

SUPPLEMENTARY ONLINE DATA

Feedback regulation by Atf3 in the endothelin-1-responsive transcriptome of cardiomyocytes: Egr1 is a principal Atf3 target

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METHODS

Reaction equations

The reaction equations governing the expression of *Egr1* and *Atf3* mRNA, Atf3 protein and subsequent suppression of *Egr1* mRNA expression by Atf3 protein are described as follows. Each of the following processes occur at the rate indicated. Further details on these can be found in Table S1. -P denotes a phosphorylated version of the protein.

The phosphorylation of MKK by ET-1 is denoted by:



which subsequently phosphorylates the unphosphorylated ERK:



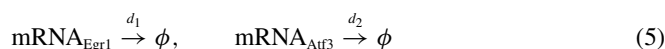
ERK-P is now free to transcribe both *Egr1* and *Atf3* mRNA such that:



and:

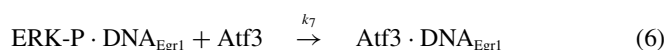


which are both degraded:



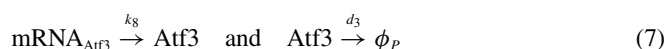
Here \cdot denotes a complex and ϕ the degraded mRNA.

The suppression of *Egr1* mRNA transcription by Atf3 is described by:



where the concentration of ERK-P is considered to be in excess.

Finally the translation of *Atf3* mRNA to Atf3 protein and subsequent degradation of the protein are denoted by:



respectively, where ϕ_p denotes degraded protein. In the present study we do not explicitly account for the degraded mRNAs or Atf3 protein.

Mathematical model

The Law of Mass Action [6] was applied to eqns (1)–(7). This led to the following system of nonlinear ordinary differential equations:

$$\frac{dm}{dt} = -k_1 e_T m \quad (8)$$

$$\frac{dm_P}{dt} = k_1 e_T m \quad (9)$$

$$\frac{dE}{dt} = -k_2 m_P E \quad (10)$$

$$\frac{dE_P}{dt} = k_2 m_P E - k_3 E_P D_E + k_{-3} T_E - k_5 E_P D_A + k_{-5} T_A \quad (11)$$

$$\frac{dD_E}{dt} = -k_3 E_P D_E + k_{-3} T_E \quad (12)$$

$$\frac{dT_E}{dt} = k_3 E_P D_E - k_{-3} T_E - k_7 T_E A \quad (13)$$

$$\frac{dM_E}{dt} = k_4 T_E - d_1 M_E \quad (14)$$

$$\frac{dD_A}{dt} = -k_5 E_P D_A + k_{-5} T_A \quad (15)$$

$$\frac{dT_A}{dt} = k_5 E_P D_A - k_{-5} T_A \quad (16)$$

$$\frac{dM_A}{dt} = k_6 T_A - d_2 M_A \quad (17)$$

$$\frac{dA}{dt} = k_8 M_A - k_7 T_E A - d_3 A \quad (18)$$

$$\frac{dS}{dt} = k_7 T_E A \quad (19)$$

Each of the variables is defined as follows: e_T represents the concentration of ET-1 (denoted $e_T = [\text{ET-1}]$), $m = [\text{MKK}]$, $m_P = [\text{MKK-P}]$, $E = [\text{ERK}]$, $E_P = [\text{ERK-P}]$, $D_E = [\text{DNA}_{\text{Egr1}}]$, $D_A = [\text{DNA}_{\text{Atf3}}]$, $T_E = \text{ERK-P} \cdot \text{DNA}_{\text{Egr1}}$, $T_A = \text{ERK-P} \cdot \text{DNA}_{\text{Atf3}}$, $M_E = [\text{mRNA}_{\text{Egr1}}]$, $M_A = [\text{mRNA}_{\text{Atf3}}]$, $S = [\text{Atf3} \cdot \text{DNA}_{\text{Egr1}}]$ and

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$A = [\text{Atf3}]$. In the present study the concentration of ET-1 is assumed to be constant. The rate of Atf3 protein to Egr1 DNA binding is assumed to be immediate and no delays are incurred in this process.

The system is closed with the initial conditions:

$$\begin{aligned} m &= m_0, m_p = 0, E = E_0, E_p = 0, D_E = D_{E0}, T_E = 0, \\ M_E &= M_{E0}, D_A = D_{A0}, T_A = 0, M_A = 0, A = 0 \text{ and} \\ S &= 0 \end{aligned} \quad (20)$$

which state that MKK and ERK are initially assumed to be unphosphorylated, the concentration of *Egr1* mRNA is non-zero whereas no *Atf3* mRNA, Atf3 protein or any of the complexes have been created.

The governing system of equations can be simplified as follows. Addition of eqns (8) and (9), integration with respect to time and application of the respective initial conditions yields the conservation relation:

$$m + m_p = m_0 \quad (21)$$

Substituting for m into eqn (9), integrating and applying the initial condition yields:

$$m_p(t) = m_0 (1 - e^{-k_1 e_T t}) \quad (22)$$

Addition of eqns (12), (13) and (19), integration with respect to t and application of the initial conditions yields.

$$D_E + T_E + S = D_{E0} \quad (23)$$

Likewise for eqns (15) and (16):

$$D_A + T_A = D_{A0} \quad (24)$$

Assuming eqn (16) is quasi-steady and substituting for D_A using eqn (24) leads to:

$$T_A \simeq \frac{D_{A0} E_p}{E_p + K_5} \quad (25)$$

where $K_5 = k_{-5}/k_5$.

Bringing all these results together gives:

$$m_p(t) = m_0 (1 - e^{-k_1 e_T t}) \quad (26)$$

$$\frac{dE}{dt} = -k_2 m_p E \quad (27)$$

$$\frac{dE_p}{dt} = k_2 m_p E - k_3 E_p (D_{E0} - T_E - S) + k_{-3} T_E \quad (28)$$

$$\frac{dT_E}{dt} = k_3 E_p (D_{E0} - T_E - S) - k_{-3} T_E - k_7 T_E A \quad (29)$$

$$\frac{dM_E}{dt} = k_4 T_E - d_1 M_E \quad (30)$$

$$\frac{dM_A}{dt} = \frac{k_6^* E_p}{E_p + K_5} - d_2 M_A \quad (31)$$

$$\frac{dA}{dt} = k_8 M_A - k_7 T_E A - d_3 A \quad (32)$$

$$\frac{dS}{dt} = k_7 T_E A \quad (33)$$

with the initial conditions:

$$\begin{aligned} E &= E_0, E_p = 0, T_E = 0, M_E = M_{E0}, M_A = 0, \\ A &= 0 \text{ and } S = 0 \end{aligned} \quad (34)$$

where $k_6^* = k_6 D_{A0}$. When Egr1 transcription is not suppressed by Atf3 protein we have $k_7 = 0$.

Parameterization

The mathematical model has been informed with data available within the literature, from our own previous studies as well the present study. A complete list of the parameter values used can be found in Table S1. In cases where parameter values have been derived these are explained as follows.

Estimation of the activation rate of MKK and ERK

The time course for activation of ERK1 was determined previously [9] and maximal activation was at 3 min. The time course for activation of MKK was determined by immunoblotting with antibodies against phosphorylated (i.e. activated) MKK (Cell Signaling Technology). A representative blot is shown in Figure S1. The time course for activation by a range of agonists (epidermal growth factor, ET-1, phorbol 12-myristate 13-acetate or platelet-derived growth factor) all showed maximal activation by 2–3 min. We therefore assumed the time to maximal activation of MKK to be 2 min with a further 1 min for maximal activation of ERK.

The concentration of MKK in cardiomyocytes was estimated by immunoblotting cardiomyocyte extracts from a known number of cells alongside known concentrations of recombinant MKK1. Antibodies against total MKK were from Cell Signaling Technology. Following densitometric analysis, a standard curve was constructed from which the amount of MKK in the myocyte extract was estimated. The concentration was calculated on the basis of the estimated volume of a neonatal myocyte. The concentration of ERK was assumed to be similar to MKK given that this lies within the range seen in other cells [4].

$k_1 e_t$: Rate of MKK activation by ET-1

The time taken for maximal activation of MKK by ET-1 is 2 min, thus:

$$k_1 e_t = \frac{1}{120 \text{ s}} = 8.30 \times 10^{-3} \text{ s}^{-1}$$

k_2 : Rate of ERK activation by MKK

The time taken to activate ERK by MKK is 60 s, so:

$$k_2 E = \frac{1}{60 \text{ s}} = 1.67 \times 10^{-2} \text{ s}^{-1}$$

The total ERK concentration is 130 nM such that:

$$k_2 = 1.28 \times 10^5 \text{ (Ms)}^{-1}$$

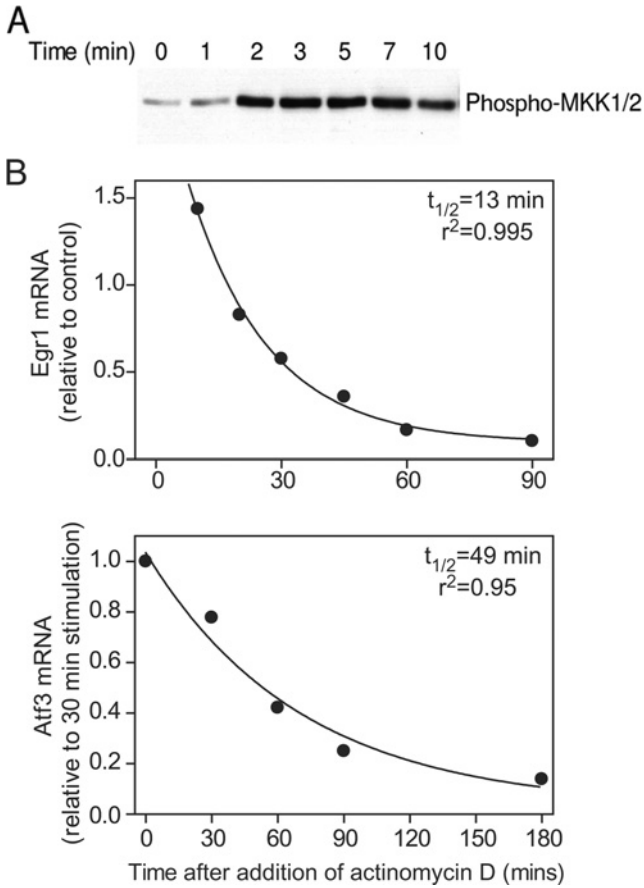


Figure S1 Activation of MKK phosphorylation by ET-1 (A) and effect of actinomycin D on Egr1 and Atf3 transcription (B)

(A) Cardiomyocytes were exposed to ET-1 for the times indicated. Protein extracts were immunoblotted with antibodies to phospho-MKK. A representative image is shown. (B) Cardiomyocytes were stimulated for 30 min before addition of actinomycin D ($4\mu\text{M}$) to inhibit transcription. Expression of Egr1 (upper panel) or Atf3 (lower panel) mRNAs were measured by qPCR at the indicated times after actinomycin D addition. A one-phase exponential curve was fitted using GraphPad Prism 4.

k_4^* , k_6^* : Egr1 and Atf3 transcription rates

The size of the Atf3 and Egr1 genes, mRNAs and proteins were for mouse (for rat, the 5' untranslated region was not defined for Egr1 and the rat genome is not well sequenced in the intronic regions for Atf3). Sequences were obtained from the NCBI. For Atf3 (Gene ID: 11910), this gives a total gene length of 13 038 base pairs (bp), a 5' untranslated region of 62 nucleotides and protein of 181 amino acids. For Egr1 (Gene ID: 13653), this gives a total gene length of 3750 bp. To estimate the rate of transcription, the total length of the gene was used allowing for an additional 200 nucleotides to be transcribed before termination. The maximum rate of transcription has been estimated recently to range from 55 bases/s to greater than 800 bases/s [1,5]. Thus to transcribe one molecule of mRNA from one gene, assuming a rate of 55 bases/s, takes:

$$\frac{3950 \text{ bases}}{55 \text{ bases/s}} = 71.82 \text{ s}$$

Per gene, this equates to 1.39×10^{-2} molecules mRNA s^{-1} . Since a cell contains two genes, we have 2.78×10^{-2} molecules of Egr1 mRNA being synthesized per cell per s. Taking the cell volume

of 6.7 pl we obtain:

$$k_4^* = \frac{2.78 \times 10^{-2} \text{ molecules} \cdot \text{s}^{-1}}{6.7 \times 10^{-9} \text{ ml}} = 4.15 \times 10^5 \text{ molecules} \cdot \text{ml}^{-1} \cdot \text{s}^{-1} = 6.89 \times 10^{-16} \text{ Ms}^{-1} \quad (35)$$

We can undertake a similar calculation for Atf3 transcription to obtain:

$$k_6^* = 5.15 \times 10^{-15} \text{ Ms}^{-1}$$

k_8 : Atf3 translation rate

The rate of translation of Atf3 was estimated on the basis of scanning of the 5' untranslated region at a rate of six nucleotides/s [11], translation of the coding sequence at a rate of 20 amino acids/s (N.B. the reported rate of translation is in the range of 4–20 amino acids/s [10,12] and we presume translation of IEGs is efficient) with five ribosomes attached simultaneously to each mRNA (N.B. the predicted occupancy is one ribosome per 32 amino acids) [7].

K_5 and k_3 , k_{-3} : ERK-P dissociation rates for Egr1 and Atf3 DNA

The model is based on the assumption that phospho-ERK bind to transcription factors that are pre-bound to the Atf3 and Egr1 promoters and this drives transcription. We presume that the ERK binding is mediated through a DEF motif with an estimated dissociation rate of $0.5 \mu\text{M}$ (the dissociation rate for Elk1 is $0.25 \mu\text{M}$; that for Fos is $1 \mu\text{M}$) [2]. Given that $K_3 = k_{-3}/k_3$, we use an initial estimate of $k_{-3} = 5 \times 10^{-2}/\text{s}$ to obtain $k_3 = 1 \times 10^5 (\text{Ms})^{-1}$.

d_1 , d_2 , d_3 : Degradation rates of Egr1 mRNA, Atf3 mRNA and Atf3 protein

To estimate the half-life of Egr1 and Atf3 mRNA, cardiomyocytes were exposed to ET-1 for 30 min then incubated without or with actinomycin D ($4 \mu\text{M}$, added directly to the culture dish). Cells were harvested at the indicated times following addition of actinomycin D and mRNA expression was measured by qPCR. GraphPad Prism 4 was used to fit a one-phase exponential decay curve to the data shown in Figure S1, giving a half-life of 13 min for Egr1 and 49 min for Atf3. The rate of degradation is defined by:

$$d = \frac{\ln 2}{t_{1/2}}$$

which leads to:

$$d_1 = 8.89 \times 10^{-4} \text{ s}^{-1} \text{ and } d_2 = \frac{\ln 2}{2580 \text{ s}} = 2.36 \times 10^{-4} \text{ s}^{-1} \quad (36)$$

We assume Atf3 protein degrades at the same rate as Atf3 mRNA.

Egr1 DNA concentration

We assume there are two molecules of DNA per cell. The volume of a neonatal myocyte was estimated given that an adult myocyte has a volume of 34 pl with a capacitance of 66 pF [3,8] and the capacitance of a neonatal myocyte is 13 pF [8]. This gives 6.7 pl per cell leading to concentration of 33.2 pM.

Table S1 Model parameter values

Parameter	Definition	Value
m_0	Total MKK	130 nM
E_0	Total ERK	130 nM
M_{E0}	Initial <i>Egr1</i> mRNA concentration	1 pM
D_{E0}	<i>Egr1</i> DNA concentration	33.2 pM
$k_1\theta_1$	Rate of MKK activation by ET-1	$8.30 \times 10^{-3} \text{ s}^{-1}$
k_2	Rate of ERK activation by MKK	$1.28 \times 10^5 (\text{Ms})^{-1}$
k_3	Rate of ERK-P activation of <i>Egr1</i> DNA	$1.00 \times 10^5 (\text{Ms})^{-1}$
k_{-3}	Rate of ERK-P reverse activation of <i>Egr1</i> DNA	$5.00 \times 10^{-2} (\text{Ms})^{-1}$
k_4^*	<i>Egr1</i> mRNA transcription rate	$6.89 \times 10^{-15} \text{ M/s}$
k_6^*	<i>Atf3</i> mRNA transcription rate	$1.03 \times 10^{-15} \text{ M/s}$
k_7	<i>Atf3</i> suppression rate	To be determined
k_8	<i>Atf3</i> translation rate	0.25 s^{-1}
K_5	ERK-P and <i>Atf3</i> DNA dissociation rate	$0.5 \times 10^{-6} \text{ M}$
d_1	Degradation rate of <i>Atf3</i> mRNA	$8.89 \times 10^{-4} \text{ s}^{-1}$
d_2	Degradation rate of <i>Egr1</i> mRNA	$2.36 \times 10^{-4} \text{ s}^{-1}$
d_3	Degradation rate of <i>Atf3</i> protein	$2.36 \times 10^{-4} \text{ s}^{-1}$

RESULTS

The governing system of nonlinear ordinary differential equations (ODEs) (eqns 27–33) was solved using Gear's method available in Matlab (The Mathworks, Version 7.11) via the solver ode15, with $m_p(t)$ given by eqn (26).

Using the parameter values detailed in Table S1 we found the 20-fold change in *Egr1* mRNA determined experimentally could not be reproduced using these values [using an initial estimate of $k_7 = 1 \times 10^5 (\text{Ms})^{-1}$]. As such we undertook a sensitivity analysis in which we varied the rates of *Egr1* and *Atf3* mRNA transcription (k_4^*, k_6^*), ERK-P reverse activation of *Egr1* DNA (k_{-3}) and the ERK-P association rate for *Atf3* DNA (K_3). The most appropriate variation in these values which gave a good fit to the data was found to be a 5-fold increase in both the transcription rates of *Egr1* and *Atf3* mRNA ($5 \times k_4^*, 5 \times k_6^*$), a 10-fold decrease in the rate of ERK-P dissociation for *Egr1* DNA ($k_{-3}/10$) and a 50-fold decrease in the ERK-P association rate for *Atf3* DNA ($K_3/50$). Such a variation in the rates of *Egr1* and *Atf3* transcription is equivalent to a rate of 275 bases/s rather than the original assumption of 55 bases/s. Such a variation lies within the range recently reported in [1] and [5].

This led to a very good fit to the experimental data in terms of the magnitude variation in *Egr1* mRNA observed experimentally and a relatively good fit (qualitatively) to the suppression of *Egr1* mRNA by *Atf3*. To further improve this model-data fit we adjusted the rate of *Atf3* suppression (k_7). Good fits to the data were obtained for the range of values $1.00 \times 10^5 (\text{Ms})^{-1} \leq k_7 \leq 6 \times 10^5 (\text{Ms})^{-1}$.

Table S2 Primers used for qPCR and sqPCR

mRNA sequences were from the Rat Genome Database (<http://www.ncbi.nlm.nih.gov/entrez>).

Gene symbol (GenBank® accession number)	Size (bp)	Forward primer (position in sequence)	Reverse primer (position in sequence)
(a) qPCR primers			
<i>Atf3</i> (NM_012912.1)	108	5'-TCGCCATCCAGAACAAGCA-3' (140–158)	5'-GGGCCACCTCAGACTTGGT-3' (229–247)
<i>Egr1</i> (NM_012551.2)	98	5'-CTACGAGCACCTGACCACAGATC-3' (204–227)	5'-GCAACCGGGTAGTTTGGCT-3' (283–301)
<i>Gapdh</i> (NM_017008.3)	93	5'-CCAAGGTCATCCATGACAACTT-3' (476–497)	5'-AGGGGCCATCCACAGTCTT-3' (550–568)
<i>Ptgs2</i> (NM_017232.3)	90	5'-GAAGAACTTACAGGAGAGAAAGAAATGG-3' (1393–1420)	5'-CAGCAGGGCGGGATACAGT-3' (1464–1482)
<i>Dusp1</i> (NM_053769.3)	62	5'-GCGCGCTCCACTCAAGTC-3' (337–354)	5'-GGGCAGGAAGCCGAAAC-3' (381–398)
<i>Dusp5</i> (NM_133578.1)	70	5'-CGACATTAGCTCCCACTTTCAA-3' (882–903)	5'-AGGACCTTGCTCCCTCTTC-3' (934–953)
<i>Areg</i> (NM_017123.1)	108	5'-CTGCTGGTCTTAGGCTCAGG-3' (218–237)	5'-CACAAGTCCACCAGCACTGT-3' (306–325)
<i>Il6</i> (NM_012589.1)	157	5'-GAGTTGTGCAATGGCAATTC-3' (202–221)	5'-ACTCCAGAAGACCAGAGCAG-3' (339–358)
<i>Il1rl1</i> (NM_013037.1)	100	5'-GCCCTTCACTCTGGGTACTACT-3' (68–88)	5'-GCAATGGCACAGGAAGGTAAC-3' (147–167)
(b) sqPCR primers			
<i>Atf3</i> (NM_012912.1)	331	5'-GCTGCCAAGTGTGAAACAAG-3' (298–318)	5'-CAGTTTCCAATGGCTTCAGG-3' (608–628)
<i>Gapdh</i> (NM_017008.3)	452	5'-ACCACAGTCCATGCCATCAC-3' (520–539)	5'-TCCACCACCCTGTTGCTGTA-3' (952–971)

Table S3 Response of cardiomyocyte transcriptome to adenoviral infection

Cardiomyocytes were uninfected (no virus) or infected with empty AdVs and gene expression profiles were examined using Affymetrix microarrays. The data were analysed using GeneSpring to identify transcripts that were significantly changed (>1.5 -fold, FDR < 0.05) by AdV infection. Transcripts are clustered according to known or probable function and are listed alphabetically with up-regulated transcripts listed first. Results are means for $n = 3$ independent hybridizations each representing three separate preparations of cardiomyocytes.

Probeset	Gene symbol	Gene description	Raw values (no virus)	Fold change induced by virus
(a) Antiviral response				
7196285	<i>Adar</i>	Adenosine deaminase, RNA-specific	204	1.80*
7315869	<i>Ddit3</i>	DNA-damage inducible transcript 3	273	2.27*
7301235	<i>Ifi27</i>	Interferon α -inducible protein 27	1785	1.98*
7072322	<i>Ifi35</i>	Interferon-induced protein 35	91	1.97*
7215722	<i>Ifi44</i>	Interferon-induced protein 44	126	3.96*
7067089	<i>Ifi47</i>	Interferon-inducible protein 47	141	7.07*
7041465	<i>Ifit2</i>	Interferon-induced protein with tetratricopeptide repeats 2	89	1.89*
7041467	<i>Ifit3</i>	Interferon-induced protein with tetratricopeptide repeats 3	57	3.38*
7170909	<i>Iigp1</i>	Interferon inducible GTPase 1	106	4.47*
7067595	<i>Irf1</i>	Interferon regulatory factor 1	154	1.72*
7131960	<i>Irf9</i>	Interferon regulatory factor 9	443	2.28*
7092176	<i>Mx1j2</i>	Myxovirus (influenza virus) resistance 1/2	55	19.37*
7087207	<i>Mx2j1</i>	Myxovirus (influenza virus) resistance 2/1	122	19.05*
7098547	<i>Oas1a/k</i>	2'-5' Oligoadenylate synthetase 1A/K	91	12.05*
7102451	<i>Oas1b/i</i>	2'-5' Oligoadenylate synthetase 1B/I	55	6.03*
7098544	<i>Oas1i</i>	2'-5' Oligoadenylate synthetase 1I	73	5.81*
7102456	<i>Oas1k/a</i>	2'-5' Oligoadenylate synthetase 1K/A	97	1.80*
7103432	<i>Oas2</i>	2'-5' Oligoadenylate synthetase 2	41	3.58*
7102992	<i>Oasl</i>	2'-5' Oligoadenylate synthetase-like	101	11.64*
7103001	<i>Oasl2</i>	2'-5' Oligoadenylate synthetase-like 2	84	9.95*
7339147	<i>Plscr1</i>	Phospholipid scramblase 1	104	1.69*
7305174	<i>Rsad2</i>	Radical S-adenosylmethionine domain-containing 2	61	12.13*
7055354	<i>Trim5</i>	Tripartite motif-containing 5	835	1.52
7263822	<i>Zc3hav1</i>	Zinc finger CCCH type, antiviral 1	124	2.05
(b) Cytokine/chemokine signalling				
7073869	<i>C1qtnf1</i>	C1q and tumour necrosis factor-related protein 1	189	1.58
7356847	<i>Ccl20</i>	Chemokine (C-C motif) ligand 20	146	1.77
7070340	<i>Ccl7</i>	Chemokine (C-C motif) ligand 7	1918	2.00
7212626	<i>Csf1</i>	Colony-stimulating factor 1 (macrophage)	122	1.84
7071906	<i>Csf3</i>	Colony-stimulating factor 3 (granulocyte)	220	2.80
7116933	<i>Cxcl10</i>	Chemokine (C-X-C motif) ligand 10	37	5.21
7116931	<i>Cxcl11</i>	Chemokine (C-X-C motif) ligand 11	472	1.81
7123570	<i>Cxcl13</i>	Chemokine (C-X-C motif) ligand 13	132	1.69

Table S3 Continued

Probeset	Gene symbol	Gene description	Raw values (no virus)	Fold change induced by virus
7055111	<i>Il18bp</i>	IL18-binding protein	155	1.92
7260080	<i>Il6</i>	IL6	149	5.13
7138335	<i>Ripk3</i>	Receptor-interacting serine-threonine kinase 3	151	1.71
7362008	<i>Stat1/4</i>	Signal transducer and activator of transcription 1/4	906	2.26
7311784	<i>Stat2</i>	Signal transducer and activator of transcription 2	157	3.36
7216733	<i>Tnf</i>	Tumour necrosis factor (TNF superfamily, member 2)	94	2.19
7327525	<i>Tnfrsf11b</i>	Tumour necrosis factor receptor superfamily, member 11b	1001	1.93
7098502	<i>Trafd1</i>	TRAF (tumour-necrosis-factor-receptor-associated factor) type zinc finger domain containing 1	192	1.50
7351276	<i>Ccr1</i>	Chemokine (C-C motif) receptor 1	123	0.53
7041124	<i>Il33</i>	IL33	100	0.64
7123902	<i>Pf4</i>	Platelet factor 4	704	0.59
(c) Immune/inflammatory response				
7149693	<i>Bst2</i>	Bone marrow stromal cell antigen 2	165	3.21*
7093567	<i>Cd80</i>	CD80 molecule	80	2.20
7093779	<i>Cd86</i>	CD86 molecule	106	1.63
7216827	<i>Cfb</i>	Complement factor B	66	2.85
7296860	<i>Cmpk2</i>	Cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	72	7.20*
7327552	<i>Enpp2</i>	Ectonucleotide pyrophosphatase/phosphodiesterase 2	127	2.31
7107717	<i>Fcgr3a</i>	Fc fragment of IgG, low affinity IIIa, receptor	160	2.86
7200128	<i>Gbp2</i>	Guanylate-binding protein 2	101	7.94*
7076940	<i>Irgm</i>	Immunity-related GTPase family, M	240	6.43
7293880	<i>Isg15</i>	ISG15 ubiquitin-like modifier	64	14.88*
7033289	<i>Isg20</i>	Interferon-stimulated exonuclease gene 20	84	2.56*
7270067	<i>Klrk1</i>	Killer cell lectin-like receptor subfamily K, member 1	28	1.72
7084895	<i>Lgals3bp</i>	Lectin, galactoside-binding, soluble, 3 binding protein	319	3.79*
7080131	<i>Lgals5/9</i>	Lectin, galactose-binding, soluble 5/9	116	2.19*
7080134	<i>Lgals9/5</i>	Lectin, galactoside-binding, soluble, 9/5	162	4.47*
7069999	<i>Nos2</i>	Nitric oxide synthase 2, inducible	65	1.87
7266324	<i>Reg3g</i>	Regenerating islet-derived 3 γ	1758	1.57
7216994		RT1 class Ia, locus A2/A1 locus A3 RT1 class Ib, locus EC2 MHC class I RT1.Aa alpha-chain	289	2.02
7220575		RT1 class I, locus1 RT1 class I, locus CE12/CE14	271	2.24
7216676		RT1 class I, locus CE10/CE7/CE11	164	1.72
7224452		RT1 class I, locus CE11/CE7 RT1 class Ib, locus EC2	271	2.00
7224458		RT1 class I, locus CE12/14 RT1 class I, locus1	244	2.31
7224429		RT1 class I, locus CE13/CE14	102	1.78
7224511		RT1 class I, locus CE15	422	1.76
7220557		RT1 class I, locus CE3/A3 RT1 class Ia, locus A1/A2 RT1 class Ib, locus EC2	116	1.75
7220541		RT1 class I, locus CE5/CE4 RT1 class Ib, locus EC2 MHC class I RT1.Aa alpha-chain mature alpha chain of MHC class Ib protein-like	327	2.04
7216505		RT1 class Ib, locus N2/N1/N3	103	1.66
7216519		RT1 class Ib, locus N3/N1/N2	189	2.17
7216562		RT1 class I, locus T24, gene 1/4 MHC class I RT1.O type 149 processed pseudogene	198	2.09
7216540		RT1 class I, locus T24, gene 1/1/4 MHC class I RT1.O type 149 processed pseudogene RT1 class Ib, locus EC2	210	3.82
7220923	<i>Tap1</i>	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	120	3.12
7220903	<i>Tap2</i>	Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	106	1.77
7221026	<i>Tapbp</i>	TAP-binding protein	239	2.78
7269637	<i>Tapbp/Vamp1</i>	TAP-binding proteinlike vesicle-associated membrane protein 1	113	1.59
7145662	<i>Tlr3</i>	Toll-like receptor 3	80	1.83
7226106	<i>Tor1b</i>	Torsin family 1, member B	154	1.51*
7112701	<i>Tor3a</i>	Torsin family 3, member A	162	1.91*
7071036	<i>Trim25</i>	Tripartite motif-containing 25	194	2.01*
7295338	<i>Xdh</i>	Xanthine dehydrogenase	155	1.56*
7226238	<i>Aif1 Lamc3</i>	Allograft inflammatory factor 1-like laminin γ 3	920	0.65*
7111837	<i>Cfh</i>	Complement factor H	122	0.60
7325371	<i>Lyz2 Lyz2</i>	Lysozyme 2 lysozyme C type 2	350	0.59
7321134	<i>Mir196a</i>	MicroRNA mir-196a	72	0.66*
(d) Agonists/receptors				
7035427	<i>Adm</i>	Adrenomedullin	210	1.52
7322881	<i>Angptl4</i>	Angiopoietin-like 4	186	1.74
7301401	<i>Bdkrb1</i>	Bradykinin receptor B1	53	1.81*
7232862	<i>Bmp2</i>	Bone morphogenetic protein 2	40	1.97
7123853	<i>Ereg</i>	Epiregulin	55	2.55
7204090	<i>Fst</i>	Follistatin	83	1.93
7144218	<i>Gdf15</i>	Growth differentiation factor 15	168	2.89

Table S3 Continued

Probeset	Gene symbol	Gene description	Raw values (no virus)	Fold change induced by virus
7261075	<i>Hgf</i>	Hepatocyte growth factor	155	1.57
7348035	<i>Htr1b</i>	5-hydroxytryptamine (serotonin) receptor 1B	33	1.60
7318105	<i>Ly6e</i>	Lymphocyte antigen 6 complex, locus E	127	2.89
7216279	<i>Olr1730</i>	Olfactory receptor 1730	42	1.64
7201157	<i>Ptger3</i>	Prostaglandin E receptor 3 (subtype EP3)	64	1.59
7055094	<i>Folr2</i>	Folate receptor 2 (fetal)	174	0.66
7364798	<i>Htr2b</i>	5-hydroxytryptamine (serotonin) receptor 2B	99	0.65
7313468	<i>Igf1</i>	Insulin-like growth factor 1	2690	0.36
7071991	<i>Igfbp4</i>	Insulin-like growth factor binding protein 4	494	0.63
7146031	<i>Msr1</i>	Macrophage scavenger receptor 1	640	0.57
7055209	<i>Olr63</i>	Olfactory receptor 63	180	0.61
7263730	<i>Ptn</i>	Pleiotrophin	825	0.37
7094158	<i>Tfrc</i>	Transferrin receptor	1300	0.58
(e) Cell adhesion/extracellular matrix				
7296103	<i>Sdc1</i>	Syndecan 1	184	1.78
7247754	<i>Sdc4</i>	Syndecan 4	1059	1.57
7213140	<i>Vcam1</i>	Vascular cell adhesion molecule	1140	1.78
7202072	<i>Vcan</i>	Versican	618	1.62
7317088	<i>Col14a1</i>	Collagen, type XIV, α 1	271	0.64
7319496	<i>Fbln1</i>	Fibulin 1	299	0.65
7260805	<i>Fgl2</i>	Fibrinogen-like 2	254	0.60
7169581	<i>Pcdhb21</i>	Protocadherin β 21	88	0.65
(f) Cell cycle/cell death				
7341999	<i>Birc3</i>	Baculoviral IAP repeat-containing 3	70	1.55
7321430	<i>Cdk2</i>	Cyclin-dependent kinase 2	229	1.57
7217282	<i>Cdkn1a</i>	Cyclin-dependent kinase inhibitor 1A	1072	2.49
7114725	<i>Ephx1</i>	Epoxide hydrolase 1, microsomal	124	2.25
7041442	<i>Fas</i>	Fas (tumour necrosis receptor superfamily member 6)	275	1.62
7055435	<i>Hpx</i>	Haemopexin	93	2.43
7325416	<i>Mdm2</i>	Mdm2 p53-binding protein homologue (mouse)	678	1.64
7211447	<i>Mlt11</i>	Myeloid/lymphoid or mixed-lineage leukaemia (trithorax homologue, <i>Drosophila</i>); translocated to, 11	121	1.62
7192589	<i>Tnfsf10</i>	Tumour necrosis factor (ligand) superfamily, member 10	53	2.00
7207681	<i>Ccna2</i>	Cyclin A2	261	0.56
7203074	<i>Ccnb1</i>	Cyclin B1	354	0.66
7218195	<i>Cdc2</i>	Cell division cycle 2, G ₁ to S and G ₂ to M	234	0.55
7132836	<i>Pbk</i>	PDZ-binding kinase	164	0.57
7338657	<i>Ttk</i>	Ttk protein kinase	88	0.64
(g) Protein synthesis/modification/folding/degradation				
7088621	<i>Dtx3l</i>	Deltex 3-like (<i>Drosophila</i>)	55	2.16
7114108	<i>Eef1g1Slamf7</i>	Eukaryotic translation elongation factor 1 γ SLAM family member 7	302	1.56
7260038	<i>Mettl20</i>	Methyltransferase like 20	114	1.52
7361254	<i>Mitd1</i>	MIT, microtubule interacting and transport, domain containing 1236	2.46	
7220914	<i>Psmb8</i>	Proteasome (prosome, macropain) subunit, β type 8 (large multifunctional peptidase 7)	62	1.76
7216935	<i>Psmb9</i>	Proteasome (prosome, macropain) subunit, β type 9 (large multifunctional peptidase 2)	170	3.44
7138231	<i>Psmc2</i>	Proteasome (prosome, macropain) activator subunit 2	1159	1.61
7103916	<i>Serpinb2</i>	Serine (or cysteine) peptidase inhibitor, clade B, member 2	180	7.32
7101220	<i>Serpine1</i>	Serine (or cysteine) peptidase inhibitor, clade E, member 1	214	1.98*
7241130	<i>Serpin1</i>	Serine (or cysteine) peptidase inhibitor, clade G, member 1	597	1.55*
7195163	<i>Serpin1</i>	Serine (or cysteine) peptidase inhibitor, clade I, member 1	113	2.12
7247744	<i>Slpi1Slpi2</i>	Secretory leucocyte peptidase inhibitor/antileukoprotease-like 2	1043	2.21
7374050	<i>Capn6</i>	Calpain 6	107	0.57
7332657	<i>Mmp12</i>	Matrix metalloproteinase 12	1365	0.44
(h) Regulation of metabolism/signalling/transcription				
7250403	<i>Abcb1b/1a</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 1B/1A	175	3.24
7318515	<i>Apol3</i>	Apolipoprotein L, 3	168	1.53
7226167	<i>Ass1</i>	Argininosuccinate synthetase 1	216	1.85
7356120	<i>Cyp27a1</i>	Cytochrome P450, family 27, subfamily a, polypeptide 1	218	1.90
7352252	<i>Enpp4</i>	Ectonucleotide pyrophosphatase/phosphodiesterase 4	243	1.72
7363922	<i>Glb1l</i>	Galactosidase, β 1-like	150	1.54
7327675	<i>Has2</i>	Hyaluronan synthase 2	256	1.78
7037881	<i>Mgmt</i>	O-6-methylguanine-DNA methyltransferase	158	1.54
7185182	<i>Nqo1</i>	NAD(P)H dehydrogenase, quinone 1	241	1.52
7093806	<i>Parp9</i>	Poly (ADP-ribose) polymerase family, member 9	80	1.81
7329573	<i>Pvalb</i>	Parvalbumin	57	1.56
7237508	<i>Ptges</i>	Prostaglandin E synthase	252	3.02
7106132	<i>Ptgs2</i>	Prostaglandin-endoperoxide synthase 2	136	1.92
7339559	<i>Rbp2</i>	Retinol-binding protein 2, cellular	93	2.28

Table S3 Continued

Probeset	Gene symbol	Gene description	Raw values (no virus)	Fold change induced by virus
7041029	<i>Rcl1</i>	RNA terminal phosphate cyclase-like 1	391	1.57
7244077	<i>Slc28a2</i>	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	174	1.57
7250503	<i>Steap1</i>	Six transmembrane epithelial antigen of the prostate 1	419	1.81
7184185	<i>Asf1b</i>	ASF1 anti-silencing function 1 homologue B (<i>S. cerevisiae</i>)	176	0.65
7115455	<i>Atf3</i>	Activating transcription factor 3	183	0.45
7197511	<i>Casq2</i>	Calsequestrin 2 (cardiac muscle)	1048	0.58
7156835	<i>F13a1</i>	Coagulation factor XIII, A1 polypeptide	237	0.34
7250763	<i>Gng11</i>	Guanine nucleotide-binding protein (G protein), gamma 11	826	0.65
7164749	<i>Hist1h2ai</i>	Histone cluster 1, H2ai-like	1978	0.62
7034549	<i>Kcne3</i>	Potassium voltage-gated channel, Isk-related subfamily, gene 3	548	0.61
7240783	<i>Pde1a</i>	Phosphodiesterase 1A, calmodulin-dependent	343	0.61
7261911	<i>Pdk4</i>	Pyruvate dehydrogenase kinase, isozyme 4	219	0.66
7288397	<i>Ptplad2</i>	Protein tyrosine phosphatase-like A domain containing 2	141	0.66
7362066	<i>Sdpr</i>	Serum deprivation response	835	0.56
7219202	<i>Smpd13a</i>	Sphingomyelin phosphodiesterase, acid-like 3A	618	0.65
7025936	<i>Tcf21</i>	Transcription factor 21	724	0.63
(i) Proteins with no known function/hypothetical proteins				
7329479	<i>Apol9a</i>	Apolipoprotein L 9a	147	4.07
7220127	<i>Ascc3</i>	Activating signal cointegrator 1 complex subunit 3	215	1.77
7070512	<i>LOC360228</i>	WDNM1 homologue	139	1.57
7333434	<i>LOC500956</i>	Unknown	150	1.53
7138718	<i>Phf11/11l</i>	PHD finger protein 11/11-like	46	2.81
7260055	<i>RGD1309621</i>	Similar to hypothetical protein FLJ10652	155	2.46
7065377	<i>RGD1561157</i>	Unknown	117	1.71
7235527	<i>Rnf114</i>	Ring finger protein 114	951	1.70*
7073915	<i>Rnf213</i>	Ring finger protein 213	51	2.54
7073928	<i>Rnf213</i>	Ring finger protein 213	65	1.99
7094771	<i>Rtp4lCtdsp1</i>	Receptor (chemosensory) transporter protein 4 CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	139	11.46*
7070419	<i>Slfn3</i>	Schlafen 3	131	5.04*
7070393	<i>Slfn5</i>	Schlafen family member 5	98	1.85*
7072336	<i>Tmem106a</i>	Transmembrane protein 106A	78	1.85
7252491	<i>Tmem140</i>	Transmembrane protein 140	206	1.83
7047575	Unknown	Unknown	55	1.57
7088625	Unknown	Unknown	88	2.72
7116103	Unknown	Unknown	71	2.16
7129836	Unknown	Unknown	38	1.54
7153410	Unknown	Unknown	96	1.74
7254427	Unknown	Unknown	119	1.55
7328454	Unknown	Unknown	46	1.64
7371101	Unknown	Unknown	54	2.67
7126637	<i>Cd38</i>	CD38 molecule	608	0.61
7191192	<i>Fam134b</i>	Family with sequence similarity 134, member B	1202	0.62
7372873	<i>Fam70a</i>	Family with sequence similarity 70, member A	534	0.51
7133474	<i>Lcp1</i>	Lymphocyte cytosolic protein 1	545	0.57
7164764	<i>LOC680097</i>	Similar to germinal histone H4 gene	6612	0.58
7367891	<i>LOC680166</i>	Unknown	204	0.61
7157871	<i>LOC682649</i>	Similar to Histone H2A type 1	647	0.53
7260779	<i>Lrrc17</i>	Leucine rich repeat containing 17	298	0.38
7060488	<i>Ms4a6b/11</i>	Membrane-spanning 4-domains, subfamily A, member 6B/11	215	0.52
7337370	<i>Ns5atp9</i>	NS5A (hepatitis C virus) transactivated protein 9	307	0.66
7035206	<i>Olfml1</i>	Olfactomedin-like 1	355	0.48
7328472	<i>Tmem71</i>	Transmembrane protein 71	128	0.59
7077262	Unknown	Unknown	100	0.66
7135004	Unknown	Unknown	194	0.62
7164849	Unknown	Unknown	845	0.66
7176278	Unknown	Unknown	208	0.65
7367340	Unknown	Unknown	67	0.57
7368283	Unknown	Unknown	409	0.63
7369003	Unknown	Unknown	1284	0.63
7370879	Unknown	Unknown	86	0.63

*Transcripts identified as part of an interferon response in other systems.

Table S4 Transcripts up-regulated by ET-1 that are regulated by Atf3, but are unaffected by No-AS virus infection

*IEG; **non-IEG.

Transcript cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	No-AS	AS-Atf3	No virus	No-AS	AS-Atf3
(a) AS Atf3 enhances response to ET-1								
Cluster A1								
7169197	<i>Egr1*</i>	289	1	0.95	1.02	1.29	1.24	4.73
7222466	<i>Egr2*</i>	85	1	0.92	0.87	1.32	1.59	2.90
7288744	<i>Jun*</i>	425	1	0.93	1.44	1.21	1.31	2.10
7320920	<i>Nr4a1*</i>	64	1	0.95	1.12	3.78	4.29	6.63
Cluster A2								
7308330	<i>Actn1*</i>	152	1	0.94	2.61	2.72	2.22	3.56
7185572	<i>Bcar1*</i>	154	1	1.05	1.94	1.56	1.41	2.23
7231595	<i>Chac1**</i>	142	1	1.01	2.04	0.96	1.08	3.03
7114850	<i>Enah*</i>	130	1	0.98	2.28	2.00	1.60	2.86
7265600	<i>Gadd45a**</i>	854	1	1.00	2.94	1.98	1.93	3.09
7243216	<i>Grem1**</i>	52	1	1.13	1.81	1.83	1.49	2.68
7179800	<i>Ier2*</i>	368	1	0.96	2.16	1.67	1.79	4.56
7354973	<i>Nop58</i>	195	1	0.95	2.86	1.93	1.62	3.39
7124933	<i>Ras11b*</i>	500	1	1.13	2.22	1.73	1.91	3.02
7096358	<i>Slc7a1**</i>	88	1	1.10	2.80	2.18	1.74	3.54
7115195	<i>Tgfb2**</i>	181	1	1.06	2.77	1.79	1.67	2.99
(b) AS Atf3 inhibits response to ET-1								
Cluster B1								
7260283	<i>Insig1</i>	2796	1	0.94	0.62	1.54	1.50	0.95
7144691	<i>Sc4mol**</i>	1376	1	0.99	0.60	1.72	1.72	0.89
7133039	<i>Stc1*</i>	560	1	1.17	0.58	1.21	1.23	0.59
Cluster B2								
7123129	<i>Agpat9</i>	65	1	0.96	1.07	2.82	2.22	1.40
7123848	<i>Areg**</i>	127	1	1.07	1.20	5.63	7.01	2.09
7043230	<i>Dusp5**</i>	178	1	0.93	0.87	3.03	2.99	1.54
7345585	<i>Fdx1**</i>	459	1	0.87	0.93	2.94	3.05	1.98
7187674	<i>Glr1x1</i>	191	1	0.94	0.82	1.66	1.92	1.14
7202670	<i>Hmgcr*</i>	527	1	1.00	0.72	1.62	1.71	1.04
7150663	<i>Mfap3l</i>	116	1	0.98	0.91	1.66	1.34	0.88
7235566	<i>Pard6b**</i>	62	1	1.06	1.19	3.20	3.62	1.48
7204067	<i>Pelo**</i>	863	1	1.12	1.19	1.76	1.90	1.24
7104906	<i>RGD1562617</i>	127	1	0.90	0.88	2.36	1.41	0.89
7214260	<i>Sgms2</i>	77	1	1.03	1.04	4.42	3.27	1.28
7141508	<i>Spry2**</i>	513	1	0.99	1.20	2.87	2.79	1.74
7297329	<i>Twist1**</i>	126	1	1.14	0.96	2.83	3.77	2.24
7350922	<i>Xirp1**</i>	217	1	1.02	1.40	4.09	3.53	1.70
7297247	Unknown	121	1	1.12	1.14	2.21	2.22	1.41

Table S5 Transcripts unaffected by No-AS virus infection that are up-regulated by ET-1 with significantly increased expression in control cells by Atf3 knockdown

*IEG; **non-IEG.

Transcript cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	No-AS	AS-Atf3	No virus	No-AS	AS-Atf3
Cluster C								
7305953	<i>Arl4a</i>	482	1	1.07	1.69	1.60	1.99	1.61
7301400	<i>Bdkrb2**</i>	104	1	1.09	1.77	1.74	1.98	2.21
7111272	<i>Btg2*</i>	311	1	0.93	1.87	3.66	4.22	3.28
7209338	<i>Ccn1*</i>	249	1	0.99	1.77	1.69	1.54	2.47
7045969	<i>Cnksr3</i>	166	1	1.14	1.96	1.61	1.63	2.21
7350918	<i>Csrnp1</i>	146	1	1.17	2.42	3.63	3.82	4.59
7044959	<i>Ctgf*</i>	342	1	0.91	3.04	3.85	4.13	5.22
7215359	<i>Cyr61*</i>	407	1	1.04	1.74	5.22	5.40	6.22
7143265	<i>Eaf1</i>	130	1	1.13	1.87	1.56	1.57	2.12
7163221	<i>Edn1*</i>	71	1	1.11	1.78	1.19	1.26	2.42
7147985	<i>Efnb2**</i>	71	1	0.99	1.74	1.42	1.45	2.15
7281135	<i>Epha2*</i>	90	1	1.16	3.52	2.89	3.29	4.32
7297136	<i>Fam110c</i>	55	1	1.09	1.92	3.70	4.75	3.76
7242382	<i>Fjx1</i>	149	1	1.07	1.74	1.74	1.71	2.10
7252051	<i>Finc**</i>	143	1	0.94	1.47	2.86	2.31	1.80
7162363	<i>Gadd45g*</i>	357	1	0.99	3.33	4.72	4.19	5.92
7174562	<i>Hbegf*</i>	100	1	0.88	2.73	3.90	4.33	3.84
7193413	<i>Hspa4l</i>	63	1	0.93	2.29	1.94	1.48	2.00
7165193	<i>Inhba**</i>	88	1	1.09	2.09	6.95	6.30	6.29
7257290	<i>Lmcd1*</i>	283	1	1.17	4.41	5.84	6.64	5.84
7100653	<i>Malik*</i>	187	1	1.00	2.06	2.23	2.25	2.36
7068314	<i>Map2k3**</i>	106	1	1.00	1.65	1.91	1.78	1.96
7194351	<i>Mei1/Tsc22d2</i>	149	1	0.99	1.84	2.45	2.43	2.43
7317471	<i>Myc*</i>	156	1	1.02	2.04	2.28	2.34	2.92
7155813	<i>Nfil3*</i>	67	1	1.04	2.71	2.57	2.83	3.71
7189518	<i>Plk2*</i>	1457	1	1.15	2.79	2.14	2.65	3.58
7051029	<i>Ppp1r15a</i>	115	1	1.02	1.93	1.57	1.57	2.26
7360736	<i>Ptp4a1</i>	638	1	1.05	2.14	2.39	2.27	2.36
7048738	<i>PVR*</i>	430	1	1.16	2.58	3.64	3.77	3.48
7340175	<i>Rassf1*</i>	163	1	0.99	1.90	1.62	1.68	2.04
7091811	<i>Rcan1</i>	2101	1	1.12	1.77	1.90	1.95	1.95
7331581	<i>Rnd1*</i>	203	1	1.20	3.24	4.78	4.76	3.58
7238766	<i>Rnd3*</i>	311	1	1.01	1.96	1.82	1.56	2.00
7029999	<i>Sertad1*</i>	217	1	1.09	1.86	1.95	2.50	2.39
7279509	<i>Stk40</i>	144	1	1.00	1.64	1.62	1.57	1.75
7075088	<i>Tnfrsf12a*</i>	750	1	1.09	2.75	3.17	3.26	3.77
7106964	<i>Tnfrsf18</i>	80	1	0.87	2.02	9.32	6.92	9.29
7199859	<i>Tspan5**</i>	217	1	1.08	1.69	2.30	2.02	1.95
7120783	Unknown	93	1	0.92	1.47	1.64	1.52	1.55
7339862	Unknown	185	1	0.84	1.53	1.62	1.14	1.60
7085544	<i>Vgll3</i>	133	1	0.96	1.59	2.87	2.52	2.78
7228236	<i>Xirp2</i>	124	1	0.93	2.14	3.84	2.73	2.17

Table S6 Transcripts affected by No-AS virus infection that are up-regulated by ET-1 and regulated by Atf3

*IEG; **non-IEG.

Transcript cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	No-AS	AS-Atf3	No virus	No-AS	AS-Atf3
(a) AS-Atf3 enhances response to ET-1								
Cluster D								
7120521	<i>Lif*</i>	82	1	1.75	3.70	2.88	3.62	7.30
7106132	<i>Ptgs2*</i>	136	1	1.92	3.93	4.94	4.44	9.84
7237652	<i>Slc25a25*</i>	94	1	1.22	1.71	1.41	1.64	2.19
7028549	<i>Il11</i>	143	1	1.20	1.74	2.88	2.82	3.87
7284153	<i>Ripk2**</i>	238	1	1.31	2.79	2.53	2.72	3.58
7261019	<i>Sema3c</i>	208	1	1.22	2.16	1.67	1.80	2.21
7035407	<i>Wee1</i>	303	1	1.21	1.89	1.74	2.23	2.26
7096947	<i>Zfand2a*</i>	288	1	1.23	1.60	1.73	2.17	2.39
(b) AS-Atf3 inhibits response to ET-1								
Cluster E								
7259100	<i>Apold1</i>	533	1	0.82	0.63	1.64	1.43	1.02
7105894	<i>B3galt2</i>	505	1	0.79	1.12	2.31	1.97	1.07
7051386	<i>Csrp3</i>	1062	1	0.75	0.72	1.53	1.37	0.72
7084788	<i>Socs3</i>	229	1	1.25	1.01	1.75	1.64	0.77
7370997	Unknown	313	1	0.69	0.81	1.83	1.43	0.80
7135004	Unknown	194	1	0.62	1.24	2.47	2.47	1.40
7305661	Unknown	81	1	0.80	1.25	3.46	3.56	2.12

Table S7 Transcripts that are down-regulated by ET-1 and regulated by Atf3

Transcript cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	No-AS	AS-Atf3	No virus	No-AS	AS-Atf3
(a) AS-Atf3 enhances response to ET-1								
Cluster F								
7279127	<i>Cited4</i>	231	1	0.91	0.48	0.52	0.59	0.47
7180333	<i>Ednra</i>	2175	1	0.99	0.60	0.58	0.64	0.41
7115625	<i>G0s2</i>	2545	1	0.86	0.49	0.30	0.26	0.12
7190436	<i>Lifr</i>	390	1	0.88	0.55	0.60	0.54	0.37
7269707	<i>Ntf3</i>	250	1	1.18	0.54	0.52	0.65	0.37
(b) AS-Atf3 inhibits response to ET-1								
Cluster G								
7222734	<i>Ddit4</i>	1553	1	1.16	2.04	0.46	0.55	0.79
7120384	<i>Pik3ip1</i>	339	1	0.83	0.57	0.29	0.34	0.46
7346730	<i>Smad6</i>	266	1	1.05	1.41	0.50	0.57	1.03
7197113	<i>Txnip</i>	2024	1	0.90	1.10	0.32	0.39	0.96
7173133	Unknown	690	1	0.74	0.15	0.23	0.16	0.18

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