

## Supplementary data

### Cardiac Actions of a Small Molecule Inhibitor Targeting GATA4–NKX2-5 Interaction

Sini M. Kinnunen<sup>1,2,+</sup>, Marja Tölli<sup>2,+</sup>, Mika Välimäki<sup>1,2</sup>, Erhe Gao<sup>3</sup>, Zoltan Szabo<sup>2</sup>, Jaana Rysä<sup>4</sup>,  
Mónica P.A. Ferreira<sup>5</sup>, Pauli Ohukainen<sup>6</sup>, Raisa Serpi<sup>2</sup>, Alexandra Correia<sup>5</sup>, Ermei Mäkilä<sup>7</sup>,  
Jarno Salonen<sup>7</sup>, Jouni Hirvonen<sup>5</sup>, Hélder A. Santos<sup>5,8</sup>, Heikki Ruskoaho<sup>1, 2,\*</sup>

<sup>1</sup> *Drug Research Program, Division of Pharmacology and Pharmacotherapy, University of Helsinki, Helsinki, Finland*

<sup>2</sup> *Department of Pharmacology and Toxicology, Institute of Biomedicine, University of Oulu, Oulu, Finland*

<sup>3</sup> *Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, United States of America*

<sup>4</sup> *School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland*

<sup>5</sup> *Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland*

<sup>6</sup> *Computational Medicine, Faculty of Medicine, University of Oulu and Biocenter Oulu, Oulu, Finland*

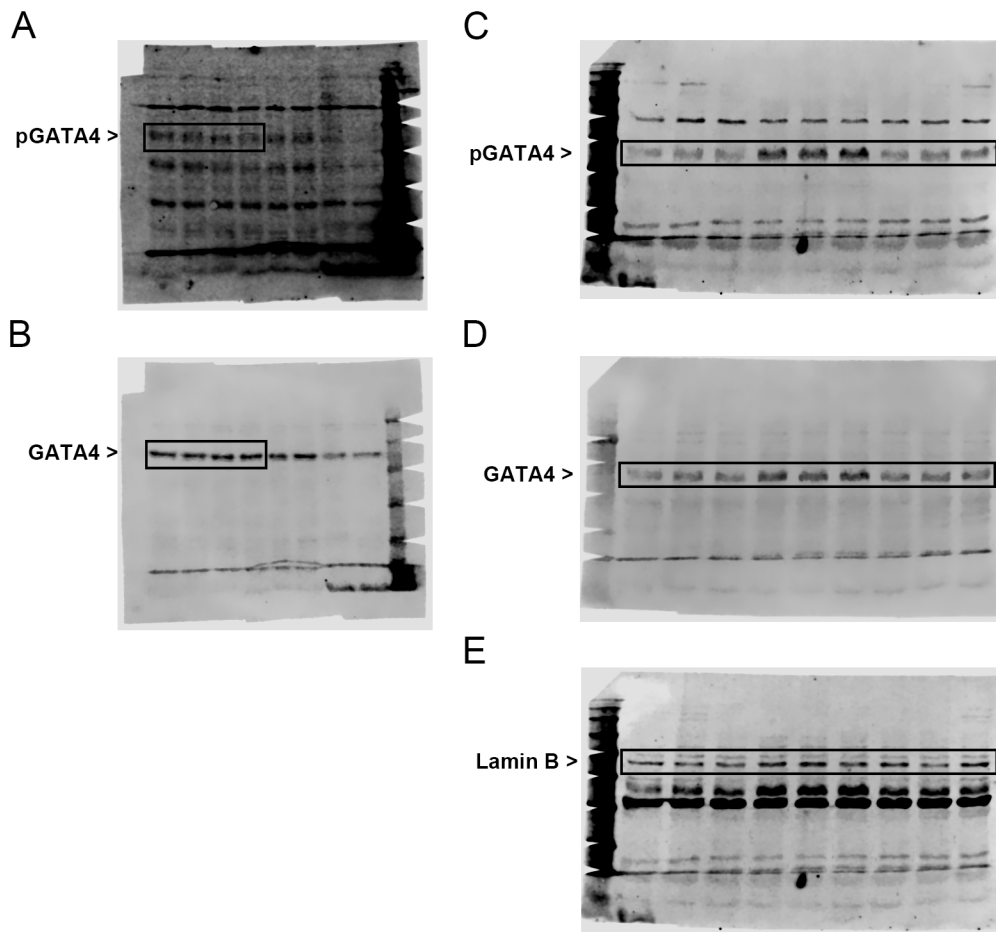
<sup>7</sup> *Laboratory of Industrial Physics, Department of Physics and Astronomy, University of Turku, Turku, Finland*

<sup>8</sup> *Helsinki Institute of Life Sciences (HiLIFE), University of Helsinki, Helsinki, Finland*

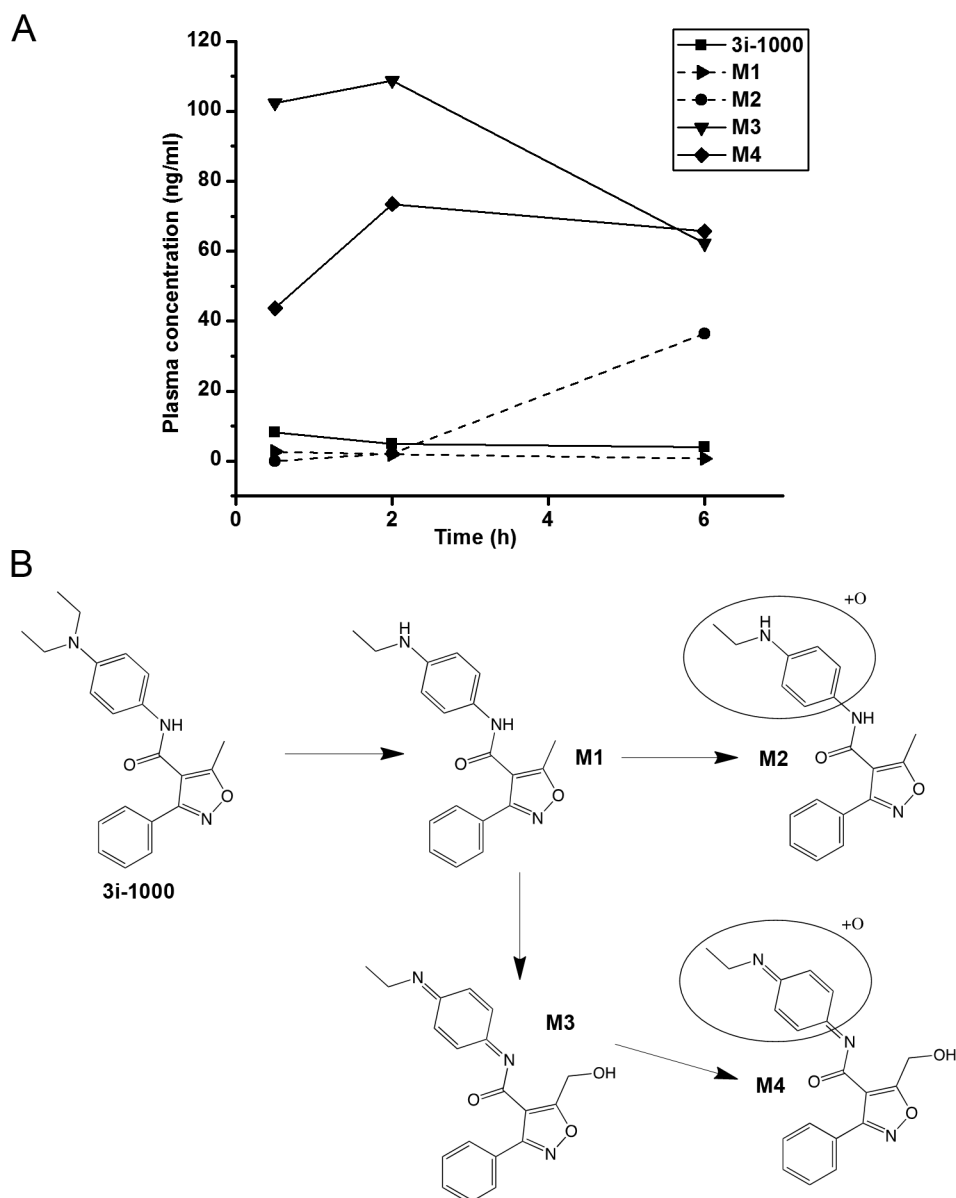
\*Corresponding author: Heikki Ruskoaho, Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki, P.O.Box 56, FI-00014 Helsinki, FINLAND

email: heikki.ruskoaho@helsinki.fi

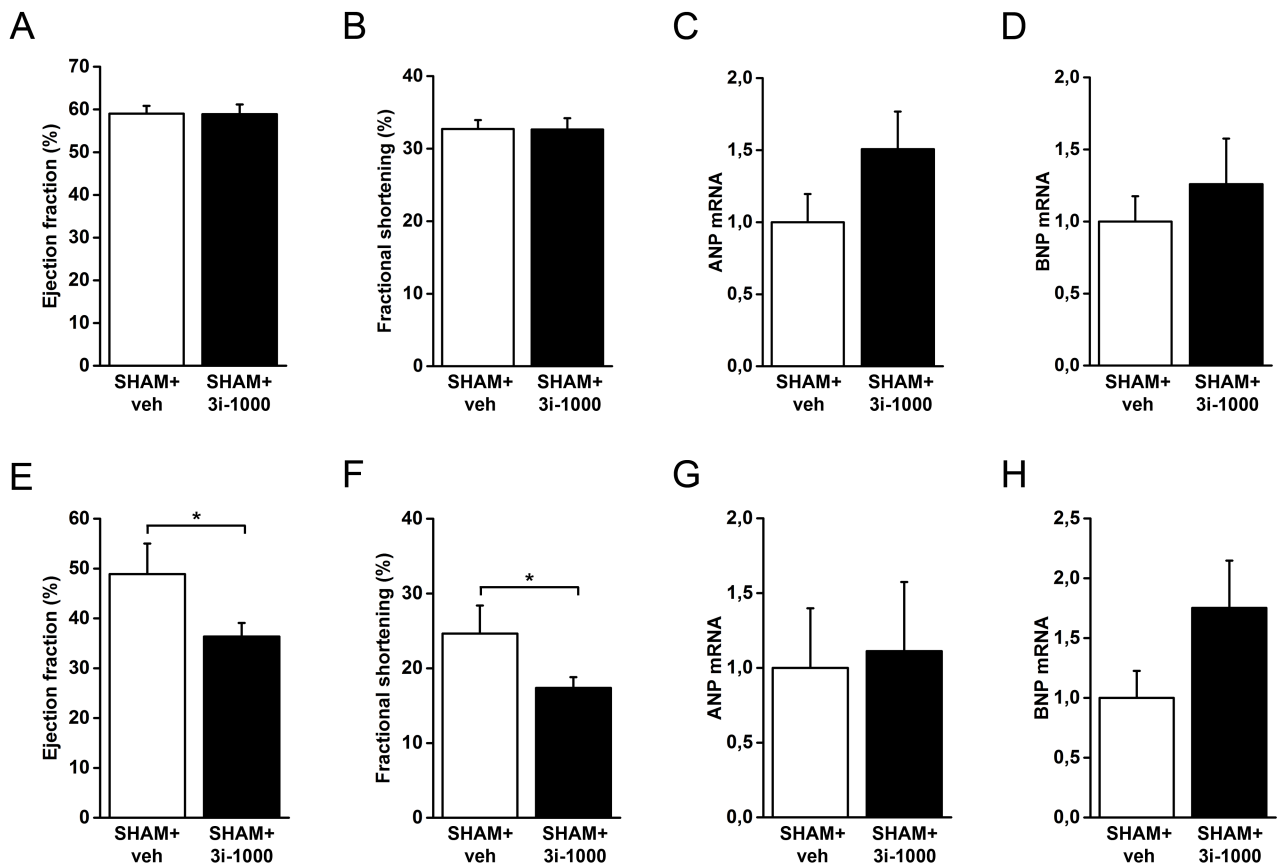
<sup>+</sup>authors contributed equally to this work



**Figure S1.** The original western blot images for Figure 3. (A, B) The bands shown in Figure 3A and (C-E) the bands shown in Figure 3B are circled by a rectangle.



**Figure S2.** Pharmacokinetics and metabolic profile of 3i-1000. **(A)** Plasma concentrations of compound 3i-1000 and its metabolites M1-M4. A single dose of compound 3i-1000 (10 mg/kg) was administered i.p. in rats and blood samples were collected from tail vein. The plasma samples from three animals were pooled and the compound concentrations were analysed from by LC-TOF mass spectrometer. **(B)** Compound structure of 3i-1000 and the most abundant metabolites.



**Figure S3. (A-D).** The echocardiographic parameters and the mRNA levels in the left ventricular tissue of rats that underwent sham-operation (SHAM) in acute myocardial infarction experiment. Rats were treated either with vehicle (veh, DMSO) or compound 3i-1000 (30 mg/kg/day i.p.) for one week. Echocardiographic measurements were performed at the end of the experiment at one week. The number of the animals was 6 in SHAM+veh and 6 in SHAM+3i-1000. mRNA levels were measured by RT-PCR and normalised to housekeeping gene 18S quantified from the same samples. The results are averages  $\pm$  SEM. **(E-H).** The echocardiographic parameters and the mRNA levels in the left ventricular tissue of rats that underwent sham-operation in ischemia-reperfusion experiment. Rats were treated either with vehicle or compound 3i-1000 (30 mg/kg/day i.p.) for one week before sham-operation. Sham-operated animals were subjected to the same surgical procedures than animals in the ischemia-reperfusion group except that the suture was passed under the LAD, but was not tied. Following 30 min of non-ischemia, the slipknot was released and the myocardium was still perfused for 24 h. Echocardiographic measurements were performed at 24 h. mRNA levels were measured by RT-PCR and normalised to housekeeping gene 18S quantified from the same samples. The number of the animals was 4 in SHAM+veh and 3 in SHAM+3i-1000 group. The results are averages  $\pm$  SEM. \* $p < 0.05$  (one-way ANOVA followed by a least significant difference post hoc test).

**Table S1.** The echocardiographic parameters of mice that underwent acute myocardial infarction (AMI) or sham-operation (SHAM) and were treated either with vehicle (V) or compound 3i-1000 (30 mg/kg/day i.p.) for 4 days. Echocardiographic measurements were performed at the end of the experiment one week after infarction. The number of animals in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison † SHAM+V vs. AMI+V; ‡ AMI+V vs. AMI+3i-1000. IVRT, isovolumic relaxation time; d, diastolic; s, systolic; BPM, beats per minute; LV, left ventricle; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall.

|                           | SHAM+V (15) |           | AMI+V (4) |           | †     | AMI+3i-1000 (3) |           | ‡     |
|---------------------------|-------------|-----------|-----------|-----------|-------|-----------------|-----------|-------|
|                           | Average     | $\pm$ SEM | Average   | $\pm$ SEM | p     | Average         | $\pm$ SEM |       |
| E' (mm/s)                 | -19,60      | 1,42      | -14,06    | 2,91      | 0,081 | -23,51          | 1,42      | 0,032 |
| A' (mm/s)                 | -22,51      | 1,03      | -13,29    | 3,21      | 0,001 | -18,27          | 1,49      | 0,151 |
| E'/A'                     | 0,89        | 0,07      | 1,21      | 0,27      | 0,096 | 1,30            | 0,14      | 0,708 |
| IVRT (ms)                 | 15,64       | 1,05      | 21,49     | 2,43      | 0,018 | 15,74           | 0,74      | 0,076 |
| Diameter;d (mm)           | 4,12        | 0,08      | 5,29      | 0,28      | 0,000 | 4,53            | 0,54      | 0,040 |
| Diameter;s (mm)           | 3,18        | 0,09      | 4,83      | 0,29      | 0,000 | 3,82            | 0,62      | 0,019 |
| Ejection Fraction (%)     | 46,22       | 2,09      | 19,19     | 1,83      | 0,000 | 34,62           | 6,91      | 0,022 |
| Fractional Shortening (%) | 22,99       | 1,30      | 8,77      | 0,85      | 0,000 | 16,56           | 3,47      | 0,046 |
| Heart Rate (BPM)          | 447,73      | 13,01     | 492,45    | 14,30     | 0,095 | 499,14          | 11,48     | 0,848 |
| LV Mass (mg)              | 107,17      | 3,88      | 186,32    | 18,65     | 0,000 | 127,88          | 18,87     | 0,003 |
| Volume;d ( $\mu$ l)       | 75,44       | 3,28      | 136,33    | 16,24     | 0,000 | 97,80           | 27,97     | 0,042 |
| Volume;s ( $\mu$ l)       | 41,00       | 2,57      | 110,94    | 15,02     | 0,000 | 67,80           | 26,93     | 0,015 |
| LVID;d (mm)               | 4,08        | 0,08      | 5,28      | 0,32      | 0,000 | 4,43            | 0,51      | 0,030 |
| LVID;s (mm)               | 3,16        | 0,10      | 4,83      | 0,32      | 0,000 | 3,72            | 0,60      | 0,014 |
| LVPW;d (mm)               | 0,74        | 0,02      | 0,71      | 0,09      | 0,491 | 0,76            | 0,07      | 0,508 |
| LVPW;s (mm)               | 1,00        | 0,03      | 0,84      | 0,07      | 0,033 | 0,91            | 0,13      | 0,456 |

**Table S2.** The mRNA and histological measurements of mice that underwent acute myocardial infarction (AMI) or sham-operation (SHAM) and were treated either with vehicle (V) or compound 3i-1000 (30 mg/kg/day i.p.) for 4 days. The left ventricular tissues were analysed at the end of the experiment one week after infarction. From SHAM+V group, 8 animals were randomly selected for mRNA measurements and 5 animals for histological analysis. The number of animals in other groups is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison  $\dagger$  SHAM+V vs. AMI+V;  $\ddagger$  AMI+V vs. AMI+3i-1000. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; COL1A1, collagen 1A1; HW/BW, heart weight to body weight.

|                   | SHAM+V  |           | AMI+V (4) |           |                | AMI+3i-1000 (3) |           |                 |
|-------------------|---------|-----------|-----------|-----------|----------------|-----------------|-----------|-----------------|
|                   | Average | $\pm$ SEM | Average   | $\pm$ SEM | $\dagger$<br>p | Average         | $\pm$ SEM | $\ddagger$<br>p |
| ANP               | 1,00    | 0,33      | 18,27     | 1,24      | 0,000          | 10,92           | 4,58      | 0,024           |
| BNP               | 1,00    | 0,24      | 6,43      | 1,58      | 0,000          | 3,16            | 0,57      | 0,034           |
| GATA4             | 1,00    | 0,12      | 0,85      | 0,12      | 0,453          | 0,59            | 0,23      | 0,307           |
| NKX2-5            | 1,00    | 0,11      | 0,83      | 0,11      | 0,393          | 0,51            | 0,23      | 0,191           |
| COL1A1            | 1,00    | 0,35      | 4,58      | 0,69      | 0,002          | 2,43            | 1,61      | 0,091           |
| Fibrosis          | 1,00    | 0,62      | 26,61     | 4,17      | 0,001          | 24,70           | 2,67      | 0,690           |
| Apoptosis (tunel) | 2,44    | 0,39      | 3,50      | 0,85      | 0,231          | 4,67            | 0,57      | 0,246           |
| Scar              | 6,61    | 3,59      | 37,57     | 2,41      | 0,000          | 26,47           | 4,56      | 0,052           |
| Ki67+ cells       | 0,76    | 0,25      | 1,35      | 0,31      | 0,330          | 1,73            | 0,84      | 0,571           |
| c-kit+ cells      | 3,11    | 1,21      | 11,11     | 7,84      | 0,306          | 13,20           | 7,25      | 0,808           |
| HW/BW             | 4,95    | 0,10      | 7,54      | 0,46      | 0,000          | 5,69            | 0,38      | 0,000           |

**Table S3.** The echocardiographic parameters of rats that underwent acute myocardial infarction (AMI) or sham-operation (SHAM) and were treated either with vehicle or compound 3i-1000 (30 mg/kg/day i.p.) for one week. Echocardiographic measurements were performed at the end of the experiment at one week. The number of the animals in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison † SHAM+V vs. AMI+V; ‡ AMI+V vs. AMI+3i-1000. IVRT, isovolumic relaxation time; d, diastolic; s, systolic; BPM, beats per minute; LV, left ventricle; IVS, intra ventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall.

|                           | SHAM+V (6) |           | AMI+V (7) |           | †     | AMI+3i-1000 (8) |           | ‡     |
|---------------------------|------------|-----------|-----------|-----------|-------|-----------------|-----------|-------|
|                           | Ave        | $\pm$ SEM | Ave       | $\pm$ SEM | p     | Ave             | $\pm$ SEM | p     |
| E' (mm/s)                 | -40,71     | 4,31      | -38,23    | 4,70      | 0,702 | -34,02          | 4,79      | 0,487 |
| A' (mm/s)                 | -20,95     | 1,33      | -20,54    | 2,21      | 0,940 | -21,55          | 1,67      | 0,844 |
| E'/A'                     | 1,99       | 0,28      | 1,91      | 0,17      | 0,797 | 1,59            | 0,20      | 0,304 |
| IVRT (ms)                 | 22,90      | 0,99      | 25,66     | 1,14      | 0,169 | 24,26           | 1,73      | 0,446 |
| Diameter;d (mm)           | 8,32       | 0,17      | 9,46      | 0,25      | 0,009 | 8,79            | 0,35      | 0,081 |
| Diameter;s (mm)           | 5,61       | 0,20      | 7,19      | 0,42      | 0,013 | 6,47            | 0,52      | 0,200 |
| Ejection Fraction (%)     | 59,04      | 1,80      | 44,68     | 6,24      | 0,042 | 49,94           | 5,01      | 0,405 |
| Fractional Shortening (%) | 32,71      | 1,25      | 24,02     | 3,80      | 0,048 | 27,09           | 3,17      | 0,436 |
| Heart Rate (BPM)          | 266,27     | 3,49      | 282,97    | 8,06      | 0,095 | 267,19          | 6,45      | 0,090 |
| LV Mass (mg)              | 921,41     | 95,47     | 1198,41   | 129,44    | 0,084 | 1057,56         | 86,55     | 0,334 |
| Volume;d ( $\mu$ l)       | 377,53     | 16,49     | 501,74    | 28,21     | 0,008 | 430,29          | 37,07     | 0,084 |
| Volume;s ( $\mu$ l)       | 155,69     | 12,33     | 278,98    | 36,54     | 0,013 | 226,89          | 39,78     | 0,235 |
| IVS;d (mm)                | 1,39       | 0,16      | 1,36      | 0,12      | 0,914 | 1,52            | 1,19      | 0,487 |
| IVS;s (mm)                | 2,08       | 0,21      | 1,83      | 0,26      | 0,472 | 2,05            | 0,28      | 0,506 |
| LVID;d (mm)               | 8,20       | 0,17      | 9,39      | 0,22      | 0,006 | 8,64            | 0,34      | 0,050 |
| LVID;s (mm)               | 5,55       | 0,18      | 7,17      | 0,43      | 0,010 | 6,38            | 0,49      | 0,152 |
| LVPW;d (mm)               | 1,51       | 0,08      | 1,49      | 0,10      | 0,879 | 1,52            | 0,12      | 0,783 |
| LVPW;s (mm)               | 2,16       | 0,09      | 2,10      | 0,17      | 0,830 | 2,09            | 0,27      | 0,962 |

**Table S4.** The mRNA and histological measurements of rats that underwent acute myocardial infarction (AMI) or sham-operation (SHAM) and were treated either with vehicle or compound 3i-1000 (30 mg/kg/day i.p.) for one week. The left ventricular tissues were analysed at the end of the experiment at one week. The mRNA measurements were performed to all animals (6 in SHAM+V, 7 in AMI+V and 8 AMI+3i-1000). For histological analysis, 4 animals were randomly selected from each group. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison † SHAM+V vs. AMI+V; ‡ AMI+V vs. AMI+3i-1000. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; HW/BW, heart weight to body weight.

|              | SHAM+V  |           | AMI+V   |           | †     | AMI+3i-1000 |           | ‡     |
|--------------|---------|-----------|---------|-----------|-------|-------------|-----------|-------|
|              | Average | $\pm$ SEM | Average | $\pm$ SEM | p     | Average     | $\pm$ SEM | p     |
| ANP          | 1,00    | 0,20      | 6,26    | 1,41      | 0,001 | 5,02        | 1,07      | 0,361 |
| BNP          | 1,00    | 0,18      | 1,28    | 0,25      | 0,426 | 1,04        | 0,20      | 0,462 |
| GATA4        | 1,00    | 0,07      | 0,55    | 0,11      | 0,013 | 0,75        | 0,14      | 0,207 |
| NKX2-5       | 1,00    | 0,08      | 0,53    | 0,13      | 0,033 | 0,87        | 0,18      | 0,093 |
| Fibrosis     | 10,33   | 3,67      | 20,28   | 0,53      | 0,006 | 18,50       | 0,28      | 0,562 |
| Ki67+ cells  | 1,40    | 0,93      | 2,00    | 0,53      | 0,487 | 3,35        | 0,47      | 0,132 |
| c-kit+ cells | 0,83    | 0,55      | 0,78    | 0,54      | 0,980 | 7,78        | 2,64      | 0,004 |
| HW/BW (%)    | 0,34    | 0,01      | 0,39    | 0,02      | 0,021 | 0,36        | 0,01      | 0,219 |

**Table S5.** The echocardiographic parameters of rats that were treated with angiotensin II (33.3 µg/kg/h s.c.) and vehicle or angiotensin II and the compound 3i-1000 (30 mg/kg/day i.p) for two weeks. Echocardiographic measurements were performed at 2 weeks. The number of animals in each group is shown in brackets. The results are averages ± SEM. Significance (p) was calculated by independent samples t-test. IVRT, isovolumic relaxation time; LV, left ventricle; d, diastolic; s, systolic; BPM, beats per minute; IVS, intra ventricular septum; LVPW, left ventricular posterior wall; MeanWT, mean wall thickness.

|                           | Vehicle (6) |       | 3i-1000 (6) |       |       |
|---------------------------|-------------|-------|-------------|-------|-------|
|                           | Average     | ±SEM  | Average     | ±SEM  | p     |
| E' (cm/s)                 | 95,17       | 5,84  | 71,17       | 2,88  | 0,004 |
| A' (cm/s)                 | 55,67       | 2,65  | 62,67       | 7,22  | 0,384 |
| E'/A'                     | 1,74        | 0,16  | 1,21        | 0,14  | 0,030 |
| IVRT (ms)                 | 28,17       | 1,40  | 24,83       | 1,05  | 0,086 |
| LV;d (mm)                 | 7,28        | 0,24  | 6,28        | 0,32  | 0,032 |
| LV;s (mm)                 | 4,23        | 0,11  | 3,25        | 0,28  | 0,016 |
| Ejection Fraction (%)     | 77,93       | 1,72  | 84,88       | 1,72  | 0,017 |
| Fractional Shortening (%) | 41,82       | 1,63  | 48,95       | 1,99  | 0,020 |
| Heart Rate (BPM)          | 247,17      | 8,52  | 295,00      | 5,86  | 0,001 |
| LV Mass (mg)              | 1054,17     | 88,18 | 960,00      | 51,38 | 0,378 |
| IVS;d (mm)                | 1,93        | 0,08  | 2,13        | 0,04  | 0,044 |
| IVS;s (mm)                | 3,45        | 0,09  | 3,50        | 0,13  | 0,759 |
| LVPW;d (mm)               | 1,90        | 0,12  | 2,08        | 0,10  | 0,260 |
| LVPW;s (mm)               | 3,15        | 0,15  | 3,53        | 0,15  | 0,103 |
| MeanWT;d (mm)             | 1,95        | 0,07  | 2,13        | 0,06  | 0,062 |
| MeanWT;s (mm)             | 3,33        | 0,08  | 3,55        | 0,11  | 0,153 |

**Table S6.** The mRNA measurements of rats that were treated with angiotensin II (33.3 µg/kg/h s.c.) and vehicle or angiotensin II and the compound 3i-1000 (30 mg/kg/day i.p) for two weeks. The left ventricular tissues were analysed at the end of the experiment at two weeks. The number of animals in each group is shown in brackets. The results are averages ± SEM. Significance (p) was calculated by independent samples t-test. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; LV mass/BW, left ventricular mass to body weight.

|                    | Vehicle (6) |      | 3i-1000 (6) |      | p     |
|--------------------|-------------|------|-------------|------|-------|
|                    | Average     | ±SEM | Average     | ±SEM |       |
| ANP                | 1,00        | 0,48 | 0,27        | 0,10 | 0,163 |
| BNP                | 1,00        | 0,20 | 0,73        | 0,21 | 0,377 |
| GATA4              | 1,00        | 0,27 | 0,54        | 0,11 | 0,170 |
| NKX2-5             | 1,00        | 0,25 | 0,68        | 0,20 | 0,339 |
| Bodyweight (g)     | 307,67      | 9,78 | 295,50      | 6,63 | 0,327 |
| LV mass/ BW (mg/g) | 3,41        | 0,23 | 3,25        | 0,18 | 0,607 |

**Table S7.** The echocardiographic parameters of rats treated either with vehicle or compound 3i-1000 (30 mg/kg/day i.p.) for one week before ischemia-reperfusion (I/R) or sham-operation (SHAM). Following 30 min of ischemia, the slipknot was released and the myocardium was reperfused for 24 h. Echocardiographic measurements were performed at 24 h. The number of animals in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison SHAM+V vs. I/R+V; † I/R+V vs. I/R+3i-1000. IVRT, isovolumic relaxation time; d, diastolic; s, systolic; BPM, beats per minute; LV, left ventricle; IVS, intra ventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall.

|                           | SHAM+V (4) |           | I/R+V (15) |           |       | I/R+3i-1000 (15) |           |       |
|---------------------------|------------|-----------|------------|-----------|-------|------------------|-----------|-------|
|                           | Average    | $\pm$ SEM | Average    | $\pm$ SEM | p     | Average          | $\pm$ SEM | p     |
| E' (mm/s)                 | -16,20     | 1,89      | -22,19     | 1,73      | 0,072 | -18,98           | 1,27      | 0,134 |
| A' (mm/s)                 | -15,86     | 2,04      | -18,34     | 1,74      | 0,500 | -18,83           | 1,70      | 0,837 |
| E'/A'                     | 1,04       | 0,10      | 1,38       | 0,19      | 0,295 | 1,09             | 0,10      | 0,168 |
| IVRT (ms)                 | 17,01      | 0,90      | 17,03      | 0,68      | 0,994 | 17,02            | 0,83      | 0,997 |
| Diameter;d (mm)           | 3,85       | 0,22      | 4,40       | 0,05      | 0,001 | 4,30             | 0,06      | 0,258 |
| Diameter;s (mm)           | 2,90       | 0,22      | 3,52       | 0,08      | 0,007 | 3,37             | 0,11      | 0,284 |
| Ejection Fraction (%)     | 48,89      | 6,13      | 40,66      | 2,76      | 0,229 | 43,41            | 3,35      | 0,532 |
| Fractional Shortening (%) | 24,64      | 3,76      | 19,99      | 1,58      | 0,238 | 21,65            | 1,91      | 0,512 |
| Heart Rate (BPM)          | 441,69     | 25,28     | 492,35     | 11,18     | 0,097 | 476,06           | 15,69     | 0,403 |
| LV Mass (mg)              | 109,48     | 10,05     | 125,20     | 6,42      | 0,316 | 128,28           | 7,98      | 0,760 |
| Volume;d ( $\mu$ l)       | 65,04      | 8,69      | 88,01      | 2,48      | 0,001 | 83,24            | 2,93      | 0,259 |
| Volume;s ( $\mu$ l)       | 33,25      | 6,57      | 52,30      | 3,03      | 0,014 | 47,52            | 3,63      | 0,320 |
| IVS;d (mm)                | 0,74       | 0,04      | 0,72       | 0,04      | 0,854 | 0,68             | 0,05      | 0,573 |
| IVS;s (mm)                | 1,03       | 0,06      | 0,92       | 0,06      | 0,456 | 0,92             | 0,06      | 0,930 |
| LVID;d (mm)               | 3,85       | 0,23      | 4,32       | 0,06      | 0,004 | 4,25             | 0,07      | 0,461 |
| LVID;s (mm)               | 2,91       | 0,21      | 3,46       | 0,09      | 0,019 | 3,32             | 0,11      | 0,327 |
| LVPW;d (mm)               | 0,78       | 0,05      | 0,72       | 0,03      | 0,287 | 0,78             | 0,03      | 0,124 |
| LVPW;s (mm)               | 0,96       | 0,07      | 0,92       | 0,04      | 0,677 | 1,01             | 0,05      | 0,196 |

**Table S8.** The mRNA and histological measurements of rats treated either with vehicle or compound 3i-1000 (30 mg/kg/day i.p.) for one week before ischemia-reperfusion (I/R) or sham-operation (SHAM). Following 30 min of ischemia, the slipknot was released and the myocardium was reperfused for 24 h. The left ventricular tissues were analysed at the end of the experiment. The number of animals in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison SHAM+V vs. I/R+V; † I/R+V vs. I/R+3i-1000. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; COL1A1, collagen 1A1.

|                   | SHAM+V (4) |           | I/R+V (15) |           | †<br>p | I/R+3i-1000 (15) |           | ‡<br>p |
|-------------------|------------|-----------|------------|-----------|--------|------------------|-----------|--------|
|                   | Average    | $\pm$ SEM | Average    | $\pm$ SEM |        | Average          | $\pm$ SEM |        |
| ANP               | 1,00       | 0,40      | 1,81       | 0,21      | 0,041  | 1,27             | 0,12      | 0,039  |
| BNP               | 1,00       | 0,23      | 1,31       | 0,13      | 0,257  | 1,09             | 0,12      | 0,225  |
| GATA4             | 1,00       | 0,20      | 0,64       | 0,05      | 0,004  | 0,59             | 0,04      | 0,547  |
| NKX2-5            | 1,00       | 0,16      | 0,63       | 0,05      | 0,003  | 0,62             | 0,04      | 0,865  |
| COL1A1            | 1,00       | 0,14      | 1,21       | 0,13      | 0,436  | 1,11             | 0,13      | 0,581  |
| Apoptosis (tunel) | 0,90       | 0,24      | 1,91       | 0,21      | 0,052  | 1,77             | 0,27      | 0,684  |

**Table S9.** The echocardiographic parameters of rats that underwent acute myocardial infarction (AMI) or sham-operation (SHAM) and were treated either with vehicle or compound 3i-1000 loaded TOPSi 7 $\mu$ m particles. Echocardiographic measurements were performed at one week. The number in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison  $\dagger$  SHAM+TOPSi vs. AMI+TOPSi;  $\ddagger$  AMI+TOPSi vs. AMI+3i-1000-TOPSi;  $\text{¥}$  SHAM+3i-1000-TOPSi vs. AMI+3i-1000-TOPSi. IVRT, isovolumic relaxation time; d, diastolic; s, systolic; BPM, beats per minute; LV, left ventricle; IVS, intra ventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall.

|                           | SHAM+TOPSi (7) |           | SHAM+3i-1000-TOPSi (7) |           | ¥     | AMI+TOPSi (6) |           | $\dagger$ | AMI+3i-1000-TOPSi (7) |           | $\ddagger$ |
|---------------------------|----------------|-----------|------------------------|-----------|-------|---------------|-----------|-----------|-----------------------|-----------|------------|
|                           | Average        | $\pm$ SEM | Average                | $\pm$ SEM | p     | Average       | $\pm$ SEM | p         | Average               | $\pm$ SEM | p          |
| E' (mm/s)                 | -39,52         | 1,11      | -38,48                 | 3,14      | 0,054 | -31,34        | 3,31      | 0,057     | -30,53                | 3,25      | 0,844      |
| A' (mm/s)                 | -18,60         | 2,33      | -34,42                 | 5,32      | 0,006 | -18,49        | 1,82      | 0,984     | -19,22                | 3,54      | 0,890      |
| E'/A'                     | 2,37           | 0,35      | 1,30                   | 0,25      | 0,179 | 1,74          | 0,16      | 0,119     | 1,82                  | 0,27      | 0,838      |
| IVRT (ms)                 | 23,64          | 0,83      | 23,15                  | 1,33      | 0,752 | 33,67         | 10,33     | 0,154     | 25,24                 | 1,76      | 0,228      |
| Diameter;d (mm)           | 8,11           | 0,19      | 8,29                   | 0,15      | 0,249 | 9,30          | 0,33      | 0,046     | 8,93                  | 0,65      | 0,515      |
| Diameter;s (mm)           | 5,64           | 0,21      | 5,56                   | 0,20      | 0,009 | 7,63          | 0,59      | 0,005     | 7,31                  | 0,62      | 0,617      |
| Ejection Fraction (%)     | 55,97          | 2,44      | 59,30                  | 2,44      | 0,002 | 35,23         | 7,61      | 0,008     | 35,08                 | 5,95      | 0,984      |
| Fractional Shortening (%) | 30,57          | 1,68      | 32,99                  | 1,85      | 0,002 | 18,47         | 4,56      | 0,011     | 18,13                 | 3,51      | 0,938      |
| Heart Rate (BPM)          | 267,74         | 8,73      | 278,97                 | 9,13      | 0,526 | 264,30        | 14,20     | 0,840     | 289,41                | 14,10     | 0,150      |
| LV Mass (mg)              | 978,99         | 46,74     | 928,47                 | 46,98     | 0,892 | 819,46        | 97,25     | 0,088     | 916,64                | 55,52     | 0,290      |
| Volume;d ( $\mu$ l)       | 356,58         | 18,43     | 373,61                 | 15,22     | 0,170 | 485,03        | 37,23     | 0,048     | 457,27                | 71,67     | 0,656      |
| Volume;s ( $\mu$ l)       | 157,82         | 13,12     | 152,70                 | 12,24     | 0,008 | 321,92        | 47,64     | 0,004     | 297,02                | 53,23     | 0,636      |
| IVS;d (mm)                | 1,54           | 0,18      | 1,45                   | 0,08      | 0,113 | 0,84          | 0,18      | 0,007     | 1,11                  | 0,19      | 0,261      |
| IVS;s (mm)                | 2,13           | 0,22      | 2,23                   | 0,08      | 0,029 | 1,18          | 0,29      | 0,007     | 1,51                  | 0,26      | 0,312      |
| LVID;d (mm)               | 8,03           | 0,21      | 8,05                   | 0,15      | 0,140 | 9,25          | 0,36      | 0,044     | 8,89                  | 0,65      | 0,531      |
| LVID;s (mm)               | 5,59           | 0,23      | 5,40                   | 0,19      | 0,006 | 7,62          | 0,61      | 0,004     | 7,28                  | 0,61      | 0,604      |
| LVPW;d (mm)               | 1,59           | 0,07      | 1,49                   | 0,07      | 0,823 | 1,35          | 0,17      | 0,141     | 1,46                  | 0,11      | 0,490      |
| LVPW;s (mm)               | 2,30           | 0,10      | 2,35                   | 0,10      | 0,085 | 1,77          | 0,27      | 0,049     | 1,91                  | 0,22      | 0,593      |

**Table S10.** The mRNA and histological measurements of rats that underwent acute myocardial infarction (AMI) or sham-operation (SHAM) and were treated either with vehicle or compound 3i-1000 loaded TOPSi 7  $\mu$ m particles. The left ventricular tissues were analysed at the end of the experiment at one week. The number of animals in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by one-way ANOVA followed by a least significant difference post hoc test. Comparison † SHAM+TOPSi vs. AMI+TOPSi; ‡ AMI+TOPSi vs. AMI+3i-1000-TOPSi; ¥ SHAM+3i-1000-TOPSi vs. AMI+3i-1000-TOPSi. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; COL1A1, collagen 1A1; OPN, osteopontin; HW/BW, heart weight to body weight.

|           | SHAM+TOPSi (7) |           | SHAM+3i-1000-TOPSi (7) |           | ¥<br>p | AMI+TOPSi (6) |           | †<br>p | AMI+3i-1000-TOPSi (7) |           | ‡<br>p |
|-----------|----------------|-----------|------------------------|-----------|--------|---------------|-----------|--------|-----------------------|-----------|--------|
|           | Average        | $\pm$ SEM | Average                | $\pm$ SEM |        | Average       | $\pm$ SEM |        | Average               | $\pm$ SEM |        |
| ANP       | 1,00           | 0,12      | 0,64                   | 0,11      | 0,006  | 4,32          | 1,20      | 0,001  | 3,08                  | 0,46      | 0,157  |
| BNP       | 1,00           | 0,11      | 1,02                   | 0,23      | 0,016  | 0,52          | 0,11      | 0,030  | 0,50                  | 0,05      | 0,921  |
| GATA4     | 1,00           | 0,16      | 1,32                   | 0,27      | 0,024  | 0,93          | 0,13      | 0,789  | 0,72                  | 0,07      | 0,420  |
| NKX2-5    | 1,00           | 0,25      | 0,43                   | 0,15      | 0,603  | 0,30          | 0,10      | 0,007  | 0,31                  | 0,11      | 0,953  |
| COL1A1    | 1,00           | 0,23      | 0,38                   | 0,08      | 0,021  | 5,06          | 1,09      | 0,000  | 2,46                  | 0,68      | 0,007  |
| TNF-alpha | 1,00           | 0,42      | 1,04                   | 0,40      | 0,674  | 1,75          | 0,58      | 0,295  | 1,32                  | 0,54      | 0,549  |
| OPN       | 1,00           | 0,23      | 0,29                   | 0,07      | 0,070  | 11,16         | 4,26      | 0,023  | 7,89                  | 4,21      | 0,442  |
| HW/BW     | 3,75           | 0,14      | 3,71                   | 0,04      | 0,006  | 4,37          | 0,27      | 0,036  | 4,54                  | 0,26      | 0,569  |

**Table S11.** The mRNA measurements of the left ventricular endocardial layer of rats in response to isoprenaline-induced myocardial ischemia after treatment with 3i-1000 loaded nanoparticles. The isoprenaline was injected s.c. 24 h before i.v administration of the control particles or 3i-1000 loaded particles. The samples were collected 4 h after particle injections. The number of animals in each group is shown in brackets. The results are averages  $\pm$  SEM. Significance (p) was calculated by independent samples t-test. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; COL1A1, collagen 1A1; IL6, interleukin-6; OPN, osteopontin; TGFB1, transforming growth factor beta 1; TNF-alpha, tumour necrosis factor alpha.

|           | Control (4-5) |           | 3i-1000 (4-5) |           | +<br>p |
|-----------|---------------|-----------|---------------|-----------|--------|
|           | Average       | $\pm$ SEM | Average       | $\pm$ SEM |        |
| ANP       | 1,00          | 0,02      | 0,92          | 0,01      | 0,010  |
| BNP       | 1,00          | 0,07      | 0,75          | 0,07      | 0,044  |
| GATA4     | 1,00          | 0,00      | 1,08          | 0,06      | 0,236  |
| NKX2-5    | 1,00          | 0,05      | 1,05          | 0,09      | 0,673  |
| COL1A1    | 1,00          | 0,00      | 0,75          | 0,06      | 0,013  |
| IL6       | 1,00          | 0,02      | 0,50          | 0,04      | 0,000  |
| OPN       | 1,00          | 0,16      | 0,55          | 0,06      | 0,024  |
| TGFB1     | 1,00          | 0,15      | 0,88          | 0,12      | 0,525  |
| TNF-alpha | 1,00          | 0,08      | 0,86          | 0,05      | 0,150  |

**Table S12.** Taqman probes used on RT-PCR. m, mouse; r, rat; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; COL1A1, collagen 1A1; TNF-alpha, tumour necrosis factor alpha; OPN, osteopontin.

| Messenger RNA | Probe             | Sequence                         |
|---------------|-------------------|----------------------------------|
| mANP          | Forward           | GAAAAGCAAAGCTGAGGGCTCTG          |
|               | Reverse           | CCTACCCCCGAAGCAGCT               |
|               | Fluorogenic probe | TCGCTGGCCCTCGGAGCCT              |
| rANP          | Forward           | GAAAAGCAAAGCTGAGGGCTCTG          |
|               | Reverse           | CCTACCCCCGAAGCAGCT               |
|               | Fluorogenic probe | TCGCTGGCCCTCGGAGCCT              |
| mBNP          | Forward           | AGGCGAGACAAGGGAGAACA             |
|               | Reverse           | GGAGATCCATGCCGAGA                |
|               | Fluorogenic probe | CATCATTGCCTGGCCCATCGC            |
| rBNP          | Forward           | TGGGCAGAAGATAGACCGGA             |
|               | Reverse           | ACAACCTCAGCCCGTCACAG             |
|               | Fluorogenic probe | CGGCGCAGTCAGTCGCTTGG             |
| m,rGATA4      | Forward           | AAGGCTATGCATCTCCTGTCACT          |
|               | Reverse           | CCAGGCTGTTCCAAGAGTCC             |
|               | Fluorogenic probe | ACATCGCAGGCCAGCTCCAAGC           |
| mNKX2-5       | Forward           | CCTCGGGCGGATAAAAAAGA             |
|               | Reverse           | CGGCTTTGTCCAGCTCCA               |
|               | Fluorogenic probe | CTGTGCGCGCTGCAGAAGGC             |
| rNKX2-5       | Forward           | CCTCGGGCGGATAAGAAAGA             |
|               | Reverse           | CGGCTTTGTCCAGCTCCA               |
|               | Fluorogenic probe | CTGTGCGCGCTGCAGAAGGC             |
| m,r18 S       | Forward           | TGGTTGCAAAGCTGAAACTTAAAG         |
|               | Reverse           | AGTCAAATTAAGCCGCAGGC             |
|               | Fluorogenic probe | CCTGGTGGTGCCCTTCCGTCA            |
| mCOL1A1       | Forward           | CCCTGGCCTTGGAGGAA                |
|               | Reverse           | CACGGAAACTCCAGCTGATTTT           |
|               | Fluorogenic probe | CTTTGCTTCCCAGATGTCCTATGGCTATGATG |
| rCOL1A1       | Forward           | CCCCTTGGTCTTGGAGGAA              |
|               | Reverse           | GCACGGAAACTCCAGCTGAT             |
|               | Fluorogenic probe | CTTTGCTTCCCAGATGTCCTATGGCTATGATG |
| rTNF-alpha    | Forward           | GACAAGGCTGCCCCGACTA              |
|               | Reverse           | CTCCTGGTATGAAGTGGCAAATC          |
|               | Fluorogenic probe | TGCTCCTCACCCACACCGTCAGC          |
| rOPN          | Forward           | AATCGCCCCACAGTCG                 |
|               | Reverse           | CCTCAGTCCGTAAGCCAAGC             |
|               | Fluorogenic probe | TGTCCTGACGGCCGAGGTGA             |