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

Article

Accepted Version

Supplemental information

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Supplemental Information Giraldo et al Fig. S1

**Figure S1** Infection with AdVs modulates expression of a subset of cardiomyocyte mRNAs

Cells (uninfected or infected with 150 MOI FLAG or AS-Atf3) were unstimulated or exposed to ET-1 for 90 min and RNA expression profiles determined using Affymetrix rat exon 1.0 ST arrays. A, Heatmaps show expression profiles for probesets that were significantly changed (>1.5-fold, FDR<0.05) by viral infection alone. Normalisation is to the gene median (range is -2.5 (cyan) through 0 (black) to +2.5 (red) on a log2 scale). Results are means for 3 hybridisations each representing 3 independent myocyte preparations. Clustering on conditions and entities used a Euclidean distance matrix and centroid linkage ratio. B, Transcripts were classified according to function. Upregulated and downregulated transcripts are in red and blue, respectively. Transcripts regulated by interferons (Ifn) in other systems are in lighter colours.
Supplemental Table 1  Response of cardiomyocyte transcriptome to adenoviral infection

Cardiomyocytes were uninfected (no virus) or infected with empty adenoviruses 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 adenovirus infection. Transcripts are clustered according to known or probable function and are listed alphabetically with upregulated transcripts listed first. Results are means for n=3 independent hybridisations each representing 3 separate preparations of cardiomyocytes. * Transcripts identified as part of an interferon response in other systems.

| Probeset | Genesymbol | Genedescription | Raw values (No virus) | Fold change induced by virus |
|----------|------------|-----------------|----------------------|-----------------------------|
| 7196285  | Adar       | Adenosine deaminase, RNA-specific | 204                  | 1.80 *                     |
| 7315869  | Ddit3      | DNA-damage inducible transcript 3 | 273                  | 2.27                        |
| 7301235  | Ifi27      | interferon, alpha-inducible protein 27 | 1785                | 1.98                        |
| 7072322  | Ifi35      | Interferon-induced protein 35 | 91                   | 1.97                        |
| 7215722  | Ifi44      | Interferon-induced protein 44 | 126                   | 3.96                        |
| 7067089  | Ifi47      | Interferon gamma inducible protein 47 | 141                  | 7.07                        |
| 7041465  | Ifi2       | Interferon-induced protein with tetratricopeptide repeats 2 | 89                   | 1.89                        |
| 7041467  | Ifi3       | Interferon-induced protein with tetratricopeptide repeats 3 | 57                   | 3.38                        |
| 7170909  | Igg1       | Interferon inducible GTPase 1 | 106                   | 4.47                        |
| 7076595  | Ifr1       | Interferon regulatory factor 1 | 154                   | 1.72                        |
| 7131960  | Ifr9       | Interferon regulatory factor 9 | 443                   | 2.28                        |
| 7092176  | Mx1/2      | Myxovirus (influenza virus) resistance 1/2 | 55                   | 19.37                       |
| 7087207  | Mx2/1      | Myxovirus (influenza virus) resistance 2/1 | 122                  | 19.05                       |
| 7098947  | Oas1a/k    | 2'-5' oligoadenylate synthetase 1A/K | 91                   | 12.05                       |
| 7102451  | Oas1b/i    | 2-5 oligoadenylate synthetase 1B/i | 55                   | 6.03                        |
| 7098944  | Oas1i      | 2'-5' oligoadenylate synthetase 1I | 73                   | 5.81                        |
| 7102456  | Oas1k/a    | 2'-5' oligoadenylate synthetase 1K/A | 97                   | 1.80                        |
| 7103432  | Oas2       | 2'-5' oligoadenylate synthetase 2 | 41                   | 3.58                        |
| 7102992  | Oasl       | 2'-5' oligoadenylate synthetase-like | 101                  | 11.64                       |
| 7103001  | Oasl2      | 2'-5' oligoadenylate synthetase-like 2 | 84                   | 9.95                        |
| 7125147  | Pilsr1     | Phospholipid scramblase 1 | 104                   | 1.69                        |
| 7305174  | Rsad2      | Radial S-adenosyl methionine domain containing 2 | 61                   | 12.13                       |
| 7055354  | Trim5      | Tripartite motif-containing 5 | 835                   | 1.52                        |
| 7263822  | Zc3hav1    | Zinc finger CCCH type, antiviral 1 | 124                   | 2.05                        |

Cytokine/chemokine signalling

| Probeset | Genesymbol | Genedescription | Raw values (No virus) | Fold change induced by virus |
|----------|------------|-----------------|----------------------|-----------------------------|
| 7073869  | C1qtnf1    | C1q and tumor necrosis factor related protein 1 | 189                  | 1.58                        |
| 7356847  | Ccl20      | Chemokine (C-C motif) ligand 20 | 146                  | 1.77                        |
| 7070340  | Ccf7       | Chemokine (C-C motif) ligand 7 | 1918                 | 2.00                        |
| 7212826  | Cs1        | Colony stimulating factor 1 (macrophage) | 122                  | 1.84                        |
| 7071906  | Cs3        | Colony stimulating factor 3 (granulocyte) | 220                  | 2.80                        |
| 7116933  | Cxcl10     | Chemokine (C-X-C motif) ligand 10 | 37                   | 5.21                        |
| 7116931  | Cxcl11     | Chemokine (C-X-C motif) ligand 11 | 472                   | 1.81                        |
| 7123570  | Cxcl13     | Chemokine (C-X-C motif) ligand 13 | 132                   | 1.69                        |
| 7055111  | Il18bp     | Interleukin 18 binding protein | 155                   | 1.92                        |
| 7260080  | Il6        | Interleukin 6 | 149                   | 5.13                        |
| 7138335  | Ripk3      | Receptor-interacting serine-threonine kinase 3 | 151                  | 1.71                        |
| 7362008  | Stat1/4    | Signal transducer and activator of transcription 1/4 | 906                  | 2.26                        |
| 7311784  | Stat2      | Signal transducer and activator of transcription 2 | 157                  | 3.36                        |
| 7216733  | Tnf        | Tumor necrosis factor (TNF superfamily, member 2) | 94                   | 2.19                        |
| 7327525  | Tnfrsf11b  | Tumor necrosis factor receptor superfamily, member 11b | 1001                | 1.93                        |
| 7098502  | Trald1     | TRAF type zinc finger domain containing 1 | 192                   | 1.50                        |
| 7351276  | Ccr1       | Chemokine (C-C motif) receptor 1 | 123                   | 0.53                        |
| 7041124  | Il33       | Interleukin 33 | 100                   | 0.64                        |
| 7123902  | Pf4        | Platelet factor 4 | 704                   | 0.59                        |

Immune/inflammatory response

| Probeset | Genesymbol | Genedescription | Raw values (No virus) | Fold change induced by virus |
|----------|------------|-----------------|----------------------|-----------------------------|
| 7149693  | Bst2       | Bone marrow stromal cell antigen 2 | 165                  | 3.21                        |
| 7093567  | Cd80       | CD80 molecule | 80                    | 2.20                        |
| 7093779  | Cd86       | CD86 molecule | 106                   | 1.63                        |
| 7216827  | Ccb       | Complement factor B | 66                   | 2.85                        |
| 7296860  | Cmpk2     | Cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial | 72                   | 7.20                        |
| 7327552  | Enpp2     | Ectonucleotide pyrophosphatase/phosphodiesterase 2 | 127                  | 2.31                        |
| 7107717  | Fgcr3a    | Fc fragment of IgG, low affinity IIla, receptor | 180                   | 2.86                        |
| 7200128  | Gbp2      | Guanylate binding protein 2 | 101                   | 7.94                        |
| 7076940  | Irgm      | Immunity-related GTPase family, M | 240                   | 6.43                        |
| 7293880  | Isg15     | ISG15 ubiquitin-like modifier | 64                    | 14.88                       |
| 7033289  | Isg20     | Interferon stimulated exonuclease gene 20 | 84                   | 2.56                        |
| 7270067  | Kikr1     | Killer cell lectin-like receptor subfamily K, member 1 | 28                   | 1.72                        |
| 7084895  | Lgals3bp  | Lectin, galactoside-binding, soluble, 3 binding protein | 319                   | 3.79                        |
| Gene ID  | Description                                                                 | Value 1 | Value 2 | P  |
|---------|------------------------------------------------------------------------------|---------|---------|----|
| 7080131 | Lgals5/9 Lectin, galactose binding, soluble 5/9                              | 116     | 2.19    | *  |
| 7080134 | Lgals9/5 Lectin, galactoside-binding, soluble, 9/5                           | 162     | 4.47    | *  |
| 7069999 | Nos2 Nitric oxide synthase 2, inducible                                      | 65      | 1.87    |    |
| 7266324 | Reg3g Regenerating islet-derived 3 gamma                                    | 1758    | 1.57    |    |
| 7216994 | RT1 class Ia, locus A2/A1 | locus A3 | RT1 class Ib, locus EC2 | 289 | 2.02 |
|         | MHC class I RT1.Aa alpha-chain                                              |         |         |    |
| 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 la, locus A1/A2 | RT1 class Ib, locus EC2 | 116 | 1.75 |
| 7220541 | RT1 class I, locus CE5/CE4 | RT1 class Ib, locus EC2 |         | 327 | 2.04 |
|         | MHC class I RT1.Aa alpha-chain | mature alpha chain of MHC class Ib protein-like |         |    |    |
| 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 |         | 198 | 2.09 |
|         | RT1.O type 149 processed pseudogene                                          |         |         |    |
| 7216540 | RT1 class I, locus T24, gene 1/4 | MHC class I |         | 210 | 3.82 |
|         | RT1.O type 149 processed pseudogene | RT1 class Ib, locus EC2 |         |    |    |
| 7220923 | Tap1 Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)            | 120     | 3.12    |    |
| 7220903 | Tap2 Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)            | 106     | 1.77    |    |
| 7221026 | Tappb TAP binding protein                                                   | 239     | 2.78    |    |
| 7269637 | Tapbp/Vamp1 TAP binding protein-like                                         | 113     | 1.59    |    |
| 7145662 | Trl3 Toll-like receptor 3                                                   | 80      | 1.83    |    |
| 7226106 | Tor1b Torsin family 1, member B                                            | 154     | 1.51    | *  |
| 7112701 | Tor3a Torsin family 3, member A                                            | 162     | 1.91    | *  |
| 7071036 | Trim25 Tripartite motif-containing 25                                       | 194     | 2.01    |    |
| 7295338 | Xdh Xanthine dehydrogenase                                                  | 155     | 1.56    | *  |
| 7222388 | Aif1| Lamc3 Allograft inflammatory factor 1-like | laminin gamma 3 | 920 | 0.85 |
| 7111837 | Cth Complement factor H                                                     | 122     | 0.60    |    |
| 7325371 | Lyz2| Lyc2 Lysozyme 2 | lysozyme C type 2 | 350 | 0.59 |
| 7321134 | Mr196a MicroRNA mir-196a                                                    | 72      | 0.66    | *  |

### Agonists/Receptors

| Gene ID  | Description                                                                 | Value 1 | Value 2 | P  |
|---------|------------------------------------------------------------------------------|---------|---------|----|
| 7035427 | Adm Adrenomedullin                                                          | 210     | 1.52    |    |
| 7322881 | Angpt4 Angiopoietin-like 4                                                 | 186     | 1.74    |    |
| 7301401 | Bdkrb1 Bradykininin receptor B1                                             | 53      | 1.81    | *  |
| 7232862 | Bmp2 Bone morphogenetic protein 2                                          | 40      | 1.97    |    |
| 7123853 | Ereg Epiregulin                                                           | 55      | 2.55    |    |
| 7204090 | Fst Follistatin                                                            | 83      | 1.93    |    |
| 7144128 | Gdf15 Growth differentiation factor 15                                      | 168     | 2.89    |    |
| 7261775 | Hgf Hepatocyte growth factor                                               | 155     | 1.57    |    |
| 7249035 | Htr1b 5-hydroxytryptamine (serotonin) receptor 1B                            | 33      | 1.60    |    |
| 7318105 | Ly6e Lymphocyte antigen 6 complex, locus E                                 | 127     | 2.89    |    |
| 7216279 | Olr1730 Octoflavin receptor 1730                                            | 42      | 1.64    |    |
| 7201157 | Ptger3 Prostaglandin E receptor 3 (subtype EP3)                              | 64      | 1.59    |    |
| 7055094 | Fxor2 Folate receptor 2 (fetal)                                            | 174     | 0.66    |    |
| 7364798 | Htr2b 5-hydroxytryptamine (serotonin) receptor 2B                           | 99      | 0.65    |    |
| 7313468 | Igf1 Insulin-like growth factor 1                                           | 2690    | 0.36    |    |
| 7071991 | Igfbp4 Insulin-like growth factor binding protein 4                         | 494     | 0.63    |    |
| 7146031 | Msr1 Macrophage scavenger receptor 1                                      | 640     | 0.57    |    |
| 7055209 | Olt3 Octoflavin receptor 6                                                 | 180     | 0.61    |    |
| 7263730 | Ptn Pleiotrophin                                                           | 825     | 0.37    |    |
| 7041587 | Tfrc Transferrin receptor                                                  | 1300    | 0.58    |    |

### Cell adhesion/extracellular matrix

| Gene ID  | Description                                                                 | Value 1 | Value 2 | P  |
|---------|------------------------------------------------------------------------------|---------|---------|----|
| 7296103 | Sdc1 Syndecan 1                                                             | 184     | 1.78    |    |
| 7247754 | Sdc4 Syndecan 4                                                             | 1059    | 1.57    |    |
| 7213140 | Vcam1 Vascular cell adhesion molecule 1                                     | 140     | 1.78    |    |
| 7202072 | Vcan Versican                                                                | 618     | 1.62    |    |
| 7317088 | Col14a1 Collagen, type XIV, alpha 1                                         | 271     | 0.64    |    |
| 7319496 | Fbn1 Fibulin 1                                                              | 299     | 0.85    |    |
| 7260805 | Fgll2 Fibrinogen-like 2                                                     | 254     | 0.60    |    |
| 7169581 | Pcdh21 Protocadherin beta 2                                                 | 88      | 0.65    |    |

### Cell cycle/cell death

| Gene ID  | Description                                                                 | Value 1 | Value 2 | P  |
|---------|------------------------------------------------------------------------------|---------|---------|----|
| 7341999 | Birc3 Baculoviral IAP repeat-containing 3                                   | 70      | 1.55    |    |
| 7321430 | Cdk2 Cyclin dependent kinase 2                                              | 229     | 1.57    |    |
| 7217282 | Cdkn1a Cyclin-dependent kinase inhibitor 1A                                 | 1072    | 2.49    |    |
| 7114725 | Ephx1 Epoxide hydrolase 1, microsomal                                        | 124     | 2.25    |    |
| 7041442 | Fas Fas (TNF receptor superfamily member 6)                                 | 275     | 1.62    |    |
| Protein | Function/Modification/Folding/Degradation | Regulation of Metabolism/Signalling/Transcription |
|---------|------------------------------------------|-------------------------------------------------|
| **Protein synthesis/modification/folding/degradation** |
| Dtx3l | Deltex 3-like (Drosophila) | Ddx3l (DDX3L) |  |
| Eef1g | Eukaryotic translation elongation factor 1 gamma (Eef1g) | Eef1g (EFTF) |  |
| Mettl20 | Methyltransferase like 20 | Mettl20 (METS) |  |
| Mitd1 | MIT, microtubule interacting and transport, domain containing 1 | Mitd1 (MITD1) |  |
| Psmb8 | Proteasome (prosome, macropain) subunit, beta type 8 | Psmb8 (PSMB8) |  |
| Psmb9 | Proteasome (prosome, macropain) subunit, beta type 9 | Psmb9 (PSMB9) |  |
| Psme2 | Proteasome (prosome, macropain) activator subunit 2 | Psme2 (PSME2) |  |
| **Protein synthesis/modification** |
| Abcb1b/1a | ATP-binding cassette, sub-family B (MDR/TAP),member 1B/1A | Abcb1b/1a (ABCB1B) |  |
| Apol3 | Apolipoprotein L, 3 | Apol3 (APOL3) |  |
| Ass1 | Argininosuccinate synthetase 1 | Ass1 (ASS1) |  |
| Cyp27a1 | Ectonucleotide pyrophosphatase/phosphodiesterase 4 | Cyp27a1 (CYP27A1) |  |
| Gbl1 | Galactosidase, beta 1-like | Gbl1 (GBL1) |  |
| Has2 | Hyaluronan synthase 2 | Has2 (HAS2) |  |
| Mgmt | O-6-methylguanine-DNA methyltransferase | Mgmt (MGMT) |  |
| **Proteins with no known function/hypothetical proteins** |
| Apol9a | Apolipoprotein L 9a | Apol9a (APOL9A) |  |
| Ascc3 | Activating signal cointegrator 1 complex subunit 3 | Ascc3 (ASCC3) |  |
| Ass1 | Activating transcription factor 3 | Ass1 (ASS1) |  |
| Cyp27a1 | Ectonucleotide pyrophosphatase/phosphodiesterase 4 | Cyp27a1 (CYP27A1) |  |
| Has2 | Hyaluronan synthase 2 | Has2 (HAS2) |  |
| Mgmt | O-6-methylguanine-DNA methyltransferase | Mgmt (MGMT) |  |
| Nqo1 | NAD(P)H dehydrogenase, quinone 1 | Nqo1 (NQO1) |  |
| Rbp2 | Retinol binding protein 2, cellular | Rbp2 (RBP2) |  |
| Slc28a2 | Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2 | Slc28a2 (SLC28A2) |  |
| Steap1 | Six transmembrane epithelial antigen of the prostate 1 | Steap1 (STEAP1) |  |
| Steap1 | Six transmembrane epithelial antigen of the prostate 1 | Steap1 (STEAP1) |  |
| Tcf21 | Transcription factor 21 | Tcf21 (TCF21) |  |
| **Proteins with no known function/hypothetical proteins** |
| Apol9a | Apolipoprotein L 9a | Apol9a (APOL9A) |  |
| Ascc3 | Activating signal cointegrator 1 complex subunit 3 | Ascc3 (ASCC3) |  |
| Ass1 | Activating transcription factor 3 | Ass1 (ASS1) |  |
| Cyp27a1 | Ectonucleotide pyrophosphatase/phosphodiesterase 4 | Cyp27a1 (CYP27A1) |  |
| Has2 | Hyaluronan synthase 2 | Has2 (HAS2) |  |
| Mgmt | O-6-methylguanine-DNA methyltransferase | Mgmt (MGMT) |  |
| Nqo1 | NAD(P)H dehydrogenase, quinone 1 | Nqo1 (NQO1) |  |
| Rbp2 | Retinol binding protein 2, cellular | Rbp2 (RBP2) |  |
| Slc28a2 | Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2 | Slc28a2 (SLC28A2) |  |
| Steap1 | Six transmembrane epithelial antigen of the prostate 1 | Steap1 (STEAP1) |  |
| Tcf21 | Transcription factor 21 | Tcf21 (TCF21) |  |

**Proteins with no known function/hypothetical proteins**

*Proteins with no known function/hypothetical proteins*
| Gene ID   | Gene Name          | Description                                                                 | Value | Score |
|----------|--------------------|------------------------------------------------------------------------------|-------|-------|
| 7073915  | Rnf213             | Ring finger protein 213                                                       | 51    | 2.54  |
| 7073928  | Rnf213             | Ring finger protein 213                                                       | 65    | 1.99  |
| 7094771  | Rtp4/Ctdsp1        | Receptor (chemosensory) transporter protein 4 | CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1 | 139   | 11.46 |
| 7070419  | Sfn3               | Schlafen 3                                                                    | 131   | 5.04  |
| 7070393  | Sfn5               | Schlafen family member 5                                                      | 98    | 1.85  |
| 7072336  | Tmem106a           | Transmembrane protein 106A                                                    | 78    | 1.85  |
| 7252491  | Tmem140            | Transmembrane protein 140                                                     | 206   | 1.83  |
| 7047575  | Unknown            | Unknown                                                                       | 55    | 1.57  |
| 7088625  | Unknown            | Unknown                                                                       | 88    | 2.72  |
| 7116103  | Unknown            | Unknown                                                                       | 71    | 2.16  |
| 7129386  | 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  | Cd38               | CD38 molecule                                                                 | 608   | 0.61  |
| 7191192  | Fam134b            | Family with sequence similarity 134, member B                                 | 1202  | 0.62  |
| 7372873  | Fam70a             | Family with sequence similarity 70, member A                                  | 534   | 0.51  |
| 7133474  | Lcp1               | Lymphocyte cytosolic protein 1                                                | 545   | 0.57  |
| 7164764  | LOC680097          | Similar to germinal histone H4 gene                                           | 6612  | 0.58  |
| 7367891  | LOC680166          | Unknown                                                                       | 204   | 0.61  |
| 7157871  | LOC682649          | Similar to Histone H2A type 1                                                | 647   | 0.53  |
| 7260779  | Lrrc17             | Leucine rich repeat containing 17                                             | 298   | 0.38  |
| 7060488  | Ms4a6b/11          | Membrane-spanning 4-domains, subfamily A, member 6B/11                        | 215   | 0.52  |
| 7337370  | Ns5atp9            | NS5A (hepatitis C virus) transactivated protein 9                             | 307   | 0.66  |
| 7035206  | Olfml1             | Olfactomedin-like 1                                                           | 355   | 0.48  |
| 7328472  | Tmem71             | 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  |
Table 2  Transcripts upregulated by ET-1 that are regulated by Atf3 but are unaffected by FLAG virus infection *, IEG; **, non-IEG

| Transcript Cluster | Gene symbol | Raw values | Control | ET-1 |
|--------------------|-------------|------------|---------|------|
|                    |             | No virus   | FLAG    | AS-Atf3 | No virus | FLAG | AS-Atf3 |
| **AS Atf3 enhances response to ET-1** |
| Cluster A1         |             |            |         |       |         |      |      |
|                     |             | 7169197    | 289    | 1 0.95 | 1.02  | 1.29  | 1.24 | 4.73 |
|                     |             | 7222466    | 85     | 1 0.92 | 0.87  | 1.32  | 1.59 | 2.90 |
|                     |             | 7288744    | 425    | 1 0.93 | 1.44  | 1.21  | 1.31 | 2.10 |
|                     |             | 7320920    | 64     | 1 0.95 | 1.12  | 3.78  | 4.29 | 6.63 |
| Cluster A2         |             | 7308330    | 152    | 1 0.94 | 2.61  | 2.72  | 2.22 | 3.56 |
|                     |             | 7185572    | 154    | 1 1.05 | 1.94  | 1.56  | 1.41 | 2.23 |
|                     |             | 7231595    | 142    | 1 1.01 | 2.04  | 0.96  | 1.08 | 3.03 |
|                     |             | 7114850    | 130    | 1 0.98 | 2.28  | 2.00  | 1.60 | 2.66 |
|                     |             | 7265600    | 854    | 1 1.00 | 2.94  | 1.98  | 1.93 | 3.09 |
|                     |             | 7243216    | 52     | 1 1.13 | 1.81  | 1.83  | 1.49 | 2.68 |
|                     |             | 7179800    | 368    | 1 0.96 | 2.16  | 1.67  | 1.79 | 4.56 |
| AS Atf3 inhibits response to ET-1 |
| Cluster B1         |             | 7260283    | 195    | 1 0.95 | 2.86  | 1.93  | 1.62 | 3.39 |
|                     |             | 7124933    | 500    | 1 1.13 | 2.22  | 1.73  | 1.91 | 3.02 |
|                     |             | 7096358    | 88     | 1 1.10 | 2.80  | 2.18  | 1.74 | 3.54 |
| Cluster B2         |             | 7115195    | 181    | 1 1.06 | 2.77  | 1.79  | 1.67 | 2.99 |

| **AS Atf3 inhibits response to ET-1** |
| Cluster B1         |             | 7123129    | 127    | 1 1.07 | 1.20  | 5.63  | 7.01 | 2.09 |
|                     |             | 7043230    | 178    | 1 0.93 | 0.87  | 3.03  | 2.99 | 1.54 |
|                     |             | 7345585    | 459    | 1 0.87 | 0.93  | 2.94  | 3.05 | 1.98 |
|                     |             | 7187674    | 191    | 1 0.94 | 0.82  | 1.66  | 1.92 | 1.14 |
|                     |             | 7202670    | 527    | 1 1.00 | 0.72  | 1.62  | 1.71 | 1.04 |
|                     |             | 7150663    | 116    | 1 0.98 | 0.91  | 1.66  | 1.34 | 0.88 |
|                     |             | 7235566    | 62     | 1 1.06 | 1.19  | 3.20  | 3.62 | 1.48 |
|                     |             | 7204067    | 863    | 1 1.12 | 1.19  | 1.76  | 1.90 | 1.24 |
|                     |             | 7104906    | 127    | 1 0.90 | 0.88  | 2.36  | 1.41 | 0.89 |
|                     |             | 7214260    | 77     | 1 1.03 | 1.04  | 4.42  | 3.27 | 1.28 |
|                     |             | 7141508    | 513    | 1 0.99 | 1.20  | 2.87  | 2.79 | 1.74 |
|                     |             | 7297329    | 126    | 1 1.14 | 0.96  | 2.83  | 3.77 | 2.24 |
|                     |             | 7350922    | 217    | 1 1.02 | 1.40  | 4.09  | 3.53 | 1.70 |
|                     |             | 7297247    | 121    | 1 1.12 | 1.14  | 2.21  | 2.22 | 1.41 |
Table 3 Transcripts unaffected by FLAG virus infection that are upregulated 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   | FLAG    | AS Atf3 | No virus | FLAG | AS Atf3 |
| Cluster C          |             |            |         |       |         |      |       |
| 7305953            | Arl4a       | 482        | 1       | 1.07  | 1.69    | 1.60 | 1.99 | 2.21 |
| 7301400            | Bdkrb2**    | 104        | 1       | 1.09  | 1.77    | 1.74 | 1.98 | 2.21 |
| 7111272            | Btg2*       | 311        | 1       | 0.93  | 1.87    | 3.66 | 4.22 | 3.28 |
| 7209338            | Ccn1**      | 249        | 1       | 0.99  | 1.77    | 1.69 | 1.54 | 2.47 |
| 7045696            | Cnkr3       | 166        | 1       | 1.14  | 1.96    | 1.61 | 1.63 | 2.21 |
| 7350918            | Csnr1p1     | 146        | 1       | 1.17  | 2.42    | 3.63 | 3.82 | 4.59 |
| 7044959            | Ctgf*       | 342        | 1       | 0.91  | 3.04    | 3.85 | 4.13 | 5.22 |
| 7215359            | Cyr61**     | 407        | 1       | 1.04  | 1.74    | 5.22 | 5.40 | 6.22 |
| 7143265            | Eaf1        | 130        | 1       | 1.13  | 1.87    | 1.56 | 1.57 | 2.12 |
| 7163221            | Edn1*       | 71         | 1       | 1.11  | 1.78    | 1.19 | 1.26 | 2.42 |
| 7147985            | Efnb2**     | 71         | 1       | 0.99  | 1.74    | 1.42 | 1.45 | 2.15 |
| 7281135            | Ephb2*      | 90         | 1       | 1.16  | 3.52    | 2.89 | 3.29 | 4.32 |
| 7297136            | Fam110c     | 55         | 1       | 1.09  | 1.92    | 3.70 | 4.75 | 3.76 |
| 7242382            | Fjx1        | 149        | 1       | 1.07  | 1.74    | 1.74 | 1.71 | 2.10 |
| 7252051            | Flnc**      | 143        | 1       | 0.94  | 1.47    | 2.86 | 2.31 | 1.80 |
| 7162363            | Gadd45g*    | 357        | 1       | 0.99  | 3.33    | 4.72 | 4.19 | 5.92 |
| 7174562            | Hbegl*      | 100        | 1       | 0.88  | 2.73    | 3.90 | 4.33 | 3.84 |
| 7193413            | Hspa4l      | 63         | 1       | 0.93  | 2.29    | 1.94 | 1.48 | 2.00 |
| 7165193            | Inhba**     | 88         | 1       | 1.09  | 2.09    | 6.95 | 6.30 | 6.29 |
| 7257290            | Lmcd1*      | 283        | 1       | 1.17  | 4.41    | 5.84 | 6.64 | 5.84 |
| 7100653            | Mafk*       | 187        | 1       | 1.00  | 2.06    | 2.23 | 2.25 | 2.36 |
| 7068314            | Map2k3**    | 106        | 1       | 1.00  | 1.65    | 1.91 | 1.78 | 1.96 |
| 7194351            | Mei1/Tsc22d2| 149        | 1       | 0.99  | 1.84    | 2.45 | 2.43 | 2.43 |
| 7317471            | Myc*        | 156        | 1       | 1.02  | 2.04    | 2.28 | 2.34 | 2.92 |
| 7155813            | Nfil3*      | 67         | 1       | 1.04  | 2.71    | 2.57 | 2.83 | 3.71 |
| 7189518            | Plik2*      | 1457       | 1       | 1.15  | 2.79    | 2.14 | 2.65 | 3.58 |
| 7051029            | Pp1r15a     | 115        | 1       | 1.02  | 1.93    | 1.57 | 1.57 | 2.26 |
| 7360736            | Ptp4a1      | 638        | 1       | 1.05  | 2.14    | 2.39 | 2.27 | 2.36 |
| 7048738            | PVR*        | 430        | 1       | 1.16  | 2.58    | 3.64 | 3.77 | 3.48 |
| 7340175            | Rassf1*     | 163        | 1       | 0.99  | 1.90    | 1.62 | 1.68 | 2.04 |
| 7091811            | Rcan1       | 2101       | 1       | 1.12  | 1.77    | 1.90 | 1.95 | 1.95 |
| 7331581            | Rnd1*       | 203        | 1       | 1.20  | 3.24    | 4.78 | 4.76 | 3.58 |
| 7238766            | Rnd3*       | 311        | 1       | 1.01  | 1.96    | 1.82 | 1.56 | 2.00 |
| 7029999            | Sertad1*    | 217        | 1       | 1.09  | 1.86    | 1.95 | 2.50 | 2.39 |
| 7279509            | Stik40      | 144        | 1       | 1.00  | 1.64    | 1.62 | 1.57 | 1.75 |
| 7075088            | Tnrsf12a*   | 750        | 1       | 1.09  | 2.75    | 3.17 | 3.26 | 3.77 |
| 7106964            | Tnrsf18     | 80         | 1       | 0.87  | 2.02    | 9.32 | 6.92 | 9.29 |
| 7199859            | Tspan5**    | 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            | Vgll3       | 133        | 1       | 0.96  | 1.59    | 2.87 | 2.52 | 2.78 |
| 7228236            | Xirp2       | 124        | 1       | 0.93  | 2.14    | 3.84 | 2.73 | 2.17 |
Table 4  Transcripts affected by FLAG virus infection that are upregulated by ET-1 and regulated by Atf3.

| Transcript Cluster | Gene symbol | Raw values | Control | ET-1 |
|--------------------|-------------|------------|---------|------|
|                    |             |            | No virus| FLAG | AS Atf3 | No virus| FLAG | AS Atf3 |
| AS Atf3 enhances response to ET-1
Cluster D            |             |            |         |      |         |         |      |         |
| 7120521            | Lif *        | 82         | 1       | 1.75 | 3.70    | 2.88    | 3.62 | 7.30    |
| 7106132            | Ptgs2 *      | 136        | 1       | 1.92 | 3.93    | 4.94    | 4.44 | 9.84    |
| 7237652            | Slc25a25 *   | 94         | 1       | 1.22 | 1.71    | 1.41    | 1.64 | 2.19    |
| 7028549            | Il11         | 143        | 1       | 1.20 | 1.74    | 2.88    | 2.82 | 3.87    |
| 7284153            | Ripk2 **     | 238        | 1       | 1.31 | 2.79    | 2.53    | 2.72 | 3.58    |
| 7261019            | Sema3c       | 208        | 1       | 1.22 | 2.16    | 1.67    | 1.80 | 2.21    |
| 7035407            | Zfland2a *   | 303        | 1       | 1.21 | 1.89    | 1.74    | 2.23 | 2.26    |
| 7096947            | Lif *        | 288        | 1       | 1.23 | 1.60    | 1.73    | 2.17 | 2.39    |

AS Atf3 inhibits response to ET-1
Cluster E            |             |            |         |      |         |         |      |         |
| 7259100            | Apold1      | 533        | 1       | 0.82 | 0.63    | 1.64    | 1.43 | 1.02    |
| 7105894            | B3galt2     | 505        | 1       | 0.79 | 1.12    | 2.31    | 1.97 | 1.07    |
| 7051386            | Csrp3       | 1062       | 1       | 0.75 | 0.72    | 1.53    | 1.37 | 0.72    |
| 7084788            | Socs3       | 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 5  Transcripts that are downregulated by ET-1 and regulated by Atf3

| Transcript Cluster | Gene symbol | Raw values | Control | ET-1 |
|--------------------|-------------|------------|---------|------|
|                    |             |            | No virus| FLAG | AS Atf3 | No virus| FLAG | AS Atf3 |
| AS Atf3 enhances response to ET-1
Cluster F            |             |            |         |      |         |         |      |         |
| 7279127            | Cited4      | 231        | 1       | 0.91 | 0.48    | 0.52    | 0.59 | 0.47    |
| 7180333            | Ednra       | 2175       | 1       | 0.99 | 0.60    | 0.58    | 0.64 | 0.41    |
| 7115625            | G0s2        | 2545       | 1       | 0.86 | 0.49    | 0.30    | 0.26 | 0.12    |
| 7190436            | Lifr        | 390        | 1       | 0.88 | 0.55    | 0.60    | 0.54 | 0.37    |
| 7269707            | Ntf3        | 250        | 1       | 1.18 | 0.54    | 0.52    | 0.65 | 0.37    |

AS Atf3 inhibits response to ET-1
Cluster G            |             |            |         |      |         |         |      |         |
| 7222734            | Ddit4       | 1553       | 1       | 1.16 | 2.04    | 0.46    | 0.55 | 0.79    |
| 7120384            | Pik3ip1     | 339        | 1       | 0.83 | 0.57    | 0.29    | 0.34 | 0.46    |
| 7346730            | Smad6       | 266        | 1       | 1.05 | 1.41    | 0.50    | 0.57 | 1.03    |
| 7197113            | Txnip       | 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    |
Table 6 Primers used for qPCR and sqPCR

Nucleotide positions in transcripts are shown in parentheses for each primer. mRNA sequences (gene symbols are given with accession numbers in parentheses) were from the Rat Genome Database viewed at [http://www.ncbi.nlm.nih.gov/entrez](http://www.ncbi.nlm.nih.gov/entrez).

| Gene symbol (accession no.) | Size (bp) | Forward primer | Reverse primer |
|-----------------------------|-----------|----------------|---------------|
| **qPCR Primers**            |           |                |               |
| **Atf3** (NM_012912.1)      | 108       | TCGCCATCCAGAACAAGCA (140-158) | GGGCCACCTCAGACTTGTTG (229-247) |
| **Egr1** (NM_012551.2)      | 98        | CTACGAGCACCTGACCAAGAGCT (204-227) | GCAACGGGGTAGGTTGCTTTG (283-301) |
| **Gapdh** (NM_017008.3)     | 93        | CCAAGGTCAATCCAGAACAACTT (476-497) | AGGGGCGATCCACAGCTTT (550-568) |
| **Ptgs2** (NM_017232.3)     | 90        | GAAGAACTTACAGGAGAAAGAAATGG (1393-1420) | CAGCACGGGCGGAGTACGTT (1464-1482) |
| **Dusp1** (NM_053769.3)     | 62        | GCGCGCTCCACTCAAGTC (337-354) | GGGCAGGAAGCGGAAAAC (381-398) |
| **Dusp5** (NM_133578.1)     | 70        | CGACATTAGCTCCCACCTTCA (882-903) | AAGGACCTTGGCCTCCCTTCC (934-953) |
| **Areg** (NM_017123.1)      | 108       | CTGCTGGTCTTAGGGCAGTC (218-237) | CAGAAGTCCCAGAGACTGTG (306-325) |
| **Il6** (NM_012589.1)       | 157       | GAGTTGTGCAATGGCAATTC (202-221) | ACTCCAGAAGAAGACACGAG (339-358) |
| **Il1f1** (NM_013037.1)     | 100       | GCCCTTCATCTGGGCTACACT (68-88) | GCAATGGCGAGGAAGGTAAAC (147-167) |
| **sqPCR PRIMERS**           |           |                |               |
| **Atf3** (NM_012912.1)      | 331       | GCTGCCAGTGCTCCAGAACAAG (298-318) | CAGTTTCCAATGGCTTCAGG (608-628) |
| **Gapdh** (NM_017008.3)     | 452       | ACCACAGCTCATGCGCATC (520-539) | TCCACCCACTGTGTCTGTA (952-971) |
Supplementary Methods - Mathematical Model

1 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 6.

The phosphorylation of MKK by ET-1 is denoted by

\[ \text{ET-1} + \text{M KK} \xrightarrow{k_1} \text{ET-1} + \text{M KK-P}, \]

which subsequently phosphorylates the unphosphorylated ERK

\[ \text{M KK-P} + \text{ERK} \xrightarrow{k_2} \text{M KK-P} + \text{ERK-P}. \]

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

\[ \text{ERK-P} + \text{DNA_{Egr1}} \xrightarrow{k_3} \text{ERK-P} \cdot \text{DNA_{Egr1}} \xrightarrow{k_4} \text{mRNA_{Egr1}}, \]

and

\[ \text{ERK-P} + \text{DNA_{Atf3}} \xrightarrow{k_5} \text{ERK-P} \cdot \text{DNA_{Atf3}} \xrightarrow{k_6} \text{mRNA_{Atf3}}, \]

which are both degraded

\[ \text{mRNA_{Egr1}} \xrightarrow{d_1} \phi, \quad \text{mRNA_{Atf3}} \xrightarrow{d_2} \phi. \]

Here \( \cdot \) denotes a complex and \( \phi \) the degraded mRNA.

The suppression of Egr1 mRNA transcription by Atf3 is described by

\[ \text{ERK-P} \cdot \text{DNA_{Egr1}} + \text{Atf3} \xrightarrow{k_7} \text{Atf3} \cdot \text{DNA_{Egr1}}, \]

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

\[ \text{mRNA_{Atf3}} \xrightarrow{k_8} \text{Atf3} \quad \text{and} \quad \text{Atf3} \xrightarrow{d_3} \phi_P, \]

respectively, where \( \phi_P \) denotes degraded protein. In this work we do not explicitly account for the degraded mRNAs or Atf3 protein.
2 Mathematical Model

The Law of Mass Action (6) was applied to equations (1)-(6). This led to the following system of nonlinear ordinary differential equations

\[
\frac{dm}{dt} = -k_1 e_t m, \\
\frac{dm_P}{dt} = k_1 e_t m, \\
\frac{dE}{dt} = -k_2 m_P E, \\
\frac{dE_P}{dt} = k_2 m_P E - k_3 E_P D_E + k_{-3} E - k_5 E_P D_A + k_{-5} T_A, \\
\frac{dD_E}{dt} = -k_3 E_P D_E + k_{-3} E, \\
\frac{dT_E}{dt} = k_3 E_P D_E - k_{-3} E - k_7 T_E A, \\
\frac{dM_E}{dt} = k_4 T_E - d_1 M_E, \\
\frac{dD_A}{dt} = -k_5 E_P D_A + k_{-5} T_A, \\
\frac{dT_A}{dt} = k_5 E_P D_A - k_{-5} T_A, \\
\frac{dM_A}{dt} = k_6 T_A - d_2 M_A, \\
\frac{dA}{dt} = k_8 M_A - k_7 T_E A - d_3 A, \\
\frac{dS}{dt} = k_7 T_E A.
\]

Each of the variables is defined as follows: \(e_T\) represents the concentration of ET-1 (denoted \(e_T=[\text{ET-1}]\)), \(m=[\text{MKK}]\), \(m_P=[\text{MKK-P}]\), \(E=[\text{ERK}]\), \(E_P=[\text{ERK-P}]\), \(D_E=[\text{DNA}_E\text{gr1}]\), \(D_A=[\text{DNA}_A\text{tf3}]\), \(T_E=\text{ERK-P-DNA}_{E\text{gr1}}\), \(T_A=\text{ERK-P-DNA}_{A\text{tf3}}\), \(M_E=[\text{mRNA}_{E\text{gr1}}]\), \(M_A=[\text{mRNA}_{A\text{tf3}}]\), \(S=[\text{Atf3-DNA}_{E\text{gr1}}]\) and \(A=[\text{Atf3}]\). Here 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

\[
m = m_0, \quad m_P = 0, \quad E = E_0, \quad E_P = 0, \quad D_E = D_{E_0}, \quad T_E = 0, \\
M_E = M_{E_0}, \quad D_A = D_{A_0}, \quad T_A = 0, \quad M_A = 0, \quad A = 0 \quad \text{and} \quad S = 0.
\]
The governing system of equations can be simplified as follows. Addition of equations (8) and (9), integration with respect to time and application of the respective initial conditions yields the conservation relation
\[ m + m_P = m_0. \] (21)
Substituting for \( m \) into equation (9), integrating and applying the initial condition yields
\[ m_P(t) = m_0 \left( 1 - e^{-k_{1c}t} \right). \] (22)
Addition of equations (12), (13) and (19), integration with respect to \( t \) and application of the initial conditions yields
\[ D_E + T_E + S = D_{E0}. \] (23)
Likewise for equations (15) and (16)
\[ D_A + T_A = D_{A0}. \] (24)
Assuming equation (16) is quasi-steady and substituting for \( D_A \) using equation (24) leads to
\[ T_A \simeq \frac{D_{A0}E_P}{E_P + K_5}, \] (25)
where \( K_5 = k_{-5}/k_5 \).
Bringing all these results together gives
\[ m_P(t) = m_0 \left( 1 - e^{-k_{1c}t} \right), \] (26)
\[ \frac{dE}{dt} = -k_2m_PE, \] (27)
\[ \frac{dE_P}{dt} = k_2m_PE - k_3E_P(D_{E0} - T_E - S) + k_{-3}T_E, \] (28)
\[ \frac{dT_E}{dt} = k_3E_P(D_{E0} - T_E - S) - k_{-3}T_E - k_7T_EA, \] (29)
\[ \frac{dM_E}{dt} = k_4T_E - d_1M_E, \] (30)
\[ \frac{dM_A}{dt} = \frac{k_5^*E_P}{E_P + K_5} - d_2M_A, \] (31)
\[ \frac{dA}{dt} = k_8M_A - k_7T_EA - d_3A, \] (32)
\[ \frac{dS}{dt} = k_7T_EA, \] (33)
with the initial conditions
\[ E = E_0, \quad E_P = 0, \quad T_E = 0, \quad M_E = M_{E0}, \quad M_A = 0, \quad A = 0 \quad \text{and} \quad S = 0, \] (34)
where \( k_5^* = k_6D_{A0} \). When Egr1 transcription is not suppressed by Atf3 protein we have \( k_7 = 0 \).
3 Parameterisation

The mathematical model has been informed with data available within the literature, from our own previous studies as well as work undertaken here. A complete list of the parameter values used can be found in Table 6. 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 minutes. The time course for activation of MKK was determined by immunoblotting with antibodies to phosphorylated (i.e. activated) MKK using primary antibodies from Cell Signaling Technology Inc. A representative blot is shown in Figure 1. 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 minutes. 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 to total MKK were from Cell Signaling Technology Inc. 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).

\[ k_1 e_t = \frac{1}{120s} = 8.30 \times 10^{-3}s^{-1}. \]

\[ k_2 = \frac{1}{60s} = 1.67 \times 10^{-2}s^{-1}. \]

The total ERK concentration is 130nM such that

\[ k_2 = 1.28 \times 10^5(Ms)^{-1}. \]

\[ 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 NCBI. For Atf3 (Gene ID: 11910), this gives a total gene length of
13038 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 b/s to greater than 800 b/s (1; 5). Thus to transcribe one molecule of mRNA from one gene, assuming a rate of 55 bases per second, takes

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

Per gene this equates to \(1.39 \times 10^{-2}\) molecules mRNA s\(^{-1}\). Since a cell contains two genes, we have \(2.78 \times 10^{-2}\) molecules of Egr1 mRNA being synthesized per cell per second. Taking the cell volume of 6.7pl we obtain

\[
k_4^* = \frac{2.78 \times 10^{-2} \text{ molecules s}^{-1}}{6.710^{-9} \text{ ml}} = 4.15 \times 10^{5} \text{ molecules ml}^{-1} \text{s}^{-1} = 6.89 \times 10^{-16} \text{ Ms}^{-1}. \quad (35)
\]

We can undertake a similar calculation for Atf3 transcription to obtain

\[
k_6^* = 5.15 \times 10^{-15} \text{ Ms}^{-1}.
\]

\(k_8 - \text{Atf3 translation rate:}\) The rate of translation of Atf3 was estimated on the basis of scanning of the 5’ untranslated region at a rate of 6 nucleotides per second (11), translation of the coding sequence at a rate of 20 amino acids per second (N.B. the reported rate of translation is in the range of 4 - 20 amino acids per second (10; 12) and we presume translation of IEGs is efficient) with 5 ribosomes attached simultaneously to each mRNA (N.B. the predicted occupancy is 1 ribosome per 32 amino acids)(7).

\(K_5\) and \(k_3, k_{-3} - \text{ERK-P dissociation rates for Egr1 and Atf3 DNA:}\) 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 0.5µM (the dissociation rate for Elk1 is 0.25µM; that for Fos is 1µM) (2). Given that \(K_3 = k_{-3}/k_3\) we use an initial estimate of \(k_{-3} = 5 \times 10^{-2}\) s to obtain \(k_3 = 1 \times 10^5\) (Ms\(^{-1}\).

\(d_1, d_2, d_3 - \text{Degradation rates of Egr1 mRNA, Atf3 mRNA and Atf3 protein:}\) To estimate the half-life of Egr1 and Atf3 mRNA, cardiomycocytes were exposed to ET-1 for 30 min then incubated without or with actinomycin D (4µM, 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 1, giving a half-life of 13 min for Egr1 and 49 min for Atf3. The rate of degradation is defined by

\[
d = \frac{\ln 2}{t_{1/2}}.
\]
| Parameter | Definition | Value         |
|-----------|------------|---------------|
| $m_0$     | Total MKK. | 130nM         |
| $E_0$     | Total ERK. | 130nM         |
| $M_{E0}$  | Initial Egr1 mRNA concentration. | 1pM           |
| $D_{e0}$  | Egr1 DNA concentration. | 33.2pM        |
| $k_{1e}$  | Rate of MKK activation by ET-1. | $8.30 \times 10^{-3}$ s$^{-1}$ |
| $k_2$     | Rate of ERK activation by MKK. | $1.28 \times 10^5$ (Ms)$^{-1}$ |
| $k_3$     | Rate of ERK-P activation of Egr1 DNA. | $1.00 \times 10^5$ (Ms)$^{-1}$ |
| $k_{-3}$  | Rate of ERK-P reverse activation of Egr1 DNA. | $5.00 \times 10^{-2}$ (Ms)$^{-1}$ |
| $k_4$     | Egr1 mRNA transcription rate. | $6.89 \times 10^{-15}$ M/s |
| $k_5$     | Atf3 mRNA transcription rate. | $1.03 \times 10^{-15}$ M/s |
| $k_7$     | Atf3 suppression rate. | To be determined. |
| $k_8$     | Atf3 translation rate. | 0.25 s$^{-1}$ |
| $K_5$     | ERK-P and Atf3 DNA dissociation rate. | $0.5 \times 10^{-6}$ M |
| $d_1$     | Degradation rate of Atf3 mRNA. | $8.89 \times 10^{-4}$ s$^{-1}$ |
| $d_2$     | Degradation rate of Egr1 mRNA. | $2.36 \times 10^{-4}$ s$^{-1}$ |
| $d_3$     | Degradation rate of Atf3 protein. | $2.36 \times 10^{-4}$ s$^{-1}$ |

Table 6: Model parameter values.

which leads to

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

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

**Egr1 DNA concentration:** We assume there are 2 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 66pF (3; 8) and the capacitance of a neonatal myocyte is 13pF (8). This gives 6.7pl per cell leading to concentration of 33.2pM.

## 4 Results

The governing system of nonlinear ordinary differential equations (ODEs) (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 equation (26).

Using the parameter values detailed in Table 6 we found the 20-fold change in Egr1 mRNA determined experimentally could not be re-produced using these values (using an initial estimate of $k_7 = 1 \times 10^5$ (Ms)$^{-1}$). As such we undertook a sensitivity analysis in which we varied the rates of Egr1 and Atf3 mRNA transcription ($k_4, k_5$), ERK-P reverse
Figure 1: 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 (4µM) 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.
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 by (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 we obtained for the range of values $1.00 \times 10^5 (\text{Ms})^{-1} \leq k_7 \leq 6 \times 10^5 (\text{Ms})^{-1}$.

References

[1] Ben-Ari, Y. et al. The life of an mRNA in space and time. *J. Cell Sci.* 123, 1761-1774 (2010).
[2] Burkhard, K.A., Chen, F., & Shapiro, P. Quantitative analysis of ERK2 interactions with substrate proteins: roles for kinase docking domains and activity in determining binding affinity. *J. Biol. Chem.* 286, 2477-2485 (2011).
[3] Cerbai, E., Pino, R., Sartiani, L., & Mugelli, A. Influence of postnatal-development on I(f) occurrence and properties in neonatal rat ventricular myocytes. *Cardiovasc. Res.* 42, 416-423 (1999).
[4] Fujioka, A. et al. Dynamics of the Ras/ERK MAPK cascade as monitored by fluorescent probes. *J. Biol. Chem.* 281, 8917-8926 (2006).
[5] Maiuri, P. et al. Fast transcription rates of RNA polymerase II in human cells. *EMBO Rep.* (2011).
[6] J.D. Murray, Mathematical Biology, Springer Verlag, 2nd ed., 1993.
[7] Qin, X., Ahn, S., Speed, T.P., & Rubin, G.M. Global analyses of mRNA translational control during early *Drosophila* embryogenesis. *Genome Biol.* 8, R63 (2007).
[8] Satoh, H., Delbridge, L.M., Blatter, L.A., & Bers, D.M. Surface:volume relationship in cardiac myocytes studied with confocal microscopy and membrane capacitance measurements: species-dependence and developmental effects. *Biophys. J.* 70, 1494-1504 (1996).
[9] Sugden, P.H. et al. Monophosphothreonyl extracellular signal-regulated kinases 1 and 2 (ERK1/2) are formed endogenously in intact cardiac myocytes and are enzymically active. *Cell. Signal.* 23, 468-477 (2011).

[10] Tinoco, I., Jr. & Wen, J.D. Simulation and analysis of single-ribosome translation. *Phys. Biol.* 6, 025006 (2009).

[11] Vassilenko, K.S., Alekhina, O.M., Dmitriev, S.E., Shatsky, I.N., & Spirin, A.S. Unidirectional constant rate motion of the ribosomal scanning particle during eukaryotic translation initiation. *Nucleic Acids Res.* 39, 5555-5567 (2011).

[12] Wohlgemuth, I., Pohl, C., & Rodnina, M.V. Optimization of speed and accuracy of decoding in translation. *EMBO J.* 29, 3701-3709 (2010).