Microarray analysis uncovers the induction of the pro-apoptotic BH3-only protein Bim in multiple models of glucocorticoid induced apoptosis

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Running Title: Dexamethasone induces expression of the BH3-only protein Bim
Summary

Despite being one of the earliest recognized and most clinically relevant forms of apoptosis, little is known about the transcriptional events that mediate glucocorticoid induced apoptosis. Therefore, we used oligonucleotide microarrays to identify the pattern of dexamethasone induced changes in gene expression in two well characterized models of glucocorticoid induced apoptosis, the murine lymphoma cell lines S49.A2 and WEHI7.2. Dexamethasone treatment induced a diverse set of gene changes that evolved over a 24 hour period preceding the onset of cell death. These include previously reported changes in expression of genes regulating pro-survival signals mediated by c-Myc and NFκB. Unexpectedly, we discovered that glucocorticoid treatment increases expression of the gene encoding bim, a BH3-only member of the Bcl-2 family capable of directly activating the apoptotic cascade. Induction of Bim was confirmed by immunoblotting, not only in S49.A2 and WEHI7.2 cells, but also in the human leukemia cell line CEM-C7, and in primary murine thymocytes. All three prototypical isoforms of Bim: BimEL, BimL, and BimS, were induced by dexamethasone. Because elevated expression of Bim initiates the execution phase of cell death, this report that Bim is induced by dexamethasone provides novel insight into the mechanism through which glucocorticoid mediated changes in gene expression induce apoptosis in lymphoid cells.

Introduction

The ability of glucocorticoid hormones to induce apoptosis in leukemia and lymphoma cells has been utilized by physicians for nearly half a century (1,2). Today, glucocorticoids are common components in many chemotherapeutic protocols for lymphoid malignancies, including multiple myeloma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and non-Hodgkin’s lymphoma (3-6). In fact, glucocorticoid therapy is essential for the successful treatment of childhood acute lymphoblastic leukemia (5).

Apoptosis is an orderly process of cell death typified by protease and endonuclease activation, and is morphologically characterized by condensed chromatin, a reduction of cell volume, and plasma
membrane blebs (7). Whether a cell will initiate the apoptotic cascade depends on the relative expression of the pro- and anti-apoptotic members of the Bcl-2 family (8). Members of the Bcl-2 family of proteins are all related by the conservation of at least one Bcl-2 Homology (BH) domain (9). Bcl-2 and Bcl-xL contain four BH domains and are capable of inhibiting apoptosis. Family members, such as Bax and Bak, share multiple BH domains with the anti-apoptotic Bcl-2 proteins, however these proteins are pro-apoptotic and are required for nearly all forms of apoptosis (10). A host of pro-apoptotic proteins containing only the BH3 domain also exist within the Bcl-2 family. These include Bim, Bbc3/PUMA, Bad, and Bid (9,11). BH3-only proteins are required for the initiation of apoptosis by multiple stimuli, and have been identified in species as primitive as *C. elegans* (12). They initiate apoptosis either by inhibiting anti-apoptotic Bcl-2 proteins (12,13), or by activating pro-apoptotic proteins such as Bax and Bak (14).

Many death-inducing signals activate constitutively expressed apoptotic machinery, capable of triggering apoptosis without altering gene expression. Unlike these signals, glucocorticoid induced apoptosis requires the activation of an incompletely defined program of transcriptional regulation initiated by the glucocorticoid receptor (15). The glucocorticoid receptor belongs to the nuclear steroid hormone receptor family of zinc finger transcription factors. Upon ligand binding, the receptor dissociates from its cytosolic chaperone complex, homodimerizes, and translocates into the nucleus. Once in the nucleus, the glucocorticoid receptor can stimulate or repress gene expression directly, by binding glucocorticoid response elements (GREs) on regulatory regions of target genes, or indirectly, through protein-protein interactions with other transcription factors, including NFκB and AP-1 (16-18).

Multiple lines of evidence demonstrate that glucocorticoid receptor mediated transcriptional regulation is required for apoptosis. Glucocorticoid induced apoptosis can be blocked by cyclohexamide and actinomycin D, demonstrating a dependence on *de novo* protein and RNA synthesis (19-21). Resistance to glucocorticoid therapy in vivo often involves loss of a functional glucocorticoid receptor, and glucocorticoid receptor mutants lacking either the domains required for transactivation or transrepression are unable to induce apoptosis (22-25). Furthermore, Reichardt and colleagues have
demonstrated that binding of the glucocorticoid receptor to DNA is required for glucocorticoid induced apoptosis in thymocytes using a knock-in mouse model expressing a glucocorticoid receptor (A458T) that is unable to dimerize and cooperatively bind palindromic GREs (26).

Because glucocorticoid induced apoptosis is mediated by regulation of gene expression, much attention has centered on the ability of glucocorticoids to repress the activity of pro-survival transcription factors such as c-Myc, NFκB, and AP-1 (27-30). These studies have elucidated transcriptional targets of the glucocorticoid receptor that regulate signal transduction pathways capable of altering the balance between life and death. While these data clearly demonstrate that repressing pro-survival signals shifts the balance in favor of death, studies to date have not identified transcriptional targets capable of directly initiating the apoptotic cascade.

To better understand the transcriptional regulatory changes that promote apoptosis, we have used oligonucleotide microarrays to identify a set of glucocorticoid regulated genes common to two glucocorticoid-sensitive models of T-cell lymphoma, the S49.A2 and WEHI7.2 murine cell lines. Using this paradigm, we have discovered that bim, a pro-apoptotic BH3 only member of the Bcl-2 family, is induced following dexamethasone treatment. This is, to our knowledge, the first report that a pro-apoptotic protein, capable of directly initiating the apoptotic cascade, is induced following dexamethasone treatment.

**Experimental Procedures**

**Cell Culture**

WEHI7.2 and S49.A2 murine T-cell lymphoma lines were gifts of Drs. Diane Dowd and Roger Miesfeld, respectively. The CEM-C7 human acute T-cell leukemia line was a gift of Dr. Brad Thompson. WEHI7.2 and S49.A2 cells were cultured in Dulbecco’s modified eagle medium, supplemented with 2 mM L-glutamine, 10% bovine calf serum (HyClone), 12.5 units/mL penicillin, and 12.5 μg/mL streptomycin. CEM-C7 cells were cultured in RPMI medium supplemented with 2 mM L-glutamine,
10% fetal bovine serum (Atlanta Biologicals), 12.5 units/mL penicillin, and 12.5 µg/mL streptomycin. Unless otherwise noted, all cell culture reagents were purchased from Gibco, BRL. All experimental cultures were started at a density of 1-2x10^5 cells/mL and grown in a humidified 7% CO₂ incubator at 37 °C. For kinetic analysis, all samples were treated simultaneously, then harvested individually at the appropriate time after treatment.

**Expression Analysis**

Gene expression analysis was performed essentially as described in the Affymetrix Expression Analysis Technical Manual (31). Total RNA was harvested from both dexamethasone treated (1 µM) and vehicle control populations at each time point by Trizol (Invitrogen) extraction. Trizol cell lysates were separated into aqueous and organic phases by the addition of chloroform to a final concentration of 20% (v/v). The aqueous phase was purified and concentrated using a Qiagen RNeasy mini-column. DNA complementary to total RNA samples were reverse transcribed using Superscript reverse transcriptase (Gibco) and a T7-(dT)₂₄ primer (Operon). This cDNA was used as a template for the synthesis of biotinylated cRNA using the T7 Megascript kit from Ambion. Biotinylated cRNA probes were fragmented and hybridized to MG-U74A(v2) GeneChips (Affymetrix) using an Affymetrix GeneChip Fluidics Station 400 and standard Affymetrix protocols. Fluorescence intensities were captured with a GeneArray Scanner (Hewlett-Packard).

GeneChip image files were processed using Microarray Analysis Suite version 5.0 (Affymetrix). Probe cells displaying irregular fluorescence intensity over the area of the cell were excluded from subsequent analyses. To facilitate comparison between samples and experiments, the trimmed mean signal of each array was scaled to a target intensity of 1500. Comparative analysis between treatment and control samples for each time point was performed by the Affymetrix statistical algorithm using default parameters. To compensate for gene expression changes occurring in the control cultures over time, each treated sample was compared to a control sample that was split and harvested in parallel with the treated
population. Metric files from expression and comparison analyses were exported to Microsoft Access XP for further filtering and analysis. In this text, genes called significantly changed were those that possessed a reliably detectable signal (absolute call ≠ “Absent” and signal ≥500 in treatment or control samples for inductions or repressions, respectively) and determined by the statistical algorithm to be changed 2-fold or greater (change call ≠ “no change”, and signal >500 in treatment or control samples for inductions or repressions respectively). To increase stringency, genes meeting the above criteria were further filtered to include only those that were also changed in the same direction (change call ≠ “no change”) in at least one adjacent time point, regardless of magnitude.

Hierarchical Clustering Analysis

Genes considered significantly changed (see Expression Analysis above) were grouped according to the similarity of their expression changes over time in both cell lines. An uncentered correlation similarity matrix and complete linkage analysis were selected to cluster signal log₂ ratios using GeneCluster v.2.11 (Stanford University). Data were visualized using TreeView version 1.5 (Stanford University).

Immunoblot Analysis

Cell cultures were harvested by centrifugation, washed twice in phosphate buffered saline, and lysed in RIPA buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% sodium dodecyl sulfate, and 0.5% sodium deoxycholate) to which complete protease inhibitor tablets were added according to the manufacturers instructions (Roche). Protein concentration in cell lysates was quantified by the Bradford method. Sample volume and protein concentration in each of the samples were normalized by the addition of RIPA buffer prior to loading. Lysates were mixed with an equal volume of 2x sample loading buffer (100 mM Tris-HCl, pH 6.8, 4% sodium dodecyl sulfate, 20% glycerol, and 0.2% bromophenol blue), then boiled for 10 minutes. Proteins were resolved on 12.5% Tris-HCl SDS-PAGE gels with a 5% stacking gel, then immobilized by electrotransfer onto
polyvinylidene fluoride membranes. Non-specific protein binding was blocked prior to incubation with primary antibodies with Tris-buffered saline containing 0.1% Tween-20 and 5% non-fat dry milk. The anti-Bim polyclonal antibody was purchased from Sigma; the anti-β-actin and anti-Bcl-2 antibodies were obtained from Pharmingen. Horseradish peroxidase-conjugated goat anti-rabbit IgG and enhanced chemiluminescence substrate (Amersham) were used for antibody detection. Results are representative of at least three independent experiments.

Northern Blot Analysis

Total RNA was extracted from cultured cells using the TRIzol reagent (Invitrogen), followed by purification through an RNeasy mini-column (Qiagen). Total RNA (10 μg) was separated in a 1.0% agarose-formaldehyde gel and transferred to a GeneScreen Plus membrane (NEN Life Science Products Inc.) in 10x SSC by capillary blotting. The RNA was fixed to the membrane by cross-linking with ultraviolet light (245 nm, 30 seconds, 1200 μJoules) using a Strata-linker UV oven (Stratagene). Membranes were hybridized with a 32P-labeled bim probe prepared from the full-length bimEL cDNA (a gift from Dr. Andreas Strasser) in QuikHyb (Stratagene) at 65 °C. Membranes were subsequently washed at 65 °C twice for 15 minutes each in 2x SSC, once for 30 minutes in 2x SSC, 0.1% SDS and once for 10 minutes in 0.1x SSC, 0.1% SDS.

Thymus Isolation

C57/BL6 mice (Jackson Laboratory) were sacrificed by CO₂ asphyxiation between 6 to 12 weeks of age. Thymi were removed, rinsed in ice cold growth medium (Dulbecco’s modified eagle’s medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 12.5 units/mL penicillin, and 12.5 μg/mL streptomycin), and then dispersed through a steel wire mesh into 5 mL fresh, cold growth medium / thymus. The suspension of thymocytes was filtered through a nylon mesh to remove connective tissue. For each experiment, thymocytes were pooled from at least three mice, diluted to 2-3 x 10⁶ cells / mL in
warm growth medium, treated with dexamethasone or vehicle control, and grown in a humidified 7% CO₂ atmosphere at 37 °C.

Apoptosis Assays

Apoptosis was measured by quantification of cellular DNA content by flow cytometry. Culture samples containing 1.5x10⁶ cells were collected by centrifugation at 200 x g for five minutes, washed once with phosphate-buffered saline, then suspended in 500 µl ice-cold methanol and incubated for a minimum of five minutes at -20 °C. Methanol-fixed samples were then collected by centrifugation at 350 x g for 2 minutes, washed once with PBS, then incubated at room temperature for one hour in propidium iodide staining solution containing 50 µg/mL propidium iodide, 0.1% NP-40, 20 µg/mL RNase A, and 0.1% sodium azide in PBS. Propidium iodide fluorescence was measured using a FACScan XL flow cytometer (Coulter). Non-aggregated whole cells having a DNA content less than that of the G₁ population were scored as apoptotic. Data analysis was performed using WinList 3D version 4.0 (Verity Software House).

Results

Microarray expression analysis of glucocorticoid regulated genes identifies bim.

Oligonucleotide microarray analysis was employed to identify glucocorticoid regulated genes in the S49.A2 and WEHI7.2 murine T-cell lymphoma cell lines. Both cell lines are well studied models of glucocorticoid induced apoptosis a phenomenon that is observed 24 to 36 hours following treatment with 1 µM of the synthetic glucocorticoid dexamethasone (Figure 1). The WEHI7.2 cell line is more sensitive to steroid induced apoptosis, likely because it expresses less of the anti-apoptotic Bcl-2 protein than the S49.A2 cells (Figure 1B, inset). To determine glucocorticoid regulated changes in gene expression that occur prior to the onset of apoptosis, RNA samples were extracted from cells treated with dexamethasone or ethanol vehicle 6, 12, 18, and 24 hours after treatment. Expression profiles of both dexamethasone
treated and control populations at each time point were determined using Affymetrix MG-U74A(v2) GeneChips. Comparison of gene expression between treatment and control populations at each time point was performed using the Affymetrix Microarray Analysis Suite version 5.0.

From over 10,000 genes or expressed sequenced tags represented on the array, 284 changed significantly after dexamethasone treatment in both cell lines (Supplementary Data, Table 1, see Experimental Procedures for selection criteria). Of the 284 genes within this set, 70% were induced and 30% repressed. Protein phosphatases, major histocompatibility complex antigens, and genes involved in free-radical metabolism were the categories of dexamethasone regulated genes whose abundance was frequently altered (data not shown).

Because both cell lines undergo apoptosis in response to dexamethasone, genes that are mediators of apoptosis are likely to be changed in the same direction in both cell lines. To identify this subset of genes, we subjected our dataset containing 284 glucocorticoid regulated genes to hierarchical clustering analysis (Figure 2A). Clustering the dataset in this manner allowed the identification of three groups of genes whose expression changed similarly in both cell lines. A predominant group of genes repressed in both cell lines contained familiar targets of glucocorticoid mediated transrepression, including phosphofructokinase, a class I major histocompatibility complex antigen, and \textit{c-myc} (Figure 2, Group I). Genes whose expression was induced by glucocorticoids in both cell lines segregated into two distinct clusters according to the kinetics of their induction (Figure 2B). Two inhibitors of pro-survival signals were induced with delayed kinetics: the NF\textit{k}B inhibitor, I\textit{k}B-\textit{α}, and the regulatory p85 subunit of phosphatidylinositol 3-kinase (Figure 2, Group II). Group III, by comparison, contained genes whose expression was induced rapidly, reaching a plateau between 12 to 18 hours. On average, expression of genes within this group were increased by approximately 2-fold as early as six hours following dexamethasone treatment. This group contained a probe for the expressed sequence tag AA796690, a sequence that is homologous to the long splice variant of the \textit{Bcl-2 Interacting Mediator of Cell Death} (\textit{bim}) (Figure 2, Group III). Because Bim is capable of initiating apoptosis at the level of the...
mitochondrion, the induction of \textit{bim} is the glucocorticoid mediated transcriptional event most directly related to apoptosis and is therefore the focus of this work.

\textit{Bim is induced by dexamethasone.} Based on the hybridization signals for the AA796690 EST, the expression of \textit{bim} was induced as early as 6 hours following dexamethasone treatment and reached a maximum induction of greater than 2-fold after 24 hours in both S49.A2 and WEHI7.2 cells (Figures 2 and 3A). Northern blotting confirmed the elevated level of \textit{bim} in WEHI7.2 cells (Figure 3B). By immunoblot analysis, we observed the induction of all three prototypical isoforms, Bim\textsubscript{EL}, Bim\textsubscript{L}, and Bim\textsubscript{S}, in both S49A2 and WEHI7.2 cells (Figures 3C and D, respectively). An increase in protein expression was apparent within 24 hours of dexamethasone treatment in both cell lines. Bim was not only induced at the time dexamethasone treated cells began to undergo apoptosis, it also was induced only by doses of dexamethasone sufficient to induce apoptosis (Figures 4A and B).

\textit{The induction of bim requires de novo transcription and translation.} Since \textit{de novo} RNA and protein synthesis is required for dexamethasone induced apoptosis in thymocytes, we tested whether the induction of \textit{bim} expression similarly required \textit{de novo} transcription and translation. WEHI7.2 cells were treated with dexamethasone in the presence and absence of the transcriptional inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide. In the presence of actinomycin D, the abundance of \textit{bim} falls to barely detectable levels. Also, cycloheximide appears to block dexamethasone induced \textit{bim} expression (Figure 5A). These results suggest that \textit{de novo} transcription and translation are required for the induction of \textit{bim} expression by dexamethasone. Furthermore, in the presence of actinomycin D, dexamethasone did not induce Bim protein expression after 36 hours (Figure 5B). These data indicate that the elevation of Bim is due to either an increase in \textit{bim} transcription, or the transcriptional induction of a protein that stabilizes the \textit{bim} transcript.

\textit{Bcl-2 overexpression does not prevent the induction of Bim and reveals the induction of Bim}\textsubscript{S}. Bcl-2 overexpression inhibits glucocorticoid induced apoptosis in the WEHI7.2 cell line (32). To verify that the induction of Bim is an event that precedes apoptosis, we tested whether Bel-2 could prevent or delay its induction following dexamethasone treatment. WEHI7.2 cells stably overexpressing Bel-2 were
treated with dexamethasone and harvested for immunoblot analysis from 6 to 24 hours following steroid treatment. As shown in Figure 5C, Bcl-2 overexpression neither prevents nor alters the kinetics of Bim induction by dexamethasone. This demonstrates that the induction of Bim occurs prior to the onset of apoptosis. These data are consistent with the evidence that Bim promotes apoptosis at a point in the apoptotic cascade that is inhibited by Bcl-2 (33). Furthermore, the protection offered by Bcl-2 overexpression enhanced the induction of BimS, the most potent Bim isoform, which is localized exclusively at the mitochondria and is capable of activating Bax (14).

*Dexamethasone induces Bim in multiple models of glucocorticoid induced apoptosis.* We next examined whether the induction of Bim by dexamethasone is limited to murine T-cell lymphoma lines or is a general characteristic of dexamethasone induced apoptosis. Immunoblot analysis revealed that three isoforms, BimEL, BimL, and BimS were induced in the human acute T-cell leukemia line, CEM-C7 as early as 24 hours after dexamethasone treatment (Figure 6A). Thus, the induction of Bim expression by dexamethasone may be relevant to therapy of human leukemia with glucocorticoids. Furthermore, we investigated whether the induction of Bim by dexamethasone is limited to transformed cells by examining its expression in primary murine thymocytes. Mouse thymocytes are acutely sensitive to dexamethasone, and are a classic model system for studying the mechanism of corticosteroid induced apoptosis (34). Freshly isolated murine thymocytes were treated with dexamethasone in vitro. Dexamethasone induced apoptosis in this model system as early as two hours following glucocorticoid treatment (Figure 6B). Immunoblot analysis revealed that Bim, mainly the BimL isoform, was induced after two hours of dexamethasone treatment (Figure 6C). Furthermore, as little as 1 nM dexamethasone was sufficient to induce Bim within eight hours (Figure 6D). The expression of Bim increased with steroid concentration up to 100 nM. Higher doses of dexamethasone resulted in a reduction of Bim, an effect that is also apparent in the kinetic analysis (see Figure 6C), and likely the result of the swift induction of apoptosis and subsequent proteolysis in glucocorticoid treated thymocytes.

**Discussion**
Using Affymetrix oligonucleotide microarrays, we have identified a set of glucocorticoid regulated genes in the S49.A2 and WEHI7.2 T-cell lymphoma lines. As expected from earlier published work, we identified several genes that are glucocorticoid regulated and capable of tipping the balance between life and death towards death. For example, glucocorticoids suppress survival signals through either the direct repression of a survival gene, c-myc, or the induction of inhibitors of survival factors, including IκB-α and the regulatory subunit of the phosphatidylinositol 3-kinase. In addition, dexamethasone induces the expression of both the type I and type II inositol (1,4,5) trisphosphate receptors, consistent with evidence for a role of intracellular calcium release in apoptosis (20,35,36). However, because of its documented ability to initiate apoptosis, the discovery that bim is induced by dexamethasone is the principal achievement of the microarray experiments described in this report.

Dexamethasone induced the expression of the pro-apoptotic BH3-only protein Bim in not only the S49.A2 and WEHI7.2 murine T-cell lymphoma cell lines, but also the human T-cell leukemia cell line CEM-C7, and primary thymocytes. Multiple laboratories have demonstrated that elevated expression of Bim, particularly the short isoform, is sufficient to induce apoptosis in a variety of cell lines (13,33). Enforced expression of Bim leads to apoptosis in hematopoietic cells, even in the presence of cytokines, demonstrating that increased expression of Bim is sufficient to induce apoptosis in the absence of other apoptotic signals (37,38). While the activity of BimEL and BimL can be regulated by interaction with the microtubule-associated dynein motor complex, no such interaction occurs with BimS (39). In addition, the BH3 domain of Bim and full-length BimS bind Bax on the mitochondria, induce its oligomerization and promote the release of cytochrome c (14,40). Thus, the elevated expression of BimS following dexamethasone treatment is a death-promoting stimulus capable of directly initiating the apoptotic cascade.

Strasser and colleagues have demonstrated that immature T cells from bim−/− mice, while not completely resistant to glucocorticoid induced apoptosis, die with delayed kinetics following dexamethasone treatment (41). Because thymocytes from Bax−/−/Bak−/− mice are completely resistant to dexamethasone induced apoptosis (10), the incomplete resistance of bim-deficient thymocytes to
Dexamethasone indicates that another BH3-only protein is compensating for the absence of \textit{bim}. Among the other known BH3-only proteins, Bim is the only one we observe to be significantly induced during glucocorticoid induced apoptosis. It has been suggested that Bbc3/PUMA may promote glucocorticoid induced apoptosis in thymocytes since its transcript is induced following treatment with dexamethasone (42). However, we have not observed a glucocorticoid mediated increase in Bbc3/PUMA protein expression in either WEHI7.2 or primary mouse thymocytes (data not shown).

Although the induction of \textit{bim} by glucocorticoids has not previously been recognized, retrospective analysis of an earlier microarray report reveals evidence of \textit{bim} induction by dexamethasone. Tonko and colleagues, identified eight genes or expressed sequence tags coordinately regulated in both proliferating and G$_1$/G$_0$ arrested human CEM-C7 leukemia cells after glucocorticoid treatment (43). We find that one of the EST’s, AA682502, has high homology with the murine \textit{bim} mRNA. Indeed, in the present report, immunoblot analysis confirmed that Bim is induced in CEM-C7 cells treated with dexamethasone.

\textit{Bim} is likely a participant in glucocorticoid mediated apoptosis, however, the mechanism of its regulation by glucocorticoids is not clear. Since the human \textit{bim} promoter does not contain a glucocorticoid response element, and since cycloheximide blocks the induction of \textit{bim}, this response may not be directly mediated by the glucocorticoid receptor. Recently, \textit{bim} has been identified as a target of the forkhead family of transcription factors (44). Forkhead proteins like FKHRL1 are transcription factors whose activity is repressed when phosphorylated by pro-survival kinases such as phosphatidylinositol 3-kinase and protein kinase B/Akt. Because we observe an induction of the regulatory phosphatidylinositol 3-kinase p85 subunit, the induction of \textit{bim} may result from decreased survival kinase activity that leads to the activation of FKHRL1 (45,46).

Because glucocorticoid induced apoptosis requires receptor mediated regulation of gene transcription, the concept that glucocorticoid induced cell death is mediated through induction of a “death gene” emerged over three decades ago (47). While the efforts of many laboratories have identified glucocorticoid induced genes capable of reducing pro-survival signals, a glucocorticoid induced “death
gene” has remained elusive. Now we report the induction of the pro-apoptotic protein Bim by dexamethasone. Expression of Bim, particularly BimS, is capable of inhibiting the pro-survival activity of Bcl-2 and directly inducing the oligomerization of Bax. As bim-deficient thymocytes are not completely resistant to dexamethasone, further experiments are necessary to uncover which BH3-only protein works in concert with Bim to promote glucocorticoid induced apoptosis. Only when all compensatory proteins are removed to generate a resistant phenotype can the role of individual BH3-only proteins be investigated. Nevertheless, the identification of bim as a glucocorticoid induced death gene provides the foundation for developing a complete model of the glucocorticoid induced apoptotic pathway. Understanding the mechanism by which glucocorticoids induce the expression of Bim will reveal novel points along the death-promoting cascade at which apoptosis may be therapeutically accelerated.

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Figure legends

Figure 1: The synthetic glucocorticoid dexamethasone induces apoptosis in the WEHI7.2 and S49.A2 murine lymphoma cell lines. The extent of apoptosis in cultures of A) WEHI7.2 or B) S49.A2 cells grown in the absence (open squares) or presence (closed squares) of 1 µM dexamethasone for the times indicated was assessed by flow cytometric analysis of cellular DNA content. Symbols represent the mean ± standard error of at least three experiments. Inset immunoblot demonstrates higher expression of Bcl-2 in protein lysates from untreated S49.A2 cells than in WEHI7.2 cells.

Figure 2: Microarray expression analysis of two models of glucocorticoid induced apoptosis reveals common glucocorticoid regulated gene changes. A) Hierarchical clustering of 284 glucocorticoid regulated genes identified three groups of genes expressed similarly in both cell lines. Horizontal distance between branches of genes in the dendrogram indicates the relatedness of gene expression, with smaller distances indicating higher similarity. Differences in expression between the dexamethasone treated- and time-matched control populations are designated by a matrix of colored rectangles. A red hue indicates an induced gene, whereas green identifies a gene whose expression was reduced following dexamethasone treatment. The magnitude of the expression difference is indicated by the color saturation: bold colors represent large differences, dark colors represent small differences, and black indicates no change. Genes annotated with bold type are referred to in the text. B) The average change in gene expression over time for groups I, II, and III. Ordinate values are given as the signal log₂ ratio of fluorescence intensity between treatment and control populations. Thus, a ratio of x represents a 2^x–fold change in expression following dexamethasone treatment.

Figure 3: Bim is induced during dexamethasone induced apoptosis in S49.A2 and WEHI7.2 cells. A) The expression of Bim is induced after dexamethasone treatment in S49.A2 and WEHI7.2 cells. Total cellular RNA was subjected to oligonucleotide array analysis as detailed in Experimental Procedures.
Bars represent the hybridization signal intensity of the probe set for the AA796690 Bim expressed sequence tag with or without 1 µM dexamethasone for the times indicated. B) Northern blot showing the kinetics of *bim* induction in WEHI7.2 cells treated with 1 µM dexamethasone. The major and minor bands migrate at 5.7 and 3.8 kb, respectively. (C and D) Immunoblot analysis of Bim expression in C) S49.A2 and D) WEHI7.2 cells treated with 1 µM dexamethasone for the times indicated. Immunoblots for β-actin confirmed equal protein loading.

Figure 4: **The dependence of Bim induction and apoptosis on dexamethasone concentration are similar.** A) Apoptosis was measured by flow cytometric quantification of DNA content in WEHI7.2 cells treated with increasing concentrations of dexamethasone for 36 hours. Apoptosis is induced in WEHI7.2 cells with an EC₅₀ of 35 ± 5.9 nM. Symbols represent mean values ± standard error of three experiments. The line represents a non-linear fit of the data to a variable-slope sigmoidal dose-response equation. B) Immunoblot of Bim induction in WEHI7.2 cells cultured with increasing concentrations of dexamethasone for 36 hours.

Figure 5: **Elevated expression of Bim by dexamethasone requires de novo transcription and translation, and is not inhibited by Bcl-2 overexpression.** A) Northern blot for *bim* expression in WEHI7.2 cells cultured in 1 µM dexamethasone for 12 hours with or without 1 µg/mL actinomycin D or 10 µg/mL cycloheximide. B) WEHI7.2 cells were treated with or without 1 µM dexamethasone in the presence or absence of 0.1 µg/mL actinomycin D for 36 hours. Whole cell protein lysates were prepared and Bim expression was examined by immunoblotting. C) Immunoblot analysis of Bim expression in WEHI7.2 cells stably overexpressing Bcl-2 treated with 1 µM dexamethasone for the times indicated.

Figure 6: **Dexamethasone induced the expression of Bim both in human CEM-C7 cells and in primary mouse thymocytes.** A) Human CEM-C7 cells were cultured in the presence of 1 µM
dexamethasone for the times indicated. Protein lysates were prepared and assayed for Bim expression by immunoblot. B) Thymocytes are acutely sensitive to dexamethasone. Freshly isolated thymocytes were cultured in 1 µM dexamethasone for the times indicated. Apoptosis was quantified by cells containing sub G1 DNA. Values are represent the mean ± standard error of three experiments, each using pools of thymocytes from at least three mice. C) Expression of Bim protein is induced in primary thymocytes treated with 1 µM dexamethasone for the times indicated. D) Thymocytes were treated with 1, 10, 100, or 1,000 nM of dexamethasone or ethanol control for eight hours. Protein lysates were analyzed for Bim expression by immunoblot.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Supplementary Data, Table 1

| Name                                                                 | Accession | Probe Set Name | WEIH7.2 Signal Log(2) Ratios | S49.A2 Signal Log(2) Ratios |
|----------------------------------------------------------------------|-----------|----------------|------------------------------|----------------------------|
|                                                                      |           |                | 6 h  | 12 h  | 18 h  | 24 h | 6 h  | 12 h  | 18 h  | 24 h |
| CD3 antigen, gamma polypeptide                                       | M18228    | 100001_at      | -0.4 | -0.8  | -1    | -0.6 | 0    | 0     | 0    |
| IL-1alpha-3 protein mRNA, complete cds                              | M63660    | 100514_at      | 0    | 0.5   | 0.5   | 1.2  | 0    | 0     | 0    |
| Mus musculus proliferation-associated protein 1 mRNA, complete cds  | AA620184  | 100543_s_at    | 0    | 0.4   | 0.5   | 1    | 0    | 0.3   | 0.3  |
| RAD50 homolog (S. cerevisiae)                                        | U66887    | 100549_at      | 0.8  | 1.1   | 1.1   | 1.0  | 0    | 0     | 0    |
| Mouse T-cell alpha beta gamma chain                                  | X55123    | 100924_at      | -1.3 | -1.9  | -2.1  | -1.9 | 0    | 0     | 0    |
| Mus musculus cdc2/CDC28-like kinase 4 (Clk4) mRNA, partial cds       | U11680    | 100187_s_at    | 0    | 0     | 0.8   | 1    | 0    | 0     | 0    |
| Cathepsin L                                                          | X06086    | 101963_at      | 0    | -1    | -1.3  | -0.8 | -0.5 | 0     | 0    |
| B23050                                                                | M23158    | 101298_g_at    | 0.7  | 1.1   | 1.3   | 0.8  | 0    | 0.9   | 0    |
| Placenta and embryos oncoid integrating factor                       | M23484    | 101368_at      | 0    | 0.1   | -0.5  | -1   | 0    | 0     | 0    |
| Mus musculus inositol triphosphate receptor type 2  (Itrp2) gene, partial cds | AF031127 | 101441_i_at    | 0.7  | 1.1   | 0.8   | 1    | 0    | 1.2   | 0.9  |
| Mouse MHC (Qa) Q2-k gene for class I antigen, exons 1-3              | X06086    | 101963_at      | 0    | -1    | -1.3  | -0.8 | -0.5 | 0     | 0    |
| Mouse MHC (Qa) Q2-k gene for class I antigen, exons 1-3              | X06086    | 101963_at      | 0    | -1    | -1.3  | -0.8 | -0.5 | 0     | 0    |
| Mouse MHC (Qa) Q2-k gene for class I antigen, exons 1-3              | X06086    | 101963_at      | 0    | -1    | -1.3  | -0.8 | -0.5 | 0     | 0    |
| Gene Name                                                                 | Accession | Probe ID | Log2 Fold Change | Adjusted p-value | Description                        |
|--------------------------------------------------------------------------|-----------|----------|------------------|------------------|------------------------------------|
| M. musculus intermediate conductance potassium channel mIK1 mRNA, complete cds | AF042487  | 102198_at| 0.91             | 0.6              | Tumor necrosis factor receptor superfamily, member 7 |
| Early lymphoid specific transcription factor                              | L05547    | 102293_at| 0.6              | 1.4              | Mouse beta Fe receptor type II (FCRII) gene, complete cds |
| Mouse beta Fe receptor type II (FCRII) gene, complete cds                | M01312    | 102337_s_at| 2.5              | 2.0              | Carboxypeptidase A3, mast cell       |
| Mouse beta Fe receptor type II (FCRII) gene, complete cds                | J05118    | 102351_at| 0.9              | 1.4              | Jun proto-oncogene related gene dl  |
| M. musculus cDNA3, 5 end                                                  | AA822174  | 102370_at| 0.7              | 0.6              | Tumor necrosis factor superfamily, member 7 |
| M. musculus ETO/MTG8-related protein ETO-2 mRNA, complete cds            | AF038029  | 102397_at| 0.9              | 0.6              | Early lymphoid specific transcription factor |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102414_i_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102415_r_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| Gap junction membrane channel protein beta 6                              | Z70023    | 102571_at| 0.7              | 0.6              | T-complex-associated testis expressed 1 |
| Mouse mRNA for TL antigen, complete cds                                   | IA842968  | 102384_at| 0.9              | 1.1              | Large multifunctional protease 7     |
| Mouse mRNA for TL antigen, complete cds                                   | IA842968  | 102384_at| 0.9              | 1.1              | Large multifunctional protease 7     |
| M. musculus cDNA3, 5 end                                                  | AA822174  | 102370_at| 0.7              | 0.6              | Tumor necrosis factor superfamily, member 7 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102414_i_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102415_r_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| Gap junction membrane channel protein beta 6                              | Z70023    | 102571_at| 0.7              | 0.6              | T-complex-associated testis expressed 1 |
| M. musculus cDNA3, 5 end                                                  | AA822174  | 102370_at| 0.7              | 0.6              | Tumor necrosis factor superfamily, member 7 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102414_i_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102415_r_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| Gap junction membrane channel protein beta 6                              | Z70023    | 102571_at| 0.7              | 0.6              | T-complex-associated testis expressed 1 |
| M. musculus cDNA3, 5 end                                                  | AA822174  | 102370_at| 0.7              | 0.6              | Tumor necrosis factor superfamily, member 7 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102414_i_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102415_r_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| Gap junction membrane channel protein beta 6                              | Z70023    | 102571_at| 0.7              | 0.6              | T-complex-associated testis expressed 1 |
| M. musculus cDNA3, 5 end                                                  | AA822174  | 102370_at| 0.7              | 0.6              | Tumor necrosis factor superfamily, member 7 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102414_i_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102415_r_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| Gap junction membrane channel protein beta 6                              | Z70023    | 102571_at| 0.7              | 0.6              | T-complex-associated testis expressed 1 |
| M. musculus cDNA3, 5 end                                                  | AA822174  | 102370_at| 0.7              | 0.6              | Tumor necrosis factor superfamily, member 7 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102414_i_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| M. musculus mRNA for NIPSNAP2 protein                                     | U28423    | 102415_r_at| 0.9              | 0.6              | Gap junction membrane channel protein beta 6 |
| Description                                                                 | Accession   | Description                      | Accession   |
|----------------------------------------------------------------------------|-------------|----------------------------------|-------------|
| Mus musculus mRNA for CD47, complete cDNA                                 | AB012693    | 103611_at                       | 0 0.4 0.7 1 -0.6 0 0 -0.4 |
| U1-M-BH1-a06-0-UI.x1 Mus musculus cDNA, 3 end                            | AW047899    | 103614_at                       | 0 -0.6 -0.9 -1.1 0 0 0 0 |
| Lymphoid enhancer binding factor 1                                         | D16503      | 103629_g_at                     | -0.4 -0.8 -1.4 -0.8 -0.3 -0.3 0 -0.5 |
| U1-M-AP0-e08-0-UI.x1 Mus musculus cDNA, 3 end                             | AI936552    | 103657_i_at                     | -1 -1.2 -1.1 -1 -0.4 -0.6 -0.9 -0.9 |
| U1-M-AP0-e08-0-UI.x1 Mus musculus cDNA, 3 end                             | AI936552    | 103658_f_at                     | -1.3 -1.2 -1.3 -1 -0.4 -0.6 -1 0 |
| U1-M-AP0-adv-d11-0-UI.x1 Mus musculus cDNA, 3 end                         | AI939232    | 103685_at                       | 0.5 0.7 0.8 1.4 0 0 0 0 |
| m1s6b06.x1 Mus musculus cDNA, 5 end                                       | AI646638    | 103699_i_at                     | -0.5 0 -0.5 0 0 0.5 1 0.6 |
| sk33d12.x1 Mus musculus cDNA, 3 end                                       | AI648965    | 103783_at                       | 0 0.5 0.7 1.2 0 0 0 0 |
| Mus musculus neural visinin-like protein 3 mRNA, complete cds             | AF085192    | 103835_f_at                     | 1.1 0.9 0.9 0.6 0.6 0.7 0.6 0 |
| UI-M-AM0-ado-g-04-0-UI.x1 Mus musculus cDNA, 3 end                        | AW108492    | 103907_at                       | 0 -1.3 -0.7 -1.3 0 0 0 -0.3 |
| M.musculus mRNA for ATP receptor                                           | X14896      | 103971_at                       | 0.8 1.2 1 0 0 0 0 0 |
| U1-M-BH1-ang-b-04-0-UI.x1 Mus musculus cDNA, 3 end                        | AW050325    | 103995_at                       | 0 0.8 0.8 1.2 0 0 0 -0.5 |
| Mus musculus viral envelope like protein (G7e) gene, complete cDNA        | U60488      | 104333_at                       | 0.4 0 0.4 0.5 0.5 0.7 0.9 1.1 |
| M. musculus mRNA for a growth factor-inducible immediate early gene (3CH134) | X61940      | 104598_at                       | 0 0 0 0 0 1 0 1.3 |
| U1-M-BH1-ang-b-08-0-UI.x1 Mus musculus cDNA, 3 end                        | AI852578    | 104578_f_at                     | 0.5 0.7 0.6 1 0 0 0.7 0.8 |
| Mouse mRNA for a growth-factor-inducible early gene (3CH134)              | X61940      | 104598_at                       | 0 0 0 0 0 1 0 1.3 |
| U1-M-BH1-ang-b-08-0-UI.x1 Mus musculus cDNA, 3 end                        | AI852578    | 104559_at                       | 1.2 1.5 1.6 1.7 0 0 0 0 |
| M. musculus viral envelope like protein (G7e) gene, complete cDNA        | AI852578    | 104559_at                       | 1.2 1.5 1.6 1.7 0 0 0 0 |
| U1-M-BH1-ang-b-08-0-UI.x1 Mus musculus cDNA, 3 end                        | AI852578    | 104578_f_at                     | 0.5 0.7 0.6 1 0 0 0.7 0.8 |
| M. musculus viral envelope like protein (G7e) gene, complete cDNA        | AI852578    | 104579_f_at                     | 0 0.8 0.4 0.7 0 0 0 0.4 |
| U1-M-BH1-ang-b-08-0-UI.x1 Mus musculus cDNA, 3 end                        | AI852578    | 104579_f_at                     | 0 0.8 0.4 0.7 0 0 0 0.4 |
| AMP deaminase 3                                                           | D88994      | 104671_at                       | 0.7 0.7 1.8 1.3 0.4 0 0.8 0 |
| M. musculus cathespin E gene, exon 1, partial                              | AI009840    | 104696_at                       | 1 1.9 2.3 2.5 0.5 1.5 2 2.1 |
| Myelocytomatosis oncogene                                                 | L00039      | 104712_at                       | -0.9 -1.1 -1.7 -1.3 -0.4 -0.9 -0.6 -0.6 |
| U1-M-BH1-ang-b-08-0-UI.x1 Mus musculus cDNA, 3 end                        | AI852578    | 104712_at                       | -0.9 -1.1 -1.7 -1.3 -0.4 -0.9 -0.6 -0.6 |
| M. musculus predicted GTP binding protein (IRG-47) mRNA, complete cDNA    | M63630      | 104750_at                       | 0 0 0 0 0 1 0 0 |
| Protein tyrosine phosphatase, non-receptor type 18                        | U39833      | 92273_at                        | 0 0 -1.1 -1.3 0 0 0 0 |
| Mus musculus predicted GTP binding protein (IRG-47) mRNA, complete cDNA  | M63630      | 104750_at                       | 0 1.1 0.8 0 0 0 0 0 |
| Mouse protein tyrosine phosphatase (70zpep) mRNA, complete cDNA           | M90388      | 92536_at                        | 0.9 1.3 0 1.3 0.8 0.7 1.4 1.6 |
| Mouse protein tyrosine phosphatase (70zpep) mRNA, complete cDNA           | M90388      | 92536_at                        | 0.9 1.3 0 1.3 0.8 0.7 1.4 1.6 |
| Mouse protein tyrosine phosphatase (70zpep) mRNA, complete cDNA           | M90388      | 92536_at                        | 0.9 1.3 0 1.3 0.8 0.7 1.4 1.6 |
| Mouse intracisternal A-particle-related retroviral elements and envelope pseudogene | M73818      | 92402_at                        | 0 1.4 1.2 1.4 0 0 0 0 |
| Zinc finger protein 1                                                      | X16493      | 92443_i_at                      | 0.5 0.5 0.5 0.7 0.7 0.8 1.1 1 |
| Zinc finger protein 1                                                      | X16493      | 92444_f_at                      | 0.8 0.9 0.5 1.2 1.1 1.1 1.4 1.4 |
| M. musculus mRNA for KRAZ-containing zinc-finger protein KRAZ1, complete cDNA | AB020404    | 92480_f_at                      | 0 0.7 0.9 2 0 0 0 0.4 |
| M. musculus mRNA for adenylate kinase isozyme 3, partial cDNA             | AB020203    | 92492_at                        | 0 0.8 0.6 1.5 0 0 0.8 0 |
| Gene Name | Accession | Gene Description | log2 Fold Change |
|-----------|-----------|------------------|-----------------|
| **M. musculus spermidine synthase gene** | Z67748 | 92540_f_at | -0.5 -0.6 -0.9 -0.4 -0.6 -0.5 -1.4 |
| **UI-M-ANI-af-g-12-0-UL.s1** | M. musculus cDNA, 3 end | A1B46545 | 92589_at | 0 0.5 0.7 1.2 0 0 0 0 |
| **M. musculus vesicle transport protein (munc-18c) mRNA, complete cds** | U19521 | 92648_at | 0 0.9 0.6 1.5 0 0 0 0.6 |
| **CD3 antigen, delta polypeptide** | X02239 | 92683_at | -0.4 -0.7 -1.1 -1 0 0 0 0 |
| **M. musculus membrane glycoprotein gene** | Z22552 | 92778_i_at | 0 0 0 0 1 0 |
| **M. musculus membrane glycoprotein gene** | Z22552 | 92779_f_at | 0 0 0 0 3.3 5.7 5.8 0 |
| **Mouse endogenous proviral superantigen (Mtv-7 sag), and envelope protein (env) gene, 3 end** | M90535 | 92780_f_at | 0 0 -0.9 0 3.9 5.6 5.5 6.2 |
| **UI-M-ANI-af-e-09-0-UL.s1** | Mus musculus cDNA, 3 end | AI846522 | 92820_at | 1.5 1.8 1.6 3 2.9 0 2.0 0 |
| **vn58c11.r1** | Mus musculus cDNA, 5 end | AA590675 | 92876_at | 0 0 0 1 0.9 1.6 2.1 2.0 |
| **UI-M-AQo-ah-03-0-UL.s1** | Mus musculus cDNA, 3 end | AI835257 | 93025_at | 0 0 0 0 0 0.6 1 1.1 |
| **M. musculus bg1 mRNA** | Z16410 | 93040_at | 0.5 1.1 1.1 1.3 0.6 1 2 2 |
| **Mouse T-cell receptor germline beta-chain gene constant region (CT)** | M26056 | 93104_at | -0.5 -1.5 -2.4 -1.6 0 0 0 0 |
| **M. musculus H-2K gene for MHC class I antigen H-2K (allele b), exons 1 to 3 and joined CDS** | V00746 | 93120_f_at | 0 0 -1 -1.1 0 0 0 0 |
| **af76803.x1** | M. musculus cDNA, 3 end | A1995146 | 93195_at | 0 1.3 1.1 1.2 0 0 0 0 |
| **Interferon activated gene 203** | AF022371 | 93321_at | 0 -0.9 -1.1 -1.1 0 0 0 0 |
| **Histidine decarboxylase cluster** | X37437 | 93328_at | 0 0 2.5 3.9 0 0 0 0 |
| **Procollagen, type VII, alpha 1** | U32107 | 93382_at | 3 1.3 0 0 0 2.4 1.7 0 |
| **Telemic repeat binding factor 2** | AF003000 | 93413_at | 0 0 0 0 0 0 1 0.7 |
| **Mouse multidrug-resistance protein (MDR1) gene, exons 1 and 2** | M60348 | 93414_at | -0.5 -1.3 -0.8 0 0 0 0 0.3 |
| **Tumor necrosis factor (ligand) superfamily, member 11** | AF019048 | 93416_at | 0.6 0.6 2.1 2 0 0 0 0 |
| **Mouse, glutathione transferase GT 7 mRNA, complete cds** | J08792 | 93500_at | 3.4 3.5 3.3 4 0 0 0 1.7 |
| **Mouse mRNA for double IIM protein-1, complete cds** | A183492 | 93511_at | 0 0 0 0 0 0 0 0 |
| **Mus musculus cosmid CMX137 containing part of Nsdhl** | A146482 | 93636_at | 0 0.4 0.5 0 0 0 0 0.9 |
| **Period homolog (Drosophila)** | X62940 | 93728_at | 2.1 1.8 0 0 3.3 1.2 4.1 1.6 |
| **mr96b07.r1** | Mus musculus cDNA, 5 end | A146482 | 93728_at | 0 0.4 0.5 0 0 0 0 0.9 |
| **Transforming growth factor beta 1 induced transcript 4** | X62940 | 93728_at | 0 0.7 0.9 1.2 -0.4 0 0 0 |
| **Gelsolin** | J04953 | 93750_at | 0 1 1 1.4 0 0 0 0 |
| **UI-M-BH2.3-ahq-d-05-0-UL.s1** | Mus musculus cDNA, 3 end | AW123952 | 93797_g_at | 0.5 0.9 1.3 0.8 0 0 0 0 |
| **Histocompatibility 2, T region locus 10** | M13244 | 93865_s_at | -0.6 -2 -2.2 -2.7 -0.3 -0.4 -0.4 -0.8 |
| **M. musculus cosmid CMX137 containing part of Nsdhl** | AL021127 | 93868_at | 1.1 1.4 0.6 0 0 0 0 0.6 |
| **Parkinje cell protein 1** | M21530 | 93895_s_at | 1 1.7 2 2.2 0 0.5 1.3 0.8 |
| **M. musculus mRNA for B-cell-specific coactivator BOB.1/BOB.1** | Z54283 | 93915_at | 0.4 1.2 0.6 1.7 0 0 0 0.5 0.9 |
| **M. musculus h2-calponin cDNA** | Z19543 | 94004_at | 0.7 1.9 1.3 1.6 0 0 0 0.4 0.6 |
| **Mouse proteoglycan core protein mRNA, complete cds** | M34603 | 94085_at | 0.4 0.6 0 0.7 0.6 1.7 3.3 2.9 |
| **T-cell receptor beta, variable 8.2** | L37871 | 94202_at | 0 -0.7 -0.6 -1.2 0 0 0 0 0 |
| **UI-M-BH1-ahq-c-08-0-UL.s1** | Mus musculus cDNA, 3 end | AW045202 | 94208_at | 0 0.5 0.5 0.4 0.7 0.4 1.3 0 |
| **UI-M-BH1-ahq-c-08-0-UL.s1** | Mus musculus cDNA, 3 end | AW045202 | 94209_g_at | 0 0.4 0.5 0.7 0.7 0.6 1.1 0 |
| **M. musculus (strain C57Bl/6) mRNA sequence** | M74123 | 94224_s_at | 0.7 -0.7 -1.3 -1.3 -0.4 0 0 0.6 |
| **UI-M-BH2.3-ahq-d-05-0-UL.s1** | Mus musculus cDNA, 3 end | A1B45237 | 94254_at | 1.3 1.9 1.6 1.8 0.5 0 0.4 0 |
| **UI-M-BH2.3-ahq-d-05-0-UL.s1** | Mus musculus cDNA, 3 end | A1B45237 | 94255_g_at | 1.2 2.3 2 2.3 0 0 0 0.5 |
| **UI-M-AH1-agc-h-01-0-UL.s1** | Mus musculus cDNA, 3 end | A1B49533 | 94256_at | 1.4 2.1 1.8 2.1 0.5 0 0.4 0.6 |
| Gene Name | Description | Accession Number | Affymetrix ID | Ratio 1 | Ratio 2 | Ratio 3 | Ratio 4 | Ratio 5 | Ratio 6 | Ratio 7 | Ratio 8 | Ratio 9 |
|-----------|-------------|-----------------|---------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| FK506 binding protein 3, 51 kDa | | U16959 | 94297_at | 1.2 | 1.4 | 1.2 | 1.6 | 0 | 0.4 | 0.4 | 1.0 |
| M. musculus gene encoding hexokinase II, exon 1 (and joined CDS) | | Y11666 | 94375_at | -0.5 | -0.2 | -0.6 | -1.2 | 0 | -0.4 | -0.4 | -1.2 |
| Interferon activated gene 102 | | M11417 | 94774_at | -1.9 | -1.7 | -1.9 | -1.6 | 0 | -0.4 | -0.4 | -1.4 |
| Mouse avian erythroblastosis virus E26 oncogene homolog 1 (ets-1) mRNA | | M97590 | 94720_at | 0 | 0.8 | -0.7 | -1.2 | 0 | 0.5 | 0.3 | 0.0 |
| Mouse tyrosine phosphatase (PTP-1), (EC2.7.1.112) mRNA | | M97590 | 94929_at | 1.1 | 1.4 | 1.5 | 0 | 0 | 0 | 0 | 0 |
| inositol 1,4,5-triphosphate receptor 1 | | X15373 | 94977_at | 1.5 | 2.3 | 3.0 | 0 | 0.9 | 1.2 | 1.4 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Mouse cDNA, 5 end | | AI84992 | 94952_at | -0.8 | -0.9 | -0.6 | -1.1 | 0 | 0 | 0 | 0 |
| Gene Name | Accession | Description | Fold Change | t-statistic | p-value | Fold Change | t-statistic | p-value | Fold Change | t-statistic | p-value |
|-----------|-----------|-------------|-------------|-------------|---------|-------------|-------------|---------|-------------|-------------|---------|
| Fatty acid desaturase 2 | U51167 | MAU musculus cDNA, 5 end | 95693_at | 0 | 0 | 0.6 | 0 | 0 | -0.6 | 0 | 0.7 |
| U1-M-BH1-2 interrupts | AW124069 | MAU musculus cDNA, 3 end | 95701_at | -1.1 | -1.6 | -1.7 | -1.8 | 0 | 0 | 0 | 0 |
| Mouse A-X actin mRNA, complete cds | J04181 | MAU musculus cDNA, 3 end | 95705_s_at | -0.5 | -1.3 | -1.3 | -2.1 | 0 | 0 | 0.3 | 0 |
| Mouse mRNA for Mac-2 antigen | X1684 | MAU musculus cDNA, 3 end | 95706_at | 0 | 0 | 0.7 | 0 | 1 | 0 | 0 | 0.8 |
| U1-M-AK1-aat-e-01-0-UL1 | AIB4106 | MAU musculus cDNA, 3 end | 95713_at | 0 | 1.4 | 1.5 | 0 | 0 | 0.8 | 0 |
| Mouse mRNA for GDH | AA822883 | MAU musculus cDNA, 3 end | 95727_f_at | 0 | -0.5 | -0.1 | 0 | 0 | -0.5 | -0.6 | 0.5 |
| Mouse mRNA for GDH | AIB48018 | MAU musculus cDNA, 3 end | 96096_f_at | 0 | 0 | 0.7 | 1 | 0 | 0 | 0 |
| U1-M-BH2-2 inserts | AIB53996 | MAU musculus cDNA, 3 end | 96238_at | -1.2 | -0.9 | -0.5 | 0 | 0 | 0 | 0 | 0 |
| Calmodulin | M19531 | MAU musculus cDNA, 3 end | 96252_at | 0.9 | 1.6 | 1.7 | 0 | 0 | 0 | 0 |
| Starfrit gene 4 | M62606 | MAU musculus cDNA, 3 end | 9642_at | 0.5 | 0.9 | 1.0 | 0 | 0 | 0 | 0 |
| U1-M-AK1-aeu-e-01-0-UL1 | U39827 | MAU musculus cDNA, 3 end | 96533_at | 2.4 | 2.6 | 3.1 | 3.5 | 1.8 | 1.7 | 2 | 2 |
| Phospholipid nitrolysin 2 kinase, regulatory subunit, poly peptide 1 (p55 alpha) | U50413 | MAU musculus cDNA, 3 end | 96592_at | 1.2 | 2.3 | 1.3 | 2.5 | 0.9 | 0 | 1.1 | 1.1 |
| U1-M-BH1-2 inserts | U20793 | MAU musculus cDNA, 3 end | 96596_at | 1.8 | 2.5 | 1.6 | 1.3 | 0 | 0 | 0 | 1.3 |
| U1-M-BH1-2 inserts | AIB787183 | MAU musculus cDNA, 3 end | 96605_at | 0 | 2.1 | 3.3 | 2.7 | 0 | 1.1 | 1.3 | 0.8 |
| U1-M-BH1-2 inserts | AIB63172 | MAU musculus cDNA, 3 end | 96623_at | 0 | 0.6 | 0.6 | 1 | 0 | -0.5 | -0.6 | 0 |
| U1-M-BH1-2 inserts | AIB64156 | MAU musculus cDNA, 3 end | 96640_at | -0.7 | -0.9 | -1.1 | -1.2 | 0 | 0 | 0 | 0 |
| Sperminde/spermine N1-acetyl transferase | L10244 | MAU musculus cDNA, 3 end | 96657_at | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| U1-M-AF1-act-a-07-0-UL1 | AIB47034 | MAU musculus cDNA, 3 end | 96662_at | 0.5 | 0 | -0.3 | 0 | 1.1 | 2.3 | 1.7 | 2 |
| U1-M-BH1-2 inserts | AW123564 | MAU musculus cDNA, 3 end | 96672_at | 1 | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| U1-M-AK1-aat-e-02-0-UL1 | AIB37110 | MAU musculus cDNA, 3 end | 96696_at | 0 | -0.4 | -0.6 | -0.5 | 0 | -0.6 | -1.1 | 0 |
| Mouse mRNA for GDH | U41765 | MAU musculus cDNA, 3 end | 96738_at | 0 | 1.1 | 1.5 | 0 | 0 | 0 | 1 |
| ak29g02.y1 | AIB76931 | MAU musculus cDNA, 5 end | 96918_at | 0 | -0.4 | -0.9 | -1.2 | 0 | 0 | 0 | 0 |
| U1-M-AK1-aeu-e-01-0-UL1 | AJ133523 | MAU musculus cDNA, 3 end | 96998_at | 0 | 0 | 0 | 1.3 | 0.6 | 1.4 | 0 |
| Prosaposin | U57999 | MAU musculus cDNA, 3 end | 97114_at | 0 | 0.8 | 1.1 | 1 | 0 | 0.4 | 0.3 | 1.1 |
| Fibrinogen B chain | U423884 | MAU musculus cDNA, 3 end | 97124_at | -0.5 | -0.4 | -1.4 | -0.6 | 0 | 0 | 0 | 0 |
| Mouse mRNA for brain tyrosine receptor, partial cds | D38218 | MAU musculus cDNA, 3 end | 97126_at | 2.5 | 3.1 | 1.7 | 5 | 0 | 0 | 0 | 0 |
| U1-M-BH1-2 inserts | AA413015 | MAU musculus cDNA, 3 end | 97154_f_at | 0.7 | 1.1 | 1.7 | 2.2 | 0 | 0 | 0 | 3.7 |
| U1-M-BH1-2 inserts | AW061318 | MAU musculus cDNA, 3 end | 97255_at | 0 | 0.5 | 0 | 0 | 0.6 | 0.8 | 1.4 |
| U1-M-AH1-agy-d-05-0-UL1 | AIB46823 | MAU musculus cDNA, 3 end | 97383_at | 0.6 | 1.2 | 1.5 | 2.1 | 0 | 0 | 0 | 0 |
| U1-M-BH1-2 inserts | AW048113 | MAU musculus cDNA, 3 end | 97429_at | 0.6 | 1.1 | 1.4 | 1 | 0 | 0 | 0 | 0 |
| U1-M-AQ9-aug-a-06-0-UL1 | AIB85461 | MAU musculus cDNA, 3 end | 97449_at | 0.8 | 0.8 | 1 | 0 | 0.5 | 0.9 | 0.5 |
| Jc74b03.y1 | AA986258 | MAU musculus cDNA, 5 end | 97450_s_at | 1 | 1.1 | 0.6 | 0.4 | 0.4 | 0.7 | 1 | 0.5 |
| HLA-A, D region locus 1 | M69906 | MAU musculus cDNA, 5 end | 97540_f_at | 0 | -0.4 | -0.9 | -1.2 | 0 | 0 | 0 | 0 |
| Mouse, glutathione transferase GT193 mRNA, 3 end | J03953 | MAU musculus cDNA, 3 end | 97681_f_at | 1.5 | 1.7 | 2.2 | 2.2 | 0 | 0 | 0 | 0 |
| Mouse mRNA for immunoglobulin superfamily member CTLA-4 | X05719 | MAU musculus cDNA, 3 end | 97718_at | 0 | 0 | 0 | 1.5 | 1.8 | 3.5 | 3.7 |
| U1-M-BH1-2 inserts | AIB53802 | MAU musculus cDNA, 3 end | 97833_at | 0 | -0.5 | -0.7 | -1.1 | 0 | -0.3 | -0.6 | 0 |
| U1-M-BH1-2 inserts | AIB53802 | MAU musculus cDNA, 3 end | 97834_g_at | 0 | 0 | -0.7 | -1.1 | 0 | -0.5 | -0.4 | -0.7 |
| vu95f08.x1 | AA762325 | MAU musculus cDNA, 3 end | 97859_at | 0 | 1.4 | 0.8 | 1.2 | 0 | 0 | 0 | 0 |
| Mouse mRNA for Clat1, complete cds | AB031386 | MAU musculus cDNA, 3 end | 97885_at | 0.8 | 1 | 1.4 | 1.6 | 0 | 0.8 | 1.1 | 1 |
| Gene Name                                                                 | Accession   | Description                                                                 | Log2 Fold Change | Expression Levels |
|--------------------------------------------------------------------------|-------------|------------------------------------------------------------------------------|------------------|-------------------|
| Mus musculus cDNA, 3 end                                                 | AW046181    | 0.6 1.4 1.4 0 0 0.6 0.7                                                     |                 |
| Mucpualus gene for KF-1, complete cDNA                                   | AI8012161   | 0 0.6 1.1 0.9 0 0 0.7                                                       |                 |
| Mucpualus friend of GATA-1 (FOG) mRNA, complete cDNA                     | AF006492    | -0.3 -0.7 -1 -1.2 -0.6 -0.4 -0.6 -0.9                                      |                 |
| Mucpualus mRNA for unc-18 homologue, complete cDNA                       | D45903      | 0.7 1.6 1.8 1.6 0 0 0 0                                                      |                 |
| Mus musculus mRNA, 3 end                                                 | AI019193    | -0.8 -1.1 -1.7 -1.6 0 0 0 0                                                  |                 |
| Transcription factor 7, T-cell specific                                  | X61385      | -0.5 -1.1 -1.1 -1.8 0 0 0.5 -0.6                                              |                 |
| Interferon concensus sequence binding protein                            | M12489      | 0.9 1.1 0.3 0.7 0 0 0 0                                                      |                 |
| Mus musculus cDNA, 5 end                                                 | AA790307    | -0.3 -1.6 -2.8 -3.2 0 0 0 0                                                  |                 |
| U3-M-BH0-air-e-09-0-UL.s1 Mus musculus cDNA, 3 end                       | AIB53375    | 0.9 1.3 1.2 1.3 0 0 0 0                                                      |                 |
| Mucpualus LIM domain transcription factor LMO4 mRNA, complete cDNA       | AF074600    | 0 0.8 0.5 0.7 0.9 1.1 1.4 1.8                                                |                 |
| Mucpualus spermidine aminopropyltransferase (Mnapruu) mRNA, complete cDNA| AA790307    | -0.8 -1.1 -1.1 -1.7 0 0 0 0                                                  |                 |
| Mouse mRNA for thymus-leukemia (TL) antigen Tla(a)-3 exons 3-6           | X03052      | -0.5 -1 -0 0 -0.2 -0.3 -0.6 -0.7                                            |                 |
| Mucpualus somatostatin receptor type 2 (sat2) gene, alternatively spliced| AF008914    | -1.5 -1.9 -2.6 -2.3 0 0 0 0                                                  |                 |
| Mucpualus somatostatin receptor type 2 (sat2) gene, alternatively spliced| AF008914    | -0.7 -1.2 -1.4 -2.5 0 0 0 0                                                  |                 |
| Mouse (strain CBA) interferon-induced mutant Mx1 protein pseudo gene mRNA, complete cDNA | M21038 | -0.9 -1.3 -1.2 -0.7 1.2 1.6 0 0                                              |                 |
| BH3 interacting domain death agonist                                     | U75506      | 0 -0.9 -1.7 -1.4 0 0 0 -0.5                                                  |                 |
| Mucpualus somatostatin receptor type 2 (sat2) gene, alternatively spliced| AF008914    | -0.7 -1.2 -1.4 -2.5 0 0 0 0                                                  |                 |
| Interferon activated gene 204                                            | M14140      | 0 0.7 0.5 0 0 0 0 0 0                                                      |                 |
| Mucpualus cyclin G2 mRNA, complete cDNA                                   | U95826      | 0 0.8 0.7 0.4 0.8 1 1 1.8                                                    |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98818       | 0 0.7 0 0 0.9 0.9 2.2 0                                                     |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98822       | 0 0 0 0 0 0 0 0 0 0                                                        |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98859       | 1.3 2 1.8 1.5 0 1.1 1.7 1.7                                                 |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98554       | 0 1.2 0 0 0 0 0.8 2.3 2.5                                                   |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98630       | 0 1 0.4 0 0 0 0 0 0                                                        |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98631_g     | 1.2 1.4 0.6 0 0 0.3 0.6 0                                                   |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98631_g     | 1.2 1.4 0.6 0 0 0.3 0.6 0                                                   |                 |
| Glucocorticoid receptor 1                                                | X04435      | 0.9 0.7 0 0 0 0 0.9 2.2                                                     |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | 98860       | 0 0 0 0 0 0 0 0 0 0                                                        |                 |
| Acid phosphatase 5, tartrate resistant                                   | M99054      | 1.3 2 1.8 1.5 0 1.1 1.7 1.7                                                 |                 |
| U3-M-ACL-ace-e-07-0-UL.s1 Mus musculus cDNA, 3 end                        | AIB6118     | 1 0.9 1.2 1.1 0 0 0 0                                                        |                 |
| U3-M-AH1-apg-e-11-0-UL.s1 Mus musculus cDNA, 3 end                        | AIB49082    | 0 1 0.6 1 0 0 0 0 0                                                        |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | U16165      | 0 1 2.3 1.8 0 0 0 1.4                                                       |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | U13295      | 0 0 0.8 0.8 0 1.3 1.5 1.1                                                    |                 |
| Mouse RNA helicase and RNA-dependent ATPase from the DEAD box family mRNA, complete cDNA | L25125 | 0 1.2 1.1 1.5 0.4 0.4 0.6 0.7                                               |                 |
| Mouse mRNA Interferon-induced 15-KDa protein                              | 99024       | 0 0 0.8 0.8 0 1.3 1.5 1.1                                                    |                 |
| Mouse mRNA Interferon-induced 15-KDa protein                              | 99025       | 0 1.2 1.1 1.5 0.4 0.4 0.6 0.7                                               |                 |
| Thyroid hormone receptor alpha                                            | U09504      | -1.2 -0.8 -0.8 0 0 0 0 0 0                                                  |                 |
| T-complex-associated testis expressed 3                                  | U21673      | 0 0 0.3 -0.7 0 0 0 0 0 0                                                    |                 |
| U3-M-BH2.3-ace-b-10-0-UL.s1 Mus musculus cDNA, 3 end                      | AW120896    | 0 0 0 0 0 0 0 0 0 0 0 0 0.8 1                                              |                 |
| U3-M-BH2.3-ace-b-10-0-UL.s1 Mus musculus cDNA, 3 end                      | AW120896    | 0 0 0 0 0 0 0 0 0 0 0 0 0.8 1                                              |                 |
| C76102 Mus musculus cDNA, 3 end                                           | C76102      | 1.2 0 0 0.9 0.4 0.7 1.1 0.8                                                  |                 |
| U3-M-BH1-apg-g-12-0-UL.s1 Mus musculus cDNA, 3 end                        | AW047012    | 0.7 1.2 0 0 0 0 0 0 0 0 0 0.8 0.8                                             |                 |
| Mucpualus mRNA Interferon-induced 15-KDa protein                          | U49507      | 1.6 2.4 3.4 3 0 2.3 2.7                                                    |                 |
| Mouse 3 mRNA for beta-galactosidase specific lectin (14kDa)               | X15986      | 0 -0.7 -1.6 -1.7 -0.3 -0.4 -0.8 -0.7                                       |                 |
| U3-M-BH2.3-ace-b-10-0-UL.s1 Mus musculus cDNA, 3 end                      | AIB52919    | 0 -0.9 0 0 -0.9 -1.3 -1.6 -1.6                                              |                 |
| Mus musculus cDNA, 3 end                                                 | AIB52919    | -0.9 1 0.8 0.7 0 0 0 0                                                      |                 |
| Mus musculus cDNA, 3 end                                                 | AIB52919    | -0.9 1 0.8 0.7 0 0 0 0                                                      |                 |
| M12481 Mouse cytoplasmic beta-actin mRNA (_5_, _M_, _3 represent transcript regions 5 prime, middle, and 3 prime respectively) | ActinMur/M12481_5_at | 0.3 | 0.4 | 0.5 | 1.2 | 0 | 0 | 0 | 0 |
| M37897 Mouse interleukin 10 mRNA, complete cds | AFFX-MurIL10_at | 0 | 2.9 | 0 | 0 | 0.8 | 0.6 | 1 | 0 |
Microarray analysis uncovers the induction of the pro-apoptotic BH3-only protein Bim in multiple models of glucocorticoid induced apoptosis
Zhengqi Wang, Michael H. Malone, Huiling He, Karen S. McColl and Clark W. Distelhorst

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