Materials and Methods

Animals

The study was approved by the regional ethical committee for animal experiments (ethic number: N192/12 and N108/14) and conformed to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health. Male db/db and WT control mice (6 weeks old) as well as Wistar rats (12 weeks old) were purchased from Charles River (Germany) or Janiver (France) and housed in the animal facility of Karolinska University Hospital (L5) until 15 weeks of age. Db/db mice were only included if they had a blood glucose level >15 mmol/l.

Subjects:

Blood samples were collected from 19 healthy subjects and 20 patients with type 2 diabetes (general information see Supplement Table 1). Patients with type 2 diabetes were recruited from the day care center of the department of Endocrinology, Karolinska University Hospital. Subjects were classified as having type 2 diabetes if fasting plasma glucose exceeded 7.0 mmol/l on two different occasions, if plasma glucose exceeded 11.1 mmol/l two hours after oral administration of 75 g of glucose or if they had medical history of type 2 diabetes. The control subjects were matched for age, were free of medication, had no medical history of any cardiovascular disease, and had a normal oral glucose tolerance test. Participants were informed about the nature, purpose, and possible risk involved in the study before giving informed oral and written consent. The investigation was conducted in accordance with the Declaration of Helsinki and was approved by the regional ethics committee (approval number 2014/463-31/3).

Heart isolation and perfusion

Hearts were isolated and perfused in a Langendorff system as described previously[1-3]. Briefly, the animals were anaesthetized (i.p.) with fentanyl (1 mg/ kg) + fluanisone (Hypnorm®; Janssen Pharmaceutica, Beerse Belgium; 50 mg/kg) and midazolam (Dormicum; Hoffman-La Roche, Lausanne, Switzerland; 25 mg/kg). Heparin (250 IU) was injected i.p. The hearts were isolated and perfused retrogradely via the ascending aorta with gassed (5% CO_2, 95% O_2) Krebs-Henseleit (KH) buffer (in mmol/L: NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.1 and $CaCl_2$ 2.4) at a constant pressure (55 mmHg for mouse hearts and 75 mmHg for rat hearts) at 37°C. A balloon connected to a pressure transducer was inserted into the left ventricle for recording of isovolumetric left ventricular developed pressure (LVDP). Left ventricular end diastolic pressure (LVEDP) was set to 5-8 mmHg by inflation of the balloon. The experimental protocol is shown in supplement Figure I. After start of perfusion, all hearts were allowed to stabilize for at least 30 min. Global ischemia (40 min for mouse hearts and 25 min for rat hearts) was induced by clamping the inflow tubing. Reperfusion was initiated by releasing the clamp and was maintained for 60 min.

RBCs from mouse were tested in isolated mouse hearts. At the onset of ischemia, 0.4 ml KH buffer or RBCs suspension (see below) was injected into the coronary circulation via a side-arm in the perfusion system, which means the RBC suspension was present in the isolated hearts only during ischemia. The buffer and RBC suspension were incubated with vehicle (KH buffer), the arginase inhibitors N^{ω}-hydroxy-nor-L-arginine (nor-NOHA, 1 and 3 mmol/L; Bachem, Bubendorf, Switzerland) and 2 (S)-

amino-6-boronohexanoic acid (ABH, 1 mmol/L; kind gift from Corridor Pharmaceuticals Inc., Baltimore, MD, USA) or the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mmol/L, Sigma Aldrich, St Louis MO, USA,) for 20 min at 37ºC before being administered to the hearts. The NOS inhibitor L-NAME was added to the RBC suspension 5 min prior to addition of nor-NOHA. All solutions were initially dissolved in saline and stored frozen until used in the experiments. According to our previous study[3], RBCs from one mouse can be given to the heart from another mouse without altering the post-ischemic cardiac functional recovery.

RBCs from human were test in isolated rat hearts. At the onset of ischemia, 3 ml RBCs suspension (see below) was injected into the coronary circulation following incubation with vehicle (KH buffer), nor-NOHA (1 mmol/L), L-NAME (0.1 mmol/L), 1400W (0.1 mmol/L, Sigma Aldrich, St Louis MO, USA) or the combination of nor-NOHA and L-NAME as described above. Pilot experiments revealed that RBCs from healthy humans did not affect cardiac function of rat hearts in comparison with rat RBCs. Exclusion criteria were hearts with irreversible ventricular tachycardia or fibrillation or macroscopic hemolysis during preparation of RBCs.

Determination of infarct size

Infarct size was measured by triphenyltetrazolium chloride (TTC) staining in separate groups of experiments. After reperfusion, the hearts were frozen in -20°C and cut into 6-7 slices perpendicular to the base-apex axis. The apex and bottom slices were excluded. All the other slices were immersed into 1% TTC solution for 7 min at 37°C followed by fixation in 4% formaldehyde for 15 min. The slices were scanned from both sides, and the extent of myocardial necrosis was determined using Adobe Photoshop CS2 (Adobe Systems, CA, USA) by an investigator (Y.T.) blinded to the group allocation.

Blood sampling and isolation of RBCs

Mouse whole blood was collected from the thoracic cavity after removal of the heart used in the experiment. Human whole blood was collected from healthy volunteers and patients with type 2 diabetes.

Blood components were separated by immediate centrifugation at +4ºC and 1000g for 10 min. RBCs were obtained by removing the plasma and buffy coat including top part of the RBC layer after which the remaining RBCs were washed 3 times and diluted with 2 times KH buffer. This resulted in a mean hematocrit (hct) value of 29$\pm$ 3% (n=16). Successful removal of white blood cells (>99%) and platelets ($\geq$98%) using this procedure has been verified previously[3]. The supernatant from the final washing step was administered to isolated hearts subjected to ischemia-reperfusion. RBCs were either used immediately for the functional experiments or stored frozen at -80ºC for determination of arginase protein expression and activity.

Glucose incubation

Washed RBCs were diluted (1:20, hct 5%) with Krebs-Henseleit (KH) buffer containing either 5 mmol/l or 25 mmol/l. The diluted RBCs were incubated in 37°C, 5% CO_2, and 21% O_2. After 24h incubation, the RBCs were harvested and analysed for arginase expression and activity.

Arginase expression

RBCs from mice were lysed using RIPA lysis buffer (Amresco, Solon, OH, USA) containing protease inhibitors (Roche, Mannheim Germany). The protocol of protein extraction and western blot was described previously[4, 5]. Briefly, proteins were separated by 10% SDS–PAGE, transferred to nitrocellulose membrane, and blocked in 5% non-fat dried milk for 2 h at room temperature. Membranes were incubated overnight at 4°C using rabbit anti-arginase 1 (1:2000, #HPA003595, Atlas, Sigma). Membranes were then washed in TBS-T and incubated with IRDye 800-conjugated goat anti-rabbit IgG (1:15,000, LI-COR Biosciences, Cambridge, UK) and immunoreactive bands were visualized using infrared fluorescence (IR-Odyssey scanner, LI-COR Biosciences, USA). Equal loading was confirmed by expression of GAPDH (1:2000, Sigma Aldrich). Band densities were analyzed with Image Studio Lite Version 3.1 (LI-COR Biosciences, USA).

Arginase activity

RBCs were lysed using RIPA lysis buffer as above. Protein concentration and arginase activity was determined by a colorimetric assay as previously described in detail[4, 6]. Each sample was incubated at 37ºC for 1 h with L-arginine (50 mmol/l Tris-HCl, pH 9.7). The concentration of the end product urea was determined using spectrophotometry. Arginase activity is expressed in absolute values or as percentage of control depending on whether the samples were analyzed in pairs or in separate runs.

Detection of reactive oxygen species using cyan flow cytometer

RBCs from WT or db/db mice were diluted to Hct 1% in PBS. The RBCs were incubated with vehicle, glucose (5–25 mmol/L), nor-NOHA (1 mmol/L), ABH (0.1 mmol/L), L-NAME (0.1 mmol/L), NAC (1 mmol/L) or L-arginine (3mmol/L) for 30 min. The fluorescent probe 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (Life Technologies, Sweden, Molecular Probes™, C6827, final concentration 10 μmol/L) was added to the diluted RBCs, and incubated for 1 h in the dark at room temperature for determination of intracellular ROS. After the incubation, the samples were washed twice with PBS, and the fluorescence (FITC) intensity was analyzed using Cyan Flow Cytometer (Cyan, Beckman Coulter, Miami, FL, USA).

Detection of reactive oxygen species using electron spin resonance (ESR)

Washed RBCs from mouse or human were diluted to Hct 1% with Krebs/HEPES-buffer (Noxygen Science Transfer & Diagnostics GmbH) as above. The RBCs were incubated with 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH, 200 μmol/L) in the presence of 25 μmol/l desferoxamine and 5 μmol/l diethyldithiocarbamate (Noxygen Science Transfer & Diagnostics GmbH) for 30 min at 37°C and 21% O_2 with vehicle, nor-NOHA (1 mmol/L), L-NAME (0.1 mmol/L), 1400W (0.1mmol/L), NAC (1 mmol/L) or L-arginine (3 mmol/L). The cell suspensions were frozen in liquid nitrogen. ROS formation was detected by ESR using the following setting: center field 1.99 g, microwave power 1 mW, modulation amplitude 9 G, sweep time 10 s, number of scans 10, field sweep 60 G. The amount of CM• was determined from the calibration using 3-carboxy-proxyl (CP•, Noxygen Science Transfer & Diagnostics GmbH).

1 **RBCs from NAC-treated mice**
2 NAC is a typical anti-oxidative compound and has direct effect on post-ischemic
3 functional recovery. Therefore, we could not test RBCs which were treated with NAC in
4 isolated hearts as the other compounds used in the present study. We pretreated WT
5 and db/db mice (11 weeks old) with 1% NAC in the drinking water for 4 weeks. The
6 RBCs from NAC-treated mice were isolated as described above and tested in the
7 isolated heart.
8
9 **Statistics**
10 Differences between multiple groups were analysed by one-way ANOVA (repeated
11 measurement) or two-way ANOVA with Bonferroni post-hoc test. Differences between
12 two groups were analysed by *t*-test or paired *t*-test as appropriate. All statistical analysis
13 was calculated using GraphPad Prism (V6.05). Data are presented as means ± SD.
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Supplemental Table

Table I. General characteristics of healthy subjects and patients with type 2 diabetes

Variables:	Type 2 diabetes n=27	Healthy subjects n=23	p-value
Age	59 ± 13	60 ± 6	0.78
No. of males	25	18	
BMI, kg/m2	30.3 ± 5.3	25.2 ± 3.1	0.0006
BP (mmHg):			
Systolic	135 ± 14	131 ± 11	0.24
Diastolic	80 ± 9	81 ± 9	0.51
Fasting glucose (mM)	11.0 ± 3.0	5.6 ± 0.6	<0.0001
No. of smokers	2	0	
HbA1c, mmol/mol	70 ± 17	36 ± 4	<0.0001
Hemoglobin, g/L	145 ± 17	143 ± 12	0.87
Creatinine, mmol/L	96 ± 56	80 ± 13	0.18
Triglycerides, mmol/L	2.3 ± 1.3	1.3 ± 0.8	0.0022
Total cholesterol, mmol/L	4.4 ± 1.2	5.6 ± 1.3	0.0009
HDL, mmol/L	1.2 ± 0.4	1.6 ± 0.5	0.0029
LDL, mmol/L	2.2 ± 0.9	3.5 ± 1.4	0.0002
Medication			
ACEi/ARB	18	-	
Aspirin	9	-	
Lipid lowering	22	-	
beta-blockers	6	-	
Calcium channel blocker	8	-	
Insulin	18	-	
Metformin	20	-	
GLP-1	9	-	
DDP-4i	7	-	
SU	3	-	
SGLT2i	4	-	
Comorbidities			
CAD	4	-	
Heart failure	2	-	
Nephropathy	3	-	
Peripheral artery disease	2	-	
Retinopathy	3	-	

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; DDP-4i, dipeptidyl peptidase-4 inhibitor; GLP-1, glucagon like peptide 1; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein;

LDL, low-density lipoprotein; SGLT2i, sodium-glucose co-transporter inhibitor SU, sulfonylurea. Data are means ± SD.

Table II. Baseline of isolated perfused mouse heart in Langendorff system

Group	n	LVSP mmHg	LVEDP mmHg	LVDP mmHg	dP/dt_{max}	Coronary Flow ml/min
WT heart + db/db RBC + vehicle	6	91.3 ± 18.0	6 ± 0.9	85.3 ± 18.0	4633 ± 833	1.70 ± 0.35
WT heart + db/db RBC+1 mmol/l nor-NOHA	8	92.8 ± 26.2	6.6 ± 0.7	63.6 ± 10.3	3629 ± 583	1.50 ± 0.27
db/db heart + WT RBC + vehicle	5	108.0 ± 19.4	5.6 ± 0.5	102.2 ± 19.4	4527 ± 1053	1.44 ± 0.26
db/db heart + WT RBC + 1 mmol/l nor-NOHA	5	108.0 ± 22.5	5.8 ± 0.8	102.8 ± 21.4	4204 ± 1140	1.41 ± 0.23
db/db heart + KH + 3 mmol/l nor-NOHA	5	104.0 ± 9.1	6.2 ± 1.3	97.6 ± 9.7	4472 ± 530	1.52 ± 0.34
WT + db/db RBC + L-NAME	5	78.4 ± 8.8	6.6 ± 1.1	71.6 ± 9.2	3958 ± 601	1.62 ± 0.25
WT heart + db/db RBC + L-NAME + 1 mmol/l nor-NOHA	5	74.0 ± 9.3	6.2 ± 1.3	67.4 ± 9.9	3897 ± 784	1.48 ± 0.35
WT heart + WT RBC	5	86.4 ± 22.4	6.6 ± 1.5	79.2 ± 22.5	3933 ± 1469	1.45 ± 0.27
WT heart + db/db RBC + 3 mmol/l nor-NOHA	4	92.5 ± 28.9	7.2 ± 0.5	85.5 ± 29.2	4906 ± 1730	2.16 ± 0.87
db/db heart + WT RBC + L-NAME + 1 mmol/l nor-NOHA	5	122.8 ± 9.4	7.0 ± 1.0	116.6 ± 10.2	4660 ± 737	1.59 ± 0.28
WT heart + db/db RBC + L-NAME + 3 mmol/l nor-NOHA	6	96.3 ± 34.9	7.0 ± 1.3	89.2 ± 35.1	4629 ± 1543	2.03 ± 0.36
WT heart + WT RBC + L-NAME + 1 mmol/l nor-NOHA	5	66.6 ± 4.7	6.6 ± 0.9	62.6 ± 5.3	3261 ± 709	1.39 ± 0.31
WT heart + WT RBC + L-NAME	5	74.8 ± 20.8	7.2 ± 0.8	68.0 ± 20.9	4093 ± 1170	1.56 ± 0.34
WT heart + WT RBC + 1 mmol/l nor-NOHA	4	82.7 ± 8.9	6.3 ± 0.9	76.5 ± 7.0	4605 ± 349	1.42 ± 0.22
WT heart + WT RBC + 3 mmol/l nor-NOHA	4	77.7 ± 15.2	6.5 ± 1.7	71.5 ± 14.4	3920 ± 616	1.69 ± 0.34
WT heart + db/db RBC + 1 mmol/l ABH	4	81.5 ± 11.5	6.7± 1.0	74.7 ± 10.5	4152 ± 533	1.55 ± 0.34
WTheart + db/db Supernatant	6	80.5 ± 12.2	6.0 ± 0.9	74.8 ± 12.5	3477 ± 693	1.60 ± 0.37

db/db heart + db/db RBC	5	127.2 ± 10.4	6.2 ± 0.8	113.3 ± 19.8	4331 ± 1111	1.49 ± 0.22
WT heart + KH	5	85.6 ± 15.6	6.0 ± 0.7	78.8 ± 16.2	4359 ± 794	1.32 ± 0.20
db/db heart +KH	8	124.5 ± 10.6	6.3 ± 0.7	117.8 ± 10.5	5075 ± 920	1.89 ± 0.50

Abbreviations: LVSP: left ventricular systolic pressure, LVDP: left ventricular developed pressure, LVEDP: left ventricular end diastolic pressure, dp/dt_{max}: the rate of left ventricle pressure, WT: wild type, RBC: red blood cell, nor-NOHA: N^{ω}-hydroxy-nor-L-arginine, L-NAME: N^{G}-nitro-L-arginine methyl ester, KH: Krebs-Henseleit buffer. Data are means ± SD.

Supplemental Figures and Figure Legends

Isolated and perfused hearts

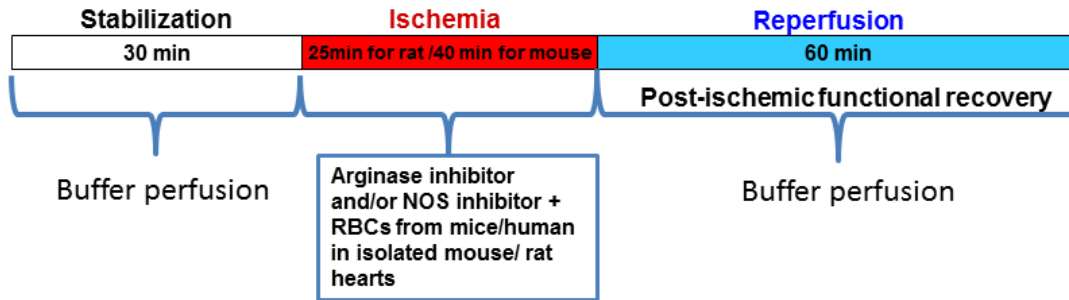

Figure S1. Experimental protocol of isolated hearts in the Langendorff model. After 30 min stabilization, the hearts were subjected to 25 min (rat) or 40 min (mouse) of ischemia followed by 60 min of reperfusion. At the onset of ischemia, KH buffer or RBC suspension (with/without arginase/NOS inhibitor) was injected into the coronary circulation via a side-arm in the perfusion system, which means that the RBC suspension was present in the isolated hearts only during ischemia.

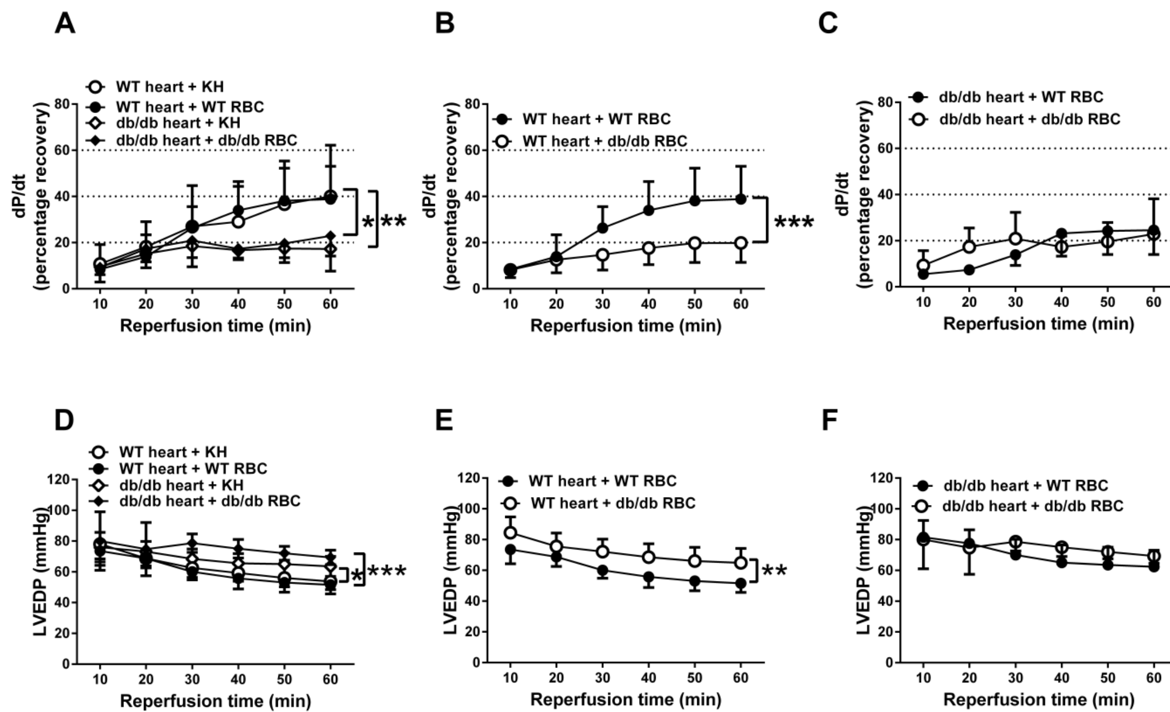

Figure S2. Effects of RBCs on post-ischemic recovery of dP/dt (A-C) and left ventricular end diastolic pressure (LVEDP; D-F). (A and D) Hearts from WT mice were given Krebs-Henseleit (KH) buffer (WT heart + KH, n=5) or RBCs from WT (WT heart + WT RBC, n=5) and hearts from db/db mice were given Krebs-Henseleit (KH) buffer (db/db heart + KH, n=4) or RBCs from db/db mice (db/db heart + db/db RBC, n=9). (B and E) Hearts from WT mice were given RBCs from either WT (n=5) or db/db (n=5) mice. (C and F) Hearts from db/db mice were given RBCs from either WT (n=5) or db/db (n=6) mice. dP/dt is expressed as percentage recovery from the pre-ischemic level and LVEDP in absolute values. Significant differences between treatments are shown *P < 0.05, **P<0.01, ***P < 0.001.

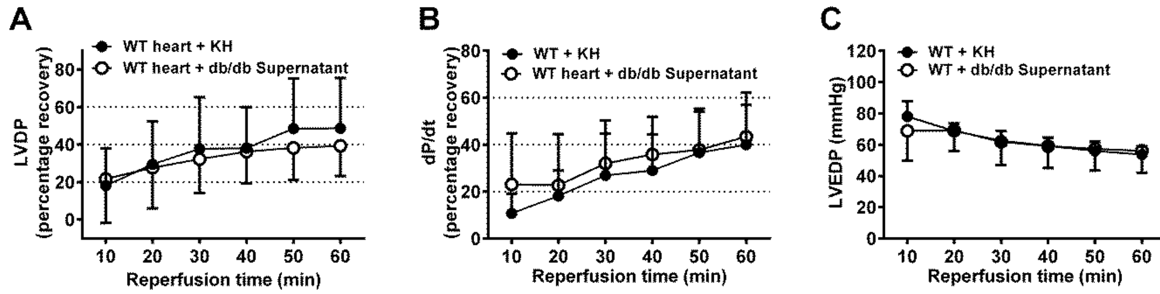

Figure S3. Effect of the supernatant from the last washing step of RBCs from db/db mice on post-ischemic recovery of (A) left ventricular developed pressure (LVDP), (B) dP/dt and (C) left ventricular end diastolic pressure (LVEDP). Hearts from WT mice were given KH buffer (n=5) or the supernatant (n=6). LVDP and dP/dt are expressed as percentage recovery from the pre-ischemic level and LVEDP in absolute values.

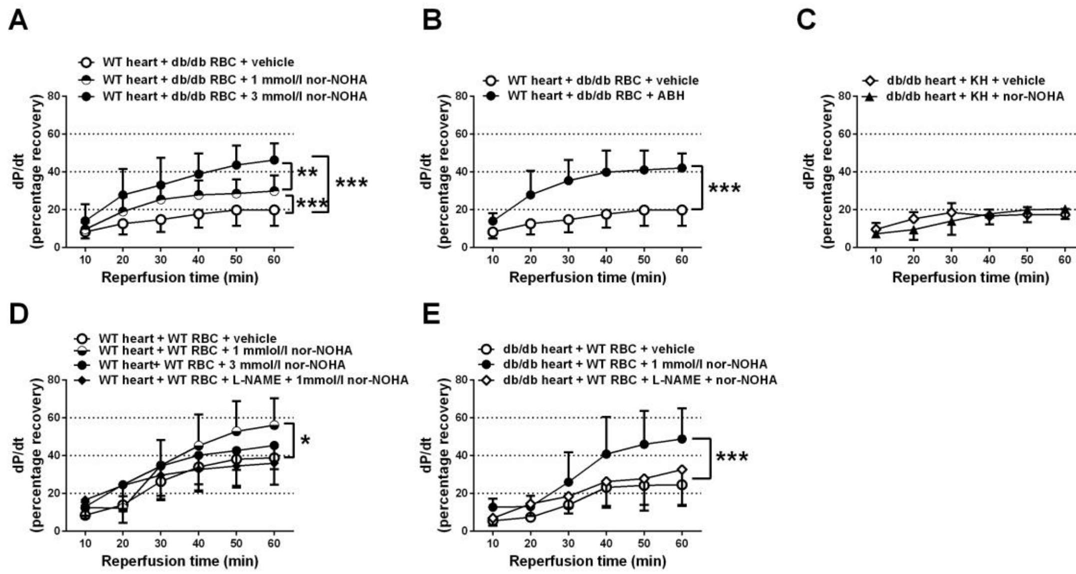

Figure S4. RBCs from db/db mice impair cardiac post-ischemic recovery (dP/dt) via an arginase-dependent pathway. (A) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5), nor-NOHA 1 mmol/L (n=8) or 3 mmol/L (n=4). (B) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5) or the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH, 1 mmol/L, n=4). (C) Hearts from db/db mice were given Krebs Henseleit (KH) buffer with either vehicle (n=8) or nor-NOHA (1 mmol/l, n=5). (D) Hearts from WT mice were given RBCs from WT mice incubated with vehicle (n=6), the arginase inhibitor nor-NOHA 1 mmol/L, (n=10), nor-NOHA 3 mmol/L (n=8) or the NOS inhibitor L-NAME (0.1 mmol/L) + nor-NOHA (1 mmol/L, n=6). (E) Hearts from db/db mice were given RBCs from WT mice incubated with vehicle (n=5), nor-NOHA (1 mmol/L, n=5) or L-NAME (0.1 mmol/L) + nor-NOHA (1 mmol/L, n=5). dP/dt is expressed as percentage recovery from the pre-ischemic level. Significant differences between treatments are shown; $*P < 0.05$, $**P<0.01$, $***P < 0.001$.

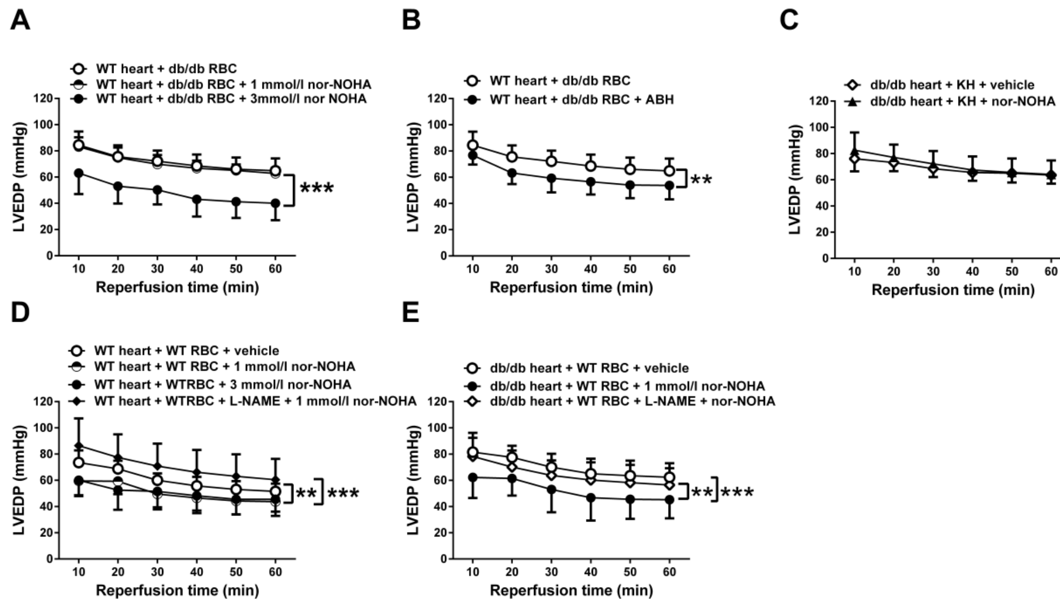

Figure S5. RBCs from db/db mice impair cardiac post-ischemic recovery (LVEDP) via an arginase-dependent pathway. (A) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5), nor-NOHA 1 mmol/L (n=8) or 3 mmol/L (n=4). (B) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5) or the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH, 1 mmol/L, n=4). (C) Hearts from db/db mice were given Krebs Henseleit (KH) buffer with either vehicle (n=8) or nor-NOHA (1 mmol/L, n=5). (D) Hearts from WT mice were given RBCs from WT mice incubated with vehicle (n=6), the arginase inhibitor nor-NOHA 1 mmol/L, (n=10), nor-NOHA 3 mmol/L (n=8) or the NOS inhibitor L-NAME (0.1 mmol/L) + nor-NOHA (1 mmol/L, n=6). (E) Hearts from db/db mice were given RBCs from WT mice incubated with vehicle (n=5), nor-NOHA (1 mmol/L, n=5) or L-NAME (0.1 mmol/L) + nor-NOHA (1 mmol/L, n=5). LVEDP is expressed in absolute values. Significant differences between treatments are shown; $^{**}P < 0.01$, $^{***}P < 0.001$.

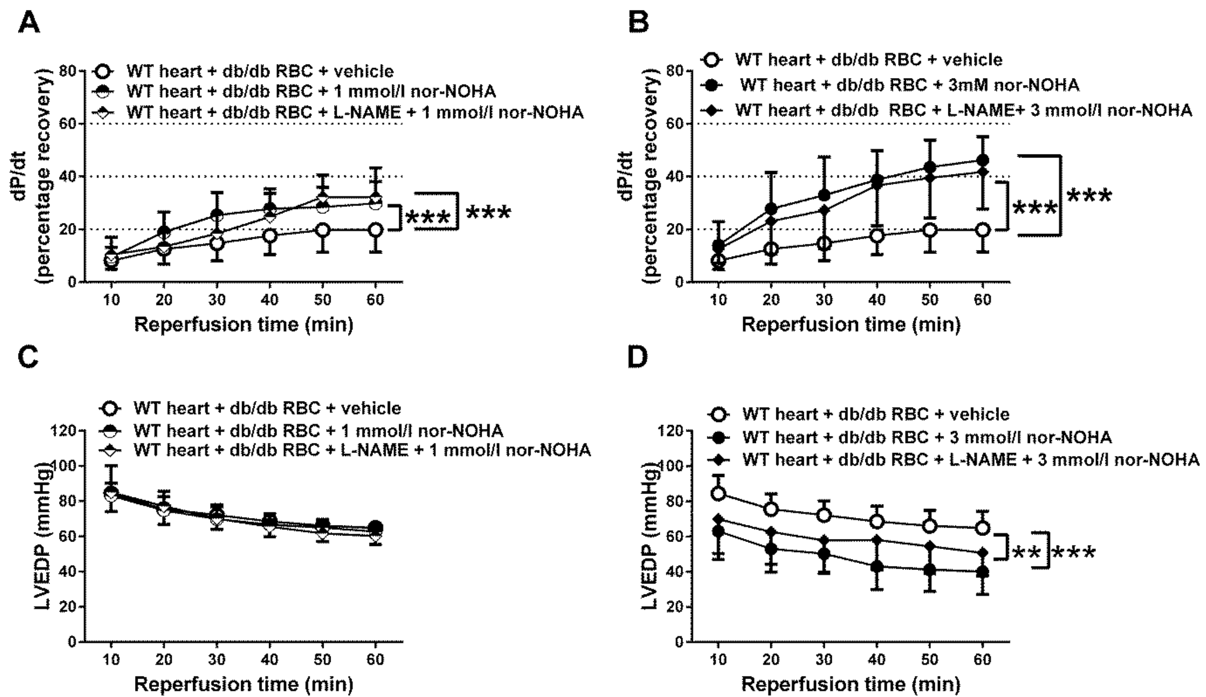

Figure S6. Effects of NOS inhibitor on improvement in recovery of dP/dt (A-B) and left ventricular end diastolic pressure (LVEDP; C-D) induced by arginase inhibition in RBCs from db/db mice. (A and C) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5), nor-NOHA, 1 mmol/L, n=8) or L-NAME (0.1 mmol/L) + nor-NOHA (1 mmol/L, n=5). (B and D) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5), nor-NOHA (3 mmol/L, n=4) or L-NAME (0.1 mmol/L) + nor-NOHA (3 mmol/L, n=6). dP/dt is expressed as percentage recovery from the pre-ischemic level and LVEDP in absolute values. Significant differences between treatments are shown; ***P* < 0.01, ****P* < 0.001.

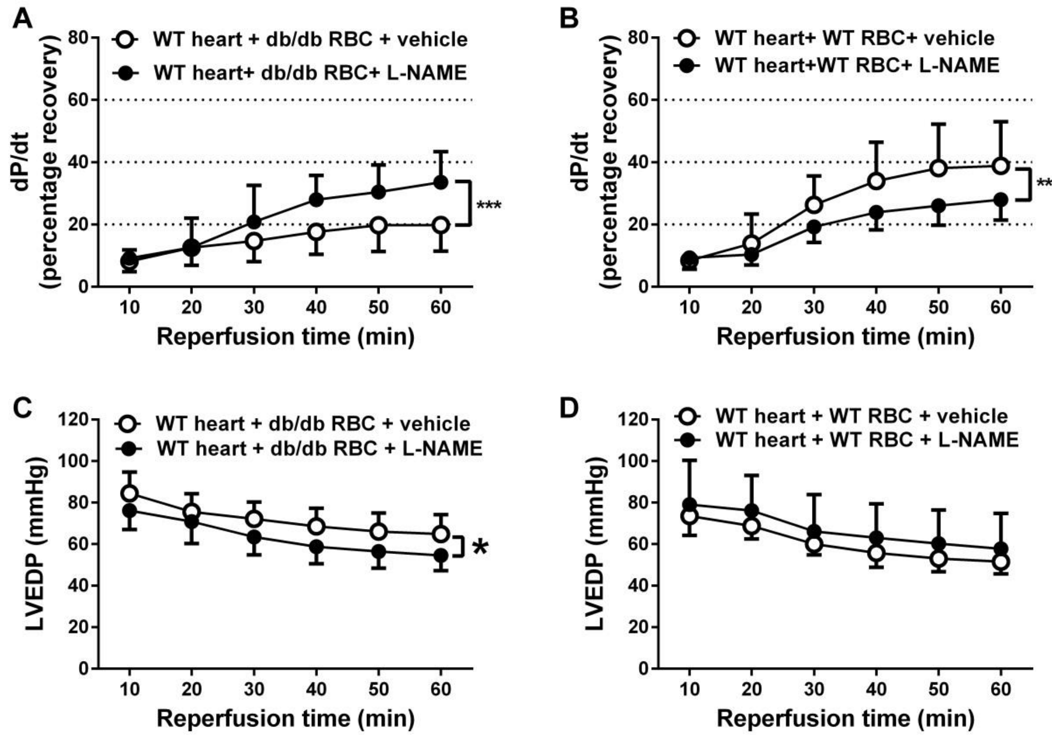

Figure S7. Effects of NOS inhibitor on the impaired recovery of dP/dt (A-B) and left ventricular end diastolic pressure (LVEDP; C-D) induced by db/db RBCs during reperfusion. (A and C) Hearts from WT mice were given RBCs from db/db mice incubated with vehicle (n=5) or L-NAME (0.1 mmol/L, n=5). (B and D) Hearts from WT mice were given RBCs from WT mice incubated with vehicle (n=6) or L-NAME (0.1 mmol/L, n=5). dP/dt is expressed as percentage recovery from the pre-ischemic level and LVEDP in absolute values. Significant differences between treatments are shown; $^{**}P < 0.01$, $^{***}P < 0.001$.

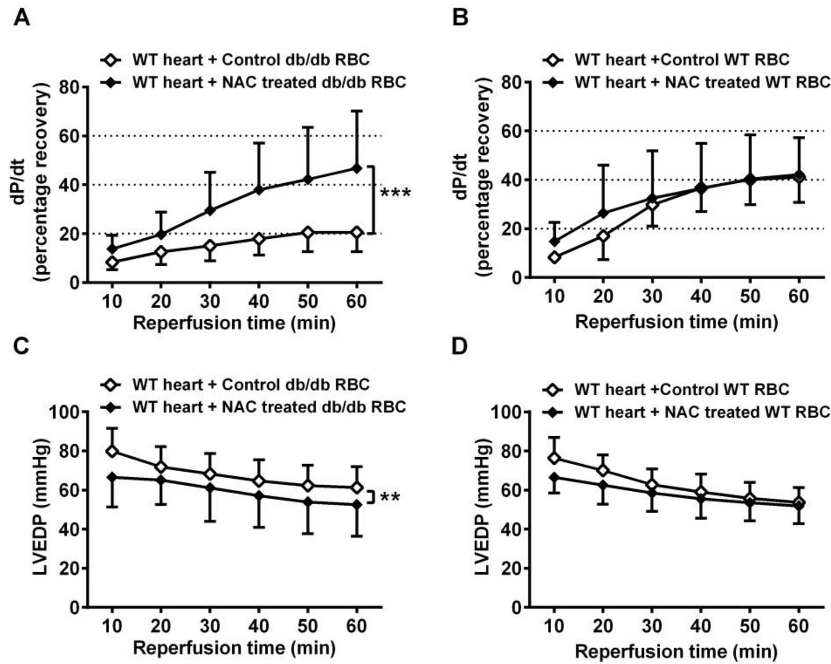

Figure S8. Effect of the antioxidant N-acetyl cysteine (NAC) on the impaired recovery of left ventricular dP/dt and LVEDP induced by db/db RBCs. (A and C) Hearts from WT mice were given RBCs from db/db mice treated with vehicle (Control, n=7) or NAC (n=7) for 4 weeks. (B and D) Hearts from WT mice were given RBCs from WT mice treated with vehicle (Control, n=6) or NAC (n=7) for 4 weeks. dP/dt is expressed as percentage recovery from the pre-ischemic level and LVEDP in absolute values. Significant differences between treatments are shown; ** $P < 0.01$, *** $P < 0.001$.

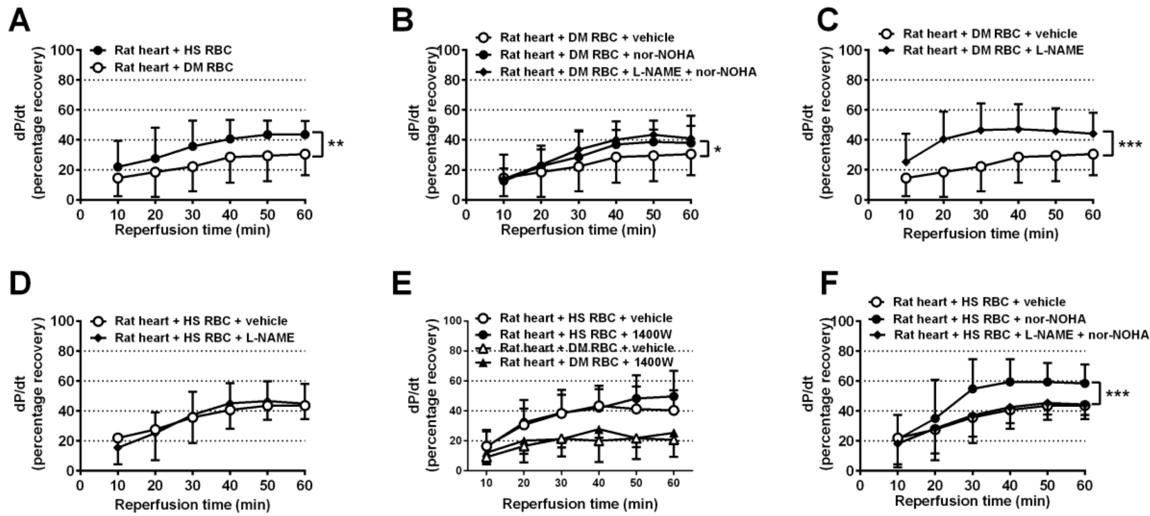

Figure S9. Effect of RBCs from patients with type 2 diabetes on left ventricular dP/dt. (A) Hearts from Wistar rats were given RBCs from either healthy subjects (HS, n=9) or patients with type 2 diabetes (DM, n=13). (B) Hearts were given RBCs from patients with DM incubated with vehicle (n=13) nor-NOHA) (1 mmol/L, n=12) or L-NAME (0,1 mmol/L) + nor-NOHA (1 mmol/L, n=6). (C) Hearts were given RBCs from patients with DM incubated with vehicle (n=13) or L-NAME (0.1 mmol/L, n=5). (D) Hearts were given RBCs from HS and incubated with vehicle (n=9) or L-NAME (0.1 mmol/L, n=9). (E) Hearts were given RBCs from HS incubated with vehicle (n=6) or 1400W (0.1 mmol/L, n=5) or RBCs from DM incubated with vehicle (n=5) or 1400W (0.1 mmol/L, n=5). (F) Hearts were given RBCs from HS incubated with vehicle (n=9), nor-NOHA (1 mmol/L, n=9) or L-NAME (0,1 mmol/L) + nor-NOHA (1 mmol/L, n=9).$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

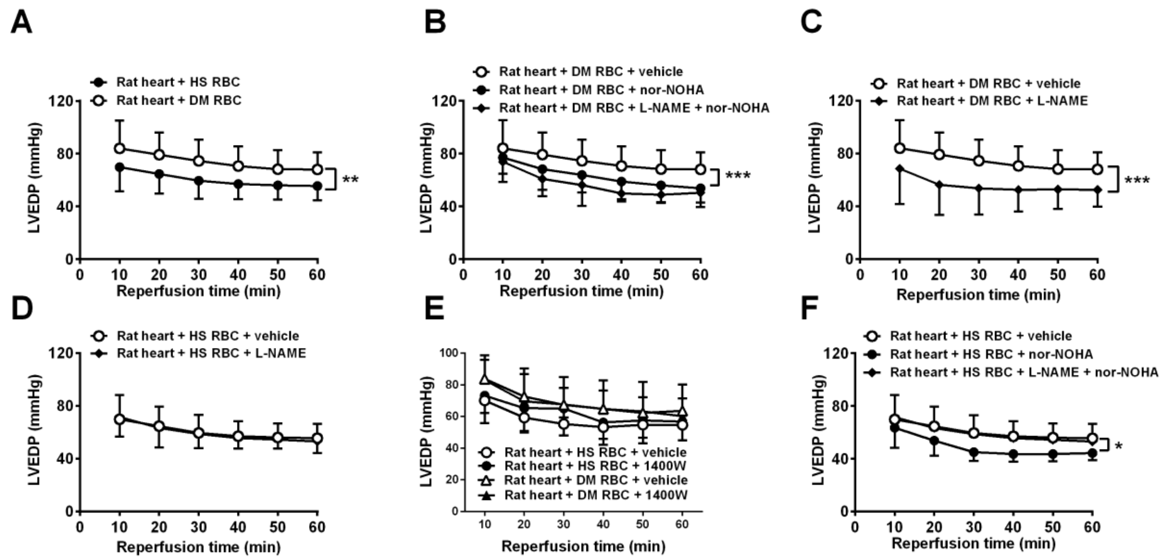

Figure S10. Effect of RBCs from patients with type 2 diabetes on left ventricular end diastolic pressure (LVEDP). (A) Hearts from Wistar rats were given RBCs from either healthy subjects (HS, n=9) or patients with type 2 diabetes (DM, n=13). (B) Hearts were given RBCs from patients with DM incubated with vehicle (n=13), nor-NOHA (1 mmol/L, n=12) or L-NAME (0,1 mmol/L) + nor-NOHA (1 mmol/L, n=6). (C) Hearts were given RBCs from DM incubated with vehicle (n=13) or L-NAME (0.1 mmol/L, n=5). (D) Hearts were given RBCs from HS incubated with vehicle (n=9) or L-NAME (0.1 mmol/L, n=9). (E) Hearts were given RBCs from HS incubated with vehicle (n=6) or 1400W (0.1 mmol/L, n=5) or RBCs from DM incubated with vehicle (n=5) or 1400W (0.1 mmol/L, n=5). (F) Hearts were given RBCs from HS incubated with vehicle (n=9), nor-NOHA (1 mmol/L, n=9) or L-NAME (0,1 mmol/L) + nor-NOHA (1 mmol/L, n=9). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

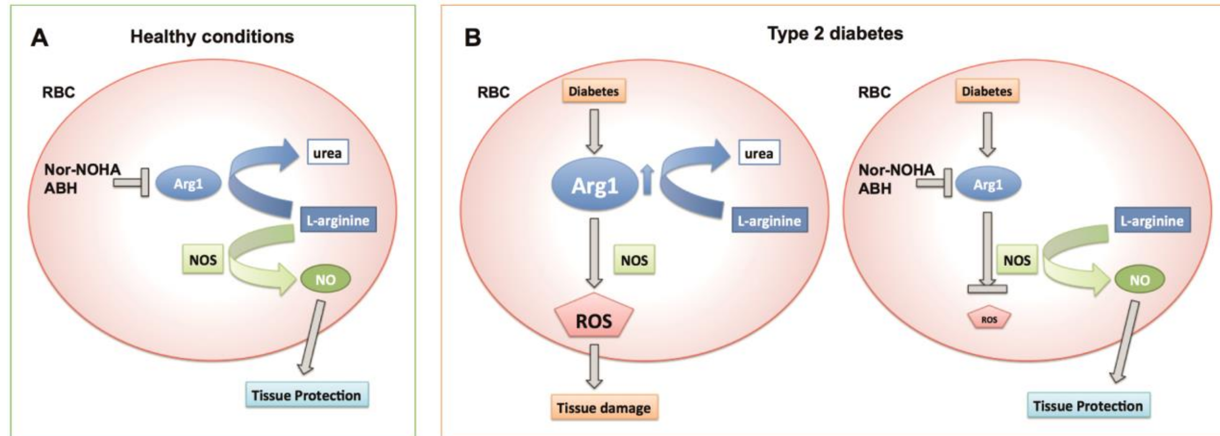

Figure S11. Role of arginase and NOS in RBCs under healthy and type 2 diabetic conditions. (A) Under healthy conditions, arginase and NOS in RBCs compete for the common substrate L-arginine, and arginase inhibition leads to increased NOS-dependent NO bioavailability which is cardioprotective during ischemia-reperfusion. (B) In type 2 diabetes, RBC arginase activity increases resulting in decreased NO bioavailability due to reduced L-arginine supply for NOS. In turn, NOS uncouples and mediates ROS production which impairs post-ischemic myocardial recovery. Inhibition of arginase or NOS in diabetic RBCs diminishes ROS production and protects the heart from ischemia-reperfusion injury. High concentrations of arginase inhibitor induce additional protection through increased NO bioavailability. Additional sources for ROS include production including NADPH oxidases, xanthine oxidase. See main text for details.