APPEEDIX E1. METHODS

Mouse Heart Tissue Collection

Each mouse was anesthetized by inhaled isoflurane before heart harvest. A U-shape incision was made for cutting both skin and peritoneum that begins at navel area and extends up to front legs. Then the chest cavity was opened by making an incision in the diaphragm and cutting the rib cage on both sides. The heart was harvested by gently lifting at the apex and cutting the great vessels. The excised heart was immediately placed into cold (4°C) Krebs buffer (119 NaCl, 25 $NaHCO_3$, 4.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 11 glucose, in mM, pH 7.4) for microvessel study or cold cell culture medium for cell isolation, respectively. The experimental protocols/groups are summarized in the Figure E1.

Microvessel Dissection and Stabilization

The distal portion of left anterior descending artery (100-150 μm in diameter) was dissected from the isolated mouse heart as previously described.[E1] Then, the microvessel was cannulated with 2 glass micropipettes and pressurized in a no-flow state in a microvessel organ chamber containing aerated (95% O_2, 5% CO_2) Krebs buffer solution, and maintained at 37^0C. After a 60-minute stabilization period in the chamber, we studied the pathophysiological change by H/R injury stimulation.

CP-H/R Model of Endothelial Cells

Mouse heart endothelial cells (MHECs) isolated from the harvested heart were cultured in EGM-2 MV medium (Lonza Biosciences, Alpharetta, GA). For developing CP-H/R model, MHECs were placed in the sealed chamber, filled by high-concentration of nitrogen (95% N_2 +5%CO_2) for 3 hours, and then re-oxygenated 2 hours by transferring

26 cells into the normoxic culture incubator. Mito-Tempo (10μM) was added to the culture
27 medium 30 minutes before H/R.[E1]
28
29 **Measurement of Intracellular Calcium**
30 Endothelial cells were loaded with 1μM Rhod-2 (Thermo Fisher Scientific) for 30
31 minutes by incubation in bath solution containing 5 KCl, 140 NaCl, 2 $MgCl_2$, 1 $CaCl_2$,
32 10 HEPES, 30 glucose (in mM). Rhod-2 AM was excited using a 545 nm laser and the
33 fluorescence emission was collected at 578 nm wavelengths. Rhod-2 fluorescence
34 signal was converted to intracellular Ca^{2+} concentration using the following equation:
35 $[Ca^{2+}]_i$= Kd($F-F_{min}$)/($F_{max}-F$), where Kd Rhod-2=1.58μM and $F_{min}=F_{max}/15$.[E2,E3] F_{max}
36 was determined by adding 100μM Rhod-2 to the 1 mM Ca^{2+} bath solution and
37 measuring the Rhod-2 fluorescence. The fluorescence quantification was obtained
38 using ImageJ (National Institutes of Health) software.
39
40 **Electrophysiological Study of Endothelial Cell K^+ Currents**
41 the patch clamp recording system are depicted in the Figure E2. Patch clamp recording
42 techniques were used to measure K^+ currents in the whole cell patch-clamp
43 configurations, as described in detail previously. [E1] The primarily cultured MHECs
44 were washed twice with Ca^{2+}-free DMEM. Whole cell K^+ currents were recorded with
45 Axon Axopatch-200B amplifier, Axon Digidata 1550B A/D converter and pClamp 11
46 software (all by Molecular Devices, Foster City, CA) in the voltage-clamp mode. The
47 bath solution containing 5 KCl, 140 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 30 glucose
48 (in mM, pH 7.4, 22°C). The patch pipette (1-3 MΩ) was filled with the pipette solution
49 containing 20 KCl, 8 NaCl, 110 K-Aspartate, 8.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 0.01

50 Niflumic acid and 10 BAPTA (in mM, pH 7.2, with calculated free Ca^{2+} 400 nmol/L).

51 For Ca^{2+} free (low Ca^{2+} group) pipette solution, no $CaCl_2$ was added. For high Ca^{2+}

52 (2μM) pipette solution, 9.7mM $CaCl_2$ was added. The free Ca^{2+} concentration was

53 calculated by using Maxchelator as described previously in detail. [E4]

54

55 Current voltage recording conditions for K^+ currents were achieved by stepping in 20

56 mV increments from a holding potential -50 mV by 150 ms test pulses in the range

57 between -100 to +100 mV. K^+ currents were expressed as current density (current

58 divided by cell membrane capacitance). Low-pass filter frequency was 2 kHz and

59 sampling rate was 10 kHz. Current time recording conditions for K^+ currents were

60 obtained by holding potential at +100 mV. The selective SK activator NS309 on the

61 whole cell K^+ currents were examined, and then both SK2/SK3 blocker apamin (10^{-7}M)

62 and the SK4(IK) blocker TRAM34 (10^{-6}M) were applied for testing the specificity of

63 SK channel activation.[E1, E5,E6]

64

65 **Western Blotting**

66 The methods for whole-cell protein purification, Western blotting, and imaging

67 quantification have been described previously. [E1,E5,E6] Membranes were incubated

68 overnight at 4°C with primary antibodies against SK3 and SK4 (Alomone Labs,

69 Jerusalem, Israel). After washing with TBST, membranes were incubated with

70 secondary antibody conjugated to horseradish peroxidase. All membranes were also

71 incubated with glyceraldehyde-3-phosphate dehydrogenase (Cell Signaling Technology,

72 Danvers, MA) as loading controls.

73

74 **Measurement of Mitochondrial Reactive Oxygen Species**

75 MHECs were stained with 5 μM MitoSox™ Red and 100 nM MitoTracker Green FM
76 (Invitrogen, Waltham, MA) according to the manufacture's protocol and previously
77 studied.[E6] Then cells were counter stained with 5μg/ml Hoechst 33342 for 10 minutes.
78 Images were taken on a Zeiss LSM710 confocal microscope (Carl Zeiss GmbH,
79 Germany). The mean values of the whole cell fluorescence of MitoSOX™ Red were
80 obtained with Image J software.[E6]

81

82 **RESULTS**

83 **Characteristics of DM and ND Mice.**

84 The age, body weight and blood glucose levels of nondiabetic (ND) and diabetic (DM)
85 mice are summarized in Figure E3.

86

87 **Dose-and Endothelium-dependent Relaxation Responses to NS309 and ADP in the**
88 **Mouse Small Coronary Arteries**

89 Following CP-H/R, the endothelium-dependent relaxation responses of mouse diabetic
90 vessels to NS309 (Figure E4A) and ADP (Figure E4B) were significantly decreased
91 compared with that of non-diabetic mice in a dose-dependent fashion. Treatment with
92 MT (10μM) improved the recovery of endothelium-dependent relaxation responses to
93 the SK activator NS309 and ADP in DM and ND mice.

94

95 **Dose-and Endothelium-independent Relaxation Responses to SNP in the Mouse**
96 **Small Coronary Arteries**

97 There were no significant differences in the dose-and endothelium-independent relaxation responses to SNP following CP-H/R between ND and DM groups. There were no significant changes in the dose-dependent relaxation response to SNP after MT (10μM) treatment between ND and DM groups (Figure E4C).

MT (10μM) Enhanced SK Channel Currents of MHECs in H/R Model from ND and DM Mice

Administration of NS309 significantly increased the total K^+ currents of MHECs in H/R model both types of cell/treatment from ND and DM mice (*P*=0.071, and *P*=0.0494, Figure E5A, C), which also seen in ND and DM treatment with MT (10μM) groups (*P*=0.0027, and *P*<0.0001, Figure E5B, D). Subsequent application of apamin (10^{-7}M) and TRAM34(10^{-6}M) abolished NS309-induced effects on K^+ currents in both cell/treatment types.

E-REFERENCES

E1. Zhang Z, Shi G, Liu Y, Xing H, Kabakov AY, Zhao AS et al. Coronary endothelial dysfunction prevented by small-conductance calcium-activated potassium channel activator in mice and patients with diabetes. *J Thorac Cardiovasc Surg*. 2020; 160(6):e263-e280.

E2. Escobar AL, Velez P, Kim AM, Cifuentes F, Fill M, Vergara JL. Kinetic properties of DM-nitrophen and calcium indicators: rapid transient response to flash photolysis. *Pflugers Arch*. 1997;434:615-31.

E3. Trafford AW, Diaz ME, O'Neill SC and Eisner DA. Comparison of subsarcolemmal and bulk calcium concentration during spontaneous calcium release in rat ventricular myocytes. *J Physiol*. 1995;488 (Pt 3):577-86.

E4. Bers DM, Patton CW and Nuccitelli R. A practical guide to the preparation of Ca(2+) buffers. *Methods Cell Biol*. 2010;99:1-26.

E5. Liu Y, Xie A, Singh AK, Ehsan A, Choudhary G, Dudley S, et al. Inactivation of Endothelial Small/Intermediate Conductance of Calcium Activated Potassium

130 Channels Contributes to Coronary Arteriolar Dysfunction in Diabetic Patients. *J Am*
131 *Heart Assoc*. 2015;4:e002062.
132
133 E6. Liu Y, Kabakov AY, Xie A, Shi G, Singh AK, Sodha NR, et al. Metabolic regulation
134 of endothelial SK channels and human coronary microvascular function. *Int J Cardiol*.
135 2020;312:1-9.