Supplementary Information for

Longitudinal shear stress response in human endothelial cells to atheroprone and atheroprotective conditions

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Other supplementary materials for this manuscript include the following:

Supplementary Information Text

SI Methods

A schematic of our experimental setup, protocol and analysis pipeline is shown in Fig. S1.

Cell Culture. HUVECs were cultured in medium M199 (Gibco) supplemented with 15% FBS (Omega), 3 ng/mL β-EC growth factor (Sigma), 4 U/mL heparin (Sigma), and 100 U/mL penicillin-streptomycin (1). HAECs (Cell Applications Cat# 03405A) were cultured in endothelial growth medium (Cell applications Cat#211-500) with 100 U/mL penicillin-streptomycin.

Shear Stress Experiments. For both HAECs and HUVECs (2), PS and OS flows were applied to ECs with shear stresses of 12 ± 4 dyn/cm^2 and 0.5 ± 4 dyn/cm^2, respectively. Samples for RNA sequencing analysis were collected at 1, 4 and 24 hr after exposure to shear. For HAECs, three biological replicates were collected for each time point for each shear condition. As described in our prior work, in HUVEC studies, two biological replicates were collected.

RNA isolation and RNA-seq Library Preparation.
HAECs: The total RNA from HAECs were extracted by using TRizol RNA isolation reagent (Thermo Fisher, Cat#15596026). The RNA quality was assessed by RNA Integrity Numbers (RIN), using an Agilent Bioanalyzer, and all RNA samples used in this study had a RIN over 9. The RNAs were ribo-depleted, fragmented, and random hexamer primed using Illumina TruSeq stranded mRNA sample preparation kit. Constructed cDNA library was subjected to single-ended 75-bp sequencing on Illumina Hi-seq 4000 instrument.

In HUVECs, RNA-seq experiments was carried out as previously described (2).

RNA-seq Data Analysis. RNA-seq fastq files for both HAEC and HUVEC datasets were aligned to the Human Reference Genome (version hg19 / Human.B37.3) and raw gene read counts (Refgene) were generated using the Omicsoft Aligner (OSA) OShell version = 9.0.8.75 (3). Only the genes with raw read count greater than 10 at any of the three time points were included in further analysis. Raw read counts were normalized and analyzed for differential expression using DESeq2 (4). Pairwise comparisons of OS vs. PS were performed for each time point for each cell type separately. The total normalized read counts after removing low expressed genes was about 17M for HAECs and about 31M for HUVECs; a gene is called differentially expressed (DE) based on a raw p-value cutoff of 0.05 and a fold-change (FC) cutoff of 1.3 (up or down), i.e., $|\log2\ FC| \geq \log2(1.3)$. Adjusted p-values were not used for differential expression analysis because of the low number of differentially expressed genes in the early hours of shear exposure.

Functional Maps and Interpretation. Pathway enrichment analysis was conducted on all DE genes at each time point for HAECs and HUVECs separately through Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (5, 6) for Kyoto Encyclopedia of Genes and Genomes (KEGG) (7), Reactome and Biocarta pathways. Functional pathways describing temporal activation were constructed through a combination of curated pathways and manual literature search.

Transcriptional Regulatory Network. Using the TRANSFAC database (Version 2020.2) (8), the transcription factors (TFs) were mapped to their published transcriptional targets. In cases where a TF was listed as a protein complex, the complex was split into genes which were represented as individual nodes. The identified transcriptional network was further filtered based on whether the TF and their targets were DE in OS vs. PS ($p \leq 0.05$ and $FC \geq 1.3$, up- or down-regulated) and the targets were DE at the same or future time points as compared to the TF. The TF-target network was simplified by (1) retaining only the TFs with node-degree ≥ 10 (except KLF2) and (2) retaining only important (EC relevant) target genes (a list of 212 genes in SI Dataset S1, List of "Important_Genes").

SI Results

RNA-seq data from HAECs and HUVECs

Total read counts were 25-37M for HAECs and 27-44M for HUVECs. Uniquely mapped read % were about 74% and 93% for HAECs and HUVECs, respectively. The total normalized read counts after removing low-expression genes were about 17M for HAECs and about 31M for HUVECs. RNA-seq in HUVECs was carried out using the poly-A selection approach, whereas in HAECs it was carried out using the ribo-depletion approach. Due to these differences, our comparison of HAECs vs. HUVECs is based on fold-changes in OS/PS (for each time point).

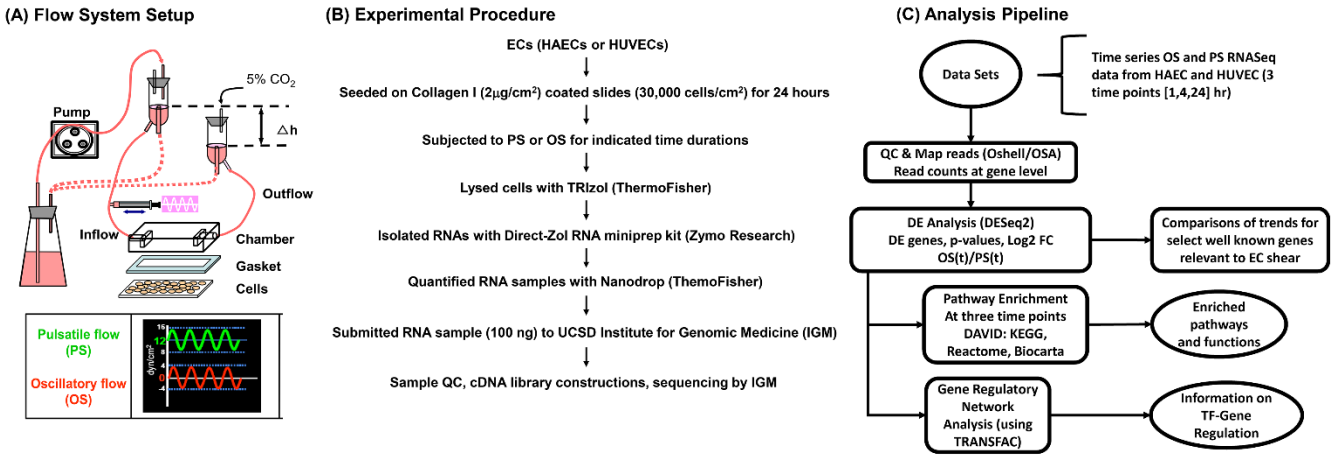
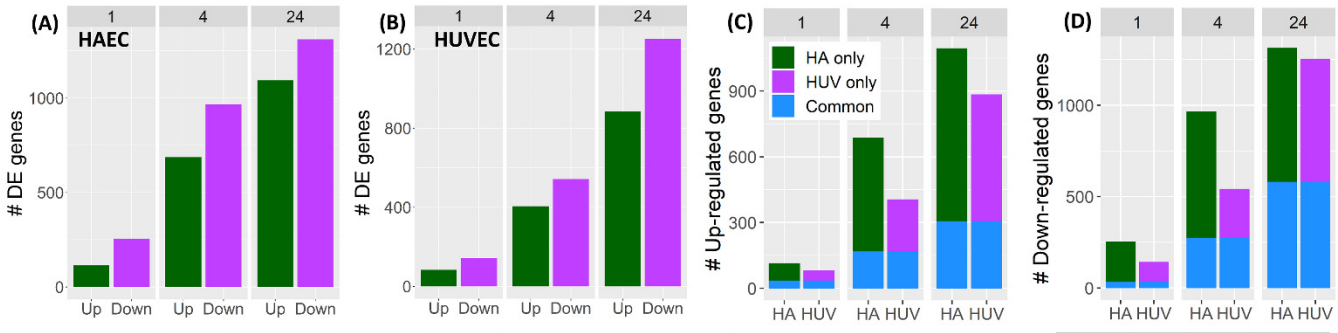


Figure S1. Schematic of experimental setup (A), protocol (B) and data analysis pipeline (C) used for comparing the response of HAECs and HUVECs. In the analysis pipeline, starting with quality check and mapping of the sequenced reads for each time point and shear condition, differentially expressed (DE) genes were identified using DESeq2 for OS vs. PS for each time point in each cell type. Functional/pathway enrichment and gene-regulatory network analysis was carried out using the DE genes.



Time (hr)	# DE genes Up	# DE genes Down
1	114	255
4	688	967
24	1094	1312

Time (hr)	# DE genes Up	# DE genes Down
1	83	143
4	405	542
24	885	1253

Time (hr)	HAEC Unique	HUVEC Unique	Common
1	79	48	35
4	520	237	168
24	788	579	306

Time (hr)	HAEC Unique	HUVEC Unique	Common
1	222	110	33
4	693	268	274
24	732	673	580

Figure S2. Global comparison of OS vs. PS differential expression in HAECs and HUVECs. The numbers of DE genes in HAECs (A) and HUVECs (B) across time are shown as bar plots; time (in hours) is shown at the top of bar plots. Panels (C) and (D) show the numbers of commonly and uniquely up-regulated (C) and down-regulated (D) DE genes in HAECs and HUVECs across time. Abbreviations: HA: HAEC; HUV: HUVEC.

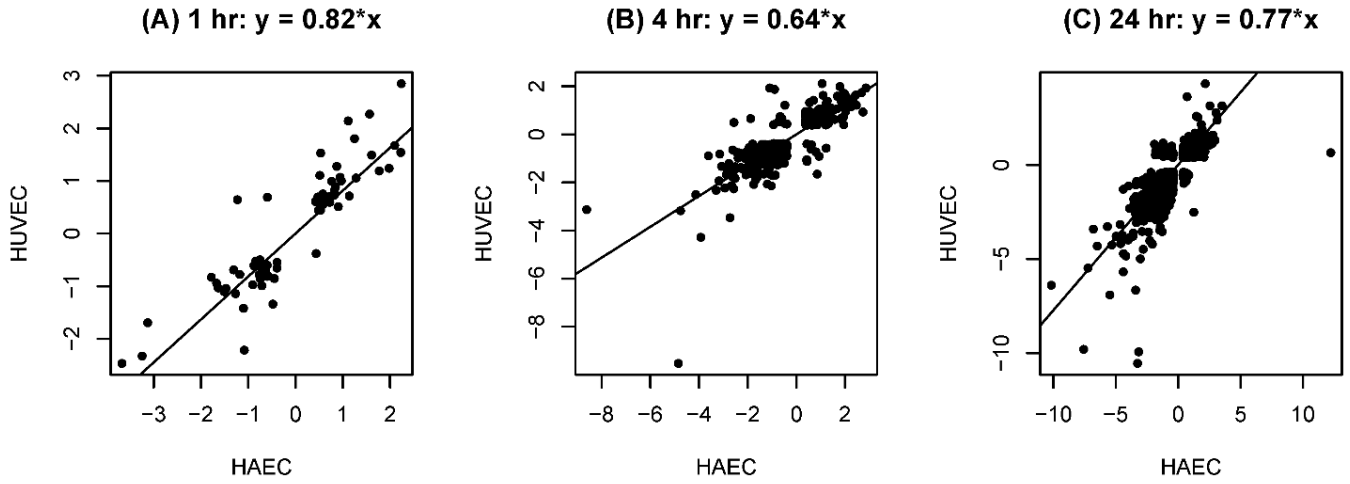


Figure S3. Scatterplot of OS/PS log2 fold-change for HAECs and HUVECs at 1, 4 and 24 hr (71 (A), 465 (B) and 959 (C) genes, respectively). Only the genes DE in both cell types at respective time points and max read counts of 10 or more at any time point (1, 4, and 24 hr) are included. As time progressed, the p-value of the Pearson correlation of linear fit decreased: 9.63E-27 (A), 4.99E-112 (B) and 1.93E-216 (C) at 1, 4 and 24 hr, respectively, suggesting that similarity of the response of HAECs and HUVECs became statistically more significant.

(A) Cell cycle: G1/S transition genes

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
CDK4	0.03	-0.04	0.16	0.11	-0.03	0.53
CDKN2D	-0.13	-0.79	-0.80	-0.24	-0.89	-0.62
CCND2	-0.09	-0.30	0.26	0.03	-0.15	0.14
CCNH	0.03	0.29	-0.17	0.18	0.23	0.43
MNAT1	-0.06	0.25	-0.05	0.07	0.16	0.46
CDK7	0.07	0.09	0.19	0.21	0.01	0.41
CDC25A	-0.04	0.18	-0.20	-0.13	0.34	0.46
E2F1	0.14	0.21	-0.71	0.04	-0.03	0.72
CDK2	0.01	-0.02	-0.25	0.04	-0.14	0.23
HDAC2	-0.03	0.04	-0.03	0.05	0.23	0.42
CCNE2	0.40	0.39	0.46	0.01	0.35	0.92
CCNA2	0.10	0.12	-1.06	-0.17	-0.08	0.39
UBE2C	0.03	-0.11	-0.82	-0.07	-0.34	0.12
CCND1	-0.04	-0.72	-0.43	-0.04	-0.20	0.02
CCNE1	0.01	0.04	-0.26	-0.08	0.01	0.45
CDC6	0.05	0.12	-0.83	0.14	0.20	0.84
POLE2	0.20	0.12	-0.38	0.11	0.13	0.74
NOTCH4	-0.13	-0.03	0.38	0.12	-0.13	-0.28

(B) Oxidative stress genes (WikiPathways)

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
MGST1	0.09	0.25	0.56	0.09	-0.04	0.32
SP1	-0.02	-0.03	-0.05	-0.22	0.03	-0.24
SOD2	0.18	0.35	0.28	0.17	0.75	0.23
NFKB1	-0.16	0.20	-0.15	-0.08	0.59	-0.07
MT1X	0.40	0.75	0.42	0.09	0.04	1.13
NFIX	-0.20	-0.52	-0.27	-0.09	0.05	-0.27
MAPK10	0.06	0.52	0.22	#N/A	#N/A	#N/A
NFE2L2	-0.04	0.01	0.17	-0.12	-0.01	-0.11
GCLC	0.15	0.29	0.05	0.15	0.22	0.17
HMOX1	0.54	0.59	0.43	0.12	0.07	-0.12
MAPK14	-0.02	-0.18	-0.08	-0.03	0.01	0.13
TXNRD2	0.14	-0.08	0.07	0.45	0.09	0.26
FOS	0.43	0.05	0.79	1.77	-0.49	0.53
CAT	0.07	-0.33	-0.56	-0.07	-0.11	-0.30
CYP1A1	-0.31	-0.31	-0.58	-1.06	-0.58	-1.38
JUNB	-0.91	-0.52	-0.30	0.10	-0.96	-0.88
GSTT2	0.51	-0.18	-0.50	1.11	-0.14	-0.32
GPX1	0.13	0.22	0.66	0.15	-0.22	0.48
NOX4	0.15	1.21	0.79	0.14	0.39	0.37
TXNRD1	0.18	0.67	0.63	0.10	0.15	0.19
GPX3	0.14	-0.33	-0.48	-0.44	-0.68	-0.74
MAOA	0.17	0.23	0.84	-0.11	-0.04	0.25
GSR	0.08	0.07	0.16	0.02	-0.03	0.01
TXN2	-0.08	0.06	0.03	0.12	-0.13	0.17
NQO1	0.05	-0.27	-0.63	-0.03	-0.32	-0.76
SOD1	0.11	-0.05	-0.12	0.18	-0.01	0.12
HIF1A	-0.02	0.26	0.32	0.02	0.39	0.27
GPX4	0.10	0.18	0.75	0.22	-0.09	0.42
GPX8	0.18	0.55	0.68	0.12	0.02	0.36

Log2 fold-change

-1.0 -0.5 0.0 0.5 1.0

(C) TGF-β signaling pathway genes

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
TGFB1	-0.24	-0.04	0.36	-0.14	-0.53	-0.13
TGFBR1	-0.12	0.52	0.32	0.00	0.76	0.58
TGFBR2	0.11	0.26	0.37	0.19	0.27	0.53
SMAD1	0.25	0.53	0.67	0.45	0.39	0.36
SMAD2	0.01	0.13	0.18	-0.12	0.18	0.14
SMAD3	-0.04	-0.08	0.19	-0.08	0.19	-0.19
SMAD4	-0.02	0.23	0.07	-0.09	0.24	-0.02
SMAD5	-0.07	-0.27	-0.25	-0.33	0.34	-0.03
SMAD9	0.29	0.81	0.07	-0.75	0.03	-0.08
MYC	-0.38	0.18	-0.07	0.44	-0.20	-0.08
ID1	-0.51	-0.49	-0.35	-0.21	-1.21	-0.76
HDAC1	0.02	-0.51	-0.63	0.05	0.04	-0.18
CDKN2B	0.14	0.60	0.93	0.39	0.01	0.58
KLF10	-0.27	-0.80	-0.74	0.59	-0.81	-0.45
TGFBR3	0.04	0.63	0.86	-0.17	-0.12	0.74
MEF2A	-0.26	-1.06	-1.40	-0.31	-0.12	-1.13
BMP2	0.22	-0.65	-0.93	0.25	-0.04	-0.20
BMP4	0.53	1.43	1.64	0.66	0.10	0.96
SMAD6	-0.71	-0.02	0.15	-0.99	-0.20	-0.76
SMAD7	-0.91	-0.31	-0.63	-0.25	-0.18	-0.41

(D) Lysosome pathway related genes

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
VWF	0.04	0.07	-0.19	0.02	0.10	-0.83
ATG2A	0.10	0.00	0.26	0.00	-0.39	-0.26
ATG9B	-0.08	-2.52	-1.49	-0.81	-1.83	-1.81
CTSH	0.31	-0.51	-0.89	-0.02	-0.28	-0.41
CTSK	-0.03	0.19	0.75	0.53	0.63	0.28
NAGA	0.05	-0.29	-0.85	-0.05	-0.23	-0.47
ACP5	-1.74	-6.58	-4.47	-0.79	-0.99	-3.74
PPT2	0.02	-0.29	-0.57	0.09	-0.21	-0.56
LAMP3	-0.05	-0.24	1.78	0.02	0.26	0.40
SLC11A1	0.28	-0.84	-0.36	0.15	-0.49	-1.83
ABCA2	0.16	-0.21	-0.25	-0.20	-0.51	-1.08
IGF2R	-0.02	-0.02	0.70	-0.15	0.11	0.02
AP1B1	0.07	0.20	0.54	0.01	-0.06	0.33
LITAF	-0.07	0.03	0.90	-0.05	0.03	0.28
ATP6V0B	-0.01	-0.11	0.16	0.06	-0.21	0.29
ATP6V0C	-0.22	-0.13	0.38	0.14	-0.48	-0.04
ATP6V1A	0.09	0.58	0.53	-0.08	0.54	0.64
ATP6V1B2	0.00	-0.03	0.23	0.00	0.09	0.37
ATP6V1C1	-0.04	0.02	0.17	-0.02	0.24	0.23
ATP6V1G1	0.04	0.17	0.30	0.12	0.16	0.33
ATP6V1H	-0.02	0.23	0.13	0.13	0.01	0.23

(E) Autophagy related genes

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
IRS2	0.84	1.89	2.07	1.68	0.47	0.76
PIK3CD	0.11	0.65	0.82	0.07	0.31	0.51
PIK3R3	-0.70	-1.31	-1.41	-0.66	-0.39	-0.14
MRAS	0.12	-1.52	-2.54	-0.27	-0.28	-0.66
DDIT4	-1.78	-1.53	-1.76	-0.83	-1.29	-1.65
BNIP3	0.01	0.26	0.39	0.36	0.71	0.53
DEPTOR	0.18	-1.69	-0.51	#N/A	#N/A	#N/A
RRAGD	-1.68	-0.04	2.22	#N/A	#N/A	#N/A
PRKAA2	0.03	-0.77	-0.62	-0.03	-0.12	-0.95
PRKACB	0.08	0.48	0.59	-0.13	0.13	0.72
BCL2	-0.13	0.24	0.74	-0.13	0.36	0.07
BCL2L1	-0.14	-1.49	-0.66	0.07	-0.53	-0.50

(F) Up-regulated TFs

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
EGR1	0.11	2.27	2.95	1.66	-0.04	1.31
HIF1A	-0.02	0.26	0.32	0.02	0.39	0.27
FOS	0.43	0.05	0.79	1.77	-0.49	0.53
EGR2	-0.65	2.47	0.23	1.50	-0.62	-0.89
SRF	-0.10	0.34	0.21	0.08	0.13	0.22
FOXO1	0.22	0.69	0.60	0.16	0.40	0.29
GABPB1	0.01	0.43	0.10	0.01	0.17	0.35
PRDM1	2.13	2.06	1.57	0.83	0.60	0.39
MITF	0.24	-0.01	-0.61	0.02	-0.24	0.44
JUN	-0.59	-0.51	0.46	0.69	-0.38	0.15
TCF4	0.04	1.18	1.09	-0.15	0.72	0.64
TFDP2	0.08	0.32	-0.05	-0.13	0.25	0.33
E2F1	0.14	0.21	-0.71	0.04	-0.03	0.72
AML1	0.03	0.43	0.48	-0.07	0.28	0.84

(G) Down-regulated TFs

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
PAX8	-0.57	-0.18	-0.19	-0.17	-0.63	-1.04
MZF1	0.01	0.13	0.09	0.02	-0.71	-0.49
NR4A1	-1.06	-2.28	-0.94	0.19	-1.38	-0.60
EPAS1	-0.05	-0.42	-0.85	-0.18	-0.15	-0.89
HEY2	-0.35	-0.69	-0.89	-1.14	-0.24	-0.05
TEAD4	-0.05	-1.28	-1.29	-0.26	-0.37	-0.98
KLF2	-3.67	-3.93	-2.38	-2.47	-4.28	-2.33
KLF4	-3.13	-4.76	-3.31	-1.69	-3.18	-2.85
GATA2	-0.38	-0.16	0.05	-0.50	-0.52	-0.47
RXRA	-0.06	-0.46	-0.45	-0.40	-0.08	-0.73
RARG	-0.22	-0.71	-0.30	-0.95	-0.93	-0.75
MAZ	-0.01	-0.28	-0.16	-0.13	-0.41	-0.07
CEBPB	-0.31	0.43	0.77	0.00	-1.13	0.15
XBP1	-0.18	0.03	0.35	0.01	-0.33	0.15
JUNB	-0.91	-0.52	-0.30	0.10	-0.96	-0.88
NFATC1	-0.01	-0.58	-0.55	-0.42	-0.53	-0.92

(H) HIF1α targets

	HAEC			HUVEC		
Symbol	1hr	4hr	24hr	1hr	4hr	24hr
CXCR4	1.11	2.86	2.54	2.14	1.93	3.14
WASF3	-0.04	1.03	0.59	0.05	0.57	-0.13
BMP4	0.53	1.43	1.64	0.66	0.10	0.96
CD36	#N/A	#N/A	#N/A	-0.32	0.94	4.14
NAMPT	0.06	0.62	0.22	0.09	0.73	0.53
ATP1B1	0.13	0.12	0.42	0.21	0.19	0.52
PTGS2	0.04	0.45	0.53	-0.26	0.58	0.07
MME	-0.04	0.14	0.28	0.23	0.78	0.35
BNIP3	0.01	0.26	0.39	0.36	0.71	0.53
NOX4	0.15	1.21	0.79	0.14	0.39	0.37
CHKA	-0.44	0.35	0.70	-0.10	0.46	0.57
PGF	0.11	1.05	1.95	0.51	0.53	1.13
EDN1	0.90	1.88	1.84	0.51	0.36	0.55
KLF8	0.07	-1.77	-1.54	-0.04	-0.62	-1.78
FGF2	0.09	-1.02	-0.87	-0.24	-0.71	-1.22
KCNMB1	-1.89	-0.92	-1.97	0.23	-0.62	-0.67
HSPA2	-0.09	-0.64	-1.28	-0.33	-0.45	-1.10
ADAMTS1	-0.33	-2.61	-3.99	0.17	-0.65	-1.13
AQP1	-0.30	-4.68	-5.32	-1.00	-0.59	-4.25
PPP1R3C	0.14	-1.76	-1.92	0.00	-0.98	-1.45
NDRG1	-0.14	-1.39	-0.95	0.06	-0.61	-1.60
IL11	-0.97	-2.10	-0.06	-0.71	-1.01	-0.06
RHOA	0.00	-0.34	-0.67	0.04	-0.14	-0.47
VEGFA	-0.43	0.16	0.27	-0.30	-0.18	-1.02
PTHLH	-0.90	-2.74	-1.91	-0.34	-1.57	-1.86

Figure S4. Heatmaps of log2 fold-change of key genes for select functions and pathways. (A) Cell-cycle G1/S transition related genes, (B) Oxidative stress genes (WikiPathways), (C) TGF-β signaling pathway genes, (D) Lysosome pathway related genes, (E) Autophagy related genes, (F) Up-regulated transcription factors (TFs), (G) Down-regulated TFs, and (H) HIF1α targets.

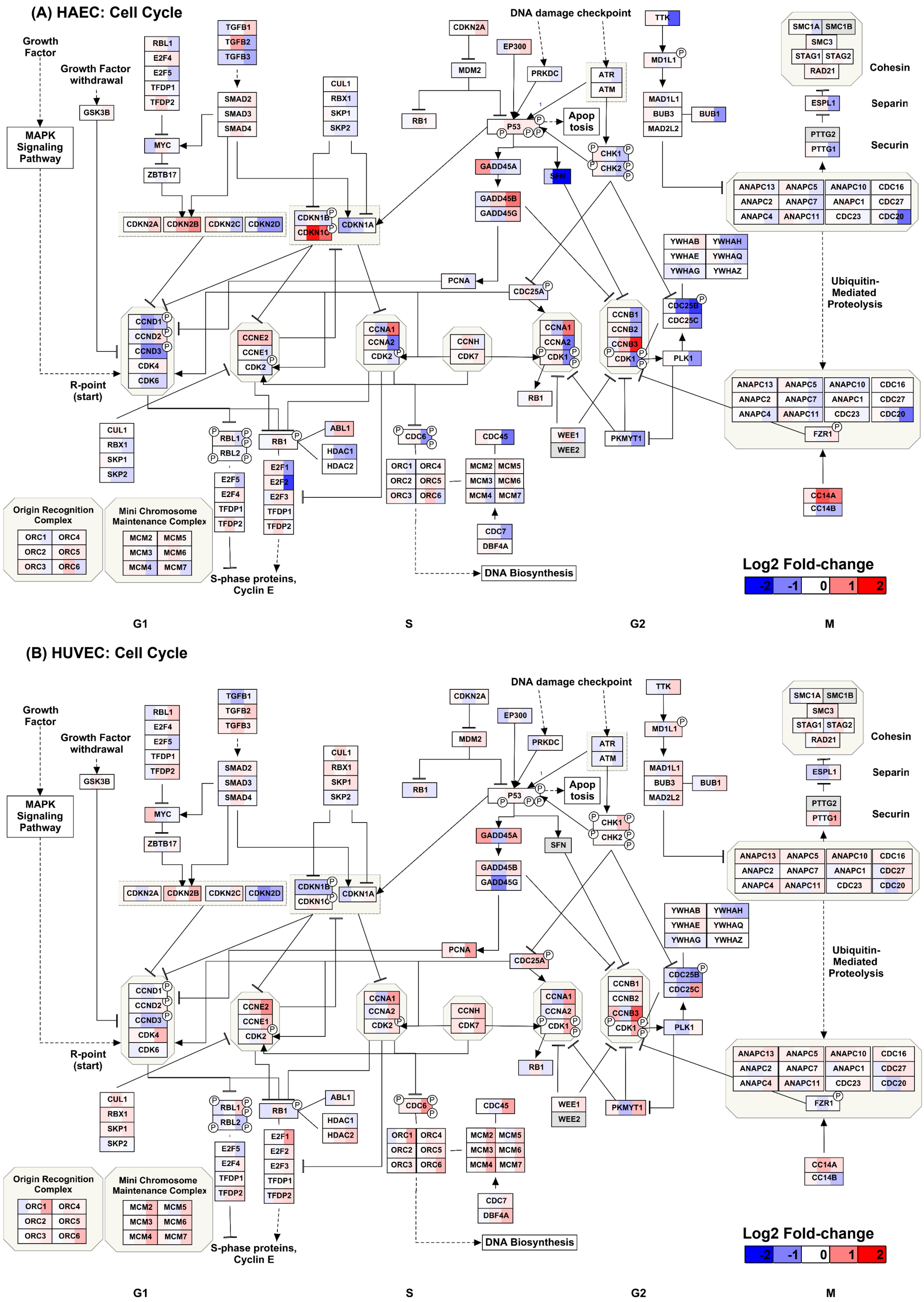
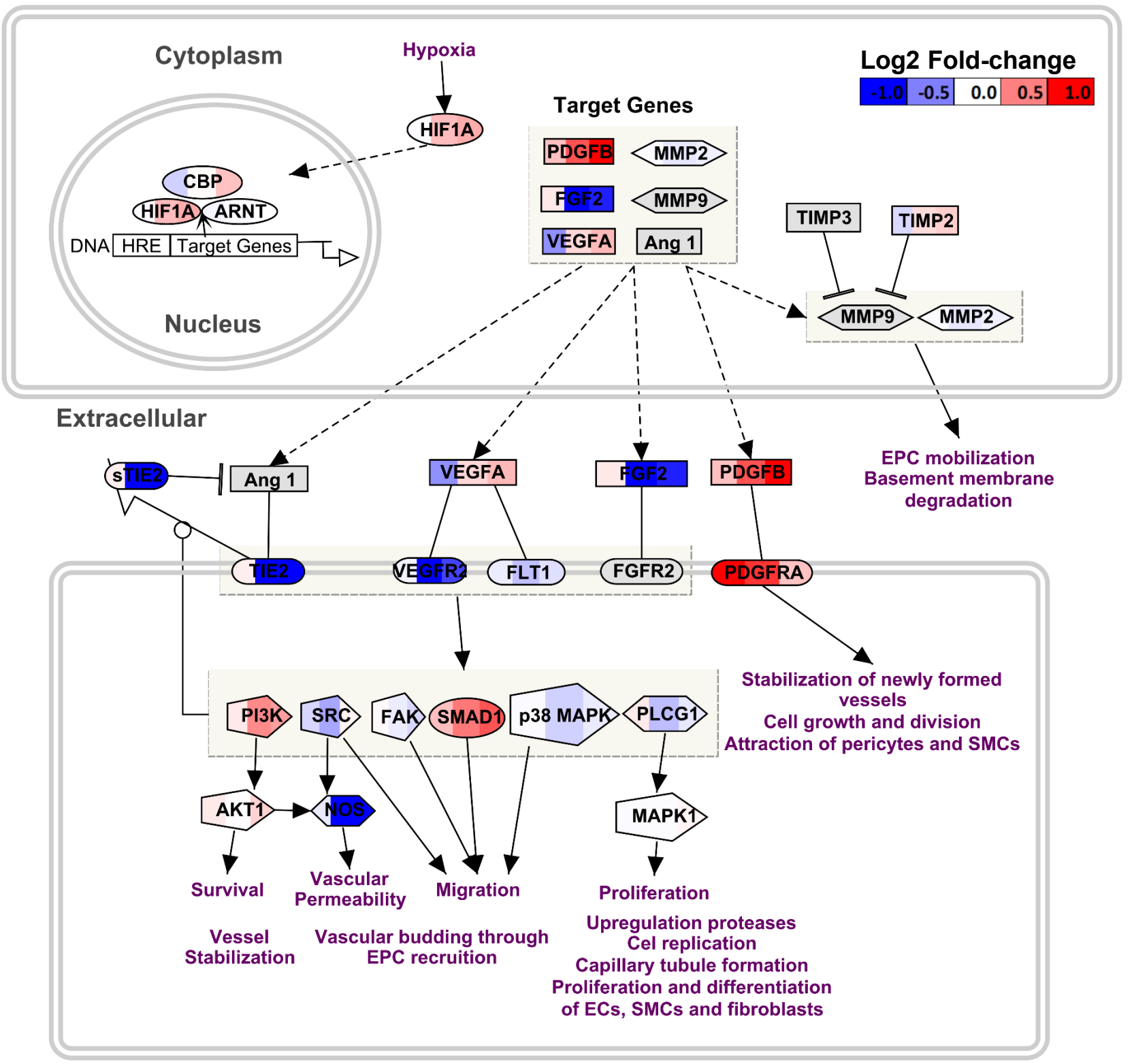


Figure S5. OS vs. PS expression changes in genes related to cell cycle pathway (WikiPathways) in HAECs (A) and HUVECs (B). Data are projected onto to the pathway genes using PathVisio (version 3.3.0). Red color represents OS/PS up-regulation and blue color represents down-regulation. Color-scale varies from -2 to +2 for log2 fold-changes. White color represents log2 fold-change of 0 (i.e., no change in expression). Overall, cell cycle genes are slightly down-regulated in HAECs, whereas many of these genes are slightly up-regulated in HUVECs. However, the fold-changes are relatively small.

(A) HAEC



(B) HUVEC

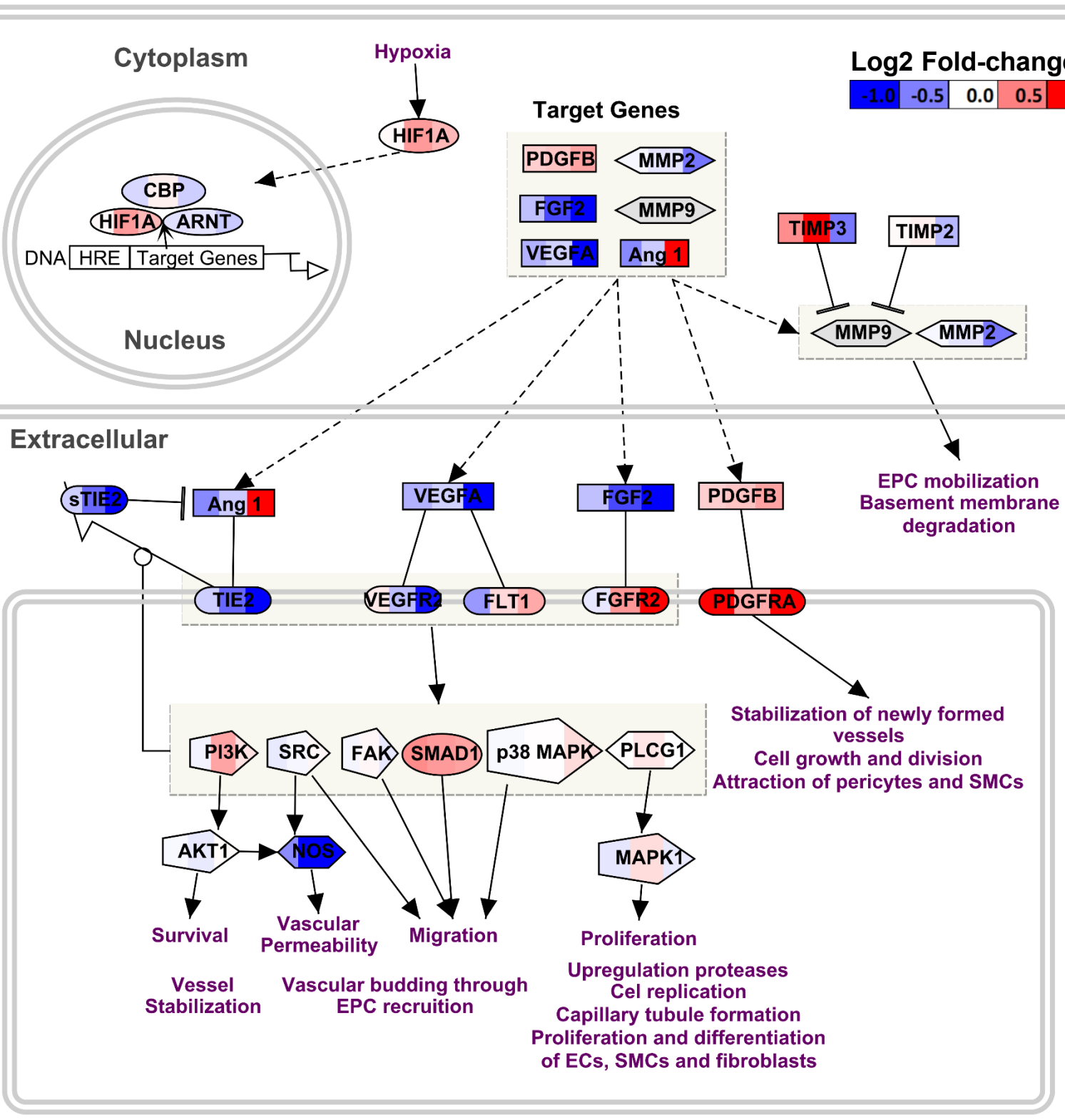


Figure S6. Temporal evolution of OS vs. PS differential expression for Angiogenesis pathway (WikiPathways) related genes in HAECs and HUVECs. Color-scale of log2 fold-change: -1 (blue) to 0 (white) to +1 (red). Several genes such as HIF1A, VEGFR2 and TIE2 show good similarities between the two cell types.

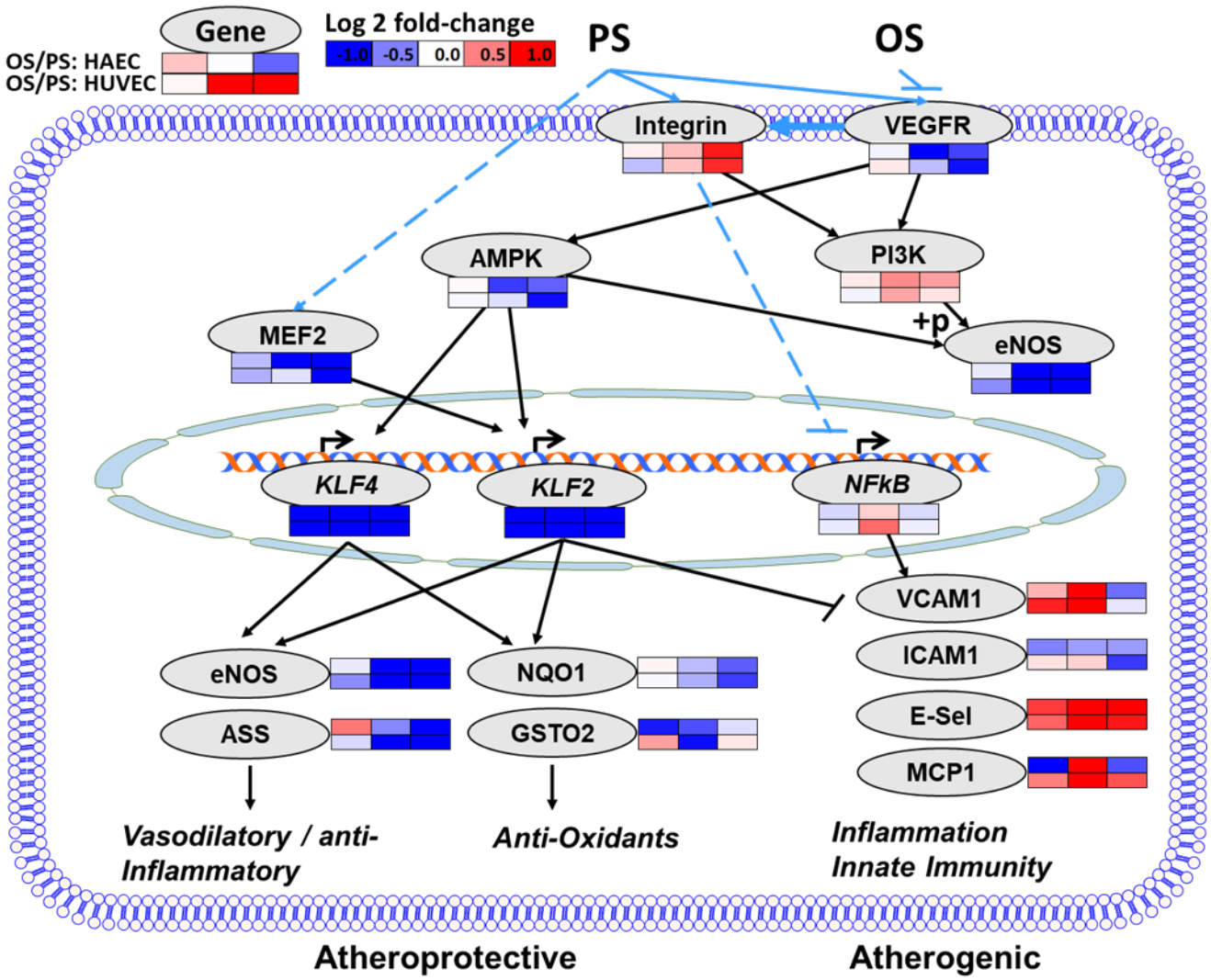


Figure S7. Temporal evolution of OS vs. PS differential expression for atheroprotective genes [KLF2, KLF4, eNOS (NOS3) and NQO1] and atherogenic genes [VCAM1, ICAM1, E-Sel (SELE) and MCP1 (CCL2)] in HAECs and HUVECs. Overall, gene programs contributing to atheroprotection (down-regulated) or atherogenesis (up-regulated) show similar OS vs. PS responses in HAECs and HUVECs.

Table S1. Results of pathway/functional enrichment for KEGG, Reactome and Biocarta pathways for DE genes ($p \leq 0.05$ and fold-change (up or down) ≥ 1.3).

Pathways		HAEC (-log10(p))			HUVEC (-log10(p))		
Category	Term	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
KEGG	hsa04015:Rap1 signaling pathway	1.77	9.48	2.30	1.12	4.61	1.91
KEGG	hsa04151:PI3K-Akt signaling pathway	1.55	5.57	3.83	0.95	3.11	2.85
KEGG	hsa04510:Focal adhesion	0.72	3.25	4.47	0.48	0.58	2.04
KEGG	hsa04014:Ras signaling pathway	0.60	7.42	2.41	1.00	4.06	1.64
KEGG	hsa04110:Cell cycle	0.38	0.59	4.43	#N/A	0.51	3.11
KEGG	hsa04512:ECM-receptor interaction	#N/A	0.93	3.64	#N/A	0.08	2.85
KEGG	hsa04668:TNF signaling pathway	5.35	8.64	2.62	3.92	7.79	1.24
KEGG	hsa04115:p53 signaling pathway	2.09	0.68	3.16	0.21	0.89	0.96
KEGG	hsa04270:Vascular smooth muscle contraction	#N/A	1.15	1.31	0.09	0.40	0.94
KEGG	hsa04350:TGF-beta signaling pathway	2.32	2.36	0.38	2.19	0.36	1.64
KEGG	hsa04068:FoxO signaling pathway	2.45	2.04	1.86	1.94	2.87	0.71
KEGG	hsa04390:Hippo signaling pathway	0.81	2.38	0.74	0.79	0.60	0.05
BIOCARTA	h_cellcyclePathway:Cyclins and Cell Cycle Regulation	#N/A	0.45	1.12	#N/A	0.08	1.81
BIOCARTA	h_g2Pathway:Cell Cycle: G2/M Checkpoint	0.26	0.09	1.12	#N/A	0.64	0.96
KEGG	hsa04514:Cell adhesion molecules (CAMs)	0.12	1.03	0.95	0.22	0.13	0.86
KEGG	hsa04210:Apoptosis	0.49	4.39	3.15	#N/A	0.40	0.47
BIOCARTA	h_g1Pathway:Cell Cycle: G1/S Check Point	#N/A	0.31	0.78	#N/A	#N/A	0.41
KEGG	hsa04066:HIF-1 signaling pathway	2.04	1.21	0.74	0.40	1.60	0.93
KEGG	hsa04370:VEGF signaling pathway	0.50	1.90	0.39	0.67	0.69	0.20
BIOCARTA	h_p53Pathway:p53 Signaling Pathway	0.38	#N/A	0.12	#N/A	#N/A	0.50
KEGG	hsa04150:mTOR signaling pathway	1.02	0.43	0.19	0.25	0.24	0.39
REACTOME	Oxidative Stress Induced Senescence	0.24	0.00	5.31	0.14	0.03	0.04
KEGG	hsa04062:Chemokine signaling pathway	0.33	0.79	0.10	0.31	0.44	0.30
	Color scale (#N/A: no genes from the pathway in our list):	0.00	0.50	1.00	1.30	1.50	2.00

Dataset S1. Sheet “OSbyPS_L2FC”: OS vs. PS log2 fold-changes (FCs) in HAECs and HUVECs at 1, 4 and 24 hr for genes with raw read counts of 10 or more in respective data sets at any time point. #N/A indicates that maximum raw read count (across the three time points in OS and PS conditions) for the gene was below 10. Sheet “Important_Genes”: A list of 212 EC relevant genes obtained by combining key genes from several EC function-related pathways.

SI References

1. Chen Z*, et al.* (2010) Shear stress, SIRT1, and vascular homeostasis. *Proc Natl Acad Sci U S A* 107(22):10268-10273.
2. Ajami NE*, et al.* (2017) Systems biology analysis of longitudinal functional response of endothelial cells to shear stress. *Proc Natl Acad Sci U S A* 114(41):10990-10995.
3. Hu J, Ge H, Newman M, & Liu K (2012) OSA: a fast and accurate alignment tool for RNA-Seq. *Bioinformatics* 28(14):1933-1934.
4. Love MI, Huber W, & Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550.
5. Huang DW, Sherman BT, & Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1):44-57.
6. Huang DW, Sherman BT, & Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 37(1):1-13.
7. Kanehisa M, Furumichi M, Tanabe M, Sato Y, & Morishima K (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 45(D1):D353-D361.
8. Matys V*, et al.* (2003) TRANSFAC: transcriptional regulation, from patterns to profiles. *Nucleic Acids Res* 31(1):374-378.