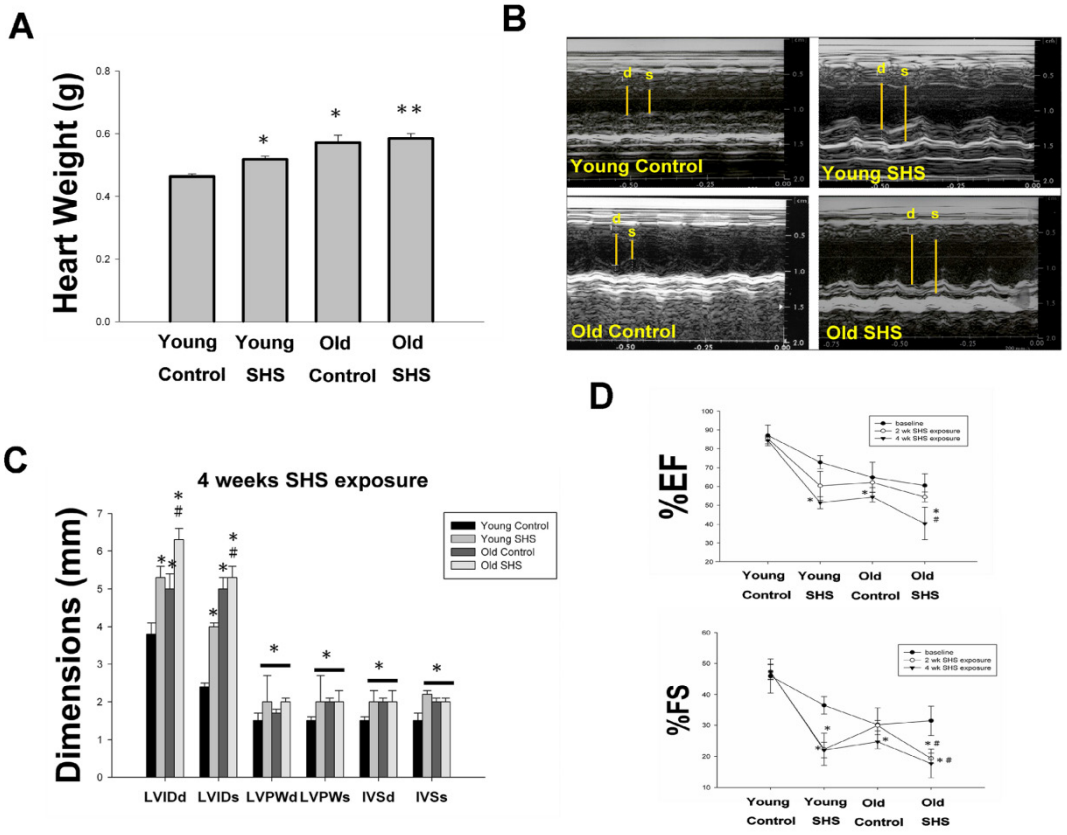
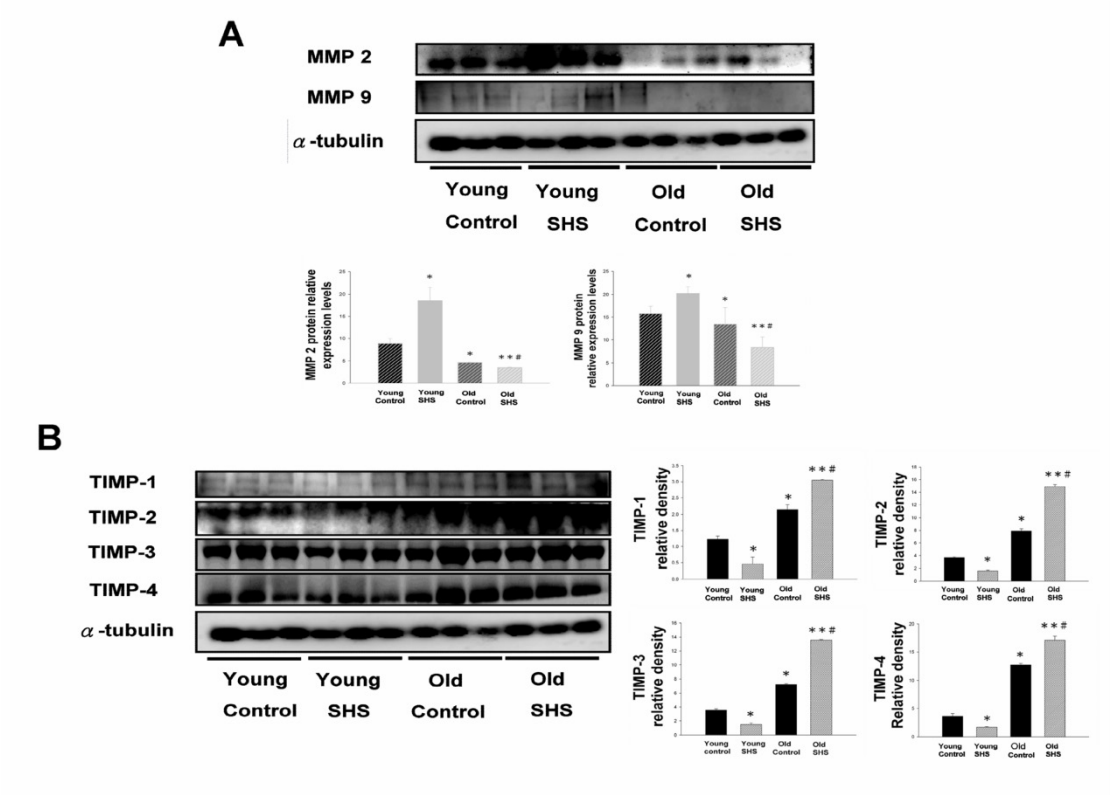

A
Heart Weight (g)
0.0
0.2
0.4
0.6
0.8
Young Control
Young SHS
Old Control
Old SHS
B
Young Control
Young SHS
Old Control
Old SHS
C
4 weeks SHS exposure
Dimensions (mm)
Young Control
Young SHS
Old Control
Old SHS
LVIDd
LVIDs
LVPWd
LVPWs
IVSd
IVSs
D
%EF
%FS
baseline
2 wk SHS exposure
4 wk SHS exposure
Young Control
Young SHS
Old Control
Old SHS

Figure S2


A
MMP 2
MMP 9
α-tubulin
Young Control
Young SHS
Old Control
Old SHS
MMP 2 protein relative expression levels
MMP 9 protein relative expression levels
B
TIMP-1
TIMP-2
TIMP-3
TIMP-4
α-tubulin
TIMP-1 relative density
TIMP-2 relative density
TIMP-3 relative density
TIMP-4 Relative density