Data Article

Time-course gene expression data on the transcriptional effects of Aminaphtone on ECV304 endothelial cells

CrossMark

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ABSTRACT

We previously showed that Aminaphtone, a drug used in the treatment of chronic venous insufficiency, modulates several vasoactive factors, such as endothelin-1 and adhesion molecules. Here, we provide data of time-course experiments about the effects of Aminaphtone on gene expression at the genome-wide level in human endothelial cells undergoing cytokine stimulation *in vitro*. ECV-304 endothelial cells were incubated with interleukin-1β (IL-1β) in the presence or absence of Aminaphtone for 1, 3, and 6 h. Gene expression profiles were analyzed by microarray. This article contains complete data on the genes significantly modulated by the drug over time. The data are supplemental to our original research article reporting detailed analysis of the actions of Aminaphtone on IL-1β stimulated endothelial cells at the molecular level, "Gene expression profiling reveals novel protective effects of Aminaphtone on ECV304 endothelial cells" (Salazar et al., 2016) [1].

Specifications Table

Subject area	*Biology*
More specific subject area	*Cellular transcriptomics*
Type of data	*Tables, figure*
How data was acquired	*Affymetrix 7G Microarray Scanner (Affymetrix, Santa Clara, CA)*
Data format	*Filtered, analyzed*
Experimental factors	*Time-course experiments of gene expression responses of confluent human ECV304 endothelial cells, stimulated with recombinant IL-1β (100 IU/ml), to treatment with Aminaphtone (6 μg/ml) vs. medium alone*
Experimental features	*Whole-genome gene expression analysis performed at 1, 3, and 6-h time points using GeneChip Gene 1.0 ST Arrays (Affymetrix, Santa Clara, CA)*
Data source location	*Milan, Italy*
Data accessibility	*Filtered, analyzed data are reported within this article. Raw and normalized data are available via NCBI's GEO accession number GEO: GSE83297 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE83297).*

Value of the data

- Previously unreported transcriptional effects of Aminaphtone, a drug currently used for the treatment of chronic venous insufficiency.
- Illustrate gene expression changes induced by Aminaphtone in a human endothelial cell line subjected to inflammatory stimulus.
- May facilitate further experiments to unveil the still unknown mechanism of action of this drug.
- May serve as a benchmark for comparison with data obtained in primary cells for further insight.
- May stimulate further research on the clinical use of this drug in other disease conditions, in which inflammation and endothelial dysfunction are key pathophysiological elements.

1. Data

Transcriptional effects of Aminaphtone treatment on human ECV304 endothelial cells stimulated with IL-1β for 1, 3 and 6 h are shown in Table 1. The complete raw and normalized data are deposited in NCBI's Gene Expression Omnibus [2] and are accessible through GEO Series accession number GEO: GSE83297 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE83297). A hierarchically clustered heatmap of the differentially expressed genes (Fig. 1) visualizes the arrangement of treatment groups into clusters. Table 2 illustrates data on the functional enrichment analysis of modulated genes, showing gene sets and molecular pathways affected by Aminaphtone in IL-1β stimulated ECV304 cells.

2. Experimental design, materials and methods

2.1. Cell cultures and treatments

Human ECV304 endothelial cells (European Collection of Authenticated Cell Cultures, ECACC No. 92091712) were seeded at $\times 10^5$/well in 6-well tissue-culture treated plates and grown to confluence

Table 1

Differentially expressed genes in Aminaphtone-treated *vs.* untreated IL-1β-stimulated ECV304 endothelial cells.

Symbol	Gene name	Gene ID	FDR	$P_{treatment}$	$P_{interaction}$	FC AMNA/IL-1β			Pairwise		
						FC 1 h	FC 3 h	FC 6 h	1 h	3 h	6 h
AAK1	AP2 associated kinase 1	22848	9.13e-02	**5.98e-03**	7.26e-01	1.55	1.66	1.31			*
ACAP1	ArfGAP with coiled-coil, ankyrin repeat and PH domains 1	9744	1.65e-02	**2.07e-04**	4.01e-01	−1.50	−1.23	−1.36	**	**	*
AGAP2	ArfGAP with GTPase domain, ankyrin repeat and PH domain 2	116986	1.73e-03	**8.86e-05**	4.53e-01	−1.39	−1.58	−1.31	**	**	*
AK4	adenylate kinase 4	205	8.79e-03	4.27e-01	**7.56e-04**	1.41	−1.01	−1.64			**
ANKRD1	ankyrin repeat domain 1 (cardiac muscle)	27063	8.02e-03	**2.75e-02**	**1.23e-02**	1.26	−1.46	−1.78			**
ANKRD13A	ankyrin repeat domain 13A	88455	8.98e-02	**2.37e-04**	1.88e-01	1.23	1.47	1.18	*	**	*
ANXA3	annexin A3	306	4.39e-04	7.76e-02	**7.05e-04**	1.48	−1.33	−1.70	**		**
AREG	amphiregulin	374	7.88e-02	**6.01e-03**	6.32e-01	−1.37	−1.39	−1.78			*
ARHGEF26	Rho guanine nucleotide exchange factor (GEF) 26	26084	7.10e-02	**1.36e-03**	9.35e-01	1.42	1.33	1.42	*		
ARL4C	ADP-ribosylation factor-like 4C	10123	2.31e-02	**2.22e-04**	8.41e-01	−1.41	−1.30	−1.39	**	**	**
ATF7IP2	activating transcription factor 7 interacting protein 2	80063	5.87e-02	**5.59e-03**	1.69e-01	1.77	1.17	1.22	**		
BCL2L11	BCL2-like 11 (apoptosis facilitator)	10018	8.59e-04	**7.06e-05**	**3.23e-02**	1.60	1.48	1.09	**	**	
BCL3	B-cell CLL/lymphoma 3	602	4.12e-03	**3.18e-02**	**5.00e-03**	−1.98	−1.05	1.13	**		
BMP6	bone morphogenetic protein 6	654	1.78e-02	**3.25e-03**	6.12e-02	−1.00	1.65	1.47		**	**
BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	664	0.00e+00	**3.64e-05**	**4.09e-06**	1.11	−1.12	−2.54			**
BRCA1	breast cancer 1, early onset	672	7.46e-02	4.42e-01	**1.64e-02**	1.96	−1.28	−1.15	*		
BUB1	BUB1 mitotic checkpoint serine/threonine kinase	699	1.40e-02	5.76e-01	**5.38e-03**	1.93	−1.28	−1.26	**		
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	701	5.11e-02	4.12e-01	**1.69e-02**	2.09	−1.32	−1.14	**		
C14orf105	chromosome 14 open reading frame 105	55195	6.13e-02	**4.75e-03**	5.28e-01	−1.22	−1.59	−1.62		**	**
C15orf48	chromosome 15 open reading frame 48	84419	5.83e-03	**7.16e-05**	**4.51e-02**	−1.61	−1.17	−1.25	**		*
C1orf63	chromosome 1 open reading frame 63	57035	3.07e-03	**1.82e-04**	3.43e-01	1.61	1.27	1.38	**		*
C2orf44	chromosome 2 open reading frame 44	80304	9.87e-03	**1.63e-03**	2.02e-01	1.79	1.35	1.20	**	*	
C3orf58	chromosome 3 open reading frame 58	205428	1.08e-05	3.22e-01	**1.35e-06**	1.54	1.17	−1.61			**
C7orf53	chromosome 7 open reading frame 53	286006	6.37e-05	**1.06e-03**	**1.05e-02**	2.19	1.05	1.27	**		
C9orf131	chromosome 9 open reading frame 131	138724	9.82e-03	**4.39e-04**	7.67e-02	1.67	1.21	1.19	**		
CACHD1	cache domain containing 1	57685	4.66e-02	**2.02e-03**	4.84e-01	1.31	1.63	1.29		**	
CBLB	Cbl proto-oncogene, E3 ubiquitin protein ligase B	868	8.71e-02	**1.29e-02**	2.19e-01	1.07	1.86	1.45		*	
CCL22	chemokine (C–C motif) ligand 22	6367	0.00e+00	**6.98e-06**	6.53e-02	−1.36	−2.20	−1.99		**	**
CD70	CD70 molecule	970	9.39e-02	**1.09e-03**	2.37e-01	−1.53	−1.22	−1.20	**		
CD83	CD83 molecule	9308	1.63e-03	**4.10e-04**	8.35e-02	1.80	1.22	1.27	**		
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	1052	6.06e-03	4.58e-01	**6.34e-04**	−1.70	1.12	1.31	**		*
CENPF	centromere protein F, 350/400 kDa	1063	8.68e-02	6.92e-01	**2.45e-02**	1.00	−1.75	−1.31			
CEP55	centrosomal protein 55 kDa	55165	4.42e-02	7.14e-01	**8.10e-03**	1.68	−1.40	−1.35	*		
CEP76	centrosomal protein 76 kDa	79959	7.67e-02	**2.29e-03**	1.52e-01	1.57	1.32	1.09	*	*	
CHCHD7	coiled-coil-helix-coiled-coil-helix domain containing 7	79145	6.62e-02	**3.19e-03**	4.05e-01	1.58	1.47	1.17	*	*	
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	10370	7.93e-02	2.38e-01	**3.69e-03**	−1.38	1.47	1.22	**		

CKAP2L	cytoskeleton associated protein 2-like	150468	1.53e-02	1.45e-01	**1.89e-02**	1.57	−1.97	−1.57
CLEC4E	C-type lectin domain family 4, member E	26253	4.03e-03	**8.42e-04**	3.66e-01	−1.25	−1.53	−1.71
CNNM4	cyclin M4	26504	1.79e-07	**9.46e-05**	**4.77e-04**	−1.11	2.00	1.57
CSF2	colony stimulating factor 2 (granulocyte-macrophage)	1437	1.02e-03	**8.79e-04**	7.21e-01	−1.68	−1.88	−1.48
CTH	cystathionase (cystathionine gamma-lyase)	1491	6.66e-03	**1.90e-04**	3.46e-01	1.29	1.59	1.31
CTSK	cathepsin K	1513	2.91e-03	**4.28e-04**	9.47e-01	1.54	1.53	1.45
CUZD1	CUB and zona pellucida-like domains 1	50624	7.05e-02	**3.72e-02**	**4.52e-02**	1.95	1.02	1.05
CX3CL1	chemokine (C-X3-C motif) ligand 1	6376	1.25e-02	**3.11e-03**	4.58e-01	−1.94	−1.48	−1.33
CXCL3	chemokine (C-X-C motif) ligand 3	2921	8.34e-02	**1.65e-02**	5.71e-02	−1.70	−1.34	1.07
CXCL6	chemokine (C-X-C motif) ligand 6	6372	2.85e-03	**1.69e-04**	6.11e-01	−1.39	−1.34	−1.58
CYB5D1	cytochrome b5 domain containing 1	124637	2.40e-03	**1.21e-03**	7.39e-02	1.78	1.50	1.07
CYP24A1	cytochrome P450, family 24, subfamily A, polypeptide 1	1591	2.62e-05	**4.54e-05**	7.89e-01	−1.52	−1.69	−1.51
DBR1	debranching enzyme homolog 1 (*Saccharomyces cerevisiae*)	51163	7.97e-02	**1.06e-03**	4.21e-01	1.52	1.26	1.24
DDIT4	DNA-damage-inducible transcript 4	54541	5.57e-04	**5.87e-04**	5.05e-01	−1.90	−1.40	−1.57
DDR1	discoidin domain receptor tyrosine kinase 1	780	1.43e-02	**1.48e-03**	8.30e-01	−1.73	−1.26	−1.13
DENND2C	DENN/MADD domain containing 2C	163259	3.14e-02	**1.33e-03**	9.57e-01	1.44	1.49	1.40
DEPDC1	DEP domain containing 1	55635	5.44e-02	9.05e-01	**1.02e-02**	1.75	−1.26	−1.44
DKK1	dickkopf 1 homolog (*Xenopus laevis*)	22943	2.94e-05	**5.83e-05**	**1.71e-02**	−1.25	−1.25	−1.94
DLEU1	deleted in lymphocytic leukemia 1 (non-protein coding)	10301	7.23e-02	**5.02e-03**	1.27e-01	1.71	1.19	1.14
DLGAP5	discs, large (Drosophila) homolog-associated protein 5	9787	4.29e-03	1.58e-01	**2.80e-03**	1.51	−1.58	−1.42
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	3337	1.36e-02	**4.99e-03**	**4.35e-02**	1.82	1.31	1.00
DUSP8	dual specificity phosphatase 8	1850	5.16e-03	**1.05e-02**	**4.26e-03**	−1.87	1.06	−1.07
EBI3	Epstein-Barr virus induced 3	10148	0.00e+00	**6.30e-06**	6.87e-01	−1.82	−1.73	−2.06
ECT2	epithelial cell transforming sequence 2 oncogene	1894	6.84e-03	5.43e-02	**1.07e-02**	1.36	−1.84	−1.45
EDN1	endothelin 1	1906	0.00e+00	**2.33e-06**	8.60e-01	−1.79	−1.93	−1.97
EFNA1	ephrin-A1	1942	4.91e-03	**1.20e-02**	**2.03e-02**	−1.74	−1.02	−1.31
EFNB2	ephrin-B2	1948	1.76e-02	**3.27e-03**	1.81e-01	1.81	1.40	1.13
EGR2	early growth response 2	1959	7.52e-03	**3.57e-03**	**6.43e-03**	−1.80	−1.04	−1.06
EIF4B	eukaryotic translation initiation factor 4B	1975	3.63e-02	4.03e-01	**9.48e-03**	1.56	−1.22	−1.69
EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2	1979	3.09e-04	**5.53e-06**	1.55e-01	1.25	1.56	1.42
ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	1999	6.05e-02	**3.62e-02**	**8.45e-03**	−1.24	1.43	1.43
ELMSAN1	ELM2 and Myb/SANT-like domain containing 1	91748	3.96e-05	**1.34e-02**	**1.00e-05**	−1.86	1.19	1.08
ELOVL4	ELOVL fatty acid elongase 4	6785	8.64e-03	**5.29e-04**	3.98e-01	1.40	1.62	1.25
ELOVL6	ELOVL fatty acid elongase 6	79071	7.35e-02	1.86e-01	**1.29e-02**	1.84	−1.08	−1.15
ENO2	enolase 2 (gamma, neuronal)	2026	6.53e-05	**1.50e-03**	**1.22e-02**	−1.27	−1.06	−2.31
ERCC6L	excision repair cross-complementing rodent repair deficiency, complementation group 6-like	54821	6.72e-02	8.17e-01	**1.39e-02**	1.75	−1.58	−1.21
ERF	Ets2 repressor factor	2077	1.91e-02	**4.84e-04**	1.05e-01	−1.62	−1.22	−1.19
EVA1A	eva-1 homolog A (*Caenorhabditis elegans*)	84141	1.25e-06	**3.14e-07**	**4.49e-03**	−1.19	−1.47	−1.74
FAM102A	family with sequence similarity 102, member A	399665	3.29e-02	6.69e-02	**5.48e-03**	−1.32	1.41	1.48
FAM111B	family with sequence similarity 111, member B	374393	7.05e-02	5.89e-01	**2.31e-02**	2.18	−1.58	−1.06
FAM46C	family with sequence similarity 46, member C	54855	5.99e-04	**5.82e-04**	**4.90e-03**	1.87	1.20	1.03
FAM72D	family with sequence similarity 72, member D	728833	2.54e-04	4.65e-01	**1.25e-03**	1.86	−1.60	−1.46
FBLIM1	filamin binding LIM protein 1	54751	1.21e-03	**1.31e-04**	5.98e-01	−1.61	−1.38	−1.39

Table 1 (*continued*)

Symbol	Gene name	Gene ID	FDR	$P_{treatment}$	$P_{interaction}$	FC AMNA/IL-1β			Pairwise		
						FC 1 h	FC 3 h	FC 6 h	1 h	3 h	6 h
FBXL20	F-box and leucine-rich repeat protein 20	84961	1.87e-04	**2.46e-04**	6.47e-02	1.19	1.91	1.40		**	*
FBXO33	F-box protein 33	254170	4.21e-02	**9.72e-04**	7.70e-02	1.22	1.62	1.13		**	
FLJ36840	uncharacterized LOC645524	645524	2.12e-02	1.52e-01	**2.64e-02**	2.66	−1.34	1.09	**		
FOS	FBJ murine osteosarcoma viral oncogene homolog	2353	4.65e-06	**4.26e-04**	**3.84e-04**	2.13	1.01	1.08	**		
FRMD6	FERM domain containing 6	122786	4.02e-02	**1.16e-02**	**3.29e-02**	1.11	−1.49	−1.57		*	**
FRMD8	FERM domain containing 8	83786	3.02e-02	**6.15e-03**	**2.25e-02**	−1.72	−1.23	1.03		**	
FST	follistatin	10468	7.49e-02	**1.12e-03**	1.27e-01	−1.07	−1.44	−1.42		**	*
FUT11	fucosyltransferase 11 (alpha (1,3) fucosyltransferase)	170384	5.23e-02	**1.79e-03**	**6.02e-03**	−1.14	1.00	−1.62			**
GAN	gigaxonin	8139	4.82e-02	**6.57e-03**	1.16e-01	1.80	1.22	1.12	**		
GAREM	GRB2 associated, regulator of MAPK1	64762	9.44e-02	**1.61e-03**	**1.88e-02**	−1.00	1.54	1.23		**	
GAS2L3	growth arrest-specific 2 like 3	283431	5.77e-02	2.78e-01	**1.30e-02**	1.92	−1.14	−1.17		**	
GCLC	glutamate-cysteine ligase, catalytic subunit	2729	3.29e-02	**2.08e-03**	7.58e-01	1.56	1.53	1.32	**		
GCLM	glutamate–cysteine ligase, modifier subunit	2730	1.37e-02	**1.51e-03**	5.69e-01	1.71	1.40	1.35	**		
GLCCI1	glucocorticoid induced transcript 1	113263	2.69e-02	**1.03e-04**	8.16e-01	1.37	1.38	1.28	**		
GPR56	G protein-coupled receptor 56	9289	1.49e-02	**3.94e-03**	7.47e-02	−1.85	−1.09	−1.27	**		
GPR89A	G protein-coupled receptor 89A	653519	5.78e-02	**3.12e-03**	3.52e-01	1.67	1.32	1.22	**		
GRB7	growth factor receptor-bound protein 7	2886	1.09e-02	**1.07e-03**	5.88e-02	−1.74	−1.18	−1.18	**		
GTF2B	general transcription factor IIB	2959	6.15e-04	**8.75e-05**	1.99e-01	1.69	1.35	1.29	**	*	*
HCAR1	hydroxycarboxylic acid receptor 1	27198	9.99e-02	**3.02e-03**	7.95e-01	−1.29	−1.45	−1.49	**		
HILPDA	hypoxia inducible lipid droplet-associated	29923	7.25e-05	**9.73e-04**	**3.21e-03**	−1.20	−1.01	−2.09			**
HIST1H2AK	histone cluster 1, H2ak	8330	3.09e-03	**2.95e-03**	1.11e-01	−1.05	−1.71	−1.85		**	*
HIST2H2BF	histone cluster 2, H2bf	440689	4.80e-03	**4.43e-05**	6.62e-01	1.36	1.32	1.47			**
HIST2H4A	histone cluster 2, H4a	8370	0.00e+00	**4.91e-07**	**1.85e-02**	1.87	1.46	1.32	**	*	
HK2	hexokinase 2	3099	1.25e-06	**4.59e-04**	**1.65e-03**	1.06	−1.37	−2.25		*	**
HMMR	hyaluronan-mediated motility receptor (RHAMM)	3161	1.23e-02	2.21e-01	**8.49e-03**	1.58	−1.43	−1.71	*	*	**
HMOX1	heme oxygenase (decycling) 1	3162	1.69e-02	**3.20e-04**	3.02e-01	−1.51	−1.19	−1.41	**		
ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	3398	5.45e-03	**1.51e-04**	**1.15e-02**	1.38	1.58	1.03	**		
IDI1	isopentenyl-diphosphate delta isomerase 1	3422	6.89e-02	**4.10e-03**	2.47e-01	1.70	1.21	1.23	**		
IGFL1	IGF-like family member 1	374918	0.00e+00	**1.06e-05**	8.05e-01	−2.11	−2.05	−2.41	**	**	**
IL1B	interleukin 1, beta	3553	2.12e-03	**1.50e-03**	3.88e-01	−1.29	−1.92	−1.69		**	**
IL32	interleukin 32	9235	3.56e-03	**8.70e-05**	1.94e-01	−1.23	−1.34	−1.59		**	**
IL7R	interleukin 7 receptor	3575	2.09e-04	**7.60e-04**	8.60e-02	−1.11	−1.95	−1.69		**	**
IRF2BP2	interferon regulatory factor 2 binding protein 2	359948	1.26e-02	7.17e-02	**2.00e-04**	−1.65	1.11	1.13	**		
ISCA1	iron–sulfur cluster assembly 1 homolog (*S. cerevisiae*)	81689	2.42e-02	**2.41e-03**	1.97e-01	1.60	1.54	1.10	**	*	
ISG20L2	interferon stimulated exonuclease gene 20 kDa-like 2	81875	8.45e-02	**4.01e-02**	**2.40e-03**	−1.62	1.08	1.03	**		
KCTD11	potassium channel tetramerisation domain containing 11	147040	4.26e-05	**1.92e-04**	3.55e-01	−1.91	−1.36	−1.65	**		
KDELC1	KDEL (Lys-Asp-Glu-Leu) containing 1	79070	5.23e-02	**7.58e-05**	3.32e-01	1.20	1.43	1.30	**	**	**
KHDRBS3	KH domain containing, RNA binding, signal transduction associated 3	10656	1.79e-03	**2.61e-06**	**7.74e-04**	1.05	1.57	1.32		**	**

KIAA0922	KIAA0922	23240	1.60e-02	**1.12e-03**	3.47e-01	1.25	1.68	1.35	••
KIAA1524	KIAA1524	57650	2.09e-02	3.06e-01	**1.10e-02**	1.64	−1.68	−1.44	•
KIF14	kinesin family member 14	9928	1.42e-02	8.55e-01	**1.29e-02**	2.46	−1.70	−1.31	•
KIF23	kinesin family member 23	9493	7.31e-02	1.00e+00	**1.64e-02**	1.88	−1.46	−1.29	•
KLRAP1	killer cell lectin-like receptor subfamily A pseudogene 1	10748	9.99e-02	9.35e-01	**2.08e-02**	1.86	−1.58	−1.22	•
KRT16	keratin 16	3868	8.89e-04	**7.06e-04**	5.60e-01	−1.73	−1.76	−1.37	••
KRT17	keratin 17	3872	1.35e-03	**6.95e-04**	2.03e-01	−1.86	−1.24	−1.43	••
KRT34	keratin 34	3885	6.82e-02	**5.18e-04**	8.08e-01	−1.27	−1.37	−1.40	•
LAMB3	laminin, beta 3	3914	9.31e-03	**7.05e-04**	8.37e-01	−1.57	−1.44	−1.39	••
LIF	leukemia inhibitory factor	3976	1.04e-03	**1.87e-02**	**1.93e-03**	−1.34	1.51	1.62	••
LMCD1	LIM and cysteine-rich domains 1	29995	7.13e-02	**2.87e-04**	8.53e-01	−1.38	−1.33	−1.28	•
LOC100130713	uncharacterized LOC100130713	100130713	8.73e-03	**3.78e-02**	**1.06e-03**	−1.33	1.51	1.34	••
LOC440896	uncharacterized LOC440896	440896	1.04e-02	**7.89e-04**	8.40e-01	−1.55	−1.49	−1.37	•
LOX	lysyl oxidase	4015	2.52e-02	**2.34e-04**	**3.94e-02**	−1.15	−1.19	−1.60	••
LPIN1	lipin 1	23175	1.40e-03	**2.40e-04**	**4.71e-02**	1.72	1.37	1.12	••
MARCH8	membrane-associated ring finger (C3HC4) 8, E3 ubiquitin protein ligase	220972	7.20e-02	**2.41e-03**	2.60e-01	1.12	1.54	1.42	••
MEF2D	myocyte enhancer factor 2D	4209	4.21e-03	9.11e-02	**1.55e-03**	−1.84	1.24	−1.00	••
MEGF9	multiple EGF-like-domains 9	1955	1.01e-04	**2.69e-04**	9.48e-01	1.62	1.76	1.69	•
MERTK	c-mer proto-oncogene tyrosine kinase	10461	6.83e-05	**2.96e-05**	6.07e-02	1.28	1.79	1.32	••
MINPP1	multiple inositol-polyphosphate phosphatase 1	9562	4.12e-02	**2.07e-03**	6.87e-01	1.51	1.54	1.28	•
MIR29A	microRNA 29a	407021	6.91e-05	**9.06e-04**	3.57e-01	1.59	1.74	2.69	••
MKI67	antigen identified by monoclonal antibody Ki-67	4288	2.28e-02	6.57e-01	**1.27e-02**	2.18	−1.58	−1.13	••
MMP1	matrix metallopeptidase 1 (interstitial collagenase)	4312	1.40e-04	**1.11e-03**	**3.61e-02**	−1.08	−1.44	−2.18	••
MMP10	matrix metallopeptidase 10 (stromelysin 2)	4319	5.96e-08	**1.29e-04**	**1.54e-03**	−1.02	−1.38	−2.37	•
MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)	4314	1.93e-05	**1.63e-02**	**2.51e-02**	−1.01	−1.63	−2.57	••
MOB3C	MOB kinase activator 3C	148932	2.40e-02	**1.54e-02**	**7.70e-03**	−1.78	1.01	−1.02	••
MYLIP	myosin regulatory light chain interacting protein	29116	0.00e+00	**9.67e-07**	**8.89e-05**	2.84	1.52	1.12	••
NAV3	neuron navigator 3	89795	7.05e-02	6.46e-01	**6.03e-03**	1.55	−1.42	−1.24	••
NCAPG	non-SMC condensin I complex, subunit G	64151	8.61e-02	7.39e-01	**1.66e-02**	1.89	−1.41	−1.18	•
NEK2	NIMA-related kinase 2	4751	5.47e-02	2.39e-01	**7.63e-03**	1.78	−1.23	−1.04	••
NINJ1	ninjurin 1	4814	6.07e-02	**5.10e-03**	8.39e-02	−1.70	−1.25	−1.06	•
NIPAL4	NIPA-like domain containing 4	348938	4.83e-03	**6.92e-04**	3.07e-01	−1.74	−1.31	−1.32	••
NR4A2	nuclear receptor subfamily 4, group A, member 2	4929	2.09e-03	5.30e-01	**1.08e-03**	−1.85	1.21	1.31	••
NUAK2	NUAK family, SNF1-like kinase, 2	81788	1.41e-02	**3.76e-04**	1.70e-01	−1.60	−1.19	−1.30	•
NXF1	nuclear RNA export factor 1	10482	1.95e-02	**4.17e-04**	7.68e-02	1.19	1.63	1.20	••
OLFM2	olfactomedin 2	93145	9.51e-02	**4.42e-03**	7.98e-02	−1.65	−1.13	−1.14	••
OSBPL11	oxysterol binding protein-like 11	114885	9.51e-02	**8.43e-03**	4.90e-01	1.84	1.30	1.37	•
OXTR	oxytocin receptor	5021	1.11e-03	**9.51e-06**	**5.52e-03**	−1.10	−1.62	−1.32	•• ••
P4HA1	prolyl 4-hydroxylase, alpha polypeptide I	5033	1.04e-04	4.09e-01	**3.61e-04**	1.58	−1.02	−1.89	••
PCF11	PCF11, cleavage and polyadenylation factor subunit, homolog (*S. cerevisiae*)	51585	2.42e-02	**3.67e-02**	**3.25e-02**	2.13	−1.01	1.06	•
PDGFB	platelet-derived growth factor beta polypeptide	5155	2.00e-04	**6.09e-04**	9.34e-02	−2.09	−1.26	−1.36	••
PDK1	pyruvate dehydrogenase kinase, isozyme 1	5163	1.91e-06	**2.69e-04**	**4.56e-04**	1.44	−1.19	−2.37	•
PDZK1IP1	PDZK1 interacting protein 1	10158	3.32e-02	**2.18e-03**	6.99e-02	−1.60	−1.03	−1.39	••
PER1	period circadian clock 1	5187	1.43e-02	6.69e-02	**3.66e-03**	−1.84	1.08	1.08	•

Table 1 (*continued*)

Symbol	Gene name	Gene ID	FDR	$P_{treatment}$	$P_{interaction}$	FC AMNA/IL-1β			Pairwise		
						FC 1 h	FC 3 h	FC 6 h	1 h	3 h	6 h
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	5210	0.00e+00	**3.32e-04**	**6.63e-05**	−1.06	1.04	−2.67			••
PHLDA3	pleckstrin homology-like domain, family A, member 3	23612	4.60e-02	**7.89e-04**	5.33e-01	−1.52	−1.27	−1.31	••	••	
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	5295	2.59e-03	**1.84e-02**	**2.19e-02**	2.40	1.14	−1.02	••		
PIK3R3	phosphoinositide-3-kinase, regulatory subunit 3 (gamma)	8503	1.86e-02	**4.86e-04**	5.59e-01	1.28	1.54	1.37		••	••
PLAT	plasminogen activator, tissue	5327	3.74e-02	**1.90e-02**	1.06e-01	−2.20	−1.18	−1.12	••		
PLCB4	phospholipase C, beta 4	5332	7.45e-06	**3.68e-02**	**2.37e-03**	1.73	−2.07	−2.14		••	••
PLEKHG2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2	64857	3.71e-02	**3.43e-03**	7.01e-02	−1.72	−1.23	−1.08	••		
PLEKHM1	pleckstrin homology domain containing, family M (with RUN domain) member 1	9842	8.05e-02	9.03e-02	**1.43e-02**	−1.28	1.65	1.26		••	
PLEKHM3	pleckstrin homology domain containing, family M, member 3	389072	3.51e-02	**4.36e-03**	9.12e-01	1.57	1.78	1.56		•	
PODXL	podocalyxin-like	5420	1.55e-02	**8.88e-04**	**9.91e-03**	−1.05	−1.16	−1.69			••
POLA1	polymerase (DNA directed), alpha 1, catalytic subunit	5422	9.69e-02	3.40e-01	**2.19e-02**	1.95	−1.35	−1.00	••		
PPP1R18	protein phosphatase 1, regulatory subunit 18	170954	2.09e-02	**7.61e-03**	**1.02e-02**	−1.77	−1.06	−1.02	••		
PPP1R3C	protein phosphatase 1, regulatory subunit 3C	5507	1.19e-02	**9.34e-03**	7.03e-02	1.04	−1.54	−1.94		••	••
PRDM1	PR domain containing 1, with ZNF domain	639	0.00e+00	**1.29e-05**	**3.46e-06**	−2.47	−1.04	−1.01	••		
PRKCA	protein kinase C, alpha	5578	3.16e-03	**6.04e-04**	9.57e-01	1.48	1.59	1.54	•	••	••
PRSS22	protease, serine, 22	64063	2.23e-02	**3.34e-04**	8.39e-01	−1.41	−1.43	−1.31	••	••	••
PTCH1	patched 1	5727	8.87e-02	**1.19e-02**	**6.15e-03**	−1.02	1.65	1.04		••	
PTP4A3	protein tyrosine phosphatase type IVA, member 3	11156	1.22e-04	**2.90e-04**	3.74e-01	−1.51	−1.95	−1.41	••	••	••
PXDC1	PX domain containing 1	221749	3.90e-02	3.71e-01	**2.02e-03**	−1.66	1.16	1.19	••		
RAI14	retinoic acid induced 14	26064	9.76e-02	7.26e-01	**1.68e-02**	1.87	−1.31	−1.26	••		
RASD2	RASD family, member 2	23551	6.21e-02	**7.34e-04**	**2.19e-02**	−1.58	−1.08	−1.15	••		
RASSF5	Ras association (RalGDS/AF-6) domain family member 5	83593	9.60e-02	**2.47e-04**	6.95e-01	−1.29	−1.39	−1.25		••	
RIPK4	receptor-interacting serine–threonine kinase 4	54101	1.33e-03	**3.07e-04**	**1.37e-02**	−1.79	−1.10	−1.23	••		
RND1	Rho family GTPase 1	27289	9.23e-03	**1.56e-02**	5.23e-02	−1.12	1.59	2.06			••
RNF103	ring finger protein 103	7844	6.68e-02	**2.52e-03**	9.20e-01	1.41	1.51	1.37	•	•	
RRM2	ribonucleotide reductase M2	6241	5.93e-02	**3.12e-03**	**1.19e-02**	1.59	1.22	−1.04	••		
S100A3	S100 calcium binding protein A3	6274	0.00e+00	**7.47e-06**	5.36e-02	−2.37	−1.37	−2.05	••		••
SAMD4A	sterile alpha motif domain containing 4A	23034	7.19e-04	**4.28e-06**	7.70e-02	1.22	1.56	1.36	•	••	••
SELE	selectin E	6401	2.45e-03	**2.50e-03**	**1.08e-02**	−1.01	1.24	1.89			••
SEMA4C	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	54910	9.90e-02	**1.27e-03**	8.89e-02	−1.53	−1.26	−1.08	••		
SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	12	2.93e-03	**4.08e-03**	1.68e-01	−2.38	−1.29	−1.37	••		
SERPINB9	serpin peptidase inhibitor, clade B (ovalbumin), member 9	5272	4.70e-02	**4.96e-03**	1.48e-01	−1.07	−1.39	−1.72			••
SERPINH1	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	871	6.23e-02	**1.47e-03**	4.28e-01	−1.27	−1.27	−1.58			••

Gene	Description	ID								
SERPINI1	serpin peptidase inhibitor, clade I (neuroserpin), member 1	5274	2.82e-02	**1.25e-03**	6.61e-01	1.54	1.28	1.46	·	·
SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1	6513	2.93e-03	**1.28e-03**	6.12e-02	−1.23	−1.19	−1.91	··	··
SLC33A1	solute carrier family 33 (acetyl-CoA transporter), member 1	9197	6.24e-02	**6.09e-04**	3.29e-01	1.42	1.42	1.16	··	·
SLC35D1	solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1	23169	5.29e-02	**3.06e-03**	6.97e-01	1.65	1.38	1.35	·	
SLC6A14	solute carrier family 6 (amino acid transporter), member 14	11254	8.97e-02	**5.67e-03**	8.88e-02	1.01	−1.46	−1.50	·	·
SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	6535	2.16e-03	**1.14e-03**	**1.69e-02**	−1.03	1.70	1.47	··	··
SMIM14	small integral membrane protein 14	201895	7.25e-02	**3.85e-03**	4.08e-01	1.19	1.64	1.41	··	·
SNHG12	small nucleolar RNA host gene 12 (non-protein coding)	85028	2.55e-04	**1.61e-05**	6.39e-02	1.66	1.38	1.21	··	··
SNORA61	small nucleolar RNA, H/ACA box 61	677838	7.99e-04	**6.54e-06**	7.40e-02	1.62	1.45	1.15	··	··
SNORD105	small nucleolar RNA, C/D box 105	692229	1.18e-02	**5.73e-03**	9.15e-02	−1.82	−1.58	−1.00	·	
SNORD13P2	small nucleolar RNA, C/D box 13 pseudogene 2	6077	1.60e-02	8.33e-02	**5.74e-03**	−1.88	1.16	1.01		·
SNORD14E	small nucleolar RNA, C/D box 14E	85391	1.44e-02	**1.01e-02**	9.15e-02	2.17	1.21	1.14	·	
SNORD22	small nucleolar RNA, C/D box 22	9304	1.49e-06	**3.21e-04**	**3.16e-02**	2.57	1.32	1.34	··	··
SNORD28	small nucleolar RNA, C/D box 28	9300	4.12e-02	**1.90e-02**	1.47e-01	1.66	1.14	1.26	·	
SNORD30	small nucleolar RNA, C/D box 30	9299	1.45e-02	**3.29e-03**	**4.36e-02**	1.82	1.11	1.16	··	··
SNORD31	small nucleolar RNA, C/D box 31	9298	5.13e-06	**9.26e-05**	**3.20e-03**	2.08	1.18	1.17	··	··
SNORD32A	small nucleolar RNA, C/D box 32A	26819	6.96e-02	**1.74e-02**	**3.10e-02**	−1.66	−1.32	1.11	·	·
SPRY4	sprouty homolog 4 (Drosophila)	81848	8.04e-02	**2.15e-03**	1.03e-01	−1.59	−1.09	−1.25	·	
SRGN	serglycin	5552	1.96e-02	**1.14e-04**	7.38e-02	−1.15	−1.28	−1.55	··	
STAT5A	signal transducer and activator of transcription 5A	6776	8.68e-02	**6.33e-04**	6.45e-01	−1.22	−1.38	−1.40	··	
STC1	stanniocalcin 1	6781	8.00e-04	**1.62e-03**	**1.37e-02**	−1.21	−1.06	−2.01	··	··
STC2	stanniocalcin 2	8614	9.30e-04	**1.85e-03**	**5.42e-03**	1.01	−1.19	−1.93	··	··
STK17B	serine/threonine kinase 17b	9262	1.23e-03	**1.93e-03**	1.18e-01	2.15	1.36	1.23	··	
STX4	syntaxin 4	6810	2.28e-03	**1.23e-04**	9.23e-01	−1.49	−1.40	−1.43	··	
SYDE1	synapse defective 1, Rho GTPase, homolog 1 (*C. elegans*)	85360	9.00e-02	**9.80e-04**	4.81e-01	−1.43	−1.19	−1.40	·	
TAGLN	transgelin	6876	3.45e-03	**3.65e-04**	6.89e-01	−1.43	−1.37	−1.61	··	
TAS2R4	taste receptor, type 2, member 4	50832	9.62e-02	**1.10e-02**	1.86e-01	1.83	1.15	1.22	·	
TFRC	transferrin receptor (p90, CD71)	7037	7.32e-02	3.99e-01	**2.32e-02**	2.18	−1.21	−1.23		·
TGFB1I1	transforming growth factor beta 1 induced transcript 1	7041	8.92e-05	**2.58e-05**	9.24e-01	−1.47	−1.48	−1.55	··	
TGFB2	transforming growth factor, beta 2	7042	1.50e-02	**2.51e-03**	**3.22e-03**	1.19	−2.14	−1.52	··	··
TICAM1	toll-like receptor adaptor molecule 1	148022	4.13e-03	**8.38e-03**	**8.32e-04**	−1.79	1.05	1.01	·	··
TLE1	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	7088	3.37e-03	**1.25e-02**	**3.72e-04**	−1.23	1.70	1.14	·	··
TM4SF18	transmembrane 4L six family member 18	116441	3.05e-02	6.72e-02	**2.10e-03**	1.31	−1.24	−1.55		··
TMEM2	transmembrane protein 2	23670	2.67e-02	**5.08e-03**	6.27e-01	2.00	1.44	1.53	··	
TNFAIP6	tumor necrosis factor, alpha-induced protein 6	7130	1.27e-02	**4.06e-02**	2.87e-01	−1.16	−1.70	−1.84	·	
TNFRSF11B	tumor necrosis factor receptor superfamily, member 11b	4982	6.74e-06	**4.09e-04**	2.27e-01	−1.49	−2.62	−1.70	··	
TNFSF18	tumor necrosis factor (ligand) superfamily, member 18	8995	0.00e+00	**3.06e-06**	3.10e-01	−2.41	−4.24	−3.41	··	
TOP2A	topoisomerase (DNA) II alpha 170 kDa	7153	4.62e-02	8.29e-01	**1.18e-02**	1.90	−1.42	−1.24		·
TRAF1	TNF receptor-associated factor 1	7185	2.49e-04	**8.97e-05**	2.32e-01	−1.69	−1.50	−1.26	··	
TRIP6	thyroid hormone receptor interactor 6	7205	8.37e-02	**9.63e-04**	6.24e-01	−1.48	−1.30	−1.26	··	
TSGA10	testis specific, 10	80705	6.19e-02	**6.67e-02**	**1.65e-02**	1.68	1.07	1.04	·	·
TTLL3	tubulin tyrosine ligase-like family, member 3	26140	3.38e-03	**1.07e-03**	**1.29e-02**	−1.04	1.65	1.45	··	··
UBALD1	UBA-like domain containing 1	124402	1.29e-02	9.81e-02	**5.00e-03**	−1.88	1.23	−1.03		··

Table 1 (*continued*)

Symbol	Gene name	Gene ID	FDR	$P_{treatment}$	$P_{interaction}$	FC AMNA/IL-1β			Pairwise		
						FC 1 h	FC 3 h	FC 6 h	1 h	3 h	6 h
ULBP3	UL16 binding protein 3	79465	5.51e-02	**3.20e-04**	1.21e-01	1.30	1.50	1.12	*	**	
ULK1	unc-51-like kinase 1 (*C. elegans*)	8408	8.48e-02	4.11e-01	**2.45e-03**	−1.44	1.33	1.27	**	*	
USP38	ubiquitin specific peptidase 38	84640	8.54e-03	**1.46e-04**	4.15e-01	1.53	1.34	1.27	**	*	*
VAMP1	vesicle-associated membrane protein 1 (synaptobrevin 1)	6843	1.08e-03	**3.77e-05**	4.65e-01	1.49	1.48	1.28	**	**	*
VLDLR	very low density lipoprotein receptor	7436	4.04e-02	**4.49e-04**	**2.18e-03**	−1.05	−1.08	−1.61			**
ZC3H7B	zinc finger CCCH-type containing 7B	23264	6.99e-02	**4.01e-04**	8.97e-01	−1.39	−1.30	−1.32	**		
ZC3HAV1	zinc finger CCCH-type, antiviral 1	56829	4.54e-02	**3.89e-03**	2.25e-01	1.74	1.29	1.17	**		
ZCCHC14	zinc finger, CCHC domain containing 14	23174	3.05e-02	**1.36e-03**	9.05e-02	1.11	1.66	1.27		**	
ZNF253	zinc finger protein 253	56242	5.52e-02	**2.43e-02**	1.07e-01	2.18	1.16	1.11	**		
ZNF426	zinc finger protein 426	79088	6.98e-02	**3.42e-03**	7.62e-01	1.55	1.51	1.30	**	*	
ZNF48	zinc finger protein 48	197407	5.05e-02	**5.04e-03**	**1.14e-02**	−1.67	−1.07	−1.03			
ZNF487P	zinc finger protein 487, pseudogene	642819	5.33e-05	**1.01e-03**	5.62e-02	1.47	2.43	1.18		**	
ZNF724P	zinc finger protein 724, pseudogene	440519	3.69e-02	1.45e-01	**1.73e-02**	2.07	−1.05	−1.15	**		
ZNF737	zinc finger protein 737	100129842	2.08e-02	**6.03e-03**	3.48e-01	2.11	1.29	1.46	**		
ZSWIM6	zinc finger, SWIM-type containing 6	57688	4.63e-02	**5.34e-03**	4.44e-01	1.55	1.78	1.22		*	

P-values in bold if significant at two-factor ANOVA (< 0.05).

FDR: false discovery rate, according to BETR analysis. $P_{treatment}$: *P*-value for treatment effect; and $P_{interaction}$: *P*-value for interaction effect, at two-factor ANOVA. FC: fold-change in Aminaphtone-treated *vs.* untreated IL-1β-stimulated endothelial cells. AMNA: Aminaphtone. Pairwise: pairwise significant at two-tailed *t*-test.
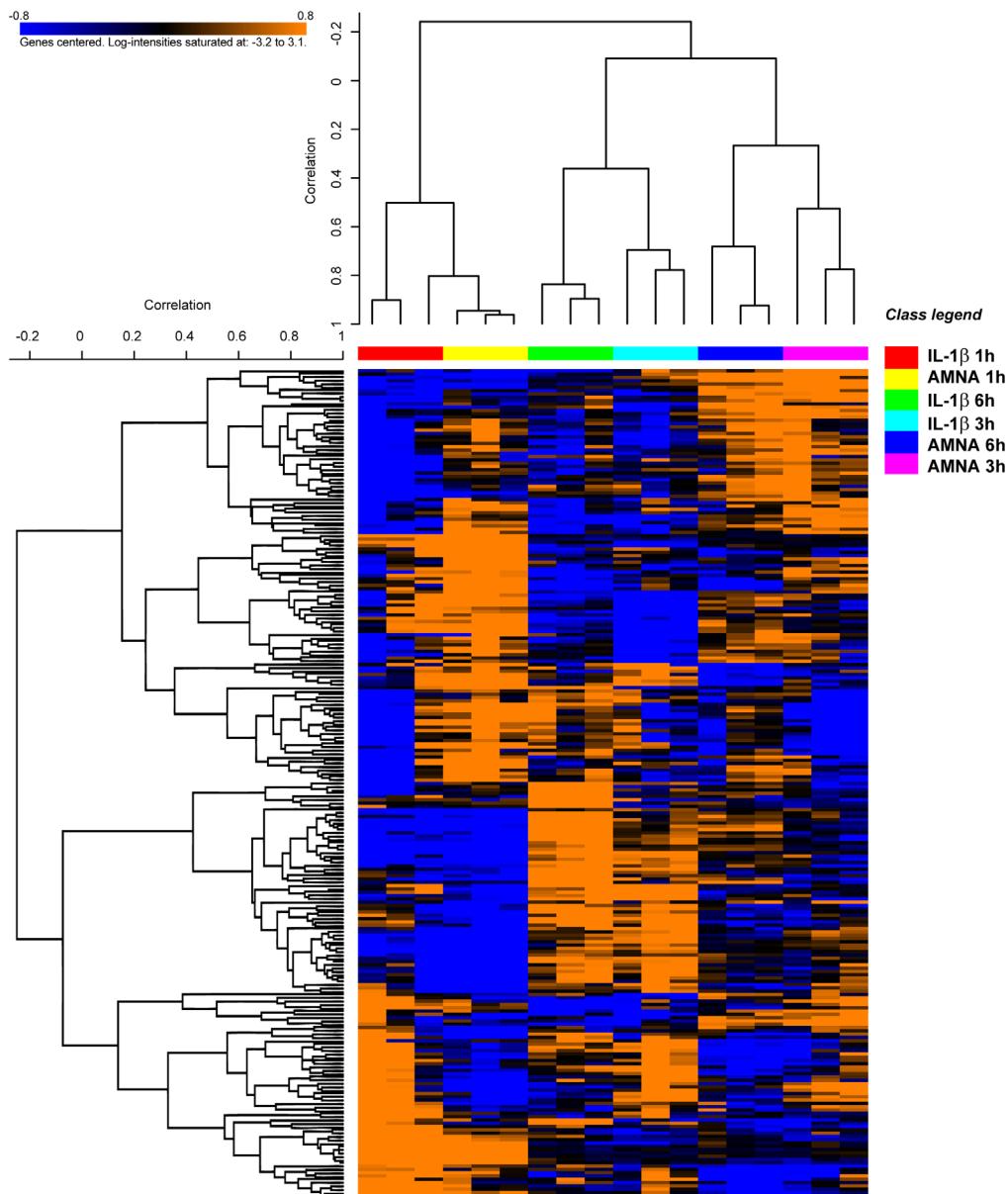
* $P < 0.05$.

** $P < 0.01$ at two-tailed *t*-test.

Fig. 1. Unsupervised hierarchical clustering of significant genes modulated over time by Aminaphtone in treated *vs.* untreated IL-1β stimulated ECV304 endothelial cells. The BETR algorithm identified 252 significantly modulated genes (FDR < 0.1). Samples and genes were clustered using Pearson's correlation (centered) and average linkage method. Each combination of treatments and time points regrouped in distinct clusters. The $\log_2$ transformed, normalized, median-centered expression level of each gene is represented with a blue, black, and orange color scale: blue indicates below median, black, equal to, and orange above median. Class legends stand for: IL-1β (IL-1β without Aminaphtone); AMNA (IL-1β with Aminaphtone).

in M199 complete medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), at 37 °C in a humidified incubator with 5% CO_2. Cells were serum starved (1% FBS) to synchronize the mitotic phase 24 h before treatment. Cells were then incubated for 1, 3, and 6 h with recombinant IL-1β 100 IU/ml (Sigma-Aldrich, St. Louis, MO) in the presence of

Table 2

Gene sets significantly modulated by Aminaphtone in treated *vs.* untreated IL-1β-stimulated endothelial cells.

GO Category[a]	GO Ontology	GO term	#genes	LS	KS	Gene list
0005975	BP	carbohydrate metabolic process	11	0.0805	**0.0028**	*DDIT4, ENO2, FUT11, GCLC, HK2, HMMR, PDGFB, PDK1, PFKFB4, PPP1R3C, SLC2A1*
0007166	BP	cell surface receptor signaling pathway	46	0.0260	**0.0020**	*AAK1, BCL2L11, BMP6, CBLB, CITED2, CX3CL1, DDIT4, DDR1, DKK1, EBI3, ECT2, EDN1, EFNA1, EFNB2, EIF4B, EIF4EBP2, FOS, FST, GAREM, GPR56, GRB7, IL1B, IL7R, KCTD11, LIF, MERTK, OXTR, PDGFB, PIK3R1, PIK3R3, PLAT, PLEKHG2, PRDM1, PRKCA, PTCH1, SEMA4C, STAT5A, STC1, STC2, TGFB1I1, TGFB2, TICAM1, TLE1, TNFSF18, ULK1, VLDLR*
0022411	BP	cellular component disassembly	7	**0.0042**	0.8859	*BNIP3, DDIT4, DDR1, MMP1, MMP10, MMP3, TOP2A*
0048732	BP	gland development	11	**0.0031**	**0.0022**	*BCL2L11, CITED2, DDR1, ELF3, HK2, ID2, OXTR, PDGFB, PTCH1, STAT5A, TGFB2*
0042592	BP	homeostatic process	26	**0.0015**	0.2886	*BCL2L11, BNIP3, CITED2, CTSK, EDN1, EGR2, GCLC, GCLM, GPR89A, HK2, HMOX1, ID2, IL1B, IL7R, MYLIP, OXTR, POLA1, PRKCA, PTCH1, SERPINA3, STAT5A, STC1, STC2, TFRC, TGFB2, TNFRSF11B*
0002376	BP	immune system process	47	**0.0015**	0.0284	*ANXA3, BCL2L11, BCL3, BMP6, BNIP3, CBLB, CCL22, CD70, CD83, CITED2, CLEC4E, CSF2, CTSK, CX3CL1, CXCL3, CXCL6, DDIT4, EBI3, EDN1, FOS, FST, GRB7, HMOX1, ID2, IL1B, IL32, IL7R, KIF23, LIF, MARCH8, MERTK, MMP1, PDGFB, PIK3R1, PIK3R3, PODXL, PRDM1, PRKCA, SELE, SERPINB9, STAT5A, TFRC, TGFB2, TICAM1, TNFSF18, ULBP3, ZC3HAV1*
0006954	BP	inflammatory response	15	0.0131	**0.0047**	*BMP6, CCL22, CX3CL1, CXCL3, CXCL6, ELF3, FOS, HMOX1, IL1B, PRKCA, SELE, SERPINA3, STAT5A, TICAM1, TNFAIP6*
0043066	BP	negative regulation of apoptotic process	20	**0.0028**	0.0677	*AGAP2, BCL3, BNIP3, CITED2, CSF2, CTH, CX3CL1, EDN1, GCLC, GCLM, HMOX1, IL1B, NR4A2, NUAK2, PIK3R1, PRKCA, SERPINB9, STAT5A, TLE1, TNFSF18*
0007389	BP	pattern specification process	6	0.0137	**0.0006**	*CITED2, DKK1, EDN1, EGR2, FST, PTCH1*
0010646	BP	regulation of cell communication	49	0.1744	**0.0034**	*AAK1, ACAP1, AGAP2, ANKRD1, ARHGEF26, BCL2L11, BCL3, BMP6, BNIP3, CBLB, CITED2, CSF2, CTH, DDIT4, DKK1, DUSP8, ECT2, EDN1, EFNA1, EGR2, FST, GAREM, GPR89A, HMOX1, IL1B, KCTD11, LIF, LMCD1, OXTR, PDGFB, PHLDA3, PLAT, PLEKHG2, PRDM1, PRKCA, PTCH1, RASD2, SEMA4C, SLC2A1, SPRY4, SYDE1, TGFB1I1, TGFB2, TICAM1, TLE1, TRAF1, TRIP6, ULK1, ZC3HAV1*
0042127	BP	regulation of cell proliferation	23	**0.0019**	0.0322	*BMP6, BRCA1, CBLB, CSF2, DDR1, EBI3, EDN1, GAREM, HILPDA, HMOX1, ID2, IL1B, KCTD11, LIF, PDGFB, PRDM1, PRKCA, PTCH1, STAT5A, TGFB1I1, TGFB2, TICAM1, TNFSF18*
0045619	BP	regulation of lymphocyte differentiation	5	**0.0009**	**0.0034**	*CD83, ID2, IL7R, PRDM1, STAT5A*
0051246	BP	regulation of protein metabolic process	38	0.0567	**0.0032**	*AGAP2, BCL3, BMP6, BRCA1, BUB1B, CBLB, CSF2, DDIT4, DKK1, DUSP8, EBI3, ECT2, EDN1, EFNA1, EIF4B, EIF4EBP2, GCLC, GRB7, HMOX1, IL1B, KRT17, LIF, LPIN1, MYLIP, PDGFB, PLAT, PRKCA, RASD2, SAMD4A, SERPINA3, SERPINB9, SERPINH1, SERPINI1, SPRY4, STAT5A, TGFB2, TICAM1, VLDLR*
0051090	BP	regulation of sequence-specific DNA binding transcription factor activity	10	**0.0020**	0.0058	*ANXA3, CTH, FOS, HMOX1, ID2, IL1B, PTCH1, TICAM1, TNFSF18, TRAF1*

GO ID	Cat	Description	#genes	LS	KS	Gene list
0006357	BP	regulation of transcription from RNA polymerase II promoter	30	**0.0035**	0.6000	ANKRD1, BCL3, BMP6, BRCA1, CITED2, DKK1, EDN1, EFNA1, EGR2, ELF3, ERF, FOS, FST, HMOX1, ID2, IL1B, LIF, LMCD1, LPIN1, MEF2D, NR4A2, PER1, PIK3R1, POLA1, PRDM1, PTCH1, RRM2, STAT5A, TOP2A, VLDLR
0036293	BP	response to decreased oxygen levels	14	**0.0024**	**0.0002**	ANKRD1, BNIP3, CITED2, DDIT4, EDN1, HMOX1, NR4A2, OXTR, PDGFB, PDK1, PLAT, STC1, STC2, TGFB2
0009605	BP	response to external stimulus	32	**0.0001**	0.0071	AGAP2, ANKRD1, BNIP3, CCL22, CITED2, CX3CL1, CXCL3, CXCL6, CYP24A1, EDN1, EFNB2, EGR2, ELOVL4, FOS, HMOX1, ID2, IL1B, NR4A2, NUAK2, PDGFB, PER1, PLAT, PRKCA, RND1, SELE, SLC2A1, STAT5A, STC1, STC2, TGFB2, TNFRSF11B, ULK1
0010033	BP	response to organic substance	48	< **0.0001**	0.0168	ANKRD1, BMP6, BRCA1, CD83, CITED2, CSF2, CTH, CX3CL1, CYP24A1, DKK1, DNAJB1, EBI3, EDN1, EGR2, EIF4B, EIF4EBP2, FOS, GAREM, GCLC, HCAR1, HMOX1, IDI1, IL1B, IL7R, LOX, LPIN1, MMP3, NR4A2, OXTR, PDGFB, PIK3R1, PIK3R3, PLAT, PRDM1, PRKCA, PTCH1, SELE, SERPINB9, SERPINH1, STAT5A, STC1, STC2, TGFB1I1, TGFB2, TICAM1, TNFRSF11B, TNFSF18, ZC3HAV1
0048511	BP	rhythmic process	6	**0.0033**	0.0194	EGR2, ID2, OXTR, PER1, STAT5A, TGFB2
0046903	BP	secretion	20	0.0238	**0.0008**	ANKRD1, ANXA3, BMP6, CLEC4E, DDR1, EDN1, FST, HK2, HMOX1, IL1B, LIF, MERTK, OXTR, PDGFB, PRKCA, SLC2A1, SRGN, STAT5A, TGFB2, VAMP1
0005125	MF	cytokine activity	15	0.0061	**0.0047**	BMP6, CCL22, CD70, CSF2, CX3CL1, CXCL3, CXCL6, EBI3, EDN1, IL1B, IL32, LIF, TGFB2, TNFRSF11B, TNFSF18
0004175	MF	endopeptidase activity	6	**0.0004**	0.1037	CTSK, MMP1, MMP10, MMP3, PLAT, PRSS22
0001071	MF	nucleic acid binding transcription factor activity	11	**0.0006**	0.0382	BCL3, CEBPD, CITED2, EGR2, ELF3, ERF, FOS, MEF2D, NR4A2, PRDM1, STAT5A
0000988	MF	protein binding transcription factor activity	11	0.0990	**0.0032**	ANKRD1, BRCA1, CITED2, ELF3, ERF, LIF, LMCD1, LPIN1, PER1, TGFB1I1, TLE1
0019904	MF	protein domain specific binding	5	**0.0031**	0.0167	ACAP1, CITED2, EGR2, IL1B, KHDRBS3
0001067	MF	regulatory region nucleic acid binding	6	**0.0040**	0.0378	BRCA1, EGR2, FOS, MEF2D, PER1, STAT5A
0031012	CC	extracellular matrix	9	**0.0034**	**0.0040**	LAMB3, LMCD1, LOX, MMP1, MMP10, MMP3, TGFB1I1, TGFB2, TNFRSF11B
0005615	CC	extracellular space	31	**0.0003**	< **0.0001**	BMP6, CCL22, CD70, CSF2, CTSK, CX3CL1, CXCL3, CXCL6, DKK1, EBI3, EDN1, HILPDA, HMOX1, IGFL1, IL1B, IL32, LIF, LMCD1, LOX, MERTK, MMP10, MMP3, PLAT, SELE, SERPINB9, SRGN, STC1, TGFB2, TNFRSF11B, TNFSF18, VLDLR

KEGG Pathway[b]	Pathway description	#genes	LS	KS	Gene list
hsa04060	Cytokine-cytokine receptor interaction	13	0.0287	0.0095	CCL22, CD70, CSF2, CX3CL1, CXCL3, CXCL6, IL1B, IL7R, LIF, PDGFB, TGFB2, TNFRSF11B, TNFSF18

Broad MSigDB Curated Gene Set[c]	#genes	LS	KS	Gene list
HINATA_NFKB_TARGETS_KERATINOCYTE_UP	13	**0.0004**	0.0095	CD83, CSF2, CXCL3, CXCL6, EFNA1, IL1B, IL32, IL7R, MMP1, PLAT, STAT5A, TNFAIP6, TRAF1
HINATA_NFKB_TARGETS_FIBROBLAST_UP	6	**0.0027**	0.1123	CD83, CXCL3, CXCL6, IL1B, MMP1, SELE
SCHOEN_NFKB_SIGNALING	5	0.0192	**0.0036**	CSF2, EDN1, IL1B, NUAK2, SERPINA3
ELVIDGE_HYPOXIA_UP	19	**0.0017**	**0.0010**	AK4, BNIP3, CITED2, DDIT4, DDR1, ELF3, ENO2, FOS, HK2, LOX, P4HA1, PDGFB, PDK1, PRKCA, SAMD4A, SLC2A1, STC1, STC2, VLDLR
ELVIDGE_HIF1A_AND_HIF2A_TARGETS_DN	14	**0.0036**	**0.0001**	AK4, BNIP3, CITED2, ENO2, FOS, HK2, LOX, P4HA1, PDGFB, PDK1, SAMD4A, SLC2A1, STC1, VLDLR
ELVIDGE_HYPOXIA_BY_DMOG_UP	17	**0.0038**	**0.0023**	

Table 2 (*continued*)

Broad MSigDB Curated Gene Set[c]	#genes	LS	KS	Gene list
				AK4, BNIP3, CITED2, DDIT4, ELF3, ENO2, FOS, HK2, LOX, P4HA1, PDK1, PRKCA, SAMD4A, SLC2A1, STC1, STC2, VLDLR
ELVIDGE_HIF1A_TARGETS_DN	13	0.0114	**0.0002**	*AK4, BNIP3, CITED2, ENO2, FOS, HK2, LOX, P4HA1, PDGFB, PDK1, SLC2A1, STC1, VLDLR*
WINTER_HYPOXIA_METAGENE	18	**0.0022**	0.0376	*BNIP3, CITED2, DDIT4, EDN1, EFNA1, ELF3, FOS, HK2, HMOX1, ID2, LOX, P4HA1, PDGFB, PFKFB4, SLC2A1, SLC6A8, STC2, TFRC*
PID_HIF1_TFPATHWAY	9	**0.0022**	0.2078	*BNIP3, CITED2, EDN1, FOS, HK2, HMOX1, ID2, SLC2A1, TFRC*
MENSE_HYPOXIA_UP	10	**0.0028**	**0.0004**	*BNIP3, CEBPD, ENO2, HK2, LOX, P4HA1, PDK1, PFKFB4, PPP1R3C, STC2*
LEONARD_HYPOXIA	11	0.0061	**0.0007**	*AK4, BNIP3, DDIT4, EFNA1, HK2, P4HA1, PDGFB, PFKFB4, PPP1R3C, SLC2A1, STC2*
JIANG_HYPOXIA_NORMAL	15	0.0134	**0.0009**	*AK4, BNIP3, CITED2, CNNM4, EIF4B, ENO2, HMOX1, KHDRBS3, LOX, P4HA1, PFKFB4, PPP1R3C, RIPK4, SLC2A1, STC2*
KRIEG_HYPOXIA_VIA_KDM3A	5	0.0182	**0.0026**	*C15orf48, CLEC4E, EDN1, HMOX1, LAMB3*
FARDIN_HYPOXIA_11	6	0.0200	**0.0029**	*AK4, BNIP3, DDIT4, FUT11, PDK1, PFKFB4*
NAGASHIMA_EGF_SIGNALING_UP	8	**0.0008**	**0.0007**	*AREG, DNAJB1, EDN1, EGR2, FOS, LIF, NR4A2, TNFRSF11B*
ZHANG_RESPONSE_TO_IKK_INHIBITOR_AND_TNF_UP	22	**0.0021**	0.0070	*BCL3, C15orf48, CD83, CXCL3, DDIT4, EDN1, EFNA1, GCLC, IGFL1, IL1B, IL32, IL7R, LAMB3, LIF, MMP10, NAV3, NINJ1, SAMD4A, SEMA4C, SERPINB9, TNFAIP6, TRAF1*
BASSO_CD40_SIGNALING_UP	8	**0.00498**	0.0093	*CCL22, CD83, DDIT4, IL1B, PTP4A3, SRGN, STAT5A, TRAF1*
WIERENGA_STAT5A_TARGETS_GROUP2	5	0.0163	**0.0036**	*CCL22, CD83, CSF2, IL7R, TRAF1*

GO ontology: BP=biological process, MF=molecular function, and CC=cellular component.

[a] GO (Gene Ontology) categories,

[b] KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways, and

[c] MsigDB (Broad Institute Molecular Signature Database) curated gene sets found to be significant at the 0.005 significance level of either the LS or KS permutation tests. For each Gene Set, the table lists the unique identifier, the number of genes differentially modulated, the Fisher (LS) and Kolmogorov–Smirnov (KS) permutation P-values (P-values < 0.005 are in bold), and the list of modulated genes.

Aminaphtone 6 μg/ml (Baldacci, Pisa, Italy) or an equal volume of medium alone. Experiments were performed in three independent replicates for each time point.

2.2. RNA isolation and whole-genome gene expression profiling

Total RNA extraction was performed with TRIzol (Life Technologies, Rockville, MD) directly added to the ECV304 culture plates. Removal of contaminating genomic DNA was done by treating RNA samples with RNase-free Turbo DNase (Life Technologies) for 15 min at room temperature. RNA quantity and quality were assessed respectively by micro-volume spectrophotometry on an Infinite 200 PRO plate reader (Tecan, Männedorf, Switzerland) and by on-chip capillary electrophoresis on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). Absorbance ratio at 260 and 280 nm was ≥ 1.9 and the RNA integrity number (RIN) was > 8 for all samples.

For each replicate, 100 ng of total RNA were amplified and labeled using the Whole-Transcript Sense Target Labeling Protocol by Affymetrix (Santa Clara, CA) without ribosomal RNA reduction. Affymetrix GeneChip Human Gene 1.0 ST arrays were hybridized with 11 μg of labeled sense DNA, washed, stained, and scanned on an Affymetrix 7G Scanner according to the manufacturer's protocols.

2.3. Data processing and probe mapping

Data were extracted using the Affymetrix Expression Console software. Background correction, log_2 transformation, quantile normalization, and median polish probeset summarization was performed using the Robust Multi-array Average (RMA) method [3] implemented in the RMAExpress software v1.0.5. The resulting dataset consisted of 28869 probesets. The BRB-ArrayTools v4.3.2 (developed by Dr. Richard Simon and BRB-ArrayTools Development Team) and Bioconductor *R* packages v2.12 [4] were used for probe filtering and annotation. Probesets were deemed as non-informative and excluded from further analysis under any of the following conditions: *P*-value of the log-ratio variation greater than 0.01, *i.e.* genes showing minimal variation across samples; 17th per-centile of intensity less than 10, *i.e.* genes with the lowest acceptable expression level at most in three samples. Multiple probesets were reduced to one per gene symbol by using the most variable probe measured by interquartile range across arrays. After applying these stringent quality control and gene filtering criteria, we analyzed the expression changes over time of 6461 genes. Project was annotated with the Bioconductor annotation package hugene10sttranscriptcluster.db v8.0.1.

2.4. Statistical and bioinformatics analysis

Differentially expressed genes were sought combining two statistics implemented in the software MultiExperiment Viewer (MeV) v4.9 [5]. To identify genes varying significantly between the two conditions across time points, we used the Bayesian Estimation of Temporal Regulation (BETR) method [6], which is a linear random-effect modeling framework that takes into account correlations within samples between sampling times. Genes assigned a False Discovery Rate (FDR) < 0.1 were deemed significantly modulated. Then, to determine which genes were mainly influenced by the effect of Aminaphtone treatment *per se* (*i.e.* irrespective of the time response) and/or by the inter-action effect of the two factors time and treatment, we applied a two-factor ANOVA to the gene list identified by BETR, given the balanced factorial design of the study. Genes were considered statisti-cally significant if the *P*-values either for treatment and/or for interaction were < 0.05. We finally performed post-hoc pairwise comparisons (2-tailed Student's *t*-test) to identify significant differences ($P < 0.05$) between treatment classes at any time points.

Unsupervised hierarchical clustering was performed using the algorithms implemented in BRB-ArrayTools, to visualize similarities and differences in gene expression profiles that could discriminate treatment classes and/or changes over time. Log_2 transformed, normalized gene expression values were median-centered, scaled, and clustered by Pearson's centered correlation and average linkage as distance metrics.

Functional analysis of significant genes identified by BETR was carried out by examining gene sets for differential expression between Aminaphtone treated and untreated samples. Gene sets were

derived from the Gene Ontology (GO) database (http://www.geneontology.org) [7], the Kyoto Ency-clopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/pathway.html), and the curated gene sets of the Broad Institute Molecular Signature Database (MSigDB) [8]. LS/KS permutation tests were used to find gene sets with more genes differentially expressed among the phenotype classes than expected by chance. The threshold *P*-value was set at 0.005. Redundant GO terms were filtered out using the web-based tool REViGO [9], allowing a similarity threshold of 0.5.

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.06.051.

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