SUPPLEMENTAL INFORMATION

SUMO1-dependent modulation of SERCA2a in heart failure

Changwon Kho1*, Ahyoung Lee1*, Dongtak Jeong1, Jae Gyun Oh2, Antoine H. Chaanine1, Eddy Kizana3, Woo Jin Park2, and Roger J. Hajjar1**

1Cardiovascular Research Center, Mount Sinai School of Medicine, New York, NY10029, USA.
2College of Life Sciences, Gwangju Institute of Science and Technology, Gwangju, South Korea.
3Westmead Clinical School, The University of Sydney, Sydney, Australia.
* These authors contributed equally to this work.
** Corresponding author; Roger J. Hajjar, MD.

[Supplemental information included Eleven figures and Nine tables.]
Supplementary Figure 1. SERCA2a complex analysis.
(a) Two-dimensional SDS-PAGE gels of SERCA2a complexes. A silver-stained SDS-PAGE gel is shown, which reveals a 12 kDa spot (arrow) that immunoprecipitated with SERCA2a. SERCA2a enrichment was confirmed by blotting the immunoprecipitated
SERCA2a complexes with a rabbit anti-SERCA2a antibody (anti-S2a). Rabbit IgG (anti-IgG) was used as a negative control.

(b) The representative peptide fingerprint for SUMO1 was identified by tandem mass spectrometric analysis. The protein sequence of SUMO1 is shown with the matched peptide highlighted in bold.

(c) *in vivo* SERCA2a SUMOylation in HEK293 cells expressing flag-tagged SUMO1. Precipitated flag-tagged SUMO1 conjugates were analyzed using an anti-SERCA2a antibody.

(d) Direct interaction between SERCA2a and Ubc9 (left panel). HEK293 cells were transfected with expression vectors for myc-tagged Ubc9 and SERCA2a or a pcDNA vector (negative control). Cell lysates were subjected to immunoprecipitation (IP) with an anti-myc antibody, and the resulting precipitates were immunoblotted with anti-SERCA2a. HC, heavy chain; LC, light chain. Endogenous interaction between SERCA2a and Ubc9 in isolated adult mouse cardiomyocytes (right). Immunoprecipitation using cardiomyocyte lysates showing Ubc9 and SERCA2a interact *in vivo*.

(e) *in vivo* SERCA2a SUMOylation in isolated adult mouse cardiomyocytes infected with adenovirus expressing SUMO1. Immunoblot analysis showed that SUMO1 has a dose-dependent affect on SERCA2a SUMOylation in cardiomyocytes.

(f) SUMOylated SERCA2a in human patient. Reverse IP experiments on SERCA2a SUMOylation *in vivo* was performed on same human patient as shown in Figure 1. IP was performed with anti-SERCA2a antibody and the SUMO1 conjugations were revealed by anti-SUMO1 (top panel). The same blot was stripped and re-probed with anti-SUMO2/3 (bottom panel).
Supplementary Figure 2. Distribution of SUMO1 expression after tail-vein injection. 
(a) Immunoblotting for SUMO1 expression. 4 weeks after tail-vein injection of either 
rAAV9/SUMO1 or rAAV9/GFP, indicated mice tissues were harvested and subjected to 
immunoblotting to detect SUMO1 expression. H: Heart; Li: Liver; Lu: Lung; B: Brain; 
K: Kidney; SM: Smooth muscle; Ctl: heart of rAAV9/GFP injected mice.
(b) Animals were injected by rAAV9/SUMO1 in a different amount of viral genomes (VG). Heart and liver tissues were then analyzed by immunoblotting to detect expression of SUMO1 transduction. GAPDH expression was examined for normalization purposes. (e) At 4 weeks after SUMO1 gene delivery, optical section images collected from cardiac tissues were double stained for SUMO1 (red) and DAPI (DNA, blue). rAAV9/GFP injected heart used as a control.
Supplementary Figure 3. Hemodynamic analyses of SUMO1 gene delivered mouse. Representative pressure volume loops at 2 months after adeno-associated virus encoding SUMO1 (rAAV9/SUMO1) delivery (4 months after TAC operation). After induction of HF, the loops shifted rightward. The end-systolic pressure-volume relationship (ESPVR) in the LV was slightly steeper in rAAV9/SUMO1 treated animals than in rAAV9/GFP treated controls, suggesting increased cardiac contractility. Sham + rAAV9/GFP: green; TAC + rAAV9/SUMO1: light blue; TAC + rAAV9/SERCA2a: blue; TAC + rAAV9/GFP: red.
Supplementary Figure 4. N-terminal domain of SERCA2a.
The protein sequences of human (AC# P16615-2), pig (AC# P11607-2), rat (AC# P11507-2) and mouse (AC# O55143-2) SERCA2a were aligned. Putative SUMO consensus motifs (ψKXE, where ψ is a hydrophobic amino acid) were detected at lysine 480 and lysine 585. The SUMO modification sites of SERCA2a are outlined in black. Hydrolase domains are outlined by a dotted line.
Supplementary Figure 5. Ubiquitination of SERCA2a.
HEK cells transfected with SERCA2a (WT or K480R/K585R) alone or together with flag-tagged SUMO1. 48 hours after transfection, cells were treated with 20 µM MG132 (proteasome inhibitor) for 3 hours. Cells were lysed in with lysis buffer (10 mM Tris–HCl, pH 7.5, 10 mM NaCl, 0.5% NP-40, 5 mM EDTA, 5 mM EGTA plus 20 mM NEM, 200 µM iodoacetamide, 1 mM sodium orthovanadate and 1 complete EDTA-free cocktail tablet (Roche Molecular Biochemicals). After centrifugation at 13,000 g for 10 minutes, supernatants were subjected to immunoprecipitation with anti-SERCA2a antibody. The ubiquitin conjugated SERCA2a were analyzed by anti-Ubiquitin antibody.
Supplementary Figure 6. Effects of tamoxifen-induced SUMO1 overexpression on Ca\textsuperscript{2+} regulatory proteins in transgenic mice.
(a) Generation of conditional SUMO1 transgenic mice. Cre/loxP conditional expression system was utilized in which administration of tamoxifen induced heart-specific SUMO1 overexpression and concomitantly inhibited EGFP expression. Cardiac SUMO1 is expressed under the control of the α-MHC promoter.
(b) Immunoblot analysis revealed that the levels of tamoxifen-induced SUMO1 expression in the transgenic mice were approximately 5-fold higher than that of wild-type littermates. WT: wild-type littermates; TG: SUMO1 transgenic mice.
(c) Representative immunoblots show alternations of cardiac protein expressions in SUMO1 transgenic mice treated with tamoxifen received in a different duration as indicated. 

(d) Effect of tamoxifen-induced SUMO1 overexpression on SUMOylations of SERCA2a. Cardiac tissues were immunoprecipitated with anti-SUMO antibodies. SUMOylated forms of SERCA2a were detected by immunoblot analysis using an anti-SERCA2a antibody. S-SERCA2a: SUMOylated SERCA2a.
Supplementary Figure 7. Hemodynamic analyses of SUMO1 transgenic mice both in sham and TAC operation groups. Systolic and diastolic function was determined in SUMO1 transgenic mice by pressure-volume analysis. At 3 month of TAC, the loops shifted rightward. The end-systolic pressure-volume relationship (ESPVR) in the LV was slightly steeper in TG animals than in WT, suggesting increased cardiac contractility. By contrast, the slope of the LV end-diastolic pressure-volume relationship (EDPVR) was decreased in TG mice, indicating a decreased end-diastolic LV chamber stiffness. WT [wild type littermate]-sham: black; TG [SUMO1 transgenic mice]-sham: blue; WT-TAC: red; TG-TAC, green.
Supplementary Figure 8. Dose-dependent effects of rAAV vectors containing shRNA against SUMO1.
(a) SUMO1 shRNA construct design. A recombinant AAV was designed to express a SUMO1 shRNA under control of the U6 promoter. As a control, a scramble expression cassette was cloned into the same viral vectors.

Kho et al.
(b) Representative immunoblot analysis of cardiac tissue extracts from mice infected with rAAV9 expressing scrambled control shRNA (rAAV9/SC) or shRNA against SUMO1 (rAAV9/shSUMO1) 3 weeks after injection (left panel). Total extracts were probed with the indicated antibodies, and the level of inhibition of cardiac SUMO1 expression was measured (5 x 10^{10} vg/mice per each group, n=5 per each group, right panel).

(e) Representative pressure-volume loops from a rAAV9/shSC and rAAV9/shSUMO1 injected mice. Pressure-volume loops are measured before and during transient inferior vena cava occlusion. SUMO1 down-regulation by rAAV9/shSUMO1 injection induced a rightward shift and decreased the slope of the linear fit line of the ESPVR. More severe effects were occurred when we injected an increased dose of rAAV9/shSUMO1.

(d) Quantitative analysis of echocardiographic assessments including internal diameters in end-diastole (LVIDd), end-systole (LVIDs), fractional shortening (FS), and ejection fraction (EF). rAAV9/shSC (5 x 10^{10} vg/mice, n=14); lower dose of rAAV9/shSUMO1 (3 x 10^{10} vg/mice, n=14); higher dose of rAAV9/shSUMO1 (5 x 10^{10} vg/mice, n=12).

(e) SUMOylation of SERCA2a. Cardiac tissues were immunoprecipitated with anti-SUMO1 antibody. SUMOylated forms of SERCA2a were detected by immunoblot analysis using an anti-SERCA2a antibody. Representative immunoblots and protein quantification results are shown (n=6 per each group).

(f) ATPase activity of SERCA2a. Ca^{2+}-dependence of SERCA2a’s ATPase activity is measured in preparations from scramble injected (●) and shRNA against SUMO1 injected hearts with 3 x 10^{10} vg/mice (●) and 5 x 10^{10} vg/mice (●) (n=3 per each group).

All data represent means ± SD. * p < 0.05; ** p < 0.001 vs. respective control using Student t-test.
Supplementary Figure 9. Effects of AAV-mediated SUMO1 gene delivery on SUMOylations of known cardiac transcriptional factors such as SRF (a), GATA4 (b), and SERCA2a (c). Cardiac tissues were immunoprecipitated with anti-SUMO1 agarose.
SUMOylated forms of SRF, GATA4, and SERCA2a were detected by immunoblotting using its primary antibodies.
Supplementary Figure 10. shRNA-mediated loss of SERCA2a impairs rodent cardiac function.
(a) Contractile properties of isolated rat cardiomyocytes. Maximal rate of contraction, relaxation and cell shortening were measured by IonOptix system. Values are means ± SD; n = 20 cells per group.
(b) Immunoblotting for SERCA2a was performed with total lysates from the isolated rat cardiomyocytes at 5 days after viral infection. Lower right hand panel shows quantification of SERCA2a in the three different groups of cells.
(c) Representative M-mode echocardiography showing dilated left ventricle end diastole and systole in SERCA2a shRNA injected hearts, without or with SUMO1 overexpression compared with sham control.
(d) Evaluation of cardiac parameters: LVIDd in mm and LVIDs in mm (n=3 rats per each group).
(e) Fractional shortening measured at two different time points.
(f) Immunoblots of SERCA2a expression. Values are mean ± SD; * p < 0.05 versus sham control; NS, no significant.
Supplementary Figure 11. shRNA-mediated loss of SERCA2a impairs murine cardiac function.
(a) Contractile properties of isolated mouse cardiomyocytes. Maximal rate of contraction, relaxation and cell shortening were measured by IonOptix system. Values are means ± SD; n = 20 cells per group.

(b) Representative M-mode echocardiography showing dilated left ventricle at end-diastole and end-systole in SERCA2a shRNA injected heart, without or with SUMO1 overexpression in comparison with sham control at 2 weeks after SERCA2a shRNA injection.

(c) Evaluation of cardiac function in six SERCA2a shRNA injected mice, four SERCA2a shRNA with SUMO1 co-injected mice and six age-matched sham control mice, showing left ventricular dimensions such as LVIDd in mm and LVIDs in mm.

(d) Fractional shortening measured at two different time points.

(e) Immunoblots of SERCA2a expression. Values are mean ± SD; ** p < 0.001 versus sham control; NS, no significant.
Supplementary Table 1. Echocardiographic parameters for SUMO1 gene transfer mice. Data represent the mean value ± SD of cardiac functional parameters after 4 month TAC operation. LVIDd, left ventricular internal dimension-diastole; LVIDs, left ventricular internal dimension-systole; FS, fractional shortening; EF, ejection fraction; HR, heart rate. * p < 0.05; ** p < 0.001 vs. rAAV9/GFP in TAC.

|          | Sham rAAV9/GFP (n=12) | TAC rAAV9/GFP (n=10) | TAC rAAV9/SUMO1 (n=14) | TAC rAAV9/SERCA2a (n=12) |
|----------|------------------------|-----------------------|-------------------------|--------------------------|
| LVIDd (mm) | 3.40 ± 0.10            | 5.13 ± 0.57           | 3.33 ± 0.38*            | 3.46 ± 0.58**            |
| LVIDs (mm) | 1.50 ± 0.30            | 4.08 ± 0.71           | 1.55 ± 0.31**           | 1.53 ± 0.30**            |
| FS (%)    | 55.13 ± 1.50           | 21.11 ± 1.71          | 53.50 ± 5.03**          | 55.42 ± 2.05**           |
| EF (%)    | 90.28 ± 1.02           | 48.50 ± 9.74          | 88.94 ± 3.70**          | 90.44 ± 1.35**           |
| HR (BPM)  | 618.18 ± 41.70         | 589.16 ± 40.33        | 658.51 ± 32.35          | 629.34 ± 48.36           |

Kho et al.
**Supplementary Table 2. Hemodynamic parameters for SUMO1 gene transfer mice.**
All values represent mean ± SD. ESPVR slope; relationship between end-systolic pressure and volume; EDPVR slope; end-diastolic pressure-volume relationship; Pmax, maximum pressure point; Pmin, minimum pressure point; Pmax, maximum dP/dt; Pmin, minimum dP/dt; EDV, left ventricular end-diastolic volume; ESV, left ventricular end-systolic volume; SV, stroke volume; HR, heart rate (beats/min). Heart weight (HW) to Body weight (BW) ratio in mice was measured. *p < 0.05 vs. rAAV9/GFP in TAC.

|                     | Sham          | TAC           | TAC           |
|---------------------|---------------|---------------|---------------|
|                     | rAAV9/GFP (n=3) | rAAV9/GFP (n=3) | rAAV9/SUMO1 (n=3) | rAAV9/SERCA2 (n=3) |
| **ESPVR slope**     | 4.48 ± 0.63   | 1.15 ± 0.58   | 4.33 ± 0.90*  | 4.56 ± 0.68*       |
| (mmHg/µl)           |               |               |               |                   |
| **EDPVR slope**     | 0.03 ± 0.02   | 0.05 ± 0.02   | 0.05 ± 0.02   | 0.05 ± 0.02        |
| (mmHg/µl)           |               |               |               |                   |
| **Pmax (mmHg)**     | 69.50 ± 2.51  | 102.50 ± 46.66| 100.11 ± 31.22| 115.66 ± 20.20     |
| **Pmin (mmHg)**     | 4.00 ± 1.41   | 13.50 ± 1.41  | 8.27 ± 6.72   | 17.70 ± 5.33*      |
| **dPmax**           | 4195.75 ± 2526.84| 5761.83 ± 2439.28| 4912.50 ± 1849.55| 5560.48 ± 1331.69 |
| **dPmin**           | -3825.25 ± 2807.56 | -4995.83 ± 9895.83 | -4240.00 ± 1849.55| -4729.75 ± 1331.69 |
| **EDV (µl)**        | 58.75 ± 10.96 | 124.00 ± 6.36 | 54.24 ± 12.12*| 50.80 ± 22.09*     |
| **ESV (µl)**        | 20.91 ± 9.31  | 98.25 ± 26.51 | 21.91 ± 12.93*| 27.94 ± 12.53*     |
| **SV (µl)**         | 39.00 ± 2.82  | 42.50 ± 16.97 | 37.76 ± 1.74* | 37.33 ± 9.01*      |
| **HR (bpm)**        | 522.50 ± 40.30| 440.25 ± 20.15| 528.12 ± 33.44| 567.71 ± 76.17     |
| **HW/BW (mg/g) (n=10)** | 3.97 ± 0.24 | 8.57 ± 2.77 | 4.15 ± 0.66* | 4.15 ± 1.24*       |
Supplementary Table 3. Echocardiographic parameters for SUMO1 transgenic mice with or without tamoxifen administration. Data represent the mean value ± SD of cardiac functional parameters after 1 month of tamoxifen treatment. WT, wild type littermate; TG, SUMO1 transgenic mice; TAM, tamoxifen; LVIDd, left ventricular internal dimension-diastole; LVIDs, left ventricular internal dimension-systole; FS, fractional shortening; EF, ejection fraction; HR, heart rate.

|                | TAM (-) (n=3) | WT (n=3) | TAM (+) (n=3) | TG TAM (-) (n=3) | TAM (+) (n=3) |
|----------------|---------------|-----------|---------------|------------------|---------------|
| LVIDd (mm)     | 3.26 ± 0.11   | 3.13 ± 0.05 | 3.33 ± 0.11   | 2.90 ± 0.34      |
| LVIDs (mm)     | 1.26 ± 0.05   | 1.30 ± 0.20 | 1.30 ± 0.10   | 1.02 ± 0.22      |
| FS (%)         | 61.46 ± 1.46  | 58.87 ± 5.00 | 61.40 ± 1.55  | 65.5 ± 3.74      |
| EF (%)         | 93.80 ± 0.69  | 92.32 ± 2.79 | 93.76 ± 0.74  | 95.46 ± 1.44     |
| HR (BPM)       | 630.43 ± 44.48 | 601.69 ± 22.35 | 562.45 ± 48.66 | 521.28 ± 35.65  |
Supplementary Table 4. Echocardiographic parameters for SUMO1 transgenic mice after TAM-induced SUMO1 overexpression at 3 month post-TAC. Data represent the mean value ± SD of cardiac functional parameters. WT, wild type littermate; TG, SUMO1 transgenic mice; TAM, tamoxifen; LVIDd, left ventricular internal dimension-diastole; LVIDs, left ventricular internal dimension-systole; FS, fractional shortening; EF, ejection fraction; HR, heart rate. ** $p < 0.001$ vs. WT in TAC.

|                | 2M post-TAC |                              | 3M post-TAC (+TAM) |
|----------------|-------------|------------------------------|--------------------|
|                | WT (n=12)   | TG (n=10)                    | WT (n=14)          |
|                |             |                              | TG (n=12)          |
| LVIDd (mm)     | 4.20 ± 0.56 | 3.76 ± 0.47                  | 4.40 ± 0.57        |
|                |             |                              | 3.24 ± 0.33**      |
| LVIDs (mm)     | 2.60 ± 0.70 | 2.33 ± 0.61                  | 2.93 ± 0.54        |
|                |             |                              | 1.44 ± 0.30**      |
| FS (%)         | 41.23 ± 7.35| 37.40 ± 8.54                 | 34.30 ± 5.56       |
|                |             |                              | 55.91 ± 6.70**     |
| EF (%)         | 77.84 ± 8.06| 73.27 ± 1.53                 | 69.57 ± 7.28       |
|                |             |                              | 90.26 ± 4.27**     |
| HR (BPM)       | 620.14 ± 30.65| 593.60 ± 65.17              | 620.72 ± 42.60     |
|                |             |                              | 550.18 ± 68.48     |
**Supplementary Table 5. Hemodynamic parameters based on in vivo pressure-volume relationships for SUMO1 transgenic mice.** All values represent mean ± SD. ESPVR slope; relationship between end-systolic pressure and volume; EDPVR slope; end-diastolic pressure-volume relationship; Pmax, maximum pressure point; Pmin, minimum pressure point; Pmax, maximum dP/dt; Pmin, minimum dP/dt; EDV, left ventricular end-diastolic volume; ESV, left ventricular end-systolic volume; SV, stroke volume; HR, heart rate (beats/min). Heart weight (HW) to Body weight (BW) ratio in mice was measured. *p < 0.05 vs. WT in TAC.

|                       | Sham (n=5) | WT (n=5) | TAC (n=3) | TG (n=3) |
|-----------------------|------------|----------|-----------|----------|
| ESPVR slope (mmHg/µl) | 4.42 ± 1.07| 6.12 ± 1.74| 2.56 ± 0.71| 4.33 ± 1.49* |
| EDPVR slope (mmHg/µl) | 0.07 ± 0.05| 0.12 ± 0.07| 0.08 ± 0.04| 0.03 ± 0.01  |
| Pmax (mmHg)           | 56.37 ± 3.90| 65.3 ± 9.68| 98.90 ±15.06| 102.66 ± 8.95* |
| Pmin (mmHg)           | 3.89±1.93  | 6.50 ± 2.01| 4.30 ± 3.30| 12.30 ± 1.50 |
| dPmax                 | 2708.73 ± 765.78| 3730.10 ± 920.805| 3751.80 ± 741.41| 4745.33 ± 475.31 |
| dPmin                 | -2035.03 ± 749.19| -3004.20 ± 918.61| -3535.80 ± 718.18| -2853.50 ± 351.98 |
| EDV (µl)              | 50.08 ± 4.17| 53.40 ± 14.34| 120.90 ±13.35| 62.09 ± 22.76 |
| ESV (µl)              | 27.89 ± 6.44| 27.10 ± 9.94| 85.40 ±23.27| 37.45 ± 26.87 |
| SV (µl)               | 27.71 ± 6.97| 33.60 ± 12.13| 46.10 ± 11.40| 26.90 ± 9.33* |
| HR (bpm)              | 437.34 ± 97.16| 448.90 ± 72.55| 441.00 ± 70.59| 384.90 ± 38.66 |
| HW/BW (mg/g) (n=10)   | 4.61 ± 0.45| 4.20 ± 0.99| 8.89 ± 0.36| 7.34 ± 0.13* |

*Kho et al.* 24
Supplementary Table 6. Echocardiographic parameters for AAV9-mediated cardiac SUMO1 silencing mice. All values represent mean ± SD. LVIDd, left ventricular internal dimension-diastole; LVIDs, left ventricular internal dimension-systole; FS, fractional shortening; EF, ejection fraction; HR, heart rate. ** p < 0.001 vs. rAAV9/SC.

|                | rAAV9/SC     | rAAV9/shSUMO1 |
|----------------|--------------|--------------|
|                | 5 x 10^10    | 5 x 10^10    |
|                | (n=14)       | (n=14)       |
| LVIDd (mm)     | 1.30 ± 0.12  | 2.02 ± 0.17**|
| LIVDs (mm)     | 3.42 ± 0.19  | 3.85 ± 0.17**|
| FS (%)         | 61.77 ± 4.77 | 47.63 ± 3.07**|
| EF (%)         | 93.85 ± 1.23 | 84.51 ± 2.63**|
| HR (BPM)       | 569.88 ± 71.35 | 639.61 ± 50.38 |
Supplementary Table 7. Hemodynamic parameters for AAV9-mediated cardiac SUMO1 silencing mice. Summary of hemodynamic parameters in each group, measured using Scisense pressure–volume catheter. The data are expressed as mean values ± S.D. ESPVR slope; relationship between end-systolic pressure and volume; EDPVR slope; end-diastolic pressure-volume relationship; Pmax, maximum pressure point, Pmin, minimum pressure point, Pmax, maximum dP/dt; Pmin, minimum dP/dt; EDV, left ventricular end-diastolic volume; ESV, left ventricular end-systolic volume; CO, cardiac output; Ea, arterial elastance; HR, heart rate (beats/min). Heart weight (HW) to Body weight (BW) ratio in mice was measured. * p < 0.05; ** p < 0.001 vs. rAAV9/SC control group.

|                          | rAAV9/SC $5 \times 10^{10}$ (n=7) | rAAV9/shSUMO1 $5 \times 10^{10}$ (n=8) |
|--------------------------|----------------------------------|--------------------------------------|
| ESPVR slope (mmHg/µl)    | 4.57 ± 0.74                      | 0.96 ± 0.76**                        |
| EDPVR slope (mmHg/µl)    | 0.06 ± 0.05                      | 0.06 ± 0.02                          |
| Pmax (mmHg)              | 73.22 ± 10.22                    | 54.00 ± 9.53*                        |
| Pmin (mmHg)              | 7.55 ± 3.20                      | 4.33 ± 2.08                          |
| dPmax (mmHg)             | 3797.02 ± 469.32                 | 2554.6667 ± 471.28*                  |
| dPmin (mmHg)             | -3264.56 ± 633.37                | -2093.67 ± 553.37                    |
| EDV (µl)                 | 48.36 ± 4.81                     | 47.33 ± 2.51                         |
| ESV (µl)                 | 24.33 ± 9.01                     | 18.83 ± 9.46                         |
| SV (µl)                  | 29.13 ± 2.23                     | 30.66 ± 4.16                         |
| HR (bpm)                 | 523.91 ± 101.88                  | 492.00 ± 42.33                       |
| HW/BW (mg/g) (n=7)       | 4.19 ± 0.01                      | 4.80 ± 0.10*                         |
Supplementary Table 8. Echocardiographic parameters for shRNA-mediated SERCA2a silencing rat. Summary of cardiac function in lentiviral SERCA2a shRNA injected rats, with either adenoviral β-gal (shS2a) or adenoviral SUMO1 (shS2a+SUMO1). Age-matched sham-operated rat served as controls (n=3 per group). The data are expressed as mean values ± S.D. * p < 0.05; ** p < 0.001 versus sham control.

|                   | Sham (n=3) | shSERCA2a (n=3) | + SUMO1 (n=3) |
|-------------------|------------|----------------|--------------|
| LVIDd (mm)        | 5.82 ± 0.48| 6.94 ± 0.68    | 7.01 ± 0.55  |
| LVIDs (mm)        | 1.68 ± 0.50| 3.13 ± 0.16*   | 3.35 ± 0.32* |
| FS (%)            | 70.91 ± 5.7| 51.56 ± 3.89*  | 52.22 ± 0.79*|
| EF (%)            | 86.52 ± 0.45| 67.31 ± 2.67**| 67.99 ± 0.63**|
| HR (BPM)          | 400.33 ± 27.53 | 437.40 ± 20.14 | 447.25 ± 42.83 |
**Supplementary Table 9. Echocardiographic parameters for shRNA-mediated SERCA2a silencing mice.** Evaluation of cardiac function in lentiviral SERCA2a shRNA injected mice, with either rAAV9/GFP (shS2a, n=6) or rAAV9/SUMO1 (SUMO1+shS2a, n=4). Age-matched sham-operated mice served as controls (n=4). The data are expressed as mean values ± S.D. ** p < 0.001 versus sham control.

|                  | Sham (n=4) | shSERCA2a (n=6) | SUMO1+shSERCA2a (n=4) |
|------------------|------------|-----------------|-----------------------|
| LVIDd (mm)       | 3.22 ± 0.11| 3.87 ± 0.14**   | 4.02 ± 0.24**         |
| LIVDs (mm)       | 1.23 ± 0.08| 2.14 ± 0.18**   | 2.29 ± 0.18**         |
| FS (%)           | 61.74 ± 1.53| 44.54 ± 3.63** | 43.11 ± 2.55**        |
| EF (%)           | 93.94 ± 0.74| 81.64 ± 3.62** | 80.30 ± 2.66**        |
| HR (BPM)         | 608.75 ± 26.57| 640.80 ± 19.05 | 615.00 ± 41.18       |