SUPPLEMENTAL MATERIAL

Supplemental Methods

Metabolic phenotyping

Body composition measurement, glucose tolerance test (GTT) and insulin tolerance test (ITT), and indirect calorimetry were performed by Comprehensive Metabolic Phenotyping Core (CMP) of City of Hope. Body composition was measured using magnetic resonance imaging (EchoMRI, Houston, TX). Each mouse was scanned for 1-2 min without anesthesia. The scores representing the total fat and total lean mass in grams were further calculated to compare the difference between different genotypes.

Mice receiving GTT and ITT were fasted for 5 hours prior to the procedure, with free access to water. Glucose (0.3 g/ml) or insulin (0.5 IU insulin/kg body weight, Humulin R U-100, Lilly) was injected into the peritoneal cavity based on the body weight (1.5 g glucose/kg body weight). Tail vein blood was drawn at 15, 30, 60, 90, and 120 mins after the injection for blood glucose measurement by using a FreeStyle Freedom Lite glucometer (Abbot Diabetes Care, Inc., Alameda, CA). The area under curve (AUC) was drawn and compared between different genotypes.

The OxyMax machine was kept at 30°C, and the oxygen sensor was heated for at least 6 hours before calibration with the reference gases: 100% nitrogen gas and a mixture of 20.5% O_2 and 0.5% CO_2. For the whole-body energy metabolism, CO_2 and O_2 levels were measured, and the data were collected every 25 mins for each mouse.

Cell culture, transfection, and treatment

Human dermal microvascular endothelial cells (HMVECs) and bovine aortic endothelial cells (BAECs) were purchased and authenticated for cell identity and negativity for mycoplasma contamination by Cell Applications Inc. HMVEC were cultured in HMVEC growth medium (Cell Applications Inc.) and BAECs in DMEM supplemented with 10% FBS under standard cell culture conditions (humidified atmosphere, 5% CO_2, 37°C). HUVECs at passages 5–8 were used for the *in vitro* studies. The normoxic cells were kept at 37°C ventilated with 5% CO_2 and atmospheric 21% oxygen. The hypoxic cells were maintained in an incubator infused with 2% O_2, 5% CO_2, and 93% nitrogen. Cells were harvested at different time points (0, 12, 24 and 48 hours).

To knock down AGO1 in HMVECs, cells were transfected with scrambled control or siRNA targeting AGO1 (TAGGCTTAACATAAAGCCGAA) as previously validated[21] using Lipofectamine-RNAiMAX transfection reagent (Invitrogen) in Opti-MEM according to manufacturer's protocol. Six hours after transfection, Opti-MEM was replaced with the complete HMVEC growth medium, and the cells were incubated for 48-72 hours.

Murine brown preadipocytes were a generous gift from Dr. Jiandie Lin (University of Michigan, Ann Arbor). The preadipocytes were maintained in DMEM supplemented with 10% fetal bovine serum (FBS). To initiate differentiation, a cocktail containing 0.5 mM IBMX, 125 μM indomethacin, and 1 μM dexamethasone was added to pre-adipocytes maintained in DMEM supplemented with 10% FBS, 20 nM insulin and 1 nM 3, 3, 5-Triiodo-L-thyronine at confluency. Cells were switched to differentiation medium (DMEM, 10% FBS, 20 nM insulin and 1 nM T3) for another two days. Complete fresh differentiation media containing 2 μg/ml recombinant mouse TSP1 (R&D Systems, Inc., Minneapolis, MN, USA) was added into the differentiated adipocytes at confluency. PBS was added as the vehicle control. Forty-eight hours post-treatment, cells were harvested and used for subsequent experimentation.

Histology, immunohistochemistry, immunofluorescent staining, and flow cytometry

Histological examinations were mainly processed by Solid Tumor Pathology Core at City of Hope. Briefly, different depots of adipose tissues, kidney, lung, liver, skeletal muscle from mice were collected and fixed in 4% paraformaldehyde overnight. The fixed tissues were later dehydrated, sectioned into 4 μm paraffin slides, and were subjected to Haemotoxylin and Eosin (HE) staining, Periodic acid-Schiff (PAS), or Masson's trichrome staining. For immunohistochemistry, adipose tissues slides were stained with UCP1 (ab23841, Abcam, 1:200 dilution), CD31 (ab56299, Abcam, 1:200 dilution) or TSP1 (MA513377, Thermo Fisher, 1:200 dilution), followed by anti-Rabbit (SK262653, Invitrogen, 1:200 dilution) or anti-Mouse HRP-conjugated secondary antibody (TB262653, Invitrogen, 1:200 dilution). The staining was detected by DAB method (TA-125QHPX, Thermo Scientific) and the images were taken using Zeiss Observer II microscope.

For immunofluorescent (IF) staining, antibodies against CD31 (ab56299, Abcam, 1:200 dilution), TSP1 (MA513398, Invitrogen, 1:100), F4/80 (14-4801-82, Invitrogen, 1:200 dilution), and GFP (#2956, CST, 1:75 dilution) were used as primary antibodies. As secondary antibodies, Alexa Fluor 488-conjugated goat anti-mouse IgG/IgM (A10680, Invitrogen, 1:200 dilution), Alexa Fluor594-conjugated goat anti-rat IgG (A-11007, Invitrogen, 1:200 dilution), or Alexa Fluor 555-conjugated goat anti-rabbit IgG (A-11037, Invitrogen, 1:200 dilution) were used as appropriate. Nuclei were stained with DAPI (P36935, Invitrogen). IF images were taken using ZEISS Axio Observer.

For TSP1 flow cytometry, SAT was enzyme-digested with collagenase D and dispase II, and the cell suspension was adjusted to 1-5x10^6 cells/mL. The cells were then fixed with 2% paraformaldehyde, washed with PBS, and incubated with a primary TSP1 monoclonal antibody

(MA5-13398, Invitrogen, 1:100 dilution) for 60 min at room temperature. Cells were then incubated with a solution containing 2% BSA-PBS for 30 min at room temperature, and then incubated with a FITC 488-conjugated goat anti-mouse IgG (H+L) secondary antibody for 40 min at room temperature in the dark. Cells incubated with primary antibody only were used as control.

Immunoblotting

Adipose tissues or cells were lysed with protein extraction reagent (NP40 Cell Lysis Buffer; Thermo Fisher) in the presence of a protease inhibitor (Cat#8340; Sigma-Aldrich) and a phosphatase inhibitor cocktail (Cat# 5870; Cell Signaling). Tissue samples (total 30 μg) along with a protein marker were loaded on an 8% SDS-PAGE gel followed by transferring onto a PVDF membrane. The membranes were blocked with 5% skim milk and were incubated with primary antibodies at 1:1000 dilution in 3% BSA overnight at 4°C. The primary antibodies used were rabbit anti-phospho-AKT (4060S, Cell Signaling), rabbit anti-AKT (4691S, Cell Signaling), rabbit anti-phospho-AMPK (2535S, Cell Signaling), rabbit anti-AMPK (2532S, Cell Signaling), mouse anti-TSP1 (MA513377, Thermo Fisher), rabbit anti-UCP1 (AB23841, Abcam), rabbit anti-AGO1 (PA5-50654, Invitrogen) and rabbit anti-β-actin (8457S, Cell Signaling). The membranes were then incubated with anti-rabbit (7074S, Cell Signaling) or anti-mouse (7076S, Cell Signaling) HRP-conjugated secondary antibody at room temperature for 1 h and developed using ECL substrate (Cat# WBKLS0500, Millipore).

Individual nucleotide resolution Crosslinking Immunoprecipitation (iCLIP)

iCLIP-seq was performed as previously described with adaptation[24]. Briefly, human microvascular ECs (HMVECs) were UV-crosslinked (400 mJ/cm^2) and lysed with a buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, and protease inhibitor cocktail (Roche). Cell lysates were subjected to RNase I and

Turbo DNase (Invitrogen) followed by IP with mouse anti-human AGO1 (Wako Chemicals; clone 2A7) at 5 μg/500 μl total lysates. The IP-ed RNA was ligated to adaptor sequence at the 3'end for library preparation and labeled with radioactive phosphate at the 5'end for visualization. RNA-bound proteins were resolved by SDS-PAGE and transferred to nitrocellulose membrane for autoradiography. The portion of the nitrocellulose blot containing the desired size of ribonuclear protein was subjected to Proteinase K (NEB). The extracted RNA was reverse-transcribed using primers containing adaptor sequences and barcodes, followed by size selection, circularization, linearization, PCR amplification, and sequencing library preparation. AGO2-CLIP was done following a similar protocol but with anti-mouse AGO2 monoclonal antibody (018-22021, Wako Chemicals).

RNA isolation and RT-qPCR analyses

RNA was extracted from cells and tissues using TRIzol (Invitrogen) following the manufacturer's instructions. The total RNA was reverse transcribed (Cat# RR036A-1, Takara), and cDNAs were used for PCR and qPCR analyses using the primers listed in Supplemental Table I. Samples were stored at −20°C and subjected to qPCR using Biorad CFX96. Each qPCR sample was performed in triplicate, with Fast SYBR Green (BioRad). β-actin and 36B4 were used as the internal controls for the *in vitro* and *in vivo* assays, respectively. For miRNA quantification, TaqMan miRNA assay was performed following the manufacturer's protocol (Life Technologies). U6 was detected as the internal control for miRNA assays. To quantify let-7e and let-7k levels in serums, a *C. elegans* miRNA, i.e. cel-miR-39 was spiked in each sample at 2 nM and detected as the spike-in control.

RNA and small RNA-seq and sequencing data analysis

RNA was extracted and sequenced as we previously described[23]. For RNA-seq, STAR[51] was used to align raw sequencing data to hg38 genome and Kallisto[52] was used to quantify transcript abundance in Transcripts per Million (TPM) values. DESeq2[53] was then used to perform differentially expressed gene analysis with default parameters (adjusted P values < 0.05 were considered significant). For CLIP-seq, CLIPSeqTools[54] was used to preprocess the raw sequencing data. Sequences were aligned to the hg38 genome, and the transcript abundance was quantified in TPM value. To identify the hypoxia- altered AGO1 binding of transcripts altered by hypoxia, P values < 0.05 from Chi-square test were considered significant. GO enrichment analysis was performed through the Gene Ontology Consortium platform[55] and Benjamini-Hochberg corrected P values < 0.05 were considered significantly enriched pathways. Small RNA-seq was performed as we described previously[56] and miRDeep2[57] was used to process the sequencing data. The counts of miRNAs were then normalized to Reads Per Million (RPM). Student's t test was performed to compare miRNA levels between wild-type and EC-AGO1-KO littermates. miRNAs with Benjamini-Hochberg corrected P values < 0.05 were considered statistically significantly different.

3'UTR luciferase reporter assay

Sequences of specific 3'UTRs were selected based on iCLIP-seq chimeric read analysis and subcloned into the pMIR-REPORT vector (Ambion) to generate pMIR-Luc-THBS1-3'UTRs. These vectors were further co-transfected with Renilla luciferase-containing vector into BAECs and cells were exposed to normoxia or hypoxia for 24 hours. Luciferase activity was measured by Dual-Glo Luciferase Reporter Assay Kit (Promega), as previously described[21, 58]. The luciferase activity was normalized to that of background Renilla luciferase expression.

Enzyme-linked immunosorbent assay (ELISA)

Cellular fractions from tissue were collected using lysis buffer containing 1% NP-40. TSP1 ESILA was performed with an ELISA kit (CSB-E08765m, Cusabio), following the manufacturer's instructions.

Subcutaneous or systemic injection of adenovirus

Adenovirus (Ad) injection was performed as described[13] with 2×10^{11} purified adenovirus particles for control (Ad-GFP) or Ad-TSP1 (Cat# 1488, Vector Biolabs). Each SAT was injected at 3 spots per injection per week for 4 weeks. For systemic administration, the same amount of virus was injected through tail vein, once per week for 4 weeks.

Measurement of blood pressure and echocardiography

Blood pressure was measured using a noninvasive computerized tail-cuff system (Visitech, Apex, NC) as previously described[59]. After the mice were placed in a plastic holder, the occlusion and sensor cuff were positioned on the base of the tail. All the mice were given at least 1 week to adapt to the system prior to blood pressure measurement. Blood pressure was measured at least 20 times in each mouse.

Echocardiography was performed as previously described[60] following recently published guidelines[61]. Mice were anesthetized with 0.5% isoflurane and underwent echocardiography using VisualSonics, SonoSite FUJIFILM, Vevo 2100 ultrasound system with a linear transducer 32–55 MHz. The measurements of heart rate, left ventricular internal dimensions at end of diastole and systole (LVIDd, LVIDs, respectively), end-diastolic interventricular septal thickness (IVSd), and LV posterior wall thickness (LVPWd) were determined from the LV M-mode tracing. Percentage fractional shortening (%FS) was used as an indicator of systolic cardiac function.

Measurement of Urinary and serum creatinine

Twenty-four-hour urine samples were collected from metabolic cages with individually housed mice. Blood was drawn and serum was isolated. Creatinine was measured in urine and serum samples by using a Mouse Creatinine Assay Kit (80350, Crystal Chem) according to the manufacturer's instructions. The ratio of urinary creatinine and serum creatinine levels was calculated to yield the glomerular filtration rate (GFR).

Cold exposure and thermoneutral treatment

Eight-week-old male mice were housed at cold temperature (6°C) or thermoneutral temperature (30°C) under environmentally controlled conditions (12 h light/dark cycle, 55 ±15% humidity) with ad libitum access to food and water for 7 days.

Supplemental Table I. Sequences of primers used

Gene/Primer ID	Species	Sequence
AGO1 loxp-loxp	mouse	Forward TGTGCCACACACTAACTGTTCA Reverse CCTGCCTGGCACCTTTACT
CDH5-Cre	mouse	Forward CCAGGCTGACCAAGCTGAG Reverse CCTGGCGATCCCTGAACA
AGO1-P2	mouse	Forward CAGCAGGTGTTTCAGGCAC Reverse GGACACTTATCCGGCTTGATG
AGO1-P3	mouse	Forward TGGAGAGGGGAGCCACATATC Reverse GTGCGTAGAGTATCCTGGTGA
AGO1-P4	mouse	Forward CCGGCATCTCAAGAACACCT Reverse GGCAGAGGTTGGACAGAGTC
AGO1-P5	mouse	Forward GCTGCCCCAGATTCTTCACT Reverse GTCCCTGCTGGGATGTTACC
AGO1-P6	mouse	Forward GGAAGACGCCAGTGTATGCT Reverse GCTGGTGTGGGACTAGGATG
UCP1	mouse	Forward GATCCAAGGTGAAGGCCAGG Reverse GTTGACAAGCTTTCTGTGG
PGC1α	mouse	Forward CGGAAATCATATCCAACCAG Reverse TGAGAACCGCTAGCAAGTTTG
Cidea	mouse	Forward TGCTCTTCTGTATCGCCCAGT Reverse GCCGTGTTAAGGAATCTGCTG
VEGFA	mouse	Forward GCACATAGAGAGAATGAGCTTCC Reverse CTCCGCTCTGAACAAGGCT
THBS1	mouse	Forward TTCTTACCCTTGACAACAACGTG Reverse CCACAGATAGCTTGGAGGTCC
eNOS	mouse	Forward CACCTACGACACCCTCAGTG Reverse CTTGACCCAATAGCTGCTCAG
PPARγ	mouse	Forward CTCCGTGATGGAAGACCACT Reverse AACCATTGGGTCAGCTCTTG
36B4	mouse	Forward AGATTCGGGATATGCTGTTGGC Reverse TCGGGTCCTAGACCAGTGTTC
AGO2	mouse	Forward CGTCCTTCCCACTACCACG Reverse CCAGAGGTATGGCTTCCTTCA
AGO3	mouse	Forward GCCCAGCCCCTATTCATCG Reverse ACTCTTCGAGGACACTTGTCT
AGO4	mouse	Forward CCAATTCGACTGTTAGCCAATCA Reverse CGAGGCCGTTTTTCTGGTTTAAT
CD36	mouse	Forward ATGGGCTGTGATCGGAACTG Reverse TTTGCCACGTCATCTGGGTTT
CD47	mouse	Forward GTCATCCCTTGCATCGTCCG Reverse GGCATCGCGCTTATCCATTTT
VEGFR2	mouse	Forward TCCAGAATCCTCTTCCATGC

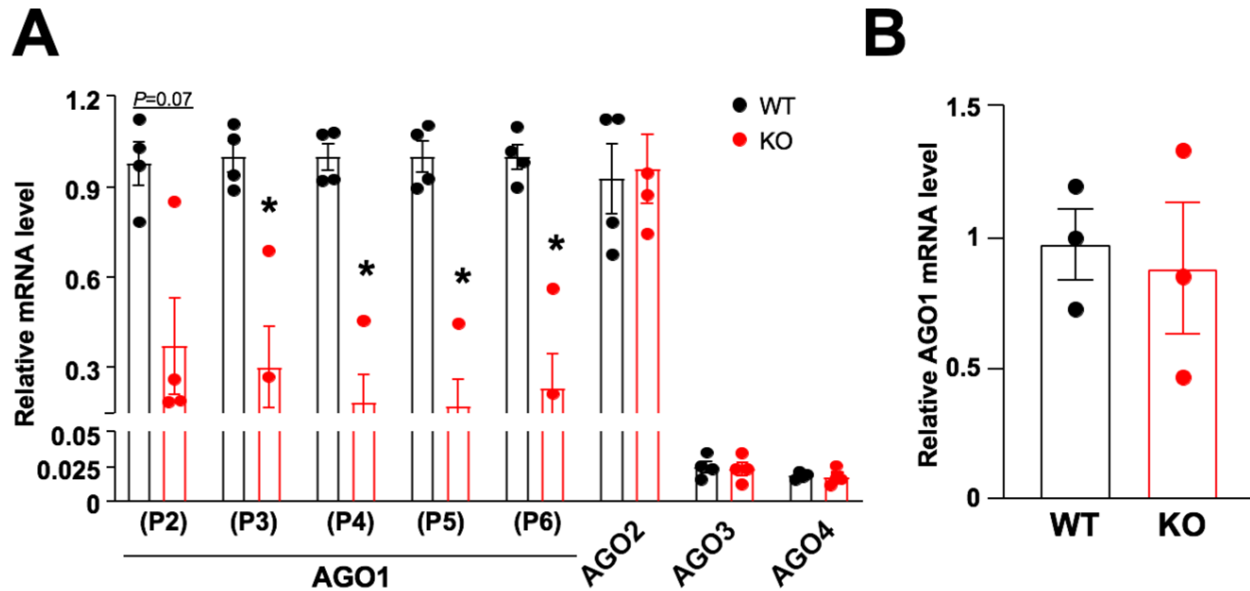
		Reverse	AAACCTCCTGCAAGCAAATG
ICAM-1	mouse	Forward Reverse	GCTACCATCACCGTGTATTCG TAGCCAGCACCGTGAATGTG
VCAM-1	mouse	Forward Reverse	AGTTGGGGATTCGGTTGTTCT CCCCTCATTCCTTACCACCC
AGO1	human	Forward Reverse	ATTGTGAGCTGGCGAATGCT CAGGCGGTGAGAAGAAGGAG
THBS1	human	Forward Reverse	AGACTCCGCATCGCAAAGG TCACCACGTTGTTGTCAAGGG
ACTB	human	Forward Reverse	CATGTACGTTGCTATCCAGGC CTCCTTAATGTCACGCACGAT
GAPDH	human	Forward Reverse	CTCCTCACAGTTGCCATGTA GTTGAGCACAGGGTACTTTATTG

Supplemental Table II. Parameters Measured in Mice by Echocardiography

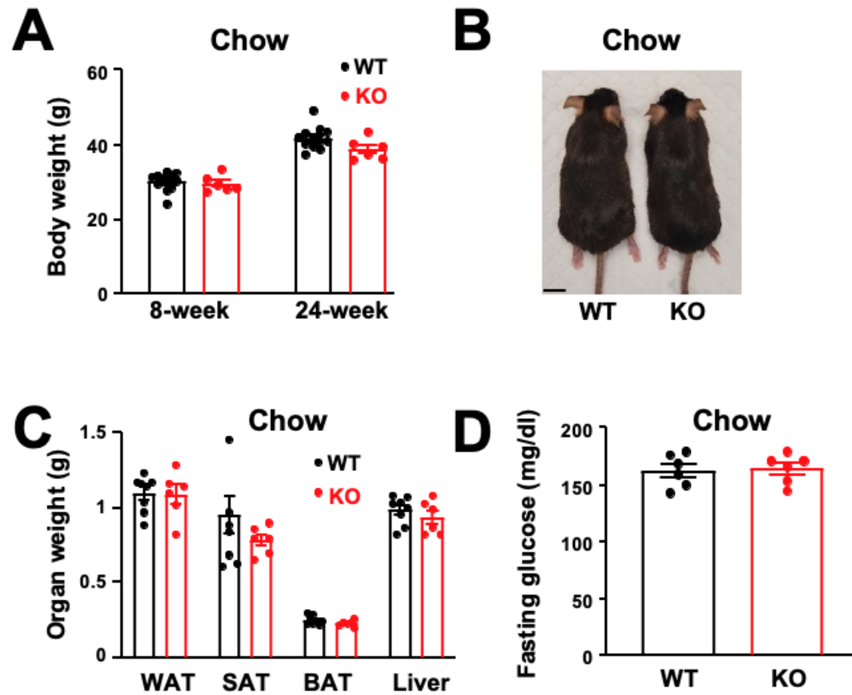
	Chow diet			HFHS diet		
	WT (n=9)	**KO (n=7)**	***p* value**	**WT (n=6)**	**KO (n=4)**	***p* value**
HR (beat/min)	549±13.6	557±13.8	0.7144	583±13.8	588±17.6	0.8276
LVM (mg)	80.41±7.3	70.96±4.6	0.2914	88.97±9.4	81.05±11.8	0.6172
LVM/BW (mg/g)	2.56±0.2	2.35±0.2	0.4394	1.95±0.2	2.37±0.3	0.2250
LVIDd (mm)	3.27±0.1	3.08±0.1	0.2733	3.45±0.2	3.29±0.3	0.6521
LVIDs (mm)	2.17±0.1	1.96±0.1	0.2424	2.31±0.1	2.07±0.2	0.3210
%FS	34.0±1.8	36.6±2.8	0.4608	33.0±2.4	36.8±1.4	0.2157

2D Echocardiography was performed with 12-week-old chow diet-fed or 24-week-old HFHS diet-fed (starting from 8-week-old) male littermates. Data are shown as mean ± SEM. P values were calculated based on Student's t test. Abbreviations: HR: heart rate, LVM: Left ventricular mass, BW: body weight, LVIDd: left ventricular internal dimension at end-diastole, LVIDs: Left ventricular internal dimension at end-systole, FS: fractional shortening.

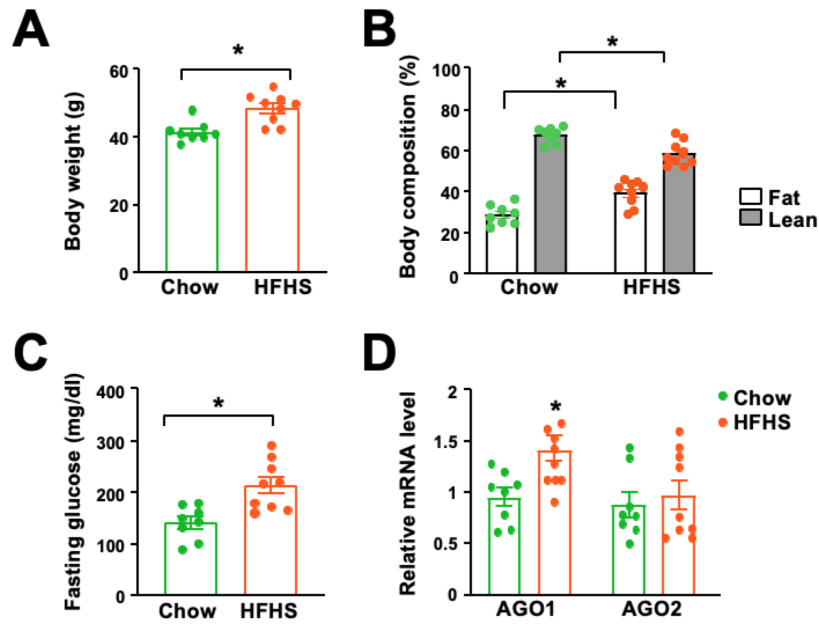
Supplemental Figures and Figure Legends



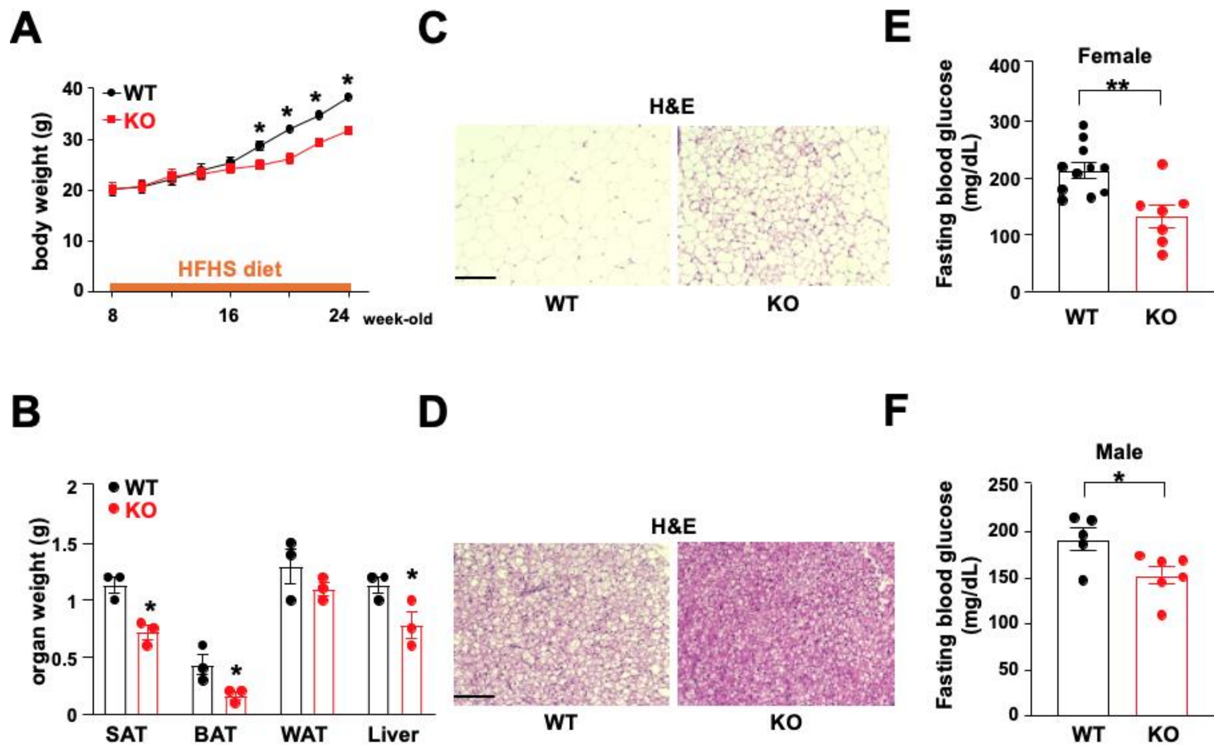
Supplemental Figure I. AGO1, but not AGO2-4 are decreased in EC-AGO1-KO mice. (A) qPCR was performed with lysates collected from the lung microvascular ECs isolated from 24-week-old male mice (n=4 mice/group). **(B)** AGO1 was detected in non-EC fractions (counter-selected with CD31) isolated from WT and EC-AGO1-KO SAT. n=3 mice/group. Data are presented as mean ± SEM. *Bonferroni-corrected $P < 0.05$ in (A).



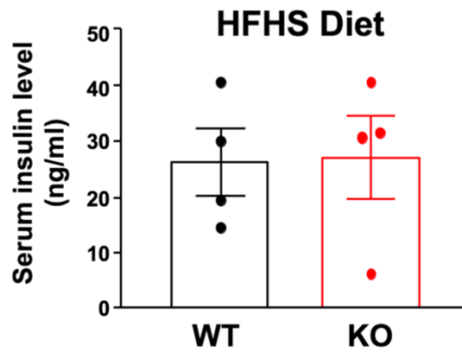
Supplemental Figure II. Lack of difference between EC-AGO1-KO and wild-type littermates fed with Chow diet. **(A)** Comparison of body weight between chow-fed WT and KO mice at 8 weeks old (WT=12 mice, KO=6 mice) and 24 weeks old (WT=8 mice, KO=6 mice). **(B-C)** Representative picture of whole body (in B) and comparison of weight of multiple organs (in C) collected from diet-fed WT and EC-AGO1-KO littermates (WT=8 mice, KO= 6 mice). Scale bar = 1 cm. **(D)** Fasting glucose levels of 24-week-old chow-fed WT and KO mice (n=6 mice/group). Data are presented as mean ± SEM.



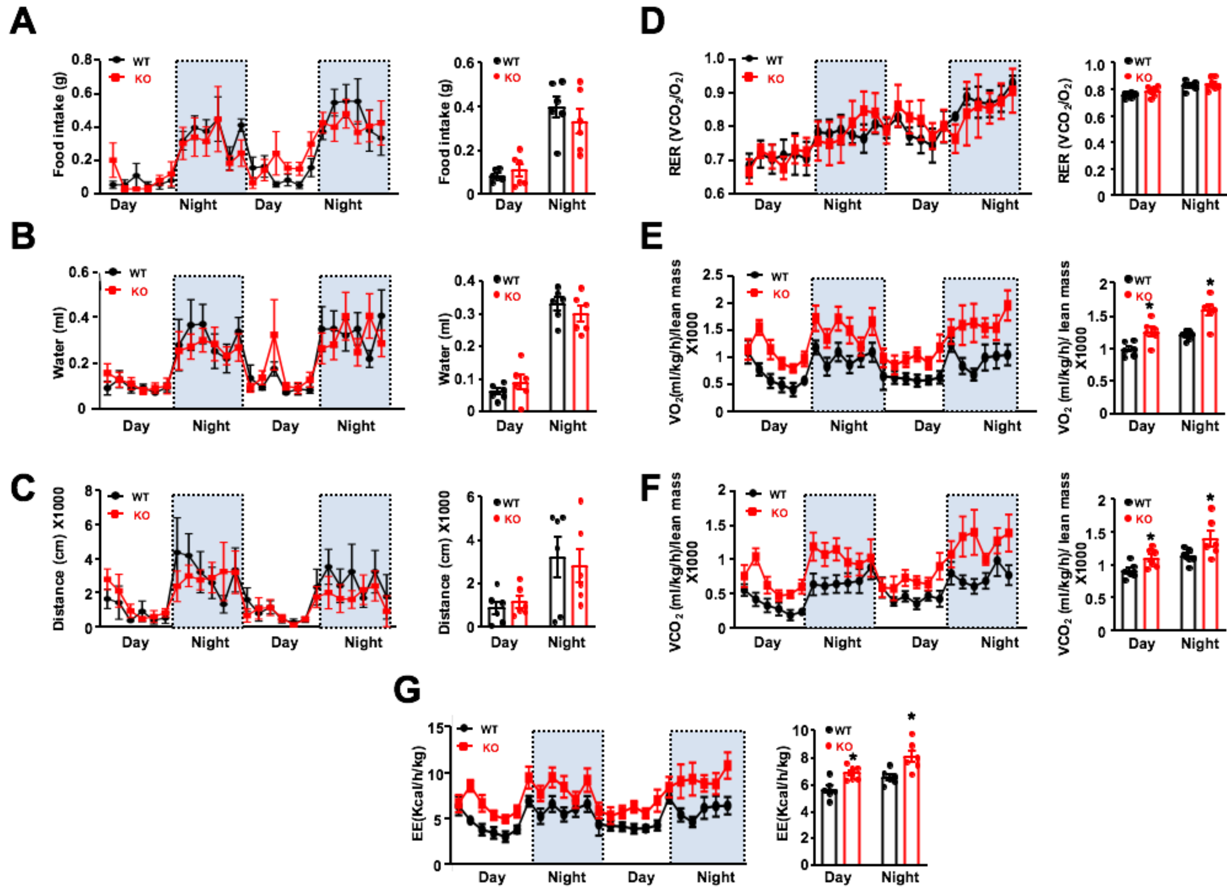
Supplemental Figure III. HFHS diet induces obesity and AGO1 expression in wild-type (WT) mice. WT mice were kept on chow (n=8 mice) or HFHS diet (n=9 mice) for 16 weeks starting at 8-week-old. Body weight **(in A)**, body composition **(in B)**, fasting glucose levels **(in C)**, and aortic AGO1 and AGO2 mRNA levels **(in D)** were measured. Data are presented as mean ± SEM. *$P <$ 0.05 based on Student's t test (in A-C). *Bonferroni-corrected $P < 0.05$ in (D).



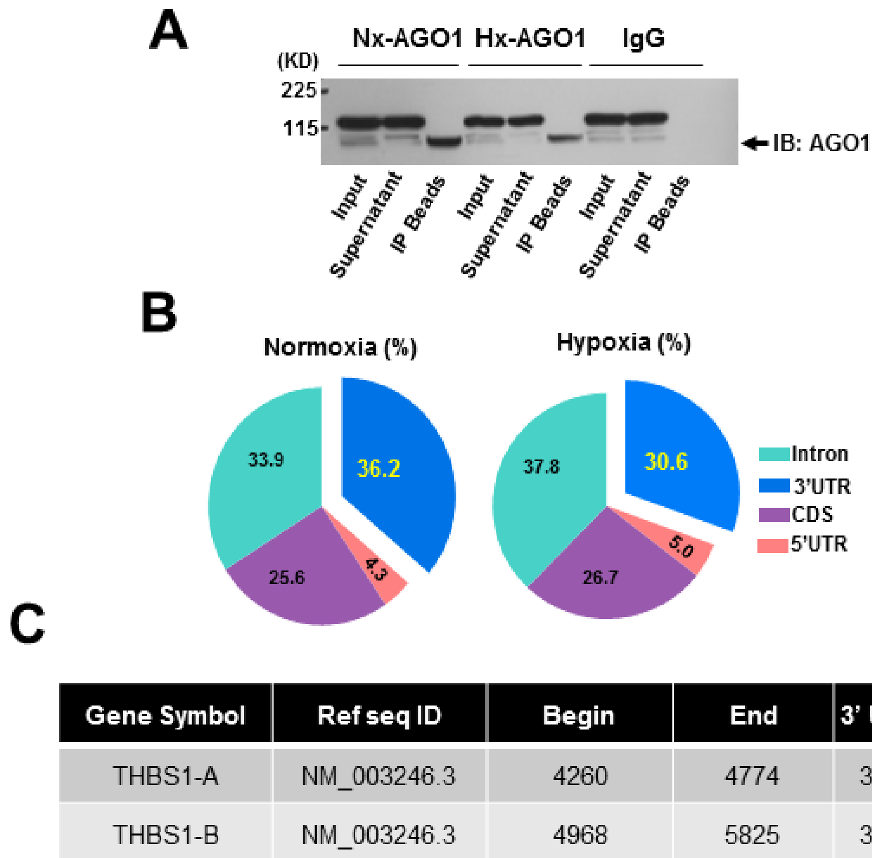
Supplemental Figure IV. Phenotypes of female EC-AGO1-KO mice. WT and EC-AGO1-KO male and female littermates were fed with HFHS diet for 16 weeks starting at 8-week-old of age. **(A)** Body weight was measured bi-weekly. **(B)** Weight of multiple organs. **(C, D)** H&E staining of SAT and BAT. Scale bars = 50 µm. n=3 mice/group in (A-D). **(E, F)** Fasting glucose levels EC-AGO1-KO mice compared to sex- and age-matched wild-type littermates. n=5-11mice/group. Data are presented as mean ± SEM. *$P < 0.05$ based on student's t test.



Supplemental Figure V. Insulin level in WT and EC-AGO1-KO mice. WT and EC-AGO1-KO male were fed with HFHS diet for 16 weeks starting at 8-week-old of age. Insulin levels were measured in serum (n=4 mice/group). Data are presented as mean ± SEM.

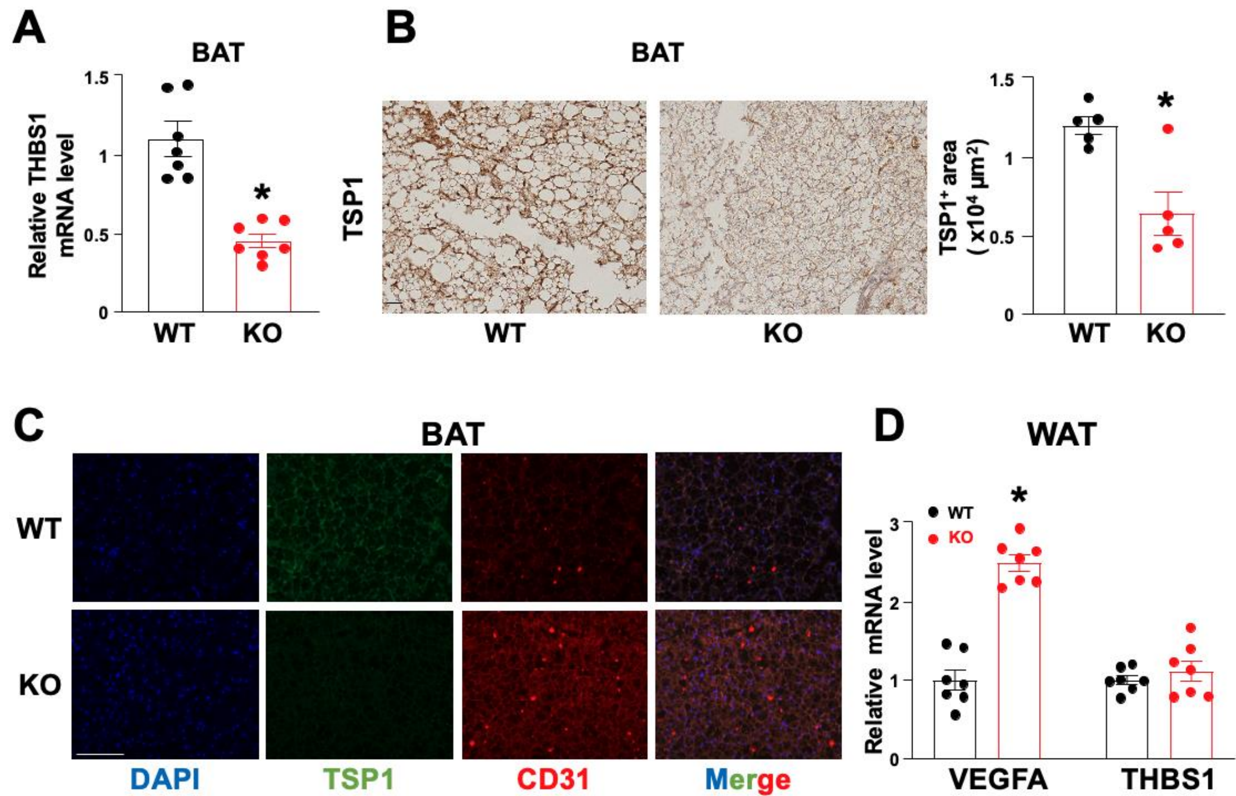


Supplemental Figure VI. Metabolic phenotyping of EC-AGO1-KO mice under HFHS diet. WT and EC-AGO1-KO littermates were fed HFHS diet for 16 weeks starting at 8-week-old of age. **(A)** Food intake, **(B)** water intake, **(C)** distance of movement, **(D)** respiratory exchange ratio (RER; *VCO_2/VO_2*), **(E-G)** oxygen consumption, carbon dioxide production and energy expenditure normalized to lean body mass (n=6 mice/group). Data are presented as mean ± SEM. *$P < 0.05$ based on Student's t test.

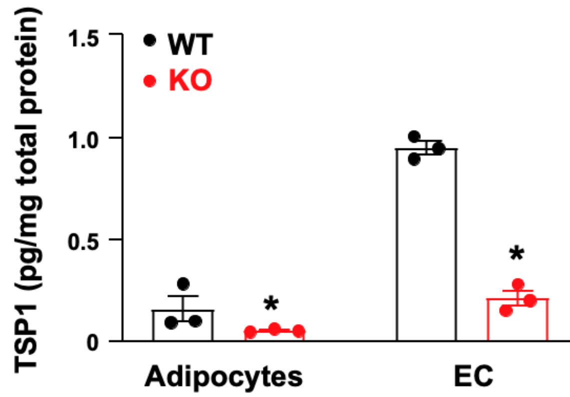


Gene Symbol	Ref seq ID	Begin	End	3' UTR range
THBS1-A	NM_003246.3	4260	4774	3693-7241
THBS1-B	NM_003246.3	4968	5825	3692-7241

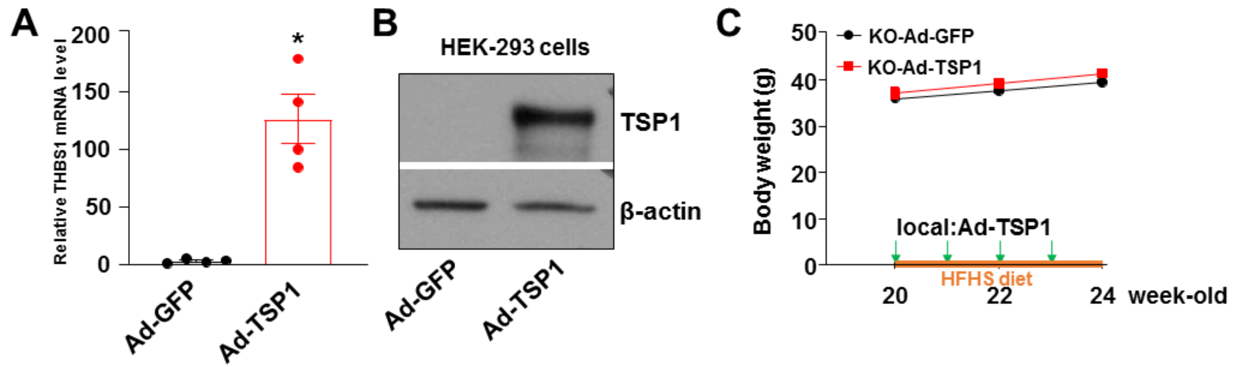
Supplemental Figure VII. AGO1 CLIP and AGO1-bound THBS1 3'UTR. (A) Immunoprecipitation of AGO1 protein under normoxia and hypoxia, with IgG as an isotype control. **(B)** Genomic distribution of AGO1-bound iCLIP-seq reads in HMVECs under normoxia (22% O2) or hypoxia (2% O2). **(C)** Locations of AGO1-bound sequences identified from iCLIP-seq.



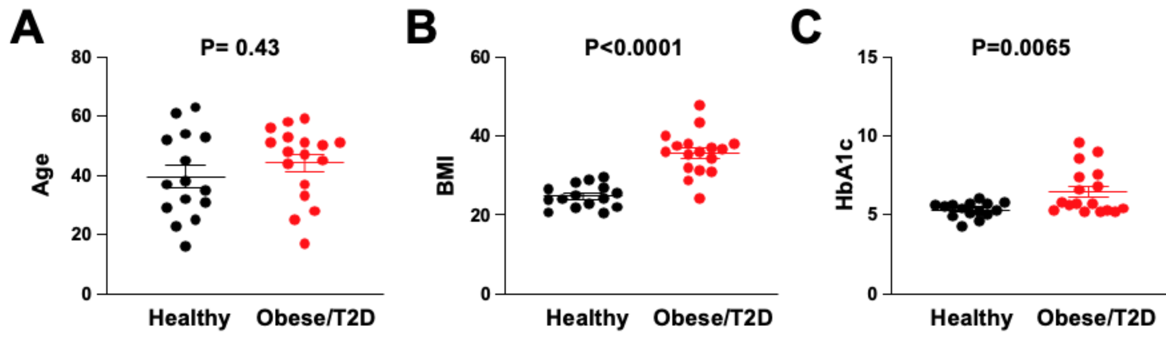
Supplemental Figure VIII. EC-AGO1-KO mice have decreased expression of TSP1 and vascular density in BAT but not in WAT. WT and EC-AGO1-KO littermates were fed HFHS diet for 16 weeks starting at 8-week-old of age. **(A)** qPCR analysis of THBS1 mRNA expression levels in BAT (n=7 mice/group). **(B)** Immunohistochemistry of TSP1 in BAT, representative images of n=5/group. Scale bar = 50 µm. **(C)** Representative co-immunostaining of TSP1 and CD31 in SAT (n=5 mice/group). Scale bars = 50 µm. **(D)** qPCR analysis of VEGFA and THBS1 mRNA levels in WAT from WT and KO mice fed HFHS diet (n=7 mice/group). Data are presented as means ± SEM. **P* based on Student's t tests (in A and B) or Bonferroni-corrected $P < 0.05$ (in D).



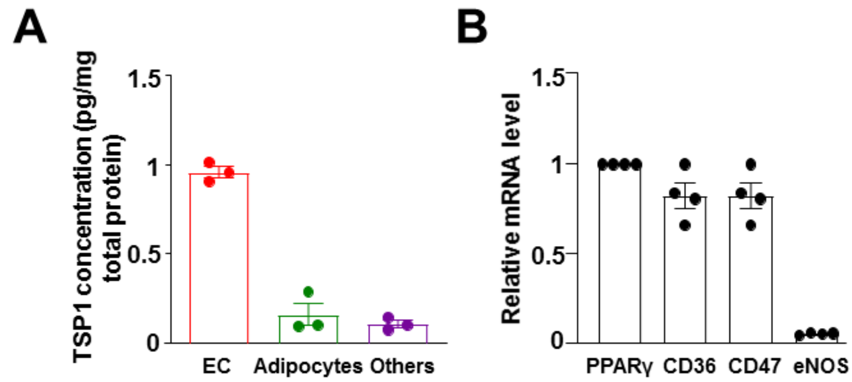
Supplemental Figure IX. TSP1 protein levels in ECs and adipocytes isolated from SAT of EC-AGO1-KO and WT littermates fed 16 weeks of HFHS diet. ELISA detection of TSP1 protein level in EC and adipocytes normalized to total protein amount (n=3 mice/group). Data are presented as mean ± SEM. *$P < 0.05$ based on Student's t test.



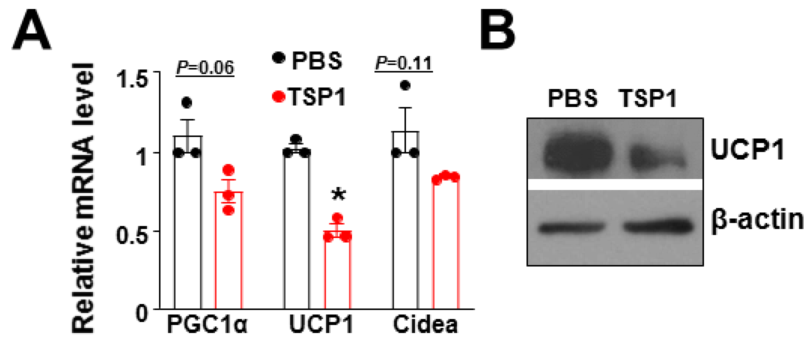
Supplemental Figure X. Adenovirus-driven ectopic expression of TSP1 *in vitro* and the effect of systemic injection of TSP1 *in vivo*. HEK-293 cells were infected either Ad-GFP or Ad-TSP1. THBS1 mRNA levels were detected by qPCR **(in A)** and TSP1 protein was detected by immunoblotting **(in B)** (n=4 samples/group). Data are presented as mean ± SEM. *$P < 0.05$ based on student's t test from 3 independent experiments. **(C)** Body weight measurements in EC-AGO1-KO mice that received local injection of Ad-GFP or Ad-TSP1 into SAT after HFHS diet feeding for 12 weeks. n=3 mice/group. Data are presented as mean ± SEM.



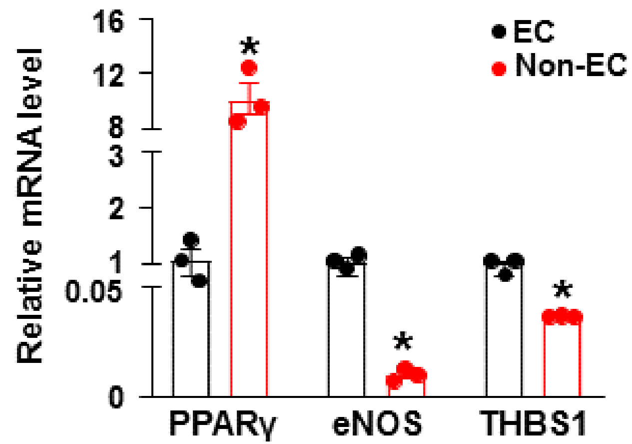
Supplemental Figure XI. Age (in A), BMI (in B), and HbA1c levels (in C) of human donors. Data are represented with mean ± SEM in the scatter plots.



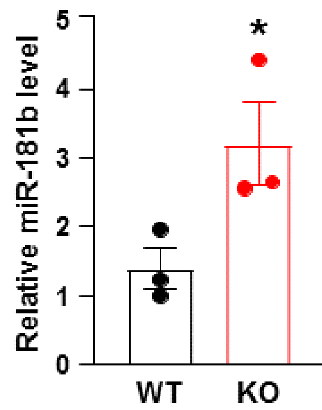
Supplemental Figure XII. Expression levels of TSP1 and its receptors in adipocytes. (A) TSP1 protein levels in cellular fractions isolated from SAT of WT mice (n=3) after 16 weeks of HFHS diet. **(B)** qPCR of indicated genes in 4 batches of differentiated murine brown adipocytes. PPARγ was detected as an adipocyte marker. Data are presented as mean ± SEM.



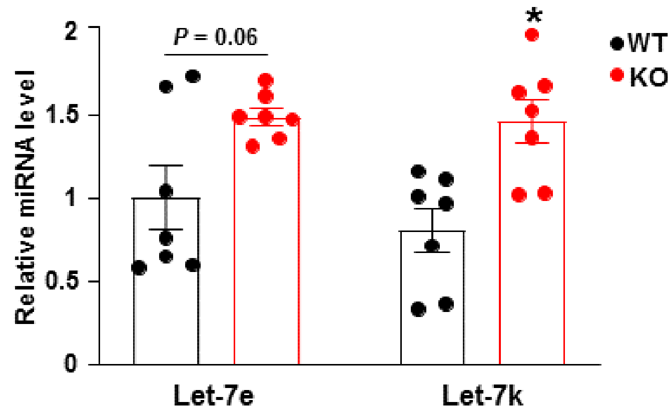
Supplemental Figure XIII. TSP1 treatment decreases the expression of browning markers. Murine brown pre-adipocytes were cultured, differentiated into mature brown adipocytes, and treated with recombinant TSP1 protein (2 μg/ml for 48 h). Browning markers were assessed by qPCR **(A)** and by immunoblotting **(B)**. Data are presented as mean ± SEM. *Bonferroni-corrected $P < 0.05$.



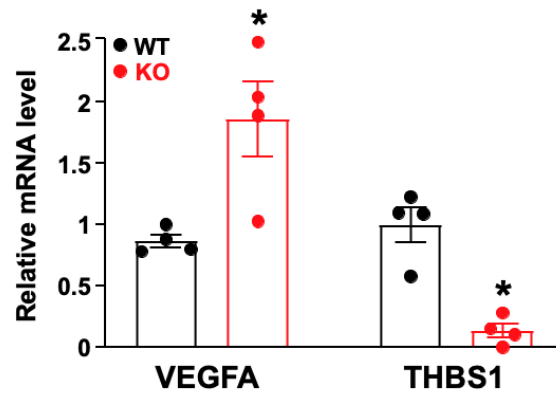
Supplemental Figure XIV. THBS1 is abundantly expressed in microvascular ECs from murine SAT. qPCR analysis THBS1, eNOS (EC marker) and PPARγ (adipocyte marker) mRNA levels of in EC and non-EC fractions isolated from SAT of WT mice (n=3 mice/group). Data are presented as mean ± SEM. *Bonferroni-corrected $P < 0.05$.



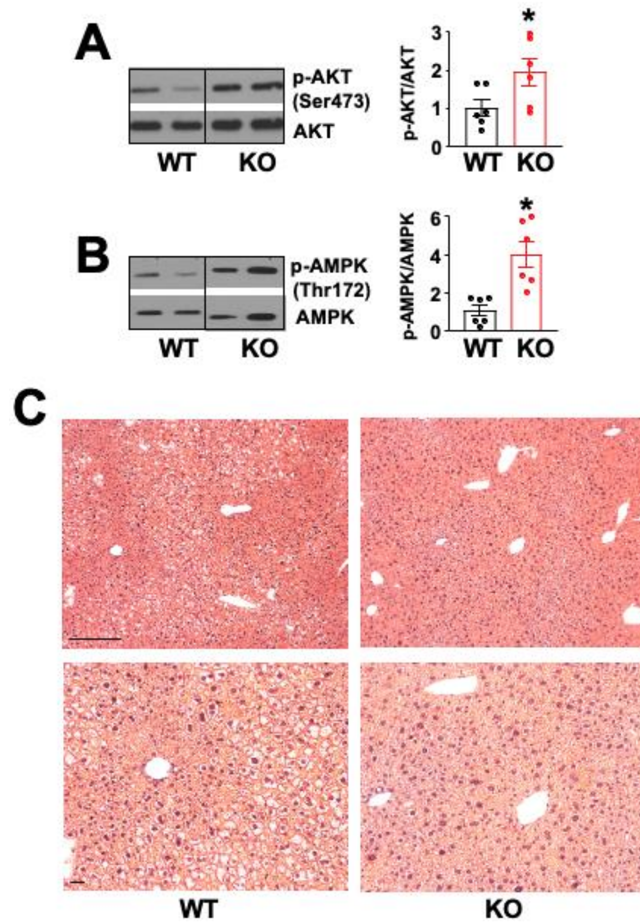
Supplemental Figure XV. Relative miR-181b level in SAT EC isolated from WT and KO mice fed HFHS diet. Taqman miRNA qPCR was performed to quantify miR-181b level (n=3 mice/group). Data are presented as mean ± SEM. *$P < 0.05$ based on Student's t test.



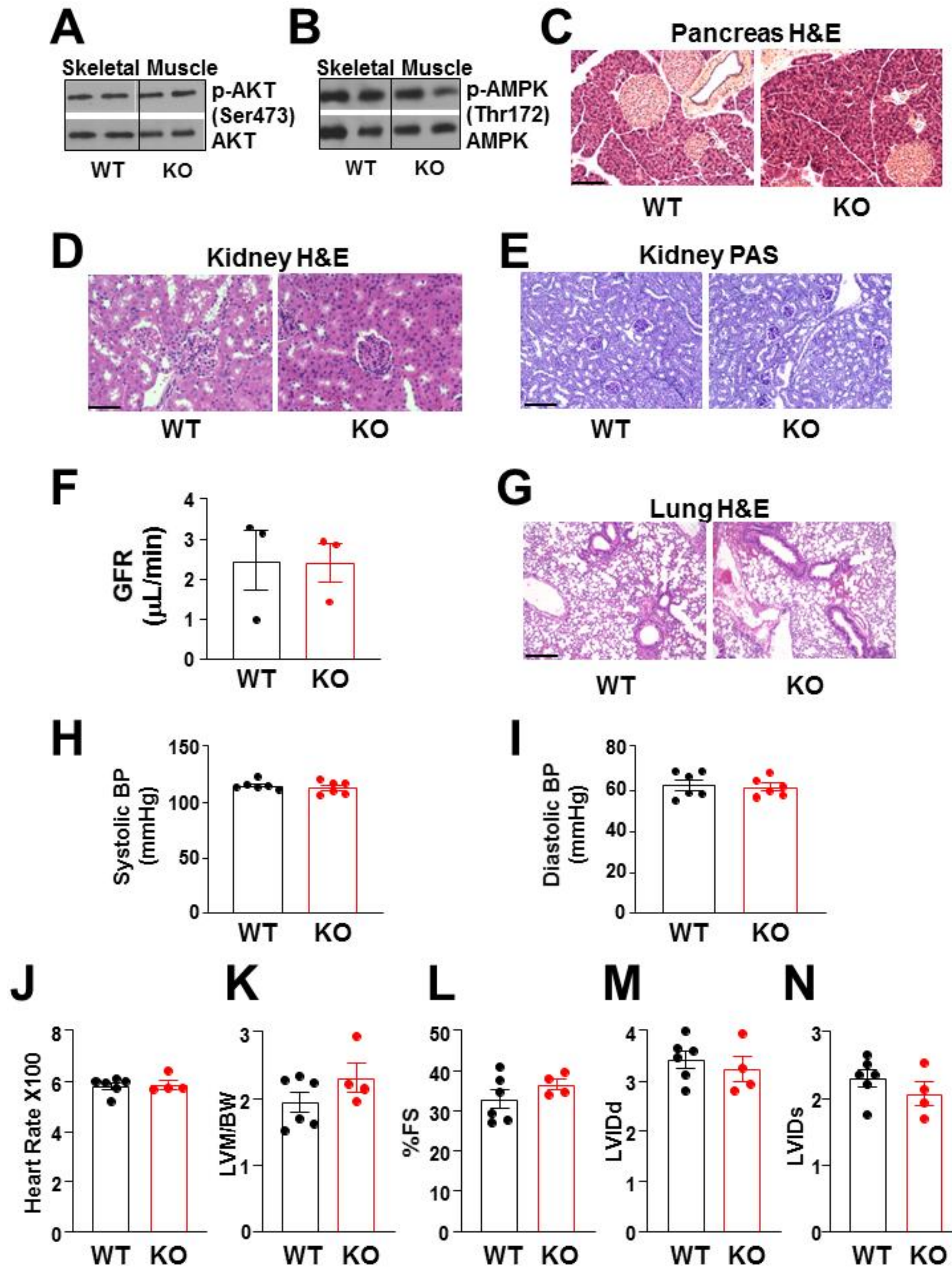
Supplemental Figure XVI. Let-7e and 7k levels are higher in the serum of EC-AGO1-KO vs WT. WT and EC-AGO1-KO littermates were fed HFHS diet for 16 weeks starting at 8-week-old of age. Taqman miRNA qPCR analysis of Let-7 levels in the serum samples from WT and KO mice (n=7 mice/group). Cel-miR-39 was added and detected as a spike-in control. Data are presented as means ± SEM. *Bonferroni-corrected $P < 0.05$.



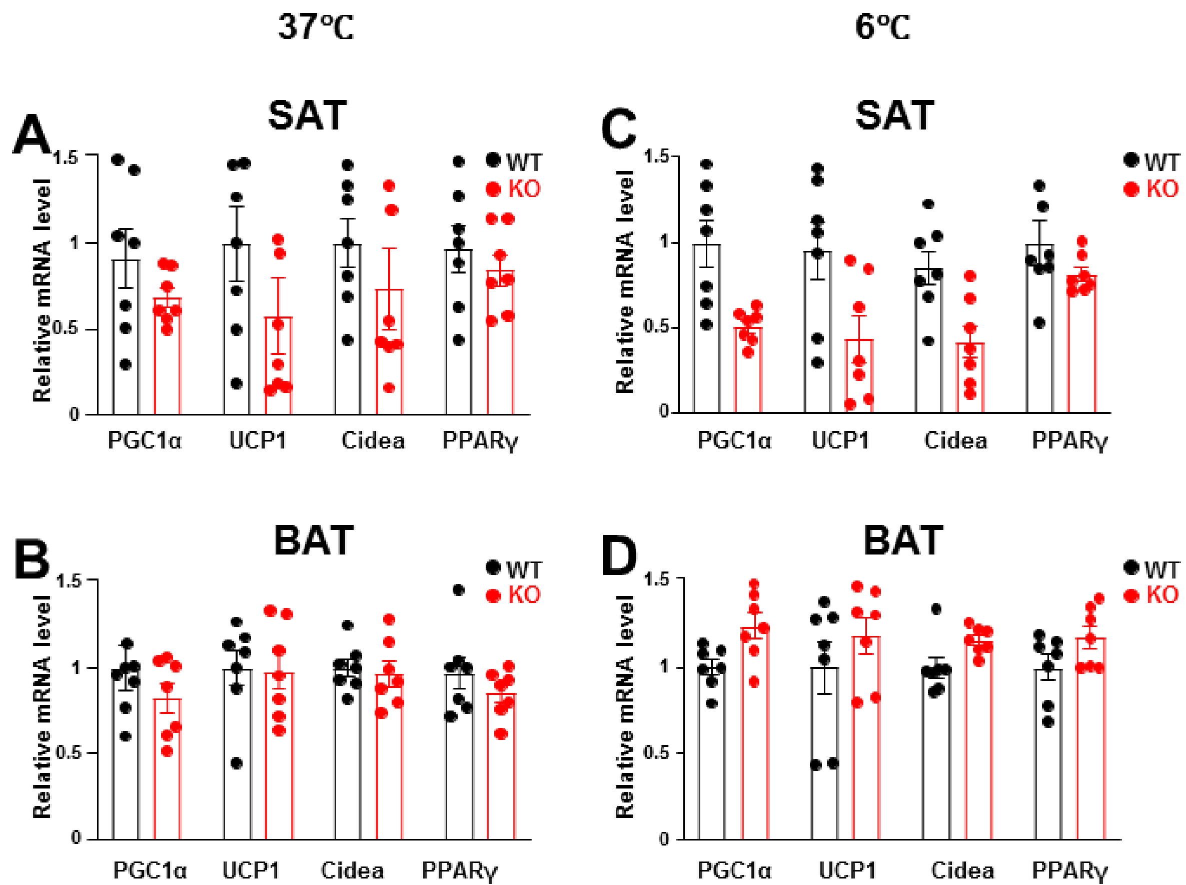
Supplemental Figure XVII. VEGFA and THBS1 expression in liver between WT and KO mice under HFHS Diet. WT and EC-AGO1-KO littermates were fed HFHS diet for 16 weeks starting at 8-week-old of age. qPCR analysis of VEGFA and THBS1 mRNA levels in liver from WT and KO mice (n=4 samples/group). Data are presented as mean ± SEM. *Bonferroni-corrected $P < 0.05$.



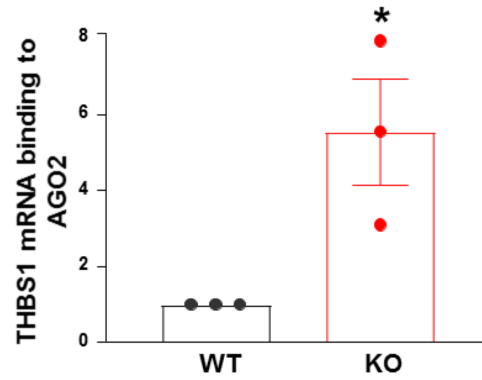
Supplemental Figure XVIII. Molecular and histological changes in the livers of EC-AGO1-KO mice. WT and EC-AGO1-KO littermates were fed HFHS diet for 16 weeks starting at 8-week-old of age. **(A and B)** Immunoblotting analysis of p-AKT (Ser473) and p-AMPK (Thr172) in livers (n=6 mice/group). **(C)** Representative HE staining of livers (n=4 mice/group). Scale bar = 200 µm in the upper images and 50 µm in the lower images. Data are presented as mean ± SEM. *P < 0.05 based on Student's t test.



Supplemental Figure XIX. Comparison in skeletal muscle, pancreas, kidney, lung, and heart between WT and KO mice under HFHS diet. (A, B) Representative immunoblotting analysis of p-AKT (Ser473) and p-AMPK (Thr172) in skeletal muscles (n=6 mice/group). **(C)** H&E staining of pancreata (n=4 mice/group). **(D-G)** Histology and function assessment of kidneys. **(D-F**) and lungs **(G)** (n=3 mice/group). Scale bars = 50 µm. **(H and I)** Blood pressure (BP). **(**n=6 mice/group). **(J-N)** Echocardiography assessment of heart rate, left ventricular mass (LVM) normalized by body weight (BW), fractional shortening (FS), left ventricular internal dimension at end-diastole (LVIDd), and left ventricular internal dimension at end-systole (LVIDs) (n=4-6 mice/group). Data are presented as mean ± SEM. More details are listed in Supplemental Table II.



Supplemental Figure XX. Lack of difference in EC-AGO1-KO mice exposed to thermoneutral or cold conditions. Eight-week-old male mice were exposed to either thermoneutrality (37°C, in A and B) or cold environment (6°C, in C and D) for 7 days (n=7 mice/group). SAT and BAT were collected for qPCR analysis of browning markers as indicated. Data are presented as mean ± SEM.



Supplemental Figure XXI. qPCR detection of THBS1 mRNA binding to AGO2. AGO2 was immunoprecipitated from ECs isolated from SAT of WT and their littermate KO mice fed with HFHS diet for 16 weeks starting at 8-week-old (n=3 mice/group). qPCR was performed to quantify the AGO2-bound THBS1 mRNA with that of 36B4 as an internal control. The relative binding in WT was set as 1. Data are presented as mean ± SEM. **P* < 0.05 based on Student's t test.