Supplemental Methods

Antibodies, Oligonucleotides, Probes, and Recombinant Proteins. Rabbit polyclonal antibody (pAb) against Ago2 was purchased from Cell Signaling Technology. Goat pAb against PCNA; rabbit pAbs against BCL2, and cyclin A; and mouse monoclonal antibodies (mAbs) against p21 and β-actin were obtained from Santa Cruz Biotechnology. Rabbit pAb against FOXO3 was from Millipore. Mouse mAb against SMα-actin was from Sigma. 5`-DIG and 3`-DIG labeled miR-126 locked nucleic acid (LNA) detection probe was from Exiqon. Dylight 594 anti-DIG antibody was from Jackson ImmunoResearch. Ago2 (eIF2C2), Dicer (DICER1), and Drosha (DROSHA)-specific and control siRNA were purchased from Santa Cruz Biotechnology. Anti-miR-126 inhibitor (AM126), miR-126 precursors or mimics (PRE126) and the respective negative control inhibitors (AMC) and mimics (PREC), with random sequences that produce no identifiable effects on known miR function, were purchased from Ambion. MiR-126 oligonucleotides were synthesized by Valuegene. Human Argonaute2 recombinant protein (rAgo2, full length) was obtained from Sino Biological.

Cell Culture. Human umbilical vein ECs (HUVECs) were cultured in medium 199 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Omega Scientific) and 10% Endothelial Growth Medium (Cell Applications). Human umbilical artery SMCs (HUASMCs) were cultured in Nutrient Mixture F12 Ham Kaighn's Modification (Sigma-Aldrich) supplemented with 10% FBS and 50% SMC Growth Medium (Cell Applications). Bovine aortic ECs (BAECs) were maintained in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% FBS.

Co-culture Modules and Parallel-plate co-culture Flow System. EC/SMC co-culture was established by plating ECs and SMCs on the lower and upper sides, respectively, of a 10-μm-thick porous polyethylene terephthalate (PET) membrane (Falcon cell culture inserts; Becton Dickinson) with 0.4-μm pores configured at a density of 1.6×10^6 pores/cm^2. ECs and SMCs were maintained in medium 199 or F12 Ham Kaighn's Modification (F12K), respectively, supplemented with 2% fetal bovine serum (FBS) until fully attached to the membrane. After the completion of EC/SMC co-culture, the inserts were incorporated into a parallel-plate flow chamber containing a polycarbonate insert holder, as previously described [1]. The chamber is connected to a perfusion loop system, which was kept in a constant-temperature controlled enclosure, with pH maintained at 7.4 by continuous gassing with a humidified mixture of 5% CO_2 in air. The flow of the perfusate in the channel is laminar. The fluid shear stress (τ) generated on the ECs seeded on the membrane can be estimated as $\tau = 6Q\mu/wh^2$, where Q is the flow rate and μ is the dynamic viscosity of the perfusate. In Laminar shear stress (LSS) experiments, ECs were exposed to LSS with a high level of mean shear stress at 12 dynes/cm^2 for indicated hours. The oscillatory shear stress (OSS) is composed of a low level of mean flow (shear stress = 0.5 dynes/cm^2) supplied by a hydrostatic flow system to provide the basal nutrient and oxygen delivery, and a superimposed sinusoidal oscillation using a piston pump with a frequency of 1 Hz and a peak-to-peak amplitude of ±4 dynes/cm^2. In some experiments, the conditioned media collected from static or 24 hr-sheared ECs seeded on glass slides pre-coated with collagen I (BD Biosciences) were used to study the paracrine effect of factor(s) released from ECs.

Preparation and Fractionation of Conditioned Media (CM), Deoxyribonuclease (DNase), Ribonuclease (RNase) and Proteinase Treatment. HUVECs with or without treatment were

cultured in medium 199 supplemented with 2% FBS for 24 hr and the media were collected and centrifuged at 1000g for 10 min to remove cell debris. The supernatant (designated as EC-CM or Total EC-CM) was transferred to a new tube and spun at 120,000 g for 120 min. The final supernatant was collected. The pellets were resuspended in medium 199 supplemented with 2% FBS of an identical volume with the Total EC-CM. For the DNase, RNase, or proteinase treatment, EC-CM were incubated with DNase I (1 unit/ml, Invitrogen) or RNase A (10 μg/ml, Invitrogen) at 37 ^{o}C for 15 min, or with proteinase K (PK, 20 μg/ml, Invitrogen) at 55 ^{o}C for 15 min and then at 95 ^{o}C for 5 min to inactivate the PK.

RNA Isolation and Quantitative RT-PCR. RNA was extracted from cultured cells by using the *mir*Vana miRNA Isolation Kit (Ambion) or TRIzol reagent (Life Technologies) according to the manufacturer's instructions. For the isolation of miRNAs from liquid samples, 5 pg of synthetic *C. elegans* miRNA *cel-miR-39* was added to each sample as a spike-in control for purification efficiency and RNA was extracted using the *mir*Vana PARIS kit (Ambion) following the manufacturer's protocol for liquid samples. Isolated RNAs were reversed-transcribed into complementary DNA with M-MLV RT system (Invitrogen) by using the Taqman primer sets for miRs (Applied Biosystems) or Oligo(dT) primers for the others. Real-time PCR was performed with the Taqman Fast Universal PCR Master Mix (Applied Biosystems) or iQ SYBR Green Supermix (Bio-Rad) by using the Taqman probes (Applied Biosystems) or specific primer pairs (FOXO3, 5'-cttgctgtatttgggtgaaca-3', 5'-tccgcttcaagacctcattt-3'; BCL2, 5'-ttggatcagggagttggaag-3', 5'- ccatgctgatgtctctggaa-3; IRS1, 5'-gtttccagaagcagccagag-3', 5'- tgaaatggatgcatcgtacc-3'). Primary and mature miR expression levels were normalized against the control RNU48 or Cel-miR-39 probes. Expressions of FOXO3, BCL2, and IRS1 were normalized against GAPDH.

Transient Transfection and Luciferase Reporter Assay. For gain- and loss-of-function studies of miRs, HUVECs or HUASMCs at 80% confluence were transfected with anti-miR-126 inhibitor (AM126), miR-126 precursors or mimics (PRE126), or the respective negative control molecules (30 nmol/L) using siPORT *NeoFX* transfection agent (Ambion) according to the manufacturer's instructions. For inhibition of gene expression, 40 nM of siRNA was used. For luciferase assay, the cells were co-transfected by using Lipofectamine 2000 reagent (Invitrogen) with 0.2 μg of respective DNA with or without the presence of AM126 or PRE126 or the negative control molecules per 2×10^5 cells, following a standard protocol. For forced expression of eGFP- or FLAH/HA-Ago2 fusion proteins, bovine aortic ECs (BAECs) were co-transfected by using Lipofectamine 2000 reagent with or without the presence of miR-126 oligonucleotides. For luciferase assay, the pSV-β-galactosidase plasmid was co-transfected with the luciferase reporter vectors to normalize the transfection efficiency. Twenty-four hours post-transfection, luciferase activity was measured using the Luciferase assay system (Promega) and normalized to the β-galactosidase activity assessed using *o*-nitrophenyl-β-D-galactopyranoside.

Plasmids. Plasmids expressing eGFP-hAgo2 (21981) [2], FLAG/HA-hAgo2 (10821) [3], FLAG-FOXO3a (8360) [4], FLAG-Bcl2 (18003) [5] were obtained from Addgene. Plasmid expressing HA-tagged human IRS1 [6] was kindly provided by Dr. Michael J. Quon from University of Maryland. To generate the luciferase reporter constructs, the 3'-UTRs of FOXO3 (6106-6672 of its mRNA, 566 bp), BCL2 (5165-5569 of its mRNA, 403 bp), or IRS1 (3735-4215 of its mRNA, 480 bp) harboring the predicted

miR-126 binding sequences were PCR-amplified from human genomic DNA and cloned into HindIII and SpeI of the pMir-Report luciferase vector (Ambion). Mutagenesis of predicted miR-126 binding sequences was performed following Quikchange site-direct mutagenesis protocol (Stratagene).

Immunoprecipitation and Co-immunoprecipitation Assay. Cells were trypsinized and lysed with Lysis Buffer containing 20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 100 U/ml RNase inhibitor, and a protease inhibitor cocktail (Roche). Protein concentration of the lysate was adjusted to be 2-3 μg/μl, and 8 μg of Ago2- or FLAG-specific antibody or control IgG was added into 100 μl of cell lysate. In some experiments using liquid samples, 200 μl of Lysis Buffer and 10 μg of Ago2-specific antibody or control IgG was added into 800 μl of conditioned media. After incubation and rotation at 4°C overnight, the immune complexes were pulled down with protein A/G Sepharose beads and washed with the lysis buffer. For immunoprecipitation assay, 50 μl of 2×SDS buffer was added into each sample, which was then subjected to Western blot analysis. For co- immunoprecipitation assay, 1 ml of TRIzol reagent was added into each sample, and RNA was extracted following a standard protocol. The purified RNA was analyzed by quantitative real-time RT-PCR.

miR Tracking. Biotinylation of miRs was carried out using BrightStar Psoralen-Biotin Kit (Invitrogen) according to manufacturer's instructions. The biotinylated miRs were transfected into HUVECs using siPORT *NeoFX* transfection agent at a concentration of 30 nmol/L. In some experiments, the ECs were washed with culture medium 8 hr post-transfection and then cocultured with SMCs for 24 hr. In other experiments, the ECs were washed with culture medium 8 hr post-transfection and incubated with fresh culture medium for another 24 hr. EC-CM were then collected from those ECs and used to treat SMCs. Direct staining of biotinylated miRs in ECs and SMCs was performed using Qdot-605 streptavidin conjugate (Invitrogen). The cells were seeded on sterilized cover slips and fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. The cells were then incubated with 10 nmol/L of Qdot-605 streptavidin conjugate in PBS and rinsed with PBS. Nuclei were stained with Hoechst 33258 (Invitrogen). The cells were dehydrated by submerging the cover slip sequentially in 30%, 50%, 70%, and 90% ethanol/water, twice in 100% ethanol, once in 100% toluene, and the final dip in 100% toluene. After mounting, the slips were visualized by fluorescence microscopy (Olympus). To capture the biotinylated miRs in cells, the transfected ECs or the cocultured SMCs were trypsinized and lysed with Lysis Buffer (as described above). The biotinylated miRs were pulled down with Streptavidin Agarose Resin (Thermo Fisher Scientific) and purified with TRIzol reagent and analyzed by quantitative real-time RT-PCR. For Northern blot of biotinylated miRs in cells, total RNA was extracted from cells with TRIzol reagent and separated by 15% denaturing urea polyacrylamide gel and then transferred to nylon membranes. The biotinylated miRs on membranes were probed with BrightStar BioDetect Kit (Ambion) according to the manufacturer's instructions.

MiR Protection and Uptake Assay. To analyze the ability of Ago2 protein in protecting miRs from RNase digestion, synthetic miR-126 or Cel-miR-39 (3 pmol) in 200 μl of medium 199 was kept alone or incubated with rAgo2 (1.5 pmol) or bovine serum albumin (BSA, 1.5 pmol) for 30 min at room temperature followed by incubated with RNase A (5 μg/ml) for another 30 min at 37 ^{o}C. The miRs from each condition were then purified using *mir*Vana PARIS kit following the manufacturer's

protocol for liquid samples and the miR levels were determined by quantitative RT-PCR. To determine the ability of Ago2 protein in facilitating uptake of miRs by the recipient cells, synthetic miR-126 or Cel-miR-39 (5 pmol) in 100 μl of medium 199 was kept alone or incubated with rAgo2 (1.5 pmol) or BSA (1.5 pmol) for 30 min at room temperature and then treated HUASMCs for 3 hr at 37 ^{o}C. The cells were then harvested and RNA was extracted from the cells and was analyzed by quantitative real-time RT-PCR.

Cell Cycle Analysis. Cells were harvested in PBS containing 2 mmol/L ethylenediaminetetraacetic acid, washed once with PBS, and fixed for 2 hr in cold ethanol (70%). They were then stained with 50 μg/mL of propidium iodide (Roche) and 1 mg/mL RNase A for 30 min. Stained cells were analyzed by flow cytometry and the data analysis were processed by FlowJo.

Annexin V-PI Staining and Flow Cytometery. Cells were trypsinized from coculture membranes and subjected to Annexin V-PI staining using FITC Annexin V Apoptosis Detection Kit (BD Pharmingen) according to manufacturer's instructions to identify apoptosis. Data were analyzed by CellQuest.

Immunofluorescence. Cells were trypsinized from coculture membranes and seeded on sterilized cover slips. After attached well, the cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS and nonspecific binding was blocked by 5% BSA in PBS. The cells were probed with primary antibody, washed and then probed with secondary antibody (Santa Cruz). Nuclei were stained with Hoechst 33258. After mounting, the slips were visualized by fluorescence microscopy.

MiR *in situ* Hybridization. 6 μm cross cryosections were prepared from 4% paraformaldehyde-fixed tissue. MiR fluorescence *in situ* hybridization (FISH) was performed on the sections as described previously [7] with minor modifications. A 5`-digoxigenin and 3`-digoxigenin labeled locked nucleic acid oligonucleotide designed to hybridize to miR-126 was incubated with the tissue sections for over 4 hr at 60 ^{o}C. MiR-126 was visualized using the Dylight 594 anti-digoxigenin antibody.

Western Blot Analysis. Cells were collected by scraping and lysed with a buffer containing 20 mmol/L Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor cocktail (Roche). The total cell lysate was separated by SDS-PAGE (10-12% running, 4% stacking) and transferred onto a polyvinylidene fluoride membrane (Immobilon P, 0.45-mm pore size). The membrane was then incubated with the designated antibodies. Immunodetection was performed using the Gel-imaging System for Life Science (Alpha-Innotech).

Animal Model. All animal studies were performed in accordance with National Institutes of Health guidelines and were approved by the UC San Diego IACUC. Male miR-126 $^{+/+}$ (WT) and miR-126 $^{-/-}$ (KO) mice (8-12 weeks old, 18-25 g) were anesthetized by intraperioneal injection (ip) of xylazine and ketamine HCl (5 mg/kg body weight and 80 mg/kg body weight, respectively). The left common carotid artery was dissected and ligated near the carotid bifurcation with the use of 5-0 silk. The wound was sutured and the animals were allowed to recover and showed no symptoms of a stroke. 43 WT and 43 KO mice were subjected to carotid artery ligation. For the systematical delivery of

EC-CM, immediately post-ligation, 15 WT and 15 KO mice were injected intravenously (iv) with 200 μl of EC-CM from 3-days cultured HUVECs twice per week for 4 weeks or with control media. For the local application of miR-126, 2 μg of PRE126 or scrambled control were preloaded into the 50 μl, 25% F-127 pluronic gel (Sigma) at 4°C. Lipofectamine 2000 reagent (Invitrogen) was added at a final concentration of 1%. Immediately after the ligation of carotid artery, the F-127 pluronic gel loaded with those oligonucleotides was applied locally to the adventitia around the ligated artery segments of 12 WT and 12 KO mice. 4 weeks after ligation, the mice were sacrificed and fixed for 5 minutes by perfusion through left cardiac ventricle with 4% *p*-formaldehyde in PBS buffer under physiological pressure. The ligated and unligated carotid arteries were isolated and immersed in fixative solution for 16 hr at 4 °C and then embedded in OCT media. 6 μm cross cryosections were prepared from those vessels using cryostat microtome and then subjected to histology or immunostaining assay.

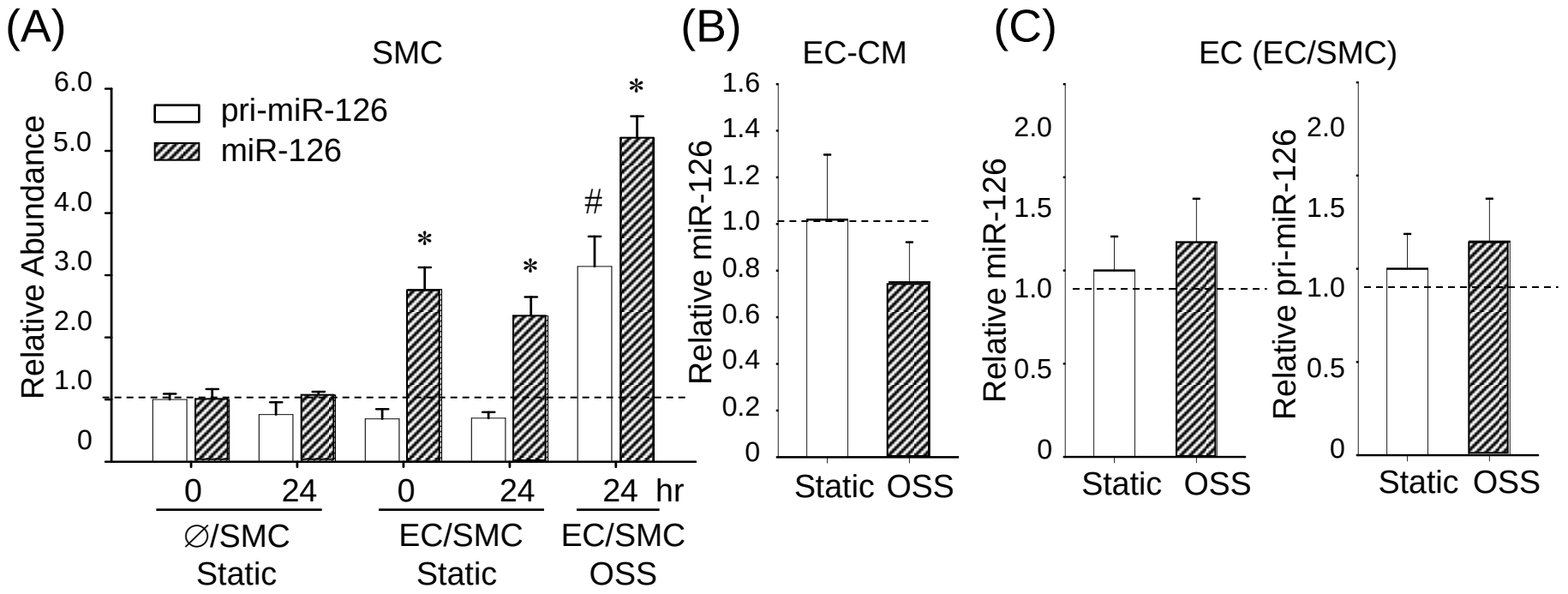
Isolation of ECs from mouse lung. Lungs from two young adult mouse were removed aseptically, rinsed in DMEM supplemented with 20% FBS, minced finely with scissors for 1min, and digested in 25 mL of type I collagenase (2 mg/mL) at 37°C for 45 minutes with occasional agitation. The cellular digest was filtered through sterile 70-μm disposable cell strainer (Falcon), centrifuged at 400*g* for 10 minutes, and washed twice with cold PBS containing 0.1% BSA; the cell pellet was resuspended in 2 mL of cold PBS containing 0.1% BSA. Dynabeads M-450 sheep anti-rat IgG (Invitrogen) pre-incubated overnight with rat anti-mouse PECAM-1 antibody were added into the cell suspension at 15 μl of beads per 1 ml of cell suspension. After 10 minutes at room temperature with occasional agitation, the bead-bound cells were separated with a magnetic separator, washed five times with DMEM supplemented with 20% FBS, and resuspended in DMEM supplemented with 20% FBS, 100 μg/ml Heparin (Sigma), 10% endothelial cell growth medium (ECGM), and 25 mol/L HEPES, and then plated in one T75 flask.

Supplementary References

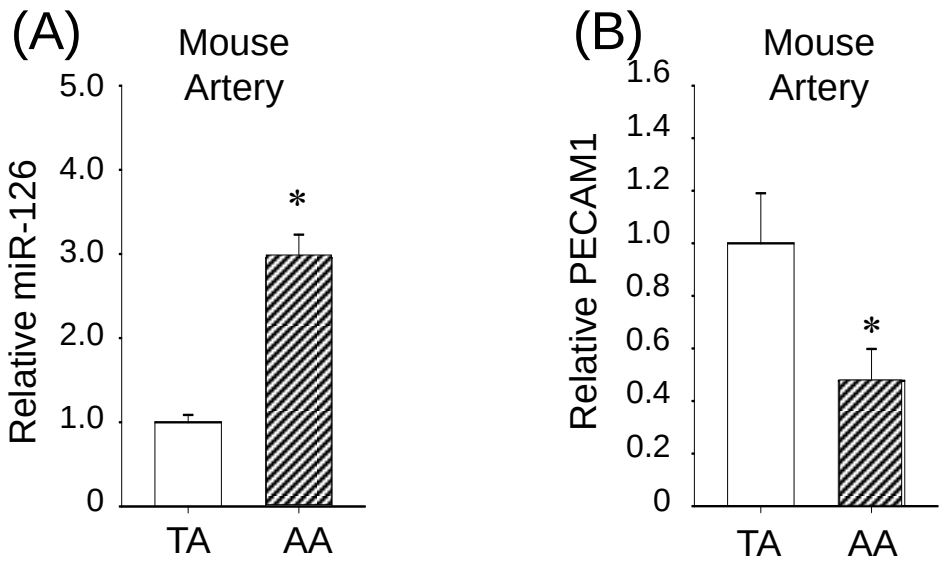
1. Chiu JJ, Chen LJ, Lee PL, Lee CI, Lo LW, Usami S, Chien S. Shear stress inhibits adhesion molecule expression in vascular endothelial cells induced by coculture with smooth muscle cells. *Blood*. 2003;101:2667-2674
2. Leung AK, Calabrese JM, Sharp PA. Quantitative analysis of argonaute protein reveals microrna-dependent localization to stress granules. *Proc Natl Acad Sci U S A*. 2006;103:18125-18130
3. Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human argonaute2 mediates rna cleavage targeted by mirnas and sirnas. *Mol Cell*. 2004;15:185-197
4. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of foxo transcription factors by the sirt1 deacetylase. *Science*. 2004;303:2011-2015
5. Wang NS, Unkila MT, Reineks EZ, Distelhorst CW. Transient expression of wild-type or mitochondrially targeted bcl-2 induces apoptosis, whereas transient expression of endoplasmic reticulum-targeted bcl-2 is protective against bax-induced cell death. *J Biol Chem*. 2001;276:44117-44128
6. Kim JA, Yeh DC, Ver M, Li Y, Carranza A, Conrads TP, Veenstra TD, Harrington MA, Quon MJ. Phosphorylation of ser24 in the pleckstrin homology domain of insulin receptor substrate-1 by mouse pelle-like kinase/interleukin-1 receptor-associated kinase: Cross-talk between inflammatory signaling and insulin signaling that may contribute to insulin resistance. *J Biol Chem*. 2005;280:23173-23183

7. Silahtaroglu AN, Nolting D, Dyrskjot L, Berezikov E, Moller M, Tommerup N, Kauppinen S. Detection of microrna in frozen tissue sections by fluorescence in situ hybridization using locked nucleic acid probes and tyramide signal amplification. *Nat Protoc*. 2007;2:2520-2528

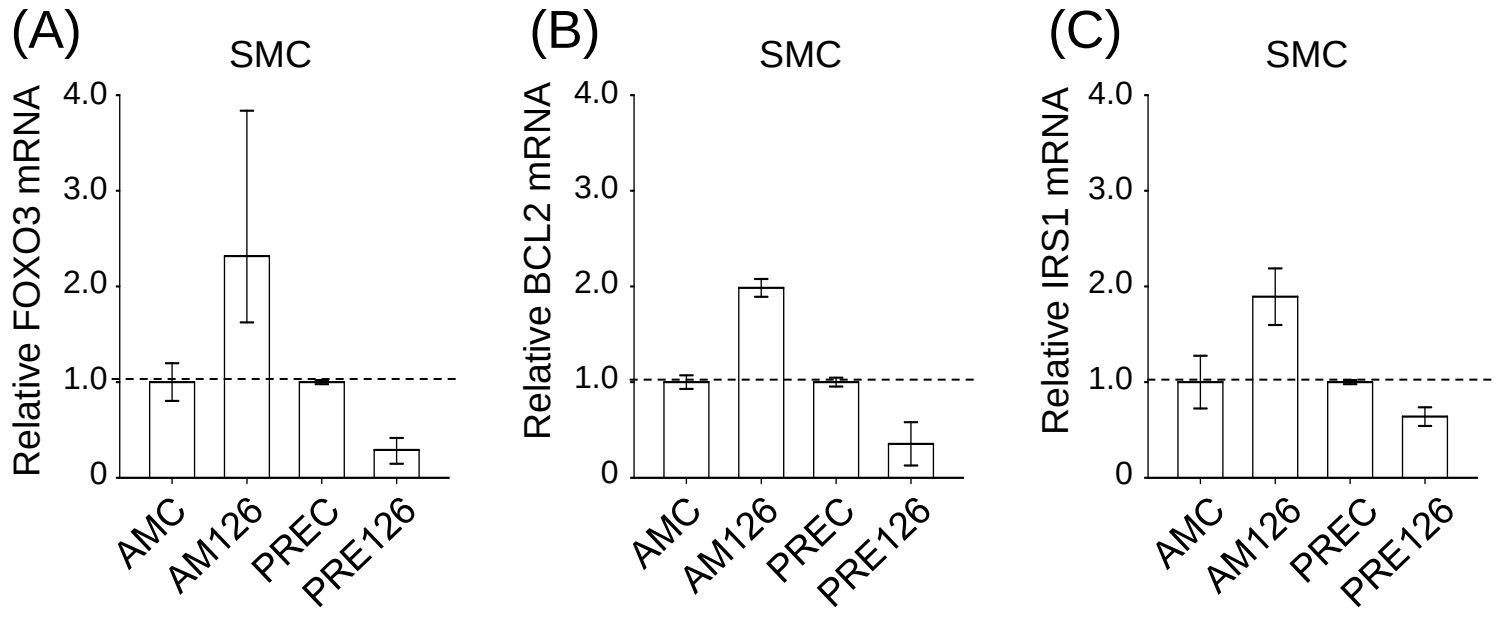
Supplemental Figures and Figure Legends



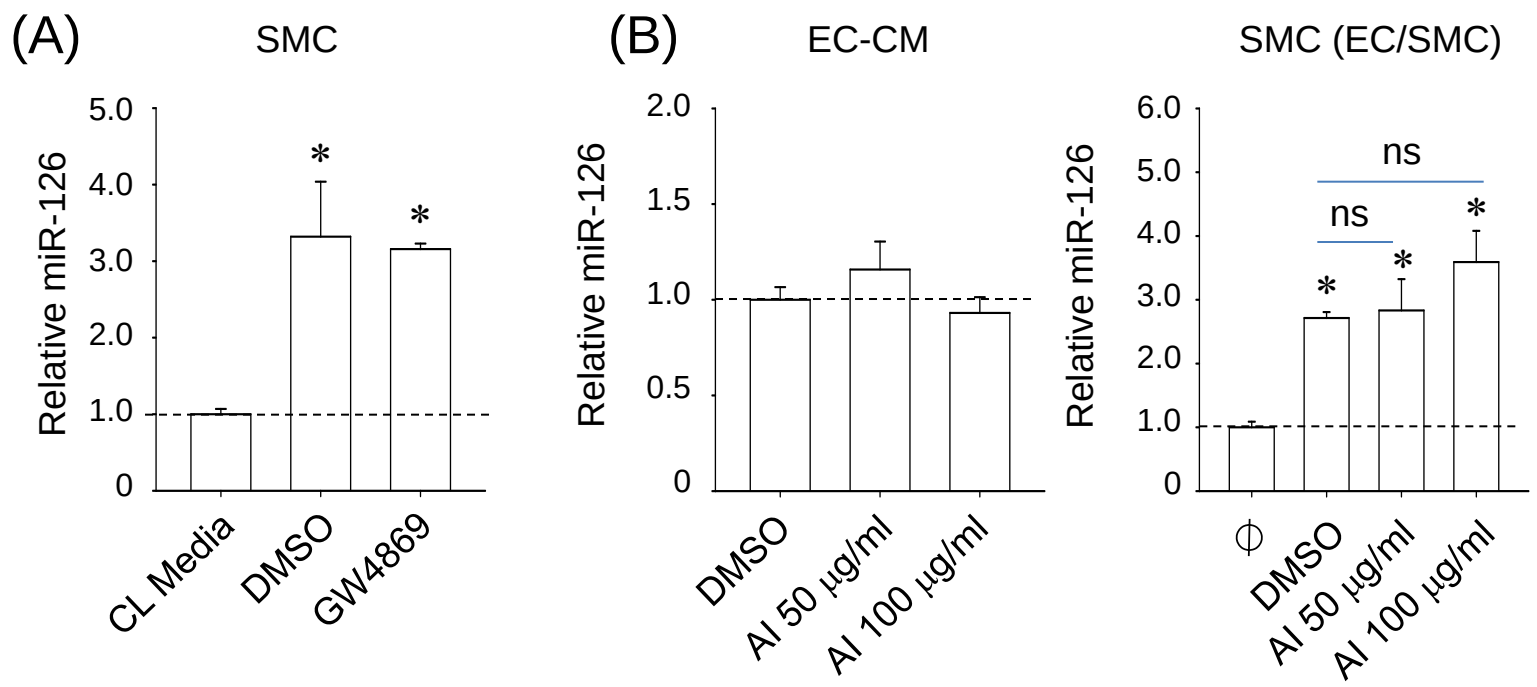
Online Figure I. OSS to ECs does not suppress miR-126 in the co-cultured SMCs. *(A)*, SMCs were monocultured or co-cultured with ECs. The ECs were kept as static controls or subjected to OSS (0.5±4 dynes/cm^2) for 24 hrs, and the levels of primary (pri-miR-126) and mature (miR-126) forms of miR-126 in SMCs were determined by quantitative RT-PCR. * $P < 0.05$ *vs*. ∅/SMC 0 hr Static, miR-126. # $P < 0.05$ *vs*. ∅/SMC 0 hr Static, pri-miR-126. *(B)* ECs were kept as static control or subjected to OSS for 24 hr and the levels of miR-126 in the static or sheared media were determined by quantitative RT-PCR. *(C)* ECs and SMCs were co-cultured and the ECs were kept as static controls or subjected to OSS (0.5±4 dynes/cm2) for 24 hrs, and the levels of primary (pri-miR-126) and mature (miR-126) forms of miR-126 were determined by quantitative RT-PCR.



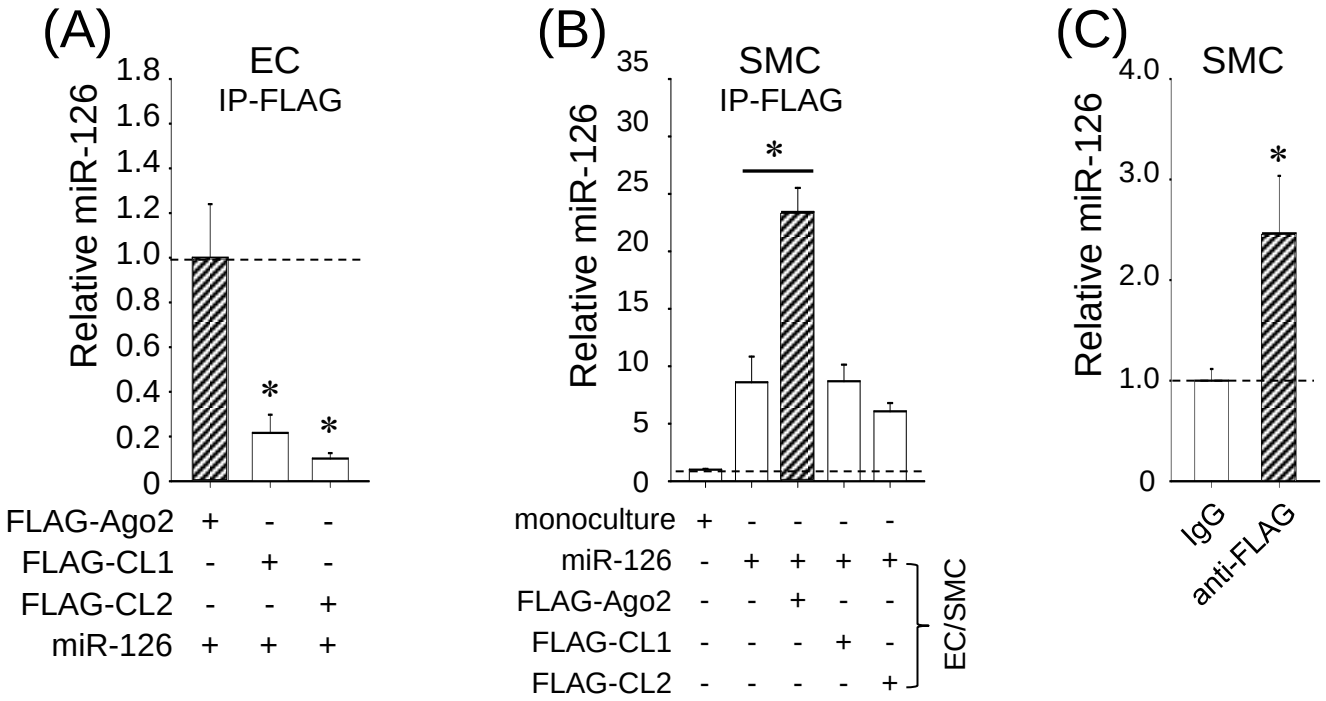
Online Figure II. The blood vessels wall of mouse aortas from the inner curvature of the aortic arch (AA) has higher level of miR-126 than that from the nearby descending thoracic aorta (TA). RNA was isolated from TA or AA regions of mouse aortas and the levels of miR-126 *(A)* and PECAM1 *(B)* were determined by quantitative RT-PCR. * $P < 0.05$ *vs*. TA.



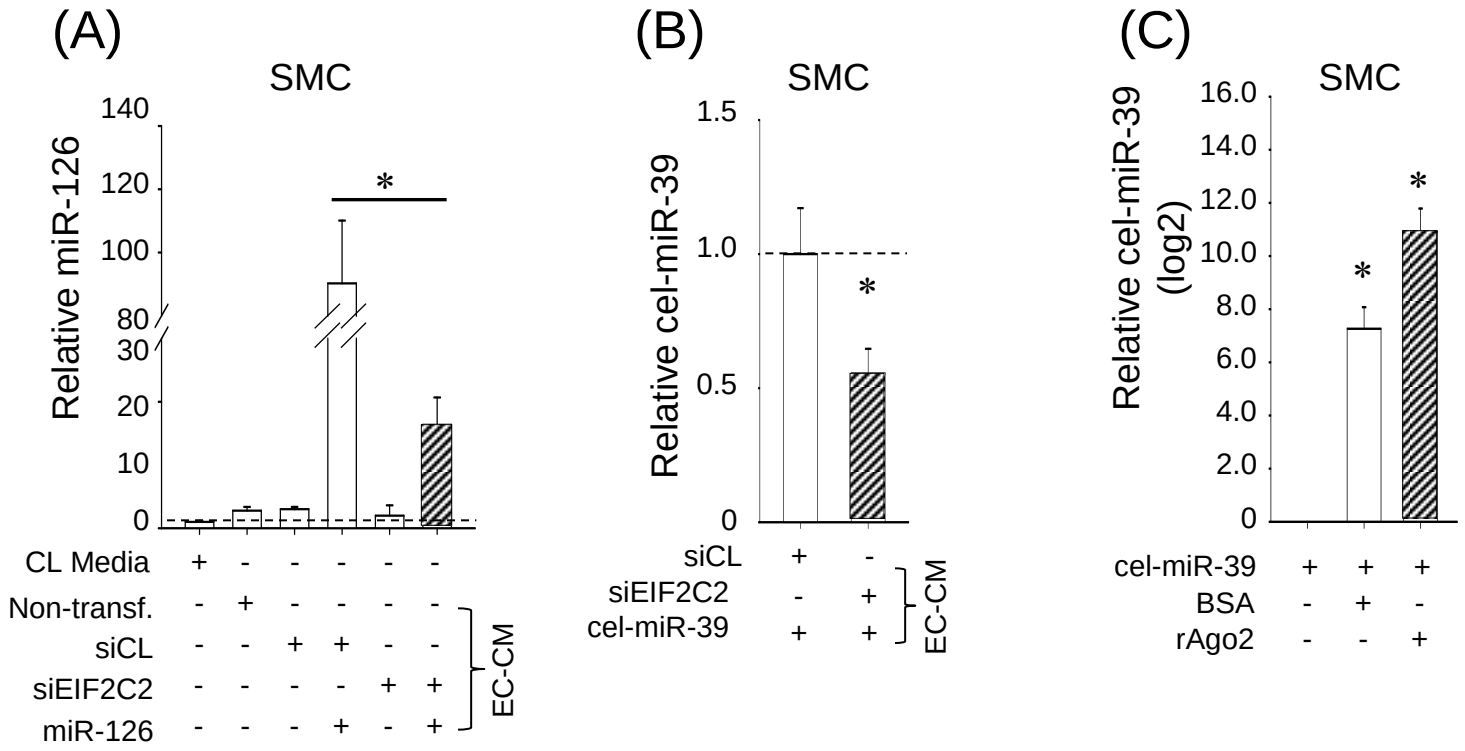
Online Figure III. MiR-126 represses FOXO3, BCL2, and IRS1 in SMCs. SMCs were transfected with AM126, PRE126 or the negative control molecules and the expressions of FOXO3, BCL2, or IRS1 were determined by quantitative RT-PCR. Error bars represent the minimum and maximum value of data sets.



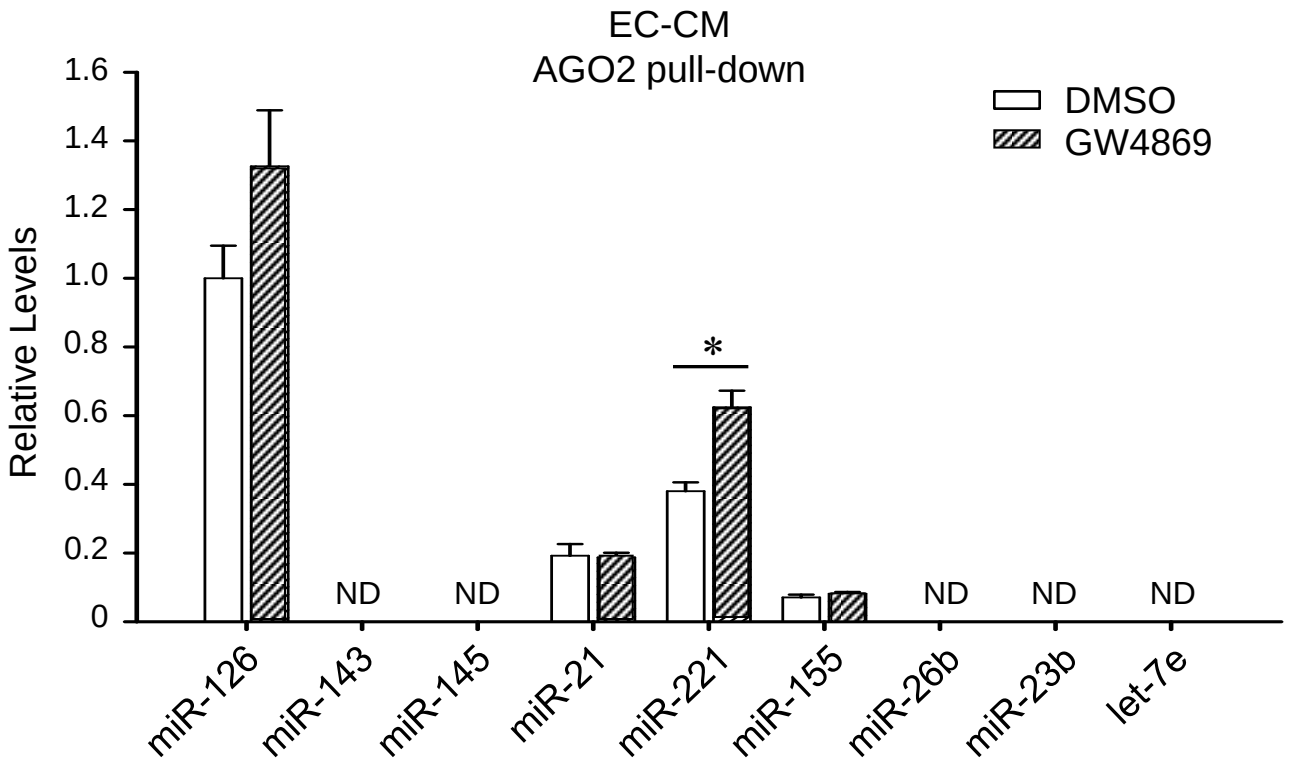
Online Figure IV. Inhibition of the ceramide signalling pathway or apoptotic body formation in ECs doesn't impair the transmission of miR-126 from ECs to SMCs. *(A),* ECs were pre-treated with nSMase2 inhibitor GW4869 (20 μmol/L) or DMSO for 2 hr and then the culture media were refreshed. SMCs were incubated with CL Media or the 24 hr-conditioned media from the pre-treated ECs for 3 hr and the levels of miR-126 in SMCs were determined by quantitative RT-PCR. * $P < 0.05$ *vs*. CL Media or DMSO control. *(B),* ECs were pre-treated with apoptotic inhibitor sc-205596 (50 μg/ml or 100 μg/ml) or DMSO for 1 hr and then kept for the collection of EC-CM *(Left)* or co-cultured with SMCs *(Right)*, the miR-126 levels in EC-CM *(Left)* or in the co-cultued SMCs *(Right)* were determined by quantitative RT-PCR 24 hr post treatment. * $P < 0.05$ *vs*. mono-culture control.



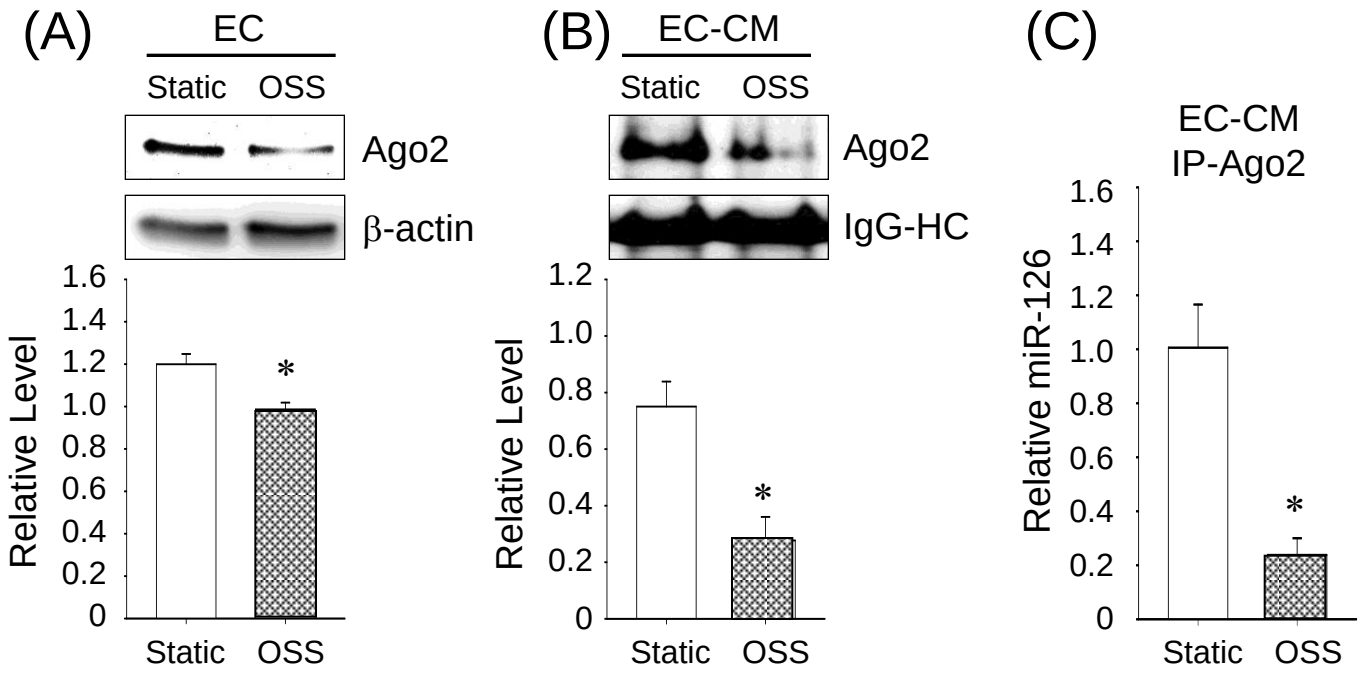
Online Figure V. Exogenous Ago2 associates with miR-126 in ECs and SMCs. SMCs were mono-cultured or co-cultured with BAECs that had been co-transfected with miR-126 and pIRESneo-FLAG/HA Ago2, a plasmid encoding the FLAG/HA-tagged human Ago2 protein (FLAG-Ago2), or two control plasmid expressing FLAG-tagged proteins (FLAG-CL1 and FLAG-CL2). Co-immunoprecipitation assay was performed on ECs *(A)* and SMCs *(B and C)* with anti-FLAG antibody or control IgG. The enrichment of miR-126 associated with FLAG-tagged proteins was determined by quantitative RT-PCR. * $P < 0.05$ *vs*. FLAG-Ago2 *(A)* or mono-culture control *(B)* or IgG control (*C)*.



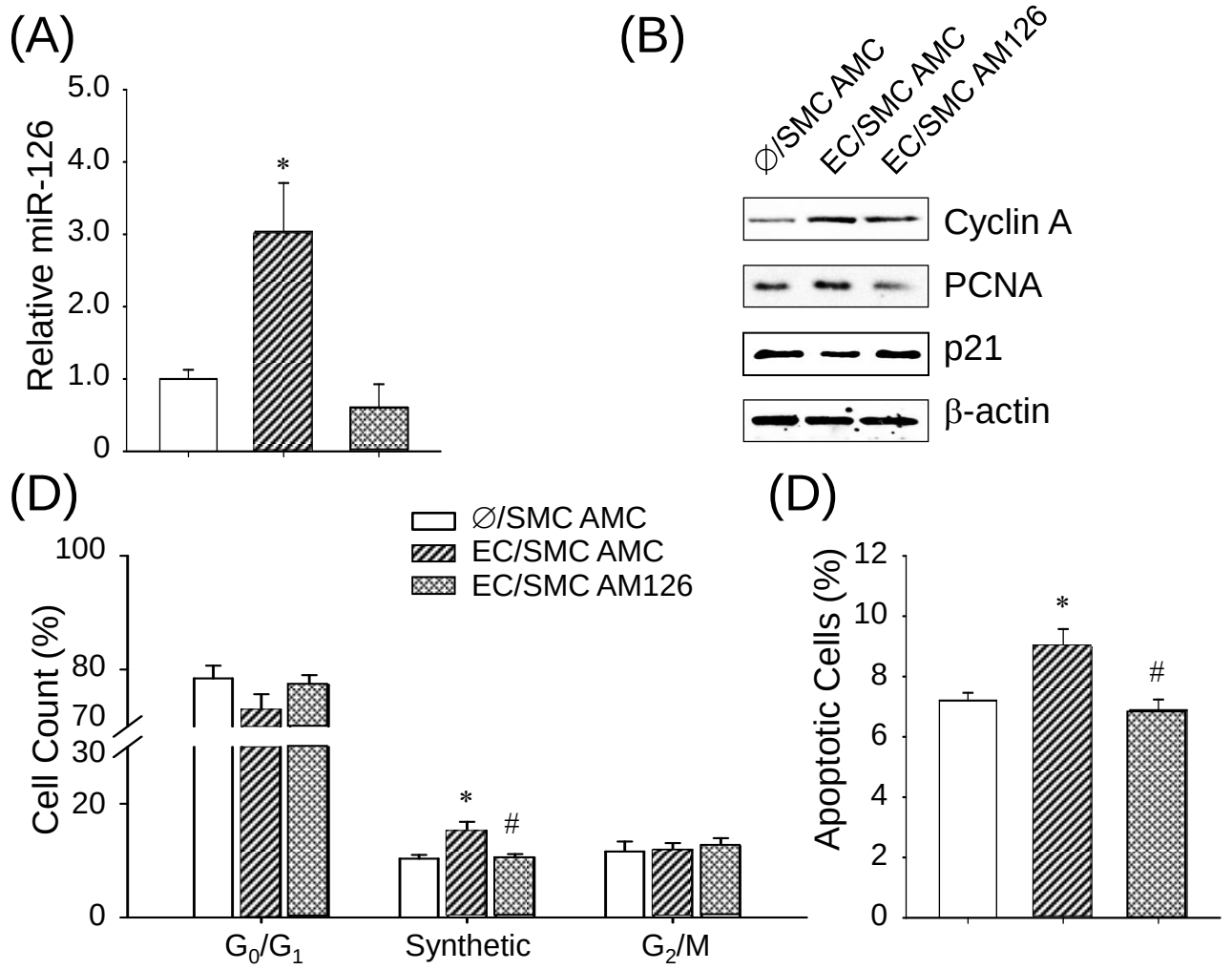
Online Figure VI. Ago2 facilitates the transmission of miR-126 and cel-miR-39 from ECs to SMCs and the uptake of cel-miR-39 by SMCs. *(A)* and *(B)*, SMCs were incubated with CL media or EC-CM from ECs co-transfected with miR-126 or cel-miR-39 and control siRNA (siCL) or specific siRNA of Ago2 (siEIF2C2), and the levels of miR-126 *(A)* or cel-miR-39 *(B)* in SMCs were determined by quantitative RT-PCR. * $P < 0.05$ *vs*. siCL. *(C)*, Synthetic cel-miR-39 was kept alone or pre-incubated with BSA or rAgo2 for 30 min, and then used to treat SMCs for 3 hr. The enrichment of cel-miR-39 in SMCs was determined by quantitative RT-PCR. * $P < 0.05$ *vs*. miRs only.



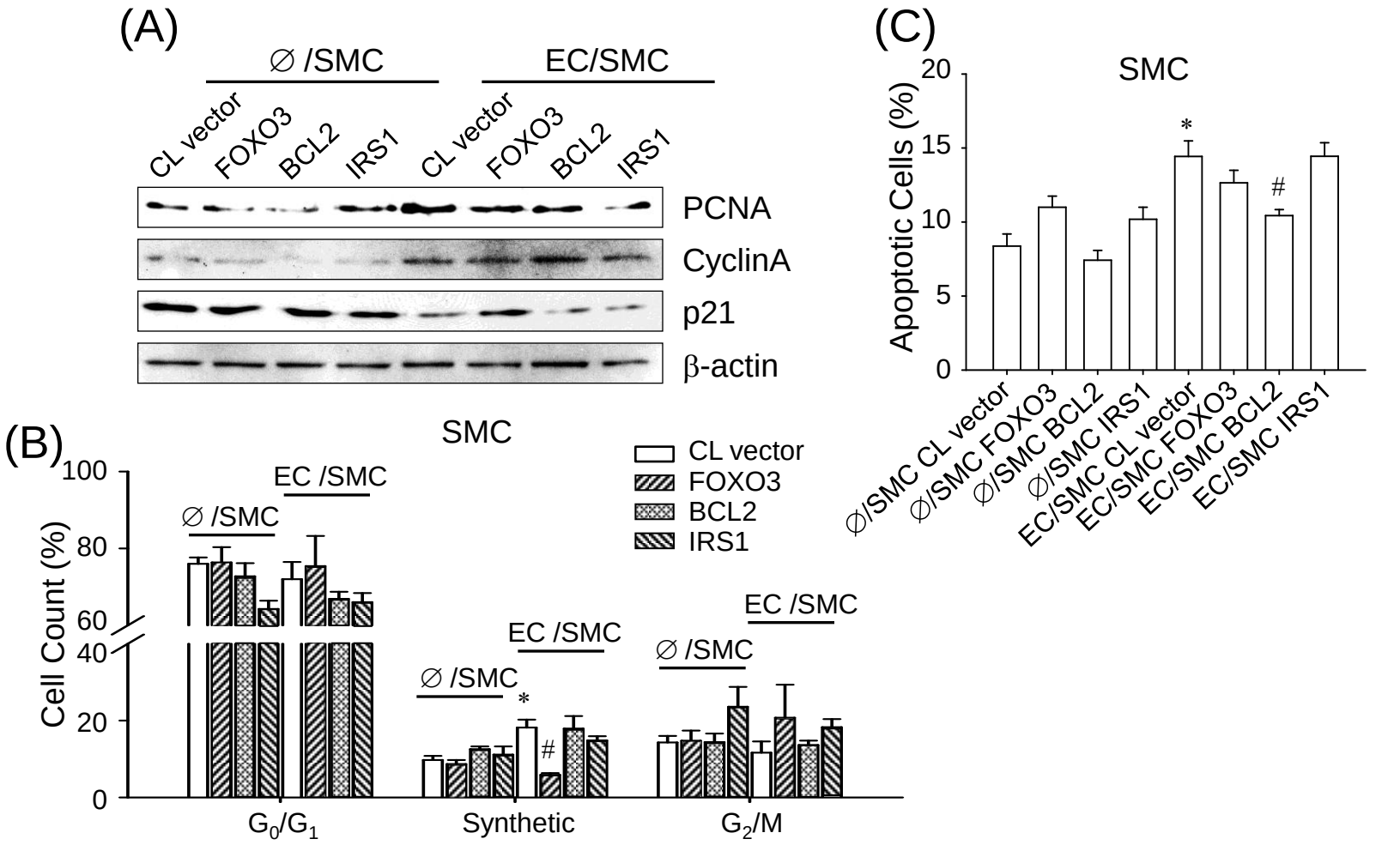
Online Figure VII. Inhibition of the ceramide signalling pathway in ECs doesn't impair the association of miRs with Ago2. ECs were pre-treated with nSMase2 inhibitor GW4869 (20 μmol/L) or DMSO for 2 hr and then the culture media were refreshed. 24 hr later, EC-CM were collected and the association of indicated miRs with Ago2 were determined by Ago2 pull-down and followed by quantitative RT-PCR. ND: undetectable. * $P < 0.05$ *vs*. DMSO control.



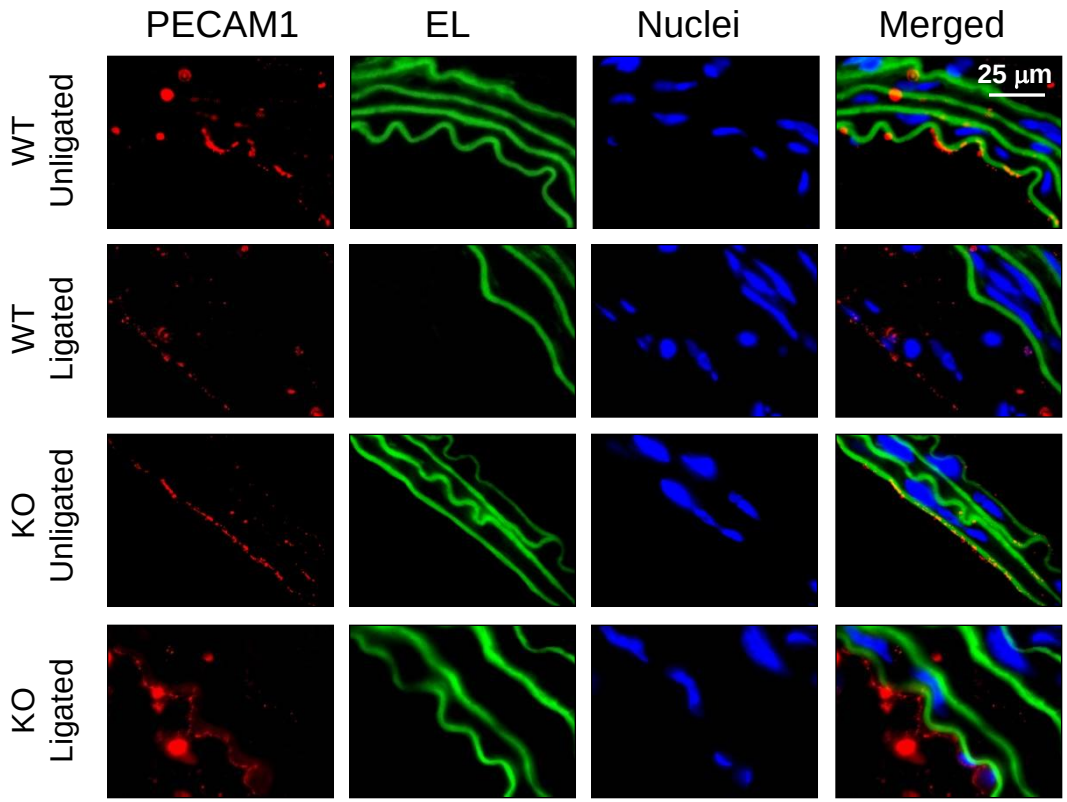
Online Figure VIII. OSS regulates expression and secretion of Ago2 in ECs. ECs were kept as static controls or subjected to OSS for 24 hr, and the levels of Ago2 in the cells *(A)* or the EC-CM *(B)* were determined by Western blot or immunoprecipitation followed by Western blot, respectively. Images are representative of triplicates with similar results. Semi-quantification results are shown in the lower panels. *(C),* The levels of miR-126 associated with Ago2 in the EC-CM were determined by co-immunoprecipitation followed by quantitative RT-PCR. * $P < 0.05$ *vs*. Static.



Online Figure IX. Inhibition of miR-126 in SMCs abolished EC co-culture-induced SMC turnover. SMCs were transfected with AMC or AM126 and then mono-cultured or co-cultured with ECs. MiR-126 levels in SMCs were determined by qRT-PCR *(A)*. Protein levels of PCNA, cyclinA, and p21 in SMCs were assayed by Western blot *(B)*. The SMCs were stained with propidium iodide and analyzed for DNA content by flow cytometry to show cell cycle distribution *(C)*, or double stained with Annexin V and propidium iodide and analyzed to show cell apoptosis *(D)*. * $P < 0.05$ *vs*. ∅/SMC transfected with AMC. # $P < 0.05$ *vs*. SMCs transfected with AMC and co-cultured with ECs.



Online Figure X. Overexpression of miR-126 targets differentially affects turnover of SMCs co-cultured with ECs. SMCs were transfected with control (CL) vector or plasmids overexpressing FOXO3, BCL2, or IRS1, and then were mono-cultured or co-cultured with ECs. The levels of PCNA, cyclinA, and p21 in SMCs were assayed by Western blot *(A)*. The SMCs were stained with propidium iodide and analyzed for DNA content by flow cytometry to show percentages of cells in G0/G1, S, and G2/M phases of the cell cycle *(B)*. The SMCs were double stained with Annexin V and propidium iodide and analyzed by flow cytometry to show percentages of cells underwent apoptosis *(C)*. * $P < 0.05$ *vs*. ∅/SMC transfected with CL vector. # $P < 0.05$ *vs*. SMCs co-cultured with ECs.



Online Figure XI. Endothelium is present in ligated or unligated mouse carotid arteries. Representative images of immunofluorescent stained PECAM1 of lighted or unligated carotid arteries from WT or KO mice.