ONLINE SUPPLEMENT

ONLINE SUPPLEMENT

MMP-2 MEDIATES ANGIOTENSIN II-INDUCED HYPERTENSION UNDER THE TRANSCRIPTIONAL CONTROL OF MMP-7 AND TACE

Authors: Jeffrey Odenbach, B.Sc; Xiang Wang, M.Sc; Stephan Cooper, B.Sc; Fung Lan Chow, M.Sc; Tatsujiro Oka, MD, PhD; Gary Lopaschuk, PhD; Zamaneh Kassiri, PhD; and Carlos Fernandez-Patron, PhD.

Affiliation: From the Departments of Biochemistry (JO, XW, SC, CF-P.), Pediatrics (TO, GL), Pharmacology (GL), Physiology (ZK), School of Molecular and Systems Medicine (JO, XW, SC, TO, GL, ZK, CF-P) and the Cardiovascular Research Group (JO, XW, SC, TO, GL, ZK, CF-P), University of Alberta, Edmonton, AB, Canada.

Corresponding author: Dr. Carlos Fernandez-Patron, Department of Biochemistry, 3-19 Medical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2H7, Canada. Phone: (780) 492 9540, Fax: (780) 492 0095, E-mail: cf2@ualberta.ca

Short title: MMP-2 mediates hypertension
MATERIALS AND METHODS

Materials
All siRNAs were synthesized by Sigma-Aldrich (Paris, France) and dissolved in PBS prior to use. The first two nucleotides of each strand were 2'-O methylated and the final two nucleotides were deoxy nucleotides to increase siRNA stability. Sequences of siRNAs are shown in Table 1 and were used as previously described 1-3. MMP-2 inhibitors (MMP-2i I / MMP-2i III) and Ang II were obtained from Calbiochem (Gibbstown, NJ, USA). Phenylephrine was obtained from Sigma (Oakville, ON, Canada).

Cell Culture
A7R5 cells (ATCC, Manassas, VA, USA) were cultured in Dulbecco’s modified eagle medium supplemented with 10% FBS at 37°C and 5% CO₂. Cells were passaged using 0.05% trypsin-EDTA solution and seeded in 6 well plates. Cells were transfected with siRNA using DharmaFECT 2 transfection reagent as per manufacturer’s protocol (Thermo Scientific, Rockford, IL, USA). Briefly, serum-starved cells were treated with a pre-mixed solution 100 nmol/L siRNA and 0.4% DharmaFECT 2 transfection reagent in serum-free media for 24 hours and MMP-2 activity was measured by gelatin zymography in conditioned media and cell lysates.

Animals
Animal protocols were conducted in accordance with institutional guidelines issued by the Canada Council on Animal Care. All animals were housed at the Animal Facility of the University of Alberta until use. Male C57BL/6 mice and Sprague Dawley Rats were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All animals were anesthetised using 2.0% Isoflurane by inhalation before and during surgical procedures. MMP-2 inhibitor I was given orally at a per diem dose of 40 mg/kg/d. Angiotensin II (1.4 mg/kg/d) and siRNA (0.4 mg/kg/d) were delivered by subcutaneously implanted ALZET osmotic minipumps (DURECT Corporation, Cupertino, CA, USA) on the posterior midsection, as described in our earlier papers 2,4. Control mice received minipumps containing PBS (vehicle), a sham operation or were left intact. All animals were euthanized by pentobarbital overdose using Euthanyl (Bimeda-MTC, Cambridge, ON, Canada).

Blood pressure measurement
Systolic blood pressure was measured indirectly using computerized tail cuff plethysmography system (Kent Scientific Corporation, Torrington, CT, USA). Conscious mice were maintained at 32-35°C using a heating pad and restrained during measurements. Averages of 10 inflation/deflation cycles were conducted to obtain mean systolic blood pressure.

Echocardiography
In vivo assessment of anatomical structures and hemodynamic function in mice was conducted by M-mode echocardiography. The animals were first anaesthetized with 2.0% Isoflurane and their cardiac function was subsequently analyzed using a Vevo 770 high-resolution imaging system (Visualsonics, Toronto, ON, Canada). Three consecutive heartbeats of each frame were analyzed to measure the wall thickness and end-diastolic internal dimension (EDD) and end-
systolic internal dimension (ESD) of the left ventricle (LV). Echocardiographic corrected LV mass (in mg) was calculated as: \( 1.05 \times 0.8 \times [(LVID;d + LVPW;d + IVS;d)^3 - (LVID;d)^3] \) on diastole (d). ID- internal diameter (in mm). PW- posterior wall thickness (in mm). IVS- interventricular septum thickness (in mm).

**Cryosectioning**
Mouse hearts were embedded in Tissue-Tek (Sakura, Torrance, CA, USA), frozen on dry ice and stored at -70 ºC. Sections (10 µm) were cut on a Leica microtome and fixed with ice-cold acetone.

**WGA-FITC staining and cardiomyocytes cross-sectional area quantification**
Sections of cardiac tissue were washed in PBS-T and incubated for 2 hrs in 50 µg/mL wheat germ agglutinin-fluorescein isothiocyanate (WGA-FITC, Invitrogen, Burlington, ON, Canada), washed and mounted. Confocal microscope images were taken with a spinning disk laser confocal microscope (Confocal Imagine Core facility, University of Alberta) and cardiomyocyte cross-sectional area was determined by averaging the sizes of at least 100 cells per section.

**Collagen staining with picrosirius red**
Sections of cardiac tissue were brought back to water and stained for 1 hr in picrosirius red staining solution (1 g/L Direct Red 80 in saturated picric acid solution), washed in acidified water and dehydrated. Slides were photographed using a DCM500 camera and ScopePhoto software (Madell Technology, Beijing, China).

**Tissue homogenization**
Heart and aorta tissues were washed in isotonic saline buffer, rinsed and weighed. Protein extraction was done in 50 mM Tris, 50 mM NaCl, 1.25 mM PMSF, 62.5 mM Glycerol-2-phosphate, 12.5 mM sodium pyrophosphate, 125 µM NaF, 6.25 µg/ml leupeptine, 312.5 µM sodium orthovanadate, 12.5% glycerol, 1% SDS, 0.1% Triton X-100 at pH 7.4. To determine the protein content, homogenates were separated by 10% SDS-PAGE followed by densitometric analysis of Coomassie blue stained bands. Equal protein loads were loaded for subsequent gelatin zymography analysis.

**Gelatin zymography**
MMP-2 enzymatic activity was determined in heart and aorta homogenates using gelatin zymography. Homogenates were subjected to electrophoresis on SDS-PAGE gels copolymerized with gelatin (2 mg/mL). Following electrophoresis, gels were washed thrice with 2.5% Triton X-100 for 20 min. Enzymatic reaction was carried out for 16 hrs at 37ºC in enzyme assay buffer (25 mM Tris, 5 mM CaCl2, 142 mM NaCl, 0.5 mM NaN3, pH 7.6) and gels were stained with coomassie blue. Enzymatic activity was visualized as clear bands against a blue background in the gel.

**RNA expression analysis by TaqMan RT PCR**
Total RNA was extracted from flash-frozen cardiac tissue using Trizol reagent (Invitrogen, Burlington, ON, Canada) and cDNA was generated from 1 µg RNA using a random hexamer.
Expression analysis of the reported genes was performed by TaqMan RT-PCR using ABI 7900 sequence detection system (Applied Biosystems, Carlsbad, CA, USA). 18S rRNA was used as an internal standard as previously described.

**Microperfusion arteriograph**

Mesenteric arteries of adult Sprague Dawley Rats (6-month old, male) were dissected and mounted on a Danish MyoTechnology arteriograph system (Aarhus, Denmark). Arteries were perfused at constant temperature (37°C) and flow rate (2 μL/min) with standard HEPES-PSS (142 mM sodium chloride, 4.7 mM potassium chloride, 1.17 mM magnesium sulfate, 1.56 mM calcium chloride, 1.18 mM potassium phosphate, 10 mM HEPES, 5.5 mM glucose, pH 7.4). Phenylephrine (10 μmol/L) was added to the bath (adventitia side) to constrict arteries for 5 min. Following constriction, MMP-2 inhibitor III (100 μmol/L) or DMSO (1%) was added to the bath for an additional 5 min. Changes in arterial diameter were recorded using Vediview acquisition software (Danish MyoTechnology, Aarhus, Denmark).

**Statistical analysis**

Results were analyzed using one-way ANOVA or t-test (Jandel SigmaStat 3.5 statistical software) as appropriate. In the echocardiography studies, between-group comparisons of the means were performed by one-way ANOVA followed by Scheffe’s F correction for multiple comparisons between means. All data are reported as means +/- sem.
SUPPLEMENTAL REFERENCES


SUPPLEMENTAL FIGURE LEGENDS

Figure S1 Quantification of increases in A) heart weight/body weight ratio and B) cardiomyocyte cross sectional area (WGA-FITC staining) in the development of cardiac hypertrophy in mice receiving Ang II (1.4 mg/kg/d) by subcutaneous osmotic minipumps. Scale bar indicates 100 µm. n=4 mice for each time point. * indicates p<0.05 vs. day 0.

Figure S2 MMP-2 is not involved in the development of Ang II-induced cardiac hypertrophy. A) Heart weight to body weight ratios of mice treated with MMP-2 inhibitor I (40 mg/kg/d) beginning 1 day prior to Ang II infusion (1.4 mg/kg/d) for 12 days. B) Histological analysis of heart sections (10 µm) stained with WGA-FITC (upper panel) to quantify average cellular cross sectional area (lower panel). Scale bar indicates 100 µm. n=4 mice for each group. * indicates p<0.05 vs. control. ‡ Indicates p<0.05 vs Ang II.

Figure S3 Validation of MMP-2 siRNA in cultured A7r5 cells. Serum starved A7r5 cell were transfected with MMP-2 siRNA (100 nmol/L) using DharmaFECT-2 transfection reagent. Conditioned medium and cell lysate were collected 24 hours after transfection. Representative images of conditioned media (upper panel) and cell lysates (middle panel) subjected to gelatin zymography. Equal loading was confirmed by SDS-PAGE (lower panel). All experiments were performed in triplicate.

Figure S4 MMP-2 siRNA attenuates Ang II-induced hypertension but not cardiac hypertrophy or fibrosis. A-D) Upper panels-Treatment protocols of mice receiving MMP-2 siRNA (0.4 mg/kg/d) given either 5 days before (pre-treatment protocol, A) or after (rescue protocol, B) Ang II (1.4 mg/kg/d). lower panels- Representative images of aorta (A, B), heart(A, B), kidney (C, D) and liver (C, D) homogenates subjected to gelatin zymography. E, F) Tail cuff plethysmography analysis of systolic blood pressure of mice subjected to pre-treatment (E) or rescue (F) protocols. G, H) Echocardiographic analysis of left ventricle mass normalized to body weight (upper panels) and qRT-PCR analysis of hypertrophy markers (lower panels) in left ventricle samples of mice subjected to pre-treatment (G) or rescue (H) protocols. I, J) Heart weight to body weight ratios (upper panels) and histological analysis of heart sections (10 µm) stained with WGA-FITC to demonstrate cellular cross sectional area (lower panels) of mice subjected to pre-treatment (I) or rescue (J) protocols. K, L) Collagen staining of heart sections (10 µm) stained with picrosirius red (upper panels) and qRT-PCR analysis of fibrosis markers (lower panels) in left ventricle samples of mice subjected to the pre-treatment (K) or rescue (L) protocols. Scale bar indicates 100 µm (WGA-FITC sections) or 250 µm (picrosirius red sections). n=3-4 mice for each group. * indicates p<0.05 vs. control. ‡ Indicates p<0.05 vs Ang II.

Figure S5 Luciferase siRNA does not attenuate Ang II-induced hypertension, cardiac hypertrophy or fibrosis. A) Experimental protocol for mice treated with Luciferase siRNA (0.4 mg/kg/d) and Ang II (1.4 mg/kg/d) by subcutaneous osmotic minipumps. B) Time course of systolic blood pressure in control mice, mice treated with Ang II or mice treated with Ang II and luciferase siRNA. C) Cardiac hypertrophy as assessed by echocardiographic analysis of left
ventricle weight to body weight ratio (upper left panel), gross pathology of heart weight to body weight ratio (upper middle panel), qRT-PCR analysis of hypertrophy marker genes (upper right panel) and histological analysis of heart sections (10 µm) stained with WGA-FITC to quantify average cellular cross-sectional area (lower panels). 

D) Fibrosis was assessed by histological analysis of heart sections (10 µm) stained with picrosirius red to determine collagen deposition (left panel) and qRT-PCR analysis of fibrosis marker genes (right panel). E) qRT-PCR (left panel) and gelatin zymography (right panel) analysis of gelatinase expression and activity in cardiac homogenates. Scale bars indicate 100 µm (WGA-FITC micrographs) or 250 µm (Picrosirius red micrographs). n=4 mice for each group. * indicates p<0.05 vs. control group.

Figure S6 MMP-7 and TACE mediate Ang II induced MMP-2 upregulation. Gelatin zymography analysis of cardiac homogenates from mice treated with or without Ang II (1.4 mg/kg/d) or siRNAs against both MMP-7 and TACE (0.4 mg/kg/d). Images are representative of 4 mice from each group.

Figure S7 Simultaneous targeting of MMP-7 and TACE attenuates Ang II-induced hypertension, hypertrophy and fibrosis. A) Experimental protocol of mice treated with MMP-7 siRNA, TACE siRNA or both (0.4 mg/kg/d) 5 days prior to Ang II (1.4 mg/kg/d) by subcutaneous osmotic minipump. B) Tail cuff plethysmography analysis of systolic blood pressure in mice subjected to the above protocol. Assessment of cardiac hypertrophy in mice subjected to the above protocol as measured by left ventricle mass to body weight ratio (M-mode echocardiography, C) and qRT-PCR analysis of hypertrophy marker genes (D). Assessment of cardiac fibrosis in mice subjected to the above protocol as measured by picrosirius red (collagen deposition, E) and qRT-PCR analysis of fibrosis marker genes (F). Scale bar indicates 250 µm. n=4 mice for each group. * indicates p<0.05 vs. control. ‡ Indicates p<0.05 vs Ang II.
Figure S1 A

**Time (days)**

- 0
- 2
- 4
- 6
- 8
- 10
- 12
- 14
- 16
- 18

**HW : BW (mg/g)**

- 3
- 4
- 5
- 6
- 7
- 8
- 9

**Control**

**Ang II**

* Significance levels: *

**A**

- **Time (days)**
- 0 2 4 6 8 10 12 14 16 18

**HW : BW (mg/g)**

- Control
- Ang II

Legend:

- Solid line: Control
- Dashed line: Ang II
**Figure S1 B**

**Cellular cross sectional area**
(WGA-FITC staining, left ventricle)

<table>
<thead>
<tr>
<th>Control day 0</th>
<th>Ang II day 4</th>
<th>Ang II day 8</th>
<th>Ang II day 12</th>
<th>Ang II day 16</th>
<th>Control day 16</th>
</tr>
</thead>
</table>

![Imagery](image1.png)

![Graph](graph.png)
Figure S2

**Ex vivo Pathology (day 12)**

- **A**
  - Bar chart showing HW : BW (mg/g) for different conditions: Control, Ang II, and MMP-2i + Ang II. Bars with * indicate statistically significant differences.

- **B**
  - Cellular cross sectional area (% of control) for WGA-FITC staining, left ventricle, day 12. Bars with * indicate statistically significant differences. Ex vivo Pathology images are also shown for Control, Ang II, and MMP-2i + Ang II.
Gelatin Zymography
(VSMC)

Conditioned medium
(Gelatin Zymography)

Cell lysate
(Gelatin Zymography)

Loading control
(SDS PAGE)

Control
Mock
MMP-2 siRNA
Figure S4 A,B

**Pre-treatment protocol**
(RNA interference)

- siRNA infusion (minipump)
- Ang II infusion (minipump)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>-5</th>
<th>0</th>
<th>5</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
</table>

**Gelatin Zymography**

(Aorta, day 11)
- Ang II: - + +
- MMP-2 siRNA: - - +
- MMP-2: 72 kDa
- Loading Control (Coomassie Blue): 55 kDa

(B) Rescue protocol
(RNA interference)

- Ang II infusion (minipump)
- siRNA infusion (minipump)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>5</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
</table>

**Gelatin Zymography**

(Aorta, day 16)
- Ang II: - + +
- MMP-2 siRNA: - - +
- MMP-2: 72 kDa
- Loading Control (Coomassie Blue): 55 kDa

(C) Gelatin Zymography

(Left ventricle, day 11)
- Ang II: - + +
- MMP-2 siRNA: - - +
- MMP-2: 72 kDa
- Loading Control (Coomassie Blue): 55 kDa

(D) Gelatin Zymography

(Left ventricle, day 16)
- Ang II: - + +
- MMP-2 siRNA: - - +
- MMP-2: 72 kDa
- Loading Control (Coomassie Blue): 55 kDa

**MMP-2 activity / total protein** (% of control)

- Control
- Ang II
- MMP-2 siRNA + Ang II

(A) Pre-treatment protocol

(B) Rescue protocol
C) Gelatin Zymography

(Kidney, day 11)

- Ang II: - + +
- MMP-2 siRNA: - - +

MMP-2 activity / total protein (% of control)

Loading Control (Coomassie Blue)

(D) Gelatin Zymography

(Liver, day 11)

- Ang II: - + +
- MMP-2 siRNA: - - +

MMP-2 activity / total protein (% of control)

Loading Control (Coomassie Blue)
Figure S4 E, F

**E**

**Pre-treatment protocol**
(RNA interference)

**Tail cuff plethysmography**
(day 10)

<table>
<thead>
<tr>
<th>MMP-2 siRNA:</th>
<th>Ang II (mg/kg/d):</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>1.0</td>
</tr>
<tr>
<td>-</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**F**

**Rescue protocol**
(RNA interference)

**Tail cuff plethysmography**
(day 15)

<table>
<thead>
<tr>
<th>MMP-2 siRNA:</th>
<th>Ang II (mg/kg/d):</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>1.4</td>
</tr>
<tr>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
**G**

Pre-treatment protocol
(RNA interference)

M-mode echocardiography
(Left ventricle, day 10)

- Control
- Ang II
- MMP-2 siRNA + Ang II

qRT-PCR
(Left ventricle, day 11)

- Control
- Ang II
- MMP-2 siRNA + Ang II

**H**

Rescue protocol
(RNA interference)

M-mode echocardiography
(Left ventricle, day 15)

- Control
- Ang II
- MMP-2 siRNA + Ang II

qRT-PCR
(Left ventricle, day 16)

- Control
- Ang II
- MMP-2 siRNA + Ang II
**Pre-treatment protocol**  
(RNA interference)

Cellular cross sectional area  
(WGA-FITC staining, left ventricle, day 11)

Control  | Ang II | MMP-2 siRNA + Ang II

**Rescue protocol**  
(RNA interference)

Cellular cross sectional area  
(WGA-FITC staining, left ventricle, day 16)

Control  | Ang II | MMP-2 siRNA + Ang II
**K**  
**Pre-treatment protocol**  
(RNA interference)

**Interstitial fibrosis**  
(Picrosirius red staining, left ventricle, day 11)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>MMP-2 siRNA + Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fn-1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**qRT-PCR**  
(Left ventricle, day 11)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>MMP-2 siRNA + Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fn-1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference

**L**  
**Rescue protocol**  
(RNA interference)

**Interstitial fibrosis**  
(Picrosirius red staining, left ventricle, day 16)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>MMP-2 siRNA + Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fn-1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**qRT-PCR**  
(Left ventricle, day 16)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>MMP-2 siRNA + Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fn-1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference

**Pre-treatment protocol**

**Rescue protocol**

**Interstitial fibrosis**

**qRT-PCR**

**Expression (% of control)**

**Col I**

**Col III**

**Fn-1**
A) Experimental protocol

- siRNA infusion (minipump)
- Ang II infusion (minipump)

Time (days): -5 0 9 11

B) Tail cuff plethysmography

Blood pressure (% of day 0)

- Control
- Ang II
- Luciferase siRNA + Ang II
M-mode echocardiography (day 10)

LV mass : BW

Control
Ang II
Luciferase siRNA + Ang II

Cellular cross sectional area (WGA-FITC staining, left ventricle, day 11)

Cellular cross sectional area (% of control)

Control
Ang II
Luciferase siRNA + Ang II

qRT-PCR, hypertrophy markers (Left ventricle, day 11)

Expression (% of control)

Control
Ang II
Luciferase siRNA + Ang II

Figure S5 C
D  
Interstitial fibrosis  
(Picosirius red staining, day 11)

Control  |  Ang II  |  Luciferase siRNA + Ang II

**Figure S5 D**

qRT-PCR, fibrosis markers  
(Left ventricle, day 11)

Expression (% of control)

<table>
<thead>
<tr>
<th></th>
<th>Col I</th>
<th>Col III</th>
<th>Fn-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>300</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Ang II</td>
<td>400</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>Luciferase siRNA + Ang II</td>
<td>500</td>
<td>400</td>
<td>300</td>
</tr>
</tbody>
</table>

* *  
*  
*  

Legend:
- Black: Control
- Light gray: Ang II
- Gray: Luciferase siRNA + Ang II
E  qRT-PCR
(Left ventricle, day 11)

F  Gelatin Zymography
(Aorta, day 11)

Ang II:  -  +  +
Luciferase siRNA:  -  -  +

MMP-2 ➔

72 kDa

Loading Control
(Coomassie Blue)
Gelatin Zymography
(Left ventricle, day 11)
Ang II: - + +
(MMP-7 + TACE) siRNA: - - +
MMP-2 → 72 kDa
Loading Control (Coomassie Blue)
Figure S7 A, B

**A**

**Experimental protocol**

- siRNA infusion (minipump)
- Ang II infusion (minipump)

| Time (days) | -5 | 0 | 9 | 11 |

**B**

**Tail cuff plethysmography**

(day 9)

- Control
- Ang II
- (MMP-7 + TACE) siRNA + Ang II

Blood pressure (% of control)
Figure S7 C, D

C  M-mode echocardiography
(Left ventricle, day 10)

qRT-PCR
(Left ventricle, day 11)

D  Interstitial fibrosis
(Picrosirius red staining, left ventricle, day 11)

qRT-PCR
(Left ventricle, day 11)