Supplementary material

Downregulation of Erythrocyte miR-210 Induces Endothelial Dysfunction in Type 2 Diabetes

Zhichao Zhou, MD, PhD[1]; Aida Collado, PhD[1]; Changyan Sun, BSc[2]; Yahor Tratsiakovich, MD, PhD[1]; Ali Mahdi, MD[1]; Hanna Winter, MSc[3], Ekaterina Chernogubova, PhD[2]; Till Seime, MSc[4], Sampath Narayanan, PhD[4, 5]; Tong Jiao, MD, MSc[1]; Hong Jin, MD, PhD[2, 4]; Michael Alvarsson, MD, PhD[5]; Xiaowei Zheng, MD, PhD[5]; Jiangning Yang, MD, PhD[1]; Ulf Hedin, MD, PhD[4], Sergiu-Bogdan Catrina, MD, PhD[5, 6]; Lars Maegdefessel, MD, PhD[2, 3] and John Pernow, MD, PhD[1, 7]

[1]Division of Cardiology, [2]Division of Molecular Vascular Medicine, Department of Medicine, Karolinska Institutet; [3]Department of Vascular and Endovascular Surgery, Technical University Munich, Munich, Germany; [4]Division of Vascular Surgery; [5]Division of Endocrinology and Diabetology, Department of Molecular Medicine and Surgery, Karolinska Institutet; [6]Centrum for Diabetes, Academic Specialist Centrum, Stockholm, Sweden; [7]Department of Cardiology, Karolinska University Hospital, Stockholm, Sweden

Short title: Erythrocyte miR-210 and endothelial dysfunction in diabetes

*Corresponding author:

Zhichao Zhou M.D., Ph.D. J8: 20, BioClinicum, Division of Cardiology, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm 17176, Sweden

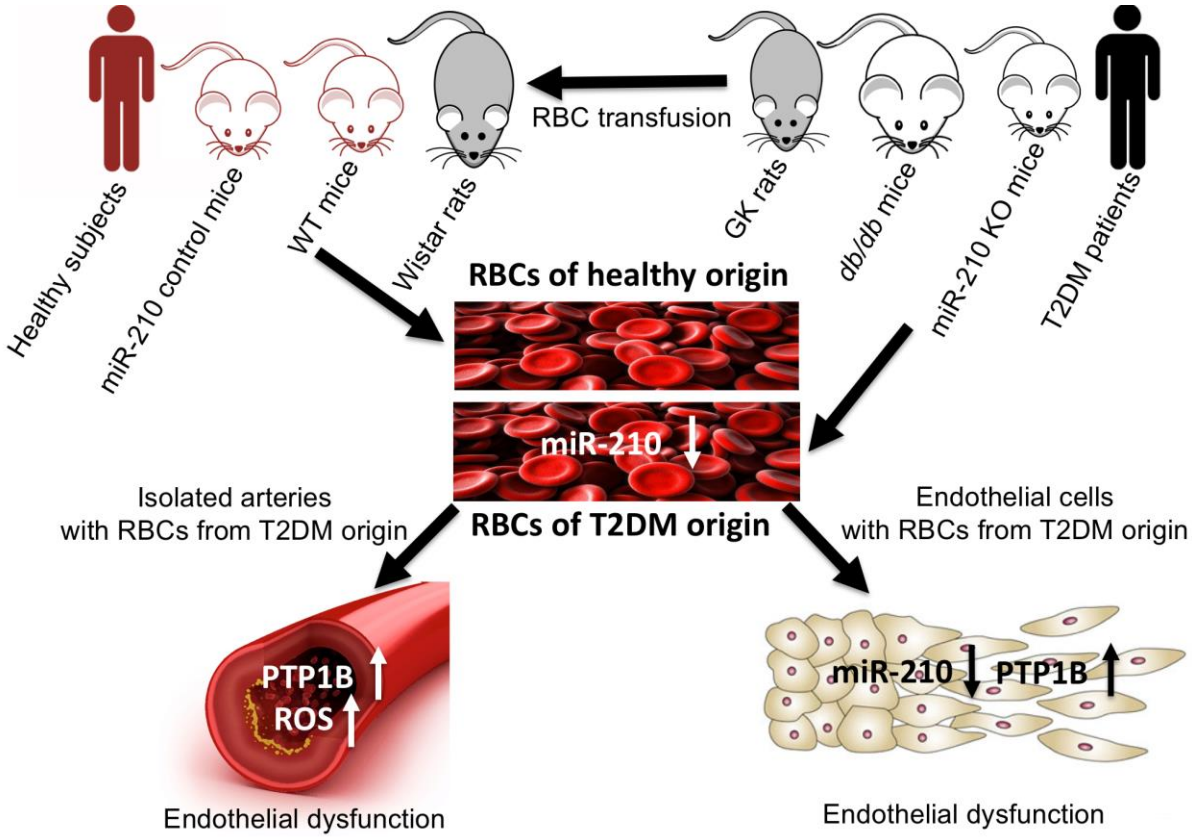
Tel: +46 851773560, Email: zhichao.zhou@ki.se ORCID: 0000-0002-5107-6529

Supplementary Table 1. Characteristics of patients whose samples are used for *in situ* hybridization

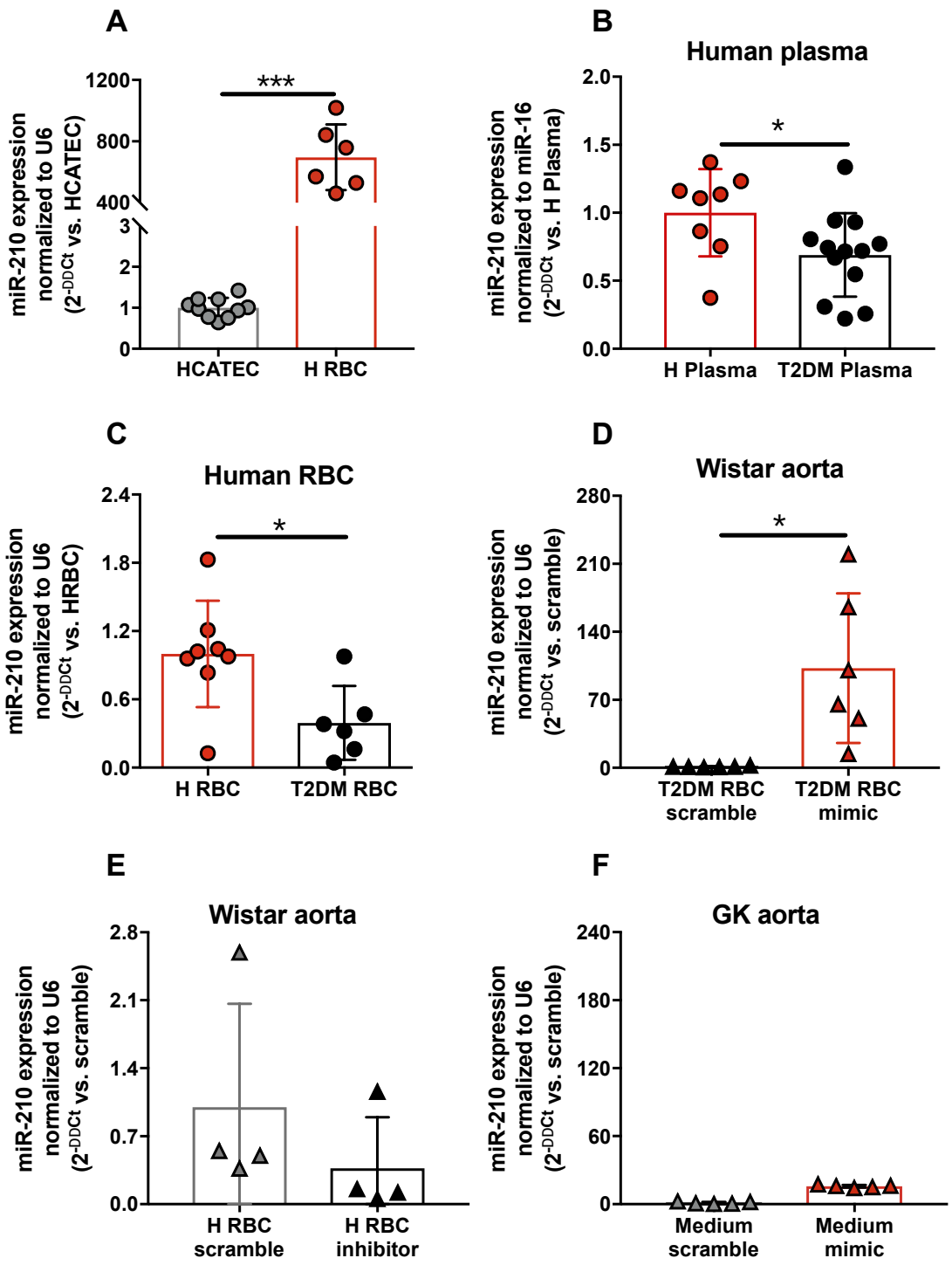
Variables:	Non-diabetes n=10	Type 2 diabetes n=10
Age, years	73 ± 5	73 ± 6 [a]
Males, no.	7	7 [b]
BMI, kg/m^2	30 ± 2	31 ± 2 [a]
Smokers, no.	6	6 [b]
Hypertension, no.	8	10
Medication, no.		
ACEi/ARB	7	9
Aspirin	9	10
Lipid-lowering	9	10
β-blocker	9	2

ACEi=angiotensin-converting enzyme inhibitor; ARB=angiotensin receptor blocker; BMI=body mass index. Data are expressed as mean ± SD; a: analyzed by Mann-Whitney; b: analyzed by Fisher's exact test. Diabetes is based on patient's history or medication (at least 1 oral anti-diabetes drug or insulin; no new onset of diabetes); hypertension is based on patient history or medication (at least 1 anti-hypertension drug).

Supplementary figures and figure legends

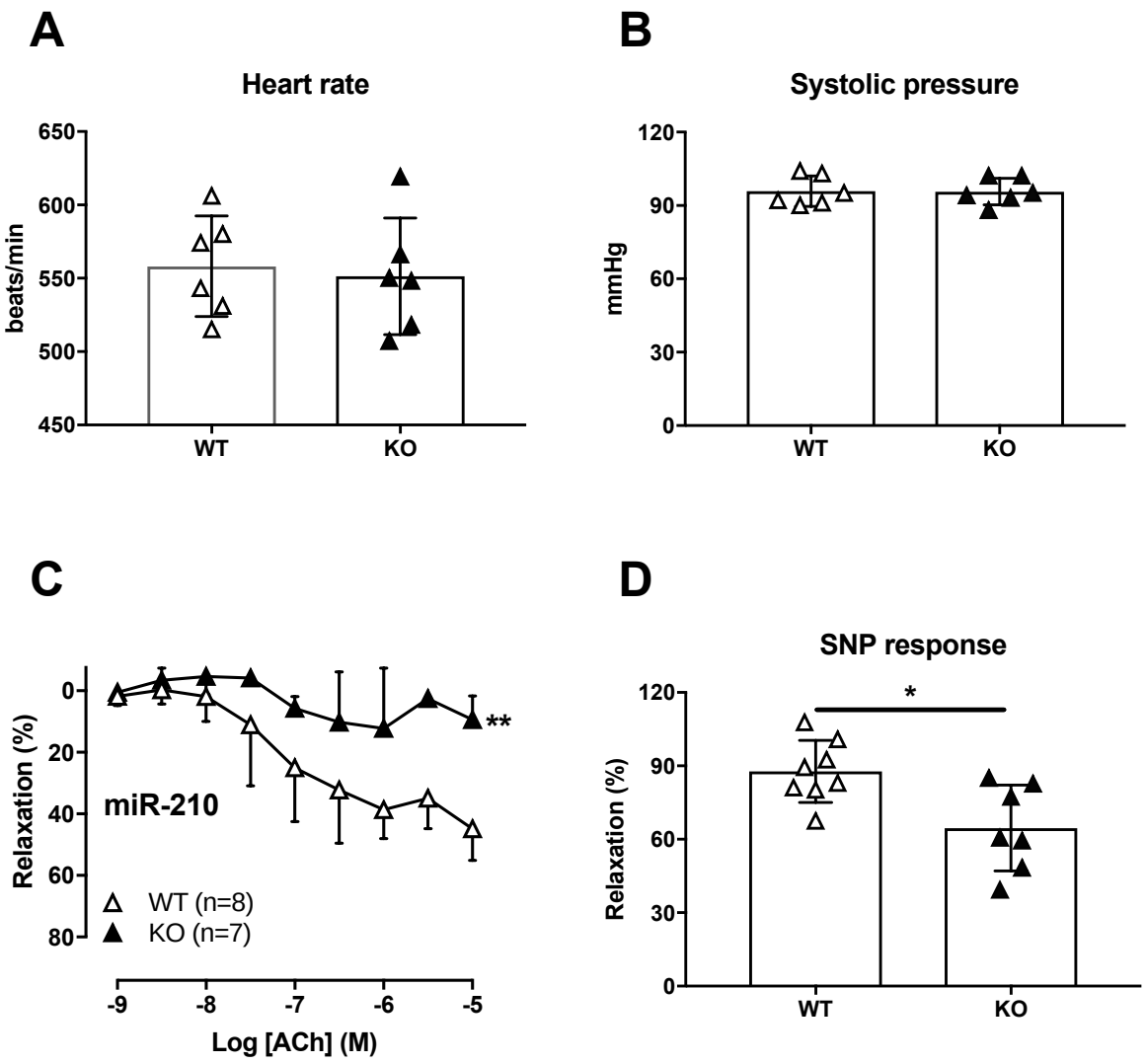


Supplementary Figure 1. Translational approaches and multiple experimental models**.** Red blood cells (RBCs) from patients and rodents with type 2 diabetes mellitus (T2DM) or miR-210 knockout (KO) mice were isolated and incubated with arteries from control rodents and human endothelial cells. RBCs from T2DM rats were transfused into the control recipient rats. Endothelial function and expression analysis were assessed in incubated arteries and endothelial cells. RBC miR-210 levels are lower in T2DM. RBCs from patients with T2DM also decrease endothelial miR-210 levels. Downregulation of RBC miR-210 promotes vascular PTP1B and ROS accounting for endothelial dysfunction. GK: Goto-Kakizaki; PTP1B: protein tyrosine phosphatase 1 B; WT: wild-type.

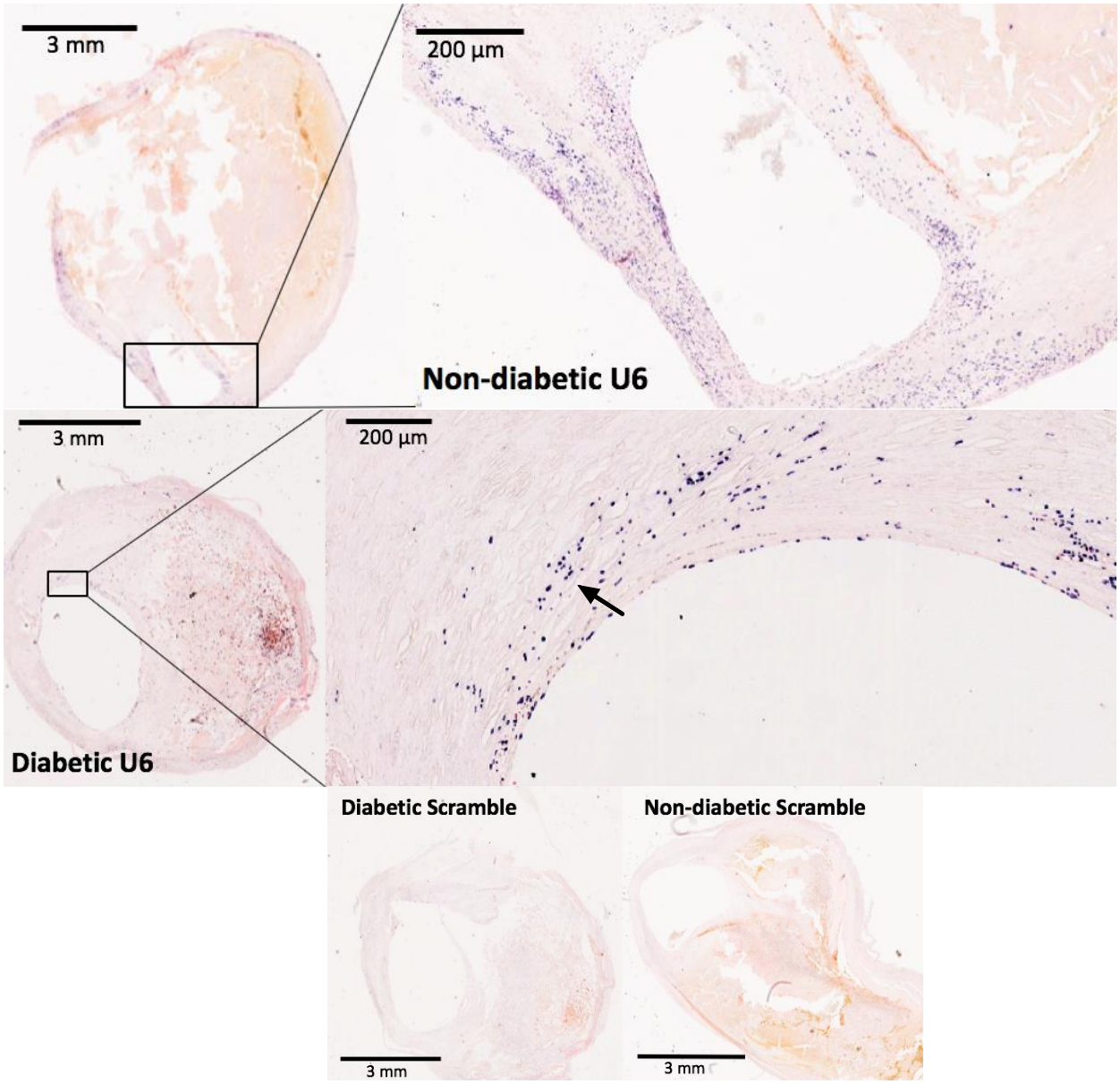


Supplementary Figure 2. Control experiments revealing functional role of red blood cell (RBC) miR-210 in endothelial dysfunction in type 2 diabetes mellitus (T2DM). (A) miR-210 expression in RBCs from healthy subjects (H RBC) and human carotid arterial endothelial cells (HCATEC).

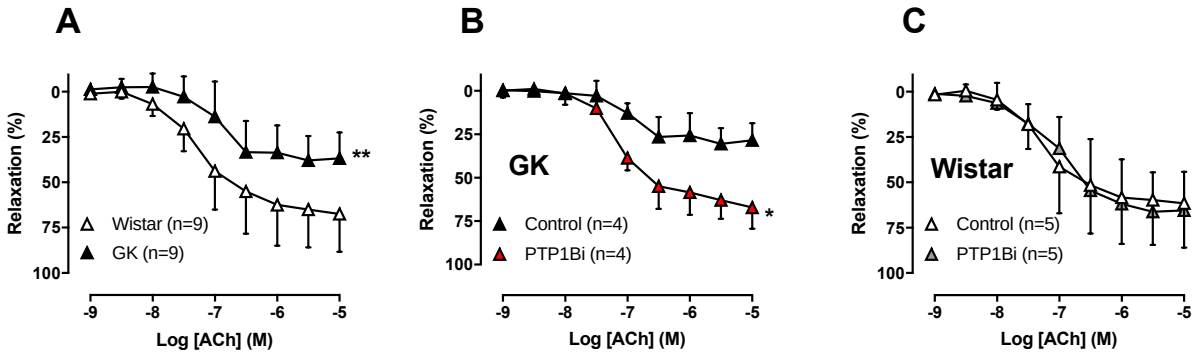
(B) miR-210 expression normalized to internal control miR-16 in plasma from patients with T2DM (T2DM plasma) and healthy subjects (H plasma). (C) miR-210 expression in T2DM RBC and RBCs from H RBC transfected with miR-210 scramble for 18h. (D) miR-210 expression in Wistar aortas co-incubated with T2DM RBC after miR-210 mimic transfection. (E) miR-210 expression in Wistar aortas co-incubated with H RBC after miR-210 inhibitor transfection. (F) miR-210 expression in Goto-Kakizaki (GK) aortas co-incubated with medium after miR-210 mimic transfection. Values are mean ± SD. *P<0.05; ***P<0.001 by Mann-Whitney test in A-G.



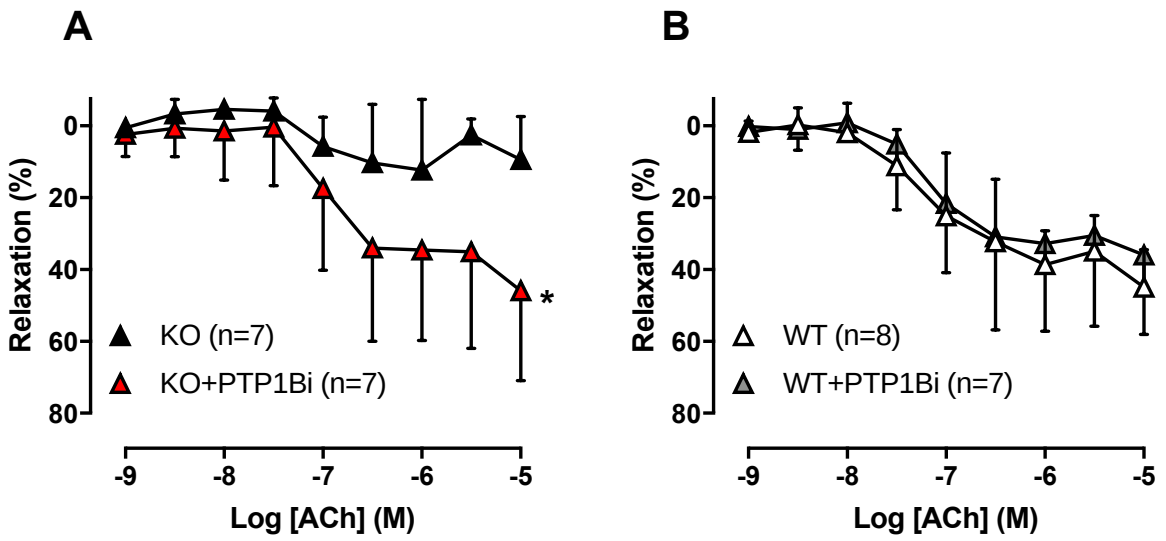
Supplementary Figure 3. Characteristics of miR-210 knockout (KO) mice. (A) Heart rate and (B) systolic blood pressure are not significantly different between miR-210 KO mice and miR-210 wild-type (WT) mice. (C) Endothelium-dependent relaxation (EDR) is impaired in aortas of miR-210 KO mice as compared to miR-210 WT controls. (D) Endothelium-independent relaxation (EIR) is impaired in aortas of miR-210 KO mice as compared to miR-210 WT mice in response to 10 μM nitroprusside (SNP). Values are mean ± SD. **P<0.01 vs. WT representing the concentration-response relation by two-way ANOVA in C. *P<0.05, NS: no significance by Mann-Whitney test in A, B and D.



Supplementary Figure 4. *In situ* hybridization imaging. *In situ* hybridization showing expression of U6 (purple) and scramble groups in carotid artery plaques from patients with and without type 2 diabetes.



Supplementary Figure 5. Role of vascular protein tyrosine phosphatase 1 B (PTP1B) in endothelial dysfunction in type 2 diabetes. (A) Endothelium-dependent relaxation (EDR) is impaired in aortas from Goto-Kakizaki (GK) rats as compared to Wistar rats. (B) Effects of PTP1B inhibition with the PTP1B inhibitor (PTP1Bi) on EDR in aortas from GK rats. (C) Effects of PTP1Bi on EDR in aortas from Wistar rats. Values are mean ± SD. *P<0.05 effect of PTP1Bi vs. Control; **P<0.01 vs. Wistar representing the concentration-response relation by two-way ANOVA.



Supplementary Figure 6. miR-210 functionally targets protein tyrosine phosphatase 1 B (PTP1B) accounting for endothelial dysfunction. (A) Effects of the PTP1B inhibitor (PTP1Bi) on endothelium-dependent relaxation (EDR) in aortas from miR-210 knockout (KO) mice. (B) Effects of PTP1Bi on EDR in aortas from miR-210 wild-type (WT) mice. Values are mean ± SD. *P<0.05 effect of PTP1Bi vs. KO representing the concentration-response relation by two-way ANOVA.