Supplemental Materials

Methods

Antibodies and reagents

The following antibodies and chemicals were acquired: Anti-p16 (orb228122; Biorbyt, San Francisco, CA), anti-MKRN1 (GTX106459; Genetex, Irvine, CA), anti-p90RSK (MAB 2056; R&D Systems, Minneapolis, MN), and anti-α-tubulin (T5168; Sigma-Aldrich, St. Louis, MO) were purchased. In addition, antibodies against TERF2IP (5433), anti-cleaved caspase3 (9664), anti-FLAG (14793), and p21 (2947) were purchased from Cell Signaling Technology (Beverly, MA). Anti-intracellular adhesion molecule-1 (ICAM-1) (8439), anti-VE-cadherin (SC-48), and anti-VCAM-1 (H-276, SC-8304) were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-TERT (ab32020) and anti-TRF2 (ab108997) were from Abcam (Cambridge, MA); anti-VCAM-1 (NBP1-4749) was from Novus Biologicals (Littleton, CO); and anti-platelet EC adhesion molecule-1 (PECAM-1) (550274) was from BD Biosciences (Franklin Lakes, NJ). Protease inhibitor cocktail (p8340), PMSF (36978), and NEM (E3876) were from Sigma-Aldrich.

The lipid profile kit (ab65390) was from Abcam (Cambridge, MA); the iQSYBR Green Supermix (1708882) and iScript cDNA synthesis kits (1708890) were from Bio-Rad (Hercules, CA); the TL PNA kit/FITC flow cytometry kit (K5327) was from Agilent Technology (Santa Clara, CA); the FITC Annexin V apoptosis detection kit (556547) was from BD Biosciences (Franklin Lakes, NJ); and the 8-hydroxy-2'-deoxyguanosine chemiluminescence detection reagent kit (NEL105001EA) was from PerkinElmer (Waltham, MA). We also purchased an anti-phospho-histone H2A.X (Ser139) antibody, clone JBW301, FITC conjugate (γ-H2A.X, 16-202A), and ApopTag peroxidase in situ apoptosis detection kit (S7100) from Millipore (Burlington, MA), FITC conjugate mouse CD31 from BioLegend (102405, San Diego, CA), TaqMan reverse transcription reagents (N808-0234; Applied Biosystems, Foster City, CA), an efferocytosis kit (4649; Essen Biosciences, Ann Arbor, MI), a dual luciferase assay kit (E1910; Promega Life Sciences, Fitchburg, WI), and Lipofectamine 2000 transfection reagent (11668027; ThermoFisher Scientific, Waltham, MA).

siRNA

Short interfering RNA (siRNA-SMARTpool) targeting human TERF2IP (L-021219-00-0010) and MKRN1 (L-006959-00-0005) was purchased from Thermo Scientific Dharmacon. TRF2 siRNA was purchased from Santa Cruz (sc-3805). Non-specific siRNA negative control was purchased from Invitrogen (#12935-112).

Generation of plasmids and adenoviruses

Plasmids containing rat WT p90RSK1 (WT-*p90rsk*) (Genebank NM031107) and dominant-negative (kinase dead) *p90rsk1* with K94A/K447A mutations (DN-*p90rsk1*) were generated as we previously described(1). pLPC-human TERF2IP full length **(#**12542**)** was from Addgene. pCMV-Flag-TERF2IP-WT was obtained by subcloning TERF2IP from pLPC-human TERF2IP full length into pCMV-Tag2B vector (Agilent Technologies, Santa Clara, CA) at sites recognized

by the restriction enzymes *Eco*RI and *Xho*I. pCMV-Flag-TERF2IP-S205A mutant was generated by site-directed mutated pCMV-Flag-MAGI1-WT using a QuikChange site-directed mutagenesis kit (Agilent Technologies) according to the manufacturer's instructions. FLAG-tagged adenoviral vectors containing TERF2IP-WT and -S205A (Ad-Flag-TERF2IP-WT and -S205A) were generated by cloning each corresponding insert from pCMV-Flag-TERF2IP-WT and -S205A into the pENTR1A vector (Life Technologies) at sites recognized by the restriction enzymes *Kpn*I and *Not*I, and then a recombinase reaction was performed to get a pDEST-based vector (#K4930-00, ViraPower Adenoviral Expression System, Promega) following the manufacturer's instructions. pACT-TERF2IP (133-191 aa),TERF2IP-(1-282 aa) and full length were obtained by subcloning TERF2IP from pENTR-Flag-TERF2IP-WT into a pACT vector at sites recognized by the restriction enzymes *BamHI* and *Xba*I. All constructs were verified by DNA sequencing by using vector specific primers. Where indicated, adenovirus containing β-galactosidase (Ad-LacZ) or green fluorescent protein (Ad-GFP) was used as a control.

Generation of human anti-phospho-TERF2IP-S205

An antibody against phosphorylated TERF2IP S205 was made by Pierce Biotechnology Inc. as follows. A peptide corresponding to amino acids (DAPVSP(pS205)SQKLKR) of the human TERF2IP sequence was synthesized. Rabbits were immunized with the synthesized peptide at a 1:1 ratio of saline and adjuvant (maximum 1 mL was used). The initial inoculation was via subcutaneous routes, and subsequent injections were spread out into a minimum of 4 different sites to avoid any lesion formation. After the primary immunization, the rabbits were checked for any adverse reactions including lesion formation, loss of appetite, and non-responsiveness. Once the rabbits passed the initial evaluation, boosts were given at weeks 2, 4, 6, 12, 18, 21, and 28 after the initial inoculation. At the time of each boost, serum was collected and tested for the presence of specific antibodies by ELISA. At the end of the immunization process, the rabbits were euthanized, and the entire serum was collected, absorbed with the same peptide with non-phosphorylated S205, and affinity purified.

Disturbed flow

We performed disturbed flow experiments as we reported previously(2). In brief, we used cones with radial grooves that were 1 mm deep. We showed tracks of fluorescent beads suspended in culture media when grooved and nongrooved cones were rotated at the same speed. Although the non-grooved cone created straight unidirectional tracks indicating steady laminar flow, tracks made by the grooved cone were short and not oriented in the same direction, indicating non-laminar (turbulent) movement of the media in the dish. Furthermore, we confirmed that in response to the flow condition improved by the grooved cone, the ECs became cobble stone-shaped recapitulating the EC morphology observed at sites of disturbed flow in vivo.

Immunofluorescence staining

Immunofluorescence staining was performed on paraffin sections, as described previously(3). In brief, the tissue sections were de-paraffinized, and heat-induced epitope retrieval was performed by boiling them in heat-induced epitope retrieval buffer containing 10 mM sodium citrate and 0.05% Tween 20 (pH 6) for 20 min. The slides were first blocked by being incubated with 10%

normal goat serum for 60 min at RT and then incubated with primary antibodies (rabbit anti-VCAM 1:200 dilution, rabbit anti-VE-cadherin 1:200 dilution, or rabbit anti-cleaved caspase-3 1:300 dilution or rabbit IgG) at 4°C overnight, followed by incubation with Alexa Fluor 546-conjugated goat anti-rabbit IgG (1:2000 dilution) for 60 min at room temperature. The expression levels of VCAM1 and cleaved caspase 3 were imaged on an Olympus FX1200 MPE confocal laser scanning microscope.

Quantification of immunofluorescent intensities

We quantified the immunofluorescent intensities of the images using ImageJ analysis. First, we selected the region of interest (we quantified the entire confocal image captured at 40X from at least 3 different fields/animal) using the selection tool located on the ImageJ selection toolbar. The l-flow and d-flow areas within the aorta were identified on the basis of published and generally accepted anatomical locations where such flow patterns are known to occur, as we and others reported previously(2, 4-6). A typical l-flow area is located in the greater curvature area(5), which is also known as a high wall shear stress area. A d-flow area is the lesser curvature area, which is also a low wall shear stress area, as described previously(2, 4-6). As we reported previously (7), we also used the EC shape, outlined by anti-VE-cadherin staining, to identify l-flow areas (elongated cell shape) and d-flow areas (irregular cell shape). We adjusted the color threshold of the region of interest (Image\Adjust\Color Threshold). From the Analyze menu, we selected "Set Measurements", and then selected the field, min and max gray values, integrated density, and mean gray value. Finally, we selected "Measure" and read the values (integrated density) from a popup box with a stack of values for those regions of interest. The same procedure was performed for regions next to regions of interest that had no fluorescence. This served as the background. The corrected total region fluorescence was calculated as integrated density - (mean fluorescence of selected fields - background readings).

En face staining of mouse aortas

We performed en face staining of mouse aortic arches at the greater curvature area, as described in our previous reports(1, 2, 7).

Serum lipid profile analysis

Mice that had fasted overnight were euthanized with CO_2. Whole blood was collected in a 1.5-mL tube, allowed to clot for 45 min at room temperature, and centrifuged at 1,500 × *g* for 30 min at 4ºC. The cholesterol levels (high- and low-density lipoprotein) were determined using cholesterol assay kits (cat #EHDL-100, BioAssay Systems, Hayward, CA).

Isolation of mouse lung endothelial cells (MLECs)

MLECs were isolated as described previously(1). One day before cell isolation, anti-PECAM-1-conjugated Dynabeads were prepared by first conjugating the beads with sheep anti-rat IgG (cat. no. 11035; Invitrogen, Carlsbad, CA) and then incubating them with rat anti-mouse PECAM-1 (purified rat anti-mouse CD31, #553370; BD Pharmingen, San Diego, CA) in a cold room overnight following the manufacturer's instructions. Whole animal perfusion was performed

using PBS. Lungs were harvested, washed thoroughly in cold PBS, minced finely with scissors, and then digested in collagenase (2 mg/mL, #4177; Worthington Biochemical Corp., Lakewood, NJ). The resulting lung tissue suspension was triturated 15-20 times using a 20-mL syringe attached to a cannula, avoiding frothing, and was filtered through a cell strainer with 70 μm pore size. The crude cell preparation was pelleted and resuspended in cold Dulbecco's PBS with Ca/Mg (#SH30264.01; HyClone Laboratories) and 0.1% BSA (#A9576; Sigma-Aldrich). The cell suspension was then incubated with anti-PECAM-1-conjugated Dynabeads (35 μL beads/mL cell suspension) at room temperature for 30 min with end-over-end rotation. After incubation, cells bound to the beads were recovered using a magnetic separator stand (#Z5410; Promega, Madison, WI), washed with DMEM containing 20% FBS, and then suspended in a complete culture medium (DMEM containing 20% FBS supplemented with 100 μg/mL porcine heparin, 100 μg/mL ECGS (#2759; Sigma-Aldrich), MEM nonessential amino acids [#25025Cl; Corning], 1 mM sodium pyruvate (#15323581; Corning), and 1% P/S and plated in 0.2% gelatin-coated Petri dishes. When the cells reached 70-80% confluence, they were detached with trypsin-EDTA to generate a single-cell suspension, pelleted, resuspended in Dulbecco's PBS with Ca/Mg and 0.1% BSA, and incubated with anti-PECAM-1–coated beads as described previously(8). The cells bound to beads were collected, washed, cultured in a complete culture medium, and used for experiments when they became confluent. Cells were used for experiments at passage 3.

Isolation of mouse aortic endothelial cells (MAoECs)

Mice, 8–12 weeks old, were anesthetized and euthanized by rapid cardiac excision. The whole aorta was carefully excised and placed in DMEM supplied with 20% FBS. The aortas were cleaned of excess fat and connecting tissues. The aortas were minced into 1 mm fragments, and dispersed in PBS with 100 mM $CaCl_2$ and $MgCl_2$ containing 5 mg/mL collagenase type 2 (cat. no LS004174; Worthington Biochemical), 2 mg/mL glucose, and 30 U/mL DNAse I (cat. number LS006331; Worthington Biochemical) under constant agitation for 45 min (180 rpm at 37°C). Enzyme activity was stopped by the addition of a 10% solution of FBS in PBS. Any remaining clumps of cells were dispersed by forcing through a sterile 18 G needle 20 times. The resulting mixture was filtered through 40 μm cell strainer (cat. no 352340; BD Biosciences, San Jose, CA), and the single-cell suspension was washed twice in DMEM with centrifugation at 400 × *g* for 6 min. The final cell pellet was re-suspended in 6 ml of DMEM. The rest of the process was the same as that for MLECs, and used for experiments when they became confluent. Cells were used for experiments at passages 2.

Human endothelial cells culture

Human umbilical vein ECs (HUVECs) were obtained from collagenase-digested umbilical cord veins(9) and collected in M200 medium supplemented with LSGS (Cascade Biologics, Inc., Portland, OR) and 2% FBS (Atlanta Biologicals, Inc., Lawrenceville, GA). HUVECs were cultured in 0.2% gelatin pre-coated dishes and used in experiments between 3 and 7 passages. Human aortic endothelial cells (HAECs) were isolated from aortic explants of heart transplant donors of anonymous origin through the UCLA transplant program as previously described (10). Our HAEC population was derived from aortic explants of anonymous heart transplant donors. Therefore, we have no individual information, including ethnicity, history, or disease

status(11). HUVECs and HAECs were cultured in Petri dishes or flasks coated with 0.2% gelatin type A (#901771; MP Biomedicals, Santa Ana, CA), in Endothelial Cell Medium (ECM, #1001, ScienCell, Carlsbad, CA) containing 465 mL of basal medium, 25 mL of FBS (#0025, ScienCell), 5 mL of Endothelial Cell Growth Supplement (ECGS, #1052, ScienCell) and 5 mL of penicillin/streptomycin solution (P/S, #0503, ScienCell). HAECs with less than 15 passages were used in this study.

Characterization and selection of ECs

Cells were grown on cover slips in a 6-well plate. Cells were washed two times with PBS and then fixed with cold methanol for 10 min at -20°C. Next, cells were incubated with blocking buffer (1% FBS in PBS) for 30 min at room temperature. Cells were incubated with FITC-anti-CD31 (2.5 mg/mL) overnight at 4°C. After incubation, cells were washed three times with wash buffer (0.1% BSA in PBS), mounted with gold antifade, and photographed using a microscope.

Primary ECs with passage 4 can respond to flow. We used HAECs to perform microarray analysis (Supplemental Figure 1) and detect the roles of p90RSK and TERF2IP in EC senescence (Figure 1A, C, D). Since we found HAECs and HUVECs to be similar in the way that flow or inflammatory cytokines affected TERF2IP-mediated EC activation and senescence between HAECs and HUVECs (Supplemental Figure 1D (HUVECs and HAECs) and E (HAECs), Supplemental Figure 2A (HUVECs), Figure 1J (HUVECs), and Figure 4C(HAECs)), we performed detailed mechanistic analyses in HUVECs, which are more facile to use (e.g. more rapid proliferation in vitro). We also compared d-flow-induced 8-oxo-dG induction in bovine aortic endothelial cells and HUVECs in Figure 1A (BAECs) and B (HUVECs) and found similar tendencies of KD-p90RSK-mediated inhibition of d-flow-induced 8-oxo-dG, also suggesting that HUVECs can recapitulate the d-flow-mediated biology in ECs isolated from the aorta.

Transfection and transduction

ECs were transfected with siRNA molecules at a final concentration of 100 nM or appropriate reporter plasmids or plasmid DNAs using Lipofectamine 2000 transfection reagent, according to the manufacturer's instructions. After transfection, cells were allowed to recover in complete medium for 24-48 h. For adenovirus transduction, we used 20 multiplicities of infection for each adenovirus. Prior to use, cells were cultured overnight in a low-serum (1% FBS) medium.

Western blotting analysis

ECs were washed twice with cold PBS, and whole cell lysates were prepared in RIPA buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40, 0.1% SDS, 1 mM dithiothreitol, 1:200-diluted protease inhibitor cocktail [P8340, Sigma-Aldrich], and 1 mM PMSF). Total lysates were resolved by SDS-PAGE and electrotransferred onto a Hybond enhanced chemiluminescence nitrocellulose membrane, which was then incubated with antibodies against each of the proteins to be detected in the lysate. Bound antibodies were visualized using enhanced chemiluminescence detection reagents (Amersham Pharmacia Biotech) according to the manufacturer's instructions and quantified by densitometry using ImageJ software. Tubulin was used as the loading control and was always probed together with

the specific protein of interest. The immunoblotted band intensities of the proteins of interest were standardized against the intensity of the anti-tubulin band. In most cases, anti-tubulin immunoblots were not shown in each figure to save space.

CheckMate mammalian two-hybrid assay

A dual-luciferase reporter assay system (#E1960; Promega)(1, 12) was used to study p90RSK-TEFR2IP interaction, in addition to a co-IP approach. ECs were co-transfected with a full-length human TERF2IP fused to the activation domain VP16 (pACT-TERF2IP), a full-length p90RSK fused to the Gal4-binding domain (pBIND-p90RSK), and a Gal4-responsive luciferase reporter (pG5-Luc). In some cases, the transfection mixture also contained pACT-TERF2IP-Myb or control vector. After 24 h of transfection, cells were harvested in 1X passive lysis buffer (cat. no. E194A; Promega), and luciferase activity in the resulting cell lysates was measured using a GloMax 20/20 Luminometer (Promega). An increase in luciferase activity, which was calculated by normalizing firefly luciferase activity according to Renilla luciferase activity (ratio of firefly luciferase activity to Renilla luciferase activity), indicates the binding of p90RSK with TERF2IP.

References

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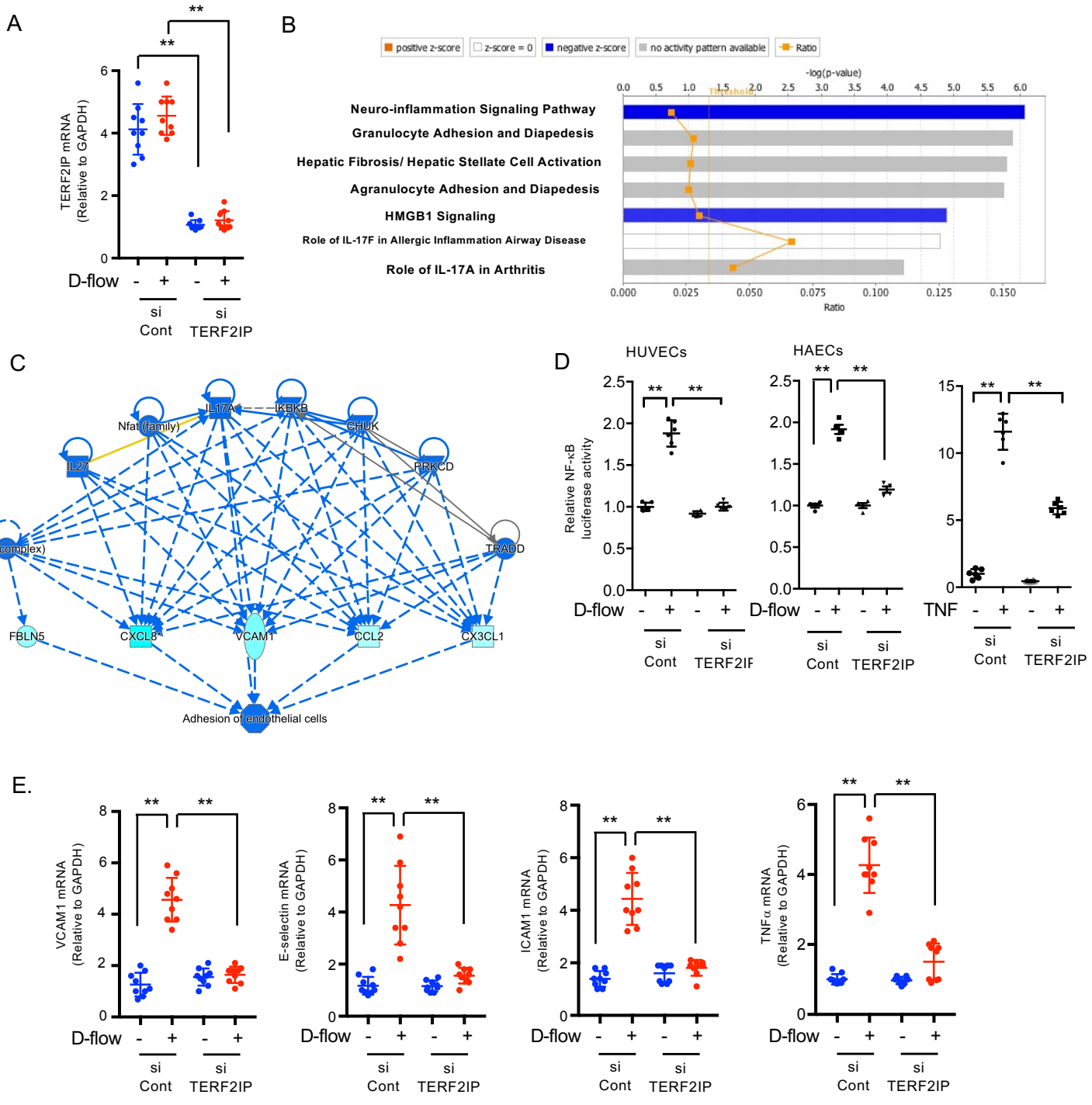
Supplemental Table 1. Abbreviations used in Supplemental Figure 1C.

Symbol	Entrez Gene Name	Expr Log Ratio	Expr p-value
Adhesion of endothelial cells			
CCL2	C-C motif chemokine ligand 2	16.95	0.018214
CHUK	conserved helix-loop-helix ubiquitous kinase		
CX3CL1	C-X3-C motif chemokine ligand 1	7.83	0.012507
CXCL8	C-X-C motif chemokine ligand 8	12.19	0.00553
FBLN5	fibulin 5	11.52	0.024327
IKBKB	inhibitor of nuclear factor kappa B kinase subunit beta		
IL17A	interleukin 17A		
IL27	interleukin 27		
Nfat (family)	Nfat factors, Nuclear factor of activated Tcells		
PI3K (complex)	Phosphatidylinositol 3 kinase		
PRKCD	protein kinase C delta		
TRADD	TNFRSF1A associated via death domain		
VCAM1	vascular cell adhesion molecule 1	9.29	0.03877

The activation of granulocytes was the highest scoring regulator effect network on the basis of the results of a right-tailed Fisher exact test (consistency core = 598.131, Supplemental Figure1C). Expr Log Ratio: Log2 ratio differential expression.

Supplemental Table 2. List of qRT-PCRT primers.

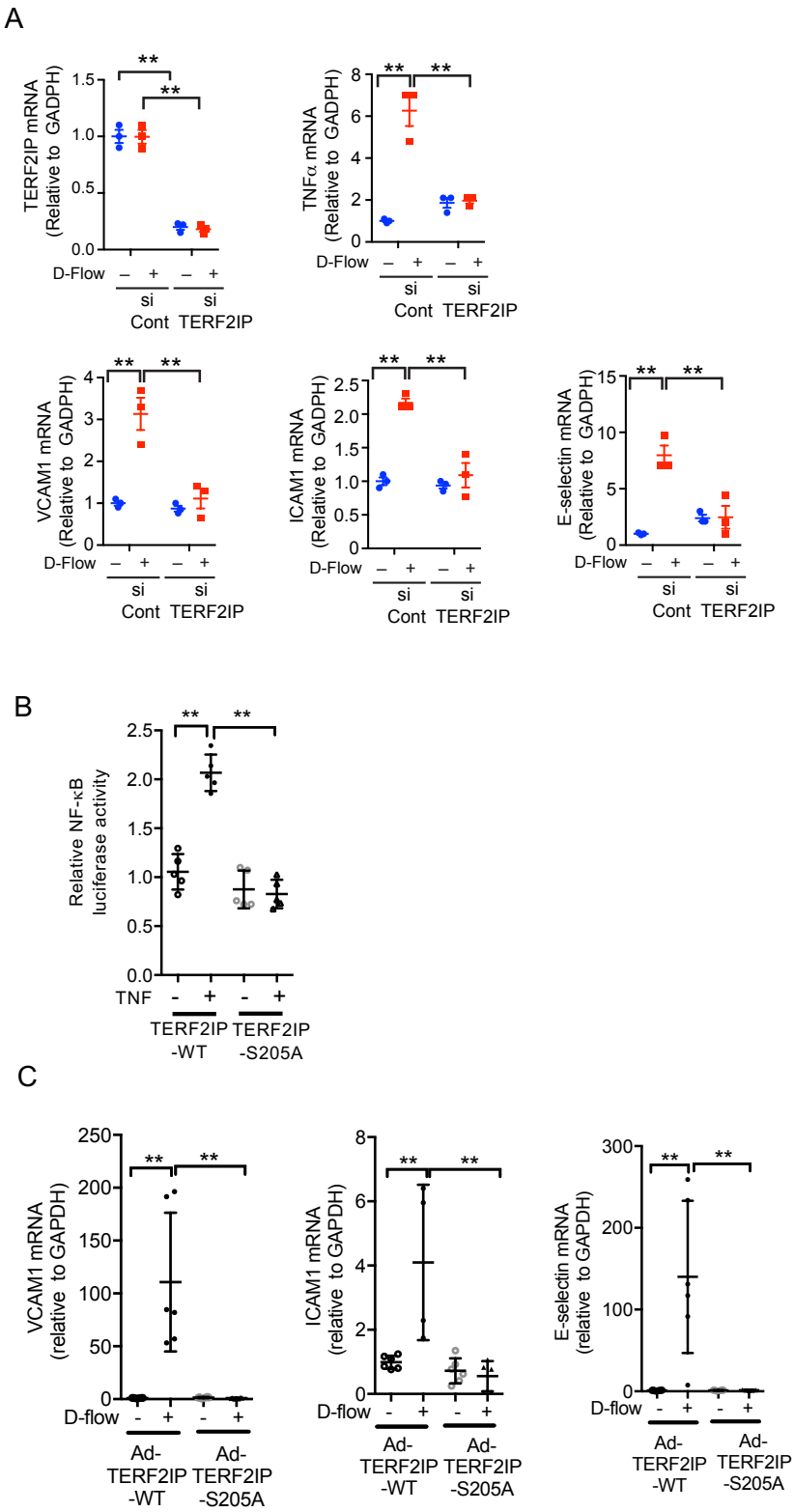
Primers	Sequences
h-GAPDH-F	5'-GGT GGT CTC CTC TGA CTT CAA CA-3'
h-GAPDH-R	5'-GTT GCT GTA GCC AAA TTC GTT GT-3'
h-VCAM1-F	5'-CCG GAT TGC TGC TCA GAT TGG A-3'
h-VCAM1-R	5'-AGC GTG GAA TTG GTC CCC TCA-3'
h-ICAM1-F	5'-GTC CCC TCA AAA GTC ATC C-3'
h-ICAM1-R	5'-AAC CCC ATT CAG CGT CAC C-3'
h-ESELECTIN-F	5'-GCT CTG CAG CTC GGA CAT-3'
h-ESELECTIN-R	5'-GAA AGT CCA GCT ACC AAG GGA AT-3'



Supplemental Fig.1

Supplemental Figure 1. Depletion of TERF2IP inhibits inflammation-related signaling and expression of genes and adhesion molecules in ECs exposed to d-flow

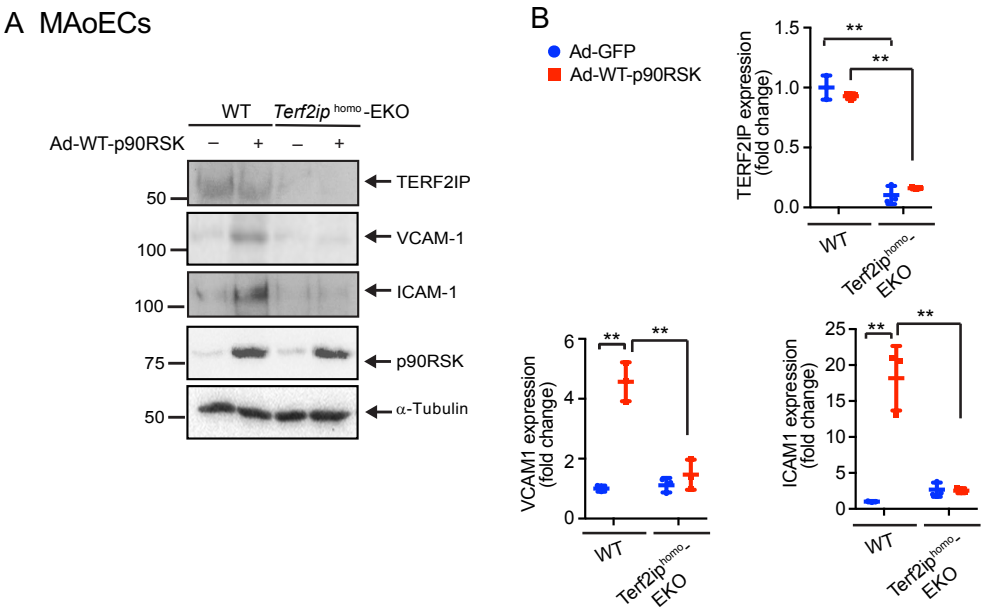
(**A**) After 48 h of control siRNA or TERF2IP siRNA transfection, HAECs were subjected to d-flow for 16 h, and expression levels of TERF2IP mRNA were analyzed by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 8-9. ** p <0.01. (**B**) Differential regulation of canonical pathways in human aortic endothelial cells (HAECs) treated with control or TERF2IP siRNA was analyzed using Ingenuity Pathway Analysis (-log[p]>4). The bar chart indicates the -log(p) value of the significance of enrichment. The line represents the ratio of differentially expressed genes from the microarray data set to the genes present in each canonical pathway. Positive z-score predicts increased pathway activity; negative z-score predicts decreased pathway activity. (**C**) The regulator effects algorithm (consistency score, 14.311) was determined by gene enrichment analyses (right-tailed Fisher exact test p-value <0.001, absolute value of z-score >2.0) according to the Ingenuity Pathway Analysis upstream regulator and downstream effects analysis (we only included categories of cardiovascular disease and cardiovascular system development and function) in our microarray data set. Light blue symbols denote genes with lower expression, and dark blue symbols denote the predicted inhibition of upstream regulator networks and adhesion of ECs as downstream functions or disease categories affected by the expression change in our data set (shown in the middle tier) in TERF2IP siRNA-treated HAECs compared with control siRNA-treated cells. The arrows with blue solid lines indicate direct (usually physical) inhibitory interactions between 2 molecules in the direction of the arrow, whereas arrows with blue dashed lines denote indirect inhibitory interactions (e.g., molecule/gene A affects molecule/gene B). The yellow line shows that the findings are inconsistent with the state of the downstream molecule. The abbreviations are defined in Supplementary Table 1. (**D**) HUVECs and HAECs were transfected with control siRNA and TERF2IP siRNA (together with an NF-κB reporter gene) for 24 h, then subjected to d-flow (left) or TNF-α (TNF, 10 μg/mL, right) for 16 h, and NF-κB activity was measured by a luciferase assay. Data represent mean ± S.D., n = 5. **p <0.01, *p <0.05. (**E**) After 48 h of control siRNA or TERF2IP siRNA transfection, HAECs were subjected to d-flow for 16 h, and expression levels of VCAM1, E-selectin, ICAM1, and TNF-α mRNA were analyzed by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 9. **p <0.01, *p <0.05. All statistical analyses in this figure were done by 1-way ANOVA followed by Bonferroni post hoc test.

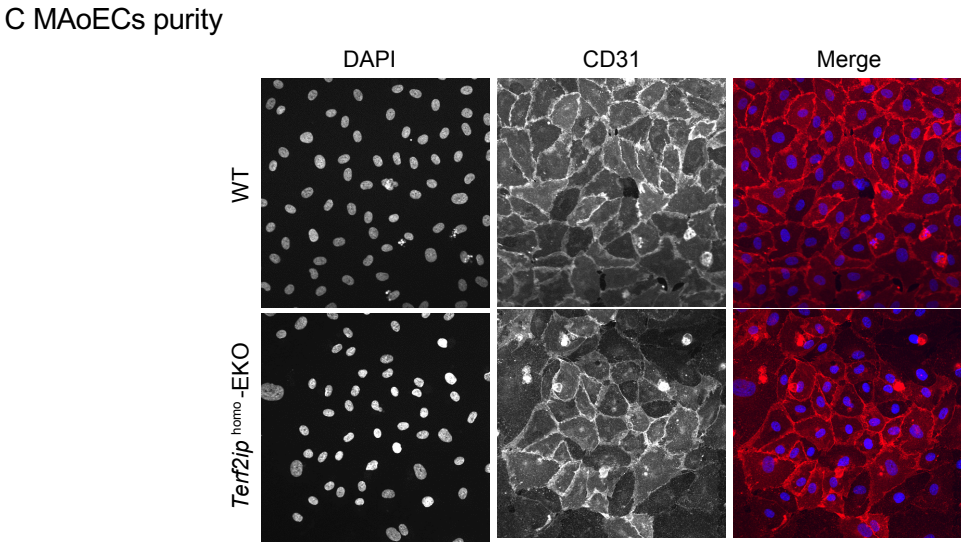


Supplemental Fig.2

Supplemental Figure 2. TERF2IP S205 phosphorylation plays a crucial role in EC activation.

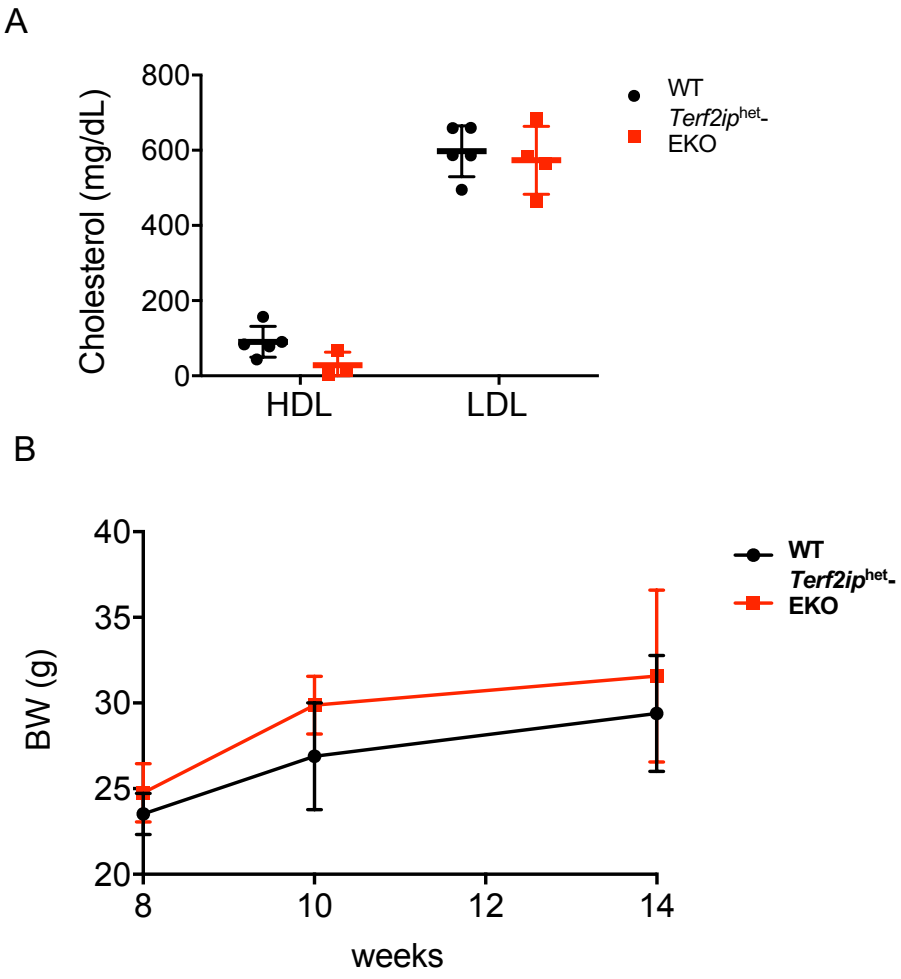
(**A**) After 48 h of control siRNA or TERF2IP siRNA transfection, HUVECs were subjected to d-flow for 16 h, and expression levels of VCAM1, E-selectin, ICAM1, and TNFα mRNA were analyzed by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 3, **p <0.01, *p <0.05. **(B**) HUVECs were transfected with an NF-κB reporter gene and also with either TERF2IP WT or S205A mutant for 24 h and incubated with TNF-α (10 μg/mL) for 24 h. NF-κB activity was detected by luciferase assay. Data represent mean ± S.D., n = 5. (**C**) HUVECs were transduced by Ad-TERF2IP S205A or Ad-TERF2IP WT for 24 h and exposed to d-flow for 16 h. Expression of VCAM1, ICAM1, and E-selectin mRNAs was determined by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 3-5, **p <0.01, *p <0.05. All statistical analyses in this figure were done by 1-way ANOVA followed by Bonferroni post hoc test.





Supplemental Figure 3. Both senescence and expression of inflammation-related molecules induced by overexpression of wild-type p90RSK are inhibited by depletion of TERF2IP in mouse aortic endothelial cells (MAoECs).

(**A, B**) MAoECs were isolated from WT or *Terf2ip*^{homo}-endothelial cell-specific knockout (EKO) mice. After transduction with Ad-p90RSK-WT or Ad-GFP as a control for 12 h, expression of certain proteins was analyzed by Western blotting using specific antibodies as indicated (**A**). Graphs represent densitometry data of immunoblots for selected proteins. Data represent mean ± S.D., n = 3. **p <0.01, *p <0.05 by 1-way ANOVA followed by Bonferroni post hoc test. (**C**) Purity of cultured MAoECs is demonstrated by immunofluorescence staining of CD31, and we observed that most of the cells were CD31 positive. To quantify the purity of the MAoECs, we also subjected the cells to flow cytometric analysis of CD31 staining. We isolated ECs from 6 mice per group, and the purity of CD31-positive cells (%) was 76 ± 2.8 for cells isolated from WT mice and 73 ± 1.4 for cells isolated from Terf2ip^{homo}-EKO mice. Mean ± S.D., n = 2 per group.



Supplemental Figure 4. Cholesterol levels and body weight in wild-type and *Terf2ip*^{het}-endothelial cell-specific knockout (EKO) mice

(**A**) High- and low-density lipoprotein (HDL and LDL) levels after 8 weeks of AAV/D377Y-mPCSK9 injection and a high-fat diet in wild-type (WT) or *Terf2ip*^{het}-EKO mice are shown. (**B**) Body weights (BW) of WT or *Terf2ip*^{het}-EKO mice by age are shown.