SUPPLEMENTARY INFORMATION

Supplementary Materials and Methods

Antibodies and Reagents: Antibodies against mTOR, raptor, Akt, phosphorylated Akt (S473), S6K1, phosphorylated S6K1 (T389), ERK1/2, phosphorylated ERK1/2 (T202/Y204), STAT1, phosphorylated STAT1 (Y701 and S727) and GAPDH were obtained from Cell Signaling Technology (Danvers, MA). Anti-rictor was purchased from Bethyl Laboratories (Montgomery, TX) and rabbit polyclonal anti-DEPTOR was purchased from Millipore (Billerica, MA). Mouse monoclonal anti-DEPTOR antibody was purchased from Novus Biologicals (Littleton, CO). Antibodies against phosphorylated SGK (S422) and SGK were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). U0126 was purchased from Cell Signaling Technology (Danvers, MA), and rapamycin was gifted to the laboratory by Wyeth-Ayest Research (Philadelphia, PA). Torin1 was purchased from R&D Systems (Minneapolis, MN) and TNFα was gifted to the laboratory by Biogen-Idec (Cambridge, MA).

Immunofluorescence microscopy: Human neonatal foreskins and discarded cardiac tissue from patients undergoing surgery were collected as approved by the Institutional Review Board (IRB) at the Brigham and Women's Hospital and Boston Children's Hospital, and were snap frozen in OCT (Sakura Finetek, Torrance, CA). Four-micron cryosections were fixed in 2% paraformaldehyde, and endogenous peroxidase activity was quenched with 3% hydrogen peroxide. After blocking with Tris-NaCl-Blocking (TNB) buffer,

sections were incubated with a mouse anti-human CD31 antibody (Dako, Carpinteria, CA) and a rabbit anti-human DEPTOR antibody (Millipore, Billerica, MA) overnight at 4°C. Control sections were incubated with a single primary antibody alone or with one primary antibody in combination with a respective mouse or rabbit IgG. After washing in PBS, sections were incubated with a secondary HRP-conjugated anti-rabbit antibody (Jackson Immunoresearch, West Grove, PA) for 30 min, and the Tyramide Signal Amplification kit (TSA Plus Cyanine 3 Kit, Perkin Elmer, Waltham, MA) was subsequently used according to the manufacturer's instructions. Finally, sections were incubated with a secondary Alexa Fluor 488-conjugated anti-mouse antibody (Life Technologies, Invitrogen, Grand Island, NY) and mounted with VectorShield reagent with DAPI (Vector Laboratories, Burlingame, CA). Immunofluorescence microscopy was performed using a Zeiss LSM700 laser scanning confocal microscope, using a 63X Zeiss plan-APOCHROMAT oil (1.4 NA) objective (Zeiss, Thornwood, NY). Each image was collected, processed and analyzed using LSM Image Brower (Version 4.2) software.

Cell proliferation assays: HUVEC were transfected with control or DEPTOR siRNAs, cultured for 72 hrs, and proliferation was assessed by [^{3}H] thymidine incorporation (1 μCi/well) in the final 18 hours of cell culture. Cells were harvested with a Tomtec automated cell harvester (Tomtec, Hamden, CT) and incorporated radioactivity was measured using a microplate scintillation counter (Perkin-Elmer, Wallac, Boston, MA).

Apoptosis assays: Apoptosis detection was performed using the Apoptosis Detection Kit from BD Pharmingen (San Jose, CA), according to manufacturer's protocol. Briefly, EC

were harvested, washed and stained with Annexin V-FITC and Propidium Iodide (PI). Fluorescence was measured on 10,000 cells/sample using a FACSCalibur® (BD Biosciences) and analyzed using FlowJo® software (Tree Star, Inc.).

Table S1. Table indicating the identity of individual kinases and proteins tested in the protein phosphokinase array, illustrated in *Figure 3*.

Coordinate	Target / Control
A1, A2	Positive control
A3, A4	p38α (T180/Y182)
A5, A6	ERK1/2 (T202/Y204, T185/Y187)
A7, A8	JNK pan (T183/Y185, T221/Y223)
A9, A10	GSK-3α/β (S21/S9)
A13, A14	p53 (S392)
A17, A18	Positive control
B3, B4	MEK1/2 (S218/S222, S222/S226)
B5, B6	MSK1/2 (S376/S360)
B7, B8	AMPKα1 (T174)
B9, B10	Akt (S473)
B11, B12	Akt (T308)
B13, B14	p53 (S46)
C1, C2	TOR (S2448)
C3, C4	CREB (S133)
C5, C6	HSP27 (S78/S82)
C7, C8	AMPKα2 (T172)
C9, C10	β-Catenin
C11, C12	p70 S6 Kinase (T389)
C13, C14	p53 (S15)
C15, C16	p27 (T198)
C17, C18	Paxillin (Y118)
D1, D2	Src (Y419)
D3, D4	Lyn (Y397)
D5, D6	Lck (Y394)
D7, D8	STAT2 (Y689)

Coordinate	Target / Control
D9, D10	STAT5a (Y699)
D11, D12	p70 S6 Kinase (T421/S424)
D13, D14	RSK1/2/3 (S380)
D15, D16	p27 (T157)
D17, D18	PLCγ-1 (Y783)
E1, E2	Fyn (Y420)
E3, E4	Yes (Y426)
E5, E6	Fgr (Y412)
E7, E8	STAT3 (Y705)
E9, E10	STAT5b (Y699)
E11, E12	p70 S6 Kinase (T229)
E13, E14	RSK1/2 (S221)
E15, E16	c-Jun (S63)
E17, E18	Pyk2 (Y402)
F1, F2	Hck (Y411)
F3, F4	Chk-2 (T68)
F5, F6	FAK (Y397)
F7, F8	STAT6 (Y641)
F9, F10	STAT5a/b (Y699)
F11, F12	STAT1 (Y701)
F13, F14	STAT4 (Y693)
F15, F16	eNOS (S1177)
F17, F18	Negative control
G1, G2	Positive control
G5, G6	Negative control

Table S2. Table summarizing the identity of regulated genes in DEPTOR siRNA-transfected EC vs. control siRNA-transfected EC, using PCR-based arrays.

	Gene Symbol	Description	Fold Induction
Genes upregulated	CXCL10	Chemokine (C-X-C motif) ligand 10 (IP-10)	356.56
	CCL5	Chemokine (C-C motif) ligand 5 (RANTES)	281.70
	IFNB1	Interferon, beta 1, fibroblast	236.22
	CX3CL1	Chemokine (C-X3-C motif) ligand 1 (Fractalkine)	107.49
	CXCL9	Chemokine (C-X-C motif) ligand 9 (MIG)	103.82
	CXCL11	Chemokine (C-X-C motif) ligand 11 (I-TAC)	96.20
	TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b	55.64
	VCAM1	Vascular cell adhesion molecule 1	24.66
	TYMP	Thymidine phosphorylase	21.92
	CCL20	Chemokine (C-C motif) ligand 20	10.11
	TGFB2	Transforming growth factor, beta 2	6.58
	IL12A	Interleukin 12A	5.46
	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	5.44
	CCL8	Chemokine (C-C motif) ligand 8 (MCP-2)	5.20
	LTA	Lymphotoxin alpha (TNF superfamily, member 1)	4.99
	CXCL12	Chemokine (C-X-C motif) ligand 12	4.65
	IL7	Interleukin 7	4.49
	IL11	Interleukin 11	4.30
	NOS2	Nitric oxide synthase 2, inducible	3.61
	CASP1	Caspase 1, apoptosis-related cystein peptidase	3.37
	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	3.35
	EDNRA	Endothelin receptor type A	3.30
	ANGPT1	Angiopoietin 1	3.26
	IL8	Interleukin 8	3.20
	FGF1	Fibroblast growth factor 1 (acidic)	3.15
	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	2.96
	ICAM1	Intercellular adhesion molecule 1	2.57
Genes downregulated	MMP9	Matrix metallopeptidase 9	- 6.26
	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide	- 5.88
	ACE	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	- 5.76
	SELE	Selectin E	- 4.68
	CCL7	Chemokine (C-C motif) ligand7	- 4.60
	PTGIS	Prostaglandin I2 (prostacyclin) synthase	- 4.33
	PF4	Platelet factor 4	- 4.22
	IL1B	Interleukin 1, beta	- 4.16
	IL16	Interleukin 16	- 4.00
	TNFRSF11B	Tumor necrosis factor (ligand) superfamily, member 11b	- 3.23
	SPP1	Secreted phosphoprotein 1	- 3.21
	ALOX5	Arachidonate 5-lipoxygenase	- 2.88
	AGT	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	- 2.80
	THBS1	Thrombospondin 1	- 2.78
	CRADD	CASP2 and RIPK1 domain containing adaptor with death domain	- 2.65
	TNFRSF10C	Tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain	- 2.39
	RPL13A	Ribosomal protein L13α	- 2.24
	PLAT	Plasminogen activator, tissue	- 2.24
	MMP1	Matrix metallopeptidase 1 (interstitial collagenase)	- 2.21
	ITGB1	Integrin, beta 1	- 2.21

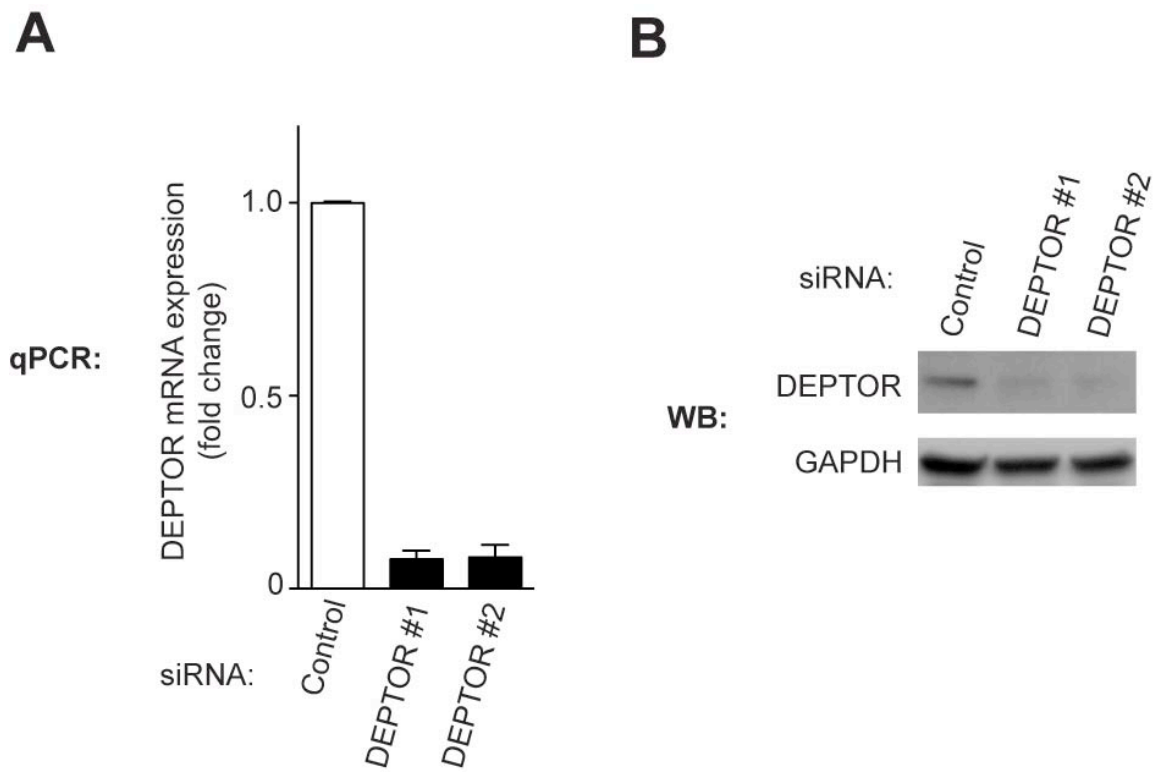

Figure S1. Assessment of DEPTOR knockdown efficiency upon siRNA transfection. HUVEC were transfected with control or two DEPTOR siRNAs, and DEPTOR expression was analyzed after 48 hrs at the mRNA and protein levels. *Panel A,* The bar graph shows the mean level of DEPTOR mRNA expression (±1SEM) from 5 independent experiments. *Panel B*, shows a representative Western blot analysis of DEPTOR and GAPDH expression 48 hrs after transfection.

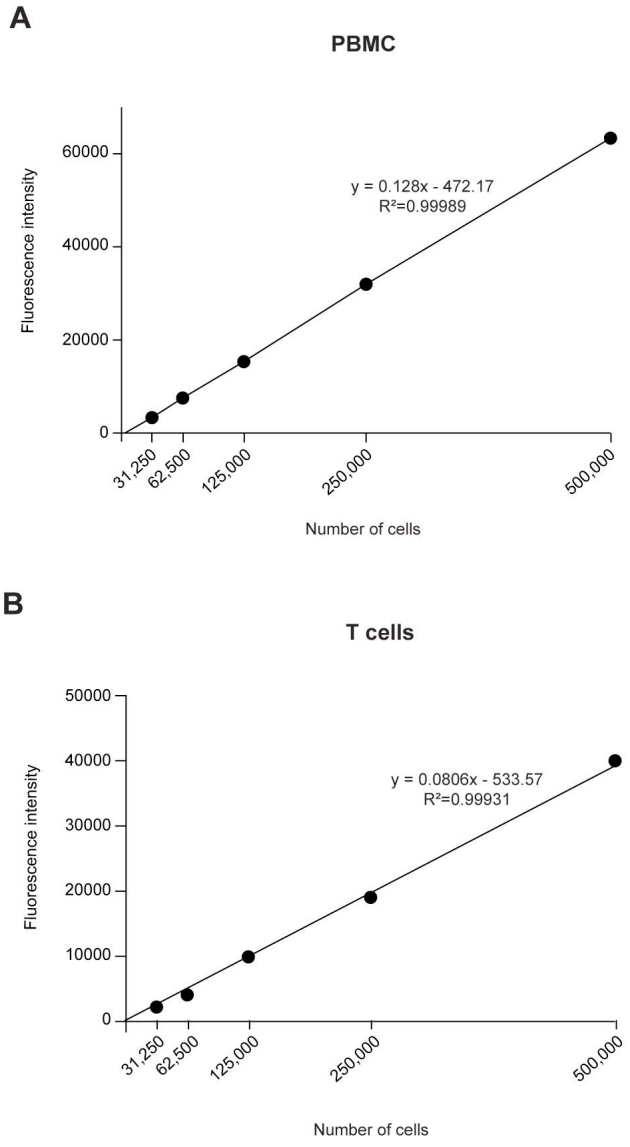

Figure S2. Representative standard fluorescence intensity curves used in the quantitative leukocyte adhesion assays. Representative curves illustrating the fluorescent intensity obtained for increasing numbers of PBMC (*Panel A*), or $CD3^+$ T cells (*Panel B*) stained with CFSE (5 μM).

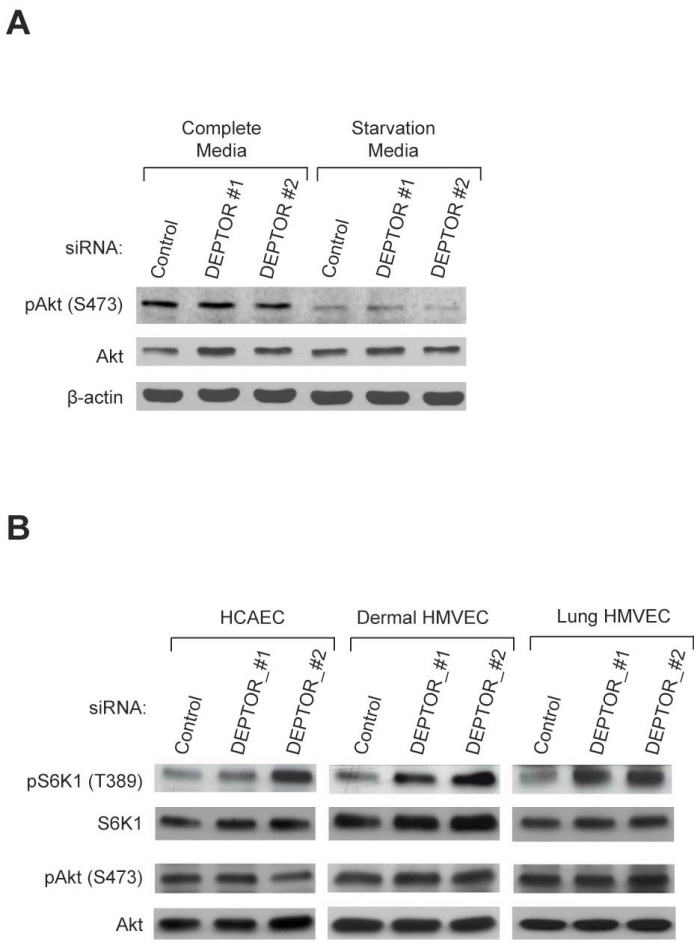

Figure S3. DEPTOR inhibits mTORC1, but not mTORC2 in vascular endothelial cells. *Panel A*, HUVEC were transfected with a control or two DEPTOR siRNAs and cultured for 48 hrs in complete growth media or in a media containing 4 times less FBS and growth supplements (labeled 'Starvation Media'). mTORC2 activity in these cells was subsequently evaluated by analyzing the expression of pAkt (S473), Akt and β-actin by Western blot. A representative blot is shown. *Panel B*, Primary cultures of human coronary artery EC (HCAEC), dermal and lung human microvascular EC (HMVEC) were transfected with control or DEPTOR siRNAs and the expression of pS6K1 (T389), S6K1, pAkt (S473), and Akt was evaluated by Western blot analysis. Data are representative of 3 independent experiments.

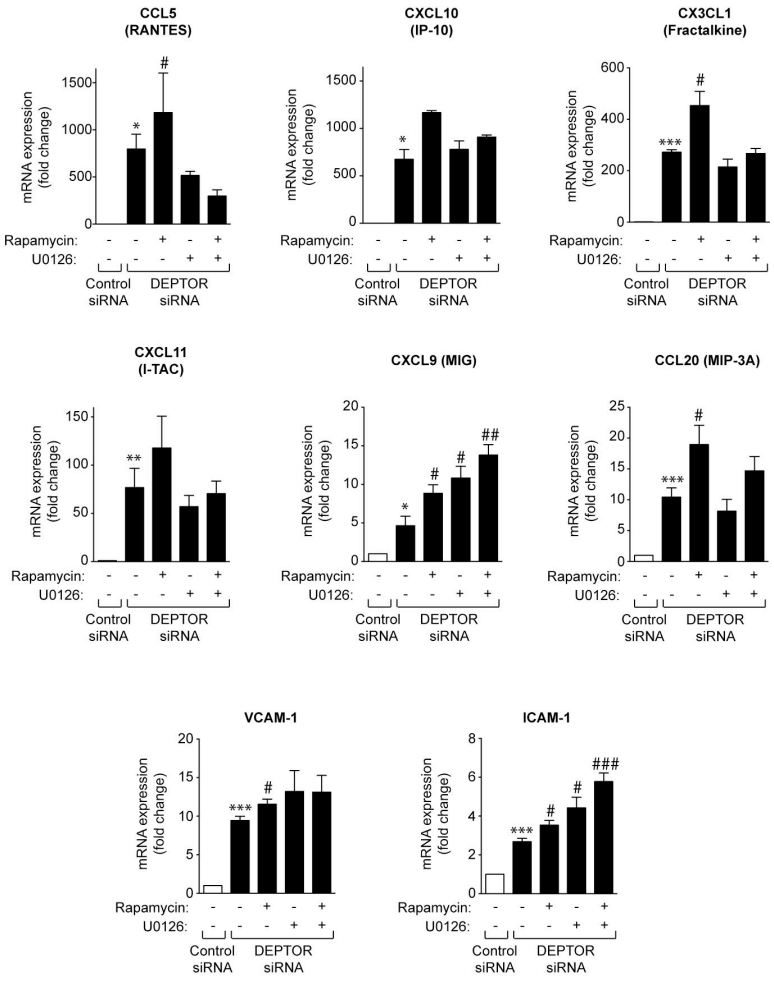

Figure S4. mTORC1 and ERK1/2-mediated signals are differentially involved in the regulation of EC activation by DEPTOR. HUVEC were transfected with control or DEPTOR siRNAs and cultured for 24 hrs with or without rapamycin (10 ng/ml) and/or U0126 (10 μM) for another 24 hrs. The mRNA expression of CCL5, CXCL10, CX3CL1, CXCL11, CXCL9, CCL20, VCAM-1 and ICAM-1 was then analyzed by qPCR. The bar graphs represent the mean fold change in mRNA expression (± 1SEM) of these 8 genes in 2 independent experiments, each using two different DEPTOR siRNAs. **P*<0.05, ***P*<0.01, ****P*<0.001 *vs.* control siRNA transfected HUVEC. #*P*<0.05, ##*P*<0.01, ###*P*<0.001 *vs.* untreated DEPTOR siRNA-transfected HUVEC.

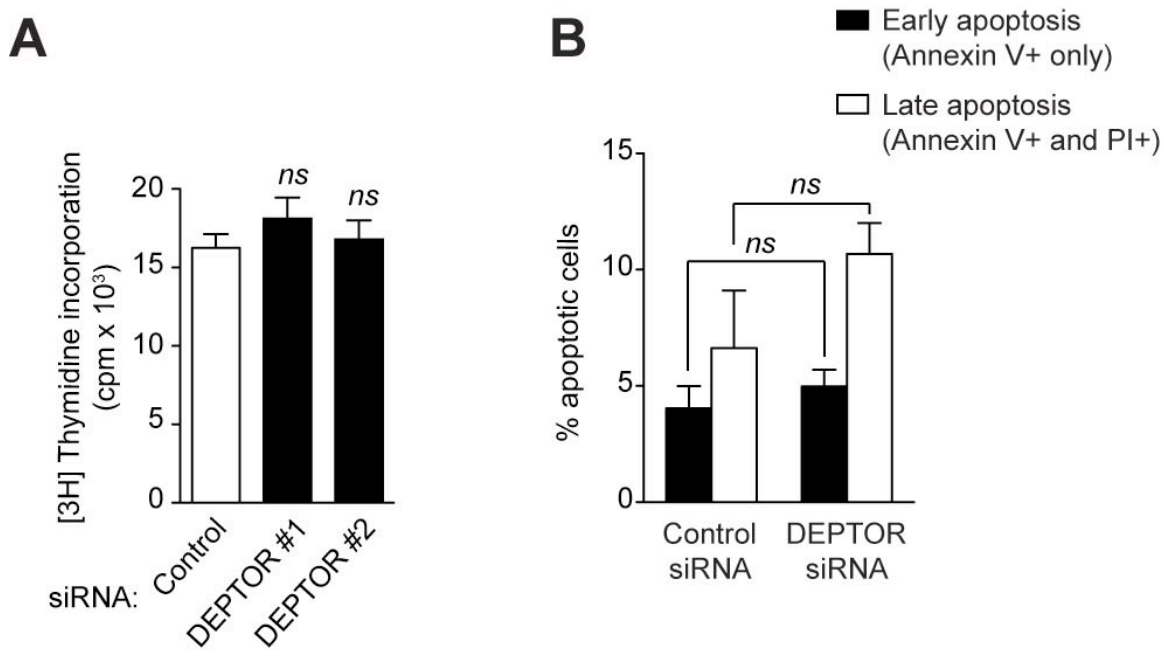

Figure S5. Effect of DEPTOR on EC proliferation and apoptosis. *Panel A*, HUVEC were transfected with control or DEPTOR siRNAs and after 72 hours, proliferation was evaluated using the [^{3}H] thymidine incorporation assay (1 μCi/well). Bar graphs illustrate the mean proliferation of EC (± 1SEM) from three independent experiments. *Panel B*, HUVEC were transfected with control siRNA or DEPTOR siRNA, and after 48 hours Annexin V and Propidium Iodide (PI) staining was evaluated by flow cytometry as an indication of apoptosis. The bar graph represents the percent apoptotic cells (± 1SEM) from 9 independent experiments.