SUPPLEMENTAL MATERIAL

Endothelial TFEB improves glucose tolerance *via* upregulation of IRS1 and IRS2

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Supplemental Methods

Cell culture

Human Coronary Artery Endothelial Cells (HCAECs) were purchased from Lonza (Cat# CC-2585) and cultured on collagen type I (354236, Corning)-coated dishes in EGM™ -2 MV Microvascular Endothelial Cell Growth Medium (Cat# CC-3202, Lonza). Mouse lung ECs were cultured on collagen type I (354236, Corning)-coated dishes and were cultured in M199 medium containing 16% fetal bovine serum (FBS), recombinant human fibroblast growth factor (F5267, Sigma-Aldrich, 1 ng/ml), heparin (H3149, Sigma-Aldrich, 90 mg/ml) and 20 mM HEPES (15630080, Gibco), and penicillin/streptomycin mix (15140122, Gibco, 50 mg/ml). Bovine aortic endothelial cells (BAECs; B304-05, Cell Applications) were cultured in DMEM medium containing 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin mix (15140122, Gibco, 50 mg/ml). Cells were cultured at 37°C with 5% CO_2 in a humidified incubator. Primary ECs were used for experiments at passages 3-5.

Isolation of mouse lung ECs.

Mouse lung ECs were isolated as described previously[1]. Dynabeads (11035, Invitrogen, 15 ul/mouse) were first precoated with CD31 antibody (1626-01, SouthernBiotech, 1:20 dilution) and ICAM2 antibody (1925-01, SouthernBiotech, 1:20 dilution) overnight at 4° C on a rotator. The antibody-conjugated beads were then washed with DPBS (without Ca^{2+}/Mg^{2+}) containing 0.1% BSA for four times in accordance with manufacturer's protocol. Lungs were harvested from 3 adult mice for each group and placed in isolation buffer (M199 medium with 20% heat-inactivated fetal bovine serum, 20 mM HEPES and 0.5 mg/ml penicillin/streptomycin on ice. Lung tissues were minced finely and digested in DPBS (with Ca^{2+}/Mg^{2+}) containing 2mg/ml collagenase A (10103586001, Sigma-Aldrich,) at 37 °C for 30 minutes. Next, a cannula attached to a 30 ml syringe was used to further triturate the tissues by aspirating the collagenase A-digested suspension for 12~15 times. The suspension was then filtered through a 70 μm cell strainer and centrifuged at 400g for 8 minutes at 4 °C. The pellets were resuspended in 3 ml DPBS (without Ca^{2+}/Mg^{2+}). In the first cell sorting, the suspension was incubated with CD31 antibody-coated beads (15ul beads/ml suspension) at room temperature for 10 minutes on a rotator. The beads and sorted ECs were washed with isolation buffer for 5-6 times until the buffer was clear, and then cultured in Collagen I-coated 10 ml dishes. When the cells achieved 90% confluence, they were detached with 0.25% trypsin-EDTA (25200072, Gibco) and spun down for a second round of sorting with ICAM2 antibody-coated beads. The primary lung ECs were split at a ratio of 1:2 for passage.

siRNA-mediated gene knockdown

HCAECs were transfected with nontargeting siRNA (Negative Control #1 siRNA, Thermo Scientific), siRNA-TFEB (D-009798-03, Dharmacon), siRNA-IRS1 (145280, Thermo Fisher Scientific), siRNA-IRS2 (137161, Thermo Fisher Scientific), or siRNA-GLUT1 (117394, Thermo Fisher Scientific) using Lipofectamine RNAiMAX Reagent (13778030, Invitrogen) in accordance with the manufacturer's protocol.

miRNA mimics transfection

HCAECs were transfected with microRNA Mimic Negative Control (miRIDIAN microRNA Mimic Negative Control #1, Horizon Discovery), miR-335-3p mimic (C-301161-01-0002, Horizon Discovery), miR-495-3p mimic (C-300762-05-0002, Horizon Discovery), or miR-548o-3p mimic (C-301394-00-0002, Horizon Discovery) using Lipofectamine RNAiMAX Reagent (13778030, Invitrogen) in accordance with the manufacturer's protocol.

Protein extracts and Western Blot

Culture cells or homogenized tissues, including soleus and gastrocnemius muscle, liver, and epididymal adipose tissue, were lysed with RIPA lysis buffer for 30 minutes on ice. The lysate was centrifuged at 12,000 rpm for 15 minutes at 4 °C. Protein samples (30μg) were resolved by the SDS polyacrylamide gel (Cat # 456-1093, Bio-Rad) in electrophoresis running buffer (25 mM Tris, 190 mM glycine, and 0.1% SDS), then transferred to 0.45 μm nitrocellulose membranes (Cat # 1620115, Bio-Rad) in transfer buffer (25 mM Tris, 190 mM glycine and 20% methanol). Membranes were blocked with 5% non-fat milk dissolved in Tris-buffered saline with 0.1 % Tween 20 (TBST) for 1 hour at room temperature. Membranes were incubated with primary antibody at 4 °C overnight. After membrane washing by TBST, the membranes were incubated with an IRDye-conjugated IgG (LI-COR Biosciences, Lincoln, NE) secondary antibody diluted 1:3000 for 1 hour. The intensity of the blot bands was quantified using an image processing program (LI-COR Biosciences, Lincoln, NE). The primary antibodies are shown in the Major Resources Table in the Supplemental Materials.

Tyrosine phosphorylation of IRS1

The tyrosine phosphorylation of IRS1 was measured as described previously[2]. Bovine aortic endothelial cells (BAECs) were infected with human IRS1 plasmid (CloneID: OHu24973, Genscript). Four hours later, cells were infected with Ad-LacZ or Ad-TFEB (10 MOI). Forty-eight hours later, cells were collected with RIPA lysis buffer (89901, Thermo Fisher Scientific, Waltham, MA) with the addition of protease inhibitor (11873580001, Roche, Penzberg, Germany) and phosphatase inhibitor (4906845001, Roche, Penzberg, Germany) cocktails. The samples were immunoprecipitated with IgG and IRS1 antibodies (3407, Cell Signaling Technology) and immobilized on magnetic beads (#70024, Cell Signaling Technology). The lysates (50 g of protein) or the precipitant were fractionated by SDS-PAGE and Western blot was performed as described above.

RNA isolation and Real-time PCR analysis

RNA was extracted using RNeasy Mini Kit (Cat# 74106, Qiagen) and reversely transcribed into cDNA using SuperScript III RT-PCR kit (18080051, Thermo Fisher Scientific) with random hexamers. qPCR was performed with iQ SYBR Green Supermix (1708880, Bio-Rad). Gene expression was normalized by the internal control 18S rRNA. The primer sequences are shown in the Major Resources Table in the Supplemental Materials.

For miRNA detection, RNA was extracted using miRNeasy Mini Kit (Cat# 217004, Qiagen) and reversely transcribed using TaqMan™ MicroRNA Reverse Transcription Kit (Cat# 4366596, Fisher scientific). miRNA expression was measured using TaqMan™ Universal PCR Master Mix (Cat# 4304437, Fisher Scientific). RNU6B and snoRNA202 were used as an internal control for human and mouse samples, respectively. The primers information is shown in the Major Resources Table in the Supplemental Materials.

Luciferase reporter assay

The IRS2 promoter fragment (500 bp) was synthesized by GENEWIZ company (South Plainfield, NJ) and cloned into the pGL4.10 vector (Cat# E6651, Promega). Hela cells were co-transfected with the IRS2 promoter-reporter plasmid (0.5 μg/well) and *Renilla* using Lipofectamine 2000 Reagent (Cat# 11668019, Thermo Fisher Scientific). Six hours later, Hela cells were infected with Ad-LacZ or Ad-TFEB (10 MOI). Forty-eight hours after infection, the

IRS2 promoter reporter activity was measured by Dual-Luciferase Reporter Assay System (Cat# E1960, Promega) in accordance with the manufacturer's protocol.

Glucose tolerance test (GTT) and Insulin tolerance test (ITT)

Mice on normal laboratory diet or HFD were administered glucose (2 g/kg, i.p.) or glucose (1 g/kg, i.p.), respectively, after overnight fasting. Blood glucose levels were measured at the indicated time points after glucose administration. Mice were administered human insulin (Humulin R, 1 U/kg, i.p.) after 6 hours fasting. Blood glucose levels were measured at the indicated time points after insulin administration.

Measurement of serum insulin

The blood samples collected from the tail vein at the indicated time points after glucose administration were centrifuged in Eppendorf tubes (5000 rpm) for 10 mins. The serum insulin levels were measured by Ultra-Sensitive Mouse Insulin ELISA Kit (90080, Crystal Chem) using mouse insulin as a standard.

Cycloheximide (CHX) chase assay

HCAECs were infected with Ad-LacZ or Ad-TFEB (10MOI) when cells were at 80% confluence. Twenty-four hours later, the cells were treated with 50 μM cycloheximide (14126, Cayman Chemical) at the indicated time points. IRS1 protein was determined by Western blot.

Glucose uptake *in vitro*

HCAECs were infected with Ad-LacZ or Ad-TFEB (10 MOI) when cells were at 80% confluence. Thirty-six hours later, the cells were treated with 50 μM LY294002 (70920, Cayman Chemical). After 12 hours, the cells were incubated with 1 mM 2-deoxyglucose for 40 mins. Glucose uptake was measured by a Glucose Uptake-Glo Assay kit (J1342, Promega) in accordance with the manufacturer's protocol.

Insulin signaling in the tissues

Mice were administered insulin (Humulin R, 5 U/kg, i.p.) after overnight fasting. The soleus and gastrocnemius muscle, liver and epididymal adipose tissue were harvested ten minutes after insulin injection. Protein samples from the same group were pooled and the Akt phosphorylation was determined by Western Blot.

Insulin transport across ECs *in vitro*

Insulin transport across ECs was determined as described previously[3]. HCAECs were seeded in the upper inserts of 12-well transwell plates (3402, costar). Seventy-two hours later, cells (100% confluence) were infected with Ad-LacZ (10 MOI) or Ad-TFEB (10 MOI). After forty-eight hours, fluorescein isothiocyanate-labeled insulin (FITC-insulin; I3661, Sigma-Aldrich; 100 nM) were added to the insert. After 20 minutes, the fluorescence intensity of FITC-insulin in the lower chamber was measured.

Measurement of Insulin uptake in ECs

HCAECs were infected with Ad-LacZ (10 MOI) or Ad-TFEB (10 MOI). After forty-eight hours, cells were incubated with FITC-insulin (100 nM) in the absence or presence of 100 nM insulin. Unlabeled insulin was used as a competitor for FITC-insulin. After 10 minutes, the fluorescence images were taken with a fluorescence microscopy and the fluorescence intensity was quantitatively analyzed.

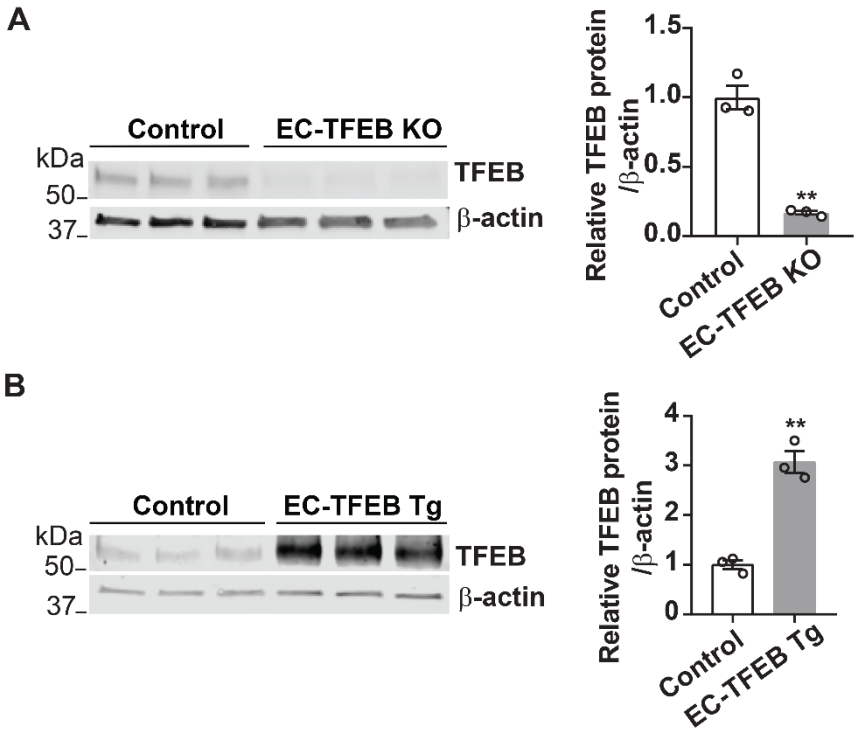
mRNA 3'UTR luciferase assay

The 3'UTR fragment (1 kb) of IRS1 mRNA was synthesized by GENEWIZ company (South Plainfield, NJ) and cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Cat# E1330, Promega). Hela cells were transfected with the IRS1 3'UTR-luciferase vector (0.1 μg/well) using Lipofectamine 2000 Reagent (11668019, Thermo Fisher Scientific). Six hours later, cells were infected with Ad-LacZ or Ad-TFEB (10 MOI). Forty-eight hours later, the luciferase reporter activity was measured by the dual-luciferase reporter assay system (E1960, Promega) in accordance with the manufacturer's protocol. Luciferase activity was normalized with respective *Renilla* activity.

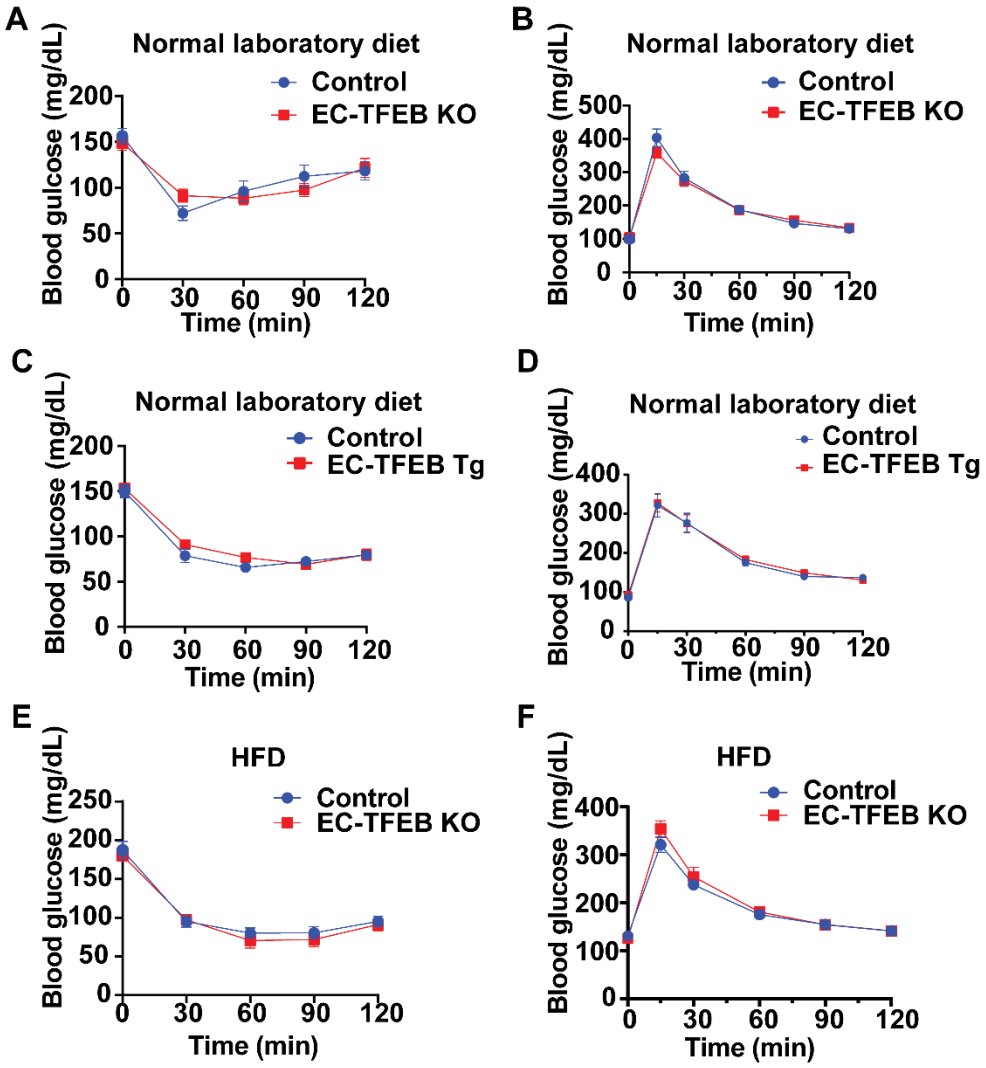
Dihydroethidium (DHE) staining and immunostaining

Gastrocnemius muscle samples were harvested from the left and right hind limbs of mice and small segments of muscle (~2 mm) were incubated with Krebs/HEPES buffer containing DHE (5 μM; Cat# D11347, Thermo Fisher Scientific) for 30 min at 37°C in the incubator[4]. Muscle samples were then embedded into optimal cutting temperature compound (23-730-571, Fisher Scientific) and cut to a 7-μm section in a Leica cryostat. The sections were fixed with 4% paraformaldehyde and blocked in 5% donkey serum for 1 hour at room temperature and then incubated with primary CD31 (# DIA-310, Dianova) at 4°C overnight. Rat IgG was used as a negative control (I-4000-1, Vector Laboratories). After washing with PBS, the sample was incubated with Alexa Fluor–labeled secondary antibody (712-547-003, Jackson ImmunoResearch Laboratories) at room temperature for 1 hour. Slides were mounted with ProLong™ Gold Antifade Mountant with DAPI (P36935, Thermo Fisher Scientific). DHE produces a red fluorescence when oxidized to ethidium bromide by superoxide anions. Images were taken with an Olympus IX73 microscope. Quantification of the mean DHE fluorescence intensity was performed with Image J. This process included 6 muscle specimens from 3 mice for each group and 4 randomly selected 20x objective fields for each specimen.

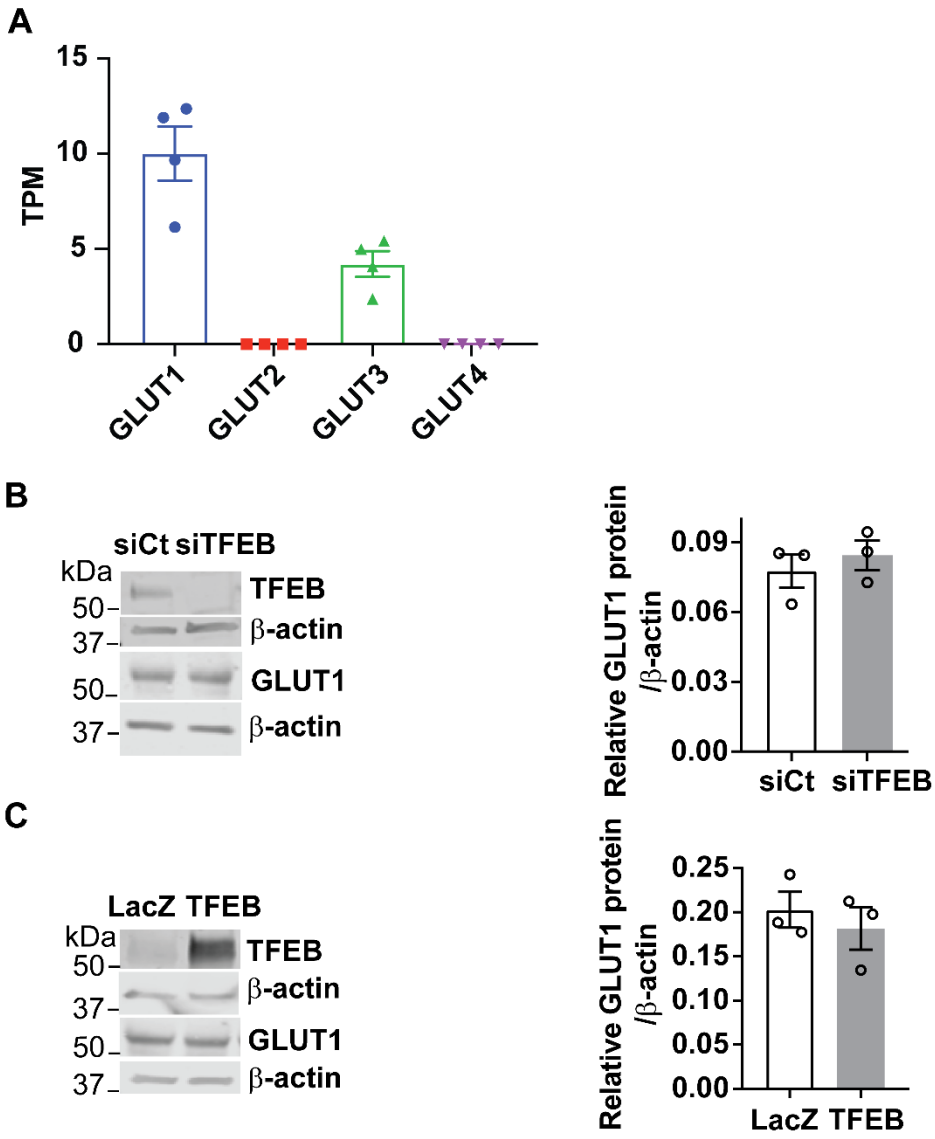
Supplemental Figures



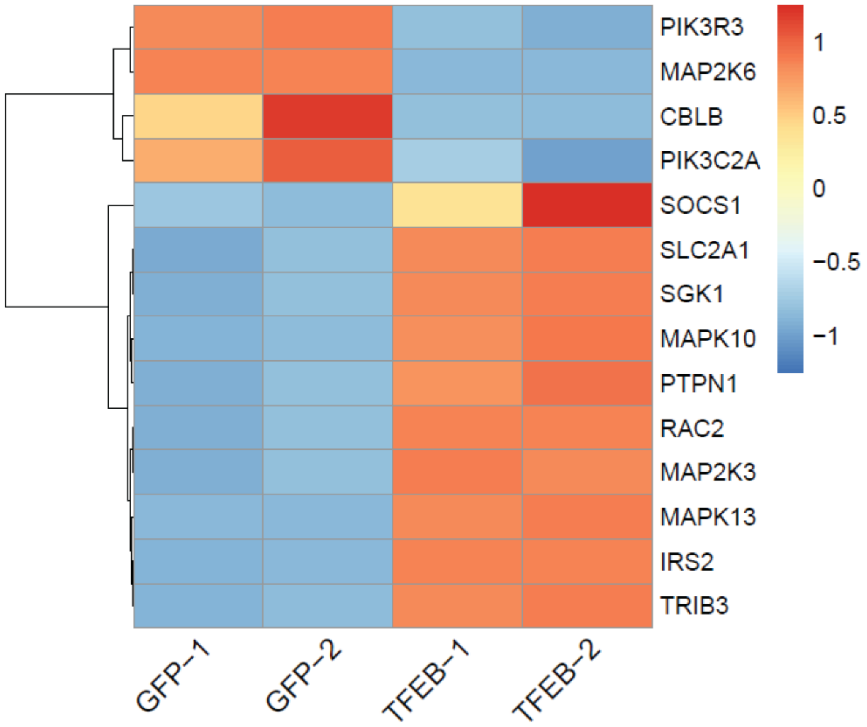
Supplemental Figure I. Determination of TFEB expression in ECs from genetically engineered EC-TFEB transgenic mice *in vivo*. A-B, Mouse lung ECs from EC-TFEB KO mice (**A**), EC-TFEB Tg mice (**B**) and their respective control mice were isolated by two rounds of cell sorting. TFEB protein was determined by Western blot. N = 3/each group. Data in **A-B** are presented as mean ± SEM; ***P* <0.01 using unpaired Student's t-test.



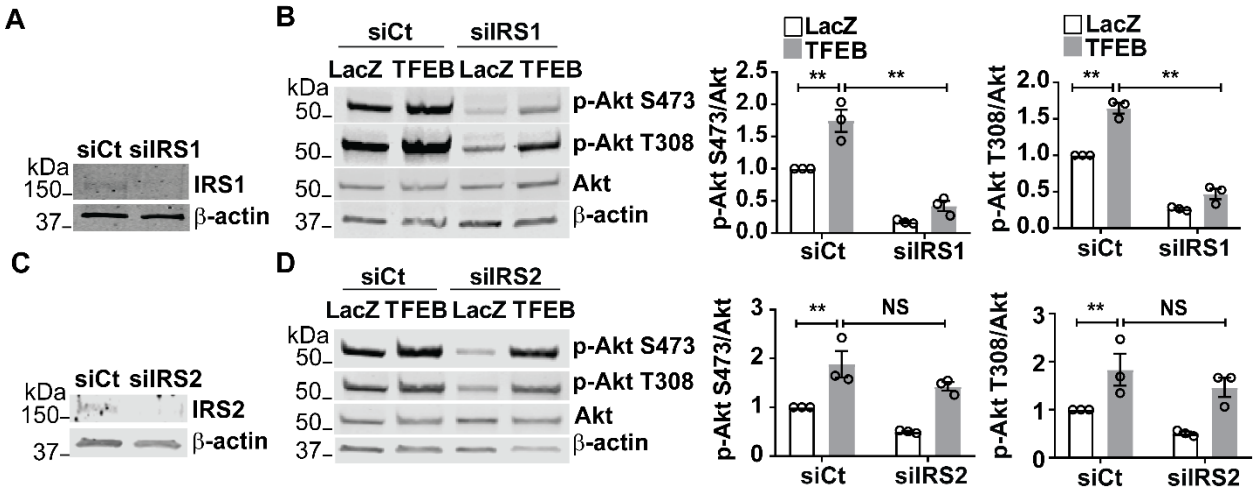
Supplemental Figure II. No changes were observed in blood glucose in male EC-TFEB genetically engineered transgenic mice on normal laboratory diet and female mice on high-fat diet (HFD). A-D, Male EC-TFEB knockout (KO) mice, EC-TFEB transgenic (Tg) mice and respective control mice were fed a normal laboratory diet for 16 weeks. Insulin tolerance test (ITT, insulin 1 U/kg, i.p.) and glucose tolerance test (GTT, glucose 2 g/kg, i.p.) were performed in the mice after overnight fasting. **A**, ITT in EC-TFEB KO and control mice (n = 7-9/each group). **B**, GTT in EC-TFEB KO and control mice (n = 7-9/each group). **C**, ITT in EC-TFEB Tg and control mice (n = 7-10/each group). **D**, GTT in EC-TFEB Tg and control mice (n = 11/each group). **E-F**, Female mice were fed an HFD for 26 weeks. ITT (insulin 1 U/kg, i.p.) and GTT (glucose 1 g/kg, i.p.) were performed in the mice after overnight fasting. **E**, ITT in female EC-TFEB KO and control mice on HFD (n = 9/each group). **F**, GTT in female EC-TFEB KO and control mice on HFD (n = 9/each group). Data in **A-F** are presented as mean ± SEM; no significance was found using 2-way ANOVA followed by Bonferroni test.



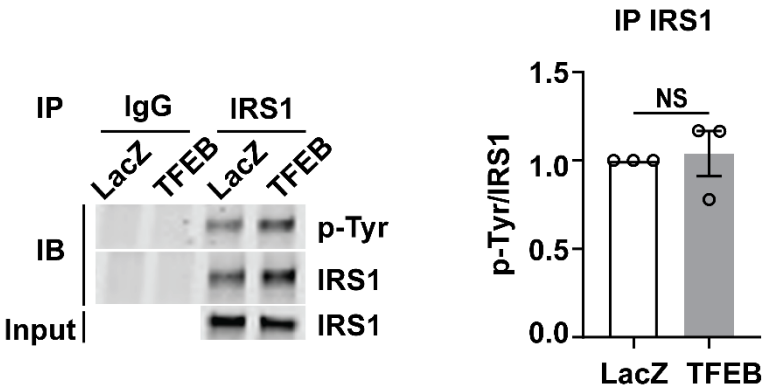
Supplemental Figure III. The effect of TFEB on GLUT1 expression. **A**, RNA sequencing data in human coronary artery endothelial cells (HCAECs) were obtained from the Gene Expression Omnibus (GEO, accession number: GSE124522). Transcripts Per Million (TPM) was used to evaluate the mRNA abundance of glucose transporters in ECs (n=4/each group). **B**, HCAECs were transfected with siRNA-control (siCt, 25 nM) or siRNA-TFEB (siTFEB, 25 nM) for 48 hours, GLUT1 and TFEB protein were measured by Western blot. **C**, HCAECs were infected with adenovirus encoding LacZ (Ad-LacZ, 10 MOI) or TFEB (Ad-TFEB, 10 MOI). Forty-eight hours later, GLUT1 and TFEB proteins were assessed by Western blot. Data are presented as mean ± SEM; no significance was found using unpaired Student's t-test in **B** and **C**.



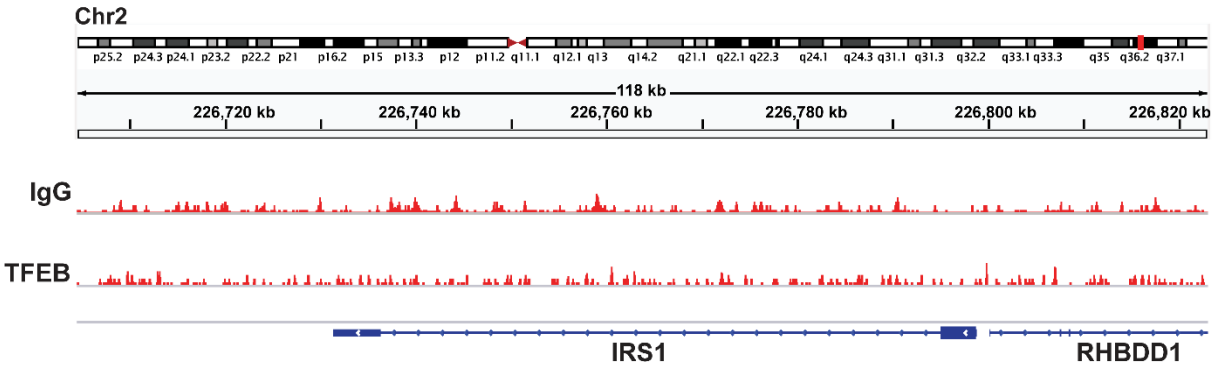
Supplemental Figure IV. Microarray analysis of AKT serine/threonine kinase (Akt)-related genes in human umbilical vein endothelial cells (HUVECs) overexpressing TFEB. Microarray data were obtained from the Gene Expression Omnibus (GEO, accession number GSE108384. HUVECs were infected with Adenovirus encoding green fluorescence protein (Ad-GFP) or Ad-TFEB (10 MOI). Twenty-four hours later, RNA was extracted for microarray analysis and Akt signaling related genes were further analyzed. Color bar [red to blue (1 to -1) vertical bar] represents the Z score of gene expression across samples.



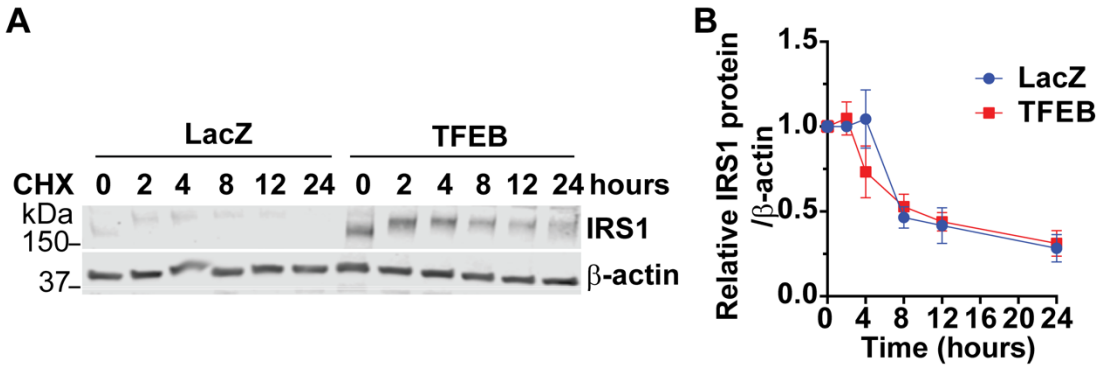
Supplemental Figure V. Insulin receptor substrate 1 (IRS1) knockdown partially blocks TFEB-activation of AKT serine/threonine kinase (Akt). A-B, Human coronary artery endothelial cells (HCAECs) were transfected with siCt (25 nM) or siIRS1 (25 nM) for 24 hours. **C-D**, HCAECs were transfected with siCt (25 nM) or siIRS2 (25 nM) for 24 hours. The cells were then infected with Ad-LacZ (10 MOI) or Ad-TFEB (10 MOI) for 48 hours. **A** and **C**, the knockdown efficiency of IRS1 and Insulin receptor substrate 2 (IRS2) was assessed by Western blot. **B** and **D**, the phosphorylation of Akt was determined by Western blot and quantitatively analyzed from three independent experiments. Data in **B and D** are presented as mean ± SEM; **P* <0.05, ***P* <0.01 using 2-way ANOVA followed by Bonferroni test.



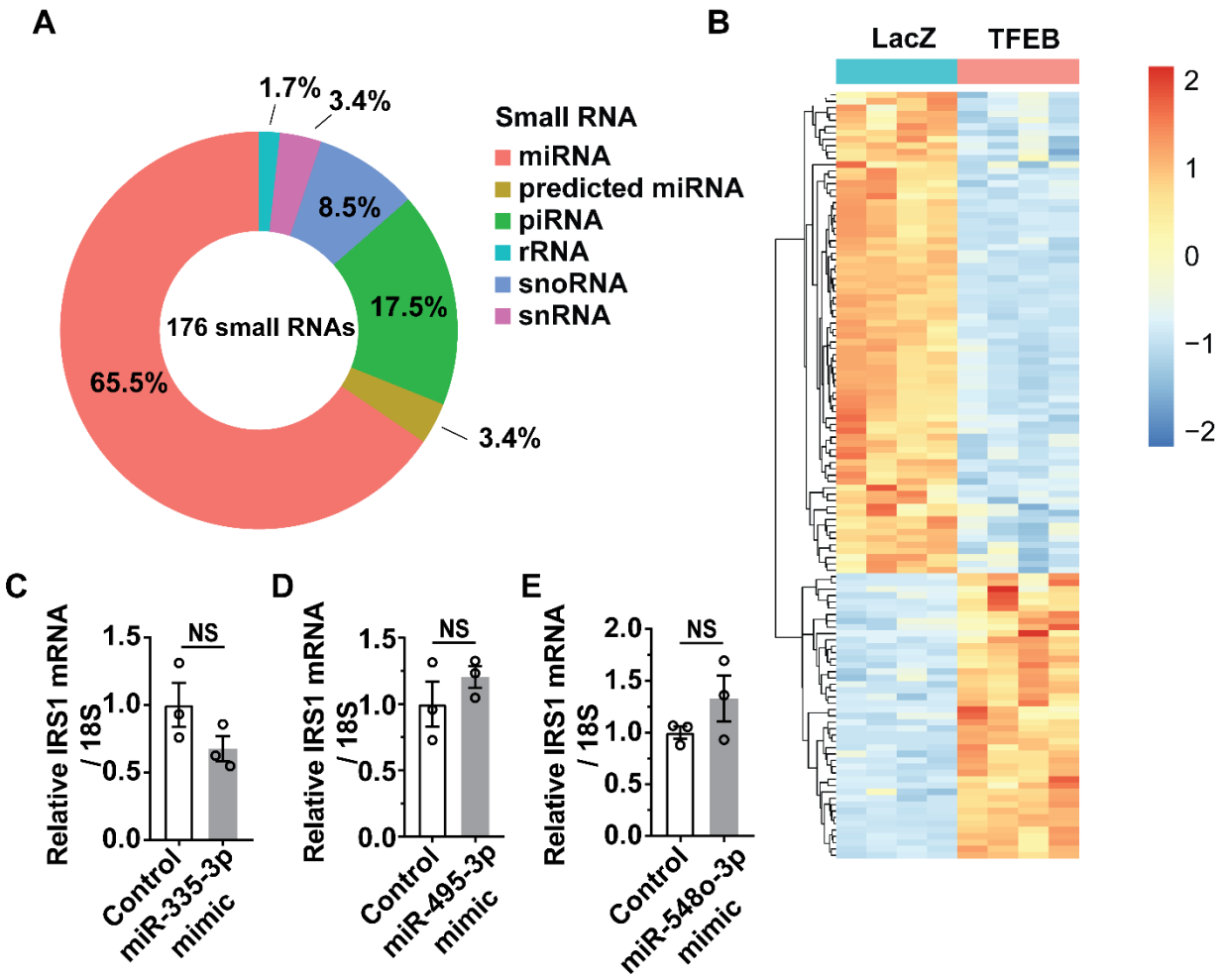
Supplemental Figure VI. Tyrosine phosphorylation of IRS1 in ECs overexpressing TFEB. Bovine aortic endothelial cells (BAECs) were transfected with human IRS1 plasmid. Four hours later, cells were infected with Ad-LacZ or Ad-TFEB (10 MOI). After 48 hours, lysates were immunoprecipitated (IP) with IgG control or IRS1 antibody and then immunoblotted for pan-tyrosine phosphorylation. The band intensity was quantitatively analyzed from three independent experiments (right panel). Data are presented as mean ± SEM; no significance was found using unpaired Student's t-test.



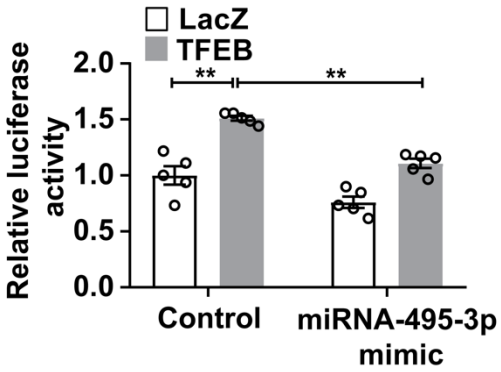
Supplemental Figure VII. No TFEB binding site was found in insulin receptor substrate 1 (IRS1) promoter region. Chromatin immunoprecipitation sequencing (ChIP-seq) data were obtained from the Gene Expression Omnibus (GEO, accession number GSE88896) and analyzed for TFEB binding peaks in the promoter of human IRS1 gene.



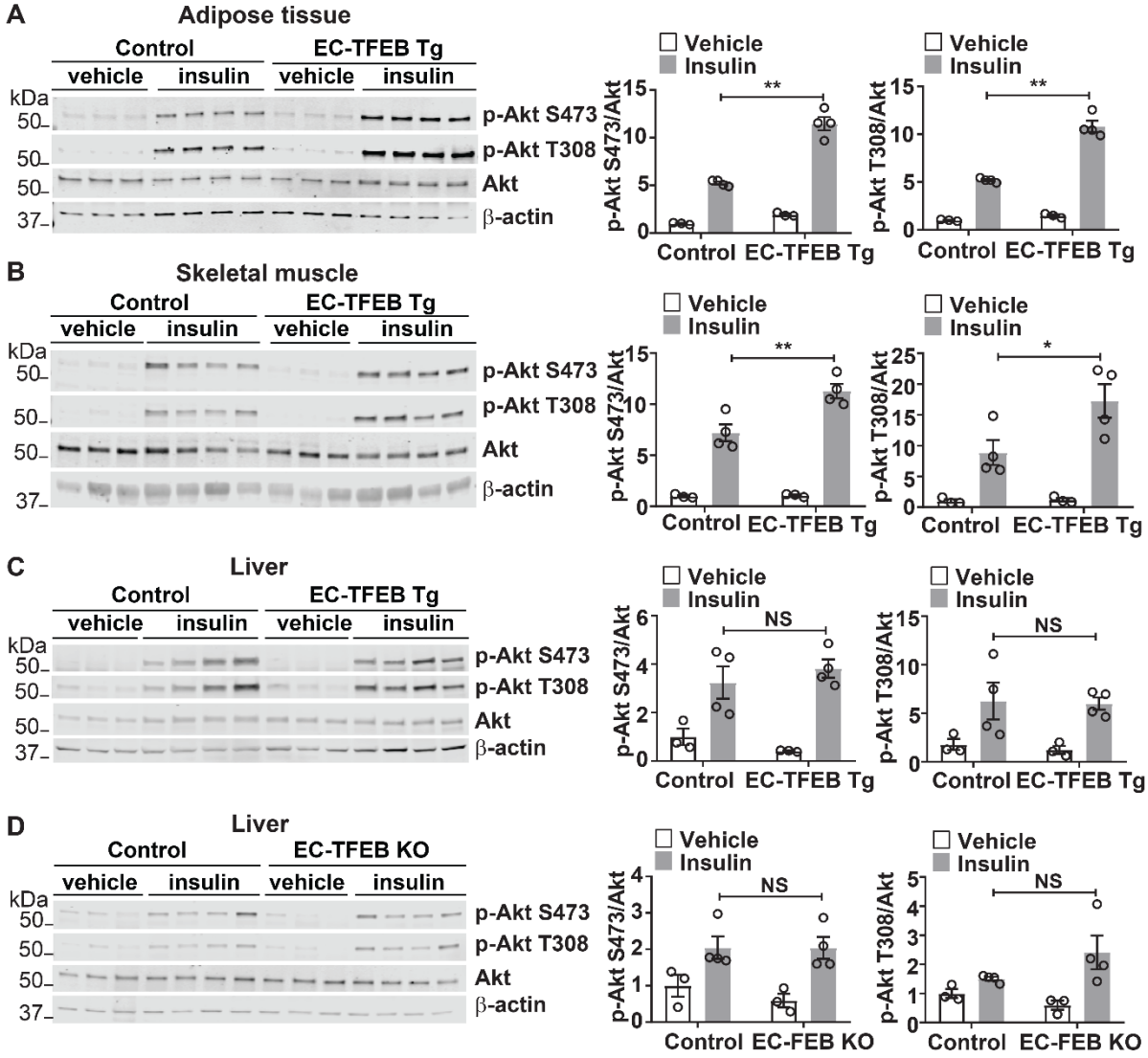
Supplemental Figure VIII. Transcription factor EB (TFEB) has no effect on insulin receptor substrate 1 (IRS1) protein stability in endothelial cells (ECs). A-B, Human coronary artery endothelial cells (HCAECs) were infected with Ad-LacZ (10 MOI) or Ad-TFEB (10 MOI). Twenty-four hours later, the cells were treated with cycloheximide (CHX; 50 μM) for the indicated time. IRS1 protein was determined by Western blot and quantitatively analyzed from three independent experiments. Data are presented as mean ± SEM; no significance was found using 2-way ANOVA followed by Bonferroni test.



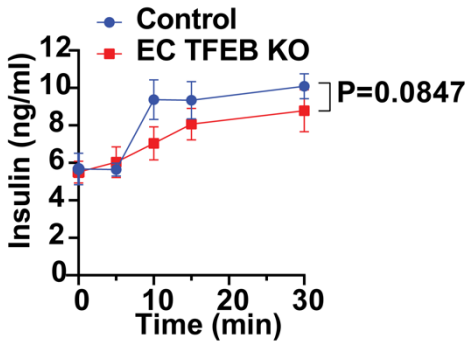
Supplemental Figure IX. The microRNA (miRNA) profile is regulated by transcription factor EB (TFEB) in endothelial cells (ECs). A, Pie chart depicting the frequency of small RNAs regulated by TFEB in human coronary artery endothelial cells (HCAECs). **B**, Heat map and hierarchical clustering of miRNAs regulated by TFEB in HCAECs. **C-E**, HCAECs were transfected with miR-335-3p mimic, miR-495-3p mimic or miR-548o-3p mimic (30 nM for each miRNA) for 72 hours. The mRNA abundance of insulin receptor substrate 1 (IRS1) was measured by qRT-PCR from three independent experiments. Data are presented as mean ± SEM; NS: no significance was found using unpaired Student's t-test.



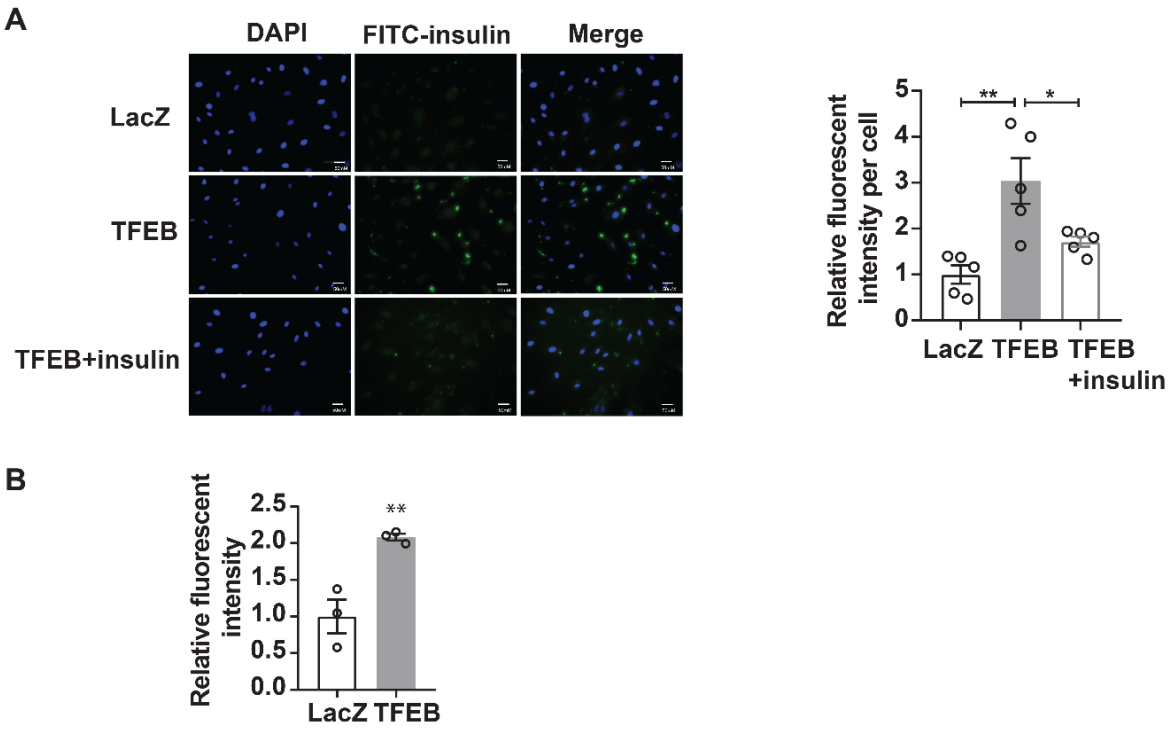
Supplemental Figure X. miR-495-3p mimic antagonizes the TFEB-induced IRS1 3'UTR luciferase activity. Hela cells were transfected with pmirGLO Dual-Luciferase miRNA reporter containing a 1 kb fragment of IRS1 mRNA 3'UTR (0~1kb) for 6 hours, and then the cells were infected with Ad-LacZ (10 MOI) and Ad-TFEB (10 MOI). Forty-eight hours later, the luciferase activity was measured and normalized with *Renilla* activity (n = 5/each group). Data are presented as mean ± SEM; ***P* <0.01 using 2-way ANOVA followed by Bonferroni test.



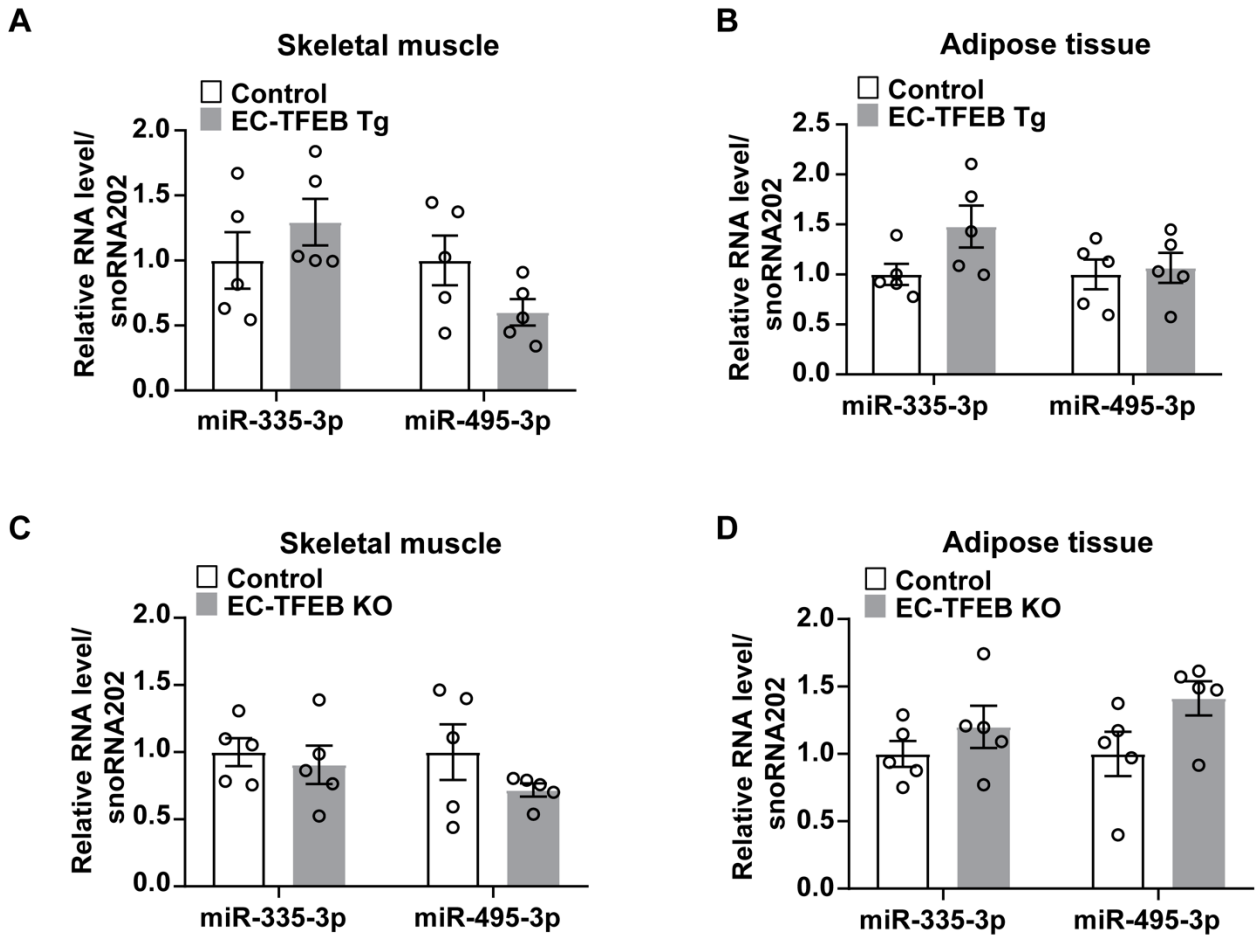
Supplemental Figure XI. The endothelial transcription factor (TFEB) transgene consistently activates AKT serine/threonine kinase 1 (Akt) signaling in the skeletal muscle and adipose tissue, but not in the liver. Male EC-TFEB Tg mice and EC-TFEB KO mice on high-fat diet (HFD) were administered insulin (5 U/kg, i.p.) after overnight fasting. Ten minutes after insulin injection, tissues were harvested for the analysis of Akt phosphorylation by Western blot. **A**-**B**, The phosphorylation of Akt in the adipose tissue (**A**) and skeletal muscle (**B**) from EC-TFEB Tg mice on HFD for 20 weeks (n = 3-4/each group). **C**-**D**, The phosphorylation of Akt in the liver from EC-TFEB Tg mice on HFD for 20 weeks (**C**) and EC-TFEB KO on HFD for 26 weeks (**D**). N = 3-4/each group. Data in **A-D** are presented as mean ± SEM; NS: no significant, **P* <0.05, ***P* <0.01 using 2-way ANOVA followed by Bonferroni test.



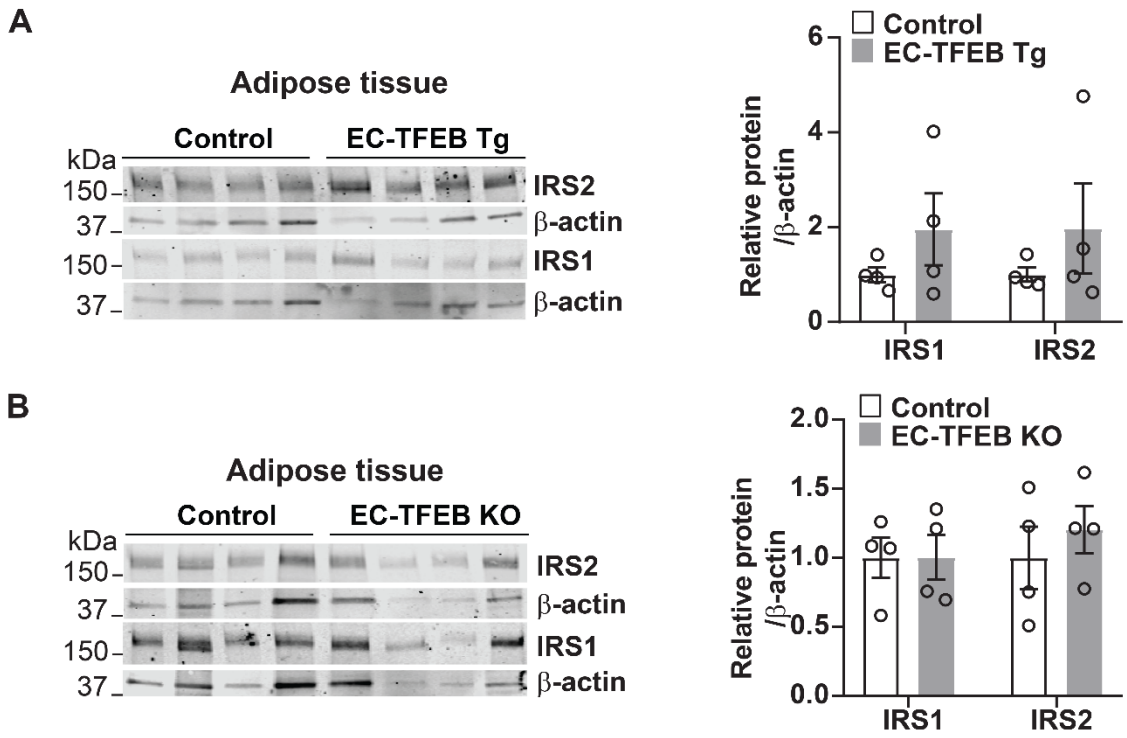
Supplemental Figure XII. No changes in serum insulin concentration in EC-TFEB KO mice. Insulin concentration was measured in male EC-TFEB KO mice (high-fat diet for 26 weeks) at the indicated time points after glucose injection (1 g/kg, i.p.). N = 7/each group. Data are presented as mean ± SEM; no significance using 2-way ANOVA followed by Bonferroni test.



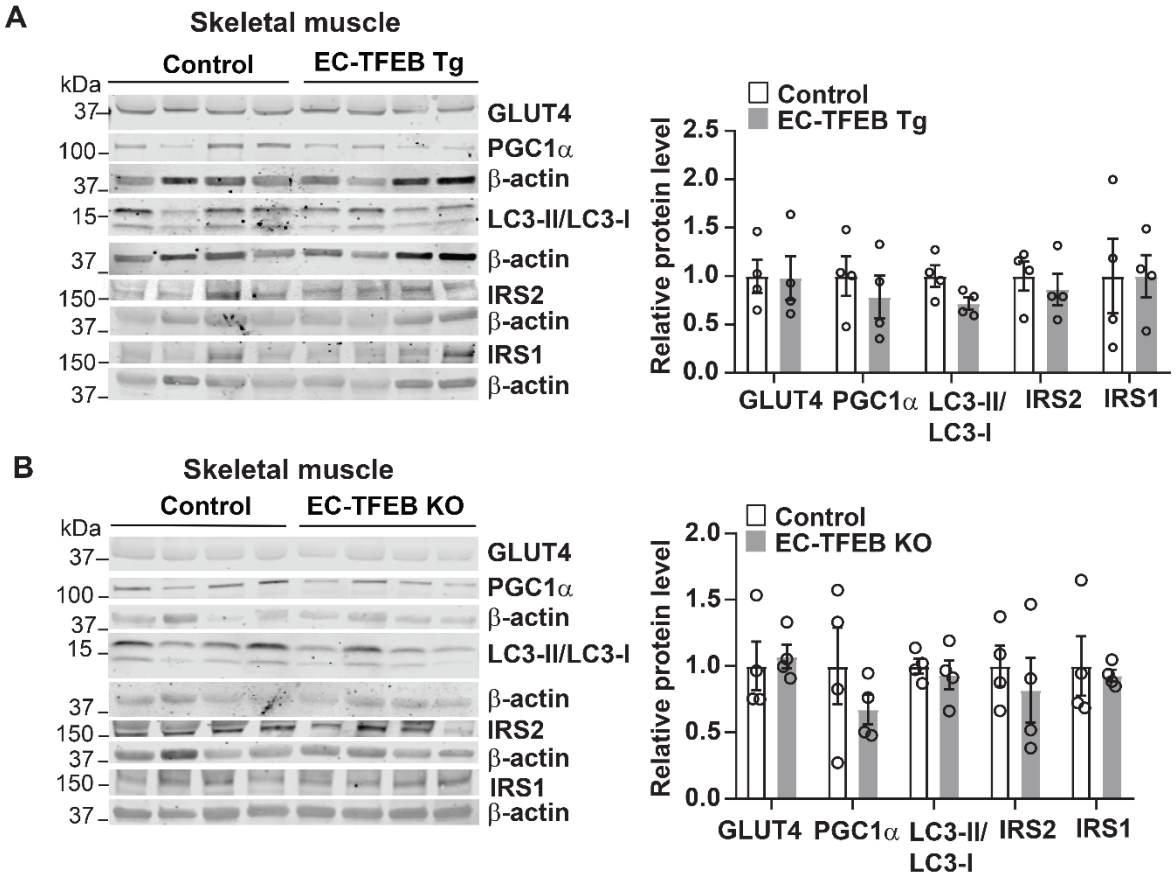
Supplemental Figure XIII. TFEB promotes insulin uptake and transport in ECs. A, HCAECs were infected with Ad-LacZ (10 MOI) or Ad-TFEB (10 MOI). After 48 hours, cells were incubated with fluorescein isothiocyanate-labeled insulin (FITC-insulin,100 nM) in the absence or presence of 100 nM insulin. Native insulin was used as a competitor for FITC-insulin. After 10 minutes, the fluorescence images were randomly taken with microscopy and the fluorescence intensity was quantitatively analyzed at right panel, n=5/each group. **B**, HCAECs were seeded in the upper inserts of transwell plates. Seventy-two hours later, cells were infected with Ad-LacZ (10 MOI) or Ad-TFEB (10 MOI). After forty-eight hours, FITC-insulin (100 nM) were added to the inserts. After 20 minutes, the fluorescence in the medium of lower chamber was measured (n=3/each group). Data are presented as mean ± SEM; **P* <0.05, ***P* <0.01 using one-way ANOVA followed by Bonferroni test in **A** and unpaired Student's t-test in **B**.



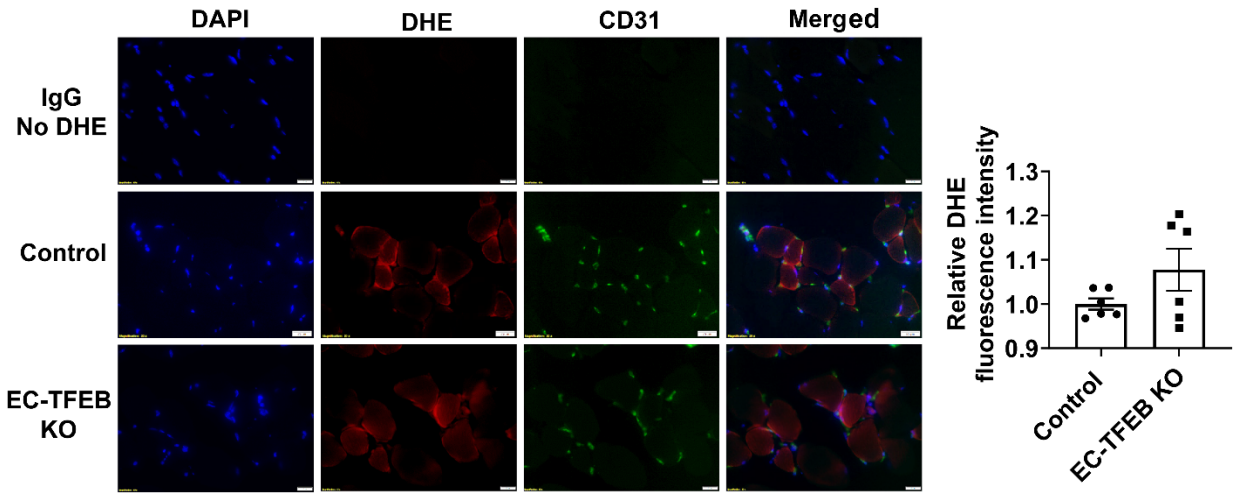
Supplemental Figure XIV. miRNAs expression in skeletal muscle and epididymal adipose tissue from male EC-TFEB transgenic mice on HFD. Tissues were harvested after overnight fasting of mice, and miRNAs were analyzed by qPCR. **A**-**B**, miR-335-3p and miR-495-3p in the skeletal muscle (**A**) and adipose tissue (**B**) from EC-TFEB Tg mice on HFD for 20 weeks (n = 5/each group). **C**-**D**, miR-335-3p and miR-495-3p in the skeletal muscle (**C**) and adipose tissue (**D**) from EC-TFEB KO mice on HFD for 26 weeks (n = 5/each group). Data in **A-D** are presented as mean ± SEM; no significant differences were found using unpaired Student's t-test.



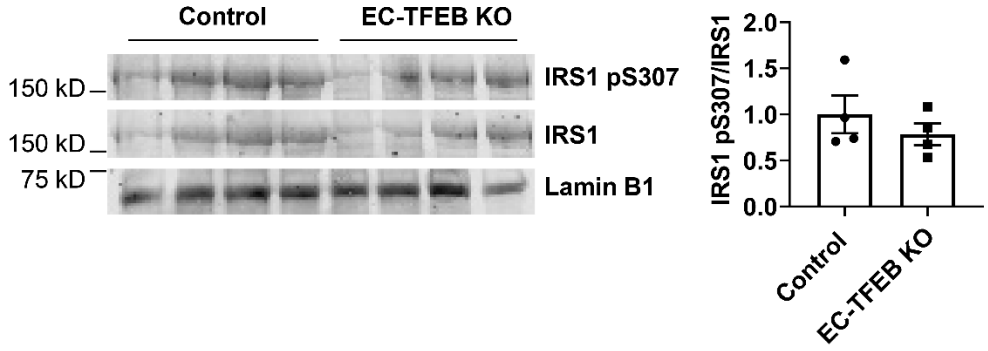
Supplemental Figure XV. IRS1 and IRS2 expression in epididymal adipose tissue of male EC-TFEB transgenic mice on HFD. A-B, Tissues of male EC-TFEB Tg mice and EC-TFEB KO mice on high-fat diet (HFD) were harvested after overnight fasting, for the analysis of IRS1 and IRS2 by Western blot. **A-B**, IRS1 and IRS2 in adipose tissues from EC-TFEB Tg mice on HFD for 20 weeks (**A,** n = 4/each group) and from EC-TFEB KO mice on HFD for 26 weeks (**B**, n = 4/each group). N = 4/each group. Data in **A-B** are presented as mean ± SEM; no significance was found using unpaired Student's t-test.



Supplemental Figure XVI. Gene expression in skeletal muscle of male EC-TFEB transgenic mice on HFD. Tissues from male EC-TFEB Tg mice and EC-TFEB KO mice were harvested after overnight fasting of mice. The expression of IRS1, IRS2, GLUT4, PGC1α and LC3 was determined by Western blot. **A**-**B**, Protein expression in skeletal muscle from EC-TFEB Tg mice on HFD for 20 weeks (**A**, n = 4/each group) and from EC-TFEB KO mice on HFD for 26 weeks (**B**, n = 4/each group). Data in **A-B** are presented as mean ± SEM; no significance was found using unpaired Student's t-test.



Supplemental Figure XVII. ROS levels in skeletal muscle of EC-TFEB KO mice on HFD. Skeletal muscle from male EC-TFEB KO mice and control mice was harvested for dihydroethidium (DHE) staining and CD31 immunostaining. Expression of CD31 (Alexa 488, displayed in green) and DHE (displayed in red) in mouse skeletal muscle were visualized by immunofluorescence staining. Rat IgG was used as a negative control. Scale bars=20 μm. Quantification of mean DHE fluorescence intensity was performed from 6 gastrocnemius muscle samples/group (n=3 mice/group), randomly selecting 4 different microscopic fields from each specimen. Data are presented as mean ± SEM; no significance was found using unpaired Student's t-test. DAPI indicates 4',6-diamidino-2-phenylindole.



Supplemental Figure XVIII. IRS1 phosphorylation at Ser307 in skeletal muscle of male EC-TFEB KO mice on HFD. After fasting overnight, the mice were administered insulin (5 U/kg, i.p.). Ten minutes later, skeletal muscle samples were harvested for the analysis of phosphorylation of IRS1. The phospho-IRS1 (Ser307) was determined by Western blot and quantitatively analyzed (n=4 mice/each group). Data are presented as mean ± SEM; no significance was found using unpaired Student's t-test.

Major Resources Table

Animals (*in vivo* studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Floxed-*Tfeb* mice	C57BL/6N-Atm1Brd/a *Tfeb*$^{tm1a(EUCOMM)Wtsi}$/BcmMmucd mice were produced at BCM from ES cells provided by the Wellcome Trust Sanger institute	C57BL/6N	Male/female	https://www.mmrrc.org/catalog/sds.php?mmrrc_id=41155
Ve-Cadherin-Cre mice	The Jackson Laboratory	C57BL/6J	Male/female	https://www.jax.org/strain/006137
Endothelial cell-selective *Tfeb* knockout mice	Generated by crossbreeding floxed-*Tfeb* mice with *Ve-Cadherin*-Cre mice	C57BL/6N	Male/female	
Endothelial cell-selective *TFEB* transgenic mice	Generated with an *mTie2* promoter–driven human *TFEB* coding region	C57BL/6J	Male	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
β-actin	Cell Signaling Technology	3700S	0.84 μg/ml
β-actin	Cell Signaling Technology	4967S	0.009 μg/ml
IRS1	Cell Signaling Technology	3407S	0.55 μg/ml
IRS2	Cell Signaling Technology	4502S	0.029 μg/ml
Phospho-IRS1 (Ser307)	Cell Signaling Technology	2381S	0.003 μg/ml
Akt	Cell Signaling Technology	9272S	0.034 μg/ml
Phospho-Akt (Ser473)	Cell Signaling Technology	4060S	0.090 μg/ml
Phospho-Akt (Thr308)	Cell Signaling Technology	13038S	1.06 μg/ml
Flag (DYKDDDDK Tag)	Cell Signaling Technology	14793S	0.141 μg/ml
Lamin B1	Cell Signaling Technology	#12586	0.691 μg/ml
LC3	Cell Signaling Technology	4108S	0.0236 μg/ml
GLUT1	Abcam	ab652	1-10 μg/ml
TFEB	Bethyl Laboratories	A303-673A	1 μg/ml
PGC1 alpha	Abcam	ab54481	0.5 μg/ml

GLUT4	Abcam	ab654	1-10 μg/ml
CD31	Dianova	DIA-310	2 μg/ml
Alexa Fluor® 488 AffiniPure Fab Fragment Donkey Anti-Rat IgG (H+L)	Jackson ImmunoResearch	712-547-003	1 μg/ml
Rat IgG	Vector Laboratories	I-4000-1	2 μg/ml

Cultured Cells

Name	Vendor or Source	lot number	Sex (F, M, or unknown)	Catalog #
Human Coronary Artery Endothelial Cells (HCAECs)	Lonza	19TL210280	46-year old male donor	Cat# CC-2585
Human Coronary Artery Endothelial Cells (HCAECs)	Lonza	0000626780	37-year old male donor	Cat# CC-2585

Quantitative Real-time PCR for microRNAs

Name	Vendor or Source	Catalog #
Taqman microRNA expression assay: miR-128-3p	Thermo Fisher Scientific	Assay ID 002216
Taqman microRNA expression assay: miR-335-3p	Thermo Fisher Scientific	Assay ID 002185
Taqman microRNA expression assay: miR-495-3p	Thermo Fisher Scientific	Assay ID 001663
Taqman microRNA expression assay: miR-548o-3p	Thermo Fisher Scientific	Assay ID 245470_mat
Taqman microRNA expression assay: miR-216a-3p	Thermo Fisher Scientific	Assay ID 475580_mat
Taqman microRNA expression assay: miR-126-3p	Thermo Fisher Scientific	Assay ID 002228
Taqman microRNA expression assay: miR-15b-5p	Thermo Fisher Scientific	Assay ID 000390
Taqman microRNA expression assay: miR-30a-5p	Thermo Fisher Scientific	Assay ID 000417

Taqman microRNA expression assay: miR-186-5p	Thermo Fisher Scientific	Assay ID 002285
Taqman microRNA expression assay: RNU6B	Thermo Fisher Scientific	Assay ID 001093
Taqman microRNA expression assay: snoRNA202	Thermo Fisher Scientific	Assay ID 001232

Primers used for Real-time PCR and ChIP

Gene	Primer sequence
TFEB	Forward:gcggcagaagaaagacaatc
	Reverse: ctgcatcctccggatgtaat
IRS1	Forward: cccaggacccgcattcaaa
	Reverse: ggcggtagataccaatcaggt
IRS2	Forward: gccaccatcgtgaaagagtg
	Reverse: ccatccgggaacaagggaaa
GLUT1	Forward: ggccaagagtgtgctaaagaa
	Reverse: acagcgttgatgccagacag
18S rRNA	Forward: catggccgttcttagttggt
	Reverse: cgctgagccagtcagtgtag
IRS2 ChIP	Forward: cgcacagtgagtaacacatcg
	Reverse: gctgtgtgtgcctgcgta

Data & Code Availability

Description	**Source / Repository**	**Persistent ID / URL**
Small RNA-Seq analysis of TFEB regulated miRNAs in endothelial cells	GEO, accession number: GSE148026	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148026
ChIP-seq TFEB and IgG in HUVEC cells	GEO, accession number: GSE88894	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE88894
Microarray analysis of TFEB-regulated genes in endothelial cells	GEO, accession number: GSE108384	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108384
RNA-Seq analysis of human coronory endothelial cells in response to nitro-conjugated linoleic acid	GEO, accession number: GSE124522	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124522
RNA-Seq analysis of human coronory endothelial cells in response to nitro-conjugated linoleic acid	GEO, accession number: GSE124522	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124522

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