SUPPLEMENTARY DATA

Inhibition of miR-200c restores endothelial function in diabetic mice through suppression of COX-2

Huina Zhang[1,2,3], Jian Liu[1], Dan Qu[1], Li Wang[1], Jiang-Yun Luo[1], Chi Wai Lau[1], Pingsheng Liu[3], Zhen Gao[1], George L. Tipoe[4], Hung Kay Lee[5], Chi Fai Ng[6], Ronald Ching Wan Ma[7], Xiaoqiang Yao[1], Yu Huang[1]

[1]Institute of Vascular Medicine, Shenzhen Research Institute, and Li Ka Shing Institute of Health Sciences , Chinese University of Hong Kong, Hong Kong, China; [2]Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing Anzhen Hospital Affiliated to the Capital Medical University, Beijing, China; [3]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; [4]Department of Anatomy, University of Hong Kong, Hong Kong, China; [5]Department of Chemistry, [6]Department of Surgery and [7]Department of Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong, China;

Additional Results

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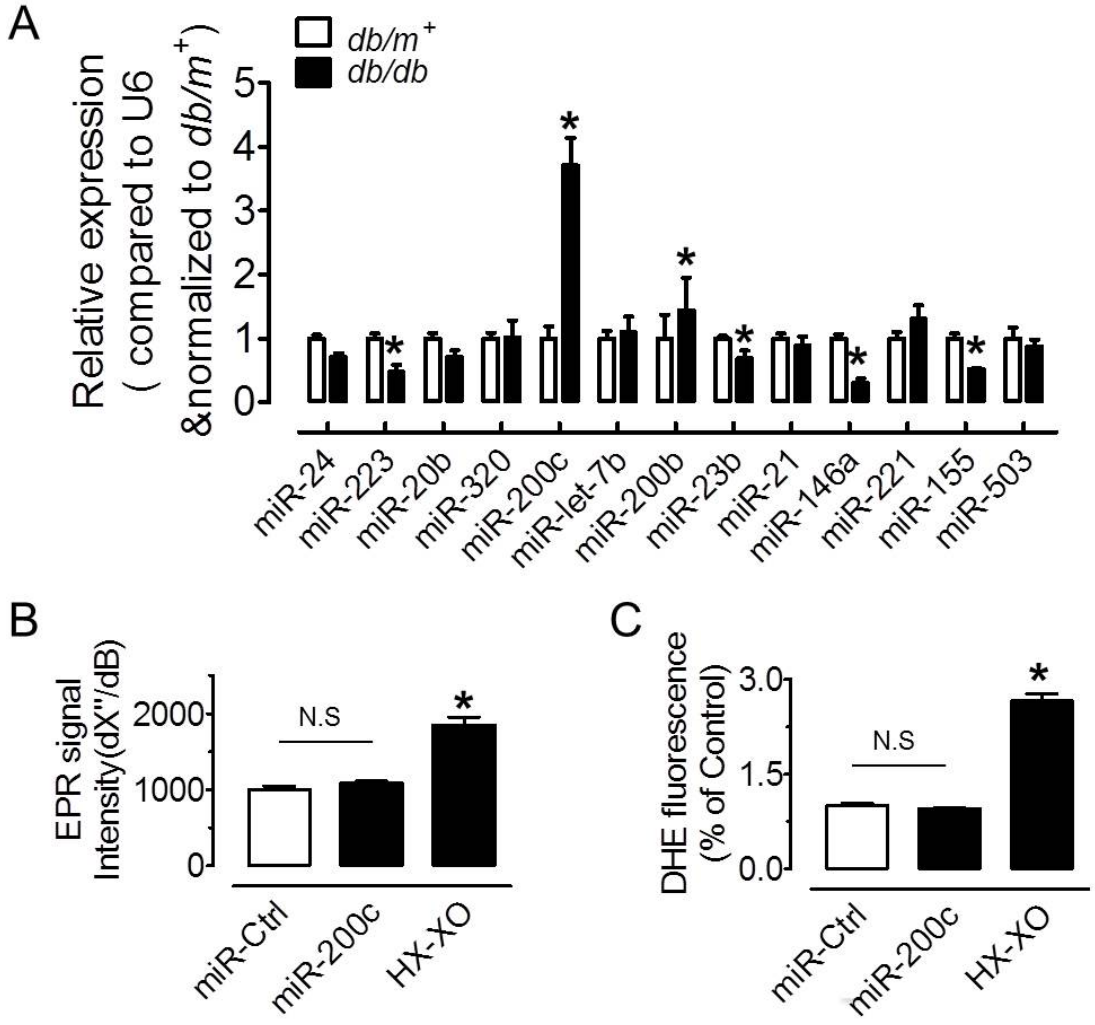
The expression of miRNAs in mouse aortas and the effect of miR-200c overexpression on ROS production in endothelial cells. The miRNA screening assay demonstrated that miR-200c was significantly higher in *db/db* mouse aortas (Online Figure IA). Overexpression of miR-200c did not induce ROS production in mouse aortic endothelial cells (MAECs) as detected by ERP spectroscopy (Online Figure IB) or by DHE staining (Online Figure IC).



Supplementary Figure 1. (A) The miRNAs screening assay comparing the expression levels of endothelium-relevant miRNAs in diabetic *db/db* and non-diabetic *db/m*$^+$ mouse aortas. **p*<0.05 vs. *db/m*$^+$. (B&C) The effect of miR-200c on ROS production in MAECs. **p*<0.05 vs. miR-Ctrl. Data are means±SEM of 4-5 experiments.

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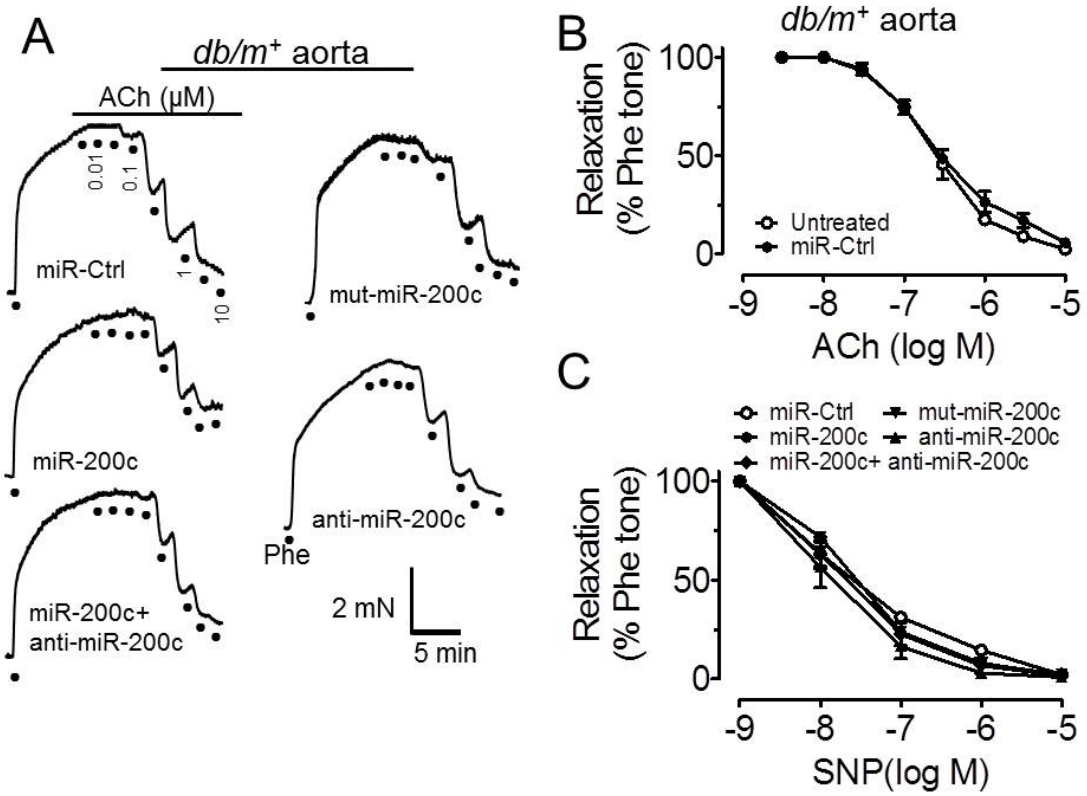
The miR-200c overexpression attenuates endothelium-dependent relaxations (EDRs) in mouse aortas. Overexpression of miR-200c (ad-miR-200) but not miR-Ctrl (ad-miR-Ctrl) by adenovirus transduction (10^7 pfu, 24 hours) reduced ACh-induced EDRs in *db/m*$^+$ mouse aortas (Online Figure IIA&B). By contrast, sodium nitroprusside (SNP)-induced endothelium-independent relaxations in *db/m*$^+$ mouse aortas were comparable in different groups of mice (Online Figure IIC).



Supplementary Figure 2. (A) Representative traces showing that ACh-induced EDRs in *db/m*$^+$ mouse aortas were attenuated following transduction with miR-200c overexpressing adenovirus. (B) Lack of the effect of ad-miR-Ctrl on EDRs. (C) SNP-induced endothelium-independent relaxations in *db/m*$^+$ mouse aortas following viral transduction. Data are means±SEM of 4-5 experiments.

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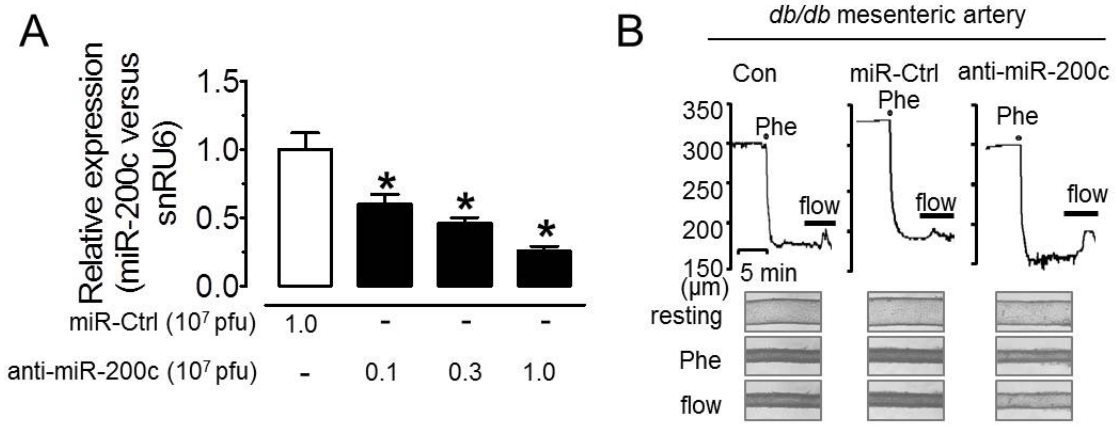
Anti-miR-200c suppresses miR-200c expression and improves flow-mediated dilatation of *db/db* mouse mesenteric arteries. Transduction of anti-miR-200 overexpressing adenovirus (24 hours, 0.1, 0.3,1 *10^7 pfu) progressively suppressed miR-200 expression in *db/db* mouse aortas (Online Figure IIIA) and augmented flow-induced dilatation in *db/db* mouse mesenteric arteries (Online Figure IIIB).



Supplementary Figure 3. (A) qPCR analysis of miR-200c expression in *db/db* mouse aortas following *ex vivo* 24-hour incubation with different dosages of ad-anti-miR-200c. *p<0.05 vs. miR-Ctrl. (B). Traces showed the effect of ad-anti-miR-200c on flow-mediated dilatation in *db/db* mouse resistance mesenteric arteries. Data are means±SEM of 4-5 experiments.

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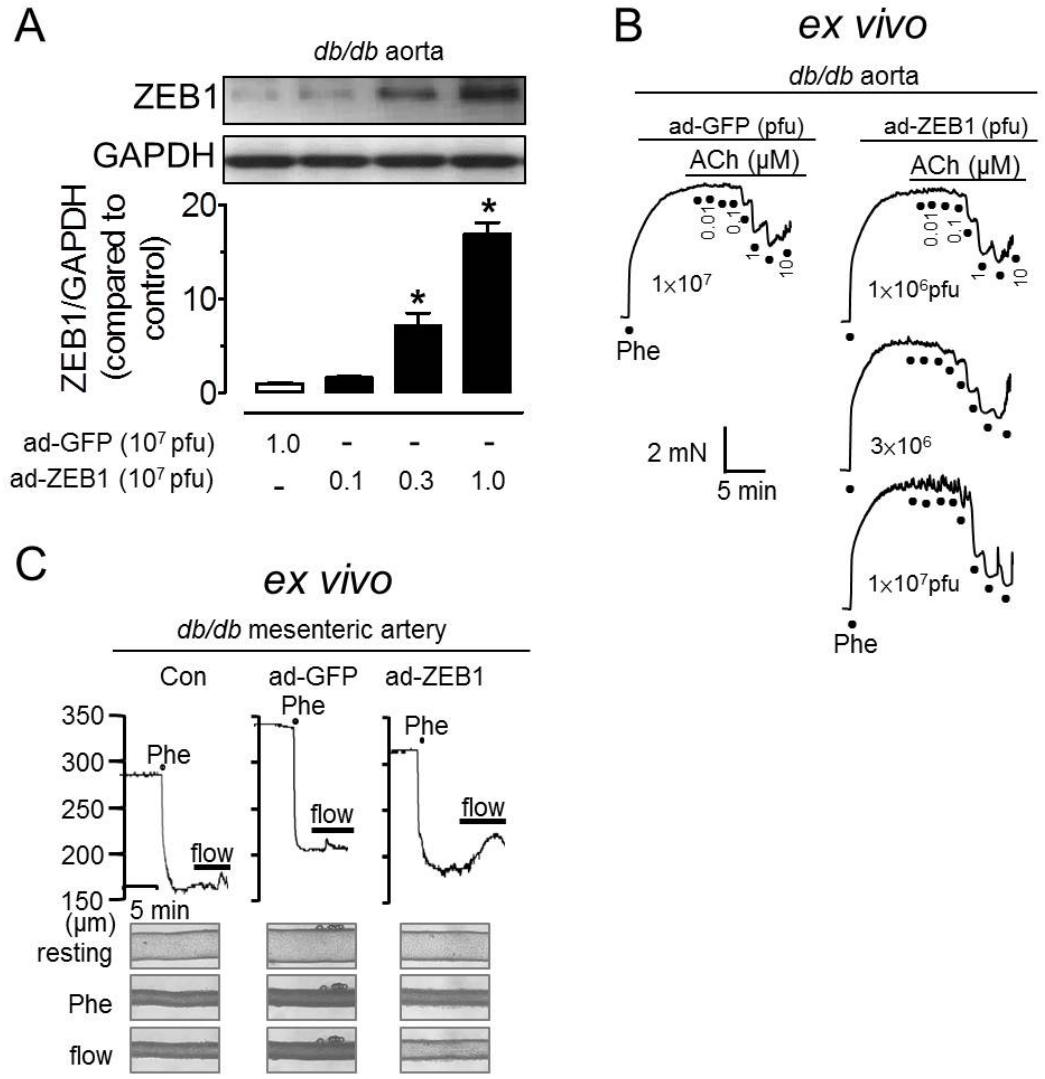
ZEB1 overexpression improves EDRs and flow-mediated dilatation of mesenteric arteries from *db/db* mice. Ad-ZEB1 transduction increased ZEB1 expression in *db/db* mouse aortas in a dose-dependent manner (Online Figure IVA) and enhanced EDRs in aortas and flow-mediated dilatation of mesenteric arteries from *db/db* mice (Online Figure IVB&C).



Supplementary Figure 4. (A) ZEB1 expression in *db/db* mouse aortas after ad-ZEB1 transduction. **p*< 0.05 vs. ad-GFP. (B&C) Traces showing the effect of ad-ZEB1 transduction on EDRs in *db/db* mouse aortas and on flow-mediated dilatation in *db/db* mouse mesenteric arteries. Data are means±SEM of 4-5 experiments.

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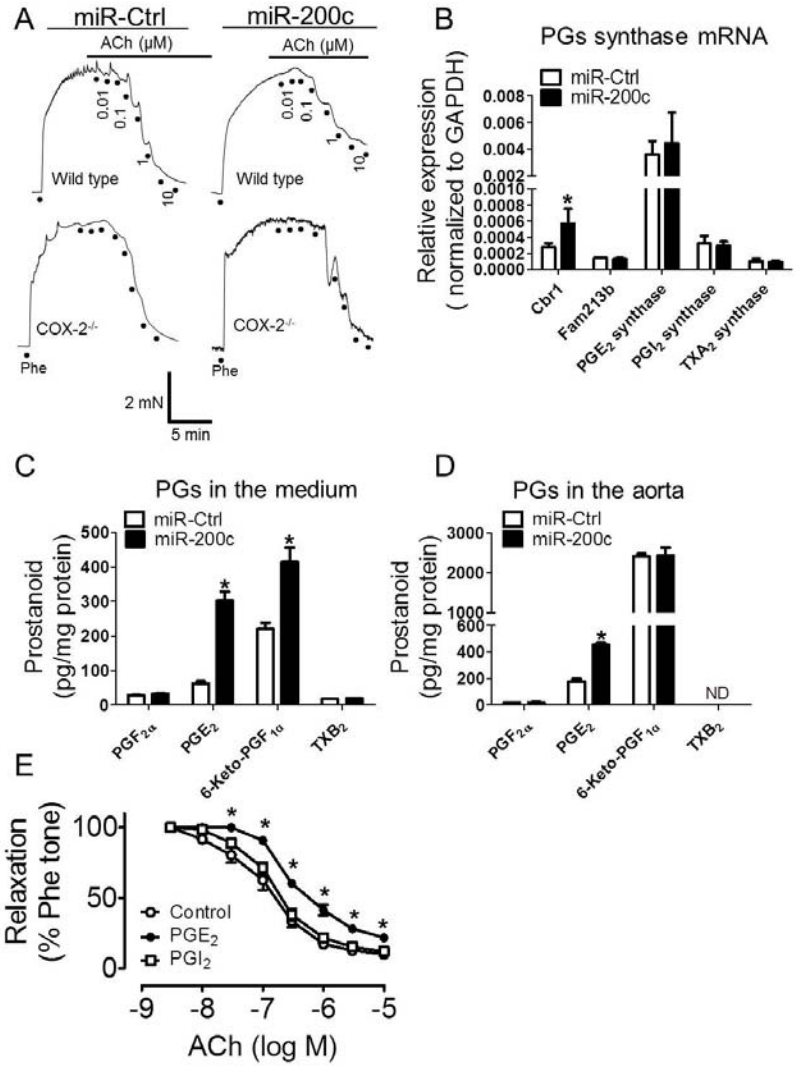
The miR-200c-induced EDR impairment may involve COX-2-dependent PGE_2 production. MiR-200c-induced endothelial dysfunction was absent in COX-2 knockout mice (Online Figure VA). MiR-200c overexpression increased Cbr1 expression (Online Figure VB), PGE_2 and PGI_2 levels in the medium (Online Figure VC) and PGE_2 level in the mouse aortas (Online Figure VD). Pretreatment with PGE_2, but not PGI_2, attenuated ACh-induced relaxation (Online Figure VE).



Supplementary Figure 5. (A) Traces showing the effect of ad-miR-200c transduction (24 hours) on EDRs in aortas from COX-2 knockout mice. (B) The mRNA level of PGs synthases in the mouse aortas after miR-200c overexpression. (C) Levels of four PGs in the medium measured by LC/MS. *p< 0.05 vs. miR-Ctrl. (D) The amount of four PGs in mouse aortas measured by LC/MS. *p< 0.05 vs. miR-Ctrl. (F) Acute inhibitory effect of PGE_2 (100 nmol/L) but not PGI_2 (100 nmol/L) on ACh-induced relaxations.*p<0.05 vs.Control. Data are means±SEM of 4-5 experiments.

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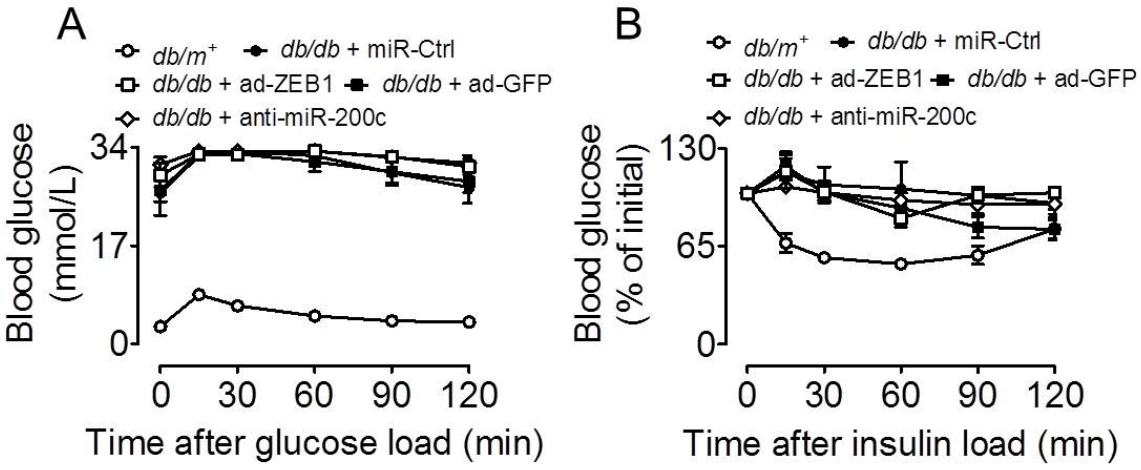
Transduction of anti-miR-200c overexpressing adenovirus or ad-ZEB1 does not affect insulin sensitivity in *db/db* mice. There was no difference in glucose tolerance (Online Figure VIA) and insulin tolerance (Online Figure VIB) in *db/db* mice after anti-miR-200c or ad-ZEB1 transduction (one week).



Supplementary Figure 6. Effects of anti-miR-200c or ad-ZEB1 transduction on glucose tolerance (A) and insulin tolerance (B) in *db/db* mice. Data are means±SEM of 4-5 experiments.