

**Supplementary Figures and Tables to Schweighofer et al. “The VEGF-induced transcriptional response comprises gene clusters at the crossroad of angiogenesis and inflammation“ (Thromb Haemost 2009; 102.3)**

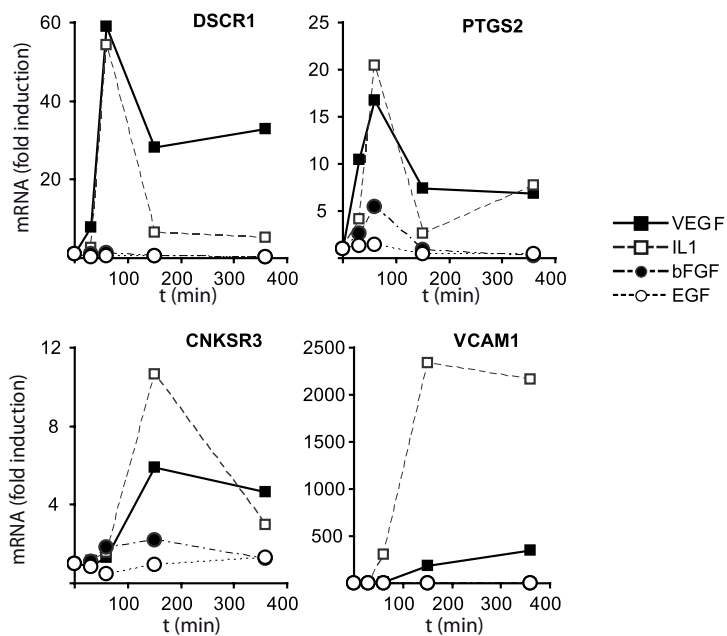
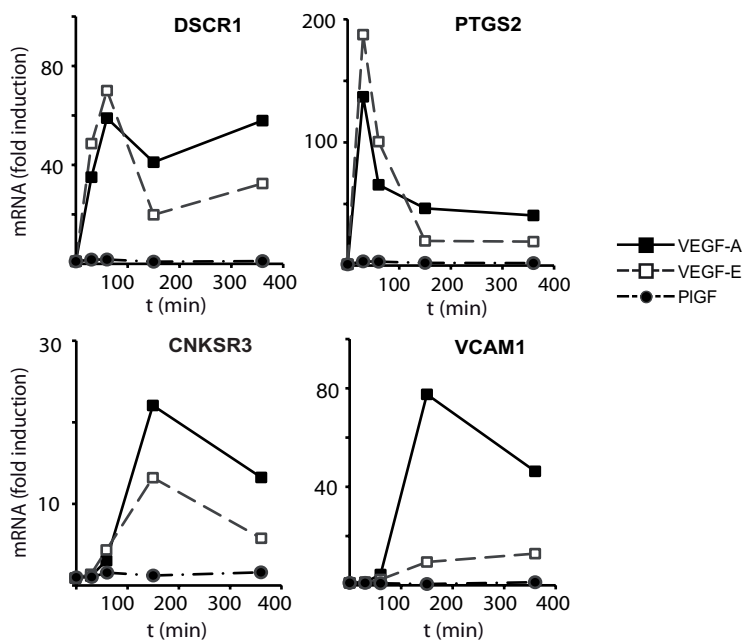
**Supplementary Figure 1. *Induction pattern and CsA sensitivity of selected genes from the VEGF/EGF/IL-1 and VEGF/IL-1 clusters.*** A) Induction pattern for VEGF, EGF, IL-1 and bFGF. HUVEC were seeded in 6-well plates, grown to confluence and stimulated in parallel with VEGF (100 ng/ml), IL-1 $\alpha$  (100 ng/ml), EGF (50 ng/ml) or bFGF (100 ng/ml) for 0.5, 1, 2.5 and 6 h. RNA was isolated and subjected to realtime RT-PCR analysis as described in the Methods. All values were normalized to  $\beta$ 2-microglobulin. One representative experiment of three performed with similar results is shown. B) Induction pattern for VEGF-A, VEGF-E and PlGF. HUVEC were stimulated by VEGF-A (100 ng/ml), VEGF-E (100 ng/ml) or PlGF (100 ng/ml) and analyzed as described above. C) Effects of CsA on induction pattern. HUVEC were seeded in 6-well plates and grown to confluence. The cells were preincubated with CsA (1  $\mu$ g/ml) for 30 minutes and induced with VEGF (100 ng/ml) for 0.5, 1, 2.5 and 6 h. All values were normalized to  $\beta$ 2-microglobulin. One representative experiment of three performed with similar results is shown.

**Supplementary Figure 2: *In contrast to IL-1 $\alpha$  VEGF fails to induce significant cytoplasmic-nuclear translocation of NF- $\kappa$ B.*** Immunofluorescence of nuclear accumulation of NF- $\kappa$ B. HUVEC were seeded on LabTek 8-well tissue culture chamber slides (NUNC, Wiesbaden, Germany) coated with 100  $\mu$ g/ml fibronectin (Becton Dickinson, Heidelberg, Germany) for 1 h. After treatment with VEGF (100 ng/ml) or IL-1 (100 ng/ml) for 20 min the cells were fixed in 4% paraformaldehyde/PBS for 10min. Permeabilization was achieved by addition of PBS containing 0.5% Triton X-100 for 5 minutes at RT, anti-NF- $\kappa$ B p65 rabbit antibodies sc-109 from Santa Cruz Biotechnology (Santa Cruz, CA) were diluted 1:500 in PBS/1% BSA and incubated for 1 h at RT. The cells were washed four times in PBS/1% BSA. Secondary Alexa 488 goat anti-rabbit antibodies from Molecular Probes (Eugene, OR), diluted 1:10000 were used for visualization followed by washing of the cells 5 times in PBS/1%BSA. For nuclear staining, Hoechst (Sigma, St.Luis, MO) diluted 1:1000 was

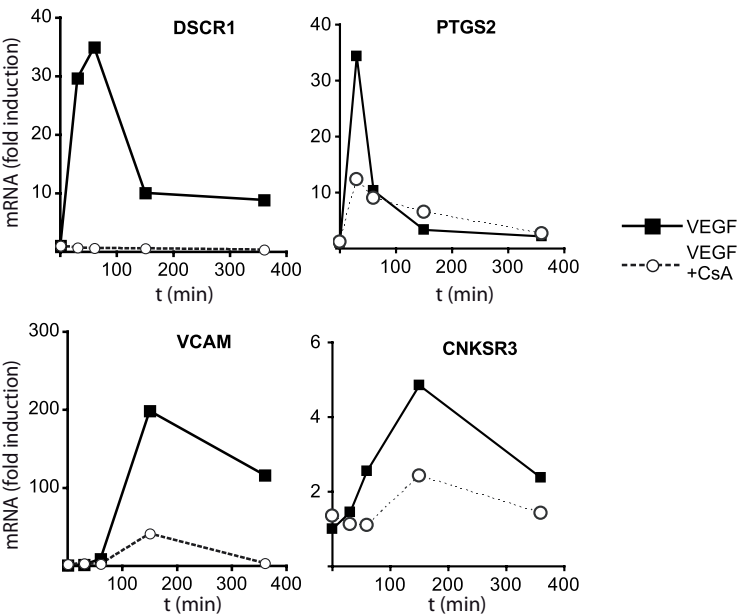
added in the last washing step. Cover slips were mounted in fluorescent mounting medium (Dako, Glostrup, Denmark) and analyzed on a Nikon Diaphot TMD microscope. Images were taken by a CCD camera (Kappa GmbH, Gleichen, Germany).

**Supplementary Table 1: List of all genes induced by VEGF over three-fold.** VEGF induced genes are sorted according to their fold induction value at the 60 min time point and dependent on their classification as *SVI* (*selectively VEGF induced*), *IR* (*inflammation related*) and *GA* (*generally activated*). Furthermore, the corresponding fold induction values for EGF and IL-1 is given. The displayed genes were selected according to the following criteria. Firstly, Affymetrix probe sets showing absolute calls of "absence", difference calls of "no change", less than 3-fold upregulation or a signal intensity of less than 60 for all VEGF-induced data points were excluded. Secondly, only genes upregulated in two arrays obtained from two independent experimental induction series were included in the selection. Thirdly, the VEGF induction of few genes could not be verified by realtime RT-PCR and these were again excluded from the selected list.

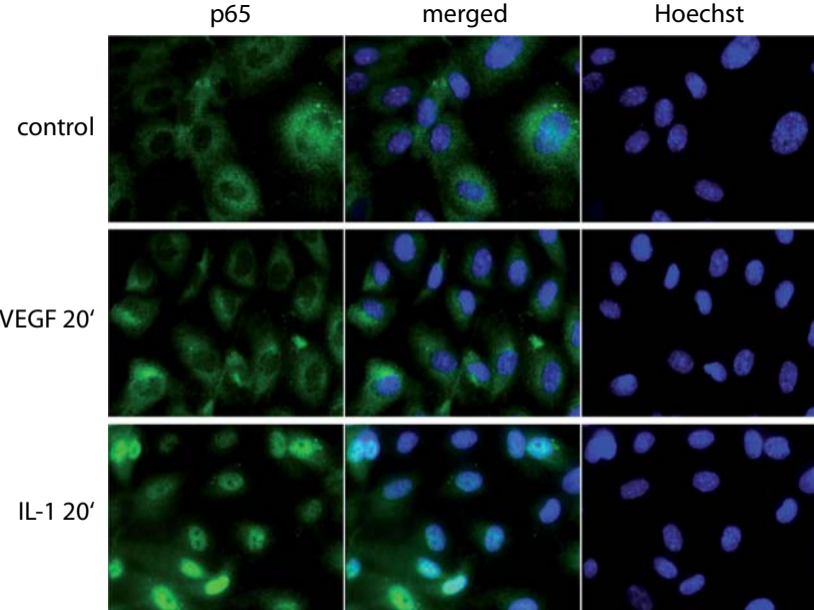
**Supplementary Table 2: Primers used for realtime RT-PCR.** Forward and reverse primers as used for realtime RT-PCR are displayed.

**A****B**

C



Supplemental Figure 1C



Supplemental Figure 2

Supplemental Table 1

UniGene ID	Entrez Gene	Gene Symbol	David Gene Name	VEGF				EGF				IL1				Classification
				30min	60min	150min	360min	30min	60min	150min	360min	30min	60min	150min	360min	
HS.165258	4929	NR4A2	Nuclear receptor subfamily 4, group A, member 2	16.0	36.8	8.6	1.1	1.1	4.3	3.0	0.7	1.0	5.3	0.9	0.4	SVI
HS.534313	1960	EGR3	Early growth response 3	6.5	21.1	1.1	1.1	1.4	1.9	0.8	0.8	8.6	24.3	1.9	2.5	SVI
HS.445534	5187	PER1	Period homolog 1 (Drosophila)	2.0	8.6	2.3	1.0	0.9	1.7	0.7	0.7	0.8	2.8	0.5	0.8	SVI
HS.450230	3486	IGFBP3	Insulin-like growth factor binding protein 3	2.3	8.0	21.1	9.2	2.0	2.6	3.7	2.8	1.2	1.0	4.3	9.8	SVI
HS.82071	10370	CITED2	Cbp/p300-interacting transactivator 2	2.8	4.3	3.5	1.4	1.5	1.6	1.7	1.1	1.1	0.7	0.8	0.5	SVI
HS.200250	1390	CREM	CAMP responsive element modulator	2.3	3.5	8.0	2.3	1.1	1.7	2.1	1.2	1.1	1.7	1.6	0.7	SVI
HS.74870	3142	HLX1	H2.0-like homeobox 1 (Drosophila)	1.3	3.5	3.5	3.2	1.1	0.9	1.6	1.1	1.1	0.7	1.4	0.9	SVI
HS.25960	4613	MYCN	V-myc myelocytomatosis viral related oncogene, neuroblastoma derived	2.8	2.5	7.5	3.5	0.4	1.3	2.3	0.9	0.9	0.3	0.4	0.4	SVI
HS.6790	4189	DNAJB9	Dnaj (hsp40) homolog, subfamily B, member 9	1.7	2.1	3.2	2.0	1.7	1.6	1.4	1.6	1.1	1.0	1.5	1.5	SVI
HS.372914	10397	NDRG1	N-myc downstream regulated gene 1	1.1	1.9	3.2	1.9	1.2	1.1	1.3	1.1	1.1	1.0	0.9	1.5	SVI
HS.2030	7056	THBD	Thrombomodulin	1.1	1.6	3.7	0.9	0.6	0.9	1.6	0.8	1.4	1.6	0.5	0.3	SVI
HS.310512	51554	CCRL1	Chemokine (C-C motif) receptor-like 1	0.9	1.5	3.7	2.0	0.8	1.1	1.4	1.0	1.1	0.6	0.6	0.8	SVI
HS.654474	4208	MEF2C	MADS box transcription enhancer factor 2, polypeptide c	1.1	1.3	4.3	1.6	1.1	1.1	1.3	0.6	0.9	1.0	0.5	0.5	SVI
HS.62192	2152	F3	Coagulation factor III (thromboplastin, tissue factor)	3.0	29.9	6.5	5.7	1.0	0.7	0.8	1.0	29.9	128.0	73.5	24.3	IR
HS.196384	5743	PTGS2	Prostaglandin-endoperoxide synthase 2	8.0	21.1	3.5	1.6	2.8	3.7	1.3	1.2	8.0	7.5	13.9	10.6	IR
HS.282326	1827	DSCR1	Down syndrome critical region gene 1	4.3	13.0	8.6	4.9	1.1	1.5	1.2	1.1	4.9	10.6	5.7	2.5	IR
HS.460	467	ATF3	Activating transcription factor 3	5.3	12.1	1.2	1.0	2.1	2.1	1.0	0.9	13.9	13.9	8.0	3.0	IR
HS.75678	2354	FOSB	Fbj murine osteosarcoma viral oncogene homolog B	4.3	11.3	1.3	1.2	1.2	3.0	1.1	0.9	24.3	36.8	1.1	1.2	IR
HS.75765	2920	CXCL2	Chemokine (C-X-C motif) ligand 2	6.5	9.8	2.3	0.9	3.0	1.9	0.8	0.8	10.6	10.6	10.6	13.0	IR
HS.319171	64332	NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta isoform A	3.5	8.0	1.1	0.9	1.9	1.9	0.7	0.9	8.0	12.1	8.6	8.6	IR
HS.444947	10221	TRIB1	Tribbles homolog 1 (Drosophila)	2.8	7.5	1.0	1.2	2.0	2.8	0.9	0.9	6.5	5.7	3.0	2.5	IR
HS.624	3576	IL8	Interleukin 8	3.5	6.5	2.0	0.4	1.6	1.2	0.7	0.7	4.9	7.5	8.6	8.6	IR
HS.432453	1326	MAP3K8	Mitogen-activated protein kinase kinase kinase 8	1.1	5.3	6.5	1.7	0.8	0.7	1.1	1.3	1.5	4.3	7.5	2.6	IR
HS.524430	3164	NR4A1	Nuclear receptor subfamily 4, group A, member 1	2.3	5.3	2.6	1.0	1.1	0.9	0.5	0.2	4.0	6.5	0.8	1.1	IR
HS.534115	9510	ADAMTS1	Adam metalloproteinase with thrombospondin type 1 motif, 1	1.6	5.3	3.2	2.3	1.1	1.9	1.2	0.9	2.1	4.0	3.0	4.3	IR
HS.23388	81575	APOLD1	Apolipoprotein I domain containing 1	2.6	5.3	1.4	0.8	1.5	1.5	0.5	0.9	1.7	1.5	1.5	2.6	IR
HS.279522	8013	NR4A3	Nuclear receptor subfamily 4, group A, member 3	0.8	4.3	1.5	1.4	0.8	0.6	0.9	0.8	0.9	24.3	9.2	14.9	IR
HS.440829	1052	CEBPD	Ccaat/enhancer binding protein (C/EBP), delta	1.4	4.0	5.3	1.5	1.2	1.4	1.3	1.0	3.5	8.0	12.1	6.1	IR
HS.89690	2921	CXCL3	Chemokine (C-X-C motif) ligand 3	1.7	3.7	2.1	1.1	1.3	1.3	0.4	1.1	24.3	42.2	36.8	39.4	IR
HS.799	1839	HBEGF	Heparin-binding EGF-like growth factor	1.9	3.5	3.7	1.6	1.4	1.6	1.6	0.9	2.3	4.0	1.6	2.8	IR
Hs.436029	---	236685_at	---	1.5	3.5	1.9	0.7	0.9	1.1	0.9	0.9	11.3	9.8	9.2	3.5	IR
HS.789	2919	CXCL1	Chemokine (C-X-C motif) ligand 1	2.0	3.2	1.5	0.8	1.3	1.9	1.0	1.2	4.6	5.7	6.1	5.7	IR
HS.1547	3759	KCNJ2	Potassium inwardly-rectifying channel, subfamily J, member 2	1.7	3.2	1.6	0.5	0.8	0.9	0.8	0.5	1.4	1.1	1.7	1.6	IR
HS.37982	4739	NEDD9	Neural precursor cell expressed, developmentally down-regulated 9	4.0	3.2	1.9	1.5	1.7	1.3	0.5	0.7	1.6	1.6	1.3	1.0	IR
HS.150557	687	KLF9	Kruppel-like factor 9	1.7	3.0	2.6	1.2	1.2	1.5	0.7	0.7	1.2	2.8	2.0	1.4	IR
HS.1722	3552	IL1A	Interleukin 1, alpha	1.7	3.0	0.9	0.8	1.3	1.4	0.3	0.8	16.0	17.1	8.6	9.2	IR
HS.73853	650	BMP2	Bone morphogenetic protein 2	1.2	2.8	2.8	1.7	0.9	1.2	1.1	1.4	1.4	2.6	2.6	2.5	IR
HS.25590	6781	STC1	Stanniocalcin 1	1.3	2.8	5.7	2.1	0.9	0.8	1.0	0.6	1.3	2.1	1.7	3.5	IR
HS.303649	6347	CCL2	Chemokine (C-C motif) ligand 2	1.4	2.6	3.2	0.6	1.5	1.3	0.8	1.0	1.7	2.1	2.1	2.1	IR
HS.89546	6401	SELE	Selectin E (endothelial adhesion molecule 1)	0.9	2.3	7.0	2.1	0.8	0.7	0.8	0.4	24.3	42.2	48.5	39.4	IR
HS.109225	7412	VCAM1	Vascular cell adhesion molecule 1	1.1	2.1	12.1	3.2	1.1	0.9	1.3	1.0	3.0	17.1	29.9	17.1	IR
HS.1048	4254	KITLG	Kit ligand	0.8	1.4	3.0	1.5	0.8	1.1	1.7	0.9	1.1	3.7	4.0	7.5	IR
HS.159223	4665	NAB2	NGFI-A binding protein 2 (EGR1 binding protein 2)	1.1	1.4	4.0	1.2	0.5	1.0	1.4	0.9	0.6	1.9	2.1	0.9	IR
HS.436873	3685	ITGAV	Integrin, alpha v (vitronectin receptor, alpha polypeptide, antigen cd51)	0.4	1.2	2.8	0.4	0.1	0.2	0.3	0.1	1.5	3.2	2.5	0.9	IR
HS.16064	154043	CNKSR3	CNKSR family member 3	1.0	1.1	4.9	1.6	1.0	0.9	1.1	0.8	1.1	3.7	8.6	2.6	IR
HS.510172	3097	HIVEP2	Human immunodeficiency virus type I enhancer binding protein 2	0.9	1.1	4.9	1.6	0.9	1.1	1.3	1.1	1.2	1.2	5.7	3.0	IR
HS.437075	9586	CREB5	CAMP responsive element binding protein 5	1.1	1.1	3.5	0.9	1.1	0.8	1.9	1.3	1.1	1.6	4.6	2.8	IR
HS.326035	1958	EGR1	Early growth response 1	26.0	22.6	1.9	0.8	18.4	9.8	0.5	0.8	19.7	5.3	1.1	1.1	GA
HS.1395	1959	EGR2	Early growth response 2	10.6	21.1	1.2	1.4	7.0	4.6	1.9	0.5	6.5	3.2	2.6	0.6	GA
HS.25647	2353	FOS	V-fos FBJ murine osteosarcoma viral oncogene homol.	32.0	13.9	1.0	1.1	42.2	4.3	0.9	0.8	90.5	13.0	1.3	1.1	GA
HS.376206	9314	KLF4	Kruppel-like factor 4 (gut)	5.7	12.1	2.8	2.1	4.9	6.1	1.7	1.9	1.7	1.6	2.6	1.5	GA
HS.534052	7538	ZFP36	Zinc finger protein 36, C3H type, homolog (Mouse)	10.6	7.5	2.5	1.5	6.1	1.4	0.2	0.4	26.0	6.5	3.2	0.9	GA
HS.171695	1843	DUSP1	Dual specificity phosphatase 1	11.3	6.1	4.0	0.6	2.8	0.6	0.7	0.6	5.3	2.5	1.1	0.3	GA
HS.171825	8553	BHLHB2	Basic helix-loop-helix domain containing, class B, 2	3.0	5.7	2.3	1.1	2.5	3.2	1.6	1.0	2.8	6.1	1.4	1.5	GA
HS.517617	23764	MAFF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog f (Avian)	2.3	4.9	2.8	2.3	2.1	2.8	1.1	1.4	1.9	4.3	1.9	2.5	GA
HS.25292	3726	JUNB	Jun B proto-oncogene	3.0	4.6	1.6	1.0	3.5	1.9	0.7	0.8	13.0	13.0	4.9	2.6	GA
HS.2128	1847	DUSP5	Dual specificity phosphatase 5	1.6	3.7	2.8	1.1	1.5	2.0	1.1	0.7	2.8	4.6	1.4	1.5	GA
HS.78944	5997	RGS2	Regulator of G-protein signalling 2, 24kda	1.6	3.5	2.5	1.1	1.6	2.6	1.2	0.9	0.7	0.7	2.6	1.4	GA
HS.435001	7071	KLF10	Kruppel-like factor 10	1.7	3.2	0.7	0.9	3.5	2.1	1.4	1.4	9.2	1.1	1.4	0.7	GA

**Supplemental Table 2: PCR primers 5'-3'**

<b>Primer</b>	<b>Sequence</b>
ATF3-forward	GTGCCGAAACAAGAAGAAGG
ATF3-reverse	TCTGGAGTCCTCCCATTCTG
beta-2-microglobulin-forward	GATGAGTATGCCTGCCGTGTG
beta-2-microglobulin-reverse	CAATCCAAATGCGGCATCT
beta-actin-forward	GTGATGGTGGGCATGGGTCA
beta-actin-reverse	TTAATGTCACGCACGATTTCCC
BHLHB2-forward	GGCATAGCACGGTAGTGGTT
BHLHB2-reverse	TCAGACCTTGGTTTGGTTCC
CCRL1-forward	CTTGGTTGCAGTGGTGCTTA
CCRL1-reverse	AGTGGTCCTGGGTACCCTTC
CNKS3-forward	GACTGGTGGCATTGTTCTT
CNKS3-reverse	GCCACGTTATTGCAAAGTCA
CREM-forward	GCCCTTTCTACCATCTCACG
CREM-reverse	GGACAGCTCCAGACCACCTA
CXCL2-forward	CTCAAGAATGGGCAGAAAGC
CXCL2-reverse	CTTCAGGAACAGCCACCAAT
DNAJB9-forward	AAAATAAGAGCCCGGATGCT
DNAJB9-reverse	TGACTGCTCAAAGAAGTCTCCA
DSCR1_E4-forward	TAGCTCCCTGATTGCCTGT
DSCR1_E5-reverse	GGAGAAGGGGTTGCTGAAGT
EGR1-forward	AGCCCTACGAGCACCTGAC
EGR1-reverse	TGGGTTGGTCATGCTCACTA
EGR2-forward	AGTTGGTCTCCAGGTTGTG
EGR2-reverse	AGCAAAGCTGCTGGGATATG
EGR3-forward	GACAATCTGTACCCCGAGGA
EGR3-reverse	TCCCAAGTAGGTCACGGTCT
EGR4-forward	ATGAGAAGAAACGGCACAGC
EGR4-reverse	GCTCAGAGAGAAGCGAAGGA
F3-forward	CCGAACAGTTAACCGGAAGA
F3-reverse	TCAGTGGGGAGTTCTCCTTC
FOSB-forward	CGTGCTGCATGAAAAACATT
FOSB-reverse	CGCACACACACACATCCATA
HLX1-forward	CTCCAACCTGCAGAGGAAAG
HLX1-reverse	GGTTCTGGAACCACACCTTC
IGFBP3-forward	GCTACAGCATGCAGAGCAAG
IGFBP3-reverse	CTGCTGGTCATGTCCTTGG
IL8-forward	CTCTTGGCAGCCTTCCTGATT
IL8-reverse	TATGCACTGACATCTAAGTTCTTTAGCA
ITGAV-forward	ACCAGAGCCTGCATCAAAAT
ITGAV-reverse	CAAGTTGCATCTGGGGAACT
JunB-forward	CCTCCACCTCGACGTTTAC
JunB-reverse	TTCCACAGTACGGTGCAGAG
KCNJ2-forward	GGGTCTTGGGAATTCTGGTT
KCNJ2-reverse	TGGGAGCCTTGTGGTTCTAC
KLF4-forward	GCGGCAAACCTACACAAAG
KLF4-reverse	CCCCGTGTGTTTACGGTAGT
MEF2C-forward	CATAACATGCCACCATCTGC
MEF2C-reverse	CGTGTGTTGTGGGTATCTCG
MYCN-forward	CGCAAAGCCACCTCTCATTA

MYCN-reverse	TCCAGCAGATGCCACATAAGG
NDRG1-forward	CTCGCTGAGGCCTTCAAGTA
NDRG1-reverse	GGGTGCCATCCAGAGAAGT
NEDD9-forward	CAGAAATTCAGG GAGCTGGA
NEDD9-reverse	GCACGTGGACAAGTTTTCTG
NFKBIZ-forward	ACTCGGAACTTGGAGAACGA
NFKBIZ-reverse	AATACGGTGGAGCTCTCTGC
NR4A2-forward	TTTCTGCCTTCTCCTGCATT
NR4A2-reverse	GTGGCACCAAGTCTTCCAAT
PER1-forward	AGCCTCGGTTTTCTGAGGAC
PER1-reverse	CCAGTCCATCCAGCTCTGA
PTGS-forward	ATCACAGGCTTCCATTGACC
PTGS-reverse	CAGGATACAGCTCCACAGCA
SELE-forward	CGCTGTAAAATCTTGGCACA
SELE-reverse	CTGTGGGCATTCAACATCTG
STC1-forward	AGCTGAATGTGTGCAGCATC
STC1-reverse	ATCACATTCCAGCAGGCTTC
THBD-forward	CGGGTTGTGTGTCTGTTAC
THBD-reverse	AGCCCTCCATGCATCTCATA
VCAM-forward	AAAAGCGGAGACAGGAGACA
VCAM-reverse	CCCTTCATGTTGGCTTTTCT