

Supplementary Information

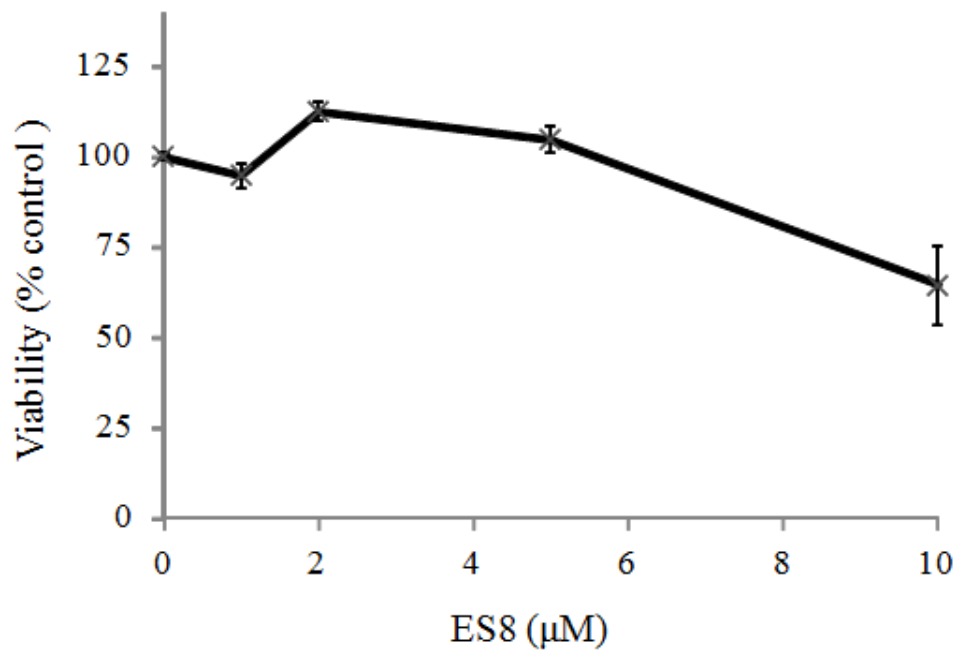


Figure S1 ES8 affects cell viability at 10 µM

The effect of ES8 (1 to 10µM) on viability of HUVEC at day 4 was measured by the metabolic MTS assay. Data is expressed in mean % ± SEM and results from three independent experiments done in triplicate.

Figure S1

a

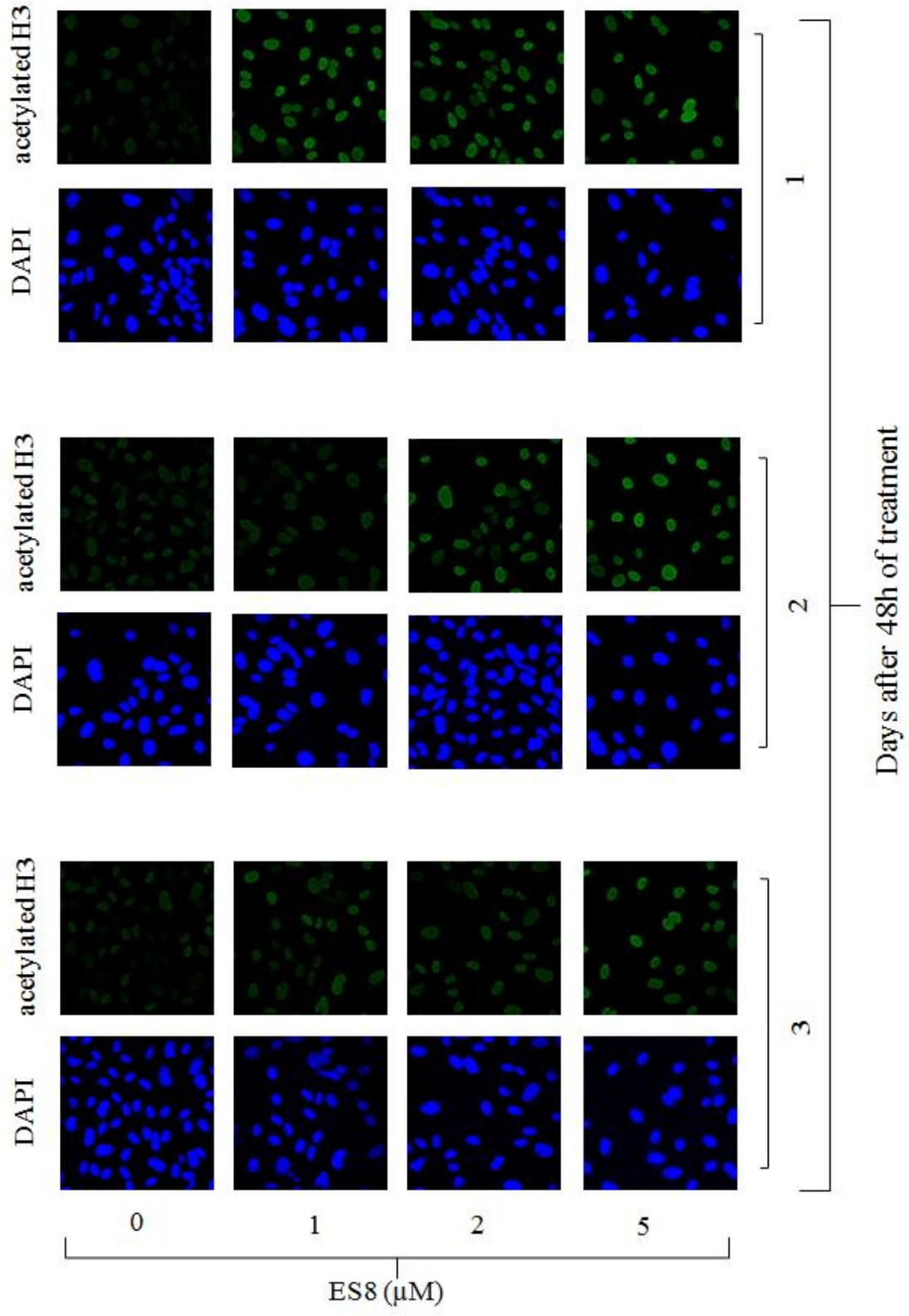


Figure S2 a

b

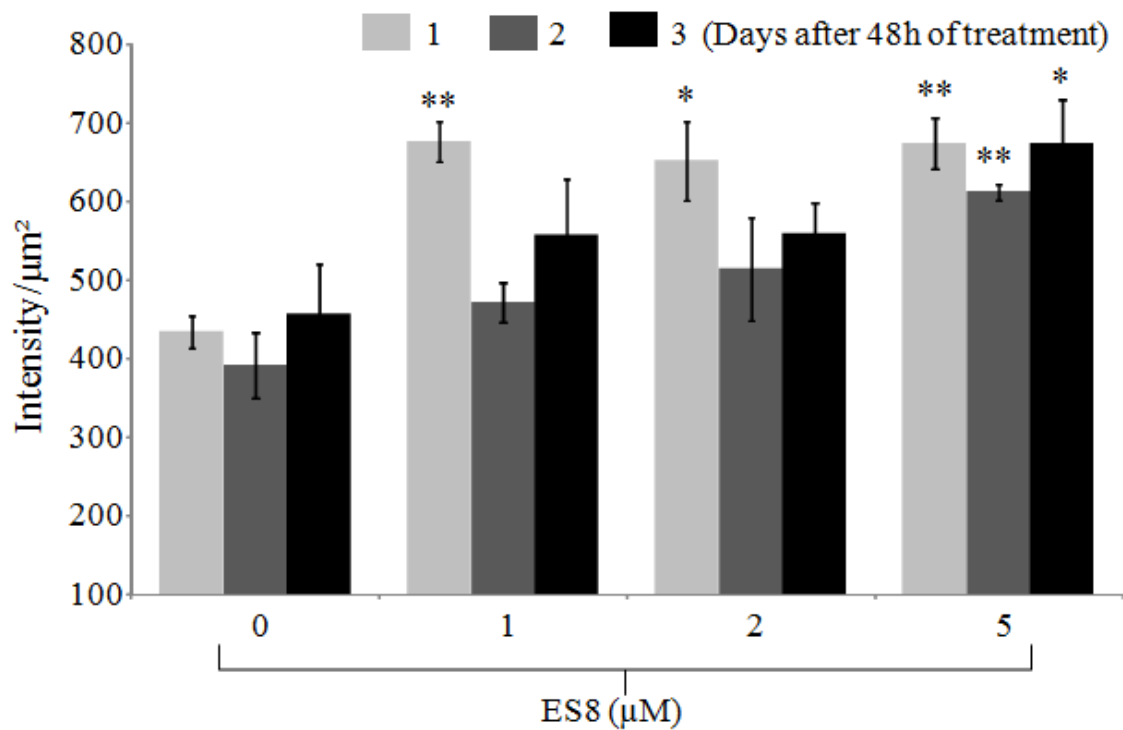


Figure S2 ES8-induced H3 acetylation is stable

To monitor changes in histone acetylation upon treatment with ES8, HUVEC were exposed during 48h to ES8 (1 to 5 μM). Cells were then cultivated for 1, 2 or 3 days in the absence of ES8, fixed and labelled with pan-acetylated histone H3 antibody and an Alexa fluor 488 conjugate. Cell nuclei were stained with DAPI. a) Representative confocal images. b) Computer assisted quantification of acetylated histone H3 immunofluorescence. Data is expressed as mean signal intensities per cell area (μm²) ± SEM of 15 images obtained from three independent experiments. * p < 0.05; **, p < 0.01 versus solvent control (0.1% DMSO).

Figure S2 b

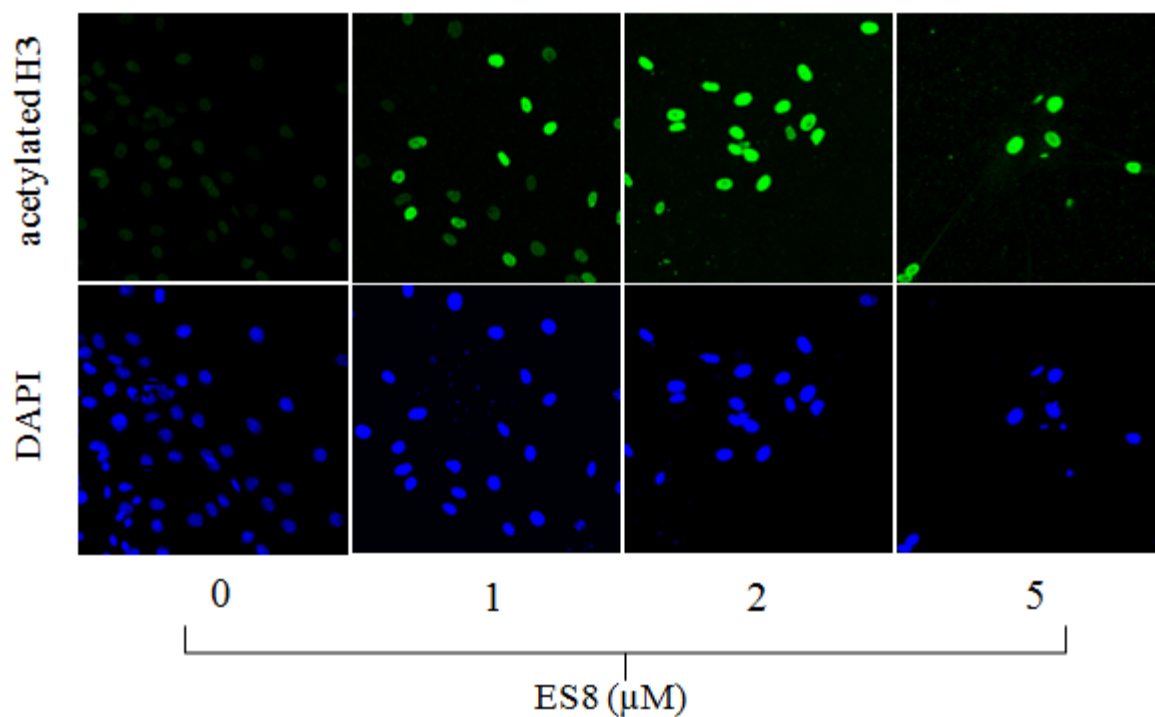


Figure S3 ES8 induces H3 acetylation in aortic rings

Thin preparations of aortic rings were made according to the previously described procedure [51], with slight modifications. Briefly, aortic rings were put on a coverslip above which 30μl of collagen solution was uniformly spread to form a thin disc. After cultivating for 5 to 6 days, the aortic rings were treated during 48h with ES8 (1 to 5μM). After fixation in paraformaldehyde (4%) and permeabilisation with Triton X-100 (1%) and BSA (1%), the aortic rings were labeled with a pan-acetyl histone H3 antibody and an Alexa fluor 488 conjugate. After mounting in Vectashield medium with DAPI (Vector Laboratories), aortic rings were examined by laser scanning confocal microscope at a magnification of 63x (Olympus Fluoview 1000).

Figure S3

Table S1 Gene transcripts involved in angiogenesis and significantly affected by two HDACi(SAHA and ES8).

Symbol	Gene name	Type(s)	Fold change with	
			SAHA	ES8
IL8	Interleukin 8	Cytokine	5.6	6.2
SERPINB2	Serpin peptidase inhibitor, clade B (ovalbumin), member 2	other	7	5.8
NQO1	NAD(P)H dehydrogenase, quinone 1	Enzyme	2.2	4.8
BEX2	Brain expressed X-linked 2	other	2.8	4.7
BMP2	Bone morphogenetic protein 2	Growth factor	4.1	3.8
CCND2	Cyclin D2	other	3.7	3.8
SGK1	Serum/glucocorticoid regulated kinase 1	Kinase	2.1	2.9
PLAT	Plasminogen activator, tissue	Peptidase	2.9	2.6
LAMB3	Laminin, beta 3	Transporter	2.1	2.2
RAB40B	RAB40B, member RAS oncogene family	Enzyme	1.5	2.1
SULF2	Sulfatase 2	Enzyme	2.1	2.1
PTX3	Pentraxin 3, long	other	2	2
BST2	Bone marrow stromal cell antigen 2	other	1.8	1.9
IGFBP7	Insulin-like growth factor binding protein 7	Transporter	2.1	1.9
ANXA1	Annexin A1	other	1.9	1.6
CALD1	Caldesmon 1	other	2	1.6
CPD	Carboxypeptidase D	Peptidase	1.6	1.6
ESM1	Endothelial cell specific molecule 1	Growth factor	1.6	1.5
APOC1	Apolipoprotein C-I	Transporter	-1.6	-1.6
FOXM1	Forkhead box M1	Transcription regulator	-1.5	-1.6
UHRF1	Ubiquitin-like with PHD and ring finger domains 1	Transcription regulator	-1.6	-1.6
BIRC5	Baculoviral IAP repeat containing 5	other	-1.7	-1.7
BRD2	Bromodomain containing 2	Kinase	-1.8	-1.7
CAPN1	Calpain 1, (μ /I) large subunit	Peptidase	-1.6	-1.8
IRAK1	Interleukin-1 receptor-associated kinase 1	Kinase	-1.6	-1.8
CD34	CD34 molecule	other	-1.7	-1.9
GPI	Glucose-6-phosphate isomerase	Enzyme	-1.8	-1.9
LDHA	Lactate dehydrogenase A	Enzyme	-1.8	-1.9
CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1	Enzyme	-2	-2
MTSS1	Metastasis suppressor 1	other	-2.2	-2
HMGB1L1	High-mobility group (nonhistone chromosomal) protein 1-like 1	Transcription regulator	-1.9	-2.1

Table S2 Gene transcripts involved in angiogenesis and significantly affected by DAC and ES8 but not by SAHA.

Symbol	Gene name	Type(s)	Fold change with	
			DAC	ES8
TFPI2	Tissue factor pathway inhibitor 2	other	3.1	10.5
TGFBI	Transforming growth factor, beta-induced, 68kda	other	1.7	2.9
COL8A1	Collagen, type VIII, alpha 1	other	1.6	2.1
IL13RA2	Interleukin 13 receptor, alpha 2	Transmembrane receptor	5.4	2.1
NEDD9	Neural precursor cell expressed,	other	1.6	1.8
EPHB1	EPH receptor B1	Kinase	1.5	1.6
HIST1H4A	Histone cluster 1, H4a	other	-1.6	-1.5
HIST1H4H	Histone cluster 1, H4h	other	-1.9	-1.7
HIST1H4C	Histone cluster 1, H4c	other	-1.7	-1.8
HIST1H2BE	Histone cluster 1, H2be	other	-1.6	-3.3
HIST1H2BC	Histone cluster 1, H2bc	other	-1.8	-3.9

Table S3. List of angiogenesis associated genes that are concomitantly affected by DAC, SAHA and ES8.

Symbol	Gene Name	Type	Fold Change with		
			DAC	SAHA	ES8
NQO1	NAD(P)H dehydrogenase, quinone 1	Enzyme	1.7	2.2	4.8
SGK1	Serum/ glucocorticoid regulated kinase 1	Kinase	1.8	2.1	2.9
SULF2	Sulfatase 2	Enzyme	2.2	2.1	2.1
BST2	Bone marrow stromal cell antigen 2	Other	1.9	1.8	1.9
IGFBP7	Insulin-like growth factor binding protein 7	Transporter	1.8	2.1	1.9
ESM1	Endothelial cell-specific molecule 1	Growth factor	1.5	1.6	1.5
GPI	Glucose-6-phosphate isomerase	Enzyme	-1.5	-1.8	-1.9
TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	Other	-1.8	-2.1	-3
TYMS	Thymidylate synthetase	Enzyme	-1.5	-2.3	-4.5

Table S4. Gene transcripts significantly affected by ES8 but not by either SAHA or DAC and involved in angiogenesis.

Symbol	Gene name	Type(s)	Fold change
DNER	Delta/notch-like EGF related receptor	TMR (Trans membrane receptor)	13.3
TMOD1	Tropomodulin 1	Enzyme	4.9

PCSK1	Proprotein convertase subtilisin/kexin type 1	Peptidase	3.8
STMN2	Stathmin-like 2	Other	3.4
PTGS1	Prostaglandin-endoperoxide synthase 1 (prostaglandin g/h synthase and cyclooxygenase)	Enzyme	3.4
ITGB3	Integrin, beta 3 (platelet glycoprotein iiiA, antigen cd61)	TMR	3.2
HSD17B2	Hydroxysteroid (17-beta) dehydrogenase 2	Enzyme	3.1
FTH1	Ferritin, heavy polypeptide 1	Enzyme	3.1
ALDOC	Aldolase c, fructose-bisphosphate	Enzyme	2.9
HOXB5	Homeobox b5	TR(Transcription regulator)	2.8
PALLD	Palladin, cytoskeletal associated protein	Other	2.4
SLIT2	Slit homolog 2 (drosophila)	Other	2.3
NPTX2	Neuronal pentraxin ii	Other	2.2
HOXB4	Homeobox b4	TR	2.2
RGS2	Regulator of g-protein signaling 2, 24kda	Other	2.2
IGFBP2	Insulin-like growth factor binding protein 2, 36kda	Other	2.2
SFRP1	Secreted frizzled-related protein 1	TMR	2.2
CCL20	Chemokine (c-c motif) ligand 20	Cytokine	2.1
TXNIP	Thioredoxin interacting protein	Other	2.1
TIPARP	Tcdd-inducible poly(adp-ribose) polymerase	Other	2.1
PTPRU	Protein tyrosine phosphatase, receptor type, u	Phosphatase	2.1
PCSK5	Proprotein convertase subtilisin/kexin type 5	Peptidase	2.1
LAMC1	Laminin, gamma 1 (formerly lamb2)	Other	2.0
SCPEP1	Serine carboxypeptidase 1	Peptidase	2.0

CDC42EP3	Cdc42 effector protein (rho gtpase binding) 3	Other	2.0
LBH	Limb bud and heart development homolog (mouse)	TR	2.0
ADM	Adrenomedullin	Other	2.0
DPP4	Dipeptidyl-peptidase 4	Peptidase	2.0
HSD11B1	Hydroxysteroid (11-beta) dehydrogenase 1	Enzyme	2.0
PAM	Peptidylglycine alpha-amidating monooxygenase	Enzyme	2.0
PDP1	Pyruvate dehydrogenase phosphatase catalytic subunit 1	Phosphatase	1.9
HLA-B	Major histocompatibility complex, class i, b	TMR	1.9
NCAM1	Neural cell adhesion molecule 1	Other	1.9
CD47	Cd47 molecule	Other	1.9
MT2A	--	Other	1.9
DKK3	Dickkopf 3 homolog (xenopus laevis)	Cytokine	1.9
TNFRSF21	Tumor necrosis factor receptor superfamily, member 21	TMR	1.9
G6PD	Glucose-6-phosphate dehydrogenase	Enzyme	1.9
CRIP1	Cysteine-rich protein 1 (intestinal)	Other	1.9
RRM2B	Ribonucleotide reductase m2 b (tp53 inducible)	Enzyme	1.9
CRIM1	Cysteine rich transmembrane bmp regulator 1 (chordin-like)	Kinase	1.8
HTRA1	Htra serine peptidase 1	Peptidase	1.8
FBLN2	Fibulin 2	Other	1.8
CD46	Cd46 molecule, complement regulatory	Other	1.8

	protein		
ITGA9	Integrin, alpha 9	Other	1.8
CHPT1	Choline phosphotransferase 1	Enzyme	1.7
SPTAN1	Spectrin, alpha, non-erythrocytic 1	Other	1.7
ENG	Endoglin	TMR	1.7
BBS2	Bardet-biedl syndrome 2	Other	1.7
MMP10	Matrix metalloproteinase 10 (stromelysin 2)	Peptidase	1.7
GNAI1	Guanine nucleotide binding protein (g protein), alpha inhibiting activity polypeptide 1	Enzyme	1.7
PFDN4	Prefoldin subunit 4	Other	1.7
PGD	Phosphogluconate dehydrogenase	Enzyme	1.7
TBPL1	Tbp-like 1	TR	1.7
MT1F	Metallothionein 1f	Other	1.7
NFE2L3	Nuclear factor (erythroid-derived 2)-like 3	TR	1.7
MTF1	Metal-regulatory transcription factor 1	TR	1.7
GLO1	Glyoxalase i	Enzyme	1.7
DYNLT3	Dynein, light chain, tctex-type 3	Other	1.6
TXNDC5	Thioredoxin domain containing 5 (endoplasmic reticulum)	Enzyme	1.6
TKT	Transketolase	Enzyme	1.6
ITGA3	Integrin, alpha 3 (antigen cd49c, alpha 3 subunit of vla-3 receptor)	Other	1.6
CTGF	Connective tissue growth factor	Growth factor	1.6
NPTN	--	Other	1.6
MICA	Mhc class i polypeptide-related sequence a	Other	1.6
POU4F1	Pou class 4 homeobox 1	TR	1.6

PMP22	Peripheral myelin protein 22	Other	1.6
DAD1	Defender against cell death 1	Enzyme	1.6
HSPA1A/HSPA1B	Heat shock 70kda protein 1a	Other	1.6
ULBP2	U116 binding protein 2	TMR	1.6
EIF2S3	Eukaryotic translation initiation factor 2, subunit 3 gamma, 52kda	Translation regulator	1.6
RGS5	Regulator of g-protein signaling 5	Other	1.6
GRB14	Growth factor receptor-bound protein 14	Other	1.6
NRP2	Neuropilin 2	Kinase	1.6
ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase 1	Enzyme	1.6
ANGPTL4	Angiopietin-like 4	Other	1.6
CADM1	Cell adhesion molecule 1	Other	1.6
MYH9	Myosin, heavy chain 9, non-muscle	Transporter	1.6
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	Other	1.6
CDKN2B	Cyclin-dependent kinase inhibitor 2b (p15, inhibits cdk4)	TR	1.6
AGRN	Agrin	Other	1.6
TXN	Thioredoxin	Enzyme	1.6
JUN	Jun proto-oncogene	TR	1.6
SLC25A4	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Transporter	1.6
LIFR	Leukemia inhibitory factor receptor alpha	TMR	1.6
SEZ6L2	Seizure related 6 homolog (mouse)-like 2	Other	1.6
APLP1	Amyloid beta (A4) precursor-like protein 1	Other	1.6

DUSP6	Dual specificity phosphatase 6	Phosphatase	1.6
CDKN2D	Cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	TR	1.6
P2RY1	Purinergic receptor P2Y, G-protein coupled, 1	G-protein coupled receptor	1.5
MAP2K1	Mitogen-activated protein kinase kinase 1	Kinase	1.5
SLC40A1	Solute carrier family 40 (iron-regulated transporter), member 1	Transporter	1.5
CXCL6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	Cytokine	1.5
GPR4	G protein-coupled receptor 4	G-protein coupled receptor	1.5
F3	Coagulation factor III (thromboplastin, tissue factor)	TMR	1.5
FLNC	Filamin C, gamma	Other	1.5
BCL2L1	BCL2-like 1	Other	1.5
LEPROT	Leptin receptor overlapping transcript	Other	1.5
PTPRB	Protein tyrosine phosphatase, receptor type, B	Phosphatase	1.5
EDNRB	Endothelin receptor type B	G-protein coupled receptor	1.5
ITGA1	Integrin, alpha 1	Other	1.5
ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	Enzyme	1.2
CHP1	Calcineurin-like EF hand protein 1	Transporter	-1.2
CD9	CD9 molecule	Other	-1.5
SET	SET nuclear oncogene	Phosphatase	-1.5
SRSF6	Serine/arginine-rich splicing factor 6	Other	-1.5
RAPGEF1	--	Other	-1.5

PTPRG	Protein tyrosine phosphatase, receptor type, G	Phosphatase	-1.5
YARS	Tyrosyl-trna synthetase	Enzyme	-1.5
RAN	RAN, member RAS oncogene family	Enzyme	-1.5
COIL	Coilin	Enzyme	-1.5
ENO1	Enolase 1, (alpha)	TR	-1.5
NFIB	Nuclear factor I/B	TR	-1.5
WASF3	WAS protein family, member 3	Other	-1.5
PIAS3	Protein inhibitor of activated STAT, 3	TR	-1.5
YES1	V-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	Kinase	-1.6
AIP	Aryl hydrocarbon receptor interacting protein	TR	-1.6
LYVE1	Lymphatic vessel endothelial hyaluronan receptor 1	TMR	-1.6
SRSF1	serine/arginine-rich splicing factor 1	other	-1.6
CNDP2	CNDP dipeptidase 2 (metallopeptidase M20 family)	peptidase	-1.6
SCARF1	scavenger receptor class F, member 1	TMR	-1.6
EIF4A1	eukaryotic translation initiation factor 4A1	translation regulator	-1.6
XPO1	exportin 1 (CRM1 homolog, yeast)	transporter	-1.6
TYK2	tyrosine kinase 2	kinase	-1.6
ECT2	epithelial cell transforming sequence 2 oncogene	other	-1.6
PTMA	--	other	-1.6
NEK2	NIMA (never in mitosis gene a)-related kinase 2	kinase	-1.6

HMMR	hyaluronan-mediated motility receptor (RHAMM)	other	-1.6
PTTG1	pituitary tumor-transforming 1	TR	-1.6
RALB	v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)	enzyme	-1.6
HSPB1	heat shock 27kDa protein 1	other	-1.7
C12orf57	chromosome 12 open reading frame 57	other	-1.7
CAV1	caveolin 1, caveolae protein, 22kDa	other	-1.7
RNH1	ribonuclease/angiogenin inhibitor 1	other	-1.8
NCL	--	other	-1.8
CAPG	capping protein (actin filament), gelsolin- like	other	-1.8
FLI1	Friend leukemia virus integration 1	TR	-1.8
RAMP2	receptor (G protein-coupled) activity modifying protein 2	transporter	-1.8
LTB	lymphotoxin beta (TNF superfamily, member 3)	cytokine	-1.8
SPHK1	sphingosine kinase 1	kinase	-1.9
EFNB2	ephrin-B2	other	-1.9
S100A13	S100 calcium binding protein A13	other	-1.9
MCM6	minichromosome maintenance complex component 6	enzyme	-1.9
RRM1	ribonucleotide reductase M1	enzyme	-2.0
DNMT1	DNA (cytosine-5-)-methyltransferase 1	enzyme	-2.0
TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B	TMR	-2.0

EFEMP2	EGF containing fibulin-like extracellular matrix protein 2	other	-2.0
ELF1	E74-like factor 1 (ets domain transcription factor)	TR	-2.0
CREBBP	CREB binding protein	TR	-2.0
MGP	matrix Gla protein	other	-2.1
ELK3	ELK3, ETS-domain protein (SRF accessory protein 2)	TR	-2.1
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	kinase	-2.2
HIST1H4B	--	other	-2.2
KIF22	kinesin family member 22	other	-2.3
RRM2	ribonucleotide reductase M2	enzyme	-2.3
GTF2I	general transcription factor Iii	TR	-2.4
SOX18	SRY (sex determining region Y)-box 18	TR	-2.5
RASIP1	Ras interacting protein 1	other	-2.9
IL33	interleukin 33	cytokine	-2.9
TFPI	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	other	-2.9
AURKA	aurora kinase A	kinase	-3.7
C10orf10	chromosome 10 open reading frame 10	other	-7.0

Table S5. List of genes associated with major angiogenic signalling pathways that are affected by ES8.

Signalling pathway	Genes modified
VEGF	BCL2L1 CAV1 HSPB1 MAP2K1 NRP2 PIK3CG PLCG2 SPHK1
FGF	MAP2K1
Notch	CCNE1 HOXB4
Semaphorin	ITGB1
Hedgehog	BMP2 FRMD6 MTSS1 SFRP1
Ephrin	ARHGEF15 PIK3CG RAPGEF1