Supplemental Material

MicroRNA-181b improves glucose homeostasis and insulin sensitivity by regulating endothelial function in white adipose tissue

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Reagents and cell culture

Pre-miR™ miRNA precursor molecules negative (non-silencing) control #1 (AM17110) and Hsa-miR-181b-5p Pre-miR™ miRNA precursor (PM12442) were ordered from Ambion. The mature miR-181b sequence is 5'- AACAUUCAUUGCUGUCGGUGGGU-3', and its miRBase Accession# is MIMAT0000257. For *in vivo* studies, oligomers with the same sequence were synthesized on a larger scale by Ambion. Anti-miR miRNA inhibitors-negative control #1 (AM17010), and miR-181b inhibitor (AM12442) were from Ambion. Lipofectamine™ 2000 reagent was from Invitrogen.

Human umbilical vein endothelial cells (HUVECs) (cc-2159) was obtained from Lonza and cultured in endothelial cell growth medium EGM®-2 (cc-4176). Cells were treated with free fatty acid as previously described,[1] or other stimuli. Silencer® Select siRNA against INPP5E, PPM1A, CTDSPL, PPAR2B, and PHLPP2 were ordered from Ambion.

Real-time quantitative PCR

Tissues were homogenized using TissueLyser II (QIAGEN). Total RNA was isolated from tissues or cells using TRIzol® Reagent (Invitrogen) according to the manufacturer's instructions. Briefly, samples were mixed with chloroform (5:1 v/v sample/chloroform) and centrifuged at 12,000 x *g* for 15 minutes at 4°C. The aqueous phase containing total RNA was collected, diluted 1:1 (v/v) in isopropanol, and precipitated by centrifugation at 12,000 x *g* for 15 minutes at 4°C. Pellets were washed with 75% ethanol, dried, and resuspended in nuclease-free water.

QuantiTect Reverse Transcription Kit (QIAGEN) was used to generate cDNA synthesized from 1 µg of total RNA and QuantiFast SYBR Green PCR Kit was used for real-time qPCR with the Mx3000P Real-time PCR system (Stratagene). For primer sequences, refer to **Online Table II**. Synthesis of stem-loop miRNA cDNAs was achieved by using TaqMan® MicroRNA Reverse Transcription Kit (PN4366596). To detect mature miRNA sequences, TaqMan® MicroRNA Assays hsa-miR-181b (Assay ID 001098), TaqMan® MicroRNA Assays hsa-miR-181a (Assay ID 000480), TaqMan® MicroRNA Assays hsa-miR-181c (Assay ID 000482), U6 snRNA (Assay ID 001973), and TaqMan® Universal PCR Master Mix No AmpErase® UNG (PN4324018) were used. The Delta-Delta CT method with formula: 2^[-(delta)(delta)Ct] was used to calculate relative gene expression values.

Western blot assay

Cells or mouse tissues were lysed in RIPA buffer (Boston BioProducts, Inc.). Protein concentration of tissue extracts or cell lysates was determined by using Pierce BCA Protein Assay Kit (Pierce). Proteins were separated by a 4–15% Mini-PROTEAN® TGX™ Precast Gel (Bio-Rad) and transferred to a methanol-activated PVDF membrane (Bio-Rad). The membrane was blocked in TBST containing 5% nonfat milk at room temperature for 1 hour and subsequently incubated with primary antibodies overnight at 4 °C. After wash, the membrane was incubated with HRP-conjugated secondary antibodies (Pierce) for 1 hour. Proteins were detected using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare). Primary antibodies used were anti-pan-Akt (#2920, Cell Signaling), anti-phospho-Akt (Ser473; #4060, Cell Signaling), anti-phospho-Akt (Thr308; #2965, Cell Signaling), anti-IRS1 (#2382, Cell Signaling), anti-insulin receptor beta (sc-711, Santa Cruz), anti-phospho-IRS1 (Ser307; 05-1087, EMD Millipore), anti-phospho-insulin receptor beta (Tyr1162/1163; sc-25103, Santa Cruz), anti-FoxO1 (#9454, Cell Signaling), anti-phospho-FoxO1 (Ser256; #9461, Cell Signaling), polyclonal Rabbit Anti-eNOS (610298, BD Transduction Laboratories), anti-phospho-eNOS (Ser1176; #9571, Cell Signaling), anti-PTEN (#9188, Cell Signaling), anti-IGF-1R (#9750, Cell Signaling), anti-GAPDH (#2118, Cell Signaling), anti-ICAM-1 (AF-796, R&D Systems), and anti-PHLPP2

(ab71973, Abcam). Quantitation was performed using the software Image J. Protein expression was normalized by a loading control.

Diet-induced obesity, glucose and insulin tolerance tests

C57BL/6J WT mice were purchased from the Jackson Laboratory. All mice were males and were maintained on a 12-hour light/dark cycle in a pathogen-free animal facility. Mice were kept on a standard chow diet or on a high-fat diet containing 60 kcal% fat (Research Diets, D12492) for 12 weeks. Mice had free access to food and water. Six weeks after HFD, mice were treated with miRNAs or siRNAs for 6 weeks (twice or once a week, i.v. 0.6 mg/kg). Systemic delivery of miRNAs or siRNAs was performed as described in our previous study.[2] ITT and GTT were performed at week 5 and 6 respectively after miR-181b treatment. All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee at Harvard Medical School, Boston, MA and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

For glucose tolerance tests (GTTs), mice were fasted for 12 hours, and then injected i.p. with d-glucose (Sigma, 1.0 g per kg of body weight). Insulin tolerance tests (ITTs) were performed on mice after 6 hours fasting. Recombinant human regular insulin (0.75 U per kg of body weight, Humulin R, Eli Lilly) was given to mice by i.p. injection. Blood glucose levels were measured before injection and at 15, 30, 60, 90, and 120 minutes after glucose or insulin injection using One Touch Ultra glucometer (LifeScan).

Luciferase activity assay and cell culture transfection

PHLPP2 3'-UTR sequences were PCR-amplified with specific primers, followed by purification and restriction enzyme digestion. Sequences were cloned into the pMIR-REPORT™-Luciferase vector between *SpeI* and *Mlu*I restriction sites. HUVECs cultured in 12-well plates were transfected in triplicates using Lipofectamine 2000 (Invitrogen) with 200 ng of the final construct per well. The next day, cells were transfected with 10nmol of either a miRNA non-silencing control or miR-181b. Cells were collected 36–48 hours after transfection and assayed using the Luciferase Reporter Assay System (Promega). Cells were co-transfected with pcDNA3.1(+), pcDNA3.1(+)-miR-181b, or pcDNA3.1(+)-miR-181b mutant. The seed sequence of miR-181b was mutated using QuikChange II XL Site-Directed Mutagenesis Kit from Agilent Technologies. Results were normalized to the amounts of protein and expressed relative to the average value of the control.

Blood chemistry

Plasma insulin was measured using the Ultra Sensitive Rat Insulin ELISA Kit (90080, Crystal Chem). Mouse Cytokine 32-Plex Discovery Assay was performed by Eve Technologies. Triglyceride levels were determined using Infinity™ Triglycerides Liquid Stable Reagent (Thermo Scientific) as described by the protocol. Total cholesterol was measured using the Infinity™ Cholesterol Reagent (Thermo Scientific). Cholesterol and triglyceride standard were from Pointe Scientific, Inc. Plasma free fatty acid was measured using Free Fatty Acid Quantification Kit (ab65341, Abcam).

Glucose uptake

Mouse mature adipocytes were serum-starved for 3–6 hours in DMEM and then incubated with KRH buffer (121 mM NaCl, 5 mM KCl, 0.33 mM CaCl2, 1.2 mM MgSO4, 12 mM Hepes, pH 7.4) with 25 nM insulin at 37 °C for 20 minutes and 2-deoxy-D-[2,6-^{3}H]glucose (0.33 μCi ml^{-1}) for an additional 10 minutes. Uptake was stopped by three rapid washes on ice with KRH containing cytochalasin B (Sigma), the cells were solubilized with KRH buffer containing 0.1% SDS, and radioactivity was measured by liquid scintillation counting.

Histological and immunohistological examinations

For immunohistology, tissues were fixed with neutral buffered 10% formalin solution (HT501128, Sigma), embedded in paraffin wax, cut into sections, and then deparaffinized. Mac-2 staining was performed on the Leica Bond III autostainer. Antigen retrieval was performed using Bond ER1 (AR9961, Leica) for 30 minutes. Sections was incubated with anti-Mac-2 (CL8942AP, Cedarlane Labs) in 1:50,000 dilutions for 30 minutes at RT. Primary antibodies binding to tissue sections was visualized using Bond Polymer Refine Detection kit (DS9800, Leica), and counterstained with hematoxylin. Images were captured by a digital system, and the staining area was measured using computer-assisted image quantification (Image-Pro Plus software, Media Cybernetics). For immunofluorescence staining, antigen retrieval was performed by boiling the slides for 10 minutes in 10mM sodium citrate pH6.0 with a pressure cooker. The sections were then incubated with 1mg/ml sodium borohydride for 5 minutes at room temperature. After three washes with TBS, the sections were incubated with 5% normal donkey serum (Jackson ImmunoResearch Lab Inc.) for 1 hr at room temperature. Slides were then incubated with Rabbit anti-Ki-67 (1:200; RM-9106-S1, Thermo Scientific) and Rat anti-F4/80 (1:100; 14-4801-85, eBioscience) overnight at 4 °C. The slides were washed three times and incubated with Alexa647 conjugated Donkey anti-rabbit and Dylight549 conjugated Donkey anti-rat secondary antibodies. Sections were then washed three times with TBS and counterstained with Hoechst (H21492, Life technology) before mounting with Prolong Gold anti-fade mounting media (P36930, Life technology). Images were acquired on an upright Carl Zeiss LSM 510 confocal microscope equipped with Plan-Neofluar 40×/1.3 oil-immersion objective using the 405 nm diode laser, the 543 nm line of a HeNe543 laser, and the 633 nm line of a HeNe633 laser. Data were analyzed in a blinded fashion, by two independent observers.

Adoptive cell transfer, Isolation of stromal-vascular fraction and ECs, and flow cytometry

Adoptive transfer of monocytes was conducted as previously described.[3] Monocytes were isolated from miR-181b- or control miRNA-treated obese mice, labeled with PKH26 in vitro, and injected into HFD-fed obese mice. Stromal vascular fractions were isolated from eWAT of the recipient mice, and subjected to FACS analysis,[4] or EC isolation.[5-8] Briefly, the perigonadal adipose tissues were isolated, minced into small pieces with scissors (1 x 2 mm2), digested with collagenase type II and dispase (1mg/ml each in DMEM/F12). Digested tissues were neutralized with DMEM/F12 medium containing 10% FBS, centrifuged at 500 xg for 10 minutes at 4 °C. Anti-Mouse CD11b Alexa Fluor® 488 (53-0112-80, eBioscience) and Anti-Mouse F4/80 Antigen APC (17-4801-80, eBioscience) were used for FACS analysis, which was performed on an LSR-II (Beckton-Deckinson, San Diego, CA) and analyzed with BD CellQuest software. For EC isolation, stromal-vascular fractions were incubated with sheep anti-rat IgG Dynabeads coated with PECAM-1 antibodies (557355, BD). The bead-bound cells were collected using a magnet and washed 3 times.

Isolation of fresh human endothelial cells and immunostaining

Isolation of EC from a forearm vein of human subjects was described previously.[9] Briefly, ECs were captured by spring-wires from cannulated forearm veins of human subjects. Wires were cut, and cells were collected from wires by centrifugation in a dissociation buffer. Isolated cells were plated on slides, and fixed for staining. Anti-PHLPP2 (ab153918), anti-vWF (#M061601, Dako), and DAPI were used for staining and quantitative immunofluorescence was used to determine protein expression relative to control staining of cultured human aortic ECs. All human subject protocols were approved by BUMC IRB and subjects provided written informed consent.

Measurement of total nitric oxide levels

To measure total NO production, the concentration of nitrate and nitrite was determined in the lysates of eWAT using a total NO detection kit (ADI-917-020, Enzo Life Sciences). Fat tissues were harvested, immediately flash frozen, and homogenized in a buffer containing 1 mM protease inhibitor cocktail. Nitrate was converted to nitrite using nitrate reductase, and total nitrite was measured according to the manufacturer's instruction.

Statistical analysis

Results were expressed as mean ± SEM. We used a paired or unpaired Student *t* tests as appropriate for statistical comparison between two groups, and ANOVA for the comparison of 3 or more groups. Differences were considered significant when $P<0.05$.

References

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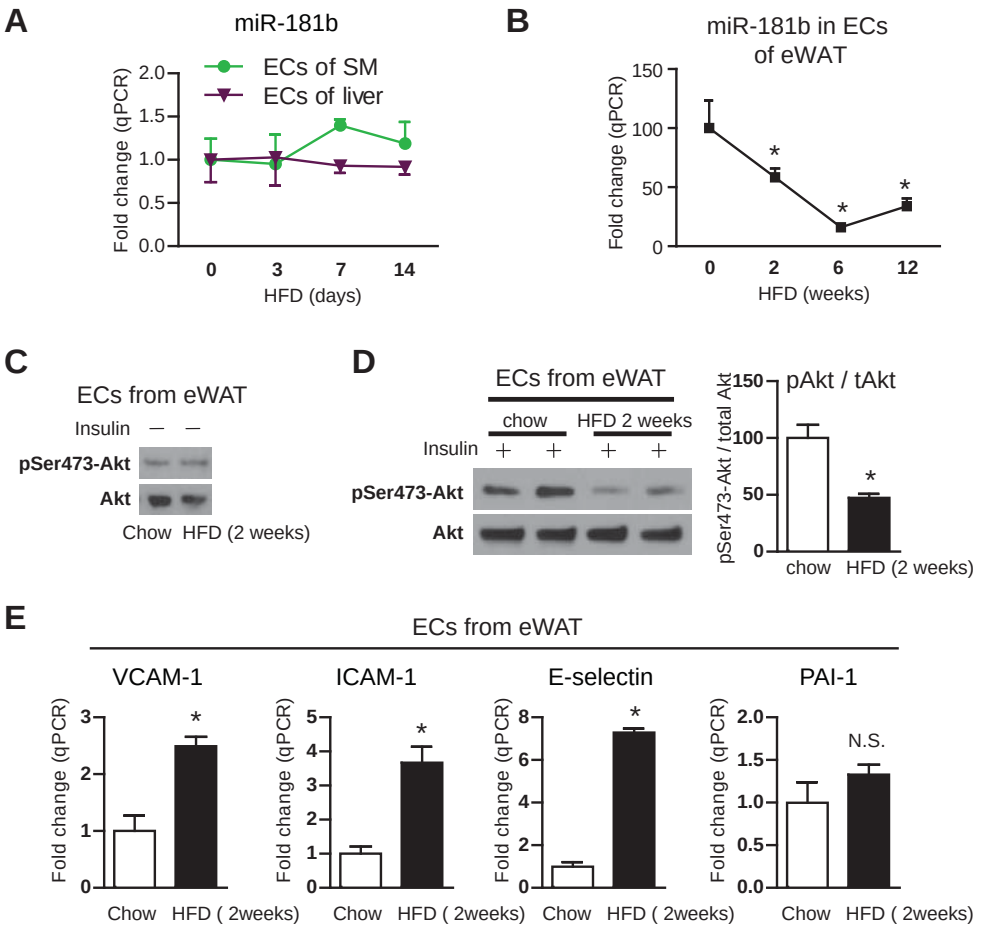
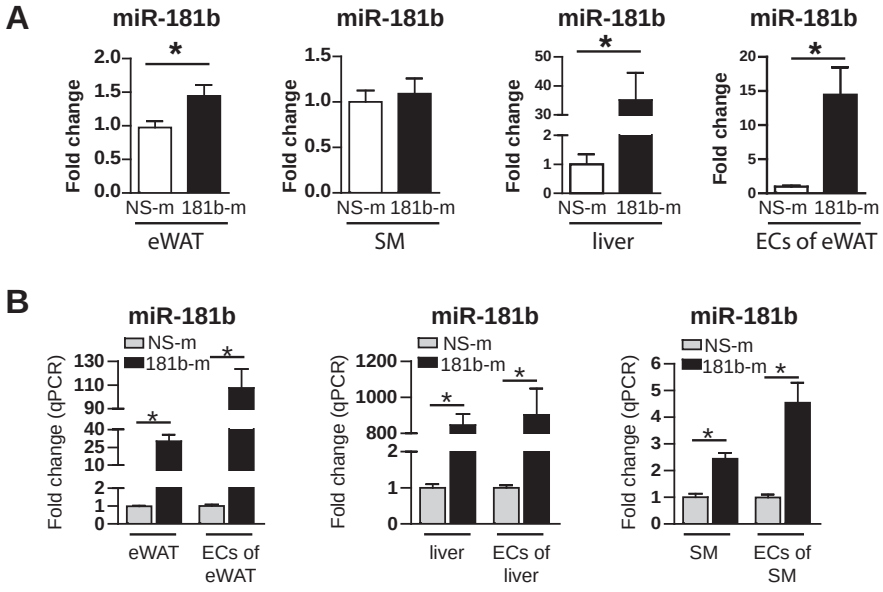
Online Figure I



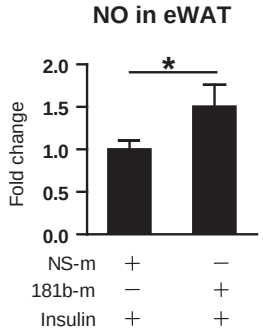
Online Figure I. A, C57BL/6 mice were fed a HFD as indicated (0, 3, 7, or 14 days). Endothelial cells (ECs) were isolated from liver and skeletal muscle for qPCR. The expression of miR-181b was normalized to small RNA U6 expression and compared to its expression in mice on chow that was subsequently set to a value of one, n=5 – 6 per group. **B**, C57BL/6 mice were fed a HFD as indicated (0, 2, 6, or 12 weeks). ECs were isolated from epididymal white adipose tissue (eWAT) for qPCR. The expression of miR-181 was normalized to small RNA U6 expression and compared to the expression of miR-181b in mice on chow that was set to a value of 100 %, n=6 –10 per group. **C**, C57BL/6 mice were fed a HFD for 0 or 2 weeks. ECs were isolated from eWAT for western blot analysis, n=6 – 9 mice per group. **D**, C57BL/6 mice were fed a HFD for 0 or 2 weeks. ECs were isolated from eWAT for western blot analysis, n=8 – 10 mice per group. Mice were fasted for 6 hours and administered with insulin 0.75 U/kg at 10 minutes before harvesting. **E**, C57BL/6 mice were fed a HFD for 0 or 2 weeks. ECs were isolated from eWAT for qPCR, n=6 – 9 mice per group. All data show mean ± SEM; *, $P < 0.05$.

Online Figure II



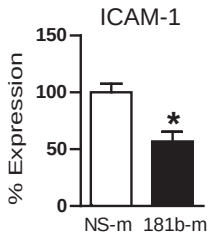
Online Figure II. A, MiR-181b expression was detected in epididymal white adipose tissue (eWAT), skeletal muscle (SM), liver, and endothelial cells (ECs) isolated from eWAT of obese mice treated with NS-m or miR-181b using the dosing regimen as outlined in Figure 2, n=8 –10 mice per group. **B**, Mice were injected with NS-m or 181b-m three times on consecutive days. The expression of miR-181b was examined by qPCR analysis in eWAT and ECs isolated from eWAT, n=3 mice per group. Data show mean ± SEM; *, $P < 0.05$.

Online Figure III



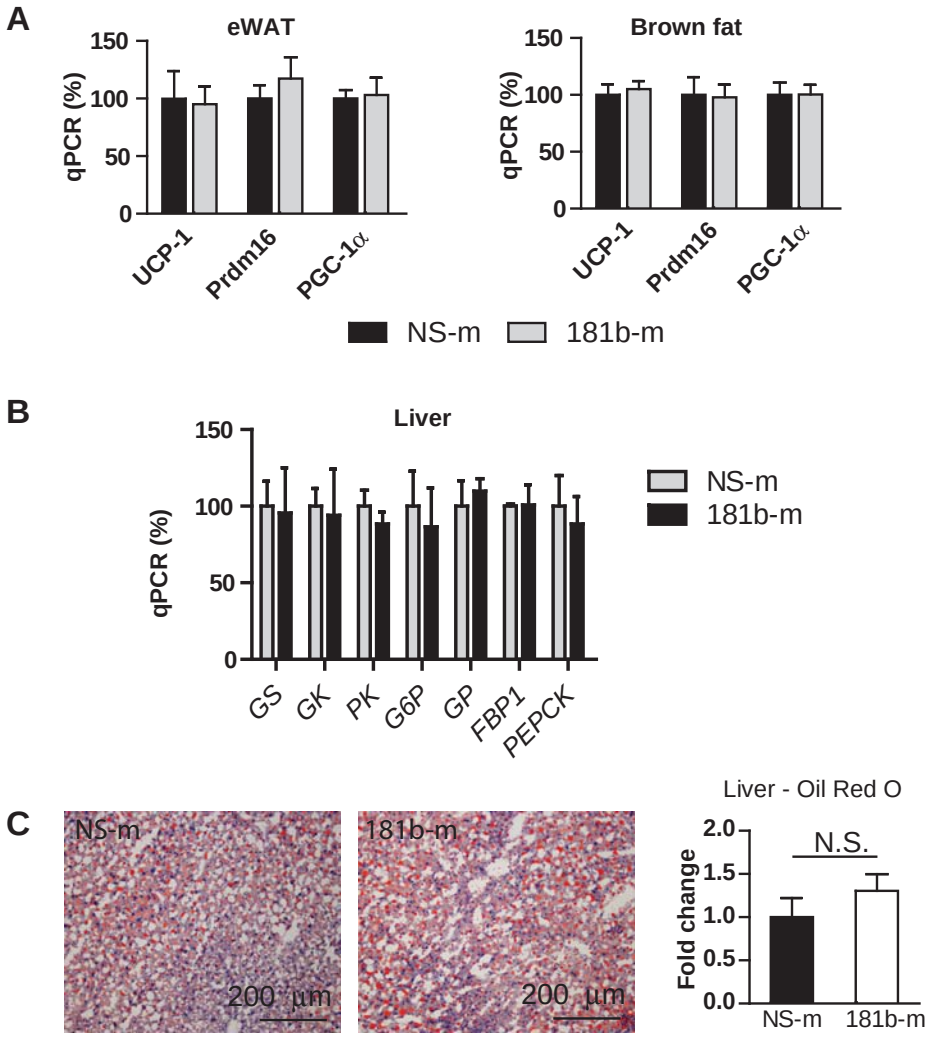
Online Figure III. Nitric oxide activity in epididymal white adipose tissue (eWAT). Obese mice were treated with NS-m or miR-181b using the dosing regimen as outlined in Figure 2 and stimulated with insulin (0.75 U per kg of body weight) before tissue harvesting, n=11 (NS-m) or n=12 (miR-181b) mice per group. Data show mean ± SEM; *, $P < 0.05$.

Online Figure IV



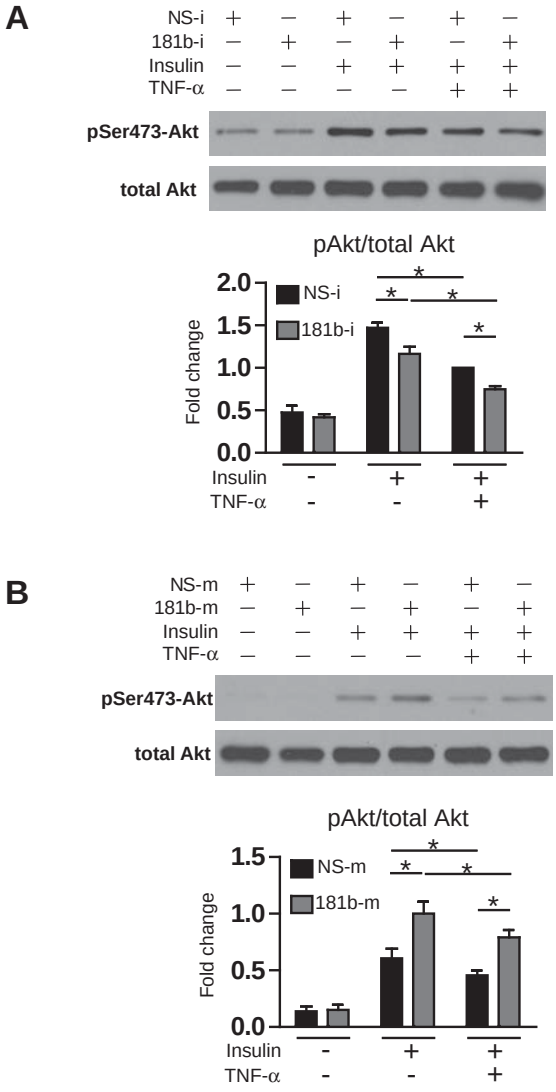
Online Figure IV. ICAM-1 expression was detected by qPCR in endothelial cells isolated from epididymal white adipose tissue of obese mice treated with NS-m or miR-181b using the dosing regimen as outlined in Figure 2, n=6 mice per group. Data show mean ± SEM; *, $P < 0.05$.

Online Figure V



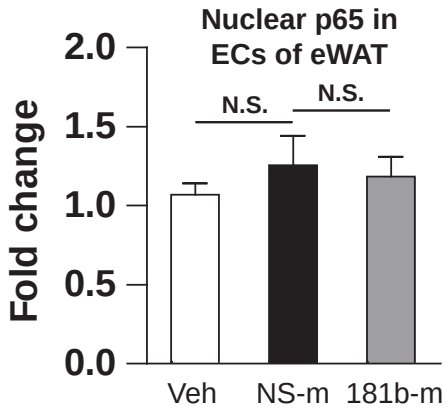
Online Figure V. A, qPCR analysis of genes involved in thermogenesis in brown adipose tissue (BAT) and epididymal white adipose tissue (eWAT) from mice described in Figure 2. **B**, qPCR analysis of gene expression in liver from mice described in Figure 2. GS: Glycogen synthase; GK: Glucokinase; PK: Pyruvate Kinase; G6P: Glucose-6-phosphatase; GP: Glycogen phosphorylase; FBP1: Fructose1,6-biphosphatase; PEPCK: Phosphoenolpyruvate carboxykinase. **C**, Frozen sections of liver from mice described in Figure 2 were stained with Oil Red O, and positive areas were quantified. All values show mean ± SEM, n=6 – 9 mice per group; *, $P < 0.05$. N.S., non-significant.

Online Figure VI



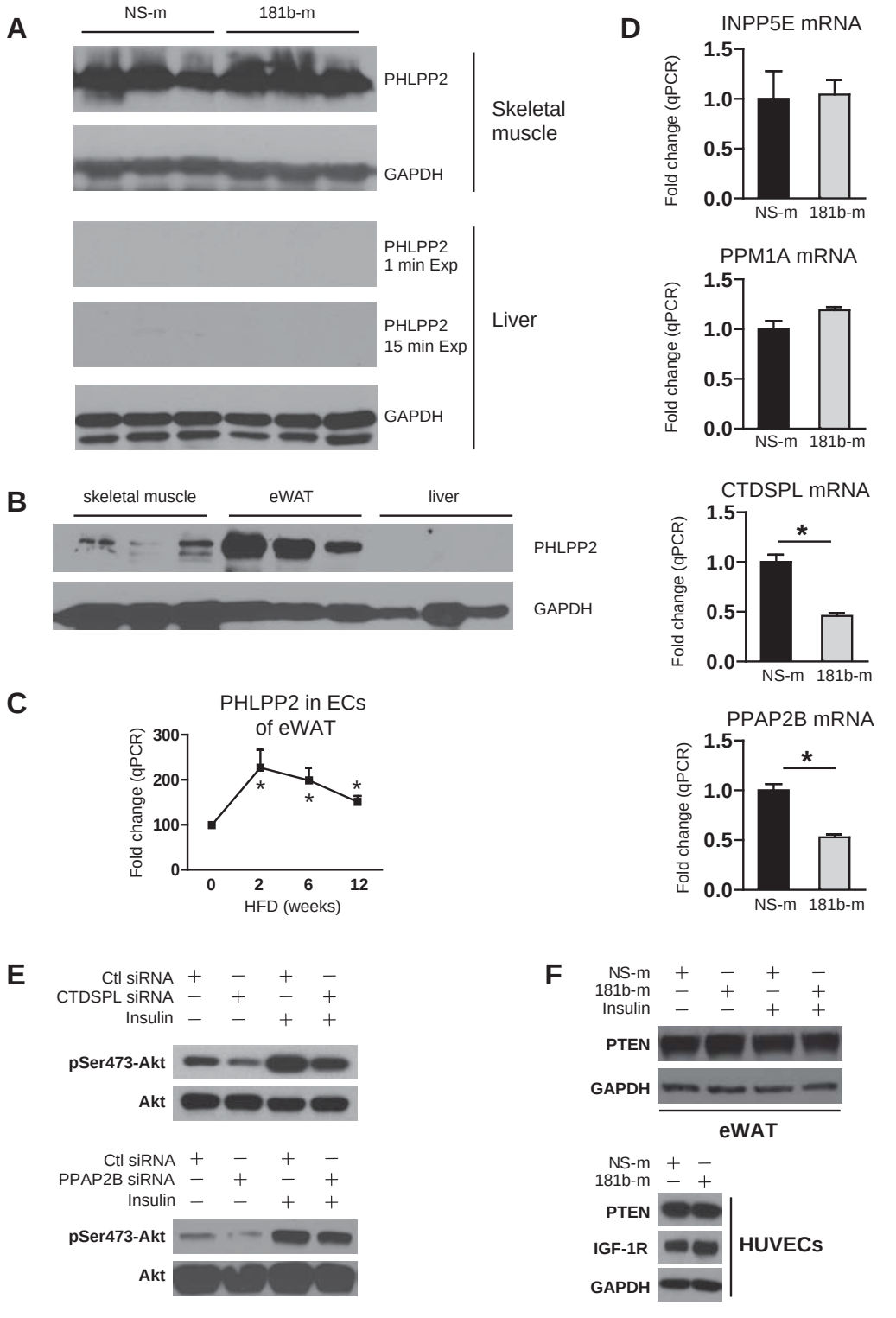
Online Figure VI. A, HUVECs were transfected with 50 nM miRNA inhibitor negative control (NS-i), or miR-181b inhibitor (181b-i) for 24 hours. Cells were starved and treated with 10 ng/ml TNF-α or PBS for 8 hours followed by 100 nM insulin for 10 minutes, and harvested for western blot analysis, n=3 independent experiments. **B**, HUVECs were transfected with 10 nM miRNA non-specific negative control (NS-m), or miR-181b (181b-m) for 24 hours. Cells were starved and treated with 10 ng/ml TNF-α or PBS for 8 hours followed by 100 nM insulin for 10 minutes, and harvested for western blot analysis, n=3 independent experiments. Data show mean ± SEM; *, $P < 0.05$.

Online Figure VII



Online Figure VII. Paraffin sections of epididymal white adipose tissue (eWAT) from mice described in Figure 2 were stained with antibodies against CD31 and p65, and nuclear accumulation of p65 was quantified in ECs reflecting vehicle (n = 39 cells), NS-m (n= 73 cells) and 181b-m (n= 94 cells). Mean ± SEM, n=6 – 9 mice per group. N.S., non-significant.

Online Figure VIII



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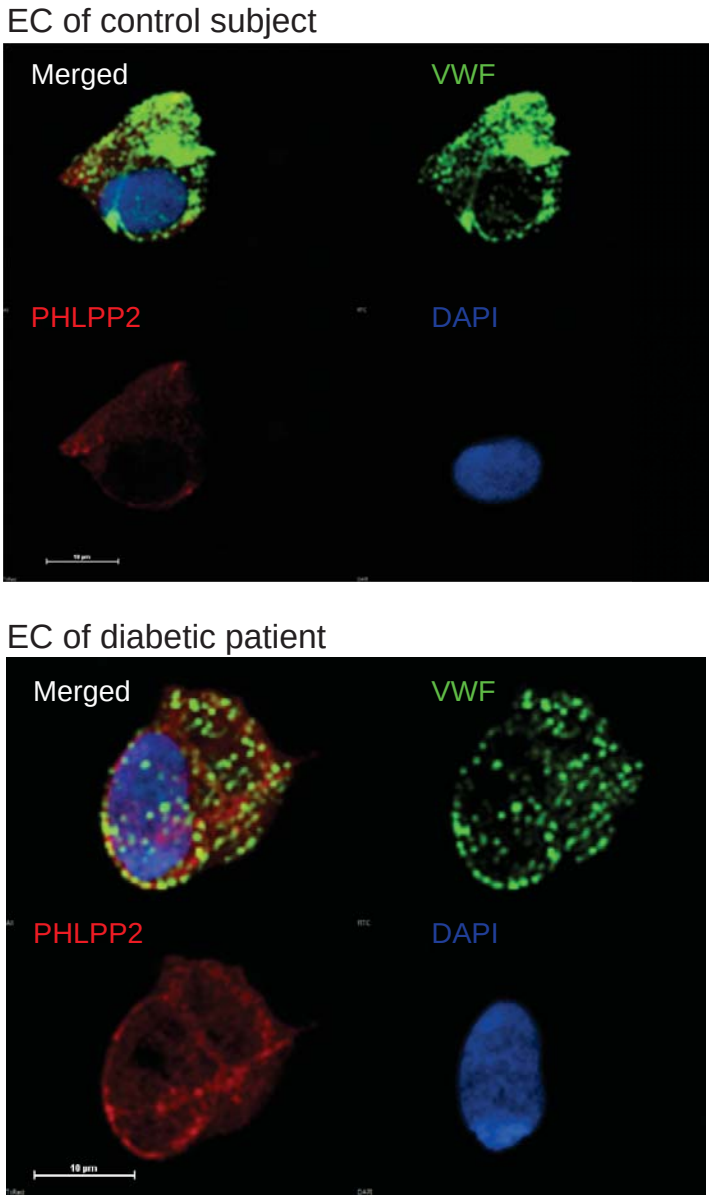
Online Figure VIII. A and **B**, Western blot analysis of PHLPP2 expression in skeletal muscle, liver, and epididymal white adipose tissue (eWAT). **C**, C57BL/6 mice were fed a HFD as indicated (0, 2, 6, or 12 weeks). Endothelial cells (ECs) were isolated from eWAT for qPCR. The expression of PHLPP2 was normalized to GAPDH expression and compared to the expression of PHLPP2 in chow-fed mice that was set to a value of 100 %, n=6 –10 per group. **D**, HUVECs were transfected with 10 nM miRNA non-specific negative control (NS-m), or miR-181b (181b-m) for 36 hours, and harvested for qPCR analysis, n=3 per group. **E**, Ctl siRNA, CTDSPL siRNA, PPAP2B siRNA-transfected HUVECs were starved and treated with 100 nM insulin for 10 minutes. Cells were collected for western blot analysis of pSer473-Akt and total Akt. **F**, PTEN expression was examined in eWAT of obese mice treated with NS-m or miR-181b using the dosing regimen as outlined in Figure 2, n=6 mice per group (upper panel). Western blot analysis of PTEN and IGF-1R in HUVECs transfected with 10 nM miRNA non-specific control or miR-181b (lower panel). Data show mean ± SEM; *, $P < 0.05$.

Online Figure IX



Online Figure IX. Representative images show PHLPP2 staining in endothelial cells (ECs) freshly isolated from control and diabetic subjects.

Online Table I

Online Table I. Systemic delivery of miR-181b does not affect lipid profile, FFAs, insulin, fat mass, or body weight of obese C57BL/6 mice.

	Units	Ctl mimics	miR-181b
Total cholesterol	mg/dl	231.5 ± 22.4	231.3 ± 8.9
Triglycerides	mg/dl	95.5 ± 4.63	106.4 ± 4.65
FFAs	nmol/ml	236.8 ± 16.7	264.1 ± 18.4
Insulin	ng/ml	1.17 ± 0.11	0.91 ± 0.18
Body weight	g	43.3 ± 4.3	40.3 ± 4.8
Brown fat mass	mg	109.1 ± 14.5	113.1 ± 14.8
Epididymal fat mass	g	2.43 ± 0.11	2.57± 0.14

All values Ctl mimics vs. miR-181b, *P*=N.S., Mean ± SEM, n=6-10.

Online Table II. Primers for real-time qPCR

Name	Sequence (5' −> 3')
mouse VCAM-1 forward:	GTTCCAGCGAGGGTCTACC
mouse VCAM-1 reverse:	AACTCTTGGCAAACATTAGGTGT
mouse ICAM-1 forward:	GTGATGCTCAGGTATCCATCCA
mouse ICAM-1 reverse:	CACAGTTCTCAAAGCACAGCG
mouse TNF-alpha forward:	CCCTCACACTCAGATCATCTTCT
mouse TNF-alpha reverse:	GCTACGACGTGGGCTACAG
mouse IL-1beta forward:	GCAACTGTTCCTGAACTCAACT
mouse IL-1beta reverse:	ATCTTTTGGGGTCCGTCAACT
mouse IL12 forward:	TGGTTTGCCATCGTTTTGCTG
mouse IL12 reverse:	ACAGGTGAGGTTCACTGTTTCT
mouse NOS2 forward:	GTTCTCAGCCCAACAATACAAGA
mouse NOS2 reverse:	GTGGACGGGTCGATGTCAC
mouse Mrc2 forward:	ATCCAGGGAAACTCACACGGA
mouse Mrc2 reverse:	GCGCTCATCTTTGCCGTAGT
mouse IL6 forward:	TAGTCCTTCCTACCCCAATTTCC
mouse IL6 reverse:	TTGGTCCTTAGCCACTCCTTC
mouse Mgl2 forward:	TTAGCCAATGTGCTTAGCTGG
mouse Mgl2 reverse:	GGCCTCCAATTCTTGAAACCT
mouse Fizz1 forward:	CCAATCCAGCTAACTATCCCTCC
mouse Fizz1 reverse:	ACCCAGTAGCAGTCATCCCA
mouse Ym1 forward:	CAGGTCTGGCAATTCTTCTGAA
mouse Ym1 reverse:	GTCTTGCTCATGTGTGTAAGTGA
mouse IL10 forward:	GCTCTTACTGACTGGCATGAG
mouse IL10 reverse:	CGCAGCTCTAGGAGCATGTG
mouse Cox2 forward:	TGAGCAACTATTCCAAACCAGC
mouse Cox2 reverse:	GCACGTAGTCTTCGATCACTATC
mouse PHLPP2 forward:	GGAGGGATTCGGGTCCTAAAG
mouse PHLPP2 reverse:	CGGGAACCAAACCTACTTCTTC
mouse UCP-1 forward:	CAAAAACAGAAGGATTGCCGAAA
mouse UCP-1 reverse:	TCTTGGACTGAGTCGTAGAGG
mouse Prdm16 forward:	CCACCAGCGAGGACTTCAC
mouse Prdm16 reverse:	GGAGGACTCTCGTAGCTCGAA
mouse PGC-1α forward:	TATGGAGTGACATAGAGTGTGCT
mouse PGC-1α reverse:	CCACTTCAATCCACCCAGAAAG
mouse GS forward:	ACCAAGGCCAAAACGACAG
mouse GS reverse:	GGGCTCACATTGTTCTACTTGA
mouse GK forward:	TGAGCCGGATGCAGAAGGA
mouse GK reverse:	GCAACATCTTTACACTGGCCT
mouse PK forward:	TCAAGGCAGGGATGAACATTG
mouse PK reverse:	CACGGGTCTGTAGCTGAGTG
mouse G6P forward:	CGACTCGCTATCTCCAAGTGA
mouse G6P reverse:	GTTGAACCAGTCTCCGACCA
mouse GP forward:	GAGAAGCGACGGCAGATCAG
mouse GP reverse:	CTTGACCAGAGTGAAGTGCAG

mouse FBP1 forward:	CACCGCGATCAAAGCCATCT
mouse FBP1 reverse:	AGGTAGCGTAGGACGACTTCA
mouse PEPCK forward:	CTGCATAACGGTCTGGACTTC
mouse PEPCK reverse:	CAGCAACTGCCCGTACTCC
mouse Hprt forward:	TCAGTCAACGGGGGACATAAA
mouse Hprt reverse:	GGGGCTGTACTGCTTAACCAG
mouse GAPDH forward:	AGGTCGGTGTGAACGGATTTG
mouse GAPDH reverse:	TGTAGACCATGTAGTTGAGGTCA
mouse SELE forward:	ATGCCTCGCGCTTTCTCTC
mouse SELE reverse:	GTAGTCCCGCTGACAGTATGC
mouse SERPINE1 forward:	TTCAGCCCTTGCTTGCCTC
mouse SERPINE1 reverse:	ACACTTTTACTCCGAAGTCGGT
human INPP5E forward:	CGCATCGTGTCTCAGATCAAG
human INPP5E reverse:	TGAAGTGGGACGTGATGAAGA
human PPM1A forward:	AGGGGCAGGGTAATGGGTT
human PPM1A reverse:	GATCACAGCCGTATGTGCATC
human CTDSPL forward:	GTGGCTGACCTCCTAGACC
human CTDSPL reverse:	TTCACGTAGTTCCCACGATGA
human PPAP2B forward:	CTACGTGGCAGCACTCTATAAG
human PPAP2B reverse:	CCCTATGGACACTTTGGCAAT
human PHLPP2 forward:	ATGGAGCAGACACTACCACTG
human PHLPP2 reverse:	GCAAAGGACGAGATGTAAGTCA